Electronic Supplementary Information(ESI) for

Theranostics of Atherosclerosis by Indole Molecule-Templated Self-Assembly of Probucol Nanoparticles

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Experimental Section

Chemicals and Materials

Probucol (PB) was purchased from TCI (Shanghai, China). Indocyanine green (ICG) was purchased from J&K Chemical (Shanghai, China). IR783, Dimethyl sulfoxide (DMSO), and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (MO, USA). Trypsin, Fetal bovine serum (FBS), and Dulbecco's modified Eagle's medium (DMEM) were obtained from Gibco (NY, USA). Cell Counting Kit-8 (CCK8) was purchased from Japan Tongren. IL-1β, IL-6, TNF-α, and GAPDH antibody were purchased from Abcam. β-acting antibody, Reactive Oxygen Species Assay Kit, 4′,6-Diamidino-2-phenylindole (DAPI) and hematoxylin were purchased from Beyotime Biotechnology (Shanghai, China). Oil Red O (ORO) was purchased from Solarbio (Beijing, China). Human high-oxidized low density lipoprotein (ox-LDL) was purchased from Yiyuan Biotechnologies (Guangzhou, China). All chemicals were of analytical reagent grade and used without further purification. Ultrapure water with a resistivity of 18.2 MΩ cm was obtained from a Millipore Mill-Q purification system and used throughout the experiment (MA, USA).

Characterization

Transmission electron microscopy (TEM) images were acquired by an S-4800 transmission electron microscopy (Hitachi Ltd., Tokyo, Japan). Hydrodynamic size distribution (DLS) was measured using ZEN3600 (Malvern Ltd., Britain). UV-Vis absorption spectra were recorded using a UV-2100 spectrophotometer (RIGOL, Beijing, China). Fourier transform infrared spectra were recorded on a Nicolet iS5 Fourier Infrared (FT-IR) spectrometer (Thermo Fisher Scientific, USA). The drug encapsulation efficacy of PB was measured using Agilent 1260 Infinity II (Agilent Technologies, USA). The fluorescence images of cells were taken on an Eclipse Ti-S inverted fluorescence microscope (Nikon, Japan). Flow cytometry analysis was carried out with Cytoflex S (Beckman Coulter Inc, CA, USA). The in vivo fluorescence imaging was performed by an using VISQUE Invivo Smart under excitation of a 750 nm filter and emission of 810 nm filter (Vieworks, Korea). The levels of TC, TG, HDL, LDL were measured by Mindray BS-220 (Mindray Bio-Medical Electronics Co, Ltd, China).

Western Blot

The protein samples were acquired from different groups, and the concentration of proteins was measured by the BCA Protein Assay Kit. After 15 μg protein per sample was separated on the 12% sodium dodecyl sulphate polyacrylamide (SDS-PAGE) gel, these samples were transferred to a polyvinylidene fluoride (PVDF) membrane at 200 mA for 1 h. PVDF membranes were blocked with 5% BSA solution at room temperature for 2 h and then incubated with primary antibodies at 4 °C overnight, followed by incubation with secondary horseradish peroxidase conjugated antibodies (1:1000, Beyotime Biotechnology, China) for 2 h at room temperature. The western blot signal was detected using an automatic chemiluminescence analysis system named Tanon-5200 (Tanon Biotechnology Co, Ltd., China).

Statistical Analysis

Statistical analysis was performed with GraphPad Prism 8 software. The results were presented as means ± SD. Statistical significance was determined by the one-way ANOVA with a multiple comparison method and one-way ANOVA with a two-tailed, unpaired t-test. The results of $p<0.05$ were considered to be statistically significant.

