Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2025, **16**, 5363

Lycopene regulates intestinal dysmotility and behavioral disorders by regulating enteric neurons and the cholinergic system†

Danna Wang,[a,b] Siyi Jiao,[a] Qianqian Guo,[a] Mengnan Zhou,[a] Wanting Ren,[a] Yuqi Zhao,[a] Dongning Li,[a] Wenyue Yang,[a] Shengquan Dai,[a] Yanqian Pei,[a] Danyixin Xiao,[a] Xuebo Liu,[a] Chunxia Xiao*[a] and Beita Zhao*[a,c]

Received 3rd January 2025,
Accepted 25th May 2025

DOI: 10.1039/d5fo00039d

rsc.li/food-function

Gut dysmotility is a prevalent gastrointestinal disorder characterized by disrupted defecation and often accompanied by depression and anxiety. Lycopene (LYC) is a type of carotenoid with strong antioxidant and anti-inflammatory properties. However, the effects of LYC on gut dysmotility and related behavioral disorders remain elusive. Herein, we found that 100 mg kg^{-1} d^{-1} LYC notably improved the gut transit time and intestinal transit rate as well as concurrently alleviated depression- and anxiety-like behaviors in a diphenoxylate (Dip, 5 mg kg^{-1} d^{-1})-induced constipated mouse model. LYC pretreatment enhanced gut barrier integrity and short-chain fatty acid (SCFA) production as well as rebalanced gut microbiota homeostasis by enriching beneficial bacteria, including *Bifidobacterium* and *Akkermansia*. Furthermore, LYC restored enteric neuronal function, as evidenced by increased HuC/D and Tuj1 expression as well as balanced neurotransmitter levels. Brain transcriptomics results suggest that LYC regulates the cholinergic synapse pathway and increases acetylcholine (Ach) content in the brain, which is associated with the alleviation of neuroinflammation. In summary, this study offers insights into functional dietary component-based nutritional strategies that target gut dysmotility comorbid behavioral dysfunction.

1. Introduction

Gastrointestinal (GI) motility is crucial for digestion and nutrient absorption, and gut dysmotility is a prevalent and burdensome GI disorder associated with constipation, diarrhea and functional GI diseases, such as irritable bowel syndrome (IBS).[1,2] Constipation, which is characterized by increased GI transit time, dry stools, and infrequent defecation, affects approximately 10.1% of adults globally, with its rising prevalence linked to imbalanced diet, chronic stress, and sedentary lifestyle.[3,4] Constipation promotes intestinal toxin accumulation, disrupts gut homeostasis and subsequently affects distant organs.[5] Research has confirmed a bidirectional correlation between constipation and behavioral disorders, including depression and anxiety.[6] A meta-analysis of 69989 patients with chronic constipation and 7179 patients with constipated IBS (IBS-C) showed that comorbid depression rates were as high as 14.6% ± 29.2% and 12.5% ± 69.2%, respectively.[7] Current therapies, such as medications and dietary fiber supplementation, can cause undesirable side effects such as irreversible intestinal damage and functional distention.[8,9] These challenges underscore the imperative need for novel nutritional approaches for managing constipation and associated behavioral disorders.

Growing evidence emphasizes gut microbiota dysbiosis as a pivotal factor in the progression of constipation and associated behavioral disorders. Clinical and preclinical studies have verified that individuals with constipation accompanied by anxiety and depression exhibit a reduced abundance of beneficial bacteria, including *Bifidobacterium* and *Lactobacillus*, along with the enrichment of pro-inflammatory taxa, such as *Sutterella* and *Oscillibacter*.[10–12] Beneficial intestinal bacteria have been shown to effectively mitigate depression- and anxiety-like behaviors in a constipated mouse model, and this was associated with restoration of the gut microbiota structure, regulation of enteric neurotransmitters, and a reduction in colonic inflammation.[11] Notably, patients with functional constipation and psychiatric symptoms have shown significant improvement in symptoms such as constipation, anxiety and depression after receiving fecal transplantation (FMT) from healthy donors.[12]

[a]*College of Food Science and Engineering, Northwest A&F University, Yangling, China. E-mail: chunxiaxiao@nwafu.edu.cn*
[b]*College of Animal Science and Technology, Northwest A&F University, Yangling, China*
[c]*College of Veterinary Medicine, Northwest A&F University, Yangling, China. E-mail: beitazhao@nwafu.edu.cn*

† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d5fo00039d

The mechanisms underlying the role of the gut microbiota in constipation and associated behavioral disorders involve bacteria-derived metabolites, such as short-chain fatty acids (SCFAs). Inulin could alleviate depression and anxiety in diphenoxylate (Dip)-induced constipated mice, and this was partly attributed to augmented SCFA production in the colon.[13] Constipation-driven enrichment of endotoxin-producing bacteria, including *Desulfovibrio* and *Oscillibacter*, compromises intestinal integrity, resulting in elevated systemic pro-inflammatory substances that subsequently trigger neuroinflammation.[14,15] The cholinergic system further bridges gut-brain communication *via* the acetylcholine (Ach)-mediated anti-inflammatory pathway, also known as the cholinergic anti-inflammatory pathway (CAP).[16] It has been demonstrated that the cholinergic system also mediates vagus nerve stimulation, which has beneficial effects on IBS and related depression, indicating the key role of the cholinergic system in regulating inflammation-related disorders.[17]

Dietary modification is an effective strategy for managing constipation and its complications.[18] Emerging evidence has highlighted bioactive dietary components as promising therapeutics for GI disorders, including constipation.[19,20] Lycopene (LYC), a natural liposoluble carotenoid enriched in various fruits, such as tomatoes and guava, exhibits potent antioxidant and anti-inflammatory properties with established human safety profiles.[21,22] Previous studies have confirmed that LYC has great potential in mitigating neuroinflammation, cognitive impairment, and gut microbiota dysbiosis while enhancing gut barrier function and mucosal immunity.[23–26] These studies emphasize the crucial role of gut microbiota in the biological activity of LYC, especially its efficacy in improving GI disorders. Nonetheless, its protective effects against constipation and related depression and anxiety disorders remain unclear and warrant mechanistic investigation.

Based on the above premises, we propose LYC as a nutritional therapeutic for constipation and related neurobehavioral comorbidities. This study demonstrates that LYC supplementation could alleviate Dip-induced gut motility dysfunction, while concurrently ameliorating depression- and anxiety-like behaviors in a constipated mouse model. LYC significantly restored gut microbiota dysbiosis and altered enteric neuron markers that play a critical role in gut motility regulation.[27] Brain transcriptomics revealed a significant enrichment of the cholinergic synaptic pathway in LYC-treated constipated mice, which is associated with the alleviation of neuroinflammation.[28] These results emphasize the potential of LYC as a promising candidate for managing gut dysmotility and associated behavioral disorders, highlighting its multifaceted benefits on gut health and brain function.

2. Materials and methods

2.1 Animal experiment design

Eight-week-old C57BL/6J male mice (SPF Biotechnology Co., Ltd., Beijing, China) were housed under standard conditions (22 ± 2 °C, 12 h/12 h light–dark cycle). All animal protocols used were in compliance with the Guide for the Care and Use of Laboratory Animals: Eighth Edition (ISBN-10:0-309-15396-4) and were approved by the Institutional Animal Care and Use Committee of Northwest A&F University (ethics permission number: XN2024-0414).

Diphenoxylate (Dip) was administrated orally to establish the constipation mouse model. The detailed experimental timeline is shown in Fig. 1A. Mice were randomly assigned into four groups (n = 10): ① Ctrl; ② Ctrl-LYC; ③ Dip; ④ Dip-LYC. The Ctrl and Dip groups were fed a standard AIN-93M diet (Jiangsu Xietong Pharmaceutical Bioengineering Co., Ltd, China), while the Ctrl-LYC and Dip-LYC mice groups were fed with an AIN-93M diet containing LYC (100 mg kg^{-1} d^{-1}, purity ≥ 95%, DASF Biotechnology Co., Ltd, Nanjing, China) for 7 weeks. In the last 14 days, the Ctrl and Ctrl-LYC groups were orally administered with normal saline, while the Dip and Dip-LYC groups were daily treated with Dip (5 mg kg^{-1} dissolved in normal saline). The behavioral tests were performed after Dip treatment before the mice were sacrificed.

2.2 Behavioral tests

2.2.1 Marble buried test (MBT). Mice were placed in a standard experimental cage with wood cushion to a depth of 4 cm and 20 glass marbles (15 mm diameter, arranged in a 5 × 4 matrix) on the top. The mice were allowed to explore for 30 min in the cage. The number of marbles buried by each mouse (two-thirds or more of their height) was recorded.

2.2.2 Elevated plus maze (EPM). The EPM comprised two opposing closed arms (30 cm × 8 cm) enclosed by 15 cm-high walls and two open arms (30 cm × 8 cm) devoid of walls. The apparatus was elevated 70 cm above the floor. The mice were placed in the center of the EPM and allowed to explore for 5 min. The time spent by mice in the open arms was recorded using a computerized video-tracking system (Shanghai Xinruan Information Technology Co., Ltd, China).

2.2.3 Tail suspension test (TST). As described previously, the TST was performed to evaluate depression-like behavior in the established model.[29] The mice were secured with adhesive tape approximately 1 cm from the tail tip and suspended at a height of 50 cm above the ground for 6 min. The immobility time of each mouse was recorded in the last 4 min.

2.2.4 Open field test (OFT). We used OFT to investigate anxiety-like and motor behaviors in constipated mice.[30] The mice were positioned at the center of a white open box (40 cm × 40 cm × 40 cm), which was partitioned into a central region and four corner regions, and engaged in spontaneous activity for 5 min. The time spent by the mice in the central region was recorded.

2.3 Fecal water content measurement

The number of feces excreted by each mouse was recorded for 1 h starting at 9:00 a.m. After measuring the fecal weight, the feces were dried in an oven at 80 °C for 48 h to determine the fecal dry weight. Fecal water content (%) was calculated as (fecal weight − dry weight) × 100/fecal weight.

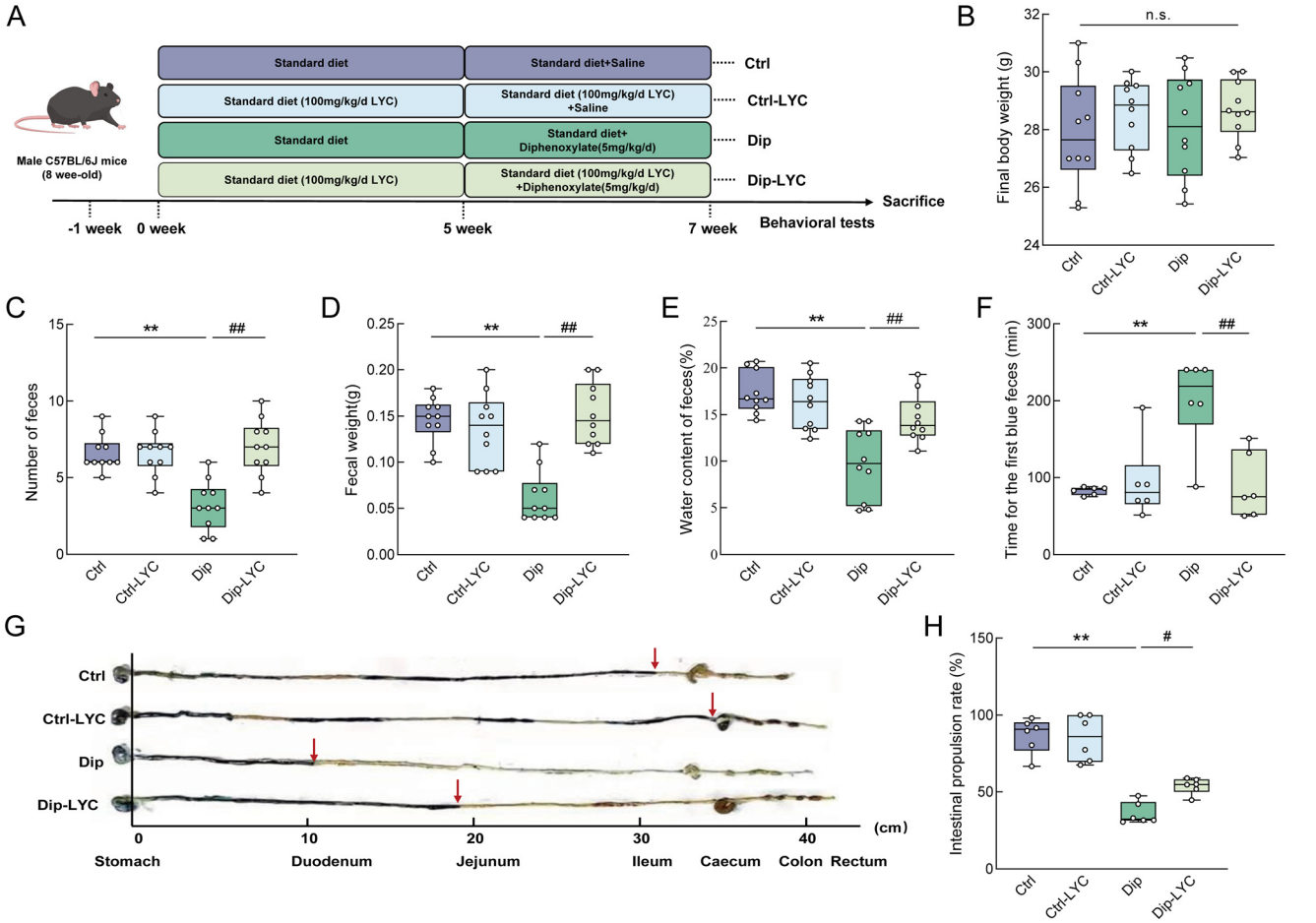

Fig. 1 Lycopene attenuates Dip-induced constipation. (A) Timeline of the animal experiment; (B) final body weight (n = 10 mice per group); (C) number of feces (n = 10 mice per group); (D) fecal weight (n = 10 mice per group); (E) water content of feces (n = 10 mice per group); (F) time for the first blue feces (n = 6 mice per group); (G) representative image of intestinal transport; (H) intestinal propulsion rate (n = 6 mice per group). Data are presented as the median ± interquartile range. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the Ctrl group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with the Dip group. Significant differences were determined through one-way ANOVA with Tukey's multiple comparison test.

