

Mechanism-guided Mn₃O₄/GDY nanoplatform intercepts neutrophil- driven oxidative amplification to treat inflammatory bowel disease

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Abstract

Abstract: Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disorder in which sustained mucosal injury increases the risk of colorectal cancer. Despite advances in biologic therapies, durable disease control remains limited, highlighting the need for mechanistically informed oral interventions. Integrative transcriptomic analysis of clinical IBD samples highlighted concurrent oxidative-stress and neutrophil-associated programs linked to tight-junction disruption and mucosal barrier injury. Guided by these findings, we engineered manganese tetroxide nanoparticle-decorated graphdiyne (Mn₃O₄/GDY) to neutralize reactive oxygen species (ROS), peroxide, and hydroxyl radicals. Following oral administration, Mn₃O₄/GDY remained stable in the gastric environment, showed inflammation-associated intestinal retention, reduced neutrophil infiltration and myeloperoxidase-associated oxidative stress, and restored epithelial barrier integrity. In a murine colitis model, Mn₃O₄/GDY markedly alleviated disease severity, with greater improvements across several assessed endpoints than those observed with pristine GDY and the clinical drug 5-aminosalicylic acid. Mechanistically, the nanoplateform attenuated the ROS–neutrophil inflammatory positive-feedback loop, thereby promoting mucosal healing and restoring intestinal homeostasis. This work supports a redox-regulated oral nanomedicine strategy for IBD treatment.

Introduction

Inflammatory bowel disease (IBD), encompassing Crohn's disease and ulcerative colitis, represents a growing global health burden characterized by chronic, relapsing inflammation, and an increased risk of colorectal cancer.^{1, 2} Despite advances in biologic therapies, a “therapeutic ceiling” persists: a substantial proportion of patients fail to achieve or maintain durable disease control with current treatments, including long-term administration of 5-aminosalicylic acid (5-ASA), corticosteroids, immunosuppressants, and biologics, and long-term treatment can be limited by adverse effects.^{3, 4} Therefore, there is an urgent demand for developing orally applicable interventions that move beyond symptomatic management to target the foundational pathogenic mechanisms driving mucosal collapse.

To better define the pathogenic processes underlying IBD, we performed an integrative transcriptomic analysis of clinical datasets and identified a prominent disease feature characterized by excessive oxidative stress accompanied by marked neutrophil enrichment. These transcriptomic features are consistent with previous evidence showing that accumulated reactive oxygen species (ROS) can directly damage epithelial cells, compromise tight junction integrity, and impair barrier function, thereby facilitating further inflammatory cell infiltration.⁵ In parallel, infiltrating neutrophils have been recognized as important amplifiers of tissue injury through the release of myeloperoxidase (MPO) and other oxidant-generating mediators, which further aggravate oxidative damage and inflammatory signaling.^{6, 7} Together, these findings support a feed-forward pathogenic framework in which oxidative stress and neutrophil-associated inflammation reinforce mucosal injury in IBD. Importantly, this framework highlights oxidative stress as a therapeutically accessible intervention point in IBD,⁸ suggesting that

sustained ROS regulation may limit epithelial injury and weaken the neutrophil-associated inflammatory amplification that perpetuates mucosal damage.

To date, various small-molecule antioxidants, including melatonin,⁹ N-acetylcysteine,¹⁰ propionyl-L-carnitine,¹¹ resveratrol,¹² curcumin,¹³ quercetin,¹⁴ and catechins,¹⁵ have been explored for IBD treatment because of their ROS-scavenging capabilities. However, the clinical use of many such compounds can be constrained by rapid clearance and poor bioavailability.¹⁶ Metal oxide nanozymes, such as cerium oxide,¹⁷ manganese oxide,¹⁸ nickel–cobalt oxide,¹⁹ and molybdenum oxide,²⁰ offer a promising alternative because of their potent ROS-scavenging capacity and physicochemical stability, and have shown therapeutic potential in preclinical models of inflammatory disease. However, their application in IBD can be limited by insufficient gastrointestinal retention and concerns regarding cumulative metal exposure during long-term administration.²¹ These challenges highlight the need for nanozyme systems that combine efficient ROS regulation with favorable gastrointestinal stability and local retention in inflamed intestinal tissue, while maintaining therapeutic efficacy at relatively low metal loading.

Graphdiyne (GDY), a two-dimensional carbon allotrope composed of sp- and sp²-hybridized carbon atoms, offers an attractive platform for meeting these demands.²² Owing to its highly conjugated and porous framework, GDY provides abundant anchoring sites for metal oxide nanoparticles and can modulate their interfacial electronic states to enhance catalytic activity.²³ In addition, GDY itself exhibits high chemical stability, broad-spectrum ROS-scavenging capability, and good biocompatibility.²⁴ Notably, GDY has shown stability in gastrointestinal environments and reported retention in injured intestinal regions, supporting its suitability for orally delivered intestinal nanomedicine.²⁵ These properties make GDY a promising scaffold for constructing redox-active nanotherapeutics with improved lesion retention and therapeutic efficacy in IBD.

In this study, guided by transcriptomic evidence highlighting oxidative stress and neutrophil-associated inflammation as prominent disease-associated processes in IBD, we rationally engineered a manganese tetroxide-decorated GDY nanopatform (Mn₃O₄/GDY) for oral redox intervention. Through interfacial electronic coupling between Mn₃O₄ and GDY, this nanopatform exhibited enhanced catalytic scavenging of multiple ROS species and functioned as an active redox modulator rather than a passive antioxidant carrier. Following oral administration, Mn₃O₄/GDY exhibited favorable gastrointestinal stability and inflammation-associated retention in colonic tissue. In a dextran sodium sulfate (DSS)-induced colitis model, Mn₃O₄/GDY effectively alleviated intestinal inflammation and restored mucosal barrier integrity, outperforming both pristine GDY and the clinical drug 5-ASA. Mechanistically, our results suggest that by broadly reducing oxidative stress, Mn₃O₄/GDY alleviates epithelial injury and attenuates the neutrophil-associated inflammatory amplification that sustains mucosal damage (Scheme 1). Together, these findings establish Mn₃O₄/GDY as a mechanism-informed oral nanomedicine and highlight interfacial redox engineering as a promising strategy for IBD treatment.

Results and Discussion

Clinical Transcriptomic Analysis Reveals an Oxidative Stress–Amplified Neutrophil Pathological Axis in IBD

Integrative analysis of the GSE193677 clinical transcriptomic dataset, comprising 2029 intestinal biopsy samples from patients with IBD and 461 healthy controls, revealed extensive transcriptional differences in inflamed mucosa (Fig. 1a). A total of 580 genes were significantly upregulated and 409 genes were downregulated in IBD tissues relative to controls (Fig. 1b), indicating a broad disruption of mucosal homeostasis rather than a limited inflammatory perturbation. Functional annotation showed that these dysregulated genes were predominantly enriched in inflammatory and immune-related processes, including inflammatory response, immune activation, neutrophil chemotaxis, and defense response to Gram-negative bacteria (Fig. 1c). Pathway analysis further revealed enrichment of cytokine–cytokine receptor interaction, IL-17, NF- κ B, TNF, NOD-like receptor, and JAK–STAT signaling pathways (Fig. 1d), consistent with sustained immune activation and cytokine dysregulation in the inflamed intestine.^{26, 27} To further delineate coordinated disease-associated programs, we performed gene set enrichment analysis (GSEA). IBD samples displayed marked enrichment of humoral immune response, acute inflammatory response, and granulocyte chemotaxis (Fig. 1e,f, **Figure S1a**), together with enrichment of JAK–STAT, cytokine–receptor interaction, and NOD-like receptor signaling (Fig. 1g, **Figure S1b**). At the individual gene level, multiple pro-inflammatory cytokines including IL-6, IL-11, and IL-33, as well as chemokines such as CCL2, CXCL1, and CXCL2, were markedly upregulated (Fig. 1h), supporting a transcriptional landscape characterized by persistent inflammatory recruitment and signaling.^{27, 28}

Beyond immune signaling, oxidative stress emerged as another defining feature of the IBD transcriptome. Genes related to ROS generation, redox regulation, and oxidative injury were significantly dysregulated (Fig. 1i), indicating that redox imbalance accompanies immune activation and may contribute to epithelial barrier dysfunction. Consistent with this notion, immune cell deconvolution using the xCell algorithm revealed increased enrichment scores for multiple immune-cell populations in IBD lesions (Fig. 1j), among which neutrophils showed the largest increase in inferred enrichment score, followed by M1 macrophages (Fig. 1k, **Figure S2**).²⁹ Memory CD4⁺ and CD8⁺ T cell subsets were also increased, consistent with broader immune remodeling. CIBERSORT analysis showed a similar directional pattern (**Figure S3**), supporting the convergence of neutrophil-associated signals across the two analytical methods.³⁰

Taken together with established neutrophil biology, these analyses support a disease-relevant framework in which oxidative stress and neutrophil-associated inflammation can form a self-amplifying feed-forward circuit in IBD. Excessive ROS disrupt epithelial redox homeostasis and barrier integrity, thereby promoting chemokine release and neutrophil recruitment. Recruited neutrophils can, in turn, generate secondary oxidants through MPO-dependent processes, further intensifying oxidative damage and inflammatory signaling.³¹ This oxidative stress–neutrophil amplification axis therefore represents both a

disease-relevant amplification mechanism and therapeutic entry point in IBD, providing the rationale for designing a redox-active nanoplatform aimed at coordinated ROS scavenging and attenuation of neutrophil-associated inflammatory amplification.³²

Synthesis and Characterization of MnO/GDY

Guided by the transcriptomic evidence highlighting concurrent ROS-related and neutrophil-associated programs in IBD, we rationally designed Mn₃O₄/GDY as a redox-active nanoplatform for attenuating this pathological amplification. Mn₃O₄/GDY was synthesized *via* a solvothermal reaction between GDY and manganese acetate tetrahydrate [Mn(CH₃COO)₂·4H₂O], during which the conjugated alkyne moieties of GDY provided nucleation and anchoring sites for in situ formation and immobilization of Mn species on the GDY framework (Fig. 2a). This synthetic strategy was designed to generate intimate interfacial contact between Mn₃O₄ and GDY, thereby favoring interfacial charge redistribution and redox coupling.