Table S1 Size change of IRPNPs before and after lyophilization

	Average size (nm)	PDI
Before	285.8	0.017
After	291.1	0.124

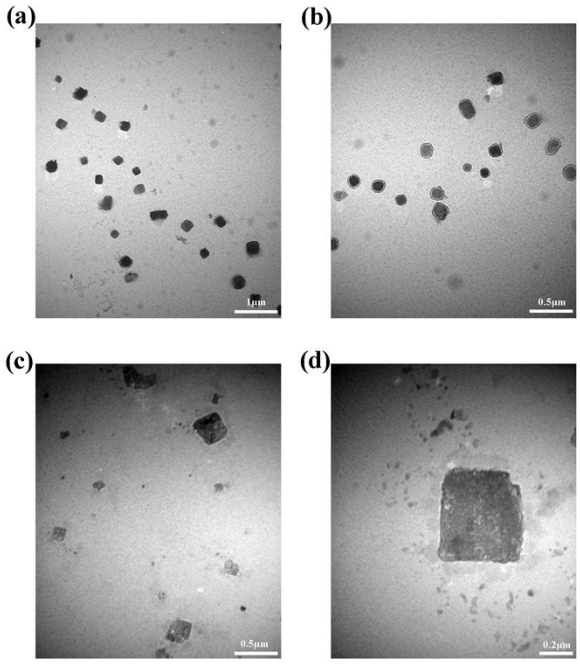

Figure S1 (a)(b) TEM images of IPNPs; (c)(d) TEM images of IRPNPs.

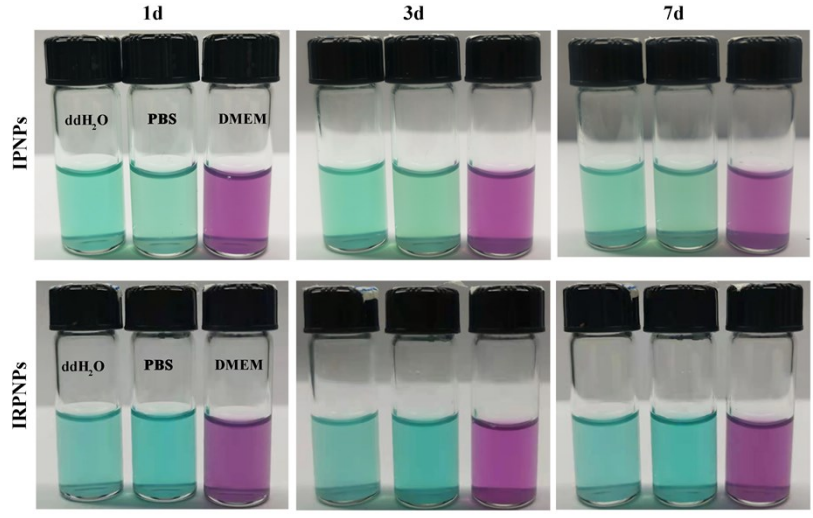

Figure S2 The stability of IPNPs and IRPNPs after incubated with different solutions for 1, 3, 7d.

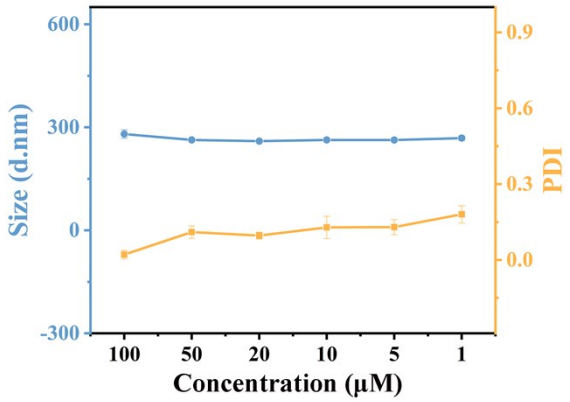

Figure S3 The size change of IRPNPs during dilution up to 1 μM of PB.

