Materials and Expression Constructs

Etoposide was purchased from Sigma, 4-hyroperoxy-cyclophosphamide (4-OH-CTX) was obtained from Niomech (IIT GmbH, Bielefeld, Germany). QVD-OPH (MP Biomedicals, Aurora, Ohio) was used in experiments at a final concentration of 25 μM and was added to cells at least 30 min prior to the addition of other reagents.

Experimental animals

All experiments with animals were performed according to the guidelines of the institutional Animal Ethics Committee. C57BL/6 and C57BL/6-Ly5.1 mice were obtained from the Walter and Eliza Hall Institute's breeding facility. Generation and genotyping of mice have been described previously: *Noxa*$^{-/-}$ [1], *Puma*$^{-/-}$[1], *Bim*$^{-/-}$[2], p53$^{-/-}$[3] and E*μ*-*Myc*[4,5] All mice were either generated on a C57BL/6 background using C57BL/6-derived ES cells or generated on a mixed C57BL/6x129SV background, using 129SV-derived ES cells and then backcrossed to C57BL/6 for > 20 generations. For the present study, E*μ*-*Myc* transgenic males were crossed with p53$^{-/-}$ or p53$^{+/-}$ females to generate E*μ*-*Myc*/*p53*$^{+/-}$ offspring and with *Puma*$^{-/-}$ or *Noxa*$^{-/-}$ or *Bad*$^{-/-}$ females to produce E*μ*-*Myc*/*Puma*$^{+/-}$ or E*μ*-*Myc*/*Noxa*$^{+/-}$ or E*μ*-*Myc*/*Bad*$^{+/-}$ males, which were then crossed with *Puma*$^{-/-}$ or *Noxa*$^{-/-}$ or *Bad*$^{-/-}$ females to yield E*μ*-*Myc*/*Puma*$^{-/-}$ or E*μ*-*Myc*/*Noxa*$^{-/-}$ or E*μ*-*Myc*/*Bad*$^{-/-}$ progeny, respectively in separate breedings. Similarly, E*μ*-*Myc*/*Noxa*$^{-/-}$ males were crossed with N*oxa*$^{-/-}$*Puma*$^{-/-}$ double knockout (DKO) females to generate E*μ*-*Myc*/*Noxa*$^{-/-}$*Puma*$^{+/-}$ males, which were then bred to *Noxa*$^{-/-}$*Puma*$^{-/-}$ females to yield E*μ*-*Myc*/*Noxa*$^{-/-}$ *Puma*$^{-/-}$ DKO mice.

E*μ*-*Myc* lymphomas and cell lines

E*μ*-*Myc* lymphomas were characterized by immunophenotype by flow cytometry and defined as either pre-B lymphomas (B220^{+}sIg^{-}) or B-cell lymphomas (B220^{+}sIgM^{+}). Frozen lymphoma stocks were thawed for either direct transplantation into C57BL/6 recipient mice for expansion and *in vivo* drug sensitivity analysis or for *in vitro* culture in order to obtain stable cell lines. To generate E*μ*-*Myc* lymphomas cell lines, single cell suspensions prepared from spleen or lymph node samples were placed in tissue culture at 37°C in a humidified 10% CO_2 incubator in high-glucose Dulbecco's Modified

Eagle's medium supplemented with 10% foetal calf serum (JRH Biosciences), 50 μM 2-mercaptoethanol (Sigma) and 100 μM asparagine (Sigma). Stable cell lines were derived in 56/71 cases, with success independent of lymphoma genotype.

Flow cytometric analysis

Flow cytometry was performed on a FACScan or LSR (Becton Dickinson) using CellQuest Pro (BD Biosciences) for analysis. For immunophenotyping of lymphomas, monoclonal antibodies (mAb) to detect sIgM, sIgD, CD19 and B220 were used as detailed previously[1]. For cytoplasmic immunofluorescent staining, cells were fixed and permeabilized in fixation/permeabilization concentrate (eBioscience) and expression of BIM protein was detected by staining for 30 min at 4°C with rat anti-BIM mAb (clone 3C5 at 5μg/mL; Enzo Life Sciences) or an isotype-matched control mAb (rat IgG2a/k; BD Pharmingen), followed by secondary staining with biotin-conjugated mouse anti-rat IgG2a Ab (BD Pharmingen), and detected with streptavidin-Cy5 (Southern Biotech). FLAG-tagged proteins were detected by cytoplasmic immunofluorescence staining with anti-FLAG antibody (M2, Sigma) as detailed previously[6].

shRNA-mediated knock-down in lymphomas and cell lines

For retroviral infection of primary lymphoma cells or cell lines, a spin-infection protocol was used[7]. Briefly, Phoenix cells were transfected with the relevant vector using Fugene (Roche, Indianopolis, IN). Target cells were then infected with viral supernatant 24-48 h later and spun together (2500 rpm, 45 minutes) in the presence of polybrene (4 mg/mL, Sigma-aldrich). Stable cell lines were produced by adding appropriate selection 24-48 h after addition of retrovirus and selecting by flow cytometry for the 5% of cells with the highest GFP expression. For knock-down of p53, the retroviral shRNA-mir construct, MSCV/LTRmiR30-PIG (LMP)-p53.1224 was previously described[8]. An LMP-Renilla construct, targeting the Renilla Luciferase gene, was used as a short hairpin control were used (Kind gifts from Dr R Dickins). The MSCV PURO-IRES-GFP (pM-PIG) retroviral construct[8] was used as a backbone for the control *Caspase-12* shRNA hairpin and for one of the *Bim* shRNA hairpin constructs utilized. The *Caspase-12* or *Bim* short hairpins (sh) were ligated into the 3'LTR region of the MSCV PURO-IRES-GFP vector construct, driven by the U6 polymerase III promoter. Alternate LMP retroviral constructs (previously described[8]) were also used, in which either the *Bim*-shRNA expression

or the *IFI16*-shRNA expression is driven by the 5' LTR polymerase II promoter. The additional independent shRNA to mouse *Bim* and non-silencing control shRNAs were kind gifts from the Victorian Centre for Functional Genomics (Melbourne, Australia). All constructs contain a puromycin resistance gene coupled to GFP, allowing for drug as well as FACS-based selection post infection.

Lymphomas derived by c-*MYC* retroviral transduction

Day E13.5 fetal liver cells from the relevant gene-targeted mice were infected with a pMIG retroviral construct[9] containing human c-*MYC* cDNA generated in our laboratory. For FLCs, viral supernatant was added every 8-12 h over 48 h and cells centrifuged (1500rpm, 15 min) after every second addition of supernatant on retronectin-coated (25μg/mL) untreated-tissue culture plates in the presence of polybrene. Following infection, cells were transferred onto OP9 stromal cells[10] for 48 h in an attempt to reduce myeloid differentiation, mixed with 1 x 10^6 *Rag-1*$^{-/-}$ bone marrow cells to support hematopoiesis post-reconstitution and then transplanted into lethally irradiated (2 x 5.5 Gy, 4h apart) congenic C57BL/6 Ly5.1 mice. Recipient mice were monitored, lymphomas harvested when spleens became morbidly enlarged and cell lines were established as described above and their genotype confirmed by allele-specific PCR (for *Noxa*, *Puma*, *Bim* and *p53*) and by Western blotting (for PUMA, BIM and p19^{ARF}). Flow cytometry confirmed that all cell lines were of B lineage immunophenotype. The majority of primary lymphomas which gave rise to these cell lines transplanted successfully (3/5 rv *c-MYC*/*Puma*$^{-/-}$*Bim*$^{-/-}$; 3/5 rv *c-MYC*/*p53*$^{-/-}$) and 1 out of 2 rv *c-MYC*/ *Noxa*$^{-/-}$*Puma*$^{-/-}$*Bim*$^{-/-}$), confirming that they were fully transformed.

