Supporting Information

An Efficient Magnetic Nanocatalyst Induced Chemo- and Ferroptosis Synergistic Cancer Therapy in Combination with T_1-T_2 Dual-Mode Magnetic Resonance Imaging through Doxorubicin Delivery.

Ling Zhu[†,#], Jingbo Wang[†,#], Xiaojie Tang[§], Caiyun Zhang[‡], Peng Wang[‡], Lizhong Wu[†], Weiqing Gao[†], Weilong Ding[†], Guilong Zhang*[,‡], and Xiaofeng Tao*[,†]

[†] Department of Radiology, School of Medicine, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University, Shanghai 200011, China

[‡] School of Pharmacy, Shandong Technology Innovation Center of Molecular Targeting and Intelligent Diagnosis and Treatment, Binzhou Medical University, Yantai, Shandong Province, 264003, P. R. China

[§] Department of Spinal Surgery, Yantai Affiliated Hospital of Binzhou Medical University, Yantai, 264000, China

Experimental Section.

Materials. All chemical reagents were used as received without further purification. Dox were obtained from Sigma-Aldrich Co. (USA). The iRGD-PEG-ss-PEG-NH_2 was purchased by Shanghai Ponsure Biotech, Inc. The gadolinium acetylacetonate, iron acetylacetonate, triethylamine (TEA), and fluorescein isothiocyanate (FITC) were purchased from Aladdin Co. (Shanghai). The ethylene glycol (EG) and diethylene glycol (DEG) were purchased from Sinopharm Co. (Shanghai). The 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) was obtained from Nanjing Jiancheng Bioengineering Institute (China). The CCK-8 kits were obtained from Dojindo (Japan). All other chemicals were analytical grade and purchased from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China).

Synthesis of GdIO. Firstly, iron acetylacetonate and gadolinium acetylacetonate were mixed into EG solution, and heated to 80 ^{o}C, and continuously stirred to until form homogeneous solution. Afterwards, the polyvinylpyrrolidone (PVP-K30) was poured into the resulting solution, and then continuously stirred for 30 min at 80 ^{o}C. Subsequently, the triethanolamine (TEA) was further added into the resulting solution, and then stirred to until form transparent orange solution. Subsequently, the obtained solution was transferred into Teflon-lined stainless steel autoclave, then heated to 230 ^{o}C, and kept for 72 h. Finally, the products were collected through magnet, and washed with the distilled water and alcohol at least three times, respectively.

Fabrication of iGdIO-Dox. The prepared GdIO particles (10 mg) were uniformly

dispersed into Dox solution (2 mg/mL, 3 mL), and the mixed solution were shook for 24 h through incubator shaker at 30 ^{o}C. After that, iRGD-PEG-ss-PEG-NH_2 (50 mg) was added into the mixture and continuously stirred for 12 h. after that, the particles were collected through magnet, and washed with the distilled water for three times. Finally, the products were dried by freezer dryer, and then reserved for the following experiment. In addition, the loading ratio of Dox in ipGdIO-Dox could be calculated through the following equation: $RR(\%)=(C_{pre}-C_{sup})\times V/M_0\times 100$ %.

Where C_{pre} and C_{sup} were the concentration of the drugs in the original and supernatant solutions, respectively. V was the volume of the solution, and M_0 was the mass of ipGdIO.

The Dox release of ipGdIO-Dox. The ipGdIO-Dox was uniformly dispersed in PBS solution containing different concentrations of GSH, and the resulting solution was sharked at room temperature. After that, the solution was centrifuged using 15000 rpm/min, and then the supernatant was collected, and measured using a UV-Vis spectrophotometer (Lambda 365, PE, USA) at a wavelength of 488 nm.

Cytotoxicity Assay. MDA-MB-231 cells were seeded into 96-well plates and adjusted to a density of 1×10^4 cells/well. Subsequently, the samples including free Dox, pGdIO-Dox, ipGdIO-Dox, and ipGdIO-Dox+NAC were used to treated MDA-MB-231 cells for 24 h. Then, MDA-MB-231 cells were washed using cold fresh PBS after removing medium, and treated with 110 μL of 10% CCK-8 at 37 °C for 2h. Finally, cell viability was measured using a microplate reader at a wavelength of 450 nm.

