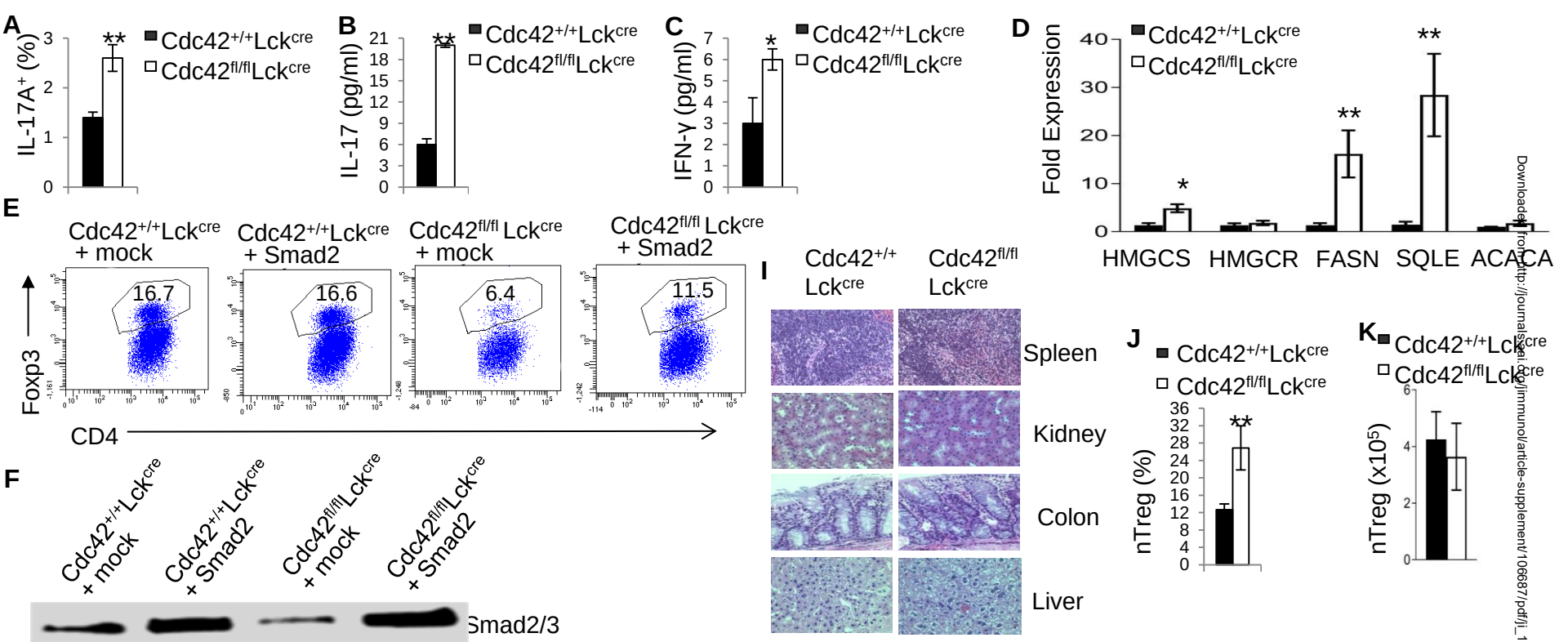
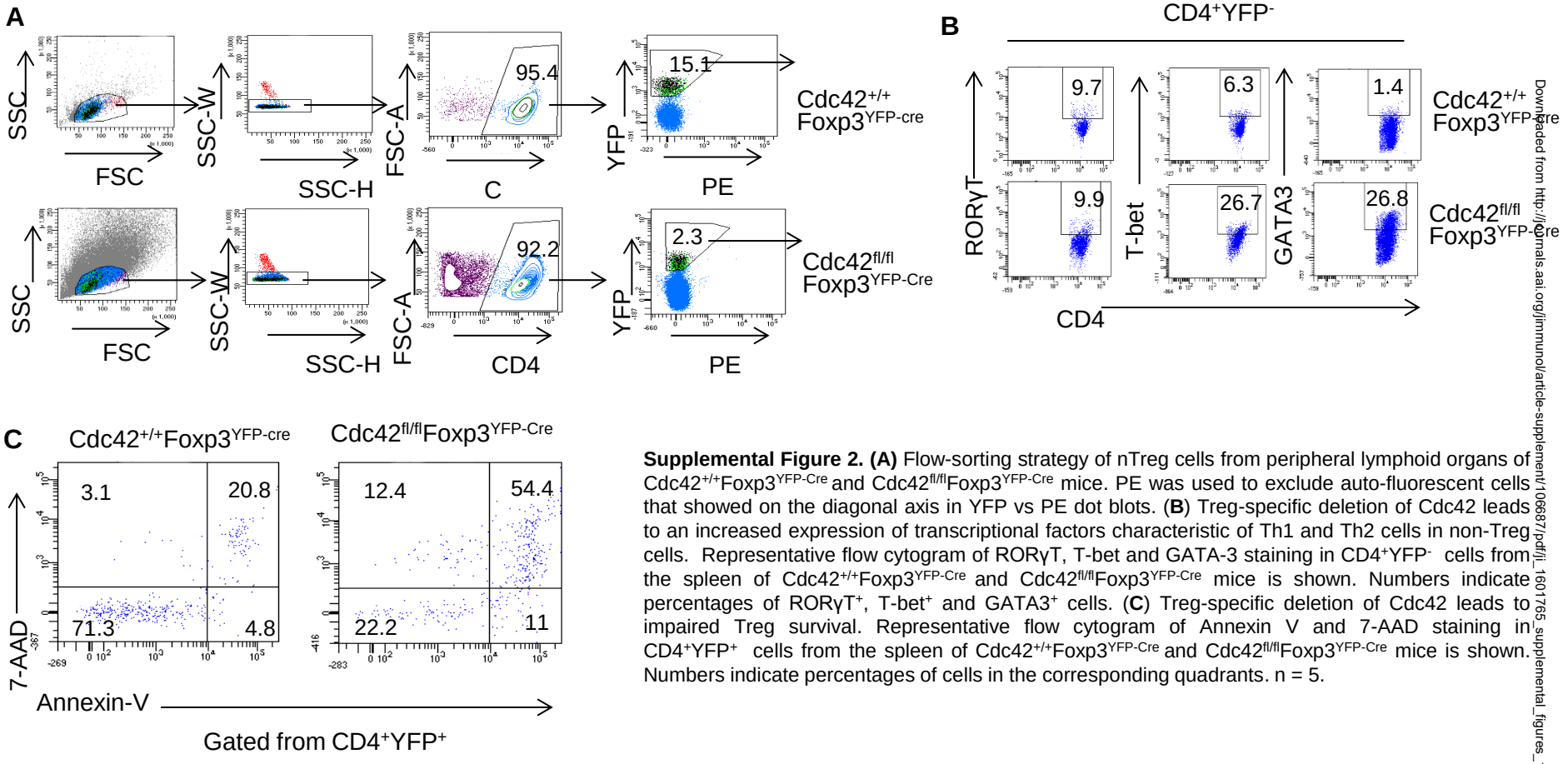
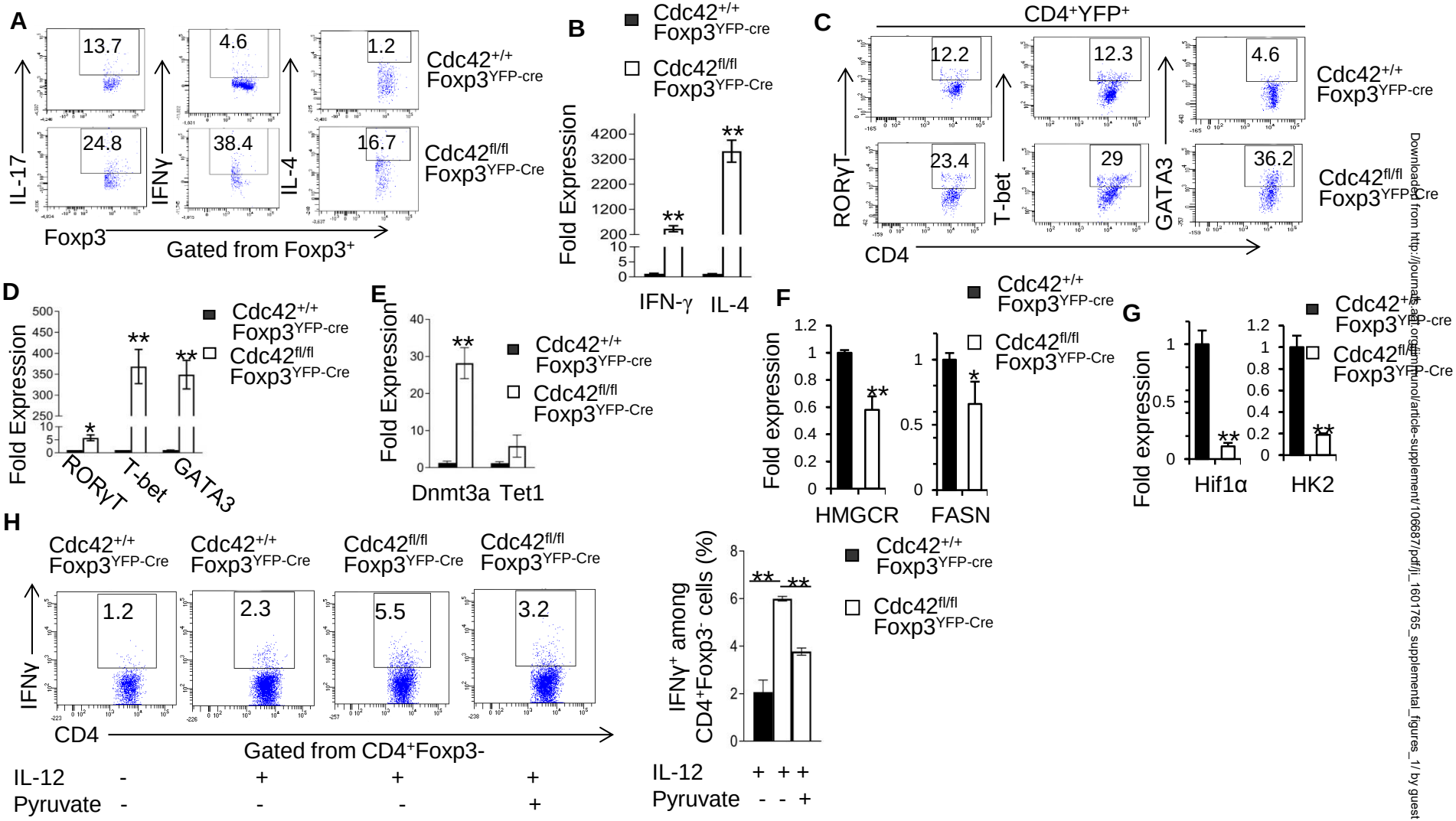


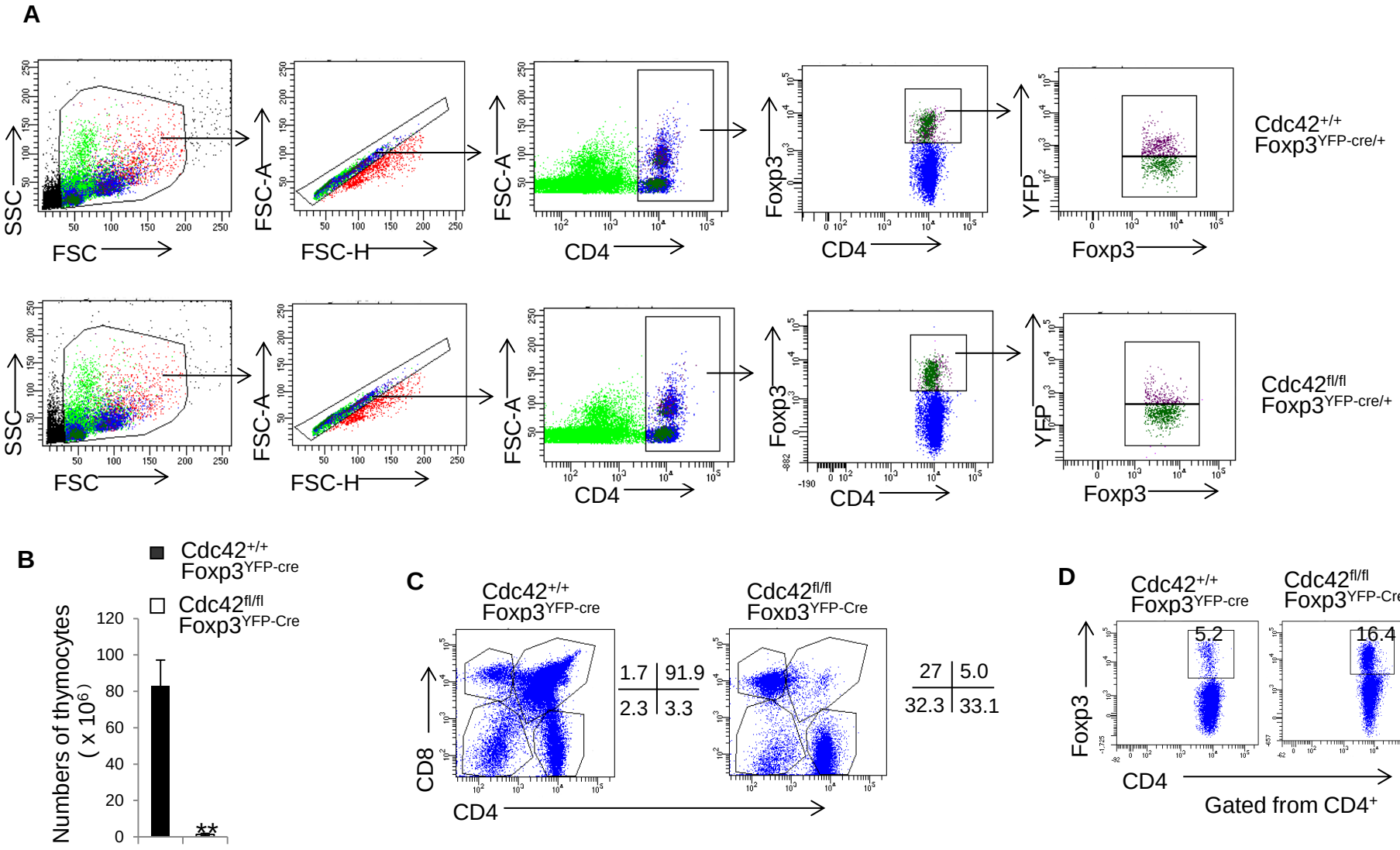
Supplemental Figure 1. (**A-D**) Cdc42 deficiency enhances Th17 cell differentiation and pathogenicity and upregulates genes involved in lipid metabolism. (**A**) Percentages of IL-17-producing cells among CD4$^+$ T cells from Cdc42$^{+/+}$Lck^{Cre} and Cdc42$^{fl/fl}$Lck^{Cre} mice cultured under Th17 polarizing condition for 2 days. (**B, C**) ELISA analysis of concentrations