Supplementary materials

1. Methods and materials

1.1 Animal experimental design

Table S1. Animal experimental design

Groups(n=8)	Treatments (8 weeks)
Control group	Sterile saline administered by gavage + sterile saline administered by subcutaneous injection
Model group	Sterile saline was administered by gavage + D-galactose was administered by subcutaneous injection (500 mg/kg body weight)
L. plantarum CCFM8661 intervention group	10^9 CFU/mL *L. plantarum* CCFM8661 was administered by gavage + D-galactose was administered by subcutaneous injection (500 mg/kg body weight)

Figure S1. Diagram of the timing of the animal intervention

1.2 Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from the hippocampus and colon (Trizol method). and its concentration and purity were determined. RT-qPCR was performed on a BioRad-CFX384 machine (Bio-Rad, California, USA) using SYBR Green Supermix. The PCR reaction was performed in a total volume of 10 μl. The thermal cycling consisted of 95℃ for 30 sec, and 40 cycles of 95 ℃ for 5 sec, 60 ℃ for 30 sec. After the PCR a melting curve (65℃～95℃, increment 0.5℃) was generated to check the specificity of the amplified fragment.

Table S2. Primers used in qPCR

Gene		5′-3′
GAPDH	F	GCAAAGTGGAGATTGTTGCCAT
	R	CCTTGACTGTGCCGTTGAATTT
Akt1	F	CATCGTGTGGCAG-GATGTGTA
	R	ACCTGGTGTCAGTCTCAGAGGTG
p65	F	GACACGACAGAATCCTCAGCATCC
	R	CCACCAGCAGCAGCAGACATG
Klotho	F	ATCTTTGACCAGACCTTGGAATGAA
	R	GAACATCATGGCATCCAAGCAC
HO-1	F	AACAAGCAGAACCCAGTCTATGC
	R	AGGTAGCGGGTATATGCGTGGGCC
Gsr	F	TGGCACTTGCGTGAATGTTG
	R	TGTTCAGGCGGCTCACATAG
ZO-1	F	CTTCTCTTGCTGGCCCTAAAC
	R	TGGCTTCACTTGAGGTTTCTG
ZO-2	F	AACGGATGCTGGAAGTTAAT
	R	TCTGCTTGCTGTCTCTCAACA
Occludin	F	CACACTTGCTTGGGACAGAG
	R	TAGCCATAGCCTCCATAGCC
Claudin-1	F	GATGTGGATGGCTGTCATTG
	R	CCTGGCCAAATTCATACCTG
TNF-α	F	CAGGCGGTGCCTATGTCTC
	R	CGATCACCCCGAAGTTCAGTAG
IL-1β	F	ACCTTCCAGGATGAGGACATGA
	R	CTAATGGGAACGTCACACACCA
IL-6	F	ACCAGAGGAAATTTTCAATAGGC
	R	TGATGCACTTGCAGAAAACA
p16	F	CTCAGCCCGCCTTTTTCTTC
	R	CGCCTTCGCTCAGTTTCTCATG
p53	F	AACTTACCAAGGCAACTATG
	R	CTTGTAGATGGCCATGGCAC

1.3 Microbial gene sequencing

Total bacterial DNA in feces was extracted from the samples following the kit's protocol (Fast DNA Stool Kit, MP Biomedicals, CA, USA), and the extracted DNA was amplified in the V3-V4 region of 16S rRNA (universal primers, 341F/806R). The amplification comprised an

initial denaturation stage at 95 °C for 5 minutes, followed by 30 cycles of 95 °C for 30 seconds, 52 °C for 30 seconds, and 72 °C for 30 seconds, and a final extension step at 72 °C. After cycling, a final extension at 72 °C for 5 minutes was performed, and the reaction was then kept at 4 °C. The obtained DNA was recovered and purified (TIANgel Mini Purification Kit, TIANGEN, Beijing, China). DNA was quantified and pooled in equal concentrations following the instructions of the Qubit dsDNA Assay Kit (Life Technologies, CA, USA). Libraries were generated using the TruSeq DNA LT Sample Preparation Kit (Illumina, CA, USA), and samples were bar-coded and finally paired-end sequenced on the Illumina MiSeq PE300 platform following the manufacturer's protocol.

