

Transcriptomic analysis of mouse ileum upon *Hymenolepis nana* infection implicates the PPAR α signaling pathway

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Abstract

Hymenolepis nana (*H. nana*) is a common zoonotic tapeworm that disrupts intestinal mucosal homeostasis; however, its impacts on ileal transcription remain unclear. In this study, we profiled the ileal transcriptome of *H. nana*-infected mice at day 14 post-infection and characterized peroxisome proliferator-activated receptor (PPAR) signaling. Differentially expressed genes (DEGs) were screened using DESeq2 with thresholds of $|\log_2(\text{fold change})| > 1$ and adjusted $P < 0.05$, followed by Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Set Enrichment Analysis (GSEA). Since PPAR α is expressed in intestinal epithelium and exerts a barrier-protective function, infected mice were treated with the PPAR α antagonist GW6471 or agonist Icaria. Intestinal pathological changes were evaluated via hematoxylin and eosin staining and immunohistochemistry. Transcriptomic analysis identified a total of 1119 DEGs, including 855 upregulated and 264 downregulated genes. GO and KEGG enrichment analyses indicated enrichment in immune and inflammatory pathways. GW6471 aggravated infection by increasing worm burden by 88.88% and worm length by 78.68%, while reducing villus height by 66.62% and MUC2 expression by 86.68%. In contrast, Icaria reduced adult worm burden by 46.67% and worm length by 45.56%, increased villus height by 82.76%, and elevated MUC2 expression by 33.75%. Collectively, these findings demonstrate that PPAR α activation ameliorates *H. nana*-induced intestinal injury, suggesting its potential therapeutic value.

Highlights

1. infection alters ileal transcriptional profiles with immune and inflammatory pathway enrichment.
2. GSEA identifies PPAR signaling pathway as a significantly enriched Pathway after infection.
3. PPAR α antagonist GW6471 aggravates infection and reduces villus height and MUC2 function.
4. PPAR α agonist Icaria reduces worm burden and alleviates intestinal damage in mice.

1. Introduction

Hymenolepis nana (*H. nana*) is a tapeworm that parasitizes the intestines of rodents and humans. It has a simple life cycle, can be transmitted through both direct and indirect routes, and is capable of causing autoinfection within the host^[1]; hosts infected with *H. nana* often experience abdominal pain, diarrhea, and vomiting; severe infections can lead to death^[2, 3]. Epidemiological studies indicate that children are the most susceptible population to *H. nana* infection, with infection rates in some endemic areas reaching 15%-20%. This parasite has become a significant public health issue affecting children's intestinal health^[2]. A survey of *H. nana* infection among 384 children in an internally displaced persons camp in Mogadishu, Somalia, found that 53.6% were male and 46.4% were female; 41.9% were under 5 years of age, and 30.2% were between 6 and 10 years of age^[4]. Furthermore, our previous study found that the prevalence of *H. nana* infection among pet hamsters in Guiyang, China was as high as 71.31%^[5].

Given the widespread prevalence and high infection rate of *H. nana* in both humans and animals, elucidating its host-parasite interaction mechanisms is of significance theoretical value for understanding pathogenesis and identifying intervention targets.

In response to *H. nana* infection, the host rapidly initiates an immune response to recognize, contain, and ultimately eliminates the parasite. This process is characterized primarily by a type 2 immune response^[6]. Intestinal epithelial cells serve as the first line of defense against *H. nana* infection. Previous research by our group has shown that, following *H. nana* infection, tuft cells initiate a type 2 immune response by secreting immunoregulatory factors. This response promotes the proliferation and differentiation of intestinal stem cells, drives intestinal epithelial remodeling, and enhances barrier function, thereby facilitating parasite clearance^[7]. The gut is not only a major immune organ but also a key site for maintaining homeostasis of nutrient absorption and energy metabolism. During parasitic infection, the host not only activates immune and inflammatory signaling pathways but also undergoes extensive metabolic remodeling. Previous studies have demonstrated that infection with *Trichinella spiralis* can induce indoleamine 2,3-dioxygenase (IDO)-dependent tryptophan depletion by regulating host tryptophan metabolic pathways, and simultaneously reshape glycerophospholipid and energy metabolism networks, thereby forming a metabolic adaptation pattern characterized by the coupling of amino acid metabolism with glucose metabolism^[8–10]. These metabolic changes not only influence host energy supply and tissue homeostasis but also modulate cellular functions, thereby contributing to the dynamic balance between host immune responses and parasite immune evasion during parasitic infection. However, current research on the interplay between host immune response and metabolic regulation during *H. nana* infection remains limited. Given the important role of the PPAR signaling pathway in lipid metabolism, energy homeostasis, and immune regulation^[11–13], investigating its involvement in the host response to *H. nana* infection is of great significance for elucidating the mechanisms of parasite-host interactions and the strategies underlying long-term coexistence.

Therefore, this study used mice infection with *H. nana* as the experimental model, employed high-throughput sequencing to systematically screen for differentially expressed genes and key signaling pathways, and functionally validated candidate pathways through animal experiments. The aim of this study was to elucidate the potential regulatory role of the PPAR signaling pathway during *H. nana* infection and to provide new insights into the prevention and treatment of *H. nana* infection.

2. Materials and Methods

2.1 Major reagents

The RNA reverse transcription kit (11141ES60), RT-qPCR kit (11201ES08), mouse tail DNA extraction kit (18700ES50), TRIeasy Total RNA Extraction Reagent (10606ES60), and tissue DNA kit ((18700ES50) were purchased from Yeasen (Shanghai, China); borax carmine and neutral balsam (822941) were purchased from Shanghai Macklin Biochemical Technology (Shanghai, China); hematoxylin and eosin (H&E) staining reagents (BL700B) was purchased from Solarbio Biotechnology (Beijing, China); GW6471

(HY-15372), Icariin (HY-N0014), Tamoxifen (HY-13757A), and the goat anti-rabbit secondary antibody (HY-P8001) were purchased from MCE (Shanghai, China); the MUC2 antibody (ab272692) was purchased from Abcam (Cambridge, UK); Neutral balsam (822941) was provided by Shanghai Macklin Biochemical Technology (Shanghai, China).

2.2 Animal and parasite sources

Fifty male hamsters (aged 4–6 weeks; weighing 16.7 ± 2.9 g) were purchased from a pet market in Nanming District, Guiyang, China, between September and October 2022. After the hamsters were euthanized, their intestines were examined for intestinal parasites; parasites were detected in 38 of the 50 hamsters, giving an infection rate of 76%. Male specific pathogen-free (SPF) C57BL/6J mice (6–8 weeks old, $n = 46$) were obtained from Saiye Model Organism Research Center (Taicang, China) [Mouse License No. SCXK (Su) 2022-0016]. The mice were maintained at 23°C under a 12-hour light / dark cycle in an SPF environment.

2.3 Morphological and molecular biological identification

Several adult worms were randomly selected, and their gravid segments were cut into small pieces to collect the eggs. Another five adult worms were randomly selected, stained with borax carmine, and mounted in neutral balsam. The eggs and stained adult worms were examined morphologically using an upright fluorescence microscope. Genomic DNA was extracted from the worms using a tissue DNA extraction kit. The cytochrome c oxidase subunit I (*COX1*) gene of *H. nana* was amplified by polymerase chain reaction (PCR) and analyzed by agarose gel electrophoresis. The *COX1* primer sequences used are shown in Table 1. The PCR products were sent to Sangon Biotech (Shanghai) Co., Ltd., for sequencing, and the obtained sequences were subjected to BLAST analysis against *COX-1* sequence deposited in GenBank (Accession No. HM536187.1).

