Cellular and regulatory roles of human DNA topoisomerase IIβ

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Abstract

DNA topoisomerase IIβ is an enzyme that manipulates DNA topology. Its mechanism of action involves introduction of transient breaks in the DNA phosphodiester backbone enabling the passage of another DNA strand through the break. TOP2β has been suggested to be implicated in chromosomal translocations associated with development of secondary malignancy induced by anti-cancer drugs that targeting TOP2 enzymes. TOP2β also plays a role in transcriptional regulation, possibly via the introduction of transient DNA breaks at promoters enabling local remodelling of chromatin.

To investigate the isoform-specific contribution of TOP2β in cytotoxicity and genotoxicity of TOP2 targeting drugs, a Nalm6 cell line previously knocked out for TOP2β and its parent cell line were utilised in this study. Their response to drugs idarubicin or daunorubicin alone or in combination with Ara-C has been investigated.

Gene expression was investigated by RNA-seq of the Nalm6 cell lines. Analysis showed that expression of a number of genes was altered and this was confirmed by RT-qPCR on the same cell line. To be sure which genes changed a new Nalm6 TOP2β knockout was generated by CRISPR cas9. In the newly created knockout, RT-qPCR was carried out to confirm which genes still had altered gene expression. Strikingly, *CBR1*, a gene implicated in doxorubicin-induced cardiotoxicity, was found to be down regulated in both Nalm6 TOP2β knockout lines. Chip followed by RT-qPCR confirmed that TOP2β was involved in regulating *CBR1* transcription.

I also generated several TOP2β knockout clones in further two cell lines (K562 and SH-SY5Y) using CRISPR. The response of wild type and three knockout SH-SY5Y clones to retinoic acid was compared in a number of ways including neurite outgrowth and RT-qPCR. Differential gene expression pattern was investigated using RNA-seq followed by IPA analysis. Results showed reduced level of ATRA-induced differentiation in the knockout cells compared with wild type. RNA-seq analysis of showed downregulation of key neuronal genes involved in neuronal functions.

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Ac-H3	acetyl-Histone H3
APL	Acute promyelocytic Leukaemia
ATP	Adenosine triphosphate
ATRA	all-trans retinoic acid
AML	Acute Myeloid Leukaemia
Ara-C	cytosine arabinoside
ALL	acute lymphoblastic leukaemia
Ara-CMP	Ara-C monophosphate
Ara-CDP	Ara-C diphosphate
Ara-DTP	Ara-C triphosphate
araU	uracil arabinoside
BCL2	B-cell lymphoma 2
BSA	Bovine Serum Albumin
CAP	catabolite gene activator protein
CBR1	Carbonyl reductase 1
CI	Combination Index
CD	Chromatin domain
CRISPR	Clustered regularly interspaced short palindromic repeats
ChIP	chromatin immunoprecipitation
CTCF	CCCTC-binding factor
CTD	C-terminal domain
DAPI	4, 6-diamidino-2-phenylindole
DAVID	Database for Annotation, Visualisation and Integrated Discovery
dCTP	deoxycytidine triphosphate
DDT	Dichlorodiphenyltrichloroethane
DEG	Differentially expressed genes
DEPBG	4′-demethylepipodophyllin benzylidene glucoside
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
DSBs	Double-Stranded Breaks

DW	Distilled water
ECL	Enhanced chemiluminescence
EDTA	Ethylene diamine tetra acetic acid
EMA	Ethidium monoazide
ESC	embryonic stem cells
FACS	Fluorescence-activated cell sorting
FBS	Fetal Bovine Serum
FDR	False Discovery Rate
FITC	Fluorescein isothiocyanate
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GC	guanidine-cytosine content
GEO	Gene Expression Omnibus
GO	gene ontology
HCl	Hydrochloric Acid
IF	immunofluorescence
IPA	Ingenuity Pathway Analysis
KEGG	Kyoto Encyclopedia of Genes and Genomes
MMS	Methyl methanesulfonate
FACS	Fluorescence Activated Cell Sorting
GFP	green fluorescent protein
gRNA	guide RNA
GyrA	subunit A of DNA gyrase
GyrB	subunit B of DNA gyrase
GHKL	Gyrase, Heat-shock protein 90, histidine Kinase and MukL
hENT-1	human equilibrative nucleoside transporter-1
LB	Lysogeny broth
mAMSA	amsacrine
MAP	molecule activity prediction
MAX	MYC Associated Factor X
MEM	minimum essential medium
MMS	Methyl methanesulfonate
MN	Micronuclei

mRNA	messenger Ribonucleic Acid
OECD	Organisation for Economic Co-operation and Development
PAGE	Polyacrylamide gel electrophoresis
Padj	Adjusted p value
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PCA	Principal Component Analysis
PI	protease inhibitor
RA	retinoic acid
RAR	Retinoic Acid Receptor
RXR	Retinoid X Receptor
RARE	Retinoic Acid Response Element
RF	Replication fork
RICC	Relative Increase in Cell Count
RIN	RNA integration number
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
RPMI	Roswell Park Memorial Institute
Rrna	ribosomal Ribonucleic Acid
RT-qPCR	Reverse Transcriptase quantitative Polymerase Chain Reaction
SDS	Sodium Dodecyl sulphate
siRNA	small interfering RNA
TAD	topologically associated domains
TBE	Tris/Borate/EDTA
TBS	Tris buffered saline
TOP1	DNA topoisomerase I
TOP2	DNA topoisomerase II
TOP3	DNA topoisomerase III
TOP1mt	mitochondrial DNA topoisomerase I
t-AL	therapy-related leukaemia
t-AML	therapy-related acute myelogenous leukaemia
UCSC	University of California Santa Cruz

USF1	upstream stimulatory factor 1
USF2	upstream stimulatory factor 2
WCE	whole cell extract

1 Chapter One: Introduction

1.1 Structure and organization of the human genome

1.1.1 Chromatin architecture

In humans, the genetic material (genome) is a deoxyribonucleic acid (DNA) double helix (Watson and Crick, 1953b; Watson and Crick, 1953a) packaged inside the nucleus as a polymer of DNA-protein complex called chromatin (McGhee and Felsenfeld, 1980). The chromatin is progressively organized across levels ranging from to the lowest scale (nucleosome core particle) to the highest scale (chromosome) organization (McGinty and Tan, 2014). The basic structure of chromatin is a repeating unit called a nucleosome (Bradbury, 1976; Kornberg, 1977; Luger *et al.*, 1997).

Each nucleosome has a nucleosome core which is an octamer histone protein (consisting of two copies of 4 core histones namely H3, H4, H2A, and H2B) (Kornberg and Lorch, 1999) wrapped by 145–147 base pairs of the DNA duplex (~1.65 times) (Luger *et al.*, 1997; McGinty and Tan, 2014). The DNA segment between each two nucleosome cores is called linker DNA. Another histone molecule H1 acts as a linker histone. H1 is added to the four histone cores which wrap 20 more base pairs of linker DNA, resulting in a total of ~166 base pairs of DNA wrapped around histone (~ 2 turns). The nucleosome core together with the linker histone H1 form a chromatosome. The chromatosome with the rest of linker DNA is called nucleosome (McGhee and Felsenfeld, 1980) (Figure 1.1).

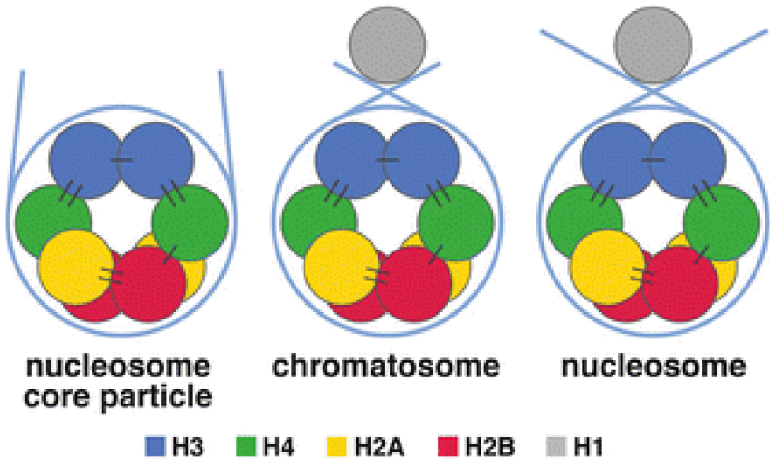


Figure 1-1 Nucleosome components

Figure shows nucleosome core, chromatosome, and nucleosome wraps DNA (blue line). This figure was reproduced from (McGinty and Tan, 2014) with permission.

The four core histones arranged as two H2A–H2B heterodimers with each heterodimer interacting with one half of the H3-H4 tetramer (Bradbury, 1976; Luger *et al.*, 1997; Kornberg and Lorch, 1999). These core histones have terminals (tails) of amino acid residues that expends between adjacent DNA gyres and around nucleosomes and are subjected to posttranslational modifications such as methylation, acetylation, phosphorylation, and ubiquitination (Luger *et al.*, 1997; Kornberg and Lorch, 1999; Wu and Grunstein, 2000).

Arrays of nucleosomes with the DNA at this level of chromatin organization have been described as fibres of "beads on a string" due to their appearance in electron micrographs (Oudet *et al.*, 1975; Kornberg and Lorch, 1999). These arrays of nucleosomes (11 nm wide) joined by DNA represent the primary structure of chromatin and occur in interphase and mitosis (McGinty and Tan, 2014).

Similar to protein structure, chromatin primary structure can be further organised to form higher levels of organization (McGinty and Tan, 2014). Linker histone H1 represents a scaffold for molecular interaction between the 11 nm fibres to reversibly form higher levels of chromatin organization into larger fibres of approximately 30 nm width (McGinty and Tan, 2014; Hansen *et al.*, 2018) (Figure 2.1). Despite the extensive research over the past decades, there is no agreement upon the structure of the 30 nm fibres (McGinty and Tan, 2014). The 30 nm fibres represent the secondary structure of chromatin. These fibres can further interact with other secondary structures to form tertiary chromatin structures (Luger *et al.*, 2012).

Further levels of chromatin organization are represented by folding of fibres to form array of loops that are structurally attached to the nuclear matrix or chromosomal scaffold components (Figure 2.1). These chromatin loops were first recognized as domains of supercoiled DNA with individual loop covering ~20-200 Kbp (Demeret *et al.*, 2001; Berezney, 2002; Cremer *et al.*, 2006).

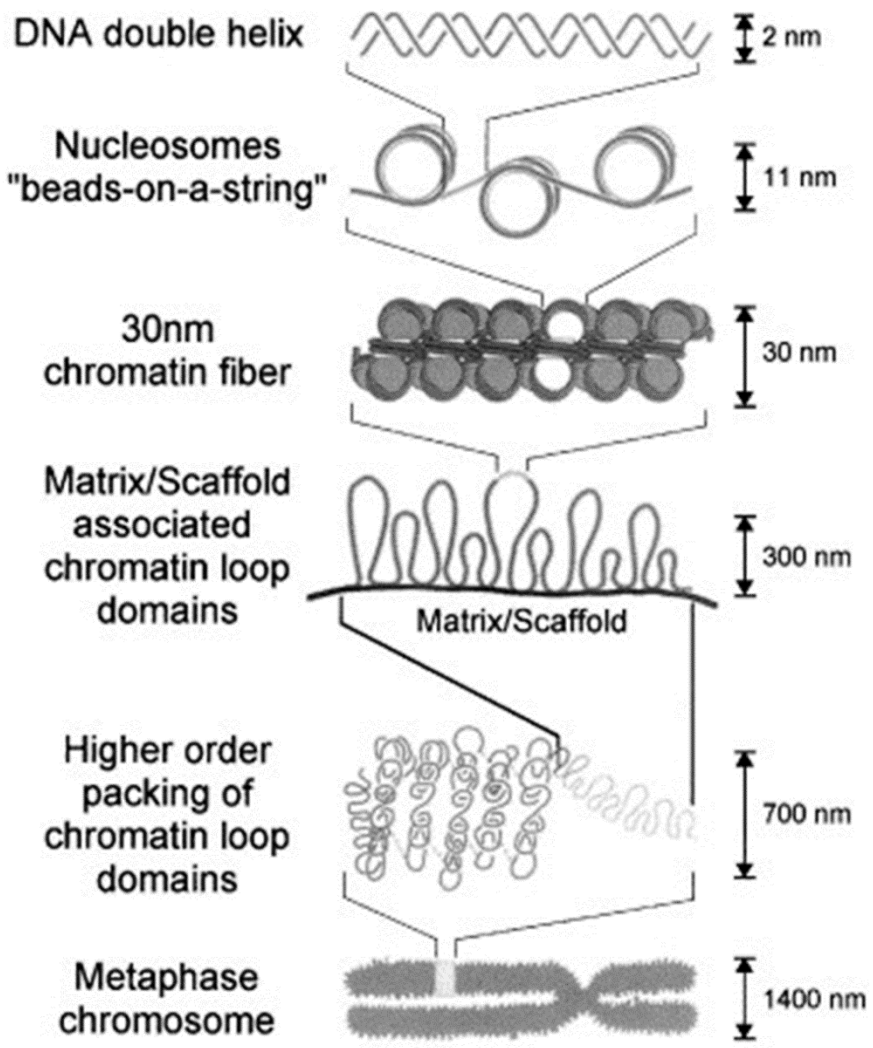


Figure 1-2 Levels of chromatin organization.

Figure shows hierarchical arrangement of chromatin inside the nucleus. This figure was reproduced from (Berezney, 2002) with permission.

With the advent of three dimensional fluorescence microscopy and quantitative computer imaging, studies of sites of DNA replication showed that these chromatin loops organized into distinct replication sites consisting of ~1 Mbp of DNA. These replication sites were shown to be unchanged during the cell cycle and remain preserved in the further cell generations as chromatin domains (CDs) with each domain containing 5-6 of the previously recognized chromatin loops (Ma *et al.*, 1998; Berezney, 2002).

Subsequently, further microscopic and biochemical studies have recognized these CDs as essential units of higher chromatin assembly (Berezney, 2002). The development of the chromatin

conformation capture (Hi-C) technique at a whole-genome scale with high resolution led to the identification of similar chromatin domains called topologically associated domains (TADs) (Dixon *et al.*, 2012; Nora *et al.*, 2012; Sexton *et al.*, 2012). Generally, TADs and the previously described 1 Mbp chromatin domains (CDs) are considered as the same chromatin structure (Dixon *et al.*, 2016). It is widely accepted now that interphase chromosomes encompassed distinct chromatin domains called topological domains or the more widely used term TADs (Dixon *et al.*, 2016). These TADs have been shown to be invariable among different cell types and conserved among species (Dixon *et al.*, 2012; Dixon *et al.*, 2016). Therefore, it has been suggested that these TADs function as a folding unit for chromosomes (Dekker, 2014).

TADs represent distinct genome compartments that are physically "insulated" from each other such that interaction between regions within one TAD is more frequent than interaction with another TAD (Dixon *et al.*, 2012; Nora *et al.*, 2012; Sexton *et al.*, 2012). It is well established that CCCTC-binding factor (CTCF) and cohesin have a key role in the formation and maintaining of TADs (Gómez-Díaz and Corces, 2014; Rao *et al.*, 2017).

CTCF is a zinc finger protein that is involved in modulation of chromatin architecture, chromatin insulation, and transcription regulation via its sequence specific binding activity (Ohlsson *et al.*, 2001; Kim *et al.*, 2007). Cohesin is a conserved protein complex composed of three subunits (RAD21, SMC1, and SMC3). Due to its ring-shaped structure (Gruber *et al.*, 2003) which can encircle chromatin fibres in a sequence-specific manner, cohesin plays a major role in various genome organization activities such as mediation of sister chromatid cohesion, gene clustering, promoter-enhancer interactions, chromatin insulation, and gene regulation (Kagey *et al.*, 2010; Watrin *et al.*, 2016). A number of mechanisms for TAD formation have been proposed with the loop extrusion model being the most attractive. Briefly, according to this model, a chromatin fibre becomes loaded with and extruded through a cohesin ring which continues to move along the fibre until it encounters a DNA-bound CTCF on both sides of the growing ring. This explains the high frequency of co-occurrence of both CTCF and cohesin at TAD boundaries (Alipour and Marko, 2012; Dekker and Mirny, 2016; Fudenberg *et al.*, 2016; Vian *et al.*, 2018).

Given that TADs are physically insulated chromatin units, it is sensible to suggest that they represent functionally insulated units. This is apparent from two aspects: coordinated gene expression of genes within the same TAD, and suppression of expansion of activities such as transcription between neighbouring regions of the genome (Dixon *et al.*, 2016).

1.1.2 DNA and chromatin supercoiling

DNA is a dynamic double-stranded helical molecule where each strand turns (coils) around the other, this occurs once per 10.5 bp of DNA in the relaxed state. Supercoiling (also called torsional stress) is the transition of the double helix from the relaxed state to either the over-wound (<10.5 bp) or the under-wound (>10.5 bp) state, termed positive or negative supercoiling respectively (Figure 1.3). Mathematically, this feature corresponds to a constant called the linking number (Lk). LK is calculated by the formula (Lk = Tw+Wr), where Tw (twist) is the number of times one strand turns around the other, and Wr (Writhe) is the number of times the duplex crosses itself (Figure 1.3). In a linear DNA duplex constrained by topological barriers or in a closed circular DNA molecule (as in plasmid or prokaryote DNA), Lk does not change upon transition in coiling state (twist, writhe, or both) unless one (or more) strand is cut, passed over the other, and rejoined again in a reaction catalyzed by topoisomerase enzymes (Bates, 2005).

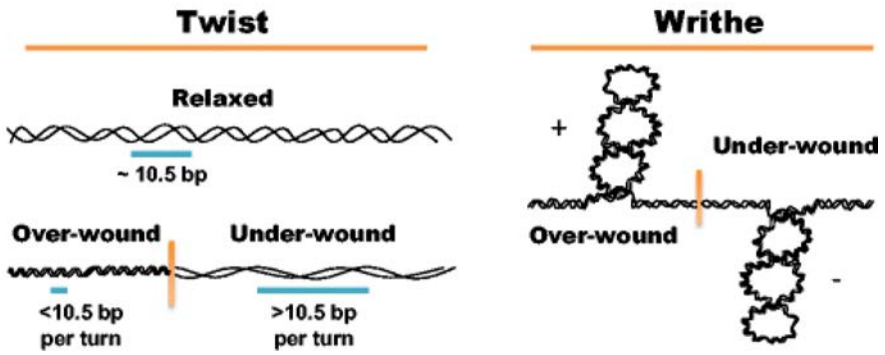


Figure 1-3 DNA supercoiling

Orange bars are barriers preventing from spreading of coiling state. This figure was reproduced from (Corless and Gilbert, 2016) under the terms of the Creative Commons CC-BY license. (https://creativecommons.org/licenses/by-nc/4.0/legalcode).

This concept of twist and writhe is further complicated and less clear in the case of chromatinized DNA partly because the majority of DNA is bound to nucleosome core units. The writhe (or supercoil) of the DNA in the chromatin exists in two states constrained and unconstrained. The constrained supercoil (nucleosome core bound) is maintained in an under-wound state while the unconstrained supercoil (nucleosome-free regions such as linker DNA) have the ability to undergo a transition in winding state and become under-wound (Corless and Gilbert, 2016; Corless and Gilbert, 2017).

Supercoiling is a fundamental feature of genome organization and function and has a key role in regulation of gene expression. Supercoiling results from separation of DNA strands from each other due to activities of DNA-binding proteins such as RNA polymerases (transcription), DNA polymerases (replication), helicases, and chromatin modelers (Bates, 2005).

A well established mechanism of generation of supercoiling is the twin domain model. According to this model, as RNA polymerase move along the transcribed DNA duplex, it generates a positive supercoiling ahead of it and a negative supercoiling behind of it (Liu and Wang, 1987; Wu *et al.*, 1988). These supercoiling patterns can be resolved via the action of topoisomerase enzymes. As supercoiling is dynamically generated during cellular activities, it is crucial to be finely tuned by the coordinated actions of both polymerases and topoisomerases (Pommier *et al.*, 2016) (see section 1.2.1.3 for further details on topoisomerases role).

1.2 Human DNA Topoisomerases

DNA Topoisomerases are a group of enzymes that are necessary for living cells because of their ability to modulate DNA topology by catalysing the passage of single or double DNA strand through another. These enzymes generate a transient break in the DNA backbone, form a covalent phosphodiester bond between their tyrosine residue and one end of the broken strand, allowing another DNA molecule to pass through the break, and then rejoin the broken strand. Therefore, these enzymes resolve topological problems such as torsional stress, DNA concatenates and knots that arise from cellular activities such as DNA replication and transcription (Wang, 1996; Champoux, 2001; Wang, 2002; Pommier *et al.*, 2016).

Generally, DNA topoisomerases are classified into type I or II families (according to the ability to break one or two DNA strands respectively) and are further classified into A or B subfamilies according to which strand is bound by the enzyme in the covalent phosphodiester state (5′ or 3′ phosphate respectively) (Wang, 1996; Champoux, 2001; Wang, 2002).

Human cells have six topoisomerase enzymes TOP2α, TOP2β, TOP3α, TOP3β, TOP1, and the most recently characterized mitochondrial TOP1 (TOP1mt)(Zhang *et al.*, 2001; Pommier *et al.*, 2016). Each topoisomerase has both specialized and shared roles with others (Champoux, 2001; Wang, 2002; Chen *et al.*, 2013) (Table 1.1).

Topoisomerase	Family	Subunit structure	Substrate and activity	ATP requirement
TOP1	IB	Monomer	Resolve supercoiling (negative and positive)	No
TOP1mt	IB	Monomer		No
TOP2α	IIA	Homodimer	Resolve supercoiling (negative and positive), concatenates, and knots.	Yes
TOP2β	IIA	Homodimer		Yes
TOP3α	1A	Monomer	Resolve supercoiling (negative only)	No
TOP3β	1A	Monomer	hypernegative supercoiling	No

Table 1-1 Human DNA topoisomerases

1.2.1 DNA Topoisomerase II (TOP2) enzymes

DNA topoisomerase II (TOP2) enzymes are vital for cell survival because of their role in replication, transcription, and chromosomal segregation during mitosis (Padget *et al.*, 2000). TOP2 allows the formation of a transient DNA double-stranded break (DSB) in one DNA segment where another DNA segment can pass through as part of its catalytic cycle. During this catalytic action, TOP2 binds covalently to two double-stranded DNA segments, allowing formation of an enzyme-enclosed double-stranded break, then religates the DSB after passing of the other DNA segment (Larsen *et al.*, 2003; Deweese and Osheroff, 2009). This process is crucial for maintaining genome integrity by preventing deleterious effects of accumulation of torsional stress and unresolved concatenates and knots. The catalytic reaction involves the formation of an intermediate TOP2-DNA covalent complex (Figure 1.5). This complex can be targeted by a group of drugs called TOP2 poisons that interfere with the catalytic cycle of TOP2, blocking the religation step leading to a cytotoxic DSB that initiates a series of molecular events culminating in ends with cell death (apoptosis) (Kaufmann, 1998; McClendon and Osheroff, 2007; Cowell and Austin, 2012) (refer to chapter 3 introduction for an extensive review).

The essential role of TOP2 enzymes and the ability of a group of compounds to target them and preventing their normal catalytic action contributes to their importance as a target for many chemotherapeutic agents (Wilstermann and Osheroff, 2003).

However, targeting TOP2 enzymes in chemotherapeutic protocols is related to the development of a secondary leukaemia called therapy-related leukaemia (t-AL) typically acute myeloid leukaemia (t-AML) (Kayser *et al.*, 2011) as well be described in chapter three introduction.

1.2.1.1 DNA TOP2 Structure and mechanism of action

DNA topoisomerase II (TOP2) plays a crucial role in modulating DNA topology during replication, transcription, and chromatin condensation (McClendon and Osheroff, 2007; Chen *et al.*, 2013; Pommier *et al.*, 2016). TOP2 enzymes are divided into two groups: TOP2α and TOP2β based on their structure and sequence (Wang, 1996; Austin *et al.*, 2018).

TOP2 enzymes are homodimeric enzymes with each monomer subdivided into three domains on the basis of sequence homology with the bacterial type II DNA gyrase. These domains are: N-terminal domain, the central domain, and the C-terminal domain (Figure 1.4A) (McClendon and Osheroff, 2007). The N-terminal domain (1-670 amino acids) is the ATPase domain; it is homologous to the B-subunit of DNA gyrase (GyrB) and contains the ATP binding site and hydrolysis.

The central domain (~ 671-1200 amino acids) is homologous to the A-subunit of DNA gyrase (GyrA) and contains the active site tyrosine (amino acid 805 for TOP2α and amino acid 821 for TOP2β) which is necessary for cleavage and ligation of DNA. The C-terminal domain (~ 1201-1521 amino acids for TOP2α and ~ 1201-1621 for TOP2β) is most variable in sequence between the two enzyme isoforms and among organisms (Figure 1.4A) (McClendon and Osheroff, 2007). The general structure of TOP2 enzymes is illustrated in (Figure 1.4B).

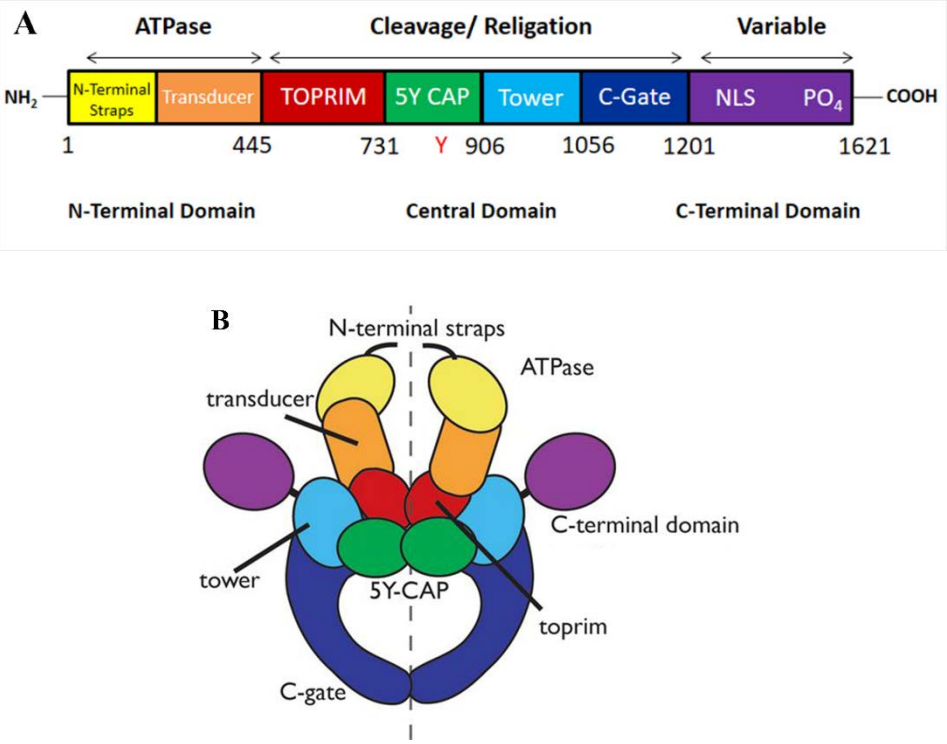


Figure 1-4 TOP2 structure

(A) TOP2 domains; the N-terminal domain (contains ATP binding site), the central domain (contains the active site tyrosine (Y) which is necessary for DNA cleavage and ligation), and the C-terminal domain which is highly variable and contains the Nuclear Localization Sequences (NLS) and phosphorylation sites (PO4). The amino acid sequence here is for TOP2β isoform. This line diagram was adapted from (Wu *et al.*, 2011) with permission. **(B)** General TOP2 elements; ATPase domains include [the GHKL (for Gyrase, Heat-shock protein 90, histidine Kinase and MukL) subdomain (yellow) and transducer subdomain (orange)]. Two 5Y-CAP (catabolite gene activator protein) domains (shown in green) and a pair of toprim folds (shown in red) form the DNA gate. The tower (shown in light blue). C-gate (shown in dark blue). C-terminal domain (shown in purple). Figure was reproduced from (Schoeffler and Berger, 2005) with permission.

The catalytic cycle of DNA topoisomerase II begins when the toprim and the 5Y-CAP domains that form the DNA gate bind to a double-stranded DNA segment, referred to as the gate segment (G-segment). Another DNA segment referred to as the transfer segment (T-segment) is bound to the ATPase domains [which consist of the GHKL (Gyrase, Heat-shock protein 90, histidine Kinase and MukL) and transducer subdomains] following dimerization of the ATPase domains induced by binding of two ATP molecules. ATP hydrolysis and release of Pi induce opening of the DNA gate and cleavage of the G-segment; this is followed by passage of the T-segment through the gap. Opening of the C-gate allows the T-segment to release. Ligation of the G-segment and release of the hydrolysis product triggers opening of ATP gate, then the enzyme enters another cycle (Figure 1.5) (Schoeffler and Berger, 2005; Cowell and Austin, 2012).

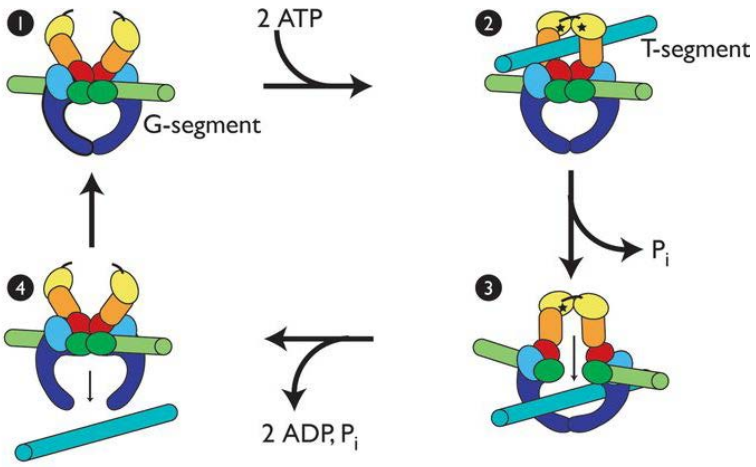


Figure 1-5 TOP2 Catalytic Cycle

Domains are coloured as figure 1.1B. The toprim and 5y-CAP domains which form the DNA gate bind to the G-segment (pale green) (step one). T-segment is bound to the ATPase domains after their dimerization with two ATP molecules (step two). ATP hydrolysis and release of Pi induce opening of DNA gate and cleavage of G-segment allowing T-segment to pass through the gap formed (step three). Releasing of T-segment upon C-gate opening (step four). Then, opening of ATP gate due to religation of G-segment and releasing of hydrolysis product allow the enzyme to enter the cycle again (Schoeffler and Berger, 2005).

1.2.1.2 TOP2 Isoforms

Human type II topoisomerases are divided into two isoforms; TOP2α and TOP2β. These isozyme forms are encoded by separate genes located on 17q21-22 and 3p34 for TOP2A and TOP2B respectively (Chung *et al.*, 1989; Austin and Fisher, 1990; Jenkins *et al.*, 1992; Tan *et al.*, 1992). Although the two isozymes differ in the molecular weight (170 kDa for α and 180 kDa for β), they have about (70 %) homology in the amino acid sequence (Austin *et al.*, 1993b; Austin and Marsh, 1998; McClendon and Osheroff, 2007). The amino acid sequence variability is very high in the C-terminal region where the amino acid identity is only 34 % (Austin *et al.*, 1993b; Austin and Marsh, 1998). These isoforms have specific immunological patterns because of the divergence in CTDs, which allows the production of antibodies specifically recognizes each form (Padget *et al.*, 2000).

TOP2α and TOP2β have different patterns of expression over the cell cycle (Padget *et al.*, 2000; Austin *et al.*, 2018). TOP2α is highly expressed during proliferation with its protein level significantly increased during S phase and maximized at G2/M phase (Adachi *et al.*, 2000; Padget *et al.*, 2000).

Furthermore, studies have shown that TOP2α protein is not detected in confluence-arrested cells or following serum deprivation (Padget *et al.*, 2000), whilst TOP2β regulation has been shown to be less dependent on the cell cycle stage and generally it is expressed in proliferating and non-proliferating cells (Zandvliet *et al.*, 1996; Austin and Marsh, 1998; Padget *et al.*, 2000; Austin *et al.*, 2018).

However, there is a controversy in the data available for TOP2β expression patterns among different cell lines (Padget *et al.*, 2000). Quantitative immunoblotting analysis of TOP2α and TOP2β expression in several cell lines has shown that TOP2β is expressed in comparable levels to TOP2α in CCRF-CEM, Raji, Molt-4, and K562 cell lines when they are in their exponential phase and the decatenation activity of TOP2β was higher than TOP2α in the exponentially growing human Raji leukaemia cells (Padget *et al.*, 2000). In addition, another study has shown that TOP2β level is increased in exponential cells (Turley *et al.*, 1997). Regulation of TOP2 protein levels over the cell cycle could be a result of changing in transcription/translation rate, mRNA or protein stability or a combination of all of these processes (Austin and Marsh, 1998).

While both isoforms have similar ATP-dependent strand-passing activities in vitro (Austin *et al.*, 1995), they have been shown to have different cellular roles. TOP2α is involved in cell division processes such as DNA replication, chromatin condensation and segregation. TOP2α remains associated with chromosomes in mitosis while TOP2β separates form chromosomes in mitosis (Deweese and Osheroff, 2009). The physiological role of the TOP2β enzyme is less clear than TOP2α but it has been shown to be involved in transcriptional regulation and to be required for neuronal differentiation (Austin *et al.*, 2018; Madabhushi, 2018) (refer to introduction of chapters 4 and 6 for extensive TOP2B review).

In vivo expression patterns have been reported for both isoforms. It has been shown that TOP2α is highly expressed in proliferating tissues such as spleen, bone marrow, thymus, and testis while TOP2β is ubiquitously expressed in tissues including post mitotic cells. Both isoforms showed higher expression levels in neoplastic than normal cells (Giovanni Capranico and Zunino, 1992; Watanabe *et al.*, 1994; Zandvliet *et al.*, 1996; Turley *et al.*, 1997; Harkin *et al.*, 2016) (see chapter 4 introduction for details).

The intracellular location of TOP2α and TOP2β over the cell cycle has been reported. TOP2α is primarily found in a nuclear location in both the nucleoli and the nucleoplasm during the interphase (Rattner *et al.*, 1996; Meyer *et al.*, 1997). During mitosis, TOP2α remains associated with metaphase chromosomes (Rattner *et al.*, 1996; Warburton and Earnshaw, 1997). TOP2β is primarily in the cytoplasm during mitosis, while its localization during interphase is less clear but there is an agreement that it has a nuclear location (Austin and Marsh, 1998; Austin *et al.*, 2018).

1.2.1.3 TOP2 cellular roles

1.2.1.3.1 DNA replication and chromosomal segregation

DNA replication starts at various regions on the chromosome called origins. These regions are primed in advance at the G1 phase. Replication is performed by a complex containing DNA polymerase with numerous proteins collectively called a replication fork (RF). Upon separation of the DNA strands by DNA helicases, two RFs are established at each origin region and move along the duplex in opposite directions. Advancing RFs along the DNA generates positive supercoiling ahead of RFs and precatenanes behind it (Peter *et al.*, 1998; Branzei and Foiani, 2010).

In the cellular environment, DNA is flanked by topological barriers such as nuclear matrix or insulators which hinders free rotation of DNA duplex around its axis. As a result, the generated supercoiling cannot be dissipated by diffusion along chromatin fibres. Hence, the accumulation of the supercoiling blocks replication progression unless it is actively removed by topoisomerase activity. Both TOP1 and TOP2 enzymes facilitate relieving of supercoiling ahead of the replication fork (RF). TOP2 also removes precatenanes behind the (RF) which affect proper chromosomal segregation if not resolved before mitosis (Lucas *et al.*, 2001; Pommier *et al.*, 2016) (Figure 1.6). This is especially important at the termination of replication where merging of the two RFs generates interlocked replicons (catenanes) (Sundin and Varshavsky, 1980; Sundin and Varshavsky, 1981) which are removed by TOP2 activity to allow chromosomal segregation (Fachinetti *et al.*, 2010; Pommier *et al.*, 2016).

1.2.1.3.2 Transcription

According to the 'twin domain model', moving of the transcription machinery (RNA polymerase, polymerase-associated proteins, and nascent transcript with associated proteins such as ribosomes) along the transcribed DNA molecule generates positive supercoiling in front of the transcription machinery and negative supercoiling behind it (Liu and Wang, 1987; Wu *et al.*, 1988; Tsao *et al.*, 1989) (Figure 1.6). A mechanism for generation of supercoiling according to this model has been proposed. The large and complex nature of the transcription machinery hinders its relative rotation while moving along the helical DNA molecule (which is also tethered to cellular components). Therefore, unwinding of DNA strands by the advancing replication machinery induces torsional stress positively and negatively ahead and behind the transcription machinery respectively (Liu and Wang, 1987; Koster *et al.*, 2010). Unless actively resolved by topoisomerases (Figure 1.6), positive supercoiling ahead of the transcription machinery hinders the progression of transcription. This is supported by studies showing that transcription significantly accumulates supercoiling upon depletion of topoisomerases (Wu *et al.*, 1988)}(Tsao *et al.*, 1989; Krasilnikov *et al.*, 1999). In addition, positive supercoiling significantly reduces mRNA level in a yeast system with mutated TOP1 and TOP2 (Gartenberg and Wang, 1992). Other studies showed that positive supercoiling can even block the initiation of transcription (Revyakin *et al.*, 2004).

Furthermore, studies have shown that TOP2 is particularly required for transcription of chromatinized DNA in vitro. One study conducted by (Mondal and Parvin, 2001) showed that TOP2 activity is necessary for transcription of chromatin templates but not for naked DNA templates. They showed that TOP2 inhibition by etoposide or ICRF-193 blocked transcription and addition of purified TOP2 recovered transcription repression. Subsequently, it has been shown that resolving of positive supercoiling is the reason for topoisomerases requirement for transcription elongation on chromatin templates (Mondal *et al.*, 2003). Another study showed a requirement for TOP2 enzymes to facilitate RNA polymerase pause-release activity at the transcription start site of selected genes (Bunch *et al.*, 2015).

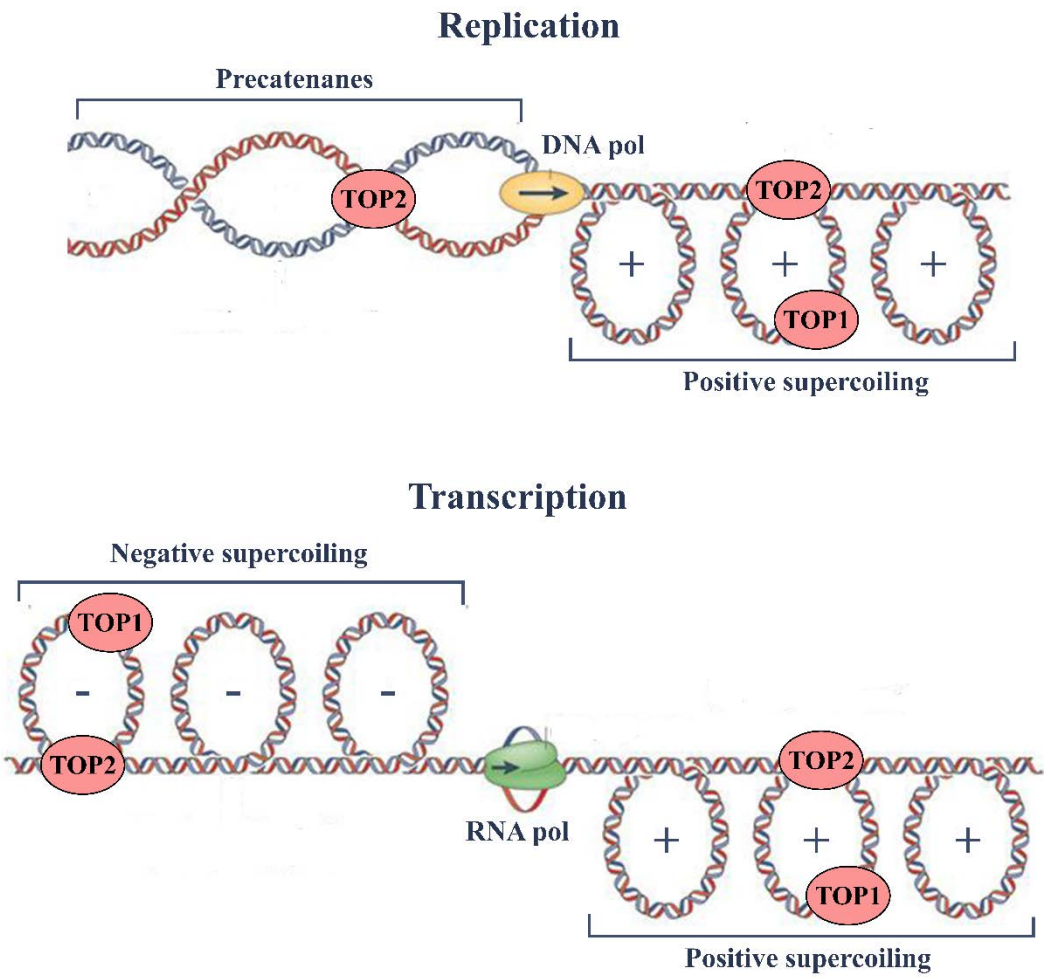


Figure 1-6 TOP2 roles in DNA replication and transcription

This figure shows torsional stress generated during replication and transcription according to the twin supercoiling domain model (Liu and Wang, 1987) and role of TOP2 in the relaxation of supercoiling and resolving precatenanes formed during replication. Figure was reproduced from (Vos *et al.*, 2011) with permission.

In addition to the global effect of TOP2 on transcription through supercoiling-relieving activity, the TOP2β isoform has been shown to further play a regulatory role on a narrower gene category, including ligand inducible and developmentally regulated genes with particular role in neuronal development (refer to introduction of chapters 4 and 6 for detailed review). In this study, the cellular and regulatory role of TOP2β was investigated (TOP2α isoform was also included in the experiments of chapters 3 and 4).

1.3 Aims

- Investigate the isoform-specific contribution of TOP2α and TOP2β as a target for cancer treatment by determining cytotoxicity and genotoxicity of TOP2 targeting drugs in three cell lines Nalm6^{WT}, Nalm6$^{B-/-}$, and Nalm6$^{A+/-}$ (chapter 3).
- Investigate the effect of (daunorubicin+Ara-C) combination at different dose ratio and/or treatment order in cancer treatment (chapter 3).
- Determine the role of TOP2β isoform in whole gene expression in Nalm6 cell line using RNA-seq analysis and RT-qPCR (chapter 4).
- Confirm the TOP2β-regulated genes in newly knockout model generated by CRISPR (chapter 5).
- Select candidate gene(s) to further study TOP2β recruitment to gene promoter by chromatin immunoprecipitation (ChIP) protocol (chapter 5).
- Investigate the role of TOP2β in gene expression and differentiation of neuronal cells (chapter 6).

2 Chapter Two: Materials and methods

2.1 Drugs and chemicals

Daunorubicin and idarubicin (Sigma-Aldrich, UK) were dissolved in deionized water as a 10 mM stock solution and stored at -20°C until used. Cytarabine (Sigma-Aldrich, UK) was dissolved in deionized water as a 50 mM stock solution and stored at -20°C until used. Methyl methanesulfonate (MMS) (Sigma-Aldrich, UK) was dissolved in Dimethyl Sulfoxide (DMSO) as a 100 µM stock solution and stored at -20°C until used as a positive control in the micronucleus tests. Rutin (Sigma-Aldrich, UK) was dissolved in Dimethyl Sulfoxide (DMSO) as an 80 mM stock and stored at -20°C until used. All-trans retinoic acid (ATRA) from (Sigma, UK, Cat. R2625) and vitamin D3 (Sigma, UK, Cat. D1530) were dissolved in ethanol as a 10 mM stock and stored as aliquots at -20°C until use. Puromycin (Sigma, UK, Cat. P9620) used for selection of cells transfected with PX459 vector was a ready-to-use 10 mg/ml solution (as puromycin dihydrochloride). Main stock was divided into aliquots and stored at -20°C until used.

2.2 Cell culture

2.2.1 Cell lines

Nalm6 is a human pre-B leukaemia cell line derived from the peripheral blood of a 19-year-old man with acute lymphoblastic leukaemia (ALL) in relapse. Nalm6 (wild type) and its genetic modified cell lines; TOP2B knockout cell line Nalm6$^{B-/-}$ and heterozygous knockout of TOP2A (Nalm6$^{A+/-}$) were kindly provided by (Noritaka Adachi) and were generated as described in (Adachi *et al.*, 2008b). K562 is a human myelogenous leukaemia cell line derived from a 53-year-old female patient with chronic myelogenous leukaemia in blast crisis.

SH-SY5Y is a human neuroblastoma cell line derived from the original SK-N-SH line which was established from the bone marrow of a four-year-old female with neuroblastoma in 1970. SK-N-SH was cloned as SH-SY. SH-SY was further subcloned as SH-SY5 which was in turn subcloned to generate SH-SY5Y (Biedler *et al.*, 1973; Ross *et al.*, 1983)

2.2.2 Cell line maintenance

All Nalm6 and K562 cell lines were maintained in (Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco by Life Technologies, Invitrogen, UK) containing 10% v/v heat-inactivated foetal bovine serum (FBS) (Gibco by Life Technologies, Invitrogen, UK) and 1% v/v Penicillin and

Streptomycin solution (10,000 units/ml penicillin, 10,000 mg/ml streptomycin, Gibco by Life Technologies, Invitrogen, UK) and incubated at 37°C and 5% CO2 under standard humidity. Cells were maintained at growing phase and kept at a density of ~1-2*10^6 cell / ml.

All SH-SY5Y clones were maintained in a 50:50 mixture of Minimum Essential Medium (MEM) and F12 (Hams) medium (Gibco by Life Technologies, Invitrogen, UK) containing 10% v/v heat-inactivated foetal bovine serum (FBS) (Gibco by Life Technologies, Invitrogen, UK) and 1% v/v Penicillin and Streptomycin solution (10,000 units/ml penicillin, 10,000 mg/ml streptomycin, Gibco by Life Technologies, Invitrogen, UK). SH-SY5Y cells were maintained at 75-80 % confluency and subcultured by trypsinization with 0.05 % Trypsin-EDTA (Gibco by Life Technologies, Invitrogen, UK) at 37°C for 5 minutes, then trypsin was neutralised with media. Cells were then pelleted by centrifugation for 5 minutes at 1000 rpm and the supernatant was discarded. Cells were then resuspended in media and a cell count was done to calculate the volume required to set a new culture. Cells were incubated at 37°C and 5% CO2 under standard humidity.

2.2.3 Cryopreservation and thawing of cell lines

Exponentially growing cells were centrifuged at 1000×g for 5 minutes (Harrier 15/80 MSE) and the supernatant medium was discarded. Cells were resuspended with fresh medium at half the final required volume (about one third the original volume). An equal volume of freezing medium containing 20 % DMSO was added and mixed. The cell suspension was then aliquoted in 1.5 ml cryogenic vials (Greiner, Bio-One GmbH, Germany) and labelled with the cell line name, passage number, date, and name of operator. Vials were cooled overnight at -80°C in a polystyrene box then transferred on the following day to liquid nitrogen storage at -192°C.

Cryogenic vials were removed from liquid nitrogen and immediately thawed at 37°C in a water bath for few minutes then 10 ml of fresh medium was added to the cells and they were centrifuged at 1000×g for 5 minutes. The supernatant was discarded and the pellet was resuspended with 10 ml of fresh medium and transferred into a small cell culture flask and incubated at 37°C and monitored for growth and subcultured.

2.3 Western blotting

2.3.1 Whole cell extraction

The whole cell extraction method was adopted from (Mirski *et al.*, 1993). Briefly, an adequate number of cells (~ 1×10^7 cells per each cell line) were centrifuged at 1000×g for 5 minutes and the media was removed and the cells were resuspended with cold phosphate buffered saline (PBS) and pelleted again to either use them directly for extraction or to store them at -80°C until use. Depending on their volumes, pellets were resuspended with 4 packed cell volumes of extraction buffer (10 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.4, 4 mM DTT, and 0.05 % of protease inhibitor (PI) cocktail [AEBSF HCl, Aprotinin, E-64, Bestatin, Leupeptin and Pepstatin]) and mixed. Then SDS was added to 0.25 % with mixing. DNase I was added to 50 µg/mL and the suspension was incubated on ice for 10-30 minutes. Once the viscosity disappeared from the suspension, an equal volume of solubilisation buffer (2% SDS, 20% glycerol, 5% β-mercaptoethanol, 0.6M Tris-HCl, pH 7.2) was added and the mixtures were heated at 68°C for 10 minutes. Then samples were either used for protein estimation and western blotting or stored as aliquots at -80°C until use.

2.3.2 Estimation of protein concentration

Bradford assays were used to estimate protein concentrations in the whole cell extracts (WCEs) using a standard curve of Bovine Serum Albumin (BSA). Samples were assayed in 96-well plates with BSA as a standard and DW as a blank. As samples contain solubilisation buffer which interferes with absorbance, BSA stock (2mg/mL) was firstly 1:1 diluted with solubilisation buffer then all samples were serially 1:1 diluted with DW to provide a range of concentrations for measurement. Then 250 µL of Bradford 1x Dye Reagent (BIO-RAD) was added to each well and incubated for 5-10 minutes at room temperature. Plate(s) were read at 595 nM on a plate reader (Bio-Rad Microplate reader 550). Standard curve(s) were created and used to determine protein concentrations of the samples.

2.3.3 SDS-acrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE (Tris-HEPES NH 4-20 %, NuSep, USA) were used with 1×Tris-HEPES-SDS running buffer (NuSep, USA) for electrophoresis. The marker (Western sure pre-stained chemiluminescent protein ladder, Li-CoR, USA) was used to analyse the molecular weight of the proteins. Samples were mixed with an equal volume of sample buffer (TruSep SDS, NuSep, USA) and heated at 68°C

(Stuart Scientific test tube heater SHT 2D) for 5-10 minutes, then the gel was run at 120 V for 1.2 hours.

2.3.4 Gel transfer

The transfer tank was filled with transfer buffer (25 mM Tris, 192mM glycine, 20% v/v methanol) and cooled with an ice block. Gels were transferred to nitrocellulose membranes (BIO-RAD, Germany) at 100 V and 500 mA for 1 hour.

2.3.5 Immunoblotting

Membranes were blocked with blocking solution (5 % (w/v) milk powder in Tris buffered saline (TBS) and 0.1 % TWEEN20 (TBS-T) for 1 hour, then incubated on a shaker with primary Antibody (Table 2.1) diluted in blocking solution either for 1 hour at room temperature or overnight at 4°C. Membranes were then washed with TBS-T (2× 1 minute + 1× 15 minutes) twice. Membranes were then incubated on a shaker with an HRP-congugated secondary antibody (Table 2.1) diluted in blocking solution either for 1 hour at room temperature or overnight at 4°C followed by washing with TBS-T (2× 1 minutes + 1× 15 minutes) twice.

2.3.6 Imaging

Blots were visualised by enhanced chemiluminescence (ECL) detection with enhancer solution (Clarity western ECL substrate, BIO-RAD, USA). Membranes were incubated with ECL reagent for 5 minutes then imaged using C-DiGid bllot scanner (Li-CoR, UK).

2.3.7 Stripping membranes

Where applicable, membranes were stripped to remove antibodies and blotted with another antibody or with an anti-actin antibody (Table 2.1) as a loading control. Briefly, membranes were washed with PBS or TBS-T then incubated with stripping buffer (Thermo Scientific, USA) for 7-15 minutes at room temperature with shaking. Stripping buffer was then removed and the membranes were washed with PBS or TBS-T then blocked with blocking solution for 30-60 minutes.

2.4 Immunofluorescence

Cells were harvested at a volume that contained approximately 2×10^5 cells for each slide. Samples were centrifuged at 1000×g for 5 minutes and resuspended with cold PBS and applied to Poly-L-

Lysine slides (VWR, UK). Cells were fixed with 4% paraformaldehyde (0.7 mL per slide) for 10 minutes, washed twice with PBS, and permeabilized with 0.8 mL KCM-T (120 mM KCl, 20 mM NaCl, 10 mM Tris-HCl, pH8.0, 1 mM EDTA, 0.1% (V/V) Triton X-100) for 15 minutes to each slide to allow for cell permeabilisation. Slides were incubated in blocking solution (KCM +T, 2% bovine serum albumin (BSA), 10% dried milk powder) for 1 hour then incubated with primary antibody (Table 2.1) for 1 hour at room temperature.

Slides were then washed with KCM-T for 3 × 10 minutes and incubated with secondary antibody (Table 2.1) for 1 hour at room temperature in the dark. Slides were further washed with KCM-T for 3 × 10 minutes in subdued light. Vectashield mounting medium with DAPI (Vector laboratories, USA) was added to each slide and the coverslips were fixed by sealing with nail polish. Immunofluorescence imaging was performed using an Olympus IX-81 microscopic imaging system and the integrated FITC fluorescence per nucleus was determined using (Volocity software V6.3, PerkinElmer, UK) and analysed using (Graphpad Prism software (v4.0), Graphpad, Inc., USA) as described by (IG *et al.*, 2011). Exposure time was optimized using autoexposure of the control samples.

2.5 Antibodies

Antibodies used in this study, their applications, and concentrations are detailed in (Table 2.1).

	Antibody	Description	Supplier	Applications and dilutions
Primary antibodies	TOP2A[1] (4566)	Rabbit polyclonal to human topoisomerase II alpha	In house	1:2000 for western blotting and 1:400 immunofluorescence
	TOP2B[2] (4555)	Rabbit polyclonal to human topoisomerase II beta	In house	1:2000 for western blotting and 1:400 immunofluorescence
	TOP2B[3] (30400)	Rabbit polyclonal to human topoisomerase II beta	In house	1:2000 for western blotting and 1:400 immunofluorescence
	TOP2B[4] (4557)	Rabbit polyclonal to human topoisomerase II beta	In house	1:1000 for western blotting
	TOP2B[5] (3535)	Rabbit polyclonal to human topoisomerase II beta	In house	5 µg per IP for ChIP
	TOP2B	Human/Mouse monoclonal to human topoisomerase II beta	R&D Systems, USA (MAB6348)	1:500 for western blotting
	USF1[6]	Mouse monoclonal to human upstream stimulatory factor 1	Santa Cruz, Germany (sc-390027)	2 µg per 500 µg of total protein per IP for ChIP
	USF2[7]	Rabbit polyclonal to human upstream stimulatory factor 2	Abcam, UK (ab125184)	5 µg per 1000 µg of total protein per IP for ChIP
	GFP[8]	Rabbit polyclonal to green fluorescent protein	Santa Cruz, Germany (sc-8334)	2 µg per 500 µg of total protein per IP for ChIP
	C-Myc[9]	Rabbit polyclonal to human C-Myc	Santa Cruz, Germany (sc-764)	5 µg per 1000 µg of total protein per IP for ChIP
	MAX	Mouse monoclonal to Human MAX	Abcam, UK (ab53570)	5 µg per 1000 µg of total protein per IP for ChIP
	Ac-H3[10]	Rabbit Polyclonal to acetyl-Histone H3	Millipore, UK (06-599B)	5 µg per IP for ChIP
	Actin	Mouse monoclonal to Actin	Abcam, UK (ab3280)	1:2000 for western blotting
	beta-Actin	Mouse monoclonal to beta-Actin	Novusbio, UK (NB600-501)	1:5000 for western blotting
	Flag	Mouse monoclonal anti-flag M2	Sigma, UK (F1804)	1:2000 for immunofluorescence
Secondary antibodies	ECL Anti-rabbit IgG	Anti-rabbit IgG horseradish peroxidase linked whole antibody	GE healthcare, UK (NA934)	1:5000 for western blotting
	Anti-mouse IgG	Anti-mouse IgG horseradish peroxidase linked whole antibody	GE healthcare, UK (NXA931)	1:5000 for western blotting
	Anti-rabbit IgG	Alexa fluor®488 goat anti-rabbit IgG (H+L)	Life Technologies, USA (A11008)	1:400 for immunofluorescence

Table 2-1 Antibody details

(1) Raised to Full C-Terminal domain (CTD) of TOPA (residue 1244-1531 of full TOP2A protein) Human TOP2A-GST fusion **(2)** Raised to Full C-Terminal domain (CTD) of TOPB (residue 1263-1621 of full TOP2B protein) Human TOP2B-GST fusion **(3)** Raised to Full C-Terminal domain (CTD) of TOPB (residue 1296-1621 of full TOP2B protein) Human TOP2B-GST fusion **(4)** Raised to the last third of C-Terminal domain (CTD) of TOPB (residue 1296-1621 of full TOP2B protein) Human TOP2B-GST fusion **(5)** Raised to the Final 116 amino acids of CTD Human TOP2B-GST fusion **(6)** Raised against amino acids 75-160 of USF-1 of human origin **(7)** Raised to a region within amino acids 296-346 of Human USF2 **(8)** Raised against amino acids 1-238 representing full length GFP of *Aequorea victoria* origin **(9)** Raised against amino acids 1-262 of c-Myc of human origin **(10)** Raised to human Histone H3 acetylated at the N-terminus

2.6 Immunophenotyping

Immunophenotyping was performed to determine the effect of vitamin D3 on expression of number of cell surface markers in Nalm6 cells. All reagents used and the protocol for cell preparation for FACS analysis are described in (Table 2.2).

	$Nalm6^{WT}$ / $Nalm6^{B-/-}$					Control (Beads)		
Conditions →	CD10 Isotype Stained	CD19 Isotype Stained	CD 38 Isotype Stained	CD10,19,38 Ab Stained	Non stained	CD10 Ab Stained	CD19 Ab Stained	CD38 Ab Stained
Sample	2×10^5 cells	2×10^5 cells	2×10^5 cells	0.5×10^6 cells	1×10^5 cells	1 drop beads	1 drop beads	1 drop beads
Washing	PBS (1% FBS)							
Resuspension	100 µl PBS (1% FBS)							
Ab/Isotype	PE Mouse IgG1, κ Isotype Control Clone MOPC-21 (RUO) 20 µl	BV421 Mouse IgG1, k Isotype Control Clone X40 (RUO) 5 µl	FITC Mouse IgG1, κ Isotype Control Clone MOPC-21 (RUO) 20 µl	PE Mouse Anti-Human CD10 Clone HI10a (RUO) 20 µl, BV421 Mouse Anti-Human CD19 Clone HIB19 (RUO) 5 µl, FITC Mouse Anti-Human CD38 Clone HIT2 (RUO) 20 µl	None	PE Mouse Anti-Human CD10 Clone HI10a (RUO) 20 µl	BV421 Mouse Anti-Human CD19 Clone HIB19 (RUO) 5 µl	FITC Mouse Anti-Human CD38 Clone HIT2 (RUO) 20 µl
Incubation	30 min at 4 □C							
Washing	PBS (1% FBS)							
Resuspension	0.5 ml PBS (1% FBS)							
Dead cells exclusion	DAPI for 5 minutes to stain dead cells.							
FACS	(Fortessa x20) machine , 20000 events/sample were measured at slow rate							

Table 2-2 Workflow of immunophenotyping of Nalm6 cells.

2.7 Determination of growth curves and doubling time of cell lines

Cell lines were seeded at a log phase and grown for up to 120 hours and cell counting was performed using the TC20 (BIO-RAD, USA) cell counter at different time points to determine growth curves. Data from 3 independent replicates were used to set up the growth curves for each cell line.

Doubling times for cell lines was determined using the following equation:

$$\textbf{Doubling population} = \frac{\boldsymbol{Log10\ (Nt/Nc)}}{\boldsymbol{Log10\ (2)}}$$

Where; (*N*t) is the number of cells at given time, (*N*c) is the initial cell number.

$$\text{Doubling time } (Td) = t/Doubling\ population$$

Where; (t) is the time at which cells were counted.

Doubling time at different time points were taken and averaged.

2.8 Cytotoxicity assays

The sensitivity of cell lines (Nalm6^{WT}, Nalm6$^{B-/-}$, and Nalm6$^{A+/-}$) to daunorubicin, idarubicin, and cytarabine as single agents and in combination were determined by cell counting and viability assays using the trypan blue exclusion method and the dose-response curve was established as Relative Increase in Cell Count (RICC) using the following formula (Fellows *et al.*, 2008):

$$\text{RICC} = \frac{\text{Increase in number of cells in treated cultures (final}-\text{starting)}}{\text{Increase in number of cells in control cultures (final}-\text{starting)}} \times 100$$

The dose response curves for single agents and in combination were analyzed and created using (Graphpad Prism software (v4.0), Graphpad, Inc., USA). Furthermore, the efficiency of drug combinations was determined for synergy analysis using CompuSyn software (ComboSyn, Inc). The CompuSyn program uses the Chou-Talalay method for drug combination analysis which is based on the median-effect equation derived from the mass-action law (Chou and Talalay, 1984; Chou, 2006; Chou, 2010) to determine the combination index (CI) which quantitatively indicates whether the combination is synergistic (CI<1), antagonistic (CI>1), or additive (CI=1) (Chou, 2006).

XTT assays were performed as follows: cells were treated with drug and incubated for 120 h. on the day of measurement, 50 µl XTT reagent (50:1 XTT reagent to electron coupling reagent, XTT

Cell Proliferation kit, Roche, UK) was added per well and cells were incubated for a further 4 hrs. A Bio-Rad 550 Microplate Reader (Bio-Rad, USA) was used to measure absorbance values and the data were analysed using (Graphpad Prism software (v4.0), Graphpad, Inc., USA). Growth inhibition values were determined by setting the values obtained with no drug as 100%.

2.9 Micronuclei assay

Micronucleus assays were used to assay the genotoxicity of drugs. *In vitro* Micronuclei test by FACS is based on flow cytometric counting of micronuclei (MN) stained fluorescently with a nucleic acid dye (CYTOX green). This protocol also allows for efficient determination of relative survival, using counting beads which can be stained and scored by the flow cytometer. The number of healthy nuclei per bead can be determined. The nuclei-to-bead ratio for the control and the treated samples can be used to determine the relative survival. The procedure was followed according to micronuclei kit (MicroFlow, Litron Laboratories, USA) instructions along with the OECD guideline (OECD, 2010) and was as follows:

Working reagents were prepared as recommended by the (MicroFlow, Litron Laboratories, USA) kit instructions and outlined in (Table 2.3). Cells were allowed to grow for (1.5-2) normal cell cycle period after drug exposure with a density of no more than $1*10^6$ cell / ml at harvesting time. In addition, it was taken into account that the cytotoxicity should be no more than 50 % at the time of harvest as this would interfere with MN endpoint and give false positive results.

Exponentially growing cells were harvested after 48 hours continuous drug exposure. Each control and treated sample was mixed to achieve homogenous suspension then a consistent volume (estimated to provide $1*10^6$ cell/ml of negative control) of each sample was transferred into separate 15 ml tubes. All samples were centrifuged at 300×g for 5 minutes to collect the cells.

1x Buffer Solution			
Number of samples*	**Volume of dH2O**	**Volume of 10x Buffer**	**Volume of FBS**
1	3.6 ml	0.4 ml	0.08 ml
After the required volumes were added and mixed, the mixture was sterilised by filtration with b Millipore filter and refrigerated.			
Nucleic Acid Dye A Working Solution			
Number of samples*	**Volume of 1x Buffer Solution**	**Volume of Nucleic Acid Dye A**	
1	0.33 ml	3.3 µl	
The required volumes were added and mixed in a polypropylene vessel and stored on ice protected from light until used.			
Complete Lysis Solution 1			
Number of Samples*	**Volume of Incomplete Lysis Solution 1**	**Volume of Nucleic Acid Dye B**	**Volume of RNase Solution**
1	0.55 ml	2.2 µl	2.75 µl
The required volumes were added and mixed in a polypropylene vessel and stored at room temperature protected from light until used.			
Complete Lysis Solution 2			
Number of samples*	**Volume of Incomplete Lysis Solution 2**	**Volume of Nuclei Acid Dye B**	
1	0.55 ml	2.2 µl	
The required volumes were added and mixed in a polypropylene vessel and stored at room temperature protected from light until used.			

Table 2-3 Working reagents preparation for Micronuclei (MN) test

*Volumes were scaled up depending on number of samples

The supernatants were discarded and the pellets were resuspended by tapping gently and placing on ice for 20 minutes then 300 µl of Ethidium Monoazide (EMA) (Dye A) working solution was added to each sample tube and the tubes were left opened to a direct light source 15 cm above the samples and submerged in about 2 cm in ice for 30 minutes. This step is required for the EMA stain to covalently bind DNA upon photoactivation. Counting beads (6 micron microspheres) were added to the 1× buffer solution (1 drop per 10 ml) and mixed well. The light was switched off and 3 ml of 1× buffer solution with beads was added to each tube. The 1× buffer solution was mixed between samples and light exposure was limited from this step onwards by covering tubes with foil. Samples were then centrifuged at 300×g for 5 minutes and the pellet were resuspended by tapping gently then immediately preceding to the next step.

500 µl of complete lysis solution 1 was added to the first sample and this was vortexed on low to medium speed for 5 seconds and this was repeated for all samples. All samples were incubated with complete lysis solution 1 for 1 hour at room temperature and away from light. Complete lysis solution 2 (500 µl was added and the previous step was repeated for all samples then the samples were incubated for 30 minutes with complete lysis solution 2 at room temperature and away from light. The samples were then transferred into flow cytometric tubes for analysis or stored for up to 24 hours at room temperature or 72 hours at 4°C.

BD fluorescence-activated cell sorting (FACS) Canto™ II cytometer (BD Biosciences, New Jersey, USA) was used for data acquisition. Samples were Excited by a 488 nm laser and Cytox Green and EMA fluorescence were collected by 585/42 and 530/30 filters of the flow cytometer respectively. The parameter Settings and gating were confirmed with both positive control methyl methanesulfonate (MMS) and negative control (DMSO and H_2O). The analysis was performed using (BD FACSDiva (v8.0), Biosciences, New Jersey, USA) software.

The stopping gate was set as 30,000 healthy nuclei counted so that for each sample, 30,000 nuclei events were counted and the relative survival was determined by the nuclei-to-beads ratio. The following parameters were calculated:

$$\text{Relative survival} = \frac{(\text{nuclei}-\text{to}-\text{beads})\ \text{ratio of treated samples}}{(\text{nuclei}-\text{to}-\text{beads})\ \text{ratio of control samples}} \times 100$$

$$\text{Micronuclei (\%)} = \frac{\text{no. of micronuclei}}{\text{no. of healthy nuclei}} \times 100$$

$$\text{Micronuclei fold change} = \frac{\text{micronuclei (\%) of treated sample}}{\text{micronuclei (\%) of control}}$$

$$\text{Apoptotic (EMA positive) cells (\%)} = \frac{\text{no. of EMA events of treated samples}}{\text{no. of EMA events of control}} \times 100$$

$$\text{Apoptotic cells fold change} = \frac{\text{Apoptotic (EMA positive)cells (\%) of treated sample}}{\text{Apoptotic (EMA positive)cells (\%) of control}}$$

2.10 Cell cycle analysis

Micronuclei kit (MicroFlow, Litron Laboratories, USA) allows for cell cycle analysis by studying histograms of nucleic acid dye (CYTOX green) fluorescence. These histograms represent the DNA content of the cell populations. DNA content histograms created by FACS were analysed using (Flowjo software (v7.6), FlowJo, LLC, USA) to quantitatively determine cell cycle phase distribution in control and treated samples.

2.11 Electroporation

Electroporation was used to transfect the cell lines with small interfering RNA (siRNA) oligonucleotides (ON-TARGET plus SMART pool, Thermo Scientific, USA) to knockdown TOP2A and TOP2B (Table 2.4). Briefly, cells were grown to a density sufficient to adjust to the required cell concentration. Appropriate volume of cell suspension was centrifuged at 300×g for 5 minutes and resuspended with 300 µl culture medium per sample such that the final density of each sample is $5*10^7$ cell/ml. Sample cell suspension from above was transferred into an electroporation cuvette (Eurogenetic, UK) and the required volume of siRNA stock (20µM) was added to the cell suspension and the cuvette was briefly swirled to mix. The cuvette was transferred into the electroporator (Fischer EPI2500, Germany) and the electroporation was performed at 10 mSec and the voltage generally was (300-370) V depending on the cell line. After electroporation, cuvette(s) with cell suspension were incubated for 15 minutes at room temperature. Cell suspension was transferred into a small culture flask with 5.7 ml of fresh medium such that the final cell concentration was $2.5*10^6$ cell/ml and incubated at 37˚C and 5% CO_2 under standard humidity. Cell viability was checked 24 hrs later with the trypan blue exclusion protocol.

siRNA	Target sequence	Molecular weight (g/mol)
ON-TARGET plus non-targeting siRNA - SMART pool, 20 nmol	Negative control siRNA with at least 4 mismatches to any human, mouse, or rat gene.	13400
ON-TARGET plus human TOP2A (7153) siRNA – SMART pool, 10 nmol	CGAAAGGAAUGGUUAACUA	13400
	GAUGAACUCUGCAGGCUAA	13430
	GGAGAAGAUUAUACAUGUA	13385
	GGUAACUCCUUGAAAGUAA	13400
ON-TARGET plus human TOP2B (7155) siRNA – SMART pool, 10 nmol	GAAGUUGUCUGUUGAGAGA	13415
	CGAAAGACCUAAAUACACA	13400
	GAUCAUAUGGGAUGUCUGA	13415
	GGUGUAUGAUGAAGAUGUA	13400
ON-TARGET plus human CBR1 (873) siRNA – SMART pool, 5 nmol	GUGAACGUAUCUAGCAUCA	13414
	CAGAGAAGAGAGUUGAACA	13414
	GAUCCCACACCCUUUCAUA	13429
	GAAGAUUGGCGUCACCGUU	13444

Table 2-4 Oligonucleotides used for siRNA knockdown

2.12 Agarose gel electrophoresis

The size of the amplification products was verified using agarose gel electrophoresis by mixing 20 µl of each PCR reaction with 5 µl of 6×DNA loading dye (Thermo Scientific). The mixtures were then loaded on 1.6% agarose gel in 1×TBE buffer with GelRed (Biotium) staining (10µl per gel) and a DNA ladder (Thermo Scientific) was used for comparison. Gels were run at 100 volts (Northumbria Biologicals Limited power pack) for 1 hour and visualised using (Gel-Doc EZ imager, Bio-Rad) system and images were analysed using (Image Lab Version 5.2) software.

2.13 PCR primers

All PCR primers listed in (Tables 2.5 and 2.6) were designed either by myself (M.K.) or from published data as stated. Target sequences were obtained from UCSC browser and primers were designed using the web tool (Primer3web version 4.0.0) and checked for specificity using both (In-Silico PCR web tool, UCSC browser) and Basic Local Alignment Search Tool (BLAST). Primers were then synthesized by Integrated DNA Technologies (IDT, Glasgow, UK). All primers were supplied from the manufacturer as a stock solution of 100 µM in a TE buffer (pH 8.0) which were used to prepare the (Forward+Reverse) working solution of 10 µM. All primers were then checked for PCR efficiency using agarose gel as detailed in section 2.12.

Generally, the following criteria were followed in designing the primers: Primer length 19-25 nucleotides (nt), GC content of 45-60% and a single amplicon size of 75-350 base pairs (bp). Melting temperatures were between 55-63°C per primer pair.

Genomic DNA primers are listed in (Table 2.5) and primers for Reverse Transcription Polymerase Chain Reaction (RT-qPCR) are listed in (Table 2.6).

Target	Primer Name	F/R	Sequence	Length (nt)	GC (%)	Amplicon Size (bp)	Application	Design
TOPKO1	TOPKO1-1F	F	CTAGGAGTGCGGCGAGTG	18	66.7	193	CRISPR	M.K.
	TOPKO1-1R	R	CCAGCCACTTACCACCCAG	19	63.2			
TOPKO19	TOPKO19-2F	F	CGCGGAGTGTGAGAAGGA	18	61.1	511	CRISPR	M.K.
	TOPKO19-2R	R	GACCAGCCACTTACCACC	18	61.1			
GAPDH-P1	GAPDH-P1F	F	TACTAGCGGTTTTACGGGCG	20	55	166	ChIP	Millipore (cat. 17-408)
	GAPDH-P1R	R	TCGAACAGGAGGAGCAGAGAGCGA	24	58.3			
CBR1-C1	CBR1-C1F	F	GAAGAAAACCCCTCAGAGAACC	22	50	189	ChIP	M.K.
	CBR1-C1R	R	CTCGCCGGGGTGCGGAGCAGGCGG	24	83.3			
CBR1-P2	CBR1-P2F	F	ACACTCACTTTGAAACCATGCA	22	40.9	172	ChIP	M.K.
	CBR1-P2R	R	GAGAATTGCTTGCTTGAACCC	21	47.6			
CBR1-P3	CBR1-P3F	F	CACAACTAAGGCATGTCTTCCA	22	45.5	160	ChIP	M.K.
	CBR1-P3R	R	TGGGTCTTGATTCTGGTGGT	20	50			
CBR1-P4	CBR1-P4F	F	TATGTTCCCACCCCAATCCC	20	55	193	ChIP	M.K.
	CBR1-P4R	R	TTGAACGACTCTCCTCAGGG	20	55			

Table 2-5 Genomic DNA primers

Table 2-6 RT-qPCR primers

Target	Primer Name	F/R	Sequence	Length (nt)	GC (%)	Amplicon Size (bp)	Design
PP1A	PP1A-1F	F	TCCTAAAGCATACGGGTCCTGGCAT	25	52	166	(Feart *et al.*, 2005)
	PP1A-1R	R	CGCTCCATGGCCTCCACAATATTCA	25	52		
CBR1	CBR1-2F	F	AGCTGGACATCGACGATCTG	20	55	187	M.K.
	CBR1-2R	R	GTGCACACATCTCGGGTAC	19	57.9		
MTMR1	MTMR1-2F	F	GACACCTACCCTGCCATCAT	20	55	176	M.K.
	MTMR1-2R	R	CAGCGCTTATCATTGGGACC	20	55		
YOD1	YOD1-2F	F	CTCCCCAAGTGTCATCCCTT	20	55	222	M.K.
	YOD1-2R	R	CTGTGCTTACCAGAACCGTG	20	55		
TOP2B	TOP2B_1F	F	ACCTGGGTGAACAATGCTGC	20	55	212	(Song *et al.*, 2012)
	TOP2B_1R	R	ACCTCCCTGCAATTCATTCCT	21	47.6		
ZNF43	ZNF43-8F	F	TGGCCAAAAGTCTTGGGTAA	20	45	110	(Neff *et al.*, 2012)
	ZNF43-8R	R	TGATCACCTGTCTGGAGCAA	20	50		
PRKACB	PRKACB-1F	F	TGGATTGGTGGGCATTAGGA	20	50	74	(Domhan *et al.*, 2008)
	PRKACB-1R	R	TGGTTGGTCTGCAAAGAATGG	21	47.6		
ZSCAN18	ZSCAN18-4F	F	GGATGGTCTCGGGTTCTTCC	20	60	162	M.K.
	ZSCAN18-4R	R	CACCTTGTCCCTGGCTAGAC	20	60		
ARMCX5	ARMCX5-3F	F	GAGCACAGTGATCCCGAAGT	20	55	172	M.K.
	ARMCX5-3R	R	GCCTCCCTCAATCAACATGG	20	55		
ZNF512B	ZNF512B-1F	F	AGTTCTGAGAGTGGCGTCAA	20	50	244	M.K.
	ZNF512B-1R	R	CCTTGTCTCTGCATCCTGGA	20	55		
ZNF626	ZNF626-6F	F	CCCTCAGTGAGCTTCCTTCAA	21	52.4	115	M.K.
	ZNF626-6R	R	GCAGACATTTGTGCGTCCC	19	57.9		
ZNF573	ZNF573-1F	F	GCCTTCAGTCGTGCTTCAAA	20	50	193	M.K.
	ZNF573-1R	R	TGTGGGCTGTCTGATGATGA	20	50		
FLT2	FLT2-2F	F	TCCCTTATGATGCCAGCAAGT	21	47.6	79	(Andersson *et al.*, 2010)
	FLT2-2R	R	CCAAAAGCCCCTCTTCCAA	19	52.6		
EMP2	EMP2-1F	F	TCCTCTCCACCATTCTCT	18	50	80	(Wadehra *et al.*, 2008)
	EMP2-1R	R	AAACCTCTCTCCCTGCTTCA	20	50		
	MAX-2R	R	GTGCATTATGATGAGCCCGTTT	22	45.5		

Continued→

ME3	ME3-1F	F	TGAAGAAGCGCGGATACGA	19	52.6	101	(Bragoszewski *et al.*, 2007)
	ME3-1R	R	GATTAGGCCGTGGATTCCAAG	21	52.4		
IL1B	IL1B-3F	F	AGATGAAGTGCTCCTTCCAGG	21	52.4	73	(Laddha *et al.*, 2014)
	IL1B-3R	R	TGGTCGGAGATTCGTAGCTG	20	55		
GZMA	GZMA-2F	F	GAAGAGACTCGTGCAATGGA	20	50	80	(Maertzdorf *et al.*, 2011)
	GZMA-2R	R	AAGGCCAAAGGAAGTGACC	19	52.6		
LILRB1	LILRB1-3F	F	GTGACGTATGCCGAGGTGAA	20	55	136	M.K.
	LILRB1-3R	R	CAGCAGCCTCAGTGTCCATC	20	60		
CYP26A1	CYP26A1-1F	F	GCAGCCACATCTCTGATCACT	21	52.4	109	(Zuchegna *et al.*, 2014)
	CYP26A1-1R	R	TGTTGTCTTGATTGCTCTTGC	21	42.9		
RARB	RARB-2F	F	GCAGAGCGTGTAATTACCTTGAA	23	43.5	145	(Poulain *et al.*, 2009)
	RARB-2R	R	GTGAGATGCTAGGACTGTGCTCT	23	52.2		
RET	RET1F	F	GCTCCACTTCAACGTGTC	18	55.6	158	(Ohshima *et al.*, 2010)
	RET1R	R	GCAGCTTGTACTGGACGTT	19	52.6		
CRABP2	CRABP1-F1	F	TCAAAGTGCTGGGGGTGAAT	20	50	168	Ian Cowell
	CRABP1-R1	R	TCTGCTCCTCAAACTCCTCCC	21	57.1		
USF1	USF1-1F	F	CACCACGGATTAGAGGTCGT	20	55	397	(Bussiere *et al.*, 2010)
	USF1-1R	R	GAGCATCACCTGTCAGCAAA	20	50		
USF2	USF2-1F	F	GATCCAAAATCCCTTCAGCA	20	45	395	(Bussiere *et al.*, 2010)
	USF2-1R	R	CTTTACTCGCTCCCGTCTTG	20	55		
C-Myc	Myc-1F	F	AATGAAAAGGCCCCCAAGGTAG	22	50	112	(Wang *et al.*, 2018)
	Myc-1R	R	GTCGTTTCCGCAACAAGTCCT	21	52.4		
MAX	MAX-2F	F	AGGTGGAGAGCGACGAAGAG	20	60	69	(Miao *et al.*, 2015)
	MAX-2R	R	GTGCATTATGATGAGCCCGTTT	22	45.5		
BCL2	BCL2-3F	F	GATTGTGGCCTTCTTTGAG	19	47.4	232	(Zucchini *et al.*, 2005)
	BCL2-3R	R	CAAACTGAGCAGAGTCTTC	19	47.4		

2.14 RNA methods

2.14.1 RNA extraction

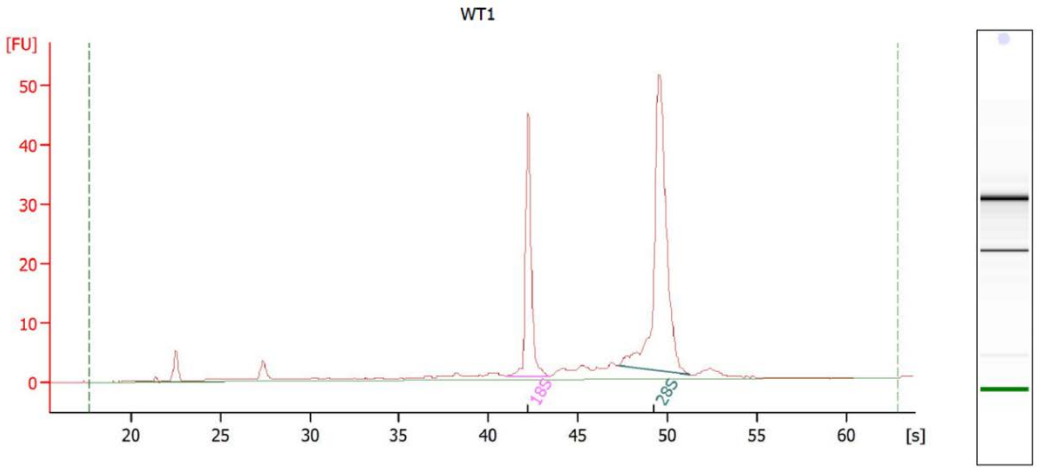
Total RNA samples were extracted and purified using silica membrane technology (RNeasy Mini kit, Qiagen, USA) according to the kit instructions. Briefly, $4*10^6$ cells per sample were harvested by centrifugation at 300×g for 5 minutes then the pelleted cells were disrupted by adding 350 µl of RLT buffer and mixed by pipetting. The resulting lysate was homogenized by pipetting directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuging at 10000×g for 2 minutes. Then one volume of 70% ethanol was added to the homogenized lysate and mixed well by pipetting. Up to 700 µl of the mixture was transferred into an RNeasy spin column placed in a 2 ml collection tube, and centrifuged at 8000×g for 15 seconds, then the flow-through was discarded and the collection tube was reused for the next step.

The spin column membrane was washed by adding 700 µl RW1 buffer and centrifuged at 8000 ×g for 15 seconds, then the flow-through was discarded and the collection tube was reused for the next step. The membrane of the RNeasy spin column was washed again with 500 µl RPE buffer and centrifuged at 8000 ×g for 2 minutes; then the flow-through was discarded and the collection tube was reused for the next step. The RPE washing step was repeated, then the RNeasy spin column tube was placed in a new 1.5 ml collection tube and 50 µl of RNase-free water was added directly to the spin column membrane and centrifuged at 8000 ×g for 1 minute to elute the RNA. The purified RNA samples were stored at -70°C for up to 1 year.

2.14.2 RNA sequencing (RNA-Seq)

2.14.2.1 RNA quality and quantity measurement

The total RNA concentration and RNA integrity number (RIN) of samples were determined using the (Agilent 2100 Bioanalyzer System, Agilent Technologies, Germany) with (Agilent RNA 6000 Nano kit, Agilent Technologies, Germany) according to the kit user manual. An example Electropherogram summary of a total RNA sample analysis using (Agilent 2100 Bioanalyzer System) is shown in (Figure 2.1).



Overall Results for sample 1 : WT1

RNA Area:	182.8	RNA Integrity Number (RIN):	9.8 (B.02.07)
RNA Concentration:	139 ng/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	2.1	Result Flagging Label:	RIN: 9.80

Fragment table for sample 1 : WT1

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	40.99	43.41	38.4	21.0
28S	47.23	51.29	80.3	44.0

Figure 2-1 Example electropherogram of a total RNA sample analysed using an Agilent 2100 Bioanalyzer.

2.14.2.2 Library preparation and sequencing platform

For Nalm6 cell lines (Chapter Four), samples of total RNA were sent to the (Institute of Genetic Medicine, Newcastle University) for Library preparation and Sequencing. TruSeq Stranded Total RNA with Ribo-Zero Globin kit (Illumina) was used which removes both cytoplasmic and mitochondrial ribosomal RNA (rRNA) as well as globin-encoding messenger RNA (mRNA). For library preparation, the High-Throughput protocol of (TruSeq® Stranded Total RNA Sample Preparation Guide, Illumina) was followed. The input RNA for library preparation was 1000 ng per sample. Sequencing was performed using (NextSeq 500, Illumina) sequencing system. A High output flow cell was used and 75 bp Paired End (PE) sequencing was performed.

For SH-SY5Y cell lines (Chapter Six), samples of total RNA were sent to the (Institute of Genetic Medicine, Newcastle University) for Library preparation and Sequencing. TruSeq Stranded mRNA kit (Illumina) was used which selects only poly-A containing mRNA molecules using poly-T oligo attached magnetic beads. For library preparation, the High Sample (HS) Protocol of (TruSeq® Stranded mRNA Sample Preparation Guide, Illumina) was followed. The input RNA for library

preparation was 1500 ng per sample. Sequencing was performed using (NextSeq 500, Illumina) sequencing system. High output flow cell was used and 75 bp single End (SE) sequencing was performed.

2.14.2.3 Analysis and interpretation of results

For Nalm6 data (Chapter Four), raw sequencing data was analysed by the (Bioinformatics Support Unit, Newcastle University). Enrichment analysis was performed in the differentially expressed genes (by > 1 log2 fold change, padj < 0.05) in Nalm6 cell line using (R software, version 3.4) as described below, as well as using Database for Annotation, Visualisation and Integrated Discovery (DAVID) web tool (version 6.7) as described by (Huang *et al.*, 2008; Sherman *et al.*, 2008). The multiple testing correction was performed using Benjamini method with a cut-off of 0.05 as a threshold of significance of over-representation of (GO) terms.

For SH-SY5Y data (Chapter six), all analysis was performed by myself under the supervision of John Casement from the (Bioinformatics Support Unit, Newcastle University). Briefly, raw sequencing files (FASTQ files) were firstly checked for sequence quality using FASTQC software. Then quantification of transcripts count was performed using Salmon, a tool that quantifies transcript expression via read abundances and it uses a gene annotation source (I used GENCODE gene sets). The following steps were performed using (R software, version 3.4). The Principal Component Analysis (PCA) was performed using DESeq2 package. Quantification data files were read using (tximport package) and analysed for differential gene expression using (DESeq2 package). Gene annotation was then added using (annotables package) with GENCODE release 29 (GRCh38.p12) as reference annotation.

Venn diagrams were generated using (gplots package). Differentially expressed genes (DEGs) were tested for biological functions and canonical pathways enrichment using QIAGEN's Ingenuity Pathway Analysis (IPA, Spring release 2019, GIAGEN Redwood City, USA, www.qiagen.com/ingenuity) using subscription provided to (Bioinformatics Support Unit, Newcastle University). IPA analysis were performed for genes which showed > 1 log2 fold change and < 0.05 padj. Analysis species parameter was set to human and apart from that IPA default parameters were used. Analysis involved downstream effects evaluation of DEGs for biological function annotations and canonical pathways. Also, analysis includes a prediction of upstream factors that may have caused the effects observed in the datasets.

IPA algorithm uses right-tailed Fisher's exact test to calculate the p-values for biological annotations or pathways. The p-value here reflects the likelihood that the association between DEGs in an experiment and a given function or pathway is due to random chance. Mathematically, it reflects robustness of overlapping between DEGs in user experiment and total number of genes allocated to the biological function or pathway. IPA then calculates the corrected p-value (padj) using Benjamini-Hochberg multiple testing correction method.

In addition to evaluation of over-representation of a biological function or pathway within a group of genes, IPA also offers a prediction of effect direction (activation or inhibition) as a value of activation score (z-score). Z-score is based on prior knowledge stored in IPA database of expected relationship between a gene and a given biological function. IPA tests genes from a user dataset that are assigned to each biological function or pathway and compares their relative expression change to what is predicted from the literature. When the noticed change direction is consistent with a biological function activation state (activation or inhibition), IPA makes a prediction for activation state of this function (as activation or inhibition).

An activation state of a biological function is inferred as a z-score value. This is based on the literature-based information about the relationship between genes and function within a molecular network. Based on differential gene expression of a gene (up or down), the activation state of a biological function is inferred from the direction of the effect correlated with (gene-function) relationship. For example, given the (gene-function) relationship is (activating), IPA makes an activation prediction for this function upon gene upregulation and an inhibition prediction upon gene downregulation. Conversely, if the (gene-function) relationship is (inhibition), IPA makes an inhibition prediction for this function upon gene upregulation and an activation prediction upon downregulation. In the biological context, a single function is associated with more than one gene. IPA creates a prediction for each single gene associated with that function and generates an overall activation state as a value of z-score. If the predicted activation state of a function is consistent for all genes associated with this function, IPA makes a prediction for that function a value of z-score.

Mathematically, overall z-score of a function or pathway is a value of whether a given function or pathway has more activation predictions than inhibition predictions such that the overall z-score of positive value indicates overall increase in function or pathway activity, while overall z-score of negative value indicates overall decrease in function or pathway activity. If activation and inhibition predictions of a given function are equal then the z-score value of the function is zero.

However, even if genes from an experiment dataset were significantly enriched for a given function or pathway, Qiagen IPA makes direction prediction score only for the pathways that are eligible for z-score calculations. For a full list of pathways eligible for z-score scoring (refer to the website: http://qiagen.force.com/KnowledgeBase/articles/Basic_Technical_Q_A/Eligible-Pathways-List). IPA describes this as follows: "QIAGEN curators assigned the molecules that should be in a particular known state when the pathway is activated…. then, the curator determined the states of additional molecules in the pathway based on the causal relationships among the connected pathway nodes, avoiding setting nodes in states that conflict with each other (again, considering the pathway in the active state). Some pathways are not amenable to being described in a single activated state, and will therefore not be eligible for z-score calculations". In this case, IPA labels the function or pathway differently indicating that this function or pathway is "currently ineligible for a prediction".

IPA also calculates the percentage of overlap between DEGs in a dataset of an experiment and the total number of genes assigned to a given pathway. Moreover, IPA provides molecule activity prediction (MAP) service as part of analysis to show downstream and upstream effects of regulation of a given gene on the related molecules within a pathway and present this as an activation or inhibition effect with labels on the pathway graph.

2.14.3 Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR)

2.14.3.1 RNA samples

RNA samples used for RT-qPCR were either extracted and purified using (RNeasy Mini kit, Qiagen, USA) as described in section 2.10.1 or prepared directly from fresh cell lysates using (SingleShot Cell Lysis, Bio-Rad) kit according to manufacturer instructions. Briefly, fresh cell suspensions containing the appropriate cell number (around 1×10^5 cells per well) were transferred into a 96-well PCR plate. Cells were centrifuged at 750×g for 5 minutes and the supernatant(s) were removed. Cells were then washed with 125 µl per well with PBS and centrifuged again at 750×g for 5 minutes and the PBS was removed.

For adherent cells, media was removed and cells were washed with 125 µl PBS then the protocol was completed similarly for both cell types. To each well, 50 µl of SingleShot cell lysis master mix (containing 48 µl SingleShot cell lysis buffer, 1 µl proteinase K solution, and 1 µl DNase solution) was added and complete resuspension was ensured by pipetting up and down. Complete lysis was

then performed by incubating the PCR plate for 10 minutes at room temperature followed by 5 minutes at 37°C and then 5 minutes at 75°C using MyiQ Single colour dual time PCR detection system (Bio-Rad). Cell lysates were then kept for up to 2 months at -20°C or for up to 12 months at -80°C.

2.14.3.2 Protocol

Gene expression analysis was performed by real-time one-step RT-PCR using SYBR Green I detection method. For this purpose, (QuantiNova™ SYBR Green RT-PCR Kit, QIAGEN, Cat. 208154) kit was used according to the manufacturer's instructions. Briefly, the total reaction volume was set to 20 µl per well in the PCR 96-well plate. In each reaction well, the following volumes were added: 10 µl (2x SYBR Green RT-PCR), 300 nM primer mix, 0.2 µl (QuantiNova SYBR Green RT Mix which contains HotStaRT-Script reverse transcriptase), 1-2 µl sample volume, and the volume was completed to 20 µl with RNase-free water. Contamination was monitored with two controls; (No reverse transcriptase) control to determine DNA contamination in the samples and (No template) control to determine other contaminations. The thermal cycler (CFX96 Touch™ , Bio-Rad) was set to the following parameters: reverse transcription reaction (50°C for 10 minutes), DNA polymerase activation and initial DNA denaturation (95°C for 3 minutes) for 1 cycle, denaturation, annealing and extension (95°C for 15 seconds, 55°C for 30 seconds, 72°C for 30 seconds) for 40 cycles.

2.15 Clustered regularly interspaced short palindromic repeats (CRISPR) technique

CRISPR technique was used to generate TOP2B knockout clones used in chapters 5 and 6. The workflow of this technique is described in the following steps.

2.15.1 Genomic editing design

Genomic editing design step involved guide RNA (gRNA) oligonucleotide design, bioinformatical testing of their possible off-target, and designing a PCR primer required for genotyping (TOPKO1 primer). Guide RNA (gRNA) oligonucleotides that target exon 1 of *TOP2B* gene were designed using the publically available CRISPR design web tools (http://crispr.mit.edu/) and (http://zifit.partners.org/ZiFiT/ChoiceMenu.aspx). Eight different gRNA oligonucleotides were designed and presented in (Table 2.7) and (Figure 2.1). Off-targets were determined using (http://www.rgenome.net/cas-offinder/) and (http://crispr.dbcls.jp/) algorithms. For each gRNA, a pair of oligos were designed in such a way that upon annealing, they have (CAAA and CCAC)

overhangs and contain gRNA sequence in the middle. In addition G nucleotides was added to gRNAs 6, 7, and 8 as it is preferable for U6 promoter. Oligonucleotides were synthesized by Integrated DNA Technologies (IDT, Glasgow, UK).

gRNA	Sequence 5' to 3'	Strand	Efficiency score*
1	GGTGGCTGCGGCGCGGGAGC	-	47
2	GGCGCGGGAGCCGGCGTGGG	-	44
3	GCGGGAGCCGGCGTGGGCGG	-	43
4	GCCGGCGTGGGCGGCGGCAA	-	68
5	GCGGCAACGGGGCACTGACC	-	79
6	CGCGCCGCAGCCACCCGACT	+	89
7	AAGTCGGGTGGCTGCGGCGC	-	83
8	CAAGTCGGGTGGCTGCGGCG	-	79

* From http://crispr.mit.edu/

Table 2-7 *TOP2B* gRNAs for CRISPR

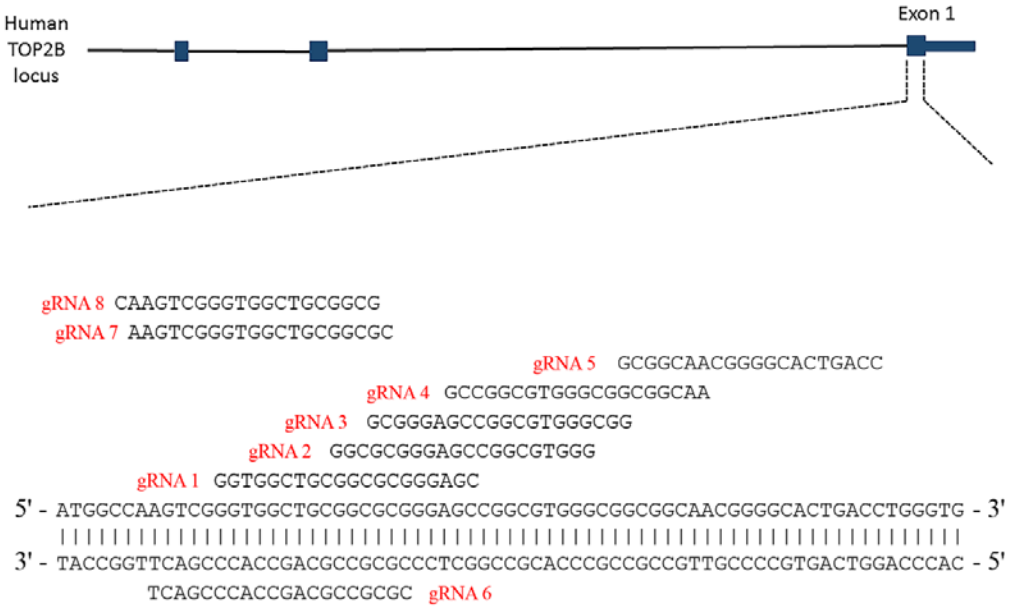


Figure 2-2 TOP2B targeting gRNAs used in CRISPR

2.15.2 Cloning

Guide RNA oligonucleotides cloned into either pSpCas9(BB)-2A-GFP (PX458) or pSpCas9(BB)-2A-Puro (PX459) V2.0 vector (Figures 2.2 and 2.3 respectively) which were a gift from Feng Zhang (Addgene plasmid # 48138 and # 62988) (Ran *et al.*, 2013).

Guide RNA (gRNA) Oligonucleotides were annealed to form the double-stranded oligos with overhangs that match the digested vector. For each (gRNA), 1 µl of each (100 µM) stock of both strands was mixed with 8 µl of nuclease free water. Annealing of the oligonucleotides was performed using MyiQ Single colour dual time PCR detection system (Bio-Rad) by applying the following incubations: 37 ºC for 30 minutes followed by 5 minutes at 95 ºC then the reaction was ramped down to 25 ºC at 5 ºC per minute.

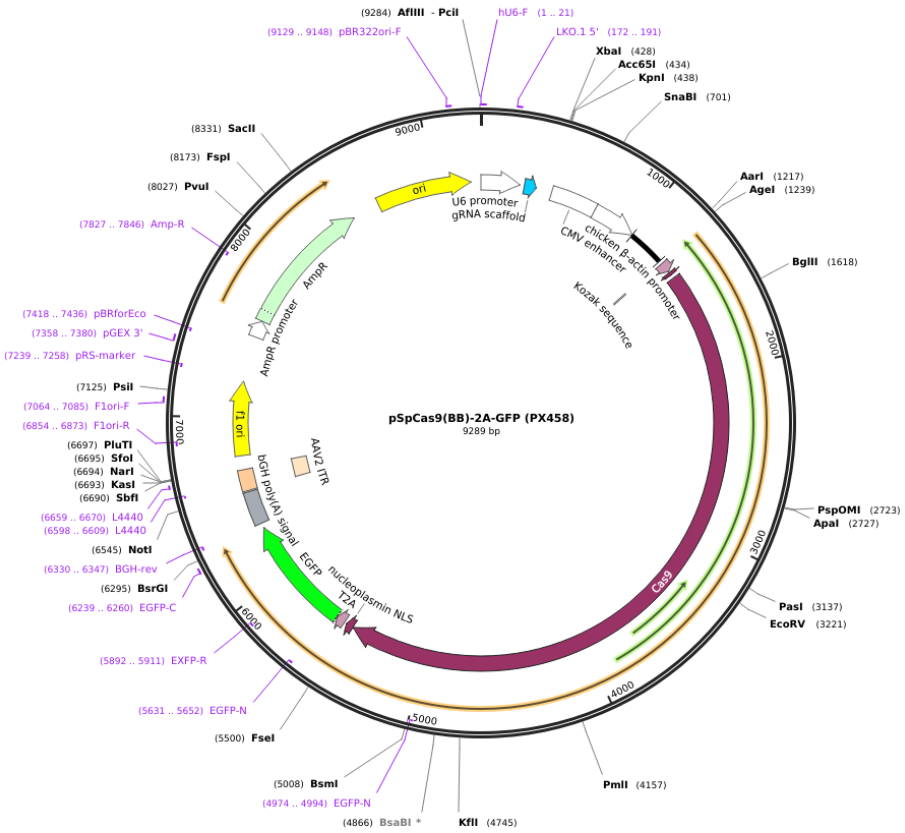


Figure 2-3 PX458 cloning vector

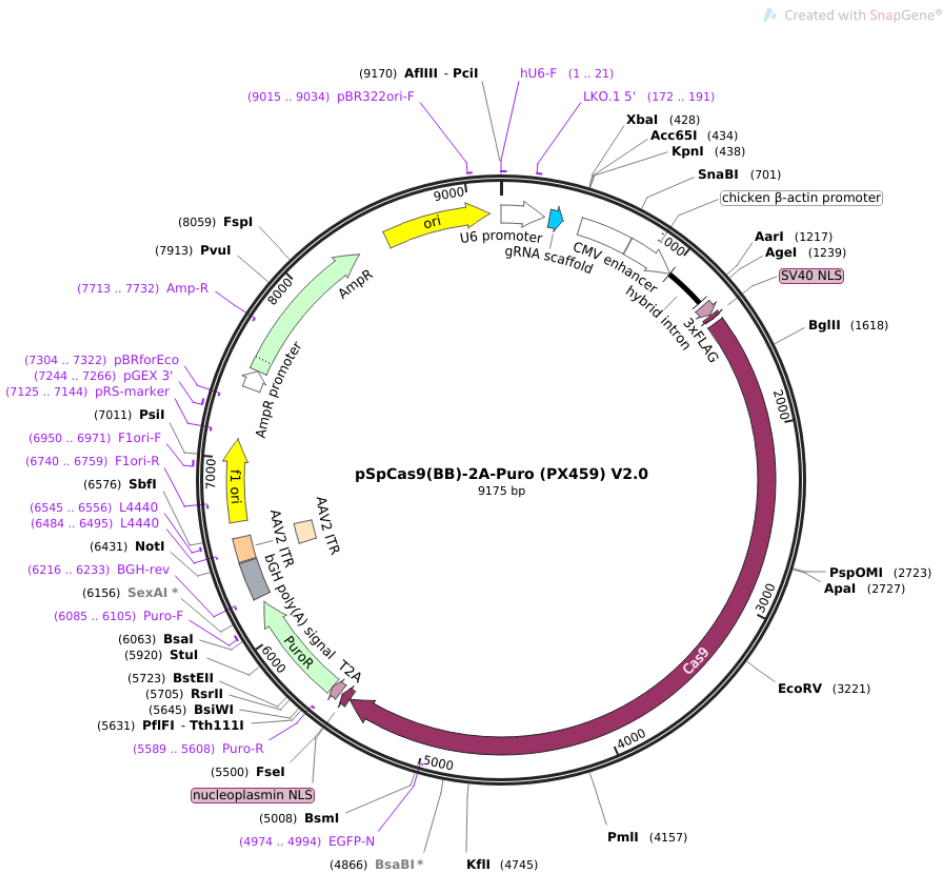


Figure 2-4 PX459 cloning vector

The Cloning vector was digested using BbsI restriction enzyme (New England Biolabs, UK, cat. R3539S) by mixing the following at 37 ºC for 1 hour: 1 µg vector, 1 µl of BbsI, 2 µl of (10X) buffer (New England Biolabs, UK, cat. B7202S), and volume was completed with nuclease-free water to 20 µl. Digested vector was loaded on a 1% agarose gel electrophoresis and run at 100 volt for 1 hour. Vector band was gel purified using the Qiaquick Gel extraction kit (Qiagen, UK, cat. 28704) according to the manufacturer's instructions.

Guide RNA (gRNA) oligonucleotides were cloned into PX458 or PX459 vector by ligation reaction for 30 minutes at room temperature. The following were mixed in the ligation reaction: 50ng of BbsI digested vector, 2 µl of diluted (1:200 in DW) annealed (gRNA), 5 µl of (2X) T7

ligase buffer (New England Biolabs, UK, cat. B0318S), and the volume was completed with nuclease-free water to 10 µl, then 1 µl of T7 ligase (New England Biolabs, UK, cat. M0318S).

Vector in which (gRNA) was inserted was transformed into a competent bacterial cell DH5α (Invitrogen, UK, cat. 18265-017) as follows:

Four microliter of ligation reaction was mixed with 40 µl of subcloning cells and left for 30 minutes on ice. The mixture was transferred to 42 °C for 20 seconds to apply heat shock. The transformation mixture was then transferred onto ice for 2 minutes, then 950 µl of pre-warmed LB media was added and sample was incubated at 37 °C for 45 minutes with shaking (180 rpm). Part of the sample (300 µl) was spread onto an LB agar plate containing 100 µg/ml Ampicillin and plates were incubated overnight at 37 °C for colony selection. On the following day, 2-5 colonies were isolated and inoculated in 5 ml LB media containing 100 µg/ml Ampicillin to prepare cultures for plasmid miniprep. Plasmid miniprep was performed using QIAprep Spin Miniprep Kit (Qiagen, UK, cat. 27104) according to manufacturer's instructions.

Plasmid samples from the miniprep were test digested to verify successful gRNA insertions. Both PX458 and PX459 have a pair of BbsI restriction sites that upon digestion, form overhangs match the (CAAA and CCAC) overhangs in the gRNA oligos. Successful insertion should destroy the BbsI restriction site which can be determined using digestion test. Therefore, a digestion test was performed using two restriction enzymes; BbsI + EcoRV for PX458 and BbsI + StuI for PX459 (New England Biolabs, UK, cat. R0195S). PX458 and PX459 have only one EcoRV and StuI restriction site respectively. Upon digestion, successful insertion should give a single band and unsuccessful insertion should give two.

In addition, for successful insertions, samples were sent for Sanger sequencing from (GATC Biotech) according to their sample requirements. Forward primer of Human U6 promoter, LKO.1 5' (5'-GACTATCATATGCTTACCGT-3') was used. Verified plasmid samples were purified again with enough quantities by midiprep using Qiagen Plasmid Midi Kit (Qiagen, UK, cat. 12143) according to the manufacturer's instructions.

2.15.3 Nucleofection

Plasmid with (gRNA) was introduced into cells by Nucleofection with Nucleofector II system (Amaxa, USA) using Amaxa® Cell Line Nucleofector® Kit V (Lonza, UK, cat. VCA-1003) for

(SH-SY5Y and K562) or Amaxa® Cell Line Nucleofector® Kit T (Lonza, UK, cat. VCA-1002) for (Nalm6) cells according to the manufacturer's instructions. Briefly, $1*10^6$ cells per sample were harvested by centrifugation at 1000 rpm for 8 minutes then resuspended in 100 µl of supplemented Nucleofector® Solution V or T. Cloning vector (PX458 or PX459) with or without gRNA was then added and mixed. Empty vector was used as a negative control and a GFP-expressing vector (PmaxGFP) (Figure 2.5), which was provided with the kit, was used as a positive control for transfection. Mixtures were then transferred into the cuvettes and Nucleofection was performed using either A-023 (for SH-SY5Y) or T-016 (for K562) or C-005 (for Nalm6) program. Samples within cuvettes were then left for about 10 minutes then 500 µl of pre-warmed media was added and total content of each cuvette was then transferred into a well of 6-well plate.

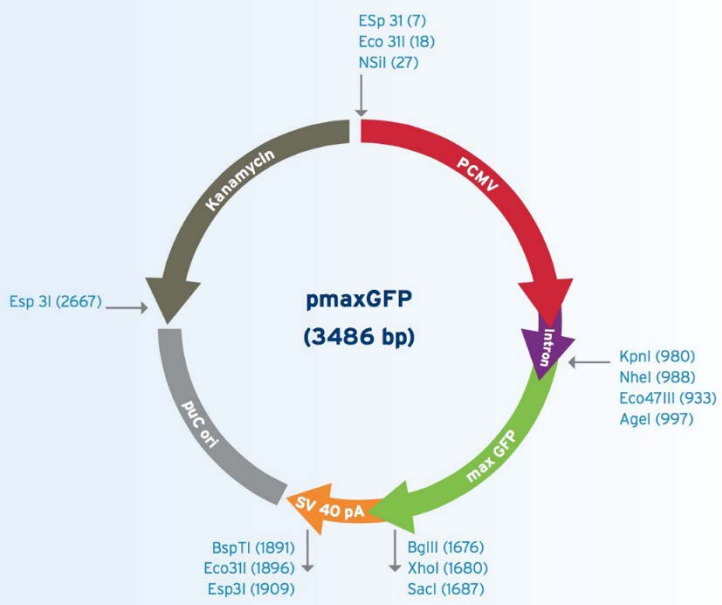


Figure 2-5 PmaxGFP cloning vector.

2.15.4 Determining transfection efficiency

Cells were firstly observed for GFP expression by live cell imaging using TiE fluorescence wide field inverted microscope (Nikon) then harvested and prepared for FACS analysis. Gating was performed as described in (Figure 2.6). Transfection efficiency represents ratio of GFP expressed cells in total population.

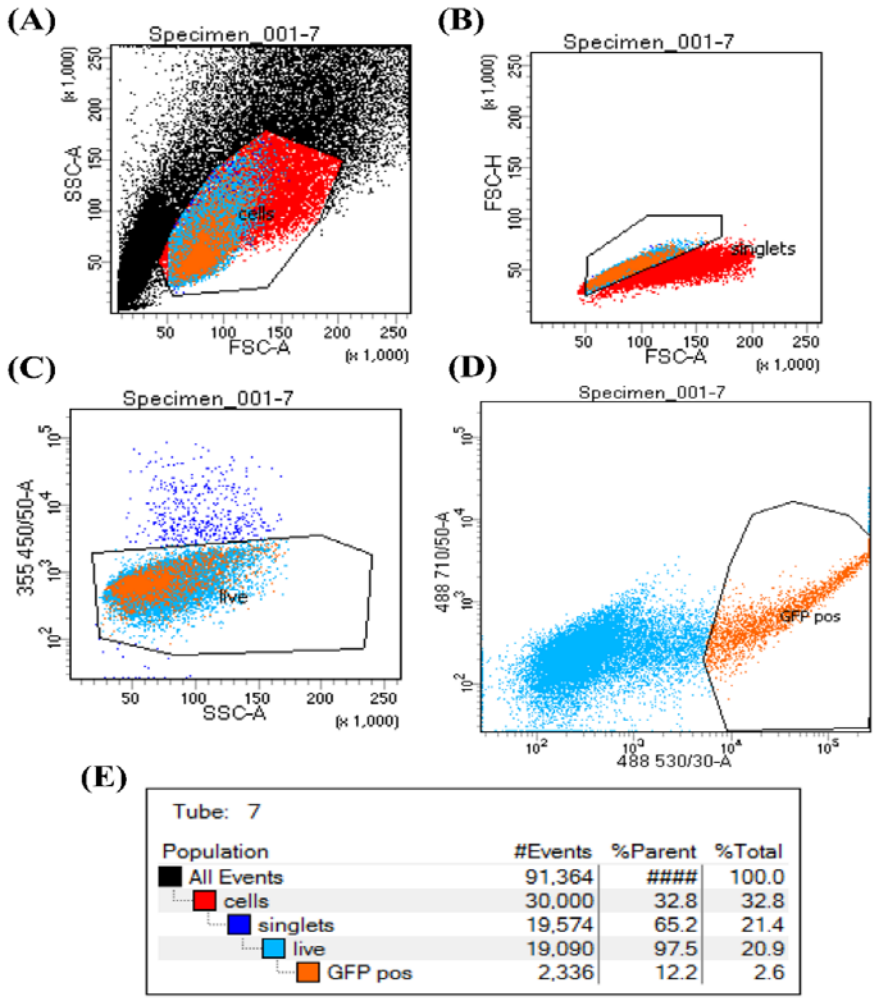


Figure 2-6 Determining transfection efficiency using FACS analysis.

This figure shows gating used in FACS for both analysis and cell sorting of transfected cells **(A)** Cells were selected from other debris **(B)** Cell clumps and doublets were excluded by selection of only singlet cell population **(C)** Dead cells were excluded by selection of only DAPI negative stained cells **(D)** Cells stained positively with GFP were selected **(E)** FACS quantitative summary table.

2.15.5 Determining gRNA efficiency

Efficiency of gRNA in generating genome editing was determined by transfection of PX458 plasmid with cloned gRNA (1-8) followed by selection of transfected cells by FACS then DNA extraction using (Wizard® SV Genomic DNA Purification System, Promega, UK) according to the manufacturer's instructions. Efficiency of gRNA in achieving mutation was measured as an insertion and/or deletion ratio (Indel %) using (EnGen mutation detection kit, New England Biolabs, UK) according to manufacturer's instructions. The principle of the kit is based on

amplification of the genomic region which is targeted by CRISPR to produce a 500-1000 bp PCR product then performing denaturation followed by annealing to produce heteroduplex DNA fragments. These duplexes were then digested with T7 Endonuclease I, a DNA nuclease that targets any mismatch of more than one base pair. Digestion products were visualized by gel electrophoresis which allows determination of the ratio of edited fragments (Figure 2.3).

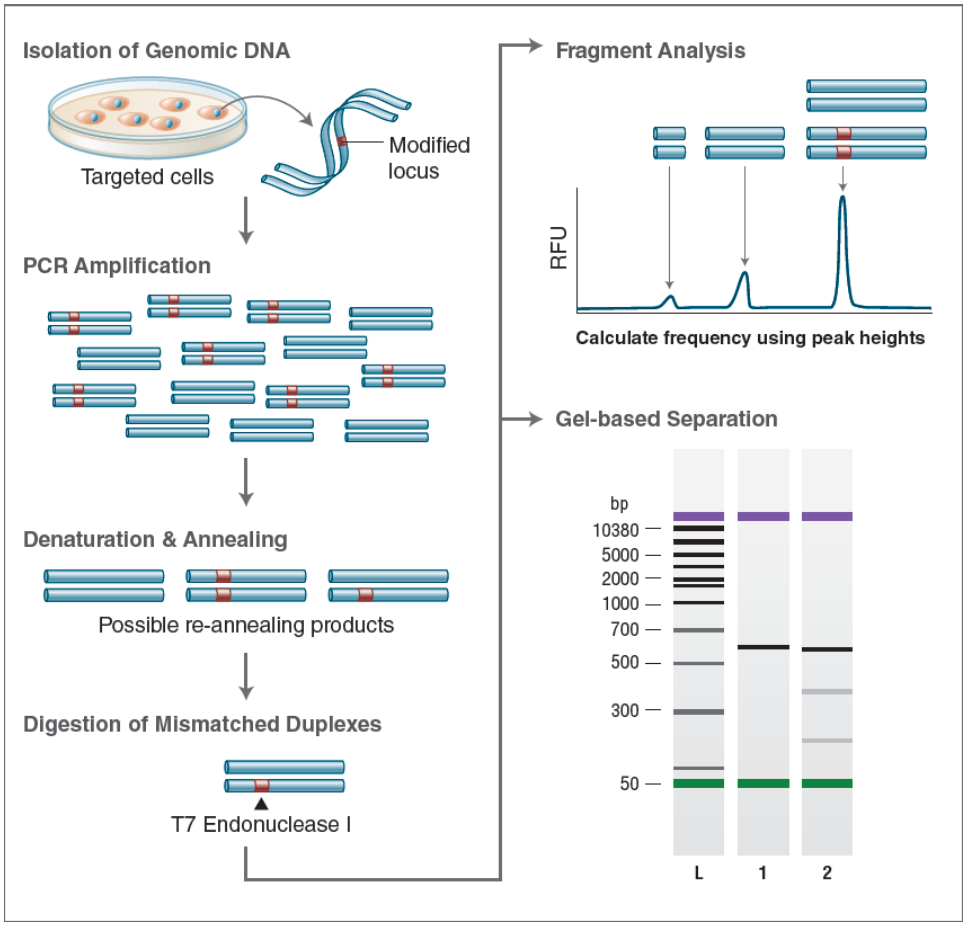


Figure 2-7 Workflow of T7 Endonuclease I digestion test

Experimentally the protocol was performed on samples which were either transfected with PX458 only (Control) or PX458 with cloned gRNAs (1-8). Briefly it was performed as the following:

The targeted region was amplified using TOPKO19 primer pair (Table 2.5) which produces a 511 bp amplicon. PCR was performed using reagents provided with the EnGen kit by mixing the following (25 µl reaction volume): 12.5 µl Q5 Hot Start High-Fidelity 2X Master Mix, 1.25 µl (F+R primer mixture 10 µM stock), 100 ng DNA, 2.5 µl DMSO (as targeted region was high GC, the PCR was optimized with 10 % DMSO) and complete volume to 25 µl with nuclease-free water.

The thermal cycler (CFX96 Touch™ , Bio-Rad) was set to the following parameters: Initial DNA denaturation (98°C for 30 seconds) for 1 cycle, denaturation, annealing and extension (98°C for 5 seconds, 58°C for 10 seconds, 72°C for 20 seconds) for 35 cycles, final extension (72°C for 2 minutes).

The product of the PCR reaction was then denatured followed by annealing to allow for heteroduplex formation. This was performed by mixing the following: 5 µl PCR reaction, 2 µl 10X NEBuffer 2, and 12 µl nuclease-free water. The mixture was then incubated in a thermal cycler with the following parameters: Initial denaturation (95°C for 5 minutes), then annealing by ramp down in 2 steps: from 95 to 85°C at 2°C/second, then from 85 to 25°C at 0.1°C/second.

Heteroduplex digestion was performed by incubating the above mixure with 1 µl T7 Endonuclease I at 37 °C for 15 minutes. Following digestion, 1 µl of Proteinase K was added and incubated at 37 °C for 5 minutes to denature and hence stop the T7 Endonuclease I.

Samples were then separated electrophoretically on a 2 % agarose gel and the efficiency were determined semiquantitatively by measuring band intensities and analyzing them with (Image lab software version 5.2).

2.15.6 Clonal selection and expansion

Cells were selected and sorted based on GFP expression of transfected cells using a BD FACSAria™ Fusion cell sorter (BD Bioscience, UK) through 488 nm wavelength channel. GFP positive cells were sorted into a single cell per well of 96-well plate and incubated at 37 ºC for 2-3 weeks until colonies formed.

2.15.7 Screening for knockout clones

2.15.7.1 Genotyping

Genotyping involved amplification of the targeted region using PCR, high percentage agarose gel separation, band extraction, then DNA purification and Sanger sequencing. Clones were harvested then centrifuged at 1000 rpm for 5 minutes. Genomic DNA was extracted using (Wizard® SV Genomic DNA Purification System, Promega, UK) according to the manufacturer's instructions.

The PCR reaction was performed using the TOPKO1 primer (Table 2.5) which amplified the region that is targeted by the gRNA oligonucleotides and produced an amplicon of 193 bp size on an agarose gel.

PCR was performed using reagents provided with the EnGen kit by mixing the following (25 µl reaction volume): 12.5 µl Q5 Hot Start High-Fidelity 2X Master Mix, 1.25 µl (F+R primer mixture 10 µM stock), 100 ng DNA, 2.5 µl DMSO (as targeted region was GC rich (76.7 %) and the PCR was optimized with 10 % DMSO) and volume was completed to 25 µl with nuclease-free water.

The thermal cycler (CFX96 Touch™ , Bio-Rad) was set to the following parameters: Initial DNA denaturation (98°C for 30 seconds) for 1 cycle, denaturation, annealing and extension (98°C for 5 seconds, 60°C for 10 seconds, 72°C for 20 seconds) for 35 cycles, final extension (72°C for 2 minutes).

Screening for Mutant clones was performed by running PCR products on a 3 % agarose gel and comparing the samples with the wild type. Clone bands that showed a difference from the wild type were purified from the gel using (QIAquick Gel Extraction Kit, Qiagen) according to the manufacturer's instructions and sent for Sanger sequencing (GATC Biotech) according to their sample requirements to confirm genome editing.

2.15.7.2 Determining TOP2B protein expression

Clones were tested for TOP2B expression by both immunofluorescence and western blotting techniques as described previously with some modifications in the immunofluorescence protocol applied specifically for clones screening. For immunofluorescence, cells were harvested and resuspended in PBS then added to poly-lysine coated multi-well slides (Marienfeld, Germany) to adhere for 10-15 minutes. Excess PBS with non-adherent cells were then removed by pipetting and samples were fixed with 4 % paraformaldehyde for 10 minutes at room temperature.

Samples were then washed twice with PBS and permeabilized with KCM with 0.1% (V/V) Triton X-100 for 15 minutes. Slides were incubated in blocking solution (KCM -T, 2% bovine serum albumin (BSA), 10% dried milk powder) for 1 hour then incubated with primary antibody (Table 2.1) for 1 hour at room temperature or at 4 °C overnight.

Slides were then washed with KCM-T for 3 × 10 minutes and incubated with secondary antibody (Table 2.1) for 1 hour at room temperature in the dark. Slides were further washed with KCM-T

for 3 × 10 minutes in subdued light. Vectashield mounting medium with DAPI (Vector laboratories, USA) was added to each slide and the coverslips were fixed by sealing with nail polish. Immunofluorescence imaging was performed using Olympus IX-81 microscopic imaging system and the integrated FITC fluorescence per nucleus was determined using (Volocity software V6.3, PerkinElmer, UK).

2.16 Chromatin immunoprecipitation (ChIP)

Chromatin immunoprecipitation is a technique used to test for interactions between DNA and proteins in biological systems and mostly in chromatin such as studying sites of interaction of transcription factors with DNA. The workflow of the ChIP protocol that was used in this study is outlined in (Figure 2.8).

2.16.1 Reagents

Preliminary solutions and buffers, which were used in preparing main ChIP buffers, are all detailed in (Appendix Table 1).

2.16.2 Cell harvesting and chromatin cross-linking

Nalm6 cells were grown to about 10^6 cells /ml in a large culture flask in a volume of 120 ml. Depending on the required number of immunoprecipitations (IPs), cell suspensions that correspond to ($2*10^7$ cells per IPs) were harvested by centrifugation at 1600 rpm for 5 minutes at 4°C and pellet were resuspended in 18 ml of ice cold PBS. Freshly prepared formaldehyde was added to 1 % (2 ml of 10 % stock) and the cell suspension was incubated for 10 minutes on ice with gentle mixing every 2 minutes. Formaldehyde was quenched by addition of Glycine to 0.125 M and cells were incubated for 5 minutes at room temperature. Cell suspension was centrifuged at 1600 rpm for 5 minutes at 4°C and the supernatant was removed. Cells were then resuspended in ice cold PBS and centrifuged at 1600 rpm for 5 minutes at 4°C. PBS was discarded carefully and pellets were frozen at -80 °C.

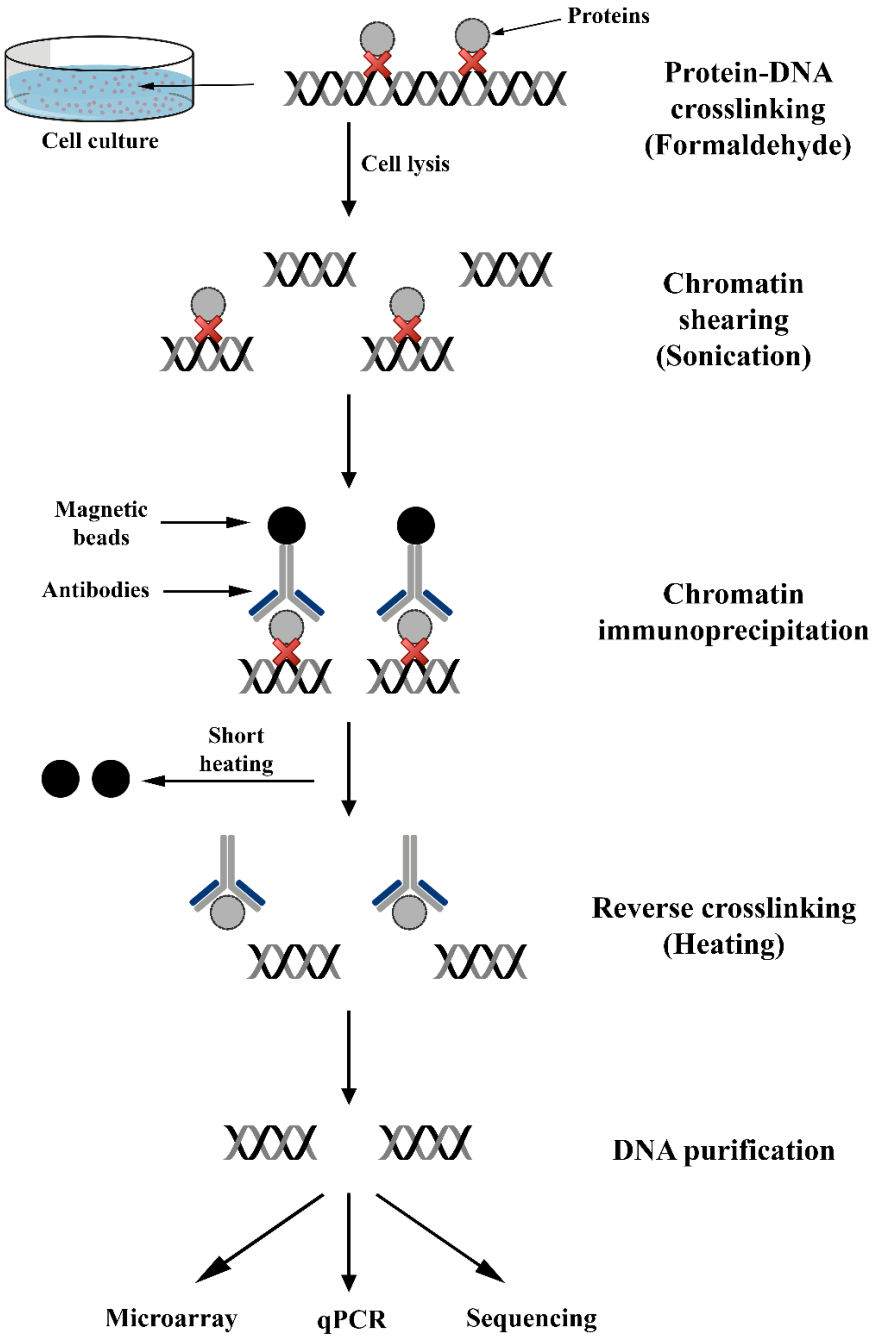


Figure 2-8 ChIP protocol workflow.

2.16.3 Chromatin extraction and fragmentation

Cell pellets were resuspended in (1 ml per $2x10^7$ cells) of ice cold 0.22 µm unit-filtered Farnham lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0.5% NP-40) with protease inhibitor cocktail (Expedeon, UK) and mixed by pipetting. Lysate was then passed 20 times through a 20 gauge needle. This treatment breaks the cells while keeping the nuclei mostly intact. Lysate was centrifuged at 2,000 rpm at 4°C for 5 minutes. Pellets were then resuspended with (300 µl per $2x10^7$ cells equivalent) of ice cold 0.22 µm unit-filtered RIPA buffer (1X PBS, 1% NP-40, 0.5% Na deoxycholate, 0.1% SDS) with protease inhibitor cocktail (Expedeon, UK) and mixed by pipetting. Lysate was then redistributed into pre-chilled eppendorf tubes depending on required number of immunoprecipitations (~$2x10^7$ cells equivalent / tube).

Chromatin fragmentation to 200-600 bp range was performed by probe sonication (Bandelin Sonopuls HD2070 sonicator) by applying 10 rounds of 7 pulsed cycle for 15 seconds and 20% power. Sonicated samples were centrifuged at 14,000 rpm for 15 minutes at 4°C in a microfuge and supernatants which came from the same sample were collected together in a pre-chilled 15 ml falcon tube (this step is to reduce sample-to-sample variation between IPs which might arise from variation in chromatin shearing level among samples). DNA and protein concentrations of chromatin samples were determined by Nanodrop ND-1000 UV-Vis Spectrophotometer (Thermo Scientific, UK) and 10 % of chromatin was taken as Input for analysis. To test fragmentation of the chromatin on a gel, 20 µl was taken and DNA was purified as described in (section 2.16.5).

2.16.4 Immunoprecipitation

ChIP-Grade Protein A/G Magnetic Beads (Thermo Scientific, UK, Cat. 26162) were used in the protocol. Beads stock was resuspended by gentle vortexing then 20 µl of resuspended magnetic bead slurry was added to a 1.5 ml eppendorf tube on ice containing 0.9 ml of ice cold 0.22 µm unit-filtered 5 mg/ml BSA (fraction V) in PBS and mixed well by brief vortexing. Eppendorf tubes were placed on a magnet and supernatants were removed. This washing step was repeated 3 more times, then beads were resuspended in 200 µl of BSA solution and antibodies were added as illustrated in (Table 2.1). Tubes were incubated on a rotator platform for 4 hrs at 4°C to allow for antibody-bead binding. Beads were washed 3 times with BSA solution and resuspended in 100 µl of BSA solution then chromatin was added (amount equivalent to 100 µg DNA per IP) and tubes were incubated on a rotator platform for 1 hour at room temperature followed by overnight incubation at 4°C.

Tubes were placed on a magnet and supernatants were removed then beads were washed with 0.9 ml of ice cold 0.22 µm unit-filtered LiCl IP Wash Buffer (100 mM Tris pH 7.5, 500 mM LiCl, 1% NP-40, 1% sodium deoxycholate) and tubes were incubated on a rotator platform for 3 minutes at room temperature. Tubes were placed on a magnet and supernatants were removed and this washing step was repeated 4 more times then beads were washed once with 0.9 ml of ice cold 0.22 µm unit-filtered TE Wash Buffer (10 mM Tris-HCl pH 7.5, 0.1 mM Na2EDTA) and tubes were incubated on a rotator platform for 1 minutes at room temperature. Beads were then resuspended in 200 µl of 0.22 µm unit-filtered IP Elution Buffer (1% SDS, 0.1 M NaHCO3) with vortexing at room temperature.

Binding between beads and chromatin-coupled antibodies was firstly destroyed by incubating tubes at 65 °C for 1 hour with vortexing every 15 minutes. Tubes were then centrifuged at 14,000 rpm for 3 minutes at room temperature and placed on the magnet for 5 minute and supernatant was collected in new tubes.

2.16.5 Reversing Chromatin cross-linking and DNA purification

For fragmentation testing samples and Input only, volume was brought up to 200 µl with 1X PBS. To each sample, 20 µl of 20 mg/ml proteinase K stock (Melford, Cat. MB2005) was added and samples were incubated for 5 minutes at room temperature followed by addition of 4 µl of 10 mg/ml RNase stock (Thermo Scientific, UK) and incubation for 20 minutes at room temperature. To purify DNA, QIAquick PCR Purification Kit (Qiagen, UK, Cat. 28104) was used according to the manufacturer's instructions. Briefly, 5 volumes (that's ~ 1000 µl) of Qiagen buffer PB was added to each sample and sample was applied to (QIAquick column in a provided 2 ml collection tube) and centrifuged at 17,900 x g (13,000 rpm) in a microfuge for 60 seconds at room temperature then flow-through was discarded. Columns were washed with 750 µl Qiagen Buffer PE then centrifuged at 17,900 x g (13,000 rpm) in a microfuge for 60 seconds at room temperature and flow-through was discarded. Centrifugation was repeated once again to allow removal of residual wash buffer.

Each QIAquick column was placed in a clean 1.5 ml microcentrifuge tube and DNA was eluted by adding 30 µl of warmed (~55°C) Qiagen Buffer EB (10 mM Tris·Cl, pH 8.5) to the center of the QIAquick membrane and allow for 2-5 minutes then column was centrifuged as before for 1 min then this step was repeated (total eluted volume was 60 µl). ChIP DNA was used in qPCR or stored at -20 °C until use.

2.16.6 PCR reaction and analysis

PCR reactions were performed using iTaq universal SYBR Green supermix (Bio-Rad) and primers described in (Table 2.5) by mixing the following (20 µl reaction volume): 10 µl iTaq universal SYBR Green supermix (2X), 1 µl (F+R primer mixture 10 µM stock), 2 µl ChIP DNA, and complete volume to 20 µl with nuclease-free water.

The thermal cycler (CFX96 Touch™ , Bio-Rad) was set to the following parameters: Initial DNA denaturation (95°C for 3 minutes) for 1 cycle, denaturation, annealing and extension (95°C for 15 seconds, 55°C for 20 seconds, 72°C for 30 seconds) for 40 cycles, melting curve analysis 65-95 °C at 0.5 °C increment for 5 seconds.

Signal of enrichment of protein of interest at primer amplification region was presented as % of Input and analysis was performed using the following formula:

$$= \left(\frac{2^{(Input\ ct - IP\ ct)}}{Input\ dilution\ factor} \right) \times 100$$

2.17 Statistical analysis

Data were analysed for significant difference with unpaired two-tailed Student's t-test using (Graphpad Prism software (v4.0), Graphpad, Inc., USA). Difference was considered to be significant when p value < 0.05 and all data represent the mean of 3 independent experiments expressed as mean ± standard error.

3 Chapter Three: TOP2 as a target for cancer treatment

3.1 Introduction

3.1.1 TOP2-targeting drugs

Drugs that act against Topoisomerases II are divided into two main groups; TOP2 poisons and catalytic inhibitors (Larsen *et al.*, 2003; Nitiss, 2009). Clinically, TOP2 poisons are the most frequently used class of anti TOP2 agents and used mainly as antineoplastic agents while catalytic inhibitors, in addition to their usage as an antitumor agent, have various therapeutic applications such as cardio-protectors and modulators of other agents (Larsen *et al.*, 2003).

Catalytic inhibitors involve a wide range of compounds that inhibit TOP2 activity by different mechanisms; for example, aclarubicin which is an anthracycline that inhibits TOP2 by interference with TOP2 catalytic action and preventing its binding to DNA because of its potent intercalating activity. Thus, it acts against the action of a group of TOP2 poisons such as etoposide, and amsacrine (Sørensen *et al.*, 1992; Petersen *et al.*, 1994; Larsen *et al.*, 2003). Similarly, Suramin acts as a TOP2 inhibitor by interfering with bounding between TOP2 and DNA and as a consequence antagonizes the cytotoxicity of some TOP2 poisons such as etoposide (Bojanowski *et al.*, 1992; Lelievre *et al.*, 1995; Larsen *et al.*, 2003).

Another catalytic inhibitor the coumarin novobiocin, inhibits TOP2 activity either by blocking ATP-binding site (Gormley *et al.*, 1996) or by interference with the enzyme capability to bind DNA (Sekiguchi *et al.*, 1996; Larsen *et al.*, 2003). On the other hand, it has been shown that merbarone has no significant effect on ATP hydrolysis nor TOP2 binding to DNA. Instead, it has a strong inhibitory effect on DNA cleavage mediated by TOP2 (Fortune and Osheroff, 1998; Larsen *et al.*, 2003). Bisdioxopiperazines such as ICRF-154, ICRF-187 and ICRF-193 exert their cytotoxicity via TOP2 by formation of noncovalent stabilized form of TOP2 around DNA (Andoh and Ishida, 1998) leading to the inhibition of ATPase activity of TOP2 (Hu *et al.*, 2002; Larsen *et al.*, 2003). However, many of the catalytic inhibitors have no clear mechanism of action but they are generally antagonistic with TOP2 poisons (Austin and Marsh, 1998).

TOP2 poisons act by stabilising TOP2-DNA covalent complexes formed as part of the catalytic cycle of the enzyme while the catalytic inhibitors exert their anti-TOP2 activity through at least one of the other steps of the catalytic action of TOP2 (Larsen *et al.*, 2003; Nitiss, 2009). TOP2

poisons increase levels of DNA-TOP2 cleavage complexes by two mechanisms; either by preventing the ligation step of the catalytic cycle of TOP2 which leads to an increase in both the level of complexes formed and their lifetime, or by increasing the rate of complex formation with limited effect on ligation efficacy of the enzyme (McClendon and Osheroff, 2007).

TOP2 poisons are classified into several groups including epipodophyllotoxins (e.g. etoposide and teniposide), anthracyclines (e.g. doxorubicin, epirubicin, daunorubicin, and idarubicin), acridines (e.g. amsacrine), the anthracenediones (e.g. mitoxantrone), and the benzo [c] phenanthridine alkaloid (e.g. NK314) (Cowell and Austin, 2012). Chemical structures of selected TOP2 poisons are depicted in (Figure 3.1).

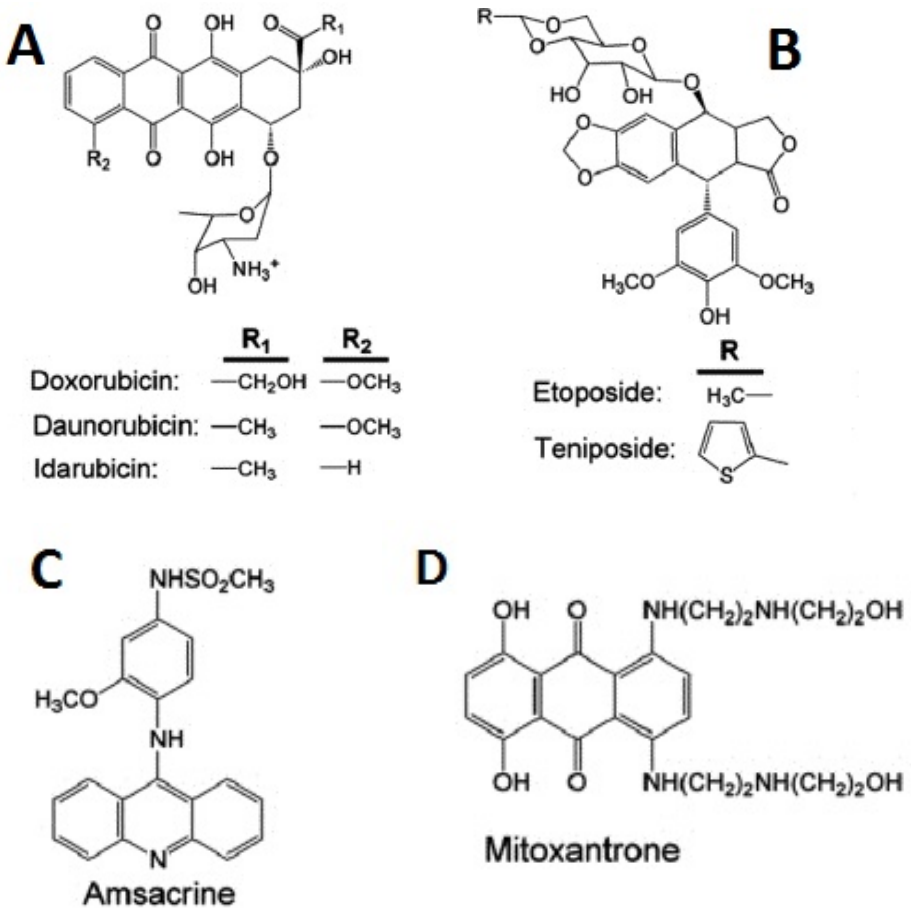


Figure 3-1 Chemical structures of selected TOP2 poisons

(A) Anthracycline **(B)** epipodophyllotoxins **(C)** Acridines **(D)** Anthracenediones. Structures are adapted from (Deweese and Osheroff, 2009) under the terms of the Creative Commons CC-BY-NC license. (https://creativecommons.org/licenses/by-nc/4.0/legalcode).

Epipodophyllotoxins are natural toxins extracted from the Indian *Podophyllum* plant and have been used for hundreds of years (Slevin, 1991). One of the most common epipodophyllotoxins is etoposide, an antitumor agent that was first synthesized in 1966 as a synthetic analogue for the natural epipodophyllotoxins 4′-demethylepipodophyllin benzylidene glucoside (DEPBG). Since this time, etoposide has been used as an antineoplastic agent but the anti TOP2 activity was not described until 1984 (Hande, 1998). Etoposide is one of the most frequently used drugs in the treatment of small-cell lung cancer and a wide range of haematological and germ-line tumours (Belani *et al.*, 1994; Hirabayashi *et al.*, 1994; Burden and Osheroff, 1998). Studies have shown that etoposide does not have potent intercalative activity and has little interaction with DNA in the absence of TOP2. It acts by binding non-covalently to TOP2 and inhibits religation of DSBs catalysed by the enzyme (McClendon and Osheroff, 2007).

A high resolution (2.16 Å) crystal structure of TOP2B (residues from 445 to 1201)-DNA complex stabilised by etoposide has been determined (Wu *et al.*, 2011). This model has shown an oligonucleotide DNA segment bound symmetrically by homodimeric TOP2B in a process that involves two etoposide molecules and six catalytic Mg^{2+}, three Mg^{2+} per monomer were clearly visible in the electron density maps. The cleavage complex was confirmed by the detection of the linkage between the scissile phosphates and the active tyrosine residues with the rupturing of phosphodiester bonds (Wu *et al.*, 2011). The crystal structure has shown that etoposide inhibits religation of the G-segment by physically keeping apart broken ends of the G-segment in a process involving insertion of two etoposide molecules in the DNA cleavage sites. In addition, this crystal structure shows relatively more opened DNA gate than those in the DNA-bound models of bacterial and yeast TOP2s and the catalytic Mg^{2+} is dissociated from the phosphotyrosyl moiety (Wu *et al.*, 2011).

Anthracyclines are a group of compounds that have been used in the treatment of haematological and solid tumours for decades (Johnson and Richardson, 1998). The anti-cancer activity of anthracyclines was first noted in the 1960s when an antibiotic Daunomycin isolated from *Streptomyces peucetius* cultures showed an anti-tumour activity against a number of cancer cell lines. Another structurally identical antibiotic rubidomycin was isolated at the same time from *Streptomyces coeruleorubidus* by another group and the compound subsequently named daunorubicin (Johnson and Richardson, 1998).

Shortly after that, doxorubicin was isolated from a mutant culture of the original strain (Johnson and Richardson, 1998). The chemical structure of doxorubicin is very similar to that of Daunomycin and it differs only by the substitution of a hydrogen atom with a hydroxyl group on the acetyl radical (Figure 2.3A). Doxorubicin showed a greater variety of activities than daunorubicin (Tan *et al.*, 1967; Bonadonna *et al.*, 1969; Johnson and Richardson, 1998).

The anti-TOP2 properties of anthracyclines were described in the mid-1980s and it is assumed that the antineoplastic properties of anthracyclines is due to their anti-TOP2 activity (Hande, 1998). Furthermore, in addition to their poisoning effect on TOP2, anthracyclines are highly intercalative compounds and have the ability to bind DNA with high affinity (Gewirtz, 1999). The intercalation properties of anthracyclines is due to the ability of their planar aglycone moiety to insert between DNA bases (Hande, 1998). Moreover, formaldehyde production upon ROS stimulation by anthracyclines leads to the formation of anthracycline DNA adducts and crosslinks which might increase their cytotoxicity (Zeman *et al.*, 1998; Parker *et al.*, 2004).

The chemical structure of anthracyclines also allows them to act as electron acceptors and produce reactive products which damage lipid membranes and macromolecules which might contribute to the cytotoxicity of anthracyclines (Gewirtz, 1999). In addition, anthracyclines interfere with DNA helicases and modify their ability to separate double-stranded DNA and obstruct separation of strands (Bachur *et al.*, 1992; Hande, 1998). However, one of the major side effects of anthracyclines is the development of cardiotoxicity (Kizek *et al.*, 2012). Several mechanisms have been suggested for anthracyclines-induced cardiotoxicity although the most common hypothesis is the oxidative stress results from free radical intermediates that primarily affect cardiac cells because of their lack of free radical scavenging system (Hande, 1998). Recently, evidence has shown that anthracyclines exert their cardiotoxicity via TOP2β (Zhang *et al.*, 2012).

Doxorubicin is one of the most common anthracyclines in clinical use. It has been prescribed widely for treatment of acute leukaemias, lymphoma, and solid tumours (Johnson and Richardson, 1998). Doxorubicin exerts its cytotoxicity by intercalation with DNA and binding to multiple nuclear targets such as TOP1 and TOP2 causing DNA damage which leads to apoptosis. In addition, doxorubicin is a known intercalative agent with the ability to inhibit both DNA and RNA polymerases and subsequently preventing DNA replication and transcription (Tacar *et al.*, 2013).

Daunorubicin is an antibiotic that acts effectively as an anti-cancer drug by inhibiting DNA synthesis and stabilizing TOP2-DNA complexes. Other mechanisms such as DNA adducts formation, free radicals generation, interference with strand separation and DNA helicase also have been reported (Gewirtz, 1999; Bertuzzi *et al.*, 2014). Daunorubicin has a narrower range of clinical use than doxorubicin (Hande, 1998), but it is the most common agent that has been used for the treatment of acute myeloid leukemia (AML) in combination with cytosine arabinoside (Ara-C) for decades (Feldman, 2011; Miyawaki *et al.*, 2011).

Idarubicin is a synthetic derivative of daunorubicin with a more lipophilic structure (Bertuzzi *et al.*, 2014). Idarubicin structure differs from daunorubicin only in the absence of the methoxy group from position 4 of the chromophore ring. Lipophilic properties of idarubicin over daunorubicin result in a higher cellular uptake and stronger DNA binding (Johnson and Richardson, 1998). Idarubicin has been introduced as an alternative for daunorubicin only for AML treatment (Hande, 1998). Several studies and clinical trials have been conducted to compare treatment outcomes of idarubicin with daunorubicin (Ohtake *et al.*, 2011). Some studies suggest more therapeutic benefits upon use of idarubicin instead of daunorubicin but these are not clinically significant and did not show an improvement in the general survival (Hande, 1998; Bertuzzi *et al.*, 2014).

Mitoxantrone is an anthracenedione that was introduced in the 1980s as an alternative for doxorubicin with less toxicity. Mitoxantrone has the same structure as doxorubicin with the exception of lacking of an amino-sugar moiety at C9 (Johnson and Richardson, 1998). Mitoxantrone has been proved to be less cardiotoxic than doxorubicin and other anthracyclines as it has a remarkably decreased potential for free radical formation (Hande, 1998). However, many studies has shown that in spite of lower cardiotoxicity of mitoxantrone, it has generally less anti tumour activity than doxorubicin (Hande, 1998). Mitoxantrone has been prescribed for the treatment of acute myeloid leukaemia, non-Hodgkin lymphoma, breast and prostate cancer (Johnson and Richardson, 1998).

Acridines are a group of compounds that have intercalation activity with DNA as a result of their planar structure. In addition, the majority of acridines have the ability to interfere with topoisomerase action. However, few agents of this group have been validated for cancer treatment in the clinic (Demeunynck, 2004). Amsacrine (m-AMSA) was introduced in the 1970s and was one of the early developed TOP2 poisons.

Amsacrine (m-AMSA) is one of the most famous antineoplastic acridines and it is prescribed for acute myeloid leukaemia (AML) treatment. It has been proposed that the anti tumour activity of (m-AMSA) is due to stabilization of TOP2 cleavage complex and prevention of the ligation process. Studies have shown that (m-AMSA) interacts with TOP2 through the methanesulfonamido side chain located on the anilino ring (Demeunynck, 2004). In vitro studies has been shown that (m-AMSA) targets both TOP2 isoforms and both enzymes form similar ternary complexes with DNA and m-AMSA. *TOP2B* deletion from murine cell lines resulted in increasing resistance to amsacrine, etoposide, and mitoxantrone suggesting that TOP2β might be a target for them in vivo (Marsh *et al.*, 1996; Errington *et al.*, 1999).

3.1.2 Relative contribution of TOP2 isoforms to the effect of TOP2 targeting drugs

Type II DNA topoisomerases are important targets in the treatment of human malignancies (Deweese and Osheroff, 2009). TOP2 targeting drugs are involved in about half of the chemotherapeutic treatments for cancer in the clinic (McClendon and Osheroff, 2007). The relative contribution of TOP2α and TOP2β isoforms in the effect of chemotherapeutic treatments of cancer is not clear (McClendon and Osheroff, 2007). Isoform-specific cellular roles and regulation patterns might reflect the contribution of TOP2 isoforms in the efficacy of TOP2 targeting drugs. As described previously in chapter one, TOP2α isoform is essential for proliferation-related activities and is highly expressed in proliferating but not in quiescent cells, whereas TOP2β isoform is expressed in all tissues even the fully differentiated cells such as cardiac cells. This suggests that TOP2α could be the isoform that primarily is responsible for anti-tumour activity of TOP2 targeting drugs while the TOP2β is associated with toxic side effects of these drugs.

Studies have been conducted to investigate the relative role of TOP2α and TOP2β in the cytotoxicity of TOP2 targeting drugs. While there is some evidence that both isoforms contribute to the cytotoxicity of etoposide, mitoxantrone, and amsacrine (mAMSA) in vivo (Willmore *et al.*, 1998; Errington *et al.*, 1999), other findings suggested that the cytotoxicity of etoposide follows TOP2α more than TOP2β (Errington *et al.*, 1999; Azarova *et al.*, 2007).

Attempts to develop TOP2 isoform specific agents are in progress. For example, NK314, a synthetic alkaloid that has been shown to specifically target TOP2α in vivo (Toyoda *et al.*, 2008). Moreover, pixantrone; a novel anthracycline and anthracenedione derivative agent which is currently under clinical trials is reported to show TOP2α selectivity (Hasinoff *et al.*, 2016). So far

there are no isoform specific drugs successfully employed in the clinic (Deweese and Osheroff, 2009).

Another important aspect is the development of therapy-related leukaemia (t-AL) typically acute myeloid leukaemia (t-AML) in patients treated with TOP2 targeting drugs which might be due to the genetic damage caused by these agents (Mauritzson *et al.*, 2002; Kayser *et al.*, 2011). The prevalence of (t-AL) is increasing due to the higher survival rates in patients with primary malignancy and the use of more intensive chemotherapeutic protocols. The development of (t-AL) is thought to be a result of non-lethal DNA damage induced in bone marrow progenitor cells by anti-cancer agents (Cowell and Austin, 2012).

It has been suggested that TOP2β is the isoform that is involved in chromosomal rearrangements that are associated with the development of (t-AL) (Azarova *et al.*, 2007; Azarova *et al.*, 2010; Haffner *et al.*, 2010; Cowell and Austin, 2012; Cowell *et al.*, 2012; Smith *et al.*, 2014).

3.1.3 Cytosine Arabinoside (Ara-C)

Cytosine arabinose (Ara-C) or its synonyms; arabinosylcytosine or cytarabine (Reiter *et al.*, 2001) is an effective anti-cancer drug that has been employed in the treatment of various types of adult and paediatric leukaemias (Hiddemann, 1991; Cline and Osheroff, 1999; Reiter *et al.*, 2001). The anticancer activity of Ara-C was determined for the first time in culture and animal models in the 1960s. This led to the first clinical trials against lymphoma and solid tumours in 1963. After beneficial outcomes of Ara-C incorporation in the treatment of acute leukaemias in 1986, it has been established that Ara-C is one of the most effective anticancer agents whether used as a single agent or in combination (Hiddemann, 1991).

It has been demonstrated that Ara-C is the most effective agent in the induction phase of AML treatment and become incorporated in all standard treatments for acute myeloid leukaemia (Kanno *et al.*, 2007). Furthermore, clinical trials have shown that Ara-C is an active drug against non-Hodgkin's lymphoma and acute lymphoblastic leukaemia (ALL) (Kanno *et al.*, 2007).

Ara-C is an antimetabolite derived synthetically from the nucleoside deoxycytidine (Hiddemann, 1991; Reiter *et al.*, 2001). It differs from the parent molecule by the addition of a *β*-hydroxyl group to the sugar moiety in the 2′ position (Hiddemann, 1991) (Figure 3.2). Despite successful clinical outcomes of treatment with Ara-C, the precise mechanism of action remains controversial (Han *et al.*, 2000; Wills *et al.*, 2000).

The overall evidence has shown that the cytotoxic effect of Ara-C is due to the combination of two mechanisms; inhibition of DNA polymerase(s), and incorporation into DNA structure and causing chain termination which stops DNA synthesis (Cline and Osheroff, 1999; Cros *et al.*, 2004; Krogh-Madsen *et al.*, 2012). Furthermore, accumulated evidence suggests that Ara-C acts in a cell cycle-specific pattern and selectively targets S phase cells leading to cell arrest at G1-S boundary (Gorczyca *et al.*, 1993; Horber *et al.*, 2000). In vitro studies have shown that the cell cycle specificity of Ara-C is influenced by dose and exposure time (Chresta *et al.*, 1992).

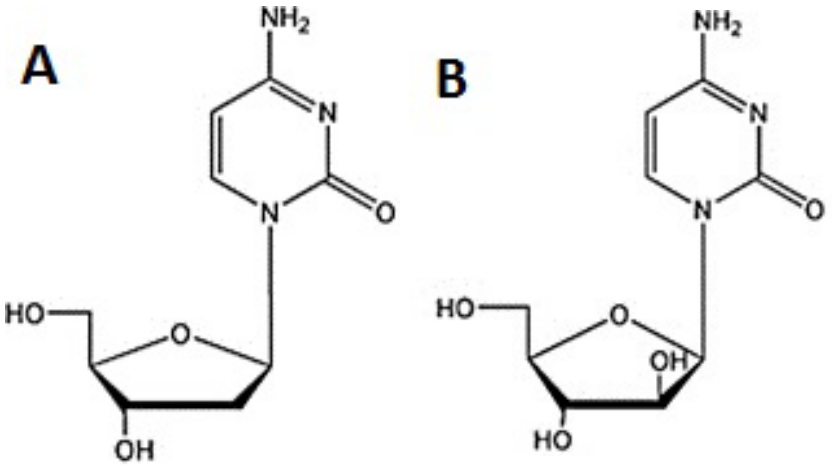


Figure 3-2 Structure of nucleoside and its derivative

Deoxycytidine (**A**) and its analogue cytosine arabinoside (Ara-C) (**B**). Adapted from (Garcia-Diaz *et al.*, 2010).

Because of the hydrophilic properties of Ara-C, it requires to be actively transported into the cell by a specific membrane transport protein; human equilibrative nucleoside transporter-1 (hENT-1) which permeates the cell membrane (Kanno *et al.*, 2007). Ara-C becomes toxic when activated by phosphorylation to its 5′-triphosphate form (Ara-CTP) by cell kinases (Cline and Osheroff, 1999; Kanno *et al.*, 2007). The (Ara-CTP) represents the key behind the cytotoxicity of Ara-C (Yamauchi *et al.*, 1996). Much evidence has shown a clear relationship between the intracellular level of Ara-CTP and the treatment outcome with Ara-C and in vitro and in vivo resistance to Ara-C (Cros *et al.*, 2004).

Phosphorylation of Ara-C to its active form (Ara-CTP) inside the cell is a process of three steps, it begins when Ara-C is converted to Ara-C monophosphate (Ara-CMP) by deoxycytidine kinase, then the latter is further phosphorylated to Ara-C diphosphate (Ara-CDP) by (deoxy)cytidylate kinase. Subsequently, Ara-CDP is phosphorylated into Ara-CTP by nucleoside diphosphate kinase

(Zhang and Kiechle, 2004). The first step of phosphorylation of Ara-C to Ara-CMP which is mediated by deoxycytidine kinase represents the rate limiting step of Ara-C activation (Gandhi and Plunkett, 1992; Zhang and Kiechle, 2004).

The activity of deoxycytidine kinase is effected by the intracellular level of deoxynucleotides and cell cycle phase (Gandhi and Plunkett, 1992), and is maximal during middle and late S phase (Hiddemann, 1991). Furthermore, this process is also controlled by the counter activity of deoxycytidine kinases and cytidine/deoxycytidine deaminase which is produced predominantly in the liver. These enzymes convert Ara-C to the nontoxic form uracil arabinoside (araU) (Hiddemann, 1991).

The (Ara-CTP) competes with deoxycytidine triphosphate (dCTP) for incorporation in the DNA by DNA polymerase during the DNA synthesis process (Zhang and Kiechle, 2004). Incorporation of the activated form (Ara-CTP) into the DNA causes chain termination and subsequent inhibition of DNA synthesis (Sarkar *et al.,* 2005). It has been determined that there is a tight relationship between the cytotoxicity of Ara-C and the level of Ara-CTP incorporation into DNA (Lacombe *et al.,* 1996). Studies have determined that Ara-CTP incorporation into DNA correlates with several events including; localized alteration in a DNA duplex, DNA polymerase and TOP 1 inhibition, and corruption of DNA elongation (Zhang and Kiechle, 2004).

