SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Amplification of the *BRD4* locus in MM samples

(A) Copy number analysis of human chromosome 19 in primary samples from human MM patients (n = 254).

(B) Global view of genome-wide copy number changes in primary samples from human MM patients. Chromosome 19, which encodes BRD4 at Chr 19p.13.1, is amplified in the majority of MM patients.

Figure S2. GSEA analyses of transcription factor target gene sets

(A) Results of GSEA analysis for transcription factor target gene sets emerging with statistically significant enrichment among JQ1 downregulated genes.

(B, C) Enrichment of genes defined by gene sets for targets of (B) E2F1 and (C) NF-κB in JQ1-treated MM cells. E2F1 target genes are downregulated by JQ1 compared to NFkB genes, which are comparatively unaffected.

(D, E) GSEA documents that JQ1 treatment in MM cells also downregulates functionally defined gene sets for E2F.

Figure S3. Concordance of multiplexed expression data across biological replicates

(A) Scatterplots of multiplexed expression data between biological replicates for MM.1S cells treated with JQ1 in a time-dependent manner (0-8hrs). R^2 values were calculated based on Pearson correlation coefficient. Average data for each transcript are presented as a heatmap in Figure 3.

(B) Scatterplots of multiplexed expression data between biological replicates for three MM cell lines (KMS11, MM.1S, OPM1) treated with JQ1 for 24 hours. R^2 values were calculated based on Pearson correlation coefficient. Replicate expression measurements exhibited high concordance among low and highly expressed genes. Average data for each transcript are presented as a heatmap in Figure S5B.

Figure S4. Myc-specific consequences of JQ1 treatment

(A) ELISA-based DNA binding assays document that JQ1 treatment (500 nM, 0 - 24 h) does not significantly affect the binding of AP-1 family members to consensus DNA binding sites. Data represent mean ± SEM.

(B) Gene expression profiling, by multiplexed transcriptional analysis, of three MM cell lines (KMS11, MM.1S and OPM1) treated with JQ1. MM cells were treated with JQ1 (500 nM) for 24 hours and assessed for effects on transcription of 230 cancer-related genes. Data are represented as a heatmap, with upregulated (red) and downregulated (blue) genes analyzed for statistical significant changes in expression. Maximum p-values of statistical significance of difference in JQ1- compared to vehicle-treated cells in all three lines are indicated. Asterisks denote genes (also presented in Figure 3H) demonstrating a statistically significant change in expression across all 3 cell lines.

(C, D) Immunoblotting analyses of (C) c-Myb and (D) E2F1 protein abundance in MM.1S cells following JQ1 treatment (0 - 24hrs, 500 nM). Measurement of GAPDH levels served as loading control.

Figure S5. Flow cytometry in MM cells infected with shRNA or treated with staurosporine

(A) Cell cycle analysis of OPM1 cells transduced with the indicated shRNAs against BRD4 (shBRD4.602 and shBRD4.1838) for 6 days. BrdU staining (APC) indicates the percentage of S-phase cells. Notably, shBRD4.1838 produces less efficient suppression of *MYC* transcription, and exhibits a diminished effect on suppressing S-phase and cell cycle progression. These data support the role of MYC suppression by genetic (shRNA) or chemical (JQ1) inhibition of BET bromdomains.

(B) Induction of apoptosis by the non-selective kinase inhibitor staurosporine in MM.1S cells (500 nM for 24 or 48 hours, as indicated). Flow cytometry was performed using PI and Annexin V staining as described in Expanded Experimental Procedures.

Figure S6. Chromatin immunoprecipitation (ChIP) studies of BRD4 binding to MYC promoter and enhancer regions. Validation of BRD4 localization to the *IgH* enhancers (and, to a lesser extent, *MYC* TSS) is provided with a second independent antibody (anti-BRD4; Sigma). JQ1 demonstrates competitive inhibition of BRD4 binding to chromatin by ChIP, consistent with data presented in Figure 4C.

Figure S7. Efficacy of BET inhibition with JQ1 in murine models of MM.

(A) BET inhibition with JQ1 has no effect on the viability of resting peripheral blood mononuclear cells (PBMCs) from normal donors (500 nM of JQ1 for 72 hours). Following PHA stimulation (5 ug/mL), a decrease in cellular proliferation was observed, supporting a selective effect of JQ1 treatment on proliferating cells.

(B) Tumor burden (based on caliper measurements) of SCID-beige mice subcutaneously xenografted with MM.1S-luc+ cells. Upon detection of engrafted tumors, mice were randomly

assigned to receive JQ-1 (50 mg/kg IP daily) or vehicle control. Data are presented as mean +/- SEM (n = 10/group).

(C) Aged Vk*MYC mice with an M-spike of at least 15 g/L were treated by intraperitoneal injections for 5 days/week with JQ1 in 10% cyclodextrine (Sigma). SPEP was performed weekly and drug response was calculated by dividing the gamma/albumin ratio at day 0 for each individual M-spike by the gamma/albumin ratio obtained post-treatment. Densitometric profiles obtained at day 0 and day 14 were overlaid to show M-spike reduction with JQ1 treatment.

EXTENDED EXPERIMENTAL PROCEDURES

Reagents

MM cell lines used were KMS-34, LR5, MOLP-8, Dox40, INA-6, MM.1S, KMS-5, KMS-12-PE, AMO-1, MR20, OPM-1, KMS-11, KMS-12-BM, KMS-26, L363, EJM, KMM1, MM.1S-myrAkt, KMS-20, MM.1S-Bcl-2, RPMI-8226/S, OCI-MY5, KMS-28-BM, KMS-18, and MM. 1R. All MM cell lines were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2mM glutamine, penicillin (100U/mL), streptomycin (50ug/mL). Additionally, the INA-6 cell line was supplemented with IL-6 (5ng/mL) and an additional 10% fetal bovine serum (final concentration of 20%). Assays were also carried out under these respective conditions. Long term culture of the MM cells occurred in a Thermo Fisher Scientific (Waltham, MA) water jacketed incubator at 37 ^{o}C and 5% CO_2. The thieno-triazolo-1,4-diazepine JQ1 compound used in assays was synthesized as previously described (Filippakopoulos et al., 2010), and diluted in DMSO to a stock concentration (10 mM) subsequently diluted to working concentrations as indicated for biological studies.

