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Ingestion of *Lacticaseibacillus rhamnosus* Fmb14 prevents depression-like behavior and brain neural activity *via* the microbiota–gut–brain axis in colitis mice†

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Large preclinical evidence suggested that colitis was one of the risk factors for depression and probiotics were effective therapeutic agents to prevent the disease. The effect of *Lacticaseibacillus rhamnosus* Fmb14 on colitis-related depression-like behavior and its possible mechanisms were investigated. One week of DSS exposure led to the following changes in male C57BL/6N mice: a reduction in the movement distance from 2218 to 1299 cm, time in central areas from 23.6 s to 11.5 s, and time in the bright box from 217 s to 103 s, which were restored to 1816 cm, 18.4 s, and 181 s, respectively, with preadministration of Fmb14 for 8 weeks. All improvements provided by Fmb14 indicated a remarkable protective effect on depression-like behavior. Fmb14 first worked to repair intestinal barrier damage and the inflammatory response in the colon through ZO1 and Ocln enhancement and IL-1β, NF-κB and IL-6 reduction, respectively. Second, dysbiosis of the gut microbiota was modulated by Fmb14, including reduction of *Akkermansia* (18.9% to 5.4%), *Mucispirillum* (0.6% to 0.1%) and *Bifidobacterium* (0.32% to 0.03%). Fmb14 supplementation ameliorates the brain inflammatory response *via* IL-18 and NF-κB reduction and improves the blood–brain barrier *via* increased levels of ZO1 and Ocln. Moreover, brain activity was facilitated by an increase in BDNF and dopamine and the downregulation of GABA in the Fmb14 group. As a consequence of the modulatory effect on the dysfunction of neurotransmitters and neuroinflammation, Fmb14 prevents neurodegeneration by inhibiting neuronal apoptosis and Nissl edema. In addition, the correlation analysis further demonstrated the preventative effect of Fmb14 on depression-like behavior through the microbiota–gut–brain axis. Together, these findings demonstrated the important role of Fmb14 in biological signal transduction over the microbiota–gut–brain axis to improve mood disorders.

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Introduction

Inflammatory bowel disease (IBD) is one of the most widely occurring diseases and affects more than 11% of the world's population[1] and the incidence of IBD is highly correlated with the prevalence of mental diseases such as anxiety disorder,[2,3] depression,[4] Parkinson's disease[5] and Alzheimer's disease.[6] The pathogenesis of IBD usually involves complex risk factors, including genetic, immunological, environmental and microbial factors, which could affect the microbiota of the gastrointestinal tract in turn.[7] Crohn's disease (CD) and ulcerative colitis (UC) are two common forms of IBD and the dextran sodium sulfate (DSS) model of chemically induced colitis in mice has been reported to lead to behavioral disorders in addition to diarrhea and mucosal ulcers.[8] Depression is a psychiatric disorder with the phenotype including depressed mood, anhedonia, feelings of guilt or worthlessness, disruptions in cognitive function and self-harm or suicide in clinical observations.[9] Depression-like behavior was also found leading to the motion of low propensity, dark environment preferment and low social requirement at the laboratory level.[10] Depression is one of the mood disorders associated with colitis both in adults and children[11] that causes high morbidity and mortality.[12] Colitis induces negative behavior through the microbiota–gut–brain axis, including immune response dysregulation,[13] gut microbiota dysbiosis and barrier

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dysfunction.[14] Colitis-related psychological disease has become a nonnegligible harmful factor to human health in modern society. Although the drug therapeutic schedules for colitis are effective and mature,[15] drug management has a negative effect on the composition of the microbiota, which could deeply aggravate behavioral dysbiosis.

Probiotics have been used as a regular microbiota-management agent to modulate gastrointestinal-related disease to improve the health of the host at both the laboratory[83] and clinical levels.[16] The beneficial effects of probiotics on psychiatric and neurologic disorders *via* the microbiota–gut–brain axis are promising and have been reported in several animal models.[17] The drug treatment schedule is not suitable for colitis-induced mood disorders because chemosynthetic drug administration aggravates gut microbiota dysbiosis.[18] *Bifidobacterium longum* and *Bifidobacterium breve* were reported to reduce live environment interference-induced anxiety-like behavior through microbiota modulation,[19] and *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus fermentum* could improve human cognition accompanied by gut metabolism system regulation.[20] *L. rhamnosus* JB-1 regulates the expression of the central GABA receptor, which could improve the behavior of mice through the vagus nerve.[21] All evidence implied an improvement in the gut microbiota of specific probiotics to ameliorate mood disorders, and considering the convincing effect of probiotics on IBD, there is potential for probiotics to ameliorate colitis-induced mood disorders.

The mechanism by which probiotics improve colitis-induced behavioral disorders is still controversial, but the bidirectionality of brain–gut interactions plays an important role.[4,22] Ingestion of probiotics restores the structure of the gut microflora and then protects the neural system, enhances the blood–brain barrier, regulates psychiatric neurotransmitters and reduces the inflammatory response.[23] Dopamine (DA) and 5-hydroxytryptamine (5-HT) are neurotransmitters that can improve depression behavior, and the administration of *Lactobacillus plantarum* PS128 was reported to alter these biomarkers significantly.[24] Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter that is opposite to DA and 5-HT; excessive levels of GABA in the brain can induce minor depression,[25] and ingestion of *L. rhamnosus* JB-1 regulates emotional behavior through GABA receptor expression modulation.[21] The composition of the gut flora also affects the development of the central nervous system for the formation of the blood–brain barrier, and brain-derived neurotrophic factor (BDNF) is essential for protecting the blood–brain barrier and preventing neuronal degeneration.[26] The cytokine-mediated association between the immune system and the central nervous system provides new insight into the alleviated effect of probiotics on IBD for the anti-cytokine ability of probiotics.[27]

DSS-induced intestinal disruption and microbiota dysbiosis through the gut–brain axis are the main causes of mental illness in mice[1] and *L. rhamnosus* Fmb14 was found to be superior in restoring gut barriers and improving the homeostasis of the internal environment in our previous research,[28,82] both of which were targeted to treat colitis. A high incidence of colitis and severe psychiatric disorders has become a public health issue for the last two decades. This work aims to explore the ability of the potential probiotic *L. rhamnosus* Fmb14 to improve mood and behavioral disorders induced by DSS in mice.

Materials and methods

Preparation of probiotics

Lacticaseibacillus rhamnosus Fmb14 (NCBI accession number: CP101845) was provided by the Food Biotechnology Laboratory, Nanjing Agriculture University. The monoclonal colony was activated in sterile de Man Rogosa Sharpe (MRS) broth (20 g glucose, 10 g beef extract, 10 g peptone, 5 g yeast powder, 5 g sodium acetate, 0.25 g manganese sulfate, 0.58 g magnesium sulfate, 2 g ammonium citrate dibasic, 2 g dipotassium phosphate, 1 mL of Tween 80 and water to 1000 mL) at 37 °C for 24 h. *L. rhamnosus* Fmb14 was incubated in sterile MRS broth culture for 24 h. Then, *L. rhamnosus* Fmb14 was adjusted to concentrations of 10^9 CFU mL^{-1}, 10^8 CFU mL^{-1} and 10^7 CFU mL^{-1} and centrifuged at 8000*g* for 10 min to collect live microorganisms. Then, three concentrations of microorganisms were washed with normal saline three times and finally resuspended to obtain the high-treatment (10^9 CFU mL^{-1}), medium-treatment (10^8 CFU mL^{-1}) and low-treatment (10^7 CFU mL^{-1}) solutions of Fmb14 for oral administration.

Effect of DSS on inducing depression-like behavior in animals

The animal experiments were conducted according to the regulations of Laboratory Animal Research Centre of Nanjing Agriculture University, Nanjing, China. All experimental protocols were approved by the ethics committee of Nanjing Agriculture University, Nanjing, China (approval number: NJAU no. 20211109165).

A four-week-old specific pathogen-free (SPF) class of male Kunming mice was purchased from Yangzhou University (Jiangsu, China) and all animals were kept under specific pathogen-free conditions.

An eight-week-old specific pathogen-free (SPF) class of male C57BL/6 mice was purchased from Yangzhou University (Jiangsu, China), and all animals were kept under specific pathogen-free conditions. Mice were housed normally for 7 days to allow environmental adaptation, illuminated with artificial light for 12 h every day (06:00 to 18:00 light cycle, 18:00 to 06:00 dark cycle), fed standard laboratory food, and allowed to freely drink water. The environmental temperature was 25 °C, and the relative humidity was 60%.

The mouse experiment was designed as shown in Fig. 1A. After the acclimation period (7 days), 40 experimental mice were divided into 5 groups (8 mice in each group): the control group, mice administered orally with saline and receiving distilled water; DSS group, mice administered orally with saline and receiving 4% DSS solution; and H-Fmb14, M-Fmb14 and L-Fmb14 groups, mice administered orally with 50 μL per 10 g

