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ORIGINAL ARTICLE

Trojan horse-inspired smart single-atom nanozyme delivery system for synergistic therapy of inflammatory bowel disease



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Abstract Systemic administration for the clinical management of inflammatory bowel disease (IBD) often leads to various side effects and toxicities, primarily due to broad therapeutic exposure of non-target tissues. Herein, a smart single-atom nanozyme (SAzymes) delivery system (Fe-SA/Cur@HAD,

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Nanozyme;
Cascade reaction;
Curcumin;
Orally;
Single atom;
Oxidative stress;
Delivery system

FCH) is constructed through coordinating iron-doped SAzymes (Fe-SA) with curcumin (Cur) for IBD synergistic therapy. Inspired by Trojan horse, FCH efficiently responds to the IBD pathological microenvironment and realizes targeted delivery *via* oral administration. In inflamed colonic tissue, FCH regulates redox homeostasis through the superoxide dismutase (SOD)-catalase (CAT) cascade reaction and releases Cur by changing the adsorption energy, thus achieving synergistic therapy. An *in vitro* IBD model of human-derived colonic organoid, along with *in vivo* IBD model of mouse, were used and demonstrated that this system could effectively reduce reactive oxygen species (ROS) levels, improve intestinal homeostasis, and promote tissue recovery. Additionally, FCH markedly suppresses the activation of inflammatory pathways and modulates the composition of the intestinal microbiota. This study innovatively modifies SAzymes, offering new perspectives on their potential applications in IBD treatment.

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1. Introduction

Inflammatory bowel disease (IBD) is a disorder characterized by chronic inflammation of the colonic mucosa and submucosa, primarily encompassing ulcerative colitis and Crohn's disease^{1,2}. Common symptoms include diarrhea, abdominal pain, and rectal bleeding. Current clinical treatment strategies are primarily based on anti-inflammatory drugs, immunomodulators, and surgical interventions, with an emphasis on symptom management³. However, their effectiveness is sometimes limited, and they are often associated with adverse side effects. Recently, a large population-based cohort study in Denmark indicated that the incidence of IBD has stabilized, whereas its prevalence continues to rise⁴. In contrast to western nations, developing countries are witnessing a rapid increase in IBD incidence, coinciding with industrialization. For example, China has seen a sharp rise in both the age-standardized incidence and prevalence rates from 1990 to 2021 (estimated annual percentage changes: 2.93 and 2.54, respectively)⁵. A recent systematic review highlighted a significant increase in hospitalization rates in newly industrialized countries, placing an increasing burden on global healthcare systems⁶. The etiology of IBD remains incompletely understood. The excessive production of reactive oxygen species (ROS), leading to oxidative stress (OS), is widely acknowledged as a contributing factor to inflammatory diseases like IBD and may represent a promising therapeutic target. Consequently, the urgent need for developing strategies to eliminate ROS in the IBD microenvironment and mitigate intestinal damage remains critical.

The advancement of nano-catalytic materials has provided novel insights into the regulation of redox homeostasis of ROS in inflammatory diseases^{7,8}. Nanozymes, which combine the physical properties of nanomaterials with the enzymatic activities of natural enzymes, have gained significant attention due to their efficiency, safety, and ease of synthesis^{9–12}. Among these, the development of self-cascade nanozymes, which exhibit multiple antioxidant activities, such as superoxide dismutase (SOD) and catalase (CAT), has emerged as an effective strategy for treating IBD by mitigating OS and improving the IBD microenvironment^{13,14}. Consequently, the preparation of nanozymes exhibiting cascade SOD-CAT activity is of great significance^{15,16}. Recently, single-atom nanozymes (SAzymes) have attracted attention because of their exceptionally high catalytic efficiency^{17–19}. These nanozymes are typically composed of individual metal atoms (*e.g.*, Fe, Pt) supported on carriers. Their primary advantage lies in their single-atom structure, which allows for a high

concentration of catalytic active sites and facilitates the exposure of these sites on the surface. Furthermore, SAzymes possess a high specific surface area and enhanced catalytic stability^{20,21}. Currently, researchers primarily utilize the electronic structure to regulate SAzyme activity, while the macroscopic coordination strategy within SAzymes has often been overlooked^{22,23}. Therefore, modifying SAzymes through a coordination strategy to construct cascade reaction complexes can enable both the delivery of natural drugs and the full exploitation of nanozyme efficacy.

Among the therapeutic strategies for IBD, oral administration facilitates direct interaction between the drug and the inflammatory lesions on the intestinal mucosa, thereby improving therapeutic outcomes while minimizing systemic effects on other organs^{24–27}. However, this method necessitates the use of drugs that exhibit high acid resistance and a specific affinity for the positively charged inflamed mucosa. The development of an acid-resistant, negatively charged coating on the surface of SAzymes can markedly enhance the efficiency of oral administration and optimize the targeted therapeutic effects²⁸. In previous studies, we synthesized hyaluronic acid-grafted dopamine (HAD), a material known for its superior biocompatibility and targeted delivery potential²⁹. Moreover, it is capable of adjusting its electronegativity in response to external environmental conditions, thus enabling acid resistance and effectively targeting intestinal mucosal lesions.

Inspired by Trojan horse, a smart SAzymes delivery system (Fe-SA/Cur@HAD, FCH) was constructed here, achieving synergistic therapy of IBD. FCH exhibits SOD-CAT cascade reaction activity, promoting antioxidant self-cascade reaction that efficiently neutralize ROS and improve the pathological environment (Fig. 1). The combination of Fe-SA with curcumin (Cur) not only enhances the anti-inflammatory effects but also overcomes the poor water solubility of Cur. HAD was incorporated into the outer layer of the system to develop an orally bioavailable single-atom antioxidant self-cascade nanozyme system characterized by high acid resistance and targeted delivery capabilities. Both *in vitro* (cellular level, human-derived colonic organoid model) and *in vivo* (IBD mouse model) experiments demonstrated that the system can effectively eliminate ROS, enhance intestinal homeostasis, and promote the recovery of damaged tissue. Furthermore, FCH effectively inhibited the expression of inflammatory pathways and modified the composition of the intestinal microbiota. This work represents an innovative modification of SAzymes and provides new insights into their potential application for IBD treatment.

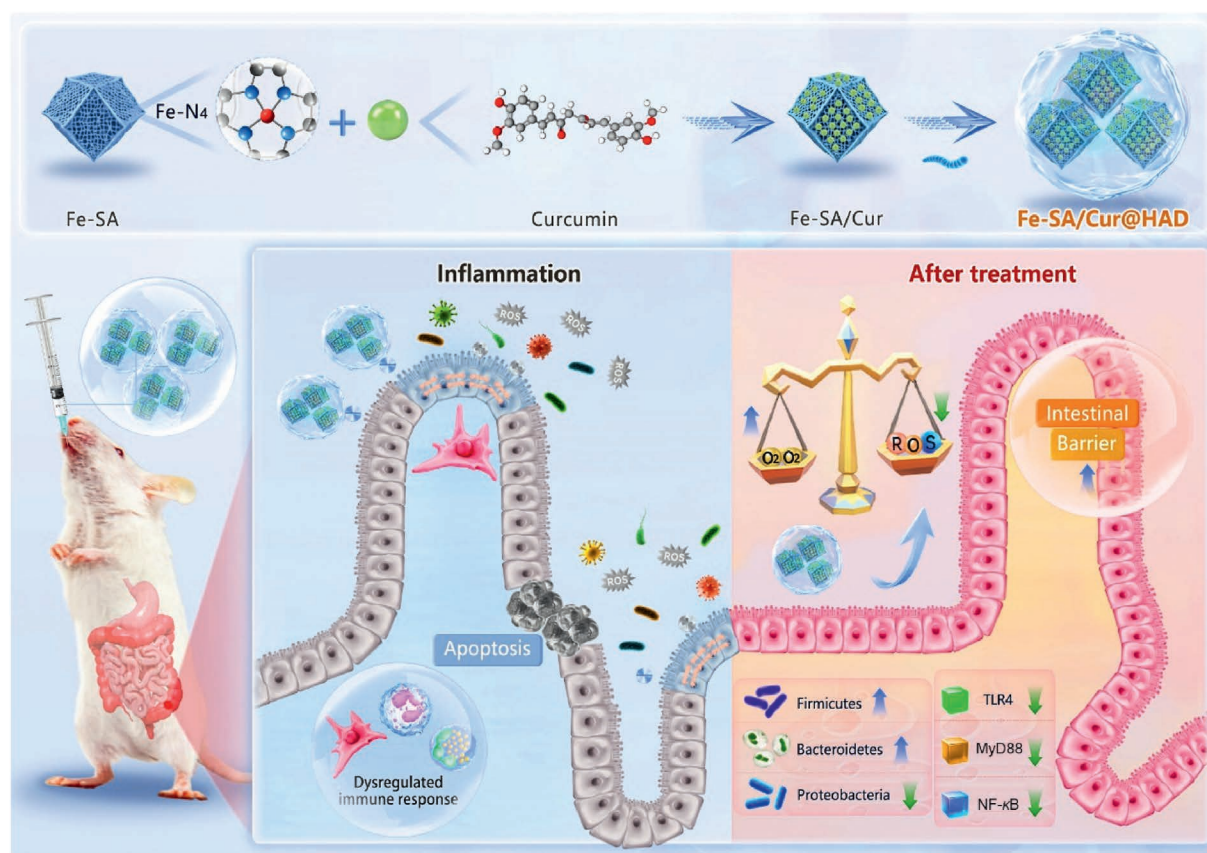


Figure 1 Trojan horse-inspired smart single-atom nanozyme delivery system for synergistic therapy of inflammatory bowel disease.

2. Materials and method

2.1. The preparation of Fe-SA

$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (1190 mg) and $\text{Fe}(\text{C}_5\text{H}_7\text{O}_2)_3$ (52 mg) were uniformly dispersed using ultrasonic vibration in a mixed solution (20 mL methanol and 10 mL tetrahydrofuran), designated as A. Meanwhile, 2-methylimidazole (1134 mg) was similarly dispersed in 5 mL mixed solution (5 mL methanol and 5 mL tetrahydrofuran), designated as B. Then, solution A was combined with solution B under ultrasonic conditions and stirred at 600 rpm (SCILOFEX, CF-1524R, Shanghai, China) for 15 min. The resulting precipitate was collected *via* centrifugation, washed three times with ethanol, and vacuum-dried to yield Fe-ZIF-8. The Fe-ZIF-8 was subsequently placed in a quartz tube, purged with N_2 , and subjected to a reaction at 800 °C for 2 h. The resultant black product was soaked in 0.5 mol/L HCl for 2 h, washed three times with ultrapure water and ethanol, and vacuum-dried to obtain Fe-SA.

2.2. The preparation of Fe-SA/Cur

Fe-SA (50 mg) was dissolved in 10 mL ethanol, then add 10 mg Cur and stir in the dark for 24 h. After rotary evaporation of the solvent, collect the residue and dialyze for 3 days to remove unreacted materials. Dry and collect Fe-SA/Cur.

2.3. The preparation of FCH

Fe-SA/Cur (50 mg), HAD (5 mg) were ultrasonically dissolved in 10 mL of ultrapure water, and stir for 48 h. Then, place the

collected product in a 3000 Da dialysis bag and dialyze for 3 days, changing the water every 6 h, followed by freeze-drying to collect FCH.

