Epigenetic dysregulation of eukaryotic initiation factor 3 subunit E (eIF3E) by lysine methyltransferase REIIBP confers a pro-inflammatory phenotype in t(4;14) myeloma

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Supplementary Materials and Methods

Cell Culture

Human myeloma cell lines (RPMI8226, KMS11, KMS34, KMS18, H929, OPM2, KMS12BM, U266) and chronic myeloid leukemia cell line K562 were cultured in RPMI-1640 medium and 293T cells were cultured in DMEM supplemented with 10% FBS and 10 mg/mL penicillin/streptomycin, and incubated at 37^0C with 5% CO_2. Cell lines were authenticated (National University of Singapore, Singapore) and tested to be *Mycoplasma*-free (Lonza, Switzerland).

Real-time qRT-PCR

Equal amount (1 µg) of RNA was used for cDNA synthesis using iScript™ Reverse Transcription Supermix (Bio-Rad). The cDNA was used for quantitative real-time PCR (qRT-PCR) analysis with iTaq™ Universal SYBR Green Supermix (Bio-Rad). All reactions were carried out in technical duplicates and biological triplicates. Calculations were based on the ΔΔCt method with GAPDH housekeeping gene for normalization. The primers used for qRT-PCR are listed below.

List of primers used for qRT-PCR

Gene	Dye	Sense	Sequence (5'-3')
GAPDH	SYBR	Forward Reverse	TGCACCACCAACTGCTTAGC GGCATGGACTGTGGTCATGAG
REIIBP	SYBR	Forward Reverse	ATGATGCGGTGTGTCCGCTGC CTATTTGCCCTCTGTGACTCT
NSD2	SYBR	Forward Reverse	AGAGGATACAGGACCCTACA GTGTTTCGTCTGCACTTTCG
ACA11	SYBR	Forward Reverse	GCGCTCACTAAGGCTCGG TGTTGTACTGAAAACCG
FGFR3	SYBR	Forward Reverse	TCCATCTCCTGGCTGAAGAACG TGTTCTCCACGACGCAGGTGTA
NSD1	SYBR	Forward Reverse	AGAGGATACAGGACCCTACA AGCAGCTGGGTTCAAATCCAA
EZH2	SYBR	Forward Reverse	CTGTTCAGAGGGAGCAAAGCTTGC TAGGTGGTGTCTTTATACGCTCAG
EZH1	SYBR	Forward Reverse	AAGCGCCATGCTATCGAAGGCAA GCCATCGATGTTGGGTGTGCACTG
KDM6A	SYBR	Forward Reverse	TACAGGCTCAGTTGTGTAACCT CTGCGGGAATTGGTAGGCTC

KDM6B	SYBR	Forward Reverse	GGAGGCCACACGCTGCTAC GCCAGTATGAAAGTTCCAGAGCTG
SETD1A	SYBR	Forward Reverse	GGCCAGATTCATCAACCACT CGATCTTCTTCTGGGACTCG
SETD1B	SYBR	Forward Reverse	CTGTTGGTGAGCTGGATGCTA CTGGAGTAAGCTGTGTCTTGG
MLL1	SYBR	Forward Reverse	AGAATCTGCGTTCGGCTGGTTC TCTGGTTCCTGCTGTCTCCACT
MLL2	SYBR	Forward Reverse	GGAATGGGTAGCTCTTTGGCGA TGCCGAATCAGCAGCTCTCGTA
MLL3	SYBR	Forward Reverse	AGATCAGCGTGGACCCTATCCT CTCTTGACTCGGCATGGTACCA
MLL4	SYBR	Forward Reverse	CCAGCGGTTCCTTCCTATC CAGTCAGAGCAGTGTCCAG
SMYD1	SYBR	Forward Reverse	GTGAAGAACGCAAGAGGCAGCT CTCCTTCACCACTTCCTGAGAG
SMYD2	SYBR	Forward Reverse	AAGGCAGAAGCCATCCGAGACA TCATCTTCTCCTGGCTGAGCTC
SMYD3	SYBR	Forward Reverse	TTACTGCGAGCAGTCCGAGACA TTGTCCTGGGTTTGGCAACGGA
SET7	SYBR	Forward Reverse	CGTATGTAGACGGAGAGCTGAAC CTCCTACAAGGCTTCCTCCATC
KDM1A	SYBR	Forward Reverse	TCAGGAGTTGGAAGCGAATCCC GTTGAGAGAGGTGTGGCATTAGC
KDM2A	SYBR	Forward Reverse	CAAGGAGAGTGTGGTGTTTGCC ACCTCTCCACAGAGGGAACATG
KDM5A	SYBR	Forward Reverse	GCTAAGGTCTGCCTACAGGCAA CCACTTTAGCGGTCCATTCTCG
DOT1L	SYBR	Forward Reverse	GTTCCTGGCATACACAAAGACCC GCTGAAACAGCCTCCTGATCTC
DICER	SYBR	Forward Reverse	TCCACGAGTCACAATCAACACGG GGGTTCTGCATTTAGGAGC
CYBB	SYBR	Forward Reverse	CTCTGAACTTGGAGACAGGCAAA CACAGCGTGATGACAACTCCAG
TLR7	SYBR	Forward Reverse	CTTTGGACCTCAGCCACAACCA CGCAACTGGAAGGCATCTTGTAG
FAIM3	SYBR	Forward Reverse	CGAACCTTCCTGCCATCCACTA CTGTGAGCCATAGTCCAGTGCT
BTK	SYBR	Forward Reverse	TACCATGGTGGAGAGCACGA CAGTTGCTCAGCCTGACTCC
PDIA2	SYBR	Forward Reverse	GCTGCTGTTTGTCAACCAGACG CCTCAGCCTTGAGTCCAAAGTAC
IFN-α	SYBR	Forward Reverse	TTCTGCACCGAACTCTACCAGC CTTCACAGCCAAGATGGAGTCC
IFN-β	SYBR	Forward Reverse	CTTGGATTCCTACAAAGAAGCAGC TCCTCCTTCTGGAACTGCTGCA

TNF-α	SYBR	Forward Reverse	CTCTTCTGCCTGCTGCACTTTG ATGGGCTACAGGCTTGTCACTC
IL-6	SYBR	Forward Reverse	AGACAGCCACTCACCTCTTCAG TTCTGCCAGTGCCTCTTTGCTG
IL-8	SYBR	Forward Reverse	GAGAGTGATTGAGAGTGGACCAC CACAACCCTCTGCACCCAGTTT
IL-10	SYBR	Forward Reverse	TCTCCGAGATGCCTTCAGCAGA TCAGACAAGGCTTGGCAACCCA
IL-12	SYBR	Forward Reverse	TGCCTTCACCACTCCCAAAACC CAATCTCTTCAGAAGTGCAAGGG
IL-23	SYBR	Forward Reverse	GAGCCTTCTCTGCTCCCTGATA GACTGAGGCTTGGAATCTGCTG
IL-27	SYBR	Forward Reverse	GGAATCTCACCTGCCAGGAGTG TGGTGGAGATGAAGCAGAGACG
IL-1β	SYBR	Forward Reverse	CCACAGACCTTCCAGGAGAATG GTGCAGTTCAGTGATCGTACAGG
VCX2	SYBR	Forward Reverse	AAGTCCTCCTCTCAGCCGAGC CTCTCCGCCTCAGGTGCCGT
SRGAP2	SYBR	Forward Reverse	CGTCAAGTCCACGGTCTCTGAA ACTTGGTGATGAGGTTCCTGCC
COMP	SYBR	Forward Reverse	GGAGATGCTTGTGACAGCGATC TGAGTCCTCCTGGGCACTGTTA
CLEC2L	SYBR	Forward Reverse	TGGAACACAGGCAGGCAGTACT ACTCTGCGTAGTCCAATCCAGG
MED12L	SYBR	Forward Reverse	GCGAGAATGACATGCCGACTCT AGCAGCGGCTAAGAGGTGTCTA
TNIK	SYBR	Forward Reverse	ACAGTGGCTGTCAGCGACATAC ATACTGCCGCTGAAACTGTCCG
ITM2A	SYBR	Forward Reverse	GGCAGGACTTATTGTTGGTGGAG CCTCAGTCACAGGCAGGAAGTT
RARRES1	SYBR	Forward Reverse	TCACGTGGTCTTCAGCACAGAG TTTCTCGATGAGCCGTGTACAAG
NUP98	SYBR	Forward Reverse	GGAACCTGTGTCTGCCTCAACA CTTTGGAAGGCAGGCGACTGAA
SOX5	SYBR	Forward Reverse	CTCGGCAAATGAAGGAGCAACTC ACTGCCAGTTGCTGAGTCAGAC
ANO5	SYBR	Forward Reverse	GCAGTGACTAAGGAGATGGAACC GTAGCAAAGACTGACAGGCGGT
CRPPA	SYBR	Forward Reverse	TCACAGCTGCTAAGGAACACGG TGTGTCTGGCACGTTCTAGCGA
BATF3	SYBR	Forward Reverse	ACCGAGTTGCTGCTCAGAGAAG AGGTGCTTCAGCTCCTCTGTCA
SLC2A7	SYBR	Forward Reverse	CTTTGGCTCAGCCTTCCAGTAC CCGTCCATGAATGTTGCGTGTC
OPRD1	SYBR	Forward Reverse	GCAACGTGCTTGTCATGTTCGG ACTTGGCACTCTGGAAAGGCAG

