Electronic Supplementary Information

A dual-catalytic nanoreactor for synergistic chemodynamic-starvation therapy toward tumor metastasis suppression

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1. Experimental Procedures

1.1 Materials and reagents. Trition X-100, cyclohexane, n-Hexanol, tetraethyl orthosilicate (TEOS), hydrofluoric acid (HF, 40%), 1-(3-diaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Alfa Aesar Chemical Ltd. (Tianjin, China); (3-aminopropyl)-triethoxysilane (APTES) was purchased from Heowns Biochemical Technology Co., Ltd.; Fe_3O_4 was purchased from Nanjing 2DNANO Tech. Co., Ltd.; glucose oxidase (GOx) and perfluorohexane (PFC) were purchased from Sigma-Aldrich. All the chemical reagents used in the experiments were analytical grade and without any purification. Mouse skin melanoma cell (B16-F10) was purchased from Shanghai Aoluo Biotechnology Co., Ltd. The water used in all experiments was Mill-Q secondary ultrapure water (18.2 $M\Omega \cdot cm^{-1}$).

1.2 Instruments. Transmission electron microscopy (TEM, HT7700, Japan) was used to characterize the morphologies of nanoparticles. Absorption spectra were obtained with a pharmaspec UV-Vis spectrophotometer (UV-1700, Shimadzu, Japan). Fluorescence spectra were measured on an Edinburgh fluorescence spectrometer with a xenon lamp (FLS980). The pH values were obtained with a pH-3c digital pH-meter (Shanghai LeiCi, China). Confocal fluorescence images were obtained with a confocal laser scanning microscope (LEICA TCS SP5, Germany). Microplate reader (Synergy 2, Biotek, USA) was used in the MTT assay. Inductively coupled plasma spectrometer (Thermo Fisher, iCAP 7400, USA) was used in the elemental analysis.

1.3 Synthesis of GOx-Fe_3O_4-HMSNs. HMSNs were compounded in line with the method of the known literature. The first step was the synthesis of SiO_2-NH_2 adopting a reverse-phase microemulsion way. 5.3 mL of Trition X-100, 22.5 mL of cyclohexane, 5.4 mL of n-hexanol, and 1 mL of water were added to the round bottom flask and stirred well for 5 min. Then 750 μL of ammonium hydroxide was added as well as stirred for 0.5 h. After fully mixing, 500 μL of TEOS and 100 μL of APTES were added to the aforementioned solution with mighty stirring. After 24 h of reaction at room temperature, the SiO_2-NH_2 was gained by centrifugation (10,000 rpm, 10 min) and washed with absolute ethyl alcohol for three times. Subsequently, the resulting SiO_2-NH_2 dissolved in 60 mL of absolute ethyl alcohol. Then 10 mL of water and 10 mL of ammonium hydroxide were injected. After stirring well for 30 min, a mixture of 0.3 mL of TEOS and 9.7 mL of absolute ethyl alcohol were added drop by drop and continuous stirred for 3 h. The obtained $dSiO_2$ was collected via centrifugation (10,000 rpm, 10 min) and washed with absolute ethyl alcohol and water for several times, then dispersed in 80 mL of water. Afterwards, a HF corrosion method was used to manufacture HMSNs. 10 mL of the above solution and 150 μL of HF solution (4%, w%) were injected with quickly stirring for 6 min. After that, the solution was centrifuged promptly and washed twice with water and absolute ethyl alcohol, finally dissolved in 40 mL of absolute ethyl alcohol.

Then, the resultant HMSNs was modified with amino groups via employing APTES. Firstly, 400 μL of NH_4OH and 16 mL of water were mixed and stirred, and then 10 μL of APTES was injected. After 12 h of reaction at room temperature, the obtained HMSN-NH_2 was collected via centrifugation (10,000 rpm, 10 min) and washed with absolute ethyl alcohol and water twice, at last dissolved in 10 mL of PBS (pH = 7.4, 0.01 M). To activate the carboxyl groups in Fe_3O_4, a PBS solution of EDC (10 mg), NHS (8 mg) and Fe_3O_4 (9 mg) was kept in dark place for 0.5 h. The same procedure was also applied to GOx (1.123mg) by using EDC (29mg) and NHS (18mg). After that, the solution of HMSNs-NH_2 was added to the mixture of Fe_3O_4 and GOx and reacted for 24 h, followed by centrifugation and washing twice to collect the GOx-Fe_3O_4-HMSNs.

