The innate sensor ZBP1-IRF3 axis regulates cell proliferation in multiple myeloma

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Supplementary methods

Cloning and lentiviral transduction

The puromycin selection gene was replaced with green fluorescent protein (eGFP) cDNA in Lentiviral pLKO.1 plasmid (Sigma). shRNA oligos (Sigma) were annealed by temperature ramp from 100°C to 25°C and cloned into pLKO.1 vector between AgeI and EcoRI sites. Doxycycline-inducible shRNAs were established using retroviral TRMPVIR vector (kind gift from Scott Lowe, Addgene plasmid #27994) as previously described.\(^1\) ZBP1-full cDNA construct was kindly gifted by Stefan Rothenburg, University of California, USA.\(^2\) The ZBP1 cDNA was amplified using forward (Forw): GGGAAATTCATGGCCCAGGCTCCTGCT and reverse (Rev): TAGCGGCCGCCTAAATCCCACCTCCCCA primers from pEGFPN.1 vector cloned into LeGO-iG2-ires-EGFP vector (Addgene plasmid #27341) between EcoRI and NotI sites followed by 5 repeats of strep-tag II (TGGAGCCATCCGCAGTTTGAAGAAA) sequences insertion between BamHI and EcoRI sites. ZBP1 ∆RHIM vector was constructed by amplifying strep-tagII-ZBP1 using forward: CCGGAATTAGGATCCATGTGGAGCCATCCG and reverse: TAGCGGCCGCTAGGCTGACTTTGCTCTTC from LeGO-strep-tagII-ZBP1 full vector. The genetic rescue experiment was performed by co-expression of ZBP1 cDNA with silent mutation at shRNA1 binding regions 5′-CAAGAGGAGCTAACTCGAGTTATATAAGATGAAGAAGGAGTGAAGTCTCCCT-3′ (here gray indicates sh1 ZBP1 target site and bold indicates mutated sequences) using LeGO-iC2 (mCherry) plasmid (addgene #27345) and anti-ZBP1 shRNA1 in MM.1S cells.

All viral particles were produced by calcium phosphate co-transfection of pRSV.REV, pMDLgpRRE and pMD2.VSVG (lentiviral) plasmids in HEK293T cells and concentrated by ultracentrifugation at 23,000 rpm for 100 minutes at 4°C. Myeloma and non-myeloma cells were treated with 8µg/ml polybrene (Sigma) and transduced with lentivirus by spinoculation at 2000 rpm, 37°C for 1hr followed by replacement of polybrene-media with appropriate culture media 24hr post-spinoculation.

shRNA sequences (5′-3′)
scramble (scr): GGCCCTCCCATCACAGTCTATA
sh1 ZBP1: CCAAGTCTCTACCGAATGAA
sh2 ZBP1: GCACAATCCAATCAACATGAT
sh3 ZBP1: GCGGATCAATAGGTCAGGAAA
sh1 IRF3: GATGAGCTACGTGAGGCATGT
sh2 IRF3: CCGCTTACCTGAGAATCTGATT
sh1 TBK1: GCAGAACGTAGTTAGCTTAT
sh2 TBK1: GCGGCAGAGTTAGGTGAAATT
sh1* ZBP1 seed control: CCAAGTCTCTCGCAATGAA

TRMPVIR-sh1 ZBP1 (5’-3’)
TCGAGAAGGTATATTGCTGTTGACAGTGAGCGCCAAGTCTCTACCGAATGAAATAGTGAA
GCCACAGATGTATTTTATCTCDGTAGAGACTTGCTCTACTGCCCTCGG

TRMPVIR-sh2 ZBP1 (5’-3’)
TCGAGAAGGTATATTGCTGTTGACAGTGAGCGCCAAGTCTCTACCGAATGAAATAGTGAA
GCCACAGATGTATTTTATCTCDGTAGAGACTTGCTCTACTGCCCTCGG

qPCR primers (5’-3’)
following primers used for ChIP and ChIP-re-ChIP
Negative: ACAGCCTAGGCCCATGGATTT (Forw) and CAGCGTCCCTCATCCAGTTC (Rev)
Peak 1: TCTCAGTTTTCACCGCTCGAT (Forw) and TCCTCTCTGATACGCTCTA (Rev)
Peak 2: TCTGACCGTGCCCACTTTAG (Forw) and AACCTCCAACCTGTGACT (Rev)
Peak 3: TGTTTGGAAGGCCACCCAGAT (Forw) and CGCTCTTACACACCACCGACTG (Rev)
E2F1: GTCTCGACTGCACCGACTTC (Forw) and GATCCGAATTTCGCGGCAC (Rev)
E2F2: GTCTCGACTGCACCGACTTC (Forw) and GATCCGAATTTCGCGGCAC (Rev)
MCM2: CTCCGTGTCCCTTCTGGTCG (Forw) and ACGATCCTCTCCGCCACTAC (Rev)
MCM3: AATCTCTCTGAGCCTCCCGC (Forw) and GTTCGGAAGTTTTCGCGCC (Rev)
MCM4: CCCTAGCACAAGCTAGAGGA (Forw) and CCCGTCGCATAACCAGAATGTA (Rev)
MCM5: GTTTTTCCCGCAACAACCTCGG (Forw) and CCAACTACACCGGAAATCCA (Rev)

Taqman probe for mouse Zbp1: Mm01247052_m1
Human ZBP1: GCCAACAACGAGGAAGA (Forw); ATCTTCTGGGCTGAAATCGT (Rev)
Human E2F1: GGACTCTTCGAGAATTTCA (Forw); TGGTGGTGACACTATGG (Rev)
Human Ki-67: CGTCCCAGTGGAAGAGTTGT (Forw); CGACCCCGCTCCTTTTGATA (Rev)
Human FOXM1: TCTTTTCTTTATGCTGCGC (Forw); CCCAGGCTGGATTTCTTCC (Rev)

Antibodies for Co-IP
anti-ZBP1 (ThermoFisher Scientific; catalog number: PA5-20455), anti-V5-Tag (ThermoFisher Scientific; catalog number: 37-7500), anti-IRF3 (BioLegend; clone number: 12A4A35).

Total cell lysis buffer
250mM NaCl, 1.5mM MgCl₂, 20mM HEPES pH 7.4, 0.5mM EDTA, 1% IGEPAL CA-630, 1% Triton X-100, 0.1% SDS, 10mM of PMSF (Sigma) supplemented with 1x halt protease & phosphatase Inhibitor cocktail (Fisher scientific).

Antibodies for immunoblot
anti-ZBP1 (1:1000; ThermoFisher Scientific; catalog number: PA5-20455), anti-ZBP1 (1:1000; SantCruz Biotech; sc-67259), anti-IRF3 (1:500; BioLegend; clone number: 12A4A35), anti-TBK1 (1:1000; SantCruz Biotech; sc-52957), anti-pTBK1(ser172) (1:250; Cell Signalling Technolog; catalog number: 5483), anti-V5-tag (1:500; Cell Signalling Technology; catalog number:132025), anti-pIRF3(S396) (1:500; ThermoFisher Scientific; Catalog number: 720012), GAPDH (1:2000; SantCruz Biotech; sc-47724).

Immunization
Zbp1⁻/⁻ animals,³ already cross-bred to C57BL/6 animals for 4-5 generations were obtained from Manolis Pasparakis, Institute of Genetics, Cologne, Germany. They were further cross-bred with wild type C57BL/6 mice for another three generations and their littermates used to study T-cell dependent humoral immune response to 4-Hydroxy-3-nitrophenylacetyl hapten conjugated to Keyhole Limpet Hemocyanin (NP-KLH) antigen (Santacruz Biotech). 6mg/kg NP-KLH prepared in Imject™ Alum Adjuvant (Thermoscientific) 3:1 ratio and injected intraperitoneally into 10-12 weeks old age-
matched Zbp1−/− and wild type littersmates. On day 4 post-immunization, 4mg/kg NP-KLH alone injected as booster dose and after 10 days post-immunization, blood samples were collected, and spleen was harvested. Single cell suspension of spleen cells were stained for B220 (BioLegend; clone: RA3-6B2), CD19 (BioLegend; clone: 6D5), CD95 (eBioscience; clone number: 15A7), GL7 (BioLegend; clone;GL7), CD138 (BioLegend; clone: 281-2) and analyzed for germinal center activated B cells (GCB), plasma cell (PC) development. GCB (B220+ CD19+ GL7+ CD95+) and PC (B220loCD138+) spleen cells were sorted using (FACSAria), and total RNA was isolated and quantified Zbp1 mRNA levels as described in qPCR methods. A standard ELISA method was used to quantify NP-KLH-specific IgG or IgM antibodies. Diluted (1:1000) serum samples used to detect levels of IgG by anti-IgG-HRP antibody (Bio-Techne) or IgM by anti-IgM-HRP antibody (Sigma) on 100μg/ml NP-KLH-coated plates. The antibody levels of immunized mice sera were normalized to their appropriate control alum-only immunized mice sera.

**ChIP-seq and ChIP-re-ChIP**

MM.1S cells were cross-linked with 1% formaldehyde (Alfa Aesar) at 10⁶ cells/ml density for 15min at room temperature with gentle mixing followed by addition of 0.125M Glycine to final volume for 5min at room temperature with gentle mixing. Cells were washed thrice with ice cold 1x PBS with 10mM phenylmethylsulfonyl fluoride (PMSF) and 10⁸ cells were lysed with hypotonic lysis buffer (10 mM Hepes-KOH, pH 7.8, 10 mM KCl, 0.1 mM EDTA, and 0.1% IGEPAL CA-630) for 15minutes on ice followed by centrifugation at 5000g for 5min. Further the cell pellet was lysed in nuclear lysis buffer (1% SDS, 50mM Tris-HCl pH 8.0, 10mM EDTA pH 8.0, 300mM NaCl supplemented with 1x halt protease & phosphatase Inhibitor cocktail (Fisher scientific) for 15min on ice. The lysate was diluted 10 times with dilution buffer (0.01% SDS, 1% Triton X-100, 1mM EDTA, 50mM Tris-HCl pH 8.0, 150mM NaCl) and sonicated to shear the chromatin DNA up to 500bp size. The lysates were precleared with 50 μl protein A/G magnetic beads (Life Technologies) and then IRF3-bound chromatins were pulled overnight, rotating at 4°C with either 5μg IRF3 antibody (BioLegend, clone:12A4A3S) or equivalent isotype control conjugated with protein A/G magnetic beads. Immunoprecipitated beads were washed twice with wash buffer A (0.1% SDS,
1% TritonX-100, 1mM EDTA, 10mM Trish-HCl pH 8.0, 150mM NaCl), buffer B (0.1% SDS, 1% TritonX-100, 1mM EDTA, 10mM Trish-HCl pH 8.0, 500mM NaCl) and buffer C (0.25M LiCl, 1% IGEPAL CA-630, 1% sodium deoxycholate, 1mM EDTA, 10mM Tris-HCl pH 8.0) for 5min, rotating at 4°C. The ChIP complex was treated with 10mg/ml RNase A, 20mg/ml proteinase K and reverse crosslinked with a buffer containing 1% SDS, 50mM Tris HCl pH 8.0, 4M NaCl, 1mM EDTA at 65°C overnight. ChIP DNA was collected with Ampure XP beads (Beckman) and quantified using Qubit High Sensitivity DNA kit (Life Technologies). 1ng of ChIP DNA was taken to prepare library using NEBNext kit for Illumina (New England Biolabs) following manufacturer’s instructions and the quality or fragment size was assessed using the Bioanalyser High Sensitivity DNA kit (Agilent). 2nM of 400-500bp DNA library was sequenced using Illumina NextSeq500 platform to obtain paired-end 150bp reads.

For ChIP-reChIP, above protocol to pull IRF3 or IRF4-bound chromatin using IRF3 antibody (BioLegend, clone:12A4A35) or IRF4 antibody (BioLegend, clone: IRF4.3E4) and their equivalent isotype control respectively was followed. The chromatin was eluted in 1% SDS with 1x halt protease & phosphatase Inhibitor cocktail (Fisher Scientific) followed by 10 times dilution with elution buffer and repeated ChIP with the appropriate antibody. The ChIP-reChIP DNA was quantified using Qubit High Sensitivity DNA kit (Life Technologies) and quantified specific DNA fragments by qPCR.

**Immunohistochemistry**

Immunohistochemistry was undertaken on one-micron formalin-fixed paraffin-embedded tissue sections on Leica Bond III automated immunohistochemistry stainer. Prior to the procedure, paraffin sections were placed in oven preheated to 60°C for 30 minutes. Ready to use antibodies were used for PAX5 (clone number: PA0552; 15 minutes incubation) and MUM.1 (IRF4) (clone number: PA0129; 15 minutes incubation) antigens. Antibodies for ZBP1 (Sigma-Aldrich; catalogue number: HPA041256; 20 minutes incubation), CD3e (clone: NCC-L-CD3-565; 20 minutes incubation) and CD21 (clone: NCC-L-CD21-269; 20 minutes incubation) were diluted 1:100. 1:100 and 1:25 respectively prior to incubation. Heat induced epitope retrieval was undertaken for 20 minutes (PAX5 (EDTA buffer), MUM.1 (EDTA buffer), ZBP1 (citrate buffer) and CD21 (citrate buffer)) and 30 minutes (CD3e (citrate buffer)).
Signal detection for single immunostains was performed using Bond Polymer Detection Kit (clone number: DS9800) with DAB (brown colour) as the chromogen. For double immunostains, the sections were initially stained for CD3e, CD21, MUM.1 or PAX5 antigens as for single immunostaining protocol. This was followed by a sequential step for staining for ZBP1 antigen, where is the ZBP1 signals were detected using Bond Polymer Refine Red Detection Kit (Red signals; catalogue number: DS9390).

**RNA-seq and ChIP-seq data analysis**

For RNA sequencing analysis, reads were aligned and transcripts were quantified using STAR (v2.5.3a), for shRNAs targeting ZBP1 in MM.1S and H929 cells; related to figure 2, against GRCh38 release 79 or with Salmon (v0.12.0), for shRNAs targeting ZBP1 or IRF3 in MM.1S; related to figure 4, against GRCh38 Gencode v28 transcript annotations. Differential expression analysis was performed in R (R Core Team, 2020) with DESeq2 (v1.24.0) from STAR output or Salmon output using tximport (v1.12.3), and limma-voom for processing CCLE data with cut off p adj <0.05. IRF3 ChIP sequencing reads were aligned with BWA MEM (v0.7.15) to GRCh38 genome (Gencode v28) with standard settings. QC and duplicate marking were performed with Picard (v2.6.0) and samtools (v1.2). Tracks were generated with Deeptools (v3.3.1), and peaks were called with MACS2 (v2.1.1). Motif enrichment was performed with Homer (v4.10). The peaks were visualized using Integrative Genomes Browser (IGV) (v2.5.2). Binding and Expression Target Analysis (BETA)-plus package (v1.0.7) was used to integrate IRF3 cistrome, with the peaks within 2kb distance to TSS, and a complete transcriptome of IRF3-depleted MM.1S cells with cut off padj <0.05. Bigwig and BED files of ATAC-seq and Pol II, H3K27ac, H3K27me3, H3K4me3, H3K4me2, H3K4me1 and IRF4 ChIP-seq files of MM.1S cells were collected from Cistrome Data Browser.

Bedtools (v2.25.0) Intersect was used to identify the common genome-wide binding of IRF3 and IRF4 factors. Deeptools computeMatrix and plotHeatmap (v3.4.1) with 2kb distance in reference to center of the region were used to visualize genome-wide binding of histone marks, Pol II, with IRF3, and IRF4 transcription factors. Homer (v4.10) was used for annotation of the genomic regions that are plotted in the
heatmap. Gene Set Enrichment Analysis (GSEA) (v4.0.3) software\textsuperscript{14} was used for pathway annotation of differentially regulated genes with p adj < 0.05 to analyse the pathways of Hallmark gene sets. Enrichr online web tool\textsuperscript{15} was used for pathways enrichment analysis of differentially regulated genes from RNA-seq and output of BETA-plus for integration of ChIP-seq and RNA-seq data. Significant pathways enriched with p adj < 0.05 were selected to create the figures and listed in the tables.

**Statistical analysis**

Data graph and statistical analysis were performed using GraphPad Prism 8.0 software under institute licence. All experiments were repeated at least three times except for RNA-seq and ChIP-seq which were performed in replicates. Fold changes for *in vivo* data that were obtained in different time points were calculated by comparing the immunized groups to median value of control group. Comparison of two groups were performed using two-tailed unpaired Student t-test. All the information on sample size, replicates, statistical method and significance are indicated in the figure legends. GraphPad Prism 8.0 or Morpheus (https://software.broadinstitute.org/morpheus) was used for heatmap creation with log2 transformed values of RNA-seq data.

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**Supplementary Figure S1 (related to Figure 1)**

(A) Heat map showing the top 30 differentially expressed genes between 27 MM and >800 non-myeloma cancer cell lines (raw data collected from the Cancer Cell Line Encyclopedia Portal (CCLE)). ZBP1 is boxed. ALL: Acute Lymphoblastic B Cell Leukaemia, BILD: Biliary Tract Carcinoma, BLCA: Bladder Carcinoma, BRCA: Breast Carcinoma, COLR: Colorectal Carcinoma, DLBC: Burkitt Lymphoma, DLHN: Lymphoma Hodgkin, ESCA: Squamous Cell Carcinoma, GBM: glioma HighGrade, HNSC: Upper Aerodigestive Tract, KIRC: Clear Cell Renal Cell Carcinoma, LAML: Acute Myeloid Leukaemia, LCML: Blast Phase Chronic Myeloid Leukaemia, LIHC: Hepatocellular Carcinoma, LGG: Glioma, LUAD: Lung Non-Small Cell Carcinoma, LUSC: Lung Squamous Cell Carcinoma, MB: Medulloblastoma, MESO: Mesothelioma, NB: Neuroblastoma, OV: Ovary Carcinoma, PAAD: Pancreas Carcinoma, PRAD: Prostate Carcinoma, SARC: Ewings Sarcoma, SCLC: Lung Small Cell, SKCM: Skin Melanoma, STAD: Stomach Gastric Carcinoma, THCA: Thyroid Carcinoma, UCEC: Endometrium Carcinoma, MM: Plasma Cell Myeloma.