Confocal Laser Scanning Microscopy (CLSM) Observation. MDA-MB-231 cells were seeded on glass coverslips placed in 12-well plates (1×10^5 cells/well) and incubated with PBS, USIO, pGdIO, ipGdIO, ipGdIO+NAC, and ipGdIO+DFO for 6 h. Then, the medium was removed, and cells were washed three times with fresh cold PBS, and further treated with trypsin for 4 h. After that, 2,7-dichlorofluorescein diacetate (DCFH-DA) prods (200 μL, dilution 1:1500) were used to incubate MDA-MB-231 cells at 37 ^{o}C for 10 min. Finally, the relative content of ROS in cell was visually observed by CLSM.

The internalization process of ipGdIO was also analyzed by CLSM and flow cytometry. According to our previous method [1], the pGdIO and ipGdIO were conjugated with FITC (green fluorescence molecules). Then, MDA-MB-231 cells were treated with pGdIO, ipGdIO, ipGdIO+amiloride (ipGdIO+A), and ipGdIO+4^{o}C for 6 h and then trypsinized, followed by staining with DAPI for additional 0.5 h at 37 °C in dark. Finally, MDA-MB-231 cells were washed with fresh cold PBS and fixed with 4% paraformaldehyde for 30 min. Then, cells were mounted on glass slides, and then analyzed through CLSM and flow cytometry.

ICP-MS Observation. MDA-MB-231 cells were seeds in 60 mm plates, and cell density was adjusted to 1×10^7 cells/well. After that, the samples including pGdIO, iPGdIO, ipGdIO+A, and ipGdIO under 4 ^{o}C condition with different concentrations were used to treat MDA-MB-231 cells for 3 and 6 h. MDA-MB-231 cells were washed with fresh PBS purging to remove extracellular particles. Then, MDA-MB-231 cells were further treated with trypsin, and then collected to 1.5 mL

of the tube. Finally, the collected cells were nitrated with concentrated nitric acid, and the gadolinium and iron ions in the solution were detected through ICP-MS analysis.

Western Blot Analysis. MDA-MB-231 cells were cultured in 6-well plates and the density was adjusted to 5×10^5 cells/well. Then, MDA-MB-231 cells were treated with saline, pGdIO, and ipGdIO for 12 h. after that, the cells were washed with cold fresh PBS, and then collected and further lysed. The total proteins were distilled through the addition of the Total Protein Extraction Kit and the protein concentrations were tested by BCA Protein Assay Kit. After that, the proteins were separated by SDS-PAGE and then transferred to poly(vinylidene difluoride) membranes. The membranes were incubated with Bax, Bcl-2, GPX4, and cleaved caspase-3 antibodies (dilution 1:1000), and β-actin (dilution 1:2000) for 4 h. Finally, the protein blots could be analyzed using the chemiluminescence system.

In Vitro and *In Vivo* MRI Study. All MR studies were performed on a 7.0 T scanner (Bruker Co., Ltd, Germany) using a volume RF coil. For tube samples, a series of spin echo images were acquired for transverse relaxation time (T_2) measurement, identical in all aspects (repetition time (TR) 2500 ms, effective echo time (TE) 5.6 ms, band width (BW) 25 kHz, slice thickness 0.8 mm, matrix 96×96, 3 average) except for 20 echoes time (TI) which was varied linearly from 10 to 2500 ms. The longitudinal relaxation time (T_1) could be achieved using a series of inversion-prepared fast spin-echo imagest. The corresponding parameters were as follows: TR 6000 ms, effective TE 5.6 ms, BW 25 kHz, slice thickness 1 mm, matrix

96×96, 3 average, TIs 20 from 10 to 2500 ms.

For the *in vivo* MR studies, the mice used in the experiment were treated in accordance with the Ethics Committee Guidelines of Binzhou Medical University. T_1- and T_2-weighted MR images of tumor were acquired with the axial and coronal orientation using a spin-echo sequence. The following acquisition parameters for T_1-weighted images 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), 3 average, and bandwidth (BW) = 50 kHz. The following acquisition parameters for T_2-weighted images were chosen: TR = 5000 ms, TE = 80 ms, TSE factor = 15, FOV = 80 mm × 80 mm, matrix size = 160 × 160, slice thickness = 1.5 mm (10 slices, gap = 0), and 5 averages.

