Supplementary Materials and Methods

Cell culture

OCI-LY1, OCI-LY7, OCI-LY8, and SU-DHL4 cell lines (a gift from Dr. Laura Pasqualucci, Columbia University) were cultured in IMDM (GE Healthcare Hyclone) supplemented with 10% FBS, 10 mM 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES), 10 mM L-glutamine, 100 I.U. penicillin, and 100 µg/ml streptomycin. OCI-AML2, OCI-AML3, and MOLM13 cell lines were cultured in RPMI (Corning) supplemented with 10% FBS, 10 mM HEPES, 10 mM L-glutamine, 100 I.U. penicillin, and 100 µg/ml streptomycin. Cells were grown in a humidified 37°C incubator with 5% CO_2. Cells were routinely tested to ensure absence of mycoplasma and were maintained at or below 2 x 10^6 cells/mL. Cell lines were authenticated through the University of Arizona Genomics Core. Human embryonic kidney (HEK) 293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Life Technologies) supplemented with 10% calf serum, 100 I.U. penicillin, and 100 µg/mL streptomycin. Human peripheral blood mononuclear cells (PBMCs) and primary CLL samples were isolated from blood samples by centrifugation through Ficoll-PaqueTM (GE Healthcare) and were grown in RPMI with 10% FBS, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10 mM L-glutamine, 100 I.U. penicillin, 100 µg/ml streptomycin. In the case of primary CLL samples, cells were grown on NK.Tert immortalized bone marrow stromal cell line.

Cell viability

Cell viability assays were performed in 96-well format as described previously (*23*). Briefly, 6 x 10^4 cells were cultured in 200 µL growth medium with inhibitors for 48 hours. Cells were harvested and stained with Annexin V, Alexa Fluor 647 conjugate, and propidium iodide (Life Technologies). Fluorescence was measured by flow cytometry using FACScalibur (Becton-Dickinson), and viability of cells was quantified using FlowJo software v10.1r7 (FlowJo LLC). In the case of primary CLL samples, stromal cells were seeded onto 96-well plates 24 hours before each experiment at 6 x 10^4 cells/well. Confluence was confirmed by phase-contrast microscopy before seeding of CLL cells at 6 x 10^5 cells/well. Cells were then treated with simvastatin for 16 hours before addition of venetoclax for an additional 8 hours. Cells were then harvested and stained for CD19, and viability was assessed using Annexin V. For the CD40L stimulations, NK.Tert stromal cells were grown to 80% confluency before trypsinization and plated at 5 x 10^4 cells/well in a 96-well plate for 20 hours. Plates were checked for confluency the following day, and unadhered cells were aspirated off and replaced with fresh medium. CLL cells were plated at 1.5 x 10^5 cells/well in medium plus vehicle or simvastatin and 2 µg/mL recombinant human CD40L (GenScript) for 16 hours, after which venetoclax was introduced at a final concentration of 10 nM for 8 hours. Viability was determined as mentioned above.

Western blotting and immunoprecipitation

Cells were lysed in radio-immunoprecipitation assay (RIPA) buffer (150 mM NaCl, 1.0% IGEPAL CA- 630, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris, pH 8.0, 2 mM EDTA, 50 mM NaF) supplemented with protease inhibitor cocktail (Calbiochem)

and phosphatase inhibitor cocktails 2 and 3 (Sigma-Aldrich). Protein concentrations were normalized using a Bradford protein assay (Bio-Rad). Lysates were prepared at 1 μg/μl concentration in 1X XT Sample Buffer (Bio-Rad) and 5% 2-mercaptoethanol (Sigma-Aldrich). Lysates were run on 4-12% Bolt Bis-Tris Plus gels (Life Technologies), and transferred onto nitrocellulose membranes. The following antibodies were used: GAPDH, PARP, caspase-9, cleaved caspase-3, MCL1, BCL-XL, BIM, BID, BAX, BAK, PUMA, COX IV, ERK (Cell Signaling Technology), BCL2 (BD Pharmingen), BAD, unprenylated RAP1A, HRK (Santa Cruz Biotechnology), and HDJ-2 (ThermoFisher). The following secondary HRP-conjugated antibodies were used: anti-mouse IgG, anti-rabbit IgG (Promega), anti-goat IgG (Santa Cruz Biotechnology), and Protein A (BD Pharmingen). For BCL-2 immunoprecipitation, lysates were incubated with 1:100 dilution of monoclonal antibody directed towards residues surrounding Gly47 of hBCL2 (D55G8;4223, Cell Signaling). Immunoprecipitates were obtained using Protein G Sepharose beads (GE Healthcare Life Sciences), and Western blots were probed with antibodies to human PUMA (D30C10;12450, Cell Signaling) or BCL2 (D17C4;3498, Cell Signaling). Blots were developed using Pierce ECL Western Blotting Substrate or SuperSignal West Femto Maximum Sensitivity Substrate (Life Technologies) and detected using a Nikon D700 SLR camera. Images were processed using Adobe Photoshop software, and densitometry was performed using ImageJ software.

Overexpression and shRNA knockdown

To generate BCL-2 and MCL-1 over-expressing lines and the stable PUMA shRNA lines, 293T HEK cells were transfected with X-tremeGene HP DNA Transfection

Reagent (Roche). 293T cells were incubated for 24 hours before replacing medium. The virus-containing medium was then harvested after an additional 24 hours and used to transduce cell lines. For lentivirus production, 293T cells were co-transfected with pCMV-VSVG (Addgene plasmid 8454) and psPAX2 (Addgene plasmid 12260). To generate retrovirus for the p53 dominant negative, 293T cells were transfected by calcium phosphate with pLXSP-GSE56 (gift from Dr. Lindsey Mayo) and pCL-Ampho. To transduce DLBCL cell lines, we incubated cells in viral supernatants for 72 hours (changing supernatant every 24 hours) with 10 μg/ml 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide (polybrene, Sigma-Aldrich). To transduce the OCI-AML3 line, 1 million cells were resuspended in viral supernatant with polybrene and spinfected at 32°C for 90 minutes at 500x g, and supernatant was changed 24 hours later. Cells were treated with puromycin (2 μg/ml) for 5 days after transduction to select for stably transduced cells. Plasmid-positive cells were maintained with puromycin (1 μg/ml).

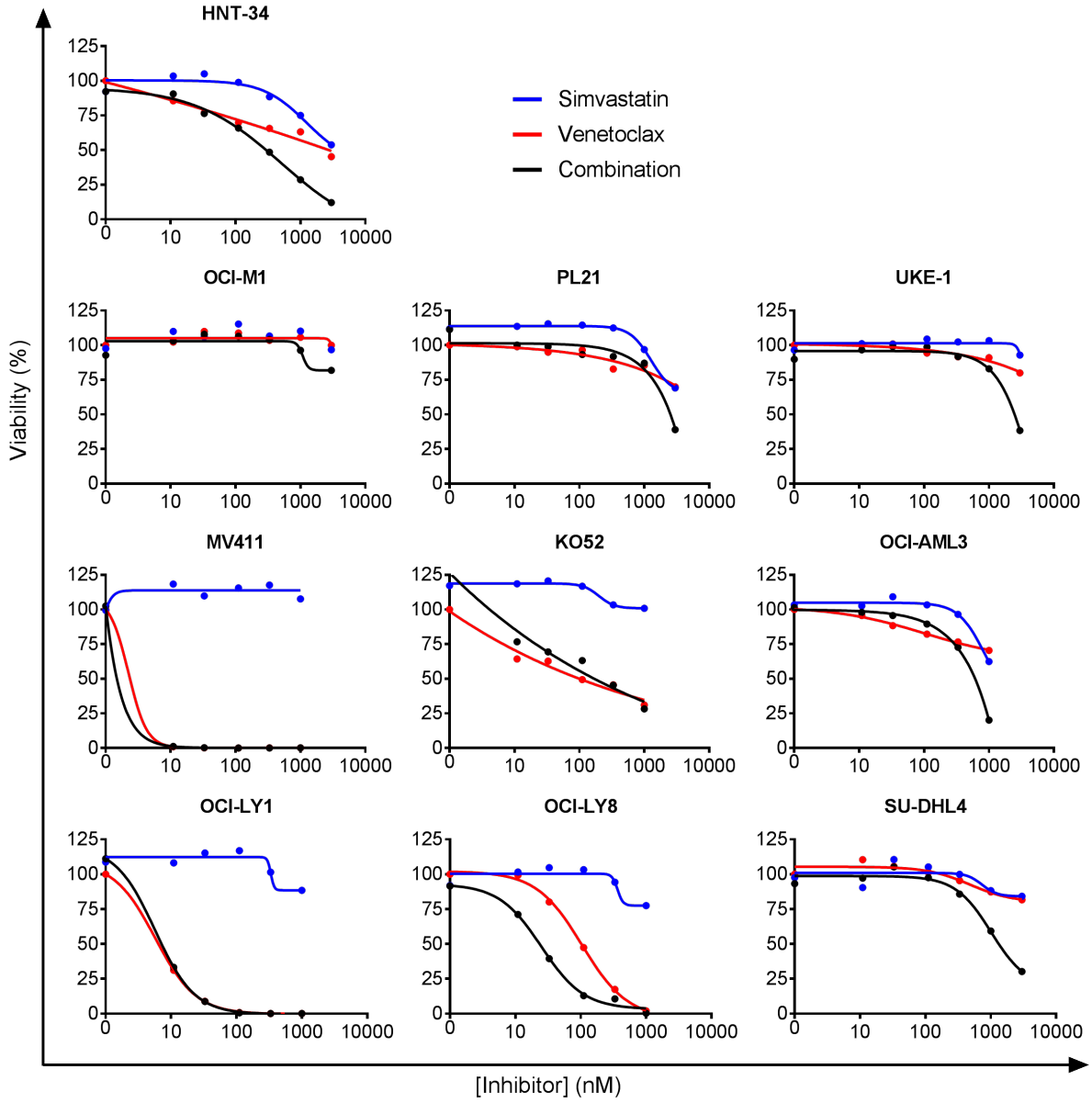
Subcellular fractionation

Cells were harvested and re-suspended in isotonic buffer [250 mM sucrose, 20 mM HEPES, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, protease inhibitor cocktail (Calbiochem), and phosphatase inhibitor cocktails 2 and 3 (Sigma-Aldrich)]. Cells were lysed by passing through 28 gauge insulin syringes, and resulting lysates were centrifuged at 800 g for 10 minutes at 4˚C six times to remove intact cells and nuclear fractions. Supernatants were then centrifuged at 10,000 g for 30 minutes at 4˚C to separate the mitochondria-

