



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Lactiplantibacillus plantarum NCU0011129 ameliorated colorectal carcinogenesis by modulating gut microbiota and serum arachidonic acid metabolism in AOM/DSS-induced mice

Xiaoyan Xu^{a, b}, Shijin Xiong^{a, b}, Tao Huang^{a, b}, Tonghao Du^d, Mingwei Zhao^{a, b}, Huiyu Li^{a, b}, Jinqing Huang^c, Tao Xiong^{a, c*}, Mingyong Xie^{a, b}

^a State Key Laboratory of Food Science & Resources, Nanchang University, Jiangxi 330047, China

^b School of Food Science & Technology, Nanchang University, Jiangxi 330006, China

^c Jiangxi Medicine Academy of Nutrition and Health Management, The First Affiliated Hospital of Nanchang University, Jiangxi, 330209, China

^d Jiangxi Hypertension Research Institute, The First Affiliated Hospital of Nanchang University, No. 17 Yongwai Zhengjie Street, Nanchang 330006, China

^e The Institute of Agricultural Products Processing, Jiangxi Academy of Agricultural Sciences, No. 602 Nanlian Road, Nanchang 330200, China

ABSTRACT: Colorectal cancer (CRC), a highly prevalent and lethal gastrointestinal malignancy, has recently seen growing interest in probiotics for its prevention and treatment. This study evaluated the mitigating effects of *Lactiplantibacillus plantarum* (NCU0011098, NCUH001084, and NCU0011129) on AOM/DSS-induced CRC mice. Phenotypic indicators and histopathological analysis demonstrated that all three strains improved the survival rates of CRC mice, reduced colonic tumor burden and shortening of colon length, lowered inflammation and oxidative stress levels in the colon, and alleviated intestinal barrier damage. Among them, NCU0011129 demonstrated the most remarkable efficacy, with a 26.19% increase in survival rate compared to the CRC group (66.7%). This strain also improved colon shortening by 68.16%. Furthermore, amplicon sequencing revealed that *L. plantarum* NCU0011129 ameliorated gut dysbiosis by decreasing the relative abundance of *Desulfovibrio*, *Dubosiella*, and *Paramuribaculum*, while promoting beneficial genera (*Akkermansia*, *Muribaculum*, and *Alistipes*). Notably, *L. plantarum* NCU0011129 modulated the serum metabolic profiles of CRC mice, altered amino acid and lipid metabolism pathways, with arachidonic acid metabolism being the most significantly affected. Overall, *L. plantarum* NCU0011129 inhibited CRC progression by modulating gut microbiota and host metabolism, with its efficacy linked to the specific attenuation of the arachidonic acid-inflammatory axis. These findings offer a promising probiotic-based strategy for CRC intervention.

Keywords: Colorectal cancer, *Lactiplantibacillus plantarum*, Gut microbiota, Serum metabolomics

1. Introduction

Colorectal cancer (CRC) has become a major disease, seriously threatening human health worldwide. Its characteristics of high incidence and high mortality make it a key area in cancer prevention and control. According to the 2022 Global Cancer Statistics, CRC has approximately 1.93 million new cases and 860,000

*Corresponding author
xiongtao0907@163.com

Received 17 October 2025
Received in revised form 1 December 2025
Accepted 9 January 2026

deaths each year, making it the third most common malignant tumor worldwide¹. Over the past few decades, the incidence and mortality rates of early-onset CRC (that is, people under 50 years old) have shown a significant and sustained upward trend, highlighting the issue of its younger onset². In China, the incidence and mortality of CRC rank second and fourth, respectively, among malignant tumors¹. Studies have shown that the occurrence, development, and metastasis of CRC were closely related to chronic intestinal inflammation, dysbiosis of the intestinal microbiota, and host metabolic disorders^{3,4}. The homeostasis of the gut microbiota is a key indicator of host health, and CRC patients commonly exhibited characteristic dysbiosis, characterized by abnormally elevated abundance of opportunistic pathogens such as *Fusobacterium nucleatum* and enterotoxigenic *Bacteroides fragilis*⁵. These pathogens can synergistically promote the occurrence and development of CRC through multiple mechanisms, including inducing persistent chronic inflammation, disrupting the integrity of the intestinal epithelial barrier, interfering with local and systemic immune homeostasis, and exacerbating host metabolic abnormalities⁶. More importantly, accompanying the increased intestinal permeability commonly seen in CRC progression, bacteria, their components, and microbial-derived metabolites are translocated into the systemic circulation, thereby regulating the physiological functions of distal organs, triggering systemic inflammatory responses, and possibly affecting the tumor microenvironment⁷. Therefore, targeted regulation of the gut microbiota to restore its homeostasis is increasingly being regarded as a promising emerging strategy for the prevention and intervention of CRC.

Functional probiotics are showing increasing potential in disease prevention and treatment. Existing research has confirmed that specific probiotic strains have positive effects in the prevention and intervention of CRC. With advantages such as high relative safety, good tolerability, and wide availability, they have become an attractive strategy. The potential mechanisms by which probiotics combat CRC are multifaceted and operate in a coordinated manner. They primarily function by (1) competitively excluding pathogens to maintain a healthy microbial balance, (2) fortifying the intestinal epithelial barrier to prevent pathogenic translocation and inflammation, and (3) orchestrating both local and systemic immune responses to suppress tumorigenic processes, thereby collectively impeding the progression of CRC⁸⁻¹⁰. For example, *Lactobacillus acidophilus* has been proven to exert anticancer effects by reducing intestinal inflammation levels, inhibiting the formation of precancerous lesions, and inducing cancer cell apoptosis¹¹. *Lacticaseibacillus rhamnosus* can upregulate the expression of tight junction proteins, promote the repair of intestinal epithelial injury, and thus significantly improve intestinal barrier function¹². *Limosilactobacillus fermentum* GR-3 can effectively regulate the structure of gut microbiota and reduce the level of intestinal oxidative stress to exert a preventive effect on CRC¹³. In addition, the physiological effects mediated by probiotics are partly attributed to their regulation of gut microbiota metabolites, especially short-chain fatty acids (SCFAs), such as butyrate, propionate, and acetate. SCFAs can reduce the risk of CRC through multiple mechanisms, such as inhibiting the proliferation of pathogens by lowering the intestinal pH, suppressing the proliferation and migration of cancer cells, alleviating the inflammatory response in the colon, and regulating the functions of immune cells^{7,14}. These

findings collectively reveal the multifaceted roles of probiotics and their metabolites in the management of colorectal cancer.

Metabolic reprogramming is one of the core hallmark features of cancer. As a direct reflection of the body's systemic metabolic state, the serum metabolome has been proven to be able to sensitively capture key biochemical changes in the occurrence and development of cancer, including lipid metabolism and amino acid metabolism disorders, etc¹⁵. In CRC, abnormalities of serum metabolites not only serve as markers of disease progression but also act as key functional mediators, directly participating in regulating tumor cell signal transduction, driving energy metabolism reprogramming, and regulating immune responses and other core biological processes¹⁶. Notably, the gut microbiota can influence the occurrence and development of CRC by reshaping the host serum metabolome. The core of this effect lies in the fact that the metabolites derived from the microbiota or regulated by the microbiota can act on the distal intestinal mucosa and regulate the systemic immune function through the blood circulation system, thus constructing a multi-dimensional interaction network of 'microbiota-metabolites-host'¹⁷⁻¹⁸. Therefore, analyzing the dynamic changes of the serum metabolome becomes the core intermediate link in revealing the association mechanism between gut microbiota and intestinal cancer.

L. plantarum NCU0011098, *L. plantarum* NCUH001084, and *L. plantarum* NCU0011129, with potential anti-colorectal cancer activity *in vitro* were screened in our previous research¹⁹. However, the anti-tumor activity and mechanisms *in vivo* of the strains remain unclear. Therefore, the present study aims to evaluate the effect of different *L. plantarum* on (1) pathological progression, (2) gut microbiota structure and diversity, and (3) the serum metabolite profiles of AOM/DSS-induced CRC model mice, and revealed its potential "microbiota-metabolism" regulatory mechanism by multi-omics analysis. The study offered a new insight into the role of probiotic-mediated intestinal microbiota regulation in CRC intervention and a novel CRC prevention and treatment strategy based on targeted microbiota and metabolic reprogramming.

2. Materials and methods

2.1. Reagents and standards

Azoxymethane (AOM) was purchased from Sigma (St. Louis, MO, USA), dextran sodium sulfate (DSS, 36-50 kDa) was obtained from MP Biomedical (Santa. Ana, CA, USA). The enzyme-linked immunosorbent assay ELISA kit (TNF- α , IL-6, IL-1 β , IL-4, and IL-10) was purchased from Elabscience Biotechnology (Wuhan, Hubei, China). Methanol, formic acid, and acetonitrile were purchased from Fisher Scientific (New Jersey, USA).

2.2 Bacteria preparation

Lyophilized probiotic powder containing three strains of *L. plantarum* (NCU0011098, NCUH001084, and NCU0011129) was obtained from the library of plant-based fermentation probiotics of the State Key Laboratory of Food Science and Resources (Nanchang, Jiangxi, China) and stored at 4 °C. The 16S rRNA

gene sequences of the three strains (Table S1) have been submitted to GenBank by our group, and the corresponding accession numbers are PQ416012, PQ416013, and PQ416017 for strains NCU0011098, NCU0011129, and NCUH001084, respectively. The strains were cultured and activated in DeMan, Rogosa & Sharpe (MRS) broth at 37 °C for 24 h. After centrifugation ($6500 \times g$, 4 °C, 10 min), the pellet was collected and washed twice with sterile phosphate-buffered saline buffer (PBS, 0.1 mol/L, pH 7.2), and then concentrated to 10^{10} CFU/mL using the same solution for further use.

2.3 Animals and experimental design

All experiment procedures were approved by the Institutional Animal Care and Use Committee of Nanchang University [SYXK (Gan) 2021-0004], and the protocols were conducted under the Guidelines for the Ethical Review of Laboratory Animal Welfare of the Chinese National Standard²⁰. All specific-pathogen-free (SPF) grade C57BL/6J male mice (6 weeks) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and housed in a pathogen-free mouse colony in a standard environment (12 h light/dark cycle, humidity $50\% \pm 10\%$; temperature, $23\text{ °C} \pm 2\text{ °C}$). The animal experimental protocol is shown in Fig.1A, seventy-two mice were evenly divided into six groups ($n=12$). After a one-week acclimatization period, all mice, except for the CTL group (which received i.p. saline), were given a single i.p. injection of AOM (10 mg/kg) and underwent three rounds of DSS exposure to establish an AOM/DSS-induced CRC model. Each round involved 7 consecutive days of drinking water containing 2.5% DSS, followed by 14 days of normal drinking water. Throughout the entire experimental period, all animals had unrestricted access to food. Post-AOM injection, mice were gavaged daily according to their group assignment: the CRC group received sterile water; the 5-ASA group received 5-aminosalicylic acid (5-ASA, 75 mg/kg); and the probiotic groups received a daily gavage of 200 μ L bacterial suspension (prepared as described in section 2.2 above) containing different *L. plantarum* strains at a dose of 10^{11} CFU/kg body weight per day (approximately 10^9 CFU per mouse). The CTL group received a single i.p. injection of saline along with daily oral gavage of sterile water. The body weight of the mice was measured twice a week, and the survival rate of each group of mice was monitored. After the experiment, mice were sacrificed through cervical dislocation after collecting the blood from the eyeballs. Before sacrifice, mice underwent a 12 h fasting period. The liver, spleen, kidney, colon, and feces of mice were collected and stored at -80 °C for a maximum of six months. After data collection, post-hoc power analysis using observed effect sizes confirmed powers > 0.8 for all primary outcomes.

