Supporting Information

Mason *et al.* 10.1073/pnas.0809957105

SI Materials and Methods

Mice. Transgenic mice used in this work had been backcrossed with C57BL/6 mice for >30 generations. Eμ-*myc* (1, 2) male mice were crossed with Eμ-*bcl*-2–22 (3) female mice and primary tumors (T0) arising in transgenic progeny were expanded by transplantation into multiple non-irradiated recipients. The tumors arising in cohorts of primary transplant recipients were pooled (T1) and cryopreserved in DMSO. The *myc*/*bcl*-2 tumors all had a primitive lymphomyeloid phenotype ($B220^+CD19^-IgM^-Thy1^{lo}CD4^+CD8^+Mac1^-Gr1^+$) (4) and *myc* tumors were either pre-B ($B220^+$ $CD19^+IgM^-$) or B ($B220^+$ $CD19^+IgM^+$) lymphomas (2).

Immunoblotting. Cell lysates were prepared in RIPA buffer (50 mM Tris, pH 8, 150 mM NaCl, 1% Triton X-100, 0.5% deoxycholic acid, and 0.1% SDS) supplemented with protease inhibitors including Pefabloc SC, soybean trypsin inhibitor, leupeptin, aprotinin, E64, and pepstatin (Roche or Sigma). Proteins were resolved by SDS:PAGE (Novex gels, Invitrogen), transferred onto nitrocellulose membranes (iBlot, Invitrogen), and detected by immunoblotting using rat monoclonal anti-Mcl-1 (clone 19C4–15, D Huang, unpublished); anti-Bid (clone 2D1–3) (5); mouse monoclonal anti-Bcl-2 (clone 7, BD Biosciences); anti-Bax (clone 5B7, Sigma); anti-Actin (clone AC-40, Sigma) or rabbit polyclonal anti-Bcl-x_L (BD Biosciences); anti-Bim (Stressgen); anti-Puma (ProSci); anti-Bad (Stressgen); and anti-Bak (Sigma). Secondary antibodies were HRP-conjugated anti-rat IgG (SouthernBiotech), anti-mouse or anti-rabbit IgG (Chemicon). The proteins were detected by enhanced chemiluminescence (ECL; GE Healthcare).

Real-Time qPCR Analysis. A total of 1–2 $\times$ 10^6 viable tumor cells were sorted by flow cytometry ($B220^+$, PI^-) from lymph node or spleen cells (stored in DMSO) obtained from mice transplanted with *myc* or *myc*/*bcl*-2 tumors. Total RNA was isolated using TRIzol (Invitrogen) and transcribed into cDNA using TaqMan Reverse Transcriptase (Applied Biosystems). For the experiments shown in Fig. S4, RNA was prepared directly after sorting, whereas for those in Fig. 4*C* and Fig. S6, the sorted cells were cultured for 3h in medium containing 25 μM of the broad-spectrum caspase inhibitor QVD-OPH (MP Biomedicals) following exposure (or not) to 500 rad γ-irradiation, or to etoposide (1 μg/ml, Mayne Pharma). Real-time qPCR was performed using an ABI Prism 7900 (Applied Biosystems) and QuantiTect SYBR Green PCR Kit (Qiagen). Data analyses were performed with the ΔCT method using β-actin as an internal control.

1. Adams JM, *et al.* (1985) The c-*myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature* 318:533–538.
2. Harris AW, *et al.* (1988) The Eμ-*myc* transgenic mouse: a model for high-incidence spontaneous lymphoma and leukemia of early B cells. *J Exp Med* 167:353–371.
3. Strasser A, *et al.* (1991) Enforced *bcl-2* expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc Natl Acad Sci USA* 88:8661–8665.
4. Strasser A, Harris AW, Bath ML, Cory S (1990) Novel primitive lymphoid tumours induced in transgenic mice by cooperation between *myc* and *bcl*-2. *Nature* 348:331–333.
5. Kaufmann T, *et al.* (2007) The BH3-Only Protein Bid Is Dispensable for DNA Damage- and Replicative Stress-Induced Apoptosis or Cell-Cycle Arrest. *Cell* 129:423–433.
6. Pezzella F, *et al.* (1990) Expression of the *bcl*-2 oncogene protein is not specific for the 14;18 chromosomal translocation. *Am J Path* 137:225–232.