CELF3	SYBR	Forward Reverse	GGTCGGATCTTTGAGCTGACTG GGAAGCGTCTTCTGTTCGTGCA
CD9	SYBR	Forward Reverse	TCGCCATTGAAATAGCTGCGGC CGCATAGTGGATGGCTTTCAGC
TMEM64	SYBR	Forward Reverse	GCCAGACTGACACCCATACCTT GGTAGGAAGCAGTCCAACCGAA
ANXA13	SYBR	Forward Reverse	CGTTGATTCGCATAGTCGTGACC CGGAGGTATCTGAGCGAACCAT
TTLL10	SYBR	Forward Reverse	CCTCACCCTTAGCCTTTACGAC CGTGTCACTGATGTAGCGGTTG
TRPV2	SYBR	Forward Reverse	CATCTTCACCGCTGTTGCCTAC CCTAGCAGGATAAGGATGTGGC
LIMA1	SYBR	Forward Reverse	AATAGCCTGGCAGTCCGTTCCA GGAATCTGGACTTAGTGGCTTGG
COL4A2	SYBR	Forward Reverse	GGATAACAGGCGTGACTGGAGT CTTTGCCACCAGGCAGTCCAAT
AK5	SYBR	Forward Reverse	TGATTGACGGCTATCCTCGGGA TTGGAGAAGGCGGTTGGTCATG
DLG2	SYBR	Forward Reverse	TGCTTCTCCCAGGCACTATTCC CTGACGAGTTGCGGTGCTATGT
STAT4	SYBR	Forward Reverse	CAGTGAAAGCCATCTCGGAGGA TGTAGTCTCGCAGGATGTCAGC
P2RY14	SYBR	Forward Reverse	GCCGCAACATATTCAGCATCGTG GCTGTAATGAGCTTCGGTCTGAC
MRPS22	SYBR	Forward Reverse	CTACGCAAAGCCTCTTGGGAAG CAACATGCCTGTCCTGGCTATAC
ZMAT3	SYBR	Forward Reverse	GCTCTGTGATGCCTCCTTCAGT TTGACCCAGCTCTGAGGATTCC
EIF3E	SYBR	Forward Reverse	CTTTCTGTCGCATCCACCAGTG TCAATCTTGGCATCCAGTCTTGC
KHDRBS3	SYBR	Forward Reverse	GACATGGACTCAGTGAGGAGAC TATGGCTGGTCTCTGTAGACGC

Western blotting analysis

Cells were wash twice in ice-cold PBS and lysed with appropriate volume of RIPA lysis buffer comprising of 50mM Tris-HCl pH7.4, 1mM EDTA, 150mM NaCl, 0.5% sodium deoxycholate, 1mM Na2VO4, 20mM NaF, and complete protease inhibitor cocktail (Roche). After which, the samples were centrifuged at 16,000rpm for 15 minutes. The supernatant was collected and lysate concentration measured using BCA assay (Thermo Scientific) according to manufacturer's instructions. Equal amounts of lysate were resolved by 10-16% sodium dodecyl sulfate-

polyacrylamide gel electrophoresis (SDS-PAGE) and subjected to immunoblotting. The respective primary antibodies were used to probe the membranes at 4°C overnight, followed by detection with the secondary antibodies tagged with horse radish peroxidase (HRP) for 1 hour at room temperature. The antibodies used are listed below.

List of antibodies used for WB and ChIP

Antibody	Catalog Number	Application
GAPDH	SantaCruz, #sc47724	WB
β-actin	SantaCruz, #sc47778	WB
NSD1/2	Abcam: #ab75359	WB
REIIBP	Cell Signaling Technology, #65127	WB
His-tag	Santa Cruz Biotechnology, #sc-8036	WB
H3K9me2	Cell Signaling Technology, #4658	WB
H3K9me3	Cell Signaling Technology, #13969	WB
H3K27me3	Cell Signaling Technology, # 9733S	WB/ChIP
H3K27me2	Cell Signaling Technology, #9728	WB
H3K27me1	Cell Signaling Technology, #84932	WB
H3K36me2	Cell Signaling Technology, #2901	WB
H3K36me3	Cell Signaling Technology, #4909	WB
H3K79me2	Cell Signaling Technology, #5427	WB
H3K4me3	Abcam, # ab8580	WB/ChIP
Histone 3	Cell Signaling Technology, #4499	WB
DOT1L	Cell Signaling Technology, #77087	WB
EZH2	Cell Signaling Technology, #3147S	WB
Flag-tag	Sigma Aldrich, F1804	WB
Dicer	Cell Signaling Technology, #5362	WB
TLR7	Cell Signaling Technology, # 5632S	WB
BTK	Cell Signaling Technology, #8547S	WB
pBTK (Y223)	Cell Signaling Technology, #5082	WB
pBTK (Y551)	Cell Signaling Technology, #18805	WB
Bcl-2	Cell Signaling Technology, #15071	WB

p-Bcl2	Cell Signaling Technology, #2827	WB
NF-κB p65	Cell Signaling Technology, #8242	WB
p-NF-κB p65	Cell Signaling Technology, #3033	WB
IκBα	Cell Signaling Technology, #4814	WB
p-IκBα	Cell Signaling Technology, #2859	WB
Erk1/2	Abcam, # ab184699	WB
p-Erk1/2	Abcam, # ab201015	WB
IL-6	Cell Signaling Technology, #12912	WB
eIF3E	Abcam #ab36766	WB/RIP
Cas9	Cell Signaling Technology, #14697S	WB
Puromycin (12D10)	Sigma-Aldrich, #MABE343	WB

shRNA and sgRNA targeting sequences

Various NSD2 shRNAs were cloned into pLV[shRNA]-Puro-U6 with the target sequence of NSD2 shR#1: CCAGAAAGAGCTTGGATATTT; NSD2 shR#2: GCACGCTACAACACCAAGTTT; NSD2 shR#3: CCTCAGTTGTTTCCACTCATT. shRNA sequences for Dicer shR#1 is GCCAAGGAAATCAGCTAAATT and Dicer shR#2 is GCTCGAAATCTTACGCAAATA. shRNA sequences for TLR7 shR#1 is GAAATGAGATTGCCCATATTT and TLR7 shR#2 is TCAGGAGTCTGACGAAGTATT. Single guide sequences for CRISPR/Cas9 knockdown of eIF3E were ATAATATTATCGCGACCTTT and AATAATATTATCGCGACCTT. Single guide sequences for eIFe3 H3K4me3 peaks are as follows: gRNA#1- GTCTTGAATTTCTCTCTGTAA; gRNA#2- GGCGGATGAGGTGCTGAGCA; gRNA#3- GACGGACACTAAGGATCTTC.

