SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURES

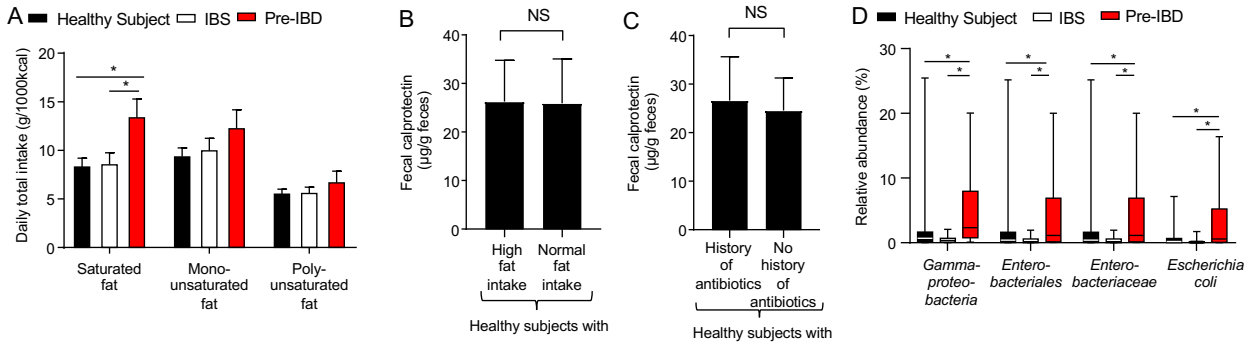

Figure S1: Related to Figure 1. Pre-IBD is associated with elevated consumption of saturated fatty acids and changes in the fecal microbial composition compared to IBS patients or healthy subjects.

(A) Daily fat intake was estimated based on the 1day recall diet diary and energy adjustment was performed by calculating nutrient density and expressed as intake / 1000kcal. (B-C) Levels of fecal calprotectin were measured in healthy subjects and correlated with reported fat intake (B) and history of recent antibiotic usage (C). (D) Relative abundance of indicated bacteria was determined by microbiota profiling of DNA isolated from feces. (A-C) Bars represent mean ± standard deviation. (D) The boxes in the Whisker blot represent the 1st to 3rd quartile ranges and the horizontal lines represent the median value. The bars in the whisker blot represent the minimum and maximum value in each group. *, $P<0.05$; NS, $P>0.05$.

