Supporting information

Mitochondria targeted cascade reaction nanosystem for improved therapeutic effect by overcoming the cell resistance

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EXPERIMENTAL SECTION

1. Materials

Chloroauric acid, Sodium hydroxide, Silver nitrate, Hexadecyltrimethylammonium chloride (CTAC), Sodium oleate, Hydroquinone, triphenylphosphonium, 3-Bromo-1-propanol, dichloroacetyl chloride were purchased from Macklin Reagent. Sodium borohydride, Sodium citrate, Hexadecyl trimethyl ammonium bromide (CTAB) were obtained from Sigma-Aldrich. 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF-AM), Lysosome Tracker Red, Mitochondria Tracker Red, Cell counting kit-8 (CCK-8), Calcein-AM/propidium iodide cytotoxicity assay kit, 2,7-Dichlorodi-hydrofluorescein diacetate (DCFH-DA), JC-1 assay kit and Western-blot relative materials were purchased from Beyotime Inst. Biotech. All the reagents were used as received without further purification.

2. Methods

2.1. Synthesis of TPP-DCA

The synthesis process of TPP-DCA was divided into two steps. For the first step, 27.5 mmol PPh_3 and 25 mmol 3-Bromo-1-propanol were added in 25 mL toluene, the reaction was refluxed for 24 h. The obtained white solids were extracted after adding diethyl ether for three times. Yield: 6.6g, 69%.

1g white power from the first step was dissolved in 40 mL dichloromethane. Then, 1.248 mL trimethylamine was added under stirring. The system was transferred to 0 ℃ ice bath. 6 mmol dichloroacetyl chloride were dropped and reacted overnight. 20 mL 0.6 mol/L HCl were used to extracted for three times. The final products were obtained after rotary evaporated. Yield: 1.53g, 54%. ALL the characterizations could be found in Supporting information.

2.2. Synthesis of AuBPs

Au seeds solutions were prepared first. 80 μL $HAuCl_4$ (25.4 mM) and 74 μL HNO_3 (0.25mol/L) were added into 8 mL CTAC solutions (66 mmol/L) and stirred gently for 15 min. Then, 100 μL of $NaBH_4$-NaOH solutions (50 mM : 50 mM) and 100 μL 1mol/L of sodium citrate solutions were added. The mixture solutions were aged in 80 ℃ water bath for 1 h.

40 mL mixture solutions containing 1.82 g CTAB, 0.076 g NaOL, 0.25 mmol/L $HAuCl_4$, 0.12 mmol/L $AgNO_3$ stirred until the solution became colorless. 0.5 mL HCl (1 mol/L), 10 mL Hydroquinone (0.1 mol/L) and 0.2 mL seeds solutions were added under the rapid stirring for 30 s. The solutions were left without disturbance for overnight. The excess surfactants were removed by centrifugation. 0.5 mL solution of mPEG-SH (15 mg/mL) were mixed with 0.5 mL AuBPs-Br (1 mg/mL) and stirred overnight. The excess PEG were removed by centrifugation (AuBPs).

2.3. Synthesis of AuBPs@TD

The different radios of AuBPs to TPP-DCA were mixed at a volume ratio of 1:1. The solutions stirred overnight followed by the centrifugation. The obtained resulting precipitate (AuBPs@TD) were dispersed in PBS for further using.

2.4. Synthesis of AuBPs@TD

1 mg/mL AuBPs-Br was stirred overnight with 5 mg/mL FITC-PEG-SH. The supernatant was removed by centrifugation and the precipitate was used to load TPP-DCA. The final product was stored under 4℃ for further use.

2.5. Photothermal effect of AuBPs

808 nm laser (0.5 W/cm^2) was used to irradiated 1 mL solution of AuBPs at different concentrations. The changes of temperature were recorded every 10 seconds. The thermal images were also captured by thermal camera.

2.6. Cellular endocytosis

HeLa cells were pre-treated with different inhibitors for 2 hours. The concentrations were as follows: 12 μg/mL chlorpromazine (CPZ), 6 μg/mL methyl-β-cyclodextrin (mβCD), 50 μg/mL genistein, 20 μg/mL amiloride. Then, the culture medium was replaced by DMEM containing inhibitors and 30 mg/mL FITC labeled AuBPs@TD. The intracellular fluorescence intensity was measured by flow cytometry and confocal laser scanning microscopy (CLSM).

2.7. Intracellular distribution

HeLa cells were incubated with 30 mg/mL FIRC labeled AuBPs@TD for different times. The lysosome tracker and mitochondria tracker were used to mark the lysosome and mitochondria, respectively. The intracellular distribution of AuBPs@TD was observed by CLSM.

2.8. The expression of resistance protein in cells.

MCF-7/ADR cells were incubated in 6-well plates for 24 h. Then, the cells were divided into six groups. I: control; II: TPP-DCA (40 ppm); III: AuBPs (80 ppm); IV: AuBPs (80 ppm) and laser (0.5 W/cm^2, 8min); V: AuBPs@TD (80 ppm); VI: AuBPs@TD (80 ppm) and laser (0.5 W/cm^2, 8min). Among them, the laser irradiation was performed after the overnight. After 4 hours, the cells were collected by lysis buffer. The expression of Hsp 90 and P-gp were measured through Western-blot methods, and β-actin proterin was used as the internal reference.

2.9. The detection of intracellular pH

HeLa cells were incubated for 24h in 6-well plates, and divided into six groups as mentioned above. 2 hours after the laser irradiation, DMEM containing 3 μM BCECF-AM probe were used for further cell culture for 30 min. The intracellular fluorescence was observed by CLSM.

2.10. Detection of intracellular ROS

The HeLa cells were treated with four methods overnight. I: control; II: TPP-DCA (50 ppm); III: AuBPs (100 ppm); IV: AuBPs@TD (100 ppm). After washed by PBS three times, DMEM containing 10 μM DCFH-DA was further incubated for 20 min. The intracellular fluorescence was monitored by CLSM.

2.11. Animal model

Balb/c mice were obtained from Liaoning Changsheng biotechnology Co. Ltd. All the relative experiments were performed according to the stipulates of the Animal Study Committee of Changchun Institute of Applied Chemistry. Various efforts were taken to alleviate the suffering of mice.

2.12. The biocompatibility of AuBPs@TD in vivo

100 μL AuBPs@TD (5.2 mg/kg) were injected intravenously in to Balb/c mice. The body weights were recorded every two days. After 5 h, 24 h, 3days, 5days of injection, the major organs were collected, and the content of Au was detected by inductively coupled plasma mass spectrometry. The major organs also collected for hematoxylin and eosin (H&E) staining. The injection of saline was used as a control.

2.13. Anticancer effect of AuBPs@TD in vivo

The tumor-bearing mice were divided into six groups. I: PBS; II: TPP-DCA (2.6 mg/kg); III: AuBPs (5.2 mg/kg); IV: AuBPs (5.2 mg/kg) and laser (0.5 W/cm^2, 10 min); V: AuBPs@TD (5.2 mg/kg); VI: AuBPs@TD (5.2 mg/kg) and laser (0.5 W/cm^2, 10 min). The size and body weight of mice were recorded every two days. The major organs and tumor sites were collected for H&E staining.

2.14. Statistical analysis

Data were expressed as mean ± SD. *Student's* two-tailed *t* test was used to assess the difference between groups. Statistical significance was denoted as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

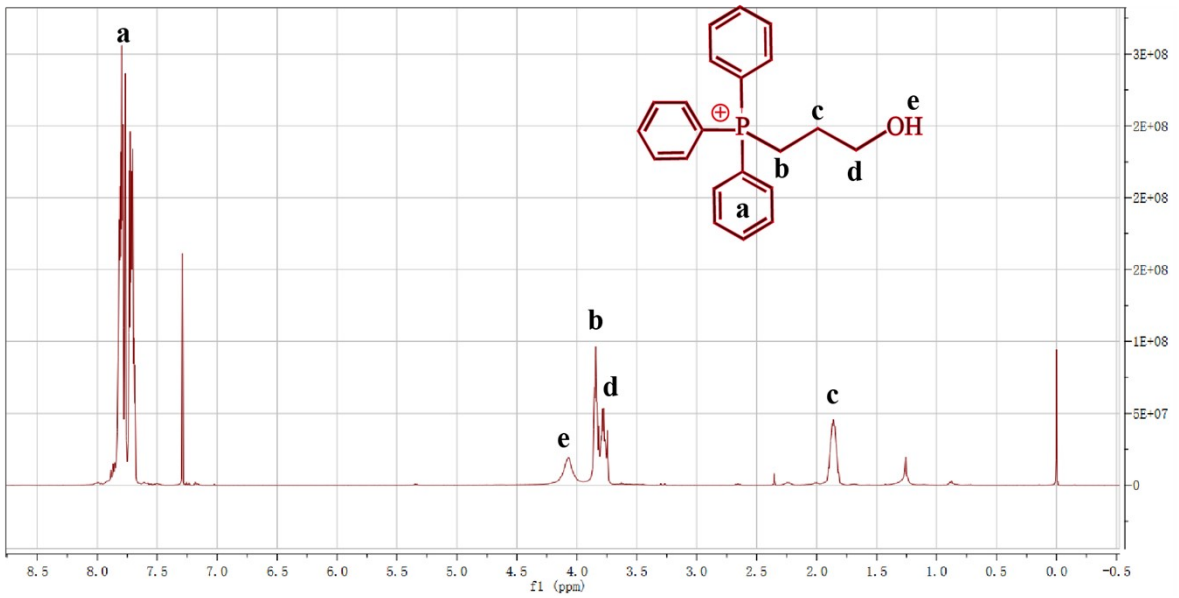

Figure S1. ^{1}H NMR spectrum of TPP derivative.