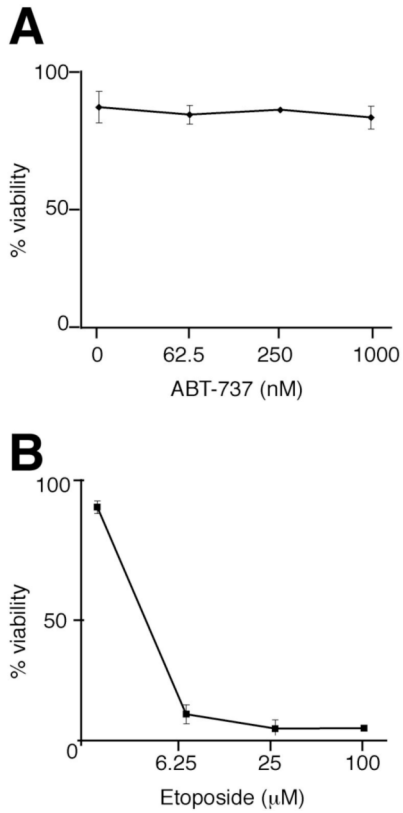

Fig. S1. *myc* lymphomas are resistant to ABT-737 *in vitro*. Viability (PI uptake analyzed by FACS) of a representative *myc* lymphoma cell line (Myc1) assessed following incubation for 24h with 0–1 μM ABT-737 (*A*) or, as a positive control, 0–100 μM etoposide (*B*). Data represents the mean $\pm$ 1 SD of three independent experiments. Six other independently-derived *myc* lymphoma-derived cell lines (AF47, AH15, A171, AH21, AF52, and Myc3) were also insensitive to ABT-737.

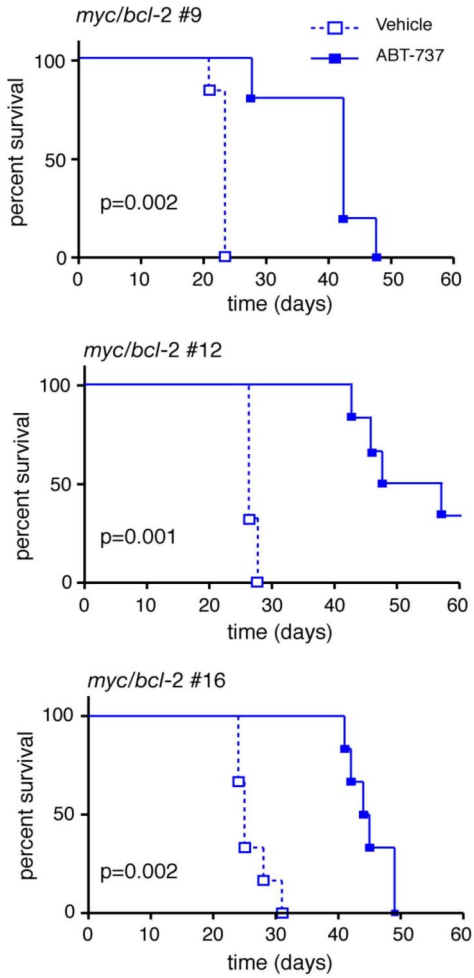

Fig. S2. Kaplan–Meier survival curves of mice transplanted (day 0) with 3 independently derived *myc/bcl-2* lymphomas and treated, starting on day 4, with ABT-737 (75 mg/kg/d for 14 days; $n = 6$, solid lines) or the vehicle ($n = 6$; dotted lines). For each lymphoma, six mice were treated with either ABT-737 or the vehicle control. Pooled data are shown in Fig. 1*B*. *P* values (log rank analysis) for vehicle vs. ABT-737 treatment are indicated.