Analysis of *Cdkn2a* locus and p53 mutation status

Genomic DNA was isolated from rv *c-MYC*-driven lymphoma cell lines. Multiplex PCR analysis of genomic DNA for the *Cdkn2a* (p16^{Ink4a}/p19^{Arf}) locus was performed to reveal gross deletions, using exon-specific primers for α-actin, exons 2, 1a and 1b as previously described[11]. Sequencing of p53 from genomic DNA was performed using two primer sets designed for each of exons 4–10 (the second set used if clarification was required). PCR products were purified and sequencing was performed using the BigDye Terminator cycle sequencing kit (Applied Biosystems) as previously described[11]. Primer sequences are available upon request.

Western Blotting

Protein samples were size-fractionated by SDS-PAGE and then blotted onto PVDF (Hybond P, Amersham Biosciences) or nitrocellulose (Invitrogen) membranes. The membranes were blocked with 5% non-fat dry milk (Devondale) in PBS with 0.1% Tween 20 (Sigma) and then probed with antibodies against BIM (clone 3C5, Enzo Life Sciences; or polyclonal Ab from Stressgen, British Columbia, Canada); BAD (Stressgen); BAX (Upstate, Lake Placid, NY); BAK (Sigma); Heat Shock Protein 70 (HSP70; used as a loading control) (N6; a gift from Dr R Anderson, Peter MacCallum Cancer Centre, Melbourne); CASPASE-3 (cleaved) (CST); CASPASE-2 (clone 11B4, Enzo Life Sciences); CASPASE-7, caspase-9 (both kind gifts from Y Lazebnik, Cold Spring Harbor Labs, Cold Spring Harbor, NY); cytochrome c (BD); p53 (CM5, Novacastra); $p19^{ARF}$ (AbCam); CHOP (clone 9C8, Enzo Life Sciences and sc-575, Santa Cruz); PUMA (Abcam); BCL-2 (clone 3F11, BD Pharmingen); BCL-x_L (clone aa 18-233, BD Biosciences); MCL-1 (Clone 19C4-15, WEHI mAb lab) and β-actin (clone AC-74, Sigma ; also used as a loading control). Detection was performed with HRP-conjugated secondary antibodies and Enhanced Chemiluminescence (ECL) (Amersham Biosciences). To date, we have not identified an antibody suitable for detection of endogenous mouse NOXA protein. Where indicated, for example for experiments including 18 h etoposide treatment time-points, QVD-OPH (25μM) was added to prevent caspase-mediated degradation of target proteins.

qRT-PCR

Approximately 0.5-1 x 10^6 cells were given a DNA damage insult (etoposide, 4-OOH-CTX or 10 Gy γ-irradiation) in the presence of QVD-OPH to prevent terminal cellular destruction, and RNA was isolated 0, 3 or 6 hr later. Total RNA was extracted using the Micro-to-Midi Total RNA Extraction Kit or TRIzol Reagent (Invitrogen, San Diego, CA) and 0.4-1 μg total RNA converted to cDNA using the Taqman RT system (Roche) or High Capacity RNA to cDNA kit (Applied Biosystems). For quantitative analysis, the resulting cDNA was subjected to PCR in 15 μL reaction volumes using primers for *Bim*, *Puma, Noxa, Bmf, Bad, Blk, β-actin (*Primer sequences provided below) with SYBR-green reagent (Qiagen) and assessed on an ABI-PRISM 7900 thermal cycler (both from Applied Biosystems). *β*-actin serves as an endogenous normalization control. Data analyses were performed by the comparative threshold cycle method[12].

Cell Death Assays

Cell death was assessed flow cytometrically by staining with propidium iodide (PI 2μg/mL) and annexin V-FITC. Cell death of GFP-expressing cells was performed following staining with PI plus either annexin V-biotin plus streptavidin-PE or annexin V-APC (Caltag). The extent of apoptosis induced specifically by DNA damage treatment (percent specific apoptosis) was calculated using the following equation: [% apoptosis - % spontaneous apoptosis]/[100 - % spontaneous apoptosis]. Each independent cell line was analyzed in at least 3 independent experiments.

***In vivo* drug response following knock-down of Bim in lymphomas**

Following retroviral transduction with control pM-PIG or sh*Bim*-pM-PIG constructs, primary lymphoma samples were transplanted into *Rag*-1$^{-/-}$ animals (as the puromycin expression from the pM-PIG vector results in selection against the vector in immuno-competent C57BL/6 mice) and lymphoma cells were harvested from the spleen and assessed for GFP expression by flow cytometry. Lymphomas were transplanted a second time into *Rag*-1$^{-/-}$ mice in order to further select for lymphoma cells expressing of the *Bim* knock-down construct (higher GFP positivity, ~50%). Following harvest, lymphoma cells were sorted by FACS for GFP and then analyzed for levels of BIM protein by Western blotting and by cytoplasmic immunofluorescence staining. For the latter, cells were fixed and permeabilized in fixation/permeabilization concentrate (eBioscience) and expression of Bim was detected by staining with rat anti-BIM mAb (clone 3C5 at 5μg/mL; Enzo Life Sciences) as described above, with both tests showing that levels of BIM were lower in sh*Bim*-GFP lymphomas compared with the paired GFP control lymphomas. For *in vivo* treatment studies, these lymphoma cells were then transplanted into *Rag*-1$^{-/-}$ mice as below and recipient mice treated with either CTX 250 mg/kg or carrier, monitored and euthanized when they became unwell or in the absence of symptoms of lymphoma at 180 days post-treatment.

Real time qRT-PCR analysis primer sequences.

qRT-PCR was performed using the following forward and reverse primers (kind gifts from Dr DCS Huang and Prof Y Haupt)

Gene	sense (5' – 3')	antisense (5' – 3')
Bad	AGTATGTTCCAGATCCCAGAGTTTG	CCTTGAAGGAACCCTCAAACTCATC

Bim	GAGTTGTGACAAGTCAACACAAACC	GAAGATAAAGCGTAACAGTTGTAAGATAACC
Blk	AGAGACGTCATCAAGACTGTTCCAC	TCAAGCTCCTGAAAATACCTCTGAC
Puma	ATGCCTGCCTCACCTTCATCT	AGCACAGGATTCACAGTCTGGA
Noxa	ACTGTGGTTCTGGCGCAGAT	TTGAGCACACTCGTCCTTCAA
Bmf	AGGCTTCTGGTATGTGCT	TCAAACTCTGGGCTCAGTTC
p21	GTTCCGCACAGGAGCAAAGT	ACGGCGCAACTGCTCAC
Mdm2	TGTGTTTGGAGTCCCGAGTTT	GCATATATTTTCCTGTGCTCCTTCA
actin	TATTGGCAACGAGCGGTTC	CCATACCCAAGAAGGAAGGCT

Genotyping

Genotyping was performed by PCR on genomic DNA using primers specific for the relevant gene:

for p53 alleles 5'TTATGAGCCACCCGAGGT, 5'TATACTCAGAGCCGGCCT and

5'TCCTCGTGCTTTACGGTATC.