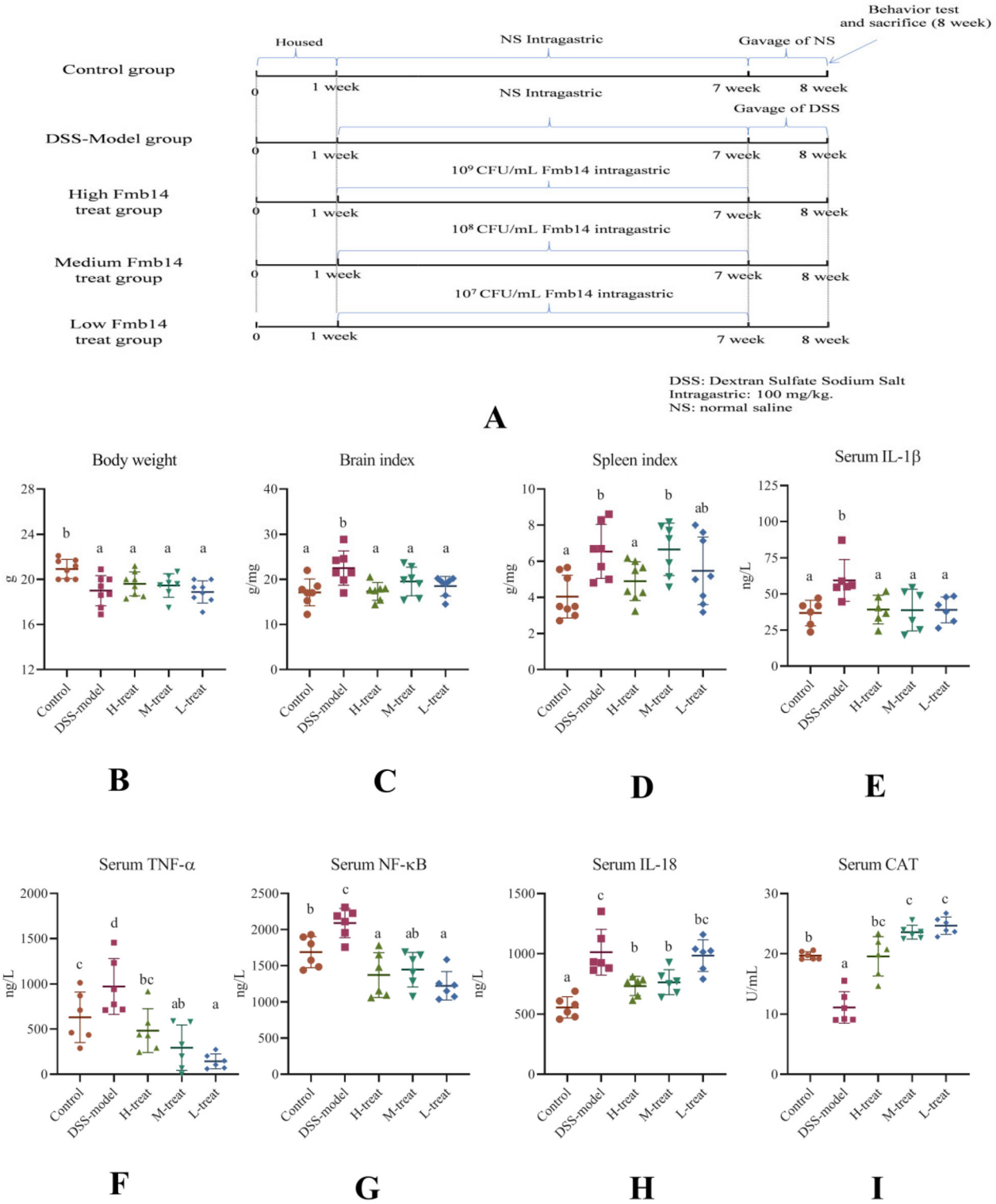

Fig. 1 Administration of *L. rhamnosus* Fmb14 ameliorated DSS-induced IBD hyperuricemia in mice. (A) *L. rhamnosus* Fmb14 experimental design for the treatment of DSS-induced depression in mice. (B–D) Effect of *L. rhamnosus* Fmb14 on the body weight and organ index in different groups of mice (B: $n = 6$, df = 4, $F = 3.950$, $p = 0.011$; C: $n = 6$, df = 4, $F = 3.982$, $p = 0.01$; D: $n = 6$, df = 4, $F = 4.45$, $p = 0.005$). (E–H) Effect of *L. rhamnosus* Fmb14 on serum cytokines in different groups of mice (E: $n = 6$, df = 4, $F = 3.933$, $p = 0.013$; F: $n = 6$, df = 4, $F = 10.150$, $p = 0.000$; G: $n = 6$, df = 4, $F = 12.433$, $p = 0.000$; H: $n = 6$, df = 4, $F = 13.871$, $p = 0.000$). (I) Effect of *L. rhamnosus* Fmb14 on serum antioxidants in different groups of mice (I: $n = 6$, df = 4, $F = 39.408$, $p = 0.000$). The mean ± SD is represented by bars, and $^*P < 0.05$ was used to indicate significance. Different lowercase letters represent significant differences at $p < 0.05$, and labels a to d represent lower to upper levels, respectively.

weight per day of different concentrations of Fmb14 solutions and receiving 4% DSS solution. The behavior tests, including the open field test, light/dark box test and three-chamber test, were conducted two days after 8 weeks of feeding.

All behavioral testing was performed between 9:00 AM and 6:00 PM two days before sacrifice.

Open field test. The open field test (OFT) was performed to measure exploratory behavior, general activity, and anxiety-like behavior.[29] The mice were placed in the center of the box (80 × 80 cm), which was divided into 16 zones, and locomotor activity was determined by quantifying the total distance, distance in the center, average speed and time spent in the inner zone. Lighting in the arena was 625 lux. All videos were captured using a camera, and the parameters were analyzed automatically using an EthoVision XT system.

Light/dark box test. The light/dark box test was modified as described by Gareau.[30] Briefly, mice were placed in a novel arena (60 × 120 cm) with a light (1/2) and a dark (1/2) compartment with a door for 5 min (the lighting in the light box was 600 lux). The time the mice stayed in each box and the number of transitions between the light and dark compartments were recorded.

Three-chamber test. The three-chamber test was modified as described by Schiavi.[31] The apparatus used was 3 connected boxes with two doors, two rectangular boxes (60 × 30 × 30 cm; $l \times w \times h$) on the sides and a square box (30 × 30 × 30 cm; $l \times w \times h$) in the middle. A small cylindrical cage was placed in the center of one rectangular box with one mouse in it, and the test mice were placed in the other rectangular box. The test lasted for 5 min, and the time when the two mice first met, the time that the two mice spent in one box and the time of every stay were recorded.

The mice were sacrificed by the method modified by our previous research.[28] All mice were fasted for 12 h before being sacrificed and then anesthetized with diethyl ether. Mouse serum was collected from the supernatant of blood, which was collected from the eye vein, left undisturbed for 30 min and centrifuged at 4000 rpm for 20 min. Meanwhile, the viscera sample was weighed after collection and stored at −80 °C until analysis.

Assessment of colitis

The degree of colitis in mice was preliminarily assessed after 3 and 7 days of DSS exposure using a fecal occult blood test kit purchased from Beyotime Biotech, Inc. (Shanghai, China). The hematochezia and stool consistency scoring system was based on previous research:[10] 0, no observable blood (negative); 1, trace blood (light blue); 2, slight blood (blue); 3, obvious blood (dark blue); and 4, gross blood (black) for hematochezia and 0, normal; 1, loose stool; 2, mild diarrhea; 3, diarrhea; and 4, gross diarrhea for stool consistency.

Biomarker measurements in tissues

The serum levels of IL-1β (MM-0040M2), TNF-α (MM-0132M2), NF-κB (MM-44130M2) and IL-18 (MM-0169M2) were detected using ELISA kits (Jiangsu Meimian Industrial Co., Ltd, Yancheng, China), and the serum levels of CAT and SOD were detected using commercial kits (Jiancheng, Nanjing, China) according to the protocols. Fresh brains were ground using a handheld grinder (Jingxin, Shanghai, China) on ice, after which the suspension of samples was obtained at low-speed centrifugation (4000 rpm, 10 min). The levels of DA (MM-0626M2), GABA (MM-0442M2), 5-HT (MM-0443M2), NE (MM-0876M2) and BDNF (MM-0204M2) in the brain were all detected using ELISA kits (Jiangsu Meimian Industrial Co., Ltd, Yancheng, China) according to the manufacturer's protocol. The level of HIAA (H411-1-1) in the brain was detected using ELISA kits (Jiancheng, Nanjing, China) according to the manufacturer's protocol. At least 6 samples from each group were selected for independent assays.

Western blotting was performed as described by Tang.[81] The colon and prefrontal cortex of brain tissues (500 mg) were placed in a mortar along with liquid nitrogen and promptly crushed into powder. The powder was added to 500 μL of RIPA lysis buffer (10 μL mg^{-1}) (Beyotime Biotech, Shanghai, China) containing PMSF and lysed on ice for 30 min after vortexing. Protein samples were extracted from the supernatant after the mixture was centrifuged at 12 000*g* for 5 min at 4 °C. The protein samples were separated on a 12% SDS-PAGE gel and transferred to NC membranes, which were blocked in TBST with 5% nonfat dry milk and treated overnight at 4 °C with primary antibodies. GAPDH, IL-1β, NF-κB, TGF-β, IL-6, IL-18, TNF-α, ZO-1, Clan, Ocln, JNK, AMPK and ERK primary antibodies were purchased from Affinity Biosciences (Nanjing, China). The membranes were then incubated for 2 h at room temperature with HRP-conjugated goat anti-rabbit IgG secondary antibodies (Beyotime Biotech, Shanghai, China) and exposed to MiniChem610 (SAGECREATION, Beijing, China). The protein bands were quantified using ImageJ software. The raw images of the western blots are shown in the ESI.†

Histological analysis

The intestine, colon and brain samples were rinsed with PBS, after which the intestine and colon samples with a length of 1 cm were prepared, while whole kidneys were used. The samples were immersed in 4% paraformaldehyde solution at room temperature for more than one day. Later, the samples were sent to Hycell Biotechnology (Nanjing, China) and stained with hematoxylin and eosin (HE), Nissl, Fluoro-Jade B (FJB) and TUNEL. The morphology of the sections in different groups was observed under a microscope (Nikon Eclipse 80i, Nikon Co., Japan).

SMRT analysis of the gut microbial composition

A total of 30 colon content samples, five in each group, were collected. The samples were immediately frozen at −80 °C until DNA extraction. A total of 0.2 g of colon content from each group was utilized for DNA isolation. The sample DNA was extracted using a Sangon Biotech (Shanghai, China) columnar soil genomic extraction kit (SK8263), and DNA samples were quantified using a NANO drop 2000 (Thermo Fisher, CA, USA). The quality of extracted DNA was assessed by

0.8% agarose gel electrophoresis and spectrophotometry (optical density at 260 nm/280 nm). The V3–V4 region of 16S rRNA genes was amplified using the universal primers forward 5′-CCTACGGGNGGCWGCAG-3′ and reverse 5′-GGACTACHVGGGTATCTAAT-3′. The PCR products were quantified and sequenced using the Personalbio platform.

All sequences were compared against the Greengenes reference database (release 13.8, **https://greengenes.secondgenome.com/**). Bioinformatic analysis was performed using Geneclouds of Personalbio (**https://www.genescloud.cn/home**) under project number MD202202252040HQZX. Briefly, QIIME2 software was used to analyze the observed species; the Shannon index, Faith's PD and other diversity indices were calculated; and a dilution curve was drawn afterward. According to the ASV table and phylogenetic tree, a UniFrac distance matrix was generated and weighed, after which the UniFrac PCoA map was drawn.[28] The potential functions of the flora were analyzed based on KEGG, COG and GO databases.

Determination of colonized *L. rhamnosus* Fmb14 in colonic materials

For the detection of *L. rhamnosus* Fmb14 colonization, feces and colonic materials were collected and subjected to PCR. The primer sequences for PCR are listed as follows: forward: 5′-TTGCGATTGCCTGTTGGTTG-3′ and reverse: 5′-CAATGCCC-CGACATATCCGA-3′. The specific primers were designed based on the coding sequence (CDS) 24 of the Fmb14 genome (the sequence is listed in the ESI†). Mouse fecal and colonic material DNA was extracted in accordance with the manufacturer's instructions using a Stool DNA Kit (Solarbio Technology, Beijing, China). Fmb14 DNA cultured in MRS for 24 h was used as a positive control. PCR was performed with 2× Taq Master Mix (Yeasen Biotech Co., Ltd, Shanghai, China) and PCR detection (Applied Biosystem, CA, USA) was performed under the following cycling conditions: 95 °C predenaturation for 10 min, 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s for 35 cycles.

