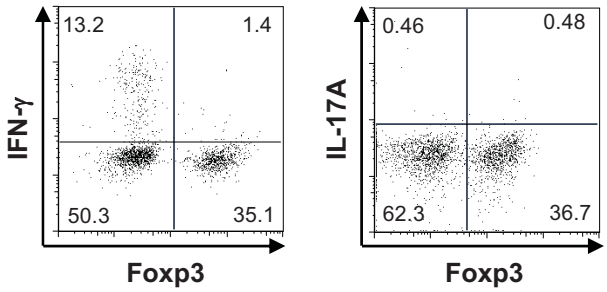
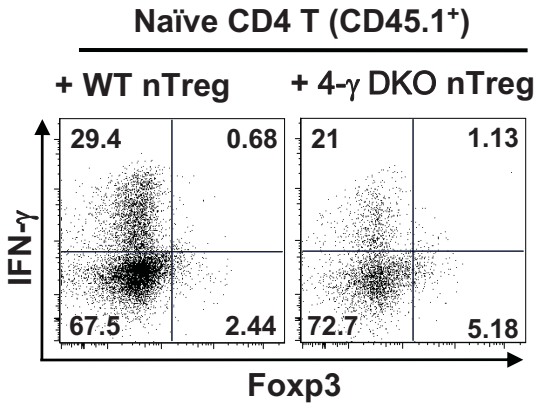
Supplementary Figure S1



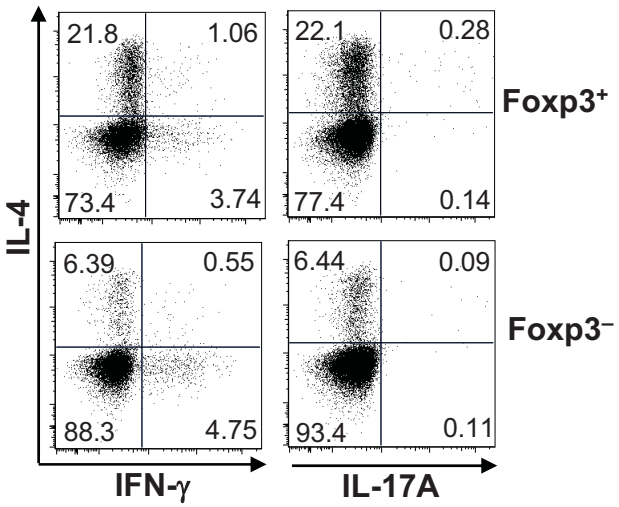
S1. IFN-γ and IL-17A production by Treg cells following Foxp3 downregulation. Sorted RFP$^+$ Treg cells from *FIR-4GET* mice were transferred into *Rag1*$^{-/-}$ mice. Eight weeks post transfer, Foxp3$^+$ and Foxp3$^-$ CD4 T cells recovered from the recipient mice were distinguished by Foxp3 intracellular staining. IFN-γ and IL-17A production was assessed by cytokine intracellular staining. Representative results from three experiments are shown.

Supplementary Figure S2



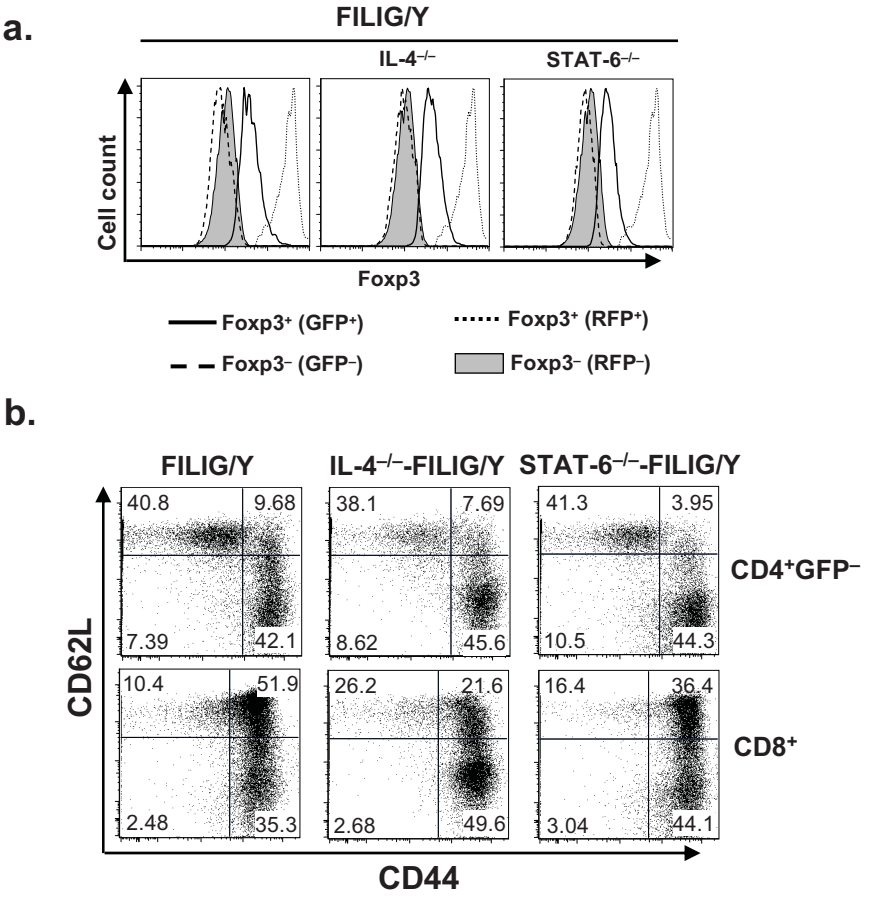
S2. The inability of Treg to produce IFN-γ modestly affected IFN-γ production by co-existing Tn cells. Sorted naïve CD4 T cells ($CD4^+CD25^-CD45RB^{high}$) were mixed with equal numbers of nTreg cells (RFP^+) purified either from *FIR* mice (+ WT nTreg) or from *FIR* mice lacking both *IL-4* and *IFN-γ* genes (+ 4-γ DKO nTreg). Congenic markers CD45.1 and CD45.2 were expressed by naïve CD4 T cells and nTreg cells respectively. Cell mixtures were then transferred into *Rag1*$^{-/-}$ recipient mice. Eight weeks post transfer, the expression of IFN-γ and Foxp3 in naïve CD4 T cells ($CD4^+CD45.1^+$) recovered from recipient mice were assessed by intracellular staining. Results are representative of three experiments.

