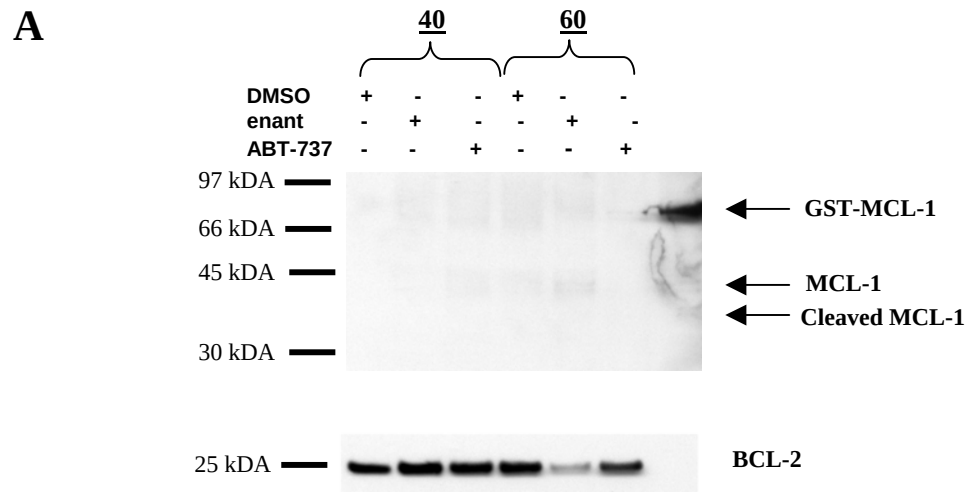
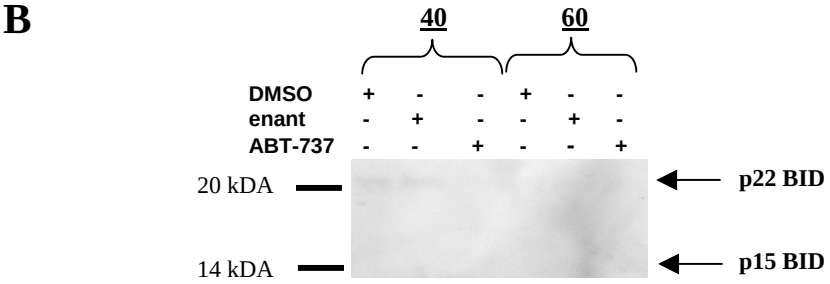
Supplemental Table 1

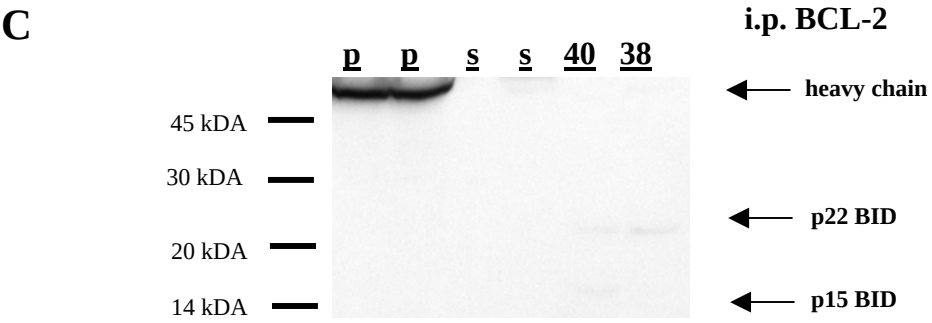
Patient	EC50 ABT-737 (nM)	EC50 NCE (nM)	Age	Rai Stage	WBC (x10E-3/ul)	CD38	LDH (313-618)	beta-2 (0-2.7)	treatment history	IgVH status
V1	7.0	1114	74	2	282	nd	918	4.1	untreated	nd
V2	3.2	473	75	1	72.5	nd	537	3.7	untreated	mut
V3	3.5	502	63	3	89.4	neg	2138	2.5	untreated	un
V4	3.6	496	67	1	184.6	nd	563	5.5	C; R	mut
V5	2.1	539	62	2	81.2	nd	431	5.3	untreated	mut
V6	3.1	493	65	2	126	neg	533	4.5	F; F+Cy; R	mut
V7	1.9	1783	58	2	47.1	nd	444	1.7	untreated	nd
V8	4.1	500	48	2	208.4	nd	398	3	untreated	mut
V9	8.7	1009	76	1	108.3	nd	434	2.6	untreated	un
V10	5.8	630	55	1	68.3	pos	613	3.2	untreated	un
V11	3.1	334	67	1	86.3	nd	501	1.9	untreated	nd
V12	8.0	579	74	2	257.1	nd	473	3.2	untreated	mut
V13	4.6	683	41	1	307.9	pos	1310	5.3	untreated	un
V14	3.3	318	59	2	233.9	neg	463	1.8	untreated	mut
V15	6.4	582	63	3	201.8	nd	431	3.2	untreated	un
V16	2.9	343	60	1	72.2	nd	444	1.9	untreated	mut
V17	9.4	861	47	3	244.1	nd	424	2.6	untreated	mut
V18	2.3	387	46	1	89.9	nd	450	2	untreated	mut
V19	6.4	572	55	1	162	nd	185	2.7	untreated	mut
V20	4.8	119	66	3	502	neg	663	5.3	S; C; F; R	mut
V21	2.1	295	68	1	107.9	nd	647	3.5	untreated	mut
V22	4.0	330	62	3	343	nd	583	4.3	untreated	mut
V23	2.4	239	64	2	140.5	nd	458	2.4	untreated	mut
V24	5.1	585	55	1	55	pos	503	2.6	untreated	mut