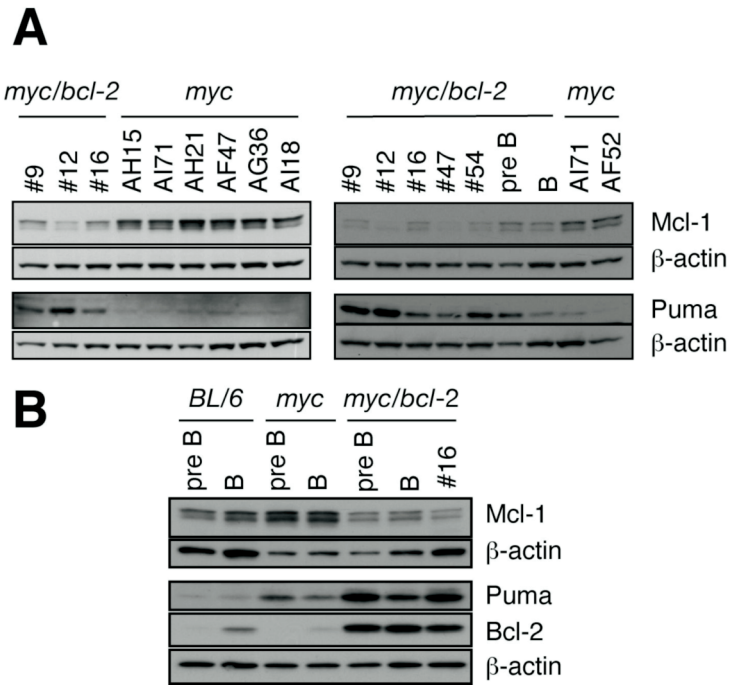

Fig. S3. Expression of Mcl-1 and Puma in (*A*) lymphomas arising in *myc/bcl-2* and *myc* transgenic mice and in (*B*) preleukemic pre-B and B cells isolated by flow cytometry from bone marrow and spleen of two healthy young *myc/bcl-2* and *myc* mice. (*A* also includes pre-B and B cell samples from *myc/bcl-2* mice and *B* includes *myc/bcl-2* tumor #16, for cross referencing between blots.) Antibodies used for Western blots are described in *Materials and Methods*.

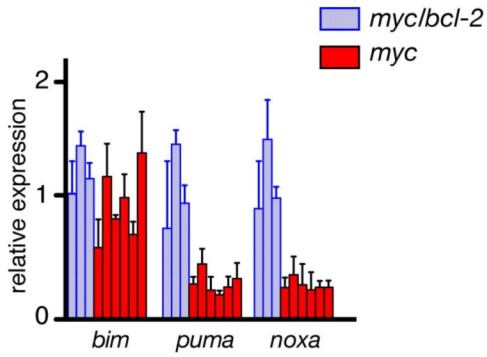

Fig. S4. Expression of *bim, puma* and *noxa* in *myc/bcl-2* and *myc* lymphomas. Real-time qPCR analysis was performed on cDNA prepared from tumor cells isolated by flow cytometry from lymphomatous tissues of transplanted mice. Data shown are means ± SEM from 2–4 independent experiments and expression levels normalized to that of *β-actin* and expressed relative to *myc/bcl-2* #16. Blue bars indicate (from left) *myc/bcl-2* tumors #9, #12, and #16 and red bars indicate (from left) *myc* tumors AH15, A171, AI18, AF47, AH21, and AF52.

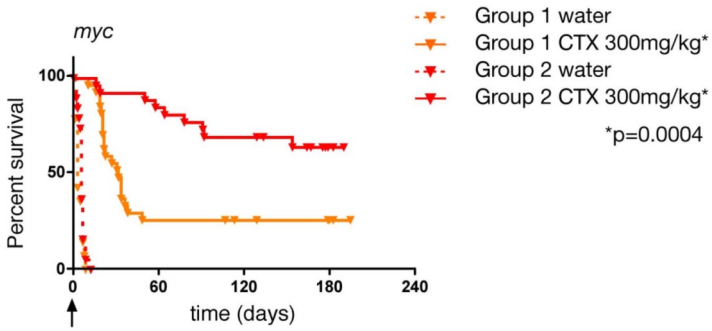

Fig. S5. Response of Myc-driven lymphomas to cyclophosphamide. Kaplan–Meier plots indicating survival of mice bearing *myc* lymphomas following treatment with a single (300 mg/kg ip) dose of cyclophosphamide or vehicle, stratified into the less responsive Group 1 (AF47, AH15, AH21, AI18, and AI71; $n = 27$, median survival 34 days) or the more responsive Group 2 (AF40, AG36, AH29, AH44, and AH66; $n = 26$, median survival undefined). *P* value (log rank analysis) for Group 1 vs. Group 2 is indicated. Treatment was initiated when tumors were palpable (≈day 12) and survival is indicated from day of treatment. For each lymphoma, 2–8 mice were treated with either cyclophosphamide or the vehicle control (water).