Cell viability

50,000 cells were seeded in 96-well plate format in 100µL of medium (with or without treatment) and viability determined using an equal volume of CellTitre-Glo Assay (Promega) according to

the manufacturer's instructions. Luminescence readings were recorded using a microplate reader (Tecan infinite M200) after 15 minutes of incubation in the dark at room temperature. Assay was performed in independent triplicates over 48 hours, and the mean values were used to plot the graph.

Cell cycle analysis

Cells were washed once in 1x PBS before resuspending 1 million cells in 0.5mL of ice-cold 1x PBS. In a drop-wise manner, the cells were added into 4.5mL of ice-cold 70% ethanol to fix for at least 24 hours at 4^0C. Before flow cytometry analysis, cells were harvested and wash with 1x PBS to remove traces of ethanol. Propidium Iodide (BD Pharmingen) Staining was performed for 15 to 30 minutes and analysed using flow cytometry (FACSAria). Assay was performed in independent triplicates and the mean values were used to plot the graph.

Colony formation assay

Cells were washed twice with 1x PBS and resuspended at 2000 cells in 2mL MACS HSC-CFU media containing human GM-CSF (Miltenyi Biotech). Cells were cultured for 14 days in 6-well plate format and pictures of colony formation were taken with microscope (Olympus SZX12). Assay was performed in independent duplicates.

Apoptosis

1 x 10^6 cells were washed with 1 x PBS and dual-stained with Annexin V-FITC and propidium iodide (PI) (BD Pharmingen) for 15 mins in the dark according to manufacturer instructions. Cells were passed through a cell mesh to obtain single cells before analysis with LSRII Flow Cytometer (BD Biosciences).

Nuclear and cytoplasmic fractionation

Nuclear and cytoplasmic fractionation was performed using NE-PER Fractionation Kit (Thermo Scientific) following manufacturer’s protocol using 10 million fresh cells for each experiment. To test the extracts for contamination between nuclear and cytoplasmic fractions, we probed with Lamin A and αTubulin, which probes for nuclear and cytoplasmic fractions, respectively.

Immunoflorescence

Cells were transfected with REIIBP-mcherry overexpression plasmid by electroporation. Transfected cells were cytospinned to obtain a monolayer of cells on slides. Slides were fixed with 3.7% formaldehyde at room temperature for 15 minutes, washed with PBS then permeabilised with 0.5% Triton-X for 10 minutes. They were blocked for 30 minutes with 3% BSA and counterstained with DAPI. Slides were visualised under the Zeiss Axio Imager M2 microscope to determine cellular localisation. Images were merged with Zeiss software v 3.8. Images are representative of technical and biological replicated experiments.

Microarray analysis

Total RNA was extracted using the Qiagen RNeasy Mini kit (Germany). Gene expression was performed using the GeneChip Human Genome U133 plus 2.0 array (Affymetrix) following the manufacturer's instructions. Data analysis was performed using GeneSpring software from Agilent Technologies. Microarray data is available as Supplementary Table 1.

Protein Synthesis Assay

Click-iT™ HPG Alexa Fluor™ 488 Protein Synthesis Assay Kit (Invitrogen) was used to detect protein synthesis between RPMI8226-VCon and RPMI8226-REIIBP according to manufacturer instructions. Imaging was done with Zeiss Axio Imager M2.

Interleukin-6 in cell culture supernatant

Myeloma cells were seeded in a 10 cm dish in 10 mL of medium at a density of 1×10^6/ml. After 24h, cells were seeded in serum-free RPMI. Supernatant was collected after 24h culture in serum-free RPMI and concentrated in Amicon Ultra-0.5 Centrifugal Filter Unit (3K filter unit) as per

manufacturer's instructions. After centrifugation, both supernatants collected in filter units and flow-through were used for detection of protein by Western Blotting.

ChIP sequencing

The purified DNA was used for ChIP-seq library preparation. The library was constructed by Novogene Corporation (Beijing, China). Subsequently, pair-end sequencing of sample was performed on Illumina platform (Illumina, CA, USA). Library quality was assessed on the Agilent Bioanalyzer 2100 system. Clean reads were aligned to the reference genome using BWA mem (v 0.7.12). After mapping reads to the reference genome, we used the MACS2 and epic2 peak calling software for H3K4me3 and H3K27me3 respectively to identify regions of IP enrichment normalized to Input control. A q-value threshold of 0.05 was used for all data sets. Data analysis were performed using deepTools (v2.0) by using either all signals from genome or signals ±5000bp before and after transcription start sites (TSS) of all non-pseudogenes. REIIBP vs VCon ChIP-seq occupancy comparison for H3K27me3 and H3K4me3 were performed using t-test of occupancy values across whole genome and genes with p-values < 0.01 were identified to be differentially-regulated by histone modifications of REIIBP. $t > 0$ indicates that average occupancy in REIIBP samples is higher than that in VCon samples. The reference genome ID used was ensembl_homo_sapiens_grch38_p12_gca_000001405_27 and the ChIP-seq tracks were visualized using Integrative Genomics Viewer (IGV, Broad Institute). The ChIP-seq data from this study was submitted to NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE198026.

RNA immunoprecipitation (RIP)

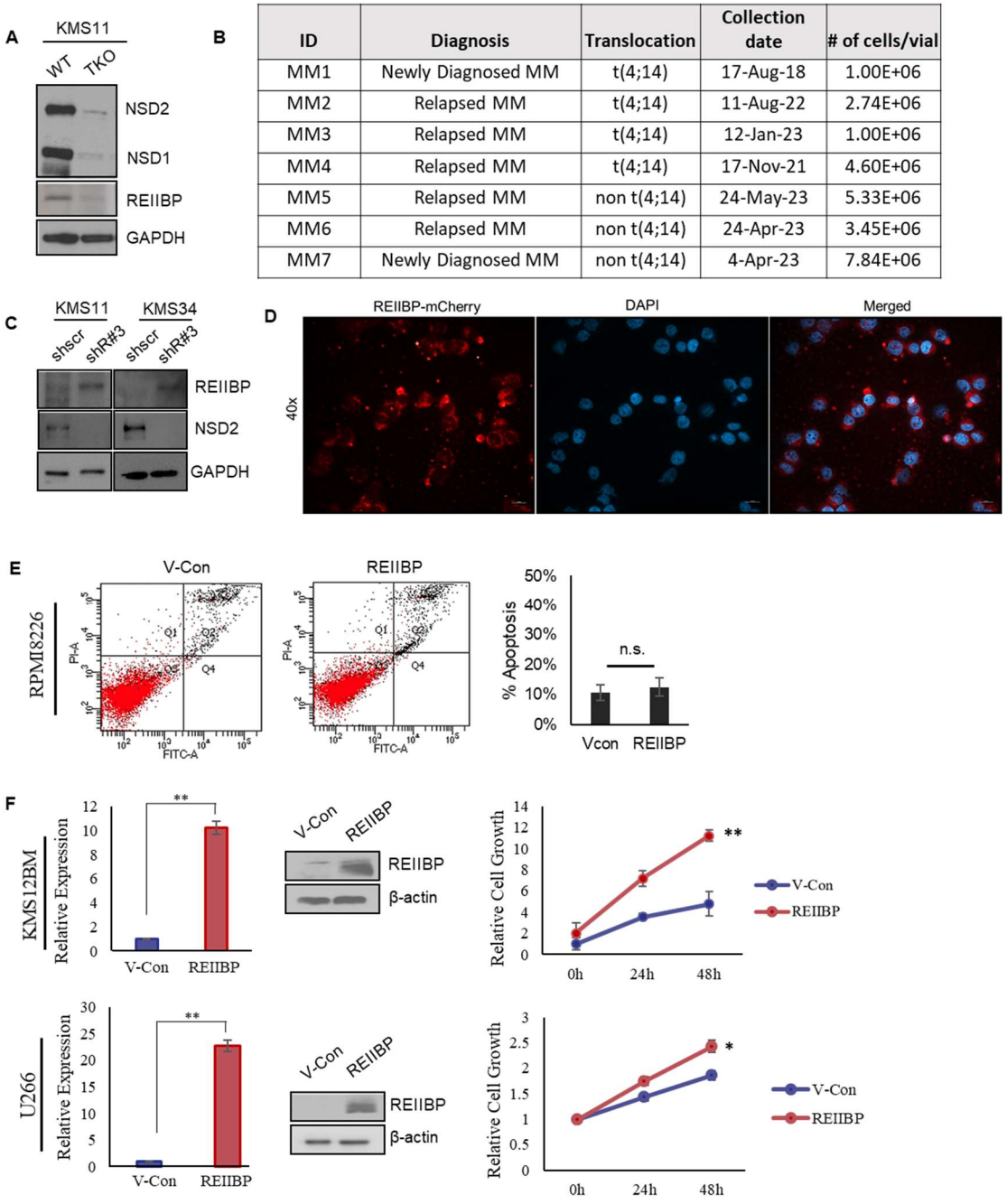
EZ-Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Sigma-Aldrich) was used to perform RNA immunoprecipitation using eIF3E or IgG (negative control) antibodies to evaluate binding to TLR7 and BTK 3'UTR mRNA with real-time RT-PCR. The TLR7 3' UTR primers used were F: 5'-CCAGAACTCCTTGTCATCAC-3' and R: 5'-AGGCAGGAGGACAGGAGT-3' and BTK F: 5'-CCTGGATGAACTGGGTGTTT-3' and R: 5'-TCTTCCCGGTCCTTCCAC-3'. The abundance of TLR7 and BTK mRNA were calculated as relative enrichment based on 10% input and normalized to housekeeping gene actin mRNA, plotted as fold change over IgG control.