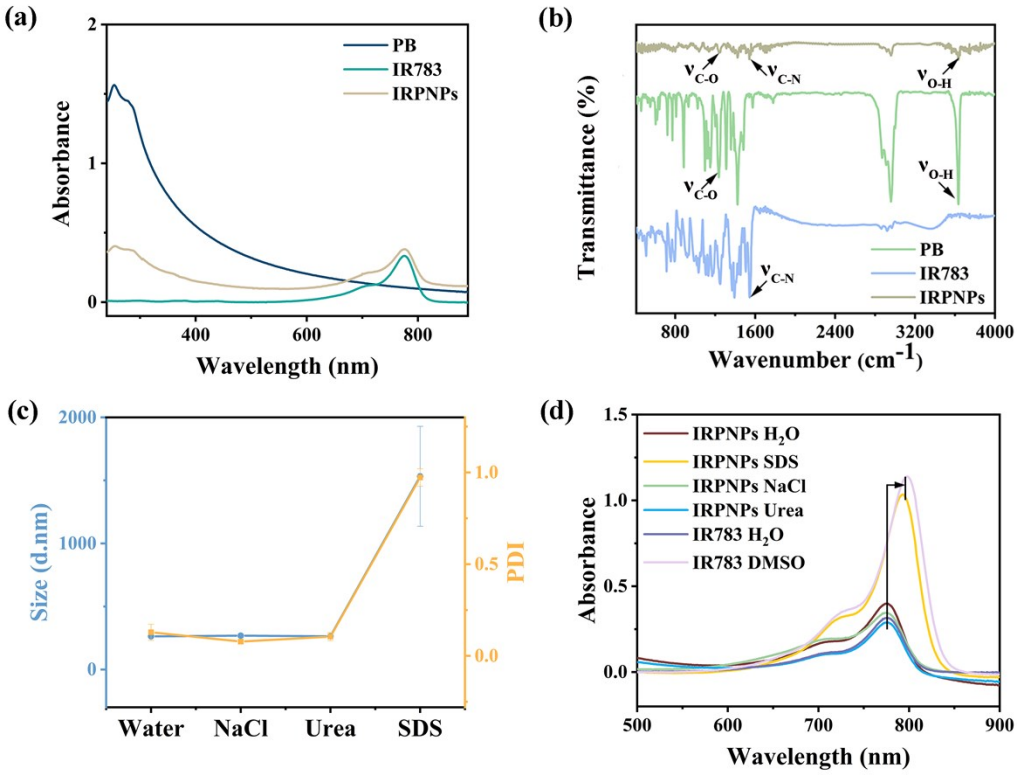

Figure S4 (a) The UV spectrum of IRPNPs; (b) The FI-IR spectrum of IRPNPs; (c) Size change and (d) UV spectrum of IRPNPs in different buffer solutions.

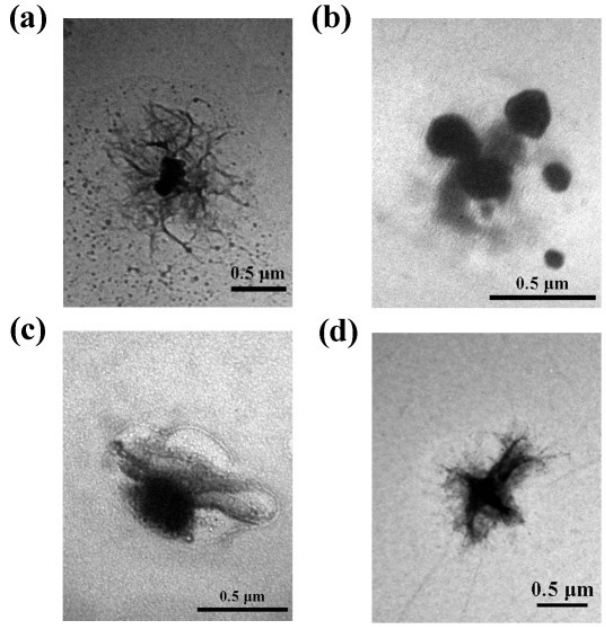

Figure S5 (a) and (b) are the TEM image of IPNPs in pH 5 acid solution and 100 μM H_2O_2 solution, respectively; (c) and (d) are the TEM image of IRPNPs in pH 5 acid solution and 100 μM H_2O_2 solution, respectively.

