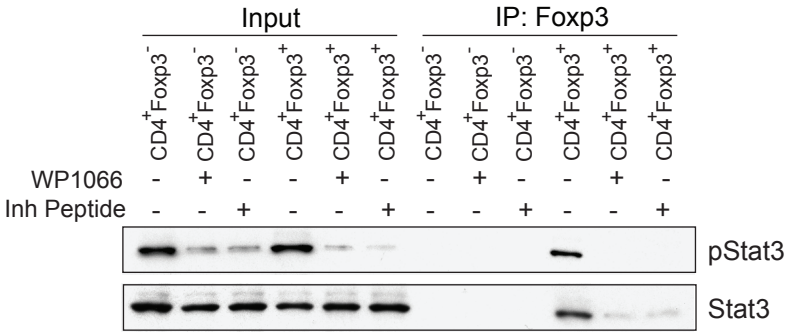
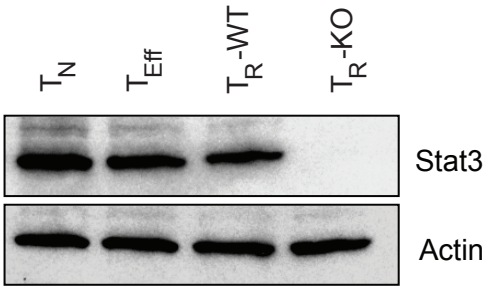
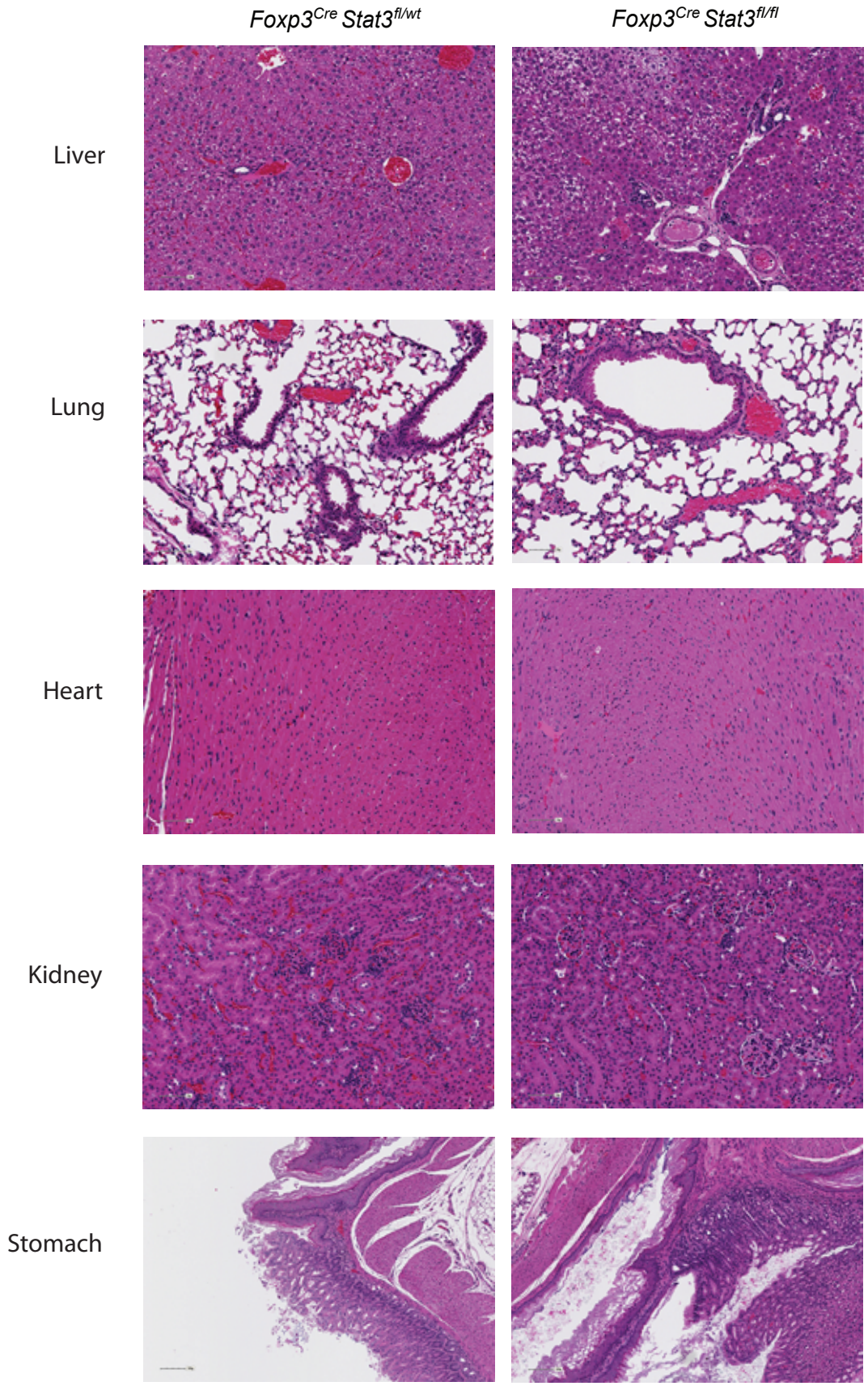


