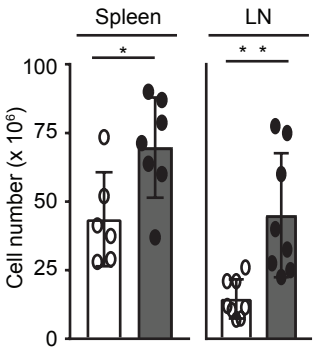
A



B

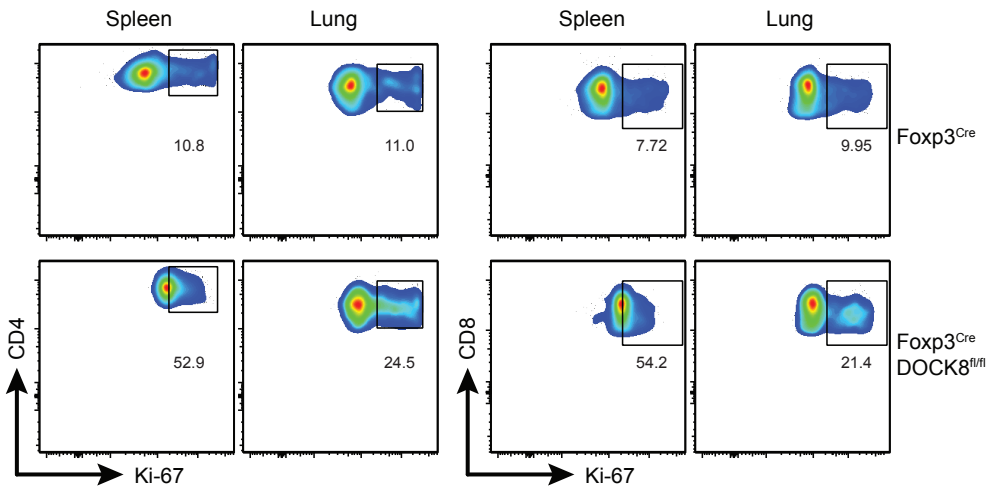


Figure S1. T_{reg} cell-specific deletion of DOCK8 causes T cells expansion, related to Figure 1.

(A) Graph showing the absolute number of lymphocytes in spleen and LN of Foxp3^{Cre} and Foxp3^{Cre} DOCK8$^{fl/fl}$ mice. Three independent experiments were performed with three or more mice per group. **(B)** Representative FACS plot showing that Foxp3^{Cre} DOCK8$^{fl/fl}$ mice have massive T cells proliferation marked by Ki-67 staining in spleen and lung in comparisons to Foxp3^{Cre} mice.Two independent experiments were performed with three mice per group. The data shown are the mean + SD. *p< 0.05, ***p< 0.001.

