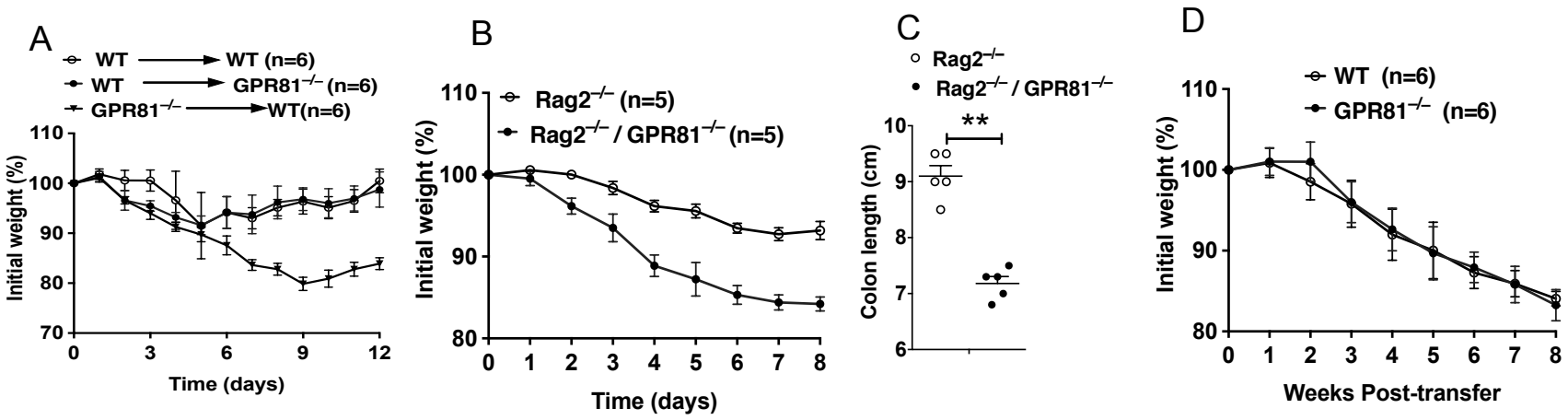
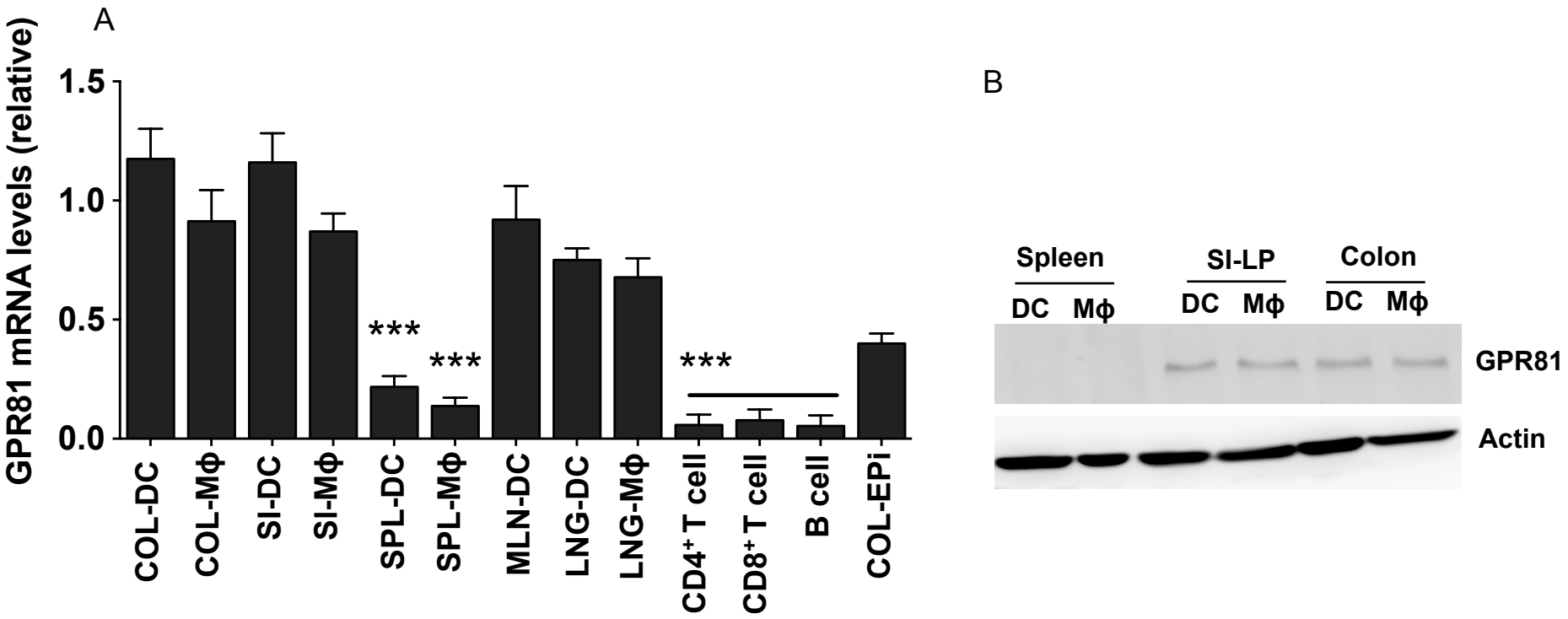
Supplementary Figure 1