2.4 Total gut transit time

After gavage administration of 200 μL Evans blue solution (5% suspended in gum Arabic; Med Chem Express) to mice, the time taken for the first appearance of blue feces was recorded as the total gut transit time.

2.5 Gastrointestinal transit rate measurement

Mice were allowed to fast overnight and then orally administered with 200 μL Evans Blue solution 30 min after the last gavage of normal saline or Dip. After 25 min, the mice were anesthetized and sacrificed, and then the whole intestine was collected. The intestine length and Evans blue propulsion length were used to calculate the gastrointestinal transit rate according to the formula: Evans blue propulsion rate (%) = Evans blue propulsion length (cm) × 100/total intestine length (cm).

2.6 H&E, immunohistochemical, and immunofluorescence staining

After fixing in 4% paraformaldehyde and embedding in paraffin, the brain and colon tissues were sliced into 5 μm sections. The tissue morphology was observed using H&E staining. Immunohistochemical and immunofluorescence staining were performed according to previously described methods.[29] After overnight incubation with primary antibodies at 4 °C, the brain and colon slices were washed with PBS and then incubated with appropriate secondary antibodies. A fluorescence microscope (Leica, Wetzlar, Germany) was used to observe the tissue sections. Antibody information is given in ESI Table S1.†

2.7 Transmission electron microscopy (TEM)

TEM was used to observe the ultrastructure of the hippocampus and colon.[31] After fixing with 2.5% glutaraldehyde (pH = 7.2) at 4 °C for 4 h, the tissues were washed with PBS and then treated with 1% osmium tetroxide for 1 h. After washing with PBS again, the tissues were dehydrated in a series of ethanol solutions (30%, 50%, 70%, 80%, 90%, and 100%, v/v) for 15-20 min and finally embedded in LR-WHITE. The ultrathin sections were stained with uranyl acetate and

lead citrate and then observed under a TEM (TH7800, Hitachi, Japan) at 80 kV.

2.8 Alcian blue staining

After dewaxing and hydration, the 5 μM colon sections were stained with Alcian blue/nuclear-fast red and washed with PBS. The colon tissues were observed using an optical microscope (Olympus, Tokyo, Japan).

2.9 Wholemount immunofluorescence staining of myenteric plexuses

Myenteric plexus separation and neuronal staining were performed as described previously.[32] After anesthetizing and euthanizing the mice, the colon was immediately collected and placed in an ice-cooled Krebs buffer. After gently removing the mesentery, the colon was cut into 2–3 cm-long pieces and cut longitudinally. The myenteric plexus samples were obtained using fine tweezers, fixed in 4% paraformaldehyde overnight and subsequently placed in 5% bovine serum albumin (SRE0096, Sigma-Aldrich, USA) containing 0.3% Triton X-100 at room temperature for 2 h to block non-specific staining. The samples were then incubated overnight with appropriate primary antibodies at 4 °C. After PBS washes, the samples were exposed to fluorescent secondary antibodies for 2 h at room temperature. Subsequently, the samples were rinsed with PBS, mounted for microscopic analysis with a fluorescence decay-resistant medium, and then covered with coverslips. All stained myenteric plexuses were observed under a Laser Scanning Confocal Microscope (Leica, TCS SP8, Germany) with a 63× oil objective, 1.00 zoom, 1.00 airy unit pinhole, and 1024 × 1024 pixels *xy* resolution. Antibody information is shown in ESI Table S1.†

2.10 ELISA analysis

Blood samples were collected through orbital bleeding under anesthesia and centrifuged at 3500*g* to obtain the serum for further analysis. The colonic and brain tissues were homogenized using RIPA lysis buffer (Solarbio, Beijing, China) and centrifuged to isolate the supernatant. The concentrations of Ach, substance P (SP), vasoactive intestinal peptide (VIP) and LPS were measured using ELISA kits (Meibiao Biotechnology, Jiangsu, China).

2.11 RT-qPCR

Total RNA was extracted from the brain and colon tissues using the Trizol reagent and reverse-transcribed into cDNA for RT-qPCR analysis using a reverse-transcription kit (Accurate Biotechnology, Hunan, China). The relative mRNA expression was determined using SYBR green reagents (Accurate Biotechnology, Hunan, China) on a QuantStudio™ 5 (Applied Biosystems, USA) and the $2^{-\Delta\Delta Ct}$ method. The sequences of specific primers are shown in ESI Table S1.†

2.12 RNA sequencing and data analysis

The total RNA from the cerebral cortex was analyzed for purity using a 5300 Bioanalyser (Agilent, USA) and quantified on an ND-2000 spectrophotometer (Thermo Fisher Scientific, USA). The transcriptome library was prepared using Illumina® Stranded mRNA Prep, Ligation (San Diego, CA), and sequenced on a NovaSeq X Plus sequencer (Majorbio Bio-pharm Biotechnology Co., Ltd, Shanghai, China). Quantitative analysis of gene expression levels was performed using RSEM software, and DESeq2 with $|\log 2FC| \geqq 1$ and FDR < 0.05 was used to identify differentially expressed genes (DEGs).[33,34] Python scipy (**https://scipy.org/install/**) was used to perform Kyoto encyclopedia of genes and genomes (KEGG) enrichment analyses of the annotated DEGs, and gene set enrichment analysis (GSEA) was conducted using the GSEA v4.1 software.

2.13 16S rDNA sequencing analysis

The E.Z.N.A.® Stool DNA extraction Kit (Omega, USA) was used to extract total microbial DNA from the fecal samples (n = 6). Primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) were used to amplify the V3–V4 region of the 16S rDNA gene by PCR. After confirmation by 2% agar-gel electrophoresis and purification using AMPure XP beads (Beckman Coulter Genomics, USA), the products were quantified by Qubit (Invitrogen, USA) and sequenced on the Illumina NovaSeq platform (LC-BioTechnology, China). Bioinformatics analyses were performed on the OmicStudio platform (**https://www.omicstudio.cn**) based on the R version 4.1.3. The Wilcoxon rank-sum test was used to determine significant differences in specific bacteria between the treatment groups.

2.14 Measurement of fecal SCFAs

The fecal SCFA content was measured using a gas chromatograph (Shimadzu Corporation, Kyoto, Japan). Approximately, 0.2 g of fresh feces was placed in a centrifuge tube, and 1 mL of distilled water was added. Then, 0.15 mL of 50% H_2SO_4 and 1.6 mL diethyl ether were added to the samples after homogenization for 10 min, and the tubes were shaken on ice for 30 min to mix the components. After centrifugation of the samples at 8000 rpm for 5 min, 1 mL of the upper organic phase was collected carefully, concentrated to 0.2 mL using a nitrogen blower and then transferred into a chromatography bottle for analysis.

2.15 Statistical analysis

Other than 16S rDNA and RNA sequencing results, the data are presented as median ± interquartile range. Significant differences among the treatment groups were analyzed by one-way ANOVA with a Tukey's *post-hoc* test in GraphPad 9.0. Image quantification was performed using the ImageJ software. The mean values were considered statistically significant if p < 0.05.

3. Results

3.1 Lycopene attenuates Dip-induced constipation

To determine whether dietary LYC affects the progression of constipation, we established a constipation mouse model by oral gavage administration of Dip (Fig. 1A). First, no statistically significant difference was observed in the final body weight among the four groups of mice (Fig. 1B), and dietary LYC notably increased the number, weight and water content of feces in Dip-induced constipated mice (Fig. 1C–E). The total gut transit time and small intestinal transport rates were measured by orally administering Evans blue solution (5% suspended in gum Arabic). The results showed that the LYC-treated mice exhibited a shorter time for the first appearance of blue feces (Fig. 1F) and a higher small intestinal transport rate compared with the Dip group (Fig. 1G and H). These findings indicate that dietary LYC has the potential to alleviate constipation-related intestinal motility dysfunction.

3.2 Lycopene restores gut microbiota dysbiosis in constipated mice

We conducted 16S rDNA sequencing of the mouse feces samples to determine the regulatory effect of LYC treatment on the gut microbiota of constipated mice. The uniFrac distance-based principal coordinate analysis (PCoA) indicated that the gut microbiota of the Dip-treated mice clustered separately from that of the Ctrl mice, indicating that constipation disrupted gut microbiota homeostasis. However, the gut microbiota profile of Dip-LYC mice displayed closer proximity to the Ctrl mice and Ctrl-LYC mice, indicating that Dip-induced gut microbiota disruption was partly restored by LYC intervention (Fig. 2A). Moreover, we observed that the Shannon index and Chao index modestly declined in the Dip group compared with the Ctrl group and increased after LYC intervention, indicating that LYC supplementation improved alpha diversity (Fig. 2B). Taxonomic profiling was further performed to determine LYC-induced alterations in the gut microbiota structure. The Dip-treated mice exhibited lower relative abundances of Firmicutes, Actinobacteriota and Verrucomicrobiota but higher relative abundances of Desulfobacterota and Proteobacteria compared with the Ctrl mice, all of which were restored after LYC administration (Fig. 2C). At the genus level, the relative abundances of *Bifidobacterium*, *Akkermansia*, C*oriobacteriaceae UCG-002* and *Allobaculum* were increased after LYC supplementation in constipated mice (Fig. 2D and E). Taxonomy indicator analysis at the genus level revealed that *Akkermansia*, Coriobacteriaceae*UCG-002*, *Bifidobacterium*, and Lachnospiraceae *NK4A136 group* were the dominant gut bacteria in the Dip-LYC group, while *Phreatobacter*, *Bradyrhizobium* and *Helicobacter* were the dominant bacteria in Dip-treated mice (Fig. 2F). To elucidate the correlation between the differential gut bacteria composition and constipation-related indexes, Pearson correlation analysis was performed for the Dip group and the Dip-LYC group (Fig. 2G). The results showed that beneficial gut bacterial genera, including *Coriobacteriaceae UCG-002*, *Akkermansia*, *Bifidobacterium* and *Allobaculum*, exhibited a significant positive correlation with the intestinal propulsion rate (r = 0.56, 0.47, 0.49, and 0.56, respectively), water content in feces (r = 0.44, 0.29, 0.36, and 0.51, respectively) and fecal weight (r = 0.49, 0.27, 0.74, and 0.53, respectively), whereas they were negatively correlated with time taken for the first appearance of blue feces (r = −0.57, −0.04, −0.64, and −0.58, respectively). However, the selected potentially harmful bacteria, including *Helicobacter*, *Elizabethkingia* and *Bradyrhizobium*, were negatively correlated with the alleviation of constipation. These findings indicate that LYC could reshape the gut microbiome structure, which may be associated with the alleviation of constipation.