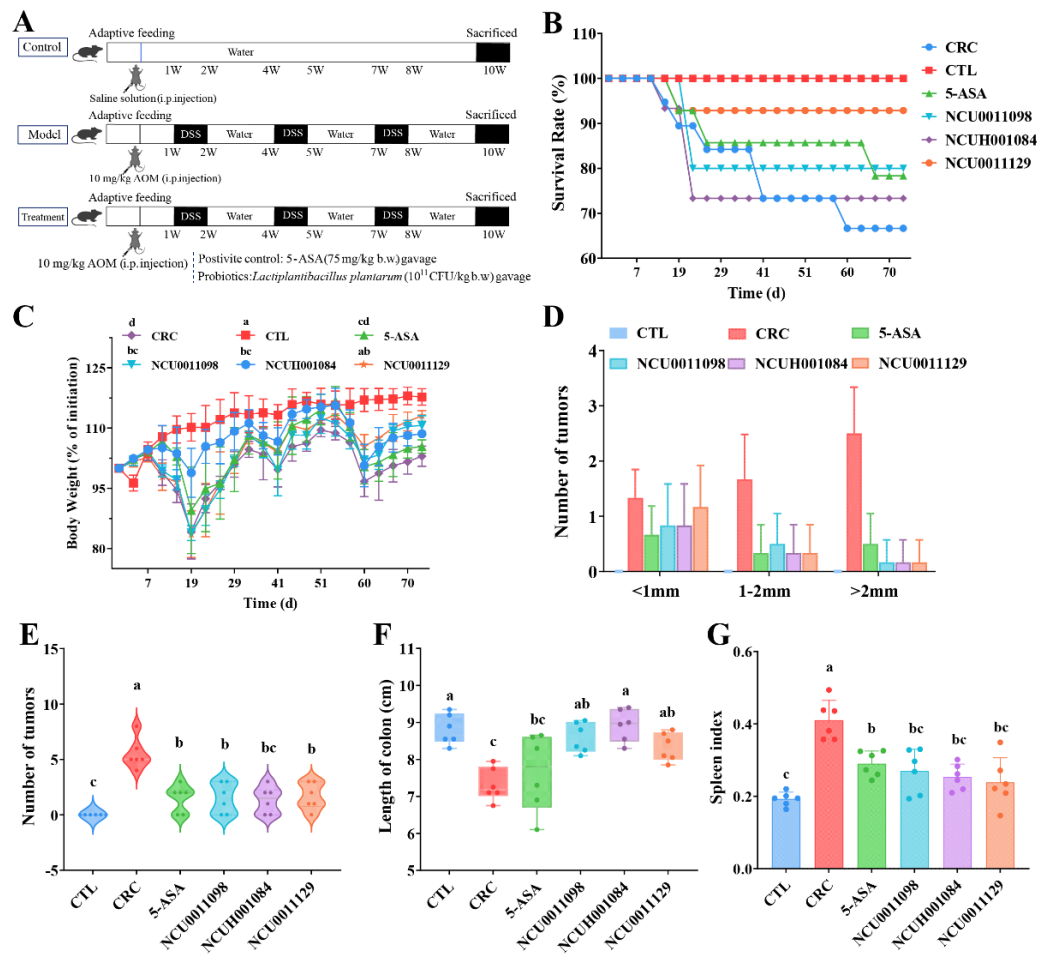


Fig. 1. The effects of *L. plantarum* on basic physiological parameters of AOM/DSS-induced CRC mice. (A) Schematic diagram of animal experiment design. (B) Change in survival rate. (C) Percentage change in weight. (D) Distribution of colon tumor diameter sizes. (E) Number of colon tumors. (F) Colon length. (G) Spleen index. Data were expressed as mean $\pm$ SEM ($n = 6$). Different lowercase letters mean statistically significant differences ($P < 0.05$). AOM/DSS, azoxymethane/dextran sodium sulfate. CTL, control. CRC, colorectal cancer. 5-ASA, Aminosalicic acid. i.p. injection, intraperitoneal injection.

2.4 Flow cytometry (FCM)

The spleen was processed according to a previous study²¹. Briefly, the spleen cells were subjected to grinding using RPMI 1640 medium containing 2% fetal bovine serum, followed by the addition of a red blood cell lysis solution. The mixture should be thoroughly agitated and then permitted to stand for a period of five minutes. The samples were then subjected to a centrifugal process ($400 \times g$, 4°C , 6 min), after which the cells were resuspended to achieve a concentration of 1×10^7 cells/mL. Fluorescently labeled antibodies were utilized to stain splenic cells, including the Zombie Aqua Fixable Viability Kit, FITC anti-mouse CD3, PE/Cyanine7 anti-mouse CD4, and PerCP/Cyanine5.5 anti-mouse CD8a. To remove non-specific binding of fluorescently labeled antibodies to Fc receptors, purified anti-mouse CD16/32 was used to block Fc receptors.

2.5 Histopathological assays and immunohistochemistry (IHC)

Formalin-fixed fresh colon samples were prepared using 4% formalin, dehydrated using an ethanol gradient, embedded in paraffin, stained with haematoxylin-eosin (HE), and observed using an Olympus BX41

microscope (OLYMPUS, Tokyo, Japan). Using the previous method, colon tissue samples were examined and scored according to the severity of inflammation²².

For IHC staining, paraffin sections were processed according to the previous method and incubated with the zonula Occludens-1 (ZO-1) antibody²³. The DAB horseradish peroxidase color development kit (Sangon Biotechnology Co., Ltd., Shanghai) was used for the chromogenic reaction. Analysis and quantification were performed using a fluorescence microscope (Axio Observer Z1, Zeiss, Oberkochen, Baden-Württemberg, Germany) and Image J v1.8.0 software (Bethesda, MD, USA). The results were expressed as integrated option density (IOD).

2.6 Real-Time Quantitative Polymerase Chain Reaction

Total RNA was extracted from colon tissue using an RNA extraction kit (Solarbio, Beijing, China), and reverse transcription was performed on the total RNA using a cDNA reverse transcription kit (Takara, Biomedicals, Kusatsu, Japan). RT-qPCR was performed using SYBR Green PCR Master Mix, and the relative expression levels of each gene were calculated using the $2^{-\Delta\Delta CT}$ method. The primer sequences used in this experiment were provided in Table S2, with β -actin as the internal reference.

2.7 Determination of oxidative stress in colon and liver tissue

Frozen colon (liver) tissue was placed on ice to thaw, and 0.1 g of the tissue sample was accurately weighed. Nine times the volume of normal saline solution was added, and the sample was homogenized in an ice bath. The homogenized sample was then centrifuged (4 °C, 3500 × g, 10 min), and the supernatant was collected. The levels of glutathione peroxidase (GSH-Px, E-BC-K096-M), total superoxide dismutase (T-SOD, E-BC-K020-M), and malondialdehyde (MDA, E-BC-K025-M) in the supernatant were measured according to the instructions of the corresponding ELISA kit.

2.8 Detection of cytokine levels in colon tissue and serum

Following the method described in Section 2.7, the colon tissue was homogenized and centrifuged (4 °C, 3500 × g, 10 min). Then, the supernatant was collected, and the total protein concentration of each colon and serum sample was quantified using the BCA protein concentration assay kit. The levels of interleukin-6 (IL-6, E-EL-M0044), tumor necrosis factor-alpha (TNF- α , E-EL-M3036), interleukin-1 β (IL-1 β , E-EL-M0037), interleukin-4 (IL-4, E-HSEL-M0002), and interleukin-10 (IL-10, E-EL-M0046) were detected using the enzyme-linked immunosorbent assay (ELISA) kits (Elabscience Biotechnology Co., Ltd.). All procedures were strictly performed according to the instructions provided in each kit manual.

2.9 Analysis of short-chain fatty acids

SCFA levels in the feces of each sample were measured using GC-Q-TOF/MS (7890B GC, 7250 Q-TOF/MS; Agilent Technologies, CA, USA). The specific steps were as follows: Fecal samples (0.1 g) were dissolved in distilled water at a ratio of 1:9 (w/v), and 2% sulfuric acid (20 μ L) and 1 mL ether (50%, v/v)

were added. After thorough mixing, 1 mmol/L 2-ethylbutyric acid was added as an internal standard. The mixture was sonicated at 4 °C for 15 min and then left to stand at room temperature for 20 min. After centrifuging ($12000 \times g$, 4 °C, 15 min), the upper organic phase was filtered through a membrane filter (0.22 μm , Polytetrafluoroethylene (PTFE), Merck Millipore, Darmstadt, Germany) and analyzed by GC-Q-TOF/MS. The temperature program and instrument parameters were detailed in Table S3. The concentration of SCFAs in each sample was determined by establishing a standard curve of the corresponding SCFA.

2.10 Characterization of gut microbiota via 16S rRNA sequencing

Gut microbiota was analyzed by 16S rRNA gene high-throughput sequencing according to a previous study²³. The OMEGA DNA isolation kit (Omega, Bio-tek, Norcross, GA, USA) was used to extract the DNA of the fecal sample. The concentration and purity of extracted bacterial DNA were determined by NanoDrop 2000 (ThermoFisher, Waltham, USA) and 1% agarose gel electrophoresis. Using the extracted DNA as a template, the V3-V4 region of the 16S rRNA gene was amplified using universal primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The amplified products were purified by 2% agarose gel electrophoresis and recovered. DNA Illumina MiSeq sequencing analysis of the DNA sample was conducted by Personal Biotechnology Co., Ltd (Shanghai, China). Alpha diversity indices (Chao1, Observed_species, Shannon, and Simpson indices) and Beta diversity indices (Bray-Curtis) were calculated using Quantitative Insights Into Microbial Ecology 2 (QIIME 2, Flagstaff, USA). Intergroup differences in microbial community structure were compared using principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) with PERMANOVA and ANOSIM, respectively. Microbial biomarkers with significant intergroup differences were screened using Linear Discriminant Analysis Effect Size (LEfSe) (LDA > 3.0).

2.11 Serum metabolome analysis

Serum metabolite extraction was performed according to the method of Zeng et al., with slight modifications²⁴. Serum samples were mixed with ice-cold methanol at a ratio of 4:1 (v/v), sonicated at 50 kHz for 30 min at 4 °C, and then the samples were placed at -20 °C for 20 min to precipitate proteins. After centrifugation ($13000 \times g$, 4 °C, 15 min), the supernatant was filtered through a 0.22 μm membrane and transferred to a sample vial for non-targeted metabolomics based on UPLC-QTOF/MS (Agilent Technologies). The elution procedures and instrument parameters were detailed in Table S4.

The raw files obtained were imported into the Agilent MassHunter workstation for peak extraction and peak alignment processing, and the data were normalized. After data preprocessing, the data were imported into SIMCA14.1 software for principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). The compounds with VIP > 1.0 and $P < 0.05$ were considered differential metabolites. The secondary data collected from Target MS/MS and Auto MS/MS were compared with the METLIN database (<http://metlin.scripps.edu>) and the Human Metabolome Database HMDB database

(<http://www.hmdb.ca/>). MetaboAnalyst 5.0 (<http://www.metaboanalyst.ca/>) was used to perform enrichment analysis and metabolic pathway analysis of the differential metabolites.

2.12 Statistical analysis

The statistical analysis was conducted by IBM SPSS Statistics (version 19.0, SPSS Inc., Chicago, IL, USA) and visualized via GraphPad Prism 9.0 (GraphPad Software Inc., Boston, MA, USA). The data were presented as mean $\pm$ standard deviation. Between-group differences were assessed using one-way analysis of variance (ANOVA), with post-hoc multiple comparisons performed using Tukey's test. The microbial co-occurrence network was constructed by R (v3.6.3) based on the SparCC algorithm and visualized in Gephi (Version 0.10.1, Web Atlas, Paris, France). The Spearman correlation coefficients for different categories of data (gut microbiota, serum metabolism, and CRC-related indicators) were calculated using R and visualized using Cytoscape 3.9.1 software. All statistical tests were performed at a significance level of $P < 0.05$.

3. Results and discussion

3.1. *L. plantarum* ameliorated the AOM/DSS-induced mortality, colon tumorigenesis, and colon shortening

The antitumor effect of different *L. plantarum* was investigated using the AOM/DSS-induced CRC mouse model, a common model of colitis-associated colon cancer²⁵. As shown in Fig. 1B, AOM/DSS-induced CRC gradually reduced the survival rate of mice, and by the end of the experiment, the survival rate of CRC mice was 66.7%. All three strains of *L. plantarum* were able to reduce the mortality rate of CRC mice to a certain extent, with the survival rate of mice ingesting *L. plantarum* NCU0011129 increased by 26.19% compared to the CRC group. The body weight of the mice decreased with the intake of DSS and gradually increased during the 2-week recovery period, *L. plantarum* effectively alleviated the weight loss in CRC mice (Fig. 1C). In terms of the number and distribution of colon tumors, the number of tumors in CRC mice was 4–10 and most of them were larger than 2 mm in diameter, whereas the number of tumors in *L. plantarum*-intervened mice was significantly reduced ($P < 0.05$) and the size of the diameter was suppressed (Fig. 1D, E). Shortened colon length in mice is a common and important morphological indicator of disease status and severity in CRC research, and measurements of colon length in mice are commonly used to assess CRC progression²⁶. The results showed that the colon length of CRC mice was significantly reduced compared to the CTL group, while the colon length of mice ingesting *L. plantarum* was not significantly different from that of the CTL group (Fig. 1F, Fig. S1). Therefore, the three strains of *L. plantarum* significantly ameliorated CRC-related phenotypes, including reduced mortality in CRC mice, alleviated the weight loss, reduced the number and diameter of colon tumors in mice, and prevented the shortening of colon length.

