Supplementary Methods

All human and animal studies were approved by the University of Michigan IRB and IACUC, respectively, in accordance with NIH guidelines.

Patients

Sixteen patients with IBS-D (9 females and 7 males), aged from 28 to 60 years, were recruited from the University of Michigan, Division of Gastroenterology and Hepatology outpatient clinic and primary care clinics. All patients with IBS-D met the Rome IV criteria and were symptomatic at the time of the study. Patients had to be free of other diseases, such as diabetes, celiac disease, and cardiovascular disorders. Biopsies of colonic tissue excluded microscopic colitis. None of study patients with IBS-D used nonsteroidal anti-inflammatory drugs, corticosteroids, histamine antagonists, or mast cell stabilizers in the last 6 months before the study. Sixteen HC (8 females and 8 males, aged 36 to 65 years) served as controls. Consents were obtained from all study subjects and the study was approved by the University of Michigan Human Research Protection Program. Healthy controls were asymptomatic subjects undergoing colonoscopy for colorectal cancer screening. All subjects had a colonoscopy, and 6 mucosal biopsies were obtained from the descending colon of each subject. One biopsy was used for immunohistochemistry to exclude microscopic colitis and 5 biopsies were used for RvD1 extraction.

Stool samples

Stool samples were obtained from five HC (two males and three females; 28 to 60 years) and eight patients with IBS-D (three males and five females; 35 to 65 years). The HC had no history of gastrointestinal diseases. The patients were diagnosed with IBS-D according to Rome IV criteria with Bristol stool form scale (≥6), and more than three bowel movements per day, and had symptoms for at least 2 years. Diarrhea was the dominant gastrointestinal symptom in the IBS-D patients included in this study. None of the patients or controls used psychotropic medications, antibiotics, or probiotics for at least 3 months before fecal sample collection. Stool samples were transported anaerobically to the laboratory and frozen at −80°C.

Fecal supernatant extraction

Fecal samples from IBS-D patients and healthy controls were diluted (1 g fecal sample/5 ml PBS), homogenized on ice, and centrifuged (10,000 *g*, 10 minutes, 4°C). Pellets were discarded, and supernatants were recovered. Bacteria were removed by 0.22-μm syringe filters. Supernatants were kept at -80°C.

Conventionalization of germ-free mice

Diluted stool or bacterial suspension in PBS was gavaged once into 8-12-week-old germ-free mice of either gender. *Lactobacillus rhamnosus GG* (ATCC 53103) and *Proteus mirabilis Hauser* (ATCC 43071) (American Type Culture Collection (ATCC), Manassas, VA) were used. Each mouse was gavaged with 0.15 mL of culture grown aerobically overnight at 37°C (~10^9 CFU grown in Luria-Bertani broth without antibiotics). After 2 weeks, mice visceromotor response to colorectal distention was

assessed. Mice were sacrificed thereafter, and tissue samples were collected. No gender effect was observed for any of the independent variables studied.

Collection of mucosal specimens and assays of supernatants

Human mucosal biopsies and experimental animal colon specimens were rapidly immersed in cryogenic plastic tubes containing ice cold acetone and stored at -80^{o} C. On the day of extraction, the samples were placed on the rotating shaker (300 rpmin) for 4h at room temperature. Liquid acetone fraction was separated from the tissue and the blot dry tissue fraction was lysed for protein measurement. The acetone from liquid fraction was evaporated by blowing a gentle nitrogen gas stream into the tubes. After evaporation of acetone the content of the tube was reconstituted with 1 ml of EIA buffer. ELISA assay of RvD1 (#500380; Cayman Chemical, Ann Arbor, MI, USA) was performed according to the instructions provided by the manufacturer. The values of the measurements were normalized to the protein concentration.

ELSA for cytokine measurement.

The colonic tissues were rapidly immersed in hard plastic tubes containing 1 ml DPBS media and continuously oxygenated (95% O_2/5% CO_2) at 37°C. After a 30-min incubation, the bathing solution was removed, filtrated, and stored at -80°C. At the end of the experiment, biopsies were weighed.

ELISA of IL6 (#583371, Cayman Chemical), TNFα (#500850, Cayman Chemical), and IL1b (#DY401, Tocris) were performed according to the instructions provided by the manufacturer.

