Supplementary: Full Methods

Cell Lines

Cell lines were grown in media at 37°C, 5% CO2, in media with antibiotics as follows. Myeloma lines: SKMM1, H929, OCI-My5, LP1, ANBL6, JIM3, U266, UTMC2, L363, EJM: ACL4 (Invitrogen) +10%FBS (Hyclone, Defined); KMS12: RPMI-1640 (Invitrogen) +10%FBS. Lymphoma lines: OCI-Ly7, OCI-Ly19, Ramos, BJAB: IMDM (Invitrogen) +10%FBS; OCI-Ly3, OCI-Ly10: IMDM +20% human plasma.

Flow Cytometry Flow cytometry was performed on a BD FACS Calibur.
For assessment of the % of gfp+ cells, cells were pelleted, resuspended in 200ul of 1xPBS+5%FBS, and the % of gfp+, lived-gated cells was measured.
For the assessment of live vs. dead cells, cells were pelleted, and resuspended in 200ul of 1xPBS with a 1:2000 dilution of SPHERO particles (SpheroTECH), a 1:2000 dilution of 0.5ug/ml calcein AM (Molecular Probes), and 5ug/ml of EtBr or propidium iodide. An equal number of bead events were collected for all samples, allowing comparison of total live (calcein+, EtBr-) and dead (calcein-, EtBr+) cells.

Gene expression

Q-RTPCR for IRF4 and its target genes was performed on cDNA as previous described in [14,47], using pre-tested Assay-on-demand probe/primer sets from Applied Biosystems using an ABI 7700 Taqman machine for 40 cycles with and annealing temperature of 60C. Gene expression was normalized to the expression of beta-2-microglobulin (B2M) for all samples. Gene expression profiling was measured using Human Lymphochips ([14,47]), comparing signal from control shRNA cells +dox (Cy3) to dox-treated cells shIRF4 cells(Cy5). Gene expression was also measured using Agilent 4x44K gene expression arrays, exactly as described by the manufacturer, comparing signal from untreated shIRF4 cells (Cy3) to dox-treated shIRF4 cells (Cy5). Exemplar array elements are shown for genes whose expression is reduced by > 1.3-fold in at least 50% of the induced time points. Array elements were filtered for those above confidence thresholds for spot size, architecture, and level above local background. These criteria

are part of the GenePix/MAdB software for human Lymphochips or a feature of the Agilent gene expression software package for Agilent 4x44k arrays.

Western blotting Western blotting was performed on cell lysates from equivalent numbers of cells, and IRF4 blotting was performed with goat anti IRF4, sc6059 (Santa Cruz), as described [48].

DNA binding Assays ELISA-based DNA binding assays were obtained from Active Motif and used to quantitate MYC DNA binding, normalized by SP1 DNA binding, in nuclear extracts prepared as described (Shaffer et al., 1996).

ShRNA target sequences-underlined.

shCONTROL (Luciferase) 5':
AGCTTAAAAAGTGGATTTCGAGTCGTCTTAATTCTCTTGAAATTAAGACGACTCGAAATCCACGG

shIRF4b 5':
AGCTAAAAAGTGCCATTTCTCAGGGAAGTATCTCTTGAATACTTCCCTGAGAAATGGCACGG

shIRF4a1 5':
AGCTAAAAACCGCCATTCCTCTATTCAAGATCTCTTGAATCTTGAATAGAGGAATGGCGGGG

shIRF4a2 5':
AGCTAAAAACCGCCATTCCTCTATTCAAGAaTCTCTTGAAtTCTTGAATAGAGGAATGGCGGGG

Achilles' Heel Screen shIRF4 5'
AGCTAAAAAGGGGCTACGATTTACCAGAACTCTCTTGAAGTTCTGGTAAATCGTAGCCCCGG

shMYC_1:5':
AGCTAAAAACGATTCCTTCTAACAGAAATGTCTCTTGAACATTTCTGTTAGAAGGAATCGGG

shMYC_2:5':

AGCTAAAAACCTATGAACTTGTTTCAAATGTCTCTTGAACATTTGAAACAAGTTCATAGGGG

shSTAG2 (1806):5':

AGCTAAAAAGAGAGTGCTCTGATTGAAATATCTCTTGAATATTTCAATCAGAGCACTCTCGG

shSTAG2 (3723):5':

AGCTAAAAAGGCACAAGCCTAATGGAAGATTCTCTTGAAATCTTCCATTAGGCTTGTGCCGG

shSUB1 (194):5':

AGCTAAAAAGAGAGCCCTGTCATCTTCTAATCTCTTGAATTAGAAGATGACAGGGCTCTCGG

shSUB1(552):5':