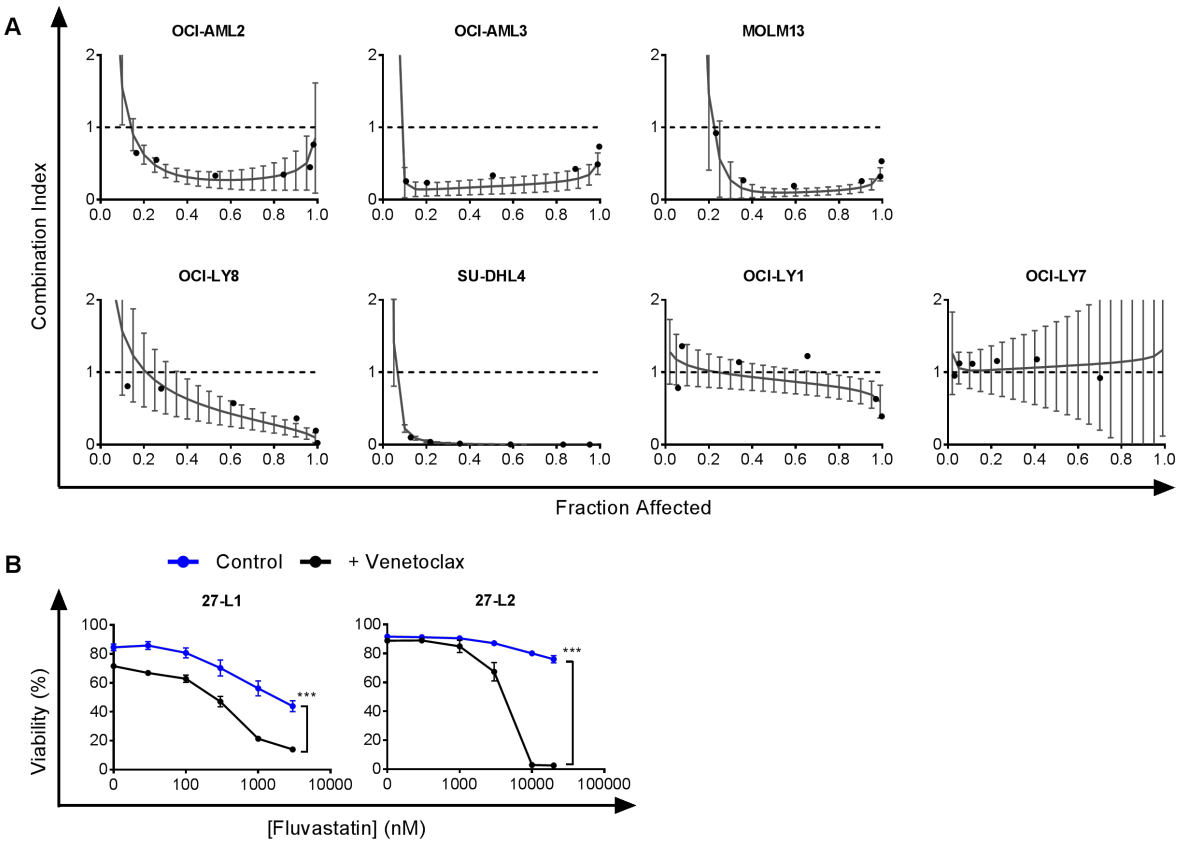
enriched heavy membrane pellet from the supernatant containing cytoplasmic fractions. Pellets were then lysed using RIPA buffer and used for western blotting as described above.

Quantitative real-time PCR

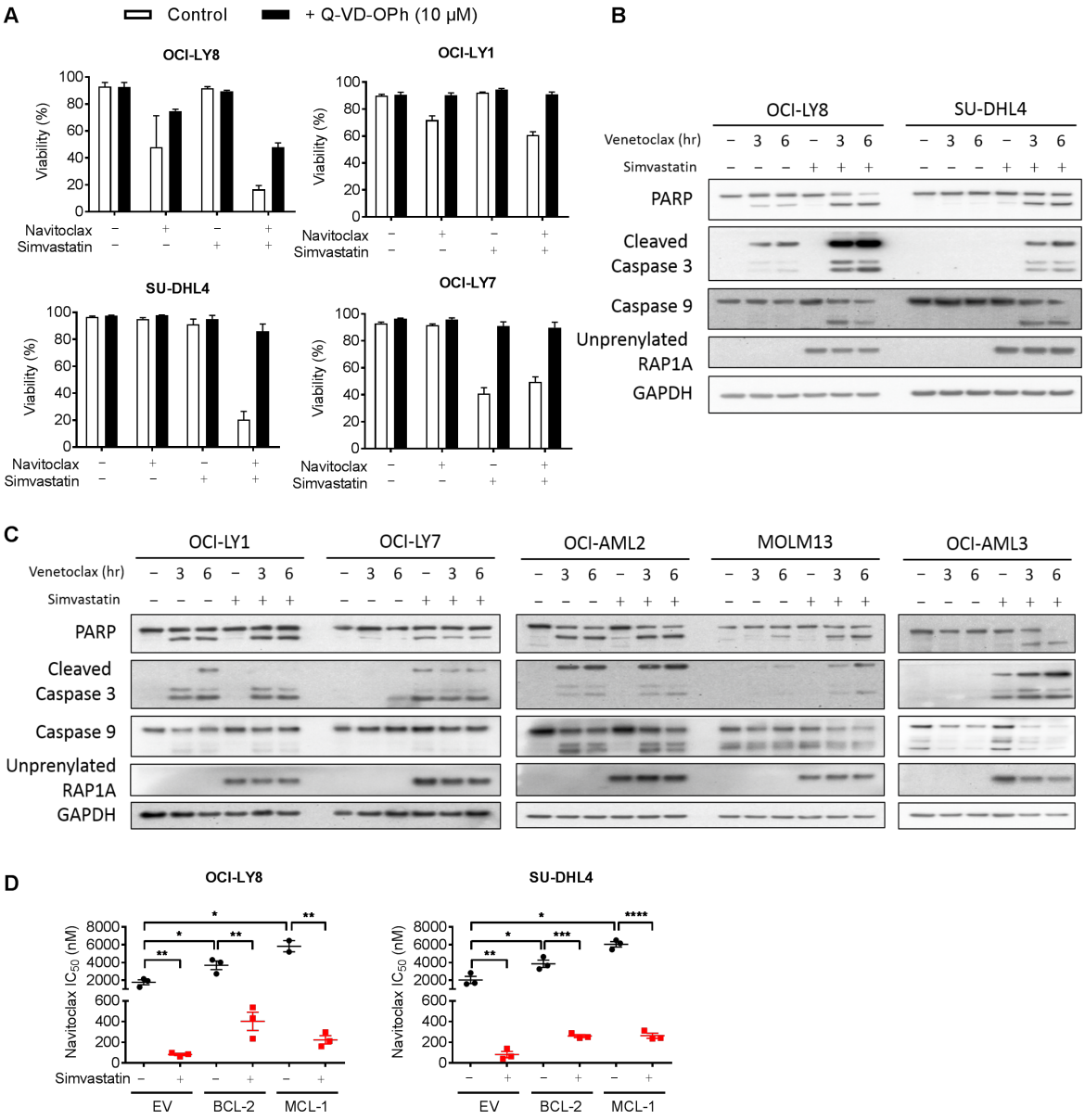
Cells were treated with inhibitors for 16 hours before harvesting and resuspending in TRIZOL (Life Technologies). RNA was extracted using Quick-RNA kits (Zymo Research) and used for cDNA synthesis using iScript cDNA Synthesis Kits (Bio-Rad) according to the manufacturer's protocols. qPCR was performed with iTaq Universal SYBR Green One-Step Kit (Bio-Rad) read on iQ5 Real-Time PCR Detection System (Bio-Rad). For CLL patient sample qPCR, NK.Tert stromal cells were grown to 80% confluency before trypsinization and plated at 5 x 10^5 cells/well in a 6-well plate for 24 hours. Plates were checked for confluency the following day, and unadhered cells were washed off once with PBS and replaced with fresh medium. CLL cells were plated on top at a concentration of 5 x 10^6 cells/well and treated with vehicle or simvastatin for 16 hours. CLL cells were gently lifted by pipetting so as to not disturb the stromal layer and collected along with two additional gentle washes with PBS to maximize CLL cell collection. Cells were centrifuged at 500 g for 5 minutes, washed once with PBS, resuspended in TRIZOL, and processed as described above.



