Electronic Supplementary Material (ESI) for Inorganic Chemistry Frontiers.

Supporting Information

A novel CuCoS Nanozyme for Synergistic Photothermal and Chemodynamic Therapy of Tumor

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Photothermal effect of CuCoS NPs

To measure the photothermal effect of CuCoS nanoparticles, different concentrations of nanoparticles (0, 25, 50, 75 and 100 μg mL^{-1}) were placed in centrifuge tubes. Then they were irradiated with an 808 nm laser (1 W cm^{-2}) for 5 min. The temperature was monitored by an infrared thermal imager and recorded every 30 s. Then the laser power was varied (0.25, 0.5, 0.75 and 1 W cm^{-2}) to investigate the effect of laser power on the photothermal effect. The photothermal conversion efficiency was calculated by Equation (1).

$$\eta = \frac{hS\left(T_{max,NP} - T_{surr}\right) - Q_{dis}}{I\left(1 - 10^{-A_{808}}\right)} \quad (1)$$

where h is the heat transfer coefficient. S is the surface area of the vessel. T_{max} is the maximum temperature of the solution. T_{surr} is the ambient temperature. Q_{dis} the heat generated by the absorption of light by water. I is the laser power density. A_{808} is the absorption value of the material at 808 nm. Where hS can be calculated by equation (2) and (3).

$$Q_{dis} = hS(T_{max,H_2O} - T_{surr}) \quad (2)$$

$$\tau_s = \frac{m_D C_D}{hS} \quad (3)$$

m_D is the mass of H_2O and C_D is the heat capacity of H_2O, τ_s is the fitted time constant, calculated by Eq. (4) and (5):

$$t = -\tau_s ln\theta \quad (4)$$

$$\theta = \frac{T_{surr} - T}{T_{surr} - T_{max}} \quad (5)$$

where t is the cooling time, T is the temperature at cooling t s, and θ is a dimensionless dynamic temperature introduced to calculate τ_s.

Detection of ROS

Methylene blue (MB) was chosen as a probe to detect hydroxyl radicals. Hydroxyl radicals will oxidize MB to discolor the solution and decrease the absorption peak at

660 nm. CuCoS (50 μg mL^{-1}), H_2O_2 (0.2 mmol L^{-1}) was added to 2 mL of PBS. MB (20 μg mL^{-1}) was then added. The solution was placed for 0, 10, 20, 30, 40 and 50 min. The change in the absorption peak at 660 nm was recorded by UV-vis spectrophotometer.

ESR was the most direct evidence for the detection of ·OH, which was captured using DMPO. CuCoS (50 μg mL^{-1}), H_2O_2 (0.2 mmol L^{-1}) was added to 2 mL of PBS (pH 5.66). DMPO (25 mM) was added afterwards. ESR spectra were recorded at 0 and 10 min of the reaction.

Cell culture

L929 fibroblasts were inoculated in DMEM medium supplemented with 10% FBS, penicillin (100 units mL^{-1}) and streptomycin (100 μg mL^{-1}), 5% CO_2 at 37°C. Breast cancer 4T1 cells were inoculated at 37°C in RPMI 1640 medium supplemented with 10% FBS, penicillin (100 units mL^{-1}) and streptomycin (100 μg mL^{-1}) and 5% CO_2.

Cell uptake assay of CuCoS NPs

4T1 cells were inoculated in 6-well plates at a density of 1×10^5 cells per well and cultured overnight. The original culture medium was replaced with fresh medium containing Cy-CuCoS (100 μg mL^{-1}). After 4 h of warming, cell uptake was detected using flow cytometry.

4T1 cells were inoculated at a density of 1×10^5 cells per well in confocal culture dishes and incubated overnight. The original medium was then replaced with fresh medium containing Cy-CuCoS (100 μg mL^{-1}). After 4 h of warming, cell uptake was observed by confocal laser scanning microscope.

Intracellular ROS detection

In vitro ROS production was detected by the cell-permeable dye 2',7'-dichlorofluorescein diacetate (DCFH-DA 10 mM). DCFH-DA itself is not fluorescent and it is readily oxidized by intracellular ROS to 2',7' dichlorofluorescein (DCF), which has green fluorescence. 4T1 cells were inoculated into 12-well plates at a density of 0.2×10^5 cells per well and incubated at 37°C, 5% CO_2 for 24 h. Then, four groups: were divided (1) CuCoS + Laser; (2) CuCoS; (3) laser; (4) control (medium) treatment. The ROS probe was then added to each well and the mixture was incubated

at 37°C for 30 min. Finally, the cells were washed repeatedly with PBS. Fluorescence detection using fluorescence microscopy.

