Supporting Information

CuO Dots Decorated Gd_2O_3@Cu Core-Shell Hierarchical Structure for Cu(I) Self-supplying Chemodynamic Therapy in Combination with MRI-guided Photothermal Synergistic Therapy

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Experimental section

Materials. All chemical reagents were used as received without further purification. $Fe(acac)_3$, $Gd(acac)_3$, $Cu(acac)_2$, 3′, 3′, 5′, 5′-tetramethylbenzidine (TMB), glutathione (GSH), N-ethyl-N′-(3-(dimethylamino)propyl) carbodiimide (EDC), 5,5-dimethyl-1-pyrroline N-oxide, sodium polyacrylate, and polyvinylpyrrolidone (PVP-K30) were purchased from Aladdin Chemical Co. Ltd. (Shanghai, China). $FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, Ethylene glycol (EG), diethylene glycol (DEG), and triethanolamine (TEOA) were provided by the Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). The Human OH ELISA Kit were purchased by Shang Hai Jianglai Biological Technology Co., Ltd. The cell counting kit-8 (CCK-8) assay was obtained from Dojindo (Japan). The transferrin-PEG-NH_2 was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. The 2,7-dichlorofluorescein diacetate (DCFH-DA) was obtained from Nanjing Jiancheng Bioengineering Institute. In addition, all other chemicals were obtained from the Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

Synthesis of CuGd core-shell nanoparticles. Firstly, copper acetylacetone (0.3 g) and gadolinium acetylacetone (0.2 g) were added into diethylene glycol (DEG), and heated to 130 ^oC under magnetic stirring for approximately 30 min. After that, 1 mL of triethanolamine (TEOA) was injected into the mixed solution and continuously stirred at 130 ^oC for 15 min. Subsequently, the resulting solution was transferred into a micro-wave reaction system, and the reaction parameter was set as follows: power (200 W), temperature (260 ^oC), running time (10 min), keeping time (20 min), and

pressure (150 psi). After the reaction, the obtained solution was centrifuged at 12000 rpm/min, and then the product was washed with alcohol for three times.

Synthesis of the malachite and USIO. The malachite was synthesized according to previously reported.[1] Briefly, 1 mL of human serum albumin was dissolved in 20 mL of the aqueous solution including 50 mM of Na_2CO_3 solution. Subsequently, the mixed solution was stirred for 20 min at room temperature. After that, $CuSO_4$ solution (25 mM) was dropwise added into the mixed solution, and then reacted for 30 min. Finally, the product was collected and washed with the distilled water for three times. In addition, the USIO was fabricated using a classical co-precipitation method. Briefly, sodium polyacrylate (MW:1000, 1.0 g) was dissolved in a mixed solution containing deionized water (40 mL), $FeCl_3 \cdot 6H_2O$ (27 mg), and $FeCl_2 \cdot 4H_2O$ (10 mg) under a N_2 atmosphere at room temperature. Then, NaOH (0.4 mL, 1M) was added to the resulting solution and stirred under a N_2 atmosphere for 30 min. After that, the black product was washed with methanol three times and collected with a magnet.

Fabrication of TRF-mCuGd. 20 mg of mCuGd were uniformly dispersed into DMSO solution containing 1mM of DMSA, and then stirred for 2 hours, making mCuGd possess abundant of carboxyl groups. After that, 2 mM of NHS was added into the mixed solution, and continuously stirred for 4 hours. Subsequently, 1 mM of transferrin-PEG-NH_2 and 2 mM of EDC were respectively added into above solution under magnetic stirring overnight at room temperature, fabricating transferrin labeled mCuGd (TRF-mCuGd). Finally, the product was centrifuged at 12000 rpm/min, and then dried using a vacuum freeze dryer.

Cell culture and cytotoxicity assay. MDA-MB-231 and cisplatin-resistant MCF7 cells were seeded into 96-well plates with a density of 4000 cells/well and further incubated in the DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in a humidified atmosphere of 5% CO_2 at 37 ^oC overnight. Then, the cells were incubated with CDDP, mCuGd, and TRF-mCuGd at a dose range from 2 μg/mL to 64 μg/mL for 24 h. In addition, under 808 nm of laser irradiation (1.0 W/cm2, 10 min), mCuGd and TRF-mCuGd at the same concentration range were used to treat cells. To block ROS-induced cell death, DFOM and NAC were respectively pretreated these cells for 1 h, and then 100 μM of DFOM and 5 mM of NAC were co-incubated with cells. After incubation for 24 h, the media were discarded and cells were washed using fresh cold PBS and then treated with 200 μL of 10% CCK-8 at 37 ^oC for 2h. The cell viability was detected using a Fluostar Optima microplate reader (BMG Labtechnologies, Germany).

ROS generation of TRF-mCuGd in vitro. The ability of TRF-mCuGd to catalyze H_2O_2 into ·OH could be detected using TMB kit. Specifically, TRF-mCuGd with different concentrations was uniformly dispersed into 1.5 mL of phosphate buffer solution (PBS, pH5.5) containing TMB (100 μL, DMSO solution, 1 mg/mL) and H_2O_2 (20 μL, 20%). Quickly, the solution was gradually blue, and hydroxyl radicals in the solution were measured via UV-vis spectroscopy at a wavelength of 650 nm.

In addition, intracellular ROS was further analyzed via flow cytometry and confocal laser scanning microscope. Firstly, the cells were seeded on glass coverslips placed in 12-well plates and treated with saline (control group), USIO, mCuGd, TRF-

mCuGd, and TRF-mCuGd+NAC at a dosage of 40 μg/mL for 4 h. Similarly, different concentrations of TRF-mCuGd were used to treat cells. After that, DCFH-DA prods were added into media, and co-incubated for 30 min. Subsequently, the cells were washed using cold PBS and fixed in 4% of paraformaldehyde solution for 30 min. Finally, intracellular ROS was observed using CLSM and FACS.