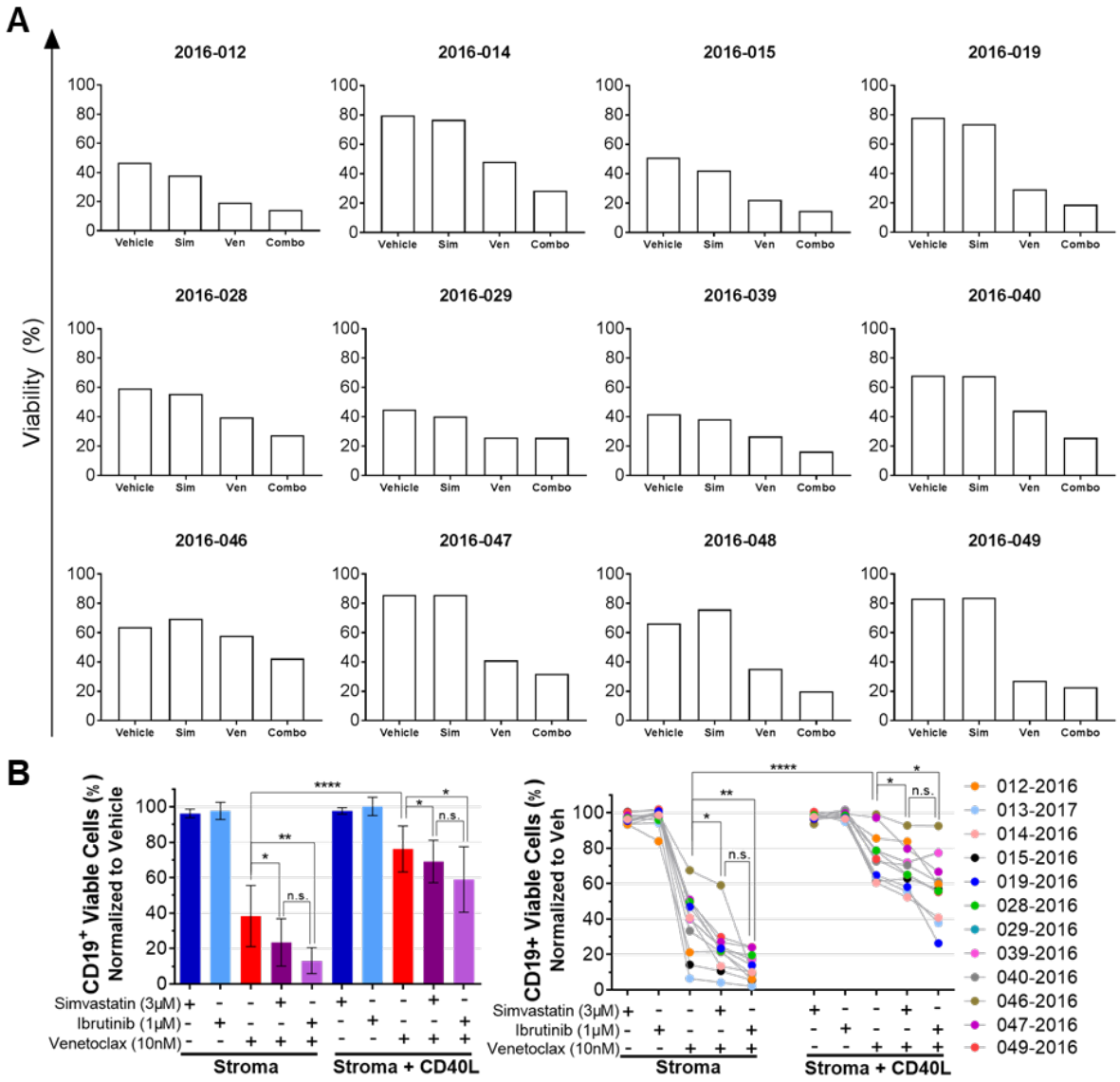
Supplementary Figure S1. Simvastatin enhances the effects of venetoclax on a subset of AML and DLBCL cell lines. Concentrations for each drug are as follows; 0, 11, 33, 111, 333, and 1000 nM for OCI-AML3, KO52, MV411, OCI-LY1, OCI-LY8; 0, 11, 33, 111, 333, 1000, and 3000 nM for SU-DHL4; 0, 3, 11, 33, 111, 333, and 1000 nM for all other cell lines, In OCI-AML3, HNT-34, UKE-1, OCI-LY8, and SU-DHL4 cells, the addition of 1000 or 3000 nM simvastatin enhanced killing by venetoclax measured after 48 hours of treatment. For all cell lines tested, n=1, with three technical replicates per condition.



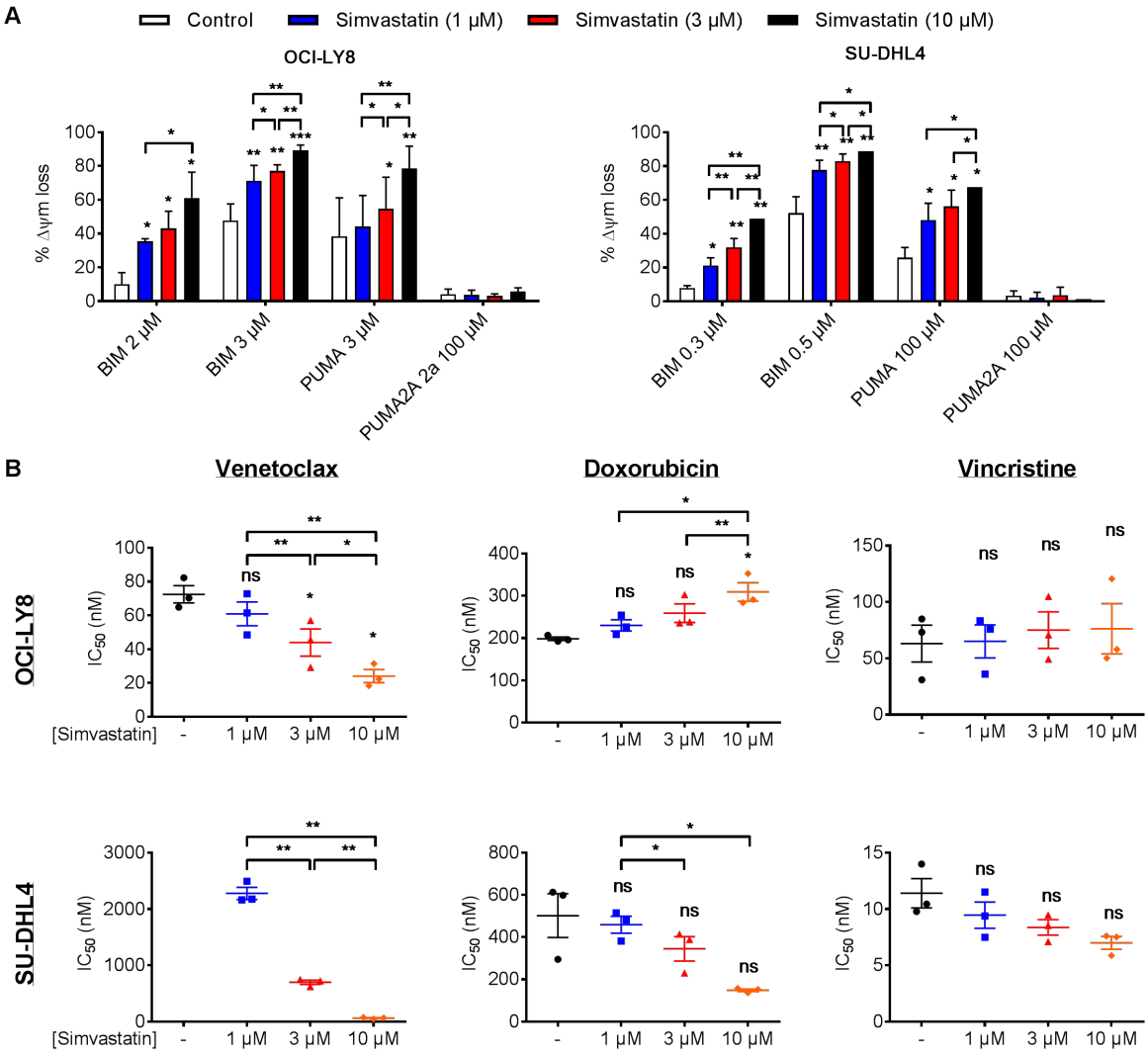
Supplementary Figure S2. **Statins synergize with venetoclax in blood cancer cells. A.** Formal synergy analysis (median-effect) of DLBCL and AML cell lines using data from Fig. 1A; n = 3. **B.** Viability of primary murine lymphoma cells co-cultured with irradiated 3T3 stroma and treated for 48 hours with the indicated inhibitors. ([venetoclax] = 10 nM for 27-L1 and 1000 nM for 27-L2). Statistics testing was done using one-tailed paired Student's t-test on the IC_{50} values of indicated treatment groups; n = 3. *** $p < 0.001$.



