**Supplemental Information**

# Homeostatic control of memory cell progenitors in the NK cell lineage

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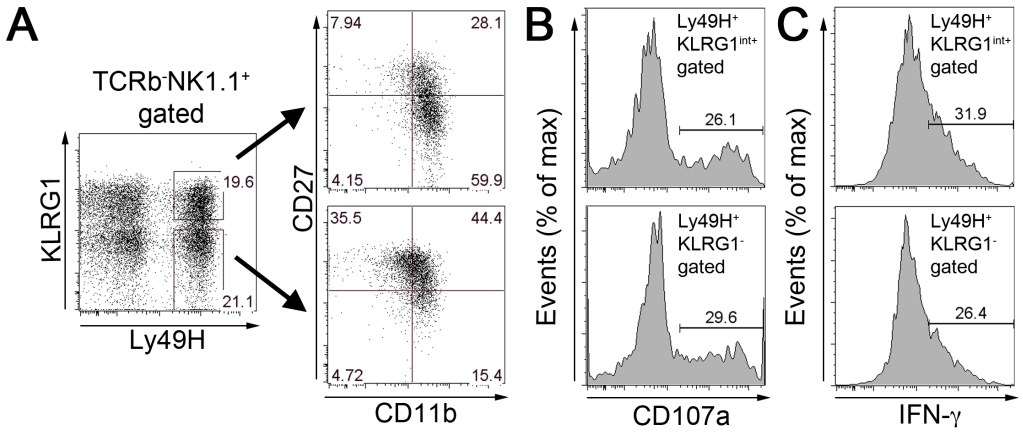


**Figure S1. Equivalent effector function between KLRG1$^{int+}$ and KLRG1$^{-}$ NK cells, related to Figure 1.**

(A) CD27 and CD11b expression by splenic naïve KLRG1$^{int+}$ and KLRG1$^{-}$ Ly49H$^{+}$ NK cells. (B) Degranulation by splenic naïve KLRG1$^{int+}$ (upper panel) and KLRG1$^{-}$ (lower panel) Ly49H$^{+}$ NK cells stimulated *ex vivo* with RMA-m157 target cells. (C) IFN-γ production by splenic KLRG1$^{int+}$ (upper panel) and KLRG1$^{-}$ (lower panel) Ly49H$^{+}$ NK cells at day 1.5 after MCMV infection. Data are representative of two independent experiments.

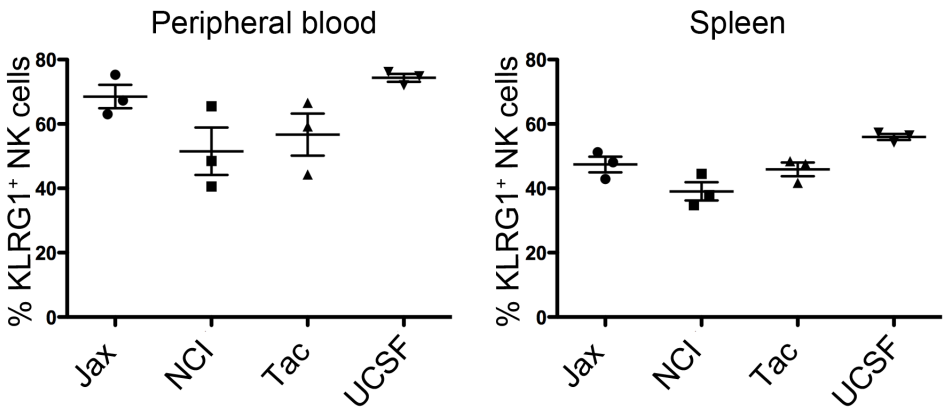


**Figure S2. Differential frequency of KLRG1$^{int+}$ NK cells in WT B6 mice from different animal vendors, related to Figure 2.**

Expression of KLRG1 on TCR$\beta^-$ NK1.1$^+$ gated NK cells in peripheral blood (left panel) and spleen (right panel) of C57BL/6 mice purchased from the Jackson Laboratory (Jax), the NCI Mouse Repository, and Taconic (Tac) and C57BL/6 mice bred in our animal facility at UCSF. All mice were 8 week-old females. Data are representative of two independent experiments with 3 mice from each strain. Error bars show S.E.M.

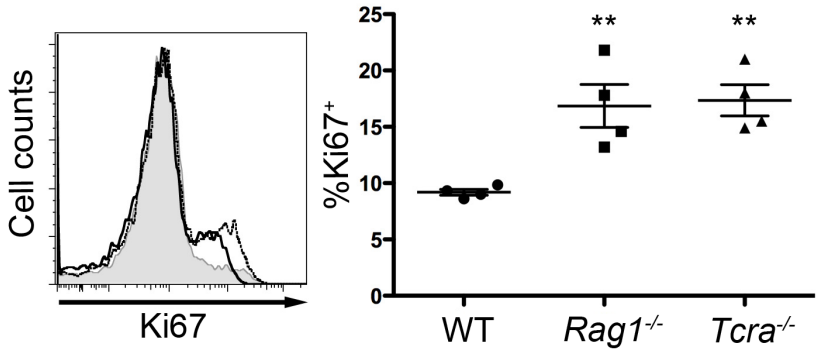


**Figure S3. Increased baseline proliferation of NK cells in T cell-deficient mice, related to Figure 3.**

Expression of Ki67 in splenic NK cells was detected by intracellular staining. A representative histogram plot (left panel; shaded: WT, bold: *Rag1*$^{-/-}$, dotted: *Tcra*$^{-/-}$) and graph summarizing results from individual mice (right panel) are shown. Data are representative of two independent experiments with 4 mice from each strain. Error bars show S.E.M.