Polysome profiling and qRT-PCR

10 million RPMI8226-VCon and RPMI8226-REIIBP cells were harvested for each polysome run in biological duplicates at a confluency of ~60% in log-phase growth. Polysome extraction and profiling were performed as previously described (Rahim AB, Vardy LA. Methods Mol Biol. 2016;1341:143-55) and twelve fractions were collected for each sample. Gradient centrifugation was performed at 36,000 rpm for 1.5 hour at 8°C (Thermo Scientific Sorvall wX + Ultra Series, Rotor sw4). RNA was extracted from each fraction and 500ng was used for cDNA synthesis and qRT-PCR with TLR-7 primers as described above. GADHP is used for normalization across fractions.

Supplementary Figures

Supplementary Figure 1



ID	Diagnosis	Translocation	Collection date	# of cells/vial
MM1	Newly Diagnosed MM	t(4;14)	17-Aug-18	1.00E+06
MM2	Relapsed MM	t(4;14)	11-Aug-22	2.74E+06
MM3	Relapsed MM	t(4;14)	12-Jan-23	1.00E+06
MM4	Relapsed MM	t(4;14)	17-Nov-21	4.60E+06
MM5	Relapsed MM	non t(4;14)	24-May-23	5.33E+06
MM6	Relapsed MM	non t(4;14)	24-Apr-23	3.45E+06
MM7	Newly Diagnosed MM	non t(4;14)	4-Apr-23	7.84E+06

Figure S1. (A) Protein were extracted from isogenic cells, KMS11-WT and KMS11-TKO (translocation knockout) cells to determine the expression of NSD1, NSD2 and REIIBP. n=2, biological repeats, representative blots are shown. (B) Details of primary patient samples used for immunoblot in Figure 1C were recorded. (C) KMS11 and KMS34 cell lines harbouring full-length NSD2 were transfected with NSD2 shRNA#3 targeting sequence and the effect on protein expression of NSD2 and REIIBP were determined by immunoblot. n=2, biological repeats, representative blots are shown. (D) 40x

magnification showing the localization of REIIBP-mCherry (red fluorescence) transfected in RPMI8226 cells. Nuclei were stained with DAPI. Image is a representative field. (E) RPMI8226 cells were transiently transfected with vector control (V-con) or REIIBP construct and FACS analysis performed for Annexin V-FITC/PI staining. The percentage of apoptotic fraction defined as Q2+Q4 were plotted. Mean ± SEM from 3 independent experiments are shown in the graph (n.s. not significant, student's t-test). (F) KMS12BM and U266 cells were transiently transfected with REIIBP construct and confirmed using qRT-PCR and WB. Cell viability was determined over 48 hours using CTG assay and plotted as fold change to 0 hours. Mean ± SEM from 3 independent experiments are shown in the graph. Asterisks represent significant differences (*$p < 0.05$, **$p < 0.01$, student's t-test).

Supplementary Figure 2

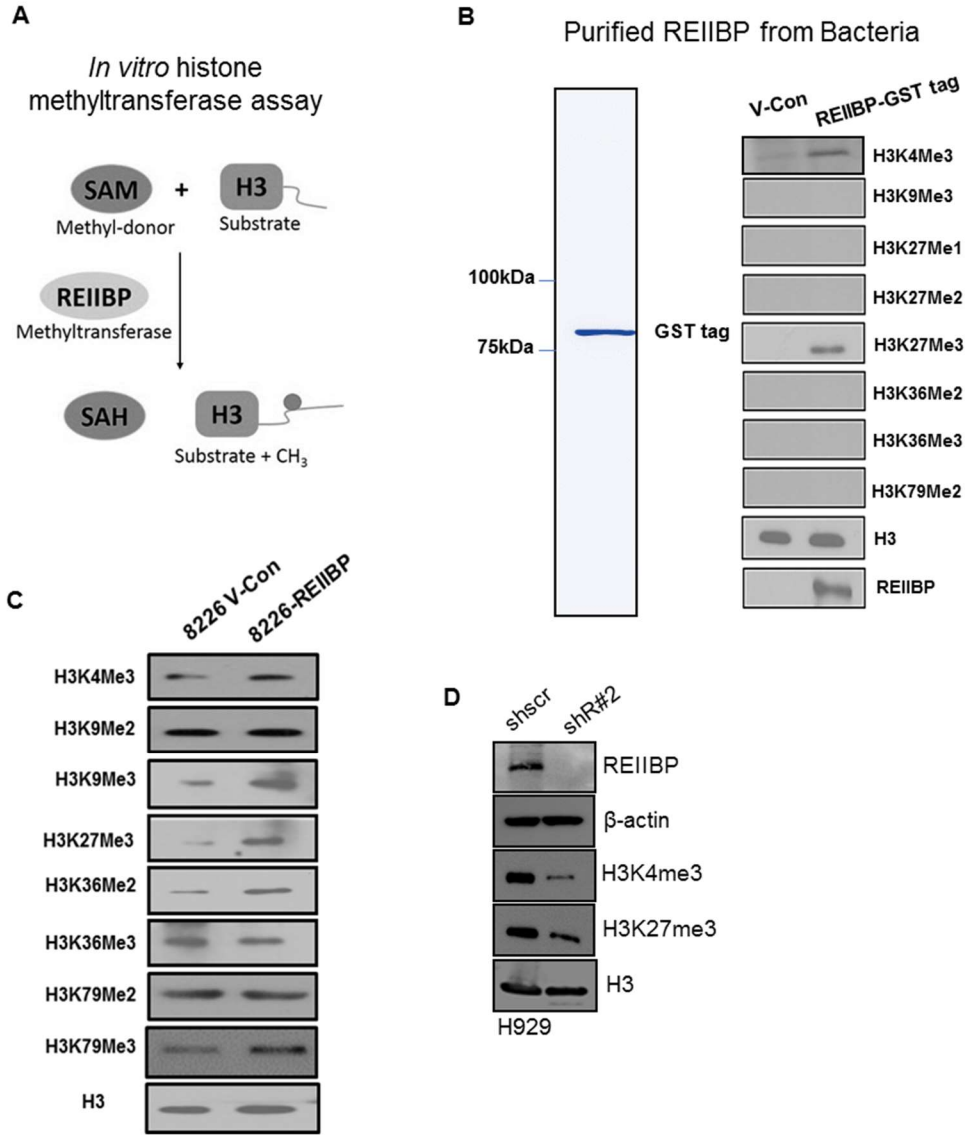


Figure S2. (A) Diagrammatic representation of the *in vitro* methylation assay with S-adenosyl-L-methionine (SAM) was shown. (B) Purified REIIBP methyltransferase from E.coli was added to SAM and H3 substrate in PKMT buffer. Western blotting was performed with the indicated antibodies. (C) 50µg of protein lysate from RPMI8226-Vector Control (V-Con) and RPMI8226-REIIBP cells were used for Western Blotting with the indicated antibodies. H3 is the loading control. (D) H929 cells were transfected with scrambled shRNA or NSD targeting shRNA#2 (from Figure 1D) for 48 hours and immunoblotting with the indicated antibodies.

