SUPPLEMENTARY MATERIALS AND METHODS

Bacteria Strains

Anaerostipes sp. and *Blautia sp.* strains were isolated from the feces of WT mice used in this study. Two fresh fecal pellets from mice were collected directly into 1 ml of BHI broth containing 5% fetal bovine serum (FBS) and 0.1% cysteine. Pellets were homogenized, and serial dilutions were plated in an anaerobic chamber onto BHI plates (pre-reduced and anaerobically sterilized) supplemented with 5% FBS, 0.1% cysteine and 2 mg/l aztreonam. After anaerobically incubating at 37°C for 72 h, individual colonies were streaked onto new BHI plates for another 48 h incubation. Then a single colony was picked up and grown in the BHI broth for 24 h. Genomic DNA was isolated from the culture, and a 16S rRNA gene segment was amplified using the primers listed in supplementary table S4 and sent for DNA sequencing to identify the taxonomy of each strain by using the Ribosomal Database Project (RDP) classifier (https://rdp.cme.msu.edu/classifier/classifier.jsp).

B. diminuta and *S. paucimobilis* were isolated from the feces of *Ang1*$^{-/-}$ mice by using BHI medium with 2 mg/l vancomycin. The identification procedure was the same as described above except being cultured under aerobic condition. *B. diminuta* and *S. paucimobilis* were maintained aerobically on BHI plates and grown aerobically in BHI medium at 37°C for 12 h.

Experimental Colitis

To generate DSS-induced colitis model, mice were administrated with DSS (2.5% w/vol; molecular weight: 36 to 50 kDa; MP Biomedicals) in drinking water for 1 week, followed by regular drinking water until the end of the study.[1] To establish TNBS-induced colitis model, mice were slowly instilled with 100 μl TNBS (1 volume of 5% w/vol TNBS solution mixed with 1 volume of absolute ethanol; Sigma-Aldrich) into the lumen of colon under anesthesia status.[2] Mice were sacrificed 4 days after TNBS treatment and colonic tissues were collected for further examination. To create a spontaneous colitis model, *Il-10*$^{-/-}$ mice (provided by Dr. Zhijian Cai, Institute of Immunology, Zhejiang University School of Medicine) were directly employed to conduct fecal microbiota transplantation experiment. *Ang1* and *Il-10* double knockout mice were generated by crossing female *Ang1*$^{-/-}$ mice with male *Il-10*$^{-/-}$ mice, and the progenies were intercrossed to generate *Ang1*$^{-/-}$; *Il-10*$^{-/-}$ offsprings. The mice were sacrificed at the 95th day after birth when the phenotype of chronic colitis was evident.

Clinical signs of mouse colitis were evaluated based on the DAI scores that include body weight loss, occult blood and stool consistency.[3] Mice were scored blindly for the colitis experiments. In brief, the weight loss score was determined as follows: 0, no weight loss; 1, loss of 1-5% original weight; 2, loss of 6-10% original weight; 3, loss of 11-20% original weight; 4, loss of >20% original weight. The bleeding score was determined as follows: 0, no blood by using Hemoccult (Beckman Coulter) analysis; 1, positive Hemoccult; 2, visible blood traces in stool; 3, gross rectal bleeding. The stool score was determined as follows: 0,

well-formed pellets; 1, semi-formed stools that did not adhere to the anus; 2, pasty semi-formed stool that adhered to the anus; 3, liquid stools that adhered to the anus.

Co-housing Experiment

3-weeks-old C57BL/6J WT mice were co-housed with age-and-gender-matched WT or *Ang1*$^{-/-}$ mice derived from the same *Ang1*$^{+/-}$ breeding pair (WT (co-WT) or WT (co-*Ang1*$^{-/-}$)). Co-housing was conducted in new cage at a 1:1 ratio for 6 weeks. Fecal samples were collected at the end of co-housing experiment and target bacterial abundance was analyzed by qPCR.