Furthermore, it is believed that the cytotoxicity of Ara-C is associated with its inhibitory effect on DNA polymerases; α, δ, and ε (Han *et al.*, 2000; Sekizawa *et al.*, 2007). Nevertheless, studies have shown that DNA polymerase α is the most sensitive polymerase isoform to Ara-CTP (Han *et al.*, 2000; Cros *et al.*, 2004). However, the mechanism by which Ara-CTP exerts its cytotoxicity might not be simply explained by the competition with deoxycytidine triphosphate (dCTP) for binding with DNA polymerase as kinetic studies have shown that Ara-CTP is a weak competitive with (dCTP) for DNA polymerase binding (Han *et al.,* 2000; Wills *et al.,* 2000).

Another studies have suggested that incorporation of Ara-CMP at the 3′ end of a replicating DNA leading to chain termination and blocking DNA synthesis (Han *et al.,* 2000). However, this again has been opposed by findings of other groups that the incorporation of Ara-C does not lead to complete chain termination and high percentage of incorporated Ara-C was located in internucleotide positions throughout the daughter DNA (Han *et al.,* 2000).

The (Ara-CTP) form also might exert toxicity on the cells which are not synthesizing DNA due to its involvement as a nucleoside alternative in glycoprotein and phospholipid metabolism (Hiddemann, 1991).

3.1.4 Combinations of TOP2 targeting drugs and Ara-C

The combination of nucleoside analogue Cytosine Arabinoside (Ara-C) and Anthracyclines such as daunorubicin or idarubicin have been commonly investigated in a wide range of studies including Acute Myelogenous Leukaemia (Hiddemann, 1991; Feldman, 2011; DiNardo and Cortes, 2015), Acute Lymphocytic Leukaemia (Bassan, 1996; Rowe, 2009), non-Hodgkin's lymphoma (Dufour *et al.*, 1996), and Acute Promyelocytic Leukaemia (Fenaux *et al.*, 2001). Despite the broad use of this combination, it has been mostly applied in the treatment of Acute Myelogenous Leukaemia (AML) over the last decades (Tardi *et al.*, 2009; Feldman *et al.*, 2012) and has proved to be an effective regimen (Krogh-Madsen *et al.*, 2012; DiNardo and Cortes, 2015).

Anthracyclines exert their anticancer activity by targeting the cellular enzyme topoisomerase II (TOP2) and prevent the ligation step of its catalytic cycle leading to a cytotoxic Double-Stranded Breaks (DSBs) that trigger cell death (Cowell and Austin, 2012). on the other hand, the effect of Ara-C is more controversial but it might be mainly due to a combination of two mechanisms (1) inhibition of DNA polymerase, and (2) incorporation into the DNA structure and causing chain termination which stops DNA synthesis (Hiddemann, 1991; Cline and Osheroff, 1999; Cros *et al.*, 2004).

The combination between an anthracycline (especially daunorubicin) and Ara-C represents the backbone treatment of AML in the majority of clinical trials over the world (Ohtake *et al.*, 2011; Bertuzzi *et al.*, 2014). Since the successful introduction of daunorubicin in the treatment of patients with AML, numerous studies and clinical trials tested the combination between daunorubicin and Ara-C that finally lead to the establishment of the (7+3) Ara-C plus daunorubicin induction regimen by Cancer and Leukaemia group B (CALGB) in 1982 (Rai *et al.*, 1981; Yates *et al.*, 1982). This combination has become the standard induction treatment of AML (Bertuzzi *et al.*, 2014).

Clinically, the first course of induction therapy for AML involves continuous infusion of 100-200 mg/m^2 of cytarabine for 7 days simultaneously with bolus injection of 45-90 mg/m^2 of daunorubicin daily for 3 days (Ohtake *et al.*, 2011; Burnett *et al.*, 2015; Lynch and Medeiros, 2015). For example, the AML17 clinical trial which is performed over 136 centres in the UK, Denmark, and New

Zealand from September 2011 to October 2013. This clinical trial involved 1206 patients who were randomized to be given 100 mg/m2 of cytarabine every 12 hours for 10 days and either 60 or 90 mg/m^2 daunorubicin on days 1, 3, and 5 (Burnett *et al.*, 2015).

Numerous attempts for increasing the clinical outcome of (daunorubicin+Ara-C) combination have successfully increased the efficiency of this regime. These attempts involved changing doses and/or timing or both (Goldstone *et al.*, 2001; Tardi *et al.*, 2009; Miyawaki *et al.*, 2011). On the other hand, it has been suggested that the combination of Ara-C with TOP2 targeting drugs has an enhanced outcome upon scheduling over concurrent treatment (Rao *et al.*, 1975; Ritch *et al.*, 1981; Minford *et al.*, 1984; Loughlin *et al.*, 1996).

3.2 Aims

Literature reviewed above involved two aspects in cancer treatment with TOP2 targeting drugs. First, the relative contribution of TOP2α and TOP2β isoforms in anti-tumour (cytotoxicity) and therapy-related leukaemia (genotoxicity) of TOP2 targeting drugs, with the suggestion that TOP2β isoform is implicated in therapy-related leukaemia. Second, the role of dose ratio/order or both in enhancing outcome of cancer treatment with TOP2 targeting drugs in combination with Ara-C. This study was conducted to investigate these two aspects. The aims were to:

- Investigate the relative contribution of TOP2 isoforms in cytotoxicity and genotoxicity of two TOP2 poisons used interchangeably: daunorubicin and idarubicin.
- Test whether using different dose ratio, scheduling, or both might enhance the efficiency of (daunorubicin+Ara-C) combination in terms of cytotoxicity and genotoxicity.

To address the aims above, the following experimental objectives were followed:

- The dose response curves were determined for each single drug; daunorubicin, idarubicin, Ara-C in Nalm6^{WT}, Nalm6$^{B-/-}$, and Nalm6$^{A+/-}$ cells lines.
- Determine genotoxicity of drugs using the in vitro micronucleus (MN) test by FACS.
- Determine the cytotoxicity and genotoxicity of different Ara-C and daunorubicin combinations.
- The cytotoxicity and genotoxicity of scheduled (daunorubicin+Ara-C) combination was compared with the concurrent treatment.
- Determine the effect of all treatments on cell cycle phase distribution in Nalm6 cell lines.

3.3 Results

3.3.1 TOP2 expression in Nalm6 cell lines

The wild type Nalm6 cell line was originally derived from a patient with acute lymphoblastic leukaemia (ALL) in relapse and it has shown that their genes can be knocked out with high efficiency by homologues recombination (So *et al.*, 2006; Adachi *et al.*, 2008a). Two TOP2 genetic variants were generated using this method by (Toyoda *et al.*, 2008); Nalm6$^{B-/-}$ and Nalm6$^{A+/-}$ and they have been shown to express different levels of the TOP2 isoforms. The expression of TOP2 in Nalm6 cell lines in this study was evaluated by both western blotting and immunofluorescence and consistently shown that the expression of TOP2B is absent in Nalm6$^{B-/-}$ and the expression of TOP2A is reduced in Nalm6$^{A+/-}$ (Figure 3.3A and B).

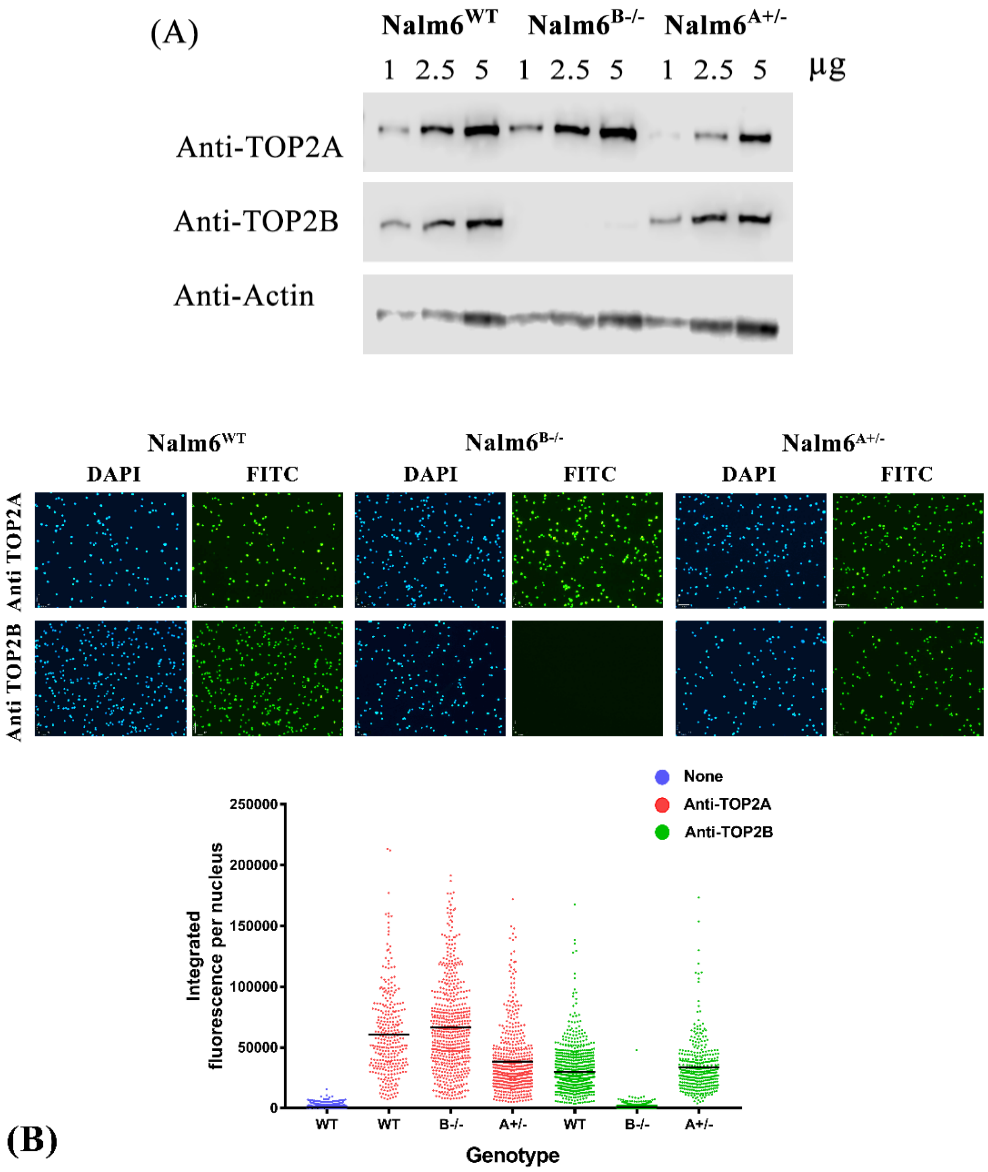


Figure 3-3 TOP2 expression in Nalm6 cell lines

Expression of both TOP2 isoforms was determined in Nalm6 cell lines; Nalm6^{WT}, Nalm6$^{B-/-}$, and Nalm6$^{A+/-}$ by western blotting (**A**) and immunofluorescence (**B**) as described in the materials and methods. For western blotting, the whole cell extracts (WCEs) were prepared for SDS-page then transferred onto nitrocellulose membranes and probed with the following antibodies: anti-TOP2A (4566), anti-TOP2B (30400), and anti-actin (ab3280). For immunofluorescence, cells were prepared for staining then probed with anti-TOP2A (4566) and anti-TOP2B (4555) antibodies. Imaging was performed using an Olympus IX-81 microscopic imaging system and the integrated FITC fluorescence per nucleus was determined using (Volocity software V6.3, PerkinElmer, UK) and analysed using (Graphpad Prism software (v4.0), Graphpad, Inc., USA) as described by (IG *et al.*, 2011). Scatter plot represents the raw integrated fluorescence for nuclei and the black line is the mean value.

3.3.2 Nalm6 cell lines have comparable Growth curves and doubling times

Growth curve of Nalm6 cell lines used in this study is shown in (Figure 3.4).

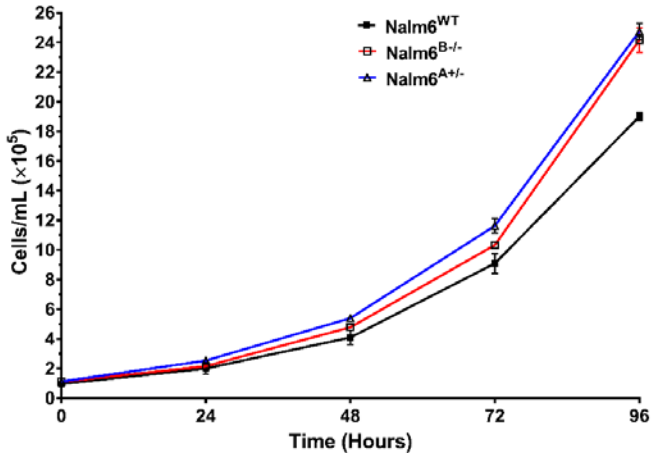


Figure 3-4 Growth curve of Nalm6 cell lines

Nalm6 cell lines were seeded at 1×10^5 cells/ml then grown for up to 4 days. Cell counting was performed every 24 hrs. Growth curves were generated from at least 3 independent experiments, error bars representing the SEM.

The doubling time of the Nalm6 cell lines was determined and the mean of 48-96 hour time points where the cells are in their exponential growth was calculated. The doubling time means were 22 hours 1 minute, 22 hours 56 minutes, and 21 hours 57 minutes for Nalm6^{WT}, Nalm6$^{B-/-}$, and Nalm6$^{A+/-}$ respectively as detailed in (Table 3.1). Statistical analysis showed no significant difference between Nalm6 cell lines in terms of doubling times. The determination of doubling times for cell lines in our study is important because in the micronuclei assay, it is crucial that the micronuclei which are formed during mitosis are scored when the cells undergo at least one cell cycle but no more than two (OECD, 2010). This time allows cells to be exposed to the agents and undergo mitosis without undergoing further cell cycles as the fate of the micronuclei formed is unknown in the additional cycles (Fenech, 2000).

Cell line	Doubling time (hrs.)	SEM (±)
Nalm6 ^{WT}	22.01	0.22
Nalm6 $^{B-/-}$	21.94	0.29
Nalm6 $^{A+/-}$	21.95	0.46

Table 3-1 Doubling times of Nalm6 cell lines

Data were generated from at least 3 independent experiments.

3.3.3 Cytotoxicity of TOP2 poisons in Nalm6 cell lines

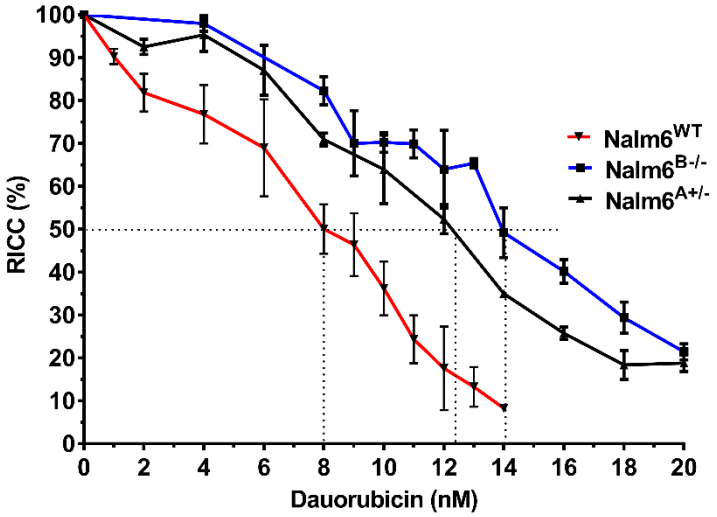
The dose response curves were determined for Nalm6 cell lines treated with two TOP2 poisons daunorubicin and idarubicin (Figure 3.5). The IC50 of drugs as well as the contribution of TOP2 isoforms to the cytotoxicity of drugs was determined by comparing the dose response curve of the wild type cells with the TOP2 null knockout for *TOP2B* ($Nalm6^{B-/-}$) and the TOP2 heterozygote (one allele) knockout for *TOP2A* ($Nalm6^{A+/-}$).

It has been well established that the TOP2 enzyme is Targeted by daunorubicin (Gewirtz, 1999). In support of this, the IC50 for daunorubicin in the Nalm6 wild type cell line was 7.77 nM which was significantly less than the IC50 in the $Nalm6^{B-/-}$ and $Nalm6^{A+/-}$ of 14.03 and 12.13 nM ($p=0.0001$ and $p=0.0004$) respectively. Similarly, there was a significant increase ($p=0.0003$) in the IC50 of idarubicin in the wild type cell line from 4.327 nM to 7.55 nM in $Nalm6^{B-/-}$ and to 6.213 nM $Nalm6^{A+/-}$ ($p=0.0247$).

In addition, it has been widely suggested that the cytotoxicity of TOP2 poisons is mainly TOP2α dependent (Azarova *et al.*, 2007). However, this might not be confirmed in our results by direct comparison between the IC50s for two reasons: firstly, both $Nalm6^{B-/-}$ and $Nalm6^{A+/-}$ showed significant increase in IC50 over the wild type cells. Secondly, both $Nalm6^{B-/-}$ and $Nalm6^{A+/-}$ cell lines showed no significant difference in terms of the IC50 of idarubicin ($p=0.0827$) (Figure 3.5).

In addition, $Nalm6^{A-/-}$ is not viable and the knockout in $Nalm6^{A+/-}$ cell line is heterozygous unlike $Nalm6^{B-/-}$ which are *TOP2B*-null cells and this makes it difficult to compare the role of both isoforms by direct comparison of IC50 values. Unlike idarubicin, however, the IC50 of daunorubicin in $Nalm6^{B-/-}$ was significantly lower than that of $Nalm6^{A+/-}$ ($p=0.0040$). Moreover, the IC50 of idarubicin was significantly less than that of daunorubicin in all cell lines (Figure 3.5). These results suggest that both TOP2 isoforms contribute to the cytotoxicity of TOP2 poisons daunorubicin and idarubicin.

	Mean IC_{50} (nM)	SEM	P values (unpaired t test)			
				WT	B-/-	A+/-
WT	7.77	0.292	**WT**			
B-/-	14.03	0.167	**B-/-**	<0.0001		
A+/-	12.13	0.273	**A+/-**	0.0004	0.004	



	Mean IC_{50} (nM)	SEM	P values (unpaired t test)			
				WT	B-/-	A+/-
WT	4.327	0.11	**WT**			
B-/-	7.55	0.245	**B-/-**	0.0003		
A+/-	6.213	0.526	**A+/-**	0.0247	0.0827	

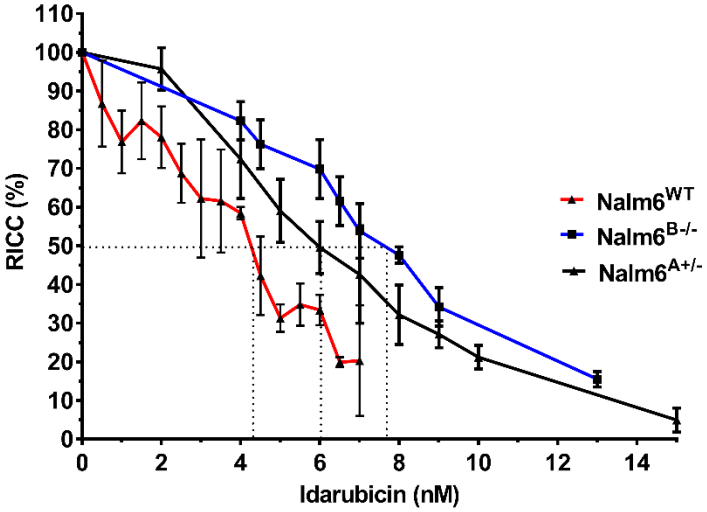
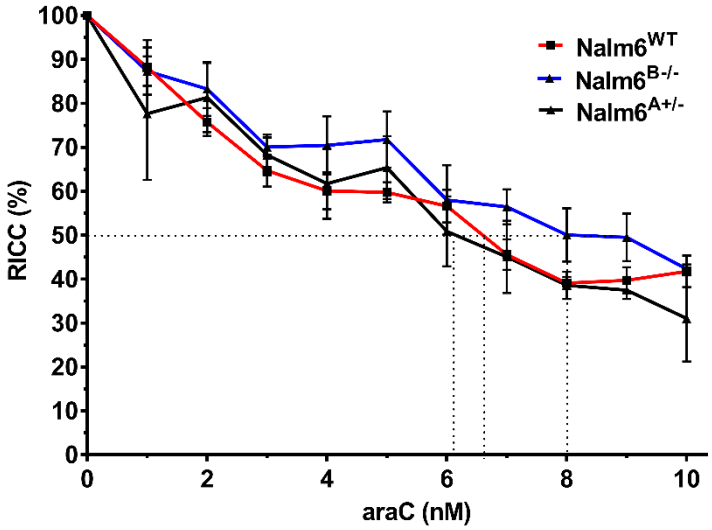


Figure 3-5 Both TOP2 isoforms comparably contribute to the cytotoxicity induced by daunorubicin and idarubicin

Cells were incubated with drugs for 48 hours continuously and the cytotoxicity was determined as the relative increase in cell count (RICC) using cell counting protocol. Data presented here is the mean of at least three independent experiments with error bars representing the SEM.

3.3.4 Cytotoxicity of Ara-C in Nalm6 cell lines

The antimetabolite Ara-C is a nucleoside analogue that interferes with DNA synthesis processes and inhibits DNA polymerase (Hiddemann, 1991; Cros *et al.*, 2004). The dose response curves of Nalm6 cell lines treated with Ara-C were determined and are presented in (Figure 3.6). The IC50 of Ara-C in Nalm6 cell lines were 6.33, 7.617, and 6.573 nM for Nalm6^{WT}, Nalm6$^{B-/-}$, and Nalm6$^{A+/-}$ respectively. As expected from the mechanism of action of Ara-C, there was no significant difference in the IC50 values between wild type cell lines and their derivatives.



	IC50 (nM)	
Cell line	**Mean**	**SEM**
Nalm6^{WT}	6.33	0.196
Nalm6 $^{B-/-}$	7.617	1.089
Nalm6 $^{A+/-}$	6.573	0.427

P values (unpaired t test)			
	WT	**B-/-**	**A+/-**
WT			
B-/-	0.2574		
A+/-	0.4356	0.7548	

Figure 3-6 Ara-C induces cytotoxicity in a TOP2-independent manner

Cells were incubated with Ara-C for 48 hours continuously and the cytotoxicity was determined as the relative increase in cell count (RICC) using cell counting protocol. Data presented here is the mean of at least three independent experiments with error bars representing the SEM.

3.3.5 Cytotoxicity of Ara-C and daunorubicin in combination

The relationship between drugs when combined with each other is either synergistic, antagonistic, or additive (Chou and Talalay, 1984; Mayer *et al.*, 2006). The cytotoxicity profile for daunorubicin and idarubicin in Nalm6 cell lines suggests that both TOP2 isoforms contribute to the cytotoxicity of these drugs. For better understanding of the combination between daunorubicin and Ara-C, selected combinations were tested in the wild type cells. Daunorubicin was selected for two reasons; firstly, it is more commonly used in the treatment of AML than idarubicin, secondly, despite the cytotoxicity of idarubicin being higher than daunorubicin, both drugs generally have the same mechanism of action.

Many studies have shown that the relationship between drugs in combination can be synergistic, antagonistic, or additive according to the ratio of the drugs when combined (Mayer *et al.*, 2006). Based on the cytotoxicity profile of drugs as a single agent, three different combinations of Ara-C and daunorubicin were used to evaluate whether the relationship between Ara-C and daunorubicin is synergistic, antagonistic, or additive using the combination Index (CI) which was calculated as described in (Materials and methods, section 2.8). The first combination involved a range of Ara-C and daunorubicin doses in a constant ratio (1:1) that covered the total cytotoxicity profile (Figure 3.7). The combination index (CI) of each combination was determined and presented in (Figure 3.7). In spite of the significant decrease in the IC50 of the combination (3.87 nM) when compared with Ara-C (6.703 nM) or daunorubicin (7.733 nM) alone, the combination index (CI) of all treatment points suggested that the inhibitory effect is additive (Figure 3.7).

Drug	Mean IC50 (nM)	SEM	P values (unpaired t test)			
				Ara-C	Daun	Ara-C + Daun
Ara-C	6.703	0.283	**Ara-C**			
Daun	7.733	0.206	**Daun**	**0.0424**		
Ara-C + Daun	3.87	0.178	**Ara-C + Daun**	**0.0011**	**0.0001**	

Ara-C+Daun (nM)	CI (mean±SEM)
2+2	**1.04 ± 0.14**
4+4	**1.10 ± 0.09**
5+5	**1.06 ± 0.11**
6+6	**1.28 ± 0.11**
8+8	**1.08 ± 0.12**
10+10	**1.02 ± 0.10**

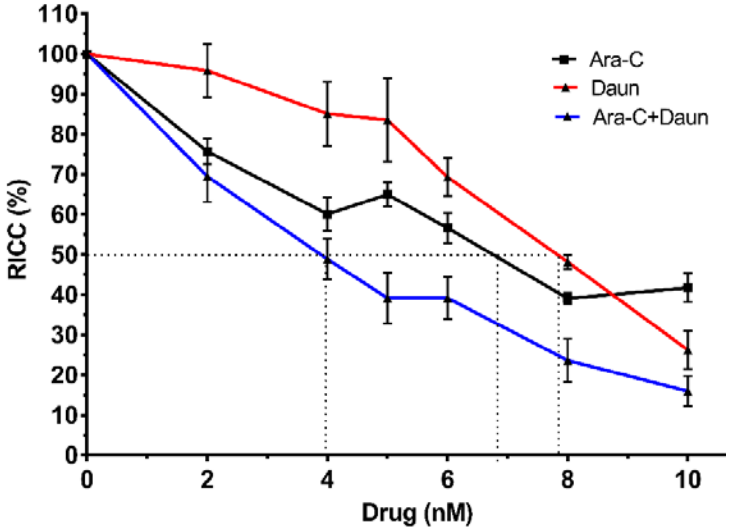
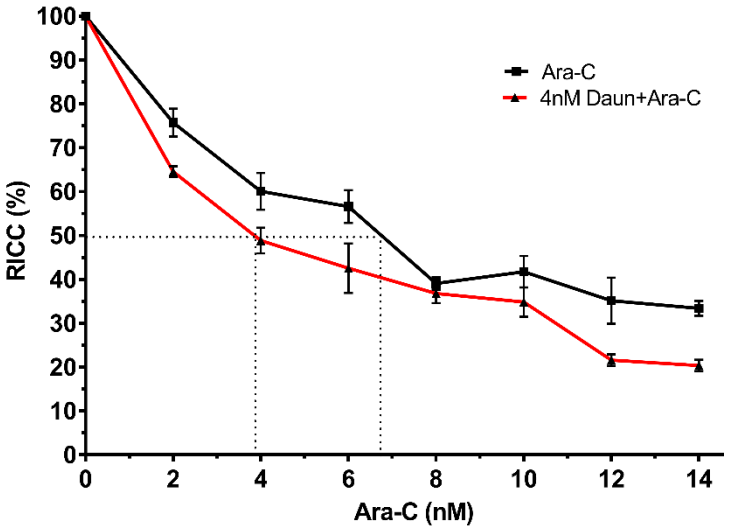


Figure 3-7 Dose response curve of (Ara-C+daunorubicin) combination at a constant ratio

Cells were incubated with Ara-C, daunorubicin, or both for 48 hours continuously and the cytotoxicity was determined as the relative increase in cell count (RICC). Data presented here is the mean of at least three independent experiments with error bars representing the SEM. (CI) the combination Index. Note: CI < 0.9 (synergistic), CI > 1.1 (antagonistic), CI = 0.9-1.1 (additive).

The second combination involved a non-constant (Ara-C+daunorubicin) molar ratio. Cells were treated with a low cytotoxic dose (IC20) of daunorubicin (4 nM) combined with a range of Ara-C doses as described in (Figure 3.8). The IC50 of Ara-C was significantly reduced (p=0.0050) from 6.713 nM to 3.973 nM when combined with daunorubicin. The combination index data for this combination showed that the relationship is synergistic at the highest Ara-C doses of 10 nM (CI=0.85 ± 0.05) and 14 nM (CI=0.89 ± 0.050**)** and this might be due to the enhanced effect of daunorubicin: Ara-C (1:3) molar ratio. Studies have shown the effect of molar ratio of drugs in combination regardless of the total dose (Tardi *et al.*, 2009).



	IC50 (nM)	
Drug	**Mean**	**SEM**
Ara-C	6.713	0.278
4nM Daun + Ara-C	3.973	0.402

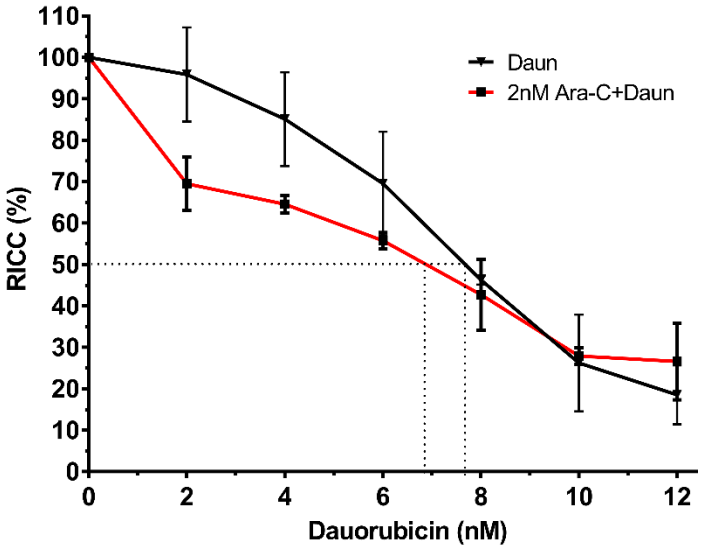
Ara-C + Daun (nM)	**CI (mean±SEM)**
2+4	**1.2 ± 0.04**
4+4	**1.1 ± 0.09**
5+4	**1.18 ± 0.19**
6+4	**1.16 ± 0.07**
8+4	**1.26 ± 0.15**
10+4	**0.85 ± 0.05**
14+4	**0.89 ± 0.05**

Figure 3-8 Dose response curve of (Ara-C+daunorubicin) combination at non-constant ratio

Cells were incubated with Ara-C, daunorubicin, or the combinations for 48 hours continuously and the cytotoxicity was determined as the relative increase in cell count (RICC). Data presented here is the mean of at least three independent experiments with error bars representing the SEM. (CI) the combination Index. Note: CI <0.9 (synergistic), CI>1.1 (antagonistic), CI =0.9-1.1 (additive).

The third combination involved non-constant Ara-C:daunorubicin molar ratios where cells were treated with a fixed low cytotoxic dose (IC20) of Ara-C (2 nM) in combination with different doses of daunorubicin that spanned the IC10 to IC80 of the cytotoxicity profile (Figure 3.9). There was no significant change (p=0.5518) in the IC50 of daunorubicin upon the addition of low cytotoxic dose of Ara-C and the combination index values indicated an additive to slightly antagonistic

relationship. The advantage of using the combination index (CI) in evaluating the relationship between drugs when combined is that it allows for a quantitative determination of synergy, antagonism, or additive relationships and provides a useful method to compare the efficiency of different combinations (Mayer *et al.*, 2006). Moreover, it allows for determining whether the enhancement of the effect of one drug by the other is due to a real synergistic relationship or simply because of the additive effect of both drugs (Chou, 2010).



	IC50 (nM)	
Drug	**Mean**	**SEM**
Daun	7.54	0.231
2nM Ara-C + Daun	7.183	0.499

Ara-C/Daun Combination (nM)	Combination Index (mean±SEM)
2/2	**1.04 ± 0.14**
2/4	**1.20 ± 0.04**
2/6	**1.25 ± 0.03**
2/8	**1.19 ± 0.13**
2/10	**1.05 ± 0.02**
2/12	**1.20 ± 0.15**

Figure 3-9 Dose response curve of (Ara-C+daunorubicin) combination at a non-constant ratio

Cells were incubated with Ara-C, daunorubicin, or the combination for 48 hours continuously and the cytotoxicity was determined as the relative increase in cell count (RICC). Data presented here is the mean of at least three independent experiments with error bars representing the SEM. (CI) the combination Index. Note: CI <0.9 (synergistic), CI>1.1 (antagonistic), CI =0.9-1.1 (additive).

Although Ara-C and daunorubicin combination is well established in the treatment of acute myeloid leukaemia, the mechanism by which both of the Ara-C and daunorubicin contribute to the therapeutic effect of this common combination in the clinic is not fully understood. Some evidence has suggested that Ara-C potentiates the cytotoxicity of a number of TOP2 poisons in a scheduling-dependent manner (Edelstein *et al.*, 1974; Ritch *et al.*, 1981; Minford *et al.*, 1984; Fountzilas *et al.*, 1990; Chresta *et al.*, 1992). These studies have shown that the cytotoxicity of TOP2 poisons was dramatically increased when the cells were pre-treated with Ara-C first and the potentiation was time-dependent. Therefore, the following step was to determine the effect of Ara-C pre-treatment

on the cytotoxicity of daunorubicin and in comparison with the concurrent treatment of the respective combination. Six selected combinations were chosen for comparison as shown in (Figure 3.10).

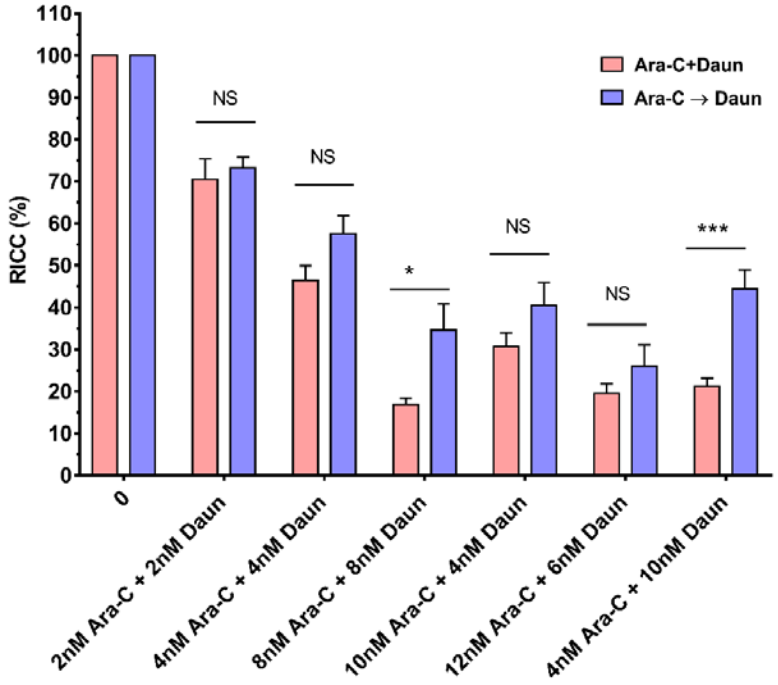


Figure 3-10 Comparison between growth inhibition effect of scheduled and concurrent (Ara-C+daunorubicin) combinations.

Cells were incubated with either Ara-C and daunorubicin at the same time for 48 hours continuously (Ara-C+Daun), or treated with Ara-C for 24 hrs then daunorubicin was added for further 24 hours (Ara-C→Daun). The cytotoxicity was determined as the relative increase in cell count (RICC). Data presented here is the mean of at least three independent experiments with error bars representing the SEM, (*P≤0.05, **P≤0.01, ***P≤0.005, NS: No significance). RICC values are also presented in Appendix table 2.

Generally, pretreatment of cells with Ara-C did not increase growth inhibition of combinations. At lower doses (2+2) and (4+4) nM, there was no significant difference between concurrent and scheduled treatment (P values: 0.6256 and 0.0808) respectively. At higher dose (8+8) nM, the difference was significant (P= 0.0247). On the other hand, statistical analysis showed that the p values for (10+4), (12+6), and (4+10) nM combinations were (0.1640, 0.2834, and 0.0017) respectively. These results were consistent with the above findings that the growth inhibition effect of Ara-C and daunorubicin in combination is additive in Nalm6 cell lines.

3.3.6 FACS analysis

The flow cytometry in vitro micronucleus assay (Litron Laboratories) was used in this study. It is a high content assay that provides a high throughput quantitative measurement of different parameters involving the determination of relative survival (%), micronuclei formation (MN), and cell cycle analysis. This analysis was performed in all three Nalm6 cell lines treated with daunorubicin, idarubicin, or Ara-C as a single agents as well as in the Ara-C+daunorubicin combinations.

3.3.6.1 Effect of drugs on relative survival of Nalm6 cell lines measured by FACS

Relative survival of the Nalm6 cell lines was determined during the micronuclei assay procedure by the addition of microsphere beads that could be stained with a nucleic acid dye (CYTOX green). These beads can be scored and counted in the flow cytometer. The ratio of healthy nuclei (which are negatively stained with EMA) per bead can be determined. Nuclei-to-bead ratio for the control and the treated samples can be used to determine the relative survival. Therefore, the relative number of healthy cells can be efficiently determined by this method which might be underestimated by other counting methods such as coulter counts. In addition, it is more rapid and allows for counting of a large number of cells (around 30000 nuclei per sample). Determining the cytotoxicity levels in the micronuclei assay is important because samples that show high levels of cytotoxicity are more likely to show false positive micronuclei results as a result of chromosomal damage as a secondary consequence of cytotoxicity (OECD, 2010) and this must be considered during the analysis of the results.

The relative increase in cell count (RICC) was determined for controls and treated samples at the time of harvesting to compare the outcome of both relative survival (%) measured by FACS and the RICC results. This was also advantageous to confirm the appropriate levels of cytotoxicity in the cell lines. The RICC was determined for all samples that underwent FACS analysis. A comparison between the relative survival measured by FACS and the RICC is shown in (Figure 3.11). Generally, both methods showed a clear similarity in the determination of cytotoxicity of agents with slightly elevated values of relative survival over the RICC which might be due the differences between methods, but statistical analysis showed that the majority of samples showed no significant difference between relative survival (%) and RICC values.

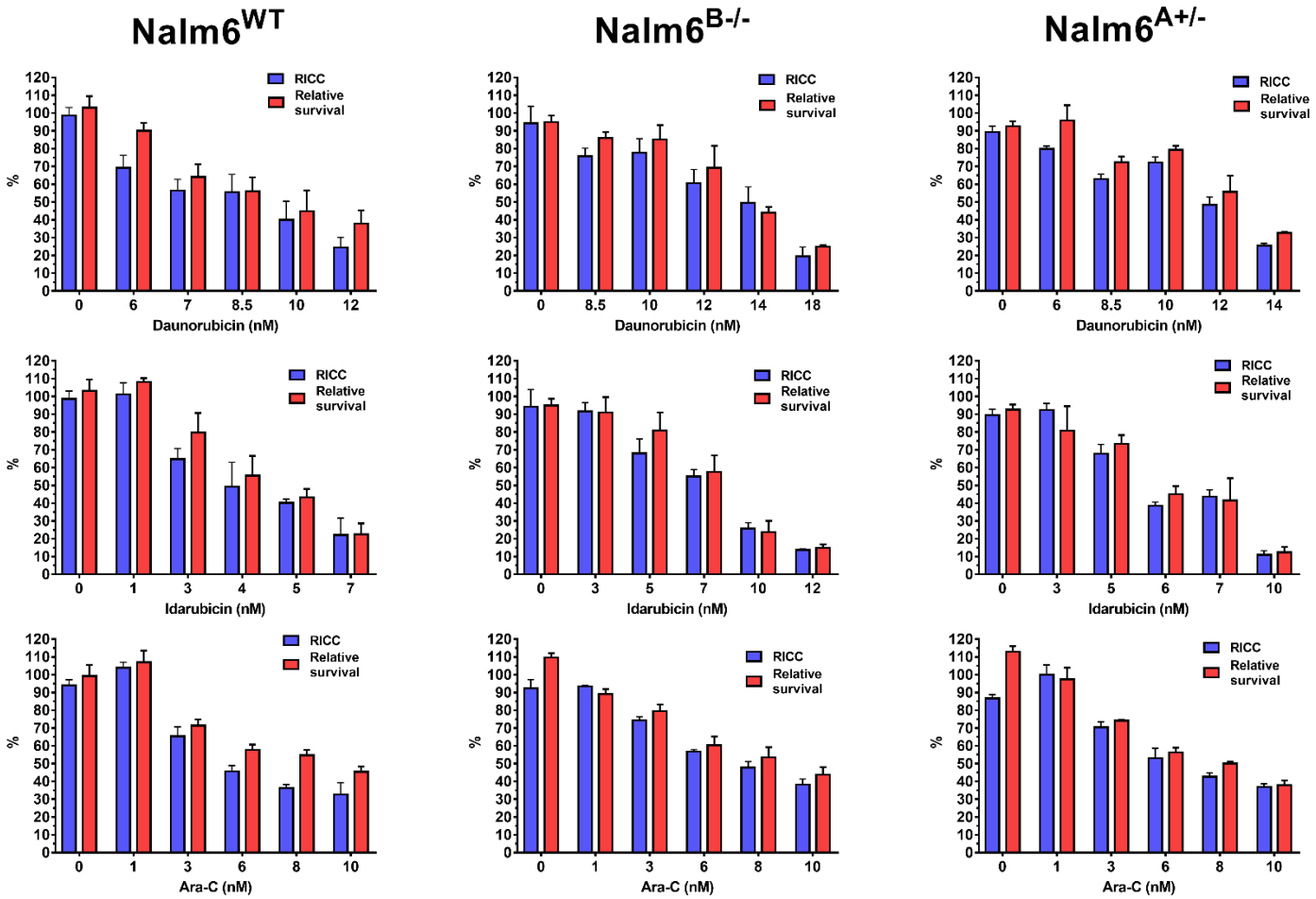


Figure 3-11 Comparison between relative survival measured with FACS and RICC.

Cells were incubated with agents for 48 hours continuously and the cytotoxicity was determined by two methods; the relative increase in cell count (RICC) using cell counting protocol and the relative survival using flow cytometer. Data presented here is the mean of at least three independent experiments with error bars representing the SEM. Relative survival was determined as described in section 2.8 of materials and methods. RICC and relative survival values are also presented in Appendix table 3.

3.3.6.2 Micronuclei (MN) and apoptosis induction by TOP2 poisons and Ara-C

Determining the genotoxicity of many drugs and especially TOP2 poisons is an important issue in the development of an effective anti-cancer treatment (Cowell and Austin, 2012). Therefore, it is important to use a sensitive method that can efficiently determine the level of genotoxicity (Pfuhler *et al.*, 2011). There are different methods to determine the genotoxicity of agents and there is a growing tendency to use micronuclei as a parameter for genotoxicity for several reasons; firstly, micronuclei scoring provides an indicator for both structural and numerical chromosomal aberrations (OECD, 2010). Secondly, this assay can be automated using immunofluorescence staining and a large number of micronuclei can be detected with a flow cytometer (Bryce *et al.*, 2007).

In our procedure, several criteria for micronuclei scoring by flow cytometer were followed to insure minimum false positive results. Firstly, very high cytotoxic doses were avoided as this might result in increased false positive MN counting. Secondly, precautions were taken into account that the MN events must exhibit $1/100^{th}$ to $1/10^{th}$ of the CYTOX Green fluorescent intensity of 2n nuclei. Thirdly, events of MN must fall within both forward and side light scatter against CYTOX Green area. Fourthly, the micronuclei events must be EMA-negative.

Samples that showed two-fold micronuclei increase over control was considered to be positive for genotoxicity. The results of micronuclei formation suggested that both daunorubicin and idarubicin induce micronuclei formation in a dose-dependent manner and the genotoxicity is linked to the cytotoxicity level and this was detected in all cell lines (Figure 3.12).

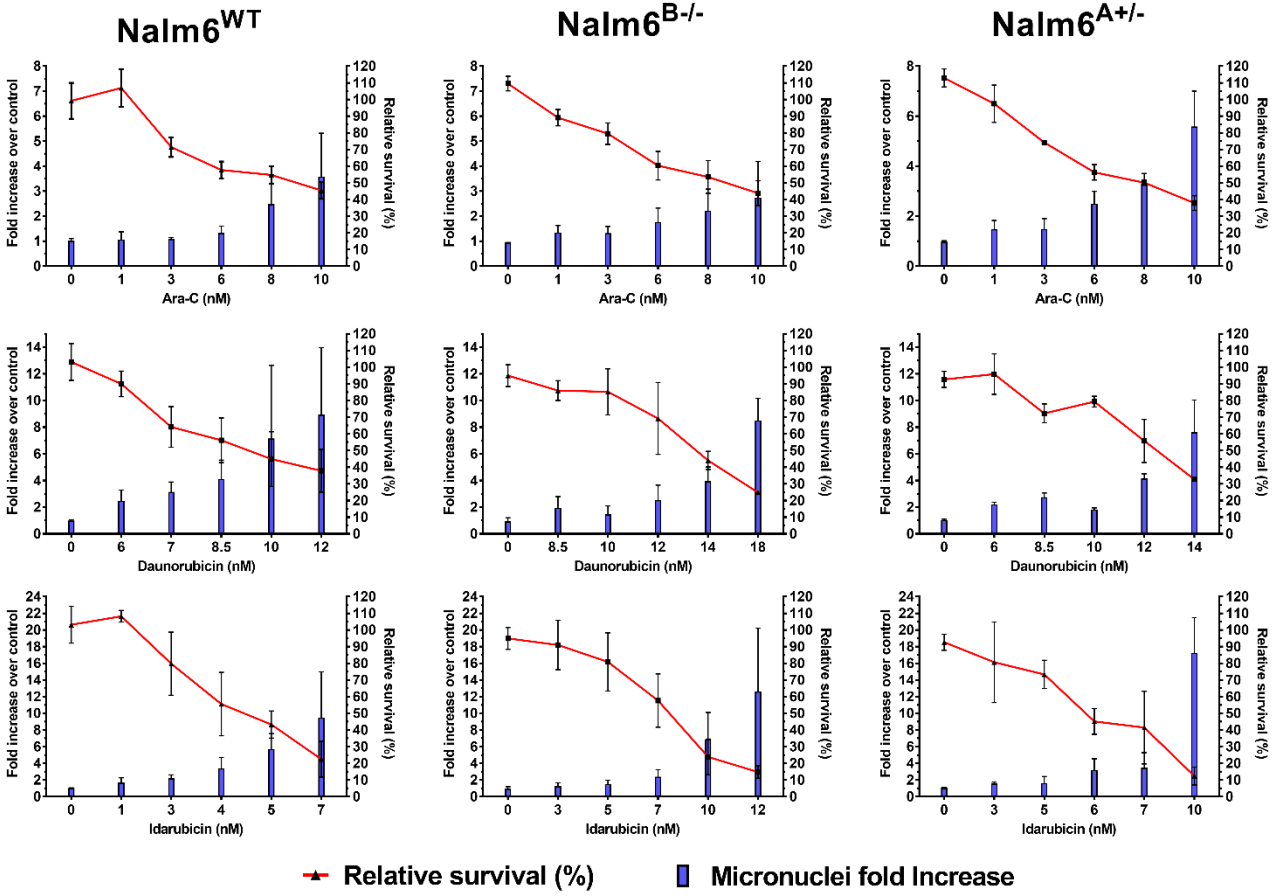


Figure 3-12 Micronuclei (MN) induction by TOP2 poisons and Ara-C in Nalm6 cells

The figure shows the relative survival against micronuclei (MN) fold increase over control for Nalm6 cell lines exposed to daunorubicin, idarubicin, and Ara-C. Cells were incubated with agents for 48 hours continuously. Data represents the mean of at least three independent experiments with error bars representing the SEM. Micronuclei (MN) fold increase was determined as described in section 2.8 of materials and methods. Average Micronuclei (%) is presented in Appendix figure 1. Micronuclei (%) and fold change values are also presented in Appendix table 4.

The results from (Figure 3.12) suggested that both TOP2 isoforms are contributing to the genotoxicity induced by treatment with daunorubicin or idarubicin because the genotoxicity was induced at comparable levels in all Nalm6 cell lines. This might be clearer by presenting the genotoxicity level at a comparable cytotoxic level in Nalm6 cell lines as in (Figure 3.13 A). Interestingly, Ara-C was less genotoxic than daunorubicin or idarubicin at the same cytotoxicity level in Nalm6^{WT} (Figure 3.13 B).

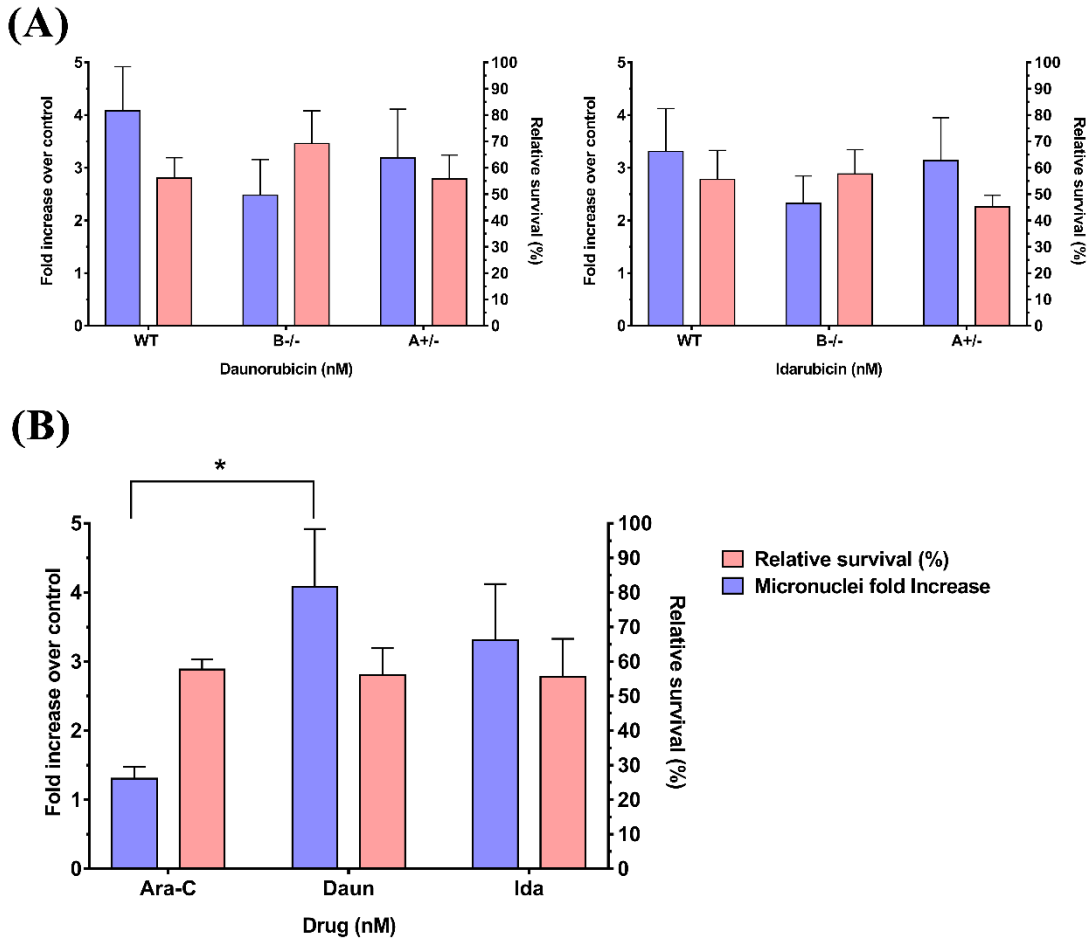


Figure 3-13 Comparison of MN induction by comparable cytotoxic doses of Drugs in Nalm6 cell lines.

This figure is derived from figure 3.12. This figure shows comparison of micronuclei induction by comparable cytotoxic doses of drugs in Nalm6 cell lines for 48 hrs **(A)** Comparison of Micronuclei induction in Nalm6 lines. For daunorubicin, doses were 8.5 nM (Nalm6^{WT}), 12 nM (Nalm6$^{B-/-}$), and 12 nM (Nalm6$^{A+/-}$). For idarubicin, doses were 4 nM (Nalm6^{WT}), 7 nM (Nalm6$^{B-/-}$), and 6 nM (Nalm6$^{A+/-}$). **(B)** Comparison of Micronuclei induction in Nalm6^{WT} by 6 nM (Ara-C), 8.5 nM (daunorubicin), and 4 nM (idarubicin). Data represents the mean of at least three independent experiments with error bars representing the SEM. (*P ≤ 0.05).

In addition, Apoptotic cells fold increase over control was also determined by FACS and it is presented in (Figure 3.14). Results showed dose-dependent induction of apoptotic cells by all drugs with idarubicin was the most potent followed by daunorubicin then Ara-C.

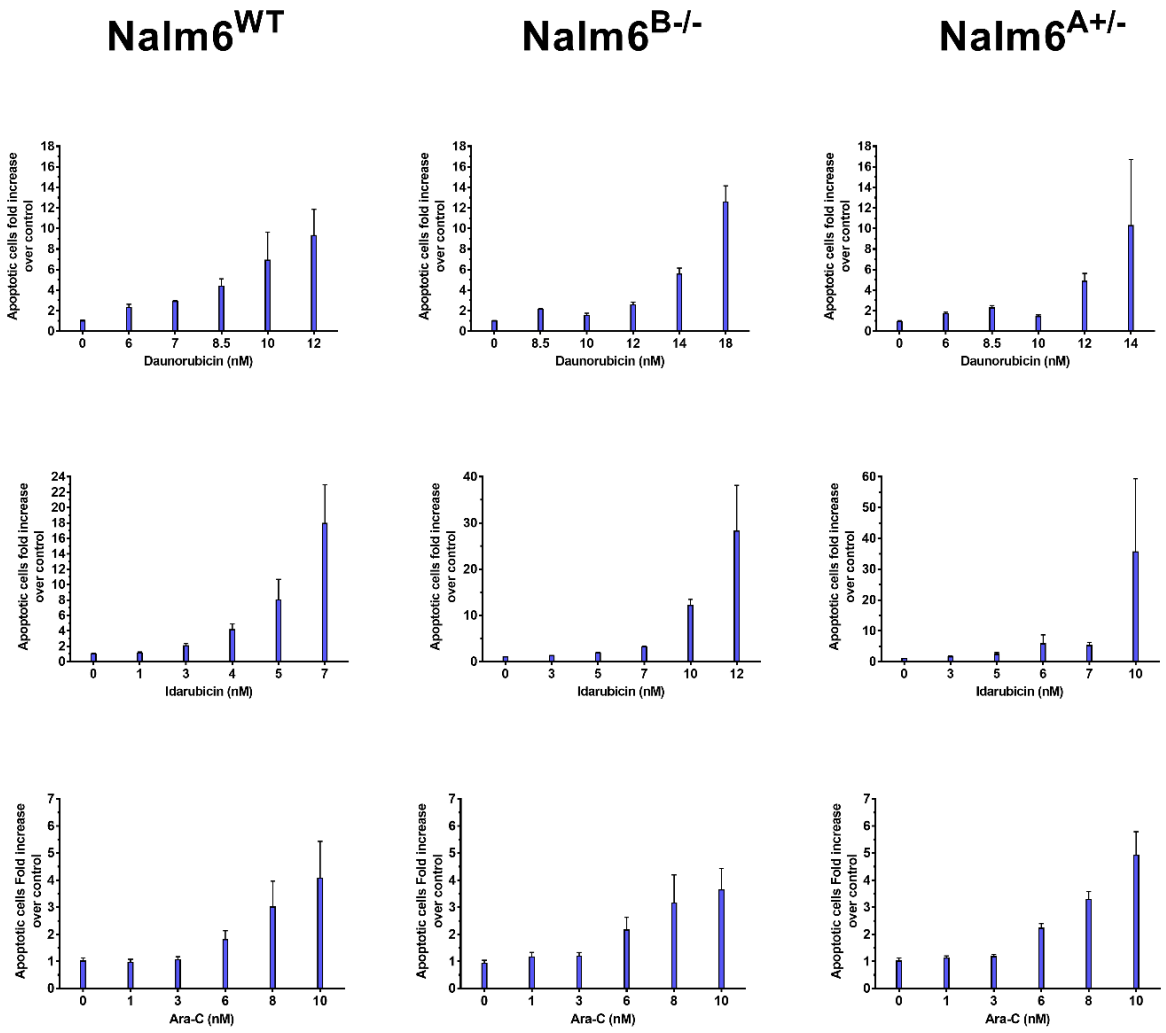


Figure 3-14 Apoptotic cells fold increase over control induced by TOP2 poisons and Ara-C in Nalm6 cell lines

This figure shows Apoptotic cells fold increase over control in Nalm6 cell lines exposed to daunorubicin, idarubicin, and Ara-C. Cells were incubated with agents for 48 hours continuously. Data represents the mean of at least three independent experiments with error bars representing the SEM. Apoptotic cells fold increase was determined as described in section 2.8 of materials and methods. Values of average apoptotic cells (%) and apoptotic cells fold increase are also presented in Appendix Table 5.

3.3.6.3 Micronuclei (MN) and apoptosis induction by daunorubicin and Ara-C combinations

In order to determine the effect of the treatment with Ara-C and daunorubicin in combination on genotoxicity, FACS analysis was conducted to compare micronuclei induction between different combinations and their respective single agents. Four different (Ara-C+daunorubicin) combinations were selected to achieve moderate cytotoxicity hence avoiding overestimation of micronuclei due to false positive as mentioned previously. Cytotoxicity and MN of these combinations are presented in (Figure 3.15). Each combination was then compared with its respective single agents of comparable level of cytotoxicity as presented in (Figure 3.16).

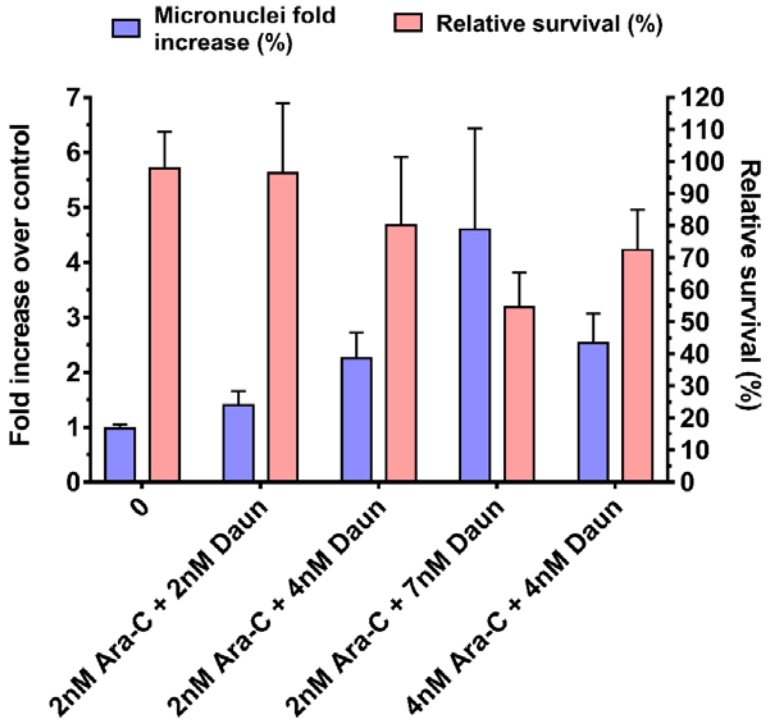


Figure 3-15 Micronuclei (MN) induction by selected (Ara-C+daunorubicin) concurrent combinations in Nalm6^{WT} cell line.

Nalm6 wild type cells were incubated with combinations for 48 hours continuously and the cytotoxicity was determined as the relative survival (%) using a flow cytometer. Data represents the mean of at least three independent experiments with error bars representing the SEM. The average micronuclei (%) and RICC are depicted in Appendix figures 2 and 3 respectively. All data values for RICC, relative survival, Average (%) micronuclei, MN fold increase, Average apoptotic (%) cells, and apoptotic cells fold change are presented in Appendix table 6.

Comparable level of cytotoxicity in (Figure 3.16) was confirmed by showing no statistical difference between each combination and the chosen doses of its members. This comparison again confirm the interesting observation that Ara-C on its own achieved less genotoxicity compared with daunorubicin at the same cytotoxicity level.

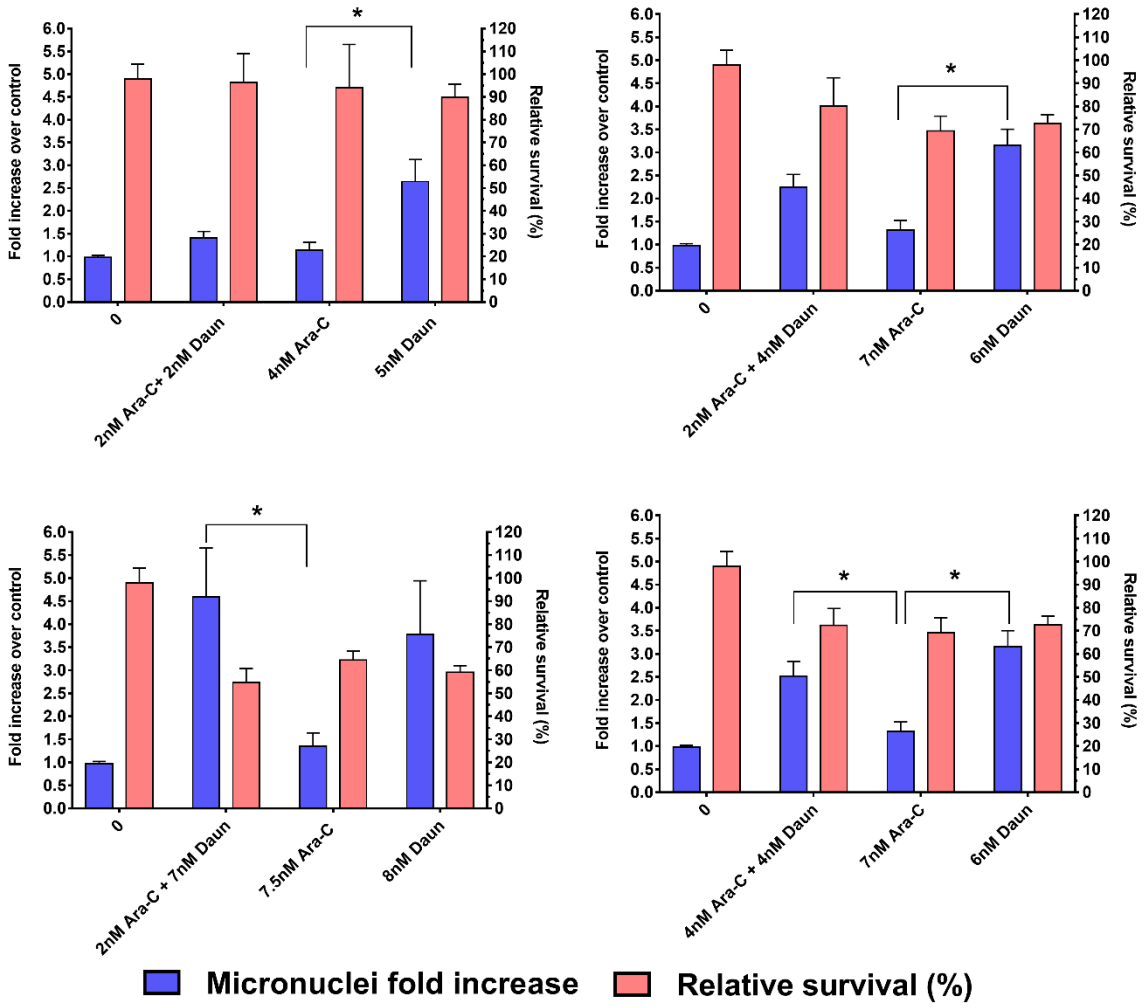


Figure 3-16 Comparison of MN induction between single agents and comparable cytotoxic doses of combinations.

Nalm6 wild type cells were incubated with drugs for 48 hours continuously and the cytotoxicity was determined using flow cytometer and presented as relative survival (%). Data represents the mean of at least three independent experiments with error bars representing the SEM. (*P ≤ 0.05).

Overall, results revealed that the Ara-C on its own induced significantly less micronuclei than daunorubicin at the same level of growth inhibition. For example, the micronuclei formation induced by 7 nM of Ara-C was significantly less than that induced by 6 nM daunorubicin ($p=0.0109$). Moreover, the genotoxicity of the combinations generally followed daunorubicin ratio within each combination, as there was no significant difference in micronuclei level between combinations and their comparable cytotoxic dose of single daunorubicin. For example, there was no statistical difference between micronuclei induction by 6 nM daunorubicin and by (4+4 nM) Ara-C+daunorubicin combination ($p=0.2517$) (Figure 3.16).

In addition, micronuclei induced by Ara-C showed no significant difference with that induced by combinations that involved low and mild daunorubicin doses; 2 and 4 nM ($p=0.3055$ and $p=0.0522$ respectively), while the difference was significant ($p=0.0422$) when compared with combinations involved higher daunorubicin dose (7 nM) although the Ara-C dose was low (2 nM) (Figure 3.16).

Apoptotic cells fold increase over control for the combination was also determined by FACS and it is presented in (Figure 3.17). (2+7) nM Ara-C+daunorubicin combination showed about 4 fold increase over control in the apoptotic cells and was significantly higher than any of the other combinations.

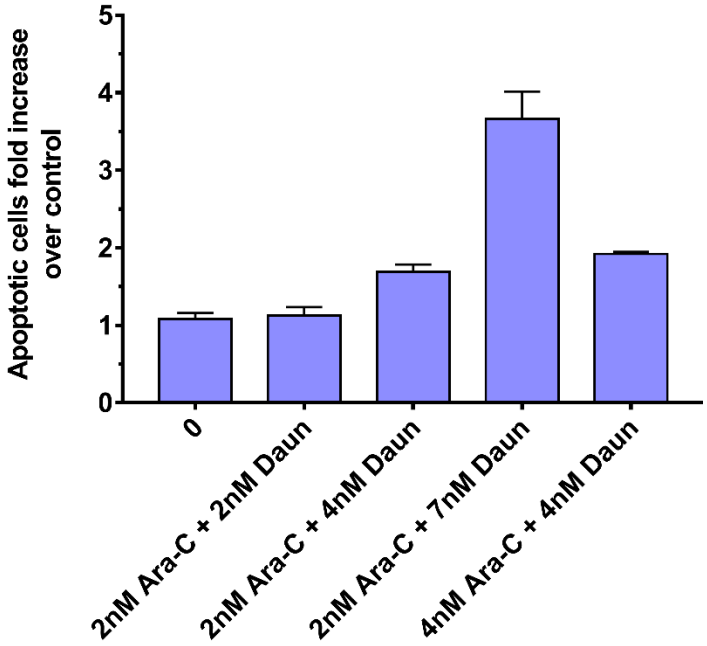


Figure 3-17 Apoptotic cells fold increase over control induced by selected (Ara-C+Daunorubicin) concurrent combination in Nalm6^{WT} cell line.

Nalm6 wild type cells were incubated with different combinations for 48 hours and the number of apoptotic cells were determined by flow cytometry. Data represents the mean of at least three independent experiments with error bars representing the SEM. All data values for RICC, relative survival, Average (%) micronuclei, MN fold increase, Average apoptotic (%) cells, and apoptotic cells fold change are presented in Appendix table 6.

On the other hand, this study also involved the effect of Ara-C pre-treatment on the outcome of Ara-C+daunorubicin combination in terms of micronuclei (MN) induction as a parameter of genotoxicity. As mentioned above, the doses for micronuclei assay by FACS should not produce more than 50% growth inhibition, as this could give false positives. The (4+4 nM) combination was chosen to assess the effect of scheduling of combination on MN induction. Results showed that when cells were treated with Ara-C first, the MN induction was significantly less than that in the concurrent treatment (P= 0.0168) at the same level of growth inhibition (Figure 3.18).

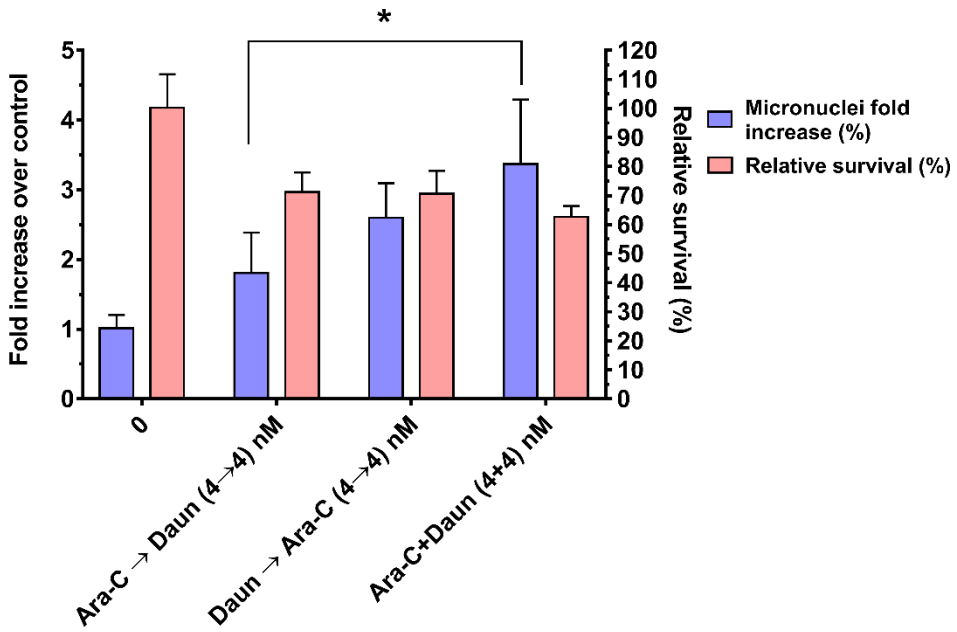


Figure 3-18 Micronuclei (MN) induction by different (Ara-C+daunorubicin) scheduled combinations in Nalm6^{WT} cell line.

Nalm6^{WT} cell were divided into three groups: **(Ara-C+Daun)** was incubated with concurrent (Ara-C+daunorubicin) combination for 48 hrs, **(Daun→Ara-C)** was incubated with daunorubicin for 48 hrs and Ara-C was added over the last 24 hrs, **(Ara-C→Daun)** was incubated with Ara-C for 48 hrs and daunorubicin was added over the last 24 hrs. The cytotoxicity was determined as relative survival (%) using flow cytometer. Data represents the mean of at least three independent experiments with error bars representing the SEM. The average micronuclei (%) and RICC are depicted in Appendix figures 4 and 5 respectively. All data values for RICC, relative survival, Average (%) micronuclei, MN fold increase, Average apoptotic (%) cells, and apoptotic cells fold change are presented in Appendix Table 7. (*$P \leq 0.05$).

Apoptotic cells fold increase over control for the combination was also determined by FACS and it is presented in (Figure 3.19). Cells exposed to Ara-C first (Ara-C→Daun) showed significantly less apoptotic cells induction than both simultaneous (Ara-C+Daun) and scheduled (Daun→Ara-C) combinations although all these combinations showed comparable growth inhibition level as in (Figure 3.18). This suggest a link between apoptotic pathway and genotoxicity seen in (Figure 3.18).

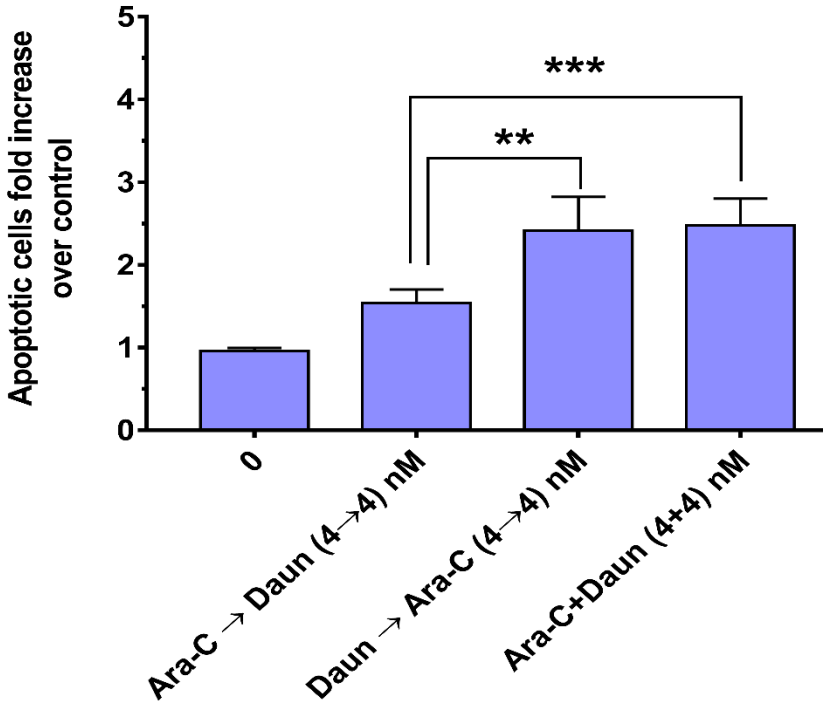


Figure 3-19 Comparison of apoptotic cells fold increase induced by selected (Ara-C+daunorubicin) scheduled combinations in Nalm6^{WT} cell line.

Nalm6^{WT} cells were treated with different combinations as explained in (Figure 3.18) and the number of apoptotic cells were determined using flow cytometry. Data represents the mean of at least three independent experiments with error bars representing the SEM. All data values for RICC, relative survival, Average (%) micronuclei, MN fold increase, Average apoptotic (%) cells, and apoptotic cells fold change are presented in Appendix table 7. (*P ≤ 0.05, **P ≤ 0.01).

3.3.6.4 Effect of treatments on Cell cycle phase distribution

Cell cycle analysis for controls and treated samples was performed along with the micronuclei assay by FACS. Nucleic acid dye (CYTOX Green) fluorescence can be determined by flow cytometer and provide a histogram of DNA content of analysed samples. These histograms were analysed by (Flowjo software (v7.6), FlowJo, LLC, USA) to quantitatively determine the relative cell population in each cell cycle phase. The advantage of using this software is that it allows for quantitative comparisons and performs statistical analysis between samples which is not be possible by direct visual comparisons of DNA content histograms. Effect of single drug treatment in all cell lines are depicted in (Figure 3.20).

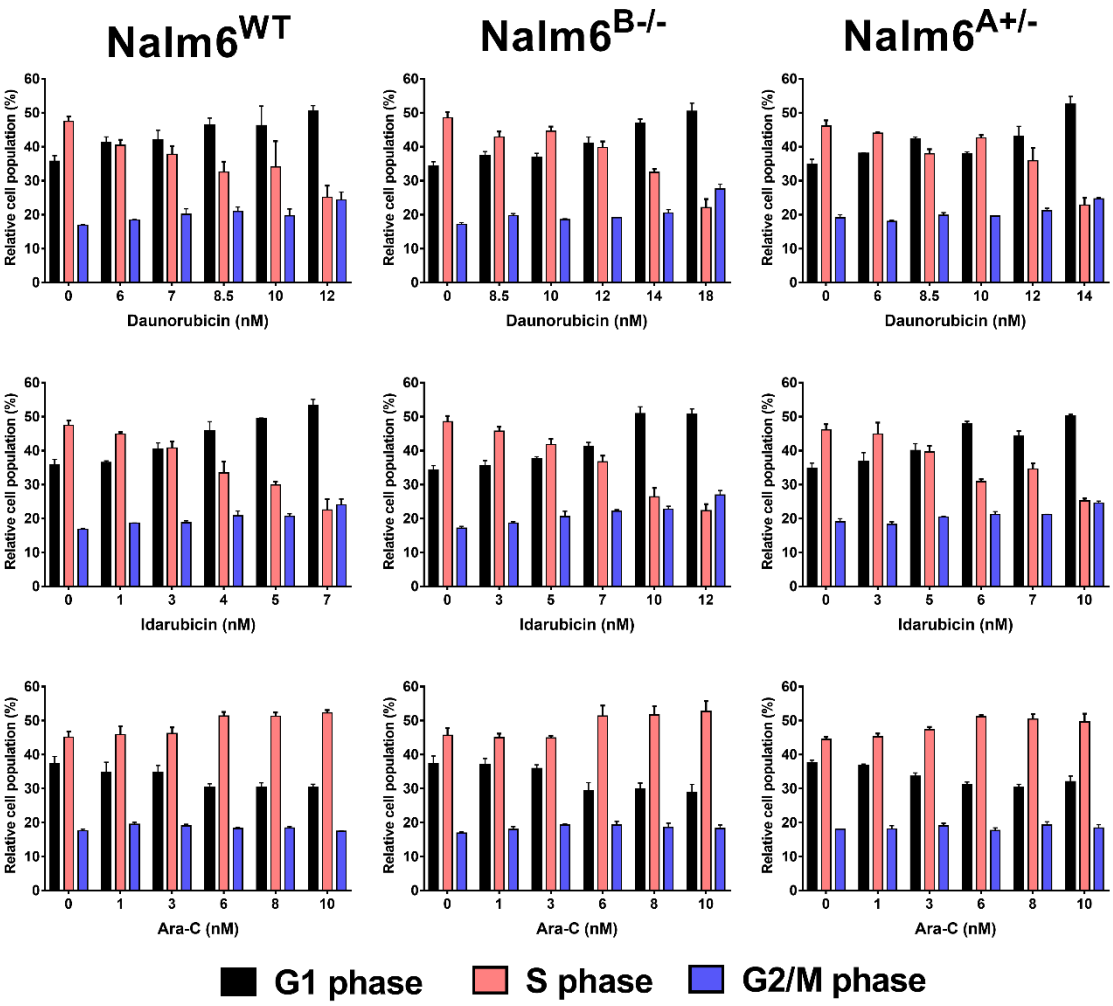


Figure 3-20 Relative cell cycle phase distribution in Nalm6 cell lines treated with daunorubicin, idarubicin, and Ara-C

Figure shows the relative cell cycle population (%) in Nalm6 cell lines incubated with TOP2 poisons. Data was collected from FACS and analysed with (Flowjo 7.6) software and bar graphs were drawn by (Prism 4) software. Data represents the mean of at least three independent experiments with error bars representing the SEM. All data values are presented in Appendix table 8.

Both daunorubicin and idarubicin caused a decrease in the S phase cell population and G1 phase arrest in a dose dependent manner in all Nalm6 cell lines (Figure 3.20). For instance, in the wild type cells the relative S phase cell population (%) was significantly reduced from (47.49±1.41) % for the control to (32.58±2.99) % and (25.08±3.47) % for samples treated with 8.5 and 12 nM daunorubicin respectively. On the other hand, the relative G1 phase cell population (%) was significantly increased from (35.70±1.68) (%) for the control to (46.46±1.99) % and (50.61±1.49) % for samples treated with 8.5 and 12 nM daunorubicin respectively (Figure 3.20). Likewise,

Nalm6^{WT} cells treated with idarubicin followed the same pattern and showed elevated G1 and G2 phase populations with decreased levels in the S phase populations. Furthermore, both Nalm6$^{B-/-}$ and Nalm6$^{A+/-}$ cell lines showed similar patterns of cell cycle phase distribution upon treatment with daunorubicin and idarubicin (Figure 3.20).

Conversely, Nalm6^{WT} cell line treated with Ara-C showed slight increase in the S phase population (%) and slight decrease in G1 phase population at the high doses (Figure 3.21). For example, relative S phase population (%) showed significant increase ($p=0.0184$) from (45.14±1.67) % for control to (52.27±0.8) % for cells treated with 10 nM. At this dose, there was a significant decrease ($p=0.0417$) in G1 phase population from (37.31±2.16) % to (30.29±0.96) %. These findings agreed with a previous data in which CCRF-CEM cell line treated with Ara-C showed an increase in S phase population (Chresta *et al.*, 1992). In addition, similar effects of Ara-C on the cell cycle phase distribution in K562 cell lines has been shown (Loughlin *et al.*, 1996). Furthermore, flow cytometry analysis of patients samples have shown S phase cell cycle arrest upon treatment with Ara-C (Caldwell *et al.,* 2014).

These results suggest that efficiency of (Ara-C + daunorubicin) combination is due to the combined effect of both drugs where Ara-C induce S phase cell accumulation which is more prone to be targeted by TOP2 poisons which target S phase cells more than other phases as (Figure 3.20) suggested. No major change was observed in the G2 phase population in all treatments.

Overall, cell cycle analysis results showed three major trends: first, both daunorubicin and idarubicin target S phase population and hence induced G1 cells accumulation. Second, in contrast to TOP2 poisons, Ara-C induced accumulation of S phase population and reduced G1 phase population. Third, dose effect of Ara-C was very similar in all cell lines suggesting completely TOP2-independent mechanism and it is presented in (Figure 3.21) which is derived from (Figure 3.20) to show a cell line based comparison. This figure also shows the cell cycle patterns behind the difference in dose response in cell lines in (Figure 3.11).

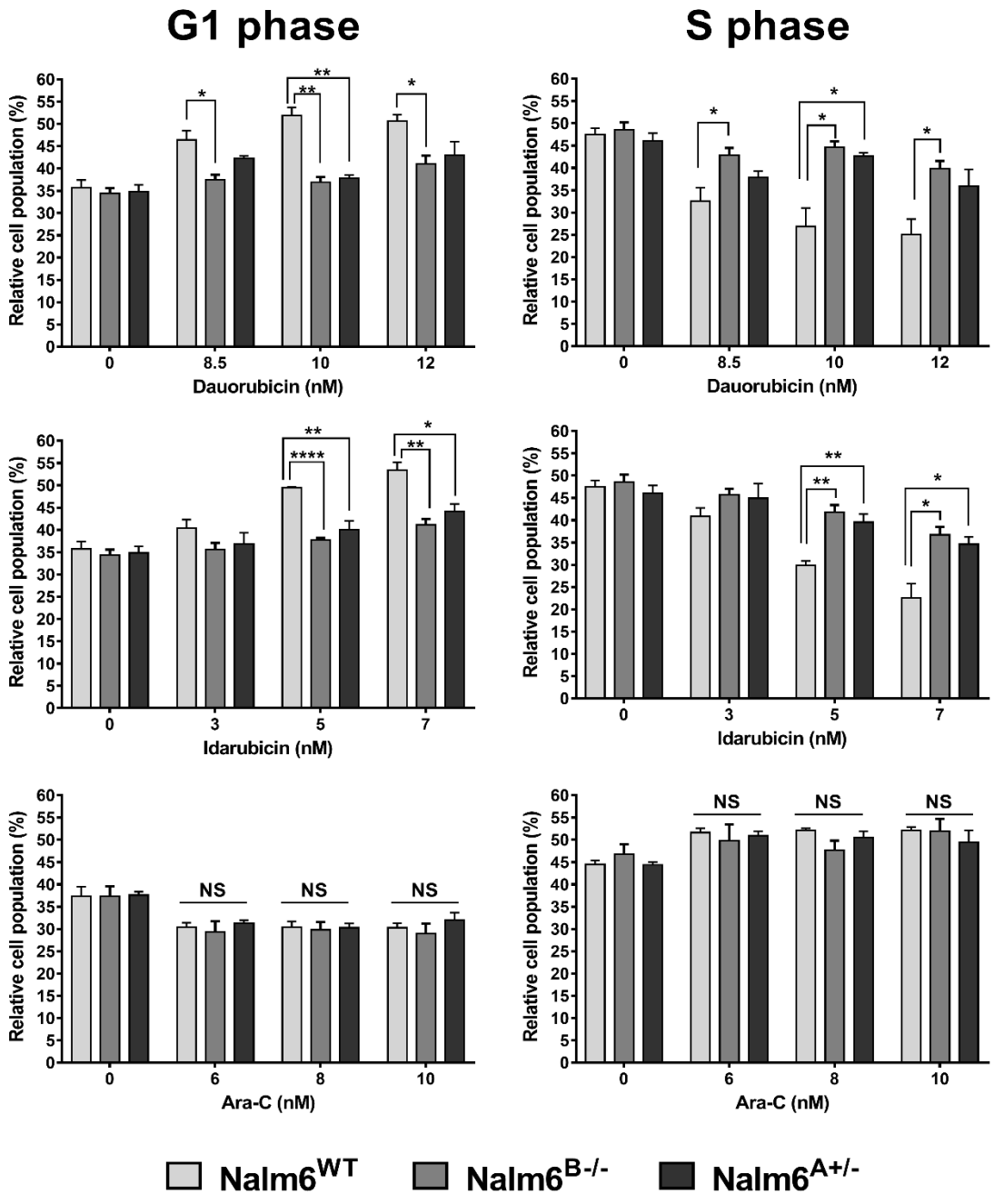


Figure 3-21 Comparison of effect of drugs on S and G1 phase cell population in Nalm6 cell lines.

This figure is derived from (Figure 3.20) to highlight differences between Nalm6 cell lines in terms of effect of drugs on S and G1 population (%). Data represents the mean of at least three independent experiments with error bars representing the SEM. All data values are presented in Appendix table 8. (NS= no significance, *P ≤ 0.05, **P ≤ 0.01, ****P ≤ 0.0001).

For (Ara-C+daunorubicin) combinations, cell cycle analysis showed no significant difference in cell cycle phase distribution between control cells and all combinations with the exception of (2+7) Ara-C+daunorubicin combination which showed significant reduction (p=0.0009) and elevation (p=0.0404) in the S and G1 phase populations respectively (Figure 3.22). In addition, no significant change was observed between combinations with the exception of the (2+7) Ara-C+daunorubicin combination which showed significant difference with all other combinations in terms of relative G1 and S phase cell populations. On the other hand, no significant change in G2 phase cell population was observed between combinations.

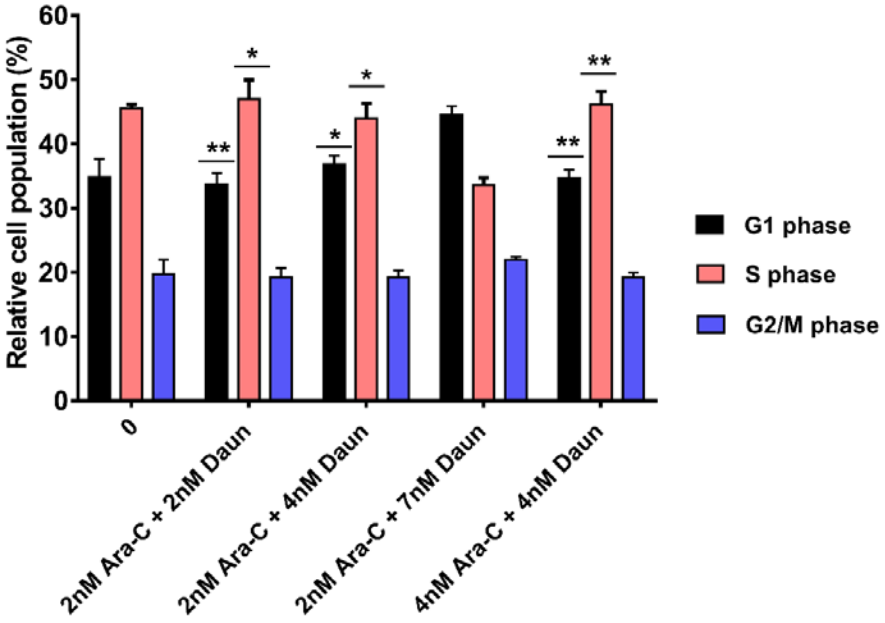


Figure 3-22 Relative cell cycle phase distribution in Nalm6^{WT} cells treated with different (Ara-C+daunorubicin) concurrent combinations.

This figure shows the relative cell cycle population (%) in Nalm6^{WT} incubated with different Ara-C+daunorubicin combinations. Data was collected from FACS and analysed with (Flowjo 7.6 software) and bar graphs were drawn by (Prism 4) software. Data represents the mean of at least three independent experiments with error bars representing the SEM. Stars (*P $\leq$ 0.05, **P $\leq$ 0.01) represent p values of difference between (2 nM Ara-C+ 7 nM Daun) combination and other combinations. Neither significant difference was seen in combinations other than (2 nM Ara-C+ 7 nM Daun) nor in G2/M population between all combinations. All data values are presented in Appendix table 9.

The cell cycle phase distribution (%) for Nalm6^{WT} exposed to three different scheduled treatments is presented in (Figure 3.23). Results showed similar cell cycle distribution between (Ara-C→Daun) and (Ara-C+Daun) combinations. This is consistent with the comparable growth inhibition level of these two combinations shown in (Figure 3.18). Notably, (Daun→Ara-C)

combination showed significant difference with the other two combinations in terms of G1 and G2/M cell population (Figure 3.23).

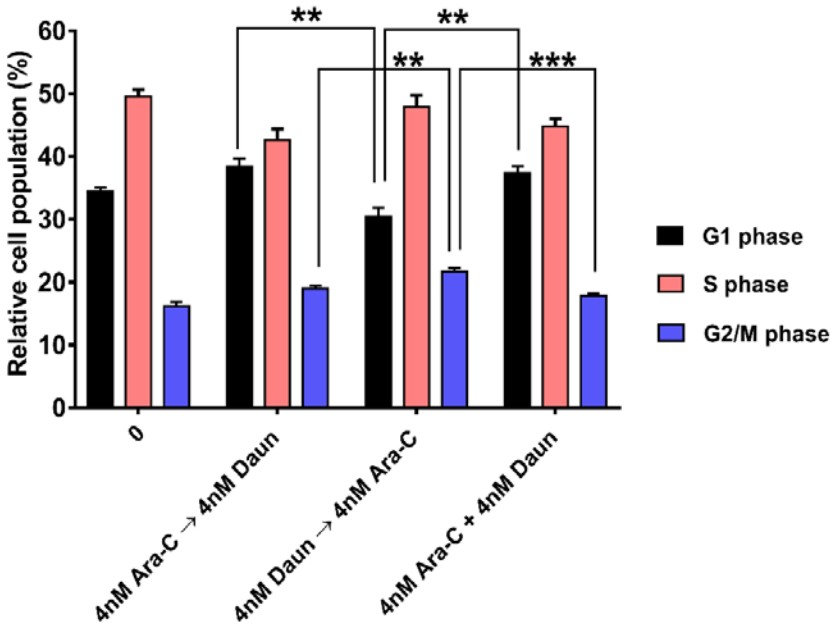


Figure 3-23 Relative cell cycle phase distribution in Nalm6^{WT} cells treated with scheduled (Ara-C+daunorubicin) combinations.

Nalm6^{WT} cells were treated with different combinations as explained in (Figure 3.18). This figure shows the relative cell cycle population (%) in Nalm6^{WT} incubated with different Ara-C+daunorubicin combinations. Data was collected from FACS and analysed with (Flowjo 7.6 software) and bar graphs were drawn by (Prism 4) software. Data represents the mean of at least three independent experiments with error bars representing the SEM. All data values are presented in Appendix table 10.

3.4 Discussion

This chapter involved studying TOP2 as a target for two anti-tumour drugs daunorubicin and idarubicin as well as testing efficiency of daunorubicin combined with Ara-C in different ratios and treatment orders. Contribution of TOP2 isoforms in the cytotoxicity of daunorubicin and idarubicin was determined using two genetic derivatives of Nalm6 cell line with isoform specific deletion of either both *TOP2B* alleles (Nalm6$^{B-/-}$) or one *TOP2A* allele (Nalm6$^{A+/-}$). Ideally, comparison of the precise contribution of both isoforms would be by deleting the same number of alleles of both isoforms. However, cells cannot survive with deletion of both *TOP2A* alleles while they survive and grow in culture without TOP2β. Growth inhibition curves of daunorubicin and idarubicin in the wild type and genetically modified Nalm6 lines showed that both TOP2α and TOP2β contribute in the cytotoxicity of both drugs. This agreed with previous studies showed that

both isoforms are targeted by TOP2 poisons in murine (Errington *et al.*, 1999) and human (Toyoda *et al.*, 2008; Lee *et al.*, 2016) cells. On the other hand, as expected, no isoform-specific contribution of TOP2 was seen in the effect of Ara-C alone.

Overall, both daunorubicin and idarubicin showed the same pattern with idarubicin being more cytotoxic. Daunorubicin is widely used in combination with Ara-C as a standard treatment for leukaemia. For this reason, daunorubicin was chosen in the rest of experiments to test the efficiency of using different combination ratio or treatment order. It has been shown that the effect of Ara-C:daunorubicin ratio in the efficiency of this combination (Tardi *et al.*, 2009). Different Ara-C:daunorubicin ratios were tested and the efficiency of combinations was evaluated for synergistic, additive, or antagonistic relationships using combination Index (CI), a parameter which was calculated as described in (Materials and methods, section 2.8). Generally, all combination ratios used showed additive relationships, while the combinations in which daunorubicin was about one-third of the Ara-C molarity (Figure 3.8), the effect was synergistic. This agreed with studies that showed synergistic effects in combinations involved more Ara-C than daunorubicin (Mayer *et al.*, 2006; Tardi *et al.*, 2009).

The genotoxicity of drugs in this study as single agents and in combination was also determined. Drug-induced genotoxicity is an important issue in cancer treatment (Cowell and Austin, 2012). In this study, the genotoxicity of drugs was measured by micronuclei (MN) assay by FACS for two reasons. First, it is easy to conduct and requires less technical experience in comparison with conventional chromosomal aberration analyses which is labour intensive and requires relatively high experience. Second, this assay can be automated using immunofluorescence staining and a large number of micronuclei can be detected with a flow cytometer (Bryce *et al.*, 2007). Furthermore, micronuclei assays by FACS are sensitive to both numerical and structural abnormalities (OECD, 2010).

Doses with high cytotoxicity were avoided during (MN) assay to avoid false positives that arise from chromosomal damage due to cytotoxicity as recommended by (OECD, 2010). For comparison between drugs as single agents, results showed that both TOP2 isoforms contribute in the genotoxicity of daunorubicin and idarubicin while no isoform specific effect was seen with Ara-C as single agent. Interestingly, genotoxicity induced by Ara-C was significantly less than that induced by daunorubicin at the same level of cytotoxicity (Figure 3.13). The same observation was seen with idarubicin but the difference did not reach significance. This would have an important

impact on modulation of this combination in clinical use to reduce genotoxicity of combinations by increasing Ara-C/daunorubicin ratio.

Consistent with the above genotoxicity profile of single agents, concurrent drug combinations showed that genotoxicity generally followed the daunorubicin ratio within the combination (Figure 3.15) and comparisons between comparable cytotoxic levels for MN induction again showed that Ara-C clearly induced less genotoxicity than TOP2 poisons at the same growth inhibition levels (Figure 3.16).

Furthermore, the effect of order of drug treatment on outcome of the combination was evaluated for both cytotoxicity and genotoxicity. Results showed that treatment with Ara-C first did not have an effect on the overall growth inhibition of combination (Figure 3.18). However, Ara-C pre-treatment significantly reduced genotoxicity as compared with simultaneous treatment of both Ara-C and daunorubicin in combination (Figure 3.18). Overall, our results suggest that genotoxicity of this common drug combination can be reduced by modulating Ara-C ratio within the combination and/or treatment with Ara-C first.

The effect of drug treatment as single agents or in combination on cell cycle distribution was also analysed using FACS. Cell cycle histograms were generated by determining DNA content of analysed samples using nuclei acid dye (CYTOX Green) fluorescence which was captured by flow cytometry. These histograms were analysed using (Flowjo software (v7.6), FlowJo, LLC, USA) to quantitatively determine the relative cell population in each cell cycle phase. The advantage of using this software is that it allows for quantitative comparisons and performs statistical analysis between samples which is not be possible by direct visual comparisons of DNA content histograms.

Generally, both daunorubicin and idarubicin treatment reduced the S phase population and increased G1 population in a dose-dependent manner and this effect was seen in all Nalm6 cell lines suggesting a non TOP2 isoform-dependent mechanism (Figure 3.20). Conversely, Ara-C treatment caused a reduction in G1 and an increase in S phase population in all cell lines (Figure 3.20). Accumulation of S phase upon Ara-C treatment was reported in the literature in an in vitro studies using CCRF-CEM and K562 cell lines (Chresta *et al.*, 1992; Loughlin *et al.*, 1996) as well as in an acute myeloid leukaemia patient samples (Caldwell *et al.*, 2014).

The cell cycle distribution in response to drugs was compared between cell lines in (Figure 3.21). This figure was derived from (Figure 3.20) to show a direct comparison between cell lines. The

cell-cycle distribution difference between cell lines in (Figure 3.21) reflects the dose-response difference between cell lines in (Figure 3.11).

Cell cycle analysis of concurrent combinations (Figure 3.22) showed that the combination with the high daunorubicin ratio (2nM Ara-C+ 7nM Daun) caused a significant reduction in the S phase population which was consistent with the previous results seen in (Figure 3.20) suggesting that daunorubicin mainly targets S phase population which require TOP2 activity to resolve topological problems accompanied by DNA synthesis that peaked at this phase.

The cell cycle phase distribution analysis of different treatment orders (Figure 3.23) showed similar cell cycle distribution between (Ara-C→Daun) and (Ara-C+Daun) combinations. This is consistent with the comparable growth inhibition level of these two combinations shown in (Figure 3.18). These results showed a consistent relationship between cytotoxicity, genotoxicity, and cell cycle distribution which collectively represent a useful strategy to study the mechanism of action, cell cycle related activity, and genotoxicity of anti-tumour agents.

Overall, these results led to the following conclusions: firstly, the cytotoxicity and genotoxicity induced by anti-tumour drugs can be rapidly and efficiently measured using in vitro micronucleus (MN) test automated with FACS, suggesting use of this method for rapid investigation of a wide range of drugs for differential targeting of TOP2 isoforms. Secondly, both TOP2 isoforms comparably contribute to the cytotoxicity and genotoxicity of daunorubicin and idarubicin in Nalm6 cell line and more drugs need to be investigated using this method. Thirdly, Ara-C is significantly less genotoxic than daunorubicin at the same level of cytotoxicity, suggesting that increasing the Ara-C ratio within the (daunorubicin+Ara-C) combination would enhance the therapeutic outcome of this combination in the treatment of leukaemia patients. Fourth, scheduled treatment of (daunorubicin+Ara-C) combination where Ara-C is administered first might be less genotoxic than simultaneous treatment (Figure 3.18). However, further experiments need to be performed to confirm this finding.

4 Chapter Four: TOP2 role in whole genome transcription of Nalm6 cell.

4.1 Introduction

Human type II topoisomerases are divided into two isoforms; TOP2α and TOP2β. These isoforms are encoded by separate genes located on 17q21-22 and 3p34 for *TOP2A* and *TOP2B* respectively (Chung *et al.*, 1989; Austin and Fisher, 1990; Jenkins *et al.*, 1992; Tan *et al.*, 1992). Although both enzymes have very similar structure (Drake *et al.*, 1989; Austin *et al.*, 1993a; Austin *et al.*, 1995), and catalytic properties in vitro (Austin *et al.*, 1995), their physiological function and expression pattern are different (Wang, 1996; Nitiss, 1998; Wang, 2002; Austin *et al.*, 2018). TOP2α is mainly expressed in proliferating cells and its level peaked at G2/M phase whereas TOP2β is expressed in proliferating and non-proliferating cells (Giovanni Capranico and Zunino, 1992; Watanabe *et al.*, 1994; Zandvliet *et al.*, 1996; Turley *et al.*, 1997; Austin and Marsh, 1998) and in terminally differentiated cells like neurons and cardiomyocytes (Tsutsui *et al.*, 1993; Zhang *et al.*, 2012).

TOP2α is essential and cannot be substituted by TOP2β for proliferation-related processes (Grue *et al.*, 1998) while TOP2β cellular function is less clear. TOP2β is not essential for cell growth and *TOP2B* null cells grow in culture normally (Dereuddre *et al.*, 1997; Errington *et al.*, 1999; Tiwari *et al.*, 2012). For the past two decades, evidence has been accumulating about the role of TOP2B in the transcription and regulation of a number of genes especially neuronal genes.

In one study on murine tissues, the two isoforms were expressed differentially with TOP2α expressed predominantly in proliferating tissues while TOP2β is expressed in a wider range of tissues (Giovanni Capranico and Zunino, 1992). In this study, both enzymes were expressed at high levels during the early but not the late stage of embryogenesis. However, one day after birth there was an interesting shifting in the pattern of expression of both enzymes in the brain and liver tissues. There was a remarkable increase in TOP2β expression in the brain but not in liver. Conversely, there was an increase in TOP2α in the liver but not in the brain indicating that the regulation of both genes is a tissue specific and developmental stage-dependent (Giovanni Capranico and Zunino, 1992).

Later on, the distribution of expression of both enzymes was studied in different developmental stages of rat brain. TOP2α expression was mainly expressed in the growing regions while TOP2β expression at the early developmental stages was higher than that at the mature stages in different

brain regions indicating that TOP2β is involved in the differentiation and maturation of neurons (Watanabe *et al.*, 1994).

Closely related findings were determined in a study on human foetal tissues. Generally, the intensity of expression of TOP2α was higher than that of TOP2β and especially elevated in the proliferation regions despite that the latter being expressed in a wider range of tissues which is further evidence that both enzymes are differentially regulated in human tissues (Zandvliet *et al.*, 1996). Similarly, this pattern of TOP2α and TOP2β expression was determined in another study on human adult tissues (Turley *et al.*, 1997). Recently, analysis of human foetal brain tissues showed that TOP2α is expressed in the proliferating layers while TOP2β is expressed in both proliferating and non-proliferating sections (Harkin *et al.*, 2016).

The role of Top2β in neural development in mice was confirmed by (Yang, 2000). This study showed that TOP2β has a crucial role in neural development. In *TOP2B* mutant mice, despite normal neurogenesis, the motor axons failed to connect to the skeletal muscle and the sensory axons did not innervate the spinal cord. The failing of motor axon to grow properly led to breathing defects and postnatal death (Yang, 2000). This phenotype was investigated by (Nur *et al.*, 2007) who suggested that the defect in the formation of neuromuscular junction in the *Top2b* mutant mice was due to blocking the neurite outgrowth in the absence of TOP2β. In this study, neurons isolated from *Top2b* mutant mice formed shorter neurites than those isolated from wild type mice. In addition, the TOP2 inhibitor ICRF-193 blocked the neurite outgrowth in cultured neurons. Furthermore, neurons from TOP2B mutant mice did not form junctions with muscle cells in co-culture (Nur *et al.*, 2007).

Consistent with (Yang, 2000), the role of TOP2β in neuronal differentiation was determined in a study on rat cerebellum. It showed a remarkable transition from TOP2α to TOP2β expression signal when cells reached their final stage of division and started to differentiate into granule or Purkinje cells, then the TOP2β level reduced sharply at the final differentiation stage (Tsutsui *et al.*, 2001a; Tsutsui *et al.*, 2001b).

The effect of TOP2β on corticogenesis in various tissues was further studied in mouse embryos. Again the *Top2b* mutant embryos showed an aberrant lamination in the cerebral cortex characterized by a defect in cerebral stratification, in addition, the neurons at late stages of corticogenesis did not migrate to the superficial layers indicating a role for TOP2β in neuronal

development (Lyu and Wang, 2003). In addition, this study suggested that TOP2β might be important not only for regulation of neuronal development genes but also for genes of other post mitotic cells.

Another study has revealed that the activity of TOP2β is especially involved in the regulation of expression of genes which are in the late stage of development (Lyu *et al.*, 2006). This study analysed the transcription profile of brains of wild type and *Top2b* null mouse embryos. Interestingly, in *Top2b* null mice, only (1-4 %) of genes were affected and all other genes responsible for general cellular function and early differentiation markers were not. However, the expression of about one-third of developmentally-regulated genes at late stage of differentiation were changed (down-regulation for most of them) in the *Top2b* null mouse embryos in comparison with the wild type group.

Furthermore, immunohistochemical staining of brain sections showed that TOP2β expression was induced at the late differentiation stage of neurons. In addition, chromatin immunoprecipitation (ChIP) analysis showed that TOP2β bound 5′ regions of a number of genes which were affected by the absence of TOP2β but not those which were TOP2β -insensitive, and the binding was especially near the promoter region (kb -0.5 to +2.5) as the study of one of those genes (*Kcnd2*) showed (Lyu *et al.*, 2006). In support of this, other studies have demonstrated that TOP2β is involved in the activation of a number of genes related to the maturation of neurons (Sano *et al.*, 2009; King *et al.*, 2013).

Because of the perinatal death phenotype in the above murine models, the effect of *TOP2B* deletion was confirmed using a non-fatal mutation model. This was studied by (Li *et al.*, 2014) using a mouse model with a retinal progenitor specific-Top2b deletion mutation. Top2b deletion led to defects in retina development characterized by; remarkable decrease in cell number, degenerative plexiform layers and outer part of photoreceptors, and delay in neuronal differentiation. In addition, RNA-seq analysis showed that genes which involve in neuronal survival and maintenance were affected in the TOP2B-truncated retinas. Later on, the same lab group revealed Top2b role in differentiation and maturation of mouse photoreceptor through regulation of expression of a network of key genes in photoreceptor function (Li *et al.*, 2017). Similarly, Top2b was shown to be one of the genes whose mutation disrupted the visual system in zebrafish as determined by both genetic screening along with immunohistochemical labelling (Nevin *et al.*, 2011)

Studies have demonstrated that TOP2β interacts with several proteins involved in transcription-regulation processes such as P53 (Cowell *et al.*, 2000), HDAC1 and HDAC2 (Tsai *et al.*, 2000; Johnson *et al.*, 2001), SNF2H (LeRoy *et al.*, 2000), UBC9 (Mao *et al.*, 2000), CD3ε (Nakano *et al.*, 1996). Histone deacetylase inhibition by agents like trichostatin A (TSA) caused a shift in TOP2β from heterochromatin to euchromatin regions in murine cells (Cowell *et al.*, 2011). In addition, interaction between topoisomerase II beta (TOP2β) and nucleophosmin (NPM) in APL cells has been reported (Nichol *et al.*, 2016).

The role of TOP2β in transcriptional regulation has been shown in Michigan Cancer Foundation (MCF)-7 cell line. This study suggested that activation of gene transcription mediated by nuclear receptors and DNA-binding transcription factors requires site-specific, intermediate double-stranded DNA break (dsDNA) mediated by TOP2β (Ju *et al.*, 2006).

Studies have shown the recruitment of TOP2β to the 5′ region of a number of genes as a result of signalling pathways induced by nuclear hormones such as androgen (Haffner *et al.*, 2010), estradiol (Ju *et al.*, 2006), retinoic acid (McNamara *et al.*, 2008). These studies involved a double stranded DNA break induced by TOP2β. In addition, it has been shown that TOP2β is involved in the expression of fatty acid synthase in liver cells in response to insulin (Wong *et al.*, 2009).

It has been confirmed that TOP2β associates with the Retinoic Acid Receptor α (RARα) and negatively modulates its transcriptional activity in acute Promyelocytic Leukaemia (APL) cell lines leading to Retinoic Acid (RA) resistance. *TOP2B* knockdown in RA-resistant clones released a transcription block and allowed RA-induced differentiation. TOP2B overexpression in RA-sensitive clones was able to induce RA resistance by reducing expression of RA target genes. In addition, Chromatin Immunoprecipitation (ChIP) analysis showed that TOP2β is specifically bound to the Retinoic Acid Response Element (RARE) region of the promoter of RARβ gene which is one of the RA target genes (McNamara *et al.*, 2008).

It has been shown a shifting from Top2a to Top2b upon differentiation of murine embryonic stem cells (ESCs) into neuronal cells (Tiwari *et al.*, 2012). Genome-scale location analysis of these neurons revealed interaction of Top2b at H3K4me sites indicating a regulatory role of Top2b and these sites were enriched preferentially at promoter regions. Top2b deletion followed by neuronal differentiation of (ESCs) did not affect the early differentiation steps, while caused losing of

survival of postmitotic neurons, an observation accompanied with the up regulation of *ngfr* p75 which is bound and down regulated by Top2b (Tiwari *et al.*, 2012).

Furthermore, it has been shown by ChIP-qPCR that, as a response to neuronal activity, Top2b mediates a double stranded DNA break in the promoter of a number of early response genes such as *Fos*, *Egr1*, and *Npas4* necessary for their expression leading to changes related to memory and learning (Madabhushi *et al.*, 2015a). Further studies added more information about TOP2β binding sites over the genome as well as interaction with other transcription factors. TOP2β occupancy testing in MCF-7 cell line using ChIP-seq showed that approximately half of TOP2β binding sites locate within genes or about 5 kb long from a transcription start sites (Manville *et al.*, 2015).

Moreover, transcription factor motif enrichment analysis of TOP2β binding sites highlighted group of transcription factors involved SP1, KLF4, TFAP2A, MYF, REST, CTCF, ESR1 and ESR2. Gene ontology analysis of genes coincided with TOP2β peaks showed significant enrichment of neuronal function terms such as axon guidance and axonogenesis (Manville *et al.*, 2015). Using ChIP-seq, it has been shown that TOP2β associates with about half of CTCF and cohesin-bound sites on mouse genome (Uusküla-Reimand *et al.*, 2016). Interaction of TOP2β binding sites with DNase I hypersensitivity and allele-specific transcription factor binding sites was also shown in this study. Interestingly, these interactions were shown to be evolutionarily conserved in a cross-species comparison involved CTCF ChIP-seq peaks mapped in human, macaque, rat, and dog (Uusküla-Reimand *et al.*, 2016).

In addition, a study showed an overlapping between CTCF/cohesin occupying sites and TOP2β binding sites at the evolutionarily conserved chromatin loop anchors and these anchors are more susceptible to DNA double-stranded breaks (DSB) mediated by TOP2β (Canela *et al.*, 2017).

4.2 Aims

The aim of this study was to determine the role of the TOP2β isoform in gene expression. Nalm6 (wild type) cell line and two genetic modified lines; *TOP2B* knockout cell line ($Nalm6^{B-/-}$) and heterozygous knockout of *TOP2A* ($Nalm6^{A+/-}$) were used in this study. To answer this question, I needed to confirm the genes that were differentially expressed in $Nalm6^{B-/-}$ which were highlighted in an RNA-seq data previously generated in our lab (cited as AROS data in this thesis). The full list of the differentially expressed genes from the AROS data are detailed in the appendix (figures 7-8 and tables 11-14). In addition, because cancer cell lines are not as genetically stable as normal cells and have higher mutation frequency (Geraghty *et al.*, 2014), taking into account that $Nalm6^{B-/-}$ cells have been cultured for long time, it is possible that the differential expression might arise from reasons other than the lack of TOP2β. Therefore, I needed to determine the differential gene expression directly after downregulation of TOP2β. I performed RNA-seq analysis using Nalm6 cells in which both TOP2 isoforms were knocked down by siRNA for 48 hrs.

To achieve the aims mentioned above, the following experimental objectives were followed:

- Optimizing electroporation conditions for Nalm6 cell line and generating cells in which *TOP2A* has been knocked down by siRNA (TOP2A-siRNA), and cells in which *TOP2B* has been knocked down by siRNA (TOP2B-siRNA).
- Determining the effect of TOP2β isoform on gene expression in Nalm6 cell line by comparing whole transcriptome data of $Nalm6^{WT}$ with that of $Nalm6^{B-/-}$ as well as with that of (TOP2B-siRNA).
- Determining genes that show a high fold change difference between $Nalm6^{WT}$ and $Nalm6^{B-/-}$ and confirming up or downregulation trends by RT-qPCR.
- In addition to TOP2β, the effect of TOP2α isoform on whole genome expression in Nalm6 cell line was determined by comparing whole transcriptome data of $Nalm6^{WT}$ with that of $Nalm6^{A+/-}$ as well as with that of (TOP2A-siRNA).

4.3 Results

4.3.1 Optimizing electroporation conditions and generating TOP2α- and β-knockdown cells.

Firstly, knockdown efficiency for our siRNA stocks (Table 2.4) was tested in $K562^{WT}$ cells as the electroporation condition for this cell line had already been optimized in our lab. Western blotting showed efficient signal reduction of TOP2B (see Appendix figure 6). Knockdown conditions in Nalm6 were optimized by performing electroporation with a range of voltages and siRNA concentrations then visualising TOP2 reduction by western blotting. Results showed that 600 nM siRNA concentration and 300-350 V was the most efficient condition in achieving reduction in both TOP2A and TOP2B after 48 hours of transfection (Figure 4.1).

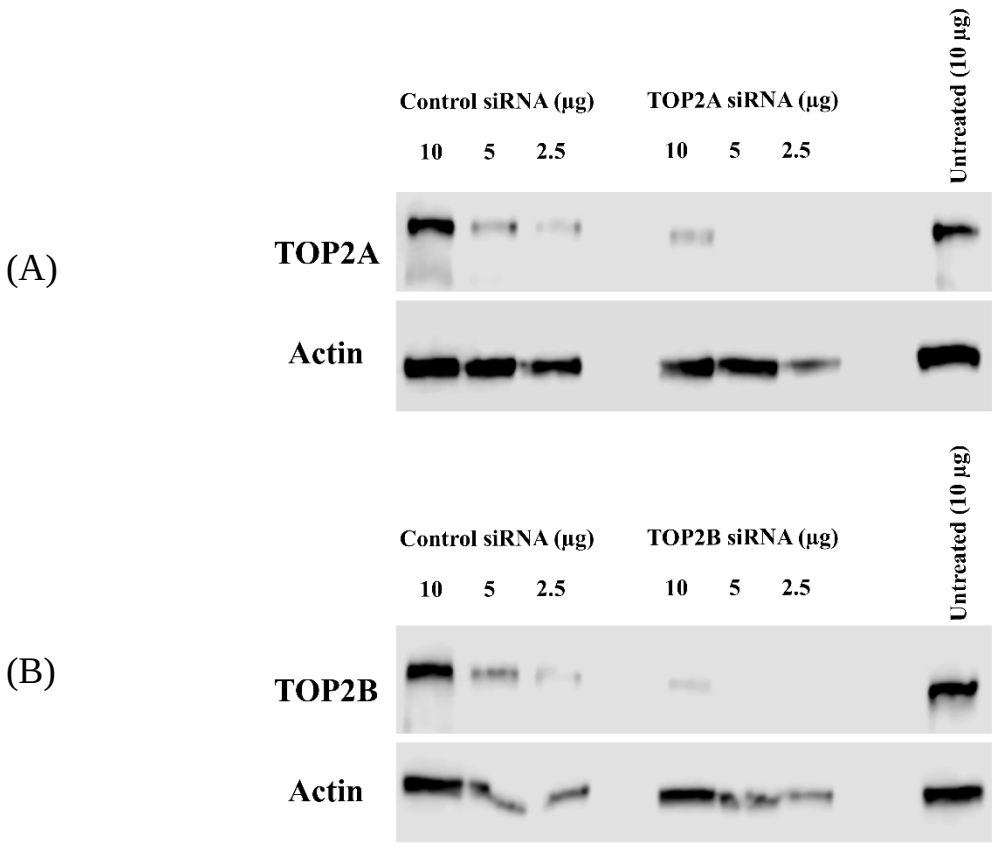


Figure 4-1 siRNA knockdown of *TOP2A* and *TOP2B* in Nalm6 cells.

Cells were transfected with 600 nM of either control or TOP2 siRNA. Electroporation conditions were (10 mSec and 300-350 V). Samples were loaded (2.5-10 µg/lane) and probed with **(A)** TOP2A (4566) or **(B)** TOP2B (30400) antibodies. Actin (ab3280) antibody was used as loading control.

4.3.2 Gene expression analysis

The role of *TOP2B* in gene expression in Nalm6 cell line was determined by whole transcriptome analysis using RNA sequencing. The analysis was performed on six different Nalm6 cell samples; wild type ($Nalm6^{WT}$), *TOP2B* knockout ($Nalm6^{B-/-}$), wild type transfected with non-targeting siRNA (Con-siRNA), wild type transfected with *TOP2B* siRNA (TOP2B-siRNA), wild type transfected with *TOP2A* siRNA (TOP2A-siRNA), and *TOP2A* knockout ($Nalm6^{A+/-}$). The study was performed on four replicates of each sample. Transfection with siRNA was performed by electroporation as described in the Materials and Methods (Section 2.11). TOP2 expression was verified in these six samples by western blotting as shown in (Figure 4.2). Total RNA samples for sequencing were extracted and evaluated as described in the Materials and Methods (Sections 2.14.1 and 2.14.2) and detailed information about the quantity and quality of RNA samples is shown in (Table 4.1).

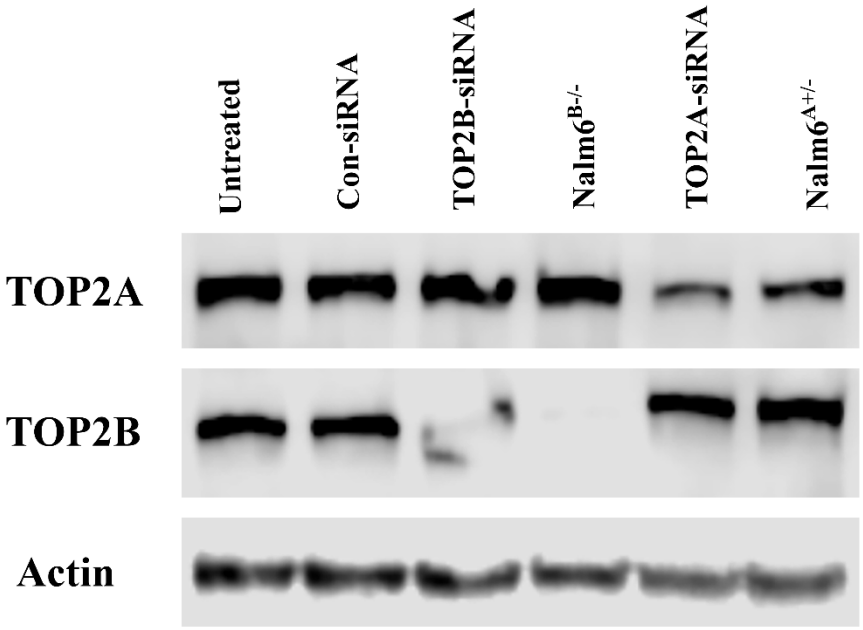


Figure 4-2 Western blotting of samples used for RNA-Seq analysis

Western blotting of Samples used for RNA-Seq analysis. Samples were loaded (10 µg/lane) on SDS-polyacrylamide gel then probed with Actin (ab3280), TOP2A (4566) or TOP2B (30400) antibodies.

Samples		Concentration. (ng/µl)	RIN
Replicate 1	WT	139	9.8
	Con-siRNA	208	9.9
	TOP2B-siRNA	219	9.9
	$Nalm6^{B-/-}$	316	9.9
	TOP2A-siRNA	213	9.9
	$Nalm6^{A+/-}$	195	10
Replicate 2	WT	340	9.9
	Con-siRNA	337	10
	TOP2B-siRNA	389	10
	$Nalm6^{B-/-}$	402	10
	TOP2A-siRNA	357	9.6
	$Nalm6^{A+/-}$	422	9.8
Replicate 3	WT	395	9.8
	Con-siRNA	318	9.9
	TOP2B-siRNA	302	10
	$Nalm6^{B-/-}$	309	10
	TOP2A-siRNA	319	9.9
	$Nalm6^{A+/-}$	370	10
Replicate 4	WT	354	10
	Con-siRNA	328	10
	TOP2B-siRNA	491	10
	$Nalm6^{B-/-}$	365	10
	TOP2A-siRNA	402	9.9
	$Nalm6^{A+/-}$	455	10

Table 4-1 Nalm6 RNA samples used in RNA-Seq study.

($Nalm6^{WT}$) untreated wild type, **(Con-siRNA)** wild type transfected with non-targeting siRNA, **(TOP2B-siRNA)** wild type transfected with *TOP2B* siRNA, **($Nalm6^{B-/-}$)** *TOP2B* knockout cells, **(TOP2A-siRNA)** wild type transfected with *TOP2A* siRNA, **($Nalm6^{A+/-}$)** *TOP2A* knockout cells.

All RNA samples were tested for quantity and quality before sequencing using Bioanalyzer system and electropherogram summary of them is presented in (Figure 4.3).

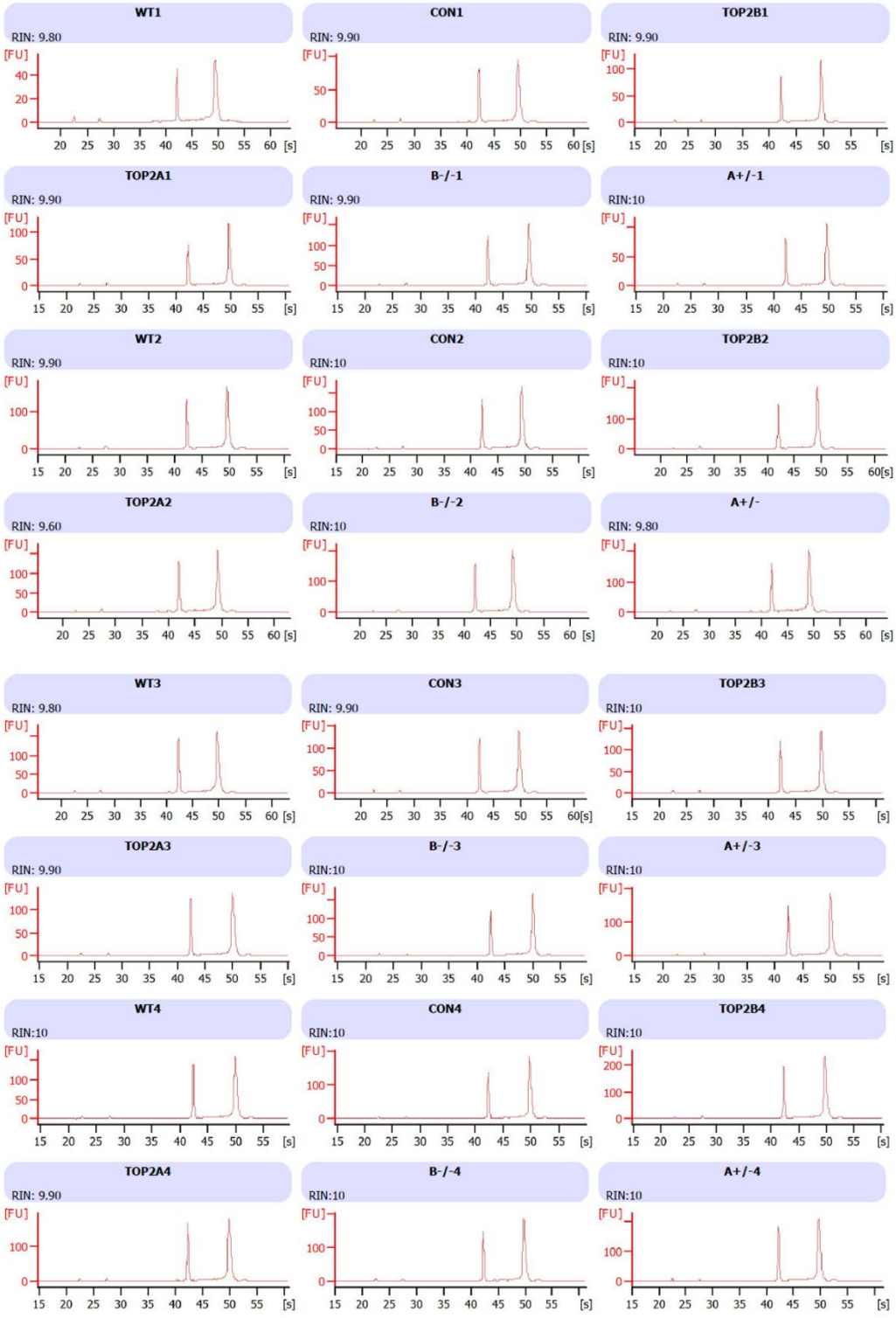


Figure 4-3 Electropherograms of Nalm6 RNA samples used in RNA-seq.

4.3.3 Gene expression in TOP2B-knockout and -knockdown cells

The comparison between the whole transcriptome of the Nalm6^{WT} and the Nalm6$^{B-/-}$ was performed using RNA sequencing (RNA-Seq) technology which is described in Materials and methods (section 2.14.2). The analysis highlighted 16 genes that were down regulated in the Nalm6$^{B-/-}$ cells by more than 1 log2 fold change (Figure 4.4 and Table 4.2). All of these genes (except *TMIGD2*) also showed high fold reduction in previous data from our lab generated by another sequencing service provider (AROS Applied Biotechnology, Denmark) (Appendix figure 7 and Appendix Table 11).

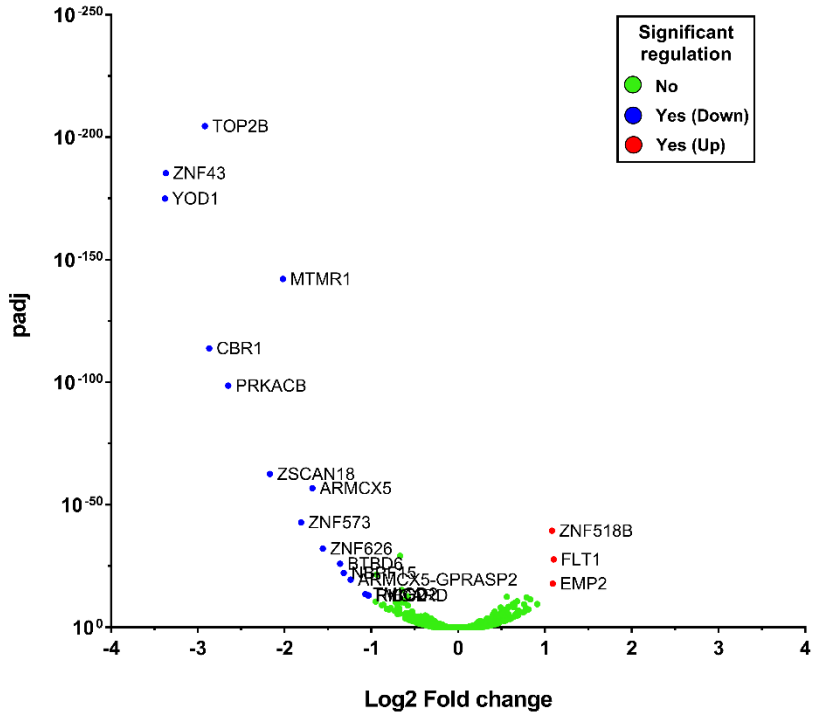


Figure 4-4 Differential gene expression in Nalm6^{WT} vs. Nalm6$^{B-/-}$

Volcano plot shows log2 fold change in gene expression. Genes that showed more than 1 log2 fold change are labelled. Y axis shows adjusted P values (padj) which represent p-value adjusted for multiple testing correction using Benjamini-Hochberg method to control the False Discovery Rate (FDR). Genes showed < 0.05 padj were considered as significantly changed.

Genes	Description	Log2 Fold change	% of control*	padj
YOD1	YOD1 deubiquitinase	3.38	9.62	9.58E-176
ZNF43	zinc finger protein 43	3.37	9.69	4.2E-186
TOP2B	Topoisomerase (DNA) II beta	2.92	13.19	2.64E-205
CBR1	Carbonyl Reductase 1	2.87	13.71	1.28E-114
PRKACB	protein kinase cAMP-activated catalytic subunit beta	2.65	15.91	2.11E-99
ZSCAN18	zinc finger and SCAN domain containing 18	2.17	22.19	2.47E-63
MTMR1	Myotubularin related protein 1	2.02	24.72	5.64E-143
ZNF573	zinc finger protein 573	1.81	28.52	1.33E-43
ARMCX5	Armadillo repeat containing, X-linked 5	1.68	31.14	1.36E-57
ZNF626	zinc finger protein 626	1.56	34.01	7.07E-33
BTBD6	BTB domain containing 6	1.36	38.96	1.05E-26
NBPF15	neuroblastoma breakpoint family member 15	1.32	40.18	5.33E-23
ARMCX5-GPRASP2	ARMCX5-GPRASP2 readthrough	1.24	42.33	2.88E-20
TMIGD2	Transmembrane and immunoglobulin domain containing 2	1.07	47.78	2.55E-14
RIBC2	RIB43A domain with coiled-coils 2	1.04	48.56	8.09E-14
PYCARD	PYD and CARD domain containing	1.03	49.10	8.09E-14

* The control ($Nalm6^{WT}$) corresponds to 100%

Table 4-2 Down regulated genes in $Nalm6^{B-/-}$ compared to $Nalm6^{WT}$

Genes are listed from the highest to the lowest log2 fold change value.

On the other hand, there were several genes that up regulated in the $Nalm6^{B-/-}$ cells although the fold change did not exceed 1.0 log2 for all of them (Figure 4.4 and Table 4.3). All of these genes (except *ZNF518B, LINC00665*, and *MT1H*) also showed high fold increase in previous data of our lab generated by another sequencing service provider (AROS Applied Biotechnology, Denmark) (Appendix figure 7 and Appendix table and 12).

The genes that showed high fold change difference between the $Nalm6^{WT}$ and the $Nalm6^{B-/-}$ were further assessed using reverse transcription quantitative polymerase chain reaction (RT-qPCR) (Figure 4.5).

Genes	Description	Log2 Fold change	% of control*	padj
FLT1	fms related tyrosine kinase 1	1.10	214.17	1.6E-28
EMP2	epithelial membrane protein 2	1.09	213.58	1.4E-18
ZNF518B	zinc finger protein 518B	1.08	211.37	3.33E-40
LINC00665	long intergenic non-protein coding RNA 665	0.91	187.61	2.95E-10
MT1H	metallothionein 1H	0.91	187.26	3.5E-10
GZMA	granzyme A	0.83	177.75	3.54E-12
TUB	tubby bipartite transcription factor	0.81	175.29	0.000000043
ME3	malic enzyme 3	0.79	172.71	5.76E-13

* The control ($Nalm6^{WT}$) corresponds to 100%

Table 4-3 Up regulated genes in $Nalm6^{B-/-}$ compared to $Nalm6^{WT}$

Genes are listed from the highest to the lowest log2 fold change value.

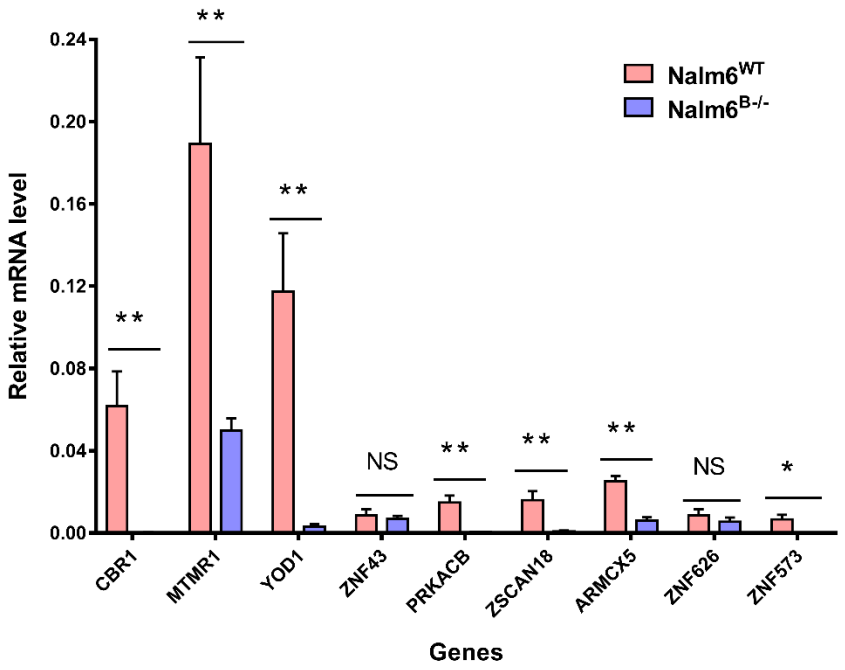


Figure 4-5 RT-qPCR analysis of genes which were down regulated in $Nalm6^{B-/-}$

Relative gene expression were determined by RT-qPCR analysis as described in section 2.14.3. PP1A gene was used as a reference gene for normalizing data. Data presented here is the mean of at least three independent experiments with error bars representing the SEM, (*P≤0.05, **P≤0.01, NS: No significance).

In addition, a number of genes that showed up regulation in both (NCL) and (AROS) data (Appendix figure 9 and Appendix Table 12) were selected for RT-qPCR analysis. Results showed the same gene expression patterns but none of the RT-qPCR differences between Nalm6^{WT} and the Nalm6$^{B-/-}$ reached significance (figure 4.6).

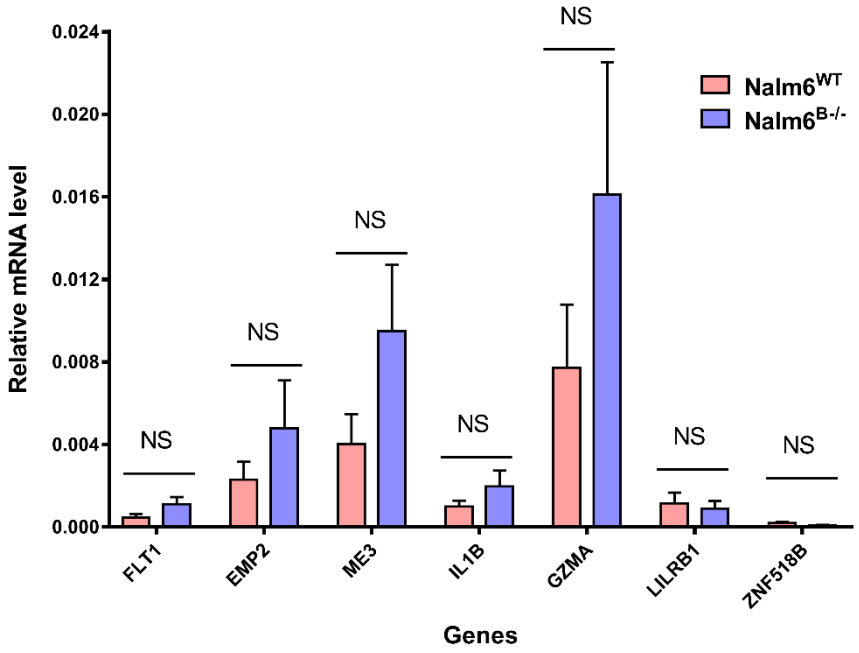
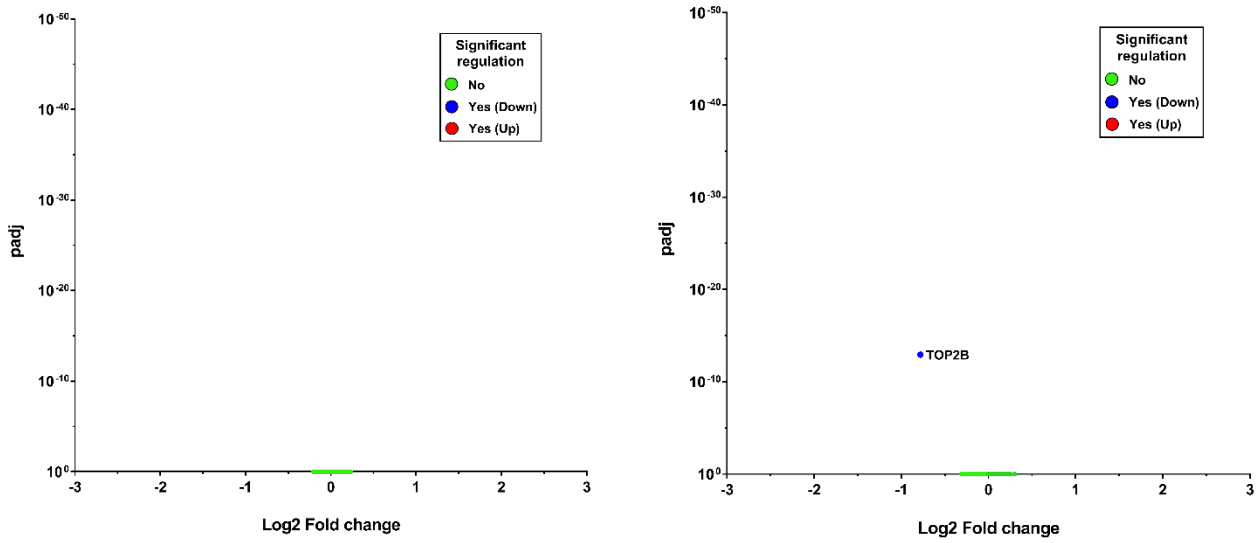


Figure 4-6 RT-qPCR analysis of genes which were Up regulated in Nalm6$^{B-/-}$

Relative gene expression were determined by RT-qPCR analysis as described in section 2.14.3. PP1A gene was used as a reference gene for normalizing data. Data presented here is the mean of at least three independent experiments with error bars representing the SEM, (*P≤0.05, **P≤0.01, NS: No significance).

Furthermore, whole transcriptome analysis was performed in Nalm6^{WT} transfected with anti *TOP2B* small interfering RNA (siRNA) for 48 hrs. Firstly, a comparison between Nalm6^{WT} and wild type transfected with non-targeting siRNA (Con-siRNA) was performed to determine if transfection condition affecting whole gene expression. Results showed that there was no difference between Nalm6^{WT} and (Con-siRNA) cells (figure 4.7A). RNA-Seq analysis showed that transfection of Nalm6^{WT} with anti *TOP2B* siRNA for 48 hrs affected only *TOP2B* gene as depicted in (figure 4.7B and Table 4.4). This might be because knockdown of *TOP2B* for 48 hours was not sufficient to induce change in gene expression shown in Nalm6$^{B-/-}$ which has grown for many passages in the absence of TOP2β or due to other reasons that need to be further addressed.



(A) (B)

Figure 4-7 Differential gene expression in Con-siRNA and TOP2B-siRNA.

Volcano plot shows log2 fold change difference in gene expression. **(A)** Comparison of Nalm6^{WT} with Nalm6 transfected with non-target siRNA **(B)** Comparison of Nalm6 transfected with non-target siRNA with Nalm6 transfected with *TOP2B* siRNA. Y axis shows adjusted P values (padj) which represent p-value adjusted for multiple correction testing using Benjamini-Hochberg method to control the False Discovery Rate (FDR). Genes showed < 0.05 padj were considered as significantly changed.

Genes	Description	Log2 Fold change	% of control*	padj
TOP2B	topoisomerase (DNA) II beta	0.78	58.11	1.11E-13

* The control (Con-siRNA) corresponds to 100%

Table 4-4 Down regulated genes in TOP2B-siRNA compared with Con-siRNA

4.3.4 Gene expression in TOP2A heterozygote-knockout and -knockdown cells

The comparison between whole transcriptome of the Nalm6^{WT} and the Nalm6$^{A+/-}$ was performed using RNA sequencing (RNA-seq) as described in the Materials and methods (section 2.14.2). The analysis highlighted 12 genes that were down regulated in the Nalm6$^{A+/-}$ cells by more than 1 log2 fold change (Figure 4.8 and Table 4.5). All of these genes (except *PRKACB*, *GVINP1*, and *OCIAD2*) also showed high fold reduction in previous data from our lab generated by another sequencing service provider (AROS) (Appendix figure 8 and Appendix table 13). On the other hand, there were several genes that up regulated in the Nalm6$^{A+/-}$ cells by more than 1 log2 (Figure 4.8 and Table 4.6). These genes (except *ZNF518B*) also showed high fold increase in previous data from our lab generated by another sequencing service provider (AROS) (Appendix figure 8 and Appendix table 14).

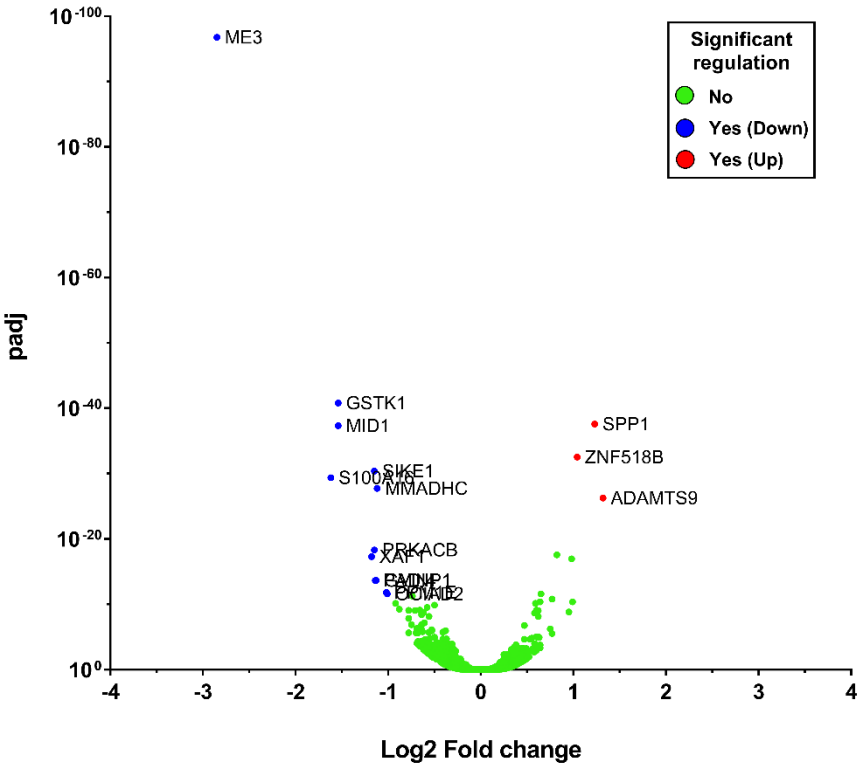


Figure 4-8 Differential gene expression in Nalm6^{WT} vs. Nalm6$^{A+/-}$

Volcano plot shows log2 fold change in gene expression. Genes that showed more than 1 log2 fold change are labelled. Y axis shows adjusted P values (padj) which represent p-value adjusted for multiple testing correction using Benjamini-Hochberg method to control the False Discovery Rate (FDR). Genes showed < 0.05 padj were considered as significantly changed.

Genes	Description	Log2 Fold change	% of control*	padj
ME3	malic enzyme 3	2.85	13.85	1.64E-97
S100A16	S100 calcium binding protein A16	1.62	32.43	4.12E-30
GSTK1	glutathione S-transferase kappa 1	1.54	34.27	1.47E-41
MID1	midline 1	1.54	34.33	4.45E-38
XAF1	XIAP associated factor 1	1.18	44.13	4.85E-18
PRKACB	protein kinase cAMP-activated catalytic subunit beta	1.15	44.93	4.77E-19
SIKE1	suppressor of IKBKE 1	1.15	45.07	3.82E-31
PADI4	peptidyl arginine deiminase 4	1.14	45.32	2.1E-14
GVINP1	GTPase, very large interferon inducible pseudogene 1	1.13	45.58	2.1E-14
MMADHC	methylmalonic aciduria and homocystinuria, cblD type	1.12	46.06	1.79E-28
PPM1E	protein phosphatase, Mg2+/Mn2+ dependent 1E	1.02	49.16	1.48E-12
OCIAD2	OCIA domain containing 2	1.01	49.69	2.46E-12
TOP2A	topoisomerase (DNA) II alpha	0.64	64.06	3.66E-09

* The control ($Nalm6^{WT}$) corresponds to 100%

Table 4-5 Down regulated genes in $Nalm6^{A+/-}$ compared to $Nalm6^{WT}$

Genes are listed from the highest to the lowest log2 fold change value.

Genes	Description	Log2 Fold change	% of control*	padj
ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif 9	1.32	249.02	5.06E-27
SPP1	secreted phosphoprotein 1	1.23	234.21	2.47E-38
ZNF518B	zinc finger protein 518B	1.04	205.78	2.73E-33

* The control ($Nalm6^{WT}$) corresponds to 100%

Table 4-6 Up regulated genes in $Nalm6^{A+/-}$ compared to $Nalm6^{WT}$

Genes are listed from the highest to the lowest log2 fold change value.

Furthermore, whole transcriptome analysis was performed in $Nalm6^{WT}$ transfected with anti *TOP2A* small interfering RNA (siRNA) for 48 hrs. RNA-seq analysis showed that transfection of $Nalm6^{WT}$ with anti *TOP2A* siRNA for 48 hrs affected *TOP2A* gene only as depicted in (Figure 4.9 and Table 4.7). This might be because knockdown of *TOP2A* for 48 hours was not sufficient to induce a change in gene expression shown in $Nalm6^{A+/-}$ which has grown for many passages with only one copy *TOP2A* or due to other reasons that need to be further addressed.

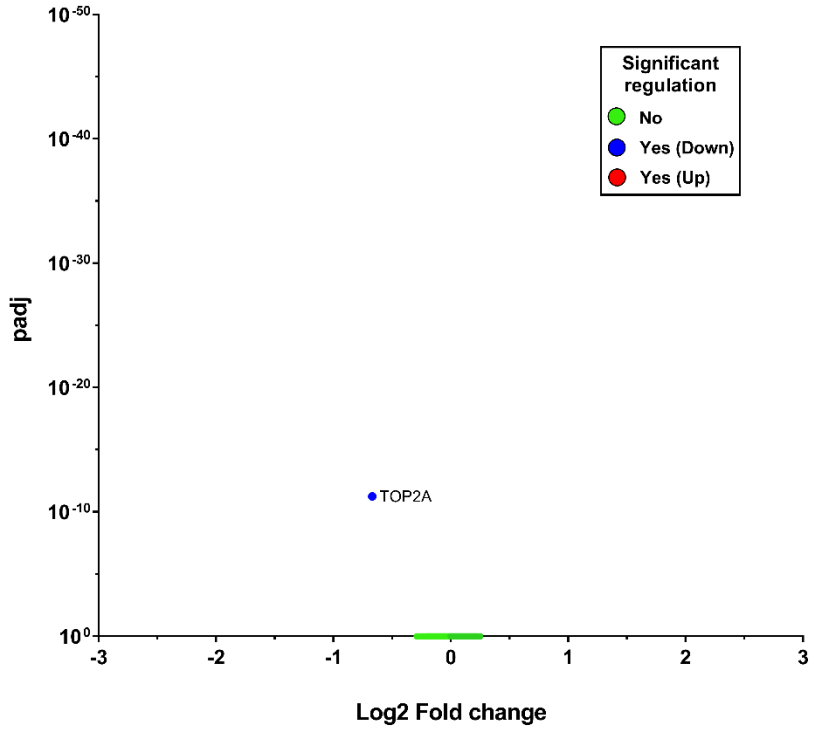


Figure 4-9 Differential gene expression in TOP2A-siRNA compared with Con-siRNA cells.

Volcano plot shows comparison of gene expression between Nalm6 transfected with non-target siRNA and Nalm6 transfected with *TOP2A* siRNA. Y axis shows adjusted P values (padj) which represent p-value adjusted for multiple testing correction testing using Benjamini-Hochberg method to control the False Discovery Rate (FDR). Genes showed < 0.05 padj were considered as significantly changed.

Genes	Description	Log2 Fold change	% of control*	padj
TOP2A	topoisomerase (DNA) II alpha	0.67	62.823	5.63658E-12

* The control (Con-siRNA) corresponds to 100%

Table 4-7 Down regulated genes in TOP2A-siRNA compared with Con-siRNA cells.

4.4 Discussion

TOP2 enzymes are required to resolve topological problems such as positive and negative supercoiling during transcription (Liu and Wang, 1987). In this chapter, the role of TOP2α and TOP2β in gene expression was tested using Nalm6$^{A+/-}$ and Nalm6$^{B-/-}$ mutated lines (provided by Noritaka Adachi). RNA-seq analysis was performed and differential gene expression was determined in both mutated lines against the parent cell (Nalm6^{WT}). Protein expression of TOP2α and TOP2β in all cell lines used in the RNA-seq analysis was confirmed by western blotting (Figure 4.2). RNA-seq results confirmed the magnitude of reduction of TOP2A and TOP2B in Nalm6$^{A+/-}$ and Nalm6$^{B-/-}$ (by 64.06 and 13.19 % of control) (Tables 4.5 and 4.2 respectively) which reflects the number of mutated alleles.

Results showed that *TOP2A* knock down and *TOP2B* knock out did affect the expression of a number of genes in Nalm6 cells. This agreed with studies that showed the effect of both isoforms in gene expression although there is more published data about TOP2β. For TOP2α, it has been shown that gene regulation and priming in embryonic stem cells requires TOP2α (Tiwari *et al.*, 2012; Thakurela *et al.*, 2013). For TOP2β, it has been reported that transcription induced by ligand involved TOP2β-mediated DSBs (Ju *et al.*, 2006; McNamara *et al.*, 2008; Haffner *et al.*, 2010). Studies have shown that TOP2β role in transcription of targeted genes involved bounding at or near promoter regions (Lyu *et al.*, 2006; Madabhushi *et al.*, 2015b; Manville *et al.*, 2015; Uusküla-Reimand *et al.*, 2016; Canela *et al.*, 2017). Further details of mechanistic of TOP2β role in gene expression will be described in discussion of chapters 5 and 6.

In Nalm6$^{B-/-}$ cells, differential gene analysis showed that 16 genes were significantly downregulated by more than one log fold change (Figure 4.4 and Table 4.2). Some of these genes are involved in DNA-binding transcription factor activity (*ZNF43, ZSCAN18, ZNF573*, and *ZNF626*). On the other hand, fewer genes were significantly upregulated as compared with Nalm6^{WT} (Figure 4.4 and Table 4.3). RT-qPCR analysis confirmed RNA-seq results although some of them did not reach significance (Figure 4.5 and 4.6). In Nalm6$^{A+/-}$ cells, 13 and 3 genes were significantly down and up regulated respectively (Figure 4.8 and Tables 4.5 and 4.6).

These genes were also differentially regulated in these cells in another RNA-seq experiment that was performed by another sequencing service provider (AROS Applied Biotechnology, Denmark). RNA-seq results from (AROS) showed more genes that differentially expressed in Nalm6$^{B-/-}$ and Nalm6$^{A+/-}$. For example, 67 or 148 genes were down or up regulated respectively in Nalm6$^{B-/-}$

(Appendix tables 11 and 12), and 27 or 48 were down or up regulated respectively in Nalm6$^{A+/-}$ (Appendix tables 13 and 14). This could be due to the more depth of sequencing applied in AROS than that the current experiment within Newcastle (NCL) (40 million (AROS) versus 30 million (NCL) paired-end (PE) reads).

Both (NCL) and (AROS) Nalm6 datasets were analysed for Gene ontology as described in section (2.14.2.3). Analysis showed no significant enrichment of GO terms or KEGG pathways in (NCL) dataset, while (AROS) dataset showed enrichment of few terms as follows: in (Nalm6^{WT} vs. Nalm6$^{B-/-}$) comparison, one molecular function (MF) term (serine-type endopeptidase activity, GO: 0004252) was highlighted. This term involves *CTSG, C1RL, GZMA, GZMK, KLK1*, and *TMPRSS3* genes. No biological process (BP) terms were significantly enriched, while five cellular component (CC) terms were significantly enriched (extracellular region [GO: 0005576], integral component of plasma membrane [GO: 0005887], proteinaceous extracellular matrix [GO: 0005578], vesicle [GO: 0031982], plasma membrane part (GO: 0044459)). In (Nalm6^{WT} vs. Nalm6$^{A+/-}$) comparison, only two (CC) terms were significantly enriched (external side of plasma membrane [GO: 0009897], tertiary granule lumen [GO: 1904724]).

Analysis also involved testing of over-representation of pathways linked to differentially expressed genes using (KEGG). Analysis of RNA-seq data from AROS data showed over-representation of two pathways (Hematopoietic cell lineage [hsa04640] and Amoebiasis [hsa05146]) in (Nalm6^{WT} vs. Nalm6$^{B-/-}$) comparison. On the other hand, no significant enrichment of KEGG pathways were seen in (Nalm6^{WT} vs. Nalm6$^{A+/-}$) comparison.

In addition, analysis was performed using samples in which both *TOP2A* and *TOP2B* were targeted by siRNA. Knockdown of *TOP2A* and *TOP2B* was performed for 48 hrs before RNA-seq analysis. The knockdown reduced expression of *TOP2A* and *TOP2B* genes by 62.8 and 58.1 % of control (Tables 4.7 and 4.4 respectively). Protein level of all samples was checked using western blotting before RNA-seq analysis as in (Figure 4.2).

However, siRNA knockdown did not show similar effect to knockout, and only the targeted genes were affected (Figures 4.9 and 4.7). The reasons might be the knockdown of TOP2 for 48 hours was not sufficient to induce a change in gene expression similar to that seen *TOP2B* knockout cells, which has grown for longer time with downregulated TOP2. Another possibility is that some of the difference in gene expression between Nalm6^{WT} and the two other modified lines might be due

to genetic instability of cells rather than TOP2 deletion. It has been reported that chromosomal content of continuous cells lines has increased mutation frequency (Geraghty *et al.*, 2014).

Over the last decade, TOP2β predominates over TOP2α in studies involved the role of TOP2 enzymes in gene expression. For this reason, we are more interested in TOP2β role in gene expression in more than one model. In this chapter, we showed how Nalm6$^{B-/-}$ cells is different from the parent Nalm6^{WT} but we still need to confirm our observations because of the reasons mentioned above as well as to further extend our understanding on genes regulated by TOP2β and the mechanism of action.

The current model (Nalm6 cell) was used as a model to study the effect of TOP2β absence on transcription. However, in many situations transcription is induced in response to triggers such as ligands. Nalm6 might undergo partial differentiation or change in expression of cell surface markers (CD10, CD19, and CD38) in response to ligands (Martin-Kleiner *et al.*, 2006; Ardekani *et al.*, 2011; Soleymani Fard *et al.*, 2012). We compared response of Nalm6^{WT} and Nalm6$^{B-/-}$ to all-trans retinoic acid (ATRA) (Appendix figure 16 and 17) or vitamin D3 (Appendix figure 14 and 15). Results showed that both Nalm6^{WT} and Nalm6$^{B-/-}$ did not show response to either D3 or ATRA and there was no difference between the two cell lines.

To generate further *TOP2B* knockout models, I used CRISPR to generate a *TOP2B* knockout in Nalm6 (Chapter 5) as well as *TOP2B* knockout model of SH-SY5Y, a neuronal cell line that can be induced to differentiate and change its transcriptional state (Chapter 6).

5 Chapter Five: TOP2β regulates expression of *CBR1, a* gene implicated in doxorubicin-induced cardiotoxicity

5.1 Introduction

Anthracyclines are a group of antibiotics that are widely used in clinic. Adriamycin (doxorubicin), daunorubicin, and epirubicin represent the most commonly used anthracyclines in cancer treatment (Mordente *et al.*, 2001). Doxorubicin is one of the most effective antineoplastic agents used for the treatment of solid and haematological tumours such as lymphoblastic and myeloblastic leukaemias, sarcoma, neuroblastoma, breast and bladder cancer (Wallace, 2003). However, efficiency of this agent is widely hampered by the development of acute or chronic cardiotoxicity following drug exposure (Ferrans *et al.*, 1997; Singal *et al.*, 1997; Singal *et al.*, 2000). This cardiotoxicity includes ventricular contractile dysfunction, pericarditis, hypertension, arrhythmias, and thromboembolism (Higgins *et al.*, 2015).

