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Supplementary Materials for

STAT1 maintains naïve CD8$^+$ T cell quiescence by suppressing the type I IFN-STAT4-mTORC1 signaling axis

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This PDF file includes:

SUPPLEMENTARY FIGURES

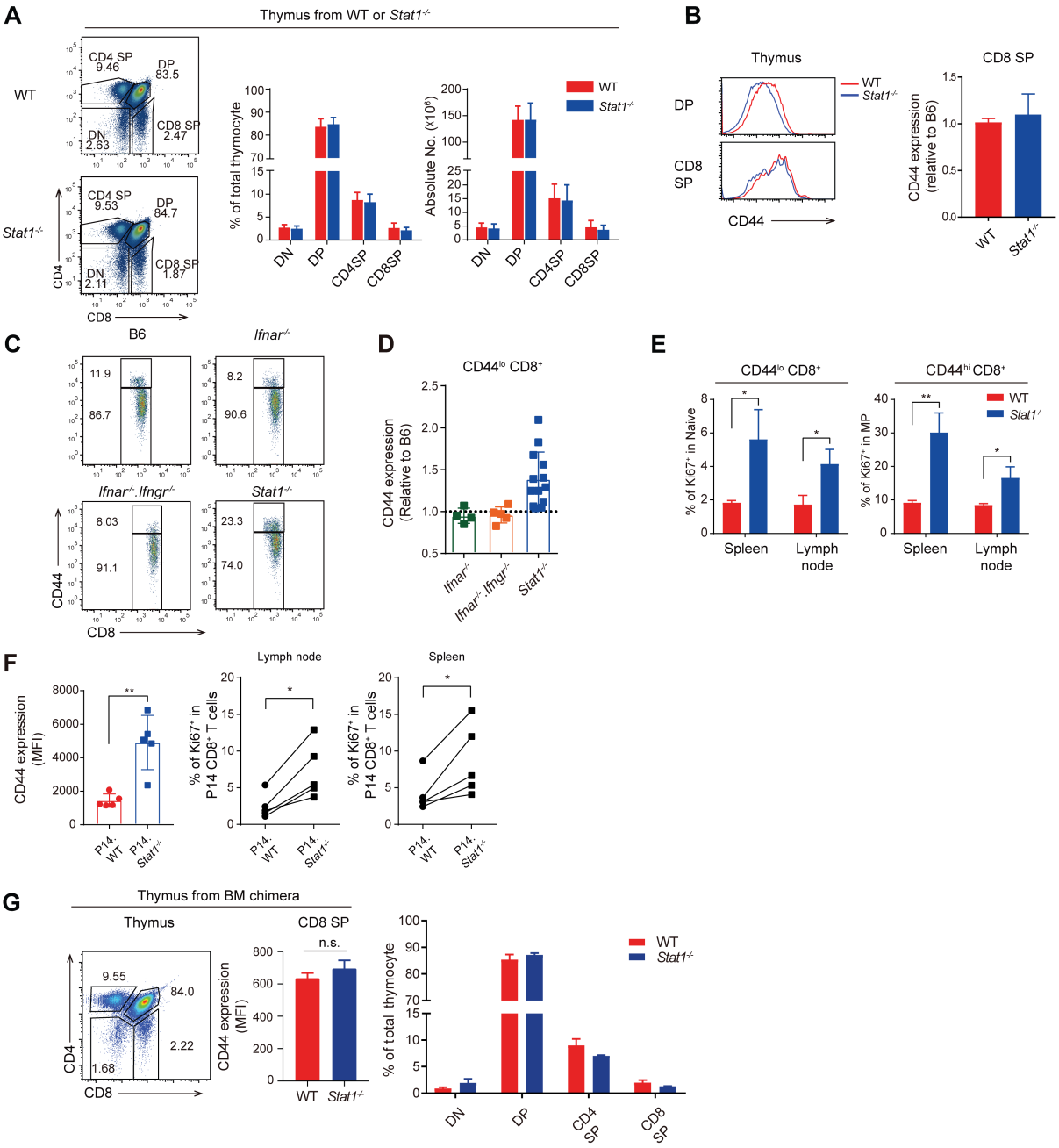

Figure S1. Effect of Stat1 deficiency on thymic T cell development. (A) Flow cytometry for CD4 and CD8 expression on thymocytes from WT and *Stat1*$^{-/-}$ mice. Bar graphs show percentage and absolute cell number of CD4^{-}CD8^{-} double-negative (DN), CD4^{+}CD8^{+} double-positive (DP), CD4^{+}CD8^{-} and CD4^{-}CD8^{+} single-positive (SP) thymocytes. **(B)** CD44 expression in CD4^{-}CD8^{+} SP thymocytes from WT and *Stat1*$^{-/-}$ mice. **(C)** Flow cytometry for

