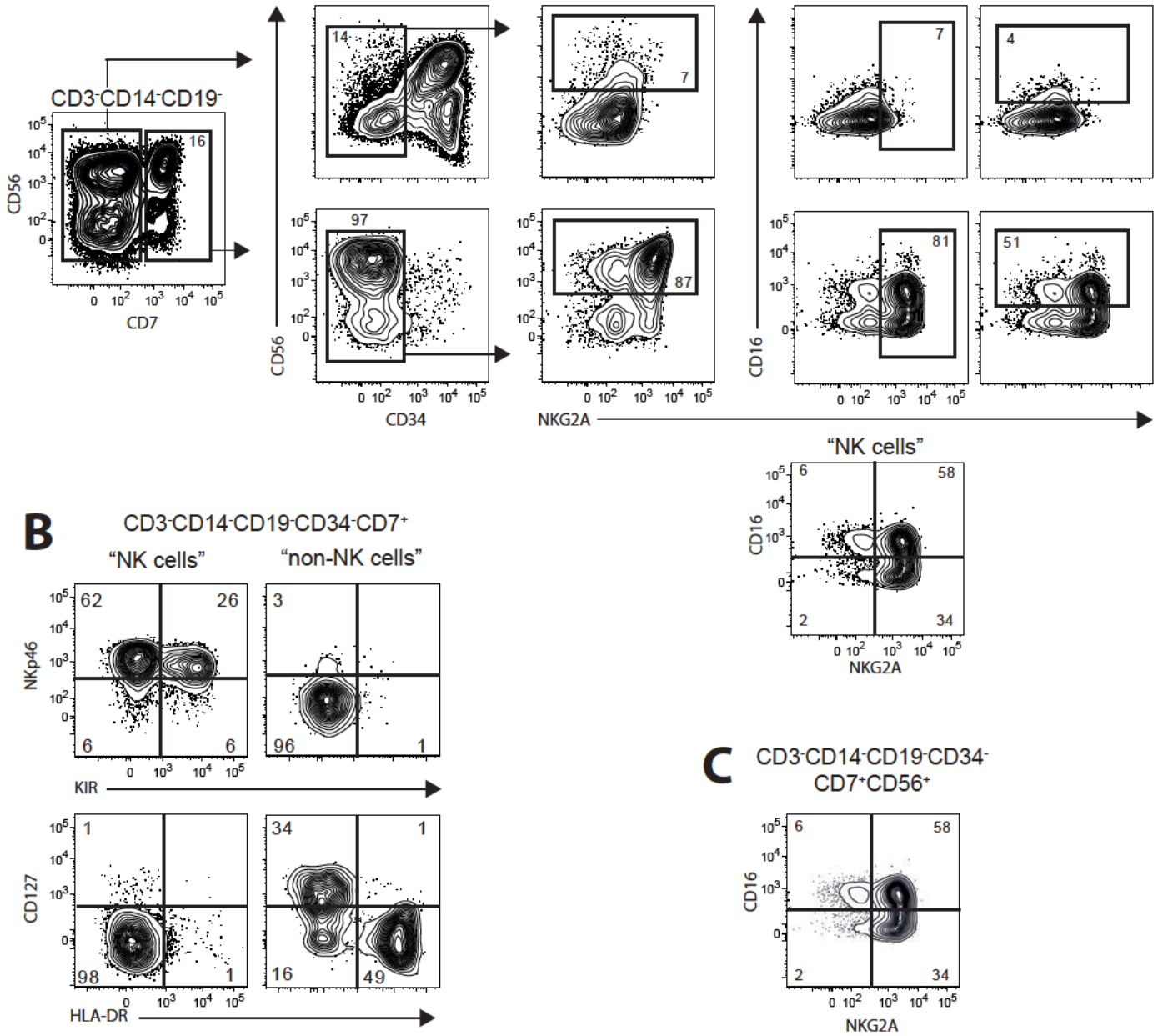
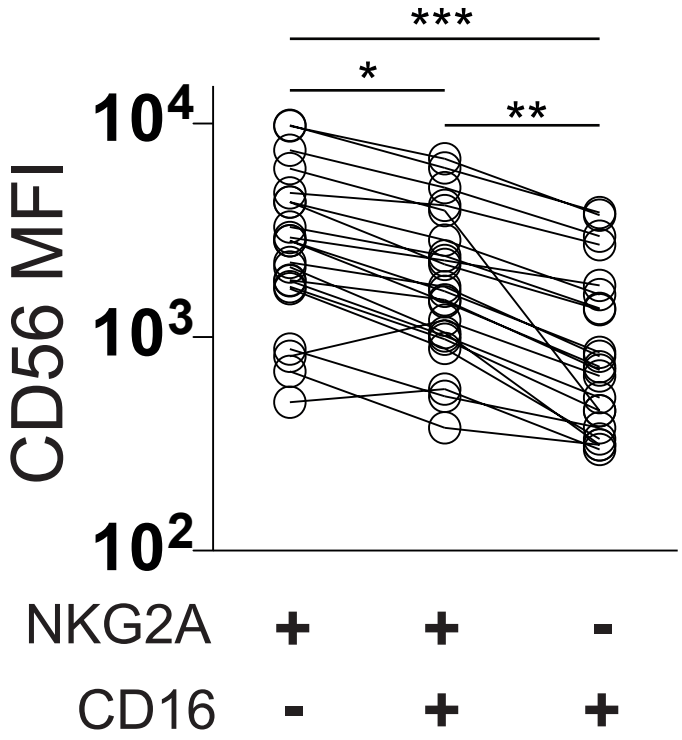