(B) mRNA expression levels of ZBP1 in >800 cancer cell lines (non-myeloma) including 27 MMCL (myeloma) from CCLE data sets. Box-whiskers plot shows minimum to maximum log2 TPM values. Elaborated form of all the cell line names shown in Online Supplementary Figure S1A legend.

(C) mRNA expression level of ZBP1 in GSE4581 data set. Expression of ZBP1 is not different between normal, MGUS and myeloma PC. However, in the hyperdiploid group expression of ZBP1 is significantly higher than normal and total myeloma PC. BMPC (n=22), normal bone marrow PC; MGUS (n=44); Myeloma (n=414). Molecular subgroups: PR: proliferative; LB: lytic bone; MS: over-expression of MMSET in t(4;14); HY: hyperdiploid; CD1 and CD2: Cyclin D1 and D2 over-expression groups; MF: MAF over-expression in t(14;16).

(D) mRNA expression levels of ZBP1 in purified myeloma PC (n=767 MM patients) compared to PC-lineage defining transcription factors IRF4, XBP1 and PRDM1 from MMRF data sets. Bar graph shows the mean values.

(E) ZBP1 mRNA expression in human common lymphoid progenitors (CLP), naïve, class switch memory (CSM) and germinal centre (GC) B cells as well as tonsillar plasma cells (data from the Blueprint DCC Portal; data shown as mean ± SEM).

(F, G) Immunoblot shows lack of ZBP1 expression in non-myeloma hematopoietic cell lines K562 (erythroleukemia), Jurkat (T cell lymphoblastic) and C1R (EBV-transformed B cell) cells, and 293T cells, and solid tumor cell lines HCC95 (squamous cell lung carcinoma), SF295 (glioblastoma), HT29 (colon adenocarcinoma), DU145 and LNCaP (prostate carcinoma) and MCF7 (breast cancer).

(H, I) ZBP1 mRNA and protein expression as assessed by qPCR (H) and immunoblotting (I) respectively in FACSPurified primary human bone marrow erythroblasts (ErythroB), peripheral blood CD14+ monocytes, CD19+ B cells, CD4+ T cells. The MMCL H929 and MM.1S are shown as positive controls.
(J) *ZBP1* mRNA expression in 53 healthy human tissues. *ZBP1* expression is only detected in organs with lymphoid tissue (data obtained from the GTEx Portal).

(K) *ZBP1* expression in lymph nodes, tonsil, and healthy bone marrow as assessed by immunohistochemistry on paraffin-embedded tissue section. Within lymph nodal and tonsillar germinal centres, *ZBP1* expression is mostly restricted to PCs. Germinal centre B cells are mostly negative. *ZBP1* is also strongly expressed in PCs outside the follicles. There is weaker *ZBP1* expression in mantle cells and other interfollicular B cells. *ZBP1* expression is mostly restricted to PCs in normal bone marrow (BM#1 & BM#2).

(L) *ZBP1* expression in tonsillar germinal centres co-stained with CD3e (T cells), CD21 (follicular dendritic cells) or MUM.1 (IRF4) as identified by immunohistochemistry on paraffin-embedded tonsil tissue section. *ZBP1* is not expressed in CD3e+ T cells or CD21+ follicular dendritic cells, but is co-expressed with IRF4 (MUM.1)+ PCs.

(M) *ZBP1* expression in bone marrow tissue sections of another two MM patients.
Supplementary Figure S2 (related to Figure 2)

*Zbp1* mRNA expression levels in murine follicular B cells (FoB), marginal-zone B cells (MZB), B1, splenic plasmablasts (splPB) and splenic and bone marrow plasma cells (splPC, BMPC) compared to PC-lineage defining transcription factors (data were reanalysed from previously published RNA-seq experiments by Shi et al., REF. #4).
**Supplementary Figure S3 (Related to Figure 3)**

(A) Schematic of ZBP1 mRNA exonic structure, positions of Za1, Za2 and RHIM domains and shRNA1 (sh1)-, shRNA2 (sh2)- and shRNA3 (sh3)-targeting sites with reference to the two main ~48 and ~40 kDa ZBP1 isoforms are shown.

(B) Immunoblotting shows shRNA1- or shRNA2-mediated depletion of ZBP1 isoforms in MMCL H929 cells on day 4 post-transduction with sh1 or sh2 or sh3 or scr control that had >90% transduction efficiency. GAPDH is shown as loading control. Two ZBP1 isoforms i.e. isoform 1 (iso-1) and isoform 2 (iso-2) are indicated.

(C) Typical example of gating strategies of flow cytometry plots (top) and applied formula (bottom) for calculation of the %GFP+ cells to analyse cytostatic effects of shRNA- or scramble-transduced cells. Live cells were determined by negative for DAPI staining.

(D) Immunoblotting for ZBP1 in MMCL MM.1S cells on day 4 post-transduction with sh1 or sh2 or scr control that had >90% transduction efficiency. GAPDH is shown as loading control.

(E) Immunoblotting for ZBP1 expression in the epithelial cancer cell line HeLa.

(F) %GFP+ cells after co-transduction of MM.1S myeloma cells with ZBP1-targeting sh1 with empty vector (mock) or ZBP1-targeting sh1 with ZBP1 cDNA with silent mutation at sh1 seed annealing sequences (ZBP1*). (n=4)

(G) ZBP1 expression assessed by immunoblotting in MM.1S cells transduced with shRNA1 targeting ZBP1 (sh1), ‘seed’ shRNA1 control (sh1*) or scr control.

(H) %GFP+ cells after transduction with ZBP1-targeting sh1, ‘seed’ shRNA1 (sh1*) control or scr control in MM.1S cells. The %GFP+ cells were normalised to day 3 %GFP expression levels for all the time points for each shRNA or scr control shown. (n=3)

(I, J) Representative flow-cytometric analysis of doxycycline inducible shRNA targeting ZBP1 in MMCL before and after dox treatment. Transduced cells are GFP+, and express dsRed+ after dox-treatment. Quantitative data for %GFP+ or %GFP+dsRed+ H929 (I) and MM.1S (J) myeloma cells without and with dox treatment in vitro. (n=3)

(K, L) Photographs of tumors explanted at sacrifice from control (i.e., non-dox-treated) or dox-treated animals engrafted with MMCL MM.1S transduced with dox-inducible shRNA1 or shRNA2 targeting ZBP1. Tumor weight at sacrifice in animals engrafted with MM.1S (K) and H929 (L) myeloma cells transduced with anti-ZBP1 shRNAs for control or dox-treated.

The error bars of all the cumulative data indicate mean ± SEM. Two-tailed unpaired t-test was applied to determine the P values. ** p≤0.01, *** p≤0.001. The number of experiments performed for the study are indicated separately in each figure legend.
Supplementary Figure S4 (Related to Figure 3)

(A) Heatmap showing log2-fold change in expression of key cell cycle genes from RNA-seq data after sh1- or sh2-mediated ZBP1 depletion in MM.1S and H929 cells (p adj <0.05).

(B) Expression of cell cycle genes FOXM1, E2F1, Ki-67 validated at mRNA level using qPCR on day 4 post-transduction with anti-ZBP1 sh1 or sh2 or scr control in MM.1S cells.

(C) Immunoblotting for indicated cell cycle proteins following dox-induction of inducible anti-ZBP1 sh1 or sh2 in MM.1S myeloma cells. Protein lysates were prepared from FACS-purified GFP+dsRed+ or GFP+ live cells on day 3 after dox-treatment or control respectively.

(D, E) A representative flow-cytometric histogram of Ki-67 expression in MM.1S cells (D) and its cumulative data shows reduction of cell proliferation marker Ki-67 in anti-ZBP1 sh1- or sh2-transduced cells as compared to scramble control cells on day 4 post-transduction in MMCL H929 and MM.1S (E). (n=3)

(F) Flow-cytometric analysis of Annexin V staining and its cumulative data shows apoptosis induction following ZBP1-depletion in MMCL MM.1S and H929. (n=3)

(G) Representative flow-cytometric example of MM patient-derived bone marrow myeloma PC purity before and after CD138+ selection using CD138 immunomagnetic microbeads.

(H) Gene set enrichment analysis with reference to Molecular Signatures Database v7.1 Hallmark gene sets for transcriptomes of ZBP1-depleted MM.1S and H929 MMCL.

(I) GSEA of highest 5% vs lowest 90% ZBP1-expressing myeloma PC. Analysis was performed using RNA-seq transcriptomes of purified myeloma PC (n=767 patients) and the inbuilt GSEA tool in the multiple myeloma research foundation (MMRF) research gateway portal.

(J) GSEA example of reactome pathway for cell cycle, mitotic genes enriched in the top 5% ZBP1-expressing cohort as compared to lowest 90% ZBP1-expressing myeloma PC cells.

The error bars of all the cumulative data indicate mean ± SEM. Two-tailed unpaired t-test was applied to determine the P values. * p≤0.05, ** p≤0.01, *** p≤0.001. The number of experiments performed for the study are indicated separately in figure legends.
Supplementary Figure S5 (Related to Figure 4)

(A) Schematic illustration of V5-tagged IRF3 and strep-tag II-tagged full length ZBP1 and its deletion mutant. RHIM, RIP homotypic interaction motif.

(B, C) Strep-tag II ZBP1-full, ZBP1-ΔRHIM and mock GFP-expressing cDNA constructs, were transiently co-expressed with V5-tagged IRF3 in HEK293T cells. Cell lysates were co-immunoprecipitated with anti-V5 antibody or anti-strep-tag magnetic beads followed by immunoblotting with anti-ZBP1 or -IRF3 antibody. IRF3 can be readily co-immunoprecipitated with only full length ZBP1.

(D) Immunoblot for pIRF3/IRF3 and pTBK1/TBK1 following dox-induction of inducible anti-ZBP1 sh1 or sh2 in MM.1S cells. Lysates were prepared from FACS-purified GFP+dsRed+ or GFP+ live cells on day 3 after dox-treatment or control respectively.
**Supplementary Figure S6 (Related to Figure 4)**

(A) Immunoblot for IRF3 in MM.1S cells on day 4 post-transduction with scr or IRF3-targetting shRNA1 or shRNA2. Lysates were prepared from the cells with >90% transduction efficiency.

(B) Flow-cytometric analysis of cell cycle in MM.1S cells on GFP+ cells day 4 post-transduction with scr or anti-IRF3 shRNA1 or shRNA2. (n=3)

(C) Annexin V staining for flow cytometric-based assessment of apoptosis in H929 and MM.1S cells on day 4 post-transduction with scr or anti-IRF3 shRNA1 or shRNA2. (n=3)

(D) %GFP+ cells after transduction with scr control or shRNA1 or shRNA2 targeting TBK1 in H929 and MM.1S cells. All the time points were normalised to day 3 %GFP expression levels for each shRNA shown. (n=3)

(E, F) A representative flow-cytometric histogram for cell cycle analysis in GFP+ cells day 4 post-transduction with TBK1-depleting shRNA1 or shRNA2 or scr control and its quantitative data show cell cycle arrest in H929 (E) and MM.1S (F) cells. (n=3-4)

(G) Annexin V staining for flow cytometric-based assessment of apoptosis in MMCL H929 and MM.1S cells on day 4 post-transduction with TBK1-depleting shRNA1 or shRNA2 or scr control. (n=3)

The error bars of all the cumulative data indicate mean ± SEM. Two-tailed unpaired t-test was applied to determine the P values. * p≤0.05, ** p≤0.01, *** p≤0.001. The number of experiments performed for the study are indicated separately in each figure legend.
Supplementary Figure S7

A

IRF3 genome-wide binding

B

activated (770 genes)

- Cell Cycle
- Mitotic G1-G1/S phases
- Cell Cycle, Mitotic
- Epigenetic regulation of gene expression
- DNA Repair
- Cell Cycle Checkpoints
- Post-translational protein modification
- Asparagine N-linked glycosylation
- Transcriptional Regulation by TP53
- Metabolism of proteins

combined score (P adj < 0.01)

C

repressed (339 genes)

- Viral mRNA Translation
- 3' -UTR-mediated translational regulation
- Eukaryotic Translation Initiation
- Nonsense-Mediated Decay (NMD)
- SRP-dependent cotranslational protein targeting to membrane
- Influenza Viral RNA Transcription and Replication
- Major pathway of rRNA processing in the nucleolus
- Ribosomal scanning and start codon recognition
- Infectious disease
- NGF signalling via TRKA from the plasma membrane

combined score (P adj < 0.05)

D

sh1 IRF3

- downregulate (0)
- upregulate (1.52e-246)
- static (background)

Cumulative Fraction of Genes

Rank of genes based on Regulatory Potential Score (from high to low)

dsh2 IRF3

- downregulate (0)
- upregulate (9.19e-261)
- static (background)

Cumulative Fraction of Genes%
Supplementary Figure S7 (Related to Figure 5)

(A) Annotation of IRF3 genome wide binding according to genomic features. ~35% and ~41% of IRF3 binding observed in promoter and intergenic genomic regions respectively.

(B, C) Enrichr pathway enrichment analysis of the 770 and 339 genes predicted to be directly activated (B) and repressed (C) by IRF3 binding to their regulatory areas.

(D) Regulatory potential prediction models display significant activating (blue) and repressive (red) function of IRF3 in MM.1S cells. Models derived from BETA-plus analysis, after integrating the IRF3 cistrome with IRF3-depleted transcriptome for anti-IRF3 shRNA1 or shRNA2.

(E) IGV browser snapshots of IRF3 and Pol II binding, chromatin accessibility and histone mark enrichment at regulatory areas of several genes promoting cell cycle progression and cell proliferation. The red block on the top indicates 5kb genomic size.

(F) Heatmap shows the downregulation of MCM2-7 complex at mRNA levels in indicated mRNA-depleted RNA-seq data (p adj <0.05).

(G, H) IRF3 binding to IFN type I pathway genes (G), the red block on the top indicates 1kb genomic size, and mRNA expression changes of indicated genes (H). Note neither IFNA1 nor IFNB1 is expressed before or after IRF3 depletion in MM.1S cells.
Supplementary Figure S8 (Related to Figure 6)

(A, B) qPCR of IRF3 ChIP or IRF3 to IRF4 ChIP-re-ChIP (A) and qPCR of IRF4 ChIP or IRF4 to IRF3 ChIP-re-ChIP (B) at the promoter regions of genes involved in cell cycle regulation.
Supplementary Table S1. Differentially expressed genes that are common in both H929 and MM.1S cells and in both shRNAs (log2 fold change >1.0 and p adj <0.05)

| down-regulated | up-regulated |
|----------------|--------------|
| NEDD1          | NCAPD3       |
| SLC1A4         | KCNQ5        |
| SURF4          | CENPK        |
| C17orf96       | RPL39L       |
| UBALD2         | C21orf58     |
| RCN2           | CENPO        |
| GPI            | FANCM        |
| TBL1X          | SUV39H2      |
| NUDT8          | PDSS1        |
| SLIT3          | IQGAP3       |
| NEDD9          | CENPU        |
| ADCY3          | OIP5         |
| MFSD2A         | PRC1         |
| KNTC1          | GGH          |
| ME2            | DUT          |
| MYH15          | MCM8         |
| ACBD7          | BRCA2        |
| YBX1           | KIAA1161     |
| DPF1           | FANCD2       |
| HNRNPUL1       | SASS6        |
| APOBEC3B       | RAD18        |
| ZNF367         | ZGRF1        |
| ACBD7          | BRCA2        |
| YBX1P1         | PTMA         |
| C12orf75       | NRGN         |
| NDC1           | ANKR18D1     |
| RTKN2          | DIAHPH       |
| RPLQ           | LRRRC8C      |
| LYAR           | TMPO         |
| AQP3           | TCOF1        |
| CHEK1          | C17orf53     |
| H2AFZ          | ANP32B       |
| NUDT1          | DMC1         |
| FBYO43         | SG01         |
| MMS22L         | CIT          |
| RANBP1         | HELS         |
| PRP38A         | DONSON       |
| YBX1P10        | C1orf112     |
| MIR1244-2      | MSH2         |
| CENPP          | DEPDC1       |

