Supplementary Information

Reactive Oxygen Species-Responsive Prodrug Nanomicelle-Functionalized *Lactobacillus Rhamnosus* Probiotics for Amplified Therapy of Ulcerative Colitis

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Experimental Section

Materials. All chemicals utilized in this study were of analytical grade, requiring no additional treatment-Hydroxysuccinimide (EDC), and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydr-ochloride (NHS) were purchased from Rhawn (Shanghai, China). 18β-Glycyrrhetinic acid (18β-GA), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 4-Morpholineethanesulfonic acid (MES), Fluorescein isothiocyanate isomer I (FITC), Rhodamine B (Rh-B) and Cyanine5.5 (Cy5.5) were all purchased from Aladdin (Shanghai, China). Man–Rogosa–Sharpe (MRS) broth and agar were purchased from hopebio (Qingdao, China). Sodium alginate (SA, 20 ~ 50 kDa) and bile salt from pig were purchased from Macklin (Shanghai, China). Chitosan oligosaccharide (COS, MW＜3000) and non-fat powdered milk were purchased from yuanye Bio-Technology, China. High glucose Dulbecco′s modified Eagle′s medium (DMEM) was obtained from Servicebio (Wuhan, China).

Synthesis and characterization of thioketal linker.

The ROS-cleavable thioketal linker (TK) was synthesized according to the previously reported method.[1] Briefly, 5 g cysteamine hydrochloride was dissolved in 40 mL hydrochloric acid at room temperature in dark, Then, acetone (100 mL) and dichloromethane (40 mL) were added into the mixture at 0 ℃. After 12 h of TK reaction, the reaction solution was filtered and washed by methylene chloride. The filter residue was re-dissolved in methanol, precipitated with ether and recrystallized at -20 ℃. After recrystallization, a white solid product was obtained by filtration. The product was dispersed evenly with an appropriate amount of dichloromethane, and the pH of the mixture was adjusted to above 10 with 5 M sodium hydroxide solution. The mixture was vigorously stirred at room temperature for 3 h and extracted three times with saturated saline solution. The lower layer liquid was collected. Subsequently, the purified product thioketal linker (TK) was collected by spin distillation. The chemical structure of TK

was characterized by ^{1}H Nuclear Magnetic Resonance spectroscopy (^{1}H NMR, JNM-ECZ600R/S1, JEOL, Japan) and Fourier transform infrared spectroscopy (FTIR, Nicolet iS50, Thermo Scientific, USA).

Synthesis and characterization of TK-grafted sodium alginate (SA-TK).

The amide reaction was based on previously reported methods.[2] Sodium alginate (SA) (1 g) was dissolved in 100 mL of 4-morpholineethanesulfonic acid buffer solution (MES, 0.1 M, pH 5). EDC (1 g) and NHS (1 g) were added to the above solution under nitrogen and stirred for 30 min before adding TK (500 mg). The pH of the solution was then adjusted to 5.0-5.5 and stirred overnight. The mixture was dialyzed (molecular weight cutoff 3500 Da) with purified water for 3 days and lyophilized to obtain SA-TK. The final product was characterized by ^{1}H NMR (JNM-ECZ600R/S1) and FTIR (Nicolet iS50).

Synthesis and characterization of SA-TK-GA (STG) conjugate.

The STG conjugate was synthesized according to the previously reported method.[3] 50 mg 18β-GA was dissolved in N,N-Dimethylformamide, then EDC (50 mg) and NHS (50 mg) were added to the above solution under nitrogen and stirred for 30 min. An aqueous solution of SA-TK (400 mg) was added dropwise at 0 ℃ to the above solution. The pH of the reaction solution was then adjusted to 5.0-5.5 by HCl and stirred overnight. The mixture was dialyzed (molecular weight cutoff 3500 Da) with purified water for 3 days and lyophilized to obtain SA-TK-GA polymeric prodrug. The final product was characterized by ^{1}H NMR (JNM-ECZ600R/S1) and FTIR (Nicolet iS50).

Preparation and characterization of STG nanomicelles.

STG nanomicelles were prepared by dispersing SA-TK-GA conjugates in pure water and

sonicated in an ice bath for 30 min. The size and Zeta potential of STG nanomicelles were measured by Malvern Zetasizer Nano ZS (ZEN3700, Malvern, England). The morphology of STG nanomicelles was observed by transmission electron microscopy (TEM, JEM-1200EX, JEOL, Japan).

Nanomicelles stability.

The stability of STG nanomicelles in aqueous media was investigated by uniformly dispersing the STG nanomicelles in PBS and storing them in a refrigerator at 4 ℃. The particle size and polydispersity indices (PDI) of STG nanomicelles were examined by Malvern Zetasizer Nano ZS (ZEN3700) for each day of a week of storage.

ROS-triggered drug release.

In vitro drug release behavior of STG nanomicelles was determined by dialysis. First, 18β-GA solutions were prepared according to different concentrations. Under the same conditions, the absorbance of 18β-GA solution was measured separately. The absorbance was analyzed by linear regression with concentration to obtain the standard curve. Then, STG nanomicelles was placed in a dialysis bag with a molecular weight cut-off of 3500 Da and then immersed in a mixture of equal volumes of PBS (pH 7.4) and ethanol (with or without H_2O_2), Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) in a 37 ℃ shaker (HNY-200F, honour, China). The released solution was collected at 0.5 h, 1 h, 2 h, 4 h, 8 h, 10 h, 12 h, 24 h and 48 h, and the absorbance of the release solution at 250 nm was detected by ultraviolet spectrometer (UV, U-3900, Hitachi, Japan). and the amount of 18β-GA released from the STG nanomicelles was determined by the standard curve method.

Anti-oxidative property of STG nanomicelles.

The scavenging efficiency of •OH by STG nanomicelles was evaluated. In brief, 4 mM $FeSO_4$, 100 mM H_2O_2, 4 mM salicylic acid and different concentrations of STG nanomicelles (100, 200, 300, 400, 600 μg mL^{-1}) were prepared. 1 mL $FeSO_4$ and 1 mL H_2O_2 were mixed well, and the supernatant was centrifuged after ten minutes incubation. A certain amount of STG nanomicelles was added to the supernatant and incubated at 37 ℃ for 30 min, and then 1 mL of salicylic acid was added, and the scavenging rate was calculated by measuring the absorbance at 510 nm by a microplate reader (Spark, TECAN, Switzerland). The hydroxyl radical scavenging rate was calculated by the following formula:

$$\bullet OH\ scavenging\ rate\ \% = (A_a - A_b)/A_a \times 100\% \quad \#(1)$$

Where, A_a and A_b are blank absorbance and STG nanoparticle absorbance, respectively.

