Experimental Section

Materials

The process of obtaining deionized (DI) water is mainly realized by a system that handles 18 MΩ cm (SHRO-plus DI). Exosome Isolation Reagent was purchased from RIBOBIO biotechnology co. LTD. (China). $CuCl_2$, PVA, phosphate buffer solution (PBS) were purchased from Sigma-Aldrich and used as received. 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA), ATP assay kit, 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) and Reactive Oxygen Species Assay Kit were purchased from Beyotime Company (China). The other solvents used in this work were purchased from Sinopharm Chemical Reagent (China) and Aladdin-Reagent (China).

Preparation of CuPy-Au

Copper-doped CuPy nanozymes were successfully synthesized according to the previous work. First, 30 mg PVA were mixed with 10 mL of deionized water and then heated at 90 °C under gentle stirring for 1.5 h. Afterward, the above mixture cooled naturally to room temperature and the freshly prepared $CuCl_2$ aqueous solution (1 g, 10 mL) was slowly added and stirred for 1 h. Subsequently, 200 μL of pyrrole monomer was added dropwise and the mixture was stirred for another 18 h at room temperature. The resulting CuPy were collected by centrifugating and washing with deionized water repeatedly, and finally dispersed in deionized water for future use. The CuPy-Au NPs were prepared via in situ growth of Au NPs on the CuPy surface. Briefly, 500 μL of CuPy (10 mg/mL) and 200 μL of $HAuCl_4$ (10 mg/mL) was added into 19 mL of ultrapure water in turn. The resultant mixture was reacted under magnetic stirring, followed by rapid addition of 300 μL of freshly prepared cool $NaBH_4$ solution. The formed CuPy-Au was then washed with ultrapure water to remove the unreacted chemicals by centrifugation. The final particulates were re-dispersed in Millipore ultrapure water.

Preparation of CuPy-Au@PEG

10 mg of CuPy-Au were dispersed in 10 mL of deionized water and further mixed with 100 mg of DSPE-PEG2000 (pre-dissolving in 1 mL of liquid formed by 0.4 mL of acetone and 0.6 mL of ethanol) under ultrasound treatment in the ice bath for 30 min and then stirred for one night. Then, the above dispersion was centrifuged and washed with H_2O to purify CuPy-Au@PEG. The obtained CuPy-Au@PEG were stored for further use.

Preparation of CuPy-Au@EM

In the preparation phase of CuPy-Au@EM, it took 3 days to focus on culturing 4T1 cancer cells, then the obtained medium was rinsed off, and 500 μg of CuPy-Au fresh medium at 5% CO_2 and 37 °C was deployed for 1 days. Furthermore, the supernatant was collected and centrifuged at 1200g for 5 min, so that debris and cells could be effectively eliminated. The exosome separation reagent manufactured and produced by China RIBOBIO Biotechnology Co. Ltd. was used for the effective separation of CuPy-Au@EM in the supernatant. The CuPy-Au@EM were deployed at a temperature of 4 °C.

Characterization

The morphology of synthesized materials was observed with field-emission TEM (JEM-F200). The high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) and corresponding energy-dispersive spectroscopy (EDS) mapping analyses were collected on a field-emission TEM (JEM-F200, Japan). Powder X-ray diffraction (XRD) patterns were conducted (Smartlab SE, Japan) with Ni-filtered Cu KR radiation (40 kV, 50 mA). The XPS experiment was carried out using a Vacuum Generators twin-crystal monochromatized Al Kα (hν = 1486.58 eV) source and a hemispherical electron analyzer (ESCALAB250Xi, USA) in a standard ultrahigh vacuum (UHV) chamber at a base pressure of ~3.0×10^{-10} Torr. The hydrodynamic diameter of the various NPs was measured by dynamic light scattering (DLS, Nano-Zen 3600, Malvern Instruments, UK). SDS-PAGE was also used to analyze the protein components onto CuPy-Au@EM.

Glucose depleting ability of CuPy-Au@EM

The assay of CuPy-Au@EM catalyzing the glucose oxidation reaction was administrated. In brief, glucose and CuPy-Au@EM were mixed, and the concentration of glucose was 1 mg/mL. At given points, the pH value of the supernatant of the mixed solutions was measured by the pH meter.

