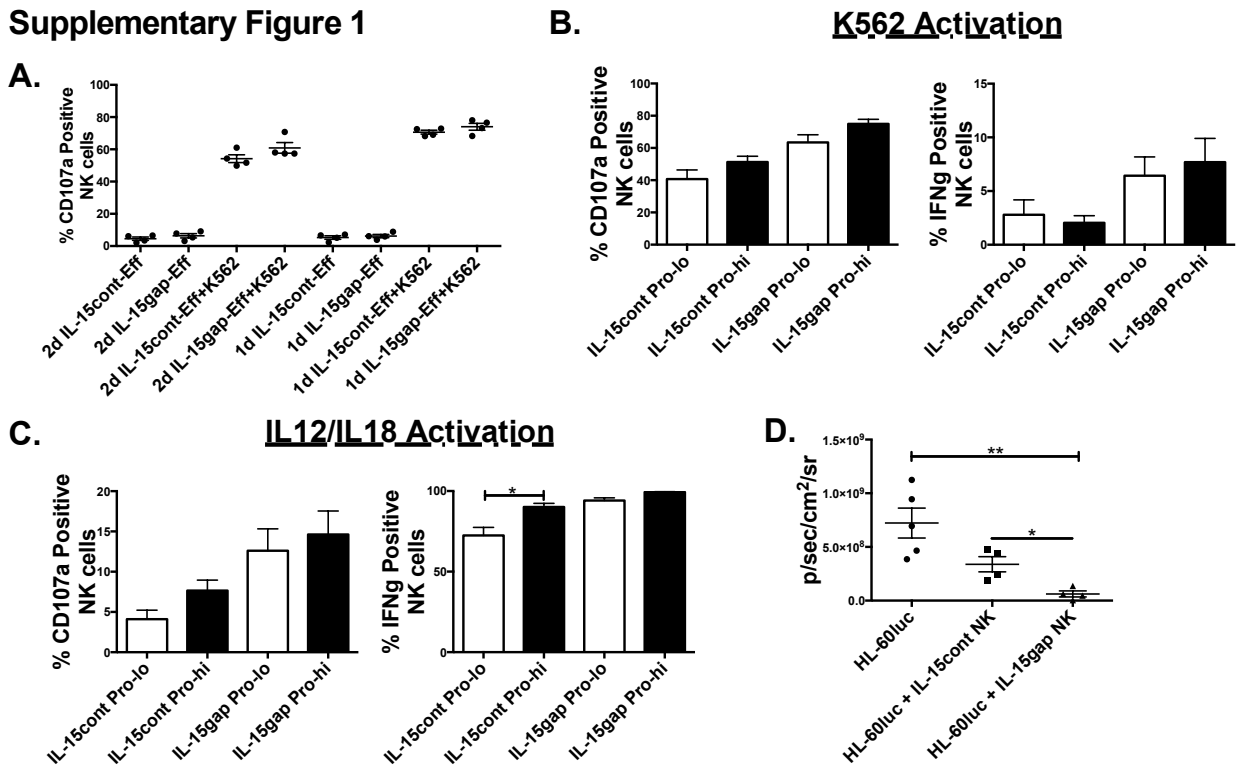