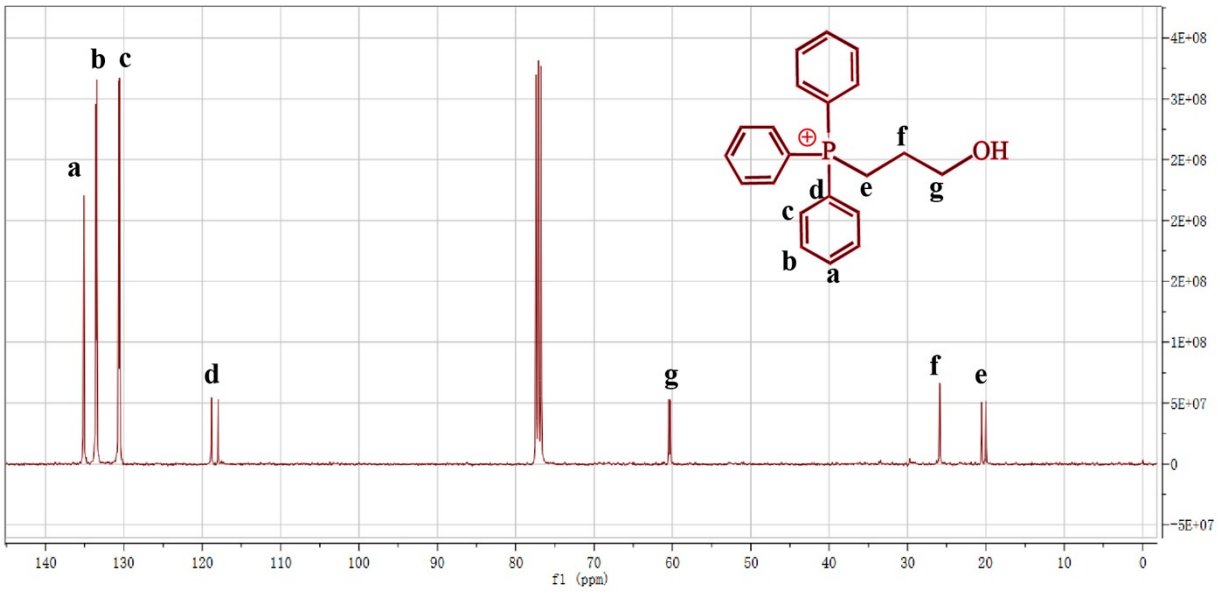

Figure S2. ^{13}C NMR spectrum of TPP derivative.

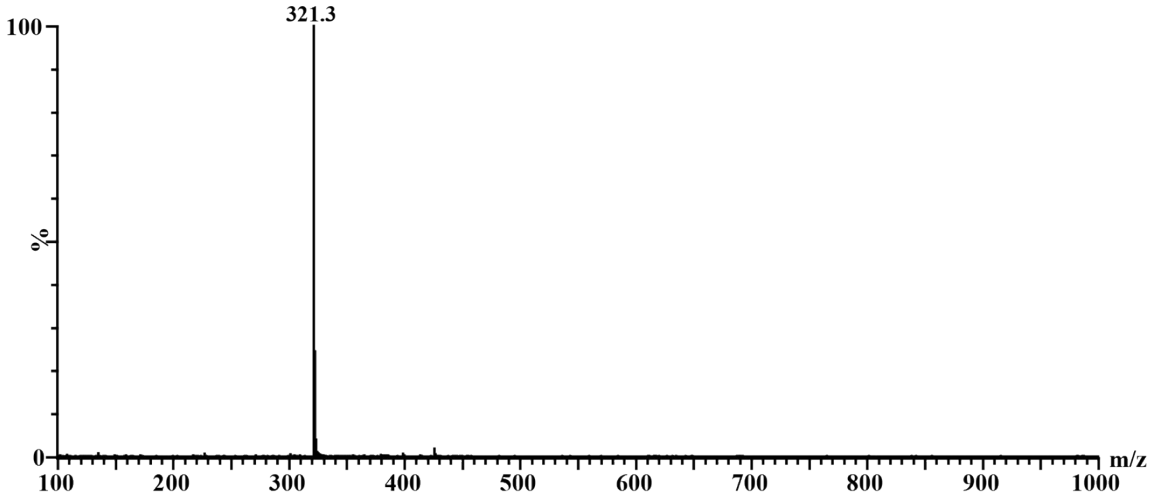

Figure S3. MALDI-TOF-MASS spectrum of TDD derivative.

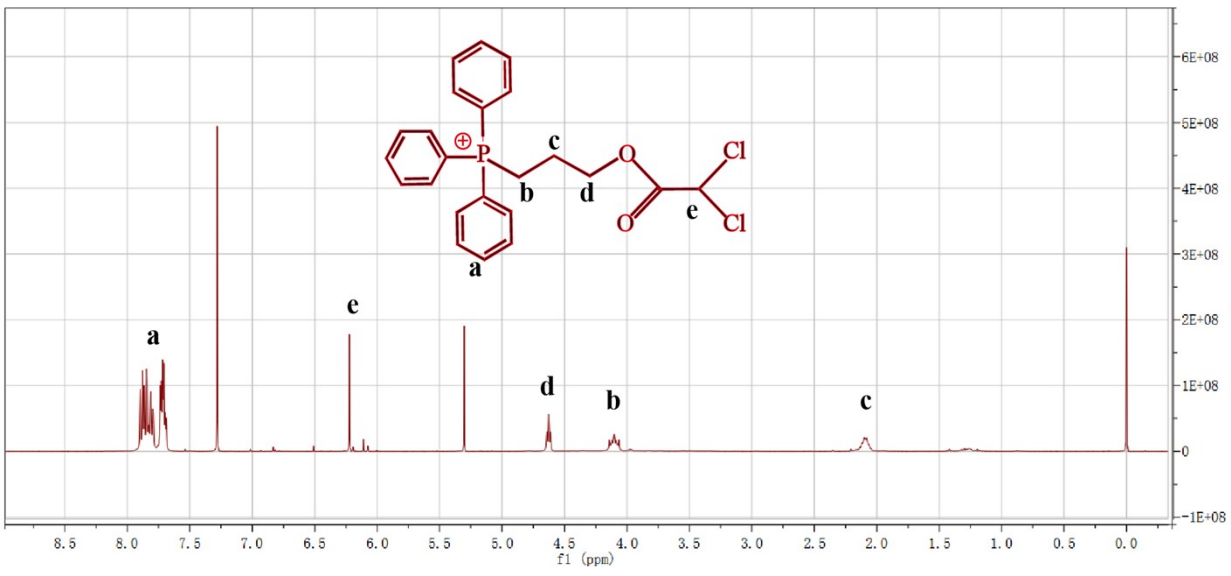

Figure S4. ^{1}H NMR spectrum of TPP-DCA.

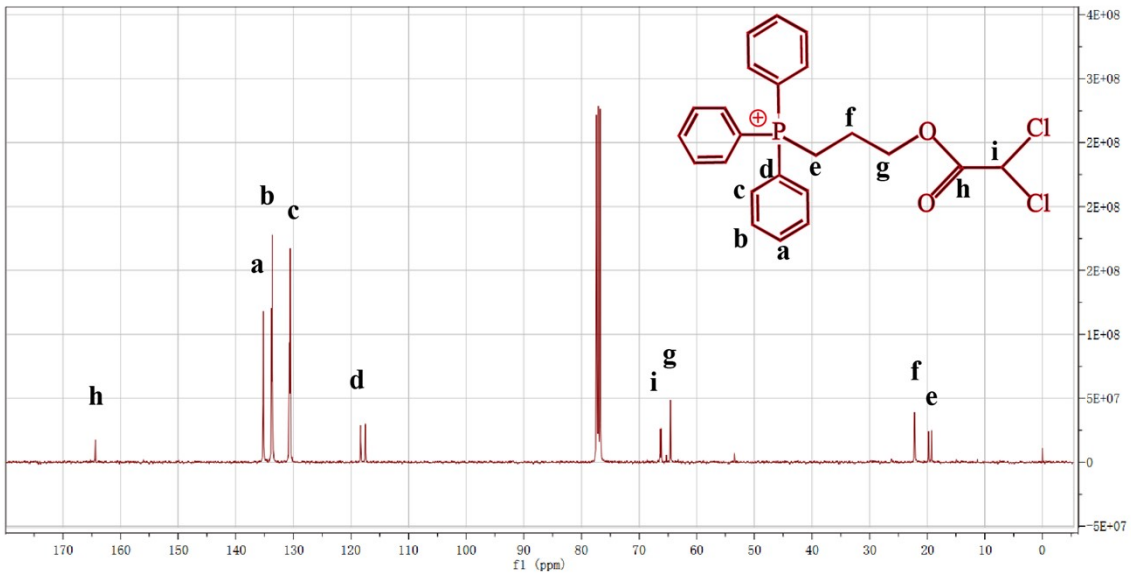

Figure S5. ^{13}C NMR spectrum of TPP-DCA.

