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Supplemental information

Suppression of adaptive NK cell expansion by macrophage-mediated phagocytosis inhibited by 2B4-CD48

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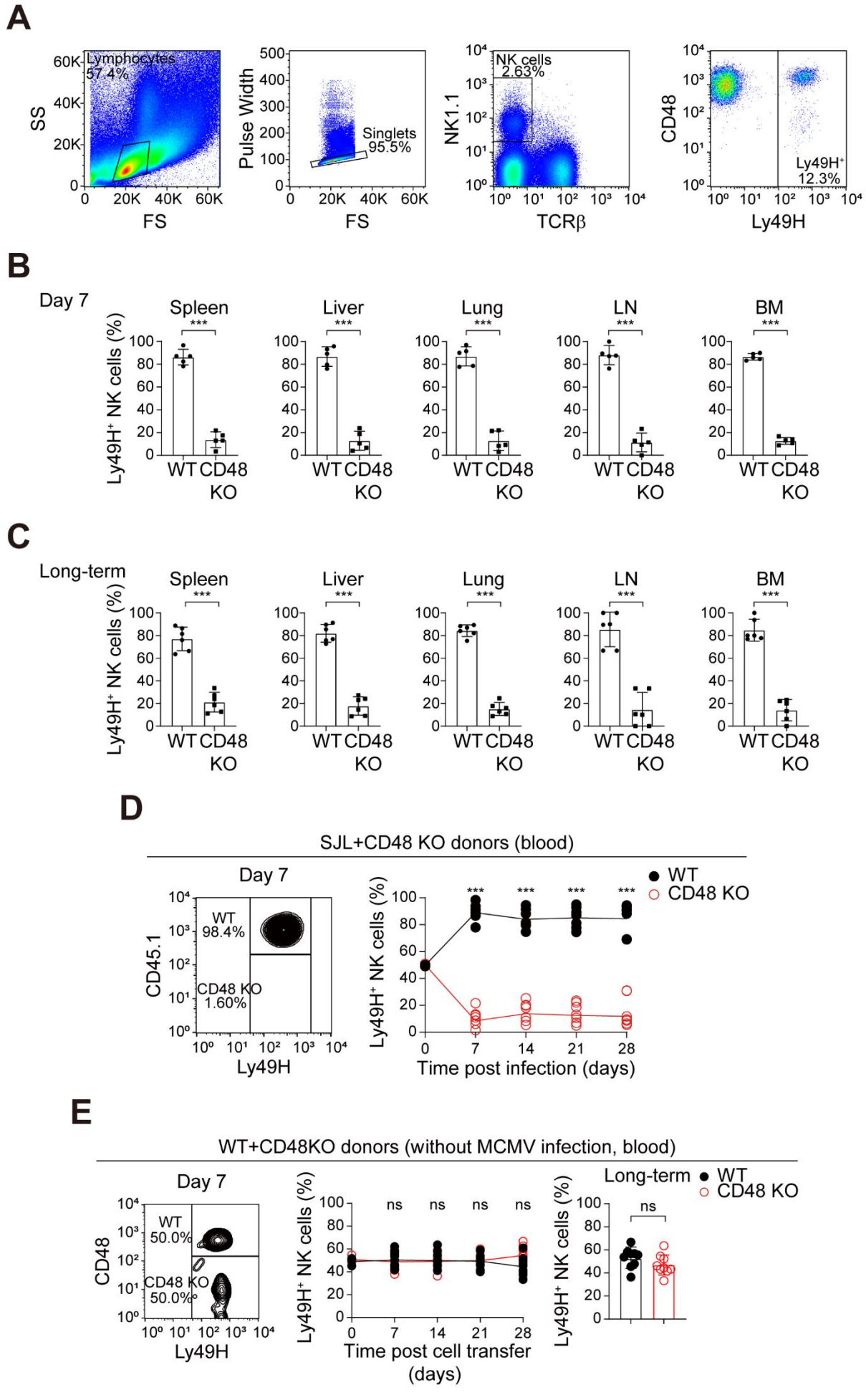
A
Lymphocytes
57.4%
SS
FS
Singlets
95.5%
Pulse Width
NK cells
2.63%
NK1.1
TCRβ
Ly49H+
12.3%
CD48
Ly49H
B
Day 7
Spleen
Liver
Lung
LN
BM
Ly49H+ NK cells (%)
WT
CD48 KO
C
Long-term
D
SJL+CD48 KO donors (blood)
Day 7
WT
98.4%
CD48 KO
1.60%
CD45.1
Ly49H
Time post infection (days)
E
WT+CD48KO donors (without MCMV infection, blood)
WT
50.0%
CD48 KO
50.0%
CD48
Time post cell transfer (days)
Long-term
ns

