

Supplementary Materials

A Multifunctional Nanotherapy for Targeted Treatment of Colon Cancer by Simultaneously Regulating Tumor Microenvironment

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Table S1. The drug loading content and encapsulation efficiency of various nanoparticles.

	The loading content [%] ^a	Encapsulation efficiency [%] ^b
CPT-11/OCD NP	14.5 ± 1.3 [*]	43.5 ± 3.9
Cy5/OCD NP	10.4 ± 1.5	33.3 ± 4.7
Cy7.5/OCD NP	8.2 ± 1.3	27.1 ± 4.3
CPT-11/PLGA NP	11.6 ± 1.1	40.6 ± 3.8
Cy5/PLGA NP	9.1 ± 1.4	30.9 ± 4.6
Cy7.5/PLGA NP	8.6 ± 1.7	26.7 ± 5.2

^a The loading content (%) = $\frac{\text{Mass of quantified drug in nanoparticles}}{\text{Mass of nanoparticles}} \times 100\%$

^b Encapsulation efficiency (%) = $\frac{\text{Mass of quantified drug in nanoparticles}}{\text{Mass of feeding drug}} \times 100\%$

Data are presented as mean ± SD (n = 3). ^{*} p < 0.05 versus CTP-11/PLGA NP.

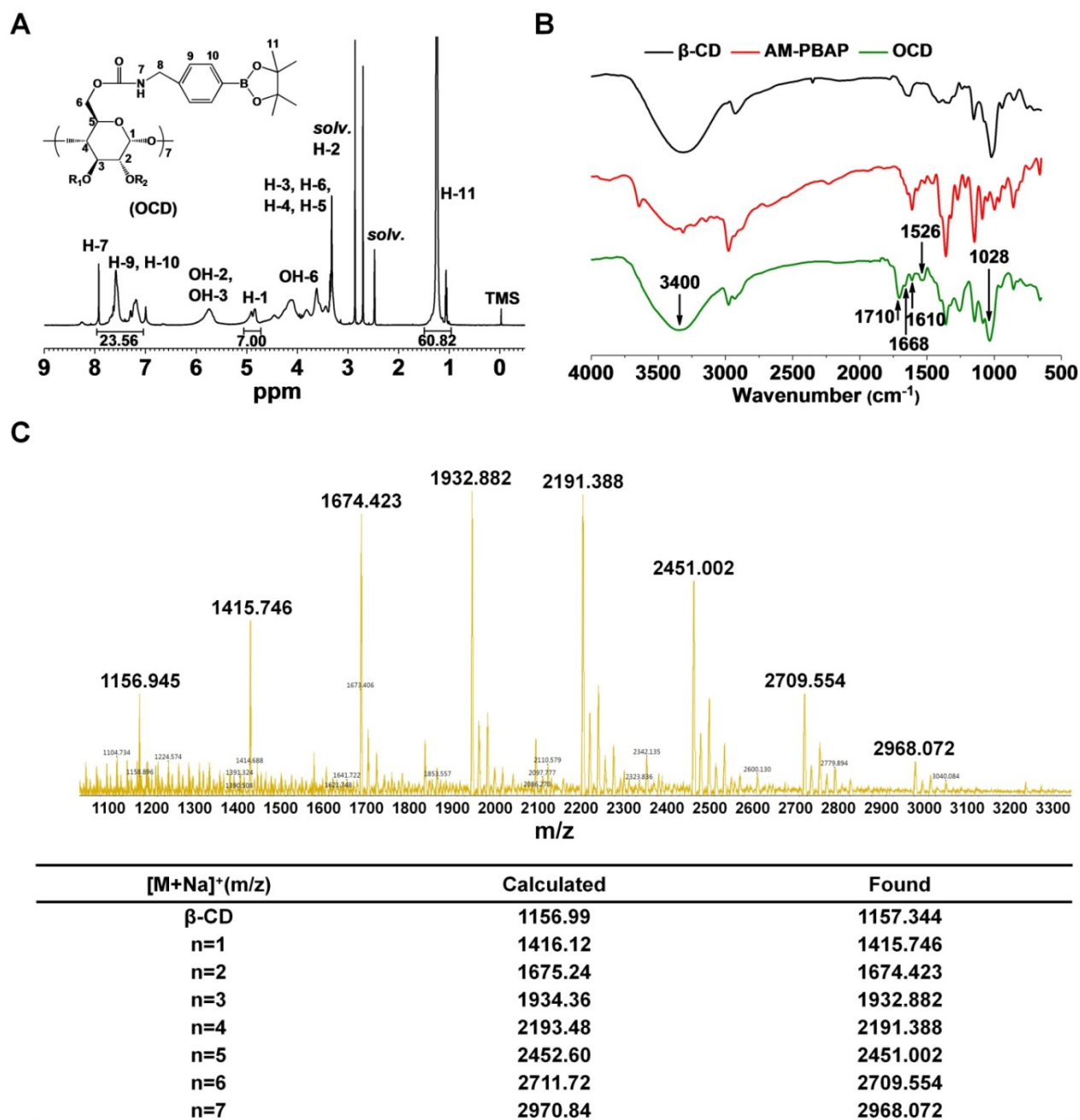


Figure S1. Structural characterization of OCD. A-C, ^1H NMR spectrum (A), FT-IR spectrum (B), and MALDI-TOF mass spectrum (C) of synthesized OCD.

