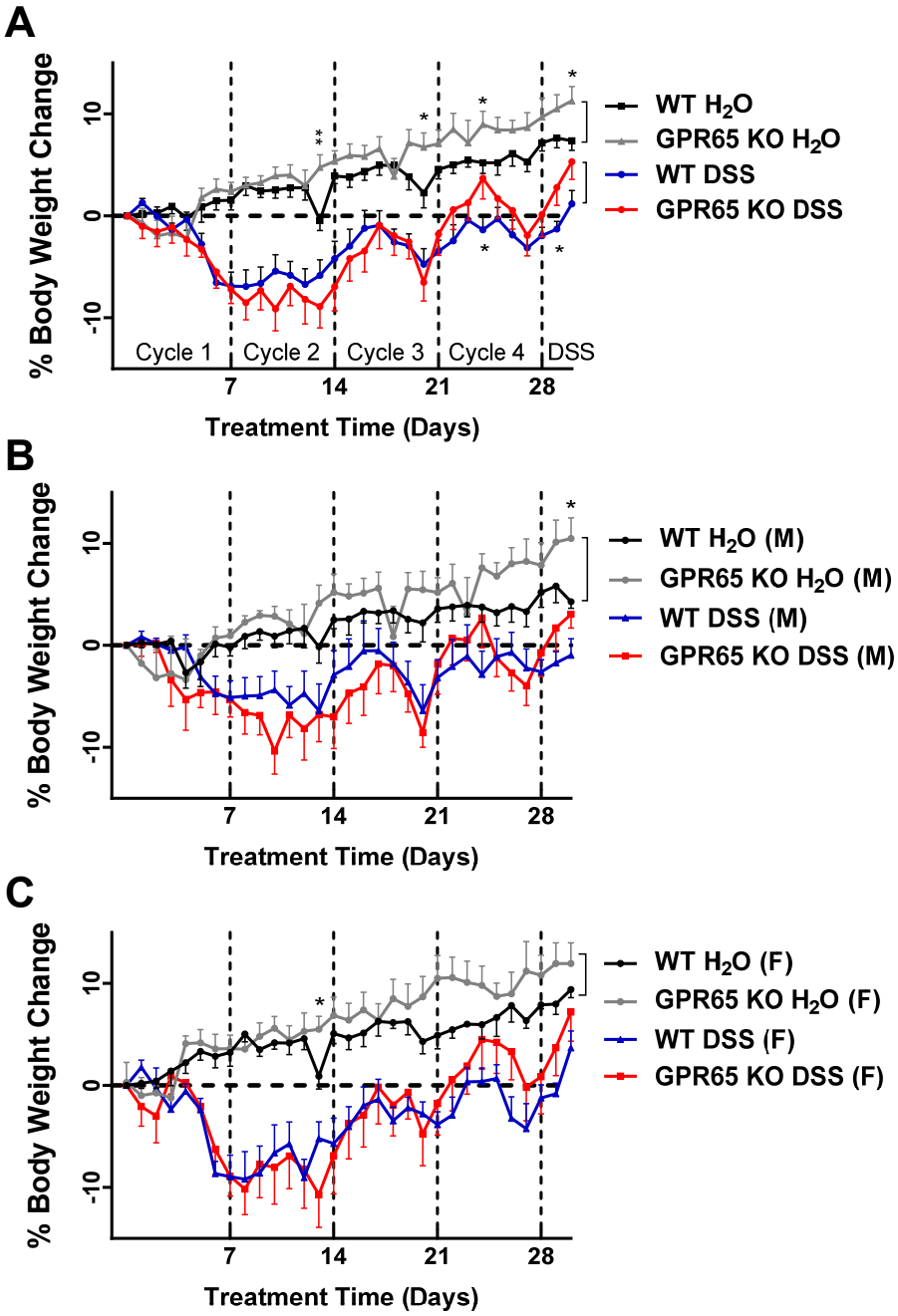
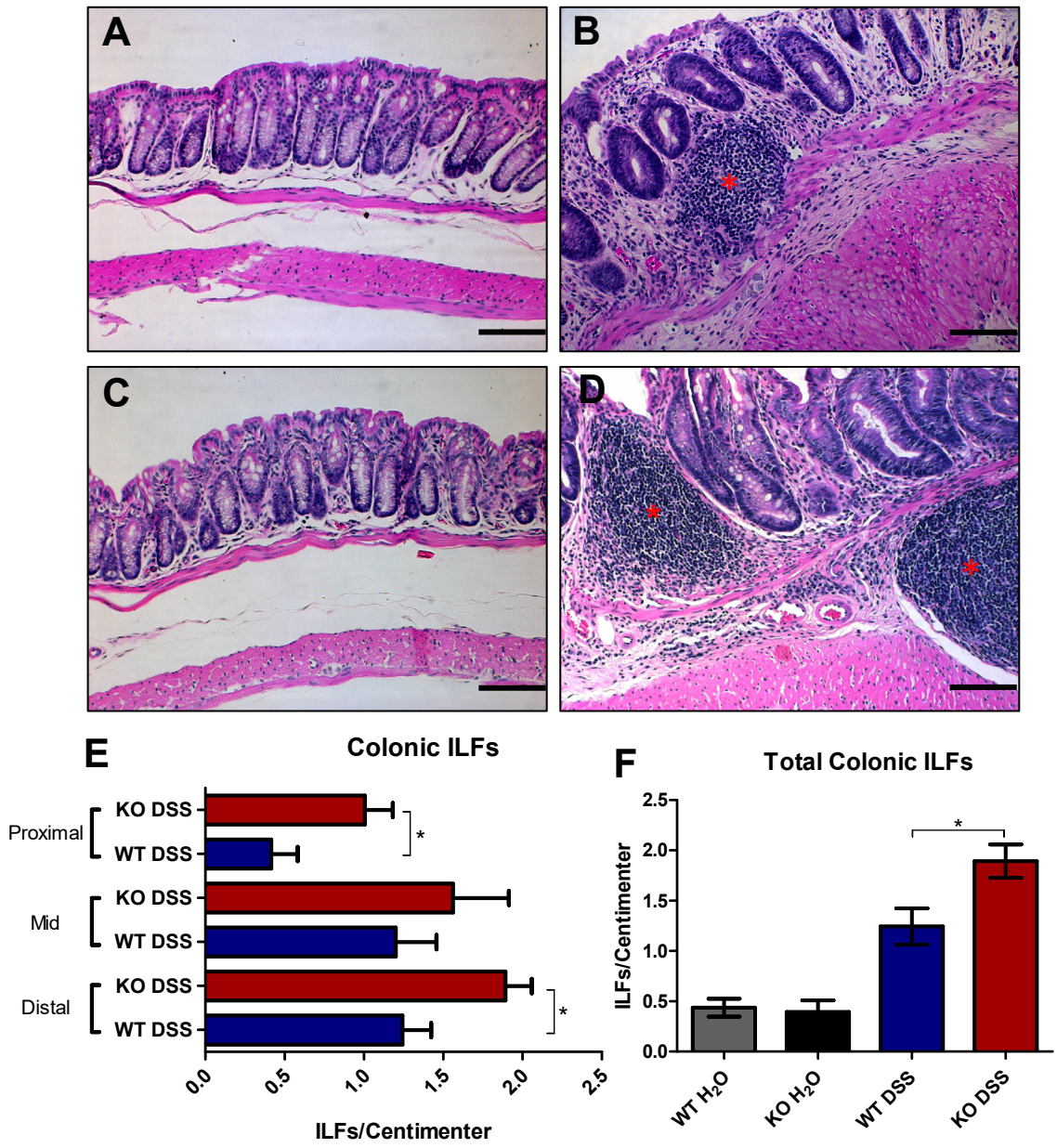
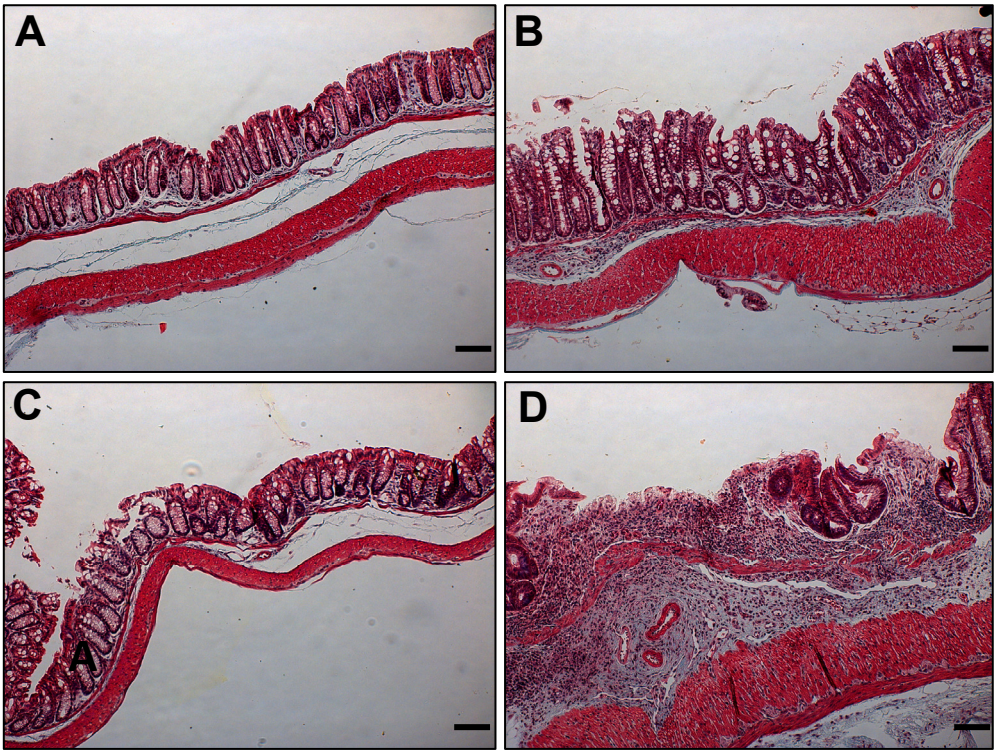


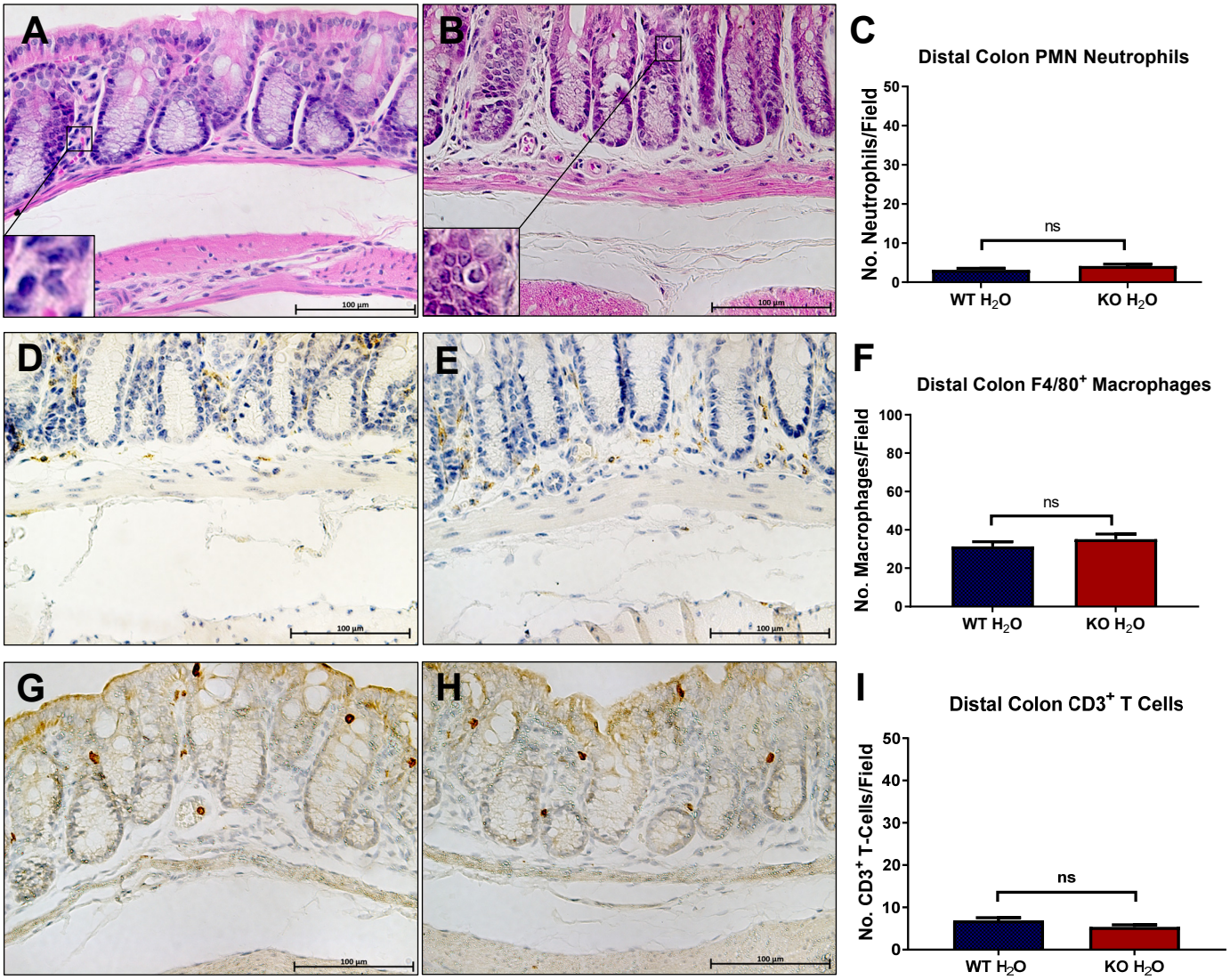
Supplementary Figure 1. Body weight change in wild-type (WT) and GPR65 knockout (KO) mice. Control GPR65 KO mice gain more weight than control WT mice during the experiment. Compared to WT-DSS mice, GPR65 KO-DSS mice trend to lose more weight in the cycle 2 and 3 but recover in the cycle 4 likely due to elevated baseline body weight gain. (A) combined, (B) male, and (C) female mouse body weight change in the control and DSS-treated WT and GPR65 KO mice. Data are presented as the mean ± SEM and