Probiotics (Lactiplantibacillus plantarum HNU082) supplementation relieves ulcerative colitis by affecting intestinal barrier functions, immunity-related genes expression, gut microbiota, and metabolic pathways in mice

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Research Article

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Abstract

Background: As a chronic relapsing disease, probiotic treatment of ulcerative colitis can avoid resistance and side effects of conventional treatment, but its mechanism of action is unknown. This study investigated the therapeutic effect and mechanism of action of potential probiotics *Lactiplantibacillus plantarum* HNU082 (Lp082) on dextran sulfate sodium-induced colitis in mice.

Results: The results showed that the intake of Lp082 increased body weight, water intake, food intake, colon length, and reduced disease activity index, immune organ index, inflammatory markers, and histopathological score. Lp082 strengthened the biological barrier by improving the diversity and composition of gut microbiota, increasing the short chain fatty acids (SCFAs) producing bacteria and SCFAs content, and enhancing the metabolic pathway of SCFAs. Lp082 improved the chemical barrier by increasing the goblet cells and mucin-2 and decreasing the ICAM-1 and VCAM. Lp082 enhanced the mechanical barrier by increasing the mRNA expression of ZO-1, ZO-2, occludin and decreasing claudin-1 and claudin-2. Lp082 strengthened the immune barrier by reducing the content of IL-1β, IL-6, TNF-α, MPO, IFN-γ and increasing the IL-10, TGF-β1, and TGF-β2. Lp082 also affected gene expression in mice, including that it inhibited the gene expression of IL-1β, IL-1α, Ereg, Cdc7, and others. Thus inhibited the biological processes such as IL-1β production, IL-6 production, acute inflammatory response, and promoted the expression of genes Isg15, Prg2, Abcc2, thus boosting the biological processes such as IL-10 production and drug transport. The differentially expressed genes in DSS group were primarily enriched in the disease-related KEGG pathways but were mainly enriched in the KEGG pathway associated with platelet activated autophagy in animals in Lp082 group. Lp082 also inhibited the mRNA expression of NF-κB1, NF-κB2, COX-2, iNOS, Toll-4, and others, thus suppressing the NK-KB pathways.

Conclusions: Shotgun metagenomic and transcriptome analysis revealed that Lp082 could relieve ulcerative colitis, protect the intestinal mucosal barrier through SCFAs, regulate gene expression and signaling pathways. The study provides new clues to reveal the complex mechanisms of the gut barriers and genes in relieving ulcerative colitis.

Background

Crohn's disease (CD) and ulcerative colitis (UC) are collectively referred to as inflammatory bowel disease (IBD) [1]. The main feature of UC is bloody diarrhea and diffused inflammation of the colonic mucosa, and its incidence increases worldwide each year, but its exact pathogenesis remains unknown [2]. In addition, traditional surgery and drug therapy have drug resistance and side effects and impose a heavy economic burden on patients [3]. Because probiotics can regulate the gut microbiota disorder of UC patients and avoid the above disadvantages, it has become a new line of treatment for UC [4]. Studies have shown that probiotics not only can optimize the composition of the gut microbiota [5] but also can strengthen the gut barrier function [6] and promote the content of short chain fatty acids (SCFAs), which can promote the stability of gut microbiota and regulate immune responses [7].

However, the mechanism of probiotics relieving UC is still unclear. So, we used *Lactiplantibacillus plantarum* HNU082 (Lp082) as a potential probiotic to study its effects and the mechanism of action in relieving UC. It is worth mentioning that Lp082 was separated from Hainan traditional naturally fermented food *Yucha*. It has good safety performance, acid and bile salt resistance, and exerts a preventive effect on hyperlipidemia through the modulation of metabolism [8]. Also, it has been found to improve the stability of the gut microbiota [9], and the whole genome sequencing result demonstrated its great potential as a probiotic.

This study used shotgun metagenomics and transcriptome analysis methods to find that Lp082 affects gut mucosal barrier, gene expression, and signaling pathway in dextran sulfate sodium (DSS)-induced colitis mice. SCFAs play an important role as mediators in regulating gut microbiota and inflammatory factors. This study provides new clues to uncover the complex mechanism by which probiotics can relieve UC and provides a reference for the development of probiotics as a potential treatment of UC in the future.

Materials And Methods

Animals and their management

A total of 32 C57BL/6J mice (male, 7 weeks old, 20–22 g) were purchased from Hunan Slac Jingda Laboratory Animal (Changsha, China). The mice were carefully placed in individually ventilated cages (Suzhou Fengshi Laboratory Animal Equipment Co., Ltd, Suzhou, China) in standard rearing conditions (temperature, 26 ± 2°C; humidity, 50% ± 5%), and maintained in 12 hours light-dark cycle. The mice were fed with standard commercial mouse food ad libitum and had free access to sterile water all the time. Mice were acclimatized to these conditions for two weeks before the experiment. After the experiment, the mice were intraperitoneally injected with 1% pentobarbital sodium solution and euthanized, and samples were collected [10]. The animal experiment and all experimental protocols were approved by the Ethics Committee of Hainan University (HNUAUCC-2021-00122) and performed according to the Guiding Principles of the Care and Use of Animals approved by the American Physiological Society.

Animal experimental design

C57BL/6J mice aged 7 weeks were randomly divided into 4 groups: control group, dextran sulfate sodium (DSS) group, *Lactiplantibacillus plantarum* HNU082 (Lp082) group, and salazosulfapyridine (SASP) group. From day 1 to 7, mice in 3 groups except the control group were given 3.5% (w/v) DSS water (36–50 kDa; Coolaber Company, Beijing, China) to establish an ulcerative colitis model. From day 8 to day 15, mice in DSS group, Lp082 group, and SASP group were given phosphate buffer saline (PBS), Lp082 (1×10^9 CFU/mL), and SASP (150 mg/kg), respectively [8] (Fig. 1a).

Physiological indexes

Physiological indexes were used to assess the health status of mice, including water intake, food intake, body weight, and disease activity index (DAI) scores modified from previous studies [11] **(Table S1)**, and fecal occult blood measured by o-toluidine method (X-Y Biotechnology, Shanghai, China). After euthanizing mice, the colon was collected for colon length measurement, and the spleen, liver, and kidney were collected and weighed for immune organ index measurement, as follows: spleen index = spleen weight (mg)/body weight (g), liver index = liver weight (mg)/body weight (g), kidney index = kidney weight (mg)/body weight (g) [12].

Inflammatory cytokines

Blood was collected from the orbital venous plexus of mice with a capillary tube before euthanasia. First, the blood sample was coagulated naturally for 30 minutes. Then, blood samples were centrifuged at 3000 rpm at 4°C for 20 min to separate and collect the serum [13]. Finally, the levels of interleukin-1beta (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-17A (IL-17A), interferon-gamma (IFN-γ), Tumor necrosis factor-alpha (TNF-α), and Myeloperoxidase (MPO) in the serum of mice were measured using the corresponding ELISA kits (X-Y Biotechnology, Shanghai, China), as previously described [14].

Pathological indicators

Colon samples were first fixed in 4% (w/v) paraformaldehyde 24h (Servicebio Company, Wuhan, China) after washing with PBS, and then were dehydrated, embedded in paraffin, and sectioned (3μm sections). Next, some colon samples were stained with hematoxylin and eosin (H&E) as previously described [15] and were observed for the histopathological changes by a light microscope (Eclipse Ci-L microscope, Nikon Corporation, Japan), as previously described [16] and was modified to assess the histological damage scores **(Table S2)**. The Mucin-2 (MUC-2) and zonula occludens-1 (ZO-1) antibodies were labeled, respectively, as previously described [17].

Fecal DNA extraction, shotgun metagenomic sequencing, and data quality control

Metagenomic DNA was extracted from fecal samples following the previously reported procedure [18]. Then, the sequencing was performed by Beijing Novogene Co., Ltd. (Beijing, China). The Raw data were processed using the sliding window method, which deletes the low-quality sequence from the original sequence, thus generating clean and high-quality data [19]. Finally, for data quality control, sickle software was used for tailoring, modification reads, and MetaPhlan3 software was employed for metagenomic species annotation. The humann2 was used to analyze the diversity of the mouse gut microbiota and the composition of the microbiota at the species levels [20]. Uniref90 database was used to annotate metagenomic functional characteristics, functional genes, and metabolic pathways [21].