1.4 Synthesis of GOx-Fe_3O_4-HMSNs-PFC/O_2. Firstly, the aforementioned GOx-Fe_3O_4-HMSNs was dried under vacuum. Then 300μL of perfluorohexane (PFC) was injected and sonicated for 1 min in ice water. The obtained material was dispersed in PBS and sonicated for 10 min in ice water. After that, the above solution was reposited in oxygen tank (O_2 flux rate = 5 L/min) for 10 min to achieve oxygen saturation.

1.5 Synthesis of GOx-Fe_3O_4-HMSNs-PFC/O_2@C. Firstly, B16-F10 cells were suspended and centrifuged (1000 rpm, 3 min), followed by washed with Tris buffer (pH = 7.4) for two times and resuspended in Tris buffer with 1% protease inhibitor. Afterwards, the cells were crushed with a homogenizer in ice water and membrane fragments were gained via differential centrifugation. The mixture of membrane fragments and GOx-Fe_3O_4-HMSNs-PFC/O_2 was stirred for 24 h in ice water. After fully coating, the product was centrifuged (10000 rpm, 10 min) and dissolved in PBS buffer.

1.6 Cell culture. B16-F10 cells were cultured in cell culture dishes with a diameter of 10 cm containing RPMI 1640 supplement with 10% fetal bovine serum and 1% 100 U/mL penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2.

1.7 Detection of ·OH. Methylene blue (MB) was used for ·OH detection. (1) Fe_3O_4 were dispersed in oxygen-free PBS at pH 5.4 with or without the addition of H_2O_2, and then incubated with MB for 2 h at room temperature. After that, UV-Vis absorption of MB was determined after centrifugation. (2) GOx-Fe_3O_4-HMSNs-PFC/O_2 were dispersed in oxygen-free PBS at pH 5.4 with or without the addition of glucose and then incubated with MB at 37 °C for 2 h. UV-Vis absorption of MB was determined after centrifugation.

1.8 Detection of H_2O_2. A H_2O_2 specific molecular probe Cy-O-Eb was used for H_2O_2 detection. Glucose was added to the GOx-HMSNs solution containing Cy-O-Eb at 37 °C, and incubated for 12 h. Then the fluorescence of supernate was determined after centrifugation.

1.9 MTT assay. (1) B16-F10 cells were cultured in 96-well plate and incubated for 24 h. The HMSNs@C of different concentration (0, 40,

80, 100, 150, and 200 μg/mL) in RPMI 1640 media were injected into the well and co-incubated for 24 h in anaerobic conditions. Then the media were discarded and 150 μL of MTT solution (0.5 mg/mL) was injected to each well. After 4 h, the MTT solution was removed and 150 μL of DMSO was injected. Then the absorbance was determined at 490 nm via a microplate reader. (2) B16-F10 cells were cultured in 96-well plate and incubated for 24 h. The cells were divided into five groups: control, Fe_3O_4-HMSNs-PFC/O_2@C, GOx-HMSNs-PFC/O_2@C, GOx-Fe_3O_4-HMSNs-PFC@C, and GOx-Fe_3O_4-HMSNs-PFC/O_2@C groups. The concentration of nanoparticles was 40 μg/mL. The cells were incubated with materials for 24 h, then their relative viabilities were tested. (3) B16-F10 cells were cultured in 96-well plate and incubated for 24 h. The cells were divided into three groups: Fe_3O_4-HMSNs-PFC/O_2@C, GOx-HMSNs-PFC/O_2@C and GOx-Fe_3O_4-HMSNs-PFC/O_2@C. The nanomaterials of different concentrations (0, 5, 10, 15, 25, 45, 65, 85, 105 μg/mL, in terms of HMSNs) in RPMI 1640 medium were injected into the well. The cells were incubated with materials for 24 h, then their relative viabilities were tested.