Fecal Microbiota Transplantation

For fecal microbiota transplantation (FMT), C57BL/6J WT or *Il-10*$^{-/-}$ mice were administered with an antibiotic cocktail (1 g/l ampicillin, 0.5 g/l vancomycin, 1 g/l neomycin, 1 g/l metronidazole in sterilized water) for 30 days. Ablation of resident microbiota was assessed by aerobic/anaerobic plating on BHI plates supplemented with 5% FBS. The antibiotic cocktail was removed 24 h prior to transplantation. Weight-, age- and gender-matched WT and littermate *Ang1*$^{-/-}$ mice were used as fecal microbiota transplant donors. Fresh fecal pellets from the donor mice were homogenized in sterile phosphate buffered saline (PBS, 100 mg/ml). Fecal suspensions were immediately gavaged into recipient mice (100 μl/mouse). Mice were transplanted every other day for 2 weeks and then subjected to DSS or TNBS administration (for WT mice), or regular feeding for 20 days for spontaneous colitis development in *Il-10*$^{-/-}$ mice.

Real-time qPCR Analysis

For tissue cytokine gene quantification, total RNAs from colonic tissue of DSS-treated mice were purified via precipitation with lithium chloride as reported previously.[4] The mRNA level was evaluated by reverse transcription reaction with Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Takara), followed by qPCR analysis with SYBR Premix Ex Taq (Takara). The qPCR was performed on a Roche 480 real-time PCR system (Roche). The primer sets are listed in table S5.

For gut bacteria analysis, bacterial DNA was extracted from feces by QIAamp DNA Stool Mini Kit (Qiagen). The quantity and quality of extracted DNA were determined by NanoDrop 2000 spectrophotometer (Thermo fisher). 10 ng extracted DNA was used as a template for qPCR analysis. The primer sets are listed in table S4.

Histological Analysis

Colon tissue was embedded in paraffin, cut into 4 μm sections, and then stained with hematoxylin and eosin (H&E) for histological analysis. Histological assessment of colitis was performed blindly by a board-certified

pathologist based on the previously described criteria that consist of the extent and severity of inflammation and ulceration of the mucosa.[5] Briefly, severity score for inflammation was as follows: 0, normal (within normal limits); 1, mild (small, focal, or widely separated, limited to lamina propria); 2, moderate (multifocal or locally extensive, extending to submucosa); 3, severe (transmural inflammation with ulcers covering >20 crypts). Score for ulceration was as follows: 0, normal (no ulcers); 1, mild (1-2 ulcers involving up to a total of 20 crypts); 2, moderate (3-4 ulcers involving a total of 20–40 crypts); 3, severe (more than 4 ulcers or over 40 crypts).

Fecal ANG Measurement

The stool samples were thawed at room temperature, and 100 mg stool was homogenized in 0.5 ml of extraction buffer (Tris 0.1 mM, citric acid 0.1 mM, urea 1.0 mM, $CaCl_2$ 0.01 mM, pH 8.0 and protease inhibitor cocktail). The samples were mixed vigorously for 30 min and centrifuged at 15,000 g for 20 min to collect the supernatant. The supernatant was filtered using a 5 μm cut-off filter. Total protein concentration was estimated using a BCA Protein Assay Kit (Thermo Fisher). Enzyme-linked immunosorbent assay (ELISA) technique was used to quantitatively measure the concentration of fecal ANG, as described previously.[6] Each sample ran with blank, standard and control. The ANG level is expressed as μg/g per sample of total protein.

16S rDNA High-throughput Sequencing and Analysis

Total fecal bacteria DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen) according to manufacturer's instruction with the addition of a bead-beating step to increase yield. The V3-V4 hypervariable region of the 16S rDNA was amplified using a universal forward sequencing primer and a uniquely barcoded reverse sequencing primer to allow for multiplexing[7] and sequenced with Realbio technology. The sequence reads were analyzed by QIIME (quantitative insights into microbial ecology, http://www.qiime.org) analysis pipeline as described.[8] In brief, FASTA quality files and a mapping file indicating the barcoded sequence corresponding to each sample were used as inputs, reads were split by samples according to the barcode, taxonomical classification was performed using the RDP-classifier, and an operational taxonomic unit (OTU) table was created. Closed reference OTU mapping was employed using the RDP database (http://rdp.cme.msu.edu). Sequences sharing 97% nucleotide sequence identity in the V3-V4 region were binned into operational taxonomic units (97% ID OTUs). α-diversity (observed species) and β-diversity analysis (unweighted UniFrac distance) were calculated using QIIME. Differential species associated with particular interventions were identified through LEfSe with effect size threshold of 2.

