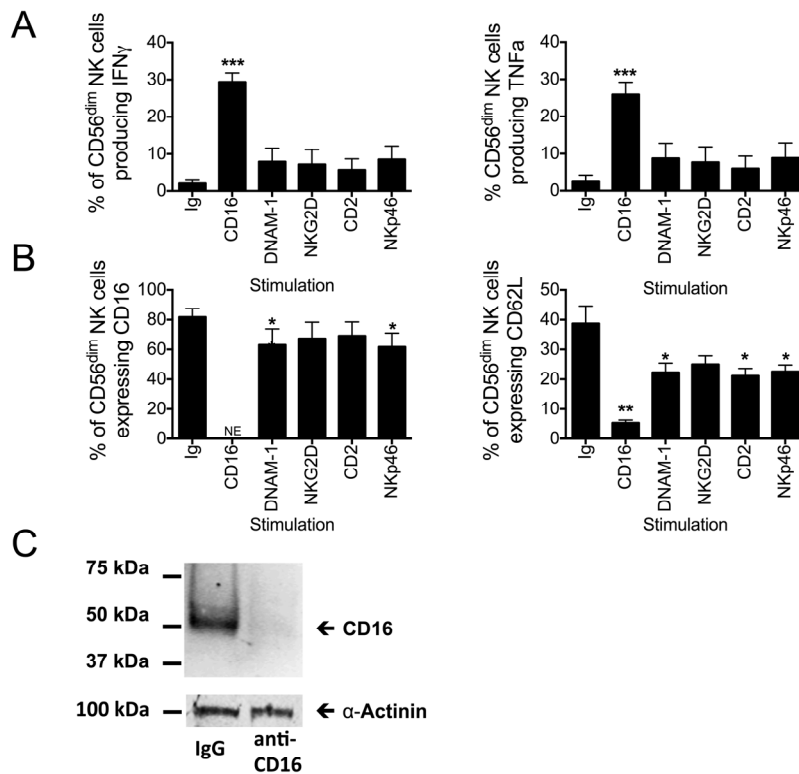


Supplemental Figure 1

**Supplemental Figure 1. IL-12 and IL-18 are both required to sufficiently activate NK cells resulting in decreased CD16 and CD62L expression.**

Purified NK cells (n=6) were incubated in either media alone, IL-12 and IL-18, IL-12 alone or IL-18 alone. CD16 and CD62L surface expression and CD69 as a marker of activation was measured on CD56<sup>dim</sup> NK cells. Bars represent the mean  $\pm$  SEM. CD69 MFI and the percentage of NK cells expressing CD16 or CD62L following cytokine treatment were compared to cells treated with media alone using the paired T test. Statistical significance is indicated as \*P $\leq$ 0.05; \*\*P<0.01. The percentage of CD56<sup>dim</sup> NK cells expressing CD16 was plotted against CD69 to determine if activation correlated with decreases in CD16 expression. The estimated regression line is shown with the r value and significance based on the Pearson correlation coefficient. Significance is calculated as \*P<0.05.



Supplemental Figure 2

**Supplemental Figure 2. Cross-linking with single activating receptors results in decreases in CD16 and CD62L expression that is not a result of CD16 internalization following CD16 activation.**

Purified NK cells (n=6) were cross-linked with plates adsorbed with IgG or antibodies to CD16, DNAM-1, NKG2D, CD2 and NKp46. Intracellular IFN $\gamma$  (left panel-A), TNF $\alpha$  production (right panel-A) and surface expression of CD16 (left panel-B) and CD62L (right panel-B) were measured after 5 hours (NE= not evaluative). Bars represent the mean  $\pm$  SEM. Cytokine production and percentage of CD16 and CD62L expression were compared to the isotype control using the paired T test. Statistical significance is indicated as \*P $\leq$ 0.05, \*\*P $\leq$ 0.01; \*\*\*P $\leq$ 0.001. (C) Representative immunoblot of CD16 protein and loading control. Purified NK cells (n=4) were cross-linked with plates adsorbed with IgG or anti-CD16 for 5 hours. Cells were then washed and lysed. CD16 protein was measured by Western blot.

## Supplemental Method

### **Western Blot**

Western blotting was performed as previously described<sup>24</sup>. Human CD16 was detected by the mAb DJ130c (Santa Cruz Biotech) and alpha-actinin was detected as a loading control using the mAb AT6/172 (EMD Millipore).