

Trichinella spiralis infection-induced ursodeoxycholic acid ameliorates colitis by regulating dendritic cells and macrophages via NLRP3 inflammasome signaling pathway

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1 ***Trichinella spiralis* infection-induced ursodeoxycholic acid ameliorates**
2 ***colitis by regulating dendritic cells and macrophages via NLRP3***
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Abstract: Gut microbiota dysbiosis contributes to inflammatory bowel disease (IBD). Helminth infections can reshape the gut microbiota and its metabolic landscape, but whether such alterations functionally ameliorate intestinal inflammation — and through which microbial species, metabolites, and immune pathways — remains poorly understood, particularly for *Trichinella spiralis* (*T. spiralis*). This study aimed to investigate the mechanistic link between *T. spiralis*-induced microbiota remodeling and colitis alleviation, and to identify key anti-inflammatory microbial species and metabolites. *T. spiralis* infection alleviated TNBS-induced colitis in mice and markedly altered the gut microbiota. Fecal microbiota transplantation from infected mice into colitic recipients reduced clinical symptoms and intestinal pathology. Integrated metagenomics and untargeted metabolomics revealed that *Bacteroides* species and bile acids were significantly increased in protected mice, with *Bacteroides acidifaciens* (*B. acidifaciens*) and ursodeoxycholic acid (UDCA) playing anti-inflammatory roles. Mechanistically, *B. acidifaciens* produced bile salt hydrolase that converted tauro-ursodeoxycholic acid into UDCA. UDCA then bound to the bile acid receptor TGR5, upregulating tight junction proteins and restoring intestinal barrier integrity. Additionally, UDCA binding to TGR5 on macrophages and dendritic cells inhibited NLRP3 inflammasome activation, reducing pro-inflammatory cytokines IL-1 β and IL-18, thereby alleviating colitis. *T. spiralis* infection ameliorates colitis by reshaping the gut microbiota, with *B. acidifaciens* as a key functional species that generates UDCA. UDCA acts via TGR5 to suppress NLRP3 inflammasome activation in dendritic cells and macrophages, reduce inflammatory cytokine production, and restore barrier integrity. Modulating *B. acidifaciens* or UDCA represents a promising microbiota-targeted strategy for treating colitis, avoiding the safety risks of direct helminth therapy.

Key words: *T. spiralis*, *B. acidifaciens*, UDCA, NLRP3 inflammasome, colitis

Introduction

Inflammatory bowel disease (IBD) is a chronic relapsing autoimmune disease of the gastrointestinal tract, characterized by rising global incidence and posing a significant threat to human health¹⁻³. Epidemiological studies have revealed a negative

correlation between the geographical distribution of autoimmune diseases (such as IBD, type I diabetes and multiple sclerosis) and endemic parasitic infection rates^{4,5}. Researchers have investigated and applied “helminth therapy” for the treatment of IBD patients⁶. During helminth infection, intricate interactions are forged among helminths, the host immune system, and the gut microbiota. Any disruption to one of these three components is likely to have a substantial impact on the other two. Helminth infection could also indirectly modify the host immune response through interactions with the gut microbiota, thus showing therapeutic potential for autoimmune diseases^{7,8}. *Trichinella spiralis* (*T. spiralis*) is a parasitic helminth with potent immunomodulatory properties. *T. spiralis* and its excretory-secretory (ES) products, along with related protein molecules, have demonstrated promising intervention effects and therapeutic efficacy in experimental models of IBD⁹⁻¹¹. However, as a pathogenic parasite, direct application of *T. spiralis* or its ES products for IBD treatment poses a risk of human infection, representing a major scientific challenge in the parasitology field for translating helminth-based therapies like *T. spiralis* into clinical practice¹².

With the development and application of microbial high-throughput sequencing technology, recent reports revealed that *T. spiralis* infection induces significant alterations in the host gut microbiota¹³⁻¹⁵. The gut microbiota, serving as the body's largest and most complex “immune organ”, plays a crucial role in the development, maturation, and regulation of the host immune system. Dysbiosis of the gut microbiota is closely associated with the occurrence and progression of multiple diseases, with gut microbiota dysregulation playing a key role in the pathogenesis of IBD. Therefore, regulating gut microbiota dysbiosis and restoring homeostasis between host immunity and the gut microbiota has emerged as a research hotspot for IBD treatment^{16,17}. The immunomodulatory effects of the gut microbiota involve the conversion and production of metabolites in the host gut. The gut microbiota mediates the conversion of primary bile acids into secondary bile acids, including UDCA and lithocholic acid^{18,19}. Bile acids, acting as critical metabolic-immune signaling molecules, play a key role in maintaining intestinal mucosal barrier function and

regulating immune homeostasis by binding to bile acid receptors²⁰. The takeda G protein-coupled receptor 5 (TGR5, also known as GPBAR1) function as important bile acid receptors and are involved in signal transduction pathways. The gut microbiota converts primary bile acids into secondary bile acids and alleviates autoimmune uveitis by modulating dendritic cells (DCs) through the TGR5 receptor²¹. At the same time, bile acid metabolites could directly regulate regulatory T cells (Tregs) and Th17 balance and ROR γ + Tregs homeostasis to control the host immune response²².

A recent study reported that adoptive transfer of *T. spiralis*-activated macrophages can ameliorate both Th1-and Th2-activated inflammation in murine models²³. *Schistosoma mansoni* infection could regulate the intestinal microbiota and relieves colitis²⁴. Moreover, *Hymenolepis diminuta* could relieve colitis by regulating the intestinal flora. However, the mechanism of how *T. spiralis* relieving colitis through intestinal flora is still poorly understood. Hence, in the present study, the alleviating effect of *T. spiralis* on colitis was first tested in a mouse model of TNBS-induced colitis. Association analysis was then performed by 16s, metagenomic and metabolomics. Finally, we further demonstrate that *T. spiralis* is attenuating colitis by modulating gut microbiota by performing with FMT and metabolite-treated mice.

2 Results

2.1 The gut microbiota is beneficial for the infection of *T. spiralis*

A model of gut microbiota dysbiosis was established with the aim of demonstrating that the gut microbiota may play an important role in the process of *T. spiralis* infection (Fig. 1A). Mice were infected with *T. spiralis* after 10 days of antibiotics gavage, and the infection differences between mice with healthy gut microbiota and those with gut microbiota dysbiosis were evaluated over the next 35 days. We found that compared with the Ts group, the body weight of mice in the Abx + Ts group decreased significantly, especially from the 14th to the 35th day after infection (Fig. 1B, $P < 0.05$). Because *T. spiralis* parasitizes in the host's intestine and multiplies in large numbers, it migrates throughout the body via the bloodstream and

eventually forms cysts in the muscles. We detected the burdens of adult worms in the intestine (Fig. 1C) and muscle larvae (Fig. 1D) , and evaluated the damage caused by *T. spiralis* to the intestine (Fig. 1E) and the number of cysts in the muscles (Fig. 1F). The results showed that compared with the Ts group, the Abx + Ts group exhibited significantly reduced parasite burden and number of cysts, along with less severe damage to muscle fiber integrity and alleviated intestinal villi injury. These results indicate that the gut microbiota facilitates *T. spiralis* establishment in the host.

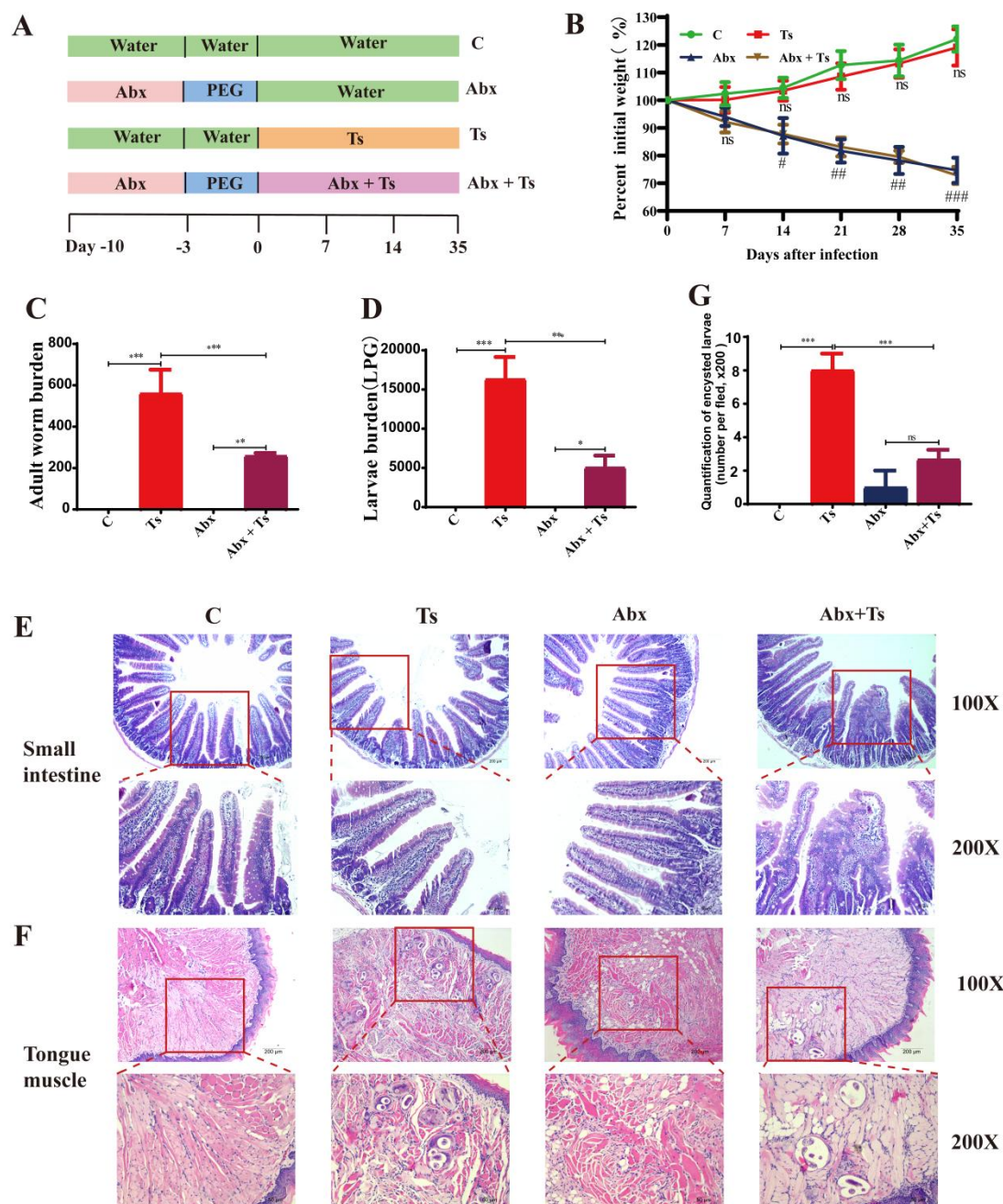


Fig. 1 The gut microbiota is beneficial for the infection of *T. spiralis*. **A** Gut microbiota dysbiosis model was established (n=5/group) (C, BALB/c mice provided with sterile water; Abx, BALB/c mice administered antibiotics; Ts, BALB/c mice challenged *T. spiralis*; Abx+Ts, BALB/c mice infected with *T. spiralis* after antibiotic administration). Comparative analysis of weight loss (**B**), adult worm burden (**C**), and muscle larvae burden (**D**) in each group of mice. Comparison of histopathological changes (H&E staining) in the small intestine (**E**) and tongue muscle (**F**) and quantification of encysted larvae in the tongue muscle (**G**) of antibiotic-treated mice subsequently infected with *T. spiralis*. The results are presented as the means $\pm$ SD, and statistical significance was calculated by t tests for two groups and one-way ANOVA for four groups. * $P < 0.05$; ** $P < 0.01$; **** $P < 0.001$.