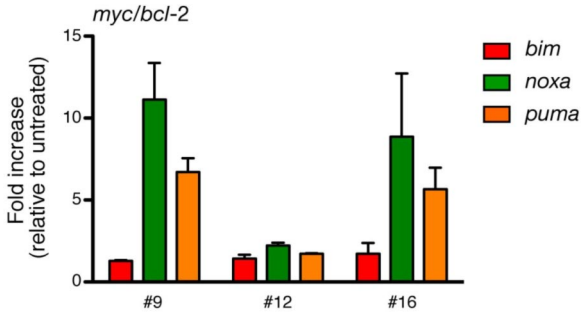

Fig. S6. *noxa* and *puma* are up-regulated by etoposide in sensitive *myc/bcl-2* lymphomas. Real-time qPCR analysis of expression of *bim*, *noxa*, and *puma* in the indicated *myc/bcl*-2 lymphoma cells treated *in vitro* for 3 h with etoposide (1 μg/ml). Expression was normalized to that of β-actin and expressed as the increase relative to untreated cells. Results shown are means $\pm$ SEM for 3–4 independent experiments.

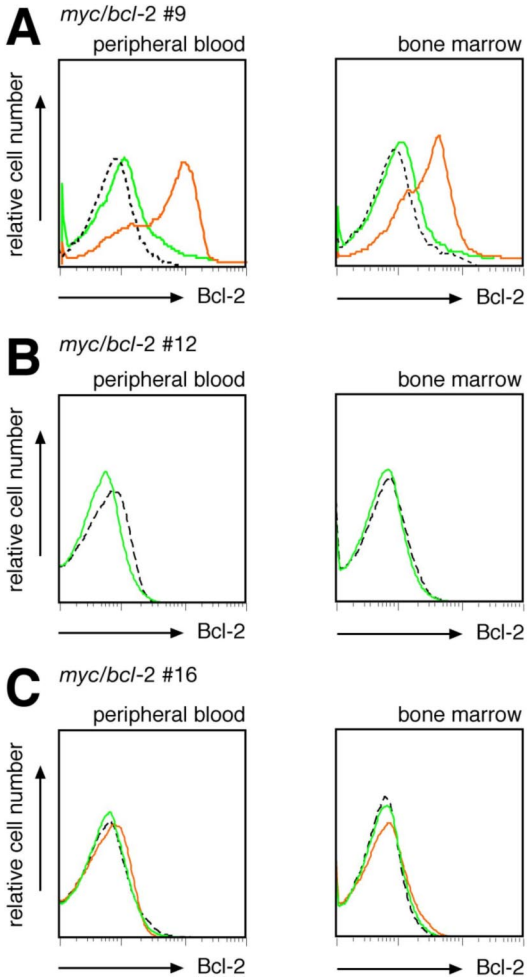

Fig. S7. Most long-term survivors are disease-free. Mice transplanted with *myc/bcl-2* lymphoma #9 (*A*), # 12 (*B*), and # 16 (*C*) were treated with ABT-737 + cyclophosphamide (50 mg/kg) or with a single high dose of cyclophosphamide (300 mg/kg) as described in *Material and Methods*. All long-term (day 150) survivors (Fig. 4) were autopsied and subjected to a full histologic analysis (Table S3) and their peripheral blood (*Left*) and bone marrow (*Right*) were assessed for residual disease by staining white cells with anti-human Bcl-2 antibody. No human Bcl-2-expressing cells were detectable by flow cytometry in any of the survivors treated with combination therapy (6/6 lymphoma #9, 6/6 lymphoma # 16, and 2/6 lymphoma #12) (green curves show representative examples) or the 3 *myc/bcl-2* #16 survivors that had been treated with high dose cyclophosphamide (orange curve in panel *C* shows a representative example). Most white cells in the sole *myc/bcl-2* #9 survivor treated with high dose cyclophosphamide, however, were human Bcl-2-positive (orange curve, *A*). Fixed, permeabilized cells were incubated with anti-human Bcl-2–100 antibody (6) (solid lines) or isotype control (dotted), followed by a FITC-conjugated anti-mouse secondary antibody (Southern Biotech).