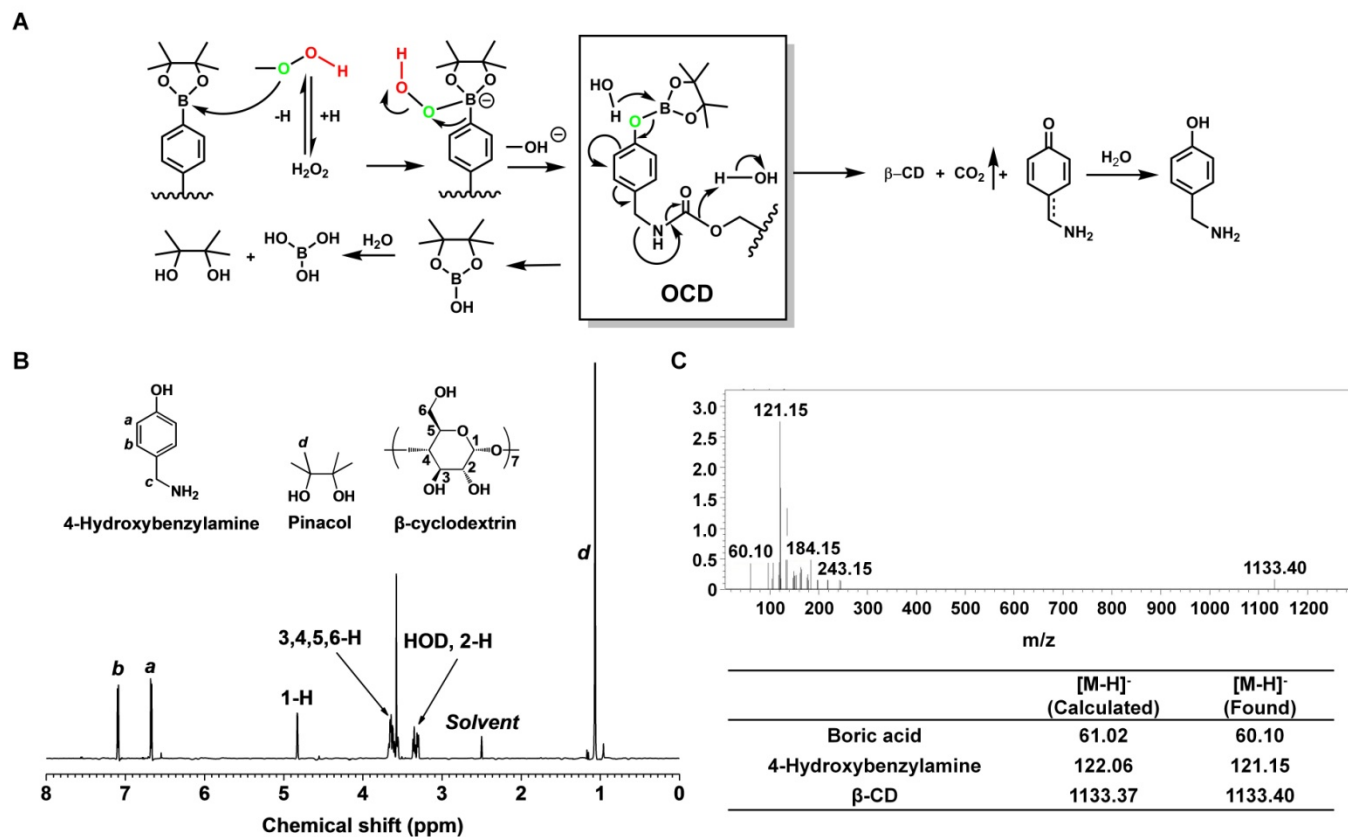


Figure S2. Hydrolysis of OCD in the presence of H_2O_2 . **A**, Schematic illustration of the hydrolysis mechanism. **B-C**, ^1H NMR spectrum (**B**) and ESI mass spectrum (**C**) of hydrolyzed products.

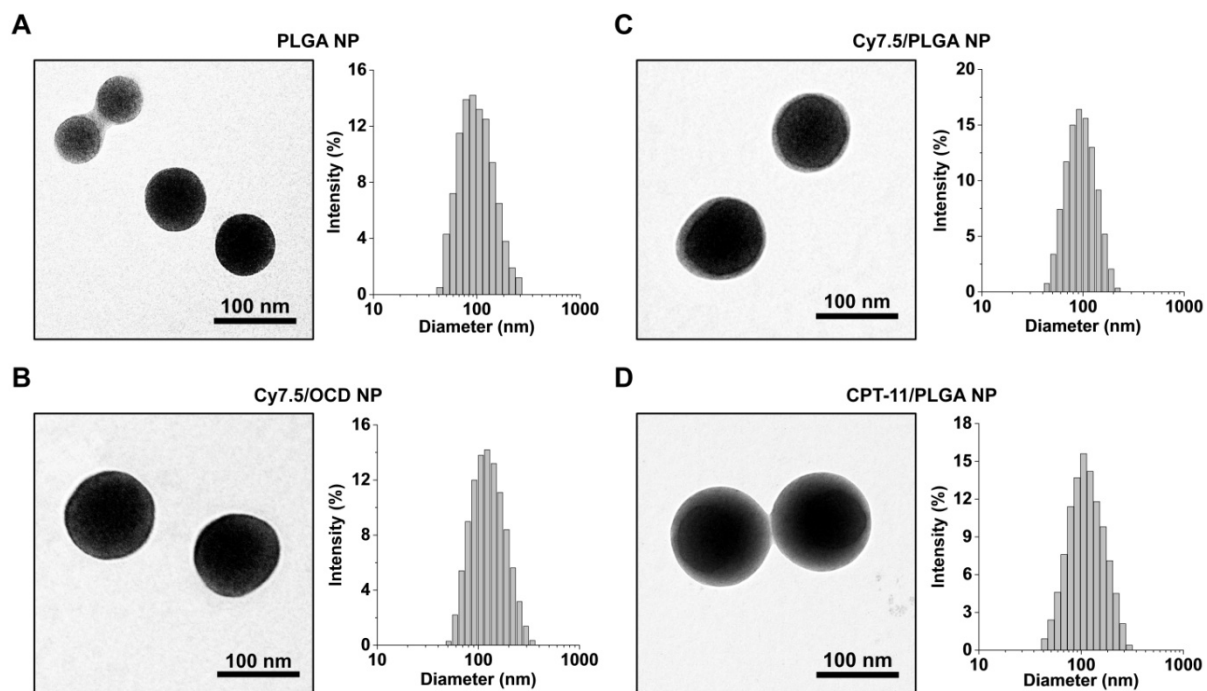


Figure S3. TEM images and size distribution profiles of different NPs. **A**, PLGA NP. **B**, Cy7.5/OCD NP. **C**, Cy7.5/PLGA NP. **D**, CPT-11/PLGA NP.

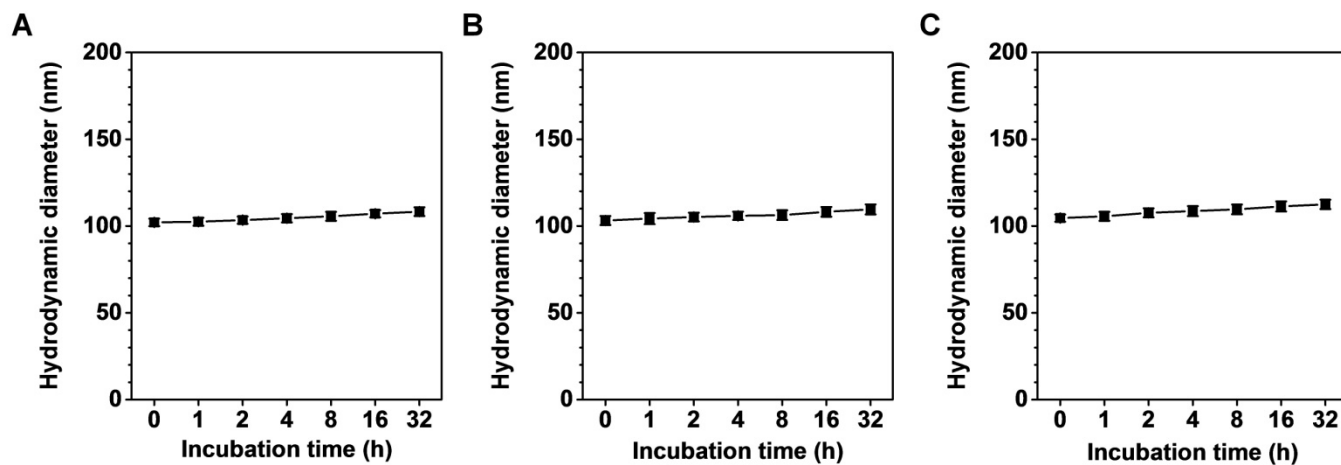


Figure S4. Time-dependent changes in mean hydrodynamic diameter of OCD NP after incubation in aqueous solutions containing typical digestive enzymes. **A**, α -Amylase. **B**, Pepsin. **C**, Trypsin. The OCD NP concentration in aqueous solution was 10 mg/mL, while the enzyme concentration was 200 U/mL. Data are presented as mean $\pm$ SD ($n = 3$).

