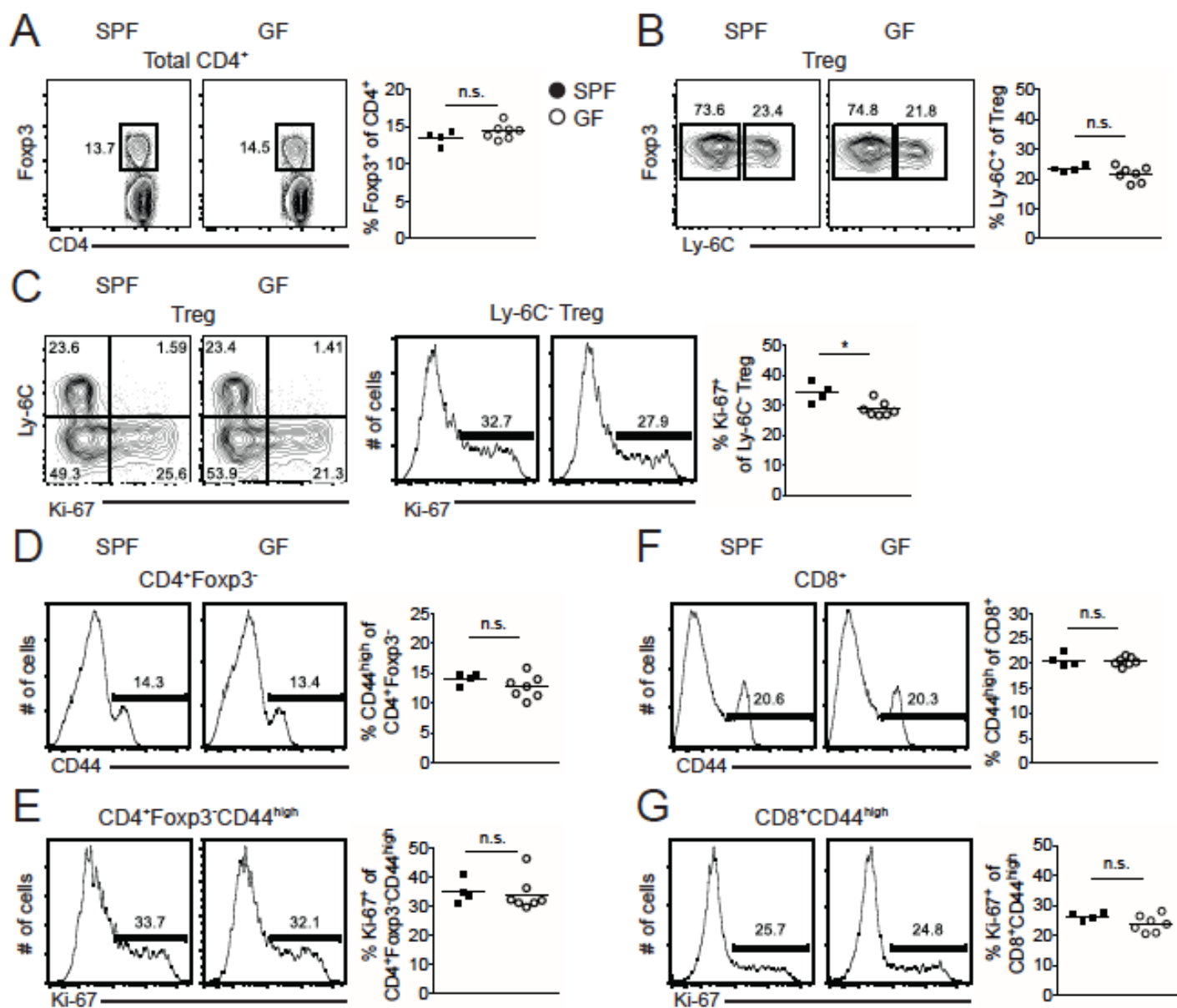
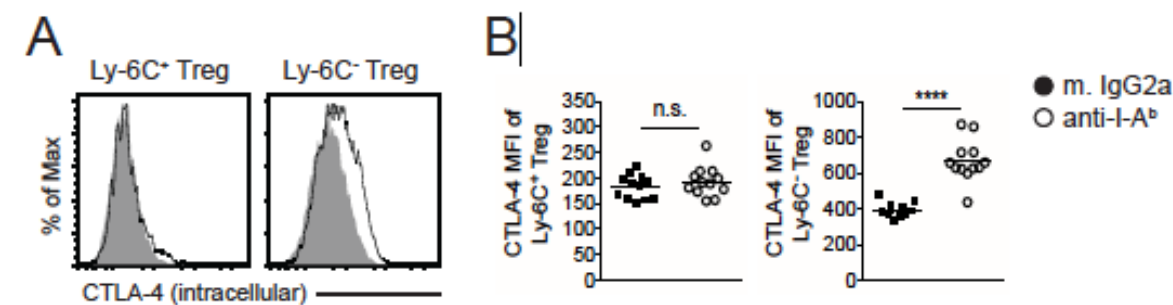


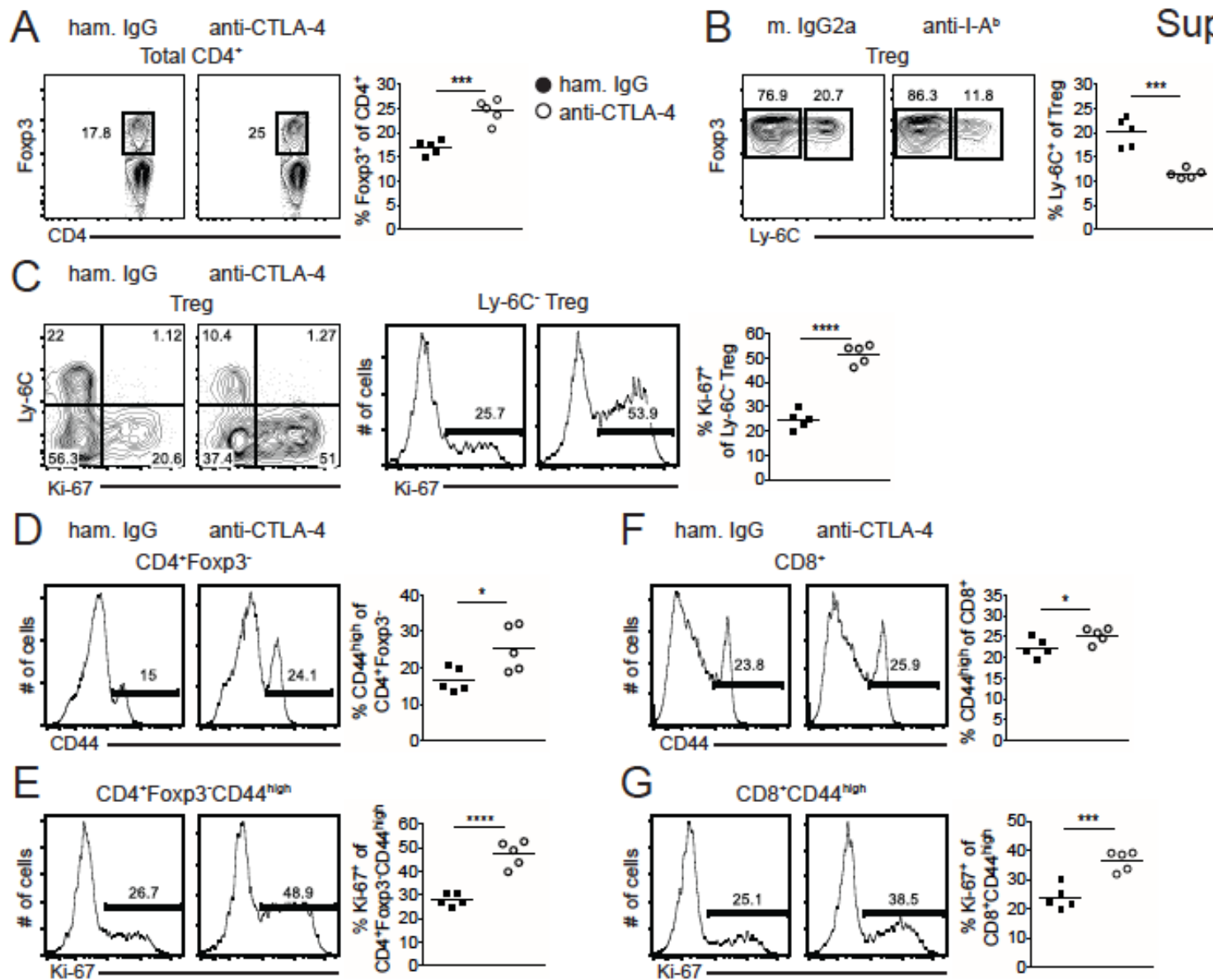
SUPPLEMENTAL FIGURE 1. Analysis of CD44 and Ki-67 expression from Ly-6C⁺ and Ly-6C⁻ Tregs in the steady state. Spleens were harvested from WT C57BL/6 mice. (A) Representative histograms of CD44 expression among CD4⁺ T cells (left) or Tregs (right). (B) Overlay of representative histograms of CD44 expression among Ly-6C⁺ (filled histogram) and Ly-6C⁻ (open histogram) Tregs. (C) Representative histograms of Ki-67 expression among Ly-6C⁺ (left) and Ly-6C⁻ (right) Tregs. Data are representative of three independent experiments with three - five mice per group per experiment (twelve mice total).



