Expanded View Figures

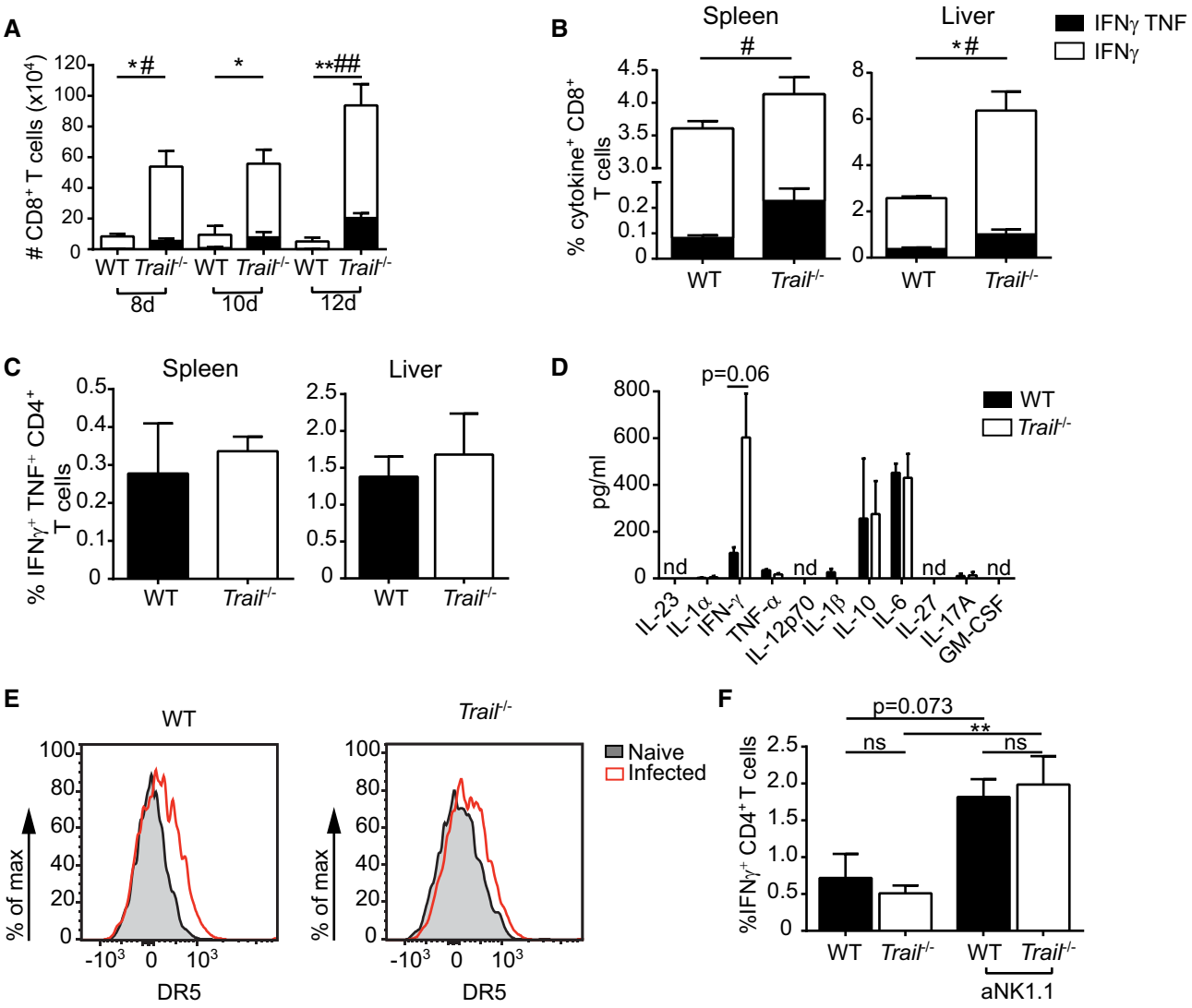

Figure EV1. *Trail* deficiency leads to an altered immune response in LCMV-infected mice.