Fluorescence *in situ* Hybridization (FISH)

A segment of the mouse distal colon was fixed in 4% paraformaldehyde solution overnight at 4°C, washed and passed through 15% and 30% sucrose solutions. Colon tissues were then embedded in optimal cutting temperature compound (O.C.T., Tissue-Tek) and cryo-sectioned into 5 μm longitudinal sections (Leica). Slides were equilibrated in hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl, 0.01% sodium dodecyl sulfate, 10% formamide, pH 7.5) and incubated in 10 ng/μl FISH probe (Genscript) for 14 h at 42°C in a humidified chamber. The probe information was listed in table S6. Slides were then incubated for 20 min in wash buffer (0.9 M NaCl, 20 mM Tris-HCl, pH 7.5) pre-heated to 42°C and washed gently three times. Samples were then incubated in the dark with 10 μg/ml Hoechst 33342 in PBS for 10 min at room temperature, washed three times with PBS and mounted in Vectashield mounting medium (Vector Labs). Images were acquired on a Nikon A1 confocal microscope.

FISH and ANG Immunostaining in Fecal Sample

The isolation and preparation of fecal bacteria for image processing were described as previously.[9] Briefly, 0.2 g of human or mouse feces was suspended in 1 ml of PBS, homogenized on a vortex mixer for 2 min, and centrifuged at low speed (35 g, 20 min) to separate larger fecal particles from bacteria. The bacteria in the supernatant (about 10^9 bacteria per μl, as determined by direct microscopic clump counts) were washed twice in 1 ml of PBS and centrifuged at 8000 g for 10 min to remove unbound soluble protein. The pellet was resuspended and fixed in 1 ml of 4% paraformaldehyde for 12 h, then centrifuged at 8,000 g for 10 min and washed twice with PBS. The bacteria in the supernatant was diluted to a final concentration of OD_{600} at 0.1, and 10 μl was pipetted on a poly-L-lysine coated slide (Thermo Fisher). After drying, the α-Proteobacteria and Lachnospiraceae were identified by FISH as described above. Then slides were blocked for 1 h at room temperature with 2% bovine serum albumin (BSA, Sigma), and incubated for 2 h with the rabbit anti-ANG (Santa Cruz) at room temperature. Secondary antibody donkey anti-rabbit IgG Alexa Fluor 488 (Thermo Fisher) was applied to slides for 1 h at room temperature in the dark. Slides were stained with Hoechst 33342 for 10 min at room temperature and mounted in Vectashield mounting medium (Vector Labs). For ANG1 distribution in intestine, the intestinal slides were directly blocked and stained by rabbit anti-ANG1 (Santa Cruz) and mouse anti-LGR5 (Thermo Fisher) at room temperature, following secondary antibodies (donkey anti-rabbit IgG Alexa Fluor 555 and donkey anti-mouse IgG Alexa Fluor 488, Thermo Fisher). Images were acquired on a Nikon A1 confocal microscope.

ANG Expression and Purification

Mouse and human recombinant ANG proteins were generated with a pET *E. coli* expression system and purified to homogeneity by high-performance liquid chromatography (HPLC) in-house.[10] Human and mouse ANG ribonuclease-inactive variants (ANG-K40I and ANG1-K40I) were generated through site-directed

mutagenesis with the Fast Mutagenesis System kit (TransGen Biotech) followed by expression in pET *E. coli* expression system and HPLC purification. Angiogenic and ribonucleolytic activities of each batch of ANG preparation were confirmed (supplementary figure S5A and S5B). [10]

Bacteria Pull-down Assay

Pull-down assay was used to confirm the interaction between ANG and bacteria *in vitro*. Human ANG, mouse ANG1 or BSA protein was coated with carrier *N*-hydroxysuccinimide (NHS)-activated magnetic beads (Thermo Fisher) according to the manufacturer's instruction. The binding abilities of each protein to different bacterial strains (*B. diminuta*, *S. paucimobilis*, *Anaerostipes sp*. or *Blautia sp*.) were evaluated by adding 10 μl of magnetic beads coated with human ANG, mouse ANG1 or BSA to a 1 ml sample containing 100 μl of bacterial solution (10^6 colony-forming unit (CFU)/ml) and 900 μl of sodium phosphate buffer (10 mM, pH 7.2). Magnetic beads were incubated for 30 min at room temperature with gentle agitation and removed from solution using a magnet separator. The binding efficiency was determined as the percent of input by qPCR analysis of bacterial DNA.