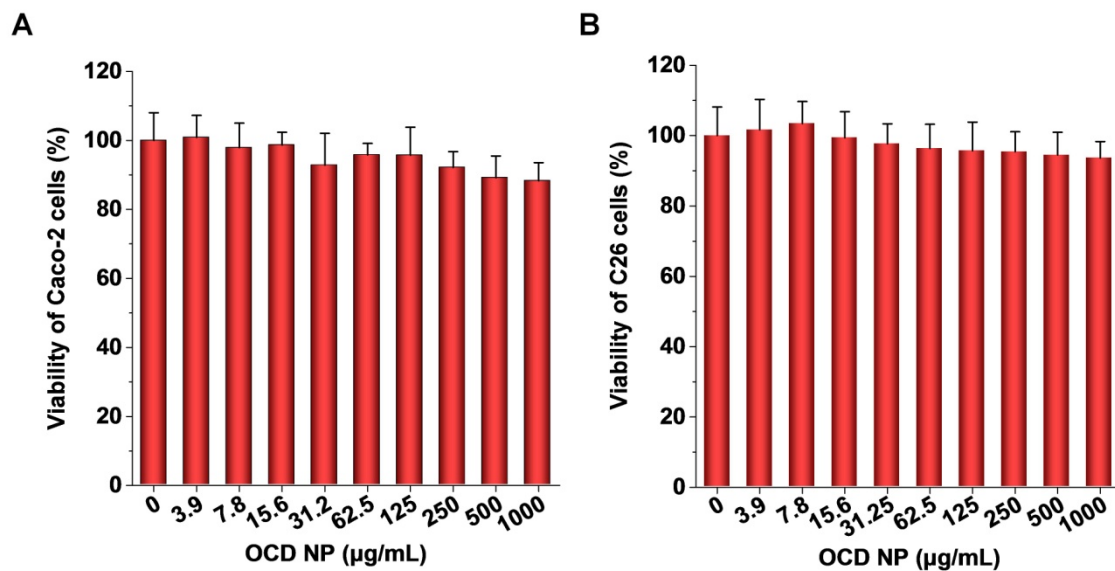


Figure S5. *In vitro* cytotoxicity of OCD NP in different cells. **A-B**, Relative cell viability data of Caco-2 cells (**A**) and C26 cells (**B**) after incubation with different concentrations of OCD NP for 12 h. All data are presented as mean $\pm$ SD (n = 6).

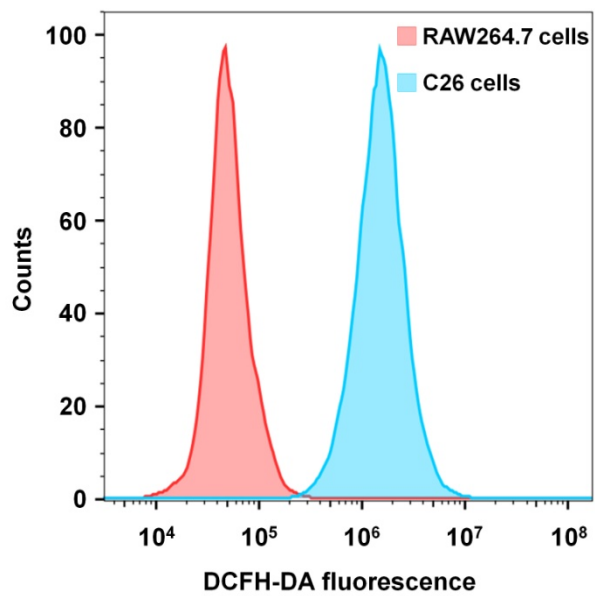


Figure S6. Comparison of intracellular ROS levels in RAW264.7 and C26 cells. Intracellular ROS levels were tested by flow cytometry using DCFH-DA as a fluorescent probe.

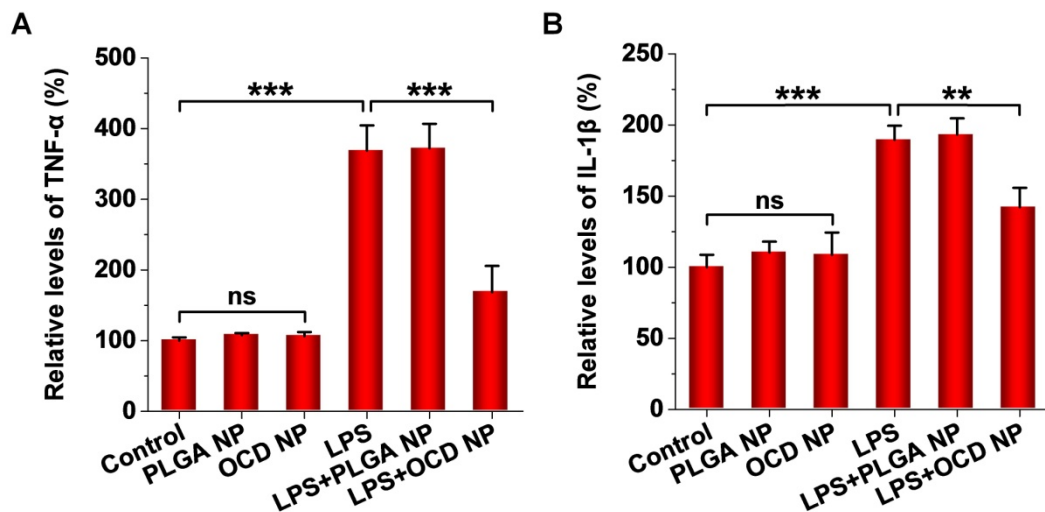


Figure S7. *In vitro* anti-inflammatory activity of OCD NP in Caco-2 cells. **A-B**, The levels of TNF- α (A) and IL-1 β (B) in LPS-stimulated Caco-2 cells after different treatments. Data are presented as mean $\pm$ SD (n = 6). **p < 0.01, ***p < 0.001; ns, no significance.