Intracellular GSH detection

4T1 cells were inoculated in 6-well plates at a density of 1×10^5 cells per well and cultured overnight. Then, they were treated in 4 groups: (1) CuCoS + Laser; (2) CuCoS; (3) laser; (4) control (medium). The amount of GSH was measured using a GSH assay kit (Abbkine, KTB1600) according to the instructions.

Hemolysis Assay

Red blood cells (RBCs) were isolated and washed from serum by centrifugation, and diluted with PBS. Then, 0.5 mL of RBC suspension was mixed with 0.5 mL of CuCoS (0.16, 0.31, 0.63, 1.25, 2.5, 5 mg mL^{-1}) in PBS. Deionized (DI) water and PBS were used as positive and negative controls, respectively. The treated suspension was cultured at 37 ℃ for 1 h. Then the samples were centrifuged and the supernatants were collected to analyze by UV-vis spectrometer.

H&E staining

The H&E staining was according to our previous report.[1] The typical steps are as follows:

1. Clear the paraffin from glass slides of tissue in two changes of xylene for 20 min per change.

2. Hydrate the glass slides of tissue through immersing it in 100% ethanol for 5 min, 100% ethanol for 5 min, 75% ethanol for 5 min, tap water for 5 min, and tap water for 5 min.

3. Stain the glass slides of tissue in hematoxylin solution for 5 min, rinse it in running tap water for 3 min, differentiate it with differentiation solution, rinse it in running tap water for 3 min, stain it with SYMPHONY B, and rinse it in running tap water for 3 min.

4. Immerse the glass slides of tissue in 85% ethanol for 5 min, 95% ethanol for 5 min, and stain it in eosin solution for 5 min.

5. Immerse the glass slides of tissue in 100% ethanol for 5 min, 100% ethanol for 5 min, 100% ethanol for 5 min, xylene for 5 min, and xylene for 5 min.

6. Seal the glass slides of tissue with neutral gum.

7. The glass slides with H&E staining are measured by a microscope.

Statistical Analysis. All experiments in our study were investigated at least three times, and the data were showed as mean ± standard deviation. Differences between different groups were calculated by f_x-T.TEXT in Microsoft Office Excel 2016 (Redmond, WA, USA). *$P < 0.05$ was considered to be a significant difference, and **$P < 0.01$ and ***$P < 0.001$ were received as highly significant difference.

References

1. P. Zheng, B. Ding, R. Shi, Z. Jiang, W. Xu, G. Li, J. Ding and X. Chen, A Multichannel Ca^{2+} Nanomodulator for Multilevel Mitochondrial Destruction-Mediated Cancer Therapy, *Adv. Mater*, 2021, **33**, 2007426.

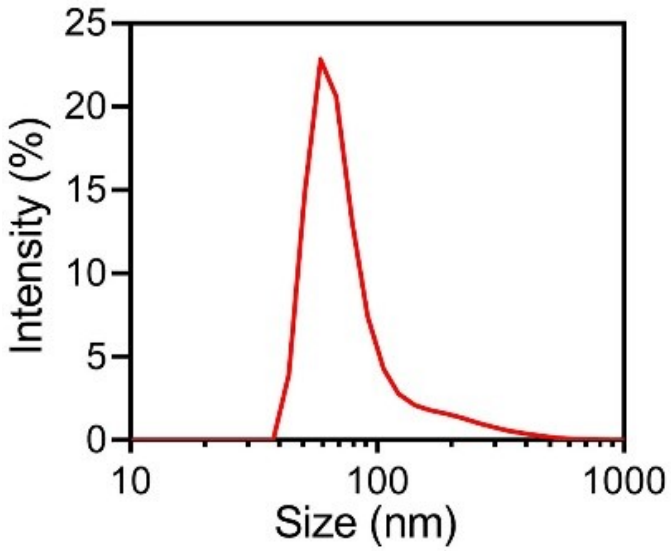

Fig. S1. The DLS data of CuCoS NPs.