NEDD1, NCAPD3, CHTF18, TTK, CDC25C, BIRC5, CENPA, IGHA1, SLC1A4, KCNQ5, GINS1, RFC5, KIF15, ORC6, CDC5A, GALC, SURF4, CENPK, DCLRE1B, CENPE, FANCI, RFC3, CDC5A, SNAP91, C17orf96, RPL39L, KIF11, RFC2, ZBP1, E2F2, NEIL3, ZBTB18, UBALD2, C21orf58, WDHD1, KIAA1524, RAD51AP, CDC3A, CDC2A, PLEKHA1, RCN2, CENPO, FAM72B, ZWINT, KIF4A, FEN1, RRM2, LRRN2, GPI, FANCM, MIS1A8A, KIF22, PTTG1, MYB, DEPD1C8, IGHA2, TBL1X, SUV39H2, SMC2, FANCB, HMMR, GINS2, CCNB1, KIF13A, NUDT8, PDSS1, BRC1A1, CKAP2L, KIF2C, ORC1, CDC20, SATB1, SLIT3, IQGAP3, C4orf46, STIL, FAM72A, SPCC5, DLGAP5, PPIC, NEDD9, CENPU, PRIM1, FANCG, NUSAP1, BR13BP, KIF20A, PLEKHH1, ADCY3, OIP5, CENPM, MCM6, POLA1, C16orf59, CDKN3, ZBTB10, MFSD2A, PRC1, PLCD1, CDC6, DTL, SKA1, CENPW, TMEM132B, KNTC1, GGH, CLSPN, ESPL1, UBE2C, CTSF, ME2, DUT, MELK, SUV39H1, MAD2L1, CDT1, FAM11B, TMEM232, MYH15, MCM8, CDC150, CHEK2, FBXO5, SHCBP1, CDC25A, NR2F2, ACBD7, BRCA2, FAM64A, WDR76, NCAPO, DDIAS, MCM10, TMEM74B, YBX1, KIAA1161, NUP35, MND1, GSG2, TRAIP, BLM, LINC0117, DPF1, FANCD2, SPDL1, BUB1B, ERCC6L, CDK1, UHRF1, ULK1, HNRNPUL1, SASS6, CHAF1B, TCF19, PARPB8, KIF23, PLK1, APOBEC3B, RAD18, POLA2, POC1A, KIF18A, AURKA, GTSE1, ZNF367, ZGRF1, CKS1B, NDC80, TUBA1C, ARHGAP11, CENPH, CNTN1, MCM3, UBE25, RRM1, SPAG5, EXO1, KIF18B, YBX1P1, PTMA, SLCO4A1, CHAF1A, TK1, NEK2, CCNF, C12orf75, NRGN, NCAPG2, KIF20B, DSCC1, INCENP, NDC1, ANKR18D1, ANLN, TUBB, SGO2, HJURP, RKN2, DIAPH3, EME1, H2AFX, MYBL2, NCAH, RPLQ, LRRRC8C, POLE2, HMGB2, NUF2, KIFC1, LYAR, TMPO, NCAPD2, PBK, CENPI, TROAP, AQP3, TCOF1, DHFR, GINS4, TONS1, PRR11, CHEK1, C17orf53, WDR62, KIAA0101, CCNA2, TUBA1B, H2AFZ, ANP32B, KNL1, FAM72D, FAM83D, MKI67, NUDT1, DMC1, ATAD2, AUNIP, MCM5, SKA3, FBXO43, SG01, BRIP1, KIF14, CDC45, UNG, MMS22L, CIT, MTFR2, TOP2A, ASPM, PKMYT1, RANBP1, HELS, UBE2T, MCM2, FOXM1, ESCO2, PRP38A, DONSON, LMNB1, PLK4, RECQL4, TPX2, YBX1P10, C1orf112, STMN1, MCM4, TACC3, RAD51, FAM72C, PSMC3IP, RACGAP1, SPCC24, CCNB2, XRCC2, MIR1244-2, MSH2, ASF1B, RFC4, TUBBP1, CEP55, CENPP, DEPDC1, CKS2, PTMAP5, AURKB, TRIP13
### Supplementary Table S2. Reactome pathways

Enrichr pathway enrichment analysis of the 270 common genes downregulated in both MM.1S and H929 cells treated with anti-ZBP1 shRNAs

| Name of the enriched pathways (Homo sapiens)                                           | Overlap | Adj P-value | Odds Ratio | Combined Score |
|----------------------------------------------------------------------------------------|---------|-------------|------------|----------------|
| Cell Cycle_R-HSA-1640170                                                                | 111/566 | 8.91E-98    | 14.527     | 3352.79        |
| Cell Cycle, Mitotic_R-HSA-69278                                                         | 95/462  | 2.25E-84    | 15.232     | 3034.86        |
| DNA strand elongation_R-HSA-69190                                                       | 18/32   | 4.48E-24    | 41.667     | 2427.56        |
| Unwinding of DNA_R-HSA-176974                                                          | 10/12   | 6.71E-16    | 61.728     | 2410.61        |
| Mitotic Prometaphase_R-HSA-68877                                                        | 36/107  | 2.88E-38    | 24.922     | 2302.45        |
| Activation of ATR in response to replication stress_R-HSA-17618                         | 19/37   | 2.29E-24    | 38.038     | 2246.47        |
| Activation of the pre-replicative complex_R-HSA-68962                                   | 16/30   | 6.64E-21    | 39.506     | 2003.07        |
| Resolution of Sister Chromatid Cohesion_R-HSA-250025                                    | 32/99   | 3.05E-33    | 23.943     | 1929.67        |
| G1/S-Specific Transcription_R-HSA-69205                                                 | 11/17   | 1.50E-15    | 47.93      | 1831.33        |
| E2F mediated regulation of DNA replication_R-HSA-113510                                 | 16/33   | 4.92E-20    | 35.915     | 1747.48        |
| M Phase_R-HSA-68886                                                                    | 50/268  | 8.22E-40    | 13.82      | 1329.89        |
| RHO GTPases Activate Formins_R-HSA-5663220                                              | 29/114  | 8.24E-27    | 18.843     | 1228.52        |
| Phosphorylation of Emi1_R-HSA-176417                                                    | 5/6     | 5.60E-08    | 61.728     | 1221.09        |
| DNA Replication_R-HSA-69306                                                             | 27/105  | 4.24E-25    | 19.048     | 1162.93        |
| Condensation of Prometaphase Chromosomes_R-HSA-2514853                                  | 7/11    | 7.92E-10    | 47.138     | 1153.01        |
| Chromosome Maintenance_R-HSA-73886                                                      | 23/86   | 6.54E-22    | 19.811     | 1052.26        |
| Mitotic Metaphase and Anaphase_R-HSA-2555396                                            | 35/174  | 1.24E-28    | 14.9       | 1039.99        |
| S Phase_R-HSA-69242                                                                    | 28/124  | 1.87E-24    | 16.726     | 992.391        |
| Synthesis of DNA_R-HSA-69239                                                           | 24/97   | 5.55E-22    | 18.328     | 977.428        |
| Mitotic Anaphase_R-HSA-68882                                                            | 34/173  | 1.57E-27    | 14.558     | 974.934        |
| Cell Cycle Checkpoints_R-HSA-69620                                                     | 35/182  | 5.56E-28    | 14.245     | 970.72         |
| G1/S Transition_R-HSA-69206                                                            | 26/112  | 4.44E-23    | 17.196     | 961.438        |
| Mitotic G1-G1/S phases_R-HSA-45327                                                     | 29/136  | 1.46E-24    | 15.795     | 942.258        |
| Polo-like kinase mediated events_R-HSA-156711                                            | 8/16    | 4.15E-10    | 37.037     | 932.413        |
| G2/M DNA replication checkpoint_R-HSA-69478                                              | 4/5     | 3.00E-06    | 59.259     | 927.032        |
| Cyclin B2 mediated events_R-HSA-157881                                                 | 4/5     | 3.04E-06    | 59.259     | 927.032        |
| Separation of Sister Chromatids_R-HSA-2467813                                           | 32/162  | 5.44E-26    | 14.632     | 924.796        |
| E2F-enabled inhibition of pre-replication complex formation_R-HSA-113507               | 6/10    | 2.84E-08    | 44.444     | 914.853        |
| G2/M Checkpoints_R-HSA-69481                                                           | 30/150  | 1.56E-24    | 14.815     | 883.889        |
| Leading Strand Synthesis_R-HSA-69109                                                    | 7/14    | 6.54E-09    | 37.037     | 820.487        |
| Polymerase switching_R-HSA-69091                                                       | 7/14    | 6.66E-09    | 37.037     | 820.487        |
| Polymerase switching on the C-strand of the telomere_R-HSA-174411                      | 7/14    | 6.78E-09    | 37.037     | 820.487        |
| CDC6 association with the ORC:origin complex_R-HSA-68689                                | 6/11    | 5.54E-08    | 40.404     | 800.287        |
| Event Description                                                                 | Set ID       | Count | p-value | Log2FoldChange | AverageLog2FoldChange |
|----------------------------------------------------------------------------------|--------------|-------|---------|----------------|----------------------|
| Cyclin A/B1 associated events during G2/M transition R-HSA-69273                 | 9/22         | 2.03E-10 | 30.303  | 785.311        |
| Telomere C-strand (Lagging Strand) Synthesis_R-HSA-174417                        | 9/22         | 2.08E-10 | 30.303  | 785.311        |
| Homologous DNA Pairing and Strand Exchange_R-HSA-5693579                         | 13/42        | 3.00E-13 | 22.928  | 748.394        |
| Telomere C-strand synthesis initiation_R-HSA-174430                              | 4/6          | 8.11E-06 | 49.383  | 718.801        |
| DNA replication initiation_R-HSA-68952                                           | 4/6          | 8.20E-06 | 49.383  | 718.801        |
| Kinesins_R-HSA-983189                                                            | 13/44        | 5.63E-13 | 21.886  | 699.358        |
| Presynaptic phase of homologous DNA pairing and strand exchange_R-HSA-5693616    | 12/39        | 3.36E-12 | 22.792  | 687.007        |
| Lagging Strand Synthesis_R-HSA-69186                                             | 8/20         | 3.33E-09 | 29.63    | 679.727        |
| Nucleosome assembly_R-HSA-774815                                                 | 14/52        | 2.55E-13 | 19.943  | 655.432        |
| Deposition of new CENPA-containing nucleosomes at the centromere_R-HSA-606279    | 14/52        | 2.63E-13 | 19.943  | 655.432        |
| M/G1 Transition_R-HSA-68874                                                      | 18/82        | 1.85E-15 | 16.26    | 616.625        |
| DNA Replication Pre-Initiation_R-HSA-69002                                       | 18/82        | 1.92E-15 | 16.26    | 616.625        |
| RHO GTPase Effectors_R-HSA-195258                                                | 36/255       | 2.93E-24 | 10.458  | 614.343        |
| Activation of NIMA Kinases NEK9, NEK6, NEK7_R-HSA-2980767                       | 4/7          | 1.77E-05 | 42.328  | 580.703        |
| HDR through Homologous Recombination (HRR)_R-HSA-5685942                         | 15/64        | 2.50E-13 | 17.361  | 570.378        |
| Extension of Telomeres_R-HSA-180786                                              | 9/28         | 2.29E-09 | 23.81    | 556.076        |
| Resolution of D-loop Structures through Synthesis-Dependent Strand Annealing (SDSA)_R-HSA-5693554 | 8/26     | 3.01E-08 | 22.792  | 467.079        |
| Homology Directed Repair_R-HSA-5693538                                           | 20/118       | 6.59E-15 | 12.555  | 459.744        |
| Resolution of D-loop Structures through Holliday Junction Intermediates_R-HSA-5693568 | 9/32    | 7.48E-09 | 20.833  | 458.353        |
| Resolution of D-Loop Structures_R-HSA-5693537                                    | 9/33         | 9.82E-09 | 20.202  | 438.268        |
| Signaling by Rho GTPases_R-HSA-194315                                            | 39/367       | 7.65E-22 | 7.8716  | 416.493        |
| DNA Repair_R-HSA-73894                                                           | 33/285       | 1.55E-19 | 8.577   | 407.105        |
| HDR through Homologous Recombination (HR) or Single Strand Annealing (SSA)_R-HSA-5693567 | 18/112   | 5.01E-13 | 11.905  | 382.139        |
| Regulation of DNA replication_R-HSA-69304                                        | 14/75        | 5.16E-11 | 13.827  | 378.627        |
| DNA Double-Strand Break Repair_R-HSA-5693532                                     | 21/145       | 2.99E-14 | 10.728  | 376.244        |
| HDR through Single Strand Annealing (SSA)_R-HSA-5685938                          | 9/37         | 2.83E-08 | 18.018  | 370.636        |
| PCNA-Dependent Long Patch Base Excision Repair_R-HSA-5651801                     | 6/19         | 2.56E-06 | 23.392  | 370.176        |
| G0 and Early G1_R-HSA-1538133                                                   | 7/25         | 6.10E-07 | 20.741  | 359.605        |
| G2/M DNA damage checkpoint_R-HSA-69473                                           | 14/78        | 8.86E-11 | 13.295  | 356.539        |
| Association of licensing factors with the pre-replicative complex_R-HSA-69298    | 5/15         | 1.83E-05 | 24.691  | 337.425        |
| Assembly of the pre-replicative complex_R-HSA-68867                              | 12/67        | 2.78E-09 | 13.267  | 307.028        |
| Recognition of DNA damage by PCNA-containing replication complex_R-HSA-110314   | 7/28         | 1.38E-06 | 18.519  | 305.024        |
| Fanconi Anemia Pathway_R-HSA-6783310                                             | 8/35         | 3.58E-07 | 16.931  | 302.829        |
| Pathway                                                                                                                                    | PPMs | PPM | PM | PNM | PNM | PPM | PPM | PM | PNM | PNM |
|-------------------------------------------------------------------------------------------------------------------------------------------|------|-----|----|-----|-----|-----|-----|----|-----|-----|-----|
| Telomere Maintenance_R-HSA-157579                                                                                                         | 11/59| 9.22E-09 | 13.81 | 300.722|
| Resolution of AP sites via the multiple-nucleotide patch replacement pathway_R-HSA-110373                                             | 6/22 | 6.41E-06 | 20.202 | 299.949|
| Regulation of TP53 Activity through Phosphorylation_R-HSA-680476                                                                         | 14/89 | 5.13E-10 | 11.652 | 290.603|
| COPI-dependent Golgi-to-ER retrograde traffic_R-HSA-6811434                                                                           | 13/81 | 2.00E-09 | 11.888 | 279.533|
| Removal of licensing factors from origins_R-HSA-69300                                                                                  | 12/72 | 6.20E-09 | 12.346 | 274.831|
| Inhibition of replication initiation of damaged DNA by RB1/E2F1_R-HSA-113501                                                            | 4/12 | 2.15E-04 | 24.691 | 274.648|
| Chk1/Chk2(Cds1) mediated inactivation of Cyclin B:Cdk1 complex_R-HSA-75035                                                               | 4/12 | 2.17E-04 | 24.691 | 274.648|
| Regulation of mitotic cell cycle_R-HSA-453276                                                                                             | 13/85 | 3.39E-09 | 11.329 | 259.251|
| APC/C-mediated degradation of cell cycle proteins_R-HSA-174143                                                                            | 13/85 | 3.46E-09 | 11.329 | 259.251|
| Condensation of Prophase Chromosomes_R-HSA-2299718                                                                                       | 8/41 | 1.30E-06 | 14.453 | 239.275|
| Orc1 removal from chromatin_R-HSA-68949                                                                                                  | 11/70 | 5.33E-08 | 11.64  | 231.184|
| Switching of origins to a post-replicative state_R-HSA-69052                                                                            | 11/70 | 5.41E-08 | 11.64  | 231.184|
| Processing of DNA double-strand break ends_R-HSA-569360                                                                                 | 12/81 | 2.23E-08 | 10.974 | 228.72|
| Golgi Cisternae Pericentriolar Stack Reorganization_R-HSA-162658                                                                         | 4/14 | 4.14E-04 | 21.164 | 220.96|
| Removal of the Flap Intermediate_R-HSA-69166                                                                                              | 4/14 | 4.18E-04 | 21.164 | 220.96|
| Resolution of Abasic Sites (AP sites)_R-HSA-73933                                                                                         | 7/35 | 6.47E-06 | 14.815 | 219.484|
| Base Excision Repair_R-HSA-73884                                                                                                       | 7/35 | 6.55E-06 | 14.815 | 219.484|
| SUMOylation of DNA replication proteins_R-HSA-4615885                                                                                     | 8/44 | 2.24E-06 | 13.468 | 215.111|
| Mitotic G2-G2/M phases_R-HSA-453274                                                                                                     | 19/175 | 1.15E-10 | 8.0423 | 213.395|
| Meiotic recombination_R-HSA-912446                                                                                                       | 9/54 | 8.32E-07 | 12.346 | 210.066|
| DNA Damage Bypass_R-HSA-73893                                                                                                           | 8/46 | 3.07E-06 | 12.882 | 201.076|
| Processive synthesis on the lagging strand_R-HSA-69183                                                                                   | 4/15 | 5.48E-04 | 19.753 | 200.313|
| Depolymerisation of the Nuclear Lamina_R-HSA-4419969                                                                                     | 4/15 | 5.54E-04 | 19.753 | 200.313|
| Termination of translesion DNA synthesis_R-HSA-5656169                                                                                  | 6/30 | 3.96E-05 | 14.815 | 190.577|
| G2/M Transition_R-HSA-69275                                                                                                             | 18/173 | 7.94E-10 | 7.7071 | 188.67|
| G2 Phase_R-HSA-68911                                                                                                                    | 2/5 | 0.01932 | 29.63  | 187.796|
| Translesion synthesis by REV1_R-HSA-110312                                                                                               | 4/16 | 7.11E-04 | 18.519 | 182.664|
| Gap-filling DNA repair synthesis and ligation in GG-NER_R-HSA-5696397                                                                     | 5/23 | 1.77E-04 | 16.103 | 182.574|
| Mitotic Prophase_R-HSA-68875                                                                                                            | 13/107 | 5.03E-08 | 8.9997 | 179.527|
| Nuclear Envelope Breakdown_R-HSA-2980766                                                                                                 | 8/51 | 6.66E-06 | 11.619 | 171.674|
| Golgi-to-ER retrograde transport_R-HSA-8856688                                                                                           | 13/110 | 6.61E-08 | 8.7542 | 171.61|
| Translesion synthesis by POLI_R-HSA-5656121                                                                                                | 4/17 | 8.96E-04 | 17.429 | 167.429|
| Translesion synthesis by POLK_R-HSA-5655862                                                                                                | 4/17 | 9.03E-04 | 17.429 | 167.429|
| Activation of APC/C and APC/C:Cdc20 mediated degradation of mitotic proteins | R-HSA-176814 | 10/75 | 1.30E-06 | 9.8765 | 163.412 |
|-----------------------------|----------------|--------|-----------|---------|---------|
| APC-Cdc20 mediated degradation of Nek2A | R-HSA-179409 | 5/25 | 2.66E-04 | 14.815 | 161.528 |
| Recruitment of NuMA to mitotic centrosomes | R-HSA-380320 | 3/11 | 0.00436 | 20.202 | 159.608 |
| TP53 Regulates Transcription of Genes Involved in G2 Cell Cycle Arrest | R-HSA-6804114 | 4/18 | 0.00113 | 16.461 | 154.165 |
| Regulation of TP53 Activity | R-HSA-5633007 | 15/151 | 5.04E-08 | 7.3584 | 146.888 |
| Assembly of the ORC complex at the origin of replication | R-HSA-68616 | 2/6 | 0.02812 | 24.691 | 146.706 |
| Phosphorylation of the APC/C | R-HSA-176412 | 4/19 | 0.00136 | 15.595 | 142.531 |
| Translesion Synthesis by POLH | R-HSA-110320 | 4/19 | 0.00137 | 15.595 | 142.531 |
| Translesion synthesis by Y family DNA polymerases bypasses lesions on DNA template | R-HSA-110313 | 6/37 | 1.41E-04 | 12.012 | 139.079 |
| Factors involved in megakaryocyte development and platelet production | R-HSA-983231 | 14/141 | 1.59E-07 | 7.3549 | 137.62 |
| AURKA Activation by TPX2 | R-HSA-8854518 | 9/72 | 8.8E-06 | 9.2593 | 133.835 |
| Meiosis | R-HSA-1500620 | 10/85 | 3.98E-06 | 8.7146 | 133.643 |
| Inactivation of APC/C via direct inhibition of the APC/C complex | R-HSA-141430 | 4/20 | 0.00165 | 14.815 | 132.256 |
| Inhibition of the proteolytic activity of APC/C required for the onset of anaphase by mitotic spindle checkpoint components | R-HSA-141405 | 4/20 | 0.00166 | 14.815 | 132.256 |
| APC/C:Cdc20 mediated degradation of mitotic proteins | R-HSA-176409 | 9/74 | 1.11E-05 | 9.009 | 128.076 |
| Mitotic Telophase/Cytokinesis | R-HSA-68884 | 3/13 | 0.00724 | 17.094 | 125.993 |
| Mitotic Spindle Checkpoint | R-HSA-69618 | 4/21 | 0.002 | 14.109 | 123.126 |
| Regulation of APC/C activators between G1/S and early anaphase | R-HSA-176408 | 9/79 | 1.84E-05 | 8.4388 | 115.219 |
| SUMO E3 ligases SUMOylate target proteins | R-HSA-3108232 | 10/96 | 1.14E-05 | 7.716 | 109.44 |
| APC/C:Cdc20 mediated degradation of Cyclin B | R-HSA-174048 | 4/23 | 0.00285 | 12.882 | 107.647 |
| Transcriptional Regulation by TP53 | R-HSA-3700989 | 23/348 | 1.05E-08 | 4.8957 | 105.782 |
| Nuclear Pore Complex (NPC) Disassembly | R-HSA-3301854 | 5/34 | 0.00114 | 10.893 | 101.815 |
| SUMOylation | R-HSA-2990846 | 10/101 | 1.77E-05 | 7.3341 | 100.55 |
| Regulation of PLK1 Activity at G2/M Transition | R-HSA-2565942 | 9/87 | 4.04E-05 | 7.6628 | 98.3472 |
| MHC class II antigen presentation | R-HSA-2132295 | 10/103 | 2.06E-05 | 7.1917 | 97.2915 |
| RHO GTPases activate CIT | R-HSA-5625900 | 3/16 | 0.01346 | 13.889 | 93.4529 |
| TP53 Regulates Transcription of Cell Cycle Genes | R-HSA-6791312 | 6/48 | 6.02E-04 | 9.2593 | 92.9528 |
| Dual Incision in GG-NER | R-HSA-5696400 | 5/39 | 0.00209 | 9.4967 | 82.3788 |
| Intra-Golgi and retrograde Golgi-to-ER traffic | R-HSA-6811442 | 13/179 | 1.64E-05 | 5.3797 | 74.2846 |
| Pathway Description | Count | p-value | Z-score | Fold Change |
|---------------------|-------|--------|---------|-------------|
| APC:Cdc20 mediated degradation of cell cycle proteins prior to satisfaction of the cell cycle checkpoint | 7/72  | 7.13E-04 | 7.2016 | 70.9443 |
| Recruitment of mitotic centrosome proteins and complexes | 7/79  | 0.00123 | 6.5635 | 60.7414 |
| Centrosome maturation | 7/79  | 0.00124 | 6.5635 | 60.7414 |
| Loss of proteins required for interphase microtubule organization?from the centrosome | 6/69  | 0.00399 | 6.4412 | 51.5046 |
| Loss of Nlp from mitotic centrosomes | 6/69  | 0.00403 | 6.4412 | 51.5046 |
| Cdc20:Phospho-APC/C mediated degradation of Cyclin A | 6/71  | 0.00456 | 6.2598 | 49.0807 |
| APC/C:Cdh1 mediated degradation of Cdc20 and other APC/C:Cdh1 targeted proteins in late mitosis/early G1 | 6/71  | 0.00459 | 6.2598 | 49.0807 |
| CDT1 association with the CDC6:ORC:origin complex | 5/58  | 0.01261 | 6.3857 | 43.4775 |
| The role of GTSE1 in G2/M progression after G2 checkpoint | 5/59  | 0.01353 | 6.2775 | 42.2533 |
| TP53 Regulates Transcription of DNA Repair Genes | 5/61  | 0.0155 | 6.0716 | 39.953 |
| Gap-filling DNA repair synthesis and ligation in TC-NER | 5/62  | 0.01655 | 5.9737 | 38.8716 |
| Dual incision in TC-NER | 5/63  | 0.01765 | 5.8789 | 37.8326 |
| Anchoring of the basal body to the plasma membrane | 6/97  | 0.02186 | 4.5819 | 28.4081 |
| Transcription-Coupled Nucleotide Excision Repair (TC-NER) | 5/76  | 0.03888 | 4.8733 | 27.3416 |
| Generic Transcription Pathway | 24/812 | 0.00346 | 2.1894 | 17.8564 |
| Membrane Trafficking | 14/420 | 0.01957 | 2.4691 | 15.6007 |
**Supplementary Table S3. Gene sets enriched in indicated reactome pathways; Gene set enrichment analysis between highest 5% and lowest 90% ZBP1-expressing myeloma PC from MMRF data sets**

