Supplementary Material

Copper Manganese Sulfide Nanoplates: A New Two-Dimensional Theranostic Nanoplatform for MRI/MSOT Dual-Modal Imaging-Guided Photothermal Therapy in the Second Near-Infrared Window

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Experimental Section

Cell Culture, Cellular Uptake of Cu_2MnS_2 NPs and Cytotoxicity Assay: MCF-7 (human breast-cancer cells), HeLa (human cervical cancer cells) and S180 (murine sarcoma cancer cells) cell lines were cultured in RPMI-1640 medium (Gibco) plus 10% fetal bovine serum (FBS, Gibco) at 37 °C in a 5% CO_2 incubator.

For cellular uptake, MCF-7, HeLa and S180 cells with a density of 1×10^5 cells/well were seeded in a 6-well tissue culture plate and incubated for 24 h. Then, the medium was replaced with fresh RPMI-1640 medium containing Cu_2MnS_2 NPs (200 μg mL^{-1}) and cultured for different times. After removing the cell culture medium, cells were gently washed with PBS buffer for 3 times, homogenized, and treated with 2 mL of HNO_3 and 0.5 mL of 30% H_2O_2 solution for 8 h. Mn content internalized by cells was determined by an ICP-OES (Optima 7000 Dual View, PerkinElmer, USA).

Cell viability was measured with CCK-8 according to the manufacture's protocol. For the cytotoxicity assay, the MCF-7, HeLa and S180 cells were first cultured in 96-well plates (100 μL, 1×10^4 cells per well) for 24 h. And cultured for another 24 h after the culture medium was replaced with 100 μL of RPMI-1640 supplemented with 10% FBS containing 20 μL of the Cu_2MnS_2 NPs at different doses. Then, followed by removal of the culture medium, 10 μL of CCK-8 solution was added to each cell well which was washed with PBS buffer twice and contained 90 μL of culture medium. The cells were further incubated for 1 h. Then the standard

CCK-8 assay was carried out to determine the cell viabilities relative to the control untreated cells.

Hemolysis Assay: The hemolysis assays were carried out to estimate whether Cu_2MnS_2 NPs could lead to any damage to red blood corpuscles (RBCs) when they were used for *in vivo* application. RBCs were isolated from serum by centrifugation and suction. The RBCs were washed five times with PBS, and the purified blood was diluted to 1/10 of its volume with PBS. 100 μL of the cell suspension were treated with different concentrations of Cu_2MnS_2 NPs (0.05, 0.1, 0.5, 1.0 and 2.0 mg mL^{-1}) and incubated at room temperature for 3 h. The cell suspensions treated with 900 μL of PBS and deionized water was used as negative and positive controls, respectively. After incubation, the samples were centrifuged and the absorbances of the supernatants were recorded at 541 nm. The percent hemolysis of RBCs was calculated as following: percent hemolysis = $[(A_{sample} - A_{negative\ control}) / (A_{positive\ control} - A_{negative\ control})] \times 100\%$.

Biodistribution: All the animal experiments were executed according to the protocol approved by the Institutional Animal Care and Use Committee of Fujian Medical University. Male BALB/c nude mice (weight ≈ 20 g) were obtained from Shanghai SLAC laboratory Animal Co., Ltd. To study the biodistribution of Cu_2MnS_2 NPs in BALB/c nude mice, ~200 μL of Cu_2MnS_2 NPs solution was intravenously injected into the tail vein (20 mg kg^{-1}). After 6 h, 12 h, 24 h and 48 h post-injection, mice were sacrificed, organs (including heart, liver, spleen, lung, kidney, tumor, intestines, stomach, brain and muscle) dissected, weighed and stored at −20 °C

before analysis. For ICP-OES experiments, 5 mL of HNO_3 and 2 mL of 30% H_2O_2 were added to each sample, and maintained at 100 °C for digestion overnight until digestion was complete, then cooled to room temperature. The solution was diluted to 15 mL with 2% HNO_3. The Mn standard solutions with different concentrations (0, 0.5, 1, 5, 10, 50, and 100 ppb) were prepared. Both standard and test samples were measured by ICP-OES (Optima 7000 Dual View, PerkinElmer, USA). The results were normalized in units of percentage of injected dose per gram (% ID per g) of tissue.

Calculation of the Photothermal Conversion Efficiency: The photothermal transduction efficiency of Cu_2MnS_2 NPs or AuNRs was performed based on the method according to a previous report [1]. The photothermal conversion efficiency η can be determined by the following eqution (1):

$$\boldsymbol{\eta = (hS\,\Delta Tmax - Q_s) / I(1-10^{-A_{1064}})} \quad (1)$$

where **h** is heat transfer coefficient; **S** is the surface area of the container; **ΔTmax** is the temperature change at the maximum steady-state temperature; $\mathbf{Q_s}$ is the heat dissipated from light absorbed bythe quartz sample cell itself, which it is measured independently to be 12.30 mW using a quartz cell containing pure water; **I** is incident laser power density (2 W cm^{-2}); $\mathbf{A_{1064}}$ is the absorbance of Cu_2MnS_2 NPs or AuNRs at 1064 nm ($\mathbf{A_{1064}}$ were 0.49 for Cu_2MnS_2 NPs and AuNRs); **hS** is determined by the following equation (2):

$$\tau_s = m_D C_D / hS \qquad (2)$$

Where τ_s is sample system time constant that can be obtained from the slope of the plot of cooling time vs -Ln(ΔT/ΔTmax), m_D and C_D are the mass (1 g for H_2O) and heat capacity (4.2 J g^{-1} for H_2O) of the used solvent. Using the above two equations (1) and (2), the photothermal conversion efficiency (η) of Cu_2MnS_2 NPs and AuNRs can be calculated.