AGCTAAAAAGAAGAATTTGTAAGATGAATATCTCTTGAATATTCATCTTACAAATTCTTCGG

Flow cytometry

IRF4 intracellular staining

For the measurement of intracellular IRF4, 500,000 cells were pelleted and washed one time with 1ml of 1xPBS with 0.5% BSA. Cells were then pelleted and fixed, with 1ml of 1% paraformaldehyde (in 1xPBS), with slow addition and constant vortexing. Cells were then allowed to sit for 10min. at RT. Cells were pelleted and washed with 1ml 1xPBS, 0.5%BSA, and 0.03% saponin. Cells were then stained (15mins RT) with 0.5ul/ 500,000 cells of primary antiIRF4 antiserum (goat anti IRF4, sc6059, Santa Cruz) and control antiserum (goat Ig sc2028, Santa Cruz) in 1xPBS, 0.5%BSA, and 0.03% saponin. Cells were washed twice with 1xPBS, 0.5%BSA, and 0.03% saponin, and stained with the secondary antibody (donkey anti-goat-FITC sc2024, or –PE sc3743, Santa Cruz) in the same buffer for 10 min. at RT. Cells were washed

once with 1xPBS, 0.5%BSA, and 0.03% saponin, and once with 1xPBS, 0.5%BSA. Samples were then resuspended in 1xPBS, 0.5%BSA, and analyzed by flow cytometry (see[14]).

Cell Cycle Analysis

For cell cycle analysis, an equal number of cells was washed with media, and placed back in culture with 10uM of BrdU for 30 minutes. Cells were then pelleted and fixed with 70% ethanol and kept on ice for 30 mins. Cells were then pelleted, and washed with 1xPBS+1%FBS. Cells were next resuspended in a 1:5 dilution of concentrated HCl for 30 mins. at RT. Cells were again pelleted and washed twice with 1xPBS+1%FBS, then once with 1xPBS+1%FBS+0.2% Tween20. 2ul of antiBrdU-FITC antibody were added (Becton Dickenson), and incubated for 20mins at RT. Cells were washed twice in 1xPBS+1%FBS+0.2%, and once with 1xPBS+1%FBS. Cells were treated with 50ul ribonuclease (100ug/ml Sigma) for 30min at 37C. Next 200ul of proidium iodide (50ug/ml) was added, cells incubated at RT for 30 mins, and cell cycle distribution was captured by flow cytometry, and phase distribution was measured using ModFit (Verity Software House, Inc.).

Gene Expression

GEO datasets:

1. IRF4 shRNA targets-Human Lymphochip -GSE8958
2. IRF4 shRNA targets-Agilent Gene Expression Array -GSE9067
3. PCDC vs. monocytes - Su, et al., PNAS | April 20, 2004 | vol. 101 | no. 16 | 6062-6067
4. primary plasma cell vs. mature B and primary myeloma vs. mature B -GSE6691
5. GCB -/+ IRF4-GSE6337
6. Activated B cells-GSE9119
7. Agilent ChIP.-on-CHIP-GSE9367
8. Primary Myeloma Patient gene expression-Zhan, et al. Blood 108, 2006:GSE2658.

