Supporting Information

Cu-based Nanoplatform for Near-infrared Light Amplified Multi-mode Prostate Cancer Specific Therapy

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Experiment section

Materials

Copper nitrate trihydrate (Cu $(NO_3)_2$•$3H_2O$) and Poly (acrylic acid) (PAA) (Sigma-Aldrich (MO, USA)). 2-Methylinmidazole (Macklin (China)). Zinc nitrate hexahydrate (Zn $(NO_3)_2$•$6H_2O$) (Fuchen Chemical Reagent (China)). 2, 2′-azobis [2-(2-imidazolin-2-yl) propane] dihydrochloride (AIPH) (Shanghai AladdinReagent (China)). Other chemicals (Beijing Chemical Reagent (China)).

Synthesis of Cu-MNCS Nanoparticles

2-Methylinmidazole (1620 mg), Cu $(NO_3)_2$•$3H_2O$ (233.2 mg) and Zn $(NO_3)_2$•$6H_2O$ (500 mg) were added to methanol (100 mL) at room temperature (RT). The mixture was stirred for 30 min. The product (Cu-ZIF-8) was centrifuged and rinsed with methanol three times. Cu-ZIF-8 was dried under vacuum for 12 h at 40 °C. Finally, Cu-ZIF -8 was pyrolyzed at 800 °C for 2 h in a tube furnace under flowing Ar gas at a heating rate of 2 °C/min and cooled down to RT naturally.

Loading of AIPH and Modification of Nanoparticles

The prepared Cu doped mesoporous carbon nanosphere (Cu-MNCS) (20 mg) was added to an aqueous solution (4 mL) containing AIPH (100 mg). The mixture was stirred away from light for 6h at RT. PAA (20 mg) was added to the mixture and was continued to stir for 6 h. The product was isolated by centrifugation and rinsed with water two times to remove any excess AIPH and PAA.

Characterization

The morphology of nanoparticles was recorded on a FEI Tecnai G2 F20 transmission electron microscope. The structure performance of nanoparticles was characterized through D8 Advance Powder X-ray Diffractometer. The absorbance of the samples was gauged by Ultraviolet-visible spectroscopy (UV-Vis). The porosity of nanoparticles was acquired using accelerated Surface Area and Porosimetry System.

Detection of hydroxyl radical

The hydroxyl radical generation ability of nanoparticles was measured by fluorometry. Briefly, Cu-MNCS@PAA were added to the terephthalic acid solution (different pH= 4.0, 5.0, or 6.5) with H_2O_2. The luminescence spectrum of the mixture was detected by fluorescence spectrophotometer and its strength was related to the hydroxyl radical level. In order to study the enhancement effect of photothermal therapy (PTT) on Fenton-like reaction, the mixture of Cu-MNCS @PAA and terephthalic acid solution (pH= 6.5) with H_2O_2 was stored in the dark at different temperatures (RT, 37°C, 47°C, or 55 °C) or treated by different irradiation time with 808 nm laser. The luminescence spectrum of the mixture was detected by fluorescence spectrophotometer.

Photothermal performance

The temperature of nanoparticles solution with different concentrations (0, 10, 20, 40

and 80 μg/mL) was gauged by a thermocouple probe each minute for 10 min under the irradiation of an 808 nm laser (0.7 W/cm^2). Thereafter, the solution was naturally cooled down. The above experiments were repeated five times. As for the thermal stability, Cu-MNCS@PAA (40 μg/mL) was irradiated with 808 nm laser (0.7 W/cm^2) in a quartz cuvette for 10 min. Then, the absorbance of the solution before and after laser irradiation was gauged by UV–vis spectrometer.

Detection of $ABTS^{+\bullet}$ free radical

The mixed solution of 2, 2 '-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (1 mg/mL, 1 mL) and Cu-MNCS-AIPH@PAA (1 mg/mL, 1 mL) was stored in the dark at 37 °C or 45 °C for various time (2, 4, and 6 h), then isolated at 12000 rpm for 5 min to get the supernatant. At last the absorbance of supernatant between 400 and 900 nm was gauged by UV–vis spectrometer.