GSH depleting ability of CuPy-Au@EM

The consumption of GSH over time was monitored by UV–vis spectroscopy. CuPy-Au@EM were mixed with GSH (1 mM) solutions under room temperature. At different time points, the solution was taken out and mixed with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (10 mg/mL), and then the UV–vis spectroscopy was applied to detect the absorbance of the above suspension.

Cell line.

4T1 mouse breast cancer cell line was obtained from the Cell Bank of the Chinese Academy of Sciences and incubated in RPMI-1640 medium supplemented with 10% FBS in a humidified atmosphere at 37 °C.

Animal tumor models

Female BALB/c mice aged 4-5 week were purchased from Vital River Company (Beijing, China). 100 μL of 4T1 cell suspension (1×10^6 cells) were subcutaneous injected into each mouse to establish the tumor models. Animal experiments have followed the guidelines of Institutional Animal Care and Use Committee of Shenzhen People's Hospital's Laboratory Animal Center

In vitro cytotoxicity of CuPy-Au@EM

Cellular apoptosis was assessed by plating 4T1 cells in 6-well plates and treating them via the different concentration of CuPy-Au@EM (0, 25, 50 and 100 μg/mL). After 48 h, cells were harvested with EDTA-free trypsin, and annexin V-FITC/PI (Beyotime

Biotechnology Co., Shanghai, China) was used to analyze cellular apoptosis via flow cytometry. For cell viability assay, 4T1 cells were plated at in 96-well plates and allowed to settle overnight for adherence. Different concentrations of CuPy-Au@EM nanozymes were then added into wells for incubation. Then the cell viability was determined using a Cell Counting Kit-8 (CCK-8).

Intracellular GSH Content

4T1 cells (5×10^6 per plate) were incubated with four different group: (1) PBS, (2) CuPy-Au@PEG and (3) CuPy-Au@EM. The CuPy-Au concentration was 50 μg/mL in group 2 and 3. After overnight of incubation, the GSH content was measured by employing a commercial colorimetric GSH assay kit from Beyotime Biotechnology, Shanghai, China. The assay was carried out according to the manufacturer's instructions. The absorbance of 340 nm was measured by a microplate reader.

Intracellular ATP detection

4T1 cells (5×10^6 per plate) were incubated with four different group (n = 3 per group): (1) PBS, (2) CuPy-Au@PEG and (3) CuPy-Au@EM. The CuPy-Au concentration was 50 μg/mL in group 2 and 3. After overnight of incubation, the supernatant was discarded and the intracellular ATP content was measured by the enhanced ATP Assay Kit according to the instructions.

Clonogenic assay.

4T1 cells were seeded in 6-well plates with a different amount (125, 250, 500, 1000 and 2000) per well and incubated at 37 ℃ for 24 h Then the cells were treated with five groups: (1) PBS; (2) RT; (3) CuPy-Au@EM; (4) CuPy-Au@PEG + RT and (5) CuPy-Au@EM + RT (n = 3 per group). The CuPy-Au concentration was 50 μg/mL in group 3, 4 and 5. The parameters were irradiated with X-rays at a dose of 4 Gy. After that, cells were washed with PBS and fresh medium was replaced every 3 days for 12 days. The colonies were fixed by 4% paraformaldehyde and then stained with Giemsa dye. Only colonies containing at least 50 cells were counted. At last, an evaluation of the

effects of different treatments was conducted by counting the survival fraction of the colonies.

In vitro immune evasion study.

RAW 264.7 were seeded in 24-well plates and cultured for 12 h. Different concentrations of CuPy-Au@PEG and CuPy-Au@EM were added the medium (n = 3 per group). Then the cells were incubated for 2 h at 37 °C, 5% CO_2, and then washed with PBS three times. The nanoparticles uptake was measured by ICP-MS as described above.

