Supporting Information

Acid-Responsive Endosomolytic Polymeric Nanoparticles with Amplification of Intracellular Oxidative Stress for Prodrug Delivery and Activation

Nannan Lu[a], Longchang Xi[b], Zengshi Zha[b], Yuheng Wang[b], Xinghua Han*[,a], and Zhishen Ge*[,b]

[a] Department of Oncology, The First Affiliated Hospital of USTC, Division of Life Science and Medicine, University of Science and Technology of China, Hefei 230001, Anhui, China.

[b] CAS Key Laboratory of Soft Matter Chemistry, Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei 230026, Anhui, China.

Corresponding Author

*E-mail: gezs@ustc.edu.cn (Z. Ge), ahslhxh@163.com (X. Han)

Materials. Cinnamaldehyde (98.0%), 1,1,1-tris(hydroxymethyl)ethane (97.0%), methacryloyl chloride (97.0%), p-toluenesulfonic acid (98.0%), nile red (97%), and benzyl methacrylate (98.0%) were obtained from Energy Chemical (Shanghai, China). 1-(1H-imidazol-4-yl)-2-(octylamino)-2-oxoethyl methacrylate (ImOAMA), the prodrug phenylboronic pinacol ester-caged CPT (ProCPT), and PEG-based macroRAFT agent (M_n = 5,000 Da, M_n/M_w = 1.06) were synthesized based on the previously reported procedures.[1-3] Benzyl methacrylate (BzMA, 98%) were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). Dichloromethane (CH_2Cl_2) was dried by using CaH_2. Tetrahydrofuran (THF) and toluene were dried by using sodium and benzophenone. Mouse breast cancer cell line 4T1, human cervical cancer cell line HeLa, and human lung cancer cell lines A549 were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). 10-Dioctadecyl-3,3,30,30-tetramethylindotricarbocyanine iodide (DiR) was obtained from Fanbo Chemicals (Beijing, China). Cell Counting Kit-8 (CCK-8), 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), LysoTracker Green probe, Annexin V-FITC, reduced glutathione (GSH) and oxidized glutathione disulfide (GSSG) Assay Kit, erminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) kit, Hematoxylin and Eosin (H&E) Stain Kit, anti-Ki-67 (nuclear-associated Antigen Ki67), anti-PCNA (proliderating cell nuclear antigen), and anti-CD3 antibodies were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), and trypsin were purchased from Gibco Company (USA). Propidium iodide (PI, 94.0%), fluorescein diacetate (FDA), and 2′,7′-dichlorofluorescin diacetate (DCFH-DA, reactive oxygen species (ROS) probe were purchased from Wako Pure Chemical Industries, Ltd. DNA Damage Detection Kit and Biochemical

Detection Kit were bought from Nanjing Jiancheng Bioengineering Institute. Mouse Elisa Kit of Interferon γ (IFN-γ), Interleukin-2 (IL-2), Interleukin-6 (IL-6) and Interleukin-10 (IL-10) purchased from Wuhan ColorfulGene Biological Technology Co., LTD. All other commercially available chemicals were ordered from Sinopharm Chemical Reagent Co., Ltd. Female 5-week old ICR mice were bought from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). The animal experiments were carried out according to Regulations for the Administration of Affairs Concerning Experimental Animals (USTCACUC1701031).

Characterization

All NMR spectra were recorded in deuterated reagent on a Bruker AV-400 MHz spectrometer operated in the Fourier transform mode. Agilent Gel permeation chromatography (GPC) system equipped with a G1310B Iso. pump, a G1316A PL gel column, and a G1362A differential refractive index detector was used to measure the molecular weights and molecular weight distributions of the polymers and calibrated with polystyrene standards. HPLC grade DMF with 1 g L^{-1} LiBr was used as eluent, and samples were run at a flow rate of 1 $mL \cdot min^{-1}$. All samples were passed over 0.22 μm PTFE membrane filters before injection. The size and size distribution of the samples were measured using dynamic light scattering (DLS) equipped a Malvern Zetasizer Nano ZS90, a He-Ne laser (633 nm), and 173° collecting optics. The morphology of samples was analyzed using a JEOL-2100F electron microscope utilizing an accelerating voltage of 200 kV. The sample for TEM observation was prepared by placing 10 μL micellar dispersion on copper grids successively coated with thin films of Formvar and carbon. UV-Vis data were obtained on a UV-1800 Shimadzu spectrometer. Reversed-phase high performance liquid chromatography (RP-