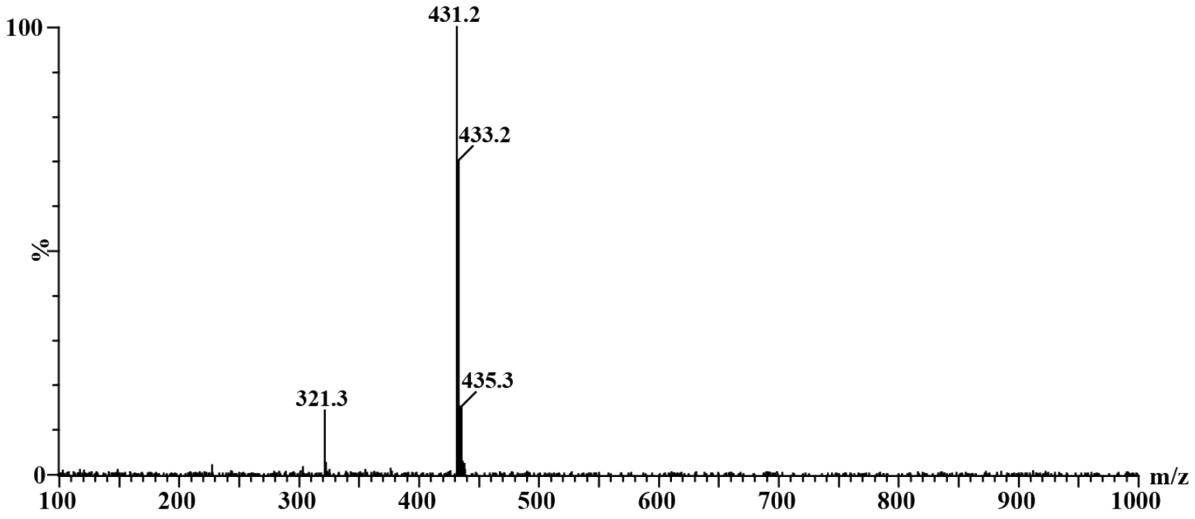

Figure S6. MALDI-TOF-MASS spectrum of TDD-DCA.

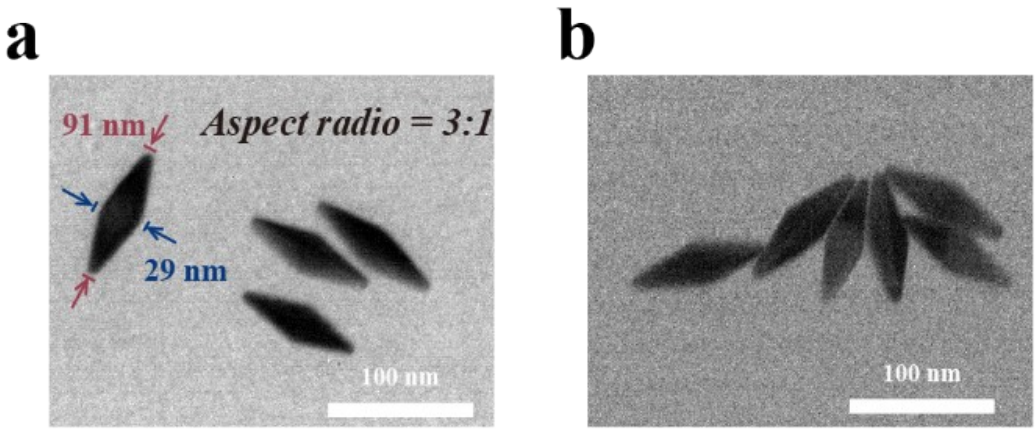

Figure S7. The TEM images of AuBPs-Br and AuBPs.

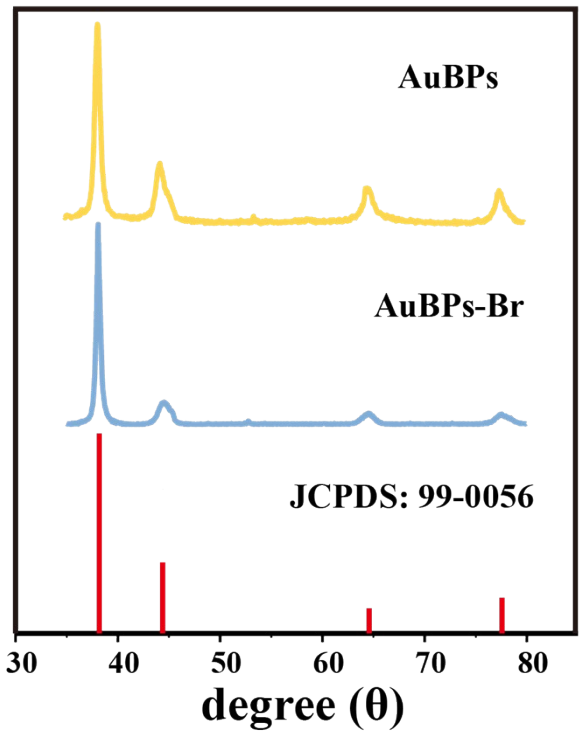

Figure S8. The XRD pattern of AuBPs-Br and AuBPs.

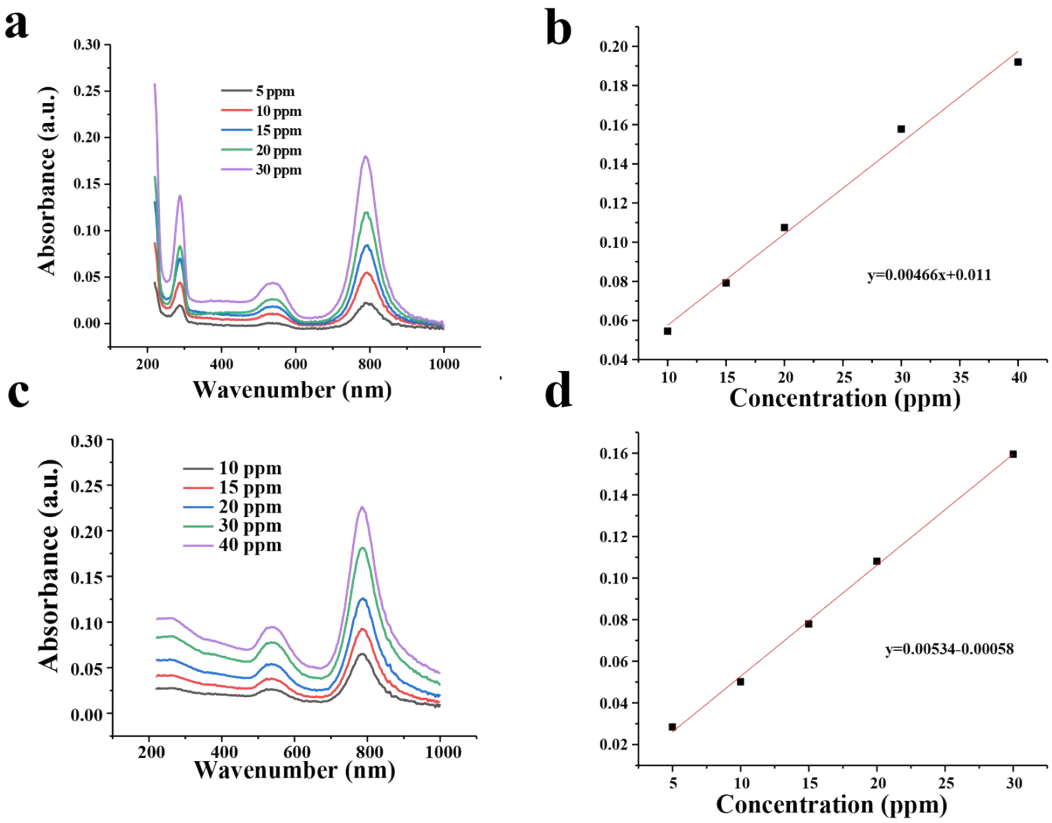

Figure S9. UV-vis absorption spectral of (a) AuBPs-Br, and (c) AuBPs. The standard curve of the Absorbance-concentration at 808 nm of (b) AuBPs-Br and (d) AuBPs.

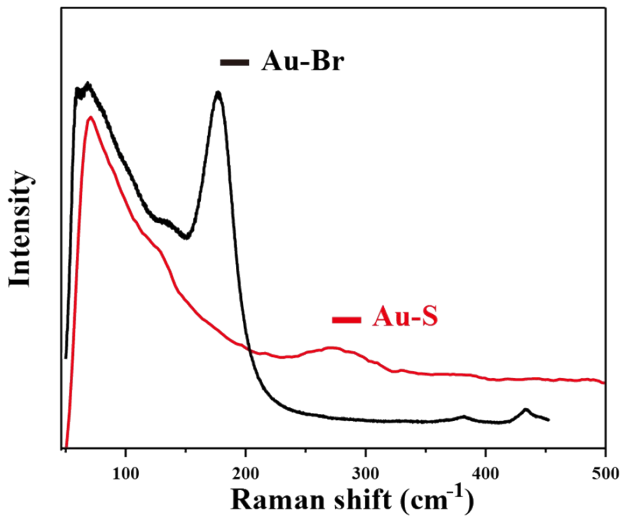

Figure S10. Raman spectra of gold bipyramids before (black) and after (red) mPEG-SH coated.