In vivo anticancer experiment. The breast cancer model was established through direct subcutaneous injection MDA-MB-231 cells with a density of 5×10^6 into the right hind leg of BALB/c nude mice. After the tumor volume reached to about 100 mm^3, cancer-bearing mice were treated with saline, Dox, pGdIO-Dox, ipGdIO-Dox, and ipGdIO-Dox+NAC through tail vein injection at a dosage of 2mg/kg (equal Dox concentration) with two days intervals. Tumor volume of mice was recorded through the following equation: $V = a \times b^2/2$, where "a" represented the longest size of tumr, and "b" represented the shortest size of tumor. Meanwhile, the body weights of mice were recorded. At the end of treatment, mice were excised, and then tumors exploited, weighed, cut for slices for histopathology analysis.

Pharmacokinetics and biodistribution of ipGdIO-Dox. Cancer-bearing mice were

intravenously injected with free Dox, pGdIO-Dox, and ipGdIO-Dox at a dosage of 2 mg/kg through tail vein. At different time intervals, the blood of mice was collected. For Dox group, the collected blood were analyzed through UV-HPLC. In addition, for pGdIO-Dox and ipGdIO-Dox groups, the collected blood were nitrated using concentrated nitric acid, and then the gadolinium and iron ions in the solution were analyzed through ICP-MS.

Cancer-bearing mice were intravenously injected with pGdIO-Dox, and ipGdIO-Dox at a dosage of 5 mg/kg through tail vein. Mice were humanely sacrificed with different time intervals (2 h, 6 h, 12 h, 24 h), the vital organs including heart, liver, spleen, lung, kidney, and tumor were dissected, weighed, homogenized. After that, the vital organs were nitrated with the concentrated nitric acid through microwave digestion system. Subsequently, the resulting solution was diluted and filtered using 0.22 μm membrane. Finally, the gadolinium and iron ions in the solution were analyzed using ICP-MS to assess the bio-distribution of ipGdIO.

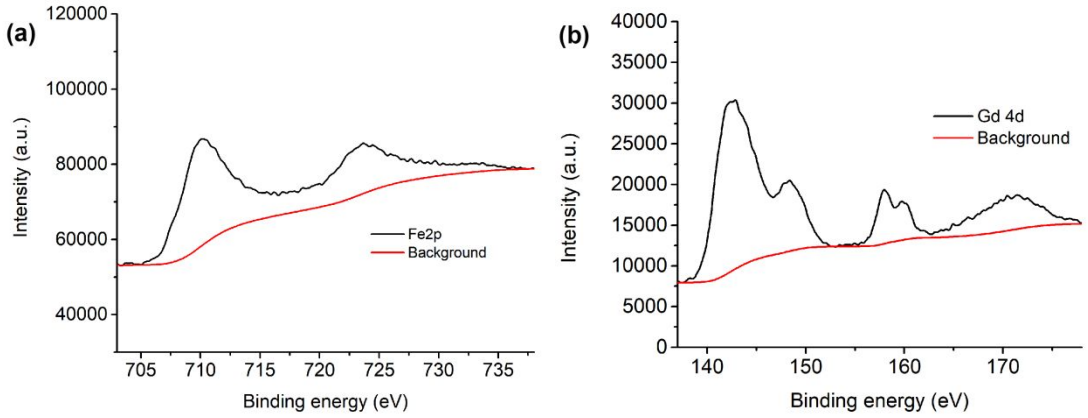

Figure S1. The XPS Fe2p and Gd4d spectra of GdIO.