Statistical analysis

Statistical analysis and Pearson's correlation analysis were performed using SPSS v.17.0 software. P value < 0.05 indicated statistical significance. Data were analyzed using one-way analysis of variance (ANOVA). Significant differences between two groups were analyzed using an independent sample t test. To determine the composition of the bacterial community, PCoA was performed using R version v.3.3.2.

Results

Fmb14 decreases diarrhea and inflammation in DSS-treated mice

Administration of Fmb14 to the mice did not decrease the weight loss of DSS (Fig. 1B) but ameliorated the diarrhea in the mice (Table 1). One week of DSS drinking increased the brain and spleen index from 17.1 to 22.5 and 4.0 to 6.5 mg

Table 1 Effect of Fmb14 administration on diarrhea and hematochezia induced by DSS

	Hematochezia					Stool consistency				
	Control	Model	H-Fmb14	M-Fmb14	L-Fmb14	Control	Model	H-Fmb14	M-Fmb14	L-Fmb14
Day 3	0.25 ± 0.46^{a}	2.87 ± 0.83^{c}	1.75 ± 0.71^{b}	2.50 ± 0.76^{c}	2.62 ± 0.74^{c}	1.00 ± 0.75^{a}	2.25 ± 0.70^{b}	1.63 ± 0.74^{ab}	2.00 ± 0.75^{b}	2.12 ± 0.64^{b}
Day 7	0.50 ± 0.53^{a}	3.25 ± 0.71^{c}	2.25 ± 0.71^{b}	2.87 ± 0.64^{bc}	3.25 ± 0.71^{c}	0.75 ± 0.70^{a}	3.12 ± 0.83^{c}	2.12 ± 0.64^{b}	2.87 ± 0.64^{c}	3.25 ± 0.71^{c}

Different lowercase letters represent significant differences at $p < 0.05$, and labels a to d represent lower to upper levels, respectively.

g^{-1}, respectively, and high Fmb14 treatment decreased the two indices to normal levels (17.3 and 4.9 mg g^{-1}, respectively) (Fig. 1C and D). In addition, DSS exposure showed no effect on the liver, intestinal and renal indices of the mice (Fig. S1†). Simultaneously, DSS dramatically increased IL-1β by 61.3%, TNF-α by 54.1%, NF-κB by 23.9% and IL-10 by 82.3% in the serum of the model group when compared with the control groups, and all Fmb14 administration groups could reduce the cytokines to or even below the normal levels (Fig. 1E–H). In addition, the effect of Fmb14 on oxidative stress was investigated, and the results showed that DSS-induced CAT increased from 19.7 to 11.1 U mL^{-1} and then recovered to 19.5 U mL^{-1} after high Fmb14 treatment, and the same trends were observed for SOD (Fig. 1I and Fig. S2†). Neither DSS nor Fmb14 treatment affected serum MDA and GSH-px (Fig. S2†).

Fmb14 alleviates colitis through tight junction enhancement

The colon lengths of H-Fmb14-treated mice were not decreased after DSS (4%) exposure for 7 days (Fig. 2B and C), and the colon index was not affected by Fmb14 treatment (Fig. 2A). Moreover, longitudinal sections of the colon and intestine were prepared for histopathological analysis, and the results showed that DSS led to a thinner crypt structure and thinner walls of the colon, which caused less glandular fluid production and an empty enteric cavity, and the destruction of the colon structure was alleviated by Fmb14 treatment (Fig. 2D). The intestinal villus structures were damaged in the DSS-treated group, and H-Fmb14 treatment restored the length of the villus structures and eliminated mucosal edema (Fig. 2E). Considering the lesion of epithelial cells, tight junction proteins of the colon, including ZO1, Clan and Ocln, were tested by western blotting. The levels of ZO1 and Ocln decreased from 1 to 0.59 and 0.66 in the DSS group and increased to 0.84 and 0.92 in the H-Fmb14 treatment group (Fig. 3A–C), respectively, but the expression of Clan was not altered by Fmb14 treatment (Fig. 3D). Additionally, cytokines in the colon were investigated, and the results indicated that DSS exposure significantly increased the levels of IL-1β, NF-κB, IL-6, IL-18 and TNF-α from 1 to 1.51, 1.55, 1.94, 2.23 and 1.58, respectively, and all cytokines were reduced to 0.73, 1.04, 1.52, 1.72 and 1.31, respectively, in the H-Fmb14 treatment group (Fig. 3E–K). These results showed that gut barriers were

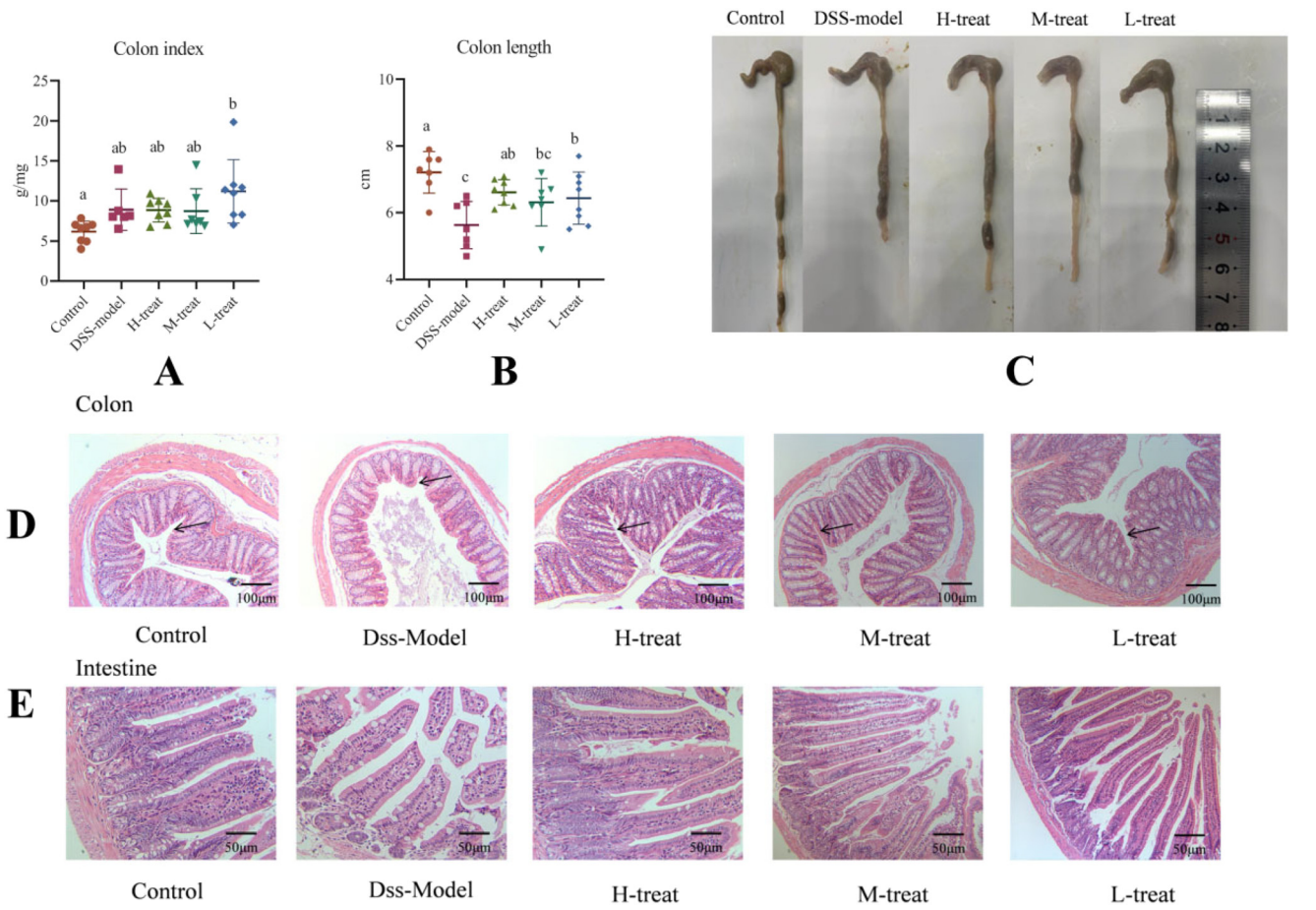

Fig. 2 Administration of *L. rhamnosus* Fmb14 alleviated the gut damage induced by DSS. (A) The colon index of every mouse (n = 6, df = 4, F = 3.723, p = 0.013). (B and C) The entire colon length of every mouse (B: n = 6, df = 4, F = 5.345, p = 0.002). (D) Hematoxylin and eosin (H–E)-stained images of the colon. (E) Hematoxylin and eosin (H–E)-stained images of the intestine. The mean ± SD and standard deviation are shown by bars, and different lowercase letters (ab) represent significant differences at $p < 0.05$. Labels a to b represent lower to upper levels, respectively.