Cytotoxicity assay and antitumor effect of Cu-MNCS-AIPH@PAA in vitro

Mouse prostate cancer RM-1 cells were seeded into 96-well plate, then incubated with nanoparticles (0, 5, 10, 20, 60, 80, and 100 μg/mL) for another 24 h. The Cell Counting Kit-8 (CCK-8) solution (10%, 100 μL) was added into the plates and maintained for 1 h. Finally, the absorbance (450 nm) was recorded to measure the cell viability by plate reader. In order to explore the therapeutic effect in vitro, RM-1 cells were incubated with the different concentrations of nanoparticles for 24 h and some of them were added with H_2O_2 (100 μM) and acid medium (pH = 6.5). Then, the cells in the 96-well plates were irradiated with 808 nm laser (0.7 W/cm^2) for 5 min and the activity of RM-1 cells was determined through CCK-8 assay. Live/Dead fluorescence staining was used to further evaluate the antitumor effect in vitro. RM-1 cells were exposed to Cu-MNCS@PAA or Cu-MNCS-AIPH@PAA (40 μg/mL) for 24 h and some of them were added with H_2O_2 (100 μM) and acid medium (pH = 6.5). Then, some were irradiated with 808 nm laser for 5 min. At last the cells were processed using a Live/Dead Viability/Cytotoxicity Kit, then observed by fluorescence microscope.

Hemolysis assay

Firstly, whole blood which was harvested from C57BL/6 mice was isolated by centrifugation (3000 rpm; 5min) to get red blood cells (RBCs). RBCs were washed with PBS three times and mixed with PBS containing different concentrations of nanoparticles. The mixed solution was maintained at 37°C for 8 h and isolated by centrifugation (12000 rpm; 5 min) to get the supernatant. The absorbance (570 nm) of supernatant was gauged by UV-Vis spectrometer. The hemolysis rate was reached according to the formula (The hemolysis rate = $(I_X\text{-}I_{0\%}) / (I_{100\%}\text{-}I_{0\%}) \times 100\%$). I_X, $I_{0\%}$, and $I_{100\%}$ represent the absorption intensity of the nanoparticles groups, DI water and PBS group, respectively.

In vitro ROS generation

2', 7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) as a fluorescent probe was employed to evaluate the ROS generation in vitro. In short, RM-1 cells were exposed

to Cu-MNCS@PAA or Cu-MNCS-AIPH@PAA (30 μg/mL) for 24 h. Then, some groups were irradiated with 808 nm laser for 5 min and all groups were stained with 10 μM of DCFH-DA for 30 min. Last, the fluorescence images were recorded via fluorescence microscope.

Blood routine examination and blood biochemistry examination

C57BL/6 mice were split into two groups, then injected with Cu-MNCS-AIPH@PAA and normal saline respectively. The whole blood was taken from C57BL/6 mice on 30 days post-injection, then subjected to laboratory examination.

Tumor inhibition in vivo

Twenty-five male C57BL/6 mice RM-1 tumor models were randomly and averagely assigned into five groups: (I) control group, (II) 808 nm laser, (III) Cu-MNCS-AIPH@PAA, (VI) Cu-MNCS@PAA+808 nm laser, and (V) Cu-MNCS-AIPH@PAA+808 nm laser. Nanoparticles saline solution or normal saline was injected into the mice. After 24 h of the injection, the tumor-bearing mice in groups (II), (VI) and (V) were exposed to 808 nm laser for 10 min. Furthermore, the tumor volumes (volume= tumor width × tumor width × tumor length / 2) and the body weight of the mice were measured every day. At last the mice were sacrificed to estimate the therapeutic efficacy on the 8th day. All operations aligned with the principles of the regulation of Jilin University for the care and use of laboratory animals.

