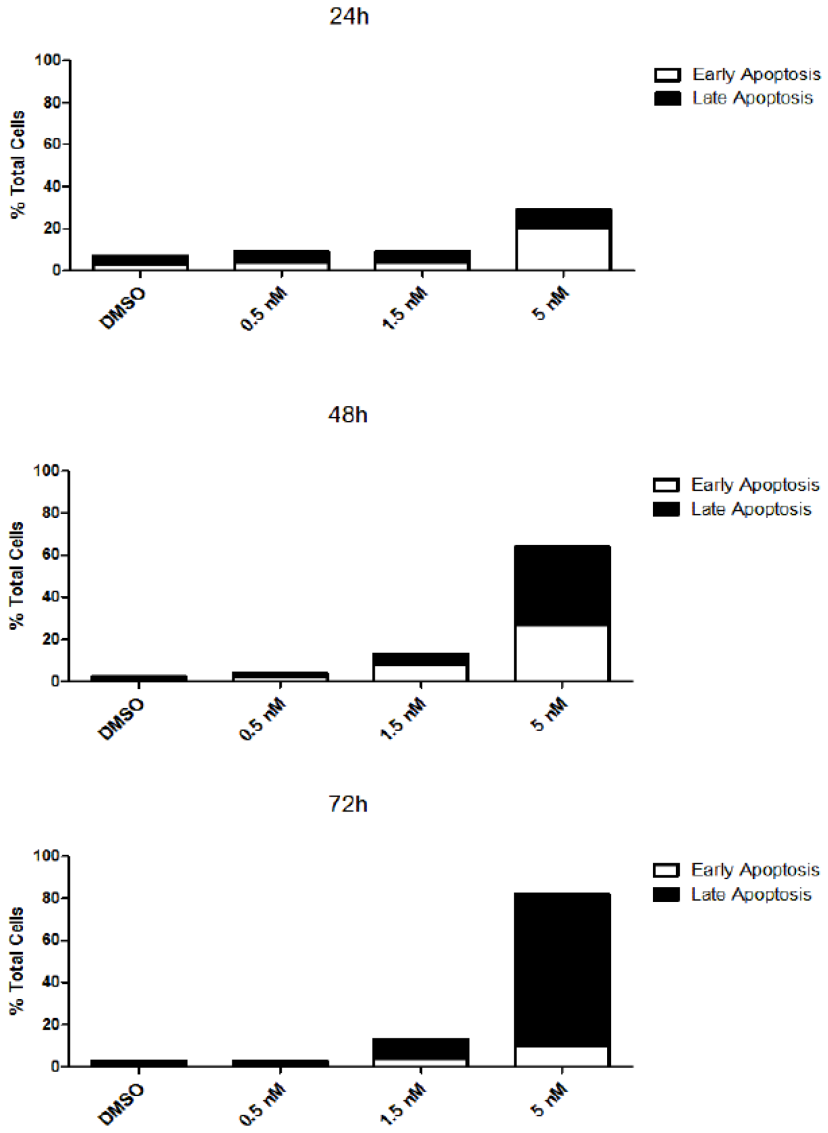
The histone deacetylase inhibitor Romidepsin induces as a cascade of differential gene expression and altered histone H3K9 marks in myeloid leukaemia cells

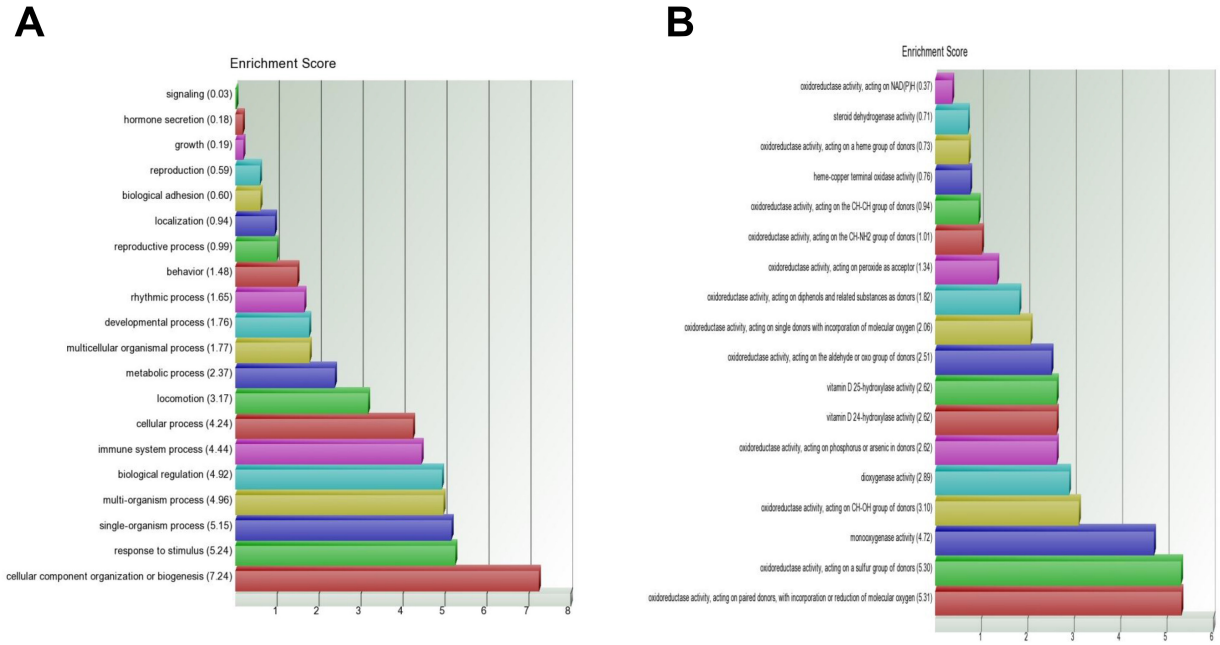
SUPPLEMENTARY MATERIALS

REFERENCES

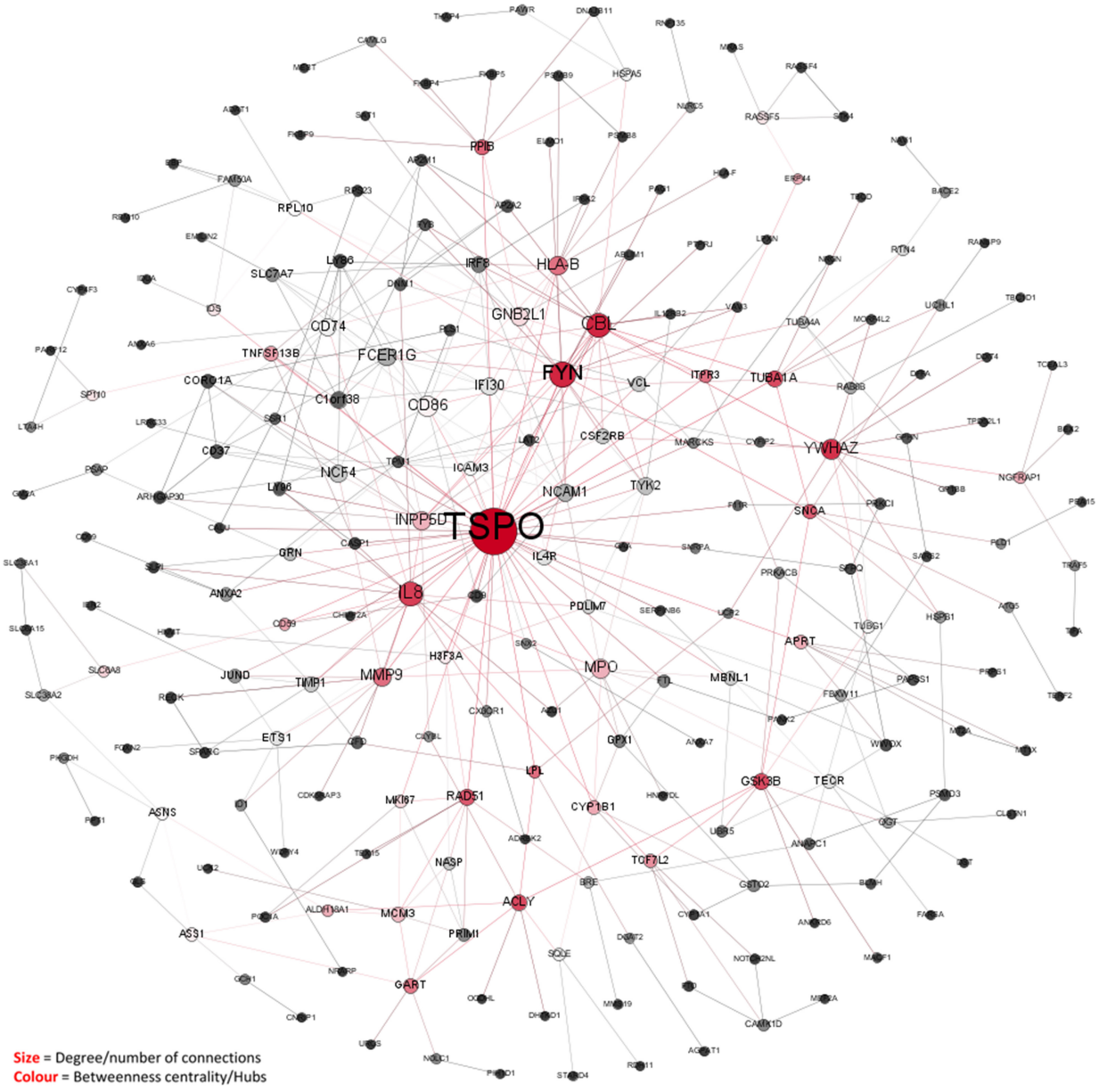
