SUPPLEMENTAL MATERIALS AND METHODS

Cell Lines, Cell Culture and Retroviral Vectors. All lymphomas used in this study were maintained in B-cell Media (BCM; 45% DMEM, 45% Iscove's Media, 55 mM β-mercaptoethanol, 10% Fetal Bovine Serum) on γ-irradiated *Arf*$^{-/-}$ MEF feeder layers. Feeder layers were comprised of ~25% confluent irradiated *Arf*$^{-/-}$ MEFs pre-incubated with BCM for 3 days prior to addition of lymphoma cells. Lymphomas were routinely split 1:3 every 2-3 days and discarded after >3 weeks in culture.

Puma$^{-/-}$Eμ-*myc* and *Noxa*$^{-/-}$Eμ-*myc* lymphomas were kindly provided by Dr. Clare Scott (WEHI, Australia). BCL-2 and MCL-1 expressing derivatives were generated by retrovirally introducing MSCV-Bcl-2/EMCV/tRFP or MSCV-Mcl-1/EMCV/tRFP into lymphomas and isolating tRFP^{+} lymphomas by sorting with a FACSAria II (BD BioSciences). In order to generate *Arf*$^{-/-}$Eμ-*myc*/Bcl-2/Mcl-1 lymphomas, *Arf*$^{-/-}$Eμ-*myc*/Bcl-2/tRFP lymphomas were transduced with an MSCV-puro based retroviral vector expressing Mcl-1 cDNA. Post-infection cells were selected with puromycin.

MSCV based vectors have been previously described [1]. For generation of MSCV-Bcl2/EMCV/tRFP, the murine Bcl-2 cDNA (a kind gift of Dr. Guido Wendel, Memorial Sloan Kettering Cancer Center, US) was subcloned into the BglII/EcoRI sites of MSCV/GFP to generate MSCV-Bcl2/EMCV/GFP. The EMCV/GFP fragment was removed using HindIII and ClaI sites and replaced with an EMCV-tRFP fragment isolated from LMHT using HindIII/SalI to generate MSCV-Bcl-2/EMCV/tRFP. MSCV-Mcl-1/EMCV/tRFP was generated by excising Bcl-2 out of MSCV-Bcl-2/EMCV/tRFP with BglII/EcoRI and dropping in a PCR-amplified mouse Mcl-1 cDNA into these sites.

MycER/3T3 and MycER/U2OS cells were generated by transduction of pBabe MycER. Infected cells were cultured 1 week in the presence of puromycin (2μg/mL) to select for MycER expressing cells. NIH3T3 were maintained in DMEM supplemented with 10% FBS and Pen/Strep. U2OS cells were obtained from the ATCC and were maintained in McCoy's 5A media supplemented with 10% FBS and Pen/Strep.

MSCV-based expression of the wild-type and mutant human Dhx9 cDNAs [2] was performed by subcloning the Dhx9 cDNA preceded by an N-terminal Myc-tag into the BglII/EcoRI sites of MSCV/Puro^R, generating MSCV-Myc-hDHX9-Puro^R. MycER/3T3 cells were then transduced with the Dhx9-expressing constructs, and expression was determined by Western blot. The Dhx9.1271 shRNA was subsequently infected into the cDNA-expressing MycER/NIH3T3 cells and assays for ABT-737 sensitivity were performed. We verified by Western blot that the human Dhx9 cDNA was not targeted by the mouse Dhx9.1271 shRNA (data not shown).

All retroviral packaging was performed using ecotropic Phoenix cells according to established protocols (http://www.stanford.edu/group/nolan/retroviral_systems/retsys.html). Lentiviral transduction was performed using 2nd generation vectors following published procedures [3]. Briefly, 10 μg of lentiviral vector pPRIME-PGK-Puro-shRNA, 7.5 μg packaging plasmid psPAX2, envelope vector and 3 μg of envelope vector, pMDG2, were mixed and transfected using calcium phosphate mediated delivery into a 60 mm dish of 293T/17 (ATCC). Media was changed the next day to 2 mL of McCoy's 5A (target cell media) and 48 hrs post-transfection lentiviral supernatant was collected every 8-12 hrs up until 72 hrs post-transfection Lentiviral supernatant (0.5 mL) was mixed with 0.5 mL media and added to a well of a 6-well dish containing target U2OS cells (ATCC) that had been seeded at ~60% confluency the

previous day. Typically, a single transduction yielded >90% GFP^+ cells. Forty-eight hours post-transduction, puromycin was added to the media (2 μg/mL) and cells were selected for stable integrants for 3 more days. Assays were commenced thereafter. A lentiviral-based shRNA targeting human p53, pLVUHshp53, was obtained from Addgene and has been previously described [4].

Solexa Sequencing. Deep sequencing template libraries were prepared by PCR amplification of the guide strand from genomic DNA isolated from viable cells that were purified through a Ficoll gradient. Primers targeted the miR30 cassette, as well as the loop region of the shRNA, and contained a unique 8 bp barcode which was used to deconvolute different experimental conditions following pooling of samples (Fig. S3). PCR products were quantitated, to ensure that equimolar amounts were combined and sequenced on an Illumina Genome Analyser using a final concentration of 8 pM. Guide strands were sequenced using a custom primer (miR30*Eco*RISeq, $^{5'}$TAGCCCCTTGAATTCCGAGGCAGTAGGCA$^{3'}$). Sequence analysis was performed using a customized Galaxy platform at Cold Spring Harbor Laboratories. We determined that barcoded shRNA sequences not present in the library were recovered at a frequency of <50 sequence reads/chip. Setting this as a baseline, we considered sequence reads that were present at least 250 times (therefore, 5x above background) in the T0 sample across all replicates. Using this standard, we recovered 1330 unique shRNAs (73% of the total library) in T0 samples across all replicates. Correlation coefficients were calculated for all replicates in each pool by comparing relative abundances of each shRNA across replicates. Quantile normalization was then used to normalize relative abundances across different barcoded samples. In T10 samples, an additional 517 shRNAs (29% of the library) dropped below the threshold cutoff and were deemed straight

lethals and filtered out. To score shRNAs that were synthetic lethal with ABT-737, the fold depletion was calculated using the normalized relative abundances of each shRNA at T10 with vehicle and divided by their respective relative abundances at T10 in the presence of ABT-737. Scores were rank ordered and those shRNAs depleted >2.5-fold were selected for validation.

shRNA Sequences. The sequences of the 97 nt miR30-shRNA template used to generate the mouse shRNA libraries used in this study are provided in Table S1. Sequences were amplified using Primer 5'XhoI ($^{5'}$CAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCG$^{3'}$) and Primer 3'EcoRI($^{5'}$CTAAAGTAGCCCCTTGAATTCCGAGGCAGTAGGCA$^{3'}$) [5], which respectively contained unique XhoI and EcoRI sites and used for cloning into MLS. The sequences of additional miR30-shRNAs (from XhoI to EcoRI site) used in this study are: human Dhx9.860,

$^{5'}$CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGACACGAGAACATGGATCAAATA
TAGTGAAGCCACAGATGTATATTTGATCCATGTTCTCGTGCTGCCTACTGCCTCGGA
ATTC$^{3'}$; human Dhx9.2296,
5'CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGACAAGGTTATTTTGTCCACAAAT
AGTGAAGCCACAGATGTATTTGTGGACAAAATAACCTTGGTGCCTACTGCCTCGGAA
TTC$^{3'}$; human Mcl-1.1840,
$^{5'}$CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCGGAAATTCTTTCACTTCATTAT
AGTGAAGCCACAGATGTATAATGAAGTGAAAGAATTCCATGCCTACTGCCTCGGAA
TTC$^{3'}$; mouse Bim.825,
$^{5'}$CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGATAGGAACAGAGAAATATGCAA
TAGTGAAGCCACAGATGTATTGCATATTTCTCTGTTCCTAGTGCCTACTGCCTCGGAA
TTC$^{3'}$; mouse Myc.1405,

5'CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCAGAAATTGATGTGGTGTCTGTT AGTGAAGCCACAGATGTAACAGACACCACATCAATTTCTTTGCCTACTGCCTCGGAA TCC3';

Western Blotting. Extracts were prepared in RIPA lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% SDS, 1% NP40, 0.25% sodium deoxycholate). Protein concentrations were determined using the DC protein assay (Bio-Rad). Total protein lysates (30 µg) were resolved by SDS-PAGE, transferred to PVDF membranes (Millipore), probed with the indicated antibodies, and visualized using enhanced chemiluminescence (ECL) detection (Perkin Elmer). The antibodies used for protein expression analysis were directed against: Bcl-2 (C-2; SC Biotech), Mcl-1 (Rockland), eEF2 (#2332; Cell Signaling), Puma (CT; ProSci), Bim (3C5; Alexis Biochemicals), murine DHX9 (ab26271; Abcam), murine p53 (NCL-p53-505; Leica Microsystems), Atr pS428 (#2853; Cell Signaling), human p53 (DO-1; SC Biotech), Atr (N-19; Santa Cruz), Chk1 pS345 (133D3; Cell Signaling), Chk1 (#2345; Cell Signaling), human Dhx9 (B-5; SC Biotech), human Mcl-1 (S-19; SC Biotech), p21 SX118; BD Biosciences), p53 pS15 (#9284; Cell Signaling), Myc-tag (9E10, McGill Hybridoma Core Facility), and anti-BrdU conjugated to Alexa 647 (MoBU01; Invitrogen).

Microarray Validation. Validation of microarray data was performed by reverse transcription (RT) - quantitative (q)PCR on total RNA isolated from *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas expressing either RLuc.713 or Dhx9.1241. cDNA was generated using random hexamer primers and Superscript II reverse transcriptase (Invitrogen). RT-qPCRs were performed according to the manufacturer's instructions using SsoFast EvaGreen reagent and a Bio-Rad CFX96 system (Bio-Rad). The following primers were used for PCR amplification; Cdkn1a (p21) fwd-5'CCTGGTGATGTCCGACCTG3', rev-5'CCATGAGCGCATCGCAATC3'; Dhx9 fwd-

$^{5'}$CCGAGGAGCCAACCTTAAAGA$^{3'}$, rev-$^{5'}$TGTCCAATTTCCATGAAGCCC$^{3'}$; Mdm-2 fwd-$^{5'}$TGTCTGTGTCTACCGAGGGTG$^{3'}$, rev-$^{5'}$TCCAACGGACTTTAACAACTTCA$^{3'}$; p53 fwd-$^{5'}$GCGTAAACGCTTCGAGATGTT$^{3'}$, rev-$^{5'}$TTTTTATGGCGGGAAGTAGACTG$^{3'}$; Mcl-1 fwd-$^{5'}$GAGGAGGAAGAGGACGACCTATACC$^{3'}$, rev-$^{5'}$AGTTTCTGCTAATGGTTCGATGAAG$^{3'}$; Puma fwd-$^{5'}$ATGCCTGCCTCACCTTCATCT$^{3'}$, rev-$^{5'}$AGCACAGGATTCACAGTCTGGA$^{3'}$; Noxa fwd-$^{5'}$ACTGTGGTTCTGGCGCAGAT$^{3'}$, rev-$^{5'}$TTGAGCACACTCGTCCTTCAA$^{3'}$; c-Myc fwd-$^{5'}$CAAATCCTGTACCTCGTCCGATTC$^{3'}$, rev-$^{5'}$CTTCTTGCTCTTCTTCAGAGTCGC$^{3'}$; Bax fwd-$^{5'}$TGAAGACAGGGGCCTTTTTG$^{3'}$, rev-$^{5'}$AATTCGCCGGAGACACTCG$^{3'}$; Sesn1 fwd-$^{5'}$GGCCAGGACGAGGAACTTG$^{3'}$, rev-$^{5'}$AAGGAGTCTGCAAATAACGCAG$^{3'}$; Plk2 fwd-$^{5'}$GACTACTGCACCATAAGCATG$^{3'}$, rev-$^{5'}$CTTCTGGCTCTGTCAACACCT$^{3'}$; Bim fwd-$^{5'}$#GAGTTGTGACAAGTCAACACAAACC$^{3'}$, rev-$^{5'}$#GAAGATAAAGCGTAACAGTTGTAAGATAACC$^{3'}$; Actin fwd-$^{5'}$TCACTATTGGCAACGAGCGGTT$^{3'}$, rev-$^{5'}$TGTCAGCAATGCCTGGGTACAT$^{3'}$; GAPDH fwd-$^{5'}$AGGTCGGTGTGAACGGATTTG$^{3'}$, rev-$^{5'}$GGGGTCGTTGATGGCAACA$^{3'}$. All signals were quantified using the comparative Ct method and normalized to the levels of Actin.

