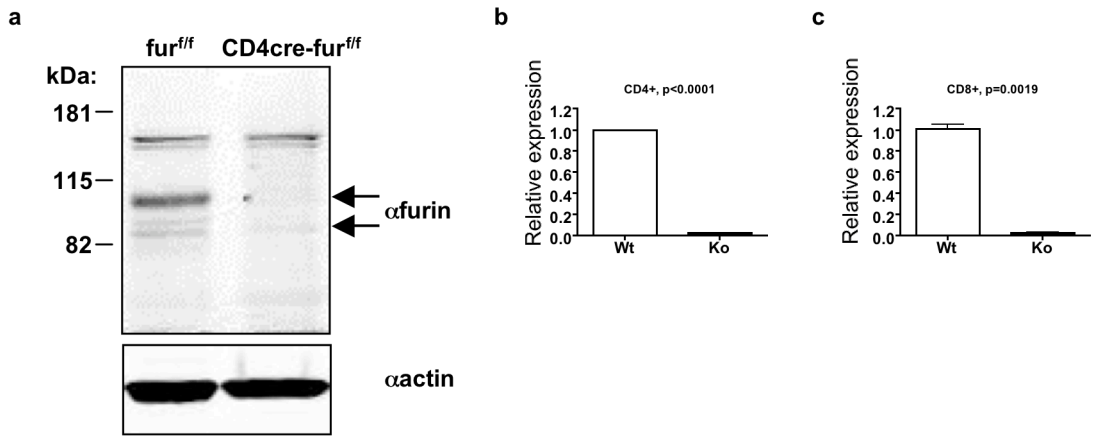
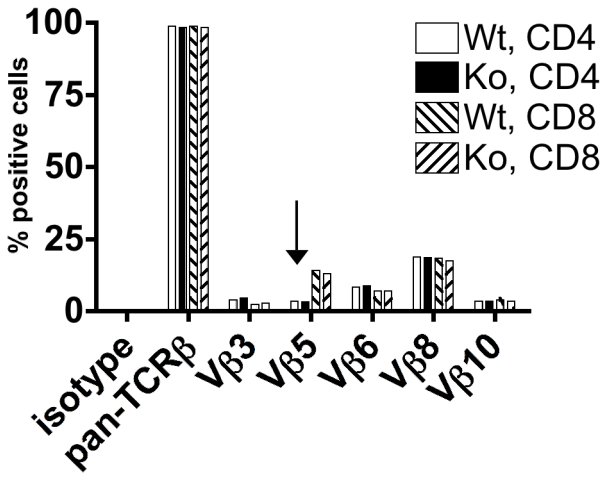
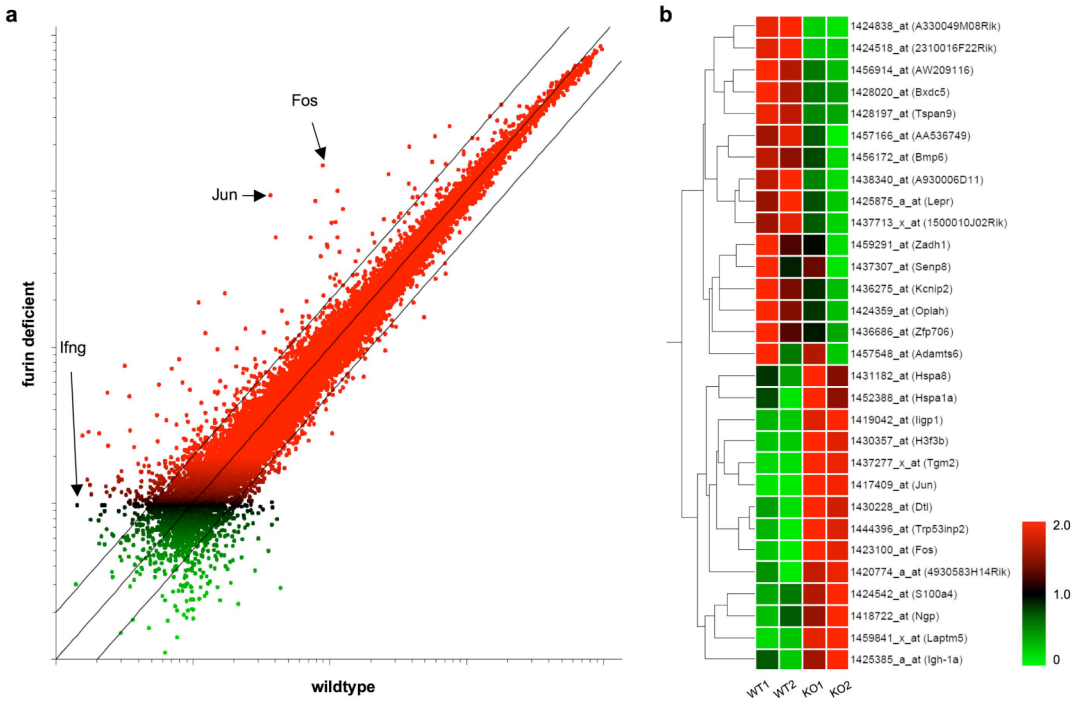