Table 1
Primer sequences used in current study.

Gene primer name	Primer sequences (5'-3')
COX1-Forward	ACCGCGTCGTGTGTGTATTT
COX1-Reverse	ACATGCAACTGGGCTCATACG
<i>Mcpt9 F</i>	AGGAACCAGGACAAGAACACA
<i>Mcpt9 R</i>	ACCACCAATAATCTCCTCAGCTC
<i>Cpa3 F</i>	CCACGGATGCTCTCTCAACT
<i>Cpa3 R</i>	GCCCAGGTCATAAACCCAGT
<i>Cma2 F</i>	GGTGGGTTTCTCATAACGCC
<i>Cma2 R</i>	CAACACATCGTCCCAGGCTT
<i>Cd55 F</i>	CCAGGAGACCAACCAACTCC
<i>Cd55 R</i>	AGTCAGACCTTCTGTTTGGACT
<i>Mcpt1 F</i>	AAACAGCATACATGGGAGACT
<i>Mcpt1 R</i>	AATCCAGGGCACATATGCAG
<i>Mcpt2 F</i>	GAGGCTAAACCACACTCCCG
<i>Mcpt2 R</i>	CCACACCTCTCCTTCGAACC
<i>S100g F</i>	ATTCAGTCAGAGTTCCCCAGC
<i>S100g R</i>	TCGCCATTCTTATCCAGCTC
<i>Muc13 F</i>	TCGTCTCTGCGAGGTCAAAG
<i>Muc13 R</i>	AAGTTGGAGAAGCCGGTCTG
<i>Zbp1 F</i>	TTGTCAGCACAATCCGGTCA
<i>Zbp1 R</i>	TGCACTTGTTGGAGCAAGGA
<i>Herc6 F</i>	GTGTATTCCTGCGGGGACAA
<i>Herc6 R</i>	CTGGCCTTTCGGTACTCTGG
<i>Tmcc3 F</i>	GTGGACCGGACCTACTCAGA
<i>Tmcc3 R</i>	GGCTGTCCGCATTGAGTTTG
<i>Rsad2 F</i>	AGCGTCACAAAGAGGTGTCC
<i>Rsad2 R</i>	ACATACTTTCCGCCACGCTT
<i>Irf7 F</i>	TTTCTAGTGATGCCGGGGAC

Gene primer name	Primer sequences (5'-3')
<i>Irf7 R</i>	CCATGCTGCATAGGGTTCCT
<i>Ubd F</i>	ACCAATGGCGGTTAATGACCT
<i>Ubd R</i>	TTTTCGATGGGGCTTGAGGA
<i>Ifit1 F</i>	CCAGCCCTTCATCTGGGTATC
<i>Ifit1 R</i>	ATGGTGTGTTCACTAGGCGG
<i>GAPDH F</i>	AGGTCGGTGTGAACGGATTTC
<i>GAPDH R</i>	GGGGTCGTTGATGGCAACA
<i>Fabp5 F</i>	GATTGCCGAGATGTGGTGTTCG
<i>Fabp5 R</i>	GGCTTCTCCAAAACCATAGCGTG
<i>Scd1 F</i>	CCCTCCGGAAATGAACGAGA
<i>Scd1 R</i>	CAGAGCGCTGGTCATGTAGT

2.4 Animal experimental design

All animal experiments were performed in three independent batches. In all batches, mice were infected with 2,000 *H. nana* eggs by oral gavage daily for 2 consecutive days^[7], and ileal tissues were collected on day 14 post-infection.

2.4.1 Transcriptomic analysis

Mice were randomly divided into two groups ($n = 3$ per group): control group (Ctrl) and *H. nana*-infected group (Infection). The control group received an equal volume of PBS. Ileal tissue samples were collected 4–9 cm proximal to the ileocecal junction for subsequent transcriptomic sequencing and related analyses.

2.4.2 PPAR α antagonist treatment

Mice were randomly divided into four groups ($n = 4$ per group): Ctrl, *H. nana*, GW6471, *H. nana* + GW6471. GW6471 (1 mg/ml, 100 μ l per mouse) was administered intraperitoneally every other day for five doses starting on the first day of infection, GW6471 is a known PPAR α antagonist^[14]. The Ctrl and *H. nana* groups received an equal volume of PBS by intraperitoneal injection.

2.4.3 PPAR α agonist treatment

Mice were randomly divided into four groups ($n = 6$ per group): Ctrl, *H. nana*, Icariin, *H. nana* + Icariin. Starting on the first day of infection, the mice were administered Icariin - a known PPAR α agonist^[15] (0.5 mg/ml, 100 μ l per mouse) by gastric gavage every other day, for a total of five doses. The Ctrl and *H. nana* groups received an equal volume of PBS by intragastric administration.

2.5 Transcriptomic analysis

Mouse ileal tissues were placed in a sterile, nuclease-free microcentrifuge tube, TRIzol reagent was added, and the sample was thoroughly homogenized ((MT-13K-L, MIULAB, Hangzhou, China) using a handheld homogenizer to extract total RNA. Transcriptomic sequencing (RNA-seq) was performed in collaboration with Chongqing Life Knowledge Source Technology. Libraries were constructed by size selection and purification using AMPure XP beads, preliminarily quantified with a Qubit 2.0 fluorometer, and diluted to 1.5 ng/μL. The insert size distribution was analyzed using an Agilent 2100 Bioanalyzer, and the effective library concentration (> 3 nM) was accurately quantified by real-time quantitative PCR(RT-qPCR) to ensure library quality. Libraries passing quality control were sequenced using the Illumina NovaSeq PE150 strategy. Raw reads were filtered to remove reads containing adapters, reads with ambiguous bases, and reads in which bases with a Q-score ≤ 20 accounted for more than 50% of the total read length, thereby obtaining clean reads. DESeq2 was then used to identify differentially expressed genes, with the screening criteria set at $|\log_2(\text{Fold Change})| > 1$ and adjusted $p < 0.05$.

2.6 Real-time quantitative fluorescence PCR (RT-qPCR)

RNA concentration and purity were measured using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and cDNA was synthesized using RNA reverse transcription kit according to the manufacturer's instructions. The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd., and the primer sequences are listed in Table 1. The RT-qPCR reaction mixture consisted of 10.0 μL of SYBR Green Master Mix, 0.8 μL of forward primer, 0.8 μL of reverse primer, 2.0 μL of cDNA template, and 6.4 μL of ddH₂O. The PCR conditions were as follows: 95°C for 5 min; 39 cycles of 95°C for 10 s and 60°C for 30 s. GAPDH was used as the internal reference gene, and relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method^[16].

2.7 Histological analysis

2.7.1 Hematoxylin and Eosin (H&E) staining

Ileal tissues from four groups of mice were rinsed with saline to remove intestinal contents, fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 4 μm thickness. The sections were deparaffinized in xylene, rehydrated through a graded series, stained with H&E, mounted with neutral mounting medium, and finally examined and photographed under a whole slide scanner (SLIDEVIEW VS200, Olympus, Japan).

2.7.2 Immunohistochemistry (IHC)

Ileal tissue sections from the four groups of mice were deparaffinized in xylene, rehydrated through a graded ethanol series, and subjected to antigen retrieval using 1× EDTA buffer. After blocking with 5% BSA at 37°C, the sections were incubated overnight at 4°C with the anti-MUC2 primary antibody. The next day, the sections were equilibrated to room temperature for 30 min, incubated with a goat anti-rabbit

secondary antibody at 37°C for 30 min, mounted with neutral mounting medium, and then examined and photographed under a whole slide scanner (SLIDEVIEW VS200, Olympus, Japan).