Animal Studies and Cell Culture work. All animal studies were approved by the Faculty of Medicine Animal Care Committee (McGill University). To generate *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 cells, *Arf*$^{-/-}$Eμ-*myc* lymphomas were isolated, expanded *ex vivo*, and transduced with MSCV-based retroviral vectors expressing Bcl-2 and turboRFP – expression of the latter driven by the EMCV IRES translational element. Retroviral infections were performed three times with freshly collected virus at 8 hrs intervals. Forty-eight hours after the last infection, RFP^{+} cells were isolated by cell sorting on a FACSAria II (BD Biosciences).

For *in vivo* competition assays *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 cells were partially transduced with MLS/shRNA. Two days post-infection, ~10^6 viable cells were injected into the tail vein of C57BL/6 recipients. Two days after, mice were treated with vehicle or 75 mg/kg ABT-737 for 7 consecutive days, at which point both spleen and lymph nodes were collected and lymphomas isolated. The RFP$^+$ cell populations were analyzed by flow cytometry. JQ1(+) was obtained from BPS Bioscience (San Diego, CA).

siRNA Knockdown. Transient knockdown was performed using siRNAs from Thermo Scientific Dharmacon's predesigned siGenome SMARTpool collection, which consisted of a mixture of four siRNAs targeting the gene of interest. NIH3T3 cells harbouring the Myc-ER construct were seeded in 6-well plates and transfected at 50% confluency with either non-targeting siRNA (D-001206-13) or siRNAs targeting mouseATR (M-062167-01) using Lipofectamine 2000 (Invitrogen). For each well, cells were plated in 2 ml of DMEM with 10% FBS and without antibiotics. 100 pmol of siRNA was diluted in 250 ml of Opti-MEM (Invitrogen) and 5 ml of Lipofectamine 2000 was diluted in 250 ml of Opti-MEM. The two solutions were mixed together and incubated at room temperature for 20 minutes before being added to the cells (40 nM final siRNA concentration). The cells were replated 24 h later and harvested for Western blot analysis 72 h after transfection.

Genomic and Nascent DNA isolation and Quantitation. Genomic DNA was isolated using the GenElute MammalianGenomic DNA Miniprep Kit (Sigma, Oakville, ON, Canada), as per instructions of the manufacturer. Nascent DNA was prepared using the λ-exonuclease method, as previously described[6] with the following modifications: The λ-exonuclease digested samples were heated at 100^{o}C for 3 min, then immediately subjected to electrophoresis on a 2% agarose gel. DNA was visualized by staining with 0.02% (w/v) methylene blue (Sigma) and the origin-

containing nascent DNA, ranging between 350 and 1,000 bp in size was excised from the gel, purified with the Sephaglas BandPrep Kit, as per instructions of the manufacturer (GE Healthcare, Piscataway, NJ), and resuspended in TE. PCRs were performed with genomic or nascent DNA using the Biorad CFX96 instrument. The sequences and amplification conditions for all primer sets are shown in Table S5. Genomic DNA from parental cells was used to generate the standard curves needed for quantification of the PCR products. A negative control without template DNA was included with each set of reactions. PCR products were also resolved on 2% agarose gels and no extraneous bands were generated with any of the primer sets.

Growth Curve Assays. Cells were plated into 24 T25 flasks at a initial concentration of 50,000 cells per dish. Each day cells from 3 individual T25 were trypsinized, collected and counted at least 3 times using an EZ coulter counter. This procedure was performed for 4 consectutive days at which point cells began to approach confluency.

Viability and Cell Death Assays. To measure viability, cells were incubated in 2 μg/mL propidium iodide prior to analysis by flow cytometry. To measure apoptosis, lymphomas were stained with AnnexinV conjugated to phycoerythrin (PE) according to the manufacturer's protocol (BD BioSciences).

Expression Microarrays and Analysis. Total RNA from 3 biological replicates were isolated from FACS sorted *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas expressing either RLuc.713 or Dhx9.1241 using TRIzol according to the manufacturers recommendations (Invitrogen). RNA quality was assessed on an Agilent 21000 Bioanalyzer (Agilent Technologies), cDNA synthesis performed, and microarray hybridizations onto an Affymetrix 1.0 ST Gene microarrays performed at the McGill University and Génome Québec Innovation Center microarray resource. We used custom CDF definitions for data extraction [7] because these provide better precision and accuracy [8] and

normalized using robust multichip averaging (RMA) in R (www.r-project.org) using the "rma" function from the "affy" package version 1.32.1 with default settings. Differentially expressed genes were identified using the RVM two-tailed t-test and the resulting p-values were adjusted for multiple testing using the Benjamini-Hochberg false discovery rate method [9]. Activity of the p53 gene set was determined using the Kolmogorov-Smirnov test comparing fold changes of p53 target genes to fold changes of non target genes (in R). Raw data has been deposited at the Gene Expression Omnibus (GEO) accession GSE36938.

H2AX Staining Assay. For γH2AX foci staining, cells were grown on glass coverslips, treated as indicated, fixed with 2% buffered paraformaldehyde for 10 min at RT, and permeabilized in PBS containing 0.2% Triton X-100 and 2 μg/mL BSA for 8 min at RT. Cells were then washed twice with PBS. Cells were then blocked 1hr in 5% FBS in PBS at RT. After washing in 1X PBS, incubated with a 1:100 dilution of mouse monoclonal anti-γH2AX antibody (clone JWB301) in 5% FBS in PBS for 1hr at RT. After washing 2X in PBS, primary antibody was then detected with a secondary Alexa 594–conjugated goat anti-rabbit IgG (Molecular Probes, Invitrogen, Eugene, OR). Cells were lastly stained with DAPI in PBS at a concentration of 50 ng/ mL for 1 min at RT. Coverslips were finally mounted in Immunomount (Thermo-Shandon). Fluorescence was visualized by Immunofluorescence was visualized using a Zeiss Observer Z1 microscope using a 20x plan apochromat lens. Experiments were performed 3 times, counting at least 200 cells from at least 5 independent fields at 10X magnification per experiment. This processes semi-automated with Multi-image processing in the Zeiss Software package.

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Supplemental Figure Legends

Supplemental Figure 1. Loss of Mcl-1 is selected against in *Arf*$^{-/-}$Eμ-*myc* Lymphomas. (**A**) *Arf*$^{-/-}$ Eμ-*myc* or *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas show differential sensitivity to RNA_i-mediated suppression of Mcl-1. *Arf*$^{-/-}$Eμ-*myc* or *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas were transduced with the indicated shRNAs. Fitness of the corresponding shRNA expressing cells was measured at T0 (T = 0 days) and T8. Error bars represent ± SEM; n=3. **(B)** *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas were transduced with the indicated shRNAs and fitness of the GFP+ cells measured on Days 2, 3, and 8. The fraction of GFP^+ cells is set relative to the values for each shRNA obtained on Day 0 (2 days after infection). Error bars represent ± SEM; n=3. (**C**) Dose-response curve of *Arf*$^{-/-}$Eμ-*myc* and *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 cells to inhibition of protein synthesis by CHX. Cell death was quantitated by propidium iodide dye staining. Error bars represent ± SEM; n=3.

Supplemental Figure 2. Mcl-1 overexpression promotes ABT-737 resistance in *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas. The fraction of PI-negative lymphomas was determined 16 h after exposure to increasing concentrations of ABT-737 and set relative to vehicle treated cells. Error bars represent SD; n=3.

Supplemental Figure 3. (A) Categories of translation associated genes evaluated in the RNAi screen. Indicated in parenthesis is the number of genes in each category. **(B)** Schematic representation of MLS retrovirus used in these studies for expression of shRNAs [1].

Supplemental Figure 4. Schematic representation of RNAi library synthesis and screening strategy. A customized shRNA library targeting genes involved in protein synthesis was synthesized using on-chip oligonucleotide synthesis and oligonucleotides were PCR-amplified to incorporate vector-compatible restriction sites and cloned in bulk into MLS. Sequence-verified shRNAs were then distributed into 3 pools and transduced into *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas. The resulting mixed lymphoma population (T0) was treated with either vehicle (0.1% DMSO) or ABT-737 (600 nM) for an additional 10 days (T10). Following isolation of genomic DNA (n = 3 for each condition), PCR was performed on the shRNA guide strands and incorporate a unique bar code identifier for the sample. The resulting PCR products were subjected to deep-sequencing to quantify the relative abundance of each shRNA in the library. The corresponding

correlation coefficients for all replicates can be found in Table S2 and the data generated in the screen can be found in Table S3.

Supplemental Figure 5. Representation of class identities of shRNAs at T0 and T10 in *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas.

Supplemental Figure 6. Dhx9 suppression does not sensitize lymphomas to etoposide. *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas were retrovirally infected with MLS expressing the indicated shRNAs. Lymphomas were then treated with increasing doses of etoposide and the percent of viable lymphomas measured 48hrs later. Cell viability was normalized to the corresponding vehicle treated lymphomas and represented as a percentage. Two independent experiments were performed and are represented as the average of triplicates for each experiment.

Supplemental Figure 7. DHX9 suppression is synthetic lethal with ABT-737. *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas were retrovirally infected with MLS expressing the indicated shRNAs. Lymphomas were then treated with 0.1% DMSO or 600 nM ABT-737 and the percentage of GFP^{+} viable lymphomas measured on the indicated days following exposure to compound. Error bars represent SEM; n=3.

Supplemental Figure 8. Scatter plots documenting correlation between p53 induction, DHX9 suppression, and ABT-737 sensitivity. **(A)** A series of epi-allelic Dhx9 shRNAs were stably expressed in *Arf*$^{-/-}$Eμ-*myc*/Bcl-2. Scatter plot comparing DHX9 knockdown to p53 levels in *Arf*$^{-/-}$ Eμ-*myc*/Bcl-2 lymphomas (R^2 = 0.72; p value < 0.02, p values were generated using a paired student's t-test; n=6). **(B)** p53 protein levels were quantitated and plotted against the ABT-737 EC_{50} calculated for each *Dhx9* shRNA (R^2=0.77; p value > 0.016, p values were generated using a paired student's t-test; n=6).

Supplemental Figure 9. p53 activation reverses resistance to ABT-737. **(A)** Eμ-*myc* lymphomas of the indicated genotype were treated overnight with vehicle, 600 nM ABT-737, 10 μM Nutlin-3 or a combination of both. Cell viability was measured by propidium iodide staining followed by flow cytometry. Error bars represent SEM; n=3. **(B)** Immunoblot analysis of cell extracts

prepared from either *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 or *p53*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas treated for 12h with 10 μM Nutlin-3.

