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Saccharomyces cerevisiae and mannan oligosaccharide alleviate adolescent dextran sodium sulfate-induced ulcerative colitis in early-life antibiotic-exposed mice through immunity-gut microbiota

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Abstract

Early-life antibiotic exposure is strongly associated with increased risk of developing ulcerative colitis (UC) during adolescence. While probiotic interventions may confer protective effects by modulating the gut microbiota and immune system, the long-term efficacy of *Saccharomyces cerevisiae* (SC) and its cell wall component mannan-oligosaccharides (MOS) in this context remains unclear. To investigate whether SC, alone or combined with MOS, provides protective effects against dextran sulfate sodium (DSS)-induced colitis in adolescent mice with prior early-life antibiotic exposure, and to elucidate the underlying regulatory mechanisms via the "immune–gut microbiota" axis.Compared with antibiotic exposure alone, SC intervention reduced inflammatory scores in juvenile colitis, downregulated pro-inflammatory mediators such as IL-6 and TNF-α in the colon, and upregulated anti-inflammatory factors including IL-10. SC also partially restored antibiotic-induced reductions in gut microbial α-diversity and promoted enrichment of beneficial bacteria such as *Akkermansia*. Furthermore, SC increased fecal SCFA concentrations (e.g.acetate, butyrate) and enhanced intestinal secretory immunoglobulin A (sIgA) levels. Long-term combined SC and MOS supplementation demonstrated synergistic effects in promoting colonization of beneficial taxa (e.g.*Parabacteroides*), maintaining SCFA homeostasis, and augmenting sIgA secretion. SC provides sustained protection against adolescent colitis by regulating antibiotic-induced immune dysregulation and gut dysbiosis. The addition of MOS further enhances this protective effect, supporting the potential of "probiotic–prebiotic" combination strategies for preventing antibiotic-associated intestinal sequelae.

1. Introduction

Ulcerative colitis (UC), the risk factors of which are mainly dysbiosis, genetics, and environment, along with Crohn's disease (CD), constitutes inflammatory bowel disease (IBD)[1–4]. The incidence of UC is mostly concentrated among adolescents and is increasing annually, making it another public health problem[5–6]. Therefore, the prevention and treatment of UC is particularly important. Antibiotics have been widely used since their discovery and have greatly extended human life expectancy[7–8]. In the early years of life, many factors, such as immunodeficiency and preterm birth, have led to a very common and widespread use of antibiotics[9]. A growing number of studies have shown that antibiotic exposure early in life affects gut bacteria and the immune system, which persist and are associated with the development of long-term diseases, including UC[9–13]. Therefore, early-life intervention to prevent adolescent colitis may become an important primary prevention strategy that can be effective in reducing the incidence of colitis. Moreover, probiotic supplementation after antibiotic exposure in early life may contribute to regulating the gut microbiota and modulating the immune response to alleviate long-term colitis[14–16]. *Saccharomyces* have shown promising preventive and therapeutic effects in diarrhea and antibiotic-induced gastrointestinal disorders[17–19]. *Saccharomyces cerevisiae* (SC), a kind of *Saccharomyces*, has long been used in food fermentation[20]. Recently, it has been highlighted that SC also has probiotic functions, such as antibacterial and anti-inflammatory properties; therefore, SC is expected to be a new probiotic species[21–23]. Additionally, its fungal properties may have benefits that

bacteria do not possess—its special cell wall component, mannan oligosaccharide (MOS), may have a stronger immunomodulatory effect[24]. Nevertheless, it remains unknown whether combined SC and MOS administration can protect against long-term colitis by modulating the "gut microbiota–immune" axis following early antibiotic exposure.

Therefore, this study establishes a mouse model of adolescent UC preceded by early-life antibiotic exposure to evaluate the long-term effects of SC intervention, alone or in combination with MOS. We hypothesize that SC ameliorates antibiotic-induced dysbiosis and immune dysregulation, thereby reducing subsequent colitis severity, while MOS addition further enhances this protective effect through synergistic mechanisms. These findings will provide novel experimental evidence supporting SC application as a preventive probiotic strategy.

2. Materials and Methods

2.1 Mice

Fourteen-day pregnant Balb/c female mice (n = 25) were purchased from Chengdu GemPharmachem Biotechnology Co., Ltd., and they were housed in the Experimental Animal Center of West China School of Public Health, Sichuan University (license number: SYXK2023-0011), under a specific pathogen-free environment.

In this study, 125 newborn mice were included and divided into six groups: NS-water; NS-DSS; Ceftri-DSS; Ceftri+SC-DSS; Ceftri+SC(l)-DSS; and Ceftri+SC+MOS(l)-DSS. All newborn pups were reared at the animal center at an ambient temperature of 23°C, 50%–70% humidity, and a 12-h light–dark cycle with free access to water and food.

This experiment was reviewed and approved by the Ethics Committee of West China Fourth Hospital and West China School of Public Health of Sichuan University (approval number: Gwll2021080).

2.2 Experiment materials

Sterile physiological sodium chloride solution (saline, NS) was purchased from Sichuan Kelun Pharmaceutical Co., LTD. Ceftriaxone (Ceftri, Aladdin Shanghai Biochemical Technology, Shanghai, China), SC CCTCC M 2019905 (active bacterial count of 2.0×10^{10} CFU/g), and MOS (degree of purity: 70%) were dissolved in sterile saline. DSS (M.W. 36–50 kDal) was purchased from MP Biomedicals, LLC (United States) and was dissolved in sterilized ultrapure water.

2.3 Treatment

From birth to 3 weeks (3w), depending on the group, all newborn mice were treated with saline, ceftriaxone (100mg/kg.bw), ceftriaxone + SC, and ceftriaxone + SC + MOS (SC and SC + MOS were administered 2 h after ceftriaxone treatment). At 3w, half of the mice in each group were euthanized

(Figure 1.A). The results of fecal dilution coating experiments indicated that the existence of very small amounts of SC in the gut and SC can survive after reaching the gut (Supplementary Figures S1).

In 4–7 weeks of age, mice in the Ceftri+SC(l)-DSS and Ceftri+SC+MOS(l)-DSS groups continued to gavage SC or SC + MOS, respectively. The remaining four groups of mice were not gavaged during this period. In 6–7 weeks, the drinking water in all "-DSS" groups was replaced with 3% DSS to induce ulcerative colitis, while, in the NS-water group, the drinking water was unchanged. At the end of the experiment, the remaining mice were euthanized (Figure 1.A). The gavage volume and intervention dose are listed in Table S1.

2.4 Histopathological analysis

At the end of the experiment, colon tissues were collected from euthanized mice; these samples were fixed in 10% neutral buffered formalin (Solarbio, Beijing, China) for 48 h, dehydrated in 70% ethanol, embedded in paraffin, frozen at −18°C, and demolded. Each specimen was then sectioned and stained with H&E. Finally, a professional pathology teacher viewed the sections under a microscope (Olympus, Tokyo, Japan) and assessed the pathological condition of the colon tissue.

Inflammation was evaluated using five aspects, and the specific scoring criteria are shown in Table S2. The final score is the sum of the scores of each dimension, with higher scores indicating more severe damage and inflammation of the colon.

2.5 mRNA expression in the colon

Weigh 10 mg of colon tissue into 1.5 mL EP tubes, add 50 mg of grinding beads (Tiangen, China) and Buffer L1 (Foregene, Chengdu, China), and grind twice at 4 M/s for 20 s on a tissue homogenizer (MP Biomedicals, United States). Extract tissue RNA according to the Animal Tissue RNA Isolation Kit instructions (Foregene, Chengdu, China). The extracted RNA was reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, United States) to obtain cDNA. The reverse transcription procedure was as follows: 25°C for 5 min, followed by 46°C for 20 min, and finally 95°C for 1 min. Then, quantitative real-time PCR (qPCR) of the cDNA was performed using a SsoAdvanced Universal SYBR Green Supermix (Bio-Rad). The qPCR protocol was as follows: reaction with an initial denaturation step at 98°C for 30 s, followed by 39 cycles of denaturation at 98°C for 15 s and temperature annealing with an extension step at 60°C for 30 s. The mRNA expression levels of the colon cytokines were normalized using GAPDH.

The expression levels of cytokines in mouse colon tissue were measured, including anti-inflammatory cytokines (i.e., interleukin [IL]-10, IL-13, and tumor growth factor-beta [TGF-b]) and pro-inflammatory cytokines (i.e., IL-5, IL-6, IL-12 (p40), IL-17A, tumor necrosis factor-alpha [TNF-a], and interferon-gamma [IFN-g]). Additionally, the expression of tumor necrosis factor ligand superfamily member 13 (APRIL), mucin 2 (MUC2), Ki67, and proteins associated with tight junctions in the gut (i.e., ZO-1, Claudin,

Occludin), was measured in colon tissue. All primers were synthesized by Sangon Biotech (Shanghai, China; Table S3).

2.6 Analysis of serum cytokine levels

Blood from mice was collected at 3w and 7w and centrifuged at 4°C and 2,000 g for 15 min. Then, the supernatant obtained in the previous experiment was centrifuged at 4°C and 2,000 g for 5 min to obtain the serum. The concentrations of IL-5, IL-6, IL-10, IL-13, IL-17a, and TNF-a in the serum samples were analyzed using Mouse Magnetic Luminex® Assays (Bio-Techne Corporation, United States) and measured using a Luminex 200TM multiplexing instrument (Merck Millipore, United States), and none of the samples were diluted. Serum TGF-b concentrations were analyzed using a Transforming Growth Factor Beta 1 enzyme-linked immunosorbent assay (ELISA) Kit (Elabscience Biotechnology Co., Ltd, Wuhan, China), and the samples were diluted 30 folds.

2.7 16S rRNA sequence

Fresh feces from mice were collected at 3w, 6w, and 7w and then frozen at 80°C. Fecal genomic DNA was extracted from fecal samples (100 mg) at each time point according to the instructions for the TIANamp Stool DNA Kit (Tiangen, Beijing, China). Extracted DNA underwent PCR amplification, product purification, library preparation, and library screening, followed by Novaseq sequencing. The raw data obtained from sequencing were spliced and filtered to obtain clean data. Then, denoising was performed using Divisive Amplicon Denoising Algorithm 2, and sequences with frequencies less than 5 were filtered out to obtain the final amplicon sequencing variants (ASVs).

For the obtained ASVs, a species annotation was made for the representative sequences of each ASV to obtain the corresponding species information and species-based abundance distribution. Simultaneously, the ASVs were analyzed for abundance and alpha diversity to provide information on species richness and homogeneity within samples. Additionally, differences in the community structure among samples or groups were examined by PCoA analysis and beta diversity calculation. To explore the differences in community structure among grouped samples, linear discriminant analysis effect size (LEfSe) was chosen to test the significance of differences in species composition and community structure from clustered samples.