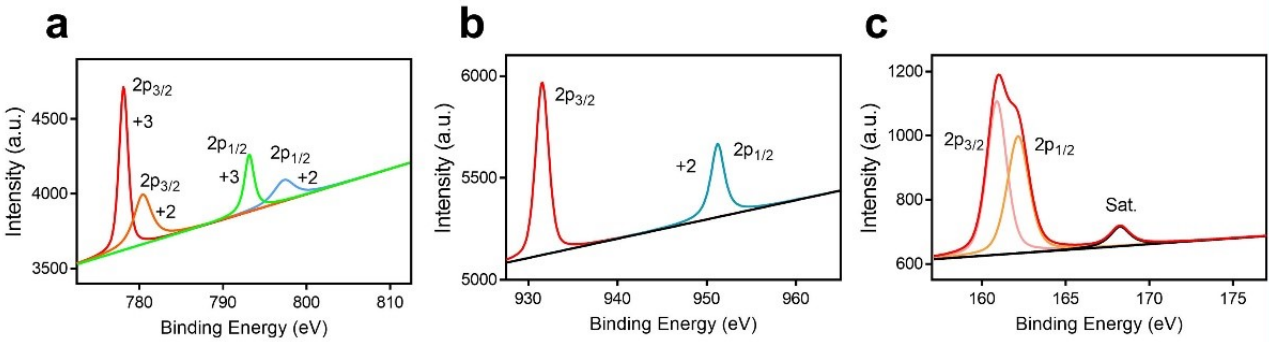

Fig. S2. Co 2p XPS (a) Cu 2p XPS (b) and S 2p XPS (c) spectra of CuCoS NPs.

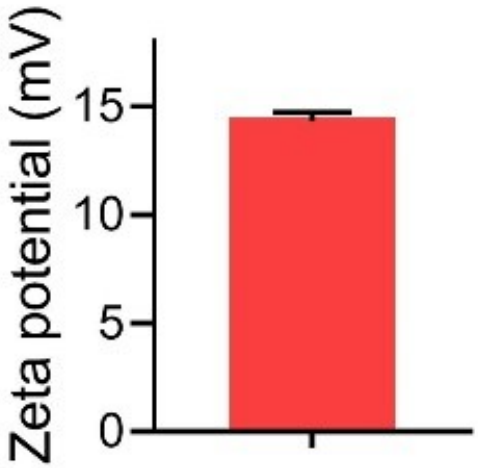

Fig. S3. The zeta potential data of CuCoS NPs.

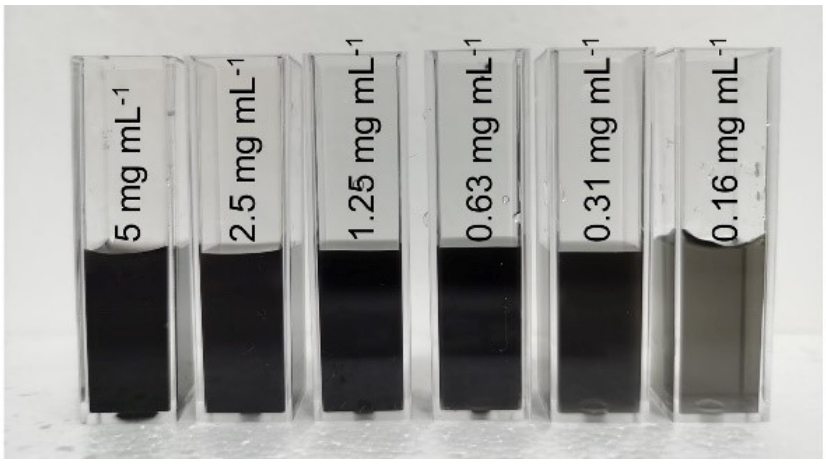

Fig. S4. The images of different concentration of CuCoS NPs dispersed in PBS.