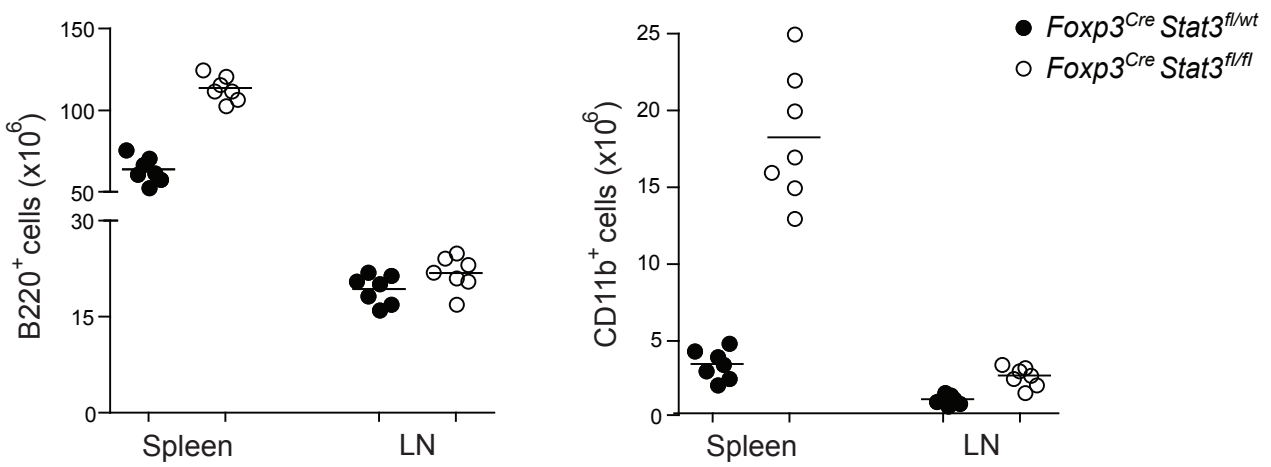
Supplementary Figure 1. Phosphorylation dependent binding of Stat3 to Foxp3. FACS sorted $CD4^{+}Foxp3^{-}$ and $CD4^{+}Foxp3^{+}$ cells were stimulated with IL-2, IL-6 (20ng/ml) and IL-10 (20ng/ml) in the presence or absence of indicated inhibitors for 6 h followed by nuclear lysis. Lysates were subjected to immuno-precipitation with Foxp3 antibody followed by western blot analysis for Stat3 or pStat3.



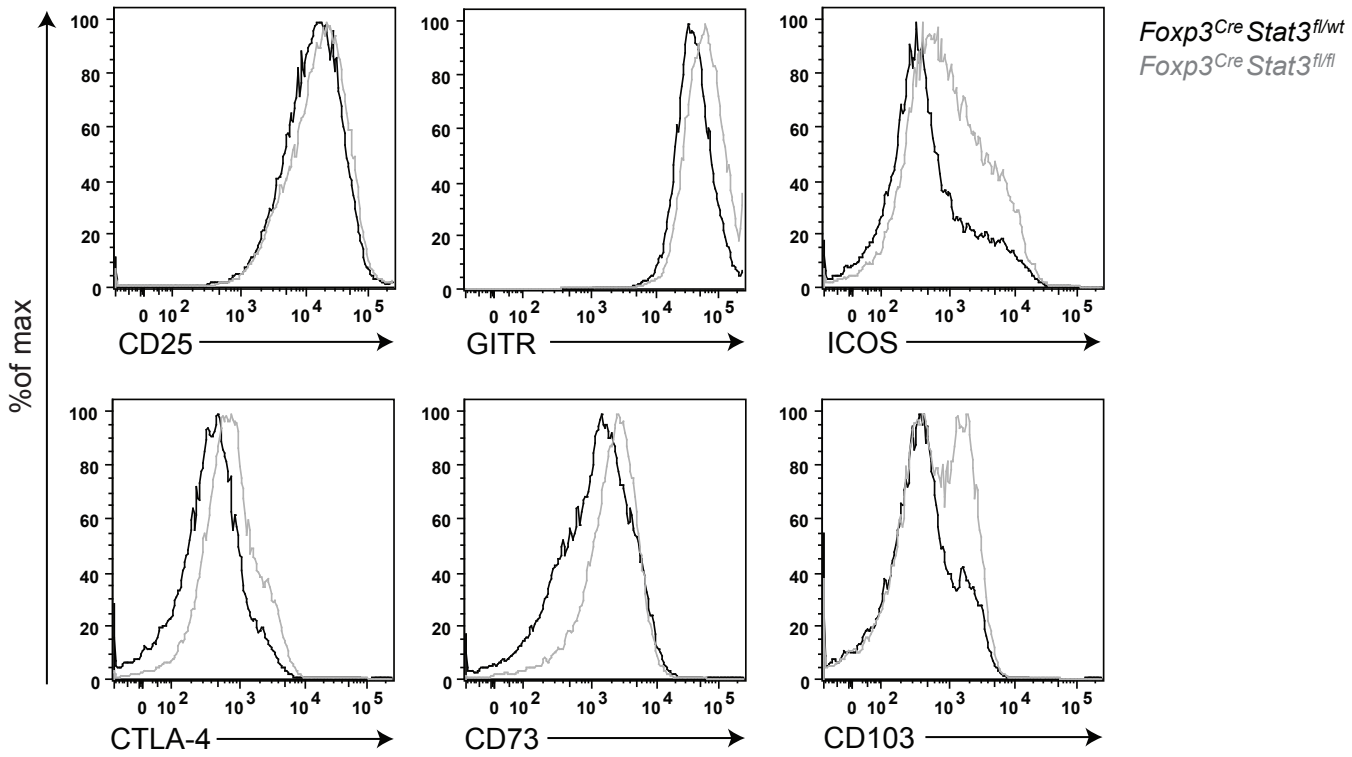
Supplementary Figure 2. Foxp3^{Cre}-mediated deletion of *Stat3^{fl}* is restricted to Treg cells. Whole cell lysates of FACS sorted naïve (T_N,CD4$^+$Foxp3$^-$CD25$^-$), activated (T_{Eff},CD4$^+$Foxp3$^-$CD25$^+$), and YFP-Cre$^+$ Foxp3$^+$ Treg cells from *Foxp3^{Cre}Stat3$^{fl/wt}$* (WT) or *Foxp3^{Cre}Stat3$^{fl/fl}$* (KO) were analyzed for Stat3 expression by western blotting.