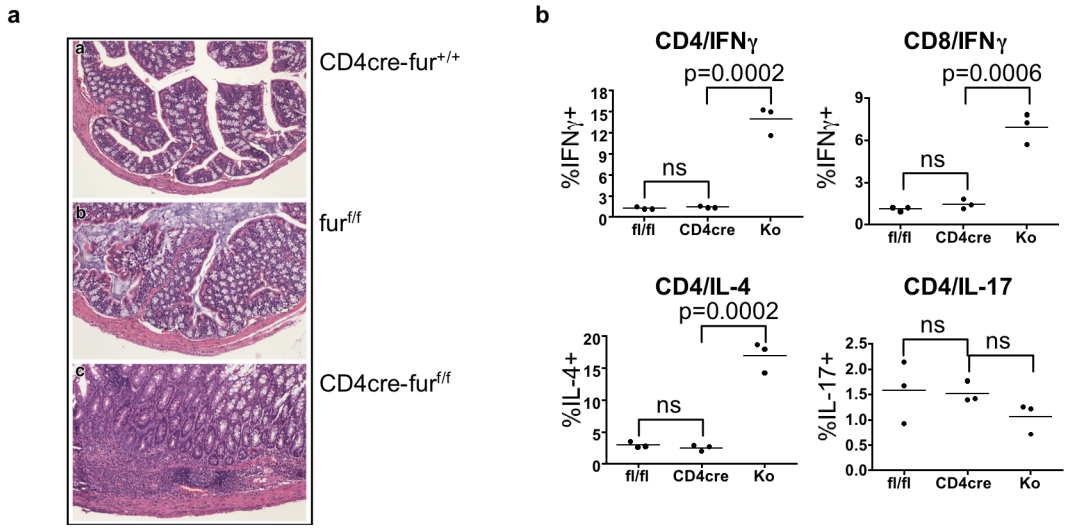
Supplemental Figure 1. Furin is efficiently deleted in CD4$^+$ and CD8$^+$ T cells. a, Western blot for furin and actin proteins in CD4Cre-fur$^{f/f}$ and fur$^{f/f}$ Th1 cells. Wild-type and furin deficient CD4$^+$ cells were cultured in Th1 polarizing conditions for 3 days (plate-bound anti-CD3 and anti-CD28 antibodies, IL-12 10 ng ml^{-1}, anti-IL-4 10μg ml^{-1}), and then expanded in IL-2 50 U ml^{-1} for 3 additional days. Arrows indicate the bands representing furin. The experiment was performed twice with similar results. **b.** Furin mRNA levels. CD4$^+$ and CD8$^+$ cells were activated with plate-bound anti-CD3 and CD28 under non-polarizing conditions for 3 days and further expanded for 3 additional days in IL-2. Experiment was performed in duplicate; relative furin mRNA expression levels normalized to 18S house-keeping gene are shown.