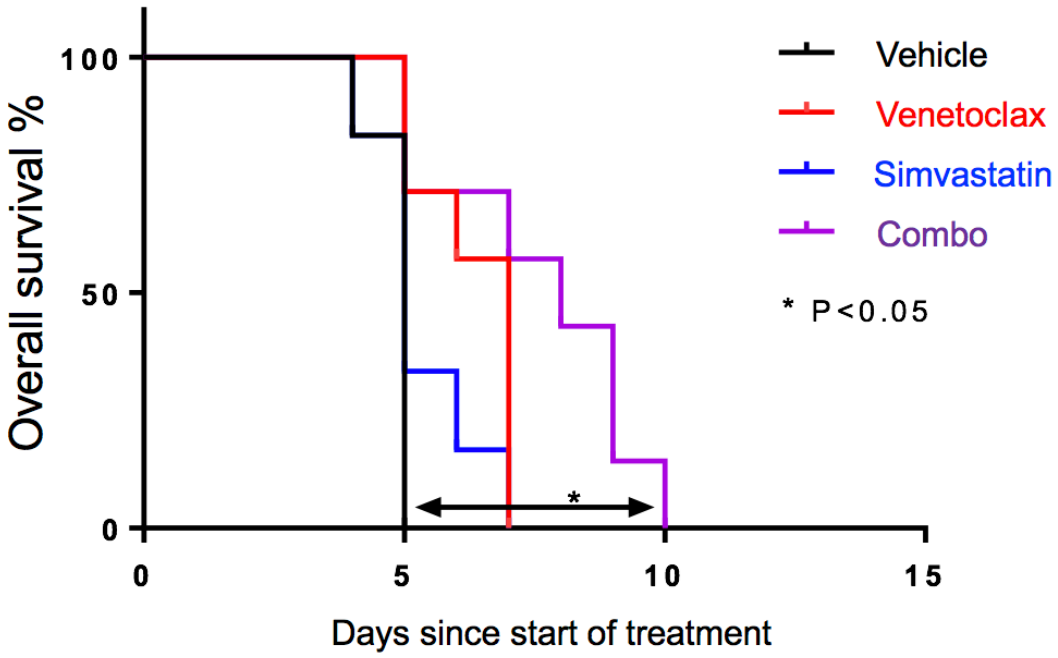
Supplementary Figure S3. **Simvastatin plus venetoclax induce apoptosis in DLBCL and AML cell lines. A.** Viability of cells treated with indicated drugs with (black bars) or without (white bars) pan-caspase inhibitor Q-VD-OPh (10 μM) for 48 hours. Doses of navitoclax are as follows: 30 nM for OCI-LY1, 300 nM for all other cell lines. **B-C.** Western blot of cells pre-treated with vehicle or simvastatin for 16 hours before addition of venetoclax for the indicated amounts of time. Caspase 9 antibody detects full length (upper band) and cleaved (lower doublet) products. GAPDH served as loading control. **B**, OCI-LY8 and SU-DLH4. **C**, OCI-LY1, OCI-LY7, and three AML lines. Doses of venetoclax are as follows: 10 nM for OCI-LY1, 30 nM for OCI-LY8, 300 nM for all other cell lines. **D.** Sensitivity of DLBCL cells over-expressing BCL-2 or MCL-1 to navitoclax with or without simvastatin (10 μM). (**A, D**) Unpaired two-tailed t-test, n = 3. In this and other supplementary figures, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ns = not significant.



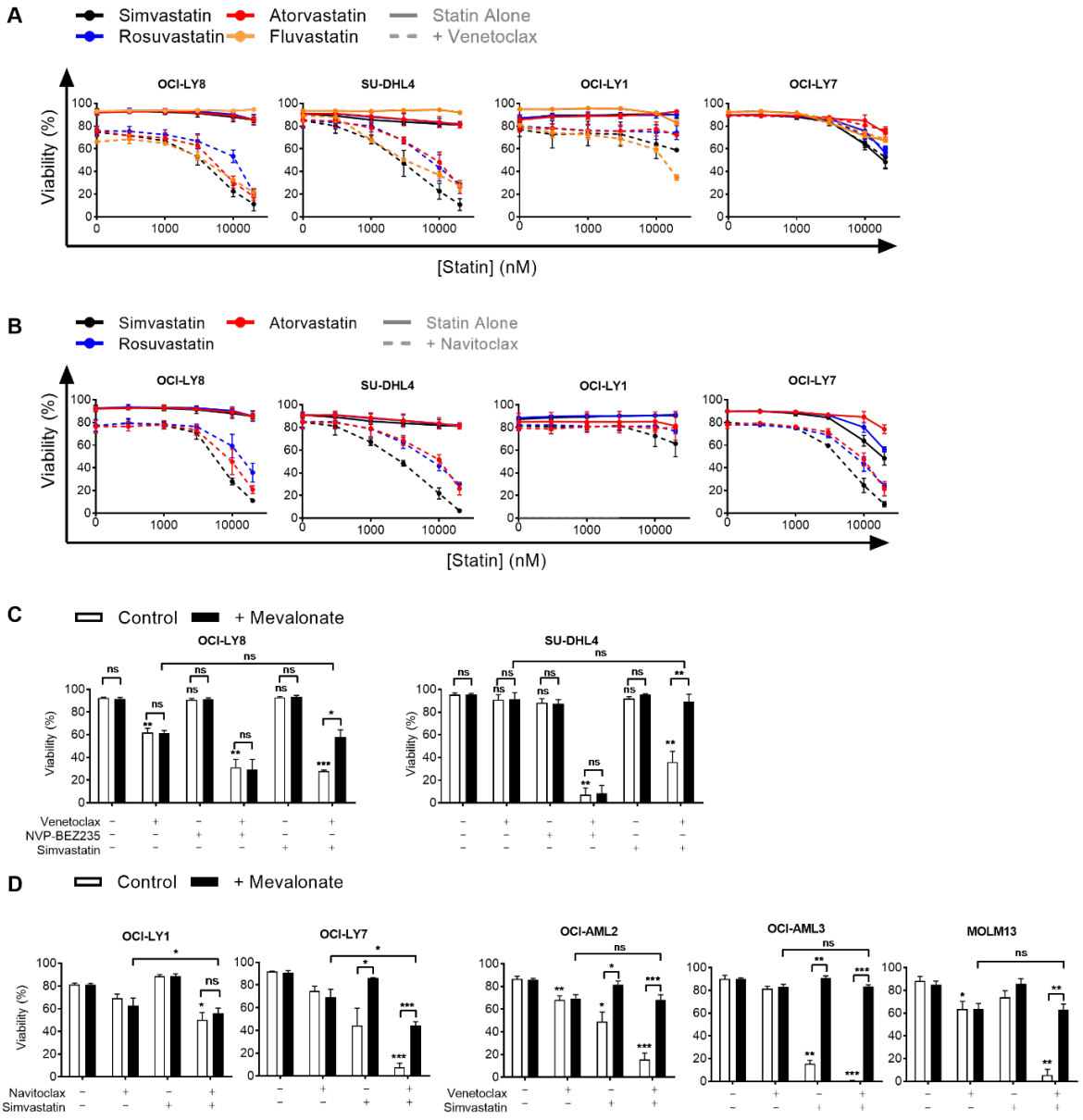
Supplementary Figure S4. **Simvastatin enhances killing of primary CLL samples cultured with stimuli from the microenvironment. A.** Individual CLL data from Fig. 1D. Sim = simvastatin (1 μM), Ven = venetoclax (3 nM), n = 1 for all. **B.** CLL survival in vitro in the absence or presence of soluble CD40L. Error bars (left panel) represent +/- SD. Right panel shows data for individual patient specimens. Significance determined by paired, two-tailed t-test with Bonferroni correction for multiple comparisons, adjusted α=0.0011. Multiplicity adjusted p-values $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), $p<0.0001$(****).



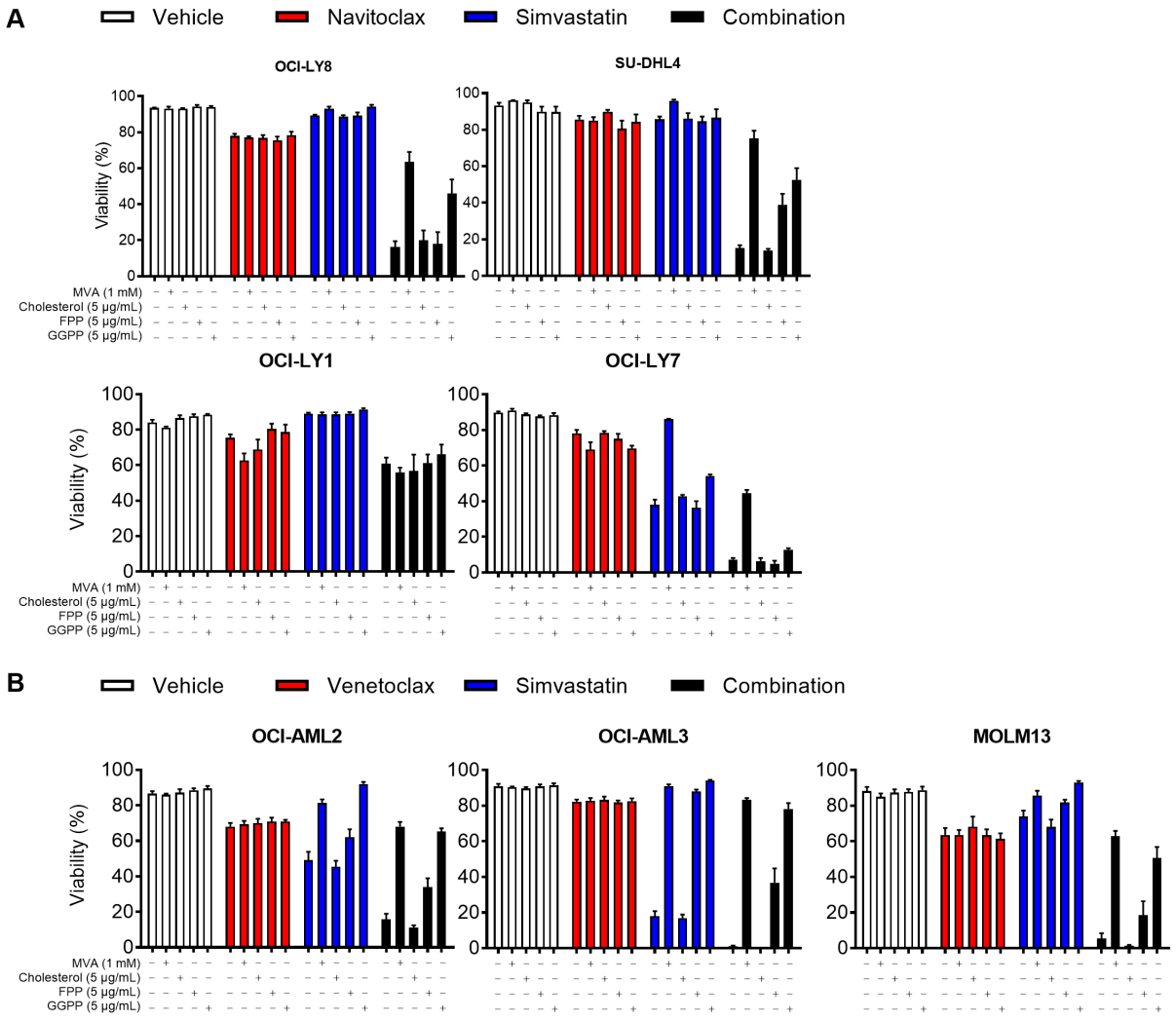
Supplementary Figure S5. **Statins induce dose-dependent increase in mitochondrial priming, but do not sensitize to chemotherapy. A.** Dynamic BH3 profiles for cells treated with indicated doses of simvastatin for 16 hours. Paired one-tailed t-test, n ≥ 3 (OCI-LY8), n = 3 (SU-DHL4). **B.** Sensitivity (IC_{50}) of two DLBCL cell lines to venetoclax or two chemotherapies. Cells were treated with inhibitors as well as the indicated doses of simvastatin for 48 hours. Paired two-tailed t-test, n = 3.



