

Probiotic-based biohybrid with gastrointestinal stress resistance and ROS-scavenging capacity to treat inflammatory bowel disease

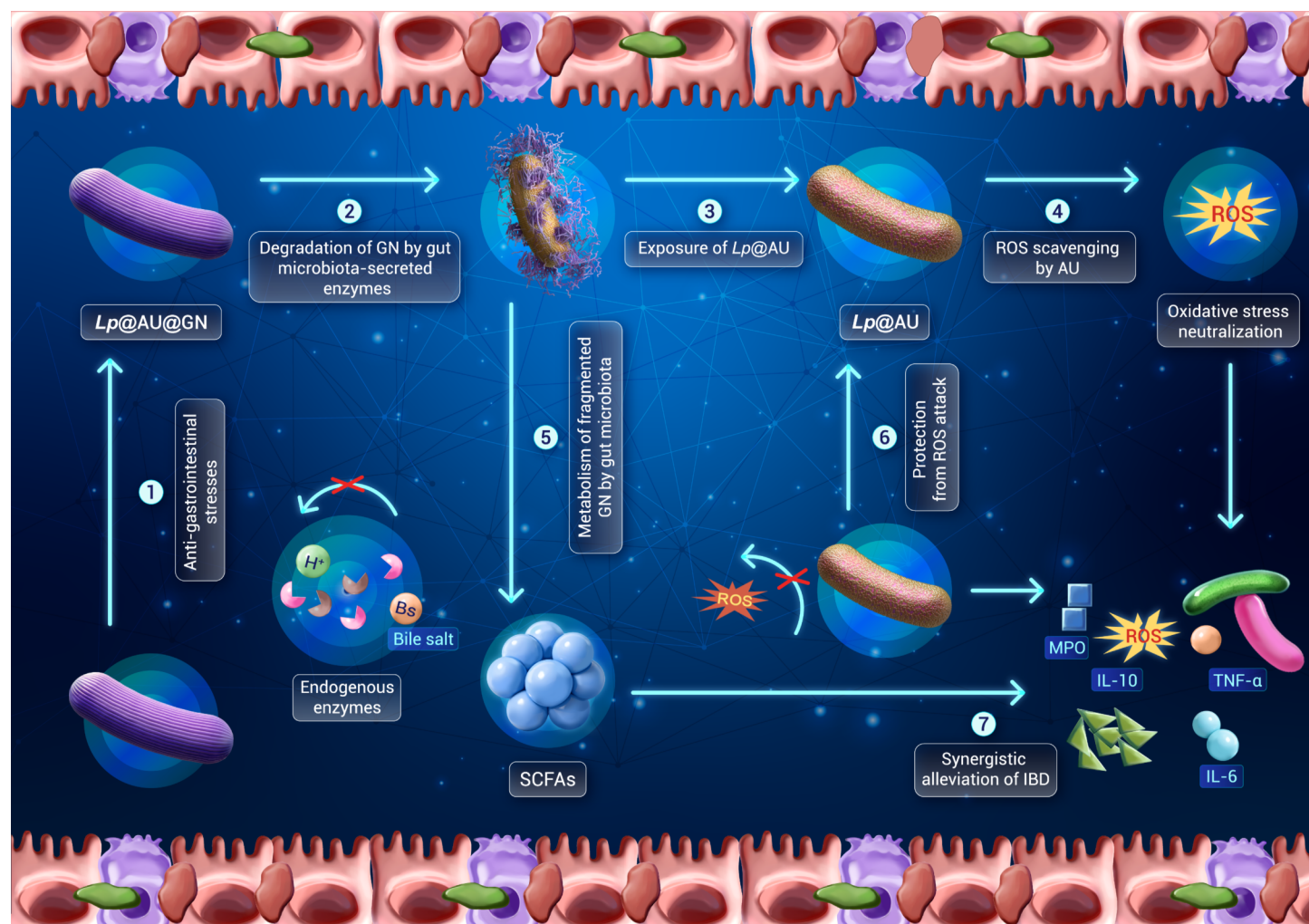
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- *Lp@AU@GN* was bio-orthogonally fabricated.
- β -glucan (GN) shielded *Lp* from digestive insults and was metabolized into short-chain fatty acids (SCFAs).
- $Au_{24}Cu_1/Au_{24}Cd_1$ mixture (AU) scavenged reactive oxygen species (ROS) to enhance *Lp* viability.
- Oral administration of *Lp@AU@GN* effectively alleviated inflammatory bowel disease (IBD).

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Oral probiotic-based therapy has emerged as a promising solution with multifaceted benefits for inflammatory bowel disease (IBD) treatment. However, their widespread and clinical utility is severely limited by the poor viability of probiotics under harsh gastrointestinal conditions and elevated oxidative stress in the inflamed intestine. To address these challenges, a probiotic-based biohybrid (*Lp@AU@GN*) was bio-orthogonally fabricated by covalently anchoring the gold nanocluster-based artificial enzyme (AU) to the probiotic *Lactobacillus plantarum* (*Lp*), a strain screened out with the IBD-alleviating potential, followed by covalent encapsulation with the prebiotic β -glucan (GN). Upon oral administration to mice with ulcerative colitis, GN performed as a shield to physically protect *Lp* from gastrointestinal stress insults. After reaching the intestine, GN was metabolized by the gut microbiota, facilitating the exposure of *Lp@AU* and the concurrent production of short-chain fatty acids (SCFAs). The incorporated AU with superoxide dismutase- and catalase-like activities efficiently scavenged excessive reactive oxygen species in situ, neutralizing oxidative stress and simultaneously enhancing *Lp* survival to synergize with SCFAs to advance the therapeutic process. Consequently, therapeutic benefits of reduced inflammation, restored intestinal barrier, and rebalanced gut microbiota were achieved. Furthermore, the therapeutic utility was extended to Crohn's disease, establishing the broad-spectrum effectiveness of *Lp@AU@GN* for addressing both major forms of IBD. Beyond IBD, our developed modular engineering strategy holds the great potential to be adapted to fabricate diverse biohybrids for other gastrointestinal or metabolic disorders' treatments by tuning the probiotic strain, antioxidant moiety, or prebiotic polymer.

INTRODUCTION

Inflammatory bowel disease (IBD) is a severe gastrointestinal disorder predominantly classified into two subtypes: ulcerative colitis (UC) and Crohn's disease (CD).^{1–3} Current pharmacological agents for IBD primarily include aminosalicylates, corticosteroids, and immunomodulators.^{4,5} However, not all patients respond effectively to these treatments, and their prolonged use can cause severe adverse effects.⁶ Furthermore, due to their distinct pathological features, most existing treatments primarily target either UC or CD individually,^{7–9} with limited strategies consistently effective across both subtypes. The development of a broadly effective strategy is challenging yet feasible by targeting the shared pathophysiology of IBD. Its core pathophysiological drivers⁹ include oxidative stress,¹⁰ immune dysregulation,¹¹ and gut microbial dysbiosis.¹² Therefore, a therapeutic strategy targeting these shared pathological features holds potential to address both UC and CD.

Oral probiotic therapy is a compelling option for IBD, owing to its natural origin, safety, and ease of administration.¹³ Critically, probiotics uniquely restore gut microbiota balance, a core aspect of IBD pathology not directly addressed by most other therapies. Their abilities to regulate metabolite production,¹⁴ outcompete pathogens,¹⁵ and modulate immune responses¹⁶ position them as a holistic alternative for treating both UC and CD. However, orally administered probiotics face major challenges in the IBD. On one hand, harsh gastrointestinal stresses, such as gastric acid and bile salts, reduce probiotic survival.¹⁷ On the other hand, elevated reactive oxygen species (ROS) in the inflamed intestinal environment pose an additional, often-overlooked stress that can impair probiotic viability and function.^{18–21} These barriers

hinder clinical applications of oral probiotic therapy, underscoring the essential for technological innovations.

To address these, encapsulation has emerged as a promising strategy.²² Among various encapsulation substrates, β -glucan (GN)—a FDA-approved polysaccharide—shows potential for protecting probiotics.²³ Its polymer structure offers physical shielding akin to synthetic polymers like Eudragit L100-55.²⁴ Moreover, GN provides biocompatibility, gastrointestinal stability, and inherent prebiotic properties.²⁵ The prebiotic property enables its selective metabolism by gut microbiota, facilitating targeted release of probiotics while promoting short-chain fatty acids (SCFAs) production,^{26–29} which may synergistically alleviate inflammation. These characteristics position GN as an ideal encapsulating substrate for oral probiotic delivery in IBD. To coat GN onto probiotics, we turn our attention to single-cell encapsulation for precise and individual protection.³⁰ While non-covalent interactions provide basic protection, covalent anchoring enhances structural stability.³¹ However, single-cell covalent encapsulation has been limited by traditional chemistry's incompatibility with live probiotics.³² Bio-orthogonal chemistry offers a powerful alternative, which enables site-specific introduction of functional groups onto live probiotic surfaces without affecting viabilities and allows highly selective covalent conjugation via biocompatible reaction like copper-free click chemistry.^{28,33}

Beyond compromising probiotic function, excess ROS in IBD also aggravate tissue damage via oxidative stress,³⁴ making ROS scavenging another crucial therapeutic goal. Recent advancements in nanozymes—nanomaterials with enzyme-like activities—have demonstrated strong potential for ROS neutralization.³⁵ Among them, gold nanoclusters (AuNCs) are notable for their excellent biocompatibility, stability, and broad-spectrum catalytic activity.³⁶ Our group previously developed $Au_{24}M_1$, a AuNC engineered by substituting a single Au atom in the Au_{25} cluster with a transition metal atom (Cu or Cd).³⁷ This atom-level precision enhances their superoxide dismutase (SOD)- and catalase (CAT)-like activities. We therefore hypothesized that co-delivering $Au_{24}M_1$ with probiotics could reduce ROS in the inflammatory microenvironment, protecting probiotic viability and neutralizing oxidative stress to synergistically improve IBD therapy.