Transmission electron microscopy (TEM) imaging showed that Mn₃O₄/GDY possessed a two-dimensional nanosheet-like morphology with an average lateral dimension of approximately 300 nm (Fig. 2b). High-magnification TEM further revealed that the Mn₃O₄ nanoparticles with an average diameter of ~ 7 nm were uniformly distributed on the GDY surface (Fig. 2c), suggesting a high density of exposed catalytic sites.³³ Elemental mapping confirmed the homogeneous spatial distribution of C, Mn, and O throughout the nanocomposite (**Figure S4**). High-resolution TEM identified lattice fringes corresponding to the (101), (103), and (112) planes of tetragonal Mn₃O₄ (Fig. 2d), which was further supported by X-ray diffraction (XRD) peaks indexed to Mn₃O₄ (Fig. 2e). These results support the formation of crystalline Mn₃O₄ on the GDY scaffold. Inductively coupled plasma mass spectrometry (ICP-MS) analysis showed a Mn loading of 5.94 wt%, while zeta potential measurement revealed a surface potential of -28.4 ± 0.67 mV, indicating good aqueous dispersibility and colloidal stability. (**Figure S5**).

To elucidate the interfacial interaction between Mn₃O₄ and GDY, Raman and X-ray photoelectron spectroscopy (XPS) were performed. The Raman spectrum of the Mn₃O₄/GDY exhibited a characteristic Mn–O vibration at ~ 648 cm⁻¹,³⁴ supporting the incorporation of Mn₃O₄ nanoparticles (Fig. 2f). More importantly, XPS analysis supported interfacial charge redistribution. The C 1s spectrum contained a distinct π – π^* satellite peak at 290.8 eV,^{35, 36} which is consistent with changes in the conjugated framework (Fig. 2g). Meanwhile, the Mn 2p spectrum exhibited features assigned to high-valence Mn species, implying that electron transfer from Mn to GDY partially stabilized oxidized Mn states at the interface (Fig. 2h). Such electronic restructuring is expected to be highly relevant to catalytic redox activity.

To further elucidate the atomic-scale origin of the Mn₃O₄–GDY interaction, first-principles density functional theory (DFT) calculations were performed. The key Mn(II) site in Mn₃O₄ involved in chemical

bonding with the GDY framework was modeled using a MnMg_3O_4 cluster; detailed considerations for the model construction are provided in the Supporting Information. Among all possible adsorption sites, the Mn center preferentially bound to the $\text{C} \equiv \text{C}$ bond of GDY. In the optimized configuration, the adsorbed $\text{C} \equiv \text{C}$ bond was elongated from 1.24 to 1.31 Å, accompanied by the formation of two Mn–C bonds (2.02 and 2.13 Å), indicating strong covalent coordination. Meanwhile, the net spin moment of Mn decreased to 2.9, in comparison to the original 4.7 of the unbound cluster, implying about two electrons of Mn was oxidized. Furthermore, localized orbital bonding analysis (LOBA) clearly identified the oxidation state of the Mn from +2 to +4 after adsorption (Fig. 2i). These results indicate pronounced interfacial electron coupling and robust Mn–C bonds, consistent with the XPS results revealing the transforming mechanism of Mn(II) to Mn(IV).

Taken together, these results indicate that $\text{Mn}_3\text{O}_4/\text{GDY}$ is not a simple physical mixture of Mn_3O_4 and GDY, but rather an interfacially coupled nanocomposite with defined Mn–C coordination and charge redistribution. This interfacial architecture provides an electronic environment compatible with multivalent Mn-mediated redox activity, providing a mechanistic basis for evaluating its multispecies ROS-scavenging activity in inflammatory microenvironments.

Multiple Antioxidative Properties of MnO/GDY

Since excessive ROS accumulation is a key driver of epithelial damage and inflammatory amplification in IBD, especially in neutrophil-rich lesions, we next examined whether the interfacial electronic coupling of $\text{Mn}_3\text{O}_4/\text{GDY}$ translates into functionally enhanced antioxidative activity. To distinguish interfacial effects from simple component coexistence, pristine Mn_3O_4 nanoparticles, GDY, and their physical mixture ($\text{Mn}_3\text{O}_4 + \text{GDY}$) were included as controls. We first assessed the scavenging of superoxide anion radicals ($\text{O}_2^{\cdot-}$), a primary ROS generated during neutrophil respiratory burst,³⁷ using the xanthine/xanthine oxidase (Xan/XOD) system coupled with electron spin resonance (ESR) detection.³⁸ Mn_3O_4 alone showed minimal suppression of $\text{O}_2^{\cdot-}$ signals (Fig. 2j), indicating limited intrinsic activity under the tested conditions. In contrast, GDY and the physical mixture reduced ESR intensity to a comparable extent, suggesting that GDY contributes substantially to $\text{O}_2^{\cdot-}$ elimination, whereas simple coexistence of the two components did not provide an additional advantage. Notably, $\text{Mn}_3\text{O}_4/\text{GDY}$ exhibited the strongest suppression of $\text{O}_2^{\cdot-}$ signals (**Figure S6**), demonstrating enhanced superoxide dismutase (SOD)-like activity. This result is particularly important because it indicates that the improved ROS-scavenging capability arises from interfacial coupling rather than mere material mixing. Mechanistically, the enhanced $\text{O}_2^{\cdot-}$ scavenging can be attributed to the Mn–C coordination-mediated electron transfer between Mn_3O_4 and GDY. As suggested by DFT and XPS analyses, interfacial charge redistribution stabilizes high-valence Mn species, which are favorable for catalytic $\text{O}_2^{\cdot-}$ dismutation. Through proton-coupled electron transfer and reversible Mn redox cycling, $\text{Mn}_3\text{O}_4/\text{GDY}$ efficiently converts $\text{O}_2^{\cdot-}$ into H_2O_2 and O_2 (**Figure S7**), functionally resembling the catalytic behavior of natural SOD.³⁹

Because H_2O_2 is both a downstream ROS intermediate and a key substrate for neutrophil-derived MPO,⁴⁰ we next evaluated its decomposition. $\text{Mn}_3\text{O}_4/\text{GDY}$ catalytically removed approximately 70% of H_2O_2 within 50 min (Fig. 2k), whereas Mn_3O_4 , GDY, and their physical mixture showed negligible activity under the same conditions. Thus, $\text{Mn}_3\text{O}_4/\text{GDY}$ not only intercepts upstream $\text{O}_2^{\cdot-}$ but also effectively eliminates H_2O_2 , a central node in oxidative amplification.

We further examined hydroxyl radicals ($\cdot\text{OH}$) scavenging using a Fenton reaction system. ESR results revealed that Mn_3O_4 alone had little effect, while GDY-containing formulations attenuated $\cdot\text{OH}$ signals to varying degrees (Fig. 2l, **Figure S8**). Again, $\text{Mn}_3\text{O}_4/\text{GDY}$ displayed the highest scavenging efficiency, further supporting its multispecies antioxidative activity.

These results support

$\text{Mn}_3\text{O}_4/\text{GDY}$ as an integrated redox-modulating platform that reduces multiple ROS species, including $\text{O}_2^{\cdot-}$, H_2O_2 , and $\cdot\text{OH}$. Importantly, this is not a passive radical-scavenging effect, but a structure-enabled catalytic process arising from interfacial electronic coupling. Notably, such enhanced broad-spectrum antioxidative activity was achieved at a relatively low Mn loading, further indicating that the improved performance of $\text{Mn}_3\text{O}_4/\text{GDY}$ arises from coupling-enhanced functional efficiency rather than simply increasing the amount of active metal species. Compared with conventional antioxidants that typically target single ROS species, $\text{Mn}_3\text{O}_4/\text{GDY}$ is able to interrupt oxidative amplification at multiple stages (Fig. 2m, **Figure S9**), which is relevant to IBD, where ROS species are continuously generated and mutually converted within inflammatory niches.¹⁶

$\text{Mn}_3\text{O}_4/\text{GDY}$ Protects Epithelial Cells and Suppresses Inflammatory Activation In Vitro

The potent cascade ROS-scavenging capacity of $\text{Mn}_3\text{O}_4/\text{GDY}$ prompted us to investigate whether these physicochemical properties could translate into cellular protection under inflammatory oxidative stress. We therefore evaluated its cytocompatibility, intracellular antioxidative activity, anti-inflammatory effects, and barrier-protective capacity in multiple *in vitro* models relevant to IBD. GDY and $\text{Mn}_3\text{O}_4/\text{GDY}$ displayed good cytocompatibility in IEC-6 intestinal epithelial cells and RAW264.7 macrophages, with cell viability remaining above 85% after 24 h exposure at concentrations up to 50 $\mu\text{g}/\text{mL}$ (**Figure S10**), supporting further evaluation in intestinal cell models. To assess intracellular ROS elimination, IEC-6 cells were challenged with H_2O_2 to induce oxidative stress. DCFH-DA staining showed marked intracellular ROS accumulation after H_2O_2 stimulation, whereas $\text{Mn}_3\text{O}_4/\text{GDY}$ treatment substantially reduced fluorescence intensity (Fig. 3a,b). GDY alone also reduced ROS to some extent, but the effect was clearly weaker. Consistent with this observation, $\text{Mn}_3\text{O}_4/\text{GDY}$ significantly improved cell viability under oxidative stress (Fig. 3c), indicating that its antioxidative effect is sufficient to confer meaningful cytoprotection.

Because oxidative stress and inflammatory signaling are tightly coupled in IBD,⁴¹ we next assessed anti-inflammatory activity in LPS-stimulated RAW264.7 macrophages. LPS markedly increased the secretion

of IL-1 β and IL-6 while suppressing IL-10 production. Both GDY and Mn₃O₄/GDY attenuated the inflammatory response, but Mn₃O₄/GDY produced a more pronounced suppression of IL-1 β and IL-6 and a stronger restoration of IL-10 (Fig. 3d–f). These data indicate that the superior redox-regulatory capability of Mn₃O₄/GDY translates into more effective control of inflammatory activation.

To further assess functional relevance at the epithelia-immune interface, we established a DSS-induced intestinal epithelial barrier injury model using Caco-2 monolayers.⁴² DSS stimulation markedly increased paracellular permeability, as shown by elevated FITC-inulin flux. Although GDY partially alleviated barrier disruption, Mn₃O₄/GDY produced a significantly greater reduction in permeability (Fig. 3g,h), indicating effective preservation of epithelial barrier function.

Taken together, these *in vitro* results show that Mn₃O₄/GDY improves complementary pathological readouts, including intracellular oxidative stress, inflammatory cytokine production in macrophages, and epithelial barrier permeability.^{43, 44} Rather than acting on a single cellular event, Mn₃O₄/GDY appears to modulate the epithelial–immune crosstalk that underlies chronic mucosal inflammation, thereby providing a functional bridge between its physicochemical redox activity and its anticipated therapeutic efficacy *in vivo*.