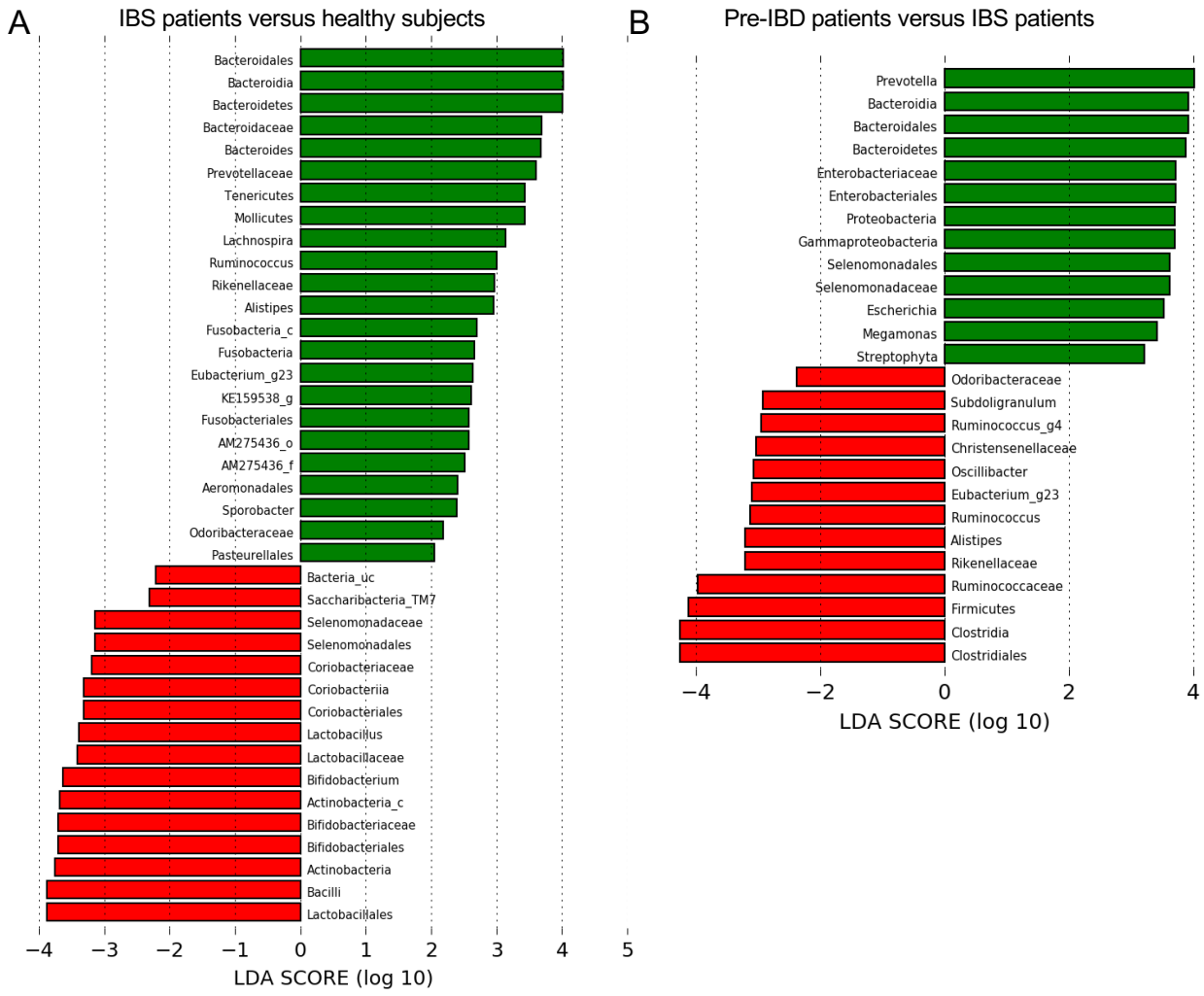

Figure S2: Related to Figure 1G. Comparison of the fecal microbiota profile of pre-IBD and IBS patients.

Microbiota profiling was performed using DNA isolated from feces of patients and differences in taxa composition were compared using the LDA Effect size algorithm. (A) Comparison of healthy subjects with IBS patients. Green, elevated in IBS patients compared to healthy subjects; red, reduced in IBS patients compared to healthy subjects. (B) Comparison of IBS patients with pre-IBD patients. Green, elevated in pre-IBD compared to IBS; Red, reduced in pre-IBD compared to IBS. An effect size >2 (on a log scale) were considered significant.