There is an extensive literature about the cause, mechanism, and management of doxorubicin-induced cardiotoxicity. Several hypotheses have been suggested to explain the molecular mechanism of doxorubicin-induced cardiotoxicity. The most common are oxidative stress (Singal and Iliskovic, 1998), disruption of intracellular Ca^{2+} homeostasis, iron complex formation, mitochondrial dysfunction and oxidative stress-independent apoptotic mechanisms (Mordente *et al.*, 2001; Octavia *et al.*, 2012). Each suggested mechanism has a limitation and, to date, the oxidative oxygen species (ROS) hypothesis is the most prevalent mechanism underlying doxorubicin-induced cardiac injury and myocardiopathy. This is supported by the fact that cardiac tissue has lower levels of catalase and superoxide dismutase activities in comparison with other tissues (Pai and Nahata, 2000). However, studies utilising treatment with (ROS) scavengers did not show protection from doxorubicin-induced cardiotoxicity (Myers *et al.*, 1983; Hasinoff *et al.*, 2003; Martin *et al.*, 2009). Therefore, alternative molecular mechanisms has been hypothesized and tested as an attempt to understand events underlying doxorubicin-induced cardiotoxicity.

TOP2β has been suggested as a possible key factor in the mechanism of doxorubicin-induced cardiotoxicity for several reasons; firstly, the well established fact that TOP2 is one of the main targets of doxorubicin (Tewey *et al.*, 1984; Cowell and Austin, 2012), together with the evidence that TOP2β but not TOP2α, is the main TOP2 isoform expressed in adult mammalian cardiomyocytes (Giovanni Capranico and Zunino, 1992) and postmitotic cells in general (Austin and Marsh, 1998; Austin *et al.*, 2018). In support of this, it has been shown that apoptosis induced

by doxorubicin in peripheral blood cells was associated with TOP2B level (Kersting *et al.*, 2006). Secondly, Top2b deletion in primary mouse embryonic fibroblasts (MEF) is reported to protect these cells from doxorubicin-induced DNA damage as shown by the reduction of γH2AX signal in $MEF^{Top2b-/-}$ compared with $MEF^{Top2b+/+}$ (Lyu *et al.*, 2007).

In addition, (Lyu *et al.*, 2007) showed that H9C2 cardiomyocytes were protected from DNA damage induced by doxorubicin, but not hydrogen peroxide or camptothecin, when treated with ICRF-187 (clinically known as dexrazoxane) which both reduced TOP2β-doxorubicin cleavage complex level and induced TOP2B degradation. ICRF-187 is a well-known catalytic inhibitor of TOP2 and has been used commonly with doxorubicin as a cardioprotective agent (Speyer *et al.*, 1988; Speyer *et al.*, 1992; Hensley *et al.*, 2009). Furthermore, (Lyu *et al.*, 2007) showed that γH2AX signal was reduced by ICRF-193, another catalytic inhibitor derived from the same family as ICRF-187 that has been shown to be more specific against TOP2β isoform (Xiao *et al.*, 2003).

The role of TOP2β in doxorubicin-induced cardiotoxicity at the molecular level has been investigated by (Zhang *et al.*, 2012). In this study, the authors generated a mouse model with targeted deletion of *Top2b* in cardiomyocytes. They showed that the mice lacking *Top2b* in cardiac tissue were protected from doxorubicin-induced cardiotoxicity. Interestingly, *Top2b*-deleted cardiomyocytes showed about 60 % decrease in doxorubicin-induced DNA double stranded breaks (DSBs) as measured by γH2AX immunostaining of heart sections as well as around 70 % reduction in the ratio of apoptotic cells as measured by TUNEL assay (Zhang *et al.*, 2012).

In addition, they showed that doxorubicin treatment induced transcriptional changes related to mitochondrial dysfunction and oxidative stress in $Top2b^{+/+}$ but not $Top2b^{\Delta/\Delta}$ cardiomyocytes. Ingenuity Pathways Analysis (IPA) of microarray data of genes that are altered by more than 1.5 fold showed that doxorubicin treatment for 16 hrs activates *Trp53inp1*, *Apaf1*, *Bax* and *Fas* in $Top2b^{+/+}$ but not $Top2b^{\Delta/\Delta}$ cardiomyocytes. These genes are involved in the p53 apoptosis pathway. Analysis 72 hrs post doxorubicin treatment showed downregulation of both *Ppargc1a* and *Ppargc1b* by 50% in $Top2b^{+/+}$ but not $Top2b^{\Delta/\Delta}$. These genes encode PGC-1α and PGC-1β, transcription coactivators that are involved in the function and the biogenesis of mitochondria. PGC-1α has been shown to be a hallmark of heart failure in another study (Sihag *et al.*, 2009). Treatment of mice with doxorubicin for 5 days followed by measuring of ejection fraction by magnetic resonance imaging (MRI) scan showed decreases in $Top2b^{+/+}$ and $Top2b^{+/\Delta}$ by 53 % and

43% respectively, while no change seen in *Top2b*$^{\Delta/\Delta}$. Reduction of ejection fraction is a clinical feature of cardiotoxicity (Zhang *et al.*, 2012).

The role of TOP2β in doxorubicin-induced cardiotoxicity in human cells was investigated in human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) by (Maillet *et al.*, 2016). This involved *TOP2B* knockout in a human pluripotent stem cell (hPSC) by CRISPR followed by induction of differentiation into cardiomyocytes. The *TOP2B* knockout hPSC-CMs showed significant reduction in cytotoxicity and DNA damage (γH2AX signal) induced by doxorubicin as compared with wild type cells.

Collectively, studies above showed a role for TOP2β in doxorubicin-induced cardiotoxicity regardless of the mechanism or pathway involved. This suggests that TOP2β could be used as a useful biomarker for early prediction of susceptibility to doxorubicin-induced cardiotoxicity. Indeed, in a study involving comparison of TOP2β levels in peripheral blood cells between two groups: anthracycline-resistant and anthracycline-sensitive patients, results showed that anthracycline-sensitive patients who had overall Left Ventricular Ejection Fraction (LVEF) <50% showed markedly higher TOP2β level (Vejpongsa *et al.*, 2013).

Another aspect of doxorubicin-induced cardiotoxicity is the potential role of the doxorubicin metabolite doxorubicinol (Figure 5.1). It has been shown that about half of the administered doxorubicin is eliminated from the body without change while the other half is metabolized mainly to the doxorubicin alcohol metabolite doxorubicinol (Joerger *et al.*, 2005). This is mainly achieved by the Carbonyl Reductase 1 (CBR1) enzyme through a two-electron reduction of the carbonyl group in the side chain of doxorubicin to generate the C13-alcohol metabolite doxorubicinol (Licata *et al.*, 2000; Kassner *et al.*, 2008).

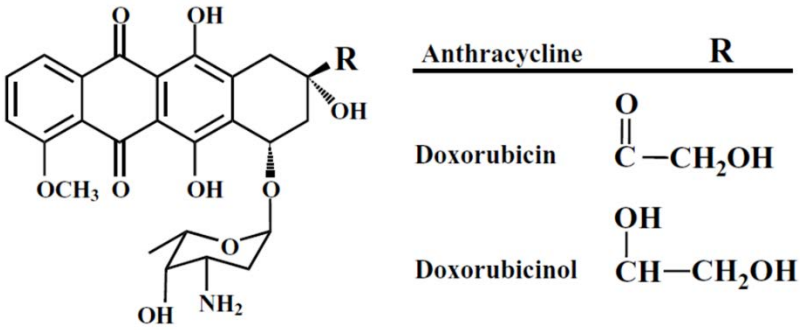


Figure 5-1 Doxorubicin and doxorubicinol chemical structures

Figure was reproduced from (Mordente *et al.*, 2003) with permission.

There is a variability in the literature as to whether the doxorubicin or doxorubicinol is the most toxic. A systematic review of the various reports suggests that doxorubicinol is more cardiotoxic but has less anti-tumour effect than doxorubicin (Edwardson *et al.*, 2015). Generally, in vivo studies suggest doxorubicinol as more cardiotoxic than doxorubicin while in vitro studies using cell lines in culture showed remarkable lower anti-tumour effect of doxorubicinol compared with doxorubicin. It has been shown that cardiac tissue has enhanced accumulation of doxorubicinol over other tissues in rats receiving multiple doses of doxorubicin (Peters *et al.*, 1981).

There is a wide range of evidence suggesting that doxorubicinol is responsible for doxorubicin-induced cardiotoxicity and is more cardiotoxic than the parent compound (Boucek *et al.*, 1987; Olson *et al.*, 1988; Mushlin *et al.*, 1993; Minotti *et al.*, 1995). The early and preclinical evidence includes different types of findings; first, it has been shown that anthracyclines with reduced cardiotoxicity form less alcohol metabolite (Menna *et al.*, 2007). Second, a correlation was shown between cardiotoxicity and the level of doxorubicinol accumulation in the heart (Olson and Mushlin, 1990; Cusack *et al.*, 1993; Stewart *et al.*, 1993; Sacco *et al.*, 2003). Third, overexpression of doxorubicin reducing enzyme CBR1 in mice heart increases cardiotoxicity (Forrest *et al.*, 2000). Fourth, deletion of one copy of *Cbr1* gene in mice significantly protects from cardiotoxicity (Olson *et al.*, 2003). Furthermore, reducing doxorubicinol formation by the inhibition of doxorubicin metabolism with phenobarbital results in less cardiotoxicity in rats (Behnia and Boroujerdi, 1999).

For these reasons, many carbonyl reductase inhibitors have been evaluated and a line of research involving the cardioprotection effect of CBR1 inhibition has emerged (Huang *et al.*, 2010; Arai *et al.*, 2015; Piska *et al.*, 2017). Some of these inhibitors were tested in clinical trials (Bruynzeel *et al.*, 2007). In a murine study, carbonyl inhibitor 23-hydroxybetulinic acid (23-HBA) reduced doxorubicin-induced cardiotoxicity and this was manifested as a selective reduction of doxorubicinol in the heart. Similar effects were seen in the cardiomyocyte H9c2 cell line (Zhou *et al.*, 2015).

In support of a role of CBR1, it has been shown that interindividual variability in susceptibility to doxorubicin-induced cardiomyopathy is correlated to single nucleotide polymorphism (SNP) in the CBR1 gene (Blanco *et al.*, 2012). The effect of SNPs of human CBR1 on the metabolism of doxorubicin has been indicated in an in vitro study (Bains *et al.*, 2009). Moreover, studies have shown that cancer patients with Down Syndrome (Trisomy 21) are at increased risk of anthracycline-induced cardiotoxicity over those without Down Syndrome (Krischer *et al.*, 1997;

O'Brien *et al.*, 2008; Quinones-Lombrana *et al.*, 2014), which might be due to higher *CBR1* expression (*CBR1* gene is located at chromosome 21) and hence may contribute to the pharmacology of doxorubicin-induced cardiotoxicity in this group.

Another strategy to reduce doxorubicin toxicity induced by its metabolites involved testing doxorubicin analogues with reduced metabolite formation. A novel doxorubicin (DOX) analogue, 13-deoxy, 5-iminodoxorubicin (DIDOX) has shown a comparable cytotoxicity profile with doxorubicin with reduced cardiotoxicity in a chronic rabbit model as measured by left ventricular fractional shortening and contractility as well as by histological examination (Frank *et al.*, 2016). Interestingly, in this study inhibition of TOP2β decatenation activity by DOX and DIDOX was compared. DOX inhibited TOP2β decatenation while DIDOX did not interact with TOP2β in a decatenation assay, consistent with other evidence that suggests TOP2β has a role in a cardiotoxicity. This drug was also tested in a clinical trial phase I to treat in patients with advanced solid tumours and no cardiotoxicity was observed (Holstein *et al.*, 2015).

On the other hand, studies have suggested that doxorubicinol has much less antitumour activity than doxorubicin (Heibein *et al.*, 2012; Bains *et al.*, 2013). These studies involved in vitro comparison of the cytotoxicity profile of doxorubicin with doxorubicinol in cell lines such as the V79/AP4 fibroblast (Bernardini *et al.*, 1991) and MCF-7 (Veitch *et al.*, 2009). Therefore, it has been suggested that doxorubicinol and reductase enzymes such as CBR1 are important factors in tumour resistance (Tak *et al.*, 2011; Heibein *et al.*, 2012). Accordingly, studies have shown increased expression of reductase enzymes in resistant tumour samples (Zhong *et al.*, 2015).

In agreement with this, inhibition of doxorubicin metabolism using aldose-reductase inhibitor (fidarestat) significantly increased doxorubicin-sensitivity of resistant colorectal cancer cells and significantly reduced cardiotoxicity in mice (Sonowal *et al.*, 2017). In this study, fidarestat treatment significantly increased sensitivity of human colon cancer cell lines HT-29 and SW480 as well as nude mice xenografts to doxorubicin. In addition, fidarestat treatment protected mice from doxorubicin-induced cardiotoxicity as determined by serum cardiotoxic biomarker troponin-I as well as heart function by echocardiography. Furthermore, fidarestat treatment of H9c2 cardiomyocyte cell line reduced cytotoxicity of doxorubicin (Sonowal *et al.*, 2017). So far, there is no reported clinical trial of fidarestat for cardioprotection against doxorubicin although it has been shown not to cause any toxicity in human in a phase III clinical trials for treatment of diabetic neuropathy (Giannoukakis, 2003; Asano *et al.*, 2004).

5.2 Aims

The literature reviewed above confirms the role of two factors in doxorubicin-induced cardiotoxicity: TOP2β and CBR1. Interestingly, both TOP2β and CBR1 play their role in the same direction, i.e. downregulation of either TOP2B or CBR1 reduce doxorubicin-cardiotoxicity. However, all existing studies have involved studying TOP2B or CBR1 independently of each other and so far, there is no published data suggesting that TOP2β mediates doxorubicin-induced cardiotoxicity through the regulation of *CBR1* gene.

In the previous chapter, RNA-seq and RT-qPCR results showed a significant regulation of a number of genes including *CBR1* upon *TOP2B* knockout (Figure 4.3, Table 4.2, Appendix Figure 9, Appendix Table 11, and Figure 4.5). However, the downregulation of *TOP2B* using siRNA did not cause similar effects as was mentioned in the discussion of previous chapter.

In this chapter, the aim was to further verify the observations seen in the previous chapter regarding the differentially expressed genes including the *CBR1*. For this reason, new *TOP2B* knockout clones in the Nalm6 cell line were generated using CRISPR. The aims of this chapter were to:

- Confirm regulation of genes seen in Nalm6$^{B-/-}$ in the new *TOP2B* knockout clones.
- Study the mechanism of *CBR1* regulation by TOP2β.

To address the aims above, the following experimental objectives were followed:

- Optimize the CRISPR technique to generate a *TOP2B* knockout model in a Nalm6 cell line.
- Use of freshly generated *TOP2B* knockout model to investigate the expression of genes which were previously either up or down regulated in Nalm6$^{B-/-}$ using RT-qPCR technique.
- Investigate the mechanism by which TOP2β regulates *CBR1* using Chromatin Immunoprecipitation (ChIP) and RT-qPCR techniques.

5.3 Results

5.3.1 Generation of a TOP2B knockout model in Nalm6 cells using CRISPR

Details for gRNA design, cloning, and verification of their cloning by sequencing are in (Sections 2.15 and 6.3.1). Briefly, eight different gRNAs were designed to target exon number 1 of the *TOP2B* gene. The sequence of these gRNAs and their predicted off-target effects are detailed in the materials and methods (figure 2.2 and table 2.7). The efficiency of these gRNAs was tested using T7 endonuclease I as described in the materials and methods (section 2.15.5). As the initial DNA samples used in the T7 endonuclease I test were from SH-SY5Y cells, the result of this test was presented in the next chapter (section 6.3.1.3). The gRNA number 6 showed the highest efficiency (figure 6.7) and the lowest off-target record as well (figure 2.7). For this reason, gRNA number 6 was chosen to generate *TOP2B* knockout clones in Nalm6 in this chapter. The gRNA number 6 was cloned into PX458 vector, a plasmid expressing both CRISPR cas9 system as well as GFP as a selective marker. Nucleofection was used to transfect Nalm6^{WT} as described in the materials and methods (section 2.15.3). Briefly, Nucleofector II system (Amaxa, USA) was used to transfect the cells using Amaxa® Cell Line Nucleofector® Kit T (Lonza, UK, cat. VCA-1002) according to the manufacturer's instructions. The optimized protocol from (Lonza, UK) for Nalm6 was followed. The following sections show the transfection and clones screening steps.

5.3.1.1 Determining the transfection efficiency in Nalm6 cells

Nalm6^{WT} cells were transfected with PmaxGFP (as a control) or PX458 cloning vectors then the transfection efficiency was determined as described in (Section 2.15.4) and presented in (Figure 5.2 A and B).

5.3.1.2 Screening for TOP2B knockout clones

Many clones were isolated and expanded for screening as described in (Section 2.15). Among these, six clones showed a different genotype pattern from wild type (Figure 5.3). PCR products of four clones (4, 6, 14, and 18) were extracted and sent for sequencing (Figure 5.3 and Table 5.1). Sanger sequencing showed that two of them had an insertion and clone number 4 had a deletion while the mutation of clone number 18 could not be sequenced although sequencing attempts were repeated several times. These four clones were further tested for TOP2B protein expression and they were all negative for TOP2B except clone 14 (in which a 3 base insertion was introduced) (Figures 5.4 and 5.5).

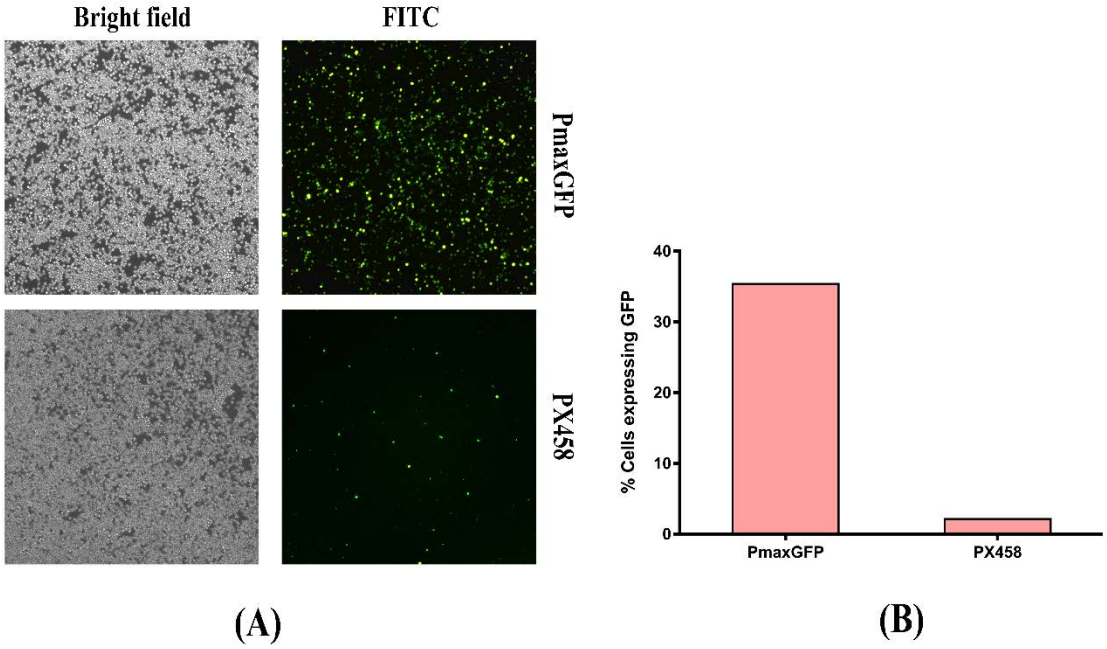


Figure 5-2 Transfection efficiency in Nalm6 cells

(**A**) Live cell imaging of Nalm6 cells transfected with the positive control (PmaxGFP) or the (PX458) vector by Nucleofection. Images were captured using TiE fluorescence wide field inverted microscope (Nikon) (10X magnification). (**B**) Transfection efficiency (%) measured by FACS.

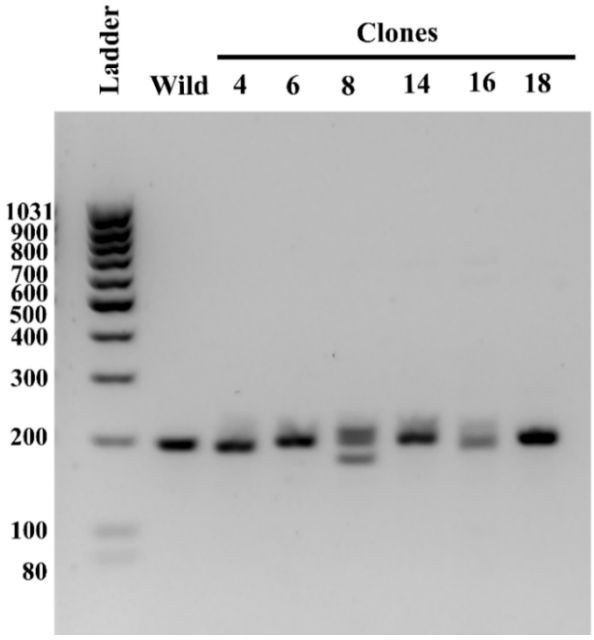


Figure 5-3 Screening for TOP2B knockout clones in Nalm6 cells by genotyping.

Samples were amplified by PCR as described in the Materials and methods section then loaded on a (3 %) agarose gel and electrophoresed to detect small size differences.

Clone	gRNA	Mutation
4	6	Wild: GGCCAAGTCGGGTGGCTGCGGCGCGGGAGCCGGCGTG Mutant: GGCCAAGT……GCTGCGGCGCGGGAGCCGGCGTG
6	6	Wild: CCATGGCCAAGT…..CGGGTGGCTGCGGCGCGGGAGCC Mutant: CCATGGCCAAGTGCCGGGTGGCTGCGGCGCGGGAGCC
14	6	Wild: ATGGCCAAGTC…….GGGTGGCTGCGGCGCGGGAGCCG Mutant: ATGGCCAAGTCCCCGGGTGGCTGCGGCGCGGGAGCCG
18	6	Mutation sequence could not be confirmed.

Table 5-1 Clones that showed bi-allelic genetic variation from wild type Nalm6 cells.

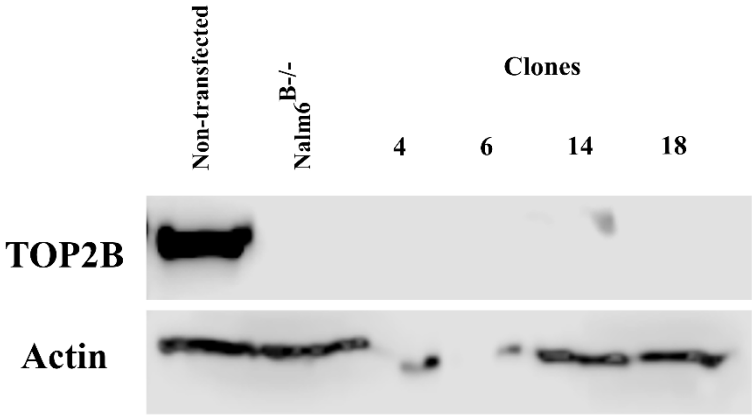


Figure 5-4 Screening for *TOP2B* knockout clones in Nalm6 cells by western blotting

Clone protein samples (5 µg / lane) were prepared for western blotting as described in (Section 2.3) and probed with anti actin (NB600-501) or anti TOP2B (MAB6348) antibodies.

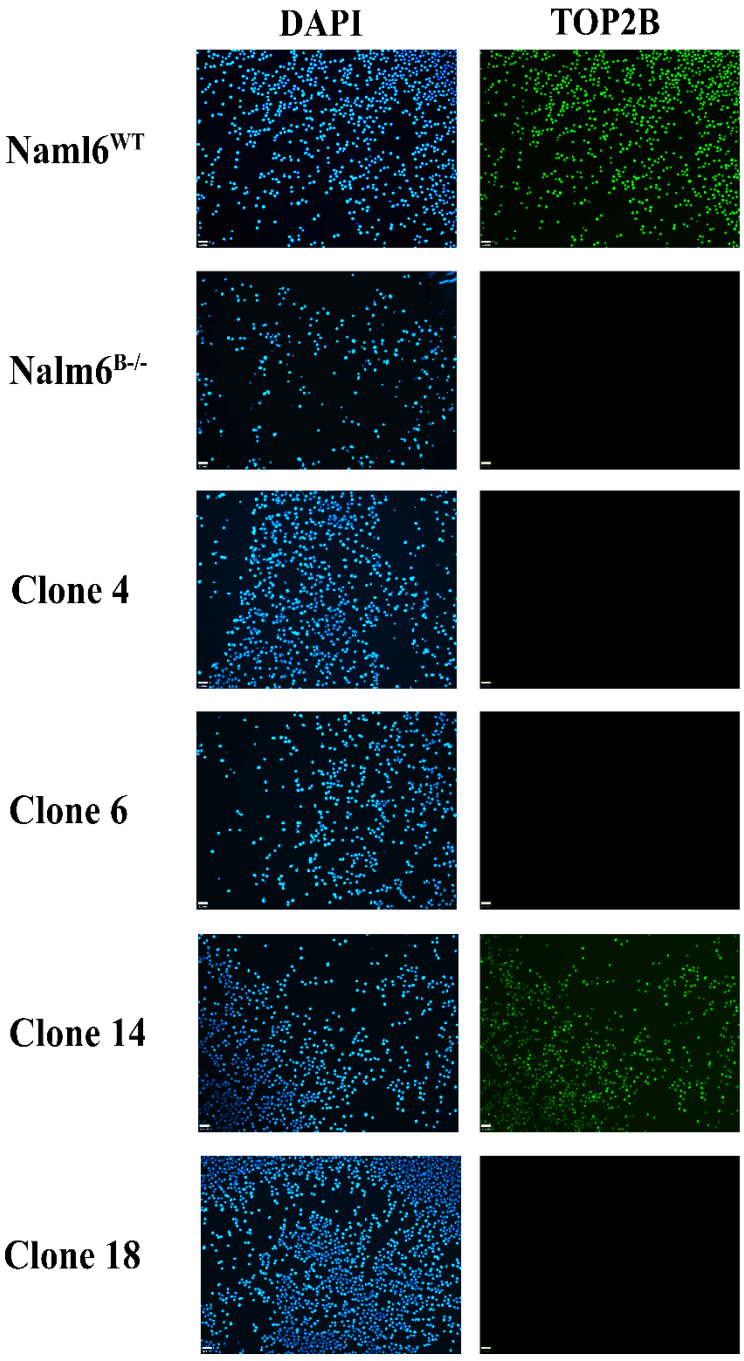


Figure 5-5 Screening for *TOP2B* knockout clones in Nalm6 cells by immunofluorescence

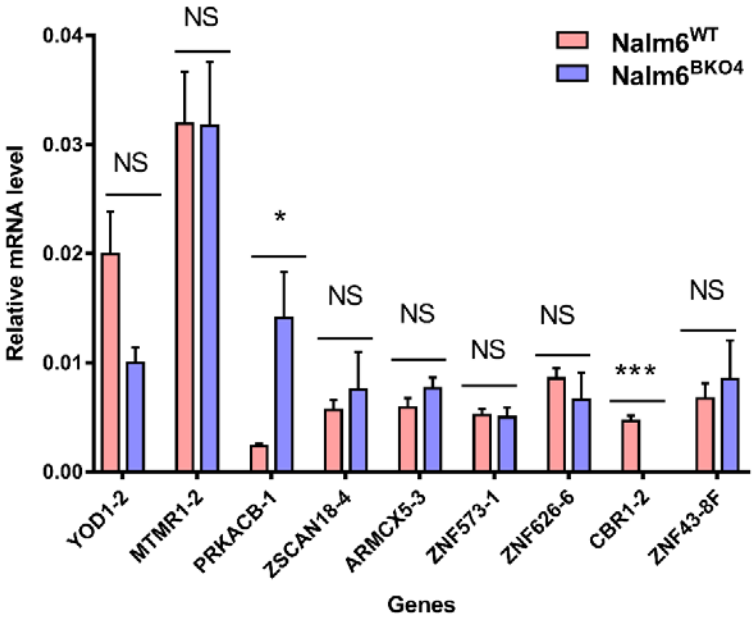
Cells were prepared for immunofluorescence as described in (Section 2.15.7.2) then probed with anti TOP2B antibodies (4555) followed by Alexa fluor®488 secondary antibodies then immunofluorescence signal was captured via FITC channel.

5.3.2 RT-qPCR analysis of selected genes

The knockout clone number 4 ($Nalm6^{BKO4}$) was selected to check expression level of genes which showed up or down regulation in $Nalm6^{B-/-}$ (Adachi) cells to confirm the expression trends in the absence of TOP2β. In addition, expression levels of the same genes were tested in a *TOP2B* knockout model of K562 cells ($K562^{BKO33}$) which were generated at the same time by me using CRISPR (details are in Appendix figures 9, 10, 11, and 12 as well as Appendix table 15). The aim was to answer the question of whether any of these genes are regulated by TOP2β in a non-cell line specific mechanism. However, none of these genes showed statistically significant changes in $K562^{BKO33}$ (Appendix Figures 13).

In $Nalm6^{BKO4}$, of 16 genes tested, 2 genes (CBR1 and PRKACB-1) showed significant change in comparison with $Nalm6^{WT}$. The *CBR1* gene was the only gene that showed significant decrease consistent with the previous results (Figure 5.6). The *CBR1* gene expression level showed tight dependency on TOP2β. Significant reduction of this gene upon *TOP2B* knockout was determined in 4 different experiments in this study: 2 independent RNA-seq experiments (Figure 4.4, Table 4.2, Appendix figure 7 and Appendix table 11) and 2 independent RT-qPCR experiments with two different Nalm6 *TOP2B* knockout cells generated by two methods (Figures 4.5 and 5.6).

(A)



(B)

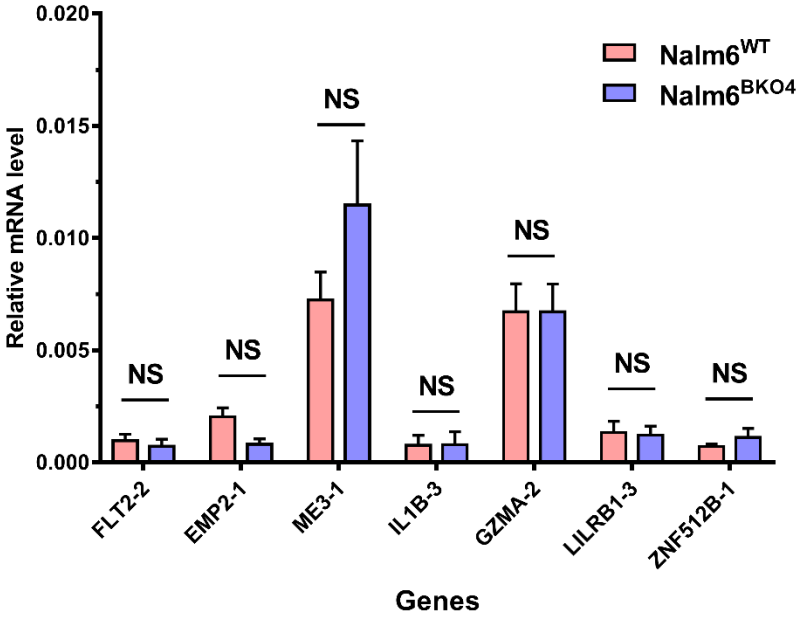


Figure 5-6 Gene expression analysis of selected genes in Nalm6^{BKO4} cells.

RT-qPCR analysis of genes which were either downregulated **(A)** or upregulated **(B)** in knockout Nalm6 from Adachi (Nam6$^{B-/-}$). RT-qPCR analysis as described in (section 2.13.4). The PP1A gene was used as a reference gene for normalizing data. Data presented here is the mean of at least three independent experiments with error bars representing the SEM, (*P≤0.05, **P≤0.01, NS: No significance).

These results were consistent and it is interesting to further study how TOP2β regulates *CBR1* gene expression because both TOP2β and CBR1 have clinical importance in cardiotoxicity associated with cancer treatment (See section 5.1).

Studies have shown the role of both TOP2β and CBR1 in doxorubicin-induced cardiotoxicity. So far, no published data suggest a model in which the cardioprotection effect seen upon TOP2β inhibition is due to decreased CBR1 activity and hence decrease in doxorubicin reduction into the cardiotoxic metabolite doxorubicinol.

5.3.3 Chromatin Immunoprecipitation analysis of CBR1 gene

To determine whether TOP2β regulates *CBR1* directly, Chromatin Immunoprecipitation (ChIP) technique was used to study the molecular events associated with *CBR1* gene landscape especially the promoter (Figure 5.7). The ChIP protocol was performed as described in (Materials and Methods, Section 2. 16). This technique was used to study TOP2β enzyme recruitment at selected regions of the *CBR1* gene in both Nalm6^{WT} and Nalm6^{BKO4} cells. In addition, ChIP was used to determine the effect of TOP2β absence on the recruitment of four transcription factors that have been shown to be recruited to regions at *CBR1* genomic locus (ENCODE database): Upstream Stimulatory Factor 1 (USF1), Upstream Stimulatory Factor 2 (USF2), C-Myc (also known as MYC), and MYC Associated Factor X (MAX) (Figure 5.7).

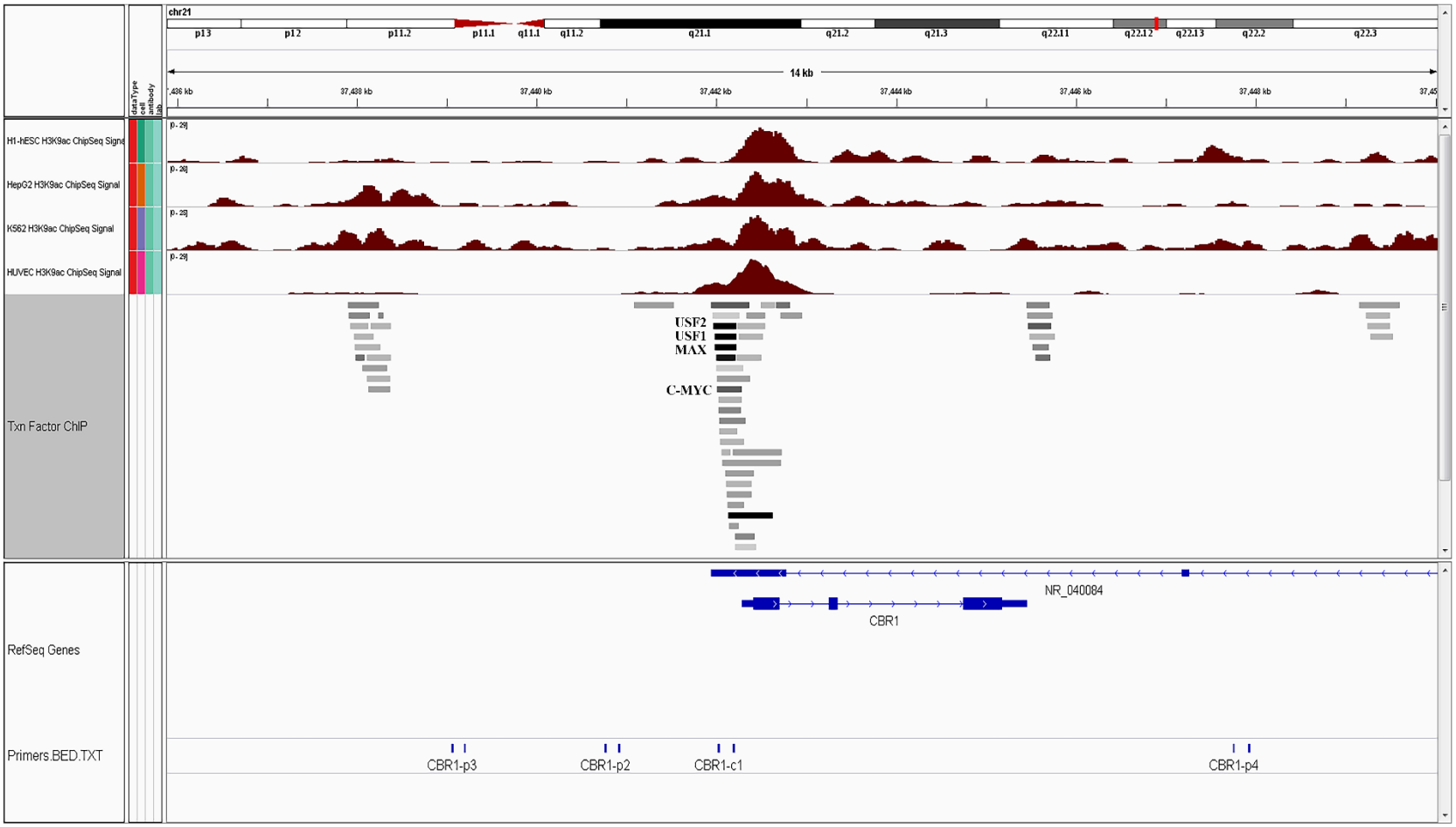


Figure 5-7 IGV viewer snapshot shows *CBR1* gene landscape.

This figure shows Acetylated Histone 3 peak signals and transcription factors ChIP data reported from (ENCODE database). PCR primers used in ChIP (CBR1-C1, CBR1-P2, CBR1-P3, and CBR1-P2) were added below RefSeq Genes track.

5.3.3.1 Testing the ChIP primers on the CBR1 gene

Based on data from (ENCODE database) which describe sites of active chromatin manifested by H3K9ac and H3K4me3 as well as regions of ChIP signal peaks of transcription factors under study, multiple primers at different regions of CBR1 gene were designed by myself as detailed in (Table 2.5) and tested for unique PCR products as described in (Figure 5.8 A and B). Four primers were selected to test ChIP signal: CBR1-C1, CBR1-P2, CBR1-P3, and CBR1-P4 as depicted in dedicated track in (Figure 5.7).

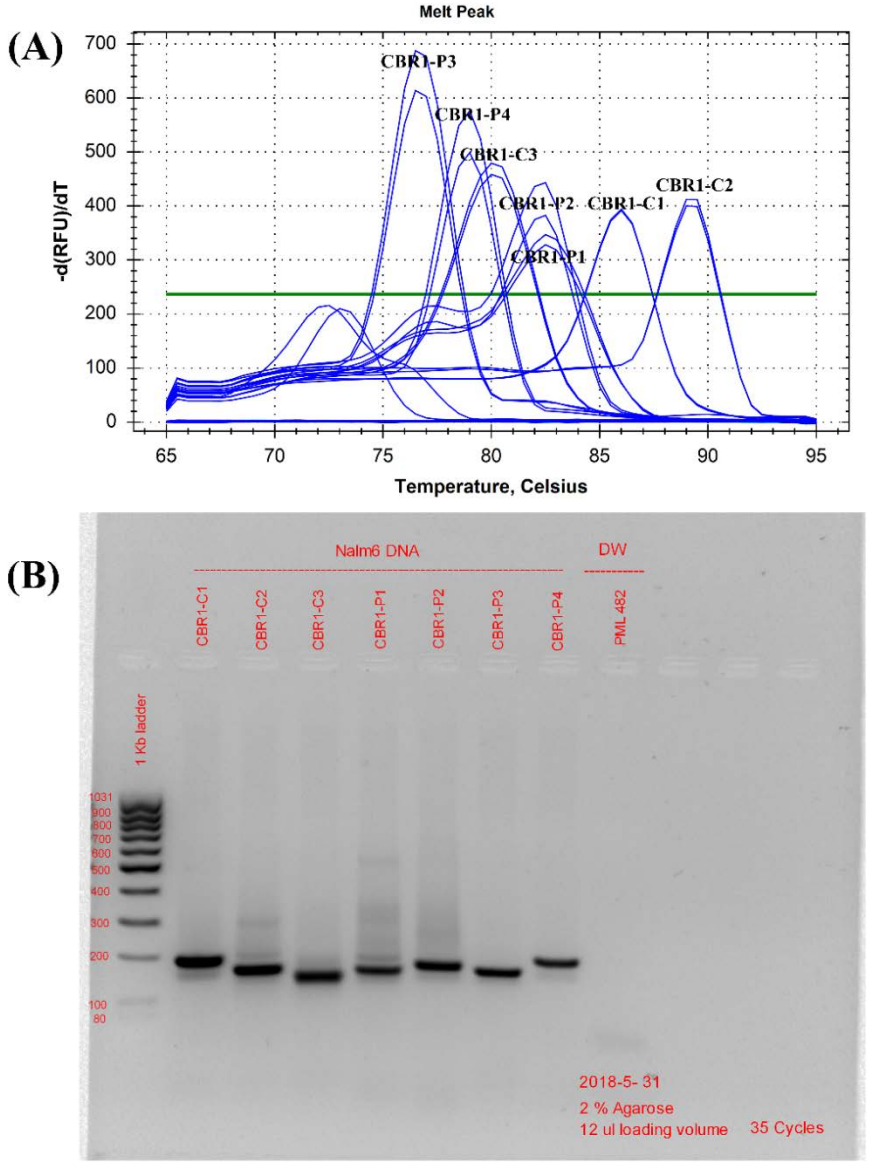


Figure 5-8 Testing ChIP primers for *CBR1* gene.

(A) Melting curve plot to test specificity of primers **(B)** Agarose gel of PCR reaction samples in A.

5.3.3.2 Optimization of Chromatin fragmentation for ChIP

Cell harvesting and chromatin fixation with 1% formaldehyde was performed as described in detail in (Materials and Methods, Section 2.16). The optimal fragmentation range of chromatin DNA depends on the purpose of experiment and ChIP resolution required to be achieved. The aim here was to achieve chromatin DNA fragments of 200-600 bp. Six chromatin samples were sonicated with a range of cycles using a Bandelin Sonopuls HD2070 probe sonicator by applying (5-10 rounds of 7 pulsed cycle for 15 seconds at 20% power) as described in (Materials and Methods, Section 2.16) and the DNA was purified as descirbed in (Materials and Methods, Section 2.16.5). DNA samples were then run on an agarose gel and visualized as in (Figure 5.9). The condition which was chosen for further ChIP experiments was (7 rounds of 7 pulsed cycle for 15 seconds at 20% power).

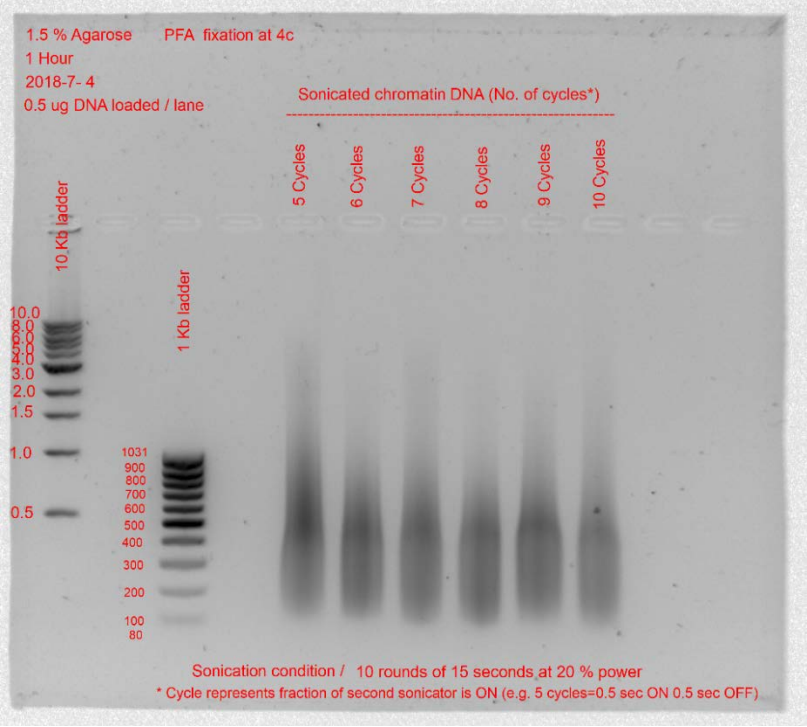


Figure 5-9 Optimization of Chromatin fragmentation for ChIP

5.3.3.3 ChIP results

To validate any ChIP protocol, it is important to have an antibody that is known to give a signal at a given genomic locus. For this purpose, Anti-acetylated Histone 3 (Ac-H3) antibody was used as a positive control in ChIP (Table 2.1) along side a primer which amplifies a 166 bp region of the *GAPDH* gene promoter (Table 2.5). In addition, all ChIP PCR primers were tested using an immunopreceipitate (IP) with an anti-GFP antibody which was used as a negative control. Furthermore, for the *CBR1* gene locus, CBR1-P4 primer was used as a negative genomic control to amplify a region where no signal is expected with the antibodies used in the experiment according to various online ChIP databases especially (ENCODE) (Figure 5.7). All antibodies and primers are detailed in (Tables 2.1 and 2.5).

ChIP was performed on both Nalm6^{WT} and Nalm6^{BKO4} and validated by showing high positive-to-negative ratio at *GAPDH* locus as well as a reasonable ratio at CBR1-C1 locus (which coresponding to the promoter region) as suggested by (ENCODE ChIP database) (Figure 5.10).

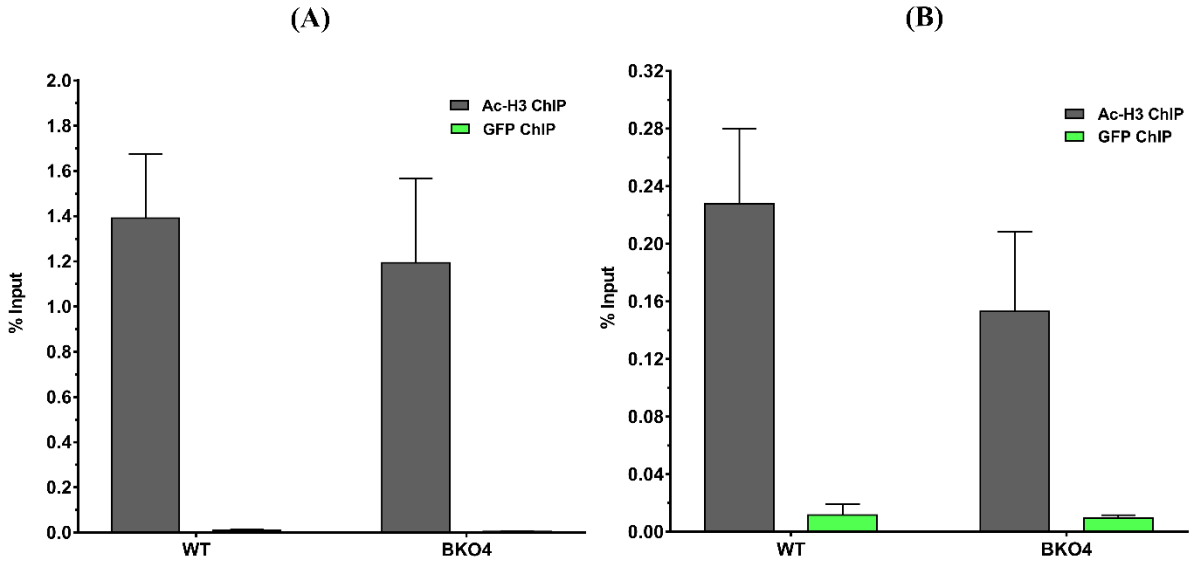


Figure 5-10 ChIP signal of negative and positive controls at two genomic loci of Nalm6 cells.

ChIP signal at **(A)** GAPDH and **(B)** CBR1-C1 primer loci. Data presented here is the mean of at least three independent experiments with error bars representing the SEM.

TOP2β signal at the four genomic loci was tested in Nalm6^{WT} and Nalm6^{BKO4} as presented in (Figure 5.11). Notably, and as expected TOP2β ChIP signal was much lower at all sites tested in the *TOP2B* knockout clone (Nalm6^{BKO4}) demonstrating another validation of our ChIP and the specificity of TOP2B antibodies used in this experiment. Results showed comparable TOP2β ChIP signal at each of the four primer locations tested in (Nalm6^{WT}) and was not concentrated specifically at the promoter site (CBR1-C1). This might suggest that TOP2β is not recruited at a specific site around the *CBR1* gene or the primers tested were not close enough to the TOP2β binding site. Another possibility that TOP2β might indirectly regulate *CBR1* expression through its topology-related activity via generating DNA double-stranded breaks (DSBs) which modulates chromatin architecture and hence either change promoter-enhancer proximity or the recruitment level of transcription factors required for transcription activation.

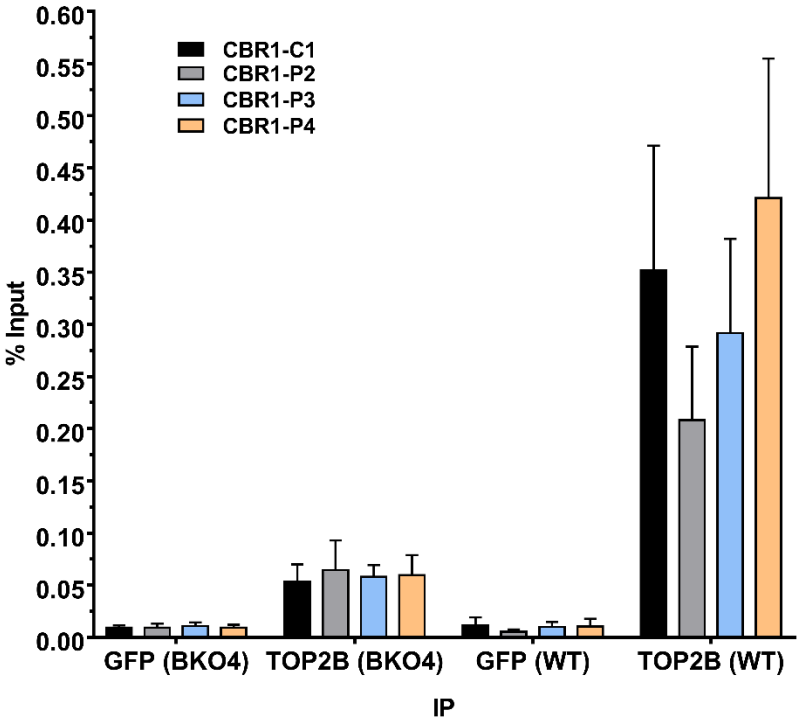


Figure 5-11 ChIP signal of TOP2β at different regions of *CBR1* gene in Nalm6 cells.

ChIP signal of TOP2B at four different regions of theCBR1 gene in Nalm6^{WT} and Nalm6^{BKO4} cells. Anti-GFP was used as a negative control. Data presented here is the mean of at least three independent experiments with error bars representing the SEM.

The regulation of *CBR1* expression by TOP2β might be indirect through regulation of the level or recruitment of other transcription factors required to induce or activate *CBR1* gene expression. To test this, four different transcription factors (USF1, USF2, MAX, and C-Myc) were tested for both ChIP signal at CBR1-C1 primer locus (Figure 5.7) and basal mRNA level in the presence or absence of TOP2β (Nalm6^{WT} compared to Nalm6^{BKO4}). ChIP signal was determined for USF1 and USF2 transcription factors at two regions of the *CBR1* gene landscape: promoter region (CBR1-C1) and a region where no signal was expected (from ENCODE DATABASE), the negative genomic control (CBR1-P4) as presented in (Figure 5.12).

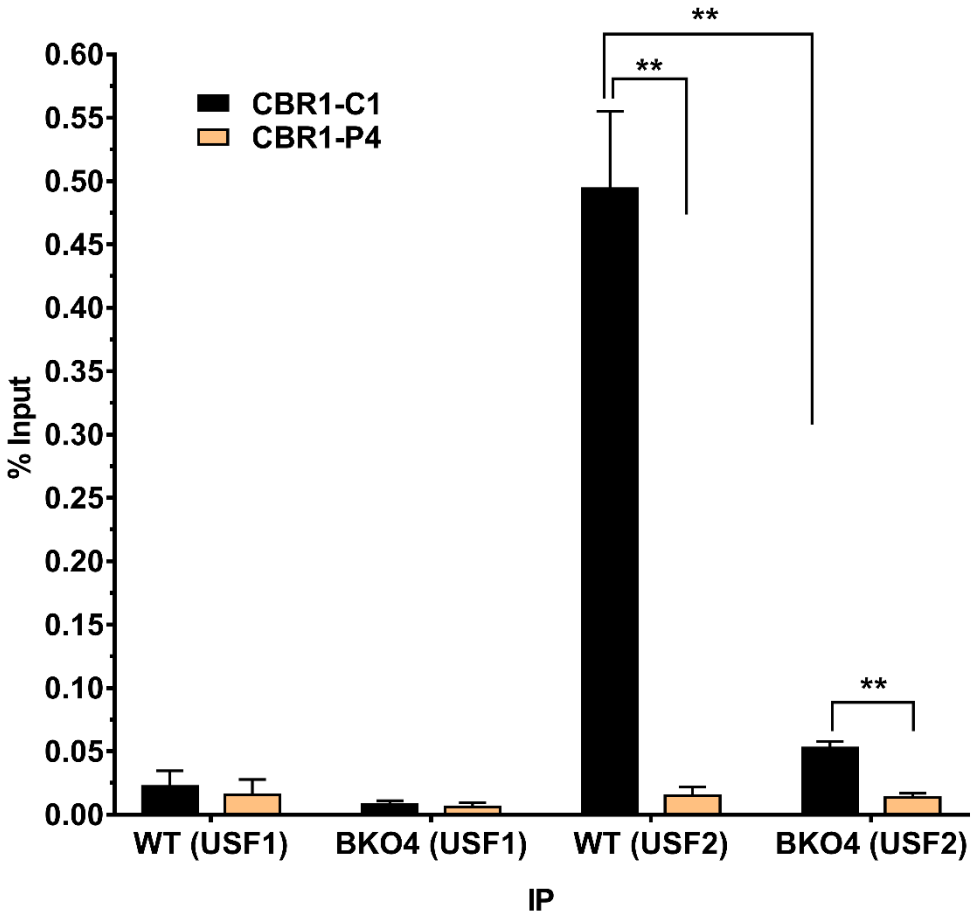


Figure 5-12 USF1 and USF2 ChIP signal on two regions of the *CBR1* gene in Nalm6 cells.

USF1 and USF2 ChIP signal at two regions of *CBR1* gene in Nalm6^{WT} and Nalm6^{BKO4} cells was tested using two primers: CBR1-C1 (promoter) and CBR1-P4 (amplify a negative genomic control where no signal is expected). Data presented here is the mean of at least three independent experiments with error bars representing the SEM, (**P≤0.01).

Overall, USF2 signal (Figure 5.12) was higher than USF1. USF2 ChIP signal at *CBR1* promoter (CBR1-C1 primer) was significantly higher than the control region (CBR1-P4) in both Nalm6^{WT} and Nalm6^{BKO4} cells, consistent with ENCODE USF2 ChIP data (presented in Figure 5.7) which added further validation to our ChIP results. Interestingly, USF2 signal was significantly (10X) reduced at the promoter region (CBR1-C1 primer) in the knockout cells (Nalm6^{BKO4}) compared to wild type (Nam6^{WT}) (Figure 5.12) without changing *USF2* basal mRNA level (Figure 5.13). This suggests a molecular mechanism where TOP2β mediates recruitment of USF2 transcription factor to *CBR1* promoter thereby regulates transcription level.

USF1, on the other hand, did not show a significant difference between Nalm6^{WT} and Nalm6^{BKO4} cells in ChIP signal (Figure 5.12) or the mRNA level (Figure 5.13).

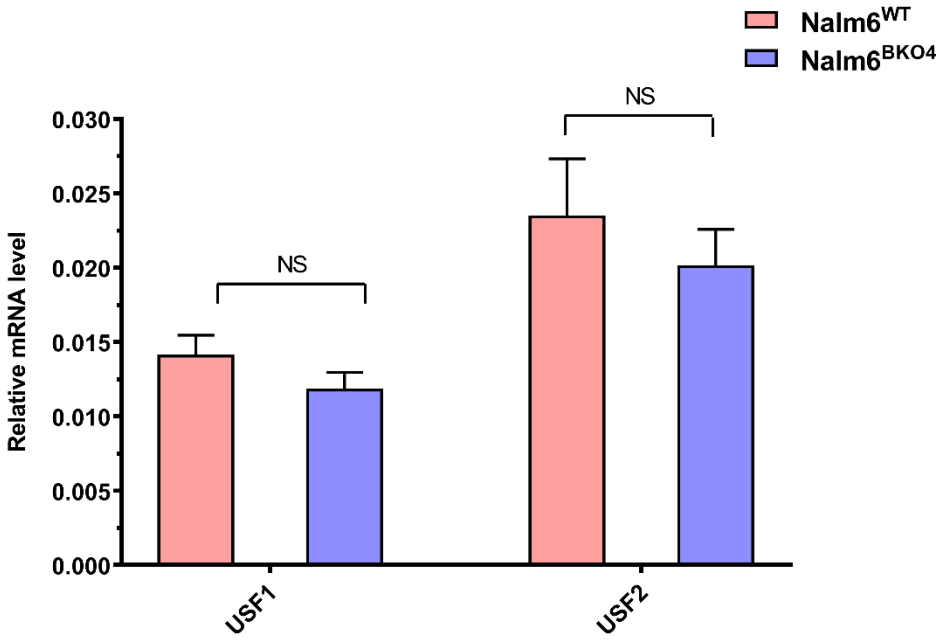


Figure 5-13 RT-qPCR analysis of *USF1* and *USF2* mRNA level in Nalm6 cells

Relative gene expression was determined by RT-qPCR analysis as described in (Materials and Methods, Section 2.14.3. The *PP1A* gene was used as a reference gene for normalizing data. Data presented here is the mean of at least three independent experiments with error bars representing the SEM, (NS: No significance).

In addition, both C-Myc and MAX transcription factors were tested for ChIP signal at the same two regions of the *CBR1* gene (CBR1-C1 and CBR1-P4). As for USF2, *TOP2B* knockout cells ($Nalm6^{BKO4}$) showed a significant reduction in ChIP signal of both C-Myc (1X) and MAX (3.5X) at the CBR1 promoter (CBR1-C1 primer) with more effect on MAX than C-Myc as shown in (Figure 5.14) with no significant change in mRNA level of both (Figure 5.15).

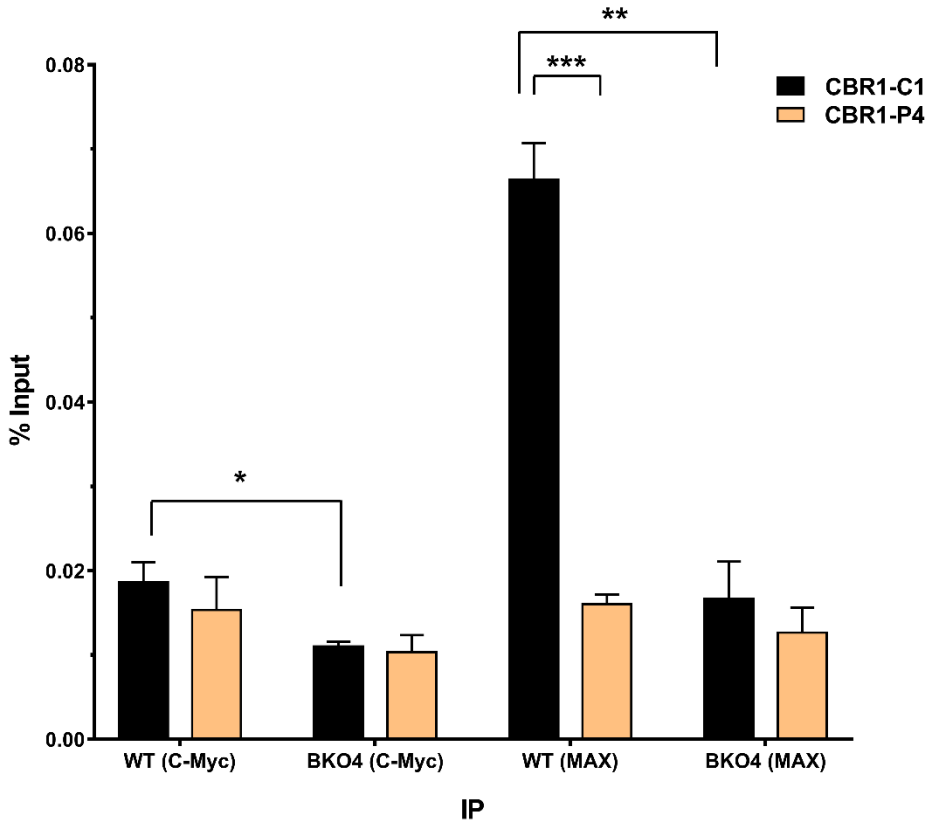


Figure 5-14 C-Myc and MAX ChIP signal at two regions of *CBR1* gene in Nalm6 cells.

ChIP signal of C-Myc and MAX at two regions of *CBR1* gene in $Nalm6^{WT}$ and $Nalm6^{BKO4}$ cells. CBR1-P4 primer was used as a negative genomic control where no signal expected. Data presented here is the mean of at least three independent experiments with error bars representing the SEM. (*P≤0.05, **P≤0.01, NS: No significance).

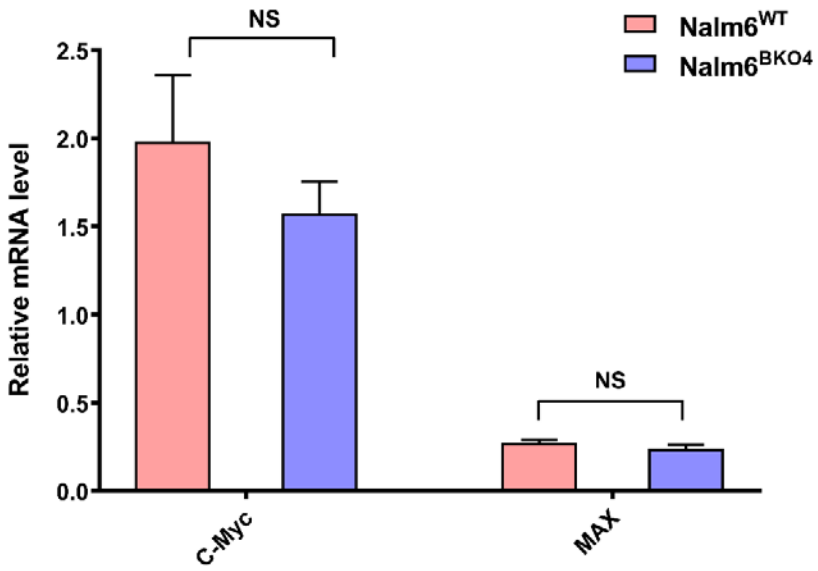


Figure 5-15 RT-qPCR analysis of *C-Myc* and *MAX* mRNA level in Nalm6 cells

Relative gene expression was determined by RT-qPCR analysis as described in (Materials and Methods, Section 2.14.3). *PP1A* gene was used as a reference gene for normalizing data. Data presented here is the mean of at least three independent experiments with error bars representing the SEM, (NS: No significance).

These results showed that *TOP2B* knockout significantly reduced the ChIP signal of C-Myc, MAX, and USF2 at *CBR1* promoter without affecting their mRNA level. This suggests that TOP2β regulates *CBR1* expression by a molecular mechanism where TOP2β through its topology-related activity creates a suitable chromatin environment (see discussion) for recruitment of transcription factors (mainly USF2 and MAX and, to less extent, C-Myc) to the *CBR1* promoter which leads to the regulation of transcription of the *CBR1* gene.

5.3.4 Effect of CBR1 inhibition on doxorubicin toxicity

Results above showed that TOP2β is required for the induction of *CBR1* expression possibly by enhancing the recruitment of USF2, MAX, and C-Myc transcription factors to the promoter. As mentioned in the introduction, in vivo studies showed that doxorubicinol is more cardiotoxic than doxorubicin while in vitro studies on tumour cell lines showed significantly less anti-tumour activity of doxorubicinol than doxorubicin. In theory, CBR1 inhibition should reduce doxorubicinol production and result in less cytotoxicity and more cardiotoxicity. Unfortunately, we could not test the latter effect as the generation of *TOP2B* knockout cardiomyocyte clones is beyond the scope of this study. However, the Nalm6 cell line was used to test the effect of CBR1 inhibition on doxorubicin cytotoxicity.

It is well established that TOP2β downregulation reduces doxorubicin cytotoxicity (Lee *et al.*, 2016). Firstly, doxorubicin cytotoxicity profile was confirmed in knockout clone (Nalm6^{BKO4}) as presented in (Figure 5.16).

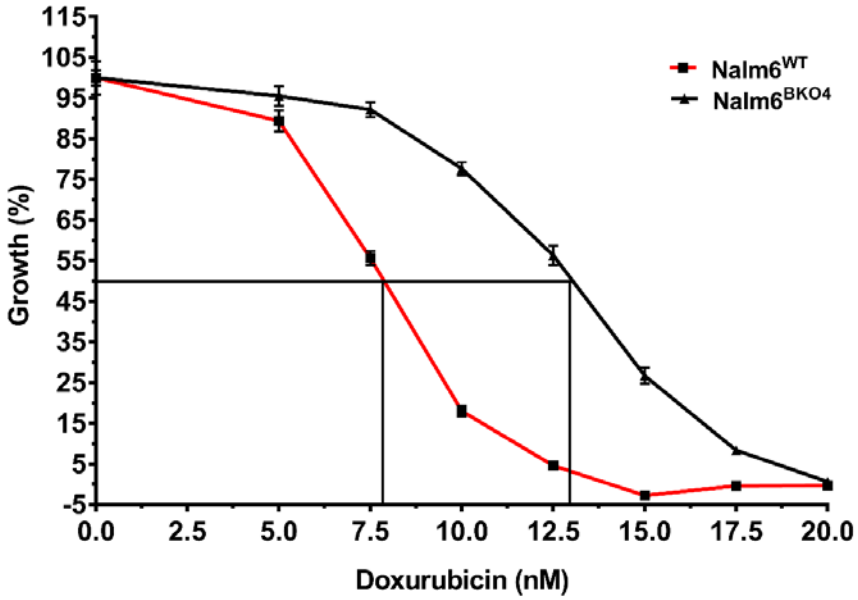


Figure 5-16 Growth inhibition of Nalm6 by doxorubicin.

Nalm6^{WT} and Nalm6^{BKO4} cells were treated with range of doxorubicin doses and growth inhibition was measured using the XTT method as described in (Materials and Methods, Section 2.8). Data presented here is the mean of at least three independent experiments with error bars representing the SEM.

The fact that TOP2 itself is a target of doxorubicin is reflected in the substantial reduction in doxorubicin cytotoxicity in Nalm6^{BKO4} (Figure 5.16), and this is likely to mask other secondary effects of TOP2β downregulation such as CBR1 inhibition. Therefore, a robust method of inhibition of CBR1 enzyme activity either chemically or by gene downregulation is required to test the effect of CBR1 on doxorubicin cytotoxicity.

In the meantime, no chemical agent that acts specifically against CBR1 is available in the literature even though a number of agents have been proposed (Abou El Hassan *et al.*, 2003; Tanaka *et al.*, 2005; Willems *et al.*, 2006; Bruynzeel *et al.*, 2007; Carlquist *et al.*, 2008; Gonzalez-Covarrubias *et al.*, 2008; Arai *et al.*, 2015; Zemanova *et al.*, 2015). Rutin, a flavonoid that has been shown to inhibit CBR1 enzyme activity in vitro (Gonzalez-Covarrubias *et al.*, 2007) was used in this study.

Results of doxorubicin treatment with or without rutin showed that rutin significantly reduced doxorubicin cytotoxicity in both Nalm6^{WT} and Nalm6^{BKO4} (Figure 5.17). This protection effect of rutin suggests either that rutin inhibits CBR1 enzyme and hence doxorubicinol formation suggesting that doxorubicinol is more cytotoxic to Nalm6 than doxorubicin or the protection effect of rutin was due to another mechanism that does not involve CBR1.

The latter would be more convincing for reasons. First, as mentioned before, no chemical agent that acts specifically against CBR1 enzyme is available and the obvious reduction in growth inhibition shown in (Figure 5.17) might reflect a protection mechanism that does not involve CBR1. Second, most published data shows that doxorubicinol has much lower anti-tumour effect than doxorubicin. Third, *CBR1* inhibition by siRNA slightly increased cytotoxicity of doxorubicin as compared with control siRNA, even the difference was insignificant, suggesting that doxorubicin is more cytotoxic than doxorubicinol (Figure 5.18 A and B).

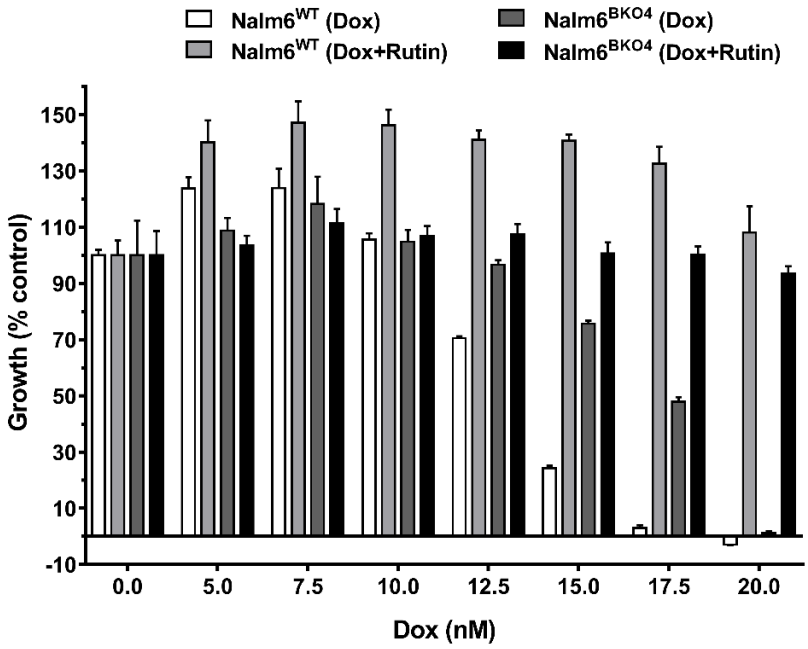


Figure 5-17 Growth inhibition of Nalm6 by doxorubicin +/- Rutin.

Nalm6^{WT} cells were treated with a range of doxorubicin doses with and without 100 µM Rutin. Growth inhibition was measured using XTT method as described in (Materials and Methods, Section 2.8). Data presented here is the mean of at least three independent experiments with error bars representing the SEM.

Currently, the best available option to inhibit CBR1 activity is to either knockout the gene or downregulate by siRNA knockdown. CBR1 downregulation was performed using anit-CBR1 siRNA (Table 2.4) followed by doxorubicin treatment. Nalm6^{WT} Cells were transfected with a control or CBR1 siRNA for 48 hours. CBR1 downregulation was determine by RT-qPCR (Figure 5.18 A). Cells were then treated with doxorubicin for 5 days and growth inhibition was measured by XTT method (Figure 5.18 B).

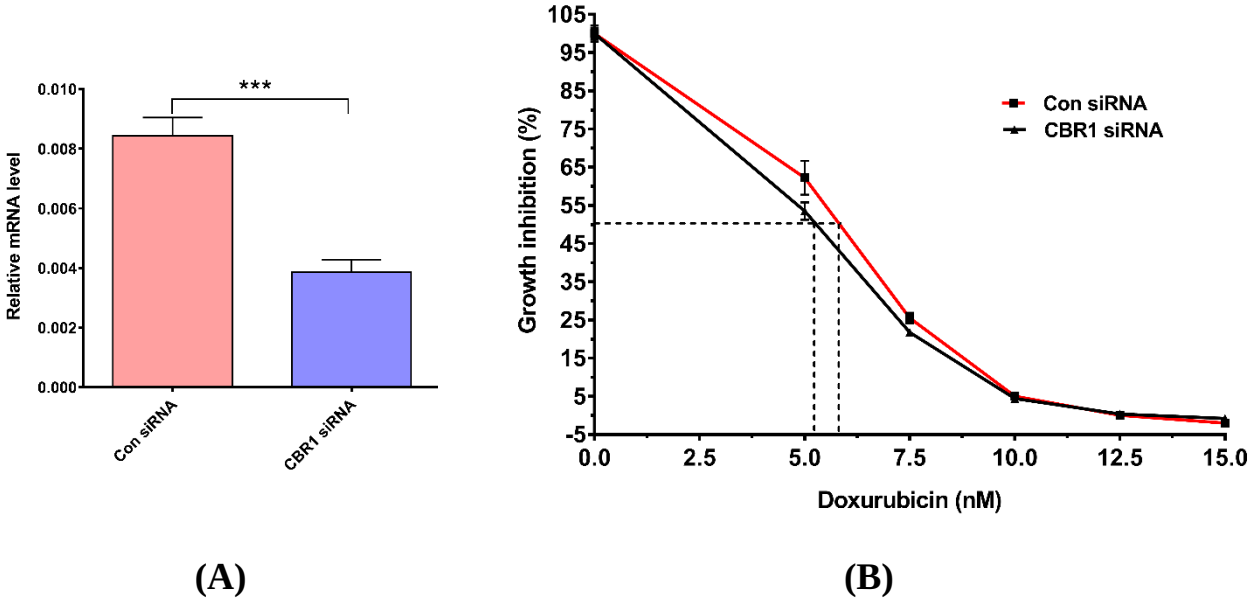


Figure 5-18 Effect of *CBR1* siRNA on doxorubicin cytotoxicity

Nalm6^{WT} cells were transfected with non-targeting or *CBR1* siRNA for 48 hrs then treated with a range of doxorubicin doses for 5 days **(A)** *CBR1* level determined by RT-qPCR **(B)** Growth curve was measured by XTT method as described in (Materials and Methods, Section 2.8). Data presented here is the mean of at least three independent experiments with error bars representing the SEM.

CBR1 mRNA level was reduced by approximately 50 % after 48 hrs of siRNA transfection. This might result in slight reduction in doxorubicin cytotoxicity which is more consistent with CBR1 role in doxorubicin activity on cancer cells. However, further optimization is needed for this experiment as CBR1 was downregulated for a short time. In summary, a robust and specific method for inhibition of CBR1 is required to further extend the observations above and this beyond the scope of this study.

5.4 Discussion

In this chapter, CRISPR was used to generate a *TOP2B* knockout model in the Nalm6 cell line. The transfection efficiency was determined using both live cell imaging and FACS analysis as in (Figure 5.2). Efficiency of transfection with the positive control PmaxGFP vector was 10.5 fold higher than the transfection efficiency with the PX458 vector. This might be due to two reasons; firstly, PX458 is ~ 3 times larger than PmaxGFP which might reduce cellular uptake. Secondly, the two vectors use different promoters (PmaxGFP uses a CMV promoter while PX458 uses a U6 promoter).

The advantage of using a GFP selective marker in a vector based CRISPR method is that selection for positively transfected cells is very efficient even with low transfection efficiency. In addition, single cell cloning can be performed using the cell sorter to efficiently allocate single cells in each collecting well (96-well plates were used). Single cell cloning by cell sorter was also verified by microscope before clone expansion. Out of total clones that were tested, screening of 4 clones are presented in the results section. Genotyping analysis at the targeted region of exon 1 of the *TOP2B* gene confirmed a mutation in 3 of 4 clones screened. Two clones have an insertion and one has a deletion (Table 5.1). All clones were confirmed for abrogation of TOP2β protein expression except clone 14 which was positive for TOP2B although it has 3 bases insertion mutation which might add one amino acid without disrupting protein expression. Clone number 4 (Nalm6^{BKO4}) was used for experiments in this chapter.

RT-qPCR analysis of genes which were either down or up regulated in Nalm6$^{B-/-}$ in the previous chapter showed insignificant change in all of the genes tested except for CBR1 (Figures 5.6). In agreement with the previous RNA-seq and RT-qPCR results, the CBR1 gene showed a 68 fold reduction in Nalm6^{BKO4} which indicates that TOP2β positively regulates CBR1 gene expression. The consistency in the downregulation of *CBR1* expression upon TOP2β inactivation in different independent analysis and knockout models of Nalm6 cells confirms that *CBR1* is regulated by TOP2β in this cell line. Whether *CBR1* is directly or indirectly regulated by TOP2β, so far there is no published data about the role of TOP2β in the expression of this gene.

A publically available RNA-seq data set might support our finding of a role of TOP2β on *CBR1* gene expression. In this data (GEO accession: GSE106297), a human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) showed downregulation of *CBR1* upon treatment of doxorubicin (Maillet *et al.*, 2016). In this data, downregulation of *CBR1* might be due to TOP2β downregulation

by doxorubicin which has been shown to downregulate TOP2β in human primary cardiomyocytes (Jiang *et al.*, 2018). This RNA-seq data was analysed by Dr. Simon Cockell (Bioinformatics Support Unit, Newcastle University) for differential gene expression analysis of *CBR1* gene. *CBR1* gene was downregulated by 2.48 (padj 0.0002931585) and 2.64 (padj 0.0002036932) fold at 1 and 2.5 μM doxorubicin respectively against control. This might be a secondary effect of TOP2β downregulation by doxorubicin, as the same data showed TOP2β downregulation by 2.24 (padj 9.448 E-08) and 2.29 (6.82292 E-08) fold at 1 and 2.5 μM doxorubicin respectively against control.

Many studies have suggested that TOP2β regulates targeted genes by binding to or near their promoter regions (Lyu *et al.*, 2006; McNamara *et al.*, 2008; Tiwari *et al.*, 2012; Madabhushi *et al.*, 2015b; Manville *et al.*, 2015; Uusküla-Reimand *et al.*, 2016; Canela *et al.*, 2017; Feng *et al.*, 2017a). Based on this, it has been proposed that TOP2β might bind a region at the *CBR1* gene. We designed four different primers at *CBR1* genomic locus (Figure 5.7). ChIP-qPCR results showed that TOP2β binds at the four regions tested with no specific preference amongst them (Figure 5.11). This assumption is aided by the fact that *CBR1* is a small gene (3,254 bases).

On the other hand, the recruitment of four transcription factors at the promoter region of *CBR1* was tested in both Nalm6^{WT} and Nalm6^{BKO4}. Results showed a significant decrease in ChIP signal for USF2 (10 fold), C-Myc (1 fold), and MAX (3.5 fold) transcription factors in the *TOP2B* knockout clone with USF2 most markedly reduced (Figures 5.12, 5.14). Next, we asked the question of whether this reduction in ChIP signal of these transcription factors in Nalm6^{BKO4} was due to lower expression or reduced recruitment by a TOP2β-mediated molecular mechanism. For this purpose, mRNA levels of *USF1*, *USF2*, C-*Myc*, and *MAX* transcription factors was determined by RT-qPCR in both Nalm6^{WT} and Nalm6^{BKO4}. RT-qPCR results indicated that *TOP2B* knockout did not affect the expression of these transcription factors (Figures 5.13 and 5.15). This suggests that TOP2β is required for *CBR1* transcriptional regulation by mediating the recruitment of transcription factors into the promoter which in turn activate gene expression.

Different mechanisms have been suggested to elucidate how TOP2β regulates the expression of genes that it acts on. One possible mechanism could be that TOP2β supports transcriptional elongation through its topological-related activity to alleviate transcription-induced supercoiling that suppress progression of transcriptional machinery. In support of this mechanism, it has been shown that TOP2 is more required for active promoters (Kouzine *et al.*, 2013; Naughton *et al.*, 2013; Bunch *et al.*, 2015). It has been shown that the catalytic activity of TOP2β in particular is

required for rendering chromatin architecture more permissive for initiation of transcription (LeRoy *et al.*, 2000; Racki *et al.*, 2009). These two mechanisms are more relevant for developmentally regulated genes.

Another mechanism is suggested for genes induced by stimuli such as ligands. This mechanism involved TOP2β-mediated DNA double stranded break (DSB) at the promoter of stimulus-induced genes leading to generate a chromatin environment that is preferable for initiation of transcription. Studies that suggest this mechanism involved the requirement of TOP2β to the promoter upon stimulation by ligands such as estradiol (Ju *et al.*, 2006), androgen (Haffner *et al.*, 2010), retinoic acid (McNamara *et al.*, 2008), glucocorticoid (Trotter *et al.*, 2015), or insulin (Wong *et al.*, 2009). However, in our study, results showed differential gene expression without induction with ligand. In addition, our model (Nalm6) did not show a response to either ATRA (Appendix figure 16 and 17) or vitamin D3 (Appendix figure 15).

Exploring the publically available ChIP data over and around *CBR1* locus showed that *CBR1* gene is flanked by two CCCTC-binding factor (CTCF) sites which are also corresponding to etoposide-induced END-seq peaks and that presumably correspond to CTCF/TOP2β binding sites (Figure 5.19). It has been shown that TOP2β interacts with CTCF at topological domains and has a role in controlling supercoiling within chromatin loops by generating DNA double-stranded breaks at these sites (Uusküla-Reimand *et al.*, 2016; Canela *et al.*, 2017). Therefore, it is possible that *CBR1* gene resides in a chromatin loop bounded by these two CTCF/TOP2β sites and thus in the absence of TOP2β, lack of supercoiling control could lead to altered expression of genes such as *CBR1* within the loop.

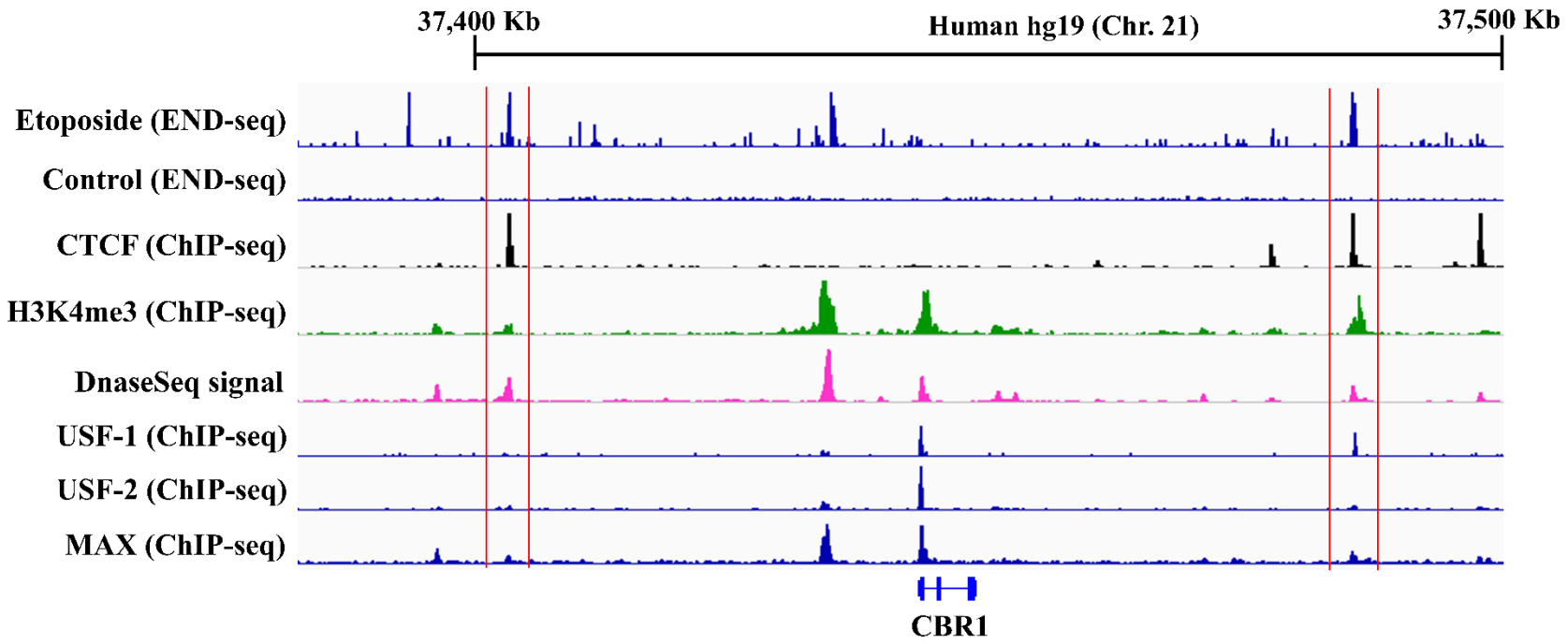


Figure 5-19 CBR1 gene is flanked by CTCF binding sites

IGV snapshot of human CBR1 gene landscape shows from top to bottom: END-seq DSBs profiles of Nalm6 cells treated with or without etoposide. CTCF ChIP signals in non-treated Nalm6. H3K4me3 ChIP signal in GM12878 cell line from ENCODE database. DnaseSeq signal in GM12878 cell line from ENCODE database. USF1, USF2, and MAX ChIP signal in GM12878 cell line from ENCODE database.

Studies have shown the role of each of TOP2β and CBR1 in doxorubicin-induced cardiotoxicity independently of each other. On one hand, *TOP2B* deletion from mouse cardiomyocyte protects from doxorubicin-induced cardiotoxicity (Zhang *et al.*, 2012). The fact that TOP2 catalytic inhibitor (ICRF-187) is clinically used as a cardioprotectant supports this role of TOP2β in cardiotoxicity (Hensley *et al.*, 2009). On the other hand, lines of evidence have shown the role of CBR1 in doxorubicin-induced cardiotoxicity. Most importantly, human *CBR1* overexpression in mouse heart increases cardiotoxicity induced by doxorubicin (Forrest *et al.*, 2000), and *Cbr1* downregulation protects mouse from doxorubicin-induced cardiotoxicity (Olson *et al.*, 2003). So far, no data links these two independent factors in cardiotoxicity induced by doxorubicin. Here, we showed a molecular mechanism whereby the cardioprotection effect seen upon TOP2β inhibition might be due to decreased CBR1 enzyme activity and hence decrease in doxorubicin reduction into the cardiotoxic metabolite doxorubicinol (Figure 5.20).

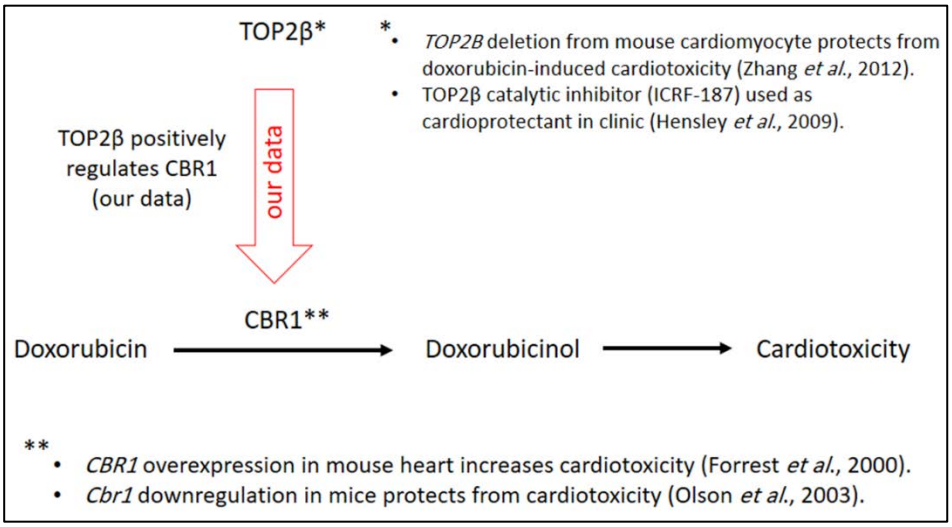


Figure 5-20 Our data links two factors implicated in doxorubicin-induced cardiotoxicity.

Unfortunately, I could not test the effect of *TOP2B* knockout on *CBR1* expression in cardiomyocytes and this would be one of the most interesting plans of future work. These results have a clinical implication as it suggests that the factors implicated in the cardiotoxicity are interconnected, highlighting a question of whether the priority would be to inhibit TOP2β (by catalytic inhibitors such as ICRF-187 or other agents) or to inhibit the CBR1 enzyme in order to achieve an effective doxorubicin antitumor activity with the least cardiotoxicity. Consideration would take into account that TOP2β itself is a target for doxorubicin for antitumour activity, while CBR1 enzyme is involved in the production of doxorubicinol, which has less antitumour but higher cardiotoxic effect than the parent compound as reviewed in the introduction of this chapter.

6 Chapter Six: TOP2β role in gene expression and ATRA-induced differentiation of SH-SY5Y cells.

6.1 Introduction

As described in chapter four introduction, TOP2β has an important role in transcription and regulation of genes in various systems with particular importance in neuronal development. The earliest evidence of TOP2β's role in neuronal development was shown by (Giovanni Capranico and Zunino, 1992). This study showed an interesting shifting in expression pattern of Top2 isoforms over development of normal murine tissue where Top2b was specifically induced in brain but not in liver at the post-natal stage. Similar findings were shown in a rat study (Tsutsui *et al.*, 1993).

This was followed by studying Top2 isoform distribution in rat brain during development (Watanabe *et al.*, 1994). This study showed that Top2a was expressed in the ventricular region at the ante-natal stage, and exclusively expressed at the mitotic regions at the first two weeks of post-natal stage. Top2a expression then rapidly decreased and completely disappeared by 21 days post-natal. On the other hand, Top2b was shown to be expressed ubiquitously in the brain at all stages (Watanabe *et al.*, 1994). Subsequently, closely related findings were shown in studies with human tissues (Zandvliet *et al.*, 1996; Turley *et al.*, 1997).

The essential role of Top2b in neuronal development was determined for the first time using Top2b murine knockout model reported by (Yang, 2000). Since then, various studies focusing on role of TOP2β in neuronal development have been conducted (Tsutsui *et al.*, 2001b; Lyu and Wang, 2003; Lyu *et al.*, 2006; Nur *et al.*, 2007; Sano *et al.*, 2009; Nevin *et al.*, 2011; Tiwari *et al.*, 2012; Li *et al.*, 2014; Madabhushi *et al.*, 2015b) as reviewed in chapter four introduction.

TOP2β has been shown to be necessary for neurite outgrowth in rat cerebellar and cortical neurons in culture, differentiating PC12 cells by neurotrophic growth factor (NGF), embryonic murine cortical neurons (Nur *et al.*, 2007), mouse dopaminergic neurons, ventral mesencephalic mouse primary culture (Heng *et al.*, 2012), and human mesenchymal stem cell-derived neurons (Isik *et al.*, 2015; Zaim and Isik, 2018).

In support of TOP2β's role in neuronal development, a *de novo TOP2B* mutation was shown to be linked to global developmental delay, intellectual disability, and autism disorder in a case study (Lam *et al.*, 2017). Indeed, a study showed that TOP2β regulates expression of long genes associated with autism in human and mouse neurons (King *et al.*, 2013). In addition to the role in neurogenesis, TOP2β has been shown to play a role in maintaining genome stability of neurons and its level of expression is reported to reduce with age in rat (Kondapi *et al.*, 2004; Mandraju *et al.*, 2011; Gupta *et al.*, 2012; Bollimpelli *et al.*, 2017)

In the context of transcription, various studies revealed TOP2β's role in the expression of genes involved in neuronal development and maintenance. For instance, Top2b inhibition in cultured rat neurons reduced expression of amphiphysin I, an essential gene for neuronal function (Tsutsui *et al.*, 2001b). Top2b deletion reduced reelin expression in mouse embryos (Lyu and Wang, 2003). Microarray analysis of mouse brains lacking Top2b revealed regulation of genes involved in neuronal activities such as axon guidance and synaptic transmission (Lyu *et al.*, 2006).

Top2b was shown to bind and regulate neuronal genes in mouse embryonic stem cell-derived postmitotic neurons (Tiwari *et al.*, 2012). Top2b deletion led to degeneration of postmitotic neurons and was accompanied by downregulation of genes highly enriched for GO terms associated with neurogenesis. Contrasting transcriptionally regulated genes in Top2b knockout neurons with Top2b chromatin occupation sites measured by ChIP followed by array highlighted 99 genes. The downregulated genes within this gene set were enriched for neuronal GO terms such as nervous system development, generation of neurons, and transmission of nerve impulse. Moreover, neuronal degeneration due to lack of Top2b was caused by up regulation of *Ngfr* p75 which was shown to be a direct target for Top2b through binding to its promoter and negatively regulating its transcription (Tiwari *et al.*, 2012). Another study showed that Top2b participates with chromatin remodeler CHD7 to regulate a set of long genes involved in neuronal function in mouse cerebellar granule neurons and Top2b inhibition led to downregulation of 203 of these genes (Feng *et al.*, 2017a).

Moreover, another study showed Top2b has a role in motor neurons (MNs) connectivity and migration in mice where terminal branching of MNs was remarkably defective in Top2b knockout mice. This was probably due to improper Hox/Pbx-dependent transcriptional activation because of downregulation of both Pbx and Hox genes upon Top2b knockout (Edmond *et al.*, 2017)

Another aspect of TOP2β's role in regulation of gene expression is mediation of transcription activation by responses such as heat shock, serum induction (Bunch *et al.*, 2015), neuronal activity induced by external stimuli (Madabhushi *et al.*, 2015b), and ligands via nuclear receptors (Ju *et al.*, 2006; Haffner *et al.*, 2010). Various studies showed that activation of expression of genes by ligands is associated with TOP2β-mediated DNA double-stranded breaks (DSBs) at the promoter of target genes as reviewed in chapter four introduction. Of those studies, (Ju *et al.*, 2006) showed that retinoic acid (RA)-induced transcription activation of *RARB* gene in MCF-7 cells involved TOP2β activity. Indeed, another study showed TOP2β recruitment and binding to the promoter of this gene in NB4 cells upon RA treatment (McNamara *et al.*, 2008). This suggests that TOP2β might be involved in transcriptional activities induced by RA in another cells as well.

SH-SY5Y is a neuroblastoma cell line that can be induced by RA to differentiate into neuronal-like cells (Brown *et al.*, 2005; Riddoch *et al.*, 2007; Bell *et al.*, 2013), making these cells a good candidate to study the role of TOP2β in both RA-induced transcription and differentiation of neuronal cells. Other advantages of using SH-SY5Y cells are their relative ease to culture in comparison with primary neurons, their human origin enables study of human specific proteins with no ethical issues unlike use of primary neurons (Kovalevich and Langford, 2013)

The SH-SY5Y cell line is a heterogeneous cell population, a characteristic feature of neuroblastoma cells (Biedler *et al.*, 1973; Biedler *et al.*, 1978; Ross *et al.*, 1983). SH-SY5Y cells involve three subpopulations: neuroblastic cells (N-type), substrate-adherent (S-type), and intermediate (I-type) cells (Ross *et al.*, 1983; Walton *et al.*, 2004). Although these subpopulations show different biochemical and phenotypic characteristics, they are not genetically distinct, as one subpopulation can be switch into another (Ciccarone *et al.*, 1989; Ross and Spengler, 2007), suggesting that these differences could be due to epigenetic reasons.

N-type cells are characterized by small rounded body shape with limited cytoplasm and short neuritic processes and grow as weakly attached cell aggregates. These cells represent the majority of SH-SY5Y population and bear neuronal biochemical properties such as neurotransmission enzyme activity (Biedler *et al.*, 1978; Ciccarone *et al.*, 1989; Walton *et al.*, 2004).

S-type cells are characterized by larger body size, expansive cytoplasm, flattened appearance, stronger attachment properties, and epithelial-like morphology (Ross *et al.*, 1983; Walton *et al.*,

2004). These cells do not behave as neuronal cells, rather they show features of Schwann, glial, and melanocytic cells (Ciccarone *et al.*, 1989; Ross *et al.*, 1995).

I-type cells represent an intermediate state between N- and S-type. These cells show morphological and phenotypical characteristics of both N- and S- type, and have moderate features with respect to cytoplasm level, attachment properties, and neuritic processes (Ross *et al.*, 1995; Walton *et al.*, 2004). I-type cells might either be a progenitor cells that can give rise to either S- or N-type (Ross *et al.*, 1995), or it represents an intermediate stage in the process of trans-differentiation between the N and S phenotypes. N- and S-type cells have the ability to switch into each other directly (Ross *et al.*, 1983; Ross and Spengler, 2007).

SH-SY5Y cells represent a well-defined model for neuronal differentiation in culture. These cells can be induced to differentiate using different agents (Teppola *et al.*, 2016) with retinoic acid (RA) representing the most prevalent agent used in differentiation protocols of SH-SY5Y cells (Kovalevich and Langford, 2013). Upon RA-induced differentiation, SH-SY5Y cells show small rounded cell body with long, straight, and less branched neurite outgrowth (Bell *et al.*, 2013; Teppola *et al.*, 2016)

Retinoic acid (RA) is an oxidation derivative of vitamin A (retinol) that plays a key role in various biological contexts including growth, development, transcriptional regulation, and differentiation (Chambon, 1996; Ross *et al.*, 2000). RA has several isoforms that either occur naturally such as all-trans-retinoic acid (ATRA), 9-cis-retinoic acid (9cRA) or synthetic such as 13-cis-retinoic acid (13cRA) (Redfern *et al.*, 1995; Ross *et al.*, 2000; Reynolds *et al.*, 2003).

Signalling pathways by which retinoic acid (RA) acts in transcriptional regulation starts upon the introduction of RA into the cell where it becomes bound by cellular retinoic acid-binding proteins 1 or 2 (CRABP1 or CRABP2) which renders RA soluble and protected in the aqueous environment of cytoplasm. CRABP1 introduces RA to the 26 subfamily of cytochrome P450 superfamily enzymes (CYP26A1, CYP26B1 and CYP26C1) which control RA level by catalysing oxidative metabolism reactions. On the other hand, CRABP2 transfers RA into the nucleus and delivers it to the retinoid receptors by direct protein-protein interactions (Rhinn and Dollé, 2012).

Within the nucleus, RA binds to two families of retinoid receptor: the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs) which act together as heterodimers. Both families have three different isotypes: α, β and γ, which are encoded by a unique gene and each isotype has

at least two different isoforms (Bastien and Rochette-Egly, 2004; Rhinn and Dollé, 2012). In the absence of RA, these RAR/RXR heterodimeric nuclear receptors bind specific DNA sequences at the RA-sensitive genes called retinoic acid response elements (RAREs) and repress transcriptional activation of target gene by recruiting repressing complexes. Upon RA binding to the RAR/RXR heterodimer receptor, a conformational change occurs leading to dissociation of co-repressors and recruitment of co-activators. These activities result in chromatin decompaction which allow transcription machinery to assemble at gene promoters and hence activate gene transcription. The extent and time of RA response is controlled by degrading of RAR RXR retinoid receptors through an ubiquitin-proteasome-dependent mechanism (Bastien and Rochette-Egly, 2004; Rhinn and Dollé, 2012).

6.2 Aims

As mentioned in the introduction, TOP2β was shown to be required for neuronal development in animal models. The first aim of this chapter was to test the role of TOP2β in a human neuronal cell model. SH-SY5Y cell line was used for this purpose. SH-SY5Y can be induced to neuronal differentiation by ATRA. So far, no data is available about the differentiation and gene expression in a *TOP2B* knockout model of this cell line. On the other hand, TOP2β has been shown to be involved in regulation of RA-targeted genes in two different cell lines (Ju *et al.*, 2006; McNamara *et al.*, 2008). This suggests that TOP2β might be involved in transcriptional activities induced by RA in other cells as well. For these reasons, SH-SY5Y represents a suitable model to test these two aspects of TOP2β roles. To address this, the following experimental objectives were followed:

- Generate a *TOP2B* Knockout model in the human neuroblastoma SH-SY5Y cell line using CRISPR technique.
- Study the effect of the *TOP2B* knockout on ATRA-induced differentiation in SH-SY5Y cells by determining neurite outgrowth formation using phase-contrast microscopy as well as using BCL gene expression as a molecular marker of differentiation.
- Study the effect of knocking out of *TOP2B* on the induction of CYP26A1, RARB, CRABP2, and RET (genes involve in ATRA-induced differentiation pathway).
- Investigate the effect of a *TOP2B* knockout on whole genome expression in SH-SY5Y cells in the presence and absence of ATRA treatment using RNA-seq.
- Perform gene enrichment analysis to highlight the biological functions and molecular pathways affected by *TOP2B* knockout in the presence and absence of ATRA.

6.3 Results

6.3.1 Generating TOP2B knockout clones by CRISPR

TOP2B knockout was performed by CRISPR technology as described in detail in the materials and methods chapter (section 2.15). As no one was using the CRISPR technique in our lab, there was a need to optimize this technique. The generation of knockout clones was not straightforward and different reagents were used through the experimental workflow before optimizing conditions for nuclefection, selection, and screening for knockout clones. The CRISPR timeline is outlined in (Figure 6.1). Once conditions were optimized, differernt *TOP2B* knockout clones of SH-SY5Y were successfully generated and used in this chapter as well as different *TOP2B* knockout clones of both Nalm6 and K562 cells which were used in the previous chapter.

Work was started by genomic editing design which includes: designing guide RNA (gRNA) oligonucleotides, bioinformatical testing of their predicted off-target effects, and designing a primer required for genotyping (TOPKO1 primer) (Figure 6.1, step 1). Transfection efficiency using nucleofection reagents was then tested using PmaxGFP plasmid as a positive control (Figure 6.1, step 2). Then I cloned the gRNA in PX459 vector (see Appendix table 16 for sequence), a plasmid encoding both cas9 system and puromycin resistance as a selection marker (Figure 6.1, steps 3 and 4). Then I established a puromycin kill curve to determine the lowest dose that killed all the cells to use it for selection of transfected cells (Figure 6.1, step 5). Cells were then transfected by nuceofection as described in the materials and methods (section 2.15.3) and treated with puromycin for selection (Figure 6.1, steps 6 and 7). The kill was very high and there was no difference between transfected and non-transfected cells in terms of survival achieved. I decided to test the transfection efficiency by immunoflouresent staining of flag (see PX459 strucure, figure 2.4) using anti-flag M2 antibody (table 2.1) (Figure 6.1, step 8). Immunoflouresence results showed very low transfection efficiency (Appendix figure 19).

Subsequently, I decided to use PX458, a cloning vector that uses GFP expression as a selection marker in order to select and sort cells using FACS which enables sorting of enough cells for cloning even if the transfection efficency was low (Figure 6.1, steps 9-18). Steps 9-18 are explained in details in this chapter in (section 6.3.1).

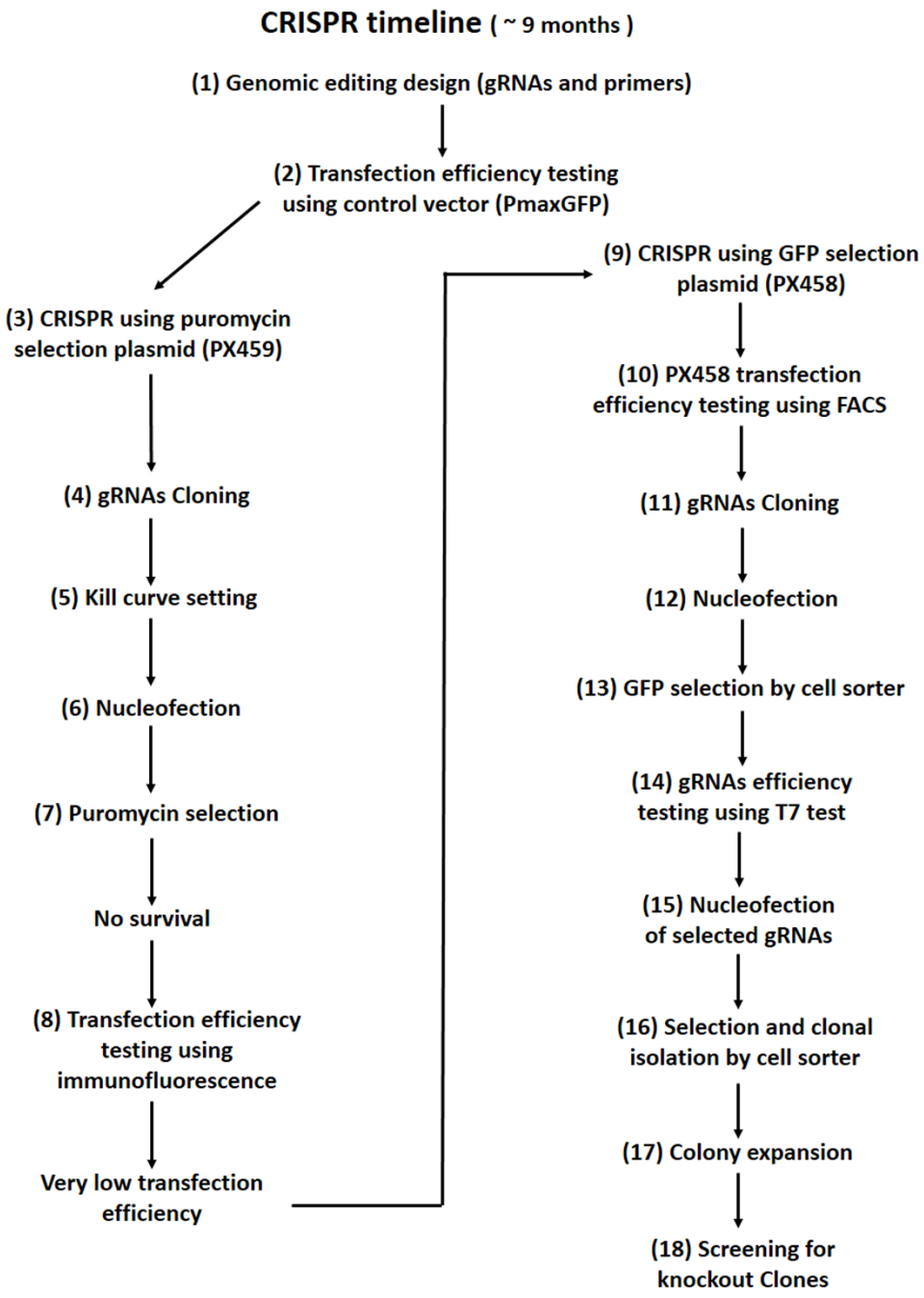


Figure 6-1 CRISPR timeline

This figure shows all successful and unsuccessful CRISPR steps followed in this study. Step **(1)** is described in section (2.15.1), **(2)** is described in section (6.3.1.1). Steps **(3 and 8)** are described in section (2.15.2) and (Appendix figure 19 and Appendix table 16). Step **(8)** is presented in Appendix figure 20. Steps (9-18) are described in section (2.15) and respective results are presented in in this chapter (for SH-SY5Y), chapter five (for Nalm6), and the appendix (for K562).

6.3.1.1 Determining the transfection efficiency in SH-SY5Y cells using nucleofection

The transfection efficiency (%) was determined by transfecting SH-SY5Y cells with a plasmid encoding the enhanced green fluorescent protein eGFP (PmaxGFP) which was provided with the nucleofection kit as a positive control. SH-SY5Y cells were transfected with 2 µg PmaxGFP as suggested by the Lonza optimized protocol for these cells. The ratio of GFP expressing cells was determined by FACS (Figure 6.2). GFP expression was visualised by live cell imaging (Figure 6.3).

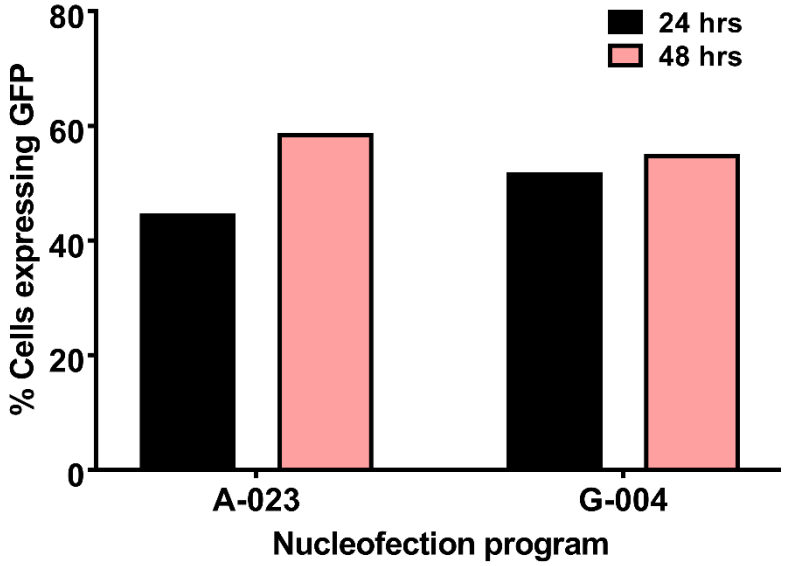


Figure 6-2 Transfection efficiency (%) in SH-SY5Y cells transfected with PmaxGFP plasmid.

SH-SY5Y was transfected with 2 µg PmaxGFP plasmid using Nucleofector II device (Lonza, UK) with two different nucleofection programs and analysed with FACS.

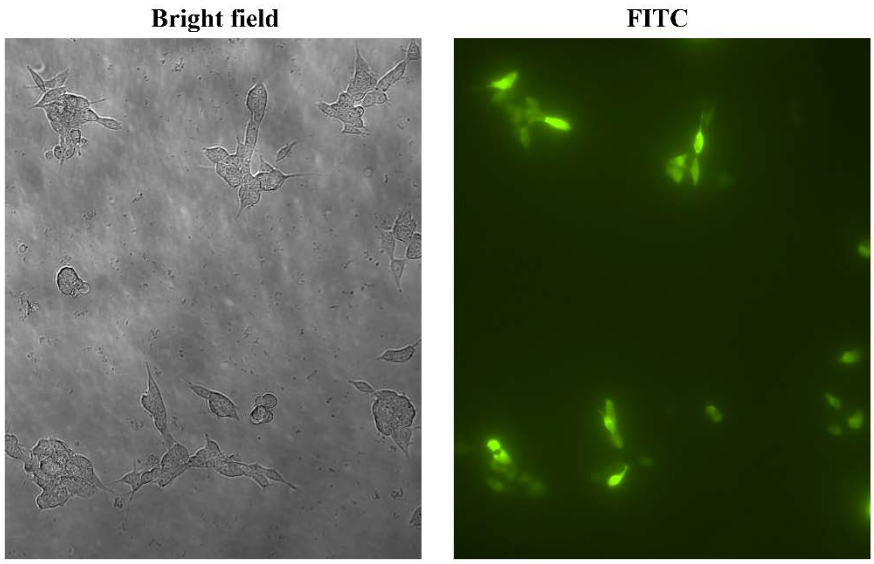


Figure 6-3 Live cell imaging of SH-SY5Y cells transfected with PmaxGFP plasmid.

SH-SY5Y cells were transfected with 2 µg PmaxGFP plasmid for 48 hrs. Images were captured using a TiE fluorescence wide field inverted microscope (Nikon) (20X magnification).

Transfection efficiency (%) with the PX458 vector was then determined by transfecting SH-SY5Y cells with six different doses (2, 4, 6, 8, 10, and 12 µg) for 48 hrs under the same condition. Cells which were transfected with 6 µg showed the best transfection efficiency of ~10% as presented in (Figure 6.4). The 6 µg dose therefore was used in further experiments. GFP expression was visualised by live cell imaging (Figure 6.5). Lower transfection efficiency with PX458 vector might be because PX458 and PmaxGFP vectors have different promoters. PmaxGFP uses the CMV promoter while PX458 uses the U6 promoter. In addition, PX458 size is (~ 9.3 Kb) compared with PmaxGFP which is (~3.5 Kb). Generally, the larger a vector the more difficult to it is to transfect.

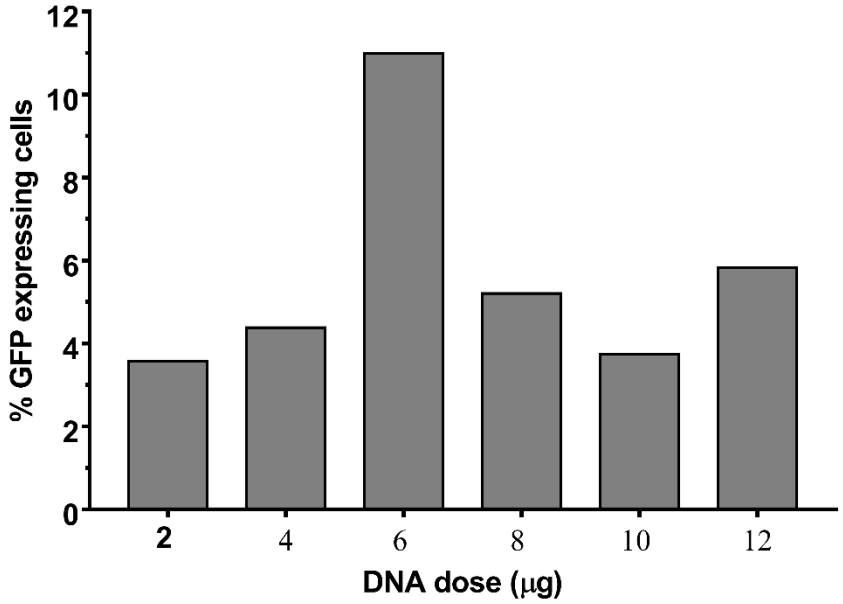


Figure 6-4 Transfection efficiency (%) in SH-SY5Y cells transfected with different doses of PX458 vector.

Transfection efficiency (%) in SH-SY5Y cells transfected with different doses of PX458 vector for 48 hrs.

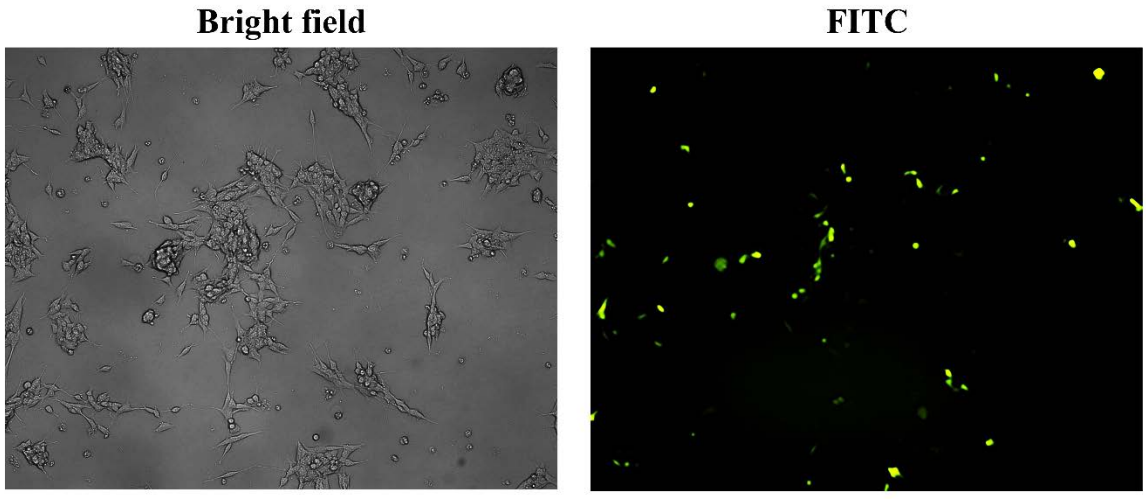


Figure 6-5 Live cell imaging of SH-SY5Y transfected with PX458

SH-SY5Y cells were transfected with 6 μg PX458 plasmid for 48 hrs. Images were captured using a TiE fluorescence wide field inverted microscope (Nikon) (10X magnification).

6.3.1.2 Verifying gRNA cloning with restriction digests and Sanger sequencing

Cloning of gRNA into PX458 vector is described in section (2.15.2). PX458 vector has a pair of BbsI restriction sites. Successful gRNA insertion should destroy the BbsI restriction site. In addition, PX458 has a unique EcoRV restriction site. Therefore, a digestion test using these two restriction enzymes should give a single band for successful insertion and two bands for unsuccessful insertion (Figure 6.6). For successful insertions, samples were sent for Sanger sequencing at (GATC Biotech) according to their sample requirements (see Table 6.1). The forward primer of Human U6 promoter, LKO.1 5' was used. Verified plasmid samples were purified again to provide sufficient quantity by midiprep using Qiagen Plasmid Midi Kit according to the manufacturer's instructions.

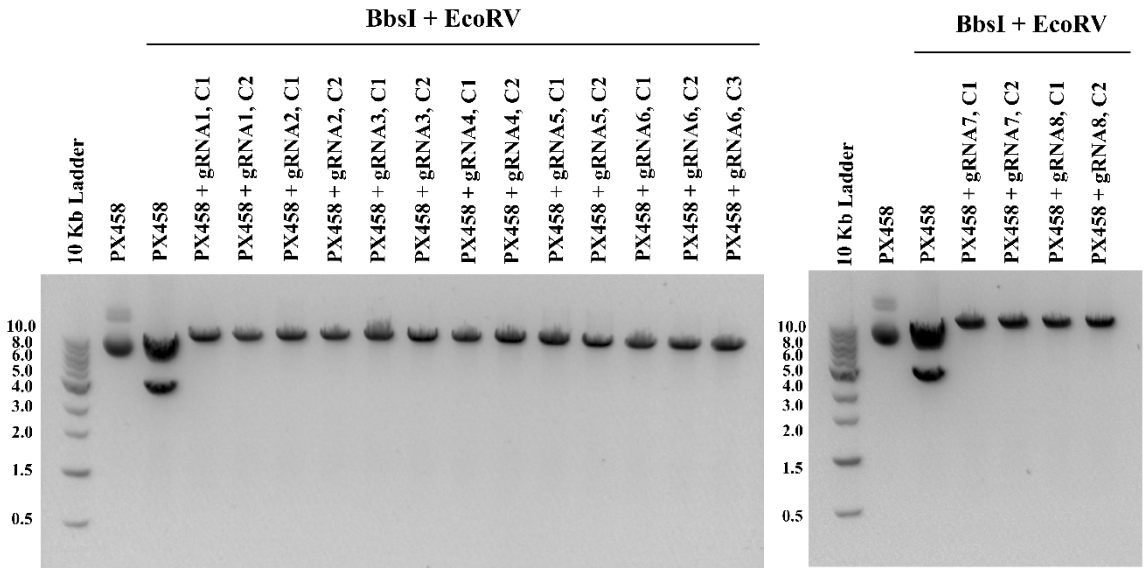


Figure 6-6 Digestion test to verify gRNA cloning into PX458 vector

Vector samples were digested and prepared for (1%) agarose gel as described in (section 2.15.2).

Sample	gRNA sequence	Sequencing result
PX458 only	None	TTATATATCTTGTGGAAGGAnGAAACACCGGGTCTTCGAGAAGACCTGTT TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX458 + gRNA1	GGTGGCTGCGGCGCGGGAGC	TTGGCTTnnTATATCTTGTGGAAGGAnGAAnCACCGGTGGCTGCGGCGCGG GAGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTA TCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX458 + gRNA2	GGCGCGGGAGCCGGCGTGGG	GGCTTnATATATCTTGTGGAAGGACGAAACACCGGCGCGGGAGCCGGCG TGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTAT CAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX458 + gRNA3	GCGGGAGCCGGCGTGGGCGG	CTTnnTATATCTTGTGGAAGGACGAAnnACCGCGGGAGCCGGCGTGGGCG GGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA ACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX458 + gRNA4	GCCGGCGTGGGCGGCGGCAA	CTTGGCTTnnTATATCTTGTGGAAGGACGAAACACCGCCGGCGTGGGCGG CGGCAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT TATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX458 + gRNA5	GCGGCAACGGGGCACTGACC	CTtnnTATATCTTGTGGAAGGACGAAACACCGCGGCAACGGGGCACTGAC CGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAA CTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX458 + gRNA6	CGCGCCGCAGCCACCCGACT	GGCTTnnTATATCTTGTGGAAGGACGAAACACCGCGCGCCGCAGCCACCC GACTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTAT CAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX458 + gRNA7	AAGTCGGGTGGCTGCGGCGC	CTTnnTATATCTTGTGGAAGGACGAAACACCGAAGTCGGGTGGCTGCGGC GCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA ACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX458 + gRNA8	CAAGTCGGGTGGCTGCGGCG	ATATCTTGTGGAAGGACGAAACACCGCAAGTCGGGTGGCTGCGGCGGTT TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..

Table 6-1 Sanger sequencing results of gRNA cloning into PX458

6.3.1.3 Determining the efficiency of gRNA in generating genome editing

The efficiency of gRNA to generate mutations was determined using the T7 endonuclease I digestion test as described in section (2.15.5) (Figure 6.7).

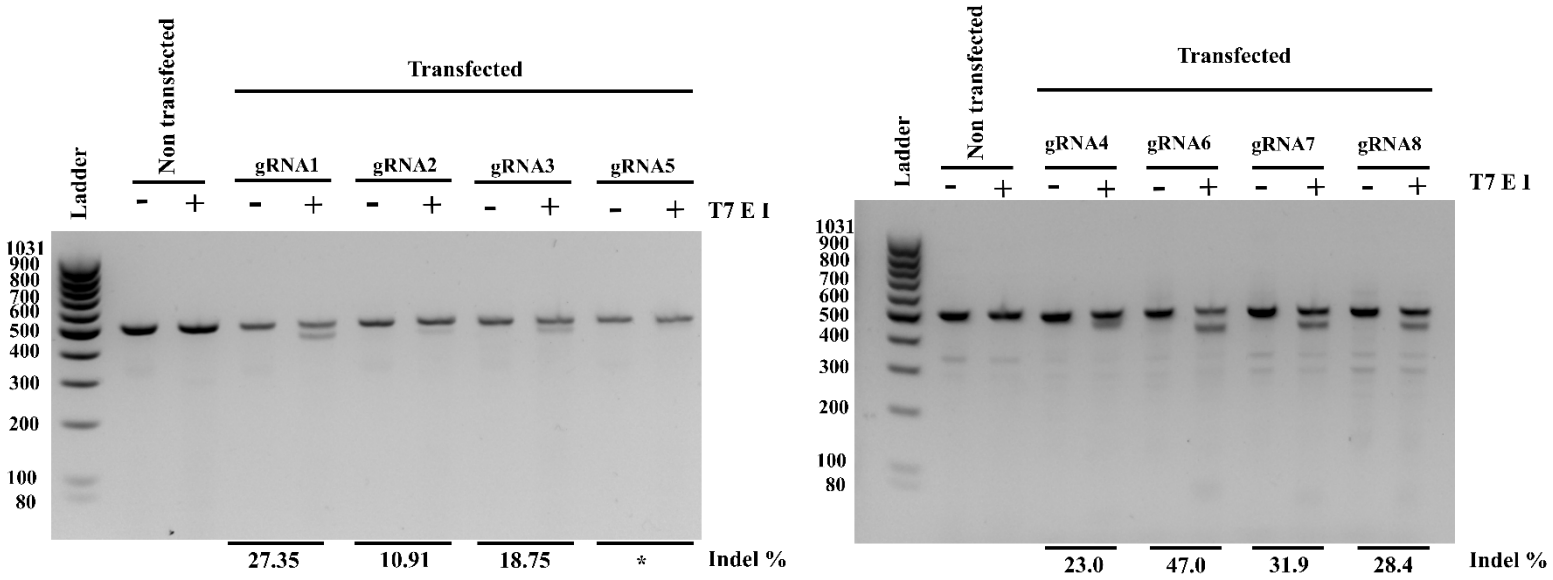


Figure 6-7 T7 Endonuclease I digestion test to determine gRNA efficiency.

Samples were prepared for (2%) agarose gel electrophoresis as described in (section 2.15.5). Briefly, successfully transfected cells were selected as a pool and DNA was extracted. The genomic region which is targeted by CRISPR was amplified by PCR. This was followed by denaturation and reannealing to produce heteroduplex DNA fragments. These duplexes were then digested with T7 Endonuclease I, a DNA nuclease that targets any mismatch of more than one base pair. Digestion products were visualized by gel electrophoresis which allows determination of the ratio of edited fragments. (*) Gel did not show a modified amplification products which might be because the difference between wild type and genetically modified species was very small and below the agarose gel resolution (2%). This is because the TOPKO19 reverse primer was only five bases long from gRNA5 due to primer design limitations in this genomic region.

6.3.1.4 Screening for TOP2B knockout clones

Out of about 168 clones which were isolated and expanded, 64 clones were checked for TOP2β expression before genotyping because they were confluent and ready to be checked at Christmas time and I could not use Sanger sequencing service for 2 weeks. Immunofluorescence was used, as it does not need as many cells as western blotting.

6.3.1.4.1 Immunofluorescence

Immunofluorescence was performed as described in (section 2.15.7.2) using anti-TOP2B antibody (4555) (Table 2.1). Nalm6$^{B-/-}$ cells, which was previously described, were included in the screening process as a negative control. Out of the 64 clones under screening, fifteen clones were negative for TOP2B. In addition, one clone gave weak signal (clone 142) so it was included in the further analysis (Figure 6.8).

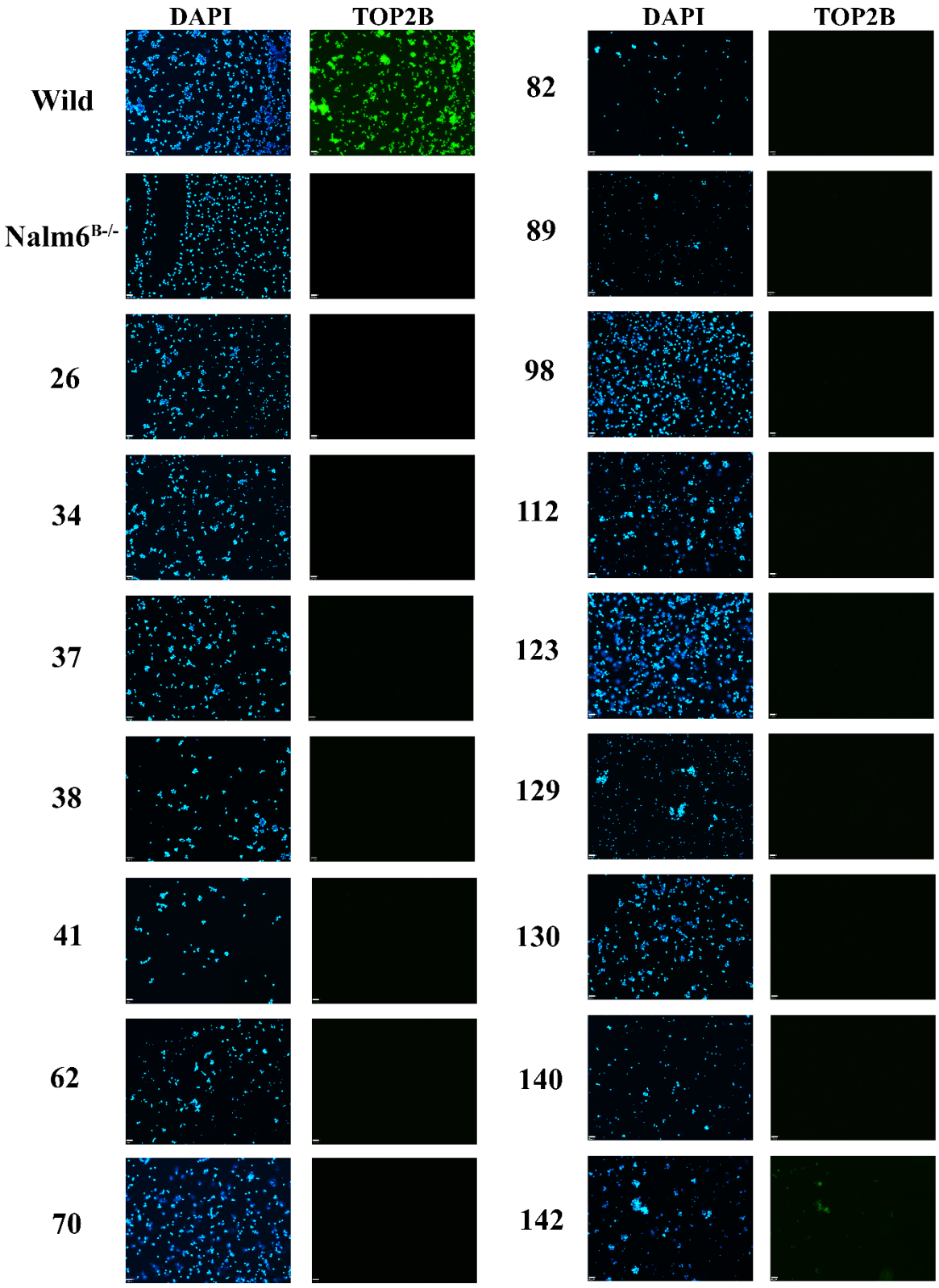


Figure 6-8 TOP2B Immunofluorescence of knockout clones

Cells were prepared for immunofluorescence as described in section (2.15.7.2) and stained with TOP2B (4555) antibody followed by Alexa fluor®488 secondary antibodies then immunofluorescence signal was captured via FITC channel.

6.3.1.4.2 Genotyping

The 16 clones were further analysed by performing DNA extraction and PCR amplification of targeted genomic region using TOPKO1 primer as described in (section 2.15.7.1). Clone bands which were different from the wild type were extracted from the gel and sent for sequencing (Figure 6.9) and (Table 6.2).

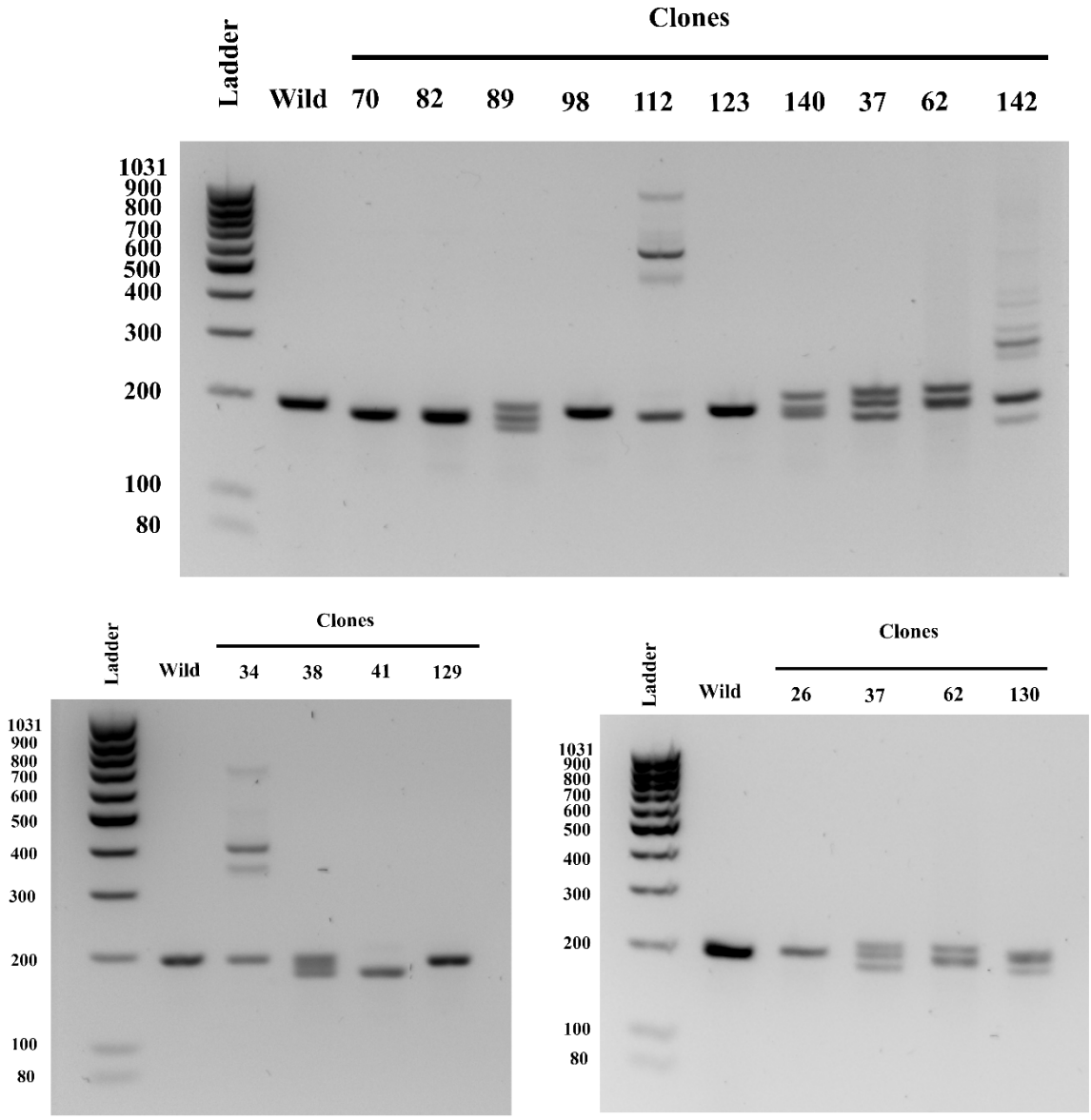


Figure 6-9 Agarose gel electrophoresis of suspected knockout clones.

Samples were prepared for genotyping as described in section (2.15.7.1) and run on (3 %) agarose gels to allow for separation of small differences.

Clone	gRNA	Mutation
26	5	Wild: CGGCGGCAACGGGGCACTGACCTGGGTGGTAAGTGGCTGGA Clone: CGGCGGCAACGGGGCACT…ACCTGGGTGGTAAGTGGCTGGA
34	6	Wild: ACTCGCCATGGCCAAGTCGGGTGGCTGCGGCGCGGGAG Clone: ACTCGCCATGGCCA…..TCGGGTGGCTGCGGCGCGGGAG
41	6	Wild: GCACTCGCCATGGCCAAGTCGGGTGGCTGCGGCGCGGG Clone: GCACTCGCCATGGC……GCGGG
70	5	Wild: GCGGCGGCAACGGGGCACTGACCTGGGTGGTAAGTGGCTGG Clone: GCGGCGGCAAC……...CTGGGTGGTAAGTGGCTGG
82	6	Mutation sequence could not be confirmed.
98	6	Wild: AGGCACTCGCCATGGCCAAGTCGGGTGGCTGCGGCGCGGGAG Clone: AGGCACTCGCCATGGCCAA…….GTGGCTGCGGCGCGGGAG
112	6	Wild: AGGCACTCGCCATGGCCAAGTCGGGTGGCTGCGGCGCGGGAG Clone: AGGCACTCGC……..GGGTGGCTGCGGCGCGGGAG
123	7	Wild: TGGCCAAGTCGGGTGGCTGCGGCGCGGGAGCCGGCGTGGGCG Clone: TGGCCAAGTCGGGTGGCTGC…….GGGAGCCGGCGTGGGCG
129	7	Wild: TCGCCATGGCCAAGTCGGGTGGCTGCGGCGCGGGAGCCG Clone: TCGCCATGGCCAAGTCGGGTGGCTGC…….GGGAGCCG

Table 6-2 Clones that showed bi-allelic genetic variation from wild type SH-SY5Y cells.

6.3.1.4.3 Western blotting

In addition to immunofluorescence, clones were then tested for TOP2β expression using western blotting as described in section (2.15.7.2). Western blotting results were consistent with immunofluorescence and confirmed TOP2B absence in knockout clones (Figures 6.10 and 6.11).

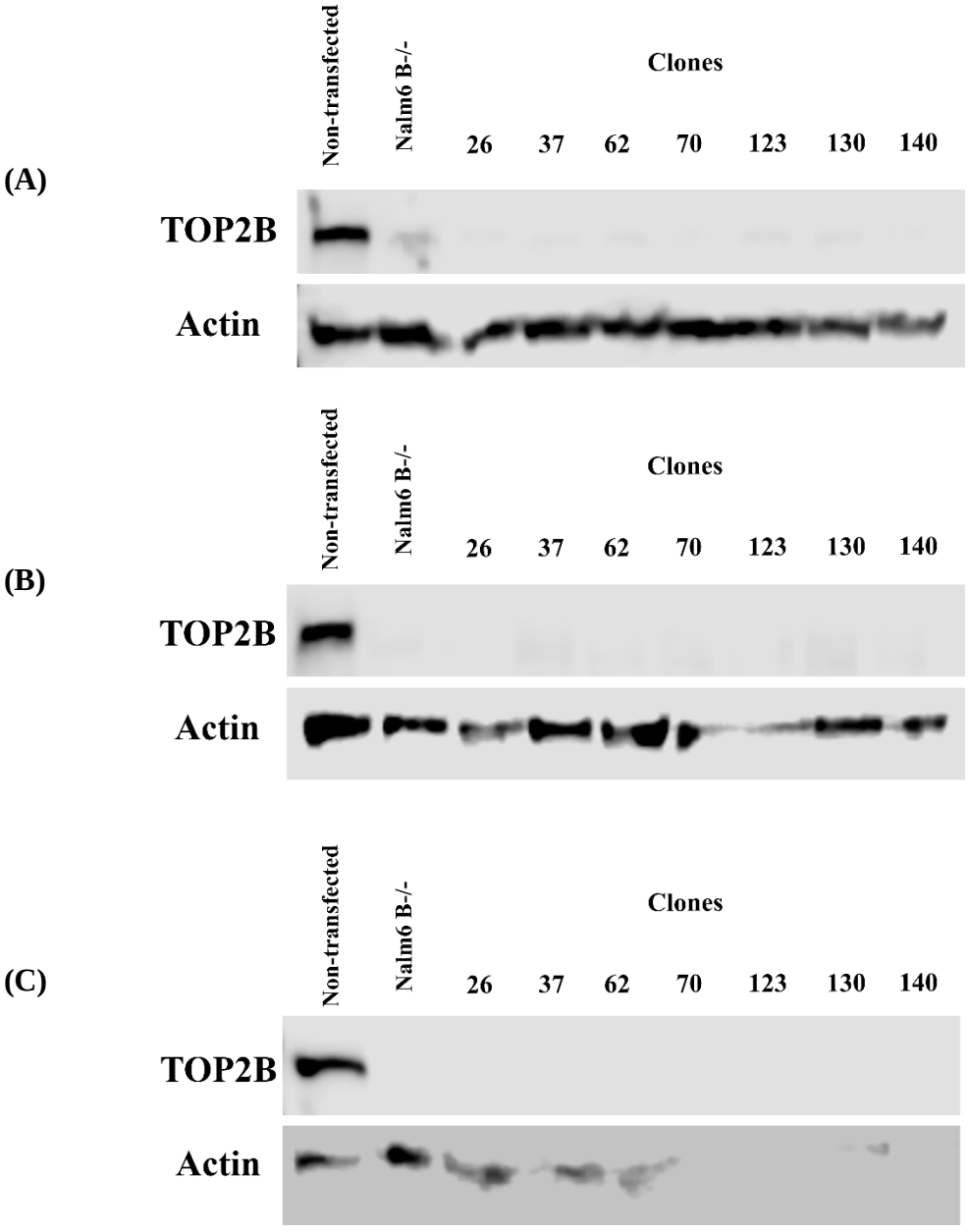


Figure 6-10 TOP2B western blotting of candidate knockout clones (first group).

Clone protein samples (5 µg of WCE / lane) were probed with **(A)** TOP2B (30400) **(B)** TOP2B (4557) or **(C)** TOP2B (MAB6348) antibodies.

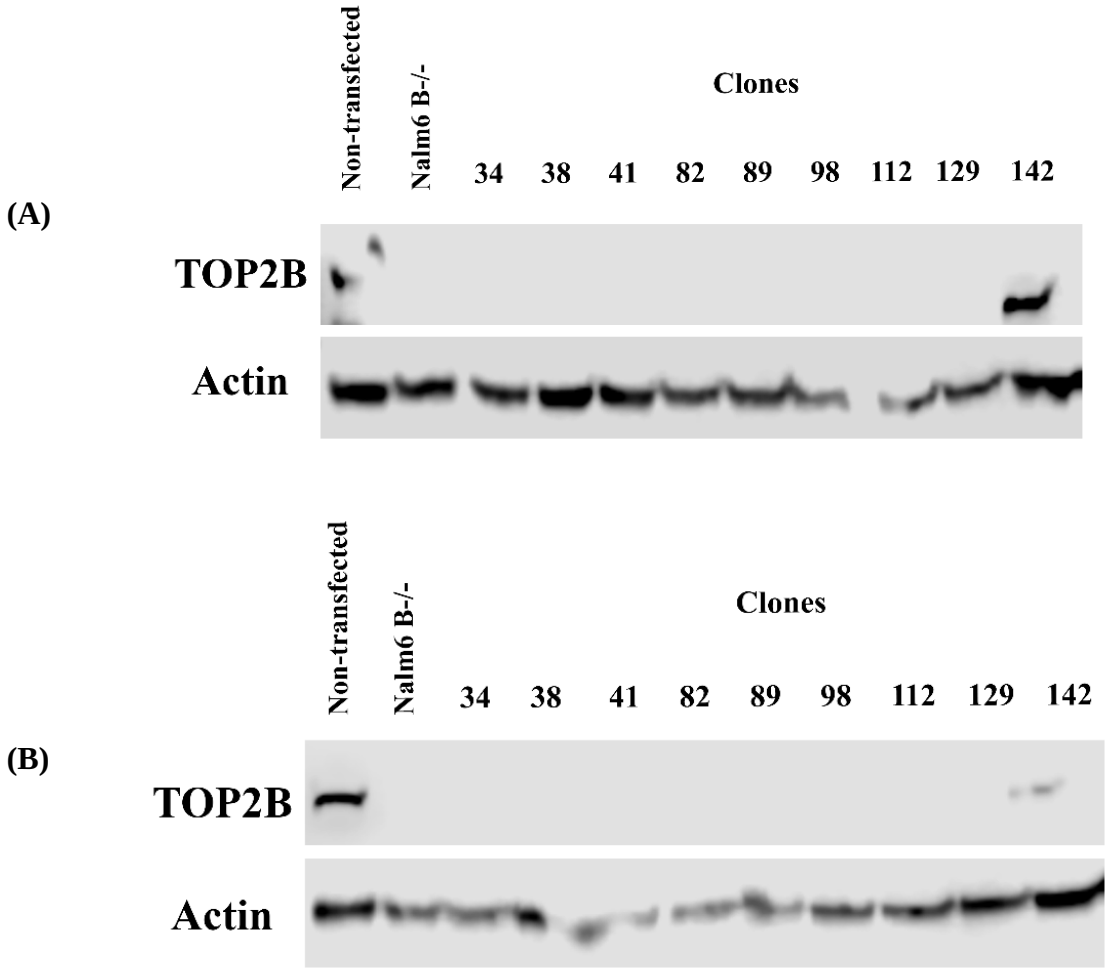


Figure 6-11 TOP2B western blotting of candidate knockout clones (second group).

Clone protein samples (5 µg of WCE / lane) were probed with **(A)** TOP2B (4557) and **(B)** TOP2B (MAB6348) antibodies.

In summary, Clones no. (26, 34, 41, 70, 98, 112, 123, and 129) were confirmed for both absence of TOP2B by IF and WB as well as confirmed to have a bi-allelic mutation by genotyping.

6.3.2 Effect of TOP2B knockout on SH-SY5Y cells

6.3.2.1 Effect of TOP2B knockout on ATRA-induced differentiation of SH-SY5Y cells

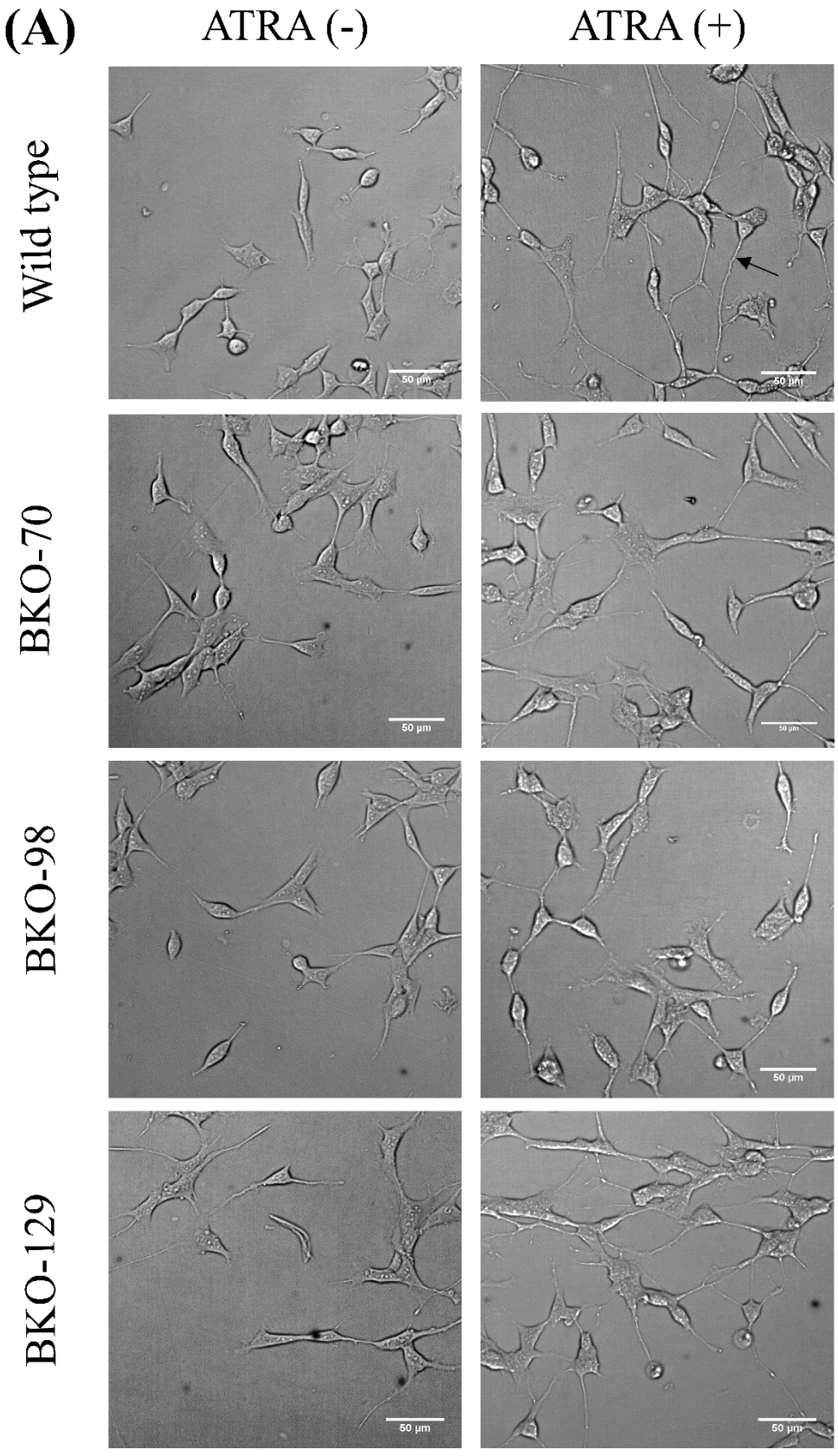
To determine the effect of TOP2β absence on ATRA-induced differentiation of SH-SY5Y, three *TOP2B* knockout clones (70, 98, and 129) were compared with the wild type for neurite outgrowth formation induced by ATRA. These clones were selected for this assay and further analysis as they derived from different gRNAs as shown in (Table 6.2). ATRA treatment reduces growth of SH-SY5Y cells compared with the untreated cells (observation confirmed in lab). As overconfluency of cells prevents accurate counting of neurite bearing cells, the initial number of seeded cells was optimized in such a way that both ATRA-treated and untreated cells have a comparable confluency by the end of time-course of differentiation (7 days). This was to ensure a comparable number of cells that can be counted in both samples. For differentiation induction, cells were treated with either (10 μM) ATRA or ethanol (0.01 % V/V) for 7 consecutive days and media was replaced every 2 days to keep ATRA concentration consistent over differentiation period. Cells were then imaged by phase-contrast microscopy using a TiE fluorescence wide field inverted microscope (Nikon). Random fields were captured for each sample and the ratio of cells showing neurite outgrowth of more than 50 μm long were determined for each sample.

Results showed that *TOP2B* knockout clones showed significant reduction in the ratio of neurite outgrowth bearing cells. Representative fields and quantification analysis of neurite outgrowth bearing cells are shown in (Figure 6.12).

In addition to morphological characterization of differentiation by neurite outgrowth assay, differentiation level was also determined biochemically by measuring the level of *BCL2*, a proto-oncogene that has been shown to be associated with neuronal development (Garcia *et al.*, 1992; Merry *et al.*, 1994) and increased in SH-SY5Y upon retinoic acid-induced differentiation (Hanada *et al.*, 1993; Lasorella *et al.*, 1995; Riddoch *et al.*, 2007; Bell *et al.*, 2013). *BCL2* level was determined at the molecular level by measuring mRNA using RT-qPCR in the presence or absence of differentiation induction for 24 hrs.

Results showed marked reduction of *BCL2* expression in *TOP2B* knockout clones compared with the wild type. These results were consistent with neurite outgrowth assay (Figure 6.13).

-Neurite outgrowth assay in wild type and *TOP2B* knockout SH-SY5Y cells.



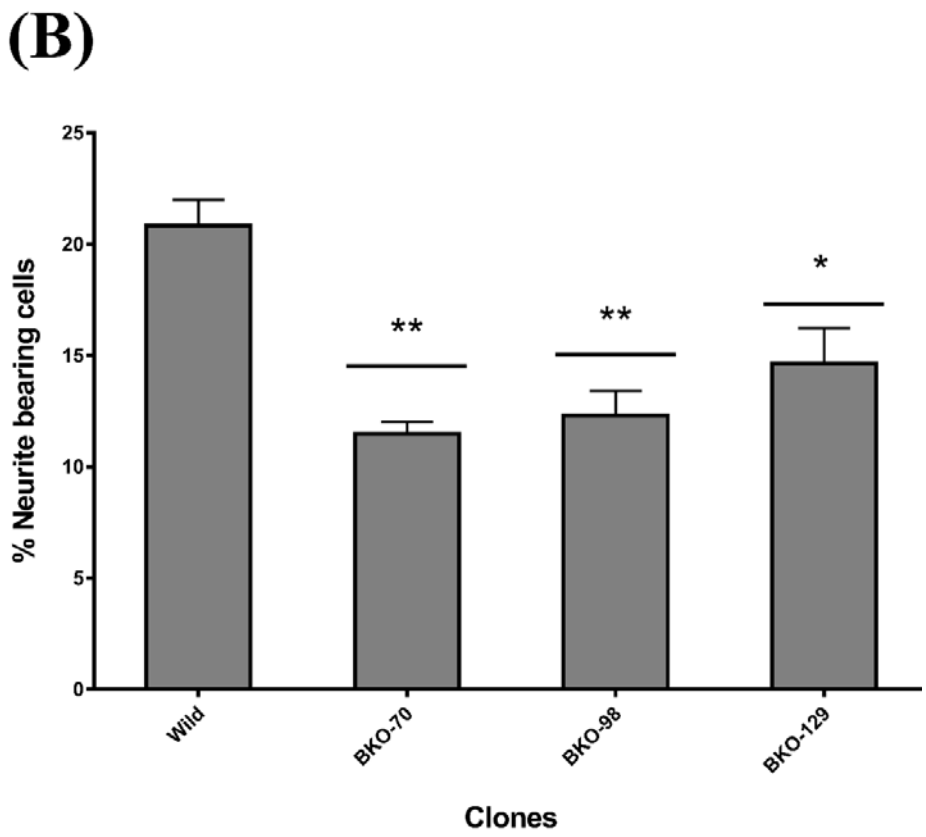


Figure 6-12 Morphological differentiation in wild type and *TOP2B* knockout SH-SY5Y clones induced by ATRA.

SH-SY5Y Cells were treated with either vehicle EtOH or 10 μM ATRA for 7 consecutive days. Cells were seeded at initial number of 4000 cells/well for control (**ATRA (-)**) cells, and at 8000 cells/well for ATRA treated (**ATRA (+)**) cells in order to have a comparable number of cells that can be counted in both samples after 7 days **(A)** Phase-contrast images of cells were captured at the end of treatment using a TiE fluorescence wide field inverted microscope (Nikon). Differentiated cells (ATRA treated) exhibit neurite extensions (arrow). Cells that exhibited neurites of ≥ 50 μm in length were counted as differentiated. Images shown are representative of at least three independent replicates. Scale bars represent 50 μm. **(B)** Differentiated-to-total cells ratio per field were calculated for both control and treated groups. Mean ratio of several random fields per sample were calculated. Basal ratio of differentiated cells in control groups were then subtracted. Data presented here is the mean of at least three independent experiments with error bars representing the SEM. (*P≤0.05, **P≤0.01), Significance of difference was tested using unpaired t-test.

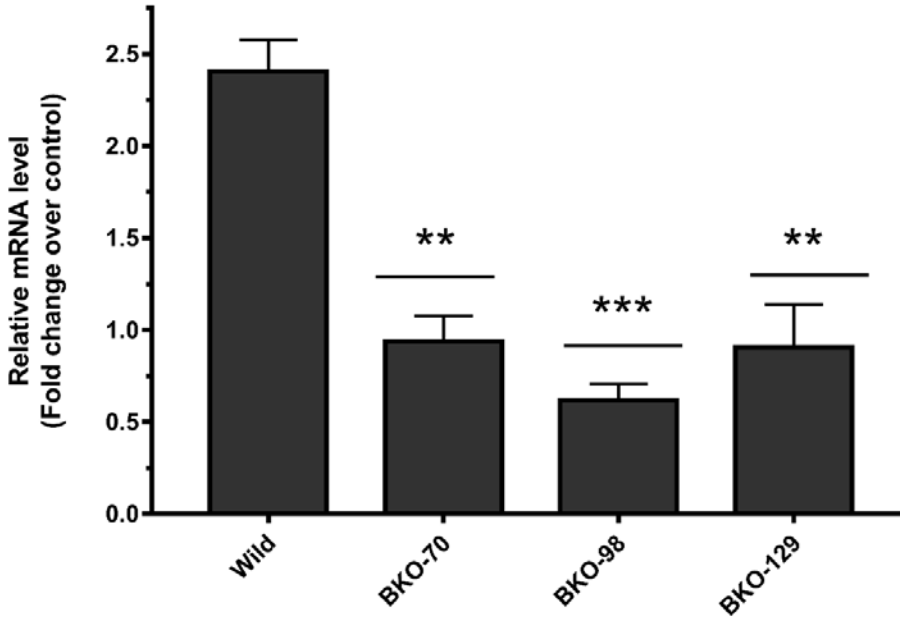


Figure 6-13 Relative *BCL2* gene induction by ATRA treatment for 24 hours.

Relative gene expression was determined by RT-qPCR analysis as described in section (2.14.3). *PP1A* gene was used as a reference gene for data normalization. Cells were treated with or without 1 μM ATRA for 24 hrs and ΔΔCt method was used to calculate fold change induction above control for each clone. Data presented here is the mean of at least three independent experiments with error bars representing the SEM. (*P≤0.05, **P≤0.01, and ***P≤0.001), Significance of difference was tested using unpaired t-test.

