

Parekh *et al.*, Supplementary materials

Supplemental Figure Legends

Supplemental Figure 1. T cell-specific deletion of Vps34. (A) Genomic DNA PCR of the Vps34 wild-type and mutant alleles in Vps34^{fl/fl};CD4-Cre or Vps34^{fl/+};CD4-Cre mice. (B) cDNA was prepared from CD4⁺ thymocytes or plate-bound anti-CD3 and anti-CD28 antibody-activated splenic CD4⁺ or CD8⁺ T cells derived from Vps34^{fl/fl};CD4-Cre or Vps34^{fl/+};CD4-Cre mice and the expression of Vps34 RNA was measured by real-time PCR assay. Data for an average of 4 individual mice is shown. (C) Cell lysates were prepared from total T cells from Vps34^{fl/fl};CD4-Cre or Vps34^{fl/+};CD4-Cre mice activated with plate-bound anti-CD3 and anti-CD28 antibodies for 36 hrs, followed by immunoblotting with anti-Vps34 or - β -actin antibody. A representative experiment of 3 individual experiments representing 4-6 mice is shown.

Supplemental Figure 2. Organ cellularity in T cell-specific Vps34-deficient mice. (A) Splenic, lymph node, liver or thymic mononuclear cells were prepared from Vps34^{fl/fl};CD4-Cre or Vps34^{fl/+};CD4-Cre mice and the total number of cells was counted. A summary of the absolute number of cells obtained from individual mice from 5-6 mice in each group is shown. *p<0.05. (B) Summary of results obtained in Fig. 4 A,B.

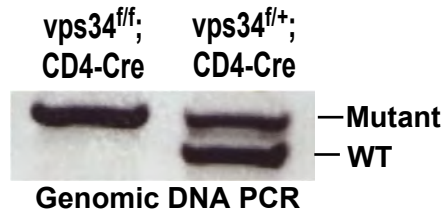
Supplemental Figure 3. Dendritic cells, B cells, $\gamma\delta$ T cells and NK cells in T cell-specific Vps34-deficient mice. Mononuclear cells were prepared from spleen, lymph nodes, liver and thymus derived from Vps34^{fl/fl};CD4-Cre or Vps34^{fl/+};CD4-Cre mice. (A) Cells were stained with anti-TCR β , -NK1.1 and -B220 antibodies. The gated cells shown in the plots are TCR β ⁻NK1.1⁺ NK cells. A summary of the frequency of NK cells and their absolute numbers calculated based on the organ cellularity with 5-6 mice analyzed in each group is shown to the right. *p<0.05. (B) Cells were stained with anti-TCR β and -TCR $\gamma\delta$ antibodies. The gated cells shown in the plots are TCR β ⁻TCR $\gamma\delta$ ⁺ T cells. A summary of the frequency of $\gamma\delta$ T cells and their absolute numbers calculated based on the organ cellularity with 5-6 mice analyzed in each group is shown to the right. *p<0.05. (C) Cells were stained with anti-CD11b and -CD11c antibodies. A summary of the frequency of DCs and their absolute numbers calculated based on the organ cellularity with

5-6 mice analyzed in each group is shown to the right. * $p < 0.05$. (D) Cells were stained with anti-B220 antibodies. A summary of the frequency of B cells and their absolute numbers calculated based on the organ cellularity with 5-6 mice analyzed in each group is shown to the right. * $p < 0.05$.