statistical significance was determined using the unpaired *t*-test between indicated groups. (*$P < 0.05$, **$P < 0.01$).



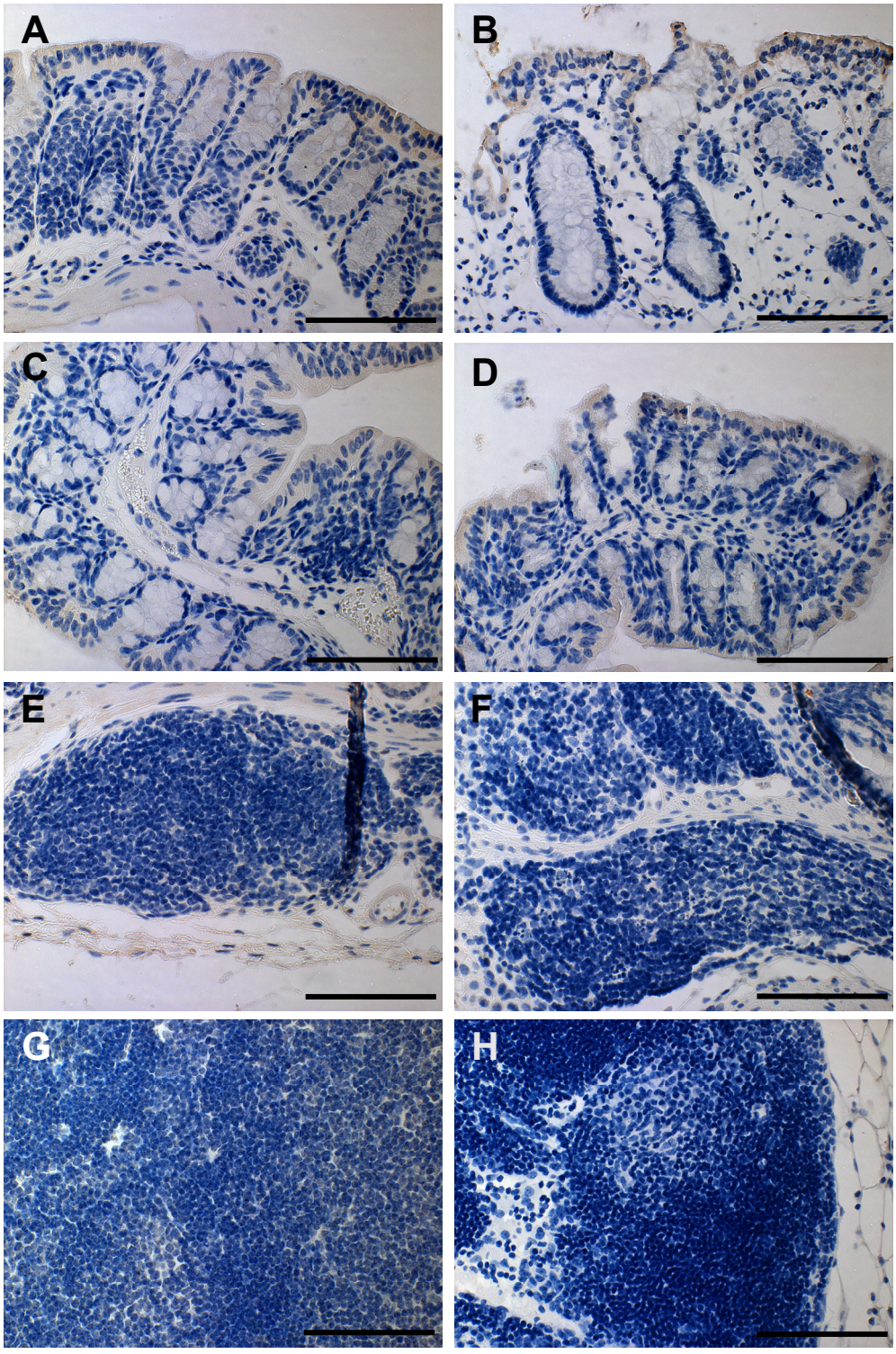
Supplementary Figure 2. Isolated lymphoid follicle (ILF) quantification in proximal, middle, and distal colon segments. ILF numbers were assessed as an indicator of intestinal inflammation. ILF numbers are highest in distal colon with reduced numbers of ILFs in the proximal colon in DSS-treated mice. GPR65 KO-DSS mice have a further increase in ILF numbers compared to WT-DSS mice. Representative pictures of ILFs in (A) WT-control, (B) WT-DSS, (C) GPR65 KO-control, and (D) GPR65 KO-DSS distal colon