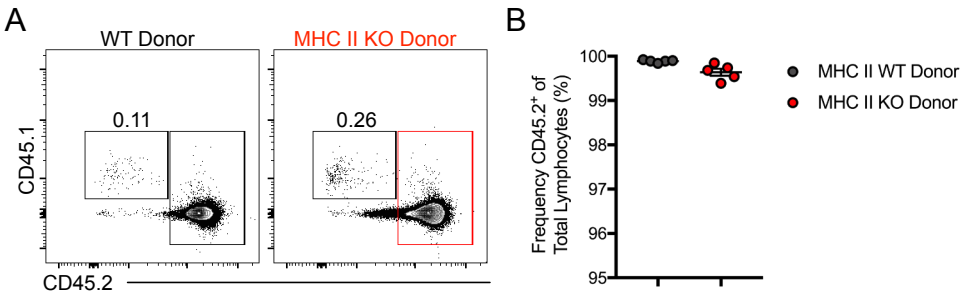
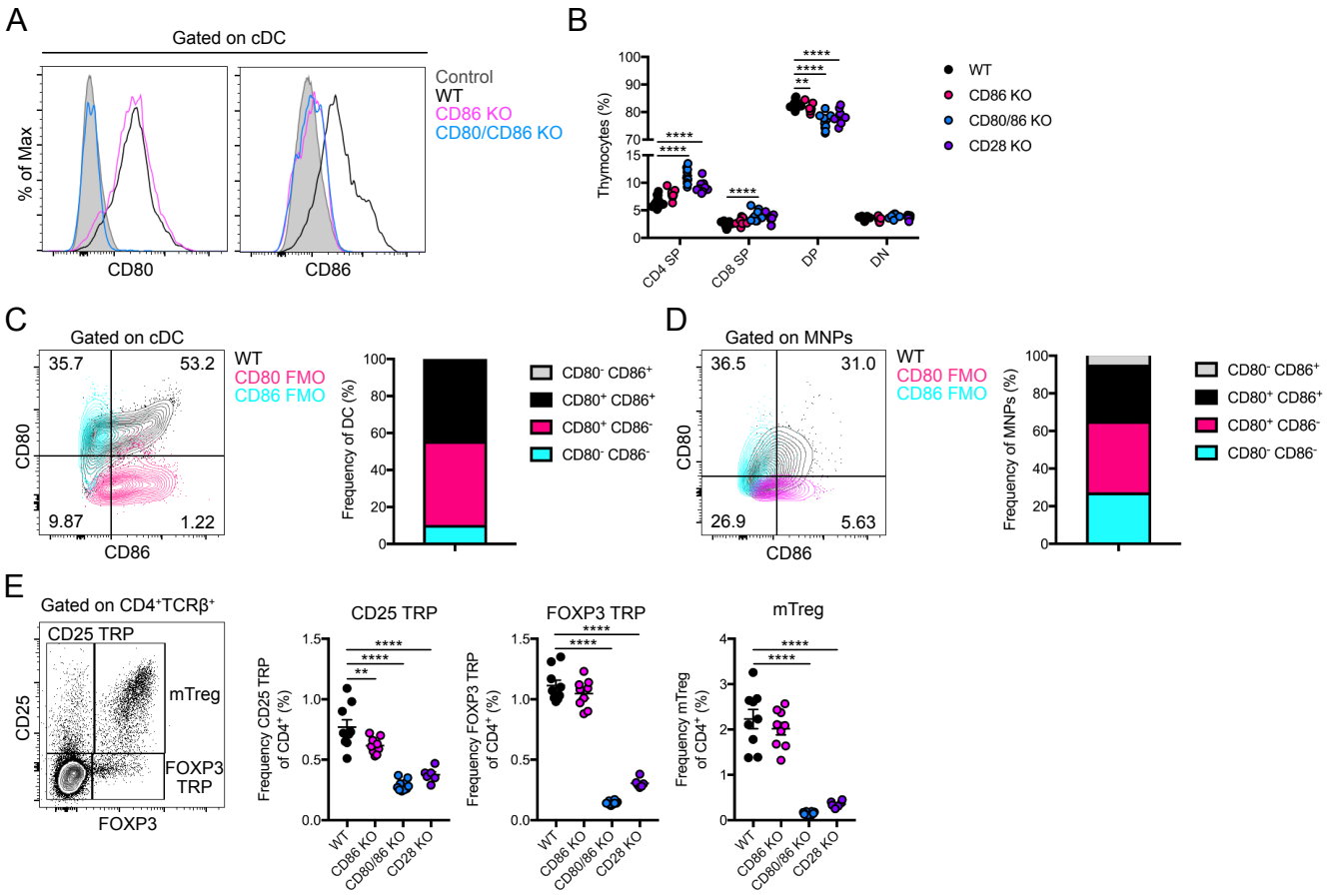
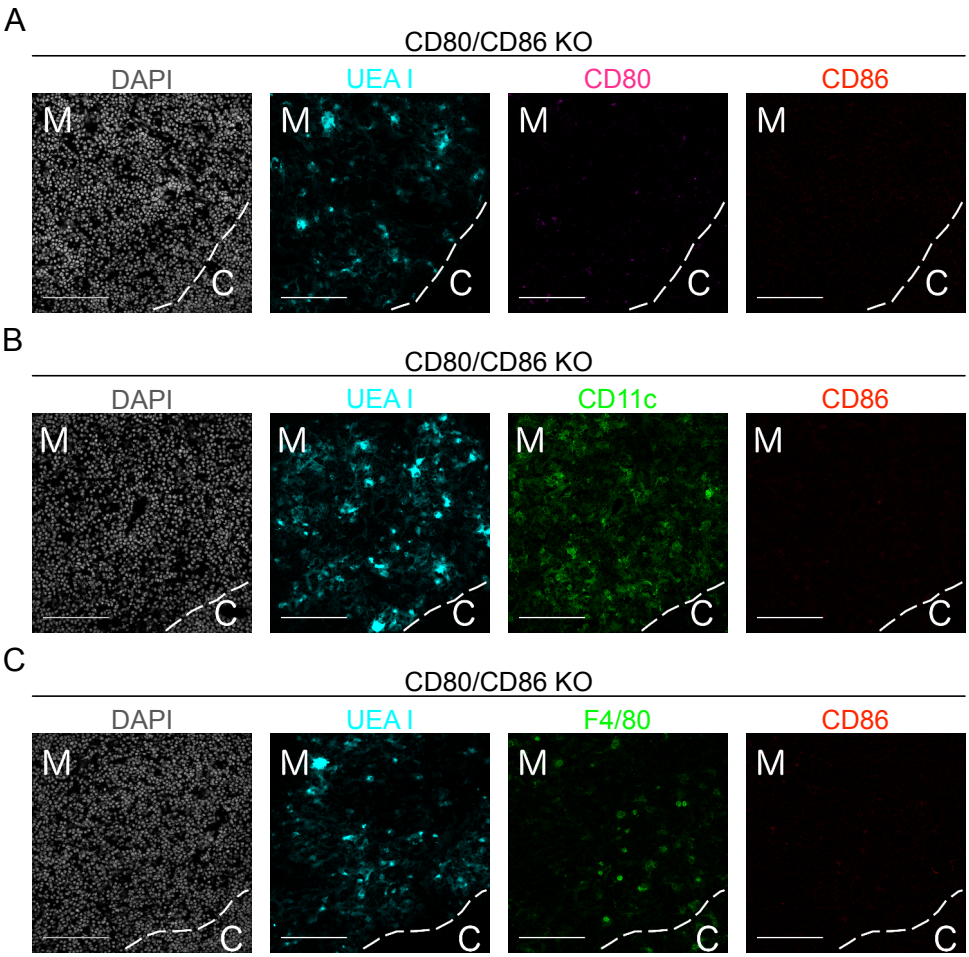