Supplementary Figures

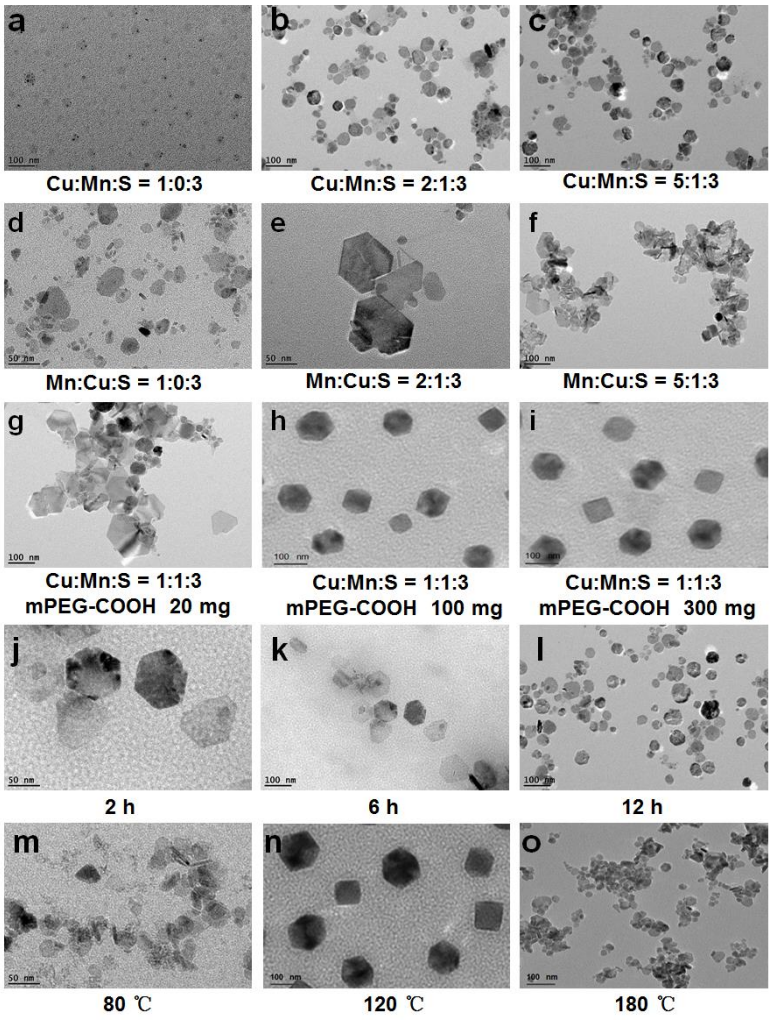

Figure S1. TEM images of Cu_2MnS_2 NPs obtained by different reaction conditions. (a-f) Conditions: 100 mg of mPEG-COOH, 120 °C, 6 h, with different Cu:Mn:S ratio (molar ratio). (g-i) Conditions: Cu:Mn:S = 1:1:3, 120 °C, 6 h, with different mass of mPEG-COOH. (j-l) Conditions: Cu:Mn:S = 1:1:3, 120 °C, 100 mg of mPEG-COOH, with different reaction time. (m-o) Conditions: Cu:Mn:S = 1:1:3, 100 mg of mPEG-COOH, 6 h, with different reaction temperature.

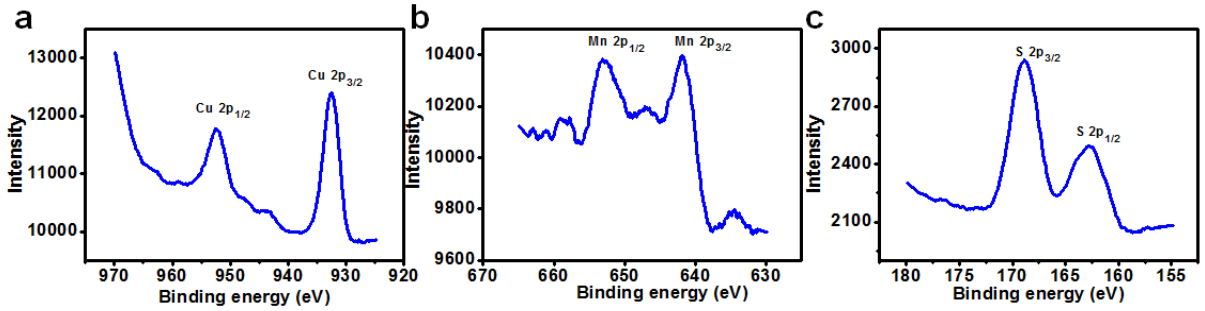

Figure S2. The XPS spectra of prepared Cu_2MnS_2 NPs. (a) Cu 2p region XPS spectrum, (b) Mn 2p region XPS spectrum, (c) S 2p region XPS spectrum.