**Cell cycle, Mitotic pathway (5% ZBP1 highest/95% ZBP1 lowest); p-value <0.05**

| Gene   | Fold-Change | Gene | Fold-Change | Gene | Fold-Change | Gene | Fold-Change | Gene | Fold-Change | Gene | Fold-Change |
|--------|-------------|------|-------------|------|-------------|------|-------------|------|-------------|------|-------------|
| PSMB9  | 1.4871      | KIF2A| 1.8556      | CDC45| 2.1065      | CEP76| 1.8712      | E2F2| 1.6004      |
| MAD1L1 | 1.5331      | PPP2R5B| 1.4041     | PPP1CB| 1.4419      | CEP192| 1.7032     | RCC2| 1.2602      |
| CDC27  | 1.4889      | NUP133| 1.4911      | CDC23| 1.8488      | PSMD10| 1.5228    | CLASP2| 1.5275     |
| DHFR   | 2.2396      | SMG6 | 1.4561      | AAAS| 1.4887      | POLA1| 1.7371     | MAD2L1| 2.2138      |
| UPF1   | 1.5695      | PABPC1| 1.4405      | PSMD5| 1.6736      | STAG2| 1.5055     | LEMD3| 1.7028      |
| DBF4   | 1.5907      | CSNK2A2| 1.4326     | NUP188| 1.7266      | EMD| 1.3580     | LEMD2| 1.3773      |
| PAFAH1B1| 1.4217     | PRKACA| 1.4529      | FGFR1OP| 1.7308     | CENPI| 2.3121     | PSMD2| 1.5048      |
| PSMB1  | 1.3729      | FBXW11| 1.7771      | PSMD8| 1.5519      | NUP93| 2.9343     | PSMD1| 1.7307      |
| CDK11A | 1.5529      | NDE1 | 1.6296      | MAPK1| 1.4827      | CENPT| 1.4832     | CDK1| 2.6002      |
| NCPD2  | 1.8065      | MCM2 | 2.1519      | NUP62| 1.6856      | PSMD7| 1.4589     | NIPBL| 1.6964      |
| PSMC4  | 1.4382      | CLASP1| 1.4175      | MCM5| 1.6225      | CCP110| 1.5745    | ANAPC10| 1.6061     |
| POLA2  | 1.9113      | NUP37| 1.5488      | EP30| 1.4826      | CEPI52| 1.8193     | PSMD6| 1.4762      |
| MNAT1  | 1.5902      | MCM6 | 1.6880      | RANAP1| 1.5512     | RAB2A| 1.3231     | PSMC3| 1.6954      |
| VRK2   | 1.4907      | DYN1C2| 1.5534      | POLE2| 2.1134      | PPP2CB| 1.4167    | DCTN2| 1.3089      |
| NUP160 | 1.7894      | POLD3| 1.7212      | PSMC6| 1.5377      | DCTN1| 1.5079     | RAD21| 1.5428      |
| CENPQ  | 1.7299      | PPP2R5C| 2.0603     | P5MA3| 1.3713      | CSNK2B| 1.3568    | ORC5| 1.7811      |
| RFC1   | 1.5251      | RBL1 | 1.6509      | VRK1| 1.6120      | CCNE1| 2.8558     | PTTG1| 2.3277      |
| TUBG2  | 1.7389      | NDC80| 2.6181      | PSMC1| 1.4951      | PSMB8| 1.5490     | RFC4| 1.7710      |
| CCDC99 | 2.0297      | XPO1 | 1.7806      | PSMB5| 1.6025      | FZR1| 1.7622     | AURKB| 2.2140      |
| PSMA4  | 1.8814      | PDS5B| 1.7431      | PSMA6| 1.3837      | LIG1| 2.0206     | CKAP5| 1.9196      |
| OFD1   | 1.4891      | SEH1L| 1.6445      | PSME2| 2.0096      | PPP2R1A| 1.5547    | FEN1| 1.8576      |
| TPR    | 1.4568      | ZW10 | 1.5717      | GINS1| 2.4646      | RPA3| 1.5353     | UBB| 1.4233      |
| RFC2   | 1.5440      | PSMC5| 1.5845      | NINL| 2.0324      | PSMA2| 1.7327     | UBE2E1| 1.8591      |
| ANAPC4 | 1.4572      | AURKA| 2.2797      | MYBL2| 2.1013      | POLD2| 1.7996     | RAB8A| 1.9369      |
| SDCAG8 | 1.3201      | DYN1L1| 1.5333     | E2F4| 1.3965      | SMC3| 1.6431     | TK2| 1.5051      |
| CUL1   | 1.4648      | ANAPC5| 1.4418     | RAE1| 1.8403      | PSMB3| 1.4316     | BUB1| 1.9658      |
| PPP1R12A| 1.4814     | BRC5 | 2.3403      | PSMA7| 1.4842      | PSMD3| 1.4378     | YWHAG| 1.6476      |
| WAPAL  | 1.4903      | NUDC | 1.5497      | CDC25B| 1.6696     | NUP88| 1.5362     | ESCO2| 2.2478      |
| PPP2R2A| 1.5342     | AC005522| 1.5583     | CSNK2A1| 1.5780     | PSMD11| 1.4532    | CDT1| 2.1051      |
| POLD1  | 1.7550      | ORC6 | 1.6600      | PSMB10| 1.8780      | YWHAE| 1.6662     | PPP2R3B| 1.5977     |
| MCM10  | 2.3261      | PSE1 | 1.6085      | MAPRE1| 1.4897      | SMG5| 1.4840     | GOLGA2| 1.3450      |
| CSNK1E | 2.0310      | NUP50| 1.6554      | E2F1| 1.9224      | CEP290| 1.4597    | PSME4| 1.3869      |
| NCPAG  | 2.7609      | CENPK| 2.3389      | TUBGCP4| 1.8073     | CENPF| 2.2820     | SMC1A| 1.5176      |
| TFD1P1 | 1.7336      | CK2 | 1.4745      | KIF23| 2.7584      | CDC8A| 2.1978     | BLZF1| 1.3958      |
| CEP164 | 1.6417      | B9D2 | 1.6184      | HAUS2| 1.9239      | KIF2C| 2.0871     | NLS1| 1.4647      |
| NUP98  | 1.6126      | NUP85| 1.6810      | CASC5| 2.1613      | PPP2R5E| 1.3319    | SGOL2| 2.0688      |
| PSM1D9 | 1.5436      | PSMF1| 1.3795      | RAB8A| 1.2818      | SKA1| 2.3352     | NUMA1| 1.6932      |
| FOXM1  | 1.9684      | MCM8 | 1.8459      | ACTR1A| 1.3089     | KS1B| 1.7487     | PMF1| 1.4223      |
| RFC5   | 1.5372      | MAX | 1.3485      | CENPO| 1.8710      | MIS12| 1.3494     | TAO1K| 1.5894      |
| Gene     | Fold-Change | Gene     | Fold-Change | Gene     | Fold-Change | Gene     | Fold-Change | Gene     | Fold-Change | Gene     | Fold-Change |
|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|
| IFNA17   | 15.513      | CAMK2G   | 1.6568      | ITGB3    | 1.6369      | KPN4     | 1.3309      | NUP62    | 1.6856      | IFNA8    | 10.747      |
| IFNA8    | 10.747      | EIF4A2   | 1.7911      | EIF4G3   | 1.6245      | DDX58    | 2.1343      | IRF1     | 1.6817      | IFNA7    | 8.4985      |
| IFNA7    | 8.4985      | EIF4A1   | 1.6722      | EIF2AK2  | 1.6181      | IRF3     | 1.8063      | UBA7     | 1.6756      | IFNA14   | 4.5365      |
| IFNA14   | 4.5365      | RNASEL   | 1.5408      | IFITM2   | 1.6173      | USP41    | 2.3186      | PTPN2    | 1.6734      | IFNA4    | 4.2438      |
| IFNA4    | 4.2438      | NUP205   | 1.7608      | RP527A   | 1.5769      | ARH1     | 1.7096      | MX2      | 1.6644      |

Interferon Signaling pathway (5% ZBP1 highest/95% ZBP1 lowest); p-value <0.05
| Gene Symbol | Expression Value | Gene Symbol | Expression Value | Gene Symbol | Expression Value |
|-------------|------------------|-------------|------------------|-------------|------------------|
| IFNB1       | 3.9058           | HERC5       | 1.566            | SUMO1       | 1.5739           | IRF5         | 1.9069         | NUP155       | 1.6571         |
| IFIT2       | 2.6502           | NCAM1       | 2.705            | NUP37       | 1.5488           | JAK2         | 1.6943         | SEH1L        | 1.6445         |
| CAMK2B      | 2.2713           | NUP85       | 1.681            | PPP2CA      | 1.5444           | PPP2CB       | 1.4167         | PIN1         | 1.8299         |
| USP18       | 2.1842           | IFNGR2      | 1.5434           | NUP107      | 1.5405           | OAS1         | 1.7109         | IRF4         | 2.2322         |
| IFI35       | 1.8975           | TTN1        | 1.3977           | KPNA1       | 1.5154           | OAS3         | 2.2781         | PPM1B        | 1.7106         |
| HLA-G       | 1.8841           | NUP1L1      | 1.5884           | NUP133      | 1.4911           | ADAR         | 1.4277         | NUP93        | 2.9343         |
| ISG20       | 1.8801           | POM121      | 1.574            | IRF9        | 1.4872           | NUP214       | 1.4859         | IFIT1        | 2.3023         |
| UBE2E1      | 1.8591           | NUP98       | 1.6126           | PTPN11      | 1.4721           | NUP35        | 1.8293         | NUP188       | 1.7266         |
| IP6K2       | 1.8488           | AAAS        | 1.4887           | STAT2       | 1.469            | PPP2R1A      | 1.5547         | MX1          | 2.3512         |
| IFNAR1      | 1.8485           | IRF2        | 1.6288           | TPR         | 1.4568           | IFNA21       | 3.7102         | NUP153       | 1.3129         |
| IFNAR1      | 1.847            | PSMB8       | 1.549            | KPNA2       | 1.4492           | UBE2L6       | 1.9286         | UBA52        | 1.3111         |
| TRIM25      | 1.7993           | TYK2        | 1.7651           | EIF4E       | 1.4464           | NUP210       | 1.9129         | UBC          | 1.3084         |
| NUP160      | 1.7894           | NUP54       | 1.4714           | EIF4A3      | 1.4336           | EIF4E2       | 1.5914         | PTPN6        | 1.2809         |
| NEDD4       | 1.7797           | RAE1        | 1.8403           | GBP3        | 1.4251           | IRF7         | 1.7838         | EIF4G1       | 1.2793         |
| NUP43       | 1.7715           | ISG15       | 2.5585           | UBB         | 1.4233           | KPNB1        | 1.6639         | MAPK3        | 1.2418         |
| OAS2        | 1.7654           | EIF4G2      | 1.5117           | RANBP2      | 1.3975           | PRKCD        | 1.8154         | JAK1         | 1.1763         |
| PLCG1       | 1.7514           | IFNA10      | 7.5309           | IFNA5       | 1.3942           | IFNA16       | 3.0585         | PML          | 2.1341         |
| PIA51       | 1.7448           | NUP50       | 1.6554           | GRB2        | 1.3373           | KPNAS        | 1.6881         | NUP7L2       | 1.7165         |
| SP100       | 1.7265           | NUP88       | 1.5362           | STAT1       | 1.3161           | XAF1         | 1.7327         | IFNAR2       | 1.7334         |
| UBE2N       | 1.7013           | KPNA3       | 1.758            | PTPN1       | 1.3146           | SRC          | 1.4247         | RAP1B        | 1.3523         |
| EIF4E3      | 1.4229           | IFI27       | 2.2101           | OASL        | 1.7928           | SOCS3        | 0.2326         |
Supplementary Table S4. Differentially expressed genes that are common between shZBP1 and shIRF3 in MM.1S cells (top50% log2 fold change and p adj <0.05)