Supplemental Figure 10. Lymphomas were transduced by a single infection with MLS expressing the indicated shRNA. *Arf*$^{-/-}$Eμ-*myc* **(A)**, *Arf*$^{-/-}$Eμ-*myc*/Bcl2 **(B)** and *p53*$^{-/-}$Eμ-*myc* **(C)** lymphomas were plated onto feeder layers at a density of 2.5 x 10^5 cells/mL and passaged every 2-3 days at a 1:3 split. The % GFP$^+$ population was measured on the indicated days (~5 x 10^4 cells analyzed/data point) and normalized to the initial value plated at Day 0. To distinguish viable from dead cells, lymphomas were stained with PI and both FSC/SSC and PI measurements were taken using a Guava Easycyte. Error bars represent ± SEM; n=3.

Supplemental Figure 11. BIM suppression does not prevent DHX9 from sensitizing lymphomas to ABT-737. **(A)** *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas were infected with either shRluc or shBIM and sorted for GFP+ lymphoma, then both *Arf*$^{-/-}$Eμ-*myc*/Bcl-2/shRluc and *Arf*$^{-/-}$Eμ-*myc*/Bcl-2/shBim cells were infected with either MLP Fluc.1309 or MLP Dhx9.1241 and selected with puromycin. The fraction of PI-negative cells was determined 16 h after exposure to increasing doses of ABT-737. Error bars are SEM; n=3. **(B)** Immunoblot analysis confirming Dhx9 knockdown and Bim knockdown in *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas expressing the indicated shRNAs.

Supplemental Figure 12. Loss of DHX9 sensitizes lymphomas to ABT-737 in a NOXA-dependent manner. (**A**) Eμ-*myc* lymphomas of the indicated genotype were infected with Fluc.1309 or Dhx.1241 and shRNA depletion induced by ABT-737 was measured for 8d. The shRNA abundance was normalized to abundance in the corresponding vehicle treated lymphomas. Error bars represent ± SEM; n=3. **(B)** The percentage of cell death induced by 600 nM ABT-737 (16h) was measured in *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 and *Noxa*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas expressing the indicated shRNA. (n=3; error bars represent ± SEM; * denotes a p value = 0.001, ** denotes a p value = 0.9, as determined by the student t-test). **(C**) Immunoblot analysis confirming DHX9 knockdown and p53 induction in *Arf*$^{-/-}$Eμ-*myc*/Bcl-2, *p53*$^{-/-}$Eμ-*myc*/Bcl-2, *Puma*$^{-/-}$Eμ-*myc*/Bcl-2 and *Noxa*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas.

Supplemental Figure 13. Targeting Dhx9 eliminates MCL-1 overexpressing lymphomas. Lymphomas of the indicated genotype were transduced with MLS expressing either Rluc.713 shRNA **(A)** or Dhx9.1241 **(B)**. These lymphomas were plated onto feeder layers at a density of 2.5 x 10^5 cells/mL and passaged every 2-3 days at a 1:3 split. The % GFP^+ population was measured on the indicated days (~5 x 10^4 cells analyzed/data point) and normalized to the initial value plated at Day 0. To distinguish viable from dead cells, lymphomas were stained with PI and both FSC/SSC and PI measurements were taken using a Guava Easycyte. Error bars represent ± SEM; n=3. **(C)** Western blotting with the indicated antibodies confirms MCL-1 overexpression and Dhx9 knockdown.

Supplemental Figure 14. Loss of DHX9 synergizes with MYC to enhance ABT-737 sensitivity in U2OS cells. (A) Cell death was measured by PI staining in U2OS cells expressing pBABE or pBABE-MycER, along with the indicated shRNAs. U2OS cells expressing pBABE or pBABE-MycER were infected with lentivirus expressing either shFLUC or shDHX9.860. Seven days post-infection, MYC activity was induced by treatment with 500 nM 4-OHT for 36h, followed by exposure to vehicle or 1 μM ABT-737 for an additional 12h. Cells were harvested, stained with PI, and analyzed by flow cytometry. Error bars represent SD; n=3. (B) Immunoblot analysis of extracts prepared from U2OS cells expressing pBABE or pBABE-MycER, infected with shFLUC or shDHX9.860, harvested 6 days post-infection.

Supplemental Figure 15. The combination of JQ1(+) and ABT-737 induces cell death regardless of Dhx9 status. *Arf*$^{-/-}$Eμ-*myc*/Bcl-2 lymphomas expressing the indicated shRNA were exposed to 150 nM ABT-737 and/or 200 nM JQ1(+) for 16 hrs and cell death quantitated by PI staining. Cell viability was determined by propidium iodide staining. n=3; Errors bars represent ±SEM.

Supplemental Figure 16. Dhx9 knockdown increases the amount of asynchoronous cells found in S-phase. Cell cycle distribution of Myc-ER/3T3 expressing the indicated shRNAs. Error bars represent SEM; n=3

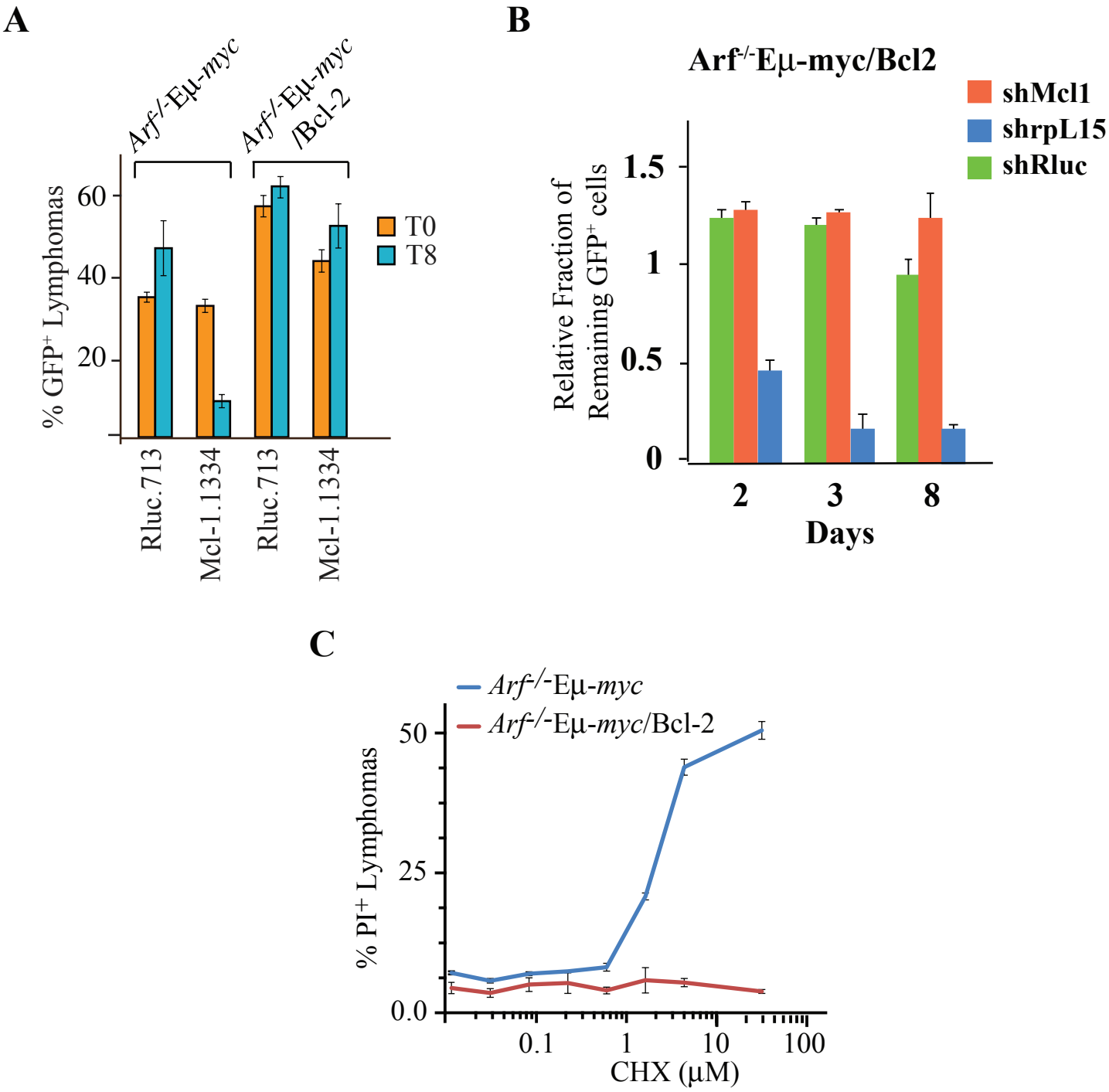
Supplemental Figure 17. Targeting DHX9 reduces H2A.X activation and sensitivity to S-phase specific killing agents. **(A)** Myc-ER/3T3 expressing the indicated shRNAs were treated with either 10 Gy of γ-IR or 2uM camptothecin for 1hr and the percentage of cells with pan nuclear H2A.X staining was counted by immunoflouresence over the indicated time periods. Experiments were performed 3 times, counting at least 200 cells from at least 5 independent fields at 10X magnification. Error bars represent SEM. **(B)** Growth curves of NIH3T3, NIH3T3/MYC-ER(-4-OHT) and NIH3T3/MYC-ER(+4-OHT), expressing the indicated shRNAs were treated with etoposide (40 uM) for 24hrs and cell death was measured with PI staining. Error bars represent SEM; n=3

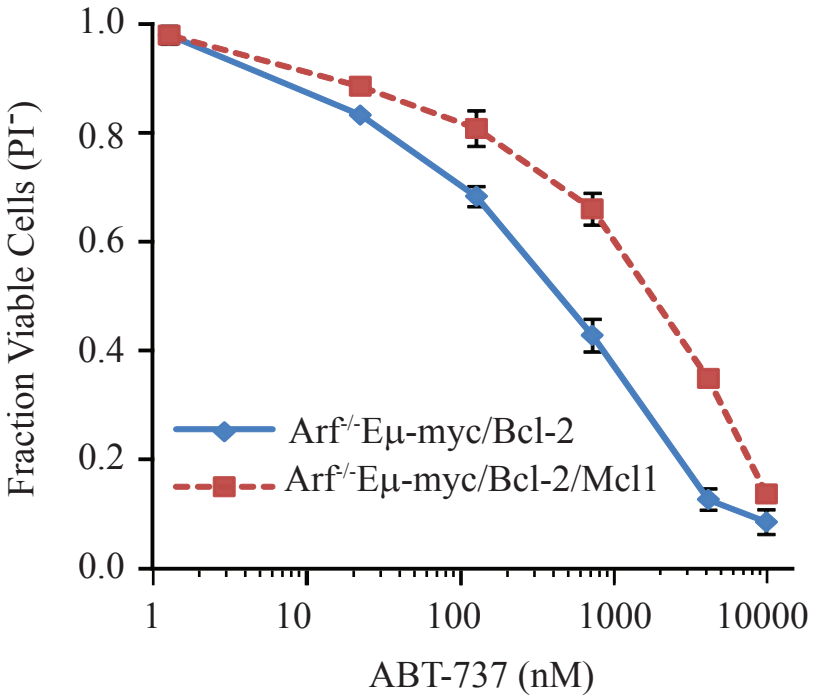
Supplemental Figure 18. (A) Representative immunoblot of cell extracts from $Arf^{-/-}$Eμ-*myc*/Bcl-2 lymphomas that had been treated with 0.5 mM HU for 24 h. **(B)** Percent of PI-positive $Arf^{-/-}$Eμ-*myc*/Bcl-2 lymphoma cells exposed to vehicle, 600 nM ABT-737, 0.5 mM HU, or a combination of both for 24h at which point they were harvested, stained with PI and analyzed by flow cytometry. Bars represent mean SEM Error; n=3.