Mouse Colonization with Specific Bacteria

Bacterial strains were freshly cultured as described above. The culture was centrifuged and the bacterial pellet was re-suspended at approximate 10^9 CFU/ml in pre-reduced BHI. WT mice were orally administrated with 200 μl *B. diminuta* or *S. paucimobilis* suspension, and *Ang1*$^{-/-}$ mice were orally administrated with 200 μl *Anaerostipes sp*. or *Blautia sp*. every other day for 2 weeks, meanwhile BHI was gavaged as vehicle control. After the final gavage, the colonization efficiency was detected by qPCR, and the mice were then challenged with DSS.

Bacterial Competition Assay

For spent medium preparation, *B. diminuta*, *S. paucimobilis*, *Anaerostipes sp*., *Blautia sp*. or *E.coli* (unrelated control) was cultured overnight, and then centrifuged at 7,000 g for 10 min. The supernatant was passed through a 0.22 μm filter, aliquoted if required, and stored at -20°C for further use. To measure the effect of spent media from *B. diminuta* or *S. paucimobilis* on the growth of *Anaerostipes sp*. or *Blautia sp*., an overnight culture of *Anaerostipes sp*. or *Blautia sp*. was diluted at 1:100 in fresh BHI media. The bacterial solution was mixed with the collected spent media at 1:1 ratio, and then incubated anaerobically at 37°C. The OD_{600} was measured at 0, 2, 4, 6, and 8 h of incubation. The procedure of measuring inhibition effect of spent media from *Anaerostipes sp*. or *Blautia sp*. on *B. diminuta* or *S. paucimobilis* was analogous to the above procedure but operated in an aerobic environment.

Scanning Electron Microscope Observation

Bacteria from the mid-logarithmic growth phase were centrifuged at 3,000 g for 5 min, then re-suspended and diluted to 0.1 OD_{600} in 10 mM sodium phosphate buffer (pH 7.2). The bacteria were incubated anaerobically/aerobically with human ANG or mouse ANG1 at different concentrations for 2 h at 37°C, then resuspended and fixed in PBS containing 2.5% glutaraldehyde for 12 h at 4°C. After centrifugation, the bacteria sample was rinsed three times in PBS buffer and post-fixed in 1% osmium tetroxide for 1 h. The sample was examined using an FEI Tencai Biotwin scanning electron microscope at 80 kV.

Microbicidal Activity Assay

Antimicrobial activity was evaluated with the colony-forming unit assay. The bacteria were grown to mid-logarithmic phase in a suitable medium, centrifuged at 3,000 g for 5 min, then resuspended and further diluted to a final concentration of 10^5 to 10^6 CFU/ml in 10 mM sodium phosphate buffer (pH 7.2). A total of 50 μl of the bacterial suspension was incubated with 50 μl recombinant ANG, ANG-K40I, ANG1, ANG1-K40I, or BSA at different concentrations for 2 h at 37 °C. Growth inhibition was analyzed after plating serial dilutions of the bacterial suspension on suitable agar plates with 48 h culture at 37°C. The rate of survival was calculated as the CFU number of ANG- or its variant-treated bacteria versus the CFU of BSA-treated bacteria. The median lethal dose (LD_{50}) was reported as the protein concentration that resulted in a survival rate of 50%.

