

SUPPLEMENTAL MATERIAL

Takahashi et al., <http://www.jem.org/cgi/content/full/jem.20110428/DC1>

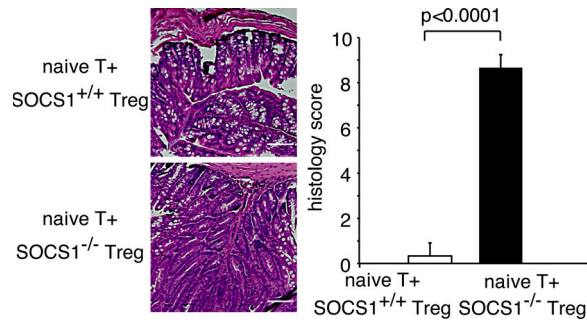


Figure S1. The suppressive activity of SOCS1-deficient T_{reg} cells is attenuated in Rag2^{-/-} mice. 2×10^5 T_{reg} cells from the LN of Ly5.2 WT-*Foxp3*^{GFP} mice (*Socs1*^{+/+} T_{reg} cells) or Ly5.2 cKO-*Foxp3*^{GFP} mice (*Socs1*^{-/-} T_{reg} cells) were cotransferred with 4×10^5 Ly5.1 naive T cells into Rag2^{-/-} mice. Histopathologies of colons stained with H&E (left) and histological scores (right) are shown. Data are representative of three independent experiments. Values represent mean \pm SD. Bars, 100 μ m.

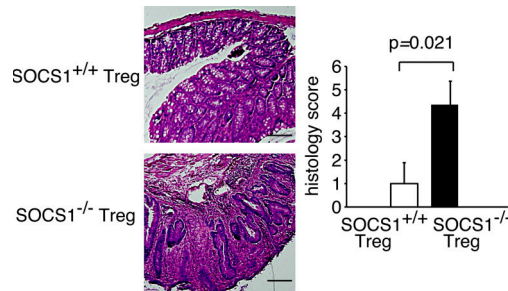


Figure S2. Colitis caused by SOCS1-deficient T_{reg} cells in Rag2^{-/-} mice. 2×10^5 *Socs1*^{+/+} or *Socs1*^{-/-} T_{reg} cells were transferred into Rag2^{-/-} mice. Histopathologies of colons stained with H&E (left) and histological scores (right) are shown. Data are representative of three independent experiments. Values represent mean \pm SD. Bars, 100 μ m.