Table S1. Response of *myc* and *myc/bcl-2* lymphoma to ABT-737

myc		Survival* (days)	Total WCC ($\times 10^3$/mL)† (normal range: 5–9)	Spleen weights, g† (normal range: 0.09–0.16)
Pre-B	Vehicle	15 ± 2	41 ± 8	0.34 ± 0.04
	ABT-737	19 ± 2	37 ± 7	0.28 ± 0.03
B cell	Vehicle	16 ± 1	46 ± 15	0.32 ± 0.05
	ABT-737	18 ± 3	42 ± 11	0.33 ± 0.05
myc/bcl-2	Vehicle	29 ± 2	232 ± 95	0.57 ± 0.14
	ABT-737	67 ± 36	302 ± 149	0.52 ± 0.22

*Survival is indicated as days from time of transplantation (d0).
†Means ± SD.

Table S2. Response of *myc* and *myc/bcl*-2 lymphomas to cyclophosphamide treatment

	myc lymphomas (10 independent lymphomas: 4 B cell, 6 Pre-B)	*myc/bcl-2* lymphomas (5 independent lymphomas)
Vehicle only	3 days (n = 43)	12 days (n = 29)
Cyclophosphamide 200 mg/kg	45 days (n = 46)	29 days (n = 29)
Cyclophosphamide 300 mg/kg	98 days (n = 53)	32 days (n = 27)

Median survival of mice transplanted with T0 or T1 lymphomas, from time of treatment (approximately d16).

Table S3. Response of *myc/bcl*-2 lymphomas to combination therapy (ABT-737 + low dose cyclophosphamide, 50 mg/kg) or high dose cyclophosphamide (300 mg/kg)

Treatment	Survivors to d150	WCC* ($\times 10^3/\mu$ L) (normal range: 5–9)	Spleen, g* (normal range: 0.09–0.16)	Bone marrow analysis	Histological analysis
myc/bcl-2 #9 combination	6/6 (100%)	4.9 ± 1.9	0.08 ± 0.01	No evidence of lymphoma	No evidence of lymphoma
myc/bcl-2 #9 cyclophosphamide	1/6 (17%)	42.9	0.57	Extensive replacement by lymphoma	Lymphomatous infiltrate in lymph nodes, liver, kidney
myc/bcl-2 #12 combination	2/6 (33%)	3.8 ± 1.6	0.07 ± 0.01	No evidence of lymphoma	No evidence of lymphoma
myc/bcl-2 #12 cyclophosphamide	0/6 (0%)	-	-	No evidence of lymphoma	No evidence of lymphoma
myc/bcl-2 #16 combination	6/6 (100%)	1.7 ± 1.6	0.09 ± 0.04	No evidence of lymphoma	No evidence of lymphoma
myc/bcl-2 #16 cyclophosphamide	3/6 (50%)	2.7 ± 0.5	0.12 ± 0.01	No evidence of lymphoma	No evidence of lymphoma

*Means ± SD.

Table S4. Primer sequences

Gene	Forward	Reverse
bim	GAGTTGTGACAAGTCAACACAAACC	GAAGATAAAGCGTAACAGTTGTAAGATAACC
puma	ATGCCTGCCTCACCTTCATCT	AGCACAGGATTCACAGTCTGGA
noxa	ACTGTGGTTCTGGCGCAGAT	TTGAGCACACTCGTCCTTCAA
p21	GTTCCGCACAGGAGCAAAGT	ACGGCGCAACTGCTCAC
β-actin	TATTGGCAACGAGCGGTTC	CCATACCCAAGAAGGAAGGCT

Table S5. Genomic PCR

Gene	Forward	Reverse
h*bcl-2*	GGATGACTGAGTACCTGAAC	CTACAGATGTGATATGGCTG
p53	TTATGAGCCACCCGAGGT	TATACTCAGAGCCGGCCT

PCR was performed on genomic DNA isolated from spleen or lymph node using the above primers.