Supporting Information

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SI Materials and Methods

Mice. *Il1rl1*$^{-/-}$, *Tbx21*$^{-/-}$, *Stat4*$^{-/-}$, and *Ifngr1*$^{-/-}$ mice were bred on a C57BL/6 background. SMARTA1 TCR-tg mice, which express a TCR specific for the LCMV glycoprotein epitope GP61-80, were crossed to Thy1.1^{+} B6.PL, *Il1rl1*$^{-/-}$, *Tbx21*$^{-/-}$, *Stat4*$^{-/-}$, and *Ifngr1*$^{-/-}$ mice to generate TCR^{tg} mice on the respective mutant backgrounds.

Bone Marrow Chimeras and Adoptive Cell Transfer. WT recipients (CD45.1^{+}) of bone marrow (1.5×10^7 cells each of WT CD45.1^{+} and *Il1rl1*$^{-/-}$ CD45.2^{+}) were lethally irradiated (11 Gy in two doses given 6 h apart) at 1 d before hematopoietic reconstitution, and residual T cells were depleted on the day of reconstitution (100 μg T24 anti-Thy1 antibody i.p.). Recipients were rested for ∼ 3 mo for hematopoietic reconstitution before the LCMV challenge was conducted. CD4^{+} T cells of Thy1.1^{+} SMARTA1 TCR-tg mice of the respective knockout backgrounds (6–8 wk old) were purified by staining with biotinylated antibodies against CD8 (53-6.7), CD11c (N418), CD11b (M1/70), CD19 (1D3), NK1.1 (PK136), Gr1 (RB6-8C5), CD25 (7D4), CXCR3 (CXCR3-173), and ST2 (DJ8), followed by antibiotin microbeads and magnetic depletion (Miltenyi Biotec). For each recipient, 2×10^5 cells of the biotin-negative fraction were transferred i.v.

Primary T-Cell Cultures. Naive CD4^{+} CD62L^{hi} CD44^{-} cells were sorted from pooled spleen and lymph node cells by FACS. Unless stated differently, T cells were cultured in RPMI 1640 + GlutaMax-I supplemented with 10% (vol/vol) FCS (Gibco; Life Technologies), penicillin (100 U/mL; Gibco; Life Technologies), streptomycin (100 μg/mL; Gibco; Life Technologies), and β-mercaptoethanol (50 ng/mL; Sigma-Aldrich) in the presence of antigen-presenting cells and 1 μg/mL GP64 (LCMV-GP$_{64–80}$). For Th1 differentiation, 5 ng/mL IL-12, 5 ng/mL IL-2, and 10 μg/mL anti–IL-4 (11B11) were added. For Th2 differentiation, 10 ng/mL IL-4, 5 ng/mL IL-2, 10 μg/mL anti–IL-12 (C17.8), and 10 μg/mL anti–IFN-γ (AN18.17.24) were added. On day 4, cells were reactivated with fresh antigen-presenting cells plus 1 μg/mL GP64, and cytokines and blocking cytokine antibodies as above. For resting conditions, cells were cultured at day 8 in fresh medium with 1 ng/mL IL-7 for 5 more days. Recombinant cytokines were purchased from R&D Systems, Peprotech, and Miltenyi Biotec. At the indicated analysis time point, cells were purified with Histopaque (Sigma-Aldrich) and high-density centrifugation (400 × *g* at 20 °C for 20 min).

Flow Cytometry Analysis. Surface receptor staining and intracellular cytokine, transcription factor, and pSTAT4 staining procedures have been described previously (1). The LCMV-specific CD8^{+} T-cell response to the dominant glycoprotein-derived epitope GP33 was assessed by MHC class I tetramer staining as described previously (2). The LCMV-specific CD4^{+} T-cell response was assessed by MHC class II tetramer staining. For these analyses, phycoerythrin-conjugated I-Ab MHC class II tetramer complexes loaded with either the LCMV glycoprotein aa66-77 epitope DIYKGVYQFKSV or the human CLIP-derived aa87-101 epitope PVSKMRMATPLLMQA were generously provided by the US National Institutes of Health's Tetramer Facility.