Determination of SCFAs in colonic contents of mouse

First, 40 mg of colonic contents of the mice were lyophilized, weighed, and then added 600 μL normal saline (85%). The sample was then placed in a shaker for 5 minutes to mix thoroughly and centrifuged (8000 RPM for 5 minutes). Then, 200 μL supernatant was taken, and 100 μL sulfuric acid (50%) was

added for acidification, then 400 μL n-hexane was added to extract SCFAs. The solution was finally passed through an organic membrane and transferred to a vial [22]. Gas chromatography–mass spectrometry (GCMS-Agilent-7890, Santa Clara, CA, USA) was used to analyze the concentrations of SCFAs, including acetic acid, propionic acid, butyric acid, isobutyric acid, and valeric acid. The concentrations of SCFAs were calculated by the external standard method, and expressed as μmol/g dry sample following the previously described method [23].

RNA sequencing

The RNA extraction mini kit (Qiagen, Hilden, Germany) was used for total RNA extraction from the mouse colon samples, and NanoDrop 2000 was used for quantification, then the library construction and the quality control were carried on, the raw RNA-seq data was filtered [24]. After constructing the RNA library, Illumina Novaseq 6000 was used for sequencing, and the FeatureCounts were used to estimate the gene expression [25].

Bioinformatics and statistical analyses

GraphPad (GraphPad Software, San Diego, CA, USA) and R software were used for statistical analysis of the data, and all data are expressed as the mean ± standard deviation (SD). Treatment groups were compared using the Wilcoxon signed-rank test [26]. The *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ were considered to indicate significant, strongly significant, and extremely significant differences, respectively.

Ade4 package and ggplot2 package were used for principal coordinate analysis (PCoA) analysis and to draw box charts and bubble charts, respectively. The “DESeq2” package was used for P-value filtering and correction in the bubble diagram of the metabolic pathway [27]. The correlation between the matrices was then analyzed according to the Corrplot package [28]. Correlation between gut microbiota, metabolic pathways, SCFAs, inflammatory factors, ZO-1, MUC-2, pathological indicators, genes, and their enriched pathways in mice were calculated by Pearson coefficient, and Cytoscape (Version 3.7.1) software was used for visualization [29].

DESeq2 was used for Gene-centric differential expression analysis, $p < 0.05$ and $|\log2FoldChange| > 1$ was used to screen for differentially expressed genes, volcano plots were used for differential gene visualization [30]. The cluster profiler software was used to perform gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis for differentially expressed genes (DEGs) [31].

Results

The probiotic Lp082 was fed with DSS to mice to evaluate the potential preventive effects of this strain on UC development. Various tissue samples, including immune organs, serum, proximal colon, fecal, cecal contents, distal colon, and other tissues, were collected to evaluate physiological indexes, inflammatory cytokines, pathological indicators, shotgun metagenomic sequencing, SCFAs analysis, and RNA sequencing (Fig. 1a).

The intake of Lp082 alleviated physiological lesions in DSS-induced colitis mice

DSS group, Lp082 group, and SASP group-under the same DSS conditions showed similar changes on days 1–7, i.e., water intake, food intake, and body weight decreased significantly on the 6th day, the 2nd and 3rd day, respectively, and DAI scores increased significantly on day 3 ($p < 0.05$) (Fig. 1b). After SASP and Lp082 intake, water and food intake increased from day 8, body weight increased from day 11, and DAI scores decreased from day 9, but the DSS group maintained the original trend on these variables (Fig. 1b).

The mice in the control group were mentally active and responsive, while the mice in the DSS group developed symptoms, such as slow response, arched back, and easy panic as the disease worsened **(Fig. S1 b).** More importantly, the immune organ index of mice in the DSS group was significantly increased ($p < 0.05$) (Fig. 1c), and the spleen became larger and blacker **(Fig. S1 c)**, shortened colon, hyperemia, and swelling of intestinal contents (Fig. 1d), and feces were not formed **(Fig. S1 a)**. It is important to note that after ingesting SASP and Lp082, the mice no longer had blood in the stool **(Fig. S1 a)**, the mental state went back to normal activities **(Fig. S1 b)**, the immune organ index decreased significantly (Fig. 1c), and the length of the colon increased significantly ($p < 0.05$) (Fig. 1d).

The above pathological indexes in Lp082 group were better than those in SASP group, suggesting that Lp082 has a better remission effect on UC.

Effects of Lp082 on inflammatory cytokines in mice with colitis

To further assess the damage in colons, the pro-inflammatory cytokines TNF-, IL-1β, IFN-α, IL-6, and MPO and anti-inflammatory cytokine IL-10 in the serum of mice were quantified. It was found that compared with the control group, the pro-inflammatory cytokines were significantly increased, and the anti-inflammatory cytokines were significantly decreased in the DSS group ($p < 0.05$), while the opposite was in the Lp082 and SASP group (Fig. 1e).

Effects of Lp082 on pathological lesions in DSS-induced colitis mice

The intake of Lp082 significantly decreased colon histopathology score and intestinal wall thickness ($p < 0.05$). The result of H&E staining paraffin section indicated that colonic tissue underwent severe damage in DSS group, including neutrophil infiltrate deep into the serosal layer (green arrow), crypt disappear (black arrows), goblet cell loss (red arrow), and inflammatory cell aggregation (blue arrow) (Fig. 2a). These were consistent with the significantly increased colon histopathology score in DSS group ($p < 0.05$) (Fig. 2b). However, the intake of Lp082 and SASP significantly improved the above situation, including increased the number of crypts (orange arrow) and goblet cells (yellow arrow), alleviated inflammatory cell foci, alleviated neutrophil infiltration, and promoted the tight junctions of intestinal glands (gray

arrow) (Fig. 2a). Moreover, a better recovery in the Lp082 group than in the SASP group was consistent with a lower histopathological score in the Lp082 group **(Figure. 2b)**. In addition, the intestinal wall was thicker in the DSS group, thinner in the SASP group, and much thinner in the Lp082 group (Fig. 2c). On the other hand, immunofluorescence results showed that the MUC-2 protein (green fluorescence) and ZO-1 protein (red fluorescence) contents were higher in the control group, almost disappeared in the DSS group, and significantly recovered in the Lp082 and SASP groups ($p < 0.05$), and even increased more than SASP in Lp082 group (Fig. 2d-e). These results were consistent with the surface density results of the two proteins (Fig. 2f-g).

Effects of Lp082 on gut microbiota

Lp082 improved the α diversity and optimized the β diversity of cecal microbiota in mice. On days 1–7 of the study, the Shannon index in DSS, Lp082, and SASP groups, under the same DSS condition, was significantly decreased with the same level (Fig. 3a) but significantly increased after the intake of Lp082 ($p < 0.05$) (Fig. 3a). The three groups (M_B, M_C, M_D) (under the same DSS molding condition) and control group (M_A) were significantly separated on day 7 ($p < 0.05$) (Fig. 3b). However, on day 15, the DSS group was still significantly separated from the control group (T_B), while the distance between Lp082 group (T_C), SASP group (T_D), and control group (T_A) was significantly reduced (p values < 0.05), and the distance between Lp082 group and control group was closer, the above results were consistent with the PCoA distance results (Fig. 3c).

Potential colitis pathogenic bacteria such as *Helicobacter hepaticus* increased significantly in DSS group but decreased in Lp082 group ($p < 0.05$), while the potential beneficial bacteria, such as *Lactobacillus plantarum*, *Bifidobacterium pseudolongum*, *Akkermansia muciniphila*, *Bacteroides ovatus*, *Parabacteroides distasonis*, *Lactobacillus reuteri*, *Anaerotruncus sp* G3 2012 significantly decreased in DSS group but significantly increased in Lp082 group ($p < 0.05$) (Fig. 3d).

The regulatory role of SCFAs

Some of the increased beneficial bacteria in Lp082 group may produce SCFAs. So, we further explored the gut microbiota metabolic pathway associated with SCFAs and found that 2 related pathways in the Lp082 group were activated: *the fermentation of pyruvate to propionate I* and *the fermentation of pyruvate to acetate and lactate II* (Fig. 4a). These two pathways directly promote the production of acetate and propionate, which was consistent with the GC-MS results of SCFAs, i.e., the contents of acetic acid, propionic acid, butyric acid were significantly decreased in the DSS group but significantly increased in the Lp082 group ($p < 0.05$) (Fig. 4b).