Mouse Oral Treatment with ANG1

3-weeks-old *Ang1*$^{-/-}$ mice were fed with ANG1 recombinant protein (20 mg/kg per mouse) in drinking water for 30 days, followed by DSS treatment. The clinical signs of colitis and fecal bacteria were analyzed in response to ANG1 treatment. To evaluate the dynamic change of bacteria in *Ang1*$^{-/-}$ mice upon ANG1 treatment, 6-weeks-old *Ang1*$^{-/-}$ mice were fed with ANG1 recombinant protein (100 mg/kg per mouse) in drinking water for 3 days. Fecal bacteria were collected and analyzed by qPCR at -1, 0, 1, 2, 3, 5, 12 days, with Day 0 as the first day of ANG1 treatment.

SUPPLEMENTARY TABLE

Table S1. Basic Information of Healthy Subjects and IBD Patients.

Characteristics	**HS (n=45)**	**IBD (n=64)**	
Age, Year (mean ± SD)	39.1 ± 13.4	36.2 ± 13.3	
Male / Female n (%)	26 (58%) / 19 (42%)	42 (65%) / 22 (35%)	
Age at diagnosis, Year (mean ± SD)	NA	30.3 ± 13.0	
Disease duration years, mean (range)	NA	5.5 (0.25 - 19)	
Montreal classification	**HS**	**UC**	**CD**
n (%)	45 (100%)	15 (23.4%)	49 (74.6%)
A1 / A2 / A3 (n)	NA	NA	3 / 39 / 7
L1 / L2 / L3 / L4 (n)	NA	NA	0 / 17 / 32 / 0
B1 / B2 / B3 (n)	NA	NA	8 / 29 / 12
E1 / E2 / E3 (n)	NA	2 / 1 / 12	NA
HBI (median, IQR)	NA	NA	8 (6-16)
PMS (median, IQR)	NA	5 (3-8)	NA

HS: healthy subjects. IBD: inflammatory bowel disease. UC: ulcerative colitis. CD: Crohn's disease. NA: not-available. Montreal classification of extent of UC: E1, ulcerative proctitis; E2, left sided UC; E3, extensive UC. Age at diagnosis by Montreal classification for CD: A1, <16; A2, 16-40; A3, >40. Localization of disease by Montreal classification: L1, ileal; L2, colonic; L3: ileocolonic; L4: upper gastrointestinal tract. Disease behavior for Montreal classification for CD: B1, non-stricturing; B2, stricturing; B3, penetrating. HBI, Harvey-Bradshaw Index; PMS, partial Mayo score; IQR, interquartile range.

Table S2. Bacteria Strains

Strain	**Classification (RDP)**
B. diminuta	Proteobacteria; α-proteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas
S. paucimobilis	Proteobacteria; α-proteobacteria; Sphingomonadales; Sphingomonadaceae; Sphingobium
Anaerostipes sp.	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Anaerostipes
Blautia sp.	Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia

Table S3. Bactericidal Activity of ANG or its Variants

Strain	**ANG (LD_{50}, μM)**	**ANG1 (LD_{50}, μM)**	**ANG-K40I (LD_{50}, μM)**	**ANG1-K40I (LD_{50}, μM)**
B. diminuta	0.81	0.85	0.79	0.67
S. paucimobilis	0.69	0.63	0.66	0.70
Anaerostipes sp.	20.75	22.50	20.05	10.82
Blautia sp.	212.9	242.9	329.5	114.2

Table S4. Primers for Bacterial Analysis

Name	Forward primer (5′→3′)	Reverse primer (5′→3′)	Reference
Universal bacterial	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC	[11]
α-proteobacteria	CIAGTGTAGAGGTGAAATT	CCCCGTCAATTCCTTTGAGTT	[12]
Lachnospiraceae	AGCTCTATCAGCAGGGAAGA	ATTACCGCGGCTGCTGGC	This study
S. paucimobilis	AAGCTCTTTTACCCGGGATGA	CCGATGTTCAAGCAATCCAGTC	This study
B. diminuta	GTGAATGATGAAGGTCTTAGG	CAGTATGTCGCCTTCGCCAC	This study
Blautia sp.	AGGAAGAAGTATCTCGGTAT	ATCAGACTTGCCACACCGTC	This study
Anaerostipes sp.	GTGAGTGAAGAAGTATTTCG	GCTTTCACTTCTGACTTACC	This study