Copy number and gene expression analysis in primary samples and MM cell lines

Gene expression data from publicly available Gene Expression Omnibus (GEO) datasets (accession numbers GSE5900 and GSE2113) were downloaded and analyzed through Oncomine 4.4 (www.oncomine.com). Differences in $\log_2$-transformed median-centered transcript levels between different groups of samples were evaluated by non-parametric Kruskal-Wallis one-way analysis of variance and Dunn's Multiple Comparison Test. Statistical analyses were performed with Prism 5 software (Graphpad). Copy number data for the human 19p13.1 locus that harbors

BRD4 were downloaded from the publically available database of the Multiple Myeloma Genomics Portal (MMGP) (www.broad.mit.edu/mmgp) for primary samples from 254 MM patients and for 45 MM cell lines. Data were analyzed using the Integrative Genomics Viewer (IGV) analysis software incorporated into MMGP. Oligonucleotide microarray data on the aforementioned MM cell lines were also downloaded from the MMGP. Previously published oligonucleotide microarray data(McMillin et al., 2010), were analyzed to compare expression of BRD4 transcript in INA6 myeloma cells cultured in vitro in the presence vs. absence of HS-5 bone marrow stromal cells.

Arrayed Lentiviral shRNA Screens

shRNA screens were performed in INA-6 cells in an arrayed format using Cell Titer-Glo (Promega) to assess proliferation/viability phenotypes as described(Boehm et al., 2007; Moffat et al., 2006). Screening data was normalized using the statistical B-Score as described (Malo et al., 2006), and further visualized in Spotfire DecisionSite.

Expression Analysis

MM.1S cells were treated with compound (JQ1 500 nM) or vehicle (DMSO < 0.2%). RNA extraction was done with TRIzol Reagent (Invitrogen, 15596-026). cDNA was reverse transcribed (Applied Biosystems, N808-0234) and subsequently underwent quantitative real time PCR (Applied Biosystems, N15597) on an Applied Biosystems 7500 Real-Time PCR system with mammalian Taqman probes from Applied Biosystems: GAPDH (Hs 02758991_g1), CDKN1A (Hs00355782_m1), and MYC (Hs00905030_m1) following the manufacturer's

protocol. Analysis was performed on triplicate PCR data for each biological duplicate normalized to GAPDH.

Nanostring data analysis

RNA was extracted as previously mentioned and added to Nanostring reagents as per manufacturer's protocol. Sample counts were first corrected for background based on negative control counts. Negative and zero values were set to 1 and the data log2-transformed. Counts were then scaled sequentially to doped-in positive controls and to 5 housekeeping genes (GAPDH, TUBB, HPRT1, GUSB, CLTC). To ensure comparable distributions, scaled counts were then quantile normalized (Bolstad et al., 2003) across samples using the Bioconductor *limma* implementation in R (Smyth, 2005). Gene values for which fewer than 25% of samples had counts above background (i.e. expressed at a reliably detectable level) were excluded from further analysis. In the time series experiment, genes were also filtered for variation to exclude those with an interquartile range < 0.5. Filtered data were used for unsupervised hierarchical clustering. For the time series experiment, samples and genes were clustered by Euclidean distance to reveal similarities in absolute expression levels. For the cell line comparison, Spearman's Rank Correlation was used to cluster genes. In both cases, color is globally normalized with blue indicating smallest fold change relative to the mean time 0 value (time series) or lowest expression level (3 cell lines). Red denotes greatest fold change or highest expression. Statistical significance of expression changes (relative to mean time 0 for the time series and treated vs untreated for the cell lines) was determined relative to a normal distribution with mean and standard deviation calculated for each comparison separately.

Oligonucleotide Microarray Profiling and Gene Set Enrichment Analysis (GSEA)

MM.1S, OPM1 and KMS11 MM cells were treated with JQ1 (500 nM, 24 h), cells were harvested, RNA was extracted and then processed for oligonucleotide microarray profiling. Affymetrix Human Gene 1.0 ST arrays (Gene Omnibus Express accession number GSE31365) were processed using the rma function of the affy Bioconductor package (Bolstad et al., 2003; Irizarry et al., 2003a; Irizarry et al., 2003b), and batch-corrected by cell type using ComBat (Johnson et al., 2007). Gene sets were downloaded from the Broad Institute's MSigDB website (Subramanian et al., 2005). Gene set permutations were used to determine statistical enrichment of the gene sets using the signal-to-noise ratio of DMSO-treated vs JQ1-treated cells.

shRNA knockdown for *BRD4*

OPM1 cells were retrovirally transduced with VSVG-pseudotyped LMS-shRNA expression vectors (Zuber et al.). The day of infection of the cells was designated as day 0. The fraction of GFP-positive cells after infection was > 95%. RNA was collected at day 4 or day5 and qRT-PCR was performed using the following primers for human BRD4: CCCCTCGTGGTGGTGAAG and GCTCGCTGCGGATGATG; GAPDH: CCTGACCTGCCGTCTAGAAA and CTCCGACGCCTGCTTCAC; and MYC: AGGGATCGCGCTGAGTATAA and TGCCTCTCGCTGGAATTACT. All signals were calculated by using deltaCt method and were normalized to GAPDH.

MYC overexpression studies

OPM1 cells were transduced using a MSCV-PGK- Puro-IRES-GFP (MSCV-PIG) construct (Hemann et al., 2003), with murine c-Myc or empty vector. 98 % of infected cells became GFP-

positive. The infected cells were then treated with 500 nM JQ1 for 24 h and processed for staining (BD, APC BrdU Flow Kit, #552598). Immunoblotting was performed to confirm Myc expression. Specifically, cells were lysed directly in Laemmli buffer. About 50,000 cell equivalents were loaded in each lane. Protein extracts were resolved by SDS-PAGE electrophoresis and transferred to nitrocellulose for blotting with anti-Myc antibody (Epitomics, #1472- 1) or anti-ß-actin HRP antibody (Sigma, #A3854).