MnO/GDY Maintains Gastrointestinal Stability and Exhibits Inflammation-Associated Intestinal Retention

For orally administered nanomaterials, therapeutic efficacy depends not only on intrinsic bioactivity but also on their ability to remain structurally stable in the gastrointestinal tract and reside long enough at diseased sites to exert local effects.^{21, 45} We therefore evaluated the gastrointestinal stability and intestinal retention behavior of Mn₃O₄/GDY. After incubation in simulated gastric fluid (SGF) for 2 h, Mn₃O₄/GDY largely retained its SOD-like activity (**Figure S11**), indicating that its redox function is resistant to acidic gastric conditions. Consistently, TEM imaging showed that the nanosheet morphology and nanoparticle distribution were well preserved after SGF incubation (**Figure S12**), supporting structural stability under the tested conditions. This stability is likely associated with the robust π -conjugated framework of GDY and the strong interfacial anchoring of Mn₃O₄ nanoparticles, which together suppress aggregation and metal ion loss under harsh conditions.²³

We next assessed gastrointestinal retention *in vivo*. Because inflamed intestinal mucosa in IBD is characterized by epithelial disruption and accumulation of positively charged inflammatory components,⁴⁶ the moderately negative surface charge of Mn₃O₄/GDY (–28.4 mV) may contribute to its interaction with inflamed tissue. *Ex vivo* fluorescence imaging using Cy5.5-labeled Mn₃O₄/GDY showed markedly higher signals in inflamed colonic tissue than in healthy controls (Fig. 4a,b). *In vivo* imaging further revealed more prolonged intestinal signals in DSS-induced IBD mice (Fig. 4c,d). To independently validate these observations, we additionally performed single-photon emission computed tomography

(SPECT) imaging using radionuclide ^{177}Lu -labeled $\text{Mn}_3\text{O}_4/\text{GDY}$. Consistent with fluorescence imaging, radiotracer imaging showed higher and more persistent signals in inflamed intestinal regions (Fig. 4e,f). These findings indicate that $\text{Mn}_3\text{O}_4/\text{GDY}$ combines two features critical for oral IBD therapy: high gastrointestinal stability and inflammation-associated local retention (Fig. 4g). This combination may increase local material exposure and provide a pharmacological basis for effective and relatively safe treatment of intestinal inflammation.

$\text{Mn}_3\text{O}_4/\text{GDY}$ Effectively Alleviates DSS-Induced Colitis In Vivo

We next investigated whether the favorable redox activity and intestinal retention of $\text{Mn}_3\text{O}_4/\text{GDY}$ translate into therapeutic benefit *in vivo*. DSS-induced colitis mice were orally treated with $\text{Mn}_3\text{O}_4/\text{GDY}$, GDY alone, or the clinically used drug 5-ASA (Fig. 5a). As expected, DSS administration induced typical manifestations of colitis, including progressive body weight loss, elevated disease activity index (DAI), and significant colon shortening (Fig. 5b–e), indicating severe intestinal inflammation and mucosal injury. In contrast, oral treatment with $\text{Mn}_3\text{O}_4/\text{GDY}$ markedly ameliorated these pathological changes. Across body weight, colon length, and DAI, $\text{Mn}_3\text{O}_4/\text{GDY}$ showed more pronounced improvement than GDY or 5-ASA under the tested conditions. Histological examination further showed extensive epithelial destruction and disorganization of mucosal architecture in DSS-treated mice, whereas $\text{Mn}_3\text{O}_4/\text{GDY}$ treatment better preserved epithelial structure and tissue integrity (Fig. 5f). In parallel, hematological analysis revealed that $\text{Mn}_3\text{O}_4/\text{GDY}$ significantly reduced circulating white blood cells, neutrophils, and platelets (Fig. 5g–i), consistent with attenuation of systemic inflammatory indices. Because oxidative injury an important effector process associated with neutrophil-rich inflammation, we next examined lipid peroxidation in colonic tissues. Immunohistochemical staining showed marked accumulation of 4-hydroxynonenal (4-HNE) in the DSS group, indicating lipid peroxidation-associated tissue injury.⁴⁷ This signal was substantially reduced by $\text{Mn}_3\text{O}_4/\text{GDY}$ treatment, with a greater effect than that observed for GDY or 5-ASA (Fig. 5j). These data show that the macroscopic improvements after $\text{Mn}_3\text{O}_4/\text{GDY}$ treatment were accompanied by lower oxidative-damage and inflammatory indices.⁴⁸

Overall, the *in vivo* therapeutic results demonstrate that $\text{Mn}_3\text{O}_4/\text{GDY}$ was associated with multi-level protection against colitis, including attenuation of clinical symptoms, preservation of colonic structure, and reduction of systemic and local inflammatory injury. These findings support ROS-directed modulation as a therapeutic strategy for attenuating inflammatory amplification in IBD.

MnO/GDY Reprograms Inflammatory and Barrier-Associated Transcriptional Landscapes in IBD

To further elucidate the molecular changes associated with the therapeutic effects of $\text{Mn}_3\text{O}_4/\text{GDY}$, we performed bulk RNA sequencing on colonic tissues from healthy control mice, DSS-treated mice, and $\text{Mn}_3\text{O}_4/\text{GDY}$ -treated mice (Fig. 6a). Hierarchical clustering showed clear separation among the three

groups (Fig. 6b), indicating substantial differences in their transcriptomic profiles. Differential expression analysis identified 679 upregulated and 312 downregulated genes in DSS-induced colitis relative to healthy controls (**Figure S13a**). Remarkably, $\text{Mn}_3\text{O}_4/\text{GDY}$ treatment reshaped this disease-associated transcriptome, with 1495 genes upregulated and 432 genes downregulated relative to the DSS group (**Figure S13b**).

To capture dynamic transcriptional trajectories, we performed Mfuzz soft clustering and identified eight gene clusters (Fig. 6c).⁴⁹ Among them, clusters 1 and 8 were strongly induced during DSS colitis and then suppressed after $\text{Mn}_3\text{O}_4/\text{GDY}$ treatment, whereas clusters 2 and 7 showed the opposite trend, being downregulated in disease but restored after treatment. Functional enrichment analysis revealed that these reciprocally regulated clusters corresponded to two opposing biological patterns. Disease-associated clusters were enriched in neutrophil migration, cytokine signaling, ROS response, and NADPH oxidase-related pathways, whereas treatment-restored clusters were associated with mitochondrial organization, TORC1 signaling, and metabolic homeostasis (Fig. 6d,e).^{50, 51} Thus, $\text{Mn}_3\text{O}_4/\text{GDY}$ treatment was associated with a transcriptional shift from inflammatory and oxidative-stress programs toward mitochondrial and metabolic-homeostasis programs. At the individual gene level, $\text{Mn}_3\text{O}_4/\text{GDY}$ suppressed a broad inflammatory module including *Il1b*, *Il6*, *Il17a*, *Ifng*, *Csf2*, *Csf3*, *Nos2*, and multiple matrix metalloproteinases (Fig. 6f).⁵² These factors are involved in neutrophil recruitment, oxidative-stress amplification, and barrier disruption. Accordingly, the transcriptomic effect of $\text{Mn}_3\text{O}_4/\text{GDY}$ is consistent with coordinated attenuation of a neutrophil-associated inflammatory network rather than isolated inhibition of individual genes.

GSEA further supported this interpretation. Pro-inflammatory and oxidative stress-related pathways, including IL-17, TGF- β , PI3K–Akt, and neutrophil-associated inflammatory signaling, were reduced, whereas pathways related to oxidative phosphorylation, ATP synthesis, and tricarboxylic acid cycle activity were restored (**Figure S14, Figure S15**).^{53, 54} These reciprocal shifts suggest that resolution of inflammation and recovery of epithelial metabolic fitness are tightly coupled during $\text{Mn}_3\text{O}_4/\text{GDY}$ therapy.

To assess the relevance to human disease, we integrated transcriptomic data from human IBD and DSS-induced colitis. Cross-species comparison identified 191 genes consistently upregulated (quadrant 1, Q1) and 23 genes consistently downregulated (Q3) in both human and mouse disease states (Fig. 6g), with enrichment in neutrophil chemotaxis, extracellular matrix remodeling, and inflammatory signaling (**Figure S16a,b**). This overlap supports the relevance of the DSS model to selected human IBD-associated programs. Integration of human IBD DEGs with $\text{Mn}_3\text{O}_4/\text{GDY}$ -responsive genes identified 351 disease-elevated genes (Q4) reduced by treatment and 60 disease-suppressed genes (Q2) increased by treatment (Fig. 6h). These genes were enriched in neutrophil chemotaxis, cytokine response, inflammatory amplification, and IL-17, and JAK–STAT signaling (Fig. 6i, **Figure S16c**), indicating that the transcriptional changes associated with $\text{Mn}_3\text{O}_4/\text{GDY}$ overlap with human IBD-associated modules.

These transcriptomic data are consistent with coordinated modulation of the disease-associated state, characterized by attenuation of neutrophil- and ROS-related inflammatory programs and increased expression of mitochondrial, metabolic, and barrier-associated programs. This systems-level pattern provides transcriptomic context linking the material's redox activity with its therapeutic effects.

Experimental Validation of Mn₃O₄/GDY–Mediated Barrier Restoration and Neutrophil Modulation

Based on the transcriptomic prediction that Mn₃O₄/GDY regulates epithelial barrier integrity and inflammatory activity, we next performed experimental validation. Immunofluorescence staining showed that DSS exposure caused marked fragmentation and loss of the tight junction proteins zona occludens-1 (ZO-1) and Claudin-1, indicating severe epithelial barrier disruption.⁵⁵ In contrast, Mn₃O₄/GDY treatment substantially restored both their expression levels and continuous junctional distribution, which was further confirmed by Western blot analysis (Fig. 7a–c). These findings show that Mn₃O₄/GDY repairs the structural basis of epithelial barrier function.

