Supplementary Information

A biomimetic MOF nanoreactor enables synergistic suppression of intracellular defense systems for augmented tumor ablation

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Reagents

Zn(NO₃)₂·6H₂O, imidazole-2-carboxaldehyde (ICA), glucose, fluorescein isothiocyanate (FITC), indocyanine green (ICG), 2',7'-dichlorofluorescin diacetate (DCFH), 2,2'-azino-bis(3ethylbenzothiazoline-6-sulphonic acid) (ABTS), D-glucose were all of analytical purity and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glucose oxidase (GOx), horseradish peroxidase (HRP), Rhodamine 123, 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Company. Lysotracker Green, Calcein-AM/PI Double Stain Kit, ATP assay kit, Reactive Oxygen Species Assay Kit (DCFH-DA), pH probe 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein,acetoxymethyl ester (BCECF AM) and Annexin V-FITC Apoptosis Detection Kit were purchased from Beyotime (Nantong, China). 4T1 cell was obtained from Aoluo Biotechnology Co., Ltd KeyGEN biotechnology (Shanghai, China). Primary antibodies for caspase-3, caspase8, caspase-9, heat shock protein-90, catalase, monocarboxylate transporter-4 and FITC-labeled secondary antibody were purchased from Wuhan Boster Biological Technology Co., Ltd. (Wuhan, China). The mouse mammary carcinoma cells (4T1 cell) were purchased from Procell Life Science Co., Ltd. (Wuhan, China). Confocal dish was purchased from Cellvis, Mountain View, CA. All the other reagents and solvents were analytical grade and used directly without further purification. Sartorius ultrapure water (18.2 M Ω ·cm⁻¹) was used for experiments.

Measurement and characterization

Transmission electron microscopy (TEM, HT7700, Japan) was employed to characterize the morphologies of the nanoreactors. Fluorescence spectra were obtained with an Edinburgh fluorescence spectrometer with a xenon lamp (FLS-920). Absorption spectra were measured on a pharmaspec UV-1700 UV-visible spectrophotometer (Shimadzu, Japan). Fourier infrared spectrometer (Nicolet iS50 FT-IR) was used to characterize the infrared spectrum. All pH measurements were made using a pH-3c digital pH meter (Shanghai Leici Equipment Engineering Co., Ltd., Shanghai, China). In the MTT assay, the absorbance was measured using a microplate reader (Synergy 2, Biotek, USA). Cell disruption was performed using a homogenizer (IKA, Germany, T10 basic ultra-turrrax). Confocal fluorescence images were captured using TCS-SP8 confocal laser scanning microscope (Leica, Germany). *In vivo* fluorescence images were captured using live animal imaging system (IVIS Lumina III, US). Inductively coupled plasma atomic

emission spectrometer (ICP-AES, iCAP 7600, Thermo Fisher, US) was used to measure the metal ion concentration. The sections were observed through a Nikon Eclipse 80i microscope.

Synthesis of GOx-ICG@ZIF, ZIF, GOx@ZIF and ICG@ZIF.

ICA (15.3 mg) was first ultrasonication dissolved in 2 mL of H₂O and stirred at room temperature, then, 1 mg GOx and 1 mg ICG were added into the solution and stirred until dissolved. Afterwards, 100 μ L Zn(NO₃)₂·6H₂O solution containing 11.9 mg Zn(NO₃)₂·6H₂O was quickly added into the mixture and stirred for another 1 h. Finally, the GOx-ICG@ZIF crystal was separated and washed with H₂O for three times *via* centrifugation at 6000 rmp for 15 min. ZIF, GOx@ZIF and ICG@ZIF were synthesized with the same procedure by changing the reagents added into the solution.

UV-Vis absorbance curves of ICG.

ICG solutions with a concentration gradient from $3-11 \ \mu g/mL$ were prepared, and the absorbance spectra from 600-900 nm were recorded.

Fluorescence curves of GOx-FITC.

GOx-FITC solutions with a concentration gradient of 10-40 μ g/mL were prepared, and the fluorescence spectra from 500-580 nm were recorded. $\lambda_{ex} = 488$ nm.