1. Yu H, Kim PM, Sprecher E, Trifonov V, Gerstein M. The importance of bottlenecks in protein networks: correlation with gene essentiality and expression dynamics. PLoS Comput Biol. 2007; 3:e59. https://doi.org/10.1371/journal.pcbi.0030059. [PubMed]



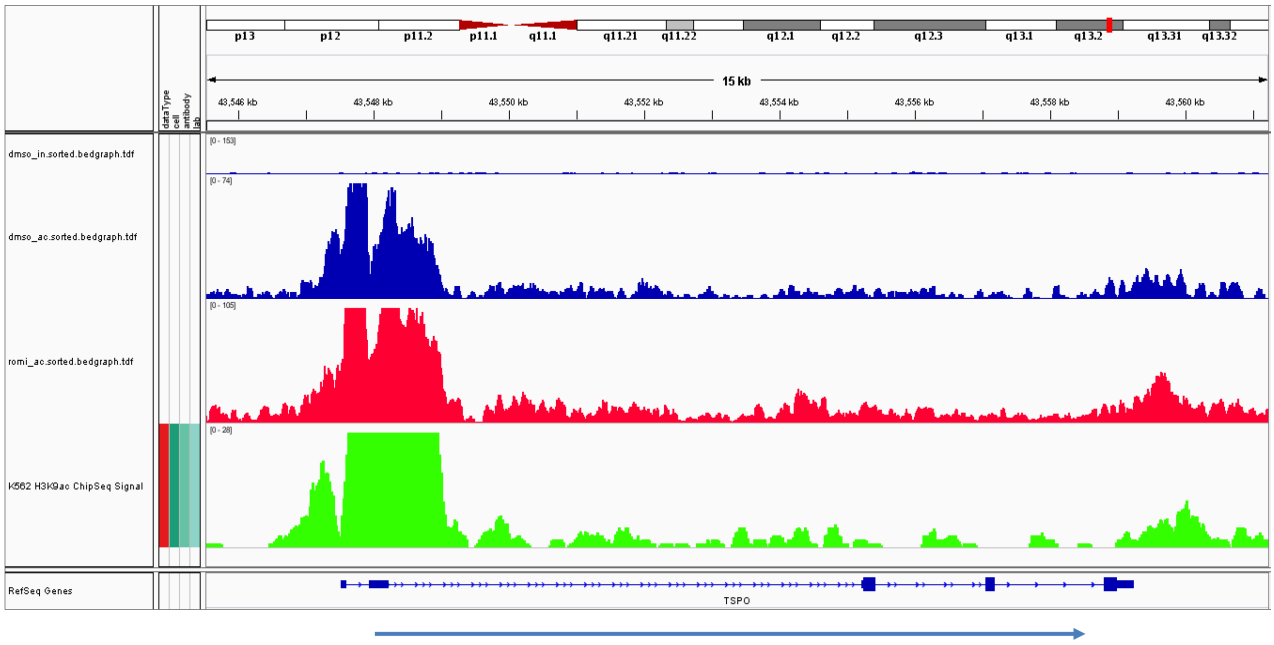
Supplementary Figure 1: Tracking of early and late apoptosis in SKM-1 cells following treatment with Romidepsin. Annexin V and PI