A–C Total numbers of cytokine-producing $GP_{33–41}$-specific $CD8^+$ T cells were counted in the spleen at the indicated time points after LCMV infection (A). Frequencies of cytokine-producing $NP_{396–404}$-specific $CD8^+$ T cells (B) or $GP_{61–80}$-specific $CD4^+$ T cells (C) were measured 8 days postinfection. Data shown are mean ± SEM of $n = 3$ mice per group and are from at least 2–3 experiments. Unpaired two-tailed *t*-test was used. $^*P < 0.05$; $^{**}P < 0.01$ between $IFN\gamma^+$ cells. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ between $IFN\gamma^+$ TNF^+ cells.

D Cytokine concentrations were measured in the serum 36 h after LCMV infection using a cytokine multiplex assay. Data indicate mean ± SEM of $n = 4$ mice per group. nd, non-detectable. One experiment was performed. Statistical analyses were performed using unpaired two-tailed *t*-test.

E Mice were infected with LCMV, and DR5 was measured on splenic monocytes (defined as $CD11b^+CD11c^-Ly6C^+Ly6G^-$ cells) 24 h after infection. Naïve monocytes were used as a control, and data show one representative of $n = 3$ infected mice per group. One experiment was performed.

F Mice were infected with LCMV, and frequencies of $IFN\gamma^+$ $GP_{61–80}$-specific $CD4^+$ T cells were measured in the spleen 8 days postinfection. When indicated (aNK1.1), NK cells were depleted. Data shown are mean ± SEM of $n = 3$ mice per group and are representative of at least two independent experiments. One-way ANOVA with Tukey post-test was used. ns, non-significant; $^{**}P < 0.01$.

Figure EV2. TRAIL signaling controls the expression of multiple genes in NK cells during early LCMV infection.

A Venn diagram showing the difference and overlap of genes that are differentially expressed in NK cells of WT versus *Trail*$^{-/-}$ mice during LCMV infection (for an adjusted *P*-value < 0.01 and absolute log2 fold change ≥ 2).

B, C Gene Ontology graph showing relationships between pathways that are associated with differentially expressed genes in NK cells of WT (B) or *Trail*$^{-/-}$ (C) mice during LCMV infection. Colored dots indicate the most semantically specific pathways, and gray dots represent connection nodes. Pathways related to inflammation are highlighted by a red ring.