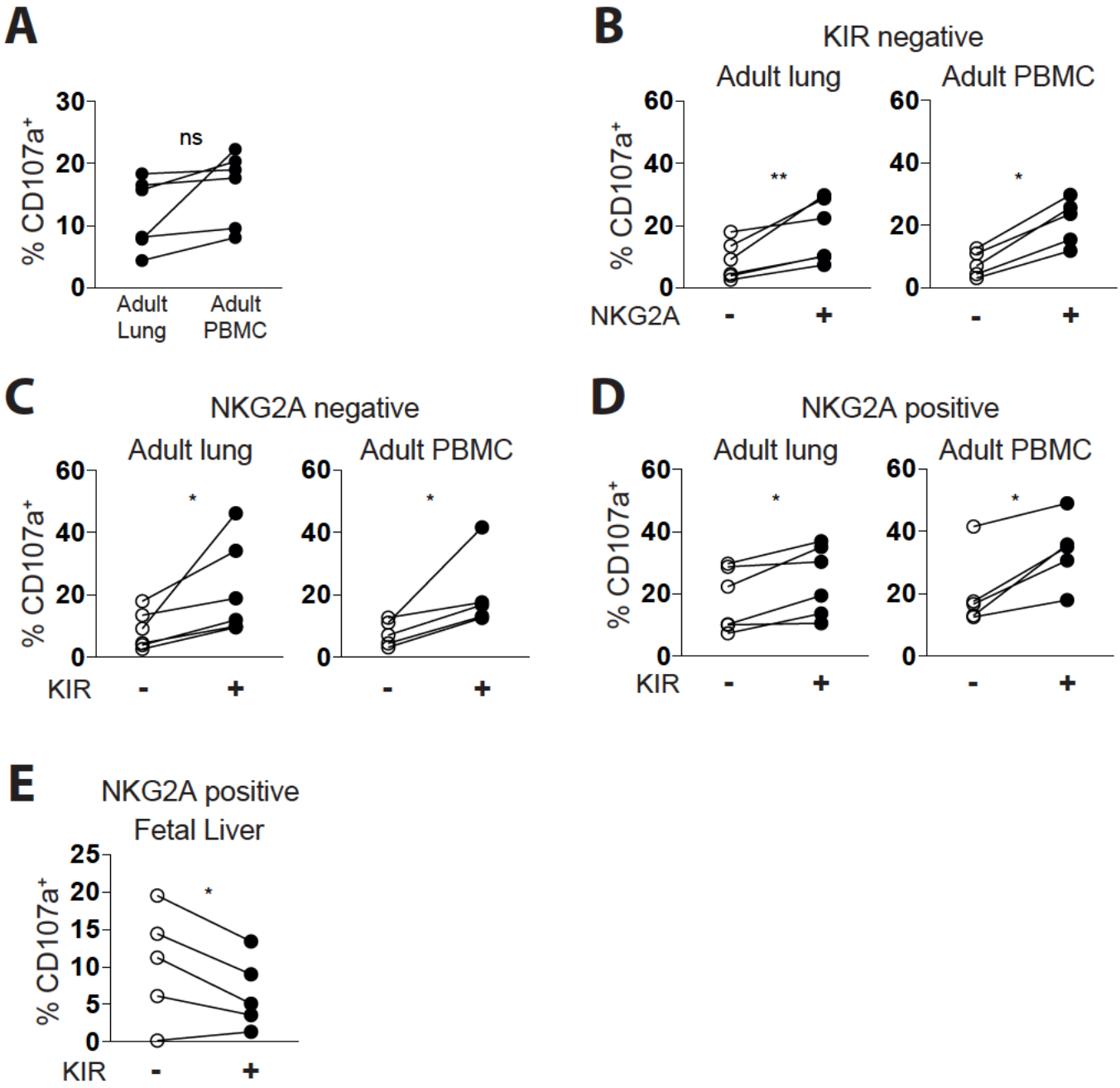
Supplementary Figure 1