1.4 Histological and immunohistochemistry examination

Brain tissues were fixed with 10% neutral formaldehyde solution and then paraffin embedded. The tissues were sectioned and mounted on clean glass slides,then baked at 60 ℃ for 2 h and treated with dimethyl benzene for 20 min each time. Each section was treated with ethanol, 95% ethanol, 80% ethanol and distilled water in sequence until it became transparent. Sections underwent hematoxylin eosin (HE) staining.

For immunohistochemistry, endogenous peroxidase activity in sectioned tissue was blocked with 3% H_2O_2 and non-specific binding sites were blocked with 3% normal goat serum. Sections were incubated with anti-Iba1 for 1 h at 37 °C. Subsequently, the sections were washed in PBS and incubated with biotinylated goat anti-rabbit IgG secondary antibody for 1 h at room temperature, followed by incubation for 1 h with an avidin–biotin–horseradish peroxidase complex. Histological features of these sections were examined under a microscope (Nikon).

2. Results

Table S3. Results of statistical analysis of indicators

Indexes	Normal distribution test (Shapiro-Wilk test)			Homogeneity test of variance (Brown-Forsythe test)		ANOVA		Kruskal-Wallis test		Corresponding graph
	Control Group	Model Group	CCFM8661 Group	P value	Are SDs significantly different (P < 0.05)?	P value	Significant diff. among means (P < 0.05)?	P value	Neous variance	
Cognitive index	Yes	Yes	Yes	0.9045	No	0.0075	Yes	-	-	Figure 1 b
Discriminant index	Yes	Yes	Yes	0.9045	No	0.0075	Yes	-	-	Figure 1 c
Alternation number	Yes	Yes	Yes	0.0671	No	<0.0001	Yes	-	-	Figure 1 d
The number of maximum alternations	Yes	Yes	Yes	0.2016	No	0.0024	Yes	-	-	Figure 1 e
Spontaneous alternate behavior score	Yes	Yes	Yes	0.7401	No	0.0002	Yes	-	-	Figure 1 f
The total distance of movement	No	Yes	Yes	-	-	-	-	0.8566	No	Figure 1 g
Movement speed	Yes	Yes	Yes	0.2298	No	0.6236	No	-	-	Figure 1 h
Time proportion	Yes	Yes	Yes	0.3503	No	0.0079	Yes	-	-	Figure 1 i
Frequency of exploration	Yes	Yes	Yes	0.9189	No	0.2784	No	-	-	Figure 1 j
Exploratory behavior score	Yes	Yes	Yes	0.2602	No	0.014	Yes	-	-	Figure 1 k
p16(qPCR)	Yes	Yes	Yes	0.1816	No	<0.0001	Yes	-	-	Figure 2 a
P53(qPCR)	Yes	Yes	Yes	0.4370	No	<0.0001	Yes	-	-	Figure 2 a
p16(ELISA)	Yes	Yes	Yes	0.1717	No	0.0251	Yes	-	-	Figure 2 a
P21(ELISA)	Yes	Yes	Yes	0.7225	No	0.0373	Yes	-	-	Figure 2 a
P53(ELISA)	Yes	Yes	Yes	0.0521	No	0.1235	No	-	-	Figure 2 a
Brain-Gsr	Yes	Yes	Yes	0.5739	No	0.0037	Yes	-	-	Figure 2 b