Chromatin immunoprecipitation methods

Approximately $1x10^8$ MM.1S cells were treated with 500 nM JQ1 or DMSO for 24 hours and cross-linked with 1.1 % formaldehyde (10 X crosslink solution contains: 11 % formaldehyde, 50 mM Hepes pH 7.3, 100 mM NaCl, 1 mM EDTA pH 8.0, 0.5 mM EGTA pH 8.0) followed by two washes with PBS. Cells were scraped and frozen in liquid nitrogen. Brd4 ChIP-PCR analysis was done following a published protocol (Rahl et al., 2010). In brief, 75 µl of Dynal magnetic beads (Sigma) were blocked with 0.5 % BSA (w/v) in PBS. Magnetic beads were bound with 6.25 µg of Brd4 antibody (Bethyl Labs, A310-985A, lot A301-985A-1 or Sigma, HPA015055-100, lot A31530). Cross-linked cells were lysed with lysis buffer 1 (50 mM Hepes pH 7.3, 140 mM NaCl, 1 mM EDTA, 10 % glycerol, 0.5 % NP-40, and 0.25 % Triton X-100) and washed with lysis buffer 2 (10 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM EDTA pH 8.0 and 0.5 mM EGTA pH 8.0). Cells were resuspended and sonicated in lysis buffer 3 (50 mM Tris-HCl pH 7.5, 140 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 % Triton X-100, 0.1 % Na-deoxycholate, 0.1 % SDS) for 10 cycles at 30 seconds each on ice (18 watts) with 60 seconds on ice between cycles. Sonicated lysates were cleared and incubated overnight at 4^oC with magnetic beads bound with antibody to enrich for DNA fragments bound by the indicated factor. Beads were washed three

times with sonication buffer, one time with sonication buffer with 500 mM NaCl, one time with LiCl wash buffer (20 mM Tris pH 8.0, 1 mM EDTA, 250 mM LiCl, 0.5 % NP-40, 0.5 % Na-deoxycholate) and one time with TE. DNA was eluted in elution buffer. Cross-links were reversed overnight. RNA and protein were digested using RNAse A and Proteinase K, respectively and DNA was purified with phenol chloroform extraction and ethanol precipitation.

Brd4 ChIP and input DNA were analyzed using SYBR Green real-time PCR analysis (Applied Biosystems). ENCODE H3K27Ac ChIP-seq data available on the UCSC genome browser (http://genome.ucsc.edu/ENCODE/) was used to identify potential tissue-specific *MYC* enhancer and *IGH* enhancer regulatory elements and oligos were designed for these regions. Fold enrichment was determined from triplicate PCR reactions at five potential enhancer regions adjacent to the *MYC* gene in non-translocated cells (MYC_E1, MYC_E2, MYC_E3, MYC_E4, MYC_E5), the *MYC* transcriptional start site (MYC_TS1, MYC_TS2), *IGH* enhancer regions (IGH_E1, IGH_E2, IGH_E3, IGH_E4), and two negative regions upstream of the *MYC* enhancers (MYC_NR2, MYC_NR3) over input DNA using ΔΔCt over the negative region MYC_NR1. The oligos used for this analysis are:

Primer Set	HG19 Region Location	F/R	Sequence
MYC NR1	chr8:127,714,271-127,788,621	FWD	GCAGCTAGATCGTTGGGAAG
		REV	GCTGGTGATTTCAGTGCAGA
MYC NR2	chr8:127,714,271-127,788,621	FWD	TCCTGGGTAGGAACCAGTTG
		REV	ACTCACCAAGAGCTCCTCCA
MYC NR3	chr8:127,714,271-127,788,621	FWD	AAGCCAACCCATCACTGAAC
		REV	TTCCCATGTTACCCACCACT
MYC_E1	chr8:127,845,547-127,846,357	FWD	TGCTAATTGTGCCTCTCCTGT
		REV	ACTCCCAGCAAATCAGCCTA
MYC_E2	chr8:128,218,650-128,218,982	FWD	TTGTCTTCCTCATGCCTTCTC
		REV	AGCTGCAATTCAGGGTTACTG

BET bromodomains and c-Myc

MYC_E3	chr8:128,237,690-128,237,903	FWD	TTTGGGCAGACACTGACTTG
		REV	AAGCAGAAACAGGACCCAGA
MYC_E4	chr8:128,307,286-128,307,531	FWD	GAAATGTGAGGGCACATCGT
		REV	ATACCTGCTGGAGCATTTGG
MYC_E5	chr8:128,568,909-128,569,272	FWD	TCAGCCTGTGGGCTCTAGTT
		REV	AGGGAAGTGCTACCCCATCT
MYC_TS1	chr8:128,747,740-128,748,029	FWD	ACACTAACATCCCACGCTCTG
		REV	GATCAAGAGTCCCAGGGAGA
MYC_TS2	chr8:128,749,029-128,749,395	FWD	GGTCGGACATTCCTGCTTTA
		REV	GATATGCGGTCCCTACTCCA
IGH_E1	chr14:106,047,824-106,049,452	FWD	TGGGGTACAAGAGGCTTCAG
		REV	TACAGGAGTGGGGACAGGAA
IGH_E2	chr14:106,047,824-106,049,452	FWD	GTTCTCTGGCTGGAACACCT
		REV	CCCCATCACCTGCAGAAATA
IGH_E3	chr14:106,167,019-106,167,760	FWD	CCACAGGGCTATTTTGGGTA
		REV	GCCATGCCGTTTGTATTCTC
IGH_E4	chr14:106,167,019-106,167,760	FWD	GGGAGGCCATGCTGTTTGTATTCT
		REV	AACAGCAGTTCTCTGGCTGGAACA

Immunoblotting

Lysates for blotting were prepared by seeding $5x10^6$ of the MM patient sample cells onto 100x20 mm tissue culture dish and JQ1 was added, at a final concentration of 500 nM for various time points. After collecting the cells the pellets were washed 3x with ice-cold 1X PBS and the pellet after the final wash was resuspended in lysis buffer containing treated with Triton X-100 lysis buffer containing 50 mM Tris HCl (pH = 8.0), 120 mM NaCl, 5 mM EDTA, 1 % Igepal, protease inhibitors (Roche Diagnostics, Indianapolis, IN), and phosphatase inhibitors (Calbiochem, Darmstadt, Germany). Protein concentrations were determined by using the Bradford reagent (Sigma-Aldrich, St. Louis, MO) and divided into 20 µg aliquots with containing LDS sample buffer (Invitrogen, Carlsbad, CA), reducing agent (Invitrogen, Carlsbad, CA). Samples were loaded into a NuPAGE Bis-Tris Gel (Invitrogen, Carlsbad, CA) and

separated by electrophoreses at 200 V. The gels were then transferred onto a PVDF membrane (Immobilon-P; Millipore, Billerica, MA) by a wet transfer system (Invitrogen, Carlsbad, CA) and blocked by incubation with 5 % dry milk in TBST (TBS with 0.2 % Tween20). Membranes were probed using antibodies raised against c-myc (with concordant results obtained with different antibodies, including 9402 (Cell Signaling Technologies, Danvers, MA), 9E10, or sc-764 (Santa Cruz Biotechnology)), β-actin (Santa Cruz Biotechnology, SC-1616), and α-tubulin (Santa Cruz Biotechnology, SC-5286). Chemiluminescent detection was performed with appropriate secondary antibodies.