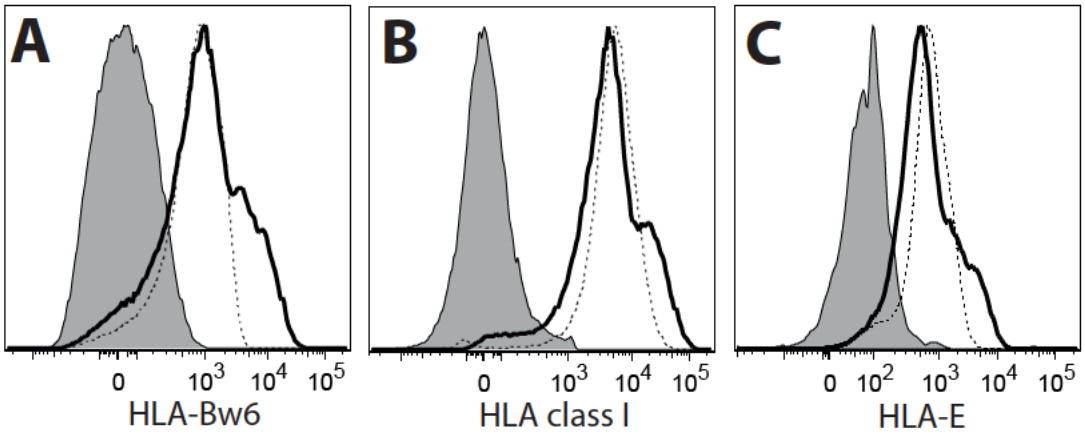
Supplementary Figure 2



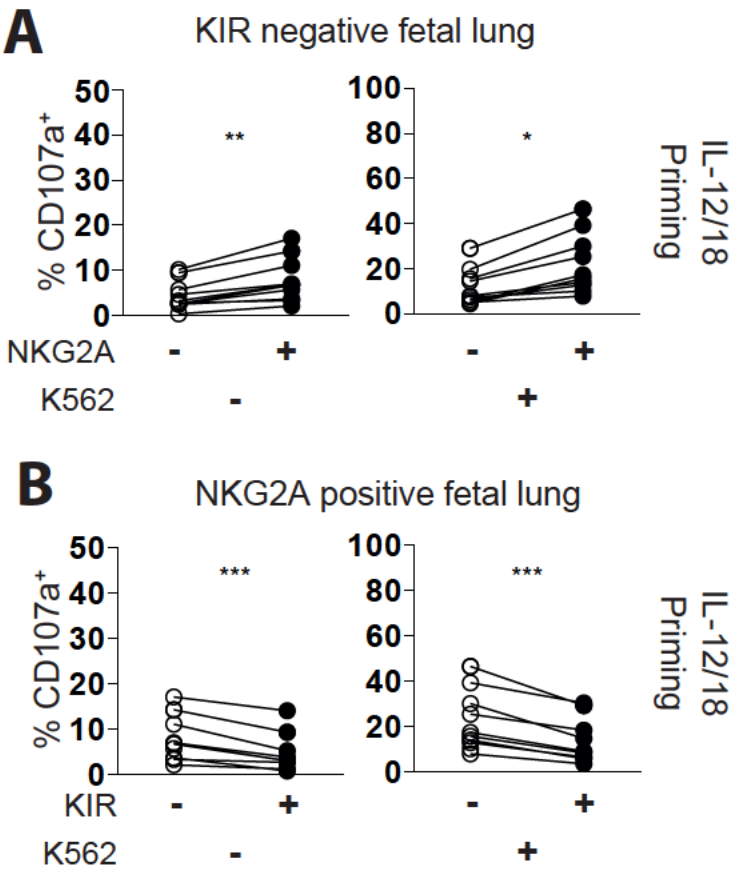
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 1