Standard method for evaluation of the GOx activity.

To determine the activity of GOx, GOx (1 μ g) or diverse nanocomposites with equiv. amount of GOx were added into 2 mL solution containing HRP (0.1 mg/mL), ABTS (2 mg/mL) and D-glucose (4 mg/mL), after incubated at room temperature for 10 min, the absorbance of the supernatant at 420 nm was recorded by the UV-Vis absorption spectra.

Evaluation the thermal stability of GOx.

To determine the thermal stability of GOx from different composites, 1 μ g GOx or other nanocomposites containing equiv. amount of GOx were treated with hyperthermia at 65 °C for 1h, subsequently, the activity of GOx from different groups were detected based on the standard method and compared with the untreated groups.

Evaluation the activity of GOx before and after trypsin treatment.

To determine the digestion resistance of GOx from different groups, 1 µg GOx or GOx-ICG@ZIF (containing the equiv. amount of GOx) were treated with trypsin under 37 °C for 1 h, subsequently, the GOx activities were compared with the GOx or GOx-ICG@ZIF based on the standard strategy. **Cell culture.**

4T1 cells were incubated in cell culture dishes with a diameter of 10 cm containing RPMI 1640 supplemented with 10% (v/v) fetal bovine serum (FBS), 1% penicillin and streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2 .

Purification of 4T1 cell membrane.

4T1 cells were first collected and treated with centrifucation (1000 rpm, 3 min). The cells were then washed Tris buffer (pH = 7.4) twice and resuspended in Tris buffer containing 1% protease inhibitor. Next, the cell disruption experiment was operated with a homogenizer in an ice-water bath. The 4T1 cell membrane was collected by differential centrifugation for several times.

Preparation of ZIF@CM, GOx@ZIF@CM, ICG@ZIF@CM, GOx-ICG@ZIF@CM.

Membrane fragments were mixed with ZIF, GOx@ZIF, ICG@ZIF and GOx-ICG@ZIF in ice-water bath, the mixtures were stirred for 24 h. After centrifugation (10,000 rpm, 10 min) and wash with H₂O for several times, the precipitates of ZIF@CM, GOx@ZIF@CM, ICG@ZIF@CM, GOx-ICG@ZIF@CM were separated and re-dispersed in H₂O and stored in 4 °C for further use.

Preparation of FITC labeled GOx (GOx-FITC).

GOx-FITC was prepared by mixing 10 mg GOx and 1 mg FITC in 10 mL PBS solution, after stirring for 24 h, the obtained solution was dialyzed in pure water until no fluorescence could be detected from the dialysate. Finally, the powder of GOx-FITC was obtained by freezing-drying.

Confocal imaging of the uptake effect of the nanoreactor.

To determine the cell uptake of the nanoreactor, 4T1 cells were seeded in the confocal dish, after the cell confluence reached about 70%, the cells were incubated with GOx-FITC-ICG@ZIF@CM nanoreactor (100 µg/mL) for 5 h, washed with fresh culture medium and finally analyzed with CLSM.

Evaluation of the immune escape effect of the nanoreactor.

To determine the immune escape effect of the nanoreactors, 4T1 cells and macrophage cells were seeded in the confocal dishes or 60 mm cell culture dishes, after the cell confluence reached about 70%, the cells were incubated with GOx-ICG@ZIF or GOx-ICG@ZIF@CM (100 µg/mL) for 3 h, washed with fresh culture medium, and finally analyzed with CLSM or imaging cytometry system.

MTT assay.

For the cell toxicity assay, 4T1 cells were seeded onto a 96-well plate (10⁴ per well) and cultured for 24 h. Then ZIF@CM, GOx@ZIF@CM, ICG@ZIF@CM or GOx-ICG@ZIF@CM with

different concentrations (10, 40, 70, and 100 μ g/mL) in RPMI 1640 media were added into the well and co-incubated for 5 h. Subsequently, fresh culture medium was added into the well. For the laser irradiation groups, the cells were irradiated with 808 nm NIR laser (1 W/cm²) for 5 min. And the cells were further cultured for 20 h. Then the media were removed and 150 μ L of MTT solution (0.5 mg/mL) was added into each well. After 4 h, MTT solution was removed and 150 μ L DMSO were added and the absorbance was monitored at 490 nm by a microplate reader.