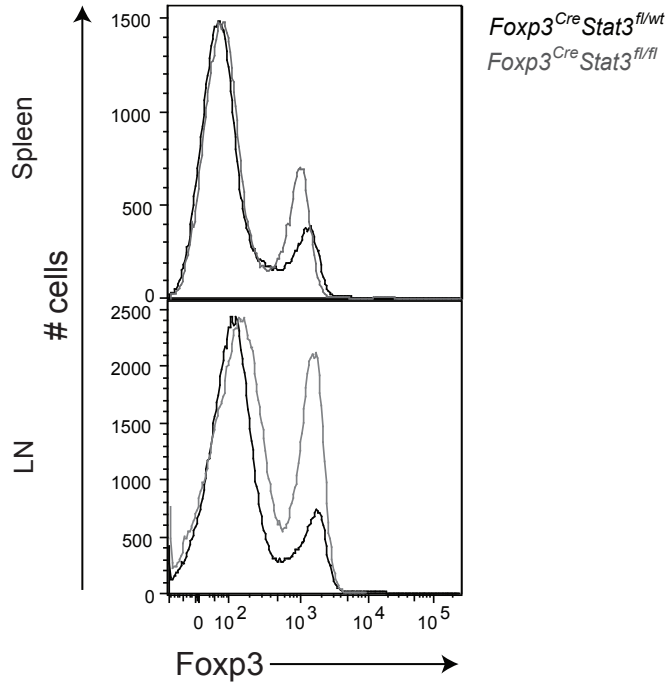
Supplementary Figure 3. H&E-stained tissue sections from *Foxp3*^{Cre}*Stat3*$^{fl/wt}$ and *Foxp3*^{Cre}*Stat3*$^{fl/fl}$ mice. The liver sections of the *Foxp3*^{Cre} *Stat3*$^{fl/fl}$ mice showed moderate lipidosis, hepatocellular necrosis, focal neutrophilic hepatitis and mild granulocytic extramedullary hematopoiesis that is most likely secondary to colitis. No noticeable lesions were observed in other tissues. Original magnification for all panels except stomach, 20x. Stomach original magnification left 4x, right 10x.



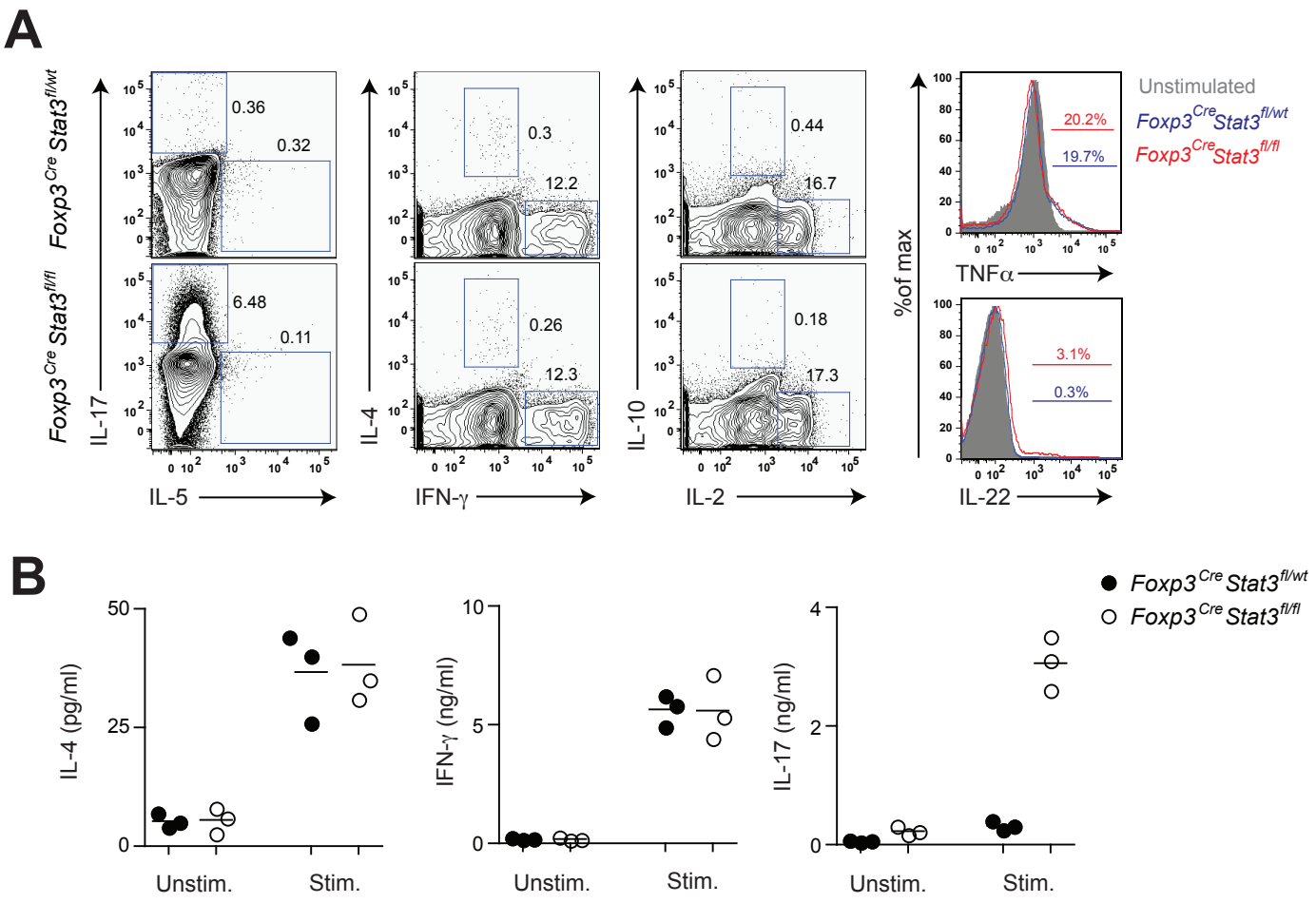
Supplementary Figure 4. Expansion of B cells and macrophages in mice harboring Stat3-deficient Treg cells. $B220^+$ and $CD11b^+$ cell numbers in the spleen and lymph nodes of *Foxp3*^{Cre} *Stat3*$^{fl/wt}$ and *Foxp3*^{Cre} *Stat3*$^{fl/fl}$ mice (n=7).