For intracellular staining of p38 protein phosphorylation, cells were starved for 4 h in serum-free medium and treated with or without IL-33 (30 ng/mL) for another 30 min. Cells were subsequently fixed with prewarmed 1× BD Phosflow Lyse/Fix Buffer for 10 min at 37 °C. Cells were permeabilized with ice-cold BD Phosflow Perm Buffer III for 30 min on ice, then stained for 30 min with PE anti-phospho-p38 (36/p38, BD Biosciences). Additional FACS analysis, not described before, included surface marker stainings for Ly6C (AL-21; BD Biosciences) and CCR6 (140706; R&D Systems), intracellular staining of IL-17A (TC11-18H10.1; Biolegend) after restimulation with PMA and ionomycin, and intracellular transcription factor staining of FoxP3 (FJK-16s; eBioscience) and RORγt (Q31-378; BD Biosciences).

Gene Expression Analysis. mRNA of in vitro-generated Th cells was isolated with the NucleoSpin RNA II Kit (Macherey Nagel) according to the manufacturer's instructions. cDNA was prepared with TaqMan reverse-transcription reagents and quantified with SYBR Select (both from Applied Biosystems). Il1rl1 (forward, CAAGTAGGACCTGTGTGCCC; reverse, CGTGTCCAACAATTGACCTG) was normalized to Hprt (forward, TCCTCCTCAGACCGCTTTT; reverse, CATAACCTGGTTCATCATCGC).

Virus. The LCMV-WE strain was propagated on L-929 cells. LCMV stocks were titrated by standard immunofocus assays on MC57G cells as described previously (3). Mice were infected i.v. with 200 or 2×10^6 PFU in 200 μL of MEM.

1. Peine M, et al. (2013) Stable T-bet(+)GATA-3(+) Th1/Th2 hybrid cells arise in vivo, can develop directly from naive precursors, and limit immunopathologic inflammation. *PLoS Biol* 11(8):e1001633.
2. Gallimore A, et al. (1998) Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. *J Exp Med* 187(9):1383–1393.
3. Löhning M, et al. (2008) Long-lived virus-reactive memory T cells generated from purified cytokine-secreting T helper type 1 and type 2 effectors. *J Exp Med* 205(1):53–61.

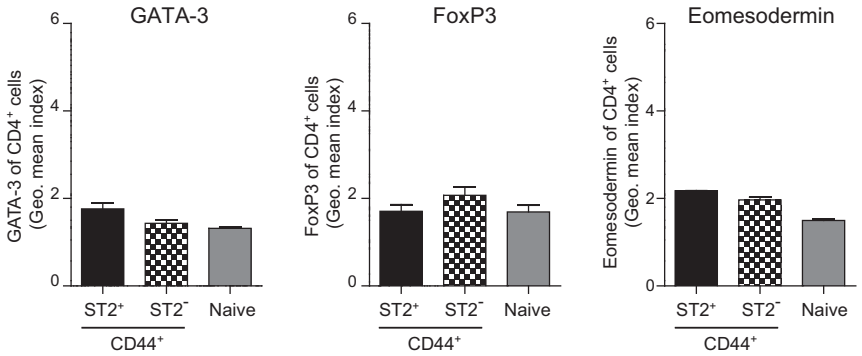

Fig. S1. ST2$^+$ and ST2$^-$ effector CD4$^+$ T cells show similar expression of GATA-3, FoxP3, and Eomesodermin. Shown is a comparison of splenic ST2$^+$ and ST2$^-$ effector CD4$^+$ T cells analyzed by flow cytometry at day 8 after LCMV infection (2×10^6 PFU). Effector CD4$^+$ T cells were defined by CD44 expression. GMIs are shown for GATA-3, FoxP3, and Eomesodermin. Naive CD4$^+$ T cells (CD62L^{hi} and CD44$^-$) served as controls. Bars represent mean + SD (n = 3). All data are representative of two independent experiments.