HPLC) analysis was conducted on an Agilent 1260 Infinity high performance liquid chromatography using HPLC-grade methanol and H_2O (9/1, v/v) as mobile phases at flow rate of 1 mL min^{-1}. Fluorescence microscopy images were performed on an inverted fluorescence microscope (Olympus IX81). Cell distribution imaging experiments were carried out by confocal laser scan microscopy (CLSM) (Zeiss LSM-710, ZEISS, Germany). A Xenogen IVIS Spectrum optical imaging device was used to study the distribution of samples in vivo.

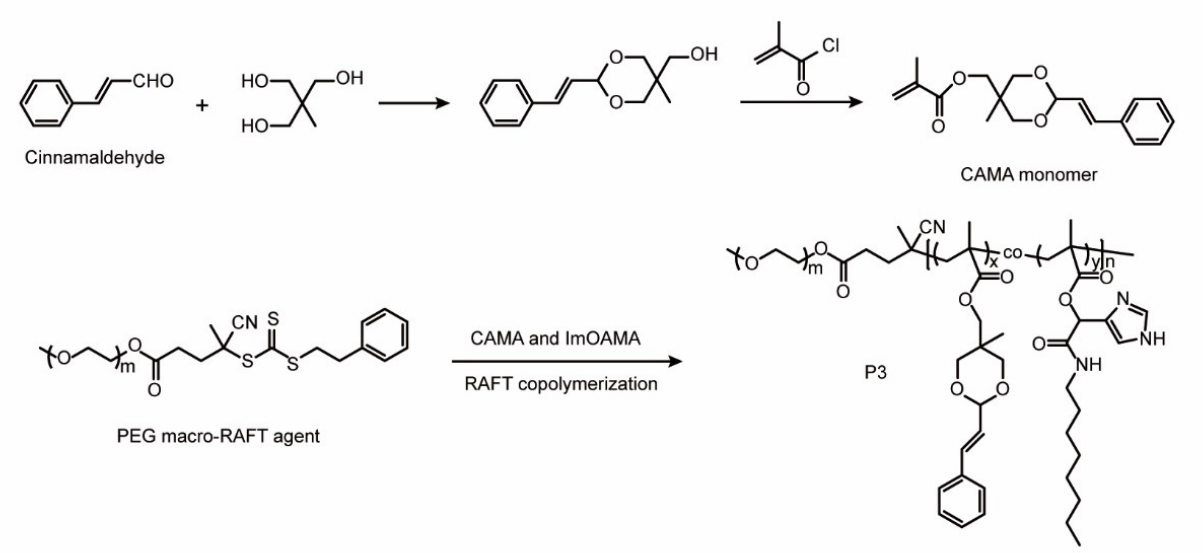

Fig. S1. The synthetic routes employed for the preparation of the block copolymer, PEG-*b*-P(CAMA-*co*-ImOAMA).