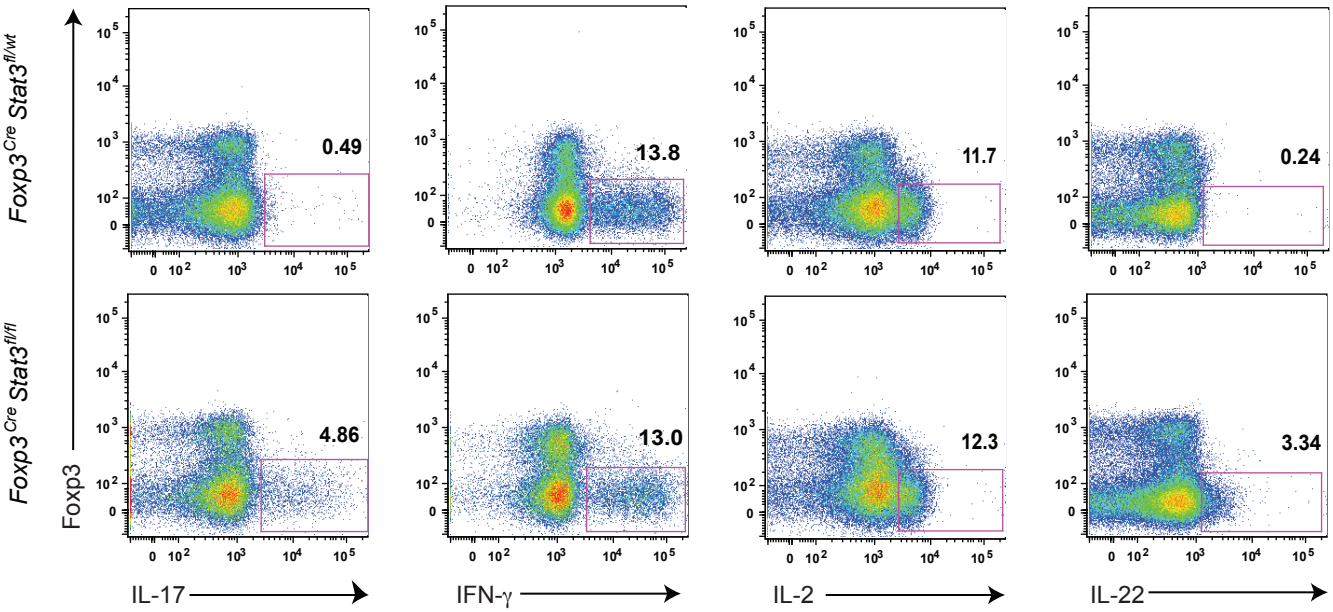
Supplementary Figure 5. Heightened activation status of Treg cells in the absence of Stat3. Flow cytometric analysis of Treg activation markers and putative effector molecules on splenic $CD4^{+}Foxp3^{+}$ T cells in *Foxp3*^{Cre} *Stat3*$^{fl/wt}$ and *Foxp3*^{Cre} *Stat3*$^{fl/fl}$ mice.



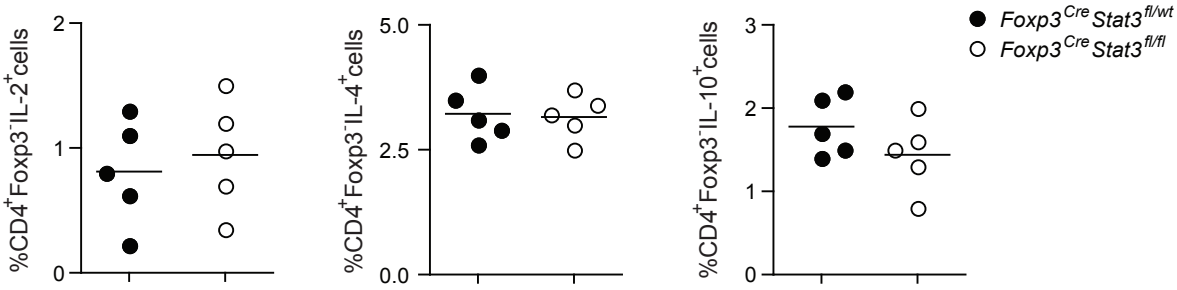
Supplementary Figure 6. Comparable levels of Foxp3 expression in Stat3-sufficient and -deficient Treg cells. Flow cytometric analysis of Foxp3 expression in $CD4^{+}Foxp3^{+}$ T cells in spleen and lymph nodes (LN) of *Foxp3*^{Cre}*Stat3*$^{fl/wt}$ and *Foxp3*^{Cre}*Stat3*$^{fl/fl}$ mice.