For Puma: 5'AGGCTGTCCCTGGGGTCATCCC, 3'GGACTGTCGCGGGCTAGACCCTCTG and

3'ACCGCGGGCTCCGAGTAGC

For Bim: 5'AAGAATCATAGGTTGACTCTAG, 3'CATTGCACTGAGATAGTGGTTGA and

3' CCCGTTGCACCACAGATGAA

For Noxa: 5' GGAGGGCATAAATGGGCAATGACAC, 3'GATGCTTCTTGGGTGCACCCACAC,

and 3'AAAGCAATCCCAAACGAC

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Eμ-*Myc* genotype	Independent lymphomas n=	Recipient mice n=	Median survival days	Range days	Mice alive at cull	Mice alive at cull %
Pre-B + B-cell, vehicle						
wt	22	2-8	4	0-13	0/89	0
Noxa$^{-/-}$	9	2-4	3	0-10	0/26	0
Puma$^{-/-}$	14	2-8	3	0-13	0/69	0
Noxa$^{-/-}$*Puma*$^{+/-}$	15	2-8	6	0-15	0/50	0
Noxa$^{-/-}$*Puma*$^{-/-}$	13	2-10	5	0-17#	0/74	0
Bim$^{-/-}$	9	2-5	6	1-15 ##	0/30	0
p53$^{-/-}$	12	2-11	4	0-12	0/40	0
Pre-B CTX 200 mg/kg						
wt	10	3-10	62	13-180	26/59	44
Noxa$^{-/-}$	4	3-5	179	11-180	9/16	56
Puma$^{-/-}$	6	2-6	23.5	14-180	4/20	20*
Noxa$^{-/-}$ *Puma*$^{+/-}$	8	2-9	176	16-180	16/31	52
Noxa$^{-/-}$ *Puma*$^{-/-}$	4	3-5	22	15-180	2/15	13*
Bim$^{-/-}$	2	2	64	26-180	1/4	25
p53$^{-/-}$	5	2-4	18	13-27	0/12	0*
Pre-B CTX 300 mg/kg						
wt	13	2-9	177	11-180	46/75	61
noxa$^{-/-}$	4	3-4	179	14-180	11/15	73
puma$^{-/-}$	5	4-7	42	8-180	10/27	37
noxa$^{-/-}$ *puma*$^{+/-}$	8	2-9	122	8-180	14/31	45
noxa$^{-/-}$ *puma*$^{-/-}$	4	3-10	177	31-180	20/25	80
bim$^{-/-}$	3###	2	183	31-180	4/6	67
p53$^{-/-}$	3	2-4	22	18-27	0/8	0*
B-cell CTX 200 mg/kg						
wt	9	2-4	49	15-180	12/26	46
noxa$^{-/-}$	5	2-3	177	14-180	6/11	55
puma$^{-/-}$	8	2-9	31	10-180	9/39	23
noxa$^{-/-}$ *puma*$^{+/-}$	7	2-7	42	14-180	10/24	42
noxa$^{-/-}$ *puma*$^{-/-}$	9	2-12	55	16-180	23/53	43
bim$^{-/-}$	6	2-8	37	10-180	1/21	5×
p53$^{-/-}$	5	2-8	21	15-47	0/25	0*
B-cell CTX 300 mg/kg						
wt	12	2-8	88	21-180	21/40	53
Noxa$^{-/-}$	5	2-3	180	18-180	8/12	67
Puma$^{-/-}$	8	3-9	177	17-180	25/48	52
Noxa$^{-/-}$*Puma*$^{+/-}$	7	2-7	92.5	14-180	11/24	46
Noxa$^{-/-}$ *Puma*$^{-/-}$	9	2-14	177	22-180	42/58	72
Bim$^{-/-}$	6	2-8	69	15-180	6/26	23
p53$^{-/-}$	7	2-10	26	8-180	1/34	3*

Table S1 Treatment outcome for mice included in the *in vivo* treatment experiments in Figs. 2, 3, and Supplemental Figure 2. Numbers of independent Eμ-*Myc* lymphomas and recipient mice analyzed with details for survival duration for each lymphoma genotype, immunophenotype and dose of CTX treatment. Mice showing no obvious symptoms of lymphoma were culled around 180 days and were diagnosed to be tumor-free by spleen size (<150 mg and lack of lymph node enlargement). In equivocal cases, a peripheral blood smear was also examined. Note that columns 3–6 give statistics for recipient mice pooled over

all lymphomas for that genotype. Significance tests between lymphomas of the different genotypes reported in the text use data summarized at the lymphoma level. # One Eμ-*Myc*/*Noxa*$^{-/-}$*Puma*$^{-/-}$ B cell lymphoma was not as aggressive when treated with carrier *in vivo*: 10 recipients, survived 2-35 days post vehicle; ## One Eμ-Myc/*Bim*$^{-/-}$ pre-B cell lymphoma was not as aggressive when treated with vehicle *in vivo*: 2 recipients, survived 27-32 days post vehicle (for both lymphomas statistical outcome was not altered by removal of this lymphoma from dataset). ### Only three Eμ-*Myc*/*Bim*$^{-/-}$ pre-B lymphomas were available for analysis, as the loss of Bim skews towards the development of B cell lymphomas[13]. * $p<0.05$ and × $p=0.08$ when compared with control (wt) Eμ-*Myc* lymphomas.

Eμ-*Myc* genotype	Independent lymphomas with no surviving recipients n=	Independent lymphomas with some surviving recipients n=	Independent lymphomas with all recipients surviving n=
Pre-B CTX 200 mg/kg			
wt	2	6	2
Noxa$^{-/-}$	1	2	1
Puma$^{-/-}$	4	2	0
Noxa$^{-/-}$*Puma*$^{+/-}$	3	2	3
Noxa$^{-/-}$*Puma*$^{-/-}$	3	1	0
Bim$^{-/-}$	1	1	0
p53$^{-/-}$	5	0	0
Pre-B CTX 300 mg/kg			
wt	2	6	5
Noxa$^{-/-}$	1	0	3
Puma$^{-/-}$	2	3	0
Noxa$^{-/-}$ *Puma*$^{+/-}$	4	1	3
Noxa$^{-/-}$ *Puma*$^{-/-}$	0	3	1
Bim$^{-/-}$	1	0	2
p53$^{-/-}$	3	0	0
B cell CTX 200 mg/kg			
wt	4	2	3
Noxa$^{-/-}$	1	2	2
Puma$^{-/-}$	3	3	2
Noxa$^{-/-}$ *Puma*$^{+/-}$	3	2	2
Noxa$^{-/-}$ *Puma*$^{-/-}$	2	4	3
Bim$^{-/-}$	5	1	0
p53$^{-/-}$	5	0	0
B cell CTX 300 mg/kg			
wt	3	4	5
Noxa$^{-/-}$	1	1	3
Puma$^{-/-}$	3	3	2
Noxa$^{-/-}$ *Puma*$^{+/-}$	2	2	3
Noxa$^{-/-}$ *Puma*$^{-/-}$	0	5	4
Bim$^{-/-}$	2	4	0
p53$^{-/-}$	6	1	0

Table S2 Numbers of independent lymphomas in the *in vivo* treatment experiments categorized by survival status of recipient mice. Each lymphoma is categorized by whether none, some or all of the mice injected with that lymphoma survive to 180 days. These counts served as input for the ordinal regression analyses to compare genotypes. Significant p values ($p<0.05$) for comparison versus wt are reported in the results section.

Figure S1. Response of Eμ-*Myc* cell lines to DNA damaging drugs *in vitro*. Eμ-*Myc* cell lines of the relevant genotypes were left untreated or treated with 4-OOH-CTX (0-10μg/mL) and cell death was assessed by flow cytometry at 24 h. Data are representative of at least 2 independent experiments from 8 Eμ-*Myc*/*p53*$^{+/+}$ cell lines; 8 Eμ-*Myc*/*p53* mutant cell lines; 2 Eμ-*Myc*/rv-*BCL-2* cell lines; 5 Eμ-*Myc*/*Puma*$^{-/-}$ cell lines; 2 Eμ-*Myc*/*Bim*$^{-/-}$ cell lines; and 2 Eμ-*Myc*/*Noxa*$^{-/-}$ cell lines.

