Supplemental Figures for manuscript "Foxp3^{+} Tregs require WASP to restrain Th2-mediated food allergy" by Lexmond *et al*.

Supplemental Figure 1. Age-dependency of IgE levels in *Was*$^{-/-}$ mice and absence of colonic inflammation in C57BL/6 and BALB/c *Was*$^{-/-}$ animals. (**A**) Serum IgE levels in 129SvEv *Was*$^{-/-}$ animals between 8 and 24 weeks. (**A**) Bi-weekly assessment of surface IgE loading on blood basophils (CD45^{Mid}CD49b^{+}) from WT (open circles, n=5) and *Was*$^{-/-}$ (gray circles, n=10) on the C57BL/6 background. (**C**) Total levels of serum IgG1 in C57BL/6 *Was*$^{-/-}$ mice. (**D**) Pearson correlation coefficients between anti-food IgE and IgG1 titers for the five main chow components in Balb/c *Was*$^{-/-}$ mice. (**E**) H&E staining of colonic cross-sections from 6-12 month old WT and BALB/c *Was*$^{-/-}$ mice (20X) and (**F**) 4-month old *Was*$^{-/-}$ mice on the C57BL/6 background (20X). (**G**) Relative gene expression of *Mcpt1* in small intestinal (SI) tissue samples and correlation of *Mcpt1* mRNA expression with anti-food IgE titer. (**H**) Pearson correlation coefficients (r) between food-specific IgE titers and baseline serum MCPT1. Symbols represent individual mice and error bars depict SEM. *p<0.05; ** p<0.01; ***p<0.001; NS: not significant as determined by two-tailed Student's t-test or paired t-test for intraindividual analyses.

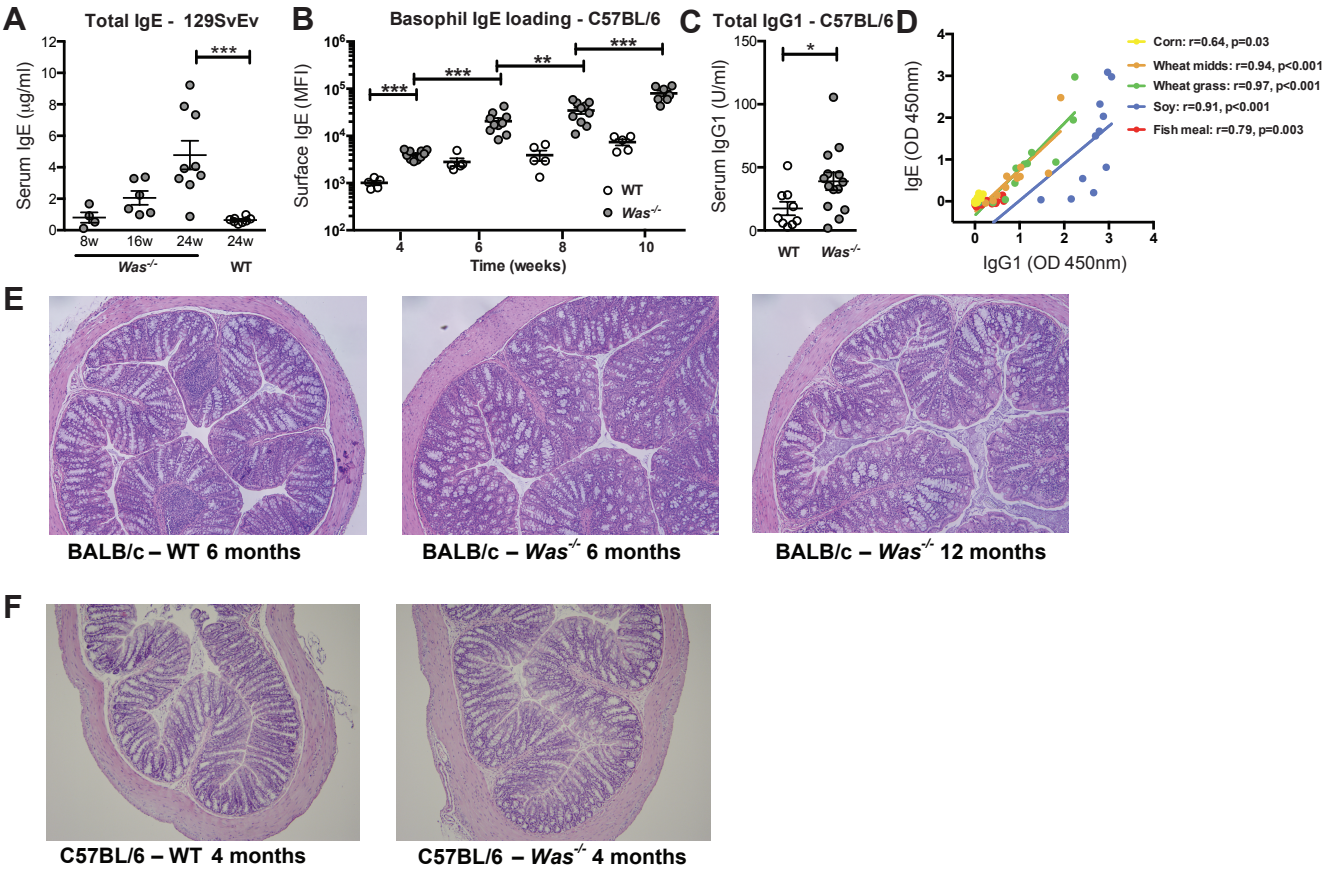
Supplemental Figure 2. Detailed analysis of food-specific titers for the five main chow constituents. Contribution of individual component-specific immunoglobulins to the cumulative anti-food isotype titers of IgG2a, IgG2b, IgG3, and IgA in SPF (n=8) and GF-housed (n=7) *Was*$^{-/-}$ mice. Bars represent results from individual mice.