Transcription Factor DNA Binding Activity Assays

NFkB, c-Myc, and AP-1 transcription factor DNA binding ELISA assays (Active Motif, Carlsbad, CA) were carried out according to the manufacturer's instructions. In brief, 2-10 ug of MM1S nuclear lysates was added to an appropriate number of wells in the assay plate and the appropriate transcription factors were allowed to bind to its respective consensus sequence. Bound protein was then probed with a primary antibody, washed to remove unbound protein, and finally probed with a secondary antibody conjugated to HRP. After a final wash, developing solution was added to each well and absorbance was measured on a spectrophotometer (Molecular Devices, Sunnyvale, California) at a wavelength of 450 nm (reference wavelength of 655 nm).

Nuclear Fractionation

The MM cell line MM.1S was seeded (5×10^6) onto a 100 x 20 mm tissue culture dish and JQ1 was added, at a final concentration of 500 nM, for 0.5, 1, 2, 4, 8, and 24 h. All assay conditions

were collected on ice at the longest duration time. Cells were washed twice with ice-cold 1 X PBS and after the final wash 1 mL of a buffer containing 10 mM HEPES, 350 mM sucrose, 5 mM EDTA, 1 mM PMSF, and protease inhibitors (Roche Diagnostics, Indianapolis, IN) was added to the cell pellet. Cells were homogenized with a B type Dounce homogenizer (10 strokes) and centrifuged at 1000 g for 10 minutes at 4^{o}C. The supernatant was then discarded and the pellets containing the nuclear cell fraction were then lysed in buffer containing 50 mM Tris HCl (pH = 8.0), 120 mM NaCl, 5 mM EDTA, 1 % Igepal, protease inhibitors (Roche Diagnostics, Indianapolis, IN), and phosphatase inhibitors (Calbiochem, Darmstadt, Germany). Protein concentration was determined by Bradford reagent (Sigma-Aldrich, St. Louis, MO) and protein was aliquoted according to transcription factor binding ELISA kit instructions.

Cell Viability Assays

MM cell lines were seeded onto 384-well tissue culture treated plates at a density of 1,000 cells/well in a volume of 50 µL of media. After seeding cells were incubated for 1 hour and during the interim a stock plate of JQ1 was thawed at room temperature in a desiccated box. The addition of JQ1 to the assay plate was done with disposable 384-well pins (V&P Scientific, San Diego, CA) that delivered 100 nL of the drug diluted in DMSO to each well of the plate. After 72 hours of incubation cells were analyzed for cell viability by the addition of CellTiter Glo (Promega, Madison, WI) to the assay plates. After 30 min incubation at 37 ^{o}C the signal from the viable cells was analyzed on a Luminoskan luminometer (Labsystems Franklin, MA).

Compartment-Specific Bioluminescence Imaging (CS-BLI) for stromal co-culture studies

Similarly to published studies (McMillin et al., 2010), the immortalized HS-5 bone marrow stromal cell line was plated at a density of 2000 cells/well in a 384-well tissue culture treated plate at Day 0. MM cell lines stably transduced with luciferase constructs were then seeded Day +1 either in the presence or absence of HS-5 and incubated at 37 ^{o}C for 1 hour prior to compound addition. At Day +4 cell viability was measured by the addition of luciferin (250 ug/mL) and final analysis on a Luminoskan luminometer (Labsystems Franklin, MA). Viability was assessed by comparing each condition to their respective drug free controls.

Flow Cytometric Studies (Cell Cycle – Apoptosis Analyses)

For cell cycle analyses, MM.1S cells ($10x10^5$ cell/mL) were treated with compound (JQ1 500 nM) or vehicle (DMSO < 0.2%) for 24 and 48 hours. Cells were spun down at 4 °C, washed with PBS, fixed with 70% ethanol overnight, and washed with PBS. RNA was degraded with RNAase (Roche, 11579681001) and DNA was stained with propidium iodide. Samples were analyzed on a BD FACS Canto II. Histograms were generated and cell cycle analysis was performed using FlowJo flow cytometry analysis software (Tree Star, Inc.).

For apoptosis/necrosis detection, MM.1S cells (10×10^5 cell/mL) were treated with compound (JQ1 500 nM) or vehicle (DMSO < 0.2%) for 24 and 48 hours. The cells were washed and resuspended in Annexin-V/propidium iodide buffer solution containing Annexin V-FITC (BD Pharmigen, 51-66211e) and Propidium Iodide (BD Pharmigen, 51-65874x). Samples were immediately analyzed on a BD FACS Canto II. Visualizations and analyses of apoptotic fractions were generated using FlowJo flow cytometry analysis software (Tree Star, Inc.).

Cellular senescence staining

BET bromodomains and c-Myc

MM.1S cells were passaged with fresh media and into a four-chamber polystyrene vessel tissue culture treated glass slides (BD Falcon= a seeding density of 2 x 10^5 cells per mL, and a final chamber volume of 500 μL. During passage, cells treated with compound (JQ1 500 nM) or vehicle (DMSO < 0.2%). After 48 hours the cells were stained for senescence using a β-Galactosidase Staining Kit (Cell Signaling Technology), and mounted for histology with 70 % glycerol.

***In vivo* xenograft studies**

Five week old female SCID-beige mice were obtained from Charles River Labs (Wilmington, MA), and were acclimated one week prior to tumor cell inoculation. A total of 5 x 10^6 MM1S-LucNeo cells were injected subcutaneously, or 2 x 10^6 cells were injected via the lateral tail vein. Mice were imaged after injection of 75 mg/kg of D-luciferin (Promega, Madison, WI) using a Xenogen IVIS Spectrum (Caliper Life Sciences, Hopkinton, MA). Two weeks after inoculation, mice with established disease were divided into treatment groups (n = 10 per group). Mice were treated daily with either JQ1 at 50 mg/kg IP or vehicle (D5W) control. Tumor burden was assessed by serial bioluminescence imaging, or tumor volume measurements in the subcutaneous tumors. Bioluminescence was quantified using the Living Images software package (Caliper Life Sciences), and statistical significance was determined by Student's t-test (and confirmed with non-parametric Mann-Whitney test). In the disseminated disease model statistical significance of survival differences was determined by log-rank test. Studies were performed under the auspices of protocols approved by the DFCI IACUC.