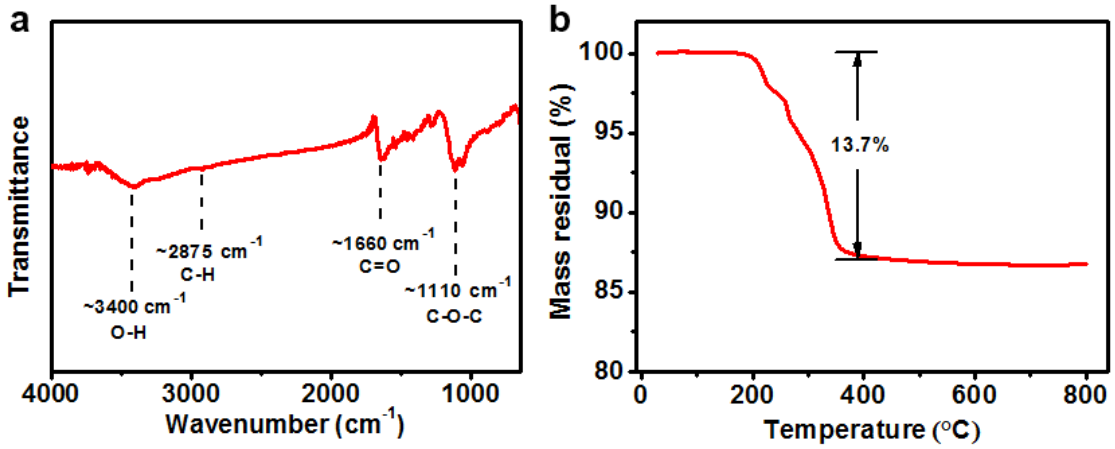

Figure S3. (a) The FTIR spectroscopy of Cu_2MnS_2 NPs (C–O–C peak at ~1110 cm^{-1}, C=O peak at ~1660 cm^{-1}, C-H peak at ~2875 cm^{-1} and O-H peak at~3400 cm^{-1} from mPEG-COOH). (b) The TGA curve of Cu_2MnS_2 NPs.

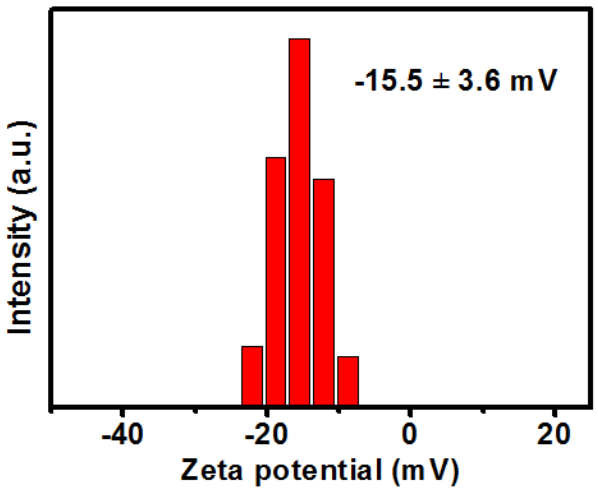

Figure S4. The zeta potential of Cu_2MnS_2 NPs.

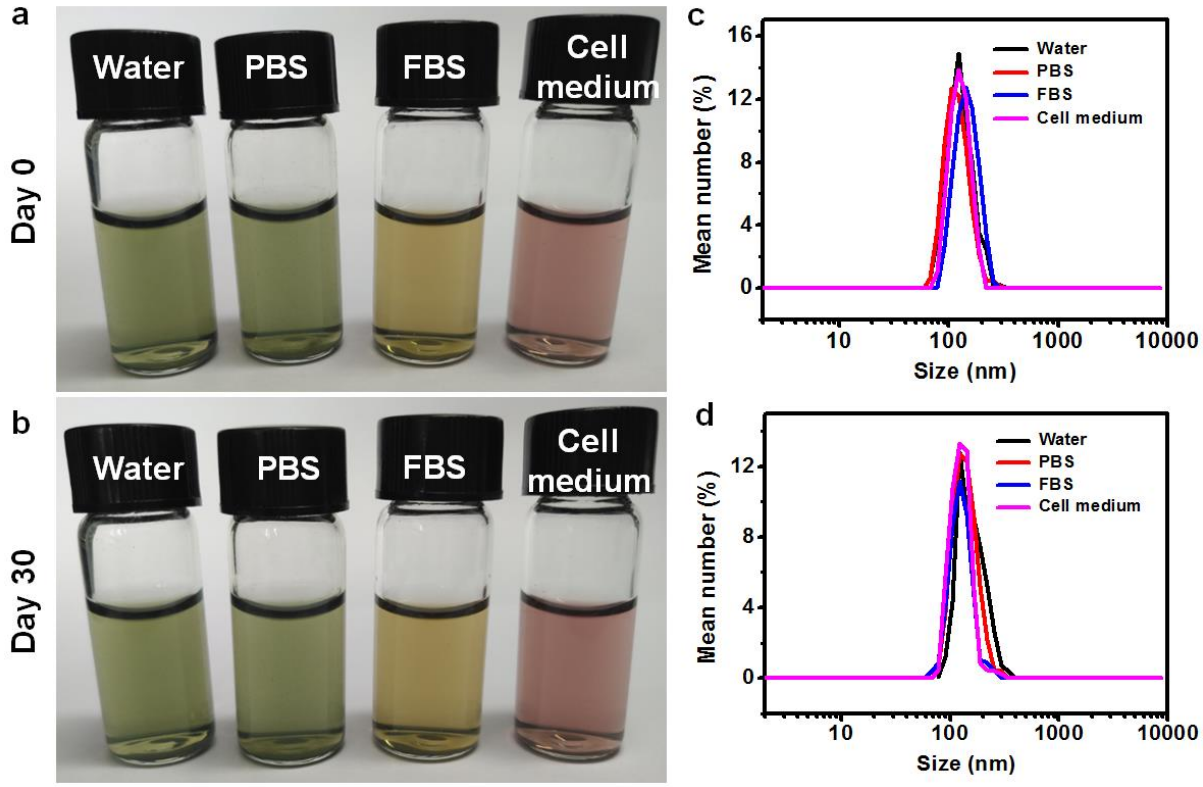

Figure S5. Photos of Cu_2MnS_2 NPs dispersed in water, PBS, fetal bovine serum (FBS), and cell culture medium at different days, (a) day 0, (b) day 30, showing good dispersibility in different media without obvious aggregation. Hydrodynamic diameters of Cu_2MnS_2 NPs in different media measured by DLS at (c) day 0, and (d) day 30, respectively.