Supplemental Figure 3. Intestinal mast cell expansion in *Was*$^{-/-}$ mice requires adaptive immunity and *Was*$^{fl/fl}$*Foxp3*-Cre mice develop Th2-type intestinal inflammation. (**A**) Comparison of serum MCPT1 in WT (open circles, n=5), *Was*$^{-/-}$ (gray circles, n=8), *Rag2*$^{-/-}$ (open triangles, n=3) and *Was*$^{-/-}$*Rag2*$^{-/-}$ (gray triangles, n=5) mice ≥ 2 months old on the 129SvEv background. (**B**) Normalized absolute mRNA counts obtained from digital mRNA profiling of jejunal tissue for indicated genes. Symbols represent individual mice and error bars depict SEM. *p<0.05; ** p<0.01; ***p<0.001; NS: not significant as determined by two-tailed Student's t-test (panel A) or one-way ANOVA with Tukey's multiple comparisons test (panel B).

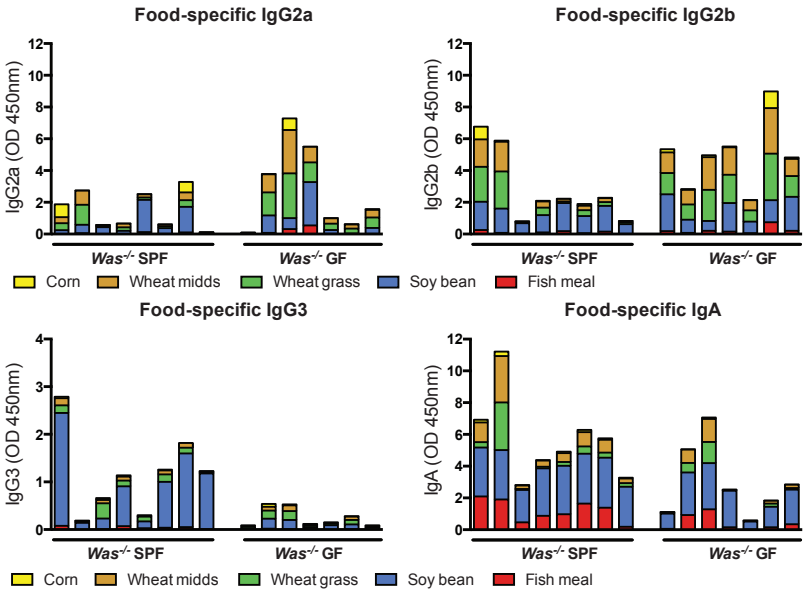
Supplemental Figure 4. WASP-deficient Foxp3^{+} Tregs fail to suppress Th2 type lymphoproliferation in vivo. (**A**) Quantification of Foxp3^{+} Tregs counts in MLNs and PPs of WT (n=5), *Was*$^{-/-}$ (n=6), and *Was*$^{fl/fl}$*Foxp3*-Cre (n=4) animals. (**B**) Gating strategy and summary statistic of the fraction of iTregs, defined as Foxp3^{+} Neuropilin1^{-}, within the total population of Tregs obtained from MLNs. (**C**) Summary statistic of the subpopulation of CD44^{Hi}CD62L^{Lo} effector memory Tregs within the total population of CD4^{+}Foxp3^{+} lymphocytes. (**D**) Absolute numbers of Th2-type, GATA3^{+}ICOS^{+} effector memory cells in MLNs and PPs. (**E**) Serum levels of total IgE and IgG1 in *Was*$^{-/-}$ mice on the 129SvEv background with either *Il4*$^{+/+}$ (n=10) or *Il4*$^{-/-}$ (n=8) alleles. Symbols represent individual mice and error bars depict SEM. *p<0.05; ** p<0.01; ***p<0.001; NS: not significant as determined by two-tailed Student's t-test or one-way ANOVA with Tukey's multiple comparisons test. Representative results of 2 independent experiments.

