Electronic Supplementary Information (ESI)

Macrophage Membrane-Camouflaged Nanoclusters of Ultrasmall Iron Oxide Nanoparticles for Precision Glioma Theranostics

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Experimental Section

Materials

All chemical reagents are commercially available and used as received without further purification unless otherwise stated. Iron (III) chloride was purchased from Adamas Reagent Co., Ltd. (Shanghai, China). Diethylene glycol (DEG) was from Sigma-Aldrich (St. Louis, MO). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were from GL Biochem. (Shanghai, China). Trisodium citrate dihydrate and sodium acetate anhydrous were from Sinopharm Chemical Reagent Ltd. (Shanghai, China). 4-(Bromomethyl)phenylboronic acid (PBA) was from Shanghai Bide Pharmatech Co., Ltd. (Shanghai, China). Gossypol was from Shanghai Macklin Co., Ltd. (Shanghai, China). GSH/GSSG Assay Kit was from Beyotime Institute of Biotechnology (Shanghai, China). Reactive oxygen species (ROS) assay kit (DCFH-DA kit) was from Beyotime Biotechnology Co., Ltd. (Shanghai, China). C6 cells (a rat glioma cell line), RAW264.7 (a murine macrophage cell line) and bEnd.3 cells (a murine brain microvascular endothelia cell line) were from Institute of Biochemistry and Cell Biology, the Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from Gibco (Carlsbad, CA). Penicillin and streptomycin were from Gino Biomedical Technology Co., Ltd. (Hangzhou, China). Cell Counting Kit-8 (CCK-8) was from 7Sea Biotech Co., Ltd. (Shanghai, China). C11-BODIPY$^{581/591}$ was from Shanghai Maokang Biotechnology Co., Ltd. (Shanghai, China). 4',6-Diamidino-2-phenylindole (DAPI) was acquired from BestBio Biotechnology Co., Ltd. (Shanghai, China). Regenerated cellulose dialysis membranes (molecular weight cut-off, MWCO = 1000 or 8000 Da) were supplied from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Water used in all experiments was purified by a PURIST UV Ultrapure Water system (RephiLe Bioscience, Ltd., Shanghai, China) with a resistivity higher than 18.2 MΩ.cm.

Synthesis of Gossypol-Crosslinked Nanoclusters of Ultrasmall Iron Oxide Nanoparticles (G-USIO NCs)

Citric acid-stabilized ultrasmall iron oxide nanoparticles (USIO NPs) were prepared according to our previous work.[1] To prepare the G-USIO NCs, USIO NPs (56 mg) were dispersed in water (16 mL),

and added with EDC (150 mg, 1 mL in water) under stirring for 30 min. Then, NHS (95 mg, 1 mL in water) was added into the above mixture solution under stirring for 3 h to activate the citric acid carboxyl groups on the surface of USIO NPs. The above solution was quickly added into an aqueous solution (2 mL) containing ethylenediamine (217 μL) under stirring for 3 days, and then the mixture was dialyzed against water for 3 days (2 L, 15 times) using a dialysis bag with an MWCO of 8000 Da. The dialysis liquid was collected to obtain the water dispersion of USIO-NH_2 NPs, which can be concentrated through an Amicon® Ultra-15 device (Merck, Darmstadt, Germany).

To prepare PBA-modified USIO NPs (USIO-PBA NPs), 4-(bromomethyl) PBA (1.916 mg) was dissolved in water (70 ℃, 5.736 mL), and then added into an aqueous solution of USIO-NH_2 NPs (9.67 mg, 10 mL) under continuous stirring (1200 rpm) for 24 h. Then, the mixture was dialyzed through a membrane with an MWCO of 8000 Da against water to obtain the USIO-PBA NPs.

Finally, gossypol (4.6 mg) was dispersed in ethanol absolute (1 mL), and added into an aqueous solution containing the USIO-PBA NPs (3.67 mg, 10 mL) with continuous stirring (1200 rpm) for 2 days to evaporate the ethanol. The above solution was centrifuged at 3000 rpm for 5 min to collect the precipitate that is related to the non-encapsulated gossypol. The precipitate was re-dissolved in ethanol to quantify the loading of gossypol according to the literature.[2] Then, the supernatant was also was centrifuged at 10000 rpm for 10 min, washed with water for 3 times, and lyophilized to obtain the G-USIO NCs. The gossypol loading content (LC) and entrapment efficiency (EE) were calculated according to the following equations:

$$LC\ (\%) = M_t / M_L \times 100\% \quad \text{(S1)}$$

$$EE\ (\%) = M_t / M_0 \times 100\% \quad \text{(S2)}$$

where M_t, M_L, and M_0 stand for the masses of encapsulated gossypol, the drug-loaded complexes, and the initial gossypol, respectively.

Macrophage Membrane Extraction

RAW264.7 cells (5×10^7 cells) were collected and suspended in 3 mL of hypotonic lysing buffer containing 50 mM Tris, 150 mM NaCl, 1% NP-40 lysis buffer, 0.25% sodium deoxycholate and 10%

(v/v%) phenylmethanesulfonyl fluoride (PMSF). Then, the macrophages were kept in an ice bath for 30 min, followed by repeated freeze-thaw processes (frozen in the liquid nitrogen and thawed at 37 ℃ in a water bath for 3-5 times). The processed cell suspension was then centrifuged at 850 g for 15 min to remove the precipitated intracellular components. The supernatant was collected and further centrifuged at 18000 g and 4 ℃ for 60 min. The final precipitate was collected as the purified macrophage membrane and the protein content was analyzed by a BCA protein assay kit. The protein content in the extracted macrophage membrane from 1×10^8 cells was determined to be 0.12 mg/mL.

Preparation of Macrophage Membrane-Coated G-USIO NCs (G-USIO@MM NCs)

The extracted macrophage membranes were used to coat the G-USIO NCs by fusing macrophage membrane with the G-USIO NCs suspension and extruding repeatedly *via* an Avanti micro-extruder (Avanti Polar Lipids, Inc., Alabaster, AL). Briefly, the macrophage membrane was mixed with the G-USIO NCs at a mass ratio of 1: 1 and extruded through an 0.8 μm polycarbonate membrane for at least 20 times to acquire the G-USIO@MM NCs.

Characterization Techniques

Dynamic light scattering (DLS) and zeta potential measurements were performed using a Malvern Zetasizer Nano ZS model ZEN3600 (Worcestershire, UK) equipped with a standard 633-nm laser. UV-vis spectroscopy was performed using Lambda 25 UV-vis spectrophotometer (Perkin-Elmer, Boston, MA). The prepared G-USIO NCs and G-USIO@MM NCs were observed using a JEM-2100F transmission electron microscopy (TEM, JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 200 kV. For TEM imaging, a typical sample of G-USIO NCs or G-USIO@MM NCs in an aqueous solution (0.5 mg/mL, 10 μL) was deposited onto a carbon-coated copper grid and air-dried before measurements. Thermal gravimetric analysis (TGA) was executed on an STA 8000 simultaneous thermal analyzer (PerkinElmer, Waltham, MA) from 50 to 800 ℃ under nitrogen atmosphere at a heating rate of 10 ℃/min. X-ray photoelectron spectroscopy (XPS) data were obtained with an Escalab 250Xi spectrometer (ThermoFisher Scientific, Waltham, MA) equipped with an analyzer mode (pass energy of 50 eV) and an Al Kα X-ray source. Fe concentrations were determined with inductively

coupled plasma-optical emission spectroscopy (ICP-OES, Leeman Prodigy, Hudson, NH) after the NCs were digested with 1 mL of *aqua regia* for 4 h, and diluted with water.

