

1 **Supplementary Data**

2 **Supplementary Methods**

- 3 1. Detailed experimental procedures of 16S rDNA gene sequencing  
4 2. Detailed data processing and analysis steps of 16S rDNA gene sequencing  
5 3. Extraction and quantification of fecal bacterial genomic DNA  
6 4. Experiment procedure of PCO fermentation broth administration

7 **Supplementary Figures**

- 8 Fig. S1. Quantification of intestinal bacteria in mice with/without gut microbiota depletion

9 **Supplementary Tables**

- 10 Table S1. Composition of gut microbiota medium  
11 Table S2. Criteria for scoring disease activity index (DAI)  
12 Table S3. List of primer sequences for RT-qPCR to determine gut microbiota abundances  
13 Table S4. List of primer sequences for RT-qPCR analysis  
14 Table S5. Statistics on raw/clean reads by 16S rDNA sequencing  
15 Table S6. Number of OTUs for single sample

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## 17 **Supplementary Methods**

### 18 1. Detailed experimental procedures of 16S rDNA gene sequencing

19 The amplification conditions were as follows: pre-denaturation at 95 °C for 3 min, 25  
20 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for  
21 45 s. Purified amplicons were pooled in equimolar and paired-end sequenced ( $2 \times 300$ ) on an  
22 Illumina MiSeq platform (Illumina, San Diego, CA, USA) by Beijing Allwegene Tech, Ltd  
23 (Beijing, China). After the sequencing, data were analyzed via the QIIME pipeline  
24 (Quantitative Insights into Microbial Ecology, USA). The raw sequences were jointed and  
25 selected (low-quality tags and chimeras that did not meet the length requirement were  
26 removed). The unique sequence with a similarity larger than 97% was classified into an  
27 operational taxonomic unit (OTU) by UCLUST (Version 1.2.22  
28 [http://www.drive5.com/uclust/downloads/1\\_2\\_22q.html](http://www.drive5.com/uclust/downloads/1_2_22q.html)). Chimeric sequences were identified  
29 and removed using Usearch (Version 8.1.1861 <http://www.drive5.com/usearch/>). The  
30 taxonomy of each 16S rRNA gene sequence was analyzed by UCLUST against the Silva 16S  
31 rRNA database (Release119 <http://www.arb-silva.de>) using a minimum threshold of 90%  
32 confidence. Then, an OTU table was generated for relative abundance plots and further  
33 analysis. Finally, we conducted the Linear Discriminant Analysis Effect Size (LEfSe) analysis  
34 to determine the noteworthy microbial taxa.

### 35 2. Extraction and quantification of fecal bacterial genomic DNA

36 Fecal samples (25 mg) were homogenized with 1% sterile PBS until the feces became  
37 mushy, followed by centrifugation at  $3,000 \times g$  for 5 min. The upper bacterial solution was  
38 treated with proteinase K, RNase, and lysozyme in a shaker at 37 °C for 1 h. After adding lysate  
39 and DNA extraction agent, the bacterial solution was incubated in a water bath at 65 °C for 10  
40 min. Then, the solution was centrifuged at  $13,000 \times g$  for 10 min. The supernatant was collected  
41 to precipitate DNA using isopropyl alcohol. After centrifugation, the DNA precipitation was  
42 washed with 75% ethanol and dissolved in sterile water after drying at room temperature. The  
43 bacterial DNA was quantified on a CFX Connect Real-time system (Bio-Rad, Hercules, CA,  
44 USA) by using a  $2 \times$  FastHS SYBR QPCR mixture with the following thermal cycle condition:

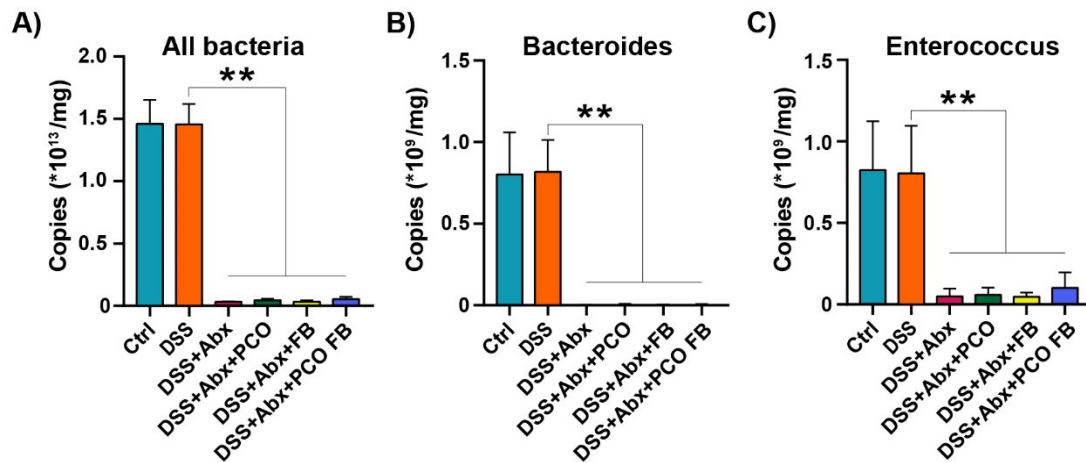
45 Initial at 95 °C for 10 min, 34 cycles of amplification (30 s at 95 °C for denaturation, 30 s at 58  
46 °C or 55 °C for annealing, 30 s at 72 °C for extension), terminal extension at 72 °C for 5 min.  
47 Two standard bacteria, i.e., *Bacteroides* and *Enterococcus* were used to represent Bacteroidetes  
48 and Firmicutes phyla, respectively. The primer sequences of target intestinal bacteria were  
49 listed in Supplementary Table 2. Bacterial contents were quantified by the standard curves  
50 using corresponding bacteria plasmids with gradient dilution.

### 51 3. Experiment procedure of PCO fermentation broth administration

52 The experiment procedure was as follows: 1) Ctrl group, received distilled water during  
53 the whole experimental period; 2) DSS group, received distilled water for the first three weeks,  
54 followed by 3% DSS (dissolved in drinking water) in the fourth week; 3) DSS + Abx group,  
55 received Abx (dissolved in drinking water) during the whole experimental period and 3% DSS  
56 in the fourth week; 4) DSS + Abx + PCO group, received Abx during the whole experimental  
57 period, PCO by gavage in the third and fourth weeks, and 3% DSS in the fourth week; 5) DSS  
58 + Abx + FB group, received Abx during the whole experimental period, FB by gavage in the  
59 third and fourth weeks, and 3% DSS in the fourth week; 6) DSS + Abx + PCO FB group,  
60 received Abx during the whole experimental period, PCO FB (200 mg/kg/d) by gavage in the  
61 third and fourth weeks, and 3% DSS in the fourth week. The concentration of PCO FB was  
62 converted into that of PCO solution before fermentation. At the end of the experiment, all mice  
63 were euthanized. Mouse tissues were collected, and all samples were stored at -80 °C for further  
64 analysis.

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66 Supplementary Figures



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68 Fig. S1. Quantification of intestinal bacteria in mice with/without gut microbiota  
69 depletion. (A) Abundances of all bacteria in mice by RT-qPCR. (B–C) Phylum abundances of  
70 gut microbiota by RT-qPCR, in which *Bacteroides* (B) and *Enterococcus* (C) were used as  
71 representative bacteria of Bacteroidetes and Firmicutes phyla, respectively. Data were  
72 represented as mean  $\pm$  SEM (n = 8). \* $p$  < 0.05, \*\* $p$  < 0.01.

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74 **Supplementary Tables**

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Table S1. Composition of gut microbiota medium

Component	Content (g/L)
K <sub>2</sub> HPO <sub>4</sub>	0.293
KH <sub>2</sub> PO <sub>4</sub>	0.176
NaCl	0.443
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.450
CaCl <sub>2</sub>	0.045
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.093
L-cysteine	0.500
Ascorbic acid	0.500
Na <sub>2</sub> CO <sub>3</sub>	4.000
Tryptone	1.000
Yeast extract	1.000

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Table S2. Criteria for scoring disease activity index (DAI)

Score	Weight loss (%)	Stool consistency	Gross bleeding
0	None	Normal	Negative
1	1–5	Loose stool	Negative
2	5–10	Loose stool	Hemoccult positive
3	10–15	Diarrhea	Hemoccult positive
4	>15	Diarrhea	Gross bleeding