2.8 Analysis of short-chain fatty acids (SCFAs)

Fifty-milligram stool samples were homogenized with 500 mL water and 100 mg glass beads for 1 min and then centrifuged at 4°C and 12,000 rpm for 10 min. Then, 200 mL of supernatant was extracted with 100 mL of 15% phosphoric acid and 20 mL of 375-g/mL 4-methylvaleric acid solution as IS and 280 mL of ether. After vortexing for 1 min, the samples were centrifuged at 4°C and 12,000 rpm for 10 min, and the supernatant was transferred to a vial before gas chromatography–mass spectrometry (GC-MS) analysis.

Regarding GC conditions, GC analysis was performed using a Trace 1300 gas chromatograph (Thermo-Fisher Scientific, USA). The GC equipment was equipped with an Agilent HP-INNOWAX capillary column (30 m × 0.25 mm ID × 0.25 mm), and helium was used as the carrier gas at a rate of 1 mL/min. The injection was performed in the split mode at a ratio of 10:1 with an injection volume of 1 mL and an injector temperature of 250°C. The temperature of the ion source and MS transfer line were 300°C and 250°C, respectively. The column temperature was programmed to ramp from an initial temperature of 90°C, to 120°C at 10°C/min, to 150°C at 5°C/min, and finally to 250°C at 25°C/min, which was maintained for 2 min. Regarding MS conditions, MS detection of metabolites was performed using an ISQ 7000 (Thermo-Fisher Scientific, USA) in the electron impact ionization mode. The single ion monitoring mode was used with an electron energy of 70 eV.

2.9 Determination of secretory immunoglobulin A (sIgA) level

Fecal samples (50 mg) were soaked in 200 mL phosphate-buffered saline, homogenized, and centrifuged at 1,000 g for 10 min at room temperature. The supernatant was used for further analysis. The sample supernatant was diluted 2,500 folds at 3w and 10,000 folds at 7w. The assay was performed according to the instructions for the Mouse sIgA ELISA kit (Elabscience Biotechnology Co., Ltd., Wuhan, China). Finally, the absorbance at 450 nm was recorded using a microplate reader (Thermo-Fisher, Shanghai, China). The data were fitted with a four-parameter logistic function.

2.10 Immunohistochemical （IHC） staining of intestinal tissue

The paraffin sections were de-waxed and hydrated by xylene I for 15 min, xylene II for 15 min, 100% ethanol I for 2 min, 100% ethanol II for 2 min, 90% ethanol for 2 min, 80% ethanol for 2 min, 70% ethanol for 2 min, ultrapure water I for 2 min, and ultrapure water II for 2 min, and then put into citrate buffer (pH 6.0) in microwave oven heating for 12 min to repair the antigen. Staining according to the 2-step plus Poly-HRP Anti Goat IgG Detection System (With DAB Solution) (Elabscience Biotechnology Co., Ltd., Wuhan, China). After hematoxylin-stained nuclei, sections were dehydrated by 70% ethanol for 2 min, 80% ethanol for 2 min, 90% ethanol for 2 min, 100% ethanol II for 2 min, 100% ethanol I for 2 min, xylene II for 10 min, and xylene I for 10 min, and then sealed with neutral resin. Air-dried for 48 h and viewed the sections under a microscope. The dilution ratio of primary antibody is 1:50 for Ki67, 1:120 for MUC2, and 1:200 for pIgR.

2.11 Statistical analysis

Data analyses were performed using GraphPad Prism 9.5.0. Data are presented as means ± standard deviations. One-way analysis of variance was used for multiple comparisons, and post hoc pairwise comparisons were performed using Tukey's test to adjust for multiple comparisons. The t-test was used for comparisons between two groups. Pearson correlation analysis was used to analyze the correlation between gut microbiota and SCFAs. P-values < 0.05 were used to denote statistical significance.

3. Results

3.1. DSS-induced ulcerative colitis in adolescence (7w)

After DSS induction, compared with the NS-water group (1.71 ± 0.76), inflammation scores in all colitis-induction groups increased apparently ($p < 0.001$) (NS-DSS: 12.71 ± 3.90; Ceftri-DSS: 9.17 ± 2.20; Ceftri+SC-DSS: 5.83 ± 2.41; Ceftri+SC(l)-DSS: 6.50 ± 2.43; and Ceftri+ SC+MOS(l)-DSS: 4.31 ± 2.18) (Figure 1.B). The Ceftri-DSS group scored lower than the NS-DSS group ($p < 0.05$) (Figure 1.B), whereas the Ceftri+SC-DSS, Ceftri+SC(l)-DSS, and Ceftri+ SC+MOS(l)-DSS groups scored lower than the Ceftri-DSS group ($p < 0.01$, 0.05, and 0.001, respectively) (Figure 1.B).

Regarding the H&E-stained sections of the colon (Figure 1.C), the NS-DSS group had the most severe inflammatory cell infiltration, mucosal detachment and necrosis, congestion, hemorrhage, and ulceration of the colon tissue. Colonic tissue structure and inflammatory infiltration were better in the Ceftri-DSS group than in the NS-DSS group, but not as good as those in the Ceftri+SC-DSS, Ceftri+SC(l)-DSS, and Ceftri+SC+MOS(l)-DSS groups.

3.2. Changes in the expression of inflammatory cytokines in the colon

At adolescent ulcerative colitis, IL-10 expression was higher in the Ceftri+SC(l)-DSS group than in the NS-DSS group ($p < 0.05$), The expression level of IL-13 was lowest in the Ceftri-DSS group ($p < 0.01$, 0.01, 0.05, 0.05, and 0.05, respectively), and the NS-DSS and Ceftri+SC+MOS(l) groups had higher TGF-b expression levels than the Ceftri-DSS group ($p < 0.05$ and 0.05, respectively) (Figure 2.A). Compared with the NS-water group, the mRNA expression levels of IL-5, IL-6, and IL-12(p40) were higher in the NS-DSS group ($p < 0.001$, all) (Figure 2.B). IL-5 and IL-6 expression was lower in all “Ceftri- “groups than in the NS-DSS group ($p < 0.001$, all), IL-12 (p40) mRNA expression was upregulated in the Ceftri-DSS group ($p < 0.001$) and downgraded in the Ceftri+SC-DSS, and Ceftri+SC+MOS(l)-DSS groups ($p < 0.01$, 0.05), the mRNA expression levels of IL-17A were lower in the Ceftri+SC-DSS, Ceftri+SC(l)-DSS, and Ceftri+SC+MOS(l)-DSS groups than in the NS-DSS and Ceftri-DSS groups ($p < 0.05$), the expression of TNF-a and IFN-g was upregulated in the Ceftri-DSS group, with higher levels than those in the NS-water group ($p < 0.01$) and in the NS-DSS group ($p < 0.05$) respectively, and the mRNA expression levels of TNF-a and IFN-g were lower in the Ceftri+SC-DSS, Ceftri+SC(l)-DSS, and Ceftri+SC+MOS(l)-DSS groups than in the Ceftri-DSS group ($p < 0.05$) (Figure 2.B).

At early life, the mRNA expression of IL-13 was higher in the Ceftri+SC group than in the Ceftri group ($p < 0.05$) and the mRNA expression of TGF-b was higher in the Ceftri+SC group than in the NS and Ceftri+SC+MOS groups ($p < 0.001$, 0.05, respectively) (Figure 2.C). The mRNA expression of IL-6 was lower in the Ceftri group than in the NS and Ceftri+SC groups ($p < 0.05$ and 0.01, respectively) and the mRNA expression of TNF-a was lower in the Ceftri group than in the NS group ($p < 0.05$) (Figure 2.D).

From early life to adolescent ulcerative colitis, cytokine expression building processes altered significantly.Colonic IL-10 and IL-13 mRNA expression was significantly elevated in Ceftri+SC(l)-DSS

group, and IL-13 and TGF-b mRNA expression was significantly reduced in Ceftri-DSS group (Figure 2.E). IL-17A and TNF-a mRNA expression was significantly reduced in Ceftri+SC+MOS(l)-DSS group,and TNF-a and IFN-g mRNA expression was significantly elevated in Ceftri-DSS group (Figure 2.F)

3.3. Changes in the levels of inflammatory cytokines in serum

At adolescent ulcerative colitis, serum IL-10 levels were lowest in the Ceftri-DSS group, and the differences were statistically significant compared with the NS-DSS, Ceftri+SC-DSS, and Ceftri+SC+MOS(l) groups ($p < 0.05$, 0.001, and 0.001, respectively), the Ceftri+SC(l)-DSS group had higher serum IL-13 levels than the other two SC intervention groups ($p < 0.001$ and 0.05, respectively), and the Ceftri+SC-DSS group had higher TGF-b level than the Ceftri-DSS group ($p < 0.01$) (Figures 3.A). Serum IL-5 levels were reduced in all DSS-induced groups compared with the NS-water group ($p < 0.05$, 0.01, 0.001, 0.001, and 0.001, respectively), Serum IL-6 levels were higher in the NS-DSS and Ceftri-DSS groups than in the NS-water group ($p < 0.001$) and serum IL-6 levels were lower in the Ceftri+SC-DSS and Ceftri+SC+MOS(l)-DSS groups than in the Ceftri-DSS group ($p < 0.01$ and 0.05, respectively), serum IL-17A level was lower in the Ceftri+SC(l)-DSS group than in the Ceftri-DSS group ($p < 0.01$), and serum TNF-a levels were higher in the NS-DSS and Ceftri-DSS groups than in the NS-water group ($p < 0.001$) and serum TNF-a levels were lower in the Ceftri+SC-DSS, Ceftri+SC(l)-DSS, and Ceftri+SC+MOS(l)-DSS groups ($p < 0.001$) (Figure 3.B).

At early life, higher serum IL-13 level was observed in the Ceftri+SC+MOS group than in the Ceftri group ($p < 0.05$), and higher serum TGF-b level were observed in the Ceftri+SC group than in the NS group ($p < 0.05$) (Figure 3.C). The serum IL-5 level in the NS group was the highest among all groups ($p < 0.05$, 0.05, and 0.01, respectively), and lower serum IL-6 and TNF-a levels were observed in the Ceftri+SC and Ceftri+SC+MOS groups than in the Ceftri group ($p < 0.05$) (Figure 3.D).

From early life to adolescent ulcerative colitis, IL-10 content was significantly increased in the Ceftri+SC+MOS(l)-DSS group,and TGF-b content was significantly increased in the Ceftri+SC-DSS group(Figure 3.E). The process of serum pro-inflammatory cytokines contents change was different from that in colonic mRNA expression, which tended more to stabilize pro-inflammatory cytokine changes, thus reducing serum pro-inflammatory cytokine content (Figure 3.F).