Protein Detection of G-USIO@MM NCs

The membrane protein of RAW264.7 cells was extracted after treatment with RIPA lysis buffer and quantitatively determined by the BCA assay kit. Then, the G-USIO NCs, macrophage membrane (MM) and G-USIO@MM NCs were investigated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the literature,[3] and the protein amount in the G-USIO@MM NCs was kept to be half as that of MM. The G-USIO NCs before membrane coating were also measured and used as control.

Evaluation of ·OH Generation

The ability of G-USIO NCs to generate ·OH was assessed through methylene blue (MB) decomposition assay. Briefly, G-USIO NCs and MB were fully mixed in phosphate buffer at pH = 6.5 with or without H_2O_2 (10 mM) under stirring in the dark (1 h) at both Fe and MB final concentrations of 10 $\mu g \cdot mL^{-1}$. The absorbance of MB was recorded by UV-vis spectroscopy at different time intervals.

***T_1* MR Relaxometry**

T_1 MR relaxometry of the G-USIO@MM NCs was performed on a 0.5-T Niumag NMI20 NMR analyzing and imaging instrument (Shanghai, China) at room temperature. Each sample was prepared by stepwise dilution with phosphate buffer (10 mM, pH = 6.5 with or without H_2O_2) to get solutions with different Fe concentrations (0.1, 0.2, 0.4, 0.8 and 1.6 mM, respectively) before measurements. The parameters were set as follows: IR sequence, point resolution of 156 mm × 156 mm, section thickness of 0.8 mm, and excitation number of 1. The r_1 relaxivity was obtained through linear fitting of the inverse T_1 relaxation time as a function of Fe concentration. Additionally, the T_1 MR images of the materials were acquired using a clinical MR system (Ingenia 3.0 T, Philips, Netherlands) and the parameters were set as follows: TE = 12 ms, TR = 523 ms, FOV = 110 × 110 mm and Matrix = 304 × 268.

Responsive Drug Release from the G-USIO@MM NCs

To study the release profile of the loaded gossypol and Fe, the G-USIO@MM NCs were suspended in 2 mL of phosphate buffer (pH = 6.5) with or without H_2O_2 (10 mM). Typically, an aqueous solution of the G-USIO@MM NCs ([Fe] = 169 μg·mL^{-1}, 2 mL) was placed in a dialysis bag (MWCO = 1000), and then exposed to 9 mL of different phosphate buffers. The entire system was kept in a constant temperature vibrator at 37 °C under gentle shaking. At different time intervals, 1 mL of solution was taken out from the outer phase and the volume of outer phase was maintained constant by adding 1 mL of the corresponding buffer solution. The amounts of released gossypol and Fe were measured *via* UV-vis spectrophotometer at 380 nm and Leeman Prodigy ICP-OES (Hudson, NH), respectively.

Cell Culture and Cellular Uptake Assay

C6 cells and bEnd.3 cells were regularly cultured and passaged using DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. RAW264.7 cells were regularly cultured and passaged using DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. The cells were cultured in a cell incubator at 37 ℃ and 5% CO_2.

To check the cellular uptake efficiency, C6 cells were seeded in a 12-well plate at a density of 1 × 10^5 cells per well in 1 mL of DMEM. After overnight incubation, the medium of each well was replaced with 1 mL of fresh medium containing USIO NPs, G-USIO NCs, or G-USIO@MM NCs ([Fe] = 5, 10, 20, 30, 40 and 50 μg·mL^{-1}, respectively), and cells were incubated for 4 h at 37 °C and 5% CO_2. Then, the cells were collected, digested with 1 mL of *aqua regia* for 4 h, and diluted with water before ICP-OES analysis of Fe contents in the cells.

Cell Viability Assay

We evaluated the *in vitro* cytotoxicity of USIO NPs, free gossypol, G-USIO NCs and G-USIO@MM NCs by standard CCK-8 assay kit. In brief, C6 cells were seeded in 96-well plates at the density of 1×10^4 cells per well with 100 μL of DMEM and cultured overnight at 37 ℃ and 5% CO_2. The next day, the medium in each well was replaced with fresh medium containing USIO NPs, gossypol, G-USIO NCs or G-USIO@MM NCs at different Fe concentrations (10, 20, 30, 40, and 50 μg·mL^{-1},

respectively, and the corresponding [gossypol] = 2.1, 4.2, 6.2, 8.3, and 10.4 μg·mL^{-1}, respectively). After 24 h culture, cells were washed three times with phosphate buffered saline (PBS), treated with 100 μL of medium containing 10% CCK-8 in each well, and regularly incubated for additional 2 h. A Thermo Scientific Multiskan MK3 ELISA reader (Waltham, MA) was used to record the absorbance of each well at 450 nm. For each sample, 6 parallel wells were analyzed to give the mean value and standard deviation (SD). Similarly, to investigate the effect of USIO NPs, free gossypol, G-USIO NCs or G-USIO@MM NCs on the viability of normal cells, bEnd.3 cells were treated and assayed under the identical experiment conditions.

Intracellular Depletion of GSH

The GSH/GSSG Assay Kit was applied to test the GSH depletion in cells after different treatments. In brief, C6 cells were seeded in 6-well plates at a density of 2.0 × 10^5 cells per well with 2 mL fresh DMEM and cultured for 12 h (37 °C and 5% CO_2). Then, the medium of each well was replaced with fresh medium containing USIO NPs, free gossypol, G-USIO NCs or G-USIO@MM NCs ([Fe] = 30 μg·mL^{-1}, the corresponding [gossypol] = 6.2 μg·mL^{-1}), and cells were cultured for additional 6 h. PBS was used as a negative control. Then, the cells were digested by trypsin and collected to detect the intracellular GSH level according to the manufacturer's instructions. The intracellular GSH level in PBS-treated C6 cells was defined as 100%.

Intracellular ROS Generation

To detect the intracellular ROS generation, DCFH-DA kit was used to observe cells under confocal laser scanning microscopy (CLSM, Carl Zeiss LSM 700, Jena, Germany). Briefly, C6 cells were cultured in confocal dishes at a density of 2 × 10^5 cells per dish with 1 mL medium for 12 h, and incubated with USIO NPs, free gossypol, G-USIO NCs or G-USIO@MM NCs ([Fe] = 30 μg·mL^{-1}) in DMEM for 6 h (cells treated with PBS were used as a negative control). Afterwards, cells in each well were treated with DCFH-DA (10 μM in 1 mL FBS-free DMEM), and cultured for another 30 min at 37 °C in the dark. The cells were washed by PBS for 3 times, fixed by glutaraldehyde (2.5% in PBS,

4 °C) for 20 min, and counterstained with DAPI (10 μg·mL^{-1}) at 37 °C for 20 min. Then, the cells were washed by PBS thrice, and visualized by CLSM. The excitation wavelengths for DCF and DAPI were 488 nm and 405 nm, respectively.