Brain-HO-1	Yes	Yes	Yes	0.087	No	0.0017	Yes	-	-	Figure 2 b
Brain-Klotho	No	Yes	Yes	-	-	-	-	0.0018	Yes	Figure 2 b
Brain-Akt1	Yes	Yes	Yes	0.5351	No	0.0063	Yes	-	-	Figure 2 b
Brain-p65	Yes	Yes	Yes	0.719	No	0.0813	No	-	-	Figure 2 b
TNF-α	Yes	Yes	Yes	0.13912	No	0.0002	Yes	-	-	Figure 2 c
IL-1β	Yes	Yes	Yes	0.1049	No	0.0002	Yes	-	-	Figure 2 c
IL-6	Yes	Yes	Yes	0.3989	No	0.0006	Yes	-	-	Figure 2 c
Brain-MDA	Yes	Yes	Yes	0.5790	No	<0.0001	Yes	-	-	Figure 2 d
Brain-GSH	Yes	No	Yes	-	-	-	-	0.0005	Yes	Figure 2 d
Brain-SOD	Yes	Yes	Yes	0.0227	Yes	-	-	0.0325	Yes	Figure 2 d
Occludin	Yes	Yes	Yes	0.8615	No	<0.0001	Yes	-	-	Figure S5 b
Claudin-1	Yes	No	Yes	-	-	-	-	0.0017	Yes	Figure S5 b
ZO-1	Yes	Yes	Yes	0.3694	No	<0.0001	Yes	-	-	Figure S5 b
ZO-2	Yes	Yes	Yes	0.8115	No	0.0003	Yes	-	-	Figure S5 b
Liver-T-AOC	Yes	Yes	Yes	0.6425	No	0.0011	Yes	-	-	Figure S5 c
Liver-GSH	Yes	Yes	Yes	0.2263	No	0.0006	Yes	-	-	Figure S5 c
Bone-BALP	Yes	Yes	Yes	0.8975	No	<0.0001	Yes	-	-	Figure S5 d
Bone-TRACP-5b	Yes	Yes	Yes	0.4166	No	<0.0001	Yes	-	-	Figure S5 d
Bone-β-CTx	Yes	Yes	Yes	0.4018	No	0.0012	Yes	-	-	Figure S5 d
Shannon index	Yes	Yes	Yes	0.2590	No	0.4363	No	-	-	Figure 4 a
Chao1 index	Yes	Yes	Yes	0.5128	No	0.9647	No	-	-	Figure 4 a
Pielou index	Yes	Yes	Yes	0.2938	No	0.3759	Yes	-	-	Figure 4 a
Relative abundance of Bacteroidetes	Yes	Yes	Yes	0.0126	Yes	-	-	0.0487	Yes	Figure 4 d
Relative abundance of Firmicutes	Yes	Yes	No	-	-	-	-	0.1234	No	Figure 4 d
Firmicutes/Bacteroidetes ratio	Yes	Yes	Yes	0.5939	No	<0.0001	Yes	-	-	Figure 4 d
Acetic acid	Yes	Yes	Yes	0.7162	No	0.0003	Yes	-	-	Figure 5 b

Propanoic acid	Yes	Yes	Yes	0.2928	No	0.0082	Yes	-	-	Figure 5 b
Isobutyric acid	Yes	Yes	Yes	0.5766	No	0.0108	Yes	-	-	Figure 5 b
Butyric acid	Yes	Yes	Yes	0.4162	No	0.006	Yes	-	-	Figure 5 b
Pentanoic acid	Yes	Yes	Yes	0.0852	No	0.0053	Yes	-	-	Figure 5 b

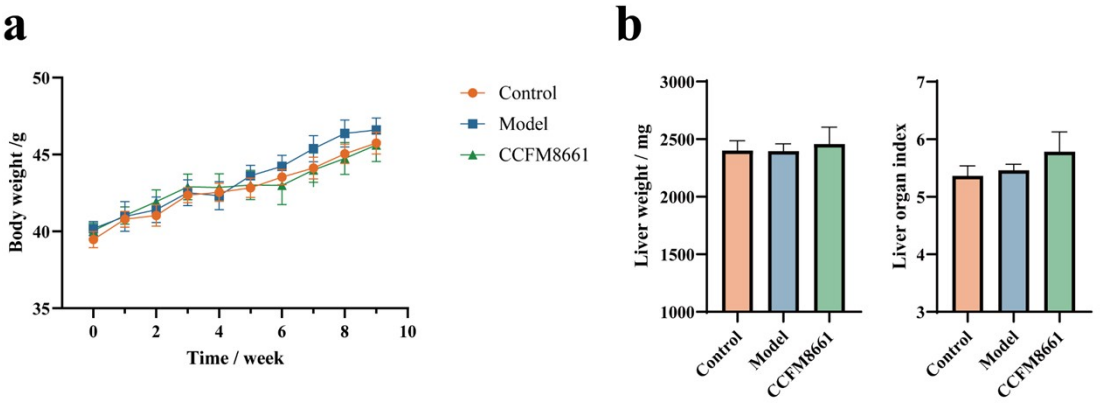

Figure S2. Effects of *L. plantarum* CCFM8661 administration on body weight and liver weight in mice: (a) Changes of body weight in mice during the intervention period. (b) Liver weight and liver organ index in mice. Data are represented as mean ± SEM..

