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Supporting Information
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**Microbial metabolites, short-chain fatty acids, restrain tissue bacterial load,
chronic inflammation, and associated cancer in the colon of mice**

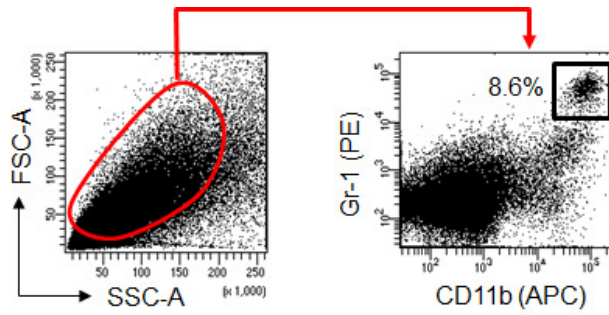
Supporting information for:

Gut microbial metabolites, short-chain fatty acids, restrain tissue bacterial load, chronic inflammation, and associated cancer in the colon of mice

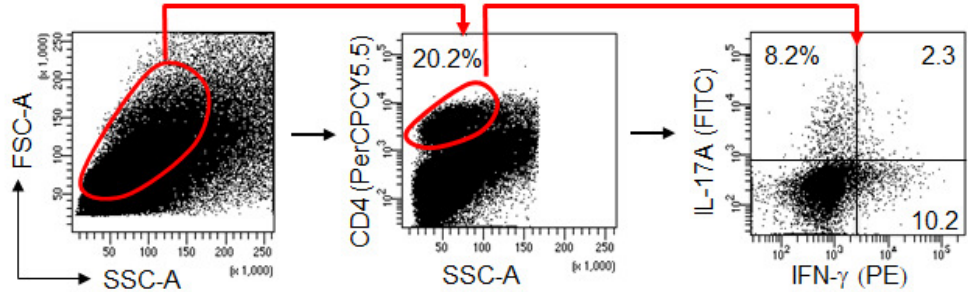
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Supporting Figure 1-5