Statistical analysis

Differences among test groups were analyzed by SPSS software (version 17.0; SPSS Institute, Chicago, IL). The statistical significance different was indicated as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

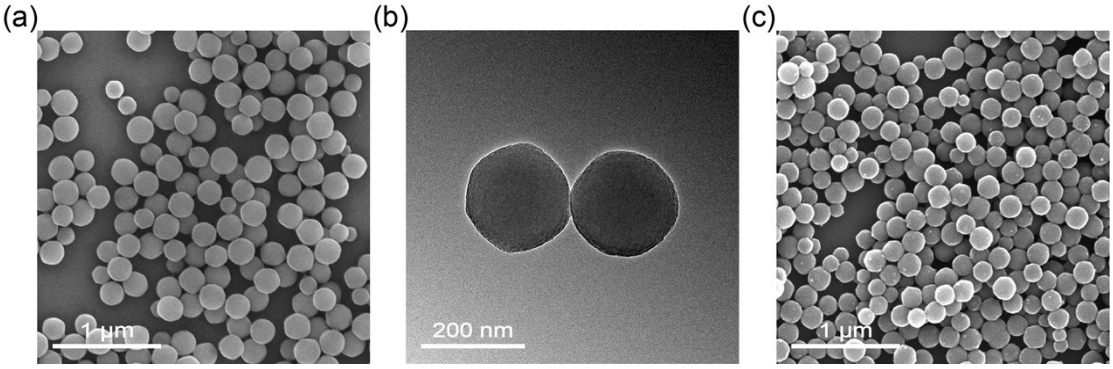

Figure S1. SEM (a) and TEM (b) images of Cu-ZIF-8. (c)SEM image of Cu-MNCS.

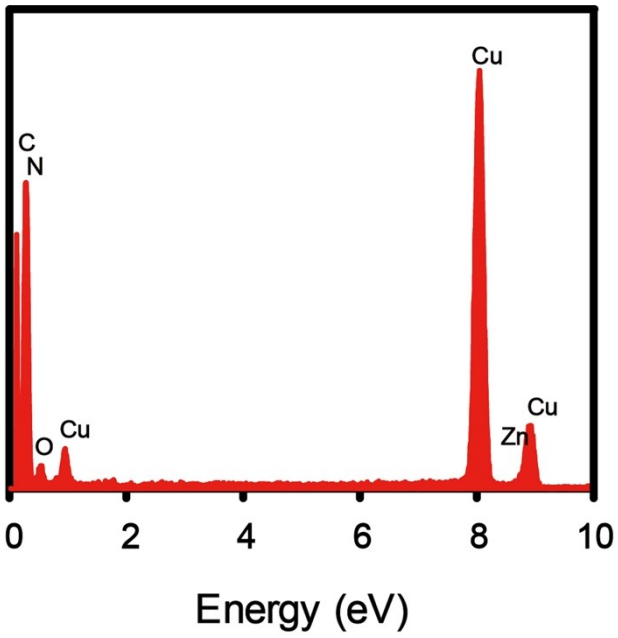

Figure S2. Energy-dispersive spectrum of Cu-MNCS.