Herein, we developed a probiotic-based biohybrid integrating gastrointestinal stress resistance and ROS-scavenging capacity for effective IBD treatment. First, through metagenomic analysis of the human gut microbiome and murine model validation, the probiotic strain *Lactobacillus plantarum* (*Lp*) was identified as having therapeutic potential for IBD. Leveraging bacterial metabolic labeling and copper-free click chemistry, dibenzocyclooctyne (DBCO)-tethered $Au_{24}M_1$ ($M = Cu$ or Cd ; designated as AU) with superior SOD/CAT-like activities were covalently anchored to the surface of *Lp* without affecting its activity, generating *Lp@AU*. To enhance stability and functionality, *Lp@AU* was encapsulated with thiol-modified GN (SmGN) through spontaneous Au–S bonding, generating the final biohybrid *Lp@AU@GN* (Figure 1A). Upon oral administration, GN shielded *Lp@AU* from the harsh gastrointestinal stresses, followed by selective metabolism by gut microbiota to liberate *Lp@AU* and produce SCFAs. The incorporated AU scavenged excess ROS, thereby neutralizing oxidative stress and improving *Lp* viability to synergize with SCFAs for enhanced therapy. In dextran sulfate sodium (DSS)-induced UC mouse model, *Lp@AU@GN* treatment significantly mitigated body weight loss, prevented colon shortening, restored colon

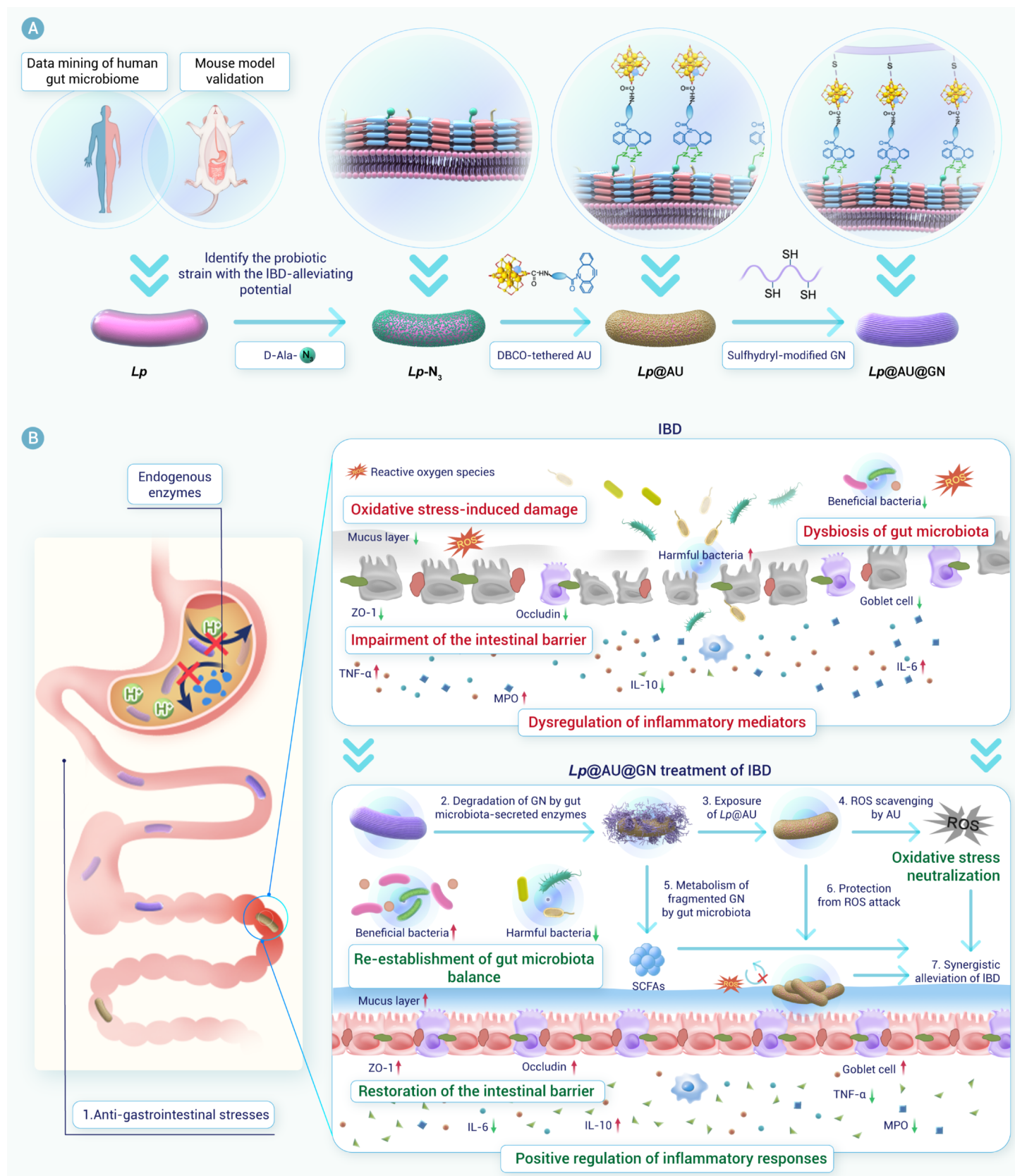


Figure 1. Schematic of *Lp*@AU@GN for synergistic IBD therapy (A) Development of *Lp*@AU@GN. (B) Therapeutic process of *Lp*@AU@GN against IBD. Following oral administration, the GN coating protected *Lp*@AU during gastrointestinal transit. Upon intestinal arrival, GN underwent gut microbiota-mediated metabolization, exposing *Lp*@AU and producing SCFAs. Concurrently, AU scavenged local excessive ROS, thereby exerting anti-oxidative stress effect and enhancing *Lp* viability to synergize with SCFAs for addressing IBD.

architecture, and reinforced intestinal barrier integrity. This was accompanied by modulation of inflammatory cytokines, suppression of inflammation-associated signaling pathways, and rebalancing of gut microbiota. Addition-

ally, the therapeutic efficacy of *Lp*@AU@GN was extended to a 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced CD mouse model, demonstrating broad-spectrum effectiveness against both major IBD forms (Figure 1B). This

study introduces a robust bacterial therapy platform, providing useful insights into the treatment of complex inflammatory diseases.

MATERIALS AND METHODS

Synthesis and characterization of $Lp@AU@GN$

To synthesize $Lp@AU@GN$, 400 μ L of Lp was added to 10 mL of culture medium containing 6 mM 3-azido-D-alanine, and the mixture was shaken at 37 °C. The Lp precipitate was collected by centrifugation (6000 rpm for 3 min) and washed twice with PBS (pH 7.4). The precipitate was subjected to an overnight click chemistry reaction using various concentrations of DBCO-tethered AU (0, 0.25, 0.5, and 1 mg/mL) to prepare $Lp@AU$. After the completion of the reaction, the optimized precipitate was dispersed in physiological saline buffer (1 $\times$ PBS, pH 7.4; 1 mL) for subsequent analyses, including NIR-II fluorescence imaging, oxygen production assessment, and SOD activity measurement. The optimized $Lp@AU$ precipitate was then conjugated with 0.08 mg/mL of SmGN through spontaneous Au-S bond formation to obtain $Lp@AU@GN$. To verify the conjugation efficacy, FITC-SmGN was used to prepare $Lp@AU@GN$ using the same procedure as above and the resulting biohybrid was analyzed by CLSM (LSM710, Germany). Before SEM and TEM analyses, the Lp and $Lp@AU@GN$ samples were fixed in a 2.5% glutaraldehyde solution to preserve their morphological integrity.

NIR-II fluorescence imaging: The NIR-II fluorescence imaging of the samples was conducted using the DeepVision system (Nirmidas Biotech, USA). Briefly, the imaging was performed under excitation with a 780 nm laser, and the fluorescence signals passing through a 1000 nm long-pass filter were collected for imaging.

Oxygen production assessment: The oxygen generation capability was evaluated using the oxygen-sensitive fluorescent dye $[Ru(dpp)_3]Cl_2$. Briefly, the sample was mixed with $[Ru(dpp)_3]Cl_2$ in a 96-well plate. The catalytic reaction was initiated by adding H_2O_2 . The decrease in fluorescence intensity (Ex: 488 nm/Em: 620 nm) due to oxygen-induced quenching was monitored using a microplate reader (Thermo Fisher Scientific, USA).

SOD activity measurement: The SOD activity was determined using a commercial Total SOD Activity Assay Kit (WST-8 method) (Beyotime Biotechnology, Shanghai, China) strictly according to the manufacturer's protocol. The absorbance was measured at 450 nm with a reference wavelength of 600 nm using a microplate reader. The inhibition rate was calculated using the corrected absorbance values (A450 nm - A600 nm) to quantify the SOD activity.

Therapeutic effect of $Lp@AU@GN$ on DSS-induced UC

C57BL/6J mice were acclimatized for 7 days and randomly divided into six cohorts: normal, model, AU@GN, Lp , $Lp@AU$, and $Lp@AU@GN$. UC was induced by administering 3% DSS in drinking water for 5 days, whereas the normal group received regular drinking water throughout the study. After DSS withdrawal, mice received daily oral gavage (200 μ L) according to their group assignments for 5 days. The Lp , $Lp@AU$, and $Lp@AU@GN$ groups were administered bacterial suspensions (1×10^{10} CFU/mL in PBS), while the AU@GN group received AU@GN (the amounts of AU and GN were equivalent to those in $Lp@AU@GN$). Normal and model groups were gavaged with PBS (200 μ L/day). After a 12-h interval following the final treatment, mice were weighed and euthanized. Colon length was measured. Distal colonic segments were fixed in 4% paraformaldehyde for histological processing. Serum was separated by centrifugation. Colonic contents and serum were cryopreserved at -80°C for further analysis.

RESULTS

Data mining combined with mouse model validation to identify the probiotic strain with the IBD-alleviating potential

To identify bacterial species possessing therapeutic benefits in IBD, we performed comprehensive analyses of the gut microbiota between UC patients and healthy individuals (HI) using sequencing datasets from the public database (GMrepo, accession code: PRJNA400072). Comparative study based on linear discriminant analysis (LDA) revealed several bacterial species exhibiting significant alterations in UC patients (LDA score > 2.0, $P < 0.05$) (Figure 2A). Among these, the LDA effect size of Lp was significantly elevated in UC patients relative to HI. Consistently, analysis of human

metagenome atlas (HMGA) database (accession code: PRJEB1220) confirmed the significantly elevated Lp abundance in UC patients versus HI ($P < 0.05$) (Figure S1). This consistent enrichment suggests a functional role of Lp in IBD. We speculate this increase might reflect a compensatory response by the host, wherein the abundance of Lp is upregulated as part of an intrinsic self-defense mechanism to mitigate damage and restore intestinal homeostasis. This finding indicates the therapeutic potential of Lp in IBD.