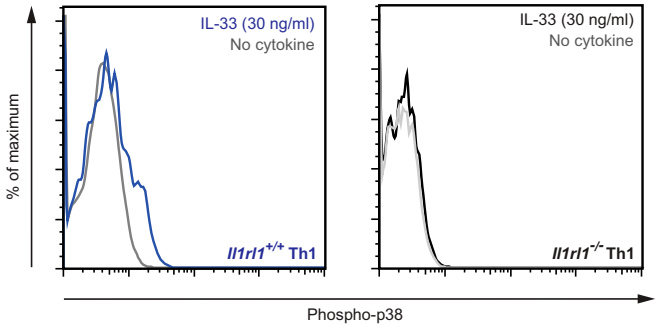

Fig. S2. ST2 downstream signaling is triggered by IL-33 in Th1 cells. Naive *Il1rl1*$^{+/+}$ and *Il1rl1*$^{-/-}$ LCMV-TCR^{tg} CD4$^+$ T cells were differentiated to Th1 cells for 8 d, starved for 4 h, and then treated with 30 ng/mL IL-33. Representative histograms are shown for intracellular phosphorylated p38 protein in *Il1rl1*$^{+/+}$ and *Il1rl1*$^{-/-}$ cells (n = 3).

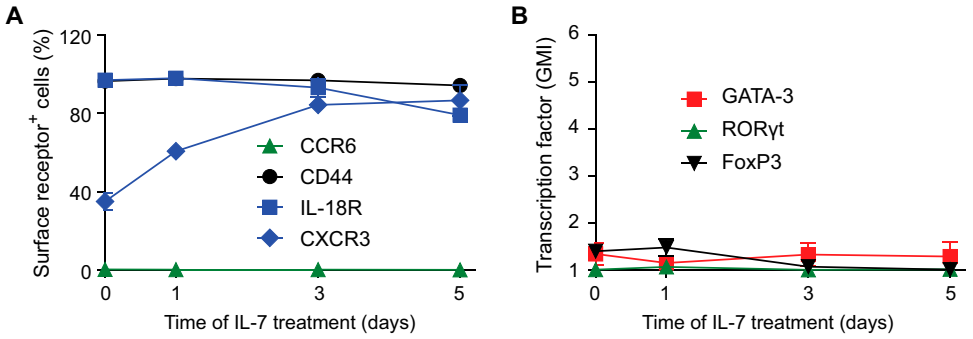

Fig. S3. Th1 cells do not lose their differentiation phenotype during resting culture conditions with IL-7. Naive LCMV-TCR^{tg} CD4$^+$ T cells were differentiated to Th1 cells for 8 d and then placed into a resting condition in fresh medium with IL-7 for another 5 d. Shown are the frequencies of cells expressing the surface markers CCR6, CD44, IL-18R, and CXCR3 (*A*) and the GMIs of GATA-3, RORγt, and FoxP3 (*B*). Symbols represent mean ± SD values of two pooled, independent experiments.

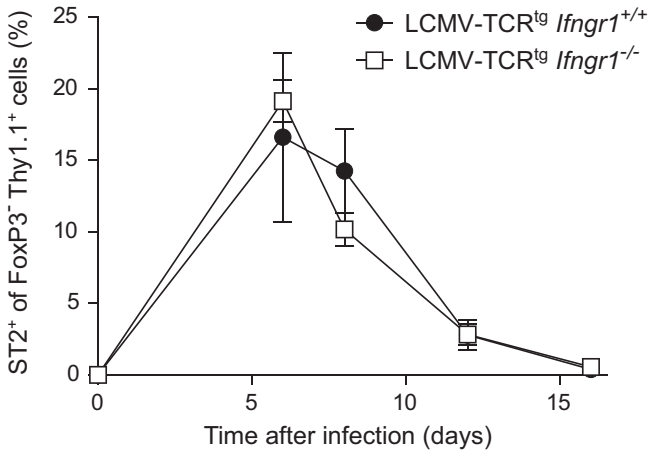

Fig. S4. IFN-γR signals are dispensable for ST2 expression on Th1 cells in vivo. Naive *Ifngr1*$^{+/+}$ and *Ifngr1*$^{-/-}$ LCMV-TCR^{tg} CD4$^+$ Thy1.1$^+$ cells were transferred into WT recipients, followed by LCMV infection (200 PFU). Shown are time course analyses of ST2 expression on FoxP3$^-$ CD4$^+$ Thy1.1$^+$ cells in peripheral blood. Symbols represent mean ± SD (n = 5).