6.3.2.2 Effect of TOP2B on genes involved in the ATRA-induced differentiation pathway

Results above showed that the absence of TOP2β affected ATRA-induced differentiation of SH-SY5Y cells. The next step was to test the effect of knocking out *TOP2B* on the expression of (*RARB, CYP26A1, CRAPB2* and *RET*), genes that have been shown to be regulated by retinoic acid and to be involved in the retinoic acid-induced differentiation pathway (Perez-Juste and Aranda, 1999; Brabender *et al.*, 2005; Oppenheimer *et al.*, 2007). The wild type SH-SY5Y cells and the knockout clones (BKO-70, BKO-98, and BKO-129) were treated with or without 1 μm of all-trans retinoic acid (ATRA) for 24 hours and RNA samples were extracted for RT-qPCR to the determine difference between the wild type and knockout clones in the relative induction of these genes (Figure 6.14).

Results showed a significant reduction in ATRA-induced expression of both *CRABP2* and *CYP26A1* genes, while no significant difference were seen between wild type and knockout clones in induction of *RARB* and *RET* genes (Figure 6.14).

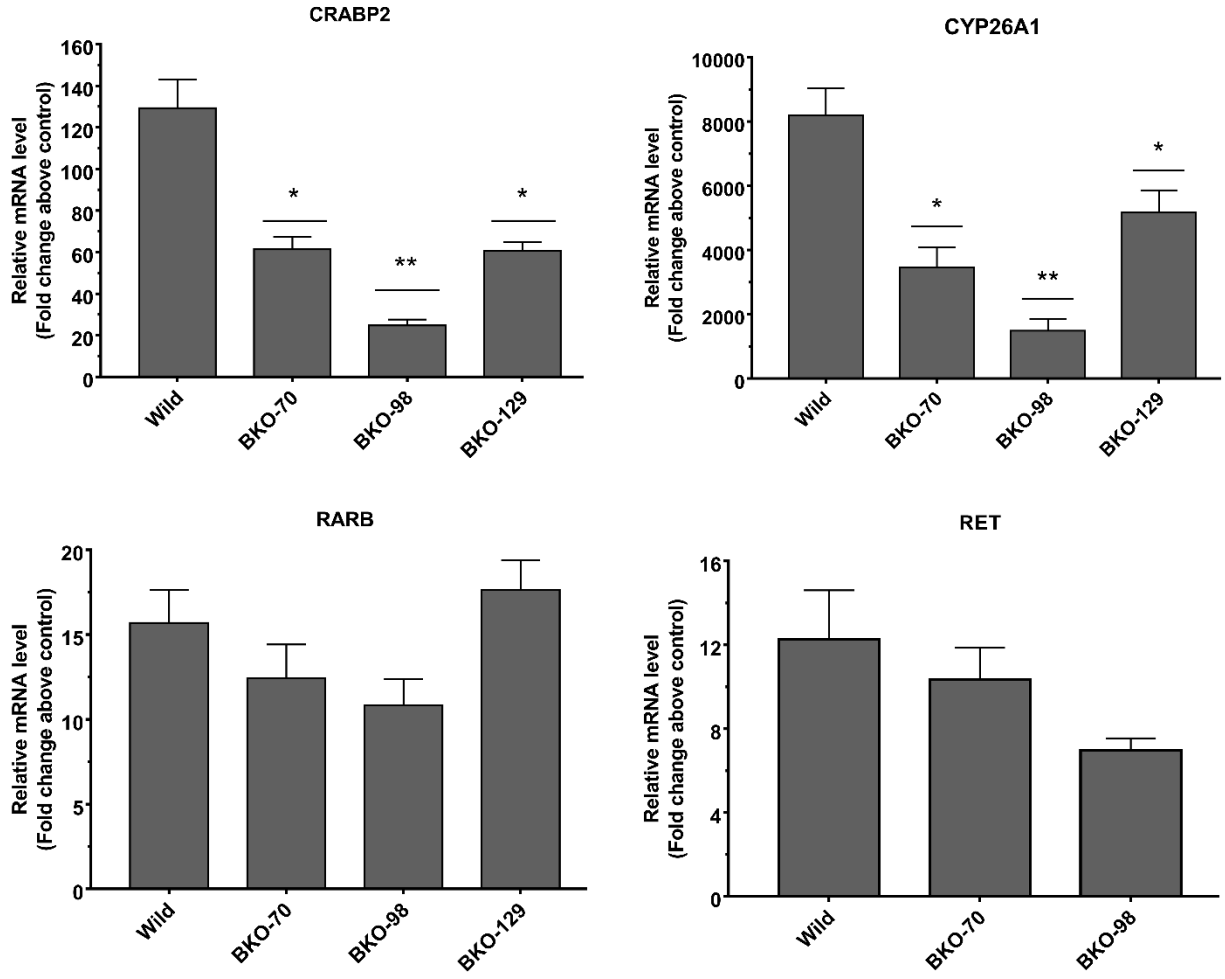


Figure 6-14 Induction of genes involved in the ATRA-induced differentiation pathway

Relative gene expression was determined by RT-qPCR analysis as described in section (2.14.3). *PP1A* gene was used as a reference gene for data normalization. Cells were treated with or without 1 μM ATRA for 24 hrs and ΔΔCt method was used to calculate the fold change in induction above control for each clone. Data presented here is the mean of at least three independent experiments with error bars representing the SEM. (*P≤0.05, **P≤0.01), Significance of difference was tested using unpaired t-test.

6.3.3 Effect of TOP2B knockout on whole transcriptome by RNA sequencing analysis

The effect of *TOP2B* knockout on whole genome expression in SH-SY5Y with and without ATRA treatment was determined using RNA-seq. The *TOP2B* knockout (BKO-98) clone was selected for RNA-seq experiment as it was generated from gRNA number 6 which was predicted to have the least off-target effect (see table 2.7). Four biological replicates of both wild type and BKO-98 clone were treated with either (10 μM) ATRA or ethanol (0.01 % V/V) for 24 hours. Cells were then harvested and total RNA samples were extracted as described in section (2.14.1). RNA samples were then tested for quantity and quality using (Agilent 2100 Bioanalyzer System) as described in section (2.14.2.1) and presented in (Table 6.3 and Figure 6.15). Library preparation for sequencing and analysis are explained in section (2.14.2).

Samples		Concentration (ng/μl)	RIN
Replicate 1	WT (Con)	634	9.9
	WT (RA)	627	9.9
	BKO-98 (Con)	624	10
	BKO-98 (RA)	555	10
Replicate 2	WT (Con)	660	10
	WT (RA)	630	9.9
	BKO-98 (Con)	612	10
	BKO-98 (RA)	577	9.9
Replicate 3	WT (Con)	739	10
	WT (RA)	782	9.9
	BKO-98 (Con)	703	10
	BKO-98 (RA)	557	10
Replicate 4	WT (Con)	661	10
	WT (RA)	735	10
	BKO-98 (Con)	652	10
	BKO-98 (RA)	590	10

Table 6-3 SH-SY5Y RNA samples used in RNA-seq analysis.

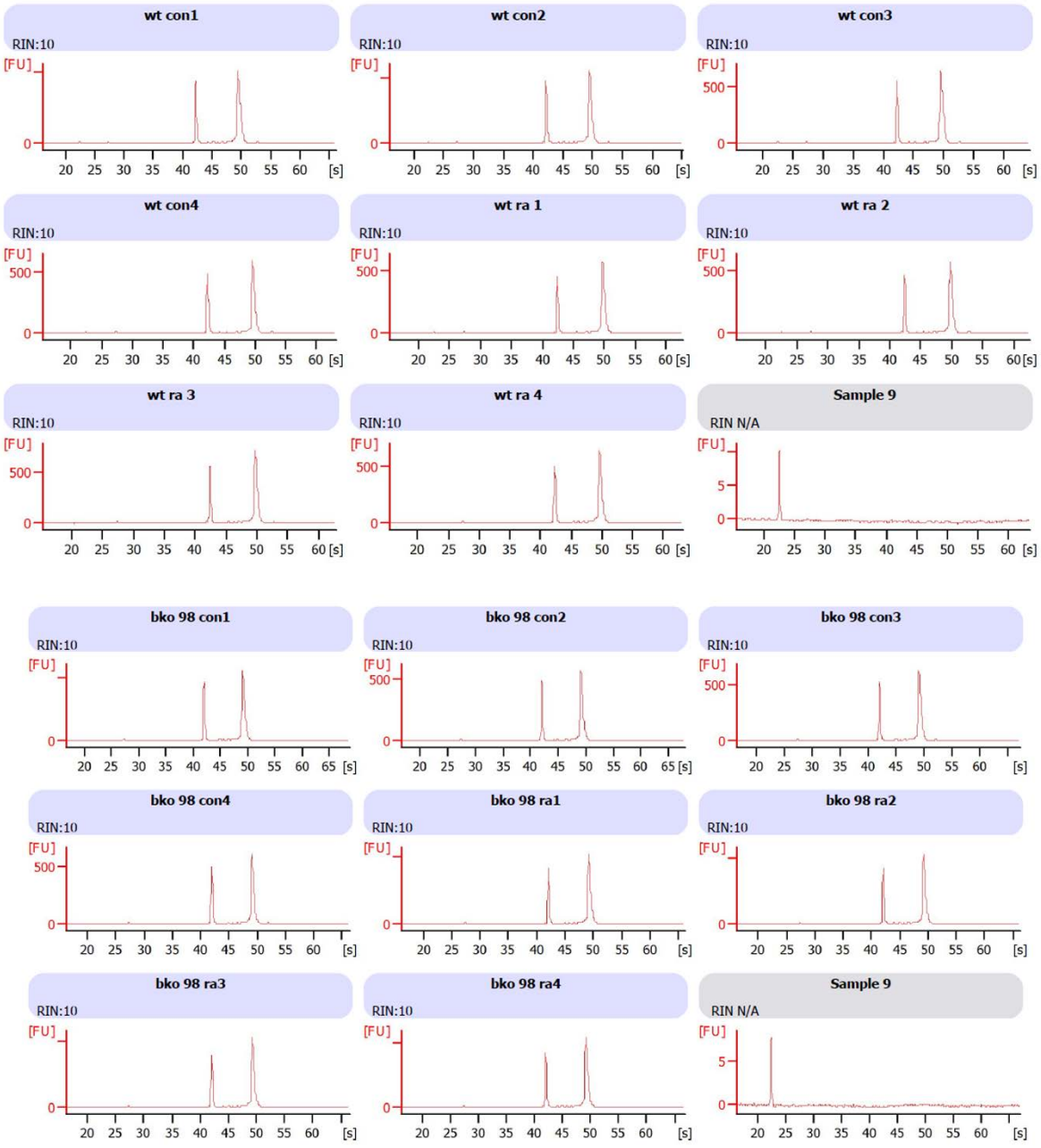


Figure 6-15 Quality of SH-SY5Y RNA samples used in RNA-seq experiment

This figure shows electropherogram summary with RIN number of RNA samples used in RNA-seq as determined using Agilent 2100 Bioanalyzer System.

RNA-seq data for the four conditions (WT (Con), WT (RA), BKO-98 (Con), and BKO-98 (RA)) were tested for Principal Component Analysis (PCA), a statistical technique used to confirm the variation and show the patterns in a dataset. PCA analysis confirmed a consistency between replicates and show the difference between conditions tested as shown in (Figure 6.16).

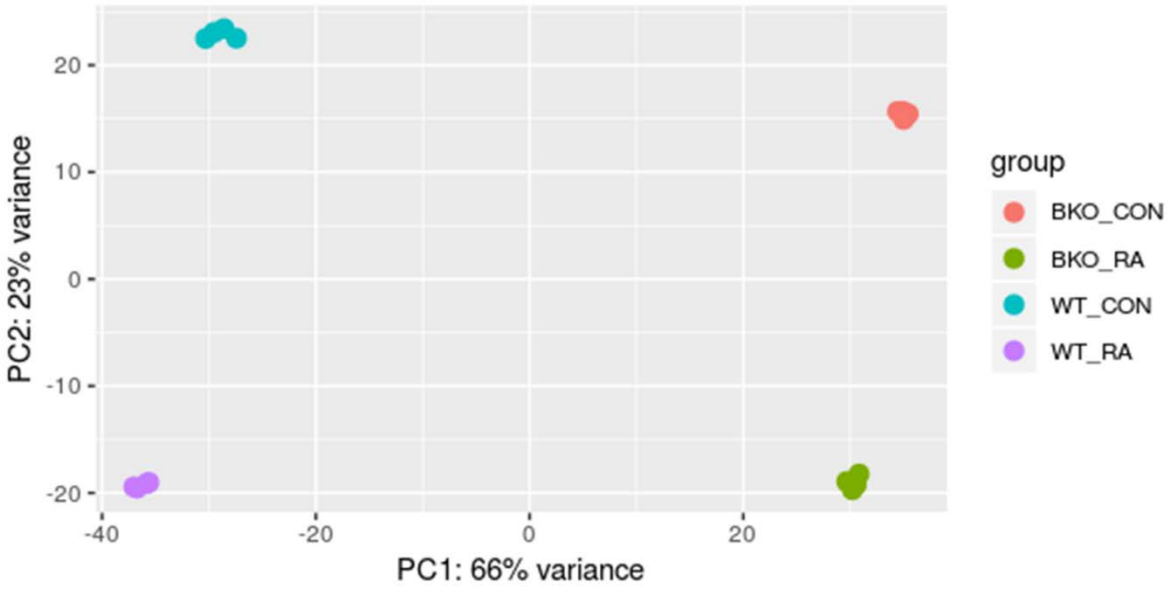


Figure 6-16 PCA analysis of conditions in RNA-seq experiment in SH-SY5Y

PCA analysis of the four conditions used in the RNA-seq experiment in SH-SY5Y cells was performed using DESeq2 package in R software (version 4.3).

6.3.3.1 Differential gene analysis

The differentially expressed genes (DEGs) in the presence or absence of TOP2β with or without ATRA were determined by RNA-seq analysis as described in section (2.14.2.3). Four comparisons were performed: wild type against BKO-98 without ATRA (WT(C) vs. BKO(C)), wild type against BKO-98 with ATRA treatment (WT(R) vs. BKO(R), untreated against treated wild type (WT(C) vs. WT(R), untreated against treated BKO-98 (BKO(C) vs. BKO(R). Differential gene analysis showed large number of genes which were significantly (padj < 0.05) either up or down regulated by more than 1 log2 fold in the four comparisons. Volcano plots of these genes are depicted in (Figure 6.17).

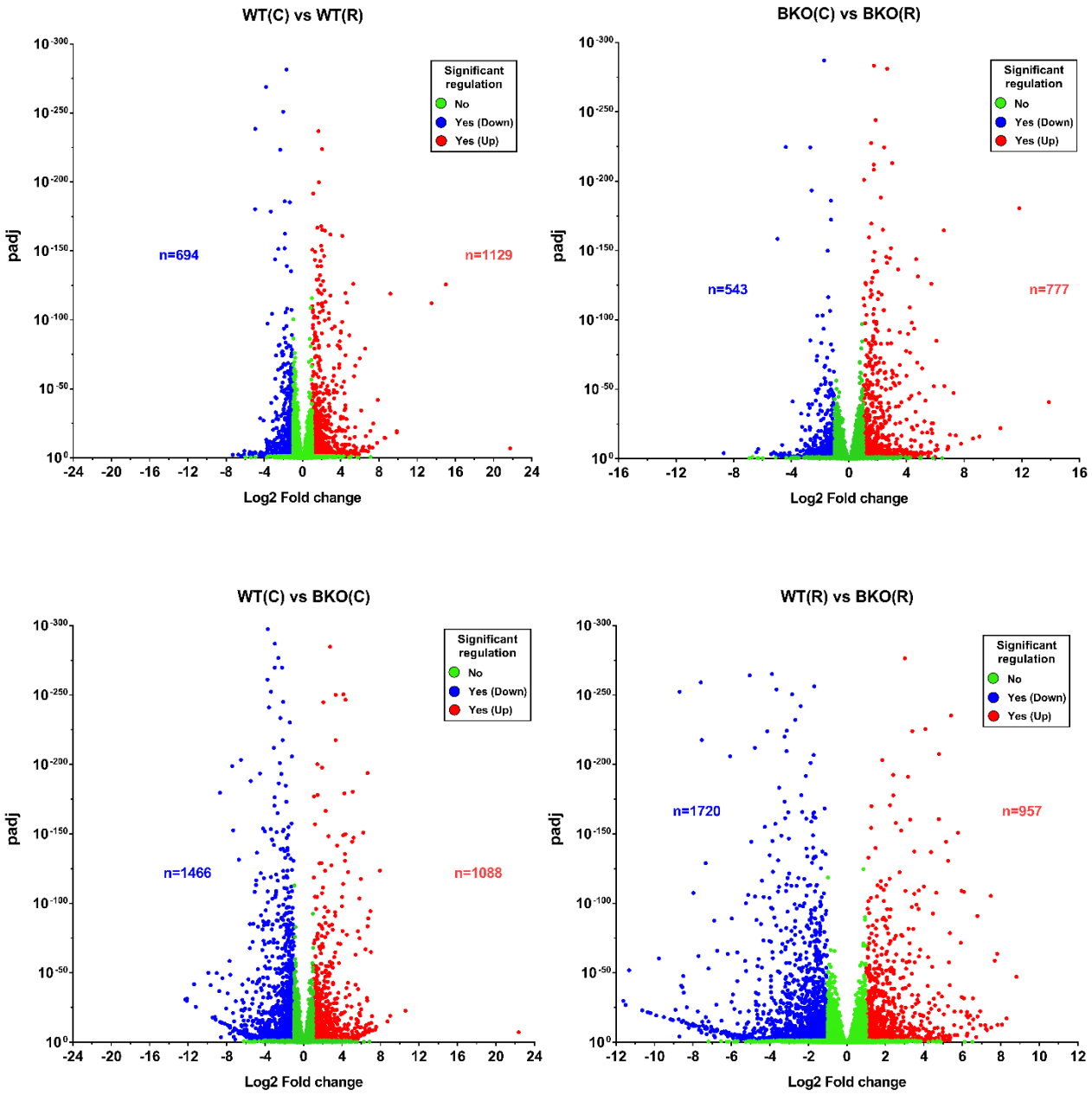


Figure 6-17 Differentially expressed genes in 4 comparisons in SH-SY5Y

Volcano plots show differential gene expression in 4 different comparisons in SH-SY5Y cells. Genes which were significantly (padj < 0.05) either up or down regulated by more than 1 log2 fold are labelled. Adjusted P value (padj) represents p-value adjusted for multiple testing correction using Benjamini-Hochberg method to control the False Discovery Rate (FDR). Plus and minus log2 values correspond to magnitude of change against control.

For listing the highly regulated genes in a summarized way, the total number of genes which were differentially expressed (whether up or down regulated) by more than 1 log2 fold in each comparison were analysed for intersection between conditions and presented as Venn diagram with genes were categorized as groups from A to O. The full list of these gene groups with padj and log2 fold values within each comparison are shown in (Appendix table 17).

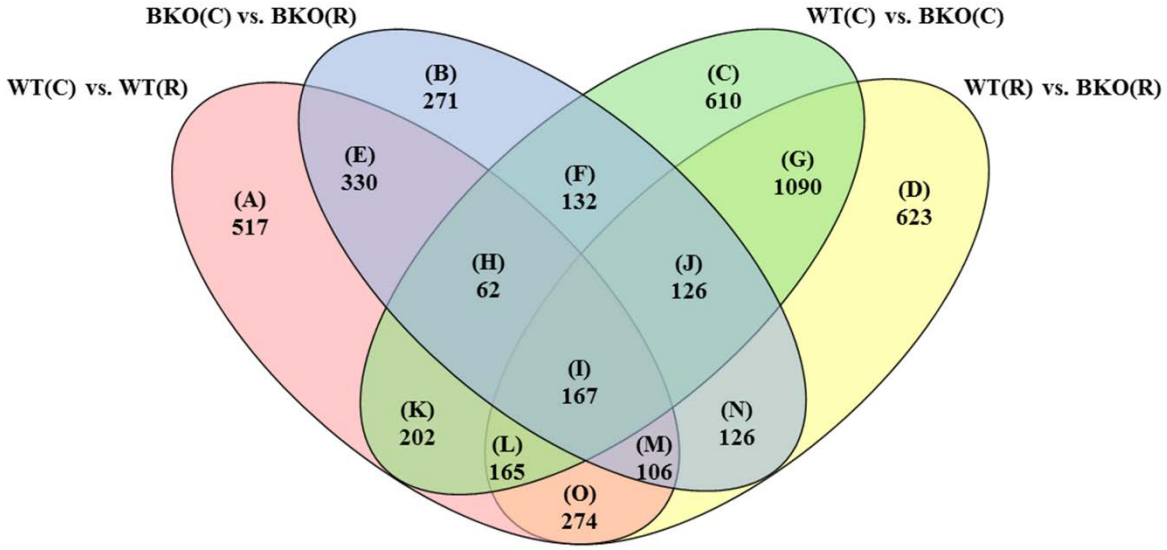


Figure 6-18 Differentially expressed genes of 4 conditions of RNA-seq in SH-SY5Y

Venn diagram shows the number of genes which were significantly (padj < 0.05) changed by more than 1 log2 fold in the four comparisons. Letters represent category allocated to gene groups to identify them in (Appendix table 17). The Venn diagram was generated using gplots package in R. the full gene lists are in (Appendix table 17).

Generally, RNA-seq analysis for differential gene expression revealed a large number of genes changed by more than 1 log2 fold in all comparisons (Figure 6.17). Notably, the number of genes which were up or down regulated in the wild type upon ATRA treatment was higher than that in the *TOP2B* knockout cells (1129 vs. 777 up- and 694 vs. 543 down-regulated) respectively (Figure 6.17). The genes which were regulated by ATRA in the wild type but not in the knockout cells are belong to the groups (A, K, L, and O) in (Figure 6.18 and Appendix table 17).

RNA-seq analysis following ATRA treatment for 24 hrs showed different levels of expression of a number of neuronal differentiation markers such as *BCL2*, *MAP2* (codes for microtubule associated protein 2), *SYP* (codes for synaptophysin), and *GAP43* (codes for growth associated protein 43) between wild type and *TOP2B* knockout cells. These markers have been shown to be induced by RA in SH-SY5Y cells in various studies (Hanada *et al.*, 1993; Cheung *et al.*, 2009; Bell *et al.*, 2013; Kovalevich and Langford, 2013; Yan *et al.*, 2015). For *BCL2*, in agreement with the previous RT-qPCR results of *BCL2* (Figure 6.13), RNA-seq results showed a significant reduction in the induction of *BCL2* gene in the *TOP2B* knockout clone by 2.2 log2 fold (padj = 1.85E-118). For *MAP2*, *SYP*, and *GAP43* expression levels, the knockout cells showed reduction (by 1.83, 1.4, and 2.5 log2 fold respectively) compared with the wild type.

Moreover, ATRA treatment led to a remarkable downregulation of *MYC*, a cell cycle release gene that is well known for activating cyclins and cyclin-dependent kinases (CDKs). In addition, MYC decreases the level/activity of proteins involved in cell cycle repression such as p21 (encoded by *CDKN1A* gene) (Garcia-Gutierrez *et al.*, 2019). *MYC* downregulation by ATRA was significantly higher in the wild type than the knockout cells (by 2.1 log2 fold, padj = 1.30E-96). This was accompanied by up regulation of *CDKN1A* (by 1.1 log2 fold, padj = 8.35E-36) in wild type cells only, and the *CCN1A* gene (encodes for cyclin A) in both wild and knockout cells with higher upregulation in the wild type by 1.2 log2 fold (padj = 3.45E-14). These results support the previous microscopic and RT-qPCR results of different levels of differentiation between wild type and knockout cells.

On the other hand, ATRA treatment for 24 hrs did not cause significant change in TOP2α expression in either the wild type or the knockout cells nor in TOP2β expression in the wild type, suggesting no direct effect of ATRA on both isoform or treatment for 24 hrs was not be enough to cause significant change. Longer ATRA treatment (~3-5 days) caused a robust downregulation of TOP2A (preliminary lab data).

RNA-seq analysis confirmed differential expression of a group of genes involved in metabolism and function of RA and regulated by RA at the same time. Differentially expressed genes (DEGs) which belong to this group are described in (Table 6.4). These genes are components of RA pathway and their induction level is controlled by RA itself (Balmer and Blomhoff, 2002; Rhinn and Dollé, 2012). Some of the genes of this group have well defined retinoic acid response elements (RAREs) (Balmer and Blomhoff, 2005) as shown in (Table 6.4).

The expression of four genes (*RARG*, *FABP5*, *AKR1C3*, and *PDK4*) in this group were induced only in the wild type cells. In addition, ATRA treatment induced expression of 8 genes in this group (*RARA, CRABP2, RARB, CYP26A1, CYP26B1, RDH10, DHRS3*, and *RET*) in both the wild type and the knockout cells but the induction was much higher in the wild type for 4 genes (*CRABP2, CYP26A1, CYP26B1,* and *DHRS3*) which were also significantly downregulated in the absence of TOP2β even without RA induction (Table 6.4). Reduced induction of two of these genes (*CYP26A1* and *CRABP2*) were previously shown in the knockout cells using RT-qPCR as shown in (Figure 6.14). *RET* oncogene also was remarkably regulated by RA (Table 6.4) and it was included to this group as it has been shown to play a key role in transcriptional events related to RA-induced differentiation (Oppenheimer *et al.*, 2007). This gene also has been shown to be involved in nervous system development as shown by RefSeq pathways web tool (O'Leary *et al.*, 2016).

Gene	WT(C) vs. WT(R)		BKO(C) vs. BKO(R)		WT(C) vs. BKO(C)		WT(R) vs. BKO(R)		RARE identified
	Log2 FC	Padj	Log2 FC	Padj	Log2 FC	Padj	Log2 FC	Padj	
RARA	1.71	7.53E-44	1.73	3.42E-44	NA	NA	NA	NA	Yes
CRABP2	5.95	0	6.11	0	-3.30	7.85E-147	-3.14	2.29E-210	Yes
RARB	4.85	0	4.11	0	NA	NA	NA	NA	Yes
RARG	1.10	1.48E-40	NA	NA	1.12	1.07E-42	NA	NA	Yes
CYP26B1	15.00	1.81E-126	11.82	3.44E-181	NA	NA	-2.37	1.32E-178	No
ALDH1A3	-1.68	9.84E-33	-1.50	6.12E-26	NA	NA	NA	NA	No
FABP5	-1.14	3.71E-26	NA	NA	1.29	2.68E-42	2.00	2.11E-84	No
CRABP1	NA	NA	NA	NA	-3.00	1.61E-270	-3.32	0	No
CYP26A1	13.50	8.83E-113	13.89	1.63E-41	NA	NA	-1.32	3.01E-105	Yes
RDH10	1.63	2.17E-06	1.99	5.71E-09	NA	NA	NA	NA	No
PDK1	NA	NA	1.54	6.42E-40	NA	NA	NA	NA	No
AKR1C3	2.58	2.91E-06	NA	NA	1.35	0.0411	NA	NA	No
PDK3	NA	NA	1.15	2.07E-21	NA	NA	NA	NA	No
PDK4	1.17	0.00624	NA	NA	2.70	1.68E-14	1.73	9.85E-08	No
DHRS3	9.20	8.75E-120	7.43	1.71E-17	-2.37	0.0354	-4.14	9.96E-225	No
PPARD	NA	NA	1.07	1.23E-19	NA	NA	NA	NA	No
RET	3.78	0.00	3.54	0.00	NA	NA	NA	NA	No

Table 6-4 Differential expression of genes involve in RA pathway and regulated by RA itself.

Table shows genes that are involved in RA metabolism and signalling pathway and were differentially expressed by (> 1 log2 fold and < 0.05 padj) in the 4 comparisons in SH-SY5Y using RNA-seq. Adjusted P values (padj) represents p-value adjusted for multiple testing correction using Benjamini-Hochberg method to control the False Discovery Rate (FDR). Padj value of zero means very low value that fall out of calculation scale. RARE information was obtained from (Balmer and Blomhoff, 2002; Balmer and Blomhoff, 2005). Plus and minus log2 values correspond to magnitude of change against control.

RNA-seq analysis showed numerous differentially expressed genes (DEGs) with various functions. The effect of this differential expression will be discussed in light of respective biological function and pathways in which these (DEGs) are involved. DEGs were evaluated for their involvement in biological functions and pathways using QIAGEN's Ingenuity Pathway Analysis (IPA, Spring release 2019, QIAGEN Redwood City, USA, www.qiagen.com/ingenuity) as described in materials and methods (section 2.14.2.3). Further details about the key genes will be described in the discussion.

6.3.3.2 Ingenuity pathway analysis (IPA) of downstream effects

IPA analysis involved downstream effects analysis of DEGs for biological function annotations and canonical pathways. Functional annotations in IPA are organised into major categories and each category contains subcategories of more specific annotations. All the major functional annotations for a given comparison are shown in the results. In addition, functional annotations which represent a key cellular and molecular events during differentiation were further scrutinized for their subcategories annotations. For canonical pathways, all significantly over-represented neurological pathways are shown in the results as well as the top 20 significantly over-represented pathways of other functions.

IPA results are presented by divided into two parts. First, shows ATRA effect on SH-SY5Y cells and in this part, the function annotations and canonical pathways over-represented in the (WT(C) vs. WT(R)) comparison are shown. Second part shows *TOP2B* knockout effects on ATRA treated and untreated SH-SY5Y and in this part, the function annotations and canonical pathways over-represented in the (WT(C) vs. BKO(C)) and the (WT(R) vs. BKO(R)) comparisons are shown.

6.3.3.2.1 ATRA effect

Biological functions and pathways correlated with RA metabolism and signalling pathway enriched in the (WT(C) vs. WT(R)) comparison are shown in (Figure 6.19). Results confirmed activation of functions and pathways related to biosynthesis and metabolism of RA as well as activation of RA receptors like RAR and RXR. ATRA treatment induced enrichment of a wide range of annotations associated with cellular, molecular, and physiological processes (Figure 6.20). These major categories were further mined for more specific function annotations which are correlated with cellular and morphological changes acquired during cell differentiation (Figure 6.21).

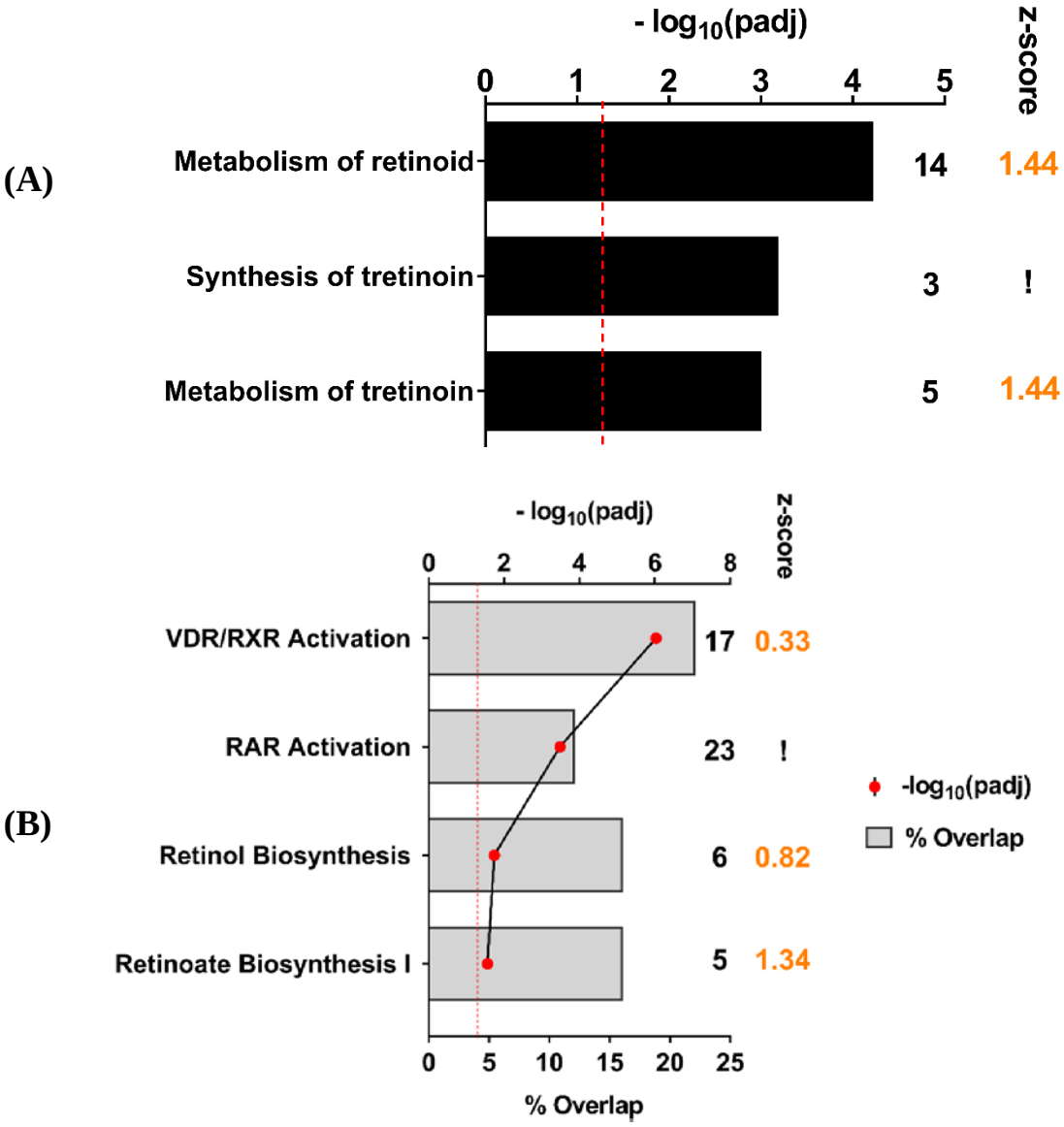


Figure 6-19 ATRA effect on functions and pathways involved in RA metabolism and signalling pathway

IPA analysis of RNA-seq data from wild type SH-SY5Y cells treated with 10 μM ATRA for 24 hrs shows **(A)** Biological function annotations and **(B)** Canonical pathways related to RA metabolism and signalling pathway. Red dots represent $-\log_{10}$ of padj values of a function or pathway and were plotted on upper axis. Bars represent percentage of overlapping between DEGs in experiment and total number of genes belong to a given function or pathway. Red dashed line represent padj cutoff of 0.05. Padj was calculated by IPA algorithm using Benjamini-Hochberg multiple testing correction method. Numbers of DEGs assigned to function or pathway are shown in black colour. Z-score values were determined as described in (materials and methods, section 2.14.2.3) and represent predicted level and direction (activation or inhibition) of a function or pathway. Z-score value of positive (orange), negative (blue), or zero (black) represents activation, inhibition, or no change respectively, while (!) function or pathway is "currently ineligible for a prediction" by IPA.

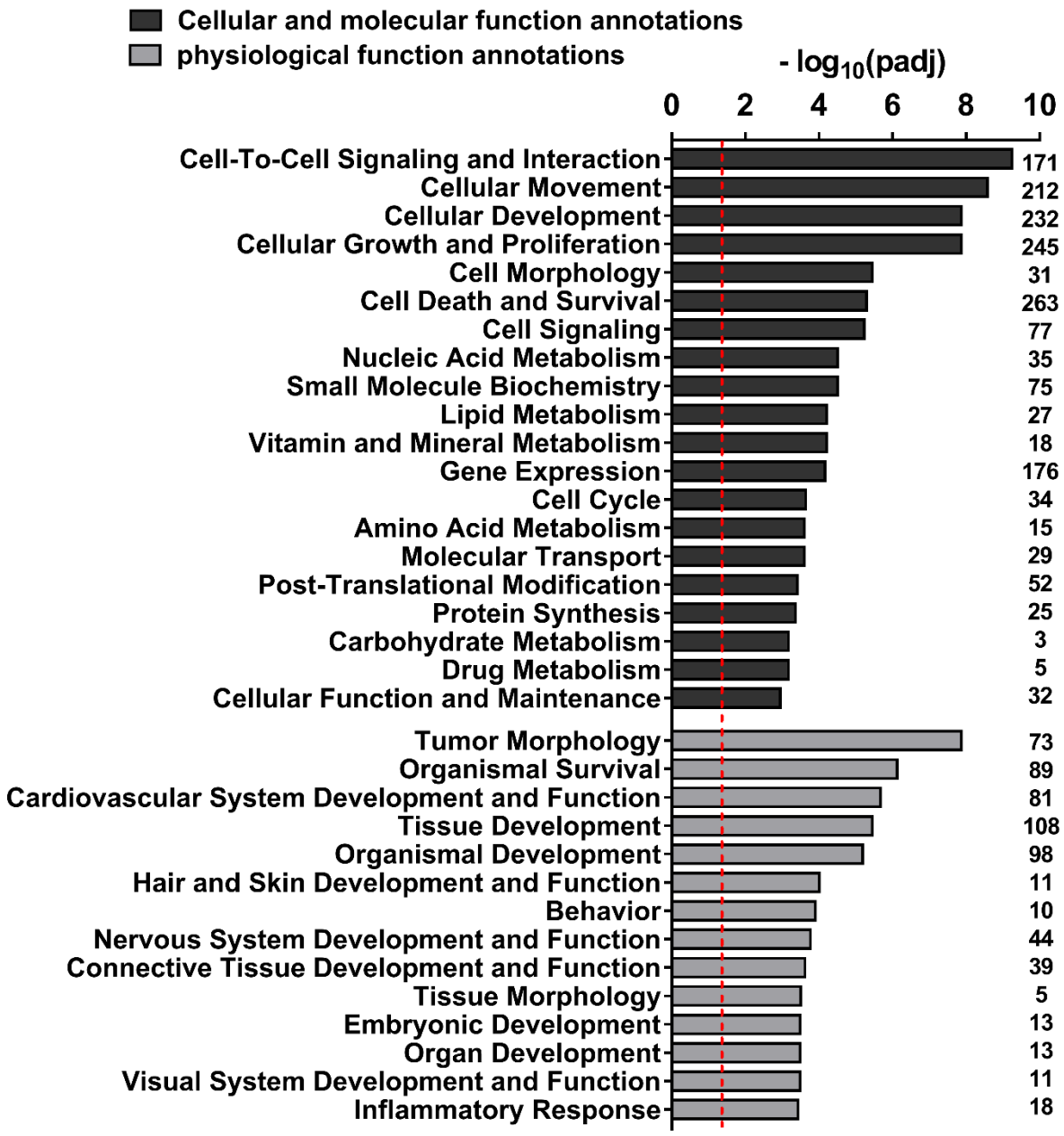


Figure 6-20 Function annotations over-represented in SH-SY5Y upon ATRA treatment.

IPA analysis of RNA-seq data from wild type SH-SY5Y cells treated with 10 μM ATRA for 24 hrs shows all major categories of function annotations enriched. Numbers of DEGs assigned to annotation are shown in black colour. Red dashed line represent padj cutoff of 0.05. Padj was calculated by IPA algorithm using Benjamini-Hochberg multiple testing correction method. Bars represent minus log10 of padj values.

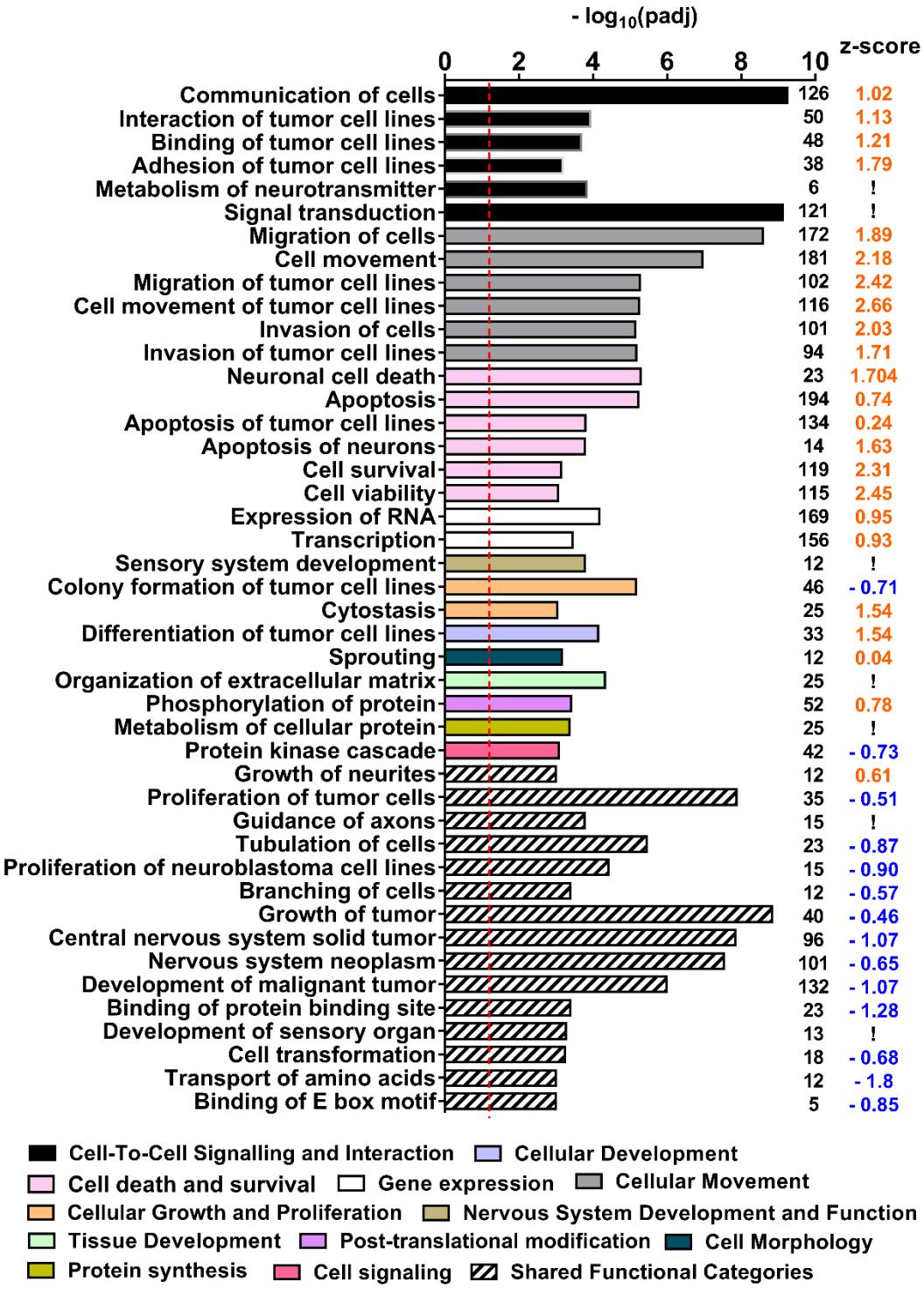


Figure 6-21 Annotation subcategories associated with RA-induced differentiation

IPA analysis of RNA-seq data from wild type SH-SY5Y cells treated with 10 μM ATRA for 24 hrs shows subcategories of function annotations related to cellular and morphological changes which associated with neurological differentiation. Numbers of DEGs assigned to annotation are shown in black colour. Red dashed line represent padj cutoff of 0.05. Padj was calculated by IPA algorithm using Benjamini-Hochberg multiple testing correction method. Bars represent minus log10 of padj values. Z-score value of positive (orange), negative (blue), or zero (black) represents activation, inhibition, or no change respectively, while (!) function or pathway is “currently ineligible for a prediction” by IPA.

Overall, results from (Figure 6.21) showed cellular and morphological changes toward neuronal differentiation. These changes included inhibition prediction of annotations for proliferation of tumor cells ($-\log_{10}$ padj = 7.8, 35 DEGs, z-score = - 0.51) and neuroblastoma cell line ($-\log_{10}$ padj = 4.4, 35 DEGs, z-score = - 0.9), colony formation of tumour cell lines ($-\log_{10}$ padj = 5.2, 46 DEGs, z-score = -0.71), growth of tumor ($-\log_{10}$ padj = 8.84, 40 DEGs, z-score = - 0.46), central nervous system solid tumor ($-\log_{10}$ padj = 7.84, 96 DEGs, z-score = - 1.07), nervous system neoplasm ($-\log_{10}$ padj = 7.54, 101 DEGs, z-score = - 0.65), development of malignant tumor ($-\log_{10}$ padj = 5.99, 132 DEGs, z-score = - 1.07), cell transformation ($-\log_{10}$ padj = 3.23, 18 DEGs, z-score = - 0.68) and activation prediction of cytostasis annotation ($-\log_{10}$ padj = 3, 25 DEGs, z-score = 1.54).

This was accompanied by activation prediction of annotations for growth of neurite ($-\log_{10}$ padj = 3, 12 DEGs, z-score = 0.61), differentiation of tumour cell line ($-\log_{10}$ padj = 4.14, 33 DEGs, z-score = 1.54), communication of cells ($-\log_{10}$ padj = 9.25, 126 DEGs, z-score = 1.02), adhesion of tumor cell lines ($-\log_{10}$ padj = 3.18, 38 DEGs, z-score = 1.79), cell movement ($-\log_{10}$ padj = 6.95, 181 DEGs, z-score = 2.18) and migration of cells ($-\log_{10}$ padj = 8.58, 172 DEGs, z-score = 1.89) (Figure 6.21). Undifferentiated SH-SY5Y cells are characterized by short processes. Once differentiated by RA, these short processes disappeared and long neurites formed. This might be supported by the inhibition of branching annotations upon ATRA treatment (Figure 6.21).

IPA analysis showed over-representation of metabolism of neurotransmitter, sensory system development, and guidance of axons annotations without directional prediction score as they were not eligible for z-score calculation (see section 2.14.2.3). The DEGs involved in growth of neurites annotation were (*APOE*, *BCL2*, *BMP4*, *CDK5R2*, *EFNB2*, *EPHA2*, *GFRA2*, *ITGA1*, *RIT1*, *SLIT2*, *TGM2*, and *TNC*). *BCL2* has been shown to be induced upon neuronal differentiation and was induced upon ATRA treatment in this study. Majority of other genes encode for neuronal receptors. For instance, *CDK5R2* encodes a neuron-specific activator of CDK5 kinase. *EFNB2* and *EPHA2* (encodes for Ephrin B2 and Ephrin Type-A Receptor 2 respectively), are members of ephrin receptor subfamily which has been shown to be involved in development of nervous system. *GFRA2* gene product is a receptor for GDNF, a neurotrophic factor has a critical role in the differentiation and survival of neuron. ITGA1 encodes for a receptor for laminin and collagen. *RIT1* product involves in neuron regeneration and development alongside the nerve growth factor. *SLIT2* encodes for a member of slit family that have a key roles in migration of neuron and axon guidance.

The DEGs involved in guidance of axons annotation were (*GFRA1, RET, SLIT2, NRXN1, OPHN1, SEMA3B, GFRA2, SPTBN2, GFRA3, PRKCA, UNC5A, DOK4, CXCR4, RELN*, and *ANOS1*). Products of these genes involved activator proteins, cell surface and neuronal receptors. *NRXN1* gene product is a member of neurexin receptor family which form a complex with neuroligins to facilitate neurotransmission at synaptic contacts. *SEMA3B* encodes for a member of semaphorin family which have a well known role in neuronal system via involvement in guidance of growth of neuronal cone. *SPTBN2* encodes for a member of spectrins which involved in membrane-cytoskeleton formation. *UNC5A* encodes for a member of family of receptors to netrin-1 which has a chemorepulsion activity to particular axons. *RELN* (Reelin) has been shown to play a key role in controlling cell-to cell interaction that required for neuronal migration.

Canonical pathways that were mostly enriched from DEGs of (WT(C) vs. WT(R)) comparison are presented in (Figure 6.22). Eight neuronal pathways were enriched from the dataset with axon guidance signalling pathway the most significant pathway including the largest number of DEGs from the comparison dataset (47 genes) among all other pathways. From the 47 DEGs encompassing the axonal guidance signalling pathway, 27 genes were significantly upregulated (*NTRK2, ENPEP, BMP4, ADAM29, PIK3CG, PRKCH, MMP28, ECEL1, SLIT2, GLI1, GNG2, MMP11, EPHB3, ADAMTS6, FZD7, ADAMTS2, NTRK1, RRAS, SEMA6C, PLXNA2, GLIS2, RAC2, SEMA3B, KALRN, ADAMTS9, PLCD3*, and *EFNB2*) and 20 genes were downregulated (*PLCH2, PLXNC1, PRKCA, EPHA2, UNC5A, ADAMTS4, SEMA3G, FGFR2, NOTUM, SEMA3C, NRP2, PAPPA, KCNJ12, WNT4, CXCR4, NTNG1, UNC5D, NTRK3, ADAMTS17*, and *NRP1*). Details for differential expression of these genes are in (Table 6.6). No prediction was made for axonal guidance pathway because there is no one activation state for this pathway as explained in the materials and methods (section 2.14.2.3).

In supporting of neuronal commitment, the synaptogenesis signaling pathway was activated upon ATRA treatment ($-\log_{10}$ padj = 1.62, 27 DEGs, z-score = 1.57) (Figure 6.22).

The visual cycle pathway was significantly over-represented with high z-score prediction for activation (z-score = 2.24, DEGs = 5). This agreed with well established role of vitamin A in visual system.

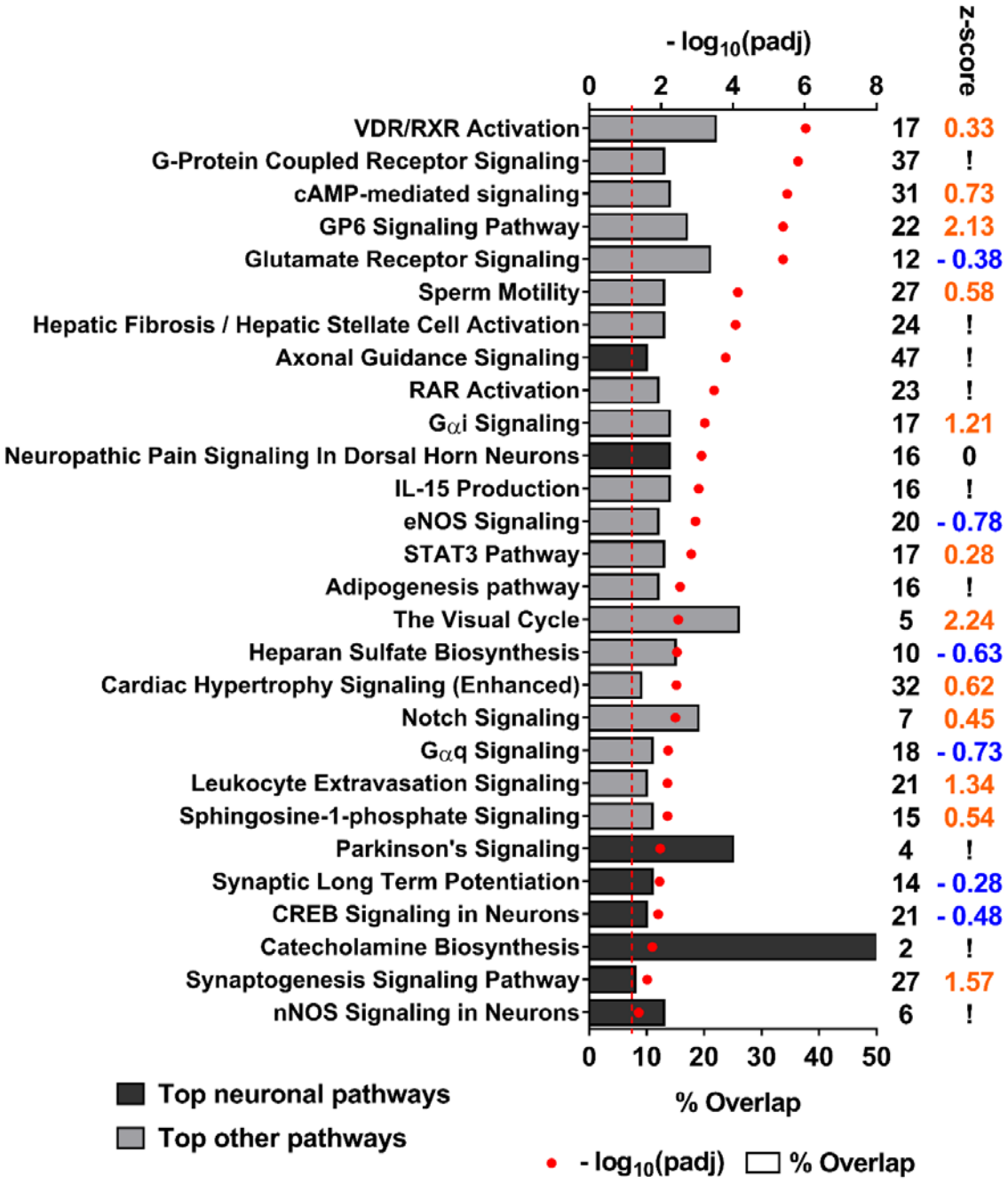


Figure 6-22 Canonical pathways over-represented in SH-SY5Y cells upon ATRA treatment

IPA analysis of RNA-seq data from wild type SH-SY5Y cells treated with 10 μM ATRA for 24 hrs shows canonical pathways enriched. Red dots represent $-\log_{10}$ of padj values of a pathway and were plotted on upper axis. Bars represent percentage of overlapping between DEGs in experiment and total number of genes belong to a pathway and were plotted on lower axis. Black bars represent neuronal pathways and grey bars represent top 20 of other non-neuronal pathways. Numbers of DEGs assigned to a pathway are shown in black colour. Red dashed line represent padj cutoff of 0.05. Padj was calculated by IPA algorithm using Benjamini-Hochberg multiple testing correction method. Z-score value of positive (orange), negative (blue), or zero (black) represents activation, inhibition, or no change respectively, while (!) function or pathway is "currently ineligible for a prediction" by IPA.

6.3.3.2.2 TOP2B effect

The effect of *TOP2B* knockout in SH-SY5Y cells was investigated in this study. IPA analysis was performed on DEGs from two comparisons: (WT(C) vs. BKO(C)) and (WT(R) vs. BKO(R) to investigate biological functions and pathways affected by absence of TOP2β.

In untreated SH-SY5Y cells, *TOP2B* knockout induced over-representation of a range of functional annotations (Figure 6.23). Within the cellular molecular and molecular annotations group, 14 major functional annotations were over-represented with cellular movement ($-\log_{10}$ = 8.37, 304 DEGs), cell-to-cell signaling and interaction ($-\log_{10}$ = 8.37, 217 DEGs), and cell morphology ($-\log_{10}$ = 7.1, 116 DEGs) the top 3 annotations enriched (Figure 6.23). Interestingly, within the physiological annotations group, nervous system development and function annotation was the most significantly over-represented ($-\log_{10}$ = 8.37, DEGs = 53).

The major categories were further investigated into the subcategories for function annotations that associated with molecular and cellular events expected to be acquired or changed during neuronal cell development (Figure 6.24). Analysis showed over-representation of a range of cellular processes associated with morphology and development of neuronal cells. Key cellular processes involved in neuronal cell morphology were inhibited upon *TOP2B* knockout included cell spreading, microtubule dynamics, organization of cytoskeleton, assembly of cells, and formations of cellular protrusions (Figure 6.24). Moreover, guidance of axons and synaptic transmission were significantly over-represented without prediction made by IPA. On the other hand, other annotations such as communication of cells and secretion of neurotransmitter were activated.

Canonical pathway analysis showed the axonal guidance signaling pathway as one of the most significant over-represented pathways ($-\log_{10}$ padj = 9.12, DEGs = 82) (Figure 6.25). Synaptogenesis signaling pathway showed the highest z-score among all pathways with remarkable inhibition prediction (z-score = - 4.24, DEGs = 50). Eleven other neuronal pathways were also over-represented (Figure 6.25). Other pathways that showed high inhibition prediction scores involved glutamate receptor signaling (z-score = - 2.31, DEGs = 180 calcium signaling (z-score = - 2.12, DEGs = 35) super pathway of serine and glycine biosynthesis I (z-score = - 2.24, DEGs = 5), and eNOS signaling (z-score = - 2.71, DEGs = 26). On the other hand, pathways that showed high activation prediction scores involved cardiac hypertrophy signaling (Enhanced) (z-score = 2.43, DEGs = 61), osteoarthritis pathway (z-score = 1.18, DEGs = 40), protein kinase A signaling (z-score = 1.41, DEGs = 54), and notch signaling (z-score = 1.13, DEGs = 9) (Figure 6.25).

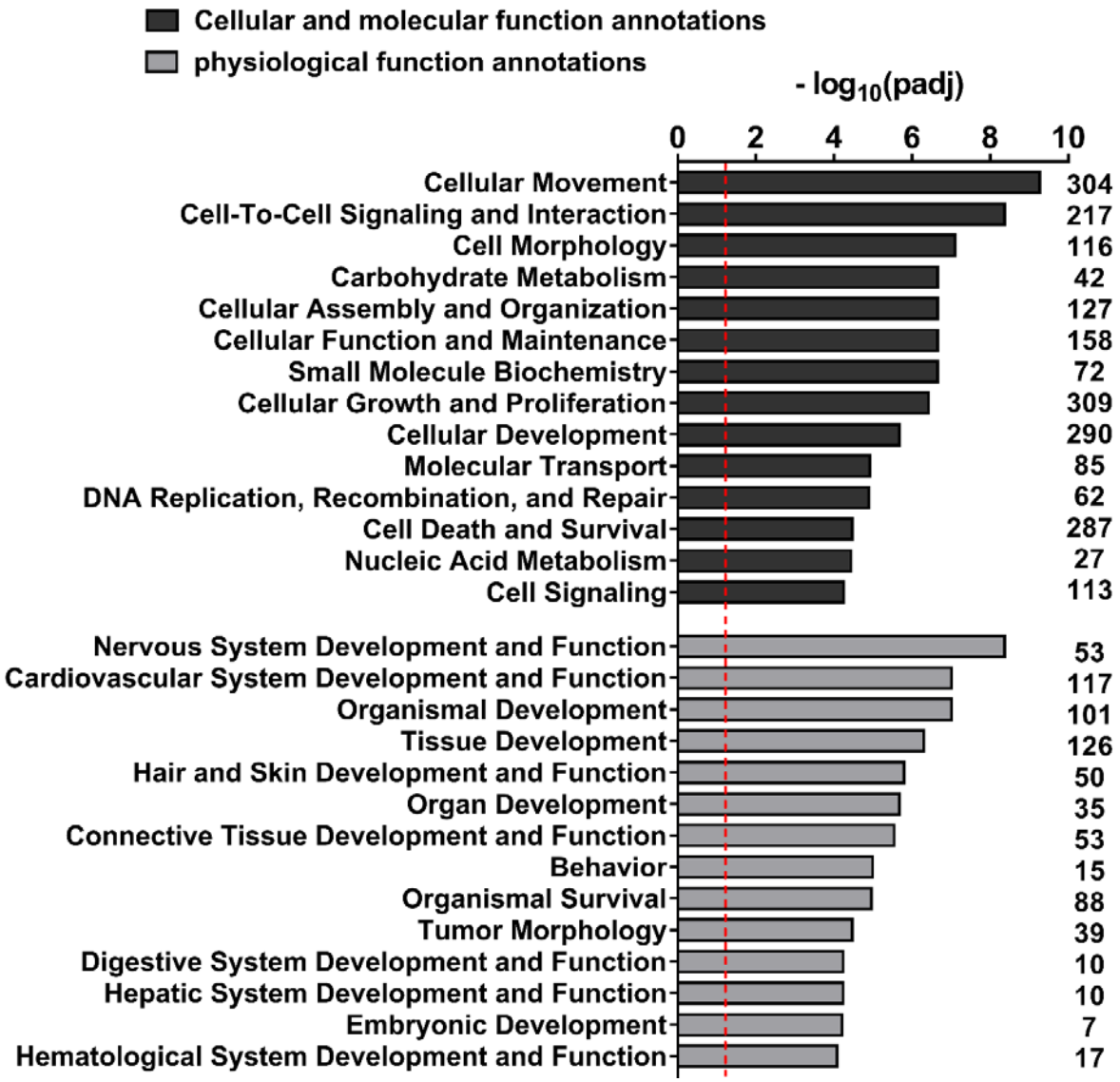


Figure 6-23 Function annotations over-represented in SH-SY5Y upon *TOP2B* knockout.

IPA analysis of DEGs in (WT(C) vs. BKO(C)) comparison shows all major categories of function annotations enriched. Numbers of DEGs assigned to annotation are shown in black colour. Red dashed line represent padj cutoff of 0.05. Padj was calculated by IPA algorithm using Benjamini-Hochberg multiple testing correction method. Bars represent minus log10 of padj values.

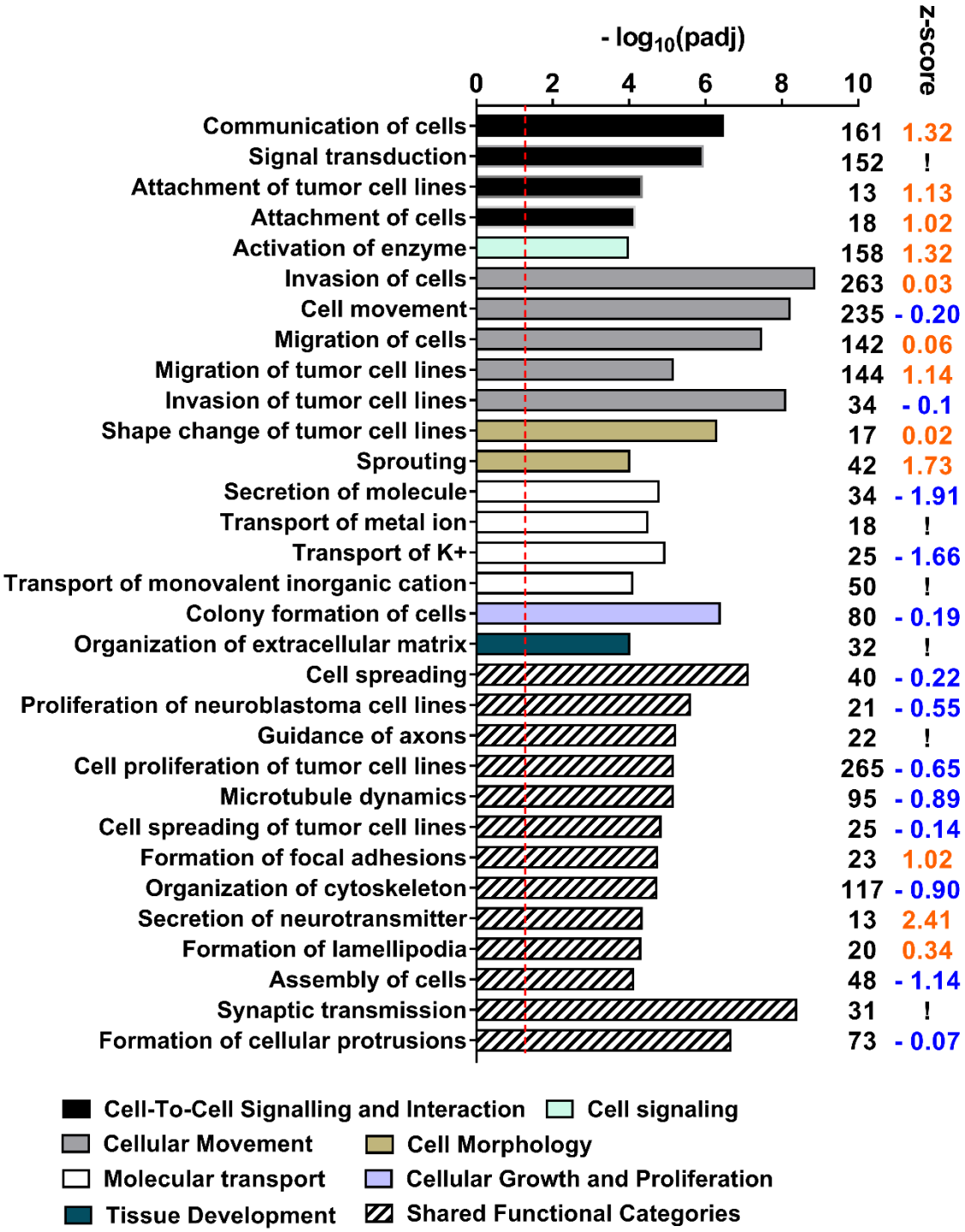


Figure 6-24 Annotation subcategories which related to neuronal development and were over-represented in the absence of TOP2β.

IPA analysis of DEGs in (WT(C) vs. BKO(C)) comparison shows subcategories of function annotations related to cellular and morphological changes which associated with neurological development. Numbers of DEGs assigned to annotation are shown in black colour. Red dashed line represent padj cutoff of 0.05. Padj was calculated by IPA algorithm using Benjamini-Hochberg multiple testing correction method. Bars represent minus log10 of padj values. Z-score value of positive (orange), negative (blue), or zero (black) represents activation, inhibition, or no change respectively, while (!) function or pathway is "currently ineligible for a prediction" by IPA.

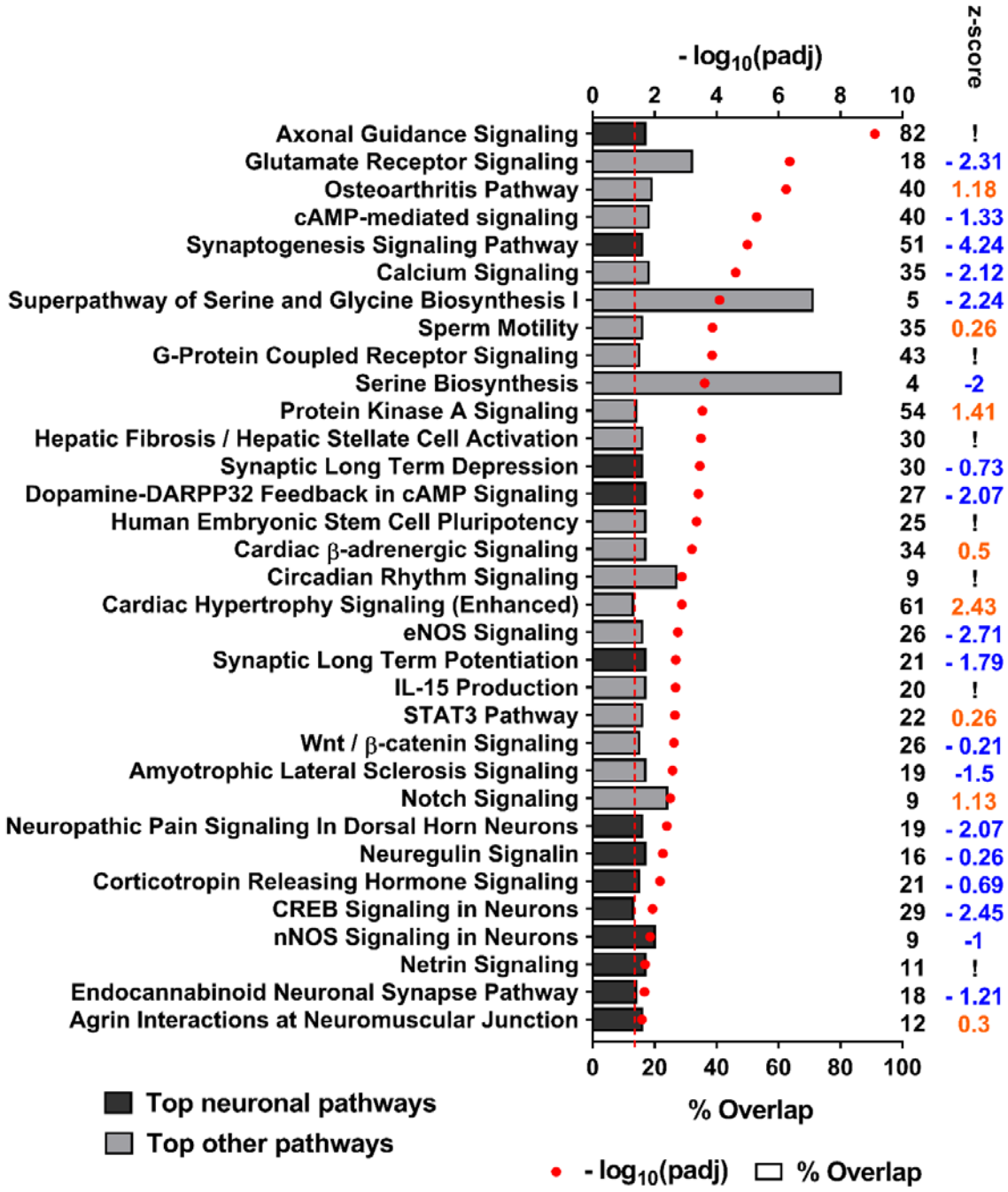


Figure 6-25 Canonical pathways over-represented in SH-SY5Y cells upon *TOP2B* knockout.

IPA analysis of DEGs in (WT(C) vs. BKO(C)) comparison shows over-represented canonical pathways. Red dots represent –log10 of padj values of a pathway and were plotted on upper axis. Bars represent percentage of overlapping between DEGs in experiment and total number of genes belong to a pathway and were plotted on lower axis. Black bars represent neuronal pathways and grey bars represent top 20 of other non-neuronal pathways. Numbers of DEGs assigned to pathway are shown in black colour. Red dashed line represent padj cutoff of 0.05. Padj was calculated by IPA algorithm using Benjamini-Hochberg multiple testing correction method. Z-score value of positive (orange), negative (blue), or zero (black) represents activation, inhibition, or no change respectively, while (!) function or pathway is “currently ineligible for a prediction” by IPA.

The role of TOP2β was studied in the context of ligand induced transcription and differentiation of SH-SY5Y cells. Wild type and *TOP2B* knockout cells were treated with (10 μM) all-trans-retinoic acid for 24 hours then RNA samples from both cell types were extracted and prepared for RNA-seq as described in (materials and methods, section 2.14.2), then the genes dataset was analysed for biological function and canonical pathway over-representation using IPA algorithm. IPA analysis showed significant over-representation of various functional annotations with (cell-to-cell signaling and interaction) and (nervous system development and function) annotations were the most significantly over-represented terms (Figure 6.26).

Further investigation of subcategories for functional annotations depicted in (Figure 6.26) showed over-representation of key events involved in neuronal cell development. For example, results showed inhibition predictions for annotations of cell spreading, development of body axis, assembly of cells (Figure 6.27). Other neuronal annotations were significantly over-represented without z-score prediction involved synaptic transmission, neurotransmission, catabolism of neurotransmitter, and guidance of axons (Figure 6.27).

Canonical pathways that were over-represented in (WT(R) vs. BKO(R)) comparison are presented in (Figure 6.28). Axonal guidance signaling pathway again come first in the top of the most significantly over-represented pathways (-log padj = 11.2, DEGs = 88) followed by synaptogenesis signaling pathway (-log padj = 9.01, DEGs = 62) and the latter showed high inhibition prediction score of (z-score = - 3.36) in the *TOP2B* knockout cells. Endocannabinoid neuronal synapse pathway was also significantly over-represented (-log padj = 3.35, DEGs = 23) with high score of inhibition prediction of (z-score = - 2.13). Eleven other neuronal pathways were over-represented and are presented in (Figure 6.28).

Results showed a range of pathways of other functions with cardiac hypertrophy signaling (Enhanced) was one of the pathways that showed high activation prediction score (z-score = 2.32, DEGs = 67).

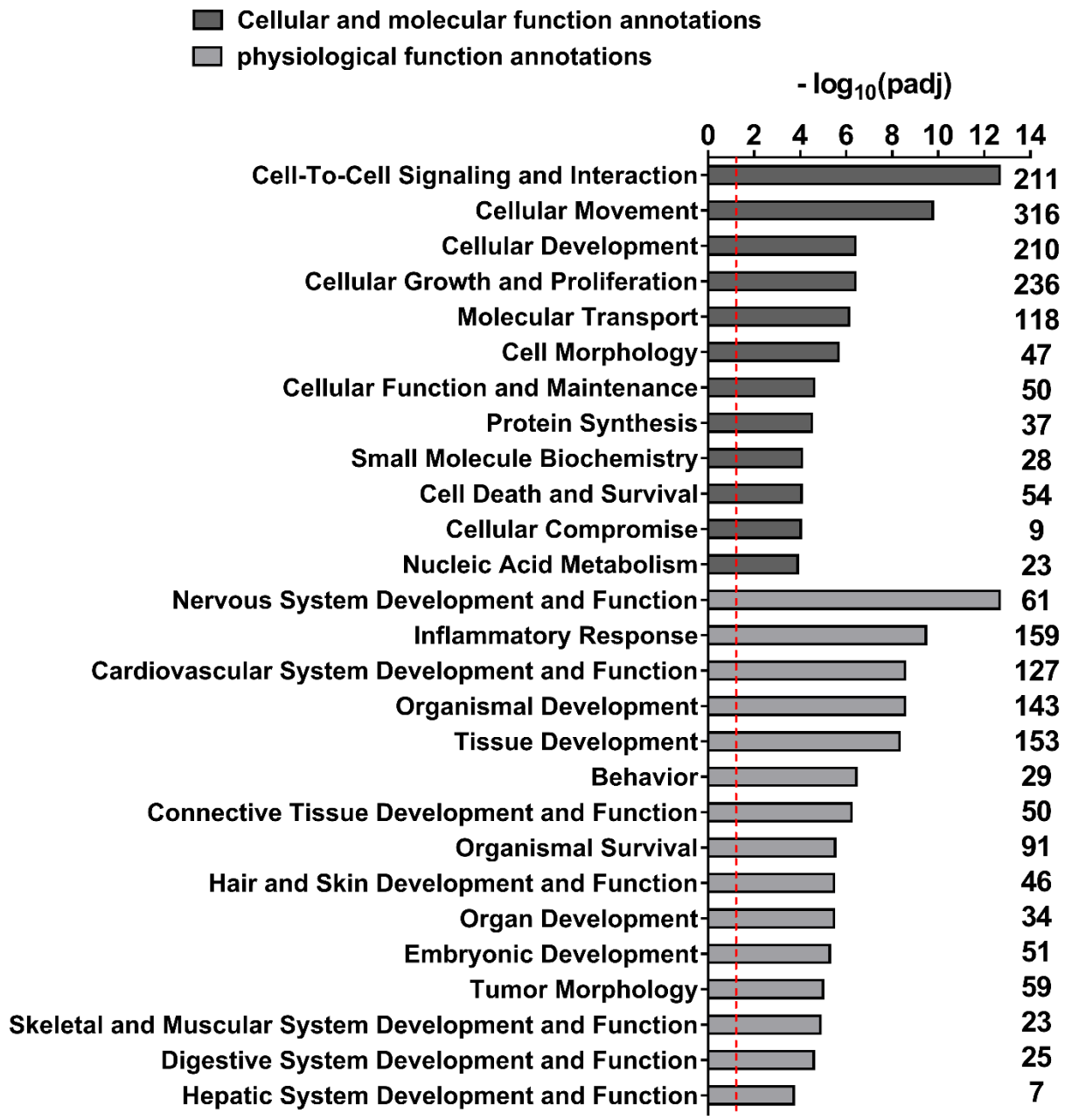


Figure 6-26 Function annotations which were over-represented upon ATRA treatment in the absence of TOP2β.

IPA analysis of DEGs in (WT(R) vs. BKO(R)) comparison shows all major categories of function annotations enriched. Numbers of DEGs assigned to annotation are shown in black colour. Red dashed line represent padj cutoff of 0.05. Padj was calculated by IPA algorithm using Benjamini-Hochberg multiple testing correction method. Bars represent minus log10 of padj values.

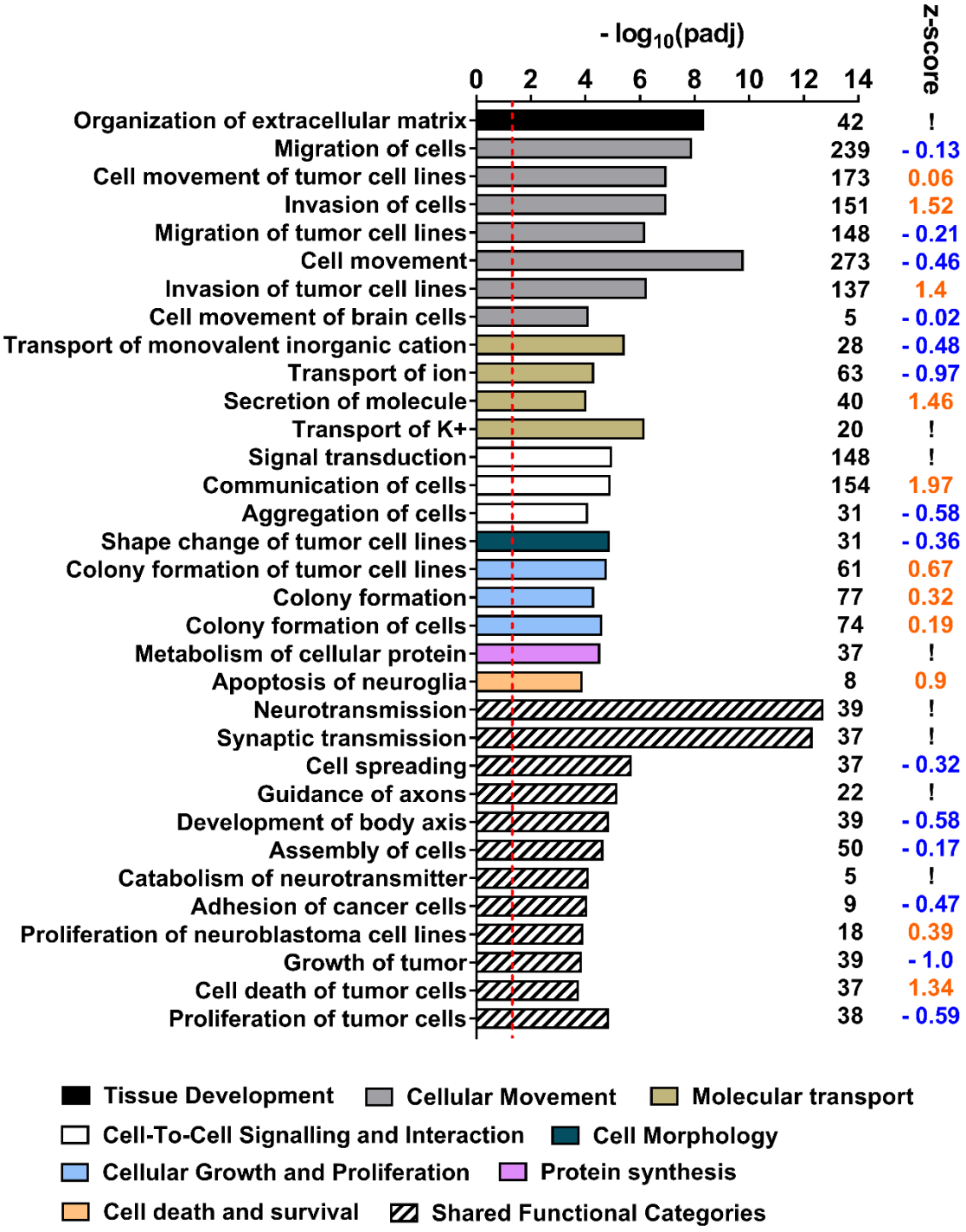


Figure 6-27 Annotation subcategories which related to neuronal development and were over-represented upon ATRA treatment in the absence of TOP2β.

IPA analysis of DEGs in (WT(R) vs. BKO(R)) comparison shows subcategories of function annotations related to cellular and morphological changes which associated with neurological development. Numbers of DEGs assigned to annotation are shown in black colour. Red dashed line represent padj cutoff of 0.05. Padj was calculated by IPA algorithm using Benjamini-Hochberg multiple testing correction method. Bars represent minus log10 of padj values. Z-score value of positive (orange), negative (blue), or zero (black) represents activation, inhibition, or no change respectively, while (!) function or pathway is "currently ineligible for a prediction" by IPA.

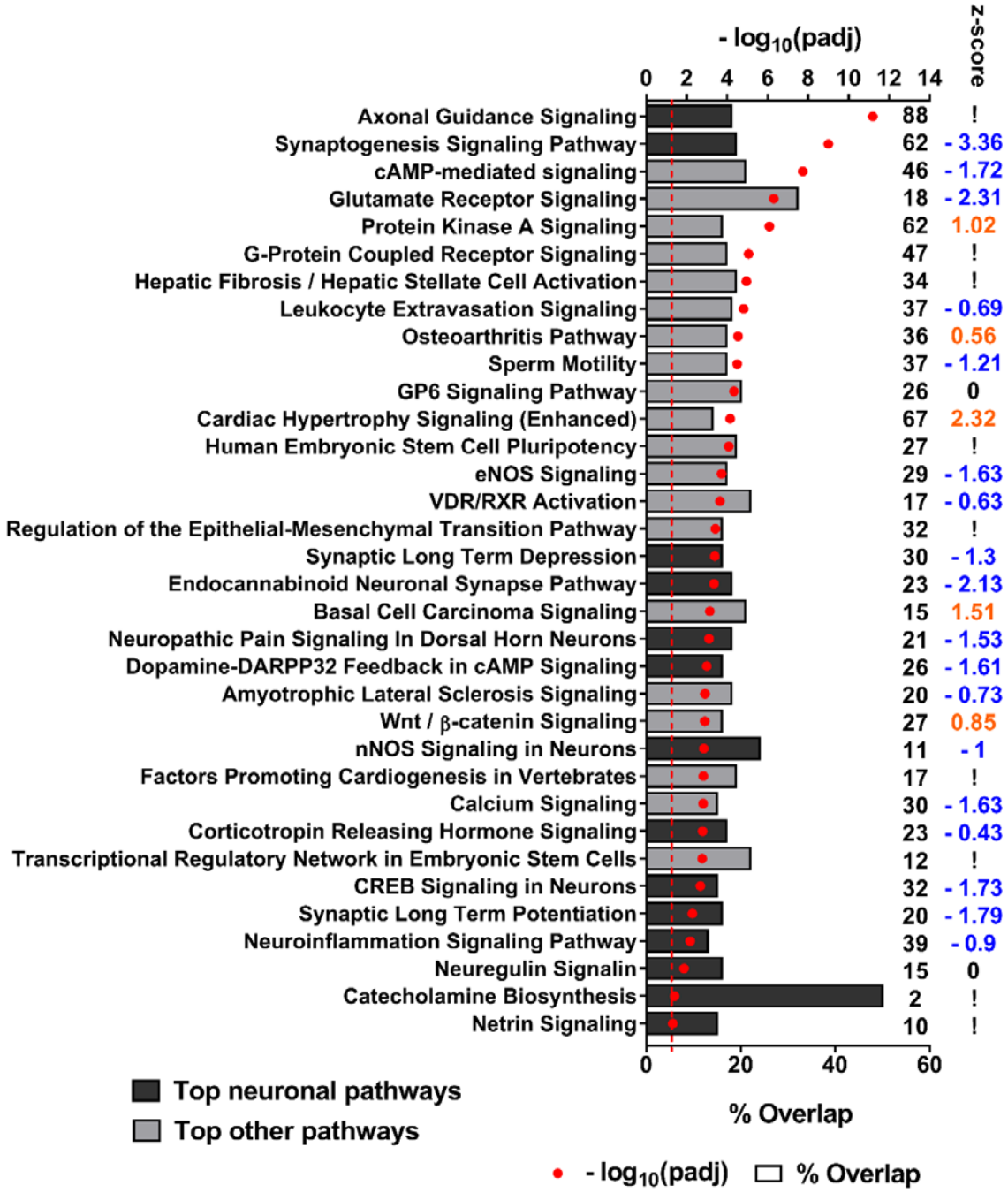


Figure 6-28 Canonical pathways over-represented upon ATRA treatment in the absence of TOP2β.

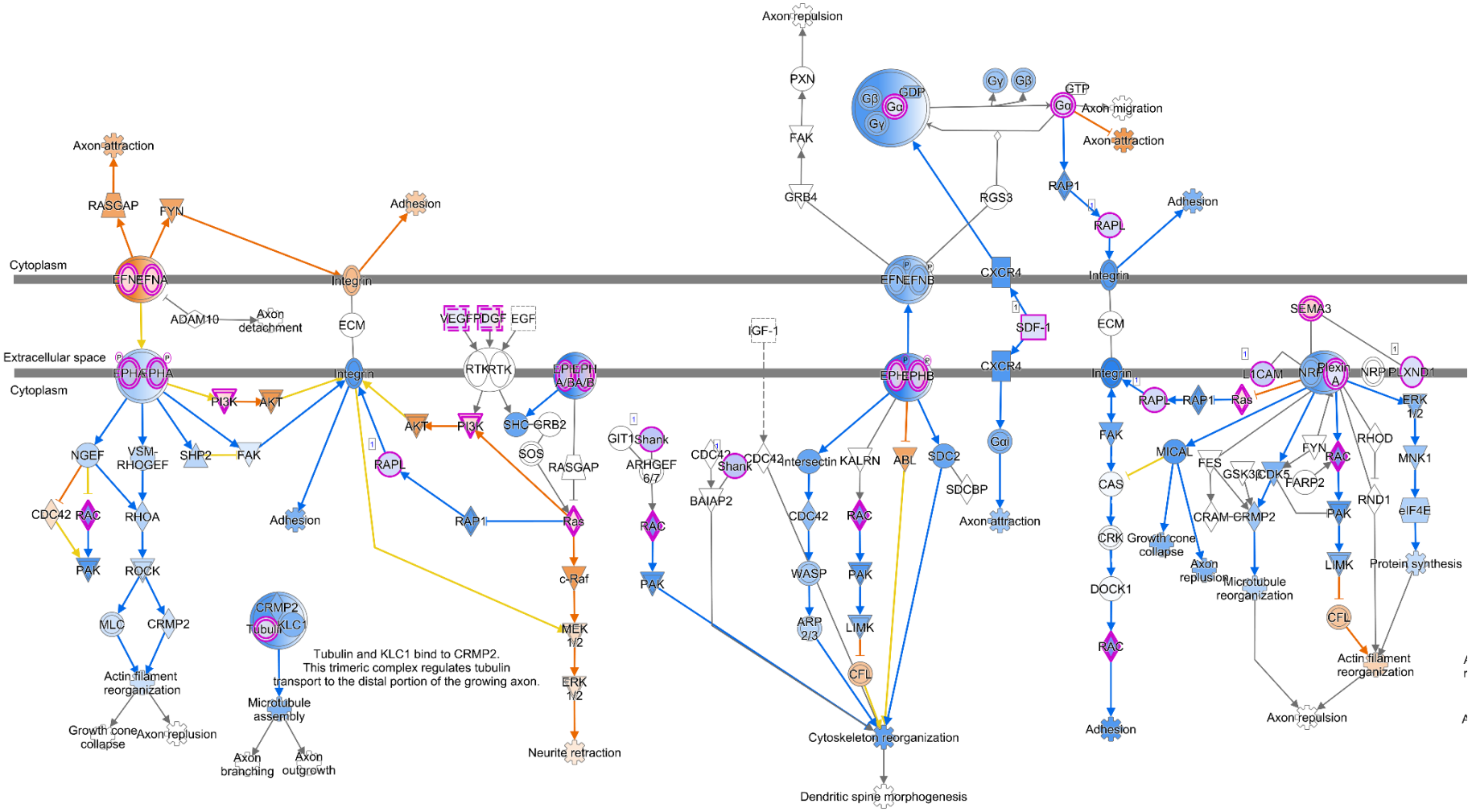
IPA analysis of DEGs in (WT(R) vs. BKO(R)) comparison shows over-represented canonical pathways. Red dots represent –log10 of padj values of a pathway and were plotted on upper axis. Bars represent percentage of overlapping between DEGs in experiment and total number of genes belong to a pathway and were plotted on lower axis. Black bars represent neuronal pathways and grey bars represent top 20 of other non-neuronal pathways. Numbers of DEGs assigned to pathway are shown in black colour. Red dashed line represent padj cutoff of 0.05. Padj was calculated by IPA algorithm using Benjamini-Hochberg multiple testing correction method. Z-score value of positive (orange), negative (blue), or zero (black) represents activation, inhibition, or no change respectively, while (!) function or pathway is “currently ineligible for a prediction” by IPA.

Axonal guidance signaling pathway was selected for detailed graph presentation as it was one of the crucial pathways in neuronal development and showed the most significant over-representation in both (WT(C) vs. BKO(C)) and (WT(R) vs. BKO(R)) comparisons. IPA graph representation of axon guidance signaling pathway in (WT(R) vs. BKO(R)) comparisons dataset is depicted in (Figure 6.29). This graph presentation includes molecules differential expression, nodes relationships, and regulation prediction of nodes as inferred by IPA using molecule activity prediction (MAP) option from overlay function in IPA software.

DEGs belong to this pathway (DEGs = 88) encode for transcription regulators, transmembrane receptors, growth factors, kinases and enzymes (Table 6.5) which altogether involve in downstream functions associated with alternation between attraction and repulsion of axon as well as other functionally related tasks such as cytoskeleton reorganization, axon branching and outgrowth, actin filament reorganization, growth cone collapse, microtubule assembly and reorganization (Figure 6.29). From these 88 DEGs, 54 and 34 genes were significantly down and up regulated respectively in the *TOP2B* knockout clone as compare with wild type (Table 6.5).

Specifically, downregulated genes in the *TOP2B* knockout cells involved neuronal growth factors, cell surface receptors and other adapter proteins. These include members of family of neurotrophic receptor tyrosine kinase *NTRK1*, *NTRK2*, and *NTRK3* (downregulated by 1.18, 3.63, and 5.11 log2 fold respectively), Three members of semaphorin family (*SEMA4B, SEMA6D*, and *SEMA7A*) were downregulated by 2, 1.69, and 1.38 log2 fold respectively with two of semaphorin receptors plexins *PLXND1* and *PLXNA2* (were downregulated by 2.11 and 1.66 log2 fold respectively). In addition, member of netrins (*NTNG2*) and nitrin receptors (*UNC5A*) were downregulated in the *TOP2B* knockout cells by 1.82 and 5.29 log2 fold respectively. Moreover, two members of slit proteins *SLIT1* and *SLIT2* and one of the related receptors *ROBO2* were downregulated in the *TOP2B* knockout cells by 3.77, 3.43, and 1.36 log2 fold respectively. Five members of Eph receptors family *EPHA10, EPHA8, EPHB3, EPHA6*, and *EPHB2* (were downregulated by 4.07, 3.63, 3.11, 1.46, and 1.06 log2 fold respectively). On the other hand, other genes were upregulated and they encode for frizzled receptors (*FZD1, FZD7*, and *FZD8*), ephrins (*EFNA4*), netrins (*NTN5* and *NTNG1*), semaphorins (*SEMA3G* and *SEMA5A*), and Wnt family members (*WNT5A, WNT16*, and *WNT7B*) (Table 6.5).

-Axonal guidance signaling pathway upon ATRA treatment in the absence of TOP2β (part 1)



-Axonal guidance signaling pathway upon ATRA treatment in the absence of TOP2β (part 2)

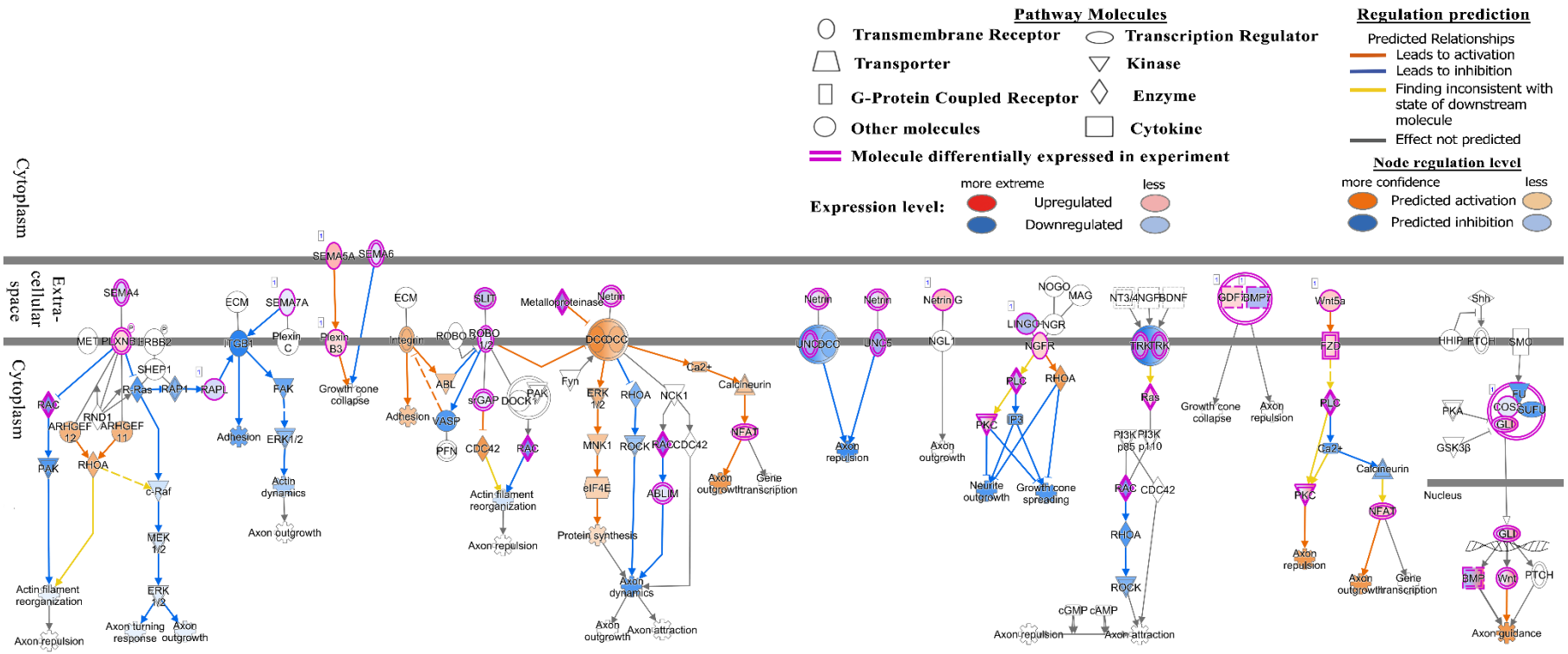


Figure 6-29 IPA graph representation of axonal guidance signaling pathway upon ATRA treatment in the absence of TOP2β

IPA graph of axonal guidance signaling pathway over-represented in (WT(R) vs. BKO(R)) comparison shows both upstream and downstream predicted effect of differential expression of molecules in experiment. Graph legends are shown in part 2 of graph.

Table 6-5 DEGs involved in axonal guidance signaling pathway regulation upon ATRA treatment in the absence of TOP2β

Padj value of zero means very low value that fall out of the calculation scale.

Symbol	Entrez Gene Name	Exp Log Ratio	Exp padj	Location	Type(s)
RAC2	Rac family small GTPase 2	-7.48	8.05E-13	Cytoplasm	enzyme
PAPPA2	pappalysin 2	-7.319	9.76E-11	Extracellular Space	peptidase
ENPEP	glutamyl aminopeptidase	-6.185	0.0000473	Plasma Membrane	peptidase
UNC5A	unc-5 netrin receptor A	-5.288	2.58E-59	Plasma Membrane	transmembrane receptor
NTRK3	neurotrophic receptor tyrosine kinase 3	-5.106	3.13E-23	Plasma Membrane	kinase
KCNJ12	potassium voltage-gated channel subfamily J member 12	-4.614	2.47E-14	Plasma Membrane	ion channel
LINGO1	leucine rich repeat and Ig domain containing 1	-4.592	1.15E-60	Plasma Membrane	other
BMP7	bone morphogenetic protein 7	-4.456	6.11E-10	Extracellular Space	growth factor
EPHA10	EPH receptor A10	-4.066	3.37E-21	Plasma Membrane	transmembrane receptor
PLCH2	phospholipase C eta 2	-3.789	8.4E-31	Cytoplasm	enzyme
SLIT1	slit guidance ligand 1	-3.768	0.000000317	Extracellular Space	other
SHANK2	SH3 and multiple ankyrin repeat domains 2	-3.675	4.91E-08	Plasma Membrane	other
EPHA8	EPH receptor A8	-3.63	1.63E-23	Plasma Membrane	kinase
NTRK2	neurotrophic receptor tyrosine kinase 2	-3.629	0	Plasma Membrane	kinase
SLIT2	slit guidance ligand 2	-3.434	1.38E-29	Extracellular Space	other
MMP24	matrix metallopeptidase 24	-3.242	1.02E-220	Plasma Membrane	peptidase
L1CAM	L1 cell adhesion molecule	-3.241	4.44E-174	Plasma Membrane	other
MMP28	matrix metallopeptidase 28	-3.196	1.11E-67	Extracellular Space	peptidase
EPHB3	EPH receptor B3	-3.105	1.42E-43	Plasma Membrane	kinase
ADAMTS17	ADAM metallopeptidase with thrombospondin type 1 motif 17	-2.916	0.0000122	Extracellular Space	other
RASSF5	Ras association domain family member 5	-2.406	1.42E-11	Plasma Membrane	other
ADAM12	ADAM metallopeptidase domain 12	-2.207	9.23E-101	Plasma Membrane	peptidase
ADAMTS7	ADAM metallopeptidase with thrombospondin type 1 motif 7	-2.15	1.86E-32	Extracellular Space	peptidase
PLXND1	plexin D1	-2.114	2.69E-61	Plasma Membrane	transmembrane receptor
TUBA4A	tubulin alpha 4a	-2.011	2.64E-14	Cytoplasm	other

SEMA4B	**semaphorin 4B**	**-1.997**	**6.06E-11**	**Plasma Membrane**	**other**
PLCB2	**phospholipase C beta 2**	**-1.992**	**0.0000001**	**Cytoplasm**	**enzyme**
CXCL12	**C-X-C motif chemokine ligand 12**	**-1.989**	**2.88E-10**	**Extracellular Space**	**cytokine**
ABLIM2	**actin binding LIM protein family member 2**	**-1.921**	**2.93E-11**	**Cytoplasm**	**other**
ADAMTS2	**ADAM metallopeptidase with thrombospondin type 1 motif 2**	**-1.838**	**2.94E-10**	**Extracellular Space**	**peptidase**
NTNG2	**netrin G2**	**-1.821**	**0.000165**	**Plasma Membrane**	**other**
SEMA6D	**semaphorin 6D**	**-1.687**	**2.97E-48**	**Plasma Membrane**	**other**
PLXNA2	**plexin A2**	**-1.659**	**2.13E-19**	**Plasma Membrane**	**transmembrane receptor**
GNAO1	**G protein subunit alpha o1**	**-1.632**	**1.62E-59**	**Plasma Membrane**	**enzyme**
MMP15	**matrix metallopeptidase 15**	**-1.612**	**6.06E-11**	**Extracellular Space**	**peptidase**
KIF7	**kinesin family member 7**	**-1.532**	**2.96E-23**	**Extracellular Space**	**other**
EPHA6	**EPH receptor A6**	**-1.459**	**2.88E-16**	**Plasma Membrane**	**kinase**
MME	**membrane metalloendopeptidase**	**-1.394**	**1.18E-16**	**Plasma Membrane**	**peptidase**
SEMA7A	**semaphorin 7A (John Milton Hagen blood group)**	**-1.381**	**0.000000123**	**Plasma Membrane**	**transmembrane receptor**
MMP11	**matrix metallopeptidase 11**	**-1.359**	**1.71E-12**	**Extracellular Space**	**peptidase**
ROBO2	**roundabout guidance receptor 2**	**-1.356**	**3.86E-33**	**Plasma Membrane**	**transmembrane receptor**
GLIS2	**GLIS family zinc finger 2**	**-1.247**	**9.04E-09**	**Nucleus**	**transcription regulator**
PRKCH	**protein kinase C eta**	**-1.226**	**1.35E-09**	**Cytoplasm**	**kinase**
WNT3	**Wnt family member 3**	**-1.221**	**1.01E-36**	**Extracellular Space**	**other**
PDGFC	**platelet derived growth factor C**	**-1.203**	**0.00143**	**Extracellular Space**	**growth factor**
NTRK1	**neurotrophic receptor tyrosine kinase 1**	**-1.179**	**9.11E-12**	**Plasma Membrane**	**kinase**
TUBB4A	**tubulin beta 4A class IVa**	**-1.165**	**2.9E-21**	**Cytoplasm**	**other**
PLCL1	**phospholipase C like 1 (inactive)**	**-1.163**	**6.08E-11**	**Cytoplasm**	**enzyme**
ADAM29	**ADAM metallopeptidase domain 29**	**-1.132**	**0.00354**	**Plasma Membrane**	**peptidase**
SRGAP1	**SLIT-ROBO Rho GTPase activating protein 1**	**-1.101**	**0.000373**	**Cytoplasm**	**other**
EPHB2	**EPH receptor B2**	**-1.061**	**9.25E-14**	**Plasma Membrane**	**kinase**
ADAMTS10	**ADAM metallopeptidase with thrombospondin type 1 motif 10**	**-1.05**	**0.0000223**	**Extracellular Space**	**peptidase**
ADAM19	**ADAM metallopeptidase domain 19**	**-1.013**	**4.95E-24**	**Plasma Membrane**	**peptidase**
PRKCE	**protein kinase C epsilon**	**-1**	**0.00000544**	**Cytoplasm**	**kinase**

RAP2B	**RAP2B, member of RAS oncogene family**	**1.029**	**7.58E-23**	**Plasma Membrane**	**enzyme**
PDGFD	**platelet derived growth factor D**	**1.031**	**3.84E-17**	**Extracellular Space**	**growth factor**
FZD1	**frizzled class receptor 1**	**1.093**	**6.84E-68**	**Plasma Membrane**	**G-protein coupled receptor**
IRS2	**insulin receptor substrate 2**	**1.111**	**2.59E-102**	**Cytoplasm**	**enzyme**
ADAMTS20	**ADAM metallopeptidase with thrombospondin type 1 motif 20**	**1.127**	**4.93E-10**	**Extracellular Space**	**peptidase**
EFNA3	**ephrin A3**	**1.252**	**1.07E-12**	**Plasma Membrane**	**kinase**
PRKCA	**protein kinase C alpha**	**1.272**	**1.14E-170**	**Cytoplasm**	**kinase**
PLXNB3	**plexin B3**	**1.31**	**0.0000432**	**Plasma Membrane**	**transmembrane receptor**
ADAMTS12	**ADAM metallopeptidase with thrombospondin type 1 motif 12**	**1.385**	**8.36E-10**	**Extracellular Space**	**peptidase**
GDF7	**growth differentiation factor 7**	**1.392**	**0.000000066**	**Extracellular Space**	**growth factor**
ADAMTS19	**ADAM metallopeptidase with thrombospondin type 1 motif 19**	**1.463**	**2.26E-58**	**Extracellular Space**	**peptidase**
PRKCD	**protein kinase C delta**	**1.545**	**4.31E-18**	**Cytoplasm**	**kinase**
NGFR	**nerve growth factor receptor**	**1.549**	**9.66E-10**	**Plasma Membrane**	**transmembrane receptor**
FZD7	**frizzled class receptor 7**	**1.561**	**1.47E-105**	**Plasma Membrane**	**G-protein coupled receptor**
SEMA3G	**semaphorin 3G**	**1.685**	**0.0000339**	**Cytoplasm**	**other**
FGFR2	**fibroblast growth factor receptor 2**	**1.686**	**2.92E-10**	**Plasma Membrane**	**kinase**
ADAMTS5	**ADAM metallopeptidase with thrombospondin type 1 motif 5**	**1.773**	**1.8E-19**	**Extracellular Space**	**peptidase**
WNT5A	**Wnt family member 5A**	**1.808**	**0.00000742**	**Extracellular Space**	**cytokine**
EFNA4	**ephrin A4**	**1.836**	**3.35E-34**	**Plasma Membrane**	**kinase**
NFATC4	**nuclear factor of activated T cells 4**	**1.857**	**1.81E-50**	**Nucleus**	**transcription regulator**
ADAM21	**ADAM metallopeptidase domain 21**	**1.867**	**0.000729**	**Plasma Membrane**	**peptidase**
NTN5	**netrin 5**	**1.957**	**0.00949**	**Other**	**other**
EPHB4	**EPH receptor B4**	**2.063**	**8.04E-46**	**Plasma Membrane**	**kinase**
FZD8	**frizzled class receptor 8**	**2.149**	**3.41E-09**	**Plasma Membrane**	**G-protein coupled receptor**
NTNG1	**netrin G1**	**2.273**	**2.99E-11**	**Extracellular Space**	**other**
PAPPA	**pappalysin 1**	**2.454**	**2.41E-08**	**Extracellular Space**	**peptidase**
SEMA5A	**semaphorin 5A**	**2.541**	**1.36E-158**	**Plasma Membrane**	**transmembrane receptor**
WNT16	**Wnt family member 16**	**2.796**	**2.6E-13**	**Extracellular Space**	**other**
ADAMTS3	**ADAM metallopeptidase with thrombospondin type 1 motif 3**	**3.204**	**1.52E-82**	**Extracellular Space**	**peptidase**

ADAMTS4	**ADAM metallopeptidase with thrombospondin type 1 motif 4**	**3.396**	**2.48E-26**	**Extracellular Space**	**peptidase**
GLI1	**GLI family zinc finger 1**	**3.752**	**1.65E-09**	**Nucleus**	**transcription regulator**
WNT7B	**Wnt family member 7B**	**4.35**	**0.000463**	**Extracellular Space**	**other**
BMP4	**bone morphogenetic protein 4**	**4.574**	**2.33E-27**	**Extracellular Space**	**growth factor**
PRKD1	**protein kinase D1**	**4.93**	**9.61E-34**	**Cytoplasm**	**kinase**

The differentially expressed genes (DEGs) that belong to axonal guidance signaling pathway in WT(C) vs. WT(R), WT(C) vs. BKO(C), and WT(R) vs. BKO(R) comparisons are shown in (Table 6.6).

-Differential expression of genes that belong to axonal guidance signaling pathway

NO.	Gene	WT(C) vs WT(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
		Log2FC	padj	Log2FC	padj	Log2FC	padj
1	RAC2	1.23	3.97E-05	-4.16	5.31E-15	-7.48	8.05E-13
2	PAPPA2	1.74	2.78E-02	-7.36	1.27E-08	-7.32	9.78E-11
3	ENPEP	5.34	3.36E-04	NA	NA	-6.18	4.73E-05
4	UNC5A	-1.47	1.07E-09	-4.29	2.85E-67	-5.29	2.60E-59
5	NTRK3	-3.00	2.06E-11	-7.62	2.31E-49	-5.11	3.15E-23
6	KCNJ12	-2.04	7.34E-24	-3.86	5.92E-54	-4.61	2.46E-14
7	LINGO1	NA	NA	-3.89	2.68E-62	-4.59	1.15E-60
8	BMP7	NA	NA	-5.78	2.62E-16	-4.46	6.10E-10
9	EPHA10	NA	NA	-2.79	2.71E-14	-4.07	3.37E-21
10	PLCH2	-1.09	1.50E-06	-2.23	7.32E-22	-3.79	8.37E-31
11	SLIT1	NA	NA	-6.22	1.84E-16	-3.77	3.16E-07
12	SHANK2	NA	NA	-4.79	1.23E-14	-3.68	4.93E-08
13	EPHA8	NA	NA	-2.27	1.00E-12	-3.63	1.63E-23
14	NTRK2	7.67	0	-3.23	3.41E-09	-3.63	0
15	SLIT2	2.52	2.60E-16	-1.39	3.64E-05	-3.43	1.39E-29
16	MMP24	NA	NA	-2.72	1.06E-165	-3.24	9.36E-221
17	L1CAM	NA	NA	-2.31	7.21E-90	-3.24	4.30E-174
18	MMP28	3.27	6.98E-66	-2.63	1.09E-09	-3.20	1.06E-67
19	EPHB3	2.27	3.53E-24	-1.61	1.77E-11	-3.10	1.43E-43
20	ADAMTS17	-3.47	1.34E-12	-3.55	2.40E-13	-2.92	1.22E-05
21	PIK3CG	4.09	8.64E-03	NA	NA	-2.90	2.79E-02
22	RASSF5	NA	NA	-1.92	8.29E-08	-2.41	1.42E-11
23	ADAM12	NA	NA	-1.48	2.25E-47	-2.21	8.75E-101
24	ADAMTS7	NA	NA	-1.68	4.40E-20	-2.15	1.87E-32
25	PLXND1	NA	NA	-1.79	1.12E-48	-2.11	2.60E-61
26	TUBA4A	NA	NA	-2.40	4.00E-17	-2.01	2.63E-14
27	SEMA4B	NA	NA	-1.32	4.08E-05	-2.00	6.07E-11
28	PLCB2	NA	NA	NA	NA	-1.99	1.11E-07
29	CXCL12	NA	NA	-6.41	1.69E-19	-1.99	2.87E-10
30	ABLIM2	NA	NA	NA	NA	-1.92	2.92E-11
31	ADAMTS2	2.05	1.71E-12	-5.63	1.47E-24	-1.84	2.94E-10
32	NTNG2	NA	NA	NA	NA	-1.82	1.65E-04
33	SEMA6D	NA	NA	-1.16	1.56E-21	-1.69	2.99E-48
34	PLXNA2	1.43	1.71E-14	-4.46	1.52E-122	-1.66	2.14E-19
35	GNAO1	NA	NA	NA	NA	-1.63	1.56E-59
36	MMP15	NA	NA	NA	NA	-1.61	6.06E-11
37	KIF7	NA	NA	-1.14	4.24E-13	-1.53	2.94E-23
38	EPHA6	NA	NA	-1.37	1.91E-13	-1.46	2.88E-16
39	MME	NA	NA	-1.45	4.97E-16	-1.39	1.18E-16
40	SEMA7A	NA	NA	-1.40	1.69E-08	-1.38	1.23E-07
41	MMP11	2.36	3.84E-35	NA	NA	-1.36	1.71E-12
42	ROBO2	NA	NA	-1.22	3.58E-27	-1.36	3.84E-33
43	GLIS2	1.25	8.78E-09	NA	NA	-1.25	9.04E-09
44	PRKCH	4.00	1.18E-91	-1.33	1.37E-09	-1.23	1.35E-09
45	WNT3	NA	NA	NA	NA	-1.22	1.01E-36
46	PDGFC	NA	NA	-1.94	8.84E-07	-1.20	1.43E-03
47	NTRK1	1.64	1.69E-21	NA	NA	-1.18	9.09E-12
48	TUBB4A	NA	NA	-1.20	1.29E-22	-1.17	2.88E-21
49	PLCL1	NA	NA	NA	NA	-1.16	6.06E-11
50	ADAM29	4.11	3.59E-20	-1.60	0.047068333	-1.13	3.54E-03
51	SRGAP1	NA	NA	NA	NA	-1.10	3.73E-04
52	EPHB2	NA	NA	NA	NA	-1.06	9.21E-14
53	ADAMTS10	NA	NA	NA	NA	-1.05	2.23E-05
54	NTN4	NA	NA	-1.45	9.22E-04	-1.03	3.15E-02
55	ADAM19	NA	NA	NA	NA	-1.01	4.86E-24
56	PRKCE	NA	NA	-1.44	1.14E-11	-1.00	5.44E-06
57	RAP2B	NA	NA	1.05	1.41E-25	1.03	7.59E-23
58	PDGFD	NA	NA	2.03	2.14E-69	1.03	3.81E-17
59	FZD1	NA	NA	NA	NA	1.09	6.40E-68
60	IRS2	NA	NA	NA	NA	1.11	2.54E-102

NO.	Gene	WT(C) vs WT(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
		Log2FC	padj	Log2FC	padj	Log2FC	padj
61	ADAMTS20	NA	NA	NA	NA	1.13	4.92E-10
62	EFNA3	NA	NA	NA	NA	1.25	1.07E-12
63	PRKCA	-1.33	4.53E-186	1.18	9.84E-158	1.27	9.31E-171
64	PLXNB3	NA	NA	1.84	5.08E-09	1.31	4.33E-05
65	LRRC4C	NA	NA	2.32	7.02E-07	1.36	1.65E-02
66	ADAMTS12	NA	NA	2.14	7.47E-22	1.38	8.37E-10
67	GDF7	NA	NA	NA	NA	1.39	6.61E-08
68	ADAMTS19	NA	NA	NA	NA	1.46	2.25E-58
69	PRKCD	NA	NA	NA	NA	1.54	4.30E-18
70	NGFR	NA	NA	1.51	4.35E-12	1.55	9.65E-10
71	FZD7	2.07	1.90E-110	1.83	1.68E-84	1.56	1.26E-105
72	ITGA5	2.31	1.74E-02	3.22	1.94E-04	1.67	3.40E-02
73	SEMA3G	-1.55	2.16E-04	1.55	1.53E-07	1.68	3.39E-05
74	FGFR2	-1.56	7.96E-09	NA	NA	1.69	2.92E-10
75	ADAMTS5	NA	NA	1.75	8.70E-24	1.77	1.80E-19
76	WNT5A	1.21	1.71E-02	3.39	3.00E-15	1.81	7.44E-06
77	EFNA4	NA	NA	2.04	1.68E-39	1.84	3.35E-34
78	NFATC4	NA	NA	1.91	7.87E-51	1.86	1.81E-50
79	ADAM21	NA	NA	1.77	4.92E-05	1.87	7.29E-04
80	NTN5	NA	NA	2.97	0.002463095	1.96	9.49E-03
81	EPHB4	NA	NA	2.20	1.76E-51	2.06	8.06E-46
82	FZD8	NA	NA	NA	NA	2.15	3.40E-09
83	NTNG1	-2.67	2.34E-15	2.33	6.57E-18	2.27	2.99E-11
84	PAPPA	-1.98	1.79E-05	1.99	2.58E-06	2.45	2.42E-08
85	SEMA5A	NA	NA	NA	NA	2.54	1.36E-158
86	WNT16	NA	NA	3.97	5.31E-22	2.80	2.60E-13
87	ADAMTS3	NA	NA	4.35	1.42E-150	3.20	1.54E-82
88	ADAMTS4	-1.49	1.58E-05	2.61	4.38E-16	3.40	2.52E-26
89	GLI1	2.41	7.22E-03	5.67	2.88E-15	3.75	1.66E-09
90	WNT7B	NA	NA	NA	NA	4.35	4.62E-04
91	BMP4	4.22	7.30E-04	3.04	0.031805785	4.57	2.31E-27
92	PRKD1	NA	NA	3.86	6.91E-21	4.93	9.60E-34
93	CXCR4	-2.26	7.55E-66	-4.13	6.47E-119	NA	NA
94	UNC5D	-2.95	1.68E-06	-4.06	1.76E-06	NA	NA
95	WNT4	-2.25	3.93E-03	-3.69	5.14E-05	NA	NA
96	NRP1	-3.52	2.28E-12	-2.90	1.33E-08	NA	NA
97	WNT5B	-1.51	1.55E-02	-2.35	3.11E-04	NA	NA
98	BDNF	NA	NA	-1.83	3.27E-25	NA	NA
99	SDC2	NA	NA	-1.57	1.03E-155	NA	NA
100	SEMA3C	-1.73	1.69E-29	-1.41	5.94E-20	NA	NA
101	KALRN	1.20	1.55E-34	-1.32	1.03E-37	NA	NA
102	NOTUM	-1.70	5.22E-03	-1.30	0.042673188	NA	NA
103	SLIT3	NA	NA	-1.24	1.04E-40	NA	NA
104	VEGFA	NA	NA	-1.22	1.03E-29	NA	NA
105	ABLIM1	NA	NA	-1.14	9.82E-42	NA	NA
106	DPYSL5	NA	NA	-1.01	1.10E-18	NA	NA
107	FZD2	NA	NA	1.01	6.10E-05	NA	NA
108	GNG12	NA	NA	1.07	1.47E-46	NA	NA
109	PTCH2	NA	NA	1.09	0.00137473	NA	NA
110	RRAS	1.63	2.65E-11	1.34	8.51E-08	NA	NA
111	ADAMTS15	NA	NA	1.66	3.37E-04	NA	NA
112	ECE2	NA	NA	5.46	1.14E-04	NA	NA
113	NRP2	-1.76	4.39E-07	NA	NA	NA	NA
114	EPHA2	-1.42	7.61E-13	NA	NA	NA	NA
115	PLXNC1	-1.13	1.11E-04	NA	NA	NA	NA
116	EFNB2	1.07	2.66E-106	NA	NA	NA	NA
117	PLCD3	1.14	1.51E-03	NA	NA	NA	NA
118	ADAMTS9	1.15	3.52E-63	NA	NA	NA	NA
119	SEMA3B	1.21	2.39E-04	NA	NA	NA	NA
120	SEMA6C	1.48	3.11E-10	NA	NA	NA	NA

NO.	Gene	WT(C) vs WT(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
		Log2FC	padj	Log2FC	padj	Log2FC	padj
121	ADAMTS6	2.11	1.04E-09	NA	NA	NA	NA
122	GNG2	2.37	0	NA	NA	NA	NA
123	ECEL1	3.14	1.35E-56	NA	NA	NA	NA

Table 6-6 Comparison profile of DEGs that belong to axonal guidance signaling pathway.

Table shows differential expression of the DEGs which were assigned to axonal guidance signaling pathway in the 3 comparisons of this study: WT(C) vs. WT(R), WT(C) vs. BKO(R), and WT(R) vs. BKO(R). Padj value of zero means very low value that fall out of the calculation scale.

6.3.3.3 Ingenuity pathway analysis (IPA) of upstream effects

Results above showed downstream effect of regulation of genes in the datasets. Using a literature-based database, IPA also provides a prediction of upstream factors that may have caused the effects observed in the datasets. This part of results show the predicted upstream regulators of each previously described comparisons. Factors that are significantly over-represented with -1 < z-score > 1 are shown. Target molecules associated with an upstream regulator within the datasets are not shown.

Table 6-7 IPA upstream regulators prediction in WT(C) vs. WT(R) comparison

Upstream Regulator	Log2 value in dataset	Molecule Type	Activation z-score	p-value of overlap
ATF4		**transcription regulator**	**-2.611**	**0.000451**
SATB1		**transcription regulator**	**-2.578**	**0.0132**
IDO1		**Enzyme**	**-2.236**	**0.0000586**
EGLN		**Group**	**-2.222**	**0.000851**
EFNA2		**Kinase**	**-2.121**	**0.00727**
CDK2		**Kinase**	**-2**	**0.0016**
BRD4		**Kinase**	**-1.995**	**4.35E-09**
BMI1		**transcription regulator**	**-1.969**	**0.00917**
FN1		**enzyme**	**-1.941**	**0.0278**
26s Proteasome		**complex**	**-1.907**	**0.0341**
EFNA4		**Kinase**	**-1.89**	**0.00844**
EFNA3		**Kinase**	**-1.89**	**0.00844**
EFNA1		**Other**	**-1.89**	**0.00917**
AR		**ligand-dependent nuclear receptor**	**-1.823**	**0.0182**
MYC	**-4.24**	**transcription regulator**	**-1.81**	**0.0292**
estrogen receptor		**group**	**-1.651**	**0.00000335**
Mek		**group**	**-1.548**	**0.0000185**
COL18A1		**Other**	**-1.387**	**0.0000673**
TAB1		**enzyme**	**-1.387**	**0.00663**
mir-29		**microRNA**	**-1.3**	**0.00408**
E2F3		**transcription regulator**	**-1.265**	**0.00893**

EFNA5		**Kinase**	**-1.238**	**0.00179**
PDLIM2		**Other**	**-1.155**	**0.00414**
NEUROG1		**transcription regulator**	**-1.069**	**0.00000453**
MAP3K7		**Kinase**	**-1.069**	**0.00961**
TERT	**-1.165**	**Enzyme**	**-1.067**	**0.0138**
Akt		**Group**	**-1.056**	**0.000000598**
ZNF217		**transcription regulator**	**-1**	**0.0000298**
RBPJ		**transcription regulator**	**-1**	**0.00208**
NONO		**transcription regulator**	**-1**	**0.0132**
TLR7/8		**Group**	**1**	**0.00031**
PAF1		**Other**	**1**	**0.00127**
TRPC1		**ion channel**	**1**	**0.00344**
PPARG	**4.651**	**ligand-dependent nuclear receptor**	**1**	**0.015**
THRB		**ligand-dependent nuclear receptor**	**1**	**0.0292**
BMP2		**growth factor**	**1**	**0.0325**
UHRF2		**Enzyme**	**1**	**0.0433**
PRL		**Cytokine**	**1.021**	**0.000904**
MAPK1		**Kinase**	**1.054**	**0.00289**
GH1		**growth factor**	**1.091**	**0.00475**
CSF3		**Cytokine**	**1.091**	**0.0163**
RARA	**1.714**	**ligand-dependent nuclear receptor**	**1.095**	**0.0000187**
NOTCH1		**transcription regulator**	**1.122**	**0.00000102**
miR-26a-5p (and other miRNAs w/seed UCAAGUA)		**mature microRNA**	**1.131**	**0.00475**
PPARA		**ligand-dependent nuclear receptor**	**1.131**	**0.0369**
NCOA2		**transcription regulator**	**1.131**	**0.0433**
mir-15		**microRNA**	**1.154**	**0.0433**
BMP4	**4.22**	**growth factor**	**1.185**	**0.000267**
IRF3		**transcription regulator**	**1.223**	**0.0262**
PRKCD		**Kinase**	**1.252**	**0.000301**
ERBB2		**Kinase**	**1.271**	**0.001**
Interferon alpha		**Group**	**1.356**	**0.000268**
EGR1		**transcription regulator**	**1.392**	**0.0154**
TRIB3		**Kinase**	**1.406**	**0.00105**
TP73		**transcription regulator**	**1.408**	**0.000125**
SMARCA4		**transcription regulator**	**1.451**	**0.000000712**
PI3K (family)		**Group**	**1.5**	**0.000142**
PRNP		**Other**	**1.503**	**0.0319**
IL13		**Cytokine**	**1.504**	**0.000306**
STAT1		**transcription regulator**	**1.573**	**0.0000458**
SREBF1	**1.372**	**transcription regulator**	**1.589**	**0.0111**
RARB	**4.849**	**ligand-dependent nuclear receptor**	**1.673**	**0.00177**
IL15		**Cytokine**	**1.693**	**0.021**
GATA6		**transcription regulator**	**1.965**	**0.00127**
FOXO4		**transcription regulator**	**1.969**	**0.0163**
TCF3		**transcription regulator**	**1.974**	**0.00108**
IGF1		**growth factor**	**1.989**	**0.0188**
PNN		**Other**	**2**	**0.00829**
ERG		**transcription regulator**	**2.086**	**0.000224**
NOTCH3		**transcription regulator**	**2.148**	**0.00337**
MXI1		**transcription regulator**	**2.236**	**0.000734**
IFNA2		**Cytokine**	**2.266**	**0.0495**
HSPA9		**Other**	**2.813**	**4.03E-09**
CST5		**Other**	**3.128**	**0.0396**

TGM2	**4.906**	**enzyme**	**3.174**	**0.0103**
RXRA		**ligand-dependent nuclear receptor**	**3.293**	**2.56E-08**
TGFB1		**growth factor**	**3.337**	**0.00000608**

Table 6-8 IPA upstream regulators prediction in WT(C) vs. BKO(C) comparison

Upstream Regulator	Log2 value in dataset	Molecule Type	Activation z-score	p-value of overlap
ATF4	**-1.207**	**transcription regulator**	**-2.88**	**4.35E-08**
WISP2		**growth factor**	**-2.61**	**0.0446**
IL13		**cytokine**	**-2.59**	**0.0000907**
MYOCD		**transcription regulator**	**-2.39**	**0.0000305**
HDAC4		**transcription regulator**	**-2.23**	**0.00213**
ESR1		**ligand-dependent nuclear receptor**	**-2.06**	**0.00000363**
PCGF2		**transcription regulator**	**-2.00**	**0.00468**
SPARC		**other**	**-1.99**	**0.00468**
SPDEF		**transcription regulator**	**-1.80**	**0.000995**
estrogen receptor		**group**	**-1.72**	**4.81E-08**
CIP2A		**other**	**-1.66**	**0.000347**
HAND2		**transcription regulator**	**-1.60**	**0.000136**
DNMT3B		**enzyme**	**-1.51**	**0.00651**
TAL1		**transcription regulator**	**-1.50**	**0.00018**
NUPR1		**transcription regulator**	**-1.48**	**0.0267**
FOXA1		**transcription regulator**	**-1.46**	**0.0221**
NR3C1		**ligand-dependent nuclear receptor**	**-1.46**	**0.0125**
IDO1		**enzyme**	**-1.41**	**0.0000255**
RXRA		**ligand-dependent nuclear receptor**	**-1.41**	**0.00522**
TCF7L2		**transcription regulator**	**-1.34**	**0.00323**
VHL		**transcription regulator**	**-1.34**	**0.0237**
TBX5		**transcription regulator**	**-1.31**	**0.0000471**
Interferon alpha		**group**	**-1.29**	**0.00434**
MAPK1		**kinase**	**-1.26**	**0.000289**
TREM1		**transmembrane receptor**	**-1.22**	**0.00233**
GATA4		**transcription regulator**	**-1.21**	**0.00271**
MDM4		**enzyme**	**-1.18**	**0.0263**
TOPBP1		**other**	**-1.18**	**0.00468**
CLCA2		**ion channel**	**-1.15**	**0.0149**
miR-34a-5p (and other miRNAs w/seed GGCAGUG)		**mature microRNA**	**-1.14**	**0.0225**
CCND1		**transcription regulator**	**-1.07**	**0.0272**
CBX7		**other**	**-1.07**	**0.0335**
BMP2		**growth factor**	**-1.07**	**0.0347**
PAK1		**kinase**	**-1.03**	**0.0116**
IL17F		**cytokine**	**-1.00**	**0.00727**
IFN Beta		**group**	**-1.00**	**0.00958**
ITGB1		**transmembrane receptor**	**1.00**	**0.00365**
CSF1	**1.329**	**cytokine**	**1.00**	**0.0383**
CTNNB1		**transcription regulator**	**1.04**	**0.00165**
S1PR3		**G-protein coupled receptor**	**1.07**	**0.000687**
ERG		**transcription regulator**	**1.11**	**0.0133**
PTTG1		**transcription regulator**	**1.13**	**0.00322**
Jnk		**group**	**1.14**	**0.0383**

TWIST1	**1.045**	**transcription regulator**	**1.17**	**0.00126**
PDGF BB		**Complex**	**1.18**	**0.0293**
TGM2		**Enzyme**	**1.20**	**0.038**
Pkc(s)		**Group**	**1.26**	**0.0326**
EZR		**Other**	**1.29**	**0.00466**
CEBPA	**-1.256**	**transcription regulator**	**1.34**	**0.0104**
RARB		**ligand-dependent nuclear receptor**	**1.34**	**0.0134**
FOXC2		**transcription regulator**	**1.45**	**0.000784**
TNF		**cytokine**	**1.45**	**0.0000276**
SMARCA4		**transcription regulator**	**1.46**	**2.96E-08**
CYR61	**3.016**	**Other**	**1.49**	**0.00451**
SMAD3		**transcription regulator**	**1.54**	**0.00025**
RELA		**transcription regulator**	**1.55**	**0.00186**
BRD7		**transcription regulator**	**1.56**	**0.00144**
PRKCD		**kinase**	**1.60**	**0.00117**
KLF6	**1.485**	**transcription regulator**	**1.60**	**0.00775**
SMARCD3		**transcription regulator**	**1.63**	**0.000971**
TBXT	**-3.495**	**transcription regulator**	**1.63**	**0.000971**
NOTCH1	**3.122**	**transcription regulator**	**1.90**	**0.0132**
SHC1		**Other**	**1.91**	**0.0302**
MUC4		**Other**	**1.95**	**0.0263**
JAK		**group**	**1.98**	**0.0347**
PTAFR		**G-protein coupled receptor**	**1.98**	**0.0418**
POSTN		**other**	**1.98**	**0.000687**
SSTR2	**-1.649**	**G-protein coupled receptor**	**1.98**	**0.00279**
PRL		**cytokine**	**1.99**	**0.0124**
GNE		**kinase**	**2.00**	**0.000246**
RLIM		**enzyme**	**2.00**	**0.00468**
mir-322		**microRNA**	**2.00**	**0.0149**
STAT1		**transcription regulator**	**2.09**	**0.0141**
PIN1		**enzyme**	**2.18**	**0.0413**
IFNL1		**cytokine**	**2.34**	**0.000695**
REST		**transcription regulator**	**2.60**	**0.000361**
TWIST2		**transcription regulator**	**2.80**	**0.0000552**
IFNG		**cytokine**	**2.81**	**0.000244**
TRIB3	**-1.387**	**kinase**	**2.95**	**0.000000727**
TGFB1	**1.235**	**growth factor**	**3.11**	**0.000172**

Table 6-9 IPA upstream regulators prediction in WT(R) vs. BKO(R) comparison

Upstream Regulator	Log2 value in dataset	Molecule Type	Activation z-score	p-value of overlap
IL1RN		**cytokine**	**-2.813**	**0.294**
EGLN		**group**	**-2.56**	**0.00713**
MAFB		**transcription regulator**	**-2.449**	**0.261**
IRF3		**transcription regulator**	**-2.433**	**0.0595**
AZGP1		**transporter**	**-2.401**	**0.000672**
SPDEF		**transcription regulator**	**-2.221**	**0.0000876**
NR1H4		**ligand-dependent nuclear receptor**	**-2.219**	**0.244**
IL13		**cytokine**	**-2.082**	**0.00249**
SIN3A		**transcription regulator**	**-2**	**0.0251**

miR-29b-3p (and other miRNAs w/seed AGCACCA)		**mature microRNA**	**-2**	**0.258**
PCGF2		**transcription regulator**	**-1.997**	**0.000447**
UPF2		**other**	**-1.982**	**0.238**
TCF3		**transcription regulator**	**-1.98**	**0.00602**
ASAH1		**enzyme**	**-1.98**	**0.182**
MYOCD		**transcription regulator**	**-1.975**	**0.00000163**
SPARC		**other**	**-1.963**	**0.0049**
PRNP	**-1.47**	**other**	**-1.88**	**0.000246**
MAPK1		**kinase**	**-1.758**	**0.0000275**
WISP2		**growth factor**	**-1.757**	**0.00513**
TAL1		**transcription regulator**	**-1.749**	**0.000227**
PTPN6		**phosphatase**	**-1.715**	**0.0049**
SLC9A3R1		**other**	**-1.486**	**0.000831**
BMP4	**4.574**	**growth factor**	**-1.472**	**0.0137**
BMP2		**growth factor**	**-1.432**	**0.00916**
RXRA		**ligand-dependent nuclear receptor**	**-1.408**	**0.00185**
HLX		**transcription regulator**	**-1.387**	**0.0433**
NANOG		**transcription regulator**	**-1.373**	**0.0133**
TP73		**transcription regulator**	**-1.332**	**0.000335**
EOMES	**3.228**	**transcription regulator**	**-1.291**	**8.03E-08**
PDCD4		**other**	**-1.214**	**0.0275**
TOPBP1		**other**	**-1.192**	**0.0049**
RHOA		**enzyme**	**-1.192**	**0.0112**
EHF		**transcription regulator**	**-1.175**	**0.00125**
ESR1		**ligand-dependent nuclear receptor**	**-1.17**	**0.0000444**
COL18A1		**other**	**-1.155**	**0.0102**
HAND2		**transcription regulator**	**-1.097**	**0.00000493**
SMARCE1		**transcription regulator**	**-1.091**	**0.0349**
CBX7		**other**	**-1.067**	**0.0349**
OGA		**enzyme**	**-1.061**	**0.00376**
HDAC4		**transcription regulator**	**-1.057**	**0.0156**
ESR2		**ligand-dependent nuclear receptor**	**-1.028**	**0.01**
RGCC		**other**	**-1**	**0.0000554**
PARP1		**enzyme**	**-1**	**0.0205**
DNMT3B		**enzyme**	**-1**	**0.0403**
PPID		**enzyme**	**1**	**0.000721**
FOXD2-AS1		**other**	**1**	**0.00157**
ROR1		**kinase**	**1**	**0.00157**
RLIM		**enzyme**	**1**	**0.0049**
SMARCD3		**transcription regulator**	**1**	**0.0349**
TBXT	**-3.808**	**transcription regulator**	**1**	**0.0349**
TWIST1	**1.112**	**transcription regulator**	**1.016**	**0.0472**
F7		**peptidase**	**1.029**	**0.021**
NRG1	**-1.784**	**growth factor**	**1.046**	**0.0083**
MYB		**transcription regulator**	**1.054**	**0.000115**
SDCBP		**enzyme**	**1.057**	**0.0156**
MAP2K1/2		**group**	**1.065**	**0.0161**
S1PR3	**1.926**	**G-protein coupled receptor**	**1.067**	**0.000721**
SOX9		**transcription regulator**	**1.067**	**0.0435**
ECM1		**transporter**	**1.068**	**0.0112**
PI3K (family)		**group**	**1.099**	**0.000834**
FOXL2		**transcription regulator**	**1.103**	**0.0108**
ST6GALNAC1		**enzyme**	**1.131**	**0.00157**

JAK		**group**	**1.131**	**0.0364**
NFkB (complex)		**complex**	**1.161**	**0.0449**
NOTCH1	**2.16**	**transcription regulator**	**1.221**	**0.000162**
PTTG1		**transcription regulator**	**1.231**	**0.00341**
LGALS1		**other**	**1.231**	**0.0122**
Lh		**complex**	**1.234**	**0.0132**
POU2F2	**-3.494**	**transcription regulator**	**1.265**	**0.00331**
MUC4		**other**	**1.383**	**0.0000705**
ZNF217		**transcription regulator**	**1.387**	**0.00000114**
DCLK1	**-1.061**	**kinase**	**1.387**	**0.000447**
MET		**kinase**	**1.391**	**0.00209**
FSH		**complex**	**1.406**	**0.00341**
DEF6		**other**	**1.422**	**0.000831**
CAV1		**transmembrane receptor**	**1.455**	**0.00221**
ID1		**transcription regulator**	**1.455**	**0.00552**
mir-34		**microRNA**	**1.456**	**0.00866**
SMARCA4		**transcription regulator**	**1.484**	**9.74E-12**
HGF	**5.191**	**growth factor**	**1.499**	**0.0139**
CD44	**2.539**	**other**	**1.556**	**0.00352**
Growth hormone		**group**	**1.606**	**0.000327**
PDGF BB		**complex**	**1.624**	**0.00578**
PTAFR		**G-protein coupled receptor**	**1.628**	**0.00154**
MAPK8		**kinase**	**1.633**	**0.0297**
HIF1A		**transcription regulator**	**1.719**	**0.000925**
IFNA2		**cytokine**	**1.757**	**0.454**
IL1A		**cytokine**	**1.771**	**0.36**
EGF		**growth factor**	**1.781**	**0.105**
RELA		**transcription regulator**	**1.791**	**0.178**
IKBKB		**kinase**	**1.934**	**0.297**
IL1B		**cytokine**	**1.945**	**0.481**
EPAS1		**transcription regulator**	**1.996**	**0.0000205**
PI3K (complex)		**complex**	**2**	**0.148**
MAPK14		**kinase**	**2**	**0.258**
TNF		**cytokine**	**2.056**	**0.0028**
SSTR2		**G-protein coupled receptor**	**2.219**	**0.000214**
TWIST2		**transcription regulator**	**2.233**	**0.0000605**
mir-322		**microRNA**	**2.236**	**0.00225**
TGFB1		**growth factor**	**2.238**	**0.000000793**
REST		**transcription regulator**	**2.241**	**0.0000104**
NEDD9	**1.693**	**other**	**2.333**	**0.00352**
STAT3		**transcription regulator**	**2.35**	**0.0128**
SYVN1		**transporter**	**2.4**	**0.00496**
EIF2AK2		**kinase**	**2.433**	**0.386**
CYR61	**3.875**	**other**	**2.529**	**0.000246**
PIN1		**enzyme**	**2.588**	**0.00252**
IFNL1		**cytokine**	**2.744**	**0.016**
EGFR	**3.175**	**kinase**	**2.757**	**0.00502**
PCDH11Y		**other**	**2.804**	**0.000222**
IFNG		**cytokine**	**3.13**	**0.0467**

6.4 Discussion

In this chapter, the CRISPR technique was optimized to generate several *TOP2B* knockout clones of the SH-SY5Y cell line. CRISPR was performed using a plasmid based method of guide RNA (gRNA) and CAS9 introduction into cells by transfection. A plasmid with a GFP-expression selection marker (PX458) was used for two reasons. Firstly, FACS selection of transfected cells is rapid and more robust than antibiotic selection especially when cells showed low transfection efficiency as was the case with the SH-SY5Y cell line in this study. Secondly, rapid and highly efficient single cell cloning can be performed using GFP-based cell sorting. Several *TOP2B* knockout clones were successfully generated and verified for the absence of TOP2β protein using both western blotting and immunofluorescence (Figures 6.8, 6.9, 6.10, 9.11, and Table 6.2). From those clones, three clones (BKO-70, BKO-98, and BKO-129) were selected for studying the role of TOP2β in gene expression and differentiation of SH-SY5Y cells. These 3 clones were generated from 3 different gRNAs targeting exon 1 of the *TOP2B* gene.

The SH-SY5Y cell line is a neuroblastoma cell line that retains the capacity to undergo neuronal differentiation by retinoic acid (RA), and/or other neural differentiation factors (Påhlman *et al.*, 1984; Encinas *et al.*, 2000; Dwane *et al.*, 2013; Teppola *et al.*, 2016). Upon RA treatment, these cells reduce proliferation and undergo morphological changes toward neuronal characteristics such as neuronal cell body and formation of extended neurites which eventually form cell axons (Constantinescu *et al.*, 2007; Bell *et al.*, 2013; Teppola *et al.*, 2016).

SH-SY5Y cells are widely used in neuronal studies because these cells retain cellular and biochemical characteristics of neuronal cells (Cheung *et al.*, 2009; Krishna *et al.*, 2014). No previous reports studied the differentiation of SH-SY5Y cells in a *TOP2B* stable knockout model. Wild type and *TOP2B* knockout clones were tested for their response to ATRA-induced differentiation using microscopic observation of morphological changes particularly neurite outgrowth as well as measurement of *BCL2* mRNA level as a differentiation marker. Neurite outgrowth assay and testing *BCL2* level are common assays to evaluate the differentiation of SH-SY5Y cells (Lasorella *et al.*, 1995; Riddoch *et al.*, 2007; Bell *et al.*, 2013).

Results showed significant reduction in the ratio of neurite outgrowth bearing cells as well as the level of Bcl2 induction in the *TOP2B* knockout clones. These results agree with previous reports that showed neuronal defects in *Top2b* knockout mice, rats, zebrafish (Yang, 2000; Lyu and Wang,

2003; Lyu *et al.*, 2006; Nur *et al.*, 2007; Nevin *et al.*, 2011; Heng *et al.*, 2012; Li *et al.*, 2014) and human mesenchymal stem cell-derived neurons (Isik *et al.*, 2015; Zaim and Isik, 2018).

It has been shown that ATRA treatment of SH-SY5Y cells rapidly induces the expression level of a number of genes such as *CYP26A1, CRABP2, RARB*, and *RET* (Perez-Juste and Aranda, 1999; Brabender *et al.*, 2005; Oppenheimer *et al.*, 2007). These 4 genes were tested for the level of induction using RT-qPCR in the wild type and *TOP2B* knockout clones (Figure 6.14). Results revealed a significant decrease in the induction of *CRABP2* and *CYP26A1* in all 3 *TOP2B* knockout clones compared with wild type.

Further to these findings, the whole transcriptome profile of SH-SY5Y cells was studied using RNA-seq technique and again this was the first study that compares total gene expression of these cells in the presence and absence of TOP2β with or without retinoic acid. For this purpose, one of the *TOP2B* knockout clones (BKO-98) was used along with the wild type cells which were transfected with empty plasmid. Both cells were grown for 24 hrs in the presence or absence of ATRA (10 μM). RNA samples were then extracted, prepared, and sequenced as described in the materials and methods (section 2.14.2). Differential gene expression was determined within 4 comparisons WT(C) vs. WT(R), BKO(C) vs. BKO(R), WT(C) vs. BKO(C), and WT(R) vs. BKO(R) (Table 6.3 and Figures 6.15 and 6,16). Results revealed large number of differentially expressed genes (DEGs) that were regulated by more than 1 log2 fold (Figures 6.17, 6.18, and Appendix table 17). There was no significant change in the expression of all known housekeeping genes such as (*GAPDH, PP1A, RPL15, ATCB, POLR2F*, and *POLR2A*) in all the 4 comparisons, confirming quality and efficiency of calculation of relative abundances of transcripts.

The effect of retinoic acid on the total transcriptomic profile of differentiated SH-SY5Y cells is well established (Oppenheimer *et al.*, 2007; Korecka *et al.*, 2013; Forster *et al.*, 2016; Pezzini *et al.*, 2017). However, to our knowledge, less information is present about the early whole genome expression changes induced by retinoic acid. This was reported in this study in the differential expression profile of the (WT(C) vs. WT(R)) comparison which showed 1202 and 2034 genes were down or up regulated respectively (Figure 6.17). RNA-seq results showed induction of the 4 genes tested previously (*CYP26A1, RARB, CRABP2*, and *RET*) as well as *BCL2* by RA. A subset of genes that were differentially expressed upon RA are involved in the metabolism and the signaling pathway of retinoic acid itself (Table 6.4), suggesting that RA regulates expression of members of its own metabolism pathway. For example, the *CYP26A1* gene is rapidly induced by RA through

two functional RA response elements (RARE) and encodes cytochrome 450 26A1, a catabolizing enzyme for RA (Loudig *et al.*, 2005). This shows how RA might take part in regulation of its level via feedback loops (White *et al.*, 2007).

IPA analysis of DEGs from the (WT(C) vs. WT(R)) comparison showed over-representation of RA-related pathways and functional annotations. Based on the IPA categorization, ATRA treatment induced a wide range of cellular and molecular changes associated with cell morphology such as (growth of neurite, guidance of axons, tabulation of cells, branching of cells, and sprouting). These changes are pivotal during the differentiation process. Canonical pathway analysis showed VDR/RXR activation as the most significant over-represented pathway.

The axonal guidance pathway was the pathway most affected in terms of the number of genes regulated (47 DEGs) (Figure 6.22 and Table 6.6). No activation state prediction was made by IPA for this pathway as it is not eligible for z-score calculation because it has more than one activation state (see section 2.14.2.3). Axonal guidance (also termed axonal pathfinding) is the process by which neuronal cells control the extension of axons towards an appropriate target. Axons extend from a part of the cell called growth cone which responds to either chemical or mechanical signals called cues and these cues can be either diffusible or nondiffusible. Axonal guidance is controlled through balance between attraction/repulsion effects of cues. These cues "instruct" an axon to follow the correct route direction through their attractive or repulsive effects. However, some cues are bifucntional so there is no single cue-to-effect relationship for these molecules (Tessier-Lavigne and Goodman, 1996; Myers *et al.*, 2011; Bearce *et al.*, 2015).

Genes that are assigned to this pathway and were regulated by ATRA included members of a family of neurotrophic receptor tyrosine kinases (*NTRK1, NTRK2*, and *NTRK3*). Particularly, *NTRK2* (also known as TrkB) was the most remarkably induced gene upon ATRA treatment (7.7 log2 fold). This agreed with previous reports showing induction of this gene during RA-induced differentiation in previous reports involving SH-SY5Y (Shiohira *et al.*, 2012) and NB1643 (Middlemas *et al.*, 1999) neuroblastoma cells. Other DEGs that were regulated by RA included members of the semaphorin family (*SEMA6C, SEMA3B, SEMA3G*, and *SEMA3C*) and related receptor plexins (*PLXNA2* and *PLXNC1*) and neuropilins (*NRP1* and *NRP2*). Semaphorins act by controlling attraction/repulsion balance (Thiede-Stan and Schwab, 2015). In addition, members of the ephrin receptor subfamily (*EFNB2*) and the related receptor (*EPHB3* and *EPHA2*), netrins

(*NTNG1* and *NTNG2*) and nitrin receptors (*UNC5A* and *UNC5D*), slit proteins (*SLIT2*), and growth factor member (*BMP4*) were significantly regulated by ATRA.

These results represent the transcriptional change upon ATRA treatment of wild type SH-SY5Y (WT(C) vs. WT(R) comparison which validates our experimental condition and for SH-SY5Y cells confirms what is expected from these cells upon RA treatment.

Next, the role of TOP2β in whole genome expression was investigated in the presence and absence of ATRA as shown in the differential gene expression and respective IPA analysis of the two comparisons: WT(C) vs. BKO(C) and WT(R) vs. BKO(R). Differential expression analysis highlighted regulation of 2554 (1466 down- and 1088 up-regulated) and 2677 (1720 down- and 957 up-regulated) genes in the *TOP2B* knockout cells in the absence and presence of ATRA respectively (Figure 6.17). Genes with neuronal function represented large proportion (603 genes, 23.6 %) or (603 genes, 22.5 %) of total regulated genes in WT(C) vs. BKO(C) and WT(R) vs. BKO(R) comparisons respectively.

Interestingly, several of the downregulated genes upon *TOP2B* knockout were also shown to be downregulated in *TOP2B* mutant models in previous reports. For instance, *MYT1L, NELL2, CDH4, GABRB3, GRIK2, ANK3, CX3CL1, GPX3, NPY, KCNMA1*, *HOXA5, RIT2, LPL*, and *TMEFF2* genes were shown to be downregulated in the brains of *Top2b* mutant murine embryos (Lyu *et al.*, 2006). These genes were all significantly downregulated in the SH-SY5Y cells lacking TOP2β in our study. In addition, results showed downregulation of *MYT1, CDH8, NELL1, ROBO2, SYT7, SYT4, SYT17, SYT11, UNC5A, UNC5D, GRIK3, GRIK4, GRIK5, TAC3*, and *NCAM1* genes, which are closely related to a group of genes (*MYT1L, CDH4, NELL2, ROBO1, SYT1, UNC5C, GRIK2, TAC1,* and *NCAM2*) in the study mentioned above. These genes have functions of transcription regulation, axon guidance, cell adhesion and migration, neuropeptide encoding, synaptic activity, and cellular response.

Further consistency with our result, several of the significantly downregulated genes in the *TOP2B* knockout SH-SY5Y cells in this study were shown to be bound by Top2b (preferentially at promoter region) in murine embryonic stem cell-derived postmitotic neurons and were also downregulated in the *Top2b* mutant derivative of these cells (Tiwari *et al.*, 2012) as determined by microarray. Gene Ontology (GO) enrichment analysis of these genes performed by the authors suggested functions related to neurogenesis. These genes were *NTRK3, POU4F2, CNTN5, FSTL5,*

UNC5D, NELL2, LPL, RALYL, MARCH4, LSAMP, NEFM, AKAP6, ZFP36L1, PRDM8, ROBO2, GRIK2, RRM2, ABLIM1, ONECUT1, NRXN1, IRX3, PLCB1, B3GALT2, and *CTNNA2*.

Moreover, five of the significantly downregulated genes upon *TOP2B* knockout in our study (*DAB1, CPNE9, SGCD, CNTN4*, and *ADAM19*) were shown to be activated by Top2b during rat cerebellar differentiation and were supressed upon Top2b inhibition (Sano *et al.*, 2009). Another 4 downregulated neuronal genes upon *TOP2B* knockout in our study (*VEGFA, GRIK2, NRXN1*, and *NRXN3*) were shown to have a role in neural differentiation of mouse retina and were downregulated upon conditional *Top2b* knockout in the retina (Li *et al.*, 2014) as determined by RNA-seq. Another 23 genes regulated by Top2b from (Li *et al.*, 2014) study also overlapped with our datasets.

More recent RNA-seq analysis from the same lab for Top2b dependent genes (TDGs) in murine photoreceptor cells at two differentiation stages: P0 and P6 revealed a group of key genes in differentiation and maturation of these cells (Li *et al.*, 2017). Consistently, group of these genes were undergone the same regulation pattern in the knockout cells in our result. In detail, the genes that showed significant downregulation in our study and in (Li *et al.*, 2017) were (*RBFOX1, FSTL5, CARTPT, PCDH7, SPON1, ROBO2, GRIK2, TOP2B, NRXN1, NRXN3, DLG2, and CTNNA2)* for P0 stage, and *(CLVS2, FSTL5, NELL2, CRABP1, SLC7A11, ASNS, MTUS2, MAN2A2, KCNIP1, STXBP2, TTC9, PRKCE, HIST1H2BJ, VEGFA, NMB, COX4I2, RRM2, UNC13A, TOP2B, GADD45A, DLG2, GNAO1, SCN3A, KIF5A, CTNNA2,* and *FAM57B*) for P6 stage. On the other hand, the genes that showed significant upregulation in both datasets were (*COL1A1, HSPB1*, and *EDNRB*) and (*RBP4, ABCG2, CSPG5, ALDH6A1, ITPKB, EFHD1, CD72, LHPP*, and *TSPO*) for P0 and P6 stages respectively.

In further agreement with our data, the requirement for Top2b in expression of a cohort genes of neuronal functions in mouse cerebellar granule neurons was shown in another report by (Feng *et al.*, 2017a). Interestingly, 37 of those genes (namely *DPP6, RBFOX1, DSCAM, CADM3, FSTL5, KCNJ3, RUNX1T1, ZMAT4, SH3GL2, PCDH7, GRIA2, CELF4, MMP24, RALYL, NRG3, ADARB2, SCG3, GRM1, CPLX1, TNIK, APBA1, EDIL3, GPM6B, FIGN, NTN4, PRKCE, PRDM8, GRIK2, NRXN1, NRXN3, TMEM198, DLG2, GNAO1, B3GALT2, SYNE1, ST8SIA4,* and *PLCL1*) were also significantly downregulated upon *TOP2B* knockout in our study.

Collectively, these results showed that TOP2β plays a role in the expression of a group of neuronal genes. Some of these genes were highlighted in common among the various reports mentioned above such as *GRIK2*, *NRXN1*, *NRXN3*, and *NELL2*. These results correspond to the reduced level of differentiation shown in the *TOP2B* knockout SH-SY5Y cells.

In this study, the differentially expressed genes in the *TOP2B* knockout cells might be either directly regulated by TOP2β or indirectly by regulation of factors that participate in their activation state.

Chromatin remodelers represent a key factor in regulation of nucleosome positioning and accessibility of chromatin and thus play a role in gene activation and silencing (Längst and Manelyte, 2015). Chromatin remodelers fall into four families: SWI/SNF, ISWI, INO80, and CHD (Tyagi *et al.*, 2016). CHD (Chromodomain-Helicase-DNA binding) is a family of chromodomain-ATPases that has been shown to play a role in the regulation of gene expression (Murawska and Brehm, 2011) particularly of neuronal genes (Egan *et al.*, 2013; Feng *et al.*, 2017a; Feng *et al.*, 2017b).

Of particular importance, CHD5 chromodomain remodeler has been shown to be essential during neurogenesis and upregulated during neuronal terminal differentiation to facilitate both activation of expression of neuronal genes and repression of poly-comb genes that are normally not expressed in neuronal tissue (Egan *et al.*, 2013). CHD5 downregulation inhibited differentiation of murine neural progenitor cells (NPCs) to neurons and this was accompanied by inhibition of activation of key neuronal genes (such as *NeuN*, *Ncam*, and *Tubb3*) and abnormal upregulation of some of the poly-comb genes such as *Hes7* (Egan *et al.*, 2013).

Interestingly, CHD5 was downregulated (~10 fold) in the *TOP2B* knockout SH-SY5Y cells. Visual inspection of publically available ENCODE ChIP data in SH-SY5Y cells showed several CTCF peaks around the 5'end of CHD5 gene with non-overlapping histone modification activation and repression markers around the promoter. Furthermore, several of the downregulated neuronal genes in this study (such as *NRTK2*, *NRTK3*, and *NRXN2*) have CHD5 ChIP-seq peaks at or near the promoter. On the other hand, other genes that have CHD5 ChIP-seq peaks at or near promoter in SH-SY5Y were shown to be significantly induced upon *TOP2B* knockout. Notably, among those induced genes were *HES7*, *VIM*, and *NGFR* (upregulated by ~ 8, 16, and 2.9 fold respectively). HES7 was shown to be upregulated upon CHD5 depletion in (Egan *et al.*, 2013) while NGFR was

shown to be upregulated in brain of Top2b lacking mouse (Tiwari *et al.*, 2012). Therefore, it is plausible that some of the transcriptional effects of *TOP2B* knockout might occur as a result of lower CHD5 expression in the *TOP2B* knockout cells.

Mechanistically, as described previously, TOP2β regulates expression of targeted genes either through its catalytic activity to modify chromatin supercoiling and change accessibility of transcriptional machinery to the susceptible genes (Madabhushi *et al.*, 2015b; Uusküla-Reimand *et al.*, 2016; Canela *et al.*, 2017) or via generation of DNA double-stranded breaks at promoters which promote gene activation (Ju *et al.*, 2006; Haffner *et al.*, 2010; Madabhushi *et al.*, 2015b; Trotter *et al.*, 2015).

In agreement with TOP2β's role in expression of many neuronal genes, IPA analysis of differentially expressed genes (DEGs) in the knockout cells highlighted the axonal guidance signaling pathway as the most significantly over-represented pathway both in the presence and absence of ATRA (Figures 6.28 and 6.25) with 88 and 82 DEGs assigned to the pathway respectively. DEGs assigned to this pathway are described with their differential expression log2 values for all comparisons in (Table 6.6). Other neuronal pathways were also significantly over-represented, with the synaptogenesis signaling pathway being the second most significantly over-represented pathway both in the presence (62 DEGs) or absence (51 DEGs) of ATRA. The results showed a remarkable inhibition prediction score for this pathway in the knockout cells both with and without ATRA (z-score of -3.36 and -4.24 respectively) (Figures 6.28 and 6.25). DEGs assigned to this pathway encode for synaptotagmins (*SYT2, 3, 4, 5, 7, 9, 11, 14, 16,* and *17*), synapsins (*SYN1, SYN2*, and *SYN3*), neurexins (*NRXN1, NRXN2*, and *NRXN3*), cadherins (*CDH2, 3, 4, 7, 15,* and *22*), *BDNF*, *NTRK3,* and various other factors such as growth factors and EPH receptors encoding genes.

IPA analysis for the prediction of upstream regulators of observed gene expression patterns in WT(C) vs. WT(R), WT(C) vs. BKO(C), and WT(R) vs. BKO(R) comparisons showed a cohort of various molecules including transcription regulators, kinases, cytokines, growth factors, and nuclear receptors (Tables 6.7, 6.8, and 6.8).