In vivo studies on Vk*MYC mice

BET bromodomains and c-Myc

Vk*MYC mice (Chesi et al., 2008) were periodically bled by tail grazing. Blood was collected into Microtainer tubes (Becton Dickinson) and was let to coagulate at room temperature, before centrifugation for 10 minutes at 2,300 g. Sera were diluted 1:2 in normal saline buffer and analyzed by serum protein electrophoresis (SPEP) on a QuickGel Chamber apparatus using pre-casted QuickGels (Helena Laboratories) according to manufacturer's instruction. Densitometric analysis of the SPEP traces was performed using the clinically certified Helena QuickScan 2000 workstation, allowing a precise quantification of the various serum fractions, including the measurements of gamma/albumin ratio. Aged Vk*MYC mice with a M-spike of at least 15 g/L were treated intra-peritoneally for 5 days/week with JQ1 in 10% cyclodextrine (Sigma). SPEP was performed weekly and drug response was calculated by dividing the gamma/albumin ratio at d0 for each individual M-spike by the gamma/albumin ratio obtained post treatment. Densitometric profiles obtained at Day 0 and Day 14 were overlaid to show M-spike reduction.

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Figure S1

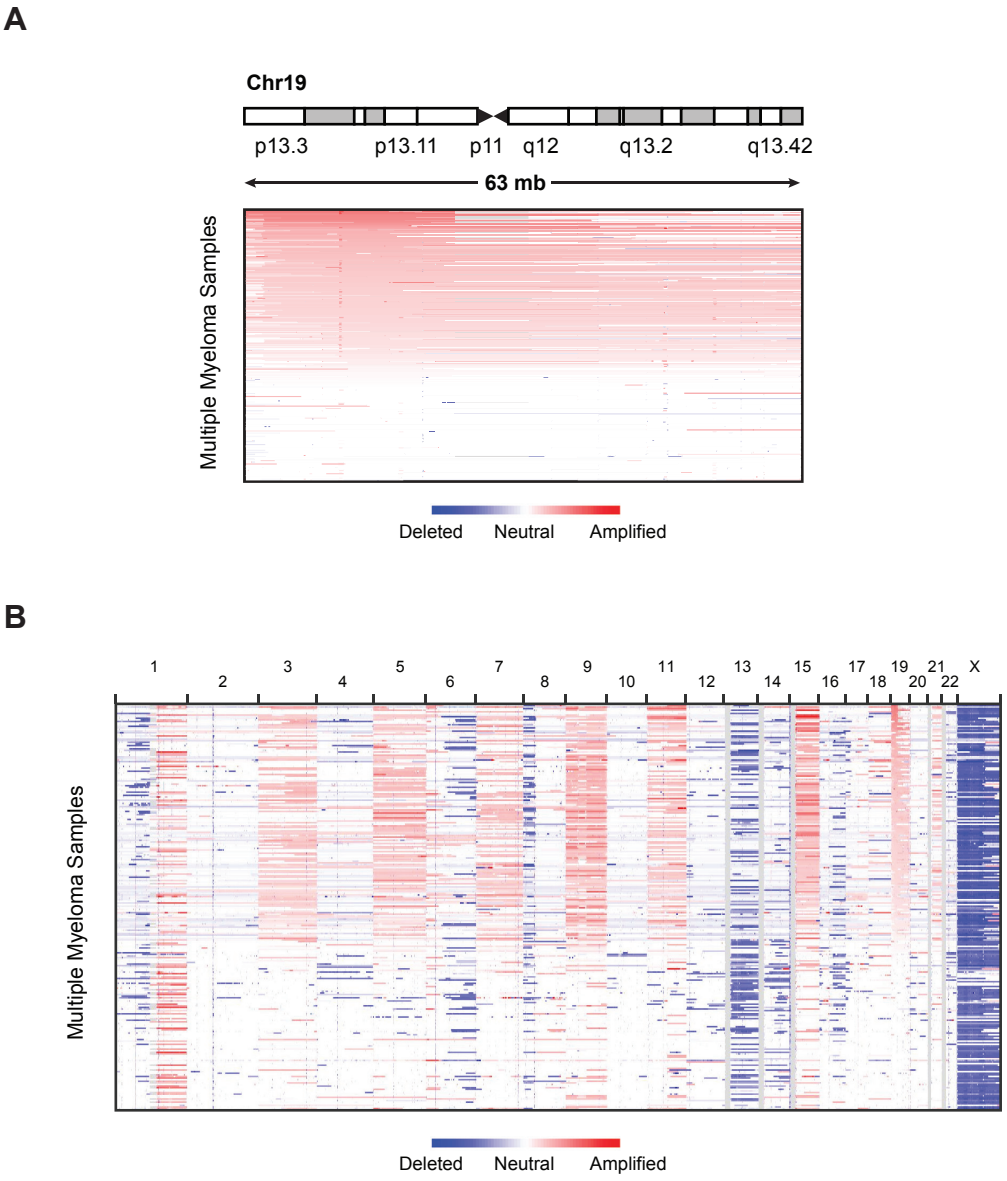
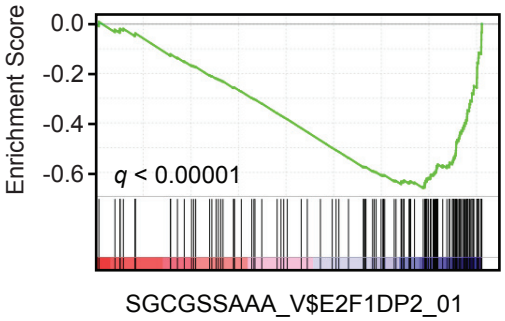

A
Chr19
p13.3
p13.11
p11
q12
q13.2
q13.42
63 mb
Multiple Myeloma Samples
Deleted
Neutral
Amplified
B
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Multiple Myeloma Samples
Deleted
Neutral
Amplified

