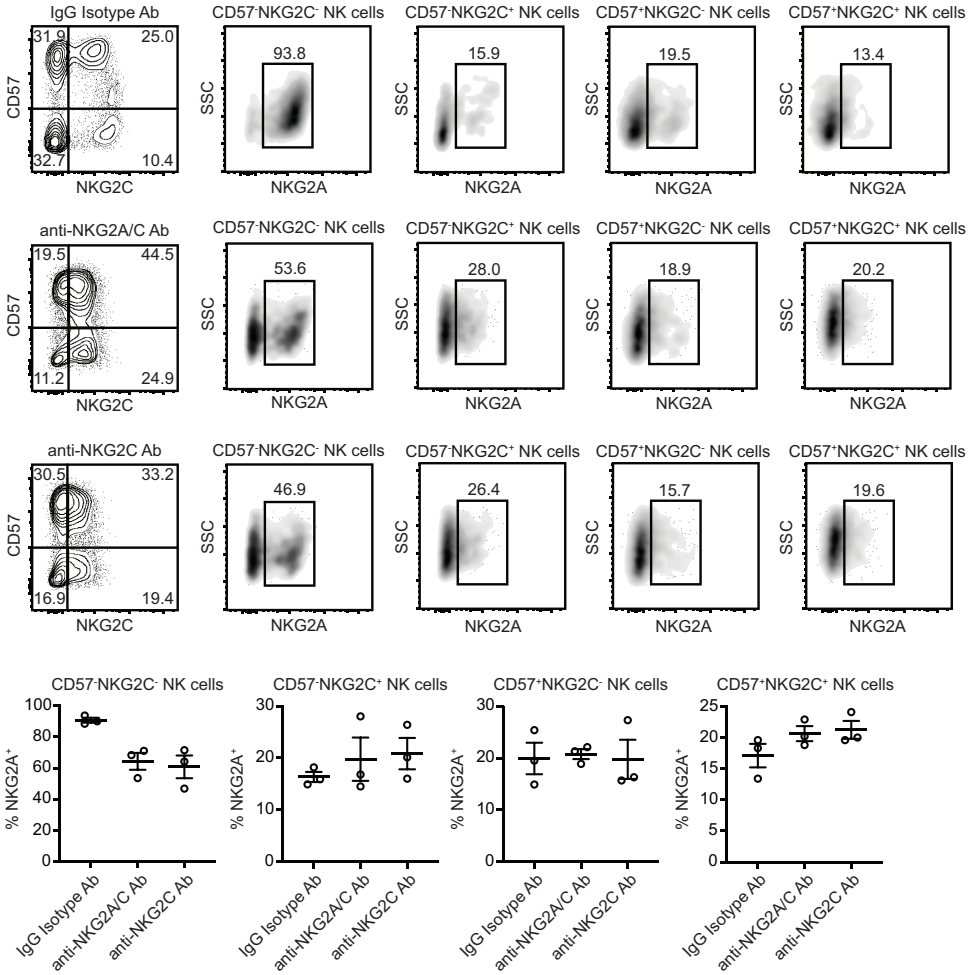
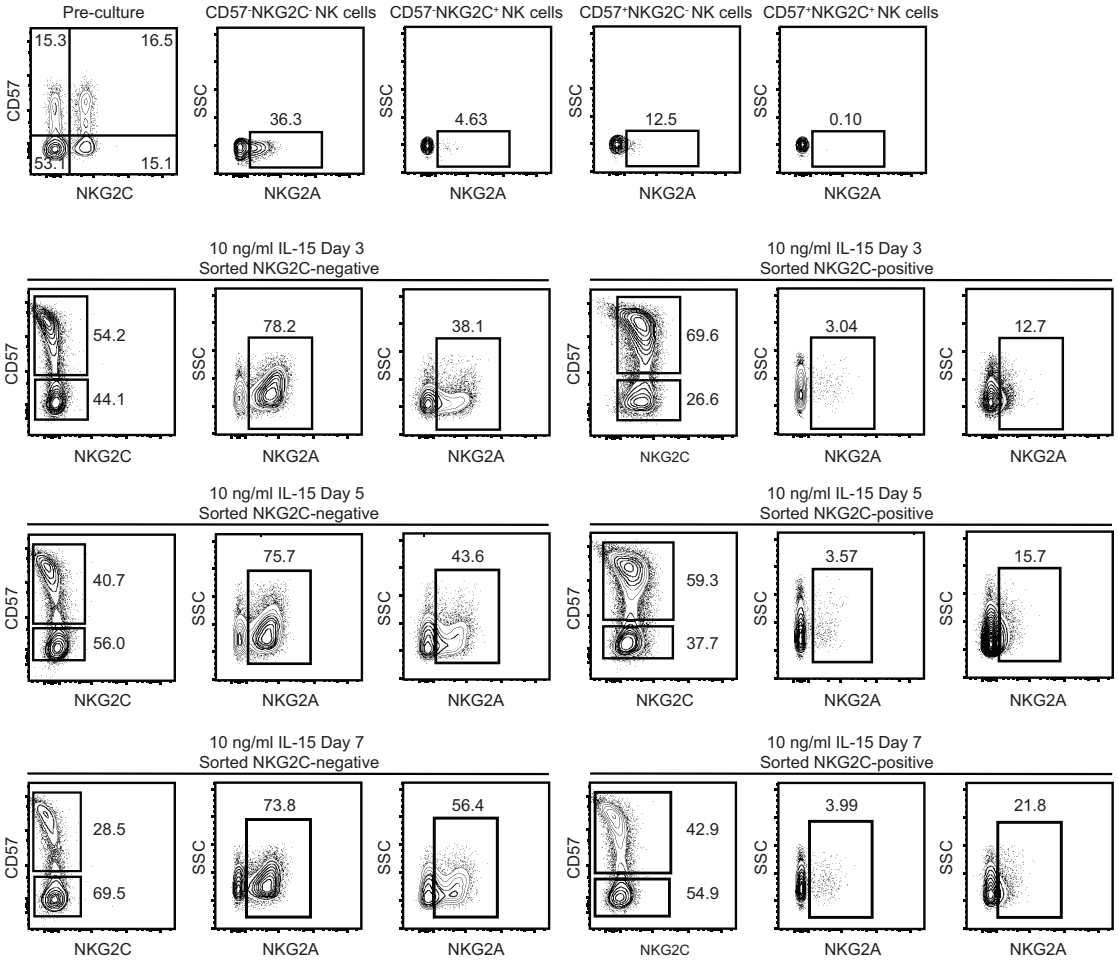
Supplemental Figure 1



