Supporting Information for

Fe/Cu-AuNP Nanocomposites as Enzyme-Like Catalysis to Modulate Tumor Microenvironment for Enhanced Synergistic Cancer Therapy

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Materials and Instruments

All chemicals used are purchased from commercial suppliers and used without further purification. 2,2′-Bipyridine-5,5′-dicarboxylic acid (bpydc), Chloroauric acid, Cupric chloride, Iron (III) chloride hexahydrate, Methylene Blue, N, N-Dimethylformamide, Tetrahydrofuran were purchased from Energy Chemical Co., Ltd. (Shanghai, China). 3,3`,5,5`-Tetramethylbenzidine, 5,5`-Dithiobis (2-nitrobenzoic acid), Glutathione reduced were purchased from Heowns Co., Ltd (Tianjin, China). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride weas purchased from Bide Pharmatech Co., Ltd. Hoechst 33342 was purchased from Invitrogen Thermo Fisher Scientific (USA). Glucose Assay Kit with O-toluidine, Calcein/PI cell Viability/Cytotoxicity Assay Kit and Reactive Oxygen Species Assay Kit (2',7, -dichlorofluorescin diacetate, DCFH-DA) were purchased from Beyotime (Nantong, China).

Transmission electron microscope (TEM) images were recorded using JEM-2100 (JEOL). The field emission scanning electron microscope (SEM) results were obtained on TESCAN MIRA LMS. X-ray photoelectron spectra (XPS) measurement was conducted on Thermo Scientific K-Alpha (Thermo Fisher Scientific, USA). The Fe, Cu, Au content was determined by inductively coupled plasma-mass spectrometry (ICP-MS, 7700s, Agilent, USA). The zeta potential and diameter distribution were obtained with Zetasizer Nano ZS90 (Malvern, UK). The NIR light was generated from the LWIRL-808nm. The UV-Vis spectra were

obtained by a UV-2700. The cellular viability was determined with a CMax Plus Microplate absorbance reader (Molecular Devices, USA). Cell imaging was obtained by Leica TCSSP8 DIVE Fluorescence Microscopy (Leica, GER).

Preparation of PEG-PMSA

254 mg mPEG-NH_2 and 8.9 mg phenazine methosulfate (PMSA) were dissolved in 7.9 mL CH_2Cl_2, and added with 9.5 μL triethylamine (TEA). After stirring at room temperature for 24 h, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (6.5 mg) and TEA (11 μL) were added every 1 h, three times, followed by another 24 h incubation. The mixture was dried via reduced pressure distillation, affording the PEG-PMSA.

Fe ion and Cu ion release

0.2 mL of Fe/Cu-AuNP-PEG was incubated in PBS at different pH conditions with GSH (0.5 mM), for different times (0, 1, 6, 12 h). Then the supernatant was collected through centrifugation and measured by ICP-MS to determine the content of Fe and Cu.

Detection of Extracellular •OH

To test the generation of •OH, colorimetric 3,3',5,5'-tetramethylbenzidine (TMB) and methylene blue (MB) degradation analysis were carried out. Firstly,

different concentrations of Fe/Cu-AuNP-PEG (Fe^{3+}: 0, 10, 20, 40, 60, 80 μg mL^{-1}) were incubated with TMB (0.5 mM) and H_2O_2 (2 mM) for 30 min. After centrifugation, the supernatants were collected and the absorption at 650 nm were measured. Next, Fe/Cu-AuNP-PEG at different concentrations (Fe^{3+}: 0, 1, 5, 10, 20, 30 μg mL^{-1}) were incubated with H_2O_2 (2 mM) and MB (10 μg mL^{-1}) for 4 h. The supernatants were collected after centrifugation, and the UV/Vis spectra at 500-800 nm were recorded.

Extracellular Depletion of GSH

To verify the GSH consumption capability of the nanoreactor, different concentrations of Fe/Cu-AuNP-PEG (Fe^{3+}: 0, 0.5, 1, 1.5, 2, 2.5 μg mL^{-1}) were reacted with 0.5 mM GSH solution. After reaction for 2.5 h, the mixed solution was centrifuged and the content of GSH in the supernatant was detected by 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) indicator according to the protocols.

Depletion of Glucose

The glucose consumption capacity of the Fe/Cu-AuNP-PEG was verified with a glucose detection kit. The Fe/Cu-AuNP-PEG (Fe^{3+}: 100 μg mL^{-1}) and glucose (100 μg mL^{-1}) were co-incubated for 0, 0.5, 1, or 2 h. The supernatants were collected by centrifugation and detected by the glucose detection reagent.

Cytotoxicity Assays

4T1 cells were seeded in a 96-well culture dish with 5000 cells/well for 24 h (37 ℃, 5 % CO_2). Then the Cells were incubated in different ways: first group (control), second groups (Fe/Cu nanoparticles), third groups (Fe/Cu-AuNP-PEG), and fourth groups (Fe/Cu-AuNP-PEG /NIR), and incubated for 6h. After that, the fourth group 4T1 cells were irradiated with an 808 nm laser (1.0 W cm^{-2}) for 10 min and incubated for 18 h. The cell viability was measured by the CCK-8 assay.

4T1 cells were seeded in a confocal microscope culture dish with 10^5 cells/well for 24 h. Then the cells were incubated in different ways: first group (control), second groups (Fe/Cu nanoparticles), third groups (Fe/Cu-AuNP-PEG), and fourth groups (Fe/Cu-AuNP-PEG /NIR), and incubated for 12 h. After that, the fourth group 4T1 cells were irradiated with an 808 nm laser (1.0 W cm^{-2}) for 10 min. Then monitored by a CLSM after staining with Calcein-AM and PI for 30 min.

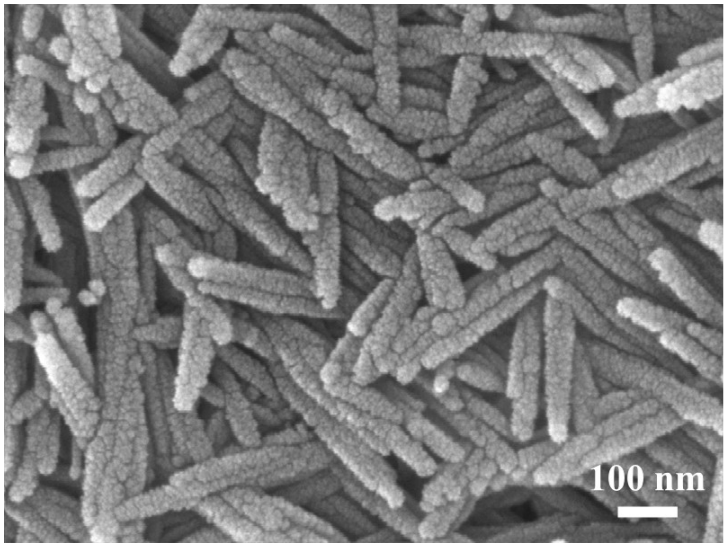

Fig. S1. SEM image of Fe/Cu nanoparticles.