| down-regulated   | up-regulated   |
|------------------|----------------|
| THRIL           | AMOTL1         |
| DHCR24          | PRPS2          |
| BLM             | NASP           |
| RRM2            | CALM3          |
| HES6            | NCAPH2         |
| RAD51           | PLEKHH2        |
| DDIAS           | FADS1          |
| CCNF            | TSPAN33        |
| CEP55           | EMLIN1         |
| AURKA           | MCC            |
| E2F8            | INSI1G         |
| PRR11           | LAM5           |
| MYB             | LRRCC1         |
| EXO1            | CCSAP          |
| SKA1            | LRP8           |
| PKMYT1          | CCNE1          |
| DTL             | MCM8           |
| SHCBP1          | MCM3           |
| BR13BP          | ITPKA          |
| TONSL           | HEMGN          |
| ERC6L           | IMPA2          |
| FAM111B         | LRRC20         |
| FEN1            | SLC16A14       |
| MAD2L1          | BARD1          |
| CDC45           | MAL2           |
| DSCC1           | CEP57L1        |
| POLA1           | EAF2           |
| PROB1           | CYP26A1        |
| RAD51AP1        | TMEFF1         |
| KIF15           | RELN           |
| WDR76           | GRB14          |
| FANCG           | RHBDL3         |
| CKAP2L          | TMEM106C       |
| CENPJ           | SCD            |
| CDC25C          | ITPRIPL1       |
| MCM6            | MYORG          |
| TP73            | SMC1A          |
| Name of the enriched pathways (Homo sapiens) | Overlap | Adjusted P-value | Odds Ratio | Combined Score |
|---------------------------------------------|--------|-----------------|------------|----------------|
| G1/S-Specific Transcription_R-HSA-69205     | 5/17   | 4.92E-06        | 53.97      | 943.0714       |
| Unwinding of DNA_R-HSA-176974               | 4/12   | 4.70E-05        | 61.16      | 901.1077       |
| DNA strand elongation_R-HSA-69190           | 7/32   | 1.80E-07        | 40.14      | 873.5304       |
| E2F mediated regulation of DNA replication_R-HSA-113510 | 6/33 | 5.71E-06        | 33.36      | 587.6216       |
| Resolution of D-loop Structures through Synthesis-Dependent Strand Annealing (SDSA)_R-HSA-5693554 | 5/26 | 3.36E-05        | 35.29      | 534.5948       |
| Activation of the pre-replicative complex_R-HSA-68962 | 5/30 | 6.12E-05        | 30.58      | 440.2043       |
| Resolution of D-loop Structures through Holliday Junction Intermediates_R-HSA-5693568 | 5/32 | 8.01E-05        | 28.67      | 403.0262       |
| POLB-Dependent Long Patch Base Excision Repair_R-HSA-110362 | 2/7 | 0.02212         | 52.42      | 388.299        |
| Resolution of D-Loop Structures_R-HSA-5693537 | 5/33 | 8.81E-05        | 27.8       | 386.366        |
| Processive synthesis on the lagging strand_R-HSA-69183 | 3/15 | 0.00348         | 36.7       | 351.9814       |
| Activation of ATR in response to replication stress_R-HSA-176187 | 5/37 | 1.41E-04        | 24.8       | 329.9524       |
| Activation of PUMA and translocation to mitochondria_R-HSA-139915 | 2/8 | 0.02742         | 45.87      | 326.7286       |
| Cell Cycle_R-HSA-1640170                   | 26/566 | 4.91E-14        | 8.429      | 320.1011       |
| Homologous DNA Pairing and Strand Exchange_R-HSA-5693579 | 5/42 | 2.56E-04        | 21.84      | 276.5402       |
| G1/S Transition_R-HSA-69206                | 9/112  | 3.20E-06        | 14.74      | 270.955        |
| Cell Cycle, Mitotic_R-HSA-69278            | 21/462 | 4.93E-11        | 8.34       | 253.3261       |
| Lagging Strand Synthesis_R-HSA-69186       | 3/20   | 0.00778         | 27.52      | 239.2529       |
| S Phase_R-HSA-69242                       | 9/124  | 5.99E-06        | 13.32      | 232.8195       |
| TP53 Regulates Transcription of Cell Cycle Genes_R-HSA-6791312 | 5/48 | 4.33E-04        | 19.11      | 229.098        |
| DNA Replication_R-HSA-69306                | 8/105  | 1.65E-05        | 13.98      | 224.2559       |
| CDC6 association with the ORC:origin complex_R-HSA-68689 | 2/11 | 0.04703         | 33.36      | 215.4542       |
| Processive synthesis on the C-strand of the telomere_R-HSA-174414 | 2/11 | 0.04797         | 33.36      | 215.4542       |
| Cell Cycle Checkpoints_R-HSA-69620         | 11/182 | 1.77E-06        | 11.09      | 212.8149       |
| Telomere C-strand (Lagging Strand) Synthesis_R-HSA-174417 | 3/22 | 0.01012         | 25.02      | 210.176        |
| Mitotic G1-G1/S phases_R-HSA-453279        | 9/136  | 9.71E-06        | 12.14      | 202.5136       |
| HDR through Single Strand Annealing (SSA)_R-HSA-5685938 | 4/37 | 0.00262         | 19.84      | 197.2621       |
| Presynaptic phase of homologous DNA pairing and strand exchange_R-HSA-5693616 | 4/39 | 0.00313         | 18.82      | 183.1741       |
| Event                                                                 | ID      | Score  | Fold   | p-value    |
|----------------------------------------------------------------------|---------|--------|---------|------------|
| Synthesis of DNA_R-HSA-69239                                        | 7/97    | 9.08E-05 | 13.24  | 182.8151   |
| G2/M Checkpoints_R-HSA-69481                                        | 9/150   | 1.85E-05 | 11.01  | 174.3159   |
| M/G1 Transition_R-HSA-68874                                         | 6/82    | 4.16E-04 | 13.43  | 162.1024   |
| DNA Replication Pre-Initiation_R-HSA-69002                           | 6/82    | 4.37E-04 | 13.43  | 162.1024   |
| HDR through Homologous Recombination (HRR)_R-HSA-5685942             | 5/64    | 0.00165  | 14.33  | 151.4058   |
| Extension of Telomeres_R-HSA-180786                                  | 3/28    | 0.01787  | 19.66  | 150.7661   |
| Resolution of Sister Chromatid Cohesion_R-HSA-250025                 | 6/99    | 0.00113  | 11.12  | 122.1646   |
| Mitotic Prometaphase_R-HSA-68877                                     | 6/107   | 0.00155  | 10.29  | 108.475    |
| Homology Directed Repair_R-HSA-5693538                                | 6/118   | 0.0026   | 9.33   | 93.21054   |
| Assembly of the pre-replicative complex_R-HSA-68867                   | 4/67    | 0.01833  | 10.95  | 83.45547   |
| Regulation of DNA replication_R-HSA-69304                            | 4/75    | 0.0262   | 9.786  | 70.36809   |
| DNA Double-Strand Break Repair_R-HSA-5693532                         | 6/145   | 0.00686  | 7.593  | 67.18344   |
| G2/M DNA damage checkpoint_R-HSA-69473                               | 4/78    | 0.02905  | 9.41   | 66.27318   |
| HDR through Homologous Recombination (HR) or Single Strand Annealing (SSA)_R-HSA-5693567 | 5/112 | 0.01573  | 8.191  | 64.72335   |
| RHO GTPases Activate Formins_R-HSA-5663220                           | 5/114   | 0.01576  | 8.048  | 62.93194   |
| DNA Repair_R-HSA-73894                                                | 9/285   | 0.00161  | 5.794  | 61.12078   |
| Chromosome Maintenance_R-HSA-73886                                   | 4/86    | 0.04094  | 8.534  | 56.99652   |
| Separation of Sister Chromatids_R-HSA-2467813                        | 6/162   | 0.01146  | 6.796  | 56.04436   |
| Regulation of TP53 Activity through Phosphorylation_R-HSA-6804756    | 4/89    | 0.04552  | 8.247  | 54.0272    |
| Mitotic Anaphase_R-HSA-68882                                         | 6/173   | 0.01541  | 6.364  | 50.24109   |
| Mitotic Metaphase and Anaphase_R-HSA-2555396                         | 6/174   | 0.01547  | 6.327  | 49.75805   |
| Transcriptional Regulation by TP53_R-HSA-3700989                     | 9/348   | 0.00602  | 4.745  | 42.76202   |
| Regulation of TP53 Activity_R-HSA-5633007                            | 5/151   | 0.04459  | 6.076  | 39.80423   |
| M Phase_R-HSA-68886                                                  | 7/268   | 0.02379  | 4.793  | 35.03605   |
## Supplementary Table S6. Commonly identified IRF3 target genes by integration of IRF3 binding within 2kb distance to TSS and both shRNAs-mediated IRF3 -depleted transcriptome of MM.1S cells

### Activated genes

| Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  | Gene  |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| CSRP1 | SEL1L | TYRO3 | Kat2b | Kpna6 | Fem1b | Sclca3 | Lsm10 | Cpox  | Arhgap11a | Akap2 | Uhrf1bp | Cs    | Lrrc20 | Irs1  | Calm3 | Hdac9 | Cdyll | Ncapg2 | Zdhhc7 | Rrbp1 | Rab3b | Hdgf  | Naa50 | Smc6  | Ezy   | Lonp1 | Eya3  | Cse1l | Hnrrnpul2b | Pgm1  | C19orf47 | Sf3b3 | Esrra | Pcnt  | Rnpepl1 | Dsn1  | Nus1  | Suclg1 | Tomm40 | Lrrc59 | Ak2    | Lrig1 | Ccl3  | Cgn   | Recql | Nxe3  | Ccm2  | Atp6ap1 | Faml11b | Clcl3 | Erc1  | Tbl1x | Hbs1l | Sipa1 | Slc2a4a | Lrrc57 | Cry1  | Stag1 | Pik3ap1 | Nudt3 | Slc37a4 | Nop9  | Hla-e | Mto1  | Slc39a7 | Pkeh2b | Npnt  | Chchd2 | Rad51ap1 | Ssrp1 | Rnf168 | Cerk  | Slc31a1 | Foxo3 | Mpc1  | Poles3 | Rap1b | Gstc4 | Cn0tl6 | Ddx2 | Mprps24 | Ybx1  | Pigx  | Crebl2 | Mis18a | Btaf1 | Mcm5  | P2x5  | Rab11fip5 | Znf275 | Cdc25c | Eif4h | Rbbp5 | Ern1  | Usf2  | Arap1 | Tmc5  | Lrrc8a | Tmem106c | Xpo7  | Golim4 | Nrn1  | Polq1 | Tchp  | Slc2a4a | Cbl   | Uqcrfs1 | Cytip  | Ug2t2b17 | Sclc38a1 | Tex261 | Rusc1 | Prps2 | Rbm3  | Amotl1 | Usf3  | Fanci  | Spats2 | Slc36a1 | Scd   | Lamp5  | M57   | Myh10 | Slk    | Dhcr24 | Wdr7  | Stk24 | Slc2a5a | Srebfb1 | Samd9l | Emilin1 | Nin   | Fkb4p | Slc4a7 | Sclca8 | Coa4  | Ubxn2a | Rad21 | Tp53inp2 | Casp3  | Feni   | Tram2 | Cd3v | Pdik1l | Glcc1  | Ddost | Spag9 | Atfip10d | Ckap2l | Hac3d  | Tpm3  | Snx12 | Eaf2  | Nek4  | Xiap  | Pkmty1 | Slc16a14 | Tln1  | Trappcb6 | Tnxrdd | Hipk2 | Smc5  | Atpp6ap2 | Mfn2  | Dpf3  | KCna3 | Fuc2a | Slc4a2 | Hnrrph2 | Ralbp1 | Imm7  | Urb1   | Skap2 | Ampd1 | Noc3l | Stard4 | Gmpmb  | Mfsd6 | Pradc1 | Myc1  | Otud7b | Arf1  | Uqccrc1 | Gde1  | Top3a | Prps1 | Prr15 | Sdf2l1 | Foxred2 | Adar  | Tec    | Ccd4c  | Dennd5 | Cd2ap | Golt1b | Soat1  | Ddrkg1 | Lyar  | Cdksrap2 | Mapk33 | Scl2a5a | Atppa | Prkci  | Atppb3 | Tcf19  | Fancg  | Snrnp25 | Endod1 | Kbtbd8 | Mprip | Synm1 | Pdia4 | Myo18a | Mcm3  | Tppp1 | Paps51 | Rad51d | Itpripl1 | Smdch1 | Gle1   | Idh3a  | Tnnt2  | Cnnf  | Mdfic  | Cobll1 | Tacc1  | Akrb1  | Var2s | Ccdc28c5 | Diaph1 | Got1   | Ngly1 | C3orf70 | Mtus1  | Znf672 | Prdm1  | Colgal1t | Mrpl27 | Mcur1 | Srpk1 | Lrp8   | Nnt   | Neus  | Rcc2  | Tex2  | Maccl  | Anln4  | Micu1  | Tor3a | Mcm8   | Chst15 | Bloc1s5 | Tnpo2 | Gltp   | Insr  | Cxox1b | Lmo7  | Rnmd5b | Sefh51 | Suz12  | Tiam1  | Mcc    | Gdf11  | Dhfr  | Cln6  | Arfgf2 | Sec24a | Stk26  | Mcm7  | Slamf7 | Foxn3  | Sptbn1 | Smtmn | Ef28  | Parg  | St3gal6 | Rad51  | Mad2l1 | Tmem214 | Nf2    | Snx30  | Jgf2bp2 | Igf2b2 | Isg20 | Ndrg1 | Wdfy1  | Msh2  | Lrrk1  | Tcbd10b | Fnip2  | Idh2   | Fbxw4  | Dst   | Ube3c | Srpk2 | Naga  | C2orf88 | Arsb  | Tnfrsf13b | Rablb6 | Micall1 | Dtl    | Dlg5  | Tym5  | Cd99l2 | Man1a1 | Cdan1  | Atg3  | Mrps16 | Pja2   | N4bp2  | Manea  | Cav1  | Hemgn  | Tp73  | Lrrc8b | Cyp26a1 | Elk4   | Ndufb10 | Cap1   | Dennd3 | Ttl    | Ocrl  | Mcm2  | Lss   | Utp23  | Dcps  | Higda1 | Cep55  | Bicd2  | Alhds1a | Syk    | Ccasp  | Skal  | Hydro1 | Pou2af1 | Znf770 | Psmg1  | Mef2d | Mast2  | Twf1   | Jrh2   | Abca5 | Smoc1  | Ankrd1 | Mrpl55 | Serpinb6 | Rgs9  | Hnrnpul2  | Irf4   | Stat2  | Afg3l2  | Paf1  | Gne  | Pigu   | Kif20b  | Ell   | Ap153  | Ccchrc1 | Znf106d | Dnacj3  | Rap2c  | Br13bp | Zbed4  | Nab1  | Otul1n | Acere3 | Dnmt1  | Zdhhc20  | Tmed5  | Polr2d  | Ab2    | Smc1a  | Ldlr  | Tcf4   | Grwd1  | Sec22c | Cd4    | Arhgold  | Dcafl2  | Mthfd2 | Uspl  | Sod2  | Rbsn  | Grhl1  | Ncaph2 | Zwi4t  | Nanp  | Rfw3  | Vapa   | Arfgf3e | Seama5a | Eif4e3  | Dvl2  | Rabla3 | Zdhhc23 | Kansl3  | Xylb  | Sigmar1 | Kiaa0044 | Pm20d2  | Pdp2  | Mcm6  | Nasp  | Shmt2 | RbBP4 | Polr3d | SMC2  | Rnaseh2a | St6gal1 | Ill10a | Uap1l1 | Ckap4  | Rrm2  | Phhl1n | Serinc5 | Aco2  | Glc18  | Kiaa0513 |
| Gene 1   | Gene 2   | Gene 3   | Gene 4   | Gene 5   | Gene 6   | Gene 7   | Gene 8   | Gene 9   | Gene 10  | Gene 11  |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| BAZ1B   | MTCH2   | HEXA    | FADS1   | POLA1   | MAL2    | HIRIP3  | DLAT    | HMGN5   | TMEM201  |
| BMP6    | REXO4   | APEH    | E2F1    | CEP57   | TONSL   | HES6    | TPD52   | ATAD5   | RAB11FIP4|
| GUSB    | KIF15   | UGTT2   | UHRF1   | PFAS    | HAUS2   | ANO8    | CEP57L1 | SULF2   | SLC25A45|
| KIF13B  | IMPA2   | SVIP    | KIF11   | LCLAT1  | NUSAP1  | JAKMIP1 | PHAX    | STRBP   | CAPD52   |
| POLH    | ABCB10  | ACO1    | CHAF1B  | NFIL3   | CCP110  | AARS    | BLVRB   | TAF9B   | ANKRD13A |
| SUB1    | CTT8    | FICD    | PSMD11  | RAD1    | DNMBP   | PASK    | KIAA0232| SNX25   | UBQLN2   |
| ZBED8   | KCTD9   | NUAK1   | CAV2    | ATP2A2  | HEATR5  | MAPK9   | PAQR4   | AYLYRE  | ARHGAP33 |
| PDXK    | COMTD1  | MLLT3   | SLC44A1 | EXOC5   | LANCL1  | CENPJ   | INPP4A  | ARFRP1  | AP1S2    |
| DSCC1   | PDI6    | LMO4    | SPAG5   | EZH2    | DD1AS   | DNAJC11 | KLF12   | NDUFA6  | LGALS17A |
| DMXL1   | EXO1    | CER56   | E2F2    | ID1     | BZW2    | LIME1   | PRKACA  | SLC20A1 | MRPS18C  |
| CDT1    | MSI2    | AAK1    | MRPS36  | SLFN13  | JADE1   | LMNB1   | KCTD1   | RARGD   | HNRNPR   |
| MCM4    | GTF2A1  | SP3     | POM121  | SHCBP1  | CCL4L2  | GB2     | KCNQ5   | NDUF51  | HMCE5    |
| MYEOV   | ZNF79I1 | ATIC    | IPO9    | USP37   | HMGN2   | TP53NP1 | NOLC1   | SLC10A7 | ANKRD33B |
| BLM     | PRRG4   | KIF4A   | HMGB3   | KANK2   | PABPC4  | TCP1    | MPC2    | CHST12  | HNRNPF   |
|POC1B | SPAG7 | FDPS | LMBRD2 | UBE2H | PPM1K | DEGS1 | HSPA8 | TMPO | SLC25A11 |
| SKIL    | ATP8B2  | PHIP    | TTL5    | RNGT    | TWILCH  | PAIP2B  | RAB26   | STIM1   | RAB27A   |
| CBX3    | WDR76   | XRCC6   | ITPKA   | BARD1   | DOCK8   | SLC7A5  | MTHFD1  | TIMM21  | TNFRSF21 |
| CDC4A7L | ACAA2   | NAPB2   | PIP5K1B | LRMP    | CDC45   | DONSON  | CYC1    | FZD5    | GAPTCH11 |
| FZD3    | BAMP8B  | AARS2   | SH3RF1  | SEL1L3  | EBP     | ZNF142  | HJURP   | MBD2    | PSMB8-AS1|
| SAE1    | DOCK9   | HEYL    | HYI     | API5    | ZFP91   | FH      | APPL1   | PRKCE   | TMEM109  |
| ZNF641A | ARID5B  | IL6ST   | STK35   | GAB2    | PYCR2   | IPO7    | SLBP    | PSMC3P1 | SUPT16H  |
| LAT2    | WNK1    | DHR5S   | AFF4    | RNF103  | PRKDC   | TBC1D30 | HS2ST1  | RASGRP3 | ARHGAP10 |
| STT3A   | XRCC5   | STK4    | FZD1    | PLCB3   | PRR11   | MFAP3   | TIMELESS | L3MBTL2 | NDUF56   |
| SOS1    | ASNSD1  | HSPAA4L | EML6    | CENPL   | ERLN1L  | LIG1    | MAN2A2  | ARL6IP6 | KATNB1   |
| TTF2    | L2GHGD  | ADD2    | PLEKHA7 | DCK     | CEPI92  | PAXIP1  | TRIM26  | SFXN5   | LRRC45   |
| NPC1    | TPP6R3  | DERA    | ADM     | HIF1X   | YWHAQ   | FNDC3B  | RAB4A   | SRGAP2  | ANKRD10  |
| MSX1    | PHYH    | TTL7    | VARS    | CCNE1   | PSMD3   | GRCP5D  | GINS3   | ERGIC1  | PGRMC2   |
| LRR8C8  | GFL1    | PIGO    | INSIG1  | POLE    | DOT1L   | ZBP1    | UBQLN1  | XRAA1   | ZMPSTE24 |
| TRIB1   | HPS3    | AURKA   | PNP     | RCAN1   | SLC38A2 | TUBB    | MIEF1   | MLEC    | DNMT3B   |
| CDH2    | THOP1   | DAZAP1  | DPYS1L  | NCAM1   | NCAPD2  | PLHPP1  | ZNF215  | MTMR12  | HNRNPAB  |
| RTEL1   | OSGN2   | ASCC3   | STK39   | JAKMIP1 | MYB     | NSFL1C  | GNPNAT1 | DOLPP1  | METTL21A |
| ACTB    | KIF18B  | SFXN4   | iTG4D   | CDS2    | LRCH3   | CP2K    | GHITM   | KNOP1   | SMARC2A  |
| KIF14   | MMS22L  | TLR4    | URB2    | SFXN2   | LIMD1   | TAPBP   | SPCS2   | PRPSAP1 | C16orf95 |
| FKBPS   | BBS7    | JPH1    | GALNT6  | LIN54   | PDK1    | SLAIN1  | MRPS1B  | LRRCC1  | DENND1B  |
| RMB47   | UBR7    | FRMD6   | ST7     | NANS    | PUS7L   | TRIM41  | SRGAP2  | SLC7A1  | TNFRSF17 |