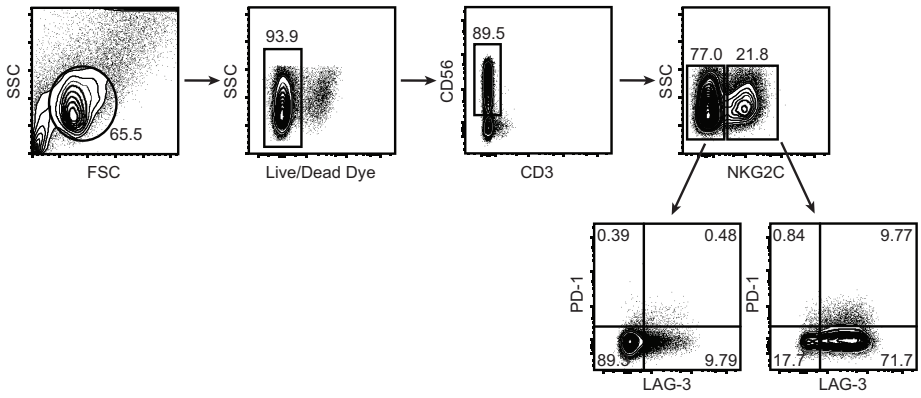
Supplemental Figure 1. NKG2A is induced at similar frequencies on NKG2C$^+$ NK cells stimulated with anti-NKG2A/C and anti-NKG2C antibodies. CD3/CD19-depleted PBMCs from HCMV seropositive donors were cultured for 7 days with 10 ng/ml IL-15 and IgG2b isotype Ab, anti-NKG2A/C Ab or anti-NKG2C Ab. Shown are FACS plots from a representative donor and summary data (n = 3) of the frequencies of CD3$^-$CD56$^+$ NK cells gated by CD57 and NKG2C that expressed NKG2A before and after culture. Results are from 2 independent experiments. Paired t tests were used; no significant results.