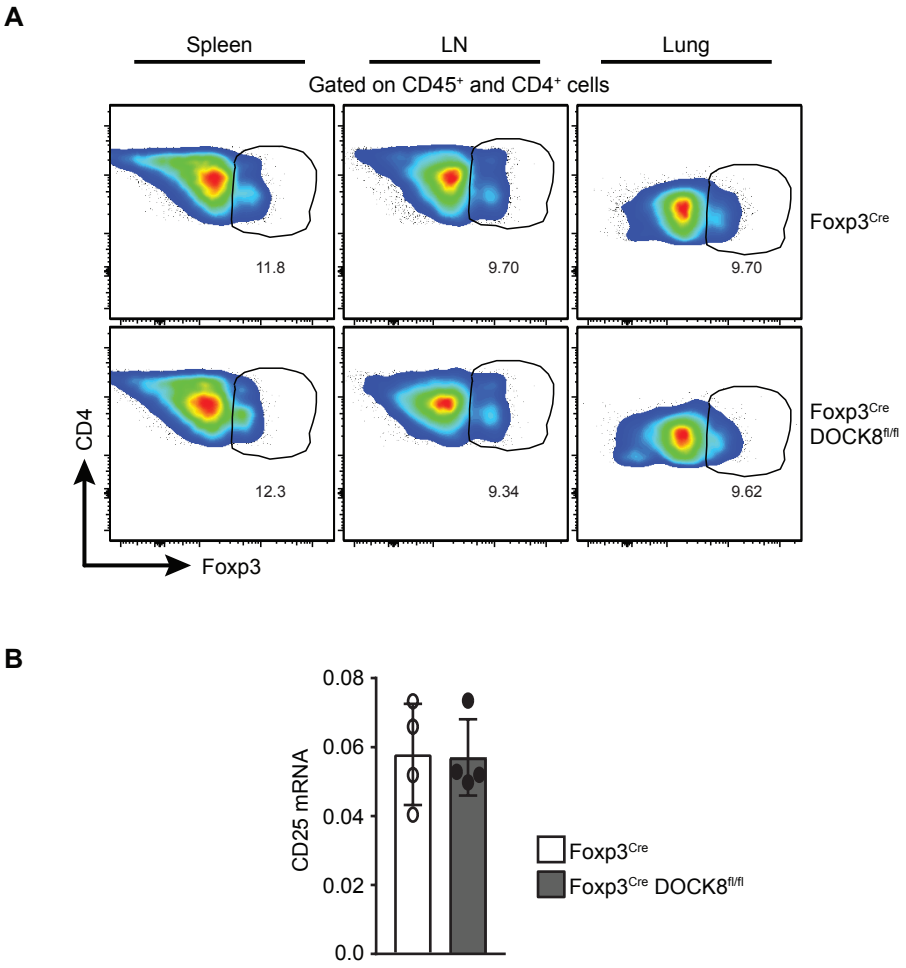


Figure S2. DOCK8 deficiency does not impact the development of T_{reg} cells and CD25 transcript level, related to Figure 3.

(A) $CD4^+$ $Foxp3^+$ T cells were analyzed in the spleen, LN and lung, isolated from eight week old $Foxp3^{Cre}$ and $Foxp3^{Cre}$ $DOCK8^{fl/fl}$ mice. Four independent experiments were performed with two or more mice per group. **(B)** DOCK8 does not directly control CD25 transcript. Histogram showing that control and DOCK8-deficient T_{reg} cells have comparable CD25 transcript levels. YFP+ Treg cells were FACS sorted from the spleen and LNs of Foxp3Cre and Foxp3Cre DOCK8fl/fl mice. Total RNA was extracted from purified cells with RNeasy kit from (Qiagen) and reverse transcribed with the iScript cDNA synthesis kit (BioRad). The Q- PCR was conducted using mouse CD25 specific primers (forward 5-CGTTGCTTAGGAAACTCCTGGA-3 and reverse 5 -GCTTTCTCGATTTGTCATGGG-3). Gene expression was normalized to the housekeeping gene RPL-19. Two independent experiments were performed with a pooled samples from three to five mice per group.

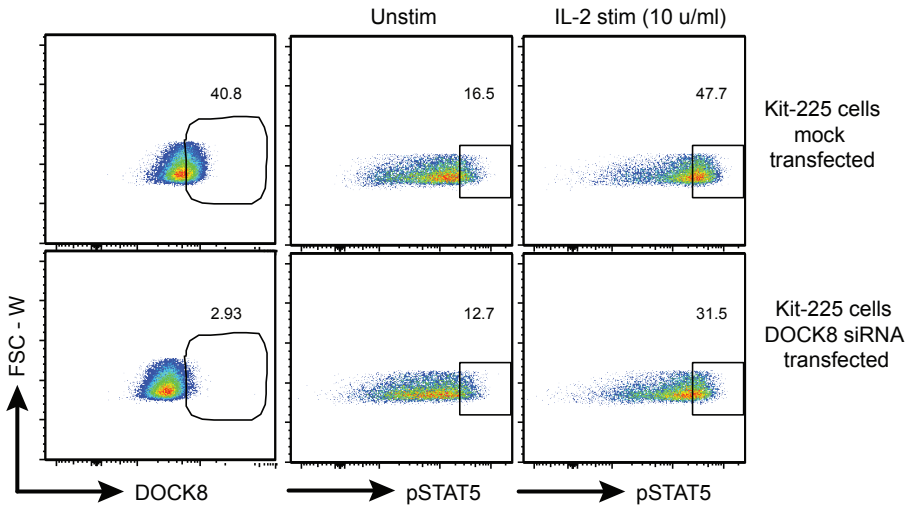


Figure S3. Kit-225 cells require DOCK8 for optimal STAT5 activation, related to Figure 5.

Control or siRNA-mediated DOCK8 knock down Kit-225 cells were stimulated with indicated concentration of IL-2 for 15 minutes and then cells were analyzed for pSTAT5 by flow cytometry. Two independent experiments were performed in triplicate.