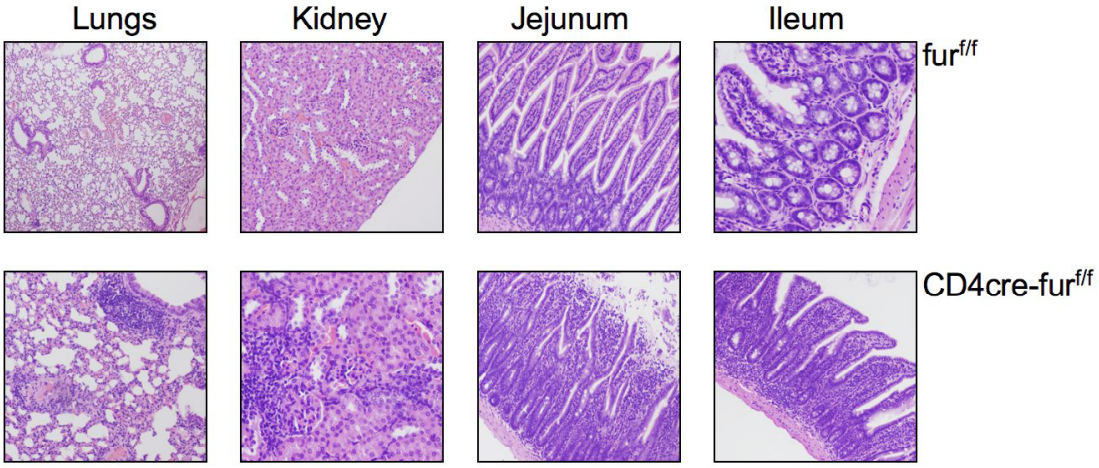
Supplemental Figure 2. Normal TCR Vβ subsets in the CD4cre-fur$^{f/f}$ animals. Lymph nodes from 6-week-old CD4cre-fur$^{f/f}$ and littermate fur$^{f/f}$ animals were stained with CD4, CD8 and isotype control, pan-TCR Vβ and indicated TCR Vβ subset specific antibodies. TCR Vβ subsets were analyzed with flow cytometry. Partial deletion of TCR Vβ5 in wild-type and furin-deficient CD4 cells is indicated with an arrow. A representative experiment of two performed is shown.



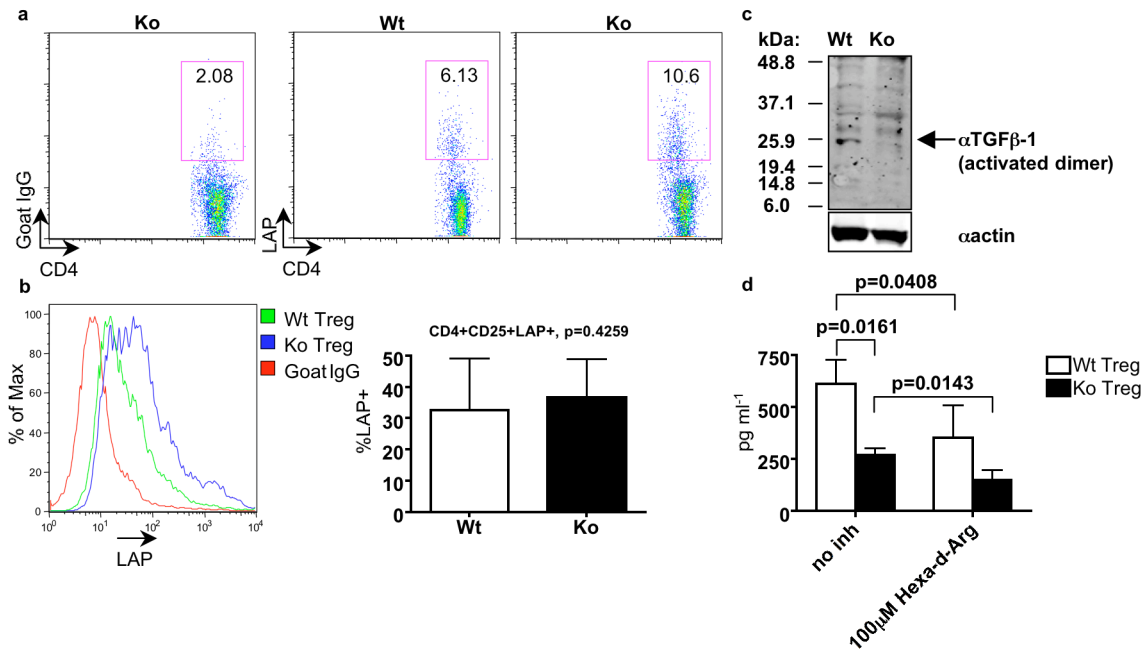
Supplemental Figure 3. Differential gene expression in naïve wild-type and furin deficient CD4^{+}CD44^{low}CD62L^{+} cells. a, Average expression values of two experiments for each of CD4Cre-fur$^{f/f}$ and fur$^{f/f}$ naïve T cells were calculated. A scatter plot view of comparison between CD4Cre-fur$^{f/f}$ (Y axis) and fur$^{f/f}$ (X axis) naïve T cells gene expression profiles is shown. The blue lines represent two fold changes. **b,** Hierarchical clustering using Pearson correlation values is shown for the 30 most differentially expressed genes in furin deficient naïve T cells. The color-coding depicts the normalized expression value for each gene (scale 0–2.0, with 1 as the median).