3.3 Lycopene regulates the ENS and gut metabolites in constipated mice

To further explore the changes in gut microbiota function after LYC intervention, a functional prediction analysis was conducted using PICRUSt based on the 16S rDNA results. The KEGG pathway analysis revealed an upregulation of the biosynthesis of nervous system markers and other secondary metabolites in LYC-treated constipated mice compared with the Dip group (Fig. 3A). The ENS, which is composed of submucosal and myenteric plexuses, is intrinsic to the gut and responsible for intestinal motility. Given the noted changes in gut motility and gut microbiota function after LYC treatment, we investigated whether these correlated with alterations in the ENS. HuC/D and βIII-Tubulin (Tuj1) were used to label the enteric neurons and nerve fibers, respectively. Wholemount immunofluorescence immunohistochemical staining of the colon indicated an increase in HuC/D and Tuj1 positives area in the myenteric plexuses of Dip-treated mice, which improved with LYC supplementation (Fig. 3B–D). ENS can regulate intestinal functions, such as gut motility, through its derived neurotransmitters.[27] Cholinergic neurons marked by choline acetyltransferase (ChAT) are the dominant neurons in the ENS and act as the main excitatory neurons driving colon contraction.[35] LYC significantly restored the downregulated ChAT mRNA expression in the colon of Dip-induced constipation mice (Fig. 3E). Ach is the main excitatory neurotransmitter that enhances intestinal muscle contraction, and its release can be stimulated by SP.[36] LYC pretreatment notably increased the levels of colonic Ach and SP in constipated mice (Fig. 3F and G). The colonic concentration of the major gastrointestinal activity inhibitor peptide VIP was significantly higher in Dip group mice, which was reduced by LYC to a level equivalent to that in Ctrl group mice (Fig. 3H). It has been demonstrated that SCFAs can stimulate enteric neurogenesis *in vivo*.[37] LYC also restored the levels of fecal SCFAs (acetic acid, propionic acid, butyric acid) in Dip-treated mice (Fig. 3I). Based on these results, we speculated that LYC can regulate the ENS and its neurotransmitters, which may be related to the increase in SCFA levels.

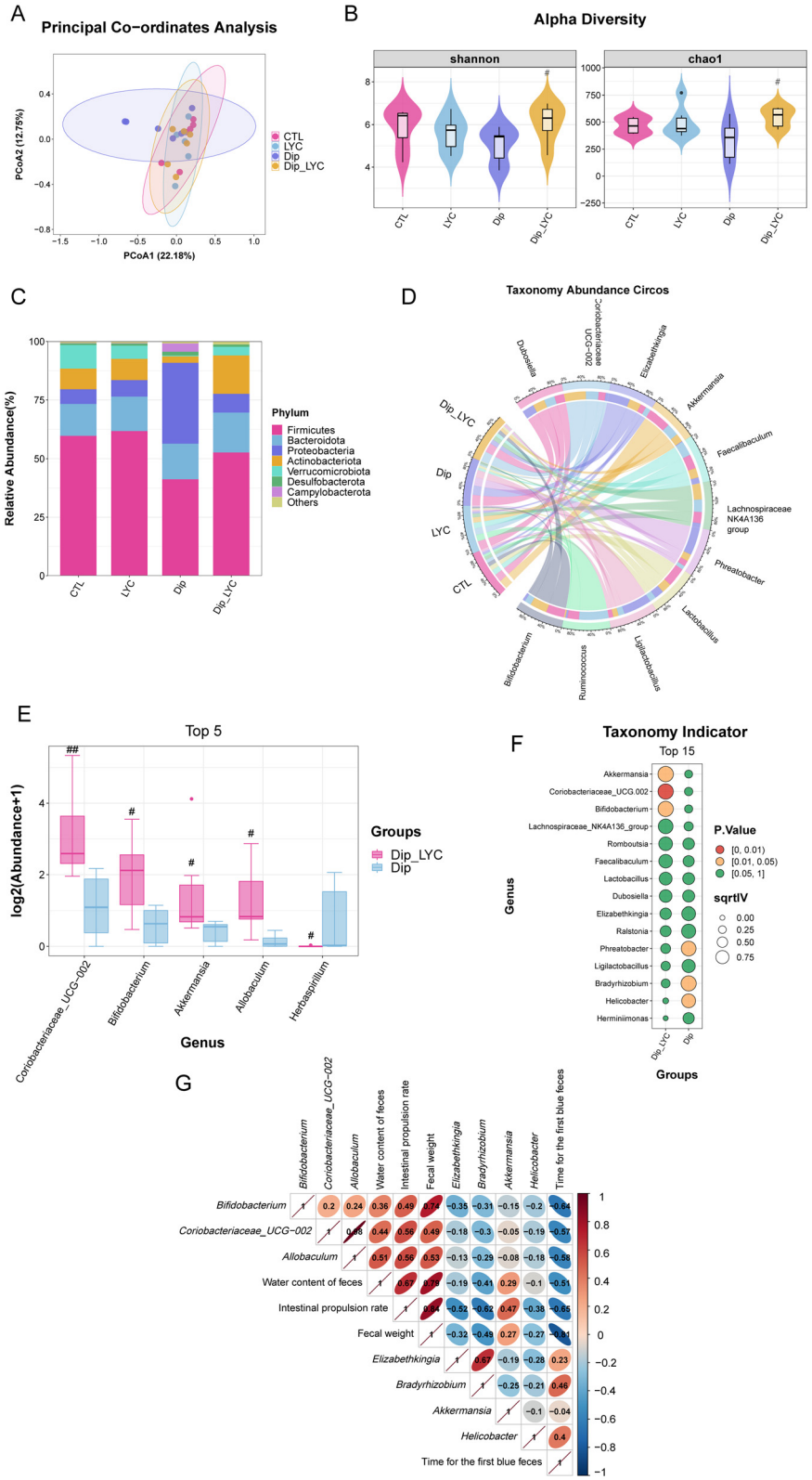

Fig. 2 Lycopene restored gut microbiota dysbiosis in constipated mice. (A) The result of principal co-ordinate analysis (PCoA) based on the Bray Curtis distance of each group; (B) α-diversity of each group; (C) relative abundance at the phylum level; (D) taxonomy abundance circus at the genus level of each group; (E) top 5 gut bacteria with significant differences at the genus level between the Dip group and Dip LYC group; (F) top 15 taxonomy indicator at the genus level between the Dip group and Dip LYC group; (G) correlation analysis between the specific gut bacteria and constipation-related indicators. Data in (E) is presented as the median ± interquartile range; $n = 6$, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with the Dip group. Statistical analyses were performed using the Wilcoxon rank-sum test for (E).

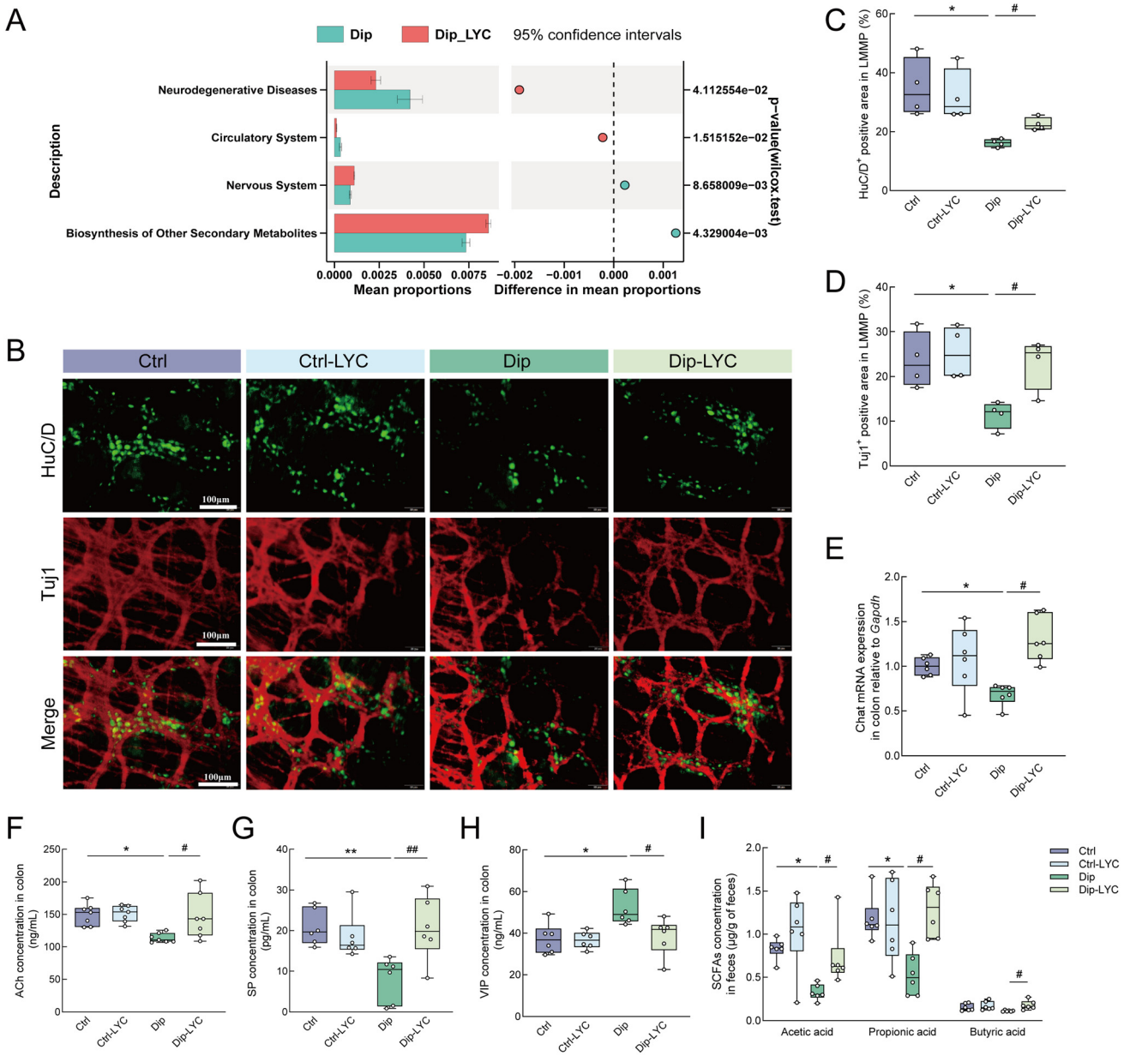

Fig. 3 Lycopene regulated ENS and gut metabolites in constipated mice. (A) KEGG pathway prediction analysis of differential gut bacteria between the Dip group and Dip-LYC group; (B) representative images of immunofluorescence staining of HuC/D and Tuj1 of colon LMMP for each group (green: HuC/D; red: Tuj1; scale bar = 100 μm); (C and D) quantification of the HuC/D and Tuj1 positive area based on immunofluorescence staining sections by ImageJ software; (E) RT-qPCR analysis of the mRNA expression of Chat in the colon for each group (n = 6 mice per group); (F–H) levels of Ach, VIP and SP in the colon (n = 8 mice per group); (I) concentration of acetic acid, propionic acid and butyric acid (n = 6 mice per group). Data in Fig. (C–I) are presented as the median ± interquartile range. *$p < 0.05$, **$p < 0.01$ compared with the Ctrl group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with the Dip group. Significant differences were determined using one-way ANOVA with Tukey's multiple comparison test.

