Supporting Information

Glucose oxidase and Polydopamine Functionalized Iron Oxide Nanoparticle: Combination of Photothermal Effect and Reactive Oxygen Species Generation for Dual-Modality Selective Cancer Therapy

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Supplementary Experimental Section：

1. Determination of loading efficiency and catalysis ability of GOx on Fe_3O_4@pDA/GOx particles

For the determination of loading efficiency of GOx on nanoparticles, in each reaction, 40 mg of magnetic nanoparticles was suspended in 20 mL of sodium phosphate buffer (10 mM, pH 8.0) with GOx at 25 mg/mL. The reaction mixtures were shaken at 150 rpm for 4, 8, 16, and 36 h, respectively. The resultant Fe_3O_4@PDA/GOx NPs were recovered by placing the container on a strong permanent magnet. The recovered particles were washed three times with 60 mL of 10 mM PBS to remove the free GOx, and then were re-dispersed and lyophilized. The reaction and washing solution was collected and analyzed. Each experimental condition was repeated in triplicate. The loading efficiency of GOx (LE %) was calculated using below formula:

$$LE\ \% = (W_t - W_r)/W_{NP} \times 100\%$$

where W_r is the amount of the remaining GOx in the reaction solution and washing solution, the W_t is the total quantity of GOx added initially in the reaction mixture, and the W_{NP} is the total quantity of magnetic nanoparticles added initially in the reaction mixture. The

concentration of GOx were determined using Bradford colorimetric assay, and the W_t and W_r were calculated accordingly.

The determination of kinetic parameters of GOx on 20 and 200 nm Fe_3O_4@pDA/GOx nanoparticles were carried out in 200 μL sodium phosphate buffer (10 mM, pH 6.8) in presence of 1 mg Fe_3O_4@PDA/GOx NPs, 7.2 mM ABTS, 1 mM HRP and different concentrations of glucose (10 μM to 1.5 mM). Reactions were incubated for 5 min at 37°C. After incubation, the nanoparticles were removed from the solution by using a magnetic separation stand. Absorption intensity was measured using Ensprit multilabel reader spectrophotometer. The corresponding H_2O_2 concentration was calculated through the H_2O_2 standard curve. Experiments were performed in triplicate and the kinetic parameters were determined by fitting the data to the standard Michaelis–Menten equation through Grapad Prism 6 (https://www.graphpad.com/scientific-software/prism/). Controls with enzyme alone and substrate alone were included in order to compensate for any interference.

2. Effects of pH and temperature on GOx activity on Fe_3O_4@pDA/GOx particles

The pH effect was determined at a constant temperature (37°C) and 5-minute incubation under different pH conditions (pH 3.0 to 11.0) in the CKBB buffer containing citric acid, KH_2PO_4, boric acid，barbitone and NaOH. The temperature effect was determined by assaying the GOx activity from 20°C to 60°C for 5-minute incubation in 10 mM sodium phosphate buffer (10 mM, pH 6.8) for 200 nm Fe_3O_4@pDA/GOx particles. The relative activity (%) was calculated by using the ratio of the activity after incubation to the activity before incubation. Each experimental condition was repeated in triplicate.

3. In vitro cytotoxicity of NPs to 4T1 cells

To quantitatively evaluate the *in vitro* cytotoxicity of the Fe_3O_4@PDA/GOx NPs to 4T1cells, cells were seeded into 96-well cell-culture plate at $1x10^4$ cells/well and maintained for 24 h. The two sizes of Fe_3O_4@PDA/GOx NPs with different concentrations (10-1000 μg/mL) were

pre-incubated with cells for 4 h. The Fe_3O_4 (50 μg/mL) and Fe_3O_4@PDA (50 μg/mL) particles were incubated with cells under the same condition as the controls. After incubation, the culture media were removed and cells were completely rinsed with PBS to eliminate the uncombined NPs. The fresh complete media were refilled and the cells were then exposed to NIR laser (808 nm, 3.6 W/cm^2) for 3 min followed by another 24 h incubation. The standard MTT assay was carried out to evaluate the cell viability relative to the control untreated cells.

4. Effects of Fe_3O_4@PDA/GOx NPs on body weight and blood glucose levels

All animal experiments were carried out in compliance with guidelines approved by the Institutional Animal Care and Use Committee of Shandong University. Animals were obtained from the Experimental Animal Centre of Shandong University. BALB/c mice were intravenously injected with PBS (as the Control) and Fe_3O_4@PDA/GOx NPs (4 mg kg^{-1}, n = 5). The body weights of the mice were measured every three days, and the blood glucose levels were analyzed the day before injection and the day after injection.