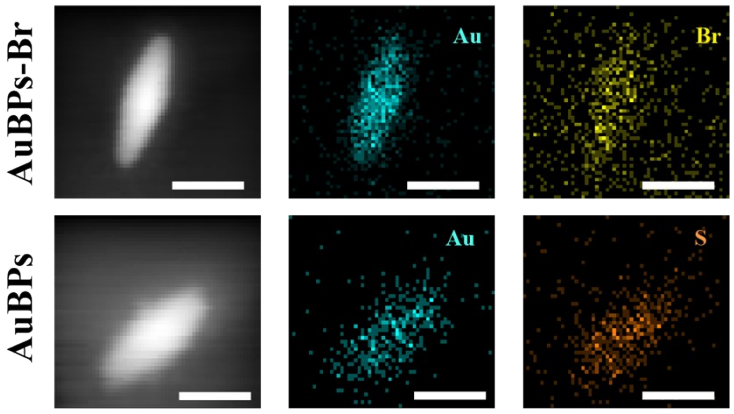

Figure S11. EDS mapping of AuBPs-Br. Scale bar: 50 μm

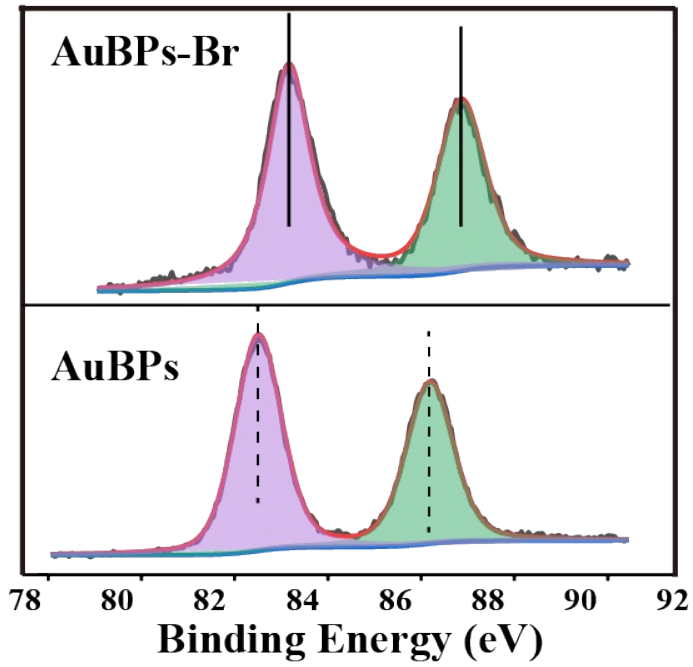

Figure S12. XPS spectra of AuBPs-Br (top) and AuBPs (below)

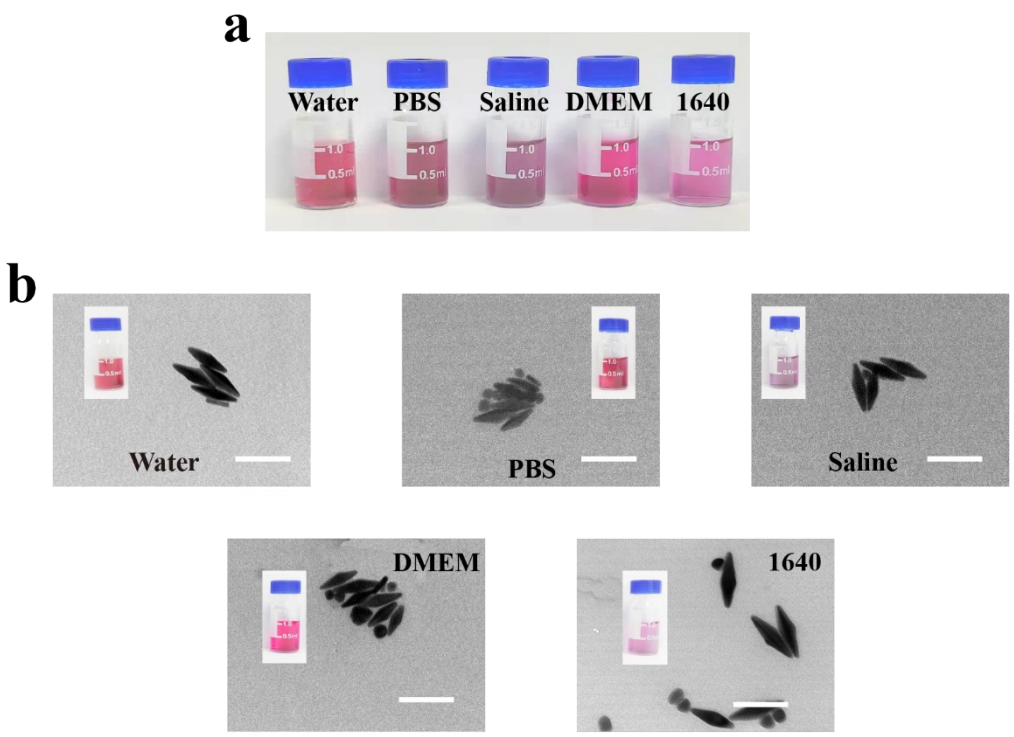

Figure S13. The long-term stability of AuBPs at room temperature in different solutions. (a) The photograph on the first day. (b) The TEM of AuBPs in different solutions on the 7th day. Insert: corresponding photograph. Scale bar: 100 nm.

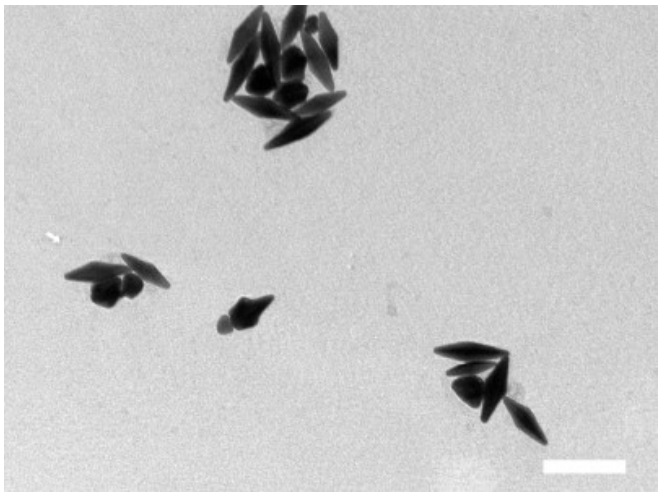

Figure S14. The TEM of AuBPs in water on the 14th day. Scale bar: 100 nm

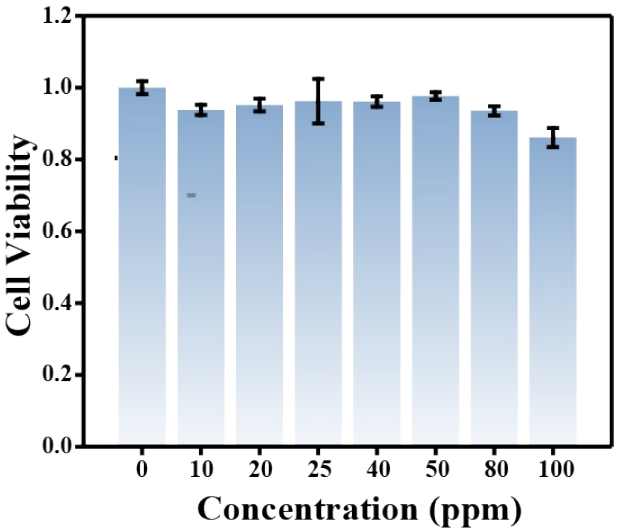

Figure S15. Cell viability after treated with various concentrations AuBPs.

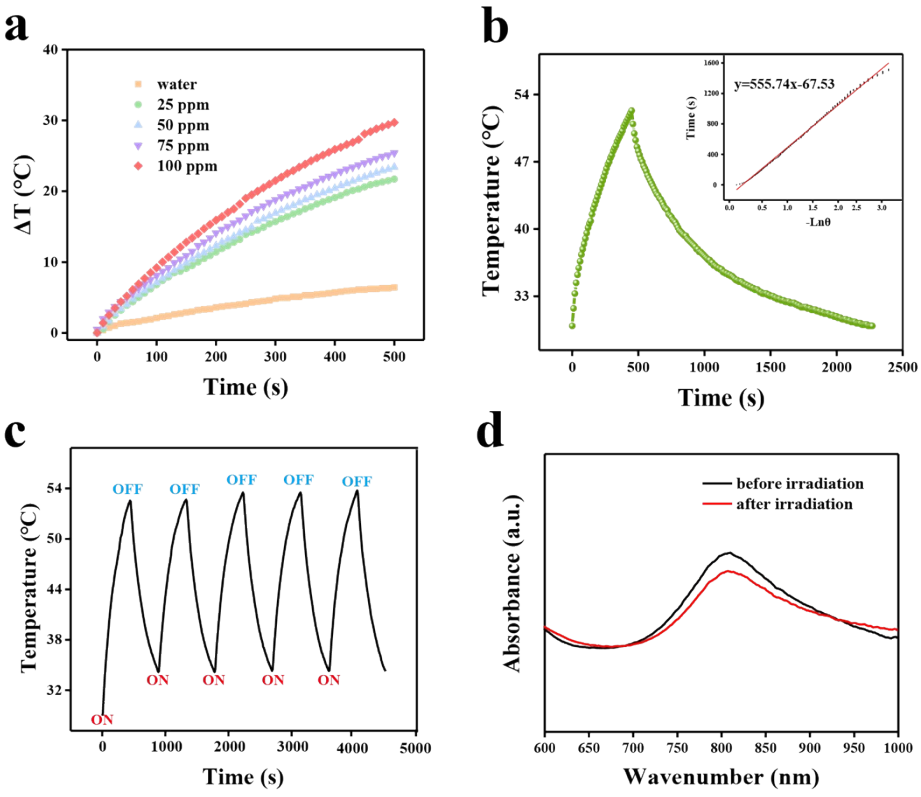

Figure S16. The photothermal capacity of AuBPs. (a) Temperature changes of AuBPs at different concentrations. (b) Heating and cooling down curves of AuBPs. Insert: the calculated photothermal conversion coefficient. (c) Photostability curve for five cycles. (d) UV-vis absorption of AuBPs before (black) and after (red) laser irradiated.