2.4. CAT-like activity of FCH

The CAT-like activity was measured in PBS (pH 7.4) containing FCH (25 $\mu\text{g/mL}$), that H_2O_2 (1 mol/L) as the substrate. The dissolved oxygen was tested using a dissolved oxygen meter. The kinetic assay for FCH was conducted at room temperature in PBS with varying concentrations of H_2O_2 (0, 0.1, 0.2, 0.3, 0.5, 1, 1.5, 2 mol/L). Michaelis-Menten constant (K_m) and maximum velocity ($V_{\max}$) values were determined using Origin 2024 software based on Eq. (1)

$$V = \frac{V_{\max} [S]}{K_m + [S]} \quad (1)$$

where V is reaction velocity, $[S]$ is substrate concentration, $V_{\max}$ is the maximal reaction velocity.

2.5. SOD-like activity of FCH

The SOD-like activity was assessed using a SOD assay kit (Beyotime, S0101S). According to the kit instructions, the SOD-like activity was calculated by comparing the colorimetric analysis of the WST-8 product.

2.6. Antioxidant activity of FCH

2.6.1. 2,2-diphenylhydrazyl radical (DPPH·) scavenging experiment

A DPPH· solution (100 $\mu\text{mol/L}$, ethanol) was mixed with various concentrations of FCH (2, 5, 12.5, 25, 50 $\mu\text{g/mL}$) in the dark for 30 min. The mixture was then centrifuged at 7000 rpm (SCILOFEX, CF-1524R, Shanghai, China) for 5 min. Then, collected the supernatant and measured the absorbance at 517 nm.

2.6.2. 2,2'-Azobis (3-ethylbenzothiazole-6-sulfonic acid) cation radical (ABTS·) scavenging experiment

Prepare the ABST solution following the kit instructions (Beyotime, S0119), and then mix the ABTS· solution with FCH (2, 5, 12.5, 25, 50 $\mu\text{g/mL}$). Incubate the mixture in the dark for 10 min, after collected the supernatant and measured the absorbance at 734 nm.

2.6.3. Hydroxyl radical ($\cdot\text{OH}$) scavenging experiment

Mix the 3,3',5,5'-tetramethylbenzidine (TMB) solution (0.416 mmol/L in DMSO), FeSO_4 solution (1.0 mmol/L in HAc-NaAc buffer, pH 4.5), and H_2O_2 solution (10 mmol/L) with FCH (2, 5, 12.5, 25, 50 $\mu\text{g/mL}$). After standing for 5 min, centrifuge the mixture for 5 min, collect the supernatant, and measure the absorbance at 652 nm.

2.7. Cell culture

NCM460 cells were cultured in high-glucose medium supplemented with 10% FBS and 1% PS. Cells were incubated at 37 °C in a 5% CO_2 environment.

2.8. Intracellular ROS scavenging activity

To Induce *in vitro* inflammatory cells, NCM 460 cells were inoculated in confocal dishes with 1×10^5 cells and incubated with a culture medium containing 3% DSS. Following this, treat the cells with Fe-SA (25 $\mu\text{g/mL}$), Fe-SA/Cur (25 $\mu\text{g/mL}$), FCH (25 $\mu\text{g/mL}$) for 24 h. Incubate in the dark with DFCH-DA (1 $\mu\text{mol/L}$) and DAPI (0.5 $\mu\text{g/mL}$), and collect images using a CLASM.

2.9. Intracellular O_2 generation

To Induce *in vitro* inflammatory cells, NCM 460 cells were inoculated in confocal dishes with 1×10^5 cells and incubated with a culture medium containing 3% DSS. Following this, treat the cells with Fe-SA (25 $\mu\text{g/mL}$), Fe-SA/Cur (25 $\mu\text{g/mL}$), FCH (25 $\mu\text{g/mL}$) for 24 h. Incubate in the dark with $\text{Ru}(\text{dpp})_3\text{Cl}_2$ (1 $\mu\text{mol/L}$) and DAPI (0.5 $\mu\text{g/mL}$), and collect images using a CLASM.

2.10. In vivo animal experiments

In *in vivo* animal experiments, male mice, aged 6–8 week (20–22 g) were purchased from Shanghai Jiesijie Laboratory Animal Co., Ltd. The acute IBD model was established through the administration of 2.5% DSS in drinking water.

The IBD model mice were subsequently randomized into four groups, while healthy mice served as a control group. The groups were designated as control, IBD + PBS, IBD + Fe-SA (25 $\mu\text{g/mL}$), IBD + Fe-SA/Cur (25 $\mu\text{g/mL}$), IBD + FCH (25 $\mu\text{g/mL}$). After 8-day treatment, all mice were euthanized, and major organs as well as colon tissues were collected for characterization.

2.11. Statistical analysis

Each experimental condition was replicated a minimum of three times. Data are presented as mean $\pm$ standard deviation (SD). Statistical analysis was conducted using Origin2024 or GraphPad Prism (version 9.0), with asterisks indicating significant differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

3. Results

3.1. Synthesis and characterization of Fe-SA

The synthesis of Fe-SA is depicted in Fig. 2A. Initially, Zn^{2+} and Fe^{3+} ions are coordinated with 2-Methylimidazole to yield Fe-doped zeolitic imidazolate framework-8 (Fe-ZIF-8). Subsequently, Fe-ZIF-8 is subjected to thermal treatment at 800 °C in a N_2 atmosphere, resulting in the thermal decomposition and evaporation of Zn^{2+} , while carbonization occurs to form a nitrogen-doped carbon structure. Finally, acid etching is performed to remove impurities potentially formed during the calcination process, thereby yielding Fe-SA. In comparison with the classic ZIF-8 structure, Fe-SA preserves the original dodecahedral framework³⁰. During high-temperature pyrolysis, its surface gradually collapses, resulting in the formation of a porous structure, as observed through transmission electron microscopy (TEM) and scanning electron microscopy (SEM) (Fig. 2B and C and Supporting Information Figs. S1 and S2). The high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) image, after aberration correction, confirmed the presence of individual Fe atoms (Fig. 2D). Additionally, the Energy dispersive spectroscopy (EDS) elemental mapping results demonstrated a uniform distribution of four elements: C, N, O, and Fe (Fig. 2E).

The content of Fe in Fe-SA is determined to be 0.0591% by inductively coupled plasma-Mass Spectrometry (ICP-MS). In the high-resolution Fe 2p X-ray Photoelectron Spectroscopy (XPS) spectrum of Fe-SA, two distinct peaks were observed, corresponding to Fe^{2+} (710.4 and 722.4 eV) and Fe^{3+} (714 and 725.5 eV) species (Supporting Information Fig. S3). To further investigate the chemical coordination of Fe and N K-edge in Fe-SA, X-ray absorption spectroscopy, including near-edge structure (XANES) and extended fine structure (EXAFS) was employed (Supporting Information Table S1). When compared to Fe foil and Fe_2O_3 , the near-edge absorption energy between Fe foil and Fe_2O_3 is evident in the XANES curves of the Fe K-edge in Fe-SA. The spatial arrangement and the symmetric characteristic peak at 7115 eV confirm the presence of a Fe- N_4 structure resembling a single Fe atom-supported framework (Fig. 2F). The Fourier-transform (FT) k^2 -weighted EXAFS spectra revealed a distinct peak at 1.5 Å, attributed to the scattering path of the Fe- N_4 coordination within the first coordination shell. Moreover, scattering signals at 2.2 and 2.6 Å, associated with the Fe-Fe structure in the outer shell, were detected. These signals differ from metallic Fe-Fe interactions, as they correspond to a more distant Fe-Fe coordination involving nitrogen (Fig. 2G and H)³¹.

The k^2 -weighted EXAFS oscillation spectrum enables the analysis of the coordination environment of Fe within the sample. As depicted in Fig. 2I, the period and frequency observed in the Fe-SA spectrum are comparable to those of natural Fe- N_4 in hemin, thereby distinguishing it from the oscillation spectra of Fe_2O_3 and Fe foil. EXAFS fitting facilitates quantitative analysis

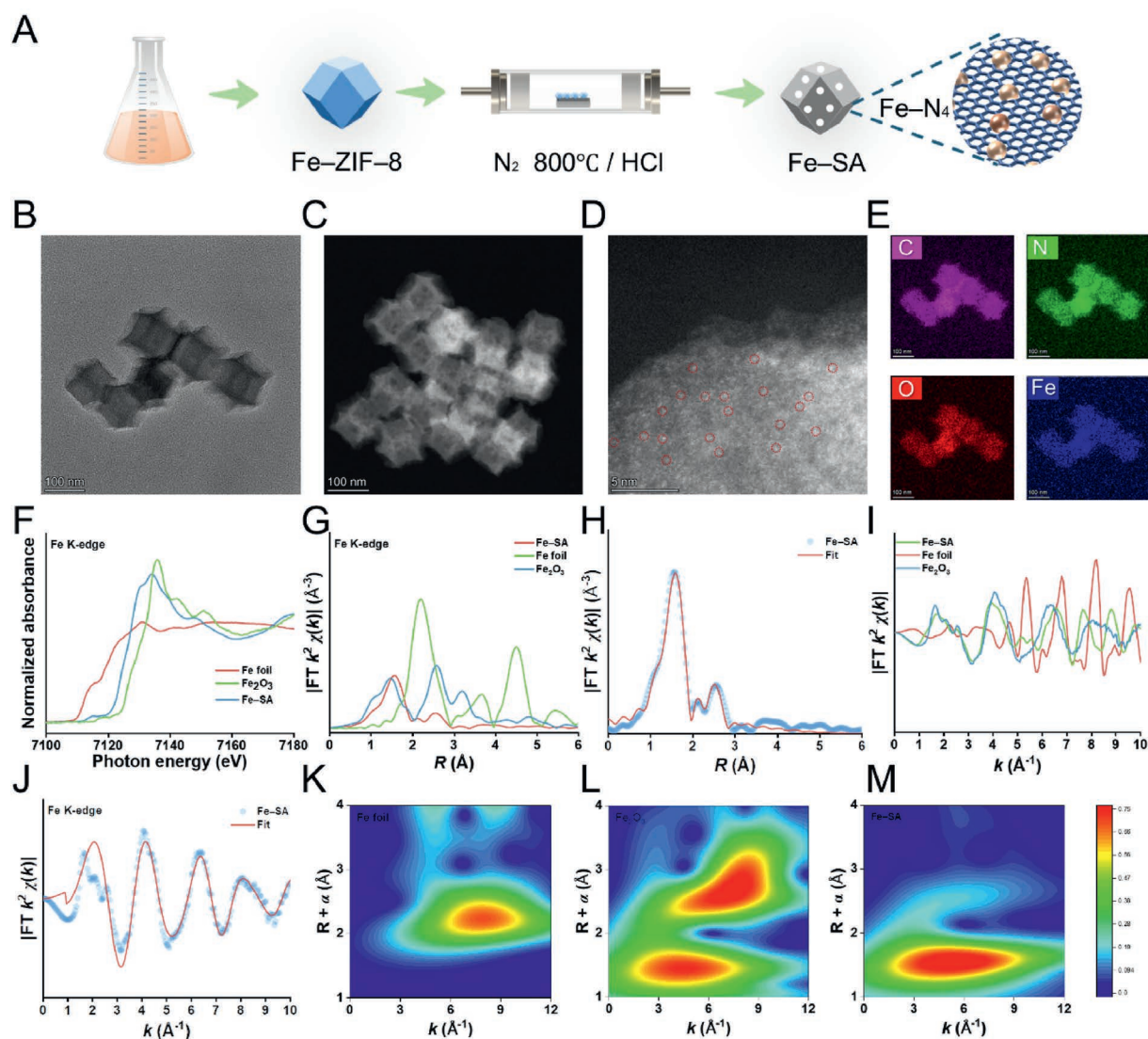


Figure 2 The synthesis and characterization of Fe-SA. (A) The synthesis scheme of Fe-SA. (B, C) The TEM images of Fe-SA. Scale bar = 100 nm. (D) The HAADF-STEM image of Fe-SA. (Fe single atoms as bright dots were marked in red). Scale bar = 5 nm. (E) The EDS mapping of Fe-SA. (F) The XANES spectra of Fe-SA. (G, H) The Fourier-transform (FT) k^2 -weighted EXAFS and the fit spectra. (I) k^2 -weighted Fourier transformed EXAFS spectra. (J) EXAFS fitting spectra. (K–M) The wavelet transforms (WT) analysis of k -edge EXAFS oscillation spectrum of Fe-foil, Fe-SA, Fe_2O_3 .

of the chemical coordination environment of a typical N -coordinated metal single-atom structure. As shown in Fig. 2J, it accurately indicates that the coordination number of nitrogen surrounding Fe is nearly four. The Fe atom is incorporated as $\text{Fe}-\text{N}_4$ co-dopants within the hierarchical carbon-based matrix of Fe-SA. Finally, the resolution in both k - and R -space was examined by employing wavelet transform analysis of the k -edge EXAFS oscillation spectrum (Fig. 2K–M). It is evident that Fe-SA exhibits the strongest absorption at $5 \text{ \AA}^{-1}$ when compared to Fe_2O_3 and Fe foil^{31–33}.