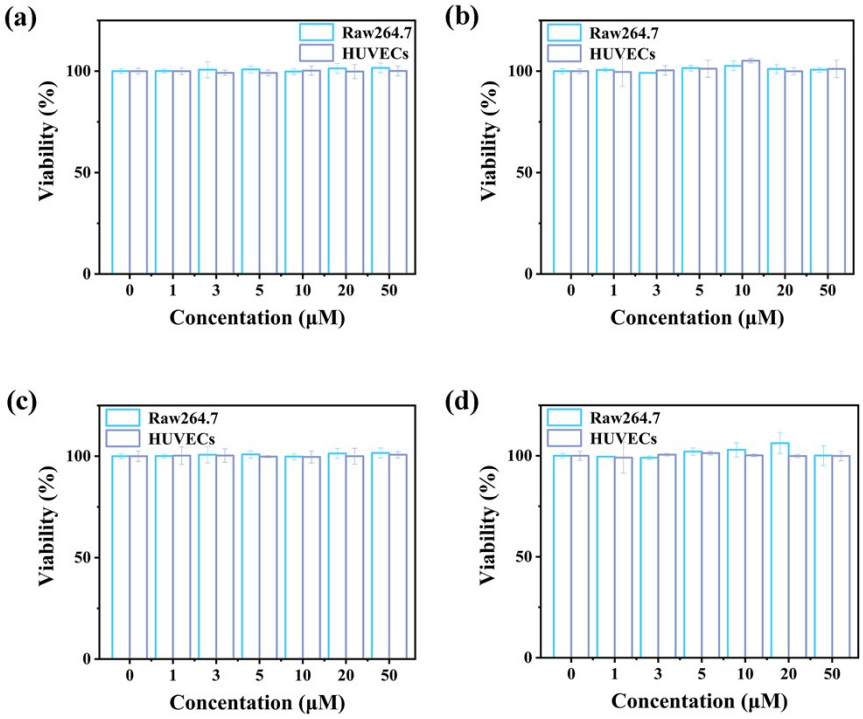

Figure S6 Cell viability assay of Raw264.7 cells and HUVECs at 24 h after incubation with various concentrations of (a) IRPNPs, (b) ICG, (c) IR783, and (d) PB.

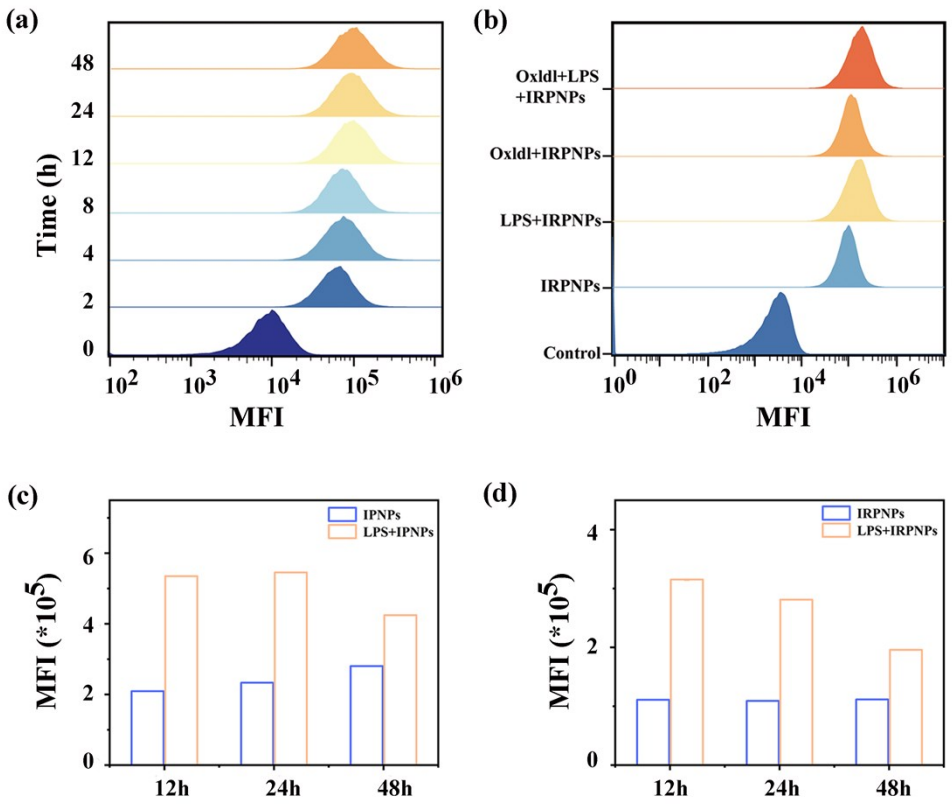

Figure S7 (a) Flow cytometric quantification of MFI internalization in Raw264.7 cells after incubation with IRPNPs for varied times; (b) Quantified MFI data showing cellular internalization of IRPNPs in Raw264.7 cells with different pre-stimulation; Quantified MFI data showing the change of (c) IPNPs and (d) IRPNPs internalization in Raw264.7 at different time after pre-stimulation with LPS.