Supplemental Figure 1. MHC II KO bone marrow chimera efficiency. (A) Flow cytometry of total thymocytes from bone marrow chimeric mice from either WT donor (left) or MHC II KO donor (right) mice (as shown in Figure 2A). Numbers adjacent to outlined areas indicate percent CD45.1$^+$ (recipient) cells in each. (B) Frequency CD45.2$^+$ (donor cells) of total thymocytes for each bone marrow chimeric donor. Each symbol represents an individual mouse (B). Six- to twelve-week-old female mice were used. Data are representative of two independent experiments (A) and pooled from two independent experiments (B).



Supplemental Figure 2. Tolerance induction by CD80/CD86. (A) Flow cytometry of CD11c$^+$ MHC II$^+$ thymic dendritic cells from WT, CD86 KO, or CD80/86 KO mice (key) stained for CD80 (left) and CD86 (right). (B) Frequency of CD4$^+$, CD8$^+$, DP, and DN among total thymocytes from WT, CD86 KO, CD80/86 KO, and CD28 KO mice. (C) Flow cytometry of CD11c$^+$ MHC II$^+$ dendritic cells from WT mice stained for CD80 and CD86 (left) and quantification (right). Numbers indicate percent cells in each quadrant. (D) Flow cytometry of FcγR1$^+$ MNPs from WT mice stained for CD80 and CD86 (left) and quantification (right). (E) Flow cytometric gating strategy for identifying CD25$^+$ Treg progenitors, FOXP3$^+$ Treg progenitors, and mature Treg (left). Frequency of CD25$^+$ (middle, left), FOXP3$^+$ (middle, right), or CD25$^+$FOXP3$^+$ (right) among CD4$^+$ thymocytes from WT, CD86 KO, CD80/CD86 KO, and CD28 KO mice. Each symbol (B, E) represents an individual mouse. Six- to twelve-week-old male and female mice were used. Small horizontal lines indicate the mean and error bars represent SEM. **P< 0.01, ***P< 0.001, ****P< 0.0001. Statistical significance was determined by ordinary one-way ANOVA with Holm-Sidak's multiple comparisons test (B, E). Data are representative of at least three independent experiments (A) or are pooled from at least three independent experiments (B, C, D, E).



Supplemental Figure 3. Immunofluorescence microscopy of CD80/CD86 KO thymus. (A) Immunofluorescence microscopy of thymic sections stained for DAPI (gray), UEA I (cyan), CD80 (magenta), and CD86 (red) in CD80/CD86 KO mice. C, cortex; M, medulla. Scale bars 100μm. (B) Immunofluorescence microscopy of thymic sections stained for DAPI (gray), UEA I (cyan), CD11c (green), and CD86 (red) in CD80/CD86 KO mice. C, cortex; M, medulla. Scale bars 100μm. (C) Immunofluorescence microscopy of thymic sections stained for DAPI (gray), UEA I (cyan), F4/80 (green), and CD86 (red) in CD80/CD86 KO mice. C, cortex; M, medulla. Scale bars 100μm. Six- to twelve-week-old male and female mice were used. Data are representative of at least three independent experiments.