3.2 *L. plantarum* attenuated systemic inflammation and immune dysregulation

Organ index (organ weight/body weight) can reflect the developmental status, physiological function, or pathological changes of organs. We calculated organ indices of spleen, liver, and kidney to assess the immune status and the effect of *L. plantarum* intake on the metabolic burden of the body in CRC mice and showed that

there was no significant difference in the kidney index among the groups (Fig. S2A). The liver index results showed that CRC mice had elevated liver indexes relative to the CTL group, while all treatment groups were able to alleviate the abnormal elevation of liver indexes (Fig. S2B). We measured oxidative stress levels in the liver, including GSH-Px, T-SOD, and MDA. The T-SOD and GSH-Px activities in CRC mice were significantly lower than those in the CTL group, while MDA levels were significantly higher. Therefore, the increase in liver indices in CRC mice may be related to the level of oxidative stress in the liver (Fig. S3). In addition, oxidative stress has been identified as a potential catalyst for liver cell damage and dysfunction, thereby exacerbating the development of liver metastasis in CRC²⁷. Furthermore, we found that the spleens of CRC mice appeared to be enlarged, and the splenic index was increased compared to the CTL group (Fig. 1G). The spleen is the largest immune organ, and spleen enlargement suggests that CRC may be a collective occurrence of tumour-associated immune stress accompanied by a systemic inflammatory response. Ingestion of *L. plantarum* and 5-ASA attenuated splenomegaly and mitigated the abnormal elevation of spleen index in CRC mice compared with the CTL group. Flow cytometric typing of splenocytes showed that CD4⁺T cells were significantly increased and CD8⁺ T cells were significantly decreased in the spleens of CRC mice, resulting in a significant elevation of CD4⁺/CD8⁺, which belongs to the CD4⁺ T-cell-dominant immune imbalance (Fig. 2A-D). On the other hand, the absence of CD8⁺ T cells may result in the body lacking anti-tumor immune function and being insensitive to tumor growth²⁸. This suggested that with the development of CRC, the systemic immune function of mice was impaired. After continuous intake of *L. plantarum* in mice, the CD4⁺/CD8⁺ T-lymphocyte ratio was corrected, and the balance of the immune system was normalized (Fig. 2D). Thus, the administration of *L. plantarum* promoted the body to exert anti-tumor effects and positively influenced the immune system's ability.

3.3 The protective effect of *L. plantarum* on the colon tissue structure of CRC mice

To assess the protective effect of *L. plantarum* on the structural integrity of colon tissue in mice with CRC, we performed HE staining on the colon tissue. The results showed that the colon structure of AOM/DSS-induced mice underwent significant changes, primarily manifested as a reduction in crypt number, shortened crypt length, atrophy, upward displacement, significantly widened crypt spacing, the crypt base no longer adhering to the mucosal muscular layer, and a decrease or disappearance of goblet cells (Fig. 2E, G). The field of view revealed submucosal connective tissue proliferation accompanied by diffuse inflammatory cell infiltration, and even the presence of colon tumors (Fig. 2E, F). As a preventive measure against inflammatory carcinogenesis models, 5-ASA effectively exerts anti-inflammatory effects, alleviating extensive inflammatory infiltration in the colon. Research indicated that 5-ASA can inhibit the growth of several CRC cell lines by interfering with the cell cycle, and is commonly used to prevent the development of inflammation-related CRC²⁹. Different *L. plantarum* significantly ameliorated CRC-associated histopathological alterations, including goblet cells depletion and crypt architectural distortion, restoring colonic morphology to near-normal levels (Fig. 2E-H). Among these, *L. plantarum* NCU0011129 demonstrated the most pronounced protective effect on colonic tissue structural integrity in CRC mice, exhibiting the lowest pathological score

and the highest number of crypt goblet cells. Therefore, *L. plantarum* NCU0011098, *L. plantarum* NCUH001084, and *L. plantarum* NCU0011129 demonstrated good protective effects on the colon tissue structure of colon cancer mice, alleviated inflammatory infiltration in the colon region of CRC mice to some extent, and prevented further epithelial lesions in the colon.

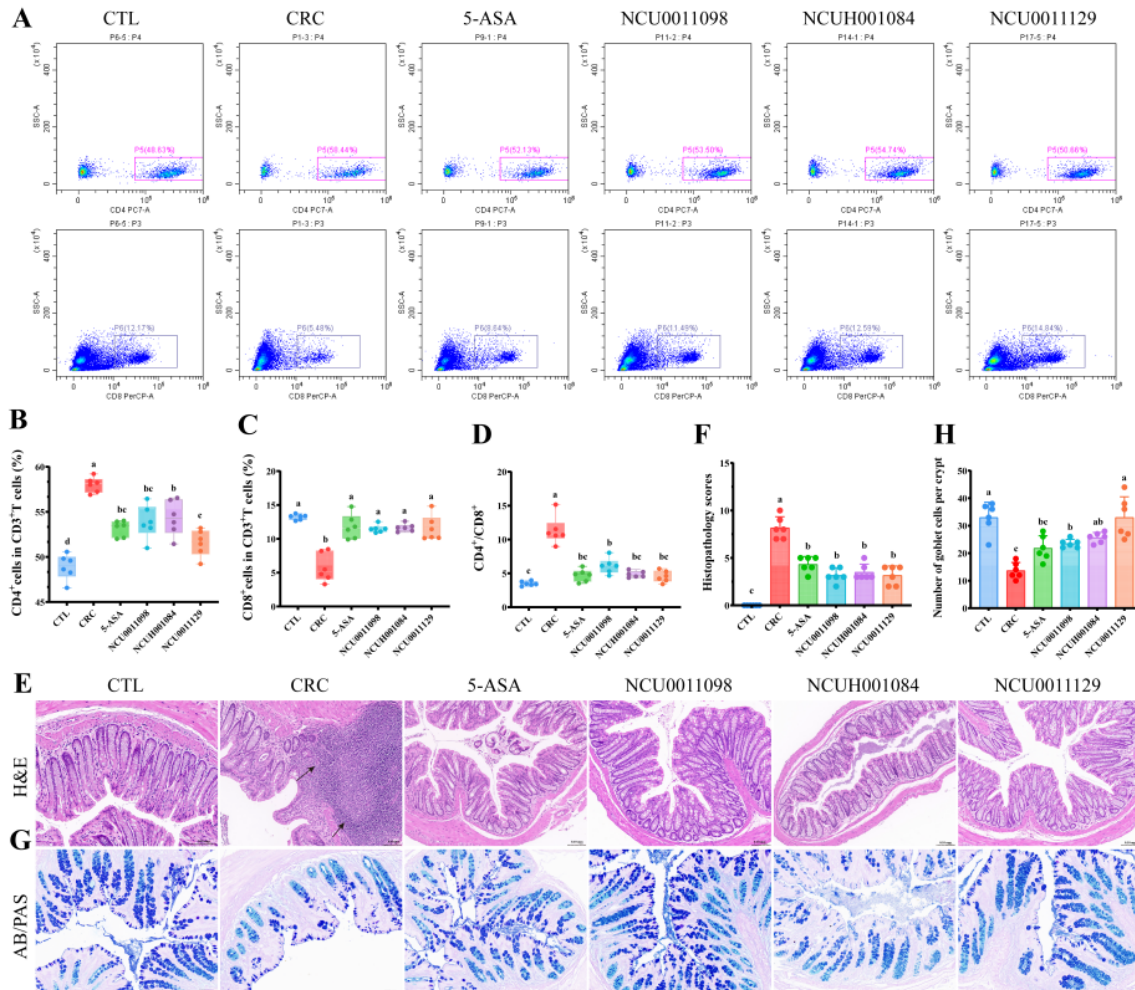


Fig. 2. The effect of *L. plantarum* on immune balance and colon tissue damage in mice with CRC. (A) Flow cytometric analysis of CD4⁺ and CD8⁺ cells. (B) Flow cytometry analysis of CD4⁺ T lymphocytes in the spleen. (C) Flow cytometry analysis of CD8⁺ T lymphocytes in the spleen. (D) Flow cytometric analysis of CD4⁺/CD8⁺ T lymphocytes in the spleen.

(E) Pathological changes of colon tissues by H&E (Hematoxylin and Eosin) staining (300× magnification). (F) Histopathology scores. (G) Visualization of the number of goblet cells per crypt by AB/PAS (Alcian Blue/Periodic Acid-Schiff) staining. (H) Number of goblet cells per crypt. Data were expressed as mean ± SEM (n = 6). Different lowercase letters mean statistically significant differences ($P < 0.05$). CTL, control. CRC, colorectal cancer. 5-ASA, Aminosalicic acid.

3.4 *L. plantarum* mitigated intestinal barrier damage and oxidative stress induced by AOM/DSS

In the AOM/DSS-induced intestinal inflammation-carcinogenesis model, oxidative stress-mediated intestinal epithelial cell damage and barrier dysfunction are key factors driving disease progression. Immunohistochemical results showed that AOM/DSS induction led to a decrease in ZO-1 protein levels in colon tissue, suggesting that colonic barrier integrity may be compromised (Fig. 3A, B). In addition, the relative expression levels of claudin-1 and occludin mRNA in CRC mice were significantly lower than those in the CTL group (Fig. 3C, D). ZO-1, claudin-1, and occludin are key proteins that constitute tight junctions and are essential for maintaining intestinal permeability³⁰. The absence of tight junction proteins in CRC mice may promote increased intestinal permeability, bacterial translocation, and impaired intestinal barrier

function^{31–33}. The results indicated that different *L. plantarum* intervention can increase ZO-1 protein expression in CRC colon, with the highest ZO-1 expression observed in the *L. plantarum* NCU0011129 group. Furthermore, all three strains significantly reversed the decrease in Claudin-1 and Occludin mRNA levels, with *L. plantarum* NCU0011098 and *L. plantarum* NCU0011129 showing the most excellent effects. The protective effect of 5-ASA on colon-tight junction proteins was similar to that of *L. plantarum* NCUH001084.

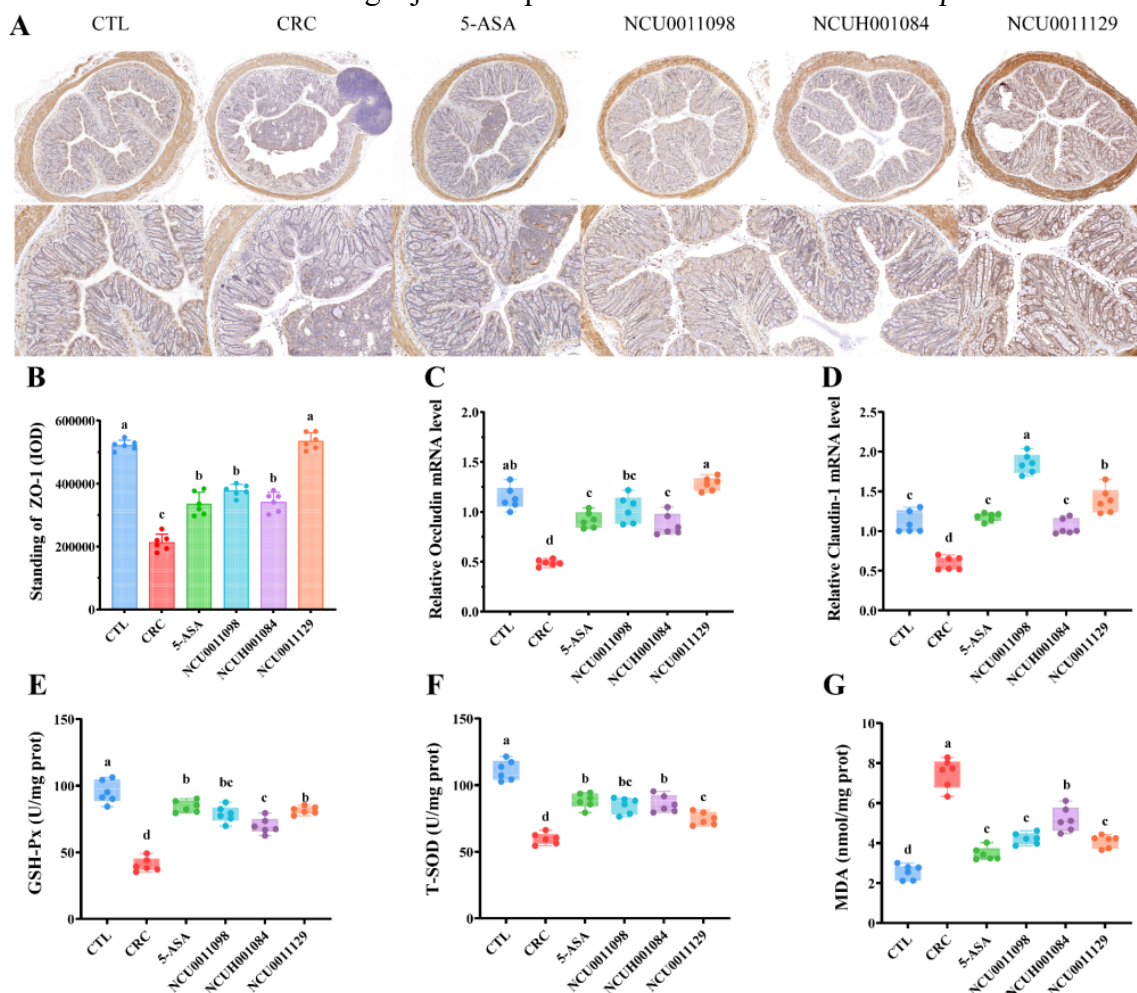


Fig. 3. The effect of *L. plantarum* on colon barrier proteins and oxidative stress levels. (A) Staining for Zonula Occludens-1 (ZO-1) in colon tissues was tested by immunohistochemistry (IHC). (B) Expression of ZO-1 in the colon with IHC staining. mRNA quantification of Occludin (C) and Claudin-1 (D). (E) Glutathione peroxidase (GSH-Px) concentration. (F) Total Superoxide Dismutase (T-SOD) concentration. (G) Malondialdehyde (MAD) concentration. Data were expressed as mean $\pm$ SEM (n = 6). Different lowercase letters mean statistically significant differences ($P < 0.05$). CTL, control. CRC, colorectal cancer. 5-ASA, Aminosalicic acid.

