

Supporting Information

for *Adv. Sci.*, DOI 10.1002/adv.202306297

Gastrointestinal Dysmotility Predisposes to Colitis through Regulation of Gut Microbial Composition and Linoleic Acid Metabolism

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Table S1. The baseline characteristics of patients with chronic constipation (CC), Crohn's diseases (CD), ulcerative colitis (UC), colorectal cancer (CRC) and healthy controls (HD).

	HD ^{#1} (n=30)	CC (n=30)	CD (n=32)	UC (n=25)	CRC ^{#2} (n=30)
Age (years)	48.62 ± 14.36	47.07 ± 17.16	33.89 ± 11.37	51.04 ± 14.72	70.40 ± 11.06
Male/female (No.)	16/14	17/13	25/7	18/7	20/10
Disease duration (CD) (months)	N/A	N/A	60.11 ± 15.56	62.08 ± 13.84	N/A
Disease location (CD) ^{#3}					
L1	N/A	N/A	7	4	N/A
L2	N/A	N/A	13	21	N/A
L3	N/A	N/A	12	0	N/A
L4	N/A	N/A	0	0	N/A
Disease location (CRC)					
Ascending colon	N/A	N/A	N/A	N/A	7
Transverse colon	N/A	N/A	N/A	N/A	1
Descending colon	N/A	N/A	N/A	N/A	0
Sigmoid colon	N/A	N/A	N/A	N/A	11
Rectum	N/A	N/A	N/A	N/A	11

Stool and tissue samples were collected in healthy controls, patients with CC, CD, and UC.

^{#1} Only stool sample collected, used as healthy controls in gut microbiota analysis.

^{#2} Only adjacent normal tissue collected, used as normal tissue control in staining and QPCR assays.

^{#3} According to the Montreal classification system.

Table S2. Primers for QPCR and probes for FISH assay.

Primers for QPCR			
Genes	Species	Forward primer	Reverse Primer
<i>IL-1α</i>	Human	CCGTGAGTTTCCCAGAAGAA	ACTGCCCAAGATGAAGACCA
<i>IL-1β</i>	Human	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTTCGTAGCTGGA
<i>IL-8</i>	Human	TTTGCCAAGGAGTGCTAAAGA	AACCTCTGCACCCAGTTTTC
<i>TNF-α</i>	Human	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
<i>GAPDH</i>	Human	CGGAGTCAACGGATTGGTC	GACAAGCTTCCCGTTCTCAG
<i>Tnf-α</i>	Mouse	GAGGTTGACTTCTCCTGGTAT	AGTGACAAGCCTGTAGCCC
<i>Il-1β</i>	Mouse	AAACCGCTTTTCCATCTTCTTCT	CGGCACACCCACCCTG
<i>Il-6</i>	Mouse	GAAGTAGGGAAGGCCGTG	CTGCAAGAGACTTCCATCCAGTT
<i>Il-17</i>	Mouse	GCTGAGCTTTGAGGGATGAT	CAGGGAGAGCTTCATCTGTGT
<i>Claudin-1</i>	Mouse	AGGTCTGGCGACATTAGTGG	CGTGGTGTGGGTAAAGAGT
<i>Claudin-2</i>	Mouse	TCTACGAGGGACTGTGGATG	TCAGATTACAGCAAGGAGTCG
<i>JAM-A</i>	Mouse	TCTCTTCACGCTATGATCCTGG	TTTGATGGACTCGTTCTCGGG
<i>Occludin</i>	Mouse	TTGAAAGTCCACCTCCTTACAGA	CCGGATAAAAAGAGTACGCTGG
<i>ZO-1</i>	Mouse	GCCGCTAAGAGCACAGCAA	TCCCCACTCTGAAAATGAGGA
<i>Gapdh</i>	Mouse	CCTGTTGCTGTAGCCGTATTCA	CCAGGTTGTCTCCTGCGACTT
<i>Bacteria</i>		TCCTACGGGAGGCAGCAGT	GGACTACCAGGGTATCTATCCTGTT
<i>Lactobacillus</i>		TGGAACAGRTGCTAATACCG	GTCCATTGTGGAAGATTCCC
<i>L. animalis</i>		CTTGCACTCACCGATAAAGAG	GTCCATTGTGGAAGATTCCC
<i>L. johnsonii</i>		GAGCTTGCCTAGATGATTTTAG	GTCCATTGTGGAAGATTCCC
<i>A. muciniphila</i>		AAGGTGGGGACTCTGGCGAG	CTTGCGGTTGGCTTCAGATAC
Probes for FISH			
Name	Species	Sequence	Modification
Lacb0722	<i>Lactobacillus (genus)</i>	YCACCGCTACACATGRAGTTCCACT	5'FAM
Muc-1437	<i>A. muciniphila</i>	CCTTGCGGTTGGCTTCAGAT	5'Cy3