2.2 *T. spiralis* can effectively intervene in mouse colitis

We established a colitis mouse model via rectal administration of TNBS to determine whether *T. spiralis* can effectively intervene in colitis (Fig. 2A). The results showed that on day 3 after establishing the colitis model, compared with the C group, mice in the TN group exhibited progressive weight loss (Fig. 2C), decreased survival rate (Fig. 2B), a greater DAI (Fig. 2D) and macroscopic score (Fig. 2G), a shorter colon length (Fig. 2E, 2F), and elevated MPO activity (Fig. 2H). Compared with the TN group, mice in the Ts + TN group exhibited significantly higher survival rate (Fig. 2B, $P < 0.05$) and notably lower DAI scores, particularly on days 2 and 3 post-modeling (Fig. 2D, $P < 0.05$). While there was a reduction in diarrhea severity, decreased defecation frequency, and a tendency toward increased body weight in the Ts + TN group, these changes did not reach statistical significance (Fig. 2C, $P > 0.069$). The colon length of mice in TN group was obviously shorter than that in the C group (Fig. 2E, $P < 0.001$); while colon shortening in the Ts+TN group was significantly alleviated (Fig. 2E, 2F) and both the colonic macroscopic score (Fig. 2G, $P < 0.05$) and MPO activity (Fig. 2H, $P < 0.05$) were remarkably reduced compared with TN group. Moreover, compared to the TN group, the Ts+TN group exhibited significantly increased numbers of Tregs and Th2-type immune cells, along with significantly elevated levels of their associated cytokines and transcription factors (Fig. 2I-2K, $P < 0.05$); conversely, this group showed significantly decreased

152 numbers of Th1 and Th17-type immune cells, as well as significantly reduced levels
153 of their associated cytokines and transcription factors (Fig. 2I-2K, $P < 0.05$). These
154 indicating that *T. spiralis* ameliorates colitis by activating Tregs and inducing a
155 Th2-type immune response, thereby suppressing inflammation-driven Th1 and
156 Th17-type immune responses. To further evaluate the intervene efficiency of *T.*
157 *spiralis*, colonic pathological injury was evaluated. The degree of colonic pathological
158 damage in the TN group was significantly higher than that in the C group, while the
159 pathological damage was significantly ameliorated in the Ts+TN group (Fig. 2L, $P <$
160 0.001).

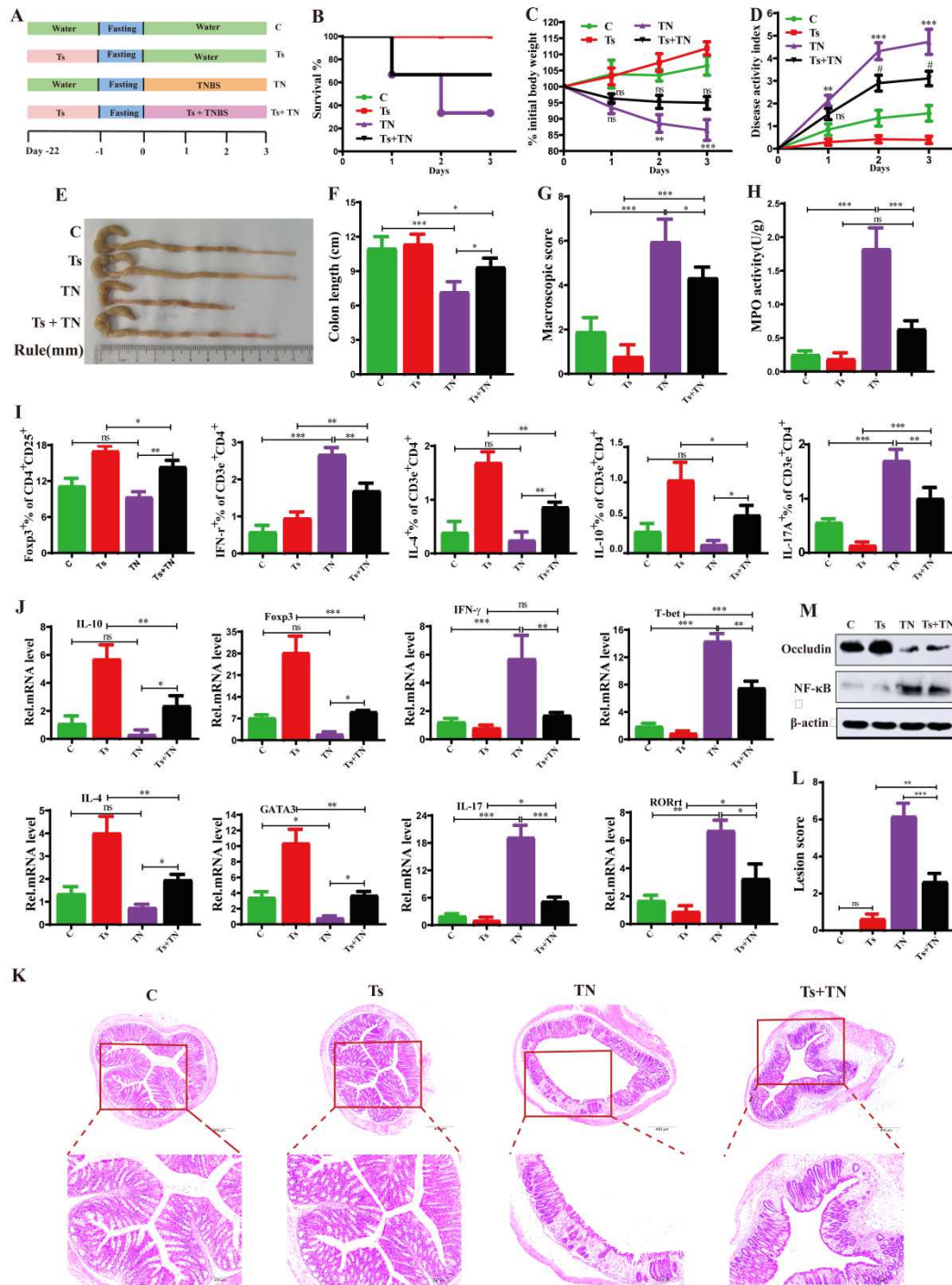


Fig. 2 *T. spiralis* can effectively intervene in mouse colitis. **A** Establishment of a murine colitis model (n=5/group) (TN, colitis was induced by intrarectal administration of TNBS; Ts+TN, colitis was induced after *T. spiralis* infection). Comparative analysis of survival (**B**), weight loss (**C**), disease activity index (**D**), colon length (**E**, **F**), macroscopic score (**G**) and MPO activity (**H**) in each group of mice. **I** The percentage of Treg (CD4⁺CD25⁺Fosp3), Th1 (CD3⁺CD4⁺IFN- γ), Th2 (CD3⁺CD4⁺IL-4 and CD3⁺CD4⁺IL-10), and Th17 (CD3⁺CD4⁺IL-17A) cells in colonic tissues was determined by flow cytometry. **J** The mRNA expression levels of cytokines and transcription

factors associated with Tregs (IL-10, Foxp3), Th1 (IFN- γ , T-bet), Th2 (IL-4, GATA3) and Th17 (IL-17A, ROR γ t) cells in colonic tissues were evaluated by RT-qPCR. **K** Comparison of histopathological changes (H&E staining) of colonic tissues. **L** Histopathological scoring of tissues. **M** The expression levels of tight junction protein (occludin) and the nuclear transcription factor regulating inflammatory responses (NF- κ B) in colonic tissues were assessed by western blot. The results are presented as the means $\pm$ SD, and statistical significance was calculated by t tests for two groups and one-way ANOVA for four groups. * P < 0.05; ** P < 0.01; *** P < 0.001.

2.3 *T. spiralis*-induced gut microbiota plays an crucial role in alleviating murine colitis

Experimental mice were allowed to freely drink water containing broad-spectrum antibiotics to deplete partial gut microbiota and establish a model of gut microbiota dysbiosis. Metagenomic sequencing was employed to validate whether the intestinal microbiota dysbiosis model was successfully established. Following antibiotic treatment, both the variety of species (Fig. S1B, S1C) and the number of ASVs (Fig. S1A) in the mice intestinal bacteria drastically decreased. The microbiota structure changed concurrently (Fig. S1D), and the diversity and abundance dropped (Fig. S1E, S1F), suggesting that the intestinal microbiota dysbiosis model in mice was successfully established after antibiotic treatment. Then, the mice were intragastrically administered PEG solution and infected with *T. spiralis*, TNBS was subsequently administered to investigate whether the intestinal microbiota plays an important role in alleviation of colitis by *T. spiralis* (Fig. 3A).

Compared with the intestinal dysbiosis model group (Abx+Ts+TN group), the Ts+TN intervention group exhibited a significantly higher survival rate (Fig. 3B, P < 0.05), concomitant with markedly reduced DAI (Fig. 3D, P < 0.05), colon macroscopic score (Fig. 3G, P < 0.05), and MPO activity (Fig. 3H, P < 0.05). The shortening of colon length was limited to merely 19.3 % in the Ts+TN intervention group (versus 41.4 % in the model group) (Fig. 3E, 3F). Relative to the Abx+Ts+TN group, the Ts+TN intervention group effectively alleviated TNBS-induced intestinal epithelial injury and crypt erosion (Fig. 3L, 3M) and restored the integrity of the colonic mucosal barrier (Fig. 3N). Mechanistic analyses revealed that the Ts+TN

199 intervention group significantly increased the levels of anti-inflammatory immune
200 cells (Tregs and Th2) and anti-inflammatory cytokines (IL-13, IL-10, IL-4 and
201 TGF- β), while reducing the abundance of pro-inflammatory immune cells (Th1 and
202 Th17) and pro-inflammatory cytokines (IL-1 β , TNF- γ and IL-17) (Fig. 3I-3K). These
203 results indicate that the gut microbiota plays a critical role in mediating the protective
204 effect of *T. spiralis* intervention against colitis.

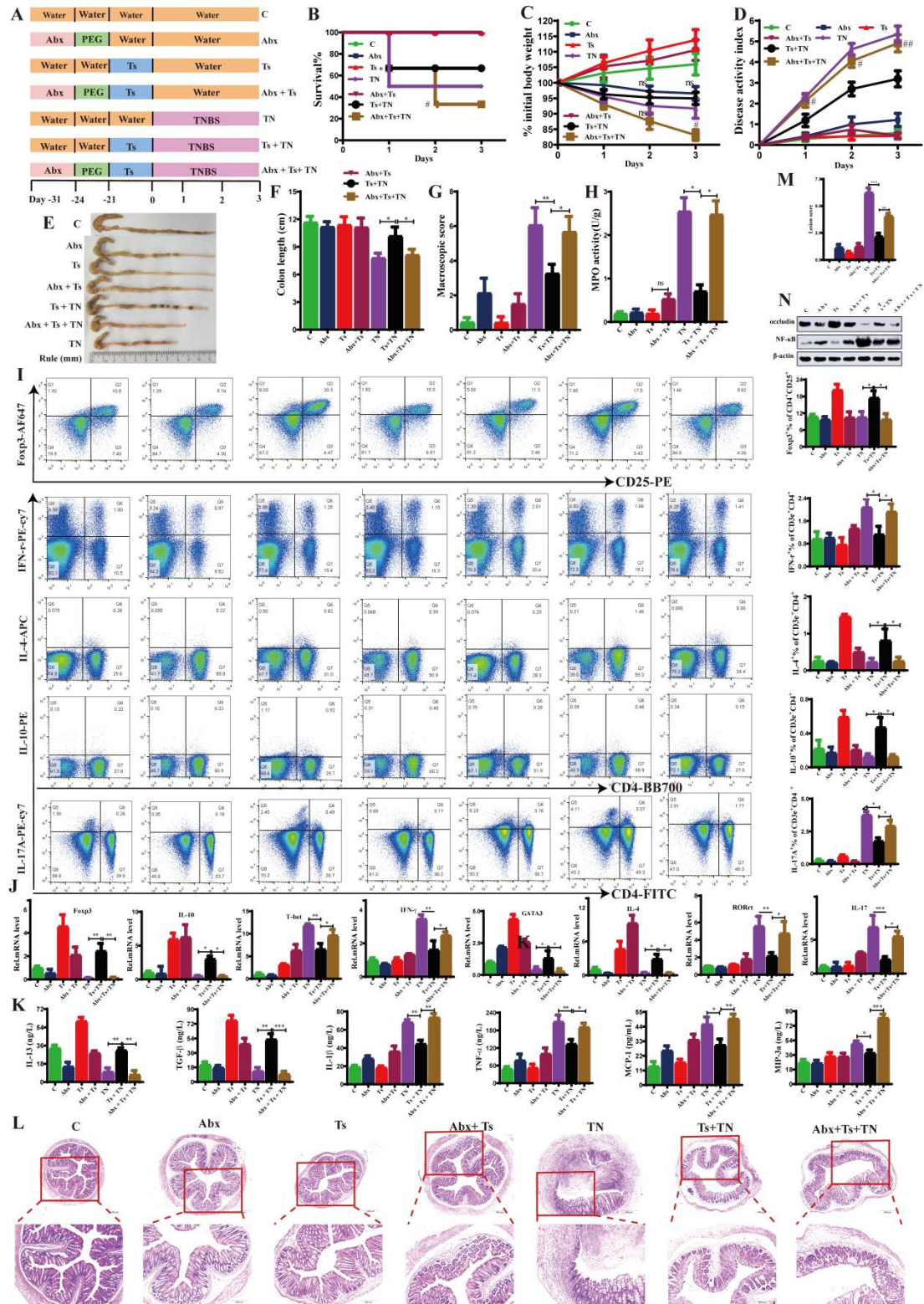


Fig. 3 *T. spiralis*-induced gut microbiota plays a crucial role in alleviating murine colitis. **A** Establishment of a gut microbiota dysbiosis model in colitis mice. (n=5/group) (Abx+Ts+TN, broad-spectrum antibiotics were provided in the drinking water for 7 d, followed by intragastric gavage with PEG solution once every 6 h for 3 consecutive days, then, the mice were infected