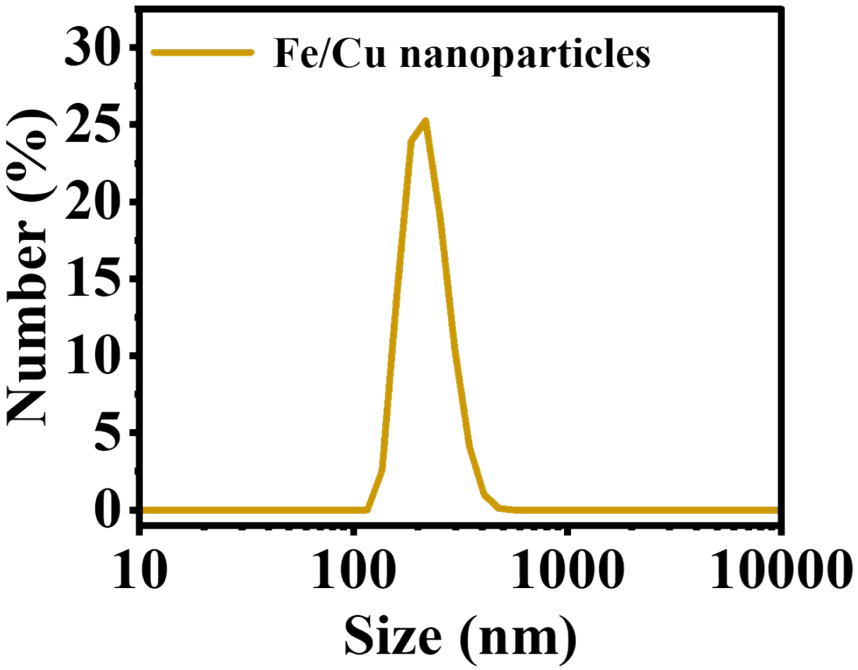

Fig. S2. DLS profile of Fe/Cu nanoparticles.

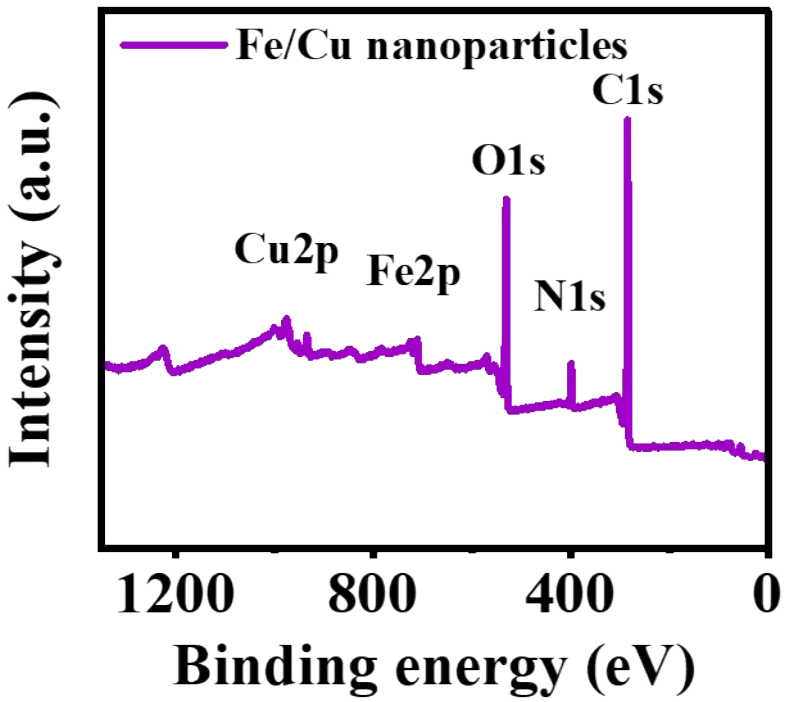

Fig. S3. XPS profile of Fe/Cu nanoparticles.

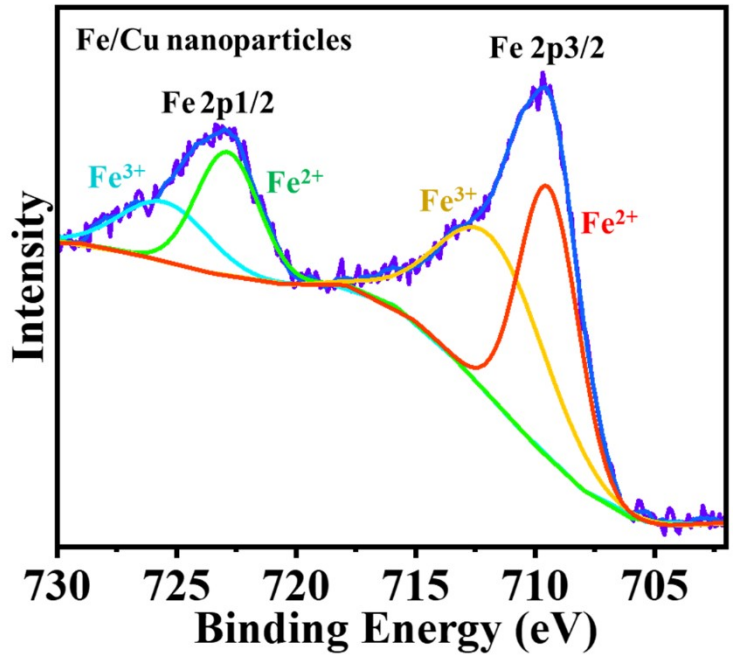

Fig. S4. Fe2p XPS spectra of Fe/Cu nanoparticles.