1.10 Confocal imaging. (1) To detect the HIF-1α of cells, B16-F10 cells were cultured in confocal dishes and incubated for 12 h. After that, HMSNs-PFC@C and HMSNs-PFC/O_2@C (40 μg/mL) in RPMI 1640 media were injected and co-incubated for 4 h in anaerobic conditions. Subsequently, B16-F10 cells were washed and fixed by use of 4% precooling paraformaldehyde at room temperature for 20 min, and treated with primary antibody for 1 h, continued to incubate with secondary antibody for 1h after washing. Then the cells were washed for three times and analyzed with CLSM (λ_{ex}=488nm, λ_{em}=500-550nm). (2) To detect the ROS generation of cells, B16-F10 cells were cultured in confocal dishes overnight, followed by incubated with PBS, Fe_3O_4-HMSNs-PFC/O_2@C, and GOx-Fe_3O_4-HMSNs-PFC/O_2@C (40 μg/mL) for 4 h in anaerobic conditions. Subsequently, DCFH was added to reduce ROS for 15 min. Then the cells were washed for three times and analyzed with CLSM (λ_{ex}=488nm, λ_{em}=515-560nm). (3) To detect the viable and dead cells, B16-F10 cells were cultured in confocal dishes and incubated for 12 h. Then PBS, HMSNs-Fe_3O_4-PFC/O_2@C, HMSNs-GOx-PFC/O_2@C and GOx-Fe_3O_4-HMSNs-PFC/O_2@C (40 μg/mL) in RPMI 1640 media were injected and co-incubated for 4 h. Then the cells were stained with 3',6'-Di(O-acetyl)-4',5'bis[N,N-bis(carboxymethyl)aminomethyl] fluorescein, tetraacetoxymethyl ester (calcein-AM) (λ_{ex}=490nm, λ_{em}=515nm) / Propidium iodide (PI) (λ_{ex}=535nm, λ_{em}=617nm) for 15 min. Finally, the cells were rinsed twice by use of PBS before confocal microscopy experiments.

1.11 Western blot. B16-F10 cells were lysed in a radioimmunoprecipitation assay buffer solution involving 20mM Tris (pH 7.5), 150mM NaCl, 50mM NaF, 1% nonidet P-40, 0.1% deoxycholic acid, 0.1% sodium dodecyl sulfate (SDS), 1mM EDTA, 1mM phenylmethylsulfonyl fluoride, and 1 μg/mL leupeptin. Then proteins were solved by use of SDS–polyacrylamide gel electrophoresis (10%) and transferred onto a polyvinylidene fluoride membrane. The membranes were shut with 5% fat-free dry milk and cultured with primary antibody for 12 h at 4 °C. Sequentially, the cells were cultured with horseradish peroxidase-conjugated secondary antibodies. Specific proteins were observed by enhanced chemiluminescence detection reagent and the blots were analyzed with a Bio-Rad imaging system.

1.12 *In vivo* target study by ICP-AES. All procedures of animal study were agreed with the Principles of Laboratory Animal Care (People's Republic of China) and were approved by the Animal Care and Use Committee of Shandong Normal University (Jinan, China). Female Balb/C mice (6-8 weeks) were fed under normal conditions. B16-F10 melanoma metastatic tumor model was utilized as an example to evaluate the therapeutic effect. 1×10^5 B16-F10 cells in 100 μL of serum-free RPMI 1640 medium were injected intravenously into the tail of mice. The mice were utilized in subsequent experiments on day 7 after tumor cells injection.

The tumor-bearing mice were intravenously injected with GOx-Fe_3O_4-HMSNs-PFC/O_2@C and GOx-Fe_3O_4-HMSNs-PFC/O_2 (10 mg/kg). After 24 h, the mice were sacrificed. Then the metastatic tumors were isolated from normal lung tissue via excising the metastatic tumors from the whole lung tissue. After that, metastatic tumors and five other major organs were dissolved to the aqua regia (HCl:HNO_3 = 3:1, v:v) for the detection of Si content by ICP-AES.