Supplementary Figure 3

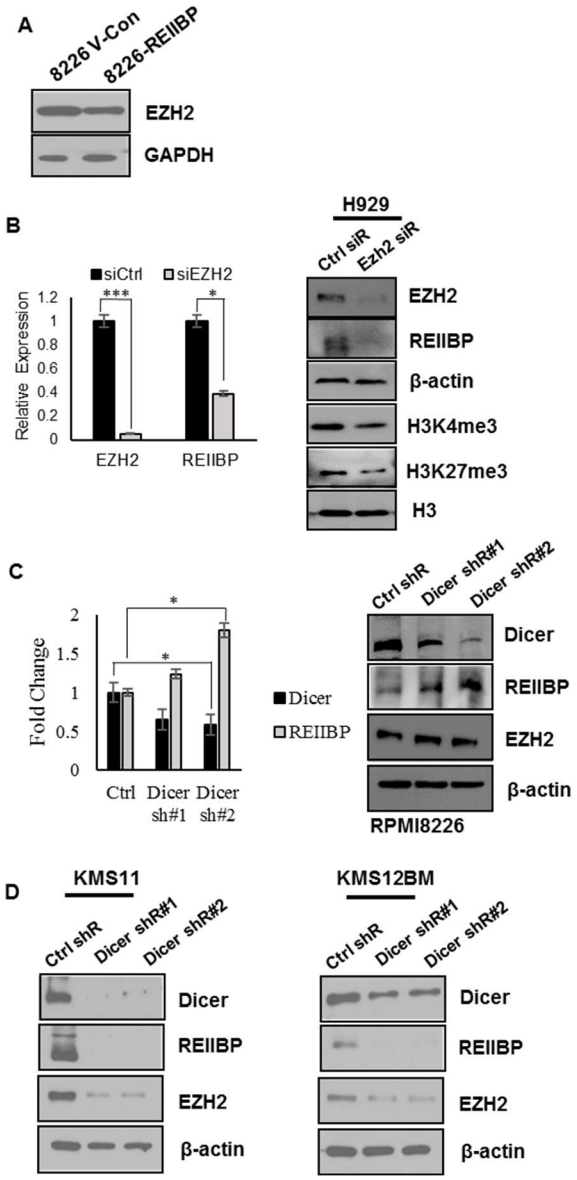


Figure S3. (A) 50µg of protein lysate from RPMI8226-Vector Control (V-Con) and RPMI8226-REIIBP cells were used to check for EZH2 levels. GAPDH is the loading control. n = 2, independent replicates. (B) H929 cells were transiently transfected with siCtrl or siEZH2 for 48hrs and the mRNA (left) and protein (right) levels of REIIBP and its associated histone marks were examined. n=2, asterisks represent significant differences (*$p < 0.05$, ***$p < 0.001$, student's t-test). (C) Two independent Dicer shRNAs were transiently transfected into RPMI8226 cells and the expression of REIIBP is determined by qRT-PCR and WB, n=2. (D) Two independent Dicer shRNAs were transiently transfected into KMS11 and KMS12BM cells and probed for the respective proteins. WB was used to assess knockdown efficiency at 48 hours. β-actin serves as the loading control.

Supplementary Figure 4

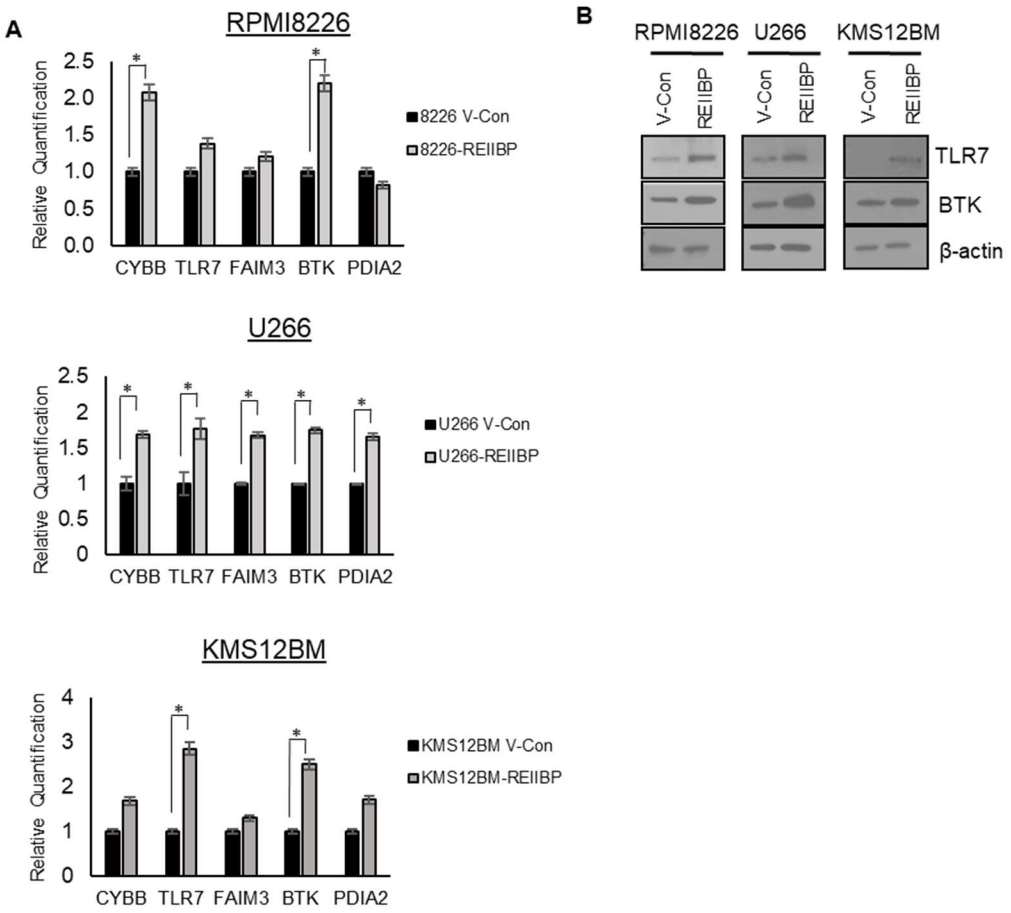


Figure S4. (A) Vector Control (V-Con) and REIIBP overexpression plasmid were transiently transfected into RPMI8226, U266 and KMS12BM to check the mRNA levels of five upregulated genes from microarray dataset, namely *CYBB, TLR7, FAIM3, BTK* and *PDIA2*. Asterisks represent significant differences (*$p < 0.05$, student's t-test) and adjusted for multiple testing. (B) Vector control or REIIBP overexpression vector were transiently transfected into RPMI8226, U266 and KMS12BM to determine the protein levels of TLR7 and BTK after 48 hours. β-actin is the loading control.