We then examined neutrophil infiltration and associated markers in colonic tissues. DSS-treated mice exhibited pronounced accumulation of Ly6G⁺ neutrophils,⁵⁵ whereas Mn₃O₄/GDY treatment significantly reduced neutrophil infiltration and showed a more pronounced effect than 5-ASA (Fig. 7d). Consistently, the expression of CXCR4 and MPO was markedly decreased after Mn₃O₄/GDY treatment (Fig. 7e,f), indicating reduced levels of markers associated with neutrophil recruitment and oxidative effector activity.⁵⁶ These findings support the transcriptomic prediction that Mn₃O₄/GDY alleviates neutrophil-associated inflammatory amplification *in vivo*. In line with this, Mn₃O₄/GDY also altered the local cytokine milieu. Levels of IL-1 β and IL-6 were significantly decreased, whereas the anti-inflammatory cytokine IL-10 was restored (Fig. 7g–i). This cytokine reprogramming was consistent with the reduced neutrophil infiltration and improved barrier integrity, supporting coordinated anti-inflammatory and mucosal protective effects of Mn₃O₄/GDY.⁵⁷

In addition, our transcriptomic analysis revealed partial restoration of mitochondrial-related pathways, including mitochondrial organization and oxidative phosphorylation, after Mn₃O₄/GDY treatment. Consistent with this trend, JC-1 staining in IEC-6 cells showed that Mn₃O₄/GDY preserved mitochondrial membrane potential under oxidative stress (**Figure S17**). Although this evidence was obtained *in vitro*, it supports the possibility that Mn₃O₄/GDY may help alleviate oxidative stress-associated mitochondrial dysfunction.

In addition, histological examination of major organs, including the heart, liver, spleen, lung, and kidney, showed no detectable tissue damage after oral administration of Mn₃O₄/GDY (**Figure S18**), supporting a favorable biosafety profile. Taken together, these validation experiments show that Mn₃O₄/GDY restores

epithelial barrier structure, reduces neutrophil-associated inflammatory responses, and improves the local cytokine milieu in colitis, supporting its therapeutic benefit in IBD.

Conclusion

In conclusion, guided by clinical transcriptomic findings, we developed an orally administrable nanotherapeutic platform, $\text{Mn}_3\text{O}_4/\text{GDY}$, for the treatment of IBD. This strategy addresses a disease-amplifying process in which oxidative stress and neutrophil-associated inflammation reinforce mucosal injury and disruption of intestinal homeostasis. By integrating Mn_3O_4 nanoparticles with a GDY scaffold, $\text{Mn}_3\text{O}_4/\text{GDY}$ forms an interfacially coupled redox system in which GDY-associated interfacial charge redistribution enhances multispecies ROS-scavenging activity, thereby attenuating oxidative amplification in the inflamed intestine. Benefiting from its high gastric stability and inflammation-associated retention in inflamed colonic tissue, $\text{Mn}_3\text{O}_4/\text{GDY}$ enables effective local intervention following oral administration. In a DSS-induced colitis model, $\text{Mn}_3\text{O}_4/\text{GDY}$ markedly reduced disease severity, and outperformed both pristine GDY and the clinical drug 5-ASA, accompanied by attenuation of oxidative stress, restoration of epithelial barrier integrity, and alleviation of neutrophil-associated inflammatory responses. Collectively, these findings support $\text{Mn}_3\text{O}_4/\text{GDY}$ as a promising oral nanomedicine for IBD and highlight the therapeutic potential of redox modulation for controlling intestinal inflammation and mucosal damage.

Declarations

Ethics approval and consent to participate

All animal experiments were approved by the ethics committee of Soochow University.

Consent for publication

Not applicable

Competing interests

The authors declare no competing financial interest.

Author Contribution

ZA, ML, JW, and XD performed the experiments, analyzed the data, wrote the original manuscript. JXW, WS, MS, YZ, and MYG conceptualized the study, revised the manuscript, supervised the work. GD, XT and LW conceived the project, designed the experiments, analyzed the data, wrote and revised the manuscript. All authors reviewed the manuscript

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Availability of data and materials

The data and materials in the current study are available from the corresponding author on reasonable request.

References

1. Hracs L, Windsor JW, Gorospe J, Cummings M, Coward S, Buie MJ, Quan J, Goddard Q, Caplan L, Markovinović A. Global evolution of inflammatory bowel disease across epidemiologic stages. *Nature*. 2025;642(8067):458–66.
2. Gros B, Kaplan GG. Ulcerative colitis in adults: a review. *JAMA*. 2023;330(10):951–65.
3. Schreiber S, Aden K, Tran F, Rosenstiel P. Rise of precision medicine: can it deliver on its promise in IBD? *Gut*. 2026;75(1):176–88.
4. Lamb CA, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK, Hayee BH, Lomer MC, Parkes GC, Selinger C. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut*. 2019;68(Suppl 3):s1–106.
5. Wan Y, Yang L, Jiang S, Qian D, Duan J. Excessive apoptosis in ulcerative colitis: crosstalk between apoptosis, ROS, ER stress, and intestinal homeostasis. *Inflamm Bowel Dis*. 2022;28(4):639–48.
6. Kang L, Fang X, Song Y-H, He Z-X, Wang Z-J, Wang S-L, Li Z-S, Bai Y. Neutrophil–epithelial crosstalk during intestinal inflammation. *Cell Mol Gastroenterol Hepatol*. 2022;14(6):1257–67.
7. Friedrich M, Pohin M, Jackson MA, Korsunsky I, Bullers SJ, Rue-Albrecht K, Christoforidou Z, Sathananthan D, Thomas T, Ravindran R. IL-1-driven stromal–neutrophil interactions define a subset of patients with inflammatory bowel disease that does not respond to therapies. *Nat Med*. 2021;27(11):1970–81.
8. Muro P, Zhang L, Li S, Zhao Z, Jin T, Mao F, Mao Z. The emerging role of oxidative stress in inflammatory bowel disease. *Front Endocrinol*. 2024;15:1390351.

9. Vaghari-Tabari M, Moein S, Alipourian A, Qujeq D, Malakoti F, Alemi F, Yousefi B, Khazaie S. Melatonin and inflammatory bowel disease: From basic mechanisms to clinical application. *Biochimie*. 2023;209:20–36.
10. Shirazi KM, Sotoudeh S, Shirazi AM, Moaddab S-Y, Nourpanah Z, Nikniaz Z. Effect of N-acetylcysteine on remission maintenance in patients with ulcerative colitis: A randomized, double-blind controlled clinical trial. *Clin Res Hepatol Gastroenterol*. 2021;45(4):101532.
11. Mikhailova T, Sishkova E, Poniewierka E, Zhidkov K, Bakulin I, Kupcinskis L, Lesniakowski K, Grinevich V, Malecka-Panas E, Ardizzone S. Randomised clinical trial: the efficacy and safety of propionyl-L-carnitine therapy in patients with ulcerative colitis receiving stable oral treatment. *Aliment Pharmacol Ther*. 2011;34(9):1088–97.
12. Gu Y, Lou Y, Zhou Z, Zhao X, Ye X, Wu S, Li H, Ji Y. Resveratrol for inflammatory bowel disease in preclinical studies: a systematic review and meta-analysis. *Front Pharmacol*. 2024;15:1411566.
13. Lang A, Salomon N, Wu JCY, Kopylov U, Lahat A, Har-Noy O, Ching JYL, Cheong PK, Avidan B, Gamus D, et al. Curcumin in Combination With Mesalamine Induces Remission in Patients With Mild-to-Moderate Ulcerative Colitis in a Randomized Controlled Trial. *Clin Gastroenterol Hepatol*. 2015;13(8):1444–9.
14. Zhang Q, Wen F, Sun F, Xu Z, Liu Y, Tao C, Sun F, Jiang M, Yang M, Yao J. Efficacy and mechanism of quercetin in the treatment of experimental colitis using network pharmacology analysis. *Molecules*. 2022;28(1):146.
15. Du Y, Ding H, Vanarsa K, Soomro S, Baig S, Hicks J, Mohan C. Low dose epigallocatechin gallate alleviates experimental colitis by subduing inflammatory cells and cytokines, and improving intestinal permeability. *Nutrients*. 2019;11(8):1743.
16. Dziąbowska-Grabias K, Sztanke M, Zając P, Celejewski M, Kurek K, Szkutnicki S, Korga P, Bulikowski W, Sztanke K. Antioxidant therapy in inflammatory bowel diseases. *Antioxidants*. 2021;10(3):412.
17. Kim YG, Lee Y, Lee N, Soh M, Kim D, Hyeon T. Ceria-based therapeutic antioxidants for biomedical applications. *Adv Mater*. 2024;36(10):2210819.
18. Wang Q, Cheng C, Zhao S, Liu Q, Zhang Y, Liu W, Zhao X, Zhang H, Pu J, Zhang S. A valence-engineered self-cascading antioxidant nanozyme for the therapy of inflammatory bowel disease. *Angew Chem Int Ed* 2022, 61 (27), e202201101.
19. Zhao N, Yang FE, Zhao CY, Lv SW, Wang J, Liu JM, Wang S. Construction of pH-Dependent Nanozymes with Oxygen Vacancies as the High-Efficient Reactive Oxygen Species Scavenger for Oral-Administered Anti-Inflammatory Therapy. *Adv Healthc Mater*. 2021;10(23):2101618.
20. Duan G, Wen L, Sun X, Wei Z, Duan R, Zeng J, Cui J, Liu C, Yu Z, Xie X. Healing diabetic ulcers with MoO₃ – X nanodots possessing intrinsic ROS-scavenging and bacteria-killing capacities. *Small*. 2022;18(10):2107137.
21. Fu W, Xu L, Chen Z, Kan L, Ma Y, Qian H, Wang W. Recent advances on emerging nanomaterials for diagnosis and treatment of inflammatory bowel disease. *J Controlled Release*. 2023;363:149–79.