To further understand the role of SCFAs, we performed a correlation analysis and found that *Helicobacter hepatica* enriched in DSS group and 7 potential probiotics enriched in Lp082 group were negatively and positively correlated with acetic acid, propionic acid, and butyric acid, respectively (Fig. 4c). These SCFAs were all negatively associated with the pro-inflammatory factors TNF-α, IL-1β, IFN-γ, IL-6, MPO but positively associated with the inflammatory suppressor IL-10 (Fig. 4d).

Comparative study on the transcriptome of intestinal epithelial cells in each group

The volcanic map showed that Lp082 significantly affected gene expression distribution (Fig. 5a-f). To further explore the impact of these differentially expressed genes (DEGs), we analyzed the pathways involved in DEGs.

The results of GO analysis showed that the DEGs of the DSS group and the control group were mainly involved in biological processes such as the humoral immune response, activation of an immune response, negative regulation of hemostasis; and cellular component such as blood microparticle, membrane attack complex; and molecular functions such as lipid binding, lipopolysaccharide-binding, thrombospondin receptor activity (Fig. 6a). On the other hand, the DEG of the Lp082 and DSS groups was mainly involved in biological processes such as blood coagulation, fibrin clot formation, regulation of humoral immune markers, regulation of inflammatory cytokines; and cellular components such as Golgi lumen, endoplasmic reticulum, and molecular functions such as endopeptidase activity and peptidase activity (Fig. 6b).

Considering that in the Lp082, the up-regulated DEGs were far more than down-regulated DEGs (Fig. 5a-f) and the DEGs have the largest proportion of participation in biological processes (Fig. 6a-c), we further conducted GO-BP analysis on significantly up-regulated DEGs. The results of GO-BP analysis showed that compared to control group, up-regulated DEGs in DSS group were mainly enriched in the 6 inflammation-related GO-BP. Among those, the genes IL-1β and IL-1α were both involved in the IL-1β production and TNF production, the oncogene Ereg were involved in the IL-1β production, the genes IL-1β and IL-1rn, oncogene Fga were all involved in positive regulation of nuclear factor kappa-B (NF-κB) transcription factor activity, the oncogene Ldlr, Dgat2, and Mfsd2a were all involved in the regulation of toll-like receptor 4 signaling pathway, the pro-oncogenes Cdc7, Dbf4 were all involved in the acute inflammatory response, the anti-tumour gene Syk and the inflammatory genes Nlrp3 as well as Syk were all involved in the pro-inflammatory factor IL-6 production (Fig. 6d). Compared to DSS group, the up-regulated genes in Lp082 group were mainly enriched in the 6 anti-inflammatory-related GO-BP. Among them, the gene Isg15, which exerted both its antiviral and anti-inflammatory effects in innate immunity, and the gene Prg2, which played an important role in wound healing, were involved in the anti-inflammatory factors IL-10 production (Fig. 6e).

The results of KEGG analysis showed that the DEGs in DSS and control groups were mainly enriched in systemic lupus erythematosus, *Staphylococcus aureus* infection, Viral carcinogenesis, Pathways in cancer, TNF signaling pathway, Cellular senescence, and mitogen-activated protein kinase (MAPK) signaling pathway **(Fig. S2a)**. However, the DEG in both Lp082 and DSS groups, SASP and DSS groups, and SASP and Lp082 groups were mainly enriched in the following five pathways: Complement and coagulation cascades, Platelet activation, Autophagy - animal, Phagosome and N-Glycan biosynthesis **(Fig. S2b-S2d)**. Besides, the DEGs in Lp082 and DSS groups, as well as SASP and DSS groups were involved in protein processing in the endoplasmic reticulum and metabolic pathways **(Fig. S2b-S2c)**.

The results of gut mucosal barrier analysis showed that gene expression of MUC-2, ZO-1, ZO-2, occludin was significantly reduced in the DSS group but significantly increased in the Lp082 and SASP groups (p values < 0.05), and the gene expression of intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM,) claudin-1, and claudin-2 increased significantly in the DSS group but decreased significantly in the Lp082 and SASP groups (p values < 0.05) (Fig. 6g-j). It is worth mentioning that MUC-2 is an essential component of gut mucosa; ICAM-1 and VCAM induce gut mucosal lesions; ZO-1, ZO-2, and occludin promote tight junctions of gut epithelial cells; claudin-1 and claudin-2 increase intestinal permeability and aggravate inflammation.

Results of gene analysis related to NF-κB pathway showed that Lp082 also inhibited the mRNA expression of NF-κB1, NF-κB2, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), Toll-4, and RelA. These genes are signaling molecules in the NF-κB signaling pathway (Fig. 6g-j).

The potential mechanism of Lp082 alleviated the DSS-induced colitis

After confirming that probiotics can help relieve UC, we explored their potential mechanisms of action. For that, correlation and interaction between Lp082 and mouse symbiotic gut microbiome were elucidated by conducting Pearson correlation analysis, and the results are summarized in 3 parts of Fig. 7a.

The upper right portion of Fig. 7a shows that Lp082 was positively correlated with six strains that are positively correlated with two metabolic pathways: pyruvate fermentation to propanoate I, Pyruvate fermentation to acetate and lactate II; and these two metabolic pathways are positively correlated with acetic acid and propionic acid; and these two acids are negatively correlated with pro-inflammatory factors such as TNF-α, IL-1β, IFN-γ, IL-6, and MPO, but positively correlated with anti-inflammatory cytokine IL-10. The pro-inflammatory factors were negatively correlated with MUC-2, ZO-1, body weight, colon length while positively correlated with histologic scores, DAI Score, spleen coefficient, but anti-inflammatory cytokine was just the opposite (Fig. 7a).

The circle in the bottom half of Fig. 7a shows that Lp082 reduces inflammation by regulating DEG and the GO-BP they participate in. Lp082 was negatively correlated with the genes Nlrp3, Syk and Cdc7, Dbf4 and IL-1β, IL-1α, P2ry2 and IL-1β, IL-1α, Ereg and Ldlr, Mfsd2a, respectively, which were involved in IL-6 production, acute response, TNF production, IL-1β production, and toll-like receptor 4 (TLR4) signaling pathway. Lp082 was positively correlated with Abcc2 and Prg2 Isg15, which were positively correlated with drug transport, drug metabolic process, and IL-10 production, respectively (Fig. 7a).

The upper left of Fig. 7a shows that Lp082 was negatively correlated with NF-κB2, NF-κB1, COX-2, Rela, Toll4, iNOS, all of which were positively correlated with NF-κB, indicating that Lp082 inhibited the NF-κB signaling pathway by down-regulating the genes of NF-κB signaling molecules, thus relieving inflammation (Fig. 7a).

In a further comprehensive analysis of the data, we found that Lp082 improves the intestinal mucosal barrier by optimizing the following 4 barriers. First, Lp082 improved the biological barrier by improving the gut microbiota diversity and optimizing species composition, increasing the bacteria that produce SCFAs, enhancing the metabolic pathway of SCFAs and the content of SCFAs. Second, Lp082 improved the chemical barrier by increasing the content of goblet cells, MUC-2, reducing the ICAM-1 and VCAM content. Third, Lp082 improved the mechanical barrier by increasing the mRNA expression of ZO-1, ZO-2, occludin, decreasing the mRNA expression of claudin-1, claudin-2. Fourth, Lp082 improved the immune barrier by reducing the content of IL-1β, IL-6, TNF-α, MPO, IFN-γ and increasing the content of IL-10, transforming growth factor-beta1 (TGF-β1), transforming growth factor-beta2 (TGF-β2) **(**Fig. 7b**)**.

