SUPPLEMENTARY MATERIALS AND METHODS

Single cell fluorescent microscopy: To determine whether ibrutinib or VAY-736 altered BAFF receptor-mediated induction of NF-κB in single OSU-CLL cells, ~2.5 x 10^5 OSU-CLL cells were transduced with either the Lenti-viral Cignal-GFP-NF-κB reporter construct (transcriptional response element (5'-GGGACTTTCC -3') or Cignal negative and positive control (pCMV promoter) constructs (~25 multiplicity of infection (MOI)) (Qiagen, Valencia, CA) according to manufacturer's instructions. The tandem repeat (TRE) element has been previously reported to activate both classical and alternative NF-κB signaling pathways by its ability to bind both p65 and p52[52]. Transduction efficiency was determined after 48 hours (~10% by fluorescence microscopy (488 nm excitation). Expired cells were excluded using 7-AAD (BD Biosciences, San Jose, CA) (561 nm excitation). After 48 hours, the cells were washed twice with PBS and re-suspended in complete RPMI medium containing 10% FBS (Atlas Biologicals, Fort Collins, CO) and 56 U/ml penicillin (Gibco), 56 µg/ml streptomycin (Gibco) and 2 mM L-glutamine (Gibco) at 1 x 10^6 cells/ml and incubated at 37°C and 5% CO_2 under ibrutinib treatment (1 uM) or DMSO for 30 minutes. The cells were washed twice with RPMI 1640 medium (Gibco) and re-suspended at ~1.0 x 10^5 cells/ml with complete RPMI 1640 in the presence of ~1.5% FBS and pretreated with VAY-736 (10 µg/ml) for 15 minutes prior to the addition of soluble BAFF (500 ng/ml) and incubated for 16 hours. After incubation, cells were plated onto 8-well imaging plates (Thermo Fisher, Waltham, MA) that had been pretreated for 5 minutes with 0.01% poly-L-lysine (Trevigen) followed by 7-AAD (1:100) (BD Biosciences) addition for viability exclusion. Cells were visualized via epifluorescence microscopy (Nikon, Eclipse Ti2) under 60X magnification where 488 nm light excitation was employed for GFP-NF-κB activity. Bright field and 561 nm excitation for 7-AAD was used to determine viable OSU-CLL cells. The level of GFP-NF-κB dependent fluorescence within single OSU-CLL cells was measured via Nikon Elements software. To identify transduced single OSU-CLL cells, relative fluorescent units (RFUs) were measured among single cells treated with the negative control GFP reporter construct for each treatment group. Similar to a flow cytometry gate, the top 5% of RFU values among the single cell population treated with the negative control GFP reporter construct were considered auto-fluorescence. The top RFU value of the remaining single cell population was used to subtract auto fluorescence among single cell populations transduced with the Cignal-GFP- NF-κB reporter construct across each treatment group. RFUs from single OSU-CLL cells containing at least 3 orders of magnitude were considered transduced. The number of cells transduced was normalized across treatment groups and expressed

on a per cell basis. The data are expressed as the mean RFU Fold Change ± SEM relative to approximately 100s of untreated cells from single OSU-CLL cells. For cell surface staining experiments, PE-VAY-736 and PE-species-/isotype-matched antibodies were added to OSU-CLL cells cultured at 1.0 x 10^6 cells/ml in 1X PBS. The cells were washed once and re-suspended in clear RPMI 1640 and visualized under 561 nm excitation via fluorescent microscopy as described above and visualized under 100X magnification. Live cell imaging time lapse experiments were performed using a stage-top incubator and objective heating system (Okolab, Naples, Italy) under a constant 37°C temperature and 5% CO_2 in a humidified environment. Images were acquired at 10-minute intervals over a 14-hour time course under 40X or 60X magnification. Background fluorescent RFUs were measured at each time point and were used to subtract auto fluorescence from single OSU-CLL cells. The data are shown as the mean RFU Fold Change ± SEM relative to each single cell at time zero and represents at least three independent experiments.