The scavenging efficiency of DPPH by STG nanomicelles was evaluated. 0.1 mM ethanol solution of DPPH and different concentrations of STG nanoparticle (2, 4, 6, 8, 10 mg mL^{-1}) were prepared. 1 mL DPPH solution and 0.3 mL STG nanomicelles were mixed and incubated for 30 min away from light, and the scavenging rate was calculated by measuring the absorbance at 517 nm by a microplate reader (Spark). The DPPH scavenging rate were calculated by the following formula:

$$DPPH\ scavenging\ rate\ \% = (A_a - A_b)/A_a \times 100\% \quad \#\ (2)$$

Where, A_a and A_b are blank absorbance and STG nanoparticle absorbance, respectively.

Cell culture.

L929 fibroblasts (L929) and mouse mononuclear macrophage leukemia cells (RAW 264.7) were purchased from the Chinese Academy of Sciences (Shanghai, China). L929 and RAW 264.7 were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM, Servicebio) supplemented with 10% fetal bovine serum (FBS, Hyclone) and 1% penicillin−streptomycin

(Servicebio) in a humidified incubator containing 5% CO_2 at 37 ℃.

For cell counting kit-8 (CCK-8) assay, a certain amount of STG conjugate was first weighed and sterilized, and STG conjugate was dispersed into Dulbecco's Modified Eagle Medium (DMEM, Servicebio) to nanomicelles with a concentration of 1 mg mL^{-1}. Before cell culture, the STG nanoparticle dispersion was diluted with DMEM medium to obtain various concentrations of 50, 100, 200, 300, and 400 μg mL^{-1}. A total of 5×10^3 L929 cells were seeded in 96-well plates with 100 μL DMEM complete medium per well. After 24 h of incubation, the DMEM contained STG nanomicelles were added and co-cultured for another 24 h. The CCK-8 (MedChemExpress, USA) assay was used to quantify cell viability after different treatments, and a microplate reader (Multiskan FC, Thermo Scientific, USA) was used to measure the absorbance at 450 nm. The cell survival rate was calculated by the formula of cell survival rate and plotted on the graph.

$$Cell\ survival\ rate = \left[(A_s - A_b)/(A_c - A_b)\right] \times 100\% \quad (3)$$

Where, A_s is the absorbance of the experimental wells, A_c is the absorbance of the control group (the absorbance of the wells without drug and containing cells), and A_b is the absorbance of the blank group (the absorbance of the wells containing only CCK-8 and DMEM).

For the live and dead staining assay, L929 cells (2×10^5 cells per well) were seeded into glass bottom cell culture dish overnight and treated with STG nanomicelles (300 μg mL^{-1}) in DMEM for another 24 h. The cells were then rinsed with PBS, stained with Calcein AM (2 μL) and PI (4 μL), and observed under a CLSM (CLSM, TCS SP8 STED 3X, Leica Microsystems).

***In vitro* ROS scavenging ability of STG nanomicelles.**

ROS scavenging ability was assessed using 2,7-dichlorofluorescein diacetate (DCFH-DA,

Solarbio, China). Firstly, 1×10^6 RAW 264.7 cells were seeded into glass bottom cell culture dish with 1 mL of DMEM complete medium per well for 12 h and then 1 mL DMEM medium containing 10 μg mL^{-1} lipopolysaccharide (LPS, Solarbio, China) was added to the cells, and the cells were incubated. After 12 h of culturing, the medium was removed, and 1 mL DMEM cell culture dispersed SA-TK (300 μg mL^{-1}) and STG nanomicelles (300 μg mL^{-1}) containing 0.1 mM H_2O_2 were added, respectively, and incubated for 8 h. Finally, cells were washed and incubated with DCFH-DA at 37 ℃ for 30 min with light protection and observed by a CLSM (TCS SP8 STED 3X).

***In vitro* anti-inflammatory ability of STG nanomicelles.**

To assess the anti-inflammatory activity of STG nanomicelles, Firstly, 80×10^5 RAW 264.7 cells were seeded into glass bottom cell culture dish with 1 mL of DMEM complete medium per well for 12 h. Then 1 mL DMEM medium containing 4 μg mL^{-1} LPS was added into the dish, and the cells were incubated for another 24 h to induce macrophage polarization from the M0 type to the pro-inflammatory M1 type. After removing medium, 1 mL DMEM containing SA-TK (300 μg mL^{-1}) and STG (300 μg mL^{-1}) were added, respectively, and incubated for 24 h. The expression level of pro-inflammatory factors (IL-1β) was detected using an enzyme-linked immunosorbent assay kit (Servicebio, China).

For immunocytochemistry, cells were first fixed with 4% paraformaldehyde for 15 min. Secondly, cells were permeabilized cells with 0.2 % (v/v) Triton X-100 for 15 min and blocked non-specific binding with 10% BSA solution. Thirdly, the cells were incubated with iNOS Rabbit pAB (1:100, Yeasen Biotechnology, China) at 4 ℃ for 12 h. After incubation with primary antibody, cells were washed with PBS and mixed with FITC-labeled goat anti-rabbit lgG (H+L) (1:100, Yeasen Biotechnology, China) and incubated for 4 h at room temperature.

Finally, the cell nucleus was stained with 4',6-diamidino-2-phenylindole (DAPI), and cells were washed three times with PBS for observation under CLSM (TCS SP8 STED 3X).

For cell flow cytometry analysis, cells in different treated groups were collected. Then the cells were incubated with CD86 (1:200, biolegend, China) and CD206 (1:200, biolegend, China) at 4 ℃ for 12 h. Next, the cells were washed three times with PBS, centrifuged, and resuspended in 300 μL of PBS. Finally, the cells were analyzed by a flow cytometry analyzer (Gallios, Beckman Coulter, USA).

Preparation of STG-CS@LGG.

The strains used in this study were *Lactobacillus rhamnosus* (LGG). LGG were purchased from Ningbo Testo Biotechnology Co., Ltd. At the beginning of each experiment, LGG were cultured in liquid MRS broth overnight at the shaking speed of 150 rpm at 37 ℃.