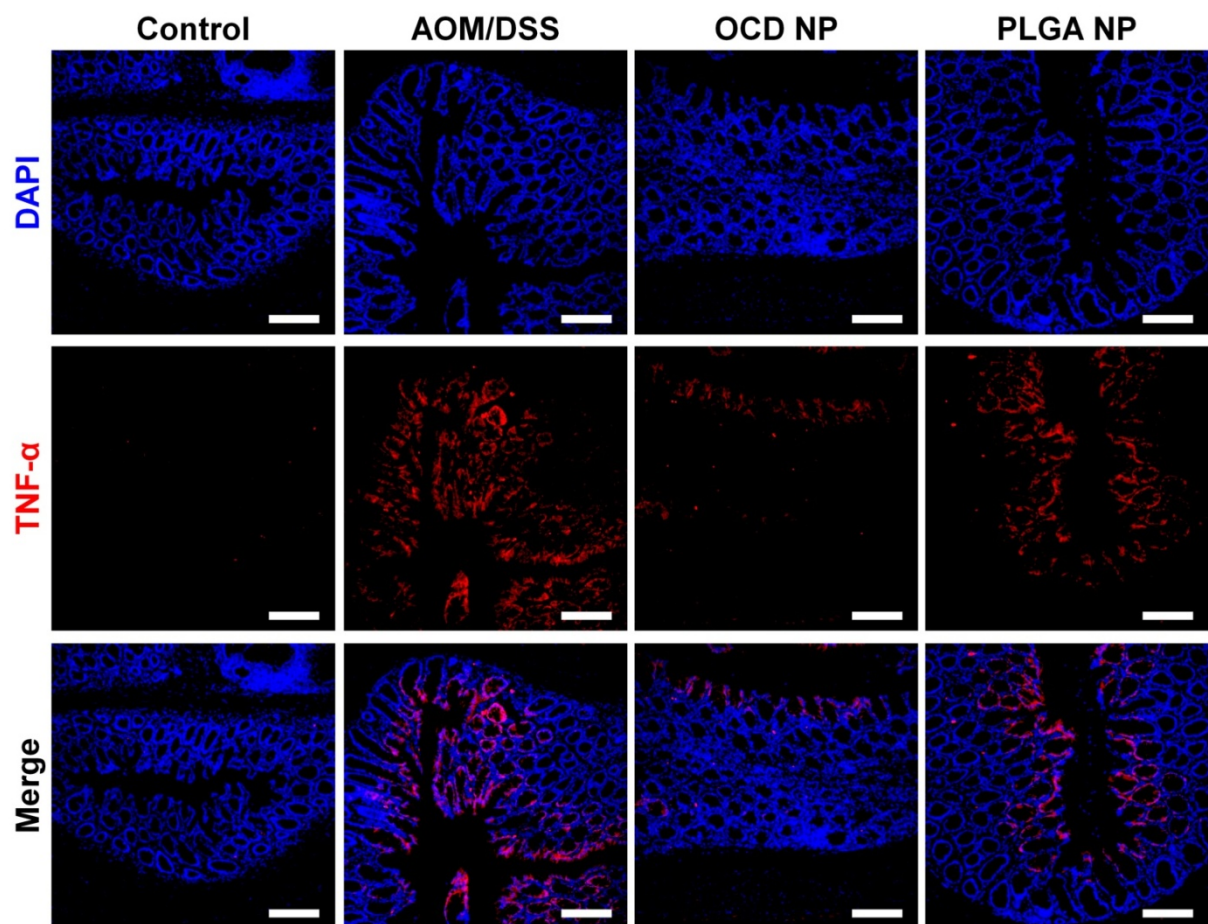


Figure S8. Immunofluorescence analysis of TNF- α expression in cryosections of colonic tissues from mice after different treatments. The nuclei were stained with DAPI (blue). Scale bars, 100 μ m.

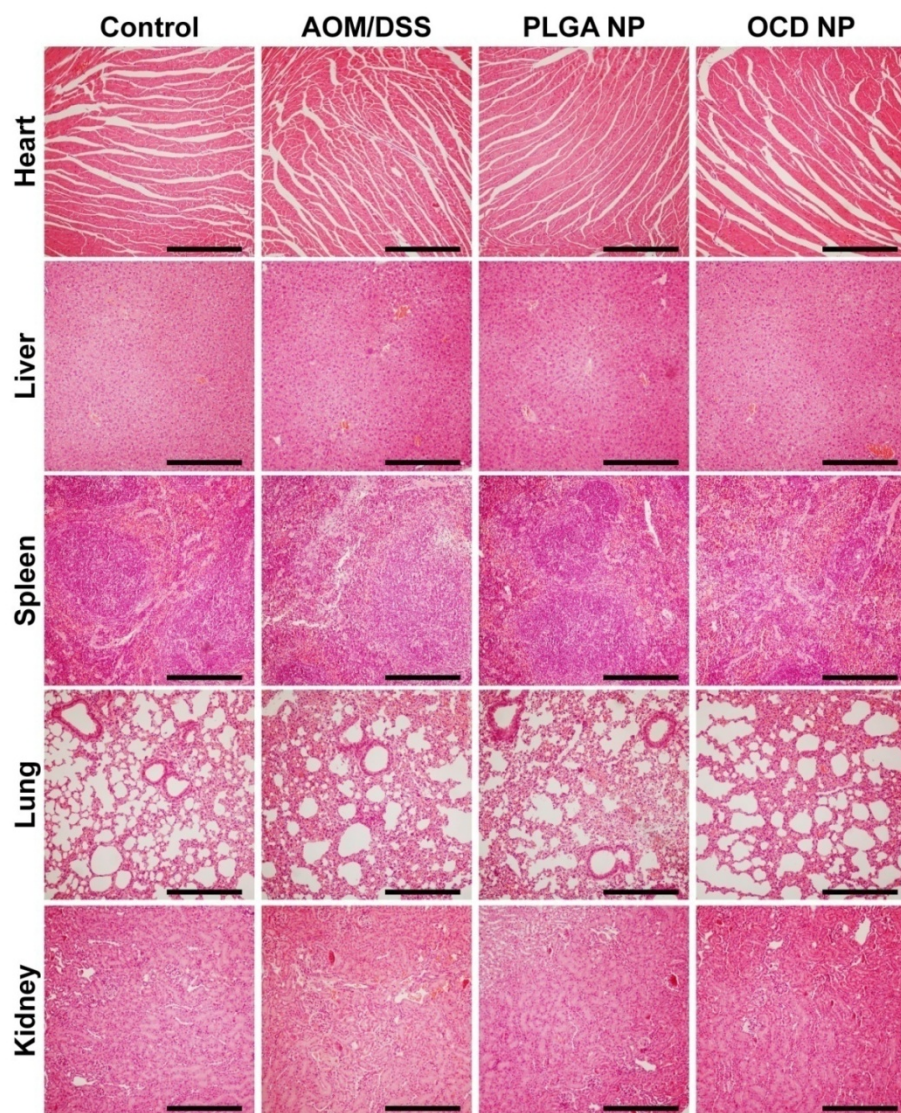


Figure S9. H&E-stained sections of major organs resected from mice after different treatments. Chronic colitis in mice was induced by AOM/DSS. Scale bars, 200 μ m.