Supplemental Figure 19. Dhx9 cDNA expression constructs used in the current study. **(A)** Schematic diagram of Dhx9 cDNA and mutant constructs used in this study. Various domains of Dhx9 are highlighted. dsRBD, double-stranded RNA Binding Domain; CTD, carboxy terminal domain harboring nuclear localization signal and single-stranded nucleic acid binding RGG box. **(B)** MycER/3T3 cells were transduced to express either Fluc or Dhx9 shRNAs in combination with the indicated DHX9 cDNAs. MYC was induced with 500 nM 4-OHT for 36 h, then treated with additional vehicle or ABT-737 for an additional 12 hrs. Cells were stained with PI and analyzed by flow cytometry. Error bars represent SEM; n=3. **(C)** Expression levels of Dhx9 and mutants expressed in MycER/3T3 cells. Immunoblot analysis of cell extracts prepared from MycER/3T3 stably expressing the indicated DHX9 cDNAs. Blots were probed with anti-Myc tag or anti-eEF2 (loading control) antibodies. Panels are from the same experiment but juxtapositioned for clarity.

REFERENCES.

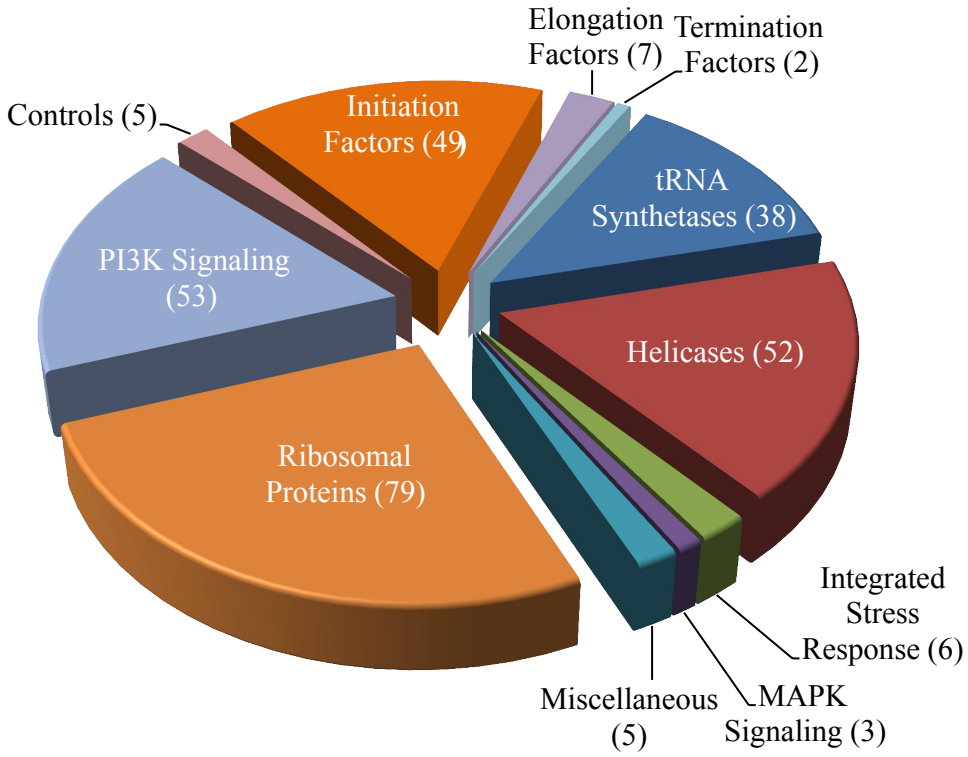
1. Dickins RA, Hemann MT, Zilfou JT, et al. Probing tumor phenotypes using stable and regulated synthetic microRNA precursors. *Nat Genet*. 2005;37(11):1289-1295.


A
Arf-/-Eμ-myc
Arf-/-Eμ-myc /Bcl-2
% GFP+ Lymphomas
60
40
20
T0
T8
Rluc.713
Mcl-1.1334
Rluc.713
Mcl-1.1334
B
Arf-/-Eμ-myc/Bcl2
shMcl1
shrpL15
shRluc
Relative Fraction of Remaining GFP+ cells
1.5
1
0.5
0
2
3
8
Days
C
Arf-/-Eμ-myc
Arf-/-Eμ-myc/Bcl-2
% PI+ Lymphomas
50
25
0.0
0.1
1
10
100
CHX (μM)

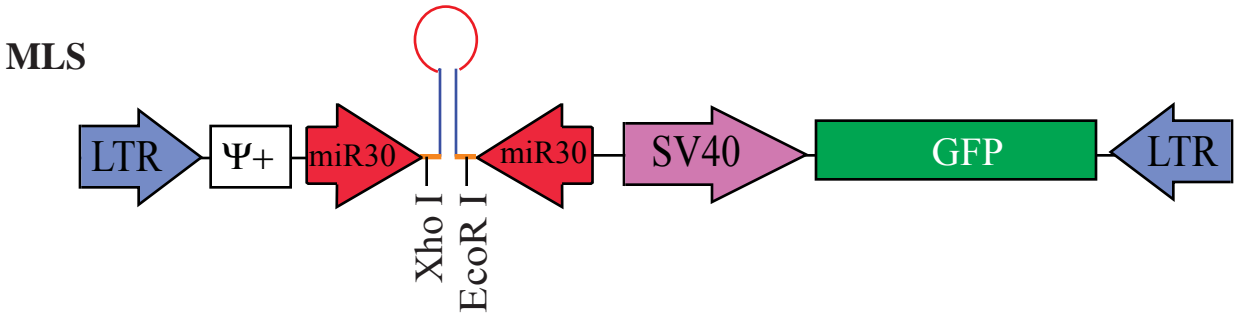

1.0
0.8
0.6
0.4
0.2
0.0
Fraction Viable Cells (PI-)
Arf-/-Eμ-myc/Bcl-2
Arf-/-Eμ-myc/Bcl-2/Mcl1
1
10
100
1000
10000
ABT-737 (nM)

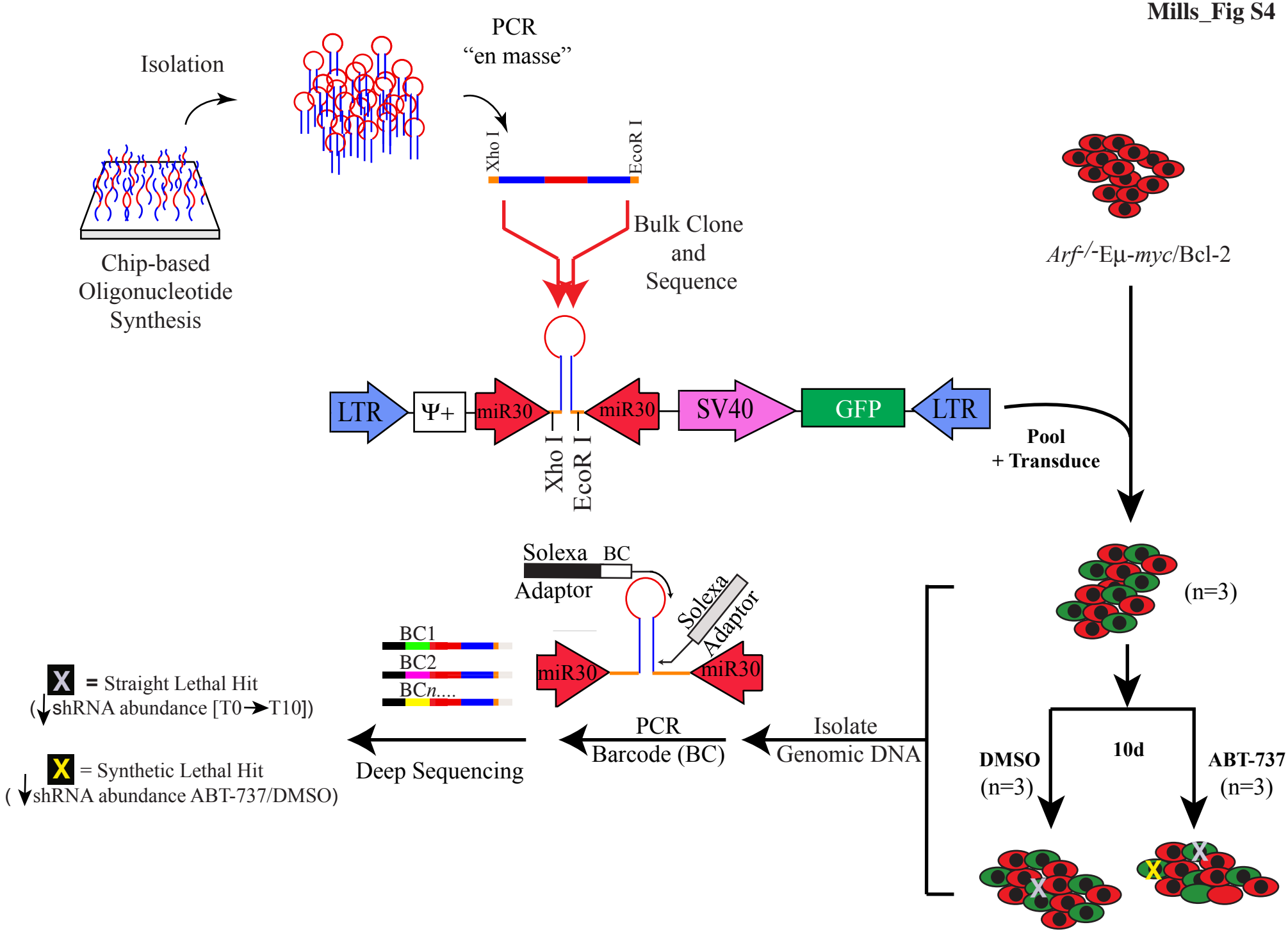
A

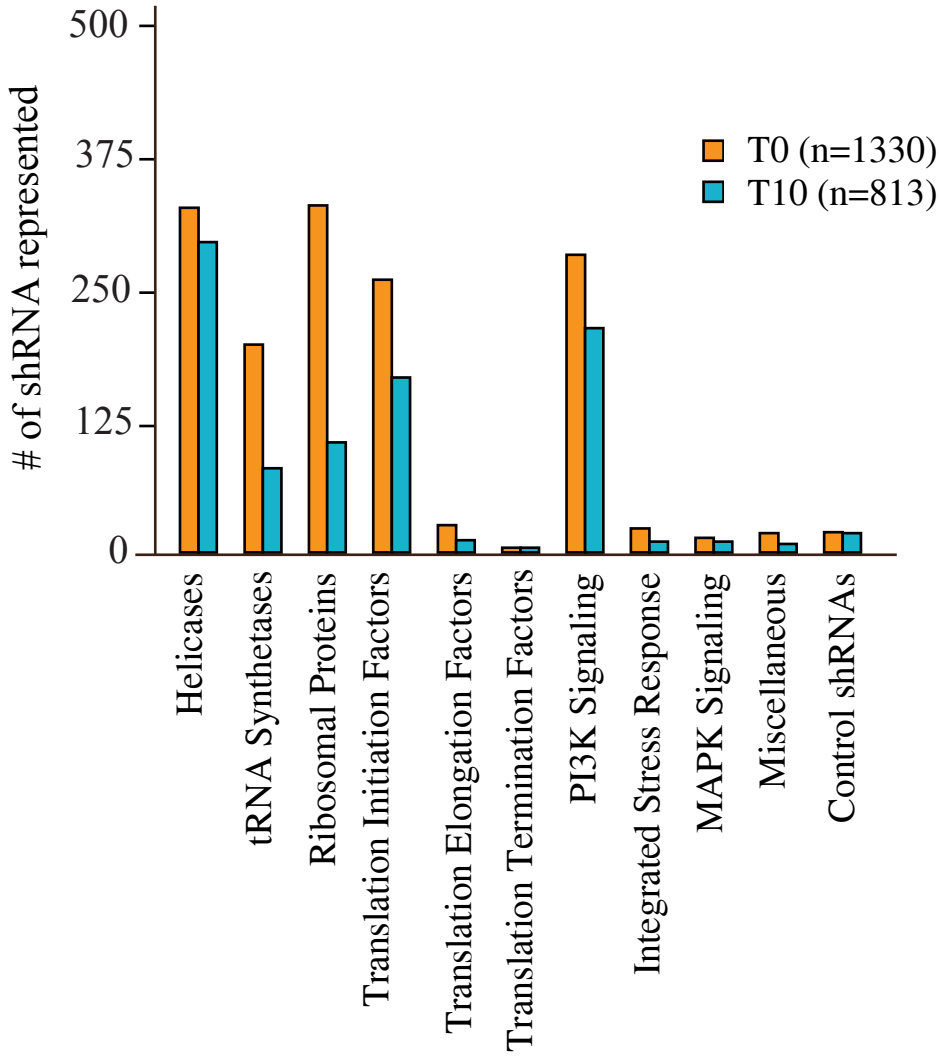
294 Genes associated with Protein Synthesis