Source data are available online for this figure

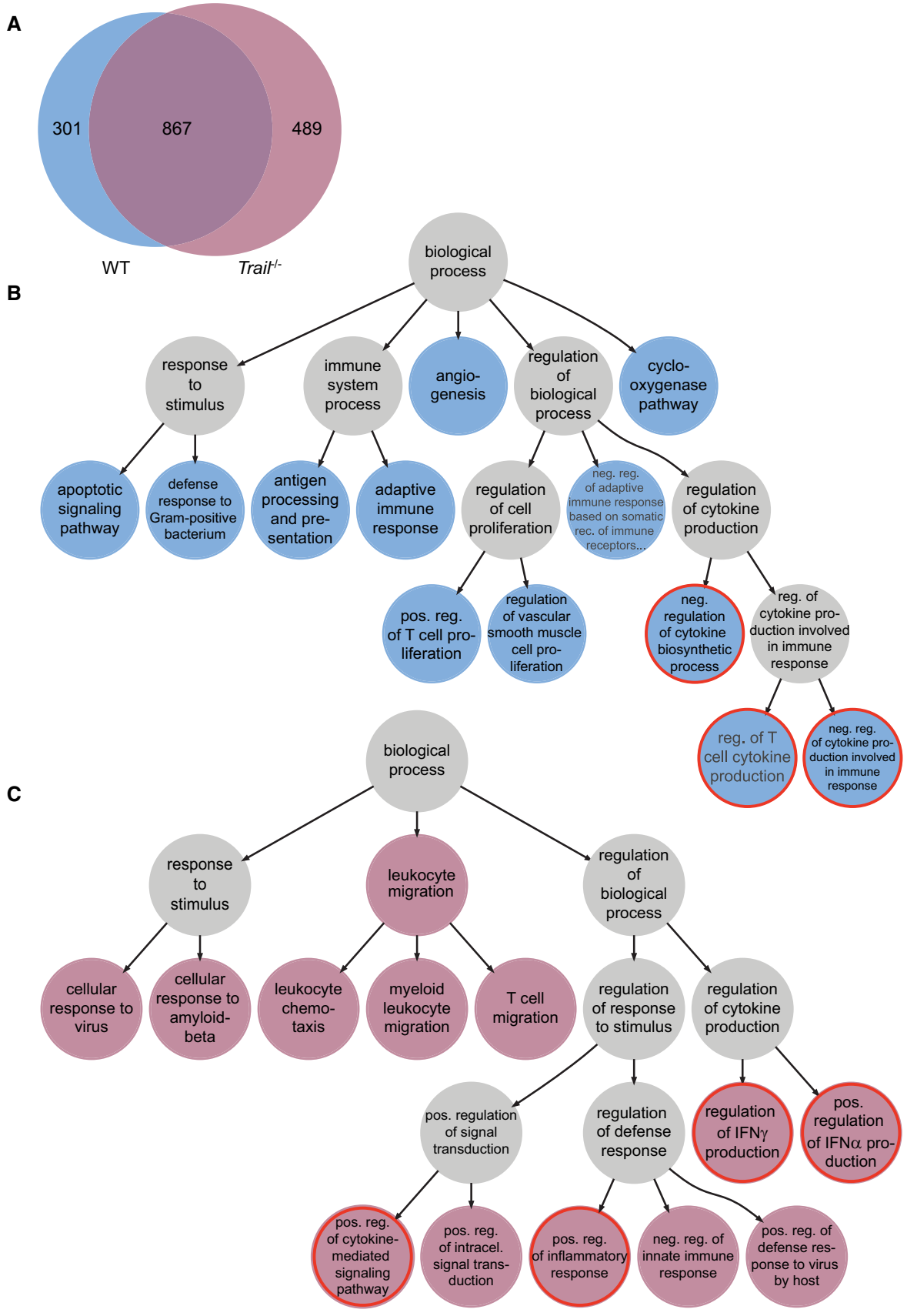

Figure EV2.