segments. Graphical representation of (E) ILF numbers in each segment of the colon and (F) combined full length colon. WT control (N=10), WT-DSS (N=13), GPR65 KO control (N=11), and GPR65 KO-DSS (N=13) mouse tissues are used for ILF quantification. Scale bar is 100µm. Data are presented as the mean ± SEM and statistical significance is determined using the unpaired *t*-test between WT-DSS and GPR65 KO-DSS groups (*$P < 0.05$).



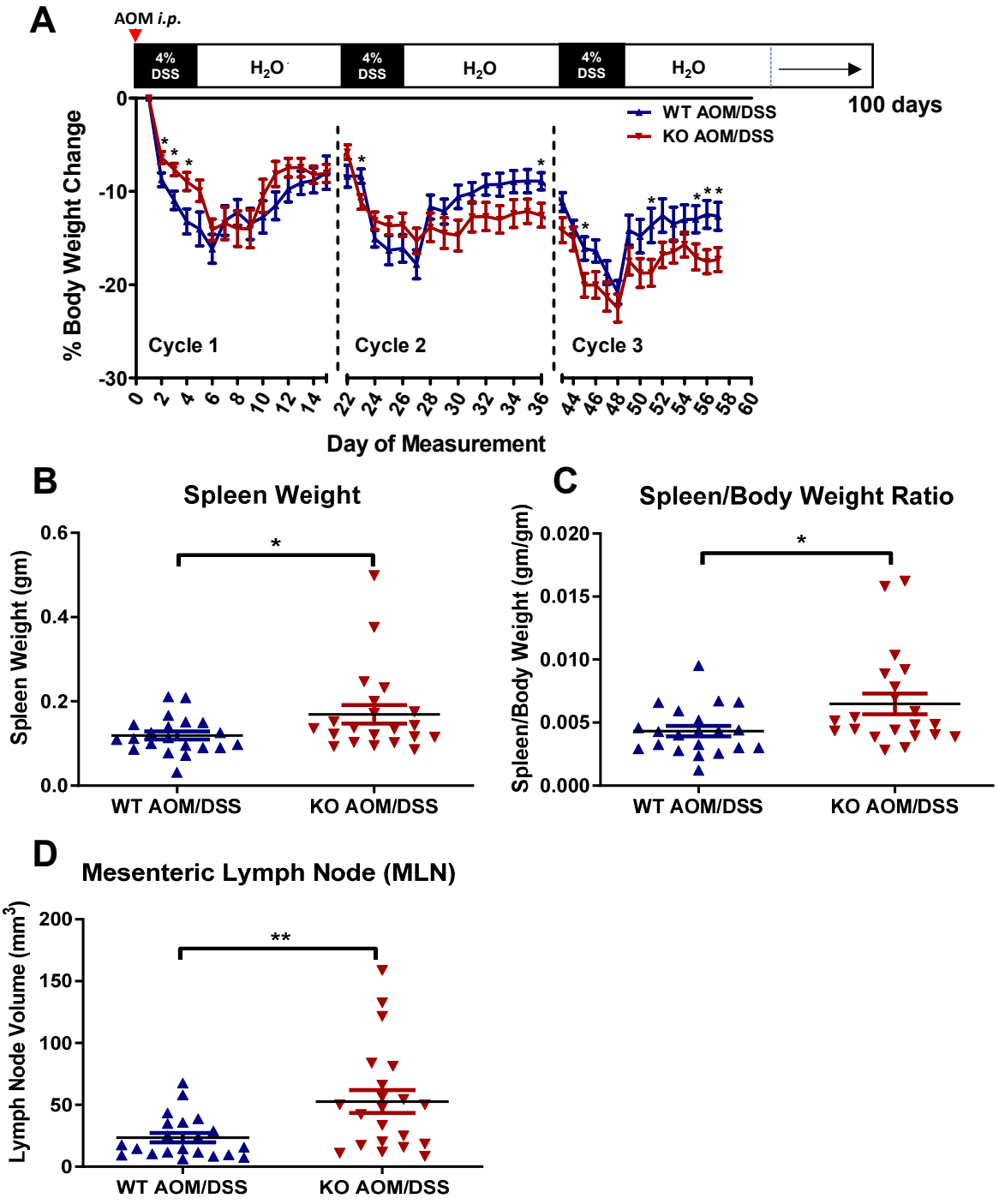
Supplementary Figure 3. Representative pictures of distal colonic fibrosis. Masson's trichrome stain indicates DSS-treated GPR65 KO mice have increased pathological fibrosis compared to WT-DSS mice. (A) WT control, (B) WT-DSS, (C) GPR65 KO control, and (D) GPR65 KO-DSS. Scale bar is 100μm.