Table S5. Primers for Mouse Cytokine Analysis

Gene	PrimerBank ID	Forward primer (5′→3′)	Reverse primer (5′→3′)
Actb	145966868c1	GTGACGTTGACATCCGTAAAGA	GCCGGACTCATCGTACTCC
Il-1β	118130747c1	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
Il-6	13624310c2	TCTATACCACTTCACAAGTCGGA	GAATTGCCATTGCACAACTCTTT
Il-17a	142367609c2	GGCCCTCAGACTACCTCAAC	TCTCGACCCTGAAAGTGAAGG
S100a8	7305453a1	AAATCACCATGCCCTCTACAAG	CCCACTTTTATCACCATCGCAA
Tnfα	133892368c2	CTGAACTTCGGGGTGATCGG	GGCTTGTCACTCGAATTTTGAGA
Cxcl1	229577225c1	ACTGCACCCAAACCGAAGTC	TGGGGACACCTTTTAGCATCTT
Cxcl2	6677885a1	CCAACCACCAGGCTACAGG	GCGTCACACTCAAGCTCTG
Ccl3	126432552c1	TGTACCATGACACTCTGCAAC	CAACGATGAATTGGCGTGGAA
Gcsf	6753536a1	ATGGCTCAACTTTCTGCCCAG	CTGACAGTGACCAGGGGAAC

Table S6. Probes Used in FISH

Probe Name	fluorophore	Sequence (5'-3')	Probe target	Reference
ALF682	Cy3	GAATTTCACCTCTACACT	α-proteobacteria	[13]
Lach435	Cy5	TCTTCCCTGCTGATAGA	Lachnospiraceae	[14]

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. The Gene Structure and Expression Pattern of ANG. (A) Tissue expression patterns of human *ANG*, mouse *Ang1* and *Ang4*. The data extracted from the Genebank Database were reorganized.[15 16] (B) Gene structures of human *ANG* and mouse *Ang1*. The human *ANG* and mouse *Ang1* loci hold a unique gene arrangement, characterized with shared promoter and 5'-untranslated regions (exon I and exon II) followed by two distinct exons encoding ANG/ANG1 (exon III) and Ribonuclease 4 (RNASE4, the fourth member of the RNase A superfamily, exon IV), respectively. The *Ang1* knockout (*Ang1*$^{-/-}$) mouse lacks the whole exon III. (C) Fecal ANG1 level was continuously declining in WT mice upon treatment with DSS. The coomassie brilliant blue staining was used as a loading control. (D) Barplot of cell cluster number (Y-axis) and cell type (X-axis) where *Ang1* expression was determined based on the single cell RNA sequencing database (PanglaoDB).[17] (E) The expression and localization of ANG in Paneth cells detected by immunofluorescence staining. The ANG1 (red), LGR5^{+} stem cell (green), nuclear (blue) were shown. Scale bar, 100 μm.

Figure S2. The Effect of *Ang1* on Colitis Progress in TNBS-induced Colitis Model. (A) Fecal ANG1 levels in TNBS-treated WT mice. The coomassie brilliant blue staining was used as a loading control. (B) Design of gut microbiota transplantation and TNBS induction in WT mice. (C) Body weight of WT, *Ang1*$^{-/-}$, WT→WT or *Ang1*$^{-/-}$→WT mice during TNBS treatment. n = 7. (D and E) Colon length (D) and histological score (E) of these mice at the end of TNBS induction. n = 7. Scale bar, 50 μm. (F) Expression levels of cytokines in the colon of WT, *Ang1*$^{-/-}$, WT→WT or *Ang1*$^{-/-}$→WT mice at the end of TNBS induction. The heatmap represents the expression of indicated genes in each mouse after normalized to the average of WT group. n = 7. Data are presented as mean ± SEM; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ by one-way ANOVA test (C-E).