with *T. spiralis*, TNBS was administered intrarectally to induce colitis at 21 dpi). Comparative analysis of survival (B), weight loss (C), disease activity index (D), colon length (E, F), macroscopic score (G) and MPO activity (H) in each group of mice. I The percentage of Treg (CD4⁺CD25⁺Foxp3), Th1 (CD3⁺CD4⁺IFN- γ), Th2 (CD3⁺CD4⁺IL-4 and CD3⁺CD4⁺IL-10), and Th17 (CD3⁺CD4⁺IL-17A) cells in colonic tissues was determined by flow cytometry. J The mRNA expression levels of cytokines and transcription factors associated with Tregs (IL-10, Foxp3), Th1 (IFN- γ , T-bet), Th2 (IL-4, GATA3) and Th17 (IL-17A, ROR γ t) cells in colonic tissues were evaluated by RT-qPCR. K The levels of pro-inflammatory cytokines (IL-13, IL-1 β , TNF- α) and chemokines (MCP-1, MIP-3 α) were determined by ELISA in mouse serum. L Comparison of histopathological changes (H&E staining) of colonic tissues. M Histopathological scoring of tissues. N The expression levels of tight junction protein (occludin) and the nuclear transcription factor regulating inflammatory responses (NF- κ B) in colonic tissues were assessed by western blot. The results are presented as the means $\pm$ SD, and statistical significance was calculated by t tests for two groups and one-way ANOVA for four groups. * P < 0.05; ** P < 0.01; *** P < 0.001.

2.4 FMT of the gut microbes of mice infected with *T. spiralis* can alleviate murine colitis

To further evaluate whether the *T. spiralis*-induced gut microbiota has a role in alleviating murine colitis, the fecal microbiota of *T. spiralis*-infected mice (donors) was transplanted into TNBS-induced colitis mice (recipients) (Fig. 4A). Compared with the TN group, the recipient mice (FMT group) showed a trend of recovery towards normal in terms of survival rate (Fig. 4B, P < 0.05), body weight (Fig. 4C, P < 0.05), DAI score (Fig. 4D, P < 0.05), colon macroscopic score (Fig. 4G, P < 0.01), MPO activity (Fig. 4H, P < 0.01), and histopathological changes (Fig. 4L, 4M). FMT not only significantly increased the levels of anti-inflammatory cytokines (IL-13, TGF- β) and decreased the levels of pro-inflammatory cytokines (IL-1 β , TNF- α) (Fig. 4K, P < 0.05), but also significantly restored the intestinal barrier function of colitis mice (Fig. 4N). More importantly, the transplantation of *T. spiralis*-induced gut microbiota increased the proportions of Tregs and Th2 immune cells, as well as the transcriptional levels of their related cytokines and transcription factors, while

decreasing the proportions of Th1 and Th17 immune cells (Fig. 4I, $P < 0.05$), along with the transcriptional levels of their related cytokines and transcription factors (Fig. 4J, $P < 0.05$). These results indicate that the *T. spiralis*-induced gut microbiota exhibits an effective role in improving colitis.

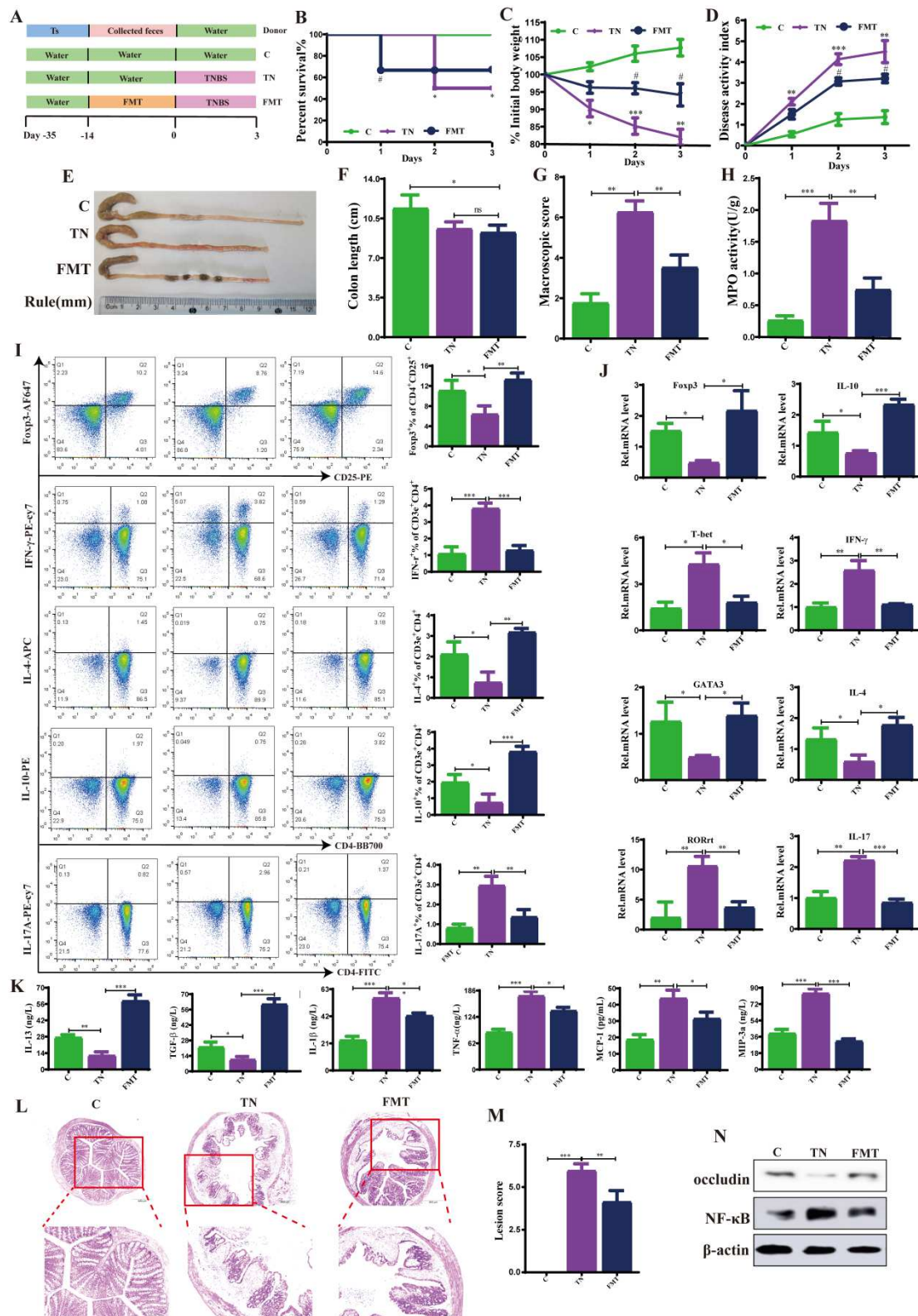


Fig. 4 FMT of the gut microbes of mice infected with *T. spiralis* can alleviate murine colitis. **A** Fecal microbiota from *T. spiralis*-infected mice was transplanted into colitis mice (Donors, mice infected with *T. spiralis*; FMT, colitis mice treated with FMT from *T. spiralis*-infected mice). Comparative analysis of survival (**B**), weight loss (**C**), disease activity index (**D**), colon length (**E**, **F**), macroscopic score (**G**) and MPO activity (**H**) in each group of mice. **I** The percentage of Treg (CD4⁺CD25⁺Foxp3), Th1 (CD3⁺CD4⁺IFN- γ), Th2 (CD3⁺CD4⁺IL-4 and CD3⁺CD4⁺IL-10), and Th17 (CD3⁺CD4⁺IL-17A) cells in colonic tissues was determined by flow cytometry. **J** The mRNA expression levels of cytokines and transcription factors associated with Tregs (IL-10, Foxp3), Th1 (IFN- γ , T-bet), Th2 (IL-4, GATA3) and Th17 (IL-17A, ROR γ t) cells in colonic tissues were evaluated by RT-qPCR. **K** The levels of pro-inflammatory cytokines (IL-13, IL-1 β , TNF- α) and chemokines (MCP-1, MIP-3 α) were determined by ELISA in mouse serum. **L** Comparison of histopathological changes (H&E staining) of colonic tissues. **M** Histopathological scoring of tissues. **N** The expression levels of tight junction protein (occludin) and the nuclear transcription factor regulating inflammatory responses (NF- κ B) in colonic tissues were assessed by western blot. The results are presented as the means $\pm$ SD, and statistical significance was calculated by t tests for two groups and one-way ANOVA for four groups. * P < 0.05; ** P < 0.01; *** P < 0.001.

2.5 Bacteroides and secondary bile acids in the mice intestine are significantly enriched after FMT

We analyzed the different species among the FMT and TN groups through metagenomics. After the genes were assembled and screened, we found that the microbial community structures between the FMT group and the TN group were significantly different (Fig.5A). Then, we calculated the relative abundances of the bacteria after species annotation. The abundances of Bacteroidota and Bacteroidales obviously increased after FMT (Fig. 5B, 5C), specifically *B. vulgatus*, *B. acidifaciens*, and *B. sartorii* (Fig. 5D, 5E), whereas the abundances of *L. taiwanensis* and *Muribaculum* sp002492595 were depleted (Fig. 5E). In addition, the LEfSe was used to analyze the differences in species and the comparison calculation results are the same, namely, the abundances of Bacteroidota, Bacteroidales and Bacteroidia markedly increased, especially *B. acidifaciens* (Fig. 5F).

