

# Microbial Changes Associated with IBD Mouse Model and Microbiota Transplantation Confers Colitis Symptom in Microbiota Deletion Mice

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## Research article

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# Abstract

Background Inflammatory bowel disease (IBD), including Crohn's disease (CD) and Ulcerative colitis (UC), are chronic and relapsing inflammation occurring among the gastrointestinal tract. Available evidence suggests that host microbiome, as well as various components of the mucosal immune system, are implicated in the pathogenesis of IBD though the exact mechanism remains unknown. Advances in DNA sequencing technologies have provided new insights on the function identification of gut microbiota. In this study, we investigated the gut microbiota response to colitis and discussed the underlying mechanisms of this alteration in combination with latest research. The function of altered microbiota was investigated through microbiota transplantation technology. Results Twenty-seven female C57BL/6J mice were fed DSS solution for 5 days and followed by 5 days normal drinking water. D 0 was considered as normal control while d 5 and d 10 were seen as disease progressive phase and recovery phase, respectively. Alpha diversity results showed that the detected microbiota composition differences among 3 phases were not due to the presence and/or absence of rare phylotypes. In IBD mouse model, community richness decreased but diversity did not change significantly. Besides, the strong negative correlation between phyla Firmicutes and Bacteroidetes decreased in IBD mouse model of which maybe one of the dysbiosis signatures. Furthermore, transplanting microbiota of IBD mouse model to antibiotic-induced microbiota depletion mice (Abx-mice) could induce inflammation similar to colitis. The proportion of the *Lactobacillus*, as well as other less abundant probiotic taxa, significantly increased in recovery phase, revealing the potential of these probiotic in IBD therapy. Conclusions IBD could induce proportional changes of gut microbiota, and these changes are capable of conferring pathogenicity in Abx-mice. This study provides updated comprehensions of the commensal microbiota alteration. Keywords Gut microbiota, dysbiosis, inflammation bowel disease, DSS, microbiota transplantation

## Background

The prevalence of inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), remains high in Europe and continues to rise across the world [1, 2]. IBD is characterized by intestinal ulcers which accumulated within whole digestive tract and may occur in other extra-intestinal organs [3]. The specific etiology of IBD is complex and remains unclear, but it was generally agreed that genetics, epigenetics, gut microbiota and host immune system factors were involved. Previous studies reported that altered composition or balance of gut bacteria were important in initiation and progression of IBD. For example, changes in abundance of *Firmicutes* and *Proteobacteria* was correlated strongly with IBD [4].

There exist significant interactions between gut microbiota and the host in several ways such as dietary energy extraction, immune system development, vitamin production and drug metabolism [5, 6], thus involved in many diseases. IBD is one of the most frequently reported diseases [4, 7, 8]. Some pathogens, such as *Vibrio cholerae* and *Clostridium perfringens*, could secrete enzymes capable of utilizing mucus or disrupt tight junctions [9-11]. StcE, a member of *E. coli* metalloprotease, could assist pathogens to reach

gut epithelium, disrupt colonic mucus and tight junctions thus contributing to IBD [12]. On the other hand, dysbiosis can lead to an imbalance in metabolites production that in turn may link to IBD pathogenesis [13]. The decrease of butyrate-producing species, such as *Roseburia hominis* and *Faecalibacterium prausnitzii*, play an important role in epithelial barrier integrity [14].

Rodent colitis models are important tools to investigate IBD pathogenesis and have contributed greatly to the identification of IBD pathogenesis process. DSS-induced IBD mouse model is one of the most widely used models due to its rapidity and reproducibility [15]. DSS is toxic to colonic epithelial cells and could destroy the mucosal barrier integrity [16, 17], allowing the entry of luminal bacteria into mucosa and the dissemination of proinflammatory intestinal contents into underlying tissue [18, 19]. Therefore, DSS-induced colitis might be mediated by gut microbiota dysbiosis and corresponding inflammatory response. Previous study using DSS-induced IBD mouse model, revealed the importance of IL-17F itself and IL-17F-induced Treg cells in protecting against colitis [20]. In other studies, germ-free mice showed a higher mortality rate than wild-type mice when given the same dose of DSS [21], and the depletion of microbiota induced by broad spectrum antibiotics also exacerbates DSS-induced colitis [22], further suggesting the important role of gut microbiota in maintaining gut health. Thus, we used a DSS-induced colitis model to further explore the interactions between gut microbiota and colon inflammation.

However, there is still no conclusion concerning how microbial dysbiosis is linked to the onset and development of IBD. Fecal microbiota transplantation (FMT) provides new option for manipulating gut microbiota, thus becoming an encouraging therapeutic approach for intestinal disease and a powerful tool for investigating mechanisms of microbiological related disease. Combining FMT and antibiotics contributed to the microbiological improvement of intestinal dysbiosis in UC patients [23]. Previous study found the microbiota could alleviate murine chronic intestinal inflammation through modulating immune cell functions in terms of FMT [24].

In our study, we established IBD mouse model by drinking DSS solution and eliminated cage effects by randomly selecting mice from multiple cages during samples collection. Colonic content samples were collected on d 0, d 5 and d 10 to investigate gut microbiota composition changes; colon tissues was assessed as research indicates that microbial abundance and diversity are directly related to the physiological state of the gastrointestinal tract segment; combination of Abx-mice model and FMT manipulation may elucidate the relationship between altered gut microbiota composition and gut inflammation responses.

## Results

### Clinical Signs of IBD mouse model

After 5 days of DSS treatment, IBD mouse model showed significant decrease in body weight, and continued to decline for the next 5 days (Fig.1). Disease activity index began to rise significantly on the 3th day and peaked on d 8 (Fig.1). Colon length was decreased compared with the normal control (Fig.1). We also found an obvious edema, severely damaged mucosal structures and abundant inflammatory cell

infiltration from H&E staining of distal colon tissue (Fig.2). Also, the inflammatory cytokine TNF- $\alpha$  in serum significantly increased (Fig.2). It was worth noting that, compared with d 5-model, the colon length of d 10-model significantly increased whereas the level of TNF- $\alpha$  in serum significantly decreased.

### Gut microbiota altered in IBD mouse model

In addition to colitis development, the DSS altered the composition of the gut microbiota by 5 days administration. PCoA revealed clear clustering by DSS-treatment time point using both unweighted-unifrac and weighted-unifrac method (Fig.3). Compared with normal control, Sobs and Chao index decreased in IBD mice (Fig.4, S1). Nevertheless, the Simpson index did not change significantly (Fig.4).

We further analyzed changes in the relative abundance of specific phylotypes. The overall abundances of the dominant phyla *Firmicutes* (37.77% in normal control and 38.16% in d 5-model) and *Bacteroidetes* (58.36% in normal control and 47.37% in d 5-model) were not significantly affected by DSS treatment but taxa within these two phyla showed dramatic changes (Fig.5). However, after a 5-day recovery period, the phyla *Firmicutes* (76.92%) significantly increased and *Bacteroidetes* (13.4%) significantly decreased (Fig.5).