Figure S2

A

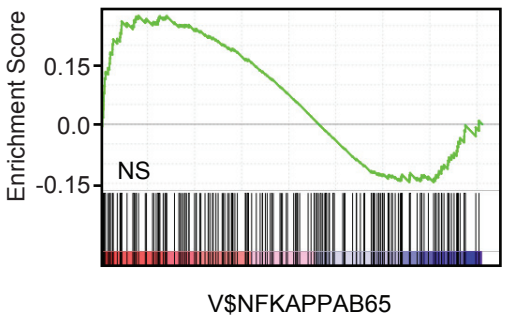
Rank	MSigDB Gene Set	NES	FDR q-val
1	SGCGSSAAA_V$E2F1DP2_01	-2.25	< 0.00001
2	V$E2F1_Q6_01	-2.20	< 0.00001
3	V$E2F_02	-2.16	< 0.00001
4	V$E2F1_Q6	-2.16	< 0.00001
5	V$E2F1DP1_01	-2.15	< 0.00001
6	V$E2F1DP2_01	-2.15	< 0.00001
7	V$E2F4DP2_01	-2.14	< 0.00001
8	V$E2F_Q6	-2.11	< 0.00001
9	V$E2F_Q4	-2.09	< 0.00001
10	V$E2F4DP1_01	-2.09	< 0.00001
11	V$MYCMAX_01	-2.08	< 0.00001
12	V$E2F1_Q4_01	-2.07	< 0.00001
13	V$E2F_Q4_01	-2.05	< 0.00001
14	V$E2F_Q3_01	-2.03	< 0.00001
15	V$E2F_Q6_01	-2.02	< 0.00001
16	V$E2F1DP1RB_01	-1.98	< 0.00001
17	KTGGYRSGAA_UNKNOWN	-1.97	< 0.00001
18	V$E2F_03	-1.92	0.00004
19	V$NMYC_01	-1.90	0.0001
20	V$E2F1_Q3	-1.85	0.00036
21	V$E2F_Q3	-1.79	0.0011
22	V$USF_C	-1.78	0.0013
23	V$E2F_01	-1.74	0.0026
24	V$MYCMAX_02	-1.73	0.0027
25	V$MYCMAX_B	-1.64	0.0095
26	V$MYC_Q2	-1.63	0.011
27	V$E2F1_Q4	-1.62	0.013
28	V$MAX_01	-1.61	0.013