To validate this, we evaluated Lp 's therapeutic efficacy in a DSS-induced UC mouse model. Mice were given drinking water containing 3% DSS to induce UC,²⁸ followed by daily oral administration of phosphate-buffered saline (PBS; model group) or Lp for 5 days (Figure 2B). Normal mice administered PBS served as the normal group. The results showed that Lp -treated UC mice had increased colon length (Figures 2C–D), decreased spleen index (Figure S2), improved colon morphology (Figure 2E), and elevated mucus expression (Figure 2F) relative to PBS-treated UC mice. Additionally, the Lp treatment reduced pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) (Figure 2G) and interleukin-6 (IL-6) (Figure 2H), as well as elevated the anti-inflammatory cytokine interleukin-10 (IL-10) (Figure 2I). Although Lp alleviated some pathology in UC mice, its efficacy was limited, as compared to the normal group. This should be because the harsh gastrointestinal conditions and the inflammatory intestinal environment (i.e., elevated ROS levels; Figure S3) would compromise Lp viability. This was indirectly evidenced by extremely low survival of Lp in simulated gastric fluid (SGF) (Figure 2J), bile salts (Figure 2K), high concentrations of H_2O_2 solution (Figure 2L; mimic the oxidative stress condition), or the simulated whole gastrointestinal digestion under IBD conditions (initial incubation with SGF, followed by exposure to simulated intestinal fluid [SIF] with 0.05 mM H_2O_2 added) (Figure 2M). Collectively, technological innovations that can enhance Lp stability and activity under such adverse conditions are needed to maximize its efficacy as an oral therapeutic toward IBD.

Bio-orthogonal preparation of $Lp@AU@GN$ and its characterization

The above findings motivated us to formulate Lp as an enhanced oral therapeutic for IBD treatment. For this, $Lp@AU@GN$ was designed and fabricated, as illustrated in Figure 1A. In brief, Lp was cultured in a medium containing 3-azido-D-alanine, which enabled the display of azide groups ($-N_3$) on the peptidoglycans (PGN) at the Lp surface via endogenous transpeptidase-mediated incorporation.³⁸ Subsequently, DBCO-tethered AU (characterization in Figure S4) were covalently linked to $Lp-N_3$ through the copper-free click chemistry reaction, resulting in the AU-equipped Lp ($Lp@AU$). Finally, a protective layer of GN was applied to encapsulate $Lp@AU$ through spontaneous covalent bond formation between the Au atom of AU and the thiol group of SmGN (characterization in Figure S5), thus $Lp@AU@GN$ was obtained.

AU comprising $Au_{24}Cd_1$ (where a single Cd atom substitutes one Au atom in the Au_{25} cluster) and $Au_{24}Cu_1$ (where a single Cu atom substitutes one Au atom in the Au_{25} cluster) at the ratio of 1:1 was first prepared based on our previous study.³⁷ Their successful synthesis was confirmed by X-ray photoelectron spectroscopy (XPS) (Figure S6) and transmission electron microscopy (TEM) (Figure S7). To maximize the anchoring of AU onto Lp , the concentration of 3-azido-D-alanine was optimized to be 6 mM, at which no adverse effects for bacterial growth were observed (Figure 3A). After covalent anchoring of varying concentrations of DBCO-tethered AU to $Lp-N_3$, as reflected by the saturated fluorescence intensity derived from intrinsic second-near infrared (NIR-II) fluorescence of AU (Figure S8), the maximum attachment efficiency was determined for subsequent experiments (Figure 3B). SOD-like activity assays demonstrated that $Lp@AU$ retained AU's superoxide scavenging capacity (Figure 3C). In CAT-like activity assessment assays based on $[Ru(dpp)_3]Cl_2$ (an oxygen-sensitive fluorescent probe), $Lp@AU+H_2O_2$ significantly reduced the probe's fluorescence intensity similar to $AU+H_2O_2$ (Figure 3D), indicating efficient H_2O_2 decomposition. To visually confirm the successful coating of GN onto $Lp@AU$, fluorescein isothiocyanate-labeled SmGN (FITC-SmGN; characterization in Figure S9) was employed. As observed by confocal laser scanning microscopy (CLSM) (Figure 3E), upon mixing FITC-SmGN with $Lp@AU$, an obvious green fluorescence signal colocalized with each individual bacterium, indicating the successful single-cell encapsulation. In striking contrast, negligible FITC-

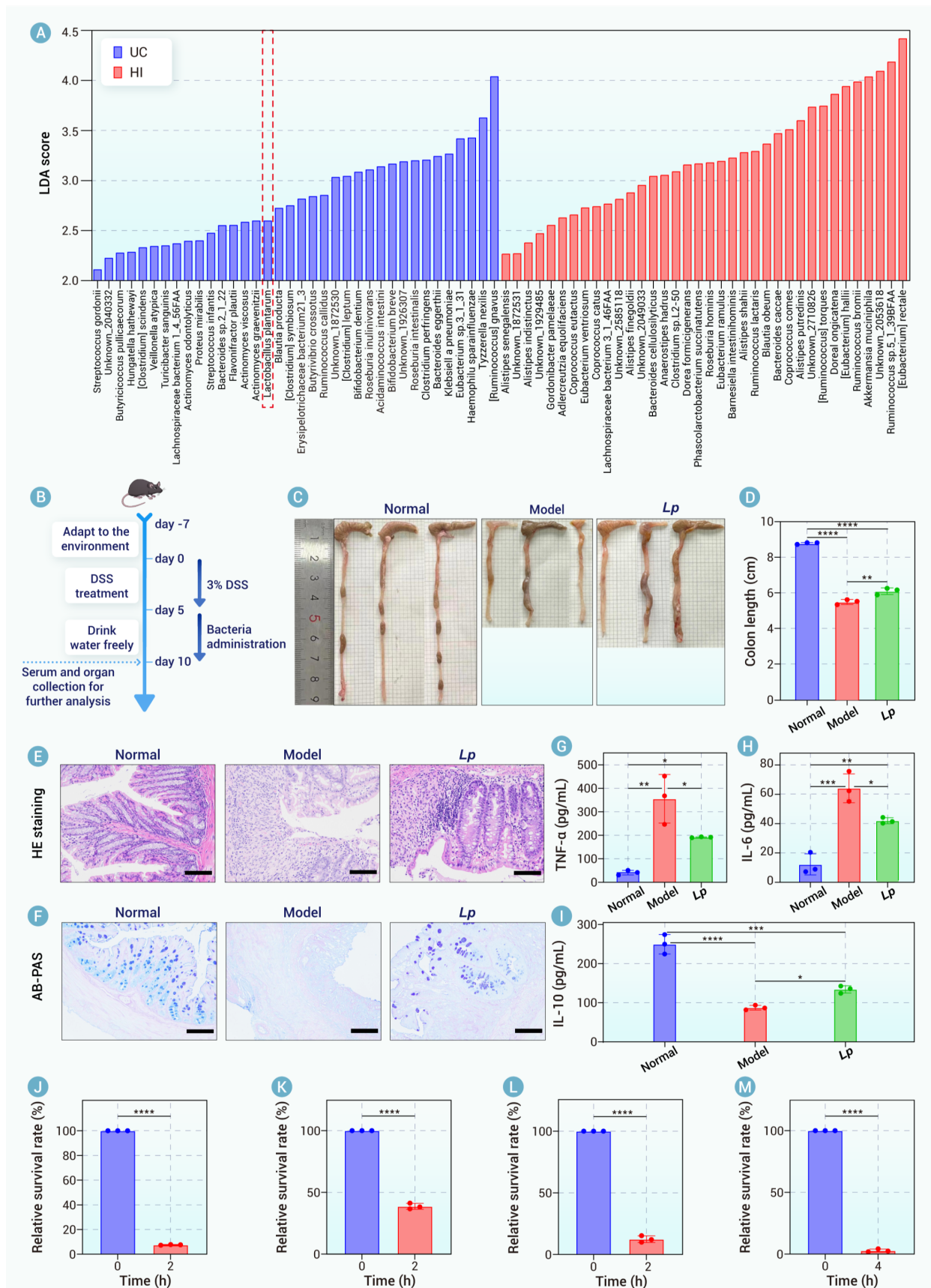


Figure 2. *Lp* demonstrated therapeutic potentials in IBD and its limitations as an oral therapeutic (A) Histogram of LDA scores (LDA > 2.0, $P < 0.05$). Colored branches represent taxa preferentially found in UC (blue) patients or HI (red). The dashed box highlights *Lp*. (B) Schematic of the experimental protocol. (C and D) Photos of the colons (C) and corresponding length quantification (D) ($n = 3$). (E) Representative hematoxylin and eosin (HE)-stained colon sections. Scale bars = 100 μ m. (F) Representative periodic acid schiff (PAS) and alcian blue (AB) staining (AB-PAS) images of colonic sections. Scale bars = 100 μ m. (G–I) Cytokine concentrations in each treatment group at endpoint ($n = 3$). (J–M) Relative survival rates of *Lp* after exposure to (J) SGF (pH 3) for 2 h, (K) 4% bile salts for 2 h, (L) 0.05 mM H_2O_2 for 2 h, or (M) simulated whole gastrointestinal conditions in IBD (sequential SGF/SIF+ H_2O_2 incubation) ($n = 3$). Data are expressed as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

