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Supplemental Information

Divergent Role for STAT5 in the Adaptive Responses of Natural Killer Cells

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Supplementary Material

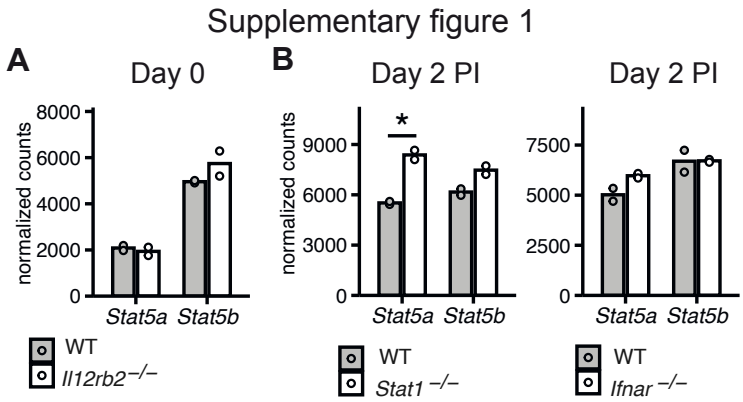

Fig. S1. IL-12- and STAT4-dependent induction of STAT5 in NK cells during MCMV infection. Related to Figure 1.
(**A**) RNA-seq on WT vs. *Il12rb2*$^{-/-}$ NK cells from mixed BMC on day 0 (uninfected). Normalized counts of *Stat5a* and *Stat5b* are displayed. (**B**) RNA-seq on WT vs. *Stat1*$^{-/-}$ or *Ifnar*$^{-/-}$ from mixed BMC on day 2 PI. Normalized counts of *Stat5a* and *Stat5b* are displayed.