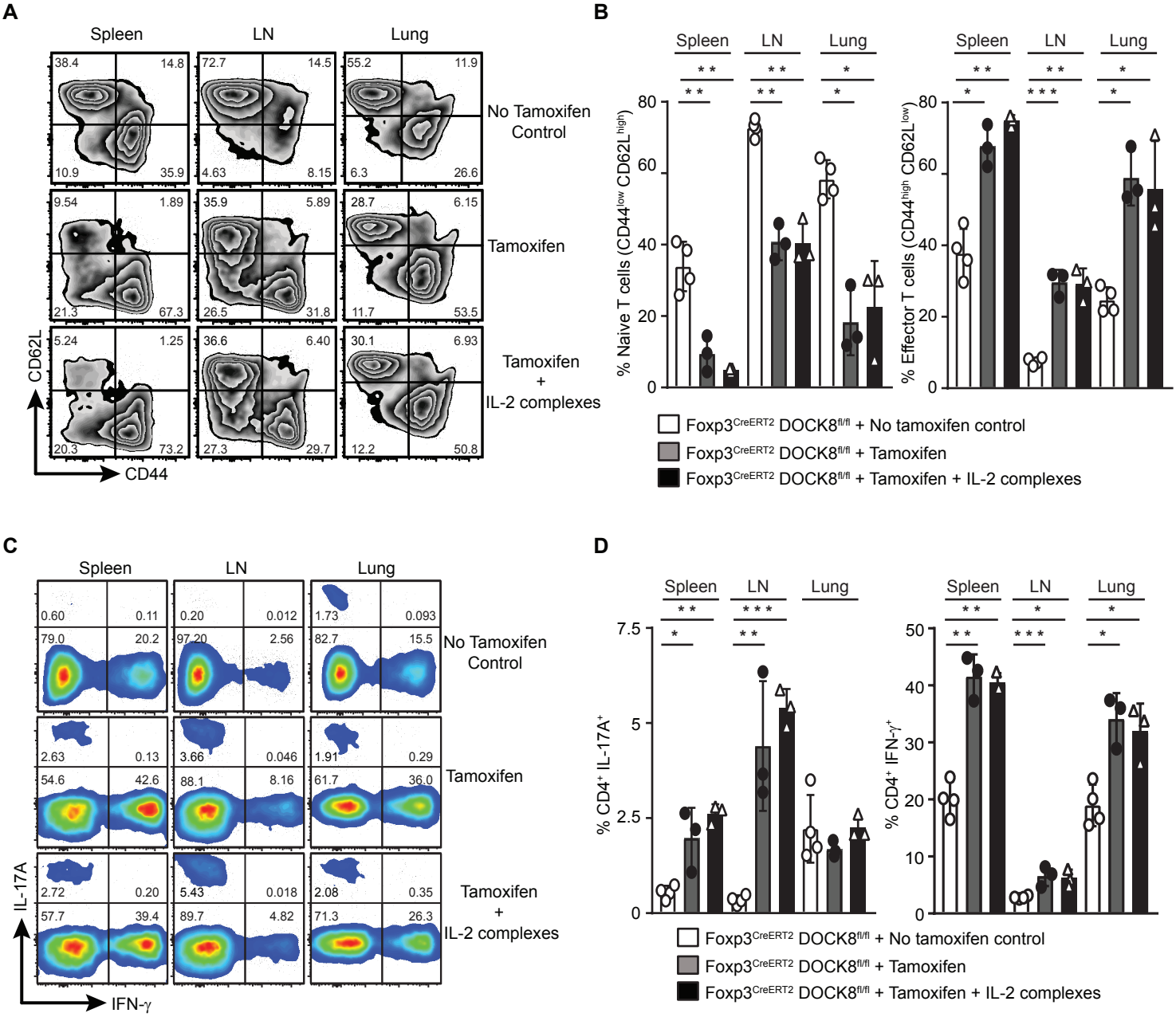


Figure S4. IL-2 complexes treatment failed to rescue T_{reg} cell functions upon acute deletion of DOCK8 in T_{reg} cells ,related to Figure 5.

(**A** and **B**) Flow cytometry analysis (**A**) and quantification (**B**) of naive/effector CD4$^+$T cells. (**C** and **D**) Flow cytometry analysis (**C**) and quantification (**D**) of intracellular IL-17A and IFN-γ expression from indicated tissues analysed from control Foxp3^{CreERT2} DOCK8$^{fl/fl}$ mice (n = 4) and Foxp3^{CreERT2} DOCK8$^{fl/fl}$ mice injected either with tamoxifen (n = 3) or tamoxifen along with IL-2 complexes (n = 3). The data are representative of two independent experiments with similar results. The data shown are the mean ± SD. Statistics were performed with Prism software by using t test* P < 0.05, * * P < 0.01, * * * P < 0.001.