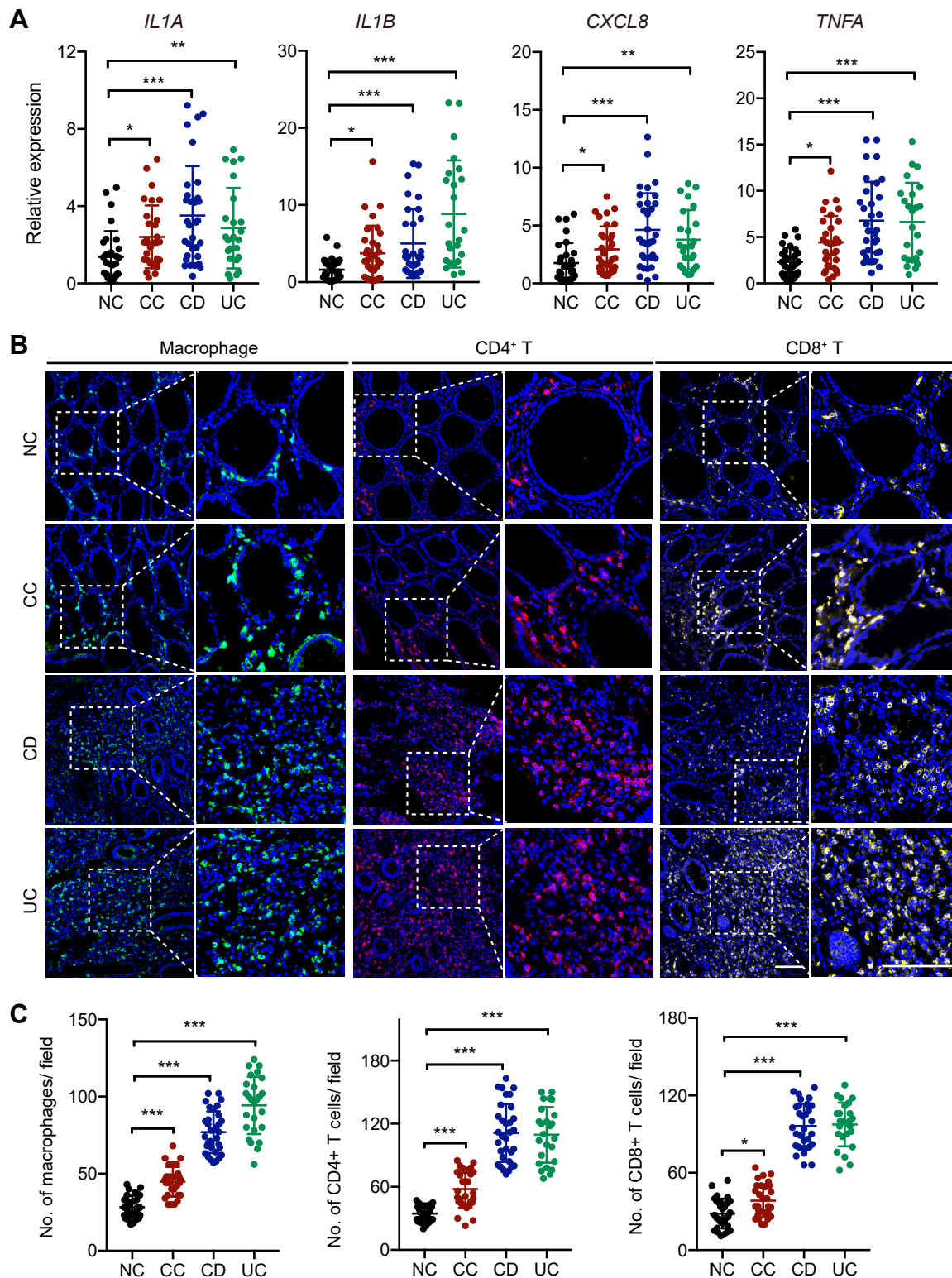


Figure S1. Colonic mucosa from patients with chronic constipation displayed increased mucosal inflammation. A) QPCR analysis of inflammatory gene expression in colonic tissues from patients with chronic constipation (CC) (n=30), inflamed region from patients with Crohn's disease (CD) (n=32), inflamed region from patients with ulcerative colitis (UC) (n=25), and normal adjacent tissue from patients with colorectal cancer served as normal control (NC) (n=30). B) Immunofluorescence staining of macrophages, CD4⁺ T and CD8⁺ T cells in tissues displayed in A. Scale bars, 100 μ m. C) Quantification of macrophages, CD4⁺ T and CD8⁺ T cells per field in B. In A and C, data represent mean $\pm$ SD; *P < 0.05, **P < 0.01, ***P < 0.001 by one-way ANOVA with Dunnett's post hoc test.