Figure S1. CD48 deficiency selectively impairs MCMV-induced expansion of Ly49H$^+$ NK cells. Related to Figure 1 and Figure 2.

(A) Representative flow cytometry analysis of the general gating strategy of Ly49H$^+$ NK cells (NK1.1$^+$, TCRβ$^-$, Ly49H$^+$) used in this study is depicted.

(B) Same as Figure 2B, except the proportions of WT and CD48 KO Ly49H$^+$ NK cells were measured in various organs, including the spleen, liver, lung, lymph node (LN) and bone marrow (BM) at day 7 (n = 5).

(C) Same as (B), except the results on day 56 are depicted (n = 6).

(D) Blood. Equal numbers of Ly49H$^+$ NK cells from WT(B6.SJL; CD45.1$^+$/CD45.2$^-$) and CD48 KO (CD45.1$^-$/CD45.2$^+$) donors were mixed and injected into Ly49H KO mice. The proportions of WT versus CD48 KO NK cells in the Ly49H$^+$ population (NK1.1$^+$, TCRβ$^-$, Ly49H$^+$) in blood of recipient mice were analyzed at indicated time points. CD45.1 was used as a marker to distinguish WT (CD45.1$^+$) and CD48 KO (CD45.1$^-$) cells. Left panel: representative flow cytometry analysis of CD45.1 expression on Ly49H$^+$ NK cells at day 7. Proportions of WT (CD45.1$^+$) and CD48 KO (CD45.1$^-$) Ly49H$^+$ NK cells are depicted. Right panel: proportions of WT and CD48 KO Ly49H$^+$ NK cells at various time points in multiple independent mice (n = 7).

(E) Same as Figure 2B, except the adoptive transfer experiments were performed without MCMV infection. From left to right: first panel, representative flow cytometry analysis of CD48 expression on Ly49H$^+$ NK cells in blood of recipient mice at day 7. Proportions of

WT ($CD48^+$) and CD48 KO ($CD48^-$) $Ly49H^+$ NK cells are depicted. Second panel: proportions of WT and CD48 KO $Ly49H^+$ NK cells at various time points in multiple independent mice (n = 9). Third panel: proportions of WT and CD48 KO $Ly49H^+$ NK cells at day 35 in blood for multiple independent mice (n = 9).

Statistical analyses were conducted using unpaired t-tests (two-tailed), except in panels displaying data with multiple time points, where multiple t-tests were employed. Each symbol represents an individual mouse; error bars depict the mean with s.d. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, ns (not significant). Data are representative of 2 (B); 2 (C); 2 (D) and 3 (E) independent experiments, two to four recipient mice were injected with purified NK cells in each independent experiment.