Intracellular LPO Accumulation

C6 cells were cultured in confocal dishes at a density of 2 × 10^5 cells per dish with 1 mL medium for 24 h, and incubated with USIO NPs, free gossypol, G-USIO NCs or G-USIO@MM NCs ([Fe] = 30 μg·mL^{-1}, the corresponding [gossypol] = 6.2 μg·mL^{-1}) in DMEM for 6 h (PBS was used as a negative control). Afterwards, the medium was substituted by FBS-free DMEM containing C11-BODIPY$^{581/591}$ (5 μM), and the cells were cultured for another 20 min at 37 °C in the dark. Then, the cells were washed by PBS for 3 times, fixed with glutaraldehyde (2.5% in PBS, 4 °C) for 20 min, washed by PBS thrice, stained with DAPI (10 μg·mL^{-1} in PBS, 37 °C) for 20 min, and washed with PBS thrice before CLSM observation. The excitation wavelengths for non-oxidized C11-BODIPY$^{581/591}$, oxidized C11-BODIPY$^{581/591}$ and DAPI were set at 581 nm, 500 nm and 405 nm, respectively.

Cell Apoptosis Assay

In brief, C6 cells were cultured in 6-well plates at a density of 2 × 10^5 cells per well with 2 mL medium for 12 h, and treated with USIO NPs, free gossypol, G-USIO NCs or G-USIO@MM NCs ([Fe] = 30 μg·mL^{-1}, the corresponding [gossypol] = 6.2 μg·mL^{-1}) in DMEM for 6 h (cells treated with PBS was used as a negative control). Afterwards, the cells were washed by PBS for 3 times, digested with trypsin, centrifuged (1000 rpm, 5 min), dispersed in binding buffer, sequentially stained with Annexin V-FITC for 10 min and PI for 5 min at 37 °C both in the dark, and analyzed by flow cytometry *via* a Becton Dickinson FACScan flow cytometer (Franklin Lakes, NJ). Each measurement was repeated for three times.

Western Blot (WB) Assay

The expression of tumor apoptosis-related proteins (Bcl-2, p53, PTEN, and Bax) was evaluated by WB assay. To be brief, C6 cells were cultured in 6-well plates at a density of 2 × 10^5 cells per well with 2 mL medium for 12 h, and treated with USIO NPs, free gossypol, G-USIO NCs or G-

USIO@MM NCs ([Fe] = 30 μg·mL^{-1}, the corresponding [gossypol] = 6.2 μg·mL^{-1}) in DMEM for 24 h (cells treated with PBS was used as a negative control). After that, cells were digested, collected, washed with PBS for 3 times and further treated with the lysis buffer containing phenylmethanesulfonyl fluoride in ice bath for 30 min to extract proteins. The lysates were centrifuged at 12000 rpm and 4 °C for 5 min. Thereafter, the supernatant for each sample was collected for WB assay according to standard protocols reported in the literature.[4]

T_1-Weighted MR Imaging of Tumors *in Vivo*

All animal experiments were carried out after approval by the Ethical Committee for Experimental Animal Care and Use of Donghua University (approval number: DHUEC-NSFC-2023-02) and also according to the policy of the National Ministry of Health of China. The orthotopic brain glioma model was established for *in vivo* MR imaging. ICR mice (6-8 weeks) were purchased from Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China). Each mouse was slowly inoculated with 1 × 10^5 C6 cells (5 μL in PBS) into the right striatum within 5 min (2.0 mm back from the bregma, 2.0 mm lateral from the sagittal suture, and 3.0 mm of depth). After inoculation for 5-7 days, MR imaging was used to monitor and confirm whether the orthotopic brain glioma model was established according to the literature.[5] Before MR imaging, each mouse was injected with Magnevist® (9.38 mg/mouse, in 100 μL PBS) as the contrast agent.

The established orthotopic glioma model was used for *in vivo* MR imaging. After anesthetized, we firstly scanned the baseline of T_1-weighted MR images before injection of the contrast agent. Then, the mice were scanned at the time points of 0, 15, 30, 45, 60 and 120 min postinjection of G-USIO NCs or G-USIO@MM NCs at the same Fe content (4.2 mg/kg, in 200 μL PBS for each mouse). Additionally, the T_1 MR images of the mice were acquired using a clinical MR system (Ingenia 3.0 T, Philips, Netherlands). The parameters of the clinical MR system were set as follows: TE = 15 ms, TR = 500 ms, FOV = 41×41 mm and Matrix = 84 × 78.

Antitumor Therapeutic Efficacy *in Vivo*

The orthotopic C6 glioma-bearing ICR mice were randomly divided into five experimental groups (n

= 5 for each group) and intravenously injected with PBS (100 μL), or PBS (100 μL) containing USIO NPs, gossypol, G-USIO NCs or G-USIO@MM NCs ([gossypol] = 5 mg/kg for all gossypol-related groups, and the concentration of USIO NPs corresponded to the gossypol-incorporated groups) for each mouse. The injection was performed every 3 days for totally 3 times. Simultaneously, MR imaging was utilized to monitor and record the tumor volume changes on day 0, day 3, day 6, day 10, respectively through the RadiAnt DICOM Viewer Version 2023 (https://www.radiantviewer.com/). Magnevist® (9.38 mg/mouse, in 100 μL PBS) was used as the contrast agent. In addition, the body weight of each mouse was measured every two days for 10 days. The tumor volume (V) was calculated according to the formula of $V = L \times W^2/2$, where L and W were the longest and shortest diameters of the tumor, respectively. The relative tumor volume was calculated based on the initial tumor volume before the first injection on the same day.

For histological analysis, the tumor-bearing mice were sacrificed on the 11th day postinjection of different materials. Vital organs (heart, liver, spleen, lung, kidney and brain) and tumor tissues were extracted, fixed in 4% paraformaldehyde overnight, and sectioned for hematoxylin and eosin (H&E) staining. Meanwhile, the tumors were sectioned for TdT-mediated dUTP Nick-End Labeling (TUNEL) and Ki67 staining. The H&E, Ki 67, and TUNEL stainings were performed according to standard protocols reported in the literature.[6]

Hemocompatibility, Biodistribution, Blood Routine and Blood Biochemical Assays *in Vivo*

In vitro hemolysis assay of the G-USIO@MM NCs was performed according to the literature.[7] Different concentrations of G-USIO@MM NCs were incubated with mouse red blood cells (RBCs), and the hemolysis rates were analyzed using UV-vis spectroscopy.

To investigate the biodistribution and metabolism of NCs, orthotopic C6 glioma-bearing mice were treated with G-USIO NCs or G-USIO@MM NCs (100 μL in PBS, [Fe] = 24.19 mg/kg for each mouse) *via* tail vein injection. At different time points postinjection, the mice were sacrificed, and the heart, liver, spleen, lung, kidney, and tumor were extracted, weighed, cut into small pieces, and digested with *aqua regia* for two days. After that, each sample was diluted by water to have a volume

of 15 mL, and analyzed by ICP-OES to quantify the Fe content. The data were expressed as mean ± SD (n = 3).

