Supplemental Figure 4. Impact of JAK2/mTOR blockade on human NK cell function and proliferation.



Human NK cells were isolated by magnetic bead purification (CD3<sup>neg</sup>, CD56<sup>+</sup>) from peripheral blood and cultured with K562 target cells at varying NK cell:K562 ratios. A) Graph shows the cytolytic activity (±SEM) of NK cells after 4 hours of culture with K562 target cells, while exposed to DMSO, ruxolitinib (1 $\mu$ M), pacritinib (1.25 $\mu$ M), sirolimus 10ng/ml, or a combination of pacritinib plus sirolimus. K562 lysis was measured using a colorimetric assay. 1 representative experiment of 2 is shown. B) Human NK cells (10<sup>5</sup>) were stimulated with a cytokine cocktail of recombinant human (rh) IL-2 (200 IU/ml) and rhIL-15 (10 ng/ml), in the presence of DMSO, ruxolitinib, pacritinib, sirolimus, or pacritinib plus sirolimus. Drugs were added once on day 0. Cytokines were replenished on day +3. Bar graph shows NK cell proliferation (±SEM) on day +5 of the culture using a colorimetric assay. n=3 independent experiments. ANOVA A and B. \**P*<0.05, \*\**P*=0.01-0.001, \*\*\**P*=0.001-0.0001, \*\*\**P*<0.0001.