$CD44^{lo}$ and $CD44^{hi}$ $CD8^+$ T cells and **(D)** relative level of CD44 expression in $CD44^{lo}$ $CD8^+$ T cells from WT, *Ifnar*$^{-/-}$, *Ifnar*$^{-/-}$*.Ifngr*$^{-/-}$, and *Stat1*$^{-/-}$ mice. **(E)** Percentage of $Ki67^+$ cells in $CD44^{lo}$ and $CD44^{hi}$ $CD8^+$ T cells from WT and *Stat1*$^{-/-}$ mice. **(F)** Level of CD44 and percentage of $Ki67^+$ cells in P14 $CD44^{lo}$ $CD8^+$ T cells from WT and *Stat1*$^{-/-}$ mice. **(G)** Flow cytometry for CD4 and CD8 expression on thymocytes derived from WT and *Stat1*$^{-/-}$ BM cells as in Fig. 2 A. The results are presented as the mean ± SEM. Data are representative of 3-4 independent experiments. *, $P < 0.05$; **, $P < 0.01$.

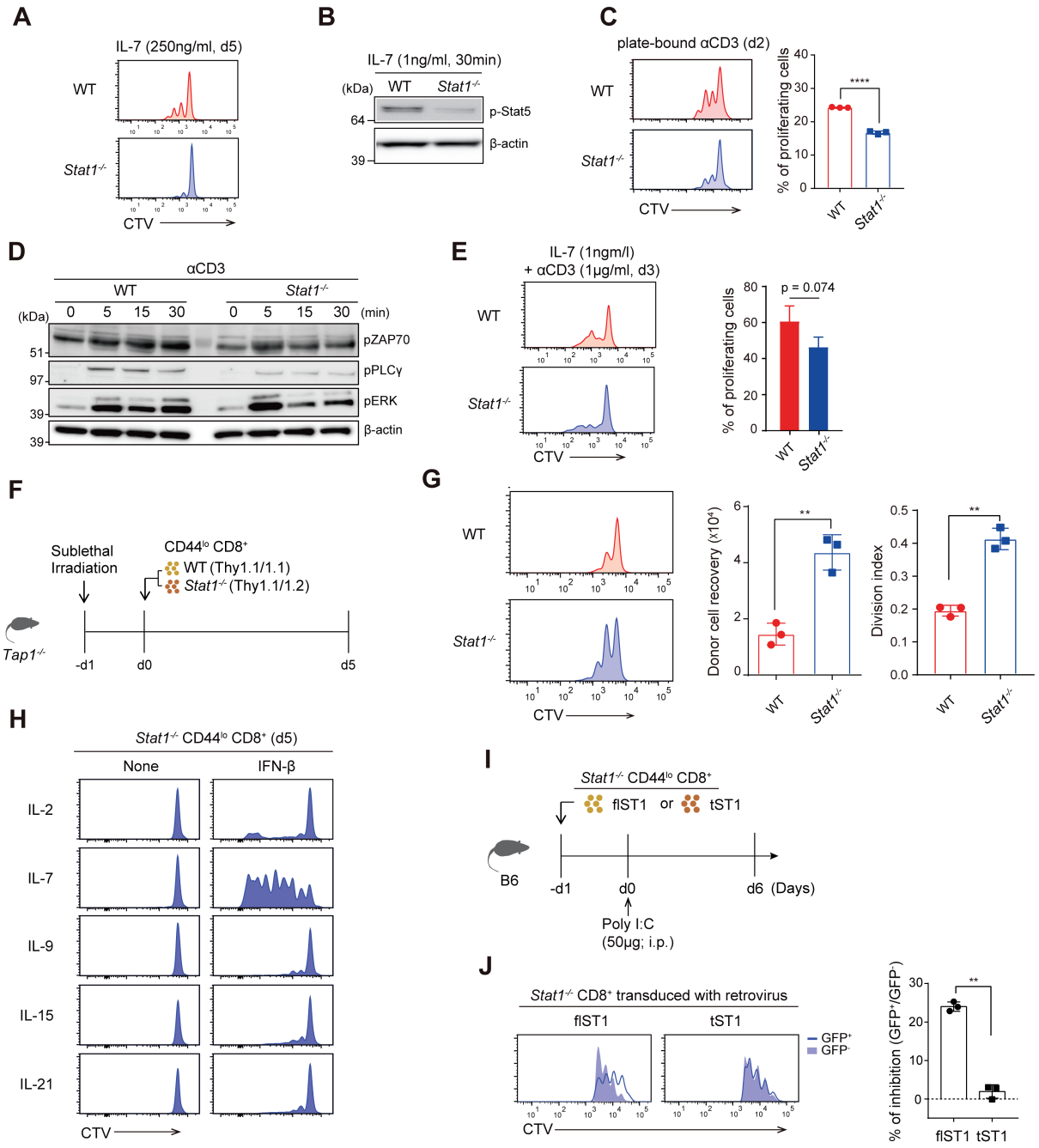

Figure S2. Effect of Stat1 deficiency on the responsiveness to T1IFN and IL-7. (A) *In vitro* proliferation and **(B)** phosphorylation of STAT5 of WT and *Stat1*$^{-/-}$ naïve CD8^{+} T cells in culture with IL-7. **(C)** *In vitro* proliferation and **(D)** phosphorylation of ZAP70, PLCγ, and ERK of WT and *Stat1*$^{-/-}$ naïve CD8^{+} T cells in culture with either plate-bound for (C) or soluble αCD3 for (D). **(E)** *In vitro* proliferation of WT and *Stat1*$^{-/-}$ naïve CD8^{+} T cells in culture with IL-7 and soluble αCD3. **(F)** Experimental scheme for adoptive transfer and **(G)** *in vivo* proliferation of WT and *Stat1*$^{-/-}$ CD8^{+} donor cells from irradiated (500 cGy) *Tap1*$^{-/-}$ recipients.

(H) *In vitro* proliferation of WT and *Stat1*$^{-/-}$ naïve CD8^{+} T cells in culture with the indicated cytokines. **(I)** Experimental scheme for adoptive transfer with retroviral transduced cells as in Fig. 4 A. **(J)** *In vivo* proliferation of *Stat1*$^{-/-}$ CD8^{+} donor cells transduced with retroviral vectors encoding either full-length (fl) or truncated (t) construct of *Stat1* gene in poly I:C-treated B6 recipient mice. The results are presented as the mean ± SEM. Data are representative of 3-4 independent experiments. **, $P < 0.01$; ****, $P < 0.0001$.