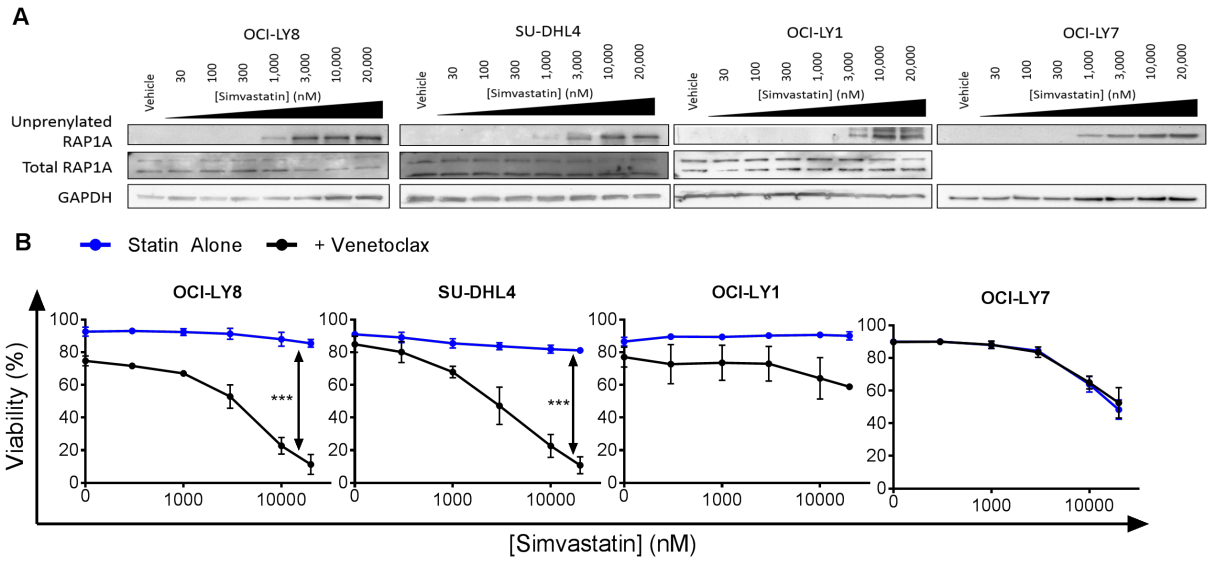
Supplementary Figure S6. **The combination of statin with venetoclax extends survival of mice with syngeneic B cell lymphoma.** Sublethally irradiated C57Bl/6 mice were transplanted with murine 27-L1 lymphoma cells. When GFP+ cells were detected in the blood, mice were randomized into groups of 6 or 7 and treated daily with vehicle, venetoclax, simvastatin, or the combination (combo). Mice were monitored daily and euthanized when clinical signs appeared. Graph shows Kaplan-Meier plot of survival with statistical analysis by log-rank text.



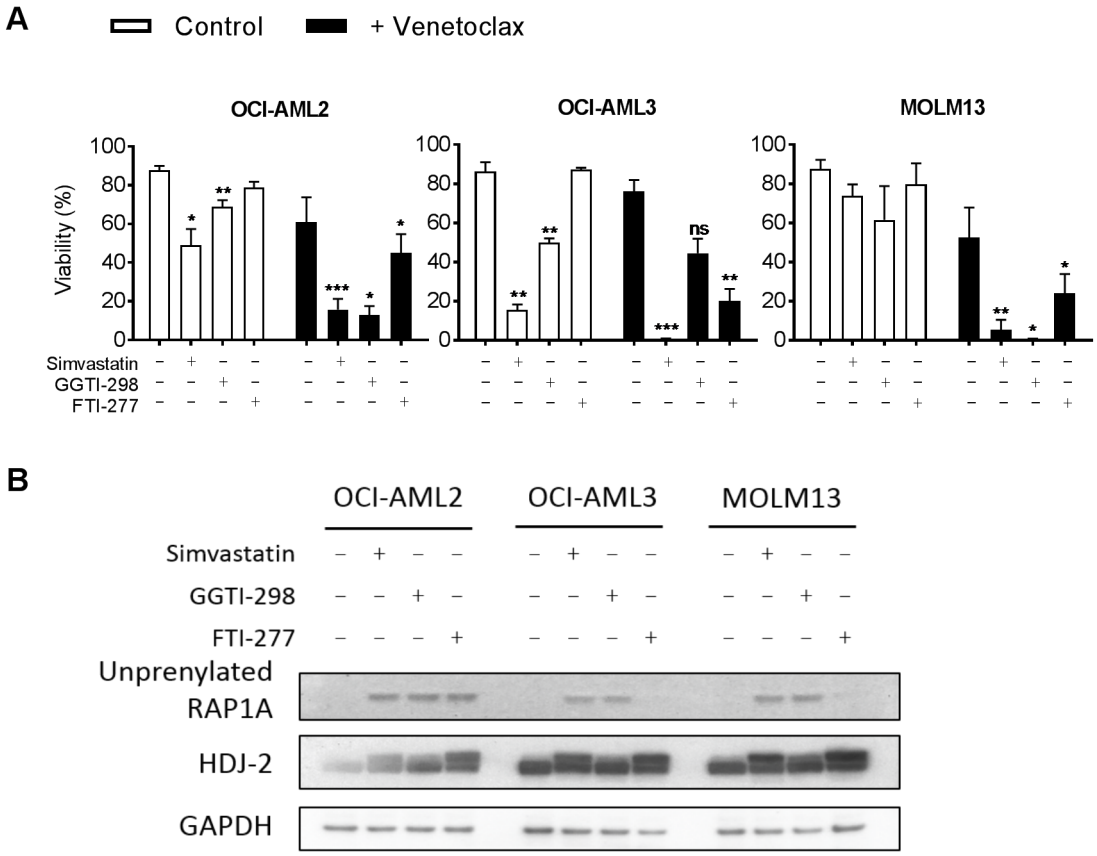
Supplementary Figure S7. **The effect of statins is due to on-target HMGCR inhibition. A.** Viability of cells treated with increasing doses of different chemically distinct statins (colors) with (dashed lines) or without (solid lines) venetoclax (30 nM for OCI-LY8, 300 nM for SU-DHL4, 10 nM for OCI-LY1, 300 nM for OCI-LY7); n = 3. **B.** Viability of cells treated as in A, but in combination with navitoclax (50 nM for OCI-LY1, 300 nM for all other cell lines); n = 3. Note that OCI-LY1 and OCI-LY7 have differential sensitivity to venetoclax vs navitoclax, such that the latter is resistant to venetoclax but not navitoclax, whereas the former is more sensitive to venetoclax. **C.** Viability of cells treated with indicated drugs with (black bars) or without (white bars) mevalonate (1 mM). Doses of drugs are 10 μM simvastatin, 30 nM (OCI-LY8) or 300 nM (SU-DHL4) venetoclax, and 50 nM NVP-BEZ235. **D.** Viability of cells treated with indicated inhibitors with (black bars) or without (white bars) 1 mM mevalonate. (**C, D**) Paired two-tailed t-tests, n = 3.