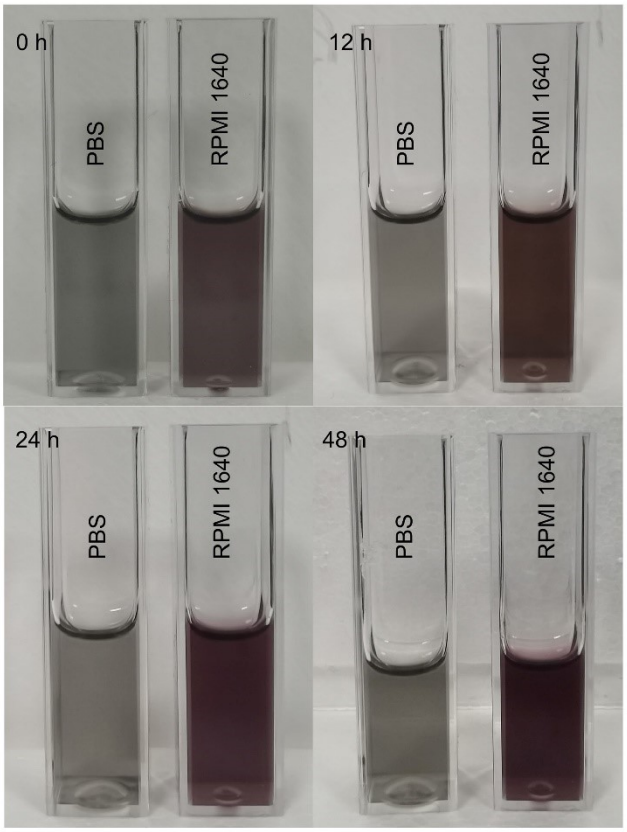

Fig. S5. The images of CuCoS NPs dispersed in PBS and RPMI 1640 medium for different time.

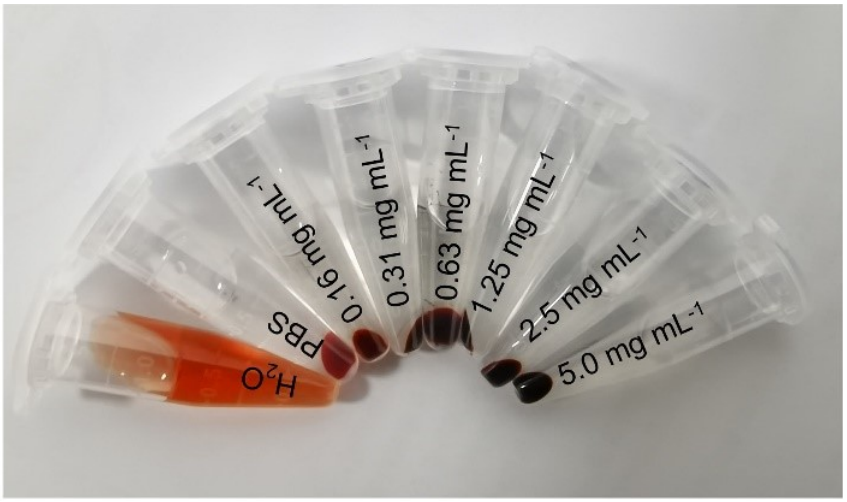

Fig. S6. The images of different concentration of CuCoS NPs-treated blood.

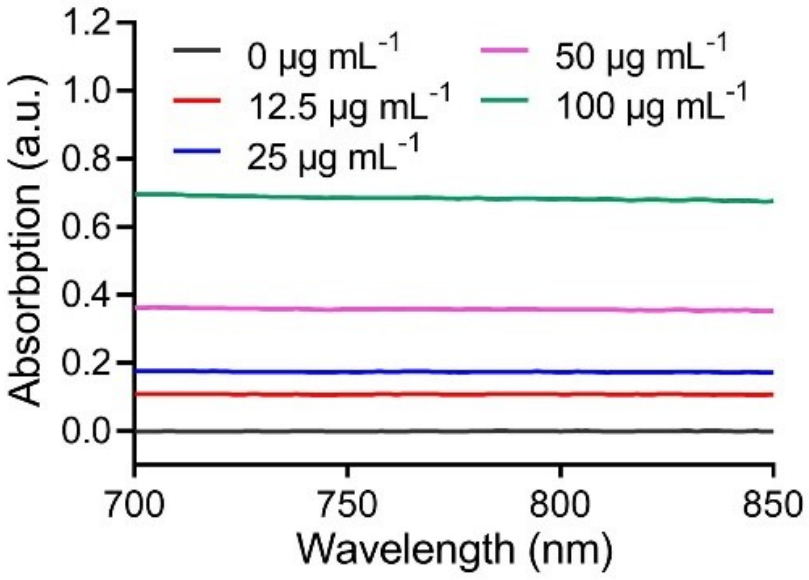

Fig. S7. The Vis−NIR absorption spectra of different concentration of CuCoS NPs.