3.4 Lycopene enhances intestinal barrier integrity and alleviates colonic inflammation in constipated mice

Damage to the intestinal epithelial barrier and mucous barrier occurs often in cases of constipation.[38] Colonic H&E and Alcian blue staining indicated that LYC alleviated pathological damages, such as obvious crypt deformation and goblet cell loss, in Dip-induced constipated mice (Fig. 4A, B and E). We observed the ultrastructure of colonic microvilli and tight junctions (TJs) using TEM and found that constipated mice displayed expanded intercellular spaces, shortened and sparse microvilli, and dysfunctional TJs (Fig. 4C, indicated by the arrows). Claudin-1 expression was also measured by immunohistochemical analysis after staining to further determine the integrity of the gut barrier. Compared with the Ctrl group mice, Dip treatment caused a notable downregulation in colonic Claudin-1 expression, which was restored by LYC treatment (Fig. 4D and F). Similarly, the colonic mRNA expression

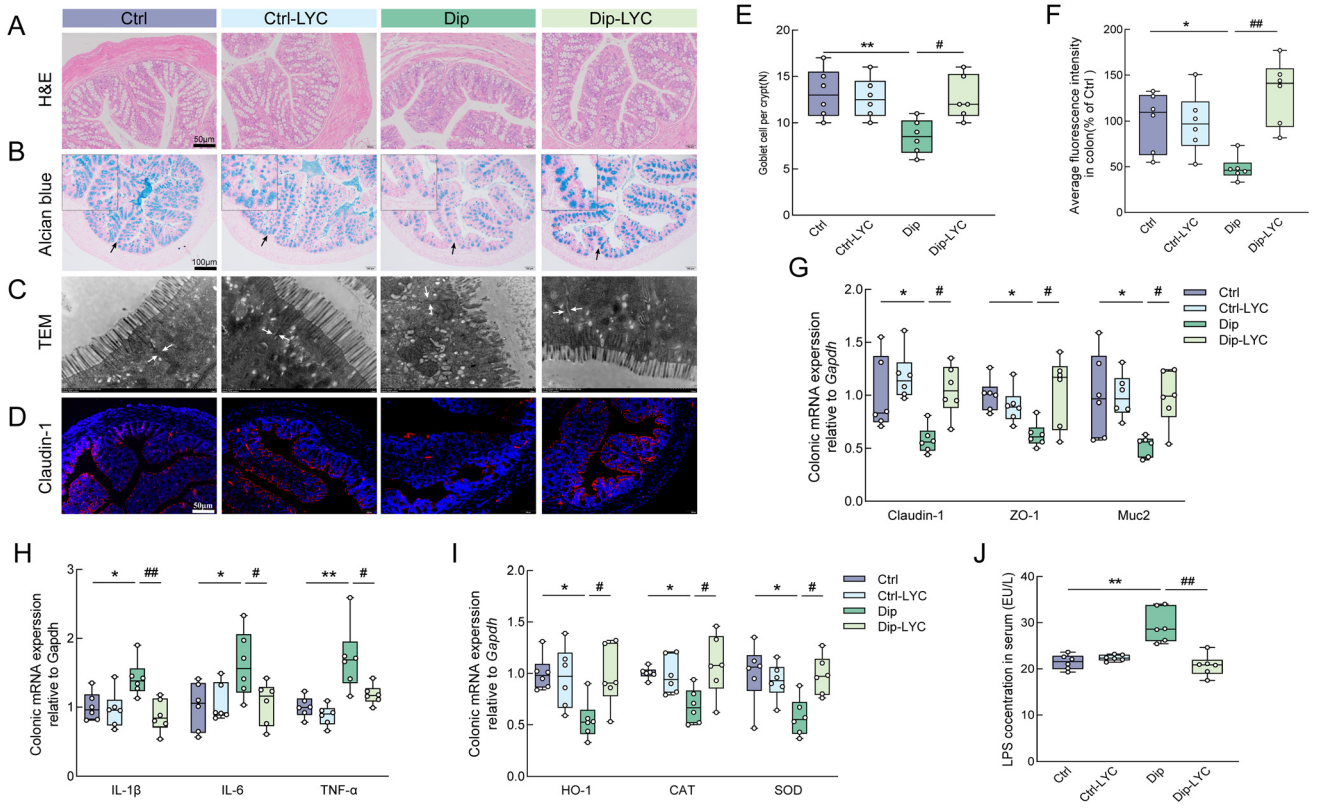

Fig. 4 Lycopene enhanced intestinal barrier integrity and alleviated colonic inflammation in constipated mice. (A) Representative images of H&E staining (scale bar, 100 μm); (B) representative images of Alcian blue staining (scale bar, 100 μm); (C) representative TEM micrographs of the colon for each group (scale bar, 1.0 μm); (D) representative images of immunohistochemistry staining of Claudin-1 in the colon for each group (scale bar, 200 μm); (E) quantification of the number of goblet cells per crypt based on Alcian blue staining; (F) quantification of the Claudin-1 positive area based on immunohistochemistry-stained sections using ImageJ software (representative images were captured from 6 slices of 3 mice per group); (G) RT-qPCR analysis of the mRNA expressions of Claudin-1, ZO-1, Muc2 in the colon for each group (n = 6 mice per group); (H–I) RT-qPCR analysis of the mRNA expressions of inflammation-related and oxidative stress-related genes in the colon for each group (n = 6 mice per group); (J) concentration of LPS in serum (n = 6 mice per group). Data in (E–J) are presented as the median ± interquartile range. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the Ctrl group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the Dip group. Significant differences were determined using one-way ANOVA with Tukey's multiple comparison test.

of ZO-1, claudin-1 and mucin 2 (Muc2) increased in LYC-treated constipated mice (Fig. 4G). Gut barrier damage causes the translocation of bacteria and endotoxins in the colonic mucosa, which in turn contributes to intestinal inflammation and oxidative stress.[39] Dip dramatically upregulated the colonic expression of proinflammatory cytokines (Il-1β, IL-6, Tnf-α) and circulating LPS levels (Fig. 4H and J), while it down-regulated antioxidant enzyme expression (HO-1, CAT, SOD), and all these were restored by LYC supplementation (Fig. 4I). These results suggest that LYC improved the constipation-related increase in intestinal permeability and inflammation.

3.5 Lycopene alleviates constipation-related depression- and anxiety-like behaviors

Considering that LYC could ameliorate colonic and systemic inflammation in constipated mice and the established link between inflammation and gut-brain axis dysregulation, we speculated that LYC may ameliorate constipation-related behavioral disorders.[40] Numerous studies have shown that depression and anxiety often coexist as comorbidities of various GI diseases, such as constipation.[12,13] We performed behavioral tests, including OFT, EPM, MBT and TST, to evaluate the impact of LYC on constipation-associated behavioral disorders. Constipated mice displayed anxiety-like behaviors, as evident from the lower instances of entering the center and the open arms in OFT (Fig. 5A–C) and EPM (Fig. 5D–F), respectively, and more buried marbles in MBT compared with the Ctrl group (Fig. 5G–I), all of which were restored by LYC treatment. Furthermore, constipated mice spent longer immobility time in TST, while LYC supplementation effectively improved this state in constipated mice (Fig. 5J and K). These data emphasize the protective effects of LYC against constipation-related depression- and anxiety-like behaviors.

3.6 Lycopene regulates the cholinergic synapse pathway and neuroinflammation in the brain of constipated mice

RNA sequencing of the cortex was performed to elucidate the potential mechanism underlying the LYC-mediated improvement of behavioral disorders in constipated mice. Principal component

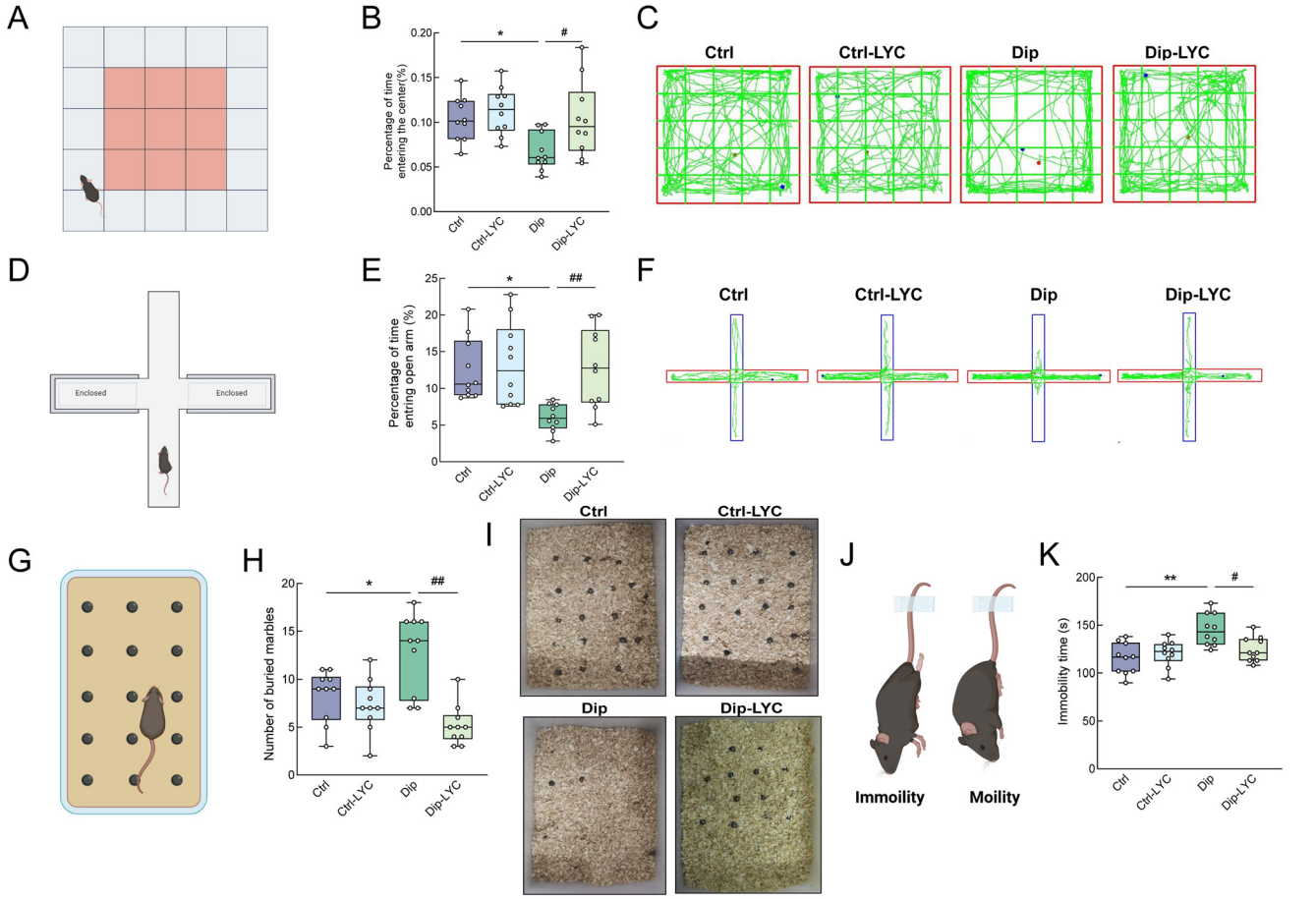

Fig. 5 Lycopene alleviated constipation-related depression- and anxiety-like behaviors. (A) Schematic of the open field test, created using FigDraw; (B) percentage of time entering the center (n = 10 mice per group); (C) representative track images of mice in the open field test; (D) schematic of the elevated plus maze., created using Biorender; (E) percent of open arm (n = 10 mice per group); (F) representative track images of mice in the elevated plus maze; (G) schematic of the marble buried test, created using Biorender; (H) number of buried marbles (n = 10 mice per group); (I) representative track images of mice in the marble buried test; (J) schematic of the tail suspension test, created using Biorender; (K) immobility time (n = 10 mice per group). Data were presented as the median ± interquartile range. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the Ctrl group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with the Dip group. Significant differences were determined using one-way ANOVA with Tukey's multiple comparison test.

analysis (PCA) revealed distinct clustering of the brain transcription profiles of the Dip group and the Ctrl group, with the Dip-LYC mice data closely approximating that of the Ctrl group (Fig. 6A). KEGG enrichment analyses of DEGs showed that the nervous-system-associated pathways, especially the cholinergic synapse pathway, underwent simultaneous changes between the Ctrl and Dip groups, as well as the Dip-LYC and Dip groups (Fig. 6B and C). Notably, the GSEA result revealed a significant upregulation of the cholinergic synapse pathway in the brain of LYC-treated constipated mice (Fig. 6D). Furthermore, we constructed a protein–protein interaction (PPI) network of the core genes in the cholinergic synapse pathway, which revealed that LYC significantly regulated genes encoding nicotinic acetylcholine receptors (Chrna7, Chrna2, Chrna3, and Chrna4) (Fig. 6E). Additionally, LYC also increased the Ach level in the brain of constipated mice (Fig. 6F).

The cholinergic synaptic pathway is closely related to inflammation in the CNS; especially, Chrna7 (known as α7nAChR) activation can reduce the production of proinflammatory cytokines.[41,42] We found that LYC significantly reduced the Iba-1 (a marker of microglial activation) positive areas in the cortex and hippocampus of Dip-treated mice (Fig. 6G–I). Additionally, there was an increase in amoeboid-type microglia in the brain of constipated mice, while the microglia phenotype changed to ramified type after LYC supplementation, which suggested that LYC inhibited the over-activation of microglia (Fig. 6G). Furthermore, LYC significantly increased the expression of antioxidant enzymes (HO-1, CAT and SOD) (Fig. 6J) and decreased the proinflammatory markers (IL-1β, IL-6 and TNF-α) in Dip-treated mice brains (Fig. 6K). Collectively, these results indicate that LYC effectively regulates the cholinergic system and mitigates neuroinflammation and oxidative stress in constipated mice.