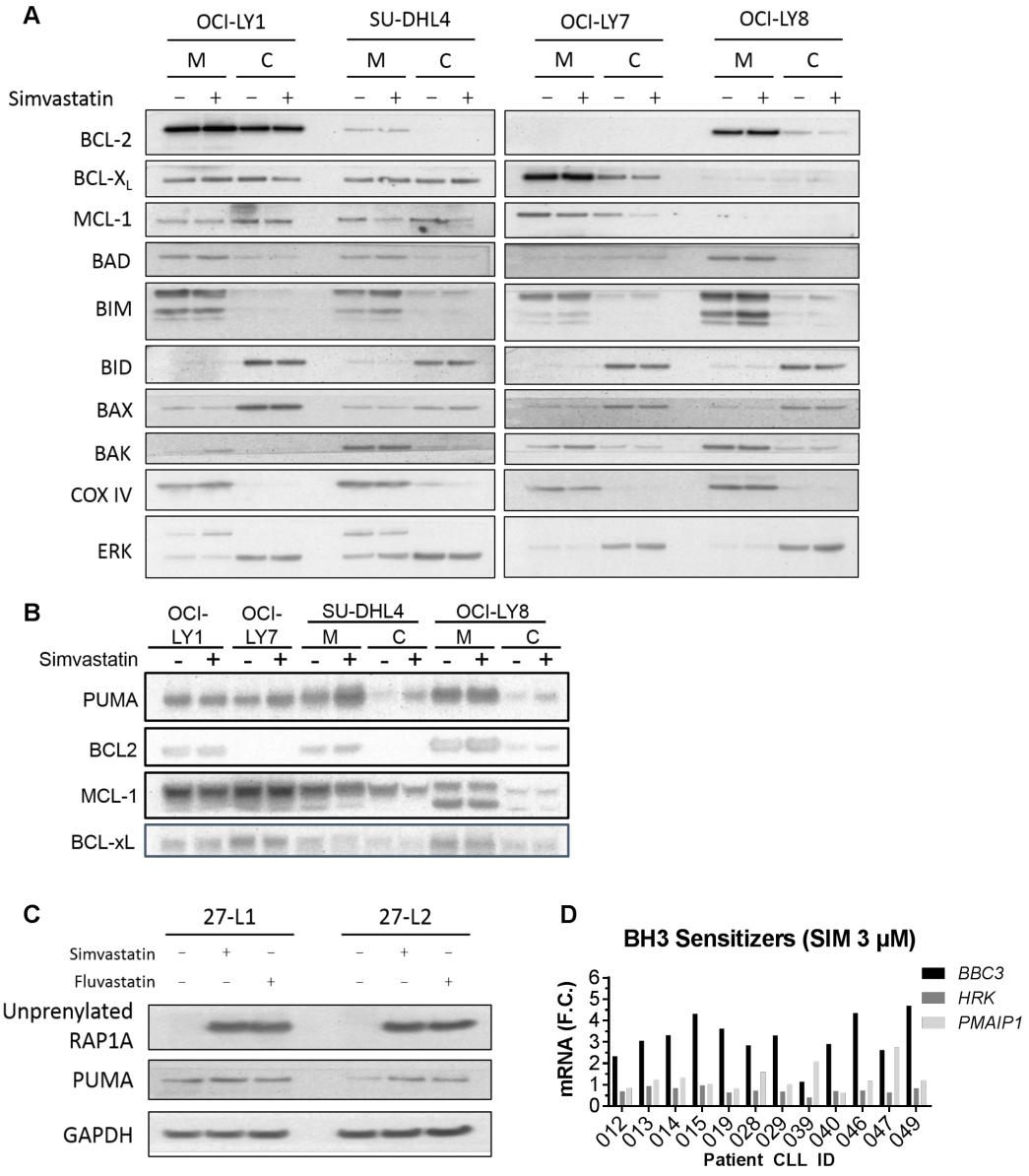
Supplementary Figure S8. **Mevalonate and geranylgeranyl pyrophosphate are sufficient to rescue from the effects of simvastatin. A.** Viability of DLBCL cell lines treated with indicated inhibitors for 48 hours with or without addition of indicated metabolites; n = 4 for control and GGPP (OCI-LY8 and OCI-LY7 cell lines only), n = 3 for all other data. **B.** Viability of AML cell lines treated with indicated inhibitors for 48 hours with or without addition of indicated metabolites; n = 3.



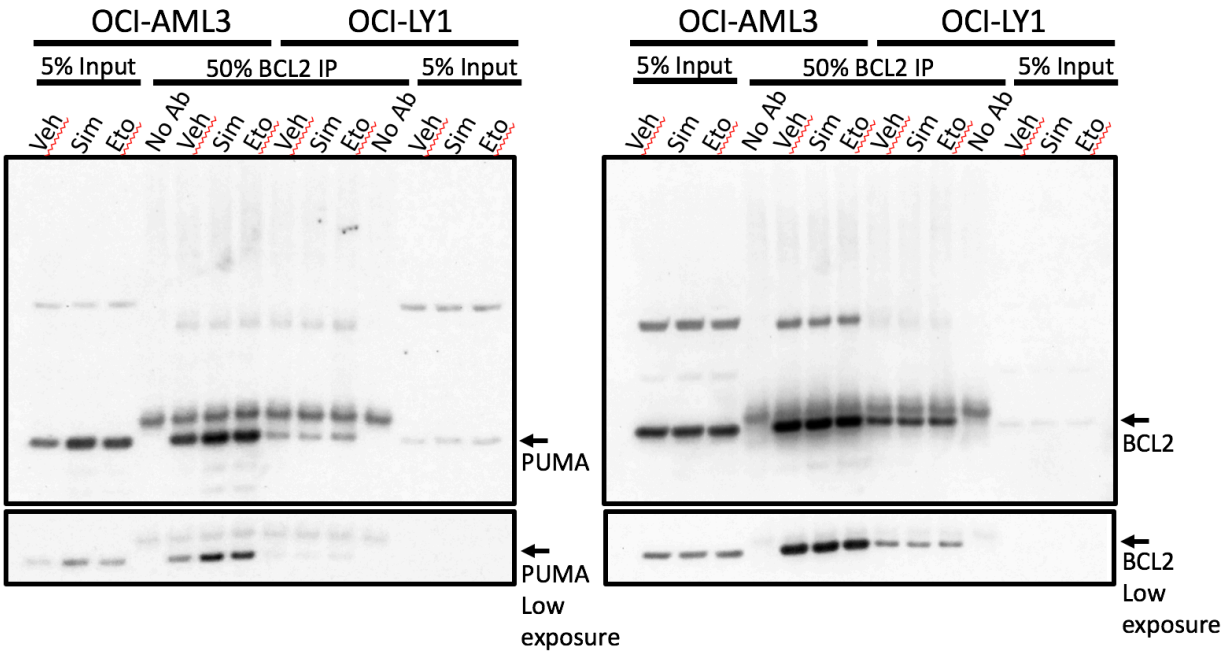
Supplementary Figure S9. **Simvastatin inhibits protein geranylgeranylation in a dose-dependent manner in DLBCL. A.** Western blots of DLBCL cell lines treated with increasing doses of simvastatin for 16 hours. **B.** Viability of cells treated with simvastatin in A for 48 hours with (black lines) or without (blue lines) venetoclax. Doses of venetoclax are as follows: 30 nM for OCI-LY8, 10 nM for OCI-LY1, 300 nM for OCI-LY7 and SU-DHL4. Significance testing was done using two-tailed paired Student's t-test on the IC_{50} values, n = 3.



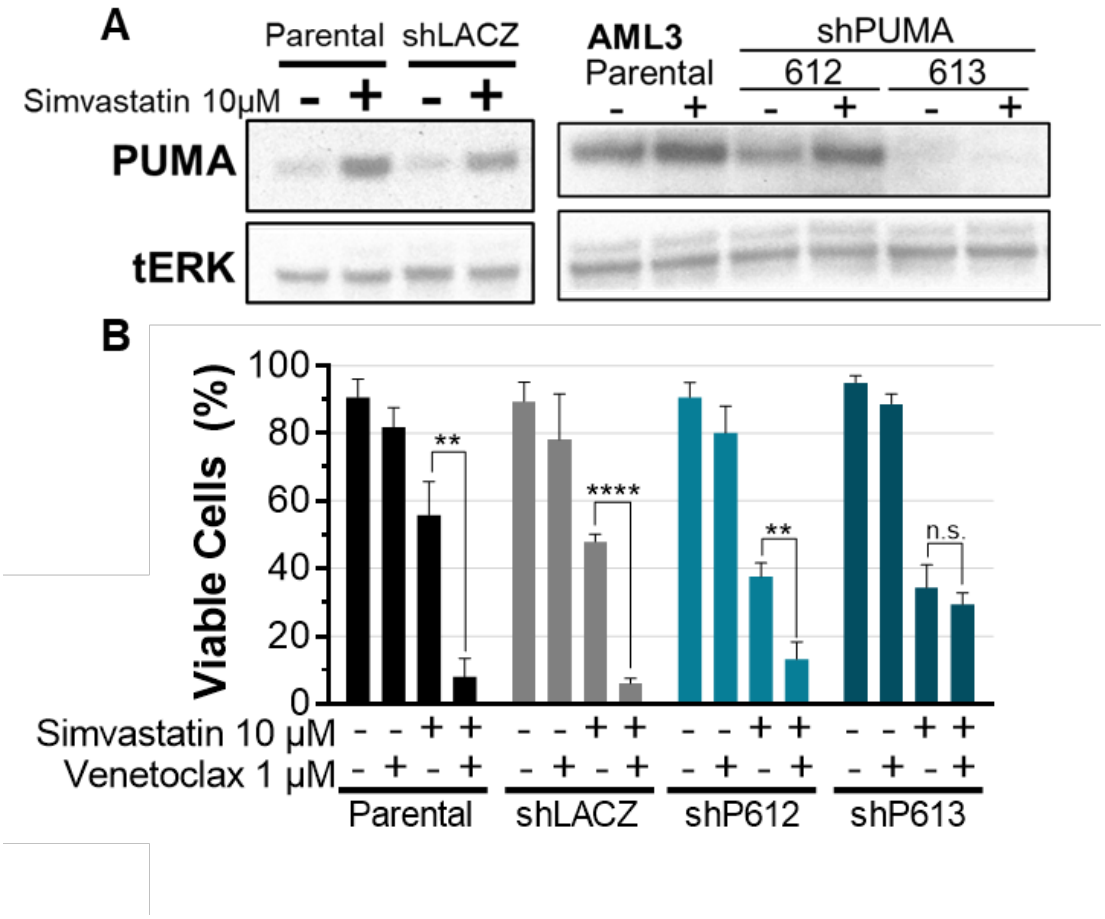
Supplementary Figure S10. **Inhibition of GGT is sufficient to recapitulate the effects of simvastatin in AML cell lines. A.** Viability of AML cell lines treated with indicated inhibitors for 48 hours. Two-tailed paired Student's t-test on the IC_{50} values, n = 3. **B.** Western blot of AML cell lines treated with 10 μM of the indicated inhibitors for 16 hours.