3.4. Changes in the gut microbiota at early life(3w), adolescence(6w), and adolescent ulcerative colitis(7w)

The principal co-ordinates analysis (PCoA) based on weighted unifrac distance (Figure 4.A). At 3w, PC1 axis contribution was 76.73% and PC2 axis contribution was 11.91%, the samples in each group were close to each other, and the groups were separated from each other. At 6w, PC1 axis contribution was 53.81% and PC2 axis contribution was 11.46%, the distance between samples in each group increased from early life, and separation of the groups was less apparent than early life. At 7w, PC1 axis contribution was 46.99% and PC2 axis contribution was 18.74%, the NS-water and NS-DSS groups were

separated slightly, the distance between samples in each group also increased from early life, and separation of the groups was also less apparent than early life.

At 3w, antibiotics decreased alpha diversity apparently and in the Ceftri+SC group, species evenness was restored to some extent (Supplementary Figures S2.A). From early life to adolescence, recovery of alpha diversity in all antibiotic-exposed groups, but did not return to normal, the effects of antibiotics on alpha diversity persisted (Figure 4.B) (Supplementary Figures S2.B). At 7w, alpha diversity was reduced by DSS-induced colitis (Figure 4.B) (Supplementary Figures S2.C).

At the phylum level, early life changes were significant the predominant phyla in the NS group were *Firmicutes*, *Bacteroidota* and *Verrucomicrobiota* (0.37, 0.40, and 0.18, respectively), in the Ceftri group were *Firmicutes* and *Bacteroidota* (0.92 and 0.06), in the Ceftri+SC group were *Firmicutes*, *Bacteroidota* and *Verrucomicrobiota* (0.87, 0.09, and 0.03, respectively), and in the Ceftri+SC+MOS group were *Firmicutes* and *Bacteroidota* (0.96 and 0.03) (Figure 4.C). Linear discriminant analysis Effect Size (LEfSe) analysis to identify biomarkers with statistical differences between groups at the phylum level at different time were *Firmicutes*, *Bacteroidota*, *Verrucomicrobiota*, and *Proteobacteria (*Supplementary Figures S3). From early life to adolescence to adolescent colitis, the construction process of *Firmicutes*, *Bacteroidota*, *Verrucomicrobiota*, and *Proteobacteria* in the antibiotic-exposed groups was different from that in the “NS- “groups (Figure 4.D).

At 3w, the relative abundance of *Lactobacillus* in the Ceftri group was significantly higher and the relative abundance of the remaining genus was lower compared to the NS group, the relative abundance of *Clostridia_vadinBB60_group*, *Akkermansia*, *Muribaculaceae*, *Staphylococcus*, *Bacteroides*, *Alloprevotella*, *Robinsoniella, Anaeroplasma, Clostridia_UCG-014, Clostridioides, Lachnoclostridium, Clostridium_innocuum_group,* and *Alistipes* was higer in the Ceftri+SC group than in the Ceftri group, and the relative abundance of *Staphylococcus*, *Clostridia_vadinBB60_group*, *Akkermansia*, *Alloprevotella*, *Robinsoniella*, *Clostridia_UCG-014*, *Prevotellaceae_UCG-001*, *Clostridioides, Lachnoclostridium, Clostridium_innocuum_group,* and *Lachnospiraceae_NK4A136_group* in the Ceftri+SC+MOS group was higher than Ceftri group.At 6w and 7w, the composition at the genus level in the antibiotic-exposed groups was different from that in the “NS- “groups (Figure 4.E).

LEfSe analysis to ideally identify biomarkers with statistical differences between groups at the genus level (Figure 4.F). At 3w, the biomarkers with statistical differences in the NS group were *Lactobacillus*, *Akkermansia*, *Muribaculaceae*, *Bacteroides*, and *Alloprevotella,* in the Ceftri group was *Lactobacillus*, and in the Ceftri+SC group were *Anaeroplasma*, *Robinsoniella*, and *Clostridia_vadinBB60_group*. At 6w, the biomarkers with statistical differences in the in the “NS-“ groups were *Muribaculaceae*, *Lactobacillus*, *Bacteroides*, *Alloprevotella*, *Alistipes*, and *Lachnoclostridium*, in the Ceftri-DSS group were *Bacteroides*, *Blautia*, *Parasutterella*, and *Ruminococcus_gnavus_group*, in the Ceftri+SC-DSS group were *Alloprevotella* and *Escherichia–Shigella*, in the Ceftri+SC(l)-DSS group were *Lachnoclostridium*, *Akkermansia*, and *Erysipelatoclostridium*, in the Ceftri+SC+MOS(l)-DSS group were *Parabacteroides*, *Dubosiella*, and *Rikenellaceae_RC9_gut_group*. At 7w, the biomarkers with statistical differences in the

NS-DSS group were *Odoribacter* and *Lactobacillus*, in the Ceftri-DSS group were *Parasutterella* and *Ruminococcus_gnavus_group*, in the Ceftri+SC-DSS group were *Clostridium_innocuum_group*, *Bacteroides*, *Escherichia–Shigella*, and *Erysipelatoclostridium*, in the Ceftri+SC(l)-DSS group *Blauti*a and *Akkermansia*, and in the Ceftri+SC+MOS(l)-DSS group was *Parabacteroides.*

3.5. Changes in the SCFAs at early life(3w), adolescence(6w), and adolescent ulcerative colitis(7w)

At 3w (Figure 5.A), total SCFAs content was reduced in Ceftri, Ceftri+SC, and Ceftri+SC+MOS groups (p < 0.001) and total SCFAs content in the Ceftri+SC+MOS group was higher than in the Ceftri group (p < 0.001). All SCFAs content was reduced in all antibiotic-exposed groups (p < 0.05), the levels of isobutyric and isovaleric acids in the Ceftri+SC group were higher than in the Ceftri group (p < 0.01), and the levels of acetic, isobutyric, isovaleric, and caproic acids were higher in the Ceftri+SC+MOS group than in the Ceftri group (p < 0.001, 0.05,0.05, and 0.001, respectively).

At 6w (Figure 5.B), no statistically significant difference in total SCFAs content was observed between the groups. The level of valeric acid in the Ceftri-DSS group was lower than in the “NS- “groups (p < 0.01), the levels of caproic acid in the Ceftri-DSS and Ceftri+SC-DSS groups were lower than in the “NS- “and Ceftri+SC(l)-DSS groups(p < 0.05), and the level of caproic acid in the Ceftri+SC+MOS(l)-DSS group was lower than in the NS-water and Ceftri+SC(l)-DSS groups(p < 0.001 and 0.05).

At 7w (Figure 5.C), total SCFAs content in the Ceftri-DSS group was higher than in the NS-DSS, Ceftri+SC-DSS, and Ceftri+SC(l)-DSS groups (p < 0.05, 0.01, and 0.05, respectively). The acetic acid content in the “NS-“ and Ceftri+SC-DSS groups, the propionic acid content in the NS-DSS, Ceftri+SC-DSS and Ceftri+SC(l)-DSS groups, and the isovaleric acid content in the NS-DSS group were lower than in the Ceftri-DSS group (p < 0.05), and the butyric acid content in the Ceftri-DSS, Ceftri+SC(l)-DSS and Ceftri+SC+MOS(l)-DSS groups were lower than in the NS-water group(p < 0.01, 0.05, and 0.01, respectively). The levels of valeric acid decreased in all DSS-induced groups compared with those in the NS-water group (p < 0.05), and the levels of caproic acid decreased in all” Ceftri- “groups compared with those in the “NS- “groups (p < 0.05).

Rikenellaceae_RC9_gut_group and *Muribaculaceae* were positively correlated with total SCFAs (r= 0.5825 and 0.6305), and *Lactobacillus* was negatively correlated with total SCFAs (r= -0.8419) (Figure 5.D). *Lactobacillus* was negatively correlated with acetic, propionic, isobutyric, butyric, and isovaleric acids (r= -0.8353, -0.8391, -0.7832, -0.6907 and -0.8282), and *Anaeroplasma* was negatively correlated with propionic acid (r= -0.5032) (Figure 5.E). *Lachnospiraceae_NK4A136_group* was positively correlated with propionic, isobutyric, butyric, isovaleric, valeric, and caproic acids (r= 0.5002, 0.5395, 0.6449, 0.5772, 0.8288, and 0.7374), *Muribaculaceae* was positively correlated with acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids (r= 0.5878, 0.6666, 0.7229, 0.8810, 0.5906, and 0.6307), *Rikenellaceae_RC9_gut_group* was positively correlated with acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids (r= 0.5634, 0.5814, 0.6231, 0.6305, 0.5024, and 0.7379), *Prevotellaceae_UCG_001* was positively correlated with valeric, butyric, and isobutyric acids (r= 0.5911, 0.6037, and 0.6244), *Alistipes* was positively correlated with butyric, valeric, and caproic acids

(r= 0.5567, 0.5213, and 0.5561), *Alloprevotella* was positively correlated with butyric acid (r= 0.5992), *Odoribacter* was positively correlated caproic acid (r= 0.5763), and *Parabacteroides* was positively correlated with acetic acid (r= 0.5123) (Figure 5.F).

3.6. Changes in intestinal mucosal barrier

At 7w, All DSS-induced groups had higher sIgA levels than the NS-water group ($p < 0.001$, 0.05, 0.01, 0.05, and 0.001, respectively), Ceftri+SC(l)-DSS group had lower sIgA level than NS-DSS group ($p < 0.05$), and sIgA levels were hihger in the Ceftri+SC+MOS(l)-DSS group than in the Ceftri-DSS, Ceftri+SC-DSS, and Ceftri+SC(l)-DSS groups ($p < 0.001$, 0.01, and 0.001, respectively) (Figure 6.A). There was no statistical difference in APRIL expression between the groups (Figure 6.B). Colonic Ki67 mRNA expression was downregulated in all DSS-induced groups ($p < 0.001$, 0.01, 0.001, 0.001, 0.001, and 0.001, respectively), Ki67 mRNA expression was higher in the Ceftri-DSS group than in the NS-DSS and Ceftri+SC-DSS groups ($p < 0.01$ and 0.05) (Figure 6.C). There was a noticeable reduction of colonic Ki67 content in all DSS-induced groups (Figure 6.D). MUC2 expression was higher in the NS-DSS group than in the NS-water, Ceftri-DSS, Ceftri+SC, and Ceftri+SC+MOS(l)-DSS groups ($p < 0.01$, 0.01, 0.001, and 0.05, respectively) (Figure 6.E). There was a reduction in the colonic content of MUC2 in the NS-DSS and Ceftri-DSS groups, and no significant variations in MUC2 content were observed among the other groups (Figure 6.F). Statistical analysis revealed no significant differences in the expression of ZO-1 in all groups, the NS-DSS group had the highest level of Claudin-1 expression ($p < 0.001$, all), and Occludin expression was downregulated in all DSS-induced groups ($p < 0.01$, 0.001, 0.001, 0.001, 0.001, and 0.001, respectively) (Figure 6.G).