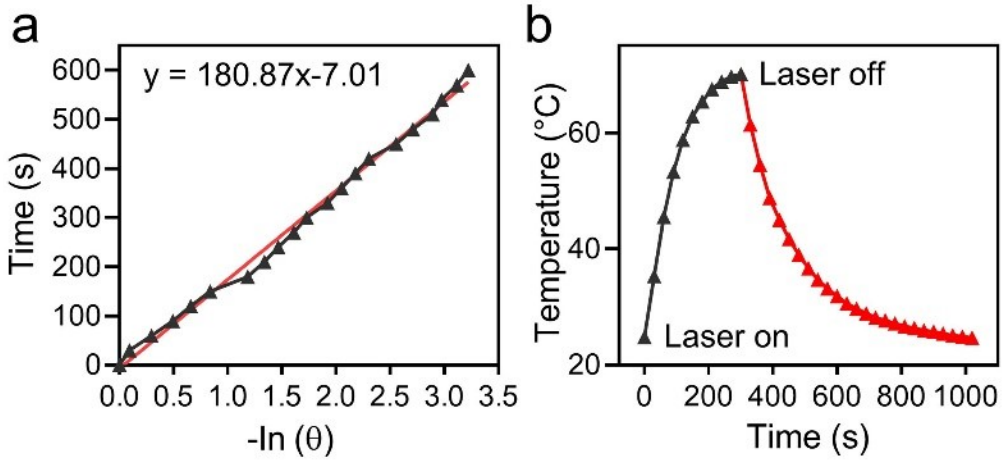

Fig. S8. (a) Linear time data obtained from the cooling period; (b) Heating and cooling curve of CuCoS NPs.

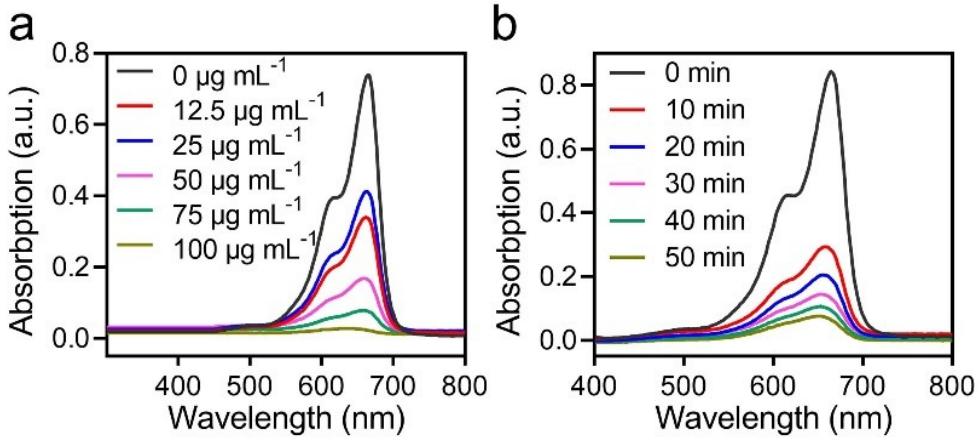

Fig. S9. (a) Absorption curves of MB degraded by different concentrations of CoCuS NPs; (b) Absorption curves of MB degraded at different time (the concentration of CuCoS NPs is 50 μg mL^{-1}).

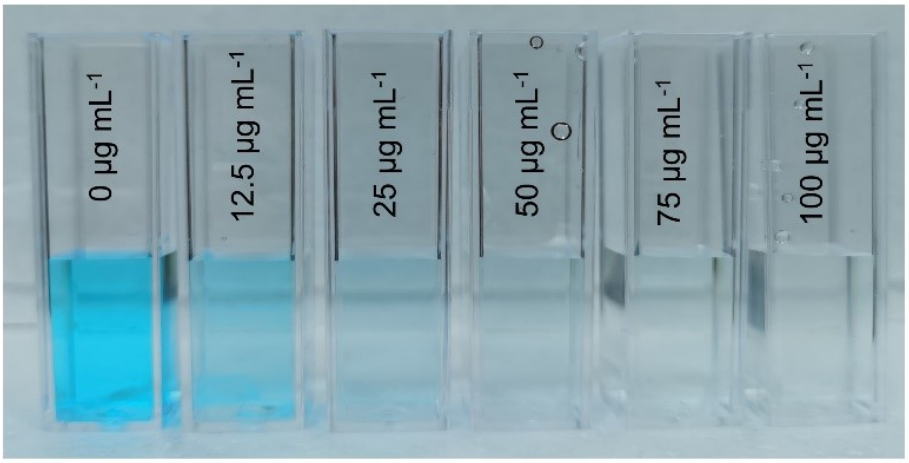

Fig. S10. The images of MB degraded by different concentrations of CoCuS NPs.