staining used to determine stages of apoptosis following treatment with DMSO, 0.5 nM, 1.5 nM and 5 nM Romidepsin. Observed is an increase in the percentage of cells entering into late apoptosis as time and dose increase.



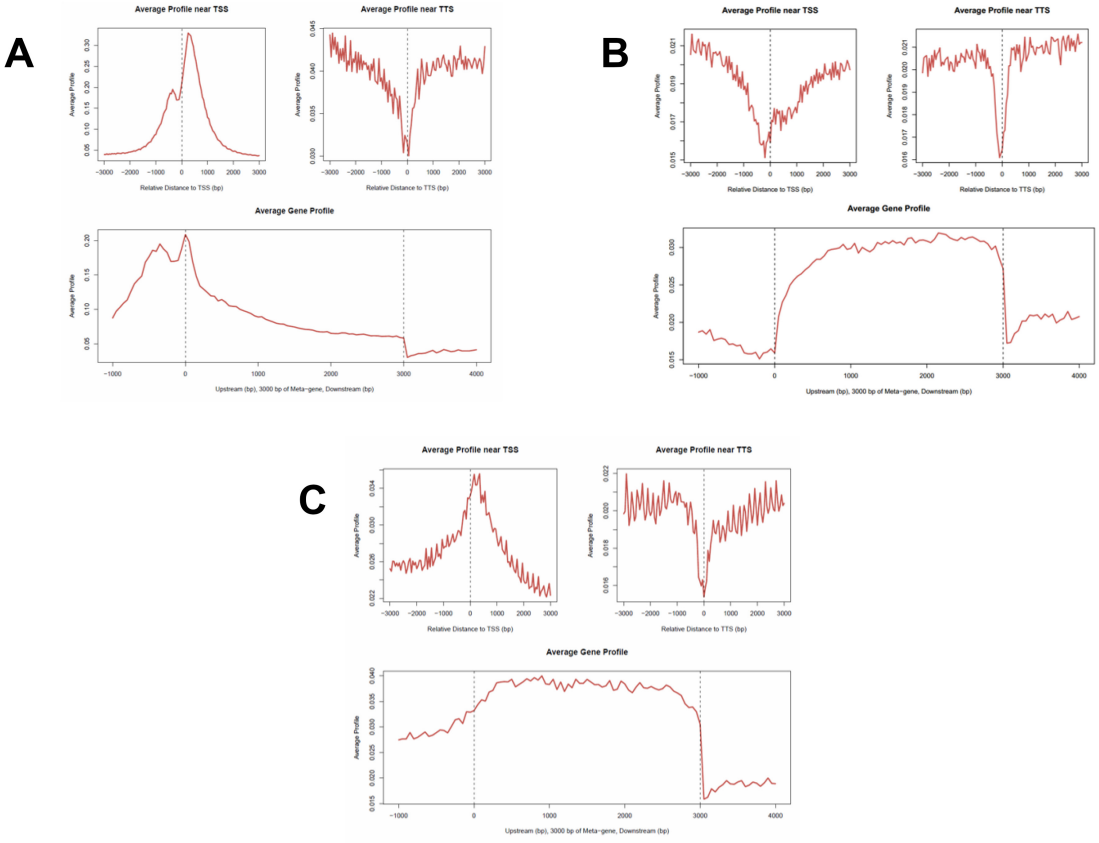
Supplementary Figure 2: Gene Ontology (GO) processes analysis. (**A**) GO biological processes analysis identifying that cellular organisation and biogenesis was the top most enriched process followed by response to stimulus with immune system process also affected. (**B**) GO molecular functions identified oxidoreductase activity as the most enriched pathway following treatment with an enrichment score of 5.31 with other related oxidoreductase activities being affected.