LGG were cultured in MRS medium at 37 ℃ for 12 h with shaking at 150 rpm, washed three times with phosphate-buffered saline (PBS, pH 7.4) and then obtained by centrifugation (6000 rpm, 5 min). First, LGG (1×10^9 CFU) were resuspended in sterile deionized water (1 mL) containing $CaCl_2$ (27.8 mM) and vortexed for 20 min, then SA (20.0 mg mL^{-1}, 2 mL) was added and shaken for 5 min, then obtained by centrifugation (6000 rpm, 5 min). S-LGG was obtained by adjusting the pH to 3.0 with HCl (0.1 M) and the strains were collected by centrifugation (6000 rpm, 5 min) and then washed three times with PBS (pH 3.0), centrifuged. CS-LGG was then resuspended in chitosan oligosaccharides (COS) solution for 30 min and centrifuged (6000 rpm, 5 min) to obtain CS-LGG. Finally, CS-LGG was then resuspended in STG nanomicelles dispersion for 30 min and centrifuged (6000 rpm, 5 min) to obtain STG-CS@LGG.

Characterizations of STG-CS@LGG.

The Zeta potentials of LGG, S@LGG, CS@LGG and STG-CS@LGG were measured by a Malvern Zetasizer Nano ZS (ZEN3700). To characterize the morphology, LGG and STG-CS@LGG were fixed with 2.5 % glutaraldehyde at 4 ℃ for 2 h. LGG and STG-CS@LGG were then dehydrated with a gradient ethanol series. Next, the LGG and STG-CS@LGG were dropped onto copper sheets air-dried and sprayed with gold, and the morphology of the LGG and STG-CS@LGG was determined by a scanning electron microscopy (SEM, VEGA3, TESCAN, China). The surface coating of LGG was observed by a CLSM (TCS SP8 STED 3X). To visualize the STG-CS@LGG under CLSM, Fluorescein isothiocyanate isomer I (FITC) was loaded into STG nanomicelles, and Rhodamine B (Rh-B) was grafted on COS.

The growth curve of STG-CS@LGG.

To detect the viability of STG-CS@LGG after coating, LGG and STG-CS@LGG were diluted with MRS medium to an optical density (OD600) at 0.2 and incubated in a 37 ℃ incubator with shaking at 150 rpm. The growth curves of LGG and STG-CS@LGG were measured at 37 ℃ every 2 h for 12 h with a microplate reader (Spark).

ROS scavenging ability of STG-CS@LGG in vitro.

The effect of coated probiotics on the scavenging of ROS (•OH) was investigated. Firstly, •OH produced by the Fenton reaction was detected with salicylic acid, which can be oxidized by •OH and produces a characteristic absorption peak at 510 nm. 1 mL $FeSO_4$ and 1 mL H_2O_2 were mixed well, and the supernatant was centrifuged after ten minutes incubation. A certain amount of LGG and STG-CS@LGG (1×10^8, 2×10^8, 3×10^8, 4×10^8 CFU) were added into the supernatant and incubated at 37 ℃ for 30 min, and then 1 mL of salicylic acid was added.

The scavenging rate was calculated by measuring the absorbance at 510 nm by a microplate reader (Spark). The hydroxyl radical scavenging rate was calculated by the following formula:

$$\bullet OH\ scavenging\ rate\ \% = (A_a - A_b)/A_a \times 100\% \quad (4)$$

Where A_a is blank absorbance, A_b are LGG and STG-CS@LGG absorbance, respectively.

External environment resistance assay for STG-CS@LGG.

The protective effect of the coatings on LGG was determined under simulated gastrointestinal conditions, including SGF with pepsin addition (pH 2.0), SIF with trypsin addition (pH 6.8), and bile salts (0.4%). Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared as described in the United States Pharmacopoeia. Briefly, SGF was prepared by dissolving 2.0 g of NaCl and 3.2 g of pepsin in 1 L of DI water, and the pH was adjusted to 2.0 with HCl. SIF was prepared by dissolving 6.8 g of KH_2PO_4 and 10 g of trypsin in 1 L of DI water, and the pH was adjusted to 6.8 with NaOH. Bile salt was prepared by dissolving 0.4 g of bile salt from pig in 100 mL of DI water. For resistance assay, equal amounts of LGG and STG-CS@LGG (1×10^9 CFU) were placed in various simulated liquids (SGF, SIF and Bile salt) and incubated at 37 ℃ at an oscillation rate of 150 rpm. At a predetermined time point, 100 μL of the samples were washed with PBS and spread on MRS agar plates at 10-fold dilution. Colonies were counted after 48 h of incubation at 37 ℃.

Meanwhile, equal amounts of LGG and STG-CS@LGG (1×10^9 CFU) were continuously cultured in SGF and Bile salt, respectively, to better simulate the harsh environment of the gastrointestinal tract. In brief, 1×10^9 CFU of LGG and STG-CS@LGG was resuspended in 1 mL of SGF following the same procedures. After 1 h of incubation, the supernatant was centrifuged at 6000 rpm for 5 min and resuspended in 1 mL of Bile salt. After 2 h of incubation,

100 μL of the samples were washed with PBS and spread on MRS agar plates in 10-fold dilution order. After another 48 h of incubation at 37 ℃, colonies were counted.

In addition, equal amounts of LGG and STG-CS@LGG (1×10^9 CFU) were resuspended in 1 mL of H_2O_2 (500 μM or 1 mM) and incubated at 37 ℃ with an oscillation rate of 150 rpm. At a predetermined time point, 100 μL of the sample was washed with PBS and spread on MRS agar plates in sequential 10-fold dilution order, and the colonies were counted after incubation at 37 ℃ for 48 h.

Stability of STG-CS@LGG in gastrointestinal tract.

To systematically evaluate the stability of STG-CS@LGG in the gastrointestinal tract and validate its ROS-responsive drug release in the inflamed colon, we conducted the following experiments:

(1) Stability validation in simulated gastrointestinal fluids: FITC-labeled STG nanomicelles (green) and RhB-labeled chitosan (red) were employed to enable dual-channel visualization under CLSM. Then the dual-labeled STG-CS@LGG (1×10^9 CFU) was sequentially incubated in SGF (1 mL, pH 2.0) for 1 h and in SIF (1 mL, pH 6.8) for 2 h to mimic physiological conditions. Finally, the sample was dropped on the central area of the slide, slowly covered with a coverslip to avoid air bubbles, and observed by a CLSM (TCS SP8 STED 3X).

(2) ROS-triggered disassembly in inflamed colon mimicking environments: STG-CS@LGG (1×10^9 CFU) was incubated in SIF containing 1 mM H_2O_2 (to simulate oxidative colitis lesions) for 6 h. Finally, the sample was dropped on the central area of the slide, slowly covered with a coverslip to avoid air bubbles, and observed by a CLSM (TCS SP8 STED 3X).

Storage Stability of STG-CS@LGG.