Increasing evidence suggests that intestinal barrier damage is closely related to oxidative stress³⁴. AOM/DSS-induced intestinal oxidative stress disrupts tight junctions between epithelial cells in colon tissue, leading to intestinal epithelial barrier dysfunction³⁴. We measured the activity of T-SOD and GSH-Px and the MDA content in mouse colon tissue. The results showed that AOM/DSS-induced CRC mice exhibited significant oxidative stress, manifested as a decrease in total antioxidant capacity (T-SOD and GSH-Px) and an increase in the lipid peroxidation product MDA (Fig. 3E-G). However, oral administration of *L. plantarum* significantly increased T-SOD and GSH-Px activity in the colon tissue of CRC mice while reducing MDA levels. Overall, the three strains of *L. plantarum* can alleviate colonic barrier damage and reduce oxidative stress levels in CRC mice, thereby suppressing the progression of CRC.

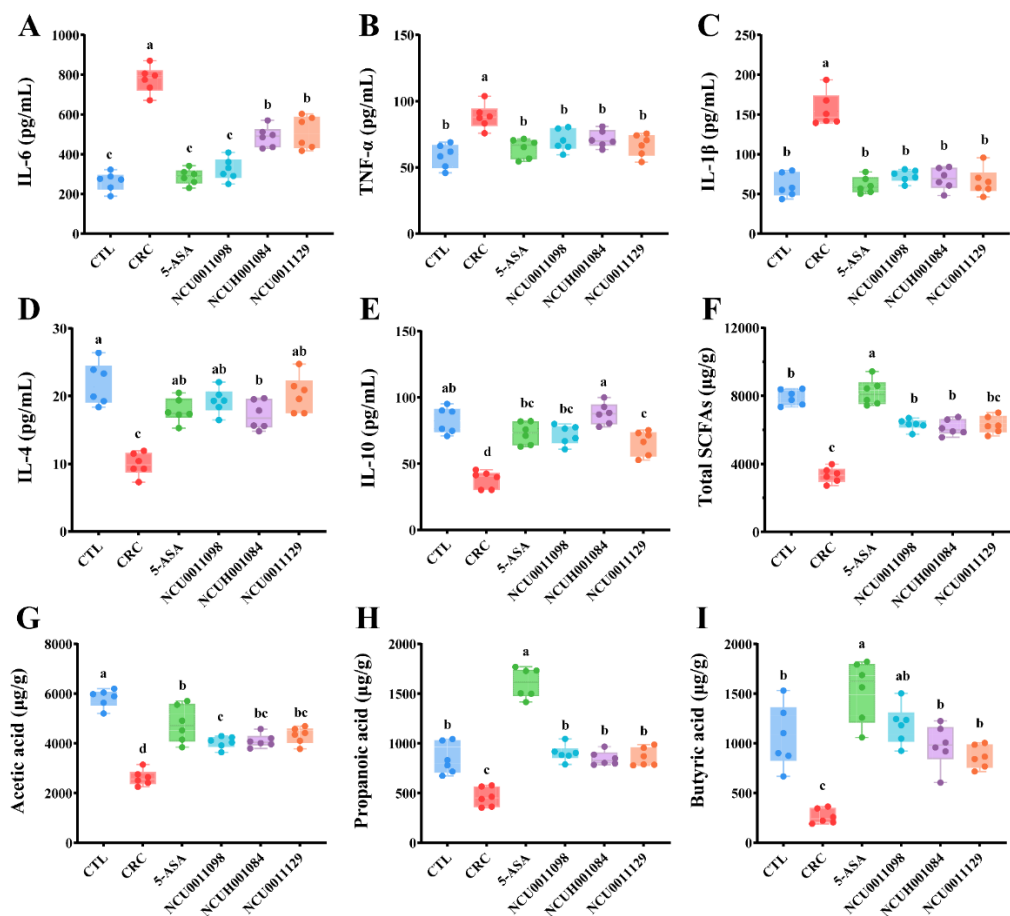
3.5 *L. plantarum* reduced inflammation levels in the colon and serum of CRC mice

Chronic inflammation is closely and complexly associated with the development of CRC, playing a key role in the initiation, progression, and metastasis of CRC. Therefore, regulating the inflammatory microenvironment of the colon is an important strategy for preventing CRC³³. Compared with the CTL group, AOM/DSS significantly upregulated the expression levels of proinflammatory cytokines IL-6, TNF- α , and IL-1 β in colon tissue (Fig. 4A-C) and significantly downregulated the expression of the anti-inflammatory cytokines IL-4 and IL-10 (Fig. 4D, E). This was consistent with the mucosal inflammatory pathological changes observed in the HE staining of CRC mice (Fig. 2E), which indicated that the inflammatory response may be correlated with the CRC progression. Notably, intervention with *L. plantarum* NCU0011098 significantly decreased the levels of pro-inflammatory cytokines (IL-6, TNF- α , and IL-1 β) and promoted the expression of anti-inflammatory cytokines IL-4 and IL-10. The strains *L. plantarum* NCUH001084 and NCU0011129 exhibited similar effects. Chronic stress-induced pro-inflammatory cytokines such as IL-6 and TNF- α in the intestines of CRC mice are key factors in CRC progression; they can promote cancer development by activating the inflammatory pathway, while the anti-inflammatory factor IL-10 maintains mucosal homeostasis and protects intestinal epithelial integrity³⁵. In addition, the levels of pro-inflammatory factors in the serum of CRC mice increased, while the levels of anti-inflammatory factors decreased (Fig. S4). The imbalance of pro-inflammatory and anti-inflammatory factors in the serum may be accompanied by a systemic inflammatory response, further leading to abnormal serum metabolism. In summary, *L. plantarum* alleviated colonic tissue and systemic inflammatory cell infiltration in CRC mice by regulating the cytokine network (inhibiting proinflammatory factors and restoring anti-inflammatory factors). Therefore, targeting the colonic inflammatory microenvironment and normalizing its inflammatory levels represents a potentially effective strategy for intervening in CRC associated with colitis.

3.6 *L. plantarum* promoted SCFA production in CRC mice

SCFAs play an important role in maintaining intestinal epithelial integrity and gut homeostasis, inhibiting inflammatory cascades, and regulating energy metabolism³⁶. The SCFA contents in the colon of different treated groups were quantified via GC-Q-TOF/MS. The results showed that, compared with the CTL group, the levels of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and total SCFAs in the feces of CRC model mice were significantly decreased (Fig. 4F-L). Notably, compared with the CRC group, the total SCFA content increased by 2.46-fold, 0.77-fold, 0.97-fold, and 1.03-fold, respectively, after intervention with 5-ASA, *L. plantarum* NCU0011098, *L. plantarum* NCUH001084, and *L. plantarum* NCU0011129 (Fig. 4F). Acetic acid and butyric acid were the main components of SCFAs, accounting for 90% of the total SCFAs³⁷. Among them, acetic acid, as the most abundant SCFA in feces, is mainly produced by the fermentation of bacteria such as *Bacteroides*. In addition to regulating the intestinal pH value, inhibiting the colonization of harmful bacteria, and maintaining the ecological balance of the gut microbiota, it also exerts anti-cancer effects by suppressing the expression of inflammatory factors³⁸⁻³⁹. Compared to the CRC group, the acetic acid levels in the 5-ASA, NCU0011098, NCUH001084, and NCU0011129 groups

significantly increased by 0.78-5.79 times, although there was no significant difference in levels among these four groups (Fig. 4G). The propionic acid levels increased by 0.55-3.27 times (Fig. 4H), and propionic acid has been confirmed to be associated with inhibiting the proliferation of CRC cells and inducing cancer cell apoptosis⁴⁰. Butyric acid, the most extensively studied SCFA, serves as the primary energy substrate for colonic epithelial cells and maintains intestinal barrier function by regulating tight junction protein expression, while also inhibiting pro-inflammatory pathways and carcinogenic signal transduction⁴¹. It is worth noting that butyric acid exhibited bidirectional regulation of colonic cell proliferation, inhibiting cancer cells' growth through distinct metabolic pathways while promoting the proliferation of normal colonic cells⁴². In this study, 5-ASA and three strains of *L. plantarum* significantly increased the levels of butyric acid and isobutyric acid in the feces of CRC mice, which was of great significance for blocking the progression of CRC (Fig. 4I, J). In addition, valeric acid, as a secondary energy substrate for colonic epithelial cells (with an energy efficiency of approximately 50% that of butyric acid), can provide energy through mitochondrial β -oxidation to maintain the homeostasis of epithelial cells⁴³. The contents of valeric acid and isovaleric acid also increased significantly after the intervention of *L. plantarum* (Fig. 4K, L). In summary, the three strains of *L. plantarum* exerted a positive regulatory effect on SCFAs metabolism in the intestines of CRC mice, while 5-ASA demonstrated the optimal efficacy in restoring the levels of six SCFAs due to its superior anti-inflammatory properties, providing experimental evidence for targeting intestinal metabolism to alleviate CRC.



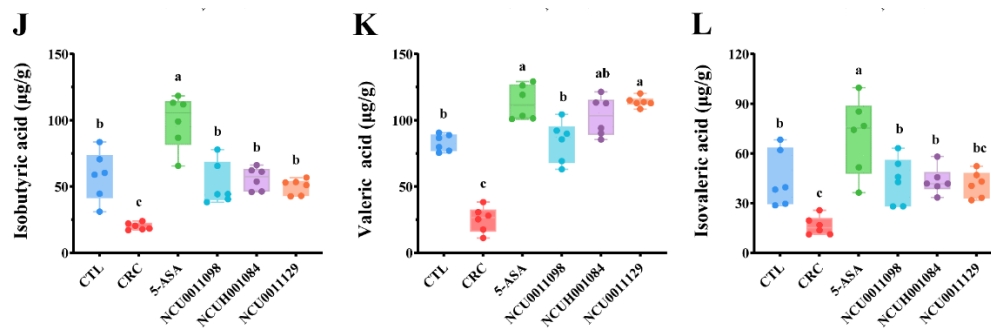
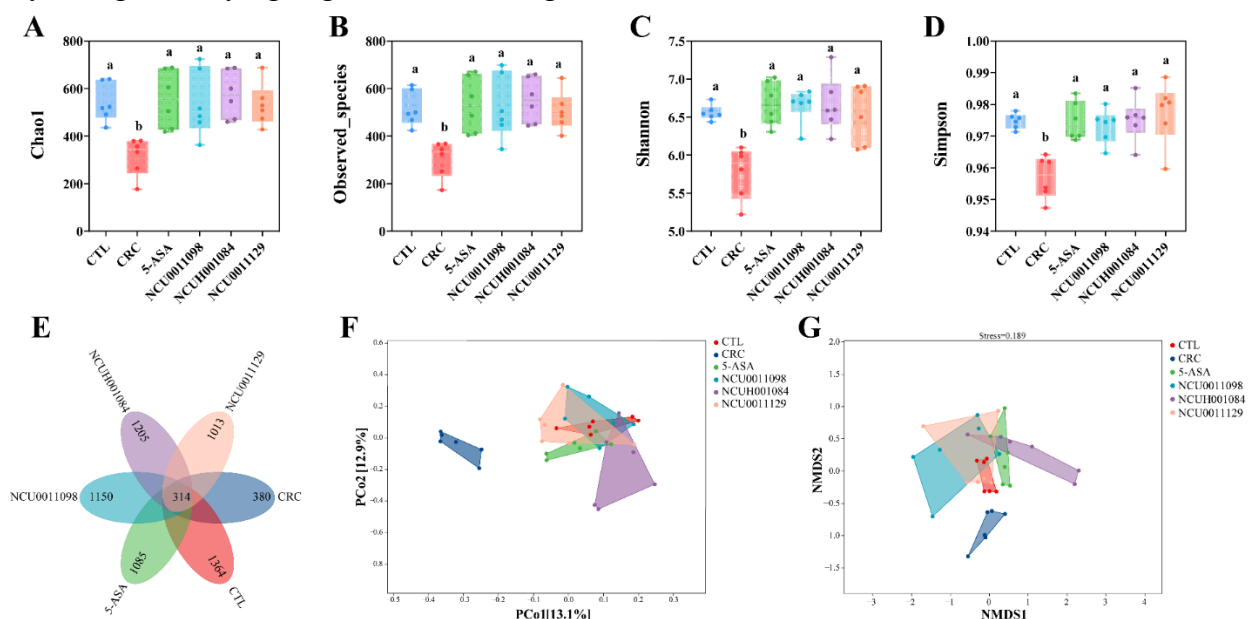


Fig. 4. The effect of *L. plantarum* on the levels of inflammatory factors and short-chain fatty acid (SCFA) content in the colon tissue. (A) Interleukin-6 (IL-6) levels. (B) Tumor necrosis factor- α (TNF- α) levels. (C) Interleukin-1 β (IL-1 β) levels. (D) Interleukin-4 (IL-4) levels. (E) Interleukin-10 (IL-10) levels. (F) Total SCFAs. (G) Acetic acid. (H) Propanoic acid. (I) Butyric acid. (J) Isobutyric acid. (K) Valeric acid. (L) Isovaleric acid. Data were expressed as mean $\pm$ SEM (n = 6). Different lowercase letters mean statistically significant differences ($P < 0.05$). CTL, control. CRC, colorectal cancer. 5-ASA, Aminosalicic acid.