22. Li H, Lim JH, Lv Y, Li N, Kang B, Lee JY. Graphynes and graphdiynes for energy storage and catalytic utilization: theoretical insights into recent advances. *Chem Rev.* 2023;123(8):4795–854.
23. Yu J, Chen W, He F, Song W, Cao C. Electronic oxide–support strong interactions in the graphdiyne-supported cuprous oxide nanocluster catalyst. *J Am Chem Soc.* 2023;145(3):1803–10.
24. Li X, Jiang H, He N, Yuan W-E, Qian Y, Ouyang Y. Graphdiyne-related materials in biomedical applications and their potential in peripheral nerve tissue engineering. *Cyborg Bionic Syst.* 2022;2022(42):1–20.
25. Xie J, Wang C, Wang N, Zhu S, Mei L, Zhang X, Yong Y, Li L, Chen C, Huang C. Graphdiyne nanoradioprotector with efficient free radical scavenging ability for mitigating radiation-induced gastrointestinal tract damage. *Biomaterials.* 2020;244:119940.
26. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature.* 2011;474(7351):307–17.
27. Neurath MF. Cytokines in inflammatory bowel disease. *Nat Rev Immunol.* 2014;14(5):329–42.
28. Nemeth ZH, Bogdanovski DA, Barratt-Stopper P, Paglinco SR, Antonioli L, Rolandelli RH. Crohn's disease and ulcerative colitis show unique cytokine profiles. *Cureus* 2017, 9 (4), e1177.
29. Smillie CS, Biton M, Ordovas-Montanes J, Sullivan KM, Burgin G, Graham DB, Herbst RH, Rogel N, Slyper M, Waldman J. Intra-and inter-cellular rewiring of the human colon during ulcerative colitis. *Cell.* 2019;178(3):714–30. e722.
30. Neurath MF. Targeting immune cell circuits and trafficking in inflammatory bowel disease. *Nat Immunol.* 2019;20(8):970–9.
31. Balmus IM, Ciobica A, Trifan A, Stanciu C. The implications of oxidative stress and antioxidant therapies in Inflammatory Bowel Disease: Clinical aspects and animal models. *Saudi J Gastroenterol.* 2016;22(1):3–17.
32. Nauseef WM. Myeloperoxidase in human neutrophil host defence. *Cell Microbiol.* 2014;16(8):1146–55.
33. Li J, Han X, Wang D, Zhu L, Ha-Thi MH, Pino T, Arbiol J, Wu LZ. Nawfal Ghazzal, M. A deprotection-free method for high-yield synthesis of graphdiyne powder with in situ formed CuO nanoparticles. *Angew Chem Int Ed* 2022, 61 (43), e202210242.
34. Thareja S, Kumar A. One-Pot In Situ Synthesis of Mn₃O₄/N-rGO nanohybrids for the fabrication of high cell voltage aqueous symmetric supercapacitors: An analysis of redox activity of Mn₃O₄ toward stabilizing the high potential window in salt-in-water and water-in-salt electrolytes. *Energy Fuels.* 2022;36(24):15177–87.
35. Elia GA, Hasa I, Greco G, Diemant T, Marquardt K, Hoeppe K, Behm RJ, Hoell A, Passerini S, Hahn R. Insights into the reversibility of aluminum graphite batteries. *J Mater Chem A.* 2017;5(20):9682–90.
36. Butenko YV, Krishnamurthy S, Chakraborty A, Kuznetsov V, Dhanak V, Hunt M, Šiller L. Photoemission study of onionlike carbons produced by annealing nanodiamonds. *Phys Rev B—Condensed Matter Mater Phys.* 2005;71(7):075420.

37. Dahlgren C, Karlsson A. Respiratory burst in human neutrophils. *J Immunol Methods*. 1999;232(1–2):3–14.
38. Campos Chiste R, Freitas M, Zerlotti Mercadante A, Fernandes E. Superoxide anion radical: generation and detection in cellular and non-cellular systems. *Curr Med Chem*. 2015;22(37):4234–56.
39. Wu A, Li M, Chen Y, Zhang W, Li H, Chen J, Gu K, Wang X. Multienzyme active manganese oxide alleviates acute liver injury by mimicking redox regulatory system and inhibiting ferroptosis. *Adv Healthc Mater*. 2024;13(11):2302556.
40. Odobasic D, Kitching AR, Holdsworth SR. Neutrophil-mediated regulation of innate and adaptive immunity: the role of myeloperoxidase. *J Immunol Res* 2016, 2016 (1), 2349817.
41. Tratenšek A, Locatelli I, Grabnar I, Drobne D, Vovk T. Oxidative stress-related biomarkers as promising indicators of inflammatory bowel disease activity: A systematic review and meta-analysis. *Redox Biol*. 2024;77:103380.
42. Toutounji M, Waness D, El-Harakeh M, El-Sabban M, Rizk S, Naim HY. Dextran Sodium Sulfate-Induced Impairment of Protein Trafficking and Alterations in Membrane Composition in Intestinal Caco-2 Cell Line. *Int J Mol Sci*. 2020;21(8):2726.
43. Neurath MF, Artis D, Becker C. The intestinal barrier: a pivotal role in health, inflammation, and cancer. *Lancet Gastroenterol Hepatol*. 2025;10(6):573–92.
44. Du J, Zhang J, Wang L, Wang X, Zhao Y, Lu J, Fan T, Niu M, Zhang J, Cheng F, et al. Selective oxidative protection leads to tissue topological changes orchestrated by macrophage during ulcerative colitis. *Nat Commun*. 2023;14(1):3675.
45. Feng Y, Luo X, Li Z, Fan X, Wang Y, He R-R, Liu M. A ferroptosis-targeting ceria anchored halloysite as orally drug delivery system for radiation colitis therapy. *Nat Commun*. 2023;14(1):5083.
46. Okumura R, Takeda K. Maintenance of intestinal homeostasis by mucosal barriers. *Inflamm Regeneration*. 2018;38(1):5.
47. Wang Y, Wang W, Yang H, Shao D, Zhao X, Zhang G. Intraperitoneal injection of 4-hydroxynonenal (4-HNE), a lipid peroxidation product, exacerbates colonic inflammation through activation of Toll-like receptor 4 signaling. *Free Radical Biol Med*. 2019;131:237–42.
48. Cao F, Jin L, Gao Y, Ding Y, Wen H, Qian Z, Zhang C, Hong L, Yang H, Zhang J, et al. Artificial-enzymes-armed *Bifidobacterium longum* probiotics for alleviating intestinal inflammation and microbiota dysbiosis. *Nat Nanotechnol*. 2023;18(6):617–27.
49. Yan Z, Yang J, Wei W-T, Zhou M-L, Mo D-X, Wan X, Ma R, Wu M-M, Huang J-H, Liu Y-J, et al. A time-resolved multi-omics atlas of transcriptional regulation in response to high-altitude hypoxia across whole-body tissues. *Nat Commun*. 2024;15(1):3970.
50. Adams CE, Rutherford DG, Jones GR, Ho GT. Immunometabolism and mitochondria in inflammatory bowel disease: a role for therapeutic intervention? *Dis Model Mech*. 2024;17(10):dmm050895.
51. Michaels M, Madsen KL. Immunometabolism and microbial metabolites at the gut barrier: Lessons for therapeutic intervention in inflammatory bowel disease. *Mucosal Immunol*. 2023;16(1):72–85.

52. Steere B, Schmitz J, Powell N, Higgs R, Gottlieb K, Liu Y, Jia B, Tuttle JL, Sandborn WJ, Sands BE, et al. Mirikizumab Regulates Genes Involved in Ulcerative Colitis Disease Activity and Anti-TNF Resistance: Results From a Phase 2 Study. *Clin Translational Gastroenterol.* 2023;14(7):e00578.
53. Kim ME, Lim Y, Lee JS. Mitochondrial Dysfunction and Metabolic Reprogramming in Chronic Inflammatory Diseases: Molecular Insights and Therapeutic Opportunities. *Curr Issues Mol Biol.* 2025;47(12):1042.
54. Yu S, Zhang M, Niu W, Huang Y, Dou Z, Tian B, Yang L, Lu J. Neutrophils in inflammatory bowel disease: disease-promoting versus protective functions. *Front Immunol.* 2026;16:1750743.
55. Yu T, Bai R, Wang Z, Qin Y, Wang J, Wei Y, Zhao R, Nie G, Han B. Colon-targeted engineered postbiotics nanoparticles alleviate osteoporosis through the gut-bone axis. *Nat Commun.* 2024;15(1):10893.
56. Xia XM, Wang FY, Xu WA, Wang ZK, Liu J, Lu YK, Jin XX, Lu H, Shen Y. Z. CXCR4 antagonist AMD3100 attenuates colonic damage in mice with experimental colitis. *World J Gastroenterol.* 2010;16(23):2873–80.
57. Wang H, Huang T, Ma Y. Mechanisms and therapeutic strategies of macrophages and neutrophils inducing ulcerative colitis progression. *Front Immunol.* 2025;16:1615340.

Scheme

Scheme 1 is available in the Supplementary Files section.

Figures

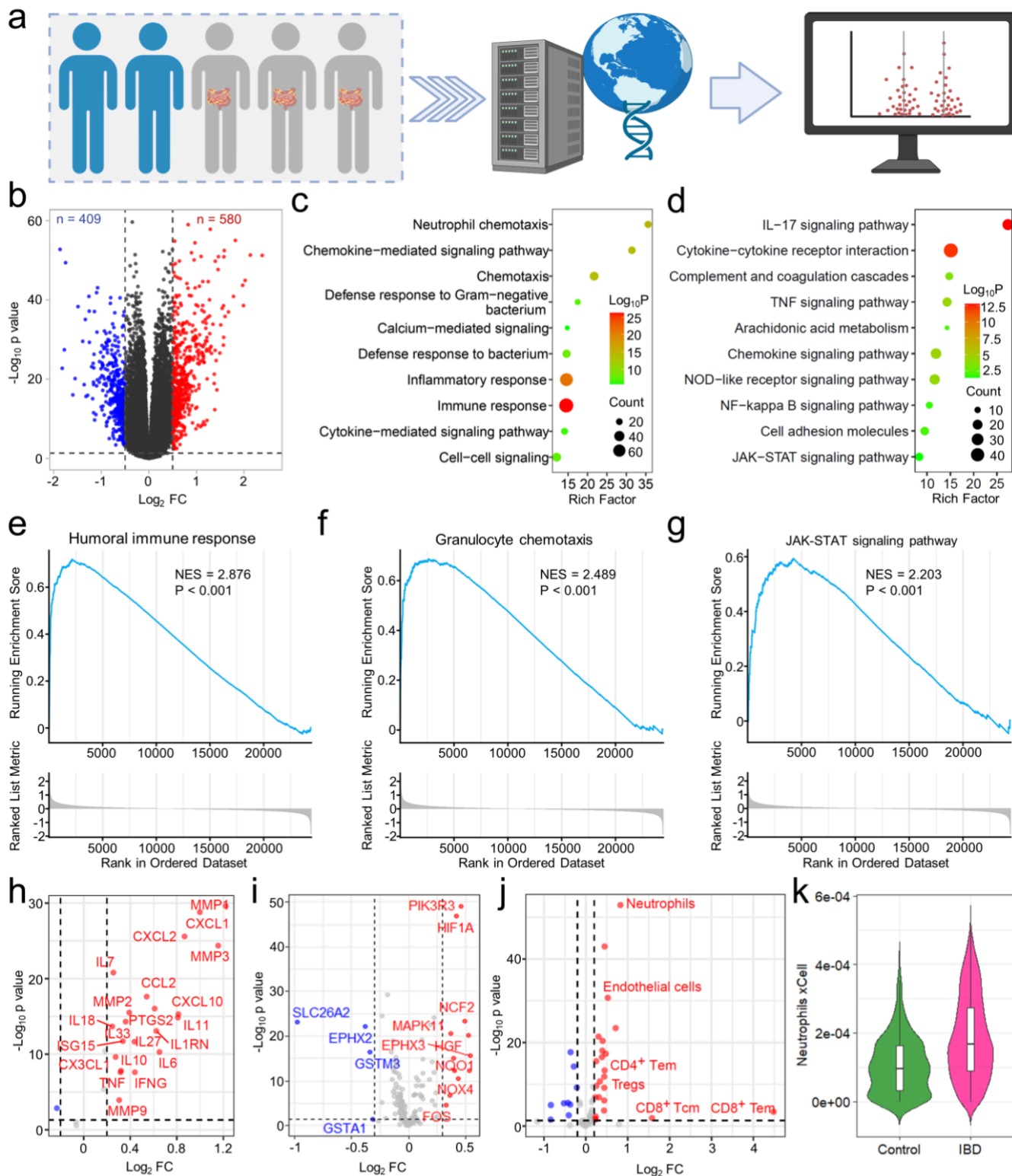


Figure 1

Transcriptomic alterations and immune microenvironment remodeling in intestinal tissues of IBD patients based on the GSE193677 dataset. (a) Schematic illustrating the overall workflow. (b) Volcano plot showing differentially expressed genes between intestinal tissues from IBD patients and healthy controls. (c) Lollipop plots display the functional enrichment results of these genes in biological processes and (d) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. GSEA plots indicate

