Supporting Information

A copper missile triggered power coalescence and death vortex within tumor cell mitochondria for synergistic cuproptosis/phototherapy/chemotherapy

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Supplement experimental section

Calculation of photothermal conversion efficiency (PCE)

The photothermal conversion efficiency of CCTH NPs (1 mL, 100 μg/mL) under 808 nm laser with a power density of 2.0 W/cm^2 was detected according to the previous method.[1, 2] Detailed calculation was given as follows:

$$\eta = \frac{hS(T_{max} - T_{sur}) - Q_{dis}}{I(1 - 10^{-A_{808\,nm}})} \times 100\% \quad (1)$$

Where h is the heat transfer coefficient, and S is the surface area of the container. T_{max} and T_{sur} are the maximum steady temperatures of the CCTH NPs' dispersion and the environmental temperature, respectively. Q_{dis} is expressed as the dissipated heat from light absorbed by the system. I is the power of the 808 nm laser, and $A_{808\,nm}$ is the corresponding absorbance of the NP dispersion at 808 nm. Among them, hS can be given by the following Equation 2~4.

$$hS = \frac{\sum_i m_i C_{p,\,i}}{\tau_s} \quad (2)$$

$$\tau_s = -\ln\theta \quad (3)$$

$$\theta = \frac{T(t) - T_{sur}}{T_{max} - T_{sur}} \quad (4)$$

Where m and C_p are the sample mass, and the specific heat capacity of the medium solution, respectively, and t is the time to cool to room temperature after irradiation.

In vitro tumor selectivity evaluation

Tumor selectivity of drug carriers (CuS-TPP-HA NPs) was measured by MTT assay (KeyGEN, China). 4T1 and LO2 cells were seeded into 96-well plates (1×10^4 cells per well) and adaptively cultured for 24 h. Then, the cells were treated with CuS-TPP-HA NPs dispersion at various concentrations (0, 10, 20, 30, 40, 50, 100 μg/mL). Every group has six parallel samples. After 24 h incubation, the cells were washed with PBS 3 times. Then, the

medium was withdrawn, and 50 μL of 1×MTT solution was added into each well and incubated for another 4 h to reduce MTT to formazan. After aspirating the supernatant, 150 μL of DMSO was added to dissolve formazan, and the plates were shaken gently for 30 min. The absorbance of the medium solution at 490 nm was detected by a microplate reader (Allsheng, China).

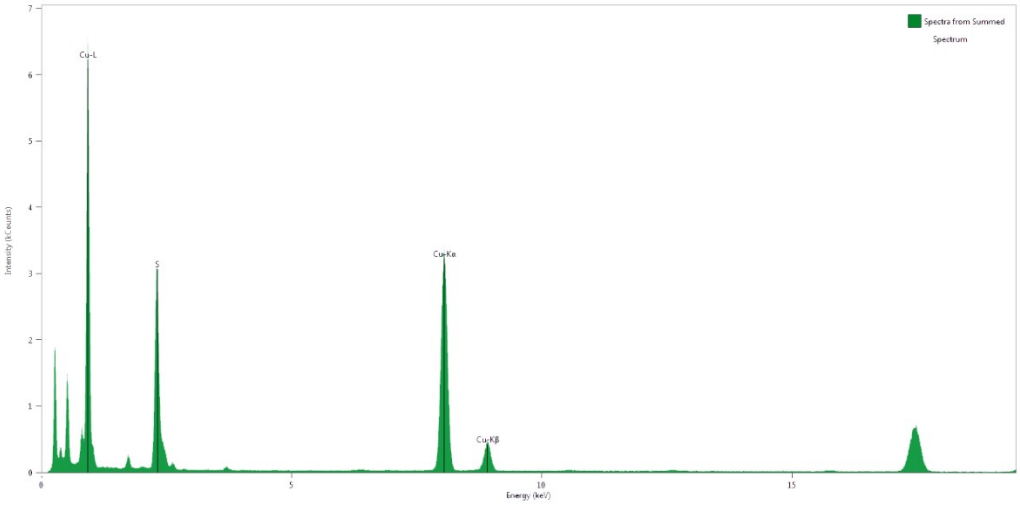

Figure S1. The XPS spectra of CuS nanoparticles.

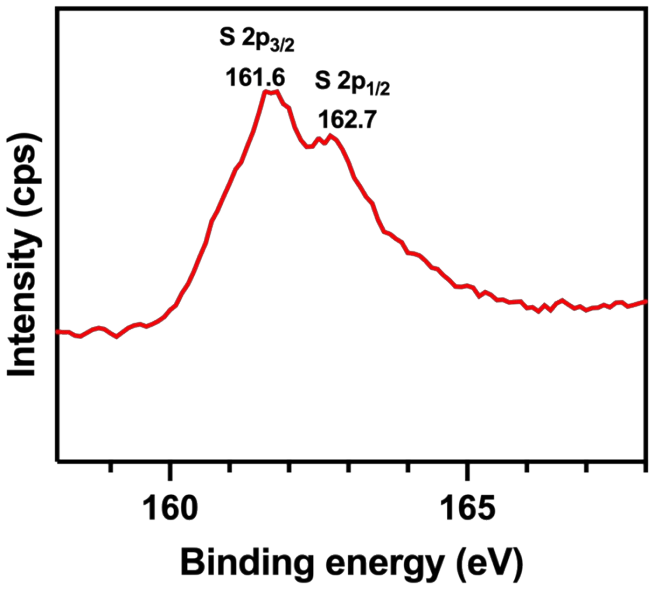

Figure S2. The S 2p XPS spectra of the CuS nanoparticles.