of IL-17 (B) and IFN-γ (C) in culture supernatant of cells from (A). (**D**) Real-time RT-PCR analysis of mRNA expression of lipid metabolism genes in Cdc42$^{+/+}$Lck^{Cre} and Cdc42$^{fl/fl}$Lck^{Cre} Th17 cells. (**E**) Representative flow cytogram of Foxp3 staining in CD4$^+$ T cells from Cdc42$^{+/+}$Lck^{Cre} and Cdc42$^{fl/fl}$Lck^{Cre} mice transduced with either mock or Smad2-expressed retrovirus followed by culturing under iTreg polarizing condition and intracellular staining for Foxp3. **(F)** Immunoblotting of total Smad2/3 in Cdc42$^{+/+}$Lck^{Cre} and Cdc42$^{fl/fl}$Lck^{Cre} iTreg cells from (E). (**G-K**) Cdc42$^{fl/fl}$Lck^{cre} mice contain increased spontaneous Th17 and Th1 cells but show no apparent autoimmune disease, associated with increased frequency of peripheral nTreg. (**G**) Representative flow cytogram of IL-17 and IFN-γ staining in splenocytes from Cdc42$^{+/+}$Lck^{Cre} and Cdc42$^{fl/fl}$Lck^{Cre} mice. Numbers indicate percentages of IL-17$^+$ and IFN-γ$^+$ cells. (**H**) Representative flow cytogram of IL-17 and IFN-γ staining in splenocytes from Cdc42$^{+/+}$Lck^{Cre} and Cdc42$^{fl/fl}$Lck^{Cre} mice after restimulation with PMA/Ionomycin for 5 h. (**I**) Representative histopathological analysis (hematoxylin and eosin staining) of spleen, kidney, colon and liver from Cdc42$^{+/+}$Lck^{Cre} and Cdc42$^{fl/fl}$Lck^{Cre} mice. (**J**) Percentages of nTreg in the spleen of Cdc42$^{+/+}$Lck^{Cre} and Cdc42$^{fl/fl}$Lck^{Cre} mice. (**K**) Numbers of nTreg in the spleen of Cdc42$^{+/+}$Lck^{Cre} and Cdc42$^{fl/fl}$Lck^{Cre} mice.n = 3-5. Error bars indicate SD. *p < 0.05, **p < 0.01.