2.8 Statistical analysis

Statistical analysis and graph generation were performed using GraphPad Prism 10 software. Data are presented as mean $\pm$ standard deviation (SD). Comparisons between two groups were performed using an unpaired two-tailed Student's *t*-test, and comparisons among multiple groups were performed using one-way analysis of variance (ANOVA). The *P* value less than 0.05 means a statistically significant difference; ns means no statistically significant.

For transcriptomic analysis, differentially expressed genes (DEGs) were identified using DESeq2, with screening criteria of $|\log_2(\text{fold change})| > 1$ and adjusted $P < 0.05$. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed for the DEGs, and GO terms or KEGG pathways with $P < 0.05$ were considered significantly enriched. Gene Set Enrichment Analysis (GSEA) was conducted to evaluate pathway enrichment, and pathways with a normalized enrichment score (NES) > 1 were considered positively enriched. Data quality and sample relationships were evaluated using Pearson correlation analysis and principal component analysis (PCA).

3. Result

3.1 Morphological and molecular biological identification of *H. nana*

Parasite were obtained from hamsters and examined under a light microscope. Morphologically, the scolex had a rostellum armed with a single circle of small hooks and was surrounded by suckers (Fig. 1A). In mature proglottids, spherical testes and lobulated ovaries were observed (Fig. 1B). The gravid proglottids were wider than the scolex, with wedge-shaped, serrated margins, and contained numerous eggs awaiting release (Fig. 1C). Microscopic examination of the eggs revealed that they were round or nearly round, with a very thin shell and a relatively thick inner embryonic membrane enclosing small hooks (Fig. 1D). PCR amplification of DNA extracted from the worms was performed using *H. nana* mitochondrial *COX1*-specific primers. Agarose gel electrophoresis of the PCR products showed a specific band of approximately 202 bp (Fig. 1E). BLAST analysis of the sequencing results revealed that the *COX1* sequence shared 99.86% sequence identity with a reference *COX1* sequence deposited in GenBank (Fig. 1F). Based on both morphological and molecular evidence, the parasites isolated from the hamster intestine were identified as *H. nana*.

3.2 Transcriptional changes in the mouse ileum following *H. nana* infection

To investigate the effects of *H. nana* infection on the host transcriptome, DESeq2 was used to analyze transcriptomic data from infected and control mice. Differentially expressed genes were identified using the criteria $|\log_2(\text{Fold Change})| > 1$ and adjusted $P < 0.05$. A total of 1,119 significantly differentially expressed genes were identified, including 855 upregulated genes and 264 downregulated genes (Fig. 2A). A hierarchical cluster heatmap showed that mucosal defense and epithelial homeostasis-related genes *Mcpt9*, *Fkbp5*, *Cd55*, and *Mcpt1* were significantly upregulated after infection (Fig. 2B), whereas genes of antiviral innate immunity including *Ifit1*, *Zbp1*, and *Herc6* were significantly downregulated (Fig. 2C). These results indicate that *H. nana* infection induces widespread transcriptional changes in the host ileal tissue, suggesting that this parasite may mediate its pathogenic effects by regulating host immune and inflammatory responses.

3.3 GO and KEGG enrichment analysis

To investigate the functions of differentially transcribed genes, GO enrichment analysis was performed ($P < 0.05$), and the top 20 enriched GO terms were selected for visualization in a bar chart (Fig. 3A). Among biological process (BP) terms, the most significantly enriched categories included immune system processes, cellular localization, stress responses, and transcription; cellular component (CC) terms were mainly associated with the extracellular space and cell surface; and molecular function (MF) terms primarily involved cation binding and receptor binding. Statistical analysis of up- and down-regulated genes within the BP category showed that the number of upregulated genes consistently exceeded that of downregulated genes. Specifically, the number of upregulated genes related to chemical responses was the highest (207 genes), whereas the number of downregulated genes related to inflammatory responses was the lowest (14 genes), suggesting that immune- and chemical response-related pathways were the primary enriched pathways (Fig. 3B). KEGG enrichment analysis ($P < 0.05$) (Fig. 3C) revealed that significantly enriched pathways included cytokine-cytokine receptor interactions, the complement and coagulation cascade, the IL-17 signaling pathway, and the chemokine signaling pathway. Analysis of upregulated and downregulated genes (Fig. 3D) indicated that the IL-17 signaling pathway, chemokine signaling pathway, FcεRI signaling pathway, PI3K-AKT signaling pathway, PPAR signaling pathway, and calcium signaling pathway were all upregulated, suggesting that these pathways may play a regulatory role in the host anti-parasitic immune response and metabolic adaptation.

3.4 Gene set enrichment analysis

PPARs are core nuclear receptors linking lipid metabolism and immune regulation, and also play an important role in parasitic infections. We found that the PPAR signaling pathway was significantly enriched in *H. nana* infection. To further validate the overall trend of this pathway during *H. nana* infection, Gene Set Enrichment Analysis (GSEA) was performed. The results showed that the PPAR signaling pathway was positively enriched in the infected group. The enrichment curve indicated a coordinated upregulation of pathway-associated genes at the global transcript level, with a normalized enrichment score (NES) of 1.196 (Fig. 4). These results suggest that the PPAR signaling pathway may

serve as a regulatory node in the host response to *H. nana* infection, potentially linking immune and inflammatory responses with metabolic changes.

3.5 Validation of transcriptomic data

To validate the reliability of the transcriptomic data, the top eight upregulated (Figs. 5A–H) and eight downregulated genes (Figs. 5I–P), ranked by fold change and statistical significance, were selected for RT-qPCR validation. The RT-qPCR results were generally consistent with the RNA-seq data in terms of transcript trends, confirming the reliability and reproducibility of the transcriptomic data.

3.6 GW6471 inhibits PPAR α signaling and aggravates *H. nana* infection

To determine whether GW6471 inhibits PPAR α signaling pathway in mice, the relative transcript levels of *Fkbp5* and *Scd1* were measured by RT-qPCR. The primer sequences are shown in Table 1. Compared with the Ctrl group, the transcript levels of both *Fkbp5* and *Scd1* were downregulated in the uninfected group treated by GW6471 (Figs. 6A&B). To further investigate the role of endogenous PPAR α in *H. nana* infection, infected mice were treated with GW6471. Compared with the *H. nana* group, GW6471 treatment increased adult *H. nana* length by 78.68% and burden by 88.88% (Figs. 6C&E). For villus height, GW6471 treatment further aggravated villus shortening, corresponding to 66.62% of the difference between the control and *H. nana* groups (Figs. 6F&G). MUC2 expression was reduced by 86.68% compared with the *H. nana* group (Figs. 6H&I). These results indicate that blocking endogenous PPAR α aggravates infection and intestinal injury.

3.7 Icariin activates PPAR α signaling and alleviates *H. nana* infection and intestinal injury

To determine whether Icariin activates the PPAR α signaling in mice, the relative transcript levels of *Fkbp5* and *Scd1* were measured by RT-qPCR. Compared with the Ctrl group, the transcript levels of *Fkbp5* and *Scd1* were significantly upregulated in the Icariin-treated uninfected. Similarly, compared with the *H. nana* group, the transcript levels of *Fkbp5* and *Scd1* were significantly upregulated in the *H. nana* + icariin group (Figs. 7A&B). To further confirm the protective role of the PPAR α , infection mice were treated with the PPAR α agonist Icariin. Compared with the *H. nana* group, Icariin treatment reduced adult *H. nana* length by 45.56% (Figs. 7C&D) and burden by 46.67% (Fig. 7E). For villus height, Icariin treatment restored villus height, corresponding to 82.76% of the difference between the control and *H. nana* groups (Figs. 7F–G). MUC2 expression was increased by 33.75% compared with the *H. nana* group (Figs. 7H–I). These results indicate that activating PPAR α alleviates infection and intestinal injury.