significant enrichment of differentially expressed genes in *humoral immune response* (e), *granulocyte chemotaxis* (f), and the *JAK–STAT signaling pathway* (g). (h) Scatter plots show differential expression patterns of cytokine-encoding genes and (i) ROS-related genes in IBD tissues. (j) Scatter plot showing immune cell infiltration scores in IBD intestinal tissues estimated using the xCELL algorithm. (k) Violin plots illustrate the differences in xCELL-derived infiltration scores of *Neutrophils* between control and IBD groups.

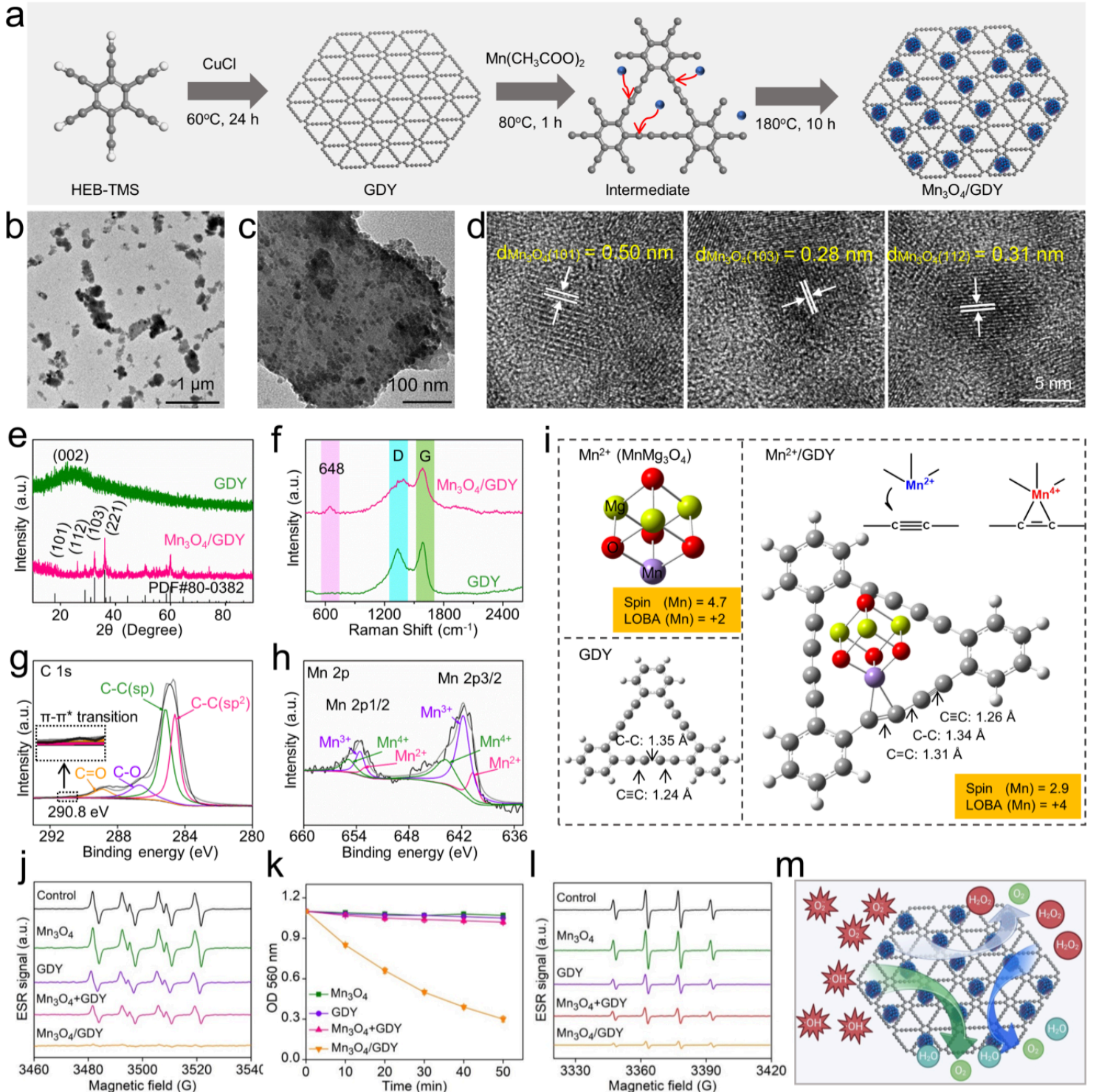


Figure 2

Synthesis and characterization of $\text{Mn}_3\text{O}_4/\text{GDY}$. (a) Schematic illustration of the synthesis procedure for $\text{Mn}_3\text{O}_4/\text{GDY}$. (b,c) TEM images of $\text{Mn}_3\text{O}_4/\text{GDY}$ under different magnifications. (d) TEM images taken from the island-like nanoparticles on a $\text{Mn}_3\text{O}_4/\text{GDY}$. (e) XRD patterns of GDY and $\text{Mn}_3\text{O}_4/\text{GDY}$. (f) Raman spectra of GDY and $\text{Mn}_3\text{O}_4/\text{GDY}$. (g,h) XPS spectra of $\text{Mn}_3\text{O}_4/\text{GDY}$. (i) Optimized structures and electronic configurations of MnMg_3O_4 (left top), GDY (left bottom), and $\text{Mn}^{2+}/\text{GDY}$ (right) obtained from DFT calculations. Mn in MnMg_3O_4 shows Spin = 4.7 and LOBA = +2. Pristine GDY exhibits $\text{C}\equiv\text{C}$ and $\text{C}-\text{C}$ bond lengths of 1.24 Å and 1.35 Å, respectively. After Mn^{2+} anchoring, bond lengths change to 1.26–1.34 Å, and Mn shows Spin = 2.9 and LOBA = +4, indicating oxidation and Mn–C coordination. (j) ESR spectra of the $\text{O}_2^{\cdot-}$ scavenging performances of various samples. (k) H_2O_2 degradation activity of various samples. (l) ESR spectra of the $\cdot\text{OH}$ scavenging performances of various samples. (m) Schematic diagram of ROS scavenging by $\text{Mn}_3\text{O}_4/\text{GDY}$.

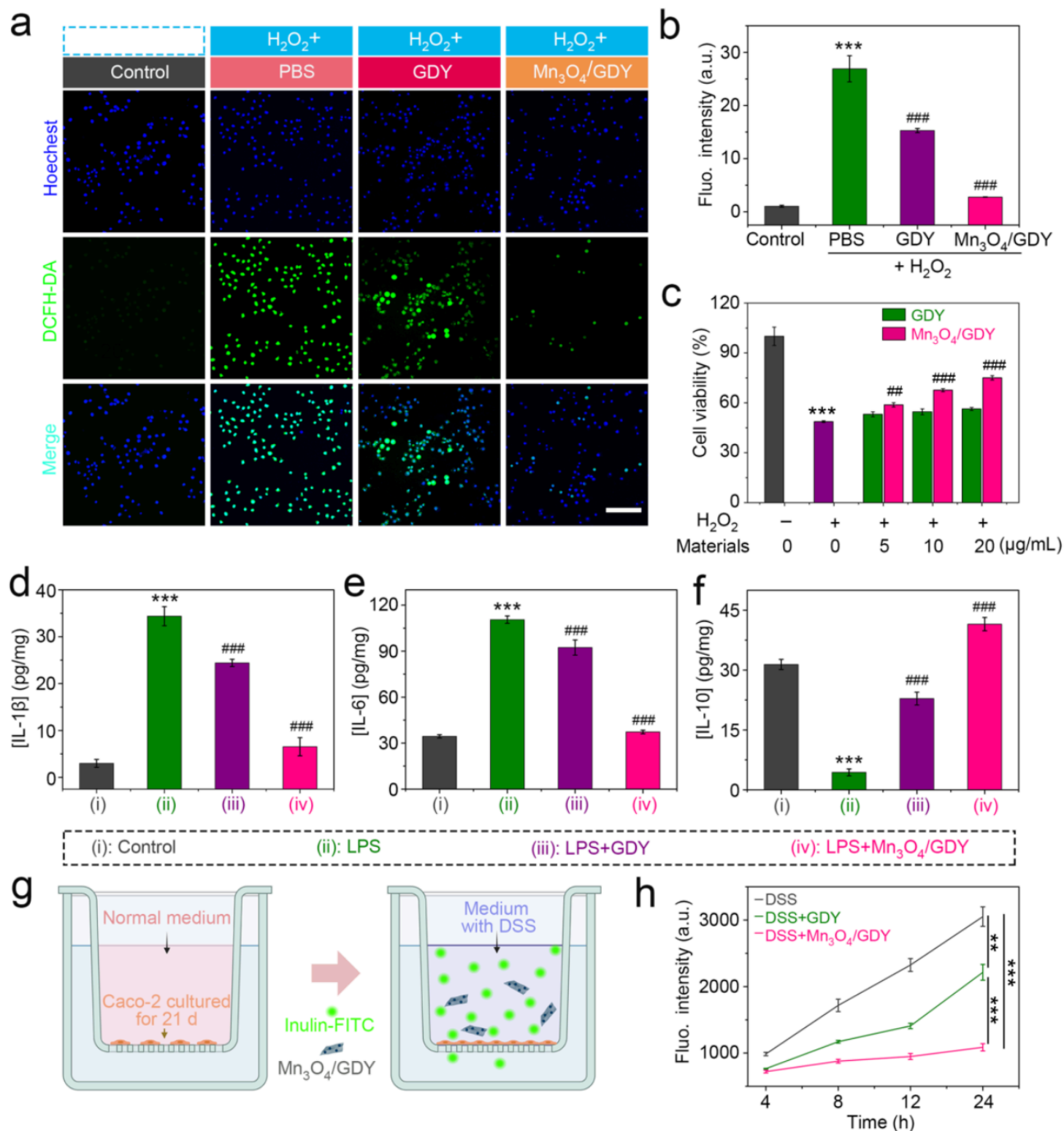


Figure 3

Antioxidant and anti-inflammatory effects of Mn₃O₄/GDY *in vitro*. (a) Representative fluorescence images and (b) quantitative fluorescence intensity of ROS levels in IEC-6 cells following treatment with H₂O₂ in the absence or presence of GDY (20 μg/mL) or Mn₃O₄/GDY (20 μg/mL). Scale bar = 10 μm. ****p* < 0.001 compared to the control group. ###*p* < 0.001 compared to the H₂O₂ group. (c) Viability of IEC-6

