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Supplemental Information

CD94 Is Essential for NK Cell-Mediated Resistance to a Lethal Viral Disease

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Supplemental Figures and Legends

Figure S1, related to Figure 3. Anti-NKG2 mAb 20D5HCmIgG1-Q blocks, but does not deplete NK cells *in vivo*.

Figure S2, related to Figure 4. Impaired NK cell control of virus loads in the absence of CD94 results in a defective $CD8^+$ T cell response.

Figure S3, related to Figure 6. Surface staining of reporter cells.

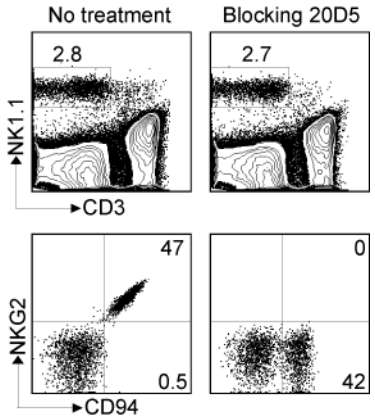

Figure S1. Anti-NKG2 mAb 20D5HCmIgG1-Q blocks, but does not deplete NK cells *in vivo*. Wild type B6 mice were treated i.p. with 150 µg 20D5HCmIgG1-Q mAb in 500 µl PBS. One day later, blood was obtained from untreated or treated mice and analyzed by flow cytometry. **Upper panel**: gated on total cells. **Lower panel:** gated on NK1.1+ CD3- NK cells.

