Figure S1

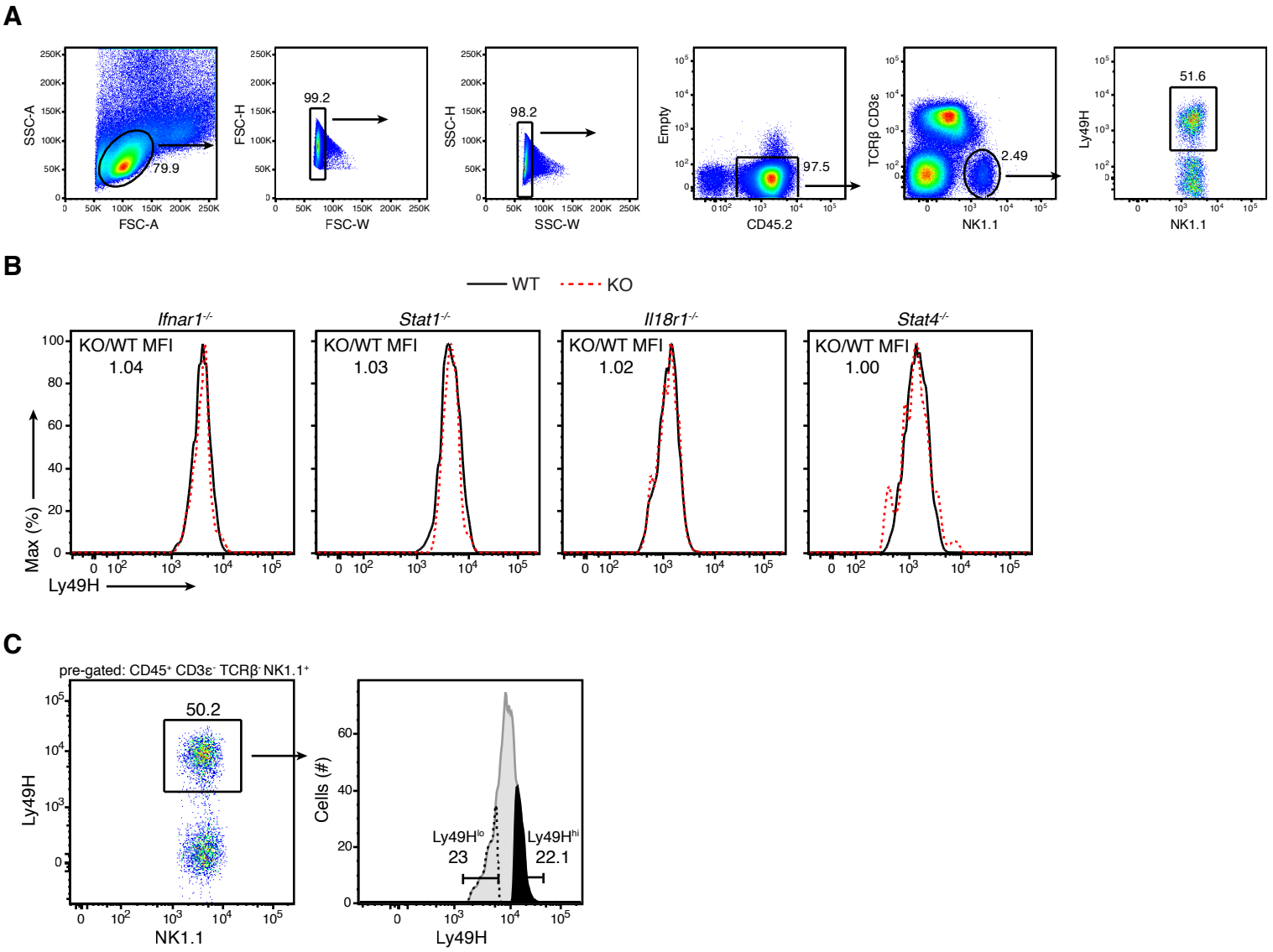

Figure S1. Gating Strategy for Mouse Ly49H$^+$ NK Cells, Related to Figures 1 and 2

(**A**) Representative flow plots from spleen of an uninfected (UI) WT mouse, illustrating the gating strategy for analysis of Ly49H$^+$ NK cells.