To verify the stability of STG-CS@LGG, STG-CS@LGG was mixed with 20% (w/v) skim milk. Then the bacteria suspension was pre-frozen at -80°C for 2 h, followed by 38 h freeze-drying to obtain the lyophilized powder of probiotics. The lyophilized powder was stored at 4°C under vacuum for 7 days. The lyophilized powder was rehydrated with saline (0.9%, w/v) at original volume. The number of viable bacteria before and after lyophilization was calculated by the dilution-coated plate method. The strain survival rates were calculated by the following formula:

$$Strain\ survival\ rate\ \% = V\ after\ lyophilization/V\ before\ lyophil$$

Where, V after lyophilization indicates the number of viable bacteria after lyophilization, and V before lyophilization indicates the number of viable bacteria before lyophilization.

Gastrointestinal retention ability of STG-CS@LGG in vivo.

All animal experimental protocols were approved by the School of Medicine and Pharmacy, Ocean University of China Animal Laboratory Animal Ethics Committee (OUC-SMP-2023-09-10). C57BL/6 mice (female, 7 weeks old) were purchased from Jinan Pengyue Laboratory Animal Breeding Co. To observe the distribution of STG-CS@LGG in the intestinal tract, Cy5.5 was used to label STG nanomicelles to construct Cy5.5-labeled STG-CS@LGG. After 12 h of starvation in mice, mice in the STG nanomicelles treated group were orally administered with 0.2 mL of Cy5.5-labeled STG nanomicelles (10 mg mL^{-1}) and mice in the STG-CS@LGG treated group were orally administered with STG-CS@LGG (10^8 CFU/mL bacteria with 10 mg mL^{-1} STG nanomicelles). Whole-body fluorescence intensity was measured under an In Vivo Imaging System (IVIS, IVScope 8200, Clinx, China) at predefined intervals (3, 6, 12, 24, and 36 h) using standardized ROIs. After the last assay, all mice were

euthanized, and major organs (heart, liver, spleen, lungs, kidneys and digestive tract) were collected and imaged.

DSS-induced ulcerative colitis model in mice.

The ulcerative colitis model was established based on the previously reported method.[4] Briefly, 7-week-old female C57BL/6 mice were randomly divided into five groups, including normal, DSS, STG, LGG, and STG-CS@LGG (n = 3 for each group). The mice in different groups were treated as follows: (1) normal: mice in the normal group were treated only with tap water; (2) DSS: mice in the DSS group were treated with 3% DSS in the tap water from day 0 to day 7 and gavage with PBS from day 2 to day 9; (3) STG nanomicelles: mice were treated with 3% DSS in the tap water from day 0 to day 7 and gavage with STG nanomicelles (300 mg/kg)/mouse/day from day 2 to day 9; (4) LGG: mice were treated with 3% DSS in the tap water from day 0 to day 7 and gavage with LGG (1×10^8 CFU)/mouse/day from day 2 to day 9; (5) STG-CS@LGG: mice were treated with 3% DSS in the tap water from day 0 to day 7 and gavage with STG-CS@LGG (1×10^8 CFU)/mouse/day from day 2 to day 9. All mice were euthanized on day 9. After treatment, the body weight, stool consistency, and fecal blood were recorded for each mouse from day 0 to day 9. The mice were dissected, and the colon was collected, and the length of the colon was measured with a straightedge.

For behavioral experiments, 7-week-old female C57BL/6 mice were randomly divided into three groups (n = 5 for each group), including Normal, DSS and STG-CS@LGG. The mice were modeled and treated according to the above steps, and the mice were assessed behaviorally on day 9 of the behavioral experiment.

Evaluation of efficacy in ulcerative colitis.

On day 9, the heart, liver, spleen, lung and kidney of mice in all groups were collected and fixed with paraformaldehyde. After paraffin embedding, the tissues were sectioned and subjected to Hematoxylin and eosin (H&E), Interleukin-1β (IL-1β), and Interleukin-6 (IL-6) staining according to standard protocols. The in vivo biocompatibility of STG-CS@LGG was determined by the results of H&E staining of heart, liver, spleen, lungs, and kidneys. The colon tissue was fixed in 4% paraformaldehyde solution, embedded in paraffin, and sectioned for H&E according to the manufacturer's instructions. Then the paraffin sections of colon tissue were deparaffinized and then used for immunofluorescence detection of ZO-1 and Occludin expression. To detect the expression of ZO-1 and Occludin, immunofluorescence staining was performed on colon sections, which were first stained for ZO-1, incubated with diluted primary antibody (ZO-1, GB115686, 1:5000) overnight at 4 ℃, and then incubated for 50 min at room temperature with HRP-labeled goat anti-rabbit IgG secondary antibody (1:5000, Servicebio, China) for 50 min at room temperature. Slides were rinsed three times with PBS and stained with Occludin, incubated with diluted primary antibody (1:5000, Servicebio, China) overnight at 4 ℃, followed by incubation with HRP-labeled goat-anti-rabbit IgG secondary antibody (1:500, Servicebio, China) for 50 min at room temperature, and finally then incubated with HRP-labeled goat anti-rabbit IgG secondary antibody (1:500, Servicebio, China) for 50 min at room temperature. Finally, DAPI was added dropwise to the sections and observed under a fluorescence microscope.

Modulation of anxiety, depression-like behaviors in UC mice.

Tail suspension test: The tail suspension test was used to evaluate depression-like behavior. The mice were suspended by their tails and then secured with tape, and the distance between the mice and the ground was about 25 cm. The test was conducted for 6 min and the resting time of the mice was calculated.

Light/dark box test: Light and dark box tests are commonly used to study the preference or anxiety level of experimental animals for light and dark environments. Mice are allowed to acclimatize in the light and dark box for approximately 30 min prior to the experiment. The mice are gently placed in the bright area of the light and dark box during the experiment. Video recordings were used to capture the mice's behavior over a 10 min period. After stopping the timer, the mice were gently removed from the box and returned to their original cages, and the light/dark boxes were promptly cleaned and disinfected after each mouse was tested.

On day 9, the brain tissues of mice in different treated groups were collected, fixed in 4% paraformaldehyde solution, embedded in paraffin, and sectioned. Subsequently, to detect Iba1 expression, brain sections were immunofluorescent stained with diluted primary antibody (IBA-1, GB113502, 1:500) incubated overnight at 4 ℃, followed by incubation with CY3 labeled goat anti-rabbit IgG secondary antibody (GB21303, 1:300) for 50 min at room temperature. Finally, the sections were incubated with DAPI for 10 min at room temperature, and then observed under a fluorescence microscope.

Gut microbiota 16S rRNA sequencing assay.