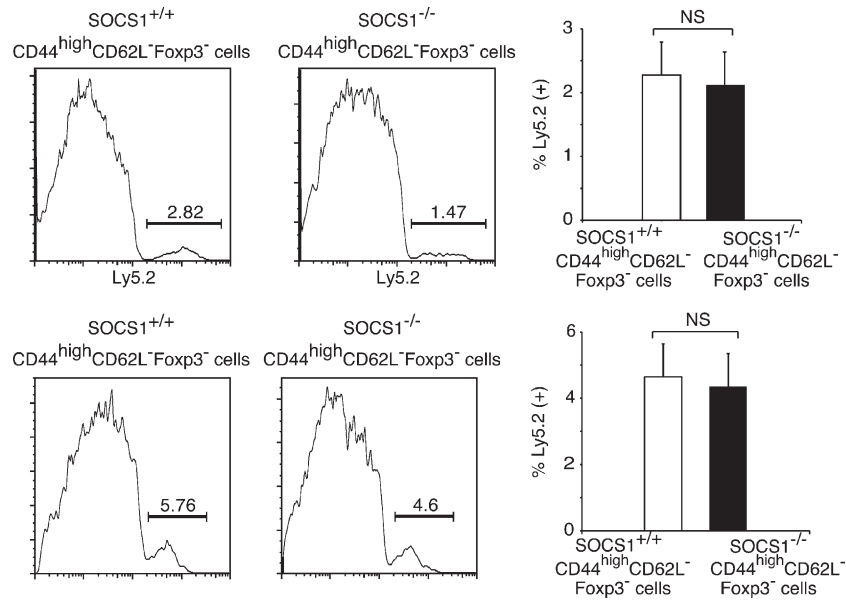


Figure S3. The loss of Foxp3 expression in T_{reg} cells is not caused by outgrowth of activated memory T cell contamination of injected donor cells in Rag2^{-/-} mice. (top) 4×10^5 Ly5.1⁺ naive T cells plus 1.8×10^5 Ly5.1⁺ WT T_{reg} cells and 2×10^4 Ly5.2⁺CD44^{high}CD62L⁻Foxp3⁻ WT or SOCS1-deficient cells were transferred into Rag2^{-/-} mice. Injected Ly5.2⁺CD44^{high}CD62L⁻Foxp3⁻ cells represented 10% of T_{reg} cells. Ly5.2 expression on CD3⁺CD4⁺ T cells from the LN in Rag2^{-/-} mice (percentage of Ly5.2 indicated in bar graph) is shown. Data are representative of three independent experiments. (bottom) 1.9×10^5 Ly5.1⁺ WT T_{reg} cells and 10^4 Ly5.2⁺CD44^{high}CD62L⁻Foxp3⁻ WT or SOCS1-deficient cells were transferred into Rag2^{-/-} mice. Injected Ly5.2⁺CD44^{high}CD62L⁻Foxp3⁻ cells represented 5% of T_{reg} cells. Ly5.2 expression on CD3⁺CD4⁺ T cells from the LN in Rag2^{-/-} mice (percentage of Ly5.2 indicated in bar graph) is shown. Data are representative of three independent experiments. Values represent mean \pm SD.

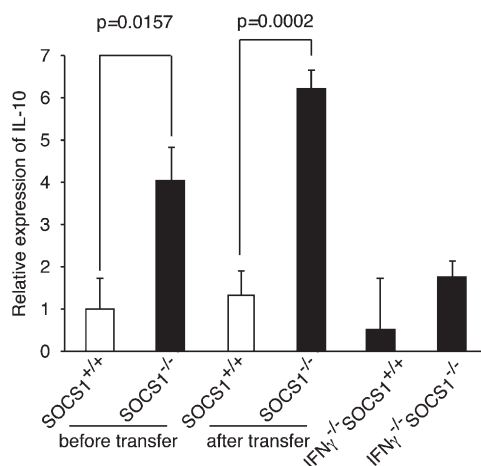


Figure S4. Expression levels of IL-10 of T_{reg} cells before and after transfer in Rag2^{-/-} mice. Before transfer (left) or 6 wk after transfer (middle), IL-10 expression levels of T_{reg} cells from WT-Foxp3^{GFP} mice (SOCS1^{+/+} T_{reg} cells) or LckCre-cKO-Foxp3^{GFP} mice (SOCS1^{-/-} T_{reg} cells) were quantified by real-time PCR assay. (right) T_{reg} cells (Ly5.2) from Ifn γ ^{-/-} SOCS1^{+/+} mice or Ifn γ ^{-/-} SOCS1^{-/-} mice were cotransferred with Ly5.1 naive T cells into Rag2^{-/-} mice. 6 wk later, Ly5.2⁺CD3⁺CD4⁺CD25^{bright} cells from the LN of recipient mice were sorted, and IL-10 expression levels were quantified by real-time PCR assay. Each expression was normalized to SOCS1^{+/+} T_{reg} cells before transfer. Data are representative of three independent experiments. Values represent mean \pm SD.

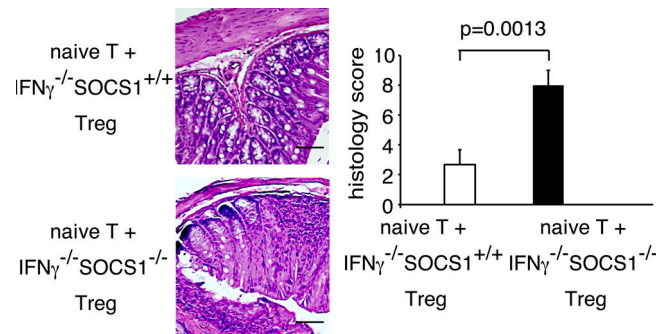


Figure S5. The suppressive activity of *Ifn γ ^{-/-}Socs1^{-/-}* T_{reg} cells is attenuated in *Rag2^{-/-}* mice. 2×10^5 Ly5.2 *Ifn γ ^{-/-}Socs1^{+/+}* or Ly5.2 *Ifn γ ^{-/-}Socs1^{-/-}* T_{reg} cells were cotransferred with 4×10^5 Ly5.1 naive T cells into *Rag2^{-/-}* mice. Histopathologies of colons stained with H&E (left) and histological scores (right) are shown. Data are representative of three independent experiments. Values represent mean \pm SD. Bars, 100 μ m.