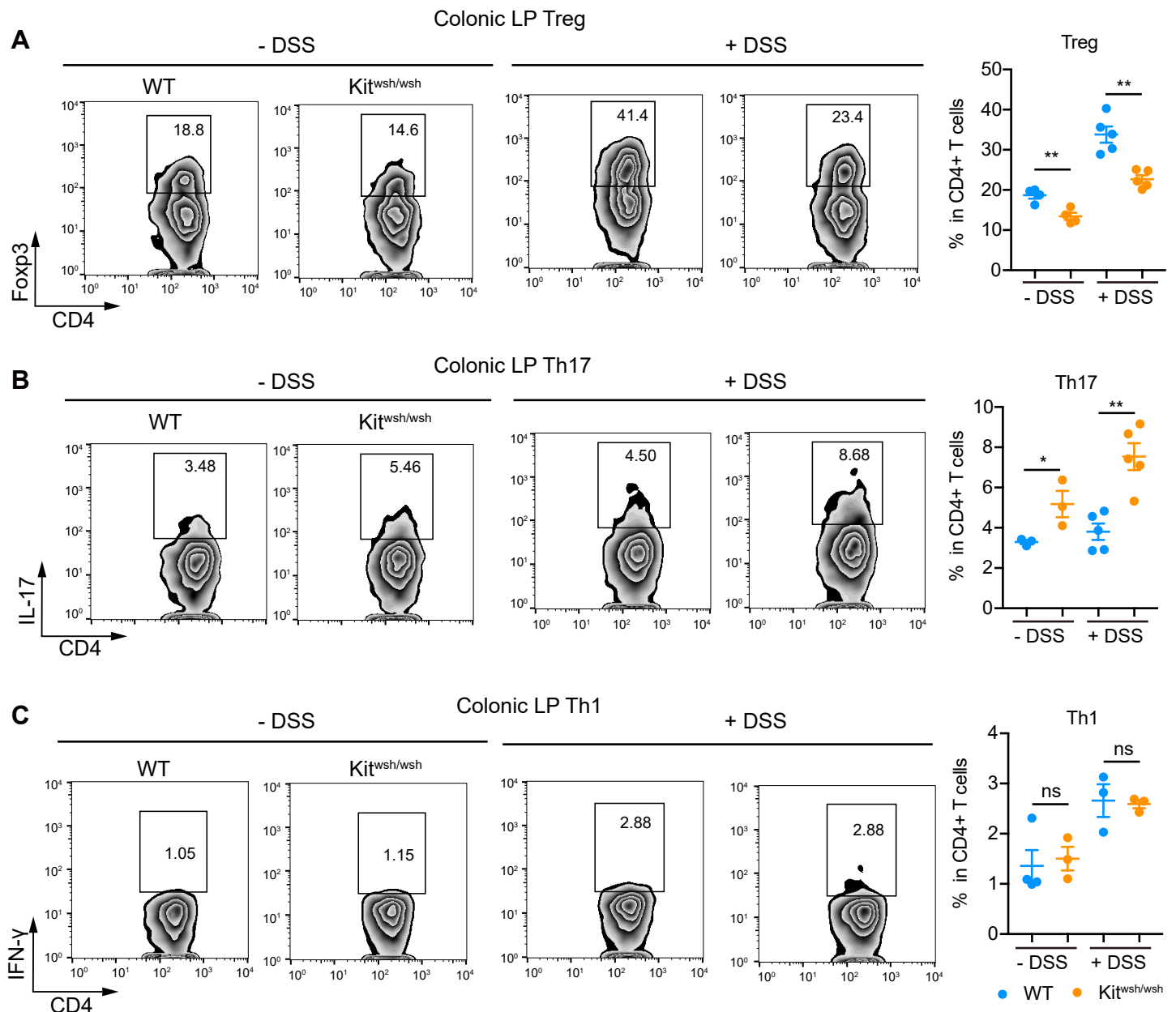


Figure S2. DSS treated *Kit^{wsh/wsh}* mice displayed altered infiltration of Treg and Th17. The abundance of Treg (A), Th17 (B), Th1 (C) cells in colonic lamina propria (LP) were analyzed by flow cytometry. Data represent mean $\pm$ SEM; ns, not significant, * $P < 0.05$, ** $P < 0.01$ by unpaired Student's *t* test.