Figure S2. Loss of Puma, and combined loss of Puma and Noxa in Eμ-*Myc* cell lines causes resistance to etoposide-induced apoptosis *in vitro* but not to the same extent as p53 deficiency. A) Eμ-*Myc* cell lines of the indicated genotypes were treated with etoposide (0.04, 0.2 or 1 μg/mL) for 6 or 24h and the extent of apoptosis determined by flow cytometry. B) Eμ-*Myc* cell lines were analyzed according to immuno-phenotype: no significant differences were observed when comparing the response of sIgM$^-$ pre-B (left hand panel) and sIgM$^+$ B (right hand panel) Eμ-*Myc* cell lines of the indicated genotypes to etoposide (0.2 μg/mL). Data represent mean ± S.E.M. from 3 to 7 independent cell lines for each genotype, with each cell line analyzed in at least 3 independent experiments.

Figure S3. No effect of loss of Noxa on response of Eμ-*Myc* lymphomas to cyclophosphamide *in vivo*. A-D) Response of Eμ-*Myc*-driven lymphomas to CTX treatment *in vivo*: Kaplan-Meier survival curves of mice transplanted (d0) with Eμ-*Myc* lymphomas and treated once lymphomas were palpable, from around d12. Pre-B (sIg$^-$) (A and C) or B cell (sIgM$^+$) (B and D) Eμ-*Myc* lymphomas including those lacking *Noxa* alone or those also lacking one or two alleles of *Puma* or those deficient for *p53* were treated with either 200 mg/kg CTX (A and B) or with 300 mg/kg CTX (C and D). Data were pooled from 4-13 independent lymphomas of each genotype; 2-14 recipient mice per treatment per independent lymphoma (see Supplemental Table 1).

Supplemental Figure 4. Box-plot analysis of ranked survival times for Eμ-*Myc* lymphomas lacking various BH3-only genes treated with cyclophosphamide *in vivo*. A median survival time was estimated for mice bearing each lymphoma. The survival times were then ranked from 1 to the number of lymphomas in increasing order. The genotypes are ordered from left to right according to

predictions from *in vitro* and *in vivo* experiments. Genotypes colored the same were expected to have similar survival times. The overall significance of the color-coded ordering was evaluated by permutation. Pair-wise comparisons with $P < 0.05$ are indicated by *. Other comparisons are non-significant. A) pre-B lymphomas lacking Puma, with or without additional loss of Noxa, displayed relative resistance to CTX at 200 mg/kg; this resistance was intermediate between $p53^{-/-}$ lymphoma cells and lymphoma cells of all other genotypes ($p = 1e^{-5}$, Supplemental Figure 4; Supplemental Table 1). C) In striking contrast, at the maximum tolerated dose (300 mg/kg) of CTX, pre-B lymphomas lacking both Puma and Noxa were markedly more sensitive than were lymphomas lacking only Puma (p=0.004). B) and D) For B cell ($sIgM^+$) Eμ-*Myc* lymphomas, those lacking Bim were intermediate between all other genotypes and those lacking p53, providing support for the importance of Bim in B cell Eμ-*Myc* lymphomas (p value 0.004 (CTX 200 mg/kg; $p = 2e^{-5}$ (CTX 300 mg/kg)).

Figure S5. Potential involvement for p53 in DNA damage-elicited Bim induction

Three independent E*μ*-Myc cell lines were transduced with either the retroviral shRNA-mir construct, LMP-p53.1224[9] or an LMP-Renilla construct, targeting the Renilla Luciferase gene, as a short hairpin control. Following selection by puromycin and flow cytometry, cell lines were treated with etoposide (1 *μ*g/mL). A) Western blotting for p53 demonstrated impaired induction of p53 post-etoposide exposure in cell lines infected with sh*p53*. B) Percent of drug-specific apoptosis was reduced in cell lines infected with sh*p53*. C) Some impairment of induction of p53 target genes, *p21* and *Mdm2* was seen in cell lines infected with sh*p53*. In two out of the three cell lines, induction of *Bim* was also diminished. For B and C, mean fold change ± S.E.M. from 3 independent experiments.

Figure S6. Role for Bim in the absence of Puma and Noxa in response to DNA-damaging chemotherapy. A) E*μ-Myc* cell lines, wt for Noxa, Puma and p53 or E*μ-Myc* lymphoma cell lines lacking both Noxa and Puma or lacking p53 were treated with etoposide (1μg/mL) for 3 h and were then assessed for levels of mRNA for the indicated BH3-only genes by qRT-PCR. B) E*μ-Myc* cell lines or E*μ-Myc* cell lines lacking both Noxa and Puma (DKO) were treated with etoposide (1 μg/mL) for 24 h and assessed for the levels of BIM by Western blotting; β-actin used as a loading control. C) Experimental protocol: Independent E*μ-Myc*/$Noxa^{-/-}$ $Puma^{-/-}$ and control E*μ-Myc* cell lines and, in parallel experiments, primary lymphomas, were retrovirally transduced with *Bim* shRNA-GFP

or control-GFP expression vectors for drug response analysis. For *in vitro* analysis, primary lymphoma samples were cultured to generate established cell lines, which were then transduced with control-GFP vectors or with one of two independent sh*Bim*-GFP vectors (see Methods), followed by *in vitro* selection (puromycin, then 2 rounds of sorting by FACS for the 5% highest GFP-expressing cells), prior to drug response analysis. For *in vivo* survival studies, primary lymphoma cells were transduced with control-GFP vectors or sh*Bim*-GFP vectors, transplanted into *Rag-1*$^{-/-}$ animals and, when tumors became palpable, lymphoma cells were harvested from the spleen. Lymphomas were transplanted a second time into *Rag-1*$^{-/-}$ mice in order to further select for GFP^{+} *Bim* knock-down-expressing lymphoma cells (higher GFP positivity, ~50%). Knock-down of BIM protein was confirmed by Western blotting and intra-cellular FACS analysis and these cells were then transplanted into Rag-1$^{-/-}$ mice for *in vivo* treatment with either CTX 250 mg/kg or carrier.

Figure S7. Confirmation of BIM knock-down in E*μ*-*Myc* lymphomas retrovirally transduced with *Bim*shRNA-GFP vector. A) Western blot analysis of BIM and β-actin (loading control) in representative E*μ*-*Myc* cell lines (wt, *Puma*$^{-/-}$ or *Noxa*$^{-/-}$*Puma*$^{-/-}$) transduced with either an sh*Bim*-GFP vector (sh*Bim*), empty vector (GFP) or (control) sh*Caspase12*-GFP vector (shCtrl). B) Primary E*μ*-*Myc*/*Noxa*$^{-/-}$*Puma*$^{-/-}$ lymphomas retrovirally transduced with sh*Bim*-GFP (sh*Bim*) or control empty vector (GFP). Representative Western blot analysis of BIM protein levels, showing reduction in BIM following transduction with the sh*Bim*-GFP vector. C) Representative histogram of intracellular BIM staining of E*μ*-*Myc*/*Bim*$^{-/-}$ lymphomas (black line, negative control) and paired samples of E*μ*-*Myc*/*Noxa*$^{-/-}$*Puma*$^{-/-}$ lymphomas retrovirally transduced with an sh*Bim*-GFP vector (green line) or with empty-GFP vector (blue line).