Cell TEM observation. MDA-MB-231 cells were seeded in 6-well plates (5×10^5 cells/well) and incubated in the DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in a humidified atmosphere of 5% CO_2 at 37 ^oC overnight. Subsequently, cells were treated with TRF-mCuGd at a dosage of 40 μg/mL for 1, 2, 3, 4, 6, 8, 12, and 24 h. After that, the cells were centrifuged and formed to sphere in tubes. Then, cells were further treated using a 0.1 M phosphate buffer containing 3% fresh glutaraldehyde overnight at 4 ^oC. In order to ensure optimal fixation, the specimens were then washed with 0.1 M phosphate buffer at 4 °C twice at 30 min intervals.

Afterwards, 2 % osmium tetroxide aqueous was used to treat cells for approximately 2 h at ambient temperature and followed by washing with buffer as above. The specimens were then dehydrated step by step through a graded series of ethanol at room temperature. Subsequently, propylene oxide was added in the specimens and treated for 15 min. The specimens were placed in a 50/50 mixture of propylene oxide/Araldite resin and then kept overnight at room temperature. After that, to ensure complete invasion of Araldite resin, the specimens was continuously placed for 48-72 h at 60 ^oC, and then left in full strength Araldite resin for 6-8 h at

room temperature. After that, the specimens were cut using a Leica ultramicrotome and stained with 1 % toluidine blue in borax. Finally, Ultra-thin sections with approximately 70-90 nm thick were cut on a Leica ultramicrotome and stained for 25 min with saturated aqueous uranyl acetate followed by staining with lead citrate for 5 min. The sections were placed on copper grid and observed using a bio-TEM.

Cell uptake of TRF-mCuGd by ICP-MS. MDA-MB-231 and CDDP-resistant MCF-7 cells were seeded in six-well culture plates at a density of 2×10^6 cells/well at 37 °C for 12 h. Afterwards, CDDP, mCuGd and TRF-mCuGd were used to incubate cells the cells for 6 h, respectively. At the end of treatment, cells were washed using fresh cold PBS for three times to remove excessive nanoparticles, and subsequent lysed by trypsin digestion. The cells were centrifuged and collected in the tubes. Finally, The cells were nitrated using concentrated nitric acid and until form transparent solution, and then Cu or Pt contents in the solution were analyzed by ICP-MS.

Western Blot Analysis. MDA-MB-231 cells were incubated with saline (control group), USIO, mCuGd, and TRF-mCuGd at a dosage of 40 μg/mL for 6 h. Subsequently, the cells were washed using cold fresh PBS three times to remove nanomaterials, and then collected and further lysed, and the corresponding protein was distilled using the Total Protein Extraction Kit and the protein concentrations were tested by BCA Protein Assay Kit. Afterwards, the proteins were separated by SDS-PAGE and transferred to poly(vinylidene difluoride) membranes. Next, specific antibodies (dilution 1:1000) including SOD-1, cleaved caspase-3, GPX4, and cleaved PARP were added to the membranes. Finally, the western blots could be observed via

the chemiluminescence system.

Anticancer Activity Investigation. The mice used in the experiment were treated in accordance with the Ethics Committee Guidelines of Binzhou Medical University, and the animal protocols were approved by Laboratory Animal Center of Binzhou Medical University. Firstly, the cancer model was established through direct subcutaneous injection of 1×10^8 MDA-MB-231 or CDDP-resistant MCF-7 cells into the left leg of BALB/c nude mice. For in vivo NIR irradiation experiment, the cancer-bearing mice were intratumorally injected with saline, AuNR+CDDP, and TRF-mCuGd with a dosage of 2.0 mg/kg at a presence or absence of laser irradiation (808nm, 0.8 W/cm^2). After injection of TRF-mCuGd for 4 hours, the laser was used to irradiate tumor site for 5 min. Cancer-bearing mice were injected with two days intervals, and tumor volumes could be recorded using a formula: $V=a\times b^2/2$. where "a" and "b" were the longest and shortest diameters of the tumor, respectively. In addition, for in vivo synergistic anticancer experiment through interaction between PTT and CDT, cancer-bearing mice were injected with saline, mCuGd, TRF-mCuGd at a presence and absence of laser irradiation (808 nm, 0.8W/cm^2) through tail vein, and the tumor sites of mice were irradiated for 10 min when post-injection 12 hours. At the end of treatment, cancer-bearing mice were sacrificed, and then tumors were weighed. In addition, the major organs were harvested for histopathology evaluation.

The detection of hydroxyl radicals in vitro and in vivo. The reaction principle of ·OH Kit was designed with double antibody sandwich enzyme-link immunoassay, and the relative ·OH concentration were measured at 450 nm absorbance. Briefly, MDA-MB-

231 cells were plated in 6 well plates at 1×10^5 cells/well overnight, and then treated the cells with USIO, mCuGd, and TRF-mCuGd at concentration of 20 ug/mL for 4 h. The culture medium were removed and washed three times with PBS buffer, the cells were collected and re-suspended with cold PBS at a density of 1×10^6 cells/ml. The cellular components were released by repeated freezing and thawing, the cells were centrifuged at 4 ^oC for 25 min at 3000 rpm/min. And the supernatant were collected, and then treated with ·OH Kit. The ·OH concentration of the reacted solution were detected using a microplate reader at a wavelength of 450 nm.

For in vivo ·OH measurement, the mCuGd, and TRF-mCuGd at the presence and absence of 808 nm laser irradiation were administrated into cancer-bearing mice at a concentration of 2 mg/kg through tail vein injection, and the tumor sites of mice were irradiated for 10 min when post-injection 12 hours. At the end of treatment, the mice were sacrificed after 24 h, and the partial tumors were collected, cut up at same weight, and then suspended with cold PBS buffer, the endogenous components were released by tissue homogenizer and then subjected to centrifuge at 4 ^oC for 25 min at 3000 rpm/min. And the supernatant were collected for ·OH concentration detection according to the manufacturer's instruction.