For blood routine and blood biochemical analysis, USIO NPs, gossypol, G-USIO NCs or G-USIO@MM NCs ([gossypol] = 5 mg/kg, in 100 μL PBS for each mouse) were intravenously injected into the healthy mice, and the PBS-treated mice were used as control. Blood samples were obtained by removing the mice eyeball after the mice were anaesthetized at 14 days postinjection (n = 3 for each group), which were then analyzed to investigate the liver function (alanine transaminase (ALT), aspartate transaminase (AST)) and kidney function (serum creatinine (CREA), uric acid (UA)). Besides, the blood routine tests including hemoglobin (HGB), RBC, white blood cell (WBC), hematocrit (HCT), mean corpusular hemoglobin concerntration (MCHC), and platelet (PLT) were also carried out.

Statistical Analysis

All experimental data were displayed as the mean ± SD (n ≥ 3). One-way analysis of variance statistical method was used to analyze the experimental results through IBM SPSS Statistic 25 software (IBM, Armonk, NY). In all cases, a p value of 0.05 was chosen as the significance level, and the data were marked with (*) for $p < 0.05$, (**) for $p < 0.01$, and (***) for $p < 0.001$, respectively.

Table S1. The EE (%) and LC (%) of gossypol in G-USIO NCs at different PBA/gossypol molar ratios. Data are shown as mean ± SD (n = 3).

Molar ratio (PBA: gossypol)	LC%	EE%
1: 3	11.9%	96.3%
1: 5	20.8%	90.5%
1: 8	15.7%	80.9%

Table S2. Zeta potentials and hydrodynamic sizes of the pristine USIO NPs, USIO-NH_2 NPs, USIO-PBA NPs, G-USIO NCs and G-USIO@MM NCs. Data are shown as mean ± SD (n = 3).

Samples	Zeta potential (mV)	Hydrodynamic size (nm)	Polydispersity index (PDI)
USIO NPs	-41.1 ± 1.4	32.3 ± 20.0	0.469 ± 0.117
USIO-NH_2 NPs	-16.4 ± 0.5	105.8 ± 16.1	0.123 ± 0.039
USIO-PBA NPs	-20.3 ± 0.9	154.7 ± 16.3	0.280 ± 0.005
G-USIO NCs	-11.0 ± 1.1	195.2 ± 38.4	0.324 ± 0.025
G-USIO@MM NCs	-26.4 ± 1.5	323.3 ± 17.0	0.470 ± 0.246

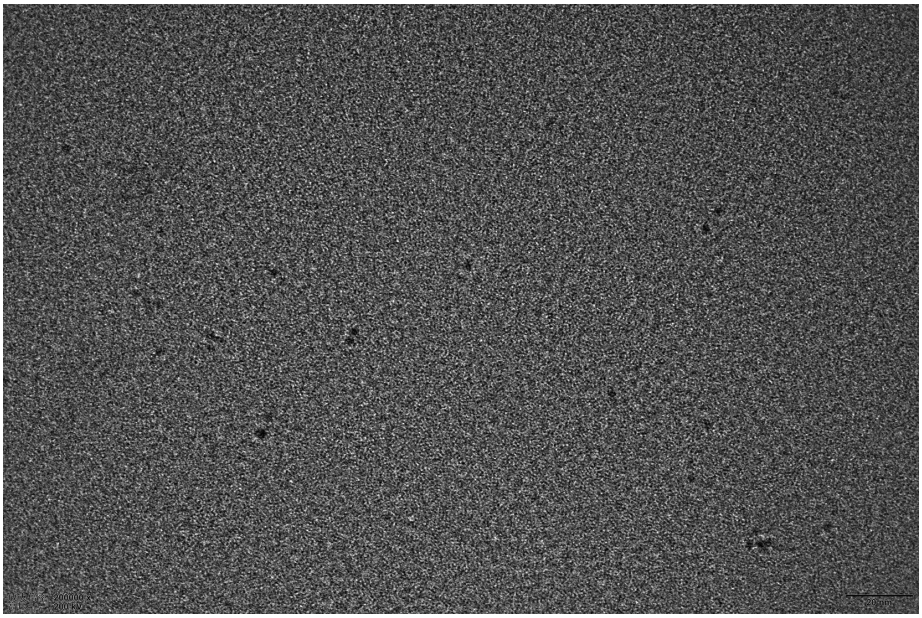

Fig. S1. TEM image of USIO NPs.

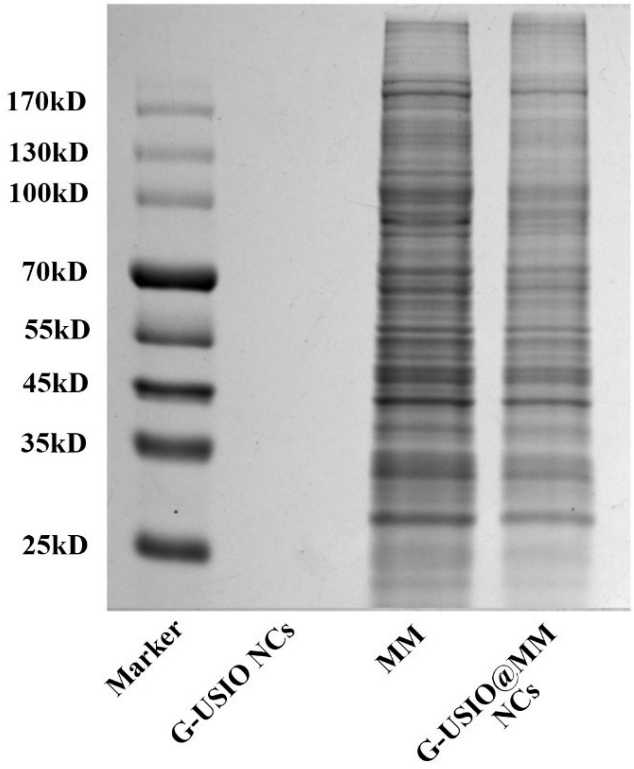

Fig. S2. SDS-PAGE analysis of G-USIO NCs, MM and G-USIO@MM NCs.