Detection of the protein expression profiles.

4T1 cells were treated with the same conditions as described in MTT assay, the incubation time was 10 h. Later, cells were lysed in a radioimmunoprecipitation assay buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 50 mM NaF, 1% nonidet P-40, 0.1% deoxycholic acid, 0.1% sodium dodecyl sulfate (SDS), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 μg/mL leupeptin. Proteins were resolved by SDS–polyacrylamide gel electrophoresis (10%) and transferred onto a polyvinylidene fluoride membrane. The membranes were blocked with 5% fat-free dry milk and incubated with primary antibody overnight at 4 °C. The membranes were incubated with horseradish peroxidase-conjugated secondary antibodies. Specific proteins were visualized with enhanced chemiluminescence detection reagent (Yuci Biotechnology, Inc., Jiangsu, China). The blots were analyzed using a Bio-Rad imaging system (Bio-Rad, Hercules, CA, USA).

Live/dead cell staining assay.

4T1 cells were seeded in confocal dishes. When the cell density approached about 70%, the cells were treated with ZIF@CM, GOx@ZIF@CM, ICG@ZIF@CM or GOx-ICG@ZIF@CM (100 μg/mL) for 5 h, and further incubated with fresh culture medium. PBS group was used as control. For the laser irradiation groups, the cells were further irradiated with 808 nm NIR laser (1 W/cm²) for 5 min. All the groups were further cultured for 20 h. Subsequently, the cells were treated with Calcein AM/Propidium Iodide (PI) for 15 min and analyzed with CLSM.

Detection of the intracellular ATP level.

4T1 cells were seeded in 60 mm cell culture dishes. When the cell density approached 70%, the cells were treated with ZIF@CM, GOx@ZIF@CM, ICG@ZIF@CM or GOx-ICG@ZIF@CM (100 μ g/mL) for 5 h, and further incubated with fresh culture medium. PBS group was used as contorl. For the laser irradiation groups, the cells were further irradiated with 808 nm NIR laser (1 W/cm²) for 5 min. All the groups were further cultured for 20 h. Then the cells were collected and counted.

Subsequently, the cells were lysed with ATP lysis buffer and the supernate was replaced into a 96well plate, after further incubated with 2 μ L of luciferin and firefly luciferase containing ATP assay Buffer, the luminescence intensity was recorded with a luminometer after 10 min.

Evaluation of the intracellular H₂O₂ level.

4T1 cells were seeded into the CLSM cell culture dish, incubated with ZIF@CM, GOx@ZIF@CM, ICG@ZIF@CM or GOx-ICG@ZIF@CM (100 μ g/mL) for 5 h, and further replaced with fresh culture medium. PBS group was used as control. For the laser irradiation groups, the cells were irradiated with 808 nm NIR laser (1 W/cm²) for 5 min. All the groups were further cultured for 20 h. Afterwards, the cells were washed with PBS and treated with DCFH-DA (10 μ M) at 37 °C for 20 min. Finally the cells were washed with PBS and analyzed with CLSM (Ex: 488 nm, Em: 500-560 nm).

Identification of cell acidification.

4T1 cells were seeded into the CLSM cell culture dish, and were incubated with ZIF@CM, GOx@ZIF@CM, ICG@ZIF@CM or GOx-ICG@ZIF@CM (100 μ g/mL) for 5 h, and further incubated with fresh culture medium. PBS group was used as control. For the laser irradiation groups, the cells were irradiated with 808 nm NIR laser (1 W/cm²) for 5 min. All the groups were further cultured for 20 h. Subsequently, the cells were washed with PBS and treated with BCECF AM probe (5 μ M) at 37 °C for 20 min. Then the cells were washed with PBS and analyzed with CLSM (Ex: 488 nm, Em: 500-560 nm).

Detection of mitochondrial membrane potential.