Overall, the results of this chapter are useful in a number of aspects. Firstly, it shows the requirement of TOP2β for neuronal differentiation in a human cell model. Secondly, it uncovers a large number of neuronal genes that regulated by TOP2β. A group of these genes are involved in canonical neuronal pathways such as axonal guidance signalling. Many of the neuronal genes regulated in the knockout cells were previously shown to be regulated by TOP2β in animal studies (overlapping with all other studies is mentioned above), suggesting a common group of genes to be focused on for more detailed studies in the future. Thirdly, it reports the genes that are regulated by retinoic acid and TOP2β at the same time. For example, the genes that were completely unchanged by ATRA in the knockout compared to the wild type cells belong to groups A, K, L, and O (Figure 6.18), while the genes that are differently regulated by ATRA in the knockout cells compared to the wild type cells belong to groups D, N, and M (see figures 6.17 and 6.18, for the full list of these genes refer to appendix table 17). These genes can be the basis for further investigation using ChIP-seq or ChIP-qPCR to uncover the genes that directly bound and regulated by TOP2β.

7 Chapter Seven: Discussion

7.1 Summary of findings

This study involved investigating of cellular and regulatory roles of TOP2β enzyme. This isoform was firstly recognized by (Drake *et al.*, 1987). Since then, many studies aimed at highlighting the isoform-specific role of TOP2β. On one hand, it has been suggested that TOP2β involved in chromosomal translocations and associated secondary malignancy or therapy-related leukaemia (Azarova *et al.*, 2007; Azarova *et al.*, 2010; Haffner *et al.*, 2010; Cowell and Austin, 2012; Cowell *et al.*, 2012; Smith *et al.*, 2014).

In this study, TOP2β was investigated for isoform-specific contribution of cytotoxicity and genotoxicity (measured by micronuclei assay by FACS) induced by TOP2 poisons (daunorubicin and idarubicin) in Nalm6 cells (Acute lymphoblastic leukaemia). Results showed comparable contribution of both isoforms in both cytotoxicity and micronuclei induction.

This study also involved investigation of cytotoxicity and genotoxicity induced by daunorubicin in combination with Ara-C. One interesting finding was the genotoxicity induced by Ara-C was significantly lower than that induced by daunorubicin at the same level of cytotoxicity (Figure 3.13).

On the other hand, TOP2β isoform has been shown to play a regulatory role in expression of ligand inducible and developmentally regulated genes (Austin *et al.*, 2018; Madabhushi, 2018). This study aimed at investigating role of TOP2β in gene expression. In Nalm6 cells, two independent RNA-seq experiments showed a number of genes that differentially expressed in both datasets (see chapter 4). RNA-seq and RT-qPCR analysis of expression of selected genes in two different knockout Nalm6 models generated by homologous recombination or CRISPR ($Nalm6^{B-/-}$ and $Nalm6^{BKO4}$) highlighted *CBR1* as one of the genes that tightly regulated by TOP2β.

ChIP analysis at CBR1 gene locus showed recruitment and binding of TOP2β at 4 locations. Moreover, ChIP showed significant reduction of signals of USF2, c-Myc, and MAX transcription factors at *CBR1* promoter region in the knockout cells. RT-qPCR analysis of these transcription factors showed no reduction in their mRNA level, suggesting reduced recruitment of these transcription factors to *CBR1* promoter region in a TOP2β dependent mechanism.

This is an important finding as although both TOP2β and *CBR1* have been reported to be implicated in doxorubicin-induced cardiotoxicity (Forrest *et al.*, 2000; Olson *et al.*, 2003; Zhang *et al.*, 2012), so far no data suggested that the TOP2β role in doxorubicin-induced cardiotoxicity might be, at least in part, due to regulation of *CBR1* gene.

TOP2β has been shown to play a key role in transcriptional regulation of neuronal genes and to be required for neuronal development (Austin *et al.*, 2018; Madabhushi, 2018). Studies have shown the role of TOP2β in expression of retinoic acid-regulated genes (Ju *et al.*, 2006; McNamara *et al.*, 2008). SH-SY5Y cells have the ability to differentiate to neuronal phenotype upon retinoic acid treatment. Therefore, these cells were chosen in this study to investigate TOP2β role in both RA-mediated transcription as well as neuronal differentiation. Nine different *TOP2B* knockout clones were successfully generated in SH-SY5Y cell line using CRISPR and verified for TOP2β protein absence (Table 6.2 and Figures 6.8, 6.10, and 6.11).

Investigations involved comparison between wild type SH-SY5Y and three selected knockout clones (generated from different gRNAs) for ATRA-induced differentiation as measured by neurite outgrowth assay as well as *BCL2* expression (Figures 6.12 and 6.13). Results showed significant reduction in neurite outgrowth and *BCL2* expression in knockout cells compared with the wild type. RT-qPCR results also showed significant reduction in ATRA-induced induction of *CYP26A1* and *CRABP2*, two genes involved in RA signalling pathway and have been shown to be induced upon RA-induced differentiation in SH-SY5Y.

In addition, analysis involved comparison between wild type SH-SY5Y and one of the knockout clones for whole genome transcription profile using RNA-seq in the presence and absence of ATRA. Results highlighted many genes that were regulated in the *TOP2B* knockout cells (Figures 6.17 and 6.18 as well as Appendix table 17). Neuronal genes represent large proportion of total genes regulated upon *TOP2B* knockout in the absence or presence of ATRA (603 genes, 23.6 %) or (603 genes, 22.5 %) respectively. IPA analysis showed axonal guidance as the most over-represented pathway. These findings consistent with previous reports showed TOP2β role in neuronal cells (Lyu and Wang, 2003; Nur *et al.*, 2007; Tiwari *et al.*, 2012; Li *et al.*, 2014; Li *et al.*, 2017). Another functions and pathways were also significantly over-represented (see section 6.3.3.2 for more details).

7.2 Conclusions

- TOP2β and TOP2α contribution in cytotoxicity and genotoxicity of daunorubicin and idarubicin in Nalm6 cells is comparable.
- Ara-C induces less micronuclei formation than daunorubicin at the same level of cytotoxicity.
- TOP2β regulates *CBR1* gene expression in Nalm6 cell line through mediation of recruitment of USF2, c-Myc, and MAX transcription factors to the promoter region.
- *TOP2B* deletion in SH-SY5Y significantly reduced ATRA-induced differentiation.
- TOP2β positively regulates CRABP2 and CYP26A1 genes in SH-SY5Y cells.
- *TOP2B* knockout in SH-SY5Y affected expression of a group of genes particularly neuronal genes.

7.3 Future work

- Investigate TOP2β contribution in cytotoxicity and genotoxicity of TOP2 poisons in K562 and SH-SY5Y cell lines using the newly generated knockout model in these cells.
- Optimize *CBR1* downregulation by siRNA or stable knockout using CRISPR to compare the effect of *CBR1* inhibition with *TOP2B* knockout on doxorubicin-induced cytotoxicity.
- Investigate whole genome binding pattern of TOP2β in Nalm6 and K562 cells using ChIP-seq to uncover genes that directly bound and regulated by TOP2β.
- Establish a *TOP2B* knockout model of human induced pluripotent stem cells then induced cardiomyocyte differentiation to test TOP2β role in *CBR1* expression in cardiomyocytes.
- Investigate whole genome binding pattern of TOP2β in SH-SY5Y cells using ChIP-seq to uncover genes that directly bound and regulated by TOP2β.

8 Chapter Eight: References

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9 Appendices

9.1 List of appendix figures

9.2 List of appendix Tables

Name	Source	MW	Stock	Storage
10 % Paraformaldehyde	Sigma (Cat. P6148)		Prepare 40 % in 0.1 M NaOH, dissolve at 70 °C, dilute to 10 % in PBS.	4 °C
1.38 M Glycine	Melford (Cat. G36050/G0707)	75.07	Dissolve 5.1798 g in 50 ml DW	RT
50 mM PIPES pH 8.0	Sigma (Cat. 1851)	302.4	Dissolve 1.512 g in 100 ml DW. Adjust pH to 8.0	RT
850 mM KCl	Melford (P41000)	74.6	Dissolve 6.341 g in 100 ml	RT
5 % NP-40	Calbiochem (Cat. 492016)		Add 5 ml to 100 ml DW	RT
5 % Na Deoxycholate	Sigma (Cat. D6750)		Dissolve 2.5 g in 50 ml DW	RT away from light
10 % SDS	Sigma (Cat. L3771)		Dissolve 5 g in 50 ml DW	RT
1.2 M Tris pH 7.5	Melford (Cat. T60040)	121.14	Dissolve 7.2684 g in 50 ml DW. Adjust pH to 7.5	RT
3 M LiCl	Sigma (Cat. L8895)	42.39	Dissolve 6.3585 g in 50 ml DW	RT
0.1 M Tris pH 7.5	Melford (Cat. T60040)	121.14	Dissolve 0.6057 g in 50 ml DW. Adjust pH to 7.5	RT
10 mM Na2EDTA	Melford (Cat. E0511)	372.2	Dissolve 0.1861 g in 50 ml DW.	RT
1 M NaHCO3	Sigma (Cat. S5761)	84.01	Dissolve 4.2005 g in 50 ml DW.	RT

Appendix table 1 Preliminary solutions used in preparing ChIP buffers

Treatment (nM)		RICC (%)			
Ara-C	**Daun**	**Ara-C+Daun**	**SEM**	**Ara-C→Daun**	**SEM**
2	**2**	70.41	4.96	73.24	2.55
4	**4**	46.44	3.52	57.57	4.31
8	**8**	16.89	1.55	34.61	6.23
10	**4**	30.76	3.19	40.49	5.50
12	**6**	19.53	2.34	26.00	5.11
4	**10**	21.24	1.94	44.37	4.61

Appendix table 2 RICC values of selected scheduled and concurrent (Ara-C+Daun) combinations.

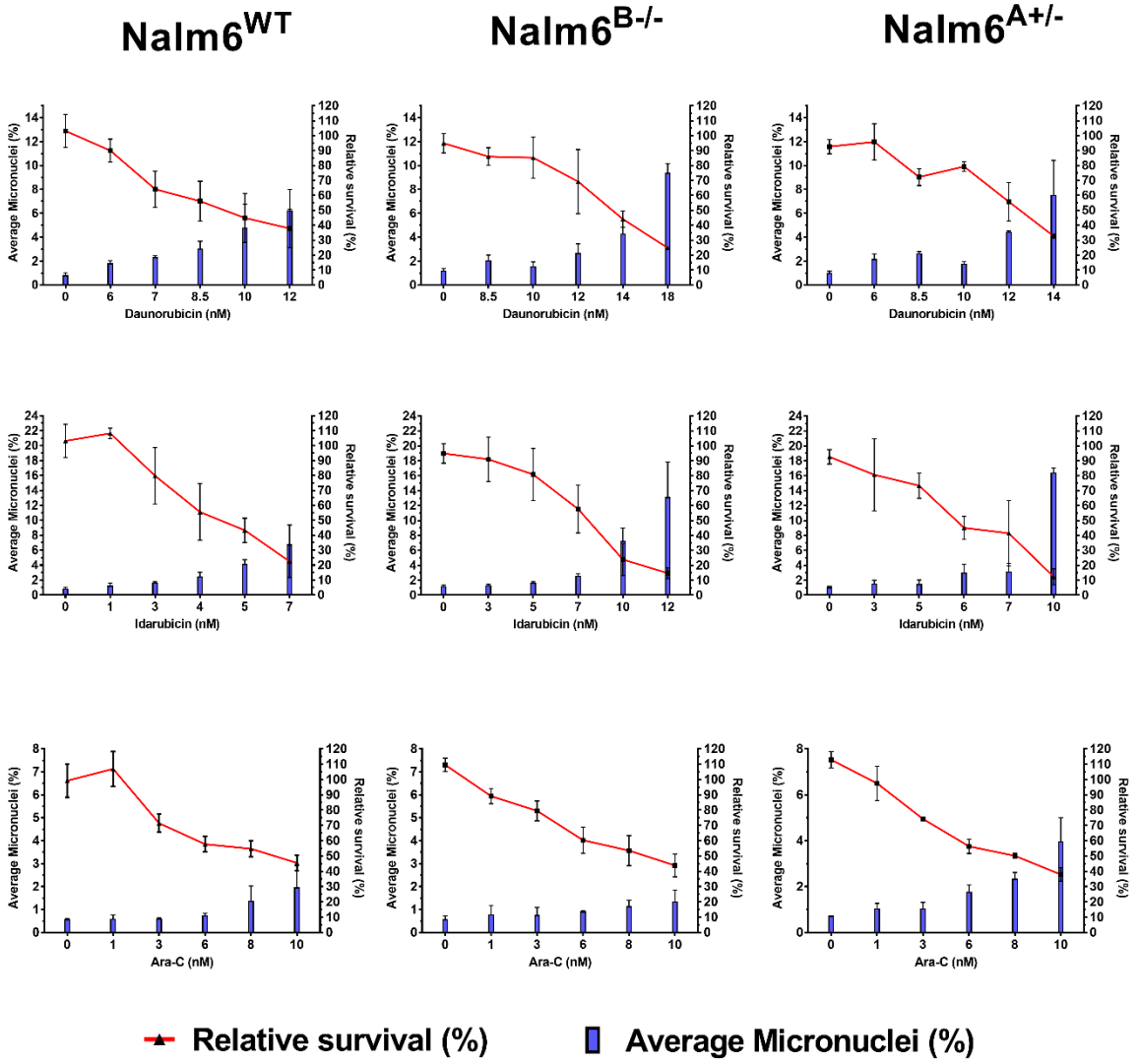
Cells were incubated with either: Ara-C and daunorubicin at the same time for 48 hours continuously (Ara-C+Daun), or treated with Ara-C first then daunorubicin 24 hours later (Ara-C→Daun). The cytotoxicity was determined as the relative increase in cell count (RICC). Data presented here is the mean of at least three independent experiments.

Drugs	Dose (nM)	Nalm6^{WT}				Nalm6$^{B-/-}$				Nalm6$^{A+/-}$			
		RICC (%)	SEM	Relative survival (%)	SEM	RICC (%)	SEM	Relative survival (%)	SEM	RICC (%)	SEM	Relative survival (%)	SEM
Daunorubicin	0	98.6	4.48	103.14	6.37	94.25	9.60	94.91	3.80	89.45	3.25	92.66	2.80
	6	69.23	6.94	89.96	4.45					79.82	1.74	95.83	8.58
	7	56.39	6.35	64.14	7.02								
	8.5	55.5	9.97	56.15	7.71	75.68	4.55	85.97	3.41	62.94	2.63	72.27	3.27
	10	39.9	10.45	44.88	11.61	77.68	7.89	85.24	7.97	72.17	3.17	79.32	2.31
	12	24.39	5.77	37.81	7.42	60.56	7.68	69.18	12.45	48.45	4.44	55.74	9.10
	14					49.58	8.96	44.08	3.15	25.50	1.32	32.68	0.63
	18					19.43	5.23	24.83	0.85				
Idarubicin	0	98.60	4.48	103.14	6.37	94.25	9.60	94.91	3.80	89.45	3.25	92.66	2.80
	1	101.21	6.38	108.20	2.03								
	3	64.73	6.02	79.76	10.92	91.57	4.88	91.02	8.59	92.38	3.77	80.64	13.94
	4	49.30	13.64	55.58	10.97								
	5	40.15	2.12	43.29	4.70	68.07	8.03	80.82	10.09	67.89	5.18	73.31	4.90
	6									38.58	2.03	45.12	4.45
	7	22.08	9.57	22.44	6.21	54.98	3.91	57.64	9.26	43.54	3.99	41.45	12.59
	10					25.69	3.36	23.88	6.20	10.91	2.41	12.33	3.08
	12					13.67	0.67	14.75	2.12				
Ara-C	0	94.00	3.24	99.23	6.24	92.43	4.88	109.55	2.52	86.73	2.02	112.91	3.14
	1	103.89	3.21	106.97	6.53	93.41	0.59	89.10	2.81	100.03	5.52	97.45	6.49
	3	65.34	5.32	71.44	3.40	74.35	2.06	79.52	3.75	70.43	3.06	74.22	0.35
	6	45.62	3.31	57.74	2.95	56.75	1.16	60.32	4.96	53.11	5.57	56.34	2.67
	8	36.32	1.89	54.72	2.97	47.81	3.30	53.55	5.70	42.65	2.26	50.08	0.93
	10	32.74	6.58	45.48	2.91	38.27	3.04	43.78	4.29	36.86	1.85	37.92	2.56

Appendix table 3 Relative survival and RICC values of Ara-C, daunorubicin, and idarubicin in Nalm6 cell lines.

Drugs	Dose (nM)	Nalm6^{WT}				Nalm6$^{B-/-}$				Nalm6$^{A+/-}$			
		MN (%)	SEM	MN Fold increase	SEM	MN (%)	SEM	MN Fold increase	SEM	MN (%)	SEM	MN Fold increase	SEM
Daunorubicin	0	0.78	0.14	0.95	0.06	1.14	0.15	0.89	0.18	0.98	0.12	0.98	0.07
	6	1.78	0.15	2.43	0.49					2.13	0.27	2.17	0.12
	7	2.29	0.11	3.10	0.46								
	8.5	3.00	0.38	4.08	0.84	2.02	0.28	1.90	0.51	2.61	0.11	2.70	0.21
	10	3.58	1.43	7.11	3.91	1.51	0.24	1.42	0.39	1.73	0.13	1.78	0.10
	12	6.21	1.03	8.89	2.93	2.63	0.47	2.48	0.68	3.30	1.12	4.10	0.30
	14					4.27	0.34	3.91	0.64	7.51	1.68	7.57	1.42
	18					9.35	0.47	8.45	0.99				
Idarubicin	0	0.78	0.14	0.95	0.06	1.14	0.15	0.89	0.18	0.98	0.12	0.98	0.07
	1	1.18	0.24	1.60	0.38								
	3	1.57	0.11	2.11	0.29	1.26	0.12	1.17	0.28	1.50	0.29	1.51	0.14
	4	2.41	0.37	3.31	0.81								
	5	4.07	0.38	5.64	1.12	1.57	0.13	1.46	0.30	1.43	0.36	1.57	0.49
	6									2.95	0.69	3.13	0.82
	7	6.75	1.51	9.43	3.22	2.51	0.22	2.32	0.52	3.06	0.69	3.37	1.10
	10					7.18	1.04	6.79	1.91	16.34	0.41	17.19	2.49
	12					13.05	2.77	12.55	4.43				
Ara-C	0	0.55	0.03	0.99	0.06	0.55	0.10	0.92	0.02	0.71	0.01	0.95	0.04
	1	0.57	0.11	1.04	0.19	0.76	0.24	1.31	0.18	1.03	0.14	1.46	0.22
	3	0.59	0.03	1.07	0.05	0.73	0.21	1.30	0.17	1.03	0.17	1.46	0.26
	6	0.71	0.07	1.30	0.17	0.89	0.04	1.73	0.34	1.75	0.19	2.48	0.30
	8	1.35	0.39	2.46	0.69	1.12	0.17	2.19	0.52	2.32	0.17	3.27	0.25
	10	1.94	0.55	3.55	1.02	1.33	0.30	2.72	0.85	3.96	0.60	5.56	0.83

Appendix table 4 Average Micronuclei (MN) and micronuclei fold increase over control induced by TOP2 poisons and Ara-C in Nalm6 cell lines.



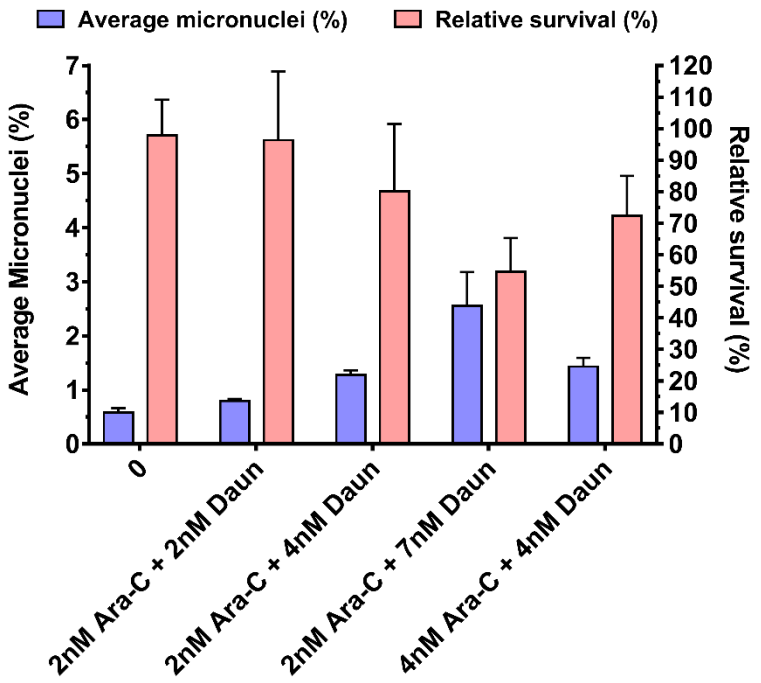
Appendix figure 1 Average Micronuclei (%) induced by daunorubicin, idarubicin, and Ara-C in Nalm6 cell lines.

Drugs	Dose (nM)	Nalm6^{WT}				Nalm6$^{B-/-}$				Nalm6$^{A+/-}$			
		Apoptotic cells (%)	SEM	Apoptotic cells Fold increase	SEM	Apoptotic cells (%)	SEM	Apoptotic cells Fold increase	SEM	Apoptotic cells (%)	SEM	Apoptotic cells Fold increase	SEM
Daunorubicin	0	3.23	0.42	0.99	0.06	3.98	0.12	0.98	0.04	2.80	0.17	0.95	0.05
	6	7.27	0.37	2.30	0.19					4.67	0.13	1.71	0.08
	7	9.22	0.95	2.88	0.08								
	8.5	14.42	3.13	4.37	0.43	8.56	0.41	2.15	0.04	6.39	0.13	2.26	0.14
	10	18.29	9.12	5.12	2.11	6.05	0.75	1.51	0.14	3.86	0.10	1.41	0.11
	12	31.29	8.89	9.32	1.47	10.22	0.95	2.56	0.17	10.23	3.64	3.59	1.30
	14					21.98	0.73	5.55	0.35	40.89	6.16	10.28	3.73
	18					49.84	3.37	12.55	0.93				
Idarubicin	0	3.23	0.42	0.99	0.06	3.98	0.12	0.98	0.04	2.80	0.17	0.95	0.05
	1	3.61	0.24	1.14	0.08								
	3	6.82	1.39	2.07	0.17	5.01	0.12	1.26	0.04	7.30	2.70	1.46	0.20
	4	13.69	3.07	4.13	0.44								
	5	24.63	2.24	7.97	1.56	7.38	0.39	1.85	0.05	9.31	1.79	2.33	0.33
	6									23.12	3.07	5.75	1.69
	7	60.25	16.91	17.92	2.91	12.44	0.93	3.12	0.15	45.46	29.19	5.19	0.63
	10					48.43	4.60	12.12	0.77	203.17	81.64	35.58	13.71
	12					113.48	26.25	28.24	5.72				
Ara-C	0	1.99	0.10	1.01	0.07	3.97	0.96	0.93	0.07	2.42	0.11	1.01	0.07
	1	1.90	0.03	0.96	0.07	4.81	1.58	1.17	0.10	2.70	0.07	1.12	0.05
	3	2.08	0.05	1.05	0.07	4.90	1.51	1.20	0.08	2.85	0.06	1.18	0.05
	6	3.57	0.25	1.81	0.19	9.06	3.35	2.16	0.27	5.37	0.23	2.22	0.10
	8	5.93	0.95	3.01	0.55	13.60	5.88	3.15	0.60	7.94	0.58	3.28	0.18
	10	8.05	1.44	4.07	0.79	15.13	5.46	3.63	0.46	11.89	1.22	4.92	0.51

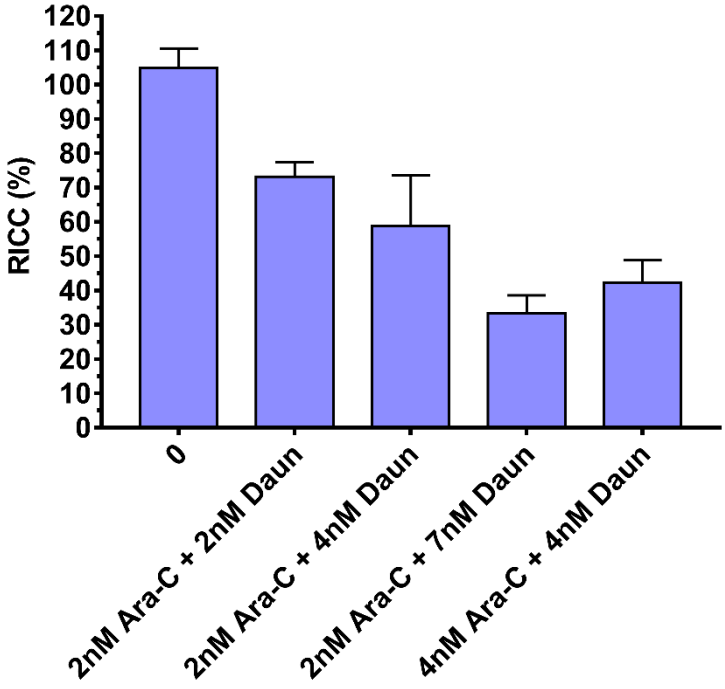
Appendix table 5 Apoptotic cells (%) and apoptotic cells fold increase over control induced by TOP2 poisons and Ara-C in Nalm6 cell lines.

combination	Growth inhibition				Micronuclei data				Apoptotic cells data-			
	RICC (%)	SEM	Relative survival (%)	SEM	MN (%)	SEM	MN Fold increase	SEM	Apoptotic cells (%)	SEM	Apoptotic cells Fold increase	SEM
0	104.92	3.28	97.89	6.52	0.58	0.05	0.98	0.04	3.94	0.24	1.08	0.04
2nM Ara-C + 2nM Daun	73.16	2.48	96.36	12.59	0.80	0.02	1.40	0.14	4.41	0.13	1.12	0.06
2nM Ara-C + 4nM Daun	58.70	8.61	80.05	12.38	1.28	0.05	2.25	0.27	6.63	0.21	1.69	0.06
2nM Ara-C + 7nM Daun	33.31	3.03	54.67	6.17	2.56	0.36	4.59	1.06	14.34	0.25	3.66	0.21
4nM Ara-C + 4nM Daun	42.35	3.74	72.39	7.30	1.43	0.09	2.52	0.32	7.57	0.49	1.92	0.02

Appendix table 6 Growth inhibition, Micronuclei, and Apoptotic cells data for selected concurrent combinations in Nalm6^{WT}



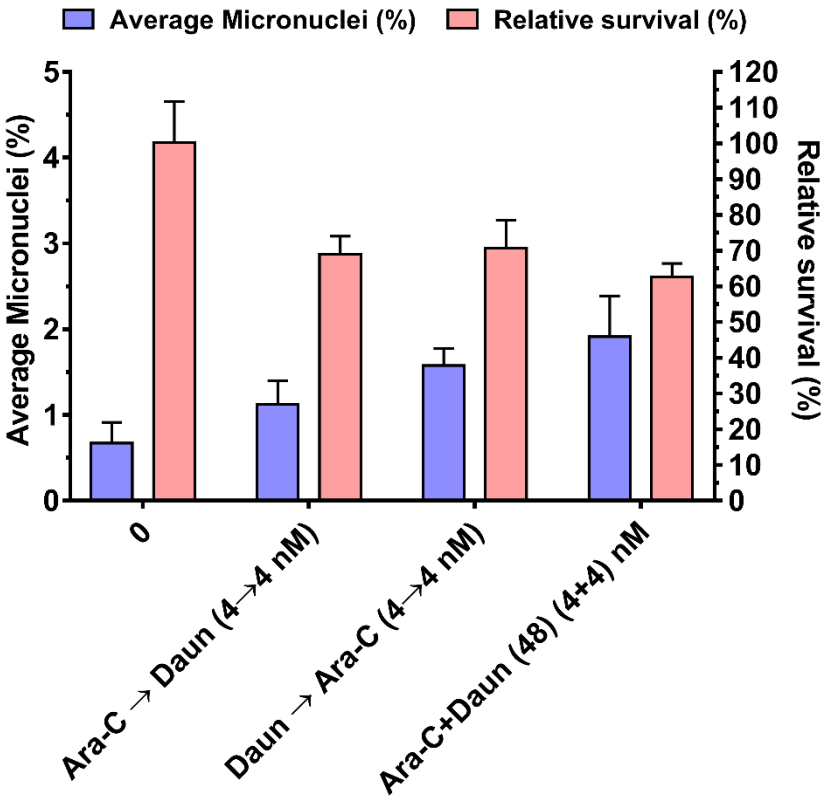
Appendix figure 2 Average Micronuclei (%) induction by selected (Ara-C+daunorubicin) concurrent combinations in Nalm6^{WT} cell line.



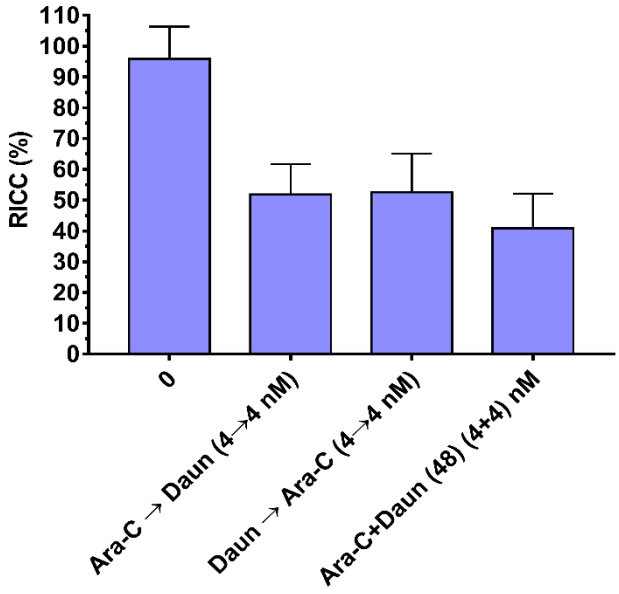
Appendix figure 3 RICC at selected (Ara-C+daunorubicin) concurrent combinations in Nalm6^{WT} cell line.

combination	Growth inhibition				Micronuclei data				Apoptotic cells data-			
	RICC (%)	SEM	Relative survival (%)	SEM	MN (%)	SEM	MN Fold increase	SEM	Apoptotic cells (%)	SEM	Apoptotic cells Fold increase	SEM
0	96.97	5.08	96.62	3.73	0.67	0.11	1.01	0.09	3.63	0.26	0.96	0.02
Ara-C → Daun (4→4 nM)	49.24	3.69	71.38	3.40	1.12	0.12	1.81	0.26	5.56	0.43	1.54	0.08
Daun → Ara-C (4→4 nM)	50.22	5.59	69.51	3.74	1.69	0.10	2.75	0.39	8.60	0.37	2.41	0.18
Ara-C+Daun (48) (4+4) nM	37.55	3.78	61.76	1.52	1.82	0.21	2.99	0.52	8.92	0.59	2.48	0.15

Appendix table 7 Growth inhibition, Micronuclei, and Apoptotic cells data for selected scheduled combinations in Nalm6^{WT}



Appendix figure 4 Average Micronuclei (%) induction by selected (Ara-C+daunorubicin) scheduled combinations in Nalm6^{WT} cell line.



Appendix figure 5 RICC at selected (Ara-C+daunorubicin) scheduled combinations in Nalm6^{WT} cell line.

Drugs	Dose (nM)	Nalm6^{WT}						Nalm6$^{B-/-}$						Nalm6$^{A+/-}$					
		G1		S		G2/M		G1		S		G2/M		G1		S		G2/M	
		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Daunorubicin	0	35.70	1.69	47.49	1.42	16.81	0.29	34.37	1.22	48.50	1.71	17.12	0.56	34.82	1.51	46.11	1.69	19.07	0.90
	6	41.17	1.72	40.39	1.63	18.43	0.14							38.06	0.10	43.96	0.31	17.97	0.41
	7	42.12	2.75	37.79	2.41	20.09	1.67												
	8.5	46.46	1.99	32.58	2.99	20.95	1.29	37.42	1.17	42.83	1.65	19.75	0.56	42.32	0.54	37.92	1.35	19.76	0.81
	10	46.25	5.77	34.07	7.57	19.67	2.01	36.87	1.19	44.60	1.33	18.53	0.27	37.86	0.65	42.60	0.83	19.55	0.19
	12	50.61	1.49	25.08	3.47	24.31	2.31	41.05	1.82	39.83	1.77	19.12	0.06	42.95	3.05	35.91	3.77	21.14	0.78
	14							47.02	1.14	32.48	0.96	20.49	1.01	52.54	2.28	22.81	2.13	24.65	0.37
	18							50.39	2.46	22.09	2.53	27.53	1.47						
Idarubicin	0	35.70	1.69	47.49	1.42	16.81	0.29	34.37	1.22	48.50	1.71	17.12	0.56	34.82	1.51	46.11	1.69	19.07	0.90
	1	36.44	0.53	44.94	0.48	18.62	0.07												
	3	40.43	1.85	40.83	1.93	18.73	0.59	35.63	1.42	45.71	1.31	18.66	0.41	36.82	2.60	44.91	3.36	18.27	0.78
	4	45.77	2.78	33.42	3.39	20.82	1.41												
	5	49.41	0.19	29.92	0.94	20.67	0.75	37.69	0.54	41.78	1.62	20.54	1.60	40.07	1.95	39.55	1.83	20.38	0.18
	6													47.98	0.68	30.84	0.71	21.17	0.84
	7	53.42	1.69	22.56	3.20	24.02	1.74	41.14	1.29	36.74	1.77	22.12	0.49	44.19	1.62	34.61	1.62	21.20	0.05
	10							50.85	2.04	26.43	2.55	22.72	0.90	50.26	0.43	25.16	0.71	24.59	0.55
	12							50.75	1.56	22.31	1.93	26.94	1.32						
Ara-C	0	37.31	2.17	45.14	1.67	17.55	0.51	37.41	2.17	45.68	2.11	16.90	0.31	37.58	0.78	44.43	0.77	17.99	0.10
	1	34.66	3.07	45.87	2.52	19.47	0.62	37.02	1.84	45.03	1.15	17.96	0.87	36.69	0.51	45.20	0.99	18.11	0.95
	3	34.75	2.07	46.25	1.76	19.01	0.48	35.91	1.07	44.89	0.66	19.20	0.42	33.64	0.97	47.35	0.79	19.01	0.76
	6	30.36	1.05	51.37	1.18	18.27	0.31	29.37	2.37	51.36	3.08	19.27	1.06	31.23	0.71	51.11	0.54	17.67	0.79
	8	30.39	1.34	51.25	1.14	18.36	0.45	29.81	1.78	51.65	2.60	18.54	1.25	30.27	0.98	50.45	1.46	19.28	0.93
	10	30.29	0.97	52.27	0.81	17.43	0.16	28.96	2.23	52.75	3.02	18.29	0.98	32.01	1.68	49.59	2.45	18.39	0.99

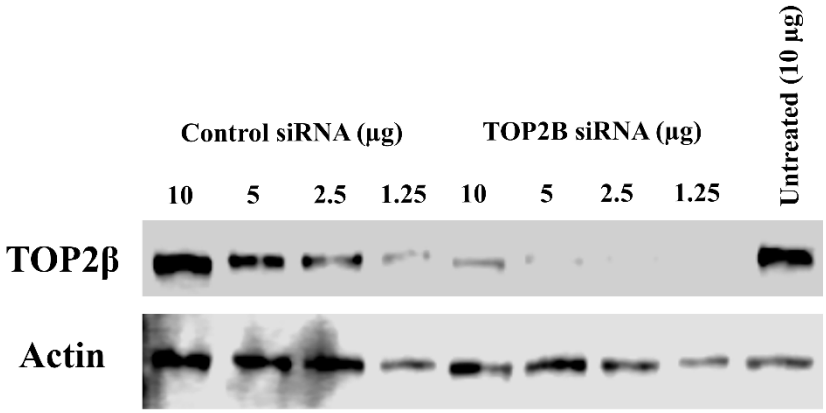
Appendix Table 8 Relative cell cycle phase distribution in Nalm6 cell lines treated with daunorubicin, idarubicin, and Ara-C.

combination	G1		S		G2/M	
	mean	SEM	mean	SEM	mean	SEM
0	34.79	2.91	45.51	0.63	19.69	2.31
2nM Ara-C + 2nM Daun	33.75	1.73	47.03	2.96	19.22	1.46
2nM Ara-C + 4nM Daun	36.83	1.32	43.94	2.38	19.23	1.07
2nM Ara-C + 7nM Daun	44.46	1.41	33.57	1.18	21.97	0.45
4nM Ara-C + 4nM Daun	34.66	1.34	46.11	2.04	19.22	0.79

Appendix table 9 Relative cell cycle phase distribution in Nalm6^{WT} cell line treated with selected (Ara-C+daunorubicin) concurrent combinations.

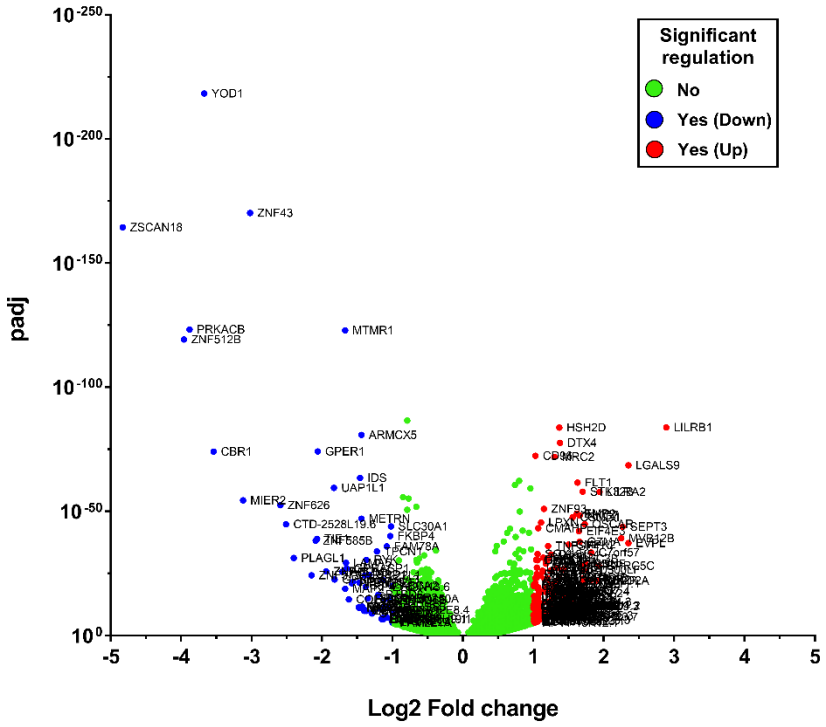
combination	G1		S		G2/M	
	mean	SEM	mean	SEM	mean	SEM
0	34.36	0.72	49.53	1.14	16.11	0.78
Ara-C → Daun (4→4 nM)	38.39	1.33	42.61	1.78	19.01	0.46
Daun → Ara-C (4→4 nM)	30.43	1.45	47.91	1.82	21.66	0.63
Ara-C+Daun (48) (4+4) nM	37.38	1.07	44.84	1.16	17.79	0.43

Appendix table 10 Relative cell cycle phase distribution in Nalm6^{WT} cell line treated with selected (Ara-C+daunorubicin) scheduled combinations.



Appendix figure 6 siRNA knockdown of *TOP2B* in K562^{WT} cell line

Samples were probed with TOP2B antibodies. Cells were transfected with 400 nM of either control or *TOP2B* siRNA. Electroporation conditions were (10 mSec and 340-360 V).



Appendix figure 7 Differential gene expression in Nalm6^{WT} vs. Nalm6$^{B-/-}$ (AROS data).

Volcano plot shows log2 fold change in gene expression. Genes showed more than 2 fold change are labelled. Y axis shows adjusted P values (padj) which represent p-value adjusted for multiple testing correction using Benjamini-Hochberg method to control the False Discovery Rate (FDR). Genes showed < 0.05 padj were considered as significantly changed.

Appendix table 11 Down regulated genes in Nalm6$^{B-/-}$ compared to Nalm6^{WT} (AROS data).

Genes	Description	Log2 Fold change	% of control*	padj
ZSCAN18	zinc finger and SCAN domain containing 18	4.83	3.53	3.64E-165
ZNF512B	zinc finger protein 512B	3.96	6.42	4.79E-120
PRKACB	protein kinase, cAMP-dependent, catalytic, beta	3.88	6.78	4.90E-124
YOD1	YOD1 deubiquitinase	3.67	7.83	4.78E-219
CBR1	carbonyl reductase 1	3.54	8.60	8.09E-75
TOP2B	topoisomerase (DNA) II beta	3.22	10.71	0*
MIER2	mesoderm induction early response 1, family member 2	3.12	11.53	3.01E-55
ZNF43	zinc finger protein 43	3.02	12.33	5.87E-171
ZNF626	zinc finger protein 626	2.59	16.62	3.04E-53
CTD-2528L19.6	RNA Gene	2.51	17.58	1.39E-45
PLAGL1	pleiomorphic adenoma gene-like 1	2.40	18.96	5.10E-32
ZNF573	zinc finger protein 573	2.15	22.60	4.64E-25
ZNF585B	zinc finger protein 585B	2.09	23.48	5.04E-39
TIE1	tyrosine kinase with immunoglobulin-like and EGF-like domains 1	2.07	23.78	1.02E-39
GPER1	G protein-coupled estrogen receptor 1	2.06	23.98	6.29E-75
ZNF432	zinc finger protein 432	1.94	26.09	1.33E-26
UAP1L1	UDP-N-acetylglucosamine pyrophosphorylase 1 like 1	1.83	28.16	3.17E-60
GPR146	G protein-coupled receptor 146	1.82	28.36	2.43E-23
ABLIM1	actin binding LIM protein 1	1.71	30.55	1.91E-26
MAP2	microtubule-associated protein 2	1.67	31.32	1.27E-19
MTMR1	myotubularin related protein 1	1.67	31.44	1.13E-123
LAMA5	laminin, alpha 5	1.66	31.60	4.91E-30
GPRASP1	G protein-coupled receptor associated sorting protein 1	1.65	31.91	9.31E-28
COL8A2	collagen, type VIII, alpha 2	1.62	32.57	2.73E-15
PTPN21	protein tyrosine phosphatase, non-receptor type 21	1.58	33.50	7.70E-22
NBPF15	neuroblastoma breakpoint family, member 15	1.56	33.91	2.01E-22
RAB39B	RAB39B, member RAS oncogene family	1.48	35.82	4.27E-12
PTMS	parathymosin [Source:HGNC	1.47	36.02	4.03E-22
MIOX	myo-inositol oxygenase	1.47	36.06	6.67E-12
IDS	iduronate 2-sulfatase	1.46	36.44	3.03E-64
ARMCX5	armadillo repeat containing, X-linked 5	1.44	36.76	1.59E-81
METRN	meteorin, glial cell differentiation regulator	1.44	36.93	8.21E-48
GP5	glycoprotein V (platelet)	1.43	37.01	2.08E-12
MIR4442	microRNA 4442	1.40	37.97	1.03E-10
PYCARD	PYD and CARD domain containing	1.39	38.09	4.74E-11
RPL22L1	ribosomal protein L22-like 1	1.39	38.13	5.53E-25
RP4-769N13.6	novel transcript, ARMCX5-GPRASP2 readthrough	1.38	38.53	2.06E-20

ZNF737	zinc finger protein 737	1.37	38.60	9.60E-11
RYK	receptor-like tyrosine kinase	1.37	38.71	3.27E-31
RIBC2	RIB43A domain with coiled-coils 2	1.35	39.27	1.23E-15
SPP1	secreted phosphoprotein 1	1.33	39.91	3.17E-25
SLC9A3R2	solute carrier family 9, subfamily A (NHE3, cation proton antiporter 3), member 3 regulator 2	1.29	40.76	5.21E-10
ST6GAL2	ST6 beta-galactosamide alpha-2,6-sialyltranferase 2	1.29	40.91	1.21E-09
CD34	CD34 molecule	1.27	41.56	1.35E-10
TPCN1	two pore segment channel 1	1.22	42.98	1.31E-34
BTBD6	BTB (POZ) domain containing 6	1.20	43.41	3.21E-17
ZNF841	zinc finger protein 841	1.15	45.10	1.15E-07
PCDH18	protocadherin 18	1.15	45.13	2.03E-07
LA16c-312E8.4	Uncategorized gene.	1.15	45.15	4.65E-11
RP11-108H9.1	RNA Gene	1.14	45.25	1.89E-07
GJA3	gap junction protein, alpha 3, 46kDa	1.14	45.27	2.50E-23
RP11-557L19.1	RNA Gene	1.14	45.51	2.10E-07
CD4	CD4 molecule	1.14	45.51	7.70E-08
ENOX1	ecto-NOX disulfide-thiol exchanger 1	1.13	45.54	2.52E-07
TMPRSS3	transmembrane protease, serine 3	1.12	45.88	2.48E-12
DENND6B	DENN/MADD domain containing 6B	1.12	46.11	5.38E-15
FAM78A	family with sequence similarity 78, member A	1.08	47.30	1.13E-36
BHLHB9	basic helix-loop-helix domain containing, class B, 9	1.08	47.43	4.92E-08
C22orf34	chromosome 22 open reading frame 34	1.07	47.63	7.44E-12
TMEM150A	transmembrane protein 150A	1.03	49.00	1.37E-15
FKBP4	FK506 binding protein 4, 59kDa	1.03	49.07	7.16E-41
SLC30A1	solute carrier family 30 (zinc transporter), member 1	1.02	49.25	9.92E-45
PLCH2	phospholipase C, eta 2	1.01	49.76	4.48E-07
ZFYVE28	zinc finger, FYVE domain containing 28	1.00	49.90	2.68E-06
ABCA2	ATP-binding cassette, sub-family A (ABC1), member 2	1.00	49.98	8.44E-21
FAM221A	family with sequence similarity 221, member A	1.00	50.00	5.66E-06
WBP5	WW domain binding protein 5	1.00	50.03	1.44E-06

* The control ($Nalm6^{WT}$) corresponds to 100%

Genes are listed from the highest to the lowest log2 fold change value. (*) *TOP2B* gene showed such a very low (padj) value that the machine rounded it to zero as the smallest non-zero normalized floating-point number is 2.225074e-308.

Appendix table 12 Up regulated genes in Nalm6$^{B-/-}$ compared to Nalm6^{WT} (AROS data).

Genes	Description	Log2 Fold change	% of control*	padj
LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	2.89	740.83	1.31E-84
EVPL	envoplakin	2.35	509.94	5.30E-38
LGALS9	lectin, galactoside-binding, soluble, 9	2.35	508.41	2.58E-69
SEPT3	septin 3	2.27	482.53	1.44E-44
MVB12B	multivesicular body subunit 12B	2.25	476.29	5.87E-40
LILRA2	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2	1.94	383.28	1.19E-58
GPRC5C	G protein-coupled receptor, class C, group 5, member C	1.93	381.05	7.59E-29
CLMP	CXADR-like membrane protein	1.91	375.07	6.39E-23
CC2D2A	coiled-coil and C2 domain containing 2A	1.89	371.92	6.91E-23
ROR2	receptor tyrosine kinase-like orphan receptor 2	1.82	352.11	3.24E-23
C7orf57	chromosome 7 open reading frame 57	1.82	351.99	3.32E-34
GDNF	glial cell derived neurotrophic factor	1.74	334.47	1.02E-29
OSCAR	osteoclast associated, immunoglobulin-like receptor	1.73	332.45	1.13E-45
TNS3	tensin 3	1.71	327.43	9.74E-22
CD300LF	CD300 molecule-like family member f	1.71	326.79	2.96E-27
ARL4C	ADP-ribosylation factor-like 4C	1.71	326.58	9.19E-24
IL1B	interleukin 1, beta	1.70	325.29	6.77E-29
STK32B	serine/threonine kinase 32B	1.70	324.80	1.01E-58
NKG7	natural killer cell granule protein 7	1.66	316.56	3.82E-49
GZMA	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	1.66	315.00	1.51E-38
EIF4E3	eukaryotic translation initiation factor 4E family member 3	1.65	313.49	8.74E-43
FLT1	fms-related tyrosine kinase 1	1.63	308.52	2.35E-62
EMP2	epithelial membrane protein 2	1.62	306.56	8.76E-50
CRAT	carnitine O-acetyltransferase	1.61	304.22	2.26E-18
CSMD1	CUB and Sushi multiple domains 1	1.56	295.86	2.30E-48
DCLK2	doublecortin-like kinase 2	1.56	294.84	4.04E-14
CTB-83J4.2	RNA gene	1.53	289.51	8.45E-13
CMPK2	cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial	1.50	283.63	2.35E-37
CTSG	cathepsin G	1.50	283.37	1.15E-12
AC004009.3	UnchAra-Cterized LOC105375207 (RNA gene)	1.48	279.18	1.62E-12
SOD1P3	superoxide dismutase 1, soluble pseudogene 3	1.47	276.34	1.57E-12
MPEG1	macrophage expressed 1	1.46	275.44	1.95E-16
TUB	tubby bipartite transcription factor	1.43	269.64	1.55E-27
ADAM19	ADAM metallopeptidase domain 19	1.42	267.72	2.08E-16
ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	1.40	263.47	2.34E-11
TSC22D3	TSC22 domain family, member 3	1.40	263.22	8.82E-13

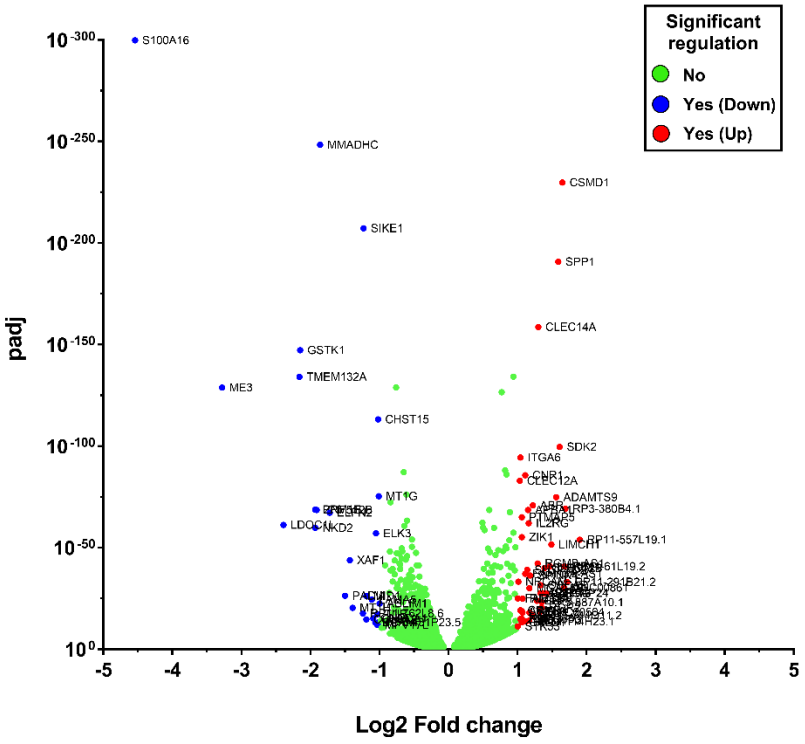
SPNS3	spinster homolog 3 (Drosophila)	1.39	261.80	2.79E-11
DTX4	deltex 4, E3 ubiquitin ligase	1.38	260.98	2.06E-78
TANC1	tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 1	1.38	259.53	6.48E-19
IGFBP4	insulin-like growth factor binding protein 4	1.37	258.45	3.76E-24
MROH3P	maestro heat-like repeat family member 3, pseudogene	1.37	258.22	6.51E-13
HSH2D	hematopoietic SH2 domain containing	1.37	257.92	1.53E-84
SLC26A9	solute carrier family 26 (anion exchanger), member 9	1.36	256.80	4.42E-20
NSUN7	NOP2/Sun domain family, member 7	1.35	254.71	2.41E-10
CD1C	CD1c molecule	1.34	252.82	2.33E-12
SULF2	sulfatase 2	1.33	252.11	4.50E-28
KALRN	kalirin, RhoGEF kinase	1.32	249.75	2.58E-22
LINC00963	long intergenic non-protein coding RNA 963	1.30	247.02	1.08E-12
MRC2	mannose receptor, C type 2	1.30	246.97	1.06E-72
RP3-416J7.4	UnchAra-Cterized LOC285766 (RNA Gene)	1.30	246.40	1.32E-18
ALOX12P2	Ara-Chidonate 12-lipoxygenase pseudogene 2	1.30	246.03	1.50E-10
BACE2	beta-site APP-cleaving enzyme 2	1.30	245.58	1.06E-17
CGNL1	cingulin-like 1	1.28	242.79	2.00E-16
ITIH3	inter-alpha-trypsin inhibitor heavy chain 3	1.28	242.43	2.76E-19
FHIT	fragile histidine triad	1.27	241.47	7.06E-11
RP4-639J15.3	RNA Gene	1.26	239.69	8.14E-09
HHIP-AS1	HHIP antisense RNA 1	1.26	238.98	2.71E-15
SMIM24	small integral membrane protein 24	1.25	238.48	3.74E-18
C1orf54	chromosome 1 open reading frame 54	1.25	238.41	1.87E-33
EBF4	early B-cell factor 4	1.25	237.86	1.07E-08
TREML2	triggering receptor expressed on myeloid cells-like 2	1.24	236.52	8.98E-11
EFEMP1	EGF containing fibulin-like extracellular matrix protein 1	1.24	236.34	7.49E-27
CTD-2313F11.1	RNA Gene	1.23	234.07	1.38E-21
MUC19	mucin 19, oligomeric	1.21	232.03	2.49E-08
ATP9A	ATPase, class II, type 9A	1.21	231.50	3.04E-08
APOBEC3B	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B	1.21	231.28	1.13E-31
TNFSF4	tumor necrosis factor (ligand) superfamily, member 4	1.21	230.82	8.19E-37
ANKRD18DP	ankyrin repeat domain 18D, pseudogene	1.20	229.13	6.58E-24
CCDC3	coiled-coil domain containing 3	1.20	229.02	2.33E-11
RP11-87G24.3	RNA Gene	1.19	228.41	2.68E-14
CTD-3035K23.7	RNA Gene	1.19	228.31	2.65E-08
CHD5	chromodomain helicase DNA binding protein 5	1.19	228.01	8.49E-19
SYNJ2	synaptojanin 2	1.19	227.85	3.88E-10
STAT5A	signal transducer and activator of transcription 5A	1.19	227.49	1.82E-30
GSN	gelsolin	1.18	226.25	2.58E-30
C1orf228	chromosome 1 open reading frame 228	1.18	226.15	2.11E-08
PHACTR3	phosphatase and actin regulator 3	1.18	225.92	1.14E-29

CIART	circadian associated repressor of transcription	1.17	225.61	8.54E-20
ZNF283	zinc finger protein 283	1.17	224.57	2.01E-18
SYPL1	synaptophysin-like 1	1.16	223.05	1.81E-23
ZNF93	zinc finger protein 93	1.15	222.01	8.45E-52
SIGLEC17P	sialic acid binding Ig-like lectin 17, pseudogene	1.15	221.58	3.24E-08
ESM1	endothelial cell-specific molecule 1	1.15	221.57	5.69E-08
RAB27B	RAB27B, member RAS oncogene family	1.15	221.30	1.70E-09
GOLGA8S	golgin A8 family, member S	1.15	221.22	1.48E-18
ME3	malic enzyme 3, NADP(+)-dependent, mitochondrial	1.14	220.76	1.12E-26
SYT11	synaptotagmin XI	1.14	220.36	3.83E-32
SYNPO	synaptopodin	1.13	219.55	8.57E-09
NCF2	neutrophil cytosolic factor 2	1.13	219.30	3.18E-08
HR	hair growth associated	1.13	218.91	1.14E-10
PVRL1	poliovirus receptor-related 1 (herpesvirus entry mediator C)	1.13	218.80	6.71E-27
NOS3	nitric oxide synthase 3 (endothelial cell)	1.13	218.74	1.95E-09
LPXN	leupaxin	1.11	216.15	2.62E-46
LRG1	leucine-rich alpha-2-glycoprotein 1	1.11	215.79	1.45E-08
RGS9	regulator of G-protein signaling 9	1.11	215.60	1.07E-13
CTC-251I16.1	TEC (To be Experimentally Confirmed) gene	1.10	214.57	1.42E-11
LRRC2	leucine rich repeat containing 2	1.10	213.98	4.62E-12
SLC16A2	solute carrier family 16, member 2 (thyroid hormone transporter)	1.10	213.87	2.18E-18
ZNF114	zinc finger protein 114	1.10	213.85	7.37E-25
GDNF-AS1	GDNF antisense RNA 1	1.09	213.58	3.32E-07
RP1-45N11.1	UnchAra-Cterized LOC105377891 (RNA gene)	1.08	211.59	1.28E-12
CCM2L	cerebral cavernous malformation 2-like	1.08	211.51	1.16E-13
RP5-1028K7.2	RNA Gene	1.08	211.44	1.13E-17
CTD-3035K23.3	Uncategorized gene.	1.08	211.23	4.73E-07
TNS1	tensin 1	1.08	211.18	1.51E-12
MGAT5B	mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase, isozyme B	1.07	210.58	3.68E-20
CTD-2368P22.1	UnchAra-Cterized LOC100128398 (RNA gene)	1.07	210.56	3.59E-13
TNFRSF8	tumor necrosis factor receptor superfamily, member 8	1.07	210.52	1.80E-08
COL5A1	collagen, type V, alpha 1	1.07	210.45	6.15E-15
SPSB1	splA/ryanodine receptor domain and SOCS box containing 1	1.07	210.36	2.46E-19
TBC1D27	TBC1 domain family, member 27	1.07	210.17	1.83E-09
LIMCH1	LIM and calponin homology domains 1	1.07	210.11	7.25E-16
CELF5	CUGBP, Elav-like family member 5	1.07	209.37	6.58E-10
LRFN2	leucine rich repeat and fibronectin type III domain containing 2	1.07	209.26	3.84E-07
CMAHP	cytidine monophospho-N-acetylneuraminic acid hydroxylase, pseudogene	1.07	209.26	5.47E-44
RNF182	ring finger protein 182	1.06	209.11	6.56E-17
ZNF831	zinc finger protein 831	1.06	209.04	2.85E-10

KLK1	kallikrein 1	1.06	208.63	1.83E-06
LST1	leukocyte specific transcript 1	1.06	208.49	1.95E-06
ULK2	unc-51 like autophagy activating kinase 2	1.06	208.34	3.99E-31
SOX13	SRY (sex determining region Y)-box 13	1.06	208.34	1.35E-33
PIK3R5	phosphoinositide-3-kinase, regulatory subunit 5	1.05	207.05	1.46E-25
VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor	1.05	207.04	4.24E-24
TMSB4Y	thymosin beta 4, Y-linked	1.05	206.46	4.13E-28
GFRA1	GDNF family receptor alpha 1	1.04	205.90	2.84E-31
LINC01588	long intergenic non-protein coding RNA 1588	1.04	205.80	1.86E-06
MYO7A	myosin VIIA	1.04	205.75	1.60E-07
ETV7	ets variant 7	1.03	204.71	3.45E-06
FBLN7	fibulin 7	1.03	204.56	4.42E-12
GZMK	granzyme K (granzyme 3; tryptase II)	1.03	203.88	6.37E-22
CD96	CD96 molecule	1.03	203.77	3.89E-73
ADAMTS10	ADAM metallopeptidase with thrombospondin type 1 motif, 10	1.03	203.70	6.53E-24
AMPD3	adenosine monophosphate deaminase 3	1.02	203.45	1.54E-12
CSPG4	chondroitin sulfate proteoglycan 4	1.02	202.86	1.43E-09
RP1-310O13.7	RNA gene	1.02	202.57	7.24E-08
RP11-13K12.1	UnchAra-Cterized LOC100507351 (RNA gene)	1.02	202.14	5.90E-06
KCNK10	potassium channel, two pore domain subfamily K, member 10	1.02	202.13	8.23E-15
DUSP4	dual specificity phosphatase 4	1.01	201.98	2.91E-13
LINC00937	long intergenic non-protein coding RNA 937	1.01	201.91	1.25E-16
C1R	complement component 1, r subcomponent	1.01	201.81	1.75E-07
ECM1	extracellular matrix protein 1	1.01	201.76	2.92E-21
S100A10	S100 calcium binding protein A10	1.01	201.74	1.80E-08
C19orf57	chromosome 19 open reading frame 57	1.01	201.42	1.38E-20
RP11-114H23.1	RNA gene	1.01	201.17	1.61E-06
OBSCN	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF	1.00	200.40	6.76E-13
C1RL	complement component 1, r subcomponent-like	1.00	200.34	7.02E-12
RP11-889L3.1	Ribosomal Protein L19 Pseudogene 9	1.00	199.98	6.40E-09
LINC00689	long intergenic non-protein coding RNA 689	1.00	199.74	2.13E-11

* The control (Nalm6^{WT}) corresponds to 100%

Genes are listed from the highest to the lowest log2 fold change value.



Appendix figure 8 Differential gene expression in Nalm6^{WT} vs. Nalm6$^{A+/-}$ (AROS data).

Volcano plot shows log2 fold change in gene expression. Genes showed more than 2 fold change are labelled. Y axis shows adjusted P values (padj) which represent p-value adjusted for multiple testing correction using Benjamini-Hochberg method to control the False Discovery Rate (FDR). Genes showed < 0.05 padj were considered as significantly changed.

Genes	Description	Log2 Fold change	% of control*	padj
S100A16	S100 calcium binding protein A16	4.54	4.30	1.32E-300
ME3	malic enzyme 3, NADP(+)-dependent, mitochondrial	3.28	10.33	1.11E-129
LDOC1L	leucine zipper, down-regulated in cancer 1-like	2.39	19.13	5.60E-62
TMEM132A	transmembrane protein 132A	2.16	22.44	6.65E-135
GSTK1	glutathione S-transferase kappa 1	2.15	22.50	5.35E-148
NKD2	naked cuticle homolog 2 (Drosophila)	1.93	26.20	1.66E-60
PPM1E	protein phosphatase, Mg2+/Mn2+ dependent, 1E	1.93	26.31	1.59E-69
ZNF512B	zinc finger protein 512B	1.91	26.64	2.57E-69
MMADHC	methylmalonic aciduria (cobalamin deficiency) cblD type, with homocystinuria	1.86	27.45	4.28E-249
ELFN2	extracellular leucine-rich repeat and fibronectin type III domain containing 2	1.72	30.31	5.37E-68
PADI4	peptidyl arginine deiminase, type IV	1.50	35.30	4.22E-27
XAF1	XIAP associated factor 1	1.43	37.03	1.28E-44
MT1H	metallothionein 1H	1.39	38.09	3.83E-21
RP11-762L8.6	Uncategorized gene	1.24	42.32	1.85E-18
SIKE1	suppressor of IKBKE 1	1.23	42.57	4.81E-208
MID1	midline 1	1.19	43.92	5.34E-27
COL8A2	collagen, type VIII, alpha 2	1.19	43.93	1.67E-15
LAMA5	laminin, alpha 5	1.11	46.46	2.91E-25
GNPDA1	glucosamine-6-phosphate deaminase 1	1.09	47.12	5.50E-16
BCAM	basal cell adhesion molecule (Lutheran blood group)	1.06	47.84	6.22E-14
ELK3	ELK3, ETS-domain protein (SRF accessory protein 2)	1.05	48.39	8.20E-58
MPV17L	MPV17 mitochondrial membrane protein-like	1.03	49.06	8.81E-13
TUB	tubby bipartite transcription factor	1.03	49.12	4.90E-18
CHST15	carbohydrate (N-acetylgalactosamine 4-sulfate 6-O) sulfotransferase 15	1.02	49.19	5.27E-114
RP3-431P23.5	RNA gene	1.01	49.53	4.22E-14
MT1G	metallothionein 1G	1.01	49.63	4.11E-76
ABLIM1	actin binding LIM protein 1	1.00	50.06	2.73E-23
TOP2A	topoisomerase (DNA) II alpha	0.76	59.06	1.11E-129

* The control ($Nalm6^{WT}$) corresponds to 100%

Appendix table 13 Down regulated genes in $Nalm6^{A+/-}$ compared to $Nalm6^{WT}$ (AROS data).

Genes are listed from the highest to the lowest log2 fold change value.

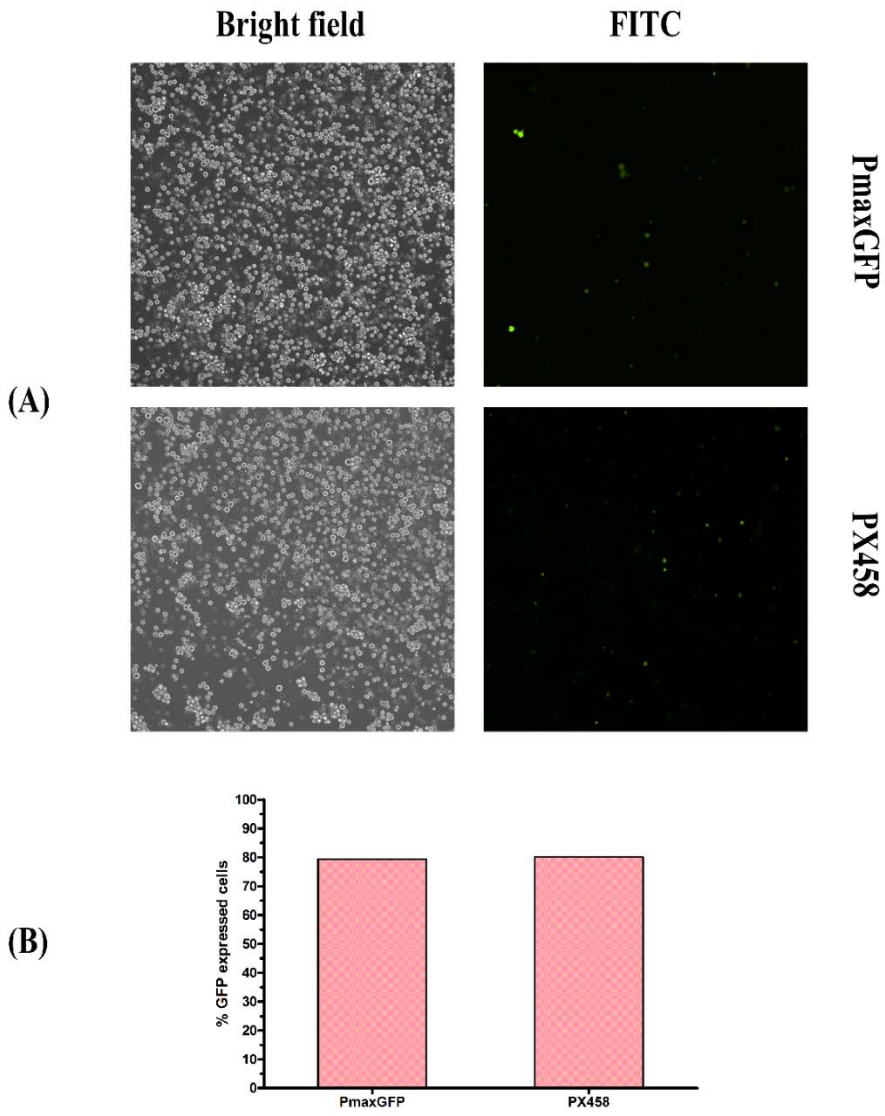
Appendix table 14 Up regulated genes in Nalm6$^{A+/-}$ compared to Nalm6^{WT} (AROS data).

Genes	Description	Log2 Fold change	% of control*	padj
RP11-557L19.1	RNA Gene	1.90	374.30	1.00E-54
RP11-291B21.2	UnchAra-Cterized LOC101060038 (RNA gene)	1.72	329.83	7.60E-34
RP3-380B4.1	Long Intergenic Non-Protein Coding RNA 1758	1.69	323.27	5.47E-70
RP11-61L19.2	RNA Gene	1.68	319.68	1.39E-41
LINC00861	long intergenic non-protein coding RNA 861	1.67	317.88	4.29E-31
CSMD1	CUB and Sushi multiple domains 1	1.65	314.24	1.39E-230
SDK2	sidekick cell adhesion molecule 2	1.61	305.00	2.06E-100
SPP1	secreted phosphoprotein 1	1.59	300.18	1.54E-191
ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif, 9	1.56	294.26	1.39E-75
LIMCH1	LIM and calponin homology domains 1	1.49	281.44	2.53E-52
LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	1.47	277.53	1.43E-41
SEPT3	septin 3	1.43	270.30	2.76E-28
CLEC12B	C-type lectin domain family 12, member B	1.39	261.40	9.58E-41
PARVG	parvin, gamma	1.37	258.92	3.38E-28
RP5-887A10.1	Long Intergenic Non-Protein Coding RNA 1781	1.35	255.12	6.60E-24
ROR2	receptor tyrosine kinase-like orphan receptor 2	1.35	254.76	3.67E-21
ARHGAP24	Rho GTPase activating protein 24	1.33	251.70	1.69E-28
OSCAR	osteoclast associated, immunoglobulin-like receptor	1.33	250.86	2.54E-32
LINC00504	long intergenic non-protein coding RNA 504	1.32	250.04	4.35E-19
CLEC14A	C-type lectin domain family 14, member A	1.30	246.10	1.80E-159
RGMB-AS1	RGMB antisense RNA 1	1.29	244.85	5.48E-43
EBF3	early B-cell factor 3	1.28	242.41	1.29E-24
ABR	active BCR-related	1.22	233.63	1.15E-71
RP11-744O11.2	DEAD-Box Helicase 5 (Pseudogene)	1.21	231.71	1.89E-17
SPINK4	serine peptidase inhibitor, Kazal type 4	1.18	226.65	4.48E-37
MGAT5B	mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase, isozyme B	1.17	225.03	7.30E-31
GDNF	glial cell derived neurotrophic factor	1.17	224.96	8.78E-19
SOD1P3	superoxide dismutase 1, soluble pseudogene 3	1.17	224.28	5.75E-15
IL2RG	interleukin 2 receptor, gamma	1.16	223.52	7.61E-63
APBA1	amyloid beta (A4) precursor protein-binding, family A, member 1	1.15	222.00	2.45E-69
SH3PXD2A	SH3 and PX domains 2A	1.14	220.84	7.37E-40
RP11-114H23.1	RNA gene	1.12	217.91	1.97E-14
LRG1	leucine-rich alpha-2-glycoprotein 1	1.12	216.90	1.55E-14
CNR1	cannabinoid receptor 1 (brain)	1.11	216.22	1.74E-86

FAM13A-AS1	FAM13A antisense RNA 1	1.11	215.70	6.14E-38
RIBC2	RIB43A domain with coiled-coils 2	1.07	210.64	8.71E-26
LYZ	Lysozyme	1.07	209.27	7.01E-19
ZIK1	zinc finger protein interacting with K protein 1	1.06	208.74	5.27E-56
PTMAP5	prothymosin, alpha pseudogene 5	1.06	208.38	7.99E-66
APELA	apelin receptor early endogenous ligand	1.06	208.34	1.43E-19
CALD1	caldesmon 1	1.06	207.95	5.18E-14
KIAA1683	KIAA1683	1.06	207.92	7.59E-16
ZNF737	zinc finger protein 737	1.04	205.27	9.67E-16
CRMP1	collapsin response mediator protein 1	1.04	205.25	5.04E-20
ITGA6	integrin, alpha 6	1.04	204.97	3.57E-95
CLEC12A	C-type lectin domain family 12, member A	1.03	204.10	9.69E-84
NRCAM	neuronal cell adhesion molecule	1.01	201.76	4.43E-34
FAM13A	family with sequence similarity 13, member A	1.00	200.67	8.47E-26
STK33	serine/threonine kinase 33	1.00	199.39	4.98E-12

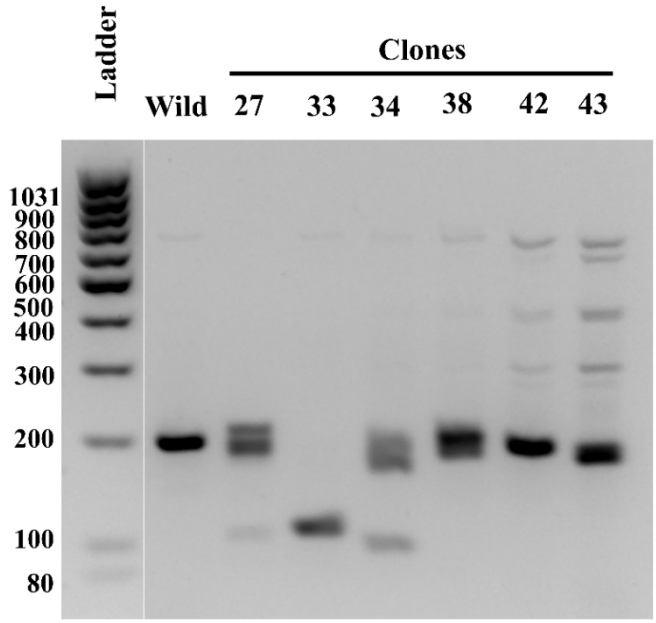
* The control (Nalm6^{WT}) corresponds to 100%

Genes are listed from the highest to the lowest log2 fold change value.



Appendix figure 9 Transfection efficiency in K562 cells.

(**A**) Live imaging of Nalm6 cells transfected with positive control (PmaxGFP) and (PX458) plasmids by Nucleofection. Images were captured using TiE fluorescence wide field inverted microscope (Nikon) (10X magnification). (**B**) Transfection efficiency (%) measured by FACS.

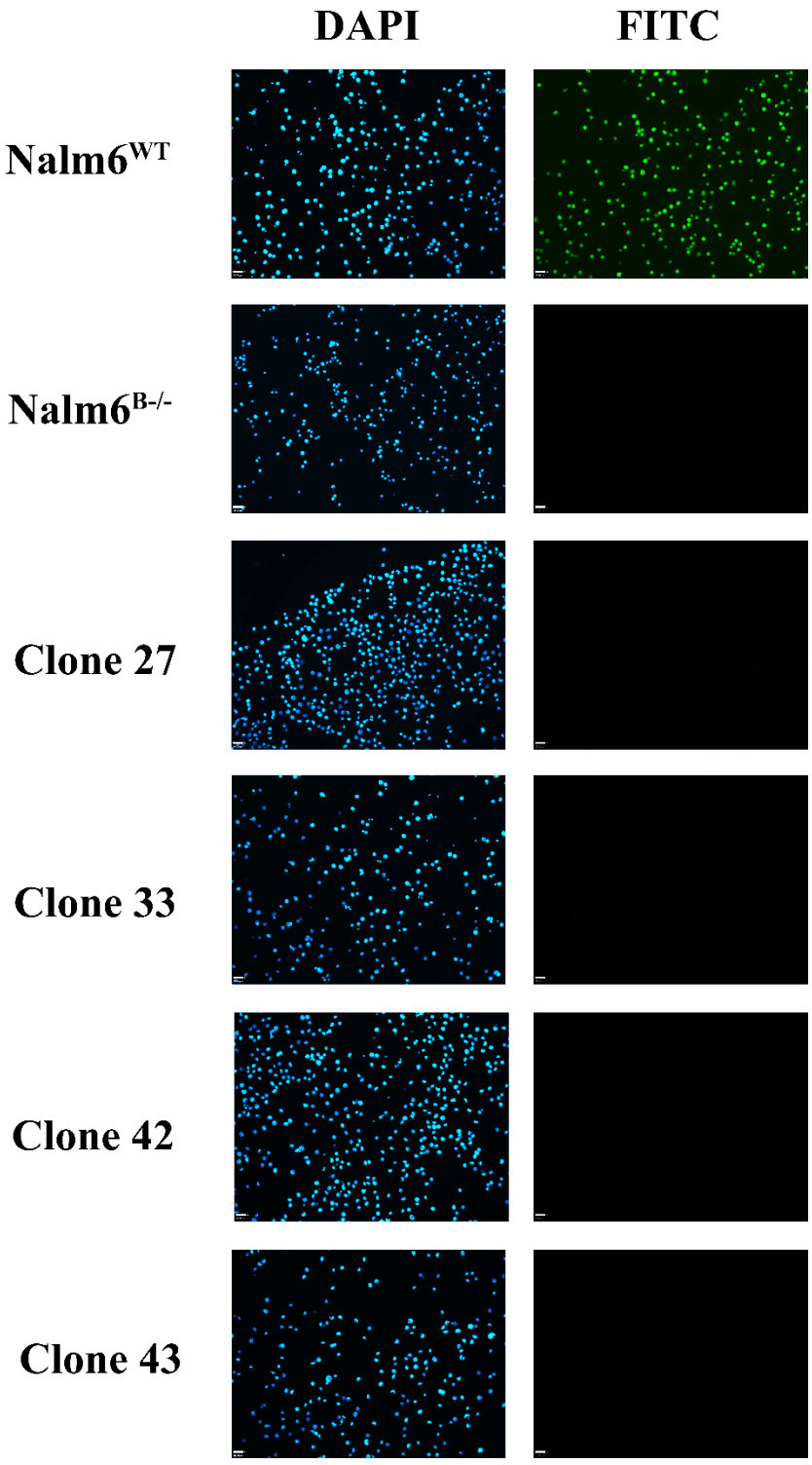


Appendix figure 10 Screening for *TOP2B* knockout clones in K562 cells by genotyping.

Samples were amplified as described in the Materials and methods and loaded on (3 %) agarose gel electrophoresis to allow for small size differences.

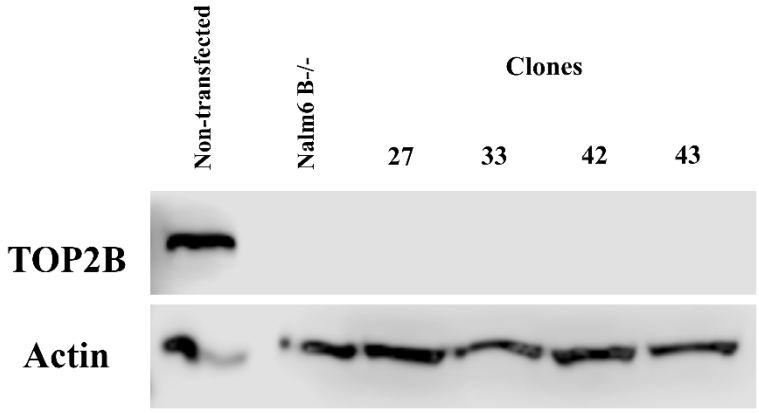
Clone	gRNA	Mutation
33	6	Wild: GCGGGAGCGGCGGCTGCGGCCTCAGGGCCTGTGAGCTGGAGG CACTCGCCATGGCCAAGTCGGGTGGCTGCGGCGCGGGAGCCG GCGTGGGCGGCGGCAACGGGGCACTGACCTGGGTGGTAAGTG GCTGG Mutant: GCGGGAGCGGCGGCTGCGGCCT…… …… …….....GACCTGGGTGGTAAGTG GCTGG
42	6	Wild: GCCATGGCCAAGT…..CGGGTGGCTGCGGCGCGGGAGC Mutant: GCCATGGCCAAGTGCCGGGTGGCTGCGGCGCGGGAGC
43	6	Mutation sequence could not be confirmed.

Appendix table 15 Clones that showed bi-allelic genetic variation from wild type K562 cells.



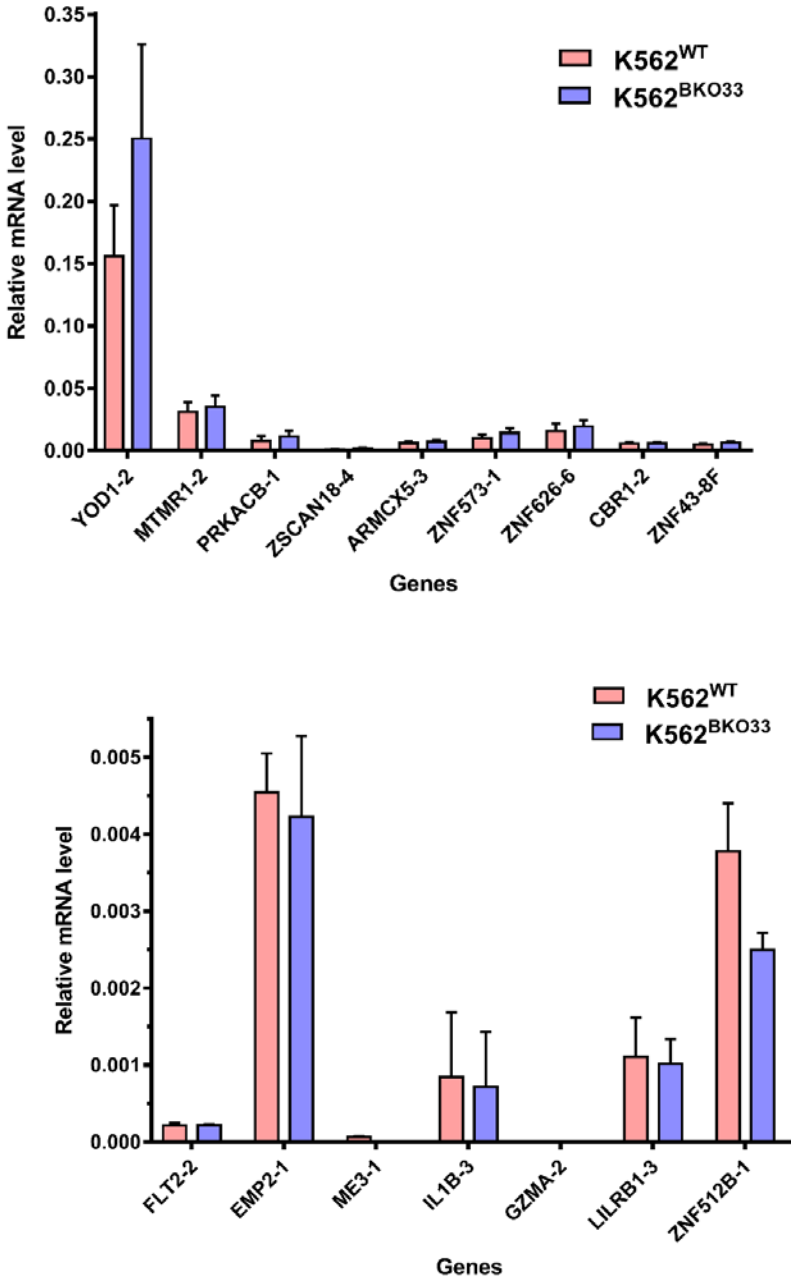
Appendix figure 11 Screening for *TOP2B* knockout clones in K562 cells by immunofluorescence.

Cells were prepared for immunofluorescence as described in (Section 2.15.7.2) then probed with anti TOP2B antibodies (4555) followed by Alexa fluor®488 secondary antibodies then immunofluorescence signal was captured via FITC channel.



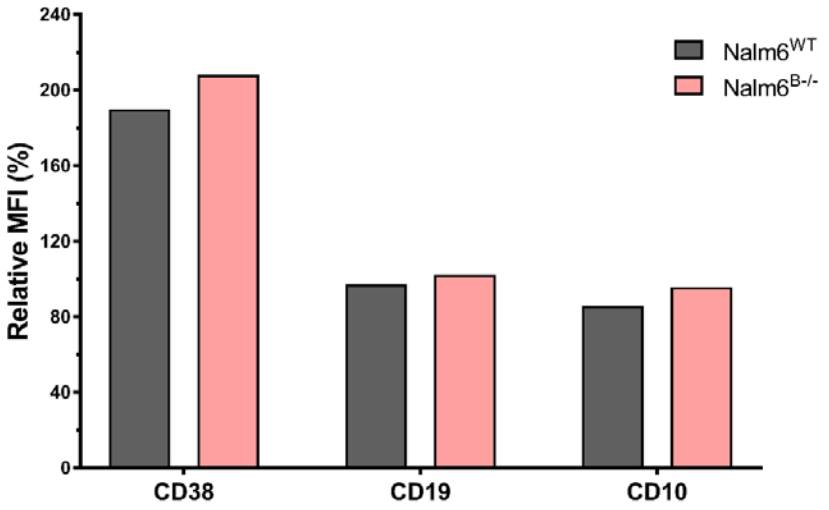
Appendix figure 12 Screening for *TOP2B* knockout clones in K562 cells by western blotting.

Clone protein samples (5 µg / lane) were prepared for western blotting as described in (Section 2.3) and probed with anti actin (NB600-501) or anti TOP2B (MAB6348) antibodies.



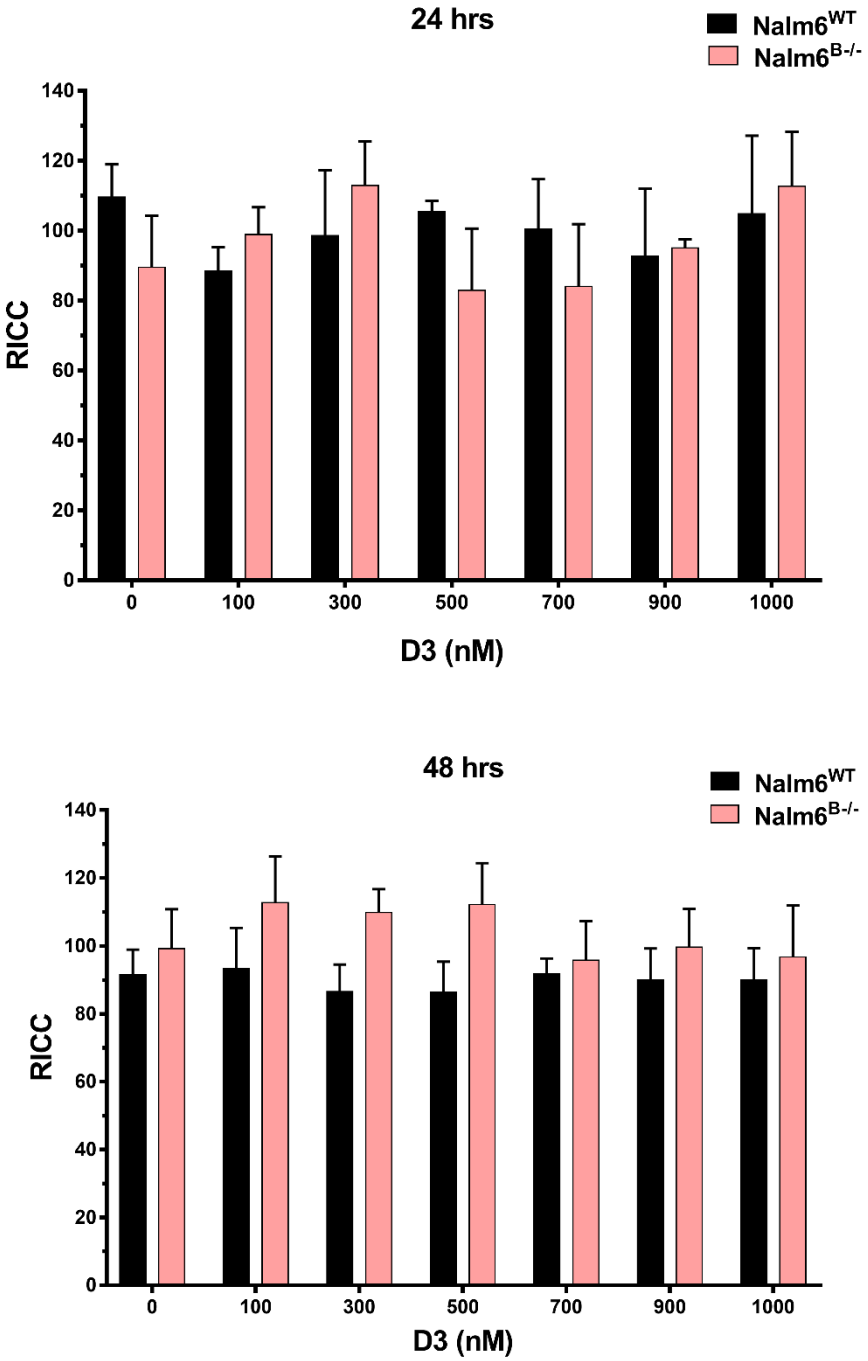
Appendix figure 13 RT-qPCR analysis of selected genes in K562^{BKO33} cells.

RT-qPCR analysis of genes which were either downregulated **(A)** or upregulated **(B)** in knockout Nalm6 from Adachi (Nam6$^{B-/-}$). RT-qPCR analysis as described in (section 2.13.4). PP1A gene was used as a reference gene for normalizing data. Data presented here is the mean of at least three independent experiments with error bars representing the SEM. No significant change was seen in ant of the genes tested.

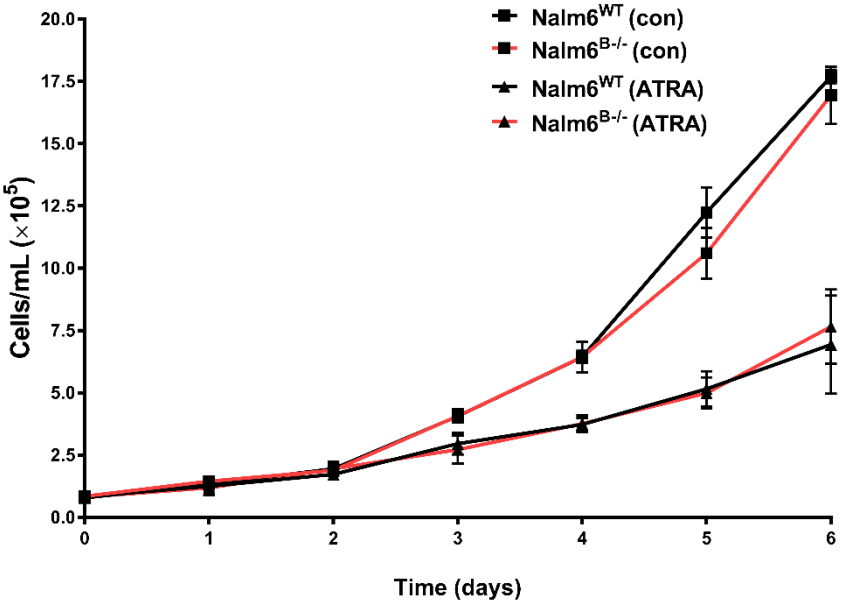


Appendix figure 14 Immunophenotyping of Nalm6 cells using FACS

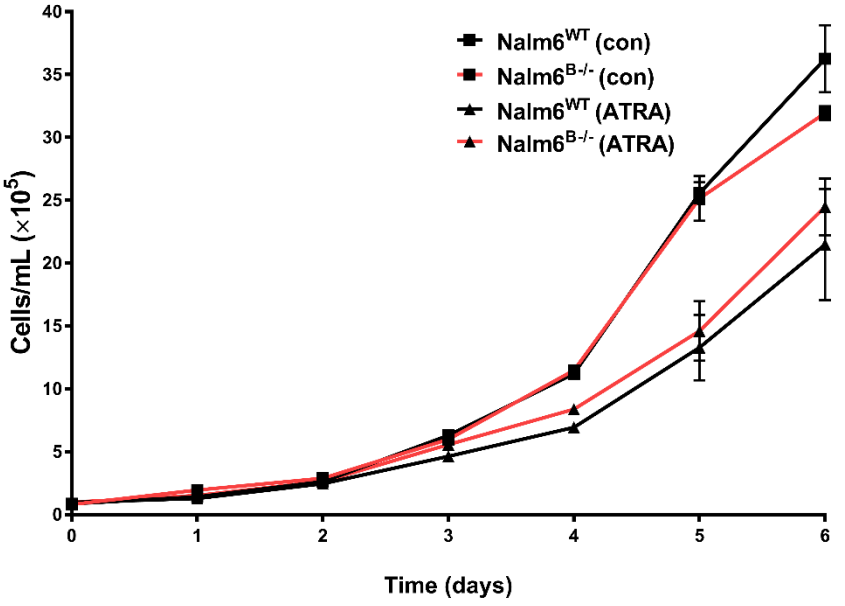
Cells were treated with (10 μM) vitamin D3 or ethanol as control for 48 hours and analysed for cell surface markers using FACS as described in materials and methods



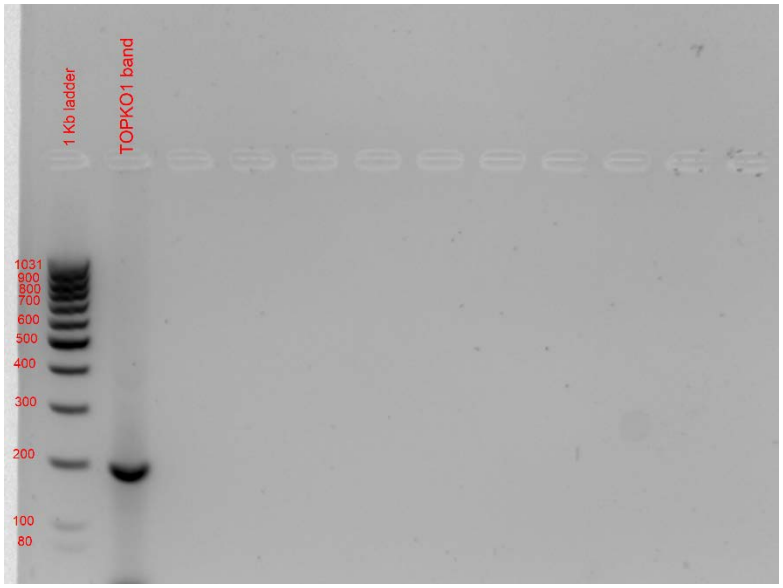
Appendix figure 15 Effect of vitamin D3 on growth of Nalm6 cells.



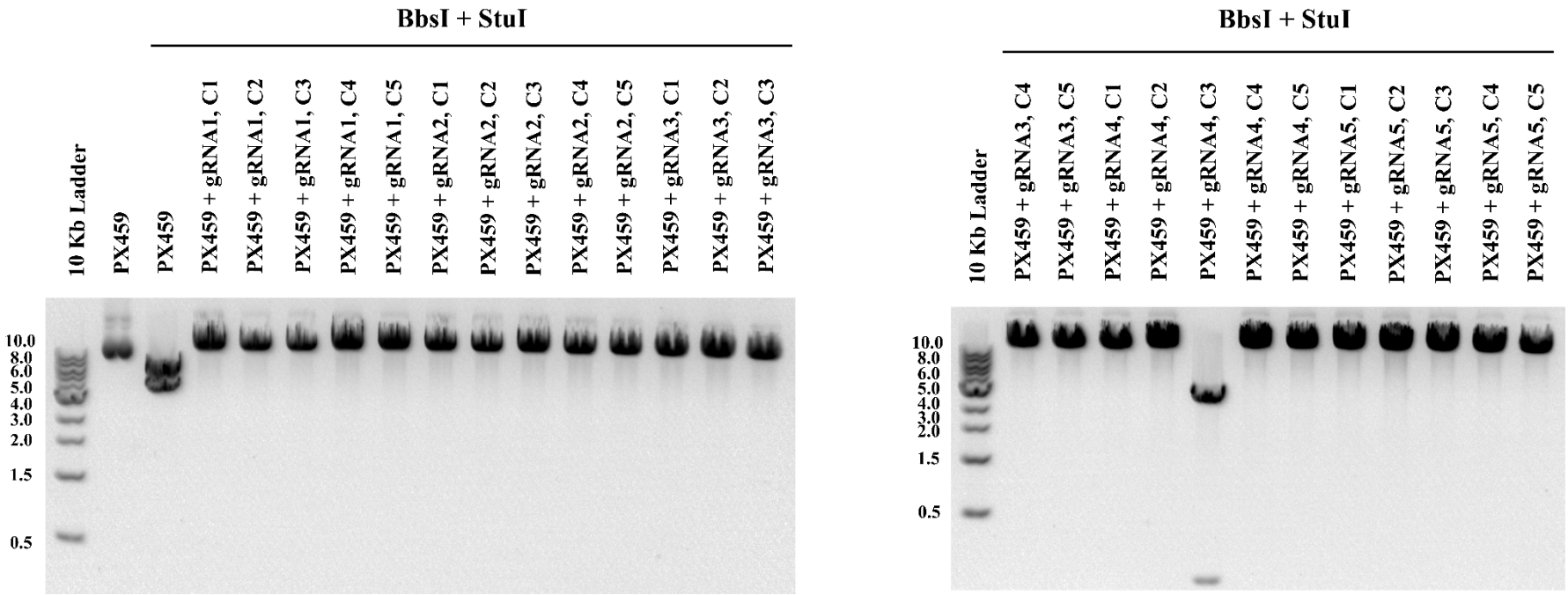
Appendix figure 16 Effect of ATRA on growth of Nalm6 cells at 2 % FBS medium.



Appendix figure 17 Effect of ATRA on growth of Nalm6 cells at 10 % FBS medium.



Appendix figure 18 TOPKO1 primer used in genotyping.



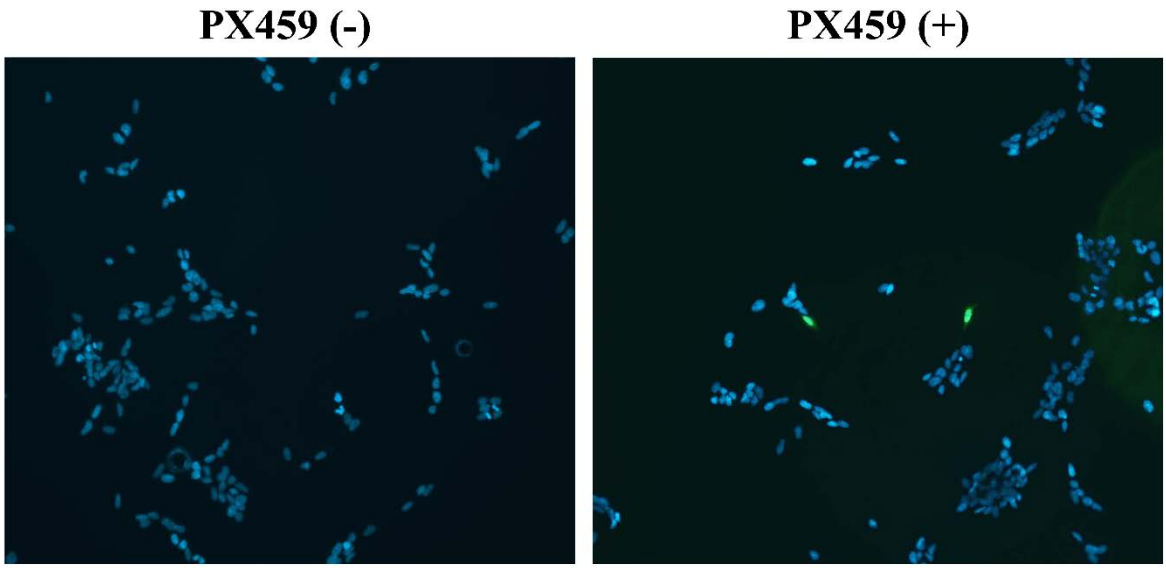
Appendix figure 19 Digestion test to verify gRNA cloning into PX459 vector.

Appendix table 16 Sanger sequencing results of gRNA cloning into PX459.

Sample	gRNA sequence	Sequencing result
PX459 only	Non	TtataTATCTTGTGGAaGGAnGAAACACCGGGTCTTCGAGAAGACCTGTTTTAG AGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX459 + gRNA1,1	GGTGGCTGCGGCGCGGGAGC	tncgatttCTTGGCTTtataTATCTTGTGGAAGGAcGAAACaCCGGTGGCTGCGGCGC GGGAGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTAT CAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT….
PX459 + gRNA1,2	GGTGGCTGCGGCGCGGGAGC	GnCTTnataTaTCTTGTGGAaGGAcGAAnnaCCGGTGGCTGCGGCGCGGgAgCGTT TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAA AAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT….
PX459 + gRNA1,3	GGTGGCTGCGGCGCGGGAGC	cttggCTTnatataTCTTGTGGAAGGACGaaacACCGGTGGCTGCGGCGCGGgaGCGT TTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGA AAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT….
PX459 + gRNA1,4	GGTGGCTGCGGCGCGGGAGC	ctTGGCTTTataTATCTTGTGGAaAGGAcGAAACACCGGTGGCTGCGGCGCGGG AGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAA CTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…
PX459 + gRNA1,5	GGTGGCTGCGGCGCGGGAGC	ttncTTGgCTTnaTaTaTCTTGTGGAaGGAnGAAaCACCGGTGGCTGCGGCGCGGga GCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAAC TTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…
PX459 + gRNA2,1	GGCGCGGGAGCCGGCGTGGG	attnctTGGcTTtataTATCTTGTGGAAGGacGAAACACCGGCGCGGGAGCCGGCGT GGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAA CTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…
PX459 + gRNA2,2	GGCGCGGGAGCCGGCGTGGG	tnGGCTTnataTaTCTTGTGgAaGGAnGAAAnaCCGGCGCGGgaGCCGGCGTGGGG TTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT….
PX459 + gRNA2,3	GGCGCGGGAGCCGGCGTGGG	attncTTGGCTTtaTATATCTTGTGGAAGGAcGAAACACCGGCGCGGGAGCCGGC GTGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATC AACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT..
PX459 + gRNA2,4	GGCGCGGGAGCCGGCGTGGG	GGCTTtataTATCTTGTGGAAGGAcGAAACACCGGCGCGGGAGCCGGCGTGGG GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT GAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..

PX459 + gRNA2,5	GGCGCGGGAGCCGGCGTGGG	GCTTtaTATATCTTGTGGAAGGAcGAAACACCGGCGCGGGAGCCGGCGTGGG GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT GAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX459 + gRNA3,1	GCGGGAGCCGGCGTGGGCGG	taTaTCTTGTGGAaGGAcGAAnnaCCGCGGgaGCCGGCGTGGGCGGGTTTTAGAG CTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGT GGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX459 + gRNA3,2	GCGGGAGCCGGCGTGGGCGG	tncTTgncTTtaTaTATCTTGTGGAaGGAcGAAACACCGCGGGAGCCGGCGTGGGC GGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAAC TTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT….
PX459 + gRNA3,3	GCGGGAGCCGGCGTGGGCGG	atttnnGgCTTtataTATCTTGTGGAAGGAcGAAACACCGCGGGAGCCGGCGTGGG CGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAA CTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT….
PX459 + gRNA3,4	GCGGGAGCCGGCGTGGGCGG	TtcTTgnCTTtataTATCTTGTGGAAGGAcGAAACACCGCGGGAGCCGGCGTGGG CGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAA CTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…
PX459 + gRNA3,5	GCGGGAGCCGGCGTGGGCGG	cTTGGCTTtataTAtCTTGTGGAAGGAnGAAACACCGCGGGAGCCGGCGTGGGC GGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAAC TTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…
PX459 + gRNA4,1	GCCGGCGTGGGCGGCGGCAA	cTTGGCTTnntaTaTCTTGTGGAaGGAcGAaacACCGCCGGCGTGGGCGGCGGCAA GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT GAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT….
PX459 + gRNA4,2	GCCGGCGTGGGCGGCGGCAA	GGCTTnntataTCTTGTGgAaGGACGAannaCCGCCGGCGTGGGCGGCGGCAAGTT TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAA AAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT….
PX459 + gRNA4,3	GCCGGCGTGGGCGGCGGCAA	agccgtGggGGCGTTTaaACTGgAtTTCgGTGttAgacgngcng
PX459 + gRNA4,4	GCCGGCGTGGGCGGCGGCAA	ggCTTtaTaTaTCTTGTGGAaGgacGAannaCCGCCGGCGTGGGCGGCGGCAAGTTT TAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAA AAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX459 + gRNA4,5	GCCGGCGTGGGCGGCGGCAA	cttGGCTTnntaTaTCTTGTGGAaGGAnGAAaCACCGCCGGCGTGGGCGGCGGCAA GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT GAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT….
PX459 + gRNA5,1	GCGGCAACGGGGCACTGACC	TTGGCTTnataTaTCTTGTGGAAaGGACGAannaCCGCGGCAACGGGGCACTGAC CGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACT TGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..

PX459 + gRNA5,2	GCGGCAACGGGGCACTGACC	cTTGGCTTnntaTaTCTTGTGGAaGGACGAAnnaCCGCGGCAACGGGGCACTGACCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..
PX459 + gRNA5,3	GCGGCAACGGGGCACTGACC	TTGGCTTtataTATCTTGTGGAaGGAcGAAACACCGCGGCAACGGGGCACTGACCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT……
PX459 + gRNA5,4	GCGGCAACGGGGCACTGACC	ggcTTnntaTaTCTTGTGGAaGGAnGAancaCCGCGGCAACGGGGCACTGACCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT……
PX459 + gRNA5,5	GCGGCAACGGGGCACTGACC	GGCTTnntaTaTCTTGTGGAAaGGAcGAAaCACCGCGGCAACGGGGCACTGACCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGTTTT…..



Appendix figure 20 Testing transfection efficiency of PX459 vector in SH-SY5Y cells using immunofluorescence.