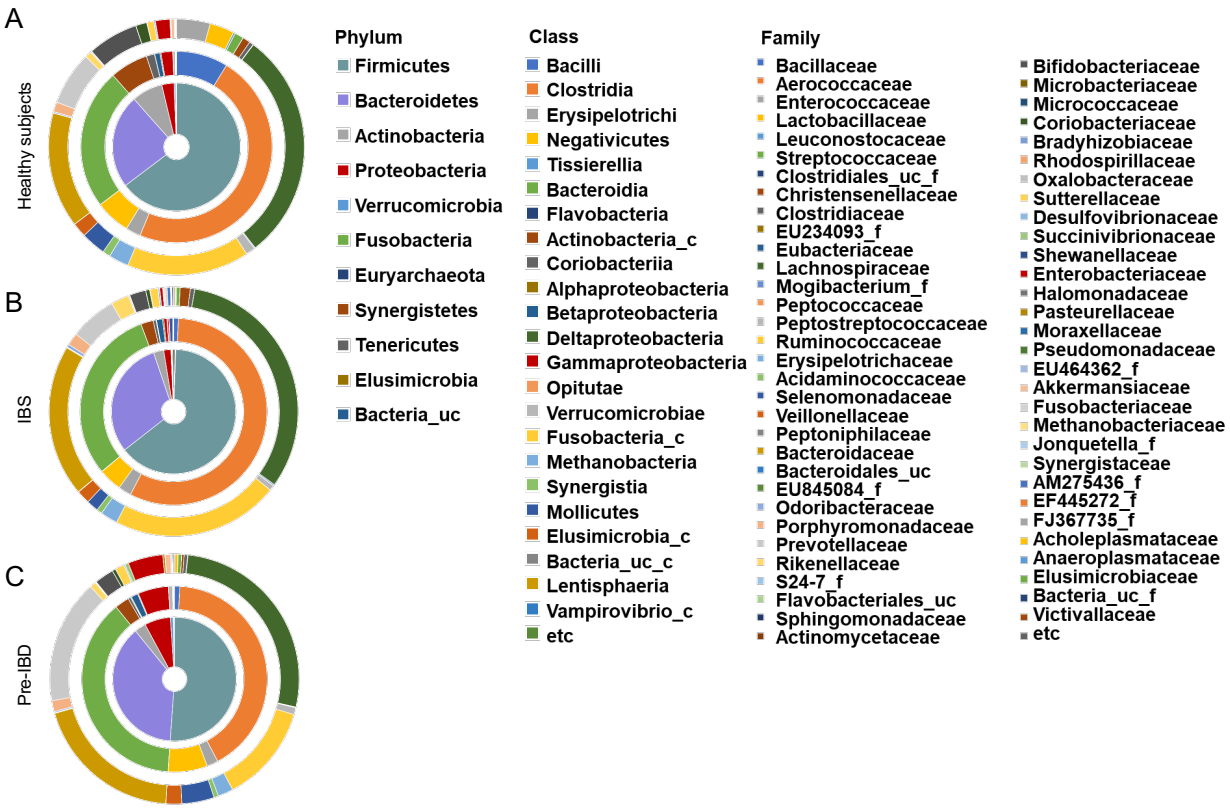

Figure S3: Related to Figure 1. Average relative abundances of phylogenic groupings at the phylum (inner circle), class (middle circle) and the family level (outer circle) determined by fecal microbiota profiling.

The abundance of phyla was determined using fecal microbiota profiling. Circles show the average relative abundance of taxa for healthy subjects (A), IBS patients (B) and pre-IBD patients (C).