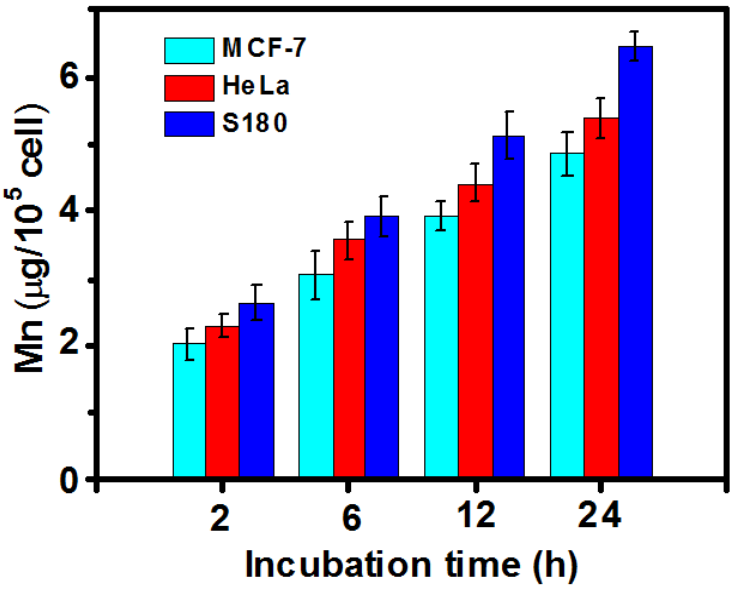

Figure S6. Time-dependent cellular uptake measured by ICP-OES.

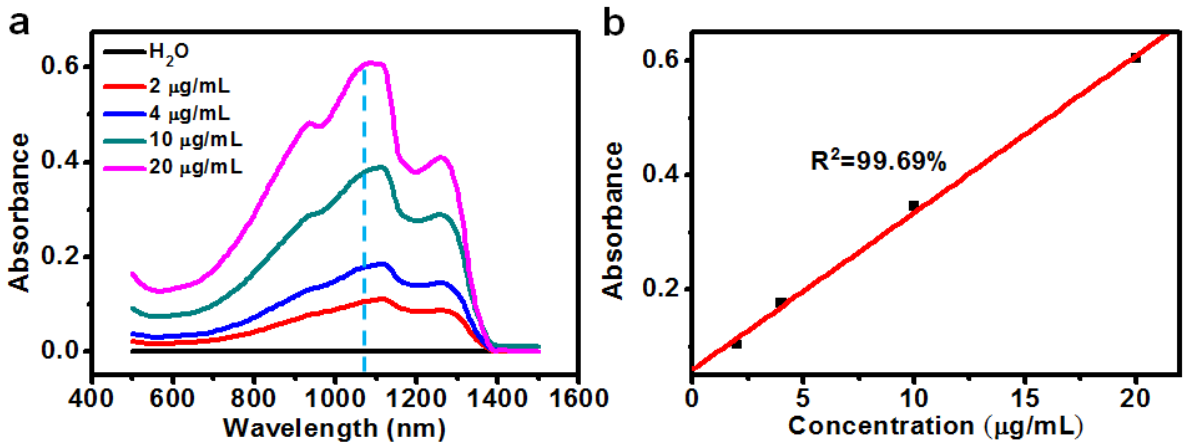

Figure S7. (a) UV-vis-NIR absorbance spectra for the aqueous solutions of Cu_2MnS_2 NPs with various concentrations (i.e., 2, 4, 10 and 20 μg mL^{-1}). (b) Plots of linear fitting absorbance at 1064 nm versus concentration for the aqueous dispersion of the Cu_2MnS_2 NPs. According to Lambert-Beer law, the molar extinction coefficient (ε) was calculated to be 1.03×10^{10} M^{-1} cm^{-1}.