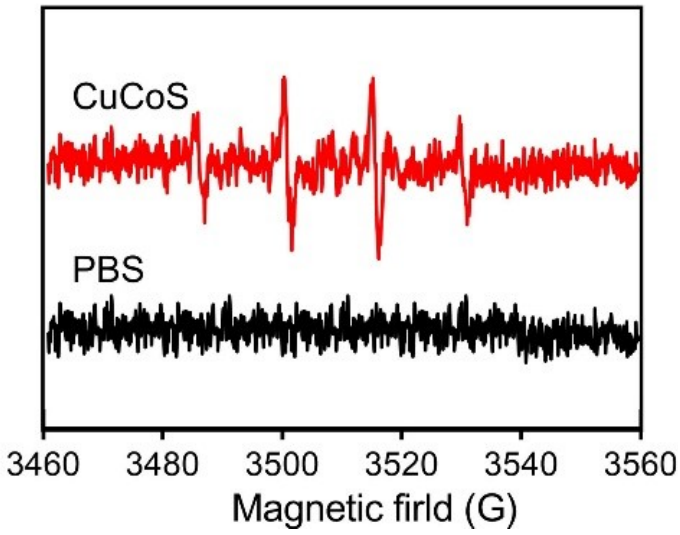

Fig. S11. ESR spectra of CuCoS NPs.

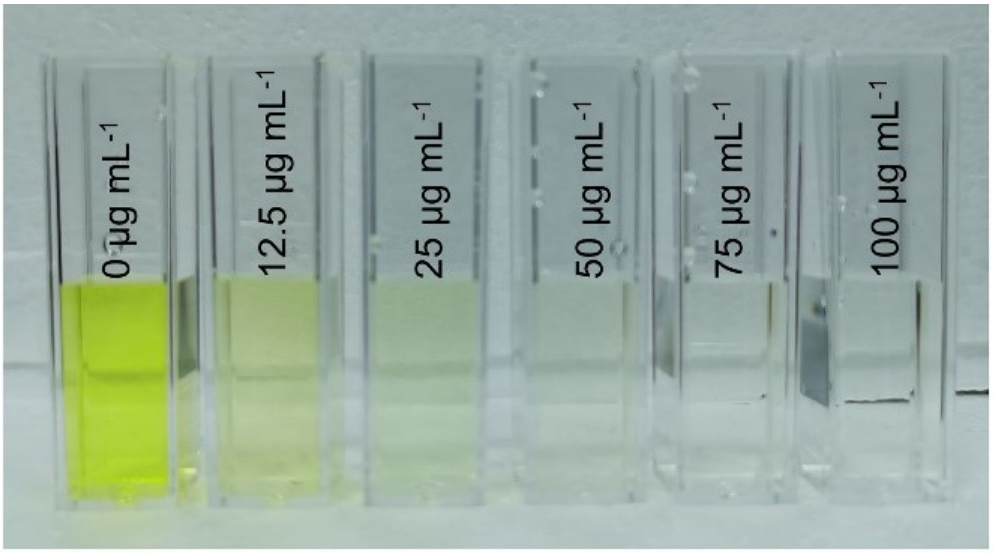

Fig. S12. The images of GSH depletion by different concentrations of CoCuS NPs.

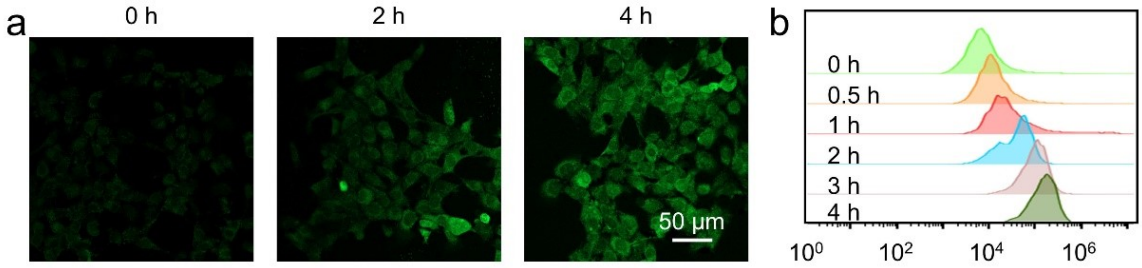

Fig. S13. (a) Fluorescent microscopy images of intracellular ROS generation treated with CuCoS NPs at different time; (b) Flow cytometry analysis of intracellular ROS generation treated with CuCoS NPs at different time.