(**B**) Histograms of Ly49H expression on WT and indicated KO Ly49H$^+$ NK cells from Figure 1F. For each histogram, the ratio of Ly49H MFI on KO compared to WT Ly49H$^+$ NK cells is shown.

(**C**) Representative flow plots from spleen of an UI WT mouse, illustrating the gating and sorting strategy for Ly49H^{lo} and Ly49H^{hi} NK cells, defined as Ly49H$^+$ NK cells in the bottom or top ~20% by Ly49H MFI, respectively.

Figure S2

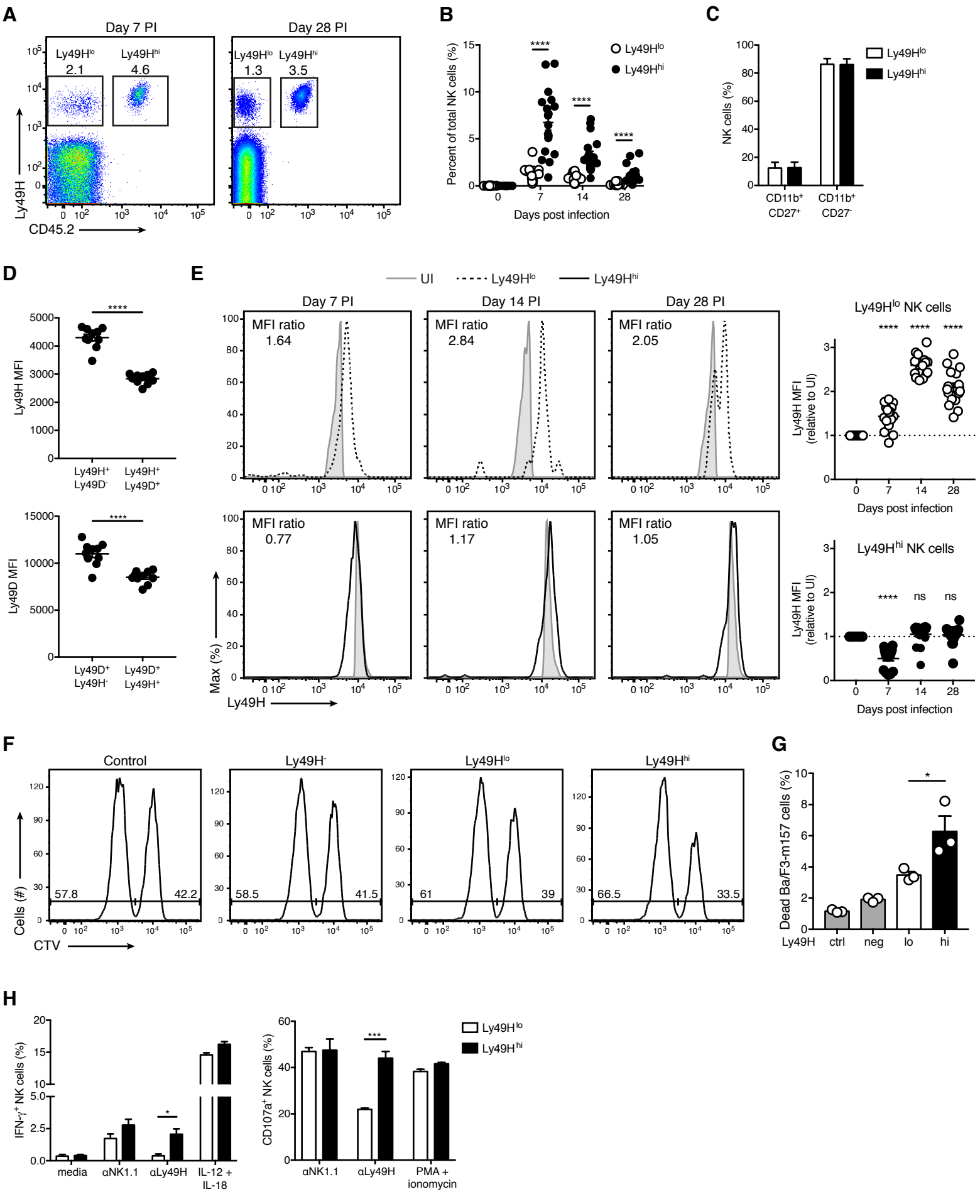
A
Day 7 PI
Day 28 PI
Ly49H^lo
Ly49H^hi
2.1
4.6
1.3
3.5
Ly49H
CD45.2
B
Percent of total NK cells (%)
Days post infection
C
NK cells (%)
CD11b+ CD27+
CD11b+ CD27-
D
Ly49H MFI
Ly49H+ Ly49D-
Ly49H+ Ly49D+
Ly49D MFI
Ly49D+ Ly49H-
Ly49D+ Ly49H+
E
UI
Day 7 PI
Day 14 PI
Day 28 PI
MFI ratio 1.64
MFI ratio 2.84
MFI ratio 2.05
MFI ratio 0.77
MFI ratio 1.17
MFI ratio 1.05
Max (%)
Ly49H
Ly49H^lo NK cells
Ly49H^hi NK cells
Ly49H MFI (relative to UI)
ns
F
Control
Ly49H-
Ly49H^lo
Ly49H^hi
Cells (#)
CTV
57.8
42.2
58.5
41.5
61
39
66.5
33.5
G
Dead Ba/F3-m157 cells (%)
Ly49H ctrl neg lo hi
H
IFN-γ+ NK cells (%)
media
αNK1.1
αLy49H
IL-12 + IL-18
CD107a+ NK cells (%)
PMA + ionomycin