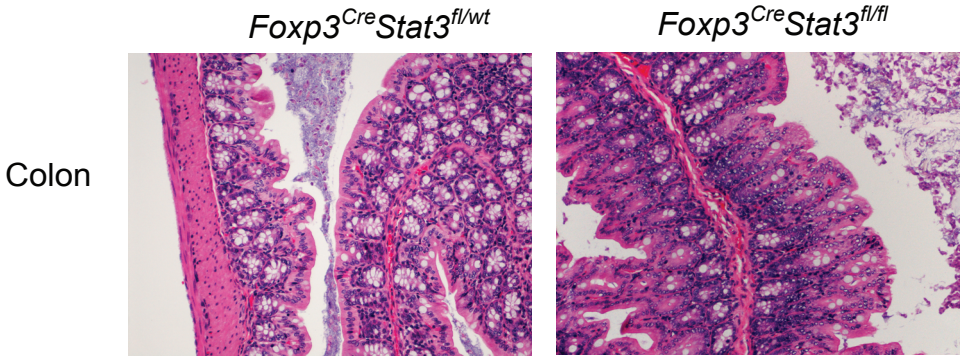
Supplementary Figure 7. Increased production of IL-17 by T cells from *Foxp3*^{Cre} *Stat3*$^{fl/fl}$ mice.
(A) Flow cytometric analysis of cytokine production by splenic CD4^{+}Foxp3^{-} T cells in *Foxp3*^{Cre} *Stat3*$^{fl/wt}$ and *Foxp3*^{Cre} *Stat3*$^{fl/fl}$ mice. Splenocytes were stimulated with PMA (50ng/ml) and Ionomycin (250ng/ml) in the presence of Golgi-Plug (1µg/ml) for 6 h prior to staining for CD4, CD8, and the indicated cytokines. A representative of four independent experiments is shown. (B) Splenic cells from *Foxp3*^{Cre} *Stat3*$^{fl/wt}$ and *Foxp3*^{Cre} *Stat3*$^{fl/fl}$ mice were cultured in a 96-well plate (1x10 cells/well), either un-stimulated or stimulated with anti-CD3 antibody for 4 days. Supernatants were collected, and IL-4, IFN-γ, and IL-17 concentrations were measured by ELISA.



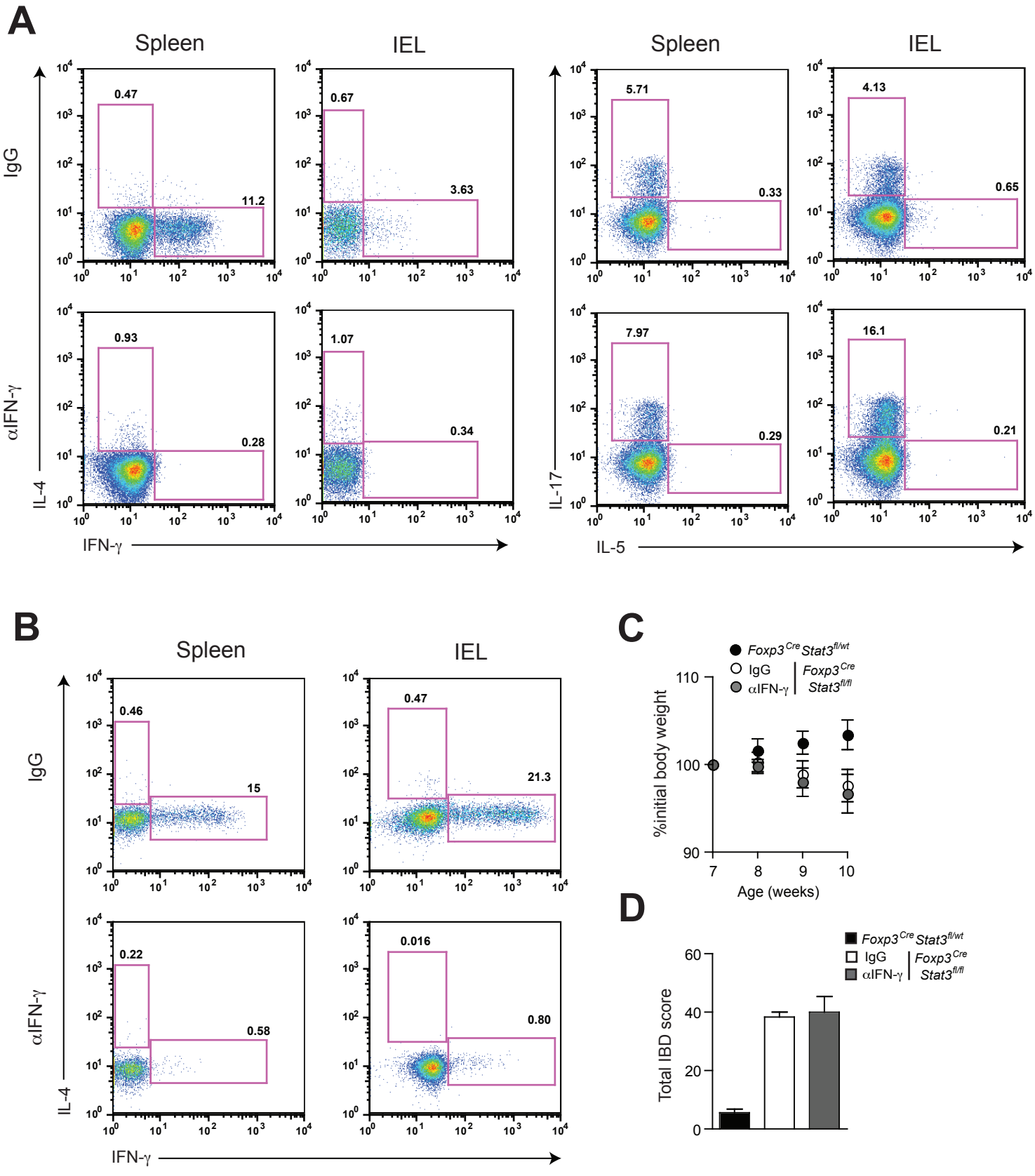
Supplementary Figure 8. Foxp3^{+} cells do not produce immune-response promoting cytokines in the absence of Stat3. Splenocytes from *Foxp3^{Cre}Stat3$^{fl/wt}$* and *Foxp3^{Cre}Stat3$^{fl/fl}$* mice were stimulated with PMA (50ng/ml) and Ionomycin (250ng/ml) antibodies in the presence of Golgi-Plug (1μg/ml) for 5 h, and stained for CD4, CD8, Foxp3, and the indicated cytokines. FACS plots were gated on CD4^{+} cells. A representative of three independent experiments is shown.