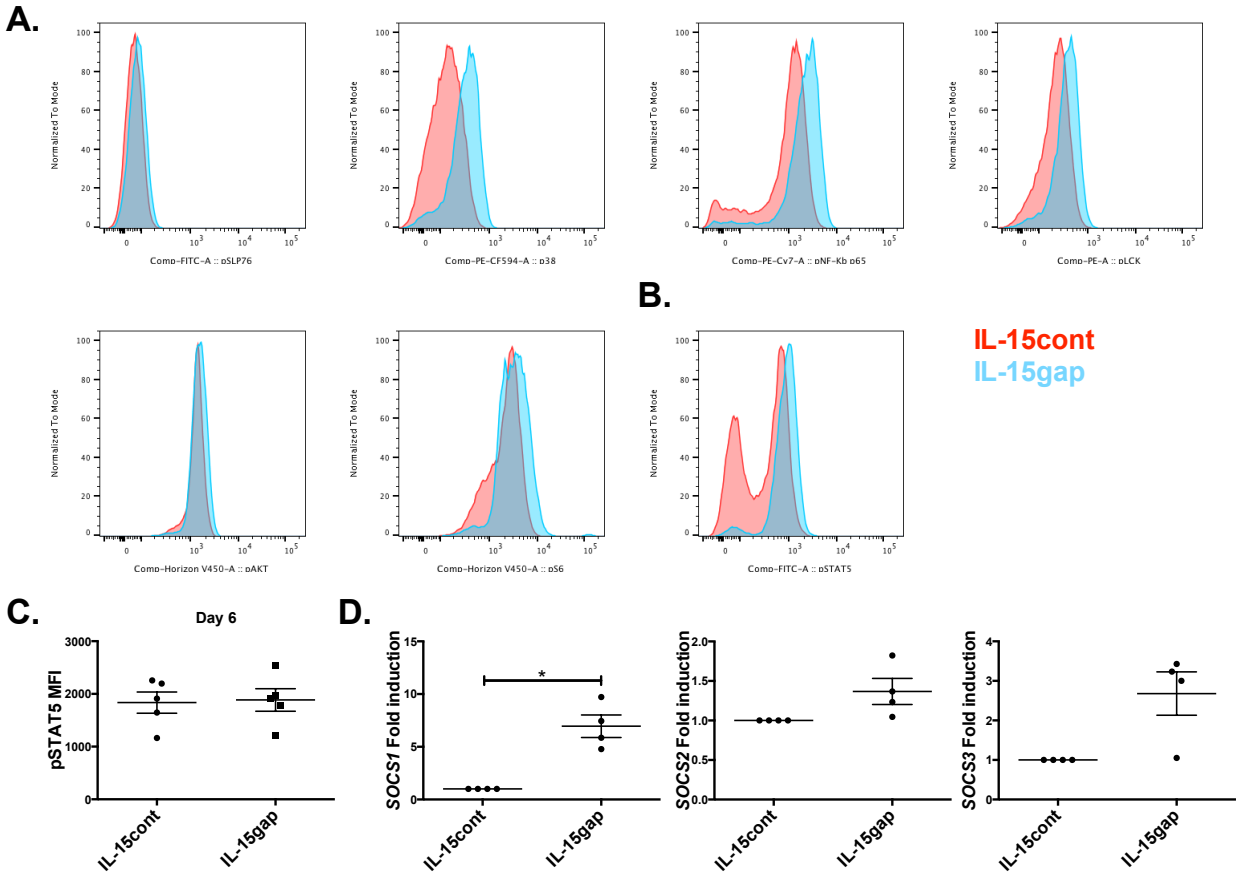
Supplementary Figure 1