Results indicated that the proportions of phylum *Firmicutes* showed a negative relationship with phyla *Bacteroidetes* ( $R^2 = 0.9883$ ,  $P \leq 0.0001$ ) in normal control, and this correlation became weaker in IBD mouse model (Fig.6). To further identify the differences of key microbiota among groups, LEfSe analysis was performed (Table.S1 and Fig.S2). Among phylum *Firmicutes*, the *Bacillales* in the normal control were more abundant than that of the IBD mice; d 5-model group increased the *Firmicutes-Erysipelotrichia-Erysipelotrichales-Erysipelotrichaceae-Turicibacter* and *Faecalibaculum* while increased *Firmicutes-Bacilli-Lactobacillales-Lactobacillaceae-Lactobacillus*, *Firmicutes-Bacilli-Lactobacillales-Enterococcaceae-Enterococcus*, *Firmicutes-Bacilli-Lactobacillales-Streptococcaceae-Streptococcus* and *Firmicutes-unclassified\_Firmicutes* were observed in d 10-model, making phylum *Firmicutes* highly dominant in d 10-model.

In order to further show the proportion and relationship of different taxon in the three groups, we performed Ternary analysis (Fig.7). Three taxa within phyla *Bacteroidetes* were enriched in normal control: *Muribaculaceae* (82.7% in normal control, 13.3% in d 5-model and 3.93% in d 10-model), *Prevotellaceae* and *Muribaculum* (both 100% in normal control, absence in d 5-model and d 10-model); meanwhile, three taxa within phyla *Bacteroidetes* concentrated in d 5-model but significantly decreased in d 10-model: *Bacteroides* (70.7% in d 5-model, 12% in normal control and 17.3% in d 10-model), *Odoribacter* (89.8% in d 5-model, 5.31% in normal control and 4.85% in d 10-model) and *Escherichia-Shigella* (87.9% in d 5-model, 0.278% in normal control and 11.8% in d 10-model); four taxa within phyla *Firmicutes* concentrated in d 5-model but significantly decreased in d 10-model: *Turicibacter* (61.2% in d 5-model, 0.626% in normal control and 38.2% in d 10-model), *Faecalibaculum* (70.2% in d 5-model, 28.3% in normal control and 1.45% in d 10-model), *Ruminococcaceae* (91.2% in d 5-model, 7.13% in normal control and 1.63% in d 10-model) and *Clostridiales* (80.9% in d 5-model, 3.65% in normal control and

15.5% in d 10-model); two taxa within phyla *Firmicutes* concentrated in d 10 model: *Lactobacillus* (62.8% in d 10-model, 29.9% in normal control and 7.35% in d 5-model) and *Ruminococcus* (100% in d 10-model, absence in d 5-model and normal control).

In addition to the bacteria concentrated in one phase mentioned above, the lower relative abundances taxa *Clostridium* and *Bifidobacterium* concentrated in d 5-model but decreased in d 10-model.

Furthermore, the abundances of *Mucispirillum*, *Helicobacter* and *Ruminiclostridium* increased in both d 5 and d 10 mouse model (Fig.S3).

### **IBD mouse model colonic commensal microbiota contributed to colitis**

Compared with vehicle-mice, colon length of FMT-mice decreased significantly (Fig.8, S4), and some colon samples of FMT-mice showed obvious blood and hygromata after microbiota transplantation (Fig.S4). However, body weight did not show significant difference and no obvious blood were observed in feces as well (data not shown). Similarly, mucosal epithelial damage and epithelial loss were observed in almost all FMT-mice, and we further found obvious inflammatory infiltration among a few of them (Fig.9).

## **Discussion**

After 5 days of DSS treatment, significant weight loss, increased DAI and reduced colon length indicated IBD mouse model was successfully established. Compared with d 5-model, the increased colon length and decreased DAI indicated the transition from progression phase to recovery phase in d 10-model.

Further, we screened response of colon bacterial flora to the DSS-induced inflammation changes. As rodents are coprophagic, gut microbiota of mice in the same cage will progressively become homogeneous over time. In order to avoid this, mice were randomly selected from multiple cages each time for sample collection. Completely different gut microbiota of IBD mouse model among d 0, d 5 and d 10 indicated gut microbiota alteration were accompanied by colitis initiation and recovery (Fig.3). Generally, commensal bacteria digested dietary fiber and produce short-chain fatty acids (SCFAs) therefore influencing gut epithelial cell and lymphocyte homeostasis and eventually contributed to the host intestine health. Germ-free mice have a higher mortality rate than wild-type mice when treated with DSS [25, 26], highlighting the important role of the flora in maintaining gut health.

However, the specific function of changed microbiota induced by inflammation remains unclear. To clarify this uncertainty, we transplant IBD mice colon bacterial flora to Abx-mice. As a result, FMT-mice showed symptoms similar to those of IBD mouse model while vehicle-mice remained healthy. This result implied that gut bacterial flora in IBD mouse shifts from normal commensal flora to harmful pathogenicity status and potentially elucidated the possible reason underlying the relapse of IBD.

We also analyzed the difference of microbiota composition between different phase of IBD mouse model and normal control. Both weighted and un-weighted unifrac PCoA separated these 3 group well (Fig.3),

indicating that the detected community differences were not due to the presence and/or absence of rarer phylotypes. Also, the decrease of community richness was observed at both progressive phase and recovery phase (Fig.4, S1) but community diversity did not change significantly (Fig.4), suggesting the losses of particular taxa while some “new” taxa rise.

Human colonic microbiota is dominated by anaerobic members within phyla *Bacteroidetes* and *Firmicutes* [27]. The situation is the same for IBD mice, but the overall abundances of these two phyla and the taxa within them showed clear changes compared with normal control. The negative relationship between phylum *Firmicutes* and *Bacteroidetes* became weaker in d 5 and d 10-mouse model which may be an indicator of disordered microbiota flora in IBD mouse.

To be specific, there was a significant increase of phylum *Firmicutes* and a significant decrease of phylum *Bacteroidetes* in d10-model compared with normal control and d 5-model. Within phylum *Firmicutes*, *Lactobacillus spp.* and *Bifidobacterium spp.* have been widely used as probiotics. Unlike the low abundance of *Bifidobacterium*, the average percentage of *Lactobacillus* could reach up to 23.1%. In fact, this bacteria specie was the primary bacteria driving the calculated multivariate discrimination in the high level of phylum *Firmicutes* in d 10-model (Fig.S2). This result also indicated the abundance of *Lactobacillus* is negatively correlated with IBD pathogenesis. If this hypothesis is correct, the supplementation of *Lactobacillus* in IBD animals and patients might confer beneficial effects. Previous studies reported similar beneficial effects for protecting gut health of *Lactobacillus*. First, *Lactobacillus* could increase intestinal anti-inflammatory cytokine level and/or reduce the proinflammatory cytokines, thus altering the immune system [28-30]; it is worth noting that *Lactobacillus* could suppress TNF- $\alpha$  expression [31], which may explain the declined TNF- $\alpha$  in IBD mouse model on d 10 (Fig.2). Second, *Lactobacillus* directly competed with pathogenic bacteria therefore contributing to gut health [32, 33]. Though whether the change of *Lactobacillus* is the cause or consequence of the disease pathogenesis remains unclear, this understanding of the ecophysiology of the commensal and probiotic *Lactobacillus* in the dysbiosis disease states provide useful information for the successful treatment and prevention of chronic intestinal inflammation.