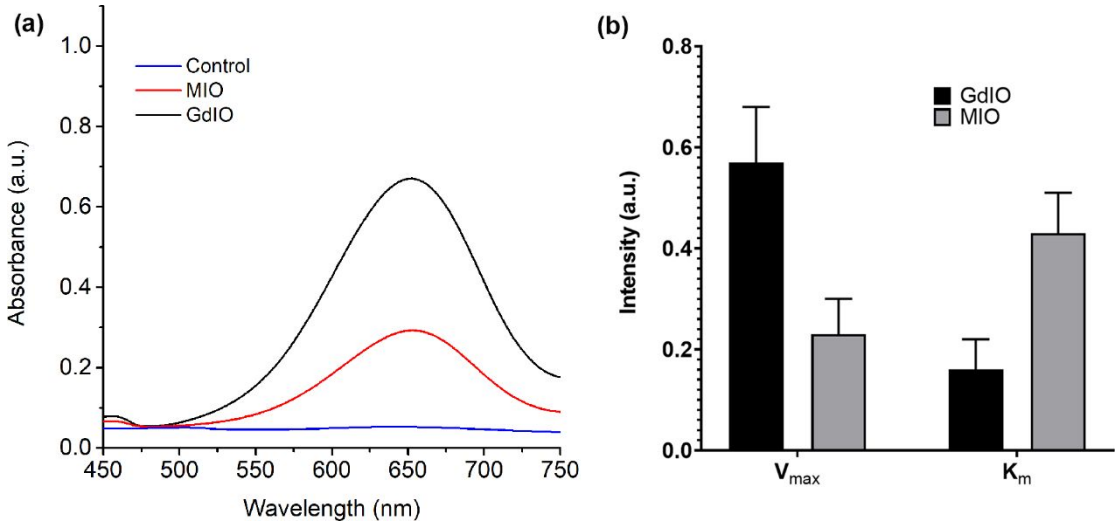

Figure S2. The catalytic activity of GdIO through TMB color test. (b) The the catalytic kinetic parameters (V_{max} and K_m) of MIO and GdIO.

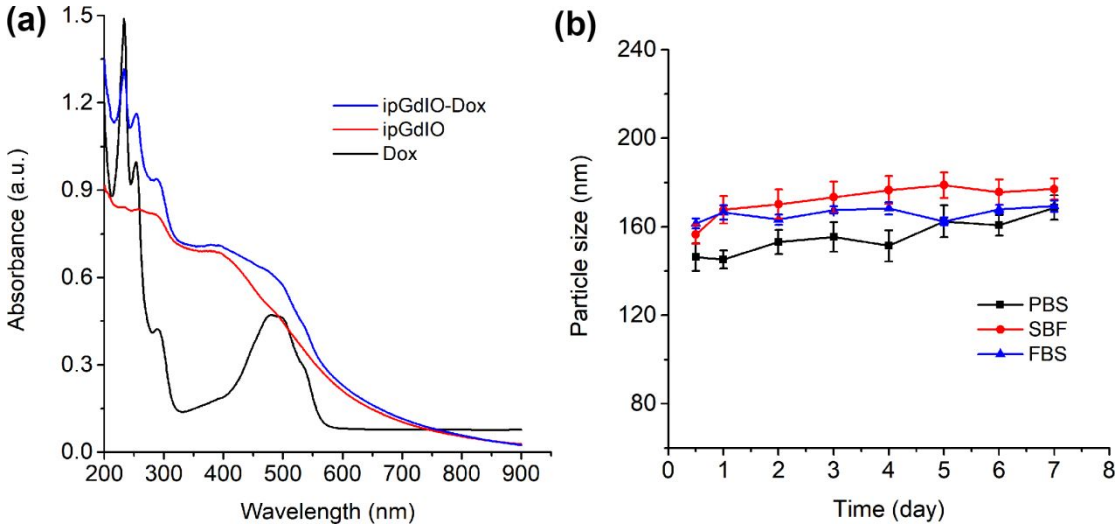

Figure S3. (a) Uv-vis spectra of Dox, ipGdIO, and ipGdIO-Dox. (b) The hydrodynamic size change of ipGdIO-Dox with standing time increase.