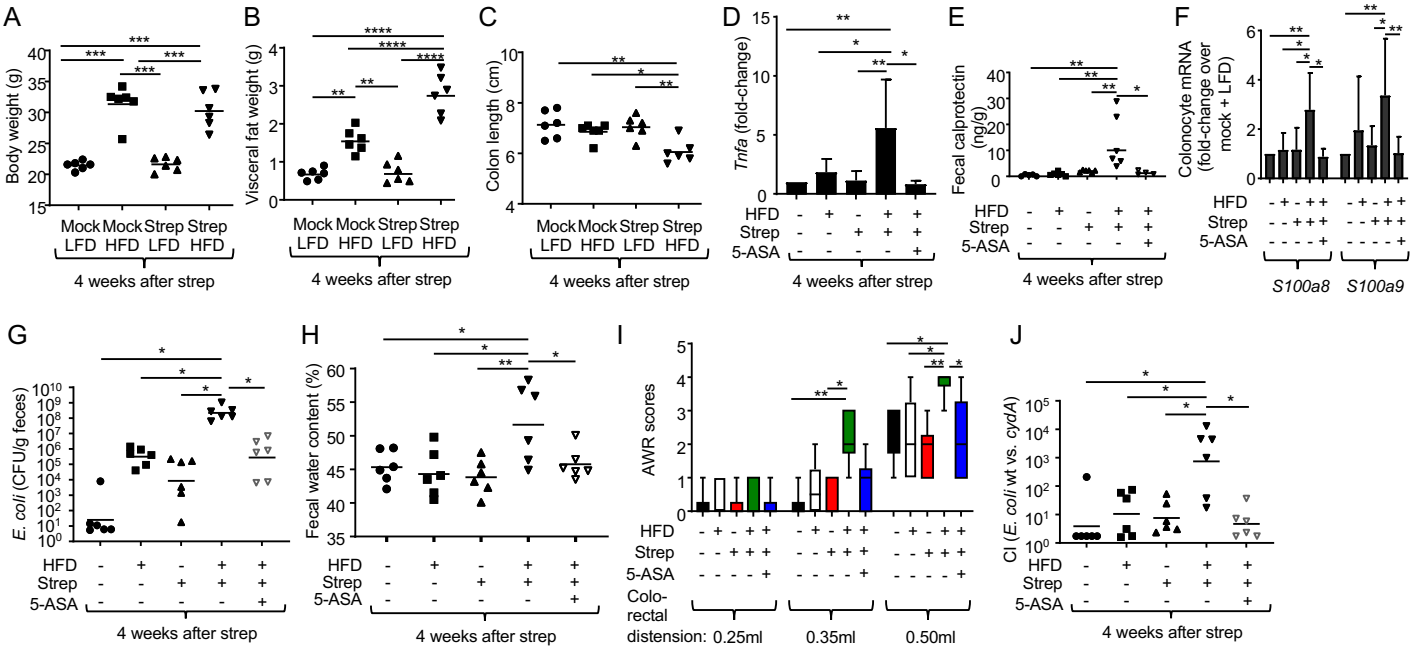

Figure S4: Related to Figure 2. Exposure of female mice to risk factors induces signs of pre-IBD. Groups of female mice (*N* = 6) were reared on a high-fat diet (HFD, 45% fat) or on a low-fat diet (LFD, 10% fat) and were mock treated or treated with a single dose of streptomycin (Strep) four weeks before necropsy. Mouse body weight (A) and visceral fat weight (B) were determined during necropsy. (C) Colon length was determined during necropsy. (D-J) Chow was supplemented with 5-ASA (5-ASA: +) or did not contain supplementation (5-ASA: -). (D) Colonocytes were isolated from the colonic mucosa for RNA isolation and transcript levels of *Tnfa* (were determined by quantitative real-time PCR. (E) Fecal calprotectin levels were determined by ELISA. (F) Transcript levels of the indicated genes were determined by quantitative real-time PCR in RNA isolated from preparations of the colonic epithelium. (G) Mice were inoculated with *E.coli* one day after streptomycin treatment and numbers in the feces determined 4 weeks later. (H) The water contents of mouse feces were measured at the indicated time point. (I) Abdominal withdrawal reflex (AWR) scores at different levels of colorectal

distension were measured during week four after antibiotic treatment. (J) Mice were inoculated with a 1:1 mixture of *E.coli* wild type (wt) and an isogenic respiration-deficient (*cydA*) mutant one day after streptomycin treatment and the competitive index (CI) in colon contents was determined 4 weeks after streptomycin treatment. (A-C, E, G-H and J) Symbols represent data from individual animals and black bars represent geometric mean. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.

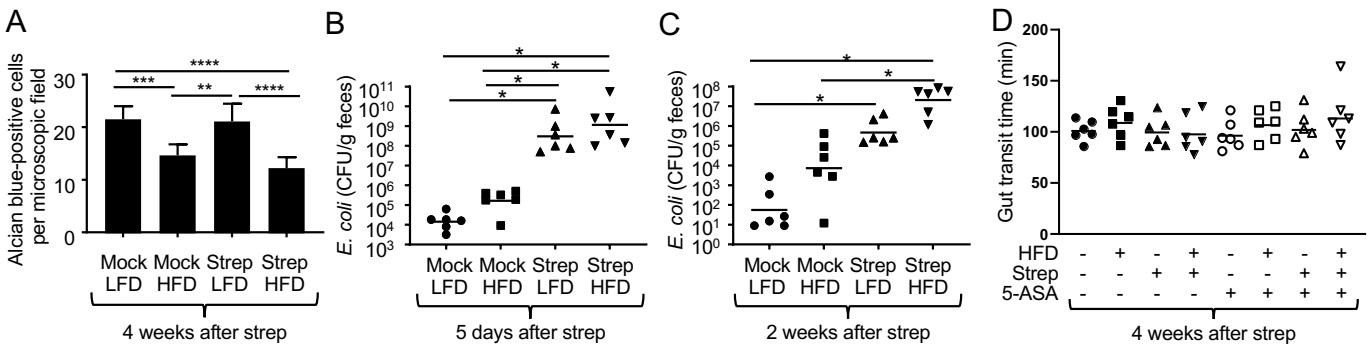

Figure S5: Related to Figure 2. Signs of disease produced by exposing male mice to risk factors for pre-IBD