1.13 *In vivo* therapeutic effect of the bio-NRs. The tumor-bearing mice were divided into six groups, and then intravenously injected with PBS, Fe_3O_4-HMSNs-PFC/O_2@C, GOx-HMSNs-PFC/O_2@C, GOx-Fe_3O_4-HMSNs-PFC/O_2, GOx-Fe_3O_4-HMSNs-PFC@C and GOx-Fe_3O_4-HMSNs-PFC/O_2@C (10 mg/kg), respectively. The body weight of each mouse was recorded every other day. After 14 days of treatment period, the mice were sacrificed for anatomy and histopathological analysis. The black B16-F10 metastatic tumors were excised from the whole lung tissue to the greatest extent. Then the mass of normal lung and the metastatic tumors were measured by use of an analytical balance. The mass ratio of metastases was obtained by the mass of metastatic tumors/mass of normal lung. Finally, the survival rate of the mice was recorded for 30 days.

2. Supplementary Figures

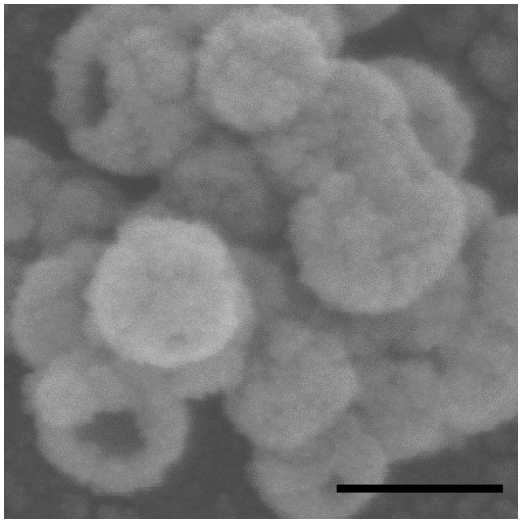

Figure S1. SEM image of HMSNs. Scale bar is 100 nm.

Figure S2. Ninhydrine color assay of HMSNs (left) and HMSNs-NH_2 (right).

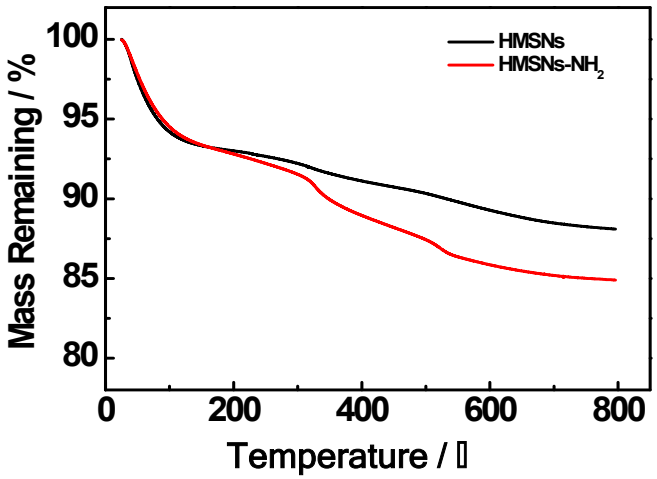

Figure S3. TGA analysis of HMSNs and HMSNs-NH_2.