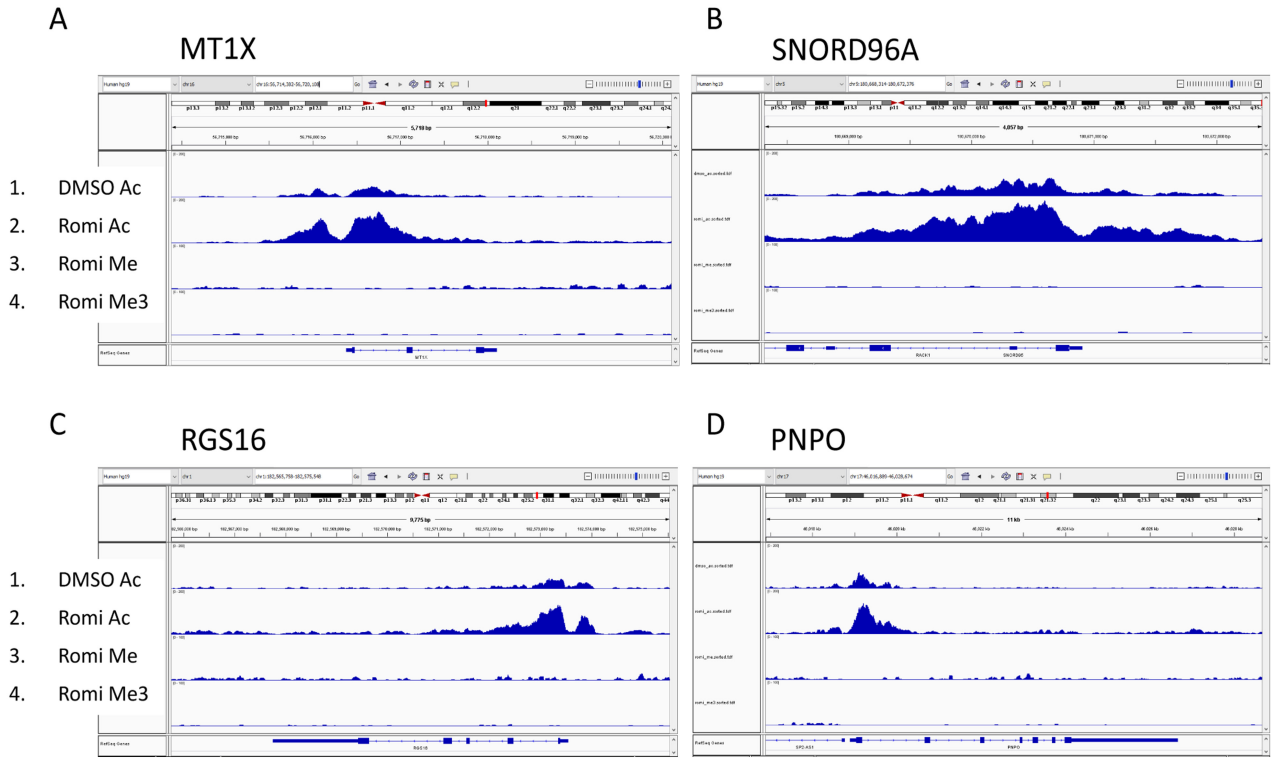
Supplementary Figure 3: STRING Known and predicted protein–protein interactions, followed by GEPHI configuration. Network analysis of 487 genes associated with differential gene expression following Romidepsin treatment. The 487 gene list was imported to STRING which enabled the formation of network matrices. These matrices could then be uploaded to a visualisation software package, Gephi 0.8.2-beta (http://gephi.org/), which enabled for the manipulation of shapes and colours to reveal and emphasise the importance of particular genes associated with hubs and bottlenecks based on their betweenness centrality (indication of a nodes centrality to a hub) and degree on connections. Bottlenecks are described as being proteins with a high degree of betweenness centrality and are believed to be "key connector proteins with surprising functional and dynamic properties" and are said to be more likely essential proteins within the network [1]. Criteria applied here included size of the node to depict degree/number of connections, whilst colour denotes betweenness centrality i.e. the larger the node, the more connected the protein is, and the more red the node, the higher the betweenness centrality.