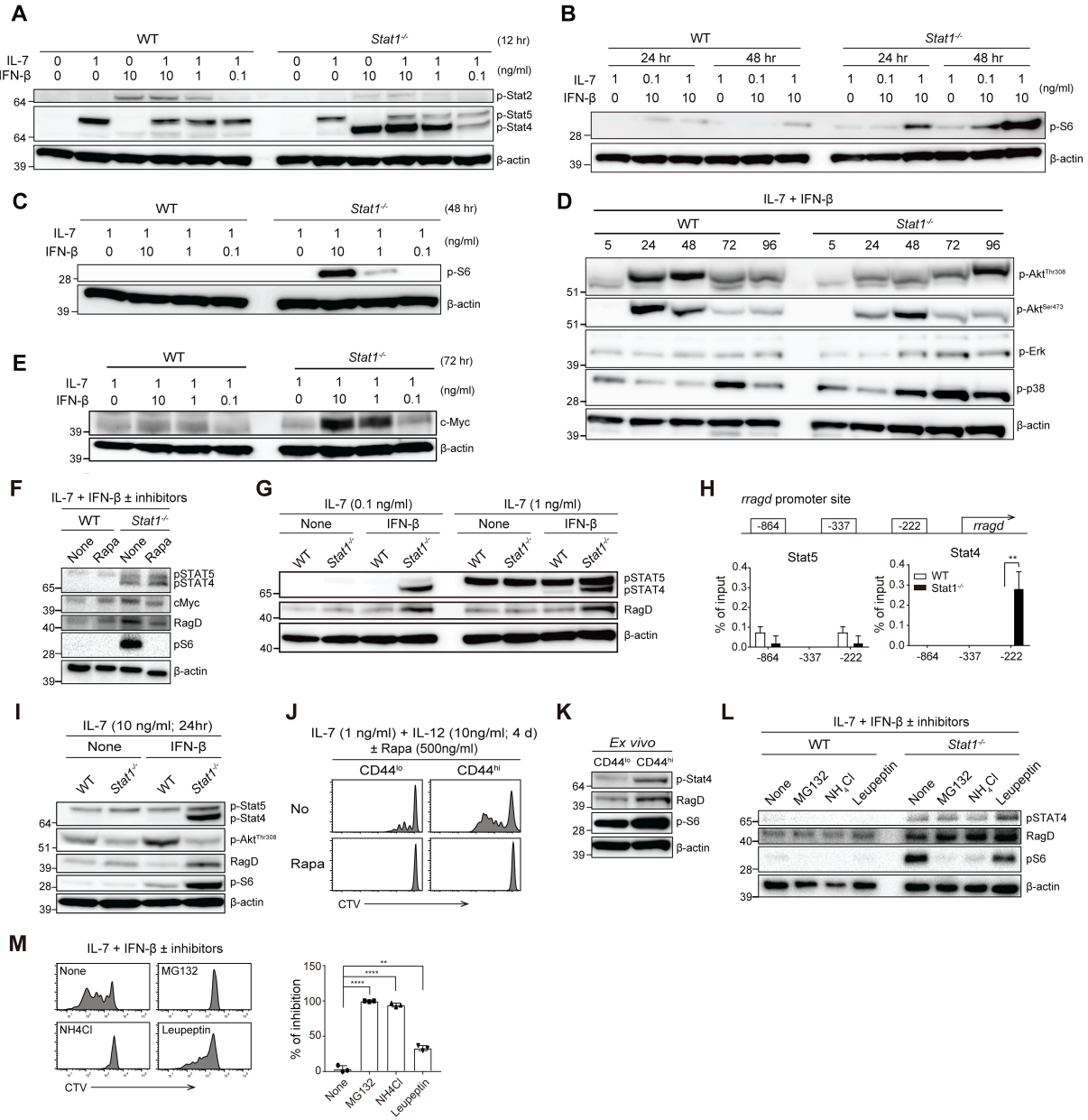

Figure S3. Effect of Stat1 deficiency on T1IFN and IL-7 signaling. (A) Phosphorylation of STAT2, STAT4, and STAT5, **(B, C)** S6, and **(D)** AKT, ERK, and p38 in WT and *Stat1*$^{-/-}$ naïve CD8^{+} T cells after culture with various concentrations of IL-7 and IFN-β for the indicated time points. **(E)** Expression of c-Myc and **(F, G)** RagD in WT and *Stat1*$^{-/-}$ naïve CD8^{+} T cells after culture with various indicated conditions. **(H)** ChIP assay for STAT4 and STAT5 binding to the indicated promoter regions of *rragd* gene analyzed in WT and *Stat1*$^{-/-}$ CD8^{+} T cells after culture with IL-7 and IFN-β for 2 days. **(I)** Immunoblotting for the various indicated signaling proteins in WT and *Stat1*$^{-/-}$ CD8^{+} T cells after culture with IL-7 and IFN-β. **(J)** *In vitro*

proliferation of $CD44^{hi}$ and $CD44^{lo}$ B6 $CD8^+$ T cells after 4 days culture with IL-7 and IL-12 in the absence or presence of rapamycin. **(K, L)** Immunoblotting for phosphorylated STAT4 and S6 and of RagD expression **(K)** in freshly isolated *ex vivo* $CD44^{hi}$ and $CD44^{lo}$ B6 $CD8^+$ T cells, and **(L)** in WT and *Stat1*$^{-/-}$ $CD8^+$ T cells after culture with IL-7 and IFN-β in the absence and presence of the indicated inhibitors. **(M)** *In vitro* proliferation of *Stat1*$^{-/-}$ naïve $CD8^+$ T cells after culture with IL-7 and IFN-β in the presence of the indicated inhibitors. The results are presented as the mean ± SEM. Data are representative of 3-4 independent experiments. Statistical significance is calculated using unpaired t-test. **, $P < 0.01$; ****, $P < 0.0001$.