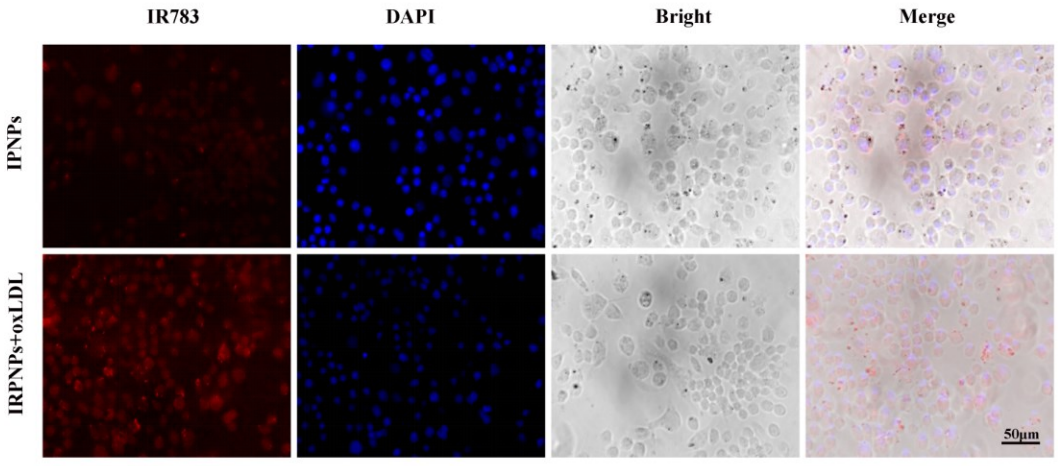

Figure S8 Fluorescence images showing the internalization of IRPNPs in Raw264.7 cells after 24 h incubation. After preincubation with fresh medium or 50 μg ml^{-1} ox-LDL for 24 h, cells were cultured with IRPNPs for 24 h, followed by Near-infrared fluorescence microscopic observation. DAPI can stain nuclei showing blue, and the IR783 fluorescence of IRPNPs was red.

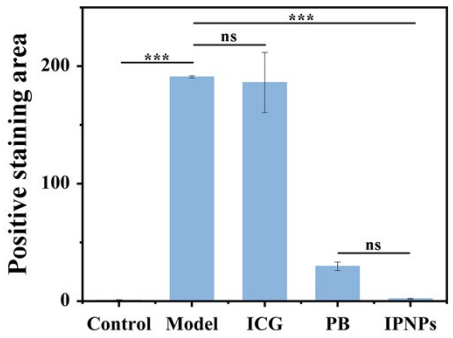

Figure S9 Quantification of positive area in Raw264.7 after ORO staining (ns $p > 0.05$; *** $p < 0.001$).