Mitochondria damage experiment

For the JC-1 assay, 4T1cells were co-incubated with five different groups (n = 3 per group) (1) PBS; (2) RT; (3) CuPy-Au@EM; (4) CuPy-Au@PEG + RT and (5) CuPy-Au@EM + RT. The cells were stained with JC-1 for 30 min before washing with PBS and harvested. Then, the mitochondrial damage/disruption was detected by fluorescence microscope (IX81, Olympus, Japan).

In vitro reactive oxygen species (ROS) generation

The ROS generation was detected in vitro on 4T1 cells by chemical method using DCFH-DA as probe. Specifically, 4T1 cells were incubated for 24 h with five different groups (n = 3 per group): (1) PBS; (2) RT; (3) CuPy-Au@EM; (4) CuPy-Au@PEG + RT and (5) CuPy-Au@EM + RT. The CuPy-Au concentration was 50 μg/mL in group 3, 4 and 5. Then, cells in group 2, 4 and 5 were exposed in radiation (4Gy). Afterward, DCFH-DA was added into the treated cells and the fluorescence images were observed by confocal laser scanning microscope (CLSM). 5, 5-dimethyl-1-pyrroline N-oxide (DMPO) was employed as a trapping probe to monitor the •OH generation by EPR.

γ-H_2AX immunofluorescence analysis

4T1 cells were seeded in 24-well plates and then cultured for 24 h at 37 °C. The experiment was divided into five groups (n = 3 per group): (1) PBS; (2) RT; (3) CuPy-

Au@EM; (4) CuPy-Au@PEG + RT and (5) CuPy-Au@EM + RT.. The CuPy-Au concentration was 50 μg/mL in group 3, 4 and 5. Then the cells were immediately packaged with sealing membrane. Next, the cells in group 2, 4 and 5 in sealed 24-well plates were subjected to X-ray irradiation at a dose of 4 Gy. After RT treatment for 2 h, the cells were fixed with 4% paraformaldehyde for 10 min, rinsed with PBS, permeabilized with methanol for 15 min at −20 °C and then rinsed with PBS again. Then the cells were exposed to a blocking buffer (1% bovine serum albumin (BSA) in PBS solution) for 1 h at room temperature and further incubated with anti- phospho-histone γ-H_2AX mouse monoclonal antibody (dilution 1:500) overnight at 4 °C. After washing with PBS, the cells were incubated with Cy5-conjugated sheep secondary antibody (dilution 1:500) for 1 h at room temperature. Excess antibody was removed by rinsing the coverslips in PBS. Cell nuclei were stained by DAPI for 5 min at room temperature. The cells were imaged via confocal fluorescence microscopy (IX81).

In vivo antitumor study

The breast tumor model was used. When tumors reached a diameter of up to about 200mm^3, tumor bearing mice were divided randomly into 5 groups (each group included 5 mice): (1) PBS; (2) RT; (3) CuPy-Au@EM; (4) CuPy-Au@PEG + RT and (5) CuPy-Au@EM + RT. The CuPy-Au concentration was 10 mg/kg in group 3, 4 and 5. Then, cells in group 2, 4 and 5 were exposed in radiation (4Gy). The treatment was conducted every 4 days for 2 times. Mice body weight was monitored every 2 days. After 16 days treatment, all the mice were sacrificed. The blood samples from these mice were collected for blood biochemistry analysis. Three important hepatic indicators (i.e., ALT: alanine aminotransferase, AST: aspartate aminotransferase, and ALP: alkaline phosphatase) and two indicators for kidney functions (i.e., BUN: blood urea nitrogen and CRE: creatinine) were measured by using a blood biochemical autoanalyzer (7080, HITACHI, Japan). Five main organs (heart, liver, spleen, lung and kidney) and tumors of all mice were harvested, washed with PBS, and fixed with paraformaldehyde for histology analysis. And the tumor tissues were weighed, and

fixed in 4% neutral buffered formalin, processed routinely into paraffin, and sectioned at 4 μm. Then the sections were stained with Terminal deoxynucleotidyl transferase-mediated deoxyuridinetriphosphate nick end labeling (TUNEL) and γ-H_2AX finally examined by using a confocal laser scanning microscope (CLSM; IX81, Olympus, Japan).