Supplemental Figure 2



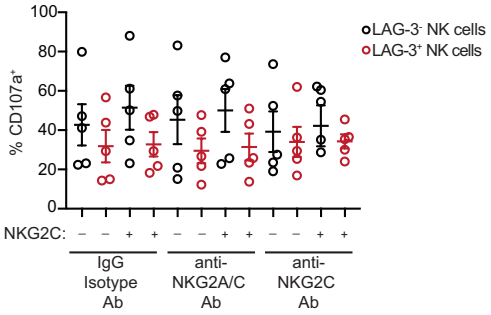
Supplemental Figure 2. NKG2A is upregulated over time on sorted NKG2C^{-} and NKG2C^{+} NK cells cultured with IL-15. CD3^{-}CD56^{dim}NKG2C^{-} and CD3^{-}CD56^{dim}NKG2C^{+} NK cells were sorted from the peripheral blood of 2 HCMV seropositive donors and cultured for 7 days with 10 ng/ml IL-15. Shown are FACS plots of NKG2A expression on NK cell subsets pre-culture and after 3, 5 and 7 days of culture from one of the donors. The second donor showed a similar trend in NKG2A expression pre- and post-culture. Each donor was sorted and cultured in an independent experiment.

Supplemental Figure 3



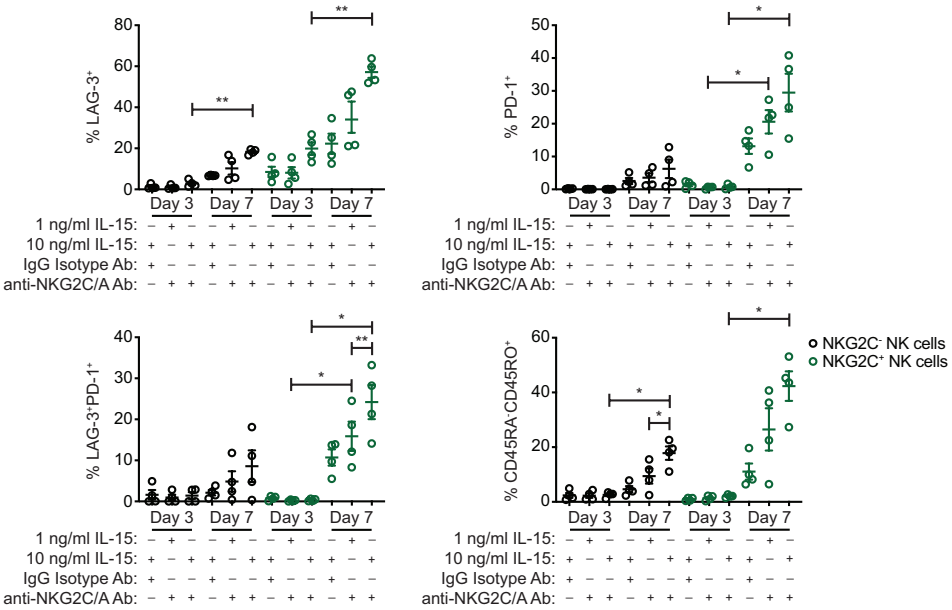
Supplemental Figure 3. Gating strategy for FACS analysis of NK cells. Shown is a representative example of a full gating path for flow cytometry analysis of NK cells from a 7-day culture with 10 ng/ml IL-15 and anti-NKG2A/C Ab.