4. Discussion

H. nana is a common intestinal parasite of rodents and humans and remains a public health concern in areas with poor sanitation^[17, 18]. Infection with this parasite not only disrupts host digestive function^[19], but also alters metabolic processes^[20] and immune response^[21]. In the present study, we identified *H. nana* by morphological and molecular biological methods. We then combined transcriptomic sequencing with agonist and antagonist intervention experiments to investigate the role of PPAR in *H. nana* infection. Our results suggest that PPAR α plays a protective role during *H. nana* infection, as pharmacological activation of PPAR α alleviated infection and intestinal injury, whereas its inhibition aggravated these outcomes.

High-throughput sequencing has become a powerful tool in parasitology, enabling the discovery of host-parasite interaction mechanisms that are difficult to capture by traditional methods. For example, RNA-seq of *Leishmania donovani* (*L. donovani*)-infected macrophages identified many novel genes associated with parasite persistence and clearance^[22], and transcriptomic profiling of *L. donovani*-infected lizards revealed immune-related signaling pathways involved in host response^[23]. However, research on the host transcriptional response during *H. nana* infection remains limited. In this study, we performed RNA sequencing on the ileal tissues of *H. nana*-infected mice and identified 1,119 DEGs. Functional enrichment analysis revealed that these DEGs were primarily enriched in immune- and inflammatory-related pathways, including the IL-17 signaling pathway, chemokine signaling pathway, and PI3K-AKT signaling pathway. Notably, GSEA showed that the PPAR signaling pathway was significantly enriched (NES = 1.1965). These findings suggest that *H. nana* infection induces widespread transcriptional changes in the host ileum, and the PPAR signaling pathway may play an important role in this process.

The transcriptomic analysis revealed enrichment of the PPAR signaling pathway but did not distinguish specific subtypes. In the context of parasitic infections, PPAR subtypes exhibit distinct roles. In *Trypanosoma cruzi* infection, activation of PPAR α or PPAR γ promotes parasite survival within macrophages by driving M2 polarization^[24]. In *Schistosoma japonicum* infection, PPAR γ agonist rosiglitazone can alleviate liver and spleen lesions^[25]. In cerebral malaria, PPAR γ activation reduces parasitemia and immunopathology by upregulating the scavenger receptor CD36^[26]. These findings indicate that the role of each PPAR subtype is highly dependent on the parasite and infection model. Among the PPAR family members, PPAR α is expressed throughout the mammalian gut, including intestinal epithelial cells and enterocytes of the villi, and functionally promotes mucus secretion and epithelial barrier integrity^[27]. In contrast, PPAR γ is primarily associated with colonic microbial regulation and M2 macrophage polarization^[28], while PPAR β/δ acts through a ligand-independent mechanism^[29]. Based on these established functions, PPAR α was selected as the candidate subtype for functional validation in *H. nana* infection. We therefore used the PPAR α antagonist GW6471 and the PPAR α agonist icariin in separate batches.

Inhibition of PPAR α with GW6471 decreased the relative transcript levels of *Fkbp5* and *Scd1* and aggravated infection, increasing adult *H. nana* burden and worm length while further reducing villus height and MUC2 expression. In contrast, activation of PPAR α with icariin increased *Fkbp5* and *Scd1*

transcript levels and alleviated infection, reducing adult *H. nana* burden by approximately 47% and worm length by approximately 46%, while restoring villus height and increasing MUC2 expression. These opposing results, together with the barrier-protective and anti-inflammatory effects of PPAR α ^[30–32], suggest that PPAR α plays a protective role in *H. nana* infection.

This study has several limitations. Transcriptomic analysis revealed enrichment of the PPAR signaling pathway but did not distinguish specific subtypes, whereas subsequent functional experiments focused on PPAR α . Whether infection specifically induces PPAR α was not verified. In addition, PPAR α -deficient models were not used. Future studies combining subtype-specific analysis and PPAR α -deficient models are needed to further confirm the role of PPAR α in *H. nana* infection.

In summary, this study demonstrates that *H. nana* infection induces transcriptomic changes in the mouse ileum involving immune, inflammatory, and metabolic pathways, with enrichment of the PPAR signaling pathway. Pharmacological inhibition and activation of PPAR α further supported a role for PPAR α in modulating infection and intestinal injury. These findings provide a foundation for further exploration of PPAR α -targeted strategies for the prevention and treatment of *H. nana* infection.

Declarations

Ethics Statement

All animal experiments of the current study were approved by the Animal Ethics Committee of Guizhou Medical University (approval No. 2100346). All animal operations and care were performed in accordance with institutional and national laboratory animal welfare guidelines.

Data availability

All data generated or analyzed from this study are included in this manuscript and supplementary materials.

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Author contributions

Wenlan Wu: Writing—original draft, Methodology, Formal analysis, Data curation. **Xiaomao Li:** Methodology, Formal analysis, Data curation. **Jing Li:** Methodology, Formal analysis, Data curation. **Tianze Ma:** Methodology. **Xuanyin Cui:** Writing—review & editing, Visualization, Supervision. **Ke Zhang:** Writing—review & editing, Visualization, Supervision, Project administration, Formal analysis,

Conceptualization. **Rong Mou:** Writing–review & editing, Visualization, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Conflicts of interest

The authors have declared that no competing interests.