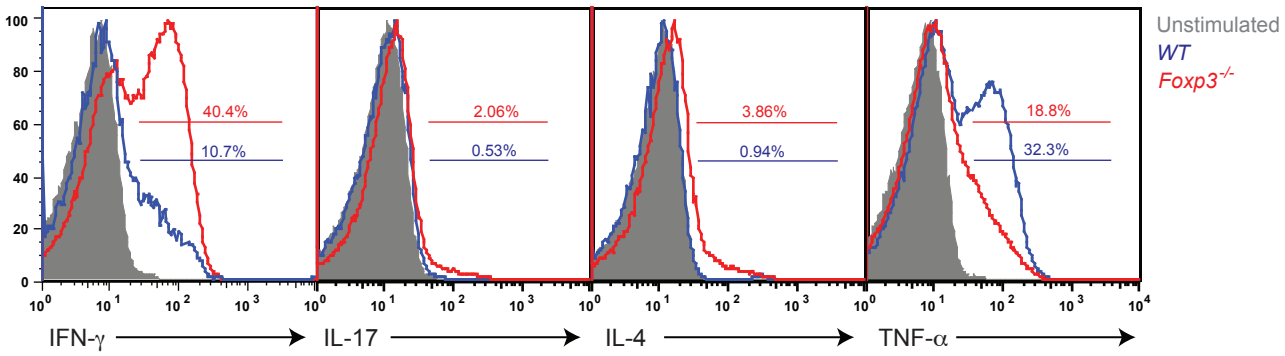
Supplementary Figure 9. Flow cytometric analysis of IL-2, IL-4, IL-10 secreting $CD4^{+}Foxp3^{-}$ T cells in the IEL subset of *Foxp3*^{Cre} *Stat3*$^{fl/wt}$ and *Foxp3*^{Cre} *Stat3*$^{fl/fl}$ mice.



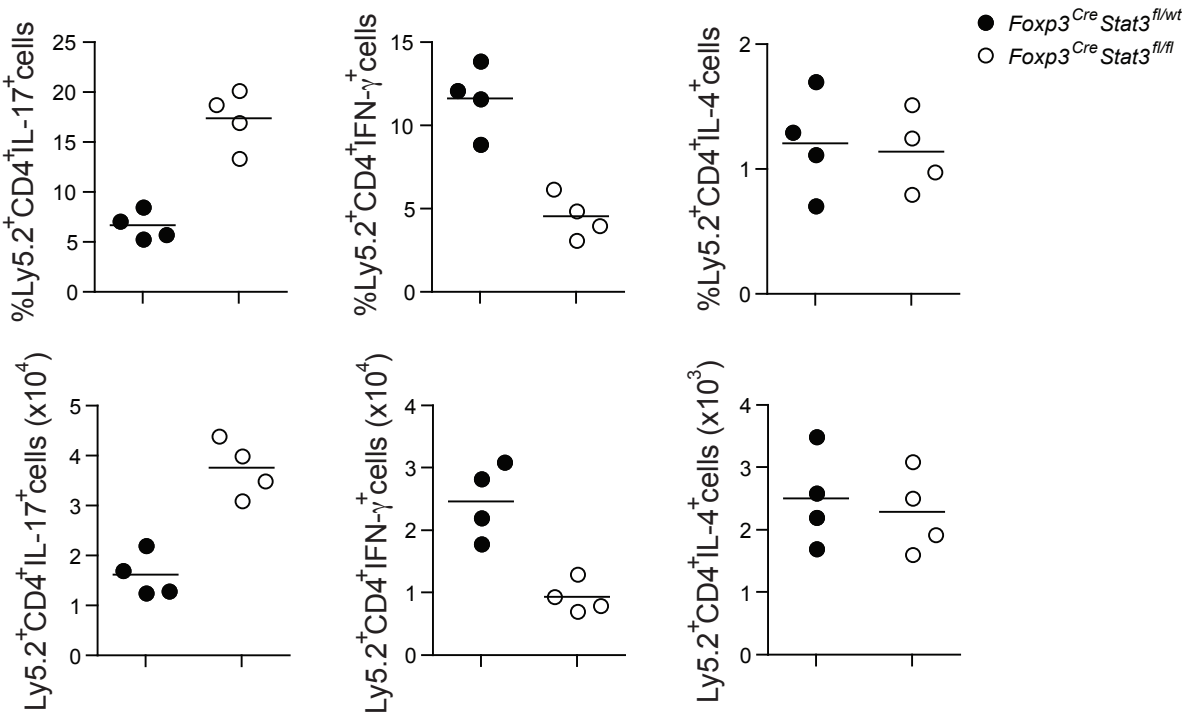
Supplementary Figure 10. Absence of IBD symptoms in young *Foxp3*^{Cre} *Stat3*$^{fl/fl}$ mice. Representative H&E stained colon sections from 3-4 week old *Foxp3*^{Cre} *Stat3*$^{fl/wt}$ and *Foxp3*^{Cre} *Stat3*$^{fl/fl}$ mice. Original magnification, 20x.