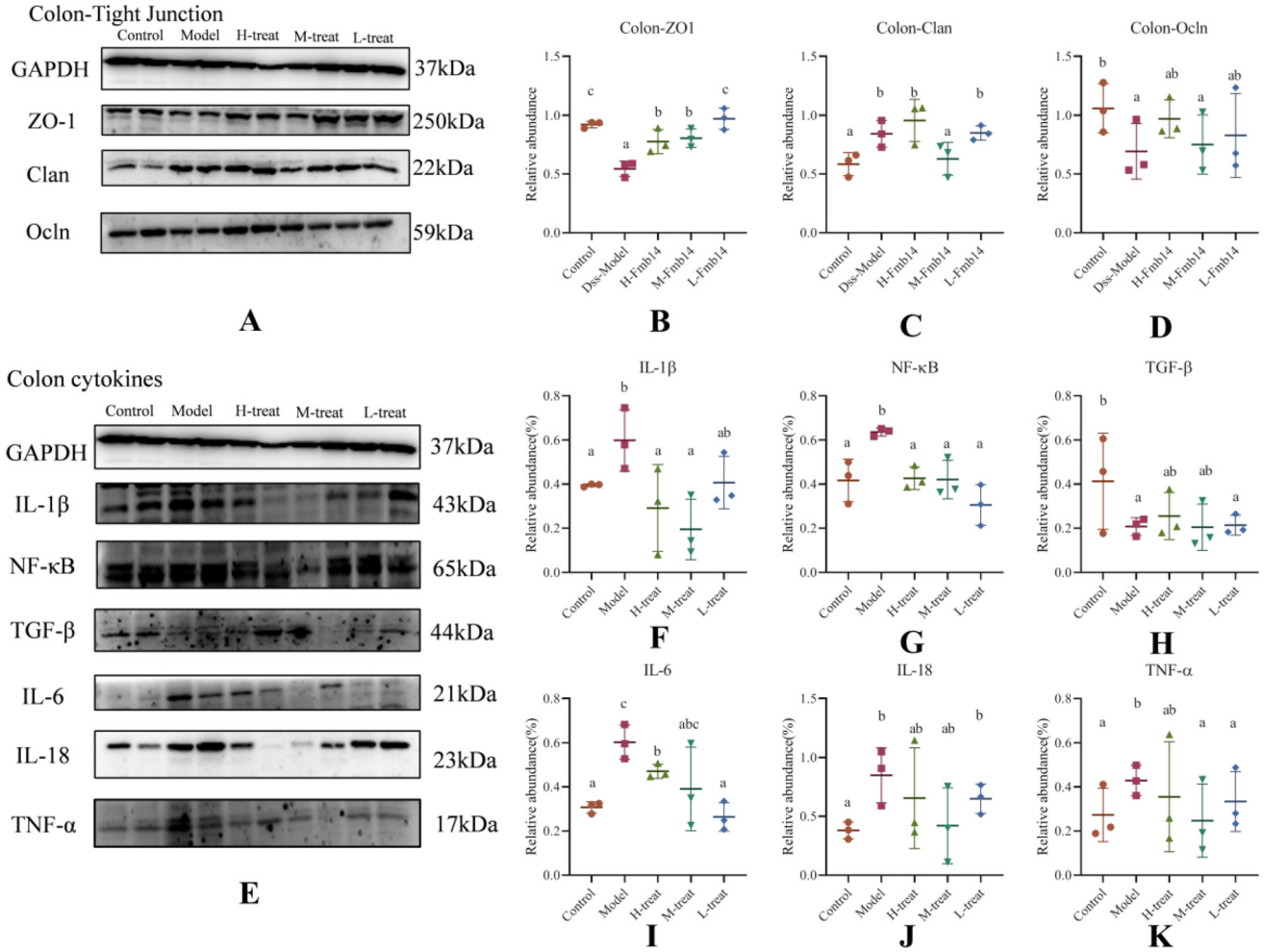

Fig. 3 Administration of *L. rhamnosus* Fmb14 restored gut barriers and reduced colon inflammation. (A–D) Western blotting of colon zonula occludens-1 (ZO1), clandin (Clan) and occludin (Ocln) (B: n = 3, df = 4, F = 13.640, p = 0.000; C: n = 3, df = 4, F = 4.825, p = 0.02; D: n = 3, df = 4, F = 1.088, p = 0.413). (E–K) Western blotting of colon cytokines (F: n = 3, df = 4, F = 3.746, p = 0.041; G: n = 3, df = 4, F = 7.549, p = 0.005; H: n = 3, df = 4, F = 1.607, p = 0.247; I: n = 3, df = 4, F = 5.732, p = 0.012; J: n = 3, df = 4, F = 1.516, p = 0.27; K: n = 3, df = 4, F = 0.603, p = 0.669). Mean ± SD is represented by bars, and different lowercase letters (abc) represent significant differences at $p < 0.05$. Labels a to c represent lower to upper levels, respectively.

damaged after 7 days of DSS exposure and preadministration of Fmb14 could partly alleviate the injury.

Fmb14 ameliorates depression-like behavior in DSS-treated mice

The open field test (OFT), light/dark box test, and three-chamber test were performed to measure general activity and depression-like behavior. DSS-treated mice presented negative locomotor activity compared with the control group for the decrease in the total movement distance from 2218 to 1299 cm and the lowering of the average speed from 8.0 to 5.3 cm s^{-1} (Fig. 4A and B). Depression-like behavior emerged in DSS-treated mice and manifested as the decrease in the distance in the central area (from 247 to 93 cm) and the lowering of the time spent at high speed (46.9 to 15.3 s) and in central areas (23.6 to 11.5 s) (Fig. 4B), and high Fmb14 treatment prevented behavioral disorders by improving the total movement distance (1816 cm), average speed (6.9 cm s^{-1}), distance in the central area (192 cm), time spent at high speed (30.6 s), and time in central areas (18.3 s) (Fig. 4B).

The L/D box test results indicated that DSS exposure led to dark environment tendencies and low exploration tendencies in mice, both of which indicate depression behaviors. Fmb14 treatment modulated the behaviors to normal levels (Fig. 4C). Moreover, the social tendency of Fmb14-treated mice was improved to the levels of the control group in terms of time to the first encounter, time in the same box, and time of every stay (Fig. 4D).

Fmb14 regulates neurotransmitters and repairs blood–brain barriers in DSS-treated mice

Previous studies have reported that depression-like behaviors are accompanied by disturbances in hormone levels or blood–brain barrier destruction. Serum levels of dopamine

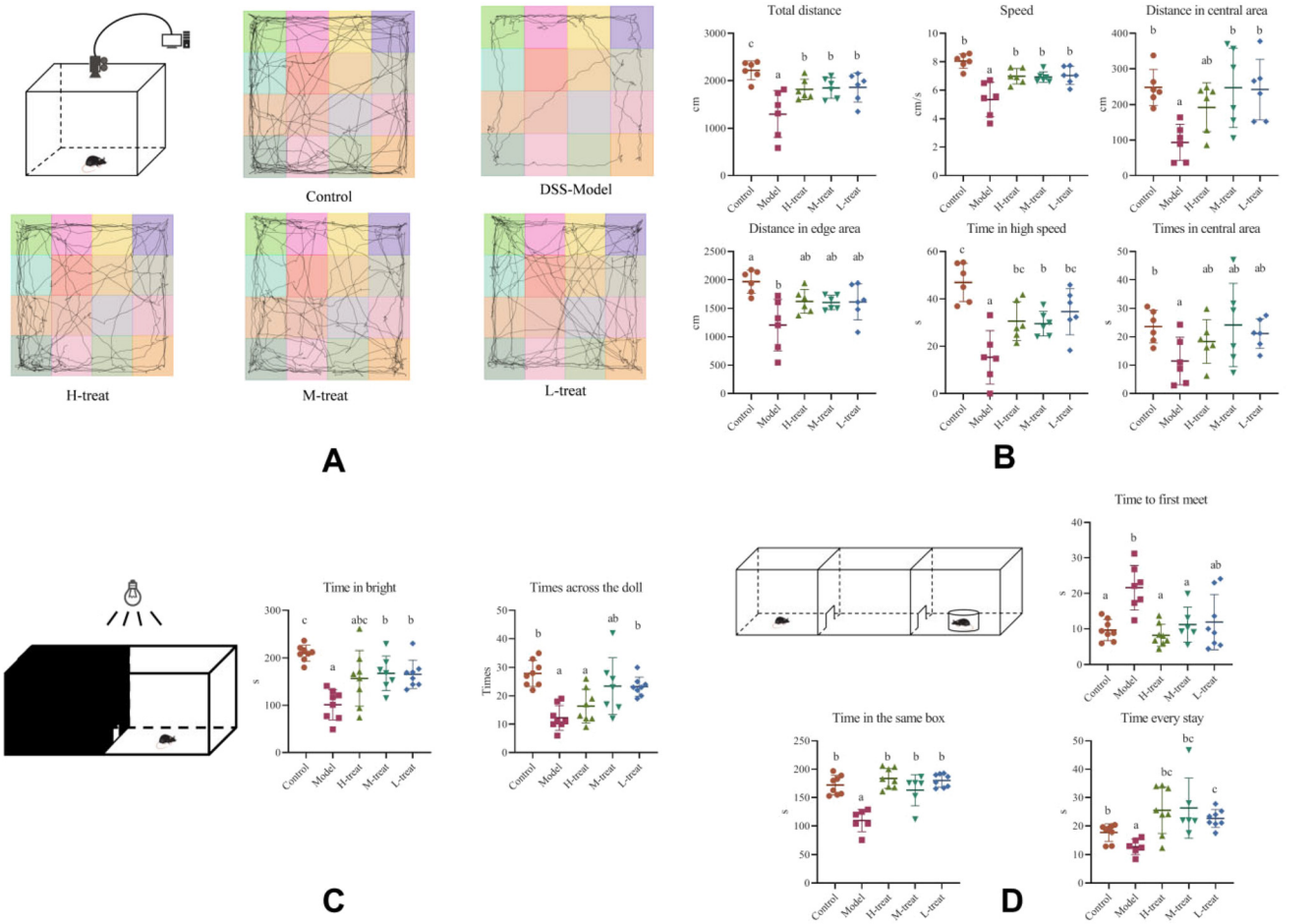

Fig. 4 Effects of different concentrations of Fmb14 preadministration on behavioral disorders in mice with DSS-induced colitis. (A) Spontaneous movement trail of mice in the open field test. (B) Brief movement data of mice in the entire area of the OFT (total distance: n = 6, df = 4, F = 6.928, p = 0.001; speed: n = 6, df = 4, F = 10.994, p = 0.000; distance in the central area: n = 6, df = 4, F = 4.474, p = 0.007; distance in the edge area: n = 6, df = 4, F = 5.388, p = 0.003; time in high speed: n = 6, df = 4, F = 10.173, p = 0.000; time in central area: n = 6, df = 4, F = 1.977, p = 0.129). (C) Spontaneous movements of mice in the light/dark test (time in light: n = 6, df = 4, F = 11.755, p = 0.000; times across the doll: n = 6, df = 4, F = 15.740, p = 0.000). (D) Spontaneous movements of mice in the three-chamber test (time to first meet: n = 6, df = 4, F = 7.115, p = 0.000; time in the same box: n = 6, df = 4, F = 16.779, p = 0.000; time every stay: n = 6, df = 4, F = 6.003, p = 0.001). Mean ± SD is represented by bars, and different lowercase letters (abc) represent significant differences at $p < 0.05$. Labels a to d represent lower to upper levels, respectively.

(DA), brain-derived neurotrophic factor (BDNF), gamma aminobutyric acid (GABA), 5-hydroxyindole acetic acid (HIAA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) in the brain were investigated using ELISA kits, and the results showed that DSS-related behavioral disorders were relevant to decreased BDNF (from 50.5 to 24.1 ng L^{-1}) and DA (33.9 to 24.0 pg mL^{-1}), as well as increased GABA (8.1 to 11.1 ng L^{-1}), when compared with the control groups (Fig. 5A–C). Treatment with a high concentration of Fmb14 increased BDNF and DA to 75.2 ng L^{-1} and 35.3 pg mL^{-1} and decreased GABA to 8.8 ng L^{-1}, and the regulatory effect of Fmb14 on BDNF, DA and GABA was dose-dependent (Fig. 5A–C). The results also indicated that DSS-induced behavioral disorders were not influenced by 5-HT, HIAA and NE, but the ameliorated effect of high concentrations of Fmb14 was synergistic with improved HIAA and NE (Fig. 5D–F).