Animals

Specific pathogen-free C57BL/6 (WT) mice were purchased from Charles Rivers laboratories (Wilmington, MA, USA), *Tlr4* KO (*C3H/HeJ* (*TLR4* $^{Lps\text{-}d}$; stock number 000659), *Tlr2* KO (B6.129-*Tlr2*^{tm1Kir}/J, stock number 004650), *Tlr4*$^{f/f}$ (stock number 024872), *MyD88*$^{f/f}$ (stock number 008888), *ALox12* KO (B6.129S2-Alox15^{tm1Fun}/J; stock number 02778), *ALox5* KO (B6.129S2-Alox5^{tm1Fun}/J; stock number 004155), ROSA26i*DTR* (*iDTR* or *C57BL/6-Gt(ROSA)26Sor*$^{tm1(HBEGF)Awai}$*/J*; stock number 007900), *Chat-cre* (*B6;129S6-Chattm2(cre)Lowl/J*; stock number 006410), *EGFP*$^{f/f}$ (B6.129(Cg)-Gt(ROSA)26Sor$^{tm4(ACTB\text{-}tdTomato,\text{-}EGFP)Luo}$/J; stock number 007676) mice were purchased from the Jackson Laboratories (Bar Harbor, ME, USA) and housed in the Animal Facility at the University of Michigan. Germ-free (C57BL/6 background) mice were obtained from the Germ-Free Animal Facility at the University of Michigan. Mice were maintained in a 12-h light/dark cycle and allowed free access to food and water. Germ-free status was checked weekly by aerobic and anaerobic culture. In all animal strains, 8–12-week-old female and male mice were used for the experiments.

Chemicals

Lyposalysacharides (Lps) from *E Coli* and diphtheria toxin were purchased from Sigma-Aldrich (St Luis, MO, USA), LTA (InvivoGen, San Diego, CA, USA). RVD1, resolvin E1, NFkB inhibitory peptide (SN50) and MK886 were purchased from Cayman chemical (Ann Arbor MI, USA), WRW4 and U0126 from Tocris (Minneapolis, MN, USA).

VMR to Colorectal Distension and Recording

The animals were anesthetized with a mixture of xylazine and ketamine (13 and 87 mg/kg body weight, respectively). Electromyography electrodes were implanted into the external oblique pelvic muscles 2 days before the beginning of the experimental procedures. Fecal supernatant from HCs or patients with IBS-D (0.15 mL) were administrated intracolonically, 30 mm proximal to the rectum, 4 h before the experiment. During the experiment, the strain gauge was connected by way of a shielded cable to a chart recorder to monitor the number of abdominal muscle contractions. A Latex balloon 0.7 mm (Edwards Lifesciences, Irvine, CA, USA) lubricated with glycerol was inserted into the colon through the rectum 30 minutes before CRD was initiated. At set times after the sample administration a series of rectal distensions were performed to generate a pressure–response curve. The responses were considered stable if there was <20% variability between 2 consecutive trials of colorectal distention (CRD). The results of electromyography were amplified and filtered (A-M Systems, Sequim, WA, USA), digitized, and integrated by using the SPIKE2/CED 1401 data-acquisition interface (Cambridge Electronic Design Limited, Cambridge, UK). Spike bursts higher than 0.3 mV were regarded as significant and were, therefore, used to estimate the response. The increase in the area under the curve of electromyography amplitude during CRD over the baseline period before CRD was recorded as the response

Lps removal

Pierce High-Capacity Endotoxin Removal Spin Columns (Thermo Fisher Scientific, Waltham, MA, USA) were used according to the manufacturers' instructions to remove Lps from fecal supernatant. The spin columns containing the resin were centrifuged at 500 x g for 1 minute to remove the storage buffer, washed with 0.2N NaOH in 95% ethanol, 2M NaCl and endotoxin-free water, and then equilibrated three times with endotoxin-free phosphate buffered saline. Filtered fecal supernatants were added to the columns and incubated at room temperature with gentle end-over-end mixing for an hour. The supernatants were collected from the columns after centrifuged at 500 x g for 1 minute.

Histology and immunohistochemistry

Colon tissues from mice were fixed with 4% paraformaldehyde for 24h and saturated with 20% sucrose solution, then embedded in Tissue-TekII OCT (Thermo Fisher Scientific). Tissue samples were cut into 7 μm thick slides and blocked with 5% donkey serum and 0.1% Triton X-100 for an hour, then incubated with rabbit Dclk1 polyclonal antibody (1:200, #109029; Abcam, Cambridge, UK) and goat Chat polyclonal antibody (1:200, #PA1-9027, Thermo Fisher Scientific) overnight. Cy3 of FITC conjugated anti-rabbit and anti-goat antibodies (1:500; Jackson Immuno Research Labs, West Grove, PA, USA) were applied for 2 hours at room temperature. Tissue samples were finally mounted in ProLong Diamond Antifade Mountant with DAPI (Thermo Fisher Scientific) and then observed under a fluorescence microscope (Olympus SZX16, Bartlett, TN, USA).

Colon organoid preparation

Human colonic biopsy and mouse colonic tissue was washed with PBS containing 1% penicillin-streptomycin (Thermo Fisher Scientific). After 3-5 washes, tissue was incubated in cold PBS containing 20 mM EDTA with DTT (Sigma-Aldrich, St Louis, MO, USA) for 5 minutes to remove mucus and in cold PBS containing 20 mM EDTA for 30 minutes for further digestion. Crypts were released by rhythmic shaking in PBS containing 0.1% BSA for 1–2 minutes. Isolated crypts were centrifuged at 300 g for 2 minutes. Colonic crypts were collected and plated in 24-well plates in a 5% CO_2 humidified incubator at 37ºC in DMEM supplemented with 10% FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin.