3.7 *L. plantarum* modulated the gut microbiota composition of CRC mice

There is a significant correlation between AOM/DSS-induced CRC and intestinal microbiota dysbiosis⁴⁴. The 16S rRNA high-throughput sequencing was applied to analyze the composition and diversity of intestinal microbiota in the different groups. The results showed that the AOM/DSS-induced CRC group exhibited a significant decrease in microbiota richness and diversity compared to the CTL group, indicating that CRC development may be associated with impaired α -diversity of the microbiota (Fig. 5A-D). It is worth noting that both the 5-ASA and *L. plantarum* intervention groups significantly improved this condition, restoring the richness and diversity of the microbiota to the levels of the CTL group. ASV-based petal plot analysis revealed that CTL, CRC, 5-ASA, and the three intervention groups (NCU0011098, NCUH001084, NCU0011129) shared 314 ASVs, with unique ASV counts of 1364, 380, 1085, 1150, 1205, and 1013, respectively (Fig. 5E). Further assessment of β -diversity using PCoA and NMDS revealed significant separation of the gut microbiota between the CRC and CTL group mice, which indicated that AOM/DSS induction led to a complete restructuring of the gut microbiota (Fig. 5F, G). Notably, all intervention groups were able to reverse this β -diversity change to varying degrees, normalizing the microbiota structure.



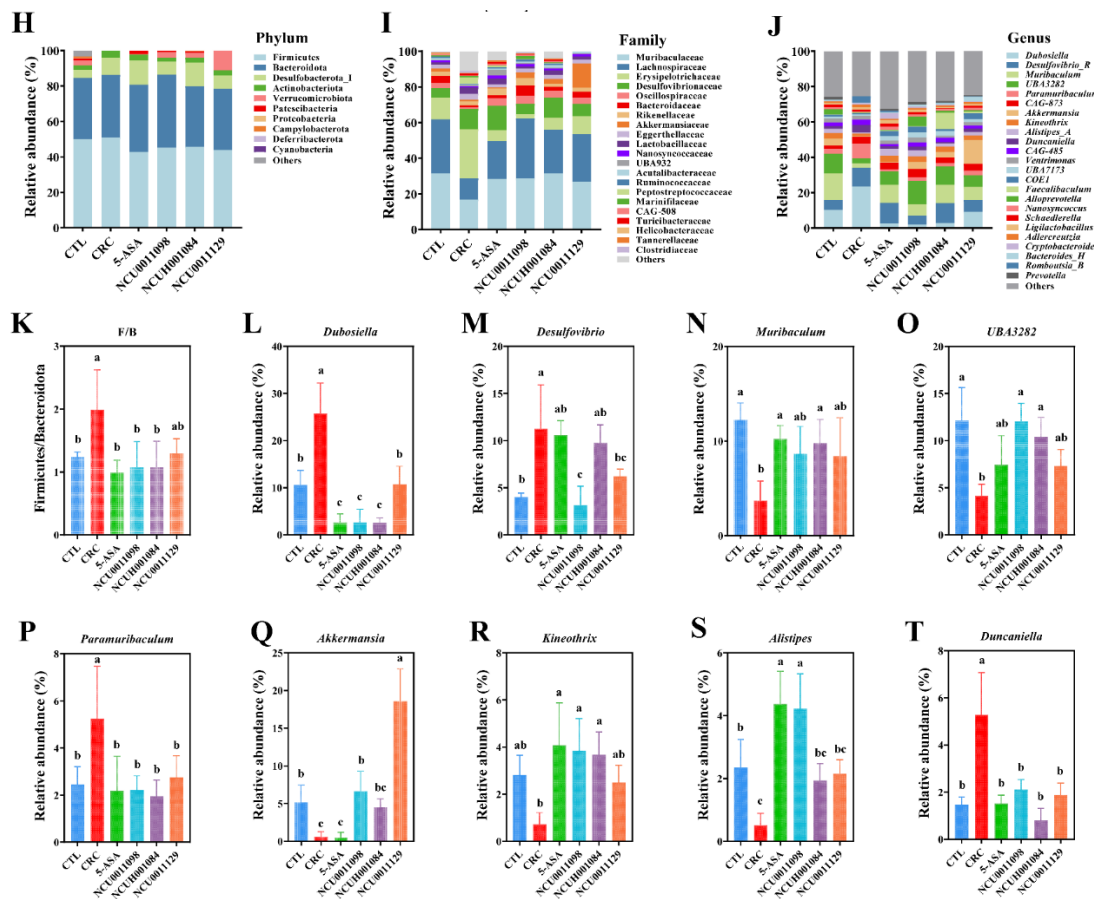


Fig. 5. The effect of *L. plantarum* on the diversity and structure of the intestinal microbiota. (A) Chao1 index. (B) Observed species. (C) Shannon index. (D) Simpson index. (E) Petal plot analysis based on the amplicon sequence variant (ASV) level. (F) The principal coordinate analysis (PCoA) is based on the Bray-Curtis algorithm. (G) The nonmetric dimensional scaling (NMDS) is based on the Bray-Curtis algorithm. Composition analysis of gut microbiota of mice in each group at the phylum level (H), family level (I), and genus level (J). (K) The Firmicutes/Bacteroidetes (F/B) ratio of the phylum in each group. The relative abundance of *Dubosiella* (L), *Desulfovibrio* (M), *Muribaculum* (N), UBA 3282 (O), *Paramuribaculum* (P), *Akkermansia* (Q), *Kineothrix* (R), *Alistipes_A* (S), *Duncaniella* (T). Data were expressed as mean $\pm$ SEM (n = 6). Different lowercase letters mean statistically significant differences ($P < 0.05$). CTL, control. CRC, colorectal cancer. 5-ASA, Aminosalicic acid.

To study the effect of *L. plantarum* on the composition of the intestinal microbiota in CRC mice, we further analyzed it at the taxonomic level (phylum, family, genus). Phylum-level analysis revealed that AOM/DSS-induced CRC mice exhibited significant dysbiosis compared with the CTL group, characterized by reduced abundance of Verrucomicrobia and increased abundance of Desulfobacterota and Actinomycetota (Fig. 5H). Notably, intervention with *L. plantarum* NCU0011098 and NCU0011129 significantly reduced the relative abundance of Desulfobacterota and Actinomycetota and restored Verrucomicrobia to normal levels. Verrucomicrobia is a bacterial phylum with important physiological functions, playing a significant role in intestinal microbiota, metabolic regulation, and disease association⁴⁵. In addition, the F/B ratio, a key indicator for assessing gut microbiota imbalance³⁰, was significantly elevated in CRC mice, while 5-ASA and *L. plantarum* intervention effectively suppressed the abnormal elevation of this ratio (Fig. 5K). At the family level, AOM/DSS induction increased the relative abundance of Erysipelotrichaceae, Desulfovibrionaceae, and Peptostreptococcaceae in the intestines of CRC mice, while it reduced the relative abundance of Muribaculaceae, Lachnospiraceae, Oscillospirales, and Akkermansiaceae (Fig. 5I). All intervention groups (5-ASA and three *L. plantarum* strains) reversed this trend to varying degrees, with *L. plantarum*

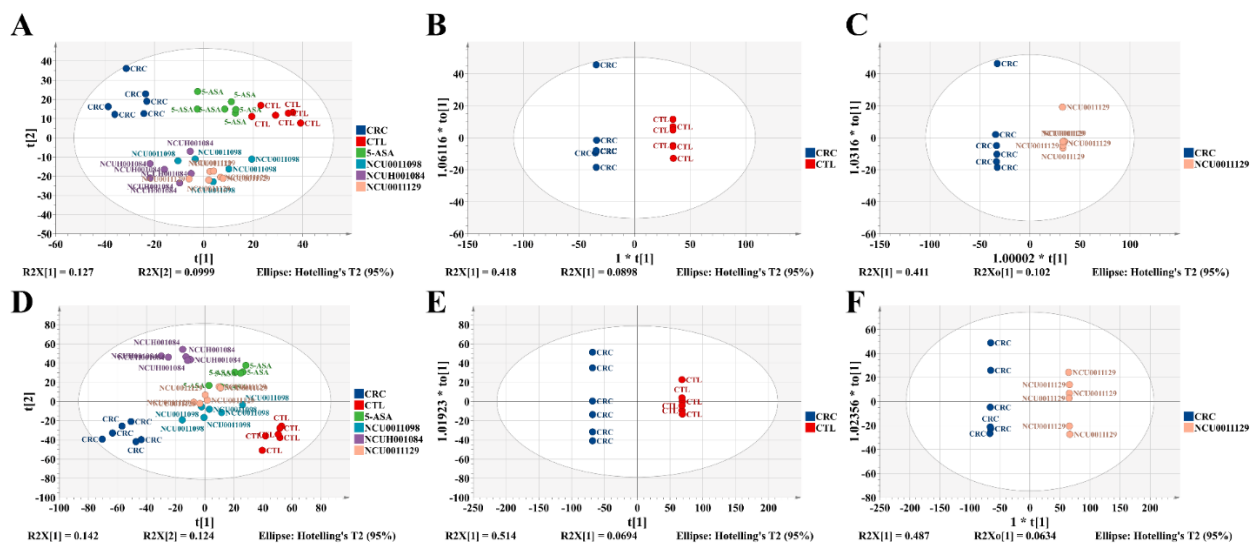
NCU0011129 intervention group having the most similar microbiota composition to the CTL group, indicating its optimal microbiota regulatory effect. At the genus level, there were obvious differences among the various groups (Fig. 5J). Specifically, AOM/DSS induction significantly increased the relative abundance of *Dubosiella*, *Desulfovibrio*, *Paramuribaculum*, and *Duncaniella*, while it decreased the relative abundance of *Muribaculum*, UBA3282, *Akkermansia*, *Kineothrix*, and *Alistipe* (Fig. 5J, L-T). Notably, *Dubosiella* was identified as a key bacterium in gut-originated Parkinson's disease model, and was enriched in the gut of mice with colorectal adenomas⁴⁶. *Alistipes* exhibits protective effects in colitis and cancer immunotherapy⁴⁵. *Akkermansia*, as next-generation probiotics, can alleviate CRC by inhibiting tumor cell proliferation, promoting apoptosis, and enhancing antitumor immune responses⁴⁷⁻⁴⁸. Results showed that *L. plantarum* NCU0011129 significantly increased the relative abundance of *Akkermansia* (Fig. 5Q). 5-ASA intervention significantly reduced the relative abundance of *Dubosiella*, *Paramuribaculum*, and *Duncaniella*, while increasing the abundance of *Muribaculum*, *Kineothrix*, and *Alistipe*, but had no significant effect on *Desulfovibrio*, *Akkermansia*. In contrast, all three strains of *L. plantarum* effectively alleviated CRC-induced dysbiosis, promoting the proliferation of beneficial bacteria and inhibiting the colonization of opportunistic pathogens. Among them, *L. plantarum* NCU0011129 demonstrated the most significant effect in increasing the abundance of *Akkermansia*. This reveals its ability to precisely regulate key beneficial bacteria, thereby synergistically strengthening the intestinal barrier, suppressing inflammation, improving metabolism, and collectively establishing an intestinal environment unfavorable for the occurrence and development of colorectal cancer.

The LEfSe analysis was conducted to identify the biomarker taxa in the mice with different treatments (Fig. 6A, B). The results showed that the characteristic microbiota of the CRC group included *f_Erysipelotrichaceae*, *g_Dubosiella*, *g_Pseudomonas*, *f_Peptostreptococcaceae*, *g_Romboutsia_B*, etc. The marker microorganisms in the CTL group were *g_Aerococcus*, *f_Aerococcaceae*, *g_Eubacterium_R*, *g_UMGS1071*, and *g_Ruthenibacterium*. The marker microorganisms in the 5-ASA group were *f_GCA_900066905*, *g_Enterococcus*. *L. plantarum* NCU0011098 group marker microorganisms include *f_Lachnospiraceae*, *f_Oscillospiraceae*. *L. plantarum* NCUH001084 group marker microorganisms include *g_Zag 111*, *g_CAG 605*, *g_UBA2730*, *g_CAG488*, and *g_Fournierella*. *L. plantarum* NCU0011129 group includes the following marker microorganisms: *g_Ruminococcus_E*, *g_Evtepia*. Each intervention group exhibited a unique microbial profile, which suggested that different *L. plantarum* strains may exert their probiotic effects by regulating specific microbial communities.