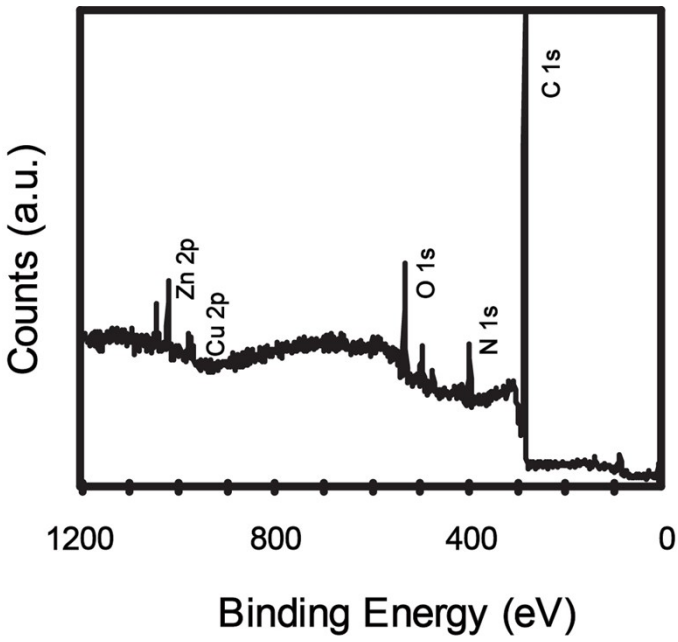

Figure S3. XPS full scan spectrum of Cu-MNCS.

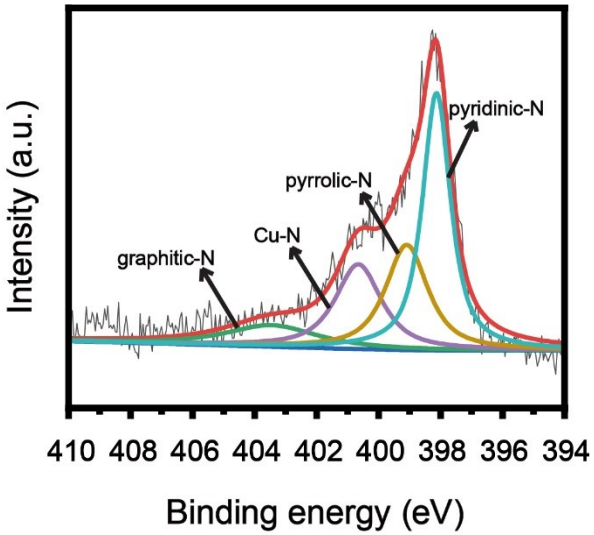

Figure S4. High-resolution XPS spectra of N 1s.

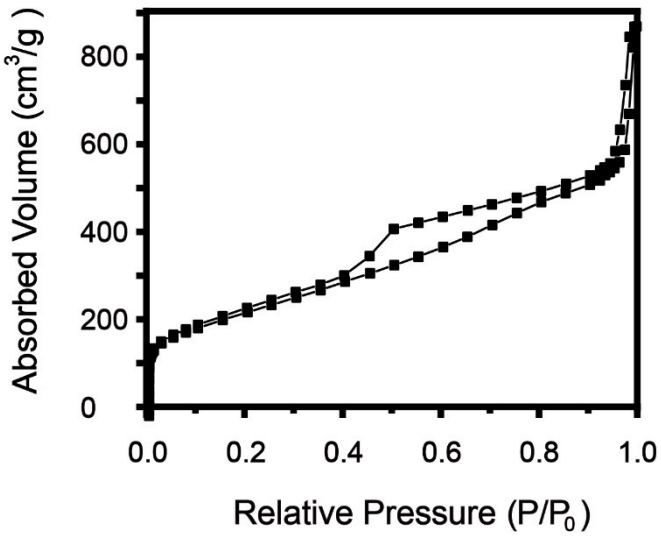

Figure S5. N_2 sorption isotherms and pore size distributions of Cu-MNCS.