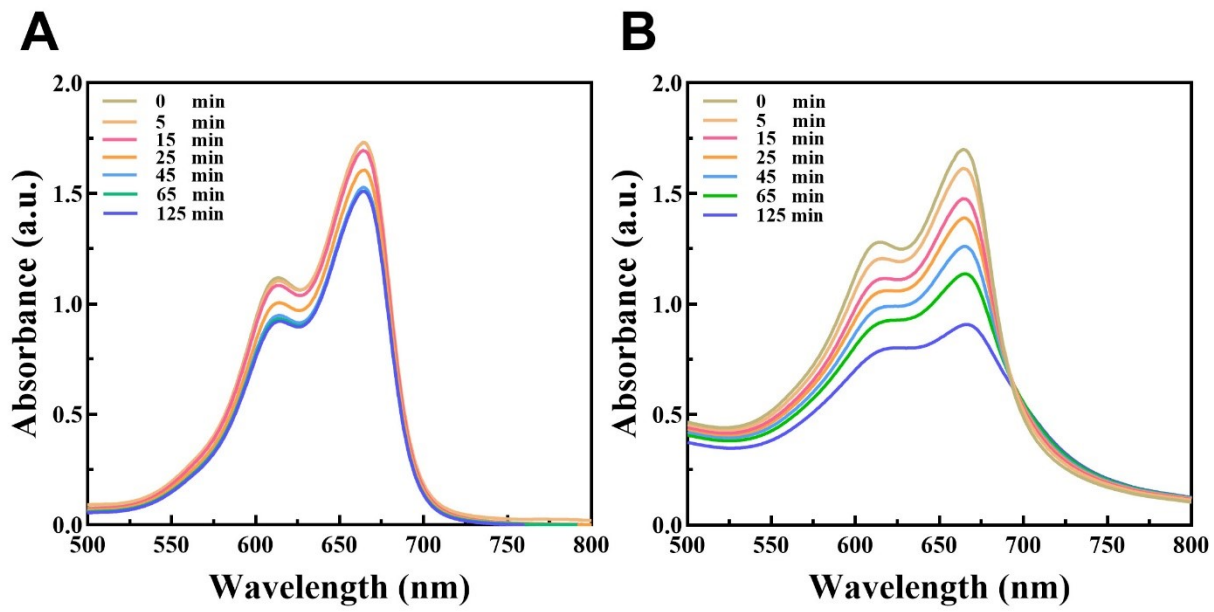

Fig. S3. Degradation of the MB (10 μg·mL^{-1}) in the absence (A) and presence (B) of G-USIO NCs ([Fe] = 10 μg·mL^{-1}) at different time points in phosphate buffer (pH 6.5).

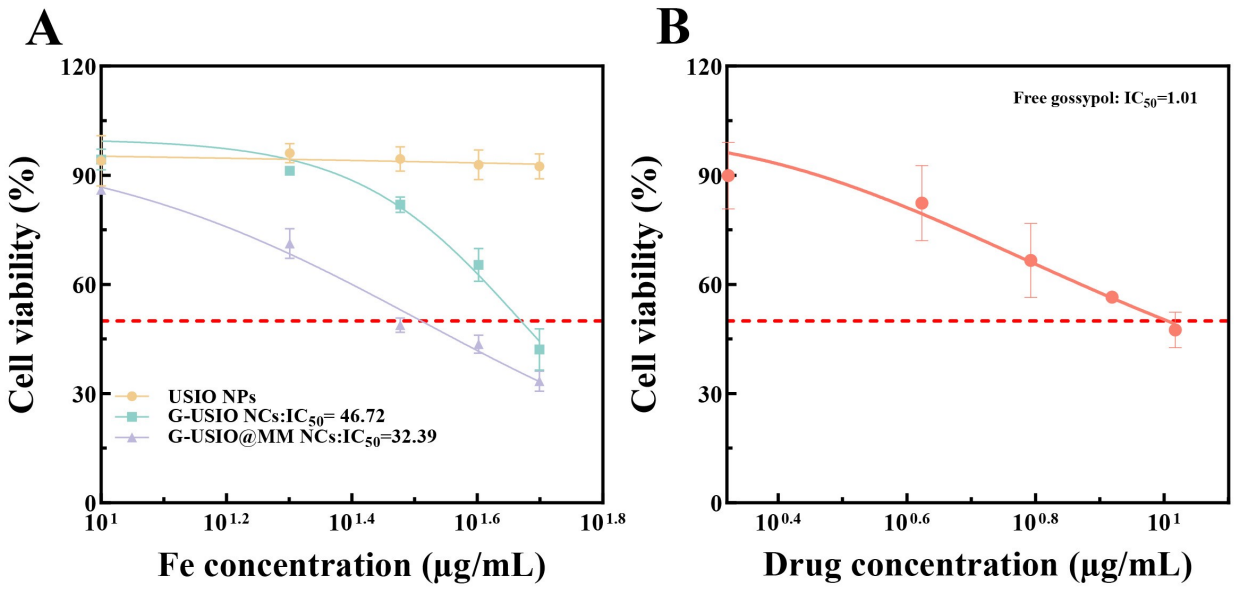

Fig. S4. (A) IC_{50} of USIO NPs, G-USIO NCs, and G-USIO@MM NCs and (B) IC_{50} of free gossypol for C6 cells treated for 24 h (n = 6).

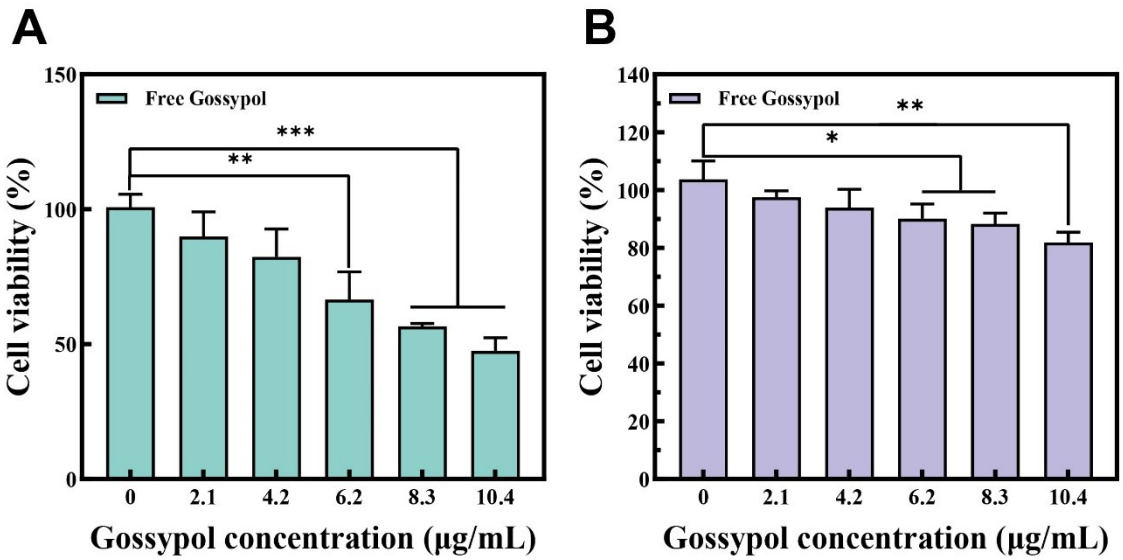

Fig. S5. Viability of C6 cells (A) and bEnd.3 cells (B) after treatment with free gossypol at different concentrations for 24 h. *, ** and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively (n = 6).

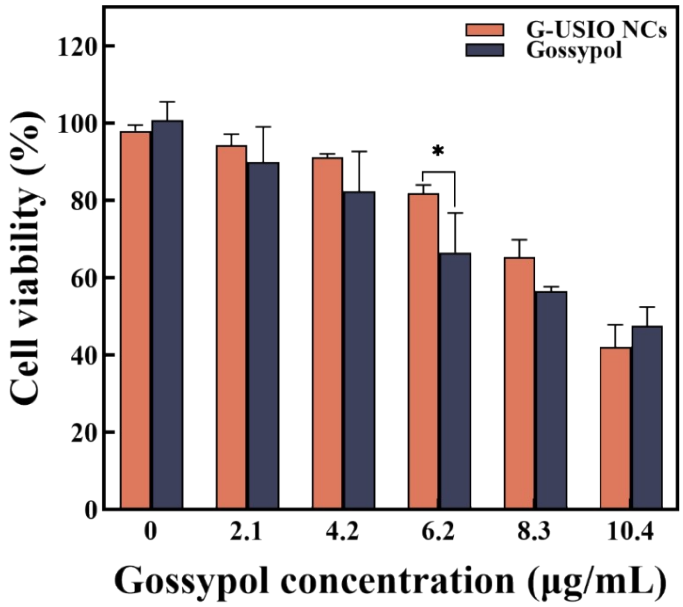

Fig. S6. Viability of C6 cells after treatment with G-USIO NCs and free gossypol at different concentrations for 24 h. * is for $p < 0.05$ (n = 6).