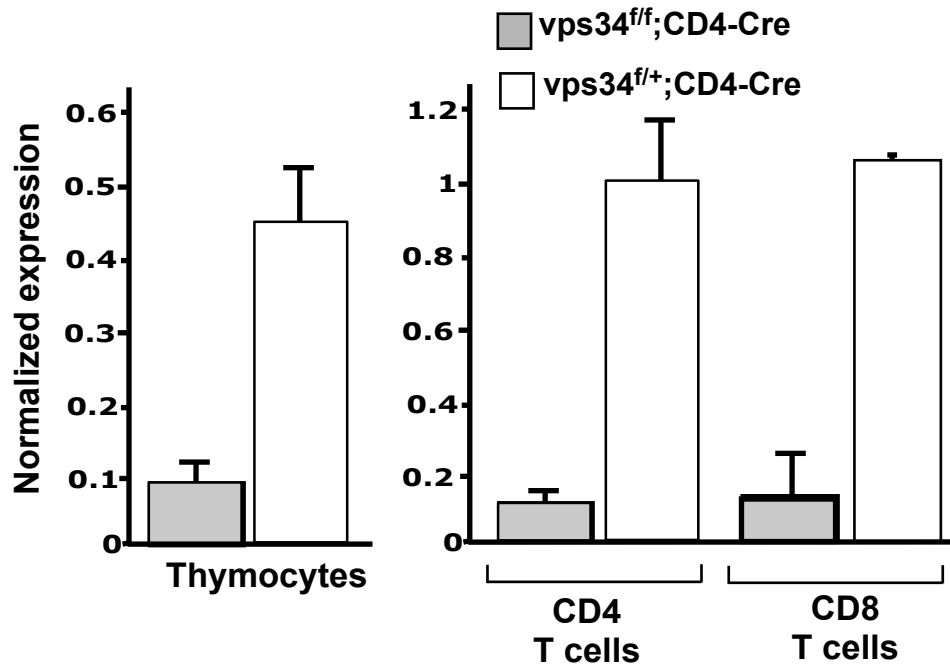
Supplemental Figure 4. Inflammatory lesions in the small intestine and colons of aged T cell-specific Vps34-deficient mice and in Rag2^{-/-} mice with colitis induced following adoptive transfer of CD4⁺CD25⁻ cells. (A) Small intestinal sections from aged Vps34^{f/f};CD4-Cre or Vps34^{f/+};CD4-Cre mice were stained with H&E. A representative section from two mice depicting a large lymphoid follicle in the small intestine of T cell-specific Vps34-deficient mice is shown. (scale bar = 100 μ m). (B) Colon sections of results depicted in Fig. 7E. Three representative pictures in each group are shown. (C) Splenic mononuclear cells from 6- to 8-week-old mice were prepared and 2×10^5 cells were activated with PMA and ionomycin (ION+PMA) in the presence of GolgiPlugTM to allow intracellular accumulation of cytokines. Cells were then harvested and surface-stained with anti-CD4 and -CD8 antibodies, followed by intracellular staining with anti-IL-17A and anti-IFN- γ antibodies, or with anti-IL-13 and anti-IFN- γ antibodies. The data shown are gated on CD4⁺ or CD8⁺ T cells. Results shown are representative of 3 individual experiments.