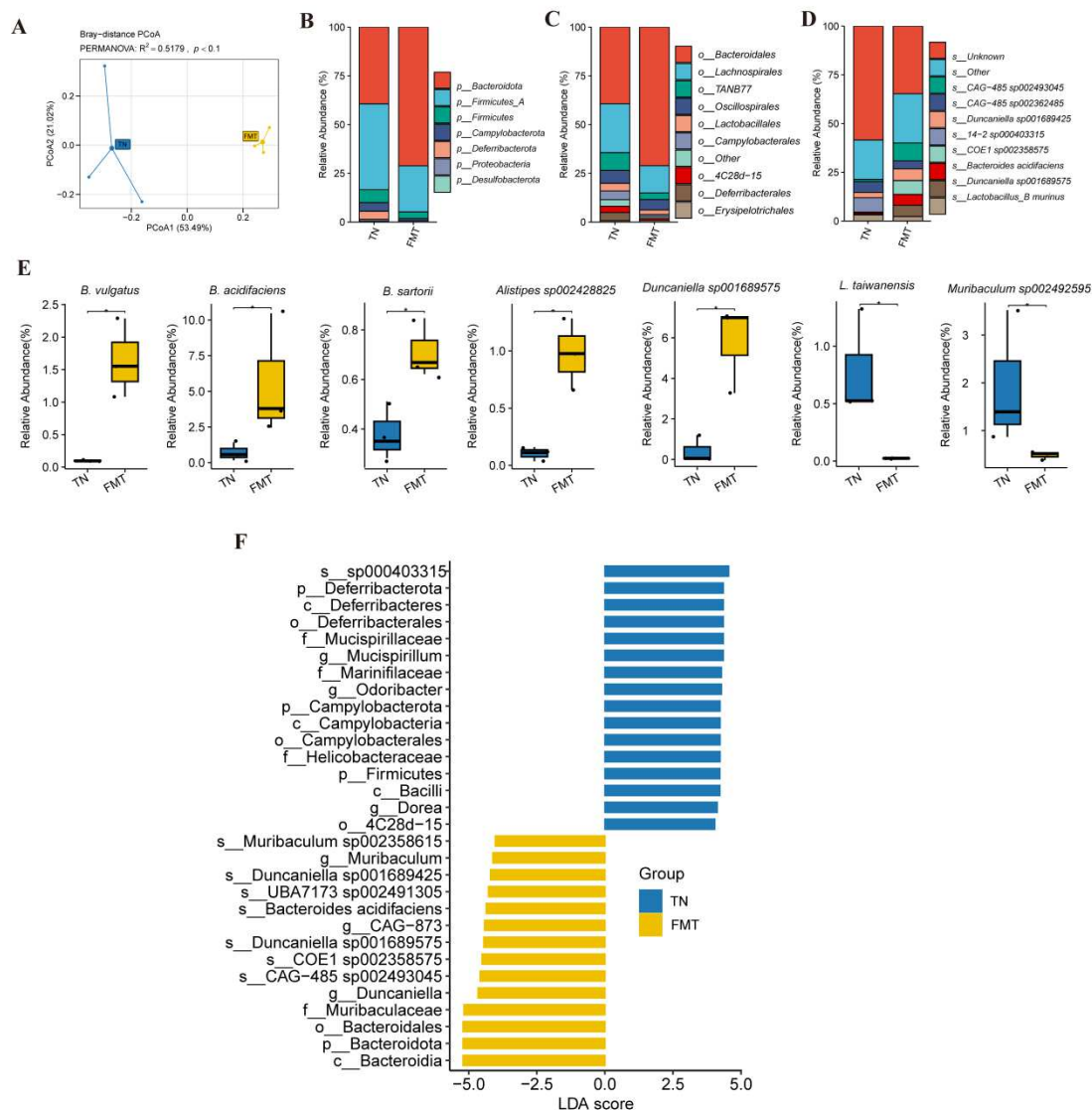


Fig. 5 Bacteroides and secondary bile acids in the mice intestine are significantly enriched after FMT. **A** PCoA score plots for mice samples. Phylum (**B**), order (**C**) and species (**D**) comparison analysis (top 10). **E** Analysis of significantly upregulated and downregulated species in the abundance table. **F** LefSe analysis (LDA score > 5). The results are presented as the means $\pm$ SD, and statistical significance was calculated by one-way ANOVA. $*P < 0.05$.

Next, we subjected intestinal contents of mice to nontargeted metabolomics. A model containing metabolic information by fitting the partial least squares discriminant analysis (PLS-DA) through 200 times random permutation testing was performed to confirm the ability of the PLS-DA to correctly classify new samples (Fig. 6A). The results demonstrated that the model was reliable and not overfitting. And the PLS-DA score plots ($p < 0.05$, Fig. 6B) confirmed that FMT significantly

286 altered the clustering of intestinal metabolites in colitis mice. The volcano plot
287 illustrates the overall distribution of different metabolites. A total of 447 metabolites
288 were identified, including 230 significantly upregulated metabolites and 217
289 significantly downregulated metabolites (Fig. 6C, $P < 0.05$). After FMT, proline,
290 isoleucine, and N-(2-Hydroxy-3-butenyl)-L-valine were significantly decreased,
291 whereas CDCA and UDCA were significantly increased (Fig. 6D, $P < 0.01$).
292 Furthermore, cluster analysis of metabolites also revealed consistent results (Fig. 6F).
293 KEGG pathway enrichment analysis of the differential metabolites was also
294 performed. After FMT, pyruvate metabolism, pyruvate metabolism, phenylalanine,
295 tyrosine and tryptophan biosynthesis were both greatly decreased, while bile secretion
296 was significantly enriched (Fig. 6E). Relevant analyses revealed that UDCA was
297 positively correlated with *B. acidifaciens* and *B. vulgatus*; CDCA was negatively
298 correlated with *B. acidifaciens* and *B. vulgatus* (Fig. 6G).

and TN mice (n=6). The vertical and horizontal dashed lines indicate the threshold for the twofold abundance difference and the p=0.05 threshold, respectively. Student's t test was performed for the comparison. Significant metabolites are presented in red (upregulated) or blue (downregulated). **D** Metastats analysis counted the significantly up and downregulated differential metabolites. **E** KEGG pathway enrichment analysis of FMT and TN mice (n=6). **F** Hierarchical cluster analysis of intestinal metabolites that were different between FMT and TN mice (n=6)

[1:Xi-7-Hydroxyhexadecanedioic	acid;	2:
(3beta,17alpha,23s)-17,23-epoxy-3,28,29-trihydroxy-27-norlanost-8-en-24-one;		3:
(3beta,17alpha,23s)-17,23-epoxy-3,28,29-trihydroxy-27-norlanost-8-en-24-one;		4:
(3beta,17alpha,23r)-17,23-epoxy-3,29-dihydroxy-27-norlanost-8-ene-15,24-dione;		5:
(4beta,5beta,6beta,14beta,15alpha,20s,22r)-5,6-epoxy-4,14,15-trihydroxy-1-oxowitha-2,24-dienolide].		

G Correlation analysis between macrogenomics and metabolomics based on pearson correlation coefficient. The results are presented as the means $\pm$ SD, and statistical significance was calculated by one-way ANOVA. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

2.6 *B. acidifaciens* and UDCA can ameliorate murine colitis

We have established a bacterial protection model to confirm that *B. acidifaciens* may plays an important role in the process of alleviating colitis in mice (Fig. 7A). To determine the colonization profile of bacterial strains in the gut, we administered *B. acidifaciens* to mice for fourteen consecutive days. Analysis of the intestinal contents was conducted on the 0 day and 7th and 14th day post-administration. The results indicated successful colonization of bacterial strains within the gastrointestinal tracts of mice (Fig. S2H). We utilized and then 16S sequencing verification to check the effectiveness of the elimination. The outcomes demonstrated that antibiotic treatment reduced the number of mice intestinal ASVs (Fig. S2A), microbial diversity (Fig. S2B, S2C), and species abundance (Fig. S2E) and changed the structure of the microbiota (Fig. S2D, S2F, S2G). Subsequently, *B. acidifaciens* was supplemented, and TNBS was rectally administered on day 0 to observe the protective effect. Following the addition of *B. acidifaciens*, there were no statistically significant differences in survival rate (Fig. 7B, $P > 0.37$) and weight loss (Fig. 7C, $P > 0.48$) of the mice. However, the disease activity index (Fig. 7D, $P < 0.05$), macroscopic score (Fig. 7G,

$P < 0.01$), MPO activity (Fig. 7H, $P < 0.01$), and colon length (Fig. 7E, 7F, $P < 0.05$) were significantly improved. Additionally, feeding *B. acidifaciens* helped reduce the area of colonic lesions, the number of inflammatory cells, as well as the degree of colonic tissue structure damage and histopathological injury (Fig. 7I, 7J).

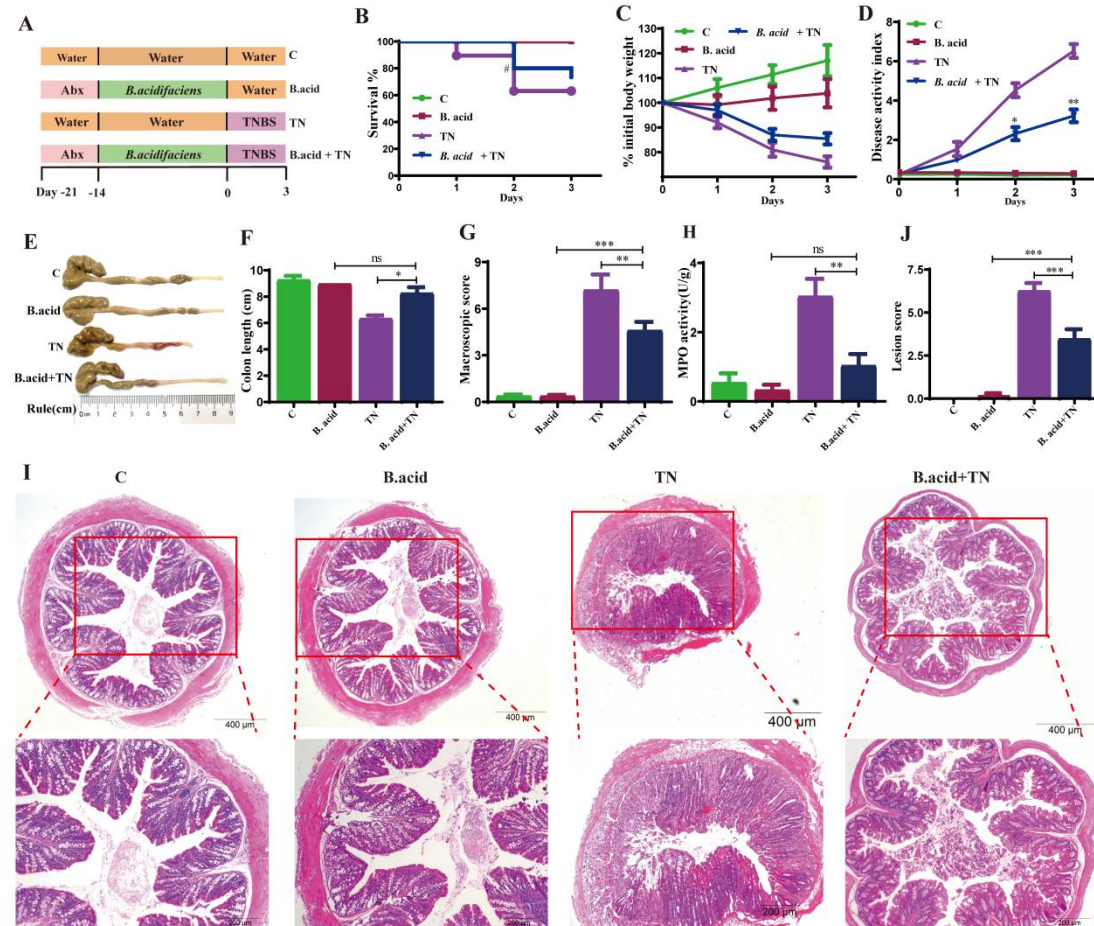


Fig. 7 *B. acidifaciens* can ameliorate murine colitis. **A** Protective model of *B. acidifaciens* in murine colitis (B. acid, mice were administered *B. acidifaciens* via oral gavage; B. acid+TN, After oral gavage with *B. acidifaciens* for 14 days, mice were administered TNBS rectally to induce colitis). Comparative analysis of survival (**B**), weight loss (**C**), disease activity index (**D**), colon length (**E**, **F**), macroscopic score (**G**) and MPO activity (**H**) in each group of mice. **I** Comparison of histopathological changes (H&E staining) of colonic tissues. **J** Histopathological scoring of tissues.

Secondary bile acids are a class of bioactive molecules that are generated through the transformation of primary bile acids mediated by the gut microbiota. For example, 3-oxoLCA suppresses Th17 cell differentiation and isoalloLCA enhances

Tregs differentiation, which are crucial in autoimmune diseases and other inflammatory conditions. Therefore, we speculate that UDCA, which are secondary bile acids, may alleviate colitis in mice. We established a supplementary UDCA protection model to confirm this presumption (Fig. S3A). These results found that there were no statistically significant differences in the survival rate (Fig. S3B, $P > 0.084$), weight loss (Fig. S3C, $P > 0.070$), and colon length (Fig. S3E, S3F, $P > 0.21$) of the mice. However, the disease activity index (Fig. S3D, $P < 0.01$), macroscopic score (Fig. S3G, $P < 0.01$), and MPO activity (Fig. S3H, $P < 0.01$) were significantly improved. Additionally, adding UDCA helped alleviate the degree of damage to the structure of colonic crypts, the degree of edema in loose connective tissues, and reduce the degree of histopathological damage (Fig. S3I, 3J).