Figure S2. Ly49H^{hi} NK Cells Exhibit Heightened Ly49H-Dependent Responses, Related to Figures 2 and 3

(**A**) Experimental design as in Figure 2A. Flow plots gated on NK cells from blood at day 7 PI and spleen at day 28 PI. Shown is an example in which Ly49H^{lo} (CD45.1) and Ly49H^{hi} (CD45.2) NK cells had the opposite congenic markers to those used in Figure 2B.

(**B**) *Klra8*$^{-/-}$ recipients received either purified splenic Ly49H^{lo} or Ly49H^{hi} NK cells and were infected with MCMV. Quantification of percent Ly49H^{lo} and Ly49H^{hi} NK cells within total NK cells in blood at indicated days PI. Data are pooled from three (Ly49H^{lo}) or four (Ly49H^{hi}) independent experiments with 3-8 mice per group per experiment.

(**C**) Experimental design as in Figure 2A. Percentage of Ly49H^{lo} and Ly49H^{hi} NK cells from blood within the CD11b^{+}CD27^{+} or CD11b^{+}CD27^{-} (terminally mature) subsets at day 7 PI. Data are representative of two independent experiments with 3-5 mice per experiment.

(**D**) Quantification of Ly49H MFI on Ly49H^{+}Ly49D^{-} and Ly49H^{+}Ly49D^{+} NK cells (top) and Ly49D MFI on Ly49H^{-}Ly49D^{+} and Ly49H^{+}Ly49D^{+} NK cells (bottom) from blood of WT mice. Data are representative of three independent experiments with 3-9 mice per experiment.

(**E**) Experimental design as in Figure S2B. Histograms (left three panels) of Ly49H expression on transferred Ly49H^{lo} (top row) or Ly49H^{hi} (bottom row) NK cells from blood compared with the bottom or top 20%, respectively, of naïve Ly49H^{+} NK cells from UI WT mice. Quantification of Ly49H MFI on Ly49H^{lo} or Ly49H^{hi} NK cells at indicated days PI relative to their corresponding naïve NK cell populations (right panels). Data are pooled from three (Ly49H^{lo}) or four (Ly49H^{hi}) independent experiments with 3-8 mice per group per experiment.