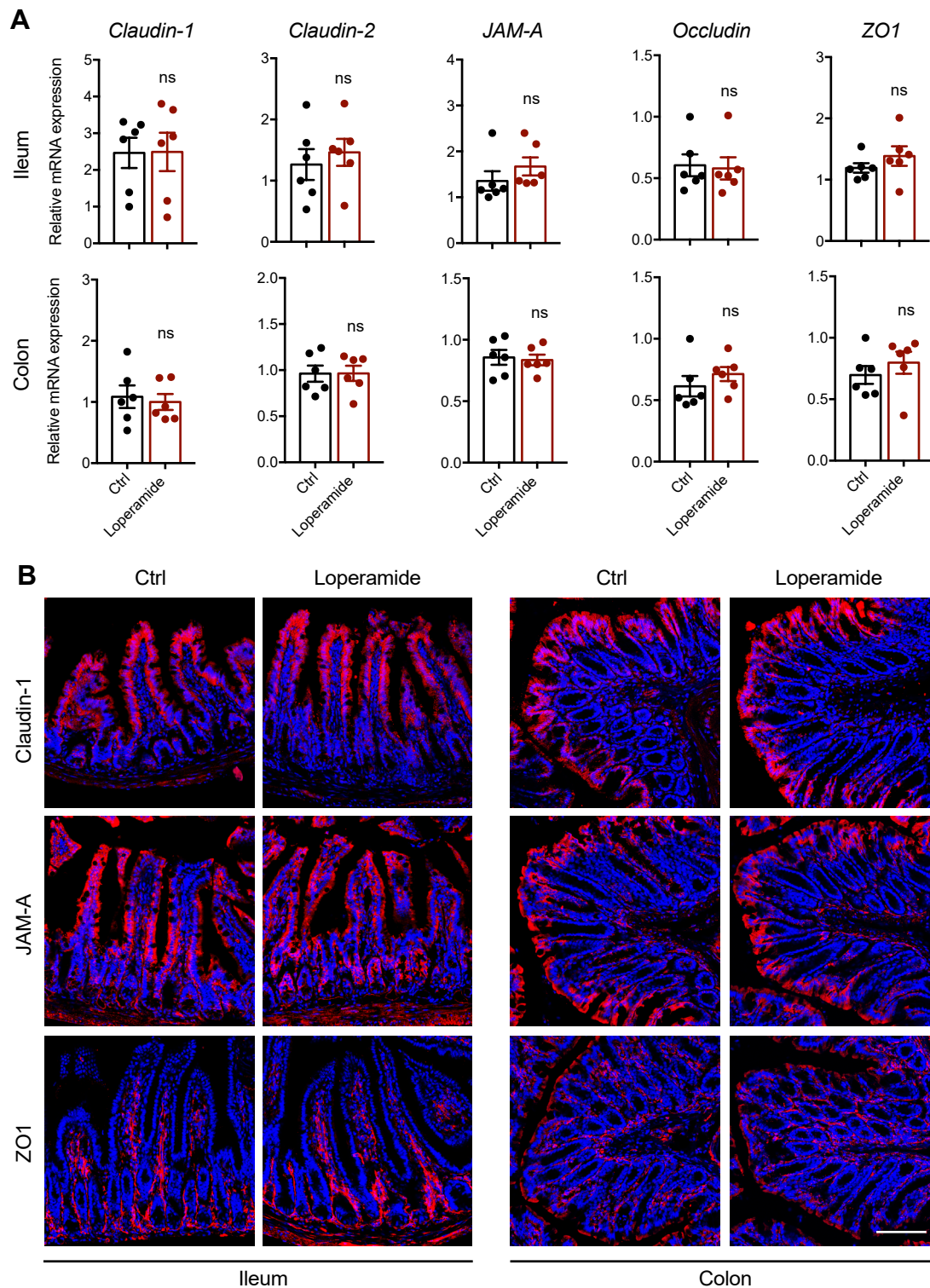


Figure S3. Loperamide treatment did not alter the expression of tight junction proteins in intestinal and colonic tissues. WT mice (n=6/group) were gavaged with 10 mg/kg body weight (b.w.) loperamide every day for 7 days. The expression of tight junction proteins including *claudin-1*, *claudin-2*, *JAM-A*, *occludin*, and *ZO-1* was examined by QPCR (A) and immunofluorescence staining (B). Scale bar, 100 μ m. Data represent means $\pm$ SEM; ns, not significant by two-sided Student *t* test.