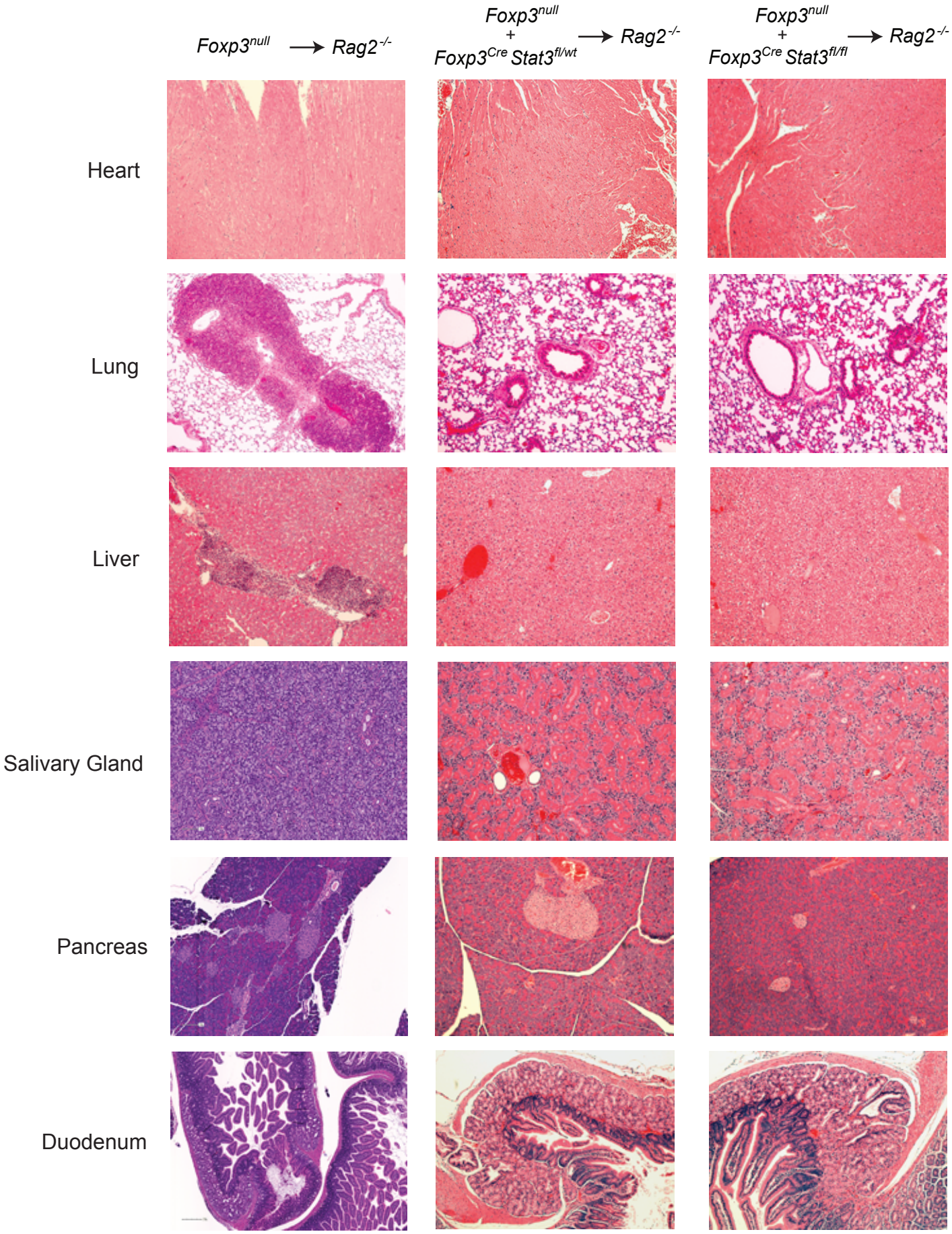
Supplementary Figure 11. IFN-γ neutralization does not prevent colitis in *Foxp3*^{Cre}*Stat3*$^{fl/fl}$ mice. (A) Flow cytometric analysis of cytokine production by CD4^{+}Foxp3^{-} and (B) CD8^{+} T cells in *Foxp3*^{Cre} *Stat3*$^{fl/fl}$ mice treated with isotype-matched IgG or IFN-γ neutralizating antibody. (C) Weight loss and (D) IBD scores in *Foxp3*^{Cre}*Stat3*$^{fl/fl}$ mice treated with isotype-matched IgG or IFN-γ neutralizating antibodies (n=5).