(**F-G**) Experimental design as in Figure 2H. (F) Histograms showing distribution of target (CTV^{hi}) and control (CTV^{lo}) cell percentages within live cells following co-culture with the indicated NK cell populations. (G) Percentage of propidium iodide-staining (dead) target cells following co-culture with the indicated NK cell populations. Data are representative of three independent experiments with 3 replicates per group per experiment. Ly49H^{lo} and Ly49H^{hi} NK cells were compared using an unpaired, two-tailed Student's t test.

(**H**) Percentage of purified splenic Ly49H^{lo} or Ly49H^{hi} NK cells producing IFN-γ (left) or degranulating (right) following 4 hour *ex vivo* culture with the indicated stimuli. Data are representative of two independent experiments with 4 replicates per stimulation group per experiment. Groups were compared using an unpaired, two-tailed Student's t test and corrected for testing multiple hypotheses.

Groups were compared using an unpaired, two-tailed Student's t test (B), a paired, two-tailed t test (C, D), or against 1 using a one sample t test (E). Data are presented as the mean ± SEM. ns, not significant; $^{*}p < 0.05$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

Figure S3

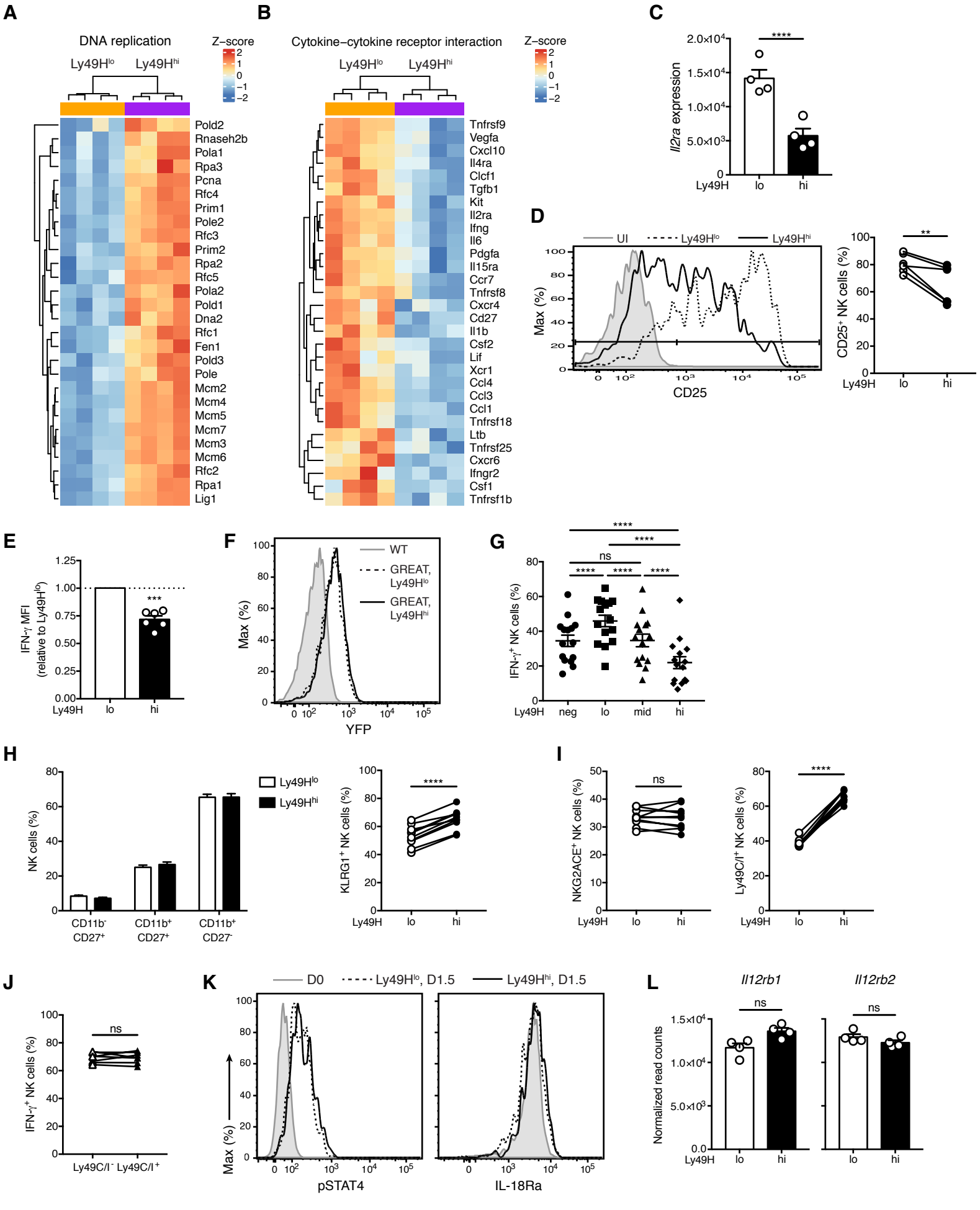
A
DNA replication
Z–score
Ly49H^lo
Ly49H^hi
Pold2
Rnaseh2b
Pola1
Rpa3
Pcna
Rfc4
Prim1
Pole2
Rfc3
Prim2
Rpa2
Rfc5
Pola2
Pold1
Dna2
Rfc1
Fen1
Pold3
Pole
Mcm2
Mcm4
Mcm5
Mcm7
Mcm3
Mcm6
Rfc2
Rpa1
Lig1