Detailed animal studies: For *in vivo* efficacy of VAY-736, Eμ-TCL1 mice were subjected to submandibular weekly bleeds and enrolled into randomized treatment groups of VAY-736 (100 mg/kg) or vehicle (PBS) after blood levels reached high leukemic burden (> 60% $CD45^+$ $CD5^+$ $CD19^+$). VAY-736 or vehicle, sterile PBS, retro-orbital injections continued once per week for two weeks and blood was obtained via submandibular vein 24 hours after injection. Weekly bleeds were performed in order to track disease progression for a maximum of 100 days.

For other efficacy studies we utilized a leukemia adoptive transfer murine model to determine *in vivo* survival advantage of VAY-736 and the efficacy of VAY-736 combined with ibrutinib. Spleens from a moribund, leukemic B6/TCL-1 donor with splenomegaly were harvested, disassociated, and purified for B-lymphocytes by Ficoll-Plaque density gradient and re-suspended in sterile phosphate-buffered saline for intravenous lateral tail vein injection of 200 μL containing 1 x 10^7 cells. “C.B17 SCID” female mice were purchased from Taconic Farms, Inc., (Hudson, NY). These mice are homozygous for the Prkdcscid spontaneous mutation (referred to as SCID) but have functional NK cells. The animals were randomly assigned to the following treatment groups (*n* = 13-15 per group): PBS injection (vehicle), VAY-736 injection, ibrutinib (Acorn PharmaTech LLC) drinking water (in 10% β-cyclodextrin, at 0.16 mg/mL, via water bottle), or combination VAY-736 injection with ibrutinib drinking water. Leukemia onset was defined when peripheral blood $CD45^+$ cells > 20% $CD5^+$ $CD19^+$ leukemic B-cells or if splenomegaly was determined by palpitation. Disease was monitored weekly by flow cytometric analysis of

leukemia populations in the blood, spleen palpitation, white blood cell counts, and weight. Study mice were euthanized by CO_2 upon reaching IACUC early removal criteria (i.e. weight loss > 20%, labored breathing, hunched posture). Blood, spleen, and bone marrow were harvested from euthanized mice and, if possible, from mice that died, to determine if leukemia was the cause of death. Criteria for leukemic death was defined as > 10% $CD5^+$ $CD19^+$ cells of the $CD45^+$ population in one of the three tissues analyzed.

PE-Antibody conjugation and Flow Cytometry immune cell sub-population panel: In order to characterize anti-BAFF-R (11C1), VAY-736, and Obinutuzumab (OBN) binding to major immune cell sub-populations in non-tumor cells, anti-BAFF-R (11C1) was purchased from BD Biosciences, while VAY-736 and OBN were labeled with PE using the Lightning-Link Antibody Labeling Kit (Novus Biologicals, Centennial, CO) according to manufacturer's instructions. Peripheral blood mononuclear cells (PBMCs) from a normal donor were used to titrate the PE-conjugated antibodies where PBMCs were incubated with varying amounts of PE-anti-BAFF-R (11C1), PE-VAY-736, and PE-OBN (10, 5, 2.5, 1.25, 0.62µg/ml) on ice for 30 minutes along with AlexaFluor 700-anti-CD19 (HIB19) (BD Biosciences). Antibody binding on the CD19+ B cell population was determined via flow cytometry (method described above using a BD LSRFortessa™ flow cytometer, BD Biosciences) where VAY-736 demonstrated higher binding affinity at equal antibody concentrations to CD19+ B cells relative to anti-BAFF-R (11C1) and OBN **(Fig. S3)**. To evaluate PE-anti-BAFF-R (11C1), PE-VAY-736, and PE-OBN binding to major immune cell sub-populations (CD3+, CD19+, CD56+, CD14+, CD11b+, and CD11c+ cells), PBMCs from normal donors (n = 3) were incubated in the absence or presence of FcR Block (Miltenyi Biotech, Somerville, MA) pre-treatment for 15 minutes followed by PE-conjugated anti-BAFF-R (11C1), VAY-736, OBN (20µg/ml) and the following antibodies to label the major immune cell subpopulations: BV786-anti-CD3 (SK7), AlexaFluor 700-anti-CD19 (HIB19) (BD Biosciences), PE-Vio 770-anti-NK1.1 (CD56) (AF12-7H3) (Miltenyi Biotech), PerCP-anti-CD14 (M(phi)P9), V450-anti-CD11b (ICRF44), APC-anti-CD11c (B-ly6), BUV395-anti-CD45 (HI30) (BD Biosciences), and Aqua LIVE-DEAD viability stain (ThermoFisher) and analyzed via flow cytometry on a BD LSRFortessa™ flow cytometer **(Fig. S4)**.