B



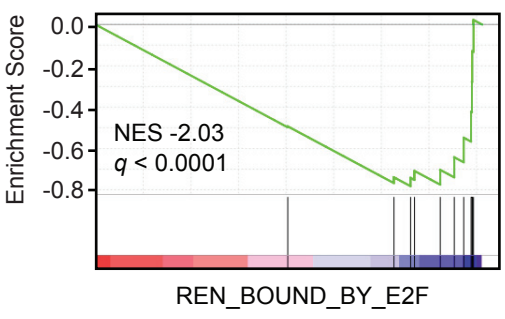
SGCGSSAAA_V$E2F1DP2_01

C



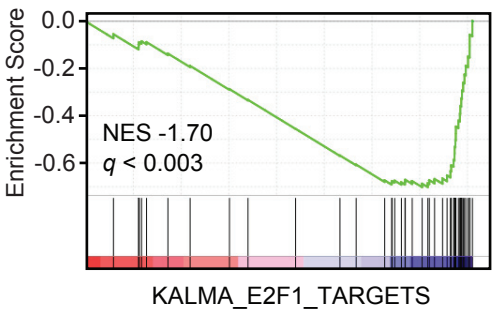
V$NFKAPPAB65

D



REN_BOUND_BY_E2F

E



KALMA_E2F1_TARGETS

Figure S3

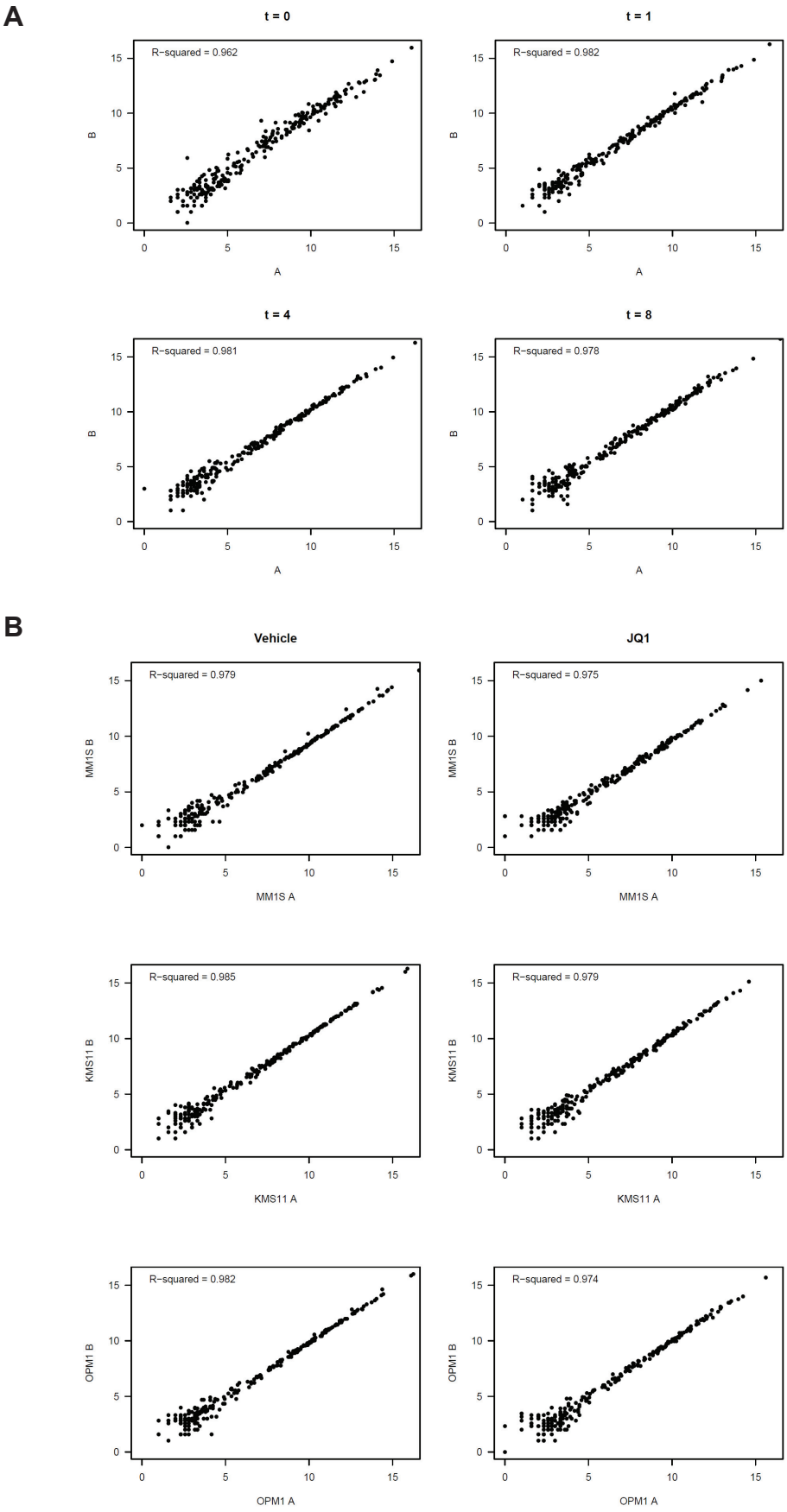

A
t = 0
R−squared = 0.962
t = 1
R−squared = 0.982
t = 4
R−squared = 0.981
t = 8
R−squared = 0.978
B
Vehicle
R−squared = 0.979
JQ1
R−squared = 0.975
R−squared = 0.985
R−squared = 0.979
R−squared = 0.982
R−squared = 0.974
A
B
MM1S A
MM1S B
KMS11 A
KMS11 B
OPM1 A
OPM1 B

Figure S4

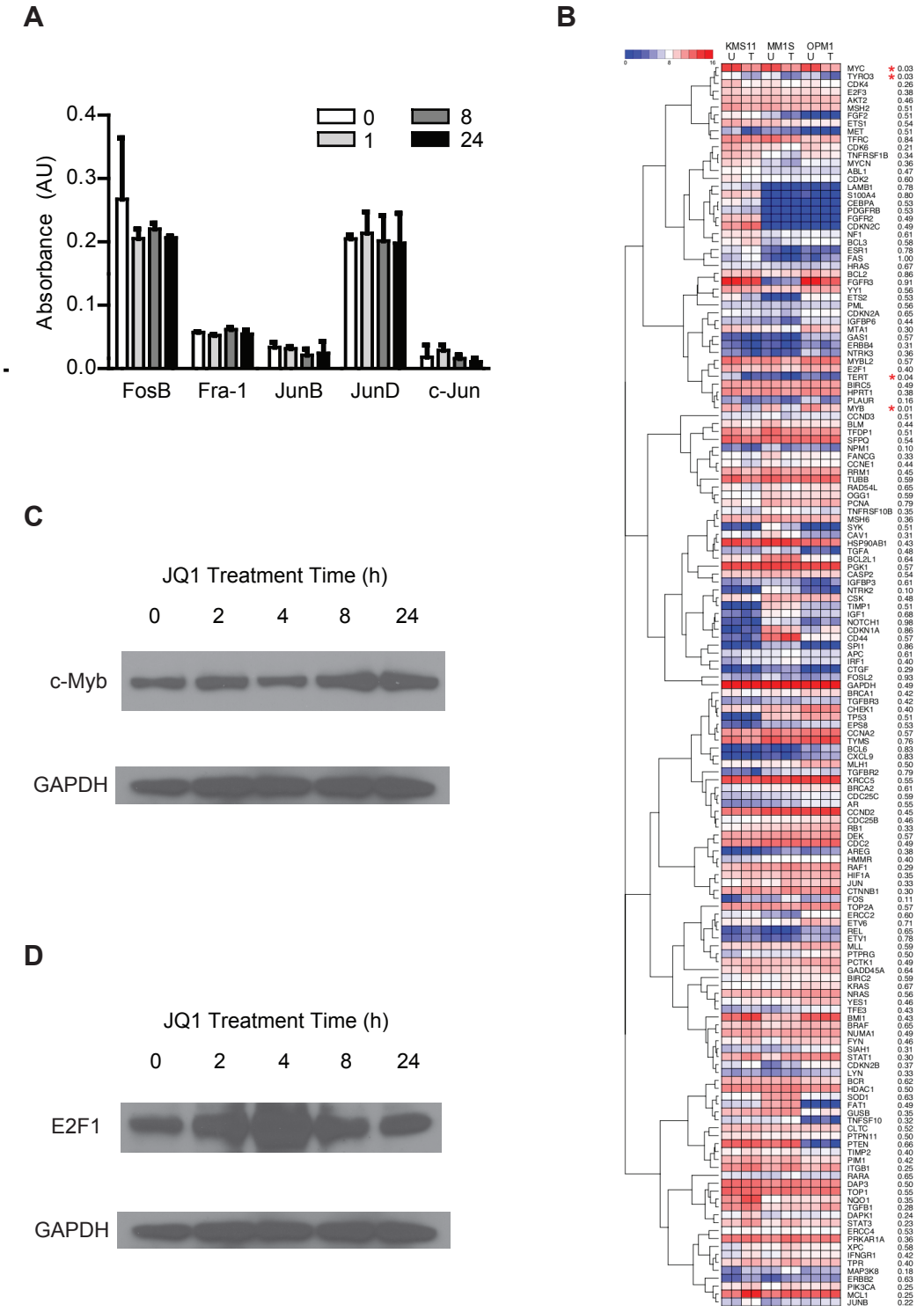

A
Absorbance (AU)
0.4
0.3
0.2
0.1
0.0
0
1
8
24
FosB
Fra-1
JunB
JunD
c-Jun
B
KMS11
MM1S
OPM1
U
T
C
JQ1 Treatment Time (h)
0
2
4
8
24
c-Myb
GAPDH
D
JQ1 Treatment Time (h)
0
2
4
8
24
E2F1
GAPDH