At 3w, all antibiotic-exposed groups had higher sIgA levels than the NS group ($p < 0.01$, 0.01, and 0.001) (Figure 6.H). APRIL expression was lower in the Ceftri group than in the NS, Ceftri+SC, and Ceftri+SC+MOS groups ($p < 0.05$, 0.01, and 0.05) (Figure 6.I). Colonic Ki67 expression tended to be lower in the Ceftri+SC+MOS group than in the NS group ($p < 0.05$) (Figure 6.J), and there was a noticeable reduction of colonic Ki67 content in all “Ceftri- “groups (Figure 6.K). MUC2 expression and content did not differ between groups (Figure 6.L, M). There were no differences in the expression of ZO-1, Claudin-1, and Occluding among the groups (Figure 6.N).

4. Discussion

Altered gut microbial composition is associated with the development of UC, and early life is the beginning and critical phase of building up the gut microbiota, in which the gut microbiota is extremely vulnerable[9–12, 25]. The incidence of UC in adolescence is high, and exposure to antibiotics can lead to disturbances in the gut microbiota, which is implicated in the development of diseases in adolescence, such as allergies, obesity, and intestinal diseases[9–12]. It has been experimentally demonstrated that probiotics can relieve symptoms and reduce susceptibility to UC[26–28]. However, evidence on the effect of SC supplementation after antibiotic exposure in early life on the development of UC in adolescence is limited. Therefore, this study is the first to apply SC and SC + MOS to an early-life antibiotic exposure

model and observe their effects on adolescent colitis, which could provide ideas on UC prevention and a theoretical basis for further application of SC.

The colonic inflammation score is the most straightforward indicator to assess the severity of colitis, and pathological H&E sections can evaluate structural changes in the colon[29]. After the DSS intervention, all "-DSS" groups scored significantly higher than the NS-water group, indicating that colitis induction was successful. Normally, DSS-induced adolescent colitis leads to a series of changes: inflammation development (increased expression of colonic pro-inflammatory cytokines [IL-5, IL-6, IL-12] and levels of serum pro-inflammatory cytokines [IL-6, TNF-a]), damage of intestinal structure, disturbances of the gut microbiota (decreased diversity, altered structure, decreased levels of SCFAs), increased sIgA secretion in response to a combination of gut microbiota and inflammation, and dysfunction of the intestinal mechanical barrier (decreased proliferative capacity of enterocytes, consumption of mucin 2, and decreased tight junction proteins). Interestingly, early life antibiotic exposure alleviated DSS-induced colitis in adolescence moderately. More importantly, antibiotic exposure followed by the administration of SC provided further protection against adolescent colitis. What's more, the protection of long-term use of SC + MOS was more pronounced.

SC regulated immune system alterations induced by antibiotic exposure in early life, and this regulation had long-term effects, alleviating adolescent UC in early-life antibiotic-exposed mice. Antibiotic exposure changed immune homeostasis early in life, and the combined effects of altered homeostatic and constructive processes led to the reduction of colonic expression of a minority of pro-inflammatory cytokines (IL-5 and IL-6) to alleviate DSS-induced UC during adolescence, but at the same time, this was accompanied by decreased colonic expression of anti-inflammatory cytokines (IL-13 and TGF-β), increased colonic expression of pro-inflammatory cytokines (IL-12(p40), TNF-a, and IFN-γ), and increased content of pro-inflammatory cytokines (IL-6 and TNF-a), which may not only aggravate inflammation but also increase intestinal permeability as colitis progresses[30]. SC further regulated the altered immune system caused by antibiotics in early life, and the process of construction from early life to adolescent UC was consequently changed. This modification of immune system and construction processes was different from that caused by antibiotic exposure alone, and it play a protective role for adolescent UC: decreases the pro-inflammatory response and strengthens the anti-inflammatory response, reducing the UC inflammatory response and protect the intestinal mucosal barrier[31–34]. Interestingly, long-term use of SC and SC + MOS had a significant effect on strengthening the anti-inflammatory response, further protecting adolescent UC.

SC regulated gut microbiota and SCFAs changes induced by antibiotic exposure in early life, and this regulation had long-term effects, alleviating adolescent UC in early-life antibiotic-exposed mice by regulating the composition of the gut microbiota and preventing an abnormal increase in SCFAs, and long-term use of SC and SC + MOS both showed a role in promoting beneficial bacterial colonization. The antimicrobial effects of antibiotics significantly changed the structure and composition of the gut microbiota and decreased the levels of SCFAs in early life. SC can restore the decrease in relative abundance of bacteria caused by antibiotics partly and increase the relative abundance of SCFAs -

producing bacteria at the same time, thereby providing the source of energy for intestinal cells to influence intestinal epithelial barrier and defense functions and modulate innate and adaptive immunity to modulate the effects of antibiotic exposure[35]. Gut microbiota constructive processes also changed caused by antibiotics: after cessation of the intervention and natural development, the gut microbiota reshaped totally, which was completely different from normal, and the relative abundance of harmful bacteria and some short-chain fatty acids increased during adolescent UC, which may promoting inflammatory responses in mice through activation of the NF-κB pathway and protein kinase signaling, etc., aggravating inflammation response in UC[36–39]. At adolescent UC, SC plays a protective role by reducing the increased relative abundance of harmful bacteria caused by antibiotic exposure and increasing the abundance of some harmless bacteria and reducing the abnormal increase in SCFAs to normal levels. Long-term use of SC and SC + MOS both increased the relative abundance of beneficial bacteria, but the species varied. Long-term use of SC significantly increased the relative abundance of *Lachnoclostridium* and *Akkermansia* at adolescence and increased the abundance of *Blauti*a and *Akkermansia* at adolescent UC. Long-term use of SC + MOS significantly increased the relative abundance of *Parabacteroides*, *Dubosiella*, and *Rikenellaceae_RC9_gut_group* at adolescence and increased the abundance of *Parabacteroides* at adolescent UC. Increased beneficial bacteria can stimulate mucin production, modulate the NF-κB pathway, and produce SCFAs, thus contributing to anti-inflammatory and maintenance of mucus barrier stability[40–42].

Long-lasting effects of the early-life gut microbiota regulation by SC to alleviate adolescent UC in early-life antibiotic-exposed mice through enhancing gut microbiota -mediated intestinal mucosal immune effects, and SC combined with MOS for long-term use strengthened this response. Early-life antibiotic exposure led to an increase in sIgA levels in the gut to regulate microbial homeostasis[43–46]. SC and SC + MOS showed a tendency to increase the expression of APRIL (a key gene that promotes specific sIgA secretion) based on this to further increase sIgA production. SC, an original non-colonizing bacterium in the gut, regulated antibiotic-induced gut microbiota changes and contributed to the intestinal tolerance to it, promoting the activation of the APRIL pathway and stimulating the production of specific sIgA, which has an impact on intestinal mucosal immunity. MOS, as a prebiotic, synergized when used in combination with SC to further enhance the response of the intestinal mucosal immune response, thereby increasing the secretion of sIgA. Increased sIgA was beneficial for bacterial colonization and strengthened intestinal mucosal immune protection[47]. After cessation of the intervention early in life, the gut microbiota completely reshaped after natural restoration, leading to the intestinal tolerance to newly colonized species. However, the species diversity of the gut microbiota in the antibiotic-exposed group had still not recovered to normal level, and therefore sIgA secretion reduced at adolescent UC, showing a lower trend than in the model group. The long-lasting effects of gut microbiota regulation by SC increased the species diversity of the gut microbiota and reduced the colonization of harmful bacteria due to antibiotic exposure and increased the abundance of some harmless genera during adolescent UC, resulting in a higher trend of sIgA secretion than that of the antibiotic group, which in turn protected the intestinal mucosal barrier and alleviated adolescent UC. With long-term use of SC, the gut gradually built up a tolerance to it over time, and at the same time changed the composition of the gut microbiota

toward the dominance of SC. Consequently, although long-term use of SC promoted the colonization of beneficial bacteria, the dominance of SC in the gut led to a decreased gut microbiota -mediated stimulation of mucosal immunity, causing a reduced secretion of sIgA. MOS, as prebiotics in combination with SC for a long period of time, were not limited to specific strains of gut microbiota composition, but promoted the growth and colonization of a wide range of bacteria, which contributed to the increase in the diversity of gut microbiota, increasing the level of sIgA and strengthening intestinal mucosal immunity in adolescent UC[48–49]. This study has several limitations. First, although we observed SCFA alterations and their microbiota correlations, we did not directly validate their causal roles through SCFA receptor knockout models or in vitro experiments. Second, the direct mechanisms by which SC and MOS influence barrier functions—such as intestinal stem cell proliferation and mucus secretion—require further investigation. Future research should focus on the SCFA–GPR signaling axis and downstream pathways.

5. Conclusions

SC can modulate immune and gut microbiota in early-life antibiotic-exposed mice, and the effects are long-lasting, making it protect against adolescent UC by attenuating inflammatory responses of the immune system, modulating the gut microbiota, and enhancing gut microbiota-mediated intestinal mucosal immune barrier. Long-term use of SC can enhance its immune and gut microbiota regulatory functions. The immunomodulatory, gut microbiota modulatory, and gut microbiota-mediated mucosal immune barrier effects further enhanced to protect adolescent UC when combined with MOS for long-term use.