The average degree reflected the connection density of nodes in the network, reflecting frequent interactions between species, which may correspond to a stable ecosystem⁴⁹. In addition, we used transitivity to represent the global clustering coefficient; the transitivity of the CTL group, *L. plantarum* NCU0011098 group, and *L. plantarum* NCU0011129 group were all greater than 0.44, indicated that these networks have modular characteristics (Fig. 6L) and suggested that their microbial communities had stronger functional synergies⁵⁰. Therefore, *L. plantarum* NCU0011129 can restore the complexity and stability of the gut microbiota co-occurrence network in CRC mice, thereby rebalancing the gut microbiota. The recovery of network topological parameters was consistent with the previously observed improvement in α/β diversity, jointly confirming the ecological regulatory efficacy of *L. plantarum* NCU0011129.

3.9 *L. plantarum* NCU0011129 attenuated the effects of AOM/DSS on serum metabolomics

Previous studies have shown that gut microbiota dysbiosis can influence serum metabolite profiles through the microbe-host co-metabolism pathway, and abnormal changes in serum metabolites were significantly associated with the progression and prognosis of CRC⁵¹. Therefore, non-targeted metabolomics analysis based on UPLC-QTOF/MS was conducted to identify the serum differential metabolites among varied groups with different treatments. In both positive and negative ion modes, PCoA showed an obvious separation trend between the CRC group and the CTL group, indicating that there were significant changes in the serum metabolites of AOM/DSS-induced CRC mice, which was consistent with the pathological phenotype of CRC mice (Fig. 7A, D). Notably, the interventions of all three strains of *L. plantarum* could alleviate the serum metabolic disorders in CRC mice to varying degrees, and the overall profile of serum metabolites of *L. plantarum* NCU0011129 was closer to that of mice in the CTL group, suggesting that it had the best metabolic regulation effect. Through OPLS-DA combined with 200 permutation tests, the significant metabolic differences between the CTL group and the CRC group, as well as between the CRC group and the *L. plantarum* NCU0011129 group, were further verified (Fig. S5, Fig. 7B-F). Eighty differential metabolites were screened out based on fold change > 2 and VIP > 1 (Fig. 7G). Among them, 43 metabolites were upregulated and 37 metabolites were downregulated after *L. plantarum* NCU0011129 intervention.



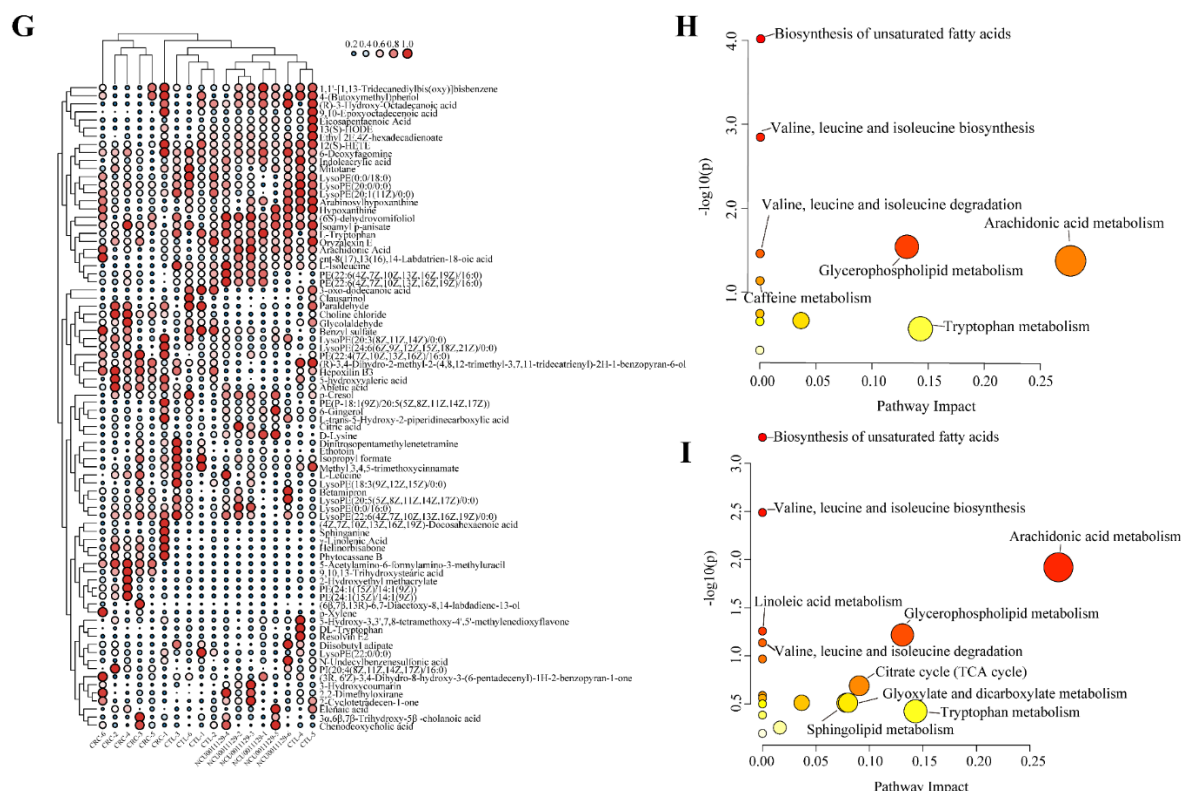


Fig. 7. The effects of *L. plantarum* on serum metabolism in CRC mice. Principal component analysis (PCA) in positive (A) and negative (D) ion mode. Orthogonal partial least squares discriminant analysis (OPLS-DA) for serum of CTL and CRC group in positive (B) and negative (E) ion mode. OPLS-DA for serum of the CRC and NCU0011129 group in positive (C) and negative (F) ion mode. (G) Heatmap of differentially expressed metabolites. Key metabolic pathways regulated by CRC (H) and NCU0011129 (I). CTL, control. CRC, colorectal cancer. 5-ASA, Aminosalicyclic acid.

To analyze the biological functions of the differential metabolites, we performed metabolic pathway enrichment analysis on the screened differential metabolites based on the KEGG database. The results showed that the differential metabolites between the CTL group and the CRC group were mainly enriched in the biosynthesis of unsaturated fatty acids, valine, leucine, and isoleucine biosynthesis, valine, leucine, and isoleucine degradation, glycerophospholipid metabolism, arachidonic acid metabolism, and tryptophan metabolism (Fig. 7H). These metabolic pathways primarily belong to amino acid and lipid metabolic pathways, which confirms the characteristics of amino acid metabolic disorders and lipid metabolic abnormalities in the pathological process of CRC⁶. Notably, the intervention of *L. plantarum* NCU0011129 not only reversed the disorders of five metabolic pathways (the biosynthesis of unsaturated fatty acids, valine, leucine, and isoleucine biosynthesis, arachidonic acid metabolism, glycerophospholipid metabolism, and tryptophan metabolism), but also regulated linoleic acid metabolism (Fig. 7I). Among them, the arachidonic acid metabolic pathway was significantly enriched ($P < 0.05$, impact > 0.1), suggesting that this pathway plays a key role in the alleviation of CRC progression by *L. plantarum* NCU0011129. The significant regulatory effect of *L. plantarum* NCU0011129 on serum metabolites is not merely an empirical observation but rather a direct and integrated manifestation of its well-documented intrinsic probiotic properties. Our team's prior research has confirmed the strain's exceptional gastrointestinal tolerance, which ensures efficient colonization and establishes the fundamental basis for sustained host-microbe interaction¹⁹. This capacity synergizes with its robust ability to produce SCFAs, directly shaping the intestinal metabolic milieu and promoting systemic

metabolic shifts. Furthermore, its potent *in vitro* antioxidant activity provides a mechanistic rationale for alleviating *in vivo* oxidative stress, thereby modulating a key upstream driver of inflammation. Consequently, the observed modulation of the arachidonic acid metabolic pathway and other systemic changes in this study represent the physiological outcome of the concerted action of these multidimensional characteristics. The strong concordance between the *in vitro* capabilities and *in vivo* functions of *L. plantarum* NCU0011129 robustly argues for the plausibility of its therapeutic efficacy, underscoring that its unique probiotic attributes constitute the fundamental basis for its superior therapeutic potential.

We used a metabolic network diagram to simply illustrate the relationship between enriched metabolic pathways and differential metabolites (Fig. 8A). Specifically, alterations in tricarboxylic acid cycle (TAC) intermediates and branched-chain amino acid metabolism suggest that *L. plantarum* NCU0011129 may disrupt the distinctive energy metabolism and biosynthetic patterns of cancer cells, thereby inhibiting their proliferative foundation. More notably, the comprehensive remodeling of the arachidonic acid (AA) metabolic pathway likely represents a key mechanism underlying the antitumor effect of this probiotic. Studies have shown that in inflammation-to-cancer models, key enzymes in the cyclooxygenase (COX) and lipoxygenase (LOX) cascades are often synergistically upregulated, with a particularly marked increase in PTGS2 (COX-2)—the core enzyme responsible for catalyzing the conversion of AA into pro-inflammatory prostaglandins (e.g., PGE₂)⁵²⁻⁵³. However, in inflammation-to-cancer models intervened with probiotics, these key enzymes can exhibit synergistic downregulation, with the expression of PTGS2 (COX-2) being significantly suppressed⁵⁴. This dual inhibition of the COX/LOX pathways strongly indicates that *L. plantarum* NCU0011129 directly mitigates the chronic inflammation driving CRC progression by attenuating the production of pro-inflammatory mediators like hepxilin B3 and prostaglandins in the arachidonic acid metabolic pathway⁵⁵. This mechanistic link between probiotic intervention and the suppression of the arachidonic acid-inflammation axis is a novel and significant finding. Simultaneously, disturbances in the synthesis of polyunsaturated fatty acids (e.g., EPA, DHA) and glycerophospholipid metabolism reflect a restructuring of cell membrane architecture and function, thereby influencing lipid-based signal transduction. Furthermore, the shift in tryptophan metabolism toward microbial-derived products such as tryptamine indicates that *L. plantarum* NCU0011129 enhances the function of beneficial gut microbiota. Importantly, the increase in indole derivative metabolites (e.g., tryptamine) creates a favorable environment for the expansion of beneficial bacteria like *Akkermansia muciniphila*. These indole derivatives collectively contribute to the maintenance of intestinal barrier integrity and immune homeostasis by aryl hydrocarbon receptor (AhR) activation⁵⁶. In summary, the intervention with *L. plantarum* NCU0011129 achieves multidimensional metabolic reprogramming. It coordinately regulates key pathways involving energy metabolism, inflammatory responses, lipid balance, and microbiota-host co-metabolism. This integrated reprogramming collaboratively establishes a systemic microenvironment that significantly suppresses tumor initiation and development.

3.10 Correlation Analysis

Studies have demonstrated that gut microbiota dysbiosis is closely associated with the initiation and progression of CRC, which can influence host serum metabolic profiles through multiple metabolic pathways⁵¹. Based on this, a correlation network was constructed in our study using Spearman's analysis, with 44 serum differential metabolites, 31 bacterial genera, and 20 phenotypic indicators integrated (Fig. 8B). The results revealed extensive significant positive and negative correlations among the three components. Within the gut microbiota, *Desulfovibrio_R*, *Duncaniella*, *Turicibacter*, *Muribaculum*, *Romboutsia_B*, *Prevotella*, and *Akkermansia* exhibited a higher degree of centrality (number of connections with other nodes) in the overall network, suggesting these bacterial taxa may serve as key regulators that critically maintain network stability and functionality (Table S5). Notably, *L. plantarum* NCU0011129 significantly reduced the abundance of *Desulfovibrio_R*, *Duncaniella*, *Romboutsia_B*, and *Turicibacter* while it increased *Muribaculum* and *Akkermansia* in CRC mice. Correlation analysis demonstrated that *Desulfovibrio_R*, *Duncaniella*, *Romboutsia_B*, and *Turicibacter* were positively associated with tumor number, pro-inflammatory cytokines, and MDA levels, but negatively correlated with colon length and SCFA content. These findings indicated that these bacterial taxa may represent potential therapeutic targets through which *L. plantarum* NCU0011129 alleviates colonic tumor burden. In contrast, *Muribaculum* and *Akkermansia* showed positive correlations with colon length, SCFA content, anti-inflammatory cytokines, T-SOD activity, and tight junction proteins (ZO-1 and Occludin), while they exhibited negative correlations with pro-inflammatory cytokines and MDA levels. A previous study has demonstrated that *Desulfovibrio* is recognized as a harmful bacterial genus associated with gallstones, obesity, and ulcerative colitis⁵⁷. *Turicibacter* has been frequently linked to host inflammation and was found to be significantly enriched in CRC tumor-bearing mice⁵⁸. Conversely, *Muribaculum* represents a beneficial commensal bacterium in the murine intestine that can enhance the efficacy of cancer immunotherapy⁵⁹. Thus, the anti-inflammatory effects of *L. plantarum* NCU0011129 in the colon appear to be mediated through remodeling of gut microbiota composition, specifically by increasing the abundance of *Muribaculum* and *Akkermansia* while reducing *Desulfovibrio_R*, *Duncaniella*, *Romboutsia_B*, and *Turicibacter*. Notably, we observed strong correlations between the altered gut microbiota and serum metabolites. Among the differential serum metabolites, 3-oxo-dodecanoic acid and PE (24:1(15Z)/14:1(9Z)) exhibited high network connectivity with degree values of 38 and 31, respectively (Table S5). Moreover, 3-oxo-dodecanoic acid showed positive correlations with *Desulfovibrio_R*, *Duncaniella*, *Turicibacter*, *Romboutsia_B*, and *Prevotella*, while demonstrating negative correlations with *Akkermansia*, *Muribaculum*, and *Alistipes_A*. Conversely, PE (24:1(15Z)/14:1(9Z)) positively correlated with *Muribaculum* but negatively correlated with *Duncaniella*, *Romboutsia_B*, and *Turicibacter*. As a microbiota-derived metabolite, 3-oxo-dodecanoic acid is primarily generated by gut bacteria through fatty acid β -oxidation and may promote inflammation by activating the NF- κ B pathway while increasing intestinal permeability. PE(24:1(15Z)/14:1(9Z)), a phosphatidylethanolamine species, participates in critical cellular processes ranging from membrane fusion to mitochondrial function and autophagy through its unique