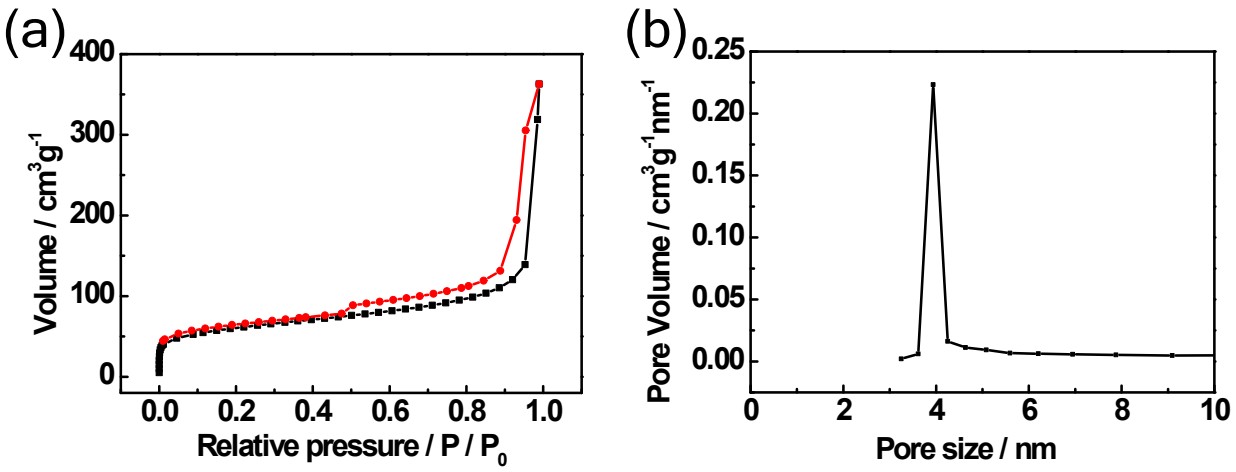

Figure S4. (a) N_2 adsorption-desorption isotherms and (b) pore size distribution of HMSNs-NH_2.

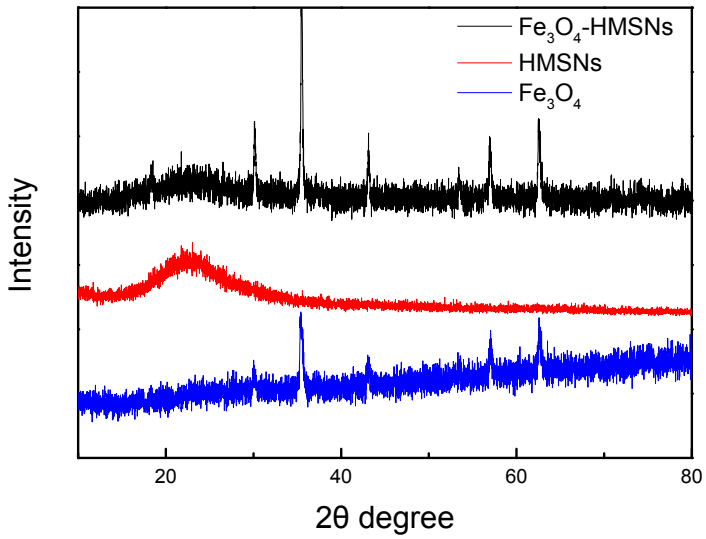

Figure S5. The XRD pattern of Fe_3O_4-HMSNs, HMSNs and Fe_3O_4.

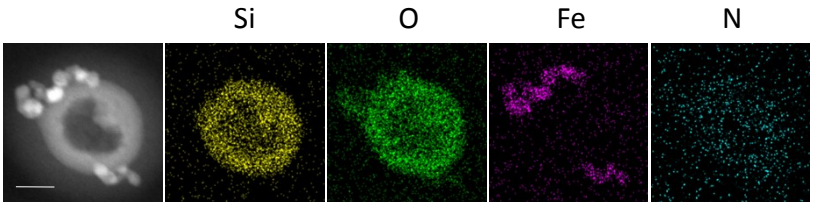

Figure S6. HAADF-STEM image and elemental mapping for GOx-Fe_3O_4-HMSN (scale bar is 30 nm).

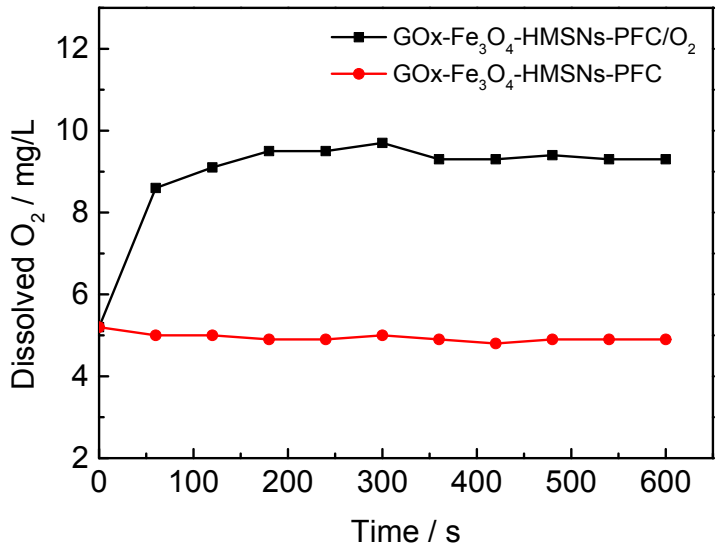

Figure S7. The oxygen release of GOx-Fe_3O_4-HMSNs-PFC/O_2 or GOx-Fe_3O_4-HMSNs-PFC.