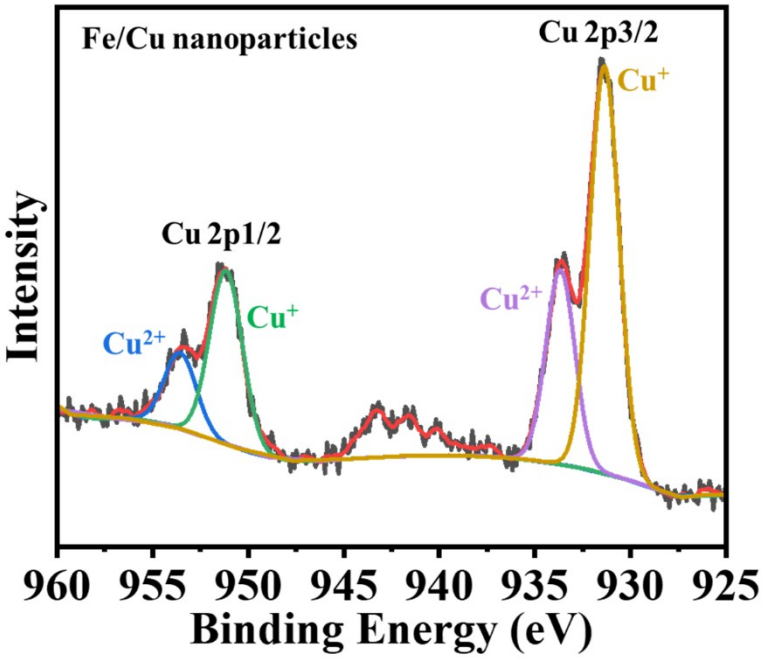

Fig. S5. Cu2p XPS spectra of Fe/Cu nanoparticles.

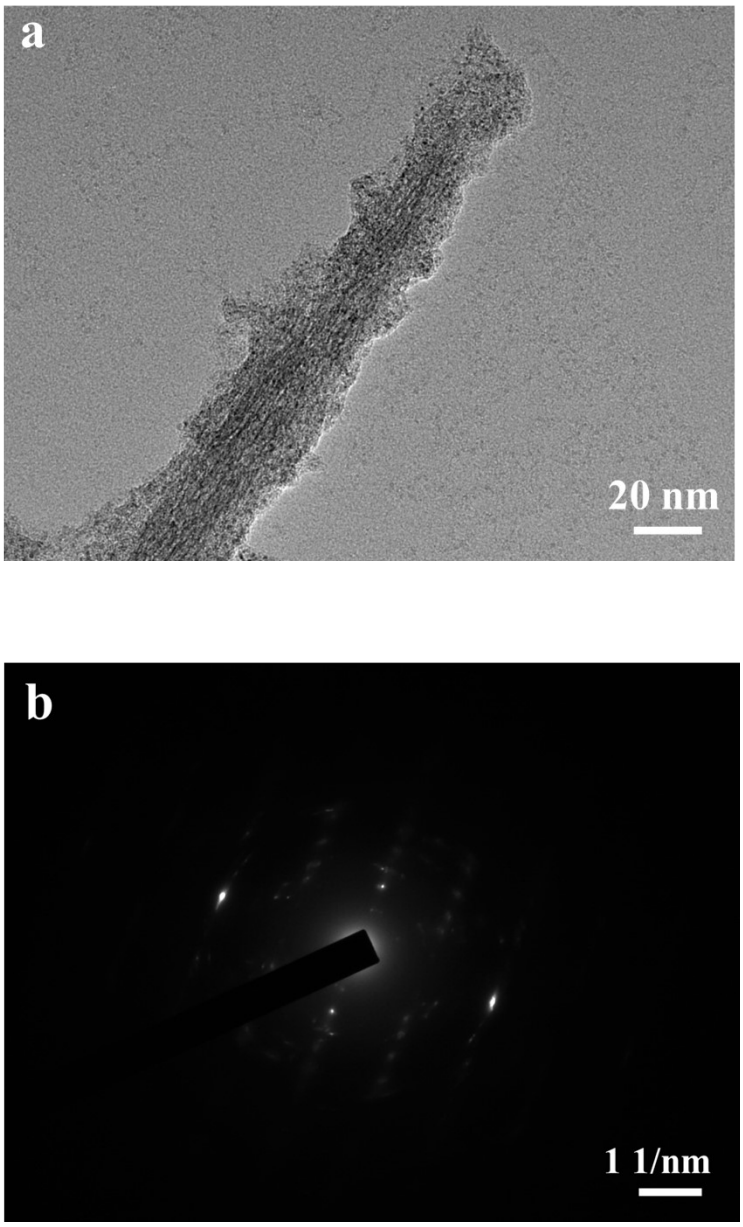

Fig. S6. TEM image of Fe/Cu-AuNP.

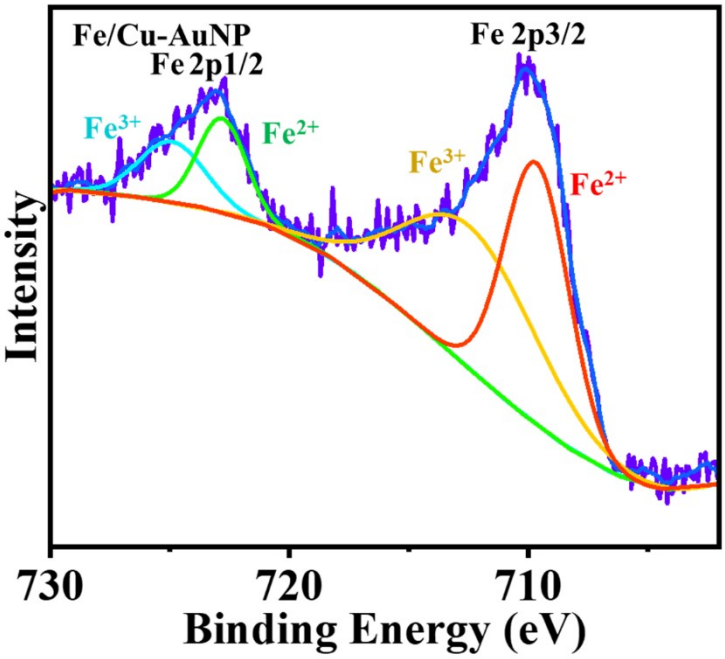

Fig. S7. Fe2p XPS spectra of Fe/Cu-AuNP.

