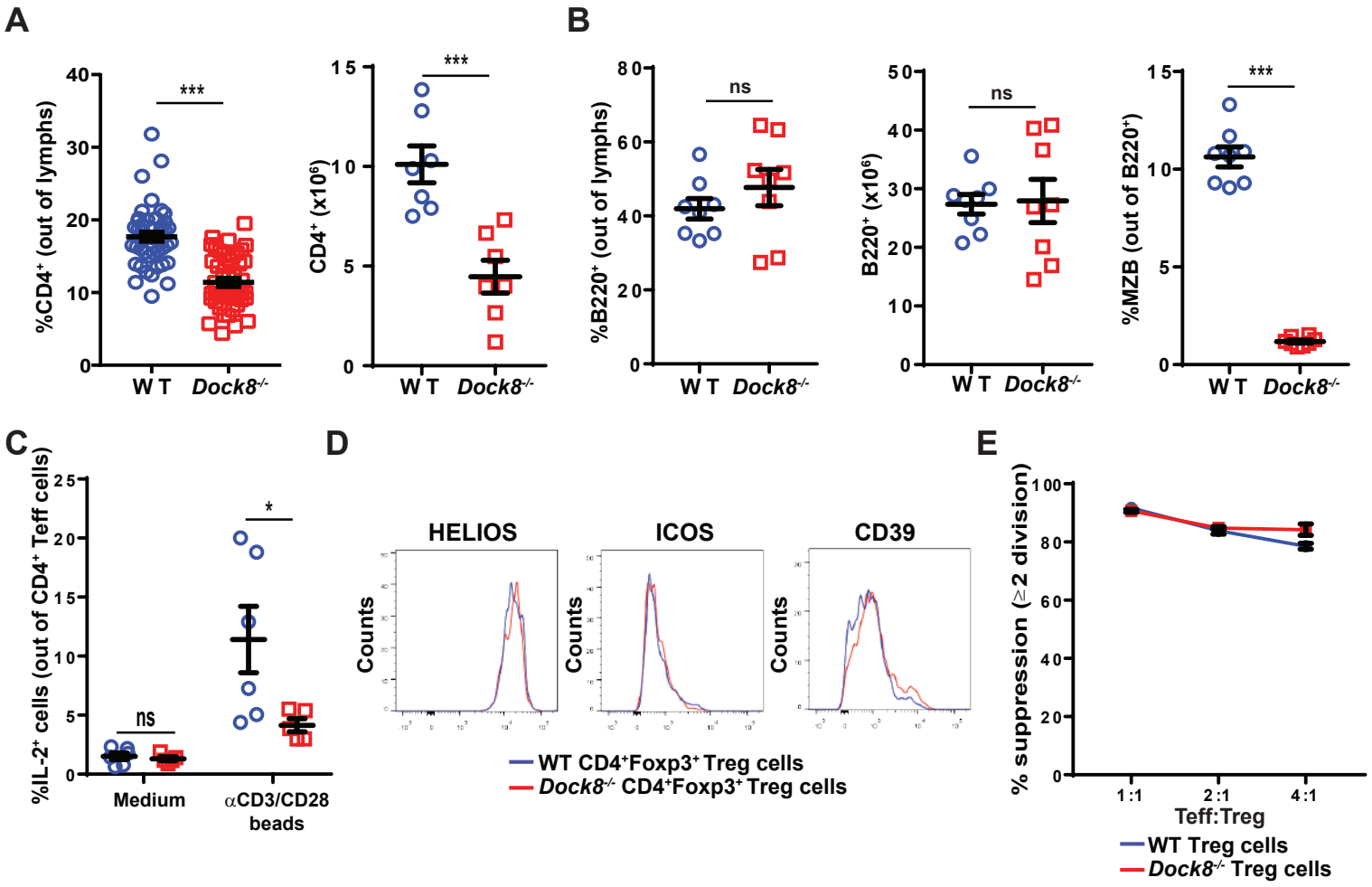
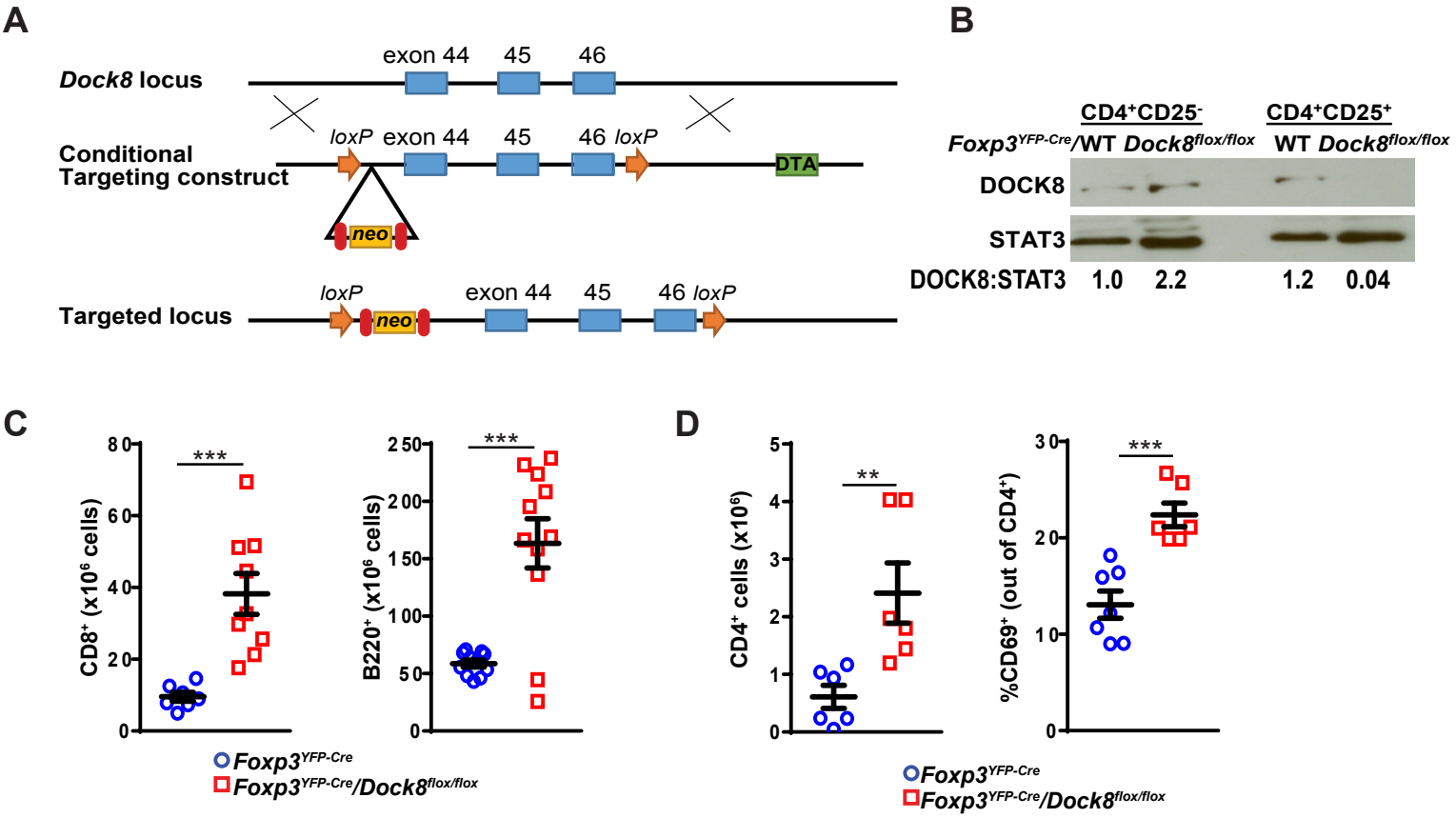
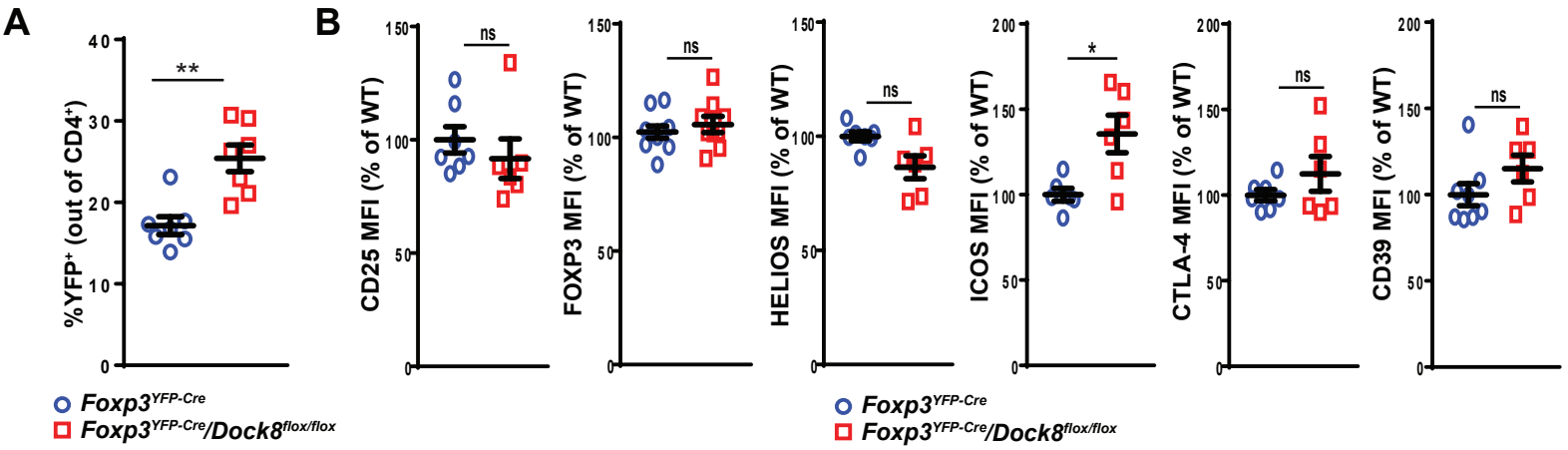


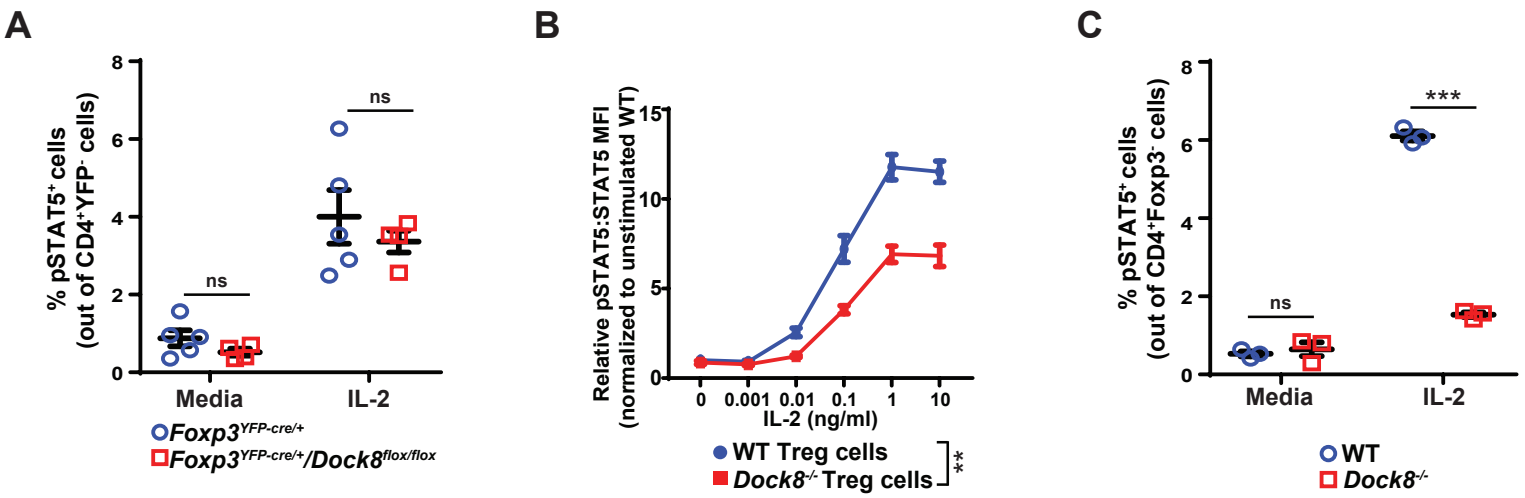
Supplementary Figure 1. Decreased CD4⁺ T cells and marginal zone B cells in *Dock8*$^{-/-}$ mice. **A.** Percentage (left, n=26 mice per group) and total number of CD4⁺ cells (right, n=7 mice per group) in the spleens of *Dock8*$^{-/-}$ mice and WT controls. **B.** Percentage and total number of B220⁺ cells and percentage of marginal zone B (MZB) cells in the spleens of *Dock8*$^{-/-}$ mice and WT controls. n=8 mice from each group. **C.** CD4⁺CD25⁻ Teff cells were isolated from the spleens of *Dock8*$^{-/-}$ and WT mice and cultured with anti-CD3+anti-CD28 coated beads. After 2 days in culture, Golgistop and Golgiplug were added to cultures for an additional 16 h. Intracellular staining for IL-2 was then performed. **D.** Expression of HELIOS, ICOS, and CD39 on/in splenic CD4⁺Foxp3⁺ cells from *Dock8*$^{-/-}$ mice and WT controls. Representative histograms are shown. **E.