Identification of fetal NK cells. (**A**) NK cells are defined as $CD56^+$ and/or $CD16^+$ and/or $NKG2A^+$ using a Boolean gate within the $CD7^+CD3^-CD14^-CD19^-CD34^-$ cells. More than 97% of the defined NK cells were $CD16^+$ or $NKG2A^+$ by using this definition. Of note, the $CD3^-CD14^-CD19^-CD34^-$ population contains a low frequency of cells that are $CD56^+$ but that do not express CD7. $CD7^-CD56^+$ cells express only low levels of NKG2A and CD16, whereas $CD7^+CD56^+$ cells express high levels of NKG2A and CD16. (**B**) NK cells defined by the Boolean gate "$CD56^+$ and/or $NKG2A^+$ and/or $CD16^+$" among $CD7^+CD3^-CD14^-CD19^-CD34^-$ cells express NKp46 and KIR (KIR2DL1/2/3/S1/S2), but lack expression of CD127 and HLA-DR. In contrast, none, or very few, "non-NK cells" (using the negative Boolean-gate for NK cells) among $CD7^+CD3^-CD14^-CD19^-CD34^-$ cells express NKp46 or KIR. Instead, the non-NK cell gate contained $HLA\text{-}DR^+$ and $CD127^+$ cells. The use of the Boolean gate "$CD56^+$ and/or $NKG2A^+$ and/or $CD16^+$" among $CD7^+CD3^-CD14^-CD19^-CD34^-$ cells thus accurately defines NK cells, and efficiently excludes e.g. $CD127^+$ innate lymphoid cells. (**C**) NK cells defined as $CD3^-CD14^-CD19^-CD34^-CD7^+CD56^+$ fetal lung cells contain 98% CD16 and/or NKG2A expressing cells. CD16 is rapidly shed from the surface of NK cells upon activation in vitro, preventing identification of NK cells based on the expression of CD16. Therefore NK cells were defined as $CD3^-CD14^-CD19^-CD34^-CD7^+CD56^+$ in functional assays. (**D**) The vast majority of $CD3^-CD14^-$

$CD19^-CD34^-CD45^+$ cells that express CD161 also express CD7. Therefore, the use of CD7 as a marker of NK cells did not exclude stage III immature NK cells from the analysis.

Supplementary Figure 2

CD56 expression decreases with fetal NK cell differentiation. The mean fluorescence intensity (MFI) of CD56 is shown for fetal lung NK cells in the differentiation stages defined by NKG2A and CD16 expression. Data are representative of all fetal organs studied. Statistical significance was tested by one-way ANOVA.

Supplementary Figure 3

NKG2A and KIR educate adult lung NK cells. (**A**) Degranulation against K562 cells by NK cells isolated from adult lung tissue and matched peripheral blood. (**B**) Degranulation by $CD57^-KIR^-NKG2A^-$ and $CD57^-KIR^-NKG2A^+$ NK cells isolated from adult lung tissue (left) and matched peripheral blood (right). (**C-D**) Degranulation by $CD57^-KIR^-$ and $CD57^-KIR^+$ NK cells. (**E**) Degranulation by $NKG2A^+$ fetal liver NK cells that express or lack KIR after stimulation with K562 cells. In all settings, background degranulation from unstimulated cells was subtracted from the responses measured against K562 cells. KIR-expressing cells were defined with a Boolean-gate for expression of KIR2DL1, KIR2DL3, and/or KIR3DL1. Statistical significance was tested using paired *t* tests.

Supplementary Figure 4

Fetal tissue cells express HLA class I. Adult PBNK cells (dashed line) and fetal lung NK cells (thick solid line). (**A**) HLA-Bw6 expression on fetal and adult cells genotyped as HLA-Bw4 negative. Fetal lung cells were also stained with anti-Bw4 antibody as negative control (filled grey). (**B**) HLA class I (HLA-A, B and C) expression and (**C**) HLA-E expression on adult and fetal lymphocytes. Isotype control is shown as filled grey.

Supplementary Figure 5

Cytokine-priming does not break fetal KIR-mediated NK cell hyporesponsiveness. Fetal lung NK cells were primed over-night with IL-12 and IL-18, and then co-cultured with K562 cells. (**A**) Degranulation by KIR^- fetal lung NK cells that express or lack NKG2A. (**B**) Degranulation by $NKG2A^+KIR^-$ and $NKG2A^+KIR^+$ fetal lung NK cells. The degranulation in wells with R10 medium alone was subtracted from responses. Statistical significance was tested using paired *t* tests.