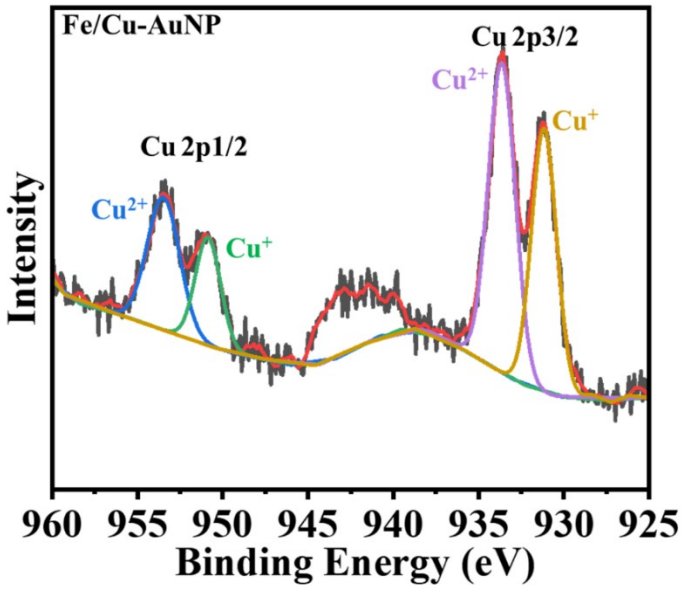

Fig. S8. Cu2p XPS spectra of Fe/Cu-AuNP.

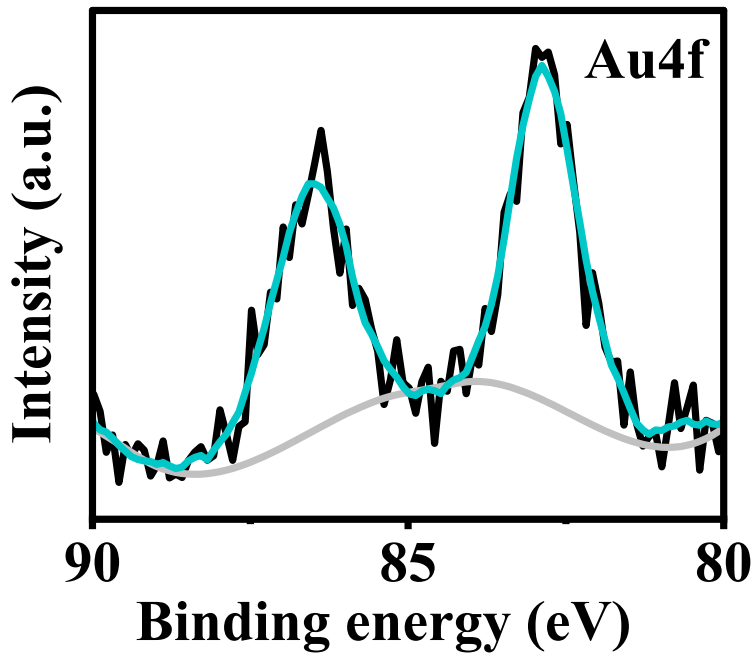

Fig. S9. Au4f XPS spectra of Fe/Cu-AuNP.

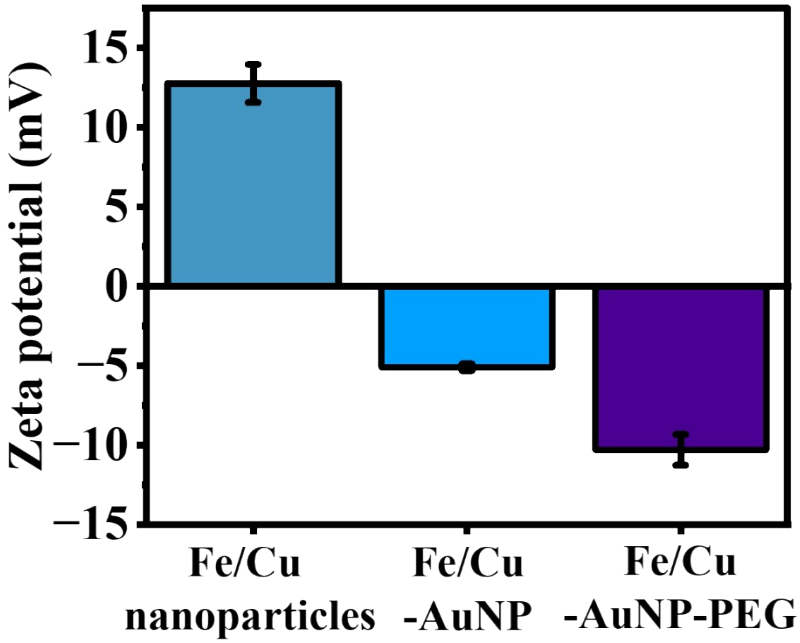

Fig. S10. Zeta potential of Fe/Cu nanoparticles, Fe/Cu-AuNP, Fe/Cu-AuNP-PEG.

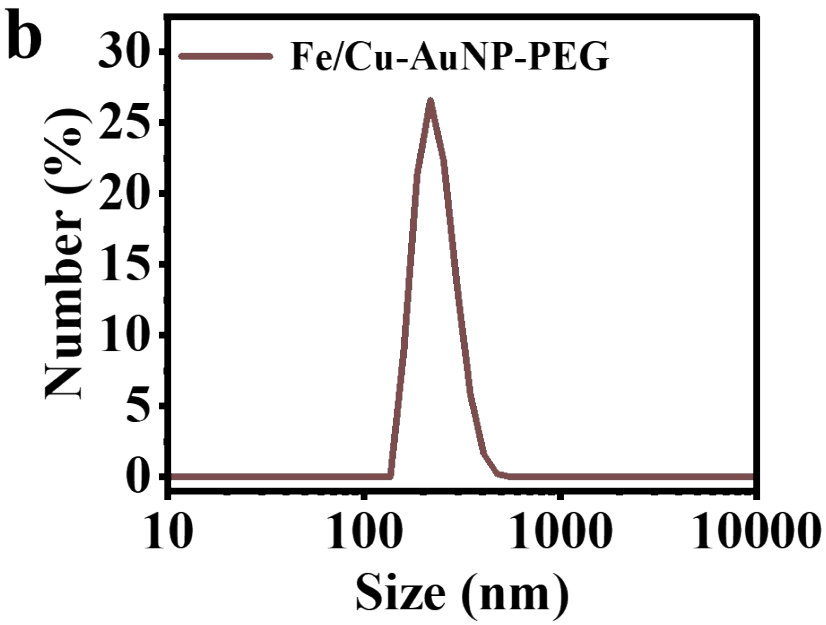

Fig.S11. DLS profile of Fe/Cu-AuNP-PEG.