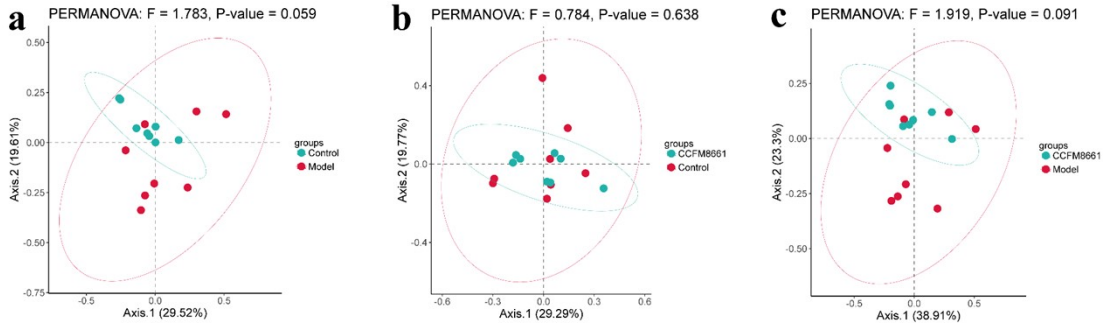

Figure S3. Comparison of β-diversity among groups. (a) Control and Model groups. (b) Control and CCFM8661 groups. (c) CCFM8661 and Model groups. β-diversity was calculated using Bray-Curtis distance, shown by principal coordinate analysis (PCoA), and differences were determined using permutation multivariate Analysis of variance (perMANOVA). Diversity maps were analyzed on the free online site (https://bioincloud.tech/standalone-task-ui/bray_pcoa).