nd = not determined

C= chlorambucil
R= rituximab
F= fludarabine
Cy= cyclophosphamide
S= splenectomy







Moore Supplimental Figure 1

Supplemental Table 1

Patient characteristics. Tabulated are clinical characteristics of patients contributing samples to Figure 1A. For cytogenetics, samples were tested, unless otherwise marked, for del 13q, trisomy 12, del 11q and del 17 p by FISH. Other cytogenetic abnormalities were obtained by standard metaphase cytogenetics, not routinely performed on all samples. CD38 surface staining was determined by FACS analysis in a subset of samples.

Supplemental Figure 1

Neither MCL-1 nor BID are present in significant amounts before or after ABT-737 treatment in CLL cells. (A and B) Primary CLL cells were cultured for 24 hours with ABT-737 (100nM), negative control enantiomer (enant, 100nM), or vehicle (DMSO, 1%). Cells were lysed in CHAPS buffer and lysates (15μg) were subjected to Western blot using antibodies to (A) full length and cleaved MCL-1 or (B) p22 and caspase-8 cleaved tBID. (C) Whole cell lysates of independent CLL samples (50μg) were subjected to immunoprecipitation with an antibody recognizing BCL-2. Lysate input (sample number), immunoprecipitation pellet (p) and proteins remaining in the supernatant (s) were loaded for comparison. Immunoblot is against full-length p22 BID or caspase-8 cleaved p15 tBID. Same blot was probed for BIM in Figure 5B. Numbers at top of blots represent patient sample numbers.

Supplemental Methods

Peptides. Peptides were synthesized by Tufts University Core Facility and purified by HPLC. Identity was confirmed by mass spectrometry. Stock solutions were made in DMSO. Sequences were taken from published sequences of BAD(LWAAQRYGRELRRMSDEFEGSFKGL) (s1), BADmu (LWAAQRYGREARRMSDEFEGSFKGL, note L-> A, a point mutation abrogating binding to BCL-2) (s2) , NOXA A (AELPPEFAAQLRKIGDKVYC) (s3), BMF (HQAEVQIARKLQLIADQFHR) (s4) BID(EDIIRNIARHLAQVGDSMDR) (s5), BIM (MRPEIWIAQELRRIGDEFNA) (s6), BIK(MEGSDALALRLACIGDEMDV) (s7), BNIP3-α (VVEGEKEVEALKKSADWVSD) (s8), HRK (SSAAQLTAARLKALGDELHQ) (s9) and PUMA (EQWAREIGAQLRRMADDLNA) (s10).

Multiple Myeloma Cell culture. LP1 and L363 cells (kind gift from Ruben Carrasco) were cultured in Iscove's modified Dulbecco's medium with 10% fetal bovine serum.