After treatment of the ulcerative colitis mouse model, two to three fecal pellets were collected from each mouse and were sent to Sangon Biotech (Shanghai) Co. for microbial classification and sequencing. The extracted DNA was used to build a 16S rDNA library and the data were analyzed on an online cloud platform (ngs.sangon.com). The 16S rRNA gene sequences were processed using the Illumina MiSeq system. Similar sequences were coded into OTUs based on 97% sequence identity. α diversity was assessed for each sample using the Shannon and Chao1 indices, and β diversity was analyzed using principal coordinate analysis (PCoA)

Supplementary Figures

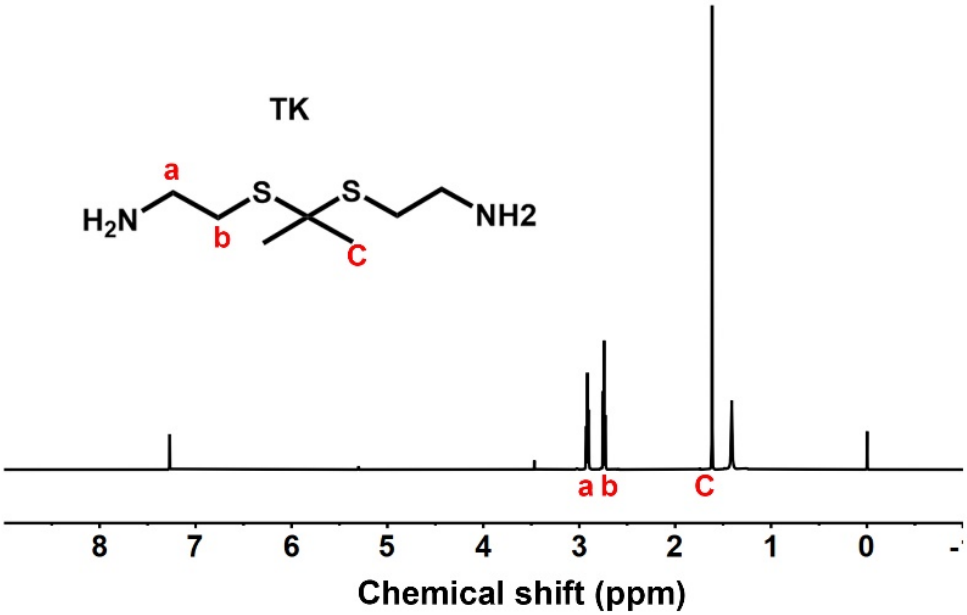

Figure S1. ^{1}H NMR spectrum of TK.

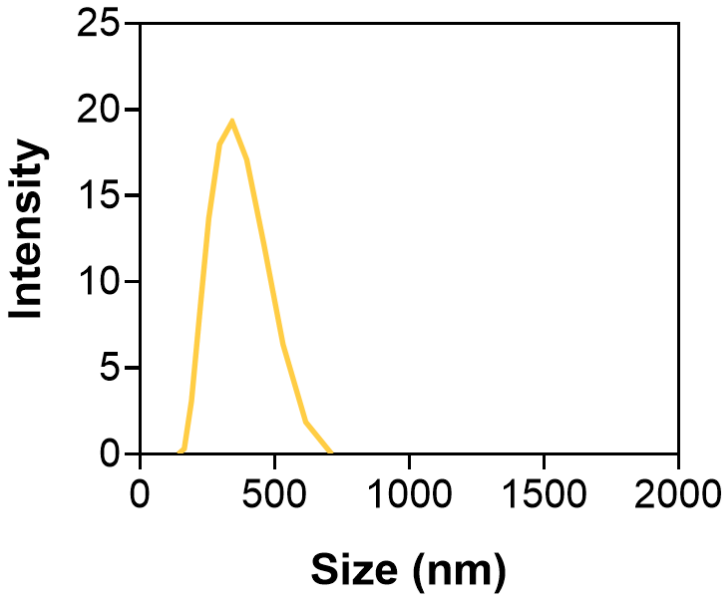

Figure S2. Hydrated particle size of STG nanomicelles.

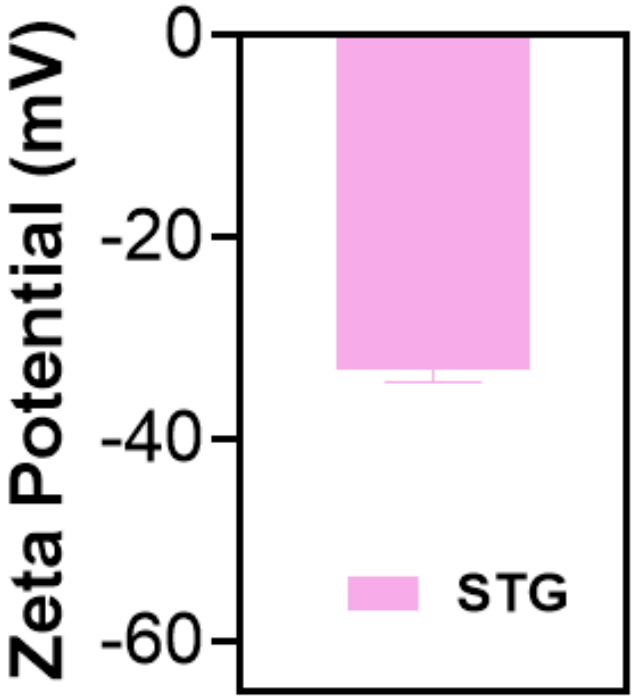

Figure S3. Zeta potentials of STG nanomicelles measured by DLS.

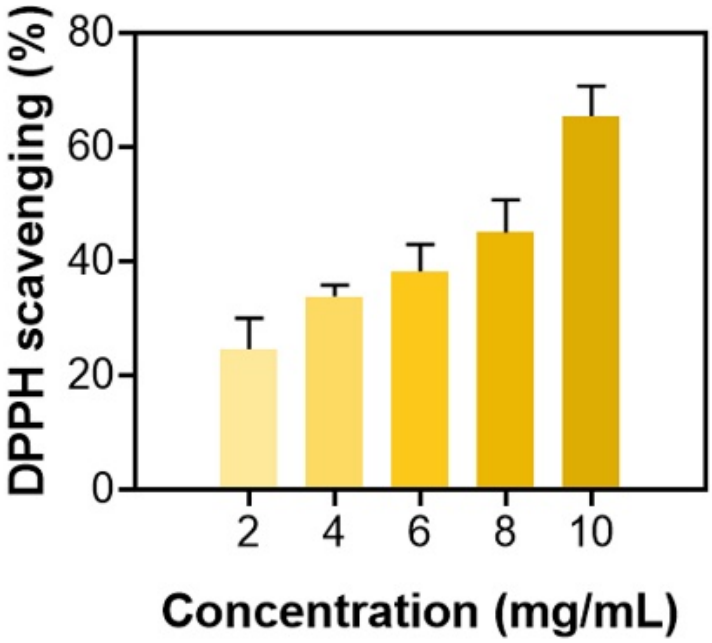

Figure S4. The DPPH scavenging efficiency of the STG nanomicelles (n = 3).