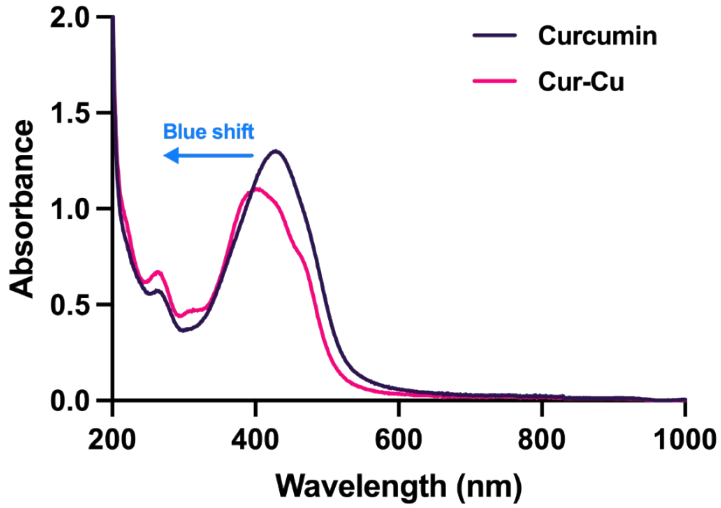

Figure S3. The UV-Vis spectra of curcumin and curcumin-copper complex.

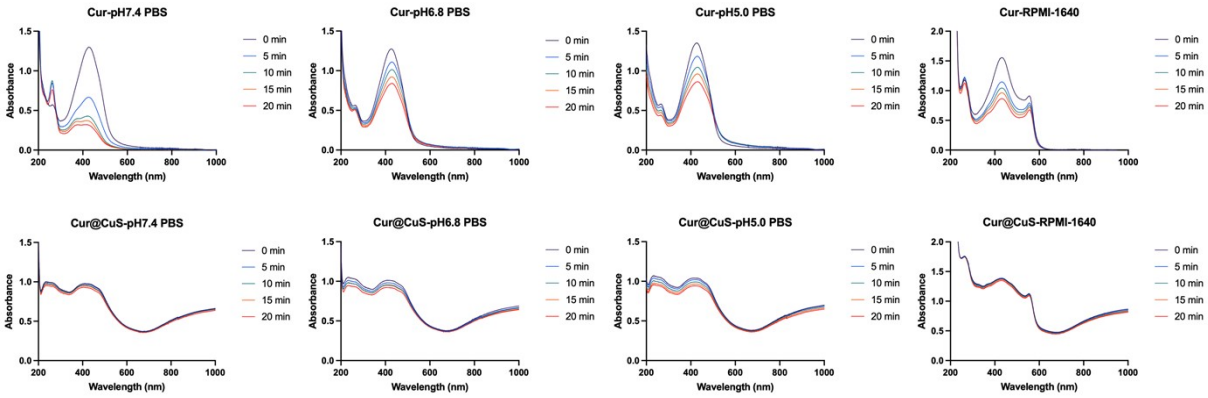

Figure S4. The UV-Vis absorption spectra of curcumin and Cur@CuS in the different PBS solutions and in the RPMI-1640 medium during a 20-minute period.