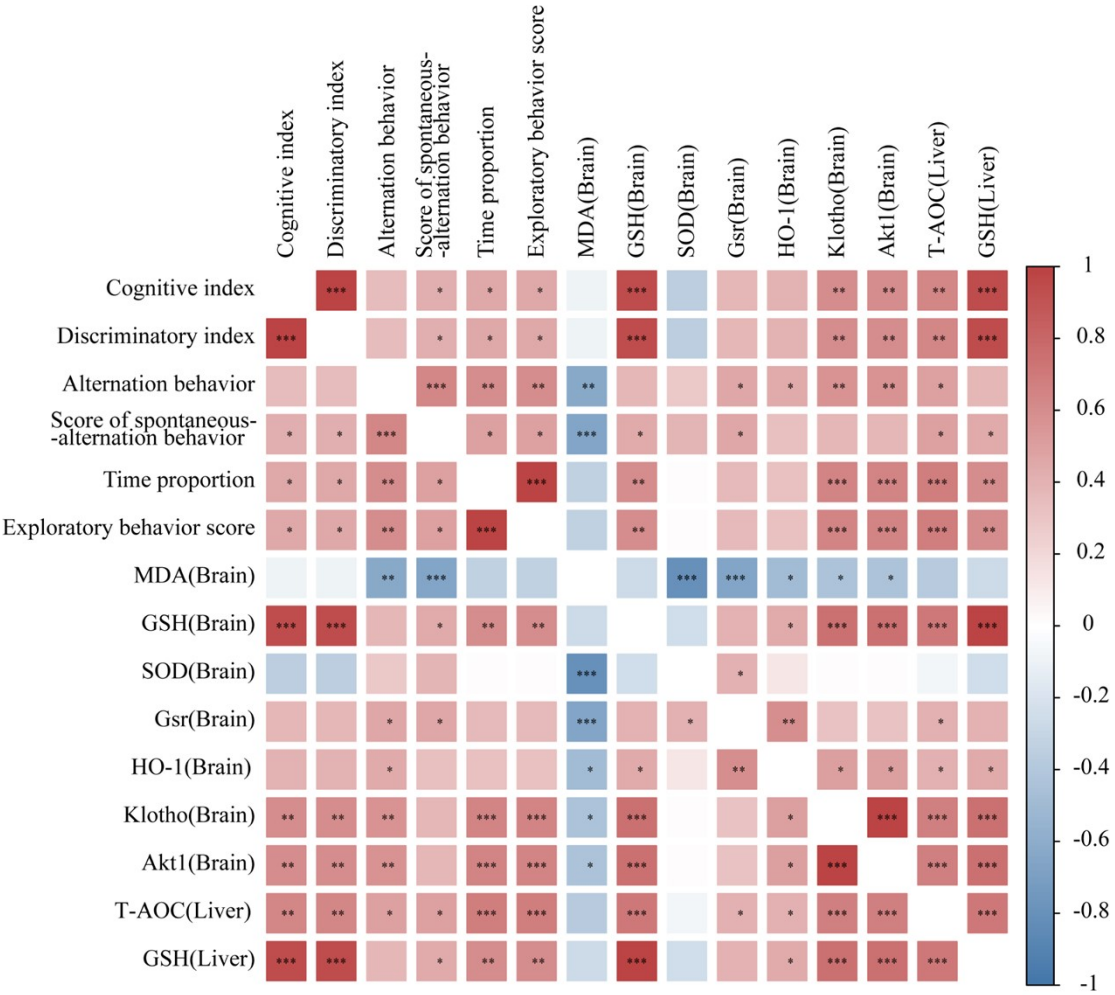

Figure S4. Correlation analysis between behavioral indicators and oxidative stress indicators. (Spearman's correlation analysis, an online website was used to create drawing: https://www.omicstudio.cn)

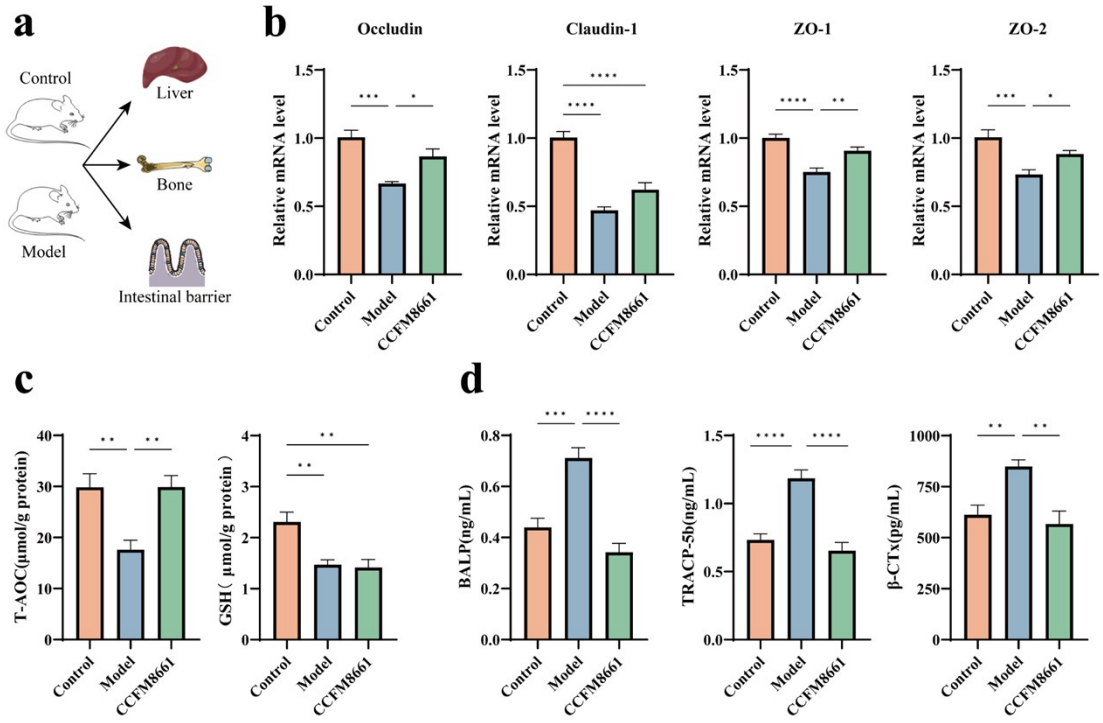

Figure S5. Effects of *L. plantarum* CCFM8661 administration on some tissues in mice: (a) Diagram of the indicators. (b) The relative expression of four TJP genes in the colon. (c) Oxidative stress in the liver, including T-AOC and GSH levels. (d) Biochemical indicators of bone metabolism. Data are presented as means ± SEM.