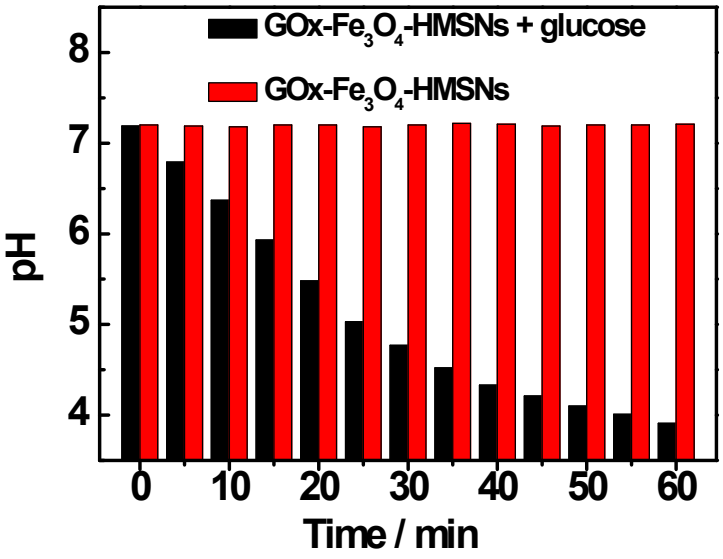

Figure S8. The pH values of GOx-Fe_3O_4-HMSNs solution treated with or without 1mM glucose.

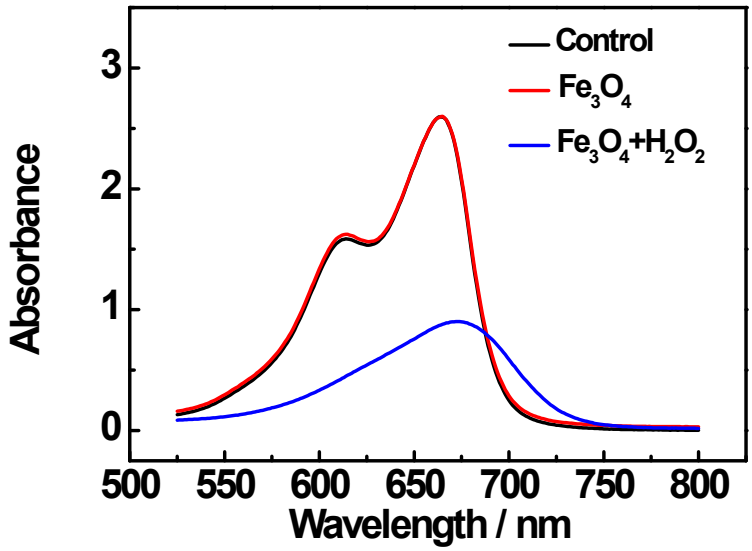

Figure S9. UV-vis absorption spectra of MB with Fe_3O_4 solution treated with or without H_2O_2 at pH 5.4.

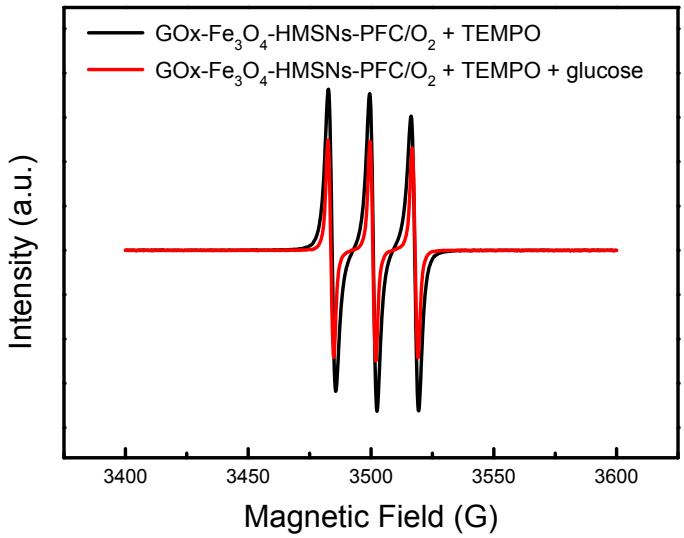

Figure S10. ESR spectra of TEMPO after incubation with GOx-Fe_3O_4-HMSNs-PFC/O_2 in the presence or absence of glucose.