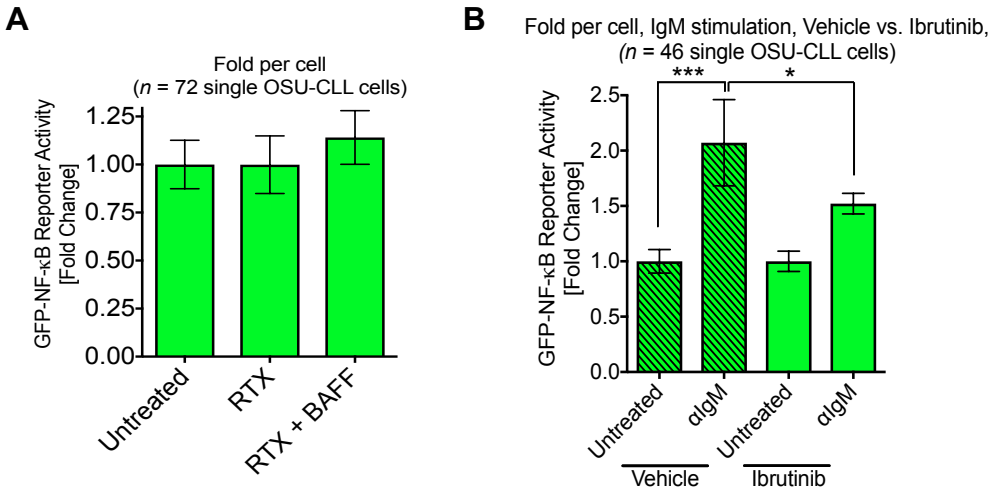

Figure S1: OSU-CLL cells transduced with NF-κB reporter.
(A) Transduced OSU-CLL cells were either pre-treated with rituximab (RTX) (10μg/mL) followed by BAFF addition or RTX alone. (B) OSU-CLL cells were transduced with either the Cignal Negative Control (neg ctrl construct) or GFP-NF-κB lentiviral vector. Cells were pre-treated with DMSO or 1 uM ibrutinib for 30 minutes followed by αIGM stimulation for 16 hours.