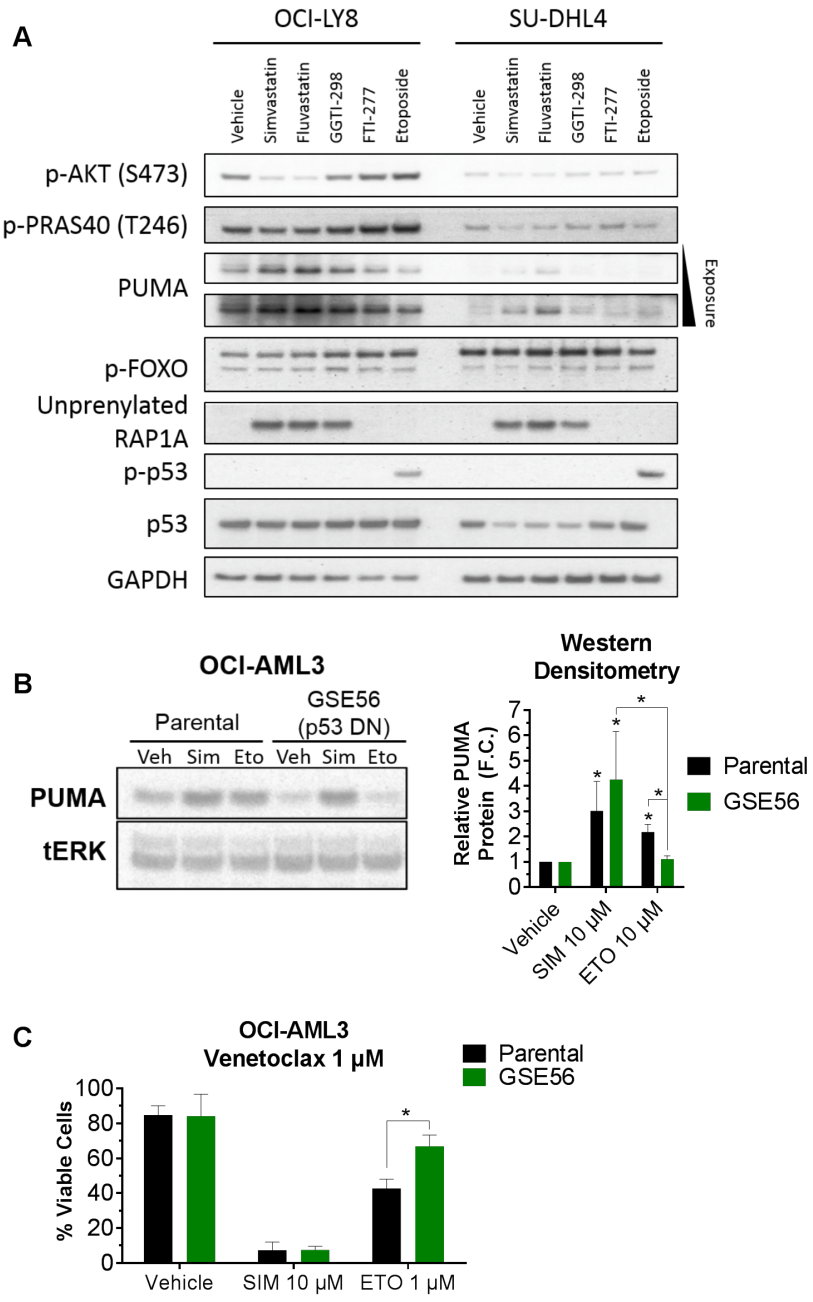
Supplementary Figure S11. **Simvastatin does not affect expression of many major BCL-2 family proteins but does increase PUMA. A.** Western blot of DLBCL cell lysate fractions enriched for heavy membranes (M) or cytoplasmic (C) proteins after 16 hours of treatment with 10 μM simvastatin. **B.** PUMA enrichment in heavy membrane fraction from sensitive DLBCL lines, SU-DHL4 and OCI-LY8. OCI-LY1 does not upregulate PUMA, and OCI-LY7 lacks detectable BCL2 expression. **C.** Western blot of primary mouse lymphoma cell lines treated with 10 μM simvastatin or fluvastatin for 24 hours. **D.** mRNA expression of BH3-only sensitizers (*BBC3* encodes PUMA, *HRK* encodes HRK, *PMAIP1* encodes NOXA) after 16 hours of treatment with 3 μM simvastatin alone, measured by qPCR in CLL patient samples and normalized to untreated. F.C. = fold change. Data are average of n = 3 for 012, 014, 015, 028, 046, 047, 049; average of n = 2 for 013, 019, 029, 039, 040.



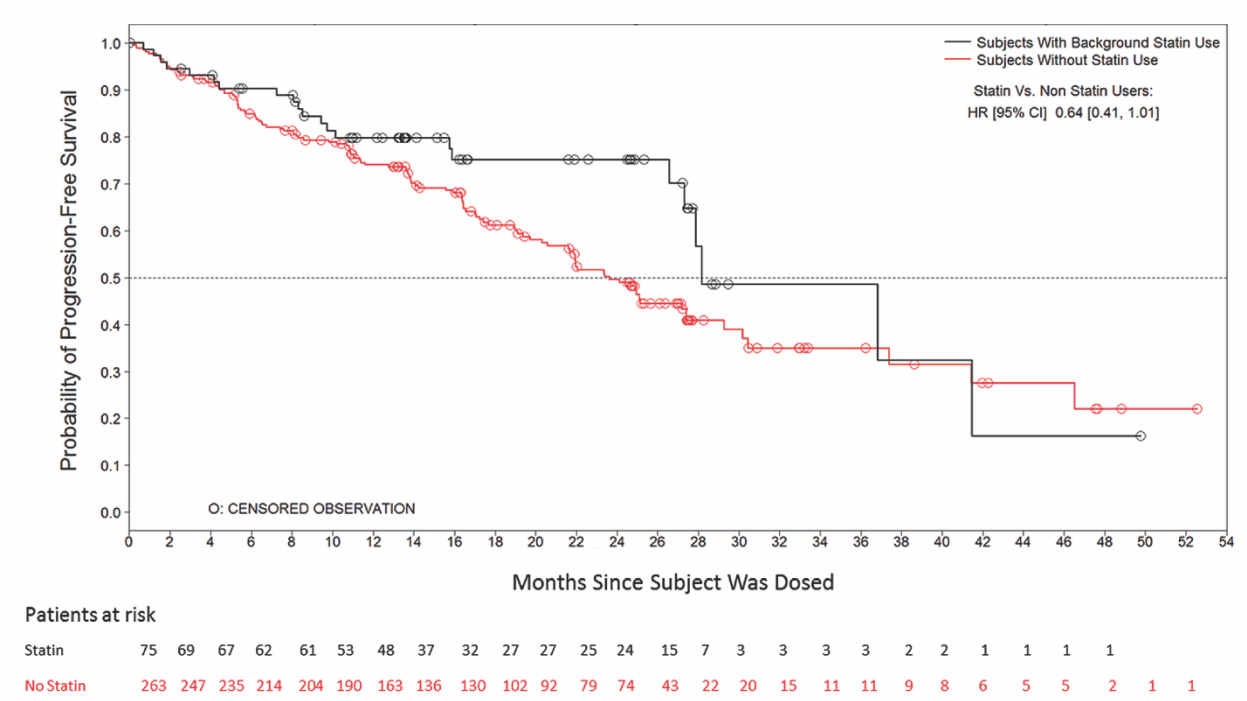
Supplementary Figure S12. **Simvastatin increases association of BCL2 with PUMA in sensitive OCI-AML3 cells but not in resistant OCI-LY1 cells.** Cells were treated for 16 hours with vehicle, simvastatin, or etoposide, as indicated. Lysates were prepared and subjected to immunoprecipitation with anti-BCL2 antibodies. Immunoprecipitates were resolved by SDS-PAGE and probed for PUMA (left) or BCL2 (right). The No Antibody controls were performed using additional vehicle-treated samples. 5% fractions of the input lysates were included to confirm increased PUMA expression in OCI-AML3 cells. Comparable results were obtained in a replicate experiment.



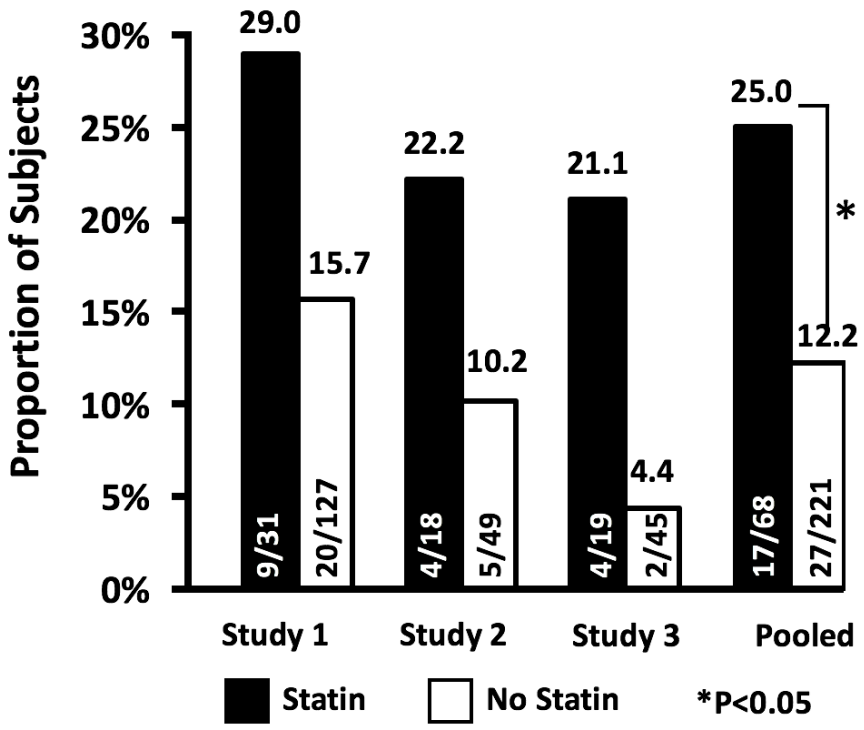
Supplementary Figure S13. PUMA knockdown in OCI-AML3 cells rescues them from sensitization to venetoclax by simvastatin. A. PUMA was stably knocked down in AML3 by shRNA (shP612: TRCN0000033612; shP613: TRCN0000033613). Blots were probed for PUMA and total ERK (tERK). **B.** AML3 lines were treated for 48 hours with simvastatin (10 μM) and/or venetoclax (1 μM), and viable cells were assessed by PI exclusion (n=3, multiple T-tests, alpha=0.05, Holm-Sidak corrected).