3.2. Synthesis and characterization of FCH

The synthesis of FCH is depicted in Fig. 3A, comprising two primary steps. Cur, a compound derived from natural ginger, possesses potent anti-inflammatory properties. However, its limited solubility in water restricts its range of applications³⁴. In

contrast to more complex delivery systems, the coordination of Fe-SA with Cur (Fe-SA/Cur) markedly enhances drug delivery, thereby improving its anti-inflammatory efficacy. The HAD coating exhibits notable stability and a negative charge, ensuring its oral bioavailability and facilitating the targeted delivery of therapeutics. The synthesis method and characterization of HAD were presented in previous work (Supporting Information Figs. S4 and S5).

TEM images reveal that the surface of Fe-SA/Cur exhibits an enhanced presence of layered structures compared to Fe-SA, as the previously collapsed architecture is progressively filled by Cur. After the HAD coating, the surface of FCH becomes enriched with additional layered materials (Fig. 3B–D). The EDS spectrum of FCH shows four elements: C (59.23%), N (32.34%), O (8.27%), Fe (0.15%) (Supporting Information Fig. S6). Furthermore, the Fourier transform infrared (FTIR) and ultraviolet-visible (UV–Vis) absorption spectra confirm the loading of Cur and its

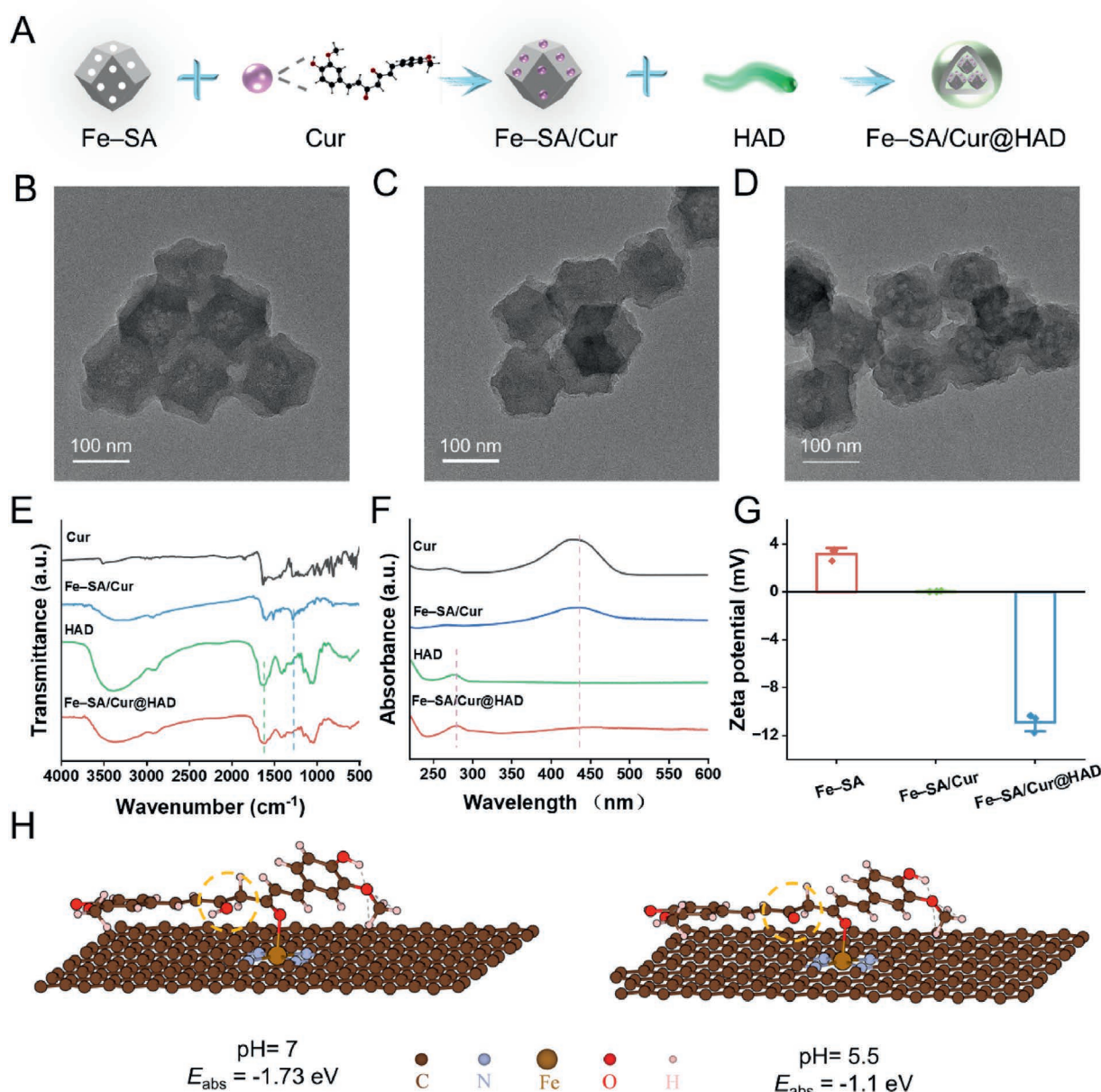


Figure 3 The synthesis and characterization of FCH. (A) The synthesis scheme of FCH. (B–D) The TEM images of Fe–SA, Fe–SA/Cur, FCH. Scale bar = 100 nm. (E) The FTIR spectra. (F) The UV–Vis spectra. (G) The zeta potential of Fe–SA, Fe–SA/Cur, FCH. (H) The binding energy of Fe–SA/Cur at different pH values by DFT computations, yellow circle: differences of structures. Data are presented as mean $\pm$ SD ($n = 3$).

encapsulation by HAD, thereby demonstrating the successful preparation of FCH (Fig. 3E and F). The zeta potential analysis shows that, following HAD loading, the zeta potential measures -10 mV (Fig. 3G). This substantial negative value establishes the foundation for targeted delivery to the positively charged regions affected by IBD³⁵.

The *in vitro* release of Cur was performed in simulated gastric, IBD microenvironments, intestinal fluids (pH 1.2, 5.5, 7), which were used to mimic different *in vivo* environments. Within 24 h, nearly 70% of Cur was released in IBD microenvironments (pH 5.5). Less than 20% of Cur was released in simulated gastric and intestinal fluids, indicating FCH could effectively pass through the stomach and small intestine (Supporting Information Fig. S7). Subsequently, FCH was incubated in simulated gastric and

intestinal fluids for 4 h to confirm its acid resistance and drug release properties. Typically, the gastric and intestinal transit times following oral administration are approximately 2 and 4 h, respectively. After immersion in gastric acid or intestinal fluid for 4 h, its structure showed negligible alteration, thereby confirming its acid resistance and targeted drug release capabilities (Supporting Information Figs. S8 and S9).

To elucidate the interaction between Cur and Fe–SA, DFT calculations were conducted on Fe–SA/Cur. Given the mildly acidic nature of the inflammatory microenvironment, the adsorption energies of Cur on Fe–N–C were calculated under acidic (pH 5.5) and physiological (pH 7) conditions (Fig. 3H). The results indicate weaker Cur–Fe–N–C interaction under acidic conditions ($E_{\text{ads}} = -1.10$ eV) than under physiological

conditions ($E_{\text{ads}} = -1.73$ eV). This notable difference suggests that the lower pH in the IBD environment may alter the protonation state of the Cur molecule, thereby affecting its charge distribution and electron cloud density. Such protonation alterations could diminish the electrostatic interactions between Fe-SA and Cur, resulting in a reduction in the overall binding energy.

3.3. Self-cascade reactions of FCH

SOD represents the initial step of the self-cascade reaction. Initially, the SOD-like activity and free radical scavenging capabilities of Fe-SA were assessed, focusing on four specific radicals: ABTS $\cdot$, DPPH $\cdot$, $\cdot$ OH, and $\text{O}_2^{\cdot-}$ ³⁶. These radicals are abundant in biological systems and are commonly associated with inflammation. As the concentration of FCH increases, its ability to scavenge these radicals improves progressively. At a concentration of 25 $\mu\text{g/mL}$, scavenging efficiencies exceeding 75% were observed for all radicals (Fig. 4A–D). Subsequently, electron spin resonance (ESR) spectroscopy was employed to visually assess the free radical scavenging efficiency. Using the

electron capture agent 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) to trap the radicals, it was found that following treatment with FCH, the free radical signals were markedly diminished, providing compelling evidence of its SOD-like activity and free radical scavenging potential (Supporting Information Figs. S10 and S11)¹³.

The self-cascade reaction of FCH is depicted in Fig. 4E. During this process, $\text{O}_2^{\cdot-}$ is converted into H_2O_2 through SOD-like activity. H_2O_2 then acts as a substrate for peroxidases, undergoing conversion into O_2 and H_2O via a CAT-like mechanism. The CAT-like activity of FCH was subsequently evaluated using a dissolved oxygen meter. As shown in Fig. 4F and G, the dissolved oxygen levels increase progressively over time with rising concentrations of H_2O_2 and FCH. This phenomenon occurs because H_2O_2 functions as the substrate in the CAT reaction and serves as a pivotal intermediate in the self-cascade reaction¹⁵. The dissolved oxygen generation per minute and H_2O_2 concentrations conform to Michaelis-Menten kinetics, indicating that this system demonstrates substantial CAT-like activity (Fig. 4H). Furthermore, we compared the activity with natural SOD, CAT enzymes. FCH shows efficiency enzymatic activity