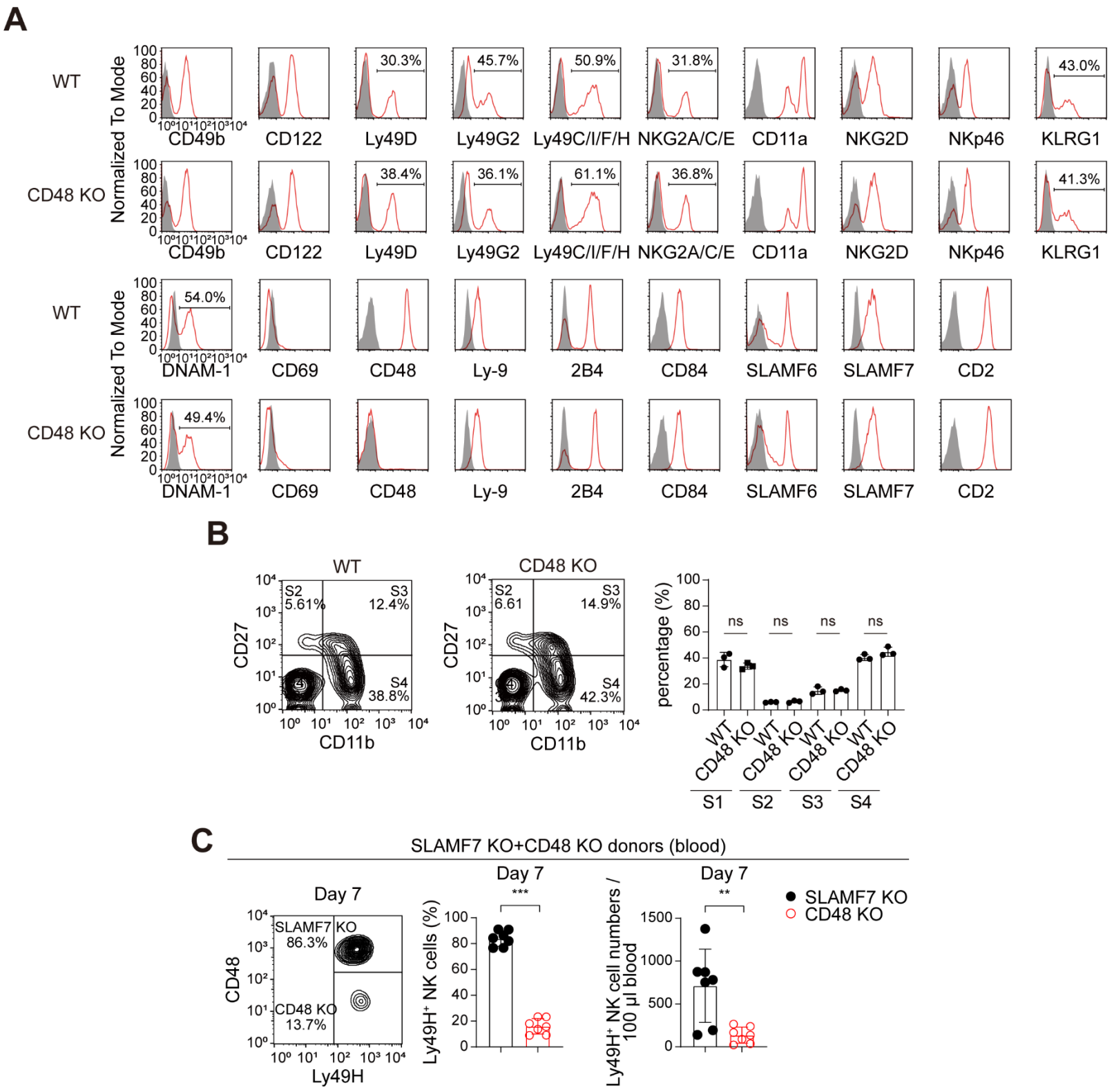
A
WT
CD48 KO
Normalized To Mode
CD49b
CD122
Ly49D
Ly49G2
Ly49C/I/F/H
NKG2A/C/E
CD11a
NKG2D
NKp46
KLRG1
30.3%
45.7%
50.9%
31.8%
43.0%
38.4%
36.1%
61.1%
36.8%
41.3%
DNAM-1
CD69
CD48
Ly-9
2B4
CD84
SLAMF6
SLAMF7
CD2
54.0%
49.4%
B
WT
CD48 KO
CD27
CD11b
S2
5.61%
S3
12.4%
S4
38.8%
6.61
14.9%
42.3%
percentage (%)
ns
S1
S2
S3
S4
C
SLAMF7 KO+CD48 KO donors (blood)
Day 7
SLAMF7 KO
86.3%
CD48 KO
13.7%
CD48
Ly49H
Ly49H+ NK cells (%)
***
Ly49H+ NK cell numbers / 100 µl blood
**
SLAMF7 KO
CD48 KO

Figure S2. CD48 deficiency does not affect NK cell development. Related to Figure 2.