Cytochrome c release. Mitochondria were purified from freshly isolated CLL cells and cell lines by mechanical disruption followed by differential centrifugation, as previously described (s11). Mitochondrial suspensions were made at 0.5 mg protein/ml, except for the case of ABT-737 and negative control enantiomer treatments, where 0.1 mg/ml was used. Release of cytochrome *c* was determined by a comparison of cytochrome *c* in the pellet and supernatant quantitated by ELISA (R&D systems).

Immunoblots. CLL protein lysates were obtained by cell lysis in Triton-X100 (142.5 mM NaCl, 5mM $MgCl_2$, 10 mM HEPES, 1 mM EGTA, 0.1% Triton-X100 (Sigma)), RIPA (150 mM NaCl, 2 mM EDTA, 0.1 M Na_2HPO_4 pH 7.2, 0.2 mM $NaVO_4$, 50 mM NaF, 1% sodium deoxycholate, 0.1% SDS, and 1% NP-40(Sigma)) or CHAPS (100 mM NaCl, 5 mM $NaPO_4$, 2.5 mM EDTA, 1% CHAPS (Sigma)) buffer supplemented with a Complete protease inhibitor cocktail tablet (Roche). C-terminal truncated GST-tagged MCL-1 and BCL-2 were prepared from bacterial lysates as previously described (s12). His-tagged caspase 8 cleaved recombinant human BID (Pharmaceutical Research Institutes S.A.) was a generous gift from Claudio Hetz (Harvard School of Public Health). The DHL4 large diffuse B cell line was a generous gift from Dr. Shipp (DFCI) and protein lysates were prepared the same as CLL lysates above. Protein samples were electrophoretically separated on NuPAGE 10% Bis-Tris polyacrylamide gels (Invitrogen). Antibodies were used to detect the following proteins on membrane: BIM (Calbiochem 22-40 or Abgent BH3 domain); BCL-2 (Pharmingen, /100); MCL-1 (Chemicon, RC-13 or Santa Cruz, S-19); BID (Santa Cruz, FL-195).

Immunoprecipitation. Cell lysates were incubated with 6C8 hamster anti-human BCL-2 antibody (3 µg) or BIM antibody (3 µg) for at least 1 hour at 4ºC in CHAPS buffer. Protein A-sepharose beads (Sigma) were added to precipitate complexes containing BCL-2 or BIM. Proteins complexed to the beads were separated from protein remaining in the supernatant by centrifugation and the fractions mixed with loading buffer prior to loading supernatant onto a 10% Bis-Tris polyacrylamide gel (Invitrogen) for analysis. For displacement reactions, 50µg of

lysate were incubated with 3μg 6C8 BCL-2 antibody for at least 1 hour at 4°C in 0.1% Triton-X100 buffer or CHAPS buffer. Protein A-sepharose beads were added and incubated for 1 hour. Then the beads were pelleted and washed 3 times and resuspended in HE buffer (1 mM EDTA and 10 mM HEPES, pH 7.4, as in (s13)). 1μM ABT-737, 1μM negative control enantiomer, or DMSO was added to the tube, incubated overnight; the supernatant was loaded onto a 10% Bis-Tris polyacrylamide gel for analysis.

Western blot protein quantification. Densitometry of protein bands were acquired using an AlphaImager EC gel documentation system (Alpha Innotec, Germany) and bands analyzed with the spot densitometry analysis tool (Alpha Ease FC software, version, 4.1.0). Quantities of relative BCL-2 and MCL-1 per CLL sample were obtained by using the densitometry of the GST standards to create a standard curve. The values of the CLL lysate bands were then used with the equation of the standard curve line to calculate a protein concentration. shRNA values were obtained by dividing either individual BIM isoform values or total BIM value by the corresponding actin value.

Subcellular Fractionation. Primary CLL cells from two independent patient samples were fractionated as previously described (s14). Equal amounts of protein from each fraction were loaded onto a NuPAGE 10% Bis-Tris polyacrylamide gel based on a Bio-Rad Protein Assay. Antibodies to verify the contents of the different fractions were as follows: heavy membrane, MnSOD (Stressgen, SOD-111); light membrane, BiP (BD Biosciences, 610978); and S100, Glucose 6 Phosphate Dehydrogenase (Abcam, ab 993).

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