Supplemental Figure 4



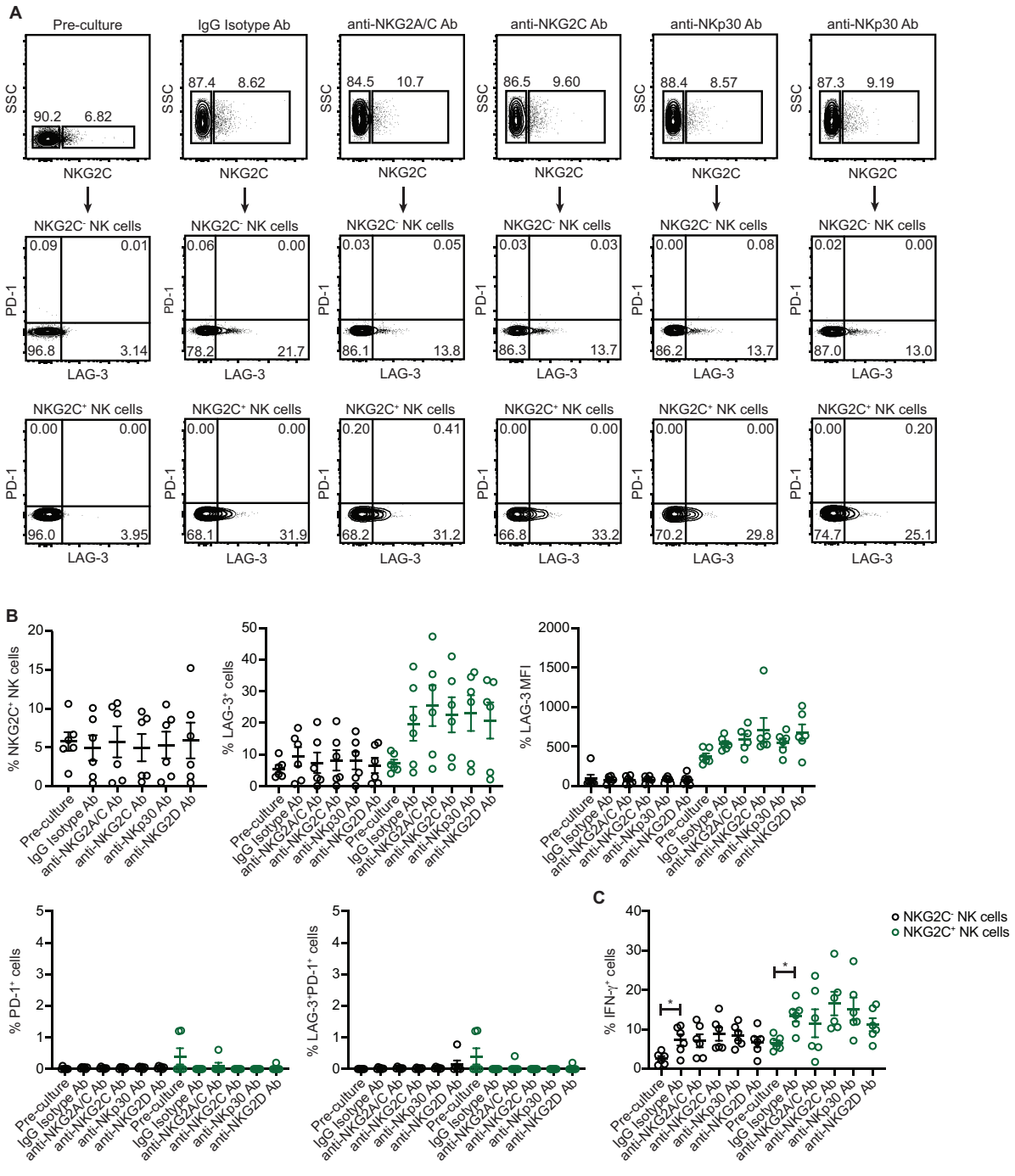
Supplemental Figure 4. Chronically stimulated adaptive NK cells expressing LAG-3 do not exhibit impaired degranulation in response to K562 targets. CD3/CD19-depleted PBMCs from HCMV seropositive donors were cultured for 7 days with 10 ng/ml IL-15 and IgG2b isotype Ab, anti-NKG2A/C Ab or anti-NKG2C Ab. NK cells post-culture were used as effectors in functional assays with K562 targets at an E:T ratio of 2:1. Shown is summary data of surface CD107a frequencies by NK cell subsets stratified by NKG2C and LAG-3 expression (n = 5). Results are from 3 independent experiments. Paired t tests were used to compare LAG-3$^-$ and LAG-3$^+$ NK cells; no significant results.