Supplementary Figures:

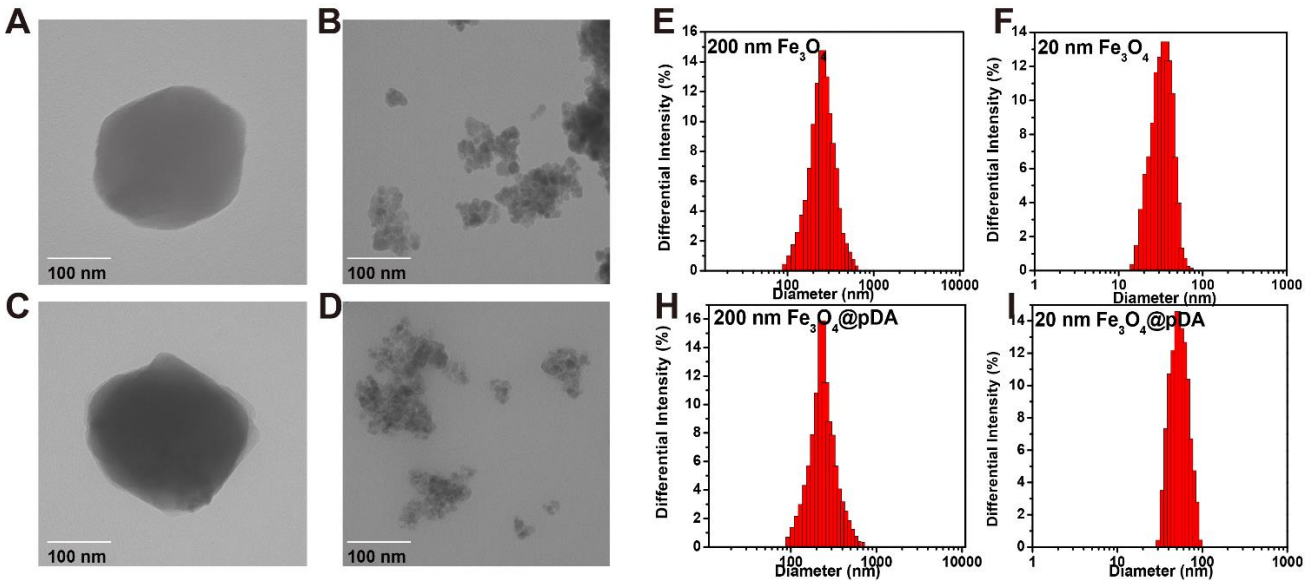

Figure S1. TEM image and hydrodynamic size of (A) and (E): 200 nm diameter Fe_3O_4 particles, (B) and (F): 20 nm diameter Fe_3O_4 particles, (C) and (H): 200 nm diameter Fe_3O_4@PDA particles, (D)

and (I) 20 nm diameter Fe_3O_4@PDA particles. According to the dynamic light scattering (DLS) analysis, the hydrodynamic diameters of two types of Fe_3O_4 particles were about 217 nm and 28 nm, and the hydrodynamic diameters of two types of Fe_3O_4@PDA particles were about 312 nm and 51 nm, respectively. The hydrodynamic diameter of the Fe_3O_4 particle increased after PDA modification and, indicating the PDA deposition and thicker hydration layer adhered to particle surfaces after PDA deposition.