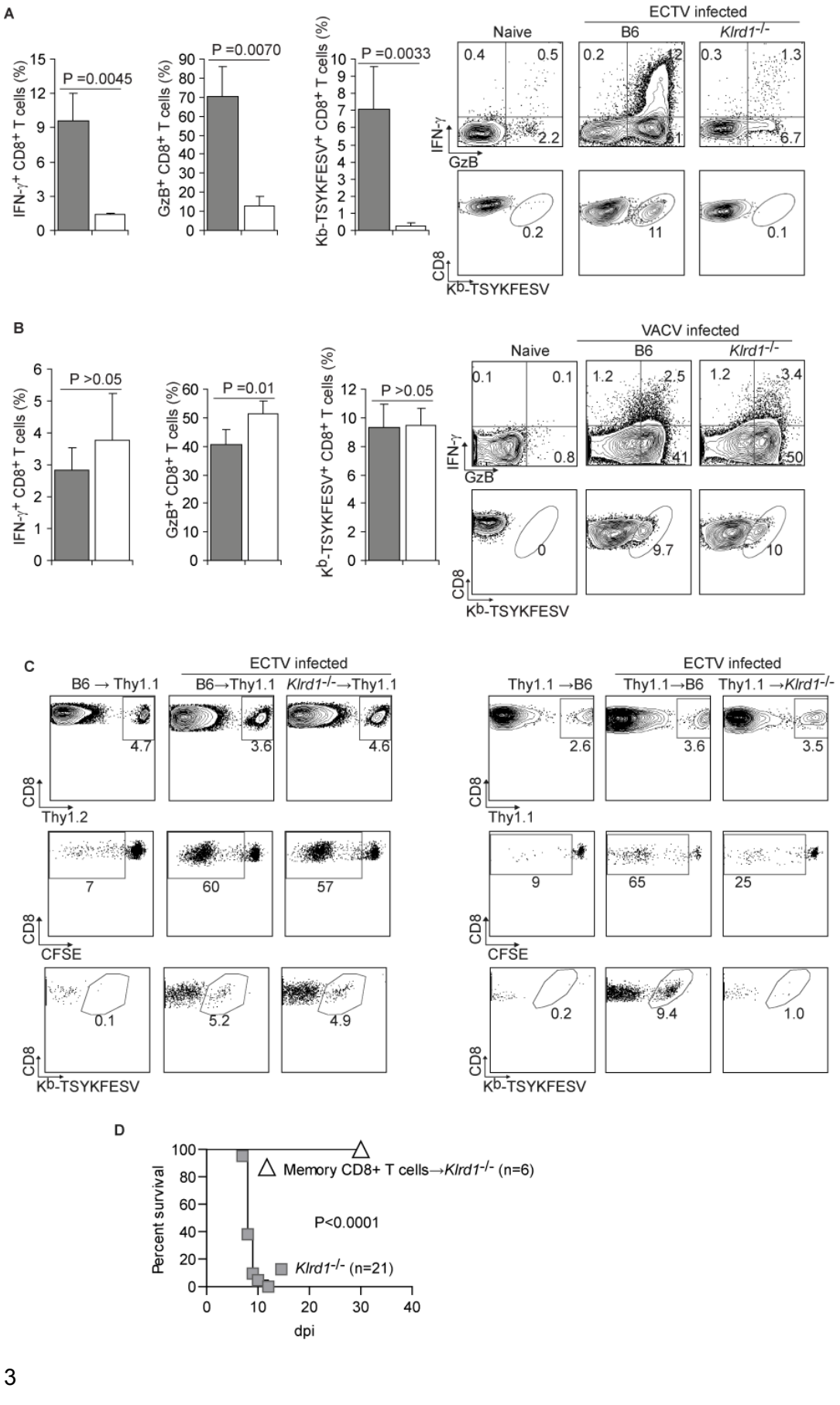
A
IFN-γ+ CD8+ T cells (%)
P =0.0045
GzB+ CD8+ T cells (%)
P =0.0070
Kb-TSYKFESV+ CD8+ T cells (%)
P =0.0033
Naive
ECTV infected
B6
Klrd1-/-
0.4
0.5
2.2
0.2
12
11
0.3
1.3
6.7
IFN-γ
GzB
0.2
11
0.1
CD8
Kb-TSYKFESV
B
IFN-γ+ CD8+ T cells (%)
P >0.05
GzB+ CD8+ T cells (%)
P =0.01
Kb-TSYKFESV+ CD8+ T cells (%)
P >0.05
Naive
VACV infected
B6
Klrd1-/-
0.1
0.1
0.8
1.2
2.5
41
1.2
3.4
50
IFN-γ
GzB
0
9.7
10
CD8
Kb-TSYKFESV
C
B6 → Thy1.1
ECTV infected
B6→Thy1.1
Klrd1-/-→Thy1.1
4.7
3.6
4.6
CD8
Thy1.2
7
60
57
CFSE
0.1
5.2
4.9
Kb-TSYKFESV
Thy1.1 →B6
Thy1.1→B6
Thy1.1 →Klrd1-/-
2.6
3.6
3.5
Thy1.1
9
65
25
0.2
9.4
1.0
D
Memory CD8+ T cells→Klrd1-/- (n=6)
P<0.0001
Klrd1-/- (n=21)
Percent survival
dpi