4T1 cells were seeded into the CLSM cell culture dish, and were incubated with ZIF@CM, GOx@ZIF@CM, ICG@ZIF@CM or GOx-ICG@ZIF@CM (100 μ g/mL) for 5 h, and further incubated with fresh culture medium. PBS group was used as control. For the laser irradiation groups, the cells were irradiated with 808 nm NIR laser (1 W/cm²) for 5 min. All the groups were further cultured for 20 h. Subsequently, the cells were washed with PBS and stained with Rhodamine 123 (1 μ M) at 37 °C for 10 min. Later, the cells were washed with PBS and analyzed with CLSM (Ex: 488 nm, Em: 500-560 nm).

Immunofluorescent staining of caspase 8, caspase 9 and caspase 3.

The cells incubated in confocal dishes were divided into 7 groups (PBS, ZIF@CM, ICG@ZIF@CM, GOx@ZIF@CM, ICG@ZIF@CM+L, GOx-ICG@ZIF@CM and GOx-

ICG@ZIF@CM+L). For each group, they were incubated with related nanoreactors (100 μ g/mL) for 5 h at 37 °C in 5% CO₂. And then, fresh culture medium was added to further culture the cells. For the laser irradiation groups, the cells were irradiated with 808 nm NIR laser (1 W/cm²) for 5 min. After further incubated for 20 h, the cells were fixation with 4% paraformaldehyde for 20 min, and stained with antibodies against caspase-3, caspase-8 or caspase-9. Then the cells were analyzed with CLSM.

Flow cytometry analysis of apoptosis.

The cell apoptosis assay of 4T1 cells was performed by flow cytometry. 4T1 cells were seeded in culture dishes with RPMI 1640 and were further divided into 7 groups (PBS, ZIF@CM, ICG@ZIF@CM, GOx@ZIF@CM, ICG@ZIF@CM+L, GOx-ICG@ZIF@CM and GOx-ICG@ZIF@CM+L). For each group, the cells were incubated with related nanoreactors (100 µg/mL) for 5 h. For the laser irradiation groups, the cells were irradiated with 808 nm NIR laser (1 W/cm²) for 5 min. After further incubated with fresh medium for another 10 h, all the cells were washed by PBS for several times and then collected. Subsequently, the cells were washed with PBS thrice and treated with Annexin V-FITC/PI for 20 min. Then the cells were analyzed by flow cytometry.

Tumor model establishment.

All animal experiments were conducted and agreed with the Principles of Laboratory Animal Care (People's Republic of China) and the Guidelines of the Animal Investigation Committee, Biology Institute of Shandong Academy of Science, China. Balb/C mice (4–6 weeks old, female, ~18 g) were fed with normal conditions of 12 h light and dark cycles and given access to food and water ad libitum. The 4T1 breast cancer model was used as an example to evaluate the therapeutic effect. To this end, 1×10^7 4T1 cells in 100 µL of serum-free RPMI 1640 medium were injected subcutaneously into the right axillary region of Balb/c mice. After the tumor size had reached approximately 75-100 mm³, the mice were used in subsequent experiments.

Plasma Concentration Analysis of the nanoreactor by ICP-AES.

4T1 breast tumor bearing Balb/c mice were prepared, and then injected with 50 μ L of GOX-ICG@ZIF@CM or GOX-ICG@ZIF (5 mg/mL) through the tail vein. At various time points, blood was obtained by the orbital sinus. Then, 0.5 mL of lysis buffer (1% SDS, 1% Triton X-100, 40 mM Tris acetate) was added to lyse the blood, and 0.5 mL of diluted HCl was added to resolve the

nanoreactors into Zn²⁺. The Zn content of each blood sample was determined by ICP-AES.

In vivo fluorescence imaging.

 $50 \ \mu\text{L}$ of 5 mg/mL nanoreactor was *i.v.* administrated into the 4T1 tumor bearing Balb/c mice. At different time points of 3, 9, 12 and 24 h post injection, the ICG fluorescence in the mice was recorded with a live body imaging system.

In vivo distribution.

4T1 tumor bearing mice were *i.v.* administrated with 50 μ L of 5 mg/mL nanoreactor. At the time points of 12 h the mice were sacrificed. Then, the ICG fluorescence of tumor and major organs were recorded with a live body imaging system.