Figure S8. c-*MYC* retrovirus-driven lymphomas lacking Puma, Noxa and Bim. A) Immuno-phenotyping of representative cell lines of the indicated genotypes, derived by infection of fetal liver with a c-*MYC* retrovirus; phenotypes were either CD19^{+}IgM^{-} or mixed CD19^{+}IgM^{-} and CD19^{+}IgM^{+}. B) Western blot analysis of levels of BCL-2, BCL-x_L, MCL-1 and HSP-70 (loading control; 2 left-hand HSP-70 blots for MCL-1 also included in Figure 5 as loading controls for p53 blots) in rv c-*MYC*-driven lymphoma cell lines. Immortalized mouse embryo fibroblasts (MEF) were included as positive controls for BCL-2. C) Cell lines from rv c-*MYC*-driven lymphomas of the

different genotypes were treated with etoposide (0.2 μg/mL; left panel, or 1 μg/mL; right panel) for 6 or 24 h. Data represent mean from 2 to 5 independent cell lines per genotype, with each cell line analyzed in at least 3 independent experiments. Error bars represent S.E.M. from five independent rv c-*MYC*-driven *Puma*$^{-/-}$*Bim*$^{-/-}$ cell lines and five independent *p53*$^{-/-}$ cell lines

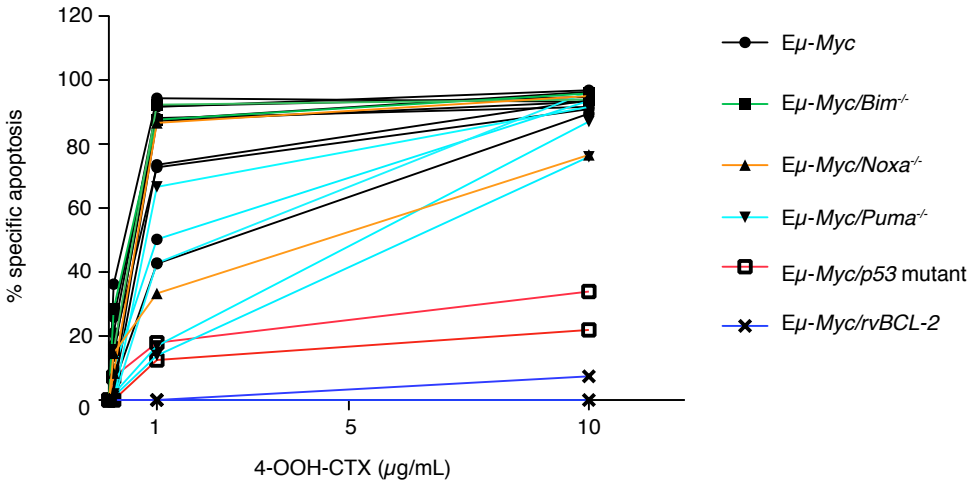

120
100
80
60
40
20
0
% specific apoptosis
1
5
10
4-OOH-CTX (μg/mL)
Eμ-Myc
Eμ-Myc/Bim-/-
Eμ-Myc/Noxa-/-
Eμ-Myc/Puma-/-
Eμ-Myc/p53 mutant
Eμ-Myc/rvBCL-2

Figure S1

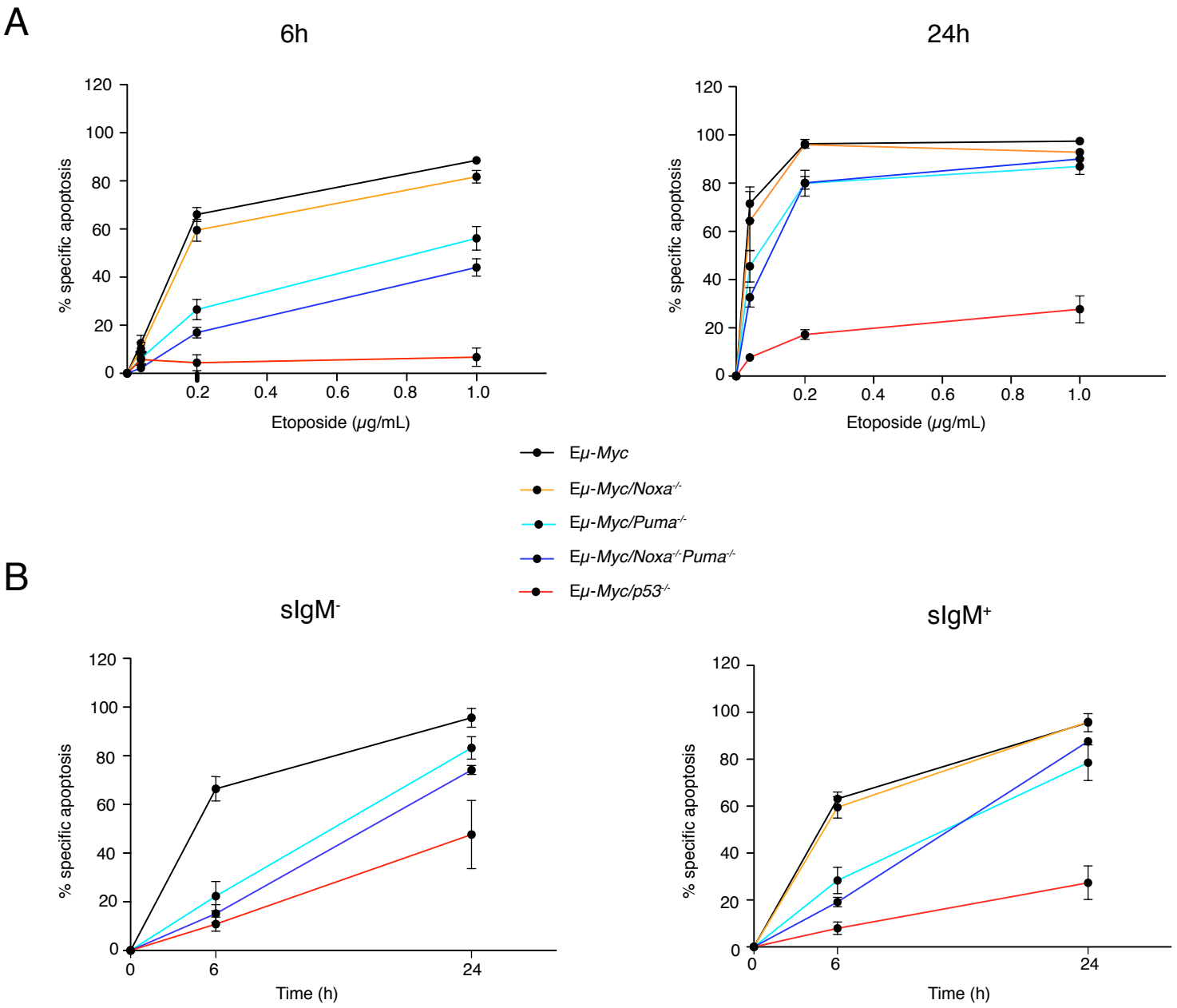

A
6h
24h
% specific apoptosis
Etoposide (μg/mL)
Eμ-Myc
Eμ-Myc/Noxa-/-
Eμ-Myc/Puma-/-
Eμ-Myc/Noxa-/-Puma-/-
Eμ-Myc/p53-/-
B
sIgM-
sIgM+
Time (h)