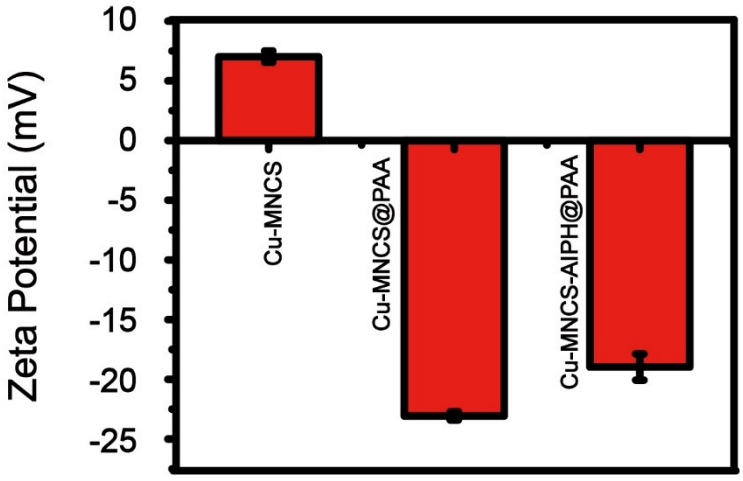

Figure S6. Zeta-potentials of as-synthesized samples at different stages.

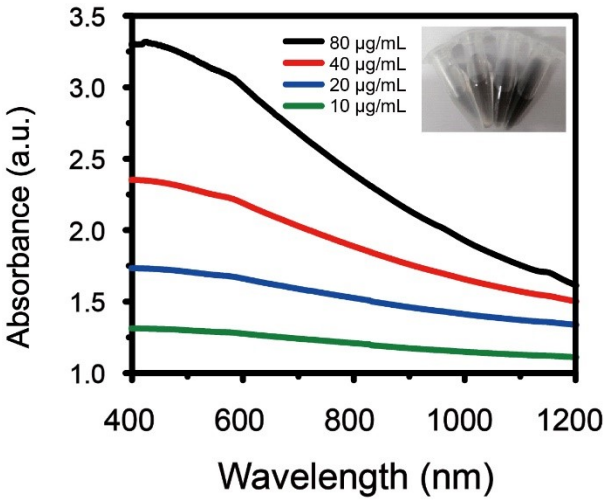

Figure S7. The UV-vis absorption spectra and photoes of Cu-MNCS@PAA with increased concentrations (from left to right).

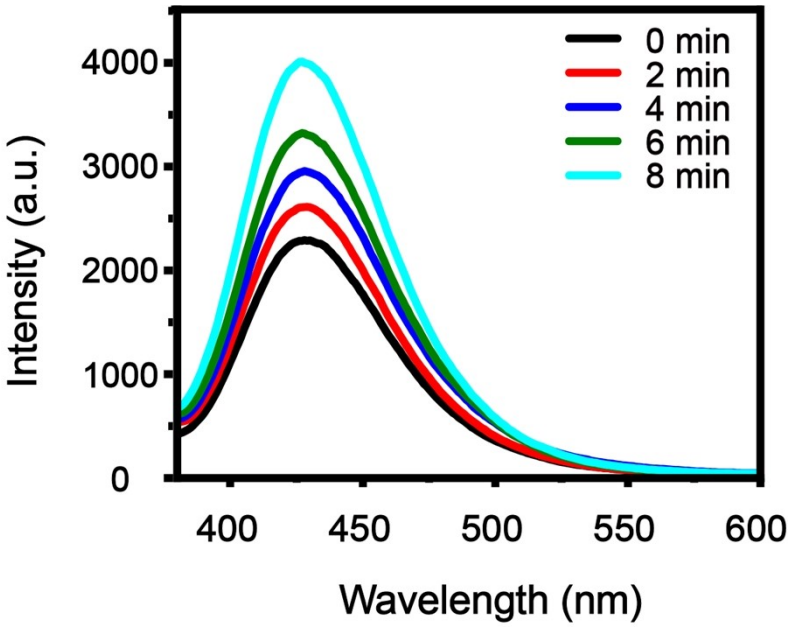

Figure S8. Fluorescence spectra of terephthalic acid oxidized by Cu-MNCS@PAA in the presence of H_2O_2 at pH = 6.5 and different irradiation time with 808 nm laser.