cells after various treatments. $***p < 0.001$ compared to the control group. $##p < 0.01$, $###p < 0.001$ compared to the H_2O_2 group. Levels of IL-1 β (d), IL-6 (e), and IL-10 (f) in LPS-activated RAW264.7 cells treated with GDY (20 μ g/mL) or Mn_3O_4 /GDY (20 μ g/mL). $***p < 0.001$ compared to the control group. $###p < 0.001$ compared to the LPS group. (g) Schematic diagram of the Caco-2 permeability assay. (h) Changes in fluorescence intensity in the Caco-2 permeability assay following various treatments. $**p < 0.01$, $***p < 0.001$. All results were presented as means $\pm$ SD (n = 3).

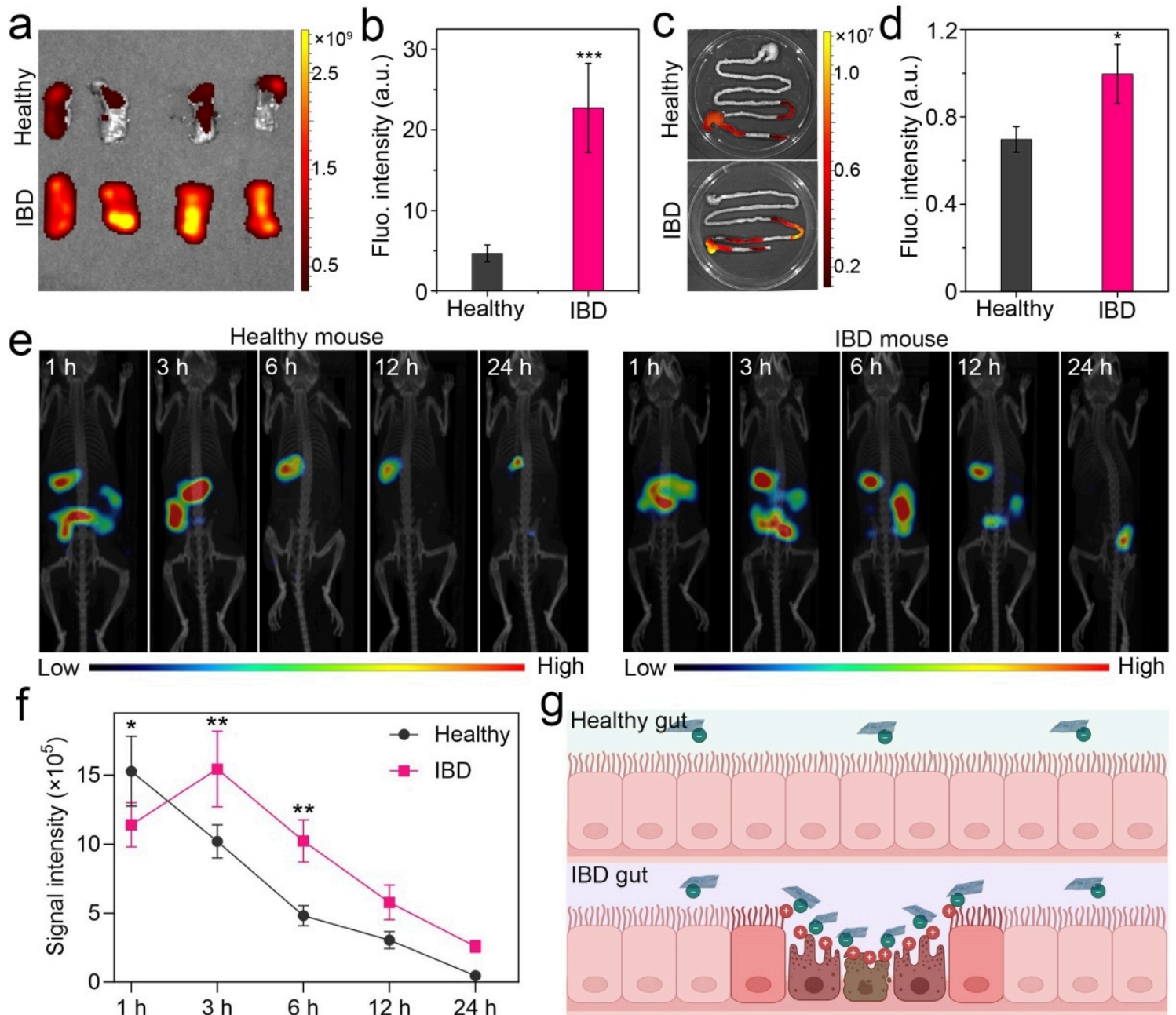


Figure 4

Mn_3O_4 /GDY shows inflammation-associated retention in colonic tissue. (a) Representative fluorescence image of colons from mice with DSS-induced IBD and healthy controls following ex vivo incubation with Mn_3O_4 /GDY for 30 min. (b) Quantification of average radiance of (a). (c) *In vivo* evaluation of Mn_3O_4 /GDY

retention after oral administration. (d) Quantitative analysis of fluorescence intensities of (c). (e) Representative SPECT/CT images of healthy and IBD mice following oral administration of Mn_3O_4 /GDY. (f) Radioactive intensities of panel (e). (g) Schematic illustration of healthy and inflamed colonic mucosa. All results were presented as means $\pm$ SD (n = 4). * p < 0.05, ** p < 0.01, *** p < 0.001 compared to the healthy control group.

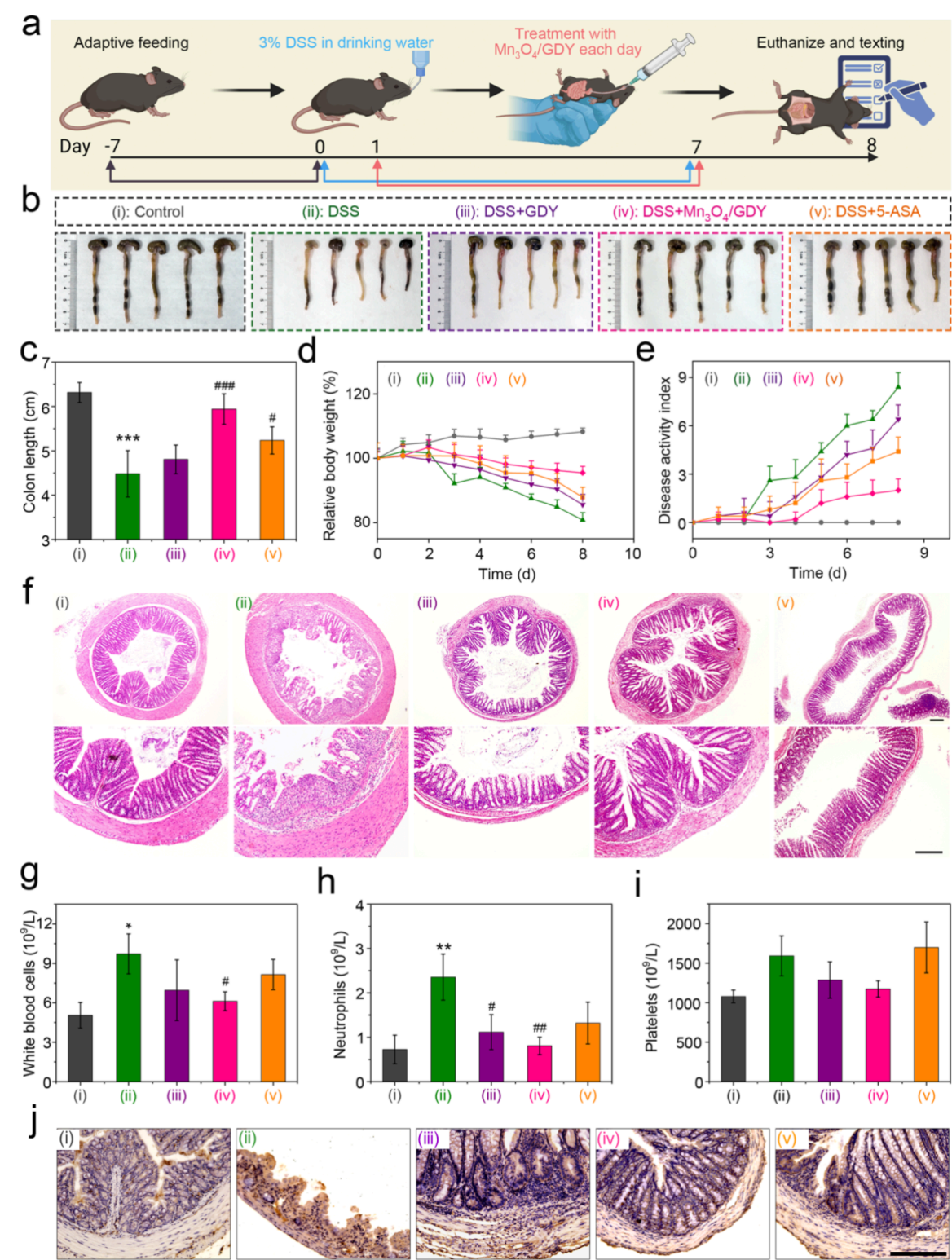


Figure 5

Therapeutic efficacy of $\text{Mn}_3\text{O}_4/\text{GDY}$ in DSS-induced IBD. (a) Schematic illustration of the experimental design for DSS-induced colitis. Mice received 3% DSS followed by daily oral administration of $\text{Mn}_3\text{O}_4/\text{GDY}$ for 7 days. (b) Representative images of colons harvested on day 8 from each treatment group. (c) Quantification of colon length in DSS-induced mice after the indicated treatments. (d,e) Changes in body weight (d) and DAI (e) during the treatment period. (f) Representative H&E staining images of colonic sections (scale bar = 100 μm). (g–i) Peripheral blood analysis of white blood cells (g), neutrophils (h), and platelets (i) after 7 days of treatment. (j) Representative immunohistochemical staining images of 4-HNE in colonic tissue sections (scale bar = 200 μm). All results were presented as means $\pm$ SD ($n = 5$). $*p < 0.05$, $***p < 0.001$ compared to the control group, $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ compared to the DSS group.