Figure S2

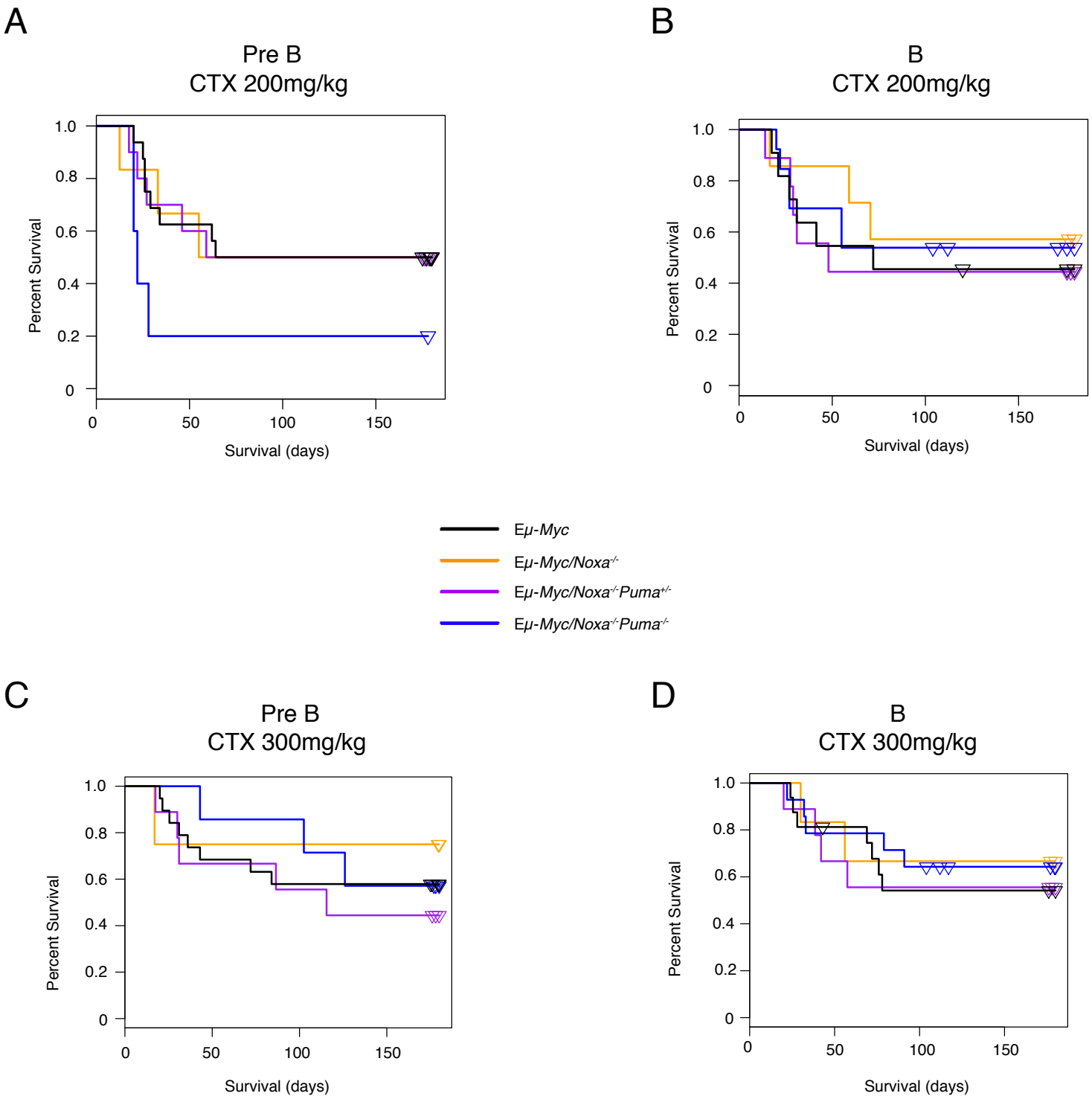
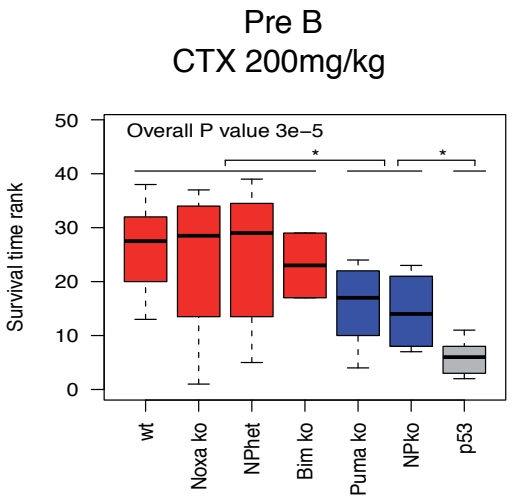
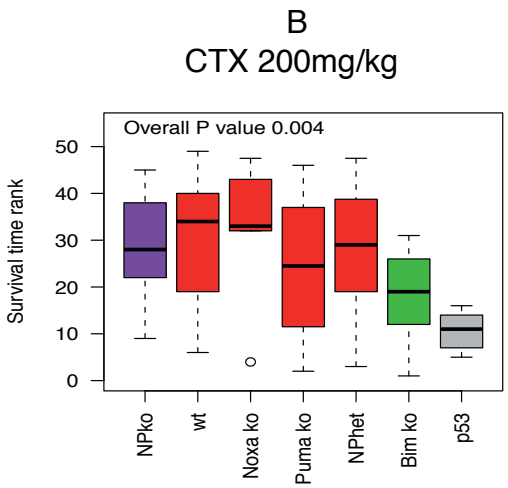

A
Pre B
CTX 200mg/kg
Percent Survival
Survival (days)
B
B
CTX 200mg/kg
Percent Survival
Survival (days)
Eμ-Myc
Eμ-Myc/Noxa-/-
Eμ-Myc/Noxa-/-Puma+/-
Eμ-Myc/Noxa-/-Puma-/-
C
Pre B
CTX 300mg/kg
Percent Survival
Survival (days)
D
B
CTX 300mg/kg
Percent Survival
Survival (days)