The development of the central nervous system was associated with blood–brain barrier (BBB) dysfunction, and downregulated trends of BDNF further proved this hypothesis. The prefrontal cortex and brain in the DSS group mice were thinner than those in the control group according to hematoxylin–eosin (H–E) staining, and the Fmb14 groups recovered to the levels of the control group (Fig. 5G). The altered tight junction proteins ZO-1 and Ocln in the different groups indicated the modified effect of Fmb14 on barrier dysfunction induced by DSS in the brain (Fig. 5H–J).

Fmb14 prevents behavioral disorders through neuronal protection and cytokine reduction in the brain

The microscopy images of the striatum in the brain showed the presence of pathological-like spots, which indicated the dysfunction of brain cells (Fig. 6A). The Nissl bodies of the DSS-treated group mice became larger and had deeper blue

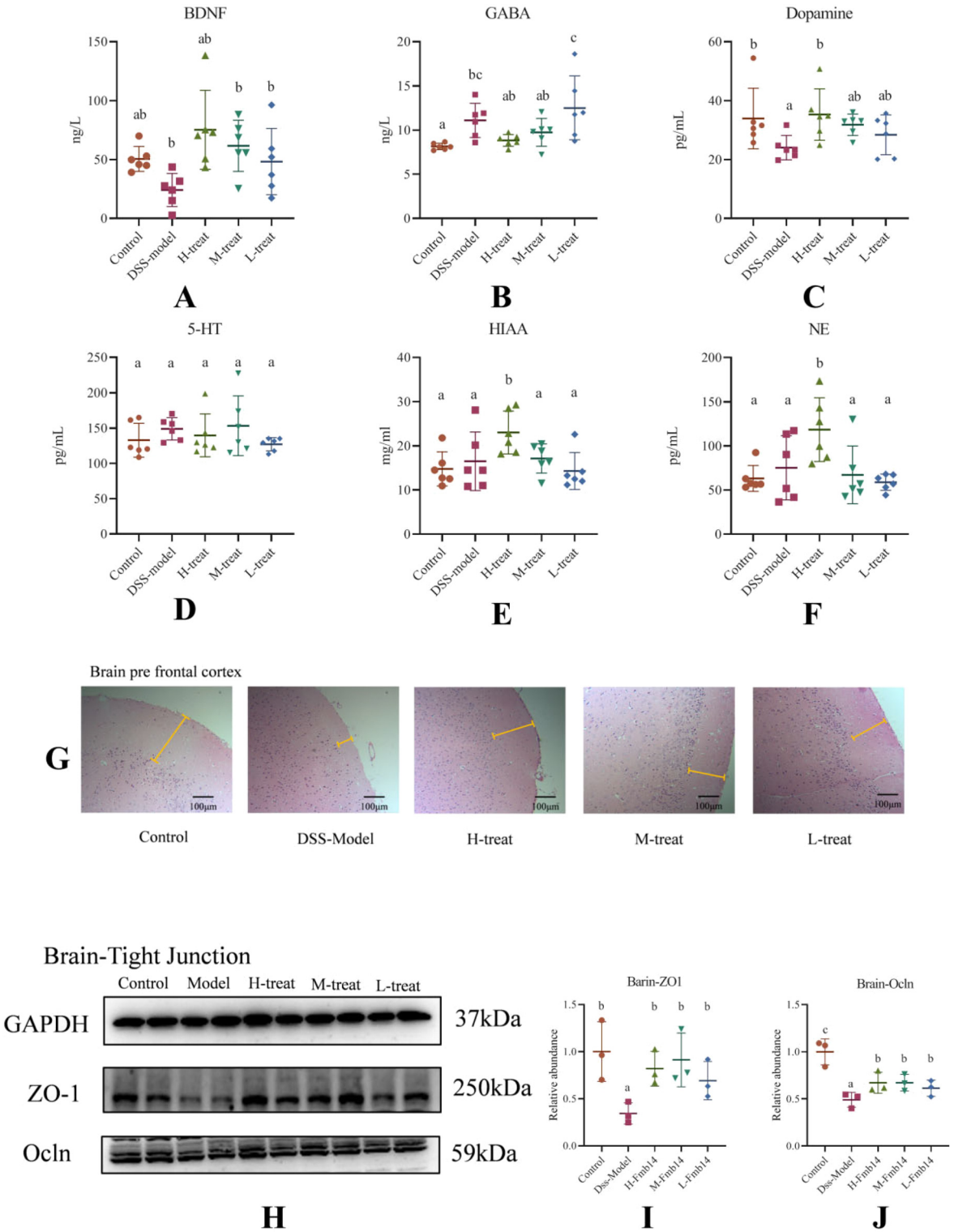

Fig. 5 Effects of different concentrations of Fmb14 preadministration on brain neurotransmitters and barriers. (A–F) Effects of different treatments on the levels of brain neurotransmitters including BDNF, GABA, DA, 5-HT, 5-HIAA and NE (A: n = 6, df = 4, F = 3.961, p = 0.013; B: n = 6, df = 4, F = 4.649, p = 0.006; C: n = 6, df = 4, F = 2.396, p = 0.077; D: n = 6, df = 4, F = 0.989, p = 0.432; E: n = 6, df = 4, F = 3.304, p = 0.026; F: n = 6, df = 4, F = 4.376, p = 0.008). (G) Hematoxylin and eosin (H–E)-stained images of the brain cortex. (H–J) Western blotting of brain zonula occludens-1 (ZO1) and occludin (Ocln) (I: n = 3, df = 4, F = 3.637, p = 0.044; J: n = 3, df = 4, F = 10.118, p = 0.002). Mean ± SD is represented by bars, and different lowercase letters (abc) represent significant differences at $p < 0.05$. Labels a to d represent lower to upper levels, respectively.

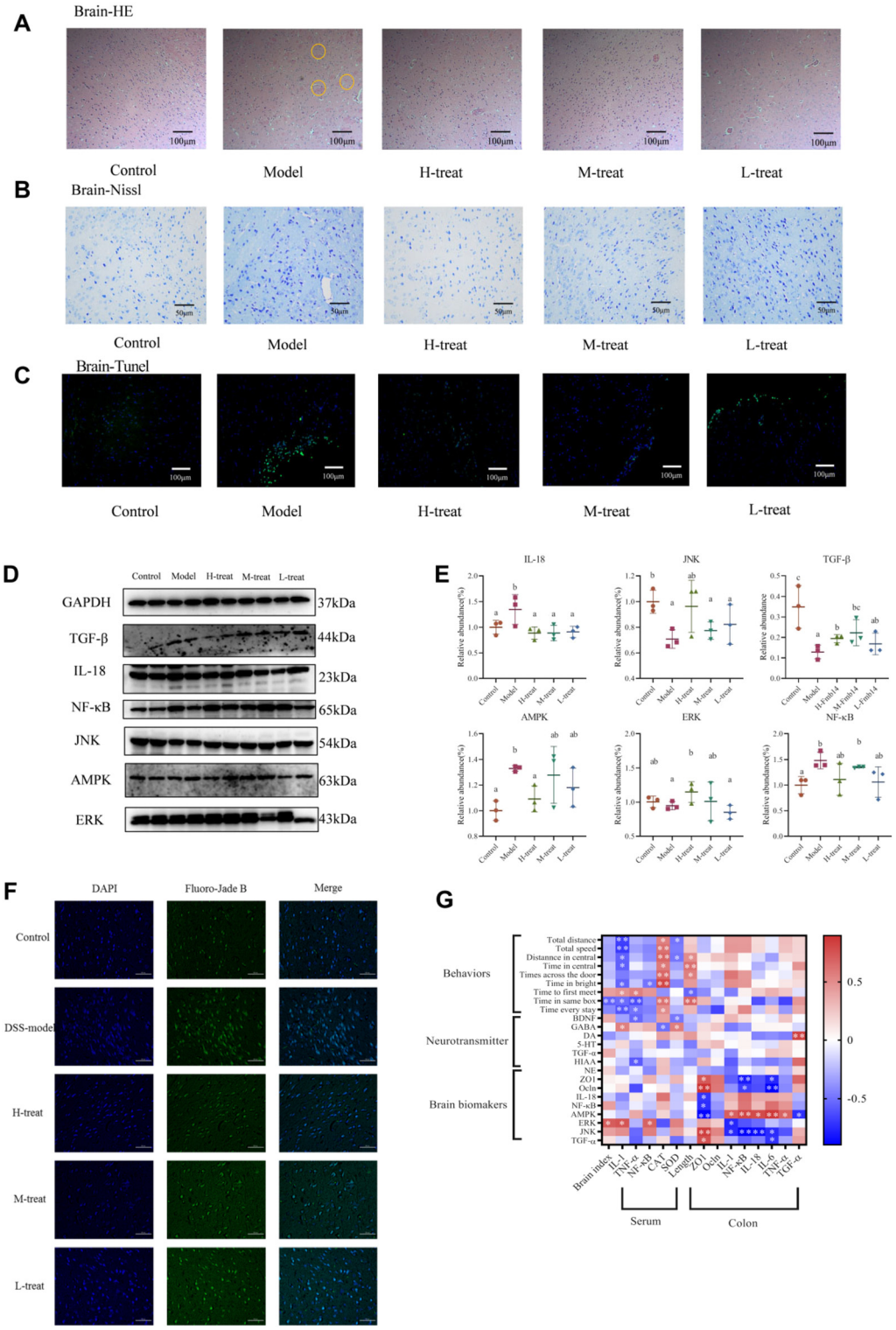

Fig. 6 Fmb14 ameliorates neuronal apoptosis and restores Nissl bodies in the brain. (A) Hematoxylin and eosin (H–E)-stained images of the brain. (B) Nissl body-stained images of the brain. (C) TUNEL-stained images of the brain. (D and E) Western blotting of brain cytokines and biomarkers (IL-18: $n = 3$, df = 4, $F = 3.823$, $p = 0.039$; JNK: $n = 3$, df = 4, $F = 2.796$, $p = 0.085$; TGF-β: $n = 3$, df = 4, $F = 5.501$, $p = 0.013$; AMPK: $n = 3$, df = 4, $F = 3.043$, $p = 0.07$; ERK: $n = 3$, df = 4, $F = 1.404$, $p = 0.301$; NF-κB: $n = 3$, df = 4, $F = 2.652$, $p = 0.096$). (F) FJB-stained images of the brain. (G) Heatmap of Pearson's correlation analysis between colon inflammation and brain-related indices. Mean ± SD is represented by bars, and different lowercase letters (abc) represent significant differences at $p < 0.05$. Labels a to d represent lower to upper levels, respectively.

staining than those of the control group, and H-Fmb14 administration restored the Nissl bodies to normal numbers and morphology, demonstrating that Fmb14 ameliorated the damage to the Nissl bodies across the gut–brain axis (Fig. 6B). Then, the protective effect of Fmb14 on brain function was also confirmed by TUNEL and FJB staining, and the results showed that Fmb14 significantly reduced the number of dead cells and denatured neurons mediated by DSS in the brain (Fig. 6C and F). The increase in cytokines IL-18 and NF-κB partly contributed to brain damage, and the increased expression of AMPK and decreased expression of ERK, JNK and TGF-β were the other altered pathways in the DSS mice when compared with the control group (Fig. 6D and E). The administration of Fmb14 restored the levels of IL-18, NF-κB, AMPK, ERK and JNK to the control levels (Fig. 6E). Pearson's correlation heatmaps of the relationships between inflammation and oxidative stress with behaviors, neurotransmitters and brain biomarkers are shown in Fig. 6G. The results showed that serum IL-1β, TNF-α, CAT, SOD and colon length were highly correlated with the behaviors of mice. In addition, the cytokines in the colon were positively correlated with brain AMPK and negatively correlated with brain JNK. The results also showed that the downregulation of ZO1 was highly correlated with alterations in brain biomarkers, including ZO1, Ocln, IL-18, NF-κB, AMPK, JNK and TGF-α (Fig. 6G).