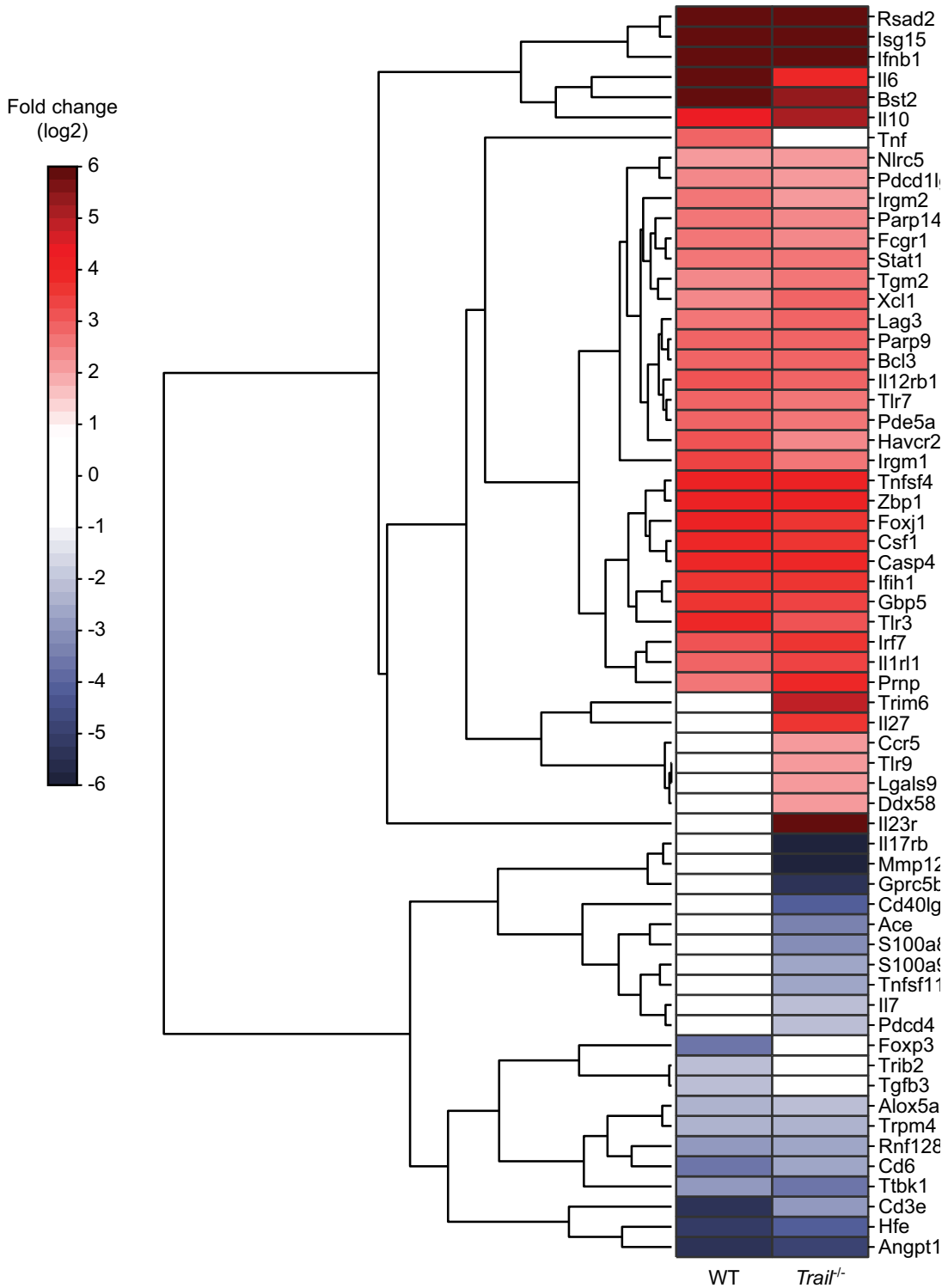

Figure EV3. TRAIL signaling controls the expression of multiple genes in NK cells during early LCMV infection.

Genes listed in the pathways highlighted by a red ring in Fig EV2 were clustered hierarchically according to their expression pattern. Each row in the dendrogram depicts the gene log2 fold changes during infection of WT or *Trail*$^{-/-}$ mice. In each sample group, dark red indicates higher and dark blue lower transcript expression.

Source data are available online for this figure

Figure EV4. Numbers and surface markers of NK and dendritic cells are unaltered in LCMV-infected *Trail*$^{-/-}$ mice.

A Mice were infected with LCMV and analyzed 24 h postinfection. Total numbers of NK cells were measured in the spleen and liver. Data shown are mean ± SEM of $n = 3$ mice per group and are representative of at least three independent experiments. Unpaired two-tailed *t*-test was used.

B–E Mice were infected with LCMV and analyzed 24 h postinfection. Frequencies (B) and total numbers of CD11c$^+$ dendritic cells (C) were analyzed in the spleen. Frequencies of CD11c$^+$ CD86$^+$ cells and CD86 expression on CD11c$^+$ cells (D), and frequencies of CD11c$^+$ I-A^b MHCII$^+$ cells and I-A^b MHC II expression on CD11c$^+$ cells (E) were quantified in the spleen. Data shown are mean ± SEM of $n = 3$ mice per group and are representative of at least two independent experiments. MFI, mean fluorescence intensity. Unpaired two-tailed *t*-test was used.

F–J Mice were infected with LCMV, and NK cells were analyzed 24 h postinfection. Frequencies of splenic NK cells expressing the indicated markers and mean fluorescence intensity (MFI) levels of these markers were assessed in spleen (F) and liver (G). Frequencies of Ly49H-positive NK cells were quantified by flow cytometry in the spleens of naïve mice (H). Eomes (I) or T-bet (J) expression was analyzed by measuring frequencies of positive NK cells or MFI levels 24 h postinfection. (F, G, I, J) Data shown are mean ± SEM of $n = 3$ mice per group from three independent experiments. (H) Data show one representative of two independent experiments, with 2 representative mice out of 3 tested per strain. Unpaired two-tailed *t*-test was used. *$P < 0.05$; **$P < 0.01$.

K NK cells were isolated from naïve mice, and *Gzmb* transcript levels were quantified. Data are represented as fold induction relative to *Gapdh*. Data shown are mean ± SEM of $n = 3$ mice per group from a unique experiment. Unpaired two-tailed *t*-test was used.