Declarations

Author Contributions: Yunyi Wang: methodology, software, validation, investigation, data curation, writing—original draft preparation, visualization. Zhixian Chen: conceptualization, methodology, writing—review and editing. Chang Lu: validation, investigation. Chenrui Peng: methodology, investigation. Lunpu Zhang: investigation, visualization. Yao Zeng: investigation, visualization. Yan Zhang: writing—review and editing. Fang He: conceptualization, writing—review and editing, supervision. Xi Shen: conceptualization, methodology, writing—review and editing, supervision, project administration, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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References

1. Ananthakrishnan, A. N., Epidemiology and risk factors for IBD. *Nat Rev Gastroenterol Hepatol* **2015,** *12* (4), 205-17.
2. Torres, J.; Mehandru, S.; Colombel, J. F.; Peyrin-Biroulet, L., Crohn's disease. *Lancet* **2017,** *389* (10080), 1741-1755.
3. Ungaro, R.; Mehandru, S.; Allen, P. B.; Peyrin-Biroulet, L.; Colombel, J. F., Ulcerative colitis. *Lancet* **2017,** *389* (10080), 1756-1770.
4. Piovani, D.; Danese, S.; Peyrin-Biroulet, L.; Nikolopoulos, G. K.; Lytras, T.; Bonovas, S., Environmental Risk Factors for Inflammatory Bowel Diseases: An Umbrella Review of Meta-analyses. *Gastroenterology* **2019,** *157* (3), 647-659.e4.
5. Torres, J.; Halfvarson, J.; Rodríguez-Lago, I.; Hedin, C. R. H.; Jess, T.; Dubinsky, M.; Croitoru, K.; Colombel, J. F., Results of the Seventh Scientific Workshop of ECCO: Precision Medicine in IBD-Prediction and Prevention of Inflammatory Bowel Disease. *J Crohns Colitis* **2021,** *15* (9), 1443-1454.
6. Mak, W. Y.; Zhao, M.; Ng, S. C.; Burisch, J., The epidemiology of inflammatory bowel disease: East meets west. *J Gastroenterol Hepatol* **2020,** *35* (3), 380-389.
7. Hutchings, M. I.; Truman, A. W.; Wilkinson, B., Antibiotics: past, present and future. *Curr Opin Microbiol* **2019,** *51*, 72-80.
8. Clardy, J.; Fischbach, M. A.; Currie, C. R., The natural history of antibiotics. *Curr Biol* **2009,** *19* (11), R437-41.
9. Wang, T.; Udomkittivorakul, N.; Bonfield, M.; Nadeem, A.; Gray, J.; Deshmukh, H., Early life antibiotic exposure and host health: Role of the microbiota-immune interaction. *Semin Perinatol* **2020,** *44* (8), 151323.
10. Zheng, H.; Zeng, X.; Xie, Q.; Wu, Y.; Liu, Q.; Chen, Q.; Huang, L.; Zhang, W., Early life environmental antibiotic exposure and preschool allergic diseases: A biomonitoring-based prospective study in eastern China. *Front Public Health* **2022,** *10*, 1043942.
11. Li, P.; Chang, X.; Chen, X.; Wang, C.; Shang, Y.; Zheng, D.; Qi, K., Early-life antibiotic exposure increases the risk of childhood overweight and obesity in relation to dysbiosis of gut microbiota: a birth cohort study. *Ann Clin Microbiol Antimicrob* **2022,** *21* (1), 46.
12. Vandenplas, Y.; Carnielli, V. P.; Ksiazyk, J.; Luna, M. S.; Migacheva, N.; Mosselmans, J. M.; Picaud, J. C.; Possner, M.; Singhal, A.; Wabitsch, M., Factors affecting early-life intestinal microbiota development. *Nutrition* **2020,** *78*, 110812.

13. Agrawal, M.; Sabino, J.; Frias-Gomes, C.; Hillenbrand, C. M.; Soudant, C.; Axelrad, J. E.; Shah, S. C.; Ribeiro-Mourão, F.; Lambin, T.; Peter, I.; Colombel, J. F.; Narula, N.; Torres, J., Early life exposures and the risk of inflammatory bowel disease: Systematic review and meta-analyses. *EClinicalMedicine* **2021,** *36*, 100884.
14. Zhu, Y.; Xu, Y.; Wang, X.; Rao, L.; Yan, X.; Gao, R.; Shen, T.; Zhou, Y.; Kong, C.; Zhou, L., Probiotic Cocktail Alleviates Intestinal Inflammation Through Improving Gut Microbiota and Metabolites in Colitis Mice. *Front Cell Infect Microbiol* **2022,** *12*, 886061.
15. Wang, S.; Peng, C.; Li, J.; Cheng, R.; He, F.; Yang, L.; Lu, J.; Shen, X., [Protective effect and mechanism of Bifidobacterium bifidum TMC3115 on long-term colitis in mice which exposed to antibiotic in early life]. *Wei Sheng Yan Jiu* **2022,** *51* (4), 624-644.
16. Korpela, K.; Salonen, A.; Vepsäläinen, O.; Suomalainen, M.; Kolmeder, C.; Varjosalo, M.; Miettinen, S.; Kukkonen, K.; Savilahti, E.; Kuitunen, M.; de Vos, W. M., Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. *Microbiome* **2018,** *6* (1), 182.
17. Rodríguez-Nogales, A.; Algieri, F.; Garrido-Mesa, J.; Vezza, T.; Utrilla, M. P.; Chueca, N.; García, F.; Rodríguez-Cabezas, M. E.; Gálvez, J., Intestinal anti-inflammatory effect of the probiotic Saccharomyces boulardii in DSS-induced colitis in mice: Impact on microRNAs expression and gut microbiota composition. *J Nutr Biochem* **2018,** *61*, 129-139.
18. Kelesidis, T.; Pothoulakis, C., Efficacy and safety of the probiotic Saccharomyces boulardii for the prevention and therapy of gastrointestinal disorders. *Therap Adv Gastroenterol* **2012,** *5* (2), 111-25.
19. Sivananthan, K.; Petersen, A. M., Review of Saccharomyces boulardii as a treatment option in IBD. *Immunopharmacol Immunotoxicol* **2018,** *40* (6), 465-475.
20. Belda, I.; Ruiz, J.; Santos, A.; Van Wyk, N.; Pretorius, I. S., Saccharomyces cerevisiae. *Trends Genet* **2019,** *35* (12), 956-957.
21. Scott, B. M.; Gutiérrez-Vázquez, C.; Sanmarco, L. M.; da Silva Pereira, J. A.; Li, Z.; Plasencia, A.; Hewson, P.; Cox, L. M.; O'Brien, M.; Chen, S. K.; Moraes-Vieira, P. M.; Chang, B. S. W.; Peisajovich, S. G.; Quintana, F. J., Self-tunable engineered yeast probiotics for the treatment of inflammatory bowel disease. *Nat Med* **2021,** *27* (7), 1212-1222.
22. Siesto, G.; Pietrafesa, R.; Infantino, V.; Thanh, C.; Pappalardo, I.; Romano, P.; Capece, A., In Vitro Study of Probiotic, Antioxidant and Anti-Inflammatory Activities among Indigenous Saccharomyces cerevisiae Strains. *Foods* **2022,** *11* (9).
23. Decherf, A.; Dehay, E.; Boyer, M.; Clément-Ziza, M.; Rodriguez, B.; Legrain-Raspaud, S., Recovery of Saccharomyces cerevisiae CNCM I-3856 in Vaginal Samples of Healthy Women after Oral Administration. *Nutrients* **2020,** *12* (8).
24. Jana, U. K.; Suryawanshi, R. K.; Prajapati, B. P.; Kango, N., Prebiotic mannooligosaccharides: Synthesis, characterization and bioactive properties. *Food Chem* **2021,** *342*, 128328.
25. Agrawal, M.; Allin, K. H.; Petralia, F.; Colombel, J. F.; Jess, T., Multiomics to elucidate inflammatory bowel disease risk factors and pathways. *Nat Rev Gastroenterol Hepatol* **2022,** *19* (6), 399-409.

26. Elian, S. D.; Souza, E. L.; Vieira, A. T.; Teixeira, M. M.; Arantes, R. M.; Nicoli, J. R.; Martins, F. S., Bifidobacterium longum subsp. infantis BB-02 attenuates acute murine experimental model of inflammatory bowel disease. *Benef Microbes* **2015,** *6* (3), 277-86.
27. Chae, J. M.; Heo, W.; Cho, H. T.; Lee, D. H.; Kim, J. H.; Rhee, M. S.; Park, T. S.; Kim, Y. K.; Lee, J. H.; Kim, Y. J., Effects of Orally-Administered Bifidobacterium animalis subsp. lactis Strain BB12 on Dextran Sodium Sulfate-Induced Colitis in Mice. *J Microbiol Biotechnol* **2018,** *28* (11), 1800-1805.
28. Yan, F.; Liu, L.; Cao, H.; Moore, D. J.; Washington, M. K.; Wang, B.; Peek, R. M.; Acra, S. A.; Polk, D. B., Neonatal colonization of mice with LGG promotes intestinal development and decreases susceptibility to colitis in adulthood. *Mucosal Immunol* **2017,** *10* (1), 117-127.
29. Christensen, B.; Hanauer, S. B.; Erlich, J.; Kassim, O.; Gibson, P. R.; Turner, J. R.; Hart, J.; Rubin, D. T., Histologic Normalization Occurs in Ulcerative Colitis and Is Associated With Improved Clinical Outcomes. *Clin Gastroenterol Hepatol* **2017,** *15* (10), 1557-1564.e1.
30. Meyer, F.; Wendling, D.; Demougeot, C.; Prati, C.; Verhoeven, F., Cytokines and intestinal epithelial permeability: A systematic review. *Autoimmun Rev* **2023,** *22* (6), 103331.
31. Okada, Y.; Tsuzuki, Y.; Sugihara, N.; Nishii, S.; Shibuya, N.; Mizoguchi, A.; Itoh, S.; Tanemoto, R.; Inaba, K.; Hanawa, Y.; Horiuchi, K.; Wada, A.; Higashiyama, M.; Watanabe, C.; Kurihara, C.; Komoto, S.; Tomita, K.; Miura, S.; Hokari, R., Novel probiotic yeast from Miso promotes regulatory dendritic cell IL-10 production and attenuates DSS-induced colitis in mice. *J Gastroenterol* **2021,** *56* (9), 829-842.
32. Zeng, X.; Li, X.; Yue, Y.; Wang, X.; Chen, H.; Gu, Y.; Jia, H.; He, Y.; Yuan, Y.; Yue, T., Ameliorative Effect of Saccharomyces cerevisiae JKSP39 on Fusobacterium nucleatum and Dextran Sulfate Sodium-Induced Colitis Mouse Model. *J Agric Food Chem* **2022,** *70* (44), 14179-14192.
33. Meng, Y.; Zhang, L.; Li, P.; Yu, J.; Mu, G.; Li, X.; Tuo, Y., Saccharomyces cerevisiae I4 Showed Alleviating Effects on Dextran Sulfate Sodium-Induced Colitis of Balb/c Mice. *Foods* **2022,** *11* (10).
34. Minton, K., Intestinal barrier protection. *Nat Rev Immunol* **2022,** *22* (3), 144-145.
35. den Besten, G.; van Eunen, K.; Groen, A. K.; Venema, K.; Reijngoud, D. J.; Bakker, B. M., The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* **2013,** *54* (9), 2325-40.
36. Koenig, J. E.; Spor, A.; Scalfone, N.; Fricker, A. D.; Stombaugh, J.; Knight, R.; Angenent, L. T.; Ley, R. E., Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U S A* **2011,** *108 Suppl 1* (Suppl 1), 4578-85.
37. Clemente, J. C.; Ursell, L. K.; Parfrey, L. W.; Knight, R., The impact of the gut microbiota on human health: an integrative view. *Cell* **2012,** *148* (6), 1258-70.
38. Kim, M. H.; Kang, S. G.; Park, J. H.; Yanagisawa, M.; Kim, C. H., Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology* **2013,** *145* (2), 396-406.e1-10.
39. Al-Roub, A.; Akhter, N.; Al-Sayyar, A.; Wilson, A.; Thomas, R.; Kochumon, S.; Al-Rashed, F.; Al-Mulla, F.; Sindhu, S.; Ahmad, R., Short Chain Fatty Acid Acetate Increases TNFα-Induced MCP-1 Production in Monocytic Cells via ACSL1/MAPK/NF-κB Axis. *Int J Mol Sci* **2021,** *22* (14).