Fmb14 prevents behavioral disorders through microbiota dysbiosis modulation in the gut

Lacticaseibacillus rhamnosus Fmb14 was first proven to colonize the colon of mice, and the PCR results showed that Fmb14 tested positive in colonic materials in H-Fmb14, M-Fmb14 and L-Fmb14 treated mice (Fig. 7A). The effect of Fmb14 administration on the diversity and abundance of gut microbiota in DSS-induced mice was obtained from MiSeq sequencing analysis of the V3–V4 region of 16S rRNA. The abundances of microbial flora (Shannon index, Simpson index and Chaos index) were lower in the DSS group than in the control group, as regulated by Fmb14 treatment (Fig. 7B). Additionally, the principal coordinates analysis (PCoA) results showed that phylogenetic community structures were markedly different between the DSS-treated and control samples and that the H-Fmb14 group covered both the DSS-treated and control groups, indicating an obviously regulated effect on gut microbiota dysbiosis (Fig. 7C). Specifically, the relative abundance at the phylum level was analyzed. The levels of *Verrucomicrobia* and *Proteobacteria* were increased from 0.8% and 0.7% to 20.1% and 2.6% after DSS exposure, respectively, and Fmb14 administration significantly reduced them to 5.4% and 1.2%, respectively (Fig. 7D and Fig. S4†). Moreover, the abundance of *Firmicutes* in the DSS-treated group decreased to 30.9% from 38.3% in the control group, and the abundance of *Firmicutes* and *Verrucomicrobia* in the Fmb14 administration group increased in a dose-dependent manner (Fig. 7D). At the genus level, the top 10 genera are shown in Fig. S4,† and Fmb14 treatment restored the increased abundance of *Akkermansia*, *Mucispirillum*, *Bacteroides* and *Bifidobacterium* caused by DSS exposure (Fig. 7E). Pearson's correlation heatmaps of the relationships between the microbiota and behaviors, neurotransmitters and brain biomarkers are shown in Fig. 7F, and the results indicated a strong negative relationship between mouse behaviors and *Verrucomicrobia* and *Proteobacteria* at the phylum level. *Akkermansia* was negatively correlated with the time to cross the door, time in the light, time in the same box and BDNF and positively correlated with GABA. Another highly negatively correlated family with behavioral disorders was *Bifidobacterium* (Fig. 7F).

Venn analysis showed that DSS exposure significantly affected the structure of the intestinal flora and decreased the species and quantity of the gut microbiota (Fig. 8A). The random forest analysis indicated that *Akkermansia* and *Bacteroides* were the target biomarkers and that Fmb14 administration alleviated DSS-induced dysbiosis (Fig. 8B). LefSe (Fig. 8C) was conducted to identify biological markers for further study of the indicator bacteria in each group, and the results showed 14 biomarkers in the DSS-treated group and 10 biomarkers in the H-Fmb14-treated group. In addition, PERMANOVA and hierarchical clustering analysis showed that DSS exposure significantly affected the structure of the intestinal flora and that H-Fmb14 treatment had a positive effect on the gut microbiota, altering the structure from the DSS to the control group (Fig. 8D). Moreover, the metabolic function of the gut microbiota predicted by sequencing data was determined and there were 50 and 3 significant differential metabolic pathways between the control group and the DSS and H-treated groups, respectively (Fig. S5†). In detail, 8 amino acid related pathways were significantly downregulated in the DSS-treated group when compared to the control group and the altered pathways were repaired when Fmb14 was treated (Fig. 8E). In the altered pathways although there were no significant differences in metabolic pathways between the H-treated and DSS groups, the administration of Fmb14 also modulated the metabolic dysbiosis induced by DSS. The specific altered predicted metabolic pathways are shown in ESI Table S1.†

Discussion

IBD has been reported to be associated with anxiety or depression through some longitudinal prospective studies of patients, and the interaction between IBD and mood disorders was investigated for bidirectionality.[4] Mental disorders such as anxiety or depression have commonly been linked to more severe IBD symptoms.[32] Dextran sulfate sodium was used to induce colitis and related mood disorders in this research (Fig. 1A), and the body index of mice, including body weight and brain and spleen indices, was altered after 7 days of DSS exposure (Fig. 1B–D). Diarrhea and increased levels of serum IL-1β, TNF-α, NF-κB and IL-18 (Fig. 1E–H) indicated the formation of colitis in the DSS-treated group.[33] The increased levels of oxidative stress were another hallmark of colitis[34] and the altered serum CAT and SOD in the DSS model were consist-

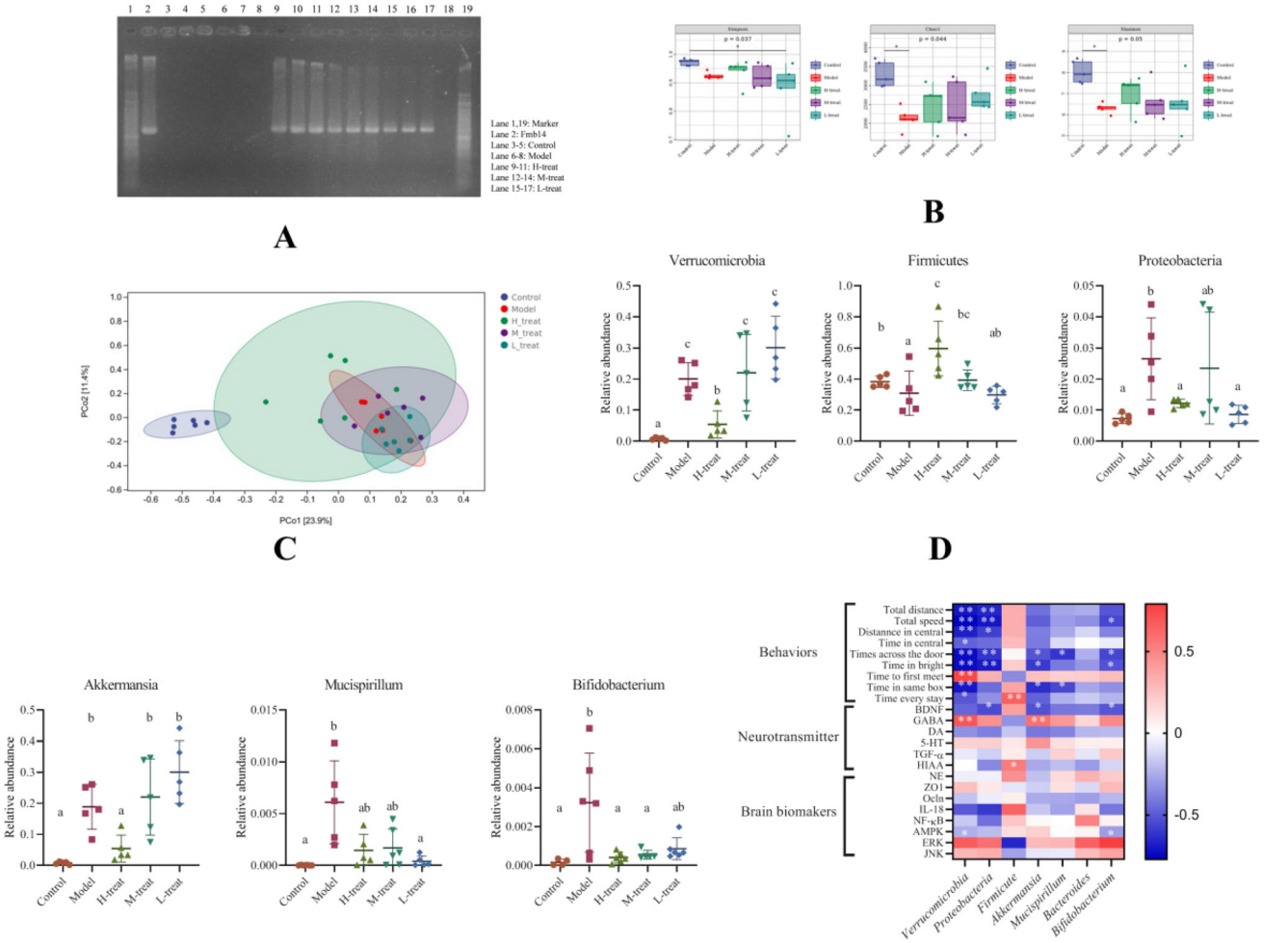

Fig. 7 Effects of *L. rhamnosus* Fmb14 treatment on the microbial diversity of the gut microbiota in mice. (A) Fmb14 colonization results by PCR in mouse colonic materials. Lanes 1 and 19 represent the 100 bp DNA ladder, lane 2 represents the positive (Fmb14 in MRS), 3–5, 6–8, 9–11, 12–14, and 15–17 represent the control, model, H-treatment, M-treatment and L-treatment groups, respectively, and lane 18 represents the blank. (B) Box plot of distinct groups based on the Simpson, Chao1, and Shannon indices. (C) Principal coordinates analysis (PCoA) of the overall diversity based on the Bray–Curtis distances. Scatter plot of PCoA scores depicting variances derived from bacterial communities in the three groups. (D) Phylum-level relative abundance of the gut microbiota (*Verrucomicrobia*: n = 5, df = 4, F = 12.491, p = 0.000; *Firmicutes*: n = 5, df = 4, F = 5.983, p = 0.002; *Proteobacteria*: n = 5, df = 4, F = 3.838, p = 0.018). (E) Genus-level relative abundance of the gut microbiota (*Akkermansia*: n = 5, df = 4, F = 11.318, p = 0.000; *Mucispirillum*: n = 5, df = 4, F = 6.692, p = 0.001; *Akkermansia*: n = 5, df = 4, F = 7.631, p = 0.001). (F) Heatmap of Pearson's correlation analysis between the altered gut microbiota and brain indicators. The mean ± SD and standard deviation are shown by bars, and different lowercase letters (abc) represent significant differences at $p < 0.05$. Labels a to c represent lower to upper levels, respectively, and *represents $P < 0.05$.

ent with previous research (Fig. 1I and Fig. S2†). Probiotic administration has become a regular therapeutic schedule for colitis for two reasons: gut microbiota modulation[35] and metabolite regulation.[36] *Lacticaseibacillus rhamnosus* Fmb14 reduced the inflammatory response and oxidative stress levels in purine-induced hyperuricemia, which indicated its superior ability to improve the immunity of the host.[28] The administration of Fmb14 significantly decreased the serum IL-1β, TNF-α, NF-κB and IL-18 levels induced by DSS, which demonstrated the protective effect of Fmb14 on colitis (Fig. 1D–H).

