MTS cell viability assays

The relative viable cell number was determined using a Cytation-3 Multi-Detection Microplate reader (Bio-TEK). Briefly, 30,000 cells were seeded in each well of 96-well plates in 100 µL of tissue culture medium. Cells were treated with varying concentrations of bortezomib, ixazomib or vehicle for 24 or 48 hours. CellTiter reagent (20 µl/well) was added and plates incubated for 1 - 3 hours. All samples were assayed in triplicate and each experiment was repeated at least twice. EC50 values for bortezomib and ixazomib were calculated using nonlinear regression in GraphPad Prism (v. 7.0).

Quantitative real-time PCR

Briefly, total RNA was extracted using TRIZOL reagent (Invitrogen) and reverse transcription was performed using an iScript cDNA synthesis kit (Bio-Rad). For qPCR, 100 ng of RNA template and 0.5 µl of pre-validated primePCR SYBR Green TNFSF10 (TRAIL) human primer with iTaq Universal SYBR Green Supermix (Bio-Rad) were combined in 20 µl of reaction volume. All reactions were performed on TRAIL mRNA level was normalized to the housekeeping genes B2M and HPRT1 (primePCR assays, Bio-Rad). Fold increase was calculated for the normalized target gene expression of the bortezomib- or ixazomib-treated samples relative to samples treated with DMSO vehicle set as 1.

Western blot analysis

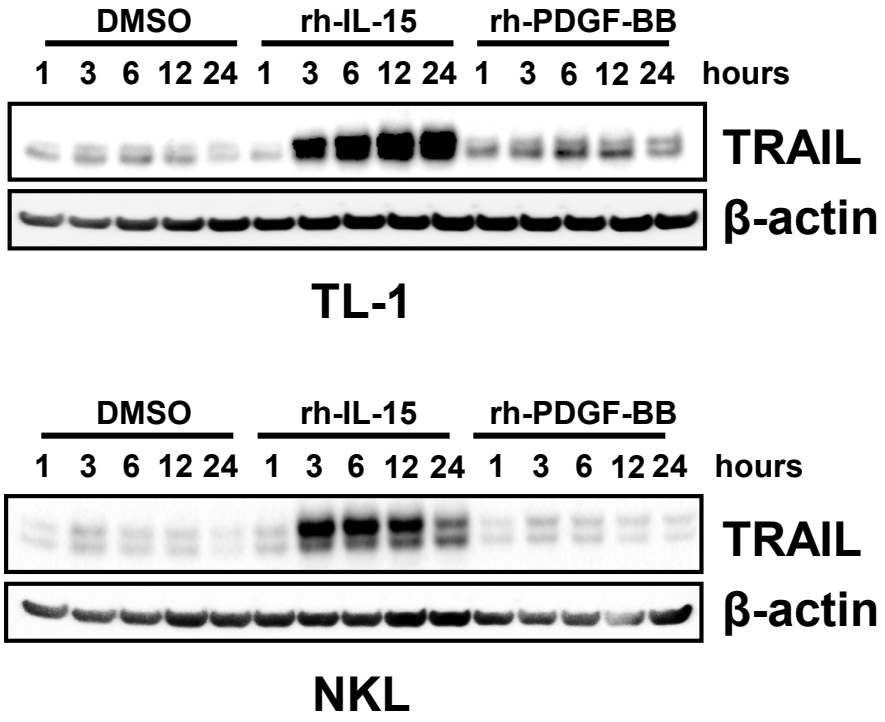
Briefly, whole cell lysates were harvested using 1X RIPA buffer, centrifuged at 17,000 xg for 15 minutes at 4˚C to pellet cell debris and protein concentrations were determined by BCA assay (Pierce). Equal amounts of protein were loaded onto Bolt 4-12% Bis-Tris gels (Life Technologies), separated by electrophoresis and transferred to PVDF membranes using a Trans-Blot Turbo RTA transfer kit (Bio-Rad). Membranes were blocked in 5% BSA/TBS-Tween buffer and then incubated overnight at 4˚C with primary antibody. Immunodetection was performed using ECL reagent (Bio-Rad) on a ChemiDOC XP (Bio-Rad) after incubation with appropriate IgG HRP-linked secondary antibodies (1:3000) (Cell Signaling).

Supplemental Figure 1: IL-15, but not PDGF-BB, induces TRAIL expression in TL-1 and NKL cells. TL-1 (top) or NKL (bottom) cells were treated with DMSO, rh-IL-15 (5 IU / mL) or rh-PDGF-BB (50 ng / mL) for the indicated times and assessed for TRAIL protein expression. Total protein samples were harvested as indicated. TRAIL protein levels were determined by immunoblotting assay. Equal loading for was confirmed by probing with β-actin antibody.

