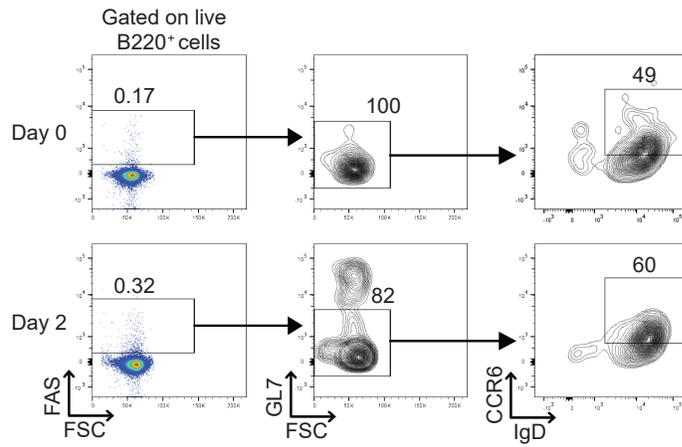
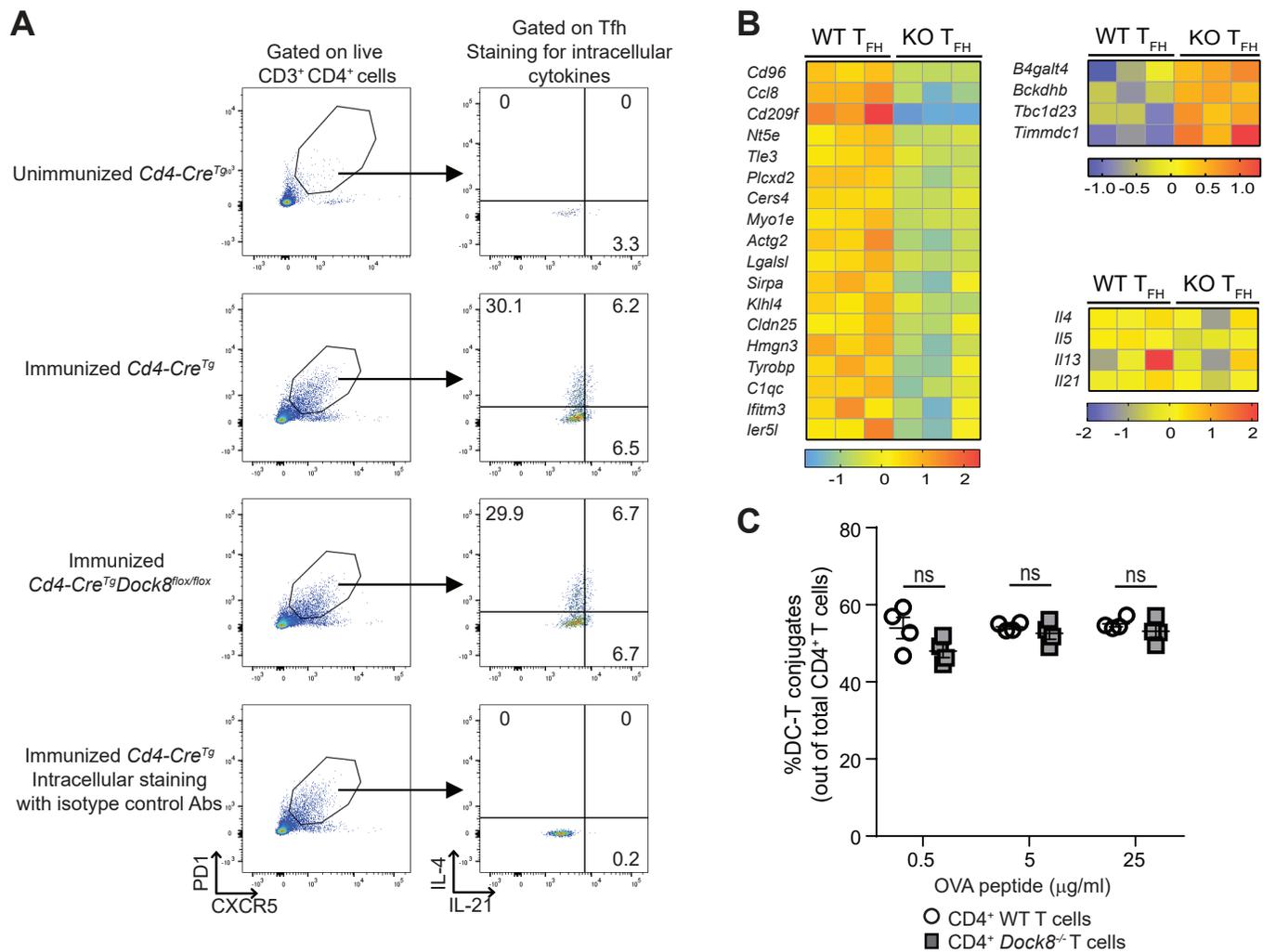


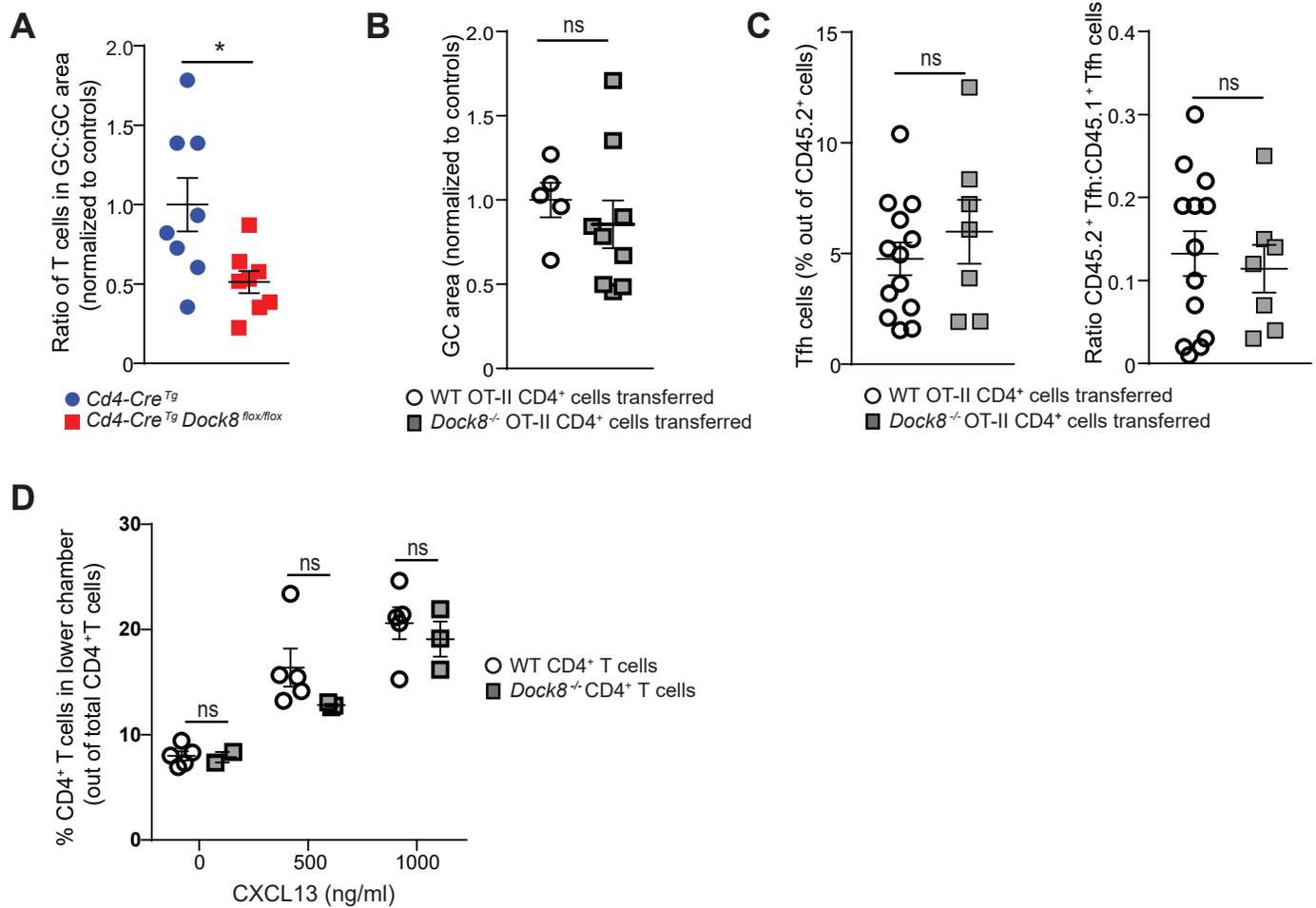
Supplemental Figure 1. *Cd4-Cre^{Tg}Dock8^{flox/flox}* mice have total and antigen-specific serum IgE levels, antigen-driven T cell proliferation, and cytokine secretion comparable to controls. **A. Immunoblots of purified B and T cell lysates from *Cd4-Cre^{Tg}Dock8^{flox/flox}* and controls. Lysates were immunoblotted for DOCK8 and β -actin for protein loading. **B-E.** *Cd4-Cre^{Tg}Dock8^{flox/flox}* mice and controls were immunized with TNP-KLH in the bilateral hocks on day 0 and 14. Serum was obtained and draining LNs were harvested on day 21 and cultured with KLH antigen *in vitro*. **B.** Serum TNP-specific IgM. **C.** Serum TNP-specific IgE (left) and total IgE levels (right). **D.** CD4⁺ T cell proliferation measured by Cell Trace Violet dilution. **E.** IL-2 (left) and INF- γ (right) secretion in culture supernatants by T cells. The graphs in B-E shows data from one representative experiment out of two. n=3-5 mice/group. Data in B-E are presented as mean \pm SEM.**



Supplemental Figure 2. Gating strategy for examining pre-GC B cells. *Cd4-Cre^{Tg}Dock8^{fllox/fllox}* and control mice were immunized in the hocks with TNP-KLH. Draining LNs were examined by flow cytometry. The gating strategy for examining pre-GC B cells pre-immunization (day 0) and on day 2 are illustrated above.