DSS-induced colon shortening and gut barrier damage in mice[37] and probiotic administration can alleviate intestinal dysfunction.[38] Preadministration of Fmb14 for 7 weeks improved gut barrier damage induced by DSS exposure through crypt and villi structure enhancement in the colon and intestine, respectively (Fig. 2D and E). Fmb14 colonization leads to a deep crypt structure, which means that more intestinal juice is secreted to maintain the biochemical metabolic reaction in the colon,[39] and a longer villi structure maintains the absorption function in the intestine.[40] Moreover, the tight junction proteins ZO-1 and Ocln were upregulated in the preadministration of Fmb14 groups compared with the DSS exposure groups (Fig. 3A–D), indicating that Fmb14 reshapes and refreshes the mucus layer, creates a healthy environment for epithelial cells and maintains the integrity of the intestinal barrier, as previously reported.[41] The alleviated effect of Fmb14 on colon cytokines, including IL-1β, NF-κB, IL-6, IL-18 and TNF-α, contributes to the reduction in inflammatory

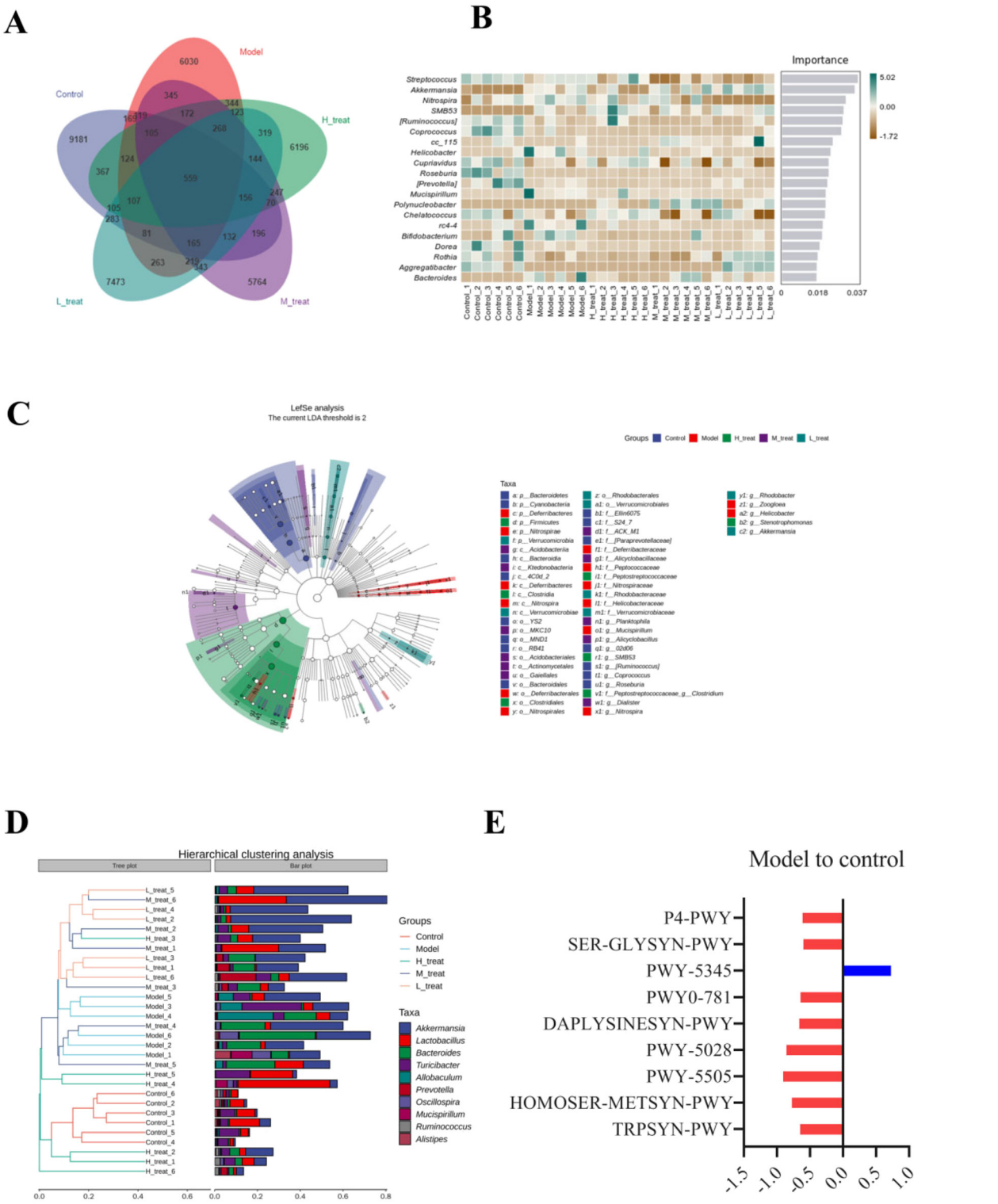

Fig. 8 Microbial markers and functional regulatory targets in depression-like behavior prevention by *L. rhamnosus* Fmb14. (A) Venn diagram examination of species differences in each group. (B) Random forest species importance analysis in each group. (C) Microbial markers screened based on the LEfSe score. (D) Hierarchical clustering analysis of microbial markers in each group. (E) Differentially altered amino acid related metabolism prediction between the control and model groups.

storms in DSS-treated mice, as shown in some previous research on *Lacticaseibacillus rhamnosus.*[42] *L. rhamnosus* showed outstanding performance in improving intestinal barrier disruption induced by multiple factors,[43–45] and Fmb14 increased intestinal permeability and the expression of Ocln and ZO-1 beyond the control group (Fig. 5B). The restorative effect of Fmb14 on tight junction proteins was mainly promoted by producing SCFAs, which could be efficiently utilized by the intestinal epithelium as an energy material.[46,47]

Behavioral disorders such as depression or anxiety are commonly mediated by "gut leak"[48] and neuroinflammation,[49] both of which are symptoms of colitis in mice; thus, the behavioral trends of the mice were tested. In the DSS model of colitis, the behaviors of mice were altered, including the motion of low propensity, marginal preferring motion and low speed (Fig. 4A and B), indicating a depression-like activity of mice. As important markers of depression-like behaviors, individuals prefer dark environments[50] and show low interest in exploring unknown regions.[51] Both the time in bright and times across the doll in the L/D box tested were improved in the preadministration Fmb14 groups when compared with the DSS group, even though the levels did not reach the same levels as those in the control group (Fig. 4C). Considering the moderate physiological effects of DSS-induced colitis on depressive-like behavior, OF and L/D may be interpreted as the impact of abdominal pain caused by colitis.[11] A three-chamber test was further used to investigate the modulatory effect of Fmb14 on the propensity of mice to communicate with each other, and the results support our hypothesis that probiotics could increase social activities (Fig. 4D). These findings were consistent with the viewpoint that probiotics are a substantial contributor to human mood enhancement.[52] Research on the improvement of behavioral disorders by probiotics is not limited, and a variety of *Lactobacillus* and *Bifidobacterium* from human feces or natural fermented foods have been shown to have the ability to reduce depression or anxiety disorders *in vivo.*[53] Moreover, *Lactobacillus rhamnosus* JB-1 regulates emotional behavior by a beneficial effect of the commensal gut microbiota on the central nervous system in normal and healthy mice.[21] Therefore, the excellent performance of Fmb14 on behavioral disorder improvements is worth noting, and the hormone levels, blood–brain barrier and gut microbiota structure need to be further understood.

The mammalian complex behaviors are controlled by the central nervous system, which specializes in and is precisely regulated by different neurotransmitters secreted from the gut.[54] GABA, NE, DA, 5-HT and 5-HIAA are positive neurotransmitters secreted by gut cells that are correlated with emotions such as happiness in animals.[55] The altered availability of 5-HT and 5-HIAA, both of which are inhibitory neurotransmitters, in the brain is a key feature of depression pathogenesis.[56] Although the levels of 5-HT in all tested groups were not significantly different, they also exhibited increasing trends in the DSS groups. GABA is another important inhibitory neurotransmitter mediating neural inhibition to balance the excitatory neurotransmission of neuronal systems in the brain,[57] and the levels of GABA were regulated by Fmb14 preadministration (Fig. 5B). Preadministration of Fmb14 alleviated the reduction in dopamine levels induced by DSS in the brain in a dose-dependent manner; as dopamine is a neurotransmitter that transmits excitement, dopamine level restoration can greatly improve depressive symptoms.[58] In addition to neurotransmitters, the modulatory effect of Fmb14 on BDNF levels in the brain was the main factor ameliorating depression-like behavior induced by DSS based on our results (Fig. 5A). BDNF is one of the most widely distributed neurotrophins in the mammalian brain that controls neuronal and glial development, and BDNF affects the development of brain tissue both in signal transduction activated under physiological and pathological conditions.[59] Low levels of BDNF in the brain were associated with a thinner frontal cortex in the brain (DSS group), as shown by our results, and Fmb14 preadministration alleviated this injury (Fig. 5G). Interestingly, the levels of NE and HIAA in the brain were not altered in the DSS group when compared to the control group but it is significantly increased after high concentrations of Fmb14 administration. High levels of NE and HIAA in the brain could resist depression,[60] but these two positive neurotransmitters could not be secreted by probiotics directly so probiotic administration may increase NE and HIAA through gut microbiota modulation. Oral administration of *L. helveticus* NS8, heat-killed *Enterococcus fecalis* (EC-12), and *Bifidobacterium* CECT 7765 increased the levels of NE to ameliorate anxiety like behavior[61] and our results showed the same phenomenon. A preliminary study on the cognition of children in Thailand found that *Lactobacillus paracasei* HII01 increased the levels of HIAA in urine after 12 weeks of supplementation and the attention state of children significantly improved.[62] Additionally, the decreased levels of ZO-1 and Ocln indicated that DSS exposure caused the tight junctions of the brain to disappear, which indicated the disordered permeability of the blood–brain barrier,[63] and the ZO-1 levels were restored to those of the control group in the Fmb14 group. Alterations in the blood–brain barrier lead to the loss of protection against exposure of the brain to molecules and bacterial metabolites in blood, which may cause damage to astrocytes or pericytes in the brain.[64]

