

Supplemental Figure 1. CD4⁺ FoxP3⁻ ICOS, CCR7, and CD69 expression change with age.

Splenocytes from young (3 months, black), middle-aged (12 months, gray), and old (18 months, white) mice were stained for CD4, CD44, CD62L, ICOS, CCR7, CD69, and FoxP3 and analyzed by flow cytometry. Data show the average ICOS MFI, CCR7 MFI, and frequency that are CD69⁺ from naïve (FoxP3⁻ CD44^{lo} CD62L^{hi}) and memory (FoxP3⁻ CD44^{hi} CD62L^{lo}) CD4⁺ T cells (±SE). *p≤0.05, **p≤0.01 (Student's *t* test).



Supplemental Figure 2. Deletion of Bim in FoxP3-Cre Bim^{f/f} and BimKO mice.

Splenocytes from young (2 months) FoxP3-Cre⁻ Bim^{f/f}, FoxP3-Cre⁺ Bim^{f/f}, and BimKO mice were stained for CD4, CD44, CD62L, FoxP3 and Bim and analyzed by flow cytometry. Representative histograms show the expression of Bim in effector Treg (eTreg, CD4⁺ FoxP3⁺ CD44^{hi} CD62L^{lo}, gray) and central Treg (cTreg, CD4⁺ FoxP3⁺ CD44^{lo} CD62L^{hi}, black).



Supplemental Figure 3. Characterization of Treg in the non-lymphoid tissues of young and old mice. Cells from the spleen, peripheral lymph nodes (pLN: inguinal, axillary, brachial), mesenteric LN (mLN), lung, liver, and intestinal intraepithelial (IEL) were isolated from young (3 months) and aged (14-16 months) WT and IL-6KO mice. Cells were stained for CD4, CD44, CD62L, FoxP3 and analyzed by flow cytometry. *A*, Data show the frequency of CD4+ FoxP3+ cells that are effector Treg (CD4⁺ FoxP3⁺ CD44^{hi} CD62L^{lo}, gray) or central Treg (CD4⁺ FoxP3⁺ CD44^{lo} CD62L^{hi}, black) (±SE). *B*, Data show the total number of CD4⁺ FoxP3⁺ cells (±SE). *C*, Data show the total number of effector Treg (CD4⁺ FoxP3⁺ CD44^{hi} CD62L^{lo}) (±SE). *p≤0.05, **p≤0.01 (Student's *t* test).