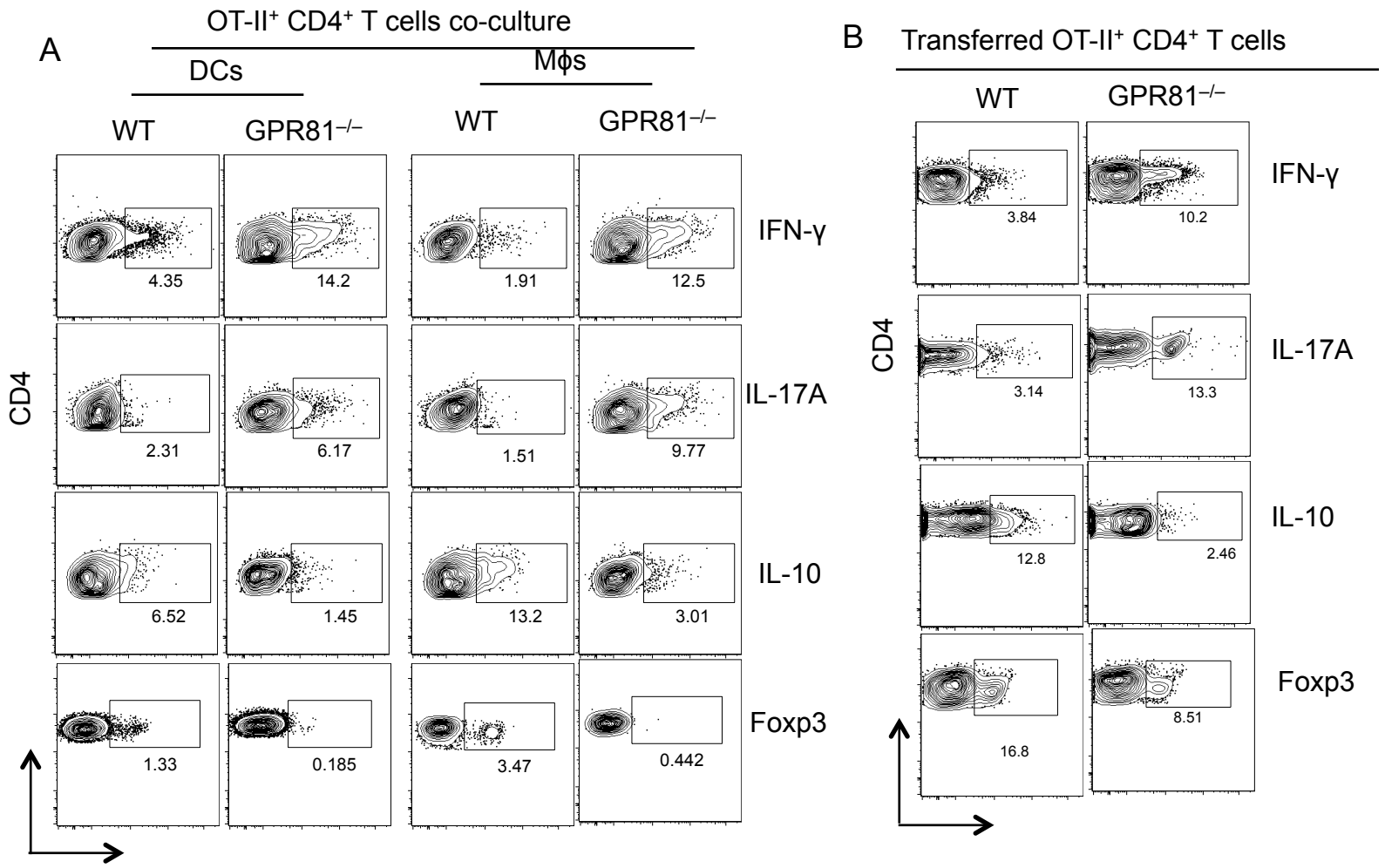
Supplementary Figure 1: GPR81-signaling in immune cells limits colitis in mice. (A) Reciprocal bone marrow chimeras of WT (CD45.1) and GPR81$^{-/-}$ (CD45.2) mice were treated with 2.5% DSS in drinking water for 6 days . Weight loss in WT → *WT,* WT → GPR81$^{-/-}$ and GPR81$^{-/-}$ → WT group mice in response to DSS treatment. **(B, C)** Rag2$^{-/-}$/GPR81$^{-/-}$ and Rag2$^{-/-}$ mice (littermate control) were treated with 2.5% DSS in drinking water for 5 days and at day 9 colons of mice were analyzed for inflammation. **(B, C)** Change in body weight and colon length (day 9) of Rag2$^{-/-}$/GPR81$^{-/-}$ and Rag2$^{-/-}$ mice . **(D)** CD45RB^{hi}CD4^{+} T cells isolated from WT or GPR81$^{-/-}$ mice were adoptively transferred into Rag2$^{-/-}$ mice. Weight loss in Rag2$^{-/-}$ mice at indicated time points (weeks) post naïve CD4^{+} T cell adoptive transfer. Data are representative of two independent experiments. Error bars indicate mean ± SEM.