Supplemental Figure 4. Colon histology and intracellular T cell cytokine production in older CD4cre-fur$^{+/+}$ (CD4cre), fur$^{f/f}$ (fl/fl) and CD4cre-fur$^{f/f}$ (Ko) mice. a, Hematoxylin and eosin staining of colons of 7-8 months old mice. **b,** Intracellular cytokine staining. Splenocytes from 7-8 months old mice were activated for 4 h with phorbol myristate acetate and ionomycin, golgiplug (BD Pharmingen) was added to cultures after 2 h. Intracellular staining was done with intracellular cytokine staining kit (BD Pharmingen). Three mice per group were analyzed, ns = not significant.

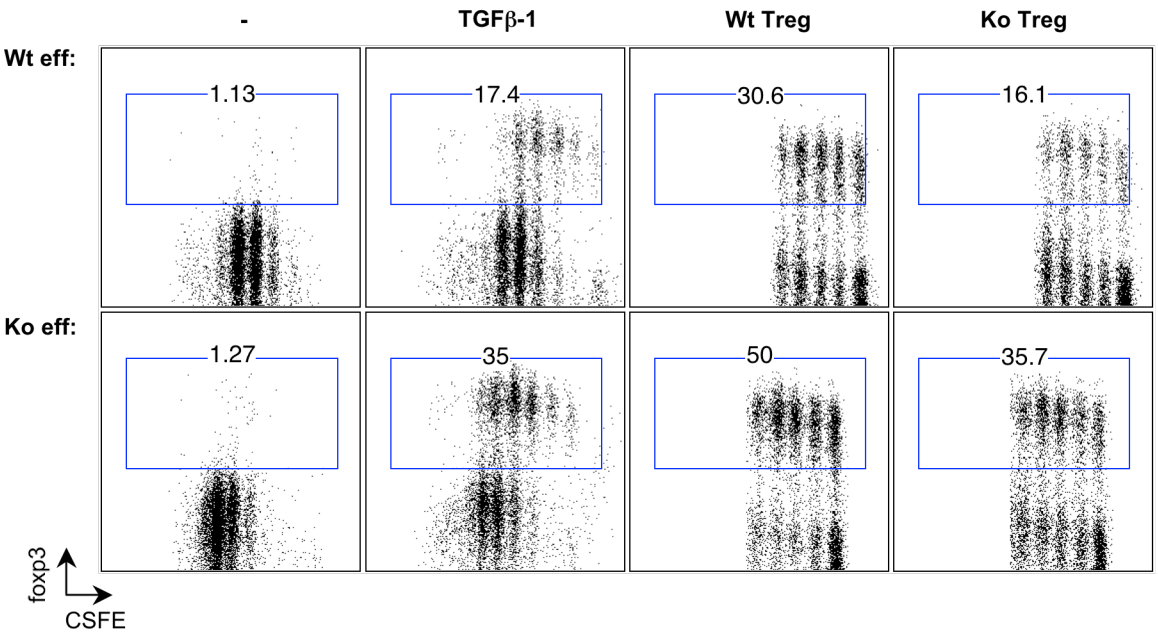


Supplemental Figure 5. Multifocal autoimmunity disease in CD4cre-fur$^{f/f}$ animals. Hematoxylin and eosin staining of lungs, kidney, jejunum and ileum of CD4cre-fur$^{f/f}$ and littermate fur$^{f/f}$ mice at 6 months old.