Supplemental Figure 1

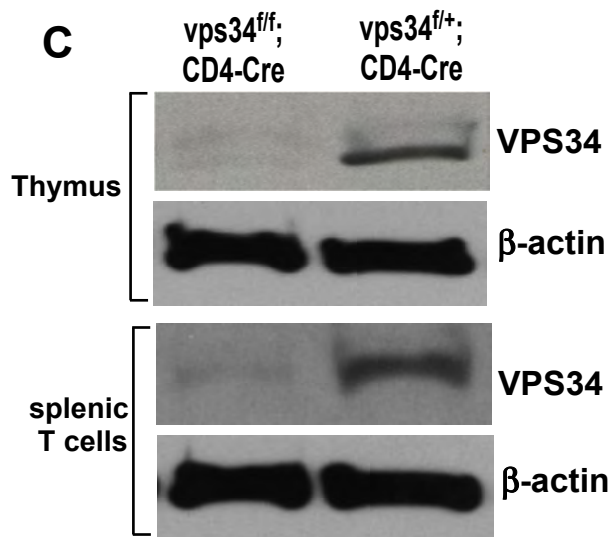
A



B

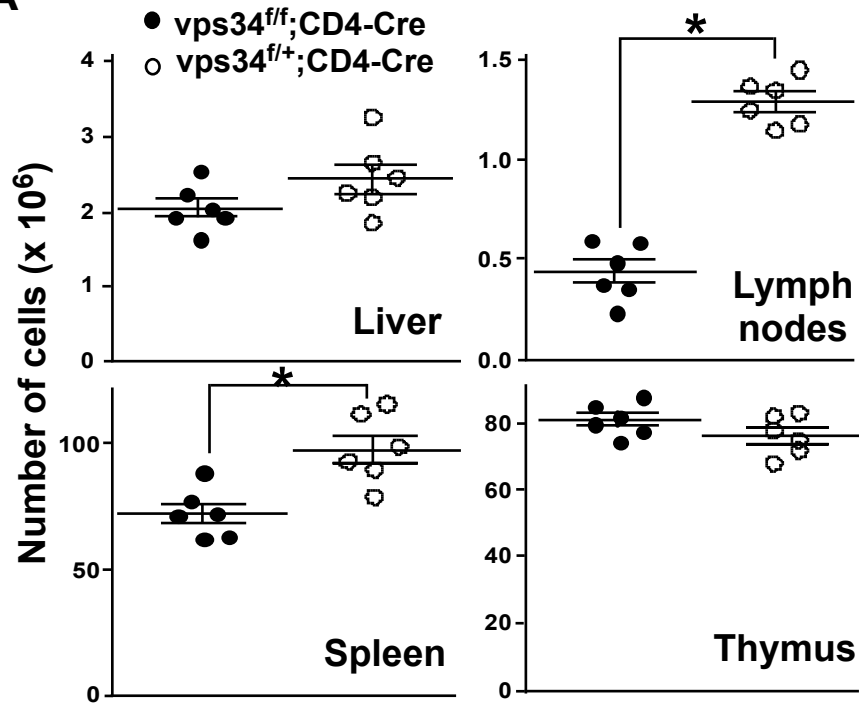


C

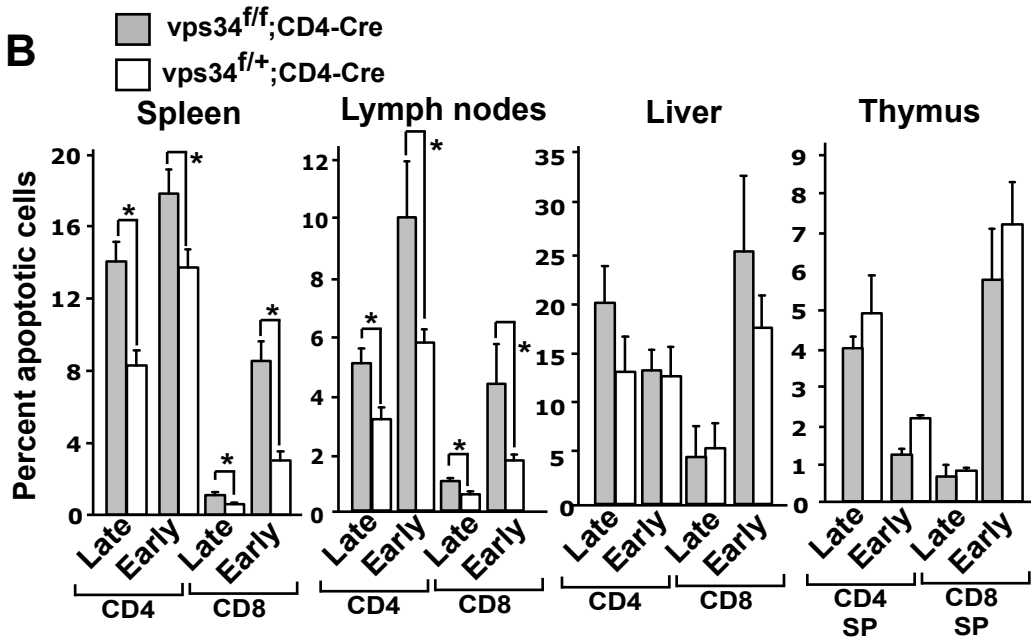


Supplemental Figure 2