The second most abundant abundance bacteria, *Muribaculaceae*, changed in manner quite differently from *Lactobacillus*. The highest abundance was observed in normal control (82.7%) and nearly disappeared in IBD mouse. This phenomenon was similar to that observed in the context of feeding trials using high-calorie and/or carbohydrate-enriched diets [34-36]. This is probably the consequence of its ability to degrade particular types of polysaccharides: plant glycans, host glycans, and  $\alpha$ -glucans [37, 38]. Also, the ability of degrading dietary carbohydrates produces succinate, acetate, and propionate [37], which may lead *Muribaculaceae* to occupy overlapped niches as *Bacteroides* does. *Bacteroides* also specialize in the fermentation of polysaccharides [39], and members of *Bacteroides* are known to produce succinate, acetate, and propionate by polysaccharides fermentation [40-42]. It may explain why *Bacteroides* exhibit opposite abundance trends compared with *Muribaculaceae*. The abundance of *Bacteroides* was low in both normal control (12%) and IBD mouse model recovery phase (17.3%) but became concentrated in IBD mouse model progressive phase (70.7%). This phenomenon may attribute to

the ability of *Bacteroides* to sense and adapt to gut environmental changes and stress. Firstly, species within *Bacteroides* could utilize host cell surface glycoproteins and glycolipids as nutrients source at sites of infection [43-50], therefore have a superb ability to access nutrients; on the other hand, *Bacteroides* could modulate its surface polysaccharides to an “on” or “off” position [51], thus avoiding the host immune response. These systems used by *Bacteroides* may explain its high abundance in the gut inflammatory environment. Previous study also reported *Bacteroides* species were found in most of anaerobic infections [52]. *Enterotoxigenic B. fragilis*, a member of *Bacteroides*, could secrete B. fragilis enterotoxin [53] and induce cyclooxygenase 2 and fluid secretion in intestinal epithelial cells [54], thus may be associated with occurrence of colorectal cancer and IBD [55, 56].

Another notable change occurred at *Akkermansia*. It has been isolated in 2004 [57] and classified as mucin-utilizing bacteria [58]. However, recent studies indicated that *Akkermansia* may also exert positive regulation on intestinal mucosal thickness and intestinal barrier integrity [59, 60] and be praised as “the next generation of probiotics” [61]. With the deepening of research, *Akkermansia* was thought to act as both “friend and foe”, so it would be unwise to advocate the efficacy of *Akkermansia* until more research and clinical data emerged. A research in China indicated that *Akkermansia* was positively correlated with the risk of Chinese type 2 diabetes [62]. Later, Ijssennagger et al found antibiotic treatment enhanced intestinal barrier function by eliminating sulphide-producing and mucus-degrading bacteria such as *Akkermansia* [63]. Besides, *Akkermansia* also triggers an excessive immune response that disrupts mucus secretion and damage intestinal barrier in IL-10<sup>-/-</sup> mice [64]. In the present study, our data suggested *Akkermansia* was highly enriched in d 5-model (68.4%), and 5 days recovery phase led to normalization of *Akkermansia* bacteria in d 10-model (15.2%), resulting in similar levels to those found in normal control (16.4%). Given this plus results of clinical signs, we could speculate reasonably that the gut microbiota of d10-model tended to restore homeostasis since the *Akkermansia* was considered a marker of gut homeostasis and restoration of the mucus layer in the gut [65].

Though transplantation of colon microbiota of d 5-model to Abx-mice could induce symptoms similar to colitis, it was not enough to infer that *Akkermansia* played as “foe” role in d 5-model. We inclined that pathogenicity was induced by a consortium of microbiota. The presence of *Akkermansia* or any other bacteria alone is not enough to induce these effects. In contrast, the composition and relative abundance of several specific microbes play an important role. The source or underlying cause of the increase of *Akkermansia* is still unclear. Previous study indicated that acetate and/or lactate produced by *Bifidobacterium* could stimulate the growth of *Akkermansia* [66]. Consistent with this, *Bifidobacterium* showed the same trend as *Akkermansia* in our result which may partly explain this elevation.

This study does have limitation because the microbiota composition during self-recovery phase (or return to initial weight) was not measured or the key bacteria may be highlighted to great extent. Secondly, the pathogenicity of altered microbiota community of IBD mouse model could be further interpreted if the microbiota composition in Abx-mice receiving FMT was monitored.

## Conclusions

Overall, our study investigated the alterations of gut microbiota in IBD mouse model in combination with updated studies, and proved the changes conferred the pathogenic effect. Further studies are necessary to determine which species play a key role in pathopoiesis. Our results also provided hint to find keystone in terms of ternary plot of microbiota among treatments, such as analysis of one or one group microbiota enriched in d 5-model. *Lachnospiraceae* and *Lactobacillus* may be of great importance during the recovery of IBD.

## Methods

### Animals

Six to eight-week-old female C57BL/6 mice used in present study were purchased from SPF (Beijing) Biotechnology Co., Ltd. Mice were housed under the same condition (specific pathogen-free conditions: temperature,  $24 \pm 1^\circ\text{C}$ ; lighting cycle, 12 h:12 h light/dark) and had free access to food and drinking water. Experiments on animals were performed in accordance with the Animal Care and Use Committee of China Agricultural University (Beijing, China).

### Mouse models

8 weeks aged female C57BL/6J mice were provided with 5% DSS (36-50 kDa; MP Biomedicals) in drinking water ad libitum for 5 days to established DSS-induced IBD mouse model. Then they received normal drinking water for additional 5 days. The mice were euthanized by  $\text{CO}_2$  asphyxiation on d 0 (as normal control mice,  $n = 9$ ), d 5 (d 5-model,  $n = 9$ ; donor mice for microbiota transplantation,  $n = 9$ ) and d 10 (d 10-model,  $n = 9$ ). Blood, colon tissue and colonic contents were collected; body weight, stool consistency and degree of intestinal bleeding were measured daily. The scoring system displayed in table S2 was described by Wirtz S et.al [18].

For the antibiotic-induced microbiota depletion mouse model (Abx-mice), we used the method as described previously with slight modifications [20, 67-70]. In brief, 6 weeks aged female C57BL/6J mice were delivered with a cocktail containing ampicillin, neomycin, metronidazole and vancomycin (1 g/L, 1 g/L, 1 g/L and 500 mg/L, respectively and all were obtained from Sigma) in drinking water ad libitum for 2 weeks, then had a “rest” for 2 days to prepare for microbiota transplantation.

### Microbiota transplantation

Colonic content samples were freshly collected from donor mice (IBD mouse model on d 5,  $n = 9$ ) and homogenized in sterile PBS (50 mg/mL). Homogenates were passed through a 40  $\mu\text{m}$  cell strainer and then centrifuged at 8500 rpm for 5 min. Precipitation was resuspended with the same volume of 10% glycerol/PBS solution and stored at  $-80^\circ\text{C}$  until used for microbiota transplantations.