In vivo IR thermographic imaging.

 $50 \ \mu\text{L}$ of 5 mg/mL nanoreactor was *i.v.* administrated into the 4T1 tumor bearing Balb/c mice. After 12 h, the tumor region was irradiated with 1 W/cm² 808 nm NIR laser, and the body temperature change of the mice was recorded with an IR camera every 2 min. PBS injected mice was used as the control group.

In vivo therapeutic effect of the nanoreactors.

The 4T1 tumor bearing mice were divided into seven different groups: (i) PBS, (ii) ZIF@CM, (iii) ICG@ZIF@CM, (iv) GOx@ZIF@CM, (v) ICG@ZIF@CM+L, (vi) GOx-ICG@ZIF@CM and (vii) GOx-ICG@ZIF@CM+L. The NPs were diluted in physiological saline to 5 mg/mL, and 50 μ L was administered to each mouse by intravenous injection. Next, 1 W/cm² of 808 nm NIR laser was used to irradiate on the tumor site after injection for 12 h, the irradiation time was 20 min. The tumor sizes and body weights were measured every other day for 20 days after treatment (tumor volume = W²×L/2, W = width, L = length). For the survival rate, the mice were identified as death as the tumor volume reached 350 mm³. (2) H&E staining of tumor slides: 12 h after different treatments, the mice were sacrificed, and the tumors were harvested to use for hematoxylin and eosin (H&E) staining; H&E staining of five major organs (liver, lung, spleen, kidney, and heart): 7 days after different treatments, the mice were sacrificed, and five major organs (liver, lung, spleen, kidney, spleen, kidney, and heart) were harvested to use for H&E staining.



Fig. S1. Fluorescence spectra of ZIF, GOx@ZIF, GOx-ICG@ZIF, GOx-FITC, GOx-FITC@ZIF and GOx-FITC-ICG@ZIF.



Fig. S2. UV-Vis absorption spectra of ZIF, GOx@ZIF, ICG@ZIF and GOx-ICG@ZIF.



Fig. S3. Fluorescence spectrum and linear standard curve of GOx-FITC from 10-40 μ g/mL. $\lambda_{em} = 520$ nm.



Fig. S4. UV-Vis spectrum and standard curve of ICG from 3-11 mg/mL.



Fig. S5. PXRD patterns of simulated ZIF and as prepared ZIF.



Fig. S6. TGA curves of ZIF, GOx@ZIF, ICG@ZIF and GOx-ICG@ZIF.



Fig. S7. FT-IR spectra of ZIF, GOx@ZIF, ICG@ZIF and GOx-ICG@ZIF.



Fig. S8. Fluorescence spectra of DCFH with different treatments.



Fig. S9. The UV-Vis spectrum of ABTS after treated with GOx from different groups in the existence of glucose and HRP. The absorption peak at 420 nm indicating the activity of GOx.



Fig. S10. Relative activity of GOx or GOx-ICG@ZIF before and after hyperthermia treatment.



Fig. S11. The relative activity of GOx-ICG@ZIF under 808 nm laser irradiation for different times. The power density of the laser was 1 W/cm².



Fig. S12. Relative reactivity of GOx before and after trypsin digestion.



Fig. S13. TEM images of (A) ZIF@CM, (B) GOx@ZIF@CM, (C) ICG@ZIF@CM and (D) GOx-

ICG@ZIF@CM after stained with phosphotungstic acid. Scale bars=100 nm.



Fig. S14. DLS of (A) ZIF@CM, (B) GOx@ZIF@CM, (C) ICG@ZIF@CM and (D) GOx-ICG@ZIF@CM.



Fig. S15. Fluorescence spectra of the GOx-FITC-ICG@ZIF@CM nanoreactor (Total) and the supernatant during incubation for 0-7 day.



Fig. S16. UV-Vis spectra of the GOx-FITC-ICG@ZIF@CM nanoreactor (Total) and the supernatant during incubation for 0-7 day.