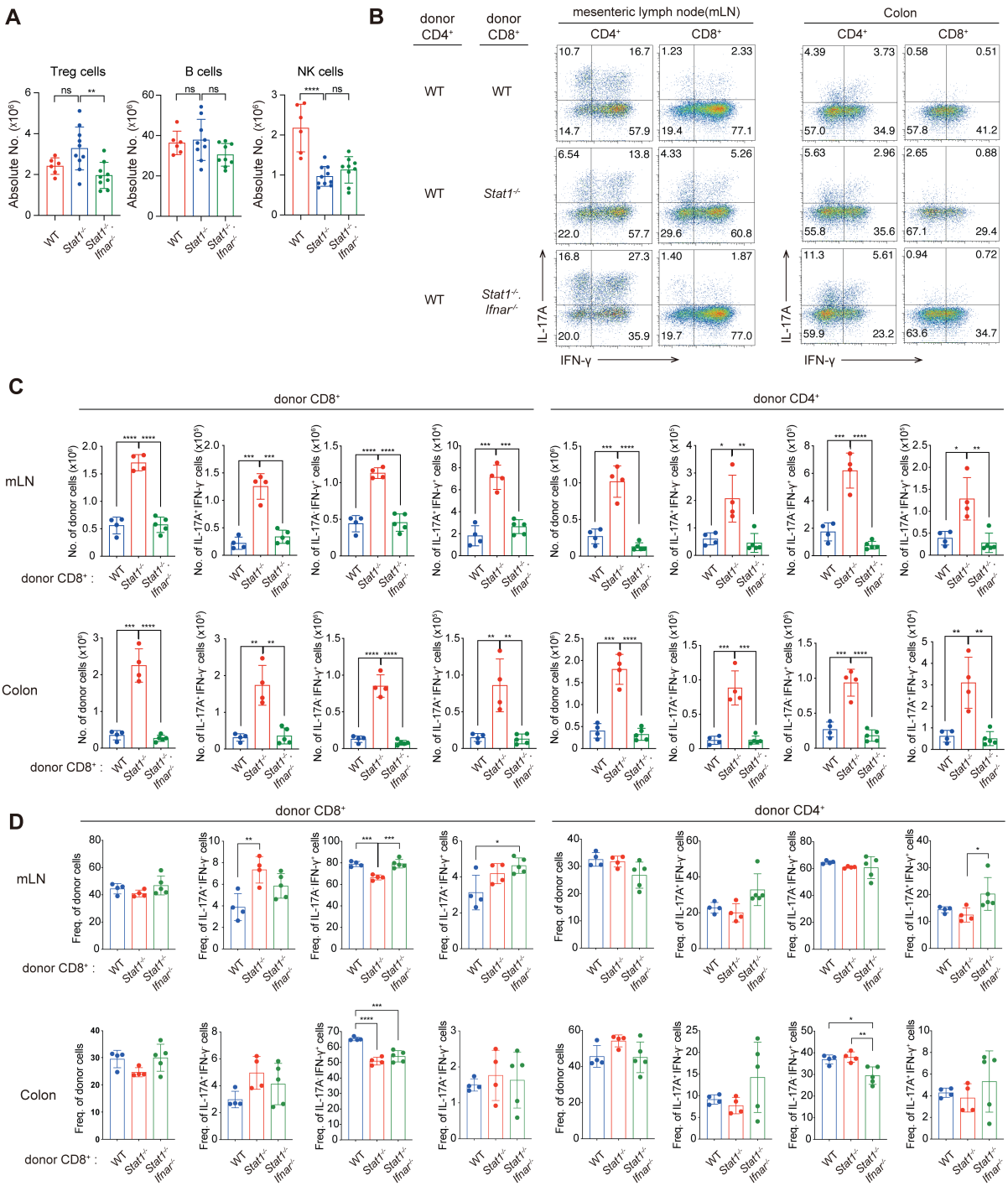

Figure S4. Pathologic nature of STAT1-deficient T cells in an IBD setting. (A) Absolute number of CD4$^+$ T regulatory cells (Treg; CD25$^+$Foxp3$^+$), B cells (B220$^+$), and NK cells (NK1.1$^+$) from WT, *Stat1*$^{-/-}$, and *Stat1*$^{-/-}$.*Ifnar*$^{-/-}$ mice as in Fig. 5 A-D. **(B)** Flow cytometry and **(C)** absolute cell number and **(D)** percentage for intracellular IL-17A and IFN-γ production from donor CD4$^+$ (from WT) and CD8$^+$ T cells (from WT, *Stat1*$^{-/-}$, or *Stat1*$^{-/-}$.*Ifnar*$^{-/-}$ mice) adoptively transferred into *Rag1*$^{-/-}$ recipient mice as in Fig. 5 E. The results are presented as

the mean ± SD. Data are representative of 3-4 independent experiments. Statistical significance is calculated using unpaired t-test and two-way ANOVA multiple comparisons. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.