Supplementary Figure 5

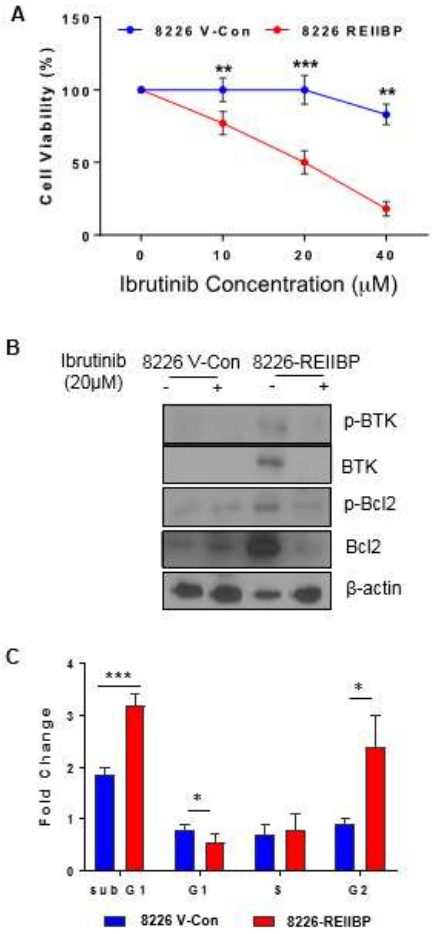


Figure S5. (A) RPMI8226 cells stably expressing control or REIIBP plasmid were treated with increasing concentrations of Ibrutinib (0, 10, 20, 40 μM) and cell viability was determined at 48 hours. REIIBP-overexpressing cells were more sensitive to Ibrutinib as compared to control. Mean ± SEM from 3 independent experiments are shown in the graph. (B) RPMI8226 cells stably expressing control or REIIBP plasmid were treated with or without 20 μM Ibrutinib and probed with the indicated antibodies. Treatment with Ibrutinib reduces BTK and p-BTK, and pro-survival protein Bcl2. (C) RPMI8226 cells stably expressing control or REIIBP plasmid were treated with 20 μM Ibrutinib and cell-cycle fractions was determined using flow cytometry at 48 hours. RPMI8226-REIIBP cells showed increased sub-G1 population indicating increased apoptosis. Mean ± SEM from 3 independent experiments are shown in the graph. Asterisks represent significant differences (*$p < 0.05$, ***$p < 0.001$, student's t-test).

Supplementary Figure 6

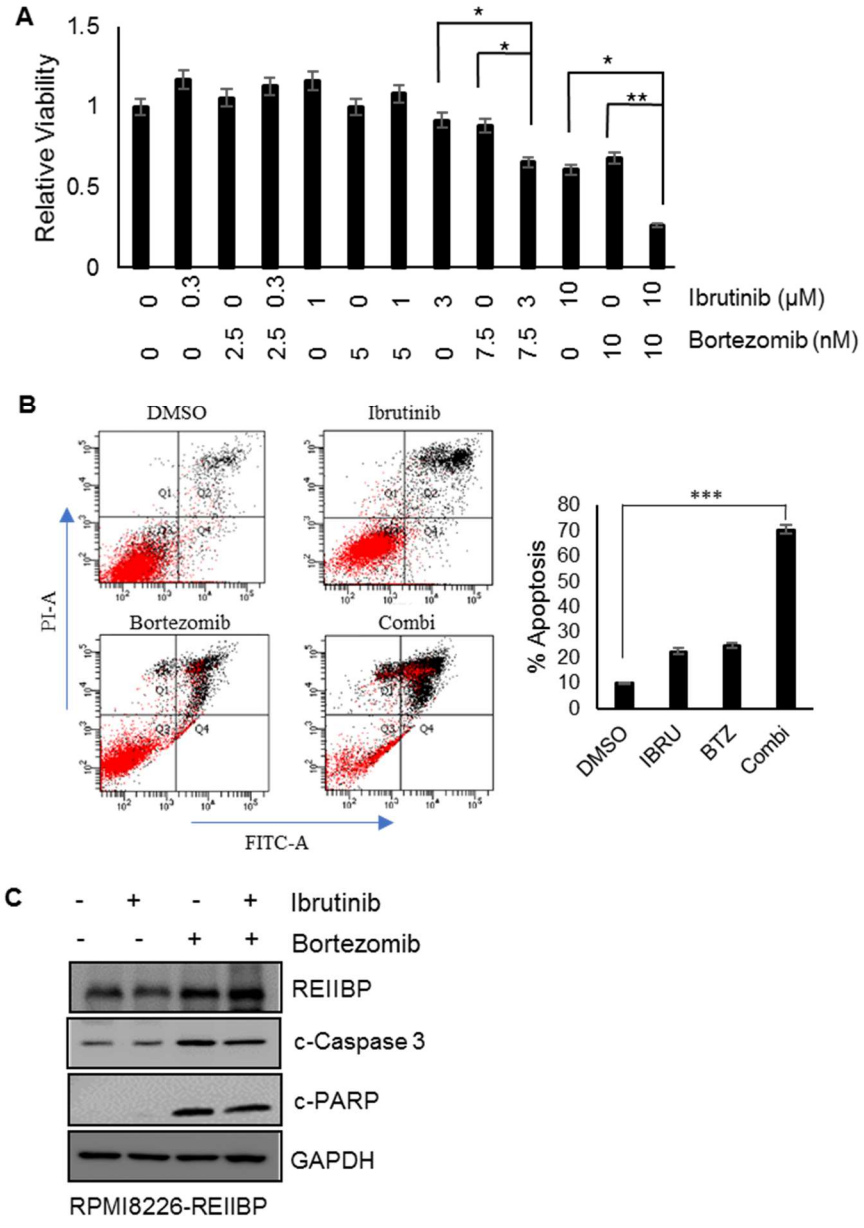


Figure S6. (A) RPMI8226-REIIBP cells were treated with single or combination of fixed-ratio concentrations of Ibrutinib and Bortezomib and viability was determined at 48 hours. Experiments were performed with three biological repeats and a representative experiment was shown. (B) RPMI8226-REIIBP cells treated with DMSO, 10 μM Ibrutinib, 10 nM Bortezomib or combination were subjected to Annexin V-PI flow cytometry to determine apoptosis. The percentage of apoptotic fraction (defined as Q2+Q4) were plotted. (C) The expression of apoptotic markers such as cleaved caspase-3 and cleaved PARP is determined by Western Blotting in RPMI8226-REIIBP cells treated with DMSO, 10 μM Ibrutinib, 10 nM Bortezomib or combination after 48 hours. The treatments had no effect on REIIBP expression. GAPDH is the loading control.