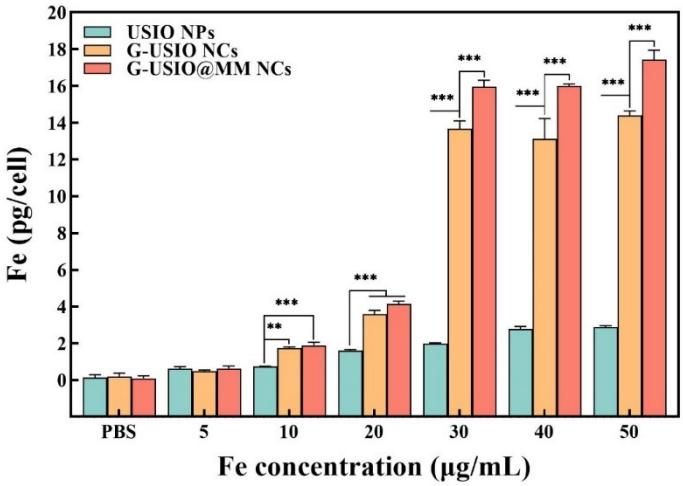

Fig. S7. Cellular uptake of Fe contents in C6 cells after cells were incubated with USIO NPs, G-USIO NCs or G-USIO@MM NCs for 4 h at different Fe concentrations as analyzed by ICP-OES. Here, ** and *** represent $p < 0.01$ and $p < 0.001$, respectively (n = 3).

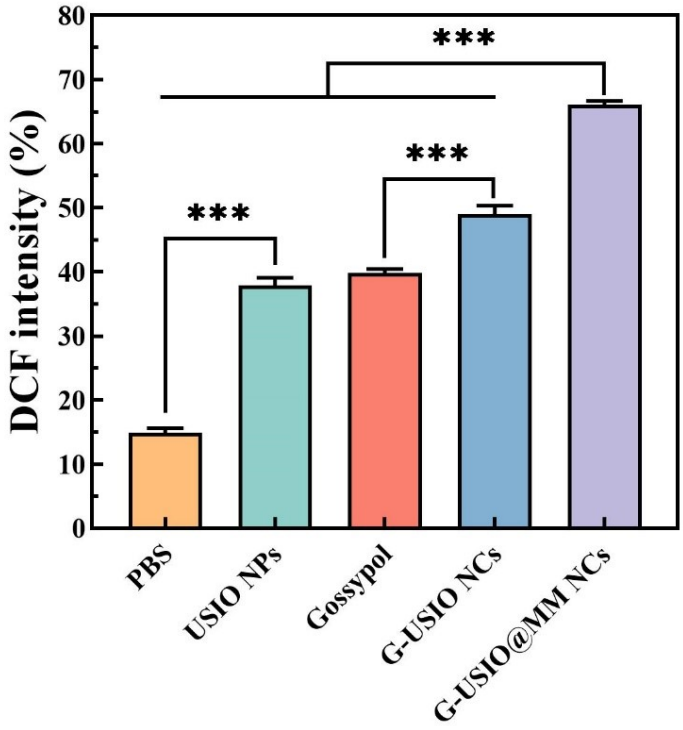

Fig. S8. The quantified DCF fluorescence intensity in C6 cells in the CLSM images as analyzed by Image J software ([Fe] = 30 μg·mL^{-1}). Here, *** is for $p < 0.001$ (n = 3).

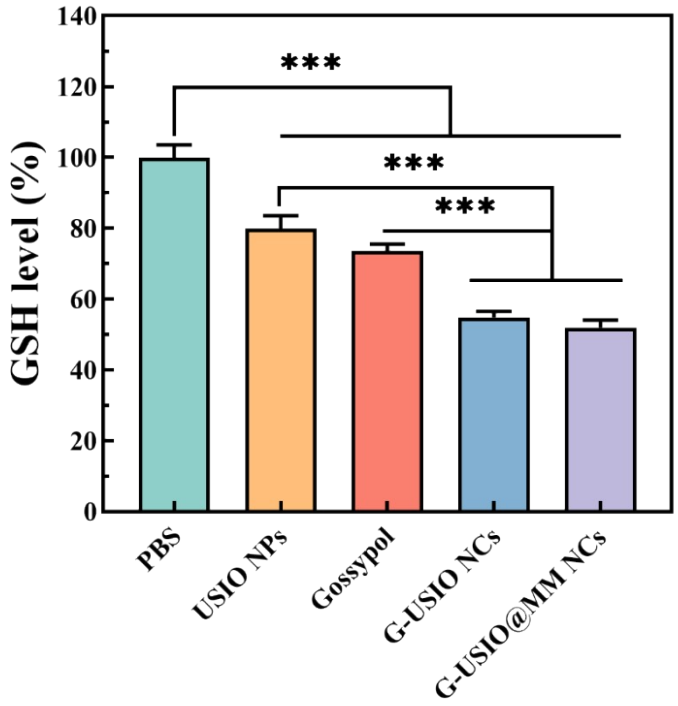

Fig. S9. The GSH level in C6 cells after treatment with different materials ([Fe] = 30 μg·mL^{-1}) for 6 h. Here, *** is for $p < 0.001$ (n = 3).