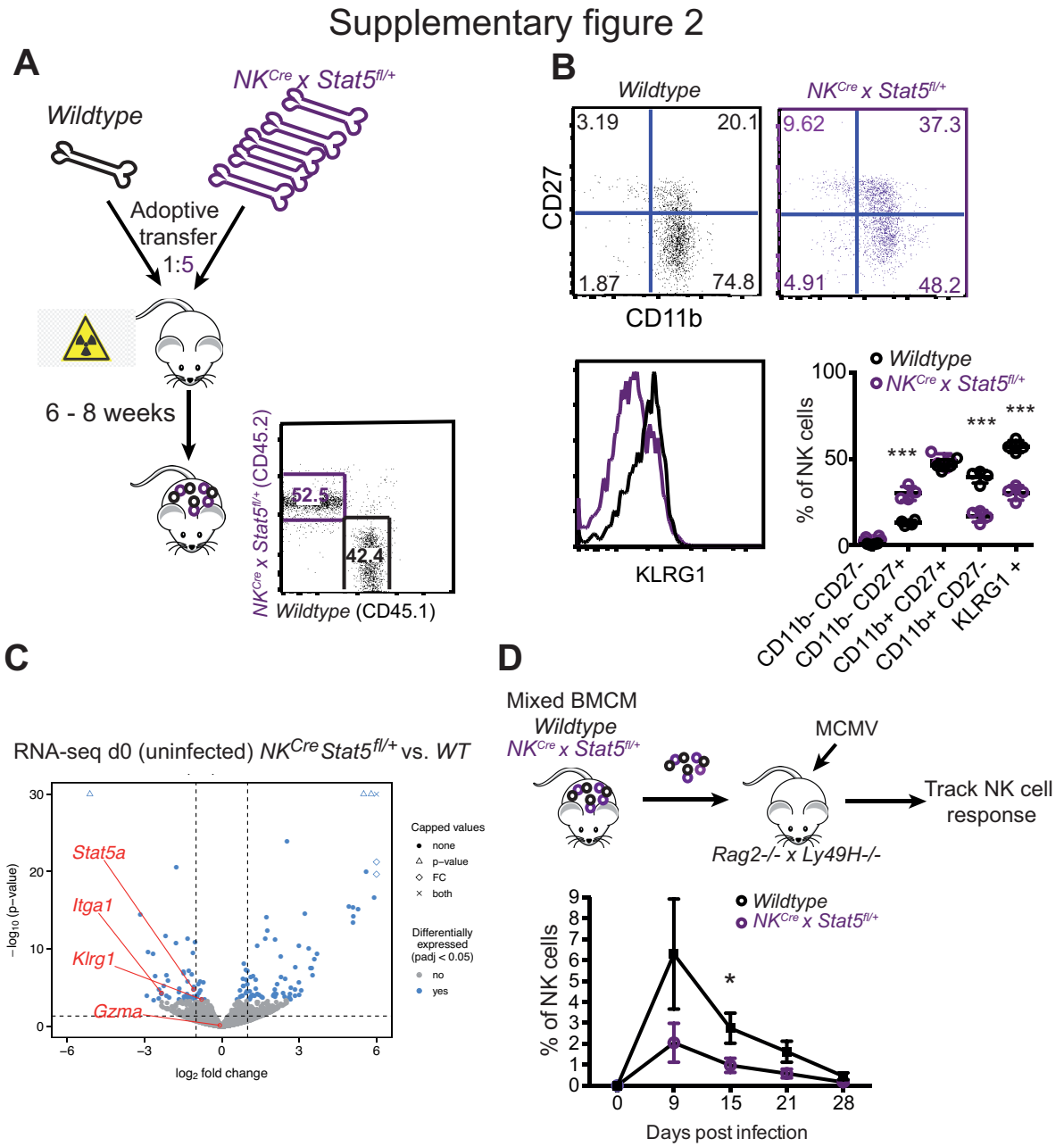

Fig. S2. STAT5-dependent anti-viral NK cell response. Related to figure 2.
(**A-C**). Mixed bone marrow chimeras (mBMC) were generated by lethal irradiation (900 cGy) of host mice, which were then reconstituted with a 1:5 mixture of bone marrow cells from WT and *NK^{Cre} x Stat5$^{fl/+}$* donor mice. (**A**) Experimental schematic of mBMC generation. Representative flow blot of NK cell reconstitution 8 weeks after reconstitution. (**B**) Analysis of NK cell maturation markers on WT and *NK^{Cre} x Stat5$^{fl/+}$* NK cells in mBMC 8 weeks post reconstitution. (Data is representative of at least 3 experiments). (**C**) Volcano blot of RNA-seq data on uninfected (d0) Ly49H^{+} WT or *NK^{Cre} x Stat5$^{fl/+}$* NK cells from mBMC. Blue dots show differentially expressed (FDR < 0.05) genes. Horizontal line indicates p = 0.05, and vertical lines show absolute log2 fold change = 1. (**D**) Splenocytes from mixed WT : *NK^{Cre} x Stat5$^{fl/+}$* BMC where adoptively transferred into *Rag2$^{-/-}$ x Ly49h$^{-/-}$* mice and infected with MCMV. Graph shows percentage of Ly49H+ WT or KO NK cells of total NK cells over the course of infection. Data is representative of 2 independent experiments (n=3-4). All error bars indicate SEM.