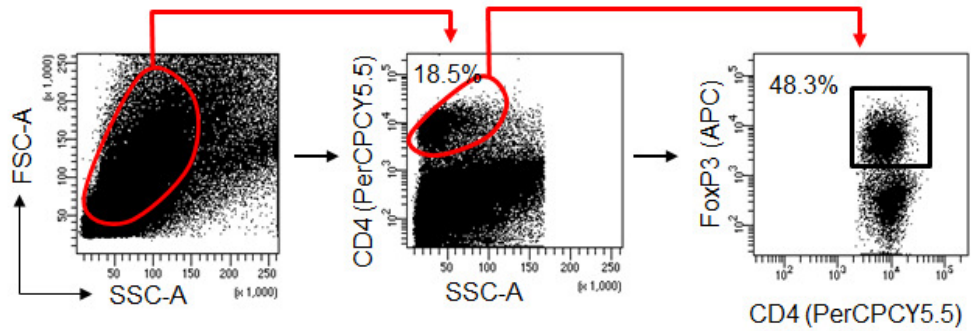
Neutrophils



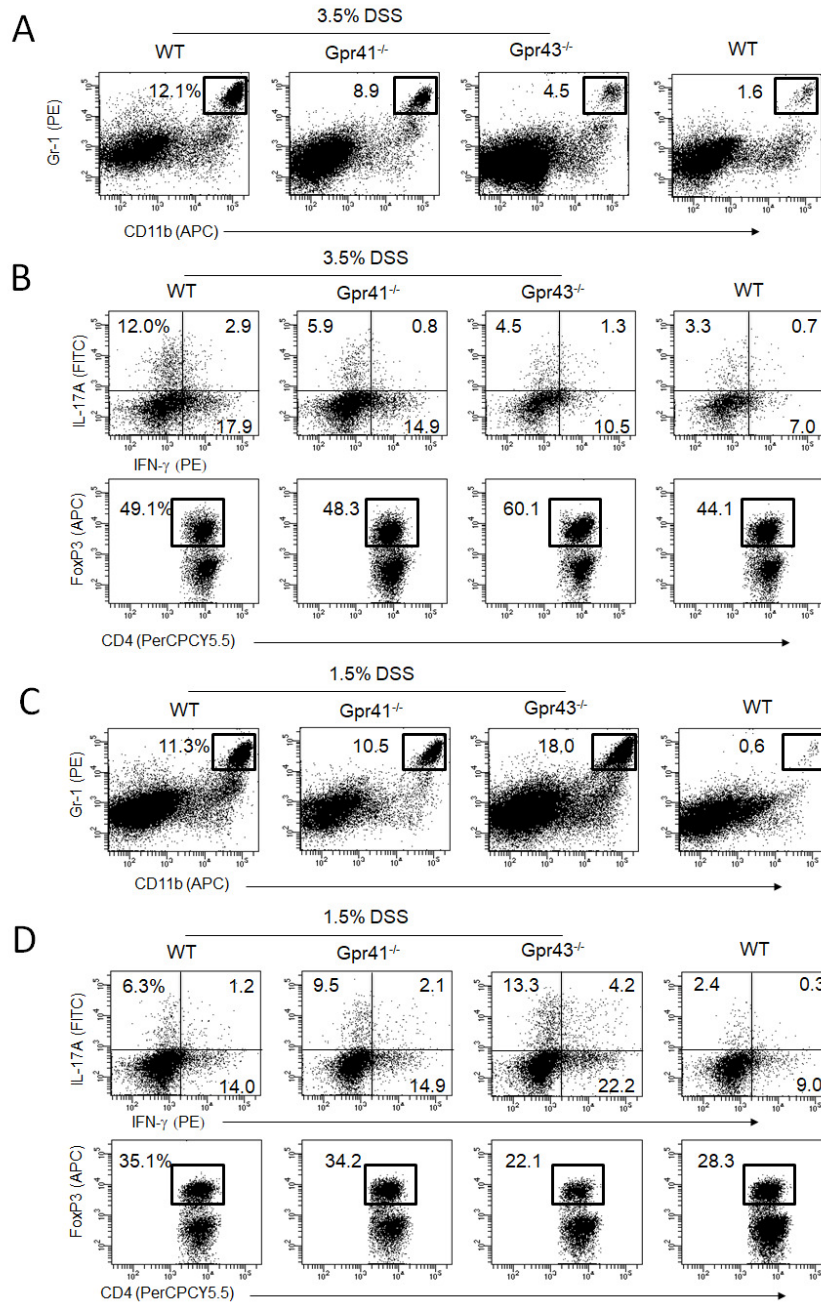
Th1/Th17



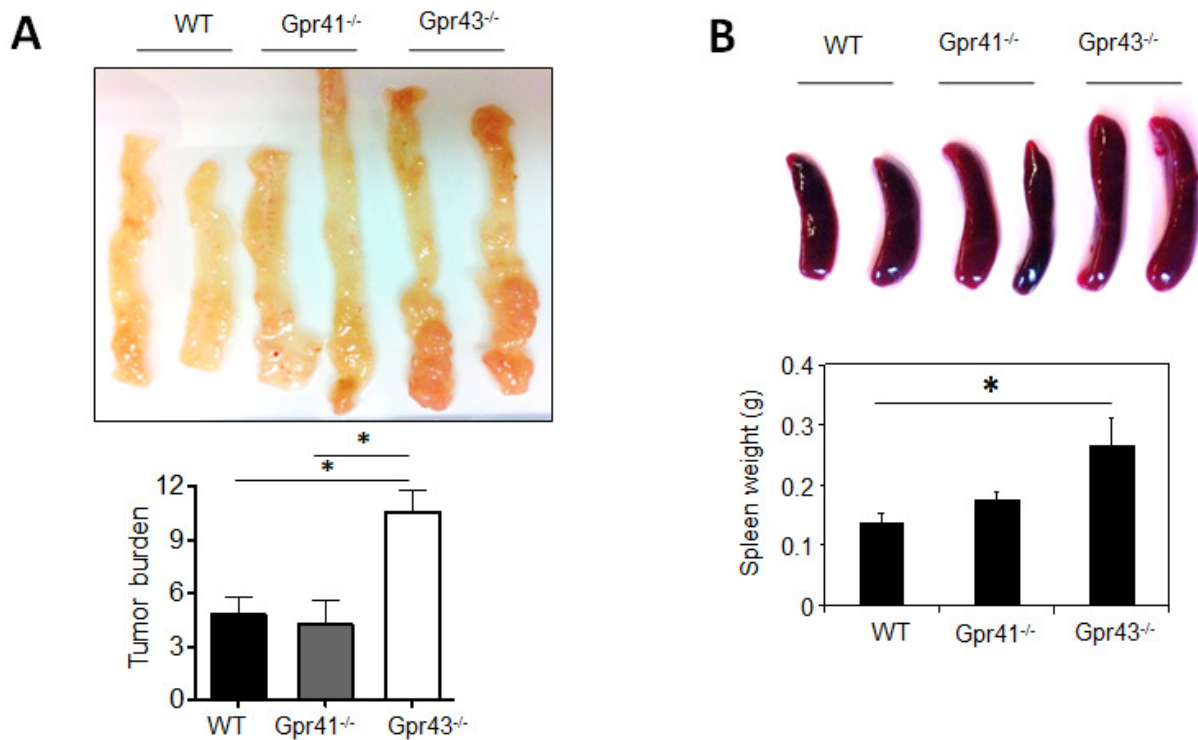
FoxP3⁺ T cells



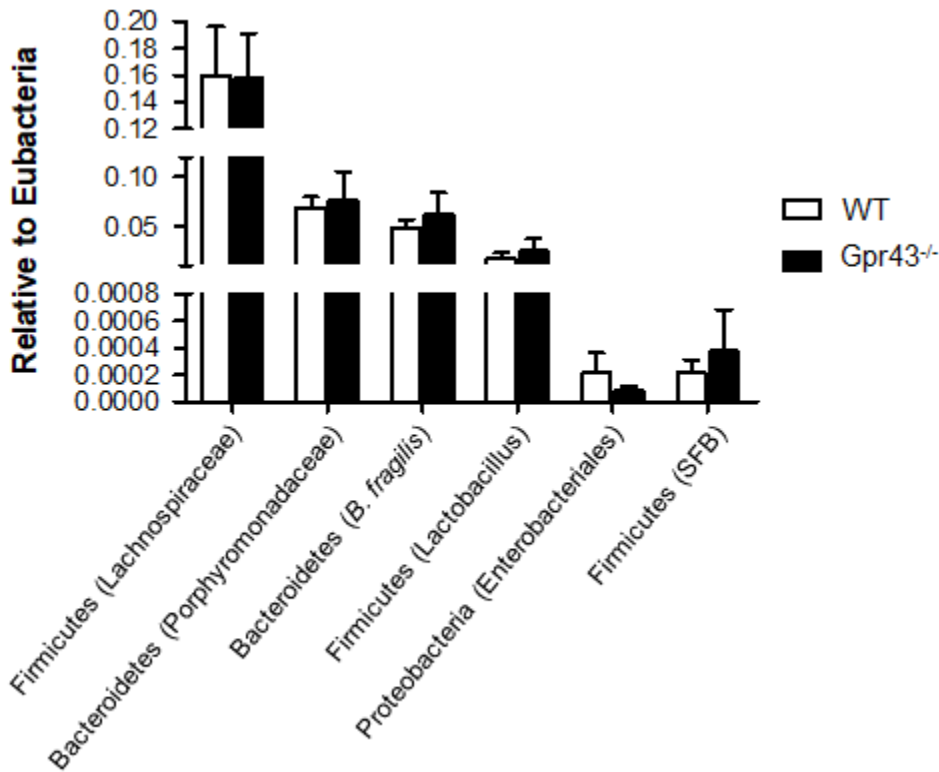
Supporting Fig. 1. Gating strategies to identify neutrophils and T helper cell subsets, such as Th1, Th17 and FoxP3⁺ T cells, in *Gpr43*^{-/-} and *Gpr41*^{-/-} mice. Colon lamina propria cells were stained with indicated antibodies. PE, Phycoerythrin; APC, Allophycocyanin; FITC, Fluorescein.