(A) Representative flow cytometry analyses of NK cell receptor repertoires on freshly isolated splenic NK cells ($NK1.1^+$, $TCR\beta^-$) from WT or CD48 KO mice (n = 3).

(B) Flow cytometry analyses of development of freshly isolated splenic NK cells ($NK1.1^+$, $TCR\beta^-$) from WT or CD48 KO mice. CD11b and CD27 were stained to determine the development status of NK cells. Stage 1 (S1), $CD11b^-/CD27^-$; Stage 2 (S2), $CD11b^-/CD27^+$; Stage 3 (S3), $CD11b^+/CD27^+$; Stage 4 (S4), $CD11b^+/CD27^-$. From left to right: first two panels: representative analyses of CD11b and CD27 expression on WT and CD48 KO NK cells. Third panel: proportions of various developmental stages for WT and CD48 KO NK cells (n = 3).

(C) Equal numbers of $Ly49H^+$ NK cells from SLAMF7 KO and CD48 KO donors were mixed and injected into Ly49H KO mice. The proportions of SLAMF7 KO versus CD48 KO NK cells in the $Ly49H^+$ population ($NK1.1^+$, $TCR\beta^-$, $Ly49H^+$) in blood of recipient mice were analyzed at day 7. CD48 was used as a marker to distinguish SLAMF7 KO ($CD48^+$) and CD48 KO ($CD48^-$) cells. From left to right: first panel, representative flow cytometry analysis of CD48 expression on $Ly49H^+$ NK cells at day 7. Proportions of SLAMF7 KO ($CD48^+$) and CD48 KO ($CD48^-$) $Ly49H^+$ NK cells are depicted. Second panel: statistical analysis of the proportions for multiple independent mice (n = 7). Third panel: numbers of SLAMF7 KO and CD48 KO $Ly49H^+$ NK cells per 100 μl blood (n = 7).

Statistical analyses were conducted using unpaired t-tests (two-tailed). Each symbol represents an individual mouse; error bars depict the mean with s.d. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, ns (not significant). Data are representative of 3 independent experiments.