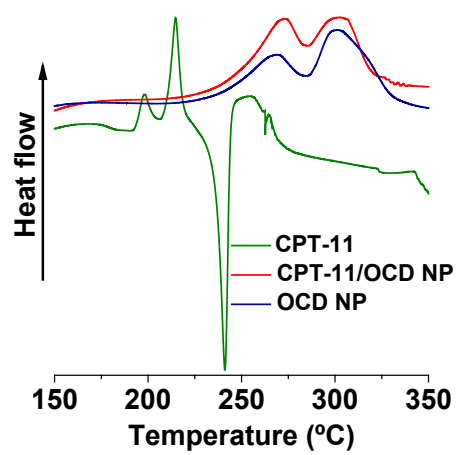


Figure S10. DSC curves of CPT-11, blank OCD NP, and CPT-11/OCD NP.

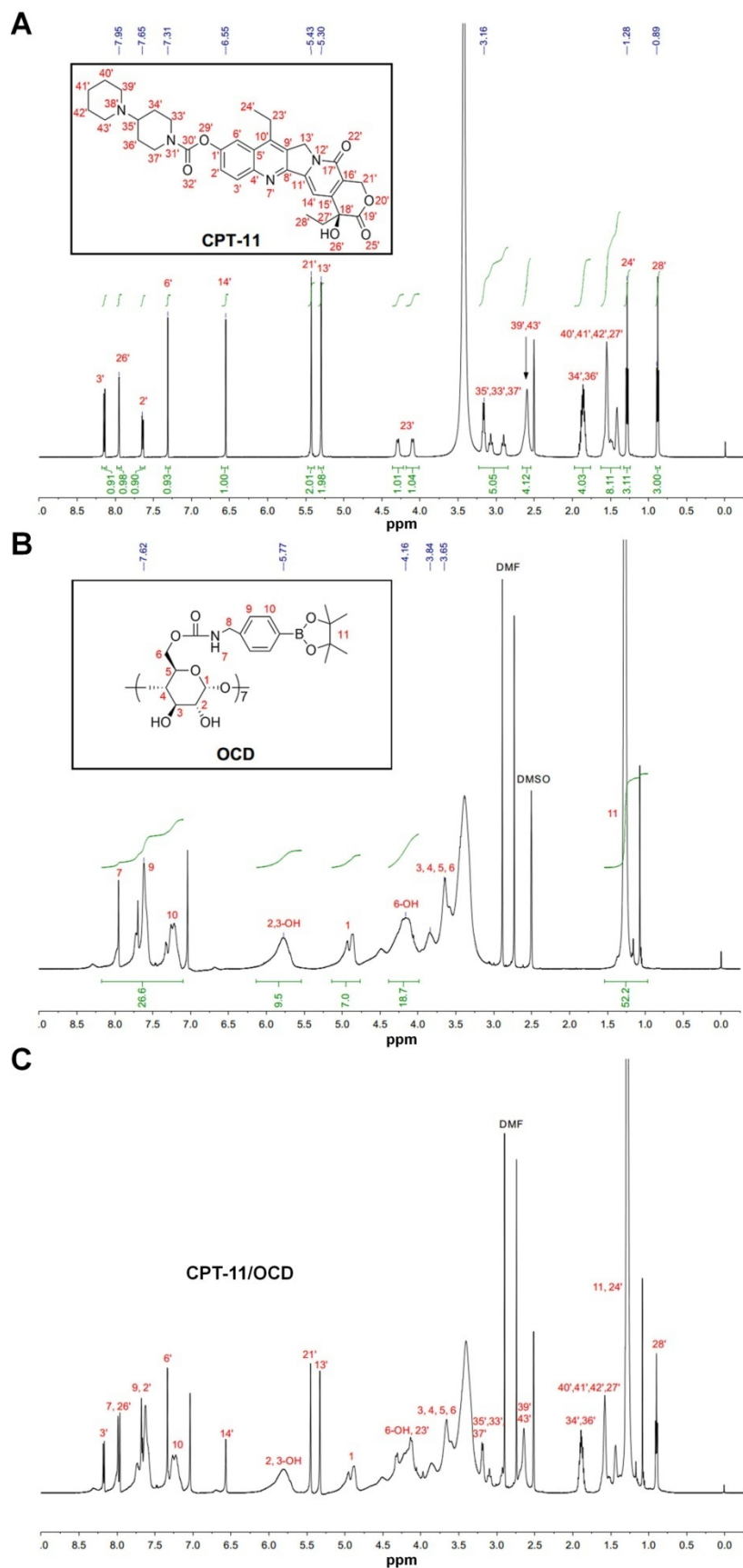


Figure S11. ^1H NMR spectra of different samples in $\text{DMSO-}d_6$. **A**, CPT-11. **B**, OCD. **C**, CPT-11/OCD.