Groups of male mice (*N* = 6) were reared on a high-fat diet (HFD: +) or on a low-fat diet (HFD: -) and were mock treated (Strep: -) or treated with a single dose of streptomycin (Strep: +) four weeks before necropsy. (A) Quantification of Alcian blue staining in blinded sections from the colon. Bars represent geometric mean± standard deviation. (B-C) Mice were inoculated with *E.coli* one day after streptomycin treatment and numbers in the feces determined 5 days (B) and 2 weeks (C) after streptomycin treatment. (D) Chow was supplemented with 5-ASA (5-ASA: +) or did not contain supplementation (5-ASA: -). The gut transition time was measured at the indicated time point. (B-D) Dots represent data from individual animals and bars represent geometric mean. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.

SUPPLEMENTARY TABLES

Table S1: Related to Figure 1. Basic & clinical characteristics of the subjects among the healthy subjects, IBS and pre-IBD groups.

Characteristics	Healthy subjects (n=43)	IBS (n = 30)	Pre-IBD (n = 19)	P value
Age (year)	40.28±12.38	44.00±14.47	43.26±14.80	0.48
Gender [n (%)]				0.42
Male	13 (30.2)	5 (16.7)	5 (26.3)	
Female	30 (69.8)	25 (83.3)	14 (73.7)	
Regular exercise [n (%)]	17 (40.5)	11 (36.7)	12 (63.2)	0.16
Regular alcohol Drinking [n (%)]	15 (34.9)	8 (26.7)	6 (31.6)	0.76
Current smoking [n (%)]	0(0)	0 (0)	0 (0)	NS
Antibiotics use (<1 year) [n (%)]	9 (20.9)	4 (13.3)	9 (46.7)	0.02
BMI (kg/m^2)	22.20±3.16	22.19±3.16	22.31±3.26	0.99
Body fat (%)	28.57±5.81	27.85±6.64	29.48±7.04	0.69
Lean body mass (gram)	26.19±9.40	25.78±4.89	25.03±2.59	0.37
SBP (mmHg)	110 (100-125)	117 (103-130)	117 (110-122)	0.19
DBP (mmHg)	67 (62-74)	75 (63-85)	77 (71-83)	0.32

Data are shown as the mean ± standard deviation for normally distributed data, median (25-75 percentile) for non-normally distributed data or the number (percentage) for categorical data . Abbreviation: IBS, Irritable bowel syndrome; IBD, inflammatory bowel disease; BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; NS, no statistically significant difference.

Table S2: Related to Figure 1. Nutritional characteristics among the healthy subjects, IBS and pre-IBD groups.

Nutritional variables	Healthy subjects (n=43)	IBS (n = 30)	Pre-IBD (n = 19)	P value
Total calorie intake (kcal/day)	1866 (1216-2787)	1864 (1067-3326)	2369 (1575-3387)	0.06
Carbohydrate (g/1000kcal/day)	154.44±6.73	147.15±10.33	128.57±13.61	0.20
Fiber (g/1000kcal)	11.72±0.82	11.20±0.85	9.75±1.12	0.37
Protein (g/1000kcal/day)	36.88±1.56	38.90±3.05	42.77±3.20	0.29
Fat (g/1000kcal/day)	25.65±2.18	28.88±3.22	39.69±4.94	0.01
Saturated fat (g/1000kcal/day)	8.45±0.89	8.65±1.24	13.51±1.93	0.03
Mono-unsaturated fat (g/1000kcal/day)	9.48±0.93	10.10±1.29	12.37±1.95	0.12
Poly-unsaturated fat (g/1000kcal/day)	5.64±0.50	5.70±0.65	6.81±1.20	0.10

Data are shown as the mean ± standard deviation for normally distributed data or median (25-75 percentile) for non-normally distributed data.