2.7 *B. acidifaciens* decomposes tauro ursodeoxycholic acid (TUDCA) into UDCA by producing bile salt hydrolase (BSH)

Because *Bacteroides* species exhibit a conserved high expression of BSH, conjugated bile acids can be hydrolyzed by BSH to unconjugated bile acids and glycine or taurine²⁵. Therefore, we hypothesize that *B. acidifaciens* also harbors the BSH gene, which hydrolyzes TUDCA to yield UDCA and taurine. TUDCA was separately supplemented into the culture systems of *B. acidifaciens*, *Lactobacillus reuteri* and *Lactobacillus bulgaricus* during their cultivation. The result demonstrated that UDCA was exclusively detected in the culture of *B. acidifaciens* (Fig. 8A). Next, we measured BSH levels in the intestinal contents of each group, and found that BSH levels were increased in the *B. acidifaciens* group (B. acid) compared with *Lactobacillus reuteri* group (L. reut) and *Lactobacillus bulgaricus* group (L. bulg) (Fig. 8B). The BSH activity of *B. acidifaciens* was confirmed by incubating bacterial lysates with conjugated bile acids and measuring the level of UDCA released from TUDCA (Fig. 8C). To further evaluate the critical role of *B. acidifaciens* in alleviating colitis through the hydrolysis of conjugated bile acids into UDCA by BSH, we constructed recombinant plasmids with the BSH sequence from *B. acidifaciens* and transformed them into *Escherichia coli* (Fig. S4A, S4B). Mice were treated with pBAD-BSH-transformed *E. coli* (E+BSH) or pBAD-vector transformed *E. coli* (E+V)

to verify the ability of BSH to hydrolyze bile acids into UDCA. Compared with the E+V group, the content of UDCA in the intestinal contents of mice in the E+BSH group was significantly increased (Fig. 8D, $P < 0.001$). Furthermore, our results verified that E+BSH can also obviously decrease the serum pro-inflammatory cytokines IL-6, IL-1 β , and IL-18 levels in colitis mice (Fig. 8E-8G, $P < 0.05$) and reduce colonic pathological lesions (Fig. 8H, 8I, $P < 0.05$).

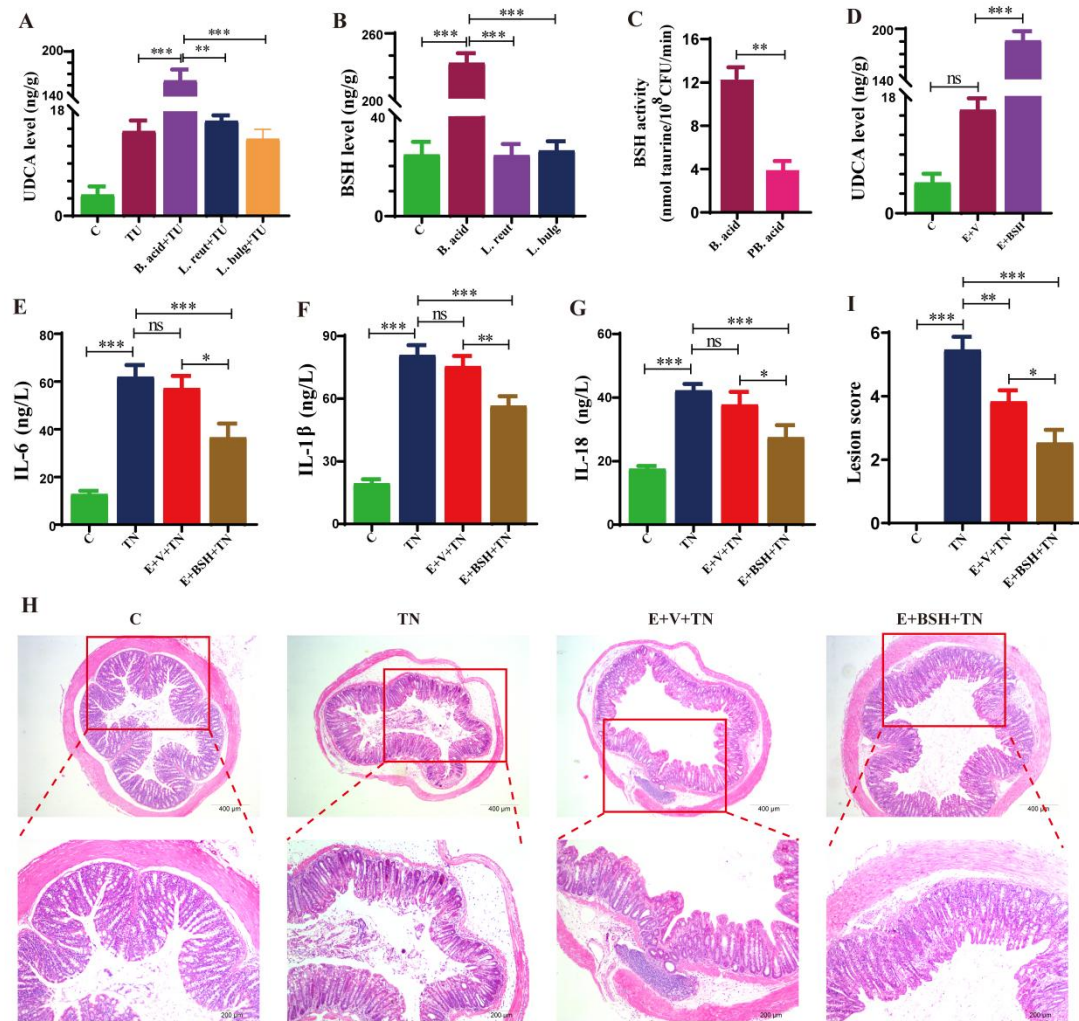


Fig. 8 *B. acidifaciens* decomposes TUDCA into UDCA by producing BSH. **A** UDCA levels in cultures were measured by ELISA (TU, only TUDCA was added to the culture; B. acid+TUDCA, TUDCA was supplemented into the culture of *B. acidifaciens*; L. reut+TUDCA, TUDCA was supplemented into the culture of *L. reuteri*; L. bulg+TUDCA, TUDCA was supplemented into the culture of *L. bulgaricus*). **B** BSH levels in intestinal contents were measured by ELISA. **C** A ninhydrin assay was used to detect the release of taurine from conjugated bile acids as an index of BSH activity (B. acid, supplemented with live *B. acidifaciens*; PB. acid, supplemented with

pasteurized *B. acidifaciens*). **D** UDCA levels in cultures were measured by ELISA (E+V, mice were treated with pBAD-vector transformed *E. coli*; E+BSH, mice were treated with pBAD-BSH-transformed *E. coli*). The levels of pro-inflammatory cytokines IL-6 (**E**), IL-1 β (**F**), and IL-18 (**G**) in the serum of colitis mice were measured by ELISA (E+V+TN, after treated with pBAD-vector, TNBS was administered rectally to mice for the induction of colitis; E+BSH+TN, after treated with pBAD-BSH, TNBS was administered rectally to mice for the induction of colitis). **H** Comparison of histopathological changes (H&E staining) of colonic tissues. **I** Histopathological scoring of tissues. The results are presented as the means $\pm$ SD, and statistical significance was calculated by one-way ANOVA. * P < 0.05; ** P < 0.01; *** P < 0.001.

2.8 UDCA inhibits NLRP3 inflammasome activation

We detected that treatment of LPS-primed bone marrow-derived macrophages (BMDMs) with UDCA blocked caspase-1 maturation and IL-1 β or IL-18 secretion in a dose-dependent manner (Fig. 9A-9C). Similar inhibitory effects of UDCA on NLRP3 inflammasome activation were also observed in bone marrow-derived dendritic cells (BMDCs) (Fig. 9D-9F). Next, we observed that UDCA had no effect on LPS-induced TNF- α production when BMDMs were treated with UDCA after 3 h LPS priming, indicating that UDCA did not affect the priming stage under these conditions. In contrast, UDCA inhibited LPS-induced TNF- α production when BMDMs were treated with UDCA for 3 h before LPS priming (Fig. 9G-9I). We further noted that UDCA had a similar effect on NLRP3 inflammasome activation independent of the pretreatment duration before nigericin stimulation (Fig. 9J), indicating its prompt inhibitory effect on the inflammasome activation stage.

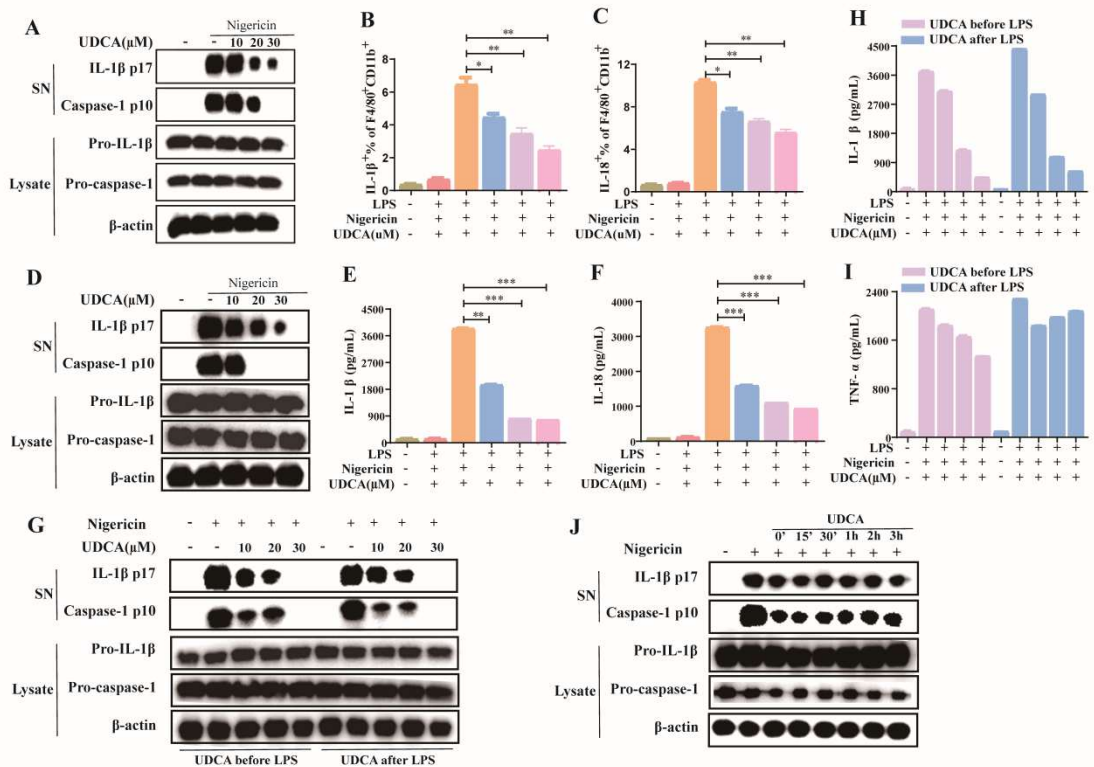


Fig. 9 UDCA inhibits NLRP3 inflammasome activation. LPS-primed BMDMs (A) and BMDCs (D) were treated with different doses of UDCA and then stimulated with nigericin for 40 min. Supernatants (SN) and cell extracts (Lysate) were analyzed by immunoblotting (A and D). The levels of IL-1 β (B) and IL-18 (C) cytokines in stimulated BMDMs were measured by flow cytometry. The levels of IL-1 β (E) and IL-18 (F) in the cell culture supernatant of stimulated BMDCs were determined by ELISA. BMDMs were treated with different doses of UDCA for 3 h and then stimulated with LPS for 3 h, or BMDMs were primed with LPS for 3 h and then treated with different doses of UDCA for 3 h, then, the cells were stimulated with nigericin. Supernatants (SN) and cell extracts (Lysate) were analyzed by immunoblotting (G). Supernatants were analyzed by ELISA for IL-1 β (H) and TNF- α (I) release. BMDMs were primed with LPS for 3 h and then treated with 50 μ M UDCA for different times as indicated, then, the cells were stimulated with nigericin. SN and Lysate were analyzed by immunoblotting (J).

2.9 UDCA inhibit NLRP3 inflammasome activation via TGR5 signaling

Recent studies have demonstrated that BAs activate distinct receptors expressed in various tissues and cell types, and TGR5 has been identified as a membrane receptor for activated bile acids. Given that UDCA exerts an activating effect on the NLRP3 inflammasome, we hypothesize that UDCA may inhibit this activation via

431 TGR5 signaling. To substantiate this mechanism, we administered UDCA to TGR5
432 knockout (TGR5^{-/-}) and wild-type (WT) mice for four weeks prior to TNBS-induced
433 colitis. As expected, UDCA significantly ameliorated TNBS-induced clinical
434 manifestations of colitis in WT mice, as evidenced by reduced DAI (Fig. S3D, $P <$
435 0.01), MPO activity (Fig. S3H, $P <$ 0.01), and histopathological scores (Fig. 3J). In
436 contrast, this protective effect was largely abrogated in TGR5^{-/-} mice (Fig. 10A-10E).
437 To further dissect the cellular basis of this observation, we isolated colonic DCs and
438 Mφ from the respective mouse groups. UDCA treatment potently suppressed IL-1β
439 and IL-18 secretion in both DCs and Mφ derived from WT mice, whereas this
440 inhibitory effect was completely lost in cells isolated from TGR5^{-/-} mice (Fig.
441 10F-10J). Taken together, these results demonstrate that UDCA inhibits NLRP3
442 inflammasome activation and alleviates colitis in a TGR5-dependent manner.