physicochemical properties and diverse biosynthetic pathways, its deficiency may impair cellular membrane function, blood coagulation, and immune system regulation⁶⁰. Furthermore, both 3-oxo-dodecanoic acid and PE (24:1(15Z)/14:1(9Z)) exhibited extensive positive and negative correlations with CRC-related indicators, respectively, suggesting their potential as biomarkers for early diagnosis of CRC and potential targets for CRC treatment. These results suggested that the beneficial effects of *L. plantarum* NCU0011129 in alleviating AOM/DSS-induced CRC may be mediated through modulating gut microbiota composition and serum metabolite levels, thereby reshaping microbial-associated metabolic pathways, altering host biochemical phenotypes, and ultimately mitigating colorectal carcinogenesis and progression (Fig. 9).

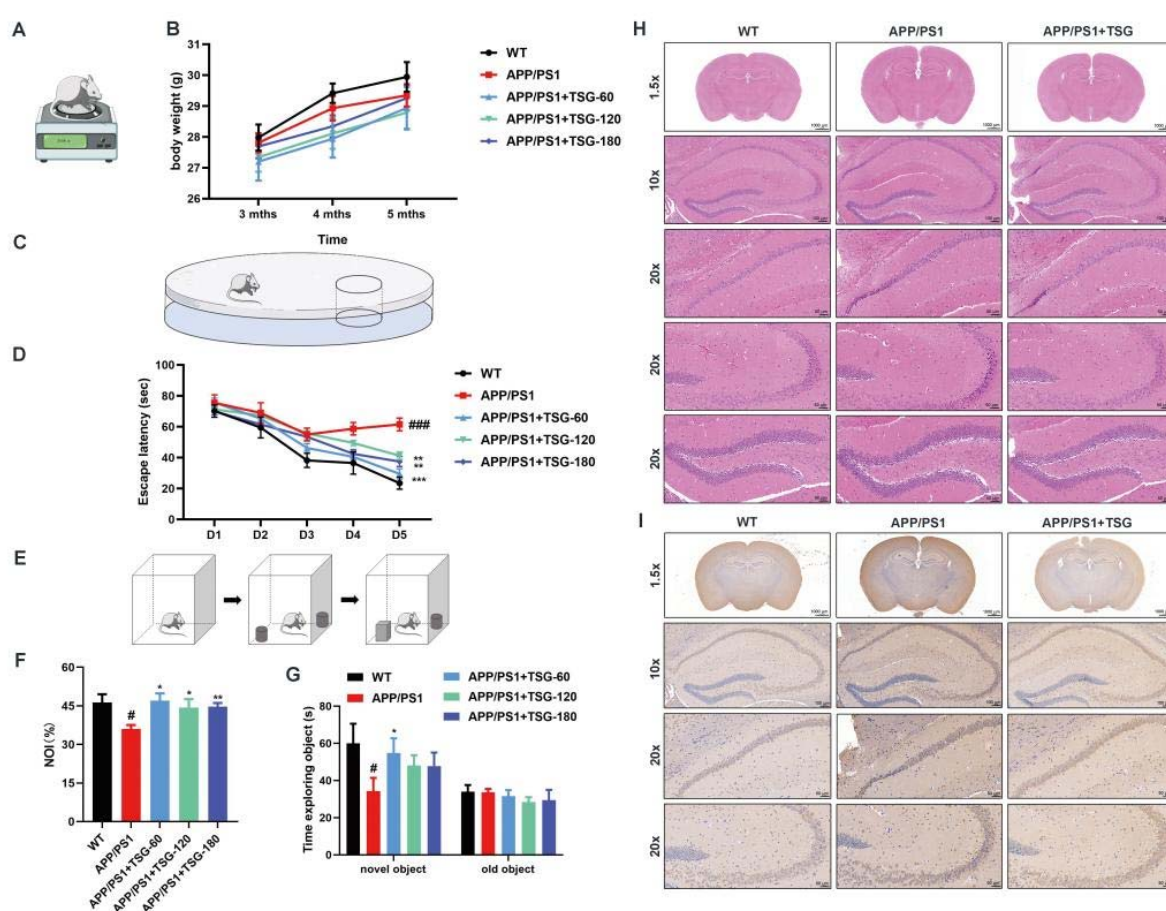


Fig. 9. TSG improved cognitive deficits without causing toxicity in AD mice. (A) Schematic diagram of body weight. (B) Body weight during 8 weeks. (C) Schematic diagram of MWM test. (D) Line chart of the changes in escape latency during training period. (E) Schematic diagram of NOR test. (F) Representative diagram of NOI. (G) Histogram of the percentage of exploring time of mice in each group. (H) Brain H&E staining to observe the effect of TSG on inflammation infiltration. (I) Aβ to observe the effect of TSG on decreasing the number and area of plaque. Data are presented as mean ± SEM. #*P* < 0.05, ###*P* < 0.001 vs control group; **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs APP/PS1 model group. MWM, morris water maze; NOR, novel object recognition; NOI, novel recognition index.

4. Conclusion

Research findings demonstrated that three *L. plantarum* strains significantly alleviated AOM/DSS-induced CRC progression. Specifically, *L. plantarum* markedly reduced CRC-associated mortality and tumor burden, ameliorated colon shortening, attenuated inflammatory infiltration and histopathological damage, while maintaining immune homeostasis in the murine model. Integrated multi-omics analysis revealed that *L. plantarum* NCU0011129 exerted its protective effects by modulating serum metabolic profiles

and restoring gut microbiota composition, thereby mitigating AOM/DSS-induced metabolic dysregulation and microbial dysbiosis. These results suggested that *L. plantarum* NCU0011129, as a probiotic supplement, may confer CRC protection via the "microbiota-metabolite-immune" axis. To translate these correlations into mechanistic insights, our future research will prioritize establishing causal linkages, for instance, through fecal microbiota transplantation experiments to confirm the indispensability of gut microbiota, and functional studies on key altered metabolites like arachidonic acid derivatives. Furthermore, exploring its synergistic potential with conventional therapies and advancing towards human clinical trials will be crucial steps to validate its promise as a novel probiotic-based intervention for CRC.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

- 1 F. Bray, M. Laversanne, H. Sung, J. Ferlay, R. L. Siegel, I. Soerjomataram, A. Jemal. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 2024, 74, 229–263.
- 2 M. Song, A. T. Chan, J. Sun. Influence of the gut microbiome, diet, and environment on risk of colorectal cancer. *Gastroenterology*, 2020, 158, 322–340.
- 3 S. La Vecchia, C. Sebastián. Metabolic pathways regulating colorectal cancer initiation and progression. *Seminars in Cell & Developmental Biology*, 2020, 98, 63–70.
- 4 S. C. Shah and S. H. Itzkowitz. Colorectal cancer in inflammatory bowel disease: mechanisms and management. *Gastroenterology*, 2022, 162, 715–730.
- 5 P. Ambalam, M. Raman, R. K. Purama, M. Doble. Probiotics, prebiotics and colorectal cancer prevention. *Best Practice & Research Clinical Gastroenterology*, 2016, 30, 119–131.
- 6 K. Cai, X. Y. Cao, F. Chen, Y. Zhu, D. D. Sun, H. B. Cheng, J. A. Duan, S. L. Su. Xianlian jiedu decoction alleviates colorectal cancer by regulating metabolic profiles, intestinal microbiota and metabolites. *Phytomedicine*, 2024, 155385.
- 7 C. Guo, D. Guo, L. Fang, T. Sang, J. Wu, C. Guo, Y. Wang, Y. Wang, C. Chen, J. Chen, R. Chen, X. Wang. *Ganoderma lucidum* polysaccharide modulates gut microbiota and immune cell function to inhibit inflammation and tumorigenesis in colon. *Carbohydrate Polymers*, 2021, 267, 118231.
- 8 H. K. M. Leung, E. K. K. Lo, C. Chen, F. Zhang, Felicianna, M. J. Ismaiah, H. El-Nezami, Probiotic mixture attenuates colorectal tumorigenesis in murine AOM/DSS model by suppressing STAT3, inducing apoptotic P53 and modulating gut microbiota. *Probiotics & Antimicro. Prot.*, 2024.
- 9 Q. Li, D. Liu, M. Liang, Y. Zhu, M. Yousaf, Y. Wu, Mechanism of probiotics in the intervention of colorectal cancer: A review. *World J Microbiol Biotechnol*, 2024, 40 (10), 1–15.
- 10 S. Y. Lee, D. Y. Lee, J. H. Kang, J. H. Kim, J. W. Jeong, H. W. Kim, D. H. Oh, S. H. Yoon, S. J Hur, Relationship between gut microbiota and colorectal cancer: Probiotics as a potential strategy for prevention. *Food Research International*, 2022, 156, 111327.
- 11 Y. Hu, P. Zhou, K. Deng, Y. Zhou, K. Hu, Targeting the gut microbiota: A new strategy for colorectal cancer treatment. *Journal of Translational Medicine*, 2024, 22, 915.