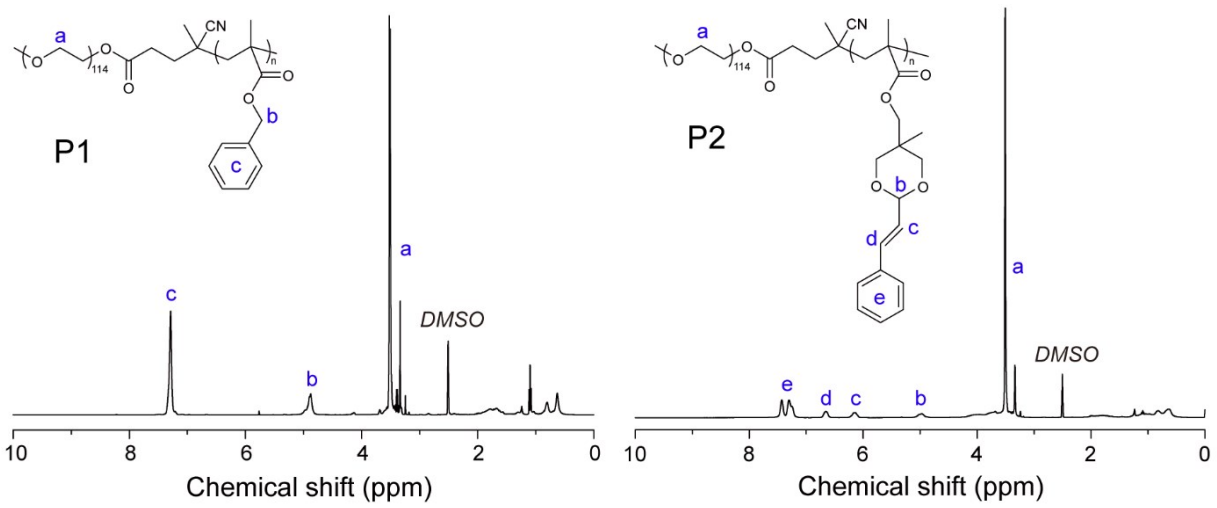

Fig. S2. ^{1}H NMR spectra recorded for polymers P1 and P2 in DMSO-d_6.

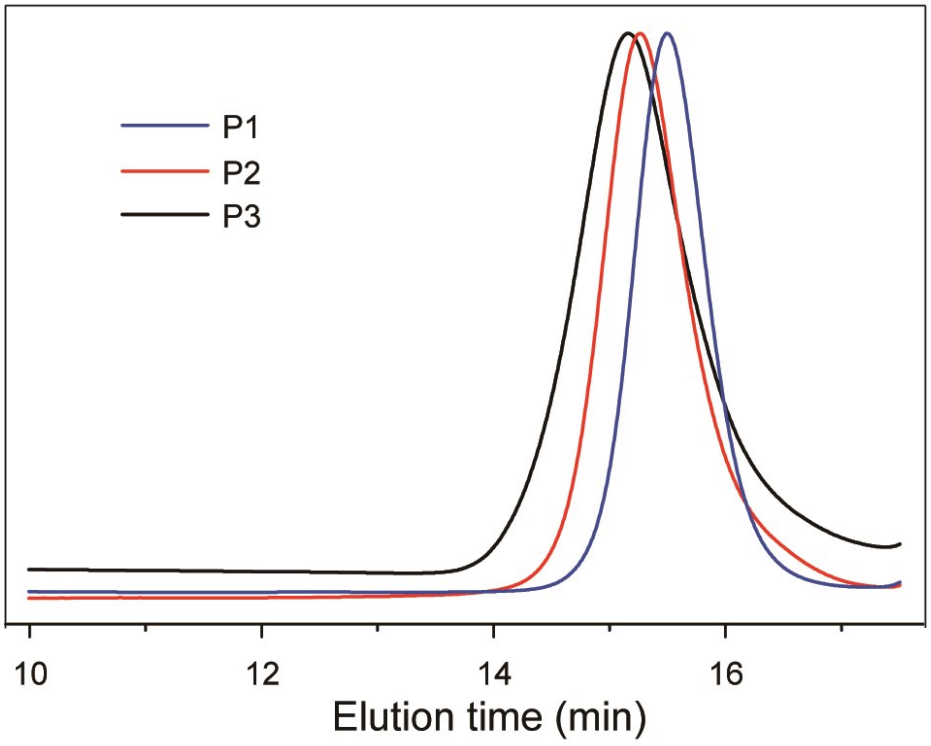

Fig. S3. GPC traces recorded for the polymers, P1, P2, and P3 by using DMF as the elution solvent.