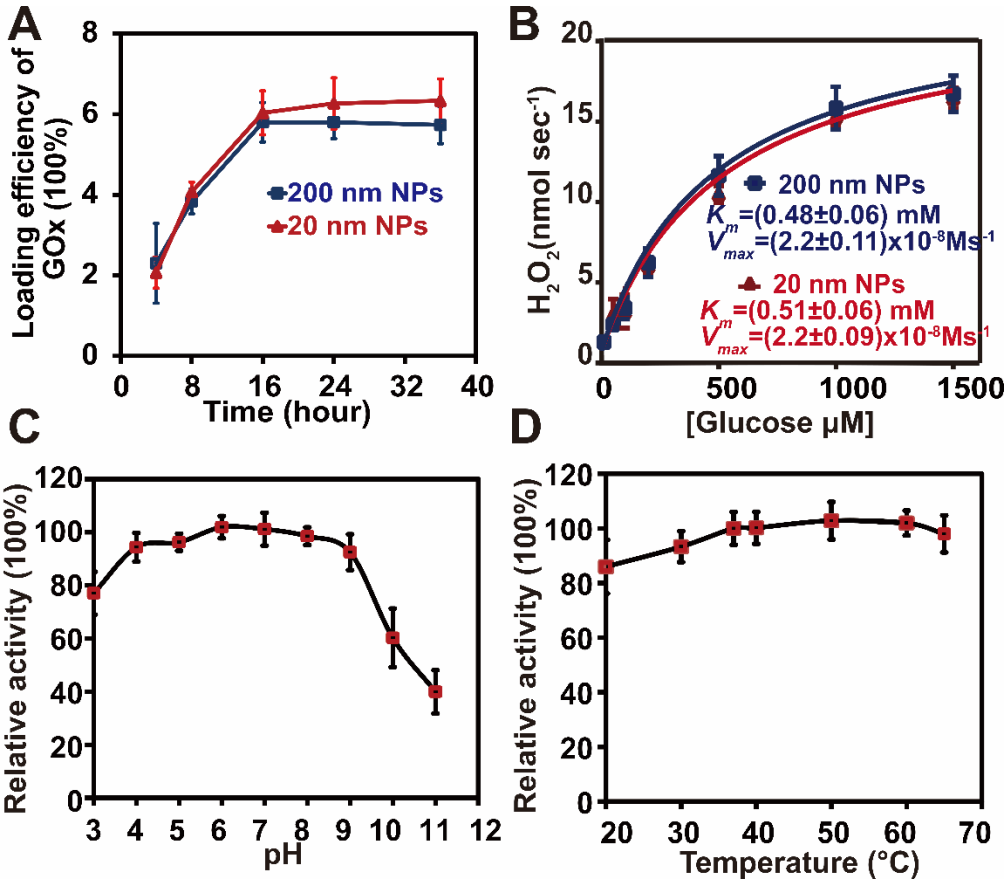

Figure S2. Characterizations of Fe_3O_4@pDA/GOx nanoparticles. A: GOx loading efficiency of 20 and 200 nm Fe_3O_4@pDA/GOx nanoparticles; B: Michaelis-Menten (steady-state) kinetics curve of 20 and 200 nm Fe_3O_4@pDA/GOx using glucose as the substrate, the kinetic parameters were calculated by fitting the data to the standard Michaelis–Menten equation through GraphPad Prism 6 (http://www.graphpad.com/scientificsoftware/prism/); C and D: pH and temperature effects on GOx activities of 200 nm Fe_3O_4@pDA/GOx.