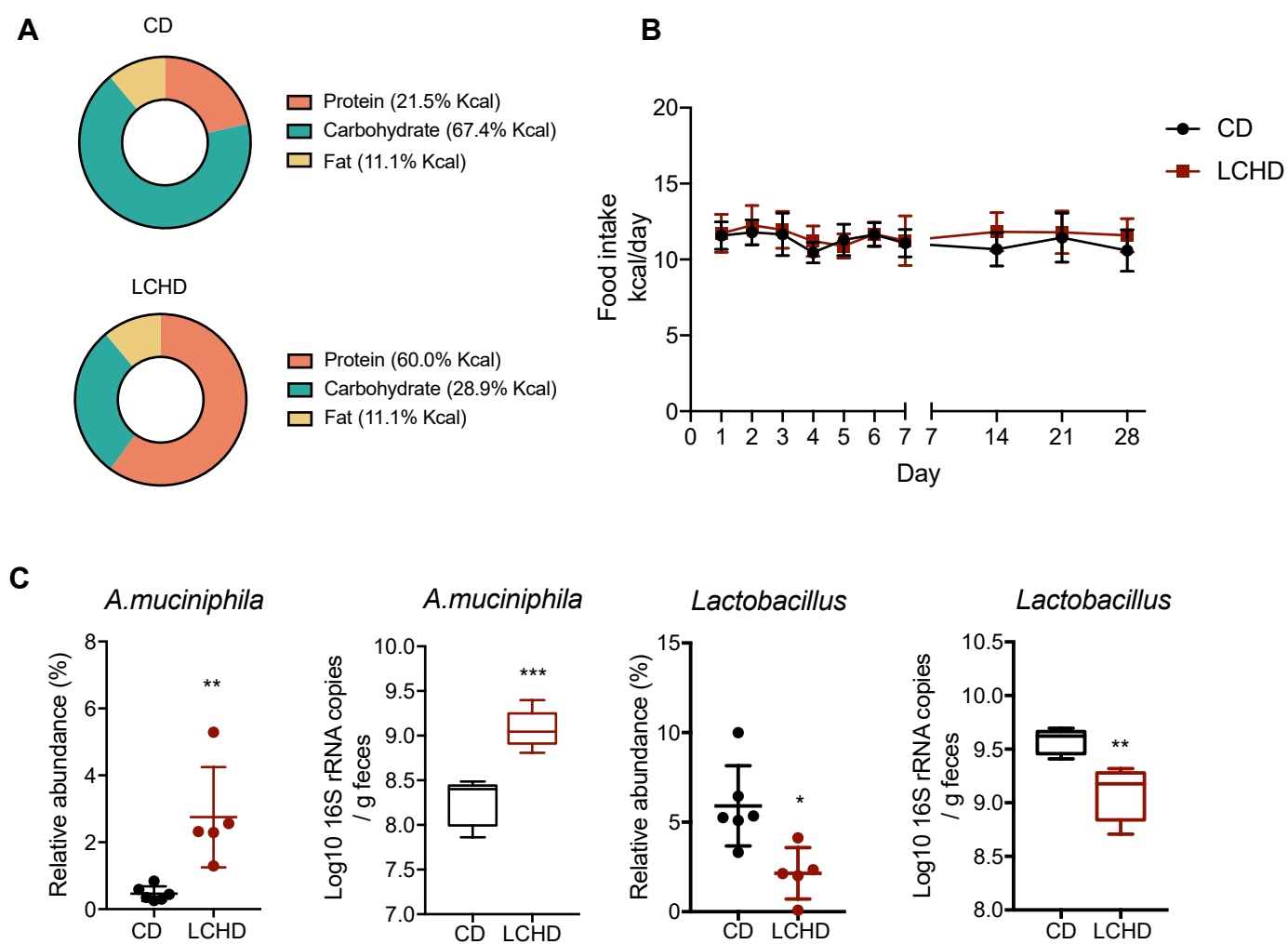


Figure S4. Mice fed with low-carbohydrate and high-protein diet displayed an elevated abundance of *A. muciniphila*, while decreased abundance of *Lactobacillus*. A) Composition of the control diet (CD) and the low-carbohydrate and high-protein diet (LCHD) represented in percent calories. B) Food intake of mice fed with CD or LCHD. C) QPCR analysis of the abundance of *A. muciniphila* and *Lactobacillus* in fecal sample from mice fed with CD or LCHD for 4 weeks. Data represent mean $\pm$ SD; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, not significant by two-sided Student *t* test.