Five Abx-mice were orally intragastric administration with 200  $\mu\text{L}$  suspension above once a day for 5 consecutive days (FMT-mice), while 5 mice were orally intragastric administration with the same volume



of 10% glycerol/PBS vehicle as control (Vehicle-mice). Then mice were euthanized and colon samples were collected. Body weight, stool consistency and degree of intestinal bleeding were measured daily.

## **Elisa**

Blood (collected on d 0, d 5 and d 10 of IBD mouse model) was centrifuged to obtain serum. The levels of TNF- $\alpha$  was determined using ELISA kits (SLCY Biotech, Beijing, China). The kit assays were carried out according to the protocol supplied by the manufacturer. The absorbance was read at 450 nm using a multimode microplate reader (iMark, BIORAD, USA).

## **Histology**

Colon sections were removed, the contents were gently extruded and then washed in saline, fixed in 4% paraformaldehyde and embedded in paraffin. After being cut into 4  $\mu$ m thickness slices, tissue sections were stained with hematoxylin and eosin and then examined using a light microscope (Nikon Eclipse Ci, Japan). Photomicrographs were captured using a digital camera attached to the microscope (Nikon digital sight DS-Fi2, Japan). Histological damage was quantitatively assessed as described by Wirtz S et.al [18]. The sum of two sub-scores results in a combined score ranging from 0 (no changes) to 6 (widespread cellular infiltrates and extensive tissue damage). (Table S3)

## **Microbiota composition by 16S rRNA sequencing analysis**

Colonic contents were collected from IBD mouse model on d 0, d 5 and d 10. Genomic DNA was extracted using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). The V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by thermocycler PCR system (GeneAmp 9700, ABI, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2  $\times$  300) on an Illumina MiSeq platform (Illumina, San Diego, USA). Random sampled of all sample effective sequences by the minimum number of effective sequences in the sample. Raw fastq files were demultiplexed, quality-filtered using QIIME (version 1.17). Operational taxonomic units (OTUs) were clustered with 97% similarity by UPARSE62(version 7.1 <http://drive5.com/uparse/>) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each sequence was analyzed by RDP Classifier (<http://rdp.cme.msu.edu/>) against the Silva (SSU115) 16S rRNA database with 70% confidence threshold [71].

## **Statistical analysis**

Statistical analysis was performed using Prism software (GraphPad 7.0). Data were first checked for normal distribution and plotted in the figures as mean  $\pm$  SD. For each figure, n = the number of independent biological replicates. No samples or animals were excluded from the analyses. Differences between two treatment groups were assessed using two-tailed, unpaired Student t test with 95% confidence level. Differences among > 2 groups with only one variable were assessed using one-way ANOVA with Tukey post hoc test. Taxonomic comparisons from 16S rDNA sequencing analysis were

analyzed by Kruskal-Wallis H test with *fdr* post hoc test. Ternary Plot was made using GGTERN software (<http://www.ggtern.com/>). LEfSe was performed to discover bacteria indicator that distinguished the treatment-specific microbiota features, LDA value was used to estimate the effect size of each feature. A significance level (*alpha*) of 0.05 and an effect size threshold of 2 was used for all indicators discussed in this study.

## Abbreviations

Inflammatory bowel disease: IBD; Operational taxonomic units: OTUs; Crohn's disease: CD; Ulcerative colitis: UC; Fecal microbiota transplantation: FMT; short-chain fatty acids: SCFAs.

## Declarations

### Ethics approval

All care, maintenance, and experimental protocols were performed in accordance with the Animal Care and Use Committee of China Agricultural University (Beijing, China).

### Consent for publication

Not applicable.

### Availability of data and materials

All data generated or analyzed during this study are included in this manuscript. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

SYQ and XFZ conceived and designed the experiments. HBL provides help in the design of experiment. ZQD analyzed and interpreted the ELISA data, JL and MXC analyzed and interpreted colon histology

photograph, LJS performed the experiments and wrote the article. All authors have read and approved the version to be published and agreed to be accountable for all aspects of this work.

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# Supplementary File Legends

**Figure S1.** Alpha diversity changes in the process of IBD mouse model.

**Figure S2.** The contribution of taxa on the difference between groups. The different color nodes represented the taxa that were significantly enriched in the corresponding groups and had significant effects on the differences between the groups; taxa that was not associated with differences between groups, or no significant differences between groups were annotated in yellow.

**Figure S3.** Taxonomic distributions of bacteria from 16S rDNA sequencing data.

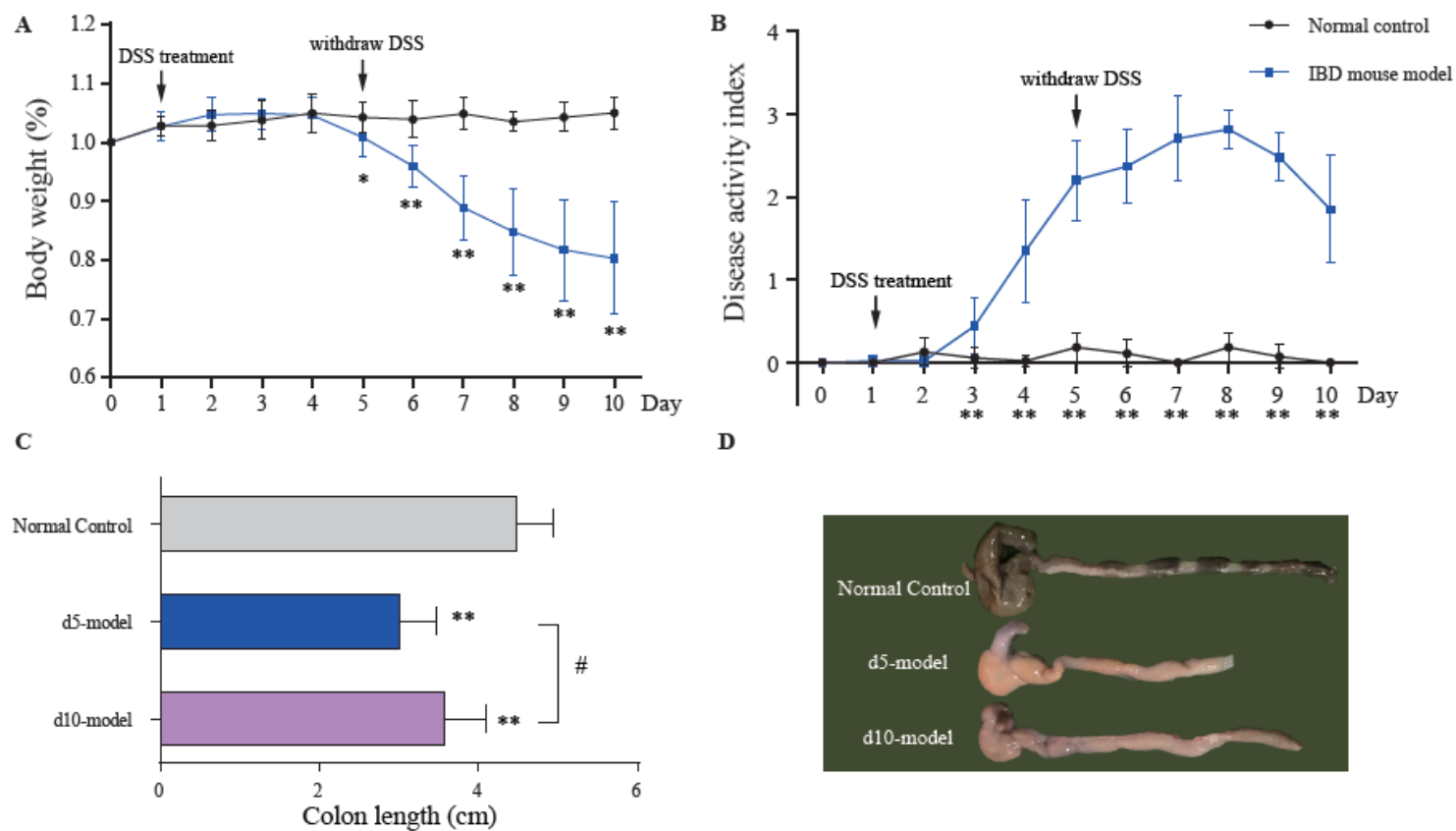
**Figure S4.** The pictures of colon after FMT.