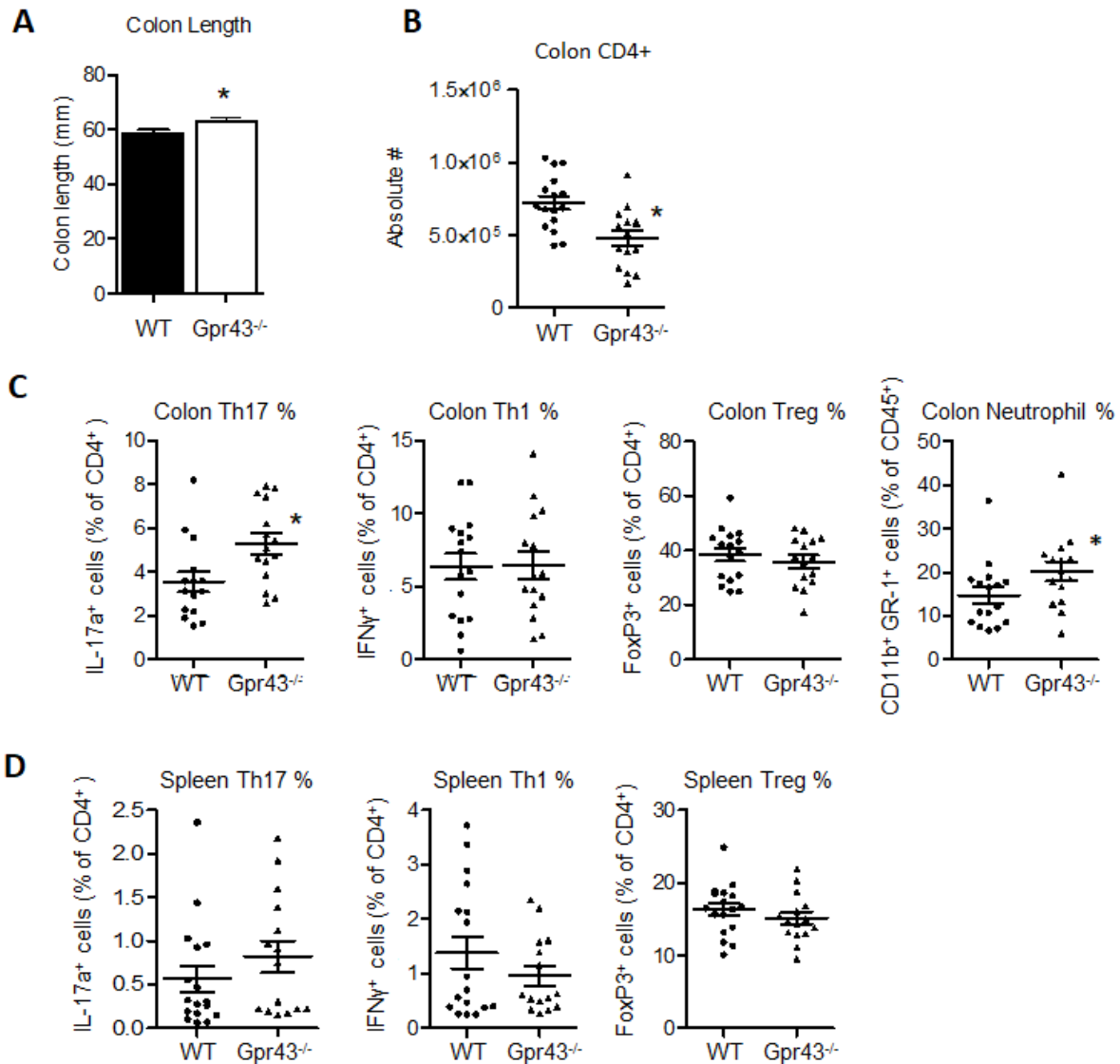
Supporting Fig. 2. Representative flow cytometry dot plots showing the frequencies of neutrophils and T helper cell subsets, such as Th1, Th17 and FoxP3⁺ T cells, in the colon lamina propria of *Gpr43*^{-/-} and *Gpr41*^{-/-} mice FOLLOING acute and chronic DSS treatments. (A) Neutrophils after acute DSS treatment. (B) Th1, Th17 and FoxP3⁺ T cells after acute DSS treatment. (C) Neutrophils after chronic DSS treatment. (D) Th1, Th17 and FoxP3⁺ T cells after chronic DSS treatment. Please see Supporting Figure 2 for gating information.