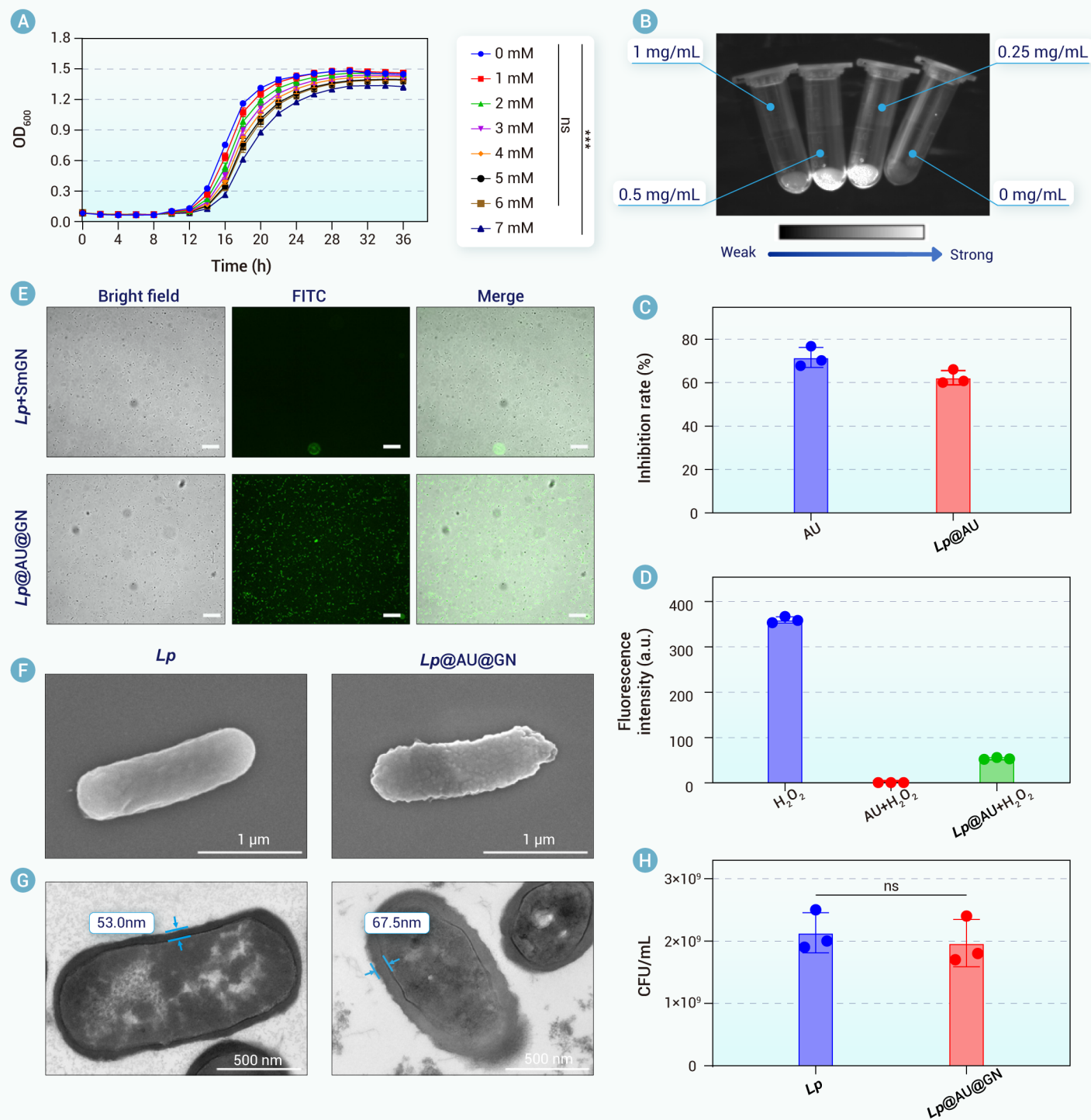


Figure 3. Characterization of *Lp@AU@GN* (A) Growth kinetics of *Lp* cultured under increasing concentrations of 3-Azido-D-alanine ($n = 3$). (B) NIR-II imaging of *Lp@AU* anchored with varying concentrations of DBCO-tethered AU (i.e., 0, 0.25, 0.5, or 1 mg/mL). (C) SOD-like activity of AU and *Lp@AU* ($n = 3$). (D) CAT-like activity of AU and *Lp@AU* ($n = 3$). (E) CLSM images of *Lp* + SmGN (without the incorporation of AU) and *Lp@AU@GN*; Green: FITC-labeled SmGN. Scale bars, 20 μm . (F) Representative SEM analysis of *Lp* and *Lp@AU@GN*. Scale bars, 1 μm . (G) Representative TEM analysis of *Lp* and *Lp@AU@GN*. Scale bars, 500 nm. (H) CFU of *Lp* and *Lp@AU@GN* after 36 h of cultivation ($n = 3$). Data are expressed as mean $\pm$ SD. *** $P < 0.001$ and ns (non-significance).

SmGN was coated onto naive *Lp*, as revealed by extremely low green fluorescence. This marked difference strongly supports the spontaneous formation of covalent Au-S bonds between SmGN and AU for coating, rather than nonspecific physical adsorption, thereby enhancing the structural integrity and stability. Correspondingly, scanning electron microscopy (SEM) and TEM analyses clearly revealed that the surface of *Lp@AU@GN* was rougher, with more folds and increased thickness compared to naive *Lp*, directly confirming the successful preparation of *Lp@AU@GN* (Figures 3F–G). Eventually, to investigate the impact of the covalent modification on the growth of *Lp*, the

viability of *Lp@AU@GN* was compared with *Lp* via colony forming unit (CFU) counting. No significant difference in CFU counts was observed between the two groups (Figure 3H), verifying the bio-orthogonal property of our engineering strategy.

Resistance of *Lp@AU@GN* to gastrointestinal and oxidative stresses

To ensure the effective functionality of *Lp* for regulating IBD, *Lp@AU@GN* must withstand the harsh gastrointestinal conditions and protect itself from the detrimental effects of excess ROS present in the inflamed intestine. With

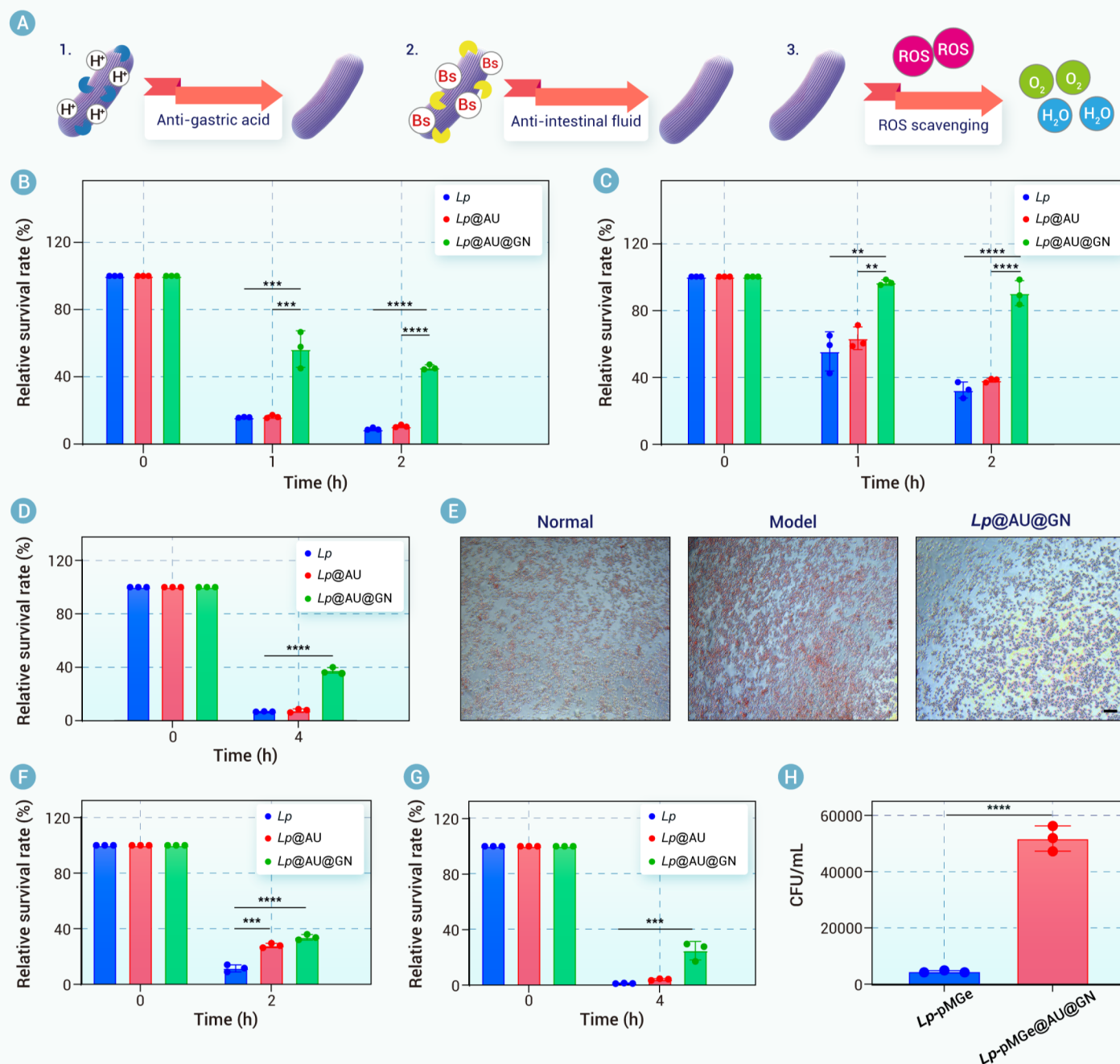


Figure 4. Evaluation of *Lp*@AU@GN's resistance against diverse stresses (A) Schematic of protective mechanisms against gastrointestinal and oxidative stresses. (B–D) Relative survival rates of *Lp*, *Lp*@AU, and *Lp*@AU@GN after exposure to (B) SGF (pH 3), (C) bile salts (4%), or (D) sequential SGF/SIF incubation ($n = 3$). (E) Fluorescence images representing ROS levels in LPS-stimulated RAW264.7 cells after different treatments (stained with the ROS probe DHE, red). Scale bar, 20 μ m. (F and G) Relative survival rates of *Lp*, *Lp*@AU, and *Lp*@AU@GN after exposure to (F) H₂O₂ (0.05 mM) or (G) sequential SGF/SIF+H₂O₂ incubation ($n = 3$). (H) Assessment of viable *Lp*-pMG36e in UC mice post-administration of *Lp*-pMG36e or *Lp*-pMG36e@AU@GN ($n = 3$). Data are expressed as mean $\pm$ SD. ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

Lp@AU@GN in hand, we then performed systematic evaluations to probe its superiority in resisting these adverse conditions and to verify the structural integrity of the biohybrid during digestion, which is essential for intact delivery to the intestines (Figure 4A). Accordingly, *Lp*, *Lp*@AU, and *Lp*@AU@GN were separately incubated in SGF and their CFU were quantified at various time points. The results revealed that, after 2 h of incubation, the survival rate of *Lp*@AU@GN was significantly higher than that of *Lp* and *Lp*@AU, respectively (Figure 4B). This finding suggests that the GN coating effectively shields *Lp* from contact with SGF, thereby maintaining the structural integrity of the biohybrid in the stomach. Subsequently, the tolerance to bile salts, an endogenous intestinal assault, was assessed. As shown in Figure 4C, the survival rate of *Lp*@AU@GN significantly surpassed that of *Lp* ($P < 0.0001$) or *Lp*@AU ($P < 0.0001$) after 2 h of incubation in a bile salt environment. Build-

ing on these findings, the survival rate of each formulation under simulated whole gastrointestinal conditions was investigated. After incubation in SGF for 2 h, the samples were transferred to SIF for an additional 2 h treatment. Consistently, *Lp*@AU@GN still exhibited the highest survival rate among all formulations (Figure 4D). Collectively, these results indicate that the *Lp*@AU@GN biohybrid can maintain its structural integrity and protect the encapsulated probiotics throughout the gastrointestinal transit, ensuring viable *Lp* to reach intestine.