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Figures

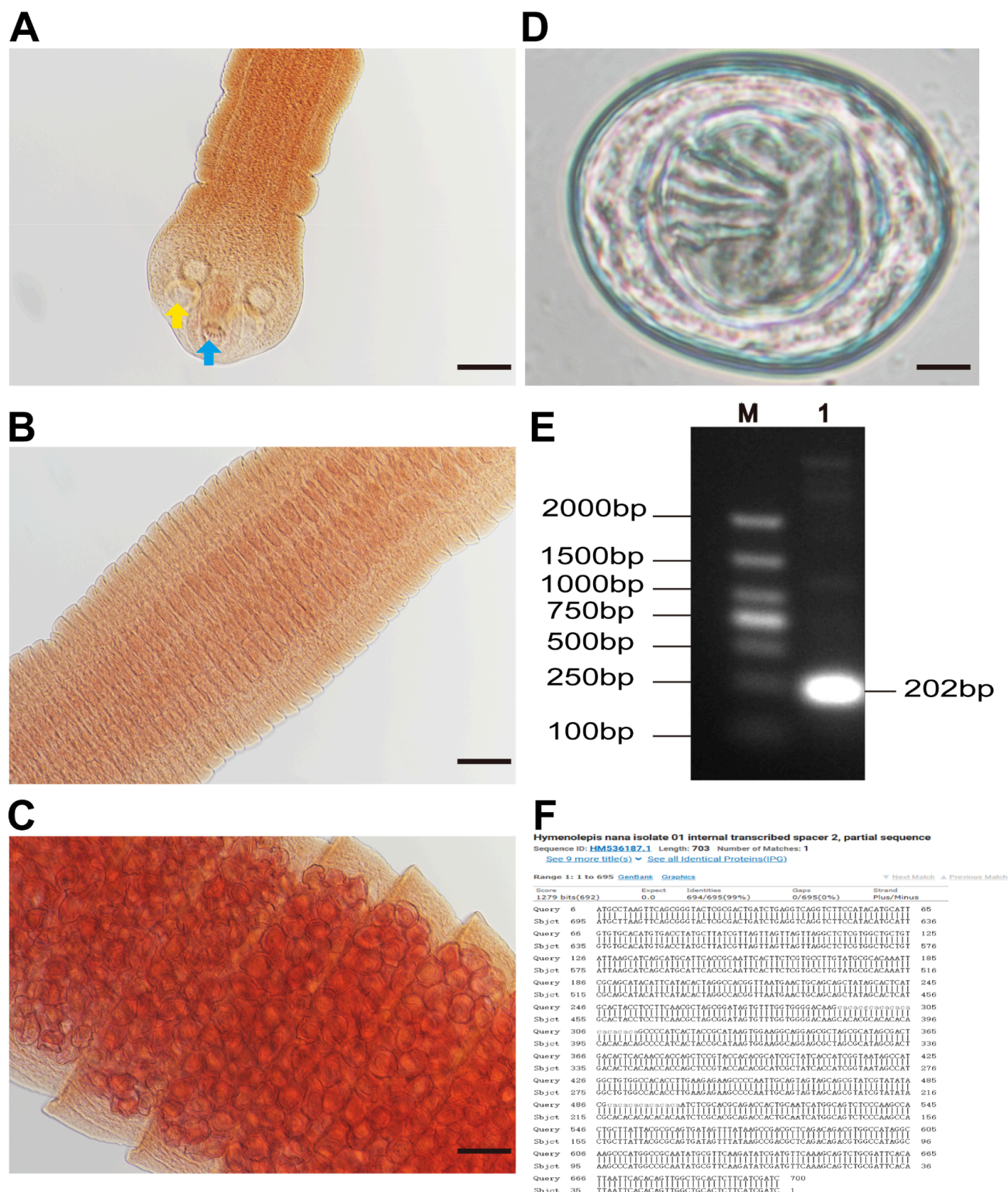


Figure 1

Morphological and molecular identification of *H. nana*. (A) Head joint of *H. nana* after staining with carboxyl borate red, the suckers indicated by yellow arrow, and the parietal protrusion is indicated by blue arrow (scale bar 100 μ m). (B) Representative image of mature proglottids of *H. nana* (scale bar 100 μ m). (C) Oviparous segment of the adult *H. nana*, with a wedge-shaped body and serrated edges, containing a large number of eggs ready for release (scale bar 100 μ m). (D) Egg obtained from *H.*

nana adults, with a thin outer shell and a thick inner embryonic membrane enclosing a small hook (scale bar 20 μ m). (E) Agarose gel electrophoresis of PCR products amplified using *H. nana* COX1-specific primers. M: DL 2000 marker, 1: COX1. (F) BLAST alignment results of the obtained COX1 sequence with reference sequence deposited in GenBank (Accession No. HM536187.1).

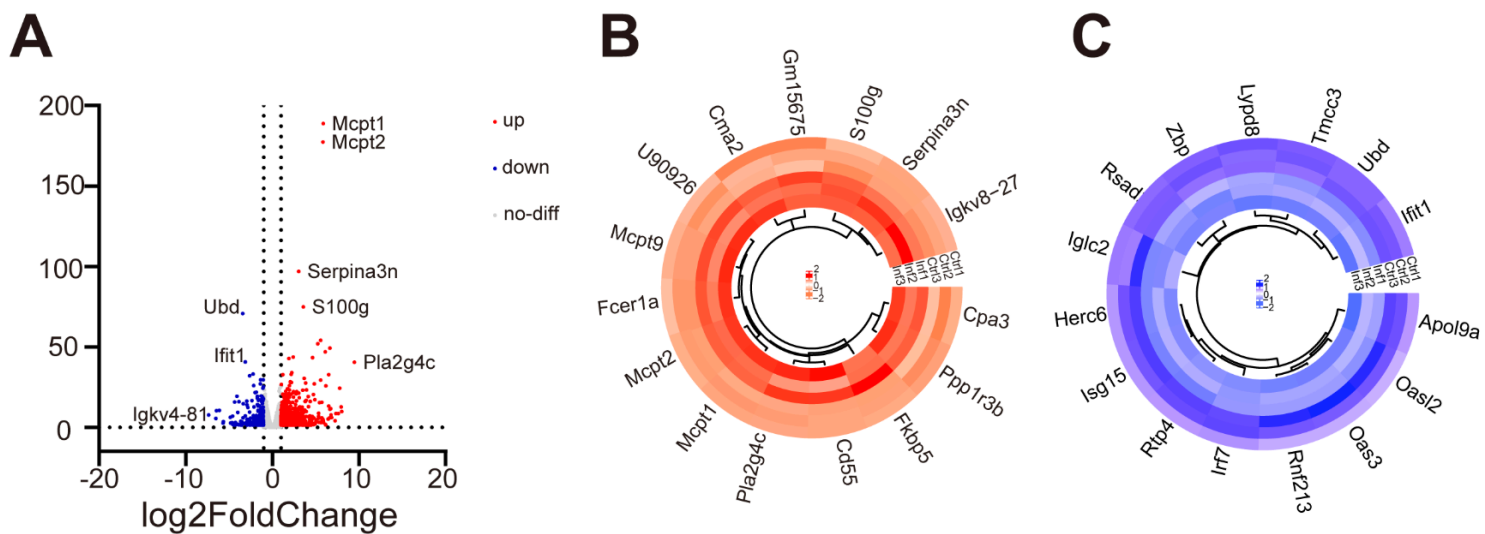


Figure 2

Data quality control and transcriptional changes. Mice were orally inoculated with or without 2,000 *H. nana* eggs daily for 2 consecutive ($n = 3$ per group). Ileal tissues were collected on day 14 post-infection for RNA sequencing. (A) Volcano plot of differentially expressed genes, red indicates significantly upregulated genes ($n = 855$), blue indicates significantly downregulated genes ($n = 264$), and gray indicates genes with no significant difference. (B) Stratified circular heatmap of upregulated genes. (C) Stratified circular heatmap of downregulated genes. DEGs were identified using DESeq2 with $|\log_2(\text{fold change})| > 1$ and adjusted $P < 0.05$.