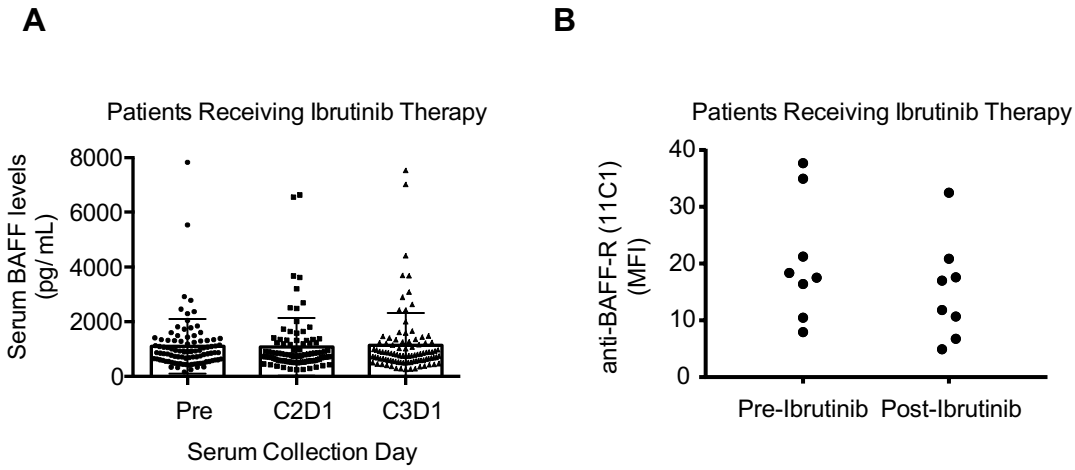

Figure S2: CLL patients increase BAFF serum levels following ibrutinib therapy.
(A) Serum from CLL patients on ibrutinib therapy was collected at the indicated time points (Pre = Cycle 1 Day 1, prior to ibrutinib therapy; C2D1 = Cycle 2 Day 1; C3D1 = Cycle 3 Day 1) and ELISA was used to determine BAFF levels *(n* = 48 CLL patients). (B) FACS analysis of PBMCs from CLL patients collected pre-ibrutinib therapy and 8-12 months post-ibrutinib therapy for surface BAFF-R (MFI) expression on CD19+ cells *(n* = 8 CLL patients).