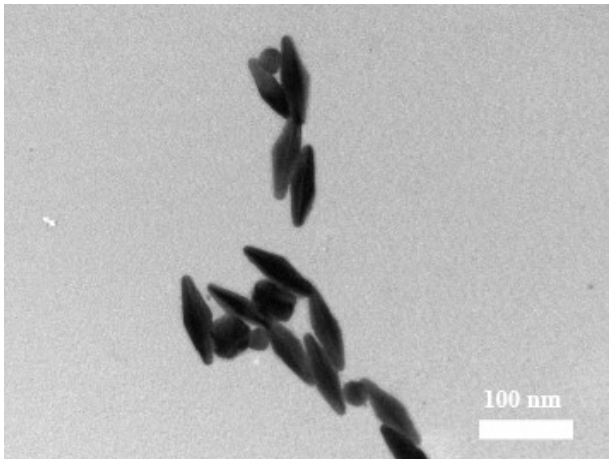

Figure S17. TEM images of. AuBPs@TD after irradiated by laser.

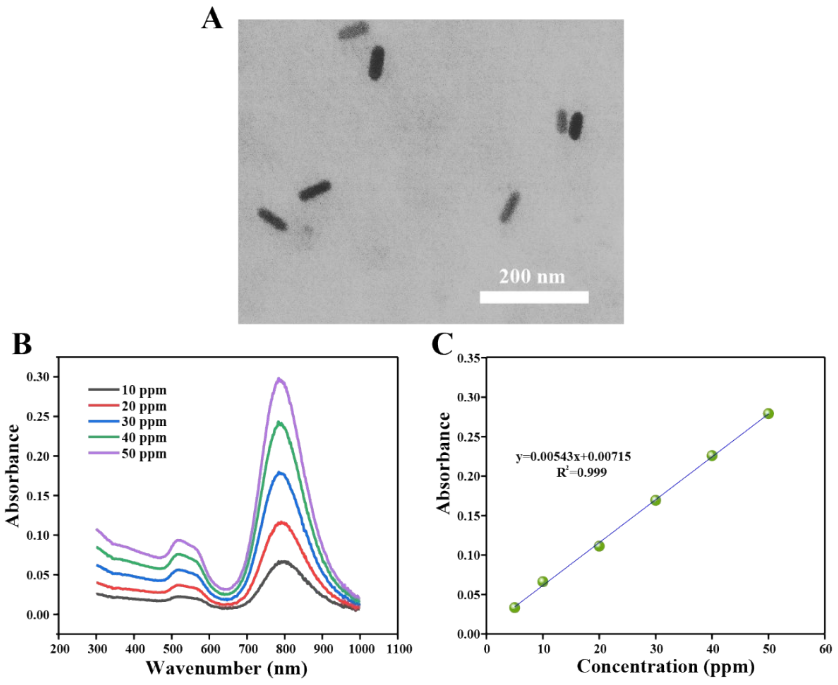

Figure 18. (A) The morphology of AuNRs. (B) The UV-vis absorption spectral of AuNRs. (C) The standard curve of the Absorbance-Concentration at 808 nm of AuNRs.

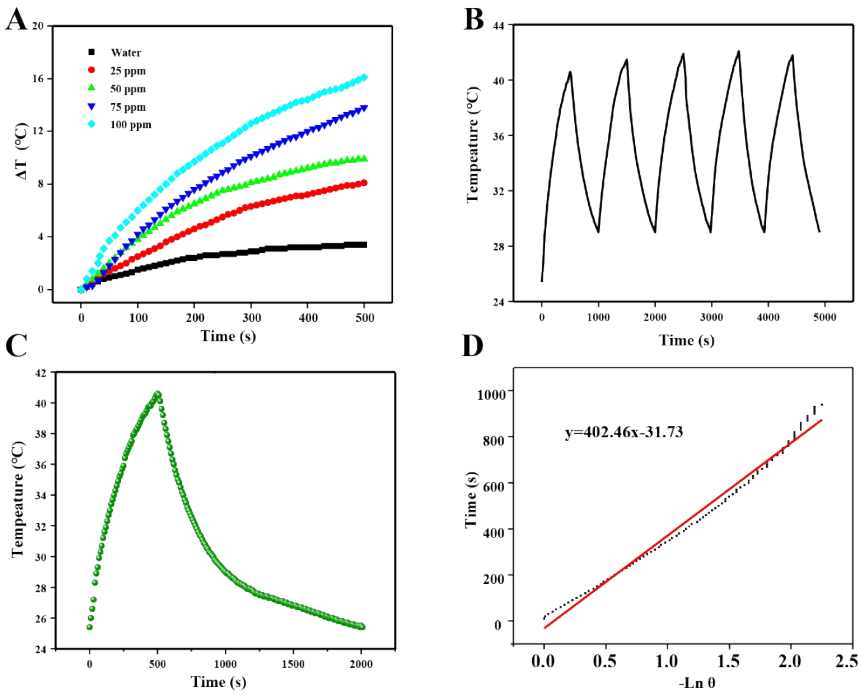

Figure 19. (A) Temperature changes of AuNRs at different concentrations. (B) Photostability curve for five cycles. (C) Heating and cooling down curves of AuNRs. (D) Time-Lnθ curves to calculate photothermal conversion efficiency.

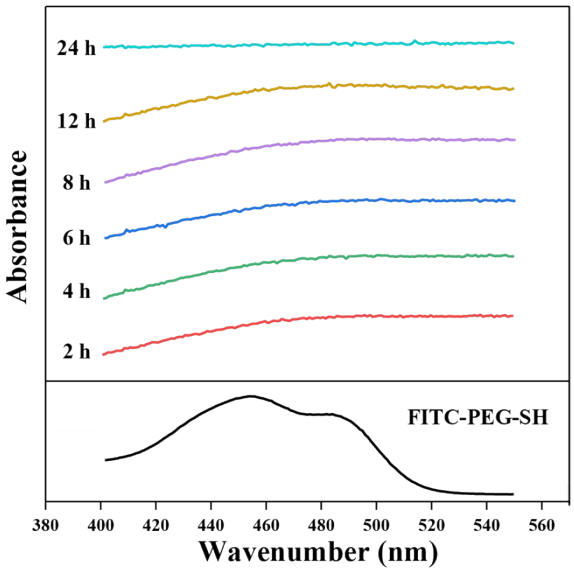

Figure S20. The absorbance of water outside of the dialysis bag.

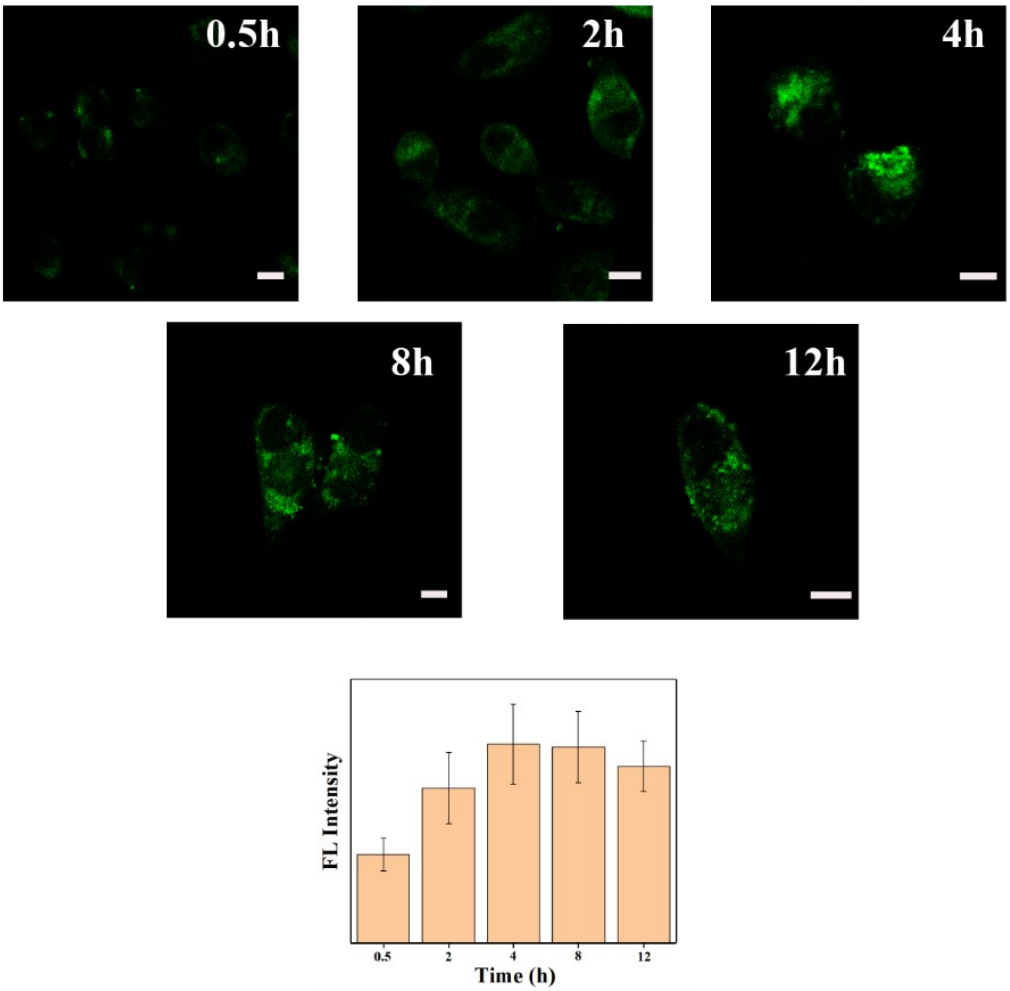

Figure S21. CLSM images of HeLa cells co-incubated with FITC labeled AuBPs@TD at different times, and the quantitative intracellular fluorescence intensity.