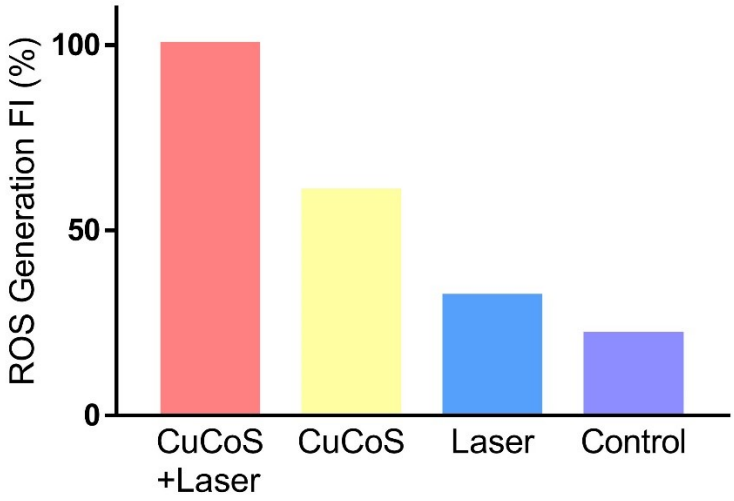

Fig. S14. The fluorescence intensity of Intracellular ROS generation.

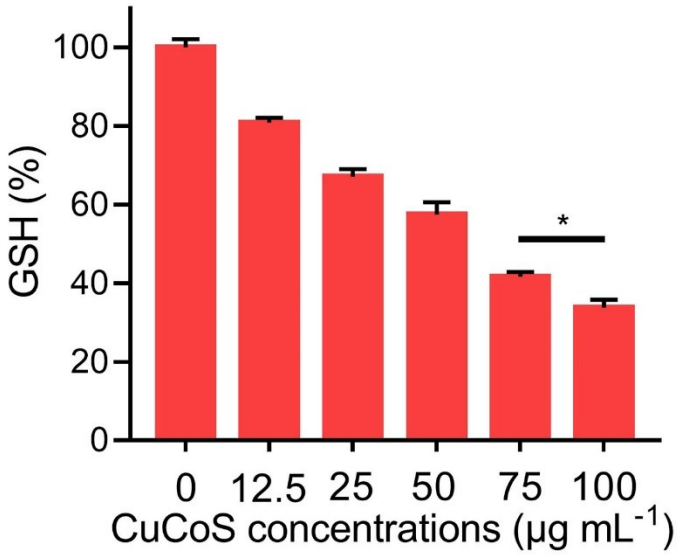

Fig. S15. The amounts of intracellular GSH treated with CuCoS NPs at different time.

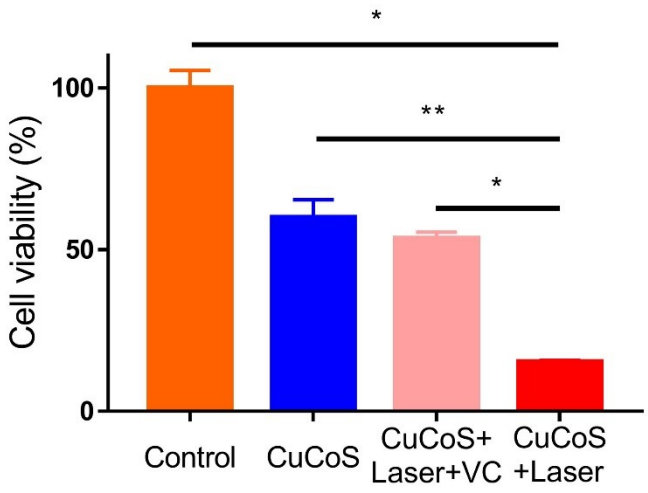

Fig. S16. Cell viabilities of 4T1 cells after different treatments.