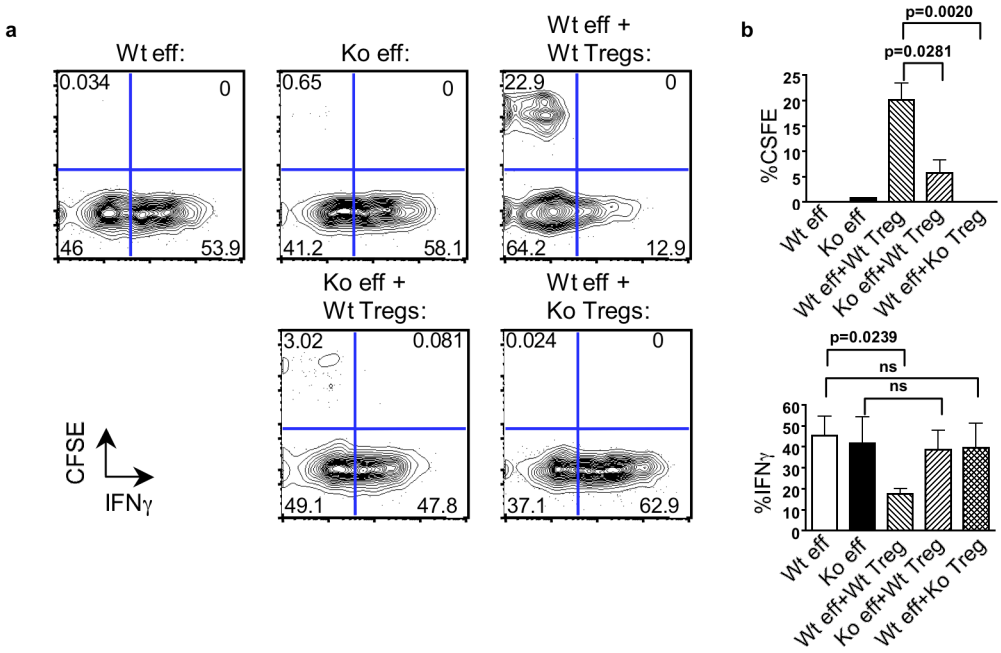


Supplemental Figure 6. Furin-deficient T cells lack processed, biologically active TGFβ-1, but express normal levels of Latency Associated Peptide on the cell surface. a. Freshly isolated mesenteric lymph nodes from CD4cre-fur$^{f/f}$ (Ko) and littermate fur$^{f/f}$ (Wt) were stained for surface expression of CD4 and TGFβ-1 Latency Associated Peptide (LAP) (biotinylated anti-LAP and biotinylated goat IgG purchased from R&D systems) and analyzed by flow cytometry. **b.** Purified wild-type of furin-deficient CD4^{+}CD25^{+} cells were activated two rounds with plate-bound anti-CD3 (10 μg ml^{-1}) and soluble anti-CD28 (2 μg ml^{-1}) antibodies as described in the methods. Surface expression of LAP on T cells was analyzed with flow cytometry. One representative experiment and average LAP expression from three independent experiments are shown. **c.** Wild-type and furin-deficient CD4^{+} cells were cultured in T regulatory cell inducing (iTreg) conditions for 3 days (plate-bound anti-CD3 10 μg ml^{-1} and soluble anti-CD28 2 μg ml^{-1} antibodies, IL-2 100 ng ml^{-1}, TGFβ-1 5 ng ml^{-1}), and then expanded in IL-2 50 U