Statistical analysis

Error bars represent the standard deviations for different parallel experiments. Significance between every two groups was calculated by the student's t-test. ***P < 0.005.

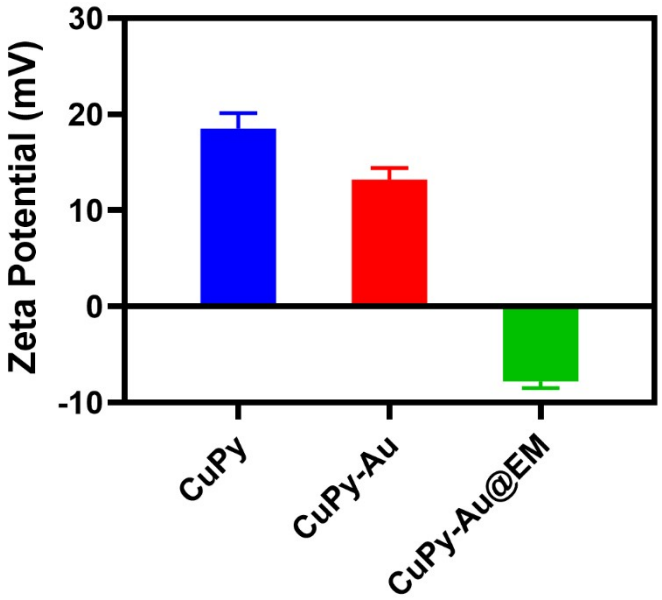

Figure S1. Zeta potential of different groups.

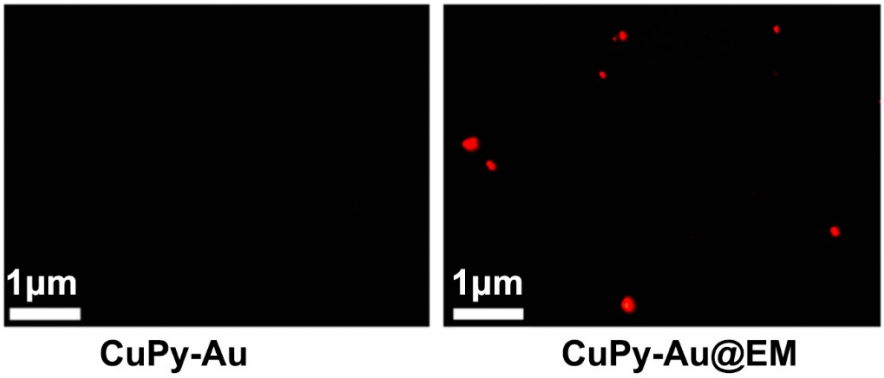

Figure S2. Fluorescence images of for Dil-labelled-CuPy-Au and -CuPy-Au@EM Dil (red).

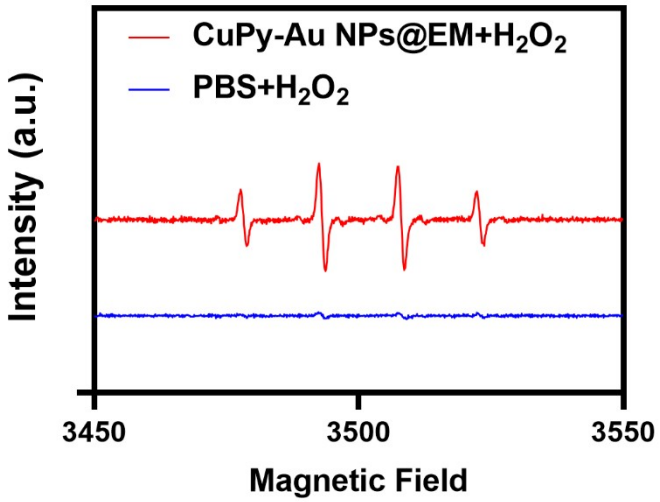

Figure S3. Electron paramagnetic resonance spectra of different groups. •OH generation was measured by using DMPO as indicators.