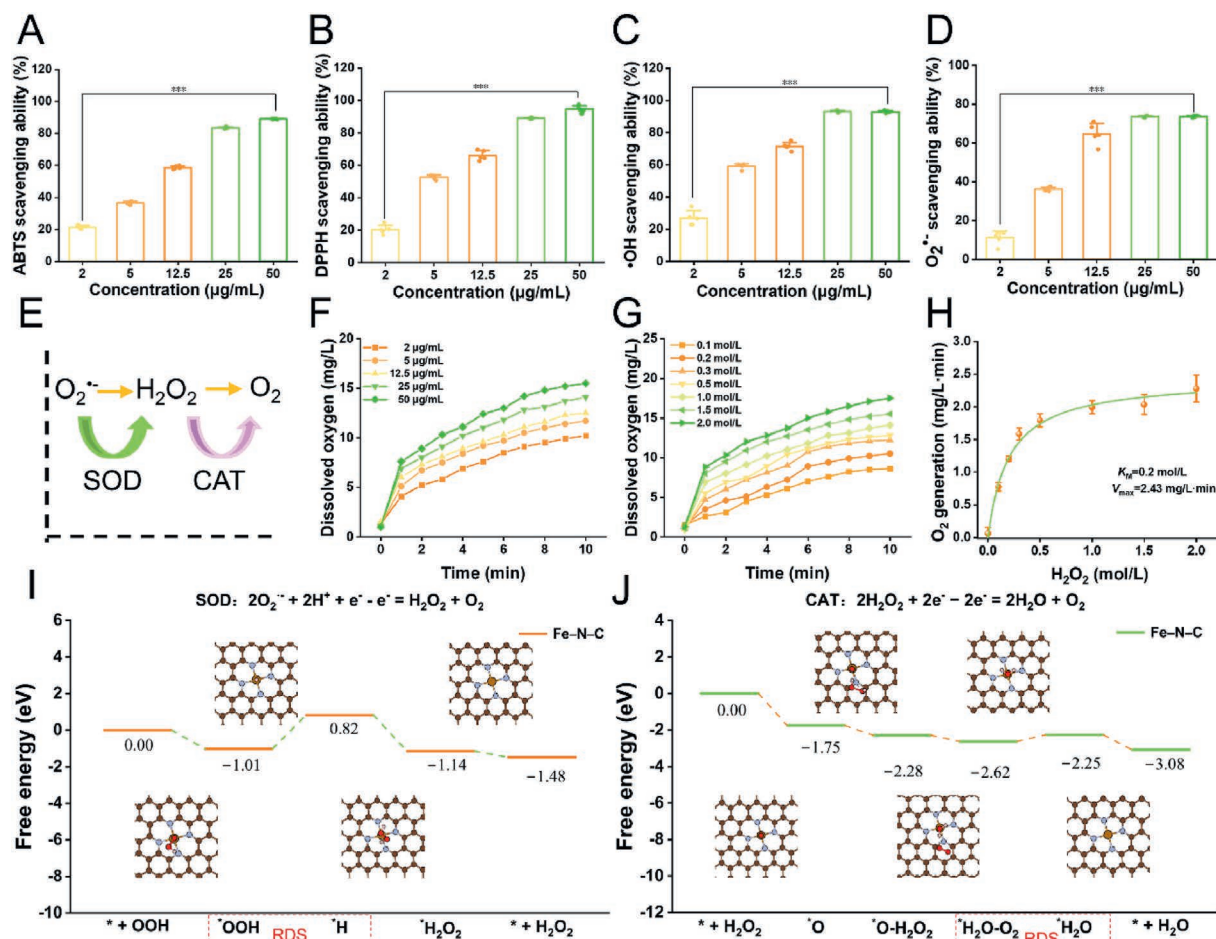


Figure 4 The self-cascade reactions of FCH. (A–D) The radicals scavenging ability of different concentrations of FCH. (E) The scheme of self-cascade reaction. (F) The dissolved oxygen within different concentrations of FCH. (G) The dissolved oxygen within different concentrations of H_2O_2 . (H) The Michaelis–Menten kinetics of CAT-like activity of FCH. (I) The reaction energy diagrams of SOD-like activity of Fe-SA/Cur. The optimized structures of key reaction steps are shown as insets. (J) The reaction energy diagrams of CAT-like activity of Fe-SA/Cur. The optimized structures of key reaction steps are shown as insets. Data are presented as mean $\pm$ SD, One-way ANOVA with Tukey's multiple comparisons test was used ($n = 5$), *** $P < 0.001$.

similar to that of natural enzymes (Supporting Information Tables S2 and S3).

Additionally, DFT calculations were performed to investigate the catalytic performance of the Fe-N-C nanozyme in two-electron SOD-like and four-electron CAT-like reactions (Fig. 4I and J)³⁷. The Gibbs free energies of all species—including reactants, intermediates, and products—were calculated. In the SOD-like reaction, adsorption of the OOH reactant is exothermic, releasing 1.01 eV of energy. The formation of *H from *OOH is the potential-limit step (PLS), requiring an energy input of 1.83 eV. *H is subsequently converted into a *H₂O₂ intermediate in an exothermic step releasing 1.96 eV. Finally, H₂O₂ desorbs from the surface an exothermic step (0.34 eV), completing the SOD-like reaction.

In the CAT-like reaction, the adsorption and conversion of H₂O₂ into *O is highly exothermic, releasing 1.75 eV. The *O intermediate then binds a second H₂O₂ molecule in another exothermic step (0.54). The subsequent transformation of *O-H₂O₂ to *H₂O-O₂ is also exothermic, releasing 0.34 eV. *H₂O-O₂ then converts into *H₂O with the release of an O₂ molecule, which is the PLS (0.36 eV). The final desorption of H₂O is exothermic, with an energy release of 0.82 eV¹³. Through the examination and

DFT calculations on the enzyme-like activities, evidence was obtained demonstrating that a self-cascade reaction may occur, thereby facilitating further investigations into its potential for anti-inflammatory biological applications.

3.4. Cellular anti-inflammation efficiency of IBD

The biosafety was evaluated through the CCK-8 assay on human epithelial cells (NCM460). As illustrated in Fig. 5A–C, NCM460 cells were exposed to various concentrations of Fe–SA, Fe–SA/Cur, and FCH for 24 h, with cell viability remaining above 80% even at the highest concentration (100 µg/mL) tested. These findings suggest a favorable safety profile with minimal toxicity. The self-cascade reaction has been confirmed in an *in vitro* buffered solution; further investigations will be conducted to explore its intracellular self-cascade reaction. Inflammation of NCM460 cells was induced with 3% dextran sulfate sodium salt (DSS), and intracellular ROS production was subsequently measured using the DCFH-DA probe. As shown in Fig. 5D, a considerable amount of ROS was generated in the cells following DSS induction, which is indicated by intense green fluorescence. However, treatment with Fe–SA, Fe–SA/Cur, or FCH markedly

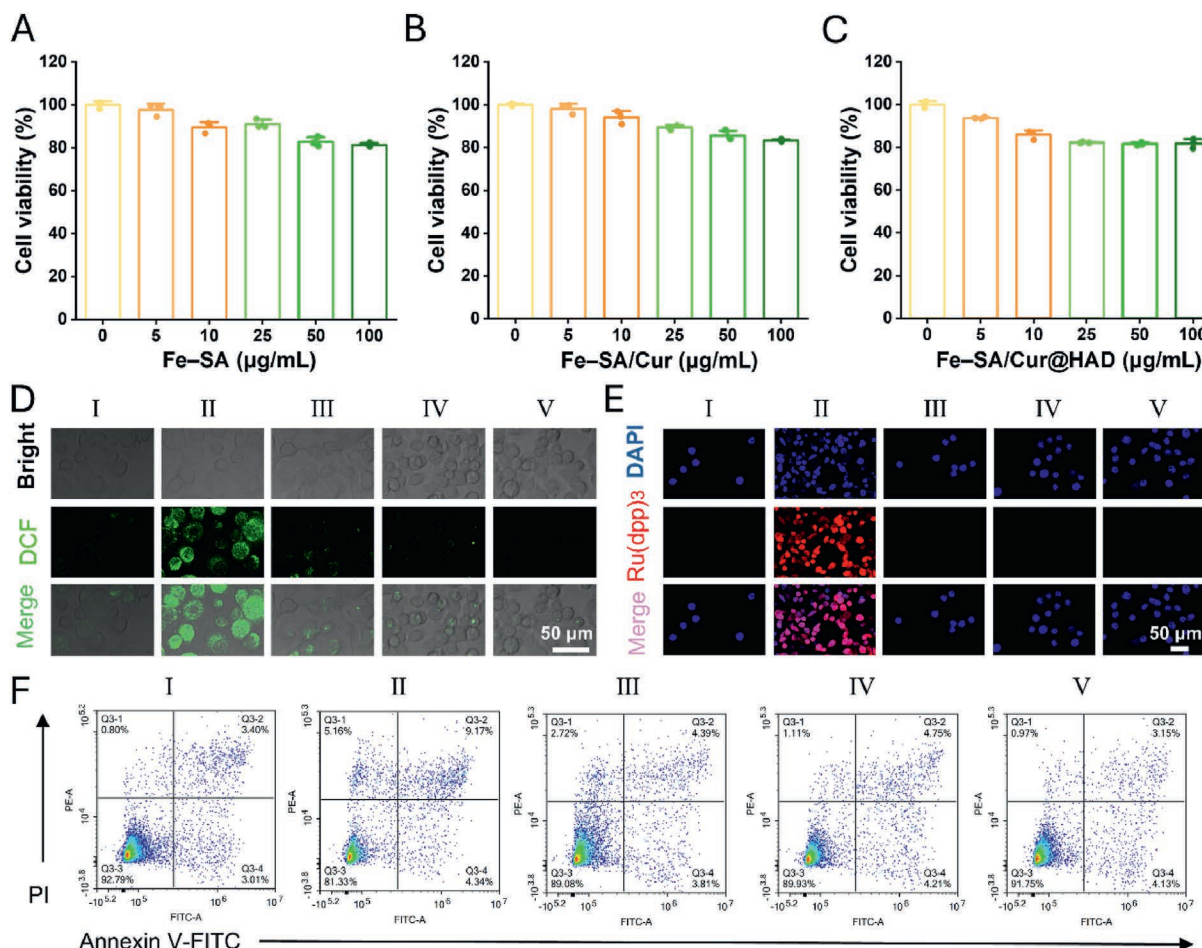


Figure 5 *In vitro* anti-inflammatory properties of FCH. (A–C) The cytotoxicity test of different concentration Fe–SA, Fe–SA/Cur and FCH with NCM 460 cells. (D) The intracellular scavenging of ROS with different treatments through the ROS probe DCFH-DA. Scale bar = 50 µm. (E) The intracellular generation of O₂ with different treatments through the oxygen-sensitive probe [Ru(dpp)₃]Cl₂. Scale bar = 50 µm. (F) The apoptosis analysis of NCM460 cells with different treatments *via* flow cytometry (flow cytometry was repeated independently three times). I: Control, II: IBD + PBS, III: IBD + Fe–SA (25 µg/mL), IV: IBD + Fe–SA/Cur (25 µg/mL), V: IBD + FCH (25 µg/mL). Data are presented as mean ± SD (*n* = 3).

reduced the DSS-induced intracellular ROS levels. Compared to the “IBD + Fe-SA” group, the green fluorescence in the “IBD + FCH” group was markedly diminished, nearly absent. Notably, FCH treatment appeared to nearly completely reverse the DSS-induced OS. This reduction is likely due to the enhanced ROS elimination by Cur, which is coordinated with the complex, thereby demonstrating a more potent synergistic anti-inflammatory effect.

Intracellular O₂ production was subsequently measured using the hypoxia probe Ru(dpp)₃Cl₂²⁹. As illustrated in Fig. 5E, intense red fluorescence was observed in the cells following 3% DSS induction, indicating the establishment of a hypoxic environment associated with inflammation. After treatment with FCH etc., the red fluorescence intensity in the cells decreased markedly, suggesting that the drug can enhance oxygen levels within the inflammatory microenvironment, thus alleviating hypoxic conditions. The intracellular fluorescence staining further supports the occurrence of the self-cascade reaction, indicating a significant improvement within the inflammatory microenvironment. To assess the effects of FCH et al. on apoptosis after DSS induction, NCM460 cells were detected using Annexin V/PI kit followed by flow cytometry analysis. As shown in Fig. 5F and Supporting Information Fig. S12, DSS treatment successfully induced significant apoptosis, while FCH et al. treatment significantly alleviated the level of apoptosis, suggesting its marked anti-apoptotic capacity.