L NK cells from naïve mice were analyzed for CD11b and CD27 expression. DN: double-negative, CD11b^{low}CD27^{low} NK cells; CD11b^{low}: CD11b^{low}CD27^{hi} NK cells; DP: double-positive, CD11b^{hi}CD27^{hi} NK cells; CD27^{low}: CD11b^{hi}CD27^{low} NK cells. Data shown are mean ± SEM of $n = 3$ mice per group from three independent experiments. Unpaired two-tailed *t*-test was used.

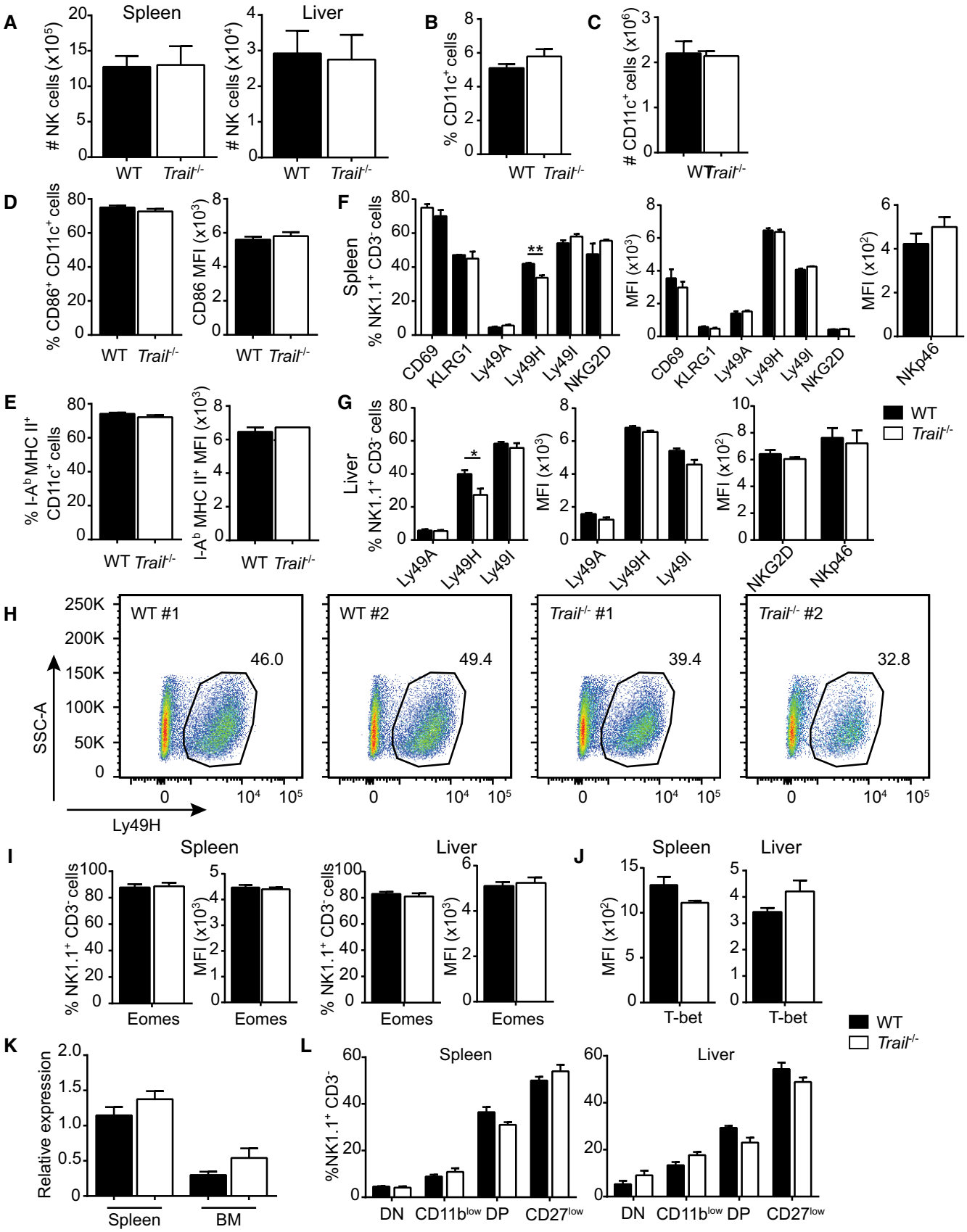
A
Spleen
NK cells (x10^5)
WT
Trail-/-
Liver
NK cells (x10^4)
B
% CD11c+ cells
C
CD11c+ cells (x10^6)
D
% CD86+ CD11c+ cells
CD86 MFI (x10^3)
E
% I-Ab MHC II+ CD11c+ cells
I-Ab MHC II+ MFI (x10^3)
F
Spleen
% NK1.1+ CD3- cells
MFI (x10^3)
MFI (x10^2)
CD69
KLRG1
Ly49A
Ly49H
Ly49I
NKG2D
NKp46
G
Liver
WT
Trail-/-
H
WT #1
46.0
WT #2
49.4
Trail-/- #1
39.4
Trail-/- #2
32.8
SSC-A
Ly49H
I
Eomes
J
T-bet
K
Relative expression
Spleen
BM
L
%NK1.1+ CD3-
DN
CD11blow DP
CD27low