** Suppression of the proliferation of Cell Trace Violet loaded CD4⁺CD25⁻ Teff cells by IL-2 preactivated CD4⁺CD25⁺CD39⁺ Treg cells from *Dock8*$^{-/-}$ mice and WT controls. Teff proliferation was measured by FACS analysis of Cell Trace Violet dilution. Results in C and E are representative of 2 independent experiments. Symbols represent individual mice (A-C) and bars in A-C and E represent mean and SEM. t-test ns $p>0.05$, * $p<0.05$, and *** $p<0.001$.



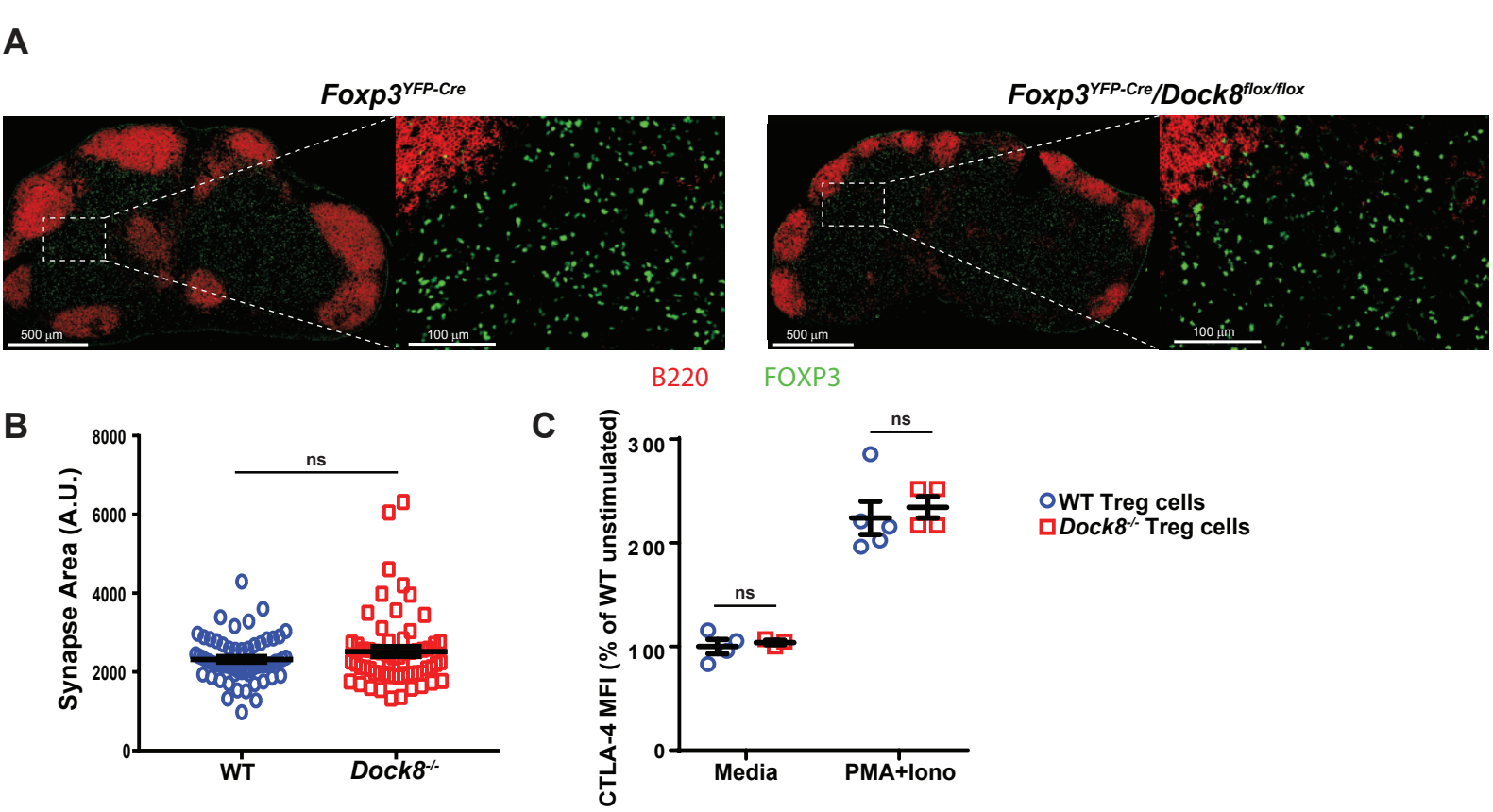
Supplementary Figure 2. Strategy for the generation of *Dock8*$^{flox/flox}$ mice. **A.** Schematic of the targeted locus and targeting vector used to generate *Dock8*$^{flox/flox}$ mice. Small red boxes indicate *Flp* sequences flanking the *neo* gene. Green box indicates *Diphtheria Toxin A* gene. Orange arrows are *loxP* sites. **B.