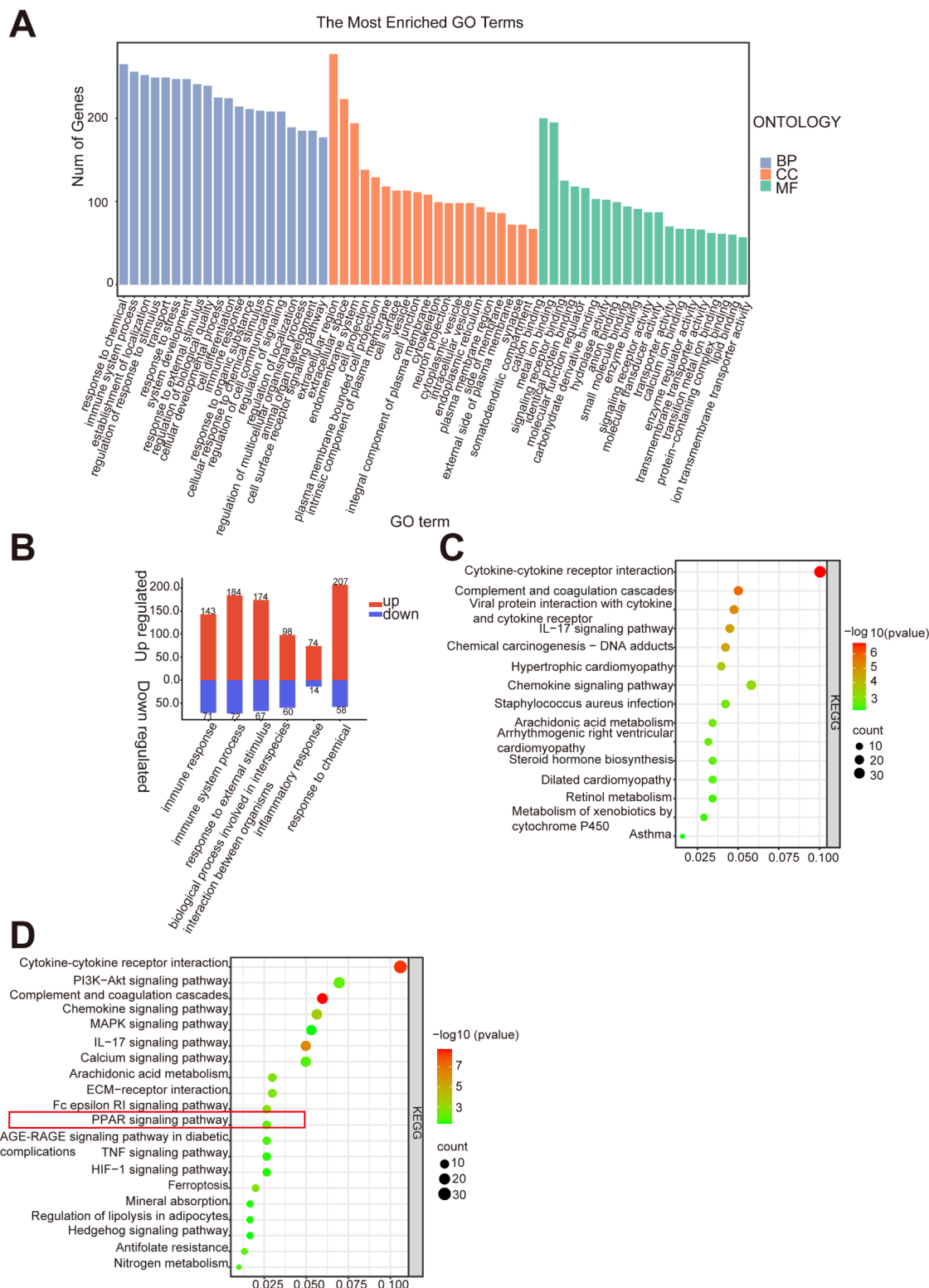


Figure 3

GO and KEGG enrichment analysis of pathways. (A) GO enrichment pathway, the horizontal axis represents the number of genes, and the vertical axis represents the names of GO terms; green indicates biological processes (BP), red indicates cellular components (CC), and blue indicates molecular functions (MF). (B) Upstream and downstream pathway analysis, red indicates an increase; blue indicates a decrease. (C) KEGG enrichment pathways. (D) Up-regulated enrichment pathways on KEGG.

PPAR signaling pathway was highlighted with red color box. Differentially expressed genes were identified using DESeq2 with $|\log_2(\text{fold change})| > 1$ and adjusted $P < 0.05$.

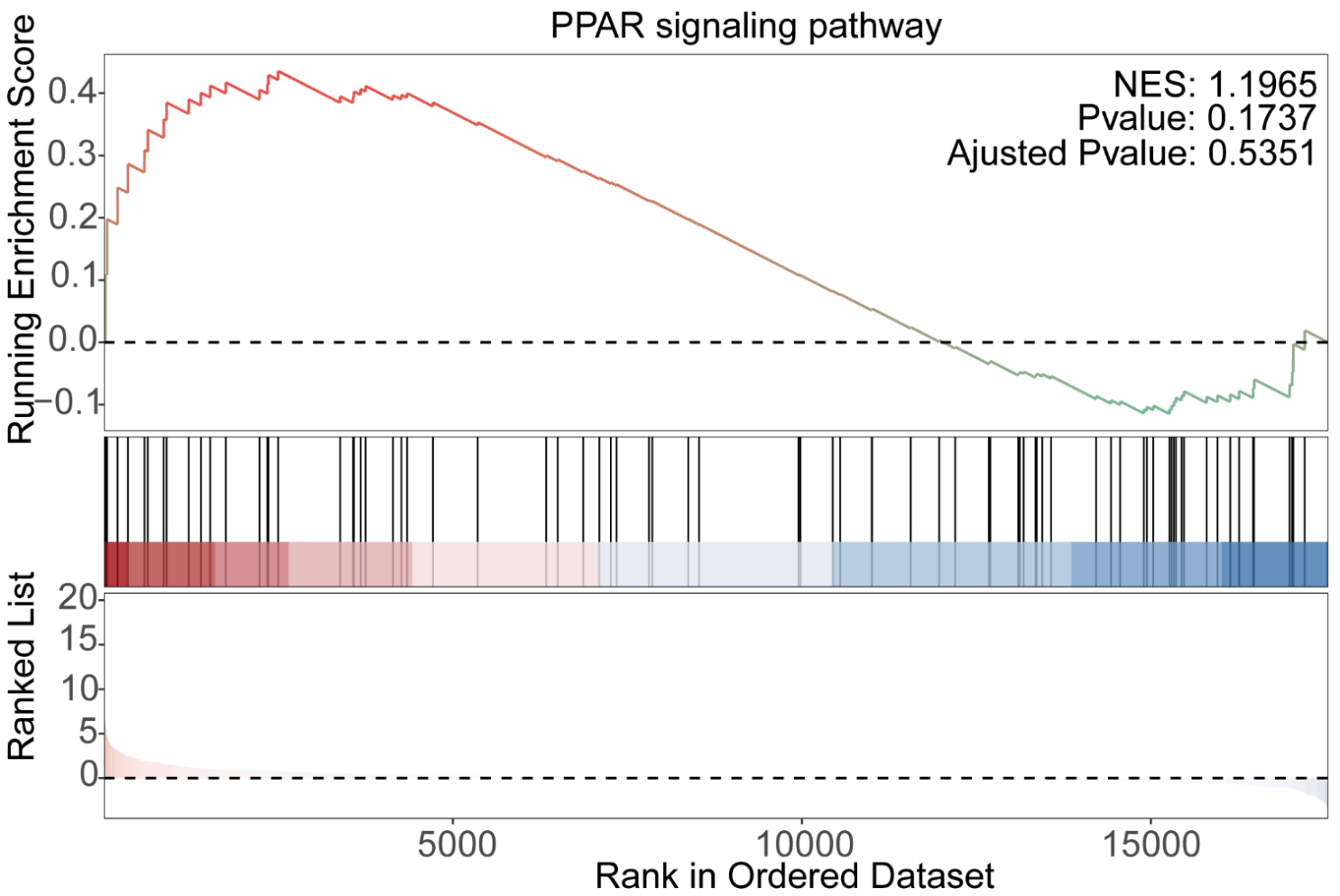


Figure 4

GSEA enrichment analysis of the PPAR signaling pathway. The horizontal axis represents gene rank, and the vertical axis represents enrichment score (ES), the green curve indicates the enrichment score over time, and the black vertical line in the middle indicates the position of PPAR pathway genes in the ranked list. NES: 1.1965; P -value: 0.5351.