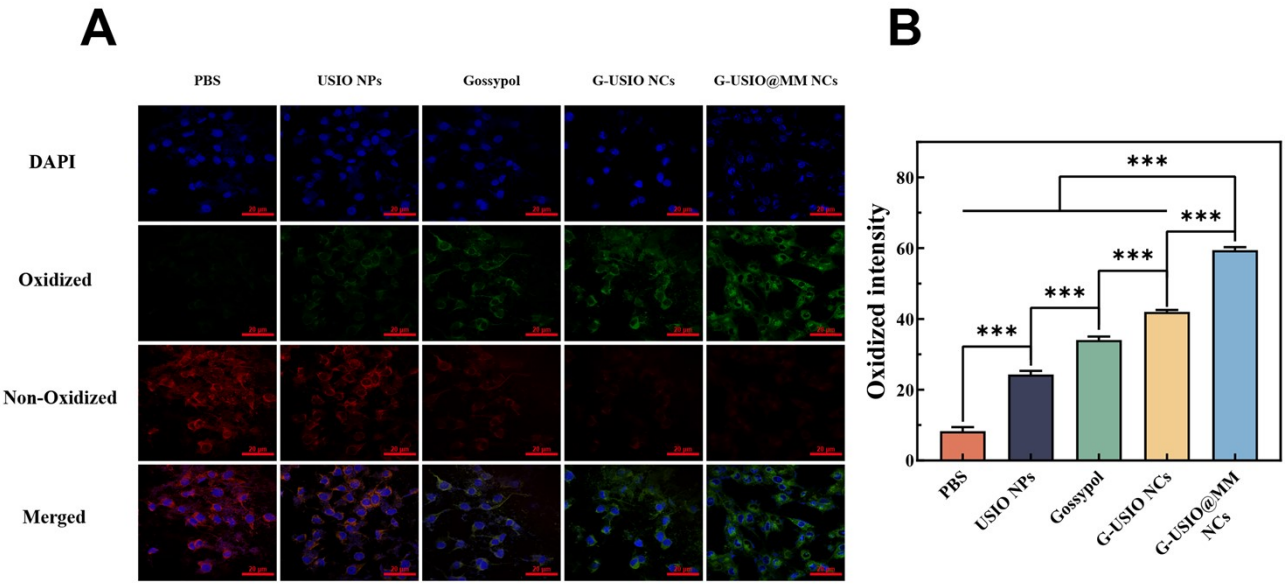

Fig. S10. (A) CLSM images (scale bar = 20 μm) and (B) quantitative fluorescence intensity of lipid peroxide (LPO) in C6 cells after treated by USIO NPs, gossypol, G-USIO NCs, or G-USIO@MM NCs. In (B), *** is for $p < 0.001$ (n = 3).

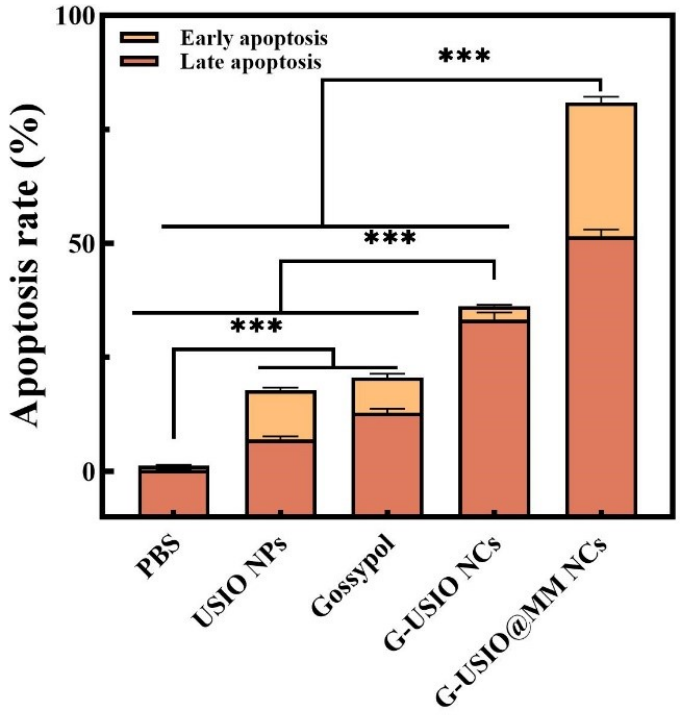

Fig. S11. Apoptosis rate of C6 cells after incubation with USIO NPs, gossypol, G-USIO NCs, or G-USIO@MM NCs ([Fe] = 30 μg·mL^{-1}, [gossypol] = 6.2 μg·mL^{-1}) for 6 h. Here, *** is for $p < 0.001$ (n = 3).

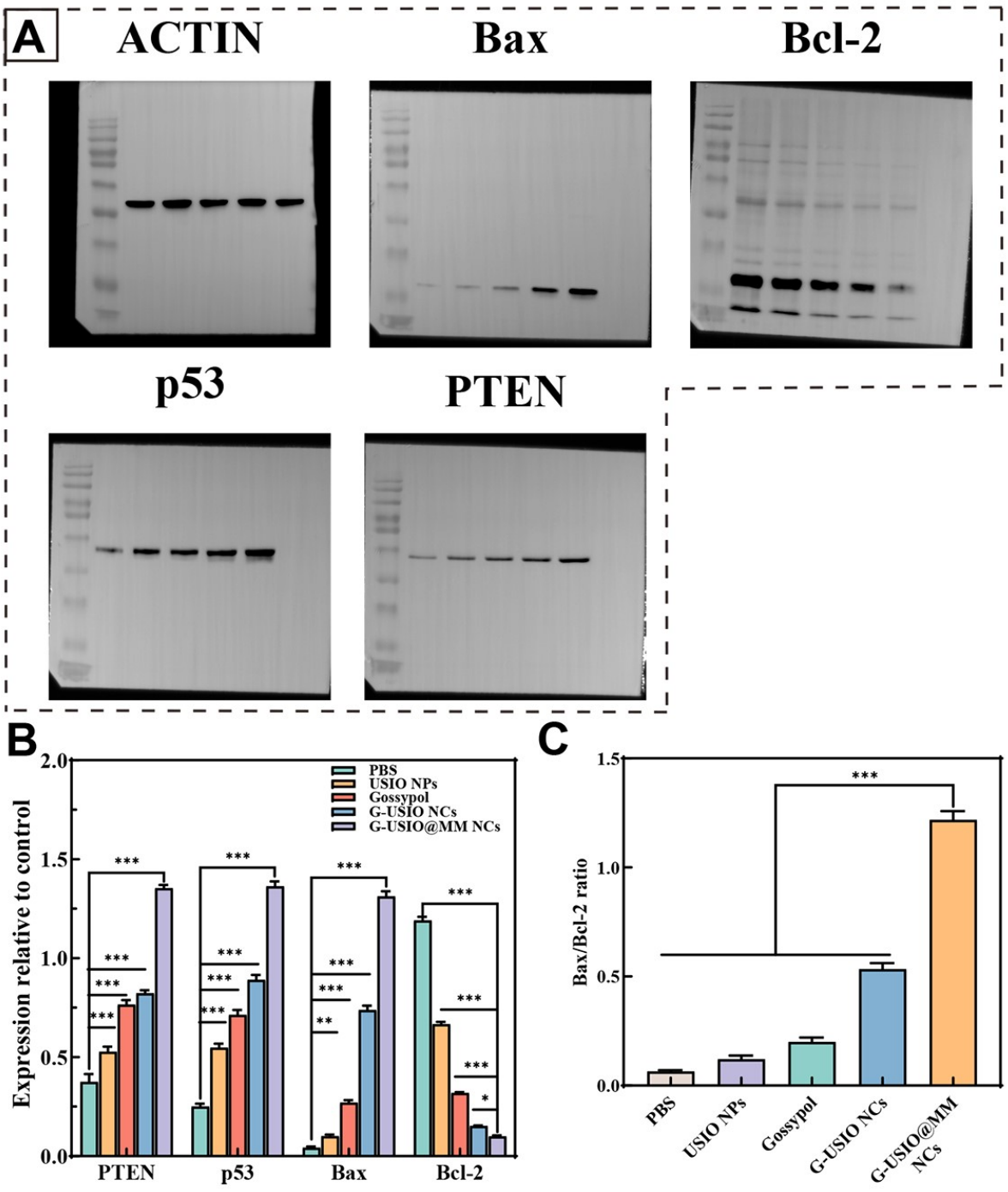

Fig. S12. (A) WB of Bax, Bcl-2, p53, and PTEN protein, (B) quantitative analysis of Bax, Bcl-2, p53, and PTEN protein expression levels and (C) Bax/Bcl-2 ratio in different groups based on the WB data. In parts A and B, *, ** and *** represent $p < 0.05$, $p < 0.01$ and for $p < 0.001$, respectively (n = 3).