3.7 Lycopene improves neuron damage and synaptic dysfunction in Dip-treated mice

Furthermore, Dip-treated mice displayed neuronal damage or nuclear atrophy in the cortex and hippocampal DG region compared with Ctrl mice, while LYC supplementation improved these morphological damages in constipated mice (Fig. 7A). Synaptic defects, including the loss of postsynaptic

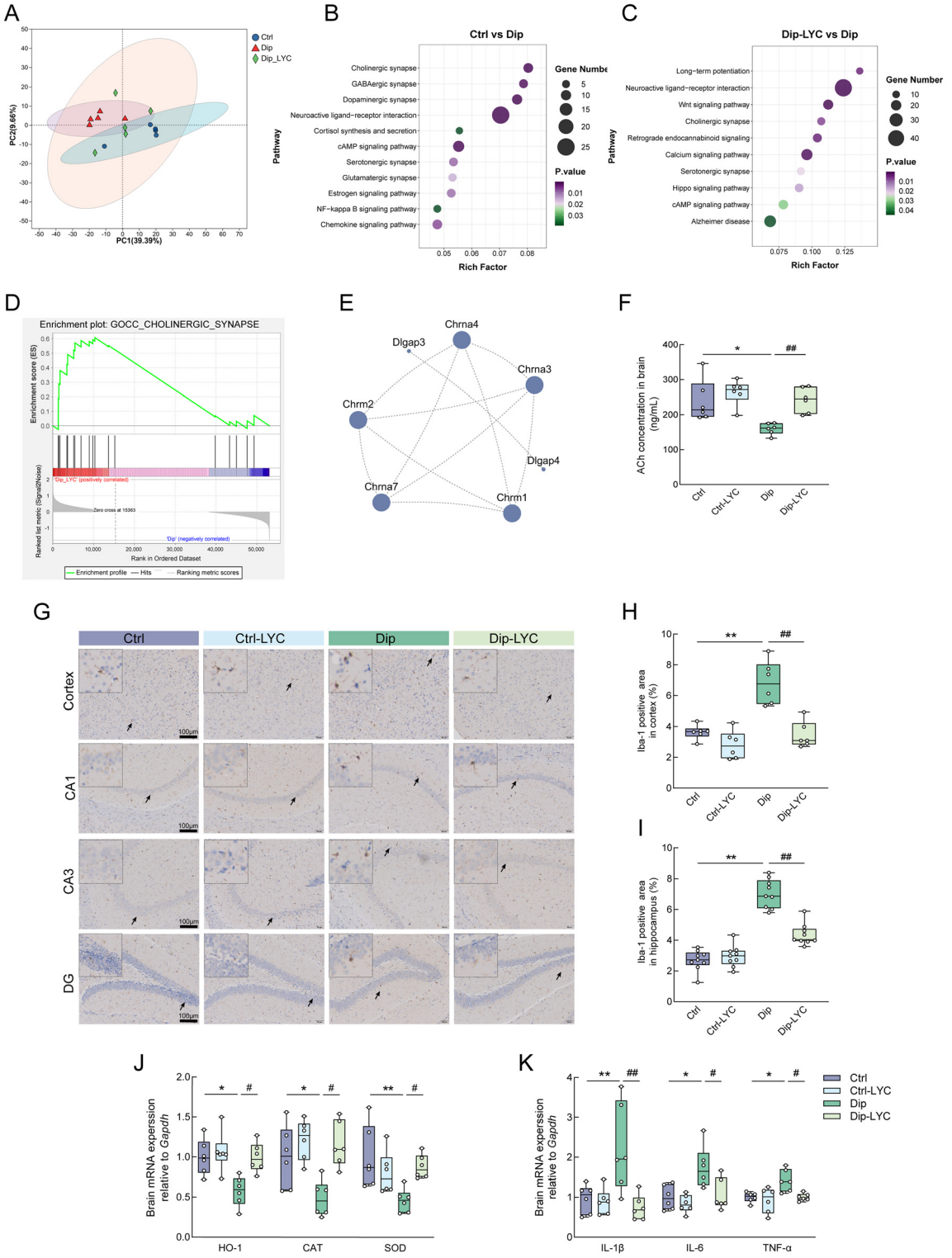

Fig. 6 Lycopene regulated cholinergic synapse pathway and neuroinflammation in the brain of constipated mice. (A) Principal-component analysis (PCA); (B and C) KEGG pathway enrichment analysis between the Ctrl group and Dip group (B) and Dip-LYC group and Dip group (C) based on RNA sequencing data; (D) enrichment plot of the cholinergic synapse pathway based on GSEA between the Dip group and Dip-LYC group; (E) PPI network of 7 hub DEGs in the cholinergic synapse pathway; (F) the level of Ach in the brain (n = 6 mice per group); (G) immunohistochemistry staining of Iba-1 in the brain (scale bar, 100 μm); (H–I) quantification of Iba-1 positive area using ImageJ software in the cortex (H) and hippocampus (I); (J and K) RT-qPCR analysis of the mRNA levels of oxidative stress-related and inflammation-related genes in the brain (n = 6 mice per group). Data in (F) and (H–K) are presented as the median ± interquartile range. *p < 0.05, **p < 0.01 compared with the Ctrl group, $^{\#}p$ < 0.05, $^{\#\#}p$ < 0.01 compared with the Dip group. Significant differences were determined using one-way ANOVA with Tukey's multiple comparison test.

density proteins (PSD), are associated with depression and anxiety.[43] TEM was used to observe the ultrastructure of mouse hippocampal synapses (Fig. 7B). LYC alleviated synaptic damages in Dip-treated mice, as evident from the increased width and length of the postsynaptic density (Fig. 7C and D) and the upregulated expression of PSD-95 (Fig. 7G). Brain-derived neurotrophic factor (BDNF), which can also be supported by PSD-95, has been reported to have antidepressant effects.[44] Immunofluorescence staining (Fig. 7E and F) and RT-qPCR results (Fig. 7G) demonstrated that LYC treatment significantly increased BDNF expression in the brain of Dip-treated mice. Thus, LYC could restore neuron damage and synaptic dysfunction in constipated mice.

4. Discussion

LYC, a natural nutritional component with proven benefits in various chronic diseases, shows a potential role in gut-brain axis regulation.[45] While prior studies suggest correlations between LYC intake and intestinal/brain homeostasis, its protective mechanisms against constipation and associated behavioral disorders remain unclear. Our findings demonstrate that LYC pretreatment significantly alleviated the constipation phenotype and associated depression- and anxiety-like behavior in mice. LYC enriched the beneficial gut bacteria, prevented gut barrier damage and promoted SCFA production in constipated mice. Importantly, LYC upregulated the nervous system pathway and restored ENS regeneration and neurotransmitter levels, which partly explains the benefits of LYC in relieving constipation. Furthermore, RNA sequencing results of the brain revealed that LYC supplementation enriched cholinergic synaptic pathways, regulated nicotinic acetylcholine receptors and increased Ach level, which could be associated with the alleviation of neuroinflammation and oxidative stress in constipated mice.

As the primary carotenoid found in the human serum, naturally sourced LYC up to 75 mg day^{-1} does not pose any risks to adults, underpinning its therapeutic potential in humans.[46] Upon ingestion, most of LYC is isomerized into the *cis* configuration, emulsified by scavenger receptor class B type 1 protein (SR-B1), and then absorbed by intestinal epithelial cells. Subsequently, LYC is converted into chylomicrons by β-carotene 15,15′-monooxygenase (BCOM1) and β-carotene 9′,10′-dioxygenase (BCO2) and then enters the blood and physical organs through portal veins and lymph circulation,[47] with detectable accumulation in the gut and brain, implying its potential role in the regulation of the gut-brain axis.[48,49] Constipation, a gastrointestinal disorder frequently prevalent with anxiety/depression as comorbidities, exhibits symptom exacerbation proportional to disease severity. Epidemiological evidence links constipation to suboptimal dietary patterns, positioning dietary modification as a first-line management

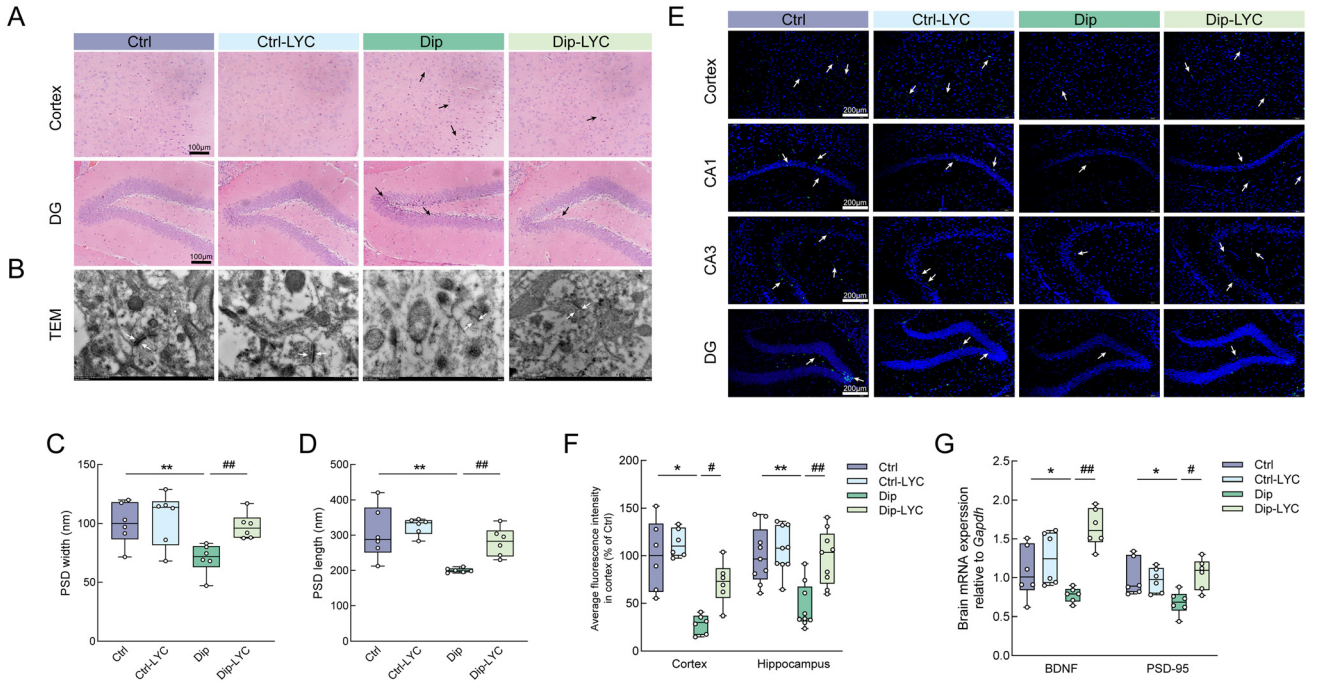

Fig. 7 Lycopene improved neuron damage, synaptic dysfunction in Dip-treated mice. (A) H&E staining of the brain (scale bar, 100 μm); (B) ultrastructure of synapses of the hippocampus (scale bar, 500 μm); (C and D) postsynaptic density (PSD) width (C) and length (D) (n = 6 mice per group); (E and F) immunofluorescence of BDNF (scale bar, 200 μm) (E) and quantification of BDNF expression using ImageJ software (F); (G) the mRNA levels of BDNF and PSD-95 in the brain (n = 6 mice per group). Data in (C and D) and (F and G) are presented as the median ± interquartile range. *p < 0.05, **p < 0.01 compared with the Ctrl group, $^{\#}p$ < 0.05, $^{\#\#}p$ < 0.01 compared with the Dip group. Significant differences were determined using one-way ANOVA with Tukey's multiple comparison test.

strategy.[4] Increasing red/orange, dark green vegetables or antioxidant intake is negatively correlated with chronic constipation incidence,[50,51] and a recent cross-sectional study indicated that LYC intake could reduce the risk of constipation in men,[52] implying that LYC may have positive effects on constipation and associated behavioral disorders. According to the body surface area calculations reported in previous studies, the human equivalent dose of LYC used in this study was 8.1 mg kg^{-1} d^{-1},[53] indicating that it may be achieved through supplementation. This study explored the preventive effect of LYC supplementation as a nutritional strategy to treat constipation induced by Dip, which may differ from clinical situations where intervention measures are applied after symptom onset. However, certain populations (such as those starting on constipation medication, elderly people or chronic opioid users prone to constipation) can benefit from preemptive dietary interventions.[54,55]