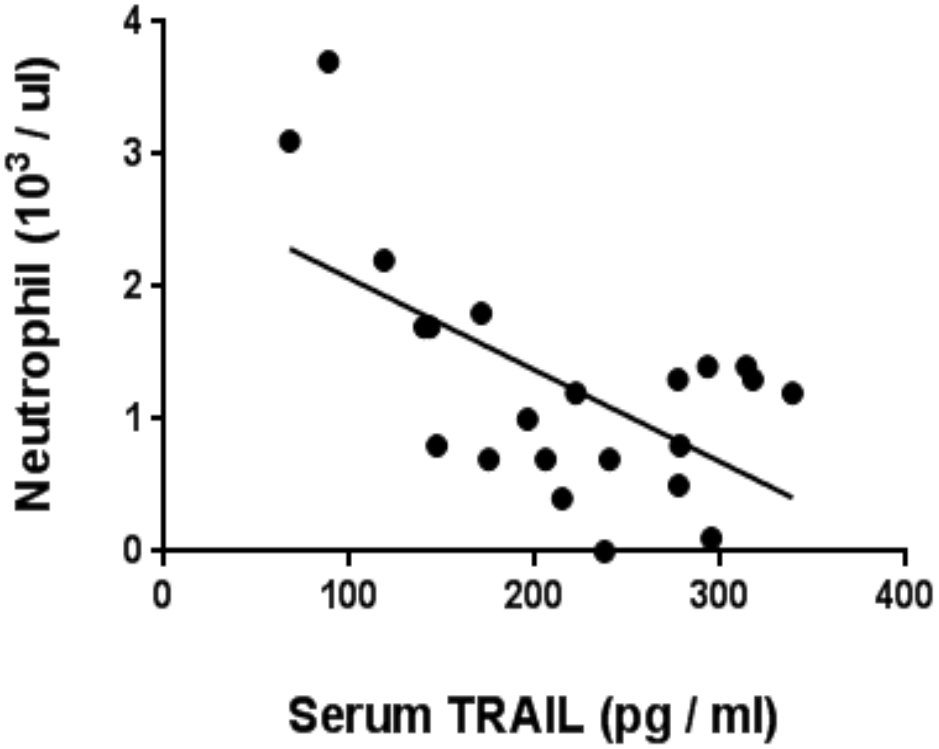
Supplemental Figure 2: TRAIL serum levels are negatively correlated with neutrophil counts. TRAIL serum levels exhibit a statistically significant ($p = 0.0028$) reverse correlation with neutrophil count in peripheral blood samples from patients with T-LGL leukemia (n = 22).

Supplemental Figure 3: Activation leads to increased TRAIL expression in PBMC and CD8+ cells. TRAIL gene expression in GSE10631 Affymetrix dataset. The TRAIL expression levels between PBMC (n = 4) and activated PBMCs (AcPBMC) (n = 3) and $CD8^+$ cells (n = 4) and activated $CD8^+$ cells ($AcCD8^+$) (n = 3) isolated from normal donors were compared. Mean $\pm$ SEM shown for PBMC versus AcPBMC and $CD8^+$ versus $AcCD8^+$ cells. *, $p < 0.001$ (ANOVA)

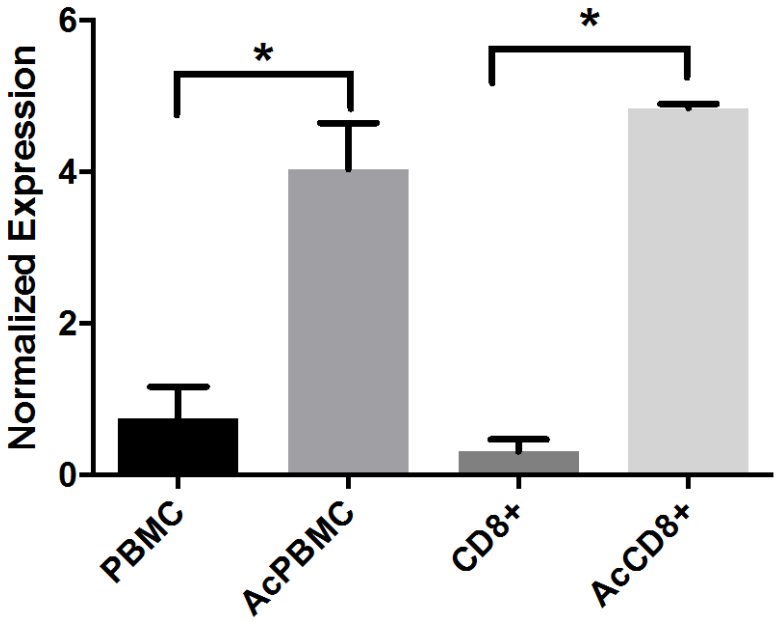
Supplemental Figure 4: Proteasome inhibitors increase apoptosis in PBMC from patients with T- and NK-LGL leukemia independent of STAT3 mutational status. A) PBMC from normal donors (n = 9), T-LGL patients with STAT3 WT (n = 10), STAT3 Y640F mutation (n = 4), STAT3 D661Y mutation (n = 4) and NK-LGL patients with STAT3 WT (n = 3), STAT3 Y640F mutation (n = 1), STAT3 D661Y mutation (n = 2) were treated with DMSO or Bortezomib (2.5 or 5 nM) for 48 hours, cells were stained for apoptosis with Annexin-V and 7-AAD and analyzed by flow cytometry (upper panel). **B)** PBMC from normal donors (n = 9), T-LGL patients with STAT3 WT (n = 10), STAT3 Y640F mutation (n = 4), STAT3 D661Y mutation (n = 4) and NK-LGL patients with STAT3 WT (n = 3), STAT3 Y640F mutation (n = 1), STAT3 D661Y mutation (n = 2) were treated with DMSO or ixazomib (100 or 200 nM) for 48 hours, cells were stained for apoptosis with Annexin-V and 7-AAD and analyzed by flow cytometry (bottom panel).