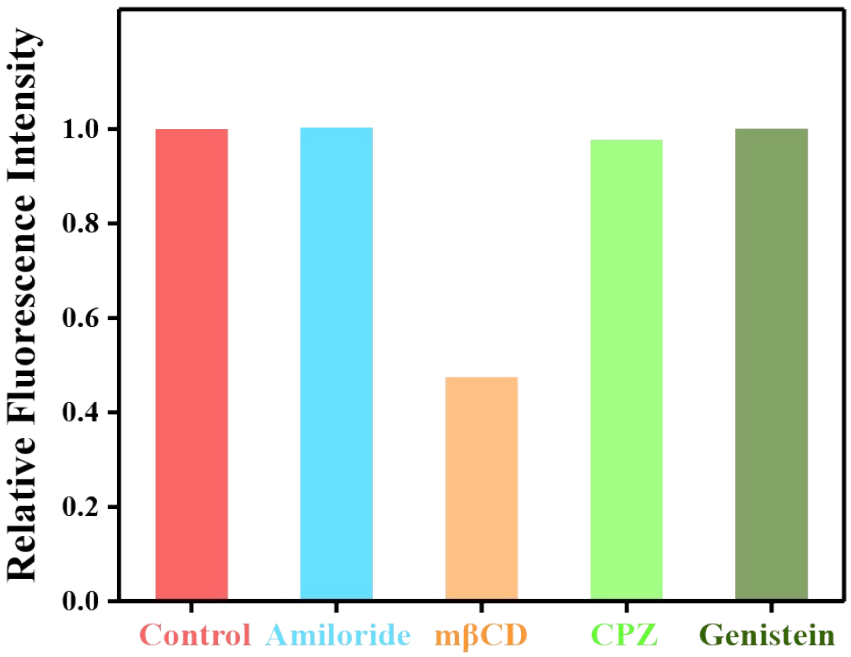

Figure S22. The quantitative fluorescence intensity in cells after treated with different endocytosis inhibitors.

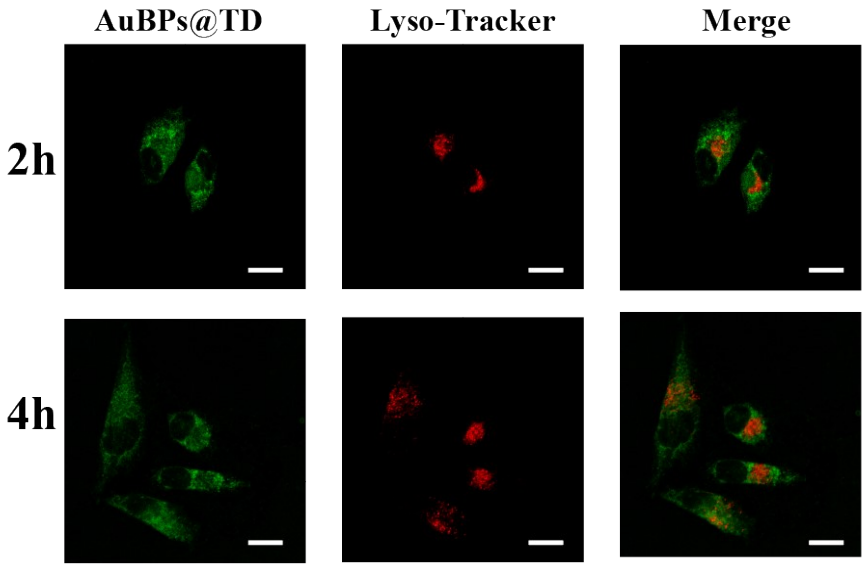

Figure S23. Co-localization experiment of FITC labeled AuBPs@TD (green) and lysosome tracker (red). Scale bar: 10 μm.

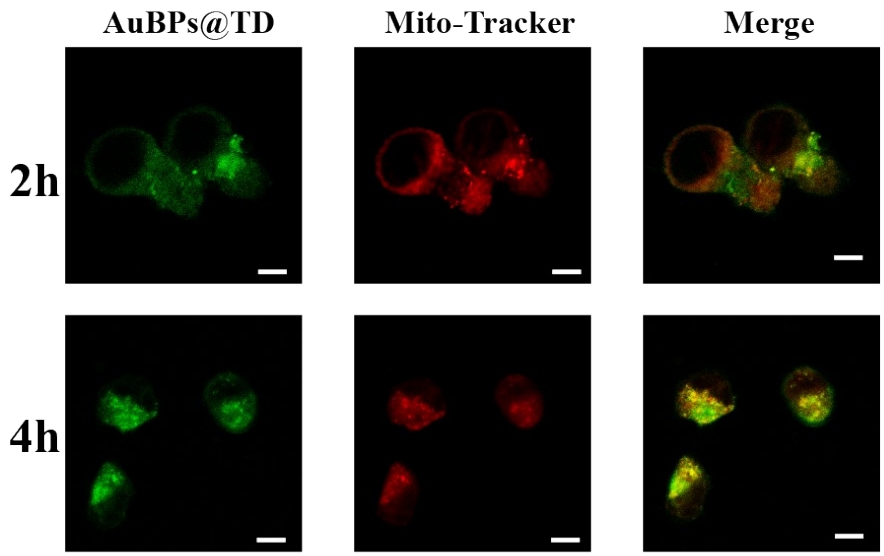

Figure S24. Co-localization experiment of FITC labeled AuBPs@TD (green) and mitochondria tracker (red). Scale bar: 10 μm

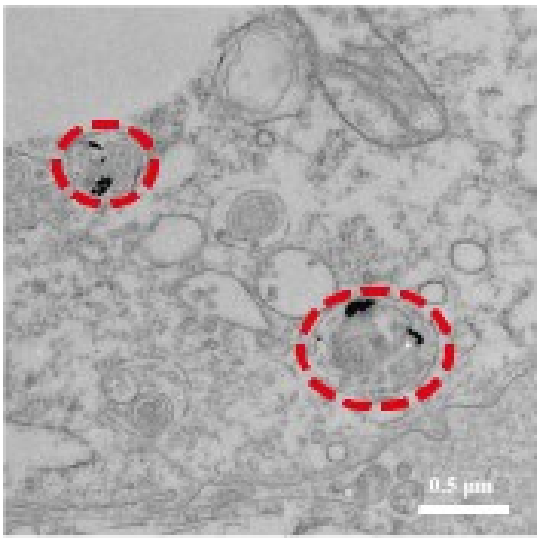

Figure S25. AuBPs@TD existed in endosomes at the early time. Red circle: endosomes.

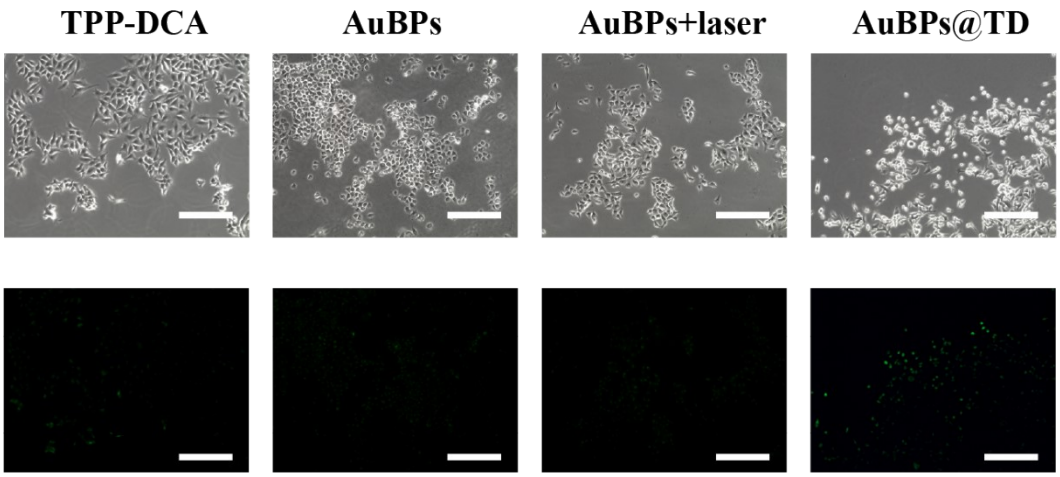

Figure S26. Intracellular pH monitored by BCECF-AM. Top line: bright filed. Below line: fluorescence filed. Scale bar: 250 μm.