40. Wang, M.; Zhou, B.; Cong, W.; Zhang, M.; Li, Z.; Li, Y.; Liang, S.; Chen, K.; Yang, D.; Wu, Z., Amelioration of AOM/DSS-Induced Murine Colitis-Associated Cancer by Evodiamine Intervention is Primarily Associated with Gut Microbiota-Metabolism-Inflammatory Signaling Axis. *Front Pharmacol* **2021,** *12*, 797605.
41. Zhang, T.; Ji, X.; Lu, G.; Zhang, F., The potential of Akkermansia muciniphila in inflammatory bowel disease. *Appl Microbiol Biotechnol* **2021,** *105* (14-15), 5785-5794.
42. Benítez-Páez, A.; Gómez Del Pugar, E. M.; López-Almela, I.; Moya-Pérez, Á.; Codoñer-Franch, P.; Sanz, Y., Depletion of Blautia Species in the Microbiota of Obese Children Relates to Intestinal Inflammation and Metabolic Phenotype Worsening. *mSystems* **2020,** *5* (2).
43. Pietrzak, B.; Tomela, K.; Olejnik-Schmidt, A.; Mackiewicz, A.; Schmidt, M., Secretory IgA in Intestinal Mucosal Secretions as an Adaptive Barrier against Microbial Cells. *Int J Mol Sci* **2020,** *21* (23).
44. Bunker, J. J.; Erickson, S. A.; Flynn, T. M.; Henry, C.; Koval, J. C.; Meisel, M.; Jabri, B.; Antonopoulos, D. A.; Wilson, P. C.; Bendelac, A., Natural polyreactive IgA antibodies coat the intestinal microbiota. *Science* **2017,** *358* (6361).
45. Rollenske, T.; Burkhalter, S.; Muerner, L.; von Gunten, S.; Lukasiewicz, J.; Wardemann, H.; Macpherson, A. J., Parallelism of intestinal secretory IgA shapes functional microbial fitness. *Nature* **2021,** *598* (7882), 657-661.
46. Bunker, J. J.; Bendelac, A., IgA Responses to Microbiota. *Immunity* **2018,** *49* (2), 211-224.
47. Nakajima, A.; Vogelzang, A.; Maruya, M.; Miyajima, M.; Murata, M.; Son, A.; Kuwahara, T.; Tsuruyama, T.; Yamada, S.; Matsuura, M.; Nakase, H.; Peterson, D. A.; Fagarasan, S.; Suzuki, K., IgA regulates the composition and metabolic function of gut microbiota by promoting symbiosis between bacteria. *J Exp Med* **2018,** *215* (8), 2019-2034.
48. Moré, M. I.; Swidsinski, A., Saccharomyces boulardii CNCM I-745 supports regeneration of the intestinal microbiota after diarrheic dysbiosis - a review. *Clin Exp Gastroenterol* **2015,** *8*, 237-55.
49. Ribeiro, M. C.; Levi, Y.; Moraschini, V.; Messora, M. R.; Furlaneto, F. A. C., Effects of Prebiotic Therapy on Gastrointestinal Microbiome of Individuals with Different Inflammatory Conditions: A Systematic Review of Randomized Controlled Trials. *Probiotics Antimicrob Proteins* **2023**.

Figures

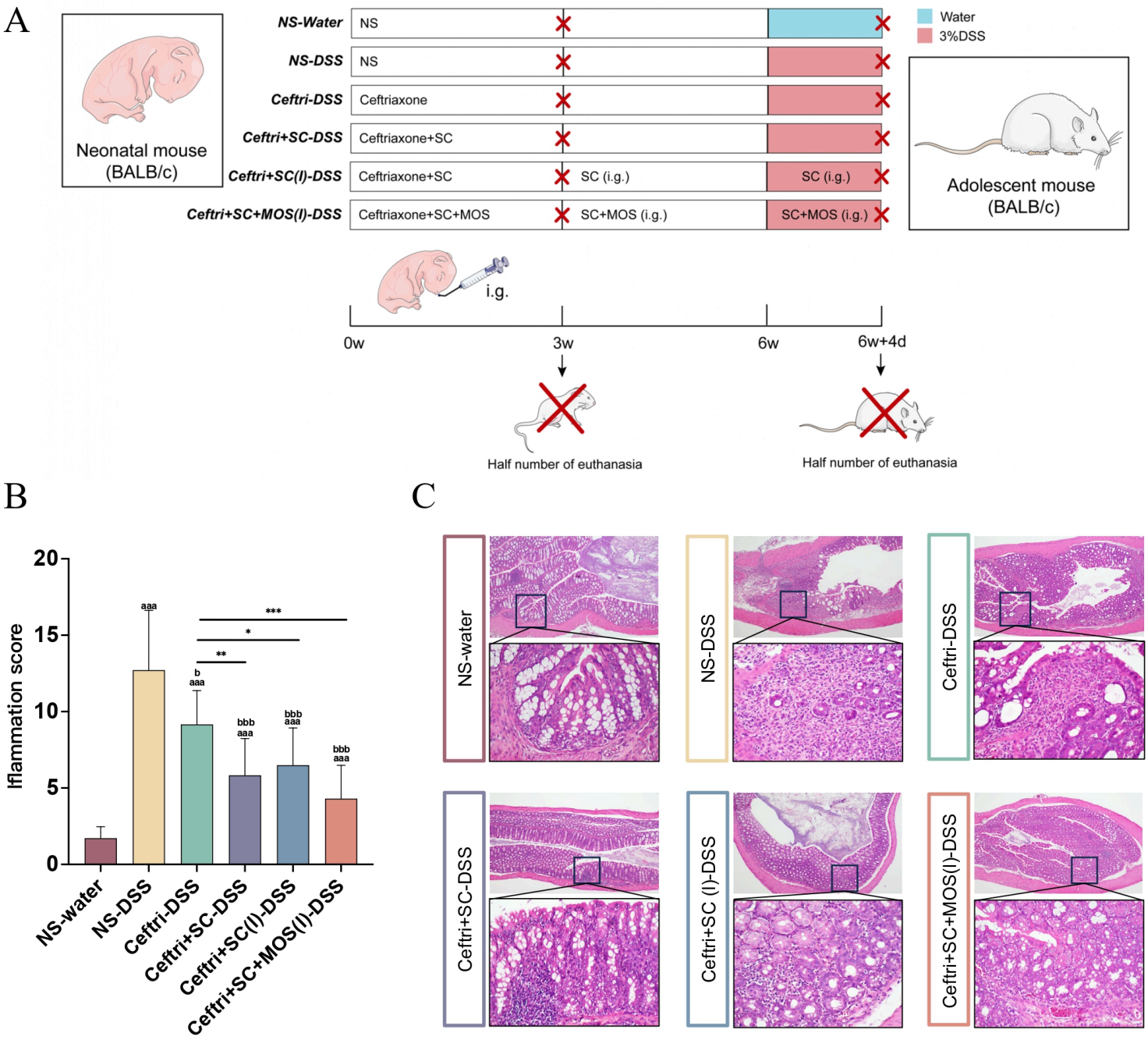


Figure 1

Adolescent DSS-induced ulcerative colitis in early-life antibiotic-exposed mice.

(A) Intervention process. (B) Inflammation score at adolescent DSS-induced UC (NS-water, n = 7; NS-DSS, n = 7; Ceftri-DSS, n = 12; Ceftri+SC-DSS, n = 12; Ceftri+SC(I)-DSS, n = 12; Ceftri+SC+MOS(I)-DSS, n = 13). (C) H&E-stained colon tissue at adolescent DSS-induced ulcerative colitis. aaa compared with the NS-water group, $p < 0.001$; b compared with the NS-DSS group, $p < 0.05$, bbb compared with the NS-DSS group, $p < 0.001$; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Microscope magnification: 40x and 200 x.

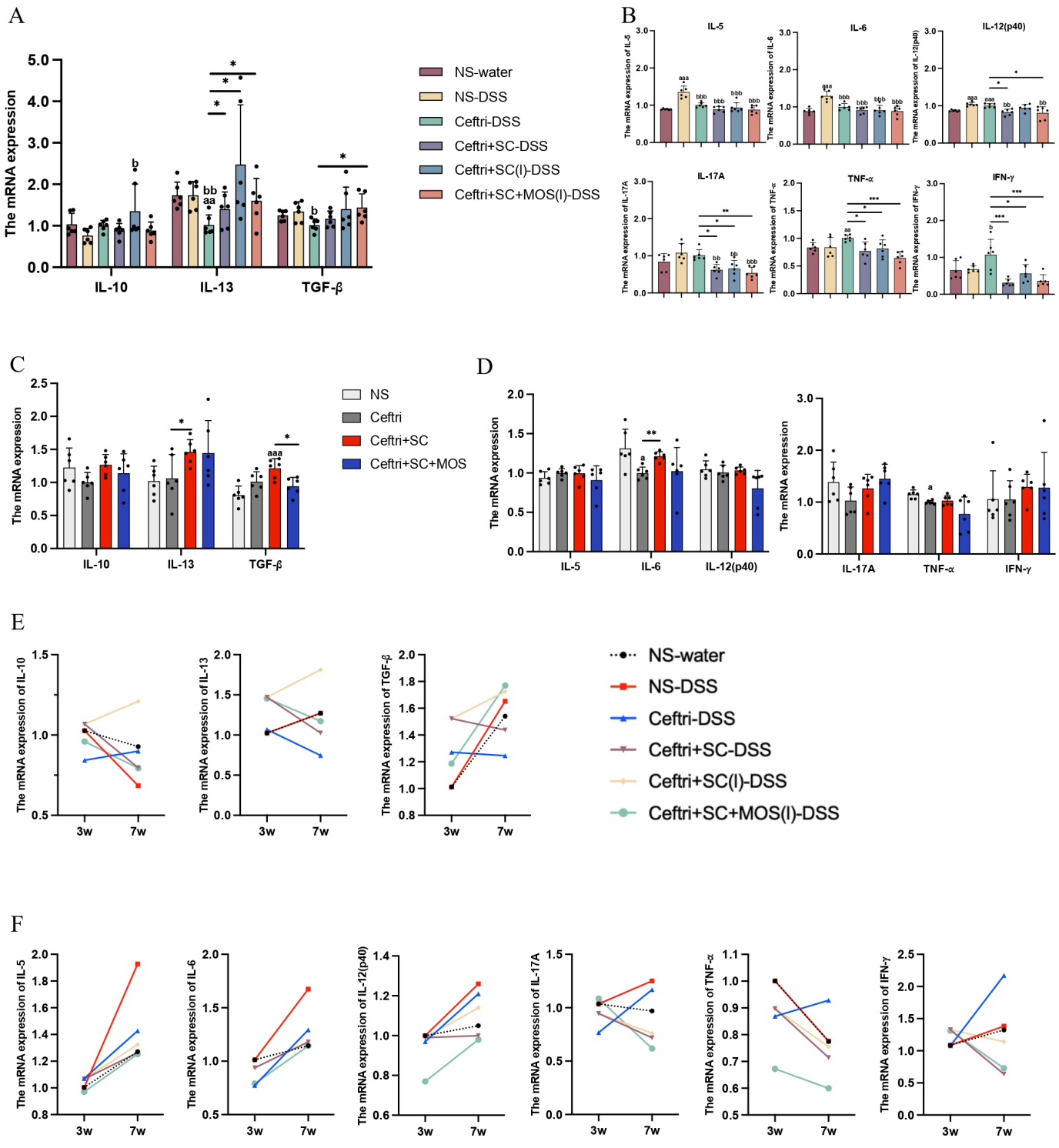


Figure 2

mRNA expression of inflammatory cytokines in the colon, n = 6.