Supplementary figure 3

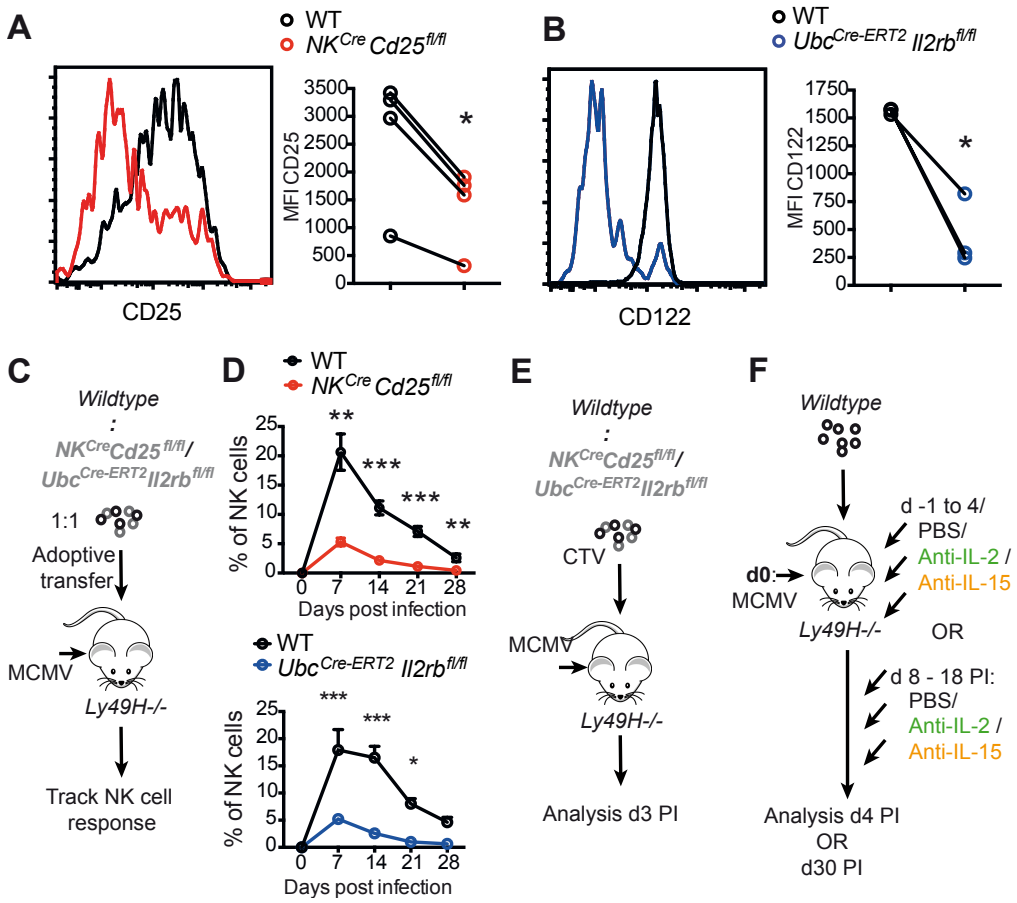

Fig. S3. Both IL-2 and IL-15 drive NK cell expansion *in vivo*. Related to figures 3 and 4. (**A-C**) Equal numbers of WT and *Ubc*$^{Cre\text{-}ERT2}$ *x Il2rb*$^{fl/fl}$ or *NK*^{Cre} *x CD25*$^{fl/fl}$ NK cells were transferred into *Ly49h*$^{-/-}$ mice. Mice transferred with *Ubc*$^{Cre\text{-}ERT2}$ *x Il2rb*$^{fl/fl}$ NK cells were treated with tamoxifen on days -3, -2 and -1 before infection with MCMV. Following MCMV infection, relative percentages of Ly49H^{+} WT and KO NK cells are displayed (n = 4-5). (**A**) Analysis of CD25 expression on WT and *NK*^{Cre} *x CD25*$^{fl/fl}$ NK cells on day 3 PI. Data is representative of 2 independent experiments. (**B**) Analysis of CD122 expression on WT *Ubc*$^{Cre\text{-}ERT1}$ *x Il2rb*$^{fl/fl}$ on day 3 PI. Data is representative of 2 independent experiments. (**C**) Experimental schematic of adoptive transfer and infection. (**D**) Graphs display percentage of Ly49H+ WT and *NK*^{Cre} *x CD25*$^{fl/fl}$ or *Ubc*$^{Cre\text{-}ERT2}$ *x Il2rb*$^{fl/fl}$ NK cells of total NK cells over the course of infection. Data is representative of at least 2 independent experiments (n=4-5). (**E**). Experimental schematic of CTV labeling and analysis: NK cells from WT mice, *NK*^{Cre} *x CD25*$^{fl/fl}$ mice, or *Ubc*$^{Cre\text{-}ERT2}$ *x Il2rb*$^{fl/fl}$ mice treated with tamoxifen on days -3, -2 and -1 were labeled with CTV and transferred into *Ly49h*$^{-/-}$ mice, followed by infection with MCMV. (**F**) Experimental schematic of antibody-mediated IL-2 and IL-15 depletion: WT Ly49H^{+} NK cells were transferred into *Ly49h*$^{-/-}$ mice treated with PBS, anti-IL-2, or anti-IL-15 on day -1 to 4 PI (early) or days 8 to 18 PI (late) and analyzed on day 4 PI (early) or day 30 PI (late). All error bars indicate SEM.