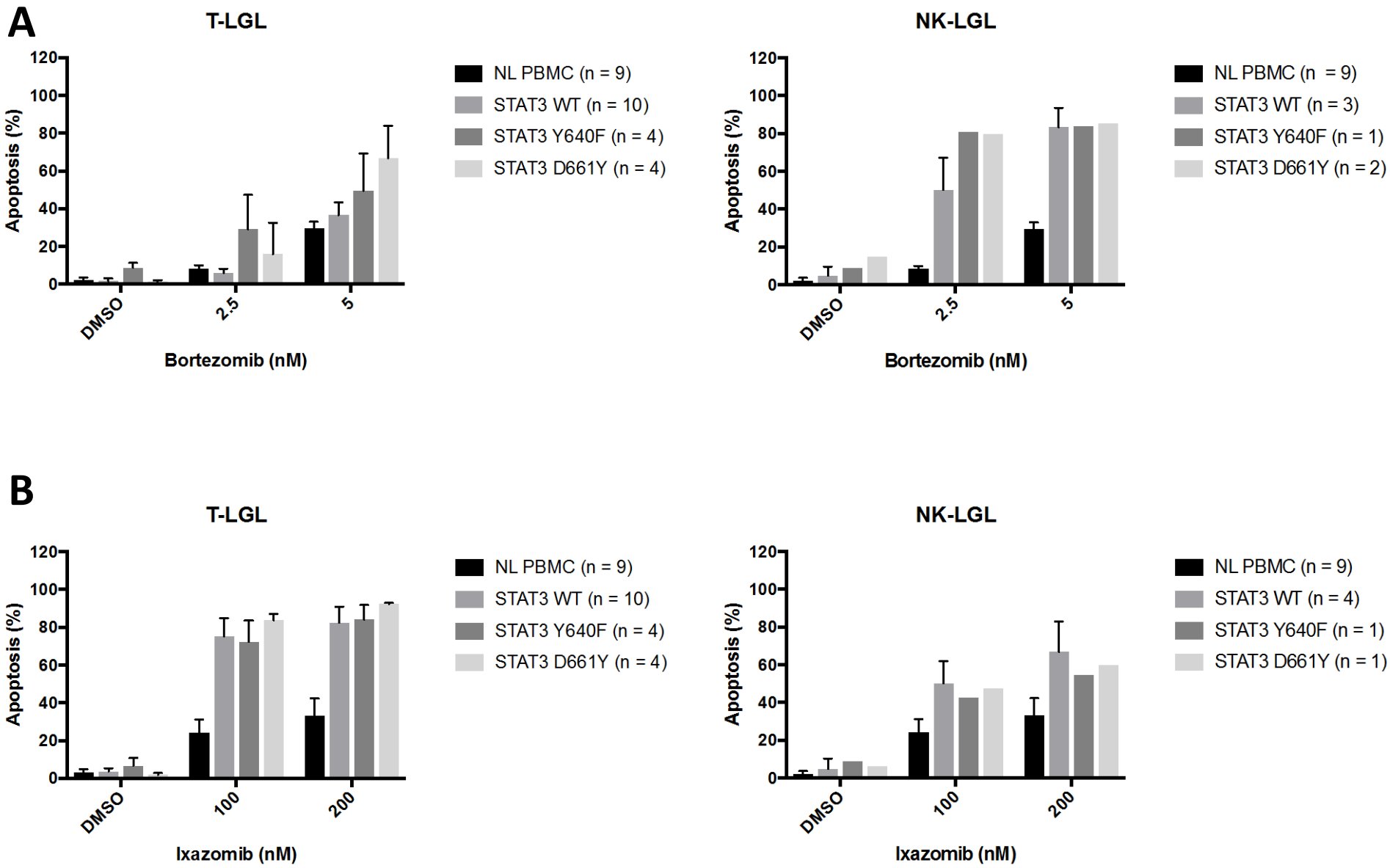
Supplemental Figure 1: IL-15, but not PDGF-BB, induces TRAIL expression in TL-1 and NKL cells.



Supplemental Figure 2: TRAIL serum levels are negatively correlated with neutrophil counts.



Supplemental Figure 3: Activation leads to increased TRAIL expression in PBMC and CD8+ cells



Supplemental Figure 4: Proteasome inhibitors increase apoptosis in PBMC from patients with T- and NK-LGL leukemia independent of STAT3 mutational status