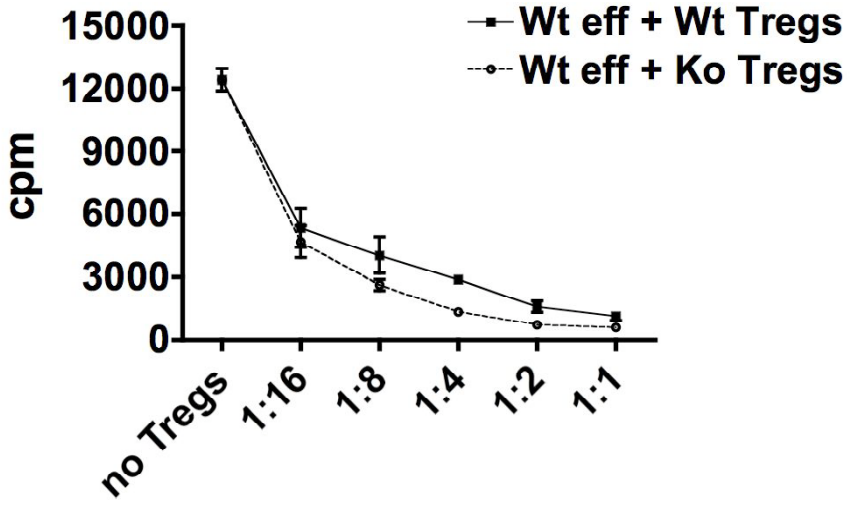
ml^{-1} for 3 additional days. Western blot with antibodies against activated/mature TGFβ-1 (Santa Cruz) and actin was performed (molecular weight of activated/mature TGFβ-1 dimer ~25 kDa). Experiment was performed four times. **d.** Wild-type and furin-deficient T regulatory cells were purified and activated as in **b.** The second activation was done in the presence or absence of furin inhibitor (furin inhibitor II, Calbiochem, Hexa-d-Arg, 100 μM) as indicated. Activated TGFβ-1 was measured in supernatants by ELISA (eBiosciences). Shown is pooled data from two independent experiments.



Supplemental Figure 7. Furin-deficient $CD4^+$ cells can upregulate Foxp3, but fail to induce Foxp3 in normal T cells. In vitro conversion assay. Purified wild-type of furin deficient $CD4^+FoxP3^+$ T regulatory cells were activated for 4 days with plate-bound anti-CD3 and IL-2 (100 U ml^{-1}). The activated Tregs were then co-cultured for an additional 4 days with CFSE-labeled wild-type or furin-deficient $CD4^+Foxp3^-$ responder cells in the presence of splenic dendritic cells at a 5:5:1 ratio ($CD4^+Foxp3^+$: $CD4^+Foxp3^-$:splenic dendritic cells), anti-CD3 (2 μg ml^{-1}) and IL-2 (100 U ml^{-1}) as indicated. Cytokine-induced conversion of wild-type or furin-deficient $CD4^+Foxp3^-$ responders was investigated in the absence of Tregs, but in the presence of dentritic cells and exogenous TGFβ-1 (5 ng ml^{-1}). $CFSE^+$ T cells were analyzed for Foxp3 expression using flow cytometry. A representative of three independent experiments performed is shown. When compared to wild-type Tregs, furin-deficient Tregs had in average 51 % reduced ability to convert wild-type effectors into $CD4^+Foxp3^+$ cells (three independent experiments, p=0.0498).



Supplemental Figure 8. Furin-deficient CD4^{+}CD25^{+} T regulatory cells have reduced suppressive activity and furin-deficient CD4^{+}CD45Rb^{hi}CD25^{-} effector cells are poorly suppressed *in vivo*. a. and b. Wild-type (Wt) or CD4cre-fur$^{f/f}$ (Ko) CD4^{+}CD45Rb^{hi}CD25^{-} naïve T cells were purified, labeled with CSFE and transferred alone or in combination with wild-type or furin-deficient CD4^{+}CD25^{+} T regulatory cells into RAG2$^{-/-}$ recipients. On day 7 effector cells were recovered and analyzed for CFSE and intracellular IFN-γ expressions on effector cells. Congenic markers CD45.1 and CD45.2 were used to distinguish transferred cell populations. (n = 3 per group, ns= not significant).



Supplemental Figure 9. Normal *in vitro* suppressive activity of furin-deficient $CD4^+CD25^+$ Treg in cells. Effector ($CD4^+CD25^-$) cells and Tregs ($CD4^+CD25^+$) were purified by flow cytometry and suppression assay was performed as described[22]. The ratios of Treg cells to $CD4^+CD25^-$ effectors are depicted in x-axis (wild-type Treg, filled square, furin-deficient Treg, open circle).