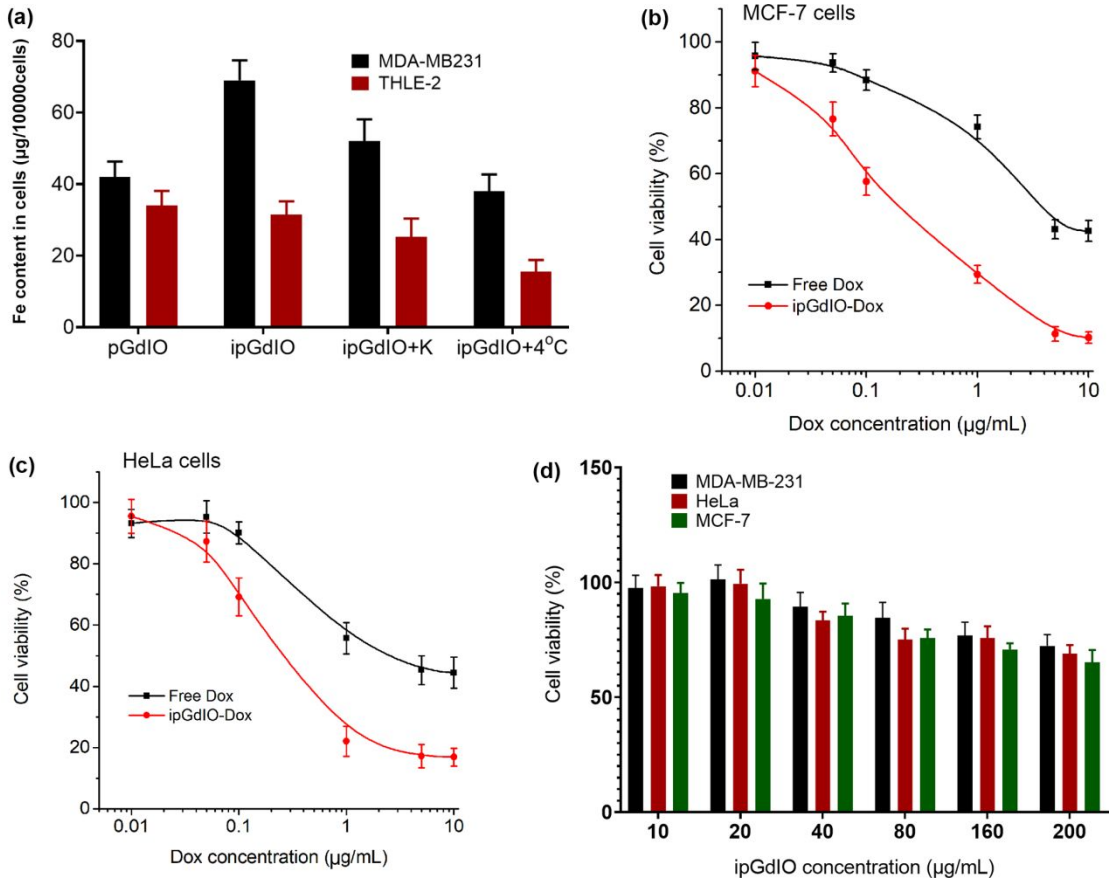

Figure S4. (a) The internalization of pGdIO and ipGdIO in MDA-MB-231 and THLE-2 cells under different conditions. (b,c) The viability of MCF-7 and HeLa cells treated with Dox and ipGdIO-Dox. (d) The viability of MDA-MB-231, MCF-7, and HeLa cells treated with different concentrations of ipGdIO particles.

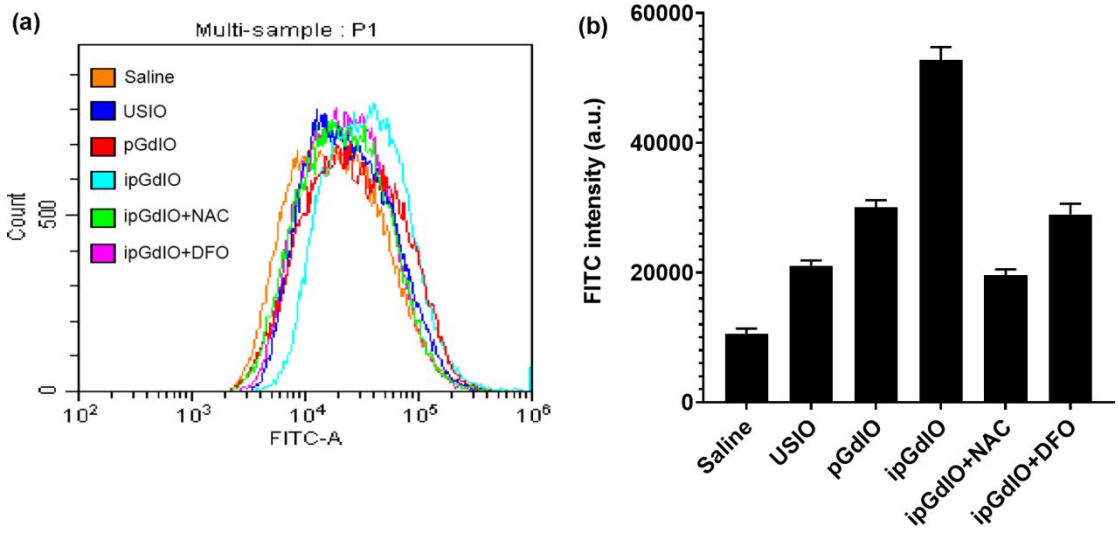

Figure S5. (a) The flow cytometry analysis of MDA-MB-231 cells treated with different samples, and (b) corresponding quantitative analysis.