Supplementary Figure 1.

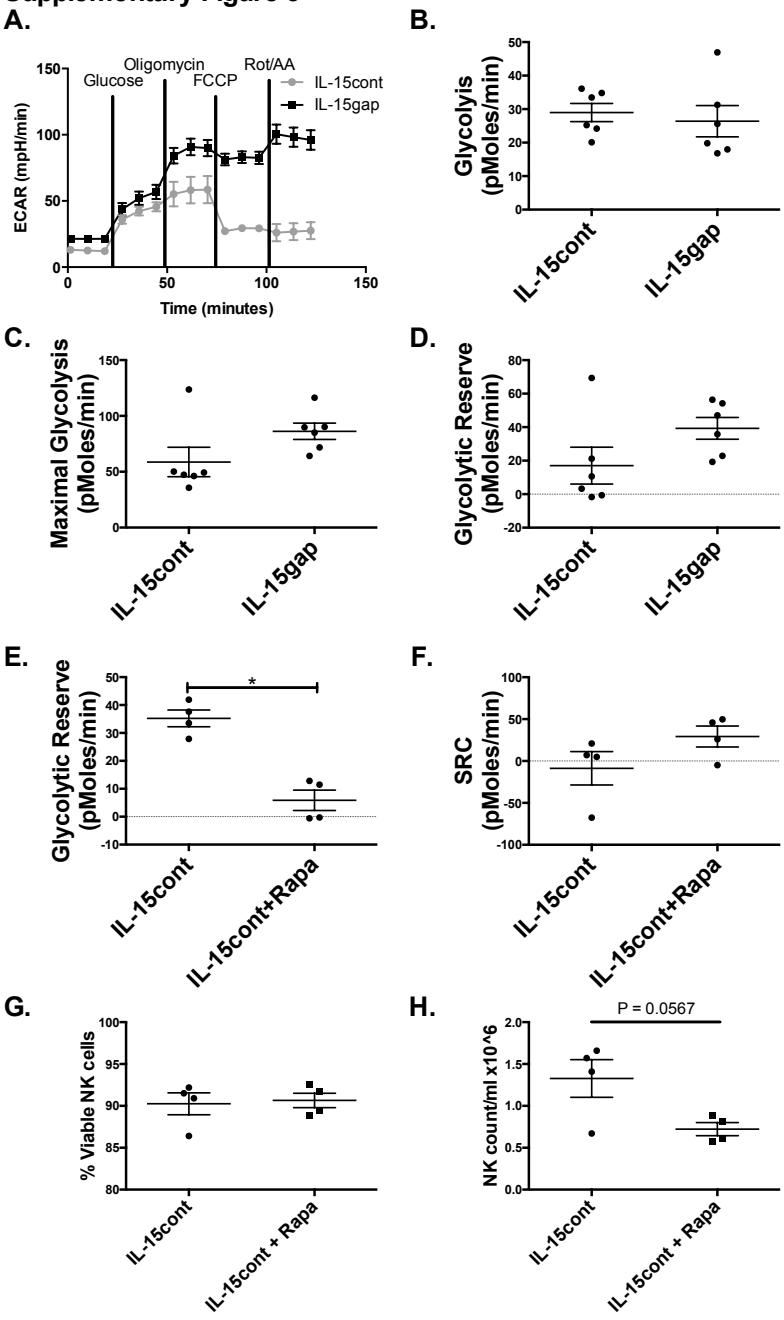
(A) Enriched NK cells were placed in 9-day cultures in which the media was changed every second day by addition of fresh media with IL-15 (2d IL-15cont) or by alternation with addition of media alone every other cycle (2d IL-15gap). The same setup was used but with changes of media (with or without IL-15) every other cycle (1d IL-15cont and 1d IL-15gap). At the end of the 9-day culture NK cell degranulation was assessed by surface expression of CD107a via flow cytometry (n = 4). (B-C) NK cells were labeled with CellTrace Dye (Molecular Probes) prior to entering culture and assessed at day 9 for function (B) against K562 Targets (2:1 E:T ratio) for 4 hours or (C) after overnight stimulation with IL-12 (10 ng/ml) and IL-18 (100 ng/ml). For the analysis the NK cells were gated on cells that proliferated and thus diluted Dye (Pro-hi (black bars)) or on cells that did not proliferate and thus did not dilute Dye (Pro-lo (white bars)). Percent $CD107a^{+}$ (left) and $IFN\gamma^{+}$ (right) NK cells noted (n = 4). Paired t test was utilized to do all internal comparisons (A and B). (D) Pooled mouse Luminescence data (p/sec/cm^2/sr) displaying differences in HL-60luc tumor load at day 14 after for mice engrafted with tumor alone (n = 5), tumor plus IL-15cont NK cells (n = 4), or tumor plus IL-15gap NK cells (n = 4). One-way ANOVA (bracket) or unpaired t test (line) was used to evaluate significance.

Supplementary Figure 2



Supplementary Figure 2.
Representative histogram plots of the indicated phospho-protein expression on IL-15cont (red) or IL-15gap (blue) NK cells activated with (A) PMA/Ionomycin or (B) recombinant IL-15. (C) NK cells were incubated in IL-15cont and IL-15gap cultures for only 6 days (first two cycles) and then intracellular STAT5 phospho-protein expression was analyzed after addition of IL-15 (n = 5). (D) Cells were cultured in IL-15cont and IL-15gap conditions for 9 days and *SOCS1-3* fold induction of the mRNA was assessed after normalization to *GAPDH* (n = 4).

Supplementary Figure 3



Supplementary Figure 3.
(A) NK cells were treated as described and harvested at day 9. Cells were immobilized on plates and the Extracellular acidification rate (ECAR) was measured (pmoles/min) real time in an XFe24 analyzer after injection of glucose, Oligomycin, FCCP plus sodium pyruvate, and Rotenone/Antimycin A (n = 6). Shown is (B) glycolysis, (C) maximal glycolysis, and (D) glycolytic reserve derived from (A) (n = 6). (E) Glycolytic reserve and (F) Spare respiratory capacity (SRC) was measured after 9 days of treatment with IL-15cont and IL-15cont plus rapamycin conditions. (G) NK cell Viability and (H) count/ml was evaluated by microscopy after 9 days of treatment with IL-15cont and IL-15cont plus rapamycin conditions (n = 4). Paired t tests were used for all comparisons.