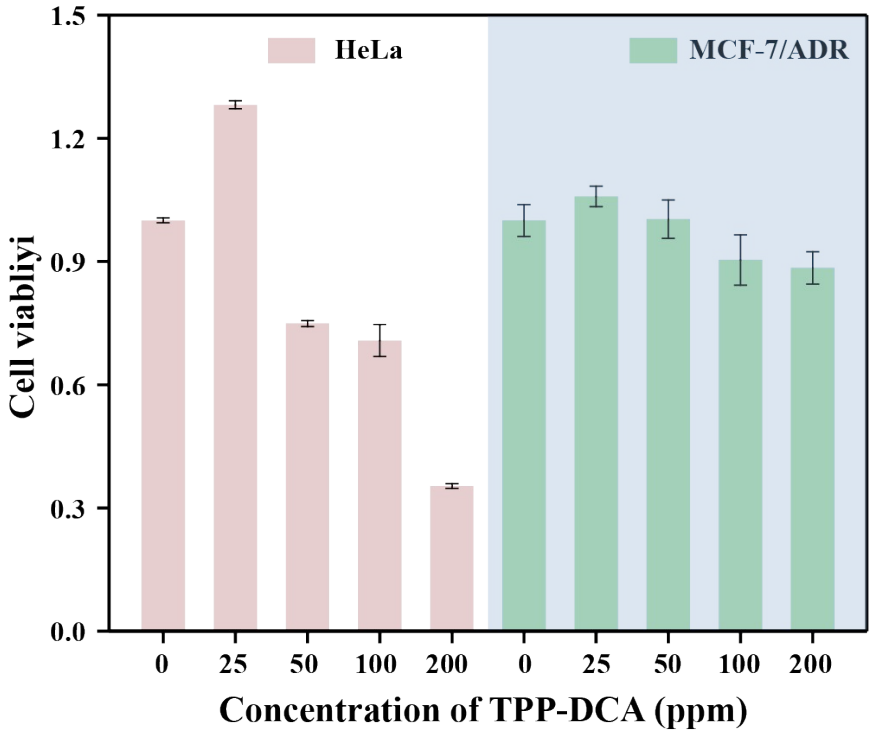

Figure S27. The cytotoxicity of TPP-DCA on different kinds of tumor cells.

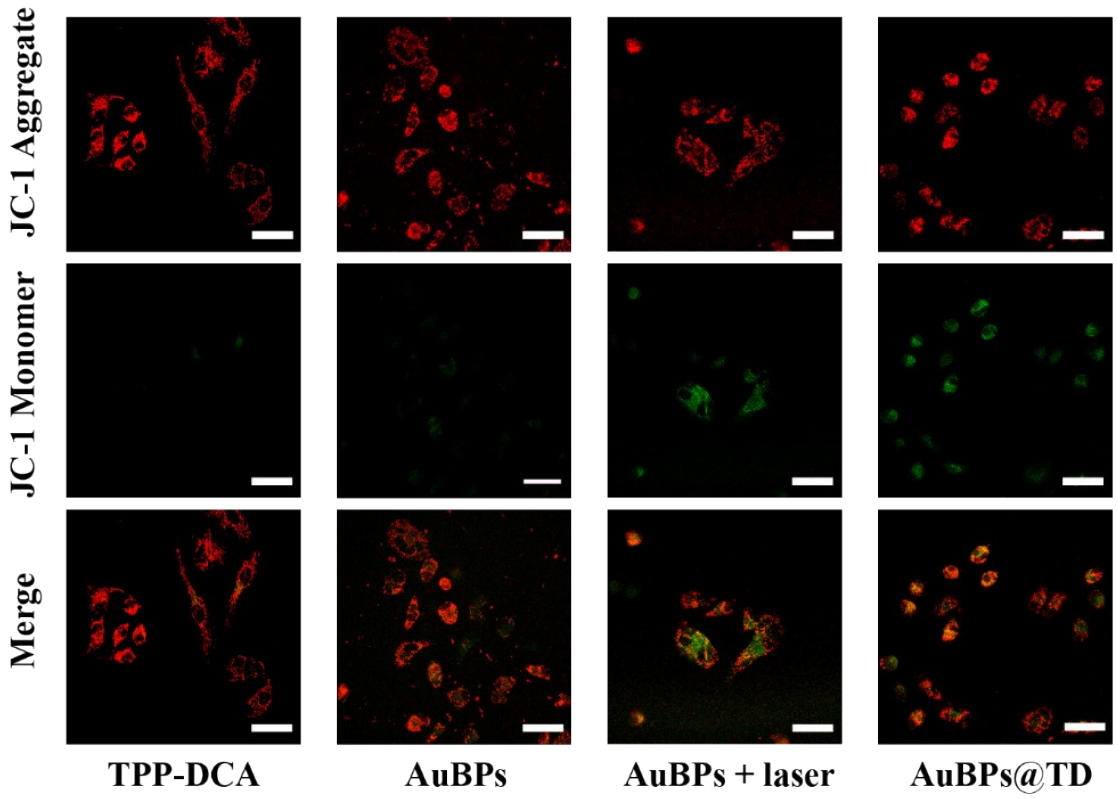

Figure S28. Confocal images of JC-1 stained HeLa cells after different treatments. Scale bar: 30 μm.

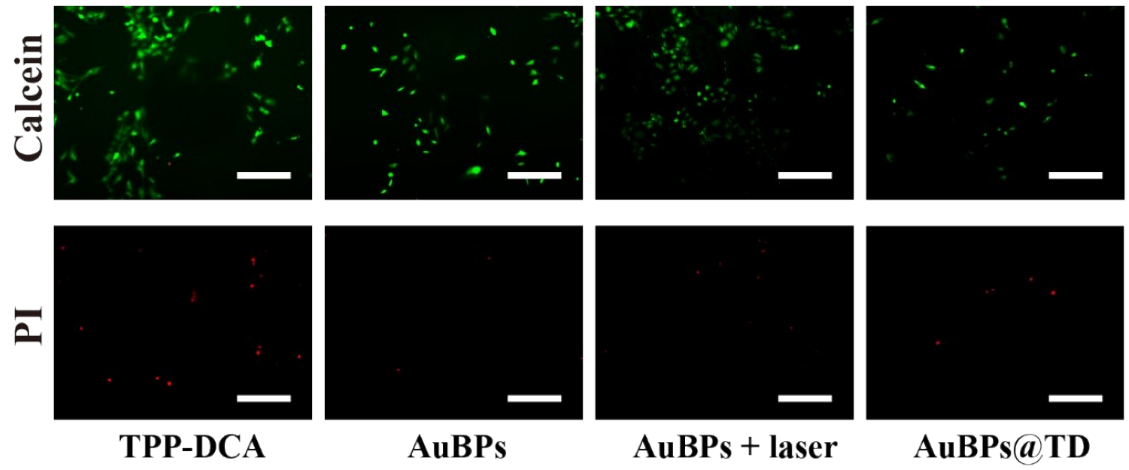

Figure S29. Fluorescence images of Calcein-AM and PI labeled HeLa cells after different treatments.

