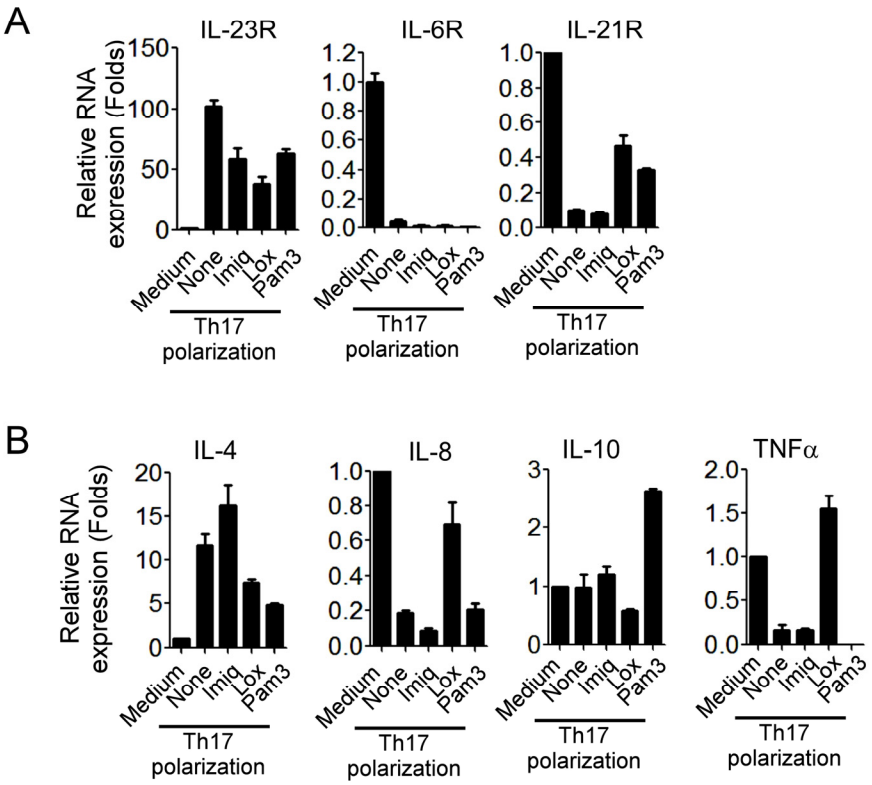
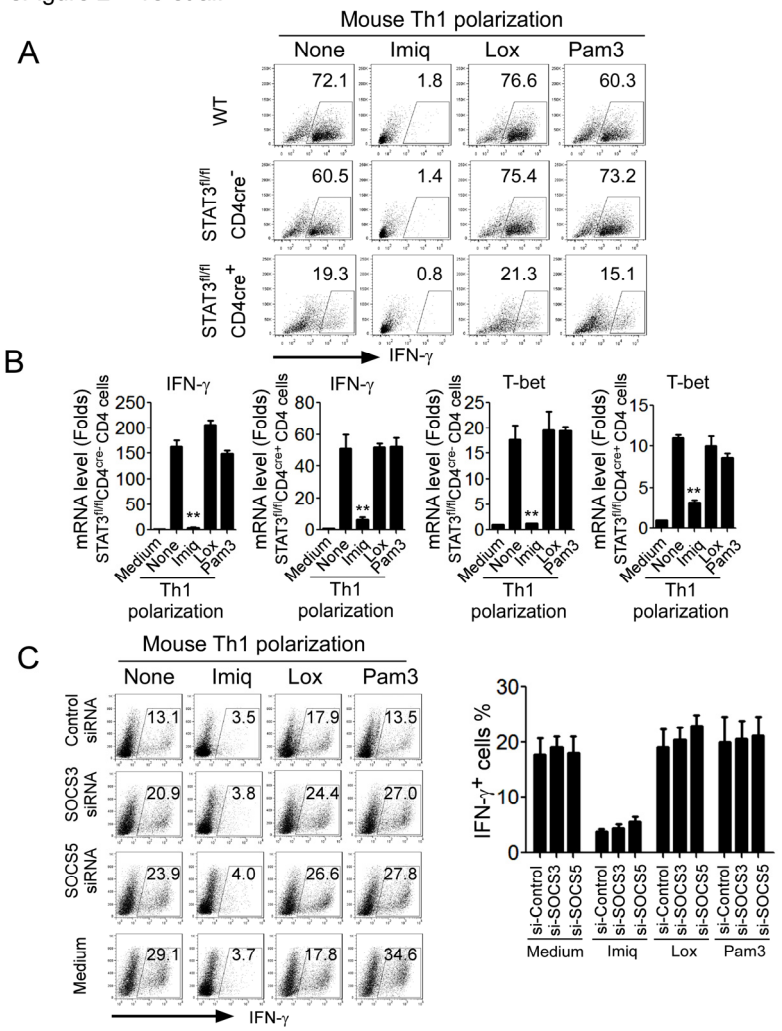
sFigure 1 Ye et al.