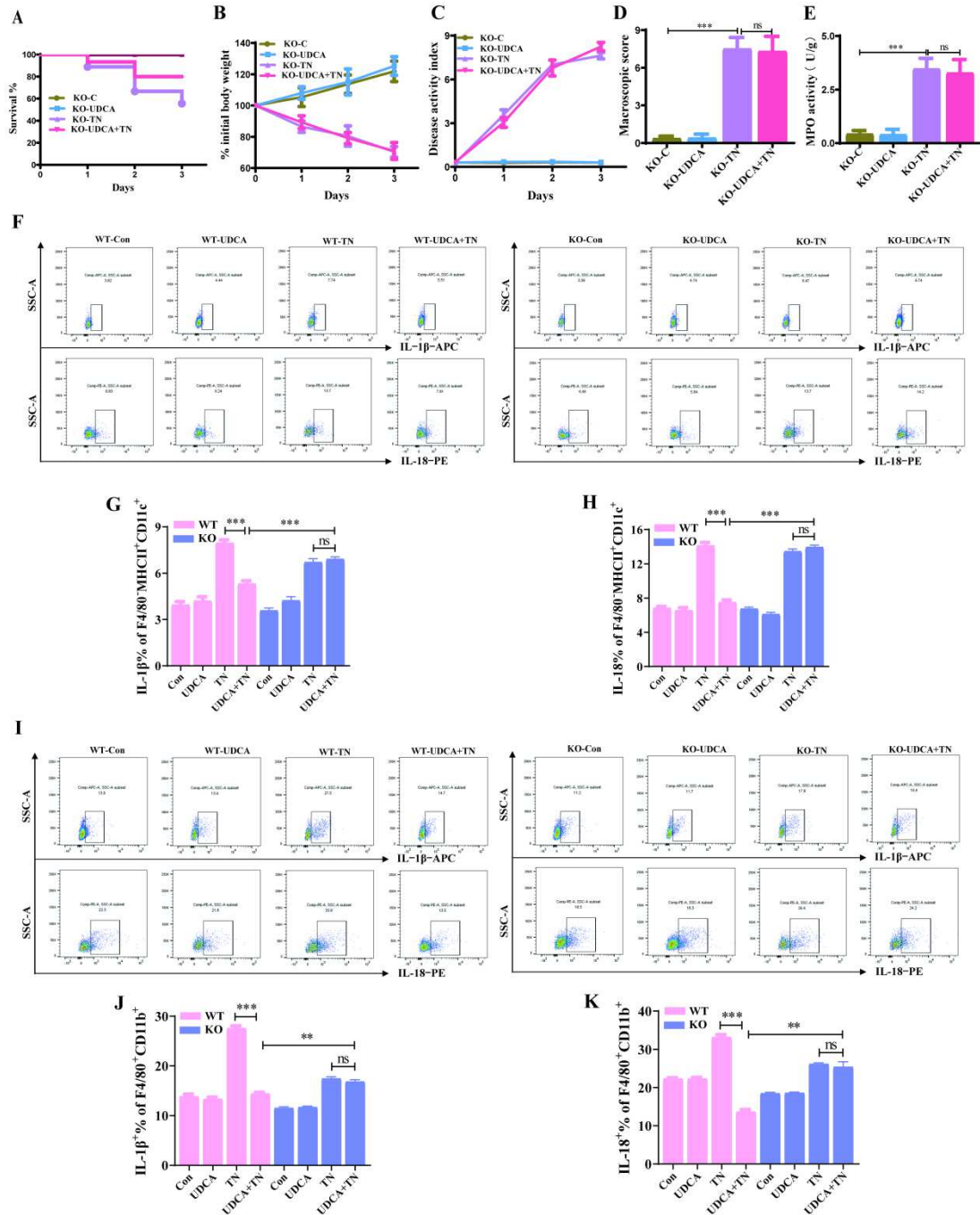


Fig. 10 UDCA inhibit NLRP3 inflammasome activation via TGR5 signaling. The $TGR5^{-/-}$ and WT mice were administered with UDCA for four weeks prior to TNBS-induced colitis. Comparative analysis of survival (A), weight loss (B), disease activity index (C), macroscopic score (D) and MPO activity (E) in each group of mice. The percentage of IL-1 β and IL-18 in DCs (F-H) and M ϕ (I-K) of $TGR5^{-/-}$ and WT mice were determined by flow cytometry.

Discussion

IBD is a chronic recurrent gastrointestinal autoimmune disease with complex pathogenesis, and the “hygiene hypothesis” suggests that the decline in parasitic infection rates is closely associated with the increasing incidence of IBD globally^{26,27}. Helminth therapy has thus emerged as a potential strategy for IBD intervention²⁸, but the direct application of pathogenic helminths such as *T. spiralis* poses safety risks, limiting its clinical translation²⁹. In this study, we systematically explored the mechanism by which *T. spiralis* alleviates TNBS-induced colitis in mice through multi-omics and functional validation experiments, and identified that *T. spiralis*-induced gut microbiota remodeling, particularly the enrichment of *B. acidifaciens* and its metabolite UDCA, mediates colitis intervention via the NLRP3 inflammasome signaling pathway in DCs and Mφ. This finding not only clarifies the key role of the “helminth-gut microbiota-metabolite-immune cell” regulatory axis in IBD, but also provides a safe and feasible alternative strategy for helminth-based therapy.

The gut microbiota is a key mediator of the crosstalk between helminths and the host immune system³⁰⁻³². Previous studies have shown that *Schistosoma mansoni* and *Hymenolepis diminuta* can alleviate colitis by regulating the gut microbiota^{24,33}, but the specific mechanism by which *T. spiralis* modulates the gut microbiota to exert anti-inflammatory effects remains unclear. Our results demonstrated that antibiotic-induced gut microbiota dysbiosis significantly weakened the ability of *T. spiralis* to alleviate colitis, as evidenced by reduced survival rate, increased DAI, and aggravated intestinal pathological damage in the Abx+Ts+TN group compared with the Ts+TN group. Moreover, FMT from *T. spiralis*-infected mice effectively recapitulated the anti-colitis effect of *T. spiralis* infection, including improved survival rate, reduced intestinal inflammation, and restored intestinal barrier function. These findings confirm that the gut microbiota is an indispensable intermediate link in *T. spiralis*-mediated colitis alleviation, which is consistent with the notion that helminths indirectly regulate host immunity through the gut microbiota.

Multi-omics analysis further identified the key functional components of the *T. spiralis*-induced gut microbiota. Metagenomic sequencing showed that FMT

significantly increased the abundance of Bacteroidota and Bacteroidales in colitis mice, especially *B. acidifaciens*, *B. vulgatus*, and *B. sartorii*. Untargeted metabolomics revealed a significant enrichment of secondary bile acids (including UDCA and CDCA) in the intestinal tract after FMT, and correlation analysis confirmed a positive correlation between UDCA and *B. acidifaciens*/*B. vulgatus*. Functional validation experiments showed that both *B. acidifaciens* supplementation and UDCA administration effectively alleviated TNBS-induced colitis, as reflected by reduced DAI, macroscopic score, and MPO activity, as well as improved intestinal histopathological damage. These results indicate that *B. acidifaciens* and UDCA are key effectors of the *T. spiralis*-induced gut microbiota in alleviating colitis.

BSH produced by gut microbiota plays a crucial role in bile acid metabolism by hydrolyzing conjugated bile acids into unconjugated forms³⁴⁻³⁶. Bacteroides species are known to express BSH highly conservedly³⁷, and our study confirmed that *B. acidifaciens* can hydrolyze TUDCA into UDCA through BSH. This was verified by in vitro culture experiments showing that UDCA was exclusively detected in *B. acidifaciens* cultures supplemented with TUDCA, and in vivo experiments demonstrating that mice treated with *E. coli* expressing *B. acidifaciens* BSH had significantly increased intestinal UDCA levels and reduced colitis severity. These findings clarify the specific metabolic pathway by which *B. acidifaciens* exerts anti-inflammatory effects, i.e., by producing BSH to regulate bile acid metabolism and increase UDCA production.

Bile acids exert immunomodulatory effects by binding to bile acid receptors such as TGR5 and FXR^{20,38,39}. Our study focused on the role of TGR5, a G protein-coupled receptor widely expressed in immune cells and intestinal epithelial cells. We found that UDCA inhibited caspase-1 maturation and the secretion of pro-inflammatory cytokines IL-1 β and IL-18 in LPS-primed DCs and M ϕ in a dose-dependent manner, indicating that UDCA suppresses NLRP3 inflammasome activation. Similar results were obtained with INT-777, a specific TGR5 agonist, suggesting that UDCA may act through TGR5 signaling. Further experiments using TGR5 knockout mice showed that TGR5 deficiency partially rescued UDCA-inhibited IL-1 β and IL-18 secretion,

indicating that TGR5 is a key mediator of UDCA's inhibitory effect on NLRP3 inflammasome activation, though other bile acid receptors may also be involved.

This study has revealed a novel mechanism by which *T. spiralis* alleviates colitis through the "gut microbiota-bile acid-NLRP3 inflammasome" axis, enriching the understanding of the interactions among helminths, gut microbiota, and host immunity; it has also identified *B. acidifaciens* and UDCA as key therapeutic targets, providing a safe alternative strategy to direct helminth infection for treating IBD. However, this study also has certain limitations. For instance, the research was based on mouse models, and its translational value to human IBD needs to be verified in clinical studies; although TGR5 has been confirmed as a key receptor for UDCA, the role of other bile acid receptors such as farnesoid X receptor in the anti-colitis effect of UDCA has not been explored in depth.

In summary, this study confirms that *T. spiralis* infection alleviates TNBS-induced colitis in mice by inducing gut microbiota remodeling (especially the enrichment of *B. acidifaciens*). *B. acidifaciens* produces BSH to hydrolyze TUDCA into UDCA, which then binds to TGR5 on the surface of DCs and Mφ, inhibits the activation of the NLRP3 inflammasome, reduces the production of pro-inflammatory cytokines, and simultaneously restores the integrity of the intestinal mucosal barrier. These findings provide new insights into the mechanisms of helminth-mediated immune regulation and offer potential strategies for IBD treatment targeting *B. acidifaciens*, UDCA, or TGR5. Future studies should focus on verifying these findings in human clinical samples, exploring the synergistic effects of multiple bile acid receptors, and clarifying the specific molecules involved in the *T. spiralis*-microbiota interactions.

3.Methods

3.1 Parasite, cells, bacteria strains, and metabolites

The isolate of *T. spiralis* (ISS534) has been maintained in the laboratory by repeated passages in SD mice. Based on Chen et al described methods⁴⁰. Briefly, *T.*

spiralis larvae were obtained by digesting mouse carcasses with 1 % HCl and 1 % pepsin (1:3000; Solarbio, Beijing, China).

Macrophages (Mφ) and DCs were cultured in RPMI-1640 culture medium (C11875500BT, Gibco, USA) supplemented with 10 % fetal bovine serum (SH30070.03, Hyclone, USA) at 37 °C and 5 % CO₂.

Bacteroides acidifaciens (*B. acidifaciens*) was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and the numbers of the strains were DSM15896. This strain was cultured in GAM medium for 48 h under anaerobic conditions at 37 °C.

Ursodeoxycholic acid (UDCA, HY-13771, purity ≥ 98 %, HPLC) was purchased from MedChemExpress (MCE).

3.2 Animals

TGR5 knockout (TGR5^{-/-}) mice was kindly provided by professor Shu Jeffrey Zhu (Zhejiang University, Hangzhou, Zhejiang, China). The 8-week-old male BALB/c and C57BL/6 mice were purchased from Hua Fu Kang Biotechnology.co.,LTD (Beijing, China). The mice were allowed to eat and drink freely and housed under specific pathogen-free (SPF) conditions at the experimental Animal Center of Jilin Agricultural University. All experimental animals were approved by the Animal Ethics Committee of Jilin Agriculture University (No.20220806026) .