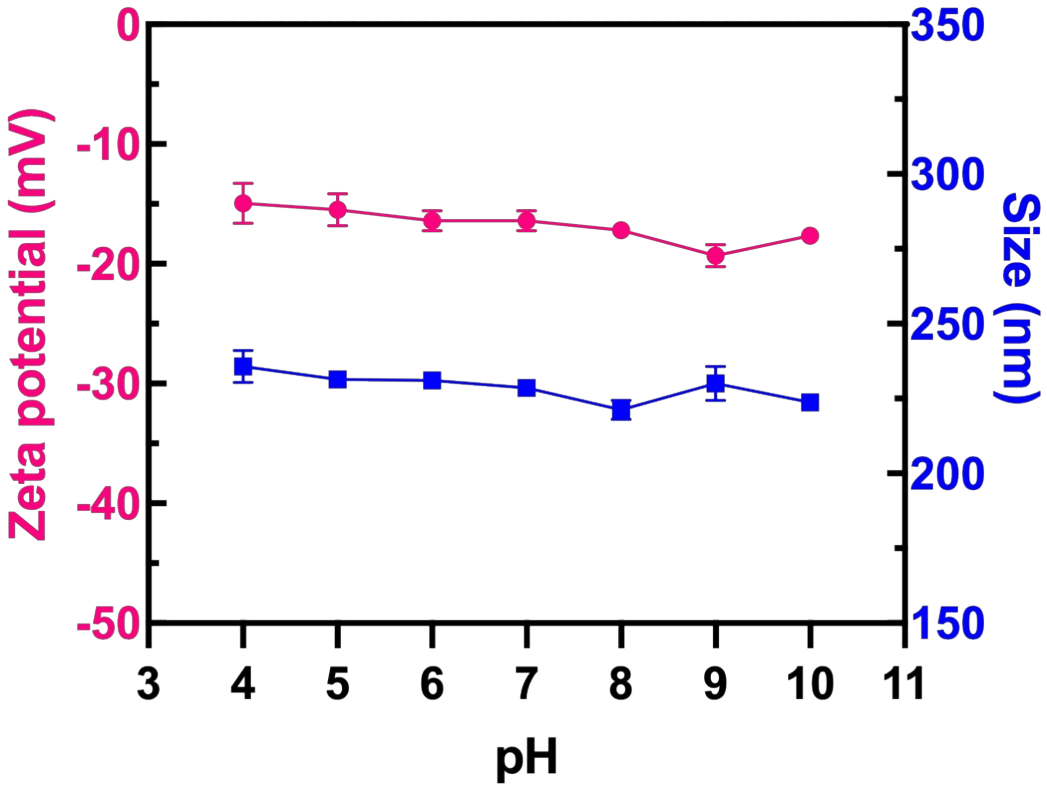

Figure S5. The particle size and zeta potential of CCTH NPs in PBS solution with pH 4 to 10 (n=3).

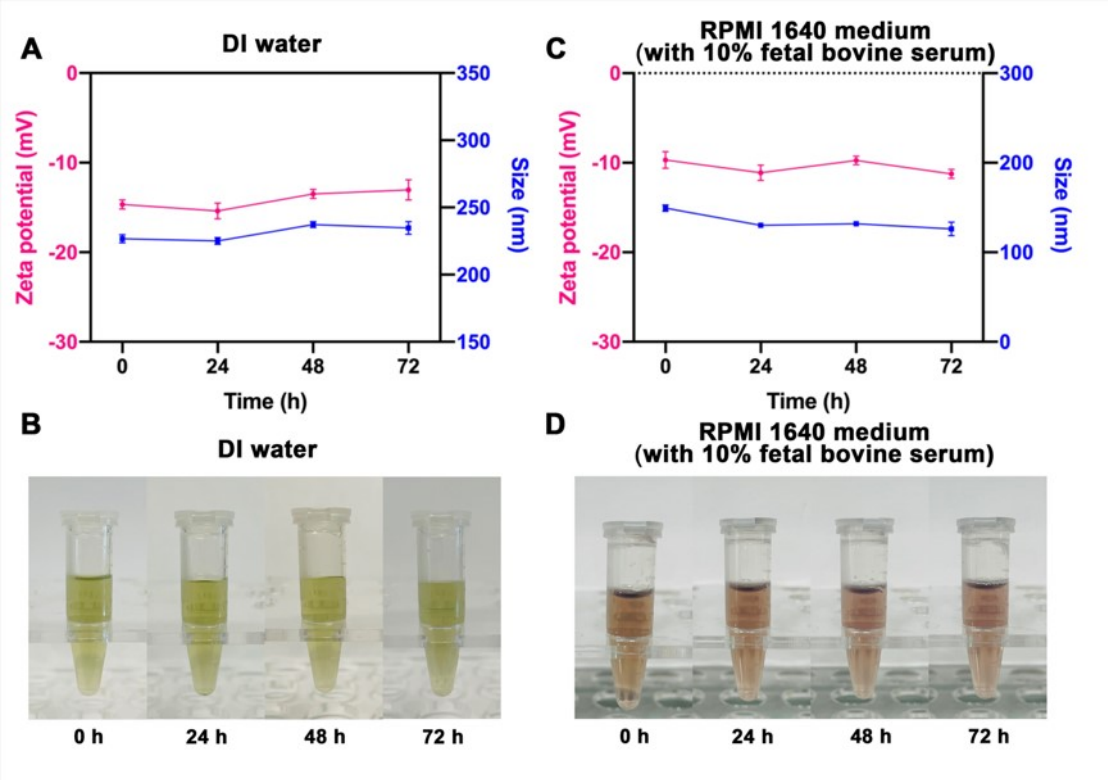

Figure S6. The particle size, zeta potential, and appearance of CCTH NPs A-B) in the deionized water and C-D) in the cell culture medium (RPMI-1640 medium with 10% fetal bovine serum) at the designated time points (n=3).