**Repressed genes**

| Gene 1 | Gene 2 | Gene 3 | Gene 4 | Gene 5 | Gene 6 | Gene 7 | Gene 8 | Gene 9 | Gene 10 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|
| CRYBB1 | IQCH   | GASS   | HIF1A  | RNF123 | TMED4  | CSTB   | WDR48  | PCYOX1 | TNFRSF10D |
| E124   | VPS9D1 | SMOX   | IQSEC1 | NAT9   | MCAT   | GALNT18| DAZAP2 | CSNK1G3| TMEM80   |
| SF3B3  | BIRC3  | HDAC10 | WNT10A | KDM3A  | ARL6IP5| MFSD10 | GRHL3  | INAFA2 | PAX8-AS1 |
| BMF    | CRTC2  | MRPL10 | IL27A  | RPS7   | HIVEP3 | LY86   | PCDH1  | TARB1  | ATP6V1C2 |
| BSPRY  | EML4   | DNAJA1 | GNA12  | POLDIP | SYTL2  | CLTA   | PCED18-A| GOLM1  | CDC42E1  |
| MIAT   | IFNGR2 | RPS6K1A| RPL7A  | P2RX1  | SLC16A3| RPS24  | RPL31  | TYW1   | FAM86C2P|
| RPS28  | IER5   | HOOK2  | KPTN   | FAH2   | ARSA   | DLGAP4 | STK17A | NEK6   | CAPRIN2  |
| RPL8   | LPIN1  | PRPSAP2| RPS19  | CD70   | STAT4  | WDR60  | FTSJ3  | CCR10  | L3MBTL1  |
| MAPT   | LRWD1  | C3AR1  | RPRG   | SGK1   | DTX3L  | IDNK   | GOLGA8A| RPS3   | ASB16-AS1|
| SYF2   | NMRK1  | RPL36  | RPL23  | FTL    | NPR3   | PIP5K1 | UNC93B1| CTNNB1 | MAPKAPK2 |
| PHPT1 | RCSD1 | APOL2 | CSK | RPL34 | SLC39A4 | TRMT1 | POMT1 | PPP1R10 | LINC00893 |
|-------|-------|-------|-----|-------|---------|-------|-------|----------|-------------|
| IL2RG | PLEKH01 | RPL15 | DEF6 | ARL2 | NARF | CASP8 | RPS23 | NFKB2 | ARHGEF40 |
| GNA12 | RPS29 | TP53I11 | ZNF644 | KY | NFKB1A | TRAF1 | RPL17 | SCNN1G | C11orf68 |
| MKNK2 | PTTG1IP | RFL | IZUMO4 | DDIT4 | EHD1 | PIGQ | ASB13 | HMOX1 | GALNT10 |
| SYNGR2 | CCDC92 | R3HDM4 | RPL27A | TMC6 | ARPC5 | GALNT2 | RNF207 | CCDC24 | APOBEC3F |
| JAK3 | STAT6 | B4GALTS | NABP1 | AZIN2 | RPL21 | ALDH1B1 | RPS27L | RPL27 | THUMP3D-AS1 |
| UBA52 | LRC1 | SSBP4 | ZBTB10 | RPS3A | RPS8 | CD86 | PARP9 | LUC7L3 | DENND5A |
| PSMB5 | VT1B | ZMIZ2 | IFI16 | RPL35A | SOAT2 | SECTM1 | RPL10 | RPL18A | SLC25A23 |
| PEA15 | SPPL2B | UNC119 | ACADVL | APOL1 | CRK | RPS9 | ZNF276 | KL3F | ATP6V0E1 |
| MX1 | PLXNA3 | OFD1 | CLSTN1 | RPL6 | PRNP | BLVRA | CTDNEP1 | COMT | NIPSNAP1 |
| SCNN1B | IGF1R | ZF3P6L1 | MRFAP1 | WASF1 | GPR137 | BAX | WDR73 | CFAP70 | SRP14-AS1 |
| BSG | RPL22 | RPS10 | OGT | SUMF2 | RPS21 | ARPC1A | CCDC86 | IKBKE | ZSCAN30 |
| MOB3A | RGS14 | AMPD3 | GPR108 | RPS6 | FAS | ARL1 | RALY-AS1 | HRAS | STX16-NPEPL1 |
| RALGDS | ELK3 | C11orf1 | VAMP3 | PARP8 | GABPB2 | TSPAN31 | EIF3F | NEK11 | FAM171A1 |
| PLXNB2 | ERC1 | LMAN2L | OXL1 | CXXC5 | GATC | RPS14 | GALNT7 | SLC2A11 | KCNQ1OT1 |
| ECE1 | NOTCH2 | NRBP1 | Dyrk2 | ARHGA | RPL38 | RGL1 | CABIN1 | Clorf52 | TMEM255B |
| SF1 | TXLB | FAU | IRF5 | GNB1L | MZF1 | GBP4 | CCDC71L | VASP | TNFRSF10B |
| SNRPA1 | DMWD | EIF3G | TMC8 | GTF3C2 | ARID5A | MAP2K3 | CSNK1G2 | CLPMT1 | DENND6A |
| SLC2A1 | LARS | RPL36A | BCL3 | UBXN8 | CYB5R1 | PPFIA4 | GRB2 | TRADD | SYNGAP1 |
| FJK1 | SLC41A3 | RPLP0 | ST14 | CUL7 | EME2 | IRF2BP2 | ABHD17A | NINJ1 | TMEM259 |
| CTSA | NMI | ZNF580 | ANXA4 | ZMAT1 | RPS15 | RPL10A | ATP6V1B2 | RPL37A | CDC42BPA |
| MR1 | VPS28 | ISCU | UBFD1 | ICK | RPL13 | ZNF467 | RNF170 | EIF3D | NAPA-AS1 |
| REL2 | POLN | EPAS1 | HSF4 | YBX3 | CC2D1A | C1RL-AS1 | TGIF2 | GRINA | HRNRPA0 |
| TRIP11 | CDK6 | TPCN1 | RHPN1 | E1F4G3 | LENG8 | C6orf62 | VEGFA | PHLD3 |
**Supplementary Table S7. Reactome pathways_Enrichr pathway enrichment analysis of the 770 genes predicted to be directly activated by IRF3 binding to their regulatory areas**

| Name of the enriched pathways (Homo sapiens)                                             | Overlap | Adjusted P-value | Odds Ratio | Combined Score |
|-------------------------------------------------------------------------------------------|---------|------------------|------------|----------------|
| Unwinding of DNA_R-HSA-176974                                                             | 9/12    | 1.79E-08         | 19.48      | 468.9          |
| DNA strand elongation_R-HSA-69190                                                         | 12/32   | 4.14E-07         | 9.74       | 201.0831       |
| G1/S-Specific Transcription_R-HSA-69205                                                  | 8/17    | 1.16E-05         | 12.22      | 199.2733       |
| Activation of the pre-replicative complex_R-HSA-68962                                     | 11/30   | 1.83E-06         | 9.524      | 178.5999       |
| Pyruvate metabolism and Citric Acid (TCA) cycle_R-HSA-71406                              | 13/48   | 3.48E-06         | 7.035      | 124.5455       |
| Citric acid cycle (TCA cycle)_R-HSA-71403                                                | 7/19    | 2.99E-04         | 9.569      | 118.7075       |
| IRS activation_R-HSA-74713                                                                | 3/5     | 0.01784          | 15.58      | 117.3632       |
| Cell Cycle_R-HSA-164017                                                                   | 65/566  | 4.39E-12         | 2.983      | 99.87958       |
| Resolution of D-loop Structures through Synthesis-Dependent Strand Annealing (SDSA)_R-HSA-5693554 | 8/26    | 3.00E-04         | 7.992      | 99.4886        |
| Activation of ATR in response to replication stress_R-HSA-176187                          | 10/37   | 7.77E-05         | 7.02       | 97.6146        |
| E2F mediated regulation of DNA replication_R-HSA-113510                                   | 9/33    | 2.40E-04         | 7.084      | 90.13903       |
| Mitotic G1-G1/S phases_R-HSA-453279                                                       | 23/136  | 6.71E-07         | 4.393      | 87.58288       |
| Cell Cycle, Mitotic_R-HSA-69278                                                           | 54/462  | 2.67E-10         | 3.036      | 87.0802        |
| G1/S Transition_R-HSA-69206                                                               | 20/112  | 2.03E-06         | 4.638      | 85.77785       |
| Signal attenuation_R-HSA-74749                                                           | 4/10    | 0.01419          | 10.39      | 81.81775       |
| S Phase_R-HSA-69242                                                                      | 21/124  | 2.03E-06         | 4.399      | 80.76056       |
| DNA Replication_R-HSA-69306                                                               | 18/105  | 1.25E-05         | 4.453      | 71.86397       |
| POLB-Dependent Long Patch Base Excision Repair_R-HSA-110362                               | 3/7     | 0.04371          | 11.13      | 70.53198       |
| M/G1 Transition_R-HSA-68874                                                               | 15/82   | 4.55E-05         | 4.751      | 69.16752       |
| DNA Replication Pre-Initiation_R-HSA-69002                                                | 15/82   | 4.86E-05         | 4.751      | 69.16752       |
| Resolution of D-Loop Structures_R-HSA-5693537                                             | 8/33    | 0.0015           | 6.297      | 66.13454       |
| Synthesis of DNA_R-HSA-69239                                                             | 16/97   | 7.73E-05         | 4.284      | 59.84078       |
| DNA Double-Strand Break Repair_R-HSA-5693532                                              | 21/145  | 2.05E-05         | 3.762      | 58.5392        |
| Assembly of the pre-replicative complex_R-HSA-68867                                       | 12/67   | 5.10E-04         | 4.652      | 54.40799       |
| IRF3-mediated induction of type I IFN_R-HSA-3270619                                       | 4/13    | 0.03282          | 7.992      | 53.88441       |
| The citric acid (TCA) cycle and respiratory electron transport_R-HSA-1428517             | 21/153  | 4.79E-05         | 3.565      | 52.19291       |
| Resolution of D-loop Structures through Holliday Junction Intermediates_R-HSA-5693568   | 7/32    | 0.00731          | 5.682      | 49.09321       |
| DNA Repair_R-HSA-73894                                                                    | 32/285  | 9.61E-06         | 2.916      | 48.36416       |
| Early Phase of HIV Life Cycle_R-HSA-162594                                               | 4/14    | 0.04018          | 7.421      | 47.76672       |
| HDR through Homologous Recombination (HRR)_R-HSA-5685942                                  | 11/64   | 0.00156          | 4.464      | 46.55633       |
| Regulation of DNA replication_R-HSA-69304                                                 | 12/75   | 0.00155          | 4.156      | 43.65912       |
| Homologous DNA Pairing and Strand Exchange_R-HSA-5842                                      | 8/42    | 0.0073           | 4.947      | 42.8895        |
| Pathway                                                                 | Overlap | Adjusted P-value | Odds Ratio | Combined Score |
|------------------------------------------------------------------------|--------|------------------|------------|----------------|
| G2/M Checkpoints_R-HSA-69481                                           | 19/150 | 3.60E-04         | 3.29       | 40.04601       |
| Synthesis of substrates in N-glycan biosynthesis_R-HSA-446193          | 10/63  | 0.00611          | 4.123      | 36.71012       |
| Cholesterol biosynthesis_R-HSA-191273                                  | 5/23   | 0.04087          | 5.647      | 36.43604       |
| Cell Cycle Checkpoints_R-HSA-69620                                     | 21/182 | 4.76E-04         | 2.997      | 35.38072       |
| Presynaptic phase of homologous DNA pairing and strand exchange_R-HSA-5693616 | 7/39   | 0.02082          | 4.662      | 34.28883       |
| Homology Directed Repair_R-HSA-5693538                                  | 15/118 | 0.00236          | 3.302      | 32.8438        |
| Sialic acid metabolism_R-HSA-4085001                                   | 6/33   | 0.03831          | 4.723      | 30.85951       |
| Regulation of TP53 Activity through Phosphorylation_R-HSA-6804756      | 12/89  | 0.00665          | 3.502      | 30.78408       |
| Orc1 removal from chromatin_R-HSA-68949                                 | 10/70  | 0.01299          | 3.711      | 29.73416       |
| Switching of origins to a post-replicative state_R-HSA-69052           | 10/70  | 0.01333          | 3.711      | 29.73416       |
| AURKA Activation by TPX2_R-HSA-8854518                                  | 10/72  | 0.01486          | 3.608      | 28.07016       |
| Removal of licensing factors from origins_R-HSA-69300                   | 10/72  | 0.01521          | 3.608      | 28.07016       |
| Regulation of cholesterol biosynthesis by SREBP (SREBF)_R-HSA-1655829   | 8/55   | 0.03296          | 3.778      | 25.59725       |
| Post-translational protein modification_R-HSA-597592                   | 42/521 | 3.74E-04         | 2.094      | 25.31411       |
| HDR through Homologous Recombination (HR) or Single Strand Annealing (SSA)_R-HSA-5693567 | 13/112 | 0.01432          | 3.015      | 23.78714       |
| Biosynthesis of the N-glycan precursor (dolichol lipid-linked oligosaccharide, LLO) and transfer to a nascent protein_R-HSA-446193 | 10/78  | 0.02488          | 3.33       | 23.75895       |
| SUMOylation_R-HSA-2990846                                              | 12/101 | 0.01708          | 3.086      | 23.44182       |
| Asparagine N-linked glycosylation_R-HSA-446203                         | 24/259 | 0.00327          | 2.407      | 23.08093       |
| Transcriptional Regulation by TP53_R-HSA-3700989                        | 30/348 | 0.0018           | 2.239      | 22.95018       |
| Loss of proteins required for interphase microtubule organization?from the centrosome_R-HSA-380284 | 9/69   | 0.03412          | 3.388      | 22.59023       |
| Loss of Nlp from mitotic centrosomes_R-HSA-380259                      | 9/69   | 0.03473          | 3.388      | 22.59023       |
| Mitotic Prometaphase_R-HSA-68877                                       | 12/107 | 0.02593          | 2.913      | 20.60454       |
| SUMO E3 ligases SUMOylate target proteins_R-HSA-310                    | 11/96  | 0.03264          | 2.976      | 20.13795       |
| Mitotic G2/G2/M phases_R-HSA-453274                                  | 17/175 | 0.01527          | 3.252      | 19.50638       |
| Sphingolipid metabolism_R-HSA-4281517                                  | 9/74   | 0.04999          | 3.159      | 19.49119       |
| Regulation of PLK1 Activity at G2/M Transition_R-HSA-2                 | 10/87  | 0.04516          | 3.867      | 18.77097       |
| Regulation of TP53 Activity_R-HSA-5633007                              | 15/151 | 0.0236           | 2.58       | 18.5986        |
| Metabolism of proteins_R-HSA-392499                                   | 69/1074| 0.00122          | 1.669      | 17.99519       |
| Epigenetic regulation of gene expression_R-HSA-212165                 | 12/115 | 0.04069          | 2.71       | 17.45593       |
| G2/M Transition_R-HSA-69275                                           | 16/173 | 0.03196          | 2.402      | 16.39538       |
| HIV Infection_R-HSA-162906                                            | 19/222 | 0.03           | 2.223      | 15.3561        |
| Metabolism_R-HSA-1430728                                              | 105/190| 0.00503          | 1.429      | 13.04787       |