**Table S1.** The significant differently species determined based on the LEfSe method.

**Table S2.** Scoring system for calculating disease activity index (DAI).

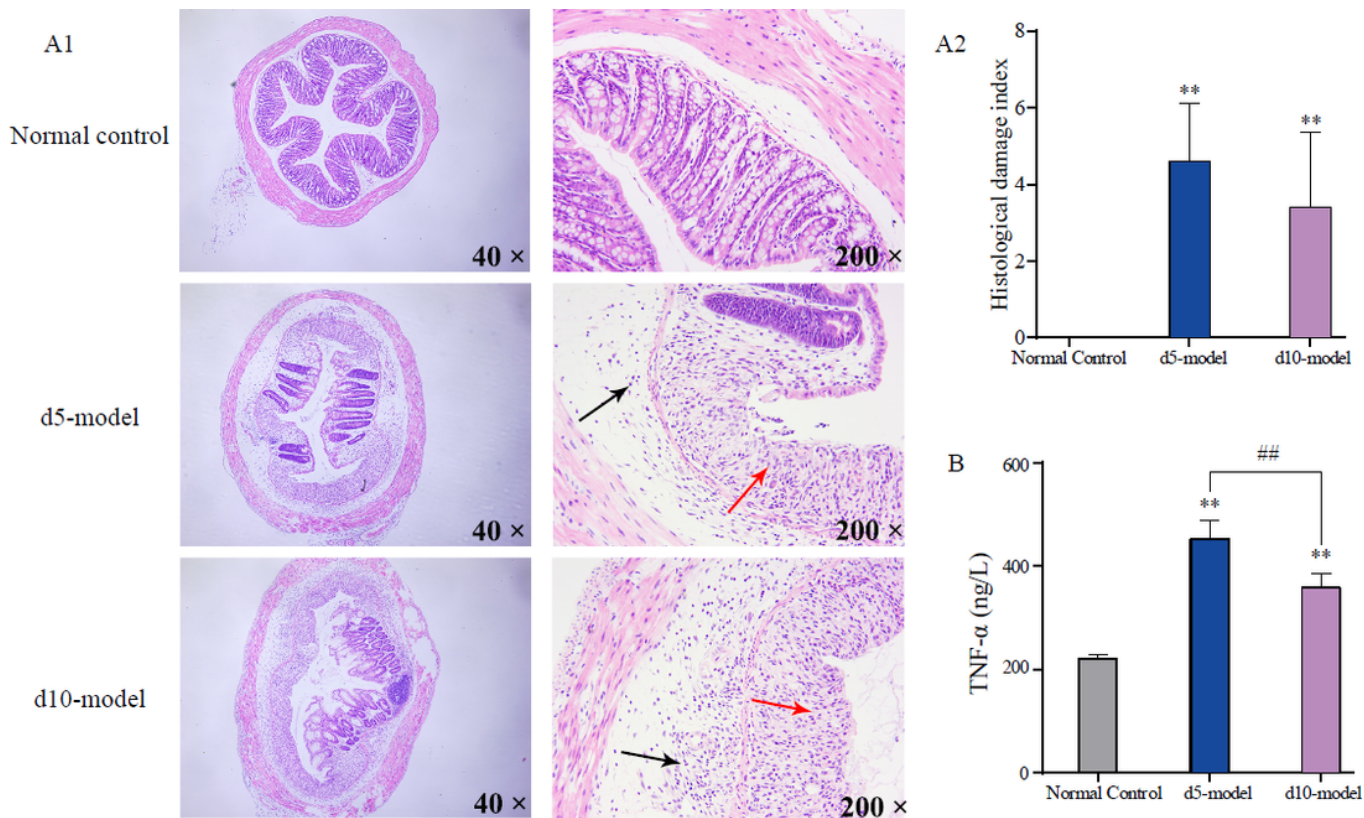
**Table S3.** Scoring system for inflammation-associated histological changes in the colon.

## Figures



**Figure 1**

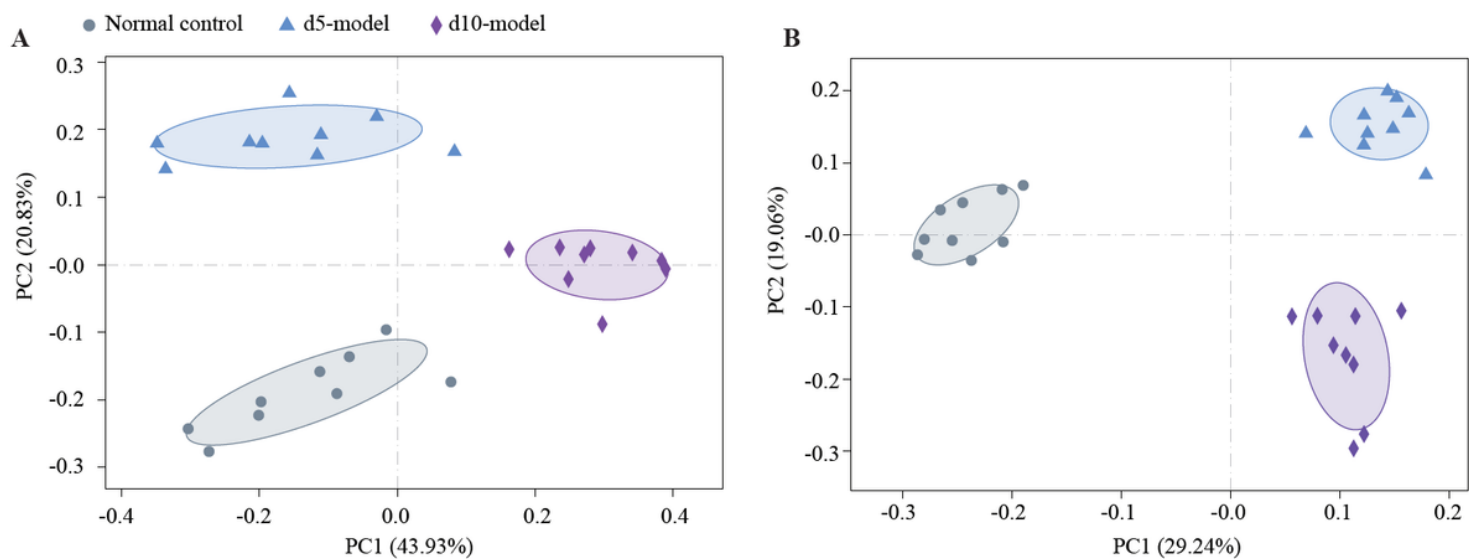
Clinical signs of IBD mouse model. (A) Body weight changes (relative to original weight, set as 100%) and (B) DAI over the whole experimental period. D 0 to d 5, n=18; d 6 to d 10, n = 9. Data were analyzed by two-way ANOVA; (C) Length of the colon between ileocecal junction and the proximal rectum. Data were analyzed by one-way ANOVA, n = 9; (D) The representative pictures of colon. Data are means  $\pm$  SD. \*p < 0.05, \*\*p < 0.01 compared with the normal control group; #p < 0.05 compared with the d 5-model group and the same below.