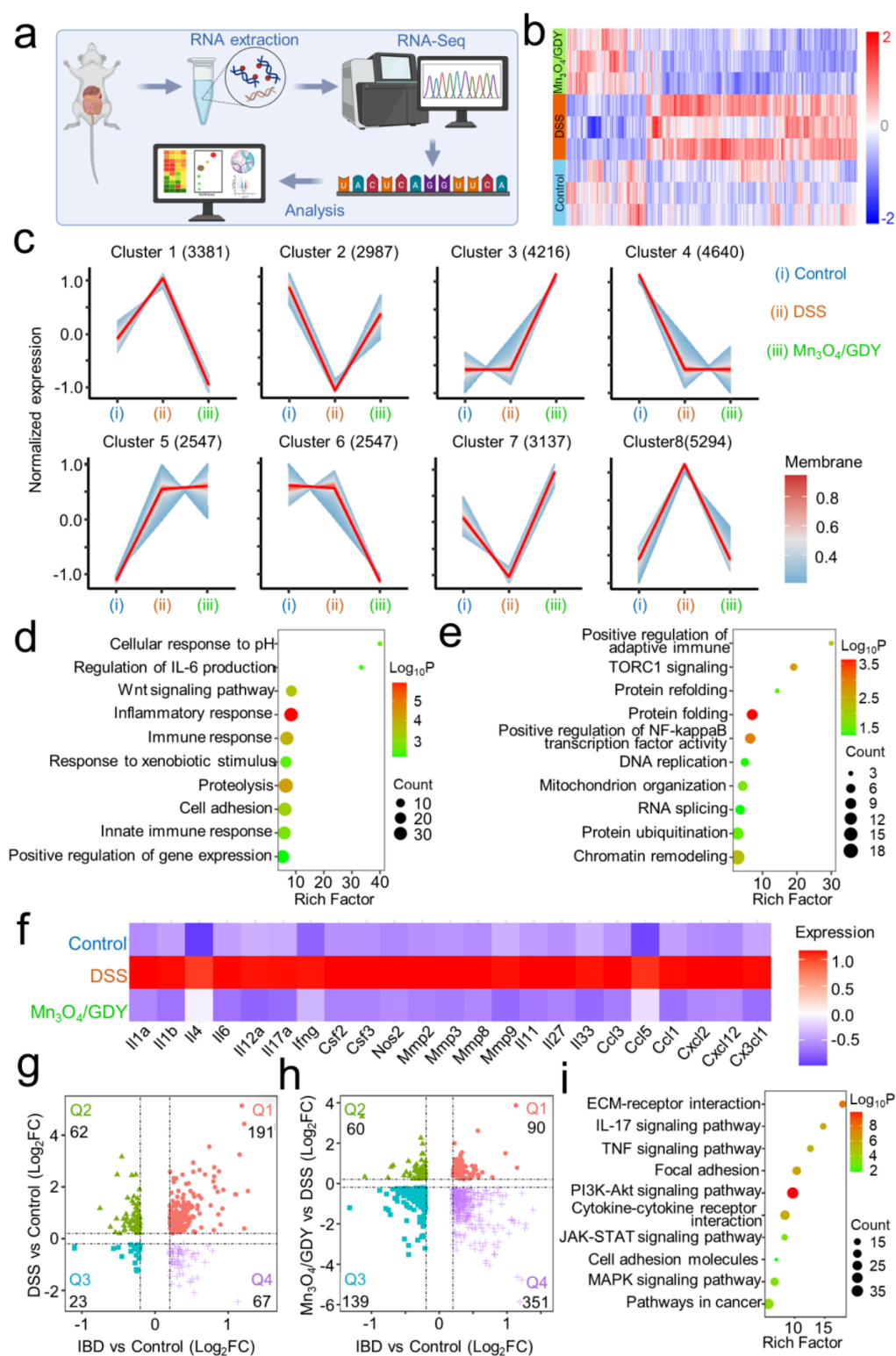


Figure 6

Transcriptomic profiling identifies key gene signatures and functional pathways associated with Mn₃O₄/GDY-mediated protection in IBD. (a) Overview of the transcriptomic analysis workflow based on RNA sequencing. (b) Heatmap showing differential gene expression in colon tissues from control, DSS, and DSS + Mn₃O₄/GDY groups. (c) Line plot showing gene expression pattern clustering of all transcripts from the three mouse groups using the Mfuzz algorithm. (d,e) Lollipop plots illustrating the enriched

Biological Process terms for clusters 1/8 (d) and clusters 2/7 (e). (f) Heatmap displaying differential expression of cytokine-related genes across the three groups. (g) Scatter plot showing integrative cross-species analysis of DEGs between human IBD colon samples versus healthy controls and DSS-induced IBD mice versus control mice, highlighting concordantly regulated genes. (h) Scatter plot showing integrative analysis of DEGs between human IBD samples versus controls and Mn₃O₄/GDY-treated mice versus IBD mice, illustrating genes whose expression changes in human IBD are reversed or attenuated by Mn₃O₄/GDY treatment. (i) Lollipop plot presenting KEGG pathway enrichment analysis of genes located in quadrants 2 and 4 identified from the cross-species comparison, representing shared pathological pathways in IBD and pathways effectively modulated by Mn₃O₄/GDY intervention.

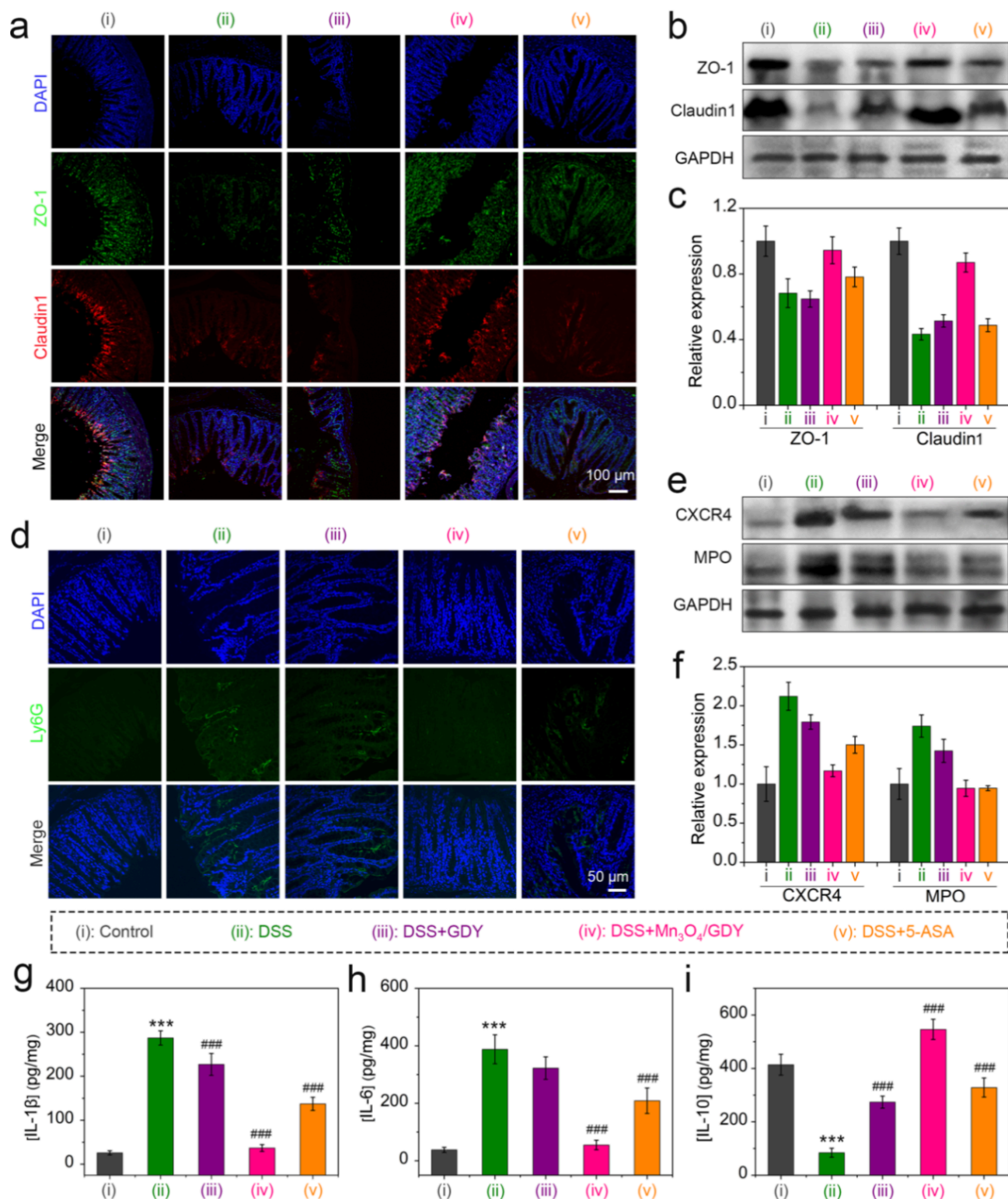


Figure 7

Mn₃O₄/GDY attenuates neutrophil infiltration, restores epithelial barrier integrity, and alleviates inflammation in DSS-induced IBD. (a) Representative immunofluorescence staining of the tight junction markers ZO-1 (green) and Claudin1 (red) in colon tissues from the five experimental groups: (i) Control, (ii) DSS, (iii) DSS + GDY, (iv) DSS + Mn₃O₄/GDY, and (v) DSS + 5-ASA. (b, c) Western blot analysis (b) and quantification (c) of ZO-1 and Claudin1 expression. (d) Representative immunofluorescence staining of

Ly6G⁺ neutrophils. (e, f) Western blot analysis (e) and quantification (f) of CXCR4 and MPO levels in colon tissues. (g-i) ELISA quantification of the inflammatory cytokines IL-1 β (g), IL-6 (h), and IL-10 (i) in colon tissues. All results were presented as means $\pm$ SD (n = 3). *** p < 0.001 compared to the control group, ### p < 0.001 compared to the DSS group.

Supplementary Files

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- [3SIMn3O4GDY260817.docx](#)
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