Supplementary Fig. 4. Leukocytes in control mouse distal colon. Polymorphonuclear (PMN) neutrophils, F4/80^{+} macrophages, and CD3^{+} T cells were evaluated in the distal colon of control mice treated with regular water. WT and GPR65 KO control mice have comparable numbers of neutrophils, macrophages, and T cells in distal colon. (A-B) Representative pictures of WT control (left) and GPR65 KO control (right) mouse neutrophils, (D-E) macrophages, and (G-H) T cells, respectively. Graphical representation of (C) neutrophils, (F) macrophages, (I) and T cells. Scale bar is 100µm. Data are presented as the mean ± SEM and statistical significance was determined using the unpaired *t*-test between WT and GPR65 KO groups. (*ns,* not significant).



Supplementary Figure 5. WT negative control for GFP signal in the intestine and intestinal associated lymphoid tissues. No GFP signal could be detected in WT mouse tissues. GFP signal could not be observed in WT-control (A) distal colon mucosa, (C) proximal colon transverse folds, (E) isolated lymphoid follicles (ILFs), and (G) mesenteric lymph nodes (MLNs). GFP signal also could not be detected in WT-DSS (B) intestinal mucosa, (D) transverse folds, (F) ILFs, and (H) MLNs. Minor non-specific staining could be seen on intestinal epithelium and ex-mural connective tissues. Scale bar is 100µm.



Supplementary Figure 6. Macroscopic parameters of colitis in WT and GPR65 KO AOM/DSS mice. GPR65 KO AOM/DSS mice have elevated inflammation parameters compared to WT AOM/DSS mice. Macroscopic colitis indicators include (A) body weight loss, (B) spleen weight, (C) spleen/body weight ratio, and (D) mesenteric lymph node (MLN) expansion. Data are presented as the mean ± SEM (WT N=21 and GPR65 KO N=21) and statistical significance was determined using the unpaired *t*-test and the Mann-Whitney test between WT and GPR65 KO groups (*$P < 0.05$, **$P < 0.01$).