Supplemental Figure 3. Examination of Tfh cytokine and gene expression and DC-T cell conjugate formation. **A.** *Cd4-Cre^{Tg}Dock8^{flox/flox}* mice and controls were immunized with TNP-KLH in the hock. Draining LNs were harvested 7 days after immunization. Isolated lymphocytes were stimulated for 4 hours with phorbol-12,13-dibutyrate and ionomycin before extracellular staining, permeabilization, and staining for intracellular IL-4 and IL-21. Representative flow cytometry plots are shown. An unimmunized *Cd4-Cre^{Tg}* control (top panel), an immunized *Cd4-Cre^{Tg}* control and a *Cd4-Cre^{Tg}Dock8^{flox/flox}* mouse (middle panels), and an immunized *Cd4-Cre^{Tg}* control stained with isotype control antibodies (bottom panel). **B.** Heatmaps of genes differentially expressed in CD4⁺CD25⁺CXCR5⁺ICOS⁺ Tfh cells from the draining LNs of *Cd4-Cre^{Tg}Dock8^{flox/flox}* mice and controls, 7 days after immunization with TNP-KLH in the bilateral hocks. Cutoffs: Fold of change >1.5, $p < 0.05$ (left and top right). Heatmap of cytokine gene expression in Tfh cells from the draining LNs of immunized *Cd4-Cre^{Tg}Dock8^{flox/flox}* mice and *Cd4-Cre^{Tg}* controls (bottom right). Scales show log₂ fold change from the geometric mean of the row. Sorted cells from three mice/group were analyzed. **C.** Percentage of T-DC conjugates out of total CD4⁺ T cells isolated from *Dock8*^{-/-} OTII and WT OTII mice incubated for 3 h with LPS stimulated WT bone marrow derived DCs loaded with OVA_{3₂₃₋₃₃₉} (n=4 mice/group) Data are presented as mean ± SEM. t-test n.s. $p > 0.05$



Supplemental Figure 4. DOCK8 deficient CD4⁺ T cells develop into Tfh cells and have normal *in vitro* migration to CXCL13. **A.** Draining LNs from *Cd4-Cre^{Tg}Dock8^{flox/flox}* and control mice immunized in the hocks with TNP-KLH on day 10. The ratio of T cells localized to the GC compared with the GC area is shown. (n=7-8 mice/group) **B-C.** WT CD4⁺CD45.2⁺OT-II or DOCK8 deficient T cells were adoptively transferred into CD45.1⁺ WT mice. Recipients were immunized with NP-OVA in the hock, and popliteal LNs were analyzed on day 9 post-immunization. (B) The GC area from immunized recipient draining LNs. (C) The percentage of CD4⁺CXCR5⁺PD1⁺ Tfh cells out of CD45.2⁺ T cells in the draining LNs (left). The ratio of CD45.2⁺ to CD45.1⁺ Tfh cells present in the draining LNs (right). (n=5-9 mice/group in B and n=7-13 mice/group in C) **D.** Transwell migration of WT CD4⁺ T cells and *Dock8^{-/-}* CD4⁺ T cells from immunized OTII mice to CXCL13. (n=3-5 mice/group) Data are presented as mean±SEM. t-test n.s. p>0.05, *p<0.05.