Supplemental Figure 1. TLR7-mediated inhibition of Th17 cells is not through interference of cytokine-cytokine receptor signaling.
(A) TLR7 ligands Lox and Imiq only partially inhibited mRNA expression of IL-23R, but not IL-6R and IL-21R. **(B)** Expression levels of cytokine IL-4, IL-8, IL-10 and TNF-α in polarizated mouse Th17 cells treated with Lox and Imiq. Mouse $CD4^+$ T cells were cultured in Th17 polarization condition medium in the presence of Imiq, Lox and Pam3 for 6 days. Indicated cytokine receptor (A) and cytokine mRNA expression levels were determined by real-time PCR. The expression level of each gene was normalized to GAPDH expression and adjusted to the level in medium only group. Data are summarized as means ± SD from three representative naïve $CD4^+$ T cells.

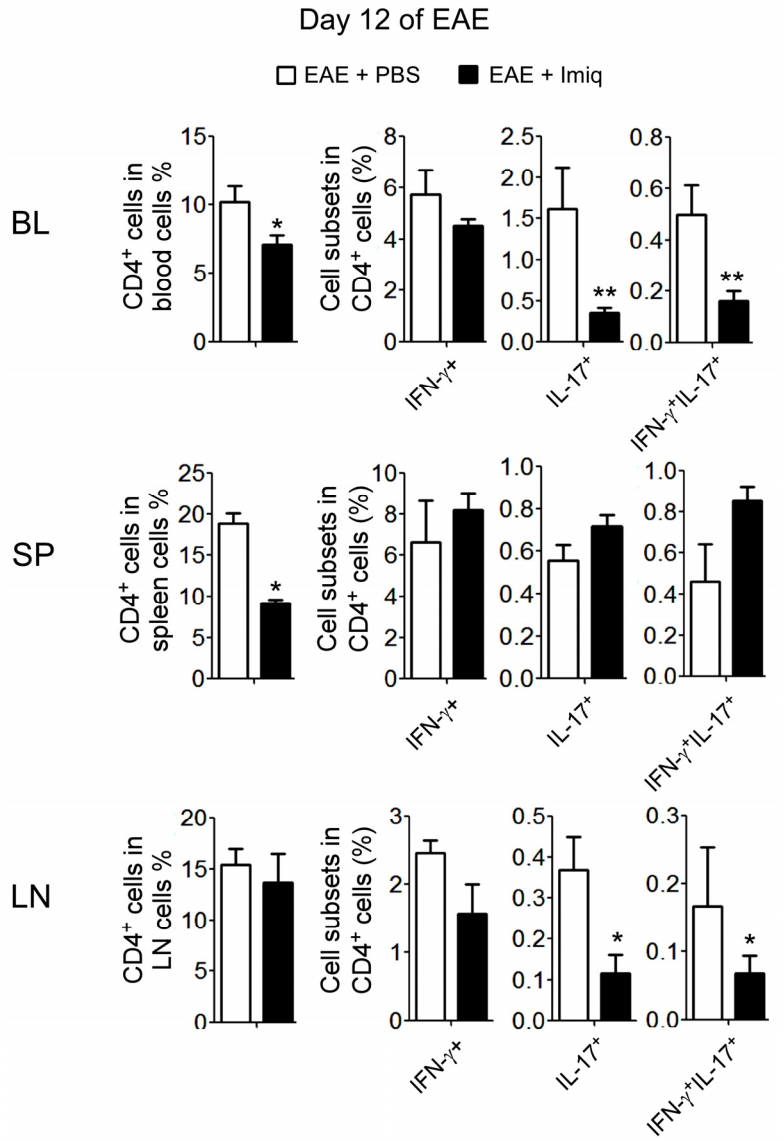
sFigure 2 Ye et al.