Figure S3

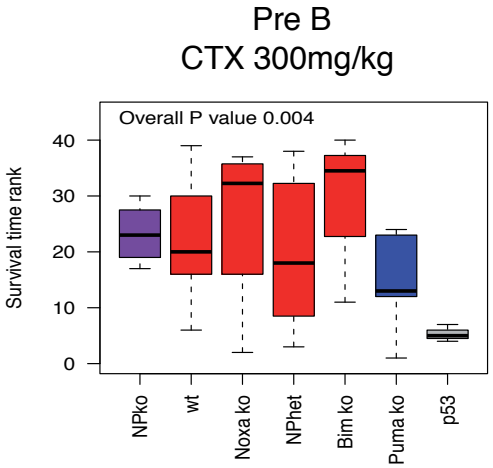
A



B



C



D

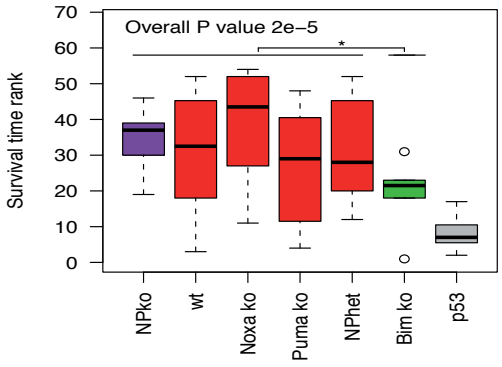


Figure S4

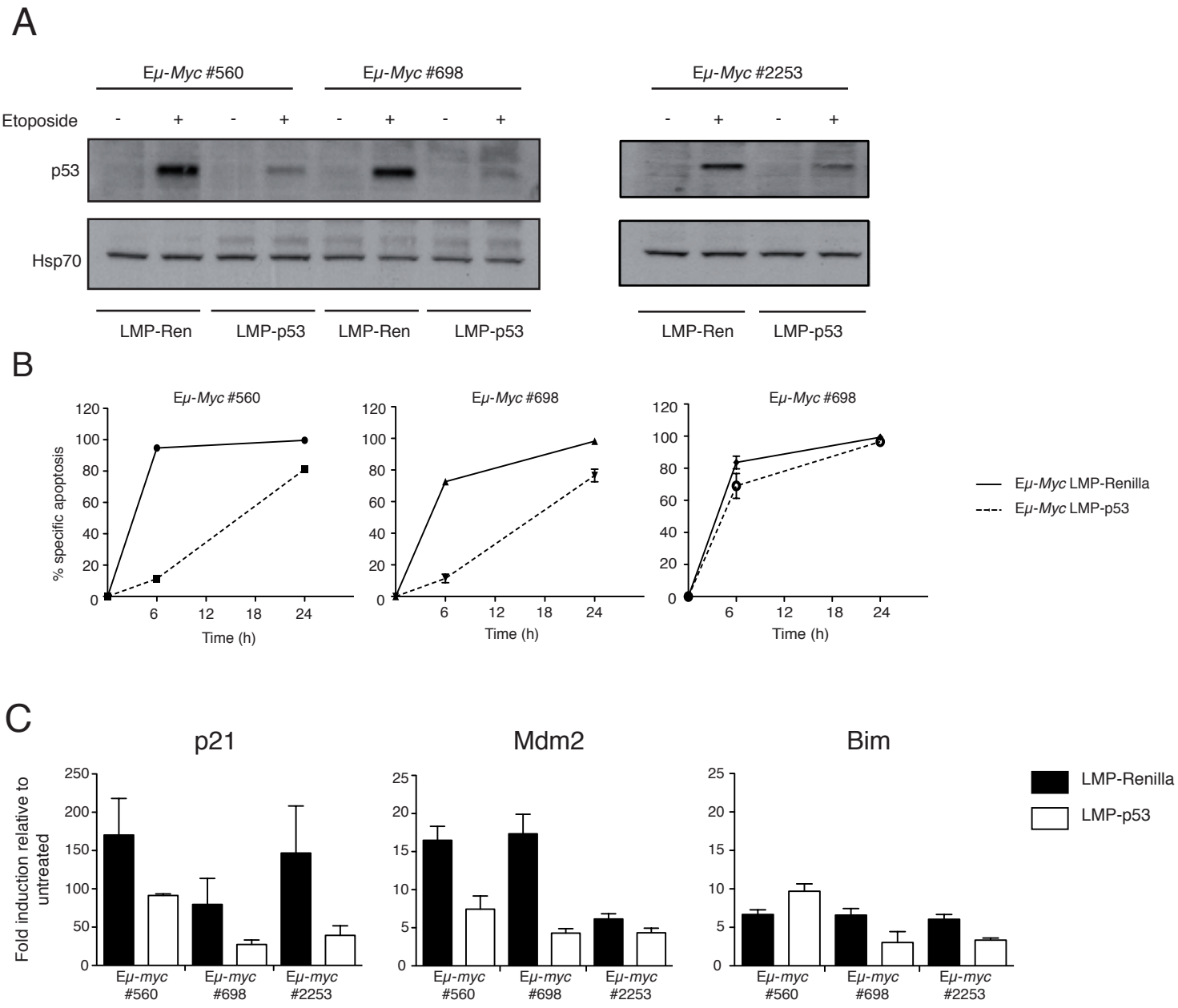

A
Eμ-Myc #560
Eμ-Myc #698
Eμ-Myc #2253
Etoposide
p53
Hsp70
LMP-Ren
LMP-p53
B
% specific apoptosis
Time (h)
Eμ-Myc LMP-Renilla
Eμ-Myc LMP-p53
C
p21
Mdm2
Bim
Fold induction relative to untreated
LMP-Renilla
LMP-p53
Eμ-myc #560
Eμ-myc #698
Eμ-myc #2253

Figure S5

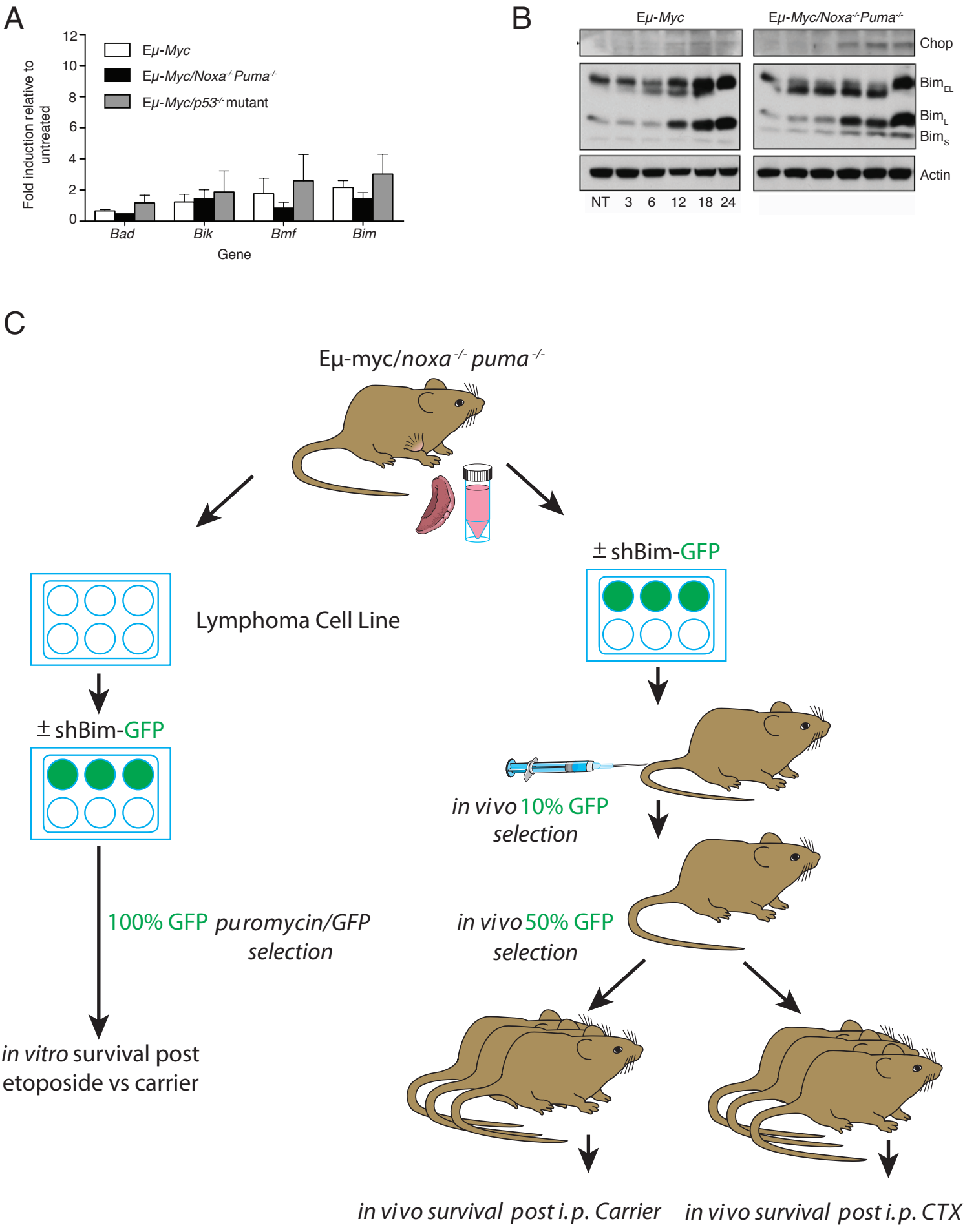
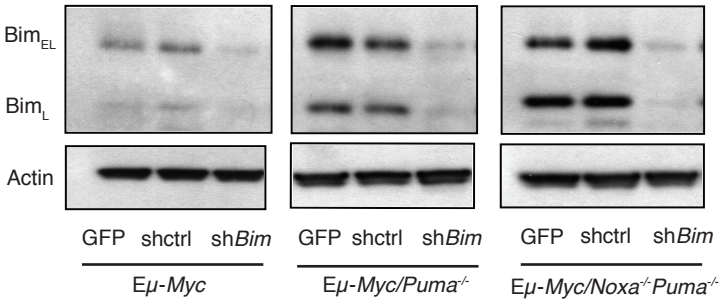
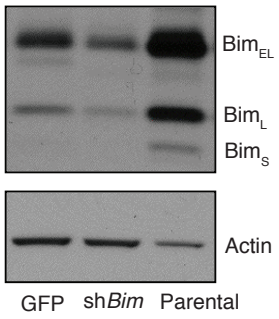