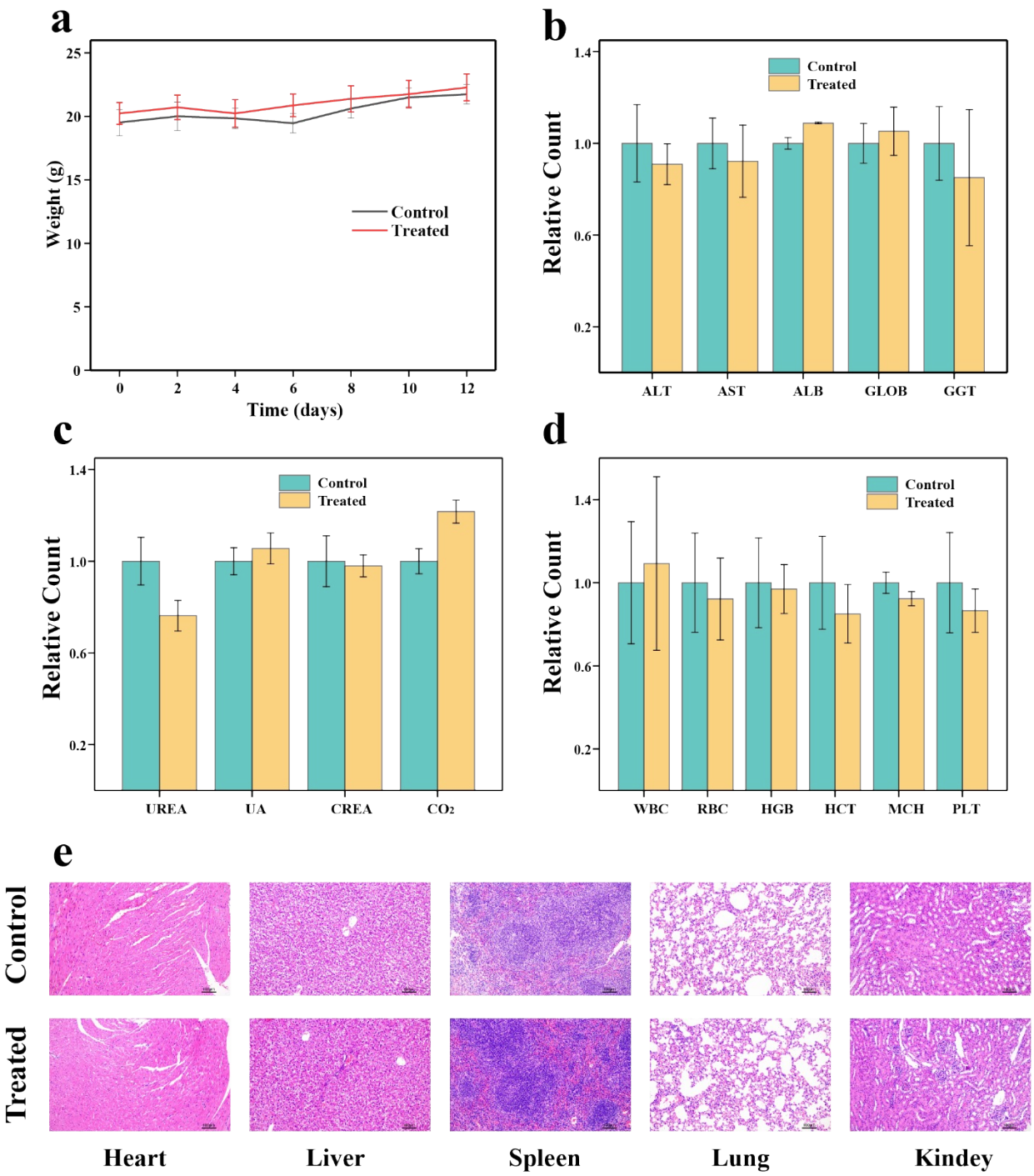

Figure S30. The biocompatibility of AuBPs@TD in vivo after intravenous injection. (a) The changes of body weight. (b) Liver functions analysis. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALB: albumin; GLOB: glob-ulin; GGT: γ-glutamyltransferase. (c) Renal functions analysis. UA: uric acid; CREA: creatinine. (d) Hematology analysis. WBC: white blood cell; RBC: red blood cell; HGB: hemoglobin; HCT: red blood cell specific volume; MCH: mean corpuscular hemoglobin; PLT: platelet. (e) The H&E staining of major organs after intravenous injection.

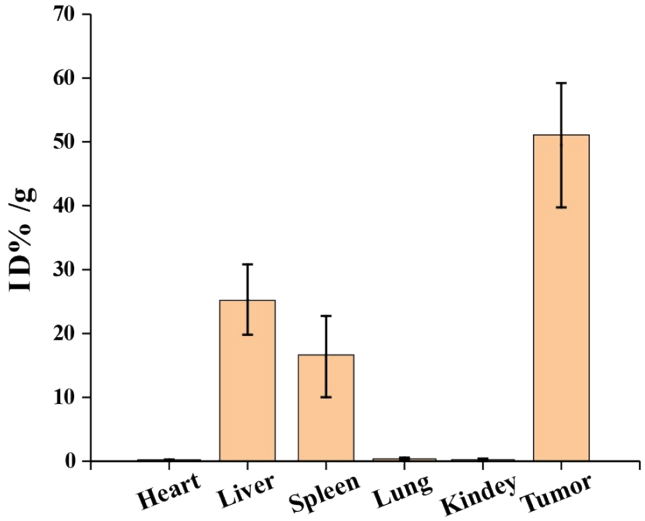

Figure S31. The residues of Au in different organs on the 14th day.

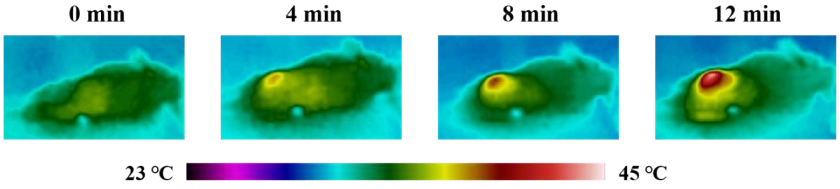

Figure S32. Thermal images of 4T-1 tumor-bearing mice after intratumoral injected with AuBPs and irradiated with laser.

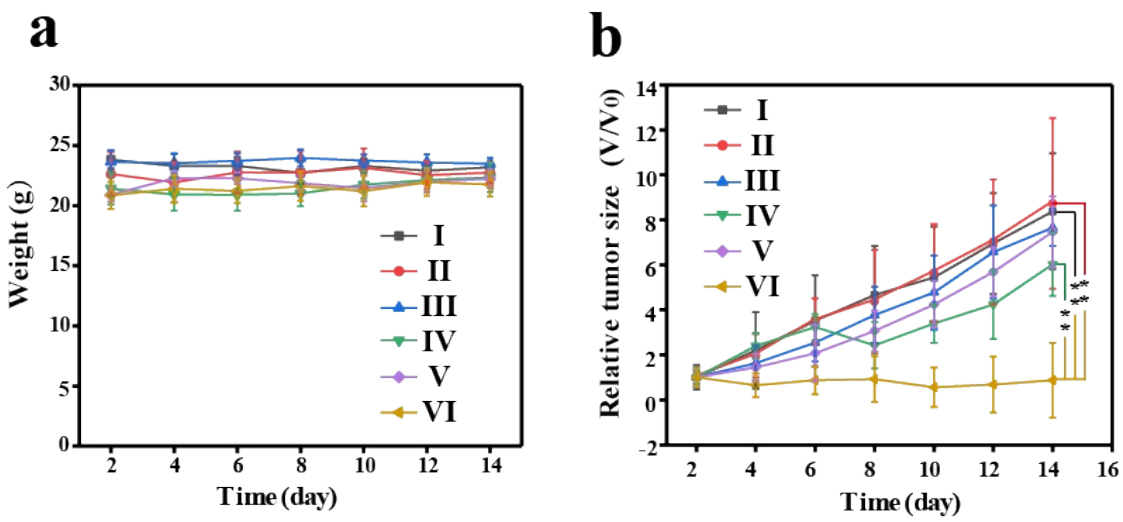

Figure S33. (a) The recorded weights of different treated mice during the therapeutic period. (b) The recorded tumor size of tumor-bearing mice.

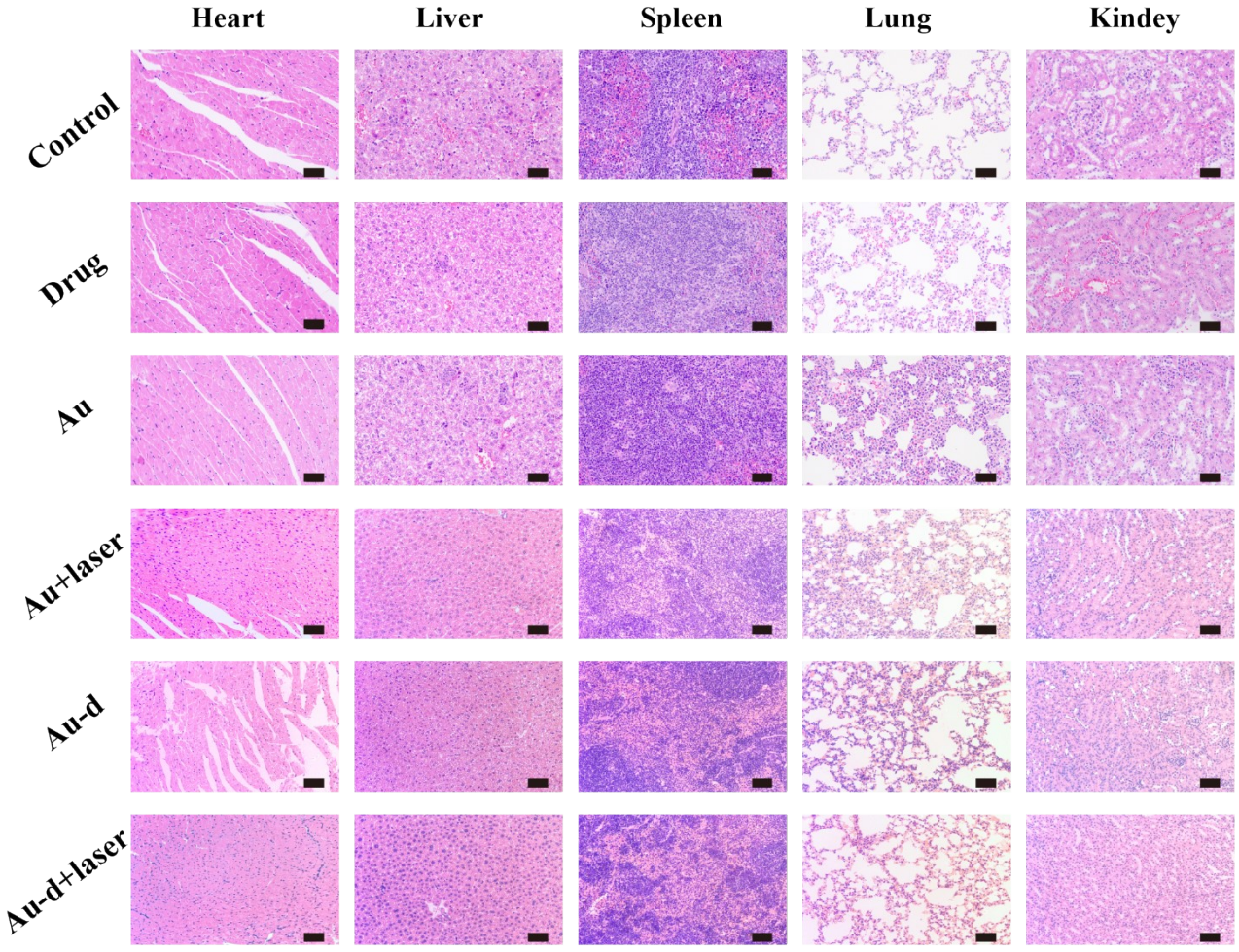

Figure S34. The H&E staining of major organs after different treatments. Scale bar: 50 μm.