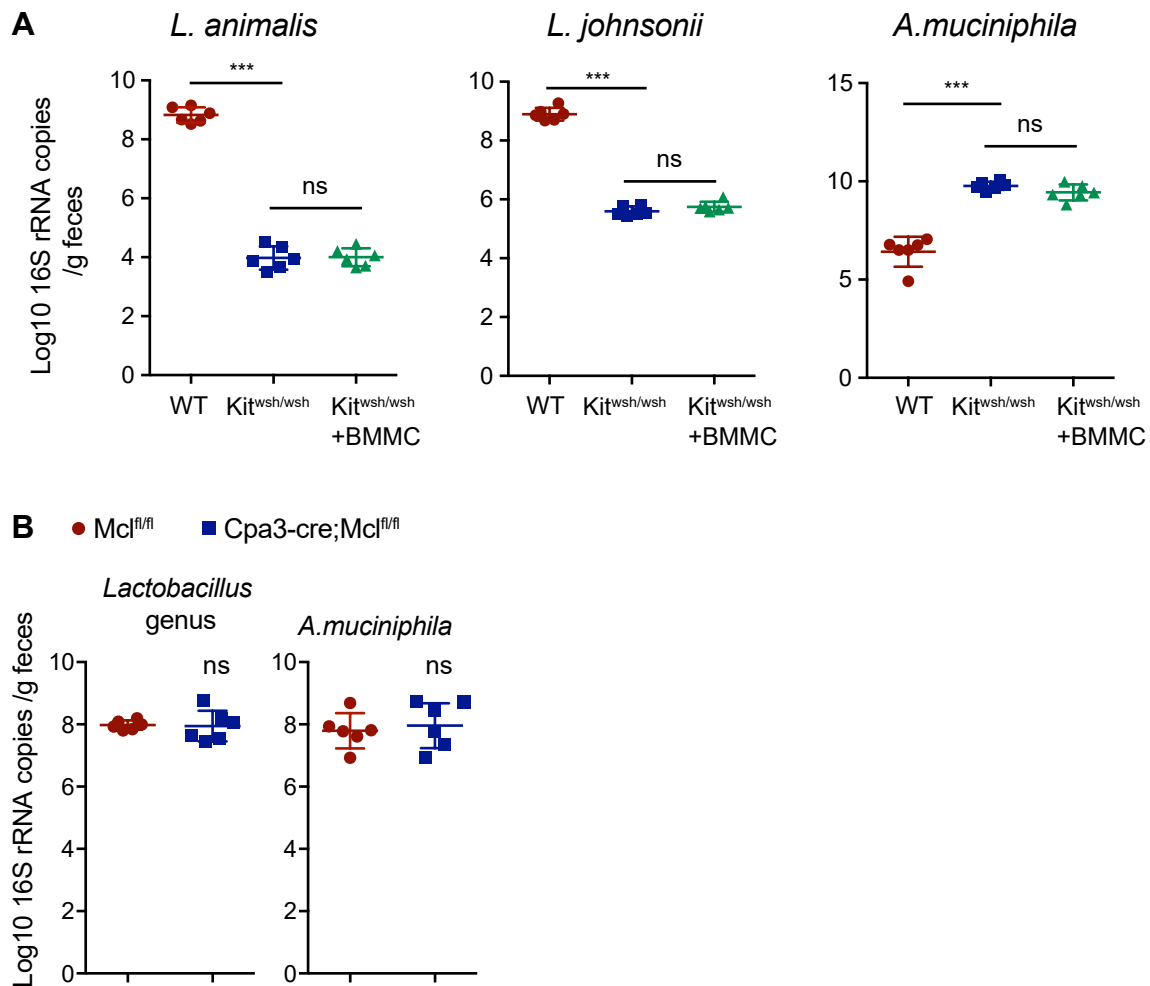


Figure S5. GI dysmotility was responsible for the altered gut microbiota in $Kit^{wsh/wsh}$ mice, rather than mast cell. A) QPCR analysis of the absolute abundance of *L. animalis*, *L. johnsonii* and *A. muciniphila* in feces of WT, $Kit^{wsh/wsh}$ and $Kit^{wsh/wsh}$ mice reconstituted with bone marrow derived mast cells (BMMC) (n=6/group). B) QPCR analysis of the absolute abundance of *Lactobacillus* genus and *A. muciniphila* in feces of $Mcl^{fl/fl}$ and $Cpa3\text{-cre}; Mcl^{fl/fl}$ mice (n=6/group). Data represent means $\pm$ SEM; ns, not significant, ***P < 0.001 by one-way ANOVA with Dunnett's post hoc test (A) and unpaired Student's *t* test (B).