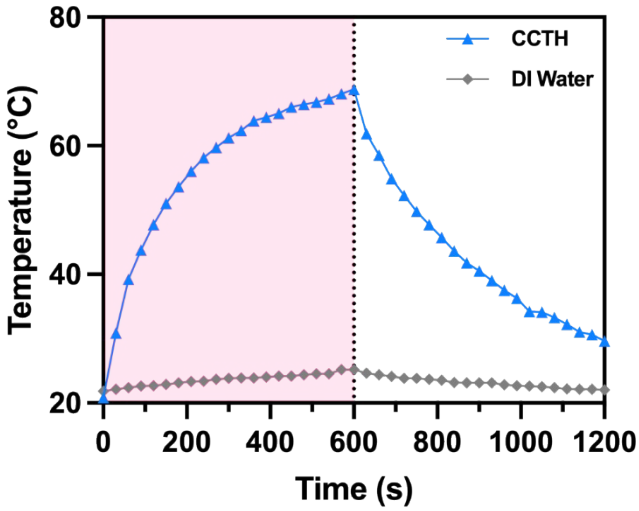

Figure S7. The heating and cooling curves of CCTH solution under NIR irradiation (808 nm, 2 W/cm^2).

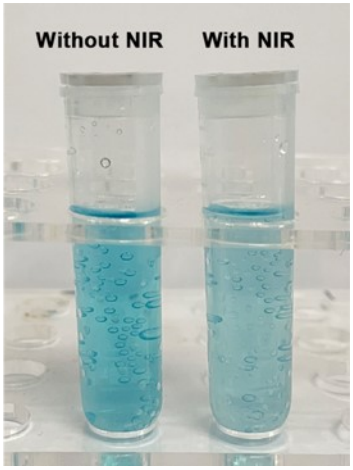

Figure S8. The MB solution after incubation with CCTH NPs for 10 min with (right) and without (left) NIR irradiation (808 nm, 1 W/cm^2, 10 min).

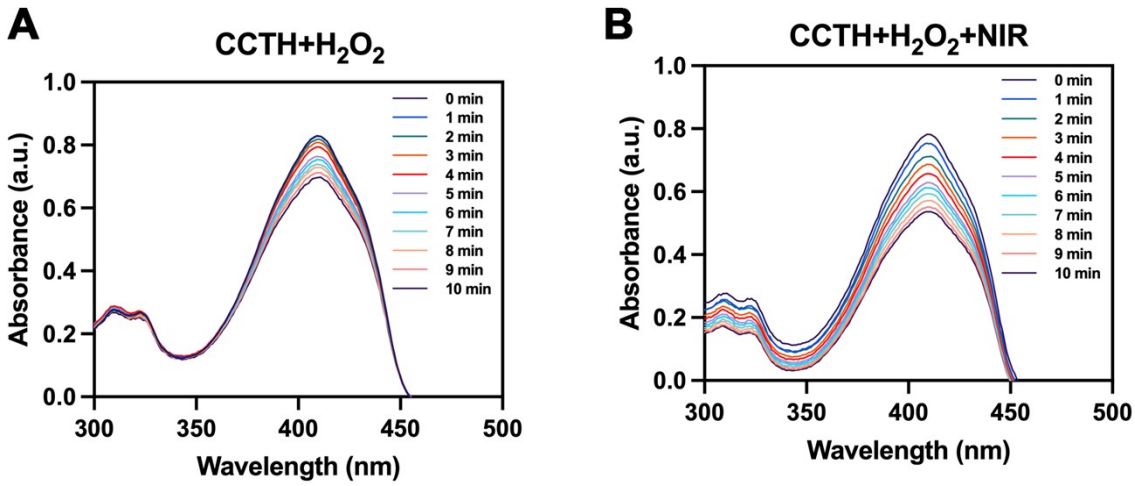

Figure S9. The UV-Vis absorption of DPBF after incubation with CCTH NPs A) with or B) without NIR irradiation (808 nm, 1 W/cm^2, 10 min).

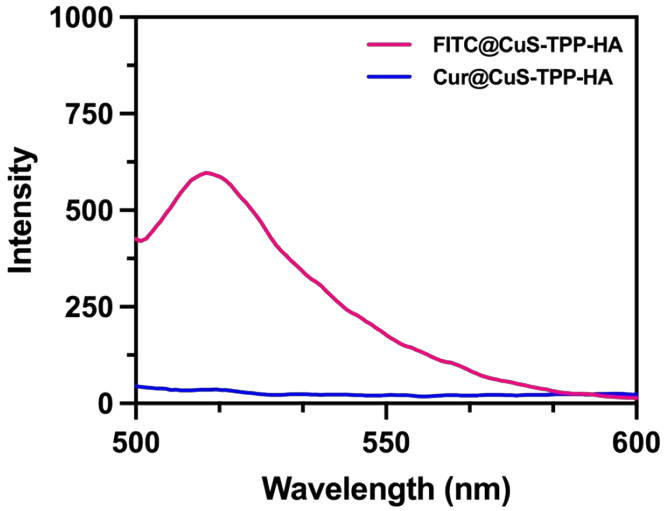

Figure S10. The fluorescence spectrum of Cur@CuS-TPP-HA NPs and FITC@CuS-TPP-HA NPs.