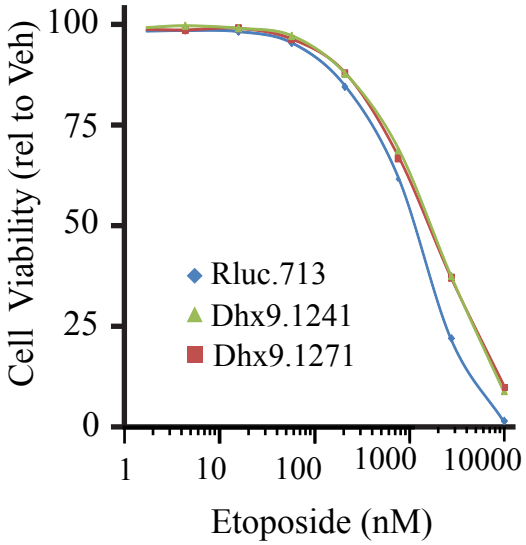


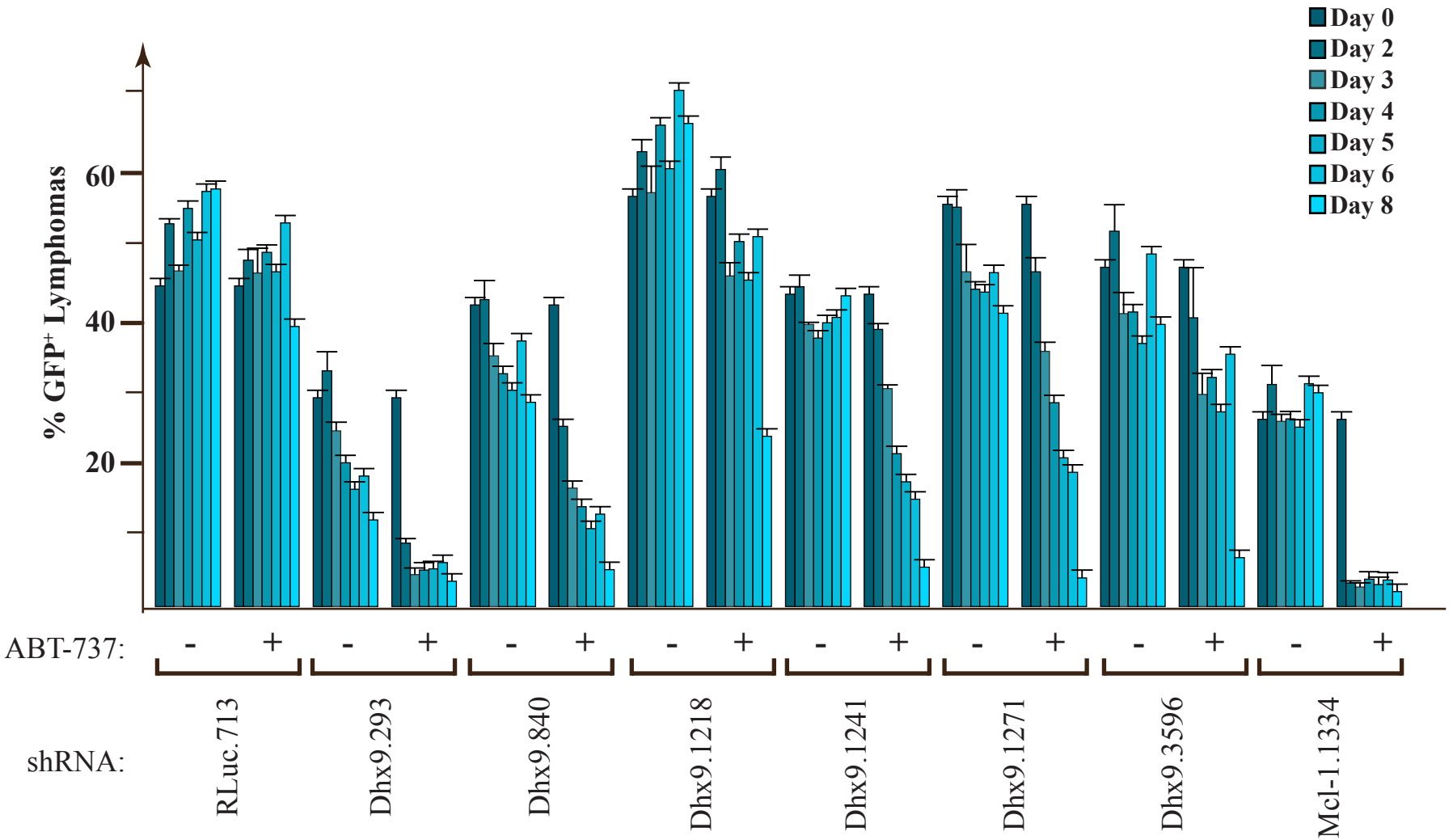
B



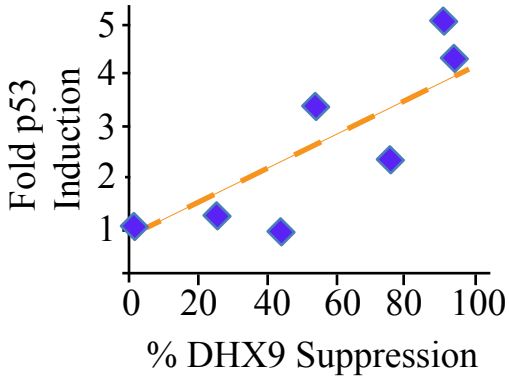

Isolation
Chip-based
Oligonucleotide
Synthesis
PCR
"en masse"
Xho I
EcoR I
Bulk Clone
and
Sequence
LTR
Ψ+
miR30
miR30
SV40
GFP
LTR
Xho I
EcoR I
Arf-/-Eμ-myc/Bcl-2
Pool
+ Transduce
(n=3)
10d
DMSO
(n=3)
ABT-737
(n=3)
Isolate
Genomic DNA
PCR
Barcode (BC)
Solexa BC
Adaptor
Solexa
Adaptor
miR30
miR30
BC1
BC2
BCn....
Deep Sequencing
X = Straight Lethal Hit
(↓shRNA abundance [T0→T10])
X = Synthetic Lethal Hit
(↓shRNA abundance ABT-737/DMSO)


500
375
250
125
0
# of shRNA represented
T0 (n=1330)
T10 (n=813)
Helicases
tRNA Synthetases
Ribosomal Proteins
Translation Initiation Factors
Translation Elongation Factors
Translation Termination Factors
PI3K Signaling
Integrated Stress Response
MAPK Signaling
Miscellaneous
Control shRNAs

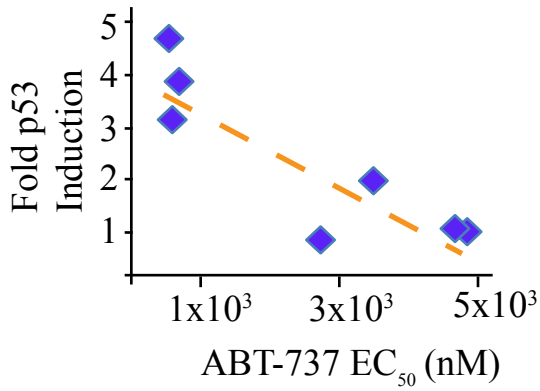

Cell Viability (rel to Veh)
100
75
50
25
0
1
10
100
1000
10000
Etoposide (nM)
Rluc.713
Dhx9.1241
Dhx9.1271


Day 0
Day 2
Day 3
Day 4
Day 5
Day 6
Day 8
% GFP+ Lymphomas
60
40
20
ABT-737:
- + - + - + - + - + - + - + - +
shRNA:
RLuc.713
Dhx9.293
Dhx9.840
Dhx9.1218
Dhx9.1241
Dhx9.1271
Dhx9.3596
Mcl-1.1334

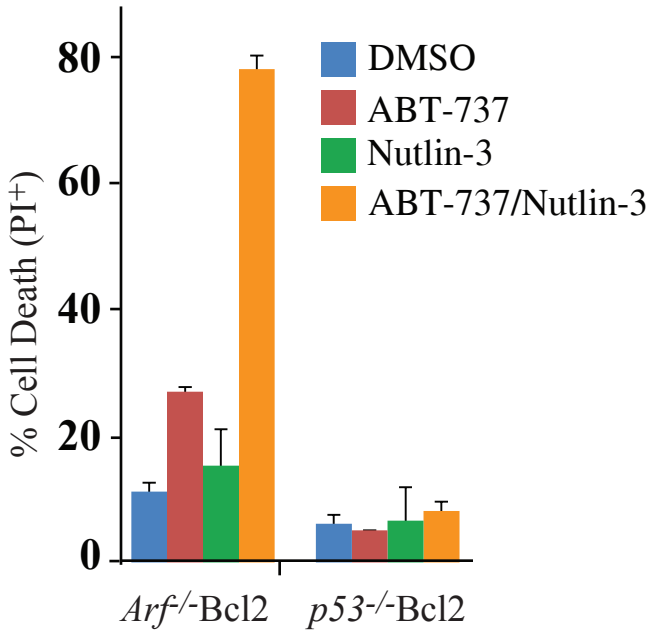
A


Fold p53 Induction
5
4
3
2
1
0
20
40
60
80
100
% DHX9 Suppression

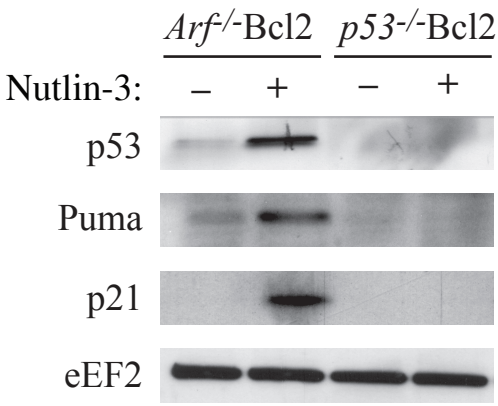
B


Fold p53 Induction
5
4
3
2
1
1x10³
3x10³
5x10³
ABT-737 EC50 (nM)