Supplementary Figure 2



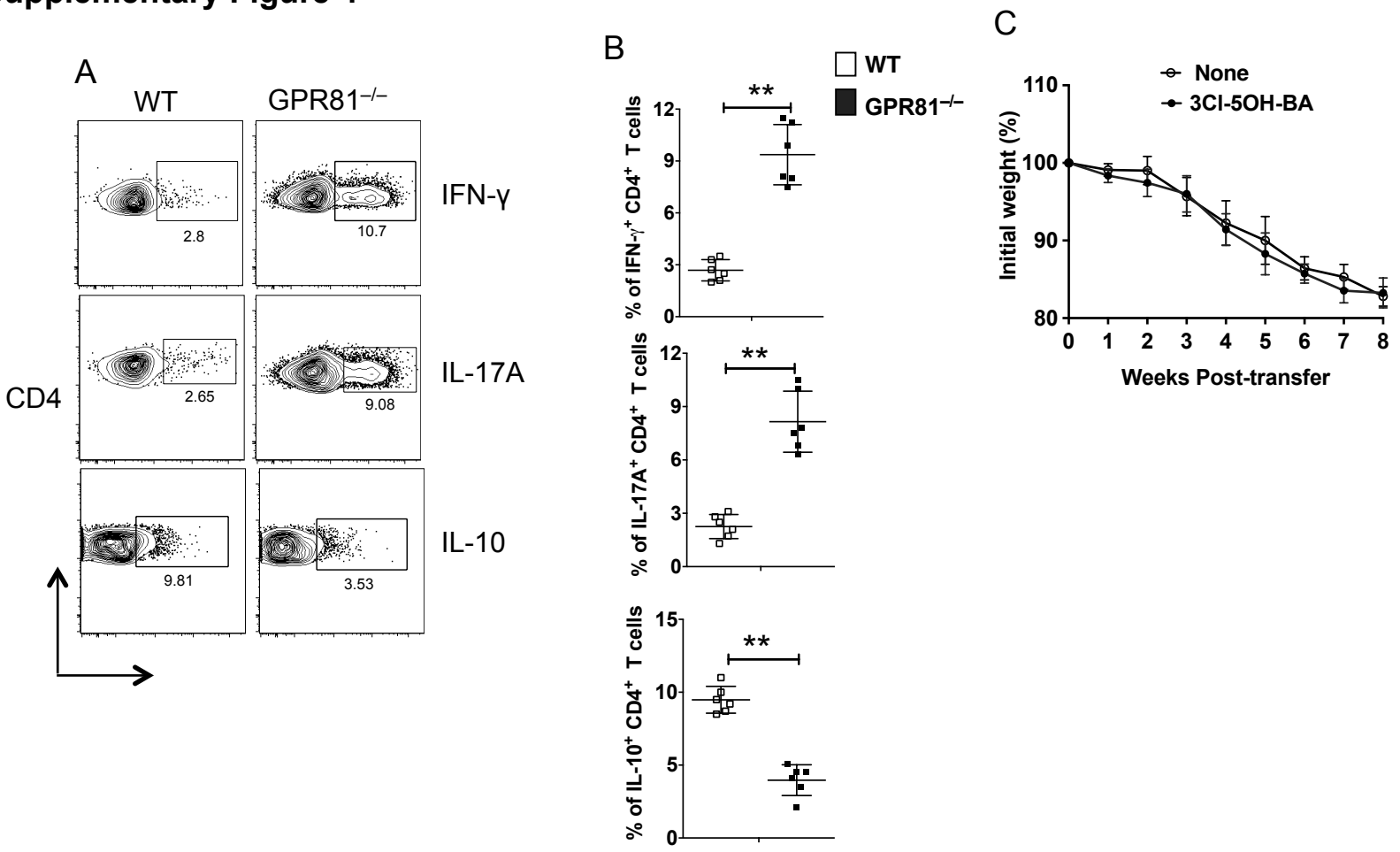
Supplementary Figure 2: GPR81 expression levels in immune cells. (**A**) Quantitative real-time PCR analysis of mRNA expression of GPR81 in DCs, macrophages (Mϕ), epithelial cells (EPi), CD4+ T cells, $CD8^+$ T cells and B cells isolated from the colon (COL), spleen (SPL), mesenteric lymph nodes (MLN) and lung (LNG) of WT mice (n=3). **(B)** Western blot analysis of GPR81 in cell lysate DC and macrophages isolated from the spleen, small intestine lamina propria (SI-LP) and colon of WT mice.

Supplementary Figure 3



Supplementary Figure 3: GPR81 signaling in intestinal APCs suppresses Th1/Th17 cell differentiation and induces regulatory T cell differentiation. (A) Representative FACS plots showing the frequencies of IL-17A$^+$, IFN-γ$^+$, Foxp3$^+$ and IL-10$^+$ OT-II CD4$^+$ T cells after culturing with colonic DCs and macrophages isolated from GPR81$^{-/-}$ and WT mice. **(B)** Representative FACS plots showing the frequencies of adoptively transferred naïve OT-II CD4$^+$ T cells positive for IFN-γ, IL-17A, Foxp3 and/or IL-10 isolated from colons of WT and GPR81$^{-/-}$ mice treated orally with OVA protein.

Supplementary Figure 4



Supplementary Figure 4: GPR81-signaling regulates balance between regulatory T cell and Th1/Th17 cell numbers in the colon. (A,B) Representative FACS plot and cumulative frequencies of endogenous $CD4^+$ T cells positive for IL-17A, IFN-γ and IL-10 in the colon of $GPR81^{-/-}$ and WT mice. (C) **GPR81 agonist treatment did not protect $Rag2^{-/-}$ /$GPR81^{-/-}$ mice from T-cell transfer model of experimental colitis.** $CD45RB^{hi}CD4^+$ T cells isolated from WT mice were adoptively transferred into $Rag2^{-/-}$ /$GPR81^{-/-}$ mice. Animals were treated with GPR81 agonist orally (3-chloro-5-hydroxybenzoic acid (3Cl-5OH-BA); 10 mg/kg; on Weeks 1, 2, 3 and 4) and monitored over a period of time for percent weight loss compared to initial weight. Data are representative of two independent experiments (n=5). Error bars indicate mean ± SEM.