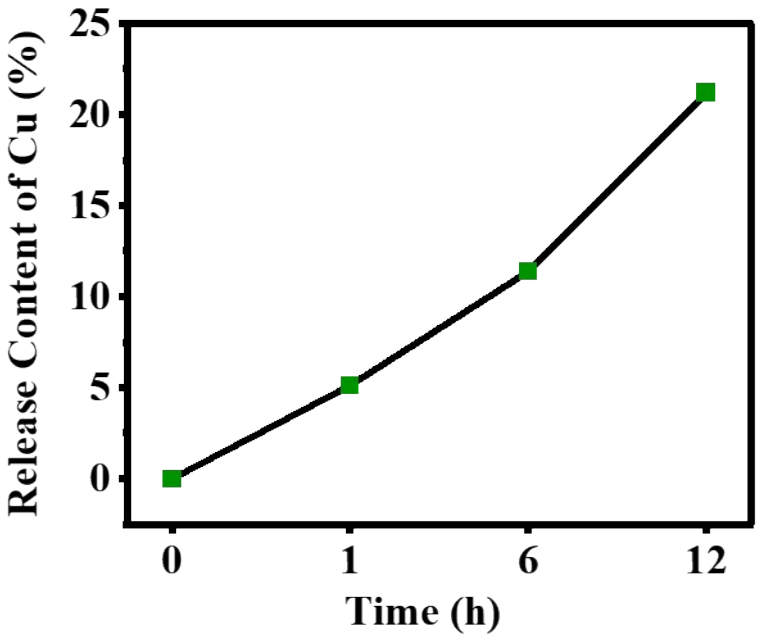

Fig. S12. The content of Cu ions released from Fe/Cu-AuNP-PEG after reacting with GSH for different time.

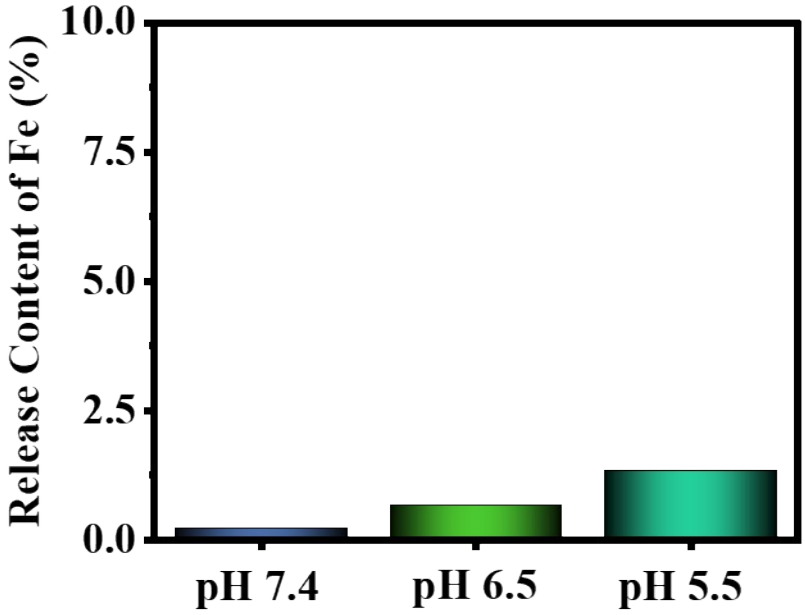

Fig. S13. The content of Fe ions released from Fe/Cu-AuNP-PEG after incubation in different pH.