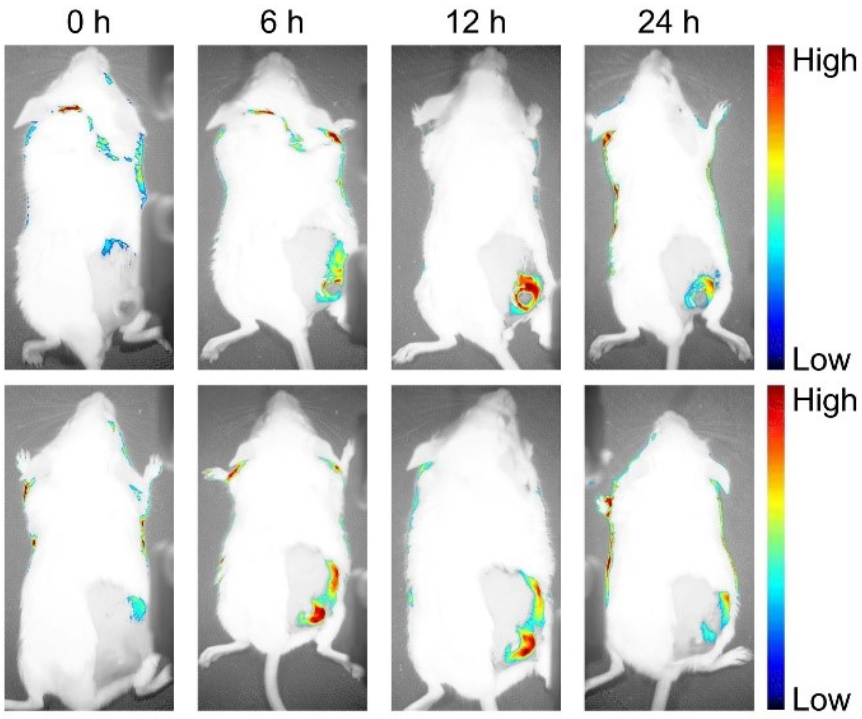

Fig. S17. The fluorescence imaging of 4T1-tumor-bearing mice i.v. injected with Cy-CuCoS NPs with the concentration of 5 mg mL^{-1}.

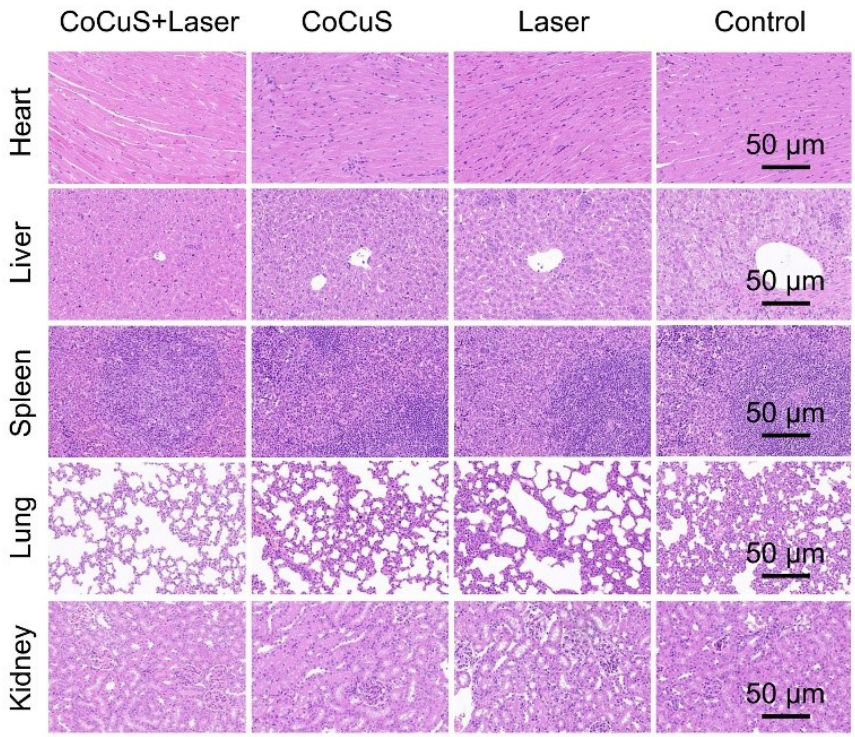

Fig. S18. Histochemical analysis of heart, liver, spleen, lung and kidney.