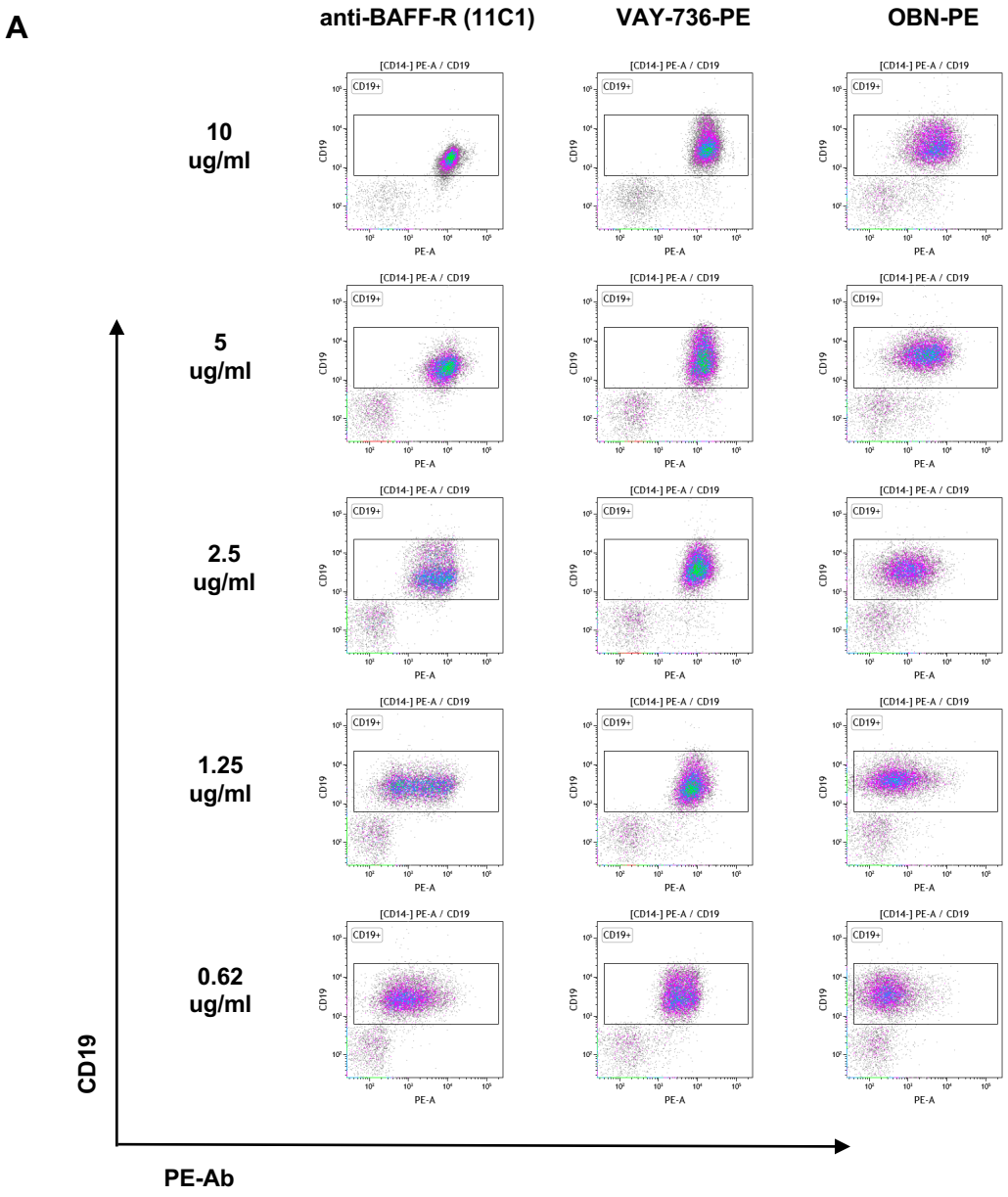

Figure S3: VAY-736 has superior binding to CD19+ cells compared to Obinutuzumab and commercially available anti-BAFF-R (11C1) antibody.
(A) Titration of anti-BAFF-R (11C1), PE labeled VAY-736, and PE labeled Obinutuzumab on CLL PBMCs demonstrating strong VAY-736 binding even at low antibody concentrations.