(A) Anti-inflammatory cytokines mRNA levels at adolescent DSS-induced UC. (B) Pro-inflammatory cytokines mRNA levels at adolescent DSS-induced UC. (C) Anti-inflammatory cytokines mRNA levels at early life. (D) Pro-inflammatory cytokines mRNA levels at early life. (E) mRNA expression changes of

anti-inflammatory cytokines from early life to adolescent DSS-induced UC. (F) mRNA expression changes of pro-inflammatory cytokines from early life to adolescent DSS-induced UC. a compared with the NS/NS-water group, $p < 0.05$, aa compared with the NS/NS-water group, $p < 0.01$, and aaa compared with the NS/NS-water group, $p < 0.001$; b compared with the NS-DSS group, $p < 0.05$, bb compared with the NS-DSS group, $p < 0.01$, and bbb compared with the NS-DSS group, $p < 0.001$; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

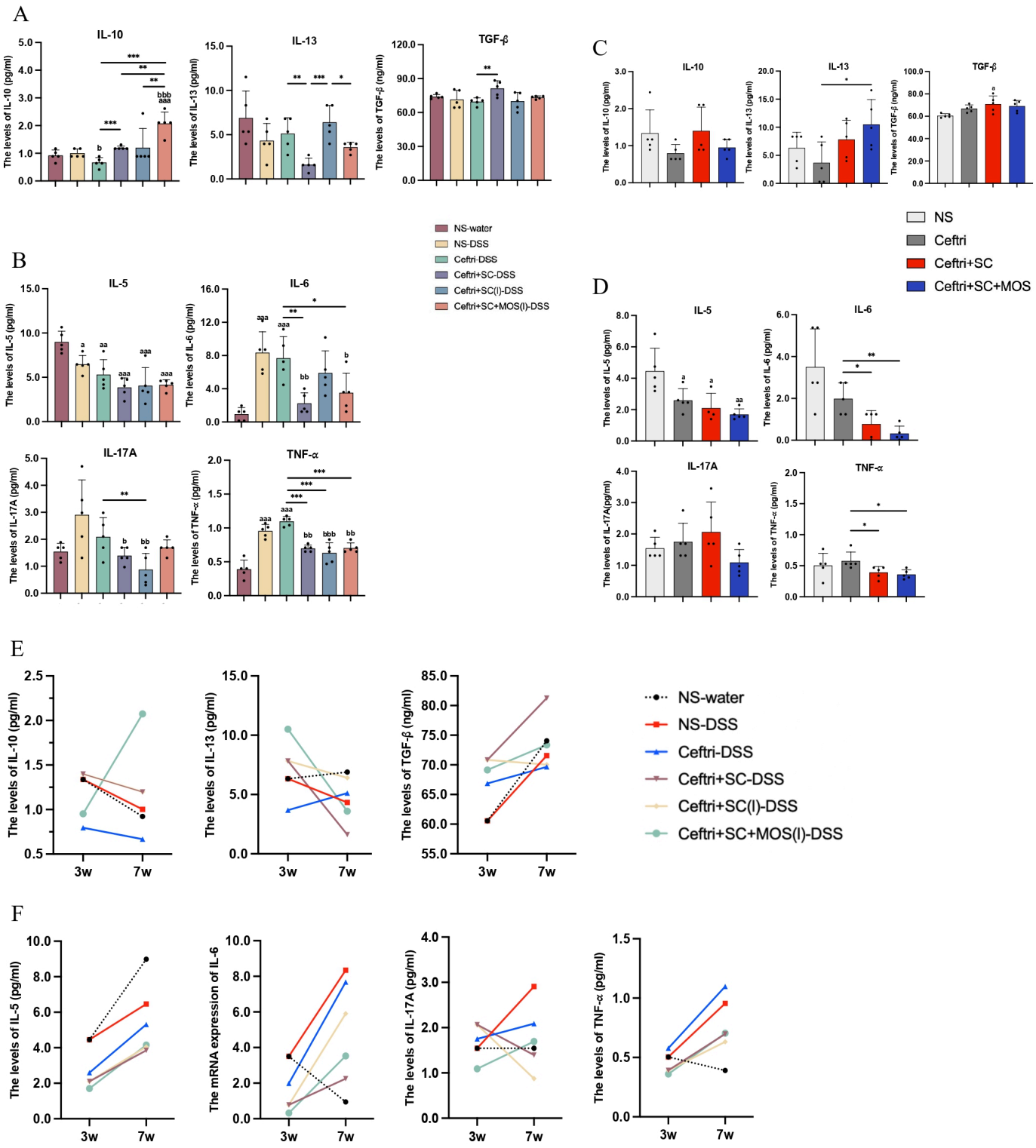


Figure 3

Serum inflammatory cytokines levels, n = 5.

(A) Serum anti-inflammatory cytokines levels at adolescent DSS-induced UC. (B) Serum pro-inflammatory cytokines levels at adolescent DSS-induced UC. (C) Serum anti-inflammatory cytokines levels at early life. (D) Serum pro-inflammatory cytokines levels at early life. (E) Changes of serum anti-inflammatory cytokines levels from early life to adolescent DSS-induced UC. (F) Changes of serum pro-inflammatory cytokines levels from early life to adolescent DSS-induced UC. a compared with the NS/NSwater group, $p < 0.05$, aa compared with the NS/NS-water group, $p < 0.01$, and aaa compared with the NS/NS-water group, $p < 0.001$; b compared with the NS-DSS group, $p < 0.05$, bb compared with the NS-DSS group, $p < 0.01$, and bbb compared with the NS-DSS group, $p < 0.001$; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

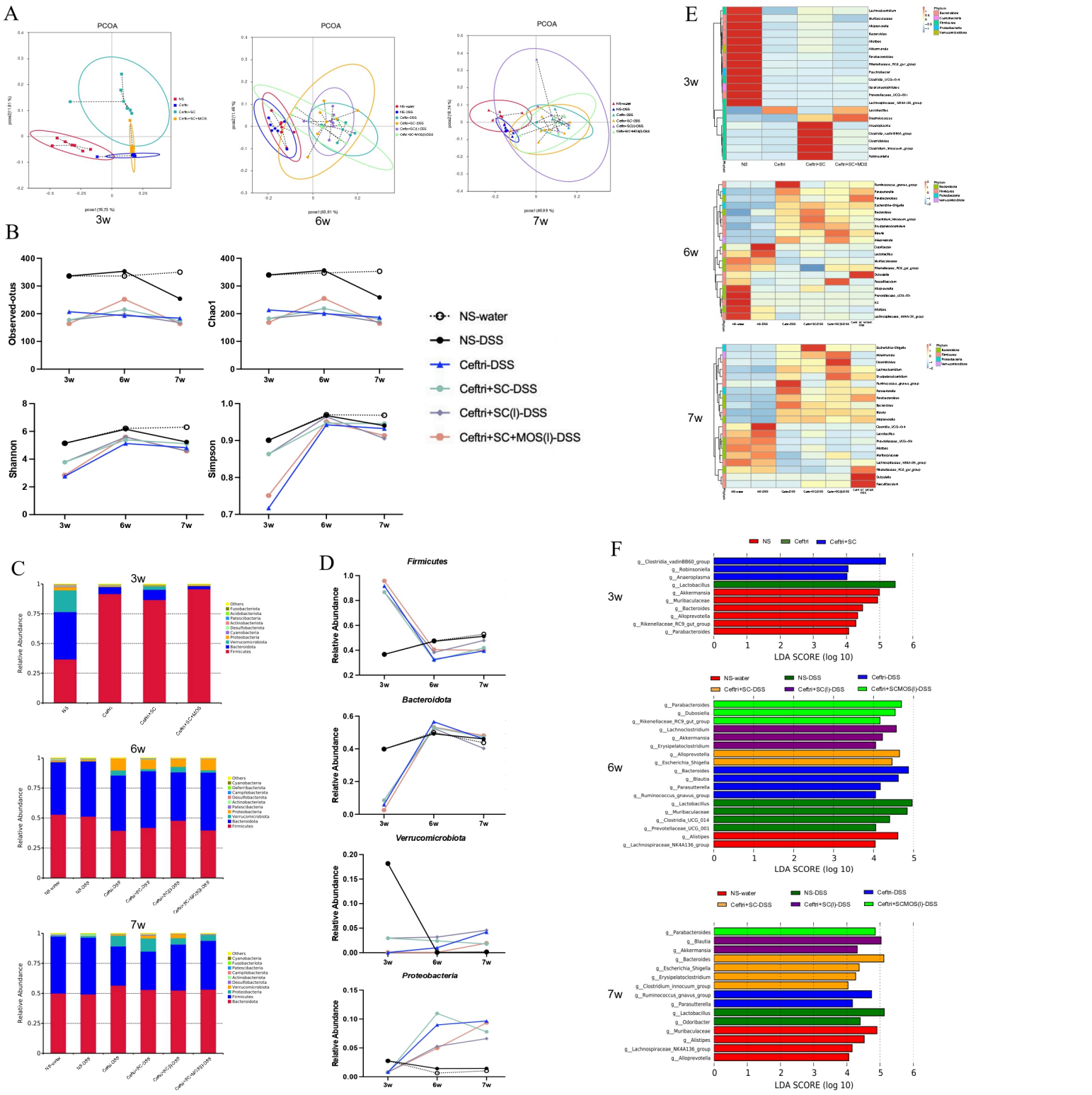


Figure 4

Changes of gut microbiota, n = 6.