Discussion

Numerous studies have shown that probiotics improve the clinical outcome of IBD patients by influencing host gut microbiota [32]. Herein, we performed a shotgun metagenomic analysis to investigate whether Lp082 can improve gut dysbiosis in the UC mice model. As expected, we observed that the intake of DSS significantly reduced the shannon value but increased PCoA distance, a finding that is consistent with Wang et al. [33]. The Shannon index reflects gut microbiota richness and uniformity and is positively correlated with gut microbiota diversity, while the PCoA distance reflects the difference in the structure of the gut microbiota between different groups; the higher the PCoA value, the greater the difference in the gut microbiota structure [34]. In particular, Lp082 treatment remarkably increased the gut microbiota diversity and reduced gut microbiota structural differences in gut microbiota, as shown by the cluster analysis and PCoA analysis. On the other hand, Lp082 also optimized species composition; that is, the abundance of pro-inflammatory microbiota decreased in the Lp082 group, such as *Helicobacter hepaticus*, a potential pathogen of colitis. Likewise, we observed an increasing trend in the abundance of potential probiotics in the Lp082 group, such as *Bifidobacterium pseudolongum* and *Bacteroides ovatus*, which reduces colonic inflammation [35], *Parabacteroides distasonis*, which is negatively associated with obesity and diabetes [36], *Akkermansia muciniphila* and *Lactobacillus reuteri*, a widely studied probiotic, *Anaerotruncus sp* G3 2012 and *Lactobacillus plantarum*, potential SCFAs-producing bacteria[37]. The above results indicate that Lp082 is beneficial to optimizing the diversity, structure, and composition of gut microbiota. After demonstrating that Lp082 can increase the abundance of potential SCFAs-producing bacteria, further analysis found that Lp082 can activate two SCFAs-producing microbial metabolic pathways and the content of SCFAs. Subsequently, correlation analysis proved that Lp082 may increase SCFAs by activating the SCFAs-producing metabolic pathway of SCFAs-producing bacteria, so as to inhibit inflammation [38] and regulate host physiological activity through SCFAs [39]. All of these suggest that Lp082 repaired the microbial barrier by regulating the gut microbiome.

Chemical barrier mainly refers to gel-like mucin layers covering the surface of gut enterocyte, which is mainly composed of MUC-2 secreted by goblet cells. It plays an important role in isolating the internal and external environment of the intestine, lubricating the intestinal mucosa, preventing harmful substances in the intestinal lumen from entering[40]. In this study, the H&E staining result showed that Lp082 significantly improved the goblet cell number. Transcriptome results and immunofluorescence

analysis revealed that ingestion of Lp082 could increase mucin 2 mRNA expression and protein content, respectively. Sun et al. [41] observed the same phenomenon when fed with *Lactobacillus plantarum* 12. Burger-van Paassen et al. [42] found that the increased mRNA expression of MUC-2 in cells was caused by SCFAs ,and Gaudier al. [43] also found that SCFAs specifically increased mucin gene expression. Lp082 has been found to increase the content of SCFAs, so we speculate that SCFAs also caused the increase in MUC-2 in the Lp082 group. This also proves that SCFAs, as the primary energy source of colon epithelial cells, are involved in protecting the intestinal epithelial barrier.

The mRNA expressions of ICAM-1 and VCAM-1 were significantly decreased after Lp082 intake. Taniguchi et al. [44] found that anti-ICAM-1 treatment significantly attenuated colonic mucosal damage, while Philpott et al. [45] found that adhesion molecules ICAM-1 & VCAM-1 induced intestinal mucosal lesions. Lp082 has been shown to be effective in relieving intestinal mucosal lesions (i.e., reduced ulceration and inflammatory cell infiltration caused by DSS). So, we speculate that Lp082 reduces mucosal lesions by reducing ICAM-1 and VCAM. The above results showed that probiotic Lp082 increased the MUC-2 content of the mucus layer, alleviated the intestinal mucosal lesions caused by ICAM-1 and VCAM-1, to repair the chemical barrier.

The aberrant structure of tight junction (TJ) proteins between intestinal epithelial cells, such as the reduction of ZO-1, ZO-2, and occludin, is one of the critical factors leading to the disruption of the gut mechanical barrier in UC patients [46]. Several studies have identified TJ protein as a new target for the current treatment of UC [47]. Because Lp082 excellently improved histopathology, we speculated that Lp082 also has a regulatory effect on TJ molecules. To this end, we analyzed major TJ proteins, including ZO-1, ZO-2, occludin. As expected, the mRNA expression and immunofluorescence protein content of ZO-1 and the mRNA expression of ZO-2 and occludin were significantly decreased in DSS-induced UC mice but improved in the Lp082 treatment group. These are consistent with the findings of Cordeiro et al. [48] that ZO-1 and ZO-2 were significantly decreased in UC but increased after probiotic Minas Frescal cheese intake, indicating that the improvement of the mechanical barrier by regulating TJ may be one of the mechanisms by which probiotic Lp082 exerts anti-UC. In addition, the mRNA expression of another particular tight junction protein, ICAM-1 and VCAM-1, was increased in the DSS group. It is consistent with the findings of elevated ICAM-1 and VCAM-1 in IBD patients in clinical studies [49]. Mitselou et al. [50] found that the adhesion molecules ICAM-1 and VCAM-1 induced intestinal mucosal injury. Taniguchi et al. [44] found that anti-ICAM-1 treatment attenuated colonic mucosal injury. It has been reported that ICAM-1 and VCAM-1 can increase the permeability of intestinal mucosa [51]. Interestingly, the mRNA expression of ICAM-1 and VCAM-1 was found to decrease after Lp082 ingestion. Therefore, it can be thought that the alleviation of UC by Lp082 may be due to down-regulation of ICAM-1 and VCAM-1 to reduce intestinal mucosal permeability, thereby inhibiting the entry of harmful bacteria and undigested food and toxins into the body and reducing inflammation. These results suggest that Lp082 repairs the intestinal mechanical barrier by regulating TJ.

Although the exact etiology of UC is complex and uncertain, studies suggest that the NF-κB pathway plays a vital role in the pathogenesis of UC [52]. Our study has proved that Lp082 inhibits the NF-κB

pathway by down-regulating the mRNA expression of NF-κB2, NF-κB1, COX-2, Rela, Toll4, iNOS, and that NF-κB can also regulate inflammation by regulating cytokines [53]. Therefore, it can be suggested that Lp082 also has a specific regulatory effect on cytokines. To confirm this, we analyzed the cytokines associated with NF-κB. As expected, we observed that the mRNA expression level and protein content of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) were significantly increased in the DSS group but significantly decreased in the Lp082 group, which is in agreement with the findings of Sugihara et al. [54]. Among them, TNF-α can promote the proliferation and differentiation of T cells and increase intestinal inflammation [55]. The upregulation of IL-1β is involved in the recruitment and retention of leukocytes in inflamed tissues and can activate innate immune lymphocytes [56]. IL-6 activates NF-κB to regulate thedextran sulfate sodium-induced colitis in mice [57]. The above results indicate that Lp082 alleviates UC by inhibiting the levels of pro-inflammatory factors (TNF-α, IL-1β, and IL-6). Interestingly, we also found that the mRNA expressions of anti-inflammatory cytokines IL10, TGF-1, and TGF-2 were significantly decreased in the DSS group but increased in the Lp082 group. Surprisingly, IL10, TGF-1, and TGF-2 were shown to activate Treg and anti-inflammatory macrophages to alleviate UC [58]. And Sato et al. [59] also found that the loss of IL-10 spontaneously gave rise to IBD, and Hume et al. [60] found that TGF-β1 and TGF-β2 could dramatically relieve intestinal inflammation in DSS-induced colitis mice. These results suggest that Lp082 alleviates UC by increasing the levels of anti-inflammatory factors IL10, TGF-1, and TGF-2. In addition to inflammatory factors, we also noticed that a heme protein, MPO, was significantly reduced in the Lp082 group. Trevisin et al. [61] found that MPO caused UC by producing cytokines and hypochlorite and that MPO in the colon of UC patients is mainly produced by neutrophil infiltration [62]. Interestingly, this is consistent with the fact that the DSS group had a severe neutrophil infiltration in this study. However, neutrophil infiltration and MPO content were significantly decreased in Lp082 group. This shows that Lp082 alleviates UC by reducing neutrophil infiltration and its secreted MPO content. In a nutshell, our results suggest that Lp082 may play an anti-UC effect by inhibiting the NF-κB pathway, down-regulating pro-inflammatory cytokines, and up-regulating anti-inflammatory cytokines, reducing MPO content, thereby maintaining immune balance and protecting the immune barrier.