Acute toxicity and biodistribution of TRF-mCuGd. For acute toxicity experiment, KM mice were randomly divided into three groups, and each group had 10 KM mice. Subsequently, KM mice were intravenously injected with cisplatin, mCuGd, and TRF-mCuGd at a dosage of 20 mg/kg. The survival period of mice treated with different groups was recorded. For the biodistribution experiment, the cancer model

was established through direct subcutaneous injection of 1×10^8 MDA-MB-231 cells into the left leg of BALB/c nude mice. About four weeks, the cancer-bearing mice were separately injected with Cu(II) ions, mCuGd, and TRF-mCuGd at a dosage of 10 mg/kg through tail vein. At different time, the cancer-bearing mice were sacrificed. After that, the vital organs and tumor tissue were cut, weighed, and then nitrated using the concentrated nitric acid through microwave digestion system. Finally, Cu contents in the digestive solution were analyzed using inductively coupled plasma mass spectrometry (ICP-MS).

In Vitro and In Vivo MRI. All MR studies were performed on a 7.0 T scanner (Bruker Technologies, USA) using a volume RF coil (inner diameter 40 mm for mice). For in vitro phantom MR experiments, the scanning procedure began with a localizer, and then a series of inversion-prepared fast spin-echo images were acquired for longitudinal relaxation time (T1) measurement. This series identical in all aspects (TR 6000 ms, effective TE 5.6 ms, BW 25 kHz, slice thickness 1 mm, matrix 96×96, 1 average) except for the 20 different inversion times (TIs) that were varied linearly from 10 to 2500 ms. Signal intensity (SI) versus TI relationships were fit to the following exponential T1 decay model by nonlinear least-squares regression: SI(TI) =A1*exp(-TI/T1)+SI(0).

For the mouse studies, the following acquisition parameters were chosen: repetition time (TR) = 370 ms, echo time (TE) = 11.6 ms, field of view (FOV) = 40 mm × 40 mm, matrix size = 192 × 192, slice thickness = 1 mm (12 slices, gap = 0), 1 average, and bandwidth (BW) = 50 kHz. In addition, the obtained images could be analyzed

using ImageJ software.

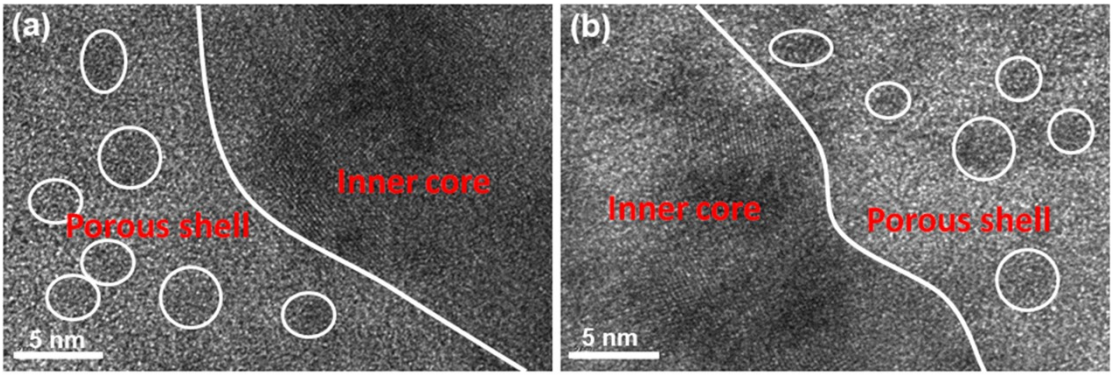

Figure S1. High-magnification TEM images of (a) sCuGd and (b) mCuGd.

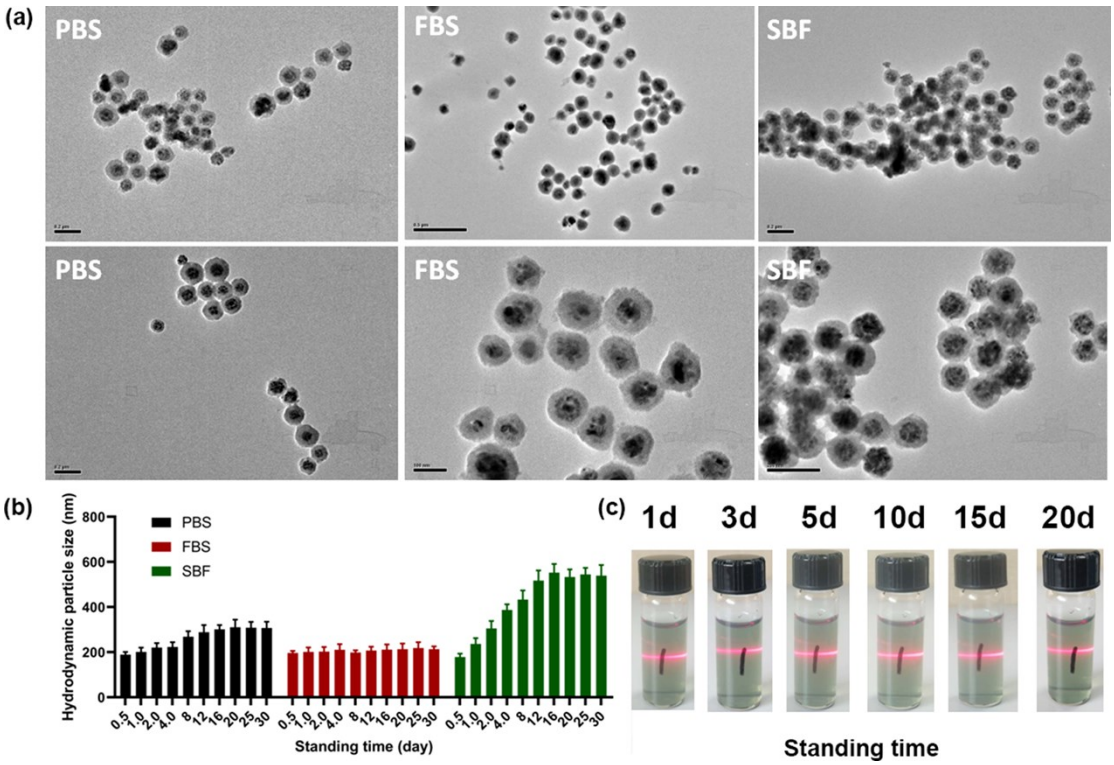

Figure S2. (a) TEM images of mCuGd dispersed by different media. (b) Hydrodynamic particle size changes of mCuGd in different media. (c) The colloidal stability of mCuGd with standing time increase.