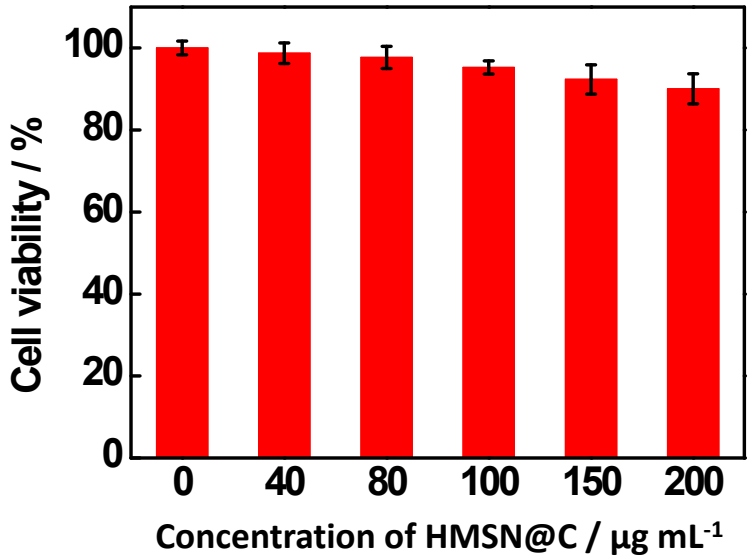

Figure S11. MTT assay of B16-F10 cells treated with various concentrations of HMSNs@C for 24 h.

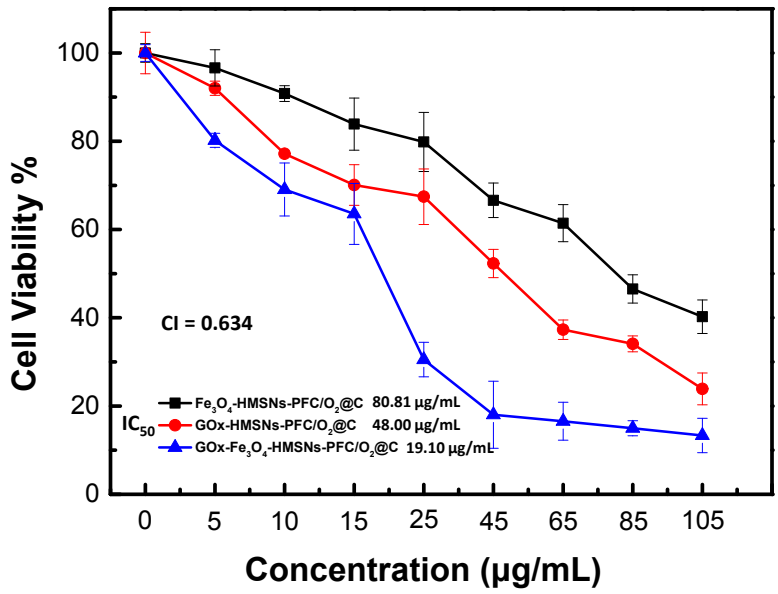

Figure S12. In vitro cytotoxicity against B16-F10 cells treated with various concentrations of Fe_3O_4-HMSNs-PFC/O_2@C, GOx-HMSNs-PFC/O_2@C and GOx-Fe_3O_4-HMSNs-PFC/O_2@C.