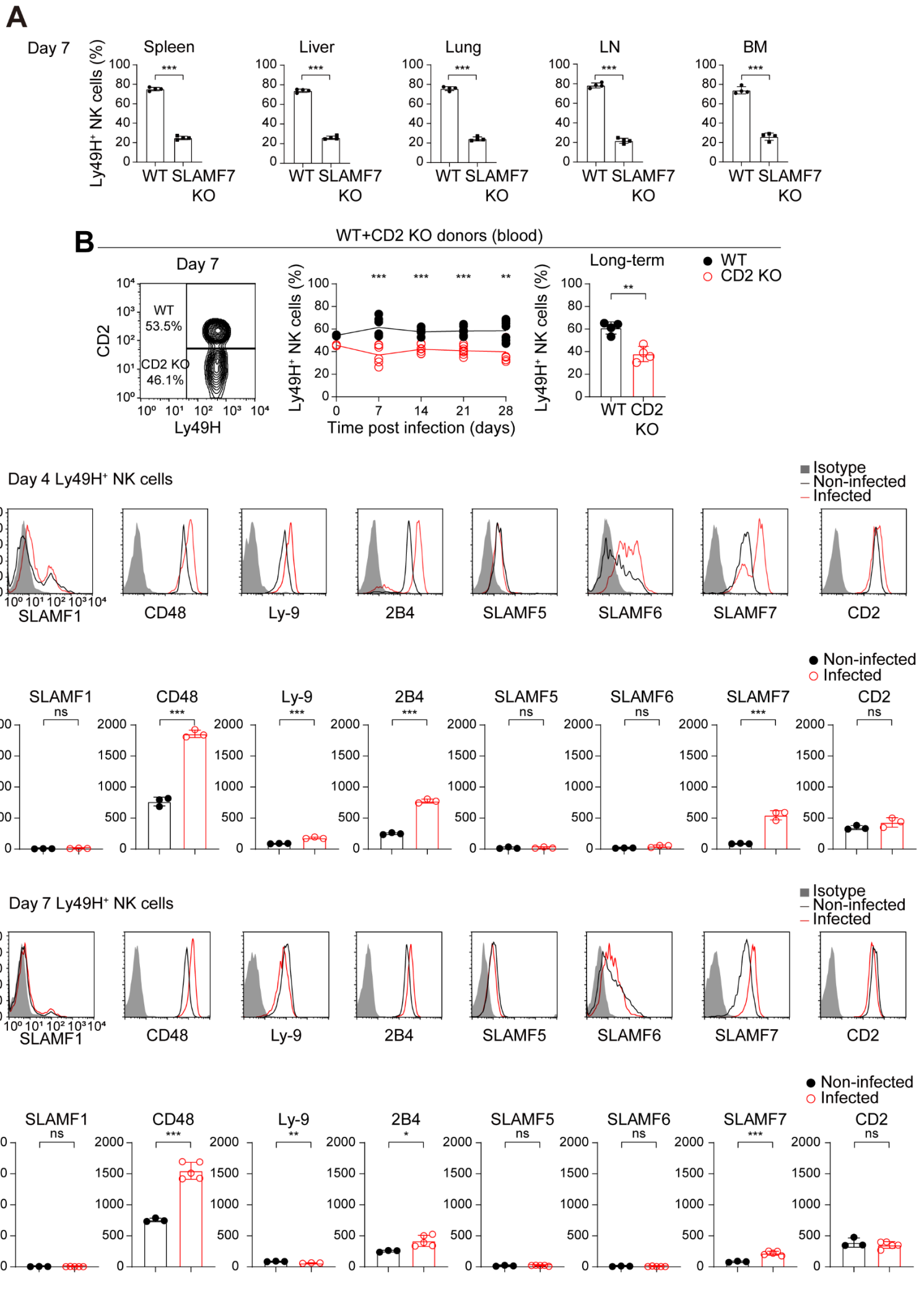
A
Day 7
Spleen
Liver
Lung
LN
BM
Ly49H⁺ NK cells (%)
WT SLAMF7 KO
B
WT+CD2 KO donors (blood)
Day 7
WT 53.5%
CD2 KO 46.1%
CD2
Ly49H
Ly49H⁺ NK cells (%)
Time post infection (days)
Long-term
WT
CD2 KO
WT CD2 KO
C
Day 4 Ly49H⁺ NK cells
Isotype
Non-infected
Infected
Normalized To Mode
SLAMF1
CD48
Ly-9
2B4
SLAMF5
SLAMF6
SLAMF7
CD2
D
Non-infected
Infected
MFI
ns
E
Day 7 Ly49H⁺ NK cells
F

Figure S3. CD48 plays a more crucial role than SLAMF7 in the expansion of Ly49H$^+$ NK cells. Related to Figure 2.

(A) Same as Figure 2C, except the proportions of WT and SLAMF7 KO Ly49H$^+$ NK cells were measured in various organs including the spleen, liver, lung, lymph node (LN) and bone marrow (BM) at day 7 (n = 4).

(B) Blood. Equal numbers of Ly49H$^+$ NK cells from WT and CD2 KO donors were mixed and injected into Ly49H KO mice. The proportions of WT versus CD2 KO NK cells in the Ly49H$^+$ population (NK1.1$^+$, TCRβ$^-$, Ly49H$^+$) in blood of recipient mice were analyzed at indicated time points. CD2 was used as a marker to distinguish WT (CD2$^+$) and CD2 KO (CD2$^-$) cells. From left to right: first panel, representative flow cytometry analysis of CD2 expression on Ly49H$^+$ NK cells at day 7. Proportions of WT (CD2$^+$) and CD2 KO (CD2$^-$) Ly49H$^+$ NK cells are depicted. Second panel: proportions of WT and CD2 KO Ly49H$^+$ NK cells at various time points in multiple independent mice (n = 6). Third panel: proportions of WT and CD2 KO Ly49H$^+$ NK cells in blood at day 56 (n = 4).

(C) On day -1 (1 day before infection), Ly49H$^+$ NK cells from WT donors were injected into Ly49H KO mice. On day 0, 7.5 x 10^2 pfu of MCMV were injected intra-peritoneally into recipients. The SLAM family receptors and CD2 expression on Ly49H$^+$ NK cells (NK1.1$^+$, TCRβ$^-$, Ly49H$^+$) in spleen of recipient mice were analyzed at day 4. Isotype controls are shown in grey, non-infected controls are shown in black, and the cells from

infected mice are shown in red. Representative flow cytometry analyses of the expression of the receptors on Ly49H$^+$ donor NK cells at day 4 are depicted.