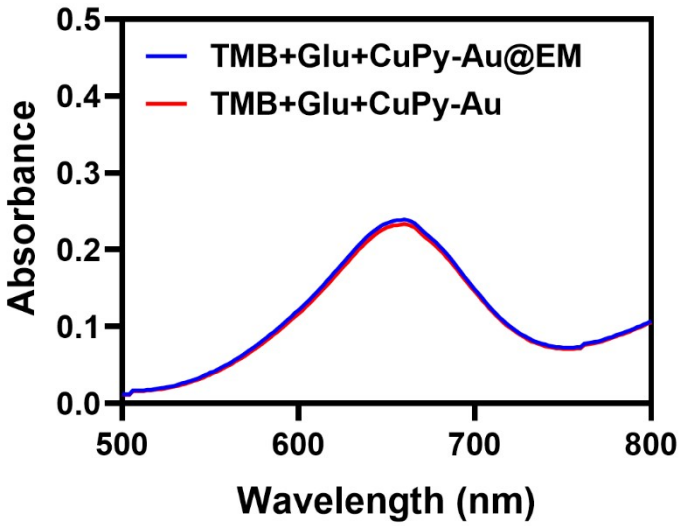

Figure S4. (E) UV−vis absorbance spectra changes of TMB in different reaction systems.

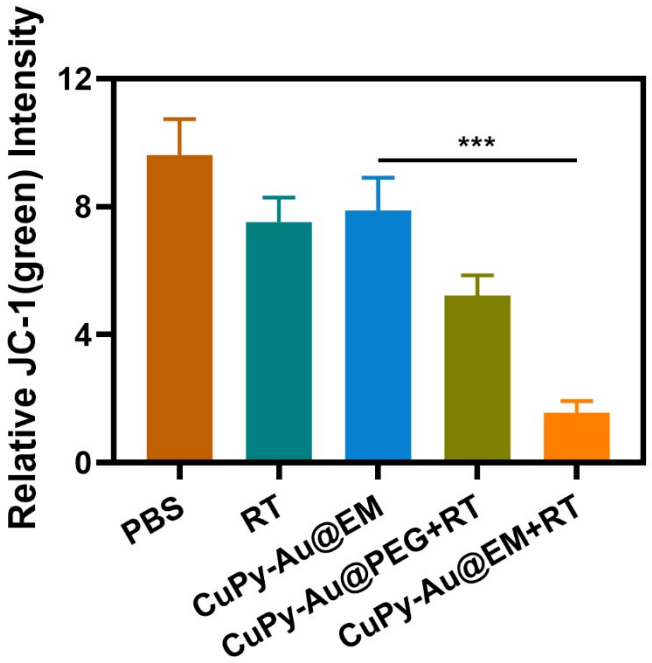

Figure S5. Relative JC-1 fluorescence intensity under different treatment.

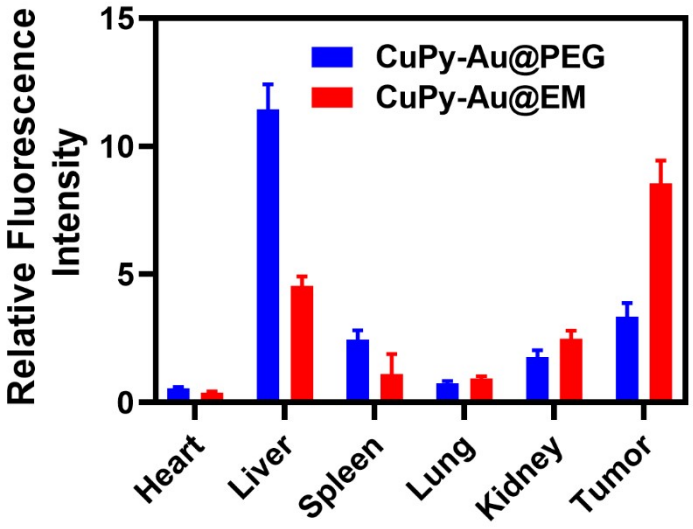

Figure S6. Semiquantitative analysis of the fluorescence imaging signal in major organs and tumors at 12 h postinjection.