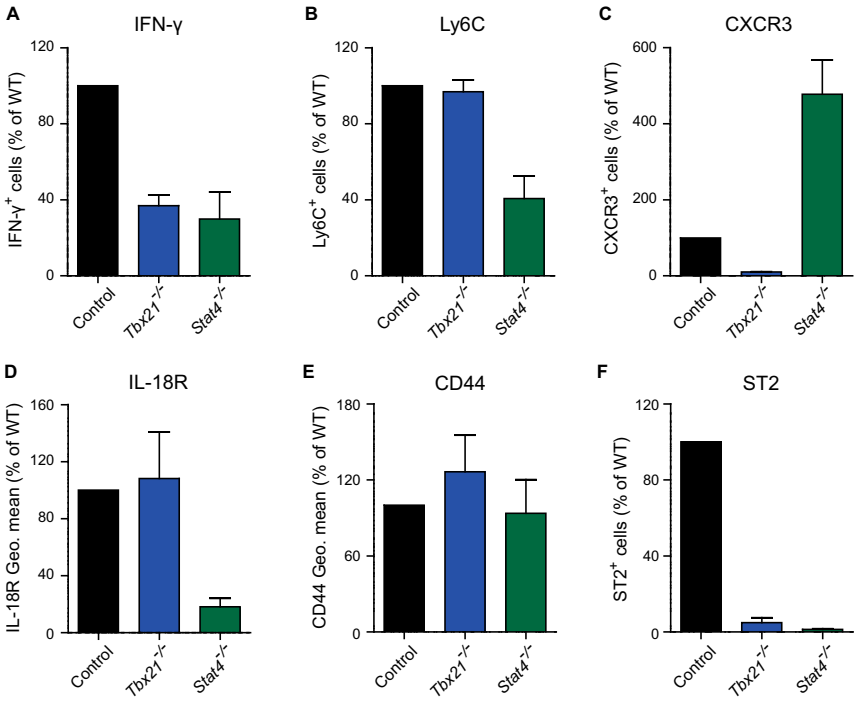

Fig. S5. In vitro-differentiated *Tbx21*$^{-/-}$ and *Stat4*$^{-/-}$ CD4$^+$ T cells exhibit Th1 characteristics, yet at lower levels than control CD4$^+$ T cells. Naive control, *Tbx21*$^{-/-}$, and *Stat4*$^{-/-}$ LCMV-TCR^{tg} CD4$^+$ T cells were differentiated in vitro for 8 d. Cells were analyzed by flow cytometry for IFN-γ production after restimulation with PMA and ionomycin (*A*), Ly6C (*B*), CXCR3 (*C*), IL-18R (*D*), CD44 (*E*), and ST2 (*F*). Bars represent mean + SD (*n* = 2–5).