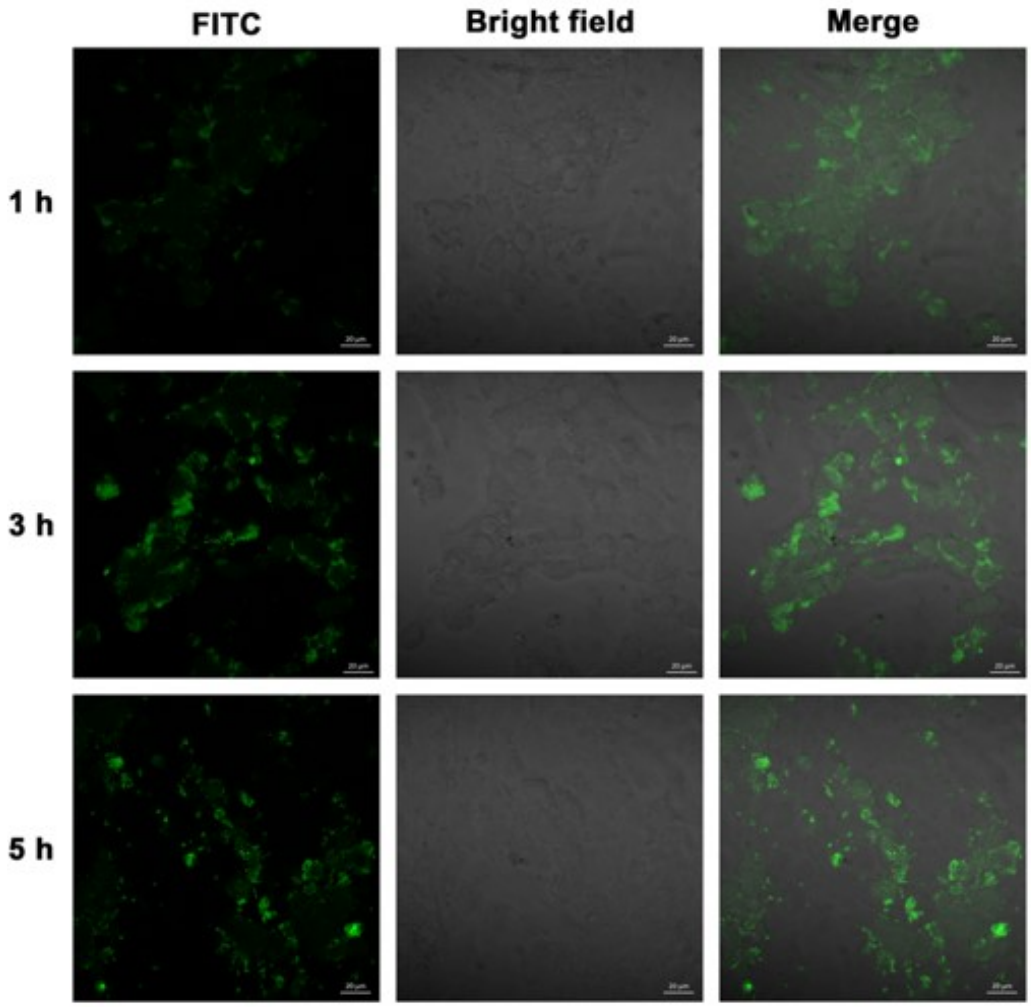

Figure S11. CLSM images of 4T1 cells incubated with FITC@CuS-TPP-HA NPs for different periods, Scale bar: 20 μm.