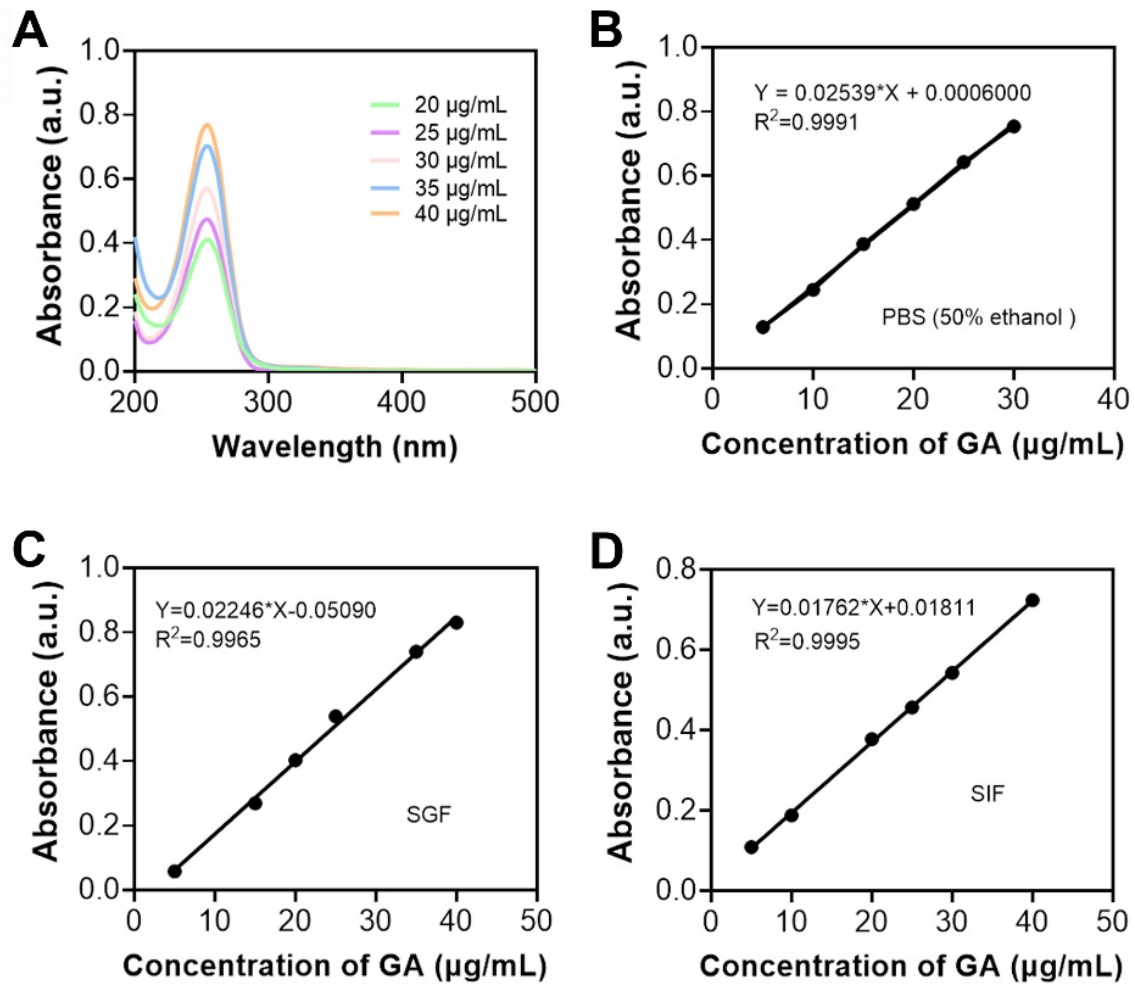

Figure S5. Characterizations of 18β-GA. (A) UV spectra of 18β-GA solutions with different concentrations. Standard curves of 18β-GA in (B) PBS (50 v% ethanol), (C) SGF and (D) SIF.

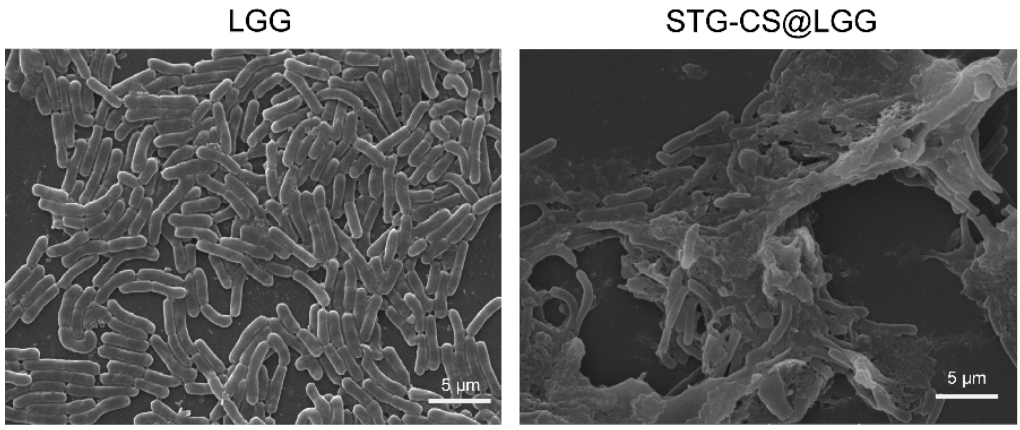

Figure S6. SEM images of LGG and STG-CS@LGG.