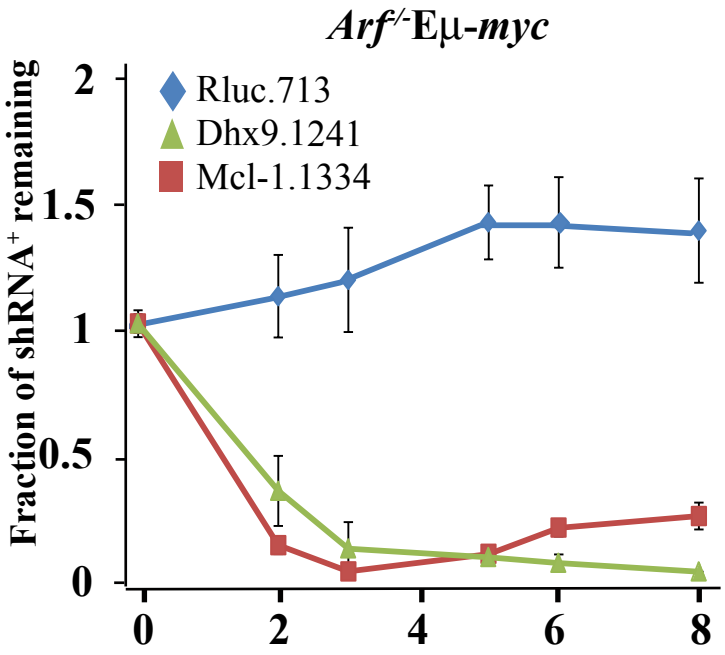
A


80
60
40
20
0
% Cell Death (PI+)
DMSO
ABT-737
Nutlin-3
ABT-737/Nutlin-3
Arf-/-Bcl2
p53-/-Bcl2

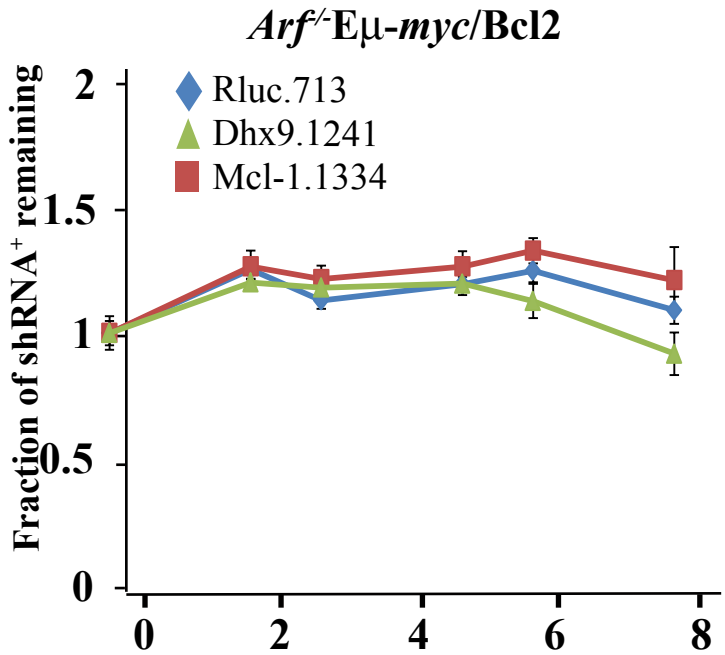
B


Arf-/-Bcl2
p53-/-Bcl2
Nutlin-3:
–
+
–
+
p53
Puma
p21
eEF2

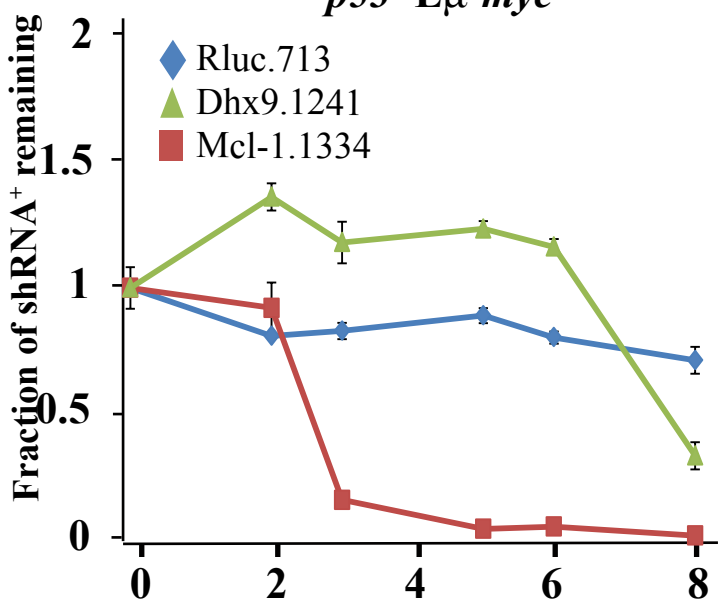
A


Arf$^{-/-}$Eμ-*myc*
Rluc.713
Dhx9.1241
Mcl-1.1334
Fraction of shRNA^{+} remaining
0
0.5
1
1.5
2
0
2
4
6
8

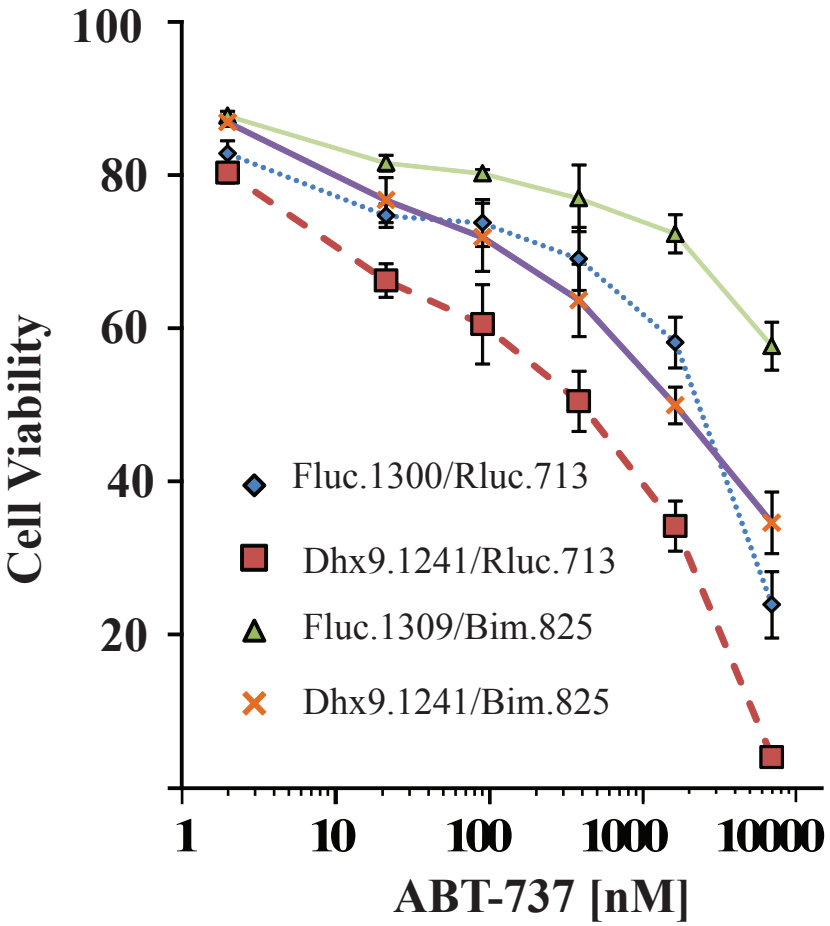
B


Arf$^{-/-}$Eμ-*myc*/Bcl2
Rluc.713
Dhx9.1241
Mcl-1.1334
Fraction of shRNA^{+} remaining
0
0.5
1
1.5
2
0
2
4
6
8

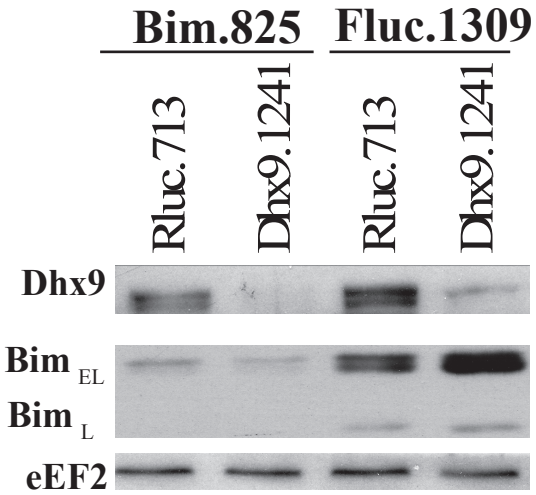
C

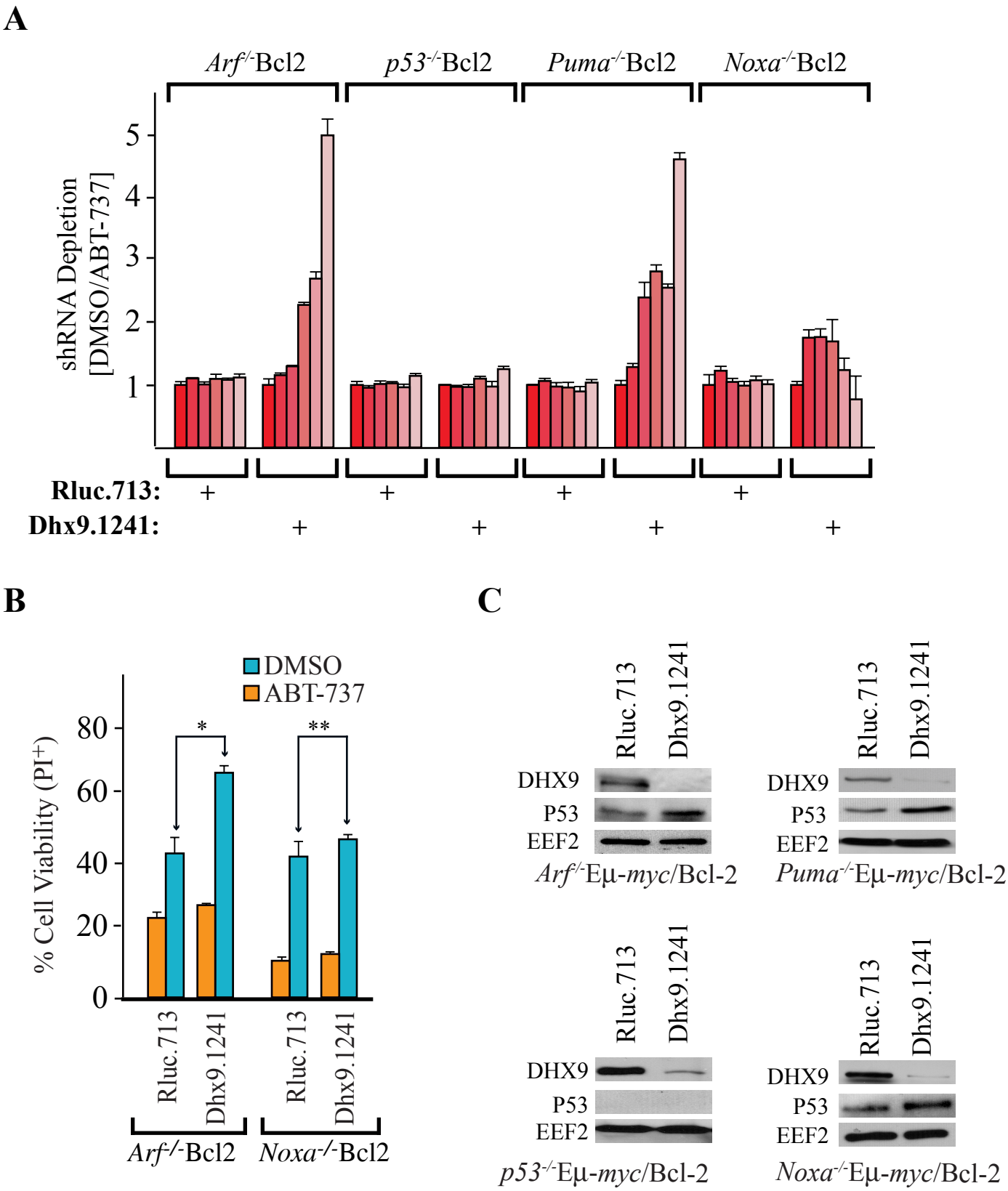

p53$^{-/-}$Eμ-*myc*
Rluc.713
Dhx9.1241
Mcl-1.1334
Fraction of shRNA^{+} remaining
0
0.5
1
1.5
2
0
2
4
6
8