Depression usually occurred as a consequence of psychological stressors or physical injury, and dysfunction of the immune system has been found as the most important contributor to the viral source of anxiety in the research of COVID-19.[65] Behavioral disorders could be alleviated after the recovery of brain function through neuroprotective therapy[66] and inflammatory factors were the therapeutic targets. Proinflammatory cytokines were reported to damage the permeability of the blood–brain barrier structure,[67] and colitis caused a cascade of reactions that culminates in the activation of transcription factors and then encodes cytokines such as IL-1β and IL-18.[68] The levels of NF-κB and IL-18 were found to be increased in the colon (Fig. 3G and J), serum (Fig. 1G and H) and brain (Fig. 6E), which were another evidence of the preventive effect of Fmb14 on depression like through the gut–brain axis. Changes in cytokines, especially the increased

levels of IL-18 and NF-κB (Fig. 6E) in the brain, are associated with neurodegeneration to induce depression like behavior. Increased neurodegeneration (Fig. 6F) and abnormal levels of Nissl bodies (Fig. 6B) lead to low neurotransmission and neurogenesis in the hippocampus, which reduces neuronal signaling transmitted to the body through the vagus nerve to exhibit disordered behaviors.[69] Depletion of brain serotonin has been proved to correlate with major depressive disorder in some clinical and preclinical evidence[49] and our results suggested that BDNF and GABA may act as therapeutic biomarkers to DSS-induced depression like behaviors through probiotic pathways. The brain usually possesses the immune privilege that defended microbial invasion or toxic materials from the blood–brain barrier[49] and the inflammatory response and apoptosis of nerve cells in the brain indicated that the immune system of the brain has broken *via* the microbiota–gut–brain axis.[70]

Dysbiosis of the microbiota is implicated in the pathogenesis of mental disorders, including depression, anxiety and autism spectrum,[71] and the use of probiotics to ameliorate behavioral disorders through gut microbiota modulation is a promising therapeutic strategy. The previously tested antidepression strains belonged predominantly to the genera *Lactobacillus* and *Bifidobacterium.*[72] Fmb14 possesses good ability to colonize the gut (Fig. 7A), which is the most basic property of probiotics in the application. DSS exposure downregulated both the abundance and diversity of the gut microbiota, and Fmb14 preadministration maintained a high α- and β-diversity when compared with the DSS group (Fig. 7B and C). At the phylum level, preadministration of Fmb14 increased the relative abundance of *Firmicutes* and decreased the relative abundance of *Proteobacteria* and *Verrucomicrobia*, which were induced by DSS exposure, and these results were in agreement with the studies on *Lactobacillus plantarum* DMDL 9010.[10] *Akkermansia* was used as an anticancer strain that degrades mucin in mucus and inhibits the occurrence of obesity.[73] However, the opposite results were found in the field of acute colitis and a model in which increased *Akkermansia* may be associated with shortened colon, higher inflammation and related depression.[10,72,74] The gut microbiota structure deter-

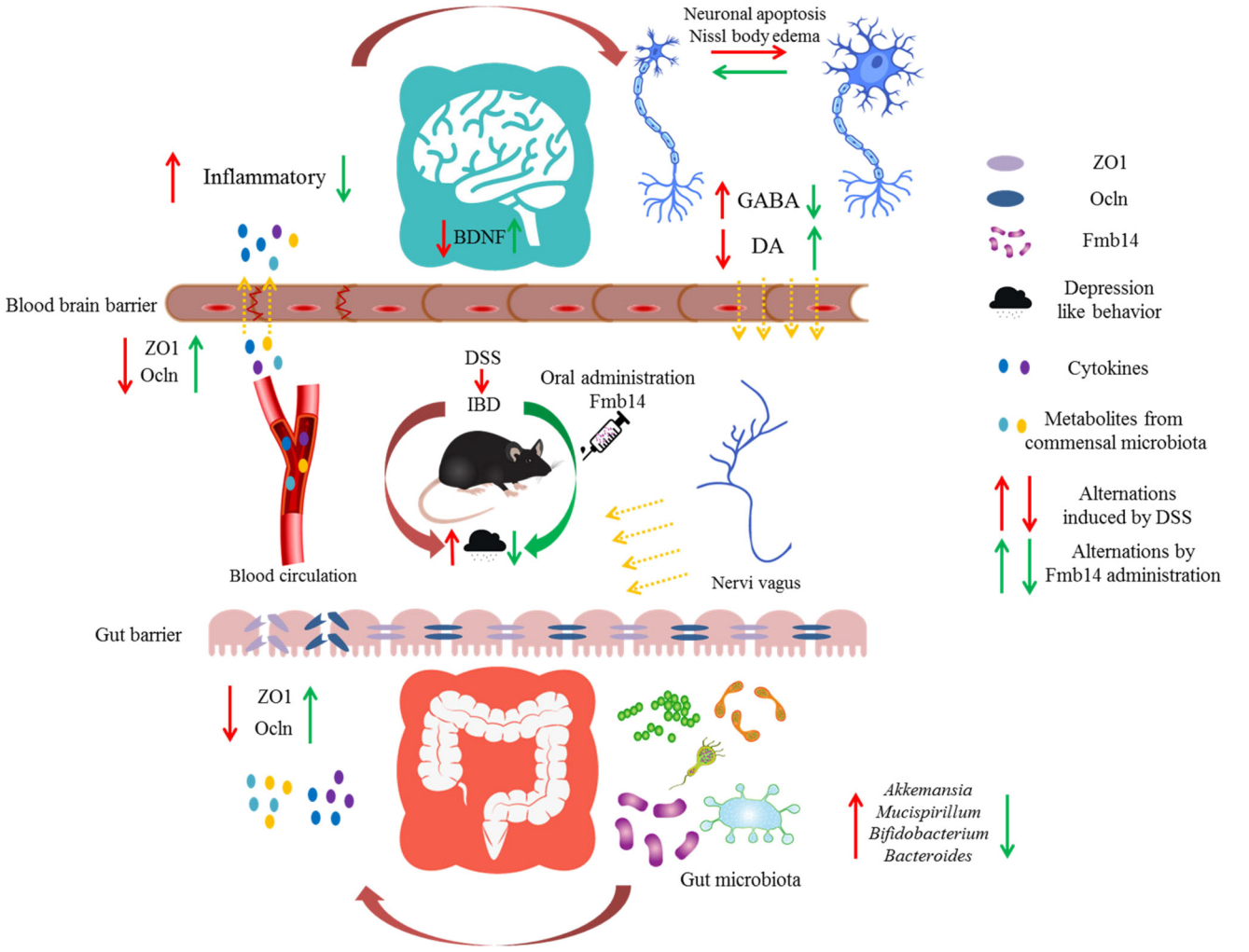

Fig. 9 Prevention mechanism of *L. rhamnosus* Fmb14 on DSS-induced depression-like behaviors.

mines the metabolic levels of the host, and the relationship between microbiology and neuroscience has been one of the most attractive research areas in recent decades.[75] The sequencing and function predicted results indicated an obvious distinction of biomarkers and metabolism pathways between the DSS and control groups, and Fmb14 administration narrowed the gap (Fig. 8). Except for the significant improvement of acetate pathways, which is characteristic of *Lactobacillus*, there were only two significantly altered pathways between the Fmb14 administration and control groups, which strongly demonstrated that Fmb14 regulated the DSS-induced metabolism of the gut microbiota. Notably, eight high gut–brain axis-related amino acid metabolism pathways, including L-tryptophan biosynthesis (TRPSYN-PWY), L-methionine biosynthesis I (HOMOSER-METSYN-PWY) and L-glutamate and L-glutamine biosyntheses (PWY-5505), were downregulated in the DSS group and restored to normal levels after Fmb14 administration (Fig. 8E). L-Tryptophan biosynthesis may be the most important because tryptophan is the sole precursor of 5-HT, which is a key monoamine neurotransmitter participating in the modulation of central neurotransmission and enteric physiological function.[76] L-Glutamate was the biosynthesis precursor of GABA and the disturbance of amino acid metabolism in the gut contributes to the abnormal alternations of neurotransmitters in the brain.

The underlying mechanism of Fmb14 administration in improving depression-like behavior induced by DSS was investigated in this manuscript. This study provides additional evidence to support the hypothesis that probiotic administration could improve the behavior of the animals[1,21] based on our observation of beneficial behavioral changes in the Fmb14-treated group. Probiotics have been reported to alleviate psychic disorders through the microbiota–gut–brain axis, including blood–brain barrier enhancement,[22] microbiota-generated metabolite regulation[77] and neuronal restoration.[78] The ameliorative effect of Fmb14 on depression-like behaviors was mainly due to complicated synergy with gut microbiota modulation and blood–brain barrier repair. A decrease in the inflammatory response was found to be an important factor affected by Fmb14, as previously reported.[79] The high inflammatory response in the brain may cause damage to Nissl bodies and lead to apoptosis of nerve cells, both of which determine the behavior of the animal through the vagus nerve.[80] The ameliorated effect of Fmb14 on DSS-induced depression-like behavior through the vagus nerve needs experimental verification in our future research for the reason that subdiaphragmatic vagotomy was not performed in this model.

In summary, our results showed that ingestion of *Lacticaseibacillus rhamnosus* Fmb14 prevents depression-like behavior and brain neural activity *via* the microbiota–gut–brain axis (Fig. 9). The protective mechanism of this biotherapy method is linked to microbial modulation and gut barrier enhancement, both of which provide positive feedback to brain metabolism and structure through the gut–brain axis. Importantly, phenotype disorders in DSS-induced mice were depression-like behaviors, and dysfunction of the brain and gut was connected with metabolism or structural dysfunction in the brain and gut. The administration of Fmb14 exhibits additional performance both in levels of behavioristics and physiological index. We propose that Fmb14 prevents DSS-induced depression-like behaviors mainly based on gut microenvironment improvement and ultimately impacts brain inflammatory reduction, blood–brain barrier enhancement, neurotransmitter regulation and neuronal repair. All findings indicate that Fmb14 administration therapies may be effective treatments for colitis-related depression-like behaviors.

Data availability

All data needed to evaluate the conclusions in the paper are present in the Supplementary Materials and all original data are present at **https://doi.org/10.7910/DVN/FTCVLV**.

Author contributions

Conceptualization: H. Zhao, Z. Lu, and Y. Lu; Methodology: H. Zhao, L. Zhou, Z. Lu, and Y. Lu; Investigation: H. Zhao, X. Chen, L. Zhang, C. Tang, and F. Meng; Visualization: H. Zhao, X. Chen, and C. Tang; Supervision: C. Tang, L. Zhang, P. Zhu and F. Meng; Writing—review & editing: H. Zhao, L. Zhou, P. Zhu, Z. Lu and Y. Lu.

Conflicts of interest

We declare that we have no financial or personal relationships with other people or organizations that can inappropriately influence our work.

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