Appendix table 17 Completed list of DEGs in SH-SY5Y by RNA-seq

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1	CTNNA3	A	5.83	5.20E-04	NA	NA	NA	NA	NA	NA
2	AL358613.3	A	5.58	9.47E-03	NA	NA	NA	NA	NA	NA
3	AC124854.1	A	4.99	5.82E-04	NA	NA	NA	NA	NA	NA
4	LINC01264	A	4.51	3.93E-03	NA	NA	NA	NA	NA	NA
5	IGSF10	A	4.29	5.03E-05	NA	NA	NA	NA	NA	NA
6	AC023794.3	A	4.27	5.19E-03	NA	NA	NA	NA	NA	NA
7	LINC00222	A	4.00	4.34E-02	NA	NA	NA	NA	NA	NA
8	AC073263.3	A	3.98	1.06E-02	NA	NA	NA	NA	NA	NA
9	HOXD12	A	3.84	1.81E-03	NA	NA	NA	NA	NA	NA
10	BAIAP2L2	A	3.79	4.98E-02	NA	NA	NA	NA	NA	NA
11	NPHS1	A	3.76	3.43E-02	NA	NA	NA	NA	NA	NA
12	YBX3	A	3.67	2.11E-02	NA	NA	NA	NA	NA	NA
13	KRT7	A	3.65	8.56E-03	NA	NA	NA	NA	NA	NA
14	SCX	A	3.61	2.09E-02	NA	NA	NA	NA	NA	NA
15	ASB12	A	3.45	1.47E-02	NA	NA	NA	NA	NA	NA
16	AC114954.1	A	3.44	2.69E-02	NA	NA	NA	NA	NA	NA
17	CRHR1	A	3.42	1.45E-02	NA	NA	NA	NA	NA	NA
18	AC063944.1	A	3.27	3.91E-02	NA	NA	NA	NA	NA	NA
19	AL138787.1	A	3.26	8.83E-03	NA	NA	NA	NA	NA	NA
20	AC113189.4	A	3.24	3.11E-02	NA	NA	NA	NA	NA	NA
21	KLK4	A	3.20	2.46E-02	NA	NA	NA	NA	NA	NA
22	IL2RB	A	3.08	4.99E-03	NA	NA	NA	NA	NA	NA
23	CICP3	A	3.08	4.18E-02	NA	NA	NA	NA	NA	NA
24	OLFML1	A	3.05	4.38E-05	NA	NA	NA	NA	NA	NA
25	AC009093.6	A	3.03	8.65E-05	NA	NA	NA	NA	NA	NA
26	HIST1H4J	A	2.99	4.99E-02	NA	NA	NA	NA	NA	NA
27	ACCSL	A	2.93	2.29E-02	NA	NA	NA	NA	NA	NA
28	DRC1	A	2.75	1.36E-02	NA	NA	NA	NA	NA	NA
29	CES5AP1	A	2.72	4.01E-02	NA	NA	NA	NA	NA	NA
30	LINC02390	A	2.68	2.94E-02	NA	NA	NA	NA	NA	NA
31	AC107918.4	A	2.63	1.38E-02	NA	NA	NA	NA	NA	NA
32	AC096536.1	A	2.61	1.97E-02	NA	NA	NA	NA	NA	NA
33	AC078883.1	A	2.54	4.50E-04	NA	NA	NA	NA	NA	NA
34	AC009336.1	A	2.54	3.49E-04	NA	NA	NA	NA	NA	NA
35	KARSP2	A	2.53	4.28E-02	NA	NA	NA	NA	NA	NA
36	AL391001.1	A	2.50	1.22E-03	NA	NA	NA	NA	NA	NA
37	FAM90A1	A	2.50	2.02E-02	NA	NA	NA	NA	NA	NA
38	PRSS50	A	2.49	1.90E-02	NA	NA	NA	NA	NA	NA
39	AC087742.2	A	2.48	1.23E-02	NA	NA	NA	NA	NA	NA
40	AC107294.2	A	2.47	1.20E-02	NA	NA	NA	NA	NA	NA
41	AC135048.1	A	2.46	3.22E-24	NA	NA	NA	NA	NA	NA
42	GBP2	A	2.44	5.42E-04	NA	NA	NA	NA	NA	NA
43	AC074194.1	A	2.43	1.93E-03	NA	NA	NA	NA	NA	NA
44	AL356750.1	A	2.42	3.57E-02	NA	NA	NA	NA	NA	NA
45	AL078590.3	A	2.40	3.74E-02	NA	NA	NA	NA	NA	NA
46	MUC4	A	2.37	4.03E-02	NA	NA	NA	NA	NA	NA
47	AC010327.3	A	2.34	4.20E-02	NA	NA	NA	NA	NA	NA
48	AC009560.1	A	2.34	1.65E-02	NA	NA	NA	NA	NA	NA
49	AL139142.2	A	2.33	7.12E-03	NA	NA	NA	NA	NA	NA
50	AC073592.1	A	2.31	1.38E-02	NA	NA	NA	NA	NA	NA
51	AL355312.1	A	2.30	1.67E-02	NA	NA	NA	NA	NA	NA
52	AC008676.2	A	2.28	3.41E-02	NA	NA	NA	NA	NA	NA
53	HOXD11	A	2.26	1.64E-28	NA	NA	NA	NA	NA	NA
54	SPRY4-AS1	A	2.23	8.27E-04	NA	NA	NA	NA	NA	NA
55	SLAMF6	A	2.23	4.30E-02	NA	NA	NA	NA	NA	NA
56	FLJ40194	A	2.21	1.91E-02	NA	NA	NA	NA	NA	NA
57	ABI3BP	A	2.20	5.53E-06	NA	NA	NA	NA	NA	NA
58	AC074029.4	A	2.19	2.77E-02	NA	NA	NA	NA	NA	NA
59	AC053503.5	A	2.19	1.50E-02	NA	NA	NA	NA	NA	NA
60	GOLGA8VP	A	2.14	2.93E-02	NA	NA	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
61	ANKRD34A	A	2.14	5.55E-37	NA	NA	NA	NA	NA	NA
62	CD248	A	2.09	8.68E-42	NA	NA	NA	NA	NA	NA
63	AC254562.3	A	2.05	2.94E-02	NA	NA	NA	NA	NA	NA
64	GPD1	A	2.05	3.37E-02	NA	NA	NA	NA	NA	NA
65	ABTB1	A	2.03	2.95E-13	NA	NA	NA	NA	NA	NA
66	AC011498.6	A	2.03	1.33E-03	NA	NA	NA	NA	NA	NA
67	RBPMS-AS1	A	2.02	1.74E-02	NA	NA	NA	NA	NA	NA
68	EDA	A	2.02	1.80E-02	NA	NA	NA	NA	NA	NA
69	SFRP4	A	2.00	1.73E-03	NA	NA	NA	NA	NA	NA
70	AL359258.3	A	1.99	3.74E-04	NA	NA	NA	NA	NA	NA
71	RASA4DP	A	1.99	2.36E-02	NA	NA	NA	NA	NA	NA
72	AC107294.3	A	1.99	4.67E-02	NA	NA	NA	NA	NA	NA
73	ADGB	A	1.98	4.70E-02	NA	NA	NA	NA	NA	NA
74	HIF1A-AS1	A	1.97	6.86E-04	NA	NA	NA	NA	NA	NA
75	AC021127.1	A	1.96	1.11E-02	NA	NA	NA	NA	NA	NA
76	PKP4-AS1	A	1.96	4.76E-02	NA	NA	NA	NA	NA	NA
77	IL10RB-AS1	A	1.95	7.44E-04	NA	NA	NA	NA	NA	NA
78	AL359694.2	A	1.93	8.12E-03	NA	NA	NA	NA	NA	NA
79	CREG2	A	1.93	4.37E-02	NA	NA	NA	NA	NA	NA
80	LINC01176	A	1.93	1.48E-03	NA	NA	NA	NA	NA	NA
81	AC010733.1	A	1.93	4.88E-02	NA	NA	NA	NA	NA	NA
82	AC005393.1	A	1.92	2.06E-03	NA	NA	NA	NA	NA	NA
83	AC087521.2	A	1.85	6.51E-03	NA	NA	NA	NA	NA	NA
84	PYGM	A	1.84	6.99E-03	NA	NA	NA	NA	NA	NA
85	IGSF22	A	1.84	2.36E-03	NA	NA	NA	NA	NA	NA
86	TP53TG3B	A	1.84	2.03E-02	NA	NA	NA	NA	NA	NA
87	AL022328.1	A	1.83	4.92E-03	NA	NA	NA	NA	NA	NA
88	FBLIM1	A	1.82	2.46E-27	NA	NA	NA	NA	NA	NA
89	EXOC6B	A	1.80	7.66E-40	NA	NA	NA	NA	NA	NA
90	CYB5R2	A	1.79	4.87E-02	NA	NA	NA	NA	NA	NA
91	FREM1	A	1.79	1.66E-21	NA	NA	NA	NA	NA	NA
92	AP003086.1	A	1.77	3.92E-05	NA	NA	NA	NA	NA	NA
93	AL359853.3	A	1.77	2.82E-02	NA	NA	NA	NA	NA	NA
94	TSPYL2	A	1.76	4.04E-39	NA	NA	NA	NA	NA	NA
95	ABTB2	A	1.75	4.78E-25	NA	NA	NA	NA	NA	NA
96	BCL2L2-PABPN1	A	1.75	4.34E-04	NA	NA	NA	NA	NA	NA
97	AC093673.1	A	1.68	2.02E-05	NA	NA	NA	NA	NA	NA
98	TNS2	A	1.68	5.60E-08	NA	NA	NA	NA	NA	NA
99	PTGS1	A	1.67	2.63E-02	NA	NA	NA	NA	NA	NA
100	IFI27	A	1.67	7.86E-03	NA	NA	NA	NA	NA	NA
101	IRF7	A	1.66	3.50E-04	NA	NA	NA	NA	NA	NA
102	FAM180B	A	1.66	4.18E-02	NA	NA	NA	NA	NA	NA
103	NPL	A	1.65	7.77E-16	NA	NA	NA	NA	NA	NA
104	AP001453.1	A	1.64	4.92E-03	NA	NA	NA	NA	NA	NA
105	TP53TG3	A	1.64	4.18E-02	NA	NA	NA	NA	NA	NA
106	SLC5A5	A	1.62	1.29E-03	NA	NA	NA	NA	NA	NA
107	AC138207.2	A	1.61	4.90E-03	NA	NA	NA	NA	NA	NA
108	SULT1A1	A	1.59	2.81E-03	NA	NA	NA	NA	NA	NA
109	AL355377.2	A	1.58	9.79E-03	NA	NA	NA	NA	NA	NA
110	ISYNA1	A	1.57	6.90E-16	NA	NA	NA	NA	NA	NA
111	COL5A1	A	1.56	1.48E-06	NA	NA	NA	NA	NA	NA
112	RIN2	A	1.55	2.99E-04	NA	NA	NA	NA	NA	NA
113	LSR	A	1.55	3.73E-02	NA	NA	NA	NA	NA	NA
114	ACADVL	A	1.55	6.35E-31	NA	NA	NA	NA	NA	NA
115	TBX4	A	1.54	1.21E-02	NA	NA	NA	NA	NA	NA
116	FNDC11	A	1.54	3.08E-03	NA	NA	NA	NA	NA	NA
117	OPHN1	A	1.53	3.08E-03	NA	NA	NA	NA	NA	NA
118	LSP1	A	1.52	2.87E-02	NA	NA	NA	NA	NA	NA
119	ASIC1	A	1.52	7.25E-32	NA	NA	NA	NA	NA	NA
120	AC026412.1	A	1.51	3.49E-02	NA	NA	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
121	AL035413.1	A	1.50	1.99E-02	NA	NA	NA	NA	NA	NA
122	FAM214B	A	1.50	4.32E-39	NA	NA	NA	NA	NA	NA
123	LINC01829	A	1.50	1.27E-02	NA	NA	NA	NA	NA	NA
124	SPACA6	A	1.50	8.99E-08	NA	NA	NA	NA	NA	NA
125	AL138781.1	A	1.49	4.99E-02	NA	NA	NA	NA	NA	NA
126	SEMA6C	A	1.48	3.11E-10	NA	NA	NA	NA	NA	NA
127	HCP5B	A	1.48	4.54E-02	NA	NA	NA	NA	NA	NA
128	HGFAC	A	1.47	1.76E-03	NA	NA	NA	NA	NA	NA
129	LTBP3	A	1.47	2.81E-13	NA	NA	NA	NA	NA	NA
130	PRICKLE4	A	1.46	1.10E-04	NA	NA	NA	NA	NA	NA
131	HSD52	A	1.44	3.14E-02	NA	NA	NA	NA	NA	NA
132	SPATA25	A	1.44	3.22E-05	NA	NA	NA	NA	NA	NA
133	RRNAD1	A	1.43	3.43E-40	NA	NA	NA	NA	NA	NA
134	LINC01695	A	1.43	8.46E-06	NA	NA	NA	NA	NA	NA
135	LINC01607	A	1.43	1.67E-03	NA	NA	NA	NA	NA	NA
136	GGN	A	1.43	2.40E-02	NA	NA	NA	NA	NA	NA
137	ARHGAP31	A	1.42	1.11E-74	NA	NA	NA	NA	NA	NA
138	TMEM86A	A	1.42	1.72E-09	NA	NA	NA	NA	NA	NA
139	TMEM159	A	1.42	2.59E-17	NA	NA	NA	NA	NA	NA
140	LCP1	A	1.42	1.00E-03	NA	NA	NA	NA	NA	NA
141	LINC00963	A	1.41	1.15E-20	NA	NA	NA	NA	NA	NA
142	SLC25A35	A	1.40	1.42E-09	NA	NA	NA	NA	NA	NA
143	OXCT1-AS1	A	1.40	1.38E-02	NA	NA	NA	NA	NA	NA
144	LINC01011	A	1.40	3.04E-07	NA	NA	NA	NA	NA	NA
145	PACSIN3	A	1.40	5.74E-10	NA	NA	NA	NA	NA	NA
146	TDH	A	1.39	8.24E-06	NA	NA	NA	NA	NA	NA
147	AC092162.3	A	1.39	3.39E-02	NA	NA	NA	NA	NA	NA
148	RCN3	A	1.38	4.32E-02	NA	NA	NA	NA	NA	NA
149	TLL2	A	1.37	2.57E-02	NA	NA	NA	NA	NA	NA
150	AC021242.3	A	1.36	7.41E-03	NA	NA	NA	NA	NA	NA
151	TVP23C	A	1.36	2.96E-04	NA	NA	NA	NA	NA	NA
152	SIGLEC10	A	1.36	3.11E-05	NA	NA	NA	NA	NA	NA
153	ROM1	A	1.36	1.14E-07	NA	NA	NA	NA	NA	NA
154	AC007876.1	A	1.35	1.12E-03	NA	NA	NA	NA	NA	NA
155	SIPA1	A	1.35	1.59E-25	NA	NA	NA	NA	NA	NA
156	SUOX	A	1.35	5.72E-10	NA	NA	NA	NA	NA	NA
157	AC004943.1	A	1.35	3.90E-02	NA	NA	NA	NA	NA	NA
158	TEX14	A	1.34	7.69E-03	NA	NA	NA	NA	NA	NA
159	LINC01816	A	1.33	2.63E-02	NA	NA	NA	NA	NA	NA
160	IRF9	A	1.32	1.55E-22	NA	NA	NA	NA	NA	NA
161	FAM182B	A	1.32	3.69E-03	NA	NA	NA	NA	NA	NA
162	DSTNP2	A	1.31	3.62E-08	NA	NA	NA	NA	NA	NA
163	AC078909.2	A	1.31	2.88E-06	NA	NA	NA	NA	NA	NA
164	AC093388.1	A	1.31	4.11E-02	NA	NA	NA	NA	NA	NA
165	SLC2A10	A	1.31	1.65E-09	NA	NA	NA	NA	NA	NA
166	POLD4	A	1.31	3.66E-04	NA	NA	NA	NA	NA	NA
167	FAM92A1P2	A	1.30	3.51E-02	NA	NA	NA	NA	NA	NA
168	NAB2	A	1.30	6.92E-47	NA	NA	NA	NA	NA	NA
169	AP003356.1	A	1.29	1.44E-05	NA	NA	NA	NA	NA	NA
170	DACT3	A	1.28	2.87E-90	NA	NA	NA	NA	NA	NA
171	NUDT6	A	1.27	9.36E-06	NA	NA	NA	NA	NA	NA
172	ACTG1P1	A	1.27	1.25E-02	NA	NA	NA	NA	NA	NA
173	AC004623.1	A	1.27	6.88E-03	NA	NA	NA	NA	NA	NA
174	ID3	A	1.27	2.03E-16	NA	NA	NA	NA	NA	NA
175	C15orf65	A	1.27	2.61E-11	NA	NA	NA	NA	NA	NA
176	TGIF1	A	1.27	1.64E-30	NA	NA	NA	NA	NA	NA
177	AL121612.2	A	1.26	2.89E-02	NA	NA	NA	NA	NA	NA
178	ABHD8	A	1.26	1.03E-04	NA	NA	NA	NA	NA	NA
179	IER5L	A	1.26	4.17E-09	NA	NA	NA	NA	NA	NA
180	AC046143.1	A	1.25	1.27E-03	NA	NA	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
181	RIT1	A	1.25	5.21E-80	NA	NA	NA	NA	NA	NA
182	OLMALINC	A	1.25	2.31E-103	NA	NA	NA	NA	NA	NA
183	MYOZ2	A	1.25	3.30E-02	NA	NA	NA	NA	NA	NA
184	RUSC1	A	1.25	2.28E-11	NA	NA	NA	NA	NA	NA
185	HSD17B1P1	A	1.25	3.88E-04	NA	NA	NA	NA	NA	NA
186	CLIP3	A	1.25	1.39E-09	NA	NA	NA	NA	NA	NA
187	GPR162	A	1.25	4.65E-23	NA	NA	NA	NA	NA	NA
188	PMEL	A	1.25	4.32E-07	NA	NA	NA	NA	NA	NA
189	AL662797.2	A	1.25	1.90E-04	NA	NA	NA	NA	NA	NA
190	ATP1B2	A	1.25	4.49E-05	NA	NA	NA	NA	NA	NA
191	AC008537.2	A	1.25	1.65E-02	NA	NA	NA	NA	NA	NA
192	MAP3K8	A	1.24	1.64E-03	NA	NA	NA	NA	NA	NA
193	ZNF763	A	1.24	1.09E-03	NA	NA	NA	NA	NA	NA
194	MMP24-AS1	A	1.23	3.16E-19	NA	NA	NA	NA	NA	NA
195	AC027176.3	A	1.23	4.76E-02	NA	NA	NA	NA	NA	NA
196	AL133517.1	A	1.23	1.46E-10	NA	NA	NA	NA	NA	NA
197	TM6SF2	A	1.22	3.74E-04	NA	NA	NA	NA	NA	NA
198	ATP6V0E2-AS1	A	1.21	1.83E-14	NA	NA	NA	NA	NA	NA
199	OBSCN-AS1	A	1.21	5.61E-07	NA	NA	NA	NA	NA	NA
200	SEMA3B	A	1.21	2.39E-04	NA	NA	NA	NA	NA	NA
201	GPSM3	A	1.21	1.79E-05	NA	NA	NA	NA	NA	NA
202	COL7A1	A	1.21	4.86E-04	NA	NA	NA	NA	NA	NA
203	KMT2E-AS1	A	1.21	2.22E-04	NA	NA	NA	NA	NA	NA
204	SHISA4	A	1.21	4.38E-14	NA	NA	NA	NA	NA	NA
205	ARHGEF3	A	1.20	4.80E-13	NA	NA	NA	NA	NA	NA
206	ABCA7	A	1.20	9.61E-05	NA	NA	NA	NA	NA	NA
207	TSPY26P	A	1.19	3.47E-37	NA	NA	NA	NA	NA	NA
208	ANPEP	A	1.19	5.43E-03	NA	NA	NA	NA	NA	NA
209	TVP23C-CDRT4	A	1.19	3.94E-02	NA	NA	NA	NA	NA	NA
210	SERTAD1	A	1.18	1.55E-06	NA	NA	NA	NA	NA	NA
211	TOB2P1	A	1.18	5.39E-03	NA	NA	NA	NA	NA	NA
212	NEAT1_1	A	1.18	1.08E-05	NA	NA	NA	NA	NA	NA
213	PHF1	A	1.18	3.84E-45	NA	NA	NA	NA	NA	NA
214	AC092687.3	A	1.18	2.66E-02	NA	NA	NA	NA	NA	NA
215	ZC2HC1C	A	1.17	3.07E-04	NA	NA	NA	NA	NA	NA
216	B3GNT7	A	1.17	2.72E-02	NA	NA	NA	NA	NA	NA
217	LINC01828	A	1.17	1.62E-02	NA	NA	NA	NA	NA	NA
218	NCOA7	A	1.17	6.68E-30	NA	NA	NA	NA	NA	NA
219	PCSK1N	A	1.17	8.71E-03	NA	NA	NA	NA	NA	NA
220	SIDT2	A	1.16	1.72E-13	NA	NA	NA	NA	NA	NA
221	AC062029.1	A	1.16	2.56E-08	NA	NA	NA	NA	NA	NA
222	NEAT1_2	A	1.16	1.99E-06	NA	NA	NA	NA	NA	NA
223	NEAT1	A	1.16	9.24E-16	NA	NA	NA	NA	NA	NA
224	CPB2-AS1	A	1.16	2.37E-02	NA	NA	NA	NA	NA	NA
225	CASP9	A	1.16	4.96E-21	NA	NA	NA	NA	NA	NA
226	AC003102.1	A	1.15	1.11E-02	NA	NA	NA	NA	NA	NA
227	SLC30A4	A	1.15	8.14E-28	NA	NA	NA	NA	NA	NA
228	SOD2-OT1	A	1.15	4.08E-03	NA	NA	NA	NA	NA	NA
229	TMEM107	A	1.14	1.23E-08	NA	NA	NA	NA	NA	NA
230	KREMEN2	A	1.14	3.88E-02	NA	NA	NA	NA	NA	NA
231	AP005329.3	A	1.14	2.41E-02	NA	NA	NA	NA	NA	NA
232	MID1	A	1.14	1.78E-15	NA	NA	NA	NA	NA	NA
233	NEAT1_3	A	1.14	3.43E-08	NA	NA	NA	NA	NA	NA
234	PLCD3	A	1.14	1.51E-03	NA	NA	NA	NA	NA	NA
235	SKIDA1	A	1.13	4.18E-32	NA	NA	NA	NA	NA	NA
236	BTG3-AS1	A	1.13	9.73E-03	NA	NA	NA	NA	NA	NA
237	EFCAB12	A	1.13	1.41E-02	NA	NA	NA	NA	NA	NA
238	AP000446.1	A	1.13	8.83E-05	NA	NA	NA	NA	NA	NA
239	PINK1	A	1.12	7.07E-19	NA	NA	NA	NA	NA	NA
240	PIEZO1	A	1.12	2.69E-04	NA	NA	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
241	SLC16A13	A	1.12	1.56E-07	NA	NA	NA	NA	NA	NA
242	PLD3	A	1.12	9.77E-10	NA	NA	NA	NA	NA	NA
243	LGALS1	A	1.12	1.46E-11	NA	NA	NA	NA	NA	NA
244	IFT22	A	1.11	7.12E-51	NA	NA	NA	NA	NA	NA
245	TMEM187	A	1.11	7.49E-03	NA	NA	NA	NA	NA	NA
246	LINC00598	A	1.11	6.18E-04	NA	NA	NA	NA	NA	NA
247	LAMB2	A	1.11	1.03E-08	NA	NA	NA	NA	NA	NA
248	SLC17A7	A	1.11	1.38E-03	NA	NA	NA	NA	NA	NA
249	AL355001.2	A	1.11	3.21E-02	NA	NA	NA	NA	NA	NA
250	NYNRIN	A	1.10	1.22E-16	NA	NA	NA	NA	NA	NA
251	SLC25A42	A	1.10	1.63E-02	NA	NA	NA	NA	NA	NA
252	AC006206.2	A	1.10	2.63E-03	NA	NA	NA	NA	NA	NA
253	SGSM3	A	1.09	7.64E-08	NA	NA	NA	NA	NA	NA
254	RABGEF1	A	1.09	2.74E-02	NA	NA	NA	NA	NA	NA
255	ITGA2B	A	1.09	3.76E-03	NA	NA	NA	NA	NA	NA
256	SHISA5	A	1.09	1.73E-04	NA	NA	NA	NA	NA	NA
257	FICD	A	1.08	5.83E-05	NA	NA	NA	NA	NA	NA
258	KLF10	A	1.08	3.44E-60	NA	NA	NA	NA	NA	NA
259	Z97192.2	A	1.08	2.06E-02	NA	NA	NA	NA	NA	NA
260	LRRC56	A	1.08	1.97E-02	NA	NA	NA	NA	NA	NA
261	ZFP36L2	A	1.08	7.86E-95	NA	NA	NA	NA	NA	NA
262	AC104836.1	A	1.07	4.81E-07	NA	NA	NA	NA	NA	NA
263	ARSA	A	1.07	1.78E-05	NA	NA	NA	NA	NA	NA
264	PRKD2	A	1.06	1.00E-22	NA	NA	NA	NA	NA	NA
265	SNX21	A	1.06	4.72E-13	NA	NA	NA	NA	NA	NA
266	GS1-124K5.4	A	1.05	2.08E-03	NA	NA	NA	NA	NA	NA
267	FCHSD1	A	1.05	1.88E-06	NA	NA	NA	NA	NA	NA
268	MOB3C	A	1.05	1.29E-09	NA	NA	NA	NA	NA	NA
269	TLE2	A	1.05	4.52E-12	NA	NA	NA	NA	NA	NA
270	THAP8	A	1.05	2.27E-05	NA	NA	NA	NA	NA	NA
271	YAF2	A	1.05	1.51E-11	NA	NA	NA	NA	NA	NA
272	MDK	A	1.05	2.80E-11	NA	NA	NA	NA	NA	NA
273	PRCP	A	1.05	9.97E-22	NA	NA	NA	NA	NA	NA
274	LYRM9	A	1.05	1.40E-02	NA	NA	NA	NA	NA	NA
275	SEC14L2	A	1.04	3.61E-04	NA	NA	NA	NA	NA	NA
276	C16orf86	A	1.04	4.49E-02	NA	NA	NA	NA	NA	NA
277	NLRP1	A	1.04	2.77E-35	NA	NA	NA	NA	NA	NA
278	PITPNM1	A	1.04	3.74E-08	NA	NA	NA	NA	NA	NA
279	TTC21A	A	1.04	4.23E-03	NA	NA	NA	NA	NA	NA
280	AC009303.4	A	1.04	6.61E-03	NA	NA	NA	NA	NA	NA
281	AC103923.1	A	1.04	2.00E-02	NA	NA	NA	NA	NA	NA
282	AC009506.1	A	1.03	3.12E-07	NA	NA	NA	NA	NA	NA
283	OR6J1	A	1.03	2.08E-02	NA	NA	NA	NA	NA	NA
284	SHD	A	1.03	1.18E-07	NA	NA	NA	NA	NA	NA
285	LINC00324	A	1.03	1.90E-02	NA	NA	NA	NA	NA	NA
286	CCDC183	A	1.03	4.88E-02	NA	NA	NA	NA	NA	NA
287	PIK3IP1	A	1.03	4.55E-05	NA	NA	NA	NA	NA	NA
288	AC107419.1	A	1.02	4.53E-02	NA	NA	NA	NA	NA	NA
289	ARIH2OS	A	1.02	8.76E-03	NA	NA	NA	NA	NA	NA
290	ESAM	A	1.02	1.09E-03	NA	NA	NA	NA	NA	NA
291	MXD3	A	1.02	2.34E-02	NA	NA	NA	NA	NA	NA
292	KAZALD1	A	1.02	2.33E-05	NA	NA	NA	NA	NA	NA
293	USP2	A	1.01	2.81E-16	NA	NA	NA	NA	NA	NA
294	ENO3	A	1.01	6.71E-06	NA	NA	NA	NA	NA	NA
295	LRRC4B	A	1.01	3.62E-03	NA	NA	NA	NA	NA	NA
296	FOXO3	A	1.01	8.71E-111	NA	NA	NA	NA	NA	NA
297	HIST4H4	A	1.01	4.49E-02	NA	NA	NA	NA	NA	NA
298	SLC25A34	A	1.01	4.59E-02	NA	NA	NA	NA	NA	NA
299	POC1B-AS1	A	1.01	5.52E-05	NA	NA	NA	NA	NA	NA
300	DLG4	A	1.01	1.42E-15	NA	NA	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
301	DQX1	A	1.00	2.00E-42	NA	NA	NA	NA	NA	NA
302	AL049844.2	A	-1.00	2.34E-03	NA	NA	NA	NA	NA	NA
303	NOL4	A	-1.00	1.55E-22	NA	NA	NA	NA	NA	NA
304	NEK6	A	-1.00	2.40E-15	NA	NA	NA	NA	NA	NA
305	MSI1	A	-1.01	4.23E-14	NA	NA	NA	NA	NA	NA
306	PAXIP1-AS2	A	-1.01	6.03E-12	NA	NA	NA	NA	NA	NA
307	RRS1	A	-1.01	5.67E-39	NA	NA	NA	NA	NA	NA
308	POLR3K	A	-1.01	7.01E-27	NA	NA	NA	NA	NA	NA
309	MRTO4	A	-1.01	3.51E-16	NA	NA	NA	NA	NA	NA
310	NOL6	A	-1.02	2.65E-68	NA	NA	NA	NA	NA	NA
311	LONRF1	A	-1.02	8.76E-12	NA	NA	NA	NA	NA	NA
312	SDAD1	A	-1.02	2.99E-14	NA	NA	NA	NA	NA	NA
313	NOC2L	A	-1.02	8.02E-10	NA	NA	NA	NA	NA	NA
314	ANK2	A	-1.02	6.75E-07	NA	NA	NA	NA	NA	NA
315	ZNF276	A	-1.02	7.50E-12	NA	NA	NA	NA	NA	NA
316	ANKRD18A	A	-1.03	1.32E-03	NA	NA	NA	NA	NA	NA
317	SNORD22	A	-1.03	6.93E-03	NA	NA	NA	NA	NA	NA
318	QTRT2	A	-1.03	2.38E-26	NA	NA	NA	NA	NA	NA
319	MAP3K1	A	-1.04	1.34E-56	NA	NA	NA	NA	NA	NA
320	AC138866.2	A	-1.04	2.63E-05	NA	NA	NA	NA	NA	NA
321	BOLA2	A	-1.04	1.33E-07	NA	NA	NA	NA	NA	NA
322	SNORA73B	A	-1.04	1.33E-02	NA	NA	NA	NA	NA	NA
323	CLUH	A	-1.04	1.20E-13	NA	NA	NA	NA	NA	NA
324	PAK1IP1	A	-1.05	7.29E-24	NA	NA	NA	NA	NA	NA
325	RETREG1	A	-1.05	2.61E-07	NA	NA	NA	NA	NA	NA
326	HAND2-AS1	A	-1.05	1.77E-47	NA	NA	NA	NA	NA	NA
327	SLC19A1	A	-1.05	2.29E-12	NA	NA	NA	NA	NA	NA
328	BMS1P17	A	-1.05	1.67E-02	NA	NA	NA	NA	NA	NA
329	SYCP2L	A	-1.05	8.95E-09	NA	NA	NA	NA	NA	NA
330	PDZD2	A	-1.05	1.49E-02	NA	NA	NA	NA	NA	NA
331	DHX37	A	-1.05	4.36E-13	NA	NA	NA	NA	NA	NA
332	ADAT2	A	-1.05	3.19E-26	NA	NA	NA	NA	NA	NA
333	MECOM	A	-1.06	4.17E-09	NA	NA	NA	NA	NA	NA
334	FAT1	A	-1.06	4.69E-17	NA	NA	NA	NA	NA	NA
335	PWAR5	A	-1.06	1.42E-07	NA	NA	NA	NA	NA	NA
336	AC025165.5	A	-1.06	2.38E-02	NA	NA	NA	NA	NA	NA
337	AGMAT	A	-1.06	3.56E-06	NA	NA	NA	NA	NA	NA
338	GATA3-AS1	A	-1.07	2.85E-17	NA	NA	NA	NA	NA	NA
339	OGFRL1	A	-1.07	5.34E-26	NA	NA	NA	NA	NA	NA
340	AC034102.4	A	-1.07	2.35E-09	NA	NA	NA	NA	NA	NA
341	CHDH	A	-1.07	3.38E-12	NA	NA	NA	NA	NA	NA
342	RAI14	A	-1.07	1.17E-03	NA	NA	NA	NA	NA	NA
343	TMOD4	A	-1.07	1.46E-02	NA	NA	NA	NA	NA	NA
344	DIEXF	A	-1.08	8.93E-50	NA	NA	NA	NA	NA	NA
345	COQ8A	A	-1.08	3.66E-19	NA	NA	NA	NA	NA	NA
346	RAPGEF5	A	-1.08	6.15E-07	NA	NA	NA	NA	NA	NA
347	KLHL35	A	-1.08	1.87E-02	NA	NA	NA	NA	NA	NA
348	NCR3LG1	A	-1.09	2.72E-26	NA	NA	NA	NA	NA	NA
349	DDX21	A	-1.09	3.88E-45	NA	NA	NA	NA	NA	NA
350	ATP8A1	A	-1.09	1.35E-11	NA	NA	NA	NA	NA	NA
351	PUS7	A	-1.09	1.25E-17	NA	NA	NA	NA	NA	NA
352	RCL1	A	-1.09	3.40E-30	NA	NA	NA	NA	NA	NA
353	CCDC86	A	-1.10	1.70E-21	NA	NA	NA	NA	NA	NA
354	RRP15	A	-1.10	5.03E-61	NA	NA	NA	NA	NA	NA
355	RPF2	A	-1.10	9.31E-35	NA	NA	NA	NA	NA	NA
356	AC093155.3	A	-1.10	3.10E-02	NA	NA	NA	NA	NA	NA
357	SPTBN2	A	-1.10	4.78E-18	NA	NA	NA	NA	NA	NA
358	BZW2	A	-1.11	4.30E-64	NA	NA	NA	NA	NA	NA
359	GPATCH4	A	-1.11	6.56E-51	NA	NA	NA	NA	NA	NA
360	EBNA1BP2	A	-1.11	3.77E-79	NA	NA	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
361	AL139339.2	A	-1.11	1.06E-02	NA	NA	NA	NA	NA	NA
362	WDR43	A	-1.11	4.37E-44	NA	NA	NA	NA	NA	NA
363	ABR	A	-1.12	2.09E-38	NA	NA	NA	NA	NA	NA
364	CES3	A	-1.12	1.64E-03	NA	NA	NA	NA	NA	NA
365	RANP1	A	-1.12	2.36E-02	NA	NA	NA	NA	NA	NA
366	AP006545.1	A	-1.12	6.28E-03	NA	NA	NA	NA	NA	NA
367	PLXNC1	A	-1.13	1.11E-04	NA	NA	NA	NA	NA	NA
368	EIF4EBP1	A	-1.13	1.21E-20	NA	NA	NA	NA	NA	NA
369	TWNK	A	-1.13	1.29E-76	NA	NA	NA	NA	NA	NA
370	ODC1	A	-1.13	5.51E-62	NA	NA	NA	NA	NA	NA
371	PHOX2B	A	-1.13	1.29E-68	NA	NA	NA	NA	NA	NA
372	PUM3	A	-1.13	1.55E-22	NA	NA	NA	NA	NA	NA
373	WWC1	A	-1.13	1.06E-08	NA	NA	NA	NA	NA	NA
374	PTPRD-AS1	A	-1.14	8.16E-03	NA	NA	NA	NA	NA	NA
375	IARS	A	-1.14	4.14E-29	NA	NA	NA	NA	NA	NA
376	TEAD4	A	-1.14	3.07E-16	NA	NA	NA	NA	NA	NA
377	PNPT1	A	-1.14	1.79E-05	NA	NA	NA	NA	NA	NA
378	BID	A	-1.14	2.99E-08	NA	NA	NA	NA	NA	NA
379	RGMB	A	-1.15	1.36E-34	NA	NA	NA	NA	NA	NA
380	UGT8	A	-1.15	2.62E-26	NA	NA	NA	NA	NA	NA
381	TMEM108	A	-1.15	2.64E-27	NA	NA	NA	NA	NA	NA
382	PER2	A	-1.15	8.27E-07	NA	NA	NA	NA	NA	NA
383	METTL1	A	-1.15	7.62E-15	NA	NA	NA	NA	NA	NA
384	AL024508.2	A	-1.15	2.25E-02	NA	NA	NA	NA	NA	NA
385	KBTBD8	A	-1.16	4.32E-07	NA	NA	NA	NA	NA	NA
386	EIF5AL1	A	-1.16	8.32E-03	NA	NA	NA	NA	NA	NA
387	GNL3	A	-1.16	1.47E-31	NA	NA	NA	NA	NA	NA
388	TRAP1	A	-1.16	5.00E-44	NA	NA	NA	NA	NA	NA
389	AC106872.5	A	-1.16	5.44E-03	NA	NA	NA	NA	NA	NA
390	TLE1	A	-1.16	4.76E-108	NA	NA	NA	NA	NA	NA
391	SRM	A	-1.16	1.56E-30	NA	NA	NA	NA	NA	NA
392	TERT	A	-1.17	1.69E-04	NA	NA	NA	NA	NA	NA
393	VSTM4	A	-1.17	3.52E-50	NA	NA	NA	NA	NA	NA
394	L3MBTL4	A	-1.17	3.80E-27	NA	NA	NA	NA	NA	NA
395	POLR3G	A	-1.17	1.07E-11	NA	NA	NA	NA	NA	NA
396	SORCS1	A	-1.17	1.16E-75	NA	NA	NA	NA	NA	NA
397	PEG10	A	-1.17	1.92E-34	NA	NA	NA	NA	NA	NA
398	CNKSR1	A	-1.17	6.75E-04	NA	NA	NA	NA	NA	NA
399	AC048344.4	A	-1.18	1.40E-02	NA	NA	NA	NA	NA	NA
400	PUS1	A	-1.18	1.16E-11	NA	NA	NA	NA	NA	NA
401	AL008638.6	A	-1.18	3.93E-02	NA	NA	NA	NA	NA	NA
402	PNO1	A	-1.18	2.94E-13	NA	NA	NA	NA	NA	NA
403	CEBPA-AS1	A	-1.18	3.04E-02	NA	NA	NA	NA	NA	NA
404	CMSS1	A	-1.18	2.74E-35	NA	NA	NA	NA	NA	NA
405	LYAR	A	-1.19	3.07E-61	NA	NA	NA	NA	NA	NA
406	MARS2	A	-1.19	4.00E-39	NA	NA	NA	NA	NA	NA
407	NOP16	A	-1.19	2.58E-37	NA	NA	NA	NA	NA	NA
408	GPD1L	A	-1.20	3.41E-22	NA	NA	NA	NA	NA	NA
409	HS6ST2	A	-1.20	6.24E-60	NA	NA	NA	NA	NA	NA
410	WDR3	A	-1.20	1.87E-66	NA	NA	NA	NA	NA	NA
411	AL391095.1	A	-1.21	1.77E-02	NA	NA	NA	NA	NA	NA
412	NSG1	A	-1.21	1.80E-31	NA	NA	NA	NA	NA	NA
413	HIST1H4K	A	-1.21	2.32E-02	NA	NA	NA	NA	NA	NA
414	LINC02012	A	-1.21	1.11E-05	NA	NA	NA	NA	NA	NA
415	PNP	A	-1.21	1.22E-66	NA	NA	NA	NA	NA	NA
416	ARHGAP22	A	-1.21	3.09E-12	NA	NA	NA	NA	NA	NA
417	HMGN2P15	A	-1.22	1.08E-02	NA	NA	NA	NA	NA	NA
418	CEP44	A	-1.22	7.01E-65	NA	NA	NA	NA	NA	NA
419	SLC29A1	A	-1.22	2.37E-48	NA	NA	NA	NA	NA	NA
420	FBXO32	A	-1.23	4.26E-05	NA	NA	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
421	AC105389.3	A	-1.24	9.25E-03	NA	NA	NA	NA	NA	NA
422	CACNA2D1	A	-1.24	3.33E-37	NA	NA	NA	NA	NA	NA
423	SH3RF3	A	-1.24	3.47E-07	NA	NA	NA	NA	NA	NA
424	ALG14	A	-1.25	3.05E-03	NA	NA	NA	NA	NA	NA
425	SERTAD4	A	-1.25	1.15E-29	NA	NA	NA	NA	NA	NA
426	MTSS1	A	-1.25	6.64E-08	NA	NA	NA	NA	NA	NA
427	MYBBP1A	A	-1.26	6.98E-32	NA	NA	NA	NA	NA	NA
428	SLC35F2	A	-1.26	9.02E-56	NA	NA	NA	NA	NA	NA
429	AC125807.2	A	-1.26	1.31E-20	NA	NA	NA	NA	NA	NA
430	TMED6	A	-1.26	3.29E-04	NA	NA	NA	NA	NA	NA
431	EXOSC5	A	-1.26	1.21E-08	NA	NA	NA	NA	NA	NA
432	HOGA1	A	-1.27	1.17E-03	NA	NA	NA	NA	NA	NA
433	AC097461.1	A	-1.27	3.34E-09	NA	NA	NA	NA	NA	NA
434	FHOD3	A	-1.27	3.87E-33	NA	NA	NA	NA	NA	NA
435	TNFRSF21	A	-1.27	1.12E-81	NA	NA	NA	NA	NA	NA
436	TTLL12	A	-1.27	1.25E-09	NA	NA	NA	NA	NA	NA
437	MYO1G	A	-1.29	1.62E-02	NA	NA	NA	NA	NA	NA
438	PLEKHA7	A	-1.30	2.89E-03	NA	NA	NA	NA	NA	NA
439	GFRA3	A	-1.30	5.43E-26	NA	NA	NA	NA	NA	NA
440	PNCK	A	-1.31	9.67E-03	NA	NA	NA	NA	NA	NA
441	PC	A	-1.33	6.25E-08	NA	NA	NA	NA	NA	NA
442	AC006111.2	A	-1.33	5.02E-04	NA	NA	NA	NA	NA	NA
443	COBLL1	A	-1.33	7.35E-12	NA	NA	NA	NA	NA	NA
444	GCH1	A	-1.34	1.46E-57	NA	NA	NA	NA	NA	NA
445	HDAC9	A	-1.34	3.26E-45	NA	NA	NA	NA	NA	NA
446	GLDC	A	-1.35	1.78E-14	NA	NA	NA	NA	NA	NA
447	COLGALT2	A	-1.36	1.34E-32	NA	NA	NA	NA	NA	NA
448	RGS10	A	-1.37	2.66E-05	NA	NA	NA	NA	NA	NA
449	MPV17L	A	-1.37	6.27E-14	NA	NA	NA	NA	NA	NA
450	LINC01315	A	-1.38	1.79E-04	NA	NA	NA	NA	NA	NA
451	B3GAT2	A	-1.39	1.43E-05	NA	NA	NA	NA	NA	NA
452	THSD7A	A	-1.40	8.01E-45	NA	NA	NA	NA	NA	NA
453	NOX1	A	-1.40	8.20E-03	NA	NA	NA	NA	NA	NA
454	OXCT2	A	-1.40	3.17E-02	NA	NA	NA	NA	NA	NA
455	EPHA2	A	-1.42	7.61E-13	NA	NA	NA	NA	NA	NA
456	SENP3-EIF4A1	A	-1.44	1.09E-03	NA	NA	NA	NA	NA	NA
457	GFOD1	A	-1.44	1.05E-11	NA	NA	NA	NA	NA	NA
458	SPOCK1	A	-1.45	3.09E-16	NA	NA	NA	NA	NA	NA
459	AC012354.1	A	-1.46	2.87E-30	NA	NA	NA	NA	NA	NA
460	ZNF503-AS2	A	-1.46	1.82E-05	NA	NA	NA	NA	NA	NA
461	ABCC4	A	-1.47	6.03E-15	NA	NA	NA	NA	NA	NA
462	SIX3-AS1	A	-1.48	2.64E-20	NA	NA	NA	NA	NA	NA
463	SNHG3	A	-1.49	1.04E-32	NA	NA	NA	NA	NA	NA
464	AC010478.1	A	-1.54	7.72E-48	NA	NA	NA	NA	NA	NA
465	SNORD78	A	-1.56	2.96E-02	NA	NA	NA	NA	NA	NA
466	SFXN2	A	-1.57	4.85E-44	NA	NA	NA	NA	NA	NA
467	LAMC3	A	-1.57	1.61E-02	NA	NA	NA	NA	NA	NA
468	LDLRAD2	A	-1.59	3.25E-02	NA	NA	NA	NA	NA	NA
469	ANKDD1B	A	-1.60	2.30E-02	NA	NA	NA	NA	NA	NA
470	CEBPB	A	-1.60	1.12E-19	NA	NA	NA	NA	NA	NA
471	PTGDR2	A	-1.61	3.99E-02	NA	NA	NA	NA	NA	NA
472	RNU6-7	A	-1.63	2.32E-02	NA	NA	NA	NA	NA	NA
473	AC090061.1	A	-1.64	4.34E-02	NA	NA	NA	NA	NA	NA
474	FAM131C	A	-1.64	2.37E-09	NA	NA	NA	NA	NA	NA
475	MUC19	A	-1.65	3.23E-02	NA	NA	NA	NA	NA	NA
476	RIPOR3	A	-1.66	8.51E-10	NA	NA	NA	NA	NA	NA
477	FRMD3	A	-1.67	1.10E-05	NA	NA	NA	NA	NA	NA
478	CA8	A	-1.68	1.68E-02	NA	NA	NA	NA	NA	NA
479	ZAR1	A	-1.70	1.46E-02	NA	NA	NA	NA	NA	NA
480	AC008808.1	A	-1.70	3.04E-03	NA	NA	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
481	DHRS2	A	-1.73	1.70E-17	NA	NA	NA	NA	NA	NA
482	LIMS2	A	-1.73	2.72E-02	NA	NA	NA	NA	NA	NA
483	SNORA71	A	-1.83	4.49E-02	NA	NA	NA	NA	NA	NA
484	LINC02269	A	-1.84	7.10E-03	NA	NA	NA	NA	NA	NA
485	PRLR	A	-1.84	2.71E-02	NA	NA	NA	NA	NA	NA
486	SIX3	A	-1.84	3.42E-163	NA	NA	NA	NA	NA	NA
487	SLC13A3	A	-1.86	2.91E-02	NA	NA	NA	NA	NA	NA
488	AP003715.1	A	-1.86	3.63E-02	NA	NA	NA	NA	NA	NA
489	KCNS2	A	-1.90	3.50E-02	NA	NA	NA	NA	NA	NA
490	AL591379.1	A	-1.97	2.93E-03	NA	NA	NA	NA	NA	NA
491	MASP1	A	-1.98	4.56E-02	NA	NA	NA	NA	NA	NA
492	AC106895.1	A	-2.03	3.89E-02	NA	NA	NA	NA	NA	NA
493	QRFPR	A	-2.08	8.31E-03	NA	NA	NA	NA	NA	NA
494	AL365223.1	A	-2.13	2.57E-02	NA	NA	NA	NA	NA	NA
495	Six3os1_2	A	-2.23	1.61E-02	NA	NA	NA	NA	NA	NA
496	AC008514.2	A	-2.23	4.23E-02	NA	NA	NA	NA	NA	NA
497	ZNF385B	A	-2.32	1.11E-03	NA	NA	NA	NA	NA	NA
498	TGFB2	A	-2.33	2.81E-03	NA	NA	NA	NA	NA	NA
499	AC073109.1	A	-2.33	1.91E-02	NA	NA	NA	NA	NA	NA
500	AC006042.1	A	-2.35	1.81E-08	NA	NA	NA	NA	NA	NA
501	MDGA1	A	-2.36	8.32E-03	NA	NA	NA	NA	NA	NA
502	ABCB10P4	A	-2.36	1.33E-02	NA	NA	NA	NA	NA	NA
503	ATP8B4	A	-2.51	3.12E-02	NA	NA	NA	NA	NA	NA
504	GVQW2	A	-2.65	4.56E-02	NA	NA	NA	NA	NA	NA
505	DCC	A	-2.73	1.02E-02	NA	NA	NA	NA	NA	NA
506	AL390835.1	A	-2.98	1.70E-02	NA	NA	NA	NA	NA	NA
507	RPL30P11	A	-3.15	4.80E-02	NA	NA	NA	NA	NA	NA
508	ARHGAP25	A	-3.33	1.07E-06	NA	NA	NA	NA	NA	NA
509	ADCY8	A	-3.57	1.08E-03	NA	NA	NA	NA	NA	NA
510	IPCEF1	A	-3.73	2.50E-02	NA	NA	NA	NA	NA	NA
511	NPM1P18	A	-3.86	7.29E-03	NA	NA	NA	NA	NA	NA
512	TNNT3	A	-3.87	1.47E-02	NA	NA	NA	NA	NA	NA
513	PROK2	A	-4.11	3.31E-03	NA	NA	NA	NA	NA	NA
514	TRABD2B	A	-4.20	4.19E-02	NA	NA	NA	NA	NA	NA
515	LINC00523	A	-4.26	1.62E-04	NA	NA	NA	NA	NA	NA
516	HECW1	A	-4.50	2.05E-02	NA	NA	NA	NA	NA	NA
517	PSPC1P1	A	-6.45	4.85E-02	NA	NA	NA	NA	NA	NA
518	CASQ2	B	NA	NA	5.98	4.14E-02	NA	NA	NA	NA
519	AF064858.1	B	NA	NA	5.66	5.48E-05	NA	NA	NA	NA
520	AL645929.2	B	NA	NA	5.50	9.84E-03	NA	NA	NA	NA
521	AL136088.1	B	NA	NA	4.79	1.03E-03	NA	NA	NA	NA
522	AC113189.3	B	NA	NA	4.52	4.84E-02	NA	NA	NA	NA
523	AC092745.3	B	NA	NA	4.47	3.35E-02	NA	NA	NA	NA
524	MAMDC2	B	NA	NA	4.03	2.85E-04	NA	NA	NA	NA
525	TCF24	B	NA	NA	3.84	8.44E-05	NA	NA	NA	NA
526	AC097634.3	B	NA	NA	3.61	2.11E-02	NA	NA	NA	NA
527	SNORD3B-1	B	NA	NA	3.39	3.19E-02	NA	NA	NA	NA
528	AC133555.5	B	NA	NA	3.37	6.86E-03	NA	NA	NA	NA
529	ARPP21	B	NA	NA	3.34	8.42E-05	NA	NA	NA	NA
530	ST8SIA6	B	NA	NA	3.33	1.29E-02	NA	NA	NA	NA
531	HMSD	B	NA	NA	3.17	4.51E-02	NA	NA	NA	NA
532	KIAA1755	B	NA	NA	3.12	4.47E-02	NA	NA	NA	NA
533	BICDL2	B	NA	NA	3.01	9.59E-04	NA	NA	NA	NA
534	EID3	B	NA	NA	2.92	9.33E-04	NA	NA	NA	NA
535	ACBD3-AS1	B	NA	NA	2.87	2.88E-02	NA	NA	NA	NA
536	AD000864.1	B	NA	NA	2.76	4.54E-02	NA	NA	NA	NA
537	HSPB6	B	NA	NA	2.70	1.04E-02	NA	NA	NA	NA
538	AL138828.1	B	NA	NA	2.56	6.14E-03	NA	NA	NA	NA
539	GRB10	B	NA	NA	2.47	2.25E-03	NA	NA	NA	NA
540	AL928711.1	B	NA	NA	2.41	3.02E-02	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
541	SAMD5	B	NA	NA	2.37	3.02E-02	NA	NA	NA	NA
542	GPNMB	B	NA	NA	2.37	3.21E-02	NA	NA	NA	NA
543	GPR146	B	NA	NA	2.14	1.32E-10	NA	NA	NA	NA
544	CMYA5	B	NA	NA	2.13	3.76E-03	NA	NA	NA	NA
545	PLXDC1	B	NA	NA	2.07	2.30E-02	NA	NA	NA	NA
546	RDH12	B	NA	NA	2.07	2.50E-02	NA	NA	NA	NA
547	C3orf58	B	NA	NA	2.06	2.71E-09	NA	NA	NA	NA
548	CPM	B	NA	NA	2.03	5.16E-03	NA	NA	NA	NA
549	AC011632.1	B	NA	NA	1.98	3.41E-02	NA	NA	NA	NA
550	AC114803.1	B	NA	NA	1.97	4.58E-03	NA	NA	NA	NA
551	AL162274.2	B	NA	NA	1.93	9.90E-04	NA	NA	NA	NA
552	uc_338	B	NA	NA	1.89	4.62E-02	NA	NA	NA	NA
553	SYTL5	B	NA	NA	1.88	1.16E-02	NA	NA	NA	NA
554	NIPAL4	B	NA	NA	1.86	1.17E-05	NA	NA	NA	NA
555	AL137918.1	B	NA	NA	1.83	5.41E-03	NA	NA	NA	NA
556	AC004832.6	B	NA	NA	1.79	8.53E-03	NA	NA	NA	NA
557	C11orf45	B	NA	NA	1.76	3.69E-02	NA	NA	NA	NA
558	SEC14L5	B	NA	NA	1.76	2.80E-03	NA	NA	NA	NA
559	AL353658.1	B	NA	NA	1.74	1.50E-02	NA	NA	NA	NA
560	NAGS	B	NA	NA	1.70	1.77E-02	NA	NA	NA	NA
561	STK4-AS1	B	NA	NA	1.68	3.17E-02	NA	NA	NA	NA
562	SYDE1	B	NA	NA	1.62	1.36E-24	NA	NA	NA	NA
563	AC008761.2	B	NA	NA	1.58	2.82E-02	NA	NA	NA	NA
564	TCAF2	B	NA	NA	1.56	5.43E-03	NA	NA	NA	NA
565	PDK1	B	NA	NA	1.54	6.42E-40	NA	NA	NA	NA
566	CHST9	B	NA	NA	1.53	2.39E-09	NA	NA	NA	NA
567	AL356309.2	B	NA	NA	1.51	5.86E-03	NA	NA	NA	NA
568	NPAS2	B	NA	NA	1.47	8.75E-05	NA	NA	NA	NA
569	FOXP1	B	NA	NA	1.46	8.93E-56	NA	NA	NA	NA
570	REST	B	NA	NA	1.45	7.77E-31	NA	NA	NA	NA
571	ERFE	B	NA	NA	1.45	3.39E-32	NA	NA	NA	NA
572	AC007283.1	B	NA	NA	1.44	4.53E-02	NA	NA	NA	NA
573	TMEM225B	B	NA	NA	1.43	1.09E-02	NA	NA	NA	NA
574	NUDT18	B	NA	NA	1.43	2.55E-07	NA	NA	NA	NA
575	TPI1P1	B	NA	NA	1.42	1.04E-02	NA	NA	NA	NA
576	SNORA59B	B	NA	NA	1.42	3.52E-02	NA	NA	NA	NA
577	KCNS3	B	NA	NA	1.41	1.70E-02	NA	NA	NA	NA
578	TSPAN4	B	NA	NA	1.41	2.27E-15	NA	NA	NA	NA
579	LDHAP3	B	NA	NA	1.40	2.82E-02	NA	NA	NA	NA
580	NXPH3	B	NA	NA	1.39	1.49E-02	NA	NA	NA	NA
581	BEND5	B	NA	NA	1.38	1.84E-31	NA	NA	NA	NA
582	RIMKLA	B	NA	NA	1.38	6.38E-30	NA	NA	NA	NA
583	RORA	B	NA	NA	1.35	1.57E-22	NA	NA	NA	NA
584	C2orf72	B	NA	NA	1.33	2.30E-19	NA	NA	NA	NA
585	BCOR	B	NA	NA	1.32	3.12E-36	NA	NA	NA	NA
586	CABP1	B	NA	NA	1.30	3.71E-10	NA	NA	NA	NA
587	TMSB4XP6	B	NA	NA	1.30	4.10E-35	NA	NA	NA	NA
588	CFAP43	B	NA	NA	1.29	1.52E-04	NA	NA	NA	NA
589	SIK2	B	NA	NA	1.29	7.64E-21	NA	NA	NA	NA
590	GALNT18	B	NA	NA	1.28	1.41E-18	NA	NA	NA	NA
591	TMEM191C	B	NA	NA	1.27	1.83E-02	NA	NA	NA	NA
592	TMSB4X	B	NA	NA	1.27	2.48E-57	NA	NA	NA	NA
593	NDNF	B	NA	NA	1.26	5.96E-17	NA	NA	NA	NA
594	ESR2	B	NA	NA	1.25	7.30E-03	NA	NA	NA	NA
595	DUSP10	B	NA	NA	1.24	2.39E-12	NA	NA	NA	NA
596	SCUBE1	B	NA	NA	1.22	3.59E-03	NA	NA	NA	NA
597	RELL1	B	NA	NA	1.22	4.65E-07	NA	NA	NA	NA
598	ARHGEF37	B	NA	NA	1.22	6.47E-08	NA	NA	NA	NA
599	INSIG2	B	NA	NA	1.22	1.80E-19	NA	NA	NA	NA
600	AC015691.1	B	NA	NA	1.21	2.98E-02	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
601	NECTIN1	B	NA	NA	1.20	3.59E-30	NA	NA	NA	NA
602	BLCAP	B	NA	NA	1.20	1.83E-54	NA	NA	NA	NA
603	TOGARAM2	B	NA	NA	1.19	6.79E-05	NA	NA	NA	NA
604	TNIP1	B	NA	NA	1.19	4.81E-23	NA	NA	NA	NA
605	CEP70	B	NA	NA	1.19	1.59E-20	NA	NA	NA	NA
606	FAM102B	B	NA	NA	1.19	2.40E-33	NA	NA	NA	NA
607	FAM13A-AS1	B	NA	NA	1.17	4.05E-03	NA	NA	NA	NA
608	SKAP2	B	NA	NA	1.17	5.21E-03	NA	NA	NA	NA
609	TRIO	B	NA	NA	1.17	4.62E-47	NA	NA	NA	NA
610	ENO1	B	NA	NA	1.16	3.41E-86	NA	NA	NA	NA
611	DDIT4	B	NA	NA	1.16	6.83E-19	NA	NA	NA	NA
612	PDK3	B	NA	NA	1.15	2.07E-21	NA	NA	NA	NA
613	VEPH1	B	NA	NA	1.15	3.97E-02	NA	NA	NA	NA
614	TSPAN2	B	NA	NA	1.14	9.35E-14	NA	NA	NA	NA
615	TCF7	B	NA	NA	1.14	2.57E-15	NA	NA	NA	NA
616	ANO7	B	NA	NA	1.14	1.16E-07	NA	NA	NA	NA
617	TESMIN	B	NA	NA	1.14	5.15E-12	NA	NA	NA	NA
618	CDKN2D	B	NA	NA	1.14	2.64E-14	NA	NA	NA	NA
619	CREB3L2	B	NA	NA	1.14	2.08E-98	NA	NA	NA	NA
620	DDIT3	B	NA	NA	1.14	2.98E-15	NA	NA	NA	NA
621	RASIP1	B	NA	NA	1.13	4.58E-02	NA	NA	NA	NA
622	FAM69A	B	NA	NA	1.13	1.43E-26	NA	NA	NA	NA
623	AC083843.2	B	NA	NA	1.13	8.20E-10	NA	NA	NA	NA
624	RASSF7	B	NA	NA	1.13	8.18E-03	NA	NA	NA	NA
625	AC022400.8	B	NA	NA	1.12	4.70E-04	NA	NA	NA	NA
626	C4orf3	B	NA	NA	1.12	5.71E-61	NA	NA	NA	NA
627	TMEM51	B	NA	NA	1.12	1.22E-40	NA	NA	NA	NA
628	P4HA1	B	NA	NA	1.11	2.25E-22	NA	NA	NA	NA
629	AC007382.1	B	NA	NA	1.11	2.19E-25	NA	NA	NA	NA
630	PLEKHH2	B	NA	NA	1.11	2.77E-03	NA	NA	NA	NA
631	MID1IP1	B	NA	NA	1.10	1.06E-11	NA	NA	NA	NA
632	LDHA	B	NA	NA	1.10	2.80E-24	NA	NA	NA	NA
633	AC097534.2	B	NA	NA	1.09	1.65E-08	NA	NA	NA	NA
634	GJC2	B	NA	NA	1.08	4.02E-02	NA	NA	NA	NA
635	GPI	B	NA	NA	1.08	2.37E-25	NA	NA	NA	NA
636	PRSS35	B	NA	NA	1.08	9.37E-05	NA	NA	NA	NA
637	HERC2P8	B	NA	NA	1.08	3.02E-02	NA	NA	NA	NA
638	GBE1	B	NA	NA	1.08	2.14E-23	NA	NA	NA	NA
639	PPARD	B	NA	NA	1.07	1.23E-19	NA	NA	NA	NA
640	AL356309.3	B	NA	NA	1.07	3.00E-03	NA	NA	NA	NA
641	ZNF823	B	NA	NA	1.06	2.31E-14	NA	NA	NA	NA
642	PHLDB2	B	NA	NA	1.05	4.15E-09	NA	NA	NA	NA
643	VAV3	B	NA	NA	1.05	9.65E-09	NA	NA	NA	NA
644	TMPOP2	B	NA	NA	1.05	2.80E-02	NA	NA	NA	NA
645	TMED9	B	NA	NA	1.05	4.12E-06	NA	NA	NA	NA
646	CRCP	B	NA	NA	1.04	3.31E-52	NA	NA	NA	NA
647	SMIM14	B	NA	NA	1.04	4.86E-20	NA	NA	NA	NA
648	KCTD19	B	NA	NA	1.04	3.02E-02	NA	NA	NA	NA
649	AP001062.3	B	NA	NA	1.04	7.56E-07	NA	NA	NA	NA
650	PLCH1	B	NA	NA	1.04	1.10E-14	NA	NA	NA	NA
651	CRISPLD1	B	NA	NA	1.03	1.92E-35	NA	NA	NA	NA
652	SCART1	B	NA	NA	1.03	4.14E-03	NA	NA	NA	NA
653	SLC44A1	B	NA	NA	1.02	1.04E-30	NA	NA	NA	NA
654	TPI1	B	NA	NA	1.02	2.99E-46	NA	NA	NA	NA
655	AC096745.2	B	NA	NA	1.02	1.25E-06	NA	NA	NA	NA
656	SLC36A1	B	NA	NA	1.01	7.99E-10	NA	NA	NA	NA
657	LINC02043	B	NA	NA	1.01	7.69E-03	NA	NA	NA	NA
658	TANC1	B	NA	NA	1.01	3.48E-116	NA	NA	NA	NA
659	AC108002.1	B	NA	NA	1.01	3.76E-03	NA	NA	NA	NA
660	LIFR	B	NA	NA	1.01	6.45E-06	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
661	LDHAP7	B	NA	NA	1.01	2.05E-03	NA	NA	NA	NA
662	GAB2	B	NA	NA	1.01	3.20E-41	NA	NA	NA	NA
663	CDC25C	B	NA	NA	1.01	5.12E-29	NA	NA	NA	NA
664	ZNF709	B	NA	NA	1.00	1.46E-03	NA	NA	NA	NA
665	CDKN3	B	NA	NA	1.00	4.86E-11	NA	NA	NA	NA
666	CCNG2	B	NA	NA	1.00	8.30E-46	NA	NA	NA	NA
667	TK1	B	NA	NA	1.00	1.23E-12	NA	NA	NA	NA
668	ZNF654	B	NA	NA	1.00	6.64E-20	NA	NA	NA	NA
669	RGS3	B	NA	NA	-1.01	5.77E-04	NA	NA	NA	NA
670	STMN2	B	NA	NA	-1.01	1.53E-38	NA	NA	NA	NA
671	FDXACB1	B	NA	NA	-1.01	6.57E-13	NA	NA	NA	NA
672	USP18	B	NA	NA	-1.01	4.07E-05	NA	NA	NA	NA
673	RABEPK	B	NA	NA	-1.01	9.39E-44	NA	NA	NA	NA
674	NAT8L	B	NA	NA	-1.02	1.73E-11	NA	NA	NA	NA
675	CRYBG1	B	NA	NA	-1.02	3.56E-02	NA	NA	NA	NA
676	LRRC7	B	NA	NA	-1.02	3.71E-03	NA	NA	NA	NA
677	PLCD4	B	NA	NA	-1.02	3.55E-22	NA	NA	NA	NA
678	AK5	B	NA	NA	-1.03	4.82E-02	NA	NA	NA	NA
679	AC083837.2	B	NA	NA	-1.03	5.50E-12	NA	NA	NA	NA
680	METAP1D	B	NA	NA	-1.04	3.01E-04	NA	NA	NA	NA
681	DUSP23	B	NA	NA	-1.04	1.27E-11	NA	NA	NA	NA
682	HAGHL	B	NA	NA	-1.05	4.02E-04	NA	NA	NA	NA
683	SERPINE2	B	NA	NA	-1.05	1.06E-15	NA	NA	NA	NA
684	HS6ST3	B	NA	NA	-1.05	2.36E-06	NA	NA	NA	NA
685	HSD17B6	B	NA	NA	-1.06	1.19E-02	NA	NA	NA	NA
686	TMEM5	B	NA	NA	-1.06	1.10E-08	NA	NA	NA	NA
687	HIST1H2AC	B	NA	NA	-1.07	2.64E-18	NA	NA	NA	NA
688	AC019069.1	B	NA	NA	-1.07	2.15E-17	NA	NA	NA	NA
689	KIAA1211L	B	NA	NA	-1.08	1.62E-02	NA	NA	NA	NA
690	ARSG	B	NA	NA	-1.09	1.72E-04	NA	NA	NA	NA
691	PWWP2B	B	NA	NA	-1.09	3.38E-04	NA	NA	NA	NA
692	EPB41L4A	B	NA	NA	-1.09	7.59E-03	NA	NA	NA	NA
693	NR1D1	B	NA	NA	-1.09	1.21E-06	NA	NA	NA	NA
694	HSPA12B	B	NA	NA	-1.09	1.84E-02	NA	NA	NA	NA
695	EMID1	B	NA	NA	-1.10	2.76E-05	NA	NA	NA	NA
696	DGKB	B	NA	NA	-1.10	2.51E-05	NA	NA	NA	NA
697	CADPS	B	NA	NA	-1.11	1.65E-03	NA	NA	NA	NA
698	AL359921.1	B	NA	NA	-1.11	1.44E-02	NA	NA	NA	NA
699	CERKL	B	NA	NA	-1.11	4.86E-07	NA	NA	NA	NA
700	SPOCK3	B	NA	NA	-1.11	1.16E-02	NA	NA	NA	NA
701	THBS4	B	NA	NA	-1.12	1.38E-03	NA	NA	NA	NA
702	DUSP4	B	NA	NA	-1.12	6.94E-79	NA	NA	NA	NA
703	AC004982.2	B	NA	NA	-1.14	8.91E-04	NA	NA	NA	NA
704	SIX6	B	NA	NA	-1.14	3.10E-12	NA	NA	NA	NA
705	EFCAB10	B	NA	NA	-1.15	6.14E-04	NA	NA	NA	NA
706	IMPDH1P8	B	NA	NA	-1.18	6.69E-03	NA	NA	NA	NA
707	ADAM11	B	NA	NA	-1.20	8.17E-04	NA	NA	NA	NA
708	TMCC1-AS1	B	NA	NA	-1.21	4.29E-10	NA	NA	NA	NA
709	ST6GALNAC4	B	NA	NA	-1.21	1.41E-03	NA	NA	NA	NA
710	SLC43A2	B	NA	NA	-1.22	5.96E-13	NA	NA	NA	NA
711	RNF112	B	NA	NA	-1.23	3.83E-06	NA	NA	NA	NA
712	AC004690.2	B	NA	NA	-1.24	1.12E-03	NA	NA	NA	NA
713	CASP16P	B	NA	NA	-1.24	3.41E-02	NA	NA	NA	NA
714	CCDC78	B	NA	NA	-1.25	9.12E-04	NA	NA	NA	NA
715	RTL5	B	NA	NA	-1.25	1.08E-186	NA	NA	NA	NA
716	AL139407.1	B	NA	NA	-1.27	4.64E-02	NA	NA	NA	NA
717	AL133372.2	B	NA	NA	-1.28	3.48E-02	NA	NA	NA	NA
718	ALG1L6P	B	NA	NA	-1.28	3.64E-02	NA	NA	NA	NA
719	CLYBL	B	NA	NA	-1.29	5.64E-13	NA	NA	NA	NA
720	MATR3	B	NA	NA	-1.30	8.13E-04	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
721	SNORD104	B	NA	NA	-1.30	2.00E-03	NA	NA	NA	NA
722	AC015802.5	B	NA	NA	-1.31	4.49E-02	NA	NA	NA	NA
723	DCX	B	NA	NA	-1.35	1.73E-64	NA	NA	NA	NA
724	FAM84A	B	NA	NA	-1.35	2.49E-26	NA	NA	NA	NA
725	ARHGAP15	B	NA	NA	-1.38	4.36E-02	NA	NA	NA	NA
726	AC100788.2	B	NA	NA	-1.39	4.47E-02	NA	NA	NA	NA
727	CDH18	B	NA	NA	-1.40	5.19E-03	NA	NA	NA	NA
728	RNF144B	B	NA	NA	-1.43	3.14E-03	NA	NA	NA	NA
729	TMEM176B	B	NA	NA	-1.44	1.36E-02	NA	NA	NA	NA
730	ZNF556	B	NA	NA	-1.44	9.20E-16	NA	NA	NA	NA
731	KHDRBS3	B	NA	NA	-1.44	1.35E-11	NA	NA	NA	NA
732	BAALC	B	NA	NA	-1.45	2.49E-08	NA	NA	NA	NA
733	LAMB4	B	NA	NA	-1.47	4.76E-02	NA	NA	NA	NA
734	NCKAP5	B	NA	NA	-1.48	6.72E-04	NA	NA	NA	NA
735	LINC01021	B	NA	NA	-1.48	3.44E-02	NA	NA	NA	NA
736	COL6A6	B	NA	NA	-1.49	1.47E-02	NA	NA	NA	NA
737	SYBU	B	NA	NA	-1.49	1.89E-05	NA	NA	NA	NA
738	COL8A2	B	NA	NA	-1.51	1.14E-05	NA	NA	NA	NA
739	AC023983.1	B	NA	NA	-1.52	3.82E-02	NA	NA	NA	NA
740	PTGES	B	NA	NA	-1.52	1.80E-12	NA	NA	NA	NA
741	SLC16A6P1	B	NA	NA	-1.53	2.41E-02	NA	NA	NA	NA
742	PLIN4	B	NA	NA	-1.56	3.89E-05	NA	NA	NA	NA
743	NUAK1	B	NA	NA	-1.58	1.00E-04	NA	NA	NA	NA
744	AC010890.1	B	NA	NA	-1.63	1.49E-02	NA	NA	NA	NA
745	NEUROD1	B	NA	NA	-1.64	1.03E-23	NA	NA	NA	NA
746	AC099568.2	B	NA	NA	-1.65	4.41E-02	NA	NA	NA	NA
747	MIR4800	B	NA	NA	-1.67	4.03E-02	NA	NA	NA	NA
748	TNFAIP6	B	NA	NA	-1.68	4.97E-02	NA	NA	NA	NA
749	AC015802.3	B	NA	NA	-1.69	2.37E-05	NA	NA	NA	NA
750	AGAP13P	B	NA	NA	-1.71	4.98E-02	NA	NA	NA	NA
751	WNT8B	B	NA	NA	-1.72	2.69E-02	NA	NA	NA	NA
752	AC131009.3	B	NA	NA	-1.72	4.22E-02	NA	NA	NA	NA
753	GBP6	B	NA	NA	-1.74	4.13E-02	NA	NA	NA	NA
754	AC122710.2	B	NA	NA	-1.75	2.04E-07	NA	NA	NA	NA
755	DNAJC8P1	B	NA	NA	-1.76	3.22E-02	NA	NA	NA	NA
756	CDX1	B	NA	NA	-1.80	1.79E-02	NA	NA	NA	NA
757	TTLL2	B	NA	NA	-1.81	2.88E-04	NA	NA	NA	NA
758	MST1R	B	NA	NA	-1.82	2.75E-02	NA	NA	NA	NA
759	GRM7	B	NA	NA	-1.83	2.08E-03	NA	NA	NA	NA
760	KIZ-AS1	B	NA	NA	-1.94	4.39E-02	NA	NA	NA	NA
761	LRMDA	B	NA	NA	-2.02	4.26E-02	NA	NA	NA	NA
762	STAR	B	NA	NA	-2.09	4.34E-02	NA	NA	NA	NA
763	IFI44L	B	NA	NA	-2.12	2.34E-02	NA	NA	NA	NA
764	KIZ	B	NA	NA	-2.12	1.16E-20	NA	NA	NA	NA
765	PAPPA-AS2	B	NA	NA	-2.25	3.74E-02	NA	NA	NA	NA
766	FOXP4-AS1	B	NA	NA	-2.43	3.48E-02	NA	NA	NA	NA
767	MGLL	B	NA	NA	-2.47	3.39E-03	NA	NA	NA	NA
768	ADGRF3	B	NA	NA	-2.58	1.15E-02	NA	NA	NA	NA
769	TMEM229B	B	NA	NA	-2.61	1.37E-03	NA	NA	NA	NA
770	VDR	B	NA	NA	-2.62	9.61E-03	NA	NA	NA	NA
771	AL035409.1	B	NA	NA	-2.69	1.89E-02	NA	NA	NA	NA
772	CHCHD4P3	B	NA	NA	-2.78	1.81E-02	NA	NA	NA	NA
773	TMEM26	B	NA	NA	-2.80	1.60E-02	NA	NA	NA	NA
774	COL2A1	B	NA	NA	-2.83	6.18E-03	NA	NA	NA	NA
775	IFI35	B	NA	NA	-2.85	3.91E-02	NA	NA	NA	NA
776	AC100793.4	B	NA	NA	-2.87	1.98E-02	NA	NA	NA	NA
777	EPHB1	B	NA	NA	-2.92	3.61E-02	NA	NA	NA	NA
778	TMEM176A	B	NA	NA	-3.24	3.09E-02	NA	NA	NA	NA
779	RNU6ATAC35P	B	NA	NA	-3.39	2.77E-02	NA	NA	NA	NA
780	FAM230A	B	NA	NA	-3.62	3.73E-02	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
781	CACNA2D3	B	NA	NA	-3.79	2.12E-02	NA	NA	NA	NA
782	AQP12B	B	NA	NA	-3.84	2.14E-02	NA	NA	NA	NA
783	FCGR1A	B	NA	NA	-3.94	2.12E-02	NA	NA	NA	NA
784	LINGO4	B	NA	NA	-4.30	5.31E-03	NA	NA	NA	NA
785	PDE1A	B	NA	NA	-4.48	1.10E-04	NA	NA	NA	NA
786	GPR179	B	NA	NA	-4.57	3.28E-02	NA	NA	NA	NA
787	C11orf87	B	NA	NA	-4.57	4.08E-03	NA	NA	NA	NA
788	ATP8A2	B	NA	NA	-4.67	1.40E-02	NA	NA	NA	NA
789	AC023509.1	C	NA	NA	NA	NA	-5.63	0.003834049	NA	NA
790	SFTPC	C	NA	NA	NA	NA	-5.19	0.031305906	NA	NA
791	AC009158.1	C	NA	NA	NA	NA	-4.98	0.018423524	NA	NA
792	PGA4	C	NA	NA	NA	NA	-4.90	0.040192265	NA	NA
793	DCAF8L1	C	NA	NA	NA	NA	-4.88	0.010172623	NA	NA
794	CNOT6LP1	C	NA	NA	NA	NA	-4.86	0.006183986	NA	NA
795	OPLAH	C	NA	NA	NA	NA	-4.79	0.002739899	NA	NA
796	AC091196.1	C	NA	NA	NA	NA	-4.72	0.002507439	NA	NA
797	PCSK1	C	NA	NA	NA	NA	-4.70	0.0027813	NA	NA
798	AP003108.5	C	NA	NA	NA	NA	-4.68	0.011099203	NA	NA
799	AP003559.1	C	NA	NA	NA	NA	-4.63	0.002535512	NA	NA
800	AC005865.1	C	NA	NA	NA	NA	-4.61	0.008648675	NA	NA
801	HK3	C	NA	NA	NA	NA	-4.55	9.75E-05	NA	NA
802	AL109613.1	C	NA	NA	NA	NA	-4.55	0.010128849	NA	NA
803	AL645608.8	C	NA	NA	NA	NA	-4.53	6.32E-04	NA	NA
804	RIPK4	C	NA	NA	NA	NA	-4.45	0.005666926	NA	NA
805	AC008758.3	C	NA	NA	NA	NA	-4.33	0.024082719	NA	NA
806	TEX41	C	NA	NA	NA	NA	-4.33	0.010352282	NA	NA
807	GOLGA6L17P	C	NA	NA	NA	NA	-4.18	0.010957223	NA	NA
808	IGHM	C	NA	NA	NA	NA	-4.05	0.013171501	NA	NA
809	AC090458.1	C	NA	NA	NA	NA	-4.05	0.012014847	NA	NA
810	AL021154.1	C	NA	NA	NA	NA	-3.97	0.007739555	NA	NA
811	AC010280.1	C	NA	NA	NA	NA	-3.95	0.033306848	NA	NA
812	SMPDL3A	C	NA	NA	NA	NA	-3.91	0.014866581	NA	NA
813	ENO1P2	C	NA	NA	NA	NA	-3.89	0.01183019	NA	NA
814	CACNA1F	C	NA	NA	NA	NA	-3.87	0.02876338	NA	NA
815	AC011270.2	C	NA	NA	NA	NA	-3.84	0.043371954	NA	NA
816	RAB17	C	NA	NA	NA	NA	-3.75	0.038628136	NA	NA
817	LINC01028	C	NA	NA	NA	NA	-3.73	0.008291989	NA	NA
818	AC069257.1	C	NA	NA	NA	NA	-3.72	0.019650959	NA	NA
819	OXCT2P1	C	NA	NA	NA	NA	-3.65	0.010466095	NA	NA
820	TH	C	NA	NA	NA	NA	-3.48	0.026544066	NA	NA
821	AC011369.1	C	NA	NA	NA	NA	-3.44	0.033037023	NA	NA
822	HOXA5	C	NA	NA	NA	NA	-3.43	0.023097169	NA	NA
823	FCRLB	C	NA	NA	NA	NA	-3.39	0.044457055	NA	NA
824	AC021739.2	C	NA	NA	NA	NA	-3.35	0.012383733	NA	NA
825	DCAF8L2	C	NA	NA	NA	NA	-3.34	0.036056034	NA	NA
826	SH3GL2	C	NA	NA	NA	NA	-3.27	0.031367237	NA	NA
827	IZUMO2	C	NA	NA	NA	NA	-3.22	0.010494109	NA	NA
828	FABP6	C	NA	NA	NA	NA	-3.15	0.009876367	NA	NA
829	AC024270.2	C	NA	NA	NA	NA	-3.12	0.037028982	NA	NA
830	CXorf57	C	NA	NA	NA	NA	-3.07	0.044261797	NA	NA
831	GPR78	C	NA	NA	NA	NA	-3.05	0.025643669	NA	NA
832	AL122058.1	C	NA	NA	NA	NA	-3.03	0.027861944	NA	NA
833	TBX21	C	NA	NA	NA	NA	-3.02	0.004659188	NA	NA
834	FOXD2-AS1	C	NA	NA	NA	NA	-2.99	0.037567031	NA	NA
835	LINC01915	C	NA	NA	NA	NA	-2.96	0.044471023	NA	NA
836	TLX1NB	C	NA	NA	NA	NA	-2.92	0.03486211	NA	NA
837	AC105339.3	C	NA	NA	NA	NA	-2.88	0.038555141	NA	NA
838	LINC01091	C	NA	NA	NA	NA	-2.86	0.006873894	NA	NA
839	AC007326.1	C	NA	NA	NA	NA	-2.83	0.023458614	NA	NA
840	AC018521.4	C	NA	NA	NA	NA	-2.79	0.03745473	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
841	SNTG2	C	NA	NA	NA	NA	-2.79	0.003134001	NA	NA
842	NCAM1-AS1	C	NA	NA	NA	NA	-2.78	6.06E-04	NA	NA
843	AC020661.2	C	NA	NA	NA	NA	-2.78	0.033989949	NA	NA
844	AC091167.8	C	NA	NA	NA	NA	-2.76	0.031063912	NA	NA
845	AL445209.1	C	NA	NA	NA	NA	-2.66	0.027517401	NA	NA
846	AC012236.1	C	NA	NA	NA	NA	-2.66	0.044325502	NA	NA
847	ADAMTS16	C	NA	NA	NA	NA	-2.64	0.032390109	NA	NA
848	CTRC	C	NA	NA	NA	NA	-2.63	0.001063883	NA	NA
849	MORF4L1P1	C	NA	NA	NA	NA	-2.59	0.02213359	NA	NA
850	FAM167A-AS1	C	NA	NA	NA	NA	-2.57	8.53E-04	NA	NA
851	KCNK12	C	NA	NA	NA	NA	-2.50	0.007797791	NA	NA
852	KRTAP5-11	C	NA	NA	NA	NA	-2.47	0.020924468	NA	NA
853	AC027243.1	C	NA	NA	NA	NA	-2.42	0.049146452	NA	NA
854	AC027176.2	C	NA	NA	NA	NA	-2.39	0.012196196	NA	NA
855	AC079385.3	C	NA	NA	NA	NA	-2.35	0.03717958	NA	NA
856	CD300E	C	NA	NA	NA	NA	-2.34	0.021619908	NA	NA
857	DUOX2	C	NA	NA	NA	NA	-2.29	0.015577346	NA	NA
858	HTR1E	C	NA	NA	NA	NA	-2.28	1.39E-61	NA	NA
859	AC010857.1	C	NA	NA	NA	NA	-2.28	0.041773991	NA	NA
860	SERF1A	C	NA	NA	NA	NA	-2.22	0.008451589	NA	NA
861	DLL1	C	NA	NA	NA	NA	-2.20	0.04400769	NA	NA
862	TPM1	C	NA	NA	NA	NA	-2.12	8.46E-100	NA	NA
863	AL356479.1	C	NA	NA	NA	NA	-2.08	0.021844541	NA	NA
864	TENM2	C	NA	NA	NA	NA	-2.08	0.022377873	NA	NA
865	TNFRSF1B	C	NA	NA	NA	NA	-2.07	0.001236302	NA	NA
866	AC087885.1	C	NA	NA	NA	NA	-2.06	0.033612479	NA	NA
867	AC104453.1	C	NA	NA	NA	NA	-1.97	0.040038124	NA	NA
868	CPNE8	C	NA	NA	NA	NA	-1.94	0.035214679	NA	NA
869	CHRNA7	C	NA	NA	NA	NA	-1.92	1.54E-18	NA	NA
870	NECAB2	C	NA	NA	NA	NA	-1.90	0.002825188	NA	NA
871	LINC01250	C	NA	NA	NA	NA	-1.87	3.60E-12	NA	NA
872	AL353795.1	C	NA	NA	NA	NA	-1.87	0.037646169	NA	NA
873	CLSTN2	C	NA	NA	NA	NA	-1.86	1.43E-70	NA	NA
874	PLIN1	C	NA	NA	NA	NA	-1.84	0.028487515	NA	NA
875	DPF1	C	NA	NA	NA	NA	-1.84	3.23E-55	NA	NA
876	KCNK9	C	NA	NA	NA	NA	-1.82	0.019846088	NA	NA
877	AC000403.1	C	NA	NA	NA	NA	-1.79	0.012030967	NA	NA
878	AC068831.3	C	NA	NA	NA	NA	-1.74	0.040044854	NA	NA
879	CICP22	C	NA	NA	NA	NA	-1.74	0.007566207	NA	NA
880	GRM1	C	NA	NA	NA	NA	-1.74	0.009310616	NA	NA
881	AC090181.2	C	NA	NA	NA	NA	-1.73	1.97E-13	NA	NA
882	TSPAN7	C	NA	NA	NA	NA	-1.73	1.65E-85	NA	NA
883	TMC3-AS1	C	NA	NA	NA	NA	-1.73	4.73E-04	NA	NA
884	SEMA6B	C	NA	NA	NA	NA	-1.71	0.029063321	NA	NA
885	ABCC3	C	NA	NA	NA	NA	-1.70	0.027490395	NA	NA
886	SV2C	C	NA	NA	NA	NA	-1.69	7.65E-55	NA	NA
887	DGKK	C	NA	NA	NA	NA	-1.69	4.28E-07	NA	NA
888	LILRB5	C	NA	NA	NA	NA	-1.69	0.037940702	NA	NA
889	APBA1	C	NA	NA	NA	NA	-1.68	7.07E-05	NA	NA
890	AC111006.1	C	NA	NA	NA	NA	-1.67	0.028308184	NA	NA
891	DGCR9	C	NA	NA	NA	NA	-1.67	7.06E-18	NA	NA
892	RIPOR2	C	NA	NA	NA	NA	-1.66	0.001668669	NA	NA
893	AC091152.2	C	NA	NA	NA	NA	-1.66	6.04E-07	NA	NA
894	ZNF774	C	NA	NA	NA	NA	-1.66	4.07E-05	NA	NA
895	SLC22A25	C	NA	NA	NA	NA	-1.65	0.00220686	NA	NA
896	AC011912.1	C	NA	NA	NA	NA	-1.63	7.17E-09	NA	NA
897	CCDC17	C	NA	NA	NA	NA	-1.63	0.04709407	NA	NA
898	RNY3P15	C	NA	NA	NA	NA	-1.61	0.043686767	NA	NA
899	GOLGA6L7P	C	NA	NA	NA	NA	-1.61	0.001582361	NA	NA
900	AC104794.2	C	NA	NA	NA	NA	-1.61	2.94E-06	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
901	AC018413.1	C	NA	NA	NA	NA	-1.61	0.008501438	NA	NA
902	CHSY1	C	NA	NA	NA	NA	-1.59	1.46E-74	NA	NA
903	STARD13	C	NA	NA	NA	NA	-1.59	1.80E-05	NA	NA
904	CACNA1C	C	NA	NA	NA	NA	-1.58	2.53E-09	NA	NA
905	THUMPD2	C	NA	NA	NA	NA	-1.57	1.02E-12	NA	NA
906	SDC2	C	NA	NA	NA	NA	-1.57	1.03E-155	NA	NA
907	AFF3	C	NA	NA	NA	NA	-1.57	6.31E-16	NA	NA
908	GPM6B	C	NA	NA	NA	NA	-1.57	1.98E-36	NA	NA
909	LINC02367	C	NA	NA	NA	NA	-1.55	6.95E-06	NA	NA
910	FIGN	C	NA	NA	NA	NA	-1.54	3.06E-42	NA	NA
911	AP000553.1	C	NA	NA	NA	NA	-1.53	0.010096695	NA	NA
912	AC103740.1	C	NA	NA	NA	NA	-1.52	0.00747748	NA	NA
913	LINC01224	C	NA	NA	NA	NA	-1.52	1.90E-55	NA	NA
914	KIF5C	C	NA	NA	NA	NA	-1.52	1.20E-26	NA	NA
915	LGI3	C	NA	NA	NA	NA	-1.51	0.011442305	NA	NA
916	PXYLP1	C	NA	NA	NA	NA	-1.51	3.44E-60	NA	NA
917	FOXD4L1	C	NA	NA	NA	NA	-1.50	0.015386304	NA	NA
918	AC141557.2	C	NA	NA	NA	NA	-1.49	0.023532222	NA	NA
919	IL21R	C	NA	NA	NA	NA	-1.48	0.039233192	NA	NA
920	PITPNM2	C	NA	NA	NA	NA	-1.46	5.96E-08	NA	NA
921	PLEKHF1	C	NA	NA	NA	NA	-1.46	0.021742194	NA	NA
922	AC104534.1	C	NA	NA	NA	NA	-1.44	0.044844177	NA	NA
923	LINC02249	C	NA	NA	NA	NA	-1.43	5.87E-10	NA	NA
924	KRT80	C	NA	NA	NA	NA	-1.43	5.25E-18	NA	NA
925	AL133461.1	C	NA	NA	NA	NA	-1.43	1.25E-13	NA	NA
926	GOLGA8IP	C	NA	NA	NA	NA	-1.41	1.03E-06	NA	NA
927	HIST1H2BJ	C	NA	NA	NA	NA	-1.41	8.37E-08	NA	NA
928	HPCAL4	C	NA	NA	NA	NA	-1.39	1.91E-78	NA	NA
929	TRIB3	C	NA	NA	NA	NA	-1.39	1.64E-50	NA	NA
930	HEG1	C	NA	NA	NA	NA	-1.38	5.40E-09	NA	NA
931	MTUS1	C	NA	NA	NA	NA	-1.38	2.33E-10	NA	NA
932	ARHGAP23	C	NA	NA	NA	NA	-1.37	1.03E-37	NA	NA
933	GALNT16	C	NA	NA	NA	NA	-1.37	6.48E-09	NA	NA
934	ELF4	C	NA	NA	NA	NA	-1.37	6.22E-15	NA	NA
935	ELAVL2	C	NA	NA	NA	NA	-1.36	5.39E-72	NA	NA
936	AC139769.1	C	NA	NA	NA	NA	-1.35	4.25E-21	NA	NA
937	OTUD7A	C	NA	NA	NA	NA	-1.35	9.01E-04	NA	NA
938	AC124312.1	C	NA	NA	NA	NA	-1.35	2.76E-04	NA	NA
939	BCAT1	C	NA	NA	NA	NA	-1.35	1.47E-52	NA	NA
940	SPECC1	C	NA	NA	NA	NA	-1.33	9.15E-04	NA	NA
941	DGCR5	C	NA	NA	NA	NA	-1.31	5.55E-13	NA	NA
942	AL603839.4	C	NA	NA	NA	NA	-1.31	0.026907753	NA	NA
943	TGFA	C	NA	NA	NA	NA	-1.31	0.001710898	NA	NA
944	AL021807.1	C	NA	NA	NA	NA	-1.30	0.009062837	NA	NA
945	AP000688.2	C	NA	NA	NA	NA	-1.30	0.02980915	NA	NA
946	C2orf48	C	NA	NA	NA	NA	-1.29	1.83E-06	NA	NA
947	KNL1	C	NA	NA	NA	NA	-1.29	8.64E-11	NA	NA
948	SPRY2	C	NA	NA	NA	NA	-1.28	2.15E-94	NA	NA
949	FAM169A	C	NA	NA	NA	NA	-1.28	5.16E-20	NA	NA
950	CERK	C	NA	NA	NA	NA	-1.28	4.86E-47	NA	NA
951	AC013468.1	C	NA	NA	NA	NA	-1.27	0.029752495	NA	NA
952	INPP5F	C	NA	NA	NA	NA	-1.27	5.71E-39	NA	NA
953	PCK2	C	NA	NA	NA	NA	-1.26	8.88E-23	NA	NA
954	ANKH	C	NA	NA	NA	NA	-1.26	1.04E-18	NA	NA
955	PMAIP1	C	NA	NA	NA	NA	-1.26	1.95E-45	NA	NA
956	CLU	C	NA	NA	NA	NA	-1.25	1.04E-11	NA	NA
957	CRYBA2	C	NA	NA	NA	NA	-1.25	0.002477618	NA	NA
958	ACSL6	C	NA	NA	NA	NA	-1.25	3.09E-05	NA	NA
959	RNF144A	C	NA	NA	NA	NA	-1.25	2.96E-158	NA	NA
960	AC068338.3	C	NA	NA	NA	NA	-1.24	0.020827884	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
961	RAD51	C	NA	NA	NA	NA	-1.24	2.51E-55	NA	NA
962	RAB39B	C	NA	NA	NA	NA	-1.24	1.95E-53	NA	NA
963	SLIT3	C	NA	NA	NA	NA	-1.24	1.04E-40	NA	NA
964	ENPP6	C	NA	NA	NA	NA	-1.23	0.041458047	NA	NA
965	IQGAP1	C	NA	NA	NA	NA	-1.23	1.62E-26	NA	NA
966	PRKXP1	C	NA	NA	NA	NA	-1.23	0.005838229	NA	NA
967	PCLAF	C	NA	NA	NA	NA	-1.23	5.18E-16	NA	NA
968	MVK	C	NA	NA	NA	NA	-1.23	7.86E-16	NA	NA
969	GDPD5	C	NA	NA	NA	NA	-1.22	0.023989773	NA	NA
970	LINC01529	C	NA	NA	NA	NA	-1.22	9.53E-05	NA	NA
971	SIPA1L2	C	NA	NA	NA	NA	-1.21	4.29E-20	NA	NA
972	SNX10	C	NA	NA	NA	NA	-1.21	3.52E-14	NA	NA
973	PPP1R1B	C	NA	NA	NA	NA	-1.21	0.025219678	NA	NA
974	ATF4	C	NA	NA	NA	NA	-1.21	7.58E-125	NA	NA
975	LINC01198	C	NA	NA	NA	NA	-1.20	0.042748141	NA	NA
976	RASD2	C	NA	NA	NA	NA	-1.20	0.035393984	NA	NA
977	PPM1E	C	NA	NA	NA	NA	-1.19	3.21E-54	NA	NA
978	CYP1B1	C	NA	NA	NA	NA	-1.19	3.05E-07	NA	NA
979	AC108025.2	C	NA	NA	NA	NA	-1.19	6.43E-05	NA	NA
980	DOCK11	C	NA	NA	NA	NA	-1.19	8.66E-17	NA	NA
981	B4GALNT1	C	NA	NA	NA	NA	-1.19	9.43E-74	NA	NA
982	IGHV1-46	C	NA	NA	NA	NA	-1.19	0.036402811	NA	NA
983	WDR76	C	NA	NA	NA	NA	-1.18	3.24E-34	NA	NA
984	AC007240.1	C	NA	NA	NA	NA	-1.17	2.24E-08	NA	NA
985	TBC1D2B	C	NA	NA	NA	NA	-1.17	8.70E-22	NA	NA
986	SHF	C	NA	NA	NA	NA	-1.17	2.36E-22	NA	NA
987	SOWAHA	C	NA	NA	NA	NA	-1.17	3.35E-07	NA	NA
988	CARS	C	NA	NA	NA	NA	-1.17	0.002268295	NA	NA
989	HECW2	C	NA	NA	NA	NA	-1.16	6.90E-09	NA	NA
990	UBASH3B	C	NA	NA	NA	NA	-1.16	0.004715636	NA	NA
991	CYP27C1	C	NA	NA	NA	NA	-1.16	1.13E-12	NA	NA
992	FLT4	C	NA	NA	NA	NA	-1.16	0.002110099	NA	NA
993	AC091305.2	C	NA	NA	NA	NA	-1.16	2.37E-06	NA	NA
994	AC012181.2	C	NA	NA	NA	NA	-1.16	0.021134444	NA	NA
995	UHRF1BP1	C	NA	NA	NA	NA	-1.15	8.61E-68	NA	NA
996	AC095055.1	C	NA	NA	NA	NA	-1.15	0.026851294	NA	NA
997	DCUN1D2	C	NA	NA	NA	NA	-1.15	2.45E-18	NA	NA
998	SCRN1	C	NA	NA	NA	NA	-1.15	9.32E-90	NA	NA
999	SLC16A14	C	NA	NA	NA	NA	-1.14	1.29E-26	NA	NA
1000	RRM2	C	NA	NA	NA	NA	-1.14	2.83E-40	NA	NA
1001	ABLIM1	C	NA	NA	NA	NA	-1.14	9.82E-42	NA	NA
1002	AC091057.7	C	NA	NA	NA	NA	-1.14	7.71E-04	NA	NA
1003	AL499616.1	C	NA	NA	NA	NA	-1.14	8.37E-11	NA	NA
1004	NEURL1	C	NA	NA	NA	NA	-1.14	6.34E-04	NA	NA
1005	AC087632.1	C	NA	NA	NA	NA	-1.14	7.04E-27	NA	NA
1006	AC104073.4	C	NA	NA	NA	NA	-1.14	4.31E-05	NA	NA
1007	AC091057.3	C	NA	NA	NA	NA	-1.13	0.006800634	NA	NA
1008	PAQR8	C	NA	NA	NA	NA	-1.13	1.70E-52	NA	NA
1009	SHMT2	C	NA	NA	NA	NA	-1.13	2.12E-26	NA	NA
1010	FSD1L	C	NA	NA	NA	NA	-1.13	4.45E-14	NA	NA
1011	FAM201A	C	NA	NA	NA	NA	-1.13	0.031156688	NA	NA
1012	CYP4F35P	C	NA	NA	NA	NA	-1.11	0.00425407	NA	NA
1013	OSBPL6	C	NA	NA	NA	NA	-1.11	5.90E-13	NA	NA
1014	43711	C	NA	NA	NA	NA	-1.11	3.65E-55	NA	NA
1015	SNURF	C	NA	NA	NA	NA	-1.11	4.86E-29	NA	NA
1016	RBMS3-AS2	C	NA	NA	NA	NA	-1.11	0.001126943	NA	NA
1017	MYO5A	C	NA	NA	NA	NA	-1.10	1.16E-27	NA	NA
1018	CDNF	C	NA	NA	NA	NA	-1.10	0.048102116	NA	NA
1019	SARS	C	NA	NA	NA	NA	-1.10	1.45E-85	NA	NA
1020	AL031985.3	C	NA	NA	NA	NA	-1.10	1.66E-29	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1021	SH3KBP1	C	NA	NA	NA	NA	-1.10	5.09E-16	NA	NA
1022	KIAA1522	C	NA	NA	NA	NA	-1.10	4.16E-16	NA	NA
1023	AP003072.2	C	NA	NA	NA	NA	-1.10	0.034730565	NA	NA
1024	ZNF609	C	NA	NA	NA	NA	-1.10	1.41E-38	NA	NA
1025	SEPT5-GP1BB	C	NA	NA	NA	NA	-1.09	0.008364198	NA	NA
1026	CEP170B	C	NA	NA	NA	NA	-1.09	8.85E-05	NA	NA
1027	SMCO4	C	NA	NA	NA	NA	-1.09	7.32E-06	NA	NA
1028	ADCY9	C	NA	NA	NA	NA	-1.09	1.22E-06	NA	NA
1029	CHKB-AS1	C	NA	NA	NA	NA	-1.08	0.002293854	NA	NA
1030	C1orf226	C	NA	NA	NA	NA	-1.08	1.62E-14	NA	NA
1031	NIPA1	C	NA	NA	NA	NA	-1.08	9.29E-54	NA	NA
1032	CELSR2	C	NA	NA	NA	NA	-1.08	3.01E-20	NA	NA
1033	ZNF887P	C	NA	NA	NA	NA	-1.08	9.95E-07	NA	NA
1034	CENPO	C	NA	NA	NA	NA	-1.08	6.15E-73	NA	NA
1035	RGS7	C	NA	NA	NA	NA	-1.07	4.96E-14	NA	NA
1036	AC068338.2	C	NA	NA	NA	NA	-1.07	0.031576954	NA	NA
1037	CORO1A	C	NA	NA	NA	NA	-1.07	3.88E-06	NA	NA
1038	AC011503.2	C	NA	NA	NA	NA	-1.07	3.33E-08	NA	NA
1039	FAM92A	C	NA	NA	NA	NA	-1.07	2.08E-24	NA	NA
1040	CAMSAP3	C	NA	NA	NA	NA	-1.07	3.74E-04	NA	NA
1041	PPP2R2B	C	NA	NA	NA	NA	-1.06	4.55E-05	NA	NA
1042	PDZD4	C	NA	NA	NA	NA	-1.06	1.90E-24	NA	NA
1043	GOLGA8S	C	NA	NA	NA	NA	-1.06	0.008270582	NA	NA
1044	TEKT4	C	NA	NA	NA	NA	-1.06	0.012388772	NA	NA
1045	MTMR9LP	C	NA	NA	NA	NA	-1.06	0.006576436	NA	NA
1046	AC005034.5	C	NA	NA	NA	NA	-1.05	0.035257111	NA	NA
1047	IRF5	C	NA	NA	NA	NA	-1.05	0.004072527	NA	NA
1048	JAG2	C	NA	NA	NA	NA	-1.05	0.036687121	NA	NA
1049	AC012618.3	C	NA	NA	NA	NA	-1.05	0.015810651	NA	NA
1050	LY6E	C	NA	NA	NA	NA	-1.04	1.15E-10	NA	NA
1051	AC018804.1	C	NA	NA	NA	NA	-1.04	5.13E-04	NA	NA
1052	SNRPN	C	NA	NA	NA	NA	-1.04	5.95E-83	NA	NA
1053	PSPH	C	NA	NA	NA	NA	-1.04	1.34E-76	NA	NA
1054	APBB2	C	NA	NA	NA	NA	-1.03	1.88E-27	NA	NA
1055	AC023355.1	C	NA	NA	NA	NA	-1.03	0.00213104	NA	NA
1056	RSL24D1	C	NA	NA	NA	NA	-1.03	2.08E-27	NA	NA
1057	SFT2D3	C	NA	NA	NA	NA	-1.03	2.78E-08	NA	NA
1058	MAGEA6	C	NA	NA	NA	NA	-1.02	4.09E-57	NA	NA
1059	GOLGA8A	C	NA	NA	NA	NA	-1.02	3.94E-17	NA	NA
1060	ZFP69B	C	NA	NA	NA	NA	-1.02	7.79E-20	NA	NA
1061	CEBPB-AS1	C	NA	NA	NA	NA	-1.02	6.96E-04	NA	NA
1062	AC098864.1	C	NA	NA	NA	NA	-1.02	8.44E-06	NA	NA
1063	MAP1B	C	NA	NA	NA	NA	-1.01	3.17E-51	NA	NA
1064	MPP3	C	NA	NA	NA	NA	-1.01	1.64E-05	NA	NA
1065	LRRC75A	C	NA	NA	NA	NA	-1.01	5.56E-07	NA	NA
1066	INAFM2	C	NA	NA	NA	NA	-1.01	9.19E-33	NA	NA
1067	DPYSL5	C	NA	NA	NA	NA	-1.01	1.10E-18	NA	NA
1068	RPH3AL	C	NA	NA	NA	NA	-1.01	0.003855026	NA	NA
1069	BCAR3	C	NA	NA	NA	NA	-1.01	7.67E-05	NA	NA
1070	EIF5A2	C	NA	NA	NA	NA	1.00	6.99E-10	NA	NA
1071	STARD4	C	NA	NA	NA	NA	1.00	1.42E-32	NA	NA
1072	YBX2	C	NA	NA	NA	NA	1.00	1.11E-05	NA	NA
1073	PSME1	C	NA	NA	NA	NA	1.00	2.63E-36	NA	NA
1074	GLMP	C	NA	NA	NA	NA	1.01	4.57E-21	NA	NA
1075	DENND2C	C	NA	NA	NA	NA	1.01	0.009544094	NA	NA
1076	IQCG	C	NA	NA	NA	NA	1.01	7.50E-13	NA	NA
1077	TEAD2	C	NA	NA	NA	NA	1.01	1.78E-56	NA	NA
1078	CCDC65	C	NA	NA	NA	NA	1.01	4.66E-04	NA	NA
1079	PRR19	C	NA	NA	NA	NA	1.01	0.013142289	NA	NA
1080	FZD2	C	NA	NA	NA	NA	1.01	6.10E-05	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1081	MITF	C	NA	NA	NA	NA	1.01	4.14E-05	NA	NA
1082	ABCD2	C	NA	NA	NA	NA	1.01	1.83E-23	NA	NA
1083	VMAC	C	NA	NA	NA	NA	1.01	7.94E-07	NA	NA
1084	ASMTL	C	NA	NA	NA	NA	1.02	1.16E-10	NA	NA
1085	GAREM1	C	NA	NA	NA	NA	1.02	3.05E-29	NA	NA
1086	SLC34A3	C	NA	NA	NA	NA	1.02	0.048419249	NA	NA
1087	ZSCAN12P1	C	NA	NA	NA	NA	1.02	3.19E-07	NA	NA
1088	TBX2	C	NA	NA	NA	NA	1.03	2.06E-10	NA	NA
1089	RGL3	C	NA	NA	NA	NA	1.03	0.001693891	NA	NA
1090	C14orf28	C	NA	NA	NA	NA	1.03	0.023808793	NA	NA
1091	NFKB2	C	NA	NA	NA	NA	1.03	8.36E-13	NA	NA
1092	LRRC34	C	NA	NA	NA	NA	1.03	1.17E-12	NA	NA
1093	AC112497.1	C	NA	NA	NA	NA	1.04	0.037340994	NA	NA
1094	SERINC2	C	NA	NA	NA	NA	1.04	5.75E-07	NA	NA
1095	TRADD	C	NA	NA	NA	NA	1.04	3.93E-07	NA	NA
1096	CHMP2B	C	NA	NA	NA	NA	1.04	3.12E-17	NA	NA
1097	CHRNB1	C	NA	NA	NA	NA	1.04	4.25E-25	NA	NA
1098	C1orf228	C	NA	NA	NA	NA	1.04	0.015227721	NA	NA
1099	CASP7	C	NA	NA	NA	NA	1.04	1.30E-26	NA	NA
1100	ZMAT3	C	NA	NA	NA	NA	1.05	6.78E-28	NA	NA
1101	LINC01719	C	NA	NA	NA	NA	1.05	6.19E-16	NA	NA
1102	SOHLH2	C	NA	NA	NA	NA	1.05	3.13E-07	NA	NA
1103	CASR	C	NA	NA	NA	NA	1.05	0.009677616	NA	NA
1104	GSTM4	C	NA	NA	NA	NA	1.05	2.11E-18	NA	NA
1105	APOL2	C	NA	NA	NA	NA	1.06	8.16E-07	NA	NA
1106	LRRN1	C	NA	NA	NA	NA	1.06	7.86E-18	NA	NA
1107	GNG12-AS1	C	NA	NA	NA	NA	1.06	0.03698338	NA	NA
1108	AC092620.2	C	NA	NA	NA	NA	1.06	0.013325804	NA	NA
1109	KATNAL2	C	NA	NA	NA	NA	1.06	4.23E-10	NA	NA
1110	PDE4A	C	NA	NA	NA	NA	1.07	3.61E-05	NA	NA
1111	IL11RA	C	NA	NA	NA	NA	1.07	2.73E-11	NA	NA
1112	WDR31	C	NA	NA	NA	NA	1.07	1.34E-05	NA	NA
1113	USP2-AS1	C	NA	NA	NA	NA	1.07	0.01416661	NA	NA
1114	GNG12	C	NA	NA	NA	NA	1.07	1.47E-46	NA	NA
1115	TSPAN10	C	NA	NA	NA	NA	1.07	3.49E-04	NA	NA
1116	WDR60	C	NA	NA	NA	NA	1.07	3.74E-12	NA	NA
1117	REEP6	C	NA	NA	NA	NA	1.08	1.27E-08	NA	NA
1118	EBF1	C	NA	NA	NA	NA	1.08	2.32E-08	NA	NA
1119	SCPEP1	C	NA	NA	NA	NA	1.08	7.56E-18	NA	NA
1120	STX4	C	NA	NA	NA	NA	1.08	1.26E-18	NA	NA
1121	POLR3GL	C	NA	NA	NA	NA	1.08	1.05E-29	NA	NA
1122	MAB21L1	C	NA	NA	NA	NA	1.08	9.01E-178	NA	NA
1123	LRRC63	C	NA	NA	NA	NA	1.09	0.044742582	NA	NA
1124	PTCH2	C	NA	NA	NA	NA	1.09	0.00137473	NA	NA
1125	AL009176.1	C	NA	NA	NA	NA	1.09	0.006298235	NA	NA
1126	TMEM67	C	NA	NA	NA	NA	1.09	4.48E-08	NA	NA
1127	LDOC1	C	NA	NA	NA	NA	1.09	3.18E-21	NA	NA
1128	IQCE	C	NA	NA	NA	NA	1.09	4.93E-10	NA	NA
1129	ATP10D	C	NA	NA	NA	NA	1.09	6.33E-11	NA	NA
1130	ANKRD7	C	NA	NA	NA	NA	1.10	1.14E-05	NA	NA
1131	OBSCN	C	NA	NA	NA	NA	1.10	1.11E-12	NA	NA
1132	SLC4A2	C	NA	NA	NA	NA	1.10	8.64E-09	NA	NA
1133	SLC2A4	C	NA	NA	NA	NA	1.10	9.22E-06	NA	NA
1134	CCDC89	C	NA	NA	NA	NA	1.10	7.63E-04	NA	NA
1135	HSPA1A	C	NA	NA	NA	NA	1.10	6.98E-11	NA	NA
1136	NBPF26	C	NA	NA	NA	NA	1.11	4.76E-06	NA	NA
1137	AL445649.1	C	NA	NA	NA	NA	1.11	0.005034569	NA	NA
1138	ABHD14B	C	NA	NA	NA	NA	1.11	1.65E-24	NA	NA
1139	RHPN1	C	NA	NA	NA	NA	1.11	0.033718483	NA	NA
1140	MICU3	C	NA	NA	NA	NA	1.11	3.79E-05	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1141	L3HYPDH	C	NA	NA	NA	NA	1.11	5.57E-11	NA	NA
1142	LRRC1	C	NA	NA	NA	NA	1.11	5.59E-05	NA	NA
1143	NEK8	C	NA	NA	NA	NA	1.11	1.09E-07	NA	NA
1144	TRPS1	C	NA	NA	NA	NA	1.11	6.06E-14	NA	NA
1145	BBOF1	C	NA	NA	NA	NA	1.12	0.001860229	NA	NA
1146	PIAS3	C	NA	NA	NA	NA	1.12	6.77E-38	NA	NA
1147	RGS14	C	NA	NA	NA	NA	1.12	1.01E-08	NA	NA
1148	AGTRAP	C	NA	NA	NA	NA	1.12	5.99E-10	NA	NA
1149	AL109976.1	C	NA	NA	NA	NA	1.12	0.003599011	NA	NA
1150	ABCC6	C	NA	NA	NA	NA	1.12	0.002097619	NA	NA
1151	PPIL6	C	NA	NA	NA	NA	1.12	5.38E-07	NA	NA
1152	LRRC27	C	NA	NA	NA	NA	1.12	5.74E-09	NA	NA
1153	C9orf72	C	NA	NA	NA	NA	1.13	1.23E-25	NA	NA
1154	HSD17B14	C	NA	NA	NA	NA	1.13	7.51E-04	NA	NA
1155	C8orf48	C	NA	NA	NA	NA	1.13	9.69E-14	NA	NA
1156	FAR2	C	NA	NA	NA	NA	1.13	3.50E-05	NA	NA
1157	MDFI	C	NA	NA	NA	NA	1.13	7.59E-04	NA	NA
1158	AC016590.1	C	NA	NA	NA	NA	1.14	0.004958609	NA	NA
1159	PPT2-EGFL8	C	NA	NA	NA	NA	1.14	9.72E-04	NA	NA
1160	LHPP	C	NA	NA	NA	NA	1.14	7.98E-04	NA	NA
1161	AC244394.2	C	NA	NA	NA	NA	1.15	0.005210565	NA	NA
1162	CBX3P2	C	NA	NA	NA	NA	1.16	0.004924833	NA	NA
1163	ANKRD20A5P	C	NA	NA	NA	NA	1.16	1.14E-05	NA	NA
1164	UNC93B1	C	NA	NA	NA	NA	1.16	2.74E-06	NA	NA
1165	AC007336.1	C	NA	NA	NA	NA	1.16	0.037542763	NA	NA
1166	DNAJC28	C	NA	NA	NA	NA	1.16	2.66E-06	NA	NA
1167	AL353622.1	C	NA	NA	NA	NA	1.16	1.26E-15	NA	NA
1168	NR1H3	C	NA	NA	NA	NA	1.16	6.19E-06	NA	NA
1169	SERTAD3	C	NA	NA	NA	NA	1.17	1.31E-12	NA	NA
1170	RHBDL1	C	NA	NA	NA	NA	1.17	0.028254249	NA	NA
1171	LINC02151	C	NA	NA	NA	NA	1.17	7.60E-04	NA	NA
1172	GSTK1	C	NA	NA	NA	NA	1.17	1.45E-105	NA	NA
1173	CAPS	C	NA	NA	NA	NA	1.17	3.18E-04	NA	NA
1174	BTN3A2	C	NA	NA	NA	NA	1.17	4.74E-13	NA	NA
1175	AL451064.2	C	NA	NA	NA	NA	1.17	0.029125056	NA	NA
1176	LGR5	C	NA	NA	NA	NA	1.18	2.36E-22	NA	NA
1177	AC063960.2	C	NA	NA	NA	NA	1.18	0.048085009	NA	NA
1178	AC068580.4	C	NA	NA	NA	NA	1.18	0.041958594	NA	NA
1179	REC8	C	NA	NA	NA	NA	1.18	3.80E-19	NA	NA
1180	METTL7A	C	NA	NA	NA	NA	1.19	1.79E-41	NA	NA
1181	RRM2B	C	NA	NA	NA	NA	1.19	1.34E-09	NA	NA
1182	BCL3	C	NA	NA	NA	NA	1.19	0.006362881	NA	NA
1183	MMAA	C	NA	NA	NA	NA	1.19	2.26E-07	NA	NA
1184	FCGRT	C	NA	NA	NA	NA	1.20	1.50E-18	NA	NA
1185	PPP1R3D	C	NA	NA	NA	NA	1.20	4.01E-15	NA	NA
1186	C6orf118	C	NA	NA	NA	NA	1.21	0.004712992	NA	NA
1187	FGF7P3	C	NA	NA	NA	NA	1.21	0.027313707	NA	NA
1188	BTN3A1	C	NA	NA	NA	NA	1.21	4.68E-21	NA	NA
1189	C19orf66	C	NA	NA	NA	NA	1.21	1.19E-04	NA	NA
1190	DFNB59	C	NA	NA	NA	NA	1.21	2.29E-05	NA	NA
1191	SERPING1	C	NA	NA	NA	NA	1.21	1.68E-22	NA	NA
1192	TMEM102	C	NA	NA	NA	NA	1.21	8.30E-09	NA	NA
1193	DOCK6	C	NA	NA	NA	NA	1.22	2.28E-08	NA	NA
1194	AL590652.1	C	NA	NA	NA	NA	1.22	0.035078337	NA	NA
1195	CELSR3	C	NA	NA	NA	NA	1.22	4.21E-10	NA	NA
1196	TAPBP	C	NA	NA	NA	NA	1.22	6.79E-40	NA	NA
1197	SIL1	C	NA	NA	NA	NA	1.23	2.06E-07	NA	NA
1198	BAG3	C	NA	NA	NA	NA	1.23	1.90E-15	NA	NA
1199	AL451123.1	C	NA	NA	NA	NA	1.23	4.45E-04	NA	NA
1200	TGFB1	C	NA	NA	NA	NA	1.24	2.84E-12	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1201	LENG9	C	NA	NA	NA	NA	1.24	0.002962706	NA	NA
1202	ARHGAP44	C	NA	NA	NA	NA	1.24	0.013751829	NA	NA
1203	GM2A	C	NA	NA	NA	NA	1.24	1.10E-43	NA	NA
1204	GPR153	C	NA	NA	NA	NA	1.24	9.64E-19	NA	NA
1205	CTSO	C	NA	NA	NA	NA	1.24	1.60E-10	NA	NA
1206	AL390879.2	C	NA	NA	NA	NA	1.25	0.002629779	NA	NA
1207	NFIA	C	NA	NA	NA	NA	1.25	1.43E-15	NA	NA
1208	GSN	C	NA	NA	NA	NA	1.25	3.68E-21	NA	NA
1209	IFT74	C	NA	NA	NA	NA	1.26	3.96E-25	NA	NA
1210	GAS2	C	NA	NA	NA	NA	1.26	0.03287525	NA	NA
1211	DEPDC7	C	NA	NA	NA	NA	1.26	1.42E-14	NA	NA
1212	CCDC18-AS1	C	NA	NA	NA	NA	1.27	6.35E-14	NA	NA
1213	SAMD15	C	NA	NA	NA	NA	1.27	0.002448321	NA	NA
1214	CASC2	C	NA	NA	NA	NA	1.27	5.55E-08	NA	NA
1215	LRGUK	C	NA	NA	NA	NA	1.27	3.29E-15	NA	NA
1216	ACBD4	C	NA	NA	NA	NA	1.27	3.09E-04	NA	NA
1217	KLHL14	C	NA	NA	NA	NA	1.27	2.39E-20	NA	NA
1218	ALX1	C	NA	NA	NA	NA	1.28	2.19E-21	NA	NA
1219	LINC00667	C	NA	NA	NA	NA	1.28	5.99E-53	NA	NA
1220	RGS20	C	NA	NA	NA	NA	1.29	3.33E-05	NA	NA
1221	FTH1P20	C	NA	NA	NA	NA	1.29	0.013348368	NA	NA
1222	DACH1	C	NA	NA	NA	NA	1.30	8.49E-97	NA	NA
1223	CD37	C	NA	NA	NA	NA	1.30	5.66E-08	NA	NA
1224	MIR34AHG	C	NA	NA	NA	NA	1.30	1.28E-04	NA	NA
1225	FAM117A	C	NA	NA	NA	NA	1.31	1.37E-15	NA	NA
1226	AC116407.3	C	NA	NA	NA	NA	1.31	0.030081415	NA	NA
1227	AP000873.1	C	NA	NA	NA	NA	1.31	1.19E-04	NA	NA
1228	AC012254.3	C	NA	NA	NA	NA	1.31	0.019692282	NA	NA
1229	MIRLET7BHG	C	NA	NA	NA	NA	1.31	9.95E-04	NA	NA
1230	HHIPL1	C	NA	NA	NA	NA	1.32	6.69E-05	NA	NA
1231	AC092164.1	C	NA	NA	NA	NA	1.32	0.021557501	NA	NA
1232	HPSE	C	NA	NA	NA	NA	1.32	0.043432379	NA	NA
1233	PPP2R3A	C	NA	NA	NA	NA	1.33	2.88E-24	NA	NA
1234	CSF1	C	NA	NA	NA	NA	1.33	0.001432218	NA	NA
1235	FSTL1	C	NA	NA	NA	NA	1.33	5.69E-51	NA	NA
1236	CCDC113	C	NA	NA	NA	NA	1.33	1.16E-07	NA	NA
1237	AC025279.1	C	NA	NA	NA	NA	1.33	0.04964615	NA	NA
1238	CEP97	C	NA	NA	NA	NA	1.34	0.018215781	NA	NA
1239	AF129075.1	C	NA	NA	NA	NA	1.34	0.04709407	NA	NA
1240	TSPAN19	C	NA	NA	NA	NA	1.34	0.00581276	NA	NA
1241	LMLN	C	NA	NA	NA	NA	1.36	2.60E-06	NA	NA
1242	WBP2NL	C	NA	NA	NA	NA	1.36	0.045668202	NA	NA
1243	SLC16A4	C	NA	NA	NA	NA	1.36	3.75E-05	NA	NA
1244	PCDH18	C	NA	NA	NA	NA	1.36	2.01E-45	NA	NA
1245	DIABLO	C	NA	NA	NA	NA	1.37	2.31E-05	NA	NA
1246	PPIC	C	NA	NA	NA	NA	1.37	1.79E-28	NA	NA
1247	DDR1	C	NA	NA	NA	NA	1.37	2.56E-06	NA	NA
1248	DUSP2	C	NA	NA	NA	NA	1.37	9.77E-09	NA	NA
1249	ZCWPW1	C	NA	NA	NA	NA	1.37	4.02E-06	NA	NA
1250	TP53INP1	C	NA	NA	NA	NA	1.37	9.25E-48	NA	NA
1251	SCN4B	C	NA	NA	NA	NA	1.38	1.40E-09	NA	NA
1252	ZBTB7B	C	NA	NA	NA	NA	1.38	0.002337922	NA	NA
1253	DNAH2	C	NA	NA	NA	NA	1.39	0.003985913	NA	NA
1254	PRR29	C	NA	NA	NA	NA	1.39	0.015121924	NA	NA
1255	HOXD13	C	NA	NA	NA	NA	1.39	5.92E-77	NA	NA
1256	TMEM200B	C	NA	NA	NA	NA	1.40	0.008341736	NA	NA
1257	ARSJ	C	NA	NA	NA	NA	1.40	4.59E-08	NA	NA
1258	ARHGAP24	C	NA	NA	NA	NA	1.40	7.17E-04	NA	NA
1259	PLTP	C	NA	NA	NA	NA	1.41	5.49E-29	NA	NA
1260	MDH1B	C	NA	NA	NA	NA	1.42	1.84E-05	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1261	TMEM53	C	NA	NA	NA	NA	1.42	6.82E-08	NA	NA
1262	PLEKHB1	C	NA	NA	NA	NA	1.42	3.83E-05	NA	NA
1263	SPEF2	C	NA	NA	NA	NA	1.43	5.04E-06	NA	NA
1264	AC009950.1	C	NA	NA	NA	NA	1.43	0.002312044	NA	NA
1265	AC011498.4	C	NA	NA	NA	NA	1.43	0.012172605	NA	NA
1266	AC004925.1	C	NA	NA	NA	NA	1.45	0.047446634	NA	NA
1267	CACNB2	C	NA	NA	NA	NA	1.45	5.38E-13	NA	NA
1268	SNRK-AS1	C	NA	NA	NA	NA	1.45	0.001705729	NA	NA
1269	HNRNPA1P49	C	NA	NA	NA	NA	1.47	0.049399209	NA	NA
1270	TMEM52	C	NA	NA	NA	NA	1.49	0.006350452	NA	NA
1271	ABCC6P2	C	NA	NA	NA	NA	1.49	0.00866232	NA	NA
1272	SMC2-AS1	C	NA	NA	NA	NA	1.50	0.015600003	NA	NA
1273	CCDC191	C	NA	NA	NA	NA	1.51	1.07E-05	NA	NA
1274	AL353593.3	C	NA	NA	NA	NA	1.51	0.031599259	NA	NA
1275	BSCL2	C	NA	NA	NA	NA	1.51	2.24E-21	NA	NA
1276	CCDC30	C	NA	NA	NA	NA	1.53	2.60E-07	NA	NA
1277	ZYX	C	NA	NA	NA	NA	1.54	2.36E-23	NA	NA
1278	CCDC96	C	NA	NA	NA	NA	1.55	6.06E-05	NA	NA
1279	AC110597.1	C	NA	NA	NA	NA	1.55	0.003094619	NA	NA
1280	RENBP	C	NA	NA	NA	NA	1.55	2.01E-05	NA	NA
1281	TAP1	C	NA	NA	NA	NA	1.56	0.048914656	NA	NA
1282	HNF4G	C	NA	NA	NA	NA	1.56	0.012898132	NA	NA
1283	PCDH10	C	NA	NA	NA	NA	1.57	1.55E-06	NA	NA
1284	CEP126	C	NA	NA	NA	NA	1.57	1.16E-24	NA	NA
1285	CFAP221	C	NA	NA	NA	NA	1.58	1.60E-04	NA	NA
1286	SCN2B	C	NA	NA	NA	NA	1.59	4.06E-04	NA	NA
1287	AL451085.2	C	NA	NA	NA	NA	1.60	0.032367506	NA	NA
1288	NME5	C	NA	NA	NA	NA	1.60	2.09E-07	NA	NA
1289	MAP3K14-AS1	C	NA	NA	NA	NA	1.61	4.62E-07	NA	NA
1290	CASTOR1	C	NA	NA	NA	NA	1.63	8.22E-07	NA	NA
1291	CD72	C	NA	NA	NA	NA	1.64	0.007776653	NA	NA
1292	B3GNT4	C	NA	NA	NA	NA	1.64	3.07E-06	NA	NA
1293	DHDH	C	NA	NA	NA	NA	1.64	0.005494764	NA	NA
1294	NAPSA	C	NA	NA	NA	NA	1.64	0.048845671	NA	NA
1295	DYNC2H1	C	NA	NA	NA	NA	1.67	9.29E-17	NA	NA
1296	COL9A3	C	NA	NA	NA	NA	1.68	3.98E-04	NA	NA
1297	AJUBA	C	NA	NA	NA	NA	1.69	9.01E-23	NA	NA
1298	AL109659.2	C	NA	NA	NA	NA	1.74	0.015496077	NA	NA
1299	SELENBP1	C	NA	NA	NA	NA	1.74	0.003622438	NA	NA
1300	PLAC9	C	NA	NA	NA	NA	1.76	0.034058361	NA	NA
1301	F10	C	NA	NA	NA	NA	1.76	5.29E-26	NA	NA
1302	MEF2B	C	NA	NA	NA	NA	1.81	8.59E-04	NA	NA
1303	GFI1	C	NA	NA	NA	NA	1.83	0.042577532	NA	NA
1304	AL360181.5	C	NA	NA	NA	NA	1.84	1.32E-05	NA	NA
1305	STX17-AS1	C	NA	NA	NA	NA	1.84	0.018374315	NA	NA
1306	ESYT3	C	NA	NA	NA	NA	1.85	0.028665648	NA	NA
1307	PTN	C	NA	NA	NA	NA	1.85	1.26E-129	NA	NA
1308	NEK10	C	NA	NA	NA	NA	1.87	0.001625421	NA	NA
1309	FBXW11P1	C	NA	NA	NA	NA	1.88	0.005769781	NA	NA
1310	AC005034.4	C	NA	NA	NA	NA	1.89	0.04188734	NA	NA
1311	AL390198.1	C	NA	NA	NA	NA	1.91	0.008208342	NA	NA
1312	LINC02202	C	NA	NA	NA	NA	1.94	0.00786019	NA	NA
1313	AL160314.2	C	NA	NA	NA	NA	1.94	0.041236503	NA	NA
1314	SEPT4-AS1	C	NA	NA	NA	NA	1.96	0.016879945	NA	NA
1315	ILDR1	C	NA	NA	NA	NA	1.97	0.015616605	NA	NA
1316	MYOZ1	C	NA	NA	NA	NA	1.97	0.042631013	NA	NA
1317	AC073073.1	C	NA	NA	NA	NA	1.97	0.035073229	NA	NA
1318	AC025259.3	C	NA	NA	NA	NA	1.99	0.017810601	NA	NA
1319	CFAP53	C	NA	NA	NA	NA	1.99	3.92E-06	NA	NA
1320	ANKRD20A1	C	NA	NA	NA	NA	2.00	0.044497015	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1321	SERPINA5	C	NA	NA	NA	NA	2.03	0.019447521	NA	NA
1322	ETV2	C	NA	NA	NA	NA	2.03	0.022405297	NA	NA
1323	AC096677.1	C	NA	NA	NA	NA	2.04	0.034878702	NA	NA
1324	TCEA3	C	NA	NA	NA	NA	2.05	0.024515139	NA	NA
1325	PLEKHA4	C	NA	NA	NA	NA	2.13	9.46E-14	NA	NA
1326	AC007249.2	C	NA	NA	NA	NA	2.13	0.006672437	NA	NA
1327	FAM124B	C	NA	NA	NA	NA	2.14	5.19E-04	NA	NA
1328	NTM	C	NA	NA	NA	NA	2.16	9.07E-08	NA	NA
1329	SPARCL1	C	NA	NA	NA	NA	2.17	0.004186539	NA	NA
1330	PYROXD2	C	NA	NA	NA	NA	2.21	0.009692427	NA	NA
1331	SYNGR4	C	NA	NA	NA	NA	2.25	0.049678879	NA	NA
1332	ZMYND12	C	NA	NA	NA	NA	2.29	0.008697355	NA	NA
1333	PLAC8	C	NA	NA	NA	NA	2.35	0.022199339	NA	NA
1334	WDR78	C	NA	NA	NA	NA	2.35	0.001665549	NA	NA
1335	AC093899.2	C	NA	NA	NA	NA	2.37	0.020072283	NA	NA
1336	AL139023.1	C	NA	NA	NA	NA	2.39	0.023989773	NA	NA
1337	LRRC37A6P	C	NA	NA	NA	NA	2.41	0.00722298	NA	NA
1338	LINC00578	C	NA	NA	NA	NA	2.41	0.037204083	NA	NA
1339	AC091563.1	C	NA	NA	NA	NA	2.41	0.044393954	NA	NA
1340	LRRC17	C	NA	NA	NA	NA	2.43	9.43E-11	NA	NA
1341	GABRR2	C	NA	NA	NA	NA	2.44	0.037116527	NA	NA
1342	AGBL2	C	NA	NA	NA	NA	2.45	0.023033006	NA	NA
1343	AC241585.2	C	NA	NA	NA	NA	2.47	7.03E-04	NA	NA
1344	CALML6	C	NA	NA	NA	NA	2.47	0.00342425	NA	NA
1345	LINC00589	C	NA	NA	NA	NA	2.47	0.009167615	NA	NA
1346	AC233702.7	C	NA	NA	NA	NA	2.48	3.37E-05	NA	NA
1347	TGFB3	C	NA	NA	NA	NA	2.49	4.52E-04	NA	NA
1348	KIF12	C	NA	NA	NA	NA	2.55	0.048084214	NA	NA
1349	AC128688.2	C	NA	NA	NA	NA	2.57	0.007938569	NA	NA
1350	ZPLD1	C	NA	NA	NA	NA	2.61	0.012702121	NA	NA
1351	AL928654.4	C	NA	NA	NA	NA	2.67	0.041461264	NA	NA
1352	SOX9-AS1	C	NA	NA	NA	NA	2.68	0.007305154	NA	NA
1353	CEBPD	C	NA	NA	NA	NA	2.70	0.005290319	NA	NA
1354	ATOH1	C	NA	NA	NA	NA	2.71	0.048374996	NA	NA
1355	AQP4-AS1	C	NA	NA	NA	NA	2.78	0.019835009	NA	NA
1356	FPR1	C	NA	NA	NA	NA	2.88	0.035119381	NA	NA
1357	AC132825.3	C	NA	NA	NA	NA	2.89	3.90E-07	NA	NA
1358	AC092611.2	C	NA	NA	NA	NA	2.90	0.019956935	NA	NA
1359	AP000721.2	C	NA	NA	NA	NA	2.90	0.014502147	NA	NA
1360	PRSS51	C	NA	NA	NA	NA	2.93	0.046827866	NA	NA
1361	AC016909.2	C	NA	NA	NA	NA	2.94	0.02571347	NA	NA
1362	IFI44	C	NA	NA	NA	NA	2.94	0.010564581	NA	NA
1363	CDC42EP2	C	NA	NA	NA	NA	3.03	0.040901666	NA	NA
1364	CDK15	C	NA	NA	NA	NA	3.08	0.015133124	NA	NA
1365	AP006333.1	C	NA	NA	NA	NA	3.25	0.043837503	NA	NA
1366	AC023043.4	C	NA	NA	NA	NA	3.27	0.039576573	NA	NA
1367	CHAD	C	NA	NA	NA	NA	3.29	0.001882877	NA	NA
1368	BX119917.1	C	NA	NA	NA	NA	3.31	0.002758111	NA	NA
1369	PLEK2	C	NA	NA	NA	NA	3.35	0.043686767	NA	NA
1370	AL359258.1	C	NA	NA	NA	NA	3.42	0.017324028	NA	NA
1371	LMOD2	C	NA	NA	NA	NA	3.42	0.035206853	NA	NA
1372	TDRD10	C	NA	NA	NA	NA	3.44	0.041219751	NA	NA
1373	IL17REL	C	NA	NA	NA	NA	3.49	0.022985474	NA	NA
1374	VWA5A	C	NA	NA	NA	NA	3.51	0.029343643	NA	NA
1375	RPS27P29	C	NA	NA	NA	NA	3.51	0.029461113	NA	NA
1376	MIR27B	C	NA	NA	NA	NA	3.59	0.022945273	NA	NA
1377	ZG16B	C	NA	NA	NA	NA	3.63	0.029769376	NA	NA
1378	AC012651.1	C	NA	NA	NA	NA	3.64	0.019544384	NA	NA
1379	IRF4	C	NA	NA	NA	NA	3.67	0.03809174	NA	NA
1380	AC116609.1	C	NA	NA	NA	NA	3.78	0.009767789	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1381	AC093726.2	C	NA	NA	NA	NA	3.96	0.010096695	NA	NA
1382	AGGF1P10	C	NA	NA	NA	NA	4.00	0.010362525	NA	NA
1383	INSL3	C	NA	NA	NA	NA	4.05	0.009012673	NA	NA
1384	ATP13A5	C	NA	NA	NA	NA	4.07	0.047602955	NA	NA
1385	AC239799.2	C	NA	NA	NA	NA	4.09	0.002304665	NA	NA
1386	PCSK9	C	NA	NA	NA	NA	4.10	0.015496077	NA	NA
1387	C1orf167	C	NA	NA	NA	NA	4.11	0.037643274	NA	NA
1388	AC005759.1	C	NA	NA	NA	NA	4.26	0.028725942	NA	NA
1389	LINC01468	C	NA	NA	NA	NA	4.40	0.002915075	NA	NA
1390	SLC5A2	C	NA	NA	NA	NA	4.40	0.01204351	NA	NA
1391	HNRNPKP3	C	NA	NA	NA	NA	4.64	0.002148618	NA	NA
1392	IKZF1	C	NA	NA	NA	NA	4.71	3.71E-04	NA	NA
1393	MROH2B	C	NA	NA	NA	NA	4.90	0.002006117	NA	NA
1394	EEF1AKMT4-ECE2	C	NA	NA	NA	NA	5.11	0.003594199	NA	NA
1395	SLC7A8	C	NA	NA	NA	NA	5.15	0.017735564	NA	NA
1396	OTOG	C	NA	NA	NA	NA	5.31	0.006438686	NA	NA
1397	EXOC1L	C	NA	NA	NA	NA	5.41	4.26E-05	NA	NA
1398	ECE2	C	NA	NA	NA	NA	5.46	1.14E-04	NA	NA
1399	SLC22A8	D	NA	NA	NA	NA	NA	NA	-5.91	7.23E-04
1400	AC100835.2	D	NA	NA	NA	NA	NA	NA	-5.70	1.07E-03
1401	VWA3B	D	NA	NA	NA	NA	NA	NA	-5.55	1.83E-02
1402	AC211486.3	D	NA	NA	NA	NA	NA	NA	-5.55	2.48E-02
1403	KCNK2	D	NA	NA	NA	NA	NA	NA	-5.53	1.92E-03
1404	LRRC55	D	NA	NA	NA	NA	NA	NA	-5.47	4.71E-03
1405	GATA6	D	NA	NA	NA	NA	NA	NA	-5.35	1.24E-03
1406	AC023824.4	D	NA	NA	NA	NA	NA	NA	-5.34	7.36E-05
1407	DOCK2	D	NA	NA	NA	NA	NA	NA	-5.27	9.42E-03
1408	ANO1	D	NA	NA	NA	NA	NA	NA	-5.27	1.56E-02
1409	GOLGA8O	D	NA	NA	NA	NA	NA	NA	-5.25	1.68E-02
1410	CD52	D	NA	NA	NA	NA	NA	NA	-5.19	2.09E-02
1411	A2M	D	NA	NA	NA	NA	NA	NA	-4.94	1.64E-02
1412	AC091951.1	D	NA	NA	NA	NA	NA	NA	-4.91	7.02E-04
1413	AL354751.2	D	NA	NA	NA	NA	NA	NA	-4.90	5.75E-03
1414	AL009172.2	D	NA	NA	NA	NA	NA	NA	-4.77	3.34E-03
1415	LY6H	D	NA	NA	NA	NA	NA	NA	-4.61	1.66E-02
1416	AC073592.3	D	NA	NA	NA	NA	NA	NA	-4.59	2.70E-02
1417	UBE2Q2P6	D	NA	NA	NA	NA	NA	NA	-4.54	5.36E-04
1418	TP53TG3HP	D	NA	NA	NA	NA	NA	NA	-4.50	2.62E-02
1419	AC093879.1	D	NA	NA	NA	NA	NA	NA	-4.44	1.00E-02
1420	AC092447.7	D	NA	NA	NA	NA	NA	NA	-4.37	9.63E-04
1421	AC018558.2	D	NA	NA	NA	NA	NA	NA	-4.23	2.13E-02
1422	MIR3186	D	NA	NA	NA	NA	NA	NA	-4.22	4.40E-02
1423	RIMBP3C	D	NA	NA	NA	NA	NA	NA	-4.19	1.70E-02
1424	BX255925.1	D	NA	NA	NA	NA	NA	NA	-4.18	1.14E-02
1425	EWSAT1	D	NA	NA	NA	NA	NA	NA	-4.16	1.88E-02
1426	AC124947.2	D	NA	NA	NA	NA	NA	NA	-4.15	4.28E-02
1427	PDCD1	D	NA	NA	NA	NA	NA	NA	-4.13	1.39E-02
1428	LINC01797	D	NA	NA	NA	NA	NA	NA	-4.13	2.56E-02
1429	AC018558.3	D	NA	NA	NA	NA	NA	NA	-4.12	2.08E-02
1430	ZNF728	D	NA	NA	NA	NA	NA	NA	-4.10	1.16E-02
1431	AL512625.3	D	NA	NA	NA	NA	NA	NA	-4.10	3.14E-03
1432	AMHR2	D	NA	NA	NA	NA	NA	NA	-4.08	1.05E-02
1433	AC138646.1	D	NA	NA	NA	NA	NA	NA	-4.05	2.07E-04
1434	AL136528.2	D	NA	NA	NA	NA	NA	NA	-4.03	4.85E-02
1435	STK32A-AS1	D	NA	NA	NA	NA	NA	NA	-4.03	7.82E-03
1436	DAO	D	NA	NA	NA	NA	NA	NA	-3.99	4.65E-02
1437	OTOF	D	NA	NA	NA	NA	NA	NA	-3.98	1.17E-02
1438	LINC01971	D	NA	NA	NA	NA	NA	NA	-3.94	5.68E-03
1439	TRIM60P17	D	NA	NA	NA	NA	NA	NA	-3.89	3.06E-02
1440	GOLT1A	D	NA	NA	NA	NA	NA	NA	-3.89	5.18E-03

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1441	AL355990.1	D	NA	NA	NA	NA	NA	NA	-3.89	2.22E-02
1442	AC024598.1	D	NA	NA	NA	NA	NA	NA	-3.88	4.72E-02
1443	ZNF723	D	NA	NA	NA	NA	NA	NA	-3.87	8.69E-03
1444	AF067845.4	D	NA	NA	NA	NA	NA	NA	-3.86	4.98E-02
1445	LINC02520	D	NA	NA	NA	NA	NA	NA	-3.78	2.70E-02
1446	AC026585.1	D	NA	NA	NA	NA	NA	NA	-3.75	4.15E-02
1447	ZBBX	D	NA	NA	NA	NA	NA	NA	-3.73	6.30E-04
1448	LMO2	D	NA	NA	NA	NA	NA	NA	-3.73	1.59E-03
1449	ONECUT1	D	NA	NA	NA	NA	NA	NA	-3.72	3.32E-03
1450	RIT2	D	NA	NA	NA	NA	NA	NA	-3.65	3.46E-02
1451	ANGPTL6	D	NA	NA	NA	NA	NA	NA	-3.62	4.21E-02
1452	AL645608.2	D	NA	NA	NA	NA	NA	NA	-3.62	1.41E-02
1453	WTH3DI	D	NA	NA	NA	NA	NA	NA	-3.59	6.45E-04
1454	AC010931.3	D	NA	NA	NA	NA	NA	NA	-3.59	1.08E-02
1455	C17orf50	D	NA	NA	NA	NA	NA	NA	-3.58	8.63E-03
1456	LHFPL3-AS2	D	NA	NA	NA	NA	NA	NA	-3.55	3.11E-02
1457	LINC00608	D	NA	NA	NA	NA	NA	NA	-3.51	1.24E-02
1458	AC026369.1	D	NA	NA	NA	NA	NA	NA	-3.48	1.56E-02
1459	AC130456.2	D	NA	NA	NA	NA	NA	NA	-3.44	6.47E-03
1460	FO681492.1	D	NA	NA	NA	NA	NA	NA	-3.34	2.07E-02
1461	IQSEC3	D	NA	NA	NA	NA	NA	NA	-3.32	6.65E-05
1462	TTLL13P	D	NA	NA	NA	NA	NA	NA	-3.26	2.64E-02
1463	AC006453.4	D	NA	NA	NA	NA	NA	NA	-3.25	1.32E-02
1464	RASA3-IT1	D	NA	NA	NA	NA	NA	NA	-3.25	4.81E-02
1465	CYP4A11	D	NA	NA	NA	NA	NA	NA	-3.24	3.76E-02
1466	COL4A3	D	NA	NA	NA	NA	NA	NA	-3.22	4.92E-02
1467	SNORD89	D	NA	NA	NA	NA	NA	NA	-3.19	4.91E-02
1468	GOLGA2P3Y	D	NA	NA	NA	NA	NA	NA	-3.17	1.67E-02
1469	AC126177.7	D	NA	NA	NA	NA	NA	NA	-3.17	2.87E-02
1470	MYRFL	D	NA	NA	NA	NA	NA	NA	-3.17	4.09E-02
1471	AL512306.2	D	NA	NA	NA	NA	NA	NA	-3.12	7.63E-03
1472	CTBP2P6	D	NA	NA	NA	NA	NA	NA	-3.04	4.35E-03
1473	C10orf10	D	NA	NA	NA	NA	NA	NA	-3.01	4.21E-02
1474	FES	D	NA	NA	NA	NA	NA	NA	-3.01	2.40E-02
1475	C2orf73	D	NA	NA	NA	NA	NA	NA	-2.98	3.19E-02
1476	AL592435.1	D	NA	NA	NA	NA	NA	NA	-2.94	1.86E-02
1477	AL032819.2	D	NA	NA	NA	NA	NA	NA	-2.92	3.27E-02
1478	ALX4	D	NA	NA	NA	NA	NA	NA	-2.90	4.73E-02
1479	CHRD	D	NA	NA	NA	NA	NA	NA	-2.90	1.00E-02
1480	CLDN5	D	NA	NA	NA	NA	NA	NA	-2.85	1.26E-03
1481	AC098820.3	D	NA	NA	NA	NA	NA	NA	-2.81	2.69E-02
1482	NRXN3	D	NA	NA	NA	NA	NA	NA	-2.81	7.91E-05
1483	AC008761.3	D	NA	NA	NA	NA	NA	NA	-2.80	8.66E-03
1484	COL4A4	D	NA	NA	NA	NA	NA	NA	-2.78	1.49E-03
1485	S100A1	D	NA	NA	NA	NA	NA	NA	-2.76	4.70E-03
1486	CP	D	NA	NA	NA	NA	NA	NA	-2.73	9.62E-03
1487	TTC24	D	NA	NA	NA	NA	NA	NA	-2.73	2.06E-02
1488	NPM2	D	NA	NA	NA	NA	NA	NA	-2.71	1.00E-02
1489	AC008687.4	D	NA	NA	NA	NA	NA	NA	-2.69	3.32E-02
1490	PRC1-AS1	D	NA	NA	NA	NA	NA	NA	-2.68	1.28E-03
1491	WNT10A	D	NA	NA	NA	NA	NA	NA	-2.66	2.63E-02
1492	AC112694.1	D	NA	NA	NA	NA	NA	NA	-2.57	1.82E-02
1493	AL831711.1	D	NA	NA	NA	NA	NA	NA	-2.57	1.84E-02
1494	TLR5	D	NA	NA	NA	NA	NA	NA	-2.56	1.93E-02
1495	CYP11A1	D	NA	NA	NA	NA	NA	NA	-2.56	3.29E-02
1496	AC092378.2	D	NA	NA	NA	NA	NA	NA	-2.56	2.93E-02
1497	CELF6	D	NA	NA	NA	NA	NA	NA	-2.53	2.72E-33
1498	FAM26E	D	NA	NA	NA	NA	NA	NA	-2.53	8.18E-03
1499	AC134682.1	D	NA	NA	NA	NA	NA	NA	-2.51	1.54E-02
1500	BX284632.1	D	NA	NA	NA	NA	NA	NA	-2.46	2.12E-04

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1501	ERICH5	D	NA	NA	NA	NA	NA	NA	-2.45	3.44E-02
1502	LINC00511	D	NA	NA	NA	NA	NA	NA	-2.45	2.72E-03
1503	PADI2	D	NA	NA	NA	NA	NA	NA	-2.44	1.03E-02
1504	EFTUD1P1	D	NA	NA	NA	NA	NA	NA	-2.44	1.09E-02
1505	LINC01132	D	NA	NA	NA	NA	NA	NA	-2.44	2.74E-03
1506	ERAP2	D	NA	NA	NA	NA	NA	NA	-2.43	2.80E-02
1507	AC009955.4	D	NA	NA	NA	NA	NA	NA	-2.41	5.96E-04
1508	CDH3	D	NA	NA	NA	NA	NA	NA	-2.36	1.29E-04
1509	AC016168.1	D	NA	NA	NA	NA	NA	NA	-2.34	2.05E-02
1510	AC013394.1	D	NA	NA	NA	NA	NA	NA	-2.33	2.07E-02
1511	AP000547.2	D	NA	NA	NA	NA	NA	NA	-2.32	2.04E-02
1512	ACSBG1	D	NA	NA	NA	NA	NA	NA	-2.32	5.07E-03
1513	AC097478.1	D	NA	NA	NA	NA	NA	NA	-2.31	3.58E-02
1514	AP001605.1	D	NA	NA	NA	NA	NA	NA	-2.31	4.56E-02
1515	AC009090.1	D	NA	NA	NA	NA	NA	NA	-2.30	4.19E-02
1516	LBX2	D	NA	NA	NA	NA	NA	NA	-2.29	5.85E-05
1517	AC007614.5	D	NA	NA	NA	NA	NA	NA	-2.28	4.27E-02
1518	AC112493.1	D	NA	NA	NA	NA	NA	NA	-2.28	8.50E-04
1519	AC243562.1	D	NA	NA	NA	NA	NA	NA	-2.26	1.25E-05
1520	AL049838.1	D	NA	NA	NA	NA	NA	NA	-2.25	1.46E-02
1521	SELENOV	D	NA	NA	NA	NA	NA	NA	-2.24	2.49E-02
1522	ICE2P2	D	NA	NA	NA	NA	NA	NA	-2.24	3.17E-02
1523	SPOCD1	D	NA	NA	NA	NA	NA	NA	-2.23	3.86E-02
1524	GRK1	D	NA	NA	NA	NA	NA	NA	-2.21	7.55E-03
1525	AC098484.2	D	NA	NA	NA	NA	NA	NA	-2.20	2.35E-02
1526	LOC101929691	D	NA	NA	NA	NA	NA	NA	-2.19	1.58E-05
1527	AC123769.1	D	NA	NA	NA	NA	NA	NA	-2.19	3.26E-02
1528	ATP8B5P	D	NA	NA	NA	NA	NA	NA	-2.18	2.13E-02
1529	TRIM54	D	NA	NA	NA	NA	NA	NA	-2.16	3.35E-02
1530	GPBAR1	D	NA	NA	NA	NA	NA	NA	-2.16	1.22E-02
1531	MSR1	D	NA	NA	NA	NA	NA	NA	-2.15	2.39E-02
1532	LINC01460	D	NA	NA	NA	NA	NA	NA	-2.13	1.05E-02
1533	STPG4	D	NA	NA	NA	NA	NA	NA	-2.12	2.15E-03
1534	CILP	D	NA	NA	NA	NA	NA	NA	-2.09	3.01E-04
1535	CSF3R	D	NA	NA	NA	NA	NA	NA	-2.09	3.01E-02
1536	LOC107984273	D	NA	NA	NA	NA	NA	NA	-2.09	1.81E-02
1537	DUOXA1	D	NA	NA	NA	NA	NA	NA	-2.08	2.83E-02
1538	MYCT1	D	NA	NA	NA	NA	NA	NA	-2.08	3.06E-02
1539	FAM212A	D	NA	NA	NA	NA	NA	NA	-2.07	3.30E-17
1540	TMEM151A	D	NA	NA	NA	NA	NA	NA	-2.06	1.56E-18
1541	AP001122.1	D	NA	NA	NA	NA	NA	NA	-2.06	3.70E-02
1542	AL606760.3	D	NA	NA	NA	NA	NA	NA	-2.04	4.64E-02
1543	AC073346.2	D	NA	NA	NA	NA	NA	NA	-2.03	3.37E-02
1544	AL513165.2	D	NA	NA	NA	NA	NA	NA	-2.03	5.62E-11
1545	CRYBB1	D	NA	NA	NA	NA	NA	NA	-2.03	4.42E-02
1546	PCDHA14	D	NA	NA	NA	NA	NA	NA	-1.99	7.69E-04
1547	NHLRC4	D	NA	NA	NA	NA	NA	NA	-1.98	2.37E-04
1548	LINC02119	D	NA	NA	NA	NA	NA	NA	-1.98	3.70E-02
1549	AC244517.6	D	NA	NA	NA	NA	NA	NA	-1.97	3.17E-02
1550	KIAA1324	D	NA	NA	NA	NA	NA	NA	-1.95	7.81E-05
1551	GPAT2	D	NA	NA	NA	NA	NA	NA	-1.94	1.17E-02
1552	ABLIM2	D	NA	NA	NA	NA	NA	NA	-1.92	2.92E-11
1553	DLX4	D	NA	NA	NA	NA	NA	NA	-1.92	4.32E-03
1554	LINC01798	D	NA	NA	NA	NA	NA	NA	-1.92	4.15E-02
1555	AC068279.1	D	NA	NA	NA	NA	NA	NA	-1.88	1.45E-03
1556	PCDHA6	D	NA	NA	NA	NA	NA	NA	-1.87	3.75E-03
1557	CRTC3-AS1	D	NA	NA	NA	NA	NA	NA	-1.87	1.34E-02
1558	TMEFF2	D	NA	NA	NA	NA	NA	NA	-1.85	1.40E-02
1559	SPINK2	D	NA	NA	NA	NA	NA	NA	-1.84	4.87E-02
1560	NTNG2	D	NA	NA	NA	NA	NA	NA	-1.82	1.65E-04

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1561	RPL4P2	D	NA	NA	NA	NA	NA	NA	-1.82	2.31E-02
1562	MAGEA5	D	NA	NA	NA	NA	NA	NA	-1.82	6.47E-06
1563	GDPGP1	D	NA	NA	NA	NA	NA	NA	-1.81	9.91E-10
1564	PCDHA8	D	NA	NA	NA	NA	NA	NA	-1.81	1.10E-24
1565	AC002472.3	D	NA	NA	NA	NA	NA	NA	-1.81	3.23E-02
1566	PACSIN1	D	NA	NA	NA	NA	NA	NA	-1.80	5.23E-04
1567	HMGN2P3	D	NA	NA	NA	NA	NA	NA	-1.80	4.36E-02
1568	PLD5	D	NA	NA	NA	NA	NA	NA	-1.79	3.05E-08
1569	RN7SL790P	D	NA	NA	NA	NA	NA	NA	-1.79	1.04E-02
1570	FGF17	D	NA	NA	NA	NA	NA	NA	-1.78	1.79E-03
1571	AC016355.1	D	NA	NA	NA	NA	NA	NA	-1.78	3.73E-12
1572	FGF5	D	NA	NA	NA	NA	NA	NA	-1.77	3.81E-03
1573	TMEM198	D	NA	NA	NA	NA	NA	NA	-1.77	1.17E-35
1574	FAM226B	D	NA	NA	NA	NA	NA	NA	-1.77	7.68E-06
1575	AC021752.1	D	NA	NA	NA	NA	NA	NA	-1.76	7.34E-03
1576	AC048382.2	D	NA	NA	NA	NA	NA	NA	-1.76	2.41E-06
1577	ABAT	D	NA	NA	NA	NA	NA	NA	-1.75	1.16E-165
1578	AL135818.1	D	NA	NA	NA	NA	NA	NA	-1.73	3.48E-02
1579	FAM3D-AS1	D	NA	NA	NA	NA	NA	NA	-1.71	3.03E-02
1580	AC026691.1	D	NA	NA	NA	NA	NA	NA	-1.70	3.10E-03
1581	SIGLEC16	D	NA	NA	NA	NA	NA	NA	-1.70	3.75E-02
1582	KCNJ10	D	NA	NA	NA	NA	NA	NA	-1.67	4.31E-02
1583	PSD	D	NA	NA	NA	NA	NA	NA	-1.67	2.22E-16
1584	MAPK8IP2	D	NA	NA	NA	NA	NA	NA	-1.66	1.35E-37
1585	PCP4L1	D	NA	NA	NA	NA	NA	NA	-1.66	4.18E-02
1586	SAXO2	D	NA	NA	NA	NA	NA	NA	-1.65	8.17E-04
1587	CCDC188	D	NA	NA	NA	NA	NA	NA	-1.64	2.09E-08
1588	SEMA6A-AS2	D	NA	NA	NA	NA	NA	NA	-1.63	9.28E-04
1589	LINC01537	D	NA	NA	NA	NA	NA	NA	-1.63	1.48E-04
1590	LINC02019	D	NA	NA	NA	NA	NA	NA	-1.63	1.21E-02
1591	AC005609.3	D	NA	NA	NA	NA	NA	NA	-1.62	6.49E-03
1592	AC127496.1	D	NA	NA	NA	NA	NA	NA	-1.61	7.23E-05
1593	AC022150.3	D	NA	NA	NA	NA	NA	NA	-1.61	1.79E-02
1594	STRC	D	NA	NA	NA	NA	NA	NA	-1.60	1.18E-03
1595	SYT14	D	NA	NA	NA	NA	NA	NA	-1.60	3.57E-44
1596	MYT1	D	NA	NA	NA	NA	NA	NA	-1.60	1.40E-02
1597	ARHGEF7-AS2	D	NA	NA	NA	NA	NA	NA	-1.60	3.70E-02
1598	RTN2	D	NA	NA	NA	NA	NA	NA	-1.60	2.20E-25
1599	MMEL1	D	NA	NA	NA	NA	NA	NA	-1.59	7.92E-03
1600	RPL12P37	D	NA	NA	NA	NA	NA	NA	-1.58	4.81E-02
1601	CR392039.2	D	NA	NA	NA	NA	NA	NA	-1.58	4.77E-03
1602	KCNJ6	D	NA	NA	NA	NA	NA	NA	-1.58	1.63E-03
1603	CEND1	D	NA	NA	NA	NA	NA	NA	-1.58	1.62E-06
1604	TPK1	D	NA	NA	NA	NA	NA	NA	-1.57	1.59E-03
1605	UNC80	D	NA	NA	NA	NA	NA	NA	-1.57	3.77E-59
1606	PLCB1	D	NA	NA	NA	NA	NA	NA	-1.57	1.94E-02
1607	AC067852.6	D	NA	NA	NA	NA	NA	NA	-1.56	3.33E-07
1608	AC009554.2	D	NA	NA	NA	NA	NA	NA	-1.56	4.57E-02
1609	LMOD3	D	NA	NA	NA	NA	NA	NA	-1.54	1.14E-02
1610	AC005150.1	D	NA	NA	NA	NA	NA	NA	-1.54	3.86E-02
1611	MYRF	D	NA	NA	NA	NA	NA	NA	-1.53	4.01E-13
1612	PCDHA5	D	NA	NA	NA	NA	NA	NA	-1.53	1.07E-03
1613	AC087386.1	D	NA	NA	NA	NA	NA	NA	-1.53	5.45E-03
1614	AC092437.1	D	NA	NA	NA	NA	NA	NA	-1.52	6.63E-03
1615	GSE1	D	NA	NA	NA	NA	NA	NA	-1.52	1.38E-56
1616	APC2	D	NA	NA	NA	NA	NA	NA	-1.52	2.84E-18
1617	ABCD1	D	NA	NA	NA	NA	NA	NA	-1.52	3.72E-13
1618	CRACR2B	D	NA	NA	NA	NA	NA	NA	-1.51	3.20E-03
1619	LOXL4	D	NA	NA	NA	NA	NA	NA	-1.51	1.87E-10
1620	TRIM17	D	NA	NA	NA	NA	NA	NA	-1.51	7.45E-16

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1621	AC068831.1	D	NA	NA	NA	NA	NA	NA	-1.51	3.26E-02
1622	MYO7A	D	NA	NA	NA	NA	NA	NA	-1.51	7.85E-04
1623	AC004466.1	D	NA	NA	NA	NA	NA	NA	-1.50	4.60E-02
1624	HDAC5	D	NA	NA	NA	NA	NA	NA	-1.49	3.46E-10
1625	TRAPPC12-AS1	D	NA	NA	NA	NA	NA	NA	-1.49	2.33E-11
1626	AC136944.2	D	NA	NA	NA	NA	NA	NA	-1.48	6.83E-13
1627	Z98257.1	D	NA	NA	NA	NA	NA	NA	-1.47	6.02E-03
1628	FBXL16	D	NA	NA	NA	NA	NA	NA	-1.46	5.01E-09
1629	AL137002.2	D	NA	NA	NA	NA	NA	NA	-1.46	1.57E-03
1630	ID2-AS1	D	NA	NA	NA	NA	NA	NA	-1.45	4.63E-16
1631	ELN	D	NA	NA	NA	NA	NA	NA	-1.45	8.12E-19
1632	ALLC	D	NA	NA	NA	NA	NA	NA	-1.45	3.16E-02
1633	TMCO4	D	NA	NA	NA	NA	NA	NA	-1.45	8.80E-03
1634	CXXC4	D	NA	NA	NA	NA	NA	NA	-1.44	4.11E-21
1635	AC087386.2	D	NA	NA	NA	NA	NA	NA	-1.44	5.17E-03
1636	CCNB3	D	NA	NA	NA	NA	NA	NA	-1.44	3.34E-02
1637	UBA7	D	NA	NA	NA	NA	NA	NA	-1.43	1.01E-06
1638	AC034198.2	D	NA	NA	NA	NA	NA	NA	-1.43	4.87E-02
1639	TTLL9	D	NA	NA	NA	NA	NA	NA	-1.43	4.60E-04
1640	AP005329.2	D	NA	NA	NA	NA	NA	NA	-1.43	1.88E-02
1641	SH2D3C	D	NA	NA	NA	NA	NA	NA	-1.42	4.58E-04
1642	TDRKH-AS1	D	NA	NA	NA	NA	NA	NA	-1.42	7.95E-05
1643	AC009802.1	D	NA	NA	NA	NA	NA	NA	-1.41	3.14E-03
1644	SLC6A1	D	NA	NA	NA	NA	NA	NA	-1.41	6.95E-03
1645	PTP4A3	D	NA	NA	NA	NA	NA	NA	-1.40	3.93E-04
1646	SYP	D	NA	NA	NA	NA	NA	NA	-1.40	3.09E-80
1647	AC048382.1	D	NA	NA	NA	NA	NA	NA	-1.40	3.45E-02
1648	AL137802.3	D	NA	NA	NA	NA	NA	NA	-1.40	1.21E-02
1649	AC092117.2	D	NA	NA	NA	NA	NA	NA	-1.39	1.83E-02
1650	MAPK8IP1	D	NA	NA	NA	NA	NA	NA	-1.39	2.24E-11
1651	MIR193BHG	D	NA	NA	NA	NA	NA	NA	-1.39	1.89E-02
1652	IL11	D	NA	NA	NA	NA	NA	NA	-1.39	4.04E-05
1653	DDX25	D	NA	NA	NA	NA	NA	NA	-1.39	1.43E-08
1654	HCN3	D	NA	NA	NA	NA	NA	NA	-1.38	1.15E-23
1655	LRG1	D	NA	NA	NA	NA	NA	NA	-1.38	3.16E-02
1656	AC090970.3	D	NA	NA	NA	NA	NA	NA	-1.37	4.68E-04
1657	CHRNB2	D	NA	NA	NA	NA	NA	NA	-1.37	1.95E-07
1658	LGR4	D	NA	NA	NA	NA	NA	NA	-1.37	6.97E-13
1659	SYN2	D	NA	NA	NA	NA	NA	NA	-1.36	4.27E-17
1660	STAT4	D	NA	NA	NA	NA	NA	NA	-1.36	1.10E-04
1661	BISPR	D	NA	NA	NA	NA	NA	NA	-1.36	3.12E-02
1662	PPFIBP2	D	NA	NA	NA	NA	NA	NA	-1.36	5.66E-22
1663	AC144831.1	D	NA	NA	NA	NA	NA	NA	-1.36	4.53E-02
1664	ENG	D	NA	NA	NA	NA	NA	NA	-1.36	3.31E-16
1665	HNRNPCP7	D	NA	NA	NA	NA	NA	NA	-1.36	4.51E-07
1666	TMX4	D	NA	NA	NA	NA	NA	NA	-1.35	3.89E-126
1667	DNM1P51	D	NA	NA	NA	NA	NA	NA	-1.34	1.06E-05
1668	MANEA-AS1	D	NA	NA	NA	NA	NA	NA	-1.34	6.09E-03
1669	NOVA2	D	NA	NA	NA	NA	NA	NA	-1.34	9.23E-100
1670	MMP17	D	NA	NA	NA	NA	NA	NA	-1.33	1.30E-02
1671	AC106869.1	D	NA	NA	NA	NA	NA	NA	-1.33	5.35E-10
1672	CYSTM1	D	NA	NA	NA	NA	NA	NA	-1.33	1.13E-13
1673	AC115284.2	D	NA	NA	NA	NA	NA	NA	-1.32	8.14E-05
1674	C1orf220	D	NA	NA	NA	NA	NA	NA	-1.32	8.18E-06
1675	NKILA	D	NA	NA	NA	NA	NA	NA	-1.32	1.96E-10
1676	BSN-AS2	D	NA	NA	NA	NA	NA	NA	-1.32	4.02E-03
1677	CNTFR	D	NA	NA	NA	NA	NA	NA	-1.31	2.46E-12
1678	KLHL32	D	NA	NA	NA	NA	NA	NA	-1.30	2.56E-03
1679	TAC3	D	NA	NA	NA	NA	NA	NA	-1.30	7.99E-04
1680	ADCY5	D	NA	NA	NA	NA	NA	NA	-1.29	6.07E-05

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1681	SYNJ2	D	NA	NA	NA	NA	NA	NA	-1.28	2.11E-10
1682	AL021937.1	D	NA	NA	NA	NA	NA	NA	-1.28	2.61E-02
1683	PTPRR	D	NA	NA	NA	NA	NA	NA	-1.28	2.89E-25
1684	TMEM169	D	NA	NA	NA	NA	NA	NA	-1.28	5.90E-41
1685	AP003068.4	D	NA	NA	NA	NA	NA	NA	-1.27	4.38E-05
1686	MAPK11	D	NA	NA	NA	NA	NA	NA	-1.26	1.86E-15
1687	STXBP5	D	NA	NA	NA	NA	NA	NA	-1.26	5.32E-12
1688	TMEM179	D	NA	NA	NA	NA	NA	NA	-1.26	2.32E-21
1689	USP20	D	NA	NA	NA	NA	NA	NA	-1.26	7.24E-11
1690	MYADML2	D	NA	NA	NA	NA	NA	NA	-1.26	2.87E-02
1691	CHD2	D	NA	NA	NA	NA	NA	NA	-1.26	4.21E-15
1692	AL390726.6	D	NA	NA	NA	NA	NA	NA	-1.25	1.51E-07
1693	FNBP1	D	NA	NA	NA	NA	NA	NA	-1.25	3.51E-26
1694	KCNH2	D	NA	NA	NA	NA	NA	NA	-1.24	3.73E-16
1695	IGHV3-43	D	NA	NA	NA	NA	NA	NA	-1.24	4.16E-02
1696	HIST2H4B	D	NA	NA	NA	NA	NA	NA	-1.24	1.51E-03
1697	CACNG8	D	NA	NA	NA	NA	NA	NA	-1.24	3.22E-37
1698	IGF2BP1	D	NA	NA	NA	NA	NA	NA	-1.24	1.20E-02
1699	FBXO6	D	NA	NA	NA	NA	NA	NA	-1.23	1.37E-04
1700	MAP7	D	NA	NA	NA	NA	NA	NA	-1.23	4.10E-31
1701	NAP1L3	D	NA	NA	NA	NA	NA	NA	-1.22	3.30E-10
1702	AL390729.1	D	NA	NA	NA	NA	NA	NA	-1.22	5.24E-04
1703	PCDHA10	D	NA	NA	NA	NA	NA	NA	-1.22	9.95E-17
1704	WNT3	D	NA	NA	NA	NA	NA	NA	-1.22	1.01E-36
1705	NLGN4X	D	NA	NA	NA	NA	NA	NA	-1.22	3.34E-51
1706	C2orf91	D	NA	NA	NA	NA	NA	NA	-1.22	1.87E-03
1707	NUDT7	D	NA	NA	NA	NA	NA	NA	-1.21	1.25E-02
1708	AC021205.3	D	NA	NA	NA	NA	NA	NA	-1.21	3.44E-02
1709	PAICSP1	D	NA	NA	NA	NA	NA	NA	-1.21	3.63E-02
1710	AC021092.1	D	NA	NA	NA	NA	NA	NA	-1.21	2.30E-12
1711	SYNE1	D	NA	NA	NA	NA	NA	NA	-1.21	6.54E-15
1712	SCUBE2	D	NA	NA	NA	NA	NA	NA	-1.21	2.51E-02
1713	MANEAL	D	NA	NA	NA	NA	NA	NA	-1.20	1.76E-34
1714	LRRC49	D	NA	NA	NA	NA	NA	NA	-1.20	2.41E-26
1715	SEZ6L2	D	NA	NA	NA	NA	NA	NA	-1.20	1.29E-08
1716	KRTCAP3	D	NA	NA	NA	NA	NA	NA	-1.20	1.39E-02
1717	CATSPER2	D	NA	NA	NA	NA	NA	NA	-1.20	1.08E-03
1718	AC087741.1	D	NA	NA	NA	NA	NA	NA	-1.20	2.18E-03
1719	ZNF716	D	NA	NA	NA	NA	NA	NA	-1.20	4.74E-02
1720	SCN3A	D	NA	NA	NA	NA	NA	NA	-1.20	1.85E-17
1721	GAREM2	D	NA	NA	NA	NA	NA	NA	-1.20	4.57E-02
1722	LHFPL4	D	NA	NA	NA	NA	NA	NA	-1.19	7.00E-64
1723	STARD5	D	NA	NA	NA	NA	NA	NA	-1.19	2.91E-04
1724	CR769775.1	D	NA	NA	NA	NA	NA	NA	-1.19	6.12E-05
1725	AC114490.3	D	NA	NA	NA	NA	NA	NA	-1.19	1.09E-02
1726	LINC01287	D	NA	NA	NA	NA	NA	NA	-1.19	3.98E-05
1727	JMJD7-PLA2G4B	D	NA	NA	NA	NA	NA	NA	-1.19	2.00E-04
1728	CACNG5	D	NA	NA	NA	NA	NA	NA	-1.18	3.27E-02
1729	KIF5A	D	NA	NA	NA	NA	NA	NA	-1.17	1.76E-39
1730	AL590235.2	D	NA	NA	NA	NA	NA	NA	-1.17	3.51E-02
1731	FBXL15	D	NA	NA	NA	NA	NA	NA	-1.17	1.97E-09
1732	PARD6A	D	NA	NA	NA	NA	NA	NA	-1.17	1.15E-06
1733	ST8SIA4	D	NA	NA	NA	NA	NA	NA	-1.17	1.03E-12
1734	C22orf24	D	NA	NA	NA	NA	NA	NA	-1.17	4.54E-03
1735	STRIP2	D	NA	NA	NA	NA	NA	NA	-1.17	1.66E-10
1736	CSRNP1	D	NA	NA	NA	NA	NA	NA	-1.16	1.72E-08
1737	PLCL1	D	NA	NA	NA	NA	NA	NA	-1.16	6.06E-11
1738	SLC2A6	D	NA	NA	NA	NA	NA	NA	-1.16	7.12E-11
1739	SPR	D	NA	NA	NA	NA	NA	NA	-1.16	2.07E-39
1740	TIMP3	D	NA	NA	NA	NA	NA	NA	-1.16	3.35E-69

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1741	SLC12A5-AS1	D	NA	NA	NA	NA	NA	NA	-1.15	1.08E-02
1742	GYG2	D	NA	NA	NA	NA	NA	NA	-1.15	1.08E-15
1743	FAM214A	D	NA	NA	NA	NA	NA	NA	-1.15	1.15E-32
1744	AC097532.2	D	NA	NA	NA	NA	NA	NA	-1.15	7.28E-11
1745	AC124312.3	D	NA	NA	NA	NA	NA	NA	-1.15	1.51E-02
1746	TMEM145	D	NA	NA	NA	NA	NA	NA	-1.14	2.86E-17
1747	GLIPR2	D	NA	NA	NA	NA	NA	NA	-1.14	1.23E-35
1748	AL512791.2	D	NA	NA	NA	NA	NA	NA	-1.14	3.48E-03
1749	AC010624.2	D	NA	NA	NA	NA	NA	NA	-1.14	4.47E-03
1750	HESX1	D	NA	NA	NA	NA	NA	NA	-1.13	1.03E-05
1751	RTN4RL2	D	NA	NA	NA	NA	NA	NA	-1.13	4.91E-02
1752	ZNF233	D	NA	NA	NA	NA	NA	NA	-1.13	2.10E-10
1753	AC106820.5	D	NA	NA	NA	NA	NA	NA	-1.13	2.17E-02
1754	LPIN1	D	NA	NA	NA	NA	NA	NA	-1.13	2.35E-08
1755	AC022966.1	D	NA	NA	NA	NA	NA	NA	-1.12	2.35E-02
1756	HYAL1	D	NA	NA	NA	NA	NA	NA	-1.12	2.26E-02
1757	PILRA	D	NA	NA	NA	NA	NA	NA	-1.12	4.74E-03
1758	DLX6	D	NA	NA	NA	NA	NA	NA	-1.12	2.70E-19
1759	RN7SL268P	D	NA	NA	NA	NA	NA	NA	-1.11	1.89E-02
1760	AC069222.1	D	NA	NA	NA	NA	NA	NA	-1.11	4.62E-02
1761	IRX5	D	NA	NA	NA	NA	NA	NA	-1.11	1.13E-11
1762	C2orf16	D	NA	NA	NA	NA	NA	NA	-1.11	1.80E-11
1763	LONRF2	D	NA	NA	NA	NA	NA	NA	-1.11	2.86E-28
1764	AL138976.2	D	NA	NA	NA	NA	NA	NA	-1.11	4.42E-02
1765	YPEL2	D	NA	NA	NA	NA	NA	NA	-1.11	5.85E-09
1766	NUAK2	D	NA	NA	NA	NA	NA	NA	-1.10	1.75E-02
1767	RPLP0P2	D	NA	NA	NA	NA	NA	NA	-1.10	5.78E-03
1768	SRGAP1	D	NA	NA	NA	NA	NA	NA	-1.10	3.73E-04
1769	AC242376.2	D	NA	NA	NA	NA	NA	NA	-1.10	2.22E-16
1770	ARHGEF10L	D	NA	NA	NA	NA	NA	NA	-1.10	1.17E-06
1771	NUP50-AS1	D	NA	NA	NA	NA	NA	NA	-1.10	8.92E-07
1772	KIF21B	D	NA	NA	NA	NA	NA	NA	-1.10	1.48E-27
1773	AP001029.2	D	NA	NA	NA	NA	NA	NA	-1.09	9.95E-03
1774	STRIP1	D	NA	NA	NA	NA	NA	NA	-1.09	1.49E-34
1775	GRAMD4	D	NA	NA	NA	NA	NA	NA	-1.09	1.46E-04
1776	LINC01134	D	NA	NA	NA	NA	NA	NA	-1.09	3.25E-02
1777	CTNNA2	D	NA	NA	NA	NA	NA	NA	-1.09	1.06E-16
1778	AL359881.1	D	NA	NA	NA	NA	NA	NA	-1.09	4.36E-03
1779	AL359881.3	D	NA	NA	NA	NA	NA	NA	-1.08	9.55E-03
1780	LINC01137	D	NA	NA	NA	NA	NA	NA	-1.08	4.91E-02
1781	LSMEM1	D	NA	NA	NA	NA	NA	NA	-1.07	3.75E-03
1782	GLRB	D	NA	NA	NA	NA	NA	NA	-1.07	2.23E-08
1783	LPAR2	D	NA	NA	NA	NA	NA	NA	-1.07	2.45E-06
1784	TLE3	D	NA	NA	NA	NA	NA	NA	-1.07	8.26E-48
1785	AC012645.1	D	NA	NA	NA	NA	NA	NA	-1.07	2.31E-02
1786	CIB2	D	NA	NA	NA	NA	NA	NA	-1.07	6.84E-63
1787	PCBP4	D	NA	NA	NA	NA	NA	NA	-1.06	5.46E-15
1788	FAM57B	D	NA	NA	NA	NA	NA	NA	-1.06	1.66E-10
1789	EPHB2	D	NA	NA	NA	NA	NA	NA	-1.06	9.21E-14
1790	DUSP15	D	NA	NA	NA	NA	NA	NA	-1.06	2.55E-04
1791	PCDHA4	D	NA	NA	NA	NA	NA	NA	-1.06	2.17E-11
1792	AC020661.1	D	NA	NA	NA	NA	NA	NA	-1.06	3.79E-02
1793	SH2B3	D	NA	NA	NA	NA	NA	NA	-1.06	1.13E-37
1794	CPEB3	D	NA	NA	NA	NA	NA	NA	-1.05	3.49E-16
1795	RASA4CP	D	NA	NA	NA	NA	NA	NA	-1.05	1.24E-06
1796	ADAMTS10	D	NA	NA	NA	NA	NA	NA	-1.05	2.23E-05
1797	RASA4	D	NA	NA	NA	NA	NA	NA	-1.05	2.73E-06
1798	CCDC144CP	D	NA	NA	NA	NA	NA	NA	-1.05	1.50E-02
1799	GUCY1A2	D	NA	NA	NA	NA	NA	NA	-1.05	1.74E-23
1800	DNAJC6	D	NA	NA	NA	NA	NA	NA	-1.05	4.15E-43

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1801	AL161729.1	D	NA	NA	NA	NA	NA	NA	-1.05	1.25E-02
1802	AC093642.2	D	NA	NA	NA	NA	NA	NA	-1.05	9.38E-03
1803	INA	D	NA	NA	NA	NA	NA	NA	-1.04	2.00E-63
1804	ATP5F1P5	D	NA	NA	NA	NA	NA	NA	-1.04	4.15E-02
1805	SYT3	D	NA	NA	NA	NA	NA	NA	-1.04	4.32E-03
1806	RUNDC3A-AS1	D	NA	NA	NA	NA	NA	NA	-1.04	1.28E-04
1807	AL731563.3	D	NA	NA	NA	NA	NA	NA	-1.04	1.51E-03
1808	AC105020.1	D	NA	NA	NA	NA	NA	NA	-1.04	1.71E-05
1809	PRORSD1P	D	NA	NA	NA	NA	NA	NA	-1.03	1.58E-02
1810	PRDM5	D	NA	NA	NA	NA	NA	NA	-1.03	5.57E-03
1811	PCDHGC4	D	NA	NA	NA	NA	NA	NA	-1.03	2.25E-34
1812	VSIG2	D	NA	NA	NA	NA	NA	NA	-1.03	3.10E-03
1813	PCGF5	D	NA	NA	NA	NA	NA	NA	-1.03	1.11E-27
1814	LINC00476	D	NA	NA	NA	NA	NA	NA	-1.03	6.73E-04
1815	PCDHA12	D	NA	NA	NA	NA	NA	NA	-1.03	4.16E-10
1816	SOGA3	D	NA	NA	NA	NA	NA	NA	-1.02	8.20E-13
1817	GDPD1	D	NA	NA	NA	NA	NA	NA	-1.02	5.30E-14
1818	ACAP3	D	NA	NA	NA	NA	NA	NA	-1.02	9.19E-04
1819	GABRA3	D	NA	NA	NA	NA	NA	NA	-1.02	5.09E-08
1820	AC012073.1	D	NA	NA	NA	NA	NA	NA	-1.02	4.21E-09
1821	EXTL1	D	NA	NA	NA	NA	NA	NA	-1.02	4.03E-02
1822	SOGA3	D	NA	NA	NA	NA	NA	NA	-1.02	3.97E-17
1823	SYT13	D	NA	NA	NA	NA	NA	NA	-1.02	1.35E-02
1824	ADAM19	D	NA	NA	NA	NA	NA	NA	-1.01	4.86E-24
1825	SLC10A5	D	NA	NA	NA	NA	NA	NA	-1.01	2.83E-02
1826	REEP2	D	NA	NA	NA	NA	NA	NA	-1.01	5.03E-21
1827	PNMA8B	D	NA	NA	NA	NA	NA	NA	-1.01	3.06E-04
1828	GDAP1	D	NA	NA	NA	NA	NA	NA	-1.00	1.73E-11
1829	STAMBPL1	D	NA	NA	NA	NA	NA	NA	-1.00	8.08E-15
1830	AC073349.2	D	NA	NA	NA	NA	NA	NA	-1.00	3.94E-14
1831	C10orf25	D	NA	NA	NA	NA	NA	NA	-1.00	1.64E-03
1832	RBMS3-AS3	D	NA	NA	NA	NA	NA	NA	1.00	3.19E-02
1833	CDCA7L	D	NA	NA	NA	NA	NA	NA	1.00	4.47E-30
1834	COL4A1	D	NA	NA	NA	NA	NA	NA	1.00	1.09E-14
1835	SNTA1	D	NA	NA	NA	NA	NA	NA	1.00	5.25E-11
1836	KLF9	D	NA	NA	NA	NA	NA	NA	1.01	2.17E-27
1837	TMEM109	D	NA	NA	NA	NA	NA	NA	1.01	3.53E-30
1838	PLOD1	D	NA	NA	NA	NA	NA	NA	1.01	8.37E-36
1839	CNNM1	D	NA	NA	NA	NA	NA	NA	1.01	3.59E-54
1840	TMEM99	D	NA	NA	NA	NA	NA	NA	1.01	5.20E-04
1841	HSP90B1	D	NA	NA	NA	NA	NA	NA	1.02	1.24E-27
1842	GRIN3A	D	NA	NA	NA	NA	NA	NA	1.02	3.81E-02
1843	USP46-AS1	D	NA	NA	NA	NA	NA	NA	1.02	4.62E-08
1844	CHPT1	D	NA	NA	NA	NA	NA	NA	1.02	3.02E-16
1845	ISOC2	D	NA	NA	NA	NA	NA	NA	1.02	1.29E-06
1846	GEMIN8	D	NA	NA	NA	NA	NA	NA	1.02	1.88E-35
1847	TMEM231	D	NA	NA	NA	NA	NA	NA	1.02	4.65E-08
1848	ADGRL2	D	NA	NA	NA	NA	NA	NA	1.02	1.95E-28
1849	TXNIP	D	NA	NA	NA	NA	NA	NA	1.03	7.20E-03
1850	NADK2	D	NA	NA	NA	NA	NA	NA	1.03	9.87E-25
1851	AC005544.1	D	NA	NA	NA	NA	NA	NA	1.04	1.86E-02
1852	PMP22	D	NA	NA	NA	NA	NA	NA	1.04	6.29E-26
1853	KLF16	D	NA	NA	NA	NA	NA	NA	1.04	7.54E-12
1854	FOXRED2	D	NA	NA	NA	NA	NA	NA	1.04	1.09E-37
1855	SHQ1	D	NA	NA	NA	NA	NA	NA	1.05	3.79E-04
1856	DERL1	D	NA	NA	NA	NA	NA	NA	1.05	1.70E-31
1857	ATL3	D	NA	NA	NA	NA	NA	NA	1.06	2.32E-50
1858	GPD2	D	NA	NA	NA	NA	NA	NA	1.06	5.39E-21
1859	CCSER1	D	NA	NA	NA	NA	NA	NA	1.07	4.92E-06
1860	TNNT1	D	NA	NA	NA	NA	NA	NA	1.07	2.13E-29

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1861	INAVA	D	NA	NA	NA	NA	NA	NA	1.07	3.09E-06
1862	AC012146.1	D	NA	NA	NA	NA	NA	NA	1.07	2.38E-13
1863	EPOP	D	NA	NA	NA	NA	NA	NA	1.07	1.99E-69
1864	PAQR7	D	NA	NA	NA	NA	NA	NA	1.07	7.61E-37
1865	CYP7B1	D	NA	NA	NA	NA	NA	NA	1.08	2.75E-15
1866	AC009054.2	D	NA	NA	NA	NA	NA	NA	1.08	4.08E-02
1867	FAIM	D	NA	NA	NA	NA	NA	NA	1.09	1.20E-13
1868	FZD1	D	NA	NA	NA	NA	NA	NA	1.09	6.40E-68
1869	NUDCD1	D	NA	NA	NA	NA	NA	NA	1.09	9.16E-29
1870	PDE3B	D	NA	NA	NA	NA	NA	NA	1.10	9.96E-37
1871	KLF11	D	NA	NA	NA	NA	NA	NA	1.10	2.88E-08
1872	UGDH	D	NA	NA	NA	NA	NA	NA	1.10	1.12E-61
1873	AC008895.1	D	NA	NA	NA	NA	NA	NA	1.10	1.44E-02
1874	HLA-B	D	NA	NA	NA	NA	NA	NA	1.10	4.08E-03
1875	PRELID2	D	NA	NA	NA	NA	NA	NA	1.10	7.25E-21
1876	LRRC3	D	NA	NA	NA	NA	NA	NA	1.11	5.71E-17
1877	MAGEA3	D	NA	NA	NA	NA	NA	NA	1.11	4.55E-84
1878	IRS2	D	NA	NA	NA	NA	NA	NA	1.11	2.54E-102
1879	GPR155	D	NA	NA	NA	NA	NA	NA	1.11	9.34E-07
1880	SGK3	D	NA	NA	NA	NA	NA	NA	1.12	7.59E-05
1881	RNF32	D	NA	NA	NA	NA	NA	NA	1.13	9.23E-07
1882	ADAMTS20	D	NA	NA	NA	NA	NA	NA	1.13	4.92E-10
1883	ESYT2	D	NA	NA	NA	NA	NA	NA	1.13	3.94E-32
1884	FHDC1	D	NA	NA	NA	NA	NA	NA	1.14	1.88E-08
1885	WWC2	D	NA	NA	NA	NA	NA	NA	1.14	1.13E-24
1886	MAGT1	D	NA	NA	NA	NA	NA	NA	1.15	8.06E-48
1887	ADAMTSL3	D	NA	NA	NA	NA	NA	NA	1.15	1.04E-07
1888	PLP2	D	NA	NA	NA	NA	NA	NA	1.16	3.79E-53
1889	XKR9	D	NA	NA	NA	NA	NA	NA	1.16	3.41E-03
1890	CC2D2B	D	NA	NA	NA	NA	NA	NA	1.16	2.96E-02
1891	PAPSS2	D	NA	NA	NA	NA	NA	NA	1.17	2.48E-06
1892	AMPD3	D	NA	NA	NA	NA	NA	NA	1.17	2.74E-04
1893	SNORA33	D	NA	NA	NA	NA	NA	NA	1.19	3.81E-02
1894	AFMID	D	NA	NA	NA	NA	NA	NA	1.19	7.65E-45
1895	SLC25A30	D	NA	NA	NA	NA	NA	NA	1.19	5.01E-12
1896	QKI	D	NA	NA	NA	NA	NA	NA	1.19	2.73E-50
1897	AC092645.1	D	NA	NA	NA	NA	NA	NA	1.20	1.18E-07
1898	TGIF2-C20orf24	D	NA	NA	NA	NA	NA	NA	1.20	1.72E-02
1899	ICMT	D	NA	NA	NA	NA	NA	NA	1.21	7.43E-38
1900	GAS7	D	NA	NA	NA	NA	NA	NA	1.21	1.05E-04
1901	CDON	D	NA	NA	NA	NA	NA	NA	1.22	1.43E-45
1902	DNAAF3	D	NA	NA	NA	NA	NA	NA	1.22	5.09E-07
1903	ALDH6A1	D	NA	NA	NA	NA	NA	NA	1.22	3.59E-33
1904	INHBA	D	NA	NA	NA	NA	NA	NA	1.22	2.66E-23
1905	C17orf67	D	NA	NA	NA	NA	NA	NA	1.23	4.24E-03
1906	HOMER3	D	NA	NA	NA	NA	NA	NA	1.24	4.67E-14
1907	MYLK	D	NA	NA	NA	NA	NA	NA	1.24	4.74E-03
1908	GAS1	D	NA	NA	NA	NA	NA	NA	1.25	3.09E-19
1909	EFNA3	D	NA	NA	NA	NA	NA	NA	1.25	1.07E-12
1910	HERC5	D	NA	NA	NA	NA	NA	NA	1.26	1.23E-29
1911	CDCP1	D	NA	NA	NA	NA	NA	NA	1.26	3.85E-02
1912	RHOBTB1	D	NA	NA	NA	NA	NA	NA	1.31	3.06E-48
1913	SULT1A2	D	NA	NA	NA	NA	NA	NA	1.31	7.51E-04
1914	AC139713.2	D	NA	NA	NA	NA	NA	NA	1.32	4.44E-02
1915	CYB561	D	NA	NA	NA	NA	NA	NA	1.34	1.87E-31
1916	HSPA5	D	NA	NA	NA	NA	NA	NA	1.35	5.59E-85
1917	GPC6	D	NA	NA	NA	NA	NA	NA	1.36	6.34E-38
1918	PRICKLE2	D	NA	NA	NA	NA	NA	NA	1.36	6.47E-03
1919	BMPR1B-AS1	D	NA	NA	NA	NA	NA	NA	1.36	1.52E-04
1920	CKS1BP2	D	NA	NA	NA	NA	NA	NA	1.38	3.65E-03

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1921	DBH-AS1	D	NA	NA	NA	NA	NA	NA	1.39	2.43E-06
1922	GDF7	D	NA	NA	NA	NA	NA	NA	1.39	6.61E-08
1923	NABP1	D	NA	NA	NA	NA	NA	NA	1.40	3.81E-14
1924	ARHGAP29	D	NA	NA	NA	NA	NA	NA	1.40	2.01E-33
1925	SLC1A1	D	NA	NA	NA	NA	NA	NA	1.43	1.36E-22
1926	SOX13	D	NA	NA	NA	NA	NA	NA	1.44	8.69E-12
1927	TAS2R4	D	NA	NA	NA	NA	NA	NA	1.44	2.93E-02
1928	PRR5L	D	NA	NA	NA	NA	NA	NA	1.45	1.10E-02
1929	CD109	D	NA	NA	NA	NA	NA	NA	1.46	4.75E-02
1930	SRPX	D	NA	NA	NA	NA	NA	NA	1.46	5.18E-07
1931	SAMD4A	D	NA	NA	NA	NA	NA	NA	1.46	4.62E-17
1932	ADAMTS19	D	NA	NA	NA	NA	NA	NA	1.46	2.25E-58
1933	PAQR5	D	NA	NA	NA	NA	NA	NA	1.47	5.75E-07
1934	C16orf46	D	NA	NA	NA	NA	NA	NA	1.49	4.08E-03
1935	ELMO1	D	NA	NA	NA	NA	NA	NA	1.50	6.40E-71
1936	AC073896.3	D	NA	NA	NA	NA	NA	NA	1.51	2.07E-02
1937	AL035078.4	D	NA	NA	NA	NA	NA	NA	1.52	2.11E-37
1938	DUSP5P1	D	NA	NA	NA	NA	NA	NA	1.54	7.46E-15
1939	PRKCD	D	NA	NA	NA	NA	NA	NA	1.54	4.30E-18
1940	EYA4	D	NA	NA	NA	NA	NA	NA	1.57	1.51E-19
1941	CD38	D	NA	NA	NA	NA	NA	NA	1.57	2.50E-02
1942	RNF152	D	NA	NA	NA	NA	NA	NA	1.57	5.52E-63
1943	DMD	D	NA	NA	NA	NA	NA	NA	1.58	3.79E-07
1944	AC136632.1	D	NA	NA	NA	NA	NA	NA	1.58	3.94E-02
1945	HK2	D	NA	NA	NA	NA	NA	NA	1.59	6.88E-54
1946	BORCS8-MEF2B	D	NA	NA	NA	NA	NA	NA	1.60	4.67E-04
1947	SLCO1A2	D	NA	NA	NA	NA	NA	NA	1.61	5.29E-05
1948	FTH1P2	D	NA	NA	NA	NA	NA	NA	1.61	2.01E-02
1949	RYR1	D	NA	NA	NA	NA	NA	NA	1.62	5.58E-03
1950	FAM47E-STBD1	D	NA	NA	NA	NA	NA	NA	1.63	1.02E-21
1951	LINC01489	D	NA	NA	NA	NA	NA	NA	1.66	2.37E-03
1952	AL590399.1	D	NA	NA	NA	NA	NA	NA	1.67	2.48E-02
1953	TMLHE-AS1	D	NA	NA	NA	NA	NA	NA	1.71	7.39E-04
1954	AC009299.1	D	NA	NA	NA	NA	NA	NA	1.72	1.51E-03
1955	RSPH9	D	NA	NA	NA	NA	NA	NA	1.73	5.45E-08
1956	AC005534.1	D	NA	NA	NA	NA	NA	NA	1.76	4.15E-03
1957	ABHD12B	D	NA	NA	NA	NA	NA	NA	1.76	4.87E-03
1958	ADORA2B	D	NA	NA	NA	NA	NA	NA	1.76	8.93E-25
1959	AMOT	D	NA	NA	NA	NA	NA	NA	1.76	1.16E-35
1960	FAM19A3	D	NA	NA	NA	NA	NA	NA	1.76	4.20E-02
1961	LAMP3	D	NA	NA	NA	NA	NA	NA	1.77	7.20E-06
1962	HNRNPA1P7	D	NA	NA	NA	NA	NA	NA	1.78	3.03E-02
1963	ROCK1P1	D	NA	NA	NA	NA	NA	NA	1.79	4.32E-03
1964	C11orf96	D	NA	NA	NA	NA	NA	NA	1.79	6.35E-53
1965	CDH15	D	NA	NA	NA	NA	NA	NA	1.83	8.84E-04
1966	IMPA2	D	NA	NA	NA	NA	NA	NA	1.84	3.55E-03
1967	TUBB8	D	NA	NA	NA	NA	NA	NA	1.86	2.90E-02
1968	AL049612.1	D	NA	NA	NA	NA	NA	NA	1.88	4.87E-02
1969	AC116667.2	D	NA	NA	NA	NA	NA	NA	1.92	1.82E-02
1970	PDIA2	D	NA	NA	NA	NA	NA	NA	1.93	7.83E-09
1971	RNA5SP18	D	NA	NA	NA	NA	NA	NA	2.06	1.06E-03
1972	ADCY2	D	NA	NA	NA	NA	NA	NA	2.07	2.90E-03
1973	5S_rRNA	D	NA	NA	NA	NA	NA	NA	2.09	1.62E-02
1974	NPM1P27	D	NA	NA	NA	NA	NA	NA	2.09	8.42E-04
1975	C1QL3	D	NA	NA	NA	NA	NA	NA	2.10	2.17E-02
1976	AC087521.1	D	NA	NA	NA	NA	NA	NA	2.13	7.06E-23
1977	FZD8	D	NA	NA	NA	NA	NA	NA	2.15	3.40E-09
1978	GUSBP5	D	NA	NA	NA	NA	NA	NA	2.16	2.88E-04
1979	CFI	D	NA	NA	NA	NA	NA	NA	2.18	2.44E-02
1980	DMC1	D	NA	NA	NA	NA	NA	NA	2.27	4.61E-03