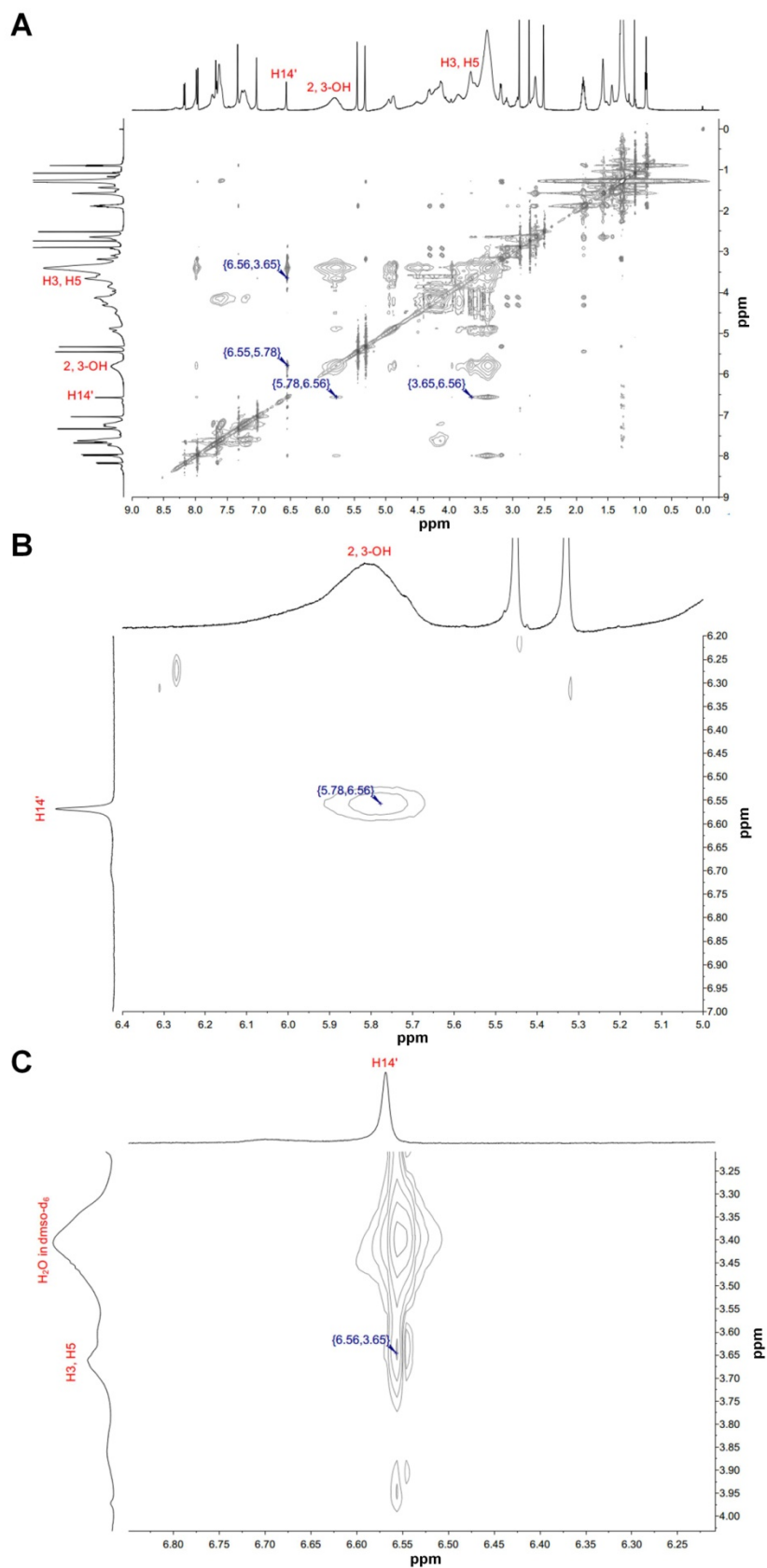


Figure S12. Characterization of inclusion interaction between CPT-11 and OCD. **A**, 2D NOESY spectrum of CPT-11/OCD in DMSO- d_6 . **B-C**, Partly zoomed in 2D NOESY spectra.

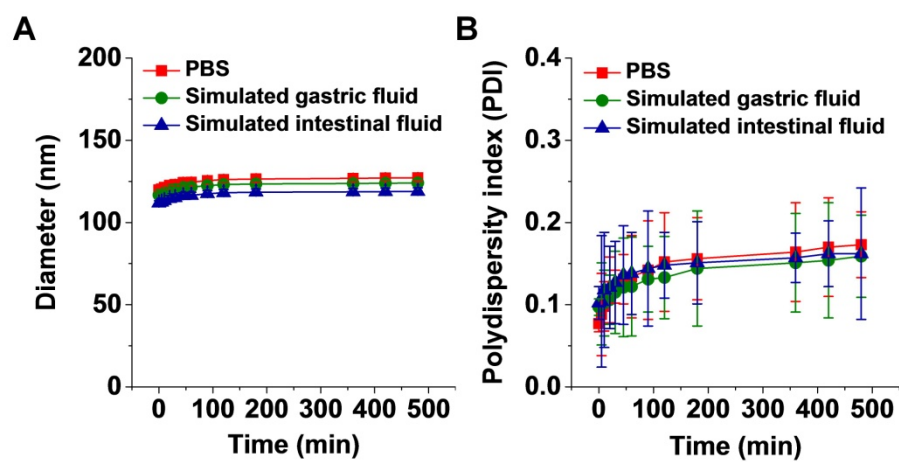


Figure S13. Stability of CPT-11/OCD NP in different solutions. **A-B**, The changes of mean hydrodynamic diameter (A) and polydispersity index (B) of CPT-11/OCD NP during 480 h incubation in PBS or simulated gastrointestinal fluids. Data are presented as mean $\pm$ SD ($n = 3$).

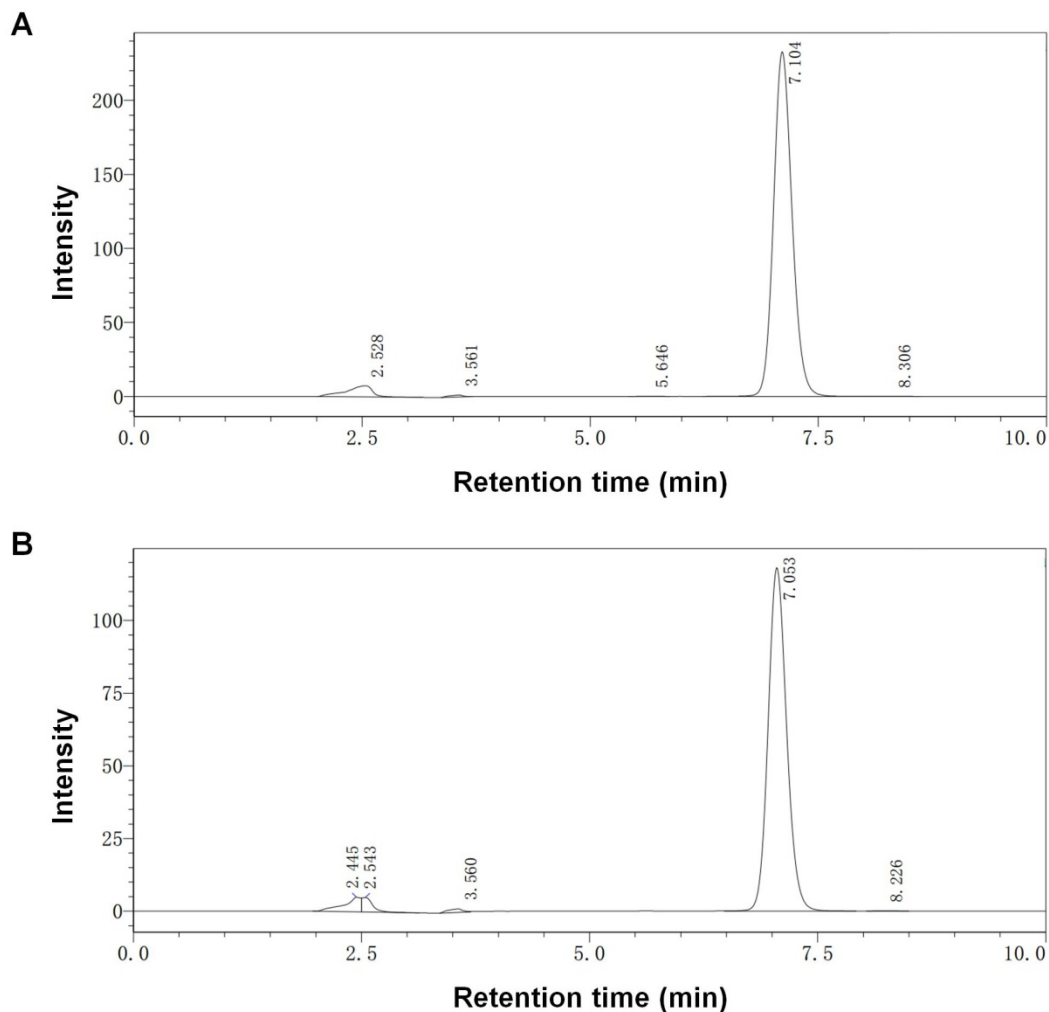


Figure S14. HPLC curves of different CPT-11 samples. **A**, Pristine CPT-11. **B**, CPT-11 released from CPT-11/OCD NP. The lactone-ring type of CPT-11 was assayed by HPLC with UV-Visible detection and a C18 reverse-phase column was used. The mobile phase was consisted of methanol and water at 60:40 (v/v). The flow rate was 1.0 mL/min. Detection was performed at 372 nm. The retention time of pristine CPT-11 with a lactone ring was 7.104 min, while it was 7.053 min for CPT-11 released from CPT-11/OCD NP.