Supplemental Figure 2. (A) Flow-sorting strategy of nTreg cells from peripheral lymphoid organs of $Cdc42^{+/+}Foxp3^{YFP\text{-}Cre}$ and $Cdc42^{fl/fl}Foxp3^{YFP\text{-}Cre}$ mice. PE was used to exclude auto-fluorescent cells that showed on the diagonal axis in YFP vs PE dot blots. (**B**) Treg-specific deletion of Cdc42 leads to an increased expression of transcriptional factors characteristic of Th1 and Th2 cells in non-Treg cells. Representative flow cytogram of RORγT, T-bet and GATA-3 staining in $CD4^{+}YFP^{-}$ cells from the spleen of $Cdc42^{+/+}Foxp3^{YFP\text{-}Cre}$ and $Cdc42^{fl/fl}Foxp3^{YFP\text{-}Cre}$ mice is shown. Numbers indicate percentages of RORγT^{+}, T-bet^{+} and GATA3^{+} cells. (**C**) Treg-specific deletion of Cdc42 leads to impaired Treg survival. Representative flow cytogram of Annexin V and 7-AAD staining in $CD4^{+}YFP^{+}$ cells from the spleen of $Cdc42^{+/+}Foxp3^{YFP\text{-}Cre}$ and $Cdc42^{fl/fl}Foxp3^{YFP\text{-}Cre}$ mice is shown. Numbers indicate percentages of cells in the corresponding quadrants. n = 5.



Supplemental Figure 3. (A-E) Treg-specific deletion of Cdc42 leads to Treg instability. (**A**) Representative flow cytogram of staining of IL-17, IFN-γ and IL-4 among Foxp3 cells from the spleen of Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre}$ mice. (**B**) Real-time RT-PCR analysis of mRNA expression of IFN-γ and IL-4 in CD4^{+}YFP^{+} cells from the spleen of Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre}$ mice. (**C**) Representative flow cytogram of RORγT, T-bet and GATA-3 staining in CD4^{+}YFP^{+} cells from the spleen of Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre}$ mice. (**D**) Real-time RT-PCR analysis of mRNA expression of RORγT, T-bet and GATA-3 in CD4^{+}YFP^{+} cells from the spleen of Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre}$ mice. (**E**) Real-time RT-PCR analysis of mRNA expression of DNMT3A and TET1 in CD4^{+}YFP^{+} cells from the spleen of Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre}$ mice. (**F-H**) Treg-specific deletion of Cdc42 downregulates lipid metabolism and glycolysis genes in nTreg cells and restoration of glycolysis rescues instability of Cdc42-deficient nTreg. (**F**) Real-time RT-PCR analysis of mRNA expression of lipid metabolism genes HGMCR and FASN in CD4^{+}Foxp3^{+} cells from the spleen of Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre}$ mice. (**G**) Real-time RT-PCR analysis of mRNA expression of glycolysis genes Hif1α and HK2 in CD4^{+}Foxp3^{+} cells from the spleen of Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre}$ mice. (**H**) CD4^{+}CD25^{+}YFP^{+} nTreg cells purified from Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre}$ mice were cultured for 36 h with or without anti-CD3/-CD28 and IL-12 (50 ng/ml), in the presence or absence of 2 mM pyruvate, and costained for CD4, Foxp3, and IFN-γ. Representative flow cytogram of IFN-γ staining among CD4^{+}Foxp3^{-} cells is shown (left). Numbers in the dot plots indicate percentages of IFN-γ^{+} cells. Average percentages of CD4^{+}Foxp3^{-}IFN-γ^{+} cells are shown in bar graph (right). n = 5. Error bars indicate SD. *p < 0.05, **p < 0.01.



Supplemental Figure 4. (A) Gating strategy of Foxp3$^+$YFP$^+$ and Foxp3$^+$YFP$^-$ cells from the spleen of Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre/+}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre/+}$ mice. (**B**) Total thymocyte numbers in Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre}$ mice. (**C**) Representative flow cytogram of thymocytes from Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre}$ mice stained for CD4 and CD8. Numbers indicate percentages of CD4$^-$CD8$^-$, CD4$^+$CD8$^+$, CD4$^+$ and CD8$^+$ cells in corresponding quadrant. (**D**) Representative flow cytogram of Foxp3 staining (gated on CD4$^+$ thymocytes) in thymocytes from Cdc42$^{+/+}$Foxp3$^{YFP\text{-}Cre}$ and Cdc42$^{fl/fl}$Foxp3$^{YFP\text{-}Cre}$ mice. Numbers indicate percentages of CD4$^+$Foxp3$^+$ cells. n = 5. Error bars indicate SD. **p < 0.01.