**Figure 2**

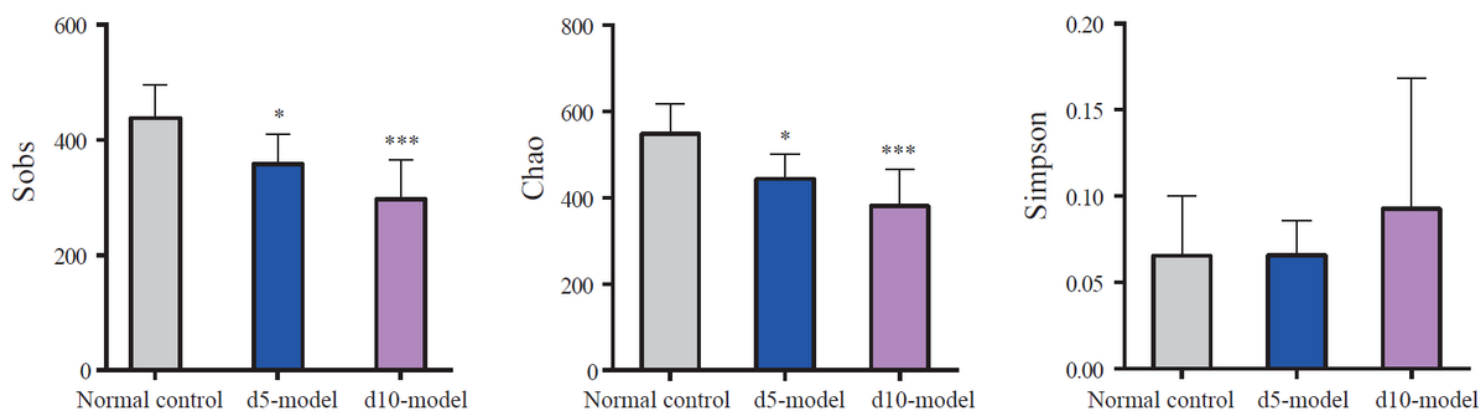
Inflammatory conditions of IBD mouse model. (A1) Representative images of the colon by H&E staining (40 $\times$  200 $\times$ ). The red arrow indicated morphological changes of mucous layer, and the black arrow indicated edema status in submucosa. (A2) Score of inflammation-associated histological changes in colon. (B) The concentration of TNF- $\alpha$  in serum. n = 9, Data are means  $\pm$  SD and analyzed by one-way ANOVA.





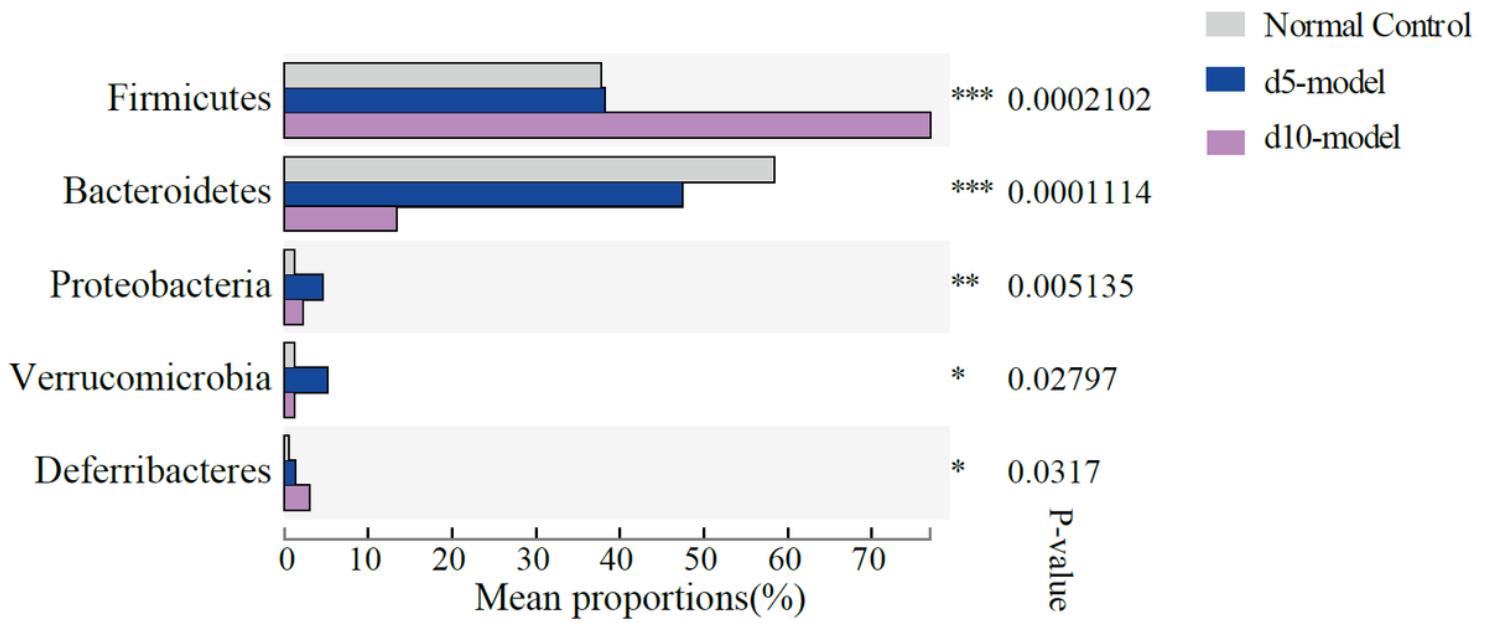
**Figure 3**

Principal coordinate analysis by weighted-unifrac (A) and unweighted-unifrac method (B).