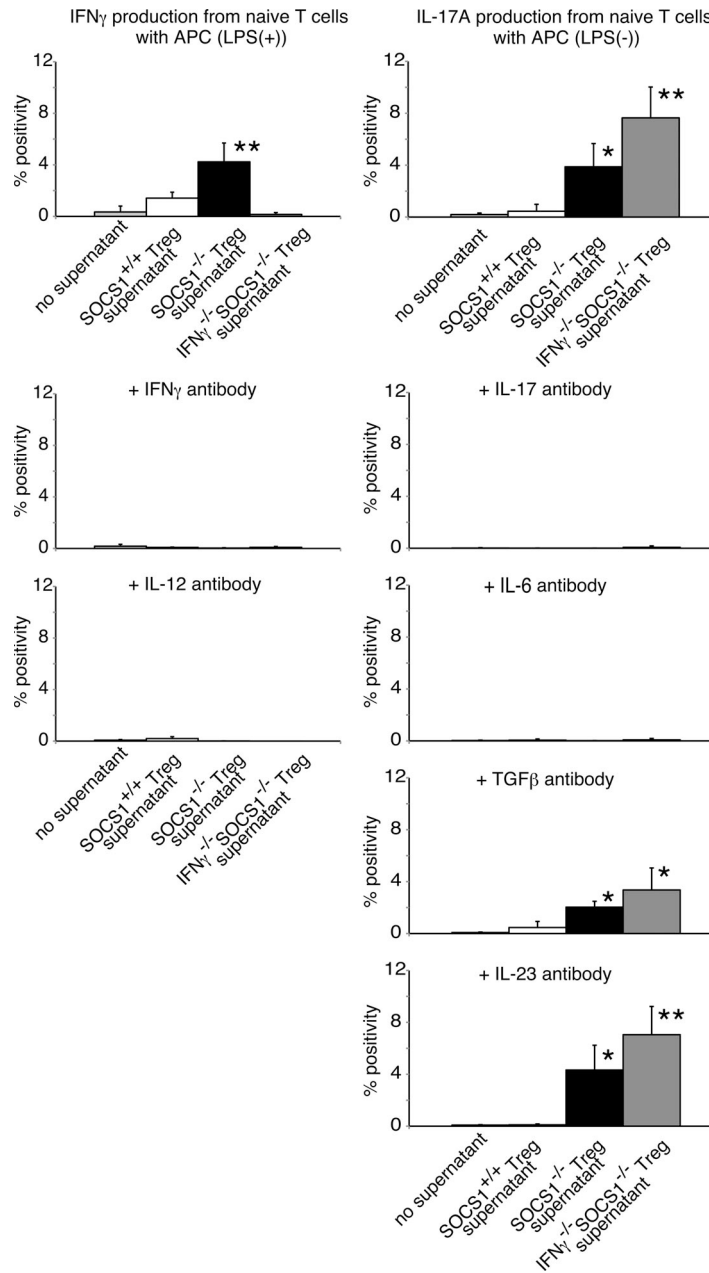


Figure S6. Anti-IFN- γ , -IL-17, -IL-12, and -IL-6 antibodies nearly completely inhibit Th1 and Th17 cell differentiation through APCs. 3×10^5 WT naive T cells/well were cultured for 5 d with LPS-stimulated (left) and LPS-untreated (right) APCs (7×10^5 T cell-depleted spleen cells/well) and supernatants from 2×10^5 *Socs1*^{+/+}, *Socs1*^{-/-}, and *Ifn γ ^{-/-} Soc1^{-/-}* T_{reg} cells/well cultured for 2 d with anti-CD3/anti-CD28 beads and 10 ng/ml IL-2 and the indicated antibodies. From flow cytometric analysis, percent positivity values of IFN- γ (left) and IL-17A (right) are shown. Data are representative of three independent experiments (*, $P < 0.05$; **, $P < 0.01$). Values represent the mean \pm SD.

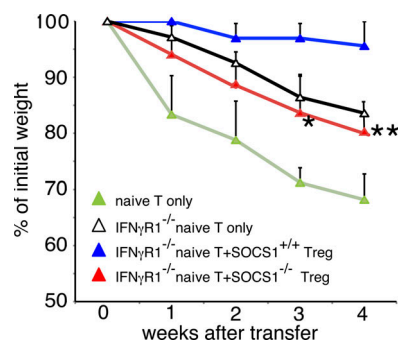


Figure S7. *Socs1* $^{-/-}$ T_{reg} cells cannot suppress colitis induced by the transfer of *Ifn γ r1* $^{-/-}$ naive T cells into *Rag2* $^{-/-}$ mice. 2×10^5 *Socs1* $^{+/+}$ or *Socs1* $^{-/-}$ T_{reg} cells were cotransferred with 4×10^5 IFN- γ receptor 1-deficient naive T cells into *Rag2* $^{-/-}$ mice. 4 wk after transfer, body weight changes of recipient *Rag2* $^{-/-}$ mice were analyzed. Data are representative of three independent experiments (*, $P < 0.05$; **, $P < 0.01$). Values represent the mean \pm SD.