Accumulating evidence has confirmed that gut microbiota dysbiosis is implicated in the pathogenesis of constipation and related behavioral disorders.[12,56] Constipation reduces the α-diversity index and induces gut microbiota imbalance, characterized by the depletion of Firmicutes and an increase in potential endotoxin producers, including Helicobacter and Desulfobacterota.[15,57–59] Additionally, probiotics, such as *Bifidobacterium bifidum*, have been shown to restore the abundance of Firmicutes in constipated mice and patients, which is consistent with our findings.[56,57] We also found that LYC increased the Chao index and Shannon index of gut microbiota in constipated mice. Helicobacter eradication therapy has been shown to ameliorate abnormal bowel symptoms in chronically constipated patients,[56] while the increased abundance of Helicobacter and Desulfobacterota has been positively associated with anxiety and depression.[57–59] Multi-strain probiotics containing *Bifidobacterium* have been demonstrated to effectively improve constipation, as well as anxious and depressive symptoms in patients with GI disorders.[60,61] *Akkermansia* species (mainly *Akkermansia muciniphila*) have shown excellent anti-depressant efficacy, correlating with the modulation of gut microbiota structure and metabolite levels.[62,63] *Coriobacteriaceae UCG-002* has recently attracted widespread attention due to its anti-inflammatory benefits and is known to promote the growth of *Akkermansia muciniphila*.[64] In this work, LYC downregulated the relative abundance of Proteobacteria, Desulfobacterota and Helicobacter and restored the depleted beneficial bacteria, including *Bifidobacterium*, *Akkermansia* and *Coriobacteriaceae UCG-002*, in constipated mice. Gut microbiota imbalance can further disrupt metabolite profiles, including SCFAs, which have significant benefits on intestinal physiology and gut-brain communication.[65] SCFAs have been confirmed to play a mediating role in the relief of constipation comorbid depression and anxiety-like behavior by dietary inulin.[13] Here, we also noticed that LYC significantly enhanced SCFA production and SCFA producers like *Lachnospiraceae NK4A136 group* and *Allobaculum* in constipated mice.[66,67] These results suggest that the improvements in gut microbiota dysbiosis and SCFA production are closely related to ameliorating depression- and anxiety-like behaviors in constipated mice treated with LYC.

As the only internal organ that has evolved an independent nervous system, the GI has a crucial role in regulating digestion and absorption through its neural regulatory system, known as ENS.[27] The ENS is a complex neural network, and the neural circuits are composed of abundant nerve cells and enteric ganglia distributed in the submucosal and myenteric plexuses that spread throughout the intestinal wall.[68] Abnormal ENS function can lead to abnormal intestinal motility, causing diarrhea or constipation. Research has shown that cases of insufficient gut motility can be related to the loss of enteric neurons in the colon, as evident from the decreased number of HuC/D^+ neurons.[69–71] Neurons in the GI tract rely on excitatory and inhibitory neurotransmitters to regulate intestinal motility, and their abnormal secretion can also affect constipation.[72] Especially, excitatory cholinergic neurons are one of the main subtypes of neurons in the ENS. Studies have shown that the absence of cholinergic neuron marker ChAT leads to proximal gastrointestinal motility disorders and a severe decline in colonic transport capacity.[35] *Lactiplantibacillus plantarum* treatment has been found to regulate $ChAT^+$ neurons in the ENS *via* microbial tryptophan metabolites, which makes it a potential management strategy for IBS with predominant diarrhea.[73] *Bifidobacterium bifidum* alleviates intestinal motility disorders by reshaping intestinal nerves by upregulating S100 β and Ach and downregulating the inhibitory neurotransmitter nNOS.[74] Additionally, chronic SCFA administration increases ChAT expression in enteric neurons, exerting a prokinetic effect on colonic motility, and butyrate can promote the synthesis of Ach and SP, all of which provide mechanistic insights into the role of SCFAs in increasing the intestinal transit rate.[75,76] We observed lesser HuC/D^+ and $Tuj1^+$ positive area and reduced expression of ChAT in constipated mice, which was significantly restored by LYC supplementation. Moreover, LYC treatment also increased the excitatory neurotransmitters Ach and SP while reducing the inhibitory neurotransmitters VIP in the colon of constipated mice. However, the involvement of the microbiota and metabolites in the mechanism of ENS regulation by LYC in constipated mice needs further exploration.

Peripheral inflammation is a prominent factor contributing to the high incidence of behavioral disorders in people with gastrointestinal diseases. The imbalance of intestinal homeostasis, including the dysbiosis of gut microbiota and metabolites, leads to gut "leakage", causing harmful bacteria and toxins to enter the blood and thereby affecting host health.[77] This study indicates that LYC can cause a significant reduction in inflammation-related bacteria enriched by Dip treatment, such as *Helicobacter* and *Herbaspirillum*.[78,79] LYC could alleviate constipation-induced colonic inflammation and oxidative stress and significantly restore downregulated colonic TJs (claudin-1, ZO-1) and Muc2 levels in Dip-treated mice, which was consistent with the decrease in circulated LPS level. Previous works have presented strong evidence emphasizing the vital role of inflammation in depression pathophysiology

and a significant increase in endotoxin-producing bacteria and colonic inflammatory factors in constipated mice, which disrupts gut barrier integrity and contributes to an increase in serum LPS, triggering systemic inflammation, including neuroinflammation.[80] A meta-analysis suggests that increased microglial activity has a positive correlation with elevated levels of IL-6, IL-8, and TNF-α in the cerebrospinal fluid of depressed patients.[81] In this study, constipated mice exhibited microglial overactivation in the cortex and hippocampus, accompanied by an increase in pro-inflammatory cytokines and a decrease in anti-oxidant stress markers, all of which notably improved after LYC administration. The cholinergic system has a significant role in regulating inflammatory responses, mainly through the CAP of the vagus nerve, which is a key neural pathway of communication between the gut and the brain.[82] Chrna7, also known as α7nAChR, an important mediator in CAP, has attracted increasing attention due to its antidepressant effect.[83] Ach inhibits the NF-κB pathway by acting on α7nAChR, consequently suppressing the proinflammatory cytokine secretion and thus suppressing neuroinflammation.[16] Research has illustrated that mice with cholinergic system dysfunction display gut microbiota dysbiosis, such as an increase in *Helicobacter ganmani*, and depressive phenotypes; meanwhile, FMT from mice with cholinergic system dysfunction caused systemic inflammation and synaptic protein loss, which contributed to depression in normal mice, indicating the critical role of the cholinergic system in regulating behavioral disorders through gut-microbiota-brain signaling.[17] Additionally, a chronic stress-induced depressive mice model exhibited decreased Ach levels in the brain.[84,85] In this work, through brain RNA sequencing, we found that LYC regulated the cholinergic synapse pathway, particularly the nicotinic acetylcholine receptors, and upregulated Ach content in the brain of constipated mice. Previous studies have also demonstrated that LYC could activate the cholinergic system in the brain, increasing the brain ACh levels by inhibiting AChE activity and ultimately improving memory impairment in rats, which may be related to the antioxidant properties of LYC.[86]

While this study provides novel insights into the therapeutic potential of LYC, it also has several limitations that warrant consideration. Firstly, although our findings suggest an association between LYC-induced gut microbiota modulation and constipation alleviation, the causal role of microbial communities requires validation through germ-free models and FMT studies to establish definitive mediation mechanisms. Secondly, given that the cholinergic system regulates inflammatory responses *via* the CAP, a process involved in the neural signaling of the gut-brain axis, future studies with Chrna7-specific conditional knockout mice are essential to elucidate the direct involvement of the cholinergic system in LYC-mediated amelioration of constipation and the comorbid anxiety- and depression-like behaviors. Lastly, elucidating the molecular mechanisms by which LYC modulates cholinergic signaling in the ENS and brain remains a critical research priority.

In conclusion, our study demonstrates that LYC effectively protects against Dip-induced constipation and associated behavioral disorders. Mechanistically, LYC promotes gut motility by inhibiting intestinal inflammation, gut microbiota dysbiosis and enteric neuron regeneration. Subsequently, LYC alleviates neuroinflammation partially by regulating the cholinergic system, which contributes to the improvement of constipation-associated depression and anxiety. Given that many neurotransmitters and anatomical characteristics are shared by ENS and CNS, ENS dysfunction is accompanied by an increase in the occurrence of CNS pathologies.[87] The interplay between intestinal motility-disorder-related ENS dysfunction and CNS disorders deserves further investigation. Overall, our findings exemplify the potential of LYC as a promising nutritional agent for managing intestinal dysmotility and related neuropsychiatric comorbidities.

Abbreviations

Ach	Acetylcholine
BDNF	Brain-derived neurotrophic factor
CAP	Cholinergic anti-inflammatory pathway
ChAT	Choline acetyltransferase
CNS	Central nervous system
Dip	Diphenoxylate
ENS	Enteric nervous system
EPM	Elevated plus maze
FMT	Fecal transplantation experiment
GI	Gastrointestinal
GSEA	Gene set enrichment analysis
KEGG	Kyoto encyclopedia of genes and genomes
IBS	Irritable bowel syndrome
LPS	Lipopolysaccharide
LYC	Lycopene
MBT	Marble buried test
Muc2	Mucin 2
OFT	Open field test
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PPI	Protein–protein interaction
PSD	Postsynaptic density proteins
SCFAs	Short-chain fatty acids
STC	Slow transit constipation
SP	Substance P
TEM	Transmission electron microscopy
TJs	Tight junctions
TST	Tail suspension test
Tuj1	βIII-Tubulin
VIP	Vasoactive intestinal peptide.

Author contributions

Danna Wang: conceptualization, investigation, methodology, data curation, and writing – original draft. Siyi Jiao: methodology, formal analysis, data curation, and software. Qianqian Guo: methodology and formal analysis. Mengnan Zhou: meth-

odology. Wanting Ren: methodology. Yuqi Zhao: methodology. Dongning Li: methodology. Wenyue Yang: methodology. Shengquan Dai: methodology. Yanqian Pei: investigation. Danyixin Xiao: software. Xuebo Liu: supervision and funding acquisition. Chunqia Xiao: conceptualization, supervision, and funding acquisition. Beita Zhao: conceptualization, supervision, and writing – review and editing. All authors have read and approved the final manuscript.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (no. 32101968), Postdoctoral Fellowship Program of CPSF under Grant Number GZB20240615, Key Research and Development project in Shaanxi Province (2022SF-024), and the Doctoral Research Fund of Northwest A&F University (no. Z1090220165). We also thank the Life Science Research Core Services of NWAFU (Zhou Min, Yang Chenghui, Huang Kerang, Liu Xiaorui, Liu Yao) and the Instrument Shared Platform of the College of Food Science & Engineering of NWAFU (Zhou Yuan, Wang Xue) for technical support.

References

1 J. Keller, G. Bassotti, J. Clarke, P. Dinning, M. Fox, M. Grover, P. M. Hellström, M. Ke, P. Layer, C. Malagelada, H. P. Parkman, S. M. Scott, J. Tack, M. Simren, H. Törnblom and M. Camilleri, Expert consensus document: Advances in the diagnosis and classification of gastric and intestinal motility disorders, *Nat. Rev. Gastroenterol. Hepatol.*, 2018, **15**, 291–308.

2 M. Simrén and J. Tack, New treatments and therapeutic targets for IBS and other functional bowel disorders, *Nat. Rev. Gastroenterol. Hepatol.*, 2018, **15**, 589–605.

3 B. Barberio, C. Judge, E. V. Savarino and A. C. Ford, Global prevalence of functional constipation according to the Rome criteria: a systematic review and meta-analysis, *Lancet Gastroenterol. Hepatol.*, 2021, **6**, 638–648.

4 M. Camilleri, A. C. Ford, G. M. Mawe, P. G. Dinning, S. S. Rao, W. D. Chey, M. Simrén, A. Lembo, T. M. Young-Fadok and L. Chang, Chronic constipation, *Nat. Rev. Dis. Primers*, 2017, **3**, 17095.

5 S. Wu, G. Yuan, L. Wu, L. Zou and F. Wu, Identifying the association between depression and constipation: An observational study and Mendelian randomization analysis, *J. Affective Disord.*, 2024, **359**, 394–402.

6 K. G. Margolis, J. F. Cryan and E. A. Mayer, The Microbiota-Gut-Brain Axis: From Motility to Mood, *Gastroenterology*, 2021, **160**, 1486–1501.

7 D. Nellesen, A. Chawla, D. L. Oh, T. Weissman, B. J. Lavins and C. W. Murray, Comorbidities in patients with irritable bowel syndrome with constipation or chronic idiopathic constipation: a review of the literature from the past decade, *Postgrad. Med.*, 2013, **125**, 40–50.