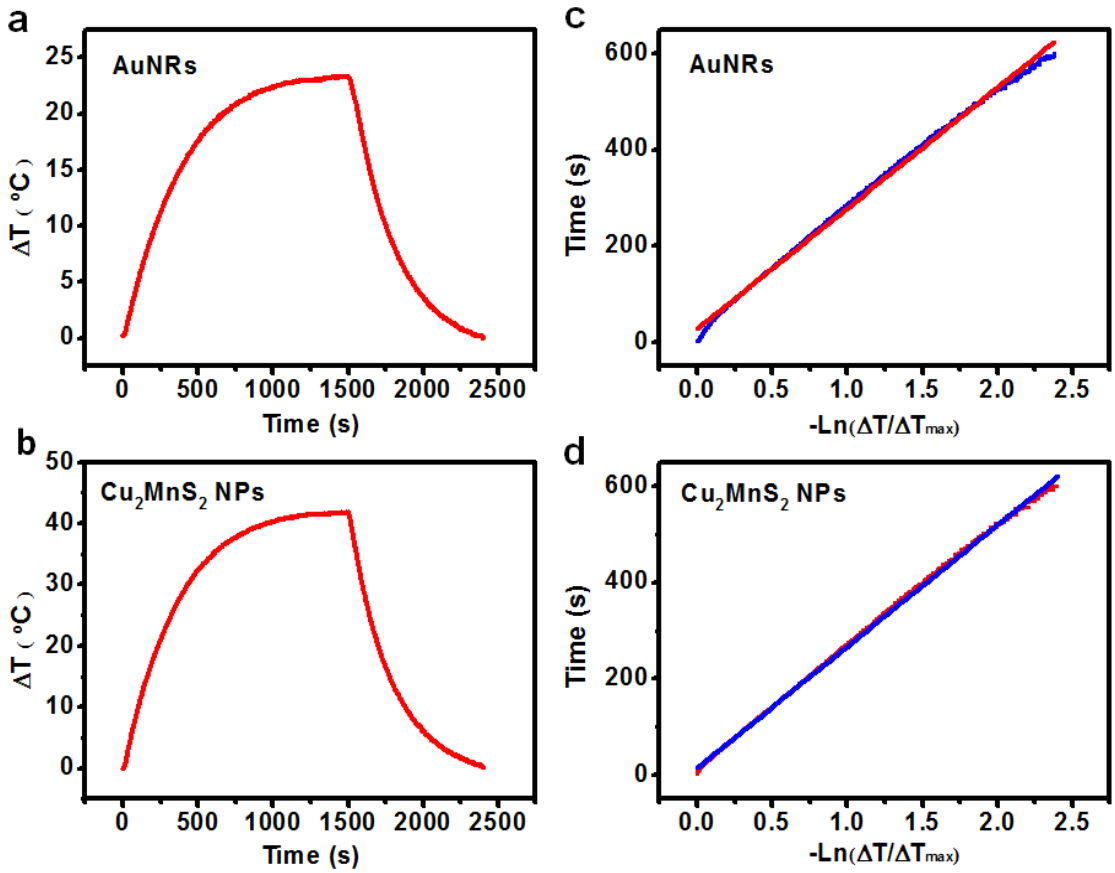

Figure S8. The temperature change of aqueous solutions of (a) AuNRs (A_{1064}= 0.49) and (b) Cu_2MnS_2 NPs (A_{1064}= 0.49) with laser irradiation for 1500 s (Laser: 1064 nm, 2 W cm^{-2}). (c) and (d) Linear time data versus -Ln(ΔT/ΔTmax) obtained from the cooling period of (a) and (b) respectively.

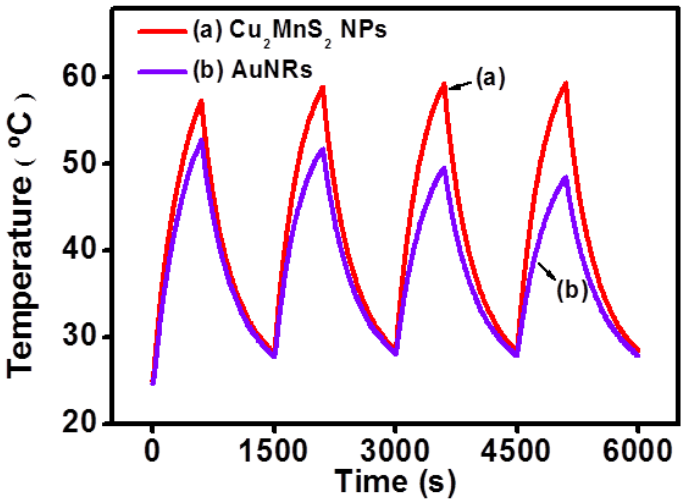

Figure S9. Photothermal heating curves of the Cu_2MnS_2 NPs (30 μg mL^{-1}) or AuNRs (30 μg mL^{-1}) over four cycles of NIR laser on/off (Laser: 1064 nm, 0.6 W cm^{-2}).

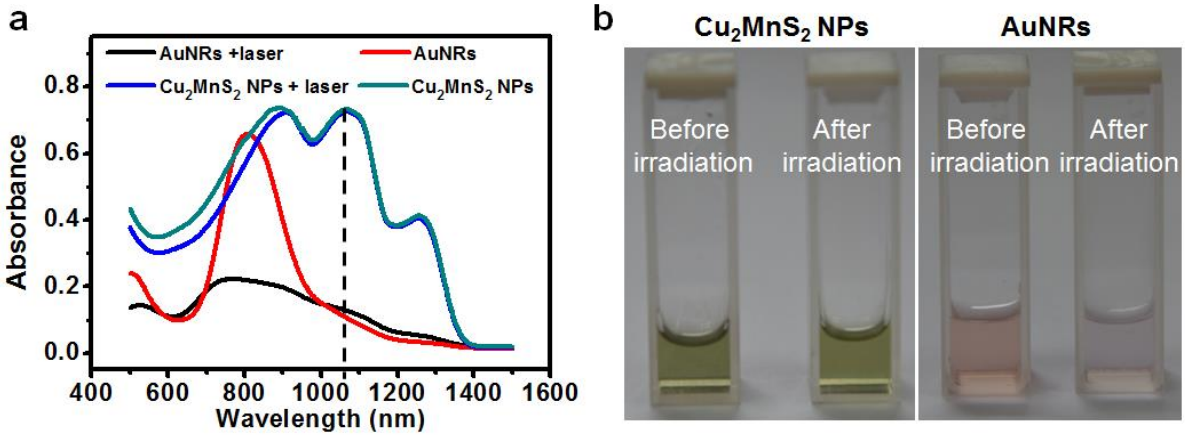

Figure S10. (a) UV-vis-NIR absorption spectra of Cu_2MnS_2 NPs (30 μg mL^{-1}) and AuNRs (30 μg mL^{-1}) before and after four laser on/off cycles of NIR laser irradiation (Laser: 1064 nm, 0.6 W cm^{-2}). (b) Photograph of the Cu_2MnS_2 NPs or AuNRs solutions before and after on/off NIR laser irradiation.

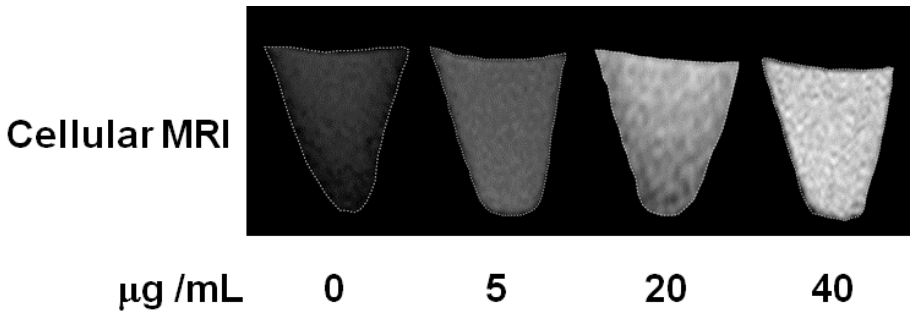

Figure S11. T_1-weighted MR images at 0.5 T of S180 cells (~1×10^5) incubated with different concentrations of Cu_2MnS_2 NPs for 24 h.