A

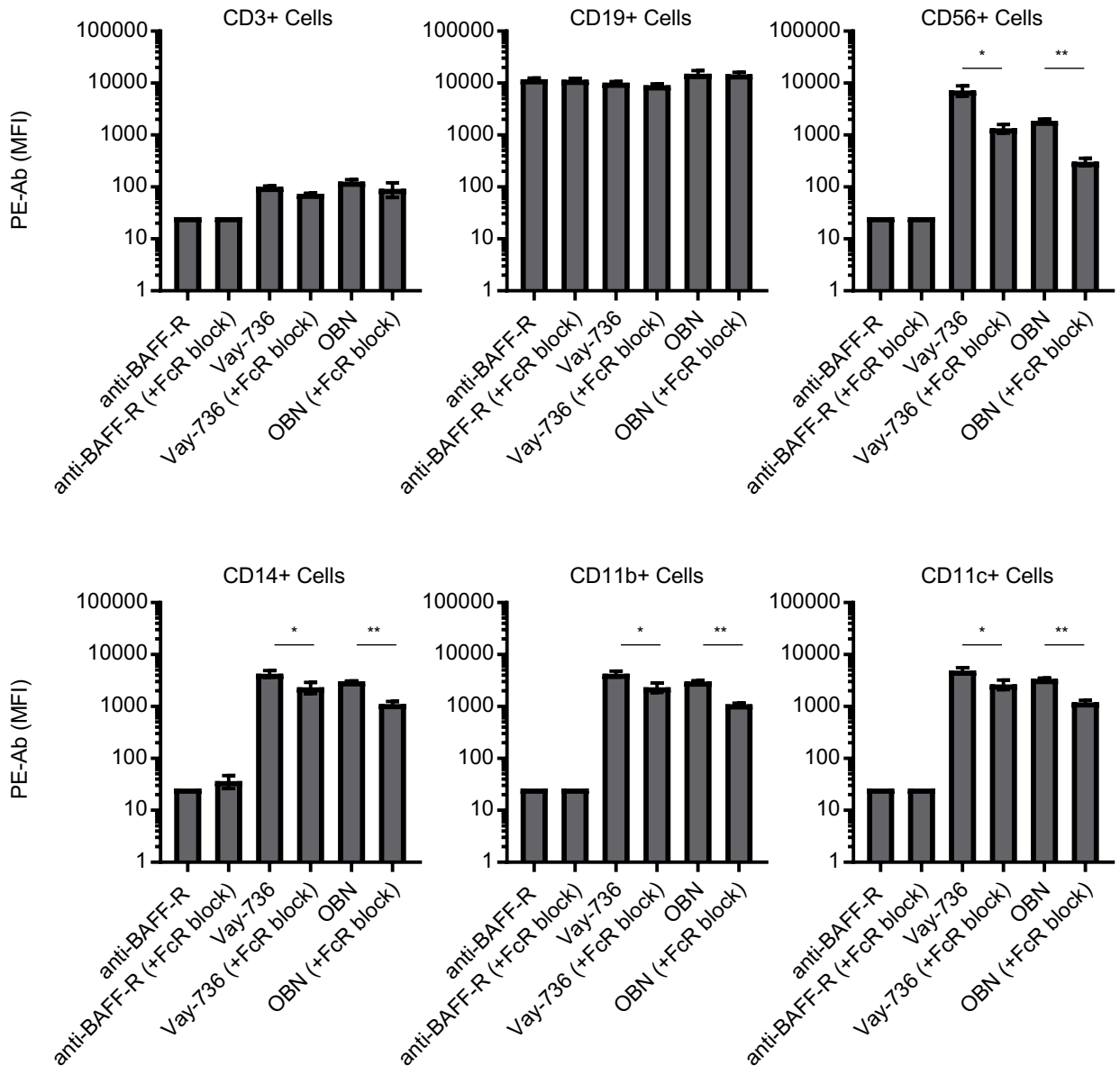

Figure S4: VAY-736, Obinutuzumab, and anti-BAFF-R (11C1) immune cell subset binding characterization.

(A) PE labeled VAY-736, PE labeled OBN, or PE anti-BAFF-R (11C1) along with antibodies defining immune cell subsets were incubated with normal donor peripheral blood with and without FcR block). Immune cell subsets were analyzed for antibody binding by median fluorescence intensity (MFI). Samples with and without FcR block were compared using paired two-sample T-test *(n* = 3).

A

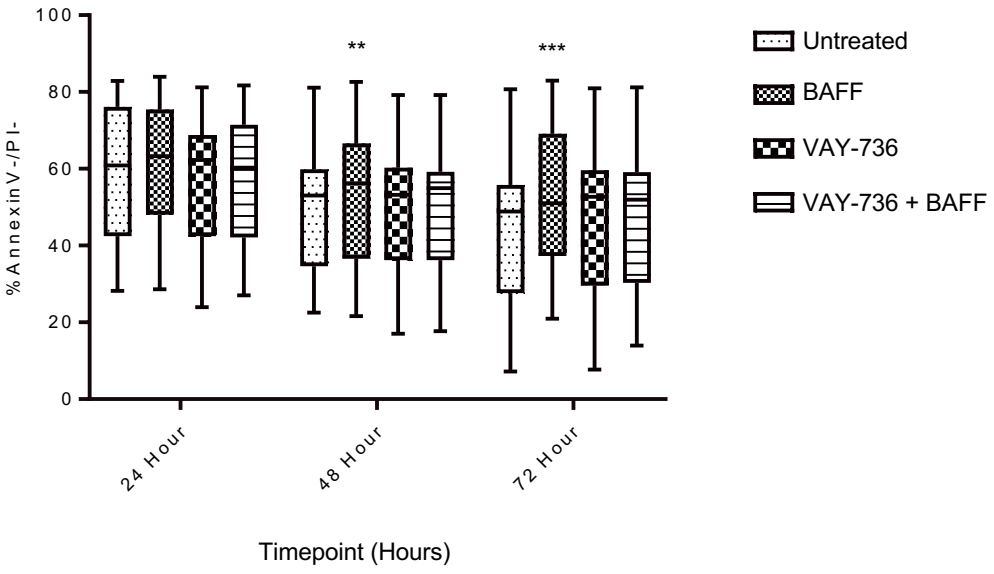

Figure S5: Raw AnnexinV/PI viability data for Figure 2B.
(A) Time course comparison of primary CLL cells' viability at 24, 48, and 72 hours treated with BAFF (500 ng/mL), VAY-736, or cells pre-treated with VAY-736 subsequently stimulated with BAFF ($p < 0.001$: 72 hours, BAFF vs. Untreated; $p < 0.01$: 48 hours, VAY-736 + BAFF vs. BAFF). Inhibitory effect of VAY-736 at each time point was tested by interaction contrast (BAFF + VAY-736 subtracting BAFF vs. VAY-736 subtracting Untreated; 72 hours, $p < 0.01$). Data were analyzed by mixed effect model and Holm's method was used to adjust multiplicity ($n = 21$ patients).