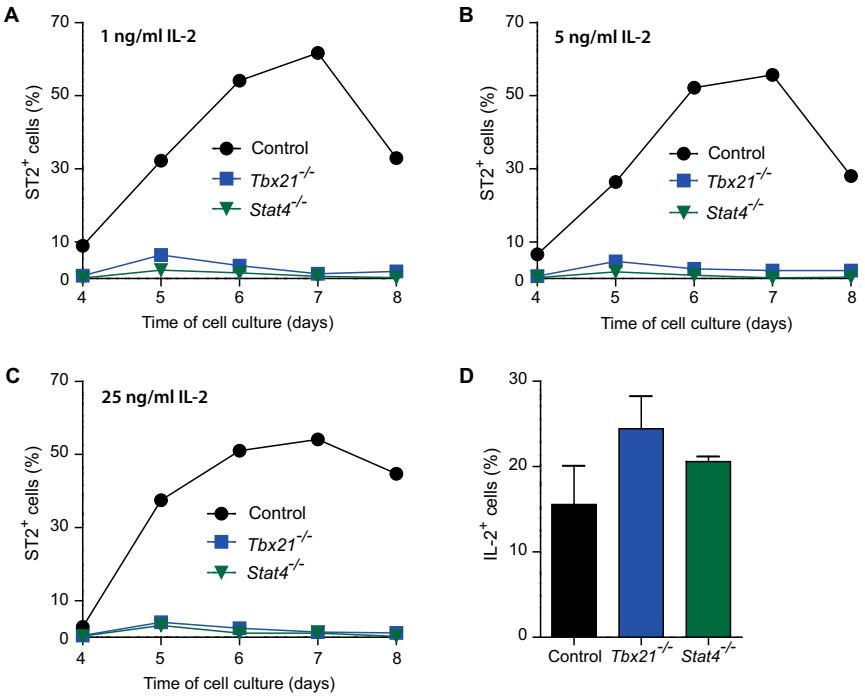

Fig. S6. IL-2 does not rescue impaired ST2 expression of *Stat4*$^{-/-}$ and *Tbx21*$^{-/-}$ Th1 cells. (*A*–*C*) Naive control (circle), *Tbx21*$^{-/-}$ (square), and *Stat4*$^{-/-}$ (triangle) LCMV-TCR^{tg} CD4^{+} T cells were differentiated to Th1 cells for 4 d, and then ST2 expression was analyzed by flow cytometry on days 4–8. Cells were cultured with 1 ng/mL (*A*), 5 ng/mL (*B*), or 25 ng/mL (*C*) recombinant IL-2. Data are representative of two independent experiments. (*D*) Naive control, *Tbx21*$^{-/-}$, and *Stat4*$^{-/-}$ LCMV-TCR^{tg} CD4^{+} T cells were differentiated under Th1-inducing conditions with 5 ng/mL IL-2 for 8 d. Shown are the respective frequencies of IL-2–producing cells after restimulation with PMA and ionomycin. Bars represent mean + SD (n = 3).