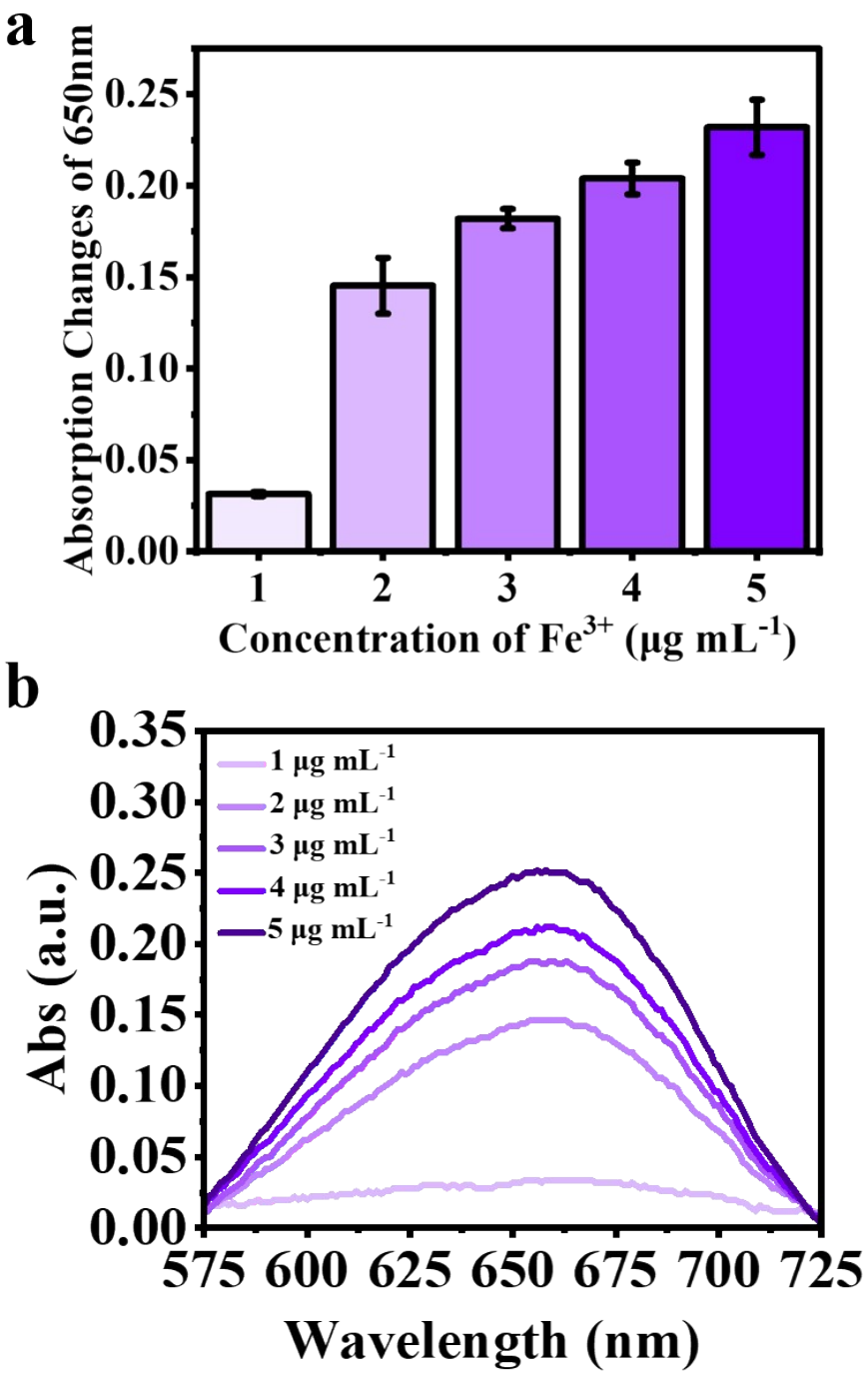

Fig. S14. The absorption changes at 650 nm of TMB after treated with different concentration of Fe/Cu-AuNP-PEG.

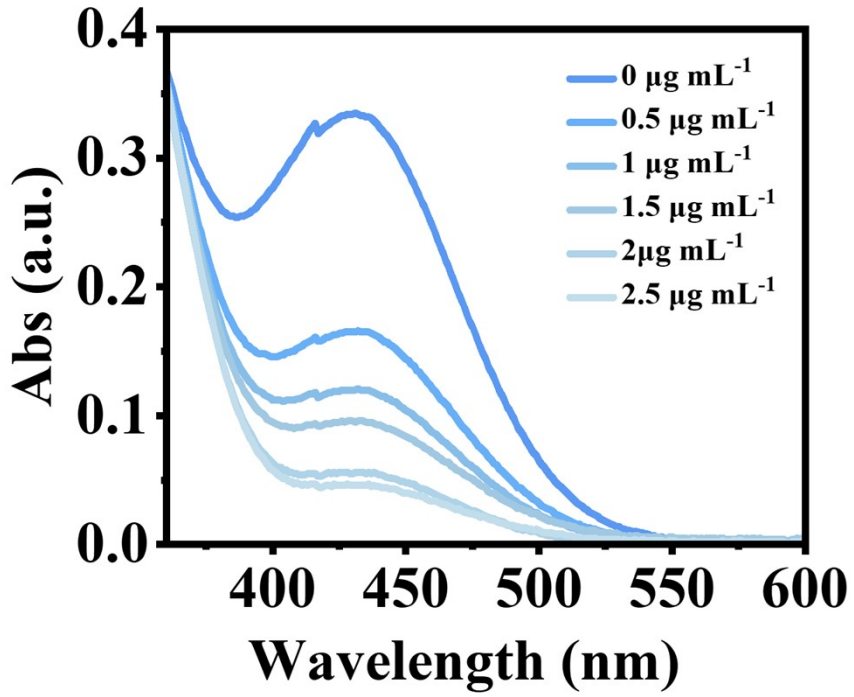

Fig. S15. GSH content after treatment with Fe/Cu-AuNP-PEG at different concentration.

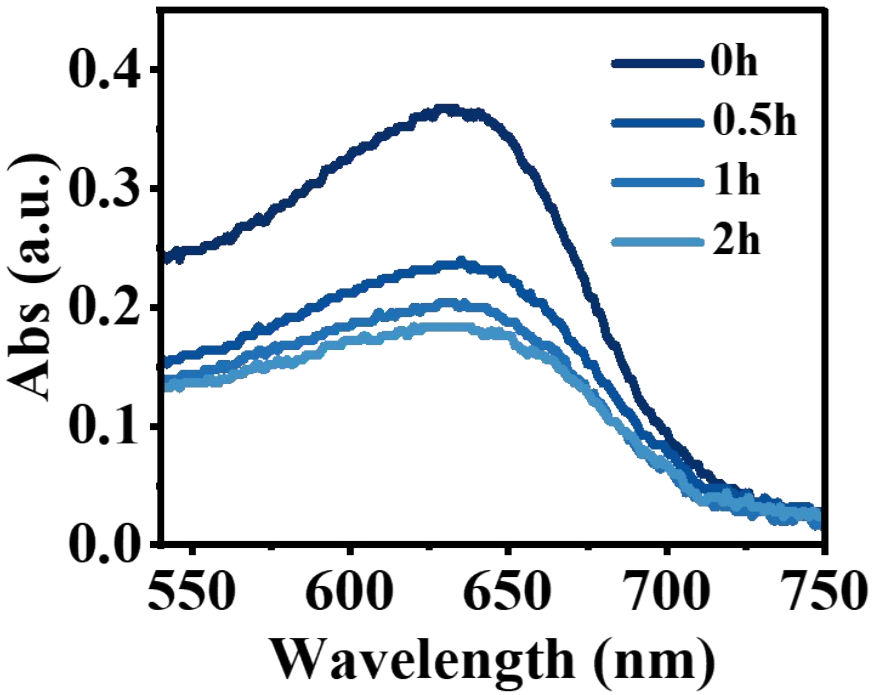

Fig. S16. Glucose content after treated with Fe/Cu-AuNP-PEG for different time.