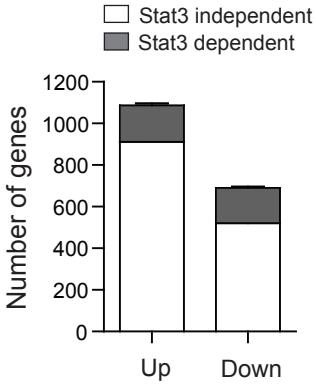
Supplementary Figure 12. CD4 T cells from *Foxp3*$^{-/-}$ mice produce both Th1 and Th2 cytokines. Splenocytes from 3 week-old *Foxp3*$^{-/-}$ and *Foxp3*$^{+/+}$ littermate control mice were stimulated with CD3 (2µg/ml) and CD28 (2µg/ml) antibodies in the presence of Golgi-Plug (1µg/ml) for 5 h, and stained for CD4, CD8, and the indicated cytokines. FACS plots were gated on CD4^{+} cells. A representative of two independent experiments is shown.



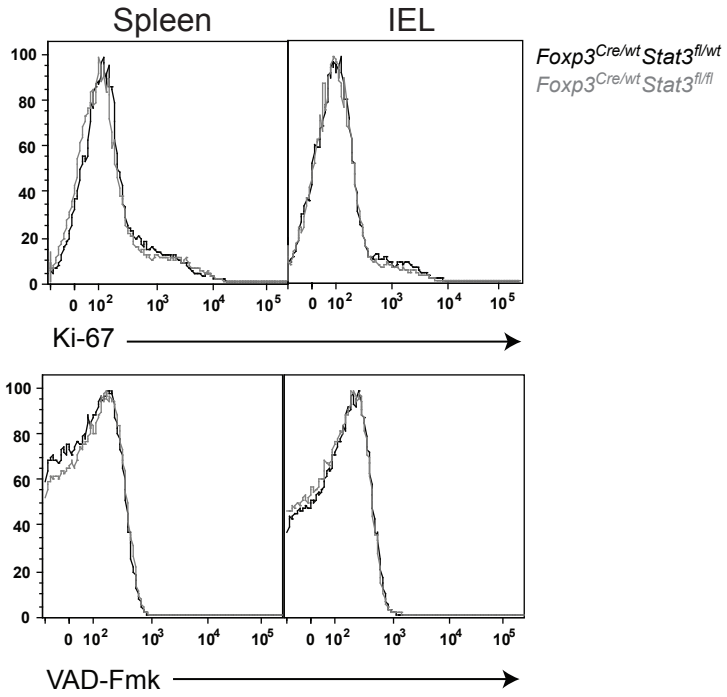
Supplementary Figure 13. Frequencies and numbers of cytokine producing LPL cells within the indicated donor derived population 4 weeks after co-transfer of either *Foxp3*^{Cre}*Stat3*$^{fl/wt}$ or *Foxp3*^{Cre} *Stat3*$^{fl/fl}$ Treg cells with *Foxp3*$^{-}$ Teff cells into *Rag2*$^{-/-}$ recipients.



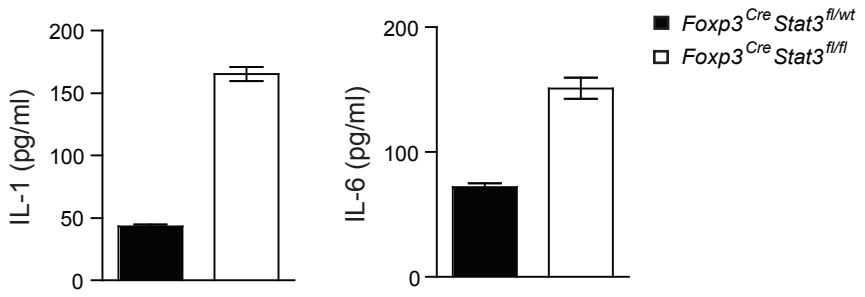
Supplementary Figure 14. H&E-stained tissue sections from recipient mice 4 weeks after co-transfer *of Foxp3*$^{-}$ T cells mixed with Treg cells from either *Foxp3*^{Cre}*Stat3*$^{fl/wt}$ or *Foxp3*^{Cre}*Stat3*$^{fl/fl}$ mice. Original magnification except duodenum, 10x. Duodenum original magnification left panel 4x, middle & right panel 10x.



Supplementary Figure 15. Stat3-dependent gene expression in Tregs. Number of Foxp3 and Stat3 -dependent genes that were up or down-regulated in Tregs as compared to Foxp3-dependent genes.



Supplementary Figure 16. Similar proliferative ability and apoptosis in Stat3-sufficient and -deficient Foxp3$^+$ cells. Splenocytes and IEL cells from either *Foxp3*$^{Cre/wt}$*Stat3*$^{fl/wt}$ or *Foxp3*$^{Cre/wt}$*Stat3*$^{fl/fl}$ mice were stained with either Ki-67 or VAD-Fmk and analysed by flow cytometry. FACS plots were gated on CD4$^+$ Foxp3$^+$ cells. A representative of two independent experiments is shown.



Supplementary Figure 17. Reduced ability of Stat3-deficient Tregs to deplete IL-1 and IL-6 in vitro. Tregs (0.5 x 10^6/well) from *Foxp3*^{Cre}*Stat3*$^{fl/wt}$ and *Foxp3*^{Cre}*Stat3*$^{fl/fl}$ mice were incubated with IL-1 (200pg/ml) or IL-6 (200pg/ml) in the presence or absence of brefeldin A for 4-6 h in duplicate cultures. Culture supernatants were assayed for the indicated cytokines by ELISA. Cultures without brefeldin A were used for IL-1 depletion whereas IL-6 depletion was performed in the presence of brefeldin A to prevent IL-6 secretion by Stat3-deficient Tregs.