81 Table S3. List of primer sequences for RT-qPCR to determine gut microbiota abundances

Name	Forward primer (5'-3')	Reverse primer (5'-3')
All bacteria	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC
<i>Bacteroides</i>	GGTTCTGAGAGGAGGTCCC	GCTGCCTCCCGTAGGAGT
<i>Enterococcus</i>	CCCTTATTGTTAGTTGCCATCATT	ACTCGTTGTACTTCCCATTGT

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Table S4. List of primer sequences for RT-qPCR analysis

Name	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
<i>Cox-2</i>	CGAGTCGTTCTGCCAATA	CTGGTCGGTTTGATGCTA
<i>Nlrp3</i>	ATTACCCGCCCAGAAAAGG	TCGCAGCAAAGATCCACACAG
<i>Il-1<math>\beta</math></i>	GGCTGGACTGTTTCTAATGC	ATGGTTTCTTGTGACCCTGA
<i>Il-6</i>	GAAACCGCTATGAAGTTCCTCTCTG	TGTTGGGAGTGGTATCCTCTGTGA
<i>Tnf-<math>\alpha</math></i>	GGGTGTTTCATCCATTCTC	GGAAAGCCCATTGAGT
<i>Mucin-1</i>	GGCATTTCGGGCTCCTTTCTT	TGGAGTGGTAGTCGATGCTAAG
<i>Mucin-2</i>	ATGCCACCTCCTCAAAGAC	GTAGTTTCCGTTGGAACAGTGAA
<i>Mucin-3</i>	CGTGGTCAACTGCGAGAATGG	CGGCTCTATCTCTACGCTCTCC
<i>Zo-1</i>	GAGCGGGCTACCTTACTGAAC	GTCATCTCTTCCGAGGCATTAG
<i>Occludin</i>	TTGAAAGTCCACCTCCTTACAGA	CCGGATAAAAAGAGTACGCTGG
<i>Mmp-9</i>	GGACCCGAAGCGGACATTG	CGTCGTCGAAATGGGCATCT



Table S5. Statistics on raw/clean reads by 16S rDNA sequencing

Sample ID	Raw_Tags	Clean_Tags
Ctrl1	62086	60570
Ctrl2	61876	58610
Ctrl3	50601	48963
Ctrl4	30008	29660
Ctrl5	63573	62480
Ctrl6	29675	28923
Ctrl7	36659	34063
Ctrl8	53665	51047
DSS1	99595	97282
DSS2	77126	75012
DSS3	51432	49744
DSS4	59814	58109
DSS5	80992	78361
DSS6	82421	80066
DSS7	70089	67621
DSS8	58850	56054
PCO1	124147	115457
PCO2	50882	49838
PCO3	95632	92169
PCO4	86807	84479
PCO5	131093	127884
PCO6	82137	80173
PCO7	82482	80036
PCO8	87117	85192
DSS+PCO1	68567	66773
DSS+PCO2	85972	83318
DSS+PCO3	74522	71952
DSS+PCO4	107312	104329
DSS+PCO5	68502	66922
DSS+PCO6	84977	82444
DSS+PCO7	84644	80812
DSS+PCO8	83325	81784
Total	2366580	2290127

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Table S6. Number of OTUs for single sample

Sample ID	Final_tags	OTUs
Ctrl1	28573	473
Ctrl2	28573	501
Ctrl3	28573	434
Ctrl4	28573	590
Ctrl5	28573	463
Ctrl6	28573	334
Ctrl7	28573	581
Ctrl8	28573	525
DSS1	28573	486
DSS2	28573	450
DSS3	28573	530
DSS4	28573	439
DSS5	28573	491
DSS6	28573	476
DSS7	28573	416
DSS8	28573	493
PCO1	28573	585
PCO2	28573	625
PCO3	28573	631
PCO4	28573	601
PCO5	28573	620
PCO6	28573	510
PCO7	28573	637
PCO8	28573	579
DSS+PCO1	28573	511
DSS+PCO2	28573	618
DSS+PCO3	28573	510
DSS+PCO4	28573	447
DSS+PCO5	28573	539
DSS+PCO6	28573	567
DSS+PCO7	28573	512
DSS+PCO8	28573	544