Supplemental Figure 5



Supplemental Figure 5. Upregulation of the checkpoint inhibitory receptors and CD45 isoform switching is influenced by IL-15 concentration and increases over time in culture. CD3/CD19-depleted PBMCs from HCMV seropositive donors were cultured for either 3 or 7 days with the indicated concentrations of IL-15 and plate-bound Ab. Shown are the percentages of $CD3^-CD56^+NKG2C^-$ and $CD3^-CD56^+NKG2C^+$ NK cells from each culture expressing LAG-3, PD-1 and CD45RO ($n = 4$). Results are from 2 independent experiments. Paired *t* tests were used, and *p*-values of multiple group comparisons (within the $NKG2C^-$ NK cells groups and within the $NKG2C^+$ NK cells groups) were adjusted using the method of Hommel. $*p \leq 0.05$, $**p \leq 0.01$

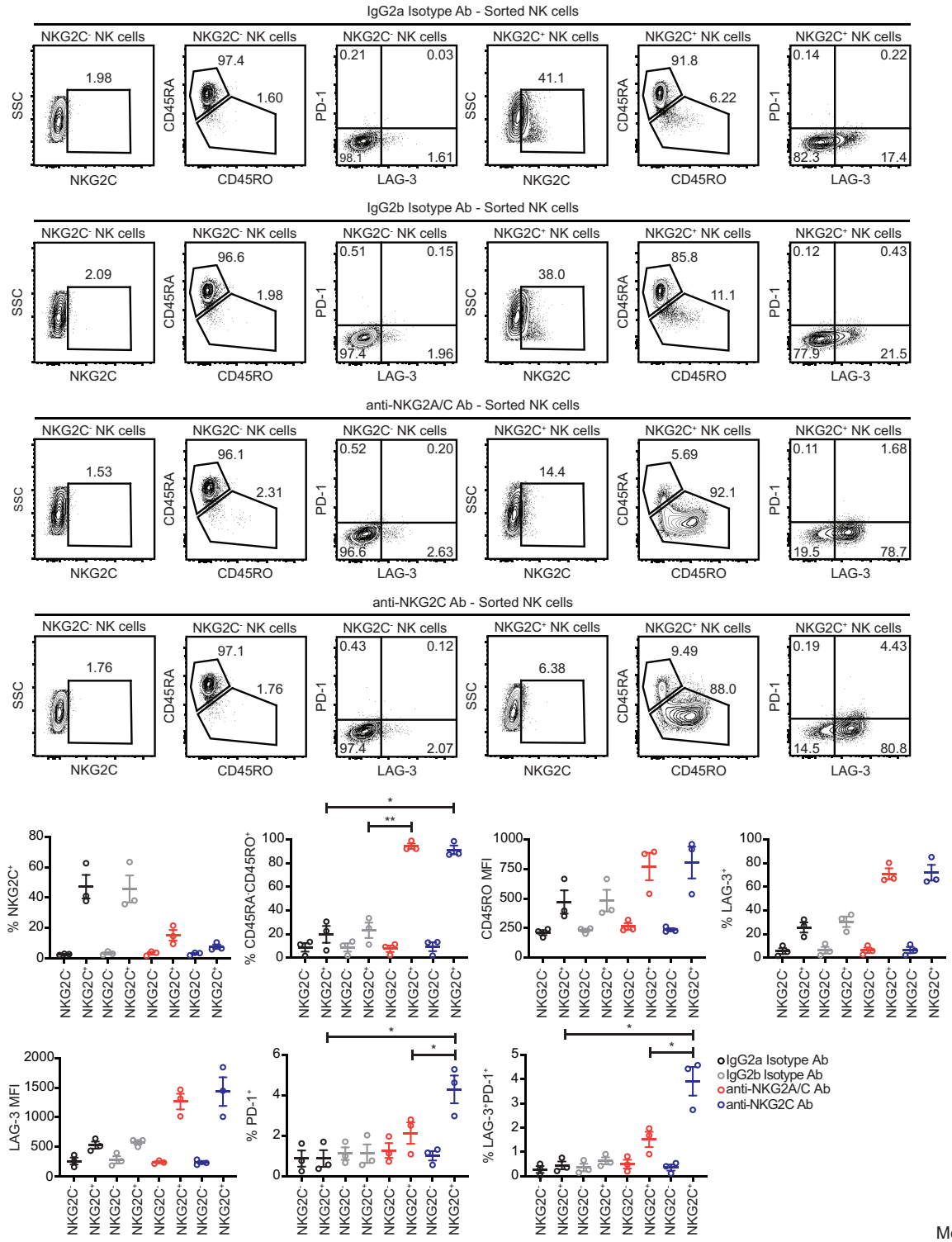
Supplemental Figure 6



Merino et al.

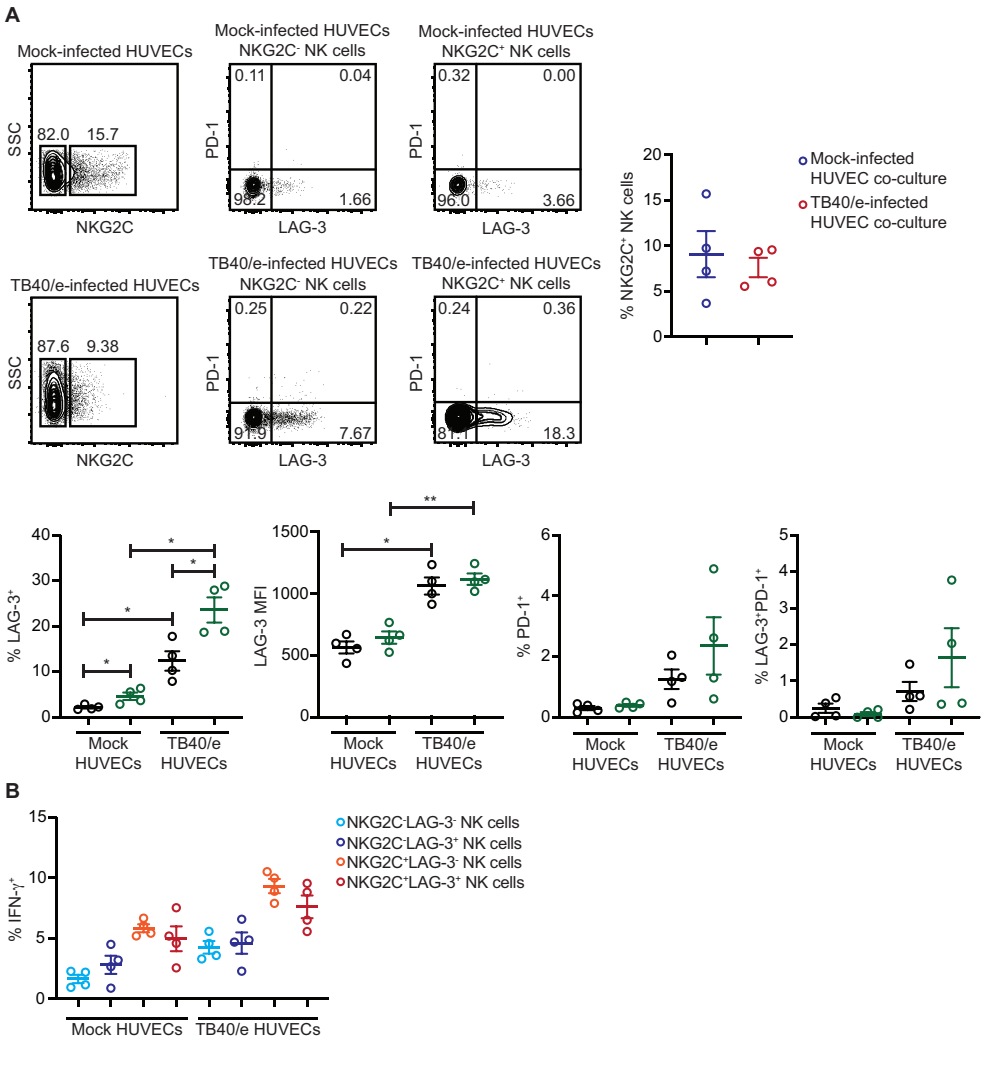