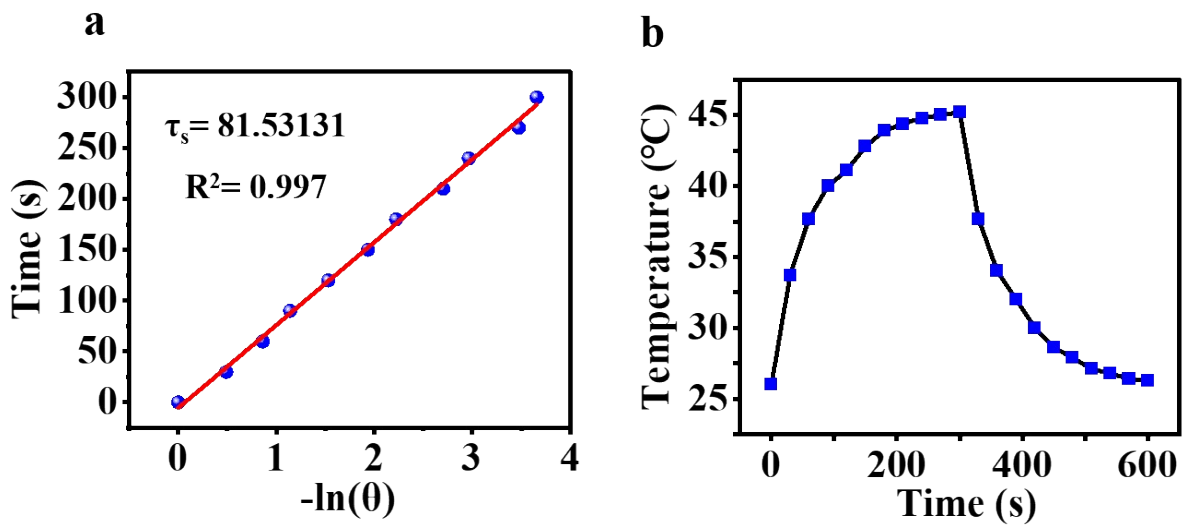

Fig. S17. The photothermal conversion efficiency of Fe/Cu-AuNP-PEG. (a) The photothermal effect of Fe/Cu-AuNP-PEG, irradiated with 808 nm laser (1.0 W/cm^2). The laser was turned off after irradiation for 5 min. (b) Time constant (τs) was calculated by the linearized energy balance to temperature versus the negative natural logarithm of temperature driving force obtained from the cooling stage in (a).

The photothermal conversion efficiency (η) was calculated using the following Equation (1).[1]

$$\eta = \frac{hS(TMax - T_{Surr}) - Q_{Dis}}{I(1 - 10^{-A_{808}})} \qquad (1)$$

Where h: the heat transfer coefficient; S: the surface area of the container;

T_{Max}: the equilibrium temperature (T_{Max} = 45.2 °C); T_{Surr}: surrounding temperature (T_{Surr} = 25.8°C);

Q_{Dis}: the heat dissipation from laser absorbed by the sample cell; I: incident laser power (1.0 W);

A_{808}: the absorbance of at 808 nm (A_{808} = 0.219).

In order to get the value of hS, a dimensionless driving force temperature (θ) and a sample system time (constant τ_s) is introduced using the Equation (2) and (3).

$$\theta = \frac{T - T_{Surr}}{T_{Max} - T_{Surr}} \qquad (2)$$

$$t = -\tau_s \ln(\theta) \qquad (3)$$

The time constant (τ_s) was determined to be 81.53131 s (Figure b). The value of hS is derived according another Equation (4),

$$hS = m_D C_D / \tau_s \qquad (4)$$

Where m_D: the mass of the solvent (m_D = 0.14 g), C_D: the heat capacity of the solvent (C_D = 4.2 J g^{-1} K^{-1}).

Thus, hS was calculated to be 0.0072.

The value of Q_{Dis} is derived according another Equation (5),

$$Q_{dis} = hS\ (T_{H_2O,\ Max} - T_{surr}) \qquad (5)$$

Q_{Dis} was measured independently to be 0.0036 W, using a sample cell containing pure water.

According to the obtained data, the photothermal conversion efficiency was calculated to be 34.36 %.

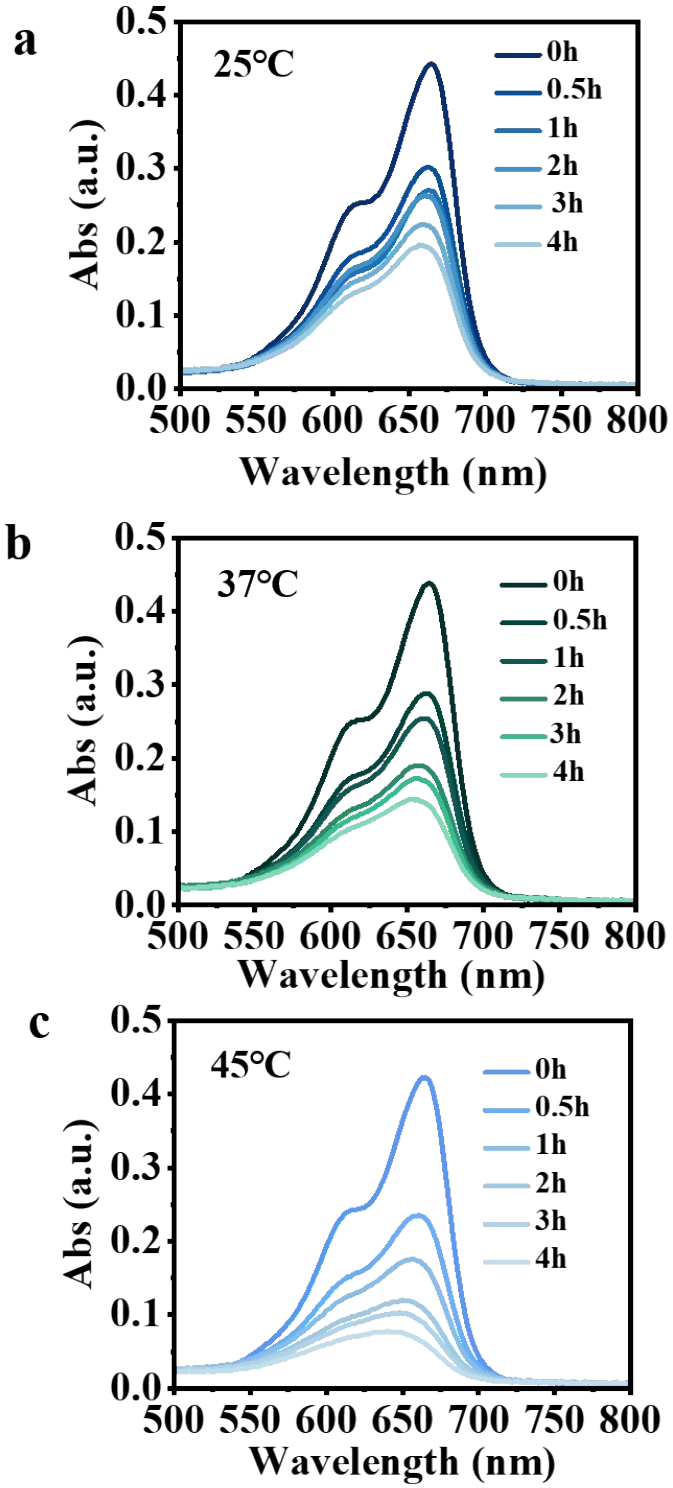

Fig. S18. Time-dependent degradation of MB by Fe/Cu-AuNP-PEG at (a) 25 ℃, (b) 37 ℃, and (c) 45 ℃.