Supplementary Figure 4: IGV tracks showing acetylation levels at the promotor of TSPO. TSPO was used as a positive control as it had been shown to be acetylated at the promoter in the closely related haematopoietic cell line K562 prior to sequencing. As can be seen in the tracks above, this was also the case in our samples (blue = SKM-1 cells treated with DMSO for H3K9ac, red = SKM-1 cells treated with Romidepsin for H3K9ac, green = control K562 H3K9 acetylation track from IGV) for validating TSPO as a positive control. Input sample shows little to no signal.



Supplementary Figure 5: Cis-regulatory Element Annotation System (CEAS) profiles of TSS, TTS and average gene profile of AcH3K9, H3K9me and H3K9me3 in Romidepsin treated samples. CEAS profiles showing (**A**) AcH3K9, (**B**) H3K9me and (**C**) H3K9me3. Both (**A**) and (**C**) display peaks around the transcriptional start site (TSS) and dips at the transcriptional termination site (TTS), whilst (**B**) portrays a dip at the TSS. Acetylation appears to taper off across the gene body, whilst methylation in both mono and tri form maintain high levels of enrichment throughout gene body.



Supplementary Figure 6: Examples of IGV tracks showing increased acetylation levels of genes with increased differential expression. Increased Acetylation marks in (**A**–**D**). Row 1 (top) is DMSO treated cells for Acetylation marks, row 2 is Romidepsin treated cells for acetylation marks; row 3 is Romidepsin treatment for mono-methylation and row 4 (bottom) is tri-methylation following Romidepsin treatment.