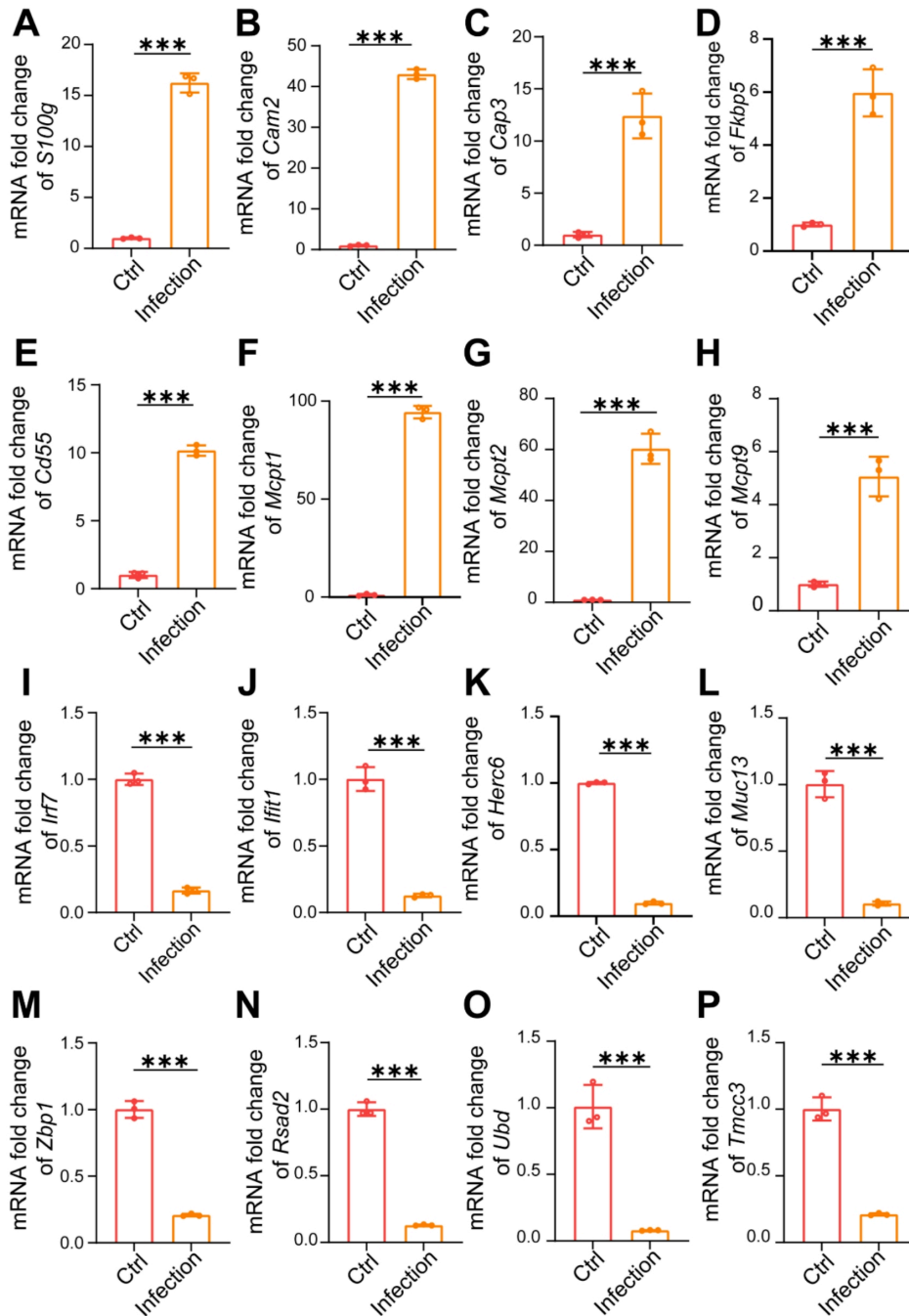


Figure 5

RT-qPCR validation of transcriptomic data. Mice were orally inoculated with or without 2,000 *H. nana* eggs daily for 2 consecutive days. Ileal tissues were collected on day 14 post-infection. Transcription levels of relative mRNAs, including *S100g* (A), *Cam2* (B), *Cap3* (C), *Fkbp5* (D), *Cd55* (E), *Mcpt1* (F), *Mcpt2* (G), *Mcpt9* (H), *Irf7* (I), *Ifit1* (J), *Herc6* (K), *Muc13* (L), *Zbp1* (M), *Rsad2* (N), *Ubd* (O), and *Tmcc3* (P), were

analyzed by the $2^{-\Delta\Delta Ct}$ method. Data are presented as mean $\pm$ SD for (A)-(P), $n = 3$ per group for (A)-(P), *** $P < 0.001$.

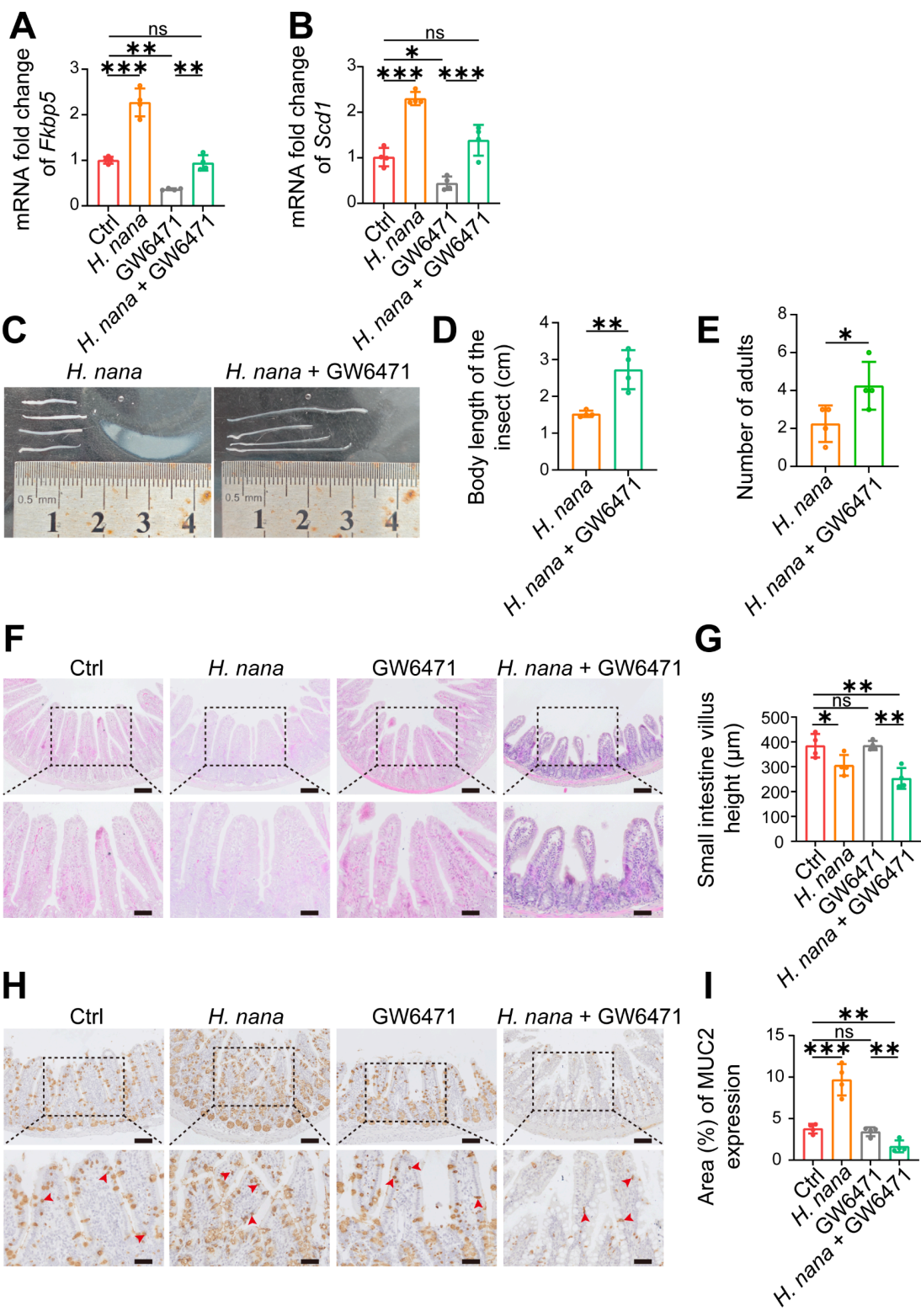


Figure 6

GW6471 inhibits PPAR α signaling and aggravates *H. nana* infection and intestinal injury. Mice were divided into four groups ($n = 4$ per group): Ctrl, *H. nana*, GW6471, and *H. nana* + GW6471. Mice in the *H.*