We further analyzed the specific regulatory effects of Lp082 on intestinal mucosal immunity. The mucosal immune system of the intestine mainly consists of Peyer’s patch and lamina propria under enterocyte [63]. The Peyer’s patch can deliver captured antigens to dendritic cells [64]. Then dendritic cells can not only trigger T cell-mediated cellular immunity and B cell-mediated humoral immunity by presenting antigens but also affect lamina propria immunity [65]. Combining previous studies, we found that DSS causes inflammation through the following six ways. First, gut permeability increases, and harmful substances enter to activate innate immunity, such as stimulating innate immune cells to produce TNF-α, IL-1β, and IL-6 [66]. Second, regulatory T cells produce less IL-10 and have a less inhibitory effect on effector T cells, resulting in the phenomenon of effector T and regulatory T cell dysregulation in UC patients [67]. Third, effector T cells promote B cell-mediated humoral immunity by promoting the secretion of IFN-γ and L-17A [68]. Fourth, effector T cells carried out immune cell recruitment and formed a vicious immune cycle with chemokines and cytokines [69]. Fifth, Peyer's patch

recognizes antigens and presents them to other immune cells through dendritic cells [64]. Sixth, antigen-activated neutrophils can both secrete MPO and recruit more immune cells from the bloodstream to the site of inflammation, further exacerbating inflammation [70] (Fig. 7b). Based on the above 6 reasons, we suggest that in addition to relieving inflammation by inhibiting the NF-κB pathway, Lp082 can also regulate inflammatory factors to maintain the balance between regulatory T cells and effector T cells to regulate intestinal mucosal immunity.

Conclusions

The Lp082 has an exciting therapeutic effect on ulcerative colitis than SASP. Also, shotgun metagenome and transcriptome analysis confirmed that Lp082 could improve gut microbiota dysbiosis, protect intestinal mucosal barrier, regulate inflammatory pathways, and affect neutrophil infiltration. These findings firmly support and advocate the clinical translation of Lp082 in the treatment of UC. It can be suggested that the application of gut microbiota and probiotics in the treatment of UC should receive more attention. The findings of this study not only provide new clues for revealing the complex mechanism of gut microbiota in relieving UC, but also provide evidence for *Lactobacillus plantarum* HN082 as a potential gut microbiota regulator to treat UC.

Abbreviations

Lp082
Lactiplantibacillus plantarum HNU082
DAI
Disease activity index
SCFAs
Short chain fatty acids
DSS
Dextran sulfate sodium
SASP
Sulfasalazine
ICAM-1
Intercellular cell adhesion molecule-1
VCAM
Vascular cell adhesion molecule
MUC-2
Mucin-2
ZO-1
Zonula occludens-1
ZO-2
Zonula occludens-2

IL-1β
Interleukin-1beta
IL-17A
Interleukin-17A
IL-6
Interleukin-6
TNF-α
Tumor necrosis factor-alpha
MPO
Myeloperoxidase
IFN-γ
Interferon-gama
IL-10
Interleukin-10
TGF-β1
Transforming growth factor-beta1
TGF-β2
Transforming growth factor-beta2
DEG
Differentially expressed genes
IBD
Inflammatory bowel diseases
UC
Ulcerative colitis
GCMS
Gas chromatography-mass spectrometry
GO
Gene Ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
TLR4
Toll-like receptor 4
PCoA
Principal Co-ordinates analysis
IECs
Intestinal epithelial cells
TJ
Tight junction
NF-κB
Nuclear factor kappa-B

PBS
Phosphate buffer saline
H&E
Hematoxylin and eosin
MAPK
Mitogen-activated protein kinase
COX-2
Cyclooxygenase-2
iNOS
Inducible nitric oxide synthase.

Declarations

Authors' contributions

The study was designed by Jiachao Zhang and Qixiao Zhai. The experiments were carried out by Yuqing Wu, Ao Li and Huanwei Liu. The data collection was performed by Yuqing Wu. The data analysis was performed by Yuqing Wu, Zeng Zhang and Chengcheng Zhang. The manuscript was written by Yuqing Wu, Rajesh Jha and Jiachao Zhang. And all of the authors perused and approved the manuscript.

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Availability of data and materials

The sequence data reported in this paper have been deposited in the NCBI database: PRJNA812271

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the College of Food Science and Engineering, Hainan University (No. HNUAUCC-2021-00122). We strictly obeyed the guidelines of the Guidelines for the Care and Use of Animals of Hainan University in the sample collection and utility protocols.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

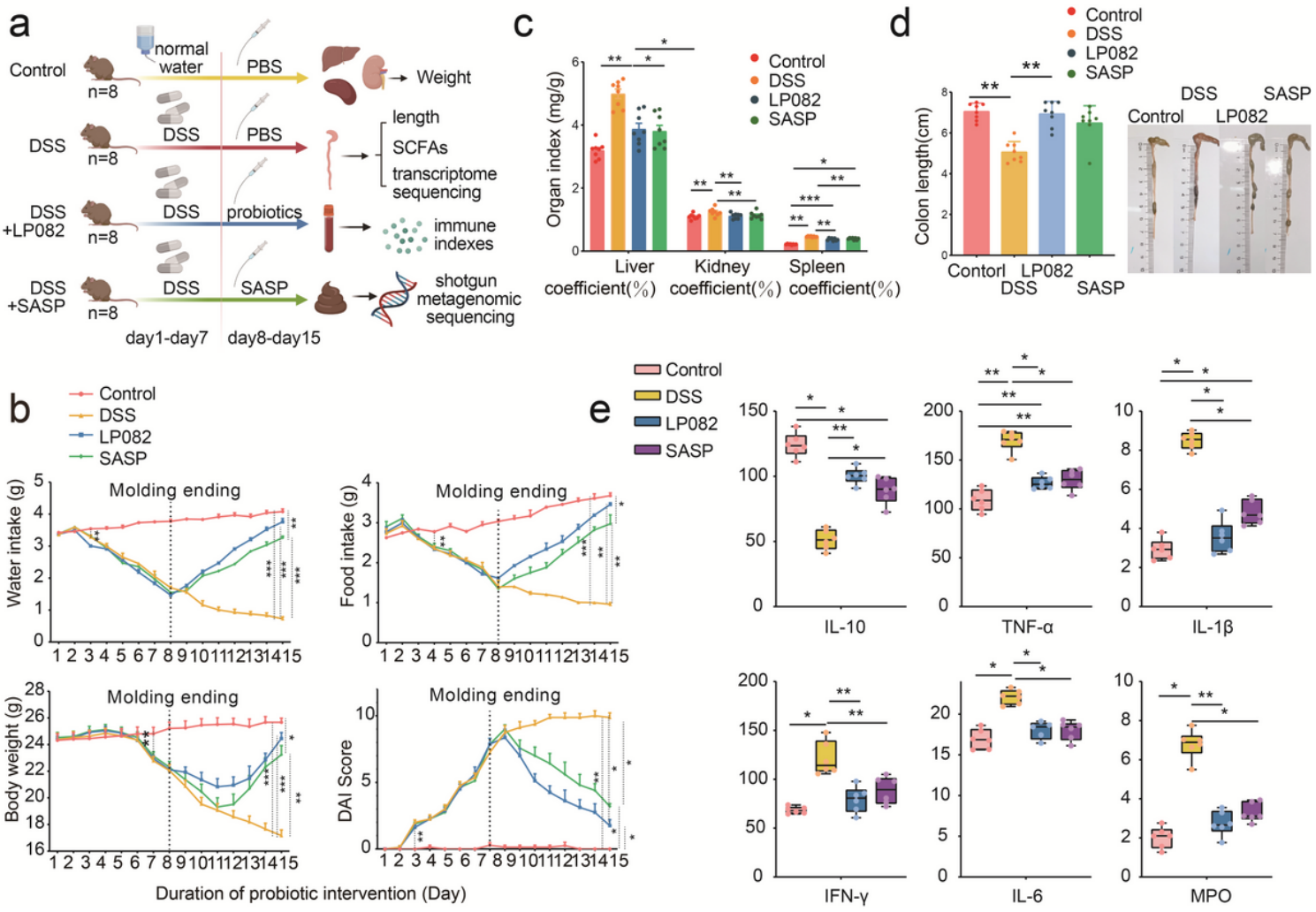

Figure 1

Effects of *Lactobacillus plantarum 082* on DSS-induced ulcerative colitis mice.

(a) Experimental design and grouping.

(b) Water intake, food intake, body weight, and disease activity index (DAI) in mice.

(c) Organ index (mg/g) of mouse spleen, liver, and kidney.

(d) Colon length (cm).

(e) Effects of Lp082 on inflammatory cytokines including IL-10, TNF-α, IL-1β, IFN-γ, IL-6 and MPO.