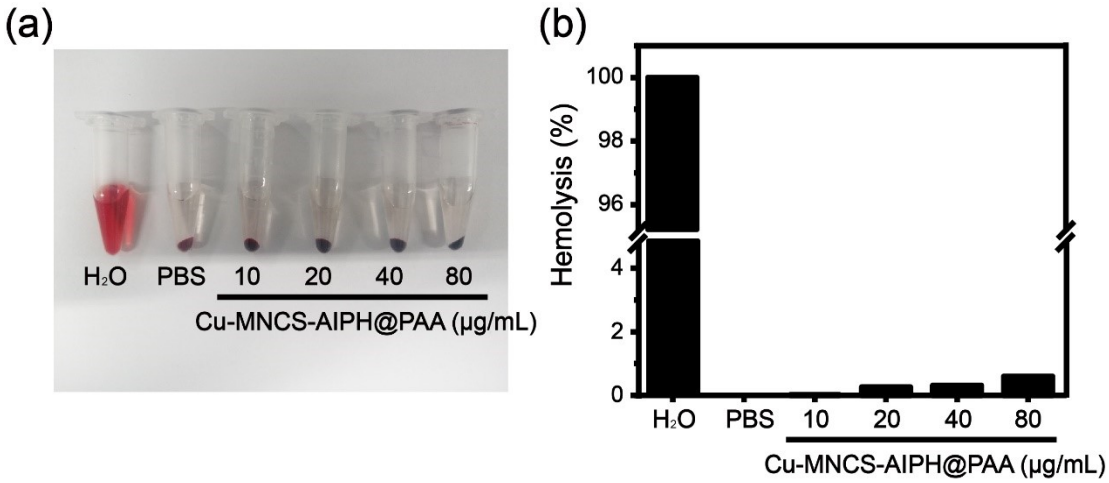

Figure S9. The hemocompatibility of DI water (positive control), PBS (negative control) or Cu-MNCS-AIPH@PAA NPs at different concentration.

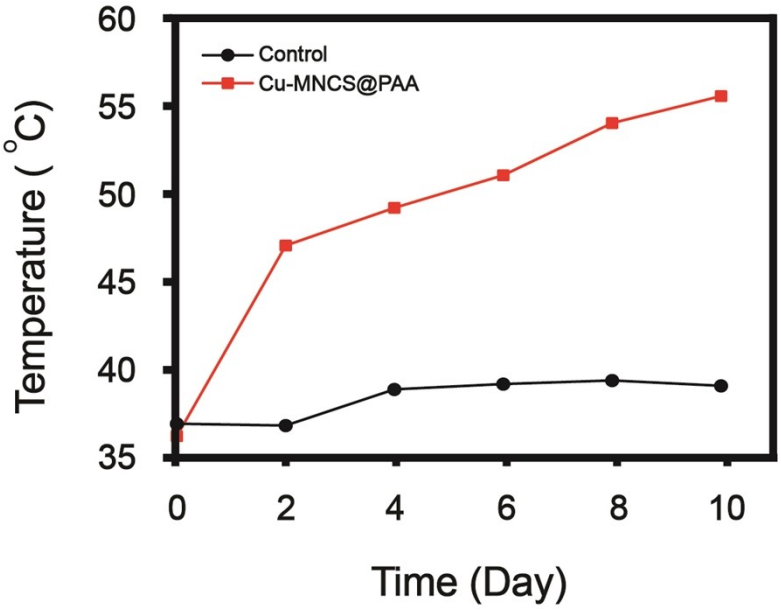

Figure S10. Temperature curve in vivo under the irradiation of 808 nm laser after injection with 24 h.