(D) Same as (C), except the statistical analyses of flow cytometry results for multiple mice are depicted (n = 3).

(E and F) Same as (C and D), except flow cytometry analysis results at day 7 are depicted. n = 3-5.

Statistical analyses were conducted using unpaired t-tests (two-tailed), except in panels displaying data with multiple time points, where multiple t-tests were employed. Each symbol represents an individual mouse; error bars depict the mean with s.d. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, ns (not significant). Data are representative of 2 (A); 2 (B) and 3 (C-F) independent experiments, one to four recipient mice were injected with purified NK cells in each independent experiment..

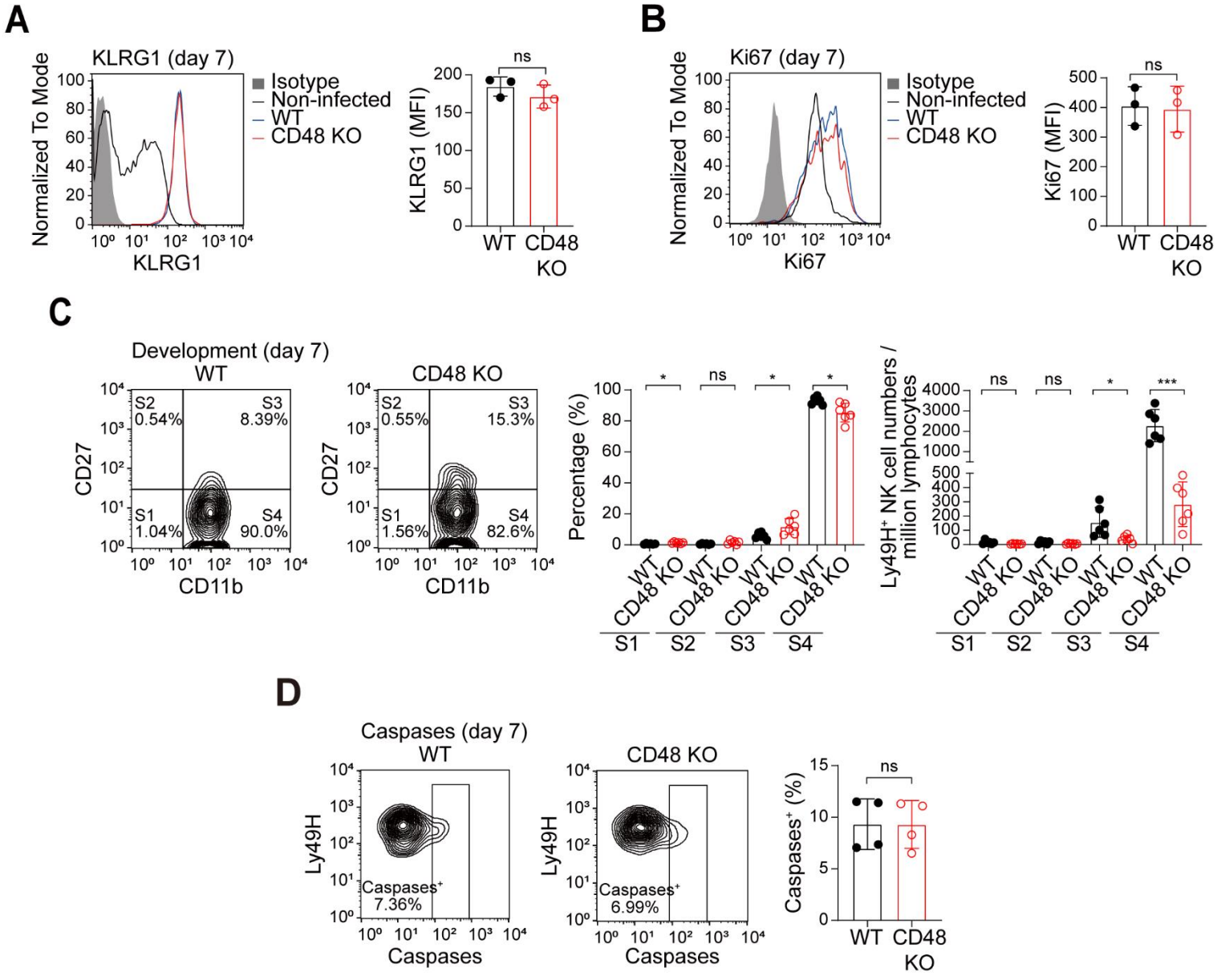
A
KLRG1 (day 7)
Normalized To Mode
Isotype
Non-infected
WT
CD48 KO
KLRG1
KLRG1 (MFI)
ns
WT
CD48 KO
B
Ki67 (day 7)
Normalized To Mode
Isotype
Non-infected
WT
CD48 KO
Ki67
Ki67 (MFI)
ns
WT
CD48 KO
C
Development (day 7)
WT
CD48 KO
CD27
CD11b
S2 0.54%
S3 8.39%
S1 1.04%
S4 90.0%
S2 0.55%
S3 15.3%
S1 1.56%
S4 82.6%
Percentage (%)
Ly49H+ NK cell numbers / million lymphocytes
ns
*
***
WT
CD48 KO
S1
S2
S3
S4
D
Caspases (day 7)
WT
CD48 KO
Ly49H
Caspases
Caspases+ 7.36%
Caspases+ 6.99%
Caspases+ (%)
ns
WT
CD48 KO