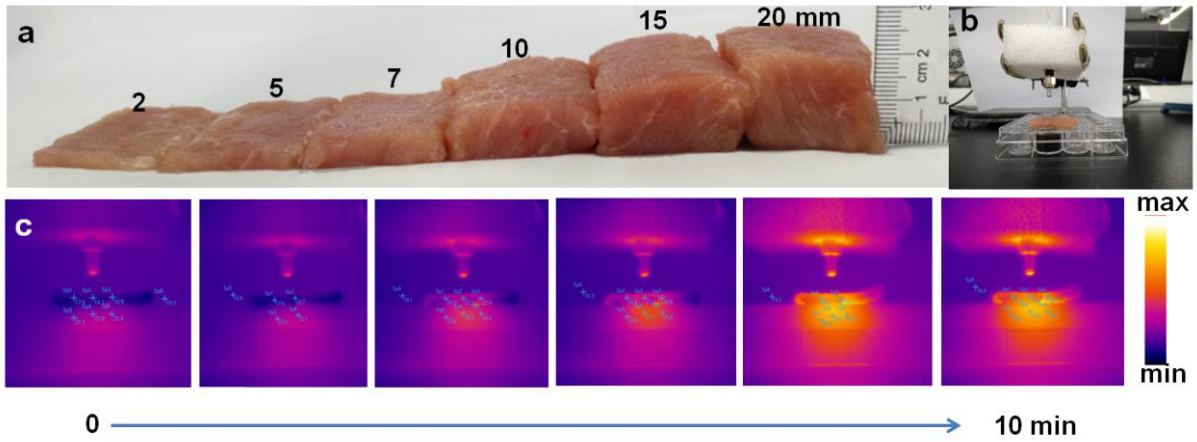

Figure S12. (a) Pork tissues with different thicknesses (2, 5, 7, 10, 15 and 20 mm) and (b) the setup for photothermal measurement under different tissue depth. (c) Representative thermal images of Cu_2MnS_2NPs solution with laser illumination (1064 nm, 1 W cm^{-2}) under 5 mm thick tissue at different time points (0, 2, 4, 6, 8 and 10 min).

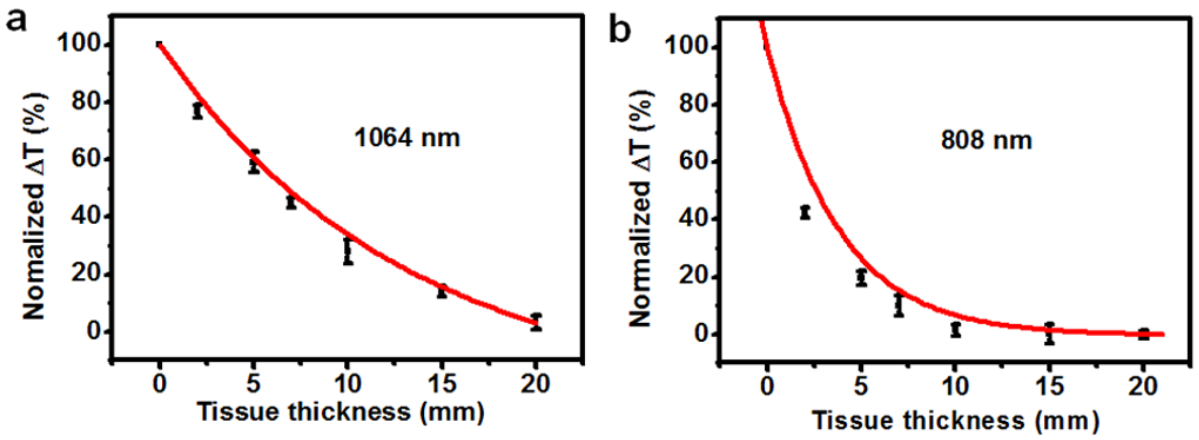

Figure S13. Scattered plot of the normalized temperature change under different tissue depth using different lasers (a) 1064 nm, 1 W cm^{-2} (b) 808nm, 1 W cm^{-2} with the fitted exponential decay curve. The error bars represent standard deviations (n = 3).

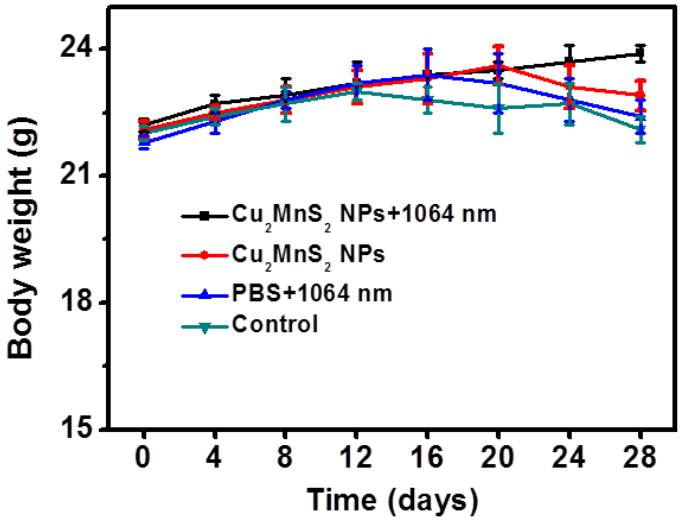

Figure S14. Body weights of mice in different groups.