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
1981	SDSL	D	NA	NA	NA	NA	NA	NA	2.28	6.58E-03
1982	AC025271.4	D	NA	NA	NA	NA	NA	NA	2.31	2.86E-02
1983	AC104248.1	D	NA	NA	NA	NA	NA	NA	2.32	1.32E-03
1984	CYSLTR2	D	NA	NA	NA	NA	NA	NA	2.34	4.77E-02
1985	LINC00886	D	NA	NA	NA	NA	NA	NA	2.35	7.74E-03
1986	HSP90B3P	D	NA	NA	NA	NA	NA	NA	2.37	3.13E-02
1987	SLCO5A1	D	NA	NA	NA	NA	NA	NA	2.50	1.19E-02
1988	SLC17A9	D	NA	NA	NA	NA	NA	NA	2.63	1.33E-02
1989	CASP4	D	NA	NA	NA	NA	NA	NA	2.65	3.64E-02
1990	RPL10P7	D	NA	NA	NA	NA	NA	NA	2.69	4.82E-03
1991	AC127071.1	D	NA	NA	NA	NA	NA	NA	2.72	6.61E-03
1992	AL117192.1	D	NA	NA	NA	NA	NA	NA	2.73	3.64E-02
1993	RBP4	D	NA	NA	NA	NA	NA	NA	2.80	1.22E-02
1994	HHIPL2	D	NA	NA	NA	NA	NA	NA	2.92	2.67E-02
1995	AC009093.1	D	NA	NA	NA	NA	NA	NA	3.07	1.87E-02
1996	JAKMIP2-AS1	D	NA	NA	NA	NA	NA	NA	3.34	3.45E-02
1997	AC124784.1	D	NA	NA	NA	NA	NA	NA	3.37	2.63E-02
1998	AL355493.1	D	NA	NA	NA	NA	NA	NA	3.42	4.56E-02
1999	AL031602.1	D	NA	NA	NA	NA	NA	NA	3.58	2.44E-02
2000	WWC2-AS2	D	NA	NA	NA	NA	NA	NA	3.60	4.95E-02
2001	AL035446.2	D	NA	NA	NA	NA	NA	NA	3.76	2.33E-02
2002	TNFRSF8	D	NA	NA	NA	NA	NA	NA	3.81	2.97E-02
2003	PRDM6	D	NA	NA	NA	NA	NA	NA	3.86	5.91E-04
2004	AC134312.3	D	NA	NA	NA	NA	NA	NA	3.94	1.50E-02
2005	KRT18P6	D	NA	NA	NA	NA	NA	NA	3.94	2.12E-02
2006	ARHGAP31-AS1	D	NA	NA	NA	NA	NA	NA	3.96	1.75E-02
2007	AC009093.10	D	NA	NA	NA	NA	NA	NA	3.96	1.95E-02
2008	EPB41L3	D	NA	NA	NA	NA	NA	NA	4.02	4.36E-02
2009	PDX1	D	NA	NA	NA	NA	NA	NA	4.10	3.63E-03
2010	AC135782.3	D	NA	NA	NA	NA	NA	NA	4.11	1.61E-02
2011	LOC254896	D	NA	NA	NA	NA	NA	NA	4.12	1.38E-02
2012	AC009237.14	D	NA	NA	NA	NA	NA	NA	4.17	2.72E-03
2013	AL163636.2	D	NA	NA	NA	NA	NA	NA	4.19	2.63E-02
2014	TRPM2	D	NA	NA	NA	NA	NA	NA	4.28	1.94E-02
2015	HGD	D	NA	NA	NA	NA	NA	NA	4.29	2.08E-02
2016	AC009052.1	D	NA	NA	NA	NA	NA	NA	4.36	3.26E-02
2017	AC092384.2	D	NA	NA	NA	NA	NA	NA	4.59	3.76E-02
2018	AC019068.1	D	NA	NA	NA	NA	NA	NA	4.99	1.35E-02
2019	LINC02068	D	NA	NA	NA	NA	NA	NA	5.08	9.45E-03
2020	AL359922.1	D	NA	NA	NA	NA	NA	NA	5.09	4.35E-02
2021	ZNF816-ZNF321P	D	NA	NA	NA	NA	NA	NA	5.25	1.41E-02
2022	AL358613.2	E	9.86	5.40E-20	9.06	9.26E-17	NA	NA	NA	NA
2023	LINC00921	E	6.35	3.61E-06	3.39	1.25E-02	NA	NA	NA	NA
2024	REG4	E	5.47	1.91E-05	4.67	3.36E-05	NA	NA	NA	NA
2025	SH3RF2	E	5.38	1.41E-04	4.30	2.20E-03	NA	NA	NA	NA
2026	AL355916.2	E	5.13	3.07E-04	4.81	1.14E-03	NA	NA	NA	NA
2027	ATP6V0A4	E	4.93	4.77E-04	4.40	4.69E-03	NA	NA	NA	NA
2028	HEPACAM2	E	4.86	2.02E-10	3.85	1.54E-06	NA	NA	NA	NA
2029	RARB	E	4.85	0	4.11	0	NA	NA	NA	NA
2030	ELFN1	E	4.75	8.76E-23	7.02	5.17E-18	NA	NA	NA	NA
2031	DAPL1	E	4.69	8.47E-04	4.54	1.48E-03	NA	NA	NA	NA
2032	PPARG	E	4.65	2.60E-113	4.75	5.03E-70	NA	NA	NA	NA
2033	OR7E158P	E	4.52	2.09E-03	3.91	1.62E-02	NA	NA	NA	NA
2034	GCSAML	E	4.40	2.21E-35	4.00	3.81E-29	NA	NA	NA	NA
2035	AL355916.1	E	4.37	3.49E-03	5.13	1.09E-04	NA	NA	NA	NA
2036	ABCA1	E	4.27	7.45E-30	5.31	1.51E-42	NA	NA	NA	NA
2037	GPRC5A	E	4.18	1.88E-29	4.63	1.25E-34	NA	NA	NA	NA
2038	GCHFR	E	4.04	9.31E-03	2.80	2.54E-02	NA	NA	NA	NA
2039	STON1-GTF2A1L	E	3.90	1.90E-05	4.47	4.65E-05	NA	NA	NA	NA
2040	GNG8	E	3.87	2.99E-81	2.39	4.66E-33	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2041	LINC00452	E	3.84	2.93E-06	2.73	1.06E-04	NA	NA	NA	NA
2042	RET	E	3.78	0	3.54	0	NA	NA	NA	NA
2043	AQP5	E	3.69	6.25E-05	2.19	1.58E-03	NA	NA	NA	NA
2044	PRSS56	E	3.65	3.89E-03	4.79	5.07E-04	NA	NA	NA	NA
2045	CNOT7P2	E	3.54	1.74E-02	5.21	1.58E-04	NA	NA	NA	NA
2046	TXK	E	3.41	1.20E-16	4.73	1.00E-24	NA	NA	NA	NA
2047	AL157395.1	E	3.35	2.52E-02	3.58	1.44E-02	NA	NA	NA	NA
2048	MROH2A	E	3.27	7.48E-04	2.67	1.88E-02	NA	NA	NA	NA
2049	RGS13	E	3.27	3.02E-60	3.97	2.15E-78	NA	NA	NA	NA
2050	CNR1	E	3.25	3.35E-48	3.25	3.78E-38	NA	NA	NA	NA
2051	LINC01117	E	3.21	1.30E-05	2.08	1.07E-02	NA	NA	NA	NA
2052	ECEL1	E	3.14	1.35E-56	3.07	3.80E-62	NA	NA	NA	NA
2053	LINC01116	E	3.08	0	3.10	0	NA	NA	NA	NA
2054	LINC01136	E	3.01	1.52E-03	3.10	2.68E-03	NA	NA	NA	NA
2055	AC097634.1	E	2.95	7.32E-08	3.13	2.71E-07	NA	NA	NA	NA
2056	RARA-AS1	E	2.93	6.65E-35	3.46	1.59E-38	NA	NA	NA	NA
2057	CTSB	E	2.91	1.70E-118	1.98	1.24E-54	NA	NA	NA	NA
2058	RAX	E	2.90	6.04E-03	3.27	1.04E-03	NA	NA	NA	NA
2059	SERTM1	E	2.87	1.24E-20	2.82	1.44E-31	NA	NA	NA	NA
2060	ERI1	E	2.83	0	2.21	4.40E-189	NA	NA	NA	NA
2061	HOXD1	E	2.79	6.57E-22	3.55	9.18E-27	NA	NA	NA	NA
2062	AC025154.2	E	2.77	1.12E-03	1.96	4.02E-03	NA	NA	NA	NA
2063	HMGN1P18	E	2.76	3.95E-09	2.39	7.51E-07	NA	NA	NA	NA
2064	LINC00887	E	2.75	3.74E-06	2.11	8.73E-04	NA	NA	NA	NA
2065	AF131216.3	E	2.71	1.05E-08	1.93	3.24E-04	NA	NA	NA	NA
2066	LINC02458	E	2.66	1.37E-04	2.27	6.62E-05	NA	NA	NA	NA
2067	AC022784.7	E	2.64	2.09E-07	1.85	1.25E-03	NA	NA	NA	NA
2068	LEMD1	E	2.62	2.25E-02	5.29	1.24E-04	NA	NA	NA	NA
2069	AL591845.1	E	2.61	6.79E-53	2.99	2.99E-64	NA	NA	NA	NA
2070	MIR210HG	E	2.60	3.37E-25	2.85	2.94E-31	NA	NA	NA	NA
2071	CNN2	E	2.59	1.67E-25	2.79	6.85E-29	NA	NA	NA	NA
2072	LRP1B	E	2.59	1.07E-04	3.15	1.91E-05	NA	NA	NA	NA
2073	PART1	E	2.56	1.09E-21	3.63	2.28E-41	NA	NA	NA	NA
2074	AC068533.3	E	2.53	5.46E-05	1.65	1.91E-02	NA	NA	NA	NA
2075	CMKLR1	E	2.52	1.29E-02	5.03	2.48E-04	NA	NA	NA	NA
2076	GABARAPL1	E	2.49	1.70E-16	1.95	1.23E-10	NA	NA	NA	NA
2077	BDKRB2	E	2.48	2.65E-21	4.19	5.61E-44	NA	NA	NA	NA
2078	AC093503.3	E	2.46	3.27E-50	2.56	1.28E-50	NA	NA	NA	NA
2079	AL034374.2	E	2.40	5.25E-06	1.13	2.74E-02	NA	NA	NA	NA
2080	RGS2	E	2.40	8.43E-114	2.64	7.54E-142	NA	NA	NA	NA
2081	THSD4	E	2.38	1.12E-26	3.31	1.10E-60	NA	NA	NA	NA
2082	LINC00933	E	2.38	1.95E-02	2.43	2.90E-02	NA	NA	NA	NA
2083	BAIAP3	E	2.37	3.12E-03	2.28	6.28E-03	NA	NA	NA	NA
2084	GNG2	E	2.37	0	1.73	4.89E-209	NA	NA	NA	NA
2085	AQP6	E	2.35	3.03E-07	1.05	3.35E-02	NA	NA	NA	NA
2086	HOXD8	E	2.34	1.94E-165	2.35	8.90E-166	NA	NA	NA	NA
2087	BHLHE40-AS1	E	2.34	7.22E-03	2.51	2.25E-03	NA	NA	NA	NA
2088	IGFBP6	E	2.33	2.10E-26	1.09	3.46E-07	NA	NA	NA	NA
2089	AC025887.2	E	2.32	4.58E-15	2.44	2.29E-16	NA	NA	NA	NA
2090	OSBPL10	E	2.32	2.53E-11	1.81	3.29E-07	NA	NA	NA	NA
2091	SH3D21	E	2.31	1.01E-34	2.90	1.50E-51	NA	NA	NA	NA
2092	HOXD9	E	2.24	4.74E-147	1.89	6.42E-98	NA	NA	NA	NA
2093	ADD3-AS1	E	2.24	6.10E-08	2.17	8.73E-08	NA	NA	NA	NA
2094	AL356235.1	E	2.23	3.42E-06	1.67	9.45E-04	NA	NA	NA	NA
2095	CGB7	E	2.17	2.60E-03	1.86	3.78E-02	NA	NA	NA	NA
2096	AC080112.4	E	2.15	7.88E-16	1.70	1.62E-09	NA	NA	NA	NA
2097	HOXD10	E	2.15	6.55E-129	1.32	8.28E-51	NA	NA	NA	NA
2098	P3H2	E	2.15	6.09E-03	2.10	6.74E-03	NA	NA	NA	NA
2099	XYLT1	E	2.14	1.45E-107	1.62	4.38E-65	NA	NA	NA	NA
2100	AC009831.1	E	2.13	1.77E-05	2.84	2.27E-07	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2101	DACT3-AS1	E	2.12	1.36E-06	1.18	8.30E-03	NA	NA	NA	NA
2102	ADAMTS6	E	2.11	1.04E-09	1.52	1.64E-05	NA	NA	NA	NA
2103	CDRT4	E	2.10	2.59E-11	1.64	3.18E-07	NA	NA	NA	NA
2104	TSPEAR	E	2.10	5.31E-55	2.17	1.84E-64	NA	NA	NA	NA
2105	C2orf70	E	2.06	1.77E-02	1.79	4.21E-02	NA	NA	NA	NA
2106	ITGB5	E	2.04	1.46E-224	2.82	0	NA	NA	NA	NA
2107	AC005790.1	E	2.04	3.10E-04	1.73	2.96E-03	NA	NA	NA	NA
2108	TSPEAR-AS2	E	2.03	1.00E-112	2.05	9.58E-121	NA	NA	NA	NA
2109	ADD3	E	2.02	4.61E-91	1.66	5.40E-62	NA	NA	NA	NA
2110	AL590666.3	E	1.99	7.25E-08	1.47	2.37E-04	NA	NA	NA	NA
2111	TSPEAR-AS1	E	1.97	3.81E-28	2.01	4.96E-30	NA	NA	NA	NA
2112	FOXC1	E	1.96	1.05E-139	1.46	1.78E-77	NA	NA	NA	NA
2113	SGK1	E	1.95	9.39E-169	1.24	2.24E-67	NA	NA	NA	NA
2114	AC005746.2	E	1.93	3.62E-07	1.50	1.13E-03	NA	NA	NA	NA
2115	ZBED5-AS1	E	1.93	1.78E-35	1.49	1.52E-21	NA	NA	NA	NA
2116	LINC01978	E	1.92	1.15E-02	1.54	4.59E-02	NA	NA	NA	NA
2117	PBX1	E	1.91	6.00E-126	1.44	1.66E-70	NA	NA	NA	NA
2118	BACH2	E	1.91	1.37E-52	1.64	1.56E-38	NA	NA	NA	NA
2119	AL449106.1	E	1.90	4.87E-02	3.70	1.24E-02	NA	NA	NA	NA
2120	EFEMP2	E	1.90	3.56E-33	1.12	4.70E-12	NA	NA	NA	NA
2121	PI15	E	1.87	4.30E-64	2.85	2.61E-145	NA	NA	NA	NA
2122	STON1	E	1.86	1.22E-26	2.21	4.21E-36	NA	NA	NA	NA
2123	PPP1R3B	E	1.85	1.91E-143	2.66	8.46E-282	NA	NA	NA	NA
2124	CGA	E	1.84	3.88E-02	3.14	5.29E-04	NA	NA	NA	NA
2125	SIGIRR	E	1.84	2.75E-13	1.32	4.28E-07	NA	NA	NA	NA
2126	AC138028.1	E	1.82	1.19E-05	1.90	3.77E-05	NA	NA	NA	NA
2127	RAMP1	E	1.79	5.34E-12	1.88	3.50E-13	NA	NA	NA	NA
2128	AC063976.2	E	1.78	3.54E-02	2.46	1.75E-03	NA	NA	NA	NA
2129	AC026904.3	E	1.77	5.88E-03	1.46	2.45E-02	NA	NA	NA	NA
2130	AC083843.3	E	1.77	1.53E-04	1.52	1.02E-02	NA	NA	NA	NA
2131	AP001065.3	E	1.73	2.34E-07	2.79	2.25E-17	NA	NA	NA	NA
2132	HAGLR	E	1.73	1.35E-67	1.69	2.37E-67	NA	NA	NA	NA
2133	PCAT6	E	1.73	1.35E-09	1.52	2.16E-08	NA	NA	NA	NA
2134	ARHGAP27	E	1.72	1.51E-03	1.97	2.40E-04	NA	NA	NA	NA
2135	RARA	E	1.71	7.53E-44	1.73	3.42E-44	NA	NA	NA	NA
2136	NIPSNAP3B	E	1.71	7.00E-26	1.50	3.44E-22	NA	NA	NA	NA
2137	NEIL2	E	1.71	3.31E-82	1.70	2.07E-87	NA	NA	NA	NA
2138	DUSP6	E	1.70	1.21E-200	1.72	9.33E-213	NA	NA	NA	NA
2139	ATP7A	E	1.70	1.20E-98	1.41	9.75E-70	NA	NA	NA	NA
2140	AL355916.3	E	1.68	3.68E-28	2.21	3.09E-41	NA	NA	NA	NA
2141	EFCAB5	E	1.68	6.33E-03	1.84	5.42E-04	NA	NA	NA	NA
2142	AC138028.2	E	1.67	1.33E-06	1.94	2.27E-08	NA	NA	NA	NA
2143	PCDH20	E	1.66	5.00E-17	1.49	1.09E-13	NA	NA	NA	NA
2144	ALDOC	E	1.66	3.30E-130	1.64	2.90E-131	NA	NA	NA	NA
2145	TPST1	E	1.64	8.62E-70	1.81	1.07E-84	NA	NA	NA	NA
2146	NFKBIZ	E	1.63	9.55E-07	1.26	3.41E-04	NA	NA	NA	NA
2147	RDH10	E	1.63	2.17E-06	1.99	5.71E-09	NA	NA	NA	NA
2148	AP002892.1	E	1.63	3.00E-04	1.14	2.47E-02	NA	NA	NA	NA
2149	AP006284.1	E	1.62	3.16E-04	1.17	1.22E-02	NA	NA	NA	NA
2150	ZBED5	E	1.61	1.10E-69	1.05	3.36E-29	NA	NA	NA	NA
2151	KCTD7	E	1.60	8.50E-43	2.07	2.95E-70	NA	NA	NA	NA
2152	ELMOD1	E	1.59	9.84E-32	2.42	8.69E-70	NA	NA	NA	NA
2153	SAT1	E	1.59	4.94E-13	1.09	1.89E-06	NA	NA	NA	NA
2154	SLCO4C1	E	1.59	3.98E-08	2.34	9.00E-16	NA	NA	NA	NA
2155	RBP1	E	1.59	2.09E-122	1.78	8.23E-150	NA	NA	NA	NA
2156	HOXC4	E	1.58	5.47E-86	1.00	1.08E-32	NA	NA	NA	NA
2157	AC024909.3	E	1.58	1.06E-03	2.18	3.65E-06	NA	NA	NA	NA
2158	LHFPL2	E	1.58	1.59E-102	1.08	1.97E-44	NA	NA	NA	NA
2159	DUSP16	E	1.56	1.57E-34	1.41	2.29E-28	NA	NA	NA	NA
2160	BLK	E	1.55	9.19E-17	1.40	3.88E-12	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2161	DARS-AS1	E	1.54	4.25E-07	1.48	3.77E-07	NA	NA	NA	NA
2162	LTBP1	E	1.54	5.83E-27	1.45	4.60E-24	NA	NA	NA	NA
2163	ASL	E	1.54	2.08E-23	1.95	9.28E-38	NA	NA	NA	NA
2164	MIR99AHG	E	1.52	3.67E-20	1.05	6.17E-10	NA	NA	NA	NA
2165	TLE6	E	1.51	6.54E-11	2.03	1.24E-15	NA	NA	NA	NA
2166	RASL10B	E	1.51	3.82E-48	1.08	2.64E-24	NA	NA	NA	NA
2167	HLX	E	1.49	9.61E-11	1.33	5.68E-08	NA	NA	NA	NA
2168	LINC01219	E	1.49	4.58E-02	1.43	4.99E-02	NA	NA	NA	NA
2169	RFX1	E	1.46	1.54E-02	1.28	4.80E-02	NA	NA	NA	NA
2170	CRB1	E	1.44	5.81E-11	1.07	3.64E-06	NA	NA	NA	NA
2171	AL137129.1	E	1.44	4.43E-47	1.98	1.32E-92	NA	NA	NA	NA
2172	ZNF395	E	1.44	1.51E-12	1.20	6.72E-09	NA	NA	NA	NA
2173	GATA4	E	1.43	1.52E-61	1.50	1.46E-67	NA	NA	NA	NA
2174	VPS13D	E	1.42	7.69E-24	1.37	3.34E-22	NA	NA	NA	NA
2175	AC007448.4	E	1.41	2.72E-05	1.45	2.03E-06	NA	NA	NA	NA
2176	AL162274.1	E	1.41	9.97E-05	1.98	6.04E-09	NA	NA	NA	NA
2177	COPZ2	E	1.40	1.31E-03	1.05	2.54E-02	NA	NA	NA	NA
2178	AP001065.1	E	1.40	4.02E-51	2.02	8.69E-116	NA	NA	NA	NA
2179	AC099494.2	E	1.39	3.54E-02	1.96	6.76E-04	NA	NA	NA	NA
2180	SMAGP	E	1.39	1.99E-09	1.24	2.94E-07	NA	NA	NA	NA
2181	IL10RB	E	1.38	7.57E-34	1.22	2.69E-26	NA	NA	NA	NA
2182	CLMN	E	1.38	3.02E-24	1.88	2.79E-43	NA	NA	NA	NA
2183	SREBF1	E	1.37	1.32E-19	1.29	5.71E-17	NA	NA	NA	NA
2184	ARHGAP28	E	1.37	6.43E-30	1.72	1.39E-45	NA	NA	NA	NA
2185	SNAPC1	E	1.36	4.33E-26	1.97	3.91E-54	NA	NA	NA	NA
2186	TMEM51-AS1	E	1.36	4.86E-34	1.34	5.74E-32	NA	NA	NA	NA
2187	AC241952.1	E	1.36	6.75E-28	1.43	2.84E-24	NA	NA	NA	NA
2188	ITPKA	E	1.36	1.50E-04	1.42	1.61E-04	NA	NA	NA	NA
2189	C17orf97	E	1.36	1.62E-10	1.02	4.88E-07	NA	NA	NA	NA
2190	NES	E	1.34	1.91E-88	1.24	2.51E-75	NA	NA	NA	NA
2191	SLC4A8	E	1.33	2.28E-07	1.57	8.22E-10	NA	NA	NA	NA
2192	SDAD1P1	E	1.32	1.54E-16	1.09	3.28E-12	NA	NA	NA	NA
2193	VASN	E	1.31	2.11E-04	1.03	7.53E-03	NA	NA	NA	NA
2194	SLC18A1	E	1.29	3.06E-144	1.18	1.86E-126	NA	NA	NA	NA
2195	AC022239.4	E	1.29	6.49E-13	1.22	1.31E-10	NA	NA	NA	NA
2196	SPAG4	E	1.27	7.32E-07	1.88	9.20E-15	NA	NA	NA	NA
2197	MYADM	E	1.27	4.22E-14	1.21	7.60E-13	NA	NA	NA	NA
2198	RAB42	E	1.26	6.21E-12	1.04	1.48E-07	NA	NA	NA	NA
2199	FDFT1	E	1.26	6.09E-86	1.49	2.97E-117	NA	NA	NA	NA
2200	AC007326.2	E	1.25	6.48E-04	2.25	2.09E-11	NA	NA	NA	NA
2201	SEPT7-AS1	E	1.23	1.25E-02	1.04	4.24E-02	NA	NA	NA	NA
2202	FUT11	E	1.18	2.79E-102	1.86	6.31E-245	NA	NA	NA	NA
2203	C8orf49	E	1.18	8.12E-09	1.27	1.07E-09	NA	NA	NA	NA
2204	ADORA2A-AS1	E	1.17	4.76E-03	1.87	4.42E-06	NA	NA	NA	NA
2205	HSD3BP5	E	1.16	9.45E-03	1.12	2.37E-02	NA	NA	NA	NA
2206	SMG6	E	1.16	9.05E-15	1.00	4.74E-11	NA	NA	NA	NA
2207	QPRT	E	1.15	2.38E-12	1.18	1.37E-12	NA	NA	NA	NA
2208	ADAMTS9	E	1.15	3.52E-63	2.64	0	NA	NA	NA	NA
2209	ANKZF1	E	1.15	3.17E-39	1.55	2.30E-72	NA	NA	NA	NA
2210	RAI2	E	1.15	5.21E-07	2.47	4.96E-28	NA	NA	NA	NA
2211	KDM3A	E	1.13	7.89E-14	1.07	2.93E-12	NA	NA	NA	NA
2212	BNIP3L	E	1.13	7.45E-48	1.15	3.38E-50	NA	NA	NA	NA
2213	AP001062.1	E	1.13	6.92E-07	1.04	2.66E-06	NA	NA	NA	NA
2214	DONSON	E	1.13	6.24E-30	1.41	8.00E-46	NA	NA	NA	NA
2215	CDKL5	E	1.12	3.44E-18	1.06	1.23E-15	NA	NA	NA	NA
2216	ARMC9	E	1.11	3.05E-08	1.11	4.04E-08	NA	NA	NA	NA
2217	CACNA1G	E	1.09	9.03E-10	1.01	1.59E-07	NA	NA	NA	NA
2218	SHB	E	1.09	1.36E-21	1.60	6.38E-39	NA	NA	NA	NA
2219	TRIOBP	E	1.09	6.53E-22	1.05	2.83E-20	NA	NA	NA	NA
2220	BNIP3	E	1.08	4.69E-108	1.73	4.05E-284	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2221	APOLD1	E	1.08	2.97E-19	1.65	8.26E-49	NA	NA	NA	NA
2222	BNIP3P1	E	1.08	1.43E-02	1.32	7.36E-04	NA	NA	NA	NA
2223	EFNB2	E	1.07	2.66E-106	1.15	1.73E-127	NA	NA	NA	NA
2224	KCNQ2	E	1.06	6.23E-10	1.18	5.20E-12	NA	NA	NA	NA
2225	AC132217.1	E	1.06	9.98E-07	1.16	8.64E-08	NA	NA	NA	NA
2226	ADORA2A	E	1.06	2.43E-18	2.19	2.74E-73	NA	NA	NA	NA
2227	AC113189.2	E	1.06	7.54E-05	1.39	1.33E-08	NA	NA	NA	NA
2228	AC068533.4	E	1.05	5.97E-10	1.49	2.40E-19	NA	NA	NA	NA
2229	HIF1A	E	1.05	4.83E-31	1.38	1.63E-53	NA	NA	NA	NA
2230	FGF11	E	1.05	1.94E-51	1.52	4.28E-122	NA	NA	NA	NA
2231	NID1	E	1.03	1.25E-151	2.09	0	NA	NA	NA	NA
2232	EGLN1	E	1.03	4.14E-54	1.67	1.20E-143	NA	NA	NA	NA
2233	TCEAL2	E	1.01	4.26E-03	1.99	3.25E-09	NA	NA	NA	NA
2234	PLIN5	E	-1.00	1.35E-02	-1.07	5.57E-03	NA	NA	NA	NA
2235	PHYHIP	E	-1.01	1.58E-04	-1.77	2.26E-10	NA	NA	NA	NA
2236	CARD9	E	-1.03	5.89E-07	-1.47	5.35E-13	NA	NA	NA	NA
2237	AC063926.3	E	-1.03	1.74E-03	-1.02	1.05E-03	NA	NA	NA	NA
2238	FGD4	E	-1.03	1.85E-09	-1.05	1.73E-09	NA	NA	NA	NA
2239	FAM86EP	E	-1.05	3.73E-05	-1.11	6.59E-06	NA	NA	NA	NA
2240	SMKR1	E	-1.05	8.77E-05	-1.31	5.39E-08	NA	NA	NA	NA
2241	MC4R	E	-1.06	2.61E-02	-1.17	5.45E-03	NA	NA	NA	NA
2242	ST6GALNAC5	E	-1.07	1.32E-12	-1.15	8.25E-14	NA	NA	NA	NA
2243	CNTN1	E	-1.07	3.09E-25	-1.61	3.50E-56	NA	NA	NA	NA
2244	STON2	E	-1.07	4.76E-20	-1.02	5.65E-15	NA	NA	NA	NA
2245	CD3EAP	E	-1.09	5.22E-16	-1.23	1.14E-22	NA	NA	NA	NA
2246	PNPO	E	-1.09	3.49E-28	-1.04	4.24E-27	NA	NA	NA	NA
2247	EML6	E	-1.09	2.78E-13	-1.42	1.18E-21	NA	NA	NA	NA
2248	DPH2	E	-1.09	1.25E-14	-1.02	6.39E-13	NA	NA	NA	NA
2249	TAF4B	E	-1.10	7.92E-23	-1.01	2.88E-20	NA	NA	NA	NA
2250	RPP40	E	-1.11	5.13E-12	-1.17	2.83E-15	NA	NA	NA	NA
2251	PRCD	E	-1.12	4.98E-32	-1.43	4.10E-45	NA	NA	NA	NA
2252	SMOC2	E	-1.12	2.74E-02	-1.20	2.08E-02	NA	NA	NA	NA
2253	CDKN1C	E	-1.13	1.21E-14	-1.79	1.87E-29	NA	NA	NA	NA
2254	METRNL	E	-1.13	3.87E-03	-1.12	3.23E-03	NA	NA	NA	NA
2255	UBE2QL1	E	-1.14	3.57E-51	-1.05	1.60E-46	NA	NA	NA	NA
2256	VPS9D1-AS1	E	-1.17	1.06E-10	-1.05	4.42E-09	NA	NA	NA	NA
2257	EEF1AKMT4	E	-1.20	7.92E-13	-1.12	8.21E-12	NA	NA	NA	NA
2258	PODXL	E	-1.21	3.02E-52	-1.19	8.49E-50	NA	NA	NA	NA
2259	SIX2	E	-1.21	7.95E-11	-1.40	4.04E-14	NA	NA	NA	NA
2260	CAMKV	E	-1.21	2.17E-33	-1.66	1.59E-54	NA	NA	NA	NA
2261	LINC01694	E	-1.22	4.63E-12	-1.04	3.49E-09	NA	NA	NA	NA
2262	RHOV	E	-1.22	1.58E-05	-1.13	5.18E-04	NA	NA	NA	NA
2263	PLLP	E	-1.23	9.29E-05	-1.08	1.41E-03	NA	NA	NA	NA
2264	GALNTL6	E	-1.23	4.83E-03	-1.07	3.70E-02	NA	NA	NA	NA
2265	CAMK4	E	-1.23	8.21E-37	-1.42	2.01E-49	NA	NA	NA	NA
2266	AL645608.5	E	-1.24	1.68E-02	-1.49	1.02E-03	NA	NA	NA	NA
2267	LRRN3	E	-1.24	8.91E-48	-1.75	2.09E-94	NA	NA	NA	NA
2268	DCAF4	E	-1.25	1.88E-12	-1.31	7.29E-14	NA	NA	NA	NA
2269	C2orf88	E	-1.25	2.70E-04	-1.12	8.48E-04	NA	NA	NA	NA
2270	SLC7A2	E	-1.25	2.06E-41	-1.18	4.45E-39	NA	NA	NA	NA
2271	SLC25A19	E	-1.26	6.14E-14	-1.04	8.60E-10	NA	NA	NA	NA
2272	ZNF365	E	-1.26	2.61E-09	-1.50	1.69E-14	NA	NA	NA	NA
2273	43525	E	-1.26	8.00E-35	-1.04	4.14E-23	NA	NA	NA	NA
2274	CITED1	E	-1.27	1.57E-05	-1.00	1.37E-03	NA	NA	NA	NA
2275	ENC1	E	-1.29	1.47E-45	-1.32	9.25E-47	NA	NA	NA	NA
2276	GRIP2	E	-1.30	2.02E-06	-1.04	6.29E-04	NA	NA	NA	NA
2277	KCNH4	E	-1.30	7.46E-05	-1.98	5.24E-09	NA	NA	NA	NA
2278	LMO1	E	-1.31	3.62E-16	-1.01	9.16E-10	NA	NA	NA	NA
2279	GRIA4	E	-1.31	9.87E-14	-2.12	6.43E-33	NA	NA	NA	NA
2280	SNHG4	E	-1.34	1.89E-31	-1.60	4.70E-46	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2281	NPY2R	E	-1.34	3.44E-22	-1.07	9.48E-18	NA	NA	NA	NA
2282	PPARGC1B	E	-1.37	3.45E-06	-1.52	8.37E-08	NA	NA	NA	NA
2283	ADGRL3	E	-1.37	6.27E-10	-1.05	7.95E-06	NA	NA	NA	NA
2284	LUZP2	E	-1.39	3.16E-06	-1.27	1.19E-04	NA	NA	NA	NA
2285	TBC1D4	E	-1.40	2.89E-37	-1.15	4.86E-26	NA	NA	NA	NA
2286	ELL2	E	-1.41	1.03E-02	-1.22	2.94E-02	NA	NA	NA	NA
2287	RRP9	E	-1.42	7.47E-35	-1.15	6.01E-24	NA	NA	NA	NA
2288	SPNS2	E	-1.42	1.06E-03	-1.69	3.20E-04	NA	NA	NA	NA
2289	TBC1D30	E	-1.44	1.33E-10	-1.24	7.85E-06	NA	NA	NA	NA
2290	LHX6	E	-1.45	1.51E-04	-1.33	1.54E-03	NA	NA	NA	NA
2291	Six3os1_1	E	-1.47	5.00E-02	-2.15	2.28E-02	NA	NA	NA	NA
2292	AL645608.1	E	-1.49	1.39E-14	-1.66	1.97E-17	NA	NA	NA	NA
2293	SLITRK6	E	-1.50	9.66E-51	-1.91	3.65E-84	NA	NA	NA	NA
2294	CABLES1	E	-1.51	7.99E-22	-1.63	3.11E-22	NA	NA	NA	NA
2295	FERMT1	E	-1.52	4.54E-04	-2.02	3.91E-07	NA	NA	NA	NA
2296	POU4F1	E	-1.55	8.23E-13	-1.51	3.36E-09	NA	NA	NA	NA
2297	WNT10B	E	-1.59	1.03E-02	-2.69	1.32E-03	NA	NA	NA	NA
2298	LURAP1L	E	-1.61	1.36E-07	-1.52	1.21E-08	NA	NA	NA	NA
2299	PLD6	E	-1.65	2.53E-46	-1.26	5.74E-32	NA	NA	NA	NA
2300	SLC6A15	E	-1.66	1.32E-80	-1.27	9.67E-51	NA	NA	NA	NA
2301	ALDH1A3	E	-1.68	9.84E-33	-1.50	6.12E-26	NA	NA	NA	NA
2302	AC005064.1	E	-1.68	6.17E-03	-1.89	6.84E-03	NA	NA	NA	NA
2303	SPATA3-AS1	E	-1.71	1.06E-02	-2.32	3.40E-03	NA	NA	NA	NA
2304	AL353746.1	E	-1.76	4.25E-18	-1.57	5.19E-15	NA	NA	NA	NA
2305	NRP2	E	-1.76	4.39E-07	-1.48	5.52E-05	NA	NA	NA	NA
2306	GUCY1A3	E	-1.77	1.88E-28	-1.14	1.23E-12	NA	NA	NA	NA
2307	TOX	E	-1.78	3.47E-106	-1.16	1.55E-48	NA	NA	NA	NA
2308	LINGO2	E	-1.85	1.13E-21	-1.60	2.24E-17	NA	NA	NA	NA
2309	AC016642.1	E	-1.89	1.40E-02	-1.79	8.17E-03	NA	NA	NA	NA
2310	FGF14-IT1	E	-1.90	1.12E-02	-2.02	1.86E-02	NA	NA	NA	NA
2311	KLRG2	E	-1.90	1.08E-03	-1.62	9.51E-04	NA	NA	NA	NA
2312	GRID1	E	-1.92	5.21E-39	-2.32	3.58E-50	NA	NA	NA	NA
2313	AC104083.1	E	-1.93	4.04E-21	-1.67	2.81E-19	NA	NA	NA	NA
2314	ABCA12	E	-1.95	1.92E-94	-1.21	6.63E-36	NA	NA	NA	NA
2315	FAM84B	E	-1.95	3.42E-02	-3.09	1.24E-03	NA	NA	NA	NA
2316	VSNL1	E	-1.98	8.10E-69	-2.20	8.51E-104	NA	NA	NA	NA
2317	ISL1	E	-2.04	1.05E-251	-1.31	2.65E-107	NA	NA	NA	NA
2318	PTCHD4	E	-2.05	1.02E-32	-2.31	1.35E-42	NA	NA	NA	NA
2319	LINC02118	E	-2.06	6.43E-03	-3.08	2.68E-03	NA	NA	NA	NA
2320	SPINT2	E	-2.06	1.66E-02	-2.63	9.70E-04	NA	NA	NA	NA
2321	GDF6	E	-2.09	1.02E-04	-2.17	3.37E-05	NA	NA	NA	NA
2322	FGF14	E	-2.10	2.44E-55	-2.68	4.51E-86	NA	NA	NA	NA
2323	STEAP3	E	-2.11	5.69E-78	-2.21	9.17E-75	NA	NA	NA	NA
2324	SORBS2	E	-2.11	5.72E-22	-1.50	1.08E-11	NA	NA	NA	NA
2325	ADCYAP1R1	E	-2.14	1.20E-66	-1.95	5.26E-54	NA	NA	NA	NA
2326	GRB14	E	-2.15	9.34E-07	-1.82	2.53E-05	NA	NA	NA	NA
2327	HPGD	E	-2.16	4.20E-07	-1.31	7.35E-04	NA	NA	NA	NA
2328	NT5C1A	E	-2.18	3.00E-14	-1.64	1.64E-07	NA	NA	NA	NA
2329	LPAR3	E	-2.20	3.55E-49	-1.13	5.24E-14	NA	NA	NA	NA
2330	NDST3	E	-2.27	2.88E-12	-1.69	7.99E-10	NA	NA	NA	NA
2331	SLC6A5	E	-2.27	2.32E-02	-2.23	5.00E-02	NA	NA	NA	NA
2332	NLRC5	E	-2.28	8.92E-05	-1.74	6.76E-04	NA	NA	NA	NA
2333	TYRP1	E	-2.31	3.05E-03	-2.44	3.76E-03	NA	NA	NA	NA
2334	ICA1	E	-2.35	3.45E-224	-1.08	1.18E-47	NA	NA	NA	NA
2335	SLC27A2	E	-2.69	4.27E-11	-1.32	4.14E-03	NA	NA	NA	NA
2336	FJX1	E	-2.76	5.29E-75	-2.00	3.26E-39	NA	NA	NA	NA
2337	AC114284.1	E	-2.80	2.74E-06	-2.95	7.98E-06	NA	NA	NA	NA
2338	PRR16	E	-2.85	5.24E-58	-2.00	1.11E-39	NA	NA	NA	NA
2339	NPAS3	E	-2.96	6.51E-04	-2.62	2.84E-03	NA	NA	NA	NA
2340	AL590132.1	E	-2.99	3.84E-09	-1.26	1.48E-02	NA	NA	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2341	NXPH1	E	-3.00	1.67E-10	-3.04	1.63E-11	NA	NA	NA	NA
2342	PLEKHN1	E	-3.10	2.04E-02	-4.14	2.59E-03	NA	NA	NA	NA
2343	AGTR1	E	-3.23	4.85E-12	-2.33	5.23E-07	NA	NA	NA	NA
2344	CCKAR	E	-3.66	2.14E-02	-4.67	1.41E-03	NA	NA	NA	NA
2345	ARC	E	-3.70	1.46E-07	-3.62	4.56E-07	NA	NA	NA	NA
2346	C10orf71	E	-3.96	4.90E-02	-5.40	3.99E-04	NA	NA	NA	NA
2347	CNTNAP4	E	-4.08	9.29E-28	-3.91	5.46E-42	NA	NA	NA	NA
2348	LRRC3B	E	-4.39	1.05E-02	-4.92	3.77E-03	NA	NA	NA	NA
2349	DRGX	E	-4.95	3.01E-239	-4.38	1.99E-225	NA	NA	NA	NA
2350	CBLN4	E	-4.97	4.66E-181	-4.96	3.46E-159	NA	NA	NA	NA
2351	ANOS1	E	-5.51	4.66E-05	-2.64	5.06E-03	NA	NA	NA	NA
2352	PPEF2	F	NA	NA	4.65	7.07E-03	-7.69	4.66E-07	NA	NA
2353	AL031651.2	F	NA	NA	4.49	2.38E-03	-4.05	0.007103402	NA	NA
2354	CNTNAP5	F	NA	NA	4.30	3.09E-14	-4.57	2.73E-16	NA	NA
2355	PCDH8	F	NA	NA	4.24	2.13E-04	-4.29	1.15E-04	NA	NA
2356	AL158211.2	F	NA	NA	3.81	5.33E-03	-3.56	0.009209079	NA	NA
2357	AL356275.1	F	NA	NA	3.71	1.44E-02	-4.18	0.003371141	NA	NA
2358	GOLGA6A	F	NA	NA	3.55	3.48E-02	-3.73	0.019369284	NA	NA
2359	C4orf50	F	NA	NA	3.55	1.13E-09	-3.67	1.76E-10	NA	NA
2360	LINC00643	F	NA	NA	3.37	4.28E-07	-1.68	0.036693356	NA	NA
2361	HORMAD2-AS1	F	NA	NA	3.36	4.62E-04	-2.18	0.04586854	NA	NA
2362	TUG1_2	F	NA	NA	3.18	2.47E-03	-3.19	0.001986057	NA	NA
2363	SLC7A11-AS1	F	NA	NA	3.07	2.63E-02	-3.31	0.011444387	NA	NA
2364	JAKMIP1	F	NA	NA	2.94	6.13E-84	-2.37	7.44E-53	NA	NA
2365	AL121749.1	F	NA	NA	2.82	1.73E-06	-2.67	6.44E-06	NA	NA
2366	AC068733.3	F	NA	NA	2.81	2.64E-03	-2.24	0.020919675	NA	NA
2367	STC2	F	NA	NA	2.48	1.33E-62	-3.40	9.78E-120	NA	NA
2368	GAL	F	NA	NA	2.43	6.68E-108	-1.29	3.50E-30	NA	NA
2369	EPAS1	F	NA	NA	2.40	8.77E-108	-1.52	1.99E-43	NA	NA
2370	MYOF	F	NA	NA	2.40	8.94E-05	-2.38	1.04E-04	NA	NA
2371	CPNE5	F	NA	NA	2.38	9.33E-04	-1.75	0.027515021	NA	NA
2372	ODAM	F	NA	NA	2.32	1.73E-02	-2.31	0.014964085	NA	NA
2373	SAMD3	F	NA	NA	2.29	2.25E-02	-2.65	0.005486591	NA	NA
2374	ETS2	F	NA	NA	2.23	1.48E-83	-1.34	1.82E-29	NA	NA
2375	BDNF	F	NA	NA	2.22	2.69E-38	-1.83	3.27E-25	NA	NA
2376	CABP7	F	NA	NA	2.22	2.25E-08	-2.35	1.68E-09	NA	NA
2377	AC022400.3	F	NA	NA	2.19	1.61E-02	-2.01	0.027737157	NA	NA
2378	CHRND	F	NA	NA	2.13	5.90E-06	-1.50	0.00328103	NA	NA
2379	PFKP	F	NA	NA	1.97	8.74E-136	-1.32	5.59E-60	NA	NA
2380	AQP10	F	NA	NA	1.90	2.10E-09	-1.03	0.004616182	NA	NA
2381	IQCJ-SCHIP1	F	NA	NA	1.89	1.38E-99	-1.16	5.79E-37	NA	NA
2382	SCHIP1	F	NA	NA	1.84	1.51E-83	-1.21	4.56E-35	NA	NA
2383	SFXN3	F	NA	NA	1.84	1.94E-69	-1.44	1.65E-42	NA	NA
2384	AC009549.1	F	NA	NA	1.84	8.86E-14	-1.56	7.03E-10	NA	NA
2385	ZNF521	F	NA	NA	1.83	7.52E-27	-1.55	1.15E-19	NA	NA
2386	PLOD2	F	NA	NA	1.77	8.60E-05	-1.42	0.002964588	NA	NA
2387	PPP1R16B	F	NA	NA	1.76	1.15E-27	-1.76	7.04E-28	NA	NA
2388	SUPT20HL2	F	NA	NA	1.74	1.81E-07	-1.46	2.25E-05	NA	NA
2389	SNCB	F	NA	NA	1.70	1.13E-06	-2.23	2.06E-11	NA	NA
2390	FAM105A	F	NA	NA	1.67	4.00E-89	-1.42	2.21E-63	NA	NA
2391	PTPRE	F	NA	NA	1.62	6.06E-04	-2.26	3.07E-07	NA	NA
2392	ATP9A	F	NA	NA	1.61	0	-1.22	1.78E-206	NA	NA
2393	SYT16	F	NA	NA	1.58	8.68E-47	-1.54	2.29E-44	NA	NA
2394	AC005828.5	F	NA	NA	1.58	6.72E-05	-2.08	1.72E-08	NA	NA
2395	AC012673.1	F	NA	NA	1.56	2.32E-02	-1.43	0.04045472	NA	NA
2396	LINC01623	F	NA	NA	1.55	3.40E-02	-2.11	0.0011301	NA	NA
2397	AF111167.2	F	NA	NA	1.55	1.51E-04	-2.20	6.46E-09	NA	NA
2398	COX4I2	F	NA	NA	1.53	1.89E-05	-1.16	0.001916954	NA	NA
2399	GLRX	F	NA	NA	1.51	1.38E-19	-1.23	3.06E-13	NA	NA
2400	VEGFA	F	NA	NA	1.49	3.52E-44	-1.22	1.03E-29	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2401	PRRG1	F	NA	NA	1.47	4.12E-18	-1.41	3.43E-17	NA	NA
2402	ZFP36L1	F	NA	NA	1.46	5.35E-09	-1.29	3.73E-07	NA	NA
2403	AL138789.1	F	NA	NA	1.46	8.81E-03	-1.64	0.001813813	NA	NA
2404	IL10RA	F	NA	NA	1.41	2.80E-02	-1.23	0.049108138	NA	NA
2405	TCF15	F	NA	NA	1.40	1.56E-05	-1.76	8.55E-09	NA	NA
2406	AL138799.1	F	NA	NA	1.39	2.97E-02	-1.34	0.033405722	NA	NA
2407	SGCE	F	NA	NA	1.36	2.16E-52	-1.05	1.88E-31	NA	NA
2408	CUX2	F	NA	NA	1.34	1.13E-73	-1.70	2.87E-119	NA	NA
2409	RNF144A-AS1	F	NA	NA	1.31	1.67E-21	-1.62	2.39E-33	NA	NA
2410	ZNF285B	F	NA	NA	1.29	1.99E-02	-1.22	0.026369582	NA	NA
2411	LRRTM1	F	NA	NA	1.29	2.90E-06	-1.84	7.09E-13	NA	NA
2412	SRC	F	NA	NA	1.26	1.18E-36	-1.01	8.29E-24	NA	NA
2413	JDP2	F	NA	NA	1.23	1.07E-17	-1.44	7.28E-25	NA	NA
2414	SPTB	F	NA	NA	1.22	1.34E-05	-2.61	1.06E-25	NA	NA
2415	IGFBP4	F	NA	NA	1.21	2.43E-104	-1.06	1.70E-78	NA	NA
2416	NANOS3	F	NA	NA	1.15	1.66E-02	-1.11	0.018870344	NA	NA
2417	ALK	F	NA	NA	1.15	3.14E-68	-2.25	1.61E-270	NA	NA
2418	BEST1	F	NA	NA	1.14	5.70E-06	-2.08	4.17E-20	NA	NA
2419	PHGDH	F	NA	NA	1.10	1.56E-51	-1.26	2.00E-68	NA	NA
2420	AC135983.1	F	NA	NA	1.10	1.24E-03	-1.97	3.42E-11	NA	NA
2421	TMEM178A	F	NA	NA	1.09	4.61E-03	-1.96	5.05E-09	NA	NA
2422	RUNDC3B	F	NA	NA	1.09	3.32E-09	-1.97	2.62E-30	NA	NA
2423	AC008543.1	F	NA	NA	1.08	1.23E-06	-1.28	2.58E-09	NA	NA
2424	ADAMTS9-AS2	F	NA	NA	1.06	2.42E-05	-1.14	3.30E-06	NA	NA
2425	PDLIM1P4	F	NA	NA	1.05	3.08E-02	-1.01	0.037897581	NA	NA
2426	AC009090.5	F	NA	NA	1.04	3.38E-02	-1.02	0.034635374	NA	NA
2427	ULK4P2	F	NA	NA	1.02	7.68E-03	-1.79	1.04E-07	NA	NA
2428	AC091057.2	F	NA	NA	1.01	3.22E-03	-1.06	0.001522249	NA	NA
2429	PDZD7	F	NA	NA	1.01	1.13E-04	-1.78	1.32E-14	NA	NA
2430	AP001347.1	F	NA	NA	1.01	2.81E-02	-1.23	0.003578187	NA	NA
2431	ZFP42	F	NA	NA	1.01	3.77E-02	-1.08	0.019730804	NA	NA
2432	SPATA18	F	NA	NA	-1.02	2.25E-04	1.42	9.97E-08	NA	NA
2433	MRAP2	F	NA	NA	-1.03	1.03E-16	1.00	7.97E-16	NA	NA
2434	ZNF157	F	NA	NA	-1.05	4.24E-06	1.15	4.96E-07	NA	NA
2435	ADGRE5	F	NA	NA	-1.07	5.08E-03	1.02	0.006762041	NA	NA
2436	SNX18P3	F	NA	NA	-1.08	5.33E-18	1.28	2.33E-24	NA	NA
2437	CCDC74B	F	NA	NA	-1.09	1.69E-12	1.29	2.24E-17	NA	NA
2438	MR1	F	NA	NA	-1.09	1.88E-02	1.70	1.19E-04	NA	NA
2439	VCAN	F	NA	NA	-1.10	1.42E-24	1.80	8.94E-65	NA	NA
2440	SNX18P12	F	NA	NA	-1.11	8.03E-08	1.78	9.98E-17	NA	NA
2441	APOL4	F	NA	NA	-1.13	1.19E-07	1.79	2.50E-17	NA	NA
2442	POU3F3	F	NA	NA	-1.13	2.88E-05	1.33	8.44E-07	NA	NA
2443	SHOX2	F	NA	NA	-1.14	3.37E-46	1.46	3.60E-75	NA	NA
2444	SOX9	F	NA	NA	-1.14	5.11E-17	1.28	1.56E-20	NA	NA
2445	BBC3	F	NA	NA	-1.14	8.41E-07	1.59	2.24E-12	NA	NA
2446	EDA2R	F	NA	NA	-1.19	3.53E-23	1.38	1.06E-29	NA	NA
2447	PSD4	F	NA	NA	-1.21	3.25E-02	1.21	0.02957198	NA	NA
2448	AC119751.3	F	NA	NA	-1.24	2.67E-06	2.26	2.55E-15	NA	NA
2449	VASH1-AS1	F	NA	NA	-1.26	3.72E-03	1.30	0.00213104	NA	NA
2450	AL645608.6	F	NA	NA	-1.26	1.51E-02	1.05	0.045437593	NA	NA
2451	MELTF	F	NA	NA	-1.27	7.31E-09	1.47	1.66E-11	NA	NA
2452	NIPAL2	F	NA	NA	-1.28	5.45E-03	1.28	0.004654492	NA	NA
2453	RSPH4A	F	NA	NA	-1.32	7.86E-06	1.74	1.18E-08	NA	NA
2454	MYOM3	F	NA	NA	-1.36	3.69E-02	1.94	7.36E-04	NA	NA
2455	IL7	F	NA	NA	-1.39	8.79E-07	1.61	6.58E-09	NA	NA
2456	ADAMTS15	F	NA	NA	-1.40	2.48E-03	1.66	3.37E-04	NA	NA
2457	ADGRE2	F	NA	NA	-1.46	4.35E-02	1.81	0.006875632	NA	NA
2458	LINC01102	F	NA	NA	-1.57	1.36E-21	1.16	6.76E-13	NA	NA
2459	LINC01237	F	NA	NA	-1.60	2.35E-04	1.15	0.010838914	NA	NA
2460	AC083837.1	F	NA	NA	-1.70	6.75E-03	1.65	0.007655532	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2461	CHGB	F	NA	NA	-1.72	5.79E-288	1.43	5.08E-201	NA	NA
2462	AOX1	F	NA	NA	-1.72	1.18E-03	1.57	0.001448645	NA	NA
2463	RSPH1	F	NA	NA	-1.76	6.33E-03	2.86	3.03E-05	NA	NA
2464	PLD1	F	NA	NA	-1.87	1.04E-02	1.95	0.00781563	NA	NA
2465	CNTN3	F	NA	NA	-1.92	1.13E-03	1.95	8.65E-04	NA	NA
2466	LEKR1	F	NA	NA	-2.01	1.46E-02	2.00	0.014332139	NA	NA
2467	MSX2	F	NA	NA	-2.11	4.96E-51	1.33	4.77E-23	NA	NA
2468	LIF	F	NA	NA	-2.27	8.62E-06	1.39	0.012667073	NA	NA
2469	SCN1A	F	NA	NA	-2.40	7.64E-16	2.62	1.17E-16	NA	NA
2470	AL022341.2	F	NA	NA	-2.49	1.75E-07	1.75	1.12E-04	NA	NA
2471	IRX6	F	NA	NA	-2.52	7.83E-07	1.66	8.95E-04	NA	NA
2472	NT5E	F	NA	NA	-2.56	4.16E-40	2.45	6.30E-35	NA	NA
2473	NALT1	F	NA	NA	-2.56	3.28E-02	4.21	0.004579911	NA	NA
2474	PGAM2	F	NA	NA	-2.70	7.08E-03	3.05	0.002629779	NA	NA
2475	PTPRVP	F	NA	NA	-2.76	4.52E-09	3.12	3.67E-11	NA	NA
2476	KCNAB1	F	NA	NA	-2.81	3.78E-40	2.14	9.22E-27	NA	NA
2477	C9orf153	F	NA	NA	-2.84	1.20E-02	2.44	0.04031912	NA	NA
2478	LCN15	F	NA	NA	-2.93	2.03E-06	3.41	3.07E-08	NA	NA
2479	CUBN	F	NA	NA	-3.37	5.99E-06	3.41	4.42E-06	NA	NA
2480	NKX3-2	F	NA	NA	-4.12	4.77E-05	4.46	5.12E-05	NA	NA
2481	LRRTM3	F	NA	NA	-4.21	7.65E-03	4.13	0.007676864	NA	NA
2482	HTR3A	F	NA	NA	-5.07	2.13E-04	3.26	6.03E-04	NA	NA
2483	AL356417.3	F	NA	NA	-5.22	2.67E-05	4.62	1.42E-04	NA	NA
2484	MAOA	G	NA	NA	NA	NA	-12.1	9.94E-31	-11.5	1.24E-27
2485	SRRM4	G	NA	NA	NA	NA	-12.1	4.00E-32	-11.6	1.22E-30
2486	RNF212	G	NA	NA	NA	NA	-11.4	1.55E-42	-11.3	1.23E-52
2487	CLVS2	G	NA	NA	NA	NA	-11.2	2.45E-26	-10.6	8.67E-24
2488	LMO3	G	NA	NA	NA	NA	-9.66	6.44E-42	-7.98	2.95E-108
2489	ASTN1	G	NA	NA	NA	NA	-9.46	1.03E-18	-10.2	9.69E-22
2490	LRRTM2	G	NA	NA	NA	NA	-9.21	5.93E-19	-5.85	8.87E-25
2491	HMX1	G	NA	NA	NA	NA	-9.14	4.35E-17	-9.17	3.07E-17
2492	KCNT1	G	NA	NA	NA	NA	-9.03	1.38E-50	-9.77	3.82E-61
2493	COL9A1	G	NA	NA	NA	NA	-8.78	1.24E-37	-7.19	6.58E-54
2494	DSCR8	G	NA	NA	NA	NA	-8.67	1.91E-15	-7.19	1.01E-15
2495	CRH	G	NA	NA	NA	NA	-8.50	9.08E-15	-9.04	1.06E-16
2496	ME3	G	NA	NA	NA	NA	-8.46	1.06E-14	-7.87	8.80E-13
2497	TMEM178B	G	NA	NA	NA	NA	-8.45	5.95E-47	-8.50	1.98E-48
2498	AL022313.4	G	NA	NA	NA	NA	-8.19	3.92E-14	-9.52	6.61E-19
2499	ADAMTS7P3	G	NA	NA	NA	NA	-7.91	4.69E-36	-8.55	1.26E-40
2500	CYTL1	G	NA	NA	NA	NA	-7.79	1.05E-12	-9.09	4.22E-17
2501	AL365361.1	G	NA	NA	NA	NA	-7.67	1.27E-12	-7.31	7.79E-12
2502	AC115220.1	G	NA	NA	NA	NA	-7.66	1.13E-12	-8.58	1.72E-15
2503	CACNG2	G	NA	NA	NA	NA	-7.34	6.69E-22	-8.74	2.87E-17
2504	ZNF729	G	NA	NA	NA	NA	-7.22	7.78E-16	-5.55	1.51E-22
2505	KCNA3	G	NA	NA	NA	NA	-7.21	8.72E-11	-7.68	3.73E-12
2506	DSCR4	G	NA	NA	NA	NA	-7.13	2.12E-10	-7.70	3.97E-12
2507	PPP2R2C	G	NA	NA	NA	NA	-7.06	2.38E-30	-8.49	1.74E-36
2508	TRH	G	NA	NA	NA	NA	-6.98	1.01E-09	-7.03	7.01E-10
2509	EBF3	G	NA	NA	NA	NA	-6.73	3.36E-132	-6.90	1.97E-88
2510	KCNA2	G	NA	NA	NA	NA	-6.58	8.04E-09	-5.20	5.51E-14
2511	AC023824.6	G	NA	NA	NA	NA	-6.54	5.25E-08	-6.51	5.80E-08
2512	GABRB3	G	NA	NA	NA	NA	-6.51	4.39E-204	-7.59	7.22E-260
2513	STAT5A	G	NA	NA	NA	NA	-6.46	4.78E-13	-4.33	3.26E-11
2514	DSC3	G	NA	NA	NA	NA	-6.44	2.92E-12	-8.08	2.75E-13
2515	PQLC2L	G	NA	NA	NA	NA	-6.38	3.61E-07	-5.33	4.14E-05
2516	MIR124-2HG	G	NA	NA	NA	NA	-6.35	8.72E-08	-6.41	5.84E-08
2517	EYA2	G	NA	NA	NA	NA	-6.33	7.51E-07	-6.53	2.99E-07
2518	VSTM2A-OT1	G	NA	NA	NA	NA	-6.28	9.41E-08	-3.48	2.71E-05
2519	ZNF727	G	NA	NA	NA	NA	-6.25	3.03E-29	-5.99	8.85E-30
2520	TNNI1	G	NA	NA	NA	NA	-6.25	7.09E-07	-2.86	7.49E-03

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2521	SLIT1	G	NA	NA	NA	NA	-6.22	1.84E-16	-3.77	3.16E-07
2522	AC116565.1	G	NA	NA	NA	NA	-6.22	3.58E-07	-6.88	9.94E-09
2523	C2orf69P1	G	NA	NA	NA	NA	-6.22	1.87E-07	-6.95	2.78E-09
2524	SLC12A7	G	NA	NA	NA	NA	-6.21	5.88E-24	-8.25	6.57E-14
2525	LINC00877	G	NA	NA	NA	NA	-6.18	3.69E-06	-7.92	1.35E-12
2526	CDH22	G	NA	NA	NA	NA	-6.16	8.55E-07	-5.58	1.30E-05
2527	RSPO4	G	NA	NA	NA	NA	-6.15	7.97E-08	-6.97	9.48E-10
2528	AC116565.2	G	NA	NA	NA	NA	-6.11	2.34E-07	-6.76	5.54E-09
2529	CNTN5	G	NA	NA	NA	NA	-6.10	2.39E-13	-7.06	6.23E-12
2530	CLVS1	G	NA	NA	NA	NA	-6.08	6.56E-25	-4.66	8.19E-18
2531	AP001148.1	G	NA	NA	NA	NA	-6.03	9.63E-07	-4.89	1.54E-04
2532	AL158055.1	G	NA	NA	NA	NA	-5.98	5.20E-07	-5.35	5.46E-06
2533	FBN3	G	NA	NA	NA	NA	-5.97	1.28E-04	-7.55	8.83E-08
2534	AC092535.2	G	NA	NA	NA	NA	-5.96	2.74E-06	-6.00	2.21E-06
2535	PROKR2	G	NA	NA	NA	NA	-5.95	1.63E-06	-5.80	2.66E-06
2536	ELFN2	G	NA	NA	NA	NA	-5.93	5.34E-07	-6.02	1.09E-05
2537	POU2F2	G	NA	NA	NA	NA	-5.92	1.02E-09	-3.49	7.85E-04
2538	ZDHHC11B	G	NA	NA	NA	NA	-5.92	6.22E-37	-8.01	5.49E-14
2539	TRIM55	G	NA	NA	NA	NA	-5.87	9.18E-06	-4.15	2.06E-03
2540	AC036111.1	G	NA	NA	NA	NA	-5.79	5.75E-06	-5.30	4.56E-05
2541	BMP7	G	NA	NA	NA	NA	-5.78	2.62E-16	-4.46	6.10E-10
2542	HSPB8	G	NA	NA	NA	NA	-5.74	2.36E-05	-6.26	1.94E-06
2543	TRIM67	G	NA	NA	NA	NA	-5.74	4.21E-46	-5.92	3.57E-35
2544	EFHC2	G	NA	NA	NA	NA	-5.65	1.79E-06	-6.88	2.50E-09
2545	AL022337.1	G	NA	NA	NA	NA	-5.62	5.81E-06	-5.64	4.93E-06
2546	LINC00654	G	NA	NA	NA	NA	-5.61	1.32E-24	-4.61	5.16E-18
2547	DSCAM	G	NA	NA	NA	NA	-5.55	4.72E-05	-3.69	1.85E-02
2548	DLGAP2	G	NA	NA	NA	NA	-5.53	0.002899269	-5.27	5.13E-03
2549	B3GAT1	G	NA	NA	NA	NA	-5.50	7.54E-189	-6.07	1.12E-206
2550	AC025575.2	G	NA	NA	NA	NA	-5.47	2.89E-06	-5.33	3.91E-08
2551	SMPD5	G	NA	NA	NA	NA	-5.39	1.42E-04	-5.46	1.04E-04
2552	AL049749.1	G	NA	NA	NA	NA	-5.38	3.24E-05	-5.80	4.90E-06
2553	HS3ST3A1	G	NA	NA	NA	NA	-5.37	8.42E-05	-2.88	3.21E-03
2554	AC114940.1	G	NA	NA	NA	NA	-5.37	6.92E-05	-5.07	2.04E-04
2555	EGFLAM	G	NA	NA	NA	NA	-5.37	3.61E-05	-2.91	4.12E-02
2556	FP700111.2	G	NA	NA	NA	NA	-5.34	6.31E-13	-7.27	1.05E-10
2557	GLT1D1	G	NA	NA	NA	NA	-5.31	6.77E-06	-3.57	1.68E-04
2558	SHANK1	G	NA	NA	NA	NA	-5.31	3.79E-44	-4.70	4.11E-36
2559	AC092070.3	G	NA	NA	NA	NA	-5.31	6.05E-06	-4.90	5.28E-07
2560	NUDT10	G	NA	NA	NA	NA	-5.29	4.36E-73	-4.41	1.31E-54
2561	KSR2	G	NA	NA	NA	NA	-5.28	5.64E-32	-4.73	1.02E-27
2562	LINC01667	G	NA	NA	NA	NA	-5.27	1.26E-15	-3.45	1.18E-16
2563	PTPRT	G	NA	NA	NA	NA	-5.20	1.23E-04	-4.57	1.11E-03
2564	AC024563.1	G	NA	NA	NA	NA	-5.17	1.15E-04	-3.90	3.30E-03
2565	C15orf32	G	NA	NA	NA	NA	-5.15	6.51E-05	-3.14	8.33E-03
2566	KLHDC7A	G	NA	NA	NA	NA	-5.14	1.53E-04	-3.89	5.12E-03
2567	XK	G	NA	NA	NA	NA	-5.12	2.31E-04	-3.24	3.17E-02
2568	SEZ6	G	NA	NA	NA	NA	-5.09	4.75E-109	-3.95	3.22E-73
2569	DSCR4-IT1	G	NA	NA	NA	NA	-5.09	1.69E-04	-5.67	1.63E-05
2570	KANK4	G	NA	NA	NA	NA	-5.05	8.21E-06	-7.37	1.14E-10
2571	AC099654.7	G	NA	NA	NA	NA	-4.99	4.41E-05	-6.46	6.18E-08
2572	RAB37	G	NA	NA	NA	NA	-4.96	7.48E-11	-3.18	2.10E-06
2573	ATP2B3	G	NA	NA	NA	NA	-4.95	4.85E-37	-4.73	3.71E-30
2574	HCN4	G	NA	NA	NA	NA	-4.93	1.66E-17	-5.31	3.36E-11
2575	SECTM1	G	NA	NA	NA	NA	-4.85	8.42E-04	-5.69	5.54E-05
2576	NPTX2	G	NA	NA	NA	NA	-4.83	0	-5.22	0
2577	SHANK2	G	NA	NA	NA	NA	-4.79	1.23E-14	-3.68	4.93E-08
2578	AP002840.1	G	NA	NA	NA	NA	-4.78	3.12E-04	-3.79	7.46E-03
2579	TPPP	G	NA	NA	NA	NA	-4.76	1.13E-44	-3.91	6.65E-33
2580	MAGEC1	G	NA	NA	NA	NA	-4.74	6.95E-84	-5.25	7.28E-101

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2581	AC005224.4	G	NA	NA	NA	NA	-4.72	4.46E-04	-2.97	4.63E-02
2582	AC005993.1	G	NA	NA	NA	NA	-4.72	0.002409283	-3.74	2.68E-02
2583	VN1R87P	G	NA	NA	NA	NA	-4.72	0.00233522	-4.59	3.21E-03
2584	CLCNKA	G	NA	NA	NA	NA	-4.69	3.69E-05	-7.60	3.57E-09
2585	RBFOX3	G	NA	NA	NA	NA	-4.69	7.35E-09	-3.77	1.47E-05
2586	COL11A1	G	NA	NA	NA	NA	-4.67	1.49E-04	-6.74	4.53E-10
2587	AGGF1P6	G	NA	NA	NA	NA	-4.66	0.007570306	-3.99	2.93E-02
2588	ZNF99	G	NA	NA	NA	NA	-4.64	1.13E-44	-4.56	5.40E-46
2589	RHCG	G	NA	NA	NA	NA	-4.61	0.003179196	-3.37	4.24E-03
2590	ZNF677	G	NA	NA	NA	NA	-4.54	3.10E-194	-4.79	8.97E-213
2591	AC090985.1	G	NA	NA	NA	NA	-4.54	7.96E-04	-4.47	9.53E-04
2592	MRVI1	G	NA	NA	NA	NA	-4.53	4.77E-10	-3.65	1.43E-05
2593	AC090204.1	G	NA	NA	NA	NA	-4.50	1.15E-14	-3.91	1.75E-16
2594	FBXL21	G	NA	NA	NA	NA	-4.47	6.47E-07	-4.77	8.08E-08
2595	SYN3	G	NA	NA	NA	NA	-4.44	1.11E-04	-5.08	9.44E-06
2596	LINC02559	G	NA	NA	NA	NA	-4.43	0.004015218	-4.53	2.93E-03
2597	AMER3	G	NA	NA	NA	NA	-4.42	3.05E-92	-4.78	1.18E-105
2598	AC092720.2	G	NA	NA	NA	NA	-4.42	9.38E-04	-5.06	8.43E-05
2599	C20orf166-AS1	G	NA	NA	NA	NA	-4.41	2.55E-06	-5.85	2.15E-06
2600	SPATA46	G	NA	NA	NA	NA	-4.38	9.28E-21	-3.11	1.94E-12
2601	KCNJ3	G	NA	NA	NA	NA	-4.37	2.07E-38	-2.95	2.08E-23
2602	AC095038.2	G	NA	NA	NA	NA	-4.34	0.01468463	-4.68	6.76E-03
2603	DPF3	G	NA	NA	NA	NA	-4.29	6.08E-11	-2.40	2.17E-04
2604	MAP7D2	G	NA	NA	NA	NA	-4.27	4.73E-82	-3.16	8.90E-48
2605	FXYD7	G	NA	NA	NA	NA	-4.25	0.008503258	-3.38	4.50E-02
2606	SYT7	G	NA	NA	NA	NA	-4.23	1.06E-154	-3.87	6.94E-97
2607	AC008945.2	G	NA	NA	NA	NA	-4.22	0.003312552	-3.66	1.31E-02
2608	LINC00599	G	NA	NA	NA	NA	-4.21	5.36E-88	-4.33	2.10E-105
2609	RUNX1T1	G	NA	NA	NA	NA	-4.20	4.23E-33	-3.09	3.95E-20
2610	TVP23A	G	NA	NA	NA	NA	-4.17	8.04E-74	-4.46	6.16E-86
2611	MYOZ3	G	NA	NA	NA	NA	-4.14	3.29E-42	-3.95	1.18E-40
2612	SORCS2	G	NA	NA	NA	NA	-4.14	1.71E-07	-3.87	1.22E-07
2613	AC099521.3	G	NA	NA	NA	NA	-4.08	1.15E-08	-3.39	8.92E-07
2614	FAM109B	G	NA	NA	NA	NA	-4.06	8.22E-22	-4.35	1.31E-22
2615	PDCD6IPP1	G	NA	NA	NA	NA	-4.05	9.89E-07	-2.11	7.60E-03
2616	NKX2-5	G	NA	NA	NA	NA	-4.05	6.31E-11	-3.51	3.17E-10
2617	ARHGAP19-SLIT1	G	NA	NA	NA	NA	-4.04	4.12E-04	-3.85	3.76E-03
2618	AC233976.1	G	NA	NA	NA	NA	-4.04	0.015883714	-3.42	4.82E-02
2619	CNTN2	G	NA	NA	NA	NA	-4.04	2.96E-71	-3.87	9.91E-66
2620	NOS1	G	NA	NA	NA	NA	-4.02	2.06E-55	-4.64	1.24E-55
2621	GATA5	G	NA	NA	NA	NA	-4.01	0.023978786	-4.21	1.53E-02
2622	AC079949.2	G	NA	NA	NA	NA	-4.00	5.61E-09	-2.94	2.81E-09
2623	HSPB7	G	NA	NA	NA	NA	-3.98	6.66E-11	-3.49	2.18E-06
2624	SUSD4	G	NA	NA	NA	NA	-3.88	4.81E-08	-5.27	4.64E-11
2625	KCNH7	G	NA	NA	NA	NA	-3.82	4.24E-22	-4.70	9.15E-24
2626	LINC02348	G	NA	NA	NA	NA	-3.82	0.008659111	-2.47	1.27E-02
2627	GRID2	G	NA	NA	NA	NA	-3.81	2.96E-06	-3.34	5.86E-05
2628	SOHLH1	G	NA	NA	NA	NA	-3.80	0.019879621	-5.92	2.99E-05
2629	CADM3-AS1	G	NA	NA	NA	NA	-3.75	0.016324768	-5.35	1.05E-04
2630	SELENOP	G	NA	NA	NA	NA	-3.75	2.62E-08	-2.41	1.75E-04
2631	STMN4	G	NA	NA	NA	NA	-3.74	1.31E-85	-4.02	1.05E-104
2632	ZNF676	G	NA	NA	NA	NA	-3.74	6.84E-12	-2.70	5.44E-08
2633	GOLGA7B	G	NA	NA	NA	NA	-3.73	2.35E-109	-4.01	1.88E-135
2634	SLC8A3	G	NA	NA	NA	NA	-3.73	3.92E-298	-4.32	0
2635	CHST2	G	NA	NA	NA	NA	-3.72	0.026257513	-3.61	3.79E-02
2636	FOXA3	G	NA	NA	NA	NA	-3.71	5.67E-04	-2.75	5.79E-03
2637	DRD2	G	NA	NA	NA	NA	-3.70	1.50E-101	-3.77	1.42E-90
2638	SLC22A10	G	NA	NA	NA	NA	-3.70	0.001941708	-4.98	2.00E-04
2639	CPA1	G	NA	NA	NA	NA	-3.70	0.041157998	-3.75	3.92E-02
2640	HES4	G	NA	NA	NA	NA	-3.67	6.09E-09	-2.44	1.83E-04

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2641	NMNAT2	G	NA	NA	NA	NA	-3.66	1.07E-87	-3.93	1.10E-97
2642	AC008397.2	G	NA	NA	NA	NA	-3.66	4.71E-10	-2.10	5.45E-05
2643	CHD5	G	NA	NA	NA	NA	-3.66	8.09E-13	-3.31	1.98E-10
2644	C19orf81	G	NA	NA	NA	NA	-3.65	1.29E-05	-3.00	1.06E-05
2645	HLA-G	G	NA	NA	NA	NA	-3.65	1.55E-07	-5.05	9.45E-07
2646	CCDC152	G	NA	NA	NA	NA	-3.65	1.63E-72	-2.87	7.86E-49
2647	CU459211.1	G	NA	NA	NA	NA	-3.63	5.63E-36	-2.96	7.57E-29
2648	BASP1	G	NA	NA	NA	NA	-3.61	6.97E-242	-4.35	0
2649	KCNK10	G	NA	NA	NA	NA	-3.60	6.34E-17	-2.83	6.25E-10
2650	AL031658.2	G	NA	NA	NA	NA	-3.58	2.22E-12	-3.02	3.27E-09
2651	TTC9B	G	NA	NA	NA	NA	-3.56	1.80E-22	-4.74	1.38E-36
2652	ALKAL2	G	NA	NA	NA	NA	-3.56	2.29E-14	-3.71	1.29E-16
2653	HOXC-AS2	G	NA	NA	NA	NA	-3.55	6.76E-06	-5.69	3.99E-14
2654	GLDN	G	NA	NA	NA	NA	-3.53	0.004229513	-2.94	3.90E-02
2655	DACT1	G	NA	NA	NA	NA	-3.51	5.55E-17	-3.45	9.76E-25
2656	AC095038.1	G	NA	NA	NA	NA	-3.51	0.020102204	-3.19	4.80E-02
2657	NEXMIF	G	NA	NA	NA	NA	-3.50	1.63E-09	-2.23	4.53E-05
2658	T	G	NA	NA	NA	NA	-3.49	8.38E-16	-3.81	6.19E-11
2659	TMEM45B	G	NA	NA	NA	NA	-3.48	1.30E-06	-2.08	7.55E-03
2660	CNGB1	G	NA	NA	NA	NA	-3.48	2.90E-06	-3.12	3.74E-05
2661	ST8SIA2	G	NA	NA	NA	NA	-3.41	3.89E-154	-2.89	1.49E-113
2662	BEND4	G	NA	NA	NA	NA	-3.40	3.43E-253	-3.14	4.45E-225
2663	AC120036.5	G	NA	NA	NA	NA	-3.37	0.02769774	-2.49	2.61E-02
2664	PEG13	G	NA	NA	NA	NA	-3.36	5.97E-06	-3.22	3.57E-04
2665	PITX1	G	NA	NA	NA	NA	-3.35	1.23E-05	-3.13	6.67E-04
2666	NKX3-1	G	NA	NA	NA	NA	-3.35	0.004288008	-5.23	2.42E-05
2667	ARHGAP8	G	NA	NA	NA	NA	-3.34	3.81E-16	-2.51	1.54E-10
2668	AC005165.1	G	NA	NA	NA	NA	-3.31	1.80E-08	-4.18	6.23E-10
2669	JPH3	G	NA	NA	NA	NA	-3.31	4.48E-82	-3.07	3.46E-73
2670	KRT19	G	NA	NA	NA	NA	-3.27	3.69E-04	-2.97	1.36E-03
2671	VSTM2A	G	NA	NA	NA	NA	-3.25	1.43E-62	-2.37	5.91E-39
2672	TMEM130	G	NA	NA	NA	NA	-3.21	3.42E-25	-3.95	8.03E-37
2673	FAM174B	G	NA	NA	NA	NA	-3.19	1.03E-05	-3.40	2.19E-06
2674	SMPD3	G	NA	NA	NA	NA	-3.18	5.74E-26	-2.74	1.17E-18
2675	CES4A	G	NA	NA	NA	NA	-3.16	1.88E-06	-3.39	8.51E-08
2676	AC015712.7	G	NA	NA	NA	NA	-3.15	1.87E-08	-4.24	5.68E-09
2677	EMILIN3	G	NA	NA	NA	NA	-3.15	2.25E-40	-3.58	7.28E-52
2678	NDN	G	NA	NA	NA	NA	-3.15	3.17E-25	-3.29	1.07E-28
2679	PPIEL	G	NA	NA	NA	NA	-3.14	4.93E-26	-3.01	8.49E-26
2680	SLCO3A1	G	NA	NA	NA	NA	-3.12	2.02E-72	-3.16	4.71E-83
2681	SPON2	G	NA	NA	NA	NA	-3.12	0.001022059	-3.78	2.43E-05
2682	CYP1A1	G	NA	NA	NA	NA	-3.11	2.47E-05	-1.91	3.87E-03
2683	KIF1A	G	NA	NA	NA	NA	-3.11	6.89E-75	-2.70	2.12E-56
2684	FGD5	G	NA	NA	NA	NA	-3.11	1.43E-08	-2.82	7.16E-09
2685	LARGE1	G	NA	NA	NA	NA	-3.10	9.27E-23	-3.15	2.37E-26
2686	IGLON5	G	NA	NA	NA	NA	-3.08	9.15E-143	-3.21	4.41E-162
2687	NCAN	G	NA	NA	NA	NA	-3.07	1.09E-40	-3.50	5.01E-51
2688	ANXA2R	G	NA	NA	NA	NA	-3.06	0.005200999	-3.16	1.63E-03
2689	SVOP	G	NA	NA	NA	NA	-3.05	9.75E-22	-3.14	2.75E-23
2690	PHACTR3	G	NA	NA	NA	NA	-3.03	4.60E-171	-3.41	1.52E-149
2691	SYT4	G	NA	NA	NA	NA	-3.01	4.67E-177	-3.66	1.00E-254
2692	PCDH1	G	NA	NA	NA	NA	-3.01	1.60E-37	-3.08	2.59E-38
2693	CRABP1	G	NA	NA	NA	NA	-3.00	1.61E-270	-3.32	0
2694	AC091544.5	G	NA	NA	NA	NA	-2.99	0.04272285	-5.61	4.46E-04
2695	PLPPR5	G	NA	NA	NA	NA	-2.99	1.08E-287	-2.68	6.42E-233
2696	MPPED1	G	NA	NA	NA	NA	-2.98	9.44E-16	-2.95	3.97E-15
2697	FAM222A	G	NA	NA	NA	NA	-2.95	3.50E-105	-3.08	4.80E-119
2698	AC093390.2	G	NA	NA	NA	NA	-2.95	0.019185269	-2.98	1.77E-02
2699	SYT17	G	NA	NA	NA	NA	-2.94	1.99E-21	-2.96	8.64E-21
2700	PRDM12	G	NA	NA	NA	NA	-2.90	0.008170176	-2.64	1.88E-02

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2701	MAGEC2	G	NA	NA	NA	NA	-2.88	2.74E-11	-3.02	7.91E-11
2702	MTHFS	G	NA	NA	NA	NA	-2.86	3.59E-27	-2.72	1.46E-20
2703	ITGA11	G	NA	NA	NA	NA	-2.86	5.67E-08	-2.45	5.35E-06
2704	SYK	G	NA	NA	NA	NA	-2.85	9.98E-110	-3.00	2.39E-122
2705	HS3ST3B1	G	NA	NA	NA	NA	-2.85	7.71E-08	-2.31	2.48E-07
2706	SHISA7	G	NA	NA	NA	NA	-2.84	1.13E-34	-2.96	9.16E-33
2707	GABRG3	G	NA	NA	NA	NA	-2.84	3.00E-07	-2.14	1.73E-04
2708	OPRD1	G	NA	NA	NA	NA	-2.81	5.16E-152	-2.44	3.80E-99
2709	FEZ1	G	NA	NA	NA	NA	-2.81	4.23E-22	-2.54	1.22E-19
2710	AC023824.7	G	NA	NA	NA	NA	-2.81	1.52E-88	-3.14	2.44E-114
2711	OR7E12P	G	NA	NA	NA	NA	-2.80	2.45E-14	-3.43	1.89E-15
2712	AC005740.1	G	NA	NA	NA	NA	-2.80	0.005268519	-4.34	7.88E-04
2713	ULBP2	G	NA	NA	NA	NA	-2.80	1.91E-04	-3.18	1.36E-07
2714	CELF4	G	NA	NA	NA	NA	-2.78	2.98E-25	-3.08	4.62E-29
2715	AL109615.3	G	NA	NA	NA	NA	-2.76	4.16E-07	-2.52	6.93E-08
2716	ISLR2	G	NA	NA	NA	NA	-2.75	9.75E-51	-2.51	3.24E-42
2717	ZNF98	G	NA	NA	NA	NA	-2.75	2.02E-08	-4.16	3.93E-15
2718	KCNF1	G	NA	NA	NA	NA	-2.75	1.64E-04	-2.97	3.78E-04
2719	CCDC177	G	NA	NA	NA	NA	-2.74	7.89E-28	-2.65	1.05E-25
2720	PPEF1	G	NA	NA	NA	NA	-2.73	2.94E-22	-3.94	2.28E-45
2721	AP3B2	G	NA	NA	NA	NA	-2.73	9.97E-69	-3.53	1.10E-112
2722	NRG1	G	NA	NA	NA	NA	-2.73	1.90E-35	-1.78	1.41E-15
2723	MMP24	G	NA	NA	NA	NA	-2.72	1.06E-165	-3.24	9.36E-221
2724	FAM86GP	G	NA	NA	NA	NA	-2.70	1.12E-04	-2.87	1.87E-04
2725	GDAP1L1	G	NA	NA	NA	NA	-2.69	3.15E-88	-3.57	9.41E-132
2726	TSSC2	G	NA	NA	NA	NA	-2.67	2.87E-93	-2.69	3.99E-88
2727	NRXN2	G	NA	NA	NA	NA	-2.66	1.65E-30	-3.18	2.70E-43
2728	RALYL	G	NA	NA	NA	NA	-2.66	4.36E-06	-3.89	4.27E-10
2729	AC110285.7	G	NA	NA	NA	NA	-2.65	2.61E-04	-3.89	5.50E-05
2730	PTPRN	G	NA	NA	NA	NA	-2.63	3.05E-41	-2.75	6.95E-46
2731	ZDHHC22	G	NA	NA	NA	NA	-2.62	8.27E-62	-3.35	1.14E-71
2732	PLA2G16	G	NA	NA	NA	NA	-2.60	1.27E-10	-2.43	1.36E-10
2733	CPNE4	G	NA	NA	NA	NA	-2.60	2.27E-36	-3.02	1.66E-35
2734	PKNOX2	G	NA	NA	NA	NA	-2.59	1.58E-07	-2.35	2.51E-07
2735	AC103739.1	G	NA	NA	NA	NA	-2.59	4.52E-04	-2.96	3.73E-04
2736	MTUS2	G	NA	NA	NA	NA	-2.58	2.61E-04	-3.77	7.44E-08
2737	AC024270.3	G	NA	NA	NA	NA	-2.58	1.55E-05	-1.66	6.50E-03
2738	RPL22L1	G	NA	NA	NA	NA	-2.58	7.30E-99	-2.11	1.18E-65
2739	AP001092.1	G	NA	NA	NA	NA	-2.57	1.15E-04	-3.73	3.43E-04
2740	LIX1	G	NA	NA	NA	NA	-2.57	2.22E-04	-2.11	1.62E-03
2741	KLHDC8A	G	NA	NA	NA	NA	-2.56	6.16E-05	-2.40	1.00E-05
2742	AL139125.2	G	NA	NA	NA	NA	-2.53	0.002569271	-1.93	3.98E-03
2743	LCN1	G	NA	NA	NA	NA	-2.53	0.021372221	-4.42	1.62E-03
2744	AL365255.1	G	NA	NA	NA	NA	-2.53	0.036867197	-2.48	2.10E-02
2745	C15orf59	G	NA	NA	NA	NA	-2.53	1.22E-14	-2.40	8.09E-16
2746	TBX3	G	NA	NA	NA	NA	-2.50	0	-2.57	0
2747	CELF5	G	NA	NA	NA	NA	-2.49	2.13E-50	-2.70	2.39E-64
2748	TRPV4	G	NA	NA	NA	NA	-2.48	0.023683346	-5.35	1.65E-05
2749	CNTN6	G	NA	NA	NA	NA	-2.48	9.30E-06	-3.68	1.01E-05
2750	AL079307.1	G	NA	NA	NA	NA	-2.47	1.19E-16	-1.82	9.30E-10
2751	AC104806.2	G	NA	NA	NA	NA	-2.47	4.45E-04	-2.93	2.68E-04
2752	LINC00592	G	NA	NA	NA	NA	-2.46	0.002718485	-1.54	2.28E-02
2753	HAPLN3	G	NA	NA	NA	NA	-2.45	1.39E-10	-2.34	8.59E-11
2754	RASGRF1	G	NA	NA	NA	NA	-2.45	0.038455701	-4.35	1.86E-04
2755	SLC7A14	G	NA	NA	NA	NA	-2.45	4.77E-24	-2.05	9.49E-18
2756	AC130466.1	G	NA	NA	NA	NA	-2.43	4.56E-04	-1.45	3.31E-02
2757	XKR7	G	NA	NA	NA	NA	-2.42	3.64E-234	-3.18	0
2758	NRG3	G	NA	NA	NA	NA	-2.42	1.33E-14	-2.54	1.96E-15
2759	FSCN1P1	G	NA	NA	NA	NA	-2.42	2.33E-07	-2.49	1.82E-07
2760	TUBA4A	G	NA	NA	NA	NA	-2.40	4.00E-17	-2.01	2.63E-14

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2761	VAT1L	G	NA	NA	NA	NA	-2.39	7.42E-51	-2.49	4.02E-54
2762	PLXNA4	G	NA	NA	NA	NA	-2.39	8.55E-140	-1.70	2.09E-73
2763	AC019254.1	G	NA	NA	NA	NA	-2.39	2.73E-04	-1.66	2.62E-02
2764	ULK4P3	G	NA	NA	NA	NA	-2.39	1.15E-12	-1.19	2.38E-04
2765	NFASC	G	NA	NA	NA	NA	-2.38	8.68E-38	-3.19	2.28E-69
2766	DISP3	G	NA	NA	NA	NA	-2.37	2.13E-23	-1.21	1.57E-06
2767	ZNF560	G	NA	NA	NA	NA	-2.33	1.72E-15	-1.36	3.46E-06
2768	AC090971.3	G	NA	NA	NA	NA	-2.33	0.001543214	-1.97	5.79E-03
2769	ADAP1	G	NA	NA	NA	NA	-2.32	4.33E-06	-1.91	3.31E-04
2770	AL138781.2	G	NA	NA	NA	NA	-2.32	0.015111803	-2.20	6.25E-03
2771	L1CAM	G	NA	NA	NA	NA	-2.31	7.21E-90	-3.24	4.30E-174
2772	LMO4	G	NA	NA	NA	NA	-2.31	7.18E-194	-2.33	1.55E-166
2773	GRM5	G	NA	NA	NA	NA	-2.30	0.004907957	-1.69	3.00E-02
2774	RAPGEF3	G	NA	NA	NA	NA	-2.28	8.24E-07	-3.62	9.48E-16
2775	ZCCHC12	G	NA	NA	NA	NA	-2.26	4.12E-24	-2.79	1.84E-44
2776	RPS6KL1	G	NA	NA	NA	NA	-2.26	3.25E-51	-2.08	1.54E-44
2777	VIPR1	G	NA	NA	NA	NA	-2.25	9.36E-05	-1.40	3.12E-02
2778	AC073551.1	G	NA	NA	NA	NA	-2.25	0.013359174	-3.63	1.30E-04
2779	CALB1	G	NA	NA	NA	NA	-2.25	5.11E-07	-2.79	3.33E-10
2780	AC092667.1	G	NA	NA	NA	NA	-2.25	0.014346152	-1.77	4.10E-02
2781	LYSMD4	G	NA	NA	NA	NA	-2.24	1.15E-43	-2.35	4.99E-52
2782	Metazoa_SRP	G	NA	NA	NA	NA	-2.24	1.25E-07	-1.75	5.94E-06
2783	AC092691.1	G	NA	NA	NA	NA	-2.23	5.46E-05	-2.38	1.35E-06
2784	MAN2A2	G	NA	NA	NA	NA	-2.22	3.65E-68	-2.43	3.09E-80
2785	MIAT_exon5_3	G	NA	NA	NA	NA	-2.22	2.67E-09	-2.03	1.15E-09
2786	BICDL1	G	NA	NA	NA	NA	-2.22	2.73E-18	-2.64	5.62E-25
2787	LRRK1	G	NA	NA	NA	NA	-2.20	9.26E-52	-2.45	1.11E-61
2788	LINC01234	G	NA	NA	NA	NA	-2.19	1.36E-39	-1.91	6.48E-26
2789	PHF21B	G	NA	NA	NA	NA	-2.19	3.47E-53	-2.41	1.23E-63
2790	TM6SF1	G	NA	NA	NA	NA	-2.19	0.023033006	-1.69	2.48E-02
2791	PRC1	G	NA	NA	NA	NA	-2.18	2.65E-218	-1.57	4.48E-114
2792	ATP2B2	G	NA	NA	NA	NA	-2.18	6.75E-07	-2.88	7.67E-11
2793	ACHE	G	NA	NA	NA	NA	-2.18	2.70E-05	-2.15	9.58E-07
2794	WHAMM	G	NA	NA	NA	NA	-2.17	9.70E-36	-1.92	1.22E-28
2795	PHACTR1	G	NA	NA	NA	NA	-2.17	5.39E-43	-2.17	1.42E-42
2796	NRG2	G	NA	NA	NA	NA	-2.17	6.55E-13	-3.12	8.74E-22
2797	SPATA41	G	NA	NA	NA	NA	-2.17	7.94E-08	-2.13	4.40E-07
2798	POU4F3	G	NA	NA	NA	NA	-2.16	4.82E-04	-1.39	4.97E-02
2799	PRICKLE1	G	NA	NA	NA	NA	-2.16	2.86E-57	-2.10	9.47E-56
2800	CTBP2P4	G	NA	NA	NA	NA	-2.16	0.021098751	-1.69	4.46E-02
2801	GIPC3	G	NA	NA	NA	NA	-2.16	6.87E-12	-2.33	1.19E-15
2802	EEF1A2	G	NA	NA	NA	NA	-2.15	3.93E-140	-1.56	9.53E-75
2803	WNT6	G	NA	NA	NA	NA	-2.15	0.014306632	-1.89	1.43E-02
2804	ARHGEF16	G	NA	NA	NA	NA	-2.15	1.28E-07	-1.51	4.31E-04
2805	PLPPR4	G	NA	NA	NA	NA	-2.15	1.06E-152	-1.65	6.83E-90
2806	ADARB2	G	NA	NA	NA	NA	-2.14	0.001363005	-2.47	1.38E-04
2807	IGDCC4	G	NA	NA	NA	NA	-2.14	3.48E-12	-1.93	1.33E-10
2808	LINC00672	G	NA	NA	NA	NA	-2.13	9.28E-12	-2.40	1.97E-17
2809	MARCH4	G	NA	NA	NA	NA	-2.13	1.23E-47	-3.09	8.06E-95
2810	ST20	G	NA	NA	NA	NA	-2.12	3.45E-26	-2.03	1.22E-25
2811	IGF2-AS	G	NA	NA	NA	NA	-2.12	4.97E-42	-1.80	6.03E-35
2812	CHRNB4	G	NA	NA	NA	NA	-2.12	3.99E-124	-1.87	2.55E-93
2813	GAP43	G	NA	NA	NA	NA	-2.12	7.92E-246	-2.53	0
2814	ACTL6B	G	NA	NA	NA	NA	-2.12	8.98E-89	-3.05	2.56E-166
2815	AC126773.2	G	NA	NA	NA	NA	-2.12	0.009692427	-2.48	3.51E-04
2816	DAB1	G	NA	NA	NA	NA	-2.11	2.60E-04	-1.53	1.58E-02
2817	AC092335.1	G	NA	NA	NA	NA	-2.11	0.004757105	-3.35	2.73E-04
2818	RIMBP3	G	NA	NA	NA	NA	-2.10	8.61E-04	-2.01	5.19E-03
2819	NYAP2	G	NA	NA	NA	NA	-2.10	2.67E-09	-2.20	2.77E-07
2820	CDH4	G	NA	NA	NA	NA	-2.10	2.82E-05	-1.74	1.87E-03

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2821	KCNH5	G	NA	NA	NA	NA	-2.10	0.009162644	-2.81	3.38E-03
2822	AKAP12	G	NA	NA	NA	NA	-2.09	8.10E-154	-1.66	2.29E-97
2823	DNM1P41	G	NA	NA	NA	NA	-2.09	2.39E-17	-2.14	1.15E-22
2824	PALMD	G	NA	NA	NA	NA	-2.09	2.35E-33	-2.40	1.28E-38
2825	AC068831.7	G	NA	NA	NA	NA	-2.08	6.09E-38	-1.80	6.98E-29
2826	BLM	G	NA	NA	NA	NA	-2.07	1.77E-119	-1.48	1.71E-61
2827	AC110285.1	G	NA	NA	NA	NA	-2.07	0.002666823	-2.62	3.21E-04
2828	ACTG1P17	G	NA	NA	NA	NA	-2.07	0.001056091	-1.48	2.27E-02
2829	U52112.1	G	NA	NA	NA	NA	-2.07	8.53E-14	-3.00	1.44E-22
2830	DGKI	G	NA	NA	NA	NA	-2.06	2.33E-25	-2.21	6.20E-29
2831	PGBD5	G	NA	NA	NA	NA	-2.05	1.72E-32	-2.11	7.76E-34
2832	ARNTL2	G	NA	NA	NA	NA	-2.05	2.59E-19	-1.13	3.72E-06
2833	TUNAR	G	NA	NA	NA	NA	-2.04	0.002465291	-2.12	8.57E-03
2834	CRTC3	G	NA	NA	NA	NA	-2.04	1.94E-85	-1.98	1.19E-82
2835	FANCI	G	NA	NA	NA	NA	-2.04	3.28E-76	-1.29	7.73E-31
2836	REEP1	G	NA	NA	NA	NA	-2.03	3.02E-27	-1.38	8.33E-13
2837	KIAA1024	G	NA	NA	NA	NA	-2.03	3.13E-63	-2.05	4.38E-51
2838	ST8SIA3	G	NA	NA	NA	NA	-2.03	8.69E-43	-2.43	1.59E-60
2839	LINC01578	G	NA	NA	NA	NA	-2.01	7.21E-112	-1.62	2.14E-73
2840	PSD2	G	NA	NA	NA	NA	-1.99	2.69E-12	-2.42	2.94E-19
2841	AC113383.1	G	NA	NA	NA	NA	-1.99	0.032423917	-2.81	1.02E-03
2842	HDDC3	G	NA	NA	NA	NA	-1.99	2.80E-37	-2.45	3.85E-54
2843	SCN3B	G	NA	NA	NA	NA	-1.98	2.51E-138	-2.85	2.86E-251
2844	AC067863.3	G	NA	NA	NA	NA	-1.97	0.020158076	-2.58	5.81E-03
2845	KCNJ15	G	NA	NA	NA	NA	-1.96	2.14E-05	-1.97	3.18E-05
2846	AC243919.1	G	NA	NA	NA	NA	-1.96	9.04E-20	-1.85	2.33E-18
2847	CARMIL2	G	NA	NA	NA	NA	-1.94	8.06E-14	-2.33	7.49E-19
2848	RASSF5	G	NA	NA	NA	NA	-1.92	8.29E-08	-2.41	1.42E-11
2849	AC005394.2	G	NA	NA	NA	NA	-1.92	0.010820215	-1.76	3.06E-02
2850	MCC	G	NA	NA	NA	NA	-1.92	2.77E-70	-2.52	4.71E-119
2851	FUNDC1	G	NA	NA	NA	NA	-1.90	1.28E-68	-1.93	2.47E-70
2852	ST8SIA1	G	NA	NA	NA	NA	-1.89	2.15E-26	-1.26	3.53E-13
2853	KCNB1	G	NA	NA	NA	NA	-1.89	0.030913419	-2.22	8.36E-03
2854	ATP1A3	G	NA	NA	NA	NA	-1.89	1.06E-18	-2.69	2.48E-37
2855	AC078842.1	G	NA	NA	NA	NA	-1.88	0.022242056	-2.60	5.77E-04
2856	IDH2	G	NA	NA	NA	NA	-1.87	8.20E-104	-1.65	1.86E-81
2857	MAP2	G	NA	NA	NA	NA	-1.87	1.50E-93	-1.84	8.22E-91
2858	HDGFL3	G	NA	NA	NA	NA	-1.87	1.62E-185	-1.75	4.50E-163
2859	AC068870.2	G	NA	NA	NA	NA	-1.87	7.68E-05	-2.25	9.63E-08
2860	AP3S2	G	NA	NA	NA	NA	-1.86	2.01E-27	-2.08	4.10E-34
2861	AC092447.5	G	NA	NA	NA	NA	-1.86	1.32E-09	-1.76	8.17E-10
2862	GRM2	G	NA	NA	NA	NA	-1.84	2.33E-08	-2.20	1.79E-11
2863	FRMD5	G	NA	NA	NA	NA	-1.84	1.75E-66	-1.67	5.47E-52
2864	HOXC9	G	NA	NA	NA	NA	-1.84	4.41E-28	-2.38	1.67E-52
2865	LINC02352	G	NA	NA	NA	NA	-1.84	2.76E-04	-2.37	1.07E-06
2866	PRR18	G	NA	NA	NA	NA	-1.84	3.85E-07	-1.44	1.78E-04
2867	SLC8A1	G	NA	NA	NA	NA	-1.84	3.92E-53	-1.88	4.55E-54
2868	SCARNA15	G	NA	NA	NA	NA	-1.83	1.45E-20	-1.24	4.52E-09
2869	GOLGA2P7	G	NA	NA	NA	NA	-1.82	1.17E-85	-1.59	3.84E-66
2870	FUT9	G	NA	NA	NA	NA	-1.81	1.84E-57	-2.62	1.78E-113
2871	TICRR	G	NA	NA	NA	NA	-1.80	6.05E-174	-1.17	3.77E-80
2872	ARNT2	G	NA	NA	NA	NA	-1.80	7.27E-81	-1.89	5.03E-89
2873	NRSN1	G	NA	NA	NA	NA	-1.80	4.75E-38	-2.07	6.66E-47
2874	SNAP91	G	NA	NA	NA	NA	-1.80	4.73E-13	-1.91	1.20E-14
2875	MORF4L1	G	NA	NA	NA	NA	-1.79	1.81E-78	-1.62	5.81E-64
2876	SGIP1	G	NA	NA	NA	NA	-1.79	1.60E-120	-2.11	1.04E-161
2877	ZNF592	G	NA	NA	NA	NA	-1.79	3.20E-14	-1.37	1.37E-08
2878	AC067863.2	G	NA	NA	NA	NA	-1.79	1.35E-09	-1.05	1.89E-03
2879	PLXND1	G	NA	NA	NA	NA	-1.79	1.12E-48	-2.11	2.60E-61
2880	RUNX3	G	NA	NA	NA	NA	-1.78	4.79E-15	-1.75	3.54E-12

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2881	PCDHGB1	G	NA	NA	NA	NA	-1.77	1.92E-09	-2.15	4.89E-17
2882	LINS1	G	NA	NA	NA	NA	-1.77	1.05E-83	-1.59	2.34E-68
2883	KRTAP5-1	G	NA	NA	NA	NA	-1.77	0.003312552	-1.63	5.61E-04
2884	AC023300.3	G	NA	NA	NA	NA	-1.77	0.008580274	-2.70	9.36E-05
2885	GOLGA6L4	G	NA	NA	NA	NA	-1.77	1.12E-26	-1.75	4.71E-30
2886	FAM135B	G	NA	NA	NA	NA	-1.77	0.018673834	-2.61	9.60E-04
2887	NCAM1	G	NA	NA	NA	NA	-1.77	5.55E-148	-1.76	9.68E-150
2888	CHRNA5	G	NA	NA	NA	NA	-1.76	1.73E-97	-1.40	5.89E-60
2889	AL589765.1	G	NA	NA	NA	NA	-1.76	8.31E-06	-2.59	1.68E-10
2890	SEC11A	G	NA	NA	NA	NA	-1.76	1.81E-111	-1.65	1.97E-97
2891	TMOD2	G	NA	NA	NA	NA	-1.76	6.03E-80	-1.84	1.40E-86
2892	SEL1L3	G	NA	NA	NA	NA	-1.74	2.53E-04	-2.54	1.79E-09
2893	SLC18A2	G	NA	NA	NA	NA	-1.74	5.21E-08	-1.68	9.61E-08
2894	GLRA3	G	NA	NA	NA	NA	-1.73	1.82E-04	-2.02	9.80E-06
2895	CASC1	G	NA	NA	NA	NA	-1.73	3.52E-04	-2.46	5.93E-09
2896	SCAND2P	G	NA	NA	NA	NA	-1.73	4.34E-11	-1.75	3.88E-13
2897	CRTAC1	G	NA	NA	NA	NA	-1.73	4.24E-07	-2.07	5.86E-09
2898	TTC39A	G	NA	NA	NA	NA	-1.73	8.72E-08	-1.06	2.76E-03
2899	RD3	G	NA	NA	NA	NA	-1.72	3.06E-44	-1.53	3.35E-35
2900	CCDC3	G	NA	NA	NA	NA	-1.72	1.09E-40	-1.73	6.98E-48
2901	AC003973.3	G	NA	NA	NA	NA	-1.72	2.31E-11	-1.88	7.01E-13
2902	AKAP13	G	NA	NA	NA	NA	-1.72	6.24E-16	-1.17	8.70E-08
2903	ACVRL1	G	NA	NA	NA	NA	-1.71	3.22E-08	-2.53	1.24E-16
2904	TARSL2	G	NA	NA	NA	NA	-1.71	3.78E-25	-2.05	2.30E-35
2905	ALPL	G	NA	NA	NA	NA	-1.71	2.52E-11	-1.34	3.04E-07
2906	SPTBN5	G	NA	NA	NA	NA	-1.71	0.029315218	-2.84	1.13E-04
2907	LRRC28	G	NA	NA	NA	NA	-1.70	2.44E-83	-1.67	1.72E-80
2908	CPLX1	G	NA	NA	NA	NA	-1.70	1.28E-19	-2.98	7.38E-58
2909	MEX3B	G	NA	NA	NA	NA	-1.70	1.42E-93	-1.57	1.77E-86
2910	TNIK	G	NA	NA	NA	NA	-1.69	2.20E-53	-1.05	5.33E-21
2911	KCTD16	G	NA	NA	NA	NA	-1.69	1.37E-09	-1.13	4.05E-05
2912	NPTN-IT1	G	NA	NA	NA	NA	-1.69	3.03E-10	-1.89	3.41E-13
2913	HOMER2	G	NA	NA	NA	NA	-1.69	2.27E-07	-1.94	1.79E-09
2914	DET1	G	NA	NA	NA	NA	-1.69	1.19E-41	-1.77	7.43E-45
2915	PTPRN2	G	NA	NA	NA	NA	-1.69	1.89E-38	-1.70	1.35E-38
2916	NAAA	G	NA	NA	NA	NA	-1.69	2.33E-07	-1.92	3.68E-08
2917	EFL1	G	NA	NA	NA	NA	-1.69	7.91E-14	-1.49	5.55E-11
2918	PRNP	G	NA	NA	NA	NA	-1.69	1.00E-55	-1.47	4.16E-44
2919	AC092919.2	G	NA	NA	NA	NA	-1.68	1.32E-04	-1.14	1.46E-02
2920	TLNRD1	G	NA	NA	NA	NA	-1.68	3.63E-98	-1.29	7.45E-63
2921	MYT1L	G	NA	NA	NA	NA	-1.68	1.15E-55	-1.33	1.21E-33
2922	MRPS11	G	NA	NA	NA	NA	-1.68	3.52E-73	-1.62	6.10E-69
2923	AC138649.1	G	NA	NA	NA	NA	-1.68	3.27E-30	-1.39	2.19E-22
2924	ADAMTS7	G	NA	NA	NA	NA	-1.68	4.40E-20	-2.15	1.87E-32
2925	RRAGD	G	NA	NA	NA	NA	-1.67	7.08E-36	-1.47	1.90E-29
2926	GRHL1	G	NA	NA	NA	NA	-1.67	1.63E-07	-1.78	1.24E-07
2927	AC118758.3	G	NA	NA	NA	NA	-1.67	3.18E-04	-2.36	8.41E-08
2928	AC091951.4	G	NA	NA	NA	NA	-1.67	2.93E-04	-1.09	1.25E-02
2929	SLC48A1	G	NA	NA	NA	NA	-1.67	1.40E-33	-1.94	1.97E-45
2930	AC090825.1	G	NA	NA	NA	NA	-1.66	4.56E-07	-1.78	1.01E-08
2931	ARID3B	G	NA	NA	NA	NA	-1.66	3.88E-139	-1.30	5.91E-91
2932	RPS17	G	NA	NA	NA	NA	-1.66	1.44E-140	-1.62	1.93E-134
2933	ADGRB3	G	NA	NA	NA	NA	-1.65	1.55E-37	-1.65	7.63E-38
2934	FAM103A1	G	NA	NA	NA	NA	-1.65	1.42E-83	-1.44	8.85E-66
2935	ABHD2	G	NA	NA	NA	NA	-1.64	4.67E-34	-1.71	7.74E-37
2936	TMEM121B	G	NA	NA	NA	NA	-1.64	1.35E-54	-2.07	6.55E-85
2937	RCCD1	G	NA	NA	NA	NA	-1.64	0.008034502	-1.74	4.36E-03
2938	ISLR	G	NA	NA	NA	NA	-1.64	1.60E-13	-2.75	3.70E-35
2939	KLHL25	G	NA	NA	NA	NA	-1.64	2.98E-23	-1.49	1.34E-19
2940	GPR176	G	NA	NA	NA	NA	-1.63	9.68E-54	-1.82	3.49E-77

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
2941	STXBP2	G	NA	NA	NA	NA	-1.63	0.014906048	-2.21	5.53E-04
2942	MYO18B	G	NA	NA	NA	NA	-1.63	0.001846942	-1.42	7.75E-03
2943	NGRN	G	NA	NA	NA	NA	-1.62	9.04E-136	-1.38	9.82E-99
2944	TTBK1	G	NA	NA	NA	NA	-1.62	9.11E-41	-1.95	2.21E-57
2945	PKN2-AS1	G	NA	NA	NA	NA	-1.61	0.025446565	-1.45	3.33E-02
2946	ABHD17C	G	NA	NA	NA	NA	-1.60	1.19E-30	-1.14	2.79E-14
2947	ZNF492	G	NA	NA	NA	NA	-1.60	4.77E-33	-1.03	1.35E-14
2948	SYN1	G	NA	NA	NA	NA	-1.60	3.92E-43	-2.61	3.66E-97
2949	BRWD1	G	NA	NA	NA	NA	-1.60	1.43E-82	-1.57	3.96E-80
2950	TM2D3	G	NA	NA	NA	NA	-1.60	6.62E-150	-1.71	2.88E-166
2951	NPTN	G	NA	NA	NA	NA	-1.60	7.00E-89	-1.66	5.49E-96
2952	ZDHHC13	G	NA	NA	NA	NA	-1.59	1.32E-54	-1.06	7.37E-25
2953	AP001972.5	G	NA	NA	NA	NA	-1.57	2.25E-79	-2.06	1.71E-134
2954	CELF3	G	NA	NA	NA	NA	-1.57	1.35E-24	-2.88	6.11E-81
2955	GOLGA6L9	G	NA	NA	NA	NA	-1.57	8.85E-109	-1.76	6.77E-141
2956	WFIKKN2	G	NA	NA	NA	NA	-1.57	0.019698895	-1.60	3.60E-02
2957	ZNF710-AS1	G	NA	NA	NA	NA	-1.57	7.99E-36	-1.55	3.42E-40
2958	SYNGR1	G	NA	NA	NA	NA	-1.56	8.31E-24	-1.66	2.17E-28
2959	TTC9	G	NA	NA	NA	NA	-1.56	1.02E-42	-1.41	5.85E-40
2960	DNM1P46	G	NA	NA	NA	NA	-1.56	2.52E-04	-1.33	1.19E-03
2961	CCDC92B	G	NA	NA	NA	NA	-1.56	2.04E-05	-1.82	8.46E-08
2962	HNRNPLP2	G	NA	NA	NA	NA	-1.56	2.49E-18	-1.18	2.53E-12
2963	CHRFAM7A	G	NA	NA	NA	NA	-1.56	0.007156901	-1.26	3.69E-02
2964	CSPG4P12	G	NA	NA	NA	NA	-1.55	1.35E-25	-1.69	2.37E-33
2965	MRPL46	G	NA	NA	NA	NA	-1.55	1.31E-34	-1.51	1.16E-30
2966	AC015871.3	G	NA	NA	NA	NA	-1.55	1.41E-14	-1.96	6.44E-23
2967	PARP6	G	NA	NA	NA	NA	-1.55	2.85E-105	-1.74	3.36E-133
2968	FAM167A	G	NA	NA	NA	NA	-1.55	4.51E-65	-1.87	5.51E-96
2969	MAP6	G	NA	NA	NA	NA	-1.54	9.39E-64	-1.56	6.06E-64
2970	BTBD1	G	NA	NA	NA	NA	-1.54	9.84E-77	-1.59	2.81E-80
2971	HPCAL1	G	NA	NA	NA	NA	-1.54	2.61E-12	-2.00	2.94E-20
2972	ADAMTS7P1	G	NA	NA	NA	NA	-1.54	0.031540724	-3.23	2.74E-04
2973	TMED3	G	NA	NA	NA	NA	-1.54	4.20E-60	-1.58	7.08E-62
2974	CRLF1	G	NA	NA	NA	NA	-1.54	7.68E-11	-1.91	1.55E-17
2975	PCDHGA9	G	NA	NA	NA	NA	-1.54	3.16E-15	-2.17	1.08E-29
2976	AC012213.5	G	NA	NA	NA	NA	-1.53	0.006051567	-1.25	3.13E-02
2977	NEFM	G	NA	NA	NA	NA	-1.53	4.56E-98	-2.40	8.45E-243
2978	MIAT	G	NA	NA	NA	NA	-1.52	1.14E-22	-2.17	2.73E-45
2979	UBE2Q2P2	G	NA	NA	NA	NA	-1.52	1.84E-23	-1.40	1.66E-21
2980	AP001107.5	G	NA	NA	NA	NA	-1.52	0.002523449	-1.98	5.92E-06
2981	AC245033.4	G	NA	NA	NA	NA	-1.51	0.007557051	-1.77	1.24E-03
2982	AC245033.3	G	NA	NA	NA	NA	-1.51	5.16E-05	-1.90	4.77E-08
2983	ZSCAN2	G	NA	NA	NA	NA	-1.50	4.68E-16	-1.86	1.97E-24
2984	C15orf40	G	NA	NA	NA	NA	-1.50	4.04E-18	-1.31	6.29E-14
2985	HOXC10	G	NA	NA	NA	NA	-1.50	2.62E-41	-2.09	3.24E-84
2986	HOXC-AS1	G	NA	NA	NA	NA	-1.50	0.001993084	-1.54	1.32E-04
2987	SNHG21	G	NA	NA	NA	NA	-1.49	7.69E-11	-1.25	8.40E-07
2988	MGAT5B	G	NA	NA	NA	NA	-1.49	8.49E-14	-1.73	4.75E-18
2989	AL136366.1	G	NA	NA	NA	NA	-1.49	0.013771576	-1.32	1.92E-02
2990	UNC45A	G	NA	NA	NA	NA	-1.48	2.70E-57	-1.63	8.35E-70
2991	ADAM12	G	NA	NA	NA	NA	-1.48	2.25E-47	-2.21	8.75E-101
2992	CBLN1	G	NA	NA	NA	NA	-1.48	8.85E-18	-1.57	2.19E-17
2993	ST20-AS1	G	NA	NA	NA	NA	-1.48	1.19E-06	-1.99	2.74E-11
2994	KLHL29	G	NA	NA	NA	NA	-1.48	2.00E-43	-1.53	5.87E-37
2995	FURIN	G	NA	NA	NA	NA	-1.47	8.36E-07	-1.63	3.14E-08
2996	ZFAND6	G	NA	NA	NA	NA	-1.47	3.44E-85	-1.81	4.75E-130
2997	ZNF718	G	NA	NA	NA	NA	-1.47	7.68E-20	-1.35	8.34E-19
2998	HEBP2	G	NA	NA	NA	NA	-1.47	2.42E-26	-1.07	3.79E-15
2999	AC027348.1	G	NA	NA	NA	NA	-1.47	0.004477953	-1.78	2.99E-04
3000	SYNM	G	NA	NA	NA	NA	-1.46	1.02E-69	-1.35	5.14E-58

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3001	GOLGA2P10	G	NA	NA	NA	NA	-1.46	2.99E-23	-1.78	1.71E-34
3002	RFLNA	G	NA	NA	NA	NA	-1.46	9.45E-04	-1.01	4.26E-02
3003	NTN4	G	NA	NA	NA	NA	-1.45	9.22E-04	-1.03	3.15E-02
3004	MME	G	NA	NA	NA	NA	-1.45	4.97E-16	-1.39	1.18E-16
3005	NDRG4	G	NA	NA	NA	NA	-1.45	1.57E-23	-2.02	8.24E-46
3006	GOLGA6L5P	G	NA	NA	NA	NA	-1.45	4.92E-21	-1.57	1.37E-26
3007	UBE2Q2L	G	NA	NA	NA	NA	-1.44	0.009453326	-1.67	6.37E-04
3008	PRKCE	G	NA	NA	NA	NA	-1.44	1.14E-11	-1.00	5.44E-06
3009	PREX1	G	NA	NA	NA	NA	-1.44	2.40E-06	-1.64	5.82E-08
3010	ELAVL4	G	NA	NA	NA	NA	-1.43	5.34E-231	-1.10	3.56E-136
3011	CSPG4P10	G	NA	NA	NA	NA	-1.43	1.08E-21	-1.76	4.17E-34
3012	TNFAIP2	G	NA	NA	NA	NA	-1.42	3.17E-11	-2.28	1.45E-25
3013	PCDHGB2	G	NA	NA	NA	NA	-1.42	2.55E-10	-1.59	3.10E-14
3014	AC027559.1	G	NA	NA	NA	NA	-1.42	0.011441497	-2.44	9.01E-07
3015	AC066613.1	G	NA	NA	NA	NA	-1.42	0.005880518	-1.22	1.69E-02
3016	SMIM18	G	NA	NA	NA	NA	-1.42	3.62E-18	-1.67	7.65E-25
3017	C19orf57	G	NA	NA	NA	NA	-1.42	6.10E-12	-1.10	7.93E-08
3018	SELENOS	G	NA	NA	NA	NA	-1.41	6.99E-55	-1.06	6.13E-32
3019	CSPG4P5	G	NA	NA	NA	NA	-1.41	1.13E-04	-1.65	1.61E-06
3020	SNX22	G	NA	NA	NA	NA	-1.41	1.52E-15	-1.12	6.89E-10
3021	AC110491.1	G	NA	NA	NA	NA	-1.41	1.97E-04	-2.00	3.56E-08
3022	RN7SL417P	G	NA	NA	NA	NA	-1.41	5.62E-04	-1.81	8.94E-07
3023	CSPG4P11	G	NA	NA	NA	NA	-1.40	5.96E-08	-1.82	1.09E-13
3024	AC000068.1	G	NA	NA	NA	NA	-1.40	3.02E-05	-1.63	2.94E-08
3025	SEMA7A	G	NA	NA	NA	NA	-1.40	1.69E-08	-1.38	1.23E-07
3026	AL590560.2	G	NA	NA	NA	NA	-1.40	0.010788448	-1.45	3.99E-03
3027	FKBP1B	G	NA	NA	NA	NA	-1.39	7.44E-40	-1.18	5.78E-32
3028	CARD10	G	NA	NA	NA	NA	-1.39	6.22E-05	-2.16	4.91E-11
3029	AC132825.2	G	NA	NA	NA	NA	-1.39	0.009644893	-1.40	5.53E-03
3030	MESD	G	NA	NA	NA	NA	-1.38	2.92E-105	-1.26	3.82E-89
3031	ANK3	G	NA	NA	NA	NA	-1.38	3.52E-09	-1.93	5.89E-17
3032	CRMP1	G	NA	NA	NA	NA	-1.38	7.49E-87	-1.58	1.34E-113
3033	FBLL1	G	NA	NA	NA	NA	-1.38	2.88E-11	-1.45	2.40E-12
3034	DPYD	G	NA	NA	NA	NA	-1.38	1.23E-27	-1.52	2.98E-30
3035	ABALON	G	NA	NA	NA	NA	-1.37	9.14E-04	-1.05	9.33E-03
3036	ABCG1	G	NA	NA	NA	NA	-1.37	8.71E-19	-1.60	1.27E-31
3037	MATN3	G	NA	NA	NA	NA	-1.37	1.18E-11	-1.24	4.54E-10
3038	LINC01105	G	NA	NA	NA	NA	-1.37	6.24E-09	-1.57	4.55E-13
3039	EPHA6	G	NA	NA	NA	NA	-1.37	1.91E-13	-1.46	2.88E-16
3040	TGM1	G	NA	NA	NA	NA	-1.36	0.004314905	-1.17	3.81E-02
3041	SLC1A2	G	NA	NA	NA	NA	-1.36	4.27E-05	-1.52	2.28E-06
3042	ZNF849P	G	NA	NA	NA	NA	-1.36	1.42E-08	-1.15	1.02E-07
3043	ASB7	G	NA	NA	NA	NA	-1.36	1.74E-46	-1.44	2.12E-53
3044	USP32P3	G	NA	NA	NA	NA	-1.36	0.002848464	-1.10	1.58E-02
3045	AC012213.1	G	NA	NA	NA	NA	-1.35	8.54E-06	-2.46	2.67E-15
3046	RN7SL239P	G	NA	NA	NA	NA	-1.35	0.042744418	-1.89	9.57E-03
3047	AC011365.2	G	NA	NA	NA	NA	-1.35	0.017488404	-1.28	4.03E-02
3048	C1QTNF1	G	NA	NA	NA	NA	-1.35	1.41E-04	-2.01	5.90E-10
3049	GPR85	G	NA	NA	NA	NA	-1.34	2.87E-15	-2.22	8.61E-40
3050	GABRP	G	NA	NA	NA	NA	-1.34	0.027093611	-1.29	2.91E-02
3051	CPEB1	G	NA	NA	NA	NA	-1.34	2.31E-08	-1.82	3.37E-14
3052	TMEM54	G	NA	NA	NA	NA	-1.34	1.08E-08	-1.77	6.58E-16
3053	EFR3B	G	NA	NA	NA	NA	-1.33	8.23E-133	-1.10	4.86E-93
3054	MEF2A	G	NA	NA	NA	NA	-1.33	3.12E-33	-1.52	2.13E-43
3055	EMILIN2	G	NA	NA	NA	NA	-1.33	7.92E-15	-1.51	1.85E-17
3056	CCM2L	G	NA	NA	NA	NA	-1.33	7.05E-04	-1.30	1.10E-03
3057	KLHL3	G	NA	NA	NA	NA	-1.32	9.61E-09	-1.29	1.24E-08
3058	SEMA4B	G	NA	NA	NA	NA	-1.32	4.08E-05	-2.00	6.07E-11
3059	ISL2	G	NA	NA	NA	NA	-1.31	1.79E-08	-1.12	2.44E-06
3060	KCNJ11	G	NA	NA	NA	NA	-1.31	0.015239825	-1.47	1.40E-02

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3061	SHC4	G	NA	NA	NA	NA	-1.31	9.04E-06	-1.68	1.93E-11
3062	ASPHD1	G	NA	NA	NA	NA	-1.31	5.14E-10	-2.46	7.66E-32
3063	AC114811.2	G	NA	NA	NA	NA	-1.31	0.009215118	-1.51	1.24E-04
3064	FOXN4	G	NA	NA	NA	NA	-1.31	1.51E-41	-1.28	7.02E-45
3065	WDR73	G	NA	NA	NA	NA	-1.31	2.22E-34	-1.43	3.35E-41
3066	CDHR2	G	NA	NA	NA	NA	-1.30	1.64E-28	-1.48	1.55E-36
3067	VPS33B	G	NA	NA	NA	NA	-1.29	5.15E-27	-1.39	4.51E-30
3068	OGDHL	G	NA	NA	NA	NA	-1.29	1.06E-31	-1.72	1.01E-53
3069	AC011767.1	G	NA	NA	NA	NA	-1.29	5.12E-05	-1.67	3.60E-08
3070	SCAMP5	G	NA	NA	NA	NA	-1.29	4.65E-49	-1.63	4.23E-78
3071	STRA6	G	NA	NA	NA	NA	-1.29	9.49E-22	-1.74	2.18E-39
3072	SFMBT2	G	NA	NA	NA	NA	-1.28	9.74E-113	-1.19	5.10E-108
3073	AC010931.2	G	NA	NA	NA	NA	-1.28	6.36E-46	-1.31	2.64E-45
3074	SH2D7	G	NA	NA	NA	NA	-1.27	5.10E-07	-1.23	1.37E-07
3075	MCF2L2	G	NA	NA	NA	NA	-1.27	5.88E-04	-1.73	1.42E-06
3076	LRRC8C	G	NA	NA	NA	NA	-1.27	3.64E-40	-1.06	3.70E-29
3077	SCRT1	G	NA	NA	NA	NA	-1.27	1.30E-06	-2.38	2.31E-21
3078	RIIAD1	G	NA	NA	NA	NA	-1.27	0.001136995	-2.96	1.56E-15
3079	FRG1KP	G	NA	NA	NA	NA	-1.27	0.023154884	-1.50	3.22E-03
3080	GABPB1-IT1	G	NA	NA	NA	NA	-1.26	4.74E-39	-1.56	7.42E-61
3081	DUSP8	G	NA	NA	NA	NA	-1.26	7.45E-13	-1.17	1.25E-11
3082	AC017076.1	G	NA	NA	NA	NA	-1.26	7.04E-04	-1.21	1.02E-04
3083	BRINP1	G	NA	NA	NA	NA	-1.26	8.70E-06	-1.83	7.39E-10
3084	CSPG4	G	NA	NA	NA	NA	-1.26	0.008464659	-1.28	9.76E-03
3085	RIMS4	G	NA	NA	NA	NA	-1.26	2.27E-52	-1.42	1.13E-63
3086	RAP1GAP	G	NA	NA	NA	NA	-1.25	8.46E-19	-1.10	1.96E-14
3087	STX1A	G	NA	NA	NA	NA	-1.24	6.86E-36	-1.54	7.77E-54
3088	POLG	G	NA	NA	NA	NA	-1.24	5.63E-05	-1.81	7.98E-10
3089	KDM6A	G	NA	NA	NA	NA	-1.24	3.19E-16	-1.03	1.53E-11
3090	PRDM8	G	NA	NA	NA	NA	-1.24	2.83E-08	-1.83	9.21E-18
3091	PDE4C	G	NA	NA	NA	NA	-1.24	0.008252065	-2.12	8.52E-07
3092	AKAP5	G	NA	NA	NA	NA	-1.24	1.78E-17	-1.25	4.88E-18
3093	AC139530.3	G	NA	NA	NA	NA	-1.24	4.84E-04	-1.66	6.96E-08
3094	AC016705.2	G	NA	NA	NA	NA	-1.24	2.97E-09	-1.41	1.43E-11
3095	AC092117.1	G	NA	NA	NA	NA	-1.24	9.34E-04	-1.38	9.70E-06
3096	AC243562.3	G	NA	NA	NA	NA	-1.23	7.37E-10	-1.40	1.71E-13
3097	DUOX1	G	NA	NA	NA	NA	-1.23	0.008160823	-1.04	3.26E-02
3098	AC013489.1	G	NA	NA	NA	NA	-1.22	2.91E-14	-1.23	2.18E-13
3099	OLFM1	G	NA	NA	NA	NA	-1.22	5.25E-62	-1.40	3.11E-78
3100	GOLGA8K	G	NA	NA	NA	NA	-1.22	4.01E-06	-1.46	2.00E-08
3101	ROBO2	G	NA	NA	NA	NA	-1.22	3.58E-27	-1.36	3.84E-33
3102	AC005696.4	G	NA	NA	NA	NA	-1.22	4.01E-33	-2.28	1.37E-98
3103	COTL1	G	NA	NA	NA	NA	-1.22	1.02E-26	-1.02	4.08E-18
3104	ZNF833P	G	NA	NA	NA	NA	-1.21	0.039254655	-1.11	4.18E-02
3105	GRIK2	G	NA	NA	NA	NA	-1.20	5.98E-19	-2.01	2.08E-51
3106	TRIM71	G	NA	NA	NA	NA	-1.20	8.72E-20	-1.37	7.76E-27
3107	NMB	G	NA	NA	NA	NA	-1.20	7.09E-13	-1.07	1.08E-10
3108	AC233266.2	G	NA	NA	NA	NA	-1.20	1.28E-11	-2.33	1.22E-40
3109	TUBB4A	G	NA	NA	NA	NA	-1.20	1.29E-22	-1.17	2.88E-21
3110	MAGEA10	G	NA	NA	NA	NA	-1.19	1.65E-39	-1.25	1.06E-42
3111	GAS6-AS2	G	NA	NA	NA	NA	-1.19	0.01633951	-1.82	3.61E-05
3112	FCHO1	G	NA	NA	NA	NA	-1.19	1.82E-04	-1.89	8.22E-10
3113	CDAN1	G	NA	NA	NA	NA	-1.19	1.02E-12	-1.03	5.33E-10
3114	MAPRE3	G	NA	NA	NA	NA	-1.19	7.62E-58	-1.69	2.53E-134
3115	UBE2Q2P1	G	NA	NA	NA	NA	-1.18	0.031156688	-1.66	1.11E-03
3116	SHC2	G	NA	NA	NA	NA	-1.18	3.93E-05	-1.73	1.75E-10
3117	PHLDA1	G	NA	NA	NA	NA	-1.18	3.29E-19	-1.11	2.94E-19
3118	AC007495.1	G	NA	NA	NA	NA	-1.17	5.23E-05	-1.31	6.88E-06
3119	SVIP	G	NA	NA	NA	NA	-1.17	1.84E-09	-1.13	6.65E-09
3120	GPER1	G	NA	NA	NA	NA	-1.17	0.031932474	-1.80	2.83E-04

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3121	KIRREL2	G	NA	NA	NA	NA	-1.17	9.70E-10	-1.55	1.55E-18
3122	POPDC3	G	NA	NA	NA	NA	-1.17	1.58E-23	-1.06	8.32E-21
3123	MFGE8	G	NA	NA	NA	NA	-1.16	9.87E-21	-1.46	2.79E-33
3124	CHN2	G	NA	NA	NA	NA	-1.16	8.00E-09	-1.79	2.03E-21
3125	SEMA6D	G	NA	NA	NA	NA	-1.16	1.56E-21	-1.69	2.99E-48
3126	GATM	G	NA	NA	NA	NA	-1.16	3.26E-04	-1.34	3.87E-05
3127	AEN	G	NA	NA	NA	NA	-1.16	6.00E-43	-1.25	3.40E-46
3128	ATCAY	G	NA	NA	NA	NA	-1.16	1.69E-48	-1.31	1.96E-62
3129	DLGAP3	G	NA	NA	NA	NA	-1.16	1.31E-06	-2.05	2.07E-18
3130	SPSB4	G	NA	NA	NA	NA	-1.15	2.74E-39	-1.07	1.04E-34
3131	SCARA3	G	NA	NA	NA	NA	-1.15	7.61E-27	-1.09	5.32E-25
3132	AC078909.1	G	NA	NA	NA	NA	-1.15	0.032315066	-1.12	1.50E-02
3133	AP001626.1	G	NA	NA	NA	NA	-1.14	0.027573933	-1.05	3.92E-02
3134	KIF7	G	NA	NA	NA	NA	-1.14	4.24E-13	-1.53	2.94E-23
3135	ASRGL1	G	NA	NA	NA	NA	-1.14	7.95E-20	-1.22	3.64E-22
3136	PDE8A	G	NA	NA	NA	NA	-1.14	8.86E-08	-1.49	4.23E-13
3137	ZDHHC11	G	NA	NA	NA	NA	-1.13	2.47E-05	-1.80	2.86E-10
3138	LBX2-AS1	G	NA	NA	NA	NA	-1.13	1.10E-05	-1.46	1.08E-10
3139	C15orf52	G	NA	NA	NA	NA	-1.12	3.05E-10	-1.42	8.92E-17
3140	MED12L	G	NA	NA	NA	NA	-1.12	8.68E-05	-1.04	2.96E-04
3141	SSUH2	G	NA	NA	NA	NA	-1.12	0.005103772	-1.16	1.94E-03
3142	FAIM2	G	NA	NA	NA	NA	-1.12	7.58E-08	-1.33	3.67E-11
3143	CORO2A	G	NA	NA	NA	NA	-1.12	6.02E-31	-1.94	5.07E-89
3144	SCD5	G	NA	NA	NA	NA	-1.11	2.24E-63	-1.12	4.79E-71
3145	LYL1	G	NA	NA	NA	NA	-1.11	1.34E-04	-1.14	1.13E-05
3146	GBP4	G	NA	NA	NA	NA	-1.11	0.029055115	-1.83	3.71E-05
3147	ZNF423	G	NA	NA	NA	NA	-1.11	3.47E-15	-1.20	2.16E-17
3148	KCNC1	G	NA	NA	NA	NA	-1.10	9.06E-11	-1.59	3.90E-22
3149	BSN	G	NA	NA	NA	NA	-1.10	2.86E-16	-2.05	6.26E-53
3150	AC006538.1	G	NA	NA	NA	NA	-1.10	1.69E-07	-1.03	1.09E-06
3151	ARRB1	G	NA	NA	NA	NA	-1.10	4.76E-10	-1.64	1.81E-21
3152	PCDHB16	G	NA	NA	NA	NA	-1.10	0.025109508	-1.58	3.18E-04
3153	FMN1	G	NA	NA	NA	NA	-1.09	2.20E-05	-1.29	1.87E-07
3154	MT-TL1	G	NA	NA	NA	NA	-1.08	8.02E-23	-1.41	1.70E-38
3155	UNC13A	G	NA	NA	NA	NA	-1.08	3.90E-17	-1.78	4.10E-45
3156	NAPB	G	NA	NA	NA	NA	-1.08	7.19E-22	-1.06	7.38E-21
3157	KIAA0513	G	NA	NA	NA	NA	-1.07	2.86E-05	-1.25	3.44E-07
3158	TOP2B	G	NA	NA	NA	NA	-1.07	1.32E-33	-1.24	1.07E-44
3159	DCLK1	G	NA	NA	NA	NA	-1.07	1.94E-27	-1.06	8.28E-28
3160	SORL1	G	NA	NA	NA	NA	-1.06	1.64E-28	-2.10	1.94E-106
3161	NACAD	G	NA	NA	NA	NA	-1.06	1.29E-11	-1.54	8.09E-24
3162	AL845472.1	G	NA	NA	NA	NA	-1.06	1.70E-18	-1.05	1.03E-17
3163	KIF26B	G	NA	NA	NA	NA	-1.06	4.91E-37	-1.27	1.68E-52
3164	FAM81A	G	NA	NA	NA	NA	-1.06	2.10E-05	-1.25	1.94E-06
3165	AC027020.2	G	NA	NA	NA	NA	-1.06	1.06E-06	-1.00	8.73E-07
3166	PEAR1	G	NA	NA	NA	NA	-1.05	6.78E-06	-1.16	3.44E-07
3167	SH3PXD2A	G	NA	NA	NA	NA	-1.05	1.98E-131	-1.16	4.54E-169
3168	ANO5	G	NA	NA	NA	NA	-1.05	9.93E-14	-1.09	6.98E-14
3169	ADD2	G	NA	NA	NA	NA	-1.04	4.10E-30	-1.02	3.09E-28
3170	TMEM59L	G	NA	NA	NA	NA	-1.04	1.43E-04	-1.88	2.56E-13
3171	CCDC33	G	NA	NA	NA	NA	-1.04	4.31E-11	-1.97	1.03E-37
3172	SULT4A1	G	NA	NA	NA	NA	-1.03	3.82E-10	-1.23	6.18E-14
3173	ZNF501	G	NA	NA	NA	NA	-1.03	1.06E-06	-1.38	1.58E-11
3174	KLHDC8B	G	NA	NA	NA	NA	-1.03	1.08E-11	-1.79	2.65E-34
3175	DMXL2	G	NA	NA	NA	NA	-1.03	2.06E-22	-1.05	1.50E-23
3176	GADD45A	G	NA	NA	NA	NA	-1.02	1.20E-22	-1.02	2.76E-25
3177	ZNF208	G	NA	NA	NA	NA	-1.02	7.47E-05	-1.49	1.29E-09
3178	HOXC11	G	NA	NA	NA	NA	-1.02	1.49E-06	-1.53	3.33E-15
3179	TBX18	G	NA	NA	NA	NA	-1.01	0.048024166	-1.63	3.35E-04
3180	FGF19	G	NA	NA	NA	NA	-1.01	0.024884742	-1.02	1.59E-02

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3181	SFRP1	G	NA	NA	NA	NA	-1.01	2.46E-44	-1.38	2.35E-82
3182	SRRM3	G	NA	NA	NA	NA	-1.01	1.56E-13	-1.50	1.11E-28
3183	SYT11	G	NA	NA	NA	NA	-1.01	2.96E-70	-1.03	3.65E-74
3184	LHFPL3	G	NA	NA	NA	NA	-1.01	1.33E-07	-1.20	1.11E-09
3185	ZBED6CL	G	NA	NA	NA	NA	-1.01	3.27E-15	-1.01	2.03E-14
3186	GRIK5	G	NA	NA	NA	NA	-1.01	5.80E-15	-1.73	1.39E-42
3187	PEX11A	G	NA	NA	NA	NA	-1.00	1.15E-14	-1.11	3.62E-19
3188	GSC	G	NA	NA	NA	NA	1.00	0.003236903	1.06	9.60E-04
3189	TMEM229A	G	NA	NA	NA	NA	1.01	1.50E-42	1.16	3.77E-54
3190	CD99P1	G	NA	NA	NA	NA	1.02	0.006930425	1.27	5.46E-04
3191	NAP1L5	G	NA	NA	NA	NA	1.02	2.63E-28	1.13	9.85E-37
3192	MOB3B	G	NA	NA	NA	NA	1.02	1.57E-12	1.24	4.44E-16
3193	AL353813.1	G	NA	NA	NA	NA	1.03	9.86E-09	1.64	5.48E-17
3194	RNASET2	G	NA	NA	NA	NA	1.04	8.39E-20	1.14	2.26E-23
3195	TWIST1	G	NA	NA	NA	NA	1.04	2.36E-119	1.11	9.91E-134
3196	EDNRB	G	NA	NA	NA	NA	1.05	0.004133662	1.05	5.55E-04
3197	RAP2B	G	NA	NA	NA	NA	1.05	1.41E-25	1.03	7.59E-23
3198	DYRK4	G	NA	NA	NA	NA	1.07	1.93E-19	1.45	8.33E-35
3199	PDIA4	G	NA	NA	NA	NA	1.07	3.87E-72	1.49	1.38E-140
3200	NFIX	G	NA	NA	NA	NA	1.07	7.86E-15	1.04	3.75E-14
3201	MAP7D3	G	NA	NA	NA	NA	1.07	5.01E-16	1.15	7.14E-18
3202	TSPO	G	NA	NA	NA	NA	1.07	8.08E-06	1.15	2.87E-06
3203	ARRDC2	G	NA	NA	NA	NA	1.08	1.69E-11	1.50	3.82E-22
3204	SARDH	G	NA	NA	NA	NA	1.08	2.43E-07	2.21	3.07E-28
3205	ST7-AS1	G	NA	NA	NA	NA	1.09	4.08E-04	1.09	4.30E-04
3206	EPHX4	G	NA	NA	NA	NA	1.10	0.001171485	1.37	5.17E-05
3207	MYO1E	G	NA	NA	NA	NA	1.11	2.81E-21	1.11	1.33E-22
3208	DIAPH2	G	NA	NA	NA	NA	1.13	1.00E-23	1.48	6.78E-39
3209	AL353759.1	G	NA	NA	NA	NA	1.13	9.24E-09	1.26	5.57E-12
3210	AL136295.1	G	NA	NA	NA	NA	1.13	3.29E-04	1.12	3.82E-04
3211	NHS	G	NA	NA	NA	NA	1.14	1.57E-18	2.26	8.99E-74
3212	PRKG1	G	NA	NA	NA	NA	1.16	3.52E-29	1.00	2.45E-22
3213	CDH2	G	NA	NA	NA	NA	1.16	3.77E-74	1.15	4.58E-72
3214	LRIG1	G	NA	NA	NA	NA	1.17	7.28E-12	1.08	1.51E-09
3215	MYLIP	G	NA	NA	NA	NA	1.17	2.23E-16	1.22	2.90E-14
3216	FAM114A1	G	NA	NA	NA	NA	1.18	5.17E-16	1.05	1.05E-14
3217	PKHD1	G	NA	NA	NA	NA	1.19	2.43E-04	1.36	5.32E-05
3218	NUDT8	G	NA	NA	NA	NA	1.19	1.94E-04	1.17	5.89E-04
3219	AP001057.1	G	NA	NA	NA	NA	1.19	0.028375294	1.44	8.55E-03
3220	HCG15	G	NA	NA	NA	NA	1.19	0.048555316	1.16	3.60E-02
3221	EYA1	G	NA	NA	NA	NA	1.20	6.35E-34	1.47	1.03E-50
3222	HSPB1	G	NA	NA	NA	NA	1.20	1.24E-06	1.15	3.71E-06
3223	MAPK15	G	NA	NA	NA	NA	1.20	6.13E-04	1.05	4.70E-03
3224	THEMIS2	G	NA	NA	NA	NA	1.21	9.51E-05	1.15	1.81E-04
3225	TLE4	G	NA	NA	NA	NA	1.21	8.65E-28	1.64	1.29E-49
3226	HRASLS	G	NA	NA	NA	NA	1.21	0.016549312	2.39	1.77E-06
3227	KCNK5	G	NA	NA	NA	NA	1.21	6.05E-10	2.40	1.98E-31
3228	ESRP1	G	NA	NA	NA	NA	1.22	0.002748614	1.87	1.07E-06
3229	SYPL1	G	NA	NA	NA	NA	1.22	5.73E-38	1.14	6.67E-34
3230	NPR1	G	NA	NA	NA	NA	1.23	0.00973445	1.58	1.51E-03
3231	AC104964.3	G	NA	NA	NA	NA	1.23	0.001090088	1.21	1.56E-03
3232	AC012307.1	G	NA	NA	NA	NA	1.24	0.009227237	1.12	1.49E-02
3233	RABL3	G	NA	NA	NA	NA	1.24	1.02E-13	1.63	1.68E-22
3234	ARMC3	G	NA	NA	NA	NA	1.24	0.021928338	1.42	1.89E-02
3235	STEAP1	G	NA	NA	NA	NA	1.25	4.26E-05	1.10	8.62E-04
3236	DTNA	G	NA	NA	NA	NA	1.25	2.05E-40	1.09	1.29E-31
3237	DSG2	G	NA	NA	NA	NA	1.26	1.14E-17	2.09	3.55E-43
3238	GXYLT2	G	NA	NA	NA	NA	1.27	3.45E-12	1.45	2.52E-16
3239	RHOB	G	NA	NA	NA	NA	1.29	3.20E-09	1.43	3.20E-11
3240	NEFH	G	NA	NA	NA	NA	1.29	5.37E-30	1.13	1.88E-20

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3241	MSRA	G	NA	NA	NA	NA	1.29	5.31E-11	1.06	1.49E-07
3242	BST1	G	NA	NA	NA	NA	1.29	1.81E-06	1.43	1.56E-07
3243	AFAP1L1	G	NA	NA	NA	NA	1.29	0.002515041	1.56	1.31E-04
3244	XKRX	G	NA	NA	NA	NA	1.30	0.004712155	1.88	1.69E-05
3245	PER3	G	NA	NA	NA	NA	1.30	5.73E-07	1.40	6.66E-08
3246	AC245297.3	G	NA	NA	NA	NA	1.30	0.010020176	1.62	2.67E-04
3247	DDN-AS1	G	NA	NA	NA	NA	1.31	9.69E-06	1.18	2.75E-05
3248	ITGA6	G	NA	NA	NA	NA	1.32	2.88E-07	1.09	3.88E-05
3249	HSPB1P1	G	NA	NA	NA	NA	1.33	2.69E-08	1.25	1.74E-07
3250	AL365295.1	G	NA	NA	NA	NA	1.33	0.005035932	1.75	2.13E-05
3251	DKK1	G	NA	NA	NA	NA	1.33	2.31E-34	1.70	2.91E-56
3252	PYGL	G	NA	NA	NA	NA	1.35	3.49E-07	1.58	6.72E-10
3253	STOM	G	NA	NA	NA	NA	1.36	0.020311393	2.10	1.03E-05
3254	AC097512.1	G	NA	NA	NA	NA	1.36	0.005334608	1.62	1.10E-03
3255	SNORA51	G	NA	NA	NA	NA	1.36	0.00982828	1.50	6.01E-03
3256	CSRP1	G	NA	NA	NA	NA	1.38	7.42E-33	1.58	1.07E-44
3257	ISPD	G	NA	NA	NA	NA	1.38	1.31E-05	1.42	7.68E-06
3258	DENND1C	G	NA	NA	NA	NA	1.38	5.87E-04	1.28	6.98E-04
3259	CXCL16	G	NA	NA	NA	NA	1.38	3.32E-04	1.91	4.98E-09
3260	KCNJ8	G	NA	NA	NA	NA	1.39	1.83E-105	2.61	0
3261	ENOSF1	G	NA	NA	NA	NA	1.39	3.74E-36	2.27	2.35E-96
3262	PARD3B	G	NA	NA	NA	NA	1.41	9.10E-12	1.77	1.46E-17
3263	GPRC5C	G	NA	NA	NA	NA	1.42	4.08E-04	1.65	5.38E-06
3264	SFT2D1	G	NA	NA	NA	NA	1.43	4.29E-38	1.39	3.47E-36
3265	GRIA3	G	NA	NA	NA	NA	1.43	2.25E-07	1.28	4.16E-08
3266	TMEM221	G	NA	NA	NA	NA	1.43	0.004418664	1.36	1.08E-03
3267	MLF1	G	NA	NA	NA	NA	1.45	6.11E-34	1.17	1.93E-21
3268	PTH2R	G	NA	NA	NA	NA	1.45	3.72E-27	1.24	3.31E-22
3269	GTF2E1	G	NA	NA	NA	NA	1.46	3.12E-26	1.89	1.05E-42
3270	OXR1	G	NA	NA	NA	NA	1.46	1.09E-20	1.29	2.51E-16
3271	TMEM134	G	NA	NA	NA	NA	1.46	7.24E-11	1.19	9.96E-08
3272	DRAM1	G	NA	NA	NA	NA	1.47	3.30E-05	1.37	8.50E-05
3273	SSPN	G	NA	NA	NA	NA	1.47	2.20E-32	1.46	1.95E-33
3274	FAM124A	G	NA	NA	NA	NA	1.47	3.38E-79	1.54	1.27E-77
3275	RPRM	G	NA	NA	NA	NA	1.47	1.57E-04	1.91	5.26E-08
3276	MAGEA1	G	NA	NA	NA	NA	1.48	0.024618784	2.02	2.67E-03
3277	IL13RA1	G	NA	NA	NA	NA	1.48	1.03E-12	1.20	1.17E-08
3278	AC099542.1	G	NA	NA	NA	NA	1.48	0.007878206	2.47	1.98E-05
3279	AC084357.3	G	NA	NA	NA	NA	1.48	4.73E-49	1.63	1.43E-48
3280	KLF6	G	NA	NA	NA	NA	1.49	1.07E-11	1.01	7.85E-06
3281	PTPN3	G	NA	NA	NA	NA	1.49	2.57E-19	1.70	1.85E-23
3282	NGFR	G	NA	NA	NA	NA	1.51	4.35E-12	1.55	9.65E-10
3283	MYO16	G	NA	NA	NA	NA	1.52	2.48E-25	1.60	6.26E-26
3284	SYTL1	G	NA	NA	NA	NA	1.53	0.018059238	1.49	2.32E-02
3285	SLC25A27	G	NA	NA	NA	NA	1.53	0.021359661	1.32	2.64E-02
3286	STK3	G	NA	NA	NA	NA	1.53	3.28E-22	1.32	5.40E-17
3287	ANKRD34B	G	NA	NA	NA	NA	1.53	0.023397274	2.78	2.34E-05
3288	APOC1	G	NA	NA	NA	NA	1.54	0.001342907	1.98	3.57E-05
3289	AC027307.2	G	NA	NA	NA	NA	1.54	7.07E-24	1.25	2.01E-20
3290	SPATC1L	G	NA	NA	NA	NA	1.54	3.78E-09	1.08	7.85E-05
3291	C1QTNF2	G	NA	NA	NA	NA	1.54	8.25E-04	1.73	1.77E-04
3292	STOX1	G	NA	NA	NA	NA	1.55	4.27E-28	1.89	3.40E-37
3293	AC003986.3	G	NA	NA	NA	NA	1.55	5.00E-04	1.66	1.58E-05
3294	AC093627.4	G	NA	NA	NA	NA	1.55	9.69E-04	1.91	2.79E-05
3295	NAT1	G	NA	NA	NA	NA	1.55	8.91E-07	1.92	1.30E-09
3296	APOBEC3C	G	NA	NA	NA	NA	1.55	1.05E-53	1.28	2.28E-36
3297	B4GALT4	G	NA	NA	NA	NA	1.56	1.03E-39	1.47	3.86E-37
3298	CDKN2A	G	NA	NA	NA	NA	1.57	1.45E-18	1.83	1.33E-23
3299	TGIF2LX	G	NA	NA	NA	NA	1.57	4.44E-20	1.08	8.23E-11
3300	GCNT4	G	NA	NA	NA	NA	1.57	0.015035181	1.49	1.77E-02

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3301	LRBA	G	NA	NA	NA	NA	1.57	6.74E-13	1.05	3.37E-06
3302	LDLRAP1	G	NA	NA	NA	NA	1.58	5.27E-04	1.40	2.38E-03
3303	PCDHGA8	G	NA	NA	NA	NA	1.59	2.91E-06	1.13	5.06E-04
3304	HSPA2	G	NA	NA	NA	NA	1.59	8.65E-14	2.37	5.78E-30
3305	CAPN12	G	NA	NA	NA	NA	1.59	0.037979208	1.41	4.19E-02
3306	COL1A1	G	NA	NA	NA	NA	1.61	2.03E-09	1.07	1.18E-04
3307	GRIN2D	G	NA	NA	NA	NA	1.62	6.06E-12	1.24	1.12E-07
3308	AL353625.1	G	NA	NA	NA	NA	1.62	1.66E-06	1.25	2.19E-04
3309	KDR	G	NA	NA	NA	NA	1.63	0.036921088	1.95	1.25E-03
3310	FOSL1	G	NA	NA	NA	NA	1.63	0.016676886	1.55	7.34E-03
3311	FAM85B	G	NA	NA	NA	NA	1.63	0.008416245	1.25	2.90E-02
3312	LEPR	G	NA	NA	NA	NA	1.64	1.42E-69	2.22	1.52E-118
3313	RNF207	G	NA	NA	NA	NA	1.64	0.002221979	1.26	2.64E-02
3314	PCDH15	G	NA	NA	NA	NA	1.64	7.41E-05	1.37	1.31E-03
3315	FN1	G	NA	NA	NA	NA	1.65	1.59E-129	1.27	3.97E-79
3316	ABRACL	G	NA	NA	NA	NA	1.65	2.48E-43	1.16	2.73E-23
3317	SYTL4	G	NA	NA	NA	NA	1.68	0.001741394	1.61	2.58E-03
3318	ST13P20	G	NA	NA	NA	NA	1.69	5.80E-08	1.66	6.51E-08
3319	CPEB2	G	NA	NA	NA	NA	1.69	2.12E-13	1.84	1.84E-15
3320	AC025766.1	G	NA	NA	NA	NA	1.70	4.30E-04	1.48	2.32E-03
3321	HSPG2	G	NA	NA	NA	NA	1.70	2.34E-12	1.75	3.84E-13
3322	KIF1C	G	NA	NA	NA	NA	1.70	1.07E-35	1.87	8.68E-43
3323	PABPC4L	G	NA	NA	NA	NA	1.72	1.05E-18	1.47	2.37E-14
3324	AC011450.1	G	NA	NA	NA	NA	1.72	5.60E-08	1.06	2.89E-04
3325	TBX20	G	NA	NA	NA	NA	1.73	1.09E-31	1.62	6.89E-24
3326	AL031847.1	G	NA	NA	NA	NA	1.73	3.04E-04	2.19	1.32E-05
3327	ADAMTS5	G	NA	NA	NA	NA	1.75	8.70E-24	1.77	1.80E-19
3328	MBL2	G	NA	NA	NA	NA	1.75	0.048882235	3.58	1.04E-03
3329	USP51	G	NA	NA	NA	NA	1.76	3.42E-27	1.20	1.87E-16
3330	DDX60	G	NA	NA	NA	NA	1.77	0.001024214	1.76	1.04E-03
3331	IQSEC2	G	NA	NA	NA	NA	1.77	3.53E-05	1.77	2.57E-05
3332	AC009227.1	G	NA	NA	NA	NA	1.78	7.29E-04	2.09	7.85E-04
3333	NEK11	G	NA	NA	NA	NA	1.78	3.51E-11	1.11	3.45E-05
3334	C6	G	NA	NA	NA	NA	1.78	0.001806073	1.62	3.55E-03
3335	MCTP1	G	NA	NA	NA	NA	1.80	2.56E-26	1.94	9.60E-30
3336	ANXA4	G	NA	NA	NA	NA	1.80	1.96E-57	1.61	1.35E-43
3337	DPT	G	NA	NA	NA	NA	1.80	0.030316309	1.85	3.32E-02
3338	BCL2L12	G	NA	NA	NA	NA	1.81	7.91E-18	1.79	2.82E-18
3339	CYP2J2	G	NA	NA	NA	NA	1.81	3.81E-22	1.86	3.74E-19
3340	CYBRD1	G	NA	NA	NA	NA	1.82	4.27E-18	2.18	2.71E-30
3341	NOTCH2NL	G	NA	NA	NA	NA	1.82	2.76E-05	2.04	1.68E-06
3342	HNRNPA3P14	G	NA	NA	NA	NA	1.82	0.008559666	1.85	1.07E-03
3343	ZNF185	G	NA	NA	NA	NA	1.83	2.77E-57	2.09	6.95E-83
3344	ITIH5	G	NA	NA	NA	NA	1.83	8.17E-04	1.87	4.35E-04
3345	RORB-AS1	G	NA	NA	NA	NA	1.83	7.48E-06	2.11	6.45E-06
3346	PLXNB3	G	NA	NA	NA	NA	1.84	5.08E-09	1.31	4.33E-05
3347	NOTCH3	G	NA	NA	NA	NA	1.85	8.40E-34	1.58	2.20E-25
3348	EFHD1	G	NA	NA	NA	NA	1.85	4.01E-38	2.04	2.27E-52
3349	MID1IP1-AS1	G	NA	NA	NA	NA	1.86	0.001256427	2.32	9.07E-06
3350	AL139384.2	G	NA	NA	NA	NA	1.88	9.98E-13	1.59	6.69E-09
3351	KLHL31	G	NA	NA	NA	NA	1.88	0.001037841	2.26	9.16E-04
3352	NBPF14	G	NA	NA	NA	NA	1.90	3.49E-13	1.89	3.32E-13
3353	NFATC4	G	NA	NA	NA	NA	1.91	7.87E-51	1.86	1.81E-50
3354	SERPINH1	G	NA	NA	NA	NA	1.91	4.41E-108	1.91	1.86E-110
3355	TREX1	G	NA	NA	NA	NA	1.91	6.42E-08	1.78	8.64E-08
3356	ZNF229	G	NA	NA	NA	NA	1.91	3.56E-15	1.86	2.75E-14
3357	FLJ36000	G	NA	NA	NA	NA	1.93	9.21E-21	1.22	6.25E-08
3358	ABCB4	G	NA	NA	NA	NA	1.93	2.78E-10	1.73	1.27E-08
3359	SPHK1	G	NA	NA	NA	NA	1.93	1.85E-04	2.05	9.95E-04
3360	CORO6	G	NA	NA	NA	NA	1.93	1.57E-18	1.23	5.20E-08

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3361	PDLIM3	G	NA	NA	NA	NA	1.94	1.15E-71	1.82	1.72E-68
3362	ANXA2	G	NA	NA	NA	NA	1.95	1.27E-82	2.02	1.30E-88
3363	AC110813.1	G	NA	NA	NA	NA	1.95	0.008992241	1.31	2.68E-02
3364	SMARCA2	G	NA	NA	NA	NA	1.97	5.40E-28	1.89	2.30E-26
3365	CHST7	G	NA	NA	NA	NA	1.97	7.62E-15	2.50	5.67E-26
3366	SMIM10L2A	G	NA	NA	NA	NA	1.98	2.96E-20	1.42	3.10E-13
3367	CNKSR3	G	NA	NA	NA	NA	2.00	1.44E-32	1.71	3.74E-24
3368	RAB38	G	NA	NA	NA	NA	2.00	7.39E-16	2.70	4.93E-33
3369	AC010595.1	G	NA	NA	NA	NA	2.00	8.42E-04	3.22	2.66E-06
3370	TENM1	G	NA	NA	NA	NA	2.01	3.85E-13	1.31	4.46E-06
3371	G0S2	G	NA	NA	NA	NA	2.02	2.37E-04	2.84	4.68E-07
3372	BOC	G	NA	NA	NA	NA	2.03	9.57E-21	1.76	2.11E-15
3373	SATB2	G	NA	NA	NA	NA	2.03	3.11E-05	1.92	3.09E-04
3374	AL512353.1	G	NA	NA	NA	NA	2.03	2.71E-11	1.29	4.28E-05
3375	PDGFD	G	NA	NA	NA	NA	2.03	2.14E-69	1.03	3.81E-17
3376	EFNA4	G	NA	NA	NA	NA	2.04	1.68E-39	1.84	3.35E-34
3377	VIM-AS1	G	NA	NA	NA	NA	2.04	4.56E-04	3.43	6.42E-07
3378	AC002351.1	G	NA	NA	NA	NA	2.06	1.54E-07	2.17	1.30E-08
3379	AIFM2	G	NA	NA	NA	NA	2.07	5.34E-27	2.23	2.28E-43
3380	AC020928.1	G	NA	NA	NA	NA	2.08	3.35E-14	2.48	3.22E-18
3381	MEIOC	G	NA	NA	NA	NA	2.08	7.91E-09	2.07	1.57E-08
3382	MIR497HG	G	NA	NA	NA	NA	2.08	0.010401085	2.35	2.82E-03
3383	LRRC37A7P	G	NA	NA	NA	NA	2.10	2.26E-09	1.52	1.84E-06
3384	GTSF1	G	NA	NA	NA	NA	2.11	9.10E-07	2.74	2.88E-09
3385	MEIOB	G	NA	NA	NA	NA	2.12	0.031376846	2.08	3.73E-02
3386	SYT9	G	NA	NA	NA	NA	2.13	6.24E-91	1.33	1.02E-39
3387	LRRC37A9P	G	NA	NA	NA	NA	2.14	1.42E-06	1.87	1.15E-06
3388	NXT2	G	NA	NA	NA	NA	2.14	3.66E-45	2.44	5.80E-59
3389	ADAMTS12	G	NA	NA	NA	NA	2.14	7.47E-22	1.38	8.37E-10
3390	MAATS1	G	NA	NA	NA	NA	2.16	6.81E-20	1.53	1.13E-10
3391	SIK1	G	NA	NA	NA	NA	2.16	4.53E-05	4.31	6.43E-15
3392	AL591895.1	G	NA	NA	NA	NA	2.17	4.40E-04	2.16	8.62E-04
3393	DDX60L	G	NA	NA	NA	NA	2.19	7.92E-08	1.08	1.74E-02
3394	EPHB4	G	NA	NA	NA	NA	2.20	1.76E-51	2.06	8.06E-46
3395	LINC00898	G	NA	NA	NA	NA	2.21	0.00721287	2.02	1.28E-03
3396	NBEA	G	NA	NA	NA	NA	2.21	5.03E-98	1.51	3.62E-46
3397	COL9A2	G	NA	NA	NA	NA	2.22	1.76E-09	1.52	7.36E-05
3398	TCF7L1	G	NA	NA	NA	NA	2.23	1.74E-44	2.26	8.94E-52
3399	PCDHGB7	G	NA	NA	NA	NA	2.25	6.64E-48	2.08	1.34E-47
3400	NBPF19	G	NA	NA	NA	NA	2.25	1.31E-76	2.04	1.76E-64
3401	CGNL1	G	NA	NA	NA	NA	2.29	2.29E-167	2.24	2.00E-171
3402	ACSF2	G	NA	NA	NA	NA	2.29	4.02E-40	1.72	1.24E-24
3403	KIAA0825	G	NA	NA	NA	NA	2.30	1.34E-52	2.32	1.61E-54
3404	BTBD19	G	NA	NA	NA	NA	2.31	4.03E-11	1.54	1.45E-05
3405	TGFBR2	G	NA	NA	NA	NA	2.31	8.80E-56	1.27	2.71E-21
3406	ABCC9	G	NA	NA	NA	NA	2.32	1.85E-33	3.24	2.43E-64
3407	DLC1	G	NA	NA	NA	NA	2.32	4.89E-17	2.32	5.71E-17
3408	BTN3A3	G	NA	NA	NA	NA	2.33	6.96E-30	1.47	3.79E-12
3409	FRMD6	G	NA	NA	NA	NA	2.34	2.75E-35	2.43	2.41E-39
3410	AKR1C2	G	NA	NA	NA	NA	2.35	2.32E-06	1.31	1.19E-02
3411	GPR156	G	NA	NA	NA	NA	2.35	2.06E-22	2.11	1.06E-20
3412	AL139317.5	G	NA	NA	NA	NA	2.35	5.24E-04	2.58	2.57E-05
3413	NEXN	G	NA	NA	NA	NA	2.38	3.09E-15	1.86	1.41E-10
3414	TLR1	G	NA	NA	NA	NA	2.40	2.01E-05	1.85	1.46E-03
3415	C1R	G	NA	NA	NA	NA	2.42	1.08E-06	2.71	3.40E-10
3416	FAM198A	G	NA	NA	NA	NA	2.43	0.00220686	3.22	1.65E-02
3417	AC093908.1	G	NA	NA	NA	NA	2.43	4.54E-20	1.99	3.14E-15
3418	MAK	G	NA	NA	NA	NA	2.45	3.84E-16	2.29	2.51E-19
3419	SOSTDC1	G	NA	NA	NA	NA	2.46	1.54E-08	1.78	1.65E-05
3420	PCDHGB4	G	NA	NA	NA	NA	2.46	1.14E-06	1.50	3.11E-03

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3421	CLUL1	G	NA	NA	NA	NA	2.47	2.64E-09	2.79	1.16E-12
3422	CC2D2A	G	NA	NA	NA	NA	2.47	2.31E-79	2.54	2.06E-77
3423	AC011468.5	G	NA	NA	NA	NA	2.47	3.36E-04	2.43	1.27E-05
3424	GAPDHP14	G	NA	NA	NA	NA	2.49	3.40E-05	3.27	2.84E-08
3425	TRIM59	G	NA	NA	NA	NA	2.50	2.39E-44	2.30	3.15E-39
3426	RDM1	G	NA	NA	NA	NA	2.51	5.93E-07	2.13	1.14E-05
3427	AMT	G	NA	NA	NA	NA	2.51	6.96E-95	2.08	4.08E-66
3428	LRP4	G	NA	NA	NA	NA	2.56	3.82E-95	2.21	1.05E-71
3429	MCTP2	G	NA	NA	NA	NA	2.56	9.25E-27	2.09	4.09E-17
3430	IL17RC	G	NA	NA	NA	NA	2.59	5.76E-26	2.30	2.69E-22
3431	CCDC88B	G	NA	NA	NA	NA	2.59	1.71E-07	2.03	1.04E-04
3432	SLC24A2	G	NA	NA	NA	NA	2.59	2.69E-20	2.92	1.80E-22
3433	TNFRSF10A-AS1	G	NA	NA	NA	NA	2.62	0.022107803	1.86	4.84E-02
3434	CPS1	G	NA	NA	NA	NA	2.63	2.44E-14	1.64	5.07E-06
3435	KDELR3	G	NA	NA	NA	NA	2.64	9.27E-51	2.01	4.77E-36
3436	LINC02084	G	NA	NA	NA	NA	2.64	6.05E-05	1.71	9.24E-04
3437	ZNF880	G	NA	NA	NA	NA	2.66	1.01E-84	2.13	1.51E-59
3438	CAPN3	G	NA	NA	NA	NA	2.67	0.012353976	2.41	1.06E-02
3439	CYTOR	G	NA	NA	NA	NA	2.67	7.36E-12	1.95	2.73E-07
3440	PRTFDC1	G	NA	NA	NA	NA	2.71	2.09E-19	2.58	4.68E-18
3441	SGCD	G	NA	NA	NA	NA	2.73	2.74E-15	2.49	1.73E-11
3442	POPDC2	G	NA	NA	NA	NA	2.73	1.24E-58	3.62	7.76E-100
3443	HMGN5	G	NA	NA	NA	NA	2.75	2.23E-28	1.95	6.43E-17
3444	FGF7	G	NA	NA	NA	NA	2.75	7.05E-14	2.37	7.93E-11
3445	C5orf17	G	NA	NA	NA	NA	2.75	8.05E-22	2.68	7.03E-21
3446	PACRG	G	NA	NA	NA	NA	2.76	5.65E-16	1.65	3.78E-06
3447	DNAJC22	G	NA	NA	NA	NA	2.77	1.97E-15	1.48	8.40E-05
3448	FAAH	G	NA	NA	NA	NA	2.77	2.16E-10	3.11	3.60E-11
3449	GSDMD	G	NA	NA	NA	NA	2.78	1.07E-04	2.82	2.45E-05
3450	ICAM3	G	NA	NA	NA	NA	2.79	7.18E-14	1.87	4.70E-07
3451	IFITM2	G	NA	NA	NA	NA	2.80	1.37E-13	2.44	1.79E-13
3452	NMI	G	NA	NA	NA	NA	2.80	0.004671002	3.12	3.11E-03
3453	LDB2	G	NA	NA	NA	NA	2.82	8.08E-56	2.78	7.12E-47
3454	ALPK1	G	NA	NA	NA	NA	2.83	1.38E-22	2.84	1.13E-23
3455	LPP	G	NA	NA	NA	NA	2.84	8.01E-18	3.58	1.78E-28
3456	WWTR1	G	NA	NA	NA	NA	2.92	3.64E-15	3.54	6.45E-22
3457	NOVA1-AS1	G	NA	NA	NA	NA	2.93	0.004257802	3.64	1.73E-04
3458	EDNRA	G	NA	NA	NA	NA	2.95	1.91E-85	2.82	3.69E-78
3459	DDIT4L	G	NA	NA	NA	NA	2.96	0.0015826	3.40	1.53E-06
3460	NTN5	G	NA	NA	NA	NA	2.97	0.002463095	1.96	9.49E-03
3461	PTX3	G	NA	NA	NA	NA	3.01	8.43E-82	3.74	5.16E-110
3462	CYR61	G	NA	NA	NA	NA	3.02	2.69E-10	3.88	4.85E-16
3463	XKR8	G	NA	NA	NA	NA	3.02	0.001062638	2.92	7.28E-03
3464	AC090136.3	G	NA	NA	NA	NA	3.03	0.021329108	3.61	1.70E-02
3465	GCFC2	G	NA	NA	NA	NA	3.04	1.86E-54	3.08	8.52E-54
3466	HES7	G	NA	NA	NA	NA	3.04	1.50E-04	2.89	8.49E-04
3467	GPR1	G	NA	NA	NA	NA	3.07	1.42E-43	2.31	2.70E-22
3468	ARHGAP42	G	NA	NA	NA	NA	3.08	1.19E-42	3.52	1.93E-58
3469	AL049737.1	G	NA	NA	NA	NA	3.08	6.98E-04	2.28	3.66E-03
3470	S100A11	G	NA	NA	NA	NA	3.09	0	2.41	1.61E-178
3471	NOTCH1	G	NA	NA	NA	NA	3.12	1.31E-07	2.16	4.02E-04
3472	SYCE1L	G	NA	NA	NA	NA	3.13	4.91E-04	2.52	4.91E-03
3473	VAMP5	G	NA	NA	NA	NA	3.24	1.12E-05	1.67	2.46E-02
3474	TRIM21	G	NA	NA	NA	NA	3.25	1.67E-16	2.25	1.43E-09
3475	TMEM63A	G	NA	NA	NA	NA	3.25	6.11E-32	3.39	7.84E-35
3476	NBPF10	G	NA	NA	NA	NA	3.29	1.47E-65	3.27	3.91E-72
3477	AASS	G	NA	NA	NA	NA	3.30	7.17E-92	2.88	2.69E-77
3478	AC092384.1	G	NA	NA	NA	NA	3.31	1.55E-04	3.43	7.62E-04
3479	KCNS1	G	NA	NA	NA	NA	3.33	0.037838126	3.38	3.38E-02
3480	LINC01168	G	NA	NA	NA	NA	3.38	0.018401257	2.62	2.43E-02