A

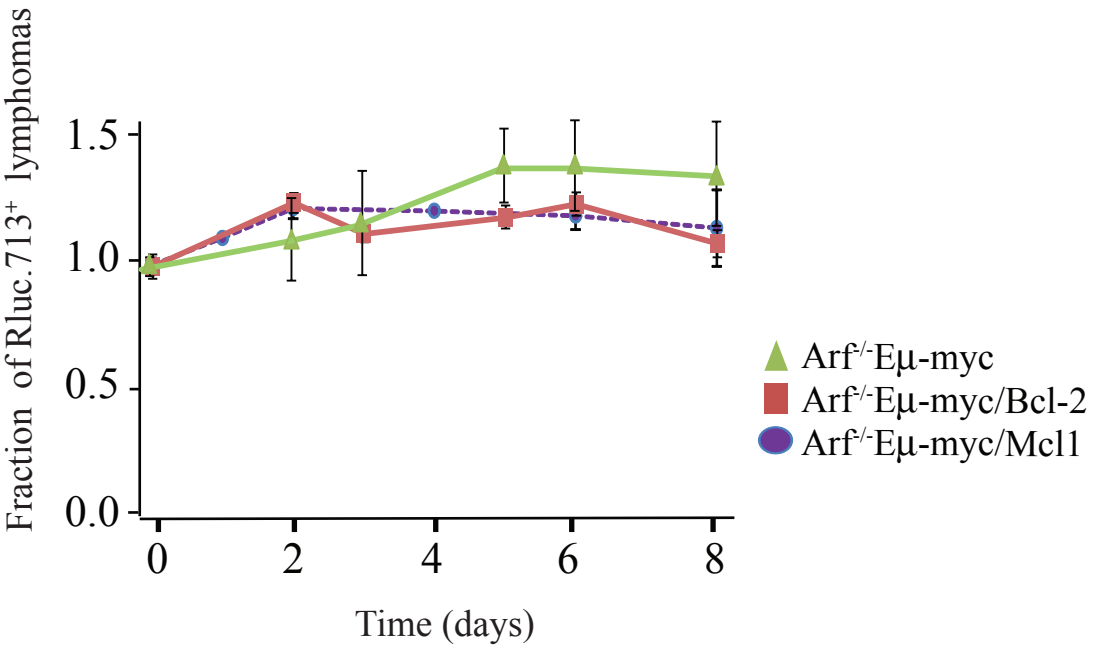

100
80
60
40
20
Cell Viability
Fluc.1300/Rluc.713
Dhx9.1241/Rluc.713
Fluc.1309/Bim.825
Dhx9.1241/Bim.825
1
10
100
1000
10000
ABT-737 [nM]

B

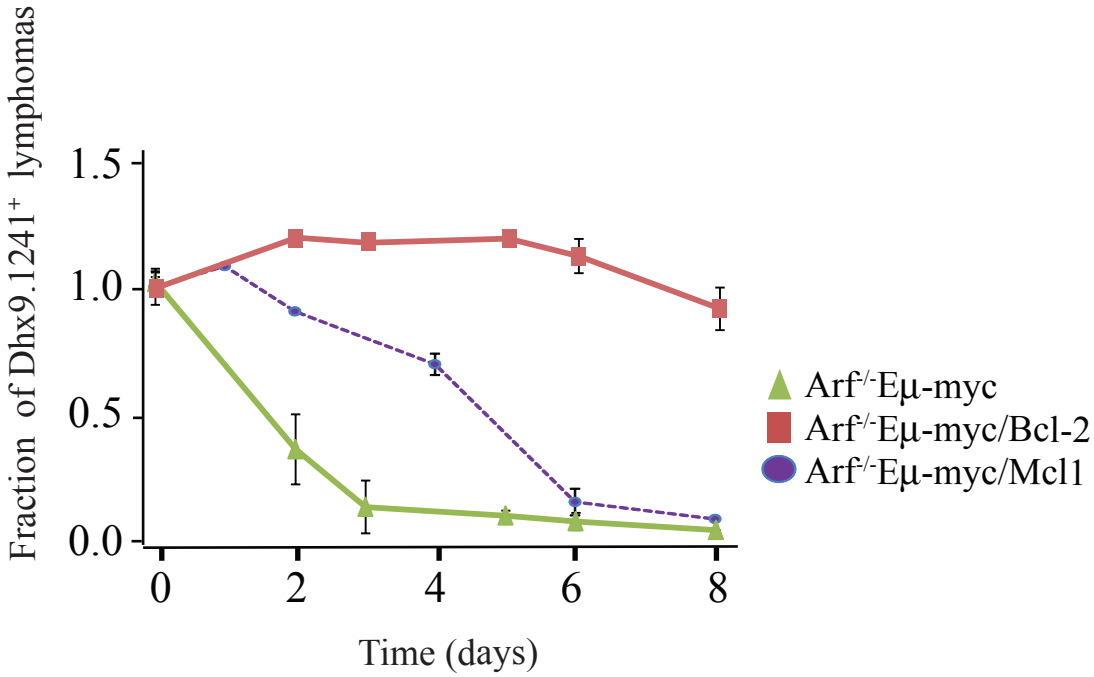

Bim.825
Fluc.1309
Rluc.713
Dhx9.1241
Rluc.713
Dhx9.1241
Dhx9
Bim EL
Bim L
eEF2


A
Arf-/-Bcl2
p53-/-Bcl2
Puma-/-Bcl2
Noxa-/-Bcl2
shRNA Depletion [DMSO/ABT-737]
1
2
3
4
5
Rluc.713: + + + +
Dhx9.1241: + + + +
B
DMSO
ABT-737
% Cell Viability (PI+)
0
20
40
60
80
*
**
Rluc.713
Dhx9.1241
Rluc.713
Dhx9.1241
Arf-/-Bcl2
Noxa-/-Bcl2
C
Rluc.713
Dhx9.1241
DHX9
P53
EEF2
Arf-/-Eμ-myc/Bcl-2
Puma-/-Eμ-myc/Bcl-2
p53-/-Eμ-myc/Bcl-2
Noxa-/-Eμ-myc/Bcl-2

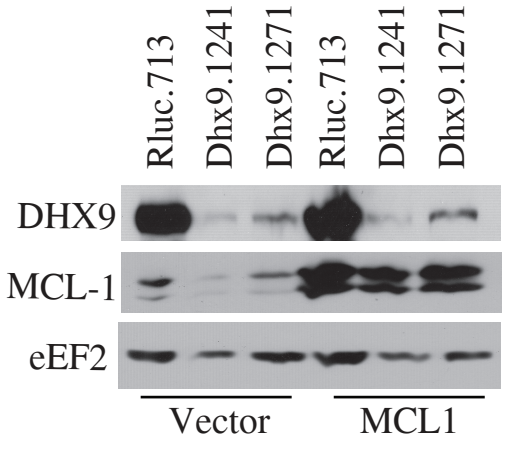
A


Fraction of Rluc.713+ lymphomas
1.5
1.0
0.5
0.0
0
2
4
6
8
Time (days)
Arf-/-Eμ-myc
Arf-/-Eμ-myc/Bcl-2
Arf-/-Eμ-myc/Mcl1

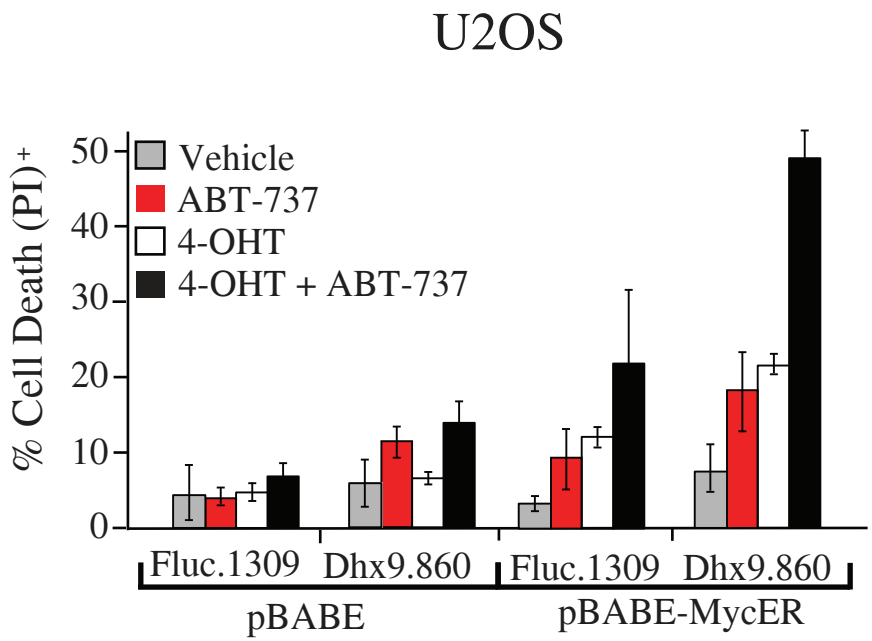
B


Fraction of Dhx9.1241+ lymphomas
1.5
1.0
0.5
0.0
0
2
4
6
8
Time (days)
Arf-/-Eμ-myc
Arf-/-Eμ-myc/Bcl-2
Arf-/-Eμ-myc/Mcl1

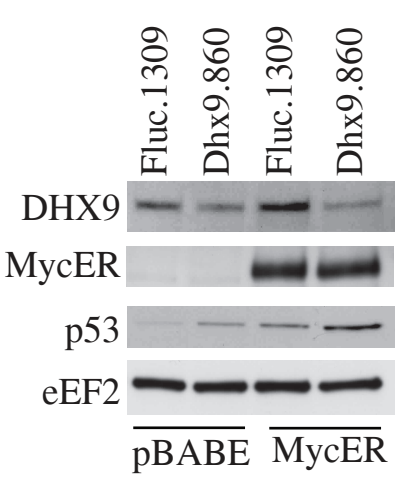
C


Rluc.713
Dhx9.1241
Dhx9.1271
Rluc.713
Dhx9.1241
Dhx9.1271
DHX9
MCL-1
eEF2
Vector
MCL1