Before further evaluating the tolerance of *Lp*@AU@GN to oxidative stress, we first investigated its ROS-scavenging capacity *in vitro*. Lipopolysaccharide (LPS)-stimulated RAW264.7 cells were adopted to mimic the high-level ROS production in IBD, as intuitively reflected by strong fluorescence intensities emitted from dihydroethidium (DHE),³⁹ a probe for detecting intracellular

O₂^{•−}. Following *Lp*@AU@GN treatment, LPS-stimulated RAW264.7 cells exhibited much weaker fluorescence, which approached intensities in untreated RAW264.7 cells, indicating the efficient scavenging of intracellular ROS (Figures 4E & S10). To further validate the ROS-scavenging capacity of *Lp*@AU@GN, we conducted additional flow cytometry-based quantitative analysis. This analysis, presented in Figure S11, confirmed the results observed in the fluorescence imaging. Encouraged by this finding, 0.05 mM H₂O₂ solution was adopted to evaluate the superiority of *Lp*@AU@GN in tolerating oxidative stress. The results revealed a significant increase in the survival rate of *Lp*@AU compared to *Lp*, directly confirming that the ROS scavenging conferred by AU protected *Lp*, which may also neutralize oxidative stress in IBD. Notably, following the additional coating of the GN shield, *Lp*@AU@GN achieved the highest survival rate, indicating the synergistic protective effect between AU and GN (Figure 4F). Finally, to simulate the journey after oral administration to IBD patients, each formulation was sequentially incubated in SGF for 2 h and in SIF containing 0.05 mM H₂O₂ for an additional 2 h. Consistently, *Lp*@AU@GN demonstrated the highest survival rate among all formulations (Figure 4G), suggesting that the enhanced formulation could comprehensively protect *Lp* from stress assaults encountered during transit after oral administration. To provide conclusive *in vivo* evidence for the enhanced intestinal delivery, *Lp* carrying an erythromycin resistance gene (*Lp*-pMG36e) and its corresponding probiotic-based biohybrid (*Lp*-pMG36e@AU@GN) were employed to evaluate their survival following oral administration to DSS-induced UC mice. The number of surviving *Lp* bacteria was determined using plate counting with erythromycin added (to eliminate the interference of other gut bacteria). As anticipated, the mice treated with *Lp*-pMG36e@AU@GN harbored significantly more viable *Lp*, showing an 11.68-fold increase compared to the *Lp*-pMG36e group (Figure 4H). This result directly confirms that the integration of the protective GN shield and ROS scavenger AU enables a substantially greater number of viable *Lp*@AU@GN biohybrids to successfully colonize the inflamed intestine. Collectively, these findings clearly suggest that the AU@GN incorporation substantially improves the bioavailability of orally administered *Lp* in the inflamed gut environment.

Selective metabolism of the GN shield in the intestine for timely awakening of encapsulated *Lp*

Given the full protection provided by the GN coating, we wondered whether this shield might act as a double-edged sword by physically restricting the proliferation of encapsulated *Lp*, which may negatively impact its therapeutic efficacy. Therefore, we further investigated the prebiotic property of GN, with a particular focus on its selective metabolism by gut microbiota. Such a targeted release is expected to relieve physical constraints on bacterial growth and facilitate the therapeutic benefits of *Lp* precisely at the inflamed intestinal sites. To mimic the situation in the gut, GN was treated with β -glucanase, a representative enzyme secreted by gut microbiota. The results from gel permeation chromatography (GPC) analysis indicated that, following treatment by β -glucanase, GN was degraded (Figure 5A). For direct observation, the morphology of *Lp*@AU@GN was investigated by SEM after different gastrointestinal conditions. After incubation with SGF for 2 h followed by SIF treatment for an additional 2 h (whole gastrointestinal digestion) (Figure 5B), *Lp*@AU@GN maintained the structural integrity, echoing the above protective results (Figure 4D). In sharp contrast, the outer layer of *Lp*@AU@GN was progressively degraded under whole gastrointestinal digestion with β -glucanase added, promoting the liberation of *Lp*@AU (Figures 5C–D). Correspondingly, the lag phase effect for the growth curve of *Lp*@AU@GN was eliminated following β -glucanase enzymatic treatment, with its activity recovered comparable to that of naive *Lp* (Figure 5E), indicating the selective degradation of the GN shield for timely “awakening” of encapsulated *Lp*. Inspired by these findings, we hypothesize that the GN shield could be metabolically utilized by the gut microbiota, a potential property that may contribute to restoring microbial homeostasis in IBD.⁴⁰ To ascertain this, GN was added to a carbon-free culture medium and co-cultured with fecal bacteria isolated from healthy mice. After 12 h of incubation, the optical density of fecal bacteria at 600 nm (OD₆₀₀) significantly increased, while the total sugar content dramatically declined (Figures 5F–G), suggesting that the gut bacteria utilized GN as a carbon source. These

results illustrate that GN not only serves as a protective barrier but also acts as a fermentable substrate for gut microbes, hopefully contributing to IBD mitigation. Furthermore, similar outcomes were observed when fecal microbiota from UC mice were cultured with GN, with OD₆₀₀ and sugar consumption levels comparable to those seen in the healthy group (Figures 5H–I). This suggests that even under inflammatory conditions, the gut microbiota retain the capacity to metabolize GN, potentially supporting metabolic benefits and microbial restoration.

By leveraging the autofluorescence of AU (Figure S8), self-tracking of *Lp*@AU@GN after oral administration was performed to visually monitor the gastrointestinal transit and integrity of the biohybrids. Initially, the fluorescence emission properties of both *Lp*@AU and *Lp*@AU@GN were verified in the NIR-II region,³⁷ confirming their suitability for subsequent *in vivo* imaging (Figure S12). Subsequently, *Lp*@AU or *Lp*@AU@GN was orally administered to mice, followed by real-time NIR-II fluorescence imaging. The results showed that mice administered *Lp*@AU@GN consistently exhibited stronger fluorescence signals than those administered *Lp*@AU at all detected time points (Figures 5J–L). We speculate that this is attributed to the GN shield, which protects *Lp*@AU from damage, thereby preserving the structural integrity and the autofluorescence of the AU core. *Ex vivo* imaging of the gastrointestinal tracts directly validated this hypothesis (Figures 5K–M). The sustained and strong NIR-II fluorescence signal from the stomach to the intestine provides direct visual evidence that *Lp*@AU@GN successfully traverses the harsh gastrointestinal tract and accumulates in the gut. In sum, the GN shield enables the efficient intestinal delivery of *Lp* with enhanced bioavailability, while simultaneously serving as a substrate for potentially supporting metabolic benefits and microbial restoration, thereby hopefully synergizing with surviving *Lp* to achieve better management of IBD.

Therapeutic efficacy of *Lp*@AU@GN toward DSS-induced UC in mice

With all the features of *Lp*@AU@GN characterized, we evaluated its therapeutic efficacy toward IBD, using the DSS-induced UC model. We first investigated the effect of dosage on therapeutic efficacy. Mice were administered 3% DSS for 5 days to establish UC,⁴¹ followed by daily oral administration of PBS, low-dose (10⁸ CFU/mL), medium-dose (10⁹ CFU/mL), or high-dose *Lp*@AU@GN (10¹⁰ CFU/mL) (Figure S13) for another 5 days. Compared to the PBS, medium- and high-dose *Lp*@AU@GN treatments significantly ameliorated the body weight loss (Figure S14), whereas only high-dose treatment markedly suppressed the elevation of disease activity index (DAI) (Figure S15). Both medium- and high-dose treatments alleviated colon shortening, with the high-dose *Lp*@AU@GN showing superior efficacy (Figures S16–S17). The low-dose *Lp*@AU@GN treatment showed no therapeutic effect. Therefore, high-dose *Lp*@AU@GN treatment was selected for subsequent experiments. To rule out the potential biotoxicity, the biocompatibility of the high-dose *Lp*@AU@GN was assessed. Normal mice received PBS or high-dose *Lp*@AU@GN daily (Figure S18). Throughout the 10-day treatment period, no significant differences in the body weight were observed among all the mice (Figure S19). After 10 days, all mice were euthanized, and the serum and major organs were collected for toxicological analysis. No significant differences were found in colon length (Figures S20–S21), spleen index (Figure S22), serum biochemical indices (Figure S23), and histopathological analysis (through HE staining, Figure S24). These findings verify the negligible toxicity of *Lp*@AU@GN at this dosage.

To verify the superiority of *Lp*@AU@GN in treating UC, we compared the therapeutic efficacy of *Lp*, AU@GN, *Lp*@AU, or *Lp*@AU@GN (denoted as the *Lp*, AU@GN, *Lp*@AU, and *Lp*@AU@GN groups). Normal and UC mice administered PBS served as the normal and model groups, respectively (Figure 6A). Compared with the model group, the *Lp*, *Lp*@AU and *Lp*@AU@GN groups showed significantly mitigated weight loss (Figure 6B), while AU@GN, *Lp*@AU, and *Lp*@AU@GN treatment significantly reduced DAI scores (Figure 6C), with the *Lp*@AU@GN group demonstrating the most pronounced effect. Colon length and spleen index revealed that all the treatment groups exhibited varying degrees of improvement in colon shortening (Figures 6D–E) and spleen enlargement (Figure 6F), with the best performance still observed in the *Lp*@AU@GN group. Notably, AU@GN treatment also demonstrated certain therapeutic benefits, attributed to AU's ability to neutralize oxidative stress coupled with GN's prebiotic functionality. The superior therapeutic effi-