B
Cytokine–cytokine receptor interaction
Tnfrsf9
Vegfa
Cxcl10
Il4ra
Clcf1
Tgfb1
Kit
Il2ra
Ifng
Il6
Pdgfa
Il15ra
Ccr7
Tnfrsf8
Cxcr4
Cd27
Il1b
Csf2
Lif
Xcr1
Ccl4
Ccl3
Ccl1
Tnfrsf18
Ltb
Tnfrsf25
Cxcr6
Ifngr2
Csf1
Tnfrsf1b
C
Il2ra expression
D
UI
Max (%)
CD25
CD25+ NK cells (%)
E
IFN-γ MFI (relative to Ly49H^lo)
F
WT
GREAT, Ly49H^lo
GREAT, Ly49H^hi
YFP
G
IFN-γ+ NK cells (%)
neg
mid
H
NK cells (%)
CD11b- CD27+
CD11b+ CD27+
CD11b+ CD27-
KLRG1+ NK cells (%)
I
NKG2ACE+ NK cells (%)
Ly49C/I+ NK cells (%)
J
IFN-γ+ NK cells (%)
Ly49C/I- Ly49C/I+
K
D0
Ly49H^lo, D1.5
Ly49H^hi, D1.5
pSTAT4
IL-18Ra
L
Il12rb1
Il12rb2
Normalized read counts
ns

Figure S3. Ly49H Expression Specifies NK Cell Effector Function During Early MCMV Infection, Related to Figure 3

(**A-C**) Experimental design as in Figures 3A-D. (A-B) Heat map and hierarchical clustering of differential KEGG pathway genes found to be significantly upregulated in Ly49H^{hi} NK cells (DNA replication, A) or Ly49H^{lo} NK cells (cytokine-cytokine receptor interaction, B) at day 1.5 PI. (C) RNA-seq reads mapping to the *Il2ra* locus. P value was calculated in DESeq2 and adjusted for testing multiple hypotheses.

(**D**) Histograms of CD25 expression on splenic Ly49H^{+} NK cells from UI and MCMV-infected WT mice at day 1.5 PI (left). Quantification of the percentage of CD25^{+} NK cells within indicated NK cell populations (right). Data are representative of three independent experiments with 6-15 mice per experiment.