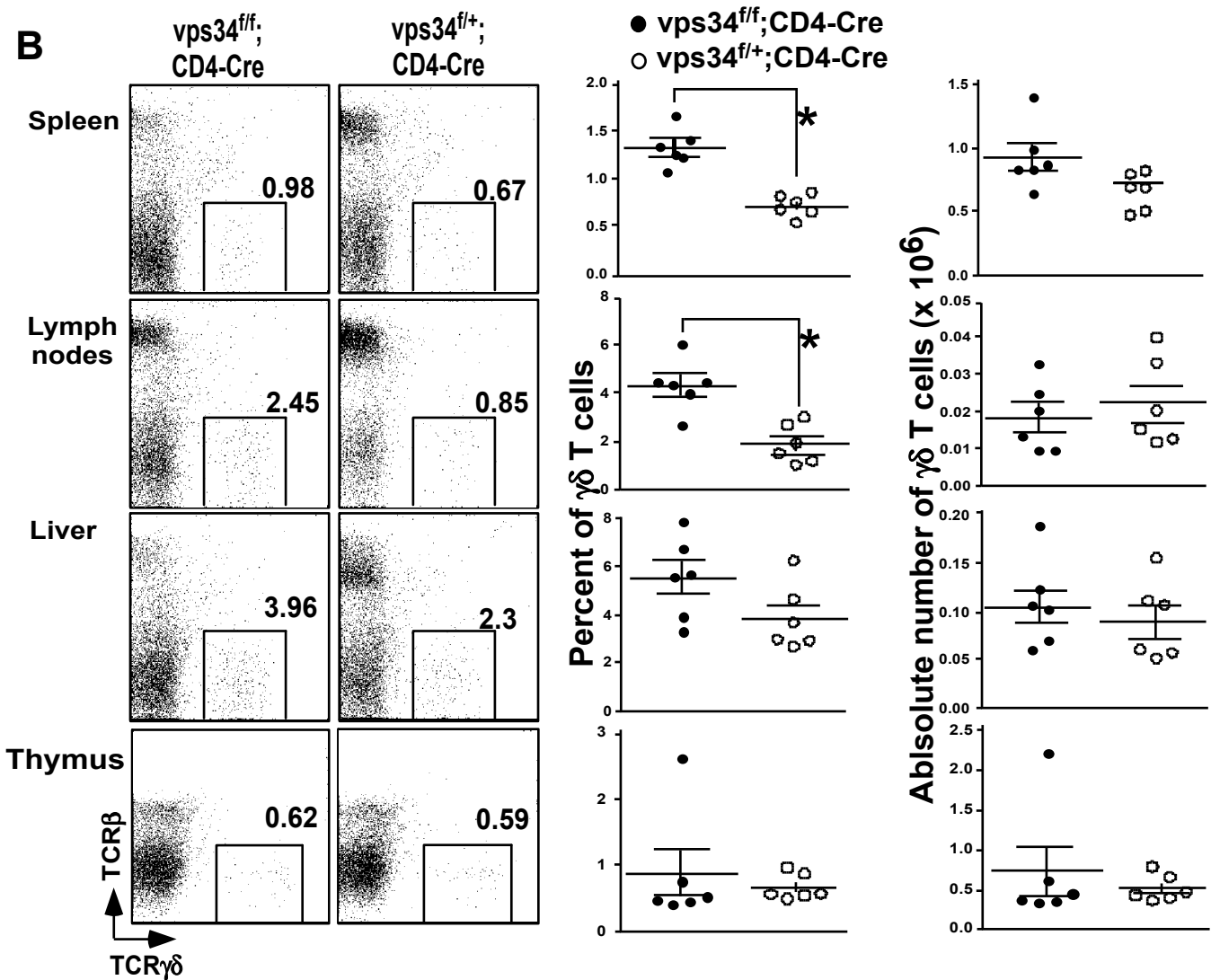
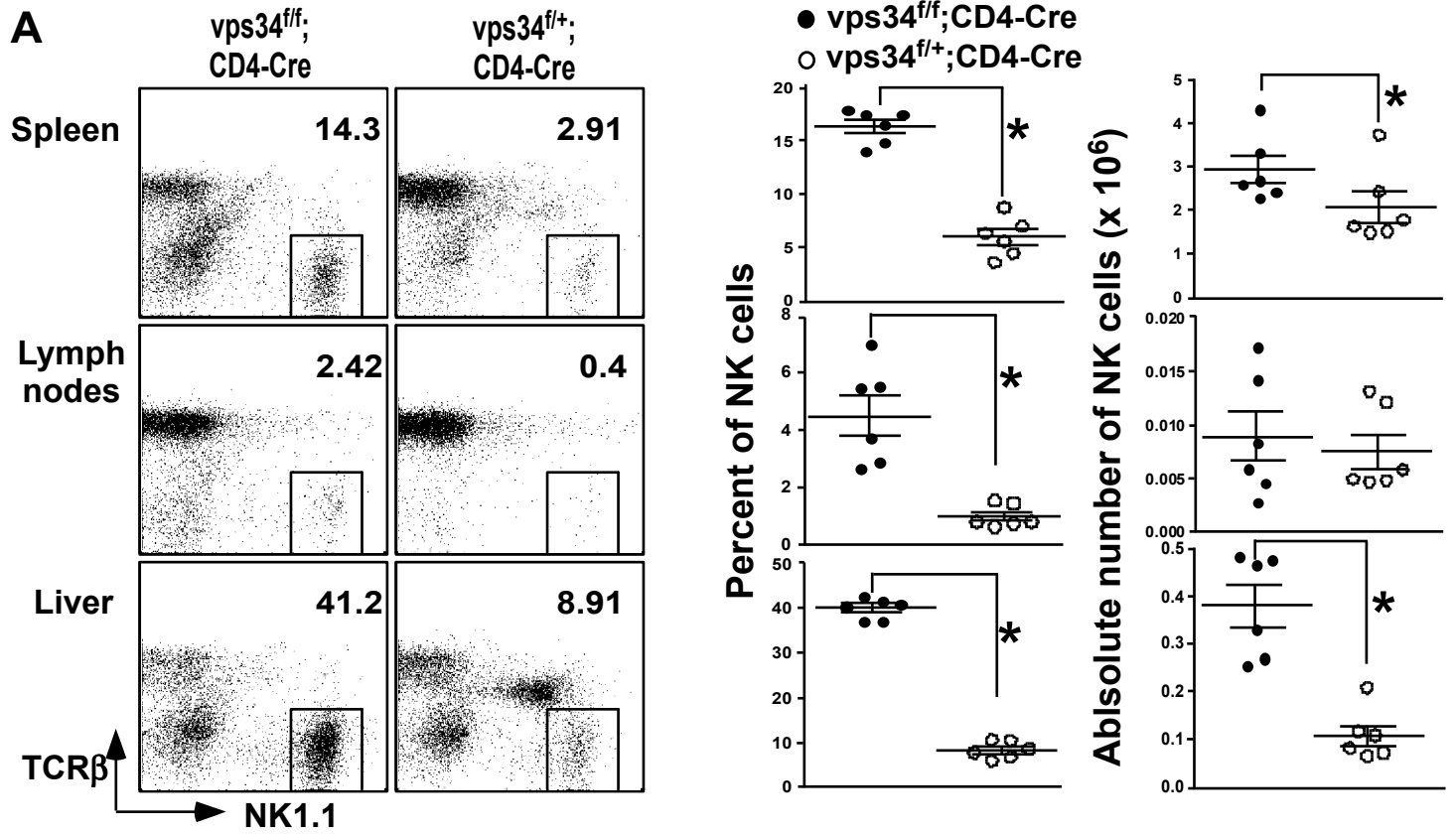
A