Figure EV4.

Figure EV5. TRAIL regulates IL-15 and NK1.1 signaling in NK cells.

A NK cells were MACS-purified from single-splenocyte suspensions from naïve donors, stimulated *in vitro* for 1 h with IL-15, and phosphorylation of S6 was measured by flow cytometry. One representative of two independent experiments is depicted (n = 1 mouse per group).

B AKT and S6 phosphorylation were measured in naive splenic NK cells. Data shown are mean ± SEM of n = 3 mice per group from at least two independent experiments. Statistical analyses were performed using unpaired two-tailed t-test.

C Splenocytes were cultured with IL-15 ± wortmannin (WRM)/LY294002, and GZMB expression was measured in NK cells. Data shown are mean ± SEM of n = 3 mice per group from at least two independent experiments. Statistical analyses were performed using one-way ANOVA with Dunn's post-test. *$P < 0.05$; **$P < 0.01$.

D, E WT splenocytes were cultured with IL-15 ± TRAIL-R2-Fc chimeric protein, and AKT phosphorylation (D) or S6 phosphorylation (E) was measured in NK cells. Values shown were normalized to unstimulated control. Data show n = 5 mice per group, pooled from two independent experiments. Statistical analyses were performed using paired two-tailed t-test. *$P < 0.05$; **$P < 0.01$.

F–H Splenocytes from naïve WT and *Trail*$^{-/-}$ mice were cultured with the indicated cytokines, and frequencies of IFNγ^+ NK cells (F), or IFNγ-expression levels in NK cells (G) were measured after 5 h. Alternatively, naïve splenocytes were cultured with IL-18/IL-12, and IFNγ was measured in the supernatant at the indicated time points (H). Data shown are mean ± SEM of n = 3–4 mice per group and are representative of two (F, G) or three (H) independent experiments. Unpaired two-tailed t-test was used. *$P < 0.05$.

I, J MACS-purified DX5$^+$ cells were cultured in wells coated with an anti-NK1.1 antibody, and frequencies of IFNγ^+ NK cells were measured after 5 h (I) or GZMB expression was measured after 24 h (J). Data shown are mean ± SEM of n = 3–4 mice per group and are representative of three (I) or one (J) independent experiments. Unpaired two-tailed t-test was used. **$P < 0.01$.

K–O Flow cytometry was applied on NK-92 cells to assess expression of TRAIL (K) and the TRAIL receptors DR4 (L) and DR5 (M). NK-92 cells were stimulated with IL-2 ± human TRAIL-R2-Fc chimeric protein, and S6 phosphorylation (N) or GZMB expression (O) was measured by flow cytometry (n = 1 per condition). (N, O) Data show one representative of 2 independent experiments.