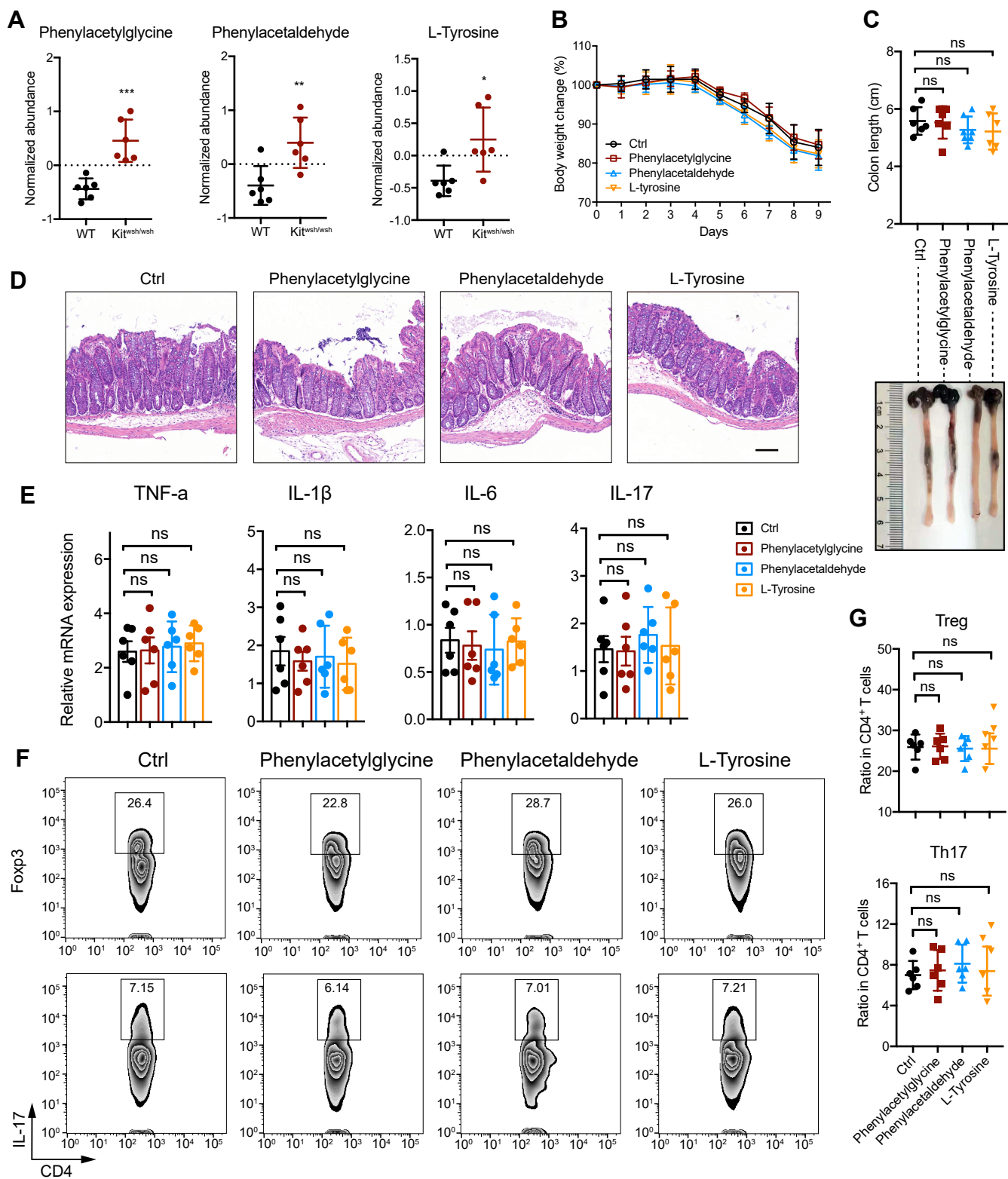


Figure S6. Phenylalanine metabolism did not significantly alter susceptibility to colitis. A) The normalized abundance of metabolites enriched in phenylalanine metabolism which includes phenylacetyl-glycine, phenylacetaldehyde, and L-Tyrosine in WT and *Kit^{wsh/wsh}* mice. B-G) WT mice (n=6/group) were treated with phenylacetyl-glycine, phenylacetaldehyde, and L-Tyrosine in drinking water at a concentration of 200 mg/kg per day for 2 weeks, followed by a 2.5% DSS treatment for 7 days and water 2 days. The body weight changes (B), colon lengths (C), representative images of H&E-stained colon sections (D), proinflammatory cytokine expression (E) and infiltration of Treg and Th17 in colonic tissue (F, G) were monitored or analyzed. Scale bar (D), 100 μ m. In A-C, E, and G, data represent mean $\pm$ SD; * P < 0.05, ** P < 0.01, *** P < 0.001, ns, not significant by one-way ANOVA with Dunnett's post hoc test.