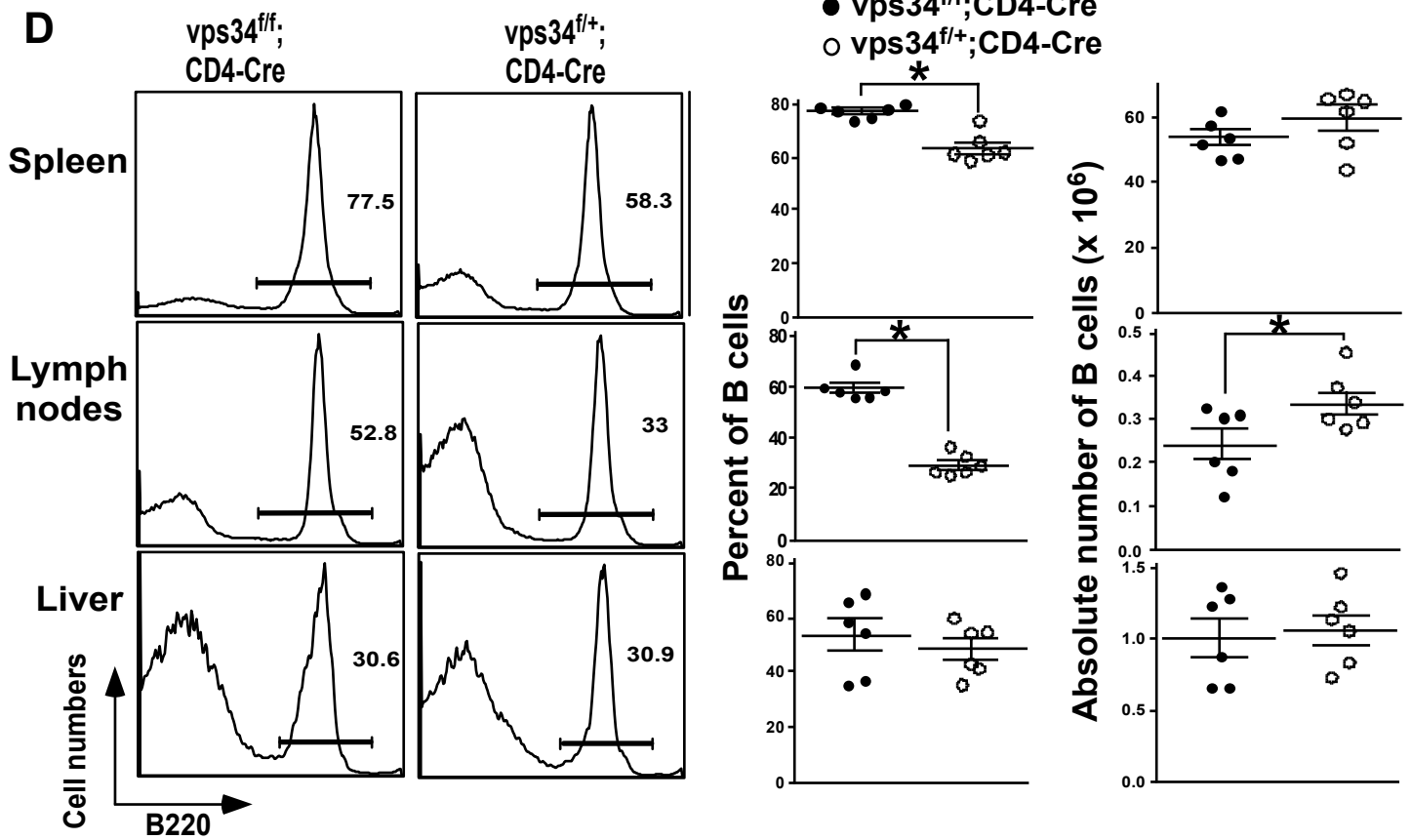