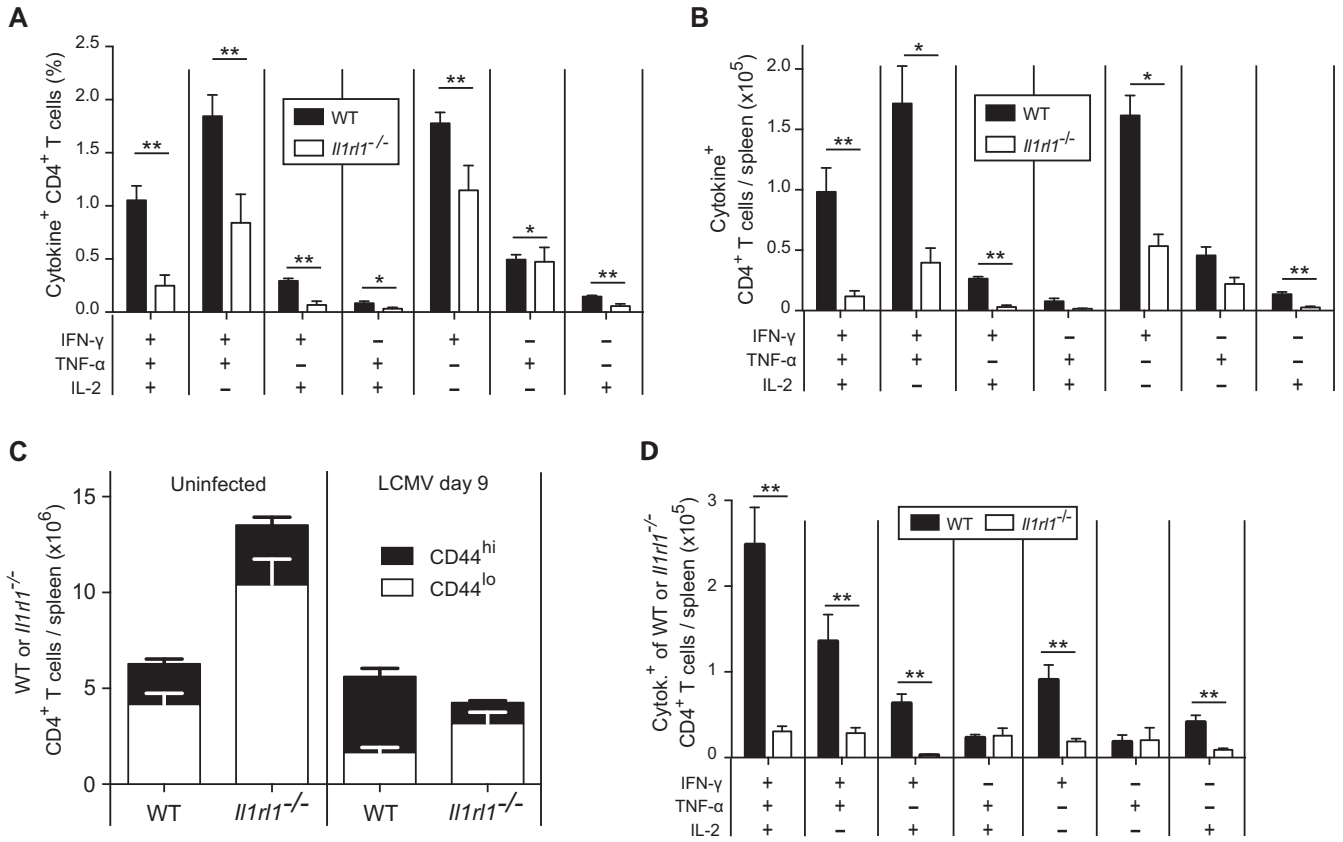

Fig. S7. Reduced numbers of cytokine-producing ST2-deficient CD4$^+$ T cells in viral infection. (*A* and *B*) WT and *Il1rl1*$^{-/-}$ mice were infected with LCMV, and on day 8 postinfection, the frequencies (*A*) and absolute numbers (*B*) of GP64-specific splenic CD4$^+$ T cells expressing IFN-γ, TNF-α, IL-2, or combinations thereof were determined. The same data were used for the analysis displayed in Fig. 4*A*. (*C* and *D*) Irradiated WT recipients were reconstituted with WT (CD45.1$^+$) and *Il1rl1*$^{-/-}$ (CD45.2$^+$) bone marrow at a 1:1 ratio. Analyses were conducted after hematopoietic reconstitution, either before (*C*, *Left*) or 9 d after (*C*, *Right* and *D*) LCMV infection. Numbers of splenic WT and *Il1rl1*$^{-/-}$ CD4$^+$ T cells, either CD44^{hi} or CD44^{lo}, were determined by flow cytometry before (uninfected) and at 9 d after LCMV infection. (*D*) Absolute numbers of splenic WT and *Il1rl1*$^{-/-}$ CD4$^+$ T cells expressing IFN-γ, TNF-α, IL-2, or combinations thereof after GP64 peptide restimulation. Frequencies calculated from the same datasets are shown in Fig. 4*E*. Bars represent mean + SEM values of four mice (*A*, *B*, and the uninfected group in *C*) or six mice (LCMV-infected group in *C* and *D*). *A*, *B*, and *D* show representative results of two independent experiments.