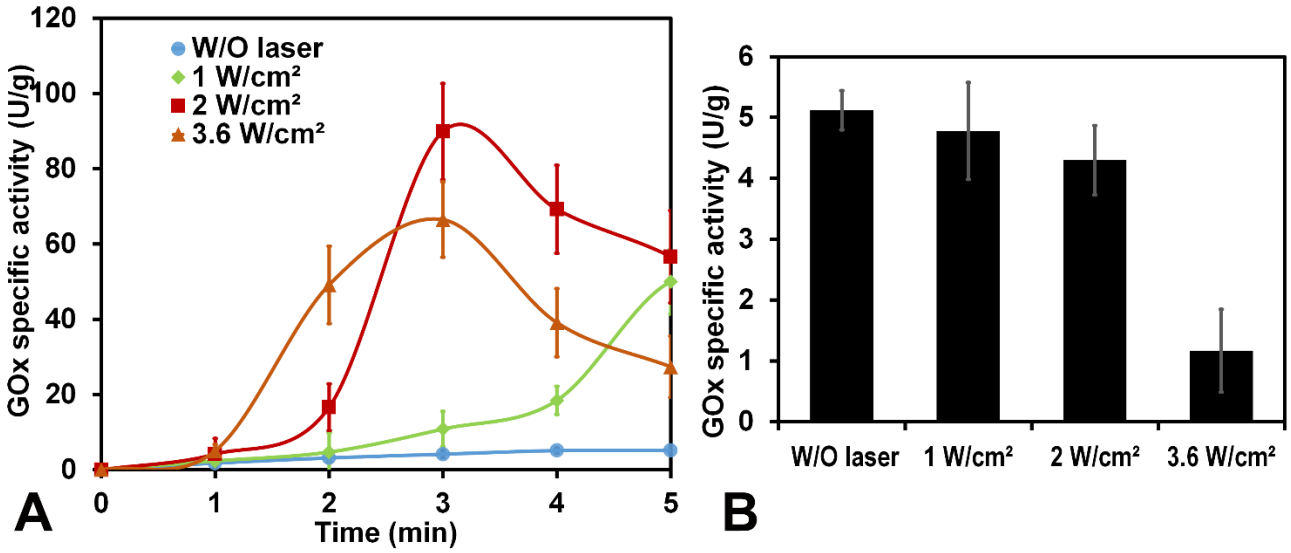

Figure S3. The effect of NIR laser irradiation on GOx activity of Fe_3O_4@pDA/GOx: A. The GOx activity during the irradiation process. The reactions were performed in 1 mL of sodium phosphate buffer (PBS, 10 mM, pH 6.8) in presence of 5 mg Fe_3O_4@PDA/GOx NPs, 7.2 mM ABTS, 1 mM HRP and 40 μM of glucose, and were either irradiated by NIR laser or incubated at 37°C. The aliquots of reaction mixtures were sampled every minute and GOx activity was analyzed using Ensprit multilabel reader spectrophotometer at 480 nm; B. The residual GOx activity after the irradiation. The NPs were incubated either at 37°C or irradiated by NIR laser in the PBS buffer for 5 min, and the resulted NPs were collected and employed for GOx activity assay. As shown in the results, the NPs performed much higher GOx activity during NIR irradiation in the system with elevated temperature, and after 5 min process, when test in the 37°C, the NPs lost 7% (using 1 W/cm^2 laser), 16% (using 2 W/cm^2 laser) and 77% (using 3.6 W/cm^2 laser) of the activity, respectively, suggesting that the NIR irradiation could only temporarily improve the GOx activity, and results in the partial loss of GOx activity of NPs. But the NPs could still reserve most of the GOx activity after 2 W/cm^2 laser treatment, suggesting that the 2 W/cm^2 laser treatment could still be a desirable condition.

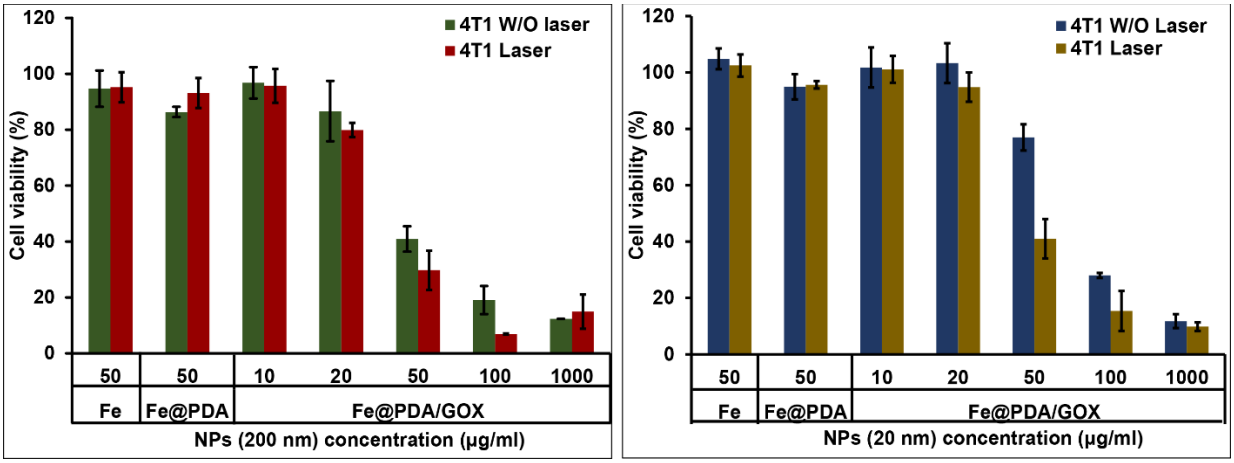

Figure S4. Cell viability studies in cancer cell 4T1 after incubation with two sizes of iron oxide (Fe),

PDA coated iron oxide (Fe@PDA), and GOx and PDA functionalized iron oxide NPs (Fe@PDA/GOx) (n = 3, mean ± s.d.)