SUPPLEMENTAL FIGURE 2. The absence of commensal microbes does not disrupt the frequency nor differential homeostasis of NP and MP T cell subsets. Spleens were harvested from SPF and GF mice in the steady state. **(A)** Representative contour plots of Foxp3 expression among total CD4⁺ T cells (left). Pooled frequency of Foxp3⁺ cells among total CD4⁺ T cells (right). **(B)** Representative contour plots of Ly-6C expression among Tregs (left); pooled frequency of Ly-6C⁺ cells among Tregs (right). **(C)** Representative contour plots of Ly-6C versus Ki-67 expression among total Tregs (left). Representative histograms show Ki-67 expression among Ly-6C⁻ Tregs; pooled frequency of Ki-67 expression among Ly-6C⁻ Tregs (right). Representative histograms (left) and pooled frequency (right) of CD44 expression among CD4⁺ T cells **(D)** or CD8⁺ T cells **(F)**. Representative histograms (left) and pooled frequency (right) of Ki-67 expression among MP CD4⁺ T cells **(E)** or MP CD8⁺ T cells **(G)**. Filled circles represent SPF mice; open circles represent GF mice. Each symbol represents an individual mouse from one experiment with four total mice (SPF) and seven total mice (GF). Small horizontal lines indicate the mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (unpaired Student's t test).



SUPPLEMENTAL FIGURE 3. Increase in intracellular CTLA-4 MFI among Ly-6C⁻ Tregs following transient and global blockade of MHC Class II. FcR γ ^{-/-} mice were administered anti-I-A^b mAb (or mouse IgG2a) on d 0, 2, and 3 and spleens harvested on d 6. **(A)** Representative histograms of intracellular CTLA-4 expression among Ly-6C⁻ and Ly-6C⁺ Tregs. **(B)** Pooled frequency of CTLA-4 MFI among Ly-6C⁻ and Ly-6C⁺ Tregs. Filled circles represent mouse IgG2a treatment; open circles represent anti-I-A^b mAb treatment. Each symbol represents an individual mouse from five independent experiments with two - five mice per group (twelve mice total). Small horizontal lines indicate the mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (unpaired Student's t test).



SUPPLEMENTAL FIGURE 4. The increased frequency and proliferation of Ly-6C⁻ Tregs following CTLA-4 blockade is not influenced by antibody-mediated deletion of T cells. FcR γ ^{-/-} mice were administered anti-CTLA-4 mAb (or hamster IgG) on d 0, 2, 4, and 6 and spleens harvested on d 8. (A) Representative contour plots of Foxp3 expression among total CD4⁺ T cells (left). Pooled frequency of Foxp3⁺ cells among total CD4⁺ T cells (right). (B) Representative contour plots of Ly-6C expression among Tregs (left); pooled frequency of Ly-6C⁺ cells among Tregs (right). (C) Representative contour plots of Ly-6C versus Ki-67 expression among total Tregs (left). Representative histograms show Ki-67 expression among Ly-6C⁻ Tregs; pooled frequency of Ki-67 expression among Ly-6C⁻ Tregs (right). Representative histograms (left) and pooled frequency (right) of CD44 expression among CD4⁺ T cells (D) or CD8⁺ T cells (F). Representative histograms (left) and pooled frequency (right) of Ki-67 expression among MP CD4⁺ T cells (E) or MP CD8⁺ T cells (G). Filled circles represent hamster IgG treatment; open circles represent anti-CTLA-4 mAb treatment. Each symbol represents an individual mouse from two independent experiments with two or three mice per group (five mice total). Small horizontal lines indicate the mean. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 (unpaired Student's *t* test).