Data in are shown as means ± SD. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

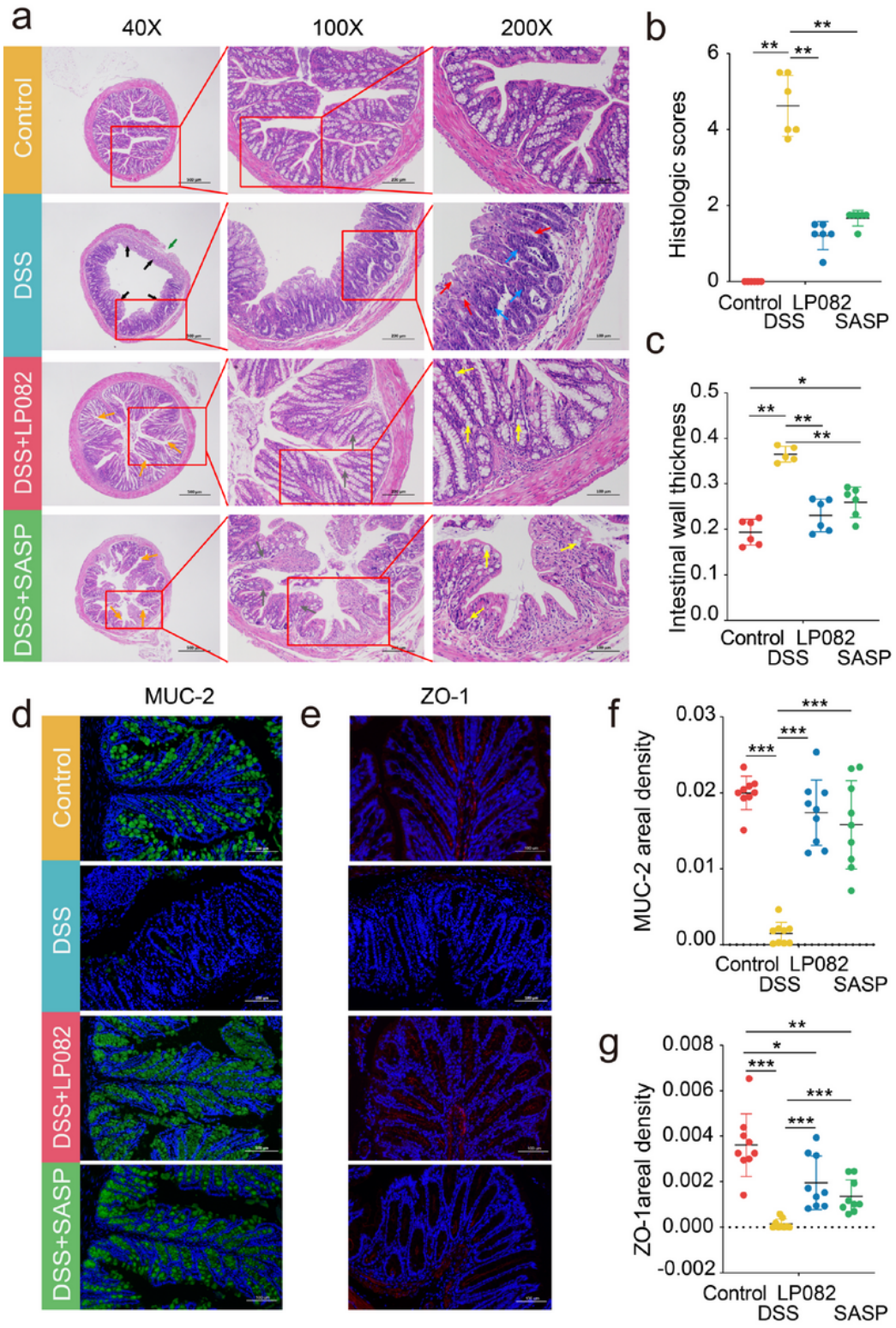

Figure 2

Effects of *Lactobacillus plantarum 082* on histological parameters and immunofluorescent proteins.

(a) Representative histological observation of hematoxylin & eosin stained mouse colon at magnification of 40×, 100×, and 200×.

(b) Histopathological scoring of colon tissue.

(c) Intestinal wall thickness.

(d) Immunofluorescence staining of MUC-2. Scale bar = 100 μm.

(e) Immunofluorescence staining of ZO-1. Scale bar = 100 μm.

(f) Areal density of MUC-2 immunofluorescence protein.

(g) Areal density of ZO-1 immunofluorescent protein.

Data are presented as mean ± SD, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

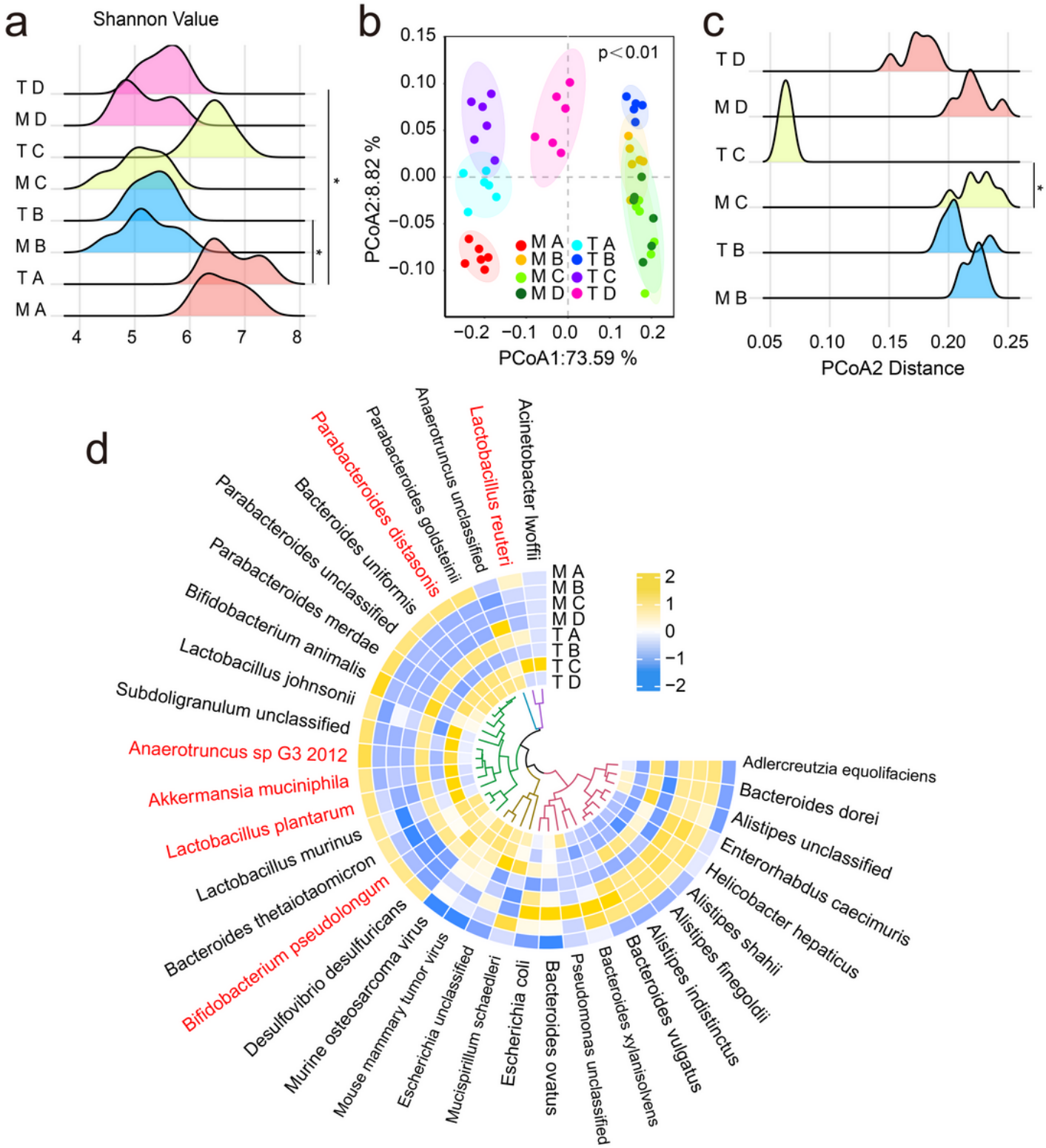

Figure 3

Effects of *Lactobacillus plantarum 082* strains on the gut microbiota in mice.

(a) The Shannon index.

(b) Principal co-ordinates analysis (PCoA) based on weighted unifrac distance at the species level.

(c) PCoA distance at the species level.

(d) Relative abundance of gut microbiota at the species level.

*: p < 0.05, **: p < 0.01, ***: p < 0.001.