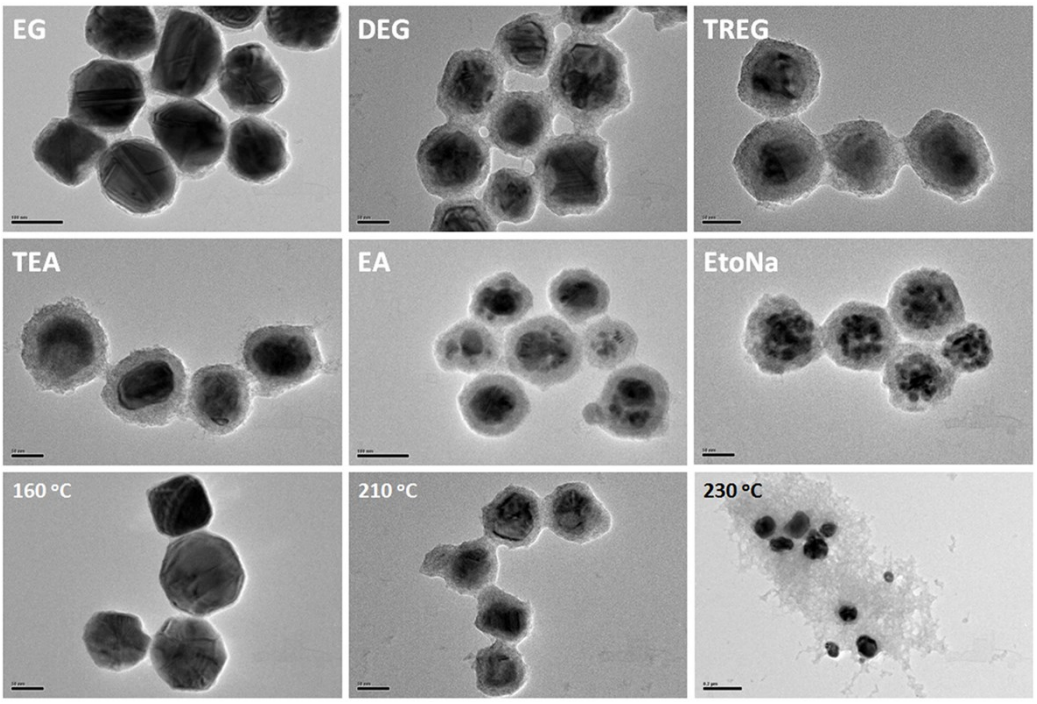

Figure S3. TEM images of CuGd nanoparticles synthesized by varying sovent, alkali, and temperature.

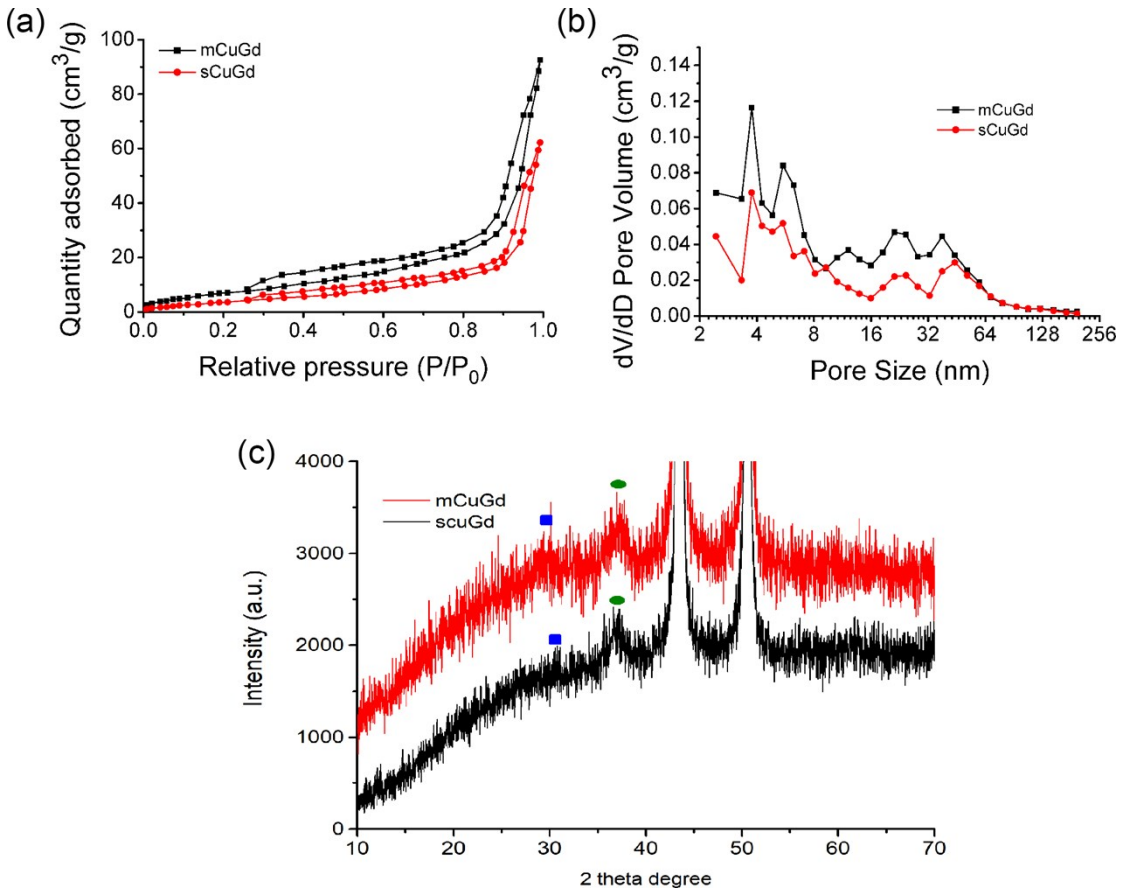

Figure S4. (a) N_2 adsorption-desorption isotherms and (b) corresponding pore size distribution of sCuGd and mCuGd. (c) The enlarged y-axis in XRD spectra of sCuGd and mCuGd.