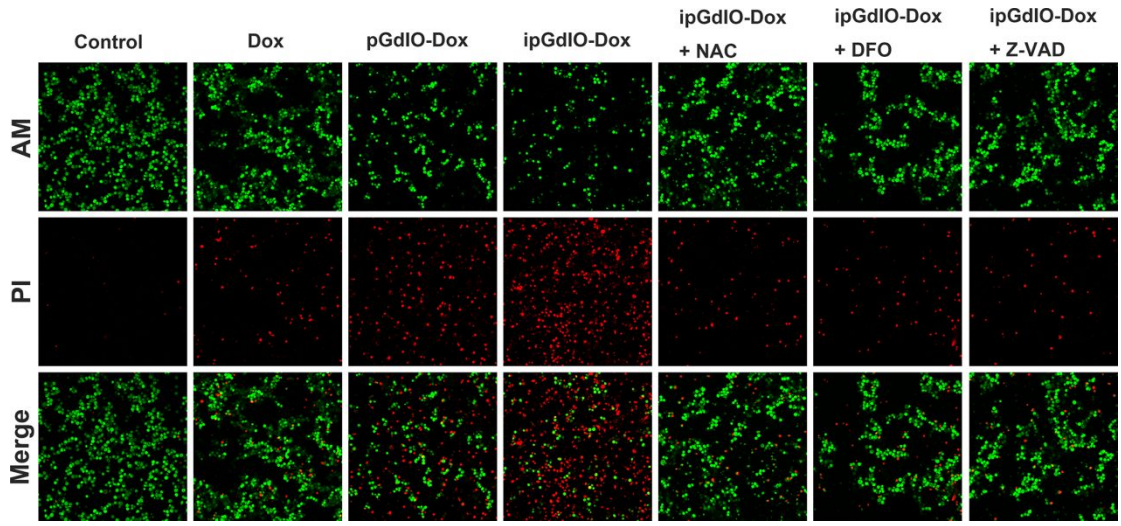

Figure S6. CLSM observation: live (green) and dead (red) of 4T1 cells stained by Calcein-AM and PI after various treatments for 24 h.

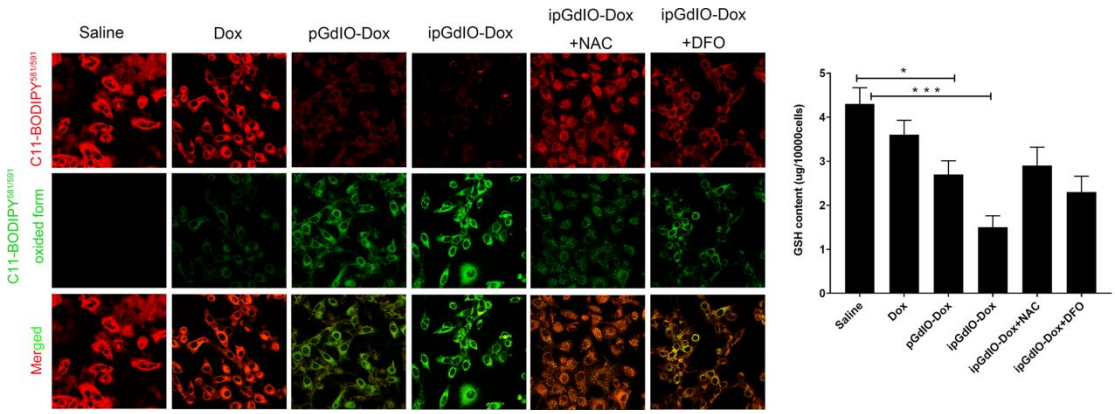

Figure S7. (a) The lipid peroxidation of cells treated with different samples. (b) GSH content in cells treated with different samples.