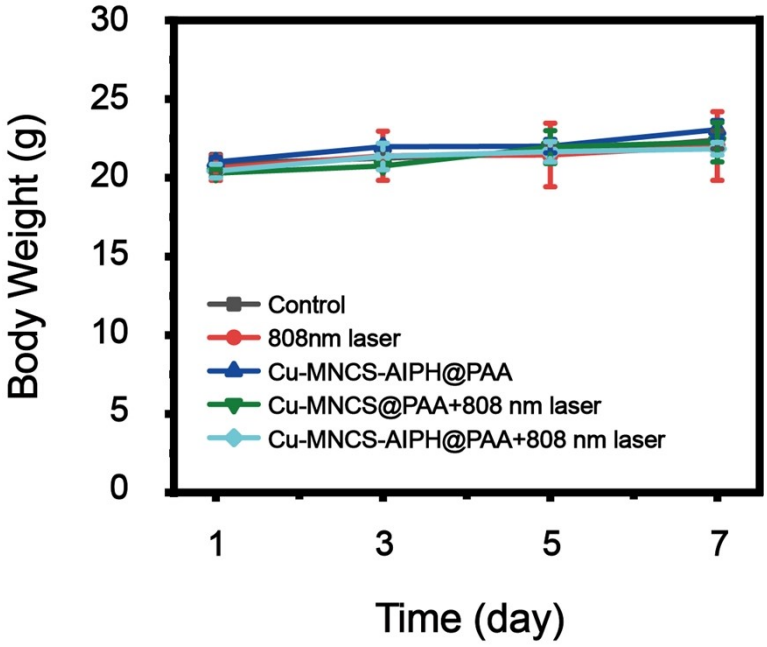

Figure S11. Body weight of tumor-bearing mice.

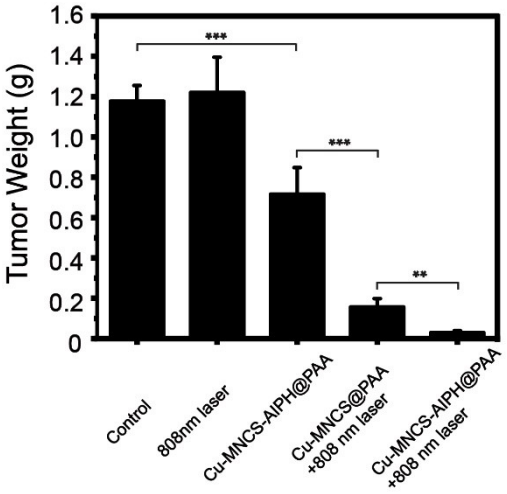

Figure S12. The final weight of tumors after treatment (**p < 0.01, ***p < 0.001).

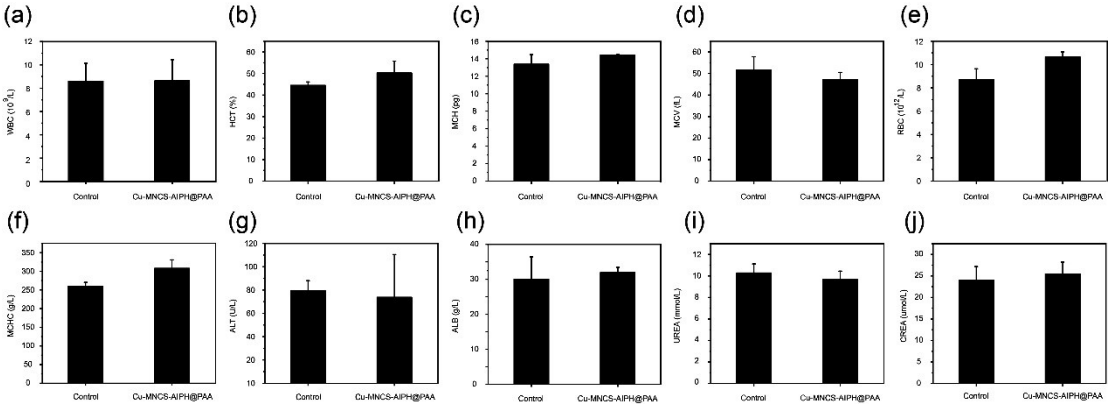

Figure S13 (a-f) Hematological index and (g-j) biochemical blood analysis of the mice on the 30th day after intravenous injection.