nana and *H. nana* + GW6471 groups were orally inoculated with 2,000 *H. nana* eggs daily for 2 consecutive days. Mice in GW6471 and *H. nana* + GW6471 groups received GW6471 (1 mg/mL) intraperitoneally every other day for five doses starting on the first day of infection. The Ctrl and *H. nana* groups received an equal volume of PBS by intraperitoneal injection. Ileal tissues were collected on day 14 post-infection. Relative mRNA transcription levels were analyzed by the $2^{-\Delta\Delta C_t}$ method. Relative transcript levels of *Fkbp5* (**A**) and *Scd1* (**B**) in the four groups. (**C**) Representative images of adult *H. nana* length. (**D**) Quantification of adult *H. nana* length. (**E**) Quantification of adult *H. nana* burden. (**F**) Representative images of H&E staining. (**G**) Statistical graph of villus height based on H&E staining. (**H**) Representative images of immunohistochemical staining for MUC2, the positive area is marked by a red arrow. (**I**) Statistical graph of MUC2 expression based on immunohistochemical staining. Scale bars are 100 μ m for the upper panel and 50 μ m for the lower panel in F and H. Data are presented as mean $\pm$ SD for (A), (B), (D), (E), (G), and (I), n = 4 per group for (A), (B), (D), (E), (G), and (I). Statistical significance was determined by unpaired two-tailed Student's t-test for D and E, and one-way ANOVA for A, B, G, and I. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, non-statistically significant.

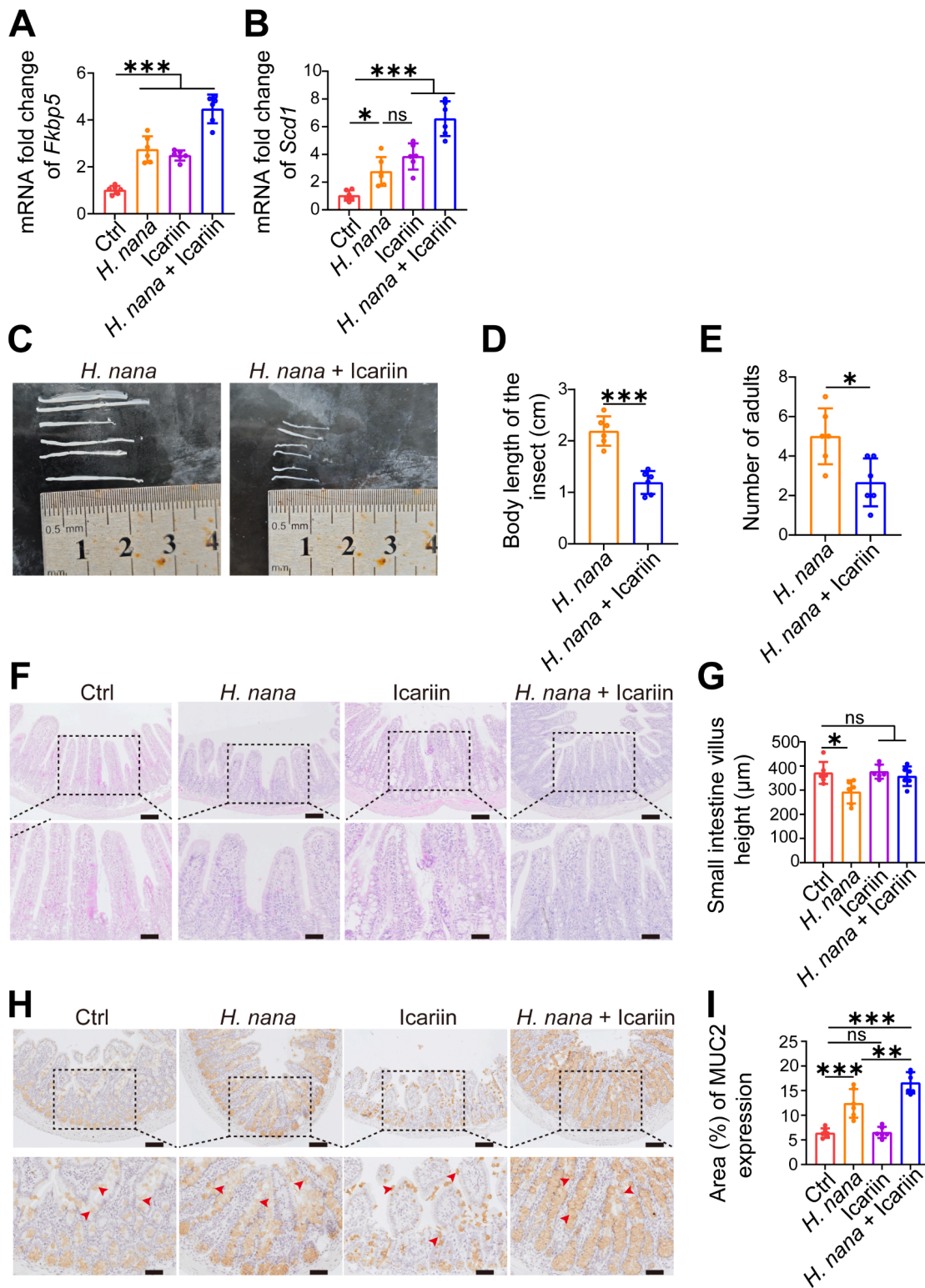


Figure 7

Icariin activates PPAR α signaling and alleviates *H. nana* infected and intestinal injury. Mice were divided into four groups ($n = 6$ per group): Ctrl, *H. nana*, Icariin, and *H. nana* + Icariin. Mice in the *H. nana* and *H. nana* + Icariin groups were orally inoculated with 2,000 *H. nana* eggs daily for 2 consecutive days. Mice in the Icariin and *H. nana* + Icariin groups received Icariin (0.5 mg/mL) intragastrically every other day for five doses starting on the first day of infection. The Ctrl and *H. nana* groups received an equal volume of

PBS by intragastric administration. Ileal tissues were collected on day 14 post-infection. Relative mRNA transcription levels were analyzed by the $2^{-\Delta\Delta C_t}$ method. Relative transcript levels of *Fkbp5* (A) and *Scd1* (B) in the four groups. (C) Representative images of adult *H. nana* worms dissected from mice in the *H. nana* and *H. nana* + Icariin groups. (D) Statistical bar chart showing the body length of adult *H. nana* worms. (E) Statistical bar chart showing the number of adult *H. nana* worms per mouse. Data are presented as means + SD. (F) Representative images of H&E staining from the four groups. (G) Statistical graph of villus height based on H&E staining. (H) Representative images of immunohistochemical staining for MUC2 from the four groups, the positive area is marked by a red arrow. (I) Statistical graph of MUC2 expression based on immunohistochemical staining. Scale bars are 100 μ m for the upper panel and 50 μ m for the lower panel in F and H. Data are presented as means $\pm$ SD, for (A), (B), (D), (E), (G), and (I), n = 6 per group for (A), (B), (D), (E), (G), and (I)., Statistical significance was determined by unpaired two-tailed Student's t-test for D and E, and one-way ANOVA for A, B, G, and I. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, non-statistically significant.

Supplementary Files

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