Figure S2. Impaired NK cell control of virus loads in the absence of CD94 results in a defective $CD8^+$ T cell response. A) Wild type B6 or CD94-deficient mice were infected with ECTV in the footpad and the splenic $CD8^+$ T cell responses were determined by flow cytometry 7 dpi. The column graphs on the right are the cumulative data for 3 experiments with 3-5 ECTV-infected mice/group. Parameters as indicated (gray columns, wild type B6 mice; white columns, CD94-deficient mice). Representative flow cytometry plots for infected and control uninfected mice as used to collect the data on the column graphs are shown on the right. **Upper plots:** IFN-γ and GzB following *ex vivo* re-stimulation with VACV-infected cells. **Lower panel:** surface staining with H2-K^b-TSYKFESV dimers. Plots are gated on $CD8^+$ $CD3^+$ cells. **B)** Wild type or CD94-deficient B6 mice were infected with VACV i.p. and the splenic $CD8^+$ T cell responses were determined by flow cytometry 7 dpi. The column graphs on the right are the summary data for 5 ECTV-infected mice/group. Parameters as indicated (gray columns, wild type B6 mice; white columns, CD94-deficient mice). The flow cytometry plots on the right are from representative individual mice used to obtain the data on the column graphs. **Upper panel:** IFN-γ and GzB following *ex vivo* re-stimulation with VACV-infected cells. **Lower panel:** surface staining with H2-K^b-TSYKFESV dimers. Plots are gated on $CD8^+$ $CD3^+$ cells. **C)** Responses of adoptively transferred $CD8^+$ T cells from wild type B6 or CD94-deficient mice into CD94-sufficient mice or CD94-deficient mice, as indicated. Total lymphocytes from naïve CD94-sufficient or CD94-deficient mice were labeled with CFSE, injected i.v. into Thy1.1 or Thy1.2 congenic mice. One dpi the recipient mice were infected with ECTV. The donor $CD8^+$ T cell responses were determined in spleens 7 dpi. **Upper panels:** gated on total $CD8^+$ cells. **Middle and lower panels**: gated on $CD8^+$ cells from donor origin with gates shown in the upper panels. The data are representative from two experiments with three pooled mice/group. **D)** CD94-deficient mice adoptively transferred with magnetically purified $CD8^+$ T cells from CD94-deficient VACV-immune mice or naive CD94-deficient were infected with ECTV and survival was monitored.

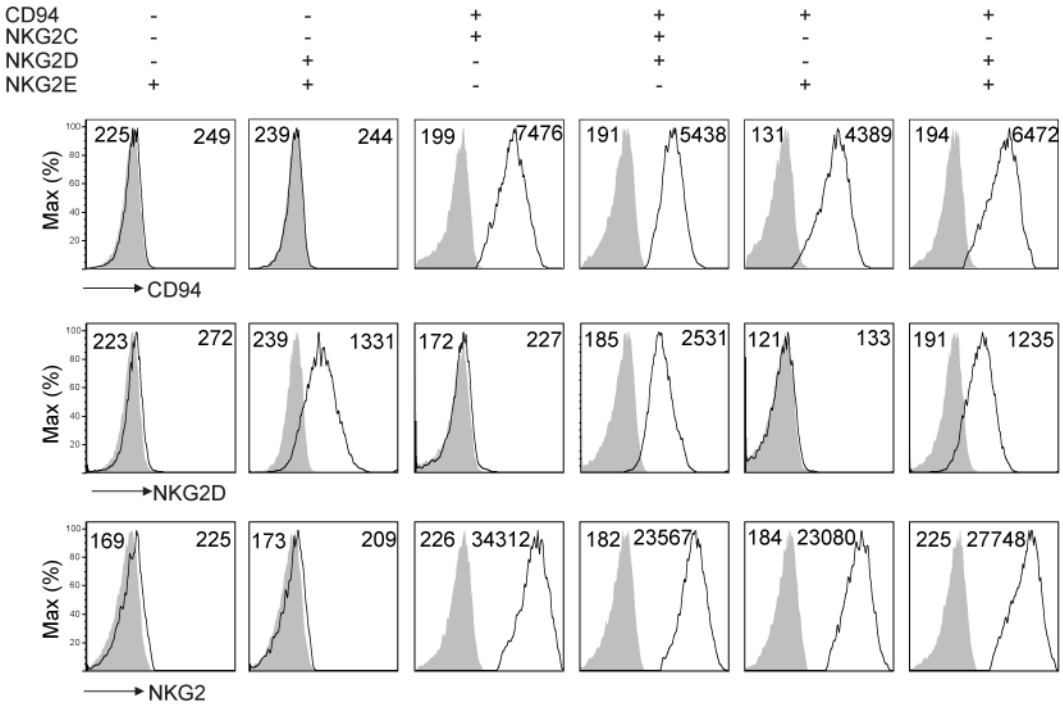

Figure S3. Surface staining of reporter cells. NFAT-GFP reporter cells expressing the indicated molecules were surface stained, as indicated, and analyzed by flow cytometry. Numbers indicated the mean fluorescence intensity of the nearest peak.