** DOCK8 expression by purified CD4$^+$CD25$^-$ (Teff) and CD4$^+$CD25$^+$ (Treg) cells isolated from spleens of *Foxp3*$^{YFP\text{-}Cre}$/*Dock8*$^{flox/flox}$ mice and *Foxp3*$^{YFP\text{-}Cre}$ controls isolated using magnetic beads. Lysates were immunoblotted for DOCK8 and STAT3 as loading control. Densitometry was performed and the relative ratio of DOCK8:STAT3 protein is noted below each lane. **C.** Number of CD8$^+$ T cells (left) and B cells (right) in the spleens of *Foxp3*$^{YFP\text{-}Cre}$/*Dock8*$^{flox/flox}$ mice and *Foxp3*$^{YFP\text{-}Cre}$ controls. **D.** CD4$^+$ cell counts and percentage of CD69$^+$ cells among CD4$^+$ T cells in the peripheral LNs from 30 week-old *Foxp3*$^{YFP\text{-}Cre}$/*Dock8*$^{flox/flox}$mice and *Foxp3*$^{YFP\text{-}Cre}$ controls. Symbols represent individual mice and bars in C and D represent mean and SEM. t-test ** p<0.01, *** p<0.001



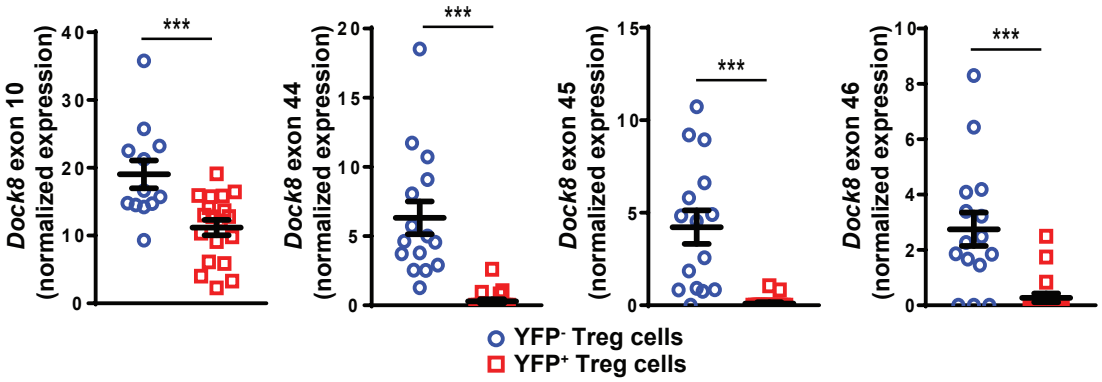
Supplementary Figure 3. Increased Treg cells in the LNs and normal or elevated expression of Treg cell markers on the Treg cells from *Foxp3*$^{YFP\text{-}Cre}$/*Dock8*$^{flox/flox}$ mice. A. Percentage of YFP$^+$ Treg cells out