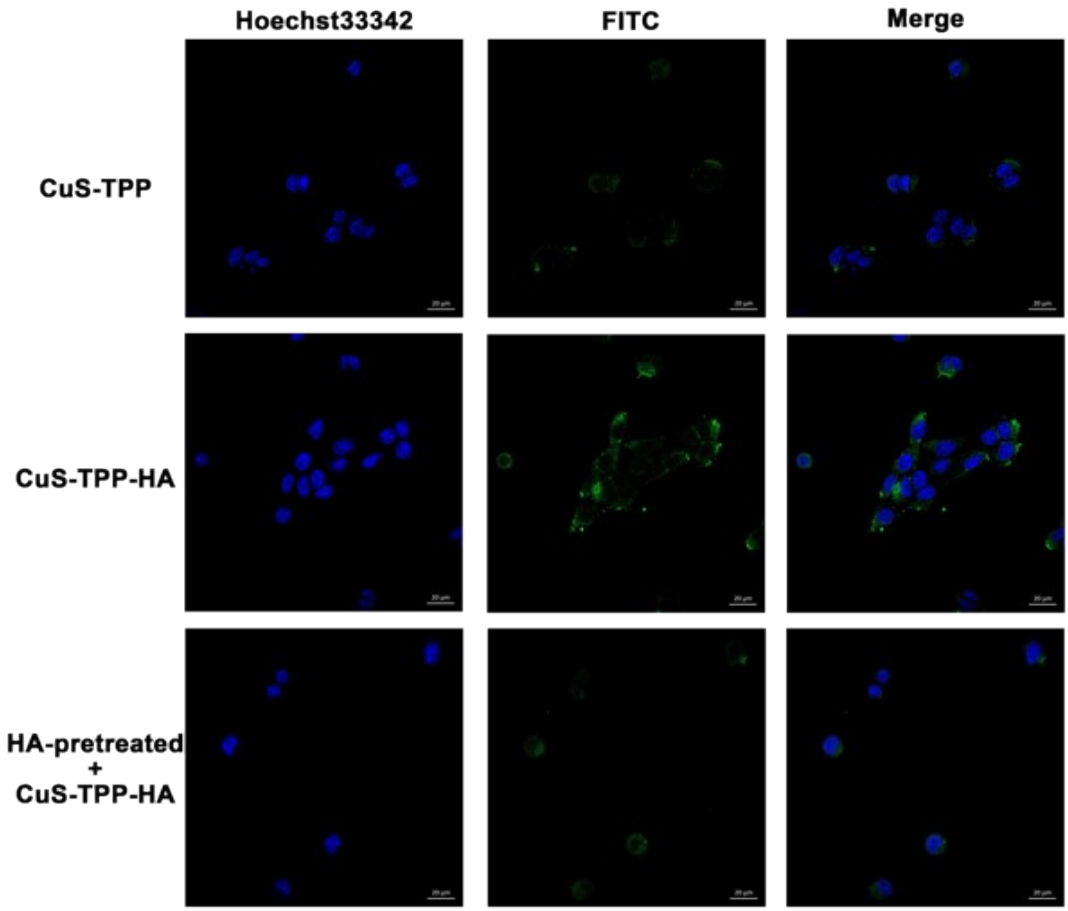

Figure S12. CLSM images of 4T1 cells incubated with FITC@CuS-TPP NPs, FITC@CuS-TPP-HA NPs, and FITC@CuS-TPP-HA NPs (with HA-pretreated) for four hours. Scale bar: 20 μm.

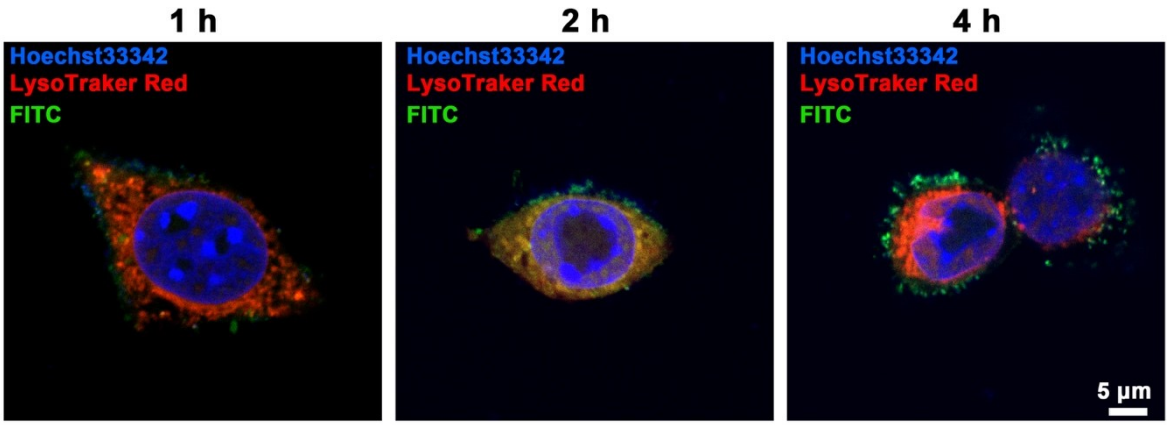

Figure S13. Intracellular localization of the F@CTH NPs and lysosome in 4T1 cells. Scale bar: 5 μm.

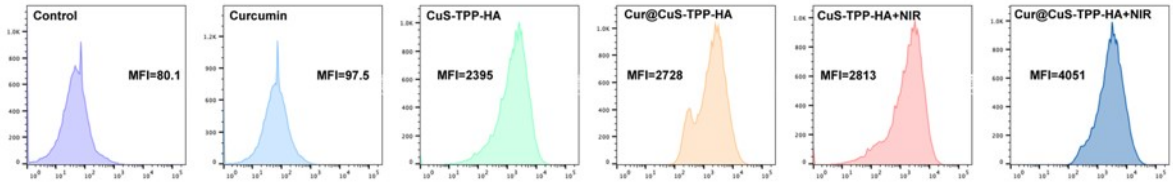

Figure S14. Flow cytometry analysis of the intracellular ROS content in different groups.