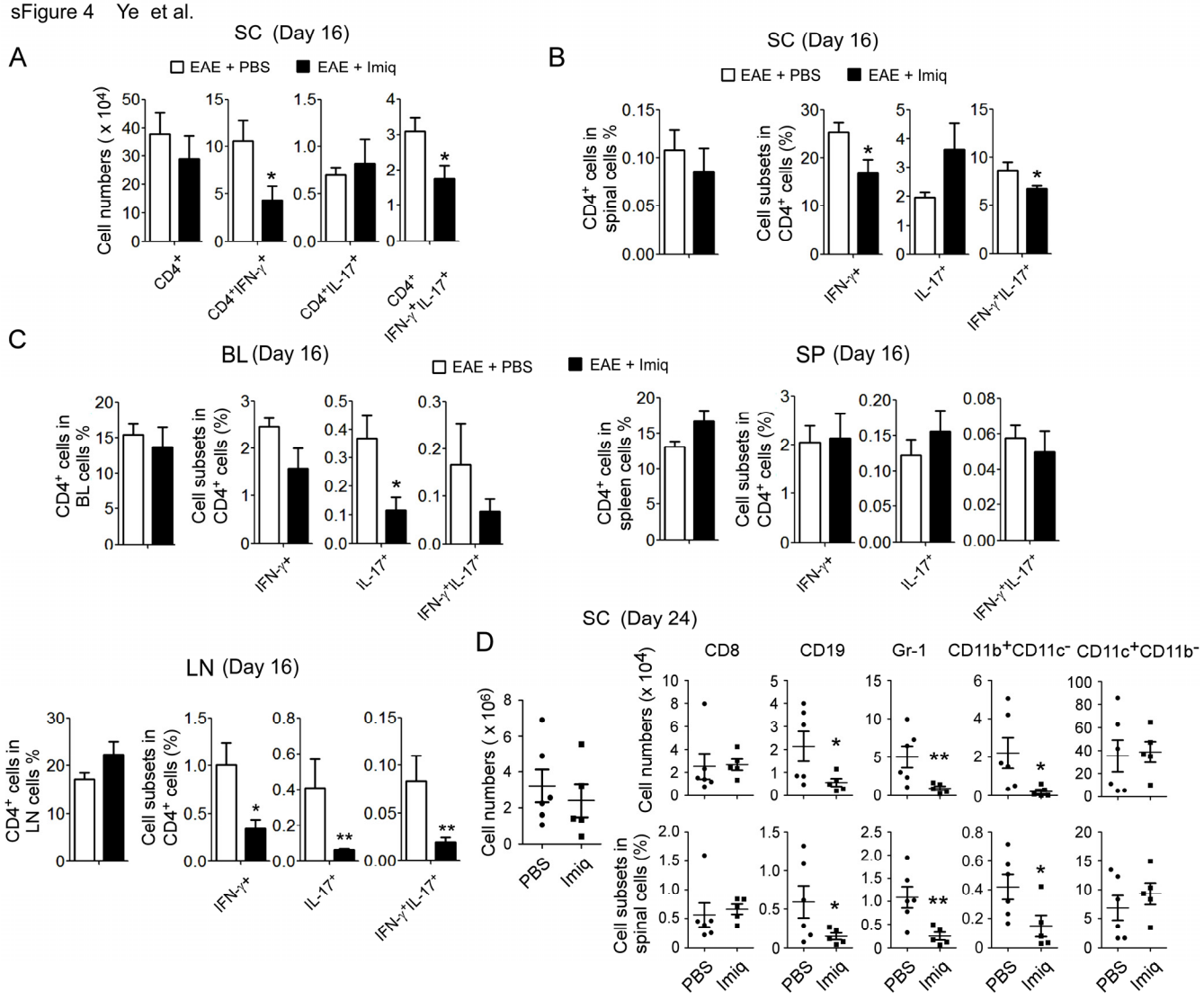
Supplemental Figure 2. STAT3-SOCS signaling is not involved in TLR7-induced inhibition of Th1 cell polarization.