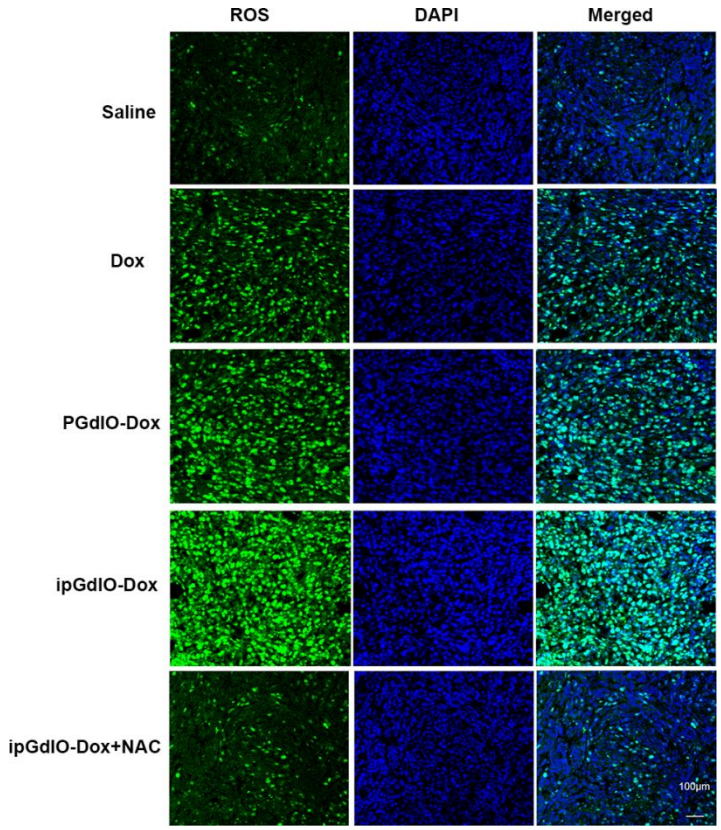

Figure S8. ROS staining of tumor slice in mice injected with different samples.

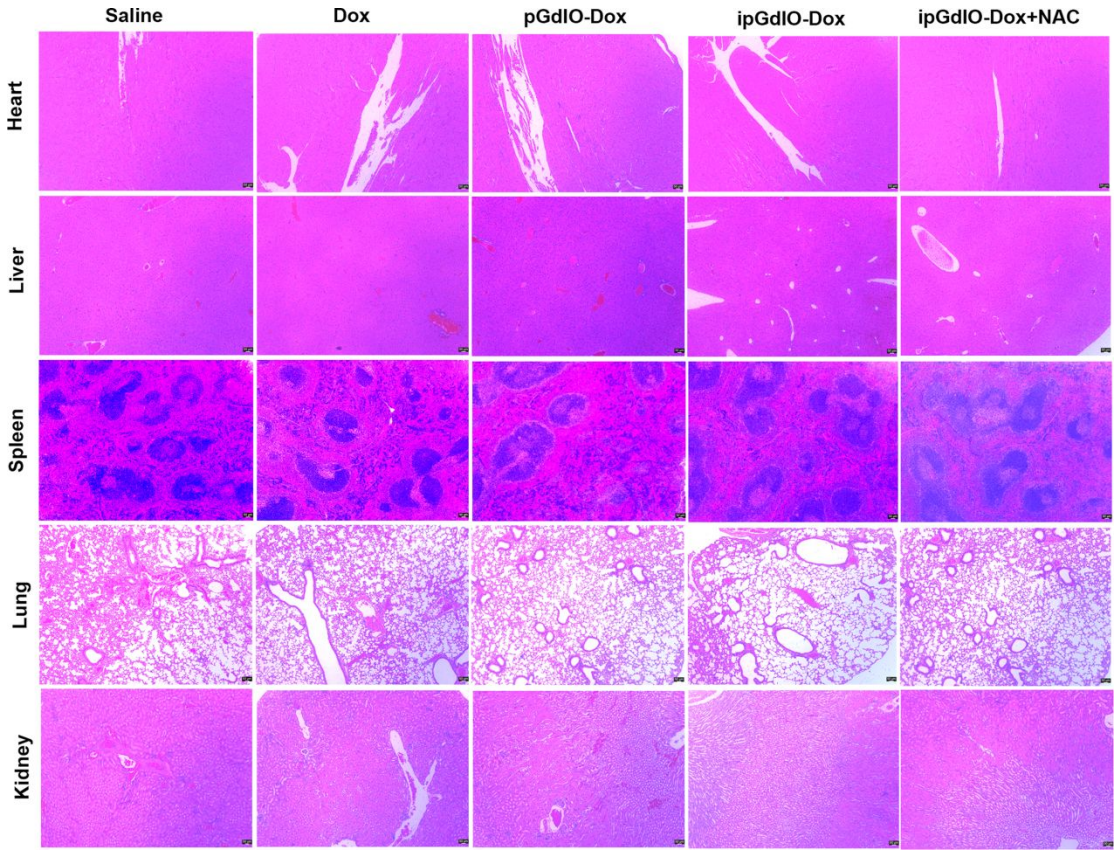

Figure S9. H&E staining analysis of vital organs in mice injected with saline, Dox, pGdIO-Dox, ipGdIO-Dox, and ipGdIO-Dox+NAC.