8 A. C. Ford and N. C. Suares, Effect of laxatives and pharmacological therapies in chronic idiopathic constipation: systematic review and meta-analysis, *Gut*, 2011, **60**, 209–218.

9 A. D. Sperber, S. I. Bangdiwala, D. A. Drossman, U. C. Ghoshal, M. Simren, J. Tack, W. E. Whitehead, D. L. Dumitrascu, X. Fang, S. Fukudo, J. Kellow, E. Okeke, E. M. M. Quigley, M. Schmulson, P. Whorwell, T. Archampong, P. Adibi, V. Andresen, M. A. Benninga, B. Bonaz, S. Bor, L. B. Fernandez, S. C. Choi, E. S. Corazziari, C. Francisconi, A. Hani, L. Lazebnik, Y. Y. Lee, A. Mulak, M. M. Rahman, J. Santos, M. Setshedi, A. F. Syam, S. Vanner, R. K. Wong, A. Lopez-Colombo, V. Costa, R. Dickman, M. Kanazawa, A. H. Keshteli, R. Khatun, I. Maleki, P. Poitras, N. Pratap, O. Stefanyuk, S. Thomson, J. Zeevenhooven and O. S. Palsson, Worldwide Prevalence and Burden of Functional Gastrointestinal Disorders, Results of Rome Foundation Global Study, *Gastroenterology*, 2021, **160**, 99–114.

10 J. Huang, B. Lin, Y. Zhang, Z. Xie, Y. Zheng, Q. Wang and H. Xiao, Bamboo shavings derived O-acetylated xylan alleviates loperamide-induced constipation in mice, *Carbohydr. Polym.*, 2022, **276**, 118761.

11 R. Li, S. Xu, B. Li, B. Zhang, W. Chen, D. Dai and Z. Liu, Gut indigenous Ruminococcus gnavus alleviates constipation and stress-related behaviors in mice with loperamide-induced constipation, *Food Funct.*, 2023, **14**, 5702–5715.

12 C. Yang, T. Hu, X. Xue, X. Su, X. Zhang, Y. Fan, X. Shen and X. Dong, Multi-omics analysis of fecal microbiota transplantation's impact on functional constipation and comorbid depression and anxiety, *BMC Microbiol.*, 2023, **23**, 389.

13 H. Zou, H. Gao, Y. Liu, Z. Zhang, J. Zhao, W. Wang, B. Ren and X. Tan, Dietary inulin alleviated constipation induced depression and anxiety-like behaviors: Involvement of gut microbiota and microbial metabolite short-chain fatty acid, *Int. J. Biol. Macromol.*, 2024, **259**, 129420.

14 Z. Yang, S. Ye, Z. Xu, H. Su, X. Tian, B. Han, B. Shen, Q. Liao, Z. Xie and Y. Hong, Dietary synbiotic ameliorates constipation through the modulation of gut microbiota and its metabolic function, *Food Res. Int.*, 2021, **147**, 110569.

15 M. Zhuang, W. Shang, Q. Ma, P. Strappe and Z. Zhou, Abundance of Probiotics and Butyrate-Production Microbiome Manages Constipation *via* Short-Chain Fatty

Acids Production and Hormones Secretion, *Mol. Nutr. Food Res.*, 2019, **63**, e1801187.
16 B. Han, X. Li and J. Hao, The cholinergic anti-inflammatory pathway: An innovative treatment strategy for neurological diseases, *Neurosci. Biobehav. Rev.*, 2017, **77**, 358–368.
17 Y. Pu, Y. Tan, Y. Qu, L. Chang, S. Wang, Y. Wei, X. Wang and K. Hashimoto, A role of the subdiaphragmatic vagus nerve in depression-like phenotypes in mice after fecal microbiota transplantation from Chrna7 knock-out mice with depression-like phenotypes, *Brain, Behav., Immun.*, 2021, **94**, 318–326.
18 P. Singh, C. Tuck, P. R. Gibson and W. D. Chey, The Role of Food in the Treatment of Bowel Disorders: Focus on Irritable Bowel Syndrome and Functional Constipation, *Am. J. Gastroenterol.*, 2022, **117**, 947–957.
19 P. Xia, X. Liu, T. Hou, F. Zhan, F. Geng, Z. Zhang and B. Li, Evaluation of the effect of prebiotic sesame candies on loperamide-induced constipation in mice, *Food Funct.*, 2022, **13**, 5690–5700.
20 L. Wu, C. Tang, L. Chen and J. Zhao, Modified dietary fiber from soybean dregs by fermentation alleviated constipation in mice, *Food Chem. X*, 2023, **19**, 100810.
21 B. Burton-Freeman and H. D. Sesso, Whole food versus supplement: comparing the clinical evidence of tomato intake and lycopene supplementation on cardiovascular risk factors, *Adv. Nutr.*, 2014, **5**, 457–485.
22 A. Hernández-Almanza, J. Montañez, G. Martínez, A. Aguilar-Jiménez, J. C. Contreras-Esquivel and C. N. Aguilar, Lycopene: Progress in microbial production, *Trends Food Sci. Technol.*, 2016, **56**, 142–148.
23 H. R. Wang, M. Z. Li, J. G. Cui, H. Zhang, Y. Zhao and J. L. Li, Lycopene Prevents Phthalate-Induced Cognitive Impairment *via* Modulating Ferroptosis, *J. Agric. Food Chem.*, 2023, **71**, 16727–16738.
24 B. Zhao, H. Liu, J. Wang, P. Liu, X. Tan, B. Ren, Z. Liu and X. Liu, Lycopene Supplementation Attenuates Oxidative Stress, Neuroinflammation, and Cognitive Impairment in Aged CD-1 Mice, *J. Agric. Food Chem.*, 2018, **66**, 3127–3136.
25 T. Silva Meneguelli, M. Duarte Villas Mishima, H. H. M. Hermsdorff, H. S. D. Martino, J. Bressan and E. Tako, Effect of carotenoids on gut health and inflammatory status: A systematic review of in vivo animal studies, *Crit. Rev. Food Sci.*, 2024, **64**, 11206–11221.
26 J. Wang, Z. Wang, B. Li, Y. Qiang, T. Yuan, X. Tan, Z. Wang, Z. Liu and X. Liu, Lycopene attenuates western-diet-induced cognitive deficits *via* improving glycolipid metabolism dysfunction and inflammatory responses in gut-liver-brain axis, *Int. J. Obes.*, 2019, **43**, 1735–1746.
27 N. J. Spencer and H. Hu, Enteric nervous system: sensory transduction, neural circuits and gastrointestinal motility, *Nat. Rev. Gastroenterol. Hepatol.*, 2020, **17**, 338–351.
28 Y. Yun, X. Wang, J. Xu, J. Chen, X. Wang, P. Yang and L. Qin, Optogenetic stimulation of basal forebrain cholinergic neurons prevents neuroinflammation and neuropsychiatric manifestations in pristane induced lupus mice, *Behav. Brain Funct.*, 2023, **19**, 11.
29 D. Wang, J. Wu, P. Zhu, H. Xie, L. Lu, W. Bai, W. Pan, R. Shi, J. Ye, B. Xia, Z. Zhao, Y. Wang, X. Liu and B. Zhao, Tryptophan-rich diet ameliorates chronic unpredictable mild stress induced depression- and anxiety-like behavior in mice: The potential involvement of gut-brain axis, *Food Res. Int.*, 2022, **157**, 111289.
30 K. Q. Fan, Y. Y. Li, H. L. Wang, X. T. Mao, J. X. Guo, F. Wang, L. J. Huang, Y. N. Li, X. Y. Ma, Z. J. Gao, W. Chen, D. D. Qian, W. J. Xue, Q. Cao, L. Zhang, L. Shen, L. Zhang, C. Tong, J. Y. Zhong, W. Lu, L. Lu, K. M. Ren, G. Zhong, Y. Wang, M. Tang, X. H. Feng, R. J. Chai and J. Jin, Stress-Induced Metabolic Disorder in Peripheral CD4 (+) T Cells Leads to Anxiety-like Behavior, *Cell*, 2019, **179**, 864–879.
31 B. Zhao, J. Wu, J. Li, Y. Bai, Y. Luo, B. Ji, B. Xia, Z. Liu, X. Tan, J. Lv and X. Liu, Lycopene Alleviates DSS-Induced Colitis and Behavioral Disorders *via* Mediating Microbes-Gut-Brain Axis Balance, *J. Agric. Food Chem.*, 2020, **68**, 3963–3975.
32 Z. Huang, L. Liao, Z. Wang, Y. Lu, W. Yan, H. Cao and B. Tan, An efficient approach for wholemount preparation of the myenteric plexus of rat colon, *J. Neurosci. Methods*, 2021, **348**, 109012.
33 B. Li and C. N. Dewey, RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome, *BMC Bioinf.*, 2011, **12**, 323.
34 M. I. Love, W. Huber and S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, *Genome Biol.*, 2014, **15**, 550.
35 C. D. Johnson, A. J. Barlow-Anacker, J. F. Pierre, K. Touw, C. S. Erickson, J. B. Furness, M. L. Epstein and A. Gosain, Deletion of choline acetyltransferase in enteric neurons results in postnatal intestinal dysmotility and dysbiosis, *FASEB J.*, 2018, **32**, 4744–4752.
36 M. Chai, L. Wang, X. Li, J. Zhao, H. Zhang, G. Wang and W. Chen, Different *Bifidobacterium bifidum* strains change the intestinal flora composition of mice *via* different mechanisms to alleviate loperamide-induced constipation, *Food Funct.*, 2021, **12**, 6058–6069.
37 F. A. Vicentini, C. M. Keenan, L. E. Wallace, C. Woods, J. B. Cavin, A. R. Flockton, W. B. Macklin, J. Belkind-Gerson, S. A. Hirota and K. A. Sharkey, Intestinal microbiota shapes gut physiology and regulates enteric neurons and glia, *Microbiome*, 2021, **9**, 210.
38 L. Wang, M. Chai, J. Wang, Q. Yu, G. Wang, H. Zhang, J. Zhao and W. Chen, *Bifidobacterium longum* relieves constipation by regulating the intestinal barrier of mice, *Food Funct.*, 2022, **13**, 5037–5049.
39 F. Shen, Q. Wang, S. Ullah, Y. Pan, M. Zhao, J. Wang, M. Chen, F. Feng and H. Zhong, *Ligilactobacillus acidipiscis YJ5* modulates the gut microbiota and produces beneficial metabolites to relieve constipation by enhancing the mucosal barrier, *Food Funct.*, 2024, **15**, 310–325.
40 T. C. Fung, C. A. Olson and E. Y. Hsiao, Interactions between the microbiota, immune and nervous systems in health and disease, *Nat. Neurosci.*, 2017, **20**, 145–155.