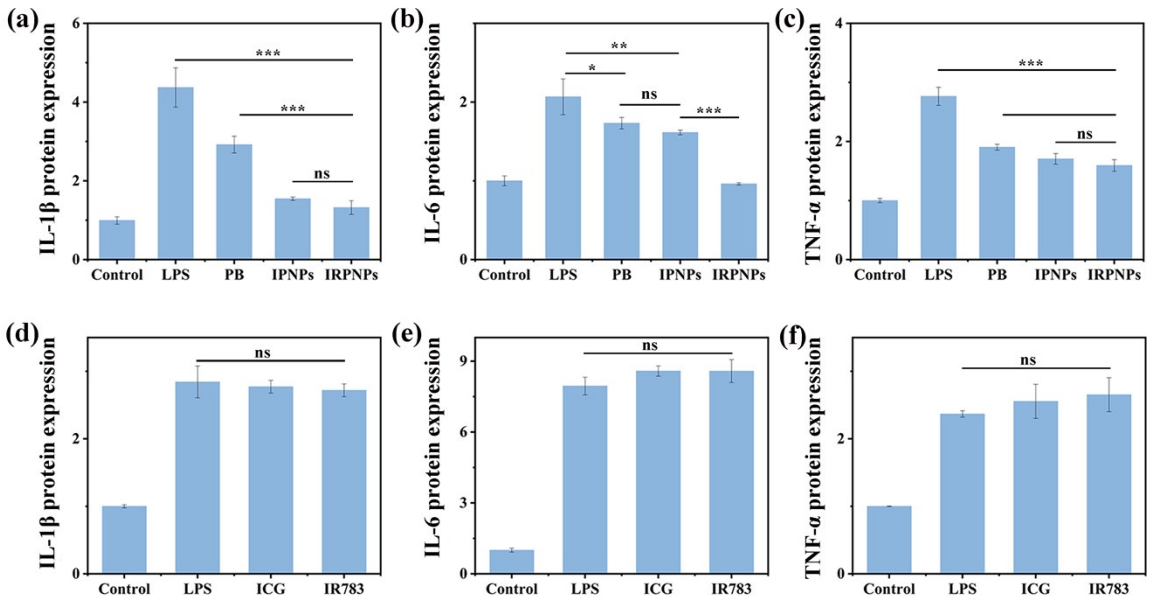

Figure S10 Quantification analysis of protein (a)(d) IL-1β, (b)(e) IL-6, (c)(f) TNF-α expression in different treatment groups (ns $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

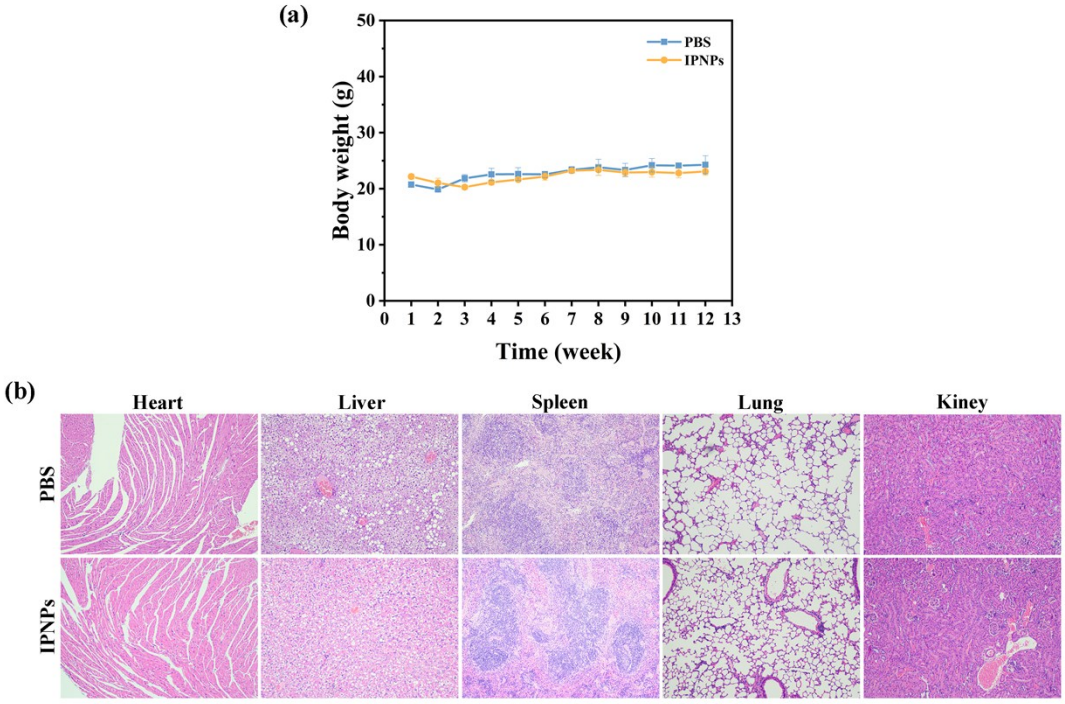

Figure S11 (a) Body weight changes, and (b) vital organs sections of AS model mice after treated with PBS or IPNPs for 12 weeks by i.v.-delivery.