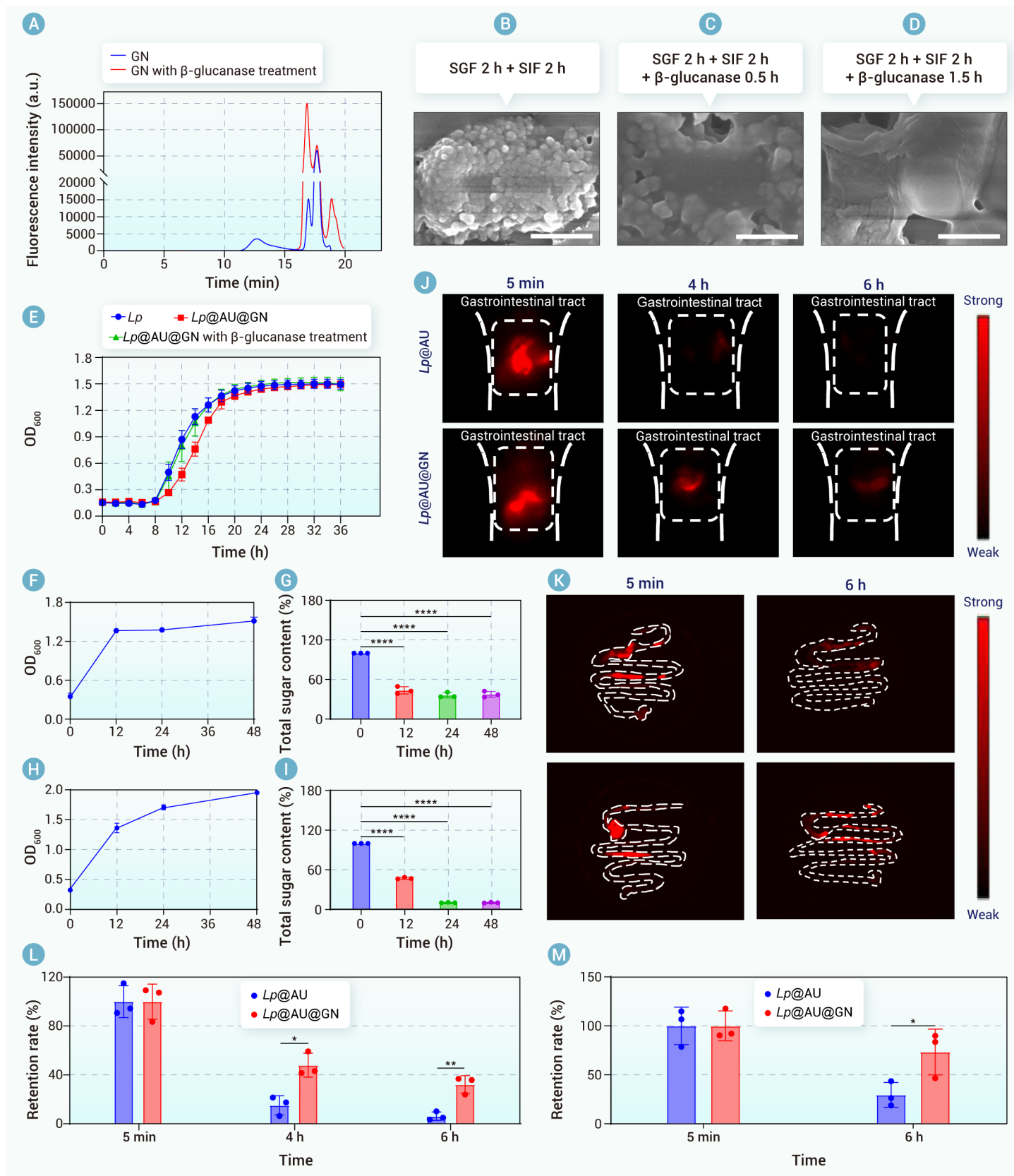


Figure 5. Evaluation of intestinal delivery of *Lp* by *Lp@AU@GN* (A) Molecular weights of GN with or without β -glucanase treatment. (B) Morphology of *Lp@AU@GN* after exposure to SGF for 2 h followed by SIF treatment. Scale bar, 500 nm. (C and D) Morphology of *Lp@AU@GN* after sequential SGF/SIF incubation with β -glucanase treatment for 0.5 (C) or 1.5 h (D). Scale bars, 500 nm. (E) Growth kinetics of *Lp*, *Lp@AU@GN*, and *Lp@AU@GN* post β -glucanase treatment (n = 3). (F and G) Bacterial density (F) and total sugar content (G) in normal mouse fecal microbiota fermentation systems over time (n = 3). (H and I) Bacterial density (H) and total sugar content (I) in UC mouse fecal microbiota fermentation systems over time (n = 3). (J and K) *In vivo* (J) and *ex vivo* (K) imaging of digestive tracts at different time points post oral administration of *Lp@AU* or *Lp@AU@GN* (n = 3). (L and M) Retention rates of *Lp@AU* or *Lp@AU@GN* within the gastrointestinal tracts at different time points (n = 3). Data are expressed as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, and **** $P < 0.0001$.

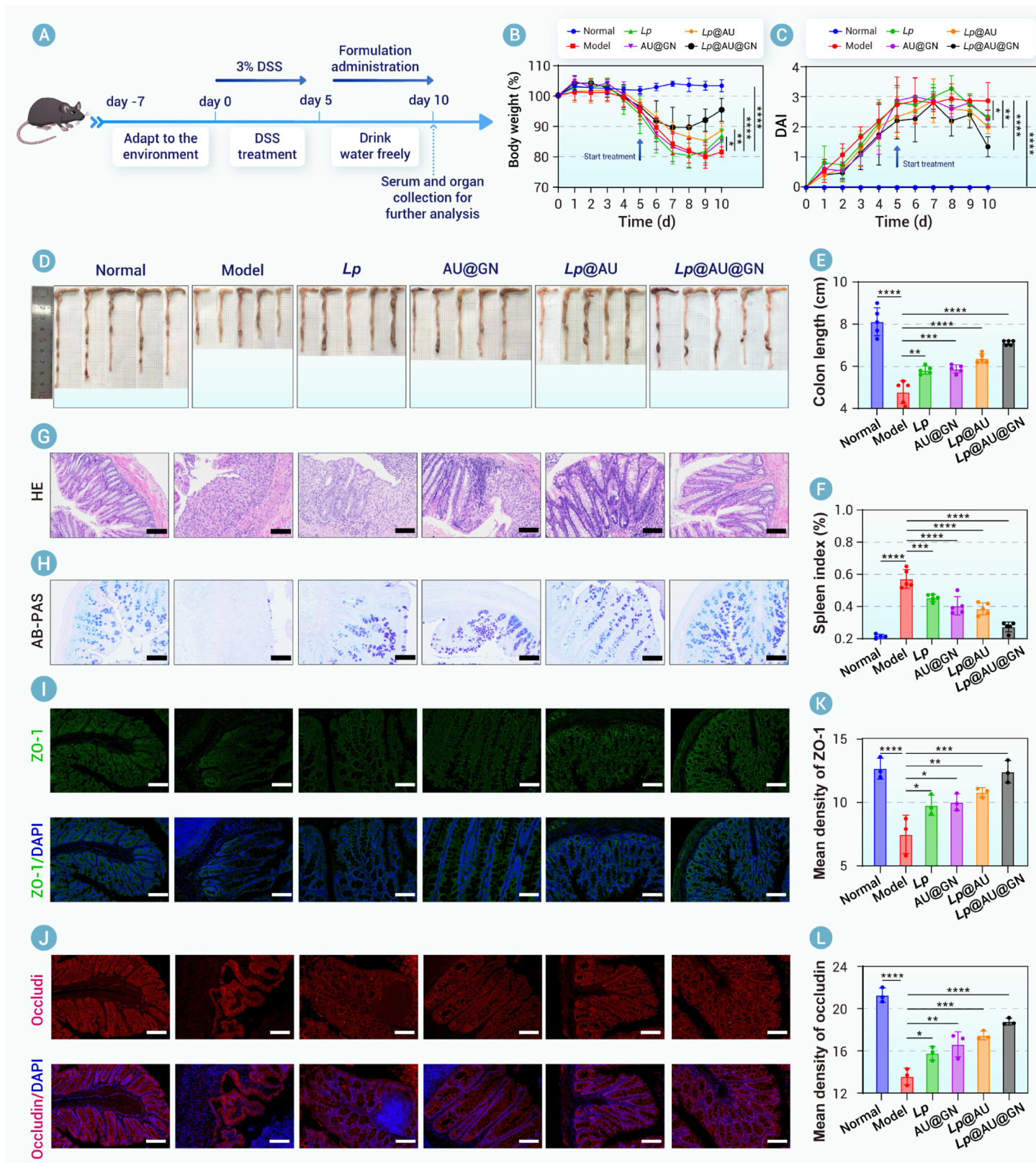


Figure 6. Therapeutic benefits of *Lp@AU@GN* in UC mice (A) Schematic of the therapeutic procedure for DSS-induced UC. (B) Body weight changes (n = 5). (C) DAI progression (n = 5). (D and E) Photos of the colons (D) and corresponding length quantification (E) (n = 5). (F) Spleen index (n = 5). (G) HE-stained colon sections. Scale bars, 100 μm. (H) AB-PAS-stained colon sections. Scale bars, 100 μm. (I and J) Immunofluorescence staining of tight junction protein (I) ZO-1 and (J) occludin in colonic tissues (n = 3). Scale bars, 100 μm. (K and L) Quantified immunofluorescence density of ZO-1 (K) and occludin (L) (n = 3). Data are expressed as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.

cacy of *Lp@AU* over *Lp* highlights the importance of protecting *Lp* from ROS. As anticipated, with the additional GN coating, *Lp@AU@GN* achieved the best therapeutic outcomes. Collectively, these findings emphasize the therapeutic

superiority of *Lp@AU@GN*, which capitalizes on the synergy among probiotic, prebiotic, and nanoenzyme components. Histological analysis of colon tissues by HE staining corroborated these findings, revealing that the

Lp@AU@GN group exhibited significantly reduced inflammatory cell infiltration, ameliorative crypt structures, and increased goblet cell numbers, which approached those observed in the normal group (Figure 6G). AB-PAS staining was performed to evaluate mucus secretion. The *Lp*, *AU@GN*, *Lp@AU*, and *Lp@AU@GN* groups all exhibited increased mucus production by varying degrees, with the *Lp@AU@GN* group still showing the most significant restoration (Figure 6H). The effect of *Lp@AU@GN* on tight junction proteins, including zonula occludens-1 (ZO-1) and occludin, was next assessed. Immunofluorescence staining revealed markedly reduced expression of ZO-1 (Figure 6I) and occludin (Figure 6J) in the model group. Treatment with *Lp@AU@GN* effectively restored the expression, comparable to the normal group (Figures 6K–L). Collectively, these results highlight the robust therapeutic efficacy of *Lp@AU@GN* in ameliorating UC.