3.5. Evaluating FCH performance in colonic organoid models

The intestinal organoid culture system was successfully established for the first time in 2009 by the Hans Clevers laboratory in the Netherlands³⁸. Intestinal organoids retain the characteristics of their distinct crypts, with cell composition and arrangement closely mirroring those of normal intestinal epithelial structures.

Currently, intestinal organoids are widely employed in the study of intestinal diseases, serving as a crucial *in vitro* model for investigating the normal physiology and pathology of intestinal epithelial cells. In this study, colon-derived distinct crypts were extracted from endoscopic biopsy samples of volunteers and cultured *in vitro* to construct 3D organoids. Subsequently, an *in vitro* IBD model was established using TNF- α (50 ng/mL) and IFN- γ (50 ng/mL), and FCH was administered to assess its therapeutic effect on IBD.

As shown in Fig. 6A, the spherical volume of the organoids progressively increased during the two-day culture period, indicating the successful establishment of intestinal organoids *in vitro*. To mimic the physiological environment, inflammatory factors (TNF- α and IFN- γ) were added into the medium of organoids, which resulted in significant morphological alterations, leading to compromised epithelial integrity and the presence of numerous black dead cells within the lumen, thereby confirming the effective induction of this *in vitro* IBD model (Fig. 6B)³⁹. Treatment with FCH markedly ameliorated the damage to colonic organoids induced by inflammatory factors (Fig. 6B and Supporting Information Fig. S13). These findings demonstrate that FCH exerts a beneficial therapeutic effect on the organoid IBD model.

To further visualize the phenotypic changes during inflammation, organoids were observed under a laser confocal microscope. Evaluation of the treatment effect of FCH yielded comparable results, as 3D reconstruction of immunofluorescence (IF)-stained

organoids revealed similar morphological alterations to those observed in the organoids under bright-field microscopy (Fig. 6C).

Zonula Occludens-1 (ZO-1) is essential for maintaining cell adhesion and the integrity of the epithelial barrier⁴⁰. It facilitates calcium-dependent intercellular adhesion and promotes the formation of tight junctions, thereby preventing the passage of bacteria and harmful substances through intercellular spaces. Occludin, another tight junction protein, serves a pivotal function in preserving epithelial barrier function.

Therefore, both ZO-1 and Occludin serve as critical indicators of epithelial function and repair efficacy. In the IBD group, both IF-stained ZO-1 and Occludin exhibited a significant reduction in expression levels and a loss of continuous structure compared to the control group, indicating that the epithelial barrier function of organoids was notably impaired during inflammation (Fig. 6C).

As anticipated, FCH treatment markedly restored intestinal barrier function. These findings thus indicate that FCH effectively promotes the recovery of intestinal barrier function in organoids, highlighting its importance in restoring damaged intestinal structures and providing robust support for further exploration of its *in vivo* applications.

3.6. In vivo treatment of IBD

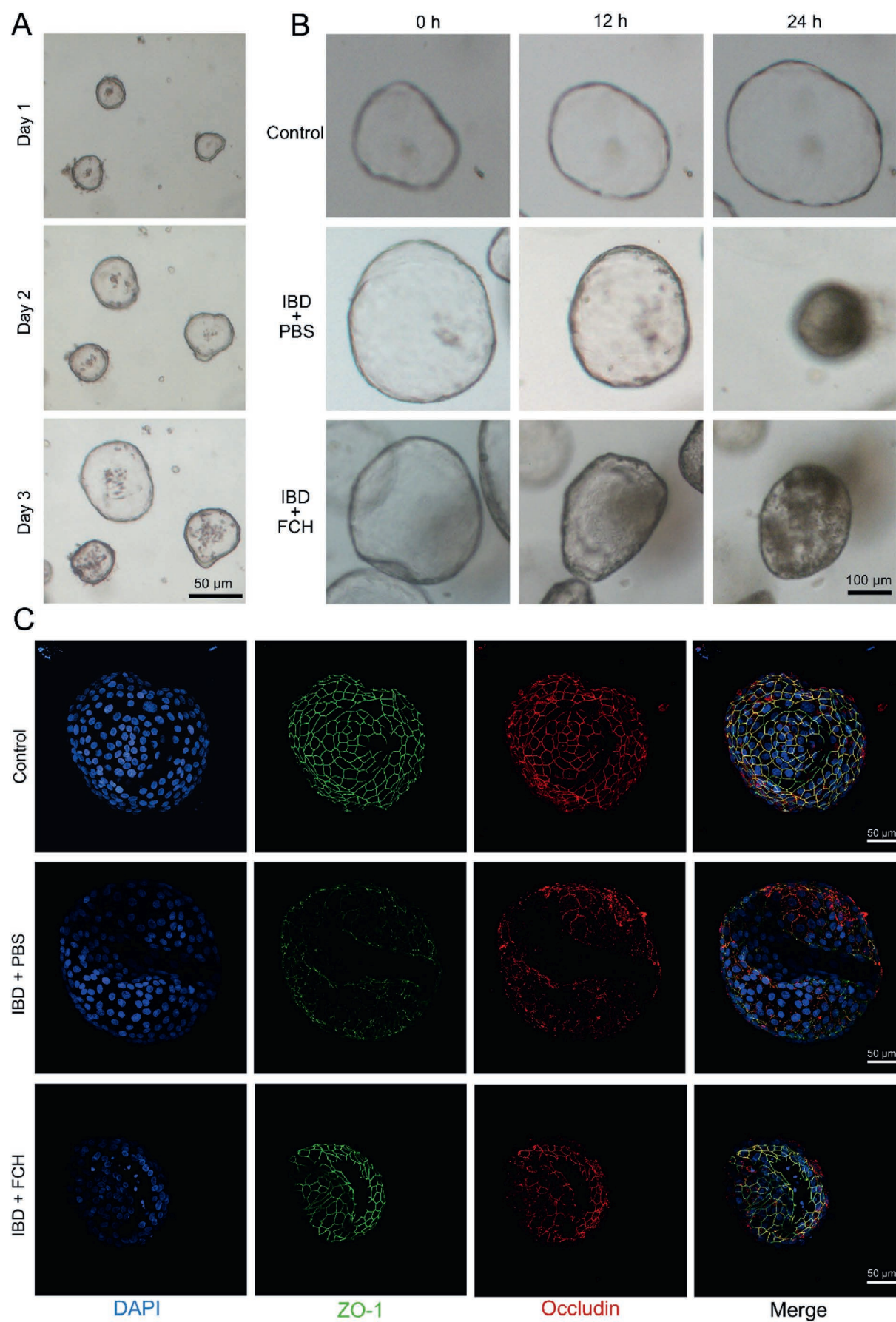
As illustrated in Fig. 7A, an acute IBD model was established by providing free access to a 2.5% w/v DSS solution in drinking water for 7 consecutive days. Simultaneously, mice in the IBD + PBS, IBD + Fe-SA, IBD + Fe-SA/Cur, or IBD + FCH groups were administered phosphate-buffered solution (PBS), Fe-SA, Fe-SA/Cur, or FCH *via* oral gavage on Days 1, 3, 5, and 7, respectively. Mice receiving normal water without drug administration served as controls. Body weight, gross bleeding, and stool consistency were monitored daily to calculate the disease activity index (DAI) scores for assessing the severity of IBD, as previously described⁴¹. The mice were subsequently sacrificed on day 8 to evaluate the therapeutic effects of the treatment on IBD *in vivo*.

Indocyanine green (ICG)-loaded materials were administered to IBD mice *via* oral gavage to evaluate the target efficacy. Notable fluorescence intensity was observed in the lower abdominal region of IBD + FCH mice at 24 h, demonstrating the target accumulation of FCH in the IBD site. To further evaluate the tissue distribution and metabolism, as well as the colon-specific adhesion efficacy of FCH, mice were euthanized after 24 h.

Then, *ex vivo* imaging of major organs was explored. As shown in Supporting Information Fig. S14, the colon tissue from “IBD + FCH” group exhibits highest fluorescence intensity, exhibiting a precise targeting capability of FCH towards the inflamed colonic regions. In contrast, no obvious fluorescence was observed in other organs, like spleen, kidney, or liver, indicating that FCH primarily acts locally within the colon and does not accumulate significantly in other organs.

Mice exposed to DSS exhibited significant weight loss compared to those in the control group (Fig. 7B)⁴². Furthermore, no significant difference in body weight change was observed between the IBD + PBS and IBD + Fe-SA or IBD + Fe-SA/Cur groups. However, FCH treatment markedly alleviated the weight loss in mice compared to the PBS treatment (Fig. 7B).

Similarly, the DAI scores indicated markedly milder intestinal inflammation in the IBD + FCH group compared to the other groups (Fig. 7C). Postmortem, the entire colon was carefully



extracted from the cecum to the anus to assess its length, which served as a critical marker of inflammation severity. Compared to the IBD + PBS group, the IBD + FCH group demonstrated significant remission in colon length shortening, thereby indicating the therapeutic effect of FCH on intestinal inflammation (Fig. 7D and E).

The therapeutic effects of Fe-SA and Fe-SA/Cur were relatively limited, potentially due to their poor targeting delivery capability. Besides, continuous administration of FCH to healthy mice for one week, followed by histological examination of the main organs using HE staining, as well as routine blood tests and serum biochemical analysis, revealed no significant toxicity of FCH (Supporting Information Figs. S15–S17).

Mouse distal colon tissues were collected from each group for histological examination. Hematoxylin and eosin staining revealed significant morphological damage of the colon following DSS treatment, including the loss of colonic crypt structure and inflammatory cell infiltration (Fig. 7F). Although Fe-SA and Fe-SA/Cur treatment showed no significant improvement, a clear histopathological remission was observed following FCH treatment (Fig. 7F). Alcian Blue with periodic acid and Schiff's solution (AB-PAS) staining, which specifically stains the mucins present in goblet cells, was employed as a key indicator of barrier function. Compared to other groups, the IBD + FCH group demonstrated a notable recovery in mucin deposition (Fig. 7G).

E-cadherin, an essential cell-cell adhesion molecule, serves a pivotal function in maintaining intestinal barrier function, and its dysfunction may compromise this integrity. As depicted in Fig. 8A and Supporting Information Fig. S18, a significant decrease in E-cadherin expression was observed in the colon of the IBD + PBS group *via* IF staining. Although no substantial recovery was noted in the colon tissue following Fe-SA or Fe-SA/Cur treatment, FCH treatment led to a marked increase in E-cadherin fluorescence intensity, suggesting that the colon tissue was undergoing rapid recovery and demonstrating further structural improvement. Subsequently, the expression of ZO-1 in colon sections was also assessed *via* IF. In comparison to the IBD + PBS group, ZO-1 protein levels were markedly higher in the IBD + FCH group (Fig. 8B, Supporting Information Fig. S19). The effect of FCH treatment on the protein expression of E-cadherin was also confirmed *via* Western blotting (WB) (Fig. 9A).

Increased apoptosis of intestinal epithelial cells represents not only a significant pathogenic factor but also a key pathological feature of IBD. Therefore, the expression of apoptosis-related proteins in colon tissues was assessed *via* WB analysis. As anticipated, DSS treatment led to elevated levels of the pro-apoptotic proteins cleaved-Caspase 3 and Bax, along with a reduction in the expression of the anti-apoptotic protein BCL2 (Fig. 9B). This pattern was notably reversed following FCH treatment (Fig. 9B). These findings provide compelling evidence for the therapeutic efficacy of FCH in the treatment of IBD. Macrophage infiltration is a hallmark of intestinal inflammation. IF staining of F4/80, a specific marker for macrophages, and Ly6G, a specific marker for neutrophils, were performed on colon sections.