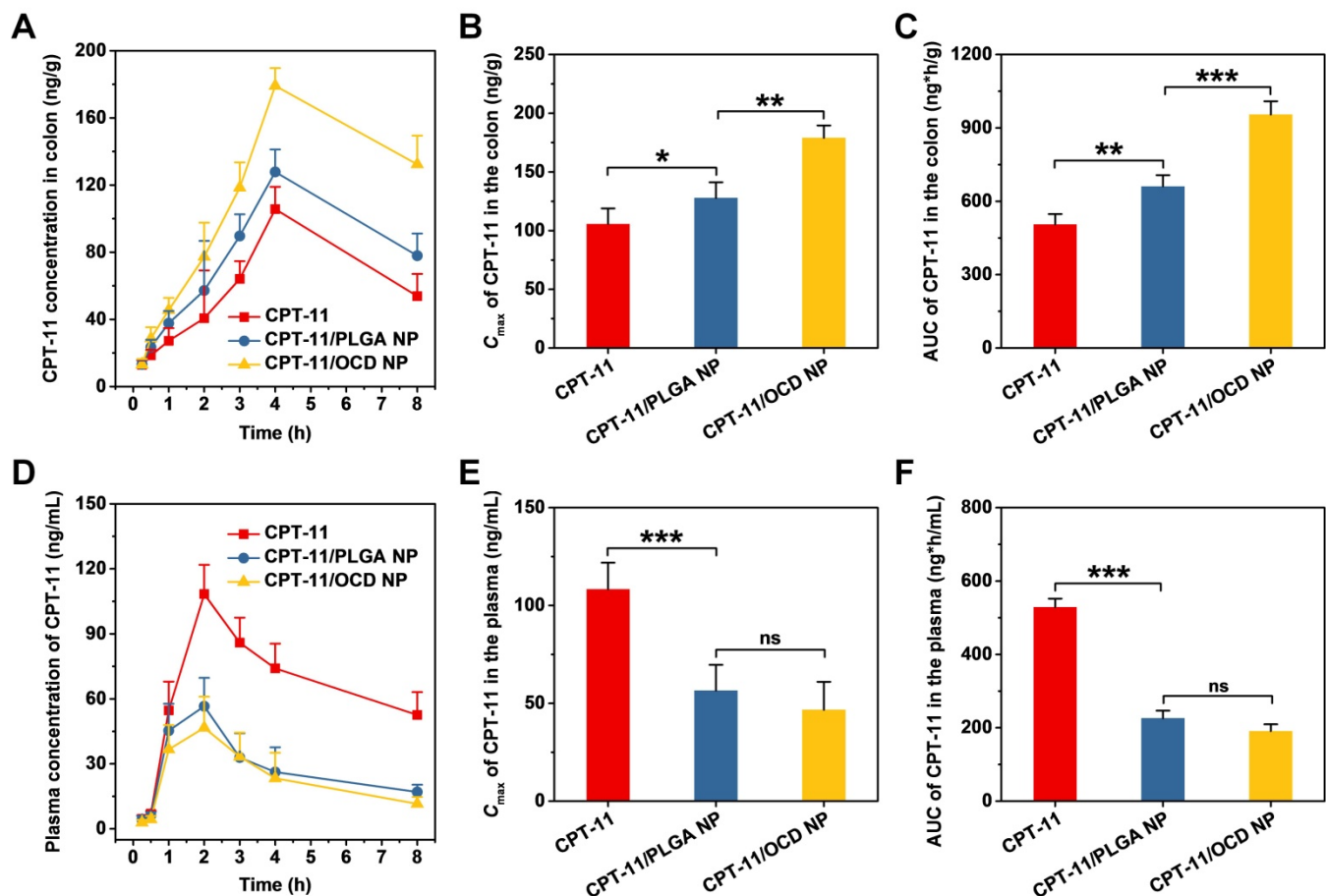


Figure S15. *In vivo* pharmacokinetic and tissue distribution studies of different CPT-11 formulations after oral administration in mice with induced colitis. **A-C**, The drug concentration-time curve (A), C_{max} (B), and AUC (C) of CPT-11 in the colon tissues. **D-F**, The drug concentration-time curve (D), C_{max} (E), and AUC (F) of CPT-11 in the plasma. Oral administration of different CPT-11 formulations at 5 mg/kg of CPT-11 was performed at day 7 after mice were induced with DSS. Data are presented as mean $\pm$ SD (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001; ns, no significance.

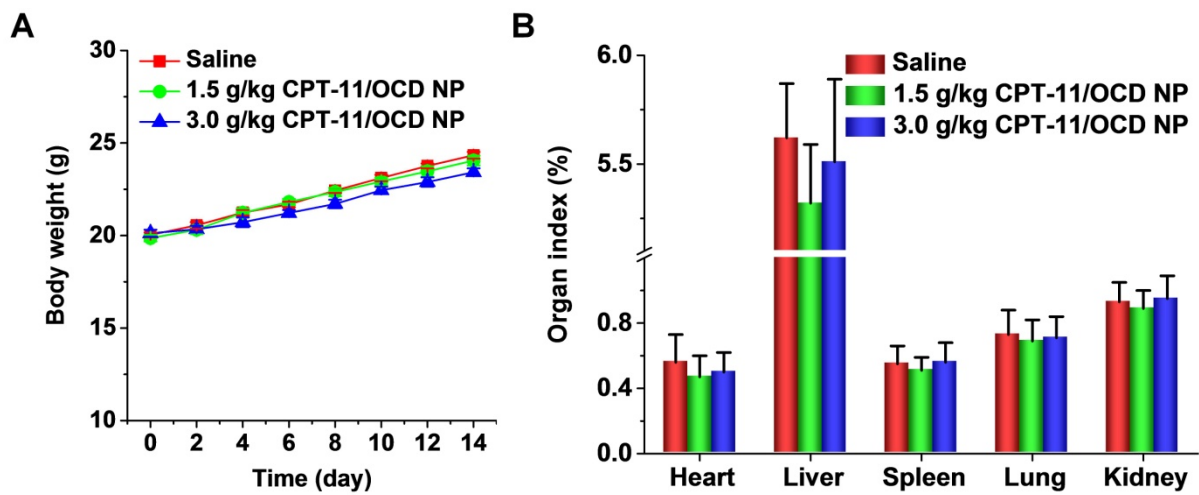


Figure S16. Changes in body weight and organ index of healthy mice treated with orally administered CPT-11/OCD NP. **A**, Changes in the body weight of mice after oral administration of various doses of CPT-11/OCD NP. **B**, The organ index of typical major organs resected from mice at day 14 after treatment. Data are expressed as mean $\pm$ SD (n = 10).