Energy adjustment was performed by calculating nutrient density and expressed as intake / 1000kcal.

Abbreviation: IBS, Irritable bowel syndrome ; IBD, Inflammatory bowel disease.

Table S3: Related to Figure 1. Comparison of IBS-related characteristics between the IBS and pre-IBD

Characteristics	IBS (n = 30)	Pre-IBD (n = 19)	P values[a]
BSSS (scores)			
Frequency	15 (11-19)	19 (14-27)	0.85
Distress	18 (12-23)	25 (16-25)	0.04
Disability	15 (9-19)	24 (19-29)	0.08
IBS-QOL (scores)	67 (56-80)	79 (51-89)	0.03
PI-IBS (%)	8 (26.7)	6 (31.6)	0.48

Data are shown as the mean ± standard deviation for normally distributed data, median (25-75 percentile) for non-normally distributed data or the number (percentage) for categorical data.

PI-IBS is defined as a group of IBS patients with a history of acute gastroenteritis before the onset of IBS symptoms.

Abbreviation: BSSS, Bowel symptom severity scales; IBS-QOL, Irritable bowel syndrome-quality of life questionnaire; PI-IBS, postinfectious-IBS

P values were analyzed with a Student's t-test for normally distributed data and with a Mann-Whitney U test for non-normally distributed data and chi square test for categorigal data.

Table S4: Related to Figure 1. Odds ratio and 95% confidence intervals for the prevalence of pre-IBD according to fat intake and antibiotics use

	OR (95% CI)	
	Model 1[a]	Model 2[b]
Fat intake (g/1000kcal/day[c])		
1st tercile	1.00	1.00
2nd tercile	1.73 (0.38-4.99)	1.86 (0.39-4.91)
3rd tercile	3.95 (1.2-8.10)	2.83 (1.09-4.67)
Antibiotics use (<1year)		
None	1.00	1.00
≥1	4.15 (1.41-12.26)	3.90 (1.26-12.26)

[a]Model 1; Unadjusted

[b]Model 2; Adjusted for age, gender, BMI, regular alcohol drinking and regular exercise

[c] Energy adjustment was performed by calculating nutrient density and expressed as intake / 1000kcal.

Fat intake: 1st tercile, <18.98 g/1000kcal/day ;2nd tercile, 18.98-40.23 g/1000kcal/day; 3rd tercile, >40.23 g/1000kcal/day.

Abbreviation: OR (95% CI), Odds ratio (95% confidence interval).

Table S5: Related to Figure 1. Interaction between fat intake and antibiotics use on pre-IBD and likelihood ratio test statistics to measure goodness of fit

Interaction test		-2 log likelihood statistics		
Wald Chi-square statistics	p-value	Reduced model[a]	Full model[b]	p-value
4.47	0.03	188.12	177.91	0.03

[a] Model without interaction term between fat intake and antibiotics use

[b] Model with interaction term between fat intake and antibiotics use

Table S6: Related to Figure 1. Odds ratio and 95% confidence intervals for the prevalence of pre-IBD relative to fat intake and antibiotics use

	OR (95% CI)	
	Model 1[a]	Model 2[b]
No high-fat diet/ no antibiotics	1.00	1.00
High-fat diet[c]/ No antibiotics	2.53 (0.65-4.86)	2.82 (0.67-4.85)
No high fat diet/ Antibiotics use	2.58 (0.53-6.63)	3.53 (0.61-7.46)
High fat diet/ Antibiotics use	7.20 (3.25-9.11)	8.63 (3.09-12.95)

[a]Model 1; Unadjusted

[b]Model 2; Adjusted for age, gender, BMI, regular alcohol drinking and regular exercise

[c]High fat diet is defined as a fat intake>40.23 g/1000kcal/day (3rd tercile group) and antibiotics use is defined as a history of antibiotics use ≥ 1 within one year.

Abbreviation: OR (95% CI), Odds ratio (95% confidence interval).