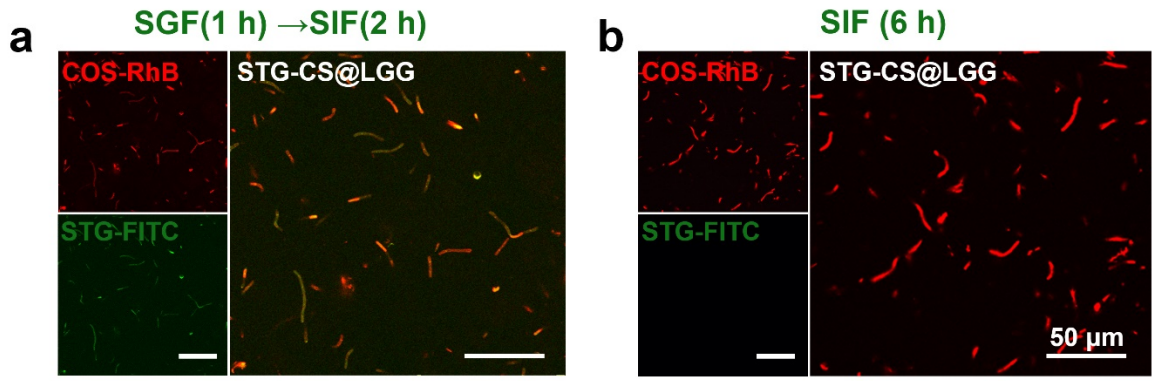

Figure S7. CLSM images of STG-CS@LGG in (a) SGF and SIF after continuous co-incubation for 3 h and (b) SIF (1 mM H_2O_2) after 6 h. Red: rhodamine B-labelled COS. Green: FITC-labelled STG nanomicelles (n= 3).

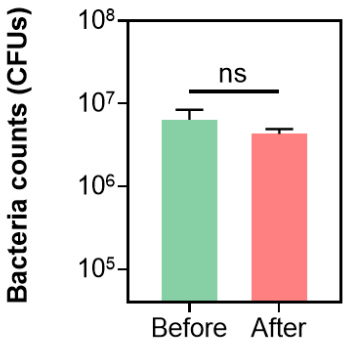

Figure S8. Number of probiotic colonies before and after lyophilization (n = 3).

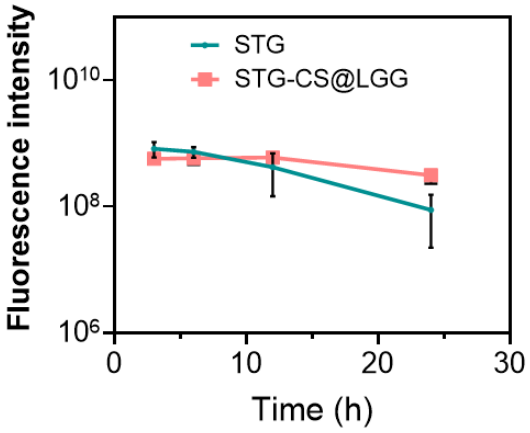

Figure S9. Fluorescence signals at the intestinal site after oral administration (n = 3).

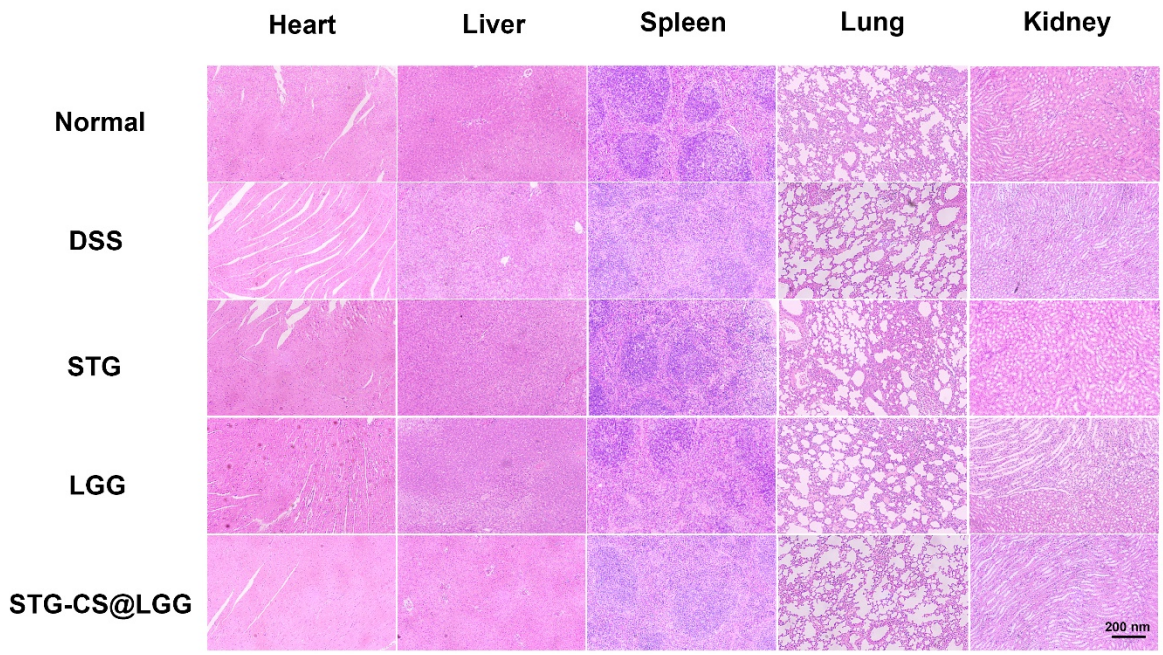

Figure S10. Representative H&E images of heart, liver, spleen, lung, and kidney in different treatment groups.