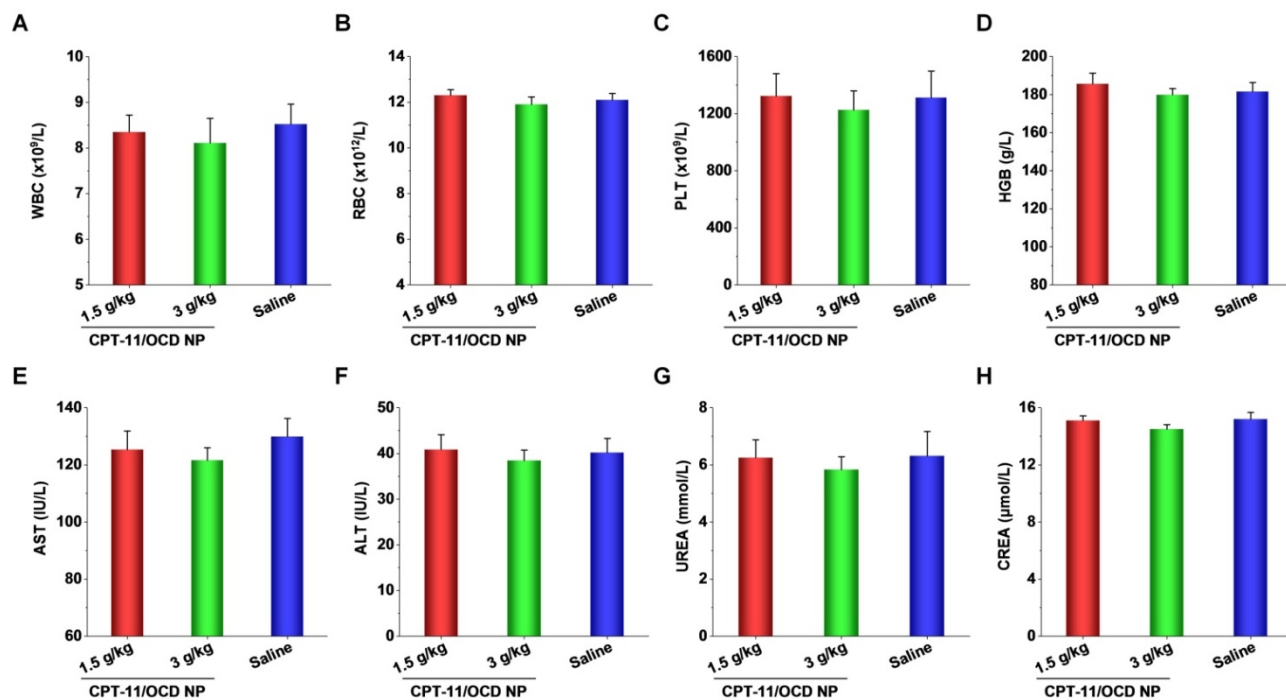


Figure S17. Hematological parameters and biomarkers relevant to liver and kidney functions. **A-D**, Complete blood count of white blood cells (A), red blood cells (B), platelets (C), and hemoglobin (D) in the blood collected from mice at day 14 after treatment with 1.5 or 3.0 g/kg of CPT-11/OCD NP. **E-H**, Typical biomarker molecules relevant to liver (E-F) and kidney (G-H) functions. WBC, white blood cells; RBC, red blood cells; PLT, platelets; HGB, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; UREA, blood urea; CREA, creatinine. Data are expressed as mean $\pm$ SD (n = 10).

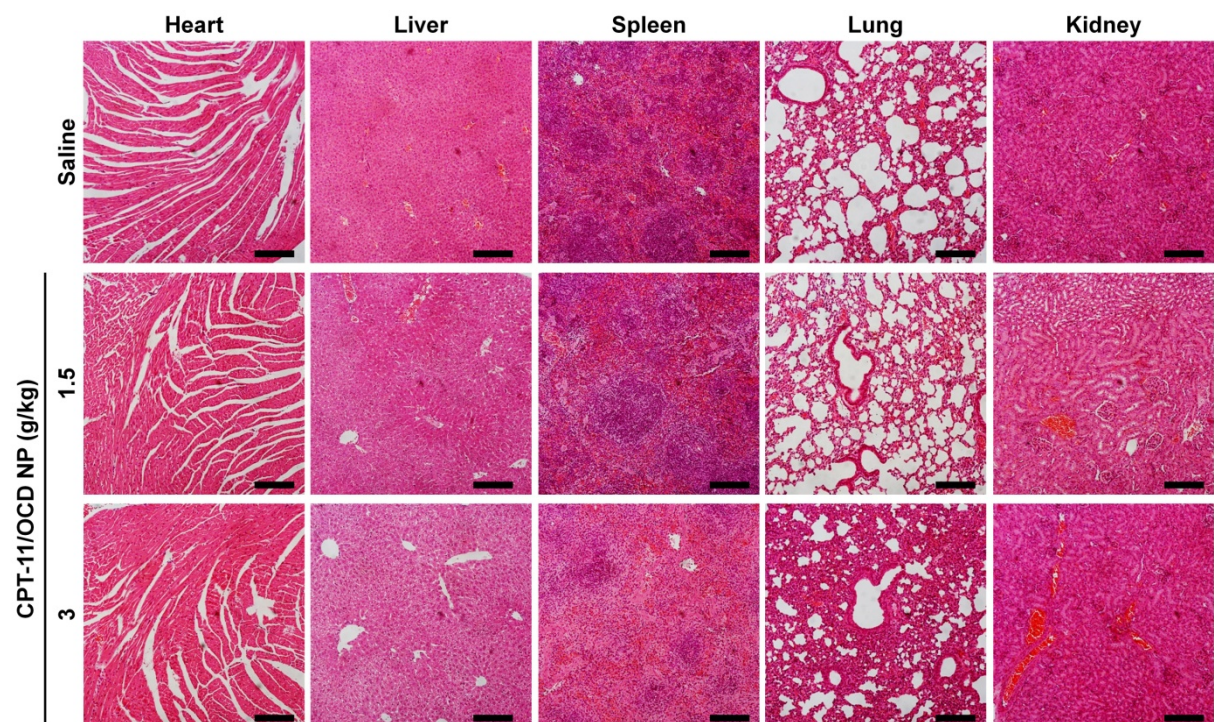


Figure S18. H&E-stained sections of major organs from mice sacrificed at day 14 after treatment with different doses of CPT-11/OCD NP. Scar bars, 100 μ m.