(A) STAT3 deficiency did not abolish TLR7-mediated suppression of Th1 cell differentiation and development in $CD4^+$ T cells. Furthermore, $CD4^+$ T cells derived from $STAT3^{fl/fl}CD4^{cre+}$mice also had lower ability to develop Th1 cells compare with those from wild-type and $STAT3^{fl/fl}$ $CD4^{cre-}$ mice. $CD4^+$ T cells derived from wild-type, $STAT3^{fl/fl}$ $CD4^{cre-}$, and $STAT3^{fl/fl}CD4^{cre+}$ were cultured in the Th1 polarization medium in the presence or absence of TLR ligands for 6 days, and IFN-γ-producing cells were evaluated using flow cytometry after stimulation with PMA and ionomycin for 5 hours. **(B)** Imiq treatment significantly inhibited IFN-γ and T-bet mRNA expression in polarized Th1 cells in $CD4^+$ T cells derived from both $STAT3^{fl/fl}CD4^{cre-}$ and $STAT3^{fl/fl}CD4^{cre+}$mice with a similar level. Cell culture and treatment were identical to (A). mRNA expression of IFN-γ and T-bet was determined by real-time PCR. Relative mRNA expression level of each gene was normalized to GAPDH expression and then adjusted to the level in naïve $CD4^+$ T cells. Data shown are mean ± SD from triplicate experiments, and paired t-test was performed. ** $p<0.01$, compared with the Th17 polarization medium only group. **(C)** Knockdown of SOCS3 and SOCS5 genes in $CD4^+$ T cells had no effect on the Imiq-mediated suppression of Th1 cell development. Mouse $CD4^+$ T cells were transfected with specific siRNAs against SOCS3 orSOCS5, as well as the scrambled siRNA control, and then cultured in Th1 polarization medium in the presence or absence of indicated TLR ligands for 6 days. IFN-γ-producing cells were evaluated using flow cytometry analysis after stimulation with PMA and ionomycin. Results shown in the right panel are the mean ± SD of IFN-γ-producing cells in the differentiated Th1 cells with indicated TLR ligand and siRNA treatments obtained from three different individual experiments.

sFigure 3 Ye et al.



Supplemental Figure 3. Effects of Imiq treatment on cell fractions of $CD4^+$, $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}17^+$, $CD4^+IFN\text{-}\gamma^+IL\text{-}17^+$ cells in blood, spleens, and lymph nodes on day 12 of EAE mice.
Cells were isolated from the blood, spleens, and lymph nodes of two treatment groups on day 12 of EAE development. Cytokine-producing $CD4^+$ T cells were evaluated using flow cytometry after stimulation with PMA and ionomycin for 5 hours. Data shown are summary of mean ± SD (n= 5 mice/group), and * $p<0.05$, ** $p<0.01$, compared with those from PBS treatment group in EAE mice using unpaired t-test.



Supplemental Figure 4. Effects of Imiq treatment on cell subsets on day 16 and 24 of EAE mice. **(A)** and **(B)** Imiq treatment decreased absolute cell numbers (in A) and cell fractions (in B) of infiltrated $CD4^+IFN\text{-}\gamma^+$ and $CD4^+IFN\text{-}\gamma^+IL\text{-}17^+$ cells in spinal cords (SC) of EAE mice. Cells were isolated from the spinal cords of two treatment mouse groups on day 16 of EAE development. Cytokine-producing $CD4^+$ T cells were evaluated using flow cytometry after stimulation with PMA and ionomycin. Data shown are summary of mean ± SD (n= 5 mice per group) and *p<0.05, compared with those from PBS administration group in EAE mice using unpaired t-test. **(C)** Effects of Imiq treatment on cell fractions of $CD4^+$, $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}17^+$, $CD4^+IFN\text{-}\gamma^+IL\text{-}17^+$ cells in blood, spleens, and lymph nodes on day 16 of EAE mice. Cells were isolated from the blood, spleens, and lymph nodes of two treatment mouse groups on day 16 of EAE development. Cytokine-producing $CD4^+$ T cells were evaluated using flow cytometry after stimulation with PMA and ionomycin for 5 hours. Data shown are summary of mean ± SD (n= 5 mice per group), and * p<0.05, ** p<0.01, compared with that from PBS administration group in EAE mice using unpaired t-test. **(D)** Imiq treatment significantly decreased both cell numbers and percentages of infiltrated $CD19^+$, $CD11b^+$ and $Gr\text{-}1^+$ cells, but did not affect total cell numbers and $CD8^+$ and $CD11c^+$ cell populations in spinal cords of EAE mice. Cells were isolated from the spinal cords of two treatment mouse groups on day 24 of EAE development. Cell populations were evaluated using flow cytometry. Data shown are summary of mean ± SD (n= 5-6 mice per group). *p<0.05 and **p<0.01, compared with those from PBS administration group in EAE mice using unpaired t-test.