Agarose bead version: ChIP/ChIP-on-CHIP Assay: Five million cells in 13ml growth media were placed in a 15ml tube, and treated with 350ul Formaldehyde (37%) to a final concentration of 1% for 10-15min@RT with gentle shaking/rolling. Crosslinking was stopped by adding 675ul Glycine (2.5M) to a final concentration of 0.125M for 5min@RT with gentle shaking/rolling. Cells were harvested by spinning 5min@1.3krpm. Cells were washed with 2x 10ml PBS, spinning 5min@1.3krpm between steps and resuspended in a 15ml tube in 1500µl ChIP Lysis Buffer (50mM HEPES, 150mM NaCl, 1% Triton-X 100, 0.1% Na-deoxycholate, 1mM EDTA--with Complete Protease Inhibitor Tablets (Roche); PMSF (100mM in alcohol; 1mM final)). Cells were lysed by sonication in a Bioruptor: High-energy setting, (30sec on, 30sec off), 15-20 min. Chromatin was transferred to 2ml tubes and cleared by spinning 5min@13krpm in a refrigerated centrifuge. Immunoprecipitation was performed either with Protein A/G coupled agarose beads (Upstate 16-157, 16-201) or Protein G coupled magnetic beads (Dynal/Invitrogen). For IP with agarose beads, supernatants were transferred to new 2ml tubes, and then precleared by incubating with 50µl of Protein A/G beads per 300µl lysate for 60min@4ºC, rotating. Supernatants were moved to a clean 1.5ml tube, and for each IP, 300µl of precleared lysate was added to a Zeba Spin Column (Pierce 89868). Antibodies were added to this lysate and incubated 16hr@4ºC, rotating. (e.g., 10µg of Normal goat or rabbit IgG (sc2028, sc2027, Santa Cruz) or antiIRF4 (sc6059 and sc11450, Santa Cruz). Immune complexes were captured by adding 50µl of Protein A/G beads to each Zeba Spin Column, and incubating 60min@4ºC, rotating. Zeba Spin Columns containing the bead-bound immune complexes were placed on a vacuum manifold and washed as follows: 0.75ml of: ChIP Lysis Buffer (twice), High Salt Buffer (ChIP Lysis with 500mM NaCl), LiCl Buffer (Upstate 20-156), TE. Washed beads were transferred to a clean 1.5ml tube by resuspending beads in 30-40µl of water. Beads were pelleted by spinning (1min@3krpm) and the liquid was discarded. For IP with Protein G magnetic beads, first, beads are washed according to manufacturer's recommendations. Then, 5-10µg antibody (per IP) is pre-bound to 50µl of washed magnetic beads (per IP) by incubating 45 min at 4ºC in 100µl Bind Buffer (0.1M NaAc; 0.2% Tween-20, 0.2% (w/v) BSA) and the antibody-bound beads are washed with 2x1ml of 0.1M NaAc. IP is performed by incubating the cross-linked lysate (300µl per IP) with the antibody-bound

beads from the previous step for 3 hours at 4ºC rotating in 1.5ml microcentrifuge tubes. Immune complexes are captured and washed (as described above) with the help of a Dynal MPC-S magnet. Unprocessed lysate (15-30μl, i.e., 5-10% of IPed amount) was now processed in parallel as "input" material along with washed beads. DNA was recovered using Chelex-100 (Biorad 142-1253 as 10% slurry in water) and Proteinase K: Add 100μl of 10% Chelex-100 suspension (in water) to each tube. Vortex briefly. Boil 10min; cool down. Add 1μl of Proteinase K (10μg/μl stock; i.e., final conc. of ~100ng/μl). Incubate 30min@55ºC. Boil 10min; cool down. Spin (1min@3krpm) and collect the supernatant which contains the DNA eluate in a new tube. Add 100μl water to beads. Vortex briefly. Spin (1min@3krpm) and collect the supernatant to combine with the previous eluate. (i.e., ~200μl total). Samples are cleaned up using QIAquick spin columns (Qiagen) according to manufacturer's instructions and eluted in 50μl. Proceed to real time PCR. Inputs can be used 1:10 to 1:100 diluted, IPed eluates can be used straight to 1:10 diluted as a starting point, or as appropriate (e.g., 1-5μl in a 20μl QPCR reaction) For most loci, quantitation was performed using SYBR green on an ABI7500 Taqman machine, 40-45 cycles, anneal @ 60C, using self-designed primers, tested for lack of primer-dimer artifacts and for single species amplification. Values for control IPs and IRF4 IPs were normalized to input DNA values, giving data in arbitrary units. Material for Agilent promoter arrays was amplified, labeled, hybridized and scanned as directed by the manufacturer.

Magnetic bead version: ChIP/ChIP-on-CHIP Assay: This method is now employed by our lab and has recapitulated the results shown in the manuscript. Chromatin immunoprecipitations were performed with Protein G magnetic beads as described above with the following modifications: cross-linked lysate amount per IP is increased to 10-20 million cell equivalents. The final clean-up of the immunoprecipitated material is performed with MinElute Columns (Qiagen) with an elution volume of 20-30μl. The ChIP and input samples are quantified with NanoDrop Spectrophotometer. 20-50ng of each sample is amplified with Whole Genome Amplification (WGA) Kit (Sigma) following manufacturer's protocol. Amplified samples are cleaned-up with QIAquick spin columns (Qiagen) and quantified with NanoDrop spectrophotometer. A second