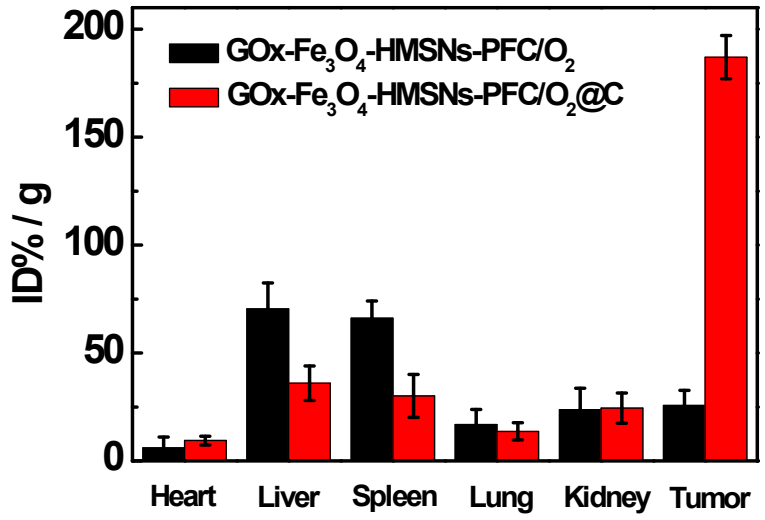

Figure S13. Si contents in different organs and metastatic tumors after the mice were injected with GOx-Fe_3O_4-HMSNs-PFC/O_2 or GOx-Fe_3O_4-HMSNs-PFC/O_2@C by ICP-AES analysis.

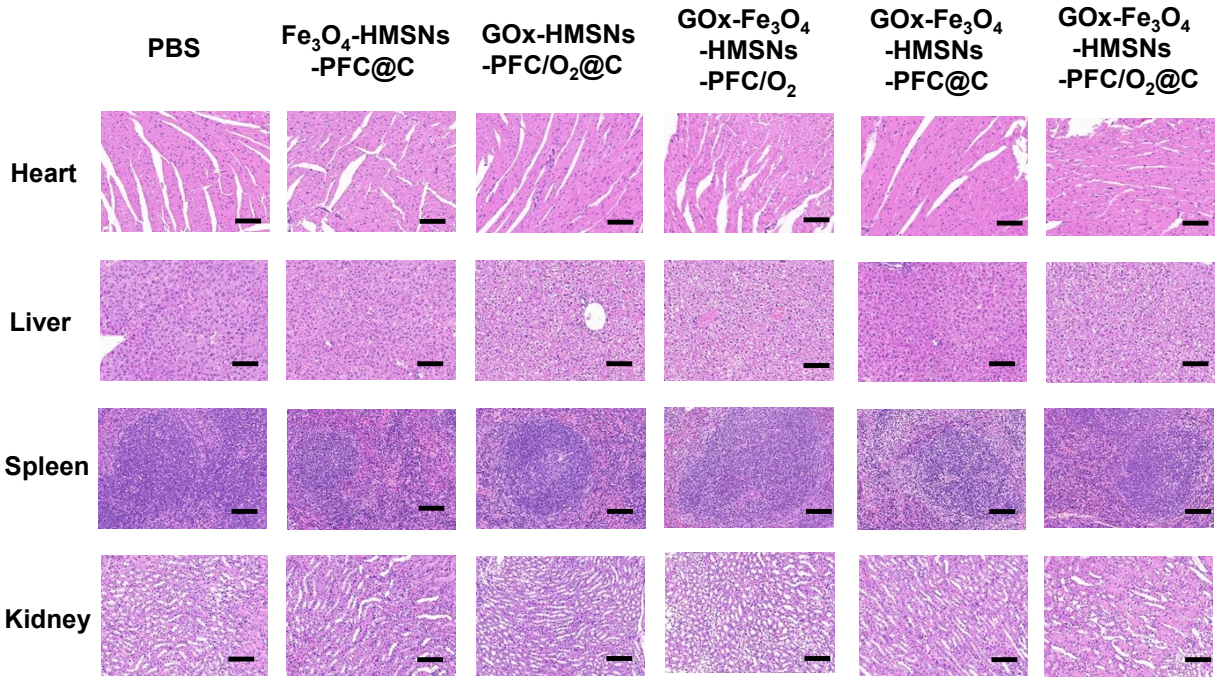

Figure S14. H&E staining images of four major organs after various treatments (scale bars are 100 μm).