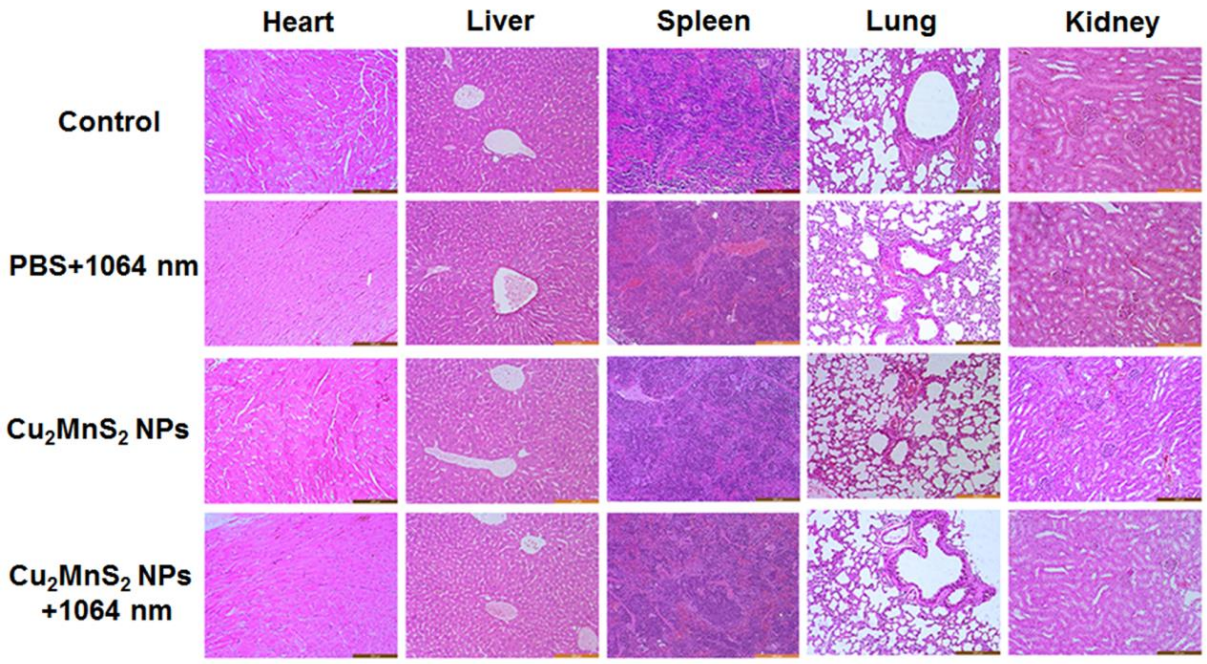

Figure S15. Representative H&E stained images of major organs collected from different groups of mice at 28th day post-treatment.