Supporting Fig. 3. AOM/DSS-induced colon cancer in *Gpr43*^{-/-} and *Gpr41*^{-/-} mice. (A) Tumor formation in the colon. (B) Enlargement of spleen following AOM/DSS treatment. Mice were treated with AOM (10 mg/Kg of body weight, i.p.) and 3 cycles of 1.5% DSS in drinking water and sacrificed at day 70 post AOM injection. Representative and pooled data (mean ± SEM, n=6-9) from 2 independent experiments are shown. *Significant differences ($P < 0.05$, one-way ANOVA followed by Tukey's multiple comparison test).



Supporting Fig. 4. Profiles of selected luminal bacterial groups in the cecum of WT and *Gpr43*^{-/-} mice after co-housing experiments. Mice were co-housed for 1-2 weeks before and during the induction of AOM/DSS-induced colon cancer. Alternatively, WT and *Gpr43*^{-/-} mice were housed in cages inoculated with mixed soiled bedding from WT and *Gpr43*^{-/-} mice (20% old mixed and 80% new bedding; changed every 3-4 days). Cecum contents were frozen at -80 °C and DNA was extracted using the FastSpin DNA for Soil kit. qPCR was performed as described previously [13, 54] using primers for indicated bacterial groups. qPCR was performed for a common Eubacteria 16S sequence using either a QuantStudio 3 or an Applied Biosystems 7300 (ThermoFisher Scientific; Waltham, Massachusetts). No significant differences between WT and *Gpr43*^{-/-} mice by Mann Whitney U-test were found after cohousing. SFB, segmented filamentous bacteria.



Supporting Fig. 5. T cell response during an early stage of AOM/DSS-induced colon cancer development in co-housed GPR43-deficient mice. (A) Colon length of co-housed WT versus *Gpr43*^{-/-} mice at 16-17 days post AOM injection. (B) Numbers of colon CD4⁺ T cells. (C) Frequencies of effector T cells, FoxP3⁺ Tregs and neutrophils in the colon of co-housed mice. (D) Frequencies of effector T cells and FoxP3⁺ Tregs in the spleen of co-housed mice. For methods to equalize microbiota, please see the legend for Figure 4. For all panels, mice were treated with AOM (10 mg/Kg of body weight, i.p.) and 3 cycles of 1% DSS in drinking water and sacrificed at day 16-17 post AOM injection. Representative and pooled data (mean \pm SEM, n=8-12) from 3 independent experiments are shown. *Significant differences from WT mice ($P < 0.05$, Mann Whitney U test). Please see Supporting Figure 2 for flow cytometry gating information.