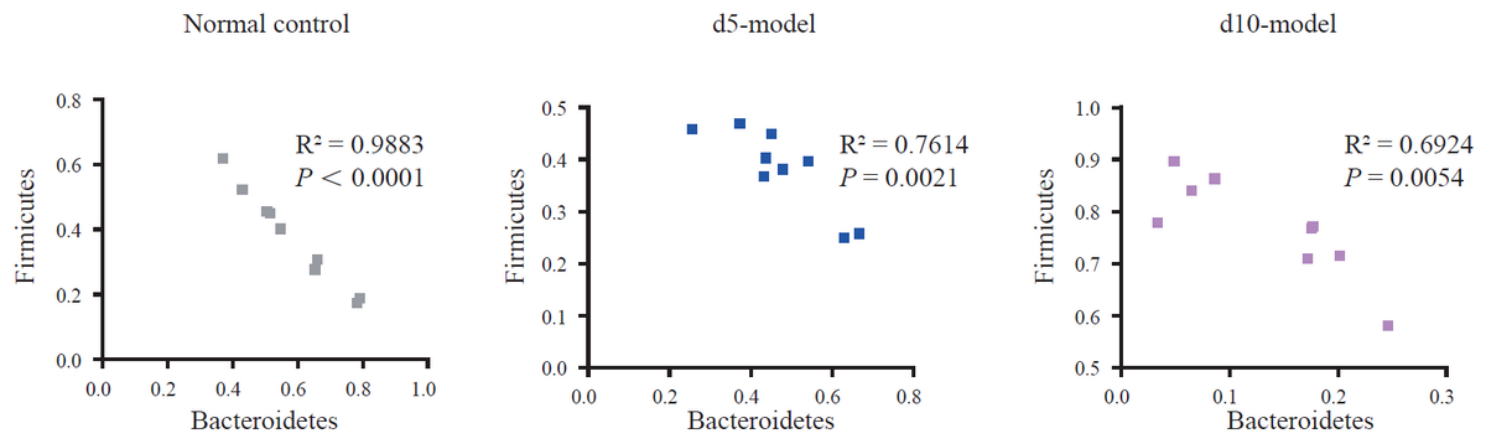


**Figure 4**

The changes of gut microbiota diversity. n = 9, Data are means ± SD and analyzed by one-way ANOVA.

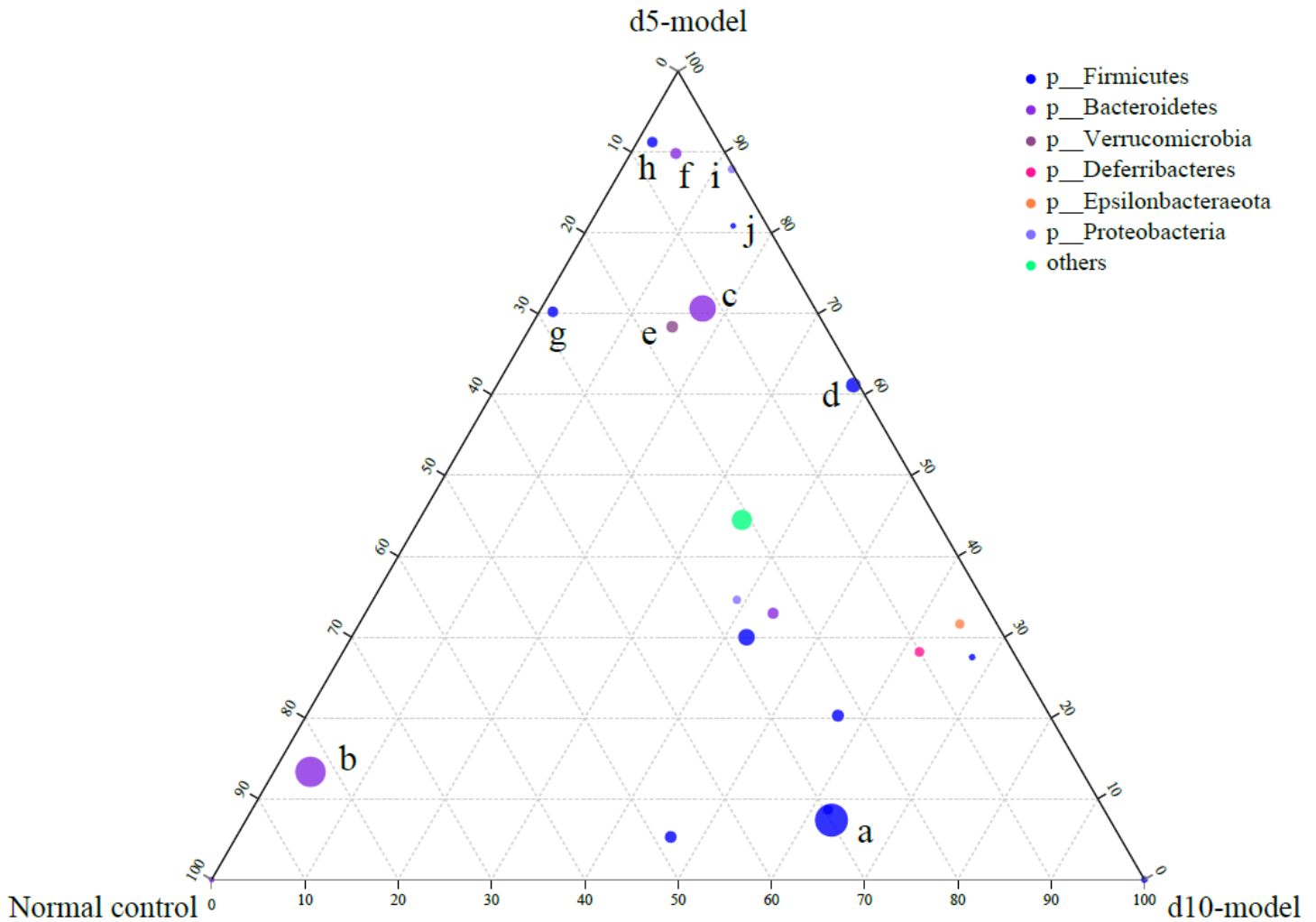


**Figure 5**  
Changes of microbial composition at the phylum level.



**Figure 6**  
The correlation between relative abundances of phylum Firmicutes and Bacteroidetes. Statistical analyses were performed by Pearson’s correlation coefficient.