Supplementary Figure S3



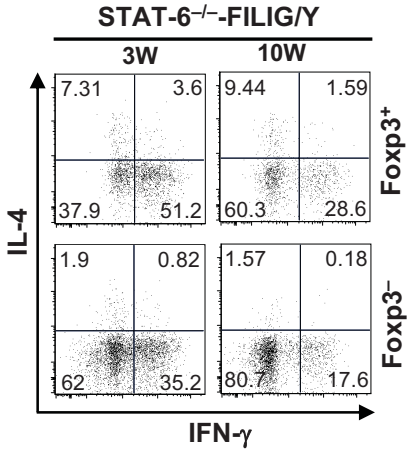
S3. IL-4 but not IFN-γ or IL-17A was highly produced by CD4 T cells from *FILIG/Y* mice. Foxp3$^+$ (GFP$^+$) and Foxp3$^-$ (GFP$^-$) CD4 T cells were purified from *FILIG/Y* mice by FACS. IL-4, IFN-γ and IL-17A production in Foxp3$^+$ and Foxp3$^-$ CD4 T cells were assessed by cytokine intracellular staining. Results are representative of at least three experiments.

Supplementary Figure S4



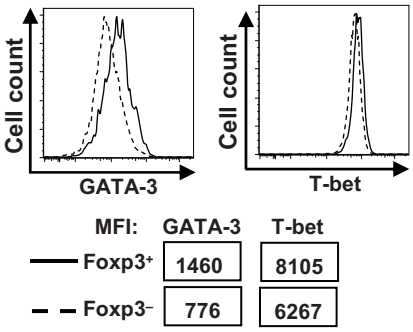
S4. Attenuated Foxp3 expression and activated T cell phenotypes in *FILIG/Y* mice deficient in *IL-4* or *STAT-6*. **a**, The comparison of Foxp3 expression levels of Foxp3$^+$ (GFP$^+$) and Foxp3$^-$ (GFP$^-$) CD4 T cells isolated from *FILIG/Y* mice, *FILIG* mice deficient in *IL-4* (IL-4$^{-/-}$-FILIG/Y) or *STAT-6* (STAT-6$^{-/-}$-FILIG/Y) to Foxp3$^+$ (RFP$^+$) and Foxp3$^-$ (RFP$^-$) CD4 T cells isolated from *FIR/Y* mice. **b**, CD62L and CD44 expression on the surface of CD4$^+$GFP$^-$ and CD8$^+$ T cells isolated from *FILIG/Y* mice and *FILIG/Y* mice deficient in *IL-4* (IL-4$^{-/-}$-FILIG/Y) or *STAT-6* (STAT-6$^{-/-}$-FILIG/Y). Representative results from at least three experiments are shown.

Supplementary Figure S5



S5. IL-4 production in Foxp3$^+$ cells in *STAT6$^{-/-}$-FILIG/Y* mice of different age. Foxp3$^+$ and Foxp3$^-$ CD4 T cells in 3- and 10-week old *STAT-6* deficient *FILIG/Y* mice were distinguished by Foxp3 intracellular staining. IL-4 and IL-13 production by Foxp3$^+$ and Foxp3$^-$ cells were determined. Results representative of two experiments are shown.

Supplementary Figure S6



S6. Ectopic Foxp3 expression promoted GATA-3 but not T-bet expression independent of STAT-6. RFP$^-$ CD4 T cells sorted from FIR mice deficient in *STAT-6* were transduced with MIG-Foxp3 as described in the Methods. GATA-3 and T-bet expression in transduced (Foxp3$^+$, solid lines) and non-transduced (Foxp3$^-$, dash lines) CD4 T cells that co-existed in the same culture were assessed by flow-cytometry. MFI of GATA-3 and T-bet staining are also shown. Results are representative of at least three experiments.