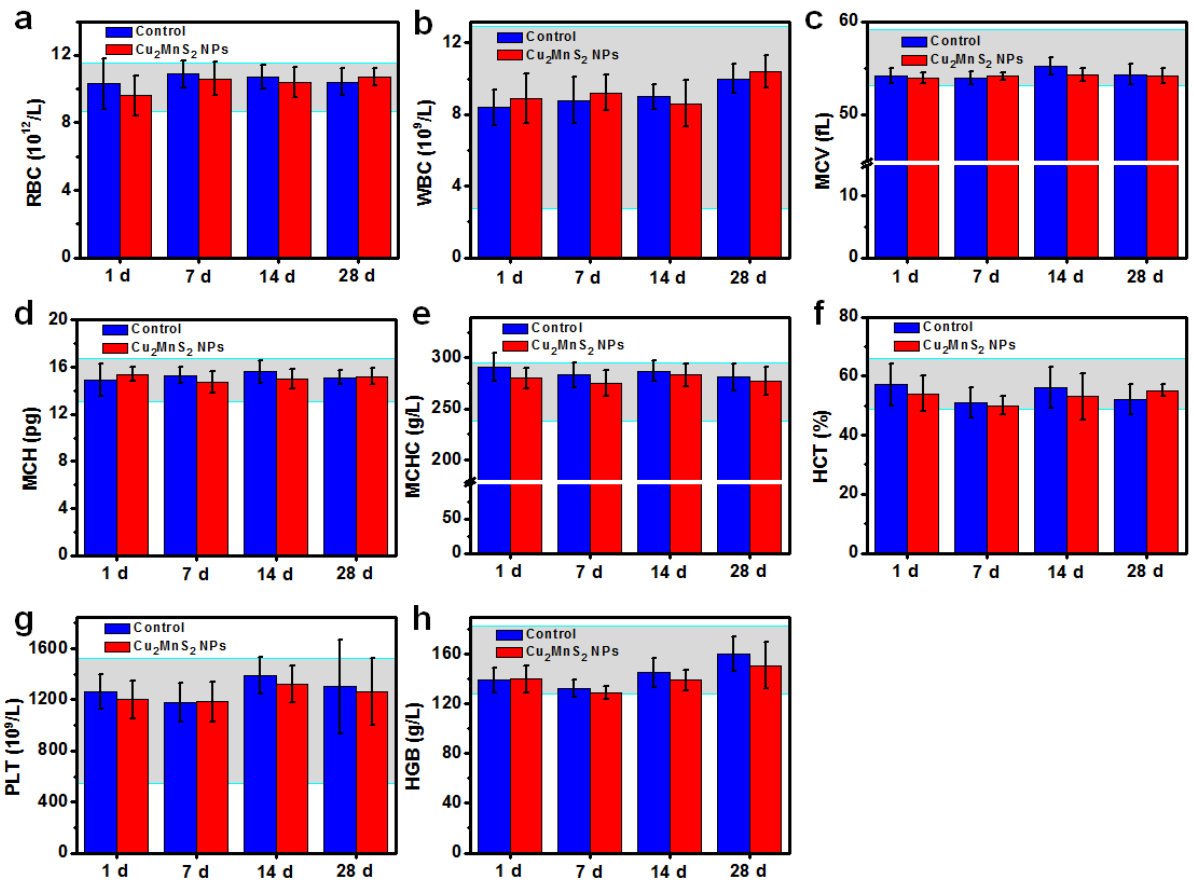

Figure S16. The hematology data from healthy control and Cu_2MnS_2 NPs treated male BALB/c nude mice. The data were collected at the different time points of 1 d, 7 d, 14 d, and 28 d after intravenous injection (20 mg kg^{-1}). The terms are as following: (a) red blood cells (RBC), (b) white blood cells (WBC), (c) mean corpuscular volume (MCV), (d) mean corpuscular hemoglobin (MCH), (e) mean corpuscular hemoglobin concentration (MCHC), (f) hematocrit (HCT), (g) platelets (PLT) and (h) hemoglobin (HGB). Heathy mice injected with PBS were used as controls. Error bars were based on five mice per data point. Gray areas in the figures show the normal reference ranges of hematology data of healthy male BALB/c nude mice [2].

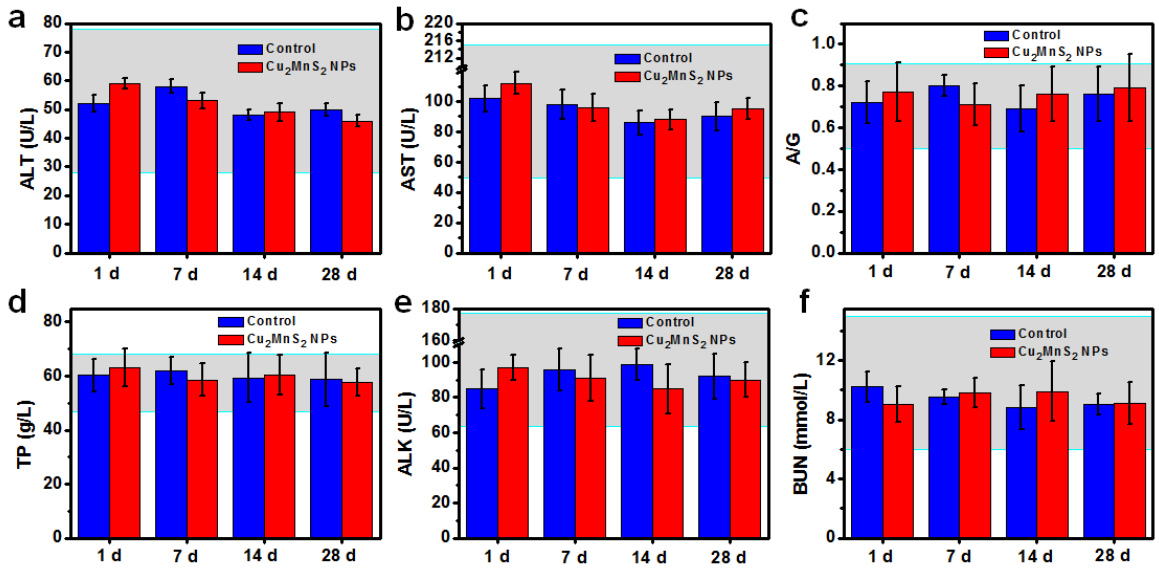

Figure S17. The serum biochemistry data from healthy control and Cu_2MnS_2 NPs treated male BALB/c nude mice. The data were collected at the different time points of 1 d, 7 d, 14 d, and 28 d after intravenous injection (20 mg kg^{-1}). The terms are as following: (a) alanine transaminase (ALT), (b) aspartate transaminase (AST), (c) time-course albumin/globulin ratios (A/G), (d) total protein (TP), (e) alkaline phosphatase (ALK) and (f) blood urea nitrogen (BUN). Heathy mice injected with PBS were used as controls. Error bars were based on five mice per data point. Gray areas in the figures show the normal reference ranges of biochemistry data of healthy male BALB/c nude mice [2].

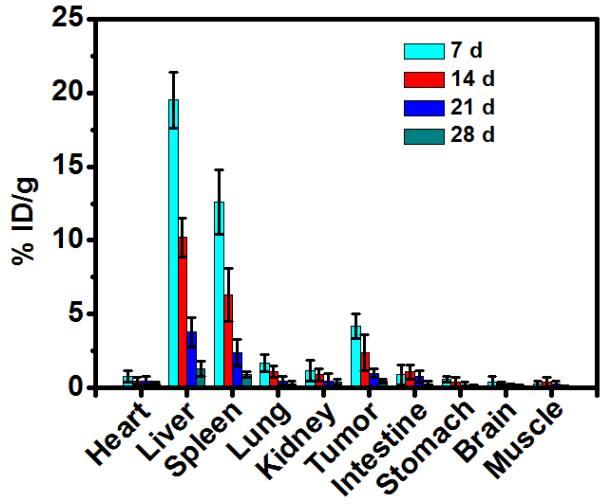

Figure S18. Long-term *in vivo* biodistribution of Cu_2MnS_2 NPs in tumors-bearing mice at 7 d, 14 d, 21 d and 28 d post-injection measured by ICP-OES analysis of Mn level in different organs (n = 5).

Supplementary References

1. Guo CS, Yu HJ, Feng B, Gao WD, Yan M, Zhang ZW, et al. Highly Efficient Ablation of Metastatic Breast Cancer Using Ammonium-Tungsten-Bronze Nanocube as a Novel 1064 nm-Laser-Driven Photothermal Agent. Biomaterials. 2015; 52: 407-16.

2. Reference Ranges of Hematology Data of Healthy Male BALB/c Nude Mice were Obtained From Charles River Laboratories: http://www.criver.com/files/pdfs/rms/balbc-nude/rm_rm_r_balb-c_nude_mouse_clinical_pathology_data.aspx