A
Fold induction relative to untreated
12
10
8
6
4
2
0
Eμ-Myc
Eμ-Myc/Noxa-/-Puma-/-
Eμ-Myc/p53-/- mutant
Bad
Bik
Bmf
Bim
Gene
B
Eμ-Myc
Eμ-Myc/Noxa-/-Puma-/-
Chop
BimEL
BimL
BimS
Actin
NT 3 6 12 18 24
C
Eμ-myc/noxa-/- puma-/-
± shBim-GFP
Lymphoma Cell Line
± shBim-GFP
in vivo 10% GFP selection
100% GFP puromycin/GFP selection
in vivo 50% GFP selection
in vitro survival post etoposide vs carrier
in vivo survival post i.p. Carrier
in vivo survival post i.p. CTX

Figure S6

A



B



C

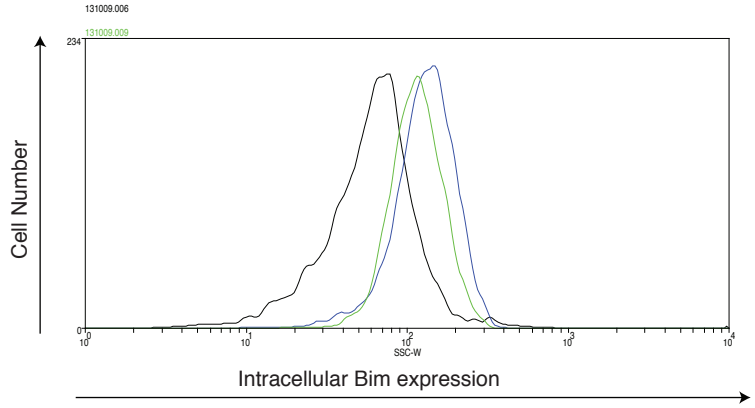


Figure S7

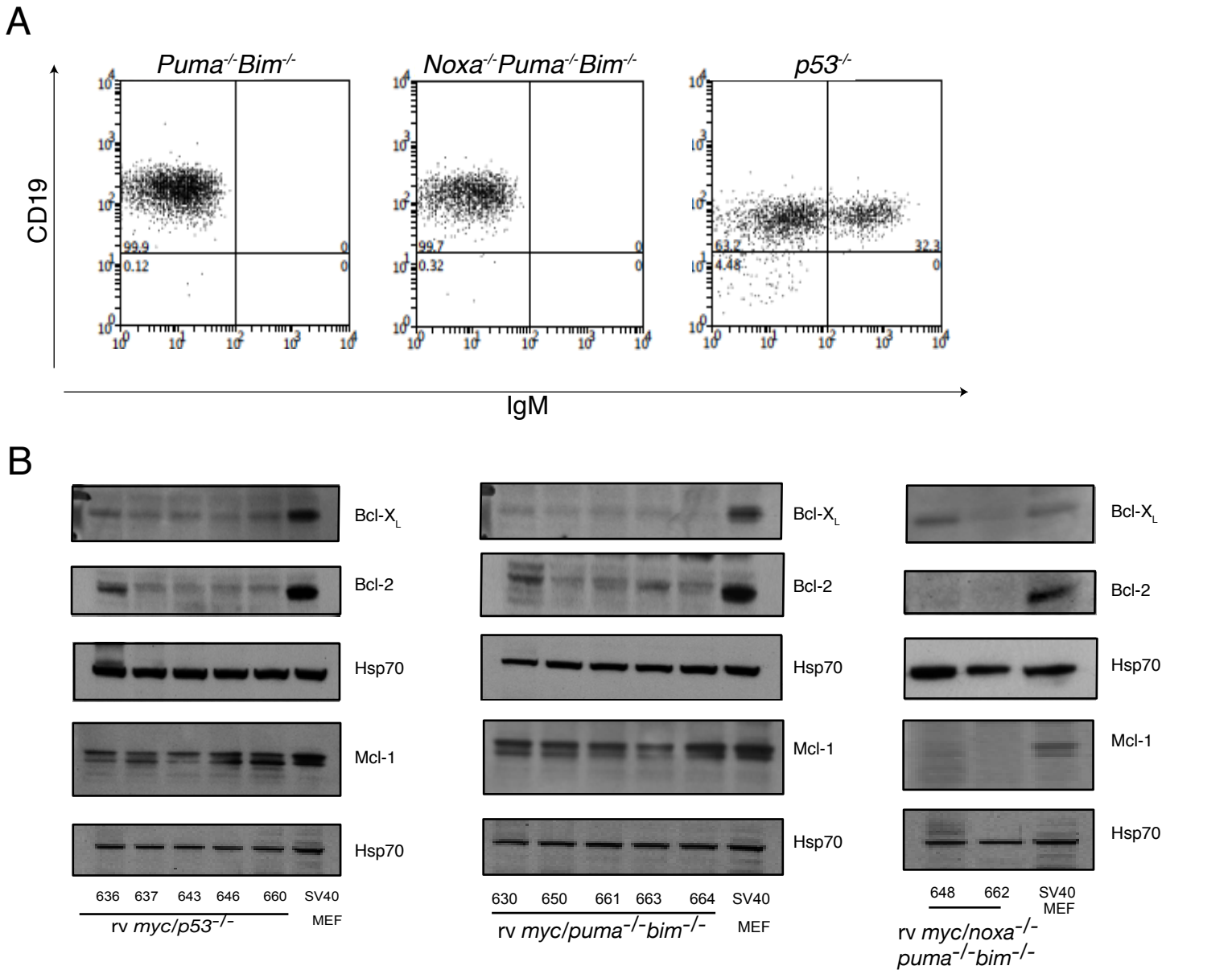

A
Puma-/-Bim-/-
Noxa-/-Puma-/-Bim-/-
p53-/-
CD19
IgM
99.9
0.12
99.7
0.32
63.2
4.48
32.3
B
Bcl-XL
Bcl-2
Hsp70
Mcl-1
Hsp70
636 637 643 646 660 SV40 MEF
rv myc/p53-/-
630 650 661 663 664 SV40 MEF
rv myc/puma-/-bim-/-
648 662 SV40 MEF
rv myc/noxa-/-puma-/-bim-/-
C
0.2 μg/mL Etoposide
1 μg/mL Etoposide
% specific apoptosis
Time (h)
Puma-/-Bim-/-
Noxa-/-Puma-/-Bim-/-
p53-/-

Figure S8