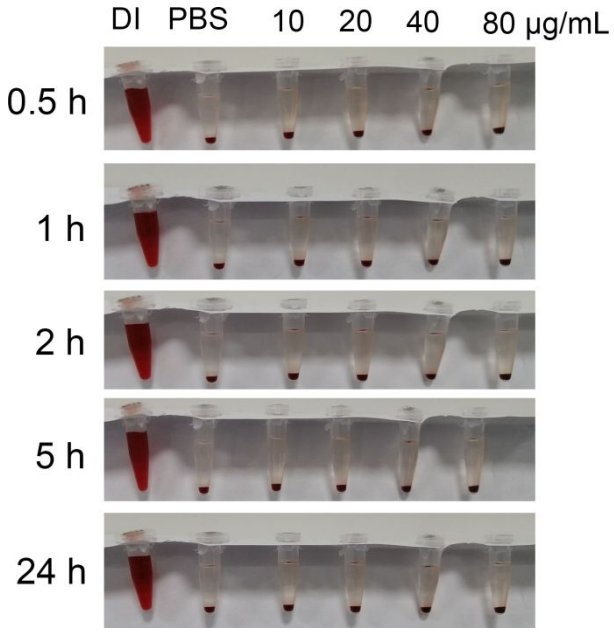

Figure S10. Hemolysis assay of ipGdIO-Dox at various concentrations.

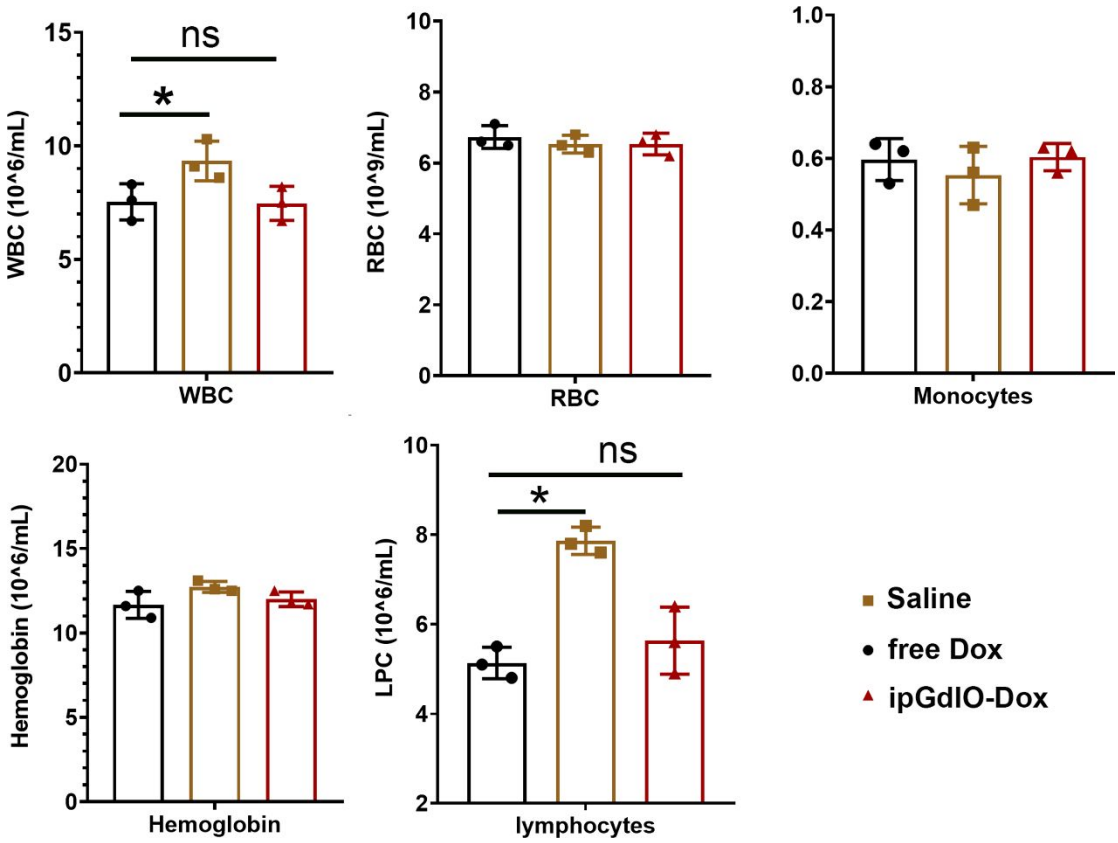

Figure S11. The blood routine analysis of mice injected with saline, Dox, and ipGdIO-Dox.