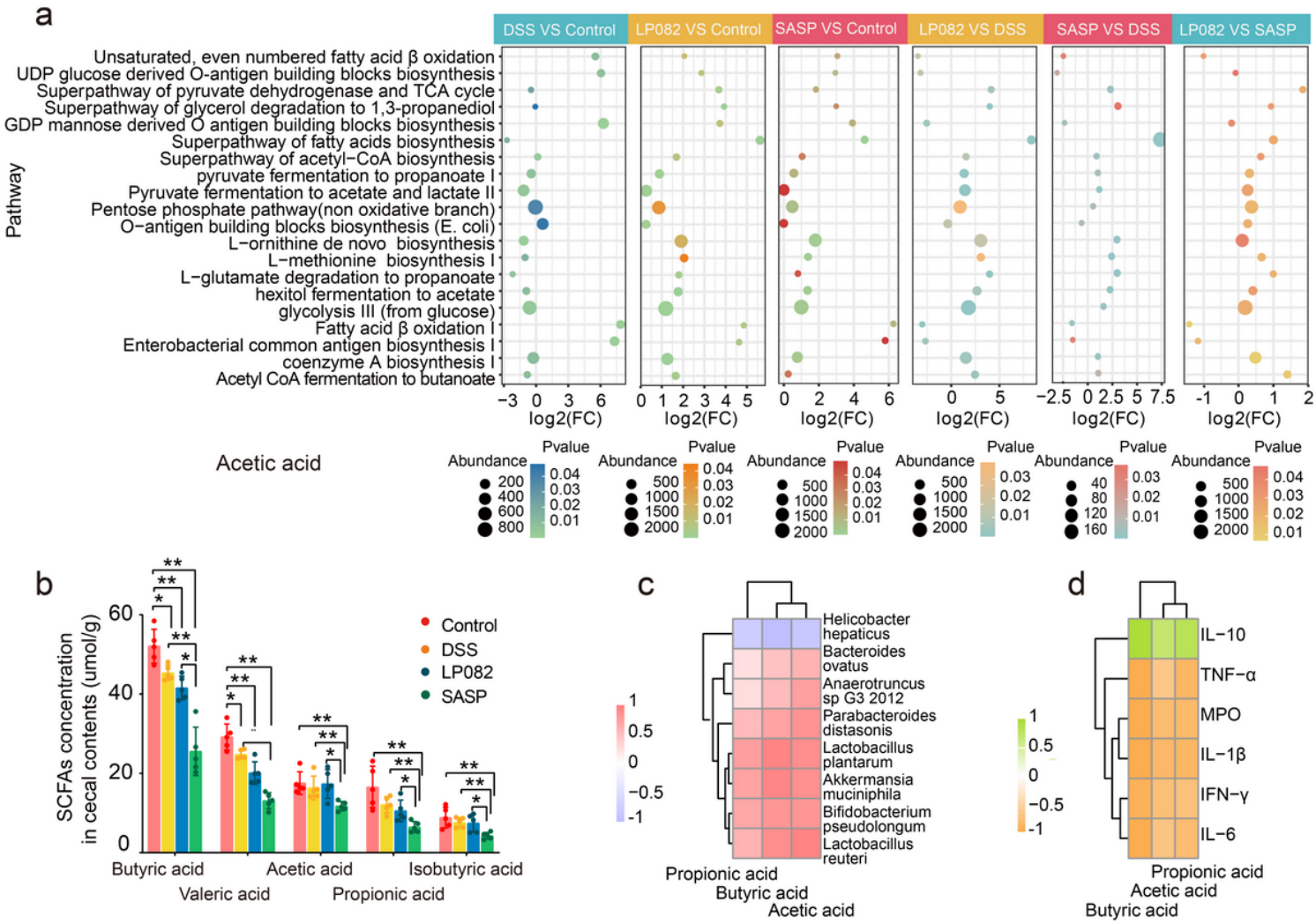

Figure 4

The important role of short-chain fatty acids (SCFAs) in alleviation of DSS-induced ulcerative colitis.

(a) Gut microbial metabolic pathways associated with SCFAs.

(b) SCFAs content determined by gas chromatography–mass spectrometry.

(c) Relationship between SCFAs and gut microbiota.

(d) Relationship between SCFAs and inflammatory cytokines.

Data in are shown as means ± SD. *: p < 0.05, **: p < 0.01, ***: p < 0.001.

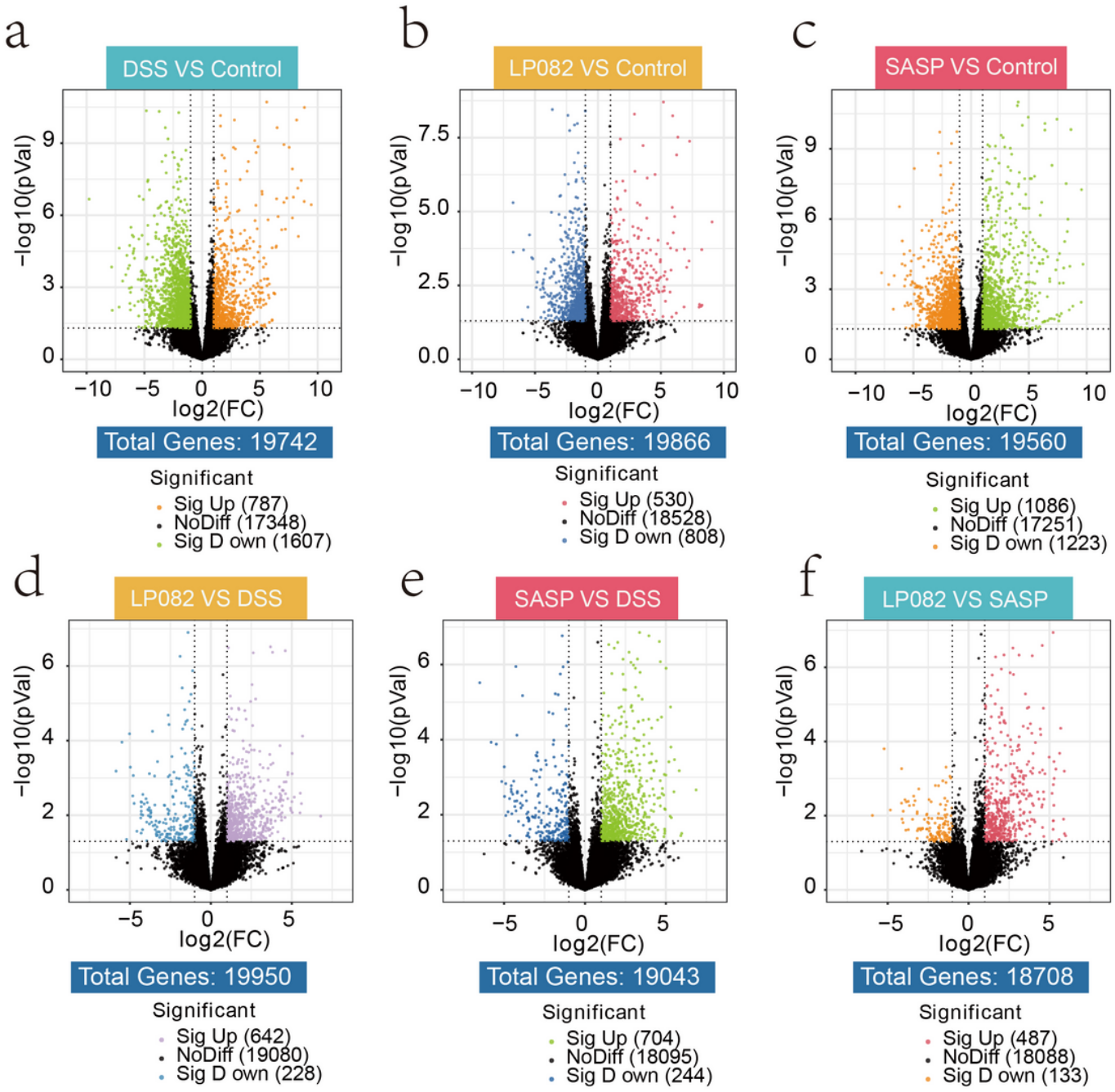

Figure 5

Effects of *Lactobacillus plantarum* Lp082 on gene expression distribution in mouse colon tissue.

(a)Gene expression distribution in the DSS group compared with the control group.

(b)Gene expression distribution in the Lp082 group compared with the control group.