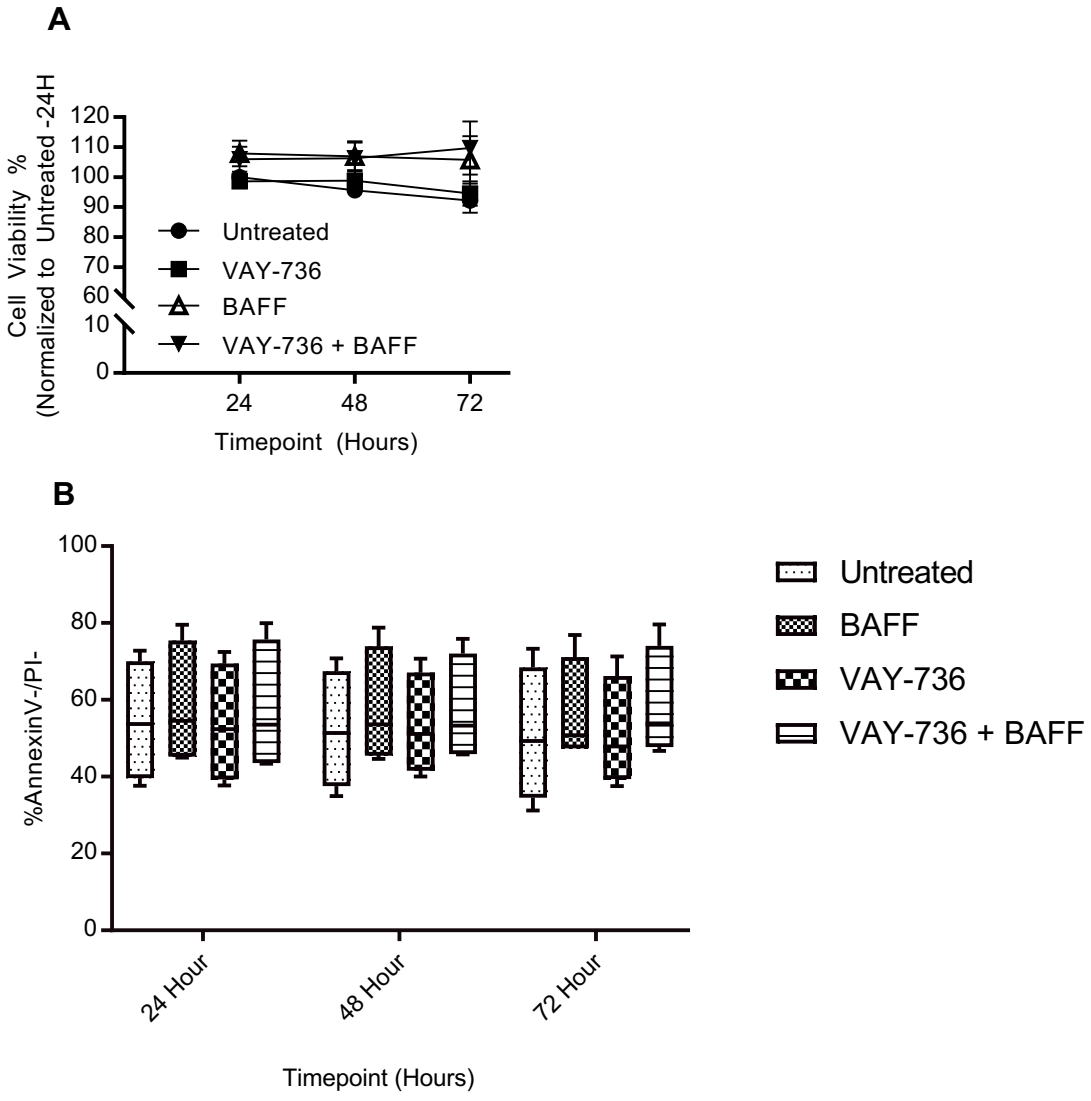

Figure S6: Time course comparison of primary TCL1 splenocytes.
AnnexinV/PI viability was taken at 24, 48, and 72 hours following treatment with BAFF (500 ng/mL), VAY-736, or pre-treatment with VAY-736 and subsequently stimulated with BAFF (*n* = 4).
(A) Normalized data showing protective effect of BAFF but minimal blocking by VAY-736. (B) Raw data representation.