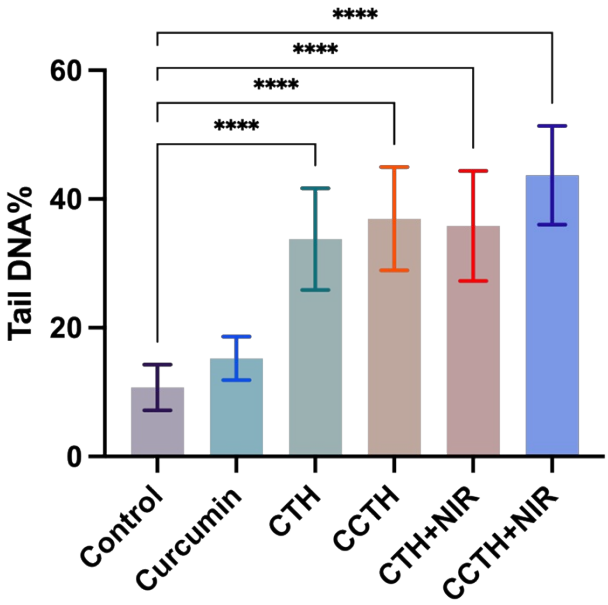

Figure S15. The content of tail DNA in 4T1 cells after different treatments was analyzed from the comet assay result (n=50). Data are shown as mean ± SD, p-values are calculated using one-way ANOVA, $^{****}p<0.0001$.

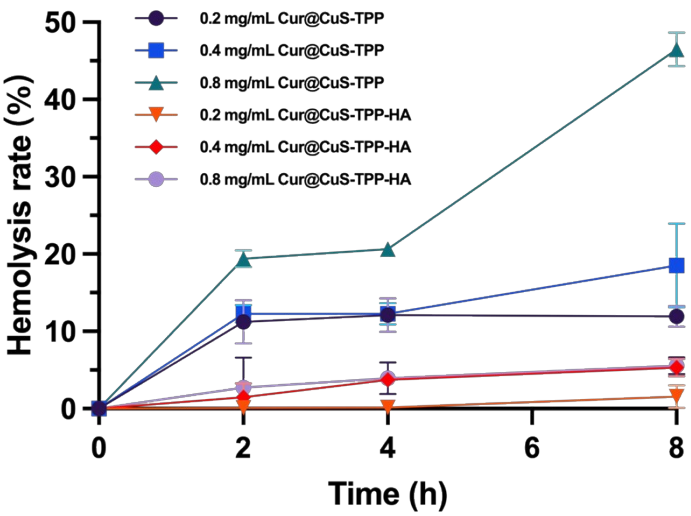

Figure S16. The hemolysis rate of Cur@CuS-TPP NPs and Cur@CuS-TPP-HA NPs at various concentrations (n=3).

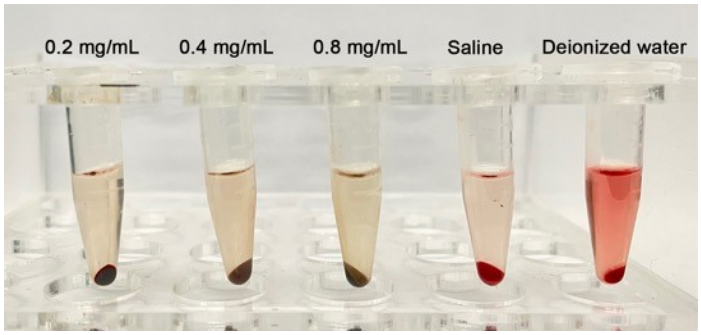

Figure S17. The photograph of RBC solutions after incubation with different concentrations of CCTH NPs. Deionized water and saline were employed as positive and negative controls, respectively.

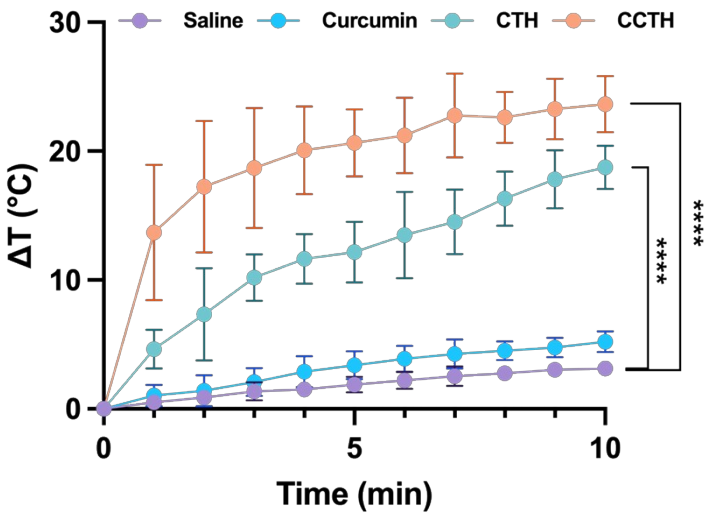

Figure S18. Temperature change curves of tumor sites in different groups under NIR irradiation (808 nm, 1 W/cm^2, 10 min) (n=3). Data are shown as mean ± SD, p-values are calculated using one-way ANOVA, $^{****}p<0.0001$.