Figure S4. Minimal effect of CD48 deficiency on NK cell activation and proliferation. Related to Figure 3.

(A-D) Same as in Figure 3B-E, except results at day 7 are depicted. Statistical analyses were conducted using unpaired t-tests (two-tailed). Each symbol represents an individual mouse; error bars depict the mean with s.d. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, ns (not significant).

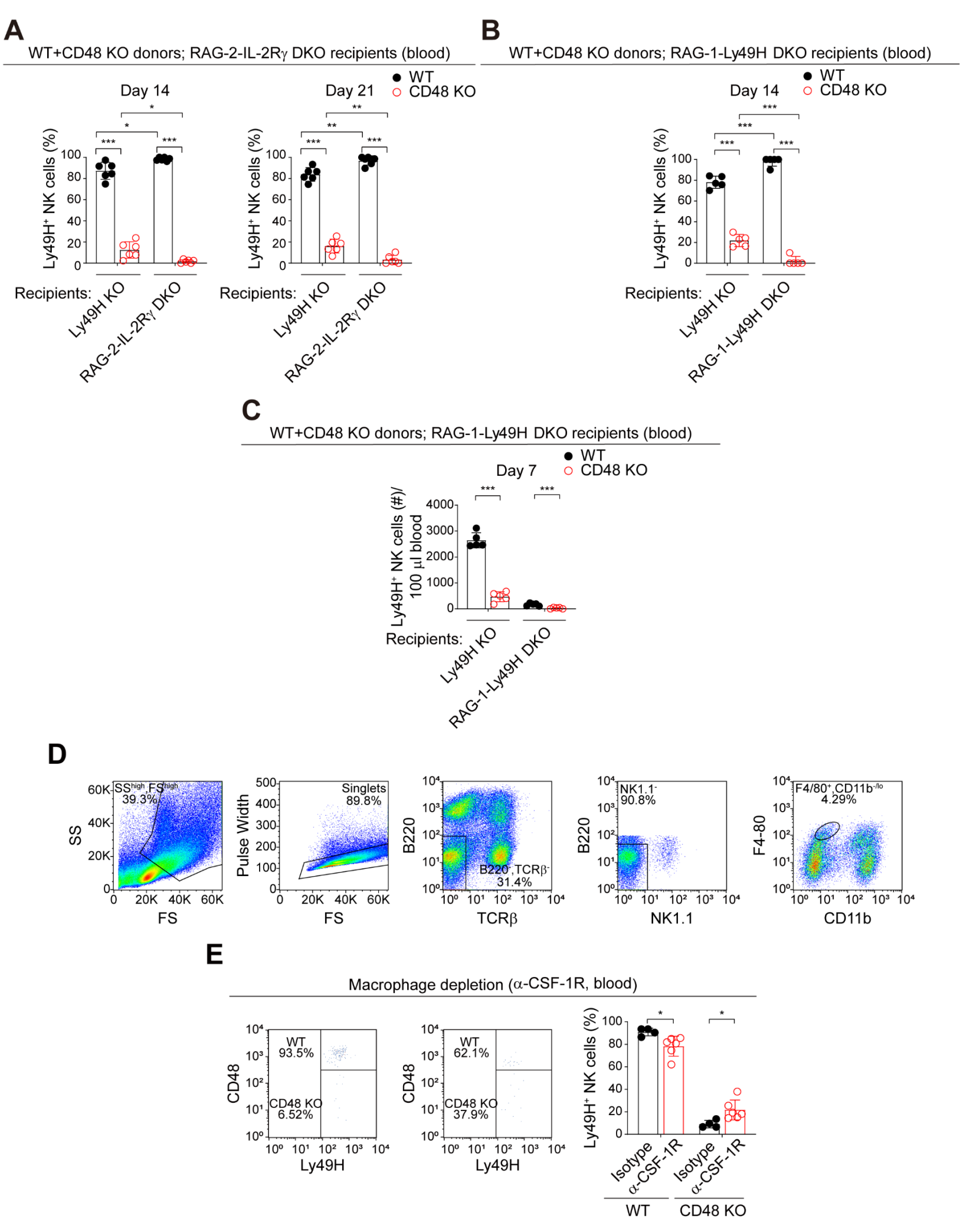
A
WT+CD48 KO donors; RAG-2-IL-2Rγ DKO recipients (blood)
Day 14
Day 21
WT
CD48 KO
Ly49H+ NK cells (%)
Recipients:
Ly49H KO
RAG-2-IL-2Rγ DKO
B
WT+CD48 KO donors; RAG-1-Ly49H DKO recipients (blood)
Day 14
WT
CD48 KO
Ly49H+ NK cells (%)
Recipients:
Ly49H KO
RAG-1-Ly49H DKO
C
WT+CD48 KO donors; RAG-1-Ly49H DKO recipients (blood)
Day 7
WT
CD48 KO
Ly49H+ NK cells (#)/ 100 µl blood
Recipients:
Ly49H KO
RAG-1-Ly49H DKO
D
SShigh FShigh 39.3%
SS
FS
Singlets 89.8%
Pulse Width
FS
B220
TCRβ
B220+TCRβ- 31.4%
NK1.1-
90.8%
B220
NK1.1
F4/80+CD11b-/lo
4.29%
F4-80
CD11b
E
Macrophage depletion (α-CSF-1R, blood)
CD48
Ly49H
WT
93.5%
CD48 KO
6.52%
WT
62.1%
CD48 KO
37.9%
Ly49H+ NK cells (%)
Isotype
α-CSF-1R
WT
CD48 KO

Figure S5. Macrophages limit the expansion of Ly49H$^+$ NK cells. Related to Figure 5.

(A) Same as in Figure 5B, except results at day 14 and day 21 are depicted (n = 6).

(B) Same as in Figure 5C, except results at day 14 are depicted (n = 5).

(C) Same as in Figure 5C, except numbers of WT and CD48 KO Ly49H$^+$ NK cells per 100 µl blood at day 7 are depicted (n = 5).

(D) Representative flow cytometry analysis of the general gating strategy of fresh isolated macrophages (B220$^-$, TCRβ$^-$, NK1.1$^-$, F4/80$^+$, CD11b$^{-/lo}$) used in this study is depicted.

(E) Same as Figure 5E, except blood was analyzed [n = 4 (isotype control); n = 6 (α-CSF-1R)].

Statistical analyses were conducted using unpaired t-tests (two-tailed), except in panels that involved comparisons of more than two groups, where one-way ANOVA followed by Tukey's multiple comparison tests were employed to assess significance. Each symbol represents an individual mouse; error bars depict the mean with s.d. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, ns (not significant). Data are representative of 2 independent experiments, two to three recipient mice were injected with purified NK cells in each independent experiment.