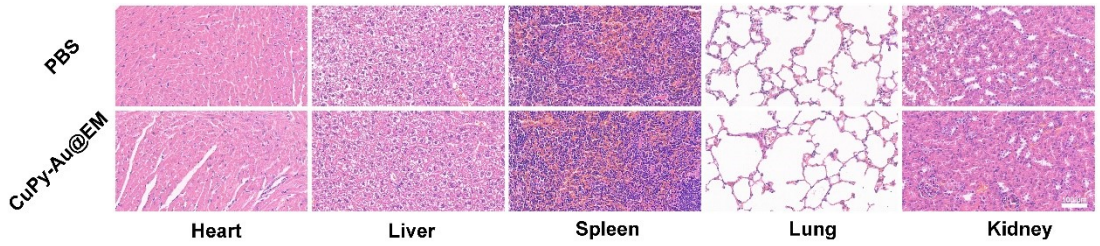

Figure S7. Histopathologic examination of the tissues including heart, liver, spleen, lung, and kidney from tumor-bearing mice after PBS or CuPy-Au@EM + RT treatment.

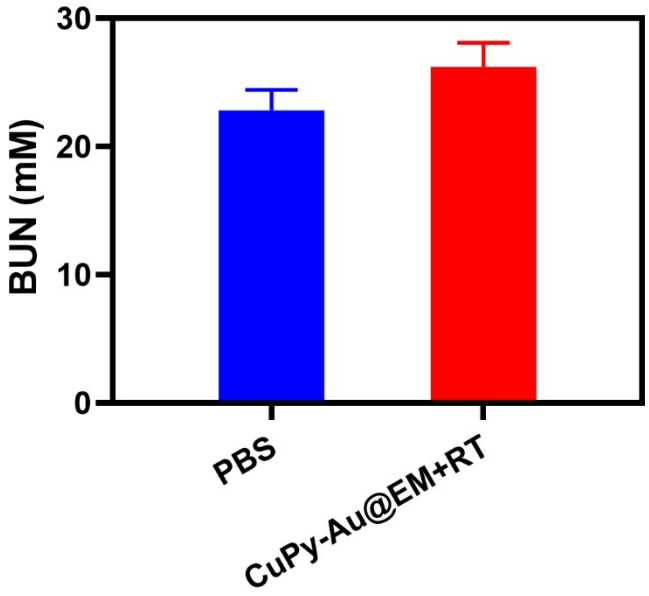

Figure S8. Blood biochemistry analysis (ALT) after various treatments.

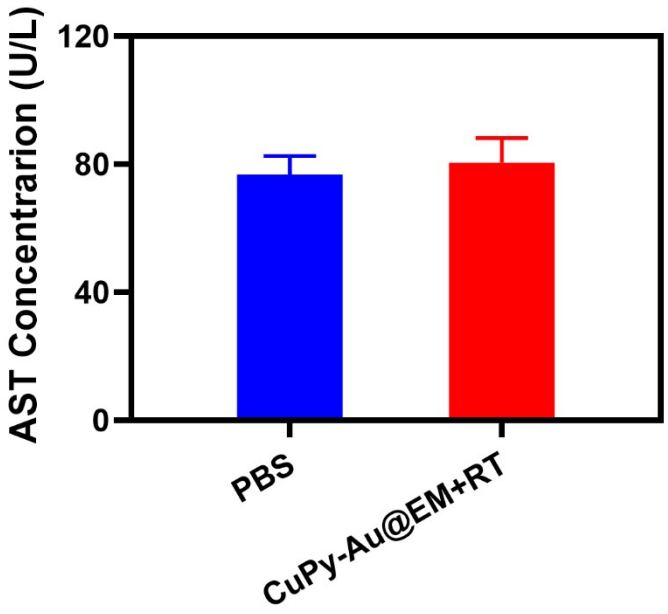

Figure S9. Blood biochemistry analysis (AST) after various treatments.