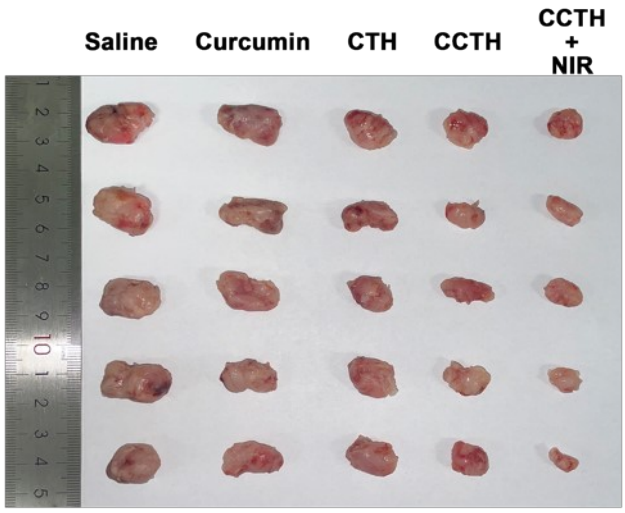

Figure S19. The photographs of tumor tissue in different groups were obtained on day 14.

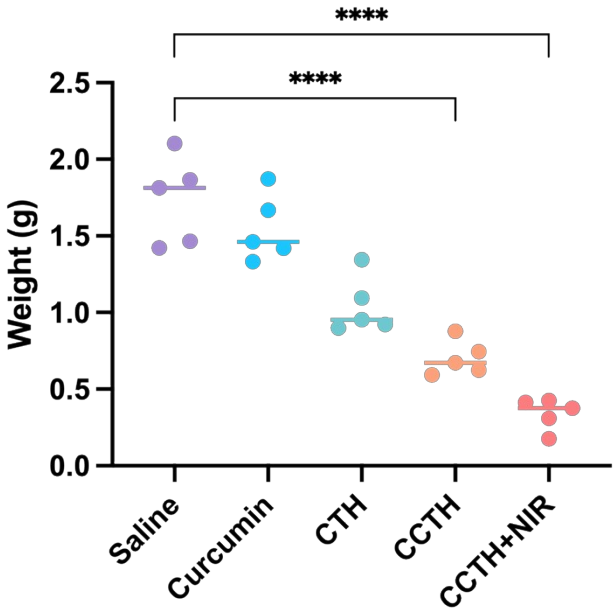

Figure S20. The weight of tumor tissue in different groups was obtained on day 14 (n=6). Data are shown as mean ± SD, p-values are calculated using one-way ANOVA, $^{****}p<0.0001$.

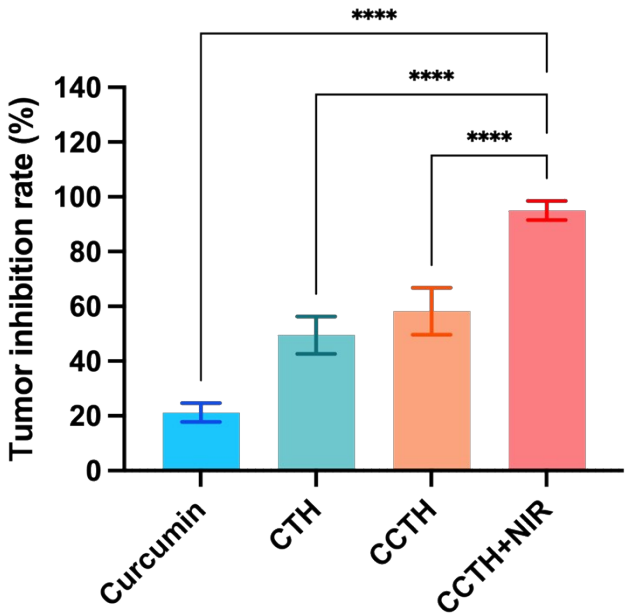

Figure S21. The tumor inhibition rate of 4T1 tumor-bearing mice in different groups after 14 days of treatment (n=6). Data are shown as mean ± SD, p-values are calculated using one-way ANOVA, $^{****}p<0.0001$.

Table S1. Specific information about Western blotting primary antibodies.

Antibody name	Molecular weight (kDa)	Dilutions	Purchasing company
Bcl-2	26	1:1000	Proteintech
Bax	21	1:2000	Proteintech
Caspase-3	17	1:1000	Abclonal
Caspase-9	46	1:1000	Abclonal
Cyto C	12~14	1:1000	Abclonal
DLAT	70	1:2000	Proteintech
FDX1	14	1:1000	Proteintech
β-actin	43	1:6500	Affinity
Tubulin	55	1:10000	Affinity

Reference

1. S. Wang, Y. Pang, S. Hu, J. Lv, Y. Lin and M. Li, *Chem. Eng. J.*, 2023, **451**, 138864.
2. S. Liang, X. Deng, Y. Chang, C. Sun, S. Shao, Z. Xie, X. Xiao, P. a. Ma, H. Zhang, Z. Cheng and J. Lin, *Nano Lett.*, 2019, **19**, 4134-4145.