Supplemental Figure 5. In vitro *suppression of polarized Th cells*. (A) Schematic of in vitro Th1, Th2, and Th17 polarization of WT naïve T cells and subsequent suppression assay using *Was*$^{+/+}$*Foxp3*$^{\text{Cre-EGFP}}$ or *Was*$^{-/-}$ *Foxp3*$^{\text{Cre-EGFP}}$. **(B)** Expression of Th-specific transcription factors following Th1/Th2/Th17 polarizing conditions. (**C**) Sorting of FOXP3-GFP^{+} cells from *Was*$^{+/+}$*Foxp3*$^{\text{Cre-EGFP}}$ or *Was*$^{\text{fl/fl}}$*Foxp3*$^{\text{Cre-EGFP}}$ mice. (**D**) Gating of CD45.1^{+} responder Th cells labeled with CFSE (left) with Th cell proliferation indicated by CFSE dilution in overlaid histograms (right). (**E**) Suppression of freshly isolated naïve CD4^{+} T cells by GFP^{+} WT Tregs isolated from *Was*$^{+/+}$*Foxp3*$^{\text{Cre-EGFP}}$ mice. Data are representative of three independent experiments.

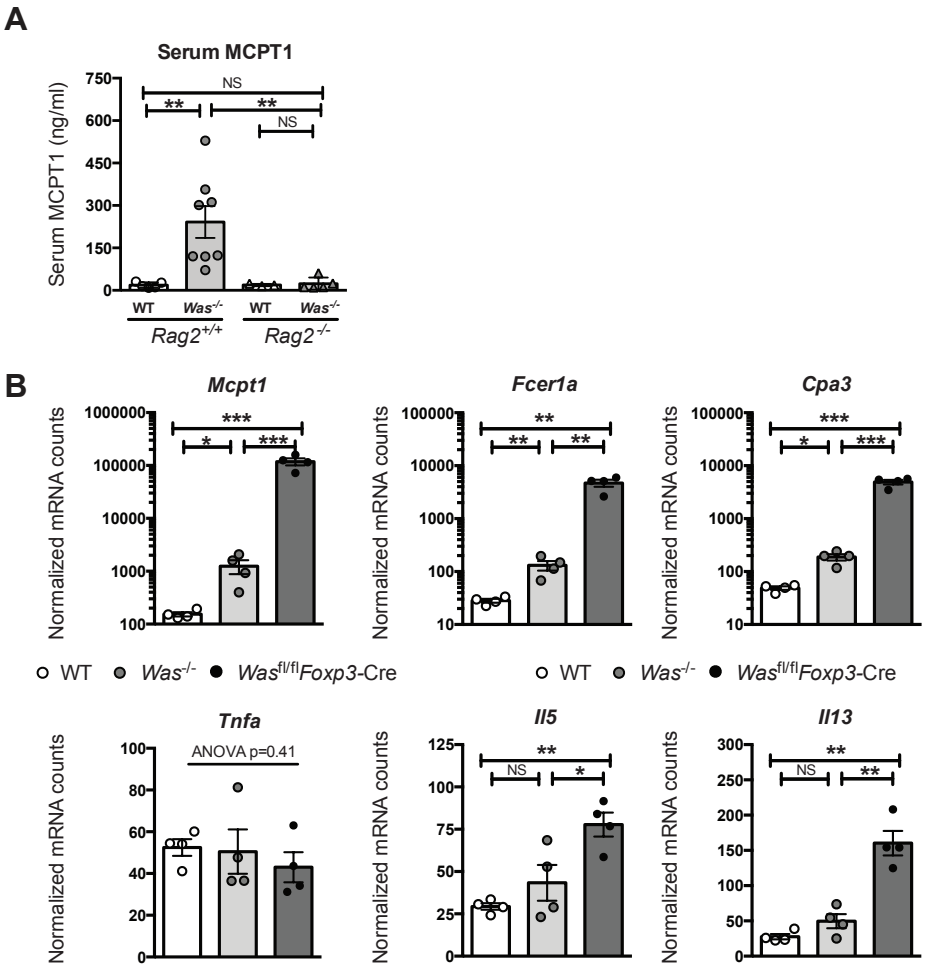
Supplemental Figure 6. WASP-deficient effector Tregs assume a Th2-like phenotype. (**A**) Fraction of total CD4^{+}Foxp3^{+} Tregs that co-express GATA3 and ICOS in WT, *Was*$^{-/-}$ and *Was*$^{\text{fl/fl}}$*Foxp3*-Cre mice. (**B**) Fraction of Foxp3^{+} Tregs amongst CD4^{+} mesenteric lymphocytes and effector memory Tregs amongst total Foxp3^{+} Tregs