Transfection with silencing RNA in colonoid cultures

siRNAs for *NFkB* p65 (sc-29411), *Erk1* (sc-29038), and *Erk2* (sc-35336) and a siRNA-A unrelated to these genes to be used as the control (sc-37007) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and diluted in Opti-MEM (Thermo Fisher Scientific). The final concentration of siRNA added to the cells was 15 nM. Colonoids were transfected with control siRNA-A, *NFkB* siRNA, or *Erk1* and *Erk2* siRNA in Opti-MEM and Lipofectamine 2000 for 4–6 h. The media were supplemented to a final volume of 2 ml of low glucose DMEM and F12 medium in equal volumes, supplemented with 10% FBS and L-glutamine, without antibiotics. The colonoids were incubated for 72 h. The media were removed and replaced with low glucose serum-free DMEM and F12 medium in equal volumes, supplemented with L-glutamine. The colonoids were stimulated for 24 h with HC-FS or IBS-FS (10 µg/ml).

Reverse transcriptase–PCR studies

Colonic tissue samples were lysed in reverse transcriptase buffer. Total RNA was extracted from cells using TRIzol reagent (Thermo Fisher Scientific). cDNAs were synthesized using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA) using 1 µg of total RNAs; the resultant cDNAs were used for PCR. PCR was performed with a CFX Connect Real-Time PCR Detection System (Bio-Rad) using SYBR Green detection. The PCR conditions were as follows: one cycle at 95°C for 10 minutes, followed by 40 two-temperature cycles at 95°C for 10 seconds and 60°C for 30 seconds. PCR amplifications were performed in a total volume of 20 µl, containing iQSYBR Green supermix (Bio-Rad). GAPDH served as an internal control. Primer sets targeting various mRNAs are listed in supplementary table 1.

Supplementary table 1. Primer sequences

Gene	Sequence	
GPR32 (H)	5’-TCAACTCTGACAATGAGACTGC-3’ 5’-AGGTGCCTATGATTGTCTAAGG-3’	NM_001506
ALOX5AP (H)	5’-GAAATCCTTTGGGCACCTTGG-3’ 5’-TCCATCTTTGCTTCAGGCT-3’	NM-001204406
COTL1 (H)	5’-GGTCCAAGTTTGCCCTCAT-3’ 5’-CCTCCAGCTCCTTCCGAT-3’	NM_021149
ALOX12 (H)	5’-GCTCCAATTCCATTTGCTGAA-3’ 5’-GCTTCGCGTGTTAATTTCCATAG-3’	NM_007440
ALOX5 (H)	5’-CCATCTGCCTGCTATAAGAACC-3’ 5’-CCAGTCGTACTTTGAATCCGT-3’	NM_09662
GAPDH (H)	5’-ACATCGCTCAGACACCATG-3’ 5’-TGTAGTTGAGGTCAATGAAGGG-3’	NM_002046
FPR2 (H)	5’-CAATATGGATTTGCACCCACTG-3’ 5’GTGTAGCCAGCAGACTCATAG-3’	NM_001462
Dclk1 (M)	5’-CACTGACATTACCGACGCTATC-3’ 5’-GGTAACGGAACTTCTCTGGTC-3’	NM_019978
Tlr4 (M)	5’-GAAGCTTGAATCCCTGCATAG-3’ 5’-AGCTCAGATCTATGTTCTTGGTTG-3’	NM_021297
Tlr2 (M)	5’-CAACTTACCGAAACCTCAGACA-3’ 5’-CCAGAAGCATCACATGACAGA-3’	NM_011905
Alox5AP (M)	5’-CAGAACTGCGTAGATGCGTA-3’ 5’-CTCCCAGATAGCCGACAAAG-3’	NM_09663
IL1β (M)	5’-GACCTGTTCTTTGAAGTTGACG-3’ 5’-CTCTTGTTGATGTGCTGCTG-3’	NM_008361
IL6 (M)	5’-AGCCAGAGTCCTTCAGAGA-3’ 5’-TCCTTAGCCACTCCTTCTGT-3’	NM-031168
TNFα (M)	5’-AGACCCTCACACTCAGATCA-3’ TCTTTGAGATCCATGCCGTTG-3’	NM_031693
Chat (M)	5’-CCTTCCTAAGCCCTCTACTGACA-3’ 5’-GACGAAGTTGCCAGATCCC-3’	NM_009891
GAPDH (M)	5’-AATGGTGAAGGTCGGTGTG-3’ 5’-GTGGAGTCATACTGGAACATGTAG-3’	NM-008084