- 12 R. Martín, C. Chamignon, N. Mhedbi-Hajri, F. Chain, M. Derrien, U. Escribano-Vázquez, P. Garault, A. Cotillard, H. P. Pham, C. Chervaux, L. G. Bermúdez-Humarán, T. Smokvina, P. Langella, The potential probiotic *Lactobacillus rhamnosus* CNCM I-3690 strain protects the intestinal barrier by stimulating both mucus production and cytoprotective response. *Sci Rep*, 2019, 9 (1).
- 13 T. Zhou, J. Wu, A. Khan, T. Hu, Y. Wang, E.-S. Salama, S. Su, H. Han, W. Jin, X. Li, A probiotic *Limosilactobacillus fermentum* GR-3 mitigates colitis-associated tumorigenesis in mice via modulating gut microbiome. *npj Sci Food*, 2024, 8, 61.
- 14 W. Fong, Q. Li, J. Yu, Gut microbiota modulation: A novel strategy for prevention and treatment of colorectal cancer. *Oncogene*, 2020, 39, 4925–4943.
- 15 Z. Hu, F. Shen, Y. Liu, Z. Zhong, Y. Chen, Z. Xia, C. Mo, H. Yu, Targeted metabolomics reveals novel diagnostic biomarkers for colorectal cancer. *Molecular Oncology*, 2025, 19, 1737–1750.
- 16 J. Gu, Y. Xiao, D. Shu, X. Liang, X. Hu, Y. Xie, D. Lin, H. Li, Metabolomics analysis in serum from patients with colorectal polyp and colorectal cancer by 1H-NMR spectrometry. *Disease Markers*, 2019, 2019, 3491852.
- 17 X. Bai, B. Liu, D. Fan, Y. Lu, X. Zhao, Modulating the gut microbiota: a novel perspective in colorectal cancer treatment. *Cancer Letters*, 2025, 612, 217459.
- 18 S. Kashyap, S. Pal, G. Chandan, V. Saini, S. Chakrabarti, N. K. Saini, A. Mittal, V. K. Thakur, A. K. Saini, R. V. Saini, Understanding the cross-talk between human microbiota and gastrointestinal cancer for developing potential diagnostic and prognostic biomarkers. *Seminars in Cancer Biology*, 2022, 86, 643–651.
- 19 X. Y. Xu, S. J. Xiong, T. H. Du, J. Y. Li, X. T. Zhao, M. W. Zhao, T. Huang, H. B. Ren, T. Xiong, M. Y. Xie, In vitro screening of *Lactiplantibacillus plantarum* with probiotic properties and anti-colon cancer potential. *Food Bioscience* 2024, 62, 105569.
- 20 MacArthur Clark, J. A.; Sun, D. Guidelines for the ethical review of laboratory animal welfare People's Republic of China National Standard GB/T 35892-2018 [Issued 6 February 2018 Effective from 1 September 2018]. *Anim. Models and Exp. Med.* 2020, 3 (1), 103–113.
- 21 F. Yang, T. L. Bai, X. Zhang, X. Meng, J. Yuan, Z. Wu, A. Yang, H. Chen, X. Li, High-fat diet-induced obesity accelerates the progression of food allergy through synergistic effect of gut microbiota and lipid metabolism. *Food Bioscience* 2025, 68, 106588.
- 22 S. Ding, Y. Ma, G. Liu, W. Yan, H. Jiang, J. Fang, *Lactobacillus Brevis* alleviates DSS-induced colitis by reprogramming intestinal microbiota and influencing serum metabolome in murine model. *Front. Physiol.*, 2019, 10, 1152.
- 23 X. Xu, S. Xiong, T. Du, Q. Liu, X. Huang, C. Liu, X. Zhao, T. Xiong, M. Xie, Fermented guava (*Psidium Guajava*) by *Lactiplantibacillus Plantarum* NCU0011129 attenuates Azoxymethane/Dextran Sodium Sulfate-induced colorectal cancer in mice: Restructuring gut microbiota and enhancing intestinal barrier function. *Food Bioscience*, 2025, 68, 106672.
- 24 X. Zeng, J. Li, X. Wang, L. Liu, S. Shen, N. Li, Z. Wang, Y. Yuan, T. Yue, Regulation of gut microbiota and microbial metabolome of kefir supernatant against *Fusobacterium nucleatum* and DSS-coinduced colitis. *J. Agric. Food Chem.*, 2024, 72, 3536–3548.
- 25 X. Fan, H. Guo, C. Teng, X. Yang, P. Qin, A. Richel, L. Zhang, C. Blecker, G. Ren, Supplementation of quinoa peptides alleviates colorectal cancer and restores gut microbiota in AOM/DSS-treated mice. *Food Chemistry*, 2023, 408, 135196.
- 26 X. Wei, F. Cheng, J. Liu, Y. Cheng, S. Yun, J. Meng, M. Chang, X. Geng, L. Xu, J. Cao, C. Feng, Sparassis latifolia polysaccharides inhibit colon cancer in mice by modulating gut microbiota and metabolism. *International Journal of Biological Macromolecules*, 2023, 232, 123299.
- 27 A. Allameh, R. Niayesh-Mehr, A. Aliarab, G. Sebastiani, K. Pantopoulos, Oxidative stress in liver pathophysiology and disease. *Antioxidants*, 2023, 12, 1653.
- 28 Q. Zhang, Q. Zhao, T. Li, L. Lu, F. Wang, H. Zhang, Z. Liu, H. Ma, Q. Zhu, J. Wang, X. Zhang, Y. Pei, Q. Liu, Y. Xu, J. Qie, X. Luan, Z. Hu, X. Liu, *Lactobacillus Plantarum*-derived indole-3-lactic acid ameliorates colorectal tumorigenesis via epigenetic regulation of CD8⁺ T cell Immunity. *Cell Metabolism*, 2023, 35, 943–960.
- 29 P. J. Koelink, M. A. C. Mieremet-Ooms, W. E. Corver, K. Wolanin, D. W. Hommes, C. B. H. W. Lamers, H. W. Verspaget, 5-aminosalicylic acid interferes in the cell cycle of colorectal cancer cells and induces cell death modes. *Inflammatory Bowel Diseases*, 2010, 16, 379–389.

- 30 L. Chen, S. Zhao, Q. Chen, P. Luo, X. Li, Y. Song, S. Pan, Q. Wu, Y. Zhang, X. Shen, Y. Chen, Poria cocos polysaccharides ameliorate AOM/DSS-induced colorectal cancer in mice by remodeling intestinal microbiota composition and enhancing intestinal barrier function. *International Journal of Biological Macromolecules*, 2025, 315, 144477.
- 31 A. A. Bhat, S. Uppada, I. W. Achkar, S. Hashem, S. K. Yadav, M. Shanmugakonar, H. A. Al-Naemi, M. Haris, S. Uddin, Tight junction proteins and signaling pathways in cancer and inflammation: A functional crosstalk. *Front. Physiol.*, 2019, 9.
- 32 G. M. De Waal, W. J. S. De Villiers, T. Forgan, T. Roberts, E. Pretorius, Colorectal cancer is associated with increased circulating lipopolysaccharide, inflammation and hypercoagulability. *Sci Rep*, 2020, 10, 8777.
- 33 Y. Kurashima, H. Kiyono, Mucosal ecological network of epithelium and immune cells for gut homeostasis and tissue healing. *Annu. Annu. Rev. Immunol.*, 2017, 35, 119–147.
- 34 M. Takami, W. Aoi, K. Matsumoto, Y. Kato, Y. Kobayashi, M. Kuwahata, High-intensity exercise impairs intestinal barrier function by generating oxidative stress. *J. Clin. Biochem. Nutr.*, 2024, 74, 136–140.
- 35 S. Yang, Y. Li, Y. Zhang, Y. Wang, Impact of chronic stress on intestinal mucosal immunity in colorectal cancer progression. *Cytokine & Growth Factor Reviews*, 2024, 80, 24–36.
- 36 D. Zhang, Y. P. Jian, Y. N. Zhang, Y. Li, L. T. Gu, H. H. Sun, M. D. Liu, H. L. Zhou, Y. S. Wang, Z.X. Xu, Short-chain fatty acids in diseases. *Cell Commun Signal*, 2023, 21, 212.
- 37 R. G. Xiong, D. D. Zhou, S. X. Wu, S. Y. Huang, A. Saimaiti, Z. J. Yang, A. Shang, C. N. Zhao, R. Y. Gan, H. B. Li, Health benefits and side effects of short-chain fatty acids. *Foods*, 2022, 11, 2863.
- 38 C. Marques, C. S. F. Oliveira, S. Alves, S. R. Chaves, O. P. Coutinho, M. Côte-Real, A. Preto, Acetate-induced apoptosis in colorectal carcinoma cells involves lysosomal membrane permeabilization and cathepsin d release. *Cell Death Dis*, 2013, 4, 507–507.
- 39 C. S. F. Oliveira, H. Pereira, S. Alves, L. Castro, F. Baltazar, S. R. Chaves, A. Preto, M. Côte-Real, Cathepsin d protects colorectal cancer cells from acetate-induced apoptosis through autophagy-independent degradation of damaged mitochondria. *Cell Death Dis*, 2015, 6, 1788–1788.
- 40 T. Ryu, K. Kim, M. Y. Son, J. K. Min, J. Kim, T. S. Han, D. S. Kim, H. S. Cho, Downregulation of PRMT1, a histone arginine methyltransferase, by sodium propionate induces cell apoptosis in colon cancer. *Oncol Rep*, 2018.
- 41 V. Singh, G. Lee, H. Son, H. Koh, E. S. Kim, T. Unno, J. H. Shin, Butyrate producers, “the sentinel of gut”: their intestinal significance with and beyond butyrate, and prospective use as microbial therapeutics. *Front. Microbiol*, 2023, 13.
- 42 A. Han, N. Bennett, B. Ahmed, J. Whelan, D. R. Donohoe, Butyrate decreases its own oxidation in colorectal cancer cells through inhibition of histone deacetylases. *Oncotarget*, 2018, 9, 27280–27292.
- 43 J. Kilner, J. S. Waby, J. Chowdry, A. Q. Khan, J. Noirel, P. C. Wright, B. M. Corfe and C. A. Evans, A proteomic analysis of differential cellular responses to the short-chain fatty acids butyrate, valerate and propionate in colon epithelial cancer cells. *Mol. BioSyst.*, 2012, 8, 1146–1156.
- 44 G. El Tekle, N. Andreeva, W. S. Garrett, The role of the microbiome in the etiopathogenesis of colon cancer. *Annu. Rev. Physiol.*, 2024, 86, 453–478.
- 45 T. Xia, C. Kang, X. Qiang, X. Zhang, S. Li, K. Liang, Y. Wang, J. Wang, H. Cao, M. Wang, Beneficial effect of vinegar consumption associated with regulating gut microbiome and metabolome. *Current Research in Food Science*, 2024, 8, 100566.
- 46 J. Wu, C. S. Li, W. Y. Huang, S. Y. Zhou, L. P. Zhao, T. Li, M. A. Li, M. X. Zhang, C. M. Qiao, W. J. Zhao, C. Cui, Y. Q. Shen, Gut microbiota promote the propagation of pathologic α -syn from gut to brain in a gut-originated mouse model of parkinson’s disease. *Brain, Behavior, and Immunity*, 2025, 128, 152–169.
- 47 Y. Jiang, Y. Xu, C. Zheng, L. Ye, P. Jiang, S. Malik, G. Xu, Q. Zhou, M. Zhang, Acetyltransferase from *Akkermansia Muciniphila* blunts colorectal tumourigenesis by reprogramming tumour microenvironment. *Gut*, 2023, 72, 1308–1318.
- 48 X. Ma, LvjunYan, X. Yu, H. Guo, Y. He, S. Wen, T. Yu, W. Wang, The alleviating effect of *Akkermansia Muciniphila* PROBIO on AOM/DSS-induced colorectal cancer in mice and its regulatory effect on gut microbiota. *Journal of Functional Foods*, 2024, 114, 106091.
- 49 Z. Zhu, M. Wang, Y. Guan, M. Li, Q. Peng, N. Zheng, W. Ma, Altered interaction network in the gut microbiota of current cigarette smokers. *Engineering Microbiology*, 2024, 4, 100138.

- 50 W. Shi, L. J. Chai, G. Y. Fang, J. L. Mei, Z. M. Lu, X. J. Zhang, C. Xiao, S. T. Wang, C. H. Shen, J. S. Shi, Z. H. Xu, Spatial heterogeneity of the microbiome and metabolome profiles of high-temperature Daqu in the same workshop. *Food Research International*, 2022, 156, 111298.
- 51 F. Chen, X. Dai, C. C. Zhou, K. Li, Y. Zhang, X. Y. Lou, Y. M. Zhu, Y. L. Sun, B. X. Peng, W. Cui, Integrated analysis of the faecal metagenome and serum metabolome reveals the role of gut microbiome-associated metabolites in the detection of colorectal cancer and adenoma. *Gut*, 2022, 71, 1315–1325.
- 52 C. Guo, D. Guo, L. Fang, T. Sang, J. Wu, C. Guo, Y. Wang, Y. Wang, C. Chen, J. Chen, R. Chen, X. Wang, *Ganoderma Lucidum* polysaccharide modulates gut microbiota and immune cell function to inhibit inflammation and tumorigenesis in colon. *Carbohydrate Polymers*, 2021, 267, 118231.
- 53 X. Wei, F. Cheng, J. Liu, Y. Cheng, S. Yun, J. Meng, M. Chang, X. Geng, L. Xu, J. Cao, C. Feng, *Sparassis Latifolia* polysaccharides inhibit colon cancer in mice by modulating gut microbiota and metabolism. *International Journal of Biological Macromolecules*, 2023, 232, 123299.
- 54 J. Liu, S. Wang, R. Yi, X. Long, X. Zhao, Effect of *Lactobacillus fermentum* ZS40 on the NF- κ B signaling pathway in an azomethane-dextran sulfate sodium-induced colon cancer mouse model. *Front. Microbiol.*, 2022, 13, 953905.
- 55 S. Ren, L. Lu, H. Su, Z. Li, S. Li, J. Pan, Y. Liu, G. Ji, H Xu, Regulating arachidonic acid metabolism: A novel strategy to prevent colorectal inflammatory cancer transformation. *J. Cancer*, 2025, 16 (14), 4155–4171.
- 56 S. A. Scott, J. Fu, P. V. Chang, Microbial tryptophan metabolites regulate gut barrier function via the aryl hydrocarbon receptor. *Proc. Natl. Acad. Sci. U.S.A.*, 2020, 117 (32), 19376–19387.
- 57 H. Zhou, D. Huang, Z. Sun, X. Chen, Effects of intestinal *Desulfovibrio* bacteria on host health and its potential regulatory strategies: A review. *Microbiological Research*, 2024, 284, 127725.
- 58 P. A. Dimitriu, G. Boyce, A. Samarakoon, M. Hartmann, P. Johnson, W. W. Mohn, Temporal stability of the mouse gut microbiota in relation to innate and adaptive immunity. *Environ Microbiol Rep*, 2013, 5, 200–210.
- 59 M. Zhang, Z. Wei, B. Wei, C. Lai, G. Zong, E. Tao, M. Fan, Y. Pan, B. Zhou, L. Shen, J. Wu, Q. Wang, Y. Peng, L. Zhen, Y. Wu, Y. Lu, G. Wang, F. Zhou, Y. Shan, Microbiota-derived urocanic acid triggered by tyrosine kinase inhibitors potentiates cancer immunotherapy efficacy. *Cell Host & Microbe*, 2025, 33, 915-931.
- 60 E. Calzada, O. Onguka, S. M. Claypool, Phosphatidylethanolamine metabolism in health and disease. *International Review of Cell and Molecular Biology*, Elsevier, 2016, 321, 29–88.