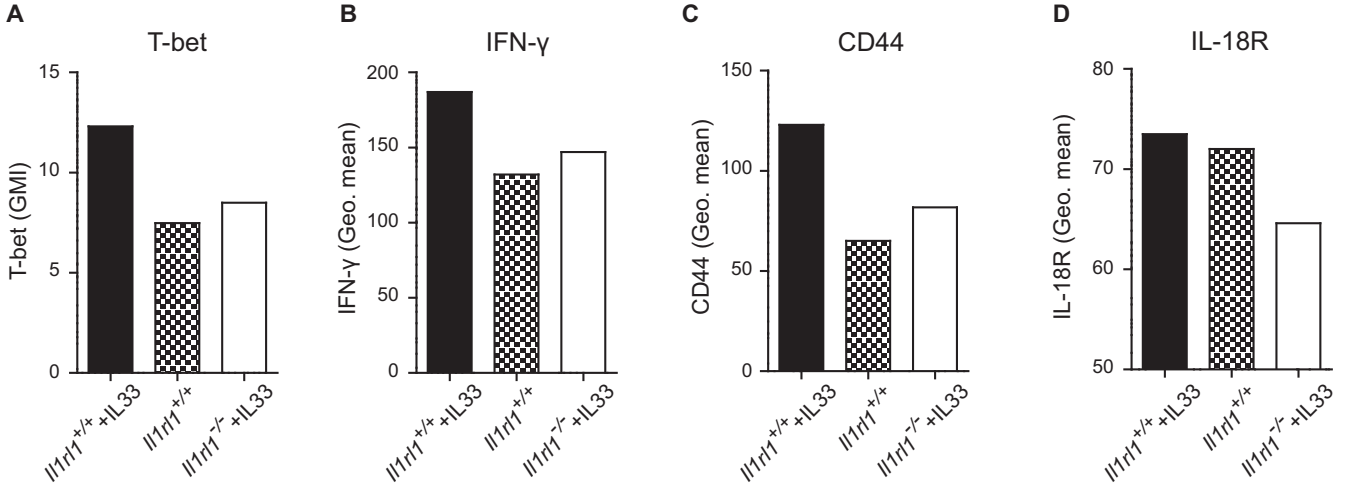

Fig. S8. Lack of IL-33 signals leads to an impaired Th1 phenotype during in vitro differentiation with low IL-12 amounts. Naive *Il1rl1*$^{+/+}$ and *Il1rl1*$^{-/-}$ LCMV-TCR^{tg} CD4$^+$ T cells were differentiated to Th1 cells with suboptimal IL-12 concentration (0.5 ng/mL) in the presence or absence of IL-33 (10 ng/mL) for 8 d. Cells were analyzed by flow cytometry for expression of T-bet (*A*), IFN-γ after restimulation with PMA and ionomycin (*B*), CD44 (*C*), and IL-18R (*D*). One representative culture is shown from two or three independent experiments.

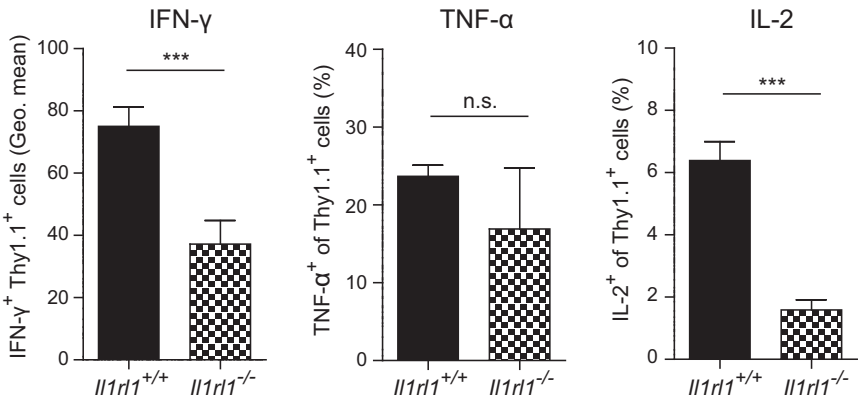

Fig. S9. Adoptively transferred ST2-deficient CD4$^+$ T cells show impaired cytokine expression in an LCMV infection. Naive *Il1rl1*$^{+/+}$ and *Il1rl1*$^{-/-}$ LCMV-TCR^{tg} CD4$^+$ Thy1.1$^+$ cells were transferred into WT recipients, followed by LCMV infection (200 PFU). Geometric means of IFN-γ and frequencies of TNF-α and IL-2 are shown after GP64 peptide restimulation of splenic *Il1rl1*$^{+/+}$ and *Il1rl1*$^{-/-}$ CD4$^+$ Thy1.1$^+$ cells at day 6 postinfection. Bars represent mean + SD ($n = 4$). All data are representative of two independent experiments.