of CD4$^+$ T cells among total Treg cells in peripheral LNs of 30 week-old *Foxp3*$^{YFP\text{-}Cre}$/*Dock8*$^{flox/flox}$ mice and *Foxp3*$^{YFP\text{-}Cre}$ controls. **B.** Quantitative analysis of surface CD25 and CD39 and total FOXP3, HELIOS, ICOS, and CTLA-4 expression in splenic CD4$^+$YFP$^+$ cells from *Foxp3*$^{YFP\text{-}Cre}$/*Dock8*$^{flox/flox}$ mice and *Foxp3*$^{YFP\text{-}Cre}$ controls (right). Symbols represent individual mice and bars represent mean and SEM. t-test ns $p>0.05$, * $p<0.05$, ** $p<0.01$



Supplementary Figure 4. CD4⁺ T cells from *Dock8*$^{-/-}$ mice had a modest impairment in IL-2-driven STAT5 phosphorylation. A. Percentage of pSTAT5⁺ cells in splenic CD4⁺CD25⁻YFP⁻ Teff cells from *Foxp3*$^{YFP\text{-}Cre}$/*Dock8*$^{flox/flox}$ mice and *Foxp3*$^{YFP\text{-}Cre}$ controls at baseline and after stimulation for 15 min with 10 ng/ml of mouse IL-2. **B.