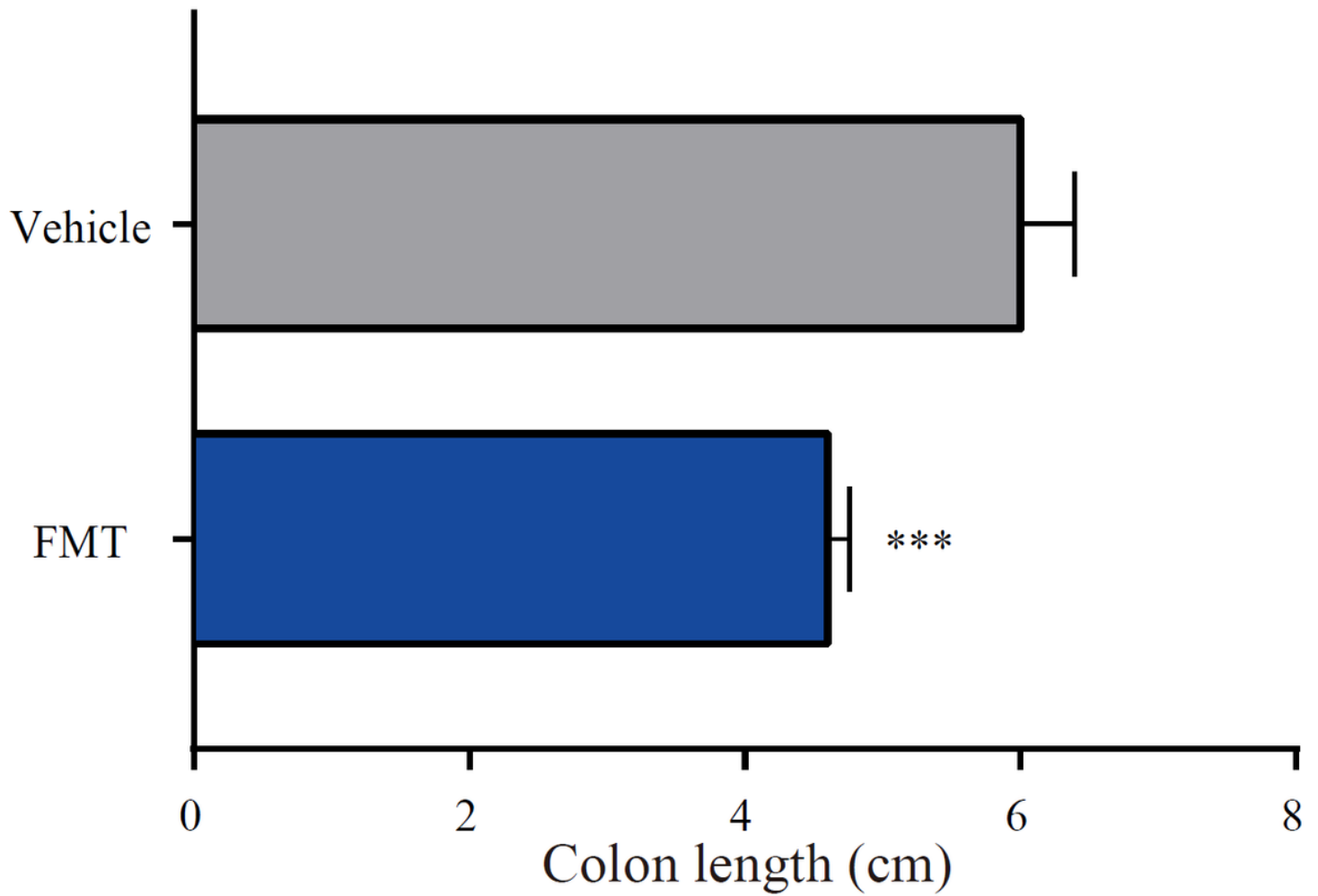
# Ternary analysis



a: Lactobacillus; b: Muribaculaceae; c: Bacteroides; d: Turicibacter; e: Akkermansia; f: Odoribacter; g: Faecealibaculum; h: Ruminocoaceae; i: Escherichia-shigella; j: norank-clostridiales

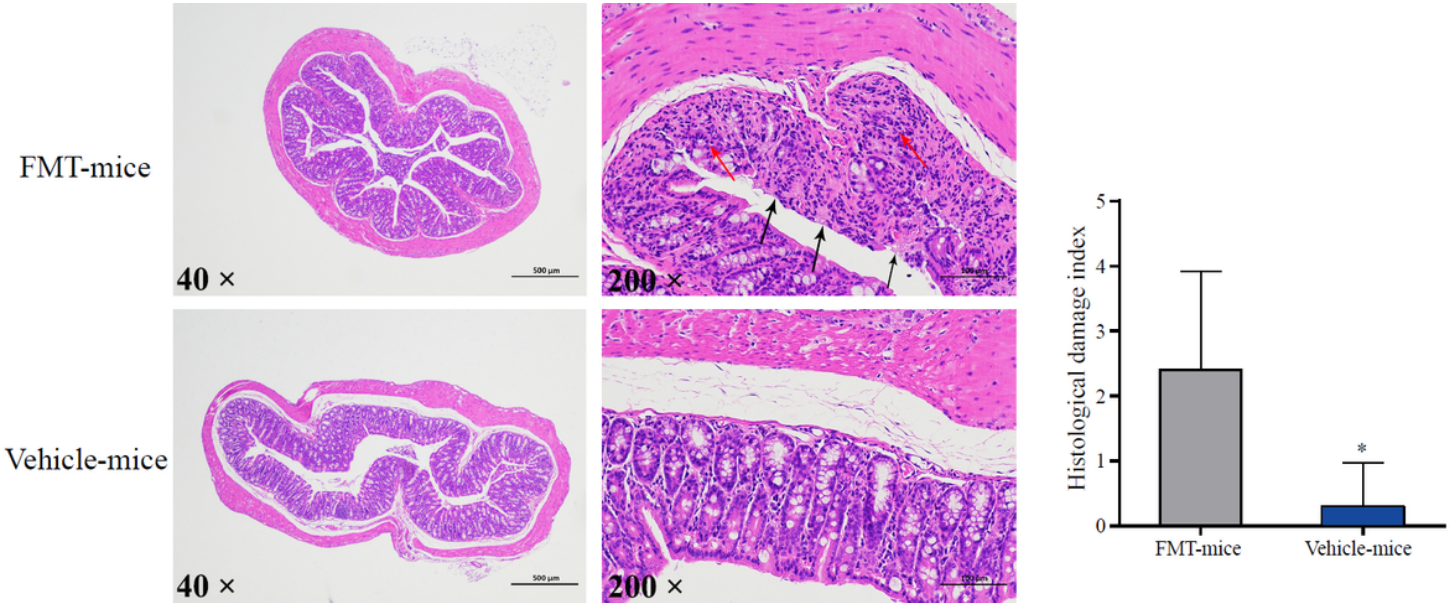
**Figure 7**

Ternary Plot of the process of IBD mouse model. The colored circles represented the phylum level classification of species to which the circles belong in the triangle chart; the circles in the triangle chart represented the genus level of species under a certain phylum level; and the circle size represented the average relative abundance of species.



**Figure 8**

Colon length after FMT. n = 5, Data are means  $\pm$  SD and analyzed by Student's t-test. \*\*\*p $\leq$ 0.0001.



**Figure 9**

Inflammatory conditions of the colon after FMT. (A) Representative images of the colon by H&E staining (40× 200×). The red arrow indicated morphological changes of mucous layer, and the black arrow indicated edema status in submucosa. (B) Score of inflammation-associated histological changes in colon. n = 5, Data are means ± SD and analyzed by Student's t-test.

## Supplementary Files

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