Supplemental Figure 6. Agonist antibody stimulation does not expand NKG2C$^+$ NK cells from HCMV seronegative donors and does not induce functional exhaustion. CD3/CD19-depleted PBMCs from HCMV seronegative donors were cultured for 7 days with 10 ng/ml IL-15 and IgG2b isotype Ab, anti-NKG2A/C Ab, anti-NKG2C Ab, anti-NKp30 Ab or anti-NKG2D Ab. Shown are (A) FACS plots from a representative donor and (B) summary data (n = 6) of the frequencies of CD3$^-$CD56$^+$NKG2C$^+$ NK cells and LAG-3 vs. PD-1 expression in the CD3$^-$CD56$^+$NKG2C$^-$ and CD3$^-$CD56$^+$NKG2C$^+$ subsets. (C) Pre- and post-culture NK cells were stimulated with K562 target cells at an E:T ratio of 2:1, and IFN-γ production in CD3$^-$CD56$^+$NKG2C$^-$ and CD3$^-$CD56$^+$NKG2C$^+$ NK cells was determined by intracellular FACS. All results are from 2 independent experiments. Paired t tests were used, and p-values of multiple group comparisons in Panel B (each group vs. IgG Isotype Ab within the NKG2C$^-$ NK cells groups and within the NKG2C$^+$ NK cells groups) were adjusted using the method of Hommel; no multiple comparison adjustment for comparing IgG Isotype Ab with the pre-culture condition.
* $p \leq 0.05$