Table S7: Related to STAR Methods. Primers for real-time PCR of mouse genes

	Forward sequence (5' – 3')	Reverse sequence (5'-3')
Actb	TGTCCACCTTCCAGCAGATGT	AGCTCAGTAACAGTCCGCCTAGA
Tnfa	AGCCAGGAGGGAGAACAGAAAC	CCAGTGAGTGAAAGGGACAGAACC
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Nos2	TTGGGTCTTGTTCACTCCACGG	CCTCTTTCAGGTCACTTTGGTAGG
Ccl2	AGGTGTCCCAAAGAAGCTGTA	ATGTCTGGACCCATTCCTTCT
Angptl4	AGGCTGGACAGTTAATTCAGAGG	ATGCTATGCTATGCACCTTCTCCAGAC
Pgc1a	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Sirt3	TCA CAA CCC CAA GCC CTT TT	GAT GGC ATG GAC TGT GGT CA
Ndufs1	AGCCGGCAGCCATCATGTTA	AGTTACTTGCTGCTGTGCCA
Ndufv1	TTTCTCGGCGGGTTGGTTC	GGTTGGTAAAGATCCGGTCTTC
Sdha	GGAACACTCCAAAAACAGACCT	CCACCACTGGGTATTGAGTAGAA

Uqcr	TGCCGAGGCCTCAGACACAG	TCCAAGGCATAAGAATAAGGTTT
Cox5a	GCCGCTGTCTGTTCCATTC	GCATCAATGTCTGGCTTGTTGAA
Atp5g1	AGTTGGTGTGGCTGGATCA	GCTGCTTGAGAGATGGGTTC
16S rRNA gene *Clostridia*	ACTCCTACGGGAGGCAGC	GCTTCTTTAGTCAGGTACCGTCAT
S100a8	CCGTCTTCAAGACATCGTTTGA	GTAGAGGGCATGGTGATTTCCT
S100a9	ATACTCTAGGAAGGAAGGACACC	TCCATGATGTCATTTATGAGGGC

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Table S8: Related to STAR Methods. Criteria used for histological grading of intestinal inflammation and damage to the epithelial surface.

Histologic feature	Score	Criteria
Infiltration by polynuclear cells	0	rare neutrophils within lamina propria
	1	occasional multifocal increased frequency of neutrophils within lamina propria
	2	diffuse infiltration of the lamina propria by low numbers of neutrophils
	3	diffuse infiltration of the submucosa and lamina propria by moderate numbers of neutrophils
	4	diffuse transmural infiltration of the submucosa, lamina propria, and muscle layers by high numbers of neutrophils with accumulation of luminal suppurative exudate
Infiltration by mononuclear cells	0	no detectable increase in mononuclear infiltration of the lamina propria
	1	mild multifocal expansion of the lamina propria by low numbers of infiltrating lymphocytes and plasma cells

	2	mild regional expansion of the lamina propria by moderate numbers of lymphocytes and plasma cells
	3	moderate regional to diffuse expansion of the lamina propria and epithelia by infiltrating lymphocytes and plasma cells
	4	marked diffuse expansion of the lamina propria and epithelia by infiltrating lymphocytes and plasma cells with distortion of the mucosal architecture
Submucosal edema	0	no observable edema
	1	mild multifocal perivascular expansion of the tunica adventitia
	2	moderate multifocal expansion of the submucosa with lymphatic dilation
	3	severe circumferential expansion of the submucosa with marked lymphatic dilation
Luminal exudate accumulation	0	no observable accumulation of suppurative exudate within the lumen
	1	mild accumulation of exudate
	2	moderate accumulation exudate
	3	severe accumulation of exudate

Superficial epithelial injury	0	no observable disruption to the brush border
	1	occasional multifocal brush border attenuation with increased apoptosis
	2	frequent to confluent brush border attenuation with abundant apoptosis
	3	marked brush border attenuation with loss of epithelial adhesion
	4	surface epithelial cell loss leading to erosive lesions of the superficial mucosa
	5	frequent erosive lesions with confluence leading to ulceration

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