Therapeutic mechanism of *Lp@AU@GN*

After validating the satisfactory therapeutic efficacy of *Lp@AU@GN*, we explored the underlying mechanism. Inflammation triggers oxidative stress, which in turn amplifies inflammatory responses, forming a vicious cycle.^{42,43} In this context, the variation of ROS levels in the colons after treatment was first assessed. DHE staining showed that ROS levels in the *Lp@AU@GN* group were significantly lower than those detected in the model group and the *Lp* group, approaching normal levels (Figures 7A & S25–S26). This observation underscores that the incorporation of AU in our design is crucial—not only to protect *Lp* from ROS but also directly neutralize oxidative stress. Concurrently, myeloperoxidase (MPO) concentration, a neutrophil-specific enzyme that catalyzes ROS production and plays a pivotal role in oxidative stress and inflammatory responses,⁴⁴ was notably reduced following *Lp@AU@GN* treatment, and this reduction was significantly greater than that observed in the *Lp* group (Figure 7B). As a result, compared to the model group, *Lp@AU@GN* treatment significantly suppressed pro-inflammatory cytokines, such as TNF- α (Figure 7C) and IL-6 (Figure 7D), while enhancing anti-inflammatory IL-10 (Figure 7E).

We next elucidated how *Lp@AU@GN* regulates UC via gut microbiota modulation. To investigate this, 16S rRNA gene sequencing was performed. The species accumulation boxplot (SAB) gradually flattened as the number of samples increased, suggesting that the sequencing depth was sufficient to reliably assess species richness (Figure S27). α -diversity analysis (Simpson's and Pielou's indices) revealed significantly increased species diversity in UC mice after *Lp@AU@GN* treatment (Figures 7F & S28). Principal coordinate analysis (PCoA, Figure 7G) and non-metric multidimensional scaling (NMDS, Figure S29) deciphered that *Lp@AU@GN* treatment notably shifted the β -diversity, bringing it closer to the normal group. At the phylum level, *Lp@AU@GN* treatment significantly increased the relative abundance of *Bacteroidota* (Figures 7H & S30). Specifically, the *Bacteroidota* play crucial roles in the digestion of complex carbohydrates, metabolizing them into essential nutrients, such as SCFAs, which provide energy to intestinal cells and help maintain the integrity of the mucosal barrier.^{45,46} Conversely, *Lp@AU@GN* treatment markedly reduced the relative abundance of *Proteobacteria* (Figures 7I & S29), whose overgrowth would compromise the intestinal barrier function and exacerbate inflammation.²⁶ At the genus level, a notable reduction in *Escherichia-Shigella*²⁴ was found after *Lp@AU@GN* intervention (Figures S31–S32). These findings were further validated through LDA effect size (LefSe) analysis (Figures 7J & S33). The model group displayed enrichment of harmful taxa (e.g., *Enterobacteriales*, *Enterobacteriaceae*, and *Gammaproteobacteria*), while the *Lp@AU@GN* group was dominated by beneficial taxa (e.g., *Bacteroidales*, *Muribaculaceae*, and *Bifidobacteriaceae*). In the LefSe pipeline, LDA was employed to identify microbial taxa with significant differences in abundance among different groups. The LDA score distribution (Figure S33) quantitatively confirmed the discriminative taxa. For instance, the model group was characterized by significantly elevated levels of *Gammaproteobacteria* and *Escherichia-Shigella*, while the *Lp@AU@GN* group showed marked enrichment of *Bacteroidales*, *Muribaculaceae*, and *Bifidobacteriaceae*. Collectively, these results demonstrate that *Lp@AU@GN* effectively re-balances gut microbiota homeostasis in UC mice.

SCFAs, primarily produced by gut microbiota through the fermentation of polysaccharide-based prebiotics in the intestine, play a crucial role in maintaining intestinal health. The effect of *Lp@AU@GN* treatment on SCFA

production was therefore investigated. *AU@GN* administration significantly increased the levels of major SCFAs in UC mice, including acetic acid (Figure 7K) ($P < 0.05$), propionic acid (Figure 7L) ($P < 0.001$), butyric acid (Figure 7M) ($P < 0.05$), isobutyric acid (Figure S34) ($P < 0.0001$), and valeric acid (Figure 7N) ($P < 0.0001$). These enhancements should be due to the prebiotic property of GN. Among these SCFAs, butyric acid is particularly noteworthy, as it serves as the primary energy source for colonic epithelial cells.⁴⁷ Propionic acid also plays a crucial role by modulating immune cell activity and maintaining immune homeostasis. As anticipated, most types of SCFAs' levels were higher in the *Lp@AU@GN* group compared to the *AU@GN* group (Figures 7K–N & S35). Such further SCFA production enhancement can be attributed to the increased abundance of certain beneficial bacteria responsible for polysaccharide metabolism, as identified above (Figures 7H, J, S30 & S33), regulated by *Lp@AU@GN* treatment. This likely facilitates the fermentation of polysaccharide-based prebiotics (e.g., GN) to promote SCFA production, highlighting the synergistic effects between GN and living *Lp* in *Lp@AU@GN* for managing UC.

To gain deeper insights, proteomic analysis was conducted on colonic tissues. Compared to the normal group, the model group exhibited 244 downregulated and 277 upregulated proteins (Figure 7O). In contrast, *Lp@AU@GN* treatment resulted in 182 upregulated and 245 downregulated proteins relative to the model group (Figure 7P). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that 17 signaling pathways were enriched among upregulated proteins, including the anti-inflammatory AMPK signaling pathway⁴⁹ (Figure 7Q). Conversely, 31 pathways were enriched among downregulated proteins (Figure 7R), including the pro-inflammatory PI3K-Akt signaling and ECM-receptor interaction pathways.^{50–51} In conclusion, by harnessing the synergistic effects of probiotic, prebiotic, and nanoenzyme, *Lp@AU@GN* effectively ameliorate gut microbiota dysbiosis and enhance SCFA production, thereby modulating the colonic protein profile toward a state favorable for the repair of UC-induced damage.

Therapeutic efficacy of *Lp@AU@GN* against TNBS-induced CD in mice

The therapeutic efficacy of *Lp@AU@GN* was subsequently evaluated in CD, another major IBD subtype. After 1% TNBS administration for pre-sensitization, mice underwent an enema with 3% TNBS to establish CD model (Figure 8A).⁵² CD mice were orally administered PBS (model group) or *Lp@AU@GN* (*Lp@AU@GN* group), and normal mice administered PBS were denoted as the normal group. As illustrated in Figure 8B, the model group exhibited remarkable body weight reduction after TNBS treatment compared to normal group, which continued to decline during the subsequent PBS gavage. *Lp@AU@GN* reversed the weight loss and gradually recovered it to the normal statue. The therapeutic effect of *Lp@AU@GN* was further reflected by the relief of colon shortening (Figures 8C–D). Histological analysis via HE staining verified the therapeutic benefits of *Lp@AU@GN*, showing restored tissue architecture that approached that observed in the normal group (Figure 8E). AB-PAS staining disclosed that TNBS-induced CD severely disrupted mucus expression, while the *Lp@AU@GN* treatment markedly enhanced it (Figure 8F), beneficial for the intestinal homeostasis. ROS levels⁵³ were next profiled. While the model group showed excessive ROS accumulation, *Lp@AU@GN* treatment effectively normalized ROS levels (Figures 8G & S36). Correspondingly, *Lp@AU@GN* intervention significantly lowered MPO concentration (Figure 8H), reduced pro-inflammatory cytokine levels (i.e., TNF- α and IL-6; Figures 8I–J), and upregulated IL-10 (Figure 8K). To conclude, *Lp@AU@GN* demonstrates the broadly effective therapeutic benefits in IBD.

DISCUSSION

IBD is driven by oxidative stress, immune dysregulation, and gut microbiota imbalance.^{54–55} This underscores the need for holistic therapeutic strategies capable of simultaneously modulating immune responses, restoring microbial homeostasis, and mitigating oxidative stress. Probiotics, particularly immunomodulatory strains like *Lp*, offer a promising alternative by modulating gut microbiota, enhancing intestinal barrier function, and regulating host immunity.^{56–58} However, a limitation is the significant loss of bacterial viability during gastrointestinal transit. Moreover, we found that *Lp* viability was also compromised under oxidative stress, a hallmark encountered after oral administration to IBD patients, thus limiting its therapeutic potential.