3.3 Antibiotic treatment and *T. spiralis* Infection

Six-week-old BALB/c mice were randomly divided into four groups: control group (CON group), the antibiotic group (Abx group), the *T. spiralis* infection group (Ts group), and the *T. spiralis* infection after antibiotic treatment (Abx + Ts group). A broad-spectrum antibiotic mixture [vancomycin (100 mg / kg), ampicillin (200 mg / kg), neomycin (200 mg / kg) or metronidazole (200 mg / kg)] was administered to Abx group and Abx + Ts group mice in their drinking water freely provided for 7 consecutive days⁴¹. In contrast, mice in the CON group and the Ts group were received sterile water. Fasted for 2 h after a 2-day interval, mice in the Abx group and the Abx + Ts group were intragastrically administered with polyethylene glycol (PEG)

mixture [PEG3350 (77.5 mg / mL), sodium bicarbonate (2.2 mg / mL), sodium chloride (1.9 mg / mL) or potassium chloride (0.98 mg / mL) and sodium sulfate (7.4 mg / mL)] and administered six times at 30-min intervals⁴². Meanwhile, mice in the CON group and the Ts group were given sterile PBS. 6 h after the last administration of the PEG mixture, mice in the Ts group and the Abx + Ts group were intragastrically administered with *T. spiralis* muscle larvae (350 *T. spiralis* per mouse), while the remaining two groups of mice were intragastrically administered with PBS.

3.4 Establishment of mouse colitis model

Colitis was induced in anesthetized mice with 2 mg 2,4,6-trinitrobenzene sulfonic acid (TNBS: Sigma Aldrich, USA) in 100 μ L of 50 % ethanol in sterile water by intrarectal administration. This colitis mouse model produces a Th1-type immune response similar to IBD^{43,44}. The day of establishing the colitis model was considered as day 0, and the mice' survival rate, weight changes, disease activity index, and occurrence of colitis were observed over the following 3 days.

3.5 FMT experiment

After 7 days of treatment with a broad-spectrum antibiotic mixture, the residual luminal microbiota and the antibiotics were flushed out using of PEG mixture as previously described. Then, fresh feces were harvested from mice infected with *T. spiralis* for 21 days to transplant gut microbiota and treated as described previously. Briefly, fresh feces from mice infected with *T. spiralis* were pooled and homogenized, diluted in sterile saline; samples were vortexed for 3 min and allowed to stand for 2 min; the optical density (OD) of the supernatant was measured, and the OD₆₀₀ was adjusted to 0.5 (approximately 10⁸ CFU bacteria), then was used for FMT treatment⁴⁵⁻⁴⁷. A total of 200 μ L of FMT were administered per mice via oral gavage for 14 consecutive days.

3.6 16S rRNA analysis of gut microbiota

Fresh fecal samples were collected and DNA extraction was carried out using the E.Z.N.A stool DNA kit. The V3-V4 hypervariable region of the 16S rRNA gene was amplified following an optimized and standardized 16S amplicon library preparation

protocol (341F: 5'-CCTAYGGGRBGCASCAG-3' and 806R: 5'-GGACTACNNGGG
TATCTAAT-3'). PCR reactions were conducted with 15 µL of Phusion High-Fidelity
PCR Master Mix (New England Biolabs), 0.2 µM of forward and reverse primers, and
approximately 10 ng of genomic DNA. The thermal cycling program included an
initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C
for 10 s, annealing at 50 °C for 30 s, elongation at 72 °C for 30 s, and a final
extension at 72 °C for 5 min. Subsequently, the PCR product was verified by
electrophoresis using an agarose gel, purified with the Qiagen Gel Extraction Kit
(Qiagen, Germany), and quantified with the DNA Quantitation Kit (Sigma, USA) to
generate a sequencing library. The quality of the library was evaluated using the Qubit
2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally,
the library underwent a 300-bp paired-end sequencing procedure on an Illumina
MiSeq platform (Illumina, San Diego, CA, USA) at Novogene Biotech Co., Ltd.
(Beijing, China).

The sequences were demultiplexed and quality-filtered using the “Quantitative
Insights Into Microbial Ecology” (QIIME, version 1.9.1) software package. Raw tags
were compared to the Silva reference database using the UCHIME algorithm to
identify and remove chimera sequences. Amplicon sequence variants (ASVs) were
clustered at 100 % sequence similarity using Uclust. Taxonomic information for each
representative sequence was annotated using the Silva Database. Alpha-diversity was
calculated with raw, unfiltered counts in QIIME v1.8.0. Principal Coordinates
Analysis (PCoA) of the ASVs was utilized to visualize differences in microbial
community structure. Partial Least Squares-Discriminant Analysis (PLS-DA) and
Venn analysis were performed using Simca-P 12.0 and R software (Version 2.15.3)
respectively. Spearman’s correlation analysis was conducted to identify relationships
between bacterial species at the genus level. Linear Discriminant Analysis (LDA)
coupled with the Effect Size (LEfSe) algorithm was employed to assess significant
microbial differences between different groups.

3.7 Metagenomic sequence

Clean data were assembled and analyzed using MEGA-HIT software (v1.0.4),

and the assembled scaffolds were then broken from the N linkage to give N-free scaffolds. Unigenes were matched against functional databases using DIAMOND software (v0.9.9.110, <https://github.com/bbuchfink/diamond/>). Functional databases included the KEGG database (version 2018-01-01, <http://www.kegg.jp/kegg/>), eggNOG database (version 4.5, <http://eggnogdb.embl.de/#/app/home>), and CAZy database (version 201801, <http://www.cazy.org/>). The top BLAST hits were selected for subsequent analysis. The relative abundance of the various functional levels was calculated based on the comparison results. Tables representing the number of genes in each sample at each taxonomic level were generated using the functional annotation results and gene abundance tables. The number of genes in a given sample for a certain function is equal to the number of genes whose abundance is not 0 among those annotated for that function. Differential function was assessed from abundance tables at each taxonomic level.

3.8 Untargeted metabolomics

Stool samples were collected from mice and processed by mixing fresh feces with methanol and H₂O, followed by homogenization using a high-throughput tissue disruptor at -20 °C for 6 min. Subsequently, the tubes containing the samples were centrifuged at 13,000 g for 15 min at 4 °C to obtain the supernatant. Metabolite extracts from mouse fecal samples were then analyzed using gas chromatography-mass spectrometry (GC-MS) following established protocols.

Additionally, the samples underwent LC-MS analysis where metabolites were separated using a reverse phase chromatographic gradient on a Thermo Fisher Hypersil GOLD TMC18 column and quantified by selected reaction monitoring (SRM). Concentrations were determined based on the ratio of isotope-labeled fragments from standard compounds to corresponding fragments from microbial metabolites, as described previously.

3.9 Functional validation of metabolites

TGR5^{-/-} mice were randomly assigned to four different groups: control group (C group), UDCA group, TNBS group (TN group), and UDCA + TNBS group (UDCA+TN group). UDCA suspensions [UDCA (10 mg / kg), PEG3350 (40 % v/v),

DMSO (10 % v/v), Tween-80 (5 % v/v) and PBS (45 % v/v)] were administered daily to mice of UDCA group and UDCA + TN group by gavage for four weeks, while groups TNBS and control received the vehicle.

3.10 Assessment of body weight, survival rate, and disease activity index (DAI)

Throughout the experiment, the body weight and survival rate of the mice were monitored. The degree of diarrhea and defecation status of the mice were observed daily. The experimental mice were scored for the DAI according to international standards: weight loss score + stool performance score + blood stool score) / 3 = DAI (Table S1)^{48,49}. Fecal occult blood was detected by the benzdine method. To evaluate the infiltration of inflammatory cells in the colon, we detected the activity of myeloperoxidase in colon tissues using commercial myeloperoxidase (MPO) assay kits according to the manufacturer's instructions.

3.11 Enzyme-linked immunosorbent assay

Pro-inflammatory cytokines IL-1 β (MM-0040M1) and TNF- α (MM-0132M1), anti-inflammatory cytokines TGF- β (MM-0135M1) and IL-13(MM-0173M1), as well as chemokines MCP-1(MM-45085M1) and MIP-3 α (MM-0151M1) in the serum and tissues of the experimental mice were detected using commercial ELISA kits (Jiangsu Meimian Industrial Co. Ltd., China).

3.12 Flow cytometry

Colonic tissues of mice were collected, cut into pieces, and digested using collagenase IV(C5138, Sigma, USA) and DNase I (DN25, Sigma, USA) to prepare a single cell suspension, cells were isolated using the 40 % and 80 % percoll solution (10317837, Cytiva, USA). Mesenteric lymph nodes (MLN) were isolated and ground into single cells. Cells were spread in a 48-well plate (1×10^6 cells / mL), and cells were co-incubated with ionomycin calcium salt, PMA, and GolgiPlugTM (brefeldin A) for 5 h^{50,51}. Cells were harvested, centrifuged and washed with FBS buffer solution. To detect viable single cells, cells were incubated with Fixable Stain 780 (565388, BD, USA) and purified rat anti-mouse CD16/CD32 (553142, BD, USA).

Subsequent, colonic single cells were stained with FITC-labeled anti-mouse CD45.2 (109805, Biolegend, USA), APC-cy7-labeled anti-mouse CD3e (152323,

Biolegend, USA), APC-cy7-labeled anti-mouse B220 (103223, Biolegend, USA), APC-cy7-labeled anti-mouse Ly6G/Ly6C (557661, BD, USA), R718-labeled anti-mouse F4/80 (285214, Biolegend, USA), PE-cy7-labeled anti-mouse CD11b (101215, Biolegend, USA), Percp-cy5.5-labeled anti-mouse MHCII (562363, BD, USA), and PE-CF594-labeled anti-mouse CD11c (565591, BD, USA) at 4 °C for 40 min. After washing with PBS buffer, the cells were stained with PE-labeled anti-mouse IL-18 (157903, Biolegend, USA) and APC-labeled anti-mouse IL-1 β (MA5-23597, Thermo Fisher, USA) at 4 °C for 40 min.

MLN single cells were equally divided into two portions. One portion was stained with Alexa 700-labeled anti-mouse CD3e (152315, Biolegend, USA) and BB700-labeled anti-mouse CD4 (566408, BD, USA) at 4 °C for 40 min, after fixation with 1 $\times$ Fixation / permeabilization solution and washing with 1 $\times$ BD perm/wash buffer, the cells were stained with PE-cy7-labeled anti-mouse IFN- γ (505849, Biolegend, USA), APC-labeled anti-mouse IL-4 (504105, Biolegend, USA), and PE-labeled anti-mouse IL-10 (505007, Biolegend, USA). The other portion was stained with FITC-labeled anti-mouse CD4 (100405, Biolegend, USA), Alexa 700-labeled anti-mouse CD3e (152315, Biolegend, USA), and PE-labeled anti-mouse CD25 (113703, Biolegend, USA) at 4 °C for 40 min, after fixation with 1 $\times$ Fix/perm buffer and washing with 1 $\times$ Perm/wash buffer, the cells were stained with AF647-labeled anti-mouse Foxp3 (126407, Biolegend, USA) and PE-cy7-labeled anti-mouse IL-17A (506921, Biolegend, USA).

After washing with FBS buffer two times, cell samples were subjected to flow cytometry. The data was analyzed by FlowJo 10.8.1 software.

3.13 Total RNA extraction and quantitative RT-qPCR

Total RNA of colon was extracted using TRIzol® Reagent and cDNA was synthesized using TransStart Tip Green qPCR SuperMix (TransGen Biotech, Beijing, China). The specific primer sequences are listed in Table S2. RT-qPCR was performed by use of ChamQ SYBR Color and RT-qPCR Master Mix on a Bio-Rad CFX96™ Real-Time system. The GAPDH served as an endogenous gene. In order to calculate the relative expression of genes using $2^{-\Delta\Delta C_t}$ method.

3.14 Western blotting

Colon tissue were lysed using RIPA buffer (Sigma-Aldrich) to extract protein as previously described²². To separate proteins were used to SDS-PAGE and combined with nitrocellulose membrane. ZO-1, claudin-1, occludin, NF- κ B, IL-1 β , caspase-1 monoclonal antibodies (Affinity Biosciences, OH, USA) were served as primary antibodies, goat anti-rabbit or rabbit anti-mouse IgG were served as secondary antibody. Protein bands were detected and captured using an Amersham Imager 600 RGB (GE, Marlborough, USA).

3.15 Histological evaluation and Immunohistochemistry

Colon tissues fixed in 4 % paraformaldehyde were dehydrated, clarified, and embedded in paraffin blocks. Colons were cut to sections, a portion of them was stained with hematoxylin and eosin (H&E) and analyzed via fluorescence microscope (DMi8, Leica, Germany).

All methods are reported in accordance with ARRIVE guidelines.