Fig. S17. Release profile of GOx from GOx-ICG@ZIF@CM in the glucose solution (1µg/mL).



Fig. S18. Average size and Zeta potential change of GOx-ICG@ZIF@CM during incubation in saline for 0-7 day.



Fig. S19. Charge and size distribution of GOx-ICG@ZIF and GOx-ICG@ZIF@CM with/without serum.



Fig. S20. CLSM images of 4T1 cell after incubated with GOX-FITC-ICG@ZIF@CM for 5 h. A: The green color is the fluorescence of FITC; B: The red color is the fluorescence of ICG; C: The picture of bright field; D: The merged picture of A, B and C. Scale bar is 50 μm.



Fig. S21. CLSM images of the uptake of GOx-ICG@ZIF@CM and GOx-ICG@ZIF against macrophages and 4T1 cells.



Fig. S22. Flow cytometry analysis of the uptake of GOx-ICG@ZIF@CM and GOx-ICG@ZIF against macrophages and 4T1 cells. The charts are the fluorescence intensity distribution of each group and the inserts are mean and median fluorescence intensity values.



Fig. S23. Relative intracellular HSP-90 expression level in different groups. 1: PBS, 2: ZIF@CM; 3: ICG@ZIF@CM; 4: GOx@ZIF@CM; 5: ICG@ZIF@CM+L; 6: GOx-ICG@ZIF@CM and 7: GOx-ICG@ZIF@CM+L.



Fig. S24. Relative intracellular CAT expression level in different groups. 1: PBS, 2: ZIF@CM; 3: ICG@ZIF@CM; 4: GOx@ZIF@CM; 5: ICG@ZIF@CM+L; 6: GOx-ICG@ZIF@CM and 7: GOx-ICG@ZIF@CM+L.



Fig. S25. Relative intracellular MCT expression level in different groups. 1: PBS, 2: ZIF@CM; 3: ICG@ZIF@CM; 4: GOx@ZIF@CM; 5: ICG@ZIF@CM+L; 6: GOx-ICG@ZIF@CM and 7: GOx-ICG@ZIF@CM+L.



Fig. S26. Relative ATP level in 4T1 cell with different treatments.



Fig. S27. Confocal images of DCFH-DA stained 4T1 cell with different treatments.



Fig. S28. Immunofluorescent staining of caspase-8, caspase-9 and caspase-3 in 4T1 cell with different treatments. Scale bar is 50 μm.



Fig. S29. Hemolytic effect analysis of the nanoreactor at concentrations from 10-100 μ g/mL on red blood cell of Balb/c mice. Triton was used as positive control and PBS was used as negative control.



Fig. S30. Enzyme-linked immunosorbent assay (ELISA) analysis of IL-6 and IL-12 after the mice were injected with GOx-ICG@ZIF or GOx-ICG@ZIF@CM (50 μL, 5 mg/mL).



Fig. S31. Retention of the nanoreactor and GOx-ICG@ZIF in the body at different time via ICP-AES analysis.



Fig. S32. *In vivo* fluorescence imaging of the 4T1 tumor bearing mice injected with the GOx-ICG@ZIF@CM nanoreactor at different time points.



GOx-ICG@ZIF

GOx-ICG@ZIF@CM

Fig. S33. *In vivo* distribution of GOx-ICG@ZIF and GOx-ICG@ZIF@CM at 12 h post injection. (H: heart, Li: liver, S: spleen, Lu: lung, K: kidney, T: tumor)



Fig. S34. IR thermographic images of 4T1 tumor bearing mice after the exposure to 808 nm laser (1 W/cm²) in PBS or GOx-ICG@ZIF@CM nanoreactor treated group at the time point of 12 h.



Fig. S35. Picture of tumors dissected on the 20 day after different treatments



Fig. S36. The body weight change curves of mice in different groups during the therapy period.



Fig. S37. H&E staining of heart, liver, spleen, lung and kidney from the mice of various groups: PBS, ZIF@CM, ICG@ZIF@CM, GOx@ZIF@CM, ICG@ZIF@CM+L, GOx-ICG@ZIF@CM and GOx-ICG@ZIF@CM+L.