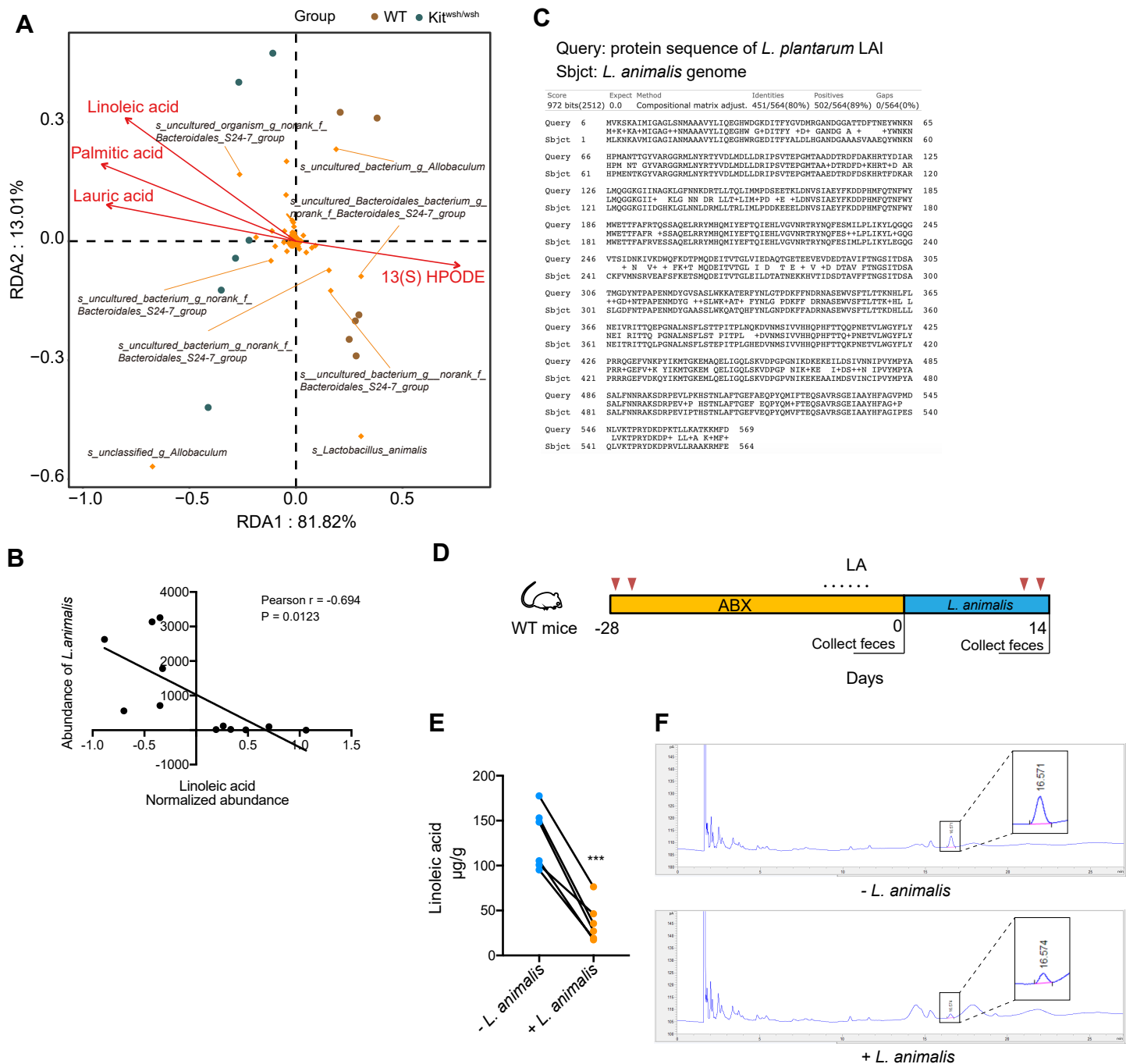


Figure S7. *L. animalis* has the ability to convert linoleic acid. A) Redundancy analysis (RDA) of species that differ between Kit^{wsh/wsh} and WT mice, and long chain fatty acids. Only top 20 different bacterial species were labeled. B) Correlation analysis of *L. animalis* and fecal levels of linoleic acid. C) BLAST search of the LAI homologous enzyme in *L. animalis* genome. D-F) WT mice were treated with a cocktail of antibiotics (ABX) for 4 weeks, and then gavaged with *L. animalis* for 2 weeks. Linoleic acid (LA) was administered during the whole process. Fecal samples were collected and the amount of LA was quantified by gas chromatography before and after *L. animalis* gavage. The quantification data was shown in E and the representative chromatogram was shown in F. *** $P < 0.001$ by paired two-sided Student *t* test.

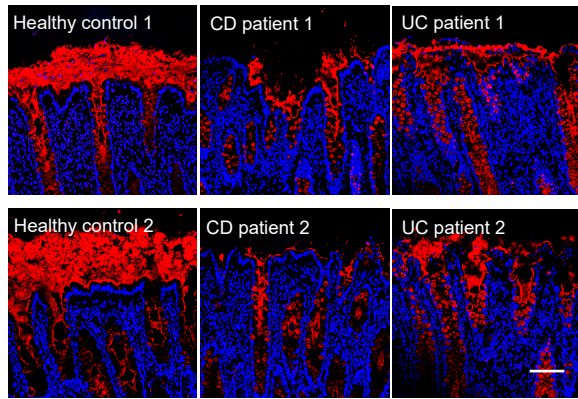


Figure S8. Representative images of mucus layer stained using anti-Mucin2 antibody in adjacent normal tissue from CRC patients and inflamed colon tissue from CD and UC patients. Scale bar, 100 μ m.