IBD exposure resulted in epithelial damage in the colon accompanied by prominent macrophage infiltration. Treatment

with FCH, rather than Fe-SA or Fe-SA/Cur, led to a significant reduction in macrophage and neutrophils infiltration (Fig. 9C, Supporting Information Figs. S20 and S21). The oxidation of dihydroethidium (DHE) by ROS yields ethidium, which emits red fluorescence upon intercalating into DNA. Thus, the efficacy of FCH in scavenging ROS was evaluated in colon tissues *via* DHE staining. As shown in the Supporting Information Fig. S22, the administration of FCH led to a marked decrease in red fluorescence in the inflamed intestinal tissues when compared to those in the PBS group, suggesting that FCH acts as a potent scavenger of free radicals.

3.7. Transcriptome analysis of the therapeutic mechanism of FCH

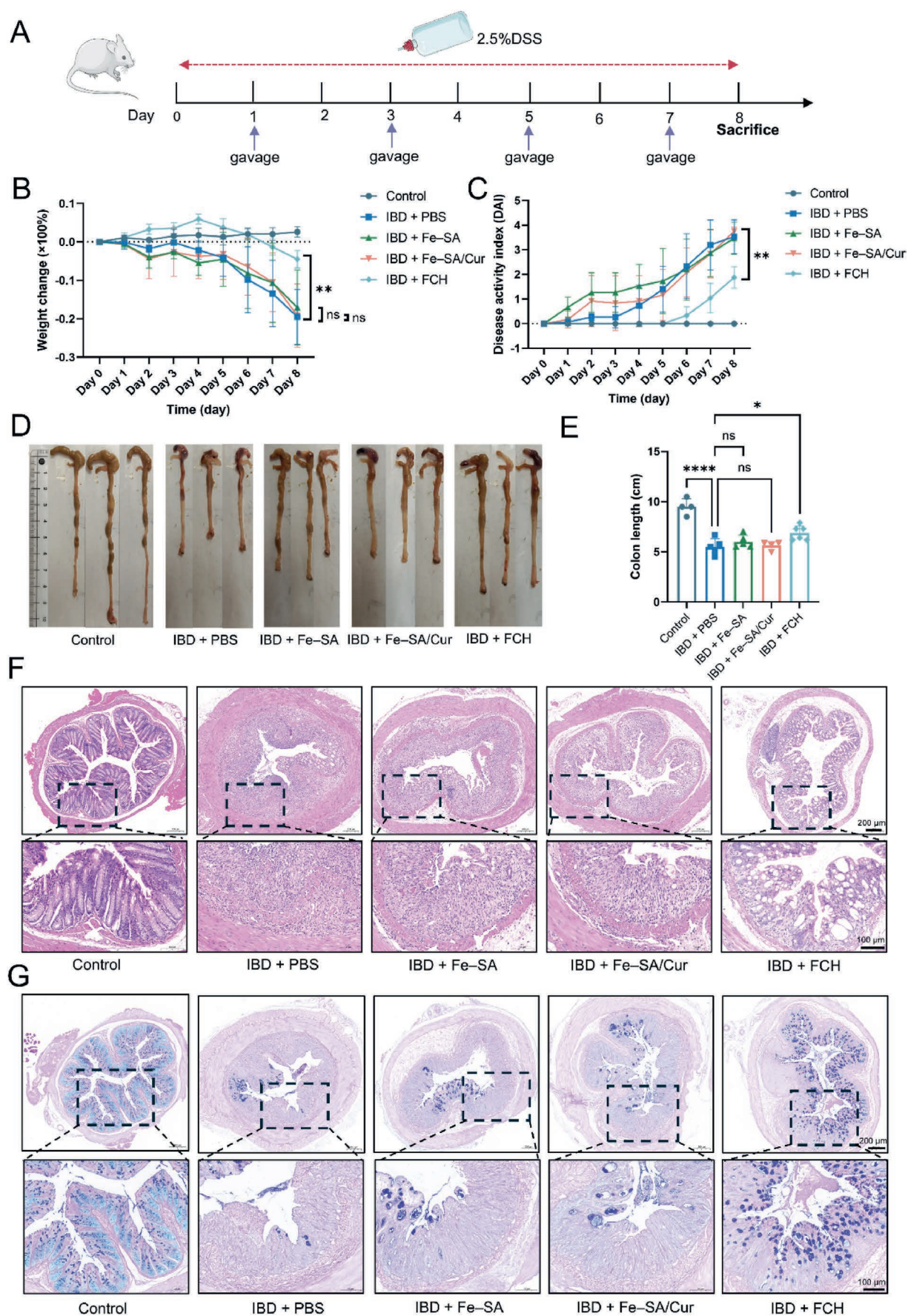
To thoroughly investigate the therapeutic mechanism of FCH in DSS-induced IBD in mice, RNA sequencing analysis of colon tissues was performed to evaluate the gene expression profiles. Differential expression analysis between the IBD + FCH and IBD + PBS groups identified 595 differentially expressed genes ($|\log_2\text{FC}| \geq 1$ & $q < 0.05$), of which 216 were up-regulated and 289 down-regulated (Fig. 10A). Gene Ontology analysis of the differentially expressed genes revealed significant enrichment in several key biological processes, including defense responses to bacteria or viruses, regulation of innate immune responses, and cellular responses to OS (Fig. 10B).

Gene-set enrichment analysis was performed to further investigate the potential mechanisms. As depicted in Fig. 10C, significant alterations were identified in pathways such as interferon signaling, the innate immune system, and the Toll-like receptor (TLR) signaling pathway. TLRs, as classic pattern recognition receptors and key responders to invading pathogens, serve a pivotal function in the innate immune system, with the TLR signaling pathway being closely implicated in various inflammatory diseases.

Additionally, Kyoto Encyclopedia of Genes and Genomes pathway analysis demonstrated strong enrichment for terms related to the NOD-like receptor signaling pathway, antigen processing and presentation, PPAR signaling pathway, Epstein-Barr virus infection, primary immunodeficiency, mucin-type O-glycan biosynthesis, and the RIG-I-like receptor signaling pathway (Supporting Information Fig. S23). These findings provide compelling evidence that FCH may ameliorate intestinal inflammation by enhancing defensive responses to microbial threats, modulating innate immune responses, and mitigating OS.

Notably, TLR4, a highly conserved protein, is widely recognized as serving a critical function in the regulation of intestinal homeostasis^{43,44}. To assess the activation of the TLR4 signaling pathway in mouse colon tissue, WB analysis was performed. As illustrated in Fig. 10D, the expression levels of MyD88 and NF- κ B were markedly increased following DSS administration. However, this trend was reversed by FCH treatment. A similar trend was observed for the expressions of TLR4, but the difference did not reach statistical significance. These results suggest that the TLR4/MyD88/NF- κ B involved inflammatory pathway may represent a key mechanism underlying the therapeutic effects of FCH in IBD treatment.

Figure 6 Evaluation of anti-inflammatory properties of FCH at organoid level. (A) Establishment and growth process of human colonic organoids. Scale bar = 50 μ m. (B) Representative images of morphological changes in organoids under different treatments. Scale bar = 100 μ m. (C) Representative images of the effects of different treatments on intestinal barrier proteins in organoids. Scale bar = 50 μ m.



3.8. Effect of FCH on the composition of intestinal flora

The ecological balance of the gut commensal microbiota regulates the phenotype of the intestinal epithelium and serves a critical function in the progression of IBD. The commensal microbiota and intestinal functions are interdependent; dysbiosis of the gut microbiota can lead to persistent intestinal inflammation and damage to the epithelial barrier, while changes in the colonic microenvironment may influence the composition of the microbiota.

To examine the characteristics of the gut microbiota in mice following DSS and FCH treatments, 16S ribosomal DNA gene sequencing was employed to analyze the microbiota composition in fecal samples. Alpha-diversity indices of the microbial communities, including the Shannon index, ACE index, coverage index, and Chao index, were analyzed across all groups. A significant reduction in the Shannon index was observed following DSS treatment (Fig. 11A), whereas no substantial changes were detected in the other indices (Supporting Information Fig. S24).

For beta-diversity, principal coordinate analysis based on operational taxonomic units revealed distinct differences in the distribution of microbiota between the control group, IBD (IBD + PBS) group, and treatment (IBD + FCH) group (Fig. 11B). Subsequently, the microbiota dysbiosis index (MDI) was employed to assess the degree of microbial imbalance⁴⁵. As depicted in Fig. 11C, the IBD group demonstrated significant dysbiosis compared to the control group. Although this difference did not reach statistical significance, a reduction in the dysbiosis index was observed in the treatment group compared to the IBD group, suggesting that FCH serves a regulatory function in maintaining microbial balance.

Compared to healthy individuals, it has been reported that patients with IBD display an intestinal microbiome characterized by a reduced relative abundance of *Firmicutes* and *Bacteroidetes*, coupled with an increase in *Proteobacteria* and *Actinobacteria*^{46,47}. Community bar plot analysis revealed alterations in the relative abundance of differential microbiota among the three groups at the phylum level (Fig. 11D). Consistent with findings in IBD patients, IBD exposure led to a decrease in the relative abundance of *Firmicutes* and an increase in *Proteobacteria*, as illustrated in the bar chart. Notably, FCH treatment markedly reversed the IBD-induced imbalance in gut microbiota composition (Fig. 11D). The community heatmap and Circos graph visually depicted the relative abundance and distribution of the fecal microbiome across different groups following IBD and FCH treatment (Fig. 11E, Supporting Information Fig. S25). Overall, these findings suggest that FCH-regulated intestinal flora may serve a pivotal function in alleviating IBD. Differences in microbiota composition across multiple groups were further analyzed. Interestingly, *Proteobacteria* and *Firmicutes* were identified as markedly altered microbiota (Fig. 11F), suggesting that these changes may be closely associated with intestinal inflammation and the therapeutic mechanisms of FCH.

4. Discussion

This study introduces for the first time a novel strategy, termed “SAzymes coordination”, which facilitates the development of an oral SAzyme platform (FCH) aimed at the effective treatment of IBD. Specifically, by leveraging SAzymes characterized by Fe–N₄ bonding, a coordination structure was formed with the well-known anti-inflammatory drug, Cur, followed by the incorporation of HAD into the outer layer to ultimately assemble FCH. Importantly, the method of preparation employed in this study was both natural and cost-effective. FCH not only capitalized on the properties of natural anti-inflammatory agents but also exploited the SOD-CAT cascade reaction of nanozymes for the efficient treatment of IBD. Experimental results revealed that FCH was capable of overcoming barriers such as gastric acid and simulated intestinal fluid, ensuring efficient accumulation in affected areas through its outer coating while optimizing the SOD-CAT cascade reaction. Furthermore, *in vitro* cellular and organoid experiments, along with *in vivo* animal trials, confirmed the efficacy of FCH in alleviating intestinal inflammation by promoting the repair of intestinal barrier damage and regulating gut microbiota.

Nanozymes, as a novel class of nanomaterials, combine the physical properties of nanomaterials with the catalytic activity inherent in natural enzymes⁹. Recently, SAzymes have garnered significant attention from researchers due to their remarkable catalytic efficiency²². In SAzymes, the individual metal atom serves as the catalytic active site, while the types and coordination numbers of surrounding atoms serve a pivotal function in determining catalytic activity, a central focus of contemporary research. As a result, macroscopic modifications have largely been disregarded. In this study, a smart SAzymes delivery system was constructed here, which integrates SAzymes with the natural drug Cur through coordination self-assembly. This strategy not only maximized the catalytic efficiency of the SAzymes but also enhanced the therapeutic effectiveness of the natural drug, further modulating the cascade reactions of nanozymes.