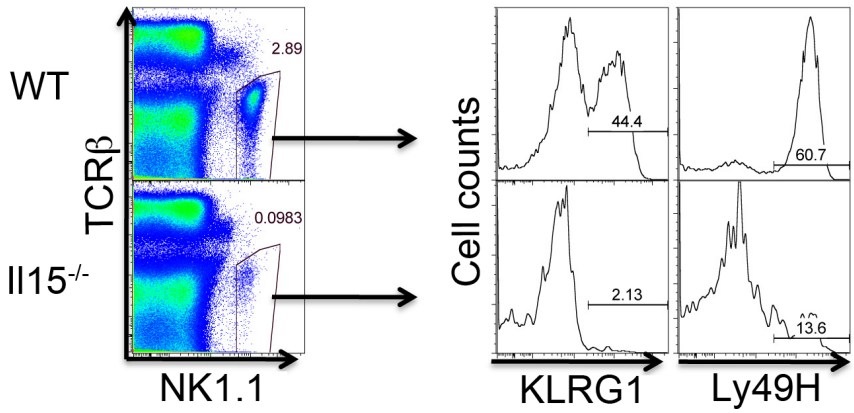


**Figure S4. Severely decreased frequency of KLRG1$^{int+}$ NK cells in *Il15*$^{-/-}$ mice, related ot Figure 4.**

Expression of KLRG1 and Ly49H by splenic NK cells isolated from WT and *Il15*$^{-/-}$ mice. Percentage of TCRβ$^{-}$NK1.1$^{+}$, KLRG1$^{+}$, or Ly49H$^{+}$ cells is shown in each panel. Data are representative of two independent experiments (n=4-6).

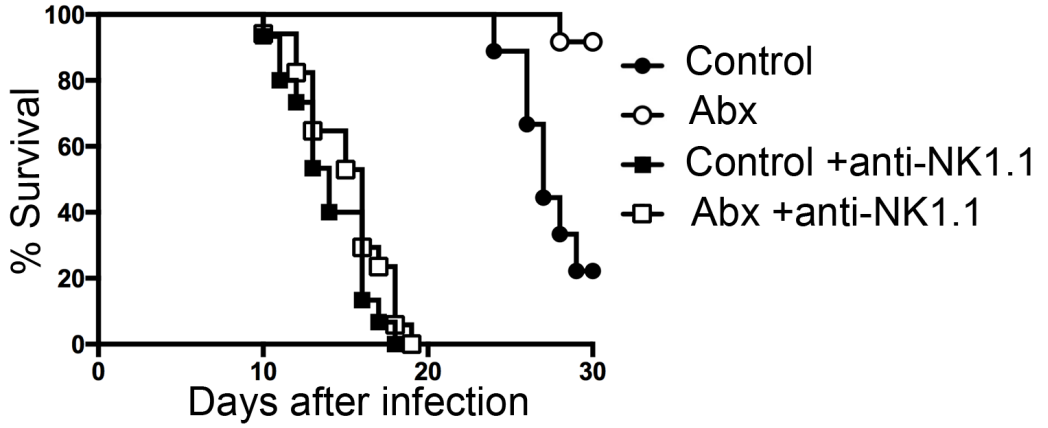


**Figure S5. Effect of antibiotics on the survival of NK cell-depleted *Rag1*$^{-/-}$ mice, related to Figure 6.**

*Rag1*$^{-/-}$ mice were treated with control or Abx-treated water for three weeks before MCMV infection (day 0) and treatment was continued throughout the experiment. Anti-NK1.1 antibody (200 μg/mouse) was injected i.p. one day before infection (day -1). Data are compiled from two independent experiments (control: n=10, Abx: n=12, control+anti-NK1.1: n=15, Abx + anti-NK1.1: n=17).

## Supplemental Experimental Procedures

### Intracellular staining

For the detection of IFN-γ, splenocytes were isolated at day 1.5 after MCMV infection and stained for cell surface expression of TCRβ (H57-597; BioLegend), NK1.1 (PK136; BioLegend), Ly49H (3D10; eBioscience), and KLRG1 (2F1; BioLegend), followed by fixation with 4% paraformaldehyde in PBS for 10 min in room temperature. Cells were permeabilized by using Permeabilization Wash Buffer (BioLegend) and stained intracellularly for IFN-γ (XMG1.2; BioLegend). For Ki67 staining, splenocytes were stained and fixed as described above. Cells were permeabilized with 0.1% Triton X-100 (Fisher Scientific) in PBS for 5 min at room temperature and then stained for Ki67 (B56; BD Biosciences). Samples were analyzed on a BD LSR II flow cytometer using FlowJo software (Tree Star).

### Degranulation assays

RMA target cells were transduced with a retroviral vector encoding MCMV 157 orf. Naive splenocytes ($1 \times 10^6$/well) were incubated for 6 hrs with target cells ($1 \times 10^5$/well) in the presence of monensin and anti-CD107a (1D4B; BD Biosciences). Cells were then collected, followed by surface staining with monoclonal antibodies to Ly49H, NK1.1, TCRβ, and KLRG1 (2F1; BioLegend).