round of amplification is performed starting with 50ng of purified amplified material and cleaned up with QIAquick spin columns. Semi-quantitative PCR (QPCR) is performed on un-amplified and amplified ChIP samples (as described in the previous section) and compared to each other in order to confirm that the amplification reactions did not introduce any observable amplification bias (data not shown). The following steps are performed essentially according to Agilent Mammalian ChIP-on-chip Protocol v9.1 (Agilent Technologies): Amplified samples are labeled with Cy5 (anti-IRF4 immunoprecipitated samples) and Cy3 (control antibody immunoprecipitated samples) with BioPrime labeling kit (Invitrogen) and hybridized to Human Promoter Set arrays (Agilent Technologies). Arrays are washed and scanned on an Agilent Scanner. Scanned images are processed with the Feature Extraction (FE) Software (Agilent Technologies) with default settings to obtain fluorescent intensity signal values for individual probes on the array. FE output files are further processed with the ChIP Analytics v1.3 Software (Agilent Technologies) to delineate and statistically evaluate IRF4 bound regions In the -5.5kb to +2.5kb (relative to transcription start sites) of the known and predicted genes in the human genome with the following parameters (as explained in detail in the ChIP Analytics 1.3 User guide [Agilent Technologies]): Default workflows are applied for pre-normalization data manipulation and normalizations. Whitehead Error Model v1.0 with default parameters was applied to define probes that show IRF4 binding. Specifically, the statistic p(Xbar), calculated by the error model, was used to evaluate significant binding events. Briefly, p(Xbar) of a given probe takes into account the p-values (for binding) associated with that probe and its immediate neighboring probes (in chromosomal context) such that low p(Xbar) values are assigned only to consecutive probes displaying significant binding, thereby selecting against (possibly spurious) isolated binding events, and selecting for “peak shaped” bound probe clusters as expected from real binding to sheared chromatin fragments. The following stringent criteria is applied to obtain a “high confidence” set of genes with IRF4-bound sequences: A gene is designated to be “IRF4-bound” if it has at least two consecutive probes with p(Xbar)<0.05 in at least one of the test (i.e., KMS12) samples where these probes show p(Xbar)>0.05 in both of the control (i.e., OCILy19) samples. Sequences of the primers used in the confirmatory ChIP-QPCR:

Gene	sequence
PRDM1-intron4	Forward-5’-CTGTTGCTGAGTGGGAGAGT
	Reverse-5’-CTCCCTAAGATGCTCTACAGGTG
SQLE-end exon1	Forward-5’-CAGAGATGGAAGAAAGGTGACAGTC
	Reverse-5’-GGATGGTCTAAGTGAAGAGTGCTTG
SCD-promoter	Forward-5’- CCGTCCAGCCTGCTTCA
	Reverse-5’- GTTGGAAGGTGCAGAATTCTTG
MYC-promoter	Forward-5’-TCCCCCGAATTGTTTTCTC
(anneal at 59C)	Reverse-5’-CCTCACCCAAAGGCATTTTA
	Probe-(FAM-TAMRA) UPL-10 (Roche)
IRF4-promoter	Forward-5’- TCACCACTGCCAGCTGCTA
	Reverse-5’- AAACTCCGGATGGCCTCAT
PRDM1-promoter	Forward-5’- GGACAGAGGCTGAGTTTGAAGA
	Reverse-5’- CGCCATCAGCACCAGAATC
CDK6-promoter	Forward-5’- GGGTGCCTCTGTAGTACCTCTACGTTT
	Reverse-5’- GTATCCAGTTAAGGGTGCTTATCATGT
CANX-promoter	Forward-5’- TCTGTTGTTCTGTGGTCACAGTGACCTT
	Reverse-5’- ACATTTGGGAAAGTCTGCTATGTAGCT CCNC-
CDK6-promoter	Forward-5’- GGGCACCTGCCCTATACCTTATCAATA
	Reverse-5’- TCTTGCGCGTACTTTATGTCTAGATGGA
ELL2-promoter	Forward-5’- GTGCCTGGCACTAGTGGGAGCTCAAC
	Reverse-5’- AAGGACACCGAGACACAGATTCACATAG
PIM2-promoter	Forward-5’- GAAGCAGAGAGGGAAGATTAGCAGTCA
	Reverse-5’- AGACTCCATCCTTCTCAGGGACCAGTAC
STAG2-promoter	Forward-5’- GCCACACCCTGTATTTGTCTCTGTGGAT
	Reverse-5’- AGCTTCGTGCTTAAGCAAGTCCCATCA
CASP3-promoter	Forward-5’- CATGCGCACATTTGCTCTCT
	Reverse-5’- CCCAGGAGTGTGGATTTGGT
CDKN1B-promoter	Forward-5’- AAAGGAGCACGCACTGGAA
	Reverse-5’- CCAGCAACCAGTAAGATCAGGTA
DDR2-promoter	Forward-5’- TAGCAGCTGCAAGATACAATAAACA
	Reverse-5’- CTCTGCAATCGGCCTGTCT
DUSP5-promoter	Forward-5’- ACAGTTCCCCTCTCTGCTAGCA
	Reverse-5’- ACCATCTACCTCTGTTAACTCTCCAA
E2F5-promoter	Forward-5’- TTGGTCAAGTCCTCTTTCCAACAAACC
	Reverse-5’- GCTTATCTTTCTGTAAGCAGGCAAGTG
EIF3S6-promoter	Forward-5’- CAAGCCAGCCTGTGAAGCT
	Reverse-5’- AGTGCGGGAGGGATTTTCG
FBXO16-promoter	Forward-5’- AACAATGTGTGGGCTCCTGAT
	Reverse-5’- CCCGTGCCCAATTTTGTAG
ISG20-promoter	Forward-5’- CCCGTGCCCAATTTTGTAG
	Reverse-5’- CCTCAAACCCTACCATGGTGTCT
NFIL3-promoter	Forward-5’- TGAGAAAAAGGGCTGATCGA
	Reverse-5’- GGACGAACCATTGTGACGTTT
PHKA1-promoter	Forward-5’- AGGAAAGATCCCAGGATAGGATT
	Reverse-5’- GAAGTGGTATTCTTGCAGTGGAA