In the current treatment of IBD, two primary issues warrant attention. The first concerns the response to and enhancement of the IBD microenvironment, while the second pertains to the efficacy of repairing damaged colonic structures²⁵. The IBD microenvironment is characterized by an abundance of ROS, and the concurrent microbial imbalance contributes to a state of hypoxia¹³. The FCH developed in this study effectively exploits the SOD-CAT cascade reaction of FCH to eliminate ROS and simultaneously generate O₂, thereby addressing and improving the hypoxic conditions within the microenvironment. Through experimental studies and DFT theoretical calculations, it has been established that FCH is capable of undergoing nanozyme SOD-CAT cascade reaction, thus providing a basis for its therapeutic effects in IBD.

Damaged intestinal structures may result in an imbalance of gut microbiota and the invasion of enteric pathogens, thereby

Figure 7 *In vivo* anti-inflammatory properties of FCH. (A) Schematic diagram of the experimental procedure. Acute experimental colitis in mice was induced by 2.5% DSS ($n = 4-7$). Gavage was performed on Days 1, 3, 5, and 7 with PBS, Fe–SA, Fe–SA/Cur, and FCH, respectively. Mice were sacrificed and tissues were collected on Day 8. (B) Percentage changes in mouse body weight. (C) Changes in the DAI scores. (D) Representative images and (E) lengths of the mouse colons from each group. (F) Representative images of H&E staining of colonic tissues from each group. Scale bar = 200 (top) and 100 μ m (bottom). (G) Representative images of AB-PAS staining of colonic tissues from each group. Scale bar = 200 (top) and 100 μ m (bottom). Data are presented as mean $\pm$ SD, repeated measures ANOVA was used to compare changes in body weight loss and DAI scores across time among groups, One-way ANOVA followed by Dunnett's test was used for multiple comparisons for colon lengths among groups, ns: $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$.

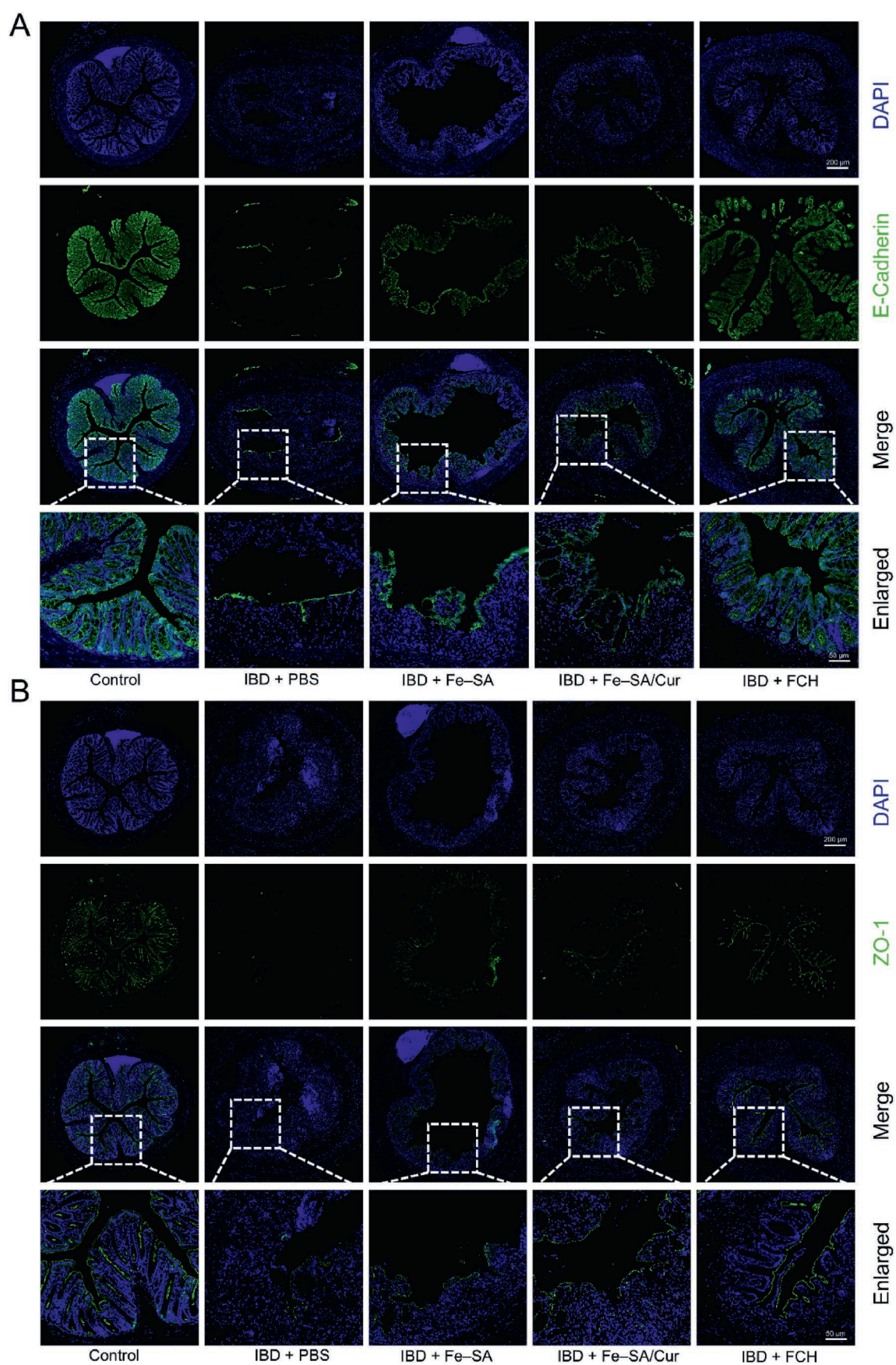
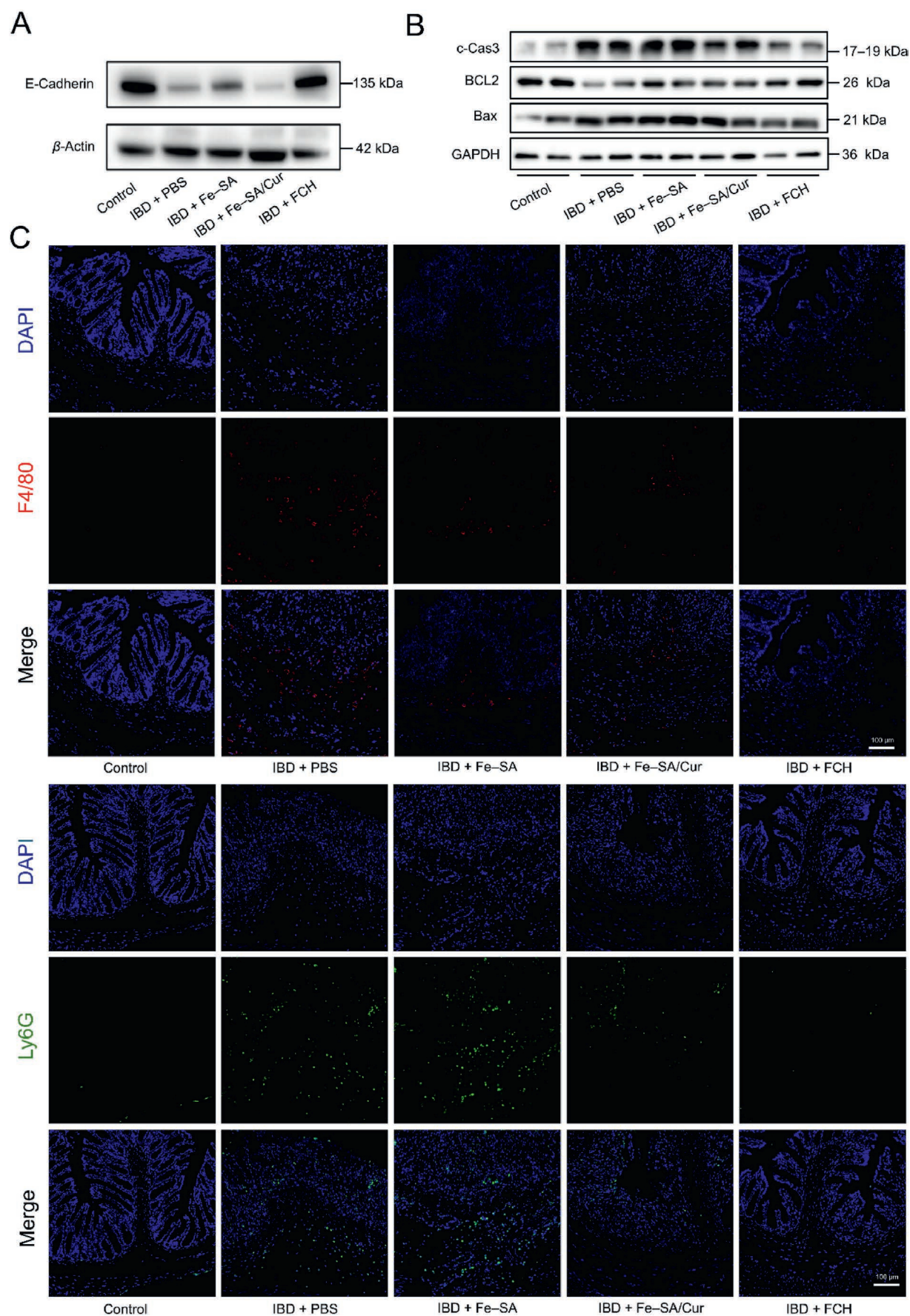


Figure 8 *In vivo* anti-inflammation property of FCH. Representative images of (A) E-Cadherin and (B) ZO-1 IF staining of colonic tissues. Scale bar = 200 µm (top), 50 µm (bottom).