(A) Principal co-ordinates analysis (PCoA) plots of fecal microbiota at early life, adolescence, and adolescent DSS-induced UC. (B) Alpha diversity changes in 3w-6w-7w. (C) Relative abundance changes at the phylum level at early life, adolescence, and adolescent DSS-induced UC. (D) Phylum level relative

abundance changes in 3w-6w-7w. (E) Heat map of relative abundance changes at the genus level at early life, adolescence, and adolescent DSS-induced UC. (F) Differentially enriched intestinal microbiota in at the genus level by linear discriminant analysis (LDA) at early life, adolescence, and adolescent DSS-induced UC. An LDA score higher than 4 represents a higher abundance in the group than that in other groups.

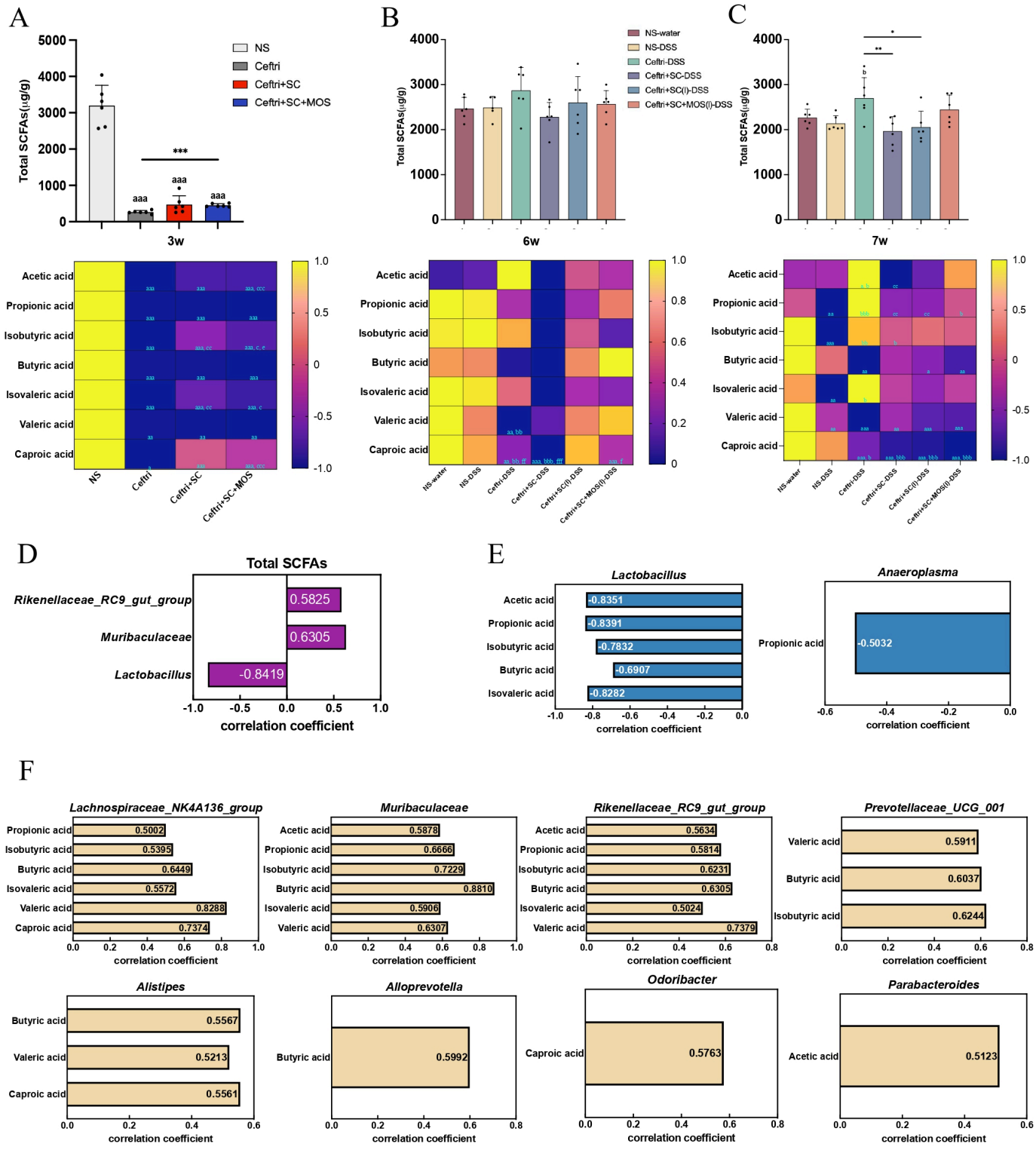


Figure 5

Changes of SCFAs, n = 6.

(A) SCFAs levels at early life. (B) SCFAs levels at adolescence. (C) SCFAs levels at adolescent DSS-induced UC. (D) Correlation of bacteria with total SCFAs. (E) Negative correlation between bacteria and SCFAs. (F) Positive correlation between bacteria and SCFAs. a compared with the NS/NS-water group, $p < 0.05$, aa compared with the NS/NS-water group, $p < 0.01$, and aaa compared with the NS/NS-water group, $p < 0.001$; b compared with the NS-DSS group, $p < 0.05$, bb compared with the NS-DSS group, $p < 0.01$, and bbb compared with the NS-DSS group, $p < 0.001$; c compared with the Ceftri/Ceftri-DSS group, $p < 0.05$, cc compared with the Ceftri/Ceftri-DSS group, $p < 0.01$, and ccc compared with the Ceftri/Ceftri-DSS, $p < 0.001$; e compared with the Ceftri+SC group, $p < 0.05$; f compared with the Ceftri+SC(l)-DSS group, $p < 0.05$, bb compared with the Ceftri+SC(l)-DSS group, $p < 0.01$, and fff compared with the Ceftri+SC(l)-DSS group, $p < 0.001$; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

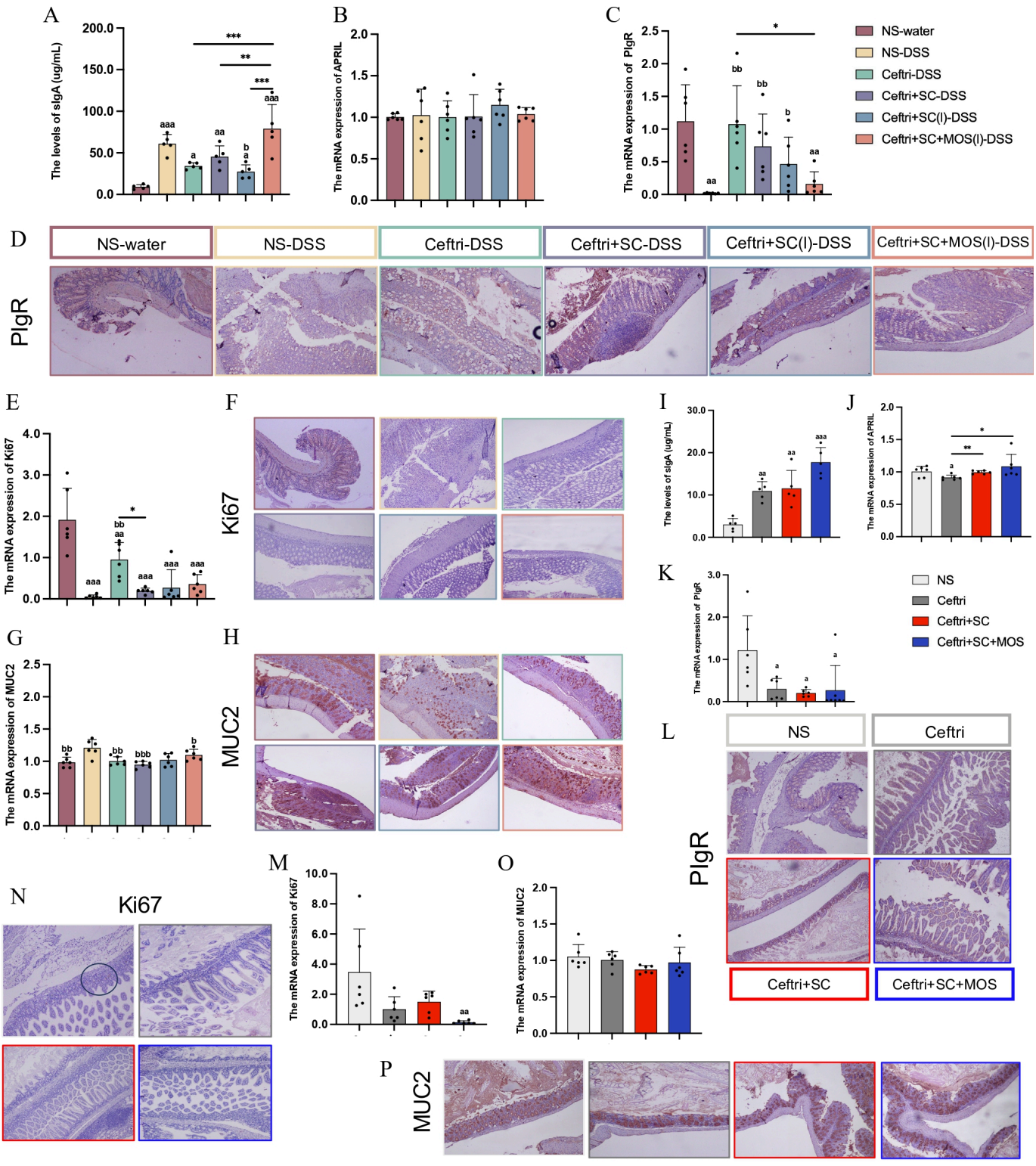


Figure 6

Changes of intestinal mucosal barrier.

(A) sIgA levels in the feces at adolescent DSS-induced UC, n = 5. (B) APRIL mRNA level at adolescent DSS-induced UC, n = 6. (C) Ki67 mRNA level at adolescent DSS-induced UC, n = 6. (D) IHC for Ki67 in intestinal tissue at adolescent DSS-induced UC. (E) MUC2 mRNA level at adolescent DSS-induced UC, n =

6. (F) IHC for MUC2 in intestinal tissue at adolescent DSS-induced UC. (G) Tight-junction protein mRNA levels at adolescent DSS-induced UC. (H) sIgA levels in the feces at early life, n = 5. (I) APRIL mRNA level at early life, n = 6. (J) Ki67 mRNA level at early life, n = 6. (K) IHC for Ki67 in intestinal tissue at early life. (L) MUC2 mRNA level at early life, n = 6. (M) IHC for MUC in intestinal tissue at early life. (N) Tight-junction protein mRNA levels at early life. a compared with the NS/NS-water group, $p < 0.05$, aa compared with the NS/NS-water group, $p < 0.01$, and aaa compared with the NS/NS-water group, $p < 0.001$; b compared with the NS-DSS group, $p < 0.05$, bb compared with the NS-DSS group, $p < 0.01$, and bbb compared with the NS-DSS group, $p < 0.001$; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Supplementary Files

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