(**E**) Experimental design as in Figure 3E. IFN-γ MFI of IFN-γ^{+} Ly49H^{hi} NK cells relative to IFN-γ^{+} Ly49H^{lo} NK cells at day 1.5 PI. Data are representative of at least five independent experiments with 3-15 mice per experiment.

(**F**) Histograms of YFP expression in naïve Ly49H^{lo} or Ly49H^{hi} NK cells from blood of *Ifng*-IRES-YFP mice. YFP MFI in Ly49H^{+} NK cells from blood of WT mice was used as a control. Data are representative of two independent experiments with 5 mice per experiment.

(**G**) Experimental design as in Figure 3E. Quantification of percent IFN-γ^{+} NK cells within indicated NK cell populations. Ly49H^{mid} NK cells are all Ly49H^{+} NK cells with Ly49H expression between that of Ly49H^{lo} and Ly49H^{hi} NK cells. Data are representative of at least five independent experiments with 3-15 mice per experiment.

(**H**) Percentage of naïve Ly49H^{lo} and Ly49H^{hi} NK cells from blood that fall within each NK cell maturation stage, defined by expression of CD11b and CD27 (left), and that express KLRG1 (right). Data are representative of at least three independent experiments with 5-10 mice per experiment.

(**I**) Percentage of naïve Ly49H^{lo} and Ly49H^{hi} NK cells from blood expressing the licensed (in C57BL/6 mice) inhibitory receptors NKG2A (left) and Ly49C/I (right). Data are representative of three independent experiments with 5-10 mice per experiment.

(**J**) Percentage of splenic Ly49C/I^{-} and Ly49C/I^{+} cells (within Ly49H^{-} NK cells) producing IFN-γ at day 1.5 PI.

(**K**) Histograms of intracellular pSTAT4 (left) and surface IL-18Ra (right) expression on splenic Ly49H^{+} NK cells from UI mice (D0) and Ly49H^{lo} and Ly49H^{hi} NK cells from MCMV-infected WT mice at day 1.5 PI. Data are representative of two independent experiments with 3-6 mice per experiment.

(**L**) Experimental design as in Figures 3A-D. Quantification of RNA-seq reads mapping to the *Il12rb1* (left) and *Il12rb2* (right) loci. P values were calculated in DESeq2 and corrected for testing multiple hypotheses.

Groups were compared using a paired, two-tailed t test (D, H, I, J), against 1 using a one sample t test (E), or an RM one-way ANOVA with Tukey's multiple comparisons test (G). Data are presented as the mean ± SEM. ns, not significant; $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Figure S4

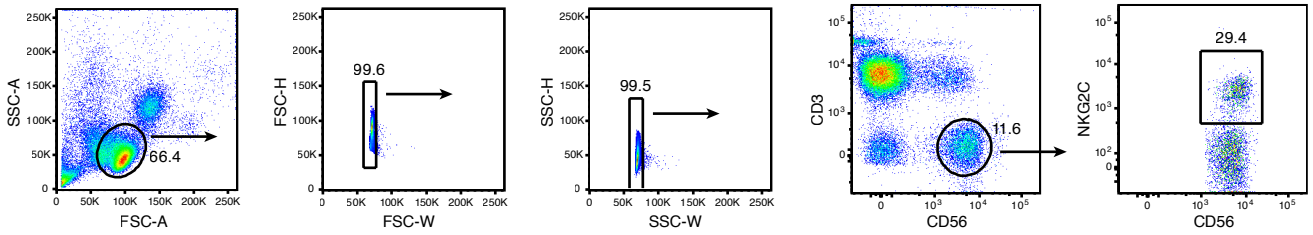

Figure S4. Gating Strategy for Human NKG2C$^+$ NK Cells, Related to Figure 4

Representative flow plots from blood of an HCMV-seropositive donor, illustrating the gating strategy for analysis of human NKG2C$^+$ NK cells.