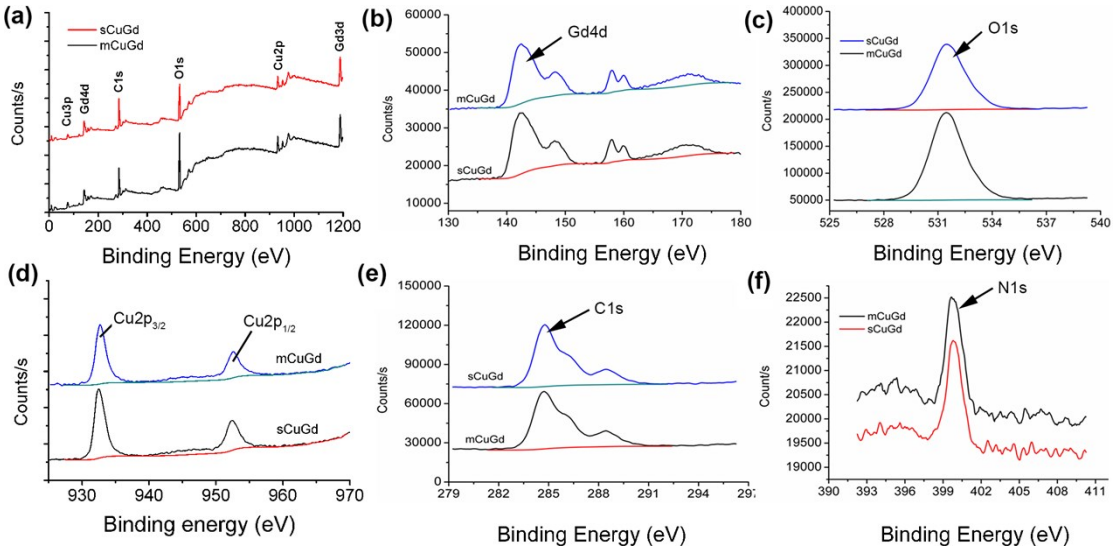

Figure S5. (a) XPS full spectra, (b) Gd4d, (c) O1s, (d) Cu2p, (e) C1s, and (f) N1s spectra of mCuGd.

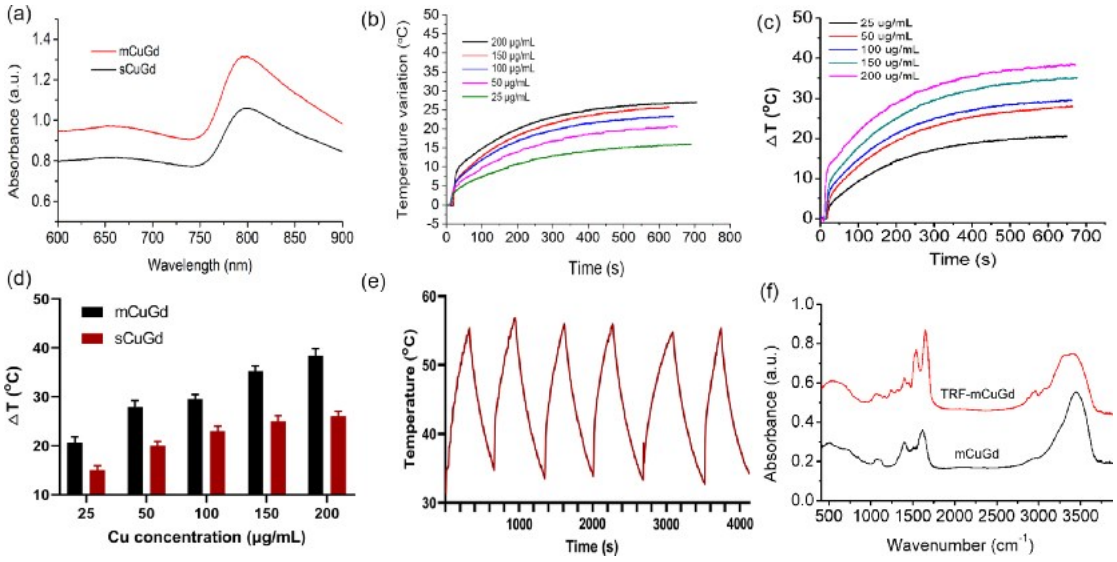

Figure S6. (a) The Uv-Vis absorption spectra of sCuGd and mCuGd. The concentration-dependent temperature curves of (b) sCuGd and (c) mCuGd under laser irradiation (808 nm, 0.8 W/cm^2). (d) The photothermal temperature changes of sCuGd and mCuGd. (e) Photothermal stability of mCuGd under laser irradiation. (f) FT-IR spectra of mCuGd and TRF-mCuGd.