3.16 Statistical analyses

The data in this study were analyzed using GraphPad Prism 8 software, and the results were reported as means $\pm$ SD. Student's t-test was employed to compare data between two groups, while comparisons among experimental groups ($n \geq 3$) were conducted using ANOVA followed by Tukey's honest significant differences post-hoc test. Statistical significance was defined as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Authors' contributions

G.L.Y. and C.F.W. designed the experiments. X.Y. and K.D.Y. were responsible for literature investigation, data visualization; H.Y. L., M.H.L., D.Y..Z., T.X.P and Y.X. were responsible original draft preparation, creation and/or publication of works, especially writing the first draft (including substantive translation and edition); H.C. L., S.Y.L., T.K.G., W.H.C., H.B.H., N.W., S.Y.Y., J.Y.G., M.X.L., G.L.Y., C.F.W and Y.X. were responsible for the overall structure and conceptual design of the article, formal analysis of data, writing-review and editing, and funding acquisition. All authors reviewed and approved the final manuscript.

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Availability of data and materials

Metagenomic sequencing data from antibiotic-treated mice for the establishment of an intestinal microbiota dysbiosis model, metagenomic data from FMT and TN mice, 16S rRNA sequencing data of antibiotic-treated mice before *B. acidifaciens* administration. And these data have been uploaded to the NCBI Sequence Read Archive database with registration numbers PRJNA1428253, PRJNA1429533, and PRJNA1419142, respectively. All data supporting the results of this study can be found in the manuscript or in the supplementary information. Source data are provided in this paper.

Declarations

Ethics approval and consent to participate

The Animal Protection and Ethics Committee of Jilin Agricultural University approved and oversaw all experimental procedures, ensuring that they were carried out in accordance with established protocols.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Data availability

Metagenomic sequencing data from antibiotic-treated mice for the establishment of an intestinal microbiota dysbiosis model, metagenomic data from FMT and TN mice, 16S rRNA sequencing data of antibiotic-treated mice before *B. acidifaciens* administration. And these data have been uploaded to the NCBI Sequence Read

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Reference

1. Rogle, G., Singh, A., Kavanaugh, A. & Rubin, D. Extraintestinal manifestations of inflammatory bowel disease: current concepts, treatment, and implications for disease management. *Gastroenterology* **161**, 1118-1132 (2021).
2. Petracco, G., Faimann, I., Reichmann, F. Inflammatory bowel disease and neuropsychiatric disorders: mechanisms and emerging therapeutics targeting the microbiota-gut-brain axis. *Pharmacol Ther.* **269**, 108831 (2025).
3. Tun, H. M. *et al.* Gut virome in inflammatory bowel disease and beyond. *Gut* **73**, 350-360 (2024).
4. Buitrago, G., Harnett, M. M. & Harnett, W. Conquering rheumatic diseases: are parasitic worms the answer? *Trends Parasitol.* **39**, 739-748 (2023).
5. Kamal, A. M. *et al.* Prevalence and associated risk factors of intestinal parasitic infections among egyptian patients with inflammatory bowel disease. *Jpn J Infect Dis.* **75**, 262-268 (2022).
6. Rapin, A. & Harris, N. Helminth-bacterial interactions: cause and consequence. *Trends Immunol.* **39**, 724-733 (2018).
7. Moyat, M., Coakley, G. & Harris, N. The interplay of type 2 immunity, helminth infection and the microbiota in regulating metabolism. *Clin Transl Immunology.* **8**, e01089 (2019).
8. Smallwood, T. B. *et al.* Helminth immunomodulation in autoimmune disease. *Front Immunol.* **8**, 453 (2017).
9. Ding, J. *et al.* *Trichinella spiralis*: inflammation modulator. *J Helminthol.* **94**, e193 (2020).
10. Ma, Z. R. *et al.* Inhibition of GSDMD-mediated pyroptosis triggered by *Trichinella spiralis* intervention contributes to the alleviation of DSS-induced ulcerative colitis in mice. *Parasit Vectors* **16**, 280 (2023).

11. Wakid, M. H., Kholy, W. E., Alsulami, M. N. & EI-Wakil, E. S. Immuno-therapeutic and prophylactic potential of *Trichinella spiralis* antigens for inflammatory bowel diseases. *Food Waterborne Parasitol.* **37**, e00248 (2024).
12. Fawzy, E. M. *et al.* The prophylactic and therapeutic impact of *Trichinella spiralis* larvae excretory secretory antigens-loaded Ca-BTC metal organic frameworks on induced murine colitis. *J Helminthol.* **98**, e41 (2024).
13. Sun, X. M. *et al.* *Trichinella spiralis*-induced immunomodulation signatures on gut microbiota and metabolic pathways in mice. *PloS Pathog.* **20**, e1011893 (2024).
14. Li, J. Q. *et al.* Galectin from *Trichinella spiralis* alleviates DSS-induced colitis in mice by regulating the intestinal microbiota. *Vet Res.* **55**, 3 (2024).
15. Long, S. R. *et al.* *Trichinella*-derived protein ameliorates colitis by altering the microbiome and improving intestinal barrier function. *Int Immunopharmacol.* **127**, 111320 (2024).
16. Danne, C., Skerniskyte, J., Marteyn, B. & Sokol, H. Neutrophils: from IBD to the gut microbiota. *Nat Rev Gastroenterol Hepatol.* **21**, 184-197 (2024).
17. Aliwa, B. *et al.* Altered gut microbiome, bile acid composition and metabolome in sarcopenia in liver cirrhosis. *J Cachexia Sarcopenia Muscle* **14**, 2676-2691 (2023).
18. Danne, C., Skerniskyte, J., Marteyn, B. & Harry, S. Neutrophils: from IBD to the gut microbiota. *Nat Rev Gastroenterol Hepatol.* **21**, 184-197 (2024).
19. Zhu, X. Q. *et al.* Quercetin activates energy expenditure to combat metabolic syndrome through modulating gut microbiota-bile acids crosstalk in mice. *Gut Microbes* **16**, 2390136 (2024).
20. Anderson, K. M. & Gayer, C. P. The pathophysiology of farnesoid x receptor (FXR) in the GI tract: inflammation, barrier function and innate immunity. *Cells* **10**, 3206 (2021).
21. Hu, J. P. *et al.* Gut microbiota-mediated secondary bile acids regulate dendritic cells to attenuate autoimmune uveitis through TGR5 signaling. *Cell Rep.* **36**, 109726 (2021).
22. Hang, S.Y. *et al.* Bile acid metabolites control TH17 and Treg cell differentiation.

- Nature* **576**, 143-148 (2019).
23. Kang, S. A. *et al.* Adoptive transfer of *Trichinella spiralis*-activated macrophages can ameliorate both Th1-and Th2-activated inflammation in murine models. *Sci Rep.* **9**, 6547 (2019).
 24. Floudas, A. *et al.* *Schistosoma mansoni* worm infection regulates the intestinal microbiota and susceptibility to colitis. *Infect Immun.* **87**, e00275 (2019).
 25. Kumar, R., Grover, S. & Batish, V. K, *et al.* Bile salt hydrolase (Bsh) activity screening of *Lactobacilli*: in vitro selection of indigenous *Lactobacillus* strains with potential bile salt hydrolysing and cholesterol-lowering ability. *Probiotics Antimicrob Proteins* **4**, 162-172 (2012).
 26. Panelli, S. *et al.* Inflammatory bowel diseases, the hygiene hypothesis and the other side of the microbiota: parasites and fungi. *Pharmacol Res.* **159**, 104962 (2020).
 27. Ledford, H. The mysteries of inflammatory bowel disease are being cracked-offering hope for new therapies. *Nature* **632**, 963-964 (2024).
 28. Shute, A. *et al.* Cooperation between host immunity and the gut bacteria is essential for helminth-evoked suppression of colitis. *Microbiome* **9**, 186 (2021).
 29. Artchayasawat, A. *et al.* Viability of *Trichinella spiralis* in traditional sour pork fermentation and its inactivation by microwave heating: implications for zoonotic risk and food safety. *Vet World.* **18**, 1660-1666 (2025).
 30. Rocha, F. A. *et al.* Microbes, helminths, and rheumatic diseases. *Best Pract Res Clin Rheumatol.* **34**, 101528 (2020).
 31. Shimokawa, C. The gut microbiome-helminth-immune axis in autoimmune diseases. *Parasitol Int.* **104**, 102985 (2025).
 32. Pandey, H., Tang, D. W., Wong, S. H. & Lal, D. Helminths in alternative therapeutics of inflammatory bowel disease. *Intest Res.* **23**, 8-22 (2025).
 33. Johnston, M. J. *et al.* Extracts of the rat tapeworm, *Hymenolepis diminuta*, suppress macrophage activation in vitro and alleviate chemically induced colitis in mice. *Infect Immun.* **78**, 1364-1375 (2010).
 34. Tang, B. *et al.* Gut microbiota alters host bile acid metabolism to contribute to

- intrahepatic cholestasis of pregnancy. *Nat Commun.* **14**, 1305 (2023).
35. Chen, Z. H. *et al.* *Bacteroides fragilis* alleviates necrotizing enterocolitis through restoring bile acid metabolism balance using bile salt hydrolase and inhibiting FXR-NLRP3 signaling pathway. *Gut Microbes* **16**, 2379566 (2024).
36. Shen, H.Y. *et al.* Dietary fiber alleviates alcoholic liver injury via *B. acidifaciens* and subsequent ammonia detoxification. *Cell host Microbe* **32**, 1331-1346 (2024).
37. Rimal, B. *et al.* Bile salt hydrolase catalyses formation of amine-conjugated bile acids. *Nature* **626**, 859-863 (2024).
38. Chiang, J. Y. & Ferrell, J. M. Bile acid receptors FXR and TGR5 signaling in fatty liver diseases and therapy. *Am J Physiol Gastrointest Liver Physiol.* **318**, G554-G573 (2020).
39. Zhang, J. *et al.* Salidroside attenuates NASH through regulating bile acid-FXR/TGR5 signaling pathway via targeting gut microbiota. *Int J Biol Macromol.* **307**, 142276 (2025).
40. Li, F., Cui, J., Wang, Z. Q. & Jiang, P. Sensitivity and optimization of artificial digestion in the inspection of meat for *Trichinella spiralis*. *Foodborne Pathog Dis.* **7**, 879-885 (2010).
41. Liu, Y. *et al.* Gut microbiome alterations in high-fat-diet-fed mice are associated with antibiotic tolerance. *Nat Microbiol.* **6**, 874-884 (2021).
42. Roy, T. L. *et al.* Comparative evaluation of microbiota engraftment following fecal microbiota transfer in mice models: age, kinetic and microbial status matter. *Front Microbiol.* **9**, 3289 (2019).
43. Zhang, Y. N. *et al.* *Dubosiella newyorkensis* modulates immune tolerance in colitis via the L-lysine-activated AhR-IDO1-Kyn pathway. *Nat Commun.* **15**, 1333.
44. Liu, X. *et al.* Deubiquitinase OTUD6A in macrophages promotes intestinal inflammation and colitis via deubiquitination of NLRP3. *Cell Death Differ.* **30**, 1457-1471 (2023).
45. Zhou, D. *et al.* Total fecal microbiota transplantation alleviates high-fat diet-induced steatohepatitis in mice via beneficial regulation of gut microbiota. *Sci Rep.* **7**, 1529 (2017).

- 899 46. Zhang, T. *et al.* Washed microbiota transplantation vs. manual fecal microbiota
900 transplantation: clinical findings, animal studies and in vitro screening. *Protein Cell*
901 **11**, 251-266 (2020).
- 902 47. Lu, G. C. *et al.* Washed preparation of faecal microbiota changes the
903 transplantation related safety, quantitative method and delivery. *Microb Biotechnol.*
904 **15**, 2439-2449 (2022).
- 905 48. Li, S. J. *et al.* Sedanolid alleviates DSS-induced colitis by modulating the
906 intestinal FXR-SMPD3 pathway in mice. *J Adv Res.* **69**, 413-426 (2025).
- 907 49. Zhang, Y. *et al.* Astragalus polysaccharides alleviate DSS-induced ulcerative
908 colitis in mice by restoring SCFA production and regulating Th17/Treg cell
909 homeostasis in a microbiota-dependent manner. *Carbohydr Polym.* **349**, 122829
910 (2025).
- 911 50. Xing, J. H. *et al.* Gut microbiota-derived LCA mediates the protective effect of
912 PEDV infection in piglets. *Microbiome* **12**, 20 (2024).
- 913 51. Qiu, J. J. *et al.* Microbiota-derived acetate is associated with functionally optimal
914 virus-specific CD8⁺ T cell responses to influenza virus infection via
915 GPR43-dependent metabolic reprogramming. *Gut Microbes* **16**, 2401649 (2024).

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