Supplementary Figure 7

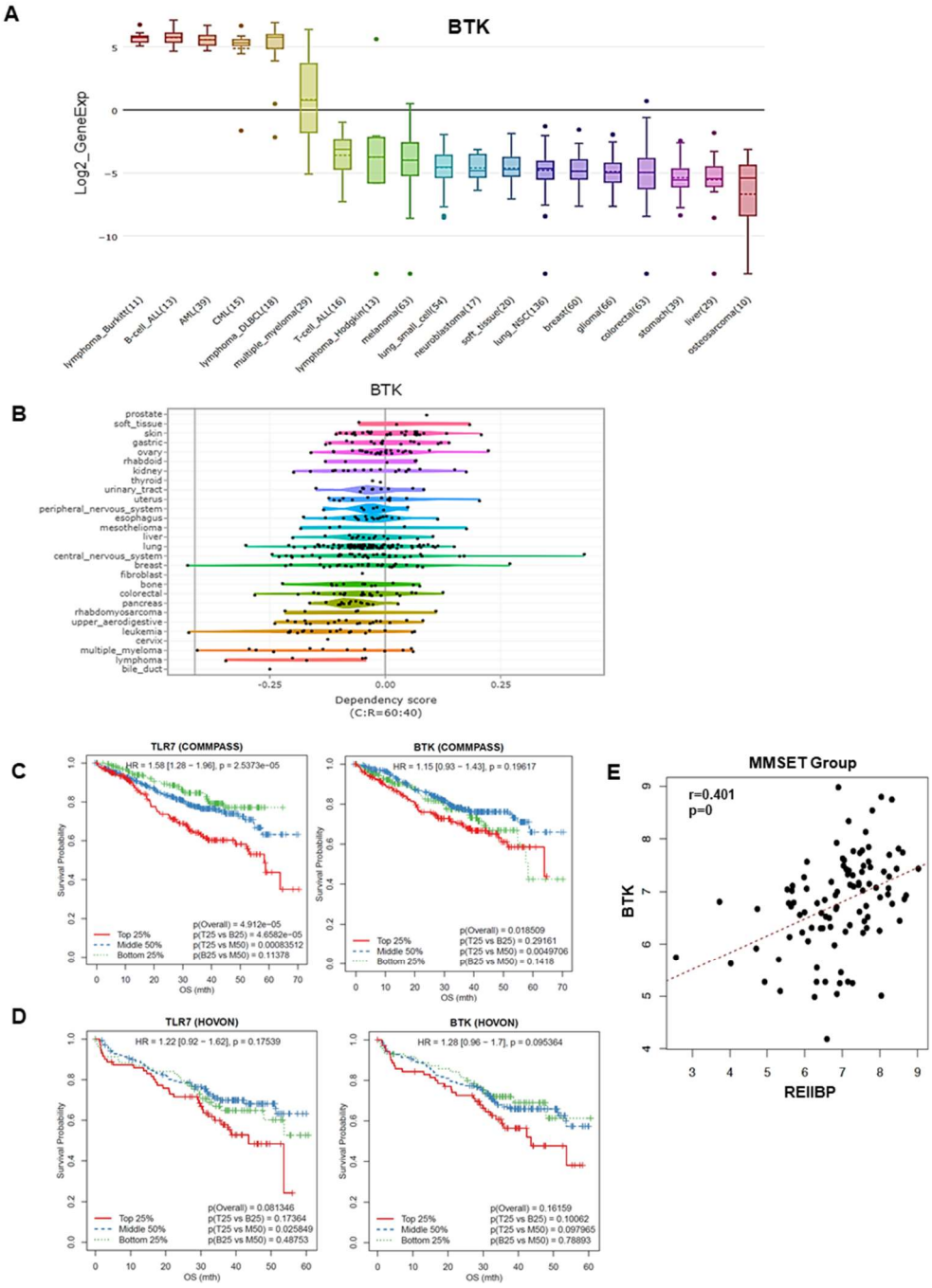


Figure S7. (A) Box plot of BTK expression values from Cancer Cell Line Encyclopedia (CCLE) and (B) scatterplot of BTK dependency scores across tissues were performed with Broad Institute's Cancer Dependency map. Survival analysis using Cox regression & Kaplan-Meier curves were performed on TLR7 and BTK in the (C) CoMMpass and (D) HOVON datasets. (E) The correlation between REIIBP and BTK in CoMMpass dataset was performed by first assigning REIIBP out of 31 registered MMSET transcripts. Correlation between REIIBP and BTK is significant in MMSET t(4;14) group (r=0.401, p =0).

Supplementary Figure 8

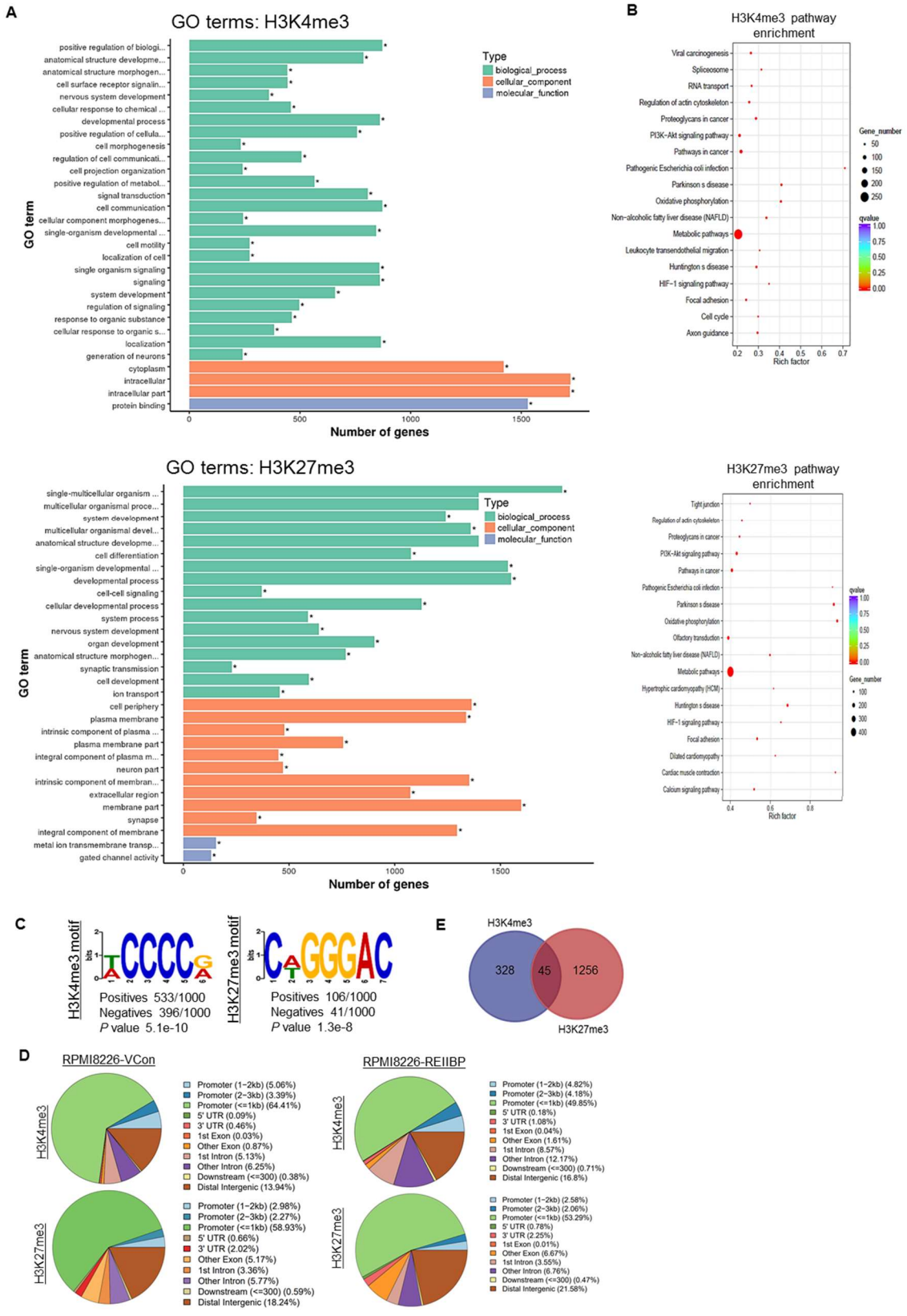


Figure S8. (A) Gene Ontology analysis was performed on differential peaks of H3K4me3 or H3K27me3 in RPMI8226-REIIBP versus RPMI8226-VCon cells. (B) KEGG pathway analysis was conducted on differential peaks of H3K4me3 and H3K27me3 from REIIBP vs Vcon samples and shown as dot plot. (C) Top enriched H3K4me3 and H3K27me3 motifs were shown from analysis using DREME motif discovery. (D) Genomic distribution of H3K4me3 or H3K27me3 peaks normalized to their respective inputs were compared between REIIBP vs Vcon samples using ChIPseeker. Peak calling was done using MACS2 and epic2. (E) Venn diagram with genes showing differential occupancy of H3K4me3 and/or H3K27me3 between REIIBP and VCon revealed an overlap of 45 doubly-marked genes.