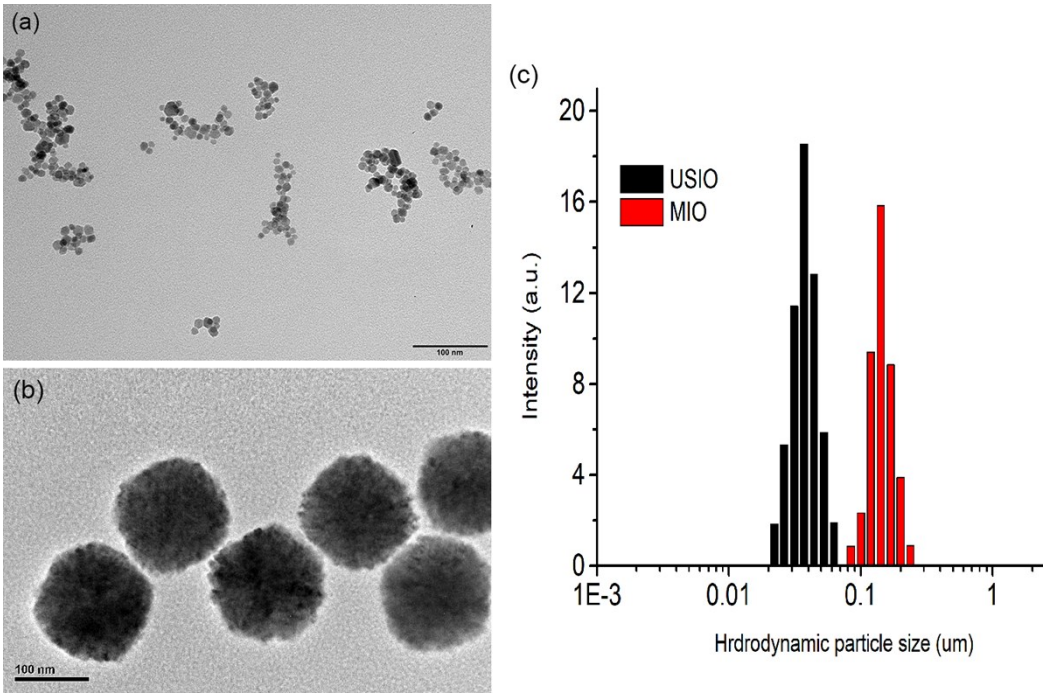

Figure S7. TEM images of (a) USIO and (b) MIO; (c) Hydrodynamic particle size of USIO and MIO.

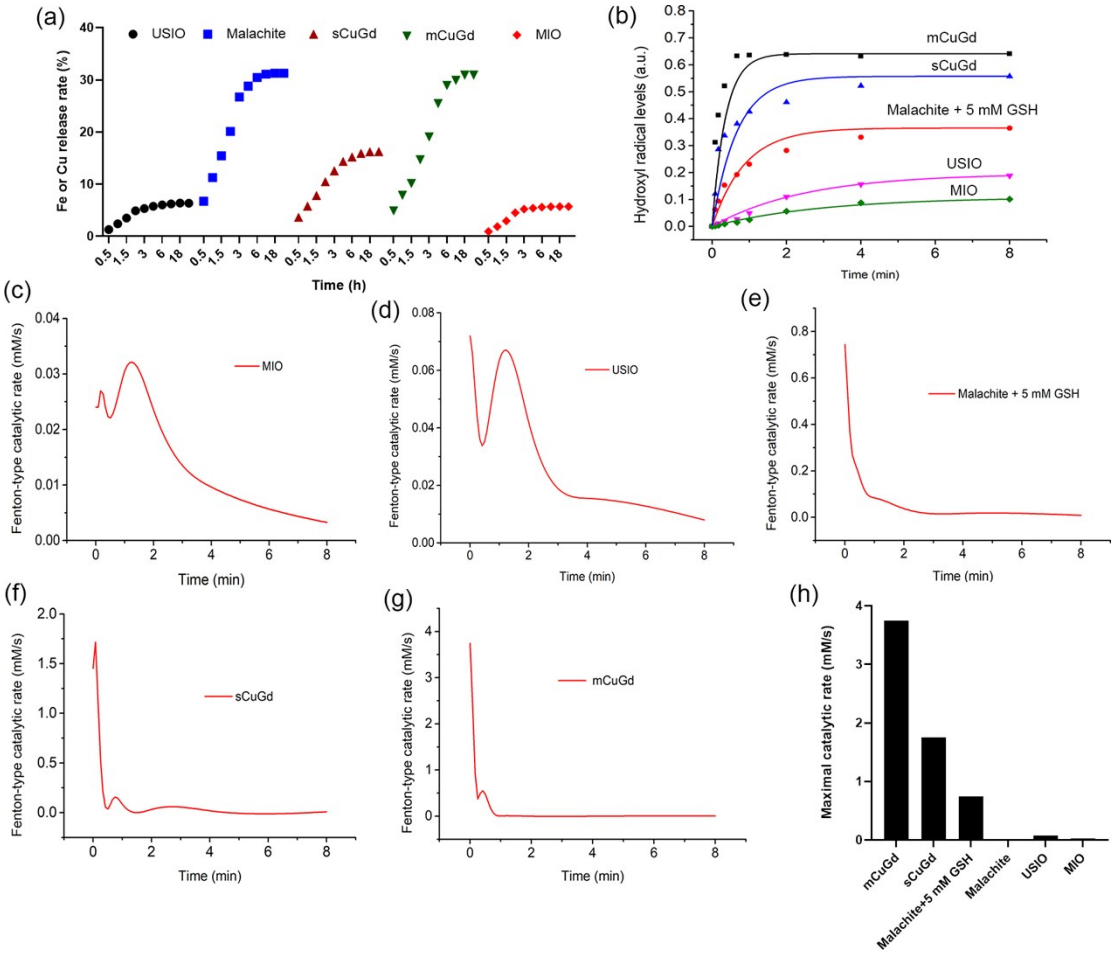

Figure S8. (a) The release behavior of metal ions from MIO, USIO, malachite, sCuGd, and mCuGd under pH 6.0. (b) The changes of hydroxyl radical level in the H_2O_2 solution containing different samples with time increasing. The maximal catalytic rate of (c) MIO, (d) USIO, (e) malachite + 5 mM GSH, (f) sCuGd, and (g) mCuGd through the first derivative of hydroxyl radical generation curves in (b). (h) Fenton-type catalytic rate of MIO, USIO, malachite, malachite + 5 mM GSH, sCuGd, and mCuGd.