Supplemental Figure 7



Supplemental Figure 7. Sorted NKG2C^{+} NK cells isolated from HCMV seropositive donors downregulate NKG2C and upregulate CD45RO, LAG-3 and PD-1 in response to agonist antibody stimulation. CD3^{-}CD56^{dim}NKG2C^{-} and CD3^{-}CD56^{dim}NKG2C^{+} NK cells were sorted from PBMCs isolated from HCMV seropositive donors (n = 3) and cultured for 7 days with 10 ng/ml IL-15 and IgG2a isotype Ab, IgG2b isotype Ab, anti-NKG2A/C Ab or anti-NKG2C Ab. Shown are FACS plots of NKG2C, CD45RA, CD45RO, LAG-3 and PD-1 staining on cultured NK cells from a representative donor as well as cumulative data. Results are from 2 independent experiments. Paired t tests were used, and p-values of multiple group comparisons (3 pairwise comparisons of interest) were adjusted using the method of Hommel. *$p \leq 0.05$, **$p \leq 0.01$

Supplemental Figure 8



Merino et al.

Supplemental Figure 8. NKG2C$^+$ NK cells from HCMV seronegative donors do not upregulate LAG-3 or PD-1 to the same extent as NKG2C$^+$ NK cells from HCMV seropositive donors in response to HCMV-infected HUVECs and do not exhibit impaired IFN-γ production. HUVECs were infected with a GFP-expressing TB40/e clinical strain of HCMV at a MOI of 0.5 or spun down in parallel without virus (mock-infected) and used for 7-day co-culture experiments with CD3/CD19-depleted PBMCs from HCMV seronegative donors (n = 4). Cultures contained 10 ng/ml IL-15. (A) Representative FACS plots and summary data of the percentages of CD3$^-$CD56$^+$NKG2C$^+$ NK cells and LAG-3 and PD-1 expression within the CD3$^-$CD56$^+$NKG2C$^-$ and CD3$^-$CD56$^+$NKG2C$^+$ NK cell subsets from mock and TB40/e co-cultures. (B) Summary data of intracellular IFN-γ in CD3$^-$CD56$^+$ NK cells gated by NKG2C and LAG-3 expression from mock and TB40/e co-cultures stimulated with K562 cells at an E:T ratio of 2:1 (n = 4). Results are from 2 independent experiments. Paired Student's t tests were used, and p-values of multiple group comparisons (Panel A: 4 pairwise comparisons as shown) were adjusted using the method of Hommel. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$