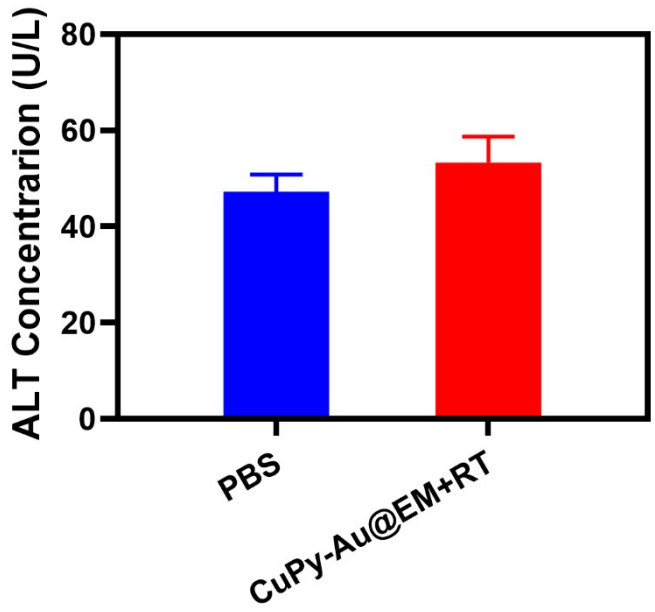

Figure S10. Blood biochemistry analysis (ALT) after various treatments.

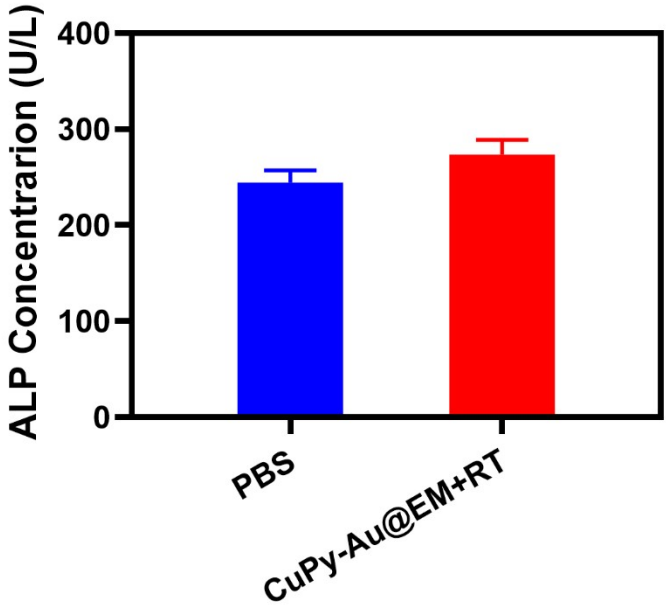

Figure S11. Blood biochemistry analysis (ALP) after various treatments.

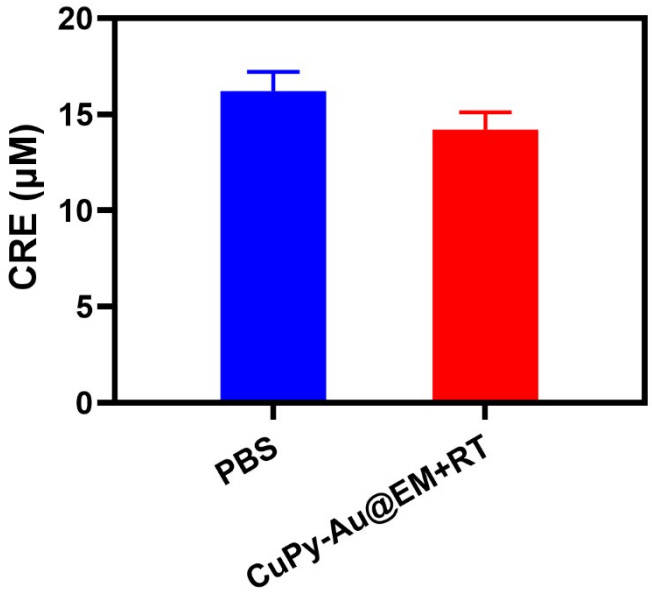

Figure S12. Blood biochemistry analysis (CRE) after various treatments.