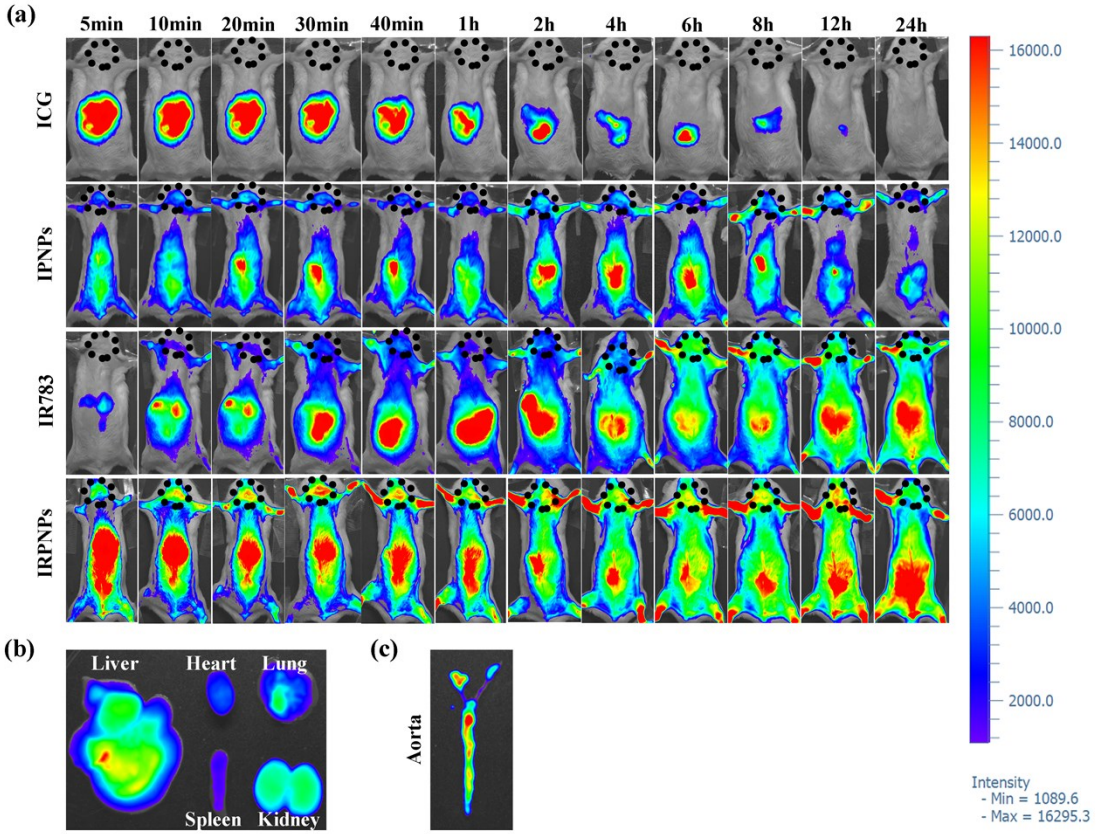

Figure S12 (a) Fluorescence images showing accumulation of different formulations by i.v.-injection in carotid artery ligation site of Kunming mice performed carotid artery ligation, the black circle is the ligation site. Isolated (b) organs imaging, and (c) aorta imaging after i.v. injected IPNPs 45 min in Kunming mice performed carotid artery ligation.

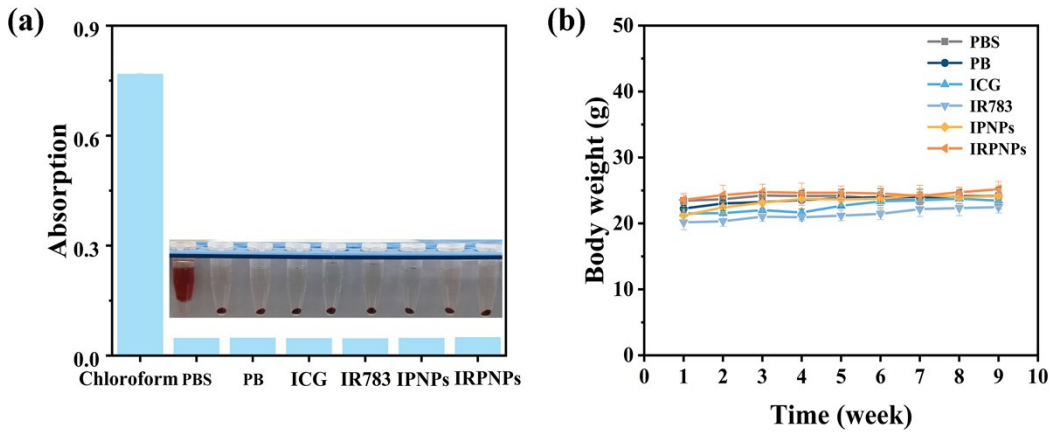

Figure S13 (a) Hemolysis analysis of hemocyte after incubation with different formulations; (b) Body weight changes of C57/BL mice after i.v. injected with different formulations.