Figure S5

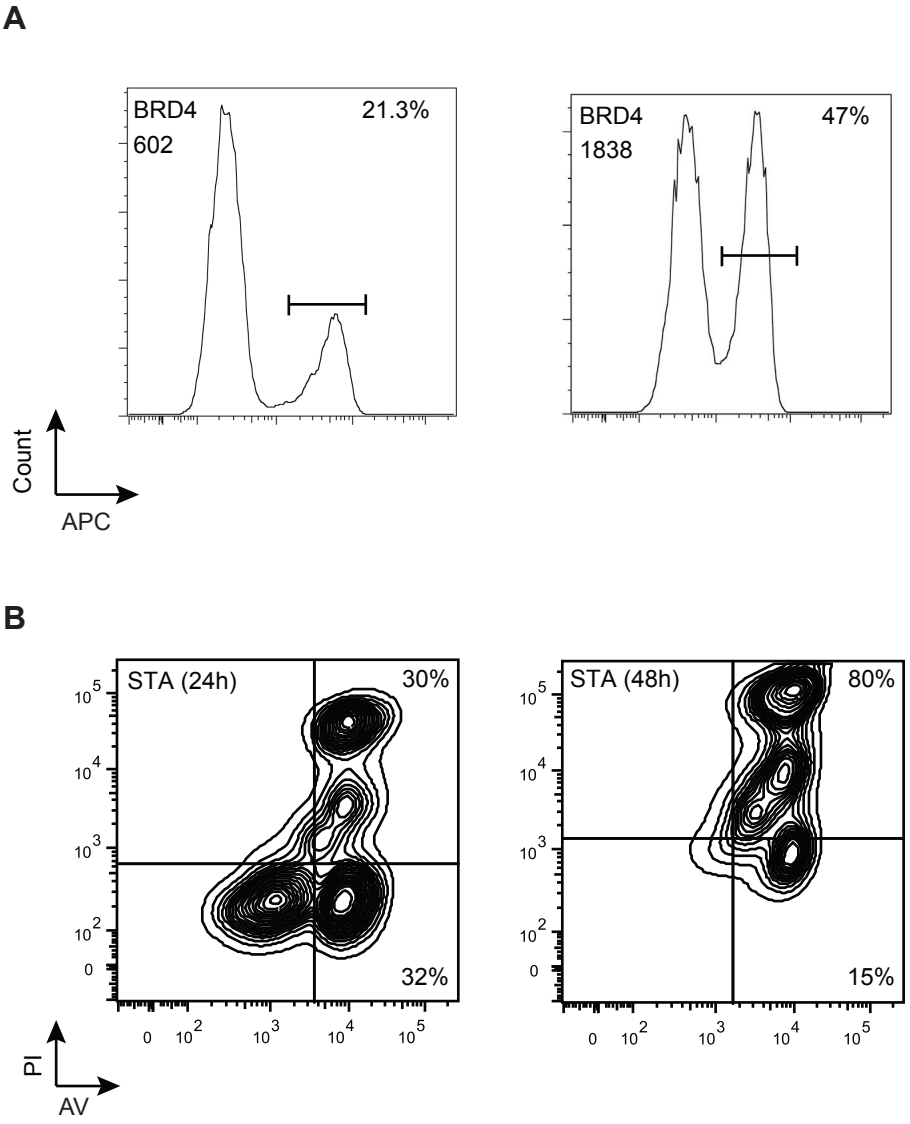

A
BRD4
602
21.3%
BRD4
1838
47%
Count
APC
B
STA (24h)
30%
32%
STA (48h)
80%
15%
PI
AV

Figure S6

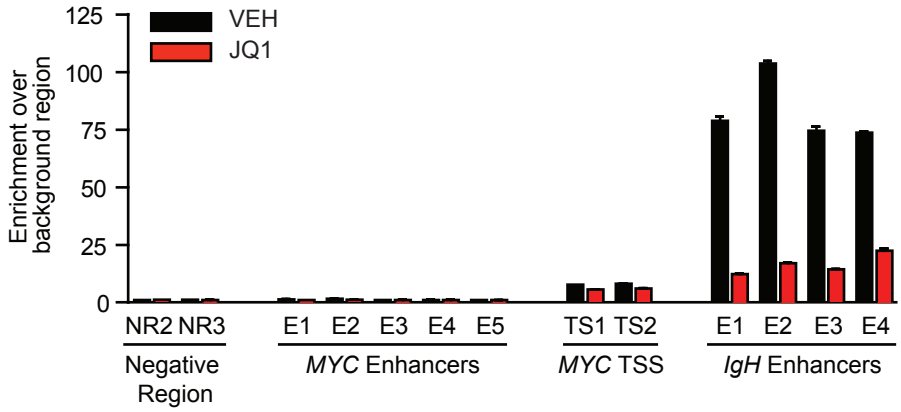
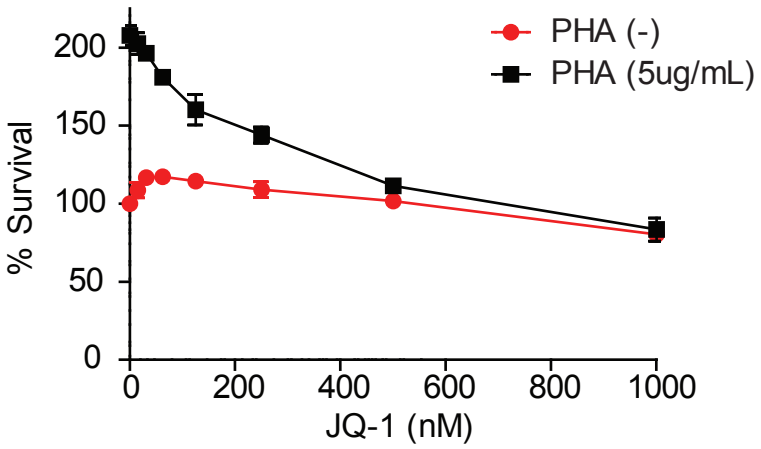
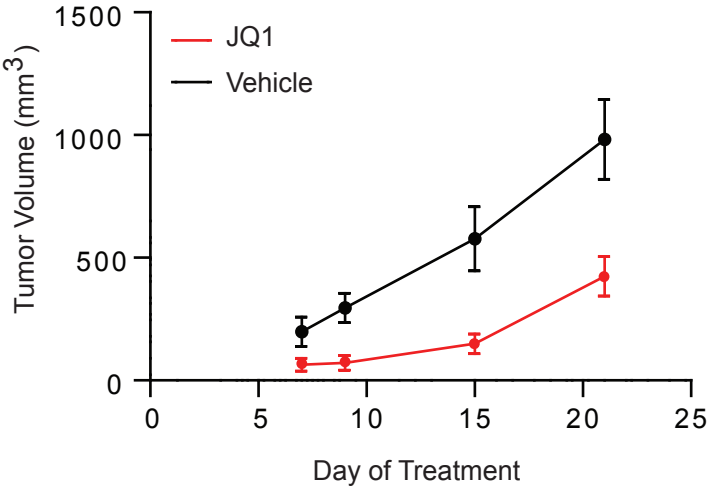

VEH
JQ1
Enrichment over background region
125
100
75
50
25
0
NR2 NR3
E1 E2 E3 E4 E5
TS1 TS2
E1 E2 E3 E4
Negative Region
MYC Enhancers
MYC TSS
IgH Enhancers

Figure S7

A



B



C