SLAMF7-promoter	Forward-5’- ACTGTCCCAGGGATGAAGGA
	Reverse-5’- CATGGCAGAACCTGGATTAGAA
SLC31A1-promoter	Forward-5’- GGCTGTCATTACCTATCCCATAATT
	Reverse-5’- TCTGTAATGTTAGGGCTCCCAAT
SUB1-promoter	Forward-5’- AGGCACACTGCCCAGGTTCCCTCAGTGA
	Reverse-5’- ATCTGCAACCCTTCCTGCTTTAACAAGT
TNFRSF17-promoter	Forward-5’- ACACAGACAGCCCCCGTAA
	Reverse-5’- GCAGCAAGAGCAGCTAGAATGT
UAP1-promoter	Forward-5’- GGCCAAGCAGAAGGATGGT
	Reverse-5’- CAAAGTTCAAGCAGCCATATGTT
UCK2-promoter	Forward-5’- TGTTTTTCCCACCGTTTAACAC
	Reverse-5’- CCAGCTCTCAGGATCCCTTGA
MYC-promoter –1.0	Forward-5’- CCTCCCATATTCTCCCGTCTAGCACCTT
	Reverse-5’- TTTGCGCCCTGTGGCGCCGGTTTGCA
MYC-promoter –1.8	Forward-5’- TTGCGGCAAAGGCCTGGAGGCAGGAGTA
	Reverse-5’- CAAGATGGGTTATTACCCGTTGAGTTT
cMYC-promoter –5.2	Forward-5’- TCTCATCTGCTGAAGAGCTTTCCTGTGT
	Reverse-5’- TTTAATGCCAAGCATGTGTTCCAACGA
cMYC-promoter –7.2	Forward-5’- CTTCATTCTCTTACCCAGTGTAGAGTCA
	Reverse-5’- TCAAGTCTGGCCCATAGTTAATGTGAA
cMYC-promoter –8.5	Forward-5’- GGCAGCCTGAGGGCCACCTCTGCCCATA
	Reverse-5’- TCAAGCCAGCAGCAGTGTGTTGGGAAA
MYC-promoter –10.5	Forward-5’- AGTAAGAGGTAACCTCTCCTTCCTTCCT
	Reverse-5’- TCTCTGCTGCTGGTGACCAGAGCAAAT

Statistical Methods Error bars in graph represent the standard deviation or standard error of measurement as indicated in the figure legends. The correlation between IRF4 and MYC expression was determined by the Pearson correlation method.

We used a modified version of Gene Set Enrichment Analysis (GSEA, [27]). We computed the t-statistics between the normal B cells and plasma cells cases for the 308 genes in the IRF4 signature, and averaged them to form a “true test statistic” for that signature. We wished to create a similar statistic for data with permuted class labels, but realized that since there were many differentially expressed genes, the t-statistics on the permuted data would have a much different scale. To deal with this we used the following method. We calculated the t-statistics between the normal B cells and plasma cells cases for all IRF4 genes, and ordered them from least to greatest. We then randomly permuted the class labels and recalculated the t-tests, and found their rank from least to greatest. We then associated with each gene of a given rank the t-statistic of the same rank from the unpermuted data. These values were than averaged for all genes in a given signature, to

arrive at a permuted test statistic for that signature. This was repeated 1000 times. Similar methods were used to estimate the enrichment of IRF4 targets between normal cells and myeloma cells, and between plasma cells and myeloma cells.