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3481	KISS1R	G	NA	NA	NA	NA	3.39	6.53E-04	4.36	1.09E-06
3482	PRRX2	G	NA	NA	NA	NA	3.44	3.06E-24	3.43	2.48E-19
3483	FAM160A1	G	NA	NA	NA	NA	3.45	3.27E-10	2.03	9.22E-04
3484	SCN9A	G	NA	NA	NA	NA	3.47	2.28E-142	3.50	3.39E-138
3485	LINC02167	G	NA	NA	NA	NA	3.48	0.010435762	3.71	8.07E-03
3486	ZNF883	G	NA	NA	NA	NA	3.48	1.89E-32	3.70	1.14E-34
3487	MTDHP4	G	NA	NA	NA	NA	3.49	0.03467006	5.37	3.67E-04
3488	AC010615.2	G	NA	NA	NA	NA	3.50	9.52E-14	3.33	1.32E-12
3489	AL160286.1	G	NA	NA	NA	NA	3.66	0.014118458	2.57	3.20E-02
3490	SSC4D	G	NA	NA	NA	NA	3.67	9.60E-58	2.90	1.74E-39
3491	MOCOS	G	NA	NA	NA	NA	3.70	1.00E-16	4.37	7.56E-25
3492	SLC46A3	G	NA	NA	NA	NA	3.75	5.25E-11	2.68	6.17E-11
3493	PRKD1	G	NA	NA	NA	NA	3.86	6.91E-21	4.93	9.60E-34
3494	AC041040.1	G	NA	NA	NA	NA	3.93	0.001012699	3.05	1.99E-03
3495	IFITM3	G	NA	NA	NA	NA	3.95	5.95E-07	2.50	7.21E-06
3496	VIM	G	NA	NA	NA	NA	3.96	0	3.96	0
3497	WNT16	G	NA	NA	NA	NA	3.97	5.31E-22	2.80	2.60E-13
3498	ZSWIM5P2	G	NA	NA	NA	NA	4.00	0.017312531	4.38	6.93E-03
3499	ADARB2-AS1	G	NA	NA	NA	NA	4.03	0.015525752	3.61	4.16E-02
3500	CRACR2A	G	NA	NA	NA	NA	4.03	1.99E-21	3.84	2.11E-20
3501	MXRA8	G	NA	NA	NA	NA	4.11	9.25E-115	3.72	9.73E-97
3502	BEX5	G	NA	NA	NA	NA	4.11	0.013419109	4.42	6.16E-03
3503	NRK	G	NA	NA	NA	NA	4.14	1.21E-06	4.55	8.19E-11
3504	TRIM14	G	NA	NA	NA	NA	4.25	5.75E-180	4.37	1.15E-137
3505	C11orf70	G	NA	NA	NA	NA	4.27	1.41E-22	3.91	3.43E-20
3506	CELSR1	G	NA	NA	NA	NA	4.30	2.15E-136	4.00	5.33E-113
3507	DCDC2	G	NA	NA	NA	NA	4.30	1.16E-04	2.31	3.03E-02
3508	ARPIN	G	NA	NA	NA	NA	4.31	1.98E-131	4.77	2.25E-161
3509	TFPI	G	NA	NA	NA	NA	4.35	1.97E-247	4.08	2.62E-226
3510	CASC9	G	NA	NA	NA	NA	4.37	4.37E-33	4.20	2.77E-41
3511	AC093274.1	G	NA	NA	NA	NA	4.38	9.88E-07	5.13	1.80E-05
3512	CLDN23	G	NA	NA	NA	NA	4.43	6.97E-06	3.63	4.24E-10
3513	ITM2A	G	NA	NA	NA	NA	4.49	2.05E-06	6.55	1.05E-10
3514	OTX1	G	NA	NA	NA	NA	4.57	1.19E-12	2.84	3.41E-09
3515	LINC00189	G	NA	NA	NA	NA	4.58	5.61E-10	3.45	4.52E-07
3516	AC069061.3	G	NA	NA	NA	NA	4.59	0.001719489	4.54	1.96E-03
3517	NBPF4	G	NA	NA	NA	NA	4.61	0.001107815	5.29	1.26E-04
3518	C1orf54	G	NA	NA	NA	NA	4.62	1.75E-43	3.71	8.19E-30
3519	AC106818.1	G	NA	NA	NA	NA	4.63	2.40E-06	3.53	1.93E-06
3520	FGFRL1	G	NA	NA	NA	NA	4.63	2.22E-123	4.47	1.82E-93
3521	AC009005.1	G	NA	NA	NA	NA	4.66	5.30E-14	3.85	4.85E-13
3522	C7	G	NA	NA	NA	NA	4.75	0	3.39	1.12E-224
3523	IL20RA	G	NA	NA	NA	NA	4.76	7.58E-35	4.77	4.68E-36
3524	PABPC5	G	NA	NA	NA	NA	4.86	8.95E-04	4.53	2.21E-03
3525	PTPRK	G	NA	NA	NA	NA	4.97	1.90E-26	4.79	4.17E-24
3526	SCAND3P1	G	NA	NA	NA	NA	5.02	3.71E-04	3.15	1.65E-02
3527	MYD88	G	NA	NA	NA	NA	5.05	3.69E-145	5.16	4.29E-145
3528	HTR7	G	NA	NA	NA	NA	5.10	1.40E-05	3.73	3.54E-05
3529	PCDHGA7	G	NA	NA	NA	NA	5.11	4.11E-181	4.78	2.65E-208
3530	HGF	G	NA	NA	NA	NA	5.16	0	5.19	0
3531	S100A6	G	NA	NA	NA	NA	5.40	6.26E-64	5.31	2.35E-58
3532	DCN	G	NA	NA	NA	NA	5.47	5.24E-14	4.71	3.11E-11
3533	MEIS3P1	G	NA	NA	NA	NA	5.49	1.89E-17	5.04	6.40E-21
3534	DPP10-AS1	G	NA	NA	NA	NA	5.53	1.06E-05	3.51	1.03E-02
3535	ZFR2	G	NA	NA	NA	NA	5.62	1.04E-16	6.22	4.15E-20
3536	SLCO1C1	G	NA	NA	NA	NA	5.64	0.001992995	6.72	1.50E-04
3537	OPCML	G	NA	NA	NA	NA	5.67	5.73E-12	7.19	4.85E-13
3538	AC129926.2	G	NA	NA	NA	NA	5.67	4.85E-39	6.55	1.86E-32
3539	CAVIN2	G	NA	NA	NA	NA	5.68	3.42E-04	5.01	2.14E-03
3540	AC093726.1	G	NA	NA	NA	NA	5.68	1.01E-04	5.00	8.84E-04

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3541	SP140L	G	NA	NA	NA	NA	5.79	4.00E-28	5.96	2.01E-33
3542	DYNLT3	G	NA	NA	NA	NA	5.81	2.11E-104	6.07	3.96E-109
3543	ERVMER61-1	G	NA	NA	NA	NA	5.82	1.12E-75	5.35	2.26E-79
3544	GPX8	G	NA	NA	NA	NA	5.82	6.41E-22	6.08	1.93E-32
3545	MAP10	G	NA	NA	NA	NA	5.88	4.82E-20	5.25	6.15E-27
3546	SP110	G	NA	NA	NA	NA	5.89	2.30E-11	5.94	1.36E-11
3547	AC109588.1	G	NA	NA	NA	NA	5.99	1.53E-24	4.52	3.25E-32
3548	RGPD1	G	NA	NA	NA	NA	6.15	6.51E-07	6.26	3.91E-07
3549	FAM136BP	G	NA	NA	NA	NA	6.17	2.08E-07	5.33	1.03E-05
3550	SLC25A43	G	NA	NA	NA	NA	6.27	2.39E-06	4.13	4.30E-05
3551	AC131532.1	G	NA	NA	NA	NA	6.28	3.58E-07	4.22	1.19E-05
3552	AC015922.3	G	NA	NA	NA	NA	6.32	7.78E-08	5.00	2.65E-05
3553	AC005538.2	G	NA	NA	NA	NA	6.37	4.55E-11	7.22	2.31E-10
3554	TDRD5	G	NA	NA	NA	NA	6.38	3.29E-07	5.74	6.69E-06
3555	GTF2IP6	G	NA	NA	NA	NA	6.39	1.01E-08	7.50	2.53E-11
3556	BCL6B	G	NA	NA	NA	NA	6.40	1.31E-07	5.59	6.21E-06
3557	ARMCX1	G	NA	NA	NA	NA	6.43	8.13E-68	5.92	2.13E-72
3558	HOXA13	G	NA	NA	NA	NA	6.52	5.61E-13	6.41	5.29E-20
3559	AC087499.1	G	NA	NA	NA	NA	6.62	1.75E-08	4.79	1.77E-06
3560	CCDC80	G	NA	NA	NA	NA	6.64	1.28E-194	5.77	1.17E-151
3561	AC092100.1	G	NA	NA	NA	NA	6.67	2.39E-08	4.96	4.87E-05
3562	PNPLA4	G	NA	NA	NA	NA	6.71	5.33E-90	5.95	8.23E-110
3563	AC015922.4	G	NA	NA	NA	NA	6.76	4.76E-14	6.25	1.32E-22
3564	BTN1A1	G	NA	NA	NA	NA	6.83	1.60E-10	8.06	1.85E-13
3565	PTGS2	G	NA	NA	NA	NA	6.83	8.30E-09	4.98	3.81E-08
3566	DNAJC15	G	NA	NA	NA	NA	6.95	3.39E-95	7.47	3.34E-106
3567	FRRS1	G	NA	NA	NA	NA	7.03	1.09E-14	6.37	4.65E-14
3568	AC069061.4	G	NA	NA	NA	NA	7.10	1.20E-19	6.28	7.93E-19
3569	MSL3P1	G	NA	NA	NA	NA	7.14	3.96E-09	7.28	1.72E-09
3570	NKAPP1	G	NA	NA	NA	NA	7.43	8.82E-10	4.98	9.30E-11
3571	TNFRSF10A	G	NA	NA	NA	NA	7.55	5.72E-12	6.57	3.16E-17
3572	AC069061.2	G	NA	NA	NA	NA	8.73	9.30E-16	6.93	3.69E-23
3573	IGF2BP2	G	NA	NA	NA	NA	9.00	7.71E-20	8.30	5.49E-18
3574	SAMD9L	H	6.62	1.52E-16	1.49	1.63E-03	5.30	9.14E-11	NA	NA
3575	DKK2	H	6.00	6.23E-73	2.12	5.78E-41	4.47	8.41E-40	NA	NA
3576	IGSF11	H	4.10	3.10E-52	1.63	4.93E-22	2.14	9.52E-14	NA	NA
3577	ADAMTSL1	H	3.94	2.20E-21	5.08	1.61E-19	-1.94	0.004758143	NA	NA
3578	CRYM	H	3.93	3.46E-80	2.14	8.13E-46	1.63	2.35E-12	NA	NA
3579	WDR11-AS1	H	3.67	1.75E-02	-3.47	2.90E-02	4.01	0.006643147	NA	NA
3580	EVA1B	H	3.35	2.91E-09	1.78	6.15E-04	1.54	0.022868881	NA	NA
3581	LINC00694	H	3.30	9.00E-13	1.32	1.01E-03	1.55	0.005293249	NA	NA
3582	SLC2A12	H	3.28	5.39E-50	1.12	1.48E-10	2.35	1.00E-24	NA	NA
3583	OSGIN1	H	3.21	1.99E-12	1.83	1.29E-06	1.27	0.029376984	NA	NA
3584	CPED1	H	3.08	1.86E-94	1.55	6.84E-25	1.16	9.69E-14	NA	NA
3585	AL365199.1	H	2.92	1.10E-162	3.42	2.59E-137	-1.38	1.60E-19	NA	NA
3586	ORAI3	H	2.83	1.26E-37	1.24	7.56E-09	1.03	3.20E-05	NA	NA
3587	MPPED2	H	2.74	1.91E-49	4.35	7.92E-99	-2.29	5.73E-27	NA	NA
3588	NQO1	H	2.28	2.55E-122	1.53	1.01E-79	1.73	7.31E-68	NA	NA
3589	BTG2	H	2.26	1.50E-22	1.82	5.13E-15	1.25	2.33E-07	NA	NA
3590	CARTPT	H	2.24	8.53E-06	6.15	1.81E-07	-3.99	0.001655441	NA	NA
3591	GREM2	H	2.19	2.48E-58	4.66	1.27E-144	-1.55	1.59E-13	NA	NA
3592	S1PR1	H	2.16	2.19E-07	1.07	1.81E-03	1.80	3.71E-05	NA	NA
3593	AL354719.2	H	2.04	1.23E-16	1.02	3.44E-06	1.14	3.77E-05	NA	NA
3594	PLEKHA2	H	1.90	1.21E-41	1.69	1.46E-34	1.20	7.59E-17	NA	NA
3595	PANX2	H	1.81	1.82E-09	2.18	1.05E-09	-1.28	0.001247026	NA	NA
3596	DNM1P47	H	1.78	6.23E-04	2.55	1.06E-06	-1.66	0.002335998	NA	NA
3597	ADGRD1	H	1.69	8.14E-03	3.63	5.74E-07	-2.61	2.52E-04	NA	NA
3598	PDZRN3	H	1.68	5.88E-44	2.02	1.44E-56	-1.15	7.62E-18	NA	NA
3599	NOTCH2	H	1.66	1.33E-237	1.08	2.90E-111	1.45	7.43E-179	NA	NA
3600	PDGFRA	H	1.63	1.75E-112	1.36	3.90E-84	1.22	1.06E-62	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3601	TNFRSF19	H	1.55	7.76E-25	5.08	7.43E-66	-3.67	2.82E-33	NA	NA
3602	HIVEP3	H	1.51	3.97E-18	2.22	1.60E-30	-1.18	3.46E-09	NA	NA
3603	CPNE9	H	1.48	1.52E-03	2.77	8.17E-08	-1.52	0.010780386	NA	NA
3604	NBL1	H	1.32	1.15E-22	1.96	4.19E-45	-1.49	6.99E-26	NA	NA
3605	OLFML3	H	1.28	1.09E-13	1.94	4.00E-18	-1.36	1.09E-08	NA	NA
3606	JAK3	H	1.24	5.05E-04	-1.10	3.64E-05	2.52	3.14E-16	NA	NA
3607	KALRN	H	1.20	1.55E-34	1.85	8.32E-74	-1.32	1.03E-37	NA	NA
3608	LINC00327	H	1.08	9.96E-03	3.27	1.69E-12	-1.78	0.001061789	NA	NA
3609	GAS6-AS1	H	1.08	4.04E-02	-1.77	1.06E-06	3.02	3.84E-13	NA	NA
3610	CDKN1A	H	1.06	8.35E-36	-1.06	2.43E-40	1.61	1.31E-85	NA	NA
3611	CHRM3	H	-1.02	3.96E-10	1.09	5.36E-11	-1.41	2.34E-18	NA	NA
3612	SLC1A4	H	-1.08	8.65E-14	1.00	1.50E-11	-1.57	2.43E-28	NA	NA
3613	ATF3	H	-1.09	1.05E-05	1.28	3.92E-07	-2.21	1.86E-20	NA	NA
3614	ADM2	H	-1.15	2.93E-08	1.69	2.70E-13	-2.89	1.01E-39	NA	NA
3615	ALDH1L2	H	-1.27	1.05E-44	1.28	1.26E-35	-2.01	2.85E-92	NA	NA
3616	DSCAML1	H	-1.33	3.46E-02	-3.32	2.49E-14	2.54	1.11E-09	NA	NA
3617	PTGFR	H	-1.36	6.41E-15	1.90	4.56E-11	-3.73	8.86E-47	NA	NA
3618	SHISA3	H	-1.38	4.19E-04	-1.09	3.64E-02	-1.11	0.005320231	NA	NA
3619	AC107398.3	H	-1.42	4.13E-02	-2.63	1.11E-05	1.20	0.031276392	NA	NA
3620	HR	H	-1.48	2.02E-14	-1.69	8.65E-20	1.20	1.54E-10	NA	NA
3621	DAND5	H	-1.56	5.17E-04	-2.11	3.63E-08	1.19	0.001537575	NA	NA
3622	SYNPR	H	-1.68	3.06E-05	-2.68	8.44E-15	1.46	2.88E-05	NA	NA
3623	AL137024.1	H	-2.05	1.21E-03	-1.78	3.57E-02	-1.27	0.049160093	NA	NA
3624	LFNG	H	-2.07	5.30E-20	-1.35	1.45E-07	-1.14	2.27E-07	NA	NA
3625	CXCR4	H	-2.26	7.55E-66	2.33	4.39E-35	-4.13	6.47E-119	NA	NA
3626	CHAC1	H	-2.27	3.49E-31	2.29	1.67E-24	-4.94	2.25E-117	NA	NA
3627	ZBTB7C	H	-2.32	1.99E-06	-2.87	3.04E-10	1.49	0.001810964	NA	NA
3628	PNMA3	H	-2.48	8.34E-11	-1.08	2.82E-02	-1.38	1.13E-04	NA	NA
3629	IRF6	H	-2.50	1.88E-18	-3.32	3.28E-33	1.59	6.79E-14	NA	NA
3630	SULF2	H	-2.74	5.53E-44	1.98	1.74E-21	-3.96	1.72E-86	NA	NA
3631	ZNF804A	H	-2.88	1.02E-144	-1.02	7.49E-18	-1.63	4.23E-52	NA	NA
3632	NRP1	H	-3.52	2.28E-12	-1.80	3.12E-03	-2.90	1.33E-08	NA	NA
3633	ASCL1	H	-3.82	9.89E-270	-1.00	3.13E-13	-3.76	8.07E-262	NA	NA
3634	SSTR1	H	-3.83	1.80E-04	-4.49	1.39E-03	-1.79	0.028727244	NA	NA
3635	IGFBP7-AS1	H	-4.97	1.02E-04	-4.74	1.40E-04	1.41	0.038835634	NA	NA
3636	DHRS3	I	9.20	8.75E-120	7.43	1.71E-17	-2.37	0.035488788	-4.14	9.96E-225
3637	NTRK2	I	7.67	0	7.27	4.53E-48	-3.23	3.41E-09	-3.63	0
3638	GPR35	I	6.56	5.89E-80	2.58	2.85E-15	1.16	0.007793535	-2.82	2.03E-22
3639	CRABP2	I	5.95	0	6.11	0	-3.30	7.85E-147	-3.14	2.29E-210
3640	RAB6C-AS1	I	5.63	4.53E-04	-3.97	1.64E-02	5.42	8.84E-04	-4.18	7.95E-03
3641	BHLHE40	I	5.52	1.32E-67	6.08	1.01E-85	1.34	0.002746084	1.89	1.75E-18
3642	SMOC1	I	5.39	6.88E-60	3.11	3.19E-04	-3.93	9.91E-07	-6.20	2.31E-64
3643	ENPP2	I	5.28	0	2.53	3.20E-63	-1.15	1.47E-11	-3.90	7.72E-266
3644	RGS8	I	4.97	0	4.36	1.95E-23	-4.58	4.05E-26	-5.20	0
3645	CD226	I	4.60	7.07E-29	2.23	5.18E-07	1.25	0.017260005	-1.12	1.05E-02
3646	FER1L4	I	4.50	1.24E-36	2.65	3.74E-16	2.95	1.55E-15	1.10	1.55E-03
3647	HTR2B	I	4.34	4.75E-56	5.71	7.79E-22	-2.70	6.33E-05	-1.33	2.20E-12
3648	SVOPL	I	4.29	4.86E-07	2.72	2.68E-09	2.81	0.002758148	1.24	5.02E-03
3649	BMP4	I	4.22	7.30E-04	5.76	2.87E-26	3.04	0.031805785	4.57	2.31E-27
3650	PDE11A	I	4.13	2.84E-99	1.13	1.67E-08	1.30	1.64E-09	-1.71	3.60E-21
3651	ARHGAP20	I	4.12	3.53E-10	2.98	2.54E-59	4.25	7.37E-11	3.10	1.80E-58
3652	ADAM29	I	4.11	3.59E-20	4.57	9.95E-14	-1.60	0.047068333	-1.13	3.54E-03
3653	TNR	I	4.02	1.51E-30	2.07	7.31E-05	-1.75	0.001233573	-3.70	1.33E-28
3654	PRKCH	I	4.00	1.18E-91	4.10	1.23E-90	-1.33	1.37E-09	-1.23	1.35E-09
3655	AC109446.3	I	3.72	6.47E-07	3.56	1.52E-02	-3.28	0.029345747	-3.44	7.16E-07
3656	HES1	I	3.69	1.89E-20	1.68	1.21E-05	3.52	1.27E-18	1.51	7.83E-05
3657	SLC6A17	I	3.54	1.31E-53	1.89	6.67E-10	-1.54	9.37E-07	-3.19	2.25E-46
3658	PTPRH	I	3.31	5.49E-44	2.32	7.71E-18	-1.07	3.38E-04	-2.07	5.67E-19
3659	MMP28	I	3.27	6.98E-66	2.70	3.63E-10	-2.63	1.09E-09	-3.20	1.06E-67
3660	SNCAIP	I	3.00	6.16E-25	1.27	1.37E-03	-1.70	3.12E-06	-3.43	8.51E-31

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3661	BHLHE41	I	3.00	1.15E-02	2.82	1.73E-03	4.45	1.09E-04	4.28	4.27E-08
3662	RAB6C	I	2.94	4.29E-05	-1.90	3.49E-03	3.30	2.61E-06	-1.54	2.18E-02
3663	C8orf31	I	2.84	1.17E-36	5.24	1.09E-27	-4.68	2.78E-22	-2.29	7.31E-25
3664	GIPR	I	2.80	4.94E-09	-1.95	1.39E-04	2.31	3.24E-06	-2.43	3.62E-07
3665	AC022784.1	I	2.77	1.08E-105	1.73	6.02E-27	-1.25	6.50E-14	-2.28	4.38E-76
3666	SPRY1	I	2.75	1.16E-101	3.50	2.58E-55	-3.46	3.42E-54	-2.72	3.65E-100
3667	DEFB131E	I	2.73	2.02E-05	2.85	1.47E-05	-2.21	0.001651823	-2.10	9.47E-04
3668	TMEM215	I	2.71	4.10E-82	3.23	5.75E-26	-3.22	6.61E-26	-2.70	1.62E-83
3669	FANK1	I	2.71	5.29E-12	1.20	4.89E-07	2.91	4.79E-14	1.41	3.77E-09
3670	FAM46A	I	2.68	5.27E-26	1.71	2.05E-11	2.36	3.50E-20	1.39	6.43E-08
3671	VGF	I	2.68	6.45E-32	1.93	1.48E-16	-1.67	9.07E-13	-2.42	2.41E-26
3672	BTK	I	2.63	1.13E-03	1.53	1.00E-02	4.46	3.13E-10	3.36	7.08E-09
3673	HOXD4	I	2.58	6.90E-48	2.81	1.03E-37	-1.26	3.33E-07	-1.03	2.23E-10
3674	LINC00525	I	2.58	4.88E-07	2.67	8.10E-04	-1.92	0.028607148	-1.82	1.70E-04
3675	JADE2	I	2.56	7.08E-23	2.06	3.50E-16	1.54	1.26E-08	1.04	8.49E-05
3676	RAB43P1	I	2.32	1.55E-02	1.63	3.35E-02	2.36	0.014189646	1.67	2.35E-02
3677	CALCB	I	2.31	1.86E-100	1.25	1.89E-13	-1.96	3.57E-35	-3.02	2.20E-147
3678	NEDD9	I	2.31	9.28E-59	2.67	9.26E-84	1.33	1.58E-19	1.69	1.27E-34
3679	FOXI3	I	2.26	1.37E-28	2.44	3.77E-225	4.00	1.05E-98	4.18	0
3680	SLC32A1	I	2.25	8.02E-03	3.67	1.39E-02	-3.35	0.027249742	-1.93	2.15E-02
3681	SLC22A31	I	2.23	3.12E-03	1.07	9.50E-03	3.80	8.17E-09	2.64	7.11E-10
3682	ISY1-RAB43	I	2.23	8.43E-18	1.11	1.78E-05	2.28	1.02E-18	1.17	4.14E-06
3683	AL365181.3	I	2.15	8.76E-12	1.53	2.87E-02	-2.92	2.35E-07	-3.54	6.96E-22
3684	EPB41L2	I	2.11	7.93E-04	2.54	5.22E-05	-1.65	0.034110678	-1.22	3.57E-02
3685	CCDC68	I	2.09	1.97E-16	5.90	5.78E-53	-2.50	4.39E-09	1.31	2.92E-09
3686	NPPA	I	2.08	1.58E-15	1.76	1.17E-02	-3.15	5.00E-08	-3.47	6.77E-26
3687	FZD7	I	2.07	1.90E-110	1.80	2.20E-135	1.83	1.68E-84	1.56	1.26E-105
3688	ADAMTS2	I	2.05	1.71E-12	5.84	3.11E-26	-5.63	1.47E-24	-1.84	2.94E-10
3689	ASS1	I	2.03	1.78E-24	5.77	3.45E-19	-5.30	2.56E-16	-1.56	4.27E-16
3690	S100A10	I	2.03	3.06E-05	2.78	2.40E-93	4.51	5.73E-27	5.26	2.03E-131
3691	HMCN2	I	2.03	1.45E-07	4.46	8.58E-22	-3.73	1.22E-15	-1.31	1.43E-03
3692	YAP1	I	1.96	4.94E-18	1.59	1.33E-19	3.82	1.10E-71	3.45	2.34E-82
3693	AC102945.2	I	1.93	2.68E-12	1.62	1.40E-05	-1.22	0.00214938	-1.53	7.56E-09
3694	RAB26	I	1.92	7.81E-15	1.13	1.03E-03	-1.13	5.53E-04	-1.92	7.63E-14
3695	GPX3	I	1.89	5.98E-18	1.91	2.08E-17	-1.13	2.17E-06	-1.11	7.11E-07
3696	C9orf135	I	1.85	6.09E-05	3.40	1.37E-03	-3.10	0.003859599	-1.55	5.40E-04
3697	LBH	I	1.85	8.88E-32	3.09	1.74E-55	-3.51	4.21E-73	-2.27	2.24E-46
3698	TNS3	I	1.84	1.17E-71	2.92	1.63E-152	-2.28	1.59E-90	-1.20	6.49E-32
3699	COL26A1	I	1.79	2.80E-17	2.59	9.69E-15	-3.70	6.95E-33	-2.90	4.68E-36
3700	TOX2	I	1.77	2.82E-40	2.01	3.11E-18	-4.12	6.23E-85	-3.88	8.82E-146
3701	ARHGDIB	I	1.72	9.02E-06	3.99	1.98E-60	1.11	0.010756495	3.37	5.06E-50
3702	ANGPT2	I	1.72	1.97E-25	1.98	8.09E-24	-1.69	3.14E-17	-1.43	3.57E-18
3703	ZNF703	I	1.66	3.57E-13	1.52	2.87E-04	-3.05	1.69E-17	-3.19	1.84E-35
3704	FAM30A	I	1.62	1.46E-02	3.42	5.23E-07	-5.21	5.90E-16	-3.41	1.27E-08
3705	OSBPL5	I	1.62	4.34E-04	2.03	2.05E-04	-1.63	0.003725935	-1.22	1.09E-02
3706	AL162431.1	I	1.60	5.23E-03	-1.38	1.04E-02	1.82	9.72E-04	-1.16	3.76E-02
3707	KRTAP5-AS1	I	1.47	6.42E-21	1.39	2.65E-09	-2.01	3.60E-20	-2.09	1.10E-37
3708	FIGNL2	I	1.46	1.02E-32	1.70	2.60E-20	-1.96	1.82E-27	-1.72	2.96E-43
3709	NAV2	I	1.46	1.06E-17	1.50	3.47E-18	-1.07	1.21E-09	-1.03	3.39E-09
3710	PRSS12	I	1.44	4.27E-52	1.07	4.96E-30	2.22	2.59E-124	1.85	2.06E-89
3711	PLXNA2	I	1.43	1.71E-14	4.23	8.60E-110	-4.46	1.52E-122	-1.66	2.14E-19
3712	C4orf47	I	1.38	1.60E-03	1.92	1.71E-13	1.81	8.43E-06	2.35	3.72E-18
3713	AC022336.2	I	1.37	1.90E-23	1.76	1.13E-19	-2.15	6.01E-30	-1.75	3.65E-36
3714	CDK5R2	I	1.37	1.43E-33	1.99	1.24E-62	-1.74	8.86E-48	-1.12	6.72E-23
3715	ARHGAP6	I	1.36	1.25E-06	1.29	7.73E-08	1.21	2.36E-05	1.14	1.71E-06
3716	H19	I	1.31	1.48E-06	2.57	6.19E-27	1.98	3.06E-14	3.23	8.29E-42
3717	AC027601.3	I	1.31	2.40E-05	1.16	7.91E-03	-1.36	8.45E-04	-1.51	9.12E-07
3718	DNAH10	I	1.29	4.64E-11	1.03	2.54E-05	-1.25	8.09E-08	-1.52	1.09E-14
3719	ETS1	I	1.29	1.11E-73	1.65	1.19E-85	-2.49	8.40E-202	-2.14	1.55E-192
3720	CTSH	I	1.28	2.20E-15	1.39	4.90E-14	-1.74	2.27E-22	-1.63	2.54E-24

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3721	CAMK2N1	I	1.25	5.25E-51	1.95	3.53E-09	-7.31	2.61E-153	-6.62	0
3722	PTPN14	I	1.21	6.65E-03	1.22	6.91E-03	1.35	0.002121461	1.35	1.71E-03
3723	HS3ST2	I	1.21	1.56E-07	2.28	1.28E-06	-4.17	3.52E-23	-3.10	1.70E-33
3724	FSTL4	I	1.21	5.06E-08	3.57	2.28E-06	-4.57	1.50E-10	-2.22	8.71E-19
3725	LINC01679	I	1.20	2.27E-05	1.16	4.36E-12	2.49	8.91E-24	2.45	1.95E-39
3726	NNAT	I	1.20	1.91E-29	2.14	1.47E-09	-8.71	2.14E-180	-7.76	0
3727	MN1	I	1.17	1.61E-03	1.93	1.05E-09	1.66	1.97E-06	2.41	9.71E-15
3728	GFRA2	I	1.17	1.76E-07	5.09	3.08E-05	-7.88	5.66E-13	-3.97	1.32E-23
3729	COL3A1	I	1.15	3.49E-03	1.29	6.76E-04	6.65	3.05E-84	6.78	1.04E-91
3730	ATP8B3	I	1.13	1.15E-14	1.09	1.59E-20	1.34	4.30E-20	1.30	5.46E-30
3731	CADM3	I	1.13	1.19E-09	-2.44	4.64E-02	-5.35	1.52E-28	-8.91	3.00E-24
3732	PDE10A	I	1.11	2.06E-192	1.05	8.12E-202	1.45	0	1.38	0
3733	ITPKB	I	1.05	1.48E-18	1.60	4.74E-119	3.33	2.65E-218	3.88	0
3734	APLNR	I	1.04	2.29E-02	1.78	1.15E-02	-1.77	0.009696179	-1.02	2.80E-02
3735	TNFRSF12A	I	1.03	9.99E-05	1.14	3.27E-08	1.28	4.61E-07	1.39	8.40E-12
3736	CX3CL1	I	-1.04	1.06E-20	-1.22	3.34E-21	-1.14	4.91E-24	-1.31	1.70E-25
3737	AC073365.1	I	-1.06	2.81E-02	-2.29	1.22E-03	-2.35	3.19E-06	-3.57	1.98E-09
3738	SOX2	I	-1.06	1.24E-05	-2.24	1.63E-71	4.11	1.00E-149	2.93	1.30E-47
3739	SCRT2	I	-1.06	9.88E-21	-1.55	2.60E-19	-2.40	6.95E-84	-2.89	1.92E-74
3740	BRSK2	I	-1.08	2.76E-11	-1.36	2.04E-06	-4.85	3.03E-137	-5.14	6.68E-107
3741	ANK1	I	-1.08	8.69E-14	-1.02	1.48E-05	-3.21	1.47E-85	-3.15	6.88E-58
3742	PLCH2	I	-1.09	1.50E-06	-2.65	3.08E-14	-2.23	7.32E-22	-3.79	8.37E-31
3743	NHSL2	I	-1.12	3.48E-54	-1.44	3.31E-117	1.91	1.79E-198	1.59	9.18E-114
3744	PSTPIP1	I	-1.12	4.19E-02	-1.86	1.00E-02	-1.87	3.26E-04	-2.62	5.01E-05
3745	PLCXD3	I	-1.13	3.16E-23	-1.65	8.49E-68	3.33	6.29E-251	2.82	4.35E-153
3746	GABRD	I	-1.14	6.95E-03	-1.17	1.55E-02	-1.81	7.24E-06	-1.84	1.47E-05
3747	BX005019.1	I	-1.14	1.18E-02	-1.98	1.48E-02	-2.50	9.03E-08	-3.33	9.86E-07
3748	NSG2	I	-1.18	2.74E-74	-1.28	2.44E-55	-3.77	0	-3.88	0
3749	MIR137HG	I	-1.18	2.85E-12	-1.55	5.29E-09	-2.28	2.94E-36	-2.64	4.29E-28
3750	DYDC2	I	-1.20	2.10E-03	-1.00	3.78E-04	2.73	2.26E-22	2.93	6.15E-19
3751	RNF125	I	-1.21	2.66E-03	-1.41	1.31E-04	1.43	8.46E-05	1.23	2.15E-03
3752	AC016717.2	I	-1.23	4.73E-06	-1.43	8.63E-04	-2.21	6.37E-15	-2.42	8.02E-11
3753	SERPINF1	I	-1.25	2.10E-22	-1.23	3.36E-55	5.03	0	5.05	0
3754	GPC3	I	-1.31	1.63E-27	-1.05	1.31E-26	1.89	2.78E-77	2.14	2.92E-79
3755	KCNG1	I	-1.32	8.36E-09	-1.49	4.31E-12	1.42	4.22E-11	1.25	5.44E-08
3756	PRKCA	I	-1.33	4.53E-186	-1.24	5.88E-173	1.18	9.84E-158	1.27	9.31E-171
3757	POU4F2	I	-1.38	2.41E-11	-3.49	1.34E-02	-6.25	1.55E-29	-8.36	1.41E-14
3758	EGFR	I	-1.46	1.76E-62	-1.04	6.06E-44	2.76	1.75E-285	3.18	0
3759	UNC5A	I	-1.47	1.07E-09	-2.47	3.35E-12	-4.29	2.85E-67	-5.29	2.60E-59
3760	SEMA3G	I	-1.55	2.16E-04	-1.42	1.22E-06	1.55	1.53E-07	1.68	3.39E-05
3761	PRLHR	I	-1.64	8.71E-25	-2.70	7.47E-23	-1.52	1.12E-21	-2.58	4.08E-21
3762	ALOX12B	I	-1.65	3.46E-02	1.60	3.25E-02	-1.55	0.037553651	1.70	2.72E-02
3763	DOK4	I	-1.70	1.09E-29	-2.20	2.28E-48	-1.20	3.33E-15	-1.70	3.10E-29
3764	LARGE2	I	-1.71	1.10E-06	-1.48	4.39E-06	1.13	4.56E-04	1.36	1.82E-04
3765	ARHGEF28	I	-1.72	8.52E-06	-2.33	1.52E-10	1.84	5.18E-07	1.23	2.55E-03
3766	LINC00237	I	-1.78	7.55E-12	-1.79	8.55E-09	-1.34	2.34E-07	-1.35	3.29E-05
3767	LINC00639	I	-1.80	4.73E-04	-1.91	1.59E-05	1.38	0.001342342	1.27	2.82E-02
3768	AL645608.7	I	-1.83	3.12E-08	-1.39	2.38E-07	1.10	4.07E-05	1.54	5.79E-06
3769	CHRM2	I	-1.83	8.60E-17	-1.75	2.40E-13	-1.21	7.53E-08	-1.14	4.72E-06
3770	HRH3	I	-1.86	2.26E-12	-1.40	1.38E-03	-3.43	4.04E-33	-2.97	1.18E-15
3771	CD44	I	-1.87	1.29E-02	-2.61	7.10E-06	3.27	7.94E-09	2.54	3.20E-04
3772	FLRT3	I	-1.89	9.17E-88	-1.27	2.35E-54	1.29	1.02E-55	1.91	1.46E-90
3773	TRHDE	I	-1.93	3.82E-05	-1.64	2.05E-04	2.38	2.03E-08	2.67	1.68E-09
3774	SPTSSB	I	-1.93	7.03E-08	-1.11	1.01E-03	1.60	1.00E-06	2.42	9.41E-13
3775	EOMES	I	-1.96	5.37E-04	-1.92	4.88E-12	3.19	6.59E-24	3.23	1.26E-10
3776	ADGRG1	I	-1.97	1.93E-03	-1.87	4.82E-02	-3.27	4.70E-07	-3.17	4.25E-05
3777	PAPPA	I	-1.98	1.79E-05	-1.51	5.65E-04	1.99	2.58E-06	2.45	2.42E-08
3778	TPTE2P6	I	-1.98	4.01E-02	-1.84	3.02E-03	2.39	7.43E-05	2.54	4.90E-03
3779	LRFN2	I	-2.02	1.74E-22	-2.35	1.96E-13	-1.90	3.09E-20	-2.23	2.80E-12
3780	KCNJ12	I	-2.04	7.34E-24	-2.79	2.92E-05	-3.86	5.92E-54	-4.61	2.46E-14

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3781	NKD2	I	-2.18	1.07E-02	-3.39	4.55E-02	-3.11	0.001786648	-4.32	2.43E-03
3782	SLC7A11	I	-2.19	4.67E-77	1.97	3.88E-57	-2.85	1.77E-121	1.31	1.59E-27
3783	TNC	I	-2.37	1.44E-10	-2.37	3.01E-14	2.24	1.13E-12	2.24	1.18E-09
3784	TMEM100	I	-2.39	1.11E-82	-1.69	7.06E-67	2.59	3.70E-149	3.29	4.40E-161
3785	IGSF1	I	-2.50	1.29E-19	-1.69	1.94E-09	1.15	7.96E-05	1.96	2.64E-12
3786	LRRC4	I	-2.55	1.09E-28	-1.97	2.40E-15	-1.80	6.22E-15	-1.21	3.60E-06
3787	RPH3A	I	-2.56	1.36E-64	-1.99	1.46E-35	-2.73	1.60E-72	-2.16	4.22E-42
3788	FOLR1	I	-2.61	2.61E-02	-2.32	1.34E-05	2.63	1.79E-06	2.92	9.71E-03
3789	ULBP1	I	-2.66	1.71E-31	1.06	3.54E-02	-4.87	6.64E-43	-1.15	6.19E-04
3790	NTNG1	I	-2.67	2.34E-15	-2.72	9.92E-24	2.33	6.57E-18	2.27	2.99E-11
3791	NR4A3	I	-2.76	2.36E-22	-1.88	4.15E-35	1.93	2.24E-36	2.81	1.89E-23
3792	CDH7	I	-2.81	1.08E-33	-1.95	2.73E-28	2.89	2.41E-57	3.74	1.51E-62
3793	ANKRD29	I	-2.91	6.39E-10	-2.27	1.08E-17	3.61	2.18E-36	4.25	4.94E-22
3794	LAMA3	I	-3.09	3.87E-21	-1.93	1.00E-20	2.51	2.33E-30	3.66	1.97E-31
3795	BHLHE22	I	-3.11	2.72E-02	-2.05	4.53E-07	4.27	1.83E-13	5.32	7.12E-06
3796	CHST15	I	-3.13	2.69E-27	-2.36	7.87E-05	-4.76	2.05E-53	-3.99	1.52E-13
3797	INHBE	I	-3.41	5.04E-09	2.62	7.83E-07	-2.67	3.95E-07	3.36	6.51E-09
3798	ADAMTS17	I	-3.47	1.34E-12	-2.83	2.89E-05	-3.55	2.40E-13	-2.92	1.22E-05
3799	IL12RB2	I	-3.58	1.24E-10	-1.64	4.72E-04	1.48	0.001752828	3.41	8.20E-10
3800	ERICH3	I	-3.65	9.10E-10	-6.31	1.10E-07	-1.14	0.019730804	-3.80	4.92E-03
3801	RYR2	I	-3.71	1.93E-02	5.06	1.09E-03	-5.12	7.44E-04	3.65	2.22E-02
3802	TRHDE-AS1	I	-3.80	6.99E-14	-2.09	3.28E-08	2.48	2.86E-10	4.19	5.13E-18
3803	GBX2	J	NA	NA	4.53	1.89E-94	5.04	1.59E-08	7.81	1.42E-64
3804	RAB27B	J	NA	NA	4.34	4.02E-46	-1.10	0.001742313	2.30	1.50E-14
3805	CA12	J	NA	NA	4.34	1.35E-09	-2.38	0.004211144	1.48	2.19E-02
3806	CXCL12	J	NA	NA	3.99	2.17E-07	-6.41	1.69E-19	-1.99	2.87E-10
3807	VIP	J	NA	NA	3.70	4.20E-07	-9.90	8.44E-51	-6.62	0
3808	CELF2	J	NA	NA	3.56	1.20E-02	-6.31	1.36E-07	-1.61	2.72E-02
3809	ADAMTS9-AS1	J	NA	NA	3.22	1.67E-53	-1.67	1.98E-13	1.21	9.70E-13
3810	AC023141.4	J	NA	NA	3.17	4.70E-02	-5.29	3.19E-05	-3.25	6.94E-05
3811	FAP	J	NA	NA	2.83	4.84E-11	6.69	1.22E-08	7.83	2.38E-13
3812	ANG	J	NA	NA	2.72	4.47E-06	2.39	0.003147046	3.71	3.21E-10
3813	GPR158	J	NA	NA	2.63	5.59E-07	-4.68	4.34E-23	-1.74	6.29E-11
3814	SLC12A3	J	NA	NA	2.55	7.36E-04	-4.46	9.52E-12	-1.72	1.77E-03
3815	SALL4	J	NA	NA	2.51	2.84E-26	-3.54	6.42E-54	-1.27	1.07E-09
3816	FAM131B	J	NA	NA	2.50	2.13E-02	-3.40	3.20E-04	-1.89	3.67E-03
3817	OSR2	J	NA	NA	2.45	2.59E-05	2.52	0.018636113	4.48	4.47E-08
3818	GPC5	J	NA	NA	2.30	1.44E-05	-1.66	0.003207015	1.27	2.13E-02
3819	LAYN	J	NA	NA	2.26	3.28E-52	-1.04	1.05E-10	1.17	1.40E-17
3820	P2RY6	J	NA	NA	2.11	1.34E-06	-2.81	1.34E-11	-1.41	5.76E-04
3821	NRIP1	J	NA	NA	2.09	2.64E-03	-3.81	2.89E-10	-1.26	4.31E-02
3822	SEMA5B	J	NA	NA	2.00	1.75E-08	-4.57	3.96E-46	-1.67	7.50E-11
3823	LINC02037	J	NA	NA	1.86	8.57E-04	-2.28	1.22E-05	-1.41	2.13E-04
3824	NELL2	J	NA	NA	1.82	3.08E-30	-4.02	1.42E-152	-2.12	2.06E-47
3825	ST20-MTHFS	J	NA	NA	1.65	3.63E-05	-4.42	8.23E-40	-2.53	2.88E-25
3826	SLC44A5	J	NA	NA	1.63	7.72E-41	-2.69	3.77E-117	-1.69	6.58E-64
3827	BNC2	J	NA	NA	1.62	2.17E-02	-4.04	5.85E-13	-2.34	1.53E-12
3828	VSTM2L	J	NA	NA	1.61	4.57E-11	-3.60	4.80E-59	-2.04	1.39E-26
3829	CIART	J	NA	NA	1.53	2.28E-03	2.05	0.001486013	3.48	2.31E-09
3830	FAM46C	J	NA	NA	1.52	6.70E-03	1.74	0.037947327	2.52	2.22E-05
3831	SLC18A3	J	NA	NA	1.50	4.53E-02	-6.38	2.58E-31	-4.68	2.93E-28
3832	ZMAT4	J	NA	NA	1.47	6.56E-07	-3.54	2.11E-41	-1.66	2.07E-12
3833	PDGFC	J	NA	NA	1.44	8.99E-04	-1.94	8.84E-07	-1.20	1.43E-03
3834	TRIM5	J	NA	NA	1.41	9.91E-06	1.08	0.015464201	2.82	2.85E-15
3835	FOXD1	J	NA	NA	1.36	9.43E-04	-5.59	1.69E-68	-4.02	9.17E-74
3836	MOXD1	J	NA	NA	1.26	3.08E-03	5.95	9.58E-12	5.59	6.93E-23
3837	PIPOX	J	NA	NA	1.26	5.52E-03	-3.30	1.61E-20	-2.88	2.17E-27
3838	CYB561D2	J	NA	NA	1.20	3.13E-03	1.31	0.002457361	2.30	2.58E-09
3839	BTBD11	J	NA	NA	1.19	7.64E-16	-1.66	4.23E-31	-1.25	2.25E-20
3840	FAM222A-AS1	J	NA	NA	1.19	2.28E-07	-3.94	9.64E-96	-3.72	5.93E-158

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3841	TMEM255A	J	NA	NA	1.18	7.72E-03	1.58	7.54E-04	1.75	2.63E-05
3842	LOXL2	J	NA	NA	1.17	6.70E-13	1.66	1.45E-23	2.43	1.23E-52
3843	ATXN1	J	NA	NA	1.13	3.58E-06	-1.74	2.85E-14	-1.60	1.09E-14
3844	DLK2	J	NA	NA	1.13	2.51E-05	-1.39	6.81E-08	-1.06	5.81E-06
3845	AC008115.3	J	NA	NA	1.08	1.54E-08	1.28	1.19E-07	1.39	3.31E-13
3846	LSAMP	J	NA	NA	1.05	2.23E-13	-2.10	8.69E-54	-1.29	2.72E-21
3847	CHRM5	J	NA	NA	1.04	3.46E-02	-2.93	1.93E-13	-2.69	1.21E-12
3848	AC012213.4	J	NA	NA	-1.00	1.24E-02	-1.42	5.89E-07	-2.33	2.66E-13
3849	AC092611.3	J	NA	NA	-1.01	4.12E-02	3.16	1.30E-08	3.01	1.28E-05
3850	CEBPA	J	NA	NA	-1.01	1.69E-02	-1.26	1.81E-04	-1.70	2.66E-06
3851	S100B	J	NA	NA	-1.01	1.25E-02	2.81	1.47E-10	1.99	3.03E-05
3852	AC135782.1	J	NA	NA	-1.02	3.04E-03	2.24	2.07E-09	1.44	7.99E-04
3853	MGP	J	NA	NA	-1.04	9.20E-13	5.95	1.96E-118	4.22	4.92E-62
3854	AC002525.1	J	NA	NA	-1.04	3.03E-02	1.52	9.96E-04	1.20	3.54E-02
3855	UNC79	J	NA	NA	-1.06	1.49E-63	-1.19	1.39E-109	-2.54	0
3856	ADAM21	J	NA	NA	-1.06	1.74E-02	1.77	4.92E-05	1.87	7.29E-04
3857	KCNH6	J	NA	NA	-1.06	4.30E-04	-1.05	7.67E-05	-1.95	3.45E-13
3858	MIR9-3HG	J	NA	NA	-1.06	5.42E-03	-5.23	5.65E-86	-5.98	7.33E-90
3859	SCG3	J	NA	NA	-1.09	4.37E-41	-1.90	1.20E-133	-2.94	0
3860	KCNK3	J	NA	NA	-1.10	1.16E-14	-3.09	7.65E-213	-3.52	4.76E-184
3861	SLC6A11	J	NA	NA	-1.10	3.73E-02	-5.59	6.73E-86	-5.69	1.65E-65
3862	AL359643.3	J	NA	NA	-1.10	5.65E-06	2.00	7.73E-15	1.63	1.93E-08
3863	SCG5	J	NA	NA	-1.11	2.85E-10	4.49	4.03E-78	3.04	6.56E-40
3864	GRIA2	J	NA	NA	-1.11	5.03E-09	-2.90	1.04E-106	-4.27	6.89E-156
3865	LEF1	J	NA	NA	-1.12	4.41E-08	-1.29	8.48E-13	-2.19	2.27E-31
3866	LINC00890	J	NA	NA	-1.14	1.20E-07	4.04	7.39E-81	2.70	3.69E-35
3867	ADAMTS3	J	NA	NA	-1.15	2.98E-13	4.35	1.42E-150	3.20	1.54E-82
3868	ZDHHC2	J	NA	NA	-1.15	4.93E-23	2.29	1.17E-86	1.28	2.24E-26
3869	VWDE	J	NA	NA	-1.16	9.43E-03	2.83	9.14E-12	1.87	1.18E-05
3870	DLX5	J	NA	NA	-1.17	1.27E-04	-1.54	2.93E-11	-1.93	1.46E-12
3871	ASB9	J	NA	NA	-1.17	7.03E-18	7.00	9.69E-66	6.78	2.31E-19
3872	VWA5B2	J	NA	NA	-1.18	3.84E-05	1.52	8.76E-08	1.16	6.77E-04
3873	FAM20C	J	NA	NA	-1.19	3.97E-02	-1.16	0.02057487	-2.00	4.87E-05
3874	MARCH11	J	NA	NA	-1.19	4.26E-10	-2.88	3.27E-108	-3.85	1.67E-123
3875	CR381653.2	J	NA	NA	-1.27	4.04E-17	2.50	1.13E-49	1.07	2.69E-09
3876	CACNA1B	J	NA	NA	-1.29	4.36E-07	-1.71	5.66E-13	-2.56	2.01E-27
3877	SNX18P26	J	NA	NA	-1.31	8.88E-08	2.43	3.87E-19	1.28	3.75E-05
3878	DISP2	J	NA	NA	-1.33	2.49E-10	-1.36	1.14E-11	-2.88	1.13E-48
3879	LRRC4C	J	NA	NA	-1.33	5.12E-03	2.32	7.02E-07	1.36	1.65E-02
3880	AKAP6	J	NA	NA	-1.34	2.30E-10	-1.48	1.69E-13	-2.94	1.14E-49
3881	ACPP	J	NA	NA	-1.34	3.09E-02	6.61	7.64E-31	5.93	6.44E-23
3882	ADGRB1	J	NA	NA	-1.37	4.35E-03	-3.46	5.95E-21	-4.29	1.30E-27
3883	FAM92B	J	NA	NA	-1.37	1.46E-02	-1.49	3.22E-04	-2.77	6.68E-10
3884	AC015712.2	J	NA	NA	-1.39	2.37E-11	-2.45	4.47E-62	-3.43	2.56E-81
3885	GLB1L3	J	NA	NA	-1.42	2.98E-02	-3.28	4.39E-16	-4.34	3.64E-21
3886	LHX9	J	NA	NA	-1.43	8.31E-03	2.05	3.93E-05	1.25	2.40E-02
3887	EPHA8	J	NA	NA	-1.47	8.45E-04	-2.27	1.00E-12	-3.63	1.63E-23
3888	PTGER3	J	NA	NA	-1.47	1.64E-08	1.99	4.37E-14	1.07	7.03E-04
3889	DPP10	J	NA	NA	-1.47	2.86E-02	3.89	4.96E-09	2.12	7.19E-03
3890	FAM155B	J	NA	NA	-1.50	2.85E-16	-1.13	5.99E-15	-2.00	1.70E-30
3891	SEZ6L	J	NA	NA	-1.51	5.01E-07	-6.72	0	-7.54	2.12E-218
3892	CAPN6	J	NA	NA	-1.51	1.66E-73	4.13	3.93E-251	3.04	9.07E-110
3893	SNAI1P1	J	NA	NA	-1.52	2.95E-02	-1.16	0.01675049	-3.15	1.01E-08
3894	LINGO1	J	NA	NA	-1.52	4.88E-06	-3.89	2.68E-62	-4.59	1.15E-60
3895	LRRN2	J	NA	NA	-1.54	4.27E-13	-1.60	8.24E-20	-2.36	2.53E-32
3896	NPTXR	J	NA	NA	-1.54	8.34E-34	-1.39	5.41E-42	-2.20	2.05E-72
3897	EDIL3	J	NA	NA	-1.55	4.82E-08	-1.64	5.08E-10	-3.33	4.86E-37
3898	ANGPTL1	J	NA	NA	-1.61	1.02E-03	4.70	8.81E-14	4.11	9.83E-10
3899	AKR1C1	J	NA	NA	-1.62	1.08E-32	4.00	1.73E-105	1.88	2.95E-27
3900	TLX1	J	NA	NA	-1.67	1.78E-05	-3.33	8.57E-24	-5.24	4.34E-52

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3901	AC119751.1	J	NA	NA	-1.68	7.53E-03	3.31	2.19E-07	1.91	1.03E-02
3902	EPHA10	J	NA	NA	-1.68	1.57E-03	-2.79	2.71E-14	-4.07	3.37E-21
3903	AC068057.1	J	NA	NA	-1.71	1.07E-02	1.29	0.048419249	-1.38	4.68E-02
3904	ATAD3C	J	NA	NA	-1.72	8.27E-04	1.14	0.03373892	-1.16	3.63E-02
3905	PLA2G4A	J	NA	NA	-1.72	2.22E-02	6.76	3.81E-08	3.36	4.77E-03
3906	TRIB2	J	NA	NA	-1.83	4.39E-104	-1.08	1.80E-41	-2.11	2.86E-140
3907	FAM19A5	J	NA	NA	-1.90	4.57E-02	5.96	4.42E-06	4.14	4.32E-03
3908	P2RX5	J	NA	NA	-1.90	3.97E-11	5.81	3.03E-21	4.12	2.35E-09
3909	KCNJ2	J	NA	NA	-2.03	2.23E-05	1.58	6.96E-04	-1.15	3.53E-02
3910	KCNJ9	J	NA	NA	-2.06	1.18E-02	-4.66	1.93E-45	-6.79	8.28E-28
3911	SLC37A1	J	NA	NA	-2.08	7.60E-07	-1.03	0.007434859	-3.53	6.95E-22
3912	LINC01123	J	NA	NA	-2.11	4.58E-02	5.95	1.30E-05	3.91	1.12E-02
3913	AC110285.2	J	NA	NA	-2.16	1.52E-02	-3.07	4.61E-08	-4.81	8.83E-11
3914	FGF20	J	NA	NA	-2.17	2.87E-08	6.58	1.37E-32	3.46	4.66E-09
3915	CPZ	J	NA	NA	-2.19	4.88E-02	-1.66	0.027644158	-2.84	2.29E-03
3916	UTRN	J	NA	NA	-2.24	1.15E-06	-3.22	1.28E-19	-4.60	1.89E-27
3917	SCML4	J	NA	NA	-2.28	4.47E-03	-1.49	0.031932474	-3.35	3.09E-06
3918	MIR7-3HG	J	NA	NA	-2.34	1.06E-03	-1.79	8.25E-05	-3.69	1.13E-09
3919	CHRM1	J	NA	NA	-2.35	2.85E-02	-6.37	2.80E-42	-8.32	6.34E-26
3920	AL359538.1	J	NA	NA	-2.43	1.45E-04	7.09	3.00E-09	4.73	2.73E-04
3921	PLAC9P1	J	NA	NA	-2.56	1.17E-03	5.51	1.84E-08	3.18	1.17E-02
3922	FSTL5	J	NA	NA	-2.78	1.39E-07	-4.94	5.06E-114	-7.71	6.28E-63
3923	PDE2A	J	NA	NA	-2.82	2.78E-32	-1.33	2.32E-09	-3.85	3.08E-61
3924	CA10	J	NA	NA	-3.01	4.62E-10	1.13	0.012527425	-2.78	8.46E-09
3925	BAHCC1	J	NA	NA	-3.25	9.04E-24	-3.76	2.08E-47	-7.35	9.46E-130
3926	PTPN5	J	NA	NA	-3.39	1.51E-02	-3.00	9.00E-08	-6.39	1.79E-08
3927	AL096816.1	J	NA	NA	-4.57	6.49E-04	-1.81	0.009057561	-5.56	7.52E-06
3928	PARVA	J	NA	NA	-4.64	1.24E-10	-4.03	7.35E-10	-8.61	1.48E-41
3929	AC012184.2	K	21.71	1.32E-07	NA	NA	22.36	4.80E-08	NA	NA
3930	AL139260.3	K	5.90	5.12E-03	NA	NA	5.12	0.019488571	NA	NA
3931	AC073610.3	K	5.30	1.27E-03	NA	NA	4.66	0.006438765	NA	NA
3932	NRG4	K	5.03	4.30E-06	NA	NA	3.12	0.009137063	NA	NA
3933	AC069257.3	K	4.58	4.23E-02	NA	NA	4.74	0.036036545	NA	NA
3934	ART4	K	4.45	4.99E-02	NA	NA	5.09	0.021879691	NA	NA
3935	SLC6A16	K	4.14	9.03E-05	NA	NA	4.40	1.67E-05	NA	NA
3936	AXDND1	K	4.03	6.00E-03	NA	NA	3.88	0.00955533	NA	NA
3937	AL136309.2	K	3.97	6.14E-03	NA	NA	3.14	0.048797018	NA	NA
3938	FOXO1	K	3.78	7.78E-04	NA	NA	4.08	2.34E-04	NA	NA
3939	MTND5P1	K	3.64	8.65E-03	NA	NA	3.70	0.007688298	NA	NA
3940	LRRC66	K	3.42	2.88E-02	NA	NA	3.50	0.025876686	NA	NA
3941	ZNF467	K	3.38	3.25E-33	NA	NA	2.95	4.50E-25	NA	NA
3942	AC074212.1	K	3.34	3.34E-02	NA	NA	4.44	0.002059038	NA	NA
3943	MTDHP3	K	3.33	4.36E-02	NA	NA	5.18	2.66E-04	NA	NA
3944	AL008707.1	K	3.33	4.65E-02	NA	NA	5.10	4.29E-04	NA	NA
3945	CYP2E1	K	3.27	7.81E-03	NA	NA	2.81	0.031364121	NA	NA
3946	AP000769.1	K	2.84	2.18E-04	NA	NA	3.22	1.64E-05	NA	NA
3947	SYT2	K	2.79	7.83E-47	NA	NA	1.45	4.67E-12	NA	NA
3948	NCAM2	K	2.77	5.64E-47	NA	NA	2.55	5.80E-40	NA	NA
3949	ITGB7	K	2.71	6.92E-03	NA	NA	2.55	0.01154489	NA	NA
3950	PLS3-AS1	K	2.65	6.40E-03	NA	NA	2.53	0.007633123	NA	NA
3951	BLVRB	K	2.64	5.94E-28	NA	NA	2.02	5.68E-16	NA	NA
3952	PCDHGB8P	K	2.61	3.86E-02	NA	NA	2.82	0.022782773	NA	NA
3953	AKR1C3	K	2.58	2.91E-06	NA	NA	1.35	0.041132304	NA	NA
3954	SSPO	K	2.53	1.20E-10	NA	NA	1.76	1.83E-05	NA	NA
3955	HOXD3	K	2.43	4.40E-31	NA	NA	1.57	5.67E-13	NA	NA
3956	BST2	K	2.38	1.87E-02	NA	NA	3.78	2.44E-05	NA	NA
3957	CMAHP	K	2.34	1.50E-27	NA	NA	1.46	1.71E-10	NA	NA
3958	AL157756.1	K	2.33	8.56E-03	NA	NA	2.07	0.030106828	NA	NA
3959	RIBC1	K	2.32	7.94E-04	NA	NA	2.21	0.001320686	NA	NA
3960	DSCR9	K	2.26	4.94E-02	NA	NA	2.53	0.025265829	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
3961	AP003419.2	K	2.17	1.49E-10	NA	NA	1.89	5.15E-08	NA	NA
3962	ATP2A1	K	2.16	1.13E-04	NA	NA	2.51	5.45E-06	NA	NA
3963	KCND1	K	2.14	1.24E-13	NA	NA	2.06	6.53E-13	NA	NA
3964	MIR22HG	K	2.09	1.75E-10	NA	NA	1.03	0.00502629	NA	NA
3965	EFCAB1	K	2.06	1.74E-02	NA	NA	2.04	0.01731186	NA	NA
3966	RAG1	K	2.05	4.36E-04	NA	NA	2.47	1.07E-05	NA	NA
3967	AC134043.2	K	2.05	1.65E-03	NA	NA	2.90	1.23E-06	NA	NA
3968	AC245595.1	K	2.02	2.48E-02	NA	NA	2.74	0.001188572	NA	NA
3969	TTC30B	K	2.02	3.02E-151	NA	NA	1.01	9.69E-36	NA	NA
3970	C1QTNF5	K	2.00	1.87E-09	NA	NA	1.32	2.44E-04	NA	NA
3971	ZCCHC24	K	1.93	1.50E-154	NA	NA	1.26	2.56E-62	NA	NA
3972	EHBP1L1	K	1.91	2.00E-05	NA	NA	1.20	0.014202821	NA	NA
3973	EMP3	K	1.89	1.49E-02	NA	NA	1.69	0.033614936	NA	NA
3974	AC021086.1	K	1.86	1.99E-02	NA	NA	2.36	0.001365306	NA	NA
3975	NEBL	K	1.78	2.96E-29	NA	NA	1.56	9.39E-23	NA	NA
3976	MYO15B	K	1.76	5.60E-04	NA	NA	1.69	9.39E-04	NA	NA
3977	PLBD1	K	1.73	1.43E-03	NA	NA	2.58	9.60E-08	NA	NA
3978	CCDC114	K	1.73	1.13E-02	NA	NA	2.42	1.65E-04	NA	NA
3979	SPATS2L	K	1.72	8.26E-121	NA	NA	1.14	4.87E-52	NA	NA
3980	TTC30A	K	1.72	6.55E-74	NA	NA	1.09	3.00E-29	NA	NA
3981	JCAD	K	1.70	1.31E-04	NA	NA	1.02	0.04403304	NA	NA
3982	TEAD3	K	1.67	6.26E-30	NA	NA	1.65	4.33E-29	NA	NA
3983	RRAS	K	1.63	2.65E-11	NA	NA	1.34	8.51E-08	NA	NA
3984	AC004678.2	K	1.62	1.42E-02	NA	NA	1.44	0.039250618	NA	NA
3985	RHBDF1	K	1.62	6.34E-06	NA	NA	1.25	9.66E-04	NA	NA
3986	CTF1	K	1.60	2.41E-07	NA	NA	1.81	2.41E-09	NA	NA
3987	GAPDHP21	K	1.59	4.75E-03	NA	NA	1.29	0.033720269	NA	NA
3988	IFIT1	K	1.59	1.69E-10	NA	NA	1.92	1.95E-15	NA	NA
3989	TMEM87B	K	1.58	2.63E-12	NA	NA	1.14	8.91E-07	NA	NA
3990	EHMT2-AS1	K	1.54	4.15E-02	NA	NA	1.58	0.038404479	NA	NA
3991	AL135905.2	K	1.52	4.39E-03	NA	NA	2.46	2.08E-07	NA	NA
3992	SLC22A18	K	1.52	4.80E-06	NA	NA	1.26	2.50E-04	NA	NA
3993	AL359752.1	K	1.50	3.51E-06	NA	NA	1.28	1.26E-04	NA	NA
3994	LRP10	K	1.49	3.54E-24	NA	NA	1.31	1.16E-18	NA	NA
3995	ROR1-AS1	K	1.46	3.56E-35	NA	NA	1.13	1.02E-20	NA	NA
3996	RHOC	K	1.45	1.12E-51	NA	NA	1.18	3.34E-34	NA	NA
3997	GPR22	K	1.42	5.97E-10	NA	NA	2.73	4.12E-36	NA	NA
3998	TSLP	K	1.42	2.93E-04	NA	NA	1.84	8.85E-07	NA	NA
3999	LPAR1	K	1.40	3.59E-36	NA	NA	1.05	3.54E-20	NA	NA
4000	LINC01759	K	1.40	3.92E-02	NA	NA	2.28	1.61E-04	NA	NA
4001	AC068946.2	K	1.40	2.99E-05	NA	NA	1.09	0.001881463	NA	NA
4002	MERTK	K	1.37	9.33E-18	NA	NA	1.43	2.09E-19	NA	NA
4003	SVIL	K	1.36	5.60E-12	NA	NA	1.16	6.06E-09	NA	NA
4004	TPBG	K	1.35	2.06E-46	NA	NA	1.63	1.82E-69	NA	NA
4005	FREM2	K	1.35	4.47E-02	NA	NA	3.50	3.06E-10	NA	NA
4006	C1RL	K	1.31	2.15E-07	NA	NA	1.51	1.42E-09	NA	NA
4007	PBXIP1	K	1.31	8.29E-31	NA	NA	1.23	3.18E-27	NA	NA
4008	CCDC87	K	1.30	2.46E-03	NA	NA	1.16	0.009789147	NA	NA
4009	JMJD1C-AS1	K	1.29	2.92E-05	NA	NA	1.71	5.55E-09	NA	NA
4010	TIMP1	K	1.28	1.61E-17	NA	NA	1.23	2.39E-16	NA	NA
4011	SUCLG2-AS1	K	1.27	8.90E-06	NA	NA	1.55	1.79E-08	NA	NA
4012	HS3ST5	K	1.27	2.24E-02	NA	NA	3.14	6.07E-11	NA	NA
4013	AL139412.1	K	1.23	3.52E-03	NA	NA	1.02	0.024400587	NA	NA
4014	MICALL2	K	1.23	1.15E-04	NA	NA	1.60	1.77E-07	NA	NA
4015	AC058822.1	K	1.20	8.32E-07	NA	NA	1.07	1.50E-05	NA	NA
4016	LYNX1	K	1.19	2.71E-02	NA	NA	1.71	7.58E-04	NA	NA
4017	TRPM4	K	1.19	5.45E-08	NA	NA	1.15	1.66E-07	NA	NA
4018	NME3	K	1.18	9.46E-03	NA	NA	1.03	0.031739671	NA	NA
4019	PNRC1	K	1.18	6.85E-26	NA	NA	1.10	1.50E-22	NA	NA
4020	PCSK4	K	1.16	9.88E-03	NA	NA	1.14	0.012788393	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4021	B4GALT6	K	1.16	8.41E-20	NA	NA	1.18	1.58E-20	NA	NA
4022	ZFP36	K	1.15	1.52E-04	NA	NA	1.37	2.90E-06	NA	NA
4023	CALCRL	K	1.13	4.15E-03	NA	NA	1.91	3.31E-08	NA	NA
4024	MRC2	K	1.13	1.62E-02	NA	NA	1.02	0.034612059	NA	NA
4025	NPC2	K	1.12	2.75E-22	NA	NA	1.11	8.53E-22	NA	NA
4026	FAM66B	K	1.11	1.44E-02	NA	NA	1.26	0.005013336	NA	NA
4027	NPHP3-ACAD11	K	1.11	1.22E-05	NA	NA	1.62	9.26E-12	NA	NA
4028	AMDHD1	K	1.11	4.34E-02	NA	NA	1.14	0.039829564	NA	NA
4029	GPR37	K	1.10	3.75E-15	NA	NA	1.19	1.25E-17	NA	NA
4030	RARG	K	1.10	1.48E-40	NA	NA	1.12	1.07E-42	NA	NA
4031	B3GNT9	K	1.08	5.24E-11	NA	NA	1.29	1.51E-15	NA	NA
4032	RAB11FIP1P1	K	1.07	8.22E-03	NA	NA	1.27	0.001018892	NA	NA
4033	TMEM88	K	1.07	3.12E-03	NA	NA	1.18	9.97E-04	NA	NA
4034	LCAT	K	1.05	5.41E-04	NA	NA	1.03	6.81E-04	NA	NA
4035	MIR503HG	K	1.04	3.73E-02	NA	NA	1.57	4.71E-04	NA	NA
4036	PRRT1	K	1.01	1.52E-05	NA	NA	1.04	9.64E-06	NA	NA
4037	AC023024.1	K	-1.01	1.33E-05	NA	NA	-1.75	5.07E-13	NA	NA
4038	AC023886.1	K	-1.01	4.81E-03	NA	NA	-1.78	4.45E-06	NA	NA
4039	EIF2S2P4	K	-1.02	9.84E-04	NA	NA	-1.04	8.74E-04	NA	NA
4040	SGPP2	K	-1.03	3.76E-03	NA	NA	-1.11	0.002069559	NA	NA
4041	AC124312.5	K	-1.04	1.17E-02	NA	NA	-1.09	0.008351237	NA	NA
4042	OSGEPL1-AS1	K	-1.04	2.47E-02	NA	NA	-1.45	0.001789384	NA	NA
4043	RGMB-AS1	K	-1.05	2.06E-02	NA	NA	-1.07	0.022714304	NA	NA
4044	VWCE	K	-1.06	5.48E-05	NA	NA	-1.01	1.47E-04	NA	NA
4045	AC092171.5	K	-1.07	9.02E-04	NA	NA	-1.18	2.70E-04	NA	NA
4046	YARS	K	-1.10	7.83E-90	NA	NA	-1.15	1.56E-97	NA	NA
4047	CAMKK1	K	-1.11	2.85E-12	NA	NA	-1.04	8.18E-11	NA	NA
4048	SLC7A1	K	-1.14	1.86E-51	NA	NA	-1.16	3.62E-54	NA	NA
4049	GARS	K	-1.14	2.69E-53	NA	NA	-1.53	1.88E-94	NA	NA
4050	CGN	K	-1.15	2.98E-04	NA	NA	-1.32	2.52E-05	NA	NA
4051	CDC42EP1	K	-1.17	2.25E-02	NA	NA	-2.08	5.07E-05	NA	NA
4052	NANOS1	K	-1.18	6.98E-28	NA	NA	-1.01	4.65E-21	NA	NA
4053	AL591623.2	K	-1.19	9.57E-03	NA	NA	-1.11	0.01820226	NA	NA
4054	LINC01619	K	-1.20	2.65E-02	NA	NA	-1.20	0.031459733	NA	NA
4055	KCTD12	K	-1.21	3.56E-44	NA	NA	-1.51	3.32E-66	NA	NA
4056	SLC3A2	K	-1.21	4.94E-43	NA	NA	-1.40	1.82E-57	NA	NA
4057	FOSL2	K	-1.22	5.68E-04	NA	NA	-1.02	0.005268519	NA	NA
4058	SESN2	K	-1.25	6.20E-26	NA	NA	-1.16	1.35E-22	NA	NA
4059	F2RL1	K	-1.26	3.75E-02	NA	NA	-1.27	0.038394417	NA	NA
4060	MTHFD1L	K	-1.27	2.02E-30	NA	NA	-1.09	1.18E-22	NA	NA
4061	AC002454.1	K	-1.28	6.01E-08	NA	NA	-2.56	2.69E-20	NA	NA
4062	ZMYND10	K	-1.29	3.05E-04	NA	NA	-1.05	0.003916231	NA	NA
4063	SPON1	K	-1.30	7.64E-03	NA	NA	-1.67	4.72E-04	NA	NA
4064	SSTR2	K	-1.31	8.27E-23	NA	NA	-1.65	9.45E-33	NA	NA
4065	MAP3K15	K	-1.33	2.57E-03	NA	NA	-2.10	3.41E-06	NA	NA
4066	FAM43A	K	-1.36	1.05E-13	NA	NA	-2.02	2.36E-24	NA	NA
4067	AC022748.2	K	-1.38	4.81E-03	NA	NA	-1.77	5.94E-04	NA	NA
4068	AC129492.1	K	-1.38	7.34E-04	NA	NA	-1.13	0.006330524	NA	NA
4069	LDLRAD4	K	-1.42	1.20E-09	NA	NA	-1.98	2.81E-17	NA	NA
4070	RPS6KA2-IT1	K	-1.42	2.51E-02	NA	NA	-2.15	0.001248938	NA	NA
4071	RPS6KA2	K	-1.43	1.42E-61	NA	NA	-1.36	6.60E-56	NA	NA
4072	GPT2	K	-1.44	5.20E-31	NA	NA	-1.38	1.25E-28	NA	NA
4073	DLL3	K	-1.48	9.66E-24	NA	NA	-1.39	7.04E-21	NA	NA
4074	C3orf80	K	-1.49	7.58E-03	NA	NA	-1.40	0.014737663	NA	NA
4075	SMG1P3	K	-1.50	4.64E-11	NA	NA	-1.32	9.28E-09	NA	NA
4076	WNT5B	K	-1.51	1.55E-02	NA	NA	-2.35	3.11E-04	NA	NA
4077	LINC00682	K	-1.51	2.36E-69	NA	NA	-1.16	8.21E-43	NA	NA
4078	SPINT1	K	-1.51	2.61E-03	NA	NA	-1.54	0.002042367	NA	NA
4079	AVIL	K	-1.51	1.12E-02	NA	NA	-1.45	0.016531643	NA	NA
4080	RN7SL2	K	-1.53	4.18E-03	NA	NA	-1.16	0.042674417	NA	NA

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4081	AC124068.2	K	-1.53	3.57E-02	NA	NA	-1.66	0.028189645	NA	NA
4082	TAL2	K	-1.56	2.93E-02	NA	NA	-1.70	0.019499417	NA	NA
4083	TMEM158	K	-1.61	2.72E-05	NA	NA	-1.42	2.17E-04	NA	NA
4084	ASNS	K	-1.63	7.36E-109	NA	NA	-2.63	2.36E-277	NA	NA
4085	PSAT1	K	-1.65	8.28E-140	NA	NA	-1.25	2.14E-81	NA	NA
4086	GADD45G	K	-1.66	7.09E-04	NA	NA	-1.33	0.007103402	NA	NA
4087	FAT3	K	-1.66	7.28E-03	NA	NA	-1.84	0.002129538	NA	NA
4088	RIMS3	K	-1.67	2.71E-282	NA	NA	-1.84	0	NA	NA
4089	NOTUM	K	-1.70	5.22E-03	NA	NA	-1.30	0.042673188	NA	NA
4090	TRIM7	K	-1.72	1.91E-02	NA	NA	-2.40	6.93E-04	NA	NA
4091	TRIM29	K	-1.72	1.32E-02	NA	NA	-1.54	0.031672794	NA	NA
4092	SEMA3C	K	-1.73	1.69E-29	NA	NA	-1.41	5.94E-20	NA	NA
4093	DOC2B	K	-1.74	2.81E-43	NA	NA	-1.01	3.47E-15	NA	NA
4094	FGL1	K	-1.76	3.72E-05	NA	NA	-1.51	5.54E-04	NA	NA
4095	COBL	K	-1.76	6.53E-03	NA	NA	-2.01	0.003011731	NA	NA
4096	AC010980.1	K	-1.78	1.71E-03	NA	NA	-2.43	1.50E-04	NA	NA
4097	GABRA5	K	-1.79	8.27E-03	NA	NA	-2.81	0.002052948	NA	NA
4098	CDHR1	K	-1.80	2.06E-04	NA	NA	-1.35	0.007427504	NA	NA
4099	OR7E14P	K	-1.82	2.48E-02	NA	NA	-2.37	0.003625992	NA	NA
4100	MTHFD2	K	-1.87	1.15E-152	NA	NA	-1.34	2.25E-79	NA	NA
4101	GPR37L1	K	-1.89	1.03E-03	NA	NA	-1.96	7.85E-04	NA	NA
4102	KCNMA1	K	-1.90	5.39E-11	NA	NA	-1.26	1.99E-05	NA	NA
4103	NPY	K	-1.90	5.75E-21	NA	NA	-1.56	1.09E-14	NA	NA
4104	LINC01833	K	-1.94	2.03E-48	NA	NA	-1.07	2.27E-16	NA	NA
4105	SLC7A5	K	-1.96	1.80E-36	NA	NA	-1.89	3.95E-34	NA	NA
4106	AC005865.2	K	-2.00	1.13E-02	NA	NA	-2.40	0.003783085	NA	NA
4107	AC016397.2	K	-2.02	3.41E-05	NA	NA	-2.45	1.90E-06	NA	NA
4108	TDRG1	K	-2.15	2.77E-03	NA	NA	-1.89	0.005418419	NA	NA
4109	Six3os1_4	K	-2.25	6.53E-04	NA	NA	-1.39	0.02725498	NA	NA
4110	WNT4	K	-2.25	3.93E-03	NA	NA	-3.69	5.14E-05	NA	NA
4111	ARHGAP9	K	-2.34	3.40E-05	NA	NA	-2.48	1.41E-05	NA	NA
4112	ISG20	K	-2.37	3.68E-03	NA	NA	-4.39	1.22E-08	NA	NA
4113	LPL	K	-2.47	2.49E-03	NA	NA	-3.09	1.93E-04	NA	NA
4114	LURAP1L-AS1	K	-2.58	2.14E-02	NA	NA	-2.82	0.018546555	NA	NA
4115	TMC5	K	-2.70	3.87E-03	NA	NA	-2.55	0.004367209	NA	NA
4116	UNC5D	K	-2.95	1.68E-06	NA	NA	-4.06	1.76E-06	NA	NA
4117	AC087457.1	K	-3.05	4.60E-03	NA	NA	-3.55	0.001655982	NA	NA
4118	LINC01441	K	-3.20	2.82E-05	NA	NA	-1.42	0.025156652	NA	NA
4119	AC134878.1	K	-3.30	1.35E-03	NA	NA	-2.93	0.005019975	NA	NA
4120	CBLN2	K	-3.32	2.68E-179	NA	NA	-1.87	1.28E-71	NA	NA
4121	AC022748.1	K	-3.51	2.97E-02	NA	NA	-3.57	0.024753286	NA	NA
4122	GPR12	K	-3.70	1.90E-05	NA	NA	-6.77	1.95E-07	NA	NA
4123	CHRNA4	K	-3.88	2.77E-02	NA	NA	-7.26	1.10E-04	NA	NA
4124	SH3TC2	K	-4.21	2.82E-04	NA	NA	-2.90	0.012837964	NA	NA
4125	IGSF21	K	-4.39	9.65E-05	NA	NA	-6.66	5.06E-07	NA	NA
4126	KLHDC7B	K	-4.91	2.70E-04	NA	NA	-5.25	1.10E-04	NA	NA
4127	AC122718.1	K	-5.58	1.57E-03	NA	NA	-3.52	0.048950484	NA	NA
4128	CARD11	K	-6.08	9.08E-06	NA	NA	-2.58	0.021629944	NA	NA
4129	LINC01586	K	-6.10	3.67E-03	NA	NA	-8.58	1.82E-04	NA	NA
4130	ARX	K	-7.31	6.15E-03	NA	NA	-7.13	0.006406952	NA	NA
4131	S1PR5	L	4.42	2.25E-04	NA	NA	5.66	7.27E-07	2.23	1.49E-08
4132	SPP1	L	4.02	5.65E-04	NA	NA	-5.41	2.31E-05	-7.98	1.20E-12
4133	LINC01115	L	3.96	4.28E-03	NA	NA	5.27	3.43E-05	2.07	6.20E-03
4134	GFRA1	L	3.84	5.06E-66	NA	NA	-1.20	3.14E-04	-4.34	5.00E-74
4135	ASIC2	L	3.75	5.79E-07	NA	NA	-4.42	0.001780099	-8.22	3.57E-11
4136	TNRC6C-AS1	L	3.65	2.37E-82	NA	NA	-1.28	3.40E-05	-4.70	8.19E-89
4137	SCGN	L	3.36	4.35E-96	NA	NA	-1.88	4.84E-12	-4.41	5.00E-117
4138	TCERG1L	L	3.21	1.86E-13	NA	NA	-4.20	4.04E-04	-8.00	1.35E-12
4139	PCDH7	L	3.20	2.13E-15	NA	NA	-2.91	5.43E-05	-7.34	8.24E-19
4140	KLF2	L	3.19	2.22E-02	NA	NA	6.23	1.95E-07	3.57	1.07E-09

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4141	TMEM132E	L	3.17	1.58E-05	NA	NA	-4.47	0.00129826	-7.69	8.40E-10
4142	TNXB	L	2.92	1.30E-02	NA	NA	5.10	1.77E-06	2.87	3.55E-04
4143	KIRREL1	L	2.75	4.87E-02	NA	NA	10.58	1.57E-23	7.68	1.56E-59
4144	FNDC5	L	2.64	9.33E-07	NA	NA	-2.41	1.68E-05	-4.45	2.12E-17
4145	SLIT2	L	2.52	2.60E-16	NA	NA	-1.39	3.64E-05	-3.43	1.39E-29
4146	AL160191.3	L	2.49	5.65E-15	NA	NA	-6.24	4.79E-08	-7.34	1.11E-11
4147	HPCA	L	2.47	7.84E-11	NA	NA	-3.82	1.58E-05	-5.13	2.94E-15
4148	SCG2	L	2.42	0	NA	NA	-1.35	2.03E-81	-3.27	0
4149	GLI1	L	2.41	7.22E-03	NA	NA	5.67	2.88E-15	3.75	1.66E-09
4150	CRB2	L	2.37	1.96E-11	NA	NA	-1.57	0.00553691	-3.93	6.57E-18
4151	ITGA5	L	2.31	1.74E-02	NA	NA	3.22	1.94E-04	1.67	3.40E-02
4152	AC020571.1	L	2.31	5.09E-06	NA	NA	-3.52	0.003804065	-3.61	3.09E-10
4153	EPHB3	L	2.27	3.53E-24	NA	NA	-1.61	1.77E-11	-3.10	1.43E-43
4154	TMC6	L	2.07	2.05E-31	NA	NA	-1.20	1.27E-09	-3.67	7.79E-85
4155	PTGER2	L	2.07	5.05E-166	NA	NA	4.36	0	2.81	0
4156	AC092368.3	L	1.96	7.27E-06	NA	NA	2.33	4.26E-08	1.29	6.64E-04
4157	FAM196B	L	1.95	4.35E-03	NA	NA	-5.28	4.53E-05	-4.60	5.60E-09
4158	FZD10-AS1	L	1.93	5.55E-03	NA	NA	-2.29	0.002905737	-4.47	8.86E-11
4159	NID2	L	1.91	6.78E-25	NA	NA	2.70	4.58E-50	1.51	1.79E-18
4160	COLQ	L	1.88	8.10E-07	NA	NA	-1.18	0.007490825	-3.13	1.57E-17
4161	PDE7B	L	1.81	2.07E-48	NA	NA	2.89	3.02E-128	1.80	5.29E-64
4162	MAGEB2	L	1.79	7.29E-04	NA	NA	-5.36	1.22E-05	-6.48	3.04E-08
4163	CTSS	L	1.79	9.84E-03	NA	NA	4.93	3.30E-18	2.91	1.79E-08
4164	INSC	L	1.76	2.13E-07	NA	NA	-1.04	0.029435296	-2.42	3.39E-12
4165	PAPPA2	L	1.74	2.78E-02	NA	NA	-7.36	1.27E-08	-7.32	9.78E-11
4166	MAOB	L	1.73	2.60E-17	NA	NA	-7.83	7.50E-13	-7.52	1.49E-15
4167	FLI1	L	1.67	1.65E-02	NA	NA	-2.44	0.0067099	-3.88	3.77E-08
4168	SIX5	L	1.58	1.16E-14	NA	NA	3.17	3.86E-62	1.71	8.44E-23
4169	GRIN2A	L	1.57	7.58E-05	NA	NA	-3.67	3.23E-05	-6.29	1.54E-08
4170	MAB21L2	L	1.56	1.15E-139	NA	NA	2.06	1.61E-245	1.25	9.62E-105
4171	TMEM204	L	1.56	1.08E-04	NA	NA	-2.19	8.50E-05	-3.82	4.93E-14
4172	AP004608.1	L	1.53	1.16E-02	NA	NA	-2.20	0.027078976	-5.18	1.24E-05
4173	GRIK4	L	1.53	3.04E-05	NA	NA	-1.44	0.002268854	-3.11	4.23E-14
4174	AC131097.3	L	1.51	8.12E-05	NA	NA	-1.03	0.034426975	-2.23	1.28E-09
4175	DCDC1	L	1.50	2.89E-02	NA	NA	2.42	1.92E-04	1.30	2.44E-02
4176	FAM111A	L	1.48	1.33E-02	NA	NA	2.30	2.51E-05	1.64	2.44E-03
4177	KCNIP1	L	1.48	3.25E-03	NA	NA	-2.09	0.004947043	-3.39	8.48E-09
4178	TCP11L2	L	1.47	1.29E-02	NA	NA	1.71	0.003113005	1.25	3.70E-02
4179	GPR19	L	1.47	4.96E-25	NA	NA	-1.61	3.32E-23	-2.29	9.42E-55
4180	TMED10P2	L	1.47	1.13E-03	NA	NA	-1.49	0.013770129	-1.80	5.85E-05
4181	BCAN	L	1.46	1.10E-22	NA	NA	-1.43	9.87E-15	-2.15	2.17E-43
4182	ZSCAN1	L	1.45	5.64E-05	NA	NA	-2.49	7.07E-06	-3.00	2.20E-13
4183	NAV1	L	1.45	2.63E-23	NA	NA	-2.23	1.34E-52	-2.69	4.04E-78
4184	RGPD2	L	1.44	2.00E-03	NA	NA	5.41	2.90E-42	4.27	3.43E-29
4185	CREB3L3	L	1.44	1.14E-03	NA	NA	-3.81	1.87E-04	-4.87	2.39E-09
4186	IL17RA	L	1.43	4.54E-03	NA	NA	-1.61	0.003978655	-2.82	5.52E-09
4187	IGDCC3	L	1.42	1.28E-14	NA	NA	-2.93	1.97E-53	-4.97	3.01E-145
4188	WHRN	L	1.41	4.98E-05	NA	NA	3.43	7.00E-29	2.42	1.57E-20
4189	SLC30A3	L	1.40	3.09E-15	NA	NA	-1.65	4.13E-13	-3.41	9.39E-66
4190	BOK	L	1.40	1.94E-02	NA	NA	-2.26	2.54E-04	-3.78	7.33E-11
4191	CLDN9	L	1.39	1.02E-02	NA	NA	-1.65	0.038455701	-2.67	1.12E-05
4192	AL160191.1	L	1.39	3.20E-03	NA	NA	-1.53	0.009340605	-3.05	2.28E-09
4193	C1QTNF1-AS1	L	1.39	3.25E-03	NA	NA	-1.78	0.006654608	-1.89	9.27E-05
4194	ACKR1	L	1.34	9.66E-04	NA	NA	-6.51	1.73E-08	-7.90	1.88E-12
4195	TNFRSF1A	L	1.34	5.34E-04	NA	NA	5.65	7.87E-81	4.65	1.96E-108
4196	CORO2B	L	1.32	9.80E-04	NA	NA	-3.27	7.25E-09	-4.10	3.05E-17
4197	LINC00928	L	1.31	6.77E-05	NA	NA	-2.72	5.76E-07	-4.13	3.42E-16
4198	MTMR11	L	1.31	5.24E-05	NA	NA	2.13	1.11E-12	1.14	1.00E-04
4199	AL139130.1	L	1.30	5.37E-03	NA	NA	-1.53	0.010066309	-2.56	1.73E-07
4200	TEC	L	1.30	3.53E-02	NA	NA	2.33	1.66E-05	1.87	4.71E-06

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			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4201	MIR4435-2HG	L	1.29	3.50E-03	NA	NA	3.21	4.91E-18	1.69	4.38E-07
4202	AC013391.3	L	1.28	3.50E-10	NA	NA	-4.26	8.38E-17	-4.60	1.39E-35
4203	DPP6	L	1.27	4.54E-90	NA	NA	-7.43	1.34E-199	-8.69	4.09E-253
4204	NR2F1	L	1.27	1.36E-14	NA	NA	2.51	1.92E-55	1.49	4.34E-20
4205	AL512380.1	L	1.24	2.88E-02	NA	NA	-1.56	0.021760862	-1.42	9.04E-03
4206	RAC2	L	1.23	3.97E-05	NA	NA	-4.16	5.31E-15	-7.48	8.05E-13
4207	WNT5A	L	1.21	1.71E-02	NA	NA	3.39	3.00E-15	1.81	7.44E-06
4208	AC015522.1	L	1.21	3.71E-03	NA	NA	-3.29	7.73E-07	-3.74	1.04E-12
4209	ALS2CL	L	1.19	2.83E-02	NA	NA	2.37	4.91E-07	1.29	4.14E-03
4210	HLA-E	L	1.18	4.00E-11	NA	NA	3.28	1.65E-94	2.47	2.28E-75
4211	SIAH3	L	1.17	2.07E-03	NA	NA	-2.12	8.46E-05	-3.08	3.04E-11
4212	PDK4	L	1.17	6.24E-03	NA	NA	2.70	1.68E-14	1.73	9.85E-08
4213	PRR5	L	1.16	3.59E-10	NA	NA	-1.61	1.87E-15	-2.32	3.91E-35
4214	WSCD2	L	1.15	3.04E-02	NA	NA	-6.15	5.69E-10	-6.52	2.47E-16
4215	SAMD9	L	1.12	2.70E-04	NA	NA	6.20	8.73E-152	5.41	6.66E-236
4216	WIPI1	L	1.11	7.04E-08	NA	NA	2.99	1.24E-57	1.72	3.50E-22
4217	AGBL1	L	1.11	3.76E-02	NA	NA	-2.19	0.00381182	-4.34	6.71E-07
4218	EVX2	L	1.10	8.25E-06	NA	NA	5.19	5.04E-148	3.50	1.09E-107
4219	HOXC8	L	1.09	1.99E-14	NA	NA	-1.78	4.36E-24	-2.61	4.68E-60
4220	HOTAIR	L	1.07	2.10E-05	NA	NA	-2.58	9.37E-25	-2.86	2.21E-32
4221	AC004233.3	L	1.07	1.42E-03	NA	NA	-2.00	9.37E-06	-2.02	5.41E-09
4222	HLA-C	L	1.04	2.72E-07	NA	NA	2.64	1.83E-48	2.57	4.48E-64
4223	DNM3	L	1.04	3.18E-06	NA	NA	-1.20	8.50E-07	-1.93	3.93E-18
4224	TSHZ3	L	1.03	5.18E-68	NA	NA	-1.69	3.03E-124	-1.89	7.81E-202
4225	SCUBE3	L	1.02	3.98E-08	NA	NA	-1.20	3.90E-07	-1.73	8.96E-19
4226	AC243562.2	L	1.02	1.65E-04	NA	NA	-1.50	2.04E-06	-2.03	1.37E-13
4227	ADGRG2	L	-1.02	5.53E-06	NA	NA	-2.02	2.83E-16	-1.71	3.40E-09
4228	GJD2	L	-1.02	2.05E-05	NA	NA	-6.49	4.98E-24	-4.65	1.17E-20
4229	LINC02268	L	-1.02	4.37E-05	NA	NA	1.19	2.61E-08	1.39	4.07E-09
4230	DUSP26	L	-1.04	1.88E-11	NA	NA	-1.50	1.44E-22	-1.26	1.50E-14
4231	DLG3	L	-1.04	1.90E-11	NA	NA	-2.60	8.70E-54	-2.55	4.09E-45
4232	AC027228.2	L	-1.08	8.41E-46	NA	NA	-2.06	1.06E-153	-1.91	4.54E-114
4233	HSPA12A	L	-1.10	3.39E-38	NA	NA	-2.59	3.52E-187	-1.56	5.68E-65
4234	CPLX2	L	-1.11	9.33E-18	NA	NA	-5.19	0	-5.05	7.44E-265
4235	AC108673.2	L	-1.11	1.89E-03	NA	NA	-2.24	7.20E-09	-1.15	1.06E-02
4236	SLC4A11	L	-1.11	2.68E-03	NA	NA	1.97	3.18E-10	2.51	1.34E-14
4237	PCSK6	L	-1.13	5.32E-09	NA	NA	-2.31	1.52E-34	-1.89	2.02E-22
4238	STAP2	L	-1.14	7.22E-05	NA	NA	1.35	1.04E-07	2.29	4.53E-20
4239	FABP5	L	-1.14	3.71E-26	NA	NA	1.29	2.68E-42	2.00	2.11E-84
4240	GALNT13	L	-1.16	4.75E-25	NA	NA	1.90	2.01E-70	2.21	4.87E-91
4241	IFFO2	L	-1.17	2.92E-05	NA	NA	-1.70	8.10E-10	-1.10	2.33E-04
4242	LINC02495	L	-1.20	1.27E-02	NA	NA	-1.81	7.23E-05	-1.57	7.08E-04
4243	KCNQ3	L	-1.21	5.41E-16	NA	NA	-7.70	2.81E-59	-5.70	8.56E-45
4244	CHRNA3	L	-1.21	4.18E-136	NA	NA	-2.19	0	-1.70	5.33E-257
4245	DAAM2	L	-1.21	2.30E-04	NA	NA	1.48	2.85E-06	2.51	1.35E-16
4246	TFPI2	L	-1.25	1.29E-17	NA	NA	1.18	1.49E-17	1.81	1.42E-37
4247	SLC39A8	L	-1.26	8.50E-14	NA	NA	1.06	1.34E-10	2.00	1.27E-34
4248	FABP5P7	L	-1.26	1.15E-41	NA	NA	1.26	2.92E-55	2.03	5.83E-114
4249	ASPDH	L	-1.31	1.34E-02	NA	NA	-2.31	1.21E-05	-1.56	1.03E-02
4250	P2RY1	L	-1.31	1.62E-08	NA	NA	2.12	1.35E-41	3.24	4.25E-59
4251	CBFA2T3	L	-1.33	8.16E-03	NA	NA	3.21	7.16E-21	3.82	2.76E-20
4252	LYPD1	L	-1.44	3.93E-02	NA	NA	-2.03	0.003372218	-2.14	3.11E-02
4253	C3orf52	L	-1.45	4.68E-05	NA	NA	1.65	2.36E-10	2.39	1.82E-13
4254	DGAT2	L	-1.46	7.60E-03	NA	NA	1.90	4.03E-06	3.01	1.20E-11
4255	GPC4	L	-1.46	2.65E-05	NA	NA	3.58	5.36E-72	4.43	3.56E-55
4256	ZNF385D	L	-1.46	1.19E-09	NA	NA	1.13	3.69E-07	2.04	1.06E-18
4257	KIT	L	-1.47	1.00E-13	NA	NA	1.13	3.58E-10	1.93	6.72E-24
4258	AC023024.2	L	-1.48	3.21E-10	NA	NA	-2.44	1.09E-21	-1.58	1.95E-07
4259	ADAMTS4	L	-1.49	1.58E-05	NA	NA	2.61	4.38E-16	3.40	2.52E-26
4260	SLC35D3	L	-1.54	2.20E-34	NA	NA	-1.08	3.55E-19	1.30	2.17E-24

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4261	AC010980.2	L	-1.54	2.24E-04	NA	NA	-3.79	1.02E-13	-2.83	2.64E-06
4262	GRIN1	L	-1.54	2.58E-02	NA	NA	-6.27	1.34E-23	-3.41	3.28E-07
4263	LMX1B	L	-1.58	1.70E-03	NA	NA	3.15	6.29E-29	5.34	6.95E-42
4264	NEUROG2	L	-1.60	9.20E-51	NA	NA	-3.26	2.09E-146	-1.13	3.15E-19
4265	FAM89A	L	-1.61	8.67E-16	NA	NA	-4.21	1.42E-47	-3.34	2.84E-22
4266	AC007128.1	L	-1.62	2.97E-04	NA	NA	-2.51	1.16E-07	-2.11	8.21E-04
4267	HSPD1P6	L	-1.69	4.54E-02	NA	NA	1.39	0.029125056	2.36	1.15E-03
4268	SLC16A6	L	-1.73	1.10E-07	NA	NA	1.55	3.28E-11	2.85	4.28E-23
4269	MLKL	L	-1.77	7.01E-03	NA	NA	1.94	6.62E-04	2.77	3.33E-06
4270	MEST	L	-1.80	2.22E-04	NA	NA	1.23	0.00850692	3.26	4.06E-14
4271	SMPDL3B	L	-1.80	1.58E-02	NA	NA	2.68	7.95E-12	3.97	9.34E-11
4272	ASNSP1	L	-1.85	4.21E-10	NA	NA	-5.54	3.36E-19	-3.56	7.57E-09
4273	GJA1	L	-1.86	2.32E-02	NA	NA	7.93	2.02E-124	8.81	5.58E-48
4274	SLC30A10	L	-1.86	6.54E-06	NA	NA	-1.05	0.010304257	1.66	7.18E-05
4275	DLK1	L	-1.90	1.67E-59	NA	NA	1.16	1.47E-22	2.73	4.13E-123
4276	RERG	L	-1.95	1.09E-68	NA	NA	2.01	4.01E-112	3.17	8.28E-192
4277	PRKAG2-AS1	L	-2.00	1.40E-10	NA	NA	1.51	7.81E-10	3.04	9.28E-26
4278	GRIK3	L	-2.00	7.90E-76	NA	NA	-12.3	1.33E-31	-10.4	1.62E-22
4279	APCDD1	L	-2.01	9.23E-07	NA	NA	-2.85	5.21E-11	-1.47	9.03E-03
4280	DIO3OS	L	-2.03	7.69E-03	NA	NA	-1.68	0.020613978	1.73	4.41E-02
4281	FLRT1	L	-2.16	1.44E-20	NA	NA	2.61	1.78E-48	3.81	2.03E-67
4282	GRM8	L	-2.21	4.44E-52	NA	NA	-3.25	3.84E-104	-1.30	1.89E-15
4283	RBFOX1	L	-2.44	1.14E-08	NA	NA	-5.97	6.26E-13	-3.21	1.30E-04
4284	KIAA1644	L	-2.53	5.54E-08	NA	NA	-4.48	7.00E-12	-4.00	1.21E-03
4285	MFNG	L	-2.54	1.69E-04	NA	NA	-5.91	6.96E-08	-4.26	1.44E-03
4286	SLC6A9	L	-2.76	6.08E-29	NA	NA	-1.19	1.24E-06	1.99	4.51E-15
4287	GRIP1	L	-2.80	1.39E-02	NA	NA	4.00	5.59E-10	6.94	3.36E-13
4288	PEX5L	L	-2.91	7.55E-14	NA	NA	-4.37	1.49E-28	-1.15	1.73E-02
4289	NTRK3	L	-3.00	2.06E-11	NA	NA	-7.62	2.31E-49	-5.11	3.15E-23
4290	DIO3	L	-3.16	8.43E-04	NA	NA	-3.91	2.77E-04	-3.25	4.55E-02
4291	PDE3A	L	-3.21	4.05E-105	NA	NA	3.81	0	6.18	0
4292	ADRA2A	L	-3.28	1.11E-40	NA	NA	-2.25	1.32E-26	1.35	1.67E-06
4293	PAX7	L	-3.33	5.84E-09	NA	NA	-7.66	3.14E-11	-4.38	7.61E-04
4294	TNNT2	L	-3.78	3.90E-12	NA	NA	-8.06	4.09E-25	-2.80	2.06E-04
4295	AL359075.2	L	-4.02	2.23E-02	NA	NA	2.66	0.041784594	4.58	6.61E-03
4296	CYP26B1	M	15.00	1.81E-126	11.82	3.44E-181	NA	NA	-2.37	1.32E-178
4297	CYP26A1	M	13.50	8.83E-113	13.89	1.63E-41	NA	NA	-1.32	3.01E-105
4298	LINC01587	M	8.65	4.37E-15	6.87	1.71E-09	NA	NA	-1.79	2.00E-08
4299	C11orf53	M	8.57	2.36E-15	10.51	9.17E-23	NA	NA	1.92	2.16E-30
4300	AC254633.1	M	7.80	1.32E-11	6.00	7.25E-07	NA	NA	-1.82	1.76E-04
4301	COL13A1	M	7.31	4.08E-08	2.96	2.13E-02	NA	NA	-2.64	2.32E-03
4302	LINC00486	M	7.11	2.85E-20	4.99	1.64E-05	NA	NA	-3.14	7.84E-35
4303	COLCA2	M	6.34	7.48E-09	8.60	2.59E-15	NA	NA	1.41	1.71E-12
4304	CCBE1	M	6.31	1.81E-303	4.78	3.70E-132	NA	NA	-1.97	1.98E-52
4305	AP005202.2	M	6.30	1.51E-07	3.55	9.57E-03	NA	NA	-2.06	5.75E-04
4306	ZP2	M	5.63	4.20E-08	2.39	2.92E-03	NA	NA	-1.78	2.09E-03
4307	AADACL4	M	5.54	4.59E-06	7.78	1.65E-11	NA	NA	2.21	3.30E-06
4308	DYSF	M	5.51	4.96E-23	2.72	7.73E-07	NA	NA	-2.34	5.41E-08
4309	CRYGC	M	5.47	5.71E-16	5.00	2.03E-09	NA	NA	-1.07	1.23E-03
4310	AC023794.2	M	5.39	9.07E-06	2.93	1.61E-02	NA	NA	-1.89	3.36E-03
4311	HIC1	M	5.31	7.27E-127	4.24	3.02E-77	NA	NA	-1.37	6.36E-14
4312	AL359313.1	M	4.93	1.24E-14	2.87	8.09E-04	NA	NA	-2.27	1.47E-07
4313	TGM2	M	4.91	1.40E-89	6.59	2.67E-165	NA	NA	1.54	4.37E-14
4314	AC025857.2	M	4.57	2.78E-10	3.97	9.72E-06	NA	NA	-1.19	8.70E-03
4315	COLCA1	M	4.51	3.43E-75	5.72	8.58E-127	NA	NA	1.37	1.41E-10
4316	FLJ12825	M	4.49	2.76E-120	2.24	1.01E-22	NA	NA	-2.75	1.11E-71
4317	BCO2	M	4.49	6.40E-39	1.63	7.95E-06	NA	NA	-2.44	6.99E-15
4318	AL590666.4	M	4.46	2.49E-20	2.04	1.26E-05	NA	NA	-1.77	2.69E-08
4319	LINC00482	M	4.44	4.83E-16	3.01	1.16E-04	NA	NA	-2.48	4.43E-07
4320	LINC01686	M	4.38	1.77E-47	1.05	3.66E-02	NA	NA	-3.95	2.46E-48

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			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4321	AC036214.1	M	4.18	6.20E-21	1.85	5.63E-04	NA	NA	-3.16	1.98E-14
4322	PLK2	M	4.15	9.28E-162	2.56	2.46E-58	NA	NA	-1.91	4.95E-37
4323	COL17A1	M	4.11	1.31E-03	6.89	9.66E-09	NA	NA	1.38	2.89E-02
4324	DLG2	M	3.99	1.84E-92	2.39	7.46E-31	NA	NA	-1.64	7.81E-18
4325	AC017048.3	M	3.98	3.85E-29	3.65	2.68E-17	NA	NA	-1.13	5.27E-05
4326	LINC02143	M	3.95	5.59E-03	5.51	2.16E-05	NA	NA	2.26	5.23E-03
4327	OTUD1	M	3.88	2.73E-85	2.74	7.95E-41	NA	NA	-1.19	6.14E-13
4328	PLAT	M	3.85	1.09E-68	1.72	1.19E-12	NA	NA	-2.99	4.78E-43
4329	COL23A1	M	3.82	9.22E-23	2.01	2.85E-06	NA	NA	-2.35	1.03E-09
4330	TFAMP1	M	3.60	4.27E-11	4.33	4.81E-04	NA	NA	-1.97	3.95E-06
4331	LAMA4	M	3.58	2.70E-48	1.06	2.00E-04	NA	NA	-3.21	7.87E-41
4332	PTGIR	M	3.53	4.40E-20	2.26	1.26E-06	NA	NA	-2.14	1.69E-11
4333	ANGPTL4	M	3.48	5.54E-20	4.87	5.72E-48	NA	NA	1.15	8.35E-07
4334	RGS16	M	3.46	0	1.74	1.16E-99	NA	NA	-1.66	2.13E-109
4335	AL355990.2	M	3.35	1.13E-09	1.76	3.52E-02	NA	NA	-2.41	3.34E-07
4336	SPINK5	M	3.25	4.21E-02	6.83	1.75E-07	NA	NA	4.22	1.53E-06
4337	PNMA8C	M	3.04	0	1.25	1.57E-50	NA	NA	-1.85	1.27E-159
4338	AC009336.2	M	3.04	1.55E-19	2.13	1.94E-09	NA	NA	-1.14	1.32E-03
4339	GDF10	M	2.83	2.28E-15	6.62	3.88E-53	NA	NA	2.90	9.18E-60
4340	RAB20	M	2.81	1.02E-30	3.53	2.91E-68	NA	NA	1.54	4.60E-20
4341	ASIC4	M	2.78	3.81E-40	2.01	3.23E-20	NA	NA	-1.59	6.63E-14
4342	AC131097.1	M	2.75	2.79E-09	1.18	4.28E-02	NA	NA	-1.51	1.35E-04
4343	CCNA1	M	2.54	7.57E-48	2.21	8.98E-26	NA	NA	-1.18	3.45E-14
4344	AC012531.1	M	2.54	3.41E-16	1.34	1.77E-04	NA	NA	-1.34	4.77E-06
4345	YPEL4	M	2.35	3.86E-22	1.58	4.41E-08	NA	NA	-1.37	4.93E-09
4346	RBP3	M	2.35	5.75E-05	6.10	1.51E-10	NA	NA	1.60	4.55E-05
4347	FOXCUT	M	2.35	5.99E-05	2.31	4.70E-03	NA	NA	-1.17	3.16E-02
4348	AC117489.1	M	2.24	5.93E-08	2.12	5.92E-05	NA	NA	-1.17	2.98E-03
4349	HOXC5	M	2.23	2.00E-28	1.80	1.37E-15	NA	NA	-1.14	6.40E-09
4350	LINC00581	M	2.18	2.06E-02	5.02	1.96E-10	NA	NA	2.80	1.22E-08
4351	LINC02381	M	1.99	1.02E-119	1.32	1.03E-47	NA	NA	-1.29	7.24E-53
4352	UPK1A	M	1.97	1.45E-02	3.29	1.96E-11	NA	NA	2.62	1.55E-09
4353	LUCAT1	M	1.92	5.33E-17	1.02	6.83E-04	NA	NA	-1.67	5.72E-14
4354	PPFIA4	M	1.89	3.24E-15	2.35	1.25E-23	NA	NA	1.08	9.13E-06
4355	SKIL	M	1.79	3.12E-65	1.11	1.13E-24	NA	NA	-1.21	1.45E-30
4356	GALNT6	M	1.70	3.19E-21	3.54	7.73E-92	NA	NA	1.62	1.30E-20
4357	RBPMS	M	1.69	1.30E-31	1.23	9.61E-16	NA	NA	-1.13	9.42E-15
4358	P4HA2	M	1.67	2.56E-59	2.27	5.28E-119	NA	NA	1.11	1.82E-30
4359	PFKFB3	M	1.64	7.89E-14	2.66	2.15E-35	NA	NA	1.05	3.41E-06
4360	AC004877.1	M	1.58	2.13E-11	1.08	3.41E-04	NA	NA	-1.48	2.46E-10
4361	LINC00622	M	1.58	9.47E-07	1.04	6.41E-03	NA	NA	-1.01	2.14E-03
4362	DRP2	M	1.48	4.21E-13	1.20	2.37E-06	NA	NA	-1.09	2.43E-08
4363	PFKFB4	M	1.39	1.29E-38	2.60	3.42E-146	NA	NA	1.18	3.89E-34
4364	GPR160	M	1.37	2.51E-02	1.33	2.08E-02	NA	NA	1.12	4.59E-02
4365	COL27A1	M	1.34	2.07E-03	2.31	1.40E-09	NA	NA	1.94	4.43E-07
4366	AL160254.1	M	1.33	1.14E-02	2.07	2.28E-06	NA	NA	1.19	4.74E-03
4367	RPL17P50	M	1.33	2.23E-02	1.31	4.22E-03	NA	NA	1.07	1.71E-02
4368	UPK1A-AS1	M	1.33	9.63E-03	2.75	4.37E-14	NA	NA	2.17	5.74E-11
4369	ANKRD24	M	1.32	2.95E-02	-2.11	9.07E-04	NA	NA	-2.27	8.96E-05
4370	PPP1R3C	M	1.27	1.13E-12	1.80	1.64E-34	NA	NA	1.22	6.00E-19
4371	PPP1R13L	M	1.26	2.07E-13	1.35	8.73E-18	NA	NA	1.01	7.74E-11
4372	AK4	M	1.08	5.53E-40	1.69	5.41E-105	NA	NA	1.55	2.05E-88
4373	PGM1	M	1.05	1.12E-83	1.39	2.39E-160	NA	NA	1.07	5.97E-97
4374	S1PR3	M	1.01	9.93E-23	3.00	8.02E-214	NA	NA	1.93	1.62E-102
4375	KIAA1614	M	-1.00	2.08E-02	-2.47	1.94E-10	NA	NA	-1.23	3.48E-03
4376	IGFBP5	M	-1.06	1.74E-65	-2.75	0	NA	NA	-1.07	2.35E-54
4377	PLXDC2	M	-1.08	6.82E-05	-1.38	7.87E-07	NA	NA	-1.04	3.82E-04
4378	TACC2	M	-1.09	7.34E-31	-1.14	1.58E-29	NA	NA	-1.04	8.05E-25
4379	FAM189A1	M	-1.11	4.41E-02	-1.40	2.29E-02	NA	NA	-1.29	3.76E-02
4380	SAMD11	M	-1.18	2.30E-05	-2.22	2.03E-16	NA	NA	-1.27	6.89E-06

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			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4381	SSTR3	M	-1.43	7.31E-04	-3.16	5.63E-07	NA	NA	-2.29	6.87E-04
4382	MFAP4	M	-1.54	1.16E-63	-1.09	2.77E-33	NA	NA	1.41	1.12E-53
4383	IL12A	M	-1.54	5.85E-06	1.10	1.69E-04	NA	NA	2.17	5.97E-12
4384	SH2D5	M	-1.63	2.39E-04	-2.54	7.10E-08	NA	NA	-1.26	2.41E-02
4385	CHRDL1	M	-1.87	4.45E-50	-1.05	8.95E-26	NA	NA	1.63	3.90E-38
4386	CHGA	M	-1.87	9.24E-187	-1.24	3.07E-83	NA	NA	1.08	2.56E-62
4387	AKAP2	M	-1.88	9.84E-33	-1.31	3.33E-19	NA	NA	1.30	7.57E-16
4388	AL645608.3	M	-2.10	7.94E-75	-1.69	9.31E-59	NA	NA	1.13	4.59E-21
4389	PIK3AP1	M	-2.17	3.90E-17	-2.71	1.14E-21	NA	NA	-1.36	1.66E-05
4390	FAM19A4	M	-2.35	6.67E-05	-1.76	1.89E-05	NA	NA	1.59	1.47E-02
4391	INSM2	M	-2.41	0	-1.49	1.05E-150	NA	NA	1.84	5.01E-204
4392	PLPP3	M	-2.51	3.27E-82	-1.47	1.90E-35	NA	NA	1.68	4.64E-36
4393	TRIM47	M	-2.67	1.00E-03	-1.35	3.97E-02	NA	NA	2.00	2.43E-02
4394	TESC	M	-2.68	1.28E-08	-1.86	9.55E-08	NA	NA	1.58	3.47E-03
4395	KCNH1	M	-2.69	2.35E-22	-1.70	3.78E-10	NA	NA	1.11	2.36E-04
4396	CTB-178M22.2	M	-2.72	1.01E-04	-1.24	1.82E-02	NA	NA	2.07	6.12E-03
4397	RELN	M	-2.83	9.21E-45	-1.31	4.12E-11	NA	NA	2.42	8.68E-33
4398	NELL1	M	-3.67	4.79E-98	-1.33	2.04E-15	NA	NA	2.15	7.65E-33
4399	ARHGAP36	M	-4.07	0	-2.59	4.43E-194	NA	NA	1.12	6.74E-30
4400	MYC	M	-4.24	0	-2.68	3.45E-225	NA	NA	2.09	1.30E-96
4401	HRK	M	-4.42	1.98E-29	-3.02	3.05E-21	NA	NA	1.59	8.63E-04
4402	CA9	N	NA	NA	5.66	5.32E-13	NA	NA	5.58	4.21E-13
4403	STC1	N	NA	NA	5.44	1.21E-19	NA	NA	3.80	8.99E-21
4404	CCDC149	N	NA	NA	4.42	3.54E-04	NA	NA	2.88	1.19E-02
4405	SLC16A12	N	NA	NA	4.28	9.99E-06	NA	NA	2.78	1.37E-03
4406	ABCG2	N	NA	NA	4.12	3.02E-05	NA	NA	2.79	1.42E-03
4407	HNF1A	N	NA	NA	4.06	4.33E-04	NA	NA	2.26	4.47E-02
4408	TRPM3	N	NA	NA	3.67	1.21E-05	NA	NA	2.48	7.21E-04
4409	SCARA5	N	NA	NA	3.48	6.12E-03	NA	NA	6.23	1.47E-05
4410	SLC7A3	N	NA	NA	3.35	1.34E-02	NA	NA	2.77	2.59E-02
4411	AC134312.1	N	NA	NA	3.34	4.85E-15	NA	NA	3.97	1.14E-19
4412	CNGA1	N	NA	NA	3.24	1.60E-04	NA	NA	2.82	6.58E-04
4413	WNT7B	N	NA	NA	3.11	4.99E-03	NA	NA	4.35	4.62E-04
4414	AC073264.4	N	NA	NA	3.11	5.37E-03	NA	NA	2.58	1.88E-02
4415	COLEC12	N	NA	NA	3.06	5.67E-18	NA	NA	2.94	4.42E-16
4416	KLF5	N	NA	NA	2.64	1.19E-05	NA	NA	1.38	3.45E-02
4417	KLHL41	N	NA	NA	2.63	7.13E-04	NA	NA	3.18	2.46E-05
4418	USH2A	N	NA	NA	2.62	6.60E-04	NA	NA	2.01	1.50E-02
4419	RBM24	N	NA	NA	2.53	5.65E-04	NA	NA	1.61	1.07E-02
4420	OAS1	N	NA	NA	2.51	6.00E-10	NA	NA	1.02	2.16E-02
4421	STEAP1B	N	NA	NA	2.33	1.03E-02	NA	NA	2.36	7.53E-03
4422	OLR1	N	NA	NA	2.29	2.35E-02	NA	NA	1.98	4.89E-02
4423	NDUFA4L2	N	NA	NA	2.24	7.29E-05	NA	NA	1.36	1.97E-02
4424	AC002101.1	N	NA	NA	2.20	3.34E-02	NA	NA	2.84	9.18E-03
4425	MGARP	N	NA	NA	2.08	1.12E-05	NA	NA	1.60	3.91E-04
4426	APCDD1L	N	NA	NA	2.08	3.22E-02	NA	NA	3.01	1.39E-03
4427	PPP1R3G	N	NA	NA	2.02	2.33E-04	NA	NA	1.87	4.62E-04
4428	CAVIN1	N	NA	NA	2.01	3.07E-05	NA	NA	3.50	2.86E-11
4429	NXPH4	N	NA	NA	1.95	1.73E-09	NA	NA	2.52	1.30E-14
4430	HVCN1	N	NA	NA	1.93	3.74E-07	NA	NA	1.25	7.77E-04
4431	APOB	N	NA	NA	1.92	1.94E-02	NA	NA	3.01	3.43E-04
4432	NIPAL1	N	NA	NA	1.90	5.86E-05	NA	NA	1.43	2.66E-03
4433	TMEM45A	N	NA	NA	1.88	6.54E-69	NA	NA	1.21	3.44E-29
4434	MAFF	N	NA	NA	1.87	4.15E-43	NA	NA	1.03	2.61E-16
4435	SEMA5A	N	NA	NA	1.85	3.20E-94	NA	NA	2.54	1.36E-158
4436	VSIG10L2	N	NA	NA	1.85	3.47E-03	NA	NA	2.47	4.13E-05
4437	CORIN	N	NA	NA	1.80	7.45E-04	NA	NA	1.77	9.69E-04
4438	AL357033.1	N	NA	NA	1.78	1.26E-02	NA	NA	1.57	2.19E-02
4439	AMOTL2	N	NA	NA	1.78	9.09E-75	NA	NA	1.09	1.14E-30
4440	FAM162A	N	NA	NA	1.73	1.21E-108	NA	NA	1.50	1.03E-82

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4441	LINC01632	N	NA	NA	1.71	1.38E-04	NA	NA	1.66	1.43E-04
4442	RNASE4	N	NA	NA	1.65	1.75E-02	NA	NA	1.45	3.80E-02
4443	SNAI2	N	NA	NA	1.63	4.87E-54	NA	NA	1.35	7.00E-40
4444	LRRC2	N	NA	NA	1.58	1.29E-05	NA	NA	1.58	1.97E-05
4445	CACNA2D2	N	NA	NA	1.57	1.31E-82	NA	NA	2.41	2.80E-193
4446	AL392083.1	N	NA	NA	1.57	6.88E-128	NA	NA	1.16	7.71E-76
4447	FAM163B	N	NA	NA	1.56	1.91E-22	NA	NA	1.07	3.26E-11
4448	AC004069.1	N	NA	NA	1.56	2.13E-03	NA	NA	1.70	5.66E-04
4449	PGK1	N	NA	NA	1.56	3.13E-170	NA	NA	1.15	8.03E-94
4450	RFLNB	N	NA	NA	1.55	2.44E-228	NA	NA	1.26	3.87E-155
4451	SLC2A1	N	NA	NA	1.48	1.22E-82	NA	NA	1.39	2.23E-73
4452	POLN	N	NA	NA	1.47	6.40E-03	NA	NA	1.10	4.45E-02
4453	SULT1C4	N	NA	NA	1.41	1.06E-44	NA	NA	1.11	1.21E-28
4454	COL6A3	N	NA	NA	1.40	1.99E-02	NA	NA	1.34	2.35E-02
4455	CSPG5	N	NA	NA	1.38	9.79E-18	NA	NA	1.37	8.15E-18
4456	FAM13A	N	NA	NA	1.23	2.16E-02	NA	NA	1.43	4.70E-03
4457	STBD1	N	NA	NA	1.21	7.36E-03	NA	NA	1.10	1.29E-02
4458	FILIP1L	N	NA	NA	1.19	6.32E-05	NA	NA	1.16	7.80E-05
4459	KCNK1	N	NA	NA	1.16	7.83E-28	NA	NA	1.67	4.69E-54
4460	GPRC5B	N	NA	NA	1.16	4.80E-11	NA	NA	1.98	4.71E-30
4461	MYO10	N	NA	NA	1.15	6.19E-08	NA	NA	1.01	2.13E-06
4462	LUM	N	NA	NA	1.15	2.08E-34	NA	NA	1.52	7.04E-59
4463	ZNF488	N	NA	NA	1.12	5.58E-18	NA	NA	1.11	4.45E-18
4464	PAM	N	NA	NA	1.11	6.55E-27	NA	NA	1.06	4.84E-25
4465	ELOVL7	N	NA	NA	1.11	1.73E-05	NA	NA	1.43	7.51E-09
4466	NRN1	N	NA	NA	1.07	1.47E-27	NA	NA	1.16	5.30E-32
4467	ATOH8	N	NA	NA	1.06	5.58E-06	NA	NA	1.40	6.91E-10
4468	PXDNL	N	NA	NA	1.06	6.91E-08	NA	NA	1.85	4.73E-19
4469	CDKN1B	N	NA	NA	1.03	8.24E-54	NA	NA	1.00	1.04E-51
4470	ZNF835	N	NA	NA	-1.01	5.23E-04	NA	NA	-1.19	1.90E-05
4471	AC104521.1	N	NA	NA	-1.02	4.17E-02	NA	NA	-1.02	3.64E-02
4472	RTBDN	N	NA	NA	-1.03	5.64E-07	NA	NA	-1.43	1.50E-14
4473	DUSP9	N	NA	NA	-1.03	4.55E-02	NA	NA	-1.83	1.60E-05
4474	CARMIL3	N	NA	NA	-1.04	1.67E-06	NA	NA	-1.85	4.52E-20
4475	AC023908.3	N	NA	NA	-1.04	4.75E-02	NA	NA	-1.09	2.82E-02
4476	GNAO1	N	NA	NA	-1.04	3.27E-24	NA	NA	-1.63	1.56E-59
4477	CNTN4	N	NA	NA	-1.07	3.31E-09	NA	NA	-1.01	1.77E-08
4478	SCN8A	N	NA	NA	-1.08	4.89E-02	NA	NA	-1.80	1.61E-04
4479	SPTBN4	N	NA	NA	-1.10	9.36E-08	NA	NA	-1.65	1.37E-17
4480	AC104117.3	N	NA	NA	-1.10	3.13E-02	NA	NA	-1.22	1.07E-02
4481	RUNDC3A	N	NA	NA	-1.13	2.06E-08	NA	NA	-1.82	1.93E-21
4482	CNTNAP2	N	NA	NA	-1.13	3.46E-04	NA	NA	-1.58	9.27E-08
4483	FAM110D	N	NA	NA	-1.14	2.08E-02	NA	NA	-1.96	1.58E-06
4484	HSD11B2	N	NA	NA	-1.17	2.03E-02	NA	NA	-1.27	6.26E-03
4485	MCF2L	N	NA	NA	-1.17	1.03E-22	NA	NA	-1.12	4.99E-21
4486	MMP15	N	NA	NA	-1.18	6.67E-06	NA	NA	-1.61	6.06E-11
4487	AC245060.5	N	NA	NA	-1.19	5.80E-08	NA	NA	-1.64	6.17E-15
4488	AC009102.2	N	NA	NA	-1.20	1.02E-04	NA	NA	-1.96	1.54E-12
4489	RASGRP2	N	NA	NA	-1.20	4.30E-04	NA	NA	-1.01	3.11E-03
4490	ACTA2	N	NA	NA	-1.20	2.11E-08	NA	NA	-1.23	4.93E-09
4491	AC021188.1	N	NA	NA	-1.20	1.94E-02	NA	NA	-1.22	1.38E-02
4492	FGFBP3	N	NA	NA	-1.27	6.31E-10	NA	NA	-1.11	7.70E-08
4493	CKMT1A	N	NA	NA	-1.27	1.57E-15	NA	NA	-1.49	1.85E-21
4494	RASGEF1C	N	NA	NA	-1.30	4.86E-02	NA	NA	-1.62	6.31E-03
4495	SLC5A4-AS1	N	NA	NA	-1.32	1.01E-02	NA	NA	-1.36	5.62E-03
4496	AP003108.3	N	NA	NA	-1.36	4.92E-02	NA	NA	-1.33	4.62E-02
4497	PRPH	N	NA	NA	-1.36	1.88E-15	NA	NA	-1.93	1.86E-31
4498	HIST2H3C	N	NA	NA	-1.37	6.53E-07	NA	NA	-1.56	3.80E-09
4499	CKMT1B	N	NA	NA	-1.40	3.00E-12	NA	NA	-1.72	1.56E-18
4500	TMIGD2	N	NA	NA	-1.41	1.74E-03	NA	NA	-1.91	3.75E-06

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			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4501	HIST1H2BE	N	NA	NA	-1.42	5.30E-07	NA	NA	-2.05	7.81E-15
4502	HMGCLL1	N	NA	NA	-1.49	7.30E-04	NA	NA	-1.42	9.31E-04
4503	ABCC8	N	NA	NA	-1.50	2.95E-02	NA	NA	-2.39	8.42E-05
4504	CEMIP	N	NA	NA	-1.60	2.08E-03	NA	NA	-3.20	1.04E-13
4505	AL008723.1	N	NA	NA	-1.62	1.10E-02	NA	NA	-1.35	3.94E-02
4506	HTRA1	N	NA	NA	-1.63	2.66E-03	NA	NA	-2.00	5.79E-05
4507	C14orf180	N	NA	NA	-1.68	3.09E-02	NA	NA	-2.77	1.78E-05
4508	LRFN4	N	NA	NA	-1.69	2.51E-09	NA	NA	-1.58	1.91E-08
4509	GGTA1P	N	NA	NA	-1.69	4.52E-04	NA	NA	-1.12	3.54E-02
4510	PAX5	N	NA	NA	-1.70	1.66E-02	NA	NA	-1.92	4.07E-03
4511	OPRM1	N	NA	NA	-1.76	9.91E-30	NA	NA	-1.26	4.27E-15
4512	PLCB2	N	NA	NA	-1.88	1.04E-06	NA	NA	-1.99	1.11E-07
4513	LINC01771	N	NA	NA	-2.31	2.16E-02	NA	NA	-2.89	1.44E-03
4514	SRPK3	N	NA	NA	-2.34	1.71E-04	NA	NA	-2.29	1.47E-04
4515	PAK6	N	NA	NA	-2.36	1.74E-06	NA	NA	-1.30	1.94E-02
4516	MEGF11	N	NA	NA	-2.37	8.89E-03	NA	NA	-3.00	3.14E-04
4517	C16orf92	N	NA	NA	-2.62	1.22E-04	NA	NA	-3.07	1.51E-06
4518	B3GALT2	N	NA	NA	-2.72	1.93E-37	NA	NA	-1.34	1.30E-08
4519	FBXL22	N	NA	NA	-2.86	2.85E-02	NA	NA	-2.66	3.99E-02
4520	RUNX1	N	NA	NA	-2.93	1.12E-02	NA	NA	-2.64	1.85E-02
4521	KRT18	N	NA	NA	-3.07	3.47E-02	NA	NA	-3.57	5.71E-03
4522	AL160270.2	N	NA	NA	-3.22	3.54E-02	NA	NA	-4.31	1.21E-03
4523	TMEM173	N	NA	NA	-3.56	2.38E-03	NA	NA	-3.18	5.02E-03
4524	AL139275.1	N	NA	NA	-3.82	1.61E-02	NA	NA	-4.02	7.60E-03
4525	AC078795.3	N	NA	NA	-3.98	5.00E-02	NA	NA	-4.23	2.80E-02
4526	SDK2	N	NA	NA	-6.42	2.13E-05	NA	NA	-7.29	1.02E-06
4527	DNAAF4-CCPG1	N	NA	NA	-8.69	8.93E-05	NA	NA	-8.70	6.45E-05
4528	MAGEB3	O	9.88	3.66E-19	NA	NA	NA	NA	-8.51	3.54E-16
4529	AP003062.3	O	7.86	1.14E-42	NA	NA	NA	NA	-6.74	9.69E-67
4530	CALCA	O	7.38	1.31E-25	NA	NA	NA	NA	-7.74	5.46E-23
4531	GJA5	O	7.02	3.15E-08	NA	NA	NA	NA	-7.09	1.98E-08
4532	AP005121.1	O	6.97	4.75E-09	NA	NA	NA	NA	-7.04	2.88E-09
4533	AC005786.3	O	6.84	1.72E-05	NA	NA	NA	NA	-6.91	1.28E-05
4534	C4orf22	O	6.60	7.66E-06	NA	NA	NA	NA	-5.15	7.62E-04
4535	AC137936.2	O	6.15	7.11E-06	NA	NA	NA	NA	-3.70	3.38E-04
4536	AJAP1	O	6.11	3.87E-35	NA	NA	NA	NA	-9.91	4.31E-20
4537	RFPL1	O	5.57	1.56E-04	NA	NA	NA	NA	-4.92	1.03E-03
4538	LINC01605	O	5.55	3.13E-03	NA	NA	NA	NA	-6.85	1.86E-04
4539	AC010547.1	O	5.51	2.50E-03	NA	NA	NA	NA	-3.97	3.37E-02
4540	GABBR2	O	5.37	1.68E-09	NA	NA	NA	NA	-4.30	1.05E-06
4541	ENPEP	O	5.34	3.36E-04	NA	NA	NA	NA	-6.18	4.73E-05
4542	GABRA4	O	4.76	2.30E-02	NA	NA	NA	NA	-4.08	4.34E-02
4543	LINC01819	O	4.63	4.04E-05	NA	NA	NA	NA	-3.17	4.74E-04
4544	CSNK1A1P1	O	4.47	1.02E-02	NA	NA	NA	NA	-3.52	2.33E-02
4545	NR5A2	O	4.44	5.43E-09	NA	NA	NA	NA	-2.73	3.56E-05
4546	AP000997.2	O	4.35	2.95E-10	NA	NA	NA	NA	-3.64	2.93E-10
4547	LINC00173	O	4.25	7.22E-06	NA	NA	NA	NA	-3.51	5.93E-05
4548	BATF2	O	4.19	3.22E-03	NA	NA	NA	NA	-4.05	1.89E-03
4549	PIK3CG	O	4.09	8.64E-03	NA	NA	NA	NA	-2.90	2.79E-02
4550	CYP4F62P	O	4.09	1.12E-03	NA	NA	NA	NA	-3.14	8.07E-03
4551	FAR2P1	O	4.06	8.63E-07	NA	NA	NA	NA	-4.49	5.00E-08
4552	AC124067.2	O	3.98	2.80E-05	NA	NA	NA	NA	-6.09	1.58E-06
4553	COL22A1	O	3.93	2.04E-06	NA	NA	NA	NA	-6.14	8.38E-08
4554	MCHR1	O	3.90	2.86E-03	NA	NA	NA	NA	-4.87	3.84E-04
4555	GLYCTK-AS1	O	3.60	2.79E-08	NA	NA	NA	NA	-1.74	4.73E-03
4556	AP003174.1	O	3.56	2.22E-09	NA	NA	NA	NA	-2.63	2.81E-07
4557	SYNDIG1L	O	3.44	8.98E-03	NA	NA	NA	NA	-2.75	2.07E-02
4558	ELF5	O	3.35	6.72E-05	NA	NA	NA	NA	-2.12	3.44E-03
4559	AC022784.2	O	3.23	9.70E-03	NA	NA	NA	NA	-4.29	3.25E-03
4560	AL451069.1	O	3.14	2.90E-05	NA	NA	NA	NA	-3.62	4.87E-06

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4561	AP001025.3	O	3.03	1.78E-04	NA	NA	NA	NA	-2.29	2.13E-03
4562	UGT3A2	O	3.02	1.91E-05	NA	NA	NA	NA	-2.38	5.10E-04
4563	WSPAR	O	3.01	5.40E-03	NA	NA	NA	NA	-3.06	4.43E-03
4564	AL392046.2	O	2.99	3.56E-06	NA	NA	NA	NA	-2.01	4.59E-04
4565	AC108866.1	O	2.98	2.35E-05	NA	NA	NA	NA	-1.94	8.27E-03
4566	HEY1	O	2.93	0	NA	NA	NA	NA	-2.81	0
4567	LINC01837	O	2.86	1.46E-02	NA	NA	NA	NA	-3.27	5.63E-03
4568	AC107222.1	O	2.86	5.30E-05	NA	NA	NA	NA	-2.17	7.29E-04
4569	FLNB-AS1	O	2.84	1.49E-08	NA	NA	NA	NA	-2.07	3.12E-05
4570	AC140481.3	O	2.81	6.14E-04	NA	NA	NA	NA	-2.19	3.66E-03
4571	AC008667.1	O	2.78	2.19E-02	NA	NA	NA	NA	-4.10	5.08E-03
4572	TMC8	O	2.76	2.78E-02	NA	NA	NA	NA	-4.18	5.31E-04
4573	AL365356.5	O	2.75	7.65E-03	NA	NA	NA	NA	-2.83	5.88E-03
4574	AC078880.3	O	2.70	1.77E-02	NA	NA	NA	NA	-2.58	2.13E-02
4575	C1QTNF4	O	2.70	1.31E-03	NA	NA	NA	NA	-2.36	4.81E-03
4576	AC005162.3	O	2.65	2.41E-04	NA	NA	NA	NA	-3.52	8.20E-06
4577	GAL3ST2	O	2.60	1.01E-04	NA	NA	NA	NA	-1.78	4.06E-03
4578	HOXC-AS3	O	2.55	1.25E-03	NA	NA	NA	NA	-1.72	3.57E-02
4579	DLGAP1-AS1	O	2.50	2.54E-04	NA	NA	NA	NA	-1.49	4.75E-02
4580	NTS	O	2.50	7.51E-06	NA	NA	NA	NA	-2.29	1.29E-05
4581	MPP4	O	2.50	4.31E-03	NA	NA	NA	NA	-2.90	1.26E-03
4582	SMUG1-AS1	O	2.43	1.22E-08	NA	NA	NA	NA	-1.62	3.95E-05
4583	COL11A2	O	2.42	3.80E-15	NA	NA	NA	NA	-1.14	1.87E-04
4584	ZNF662	O	2.39	5.40E-03	NA	NA	NA	NA	-2.95	4.95E-04
4585	MMP11	O	2.36	3.84E-35	NA	NA	NA	NA	-1.36	1.71E-12
4586	PLPP4	O	2.34	1.52E-03	NA	NA	NA	NA	-4.98	5.60E-05
4587	AC012531.2	O	2.30	1.91E-43	NA	NA	NA	NA	-1.31	2.30E-17
4588	PKD1L1	O	2.29	1.58E-04	NA	NA	NA	NA	-2.30	1.45E-04
4589	DLGAP1-AS2	O	2.25	2.02E-27	NA	NA	NA	NA	-1.93	4.51E-22
4590	GDF15	O	2.22	4.51E-08	NA	NA	NA	NA	-2.10	2.23E-07
4591	KIAA1683	O	2.21	1.03E-08	NA	NA	NA	NA	-1.09	7.95E-03
4592	ACOXL-AS1	O	2.19	1.11E-02	NA	NA	NA	NA	-1.79	3.88E-02
4593	IFIT2	O	2.19	2.23E-05	NA	NA	NA	NA	-2.50	1.14E-06
4594	AQP1	O	2.17	2.54E-47	NA	NA	NA	NA	-3.58	2.82E-109
4595	LINC00163	O	2.15	1.86E-02	NA	NA	NA	NA	-1.91	3.23E-02
4596	NEU4	O	2.11	1.51E-03	NA	NA	NA	NA	-1.86	5.79E-03
4597	CEACAM1	O	2.11	1.76E-02	NA	NA	NA	NA	-1.89	4.13E-02
4598	PGPEP1	O	2.09	5.89E-31	NA	NA	NA	NA	-1.41	1.15E-14
4599	ASIP	O	2.08	4.03E-02	NA	NA	NA	NA	-3.02	5.45E-03
4600	VTN	O	2.05	1.39E-03	NA	NA	NA	NA	-1.73	7.81E-03
4601	FLNB	O	2.02	1.87E-111	NA	NA	NA	NA	-2.04	1.57E-113
4602	GRM3	O	2.01	1.28E-08	NA	NA	NA	NA	-2.86	2.22E-13
4603	CD160	O	2.00	1.11E-02	NA	NA	NA	NA	-1.79	2.71E-02
4604	RTP5	O	2.00	3.37E-02	NA	NA	NA	NA	-3.79	1.50E-03
4605	AL365181.2	O	2.00	8.29E-06	NA	NA	NA	NA	-2.40	2.18E-07
4606	AC046134.1	O	1.98	4.14E-04	NA	NA	NA	NA	-1.69	1.98E-03
4607	ABCG4	O	1.96	1.92E-06	NA	NA	NA	NA	-1.36	1.33E-03
4608	RN7SL622P	O	1.96	2.76E-02	NA	NA	NA	NA	-2.40	8.91E-03
4609	SKOR1	O	1.94	1.04E-19	NA	NA	NA	NA	-2.14	2.42E-23
4610	43712	O	1.93	7.54E-05	NA	NA	NA	NA	-1.18	2.67E-02
4611	AC103974.1	O	1.92	5.78E-04	NA	NA	NA	NA	-1.62	3.12E-03
4612	LRRC32	O	1.90	3.88E-06	NA	NA	NA	NA	-2.77	3.08E-11
4613	DNM3OS	O	1.90	6.45E-23	NA	NA	NA	NA	-1.25	2.03E-10
4614	LGI1	O	1.90	3.48E-02	NA	NA	NA	NA	-3.69	8.80E-06
4615	KRT8P30	O	1.90	9.54E-05	NA	NA	NA	NA	-1.88	9.26E-05
4616	SVEP1	O	1.89	7.45E-03	NA	NA	NA	NA	-2.55	2.83E-04
4617	CHRM4	O	1.89	1.05E-06	NA	NA	NA	NA	-3.17	1.23E-11
4618	INSRR	O	1.87	1.94E-22	NA	NA	NA	NA	-1.37	1.27E-12
4619	KIF25-AS1	O	1.86	7.35E-12	NA	NA	NA	NA	-1.20	4.10E-06
4620	NRXN1	O	1.86	5.97E-14	NA	NA	NA	NA	-2.94	1.60E-33

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4621	TP73-AS1	O	1.83	3.83E-133	NA	NA	NA	NA	-1.09	4.01E-49
4622	AC023794.5	O	1.82	2.39E-02	NA	NA	NA	NA	-1.59	4.77E-02
4623	TNFSF13B	O	1.81	1.20E-02	NA	NA	NA	NA	-1.72	1.19E-02
4624	TRIL	O	1.79	2.36E-106	NA	NA	NA	NA	-1.45	2.11E-73
4625	IRX3	O	1.78	8.63E-26	NA	NA	NA	NA	-1.77	1.97E-26
4626	LINC01351	O	1.77	8.95E-03	NA	NA	NA	NA	-1.82	7.67E-03
4627	AL592114.3	O	1.76	2.04E-02	NA	NA	NA	NA	-1.71	2.33E-02
4628	FILIP1	O	1.75	2.74E-03	NA	NA	NA	NA	-1.43	1.52E-02
4629	PLEKHA6	O	1.75	5.88E-41	NA	NA	NA	NA	-1.87	5.94E-47
4630	SNAI1	O	1.73	2.54E-19	NA	NA	NA	NA	-1.24	2.39E-10
4631	AC008676.3	O	1.73	4.90E-03	NA	NA	NA	NA	-1.32	3.18E-02
4632	AL353751.1	O	1.73	1.26E-03	NA	NA	NA	NA	-2.12	8.43E-05
4633	APOE	O	1.73	3.94E-04	NA	NA	NA	NA	-1.00	4.89E-02
4634	AL935212.1	O	1.71	3.31E-02	NA	NA	NA	NA	-1.55	4.90E-02
4635	ITGA1	O	1.70	1.61E-80	NA	NA	NA	NA	-1.37	5.15E-53
4636	TPPP3	O	1.69	3.21E-25	NA	NA	NA	NA	-1.22	4.56E-14
4637	TRAF3IP2	O	1.69	2.28E-11	NA	NA	NA	NA	-2.00	9.92E-16
4638	KIAA0319	O	1.64	2.13E-15	NA	NA	NA	NA	-1.82	3.86E-18
4639	AC025809.2	O	1.64	8.56E-03	NA	NA	NA	NA	-2.21	4.98E-04
4640	NTRK1	O	1.64	1.69E-21	NA	NA	NA	NA	-1.18	9.09E-12
4641	CYBA	O	1.64	2.60E-13	NA	NA	NA	NA	-1.30	8.86E-09
4642	LRRC24	O	1.63	4.19E-02	NA	NA	NA	NA	-2.49	1.63E-03
4643	TFEB	O	1.62	6.56E-03	NA	NA	NA	NA	-2.46	1.04E-05
4644	HOXC6	O	1.62	2.84E-31	NA	NA	NA	NA	-1.26	2.90E-20
4645	CRISPLD2	O	1.62	2.57E-15	NA	NA	NA	NA	-2.19	5.28E-25
4646	TRPM5	O	1.61	2.09E-02	NA	NA	NA	NA	-2.12	2.32E-03
4647	SYT5	O	1.61	1.12E-19	NA	NA	NA	NA	-1.68	7.04E-22
4648	AZU1	O	1.59	1.14E-02	NA	NA	NA	NA	-1.60	1.24E-02
4649	GOLGA6L10	O	1.58	1.58E-02	NA	NA	NA	NA	-1.53	2.45E-02
4650	GAS2L2	O	1.58	4.79E-03	NA	NA	NA	NA	-1.71	1.93E-03
4651	AC012313.3	O	1.57	3.04E-03	NA	NA	NA	NA	-1.28	1.97E-02
4652	AC022613.2	O	1.54	7.68E-04	NA	NA	NA	NA	-1.59	4.83E-04
4653	AL160006.1	O	1.54	1.36E-167	NA	NA	NA	NA	-1.73	1.40E-207
4654	KITLG	O	1.53	1.18E-47	NA	NA	NA	NA	-1.90	1.13E-72
4655	FAM198B	O	1.53	3.87E-03	NA	NA	NA	NA	-1.70	1.90E-03
4656	METRN	O	1.53	6.47E-05	NA	NA	NA	NA	-1.26	1.36E-03
4657	HDAC10	O	1.53	4.67E-07	NA	NA	NA	NA	-1.32	1.61E-05
4658	AC010327.4	O	1.50	1.14E-03	NA	NA	NA	NA	-2.47	9.79E-07
4659	RAPGEF4	O	1.49	2.39E-29	NA	NA	NA	NA	-3.16	9.61E-110
4660	P2RX6	O	1.48	4.12E-03	NA	NA	NA	NA	-1.53	2.86E-03
4661	MAP1A	O	1.47	5.48E-130	NA	NA	NA	NA	-1.64	2.38E-162
4662	REM2	O	1.46	2.01E-07	NA	NA	NA	NA	-1.22	9.73E-06
4663	AC008764.9	O	1.45	2.93E-02	NA	NA	NA	NA	-2.26	8.47E-04
4664	CCNDBP1	O	1.44	2.61E-88	NA	NA	NA	NA	-1.42	1.55E-86
4665	TAS1R3	O	1.44	3.87E-03	NA	NA	NA	NA	-1.04	4.07E-02
4666	TMEM91	O	1.41	1.15E-04	NA	NA	NA	NA	-1.20	1.16E-03
4667	C15orf41	O	1.40	8.48E-10	NA	NA	NA	NA	-1.15	6.59E-07
4668	CADM1	O	1.39	8.16E-54	NA	NA	NA	NA	-1.56	9.47E-68
4669	FAR2P2	O	1.37	2.16E-02	NA	NA	NA	NA	-1.89	5.09E-04
4670	TMC3	O	1.37	1.13E-03	NA	NA	NA	NA	-2.49	3.87E-08
4671	AL592424.1	O	1.35	5.43E-05	NA	NA	NA	NA	-1.03	1.60E-03
4672	JARID2	O	1.34	4.09E-150	NA	NA	NA	NA	-1.27	3.42E-138
4673	NCOR2	O	1.33	6.41E-14	NA	NA	NA	NA	-1.26	1.24E-12
4674	BAMBI	O	1.32	9.37E-92	NA	NA	NA	NA	-1.39	4.35E-102
4675	SYTL2	O	1.30	2.65E-02	NA	NA	NA	NA	-1.17	4.72E-02
4676	JAKMIP3	O	1.30	2.36E-10	NA	NA	NA	NA	-1.44	2.05E-12
4677	SLC9A3-AS1	O	1.29	1.45E-03	NA	NA	NA	NA	-1.70	1.27E-05
4678	AC012441.1	O	1.27	2.04E-02	NA	NA	NA	NA	-1.42	8.37E-03
4679	GLIS2	O	1.25	8.78E-09	NA	NA	NA	NA	-1.25	9.04E-09
4680	AC097468.2	O	1.25	2.69E-02	NA	NA	NA	NA	-1.36	1.55E-02

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4681	GATA2-AS1	O	1.25	5.26E-24	NA	NA	NA	NA	-1.25	2.12E-24
4682	AC092198.1	O	1.25	1.74E-20	NA	NA	NA	NA	-1.20	2.39E-19
4683	AC090617.6	O	1.24	8.06E-08	NA	NA	NA	NA	-1.22	8.72E-08
4684	NAT16	O	1.24	5.39E-10	NA	NA	NA	NA	-2.22	9.73E-26
4685	AL136368.1	O	1.23	2.45E-03	NA	NA	NA	NA	-1.50	2.07E-04
4686	MAPK12	O	1.23	9.44E-09	NA	NA	NA	NA	-2.35	1.34E-29
4687	SCGB1B2P	O	1.22	4.90E-02	NA	NA	NA	NA	-1.27	3.68E-02
4688	AC021321.1	O	1.22	4.54E-02	NA	NA	NA	NA	-1.87	2.48E-03
4689	RBM20	O	1.22	3.57E-19	NA	NA	NA	NA	-1.67	2.33E-35
4690	IL13RA2	O	1.21	2.42E-02	NA	NA	NA	NA	-1.42	6.40E-03
4691	FLNC	O	1.20	2.14E-09	NA	NA	NA	NA	-1.55	2.78E-15
4692	LTK	O	1.19	6.65E-07	NA	NA	NA	NA	-1.12	2.92E-06
4693	GPR68	O	1.19	2.48E-03	NA	NA	NA	NA	-1.66	5.51E-05
4694	SMAD3	O	1.19	1.07E-13	NA	NA	NA	NA	-1.07	3.14E-11
4695	ATP2B1-AS1	O	1.18	2.91E-05	NA	NA	NA	NA	-1.14	4.14E-05
4696	EEPD1	O	1.17	1.55E-21	NA	NA	NA	NA	-1.19	4.86E-22
4697	KRT8	O	1.16	4.11E-02	NA	NA	NA	NA	-1.34	2.74E-02
4698	RASA4B	O	1.16	6.66E-03	NA	NA	NA	NA	-1.35	1.11E-03
4699	D2HGDH	O	1.15	1.25E-04	NA	NA	NA	NA	-1.54	1.02E-07
4700	GPR83	O	1.14	3.95E-02	NA	NA	NA	NA	-1.58	3.33E-03
4701	BCL2	O	1.14	5.58E-32	NA	NA	NA	NA	-2.22	1.85E-118
4702	GRASP	O	1.13	3.46E-02	NA	NA	NA	NA	-1.10	4.19E-02
4703	MIR100HG	O	1.13	4.18E-05	NA	NA	NA	NA	-1.76	4.62E-10
4704	WFIKKN1	O	1.12	4.00E-02	NA	NA	NA	NA	-1.30	1.53E-02
4705	NLGN3	O	1.10	2.98E-23	NA	NA	NA	NA	-1.02	1.21E-20
4706	DNAH10OS	O	1.09	1.16E-28	NA	NA	NA	NA	-1.43	1.77E-47
4707	SNPH	O	1.09	2.52E-09	NA	NA	NA	NA	-1.66	2.85E-19
4708	ABCB1	O	1.09	2.75E-53	NA	NA	NA	NA	-1.38	1.61E-84
4709	TUBA3FP	O	1.09	1.28E-13	NA	NA	NA	NA	-1.03	1.69E-12
4710	CDC42EP3	O	1.09	3.39E-97	NA	NA	NA	NA	-1.08	2.31E-95
4711	NME9	O	1.08	2.78E-02	NA	NA	NA	NA	-1.44	2.24E-03
4712	CIB1	O	1.08	3.05E-18	NA	NA	NA	NA	-1.66	3.83E-40
4713	AZIN2	O	1.07	1.04E-02	NA	NA	NA	NA	-1.03	1.45E-02
4714	DYNC1I1	O	1.06	5.35E-08	NA	NA	NA	NA	-1.56	6.51E-15
4715	AC002472.2	O	1.05	1.05E-03	NA	NA	NA	NA	-1.20	1.40E-04
4716	PDCD4-AS1	O	1.04	2.04E-10	NA	NA	NA	NA	-1.42	6.80E-18
4717	SERPINB1	O	1.04	5.25E-04	NA	NA	NA	NA	-1.12	1.49E-04
4718	ARRDC3	O	1.03	8.18E-11	NA	NA	NA	NA	-1.10	3.02E-12
4719	CTXN2	O	1.03	3.83E-02	NA	NA	NA	NA	-1.59	1.00E-03
4720	LMOD1	O	1.03	4.39E-02	NA	NA	NA	NA	-1.65	7.41E-04
4721	AC123768.3	O	1.01	4.25E-02	NA	NA	NA	NA	-1.02	4.75E-02
4722	AC090197.1	O	1.00	2.17E-02	NA	NA	NA	NA	-1.70	3.40E-05
4723	AC125494.2	O	1.00	2.50E-02	NA	NA	NA	NA	-1.66	1.99E-04
4724	RCN1P2	O	-1.03	5.97E-15	NA	NA	NA	NA	1.51	2.31E-33
4725	S100A4	O	-1.04	2.00E-02	NA	NA	NA	NA	1.71	1.25E-05
4726	AC146944.2	O	-1.05	1.13E-02	NA	NA	NA	NA	1.06	1.01E-02
4727	SIX1	O	-1.07	4.40E-04	NA	NA	NA	NA	1.05	7.02E-04
4728	AC064850.1	O	-1.09	4.99E-02	NA	NA	NA	NA	1.49	2.93E-03
4729	SUSD5	O	-1.10	3.06E-06	NA	NA	NA	NA	1.34	3.52E-09
4730	RCN1	O	-1.11	9.81E-46	NA	NA	NA	NA	1.76	6.65E-117

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4731	TMEM56	O	-1.13	5.20E-07	NA	NA	NA	NA	1.12	5.08E-07
4732	ADAMTSL2	O	-1.14	6.56E-08	NA	NA	NA	NA	1.09	2.19E-07
4733	TXNDC5	O	-1.14	6.53E-22	NA	NA	NA	NA	1.04	2.69E-18
4734	AP002414.2	O	-1.14	1.32E-07	NA	NA	NA	NA	1.02	3.37E-06
4735	SLC47A1	O	-1.16	3.32E-08	NA	NA	NA	NA	1.70	1.66E-17
4736	DBH	O	-1.17	4.70E-13	NA	NA	NA	NA	1.91	5.35E-34
4737	PTPRD	O	-1.17	2.05E-03	NA	NA	NA	NA	1.11	3.75E-03
4738	GATA3	O	-1.17	6.90E-18	NA	NA	NA	NA	1.17	3.70E-18
4739	BDH1	O	-1.17	7.42E-40	NA	NA	NA	NA	1.03	6.78E-31
4740	LOX	O	-1.19	4.82E-15	NA	NA	NA	NA	2.20	3.37E-53
4741	TPD52	O	-1.20	1.99E-58	NA	NA	NA	NA	1.17	4.93E-56
4742	AC135178.5	O	-1.20	2.59E-02	NA	NA	NA	NA	1.21	2.42E-02
4743	AC104066.1	O	-1.22	2.71E-02	NA	NA	NA	NA	1.25	2.21E-02
4744	PIWIL1	O	-1.22	9.38E-11	NA	NA	NA	NA	2.05	5.59E-30
4745	PCOLCE2	O	-1.23	1.43E-13	NA	NA	NA	NA	1.46	1.86E-19
4746	DDC	O	-1.24	5.52E-54	NA	NA	NA	NA	1.77	4.18E-112
4747	RND3	O	-1.25	1.25E-57	NA	NA	NA	NA	1.00	6.46E-37
4748	AKNA	O	-1.26	3.10E-03	NA	NA	NA	NA	1.31	2.01E-03
4749	AC027097.1	O	-1.34	5.90E-04	NA	NA	NA	NA	1.16	3.60E-03
4750	FRZB	O	-1.38	1.22E-18	NA	NA	NA	NA	1.41	1.04E-19
4751	FBXO8	O	-1.47	2.27E-82	NA	NA	NA	NA	1.13	8.23E-49
4752	NFE2L3	O	-1.48	1.57E-52	NA	NA	NA	NA	1.20	7.81E-35
4753	AC012055.1	O	-1.48	3.65E-04	NA	NA	NA	NA	1.25	3.88E-03
4754	MEG8	O	-1.49	1.13E-02	NA	NA	NA	NA	1.67	3.31E-03
4755	SLC35F3	O	-1.50	5.47E-10	NA	NA	NA	NA	2.47	3.98E-28
4756	APLN	O	-1.52	1.20E-17	NA	NA	NA	NA	3.37	4.06E-98
4757	AC027031.2	O	-1.53	4.47E-02	NA	NA	NA	NA	1.79	1.36E-02
4758	LINC02106	O	-1.54	2.92E-03	NA	NA	NA	NA	1.23	2.63E-02
4759	AC008808.2	O	-1.54	1.74E-06	NA	NA	NA	NA	1.04	2.81E-03
4760	MAP3K21	O	-1.55	8.86E-45	NA	NA	NA	NA	1.48	4.18E-41
4761	COL16A1	O	-1.55	1.78E-04	NA	NA	NA	NA	1.28	2.76E-03
4762	TAF7L	O	-1.55	1.36E-02	NA	NA	NA	NA	1.64	8.55E-03
4763	FGFR2	O	-1.56	7.96E-09	NA	NA	NA	NA	1.69	2.92E-10
4764	COMTD1	O	-1.63	1.82E-03	NA	NA	NA	NA	1.87	2.11E-04
4765	PTPRU	O	-1.64	2.21E-27	NA	NA	NA	NA	1.17	3.17E-14
4766	RORB	O	-1.69	3.13E-96	NA	NA	NA	NA	1.41	6.58E-67
4767	OCA2	O	-1.73	2.74E-09	NA	NA	NA	NA	1.09	5.71E-04
4768	GCSAM	O	-1.75	4.13E-02	NA	NA	NA	NA	2.42	9.14E-04
4769	AC106895.2	O	-1.75	6.08E-08	NA	NA	NA	NA	1.17	6.00E-04
4770	BEGAIN	O	-1.78	7.54E-29	NA	NA	NA	NA	1.49	1.53E-20
4771	ZNF704	O	-1.78	3.16E-68	NA	NA	NA	NA	1.45	2.72E-45
4772	SATB1-AS1	O	-1.78	2.04E-84	NA	NA	NA	NA	1.33	2.50E-46
4773	SLCO4A1	O	-1.81	1.33E-40	NA	NA	NA	NA	1.68	2.00E-35
4774	CD9	O	-1.84	2.79E-28	NA	NA	NA	NA	1.17	2.47E-11
4775	FRAS1	O	-1.87	2.42E-29	NA	NA	NA	NA	2.44	3.24E-50
4776	ZNF503	O	-1.88	1.90E-62	NA	NA	NA	NA	1.36	7.99E-32
4777	SNTB1	O	-1.89	8.06E-03	NA	NA	NA	NA	2.74	1.70E-05
4778	NT5DC3	O	-1.90	2.81E-04	NA	NA	NA	NA	1.20	3.73E-02
4779	AL133163.3	O	-1.98	1.99E-35	NA	NA	NA	NA	1.61	3.38E-23
4780	GEM	O	-2.00	1.77E-14	NA	NA	NA	NA	1.94	8.11E-14

NO.	Gene	Category	WT(C) vs WT(R)		BKO(C) vs BKO(R)		WT(C) vs BKO(C)		WT(R) vs BKO(R)	
			Log2 FC	padj	Log2 FC	padj	Log2 FC	padj	Log2 FC	padj
4781	AL845552.2	O	-2.00	5.66E-04	NA	NA	NA	NA	2.12	2.00E-04
4782	SNORA73A	O	-2.03	5.66E-04	NA	NA	NA	NA	1.69	5.99E-03
4783	ACTN2	O	-2.04	3.19E-13	NA	NA	NA	NA	2.83	2.13E-26
4784	JPH1	O	-2.12	5.10E-21	NA	NA	NA	NA	1.58	9.80E-12
4785	ATP8B1	O	-2.16	3.88E-02	NA	NA	NA	NA	2.23	3.22E-02
4786	RAD23BP1	O	-2.20	9.16E-04	NA	NA	NA	NA	1.79	1.08E-02
4787	LINC00556	O	-2.25	5.06E-04	NA	NA	NA	NA	3.38	9.36E-09
4788	SNORD22	O	-2.26	6.02E-04	NA	NA	NA	NA	1.69	1.83E-02
4789	DCLK3	O	-2.48	9.10E-16	NA	NA	NA	NA	1.42	1.28E-05
4790	IGFBP3	O	-2.53	3.68E-152	NA	NA	NA	NA	1.47	5.83E-47
4791	TMEM108-AS1	O	-2.79	7.61E-08	NA	NA	NA	NA	2.37	8.67E-06
4792	LINC02506	O	-2.86	8.71E-03	NA	NA	NA	NA	2.62	1.94E-02
4793	AC011726.2	O	-3.21	3.98E-02	NA	NA	NA	NA	4.64	7.67E-04
4794	RTL1	O	-3.63	0	NA	NA	NA	NA	3.01	3.30E-277
4795	SEC16B	O	-3.88	9.59E-03	NA	NA	NA	NA	3.57	1.94E-02
4796	AC090707.1	O	-4.48	1.83E-03	NA	NA	NA	NA	3.23	4.95E-02
4797	GPX1P1	O	-4.50	9.47E-03	NA	NA	NA	NA	4.24	1.66E-02
4798	AC004678.1	O	-5.21	2.19E-04	NA	NA	NA	NA	4.02	8.45E-03
4799	AC011939.3	O	-5.23	5.55E-03	NA	NA	NA	NA	4.76	1.41E-02
4800	RGCC	O	-5.52	3.87E-03	NA	NA	NA	NA	4.69	1.55E-02
4801	AL355315.1	O	-6.72	3.94E-03	NA	NA	NA	NA	5.34	3.11E-02