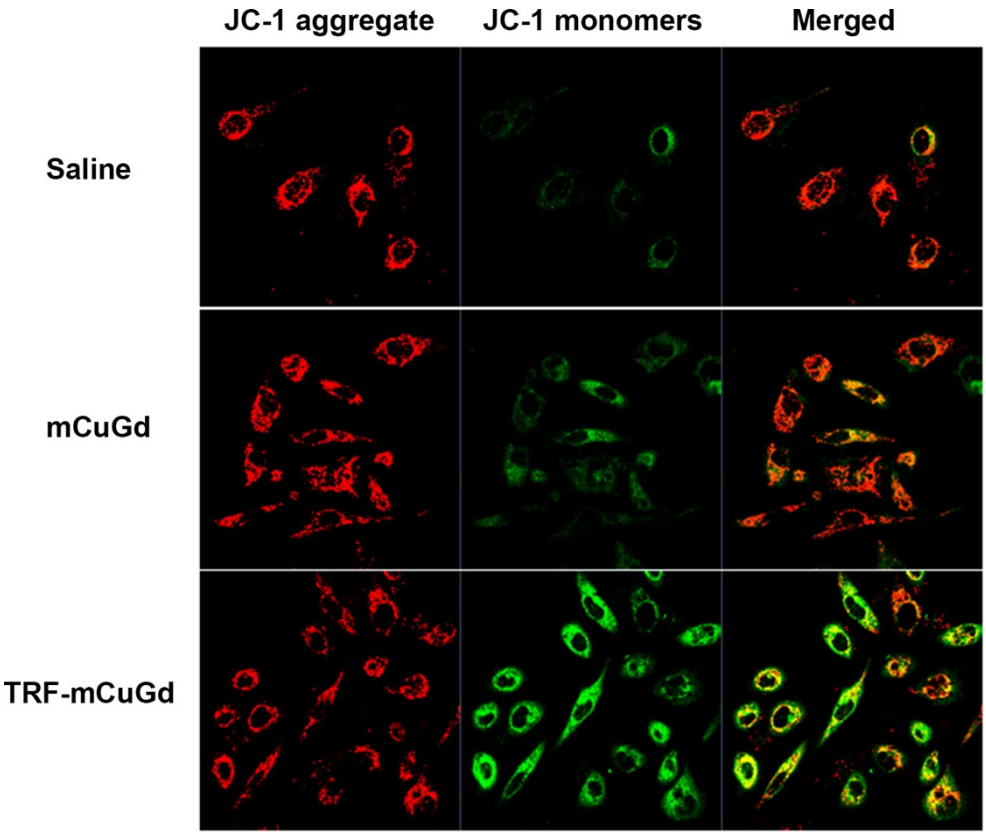

Figure S9. The mitochondrial membrane potential of cells treated with mCuGd and TRF-mCuGd was observed by CLSM.

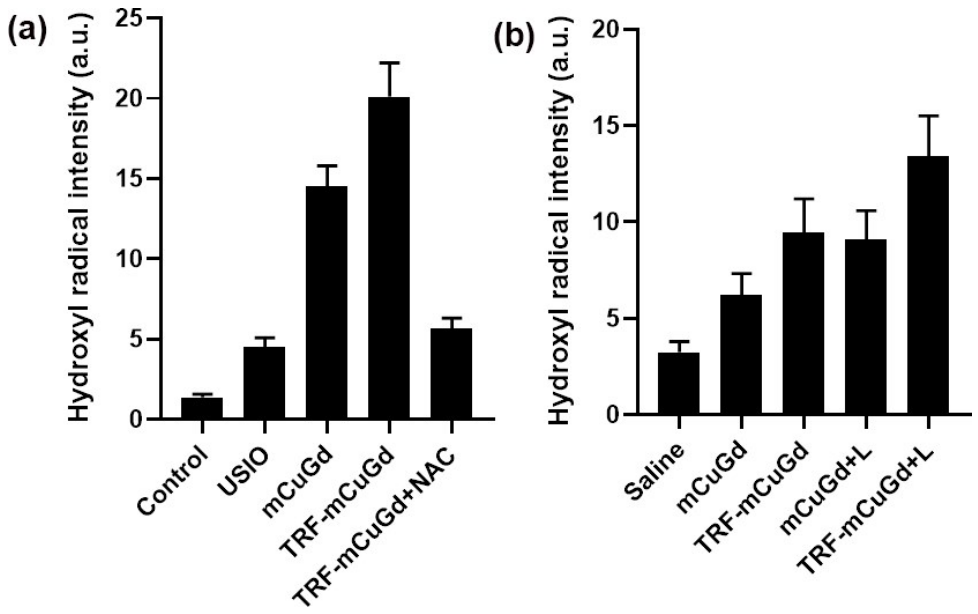

Figure S10. (a) Intracellular hydroxyl radical generation after different sample treatment for 6 hours; (b) The increased hydroxyl radical generation of tumor after post-injection different samples for 24 hours.