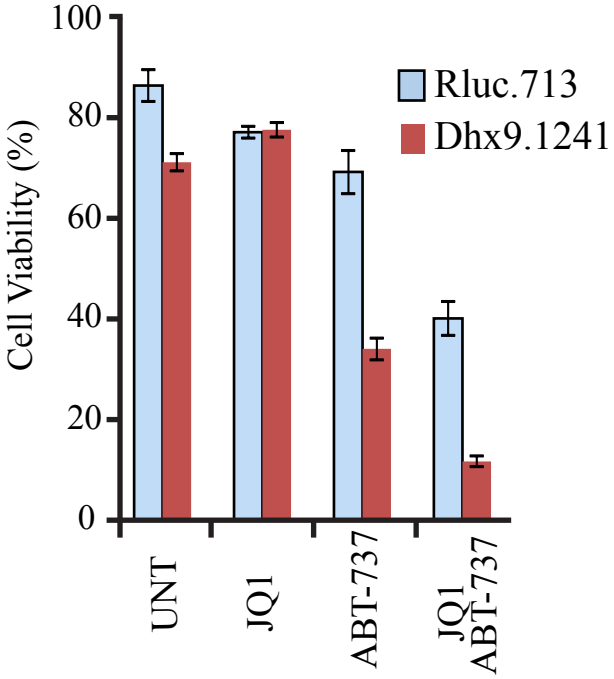
A

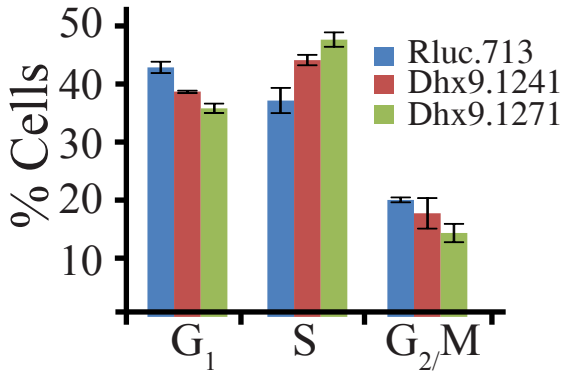

U2OS
% Cell Death (PI)+
50
40
30
20
10
0
Vehicle
ABT-737
4-OHT
4-OHT + ABT-737
Fluc.1309
Dhx9.860
Fluc.1309
Dhx9.860
pBABE
pBABE-MycER

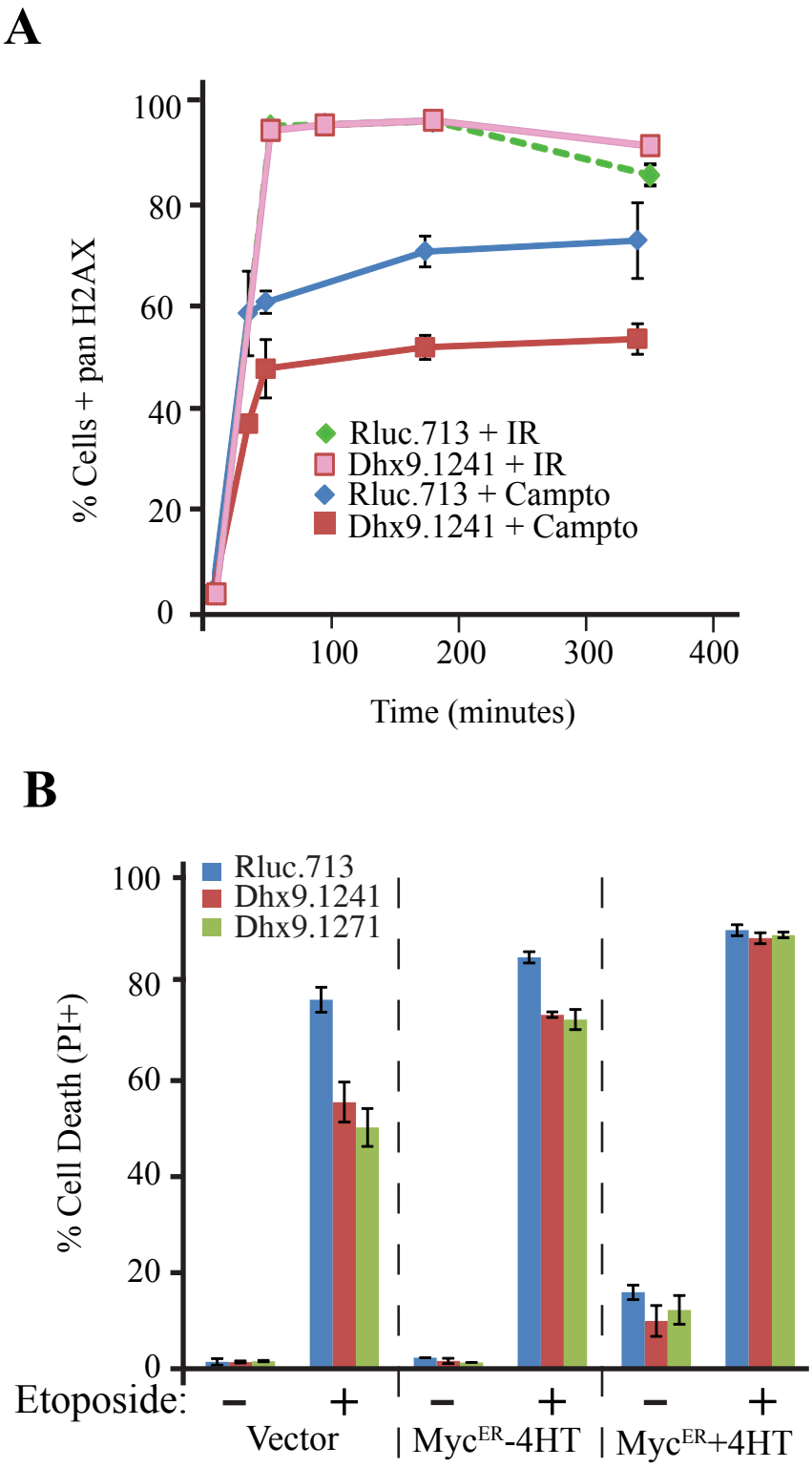
B


Fluc.1309
Dhx9.860
Fluc.1309
Dhx9.860
DHX9
MycER
p53
eEF2
pBABE
MycER

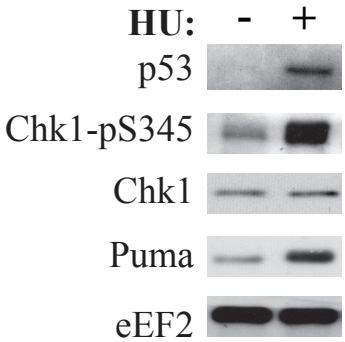
Mills_Fig S15



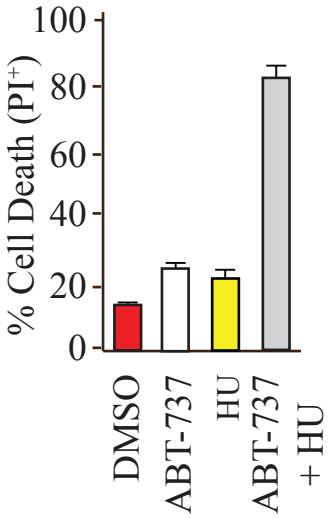

50
40
30
20
10
% Cells
Rluc.713
Dhx9.1241
Dhx9.1271
G1
S
G2/M

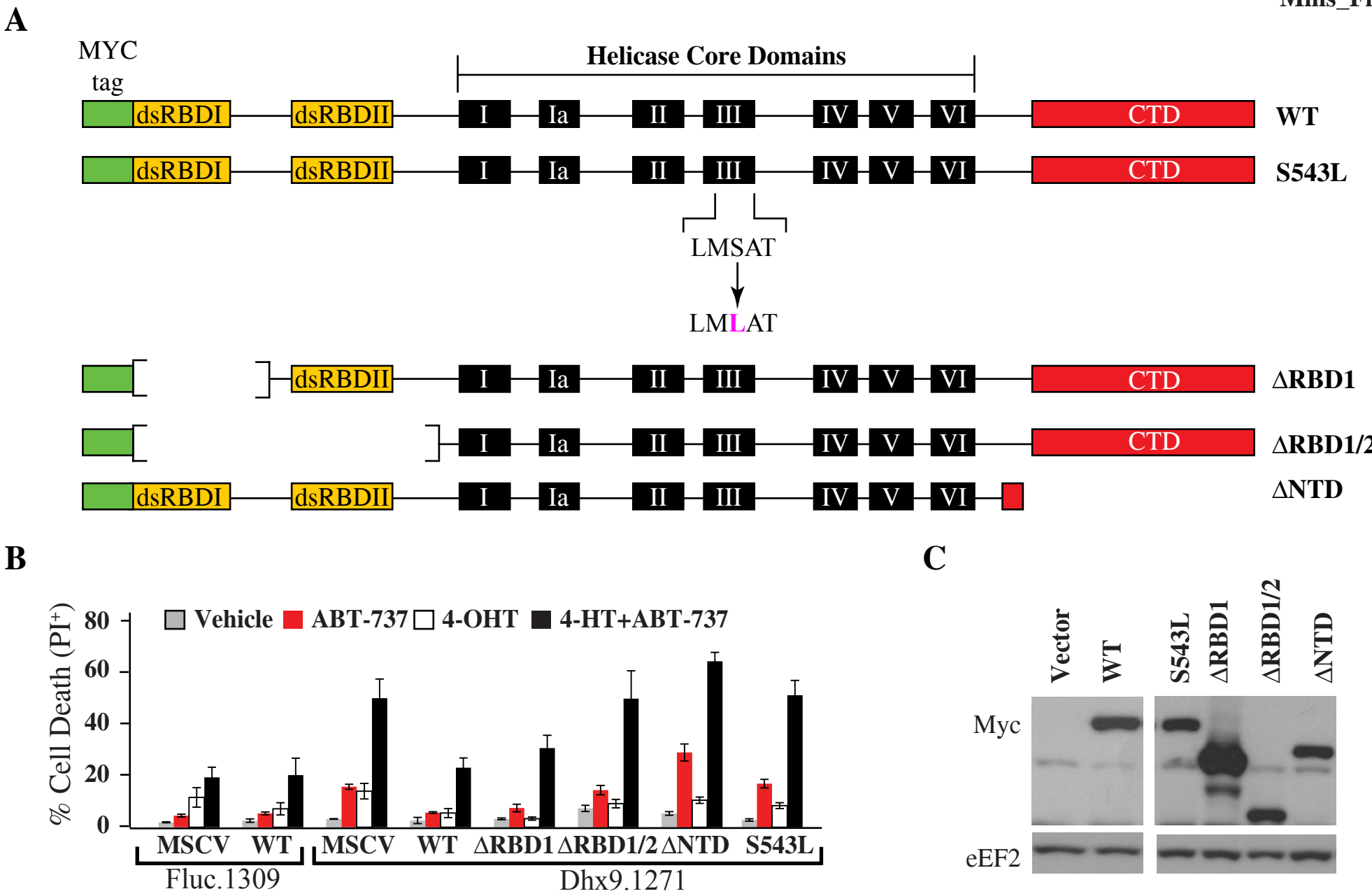

A
100
80
60
40
20
0
% Cells + pan H2AX
Rluc.713 + IR
Dhx9.1241 + IR
Rluc.713 + Campto
Dhx9.1241 + Campto
100
200
300
400
Time (minutes)
B
Rluc.713
Dhx9.1241
Dhx9.1271
100
80
60
40
20
0
% Cell Death (PI+)
Etoposide:
−
+
−
+
−
+
Vector
Myc^ER-4HT
Myc^ER+4HT

A


HU: - +
p53
Chk1-pS345
Chk1
Puma
eEF2

B


% Cell Death (PI+)
100
80
60
40
20
0
DMSO
ABT-737
HU
ABT-737 + HU


A
MYC tag
Helicase Core Domains
dsRBDI
dsRBDII
I
Ia
II
III
IV
V
VI
CTD
WT
S543L
LMSAT
LMLAT
ΔRBD1
ΔRBD1/2
ΔNTD
B
% Cell Death (PI+)
80
60
40
20
0
Vehicle
ABT-737
4-OHT
4-HT+ABT-737
MSCV
WT
ΔRBD1
ΔRBD1/2
ΔNTD
S543L
Fluc.1309
Dhx9.1271
C
Vector
WT
S543L
ΔRBD1
ΔRBD1/2
ΔNTD
Myc
eEF2