(c)Gene expression distribution in the SASP group compared with the control group.

(d)Gene expression distribution in the Lp082 group compared with the DSS group.

(e)Gene expression distribution in the SASP group compared with the DSS group.

(f)Gene expression distribution in the Lp082 group compared with the SASP group.

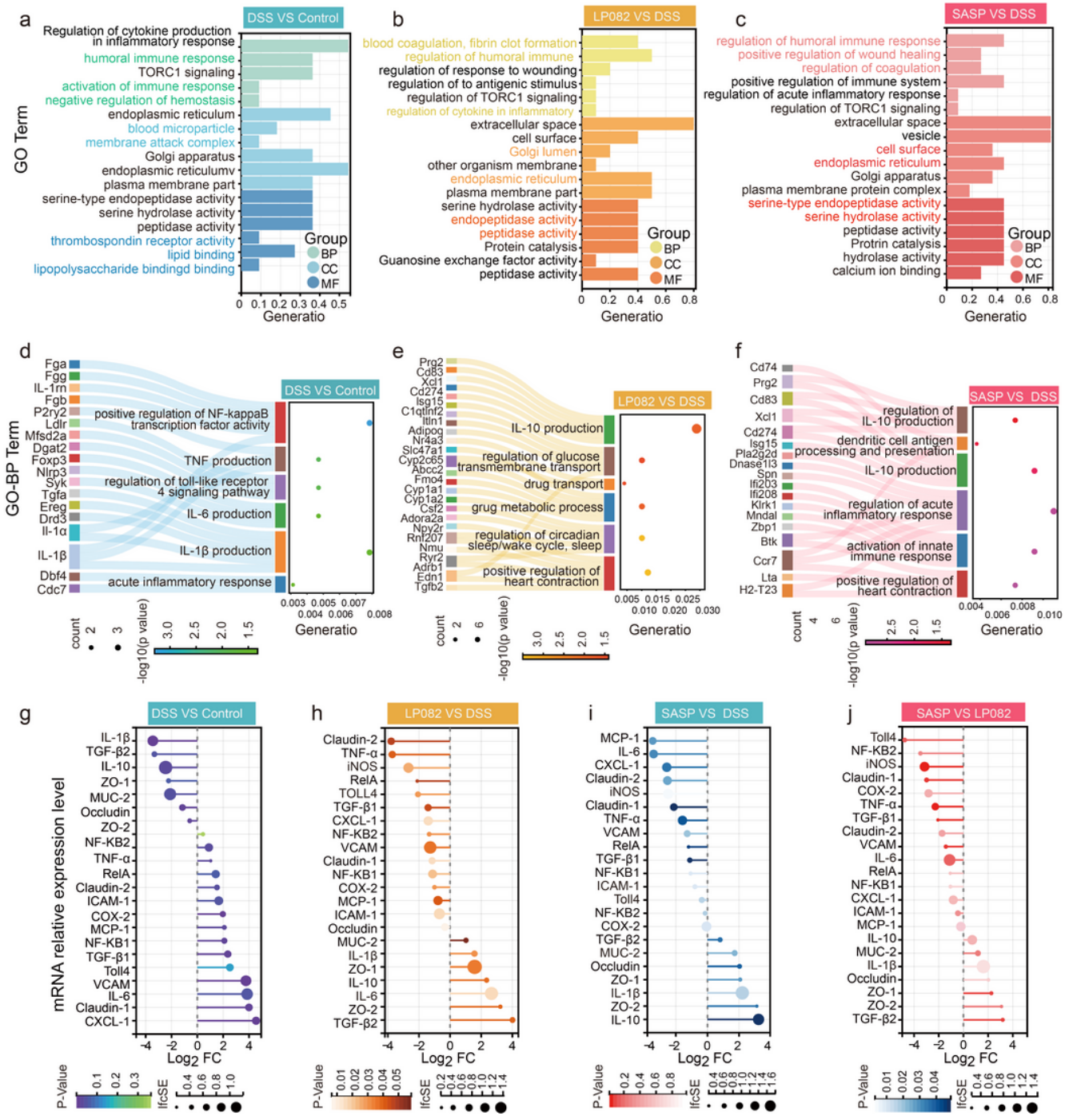

Figure 6

Comparative study of the transcriptome of intestinal epithelial cells.

(a) Gene Ontology (GO) pathway analysis of differentially expressed genes in DSS and control group.

(b) GO pathway analysis of differentially expressed genes in Lp082 and DSS group.

(c) GO pathway analysis of differentially expressed genes in SASP and DSS group.

(d) GO-BP pathway analysis of significantly up-regulated differentially expressed genes in DSS and control group.

(e) GO-BP pathway analysis of significantly up-regulated differentially expressed genes in Lp082 and DSS group.

(f) GO-BP pathway analysis of significantly up-regulated differentially expressed genes in SASP and DSS group.

(g) mRNA expression of specific genes in DSS and control group.

(h) mRNA expression of specific genes in Lp082 and DSS group.

(i) mRNA expression of specific genes in SASP and DSS group.

(j) mRNA expression of specific genes in SASP and Lp082 group.

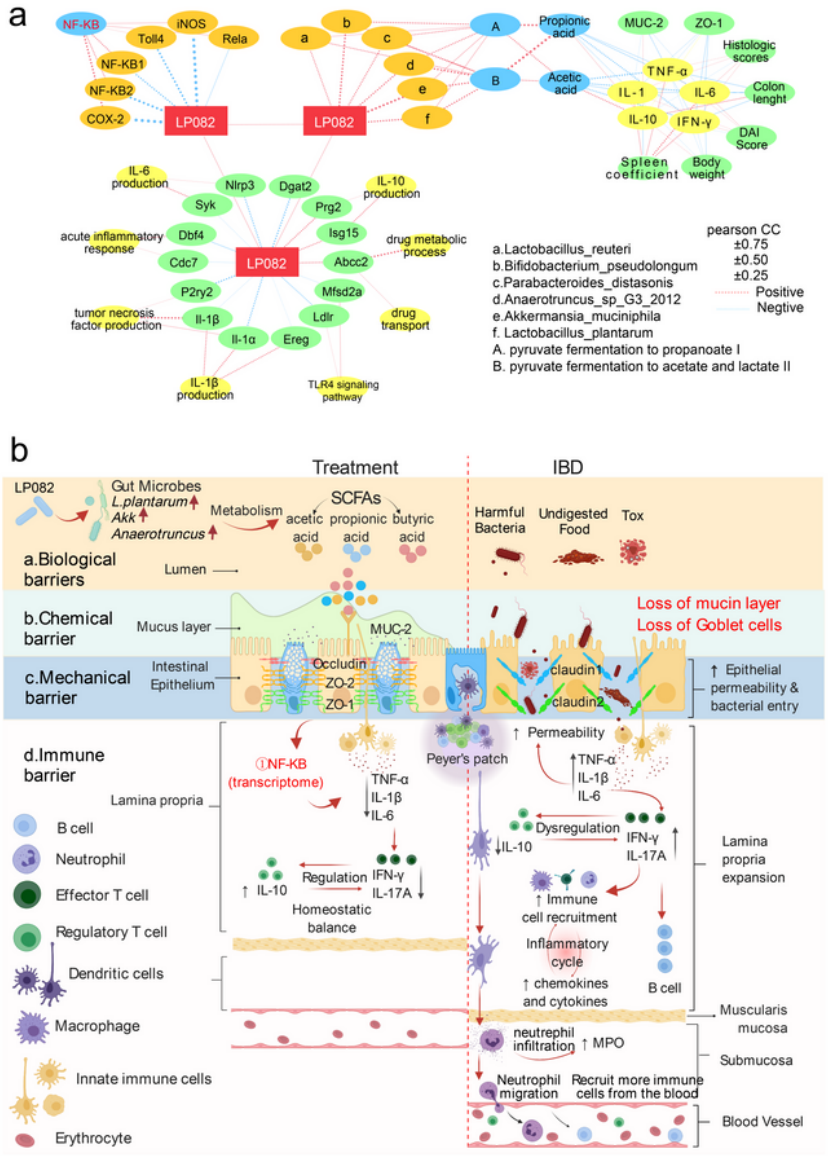

Figure 7

The mechanism of Lp082 in relieving enteritis.

(a) Correlation between Lp082 and various indicators.

(b) Potential mechanism of action of Lp082 in relieving DSS-induced ulcerative colitis.

Supplementary Files

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- Supplementarymaterial.docx