Supplementary Table 1: 130 genes showed an overlap between microarray data and increased acetylation. See Supplementary_Table_1

Supplementary Table 2: SYBR Primer sequence for qPCR

	Forward	Reverse
Genomic		
TSPO	AGACACCCCGTTTCGTAGTG	GGCCTCTAACCGGCTTTGG
MYOD1	GGGGGCGCTATTAGAACAC	CAAACCAAGGGCGGACAG
GATA2	CCTGTAATTAACCGCCAGCTC	GGCAGAACCGACCACTCATC
BCL2	AGATAGAGTTTTGTCATGTTGTGC	GGTGGGTGTATTGCTTGAGG
mRNA		
HDAC1	AATTCTTGCGCTCCATCCGT	GTTTCACAGCACTTGCCACA
HDAC2	ACTACGACGGTGATATTGGAA	CTTCGGCAGTGGCTTTATGG
DNMT1	GCCAAACGGAAACCTCAGGA	AGCGGTCTAGCAACTCGTTC
DNMT3a	ACGACCAGGAATTTGACCCT	TCCACCTGAATGCCCAAGTC
DNMT3b	GGTTTGGCGATGGCAAGTTC	TCGCACCCTAGCTTTCTCCA
FKBP5	ATGCAGCGGGAAGAACAATG	TCCCAGGATTCTTTGGCCTTT
FKBP4	TGGACATCAGCCCCAAACAG	GTCCCCAATCATGGGCATCT
DNAJB11	TGCTTATGAGGTTCTGTCAGATAGT	CGAGGGGTTCCTCCAAACAT
STK4	AGACGGTACAGCTGAGGAAC	TACGCTGCCATAGGACCCTT
CXCL8	CACCGGAAGGAACCATCTCA	GGCAAAACTGCACCTTCACAC
TSPO	CTACTCAGCCATGGGGTACG	GTGAAGCCTCCCAGCTCTTT
DNM1	GGAGCTAATCAGCACCGTTAGA	AGCATGACCTGCTCCTTAGT
YWHAZ	ACTTGACATTGTGGACATCGGA	GTGGGACAGCATGGATGACA
GSK3B	GACTAAGGATTCGTCAGGAACA	TACTCCAGCAGACGGCTACA
CBL	CGGAAGCACGTTCAGTCTGG	TTTTGGCACAGGAAGAGGTCTGG
FYN	AGCAGGATGCTGATCTAAACG	TCTAAATTCCAAAATGTCCGCCAAC
CSF2RB	CCACGGCCAATACATCGTCT	GGGCCATCTGGATGTTCACT
INPP5D	ACACCAAGCAGAAAGCGACA	CGCTGGTACTGCCATAAGACT
CD86	AGCCAAAATGGATCCCCAGTG	ATCTTCAGAGGAGCAGCACCAGA
HSPA5	GCTCAACATGGATCTGTTCCG	GAGTCGAGCCACCAACAAGA
IFR8	CAGCTCCTTCCAGACTGGTG	TCTGGGAGAATGCTGAATGGT
APRT	CTGGAGTACGGGAAGGCTGA	ACAGGCAGCGTTCATGGTT
TCIRG2	ATCACGGACTGCTTCCACTG	CTCAGGAACCGCTCTGTCTC
RPL10	TTCGGTGTGCCACTGAAGAT	TTCCGCCCCAGGTCAAAAAT
FCER1G	AGCAGTGGTCTTGCTCTTACT	TTTCGCACTTGGATCTTCAGT
CD74	CTGGAAGGTCTTTGAGAGCTG	TCTTCCTGGCACTTGGTCAG
PSMB8	CTCCTGGCTGACTTCTAGTCT	CTGAACGTTCCTTTCTCCGT
MCM3	AATGCCTCAAGTACACACTCCA	GATTGACTGCGCATGAGCTT
ACLY	GGATTTTGCGGGGTTCGTC	GACATGGCTGCAGAGAGACC
CYP1A1	GAACCTTCCCTGATCCTTGTGA	GTGTAGGGATCTTGGAGGTG
MPO	CCTGGTTAGCAGAGCTGGAC	GGGCCCATAAGTCAACCACA
SPARC	TGGCAGAGGTGACTGAGGTAT	CGTGTTTGCAGTGGTGGTTC
RASSF5	GCAGGATCCCAGAGT	GGTGAATTTACAGTTAGTGC
ELMO1	GCCGACCCAGAGTGTATAG	TATGGCCACCTTGACGATGT
GNB2L1	CTTCTGGAGGCAAGGATGGC	GATGTCCCCACCATCTAGCG
TYK2	GAAAGCCCTCAAGGCAGACT	GACTTCTCGCCTTGGTCCTC
CD59	ACCAGTTGGTGTAGGAGTTGA	TGCAGGCTATGACCTGAATGG
NCAM1	GGAGACCCCATTCCCTCCAT	CATGGTGCATGTACCTCTTGC
TNFSF13B	CACCGCGGGACTGAAAATCT	CTGCAATCAGTTGCAAGCAGT