Enrichr pathway enrichment analysis of the 339 genes predicted to be directly repressed by IRF3 binding to their regulatory areas
| Pathway                                                                                                                                  | **Gene IDs** | **Log2 Fold Change** | **Estimate** | **SE** |
|----------------------------------------------------------------------------------------------------------------------------------------|-------------|----------------------|--------------|--------|
| Formation of a pool of free 40S subunits                                                                                              | 42/96       | 6.6E-46              | 25.81        | 2874.639 |
| 3' -UTR-mediated translational regulation                                                                                              | 42/106      | 2.4E-44              | 23.38        | 2481.838 |
| L13a-mediated translational silencing of Ceruloplasmin expression                                                                          | 42/106      | 3E-44                | 23.38        | 2481.838 |
| GTP hydrolysis and joining of the 60S ribosomal subunit                                                                               | 42/107      | 3.2E-44              | 23.16        | 2447.439 |
| Peptide chain elongation                                                                                                               | 39/84       | 3.3E-44              | 27.39        | 2913.375 |
| Viral mRNA Translation                                                                                                                 | 39/84       | 4.9E-44              | 27.39        | 2913.375 |
| Eukaryotic Translation Termination                                                                                                     | 39/87       | 7.2E-44              | 26.45        | 2766.136 |
| Selenocysteine synthesis                                                                                                               | 39/87       | 8.2E-44              | 26.45        | 2766.136 |
| Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)                                                                 | 39/89       | 1.8E-43              | 25.85        | 2674.693 |
| Eukaryotic Translation Elongation                                                                                                      | 39/89       | 2E-43                | 25.85        | 2674.693 |
| Eukaryotic Translation Initiation                                                                                                       | 42/114      | 4.1E-43              | 21.74        | 2227.01  |
| Cap-dependent Translation Initiation                                                                                                     | 42/114      | 4.4E-43              | 21.74        | 2227.01  |
| Selenoamino acid metabolism                                                                                                            | 40/111      | 1.5E-40              | 21.26        | 2051.558 |
| Nonsense Mediated Decay (NMD) enhanced by the Exon Junction Complex (EJC)                                                                 | 39/106      | 5.4E-40              | 21.71        | 2063.111 |
| Nonsense-Mediated Decay (NMD) R-HSA-975957                                                                                               | 39/106      | 5.8E-40              | 21.71        | 2063.111 |
| SRP-dependent cotranslational protein targeting to membrane R-HSA-1799339                                                                | 39/107      | 7.8E-40              | 21.5         | 2034.399 |
| Translation R-HSA-72766                                                                                                                | 42/151      | 2.4E-37              | 16.41        | 1457.546 |
| Influenza Life Cycle R-HSA-168255                                                                                                       | 40/136      | 1.3E-36              | 17.35        | 1511.003 |
| Influenza Viral RNA Transcription and Replication R-HSA-168273                                                                          | 39/128      | 2.3E-36              | 17.98        | 1554.129 |
| Influenza Infection R-HSA-168254                                                                                                        | 40/147      | 3.8E-35              | 16.05        | 1342.074 |
| Major pathway of rRNA processing in the nucleolus R-HSA-6791226                                                                          | 40/166      | 7.3E-33              | 14.22        | 1112.89  |
| rRNA processing R-HSA-72312                                                                                                             | 40/180      | 2.2E-31              | 13.11        | 980.9076 |
| Metabolism of amino acids and derivatives R-HSA-71291                                                                                   | 43/335      | 1.6E-23              | 7.573        | 429.397  |
| Infectious disease R-HSA-5663205                                                                                                        | 43/348      | 7.1E-23              | 7.29         | 402.021  |
| Formation of the ternary complex, and subsequently, the 43S complex R-HSA-72695                                                          | 19/50       | 1.6E-19              | 22.42        | 1062.841 |
| Translation initiation complex formation R-HSA-72649                                                                                     | 19/57       | 2.7E-18              | 19.67        | 874.6005 |
| Ribosomal scanning and start codon recognition R-HSA-72702                                                                             | 19/57       | 2.9E-18              | 19.67        | 874.6005 |
| Activation of the mRNA upon binding of the cap-binding complex and eIFs, and subsequent binding to 43S R-HSA-72662                                                                             | 19/58       | 3.9E-18              | 19.33        | 852.1469 |
| Disease R-HSA-1643685                                                                                                                  | 51/725      | 2.4E-16              | 4.15         | 165.6816 |
| Metabolism of proteins R-HSA-392499                                                                                                     | 58/1074     | 1.7E-13              | 3.186        | 106.1788 |
| Gene Expression R-HSA-74160                                                                                                             | 66/1631     | 1.1E-09              | 2.387        | 58.50422 |
| Metabolism R-HSA-1430728                                                                                                                | 65/1908     | 1.7E-06              | 2.01         | 34.50708 |
| Regulation of necroptotic cell death R-HSA-5675482                                                                                       | 5/14        | 0.00011              | 21.07        | 272.6659 |
| RIPK1-mediated regulated necrosis R-HSA-5213460                                                                                           | 5/16        | 0.00022              | 18.44        | 224.7141 |
| Regulation                                                                 | #/Total | P-value | Log2FoldChange | CombinedScore |
|--------------------------------------------------------------------------|---------|---------|---------------|---------------|
| Regulated Necrosis_R-HSA-5218859                                         | 5/16    | 0.00023 | 18.44         | 224.7141      |
| Death Receptor Signalling_R-HSA-73887                                    | 7/48    | 0.00065 | 8.604         | 95.389        |
| Dimerization of procaspase-8_R-HSA-69416                                  | 4/11    | 0.00096 | 21.45         | 227.8933      |
| Regulation by c-FLIP_R-HSA-3371378                                       | 4/11    | 0.00098 | 21.45         | 227.8933      |
| CASP8 activity is inhibited_R-HSA-5218900                                 | 4/11    | 0.00101 | 21.45         | 227.8933      |
| Toll-Like Receptors Cascades_R-HSA-168898                                 | 11/140  | 0.00105 | 4.635         | 48.70507      |
| TRIF-mediated TLR3/TLR4 signaling_R-HSA-937061                           | 9/97    | 0.00143 | 5.474         | 55.4238       |
| Toll Like Receptor 3 (TLR3) Cascade_R-HSA-168164                         | 9/97    | 0.00146 | 5.474         | 55.4238       |
| MyD88-independent TLR3/TLR4 cascade_R-HSA-166166                         | 9/97    | 0.0015  | 5.474         | 55.4238       |
| Toll Like Receptor 4 (TLR4) Cascade_R-HSA-166016                         | 10/122  | 0.00153 | 4.836         | 48.50032      |
| Activated TLR4 signalling_R-HSA-166054                                   | 9/112   | 0.0042  | 4.741         | 42.66809      |
| Programmed Cell Death_R-HSA-5357801                                     | 11/166  | 0.00428 | 3.909         | 35.02182      |
| Ligand-dependent caspase activation_R-HSA-140534                         | 4/17    | 0.00517 | 13.88         | 121.1514      |
| Regulation of TNFR1 signaling_R-HSA-5357905                             | 5/31    | 0.00523 | 9.516         | 83.13201      |
| Innate Immune System_R-HSA-168249                                       | 28/807  | 0.00896 | 2.047         | 16.69644      |
| SHC-related events triggered by IGF1R_R-HSA-2428933                      | 3/9     | 0.0115  | 19.67         | 155.0901      |
| Apoptosis_R-HSA-109581                                                   | 10/163  | 0.01436 | 3.619         | 27.66884      |
| Immune System_R-HSA-168256                                               | 44/1547 | 0.01469 | 1.678         | 12.75707      |
| Regulation of gene expression by Hypoxia-inducible Factor_R-HSA-1234158 | 3/10    | 0.01531 | 17.7          | 133.4916      |
| TNF signaling_R-HSA-75893                                                 | 5/41    | 0.01748 | 7.195         | 53.17565      |
| Iron uptake and transport_R-HSA-917937                                   | 5/43    | 0.02144 | 6.86          | 49.17444      |
| Cellular response to hypoxia_R-HSA-2262749                               | 4/26    | 0.02381 | 9.076         | 63.62731      |
| Cytosolic sensors of pathogen-associated DNA_R-HSA-1834949               | 6/66    | 0.02395 | 5.363         | 37.75556      |
| Signaling by PTK6_R-HSA-8848021                                          | 6/67    | 0.02421 | 5.283         | 36.77103      |
| Regulation of Hypoxia-inducible Factor (HIF) by oxygen_R-HSA-1234174    | 4/26    | 0.02423 | 9.076         | 63.62731      |
| TP53 Regulates Transcription of Death Receptors and Ligands_R-HSA-6803211| 3/12    | 0.02458 | 14.75         | 102.6747      |
| Caspase activation via extrinsic apoptotic signalig pathway_R-HSA-5357769| 4/27    | 0.02623 | 8.74          | 59.98581      |
| VEGFA-VEGFR2 Pathway_R-HSA-4420097                                       | 14/320  | 0.02953 | 2.581         | 17.36617      |
| p38MAPK events_R-HSA-171007                                              | 3/13    | 0.02955 | 13.61         | 91.37597      |
| Signalling to ERKs_R-HSA-187687                                          | 12/253  | 0.03206 | 2.798         | 18.50882      |
| TRAF6 Mediated Induction of proinflammatory cytokines_R-HSA-168180       | 6/72    | 0.0326  | 4.916         | 32.36066      |
| Signaling by VEGF_R-HSA-194138                                           | 14/328  | 0.035   | 2.518         | 16.35759      |
| NGF signalling via TRKA from the plasma membrane_R-HSA-187037            | 15/374  | 0.04364 | 2.366         | 14.81257      |
**Supplementary Table S8. commonly co-regulated genes by both IRF3 and IRF4 in MM.1S cells; Integration of intersection of IRF3 and IRF4 binding within 2kb distance to TSS and both shRNAs-mediated IRF3-depleted transcriptome of MM.1S cells**