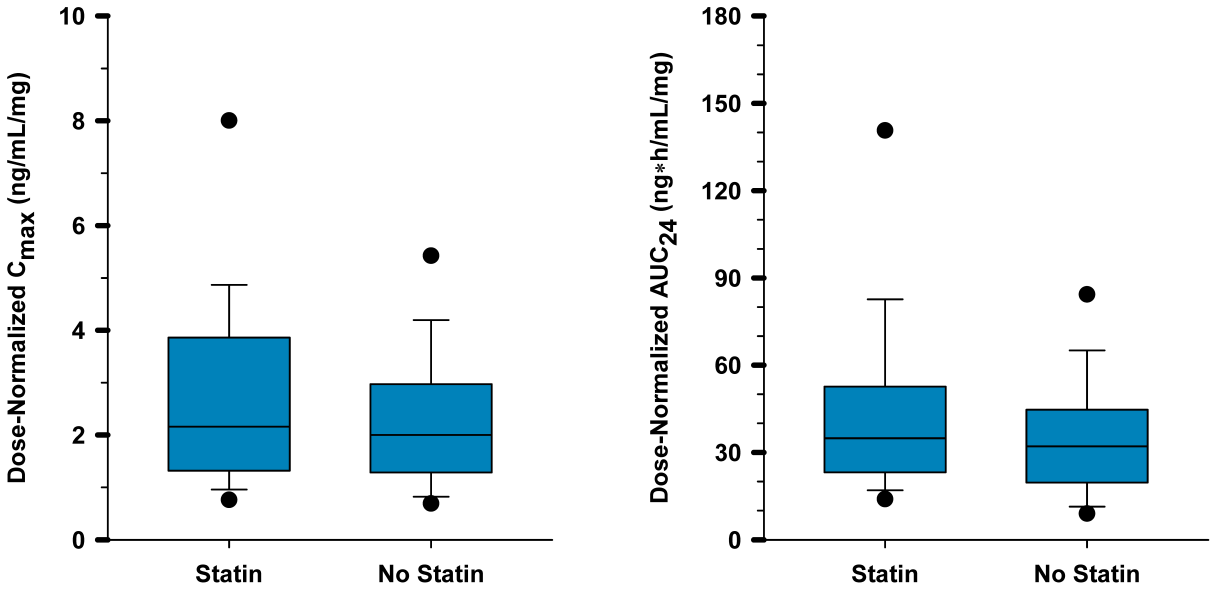
Supplementary Figure S14. **Statins increase PUMA expression through a mechanism independent of p53 in DLBCL and AML cells. A.** Western blot of DLBCL cell lines treated with 10 μM of the indicated inhibitor or vehicle (0.1% DMSO) for 24 hours. **B.** Expression of a dominant negative p53 (GSE56) in OCI-AML3 cells suppresses PUMA induction by etoposide but not by simvastatin (n=3). Significance of PUMA increase above vehicle determined by one-tailed, one-sample t-test. Significance of other comparisons determined by paired ratio t-test. **C.** GSE56 significantly reduced killing by the combination of etoposide and venetoclax but not simvastatin and venetoclax (n=3, two-tailed Student's T-Test, alpha=0.05).



Supplementary Figure S15. Statin use is associated with longer progression-free survival in CLL patients treated in venetoclax clinical trials. The Kaplan-Meier plot shows progression-free survival data as assessed by investigators, in subjects with background statin use compared to those without statin use. These data are pooled from Studies 1, 2, and 3. Hazard ratios (95% CIs) for the adjusted and unadjusted multivariate analyses of progression-free survival were 0.61 (0.38, 0.96) and 0.64 (0.41, 1.01), respectively.



Supplementary Figure S16. Response to venetoclax was enhanced in CLL clinical trials among patients who received the 400 mg statin dose. The proportion of CLL subjects in clinical studies of venetoclax monotherapy who received the 400 mg dose of venetoclax and achieved any CR is shown as the presence or absence of background statin use. Results are shown for individual studies and when pooled.



Supplementary Figure S17. Statins do not affect venetoclax PK. The plots show dose-normalized steady-state exposures of venetoclax with and without co-administration of statins. Box and whisker plots show the 5th, 25th, median, 75th and 90th percentiles; data points outside the percentiles are plotted as outliers. Tests of Cmax and AUC24 showed no significant differences (p = 0.22 and 0.16, respectively).

Sample ID	Previous therapies	Cytogenetics
2016-012	NA	46XY +12, t(14;18)(q32;q21), del(17p)
2016-014	Simvastatin	NA
2016-015	Ibrutinib	NA
2016-019	Bendamustine, rituximab ibrutinib	NA
2016-028	Rosuvastatin	NA
2016-029	Bendamustine, rituximab	del(17p), trisomy 12
2016-039	No CLL treatment or statin	IgVH mutated
2016-040	Losartan 25 mg daily No CLL treatment	Unmutated IgVH 13q del
2016-046	Atorvastatin 40 mg daily No CLL treatment	+12
2016-047	No CLL treatment or statin	FISH negative Mutated IgVH Zap70 negative
2016-048	No CLL treatment or statin	13q del
2016-049 (Same subject as 2016-014)	Simvastatin	NA

Table S1: Characteristics of CLL patient samples. NA = not available

	Statin (n=75)	No statin (n=263)	p-value
Age			
Years, median (range)	69 (54-85)	65 (29-86)	
≥65 years, n (%)	55 (73)	142 (54)	0.002
Sex, male/female (%)	79/21	67/33	0.022
Race, white/other (%)	93/7	94/6	0.732
Country, US/non-US[1] (%)	69/31	46/54	<0.001
Tobacco use, current or former, n (%)	30 (46)	92 (39)	0.252
ECOG ≥1, n (%)	46 (61)	147 (56)	0.240
Bulky disease (1 or more nodes)			
≥5 cm, n (%)	38 (51)	134 (51)	0.685
≥10 cm, n (%)	12 (16)	43 (16)	0.590
Absolute lymphocyte count			
≥25x10^9/L, n (%)	22 (29)	116 (44)	0.993
Previous lines of therapy			
Median (range)	3 (0-8)	3 (0-12)	
≥3 lines, n (%)	38 (51)	148 (56)	0.839
Fludarabine-refractory, n (%)	22 (43)	93 (45)	0.867
Previous BCRi therapy	24 (32)	63 (24)	0.106
TP53 mutation, n (%)	31 (45)	108 (52)	0.535
del(17p), n (%)	41 (57)	161 (65)	0.875

Table S2. Characteristics of CLL patients in three clinical trials of venetoclax monotherapy grouped by background statin use.

[1] Non-US Countries: Canada, UK, Germany, Poland, and Australia

Overall	Statin (n=75)	No statin (n=263)
Any AE	98.7%	98.9%
≥ Grade 3	82.7%	79.8%
Serious	57.3%	54.4%
Led to discontinuation	16.0%	18.6%
Deaths	12.0%	25.5%
AEs of Interest (any grade)		
Myalgia	6.7%	6.8%
TLS	2.7%	6.1%
Hyperuricemia	6.7%	5.3%
Hyperkalemia	16.0%	4.9%
Hyperphosphatemia	16.0%	12.9%
Hypocalcemia	6.7%	8.7%
Blood creatinine increased	12.0%	4.6%
AEs of Interest (≥grade 3)		
Myalgia	0.0%	0.4%
TLS	2.7%	6.1%
Hyperuricemia	1.3%	0.4%
Hyperkalemia	0%	1.1%
Hyperphosphatemia	2.7%	0.8%
Hypocalcemia	2.7%	1.5%
Blood creatinine increased	0%	0.8%

Most common (any grade, >20%)	Statin (n=75)	No statin (n=263)
Anemia	16.0%	29.7%
Neutropenia	45.3%	40.7%
Thrombocytopenia	14.7%	20.5%
Constipation	21.3%	14.8%
Diarrhea	49.3%	41.1%
Nausea	45.3%	40.3%
Fatigue	25.3%	31.2%
Pyrexia	12.0%	20.2%
URTI	26.7%	30.0%
Cough	20.0%	19.4%
Most common (≥grade 3, >10%)		
Anemia	5.3%	16.7%
Neutropenia	42.7%	37.3%
Thrombocytopenia	9.3%	14.8%
Neutrophil count decreased	10.7%	6.5%

Table S3. **Adverse events in CLL patients in three clinical trials of venetoclax monotherapy grouped by background statin use.** AE: adverse event. TLS: tumor lysis syndrome. URTI: upper respiratory tract infection.