in WT or *Was*$^{-/-}$ mice with either *Il4*$^{+/+}$ or *Il4*$^{-/-}$ alleles on the 129sv background. (**C**) Comparison of TGFβ-mediated induction of GATA3^{+} Tregs from naïve CD3^{+}CD4^{+}CD25^{-}GFP^{-} T cells from WT or *Was*$^{\text{fl/fl}}$*Foxp3*-Cre mice in vitro. Representative dot plots are shown with the percentage of GATA3^{+} iTregs depicted gated on CD3^{+}CD4^{+}GFP^{+} cells. (**D**) Mean fluorescent intensity (MFI) of GATA3 expression among total effector (CD45RO^{+}) Tregs and (**E**) percent of GATA3^{+} effector T cells among XLT (n=10) and WAS (n=11) patients compared to age and sex matched controls (n=10) or WAS patients after hematopoietic stem cell transplantation (HSCT) (n=3). Dots represent cells from individual mice or patients and error bars depict SEM. *p<0.05; ** p<0.01; ***p<0.001; NS: not significant as determined by one-way ANOVA with Tukey's multiple comparisons test. Panels A-C are representative of 2 independent experiments. Results in panels D and E are cumulative data from 1 experiment.



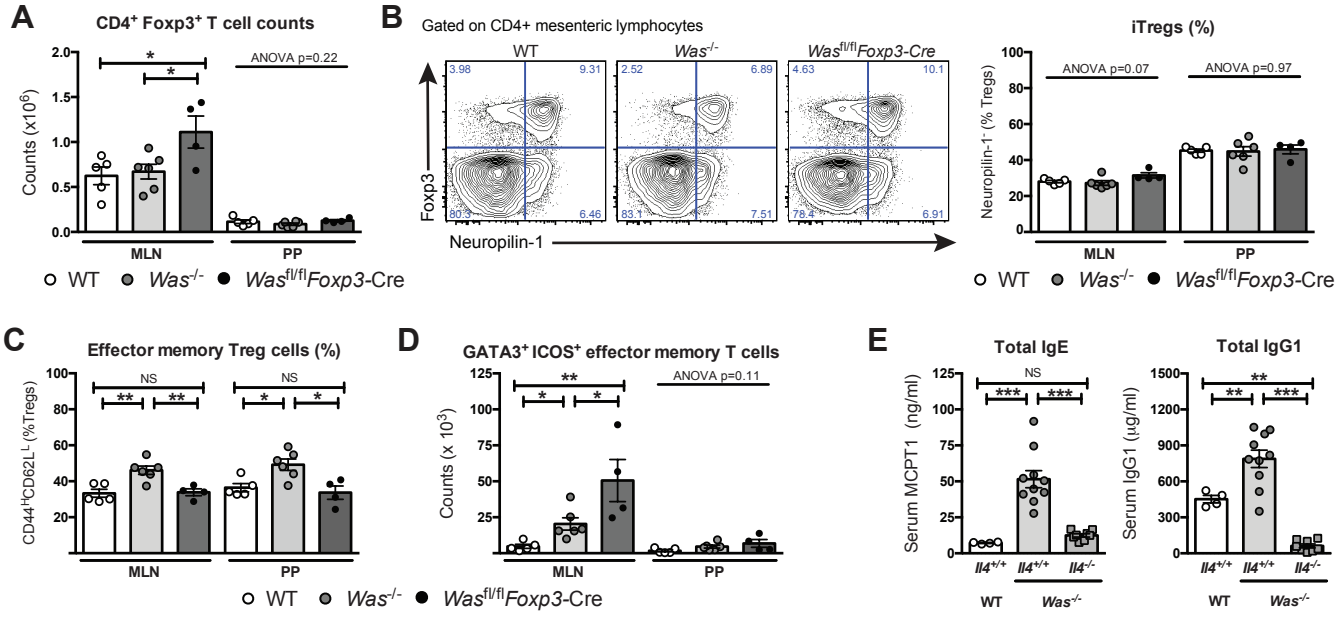
Supplemental Figure 1



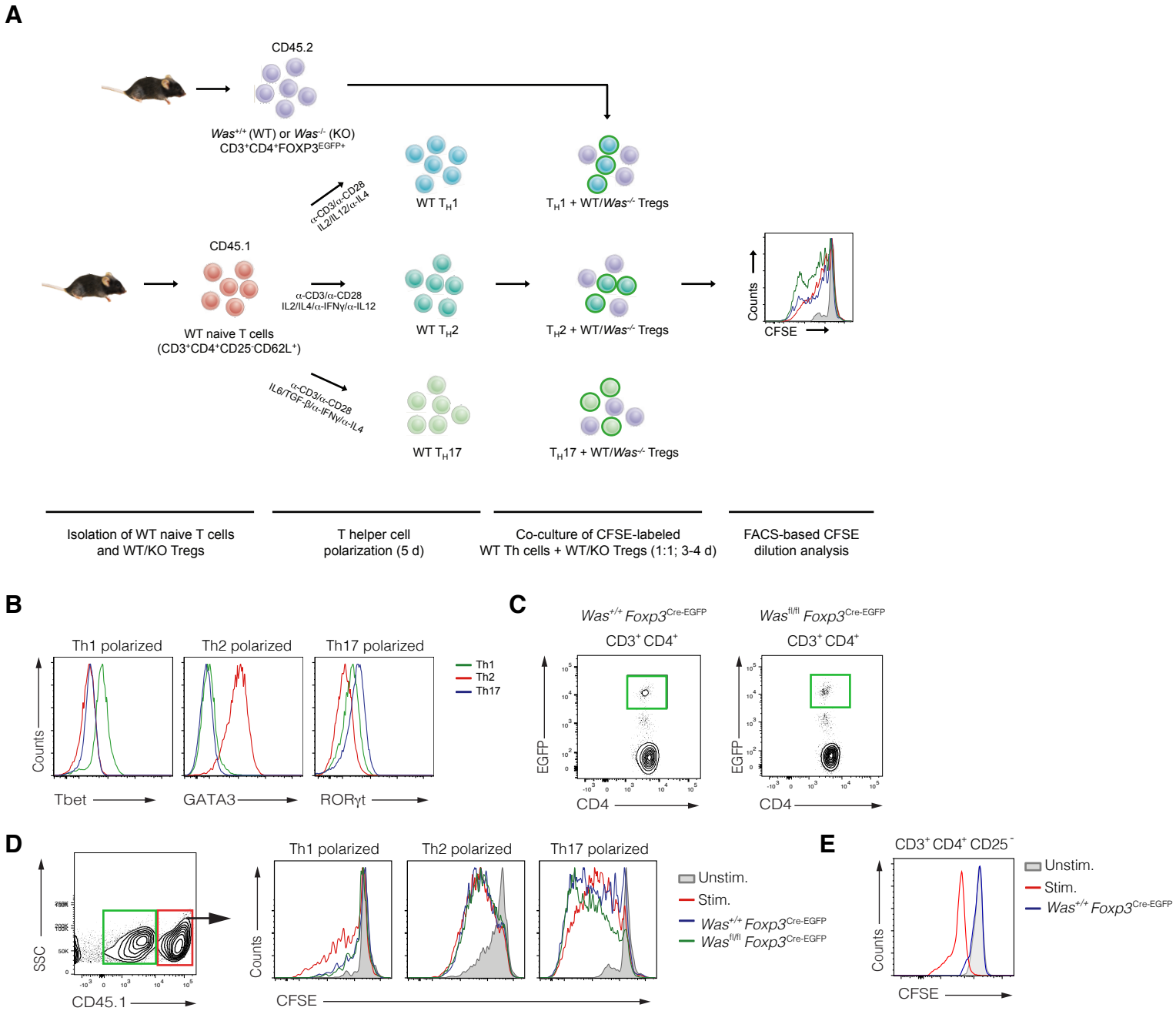
Supplemental Figure 2



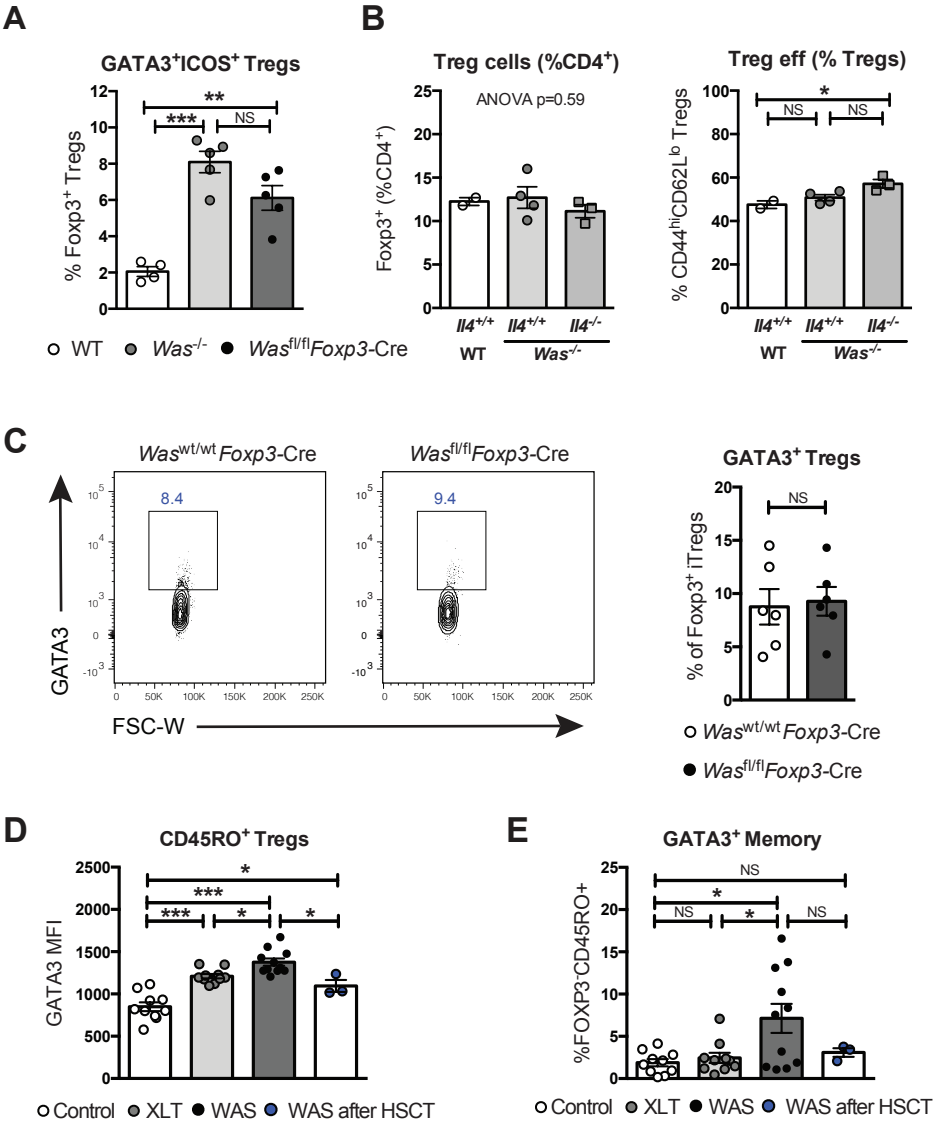
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6