| Activated genes |
|-----------------|
| HNRNP | FBXW4 | N4BP2 | LRRC8B | NANP | NASP | CCM2 | KIF11 | SNRNP25 |
| AKAP2 | MYCL | RAB3B | KCTD9 | RGS9 | FAM111B | SREBF1 | GLDC | ALYREF |
| LRRC59 | PABPC4 | DEND5B | GAB2 | GPTACH1 | CDC25C | FEM1B | DHCR24 | RDFW3 |
| RRBP1 | GFI1 | HM13 | FOXRED2 | VAR52 | ZNF672 | ATP8B2 | GDE1 | PRDM1 |
| CCL3L1 | LRRC20 | DCAF12 | SERINC5 | PRR11 | AARS | SYNM | NOC3L | EXO1 |
| PGM1 | HERPUD1 | CDC685C | WDR76 | KANS3 | NCPAH2 | LIG1 | DST | SRPK2 |
| NUDT3 | RBBP5 | UHRF1BP1 | ARKGFE2 | NCAM1 | HYOU1 | CD2AP | SMCHD1 | CCSAP |
| DOLPP1 | SKA1 | SNX30 | GRWD1 | MCUR1 | CAV1 | ARKGFE3 | BMP8B | RMDN5B |
| IRF4 | PLEKH2B | TEX261 | GOLT1B | RBBP4 | SKAP2 | NDRG1 | CSE1L | PPHLN1 |
| SSRP1 | SNX12 | C19orf47 | MPC2 | ABCB10 | POLH | TBL1X | DYSPLS2 | XRC6 |
| CASP3 | ARHGDIB | DSN1 | MIS18A | MACC1 | ZDHHH20 | SLC31A1 | UBR7 | HIGD1A |
| SLC37A4 | TRAM2 | MCM6 | HNRNPUL | CCHCR1 | RAD51AP1 | CDV3 | LYAR | SLC25A44 |
| RALBP1 | STAT2 | FOXO3 | MPC1 | ZNF791 | ATP7A | UAP1L1 | ZDHHH23 | IPO7 |
| DIAPIH1 | PDIA4 | SUZ12 | SP3 | ARFRP1 | BLOC1S5 | MCM6 | HNRNPHE2 |
| RABL6 | C3orf70 | CDDC47 | EIF4E3 | ADD2 | ST6GAL1 | NGLY1 | TMPO | SLC36A1 |
| IL10RA | FANCG | E2F1 | NAB1 | LDLR | SULF2 | CPKAP4 | DHFR | DCPS |
| SCD | KPNA6 | IRS2 | ARAP1 | BLM | SLC36A1 | PHAX | PPP6R3 | TMPO |
| MAP3K3 | UBE2H | TEC | RNASEH2 | RNGTT | DDIAAS | ID3H3A | CCL4L2 | ATIC |
| CAP1 | CREBL2 | MITCH2 | PHYH | SEMA5A | SOAT1 | H1FB | KCNA3 | PMD11 |
| TPM3 | DVL2 | AK2 | LRRC8A | MRPS18C | ESRR2 | MANEA | MANE | SVIP |
| RFX2 | TF21 | HLA-E | ANKR16 | FANC1 | AKB1B | MDFIC | ATP10D | BTA1 |
| SRPK1 | MRPS16 | HIRIP3 | MAN1A1 | HDAC9 | HEMGN | BICD2 | TCHP | COA4 |
| RNF168 | IMPA2 | LSS | TCF4 | BB57 | GLTP | PRKCI | JPH1 | ZWINT |
| SEL1L | SLC39A8 | TMEM106 | L2HGDH | PSMD3 | SLC5A3 | UQCRFC1 | JADE1 | MBD2 |
| MTHFD2 | ACTB | OTUD7B | RUSC1 | POM121 | TOR3A | GOLIM4 | MSI2 | AP13 |
| ADAR | TMEM214 | UBT1 | COMTD1 | NDUFA6 | AMPOT1 | AMPD1 | KNOF1 | GSTCD |
| FNP2 | LONP1 | HIKP2 | MCM2 | SEC24A | MTO1 | SYK | CGN | DOT1L |
| MCM8 | APH | FADS1 | NUSAP1 | SUB1 | CCT8 | GUSB | NDUF51 | LRCH3 |
| CERK | DTL | HEXA | PDP2 | SHCBP1 | ACA2A | SLK | API5 | LRRK1 |
| ERC1 | SMCA1 | TPPP | LSM10 | SLFN13 | TACC1 | SLC25A4 | CCP10 | TNFRSF13B |
| GLE1 | ZBED4 | MRPS24 | JAKMIP2 | UQCRFS1 | DHR513 | HSST1 | RAN1 | ATP6AP1 |
| PMD2RO | RTN4 | PJA2 | D2 | NDUSF1 | LRC3 |
| KIAA0040 | DONMT1 | MCM3 | PFAS | AURKA | COBL1 | USP13 | PIGU | TNFRSF17 |
| TRIM41 | INS | POUP2F1 | LCLAT1 | AFF4 | WDFY1 | SF3B3 | SLC4A1 | HNRNPUL2 |
| SAMD9L | ENDOD1 | LRRG1 | THOP1 | GHTEM | SIGMAR1 | TP53NIP2 | PPM1K | SPSB8-A51 |
| CS | P2RX5 | SLC39A7 | PHOL3 | DIA2 | KIAA0232 | PDXY | NDUF10 | KIF15 | TNFRSF21 |
| XPO7 | TCF19 | UBE3C | SMAC | PRPSAP1 | PARG | DNAJC3 | SPSMC3P | ACO1 |
| POLR2D | TONSL | SLC25A5 | LGALS17A | HSPA8 | POLQ | CLN6 | GNE | XLYB |
| MICAL1 | ATG3 | AFG3L2 | TRIM26 | PD1K1L | POLA1 | MIFN2 | DERA | NANS |
| MCC | FOXN3 | PAF1 | SIPIA1 | SH3RF1 | RAB27A | PDIA6 | PSMG1 | CSRP1 |
| Gene 1 | Gene 2 | Gene 3 | Gene 4 | Gene 5 | Gene 6 | Gene 7 | Gene 8 | Gene 9 | Gene 10 | Gene 11 | Gene 12 | Gene 13 | Gene 14 | Gene 15 | Gene 16 | Gene 17 | Gene 18 | Gene 19 | Gene 20 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| ZNF106 | ATP6AP2 | ABL2   | TEX2   | MRPL27 | RECQL  | CENPJ  | TBC1D10B | SUCLG1 | SLAMF7  | CDK4   | MAST2  | PUS7L  | CDS2   | SFXN2  | KBTBD8 | ID1    | RAB11FIP4 | FICD   | PIGO   | UGCTG7 | SMC5   | LIN54  | CER56  | KAT2B  | HP53   | DNAC11 | XIAP   | TIMELESS | METTL21A | PLCB3  | ZBED8  | SPA5G  | CDK5RAP2 | RABL3  | PAIP2B | RAP2C  | HEYL   | PYCR2  | SLCA2A4S | IL6ST  | HJURP  | PRRG4  | CRY1   | EBP    | ZNF142 | KANK2  | CAPD52 | EYA3   | POLE   | NOCL1  | SLC2A11 | CPOX   | TYMS   | VARS   | POLE3  | RAP1B  | YWHAQ  | SPA7G  | MICU1  | DNMNB2 | LMBRD2 | LRRC8C | MAN2A2 | FDPS   | STK24  | ZMPSTE2 | DOCK8  | CENPL  | RAD51D | FKB4   | UHRF1  | CEP57  | L3MBT2 | RB5N  | DCK    | RETE1  | FZD1   | MSS2L2 | SEC22C | SFXN4  | RAD51  | MEF2D  | MSX1   | DAZAP1 | CYP26A1 | SMTN   | JAKM1P1 | XRC5S  | NCPG2 | SEPS1  | HEATR5A | EXOC5  | LMO7   | ZNF770 | DEGS1  | FNDC3B | CEP55  | ANKRD13A | HES6  | APIS2  | SLC4A2 | PAPSS1 | KIF18B | NUS1   | GALNT6 | CEP57L | DNMT3B |
| NPC1   | EML6   | HAUS2  | TPDS2  | TOMM40 | GIN3S  | NSFL1C | ZFNF25 | TRIB1  | ITGA4   | POC1B  | UBXN2A | SFXN5  | ATP1B3 | BZW2   | UB2    | CDC4S  | WNK1   | RB4A8  | ACO2   | GNPNAT1 | UBQINL1 | LAT2   | ELL    | DENND1B | FDZ5   | ARHGAP10 | ZNF641 | ARL6IP6 | SLC16A14 | RBM47  | KLF12   | MFAP3  | SKIL   | NNR1   | NDF5S6 | LRRCC1 | ISG20  | KTD1T  | DLAT   | STK3S  | ST7    | MRPS18B | GAL36  | TMEM109 | TAPBP  | SLBP   | CHAF1B | PRKACA | RASGRP3 | HMGN2  | STIM1  | USF2   | APPL1  | PRKCE  | LRRCC5 | PGRMC2 | STK39  | ANKRD3S | SAIN1  | PCNT   | MCM4   | TMEM201 | PAXIP1 | C2orf88 | MRPS36 | KATNB1 | PRKDC  | SOS1   | GPRC5D | LIMD1  | PAQR4  | KIF14  | IPO9   | HBS1L  | TNPO2  | ATAD5  | DDOST  | STK4   | KIF13B | PHLPP1 |
| STT3A  | MAL2   | DMXL1  | NEK4   | PASK   | SLC2A10 | CBX3   | SDF2L1 | FH     | HMGN5  | EZH2   | UBQNL2 | ASCC3  | AAK1   | STAG1  | D5CC1  | ASNSD1 | NABP2  | CEP192 | MSH2   | CBL    | HNRNPR | NAGA   | PRADC1 | STARD4 | TLR4   | CKAP2L | TIMM2  | SMARCA2 | SPAG9  | ANO8   | ERLIN1 | ITPKA  | MTUS1  | BARD1 | PRR15  | COX6B1 | AARS2  | COLGALT1 | ZWILCH | SLC3A28 | MTMR12 | UTP23  | NCAPD2 | DONSON |

**Repressed genes**

| Gene 1 | Gene 2 | Gene 3 | Gene 4 | Gene 5 | Gene 6 | Gene 7 | Gene 8 | Gene 9 | Gene 10 | Gene 11 | Gene 12 | Gene 13 | Gene 14 | Gene 15 | Gene 16 | Gene 17 | Gene 18 | Gene 19 | Gene 20 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| CRYBB1 | ALDH1B1 | B4GALT5 | RFFL   | PPP1R10 | TARBP1 | TXLN5B | MCAT   | NAPA-AS1 | SCNN1G | ISCU   | P2RX1  | RPL36A | NFKB2  | NFKBIA | ICK    | EHD1   | IZUMO4  | EI24   | SLC2A11 | EME2   | RPL27  | PIP5K1L | OXLD1  | BLVRA  | R3HDM4 | CC2D1A | DAZAP2 | LENG8  | BAX    | RPS9   | PHPT1  | ARSA   | CCDC71L | POLDP2 | ZMAT1  | BMF    | GALNT18 | CCDC44 | CLPTM1 | SECTM1 | CFAP70 | LUC7L3 | RPL22  | ATGP61C2 | RPS28  | TP5311 | UNC119 | LRWD1  | CLTA   | RPS7   | DLGAP4 | RPL35A | TRIP11 | GNA12  | HMOMX1 | SSBP4  | STAT6  | RCD51  | UBD51  | RPS29  | RPL38  | ASB16-AS1 | UNC93B1 | FAM171A | GNBI1  | RPL6   | WDR60  | RPS5K1A | HNRNPAO | APOL1  | STX16-NPE11 | BS6    | RALGDS | RPS15  | RPS10  | GALNT2 | OGT    | RALLY-AS1 | RPL34  | SLC41A3 | SNRPA1 | IJK8E  | APOL2  | C1orf52 | HRAS   | CSNK1G2 | RPS25L1 | C3AR1  | PCEOB1-AS1 | RGS14  | CTDNEP1 | ARL6IP5 | CRK    | NINJ1  | KDM3A  | TGF2   | RPL17  | C11orf1 | SLC2A1 | RPL36  | PIGQ   | YBX3   | SF3B6  | RPL7A  | RPL15  | HIVEP3 | SRP14-AS1 | CTNNB1 | FTL    | MAP2K3 | RPS19  | VAMP3  | RNF123 | BIRC3  | GRB2  | RPL0P  | MAPT   | ECE1   | CASP8  | CRT2C  | GFP137  | HOOK2  | SGK1   | SOAT2  | TFNRSF10D | MOB3A  | EIF3D  | RPS14  | STK17A | CAPR1N2 | ZSCAN30 | CUL7   | GABBP2 | CDC42SE1 | RPS24  | PFP1A4 | GBP4   | WASF1  | CXX5C  | PAPR9  | RPL10A | ERCC1  | ARID5A | RPL8   | COMT   | LMAN2L | EIF3G   | ELK3   | DEF6   | NMI    | KY     | RNF170  | SCNN1B | LY86   | ZMIZ2  | ARPC5  | DMWD   | MIAT   | PTTG1P1 | RP58   | TFNRSF10B | MXI1   | UBA52  | NIPSNAP1 | FJX1   | IGF1R  | SYNGAP1 | ZNF580 | RPL31  | TMEM259 |
| PEA15 | ARL1 | RPL18A | RPS6 | CSNK1G3 | PARP8 | PAX8-AS1 | VPS28 | CCDC86 |
|-------|------|--------|------|---------|-------|----------|-------|--------|
| MFSD10 | WDR73 | DNAJA1 | MRPL10 | CTSA | VASP | RPS23 | RPS3 | TMEM255B |
| JAK3 | ZFP36L1 | ST14 | GTF3C2 | GAS5 | INAFM2 | HSF4 | SYF2 | APOBEC3F |
| RGL1 | TRMT1 | ARPC1A | ABHD17A | RPS3A | RPL23 | THUMPD3 | ASB13 | ARHGEF40 |
| IQCH | ARL2 | RPL37A | IRF5 | SYTL2 | ATP6V1B2 | CDK6 | SPPL2B | SLC25A23 |
| MKNK2 | NEK11 | TRADD | NAT9 | LARS | WDR48 | MAPKAPK | PCDH1 | DENND5A |
| MZF1 | LRRC1 | ZNF467 | GNAI2 | IER5 | IDNK | EIF4G3 | C11orf68 | LINC00893 |
| FTSJ3 | WNT10A | MR1 | NARF | UBXN8 | IQSEC1 | RHPN1 | IL27RA | PLEKHO1 |
| EML4 | RPGR | CYB5R1 | DTX3L | GALNT10 | EIF3F | C1RL-AS1 | RPL27A |
| MRFAP1 | KPTN | BCL3 | CSK | C6orf62 | IFNGR2 | CLSTN1 | CABIN1 |
| ZNF644 | AZIN2 | CD70 | SF1 | GRHL3 | ATP6V0E1 | GOLGA8A | LPIN1 |
### Table S9. Reactome pathways_Enrichr pathway enrichment analysis of genes predicted to be activated by IRF3-IRF4 co-binding

| Name of the enriched pathways (Homo sapiens) | Overlap | Adjusted P-value | Odds Ratio | Combined Score |
|---------------------------------------------|---------|------------------|------------|----------------|
| Cell Cycle_R-HSA-1640170                    | 53/566  | 7.12E-10         | 3.06       | 86.8934        |
| Cell Cycle, Mitotic_R-HSA-69278             | 42/462  | 1.74E-07         | 2.971      | 64.7629        |
| Unwinding of DNA_R-HSA-176974               | 8/12    | 2.50E-07         | 21.79      | 475.8844       |
| DNA Repair_R-HSA-73894                      | 29/285  | 6.33E-06         | 3.325      | 59.57776       |
| DNA strand elongation_R-HSA-69190           | 10/32   | 7.19E-06         | 10.21      | 179.3957       |
| DNA Double-Strand Break Repair_R-HSA-5693532| 19/145  | 2.51E-05         | 4.282      | 69.0833        |
| Activation of the pre-replicative complex_R-HSA-68962 | 9/30   | 3.90E-05         | 9.804      | 152.3381       |
| Resolution of D-loop Structures through Synthesis-Dependent Strand Annealing (SDSA)_R-HSA-5693554 | 8/26 | 1.35E-04 | 10.06 | 142.4407     |
| Mitotic G1-G1/S phases_R-HSA-453279         | 17/136  | 1.55E-04         | 4.085      | 56.8172        |
| Activation of ATR in response to replication stress_R-HSA-176187 | 9/37 | 1.78E-04 | 7.949 | 107.8471      |
| S Phase_R-HSA-69242                         | 16/124  | 1.89E-04         | 4.217      | 57.35635       |
| G1/S Transition_R-HSA-69206                 | 15/112  | 1.94E-04         | 4.377      | 58.28267       |
| Pyruvate metabolism and Citric Acid (TCA) cycle_R-HSA-71406 | 10/48 | 1.95E-04 | 6.808 | 91.1551       |
| G2/M Checkpoints_R-HSA-69481                | 17/150  | 3.66E-04         | 3.704      | 46.43759       |
| DNA Replication_R-HSA-69306                 | 14/105  | 3.68E-04         | 4.357      | 54.32801       |
| HDR through Homologous Recombination (HRR)_R-HSA-5685942 | 11/64 | 3.75E-04 | 5.617 | 70.67104      |
| The citric acid (TCA) cycle and respiratory electron transport_R-HSA-1428517 | 17/153 | 4.23E-04 | 3.631 | 44.54305      |
| Resolution of D-Loop Structures_R-HSA-5693537 | 8/33 | 4.40E-04 | 7.922 | 96.41805      |
| M/G1 Transition_R-HSA-68874                 | 12/82   | 5.43E-04         | 4.782      | 56.69911       |
| DNA Replication Pre-Initiation_R-HSA-69002   | 12/82   | 5.72E-04         | 4.782      | 56.69911       |
| Synthesis of DNA_R-HSA-69239                | 13/97   | 5.84E-04         | 4.38       | 51.39002       |
| Homology Directed Repair_R-HSA-5693538      | 14/118  | 0.00106          | 3.877      | 43.01631       |
| Homologous DNA Pairing and Strand Exchange_R-HSA-5693579 | 8/42 | 0.0023 | 6.225 | 63.94539      |
| HDR through Homologous Recombination (HR) or Single Strand Annealing (SSA)_R-HSA-5693567 | 13/112 | 0.00245 | 3.793 | 38.55949      |
| Resolution of D-loop Structures through Holliday Junction Intermediates_R-HSA-5693568 | 7/32 | 0.00257 | 7.149 | 72.05244      |
| Cell Cycle Checkpoints_R-HSA-69620          | 17/182  | 0.00272          | 3.053      | 30.47478       |
| Post-translational protein modification_R-HSA-597592 | 33/521 | 0.00406 | 2.07 | 19.75569      |
| G1/S-Specific Transcription_R-HSA-69205     | 5/17    | 0.00657          | 9.612      | 86.75117       |
| Presynaptic phase of homologous DNA pairing and strand exchange_R-HSA-5693616 | 7/39 | 0.0084 | 5.866 | 51.2962       |
| Assembly of the pre-replicative complex_R-HSA-68867 | 9/67 | 0.00993 | 4.39 | 37.50475      |
| Citric acid cycle (TCA cycle)_R-HSA-71403   | 5/19    | 0.0106           | 8.6        | 72.63125       |
| Metabolism of proteins_R-HSA-392499         | 54/1074 | 0.01138          | 1.643      | 13.7095        |
| Mitotic G2-G2/M phases_R-HSA-453274         | 15/175  | 0.01515          | 2.801      | 22.48218       |
| Pathway                                                                 | Rank | p-value | e-value | q-value |
|------------------------------------------------------------------------|------|---------|---------|---------|
| AURKA Activation by TPX2_R-HSA-8854518                                  | 9/72 | 0.01524 | 4.085   | 32.64071 |
| E2F mediated regulation of DNA replication_R-HSA-113510                | 6/33 | 0.01916 | 5.942   | 45.9442  |
| Regulation of DNA replication_R-HSA-69304                              | 9/75 | 0.01959 | 3.922   | 30.12717 |
| IRF3-mediated induction of type I IFN_R-HSA-3270619                    | 4/13 | 0.02059 | 10.06   | 76.47073 |
| Cholesterol biosynthesis_R-HSA-191273                                  | 5/23 | 0.02262 | 7.104   | 53.17071 |
| Epigenetic regulation of gene expression_R-HSA-212165                  | 11/115| 0.03165 | 3.126   | 22.2642  |
| HDR through Single Strand Annealing (SSA)_R-HSA-5685938                | 6/37 | 0.03173 | 5.299   | 37.59699 |
| G2/M Transition_R-HSA-69275                                            | 14/173| 0.0337  | 2.645   | 18.53759 |
| SUMOylation_R-HSA-2990846                                              | 10/101| 0.03847 | 3.236   | 22.1746  |
| Loss of proteins required for interphase microtubule organization?from the centrosome_R-HSA-380284 | 8/69 | 0.03952 | 3.789   | 25.52021 |
| Loss of Nlp from mitotic centrosomes_R-HSA-380259                      | 8/69 | 0.04039 | 3.789   | 25.52021 |
| STING mediated induction of host immune responses_R-HSA-1834941        | 4/16 | 0.04097 | 8.17    | 55.09649 |
| Organelle biogenesis and maintenance_R-HSA-1852241                     | 21/326| 0.04109 | 2.105   | 14.23895 |
| Orc1 removal from chromatin_R-HSA-68949                                | 8/70 | 0.04163 | 3.735   | 24.80183 |
| Regulation of cholesterol biosynthesis by SREBP (SREBF)_R-HSA-1655829 | 7/55 | 0.04215 | 4.159   | 27.3989  |
| Switching of origins to a post-replicative state_R-HSA-69052            | 8/70 | 0.04252 | 3.735   | 24.80183 |
| Regulation of PLK1 Activity at G2/M Transition_R-HSA-2565942           | 9/87 | 0.04253 | 3.381   | 22.30836 |
| Removal of licensing factors from origins_R-HSA-69300                   | 8/72 | 0.0471  | 3.631   | 23.44438 |
| Activation of gene expression by SREBF (SREBP)_R-HSA-2426168            | 6/42 | 0.04841 | 4.669   | 29.92483 |