** Relative pSTAT5 content in CD4⁺FOXP3⁺ Treg cells from *Dock8*$^{-/-}$ mice and WT controls at baseline and following stimulation with increasing concentrations of IL-2 for 15 minutes. The graph plots the relative pSTAT5:STAT5 MFI ratio normalized to the WT baseline ratio. **C.** Splenic CD4⁺CD25⁻ Teff cells from *Dock8*$^{-/-}$ and WT control mice at baseline and after stimulation for 15 min with 10 ng/ml of mouse IL-2. Results in A-C are representative of 3 independent experiments. Bars represent mean and SEM. Significance was determined by unpaired t-test in A and C, while ANOVA was used to compare the curves in B. ns $p>0.05$, ** $p<0.01$, *** $p<0.001$



Supplementary Figure 5. DOCK8 deficient Treg cells migrate normally to the T cell zones and are able to upregulate surface expression of CTLA-4. A. Representative immunofluorescent staining of peripheral LN sections from a 4 week old *Foxp3*$^{YFP\text{-}Cre}$/*Dock8*$^{flox/flox}$ mouse and a *Foxp3*$^{YFP\text{-}Cre}$ control. FOXP3^{+} cells are shown in green, and B220^{+} cells are red. Images are representative of three independent experiments using one mouse per group. **B.** Treg cells from *Dock8*$^{-/-}$ and WT control mice were plated on anti-CD3 and ICAM-1 coated glass chambers. After 5 min, the relative area of the immune synapse was measured. **C.** Surface expression of CTLA-4 on *Dock8*$^{-/-}$ and WT Treg cells before and after stimulation with PMA and ionomycin for 3 hours. Experiments B and C are representative of 2 independent experiments. Symbols represent individual measurements in B and individual mice in C. Bars in B and C represent mean and SEM. t-test ns $p>0.05$



Supplementary Figure 6. YFP$^+$ Treg cells from *Foxp3$^{YFP\text{-}Cre/+}$/Dock8$^{flox/flox}$* fremale mice have markedly decreased expression of *Dock8* exons 44-46. CD4$^+$CD25$^+$CD39$^+$YFP$^+$ (DOCK8 deficient) and YFP$^-$ (DOCK8 sufficient) Treg cells were sorted from *Foxp3$^{YFP\text{-}Cre/+}$/Dock8$^{flox/flox}$* female mice. mRNA expression of *Dock8* exons 10 and 44-46 are shown. Symbols represent independent samples and bars represent mean and SEM. t-test *** p<0.001