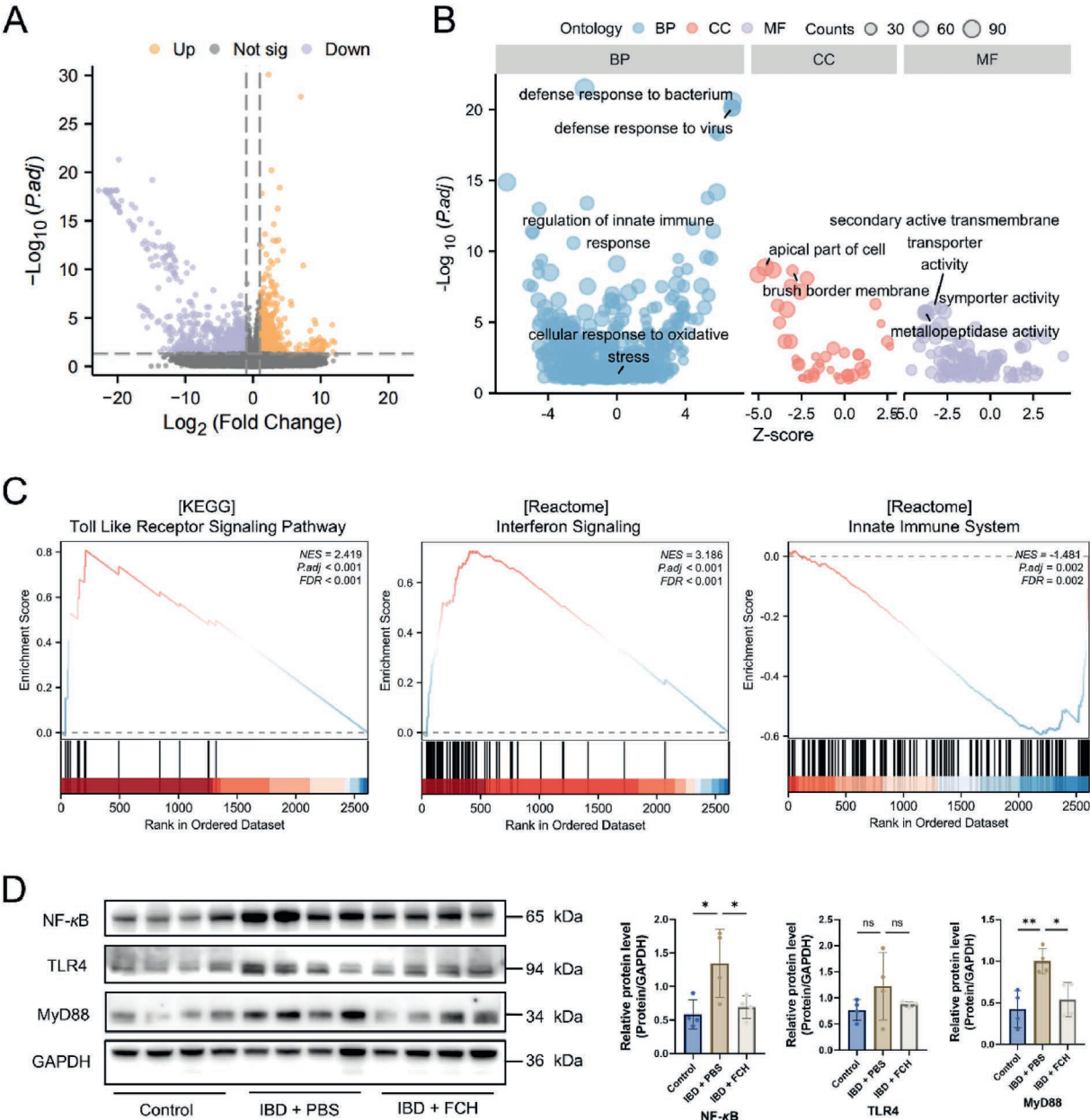


Figure 10 RNA sequencing revealing the potential mechanisms of FCH in treating IBD. (A) Volcano plot showing differential gene expression between IBD + PBS and IBD + FCH groups. (B) GO enrichment analysis of significantly differentially expressed genes categorized into Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). (C) Gene set enrichment analysis plots illustrating significantly enriched signaling pathways. (D) Western blot analysis and corresponding quantification of NF-κB, TLR4, and MyD88 protein levels in control, IBD + PBS, and IBD + FCH groups (One-way ANOVA followed by Dunnett's test was used for multiple comparisons), ns: $P > 0.05$, $*P < 0.05$, $**P < 0.01$. $n = 4$ for RNA sequencing and WB in each group.

establishing a vicious cycle. Using *in vitro* organoid models and an *in vivo* mouse IBD model, it was demonstrated that FCH effectively increased the expression of E-cadherin and ZO-1 proteins, thereby promoting the recovery of intestinal barrier function. Furthermore, the aforementioned studies showed that FCH could be efficiently targeted to the inflammation sites within the colon, reducing inflammation levels and inhibiting apoptosis, thereby exhibiting significant therapeutic effects for IBD. Regarding the

underlying mechanism, RNA-Sequencing analysis suggested that the defense response to pathogenic microorganisms, regulation of innate immune responses, and cellular response to OS may be markedly involved in FCH-mediated IBD treatment. Additionally, WB analysis further demonstrated that the TLR4/MyD88/NF-κB signaling pathway may serve as the key mechanism. Moreover, gut microbiome sequencing revealed that FCH serves a crucial function in restoring the homeostatic balance of commensal

Figure 9 *In vivo* anti-inflammatory properties of FCH. Detection of (A) E-cadherin ($n = 3$) and (B) apoptosis-related proteins by WB ($n = 3$). (C) Representative images of IF staining for F4/80 and Ly6G in colon. Scale bar = 100 μm.

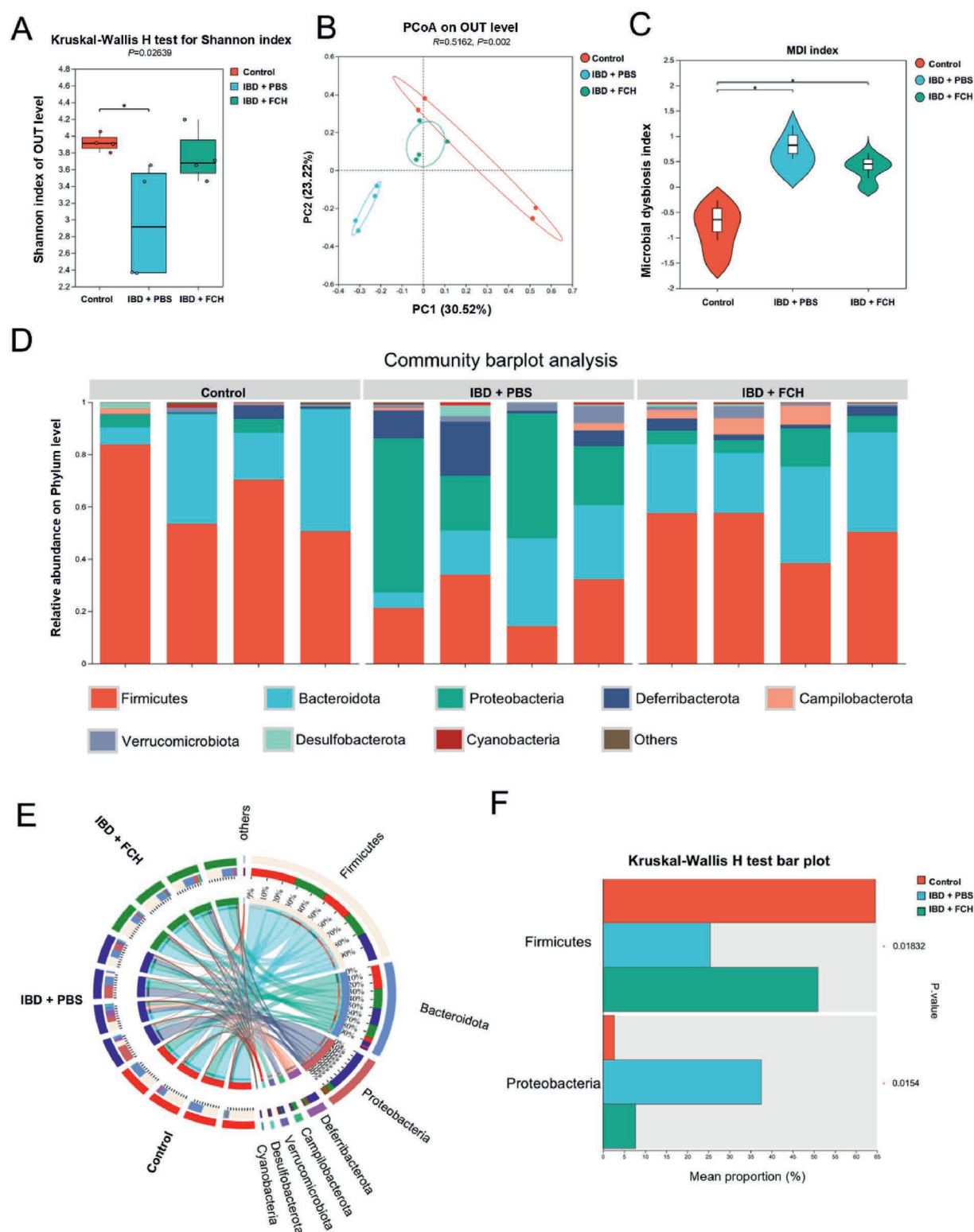


Figure 11 Fecal metabolomics analysis of the effect of FCH treatment on IBD. (A) Alpha diversity (Shannon index) analysis, comparing groups of the Control, IBD (IBD + PBS) and Treatment (IBD + FCH), $n = 4$. (B) The principal coordinate analysis (PCoA) on operational taxonomic units (OTU) levels. (C) The microbiota dysbiosis index (MDI) analysis. (D) Bar chart of the percent of community abundance of gut microbiota at the phylum level among three groups. (E) Circos plot analysis of the composition and the distribution of the fecal microbiome among different groups. (F) The analysis of species differences among multi-groups at the phylum level. $*P < 0.05$. $n = 4$ for fecal metabolomics analysis in each group.

microbes, particularly *Proteobacteria* and *Firmicutes*, thus suppressing intestinal inflammation.

Although this work provides compelling evidence for the efficacy in mitigating DSS-induced acute intestinal inflammation within a mice model, critical limitations related to clinical translation and long-term safety should be acknowledged. The pathway towards clinical translation application faces substantial hurdles⁴⁸. And we recognize that the current study has certain limitations due to the following concerns. Firstly, the complex pathogenesis of IBD is difficult to fully recapitulate in a single animal model. The 2,4,6-trinitrobenzene sulfonic acid-induced mice model of Crohn's disease could be employed to provide further corroborative evidence in subsequent studies. Besides, current results lack long-term biosafety data for FCH *in vivo*. Given that IBD is a chronic inflammatory bowel disease, treatment will also be a prolonged process. Potential risks include chronic bioaccumulation leading to persistent tissue burden, delayed organ toxicity manifesting months after exposure, sustained immune system perturbation, and immune-related adverse events^{49,50}. Therefore, it is necessary to further validate the biosafety of FCH over an extended period in the future, which is an unavoidable issue for subsequent clinical translation.

5. Conclusions

In this study, a smart single-atom nanozyme delivery system (FCH) was developed for the effective treatment of IBD. FCH demonstrated the ability to be targeted to and accumulate in intestinal regions affected by IBD, where it undergoes SOD-CAT nanozyme cascade reaction to effectively eliminate free radicals and enhance the inflammatory microenvironment. Cell line, organoid, and mouse experiments revealed that FCH could alleviate intestinal inflammation by reducing cell apoptosis, promoting the recovery of intestinal barrier function, and restoring the homeostasis of gut microbiota. This study introduces an innovative strategy for constructing SAzymes and provides new insights into novel treatments for IBD.

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Human colon samples were obtained from the Department of Gastroenterology, Affiliated Hangzhou First People's Hospital, School of Medicine, Westlake University. Informed consent was obtained from the patients or their families (38-year-old, female; 54-year-old, male; 68-year-old, female). The study was reviewed and approved by the Ethics Committee of Affiliated Hangzhou First People's Hospital (ZN-2024367-01, China) and fully complied with the principles of the Declaration of Helsinki. We would like to thank EditChecks (<https://editchecks.com.cn>) for providing linguistic assistance during the preparation of this manuscript.

Author contributions

Author Contributions: Tianliang Li: Conceptualization; Investigation; Writing-original draft; Methodology; Validation; Data curation. Zhijie Wang: Methodology; Formal analysis; Writing - original draft; Validation. Xin Chang: Investigation; Methodology. Tianhao Su: Software; Project administration. Zhaoming Wang: Investigation; Methodology. Lixing Lin: Software; Investigation. Zeyu Li: Investigation. Xiang Wang: Investigation. Yingying Chen: Validation. Zhenzhen Li: Investigation; Jianfei Yang: Validation. Yu Bai: Investigation; Writing-review & editing; Funding acquisition. Zixuan He: Investigation; Writing-review & editing; Funding acquisition. Lingyan Feng: Writing-review & editing; Methodology; Resources; Supervision; Funding acquisition.

Conflicts of interest

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

Appendix A. Supporting information

Supporting information to this article can be found online at <https://doi.org/10.1016/j.apsb.2026.01.016>.

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