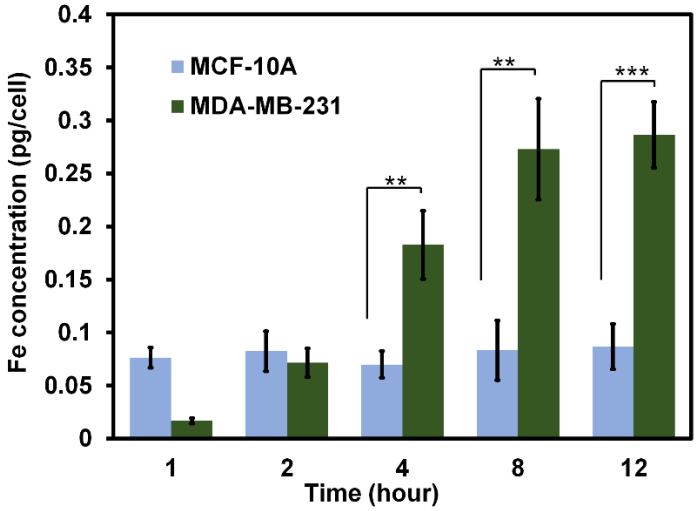

Figure S5 Quantitative analysis of the iron mass of cancer cells MDA-MB-231 and normal cells MCF-10A treated by 50 μg/ml 200 nm Fe_3O_4@pDA/GOx NPs within 12 hours (n = 3, mean ± s.d., **p<0.01, ***p<0.001).

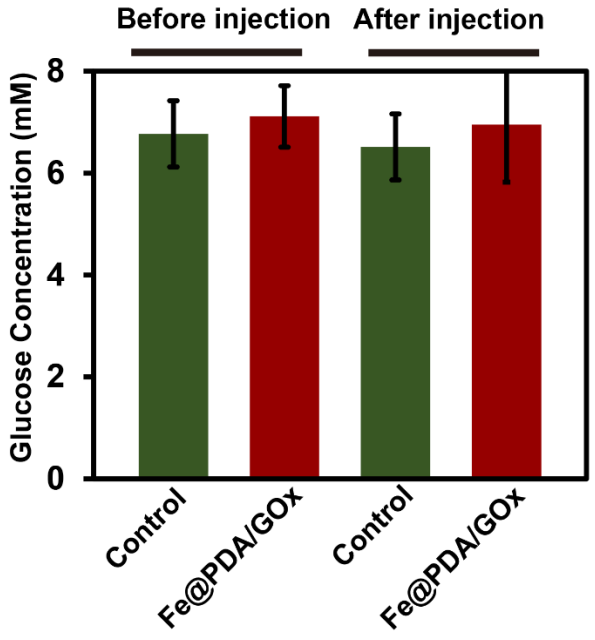

Figure S6. Blood glucose levels in of BALB/c mice before and after intravenous injection of Fe_3O_4@PDA/GOx NPs (Fe@PDA/GOx). These results suggested that the Fe_3O_4@PDA/GOx NPs did not reduces blood glucose levels in the mice after the intravenous injection. (n = 5, mean ± s.d.)

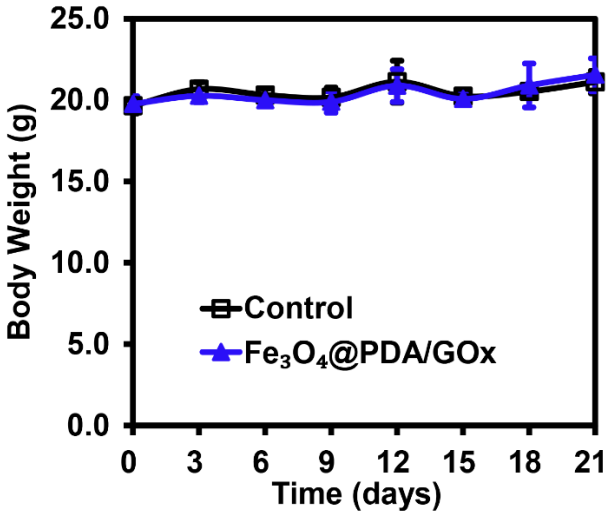

Figure S7. Body weight of the healthy mice after intravenous injection of Fe_3O_4@PDA/GOx NPs, (n = 5, mean ± s.d.).

Table S1 Elemental analysis results of the Fe_3O_4@pDA and Fe_3O_4@pDA/GOx particles.

	NPs	N (W%)	C (W%)	H (W%)	S (W%)
20 nm	Fe_3O_4	ND	0.01	ND	ND
	Fe_3O_4@pDA	0.12	0.65	0.07	ND
	Fe_3O_4@pDA/GOx	0.57	1.30	0.19	0.08
200 nm	Fe_3O_4	ND	ND	ND	ND
	Fe_3O_4@pDA	0.09	0.55	0.06	ND
	Fe_3O_4@pDA/GOx	0.51	1.18	0.17	0.08

Elemental analysis was performed on a Vario EL Elemental Analyzer.