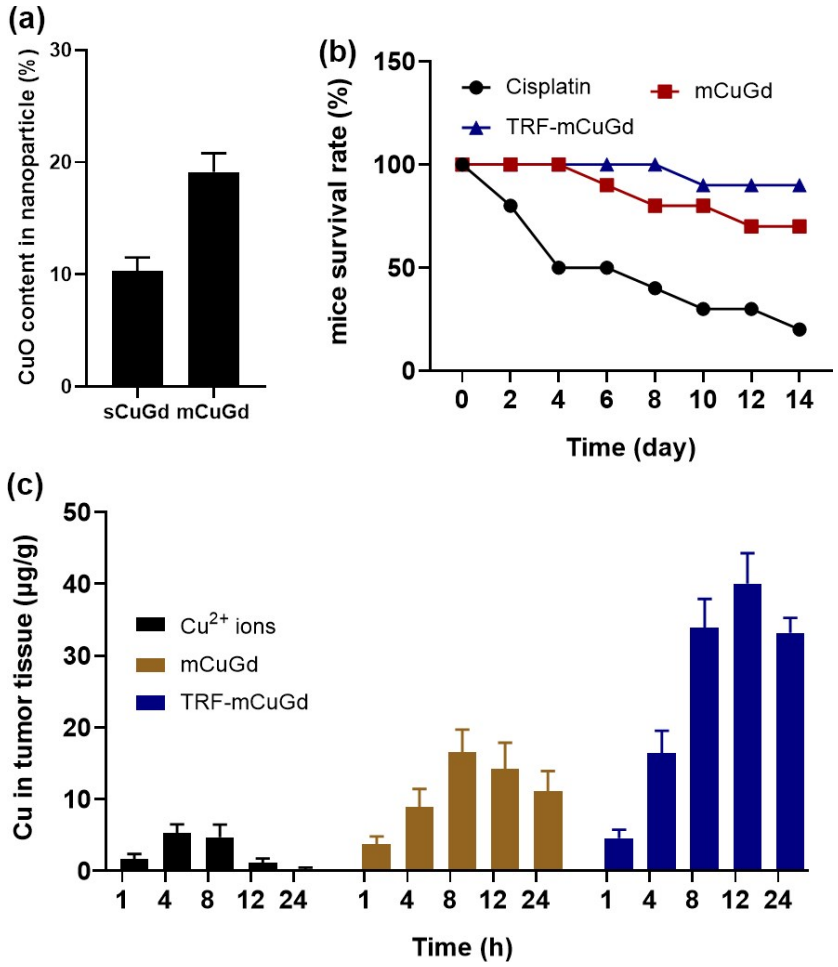

Figure S11. (a) CuO content of sCuGd and mcuGd. (b) The acute toxicity of mice intravenously injected with cisplatin, mCuGd, and TRF-mCuGd at a dosage of 20 mg/kg. (c) Cu accumulation in tumor after treatment with free Cu ions, mCuGd, and TRF-mCuGd.

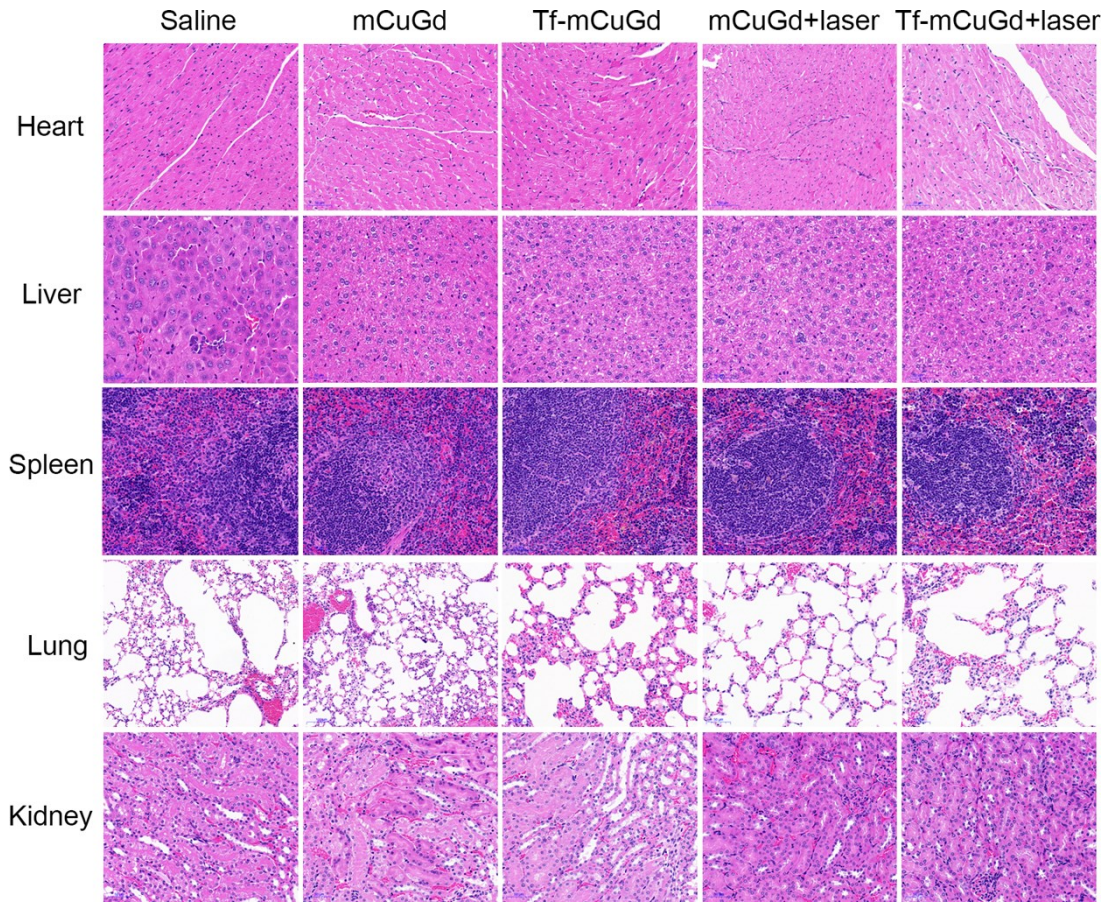

Figure S12. Pathological analysis of vital organs in mice after different samples treatment.

References.

1. L. Wang, Z. Zhang,Y. Ding, J. Wu, Y. Hu, A. Yuan, Novel copper-based and pH-sensitive nanomedicine for enhanced chemodynamic therapy, *Chem. Commun.,* **2020**, *56*, 7753-7756.