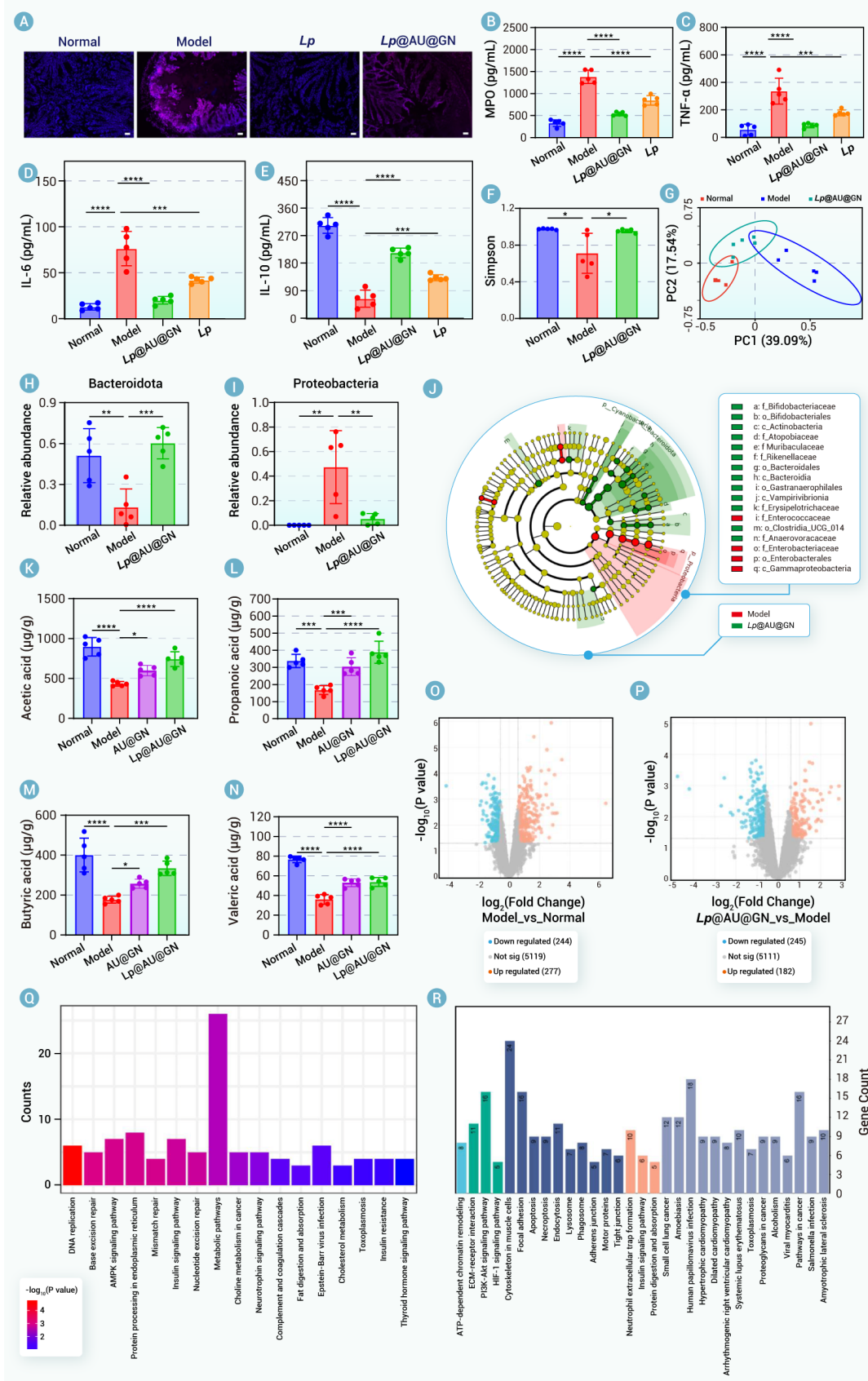


Figure 7. Mechanism of *Lp@AU@GN* in treating UC (A) Representative merged fluorescence images reflecting the ROS levels in colon tissues from each group (n = 3). ROS staining using DHE is shown in red. Nuclei are counterstained with DAPI (blue). (B) MPO concentration (n = 5). (C-E) Cytokine concentrations determined by ELISA (n = 5). (F) α-diversity of the gut microbiota based on Simpson (n = 5). (G) β-diversity analysis via PCoA (n = 5). (H and I) Relative abundance of *Bacteroidota* (H) and *Proteobacteria* (I) (n = 5). (J) Taxonomic markers identified by LEfSe analysis (LDA score > 3, $P < 0.05$) (n = 5). (K-N) Levels of SCFAs in colon contents: acetic acid (K), propionic acid (L), butyric acid (M), and valeric acid (N) (n = 5). (O and P) Volcano plot, which presents the differentially expressed genes in the comparison set of the normal group versus the model group (O) and the *Lp@AU@GN* group versus the model group (P) ($P < 0.05$; Fold change > 1.5) (n = 4). (Q and R) KEGG enrichment analysis of upregulated proteins (Q) or downregulated proteins (R) between the *Lp@AU@GN* and model groups (n = 4). Data are expressed as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

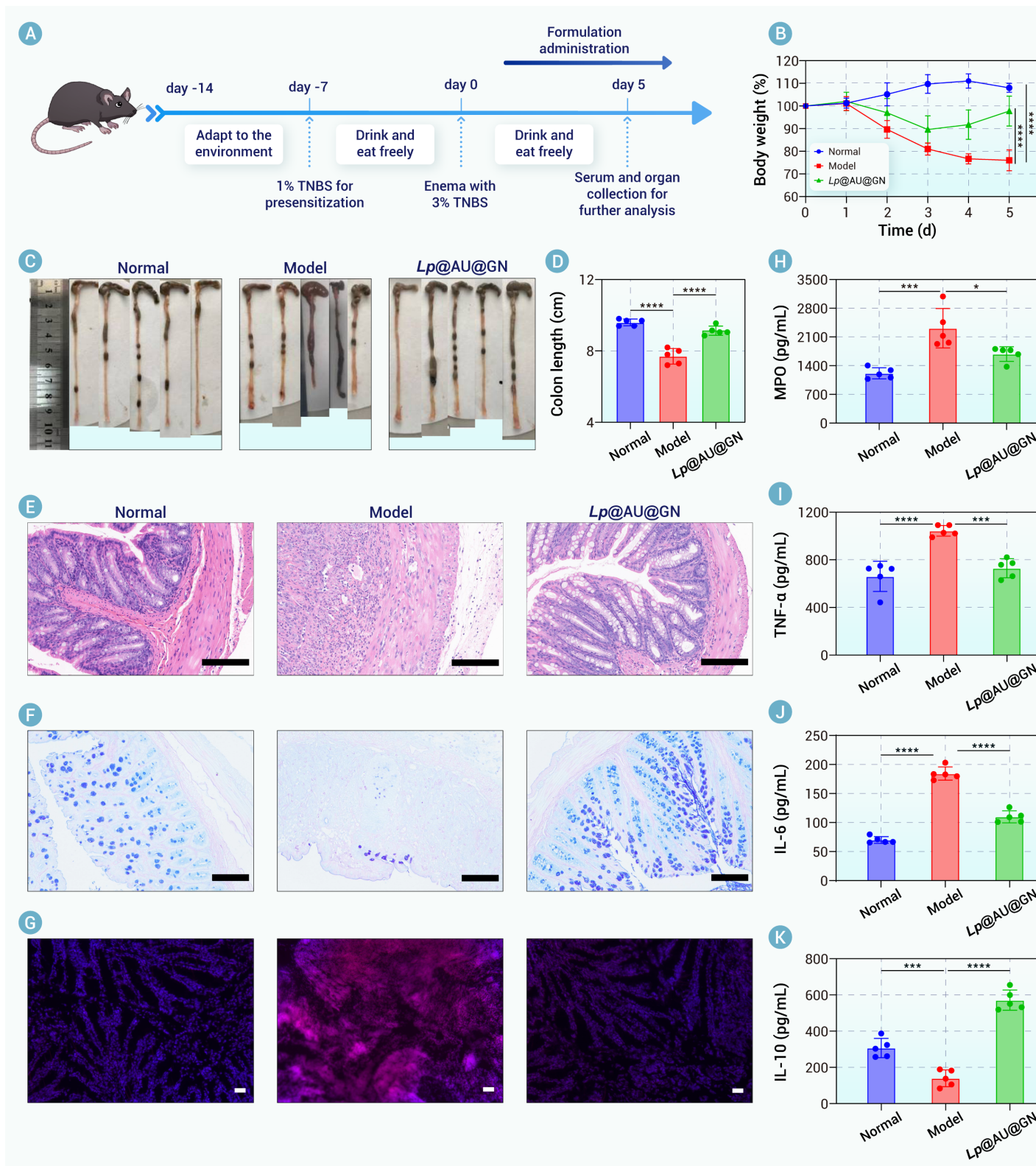


Figure 8. Efficacy of *Lp@AU@GN* in TNBS-induced CD model (A) Schematic of the therapeutic procedure. (B) Body weight changes in mice over time (n = 5). (C and D) Colon photos (C) and corresponding length (D) (n = 5). (E) HE-stained intestinal sections. Scale bars, 100 μm. (F) AB-PAS-stained intestinal sections. Scale bars, 100 μm. (G) Representative images representing ROS levels in intestinal tissues (DHE, red; DAPI, blue) (n = 3). Scale bars, 20 μm. (H) MPO concentration (n = 5). (I–K) Cytokine concentrations determined by ELISA (n = 5). Data are expressed as mean ± SD. **P* < 0.05, ****P* < 0.001, and *****P* < 0.0001.

To address these limitations, we rationally designed a probiotic-based biohybrid (*Lp@AU@GN*). This biohybrid was bio-orthogonally fabricated, featuring ROS-scavenging nanoenzymes (AU) anchored to *Lp* and a covalently attached polysaccharide-based prebiotic shield (i.e., GN). Each component plays a distinct yet complementary role. Specifically, after oral adminis-

tration, GN shielded *Lp* from harsh gastrointestinal insults. Upon reaching the intestine, GN was selectively metabolized by the gut microbiota into SCFAs (acetic, propionic, butyric, and valeric acids), which not only contributed to combating IBD but also triggered the targeted release of *Lp@AU*. This targeted release is a key advantage, ensuring that therapeutic effects are

maximized at inflamed sites while minimizing off-target exposure. In this context, AU-mediated ROS scavenging played a role in protecting both host intestines and probiotics from oxidative damage, thereby improving the *Lp* viability and amplifying its therapeutic action.

In vivo evaluation in a DSS-induced UC mouse model confirmed the superior performance of *Lp*@AU@GN. Oral administration showed superiority in attenuating body weight loss, reducing DAI, restoring colon length, normalizing spleen index, and restoring epithelial barrier integrity over other formulations (*Lp*, AU@GN, or *Lp*@AU). This comprehensive validation directly demonstrates that integration yields a synergistic effect greater than the simple sum of its parts, a claim that is often missing in conventional studies.

Mechanistically, *Lp*@AU@GN reduced ROS levels, suppressed pro-inflammatory cytokine, and upregulated the anti-inflammatory cytokine. Importantly, it restored gut microbiota balance. *Bacteroidales*-associated bacteria, involved in polysaccharide metabolism, were particularly upregulated, supporting GN incorporation and indicating metabolic benefits. These findings led to elevated SCFA levels in treated UC mice, further advancing therapy. The ability to concurrently modulate oxidative stress, immune dysfunction, and microbial dysbiosis sets it apart from previous interventions focusing on one or two of these aspects.

Given the shared pathophysiological features of UC and CD, we hypothesized our biohybrid would be effective in CD. This was validated in a TNBS-induced CD mouse model. The validation of *Lp*@AU@GN in both DSS-induced UC and TNBS-induced CD models underscores its broad-spectrum applicability across major IBD subtypes, offering a potential paradigm shift from subtype-specific treatments.

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AUTHOR CONTRIBUTIONS

All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

ETHICAL STATEMENT AND PATIENT CONSENT

GemPharmatech Co., Ltd. (Nanjing, China) provided SPF-grade male C57BL/6J mice (20 + 0.2 g, age 6–8 weeks). We housed animals at controlled ambient conditions (23 + 3°C, 55 + 10% relative humidity, 12/12 h light-dark). The animal experiments were approved by the Institutional Animal Care and Use Committee of Nanchang University (SYXK(Gan)2021-0004).

DATA AND CODE AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article and its supplemental information. The 16S rRNA data generated in this study were deposited in the NCBI SRA database (<https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1345163>, accession code PRJNA1345163).

SUPPLEMENTAL INFORMATION

It can be found online at <https://doi.org/10.59717/j.xinn-mater.2026.100203>.