Supplementary Figure 9

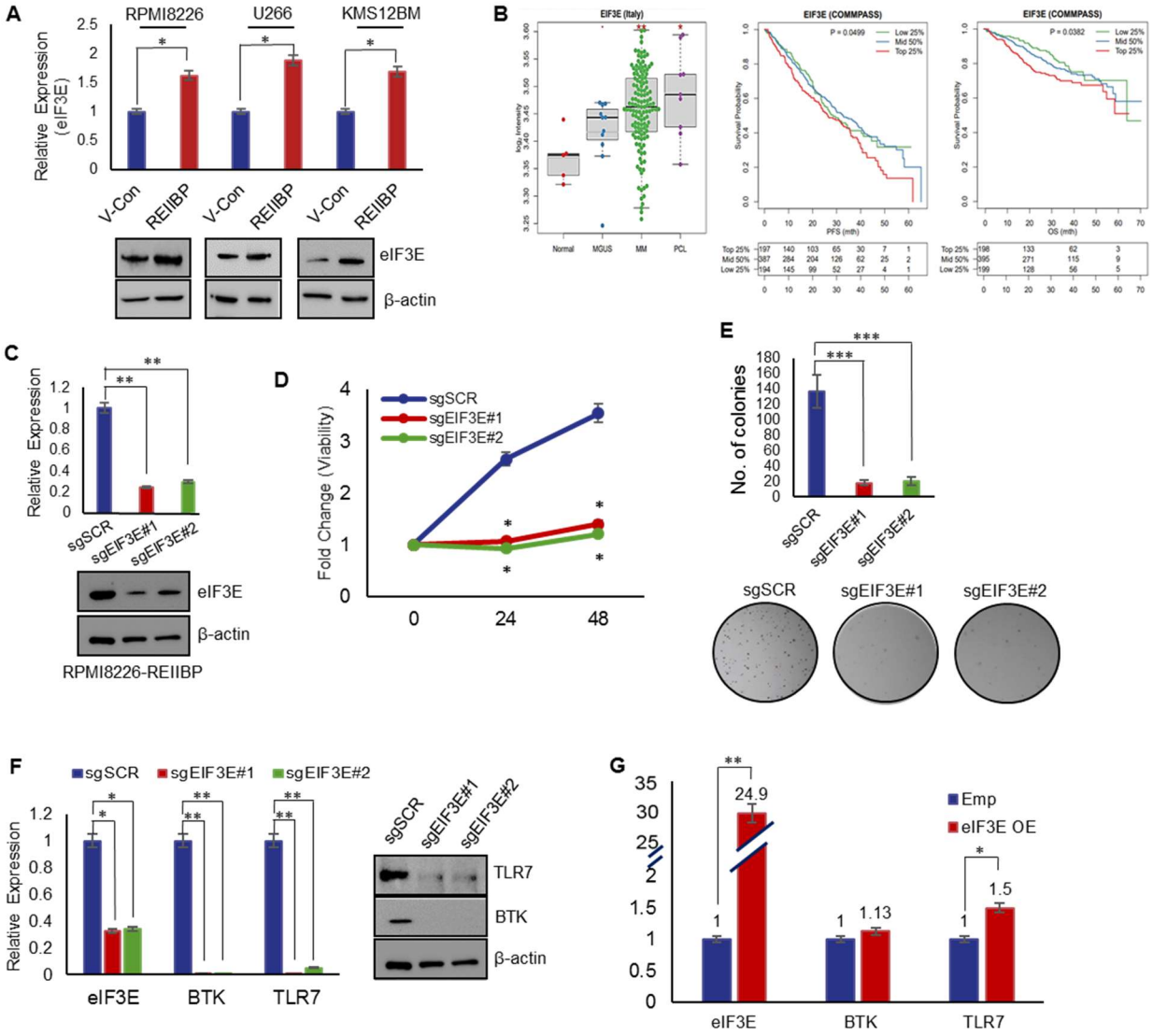


Figure S9. (A) Transient expression of REIIBP in three HMCLs RPMI8226, U266 and KMS12BM. The levels of eIF3E mRNA (n=3) and protein (n=2) were determined. Student's t-test was used to determine significance between REIIBP overexpression and its corresponding control (*, $P < 0.05$). (B) The expression of eIF3E is plotted over disease progression in Italy patient dataset and highest expression was noted in the MM and PCL groups. The expression of eIF3E was segregated into top 25%, middle 50% and low 25% and progression-free survival (PFS) and overall survival (OS) were plotted. High expression of eIF3E is significantly associated with poor survival (P<0.05). (C) Two single-guide RNAs targeting eIF3E CDS were transiently transfected into RPMI8226-REIIBP cells and qRT-PCR (n=3) and WB (n=2) were used to measure knockdown levels. Asterisks represent significant differences (**, P < 0.01; Student's t-test). (D) Viability at 24hrs and 48hrs post-transfection with eIF3E sgRNAs were determined. CTG assay was performed for three biological repeats with technical octuplicates, *, P < 0.05; Student's t-test. (E) Colony-forming assay was performed and colonies counted at 14 days after seeding (n=3, representative field shown, ***$p < 0.001$, student's t-test). (F) Knockdown of eIF3E was done with two sgRNAs and qRT-PCR (n=3) was performed at 24 hours post-transfection or WB (n=2) at 48 hours post-

transfection to check for TLR7 and BTK levels. Asterisks represent significant differences (*, $P < 0.05$; **, $P < 0.01$, Student's t-test). (G) Overexpression of eIF3E increases TLR7 and BTK (n=3, independent repeats). Asterisks represent significant differences (*, $P < 0.05$; **, $P < 0.01$, Student's t-test).

Supplementary Figure 10

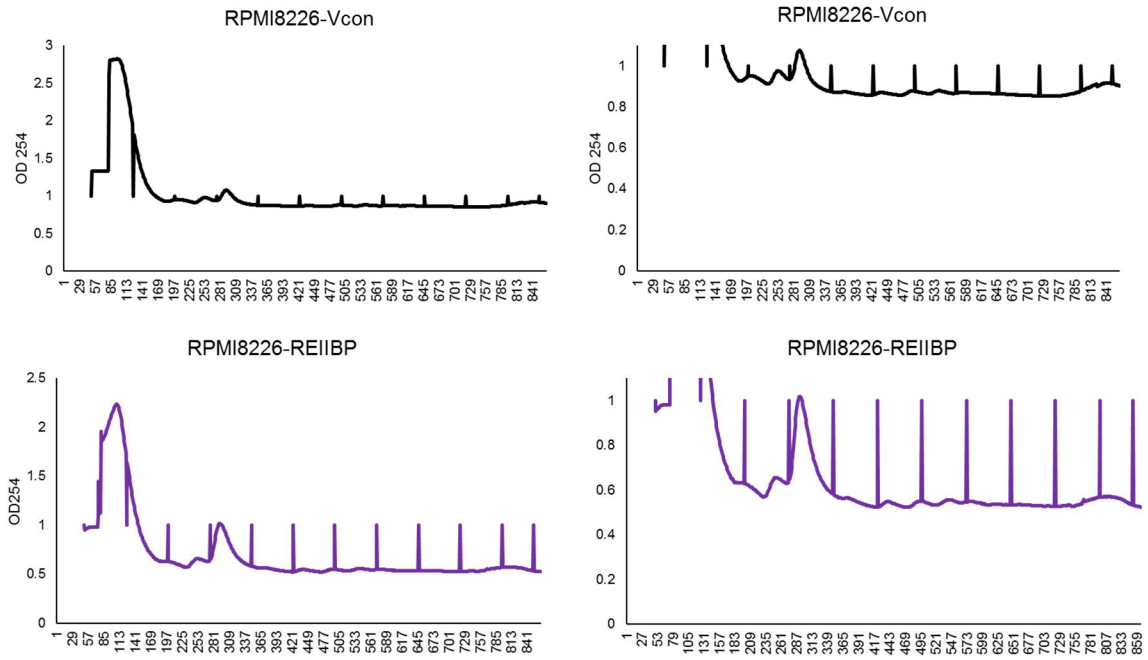


Figure S10. Representative polysome profiles of RPMI8226-VCon (top) and RPMI8226-REIIBP (bottom) performed in biological duplicates. Twelve fractions were collected for qRT-PCR.

Supplementary Tables (see Excel files)

Table S1. Full list of significant genes with fold-change > 1.3 from microarray data of RPMI8226-VCon and RPMI8226-REIIBP

Table S2. A list of genes with differential occupancy of H3K27me3 and H3K4me3 histone marks between RPMI8226-VCon and RPMI8226-REIIBP