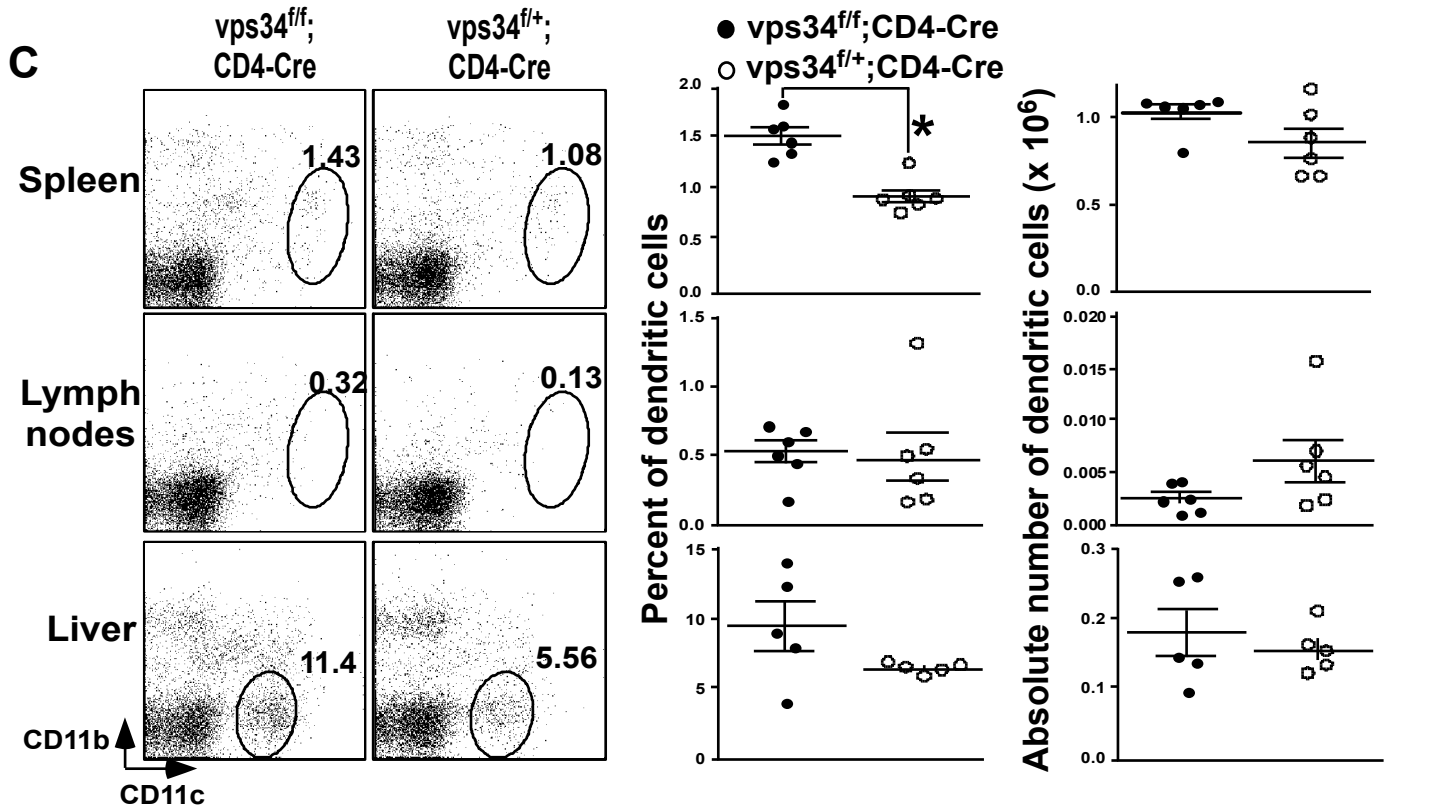
B