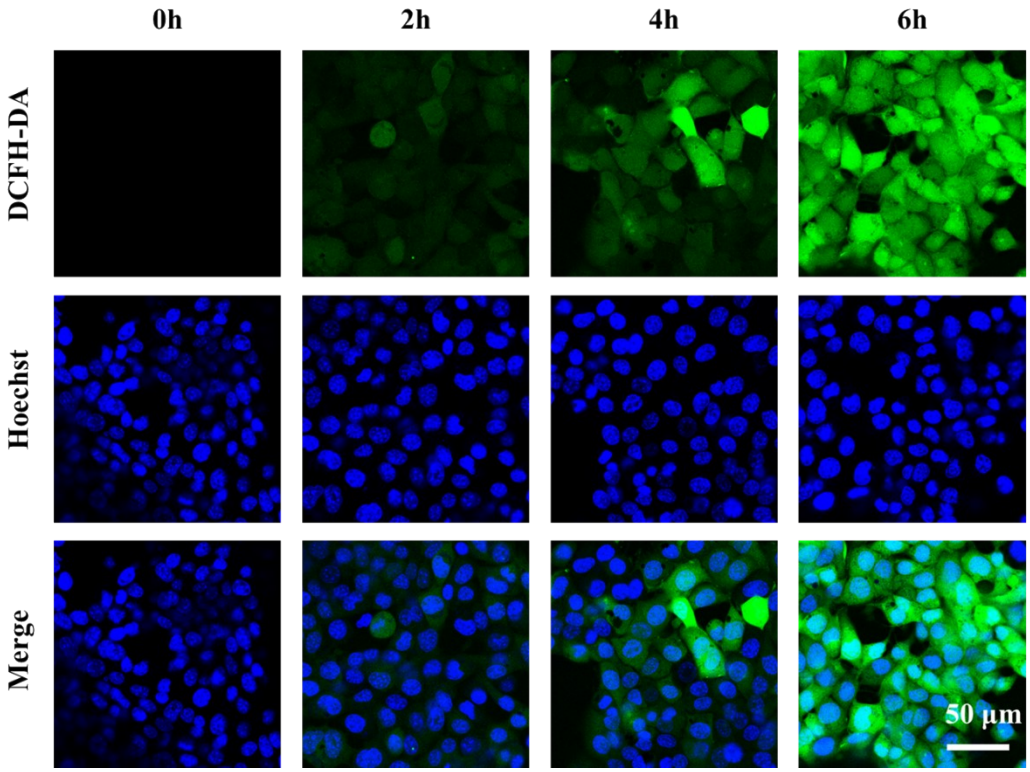

Fig. S19. Fluorescence images of 4T1 cells incubated with Fe/Cu-AuNP-PEG and stained with Hoechst (blue) and DCFH-DA (green) for intracellular ROS detection.

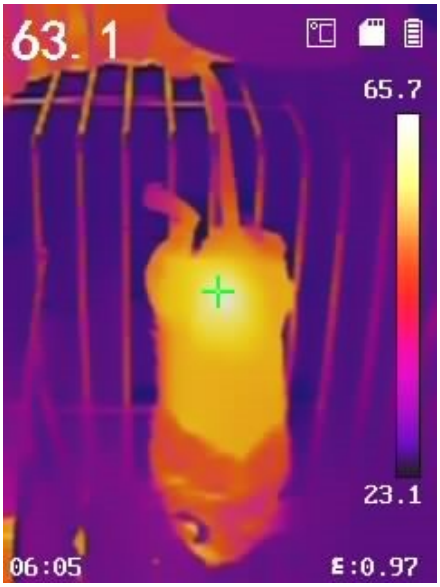

Fig. S20. Infrared thermal images of 4T1 tumor-bearing mice intratumorally injected with Fe/Cu-AuNP-PEG and exposed to NIR laser irradiation (1.0 W cm^{-2}) for 10 min.

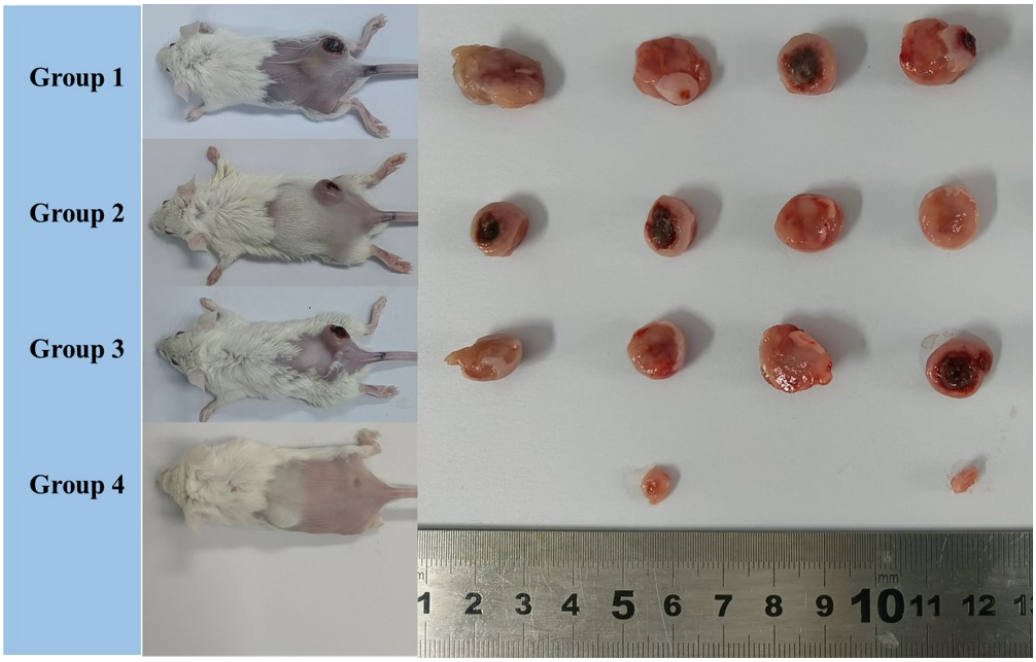

Fig. S21. Representative photos of mice and tumors on the 16th day after the treatment.

Reference

[1] S.A. Checheta, Y. Yu, X. Zhen, M. Pramanik, K. Pu, E. Miyako, Nat. Commun., 2017, 8, 15432.