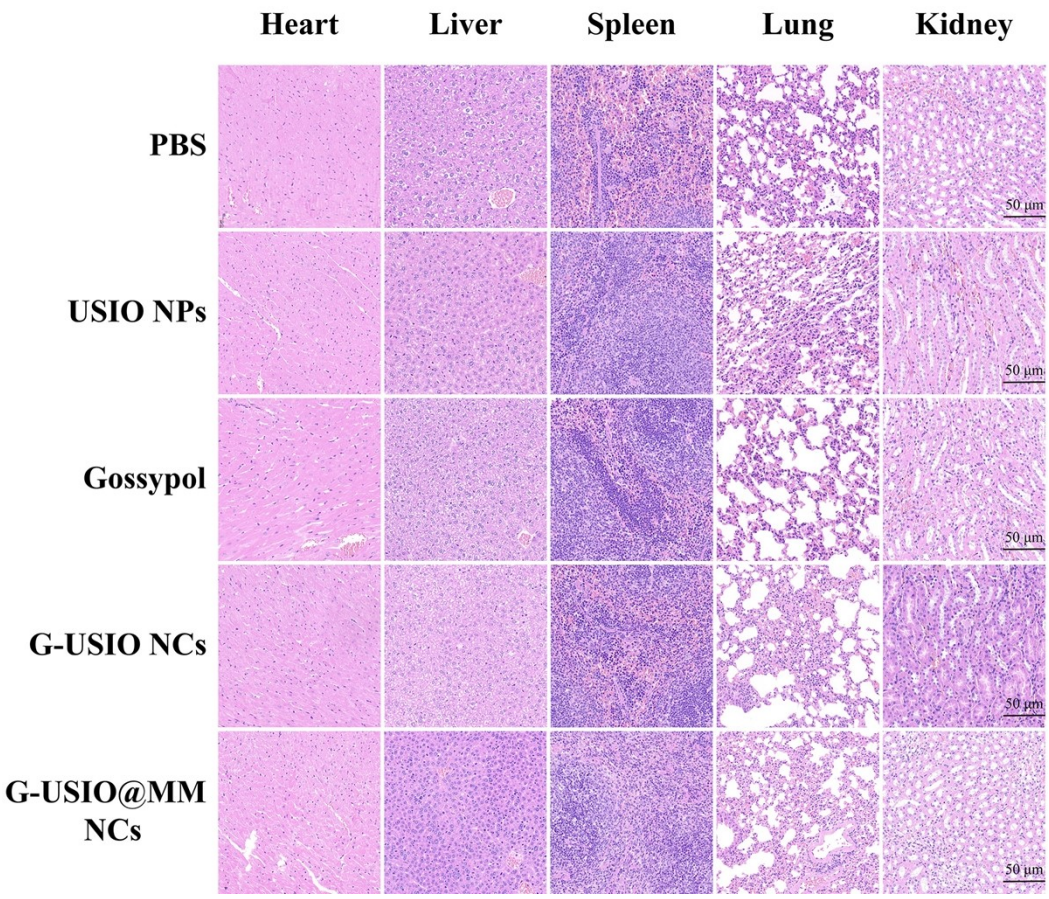

Fig. S13. H&E staining of slices of the heart, liver, spleen, lung and kidney from orthotopic C6 glioma-bearing mice after different treatments for 10 days. The scale bar in each panel represents 50 μm.

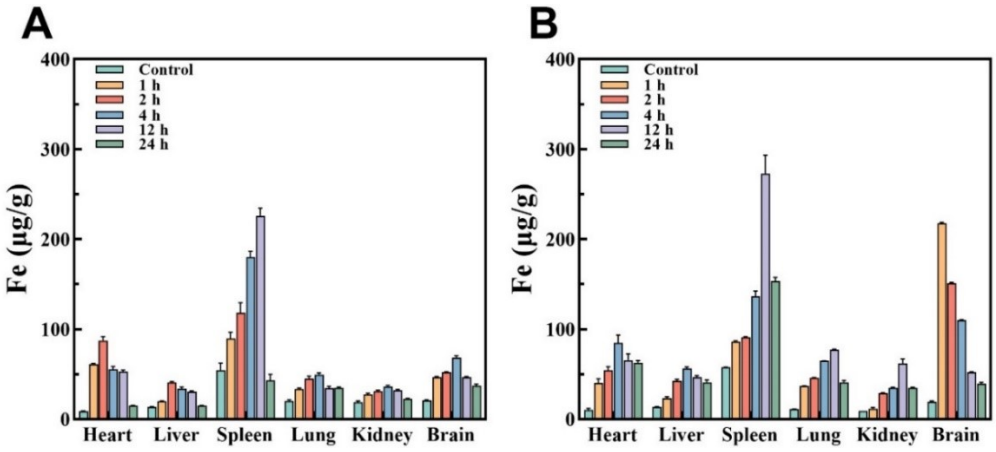

Fig. S14. *In vivo* biodistribution of Fe in the major organs and brain of the mice after tail vein intravenous injection of the G-USIO NCs (A) and G-USIO@MM NCs (B) ([Fe] = 24.19 mg/kg, 100 μL in PBS for each mouse) at different time points (0 h, 1 h, 2 h, 4 h, 12 h, and 24 h, respectively) postinjection (n = 3).

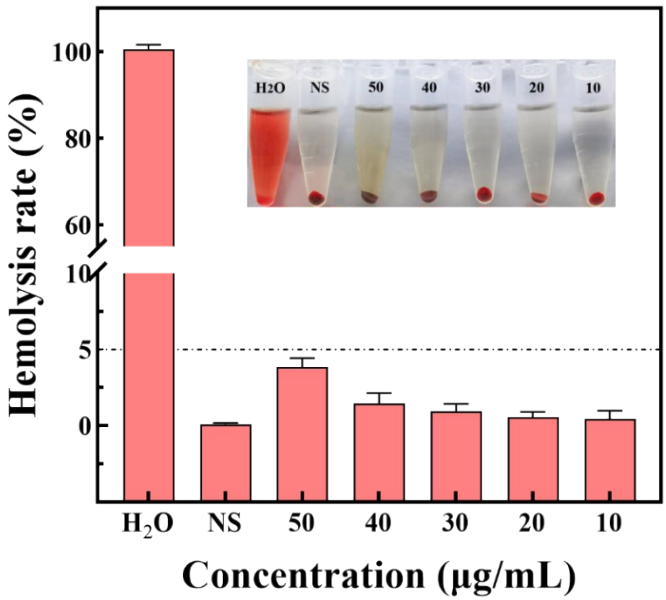

Fig. S15. Hemolysis rates of RBCs after exposed to H_2O, normal saline (NS), and NS containing G-USIO@MM NCs at different Fe concentrations for 2 h (n = 3). Inset shows the photograph of suspensions of RBCs treated with the G-USIO@MM NCs at the corresponding concentrations for 2 h, followed by centrifugation. Water and PBS were used as positive and negative controls, respectively.

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