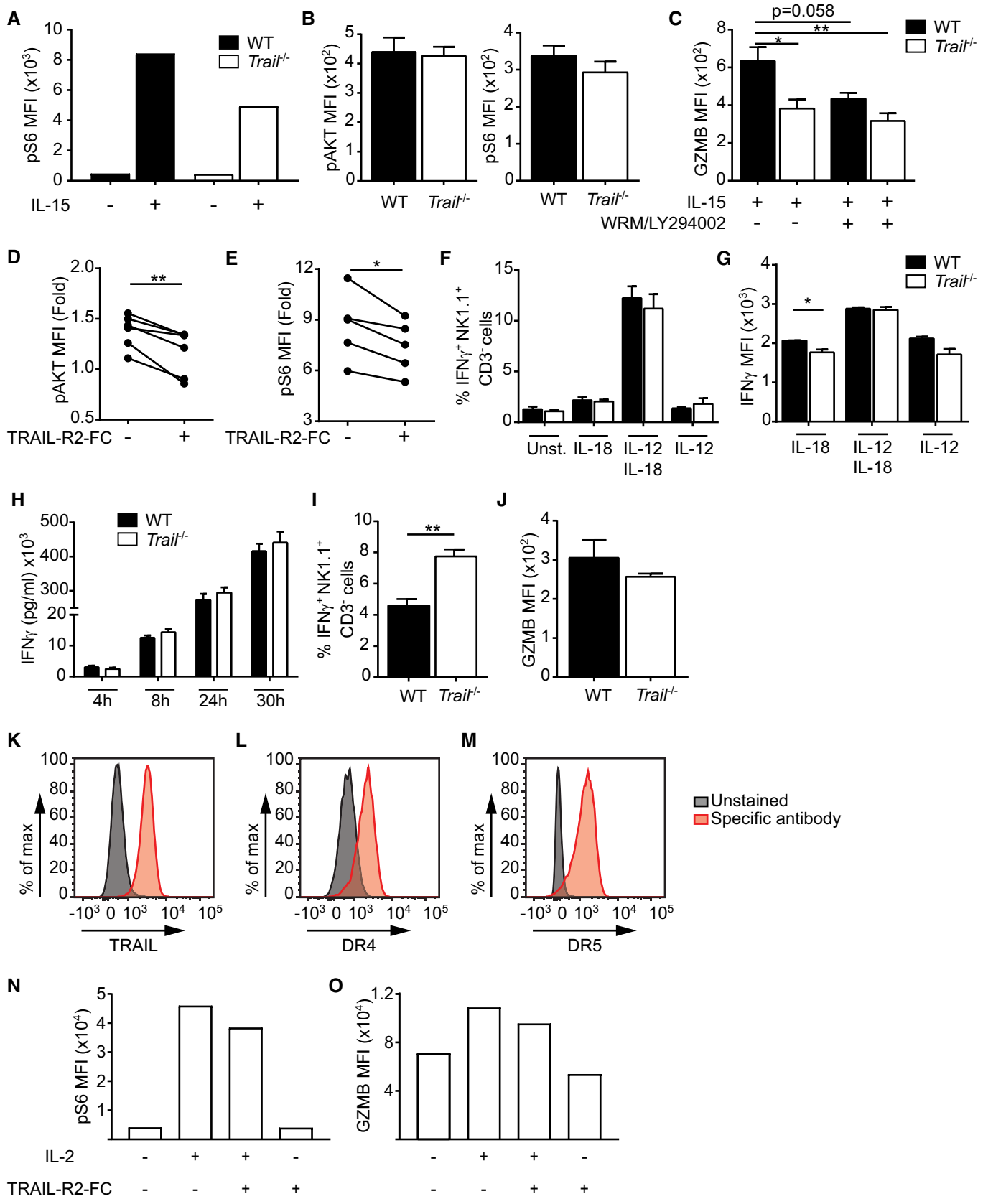
A
pS6 MFI (x10³)
WT
Trail-/-
IL-15 - + - +
B
pAKT MFI (x10²)
pS6 MFI (x10²)
WT Trail-/-
C
p=0.058
GZMB MFI (x10²)
IL-15 + + + +
WRM/LY294002 - - + +
D
pAKT MFI (Fold)
TRAIL-R2-FC - +
E
pS6 MFI (Fold)
F
% IFNγ+ NK1.1+ CD3- cells
Unst. IL-18 IL-12 IL-12 IL-18
G
IFNγ MFI (x10³)
IL-18 IL-12 IL-18 IL-12
H
IFNγ (pg/ml) x10³
4h 8h 24h 30h
I
% IFNγ+ NK1.1+ CD3- cells
J
GZMB MFI (x10²)
K
% of max
TRAIL
L
DR4
M
DR5
Unstained
Specific antibody
N
pS6 MFI (x10⁴)
IL-2 - + + -
TRAIL-R2-FC - - + +
O
GZMB MFI (x10⁴)

Figure EV5.