41 J. Y. Wang, Y. Zhang, Y. Chen, Y. Wang, S. Y. Li, Y. F. Wang, Z. X. Zhang, J. Zhang and P. Rong, Mechanisms underlying antidepressant effect of transcutaneous auricular vagus nerve stimulation on CUMS model rats based on hippocampal α7nAchR/NF-κB signal pathway, *J. Neuroinflammation*, 2021, **18**, 291.
42 D. Zhao, X. Xu, L. Pan, W. Zhu, X. Fu, L. Guo, Q. Lu and J. Wang, Pharmacologic activation of cholinergic alpha7 nicotinic receptors mitigates depressive-like behavior in a mouse model of chronic stress, *J. Neuroinflammation*, 2017, **14**, 234.
43 E. Leung, E. W. Lau, A. Liang, C. de Dios, R. Suchting, L. Östlundh, J. C. Masdeu, M. Fujita, M. Sanches, J. C. Soares and S. Selvaraj, Alterations in brain synaptic proteins and mRNAs in mood disorders: a systematic review and meta-analysis of postmortem brain studies, *Mol. Psychiatry*, 2022, **27**, 1362–1372.
44 X. Shi, X. Z. Zhou, G. Chen, W. F. Luo, C. Zhou, T. J. He, M. T. Naik, Q. Jiang, J. Marshall and C. Cao, Targeting the postsynaptic scaffolding protein PSD-95 enhances BDNF signaling to mitigate depression-like behaviors in mice, *Sci. Signaling*, 2024, **17**, eadn4556.
45 U. M. Khan, M. Sevindik, A. Zarrabi, M. Nami, B. Ozdemir, D. N. Kaplan, Z. Selamoglu, M. Hasan, M. Kumar, M. M. Alshehri and J. Sharifi-Rad, Lycopene: Food Sources, Biological Activities, and Human Health Benefits, *Oxid. Med. Cell. Longevity*, 2021, **2021**, 2713511.
46 R. A. Matulka, A. M. Hood and J. C. Griffiths, Safety evaluation of a natural tomato oleoresin extract derived from food-processing tomatoes, *Regul. Toxicol. Pharmacol.*, 2004, **39**, 390–402.
47 X. Liang, C. Ma, X. Yan, X. Liu and F. Liu, Advances in research on bioactivity, metabolism, stability and delivery systems of lycopene, *Trends Food Sci. Technol.*, 2019, **93**, 185–196.
48 N. E. Moran, J. W. Erdman Jr and S. K. Clinton, Complex interactions between dietary and genetic factors impact lycopene metabolism and distribution, *Arch. Biochem. Biophys.*, 2013, **539**, 171–180.
49 L. A. Kaplan, J. M. Lau and E. A. Stein, Carotenoid composition, concentrations, and relationships in various human organs, *Clin. Physiol. Biochem.*, 1990, **8**, 1–10.
50 C. Jiang and Y. Shao, The effects of specific vegetable subtypes on constipation incidence in the general United States population, *Front. Nutr.*, 2024, **11**, 1403636.
51 S. Wei, S. Yu, Y. Lan and Y. Jia, Association between the composite dietary antioxidant index and constipation: Evidence from NHANES 2005-2010, *PLoS One*, 2024, **19**, e0311168.
52 J. Wang, W. Kong, M. Liu, Y. Wang, Y. Zheng and Y. Zhou, Association between dietary carotenoids intake and chronic constipation in American men and women adults: a cross-sectional study, *BMC Public Health*, 2023, **23**, 1597.
53 A. B. Nair and S. Jacob, A simple practice guide for dose conversion between animals and human, *J. Basic Clin. Pharm.*, 2016, **7**, 27–31.
54 A. D. Farmer, C. B. Holt, T. J. Downes, E. Ruggeri, S. Del Vecchio and R. De Giorgio, Pathophysiology, diagnosis, and management of opioid-induced constipation, *Lancet Gastroenterol. Hepatol.*, 2018, **3**, 203–212.
55 R. De Giorgio, E. Ruggeri, V. Stanghellini, L. H. Eusebi, F. Bazzoli and G. Chiarioni, Chronic constipation in the elderly: a primer for the gastroenterologist, *BMC Gastroenterol.*, 2015, **15**, 1–13.
56 M. Murata, M. Sugimoto, S. Miyamoto and T. Kawai, Long-term improvement in constipation-related symptoms after Helicobacter pylori eradication therapy, *Helicobacter*, 2022, **27**, e12863.
57 Y. Wu, G. K. Murray, E. M. Byrne, J. Sidorenko, P. M. Visscher and N. R. Wray, GWAS of peptic ulcer disease implicates Helicobacter pylori infection, other gastrointestinal disorders and depression, *Nat. Commun.*, 2021, **12**, 1146.
58 P. Chen, X. Li, Y. Yu, J. Zhang, Y. Zhang, C. Li, J. Li and K. Li, Administration Time and Dietary Patterns Modified the Effect of Inulin on CUMS-Induced Anxiety and Depression, *Mol. Nutr. Food. Res.*, 2023, **67**, e2200566.
59 A. Takeoka, J. Tayama, M. Kobayashi, I. Sagara, S. Ogawa, T. Saigo, M. Hayashida, H. Yamasaki, S. Fukudo and S. Shirabe, Psychological effects of Helicobacter pylori-associated atrophic gastritis in patients under 50 years: A cross-sectional study, *Helicobacter*, 2017, **22**, DOI: **10.1111/hel.12445**.
60 V. H. Dao, L. B. Hoang, T. O. Trinh, T. T. T. Tran and V. L. Dao, Psychobiotics for Patients with Chronic Gastrointestinal Disorders Having Anxiety or Depression Symptoms, *J. Multidiscip. Health*, 2021, **14**, 1395–1402.
61 C. Zhang, L. Wang, X. Liu, G. Wang, X. Guo, X. Liu, J. Zhao and W. Chen, The Different Ways Multi-Strain Probiotics with Different Ratios of *Bifidobacterium* and *Lactobacillus*, Relieve Constipation Induced by Loperamide in Mice, *Nutrients*, 2023, **15**, 4230.
62 Y. Ding, F. Bu, T. Chen, G. Shi, X. Yuan, Z. Feng, Z. Duan, R. Wang, S. Zhang, Q. Wang, J. Zhou and Y. Chen, A next-generation probiotic: Akkermansia muciniphila ameliorates chronic stress-induced depressive-like behavior in mice by regulating gut microbiota and metabolites, *Appl. Microbiol. Biotechnol.*, 2021, **105**, 8411–8426.
63 H. Guo, X. Liu, T. Chen, X. Wang and X. Zhang, Akkermansia muciniphila Improves Depressive-Like Symptoms by Modulating the Level of 5-HT Neurotransmitters in the Gut and Brain of Mice, *Mol. Neurobiol.*, 2024, **61**, 821–834.
64 P. Corrales, Y. Vivas, A. Izquierdo-Lahuerta, D. Horrillo, P. Seoane-Collazo, I. Velasco, L. Torres, Y. Lopez, C. Martínez and M. López, Long–term caloric restriction ameliorates deleterious effects of aging on white and brown adipose tissue plasticity, *Aging Cell*, 2019, **18**, e12948.
65 B. Dalile, L. Van Oudenhove, B. Vervliet and K. Verbeke, The role of short-chain fatty acids in microbiota–gut–brain

communication, *Nat. Rev. Gastroenterol. Hepatol.*, 2019, **16**, 461–478.

66 P. M. Smith, M. R. Howitt, N. Panikov, M. Michaud, C. A. Gallini, Y. M. Bohlooly, J. N. Glickman and W. S. Garrett, The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis, *Science*, 2013, **341**, 569–573.

67 H. L. Greetham, G. R. Gibson, C. Giffard, H. Hippe, B. Merkhoffer, U. Steiner, E. Falsen and M. D. Collins, Allobaculum stercoricanis gen. nov., sp. nov., isolated from canine feces, *Anaerobe*, 2004, **10**, 301–307.

68 J. B. Furness, The enteric nervous system and neurogastroenterology, *Nat. Rev. Gastroenterol. Hepatol.*, 2012, **9**, 286–294.

69 K. M. Schneider, N. Blank, Y. Alvarez, K. Thum, P. Lundgren, L. Litichevskiy, M. Sleeman, K. Bahnsen, J. Kim, S. Kardo, S. Patel, L. Dohnalová, G. T. Uhr, H. C. Descamps, S. Kircher, A. M. McSween, A. R. Ardabili, K. M. Nemec, M. T. Jimenez, L. G. Glotfelty, J. D. Eisenberg, E. E. Furth, J. Henao-Mejia, F. C. Bennett, M. J. Pierik, M. Romberg-Camps, Z. Mujagic, M. Prinz, C. V. Schneider, E. J. Wherry, M. Bewtra, R. O. Heuckeroth, M. Levy and C. A. Thaiss, The enteric nervous system relays psychological stress to intestinal inflammation, *Cell*, 2023, **186**, 2823–2838.

70 G. Bassotti, V. Villanacci, C. A. Maurer, S. Fisogni, F. Di Fabio, M. Cadei, A. Morelli, T. Panagiotis, G. Cathomas and B. Salerni, The role of glial cells and apoptosis of enteric neurones in the neuropathology of intractable slow transit constipation, *Gut*, 2006, **55**, 41–46.

71 J. I. Lee, H. Park, M. A. Kamm and I. C. Talbot, Decreased density of interstitial cells of Cajal and neuronal cells in patients with slow-transit constipation and acquired megacolon, *J. Gastroenterol.*, 2005, **20**, 1292–1298.

72 L. Agrawal, M. Korkutata, S. K. Vimal, M. K. Yadav, S. Bhattacharyya and T. Shiga, Therapeutic potential of serotonin 4 receptor for chronic depression and its associated comorbidity in the gut, *Neuropharmacology*, 2020, **166**, 107969.

73 B. Xia, T. Lin, Z. Li, J. Wang, Y. Sun, D. Wang, J. Ye, Y. Zhang, R. Kou, B. Zhao, J. Yi, G. Bai and X. Liu, Lactiplantibacillus plantarum Regulates Intestinal Physiology and Enteric Neurons in IBS through Microbial Tryptophan Metabolites, *J. Agric. Food Chem.*, 2024, **72**, 17989–18002.

74 L. Wang, L. Cao, Q. Yu, M. Liang, Z. Yang, G. Wang, J. Zhao and W. Chen, *Bifidobacterium bifidum CCFM1359*, alleviates intestinal motility disorders through the BDNF-TrkB pathway, *Food Funct.*, 2025, **16**, 437–451.

75 R. Soret, J. Chevalier, P. De Coppet, G. Poupeau, P. Derkinderen, J. P. Segain and M. Neunlist, Short-chain fatty acids regulate the enteric neurons and control gastrointestinal motility in rats, *Gastroenterology*, 2010, **138**, 1772–1782.

76 L. Wang, S. Cen, G. Wang, Y. K. Lee, J. Zhao, H. Zhang and W. Chen, Acetic acid and butyric acid released in large intestine play different roles in the alleviation of constipation, *J. Funct. Foods*, 2020, **69**, 103953.

77 P. D. Cani, Metabolism in 2013: The gut microbiota manages host metabolism, *Nat. Rev. Endocrinol.*, 2014, **10**, 74–76.

78 S. E. Erdman, V. P. Rao, T. Poutahidis, A. B. Rogers, C. L. Taylor, E. A. Jackson, Z. Ge, C. W. Lee, D. B. Schauer, G. N. Wogan, S. R. Tannenbaum and J. G. Fox, Nitric oxide and TNF-alpha trigger colonic inflammation and carcinogenesis in Helicobacter hepaticus-infected, Rag2-deficient mice, *Proc. Nat. Acad. Sci. U. S. A.*, 2009, **106**, 1027–1032.

79 Y. Yang, Z. Zhou, Y. Liu, X. Xu, Y. Xu, W. Zhou, S. Chen and J. Mao, Non-Alcoholic Components in Huangjiu as Potential Factors Regulating the Intestinal Barrier and Gut Microbiota in Mouse Model of Alcoholic Liver Injury, *Foods*, 2022, **11**, 1537.

80 A. H. Miller and C. L. Raison, The role of inflammation in depression: from evolutionary imperative to modern treatment target, *Nat. Rev. Immunol.*, 2016, **16**, 22–34.

81 A. K. Wang and B. J. Miller, Meta-analysis of Cerebrospinal Fluid Cytokine and Tryptophan Catabolite Alterations in Psychiatric Patients: Comparisons Between Schizophrenia, Bipolar Disorder, and Depression, *Schizophr. Bull.*, 2018, **44**, 75–83.

82 G. Goverse, M. Stakenborg and G. Matteoli, The intestinal cholinergic anti-inflammatory pathway, *J. Physiol.*, 2016, **594**, 5771–5780.

83 H. Liu, X. Zhang, P. Shi, J. Yuan, Q. Jia, C. Pi, T. Chen, L. Xiong, J. Chen, J. Tang, R. Yue, Z. Liu, H. Shen, Y. Zuo, Y. Wei and L. Zhao, α7 Nicotinic acetylcholine receptor: a key receptor in the cholinergic anti-inflammatory pathway exerting an antidepressant effect, *J. Neuroinflammation*, 2023, **20**, 84.

84 X. Lu, Q. Ce, L. Jin, J. Zheng, M. Sun, X. Tang, D. Li and J. Sun, Deoiled sunflower seeds ameliorate depression by promoting the production of monoamine neurotransmitters and inhibiting oxidative stress, *Food Funct.*, 2021, **12**, 573–586.

85 W. Qu, S. Liu, W. Zhang, H. Zhu, Q. Tao, H. Wang and H. Yan, Impact of traditional Chinese medicine treatment on chronic unpredictable mild stress-induced depression-like behaviors: intestinal microbiota and gut microbiome function, *Food Funct.*, 2019, **10**, 5886–5897.

86 Q. Yin, Y. Ma, Y. Hong, X. Hou, J. Chen, C. Shen, M. Sun, Y. Shang, S. Dong, Z. Zeng, J. J. Pei and X. Liu, Lycopene attenuates insulin signaling deficits, oxidative stress, neuroinflammation, and cognitive impairment in fructose-drinking insulin resistant rats, *Neuropharmacology*, 2014, **86**, 389–396.

87 M. Rao and M. D. Gershon, The bowel and beyond: the enteric nervous system in neurological disorders, *Nat. Rev. Gastroenterol. Hepatol.*, 2016, **13**, 517–528.