Figure S3. The Effect of *Ang1* on Colitis Progress in Genetically Spontaneous Colitis Model. (A) Fecal ANG1 levels in *Il-10*$^{-/-}$ mice at 6, 8, 10, 12 and 14 weeks (w) of age. The coomassie brilliant blue staining was used as a loading control. (B) Body weight of *Il-10*$^{-/-}$ or *Ang1*$^{-/-}$; *Il-10*$^{-/-}$ mice during spontaneous colitis. n = 5. (C-E) DAI (C), colon length (D), and histological score (E) of *Il-10*$^{-/-}$ or *Ang1*$^{-/-}$; *Il-10*$^{-/-}$ mice at 95th day after the birth. n = 5. Scale bar, 50 μm. (F) Expression levels of cytokines in the colon of *Il-10*$^{-/-}$ or *Ang1*$^{-/-}$; *Il-10*$^{-/-}$ mice. The heatmap represents the expression of indicated genes in each mouse after normalized to the average of *Il-10*$^{-/-}$ group. (G) Diagram of gut microbiota transplantation in *Il-10*$^{-/-}$ spontaneous colitis model. (H) Body weight of WT→*Il-10*$^{-/-}$ or *Ang1*$^{-/-}$→*Il-10*$^{-/-}$ mice during spontaneous colitis. n = 7. (I-K) DAI (I), colon length (J), and histological score (K) of WT→*Il-10*$^{-/-}$ or *Ang1*$^{-/-}$→*Il-10*$^{-/-}$ mice at 95th day after the birth. n = 7. Scale bar, 50 μm. (L) Expression levels of cytokines in the colon of WT→*Il-10*$^{-/-}$ or *Ang1*$^{-/-}$→*Il-10*$^{-/-}$ mice. The

heatmap represents the expression of indicated genes in each mouse after normalized to the average of WT→*Il-10*$^{-/-}$ group. n = 7. (M and N) The abundance of α-Proteobacteria (M) or Lachnospiraceae (N) in gut microbiota of *Il-10*$^{-/-}$ or *Ang1*$^{-/-}$; *Il-10*$^{-/-}$ mice at indicated weeks (w) of age. n = 5. Data are presented as mean ± SEM; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ by unpaired Student's *t*-test (B-E, H-K, M and N).

Figure S4. The Relationship between Abundance of α-Proteobacteria or Lachnospiraceae and Colitis. (A and B) The relative abundance of α-Proteobacteria (A) or Lachnospiraceae (B) in the gut microbiota of WT mice in response to DSS treatment. n = 6. (C and D) The relative abundance of α-Proteobacteria (C) or Lachnospiraceae (D) in gut microbiota of healthy subjects (n = 45) or IBD patients (n = 64). (E and F) Body weight of WT mice with high or low abundance of α-Proteobacteria (E) or Lachnospiraceae (F) during DSS-induced colitis. n = 7. Data are presented as mean ± SEM; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$ by paired Student's *t*-test (A and B) or unpaired Student's *t*-test (C-F).

Figure S5. ANG-Regulated Bacteria Are Associated with Colitis. (A) Body weight of WT or *Ang1*$^{-/-}$ mice inoculated with *B. diminuta, S. paucimobilis, Anaerostipes sp., Blautia sp.*, or BHI. n = 6. (B and C) Representative images of colon (B) and H&E staining (C) of WT-BHI, *Ang1*$^{-/-}$-BHI, WT-*B. diminuta* or WT-*S. paucimobilis* mice on day 8 after DSS induction. Scale bar, 50 μm. (D and E) Representative images of colon (D) and H&E staining (E) of WT-BHI, *Ang1*$^{-/-}$-BHI, *Ang1*$^{-/-}$*-Anaerostipes sp.*, or *Ang1*$^{-/-}$*-Blautia sp.* mice on day 8 after DSS induction. Scale bar, 50 μm.

Figure S6. The Ribonucleolytic and Angiogenic Activities of Home-made Recombinant ANG Protein. (A) The cleavage pattern of recombinant ANG or its variant on total RNA isolated from HCT116 cells. 0.1 μM human ANG, ANG-K40I variant, mouse ANG1 or ANG1-K40I variant was added to a reaction system including 1 μg total RNA, 0.33 M HEPES, 0.33 M NaCl, pH 7.0, and 0.1 mg/ml ribonuclease free BSA. After incubation at 37 °C for 1 h, the product was analyzed in 1% agarose gel. (B) The effect of recombinant ANG or its variant on endothelial cell tube formation. The shown image is representative of at least three independent repeats. Scale bar, 200 μm.