Supplemental Figure 3

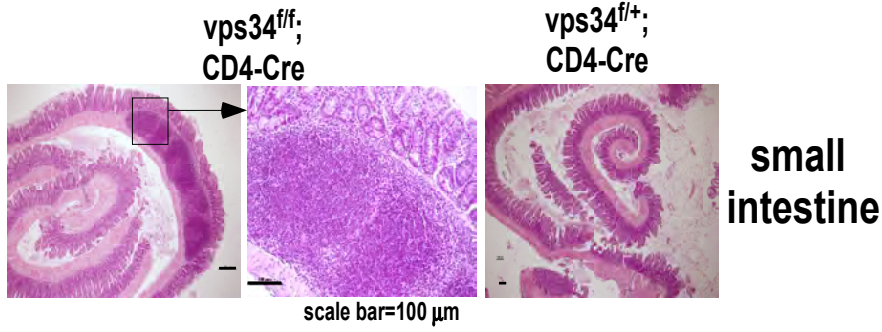


Supplemental Figure 3

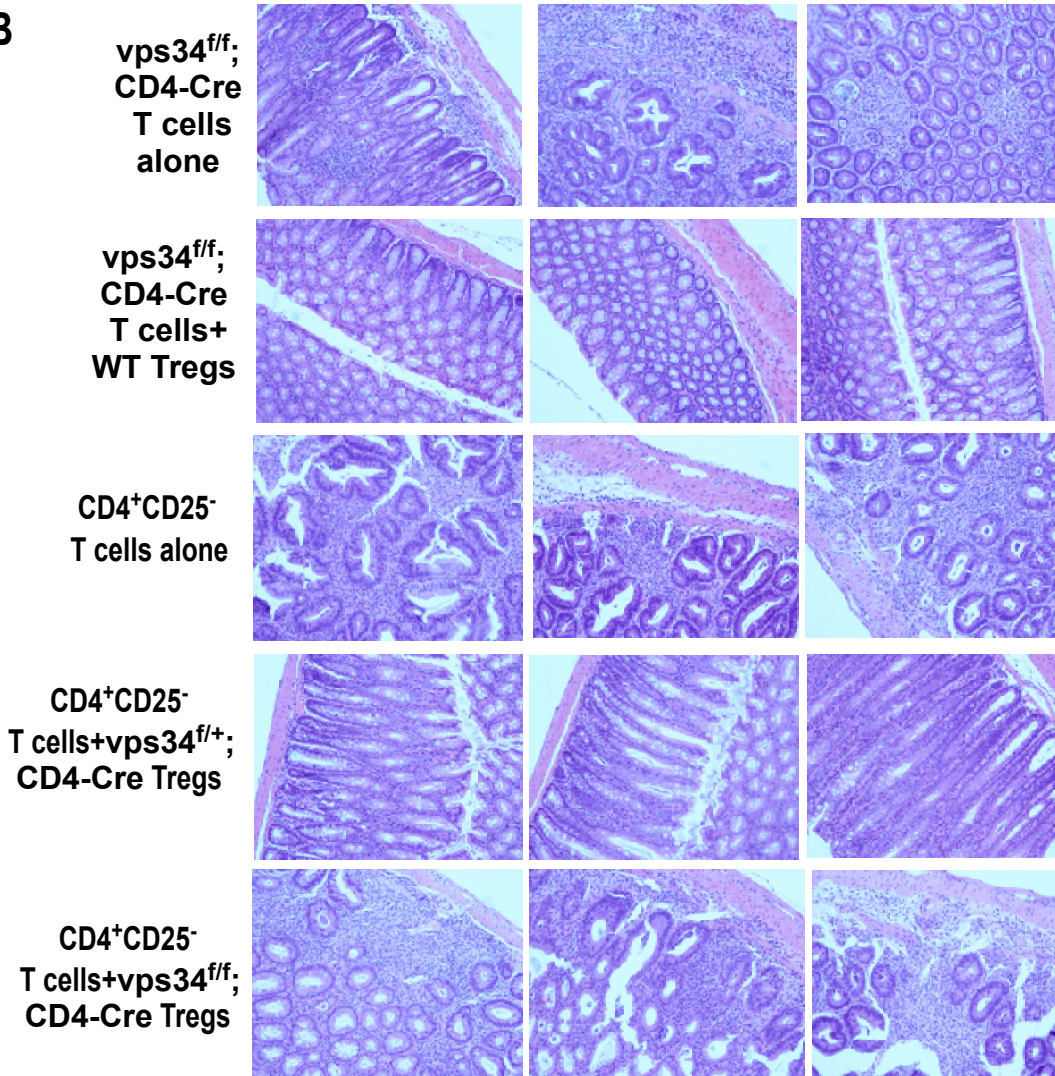


Supplemental Figure 4

A



B



C