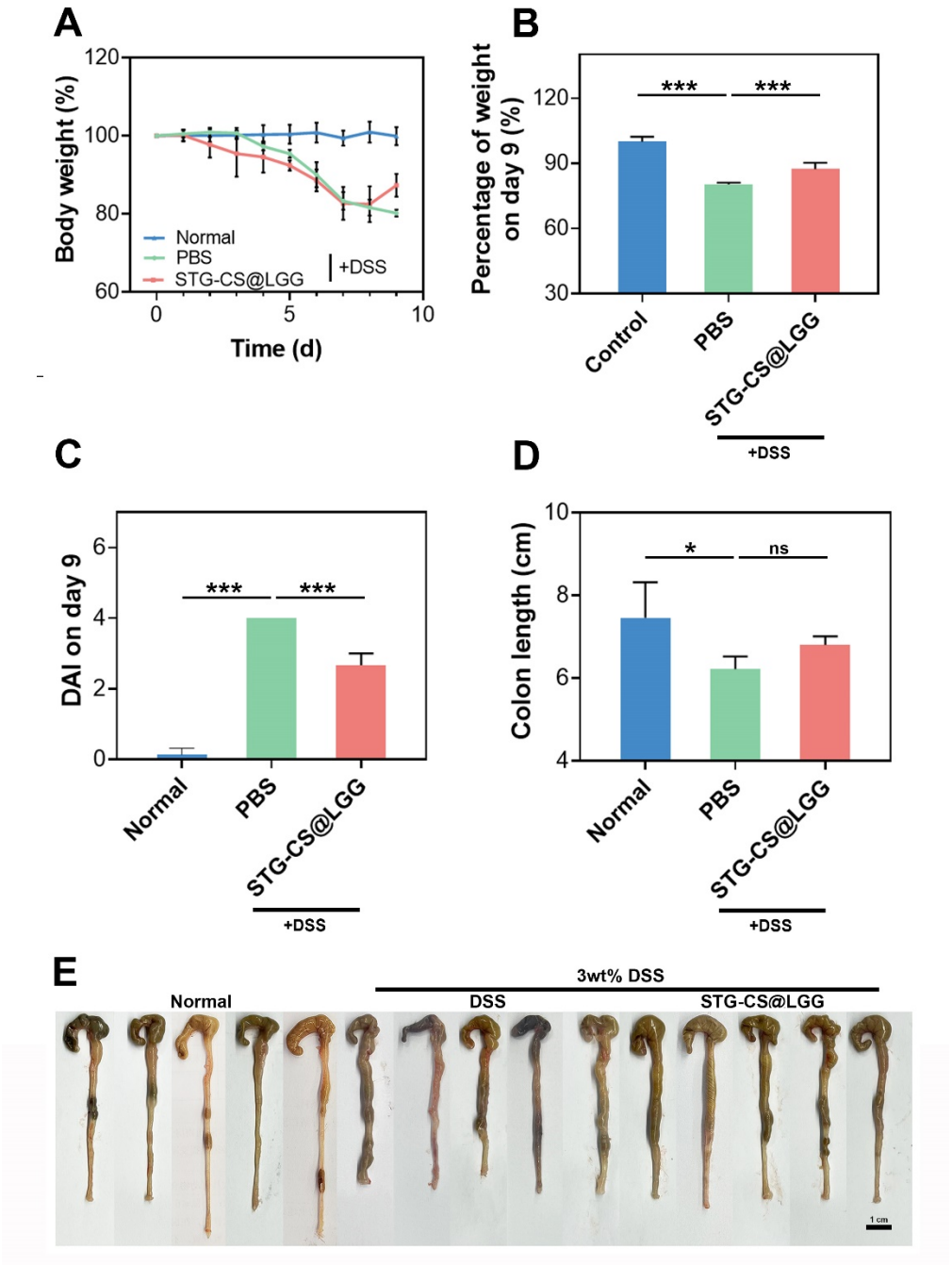

Figure S11. STG-CS@LGG effectively alleviates DSS-induced ulcerative colitis in mice. (A, B) Daily weight changes in each group over 9 days and on day 9. Data were normalized as a percentage of the body weight at day 0. (C) Changes in DAI on day 9, which is one-third of the sum of the weight loss score (0-4), stool consistency score (0-4), and blood in stool score (0-4). (D, E) Photographs of colon after 9 days of different treatments and corresponding quantitative lengths of colons for behavioral characterizations.

Table S1. Anti-depressant effects of STG-CS@LGG compared to previous studies

Comparison of behavioral treatment effectiveness	Comparison of immunofluorescence staining results	Ref.
The article investigated the depressive behavior of mice through tail suspension test, the forced swim test, the novel object recognition test, the beam walk test， and the open field test. The results of several behaviors showed that the model groups all exhibited significant depressive behaviors, while after treatment with the final material group there was no significant difference from the negative control group, and the anti-depressant treatment was extremely effective.	Immunofluorescence staining of the hippocampal region was analyzed for various markers commonly used in neuroscience research (MAP2, GFAP, Iba1, and NeuN), and the final material group demonstrated similar experimental results to the negative control group, with excellent anti-depressant effect.	[5]
The article investigated the depressive behavior of mice through tail suspension test, the forced swim test, the novel object recognition test, the hair grooming test, the sugar preference test and the open field test. The results of several behavioral studies showed that all the model groups showed significant depressive behaviors, whereas after treatment by the final material group there was no significant difference from the negative control group, and the antidepressant treatment was more effective.	Immunofluorescence staining of the hippocampal region was analyzed for various markers commonly used in neuroscience research (MAP2, DCX, and NeuN), and the final material group showed similar experimental results to the negative control group, with better antidepressant effect.	[6]
The article investigated the depressive behavior of mice through tail suspension test, the forced swim test, the novel object recognition test, the beam walk test, the open field test, and Morris water maze test. The results of several behavioral studies showed that the model group showed significant depressive behaviors, and the	Immunofluorescence staining of the hippocampal region was analyzed for various markers commonly used in neuroscience research (MAP2, GFAP, and Iba1), and the final material group showed similar experimental results to the negative control group, with better antidepressant	[7]

final material had some antidepressant effects.		
The article investigated the depressive behavior of mice through tail suspension test, the forced swim test, the open field test, and the elevated plus test. Several behavioral results indicate that the final material is more effective in antidepressant effects.	Immunofluorescence staining of the hippocampal region was analyzed for various markers commonly used in neuroscience research (DCX, NeuN and Iba1), and the final material is more effective in antidepressant effects.	[8]
We studied the depressive behavior of mice by tail suspension test and light and dark box test. Behavioral results showed that the model group showed significant depressive-like behaviors, which were like the model group after treatment with the final material, with better antidepressant effects.	Immunofluorescence staining of the hippocampal region was analyzed for markers commonly used in neuroscience research (Iba1), The anti-depressant effect of the final material was generally	**Our work**

Table S2. Performance comparisons with existing systems

Existing Systems	Performance parameters	Ref.
Mesoporous silica (MSN)-based ICANs loaded with CeNPs and coated with polyacrylic acid (PAA).	Efficient ROS scavenging and inflammatory tissue targeting.	[9]
Whey protein isolate (WPI)-based astaxanthin nanoparticles (AST NPs).	Solubilization of hydrophobic astaxanthin without cross-linkers.	[10]
Yeast microcapsules (YM) encapsulate curcumin-loaded metal-polyphenol networks (Cur-MPN).	Gastric acid resistance and MPN-mediated ROS scavenging.	[11]
Layer-by-layer (LBL)-coated MPDA nanoparticles for CO delivery.	M2 macrophage polarization via CO, and LBL-enhanced targeting.	[12]
Macrophage membrane-camouflaged polydopamine nanoparticles (PCM NPs).	innate targeting via macrophage membranes; ROS scavenging by PDA.	[13]

Movie S1. The behavior of normal mice during the light-dark box test.

Movie S2. The behavior of DSS-treated mice during the light-dark box test.

Movie S3. The behavior of STG-CS@LGG-treated mice during the light-dark box test.

Movie S4. The behavior of normal mice during the tail suspension test.

Movie S5. The behavior of DSS-treated mice during the tail suspension test.

Movie S6. The behavior of STG-CS@LGG-treated mice during the tail suspension test.

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