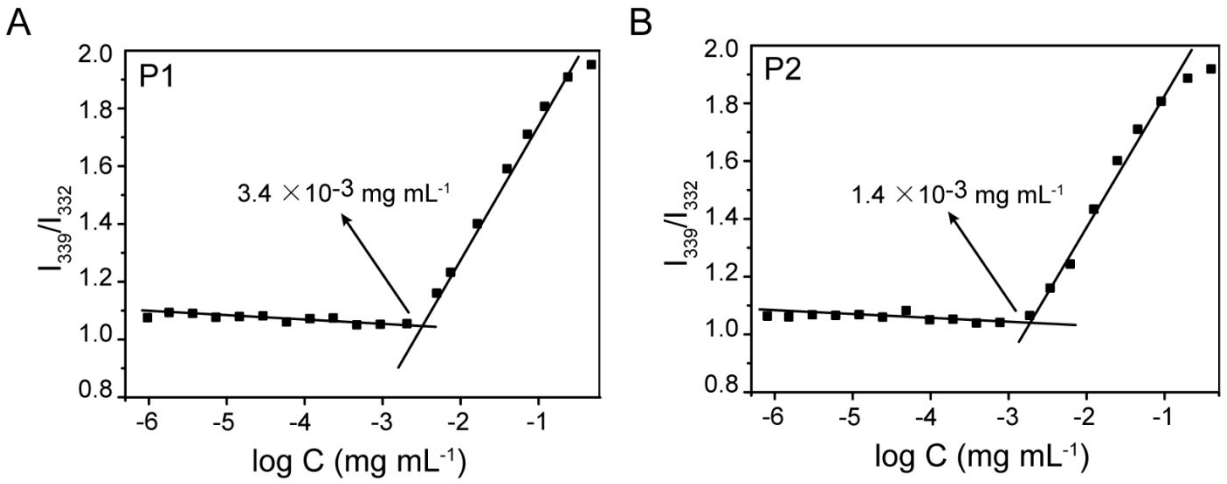

Fig. S4. CMC measurements of the polymers P1 and P2 in aqueous solution by using pyrene as the probe.

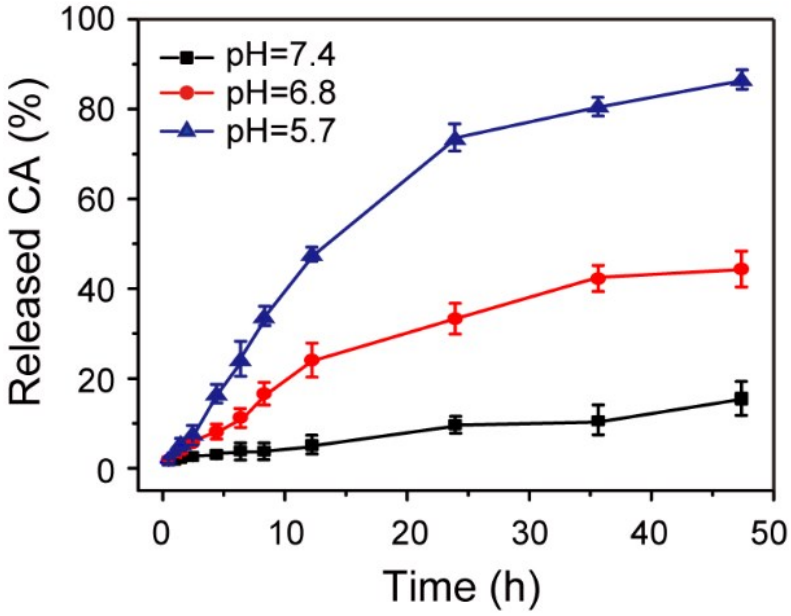

Fig. S5. Qualitative analysis of CA release from Procpt@P2 by RP-HPLC at pH 5.7, 6.8, and 7.4. Data are expressed as mean ± s.d. (n = 3).

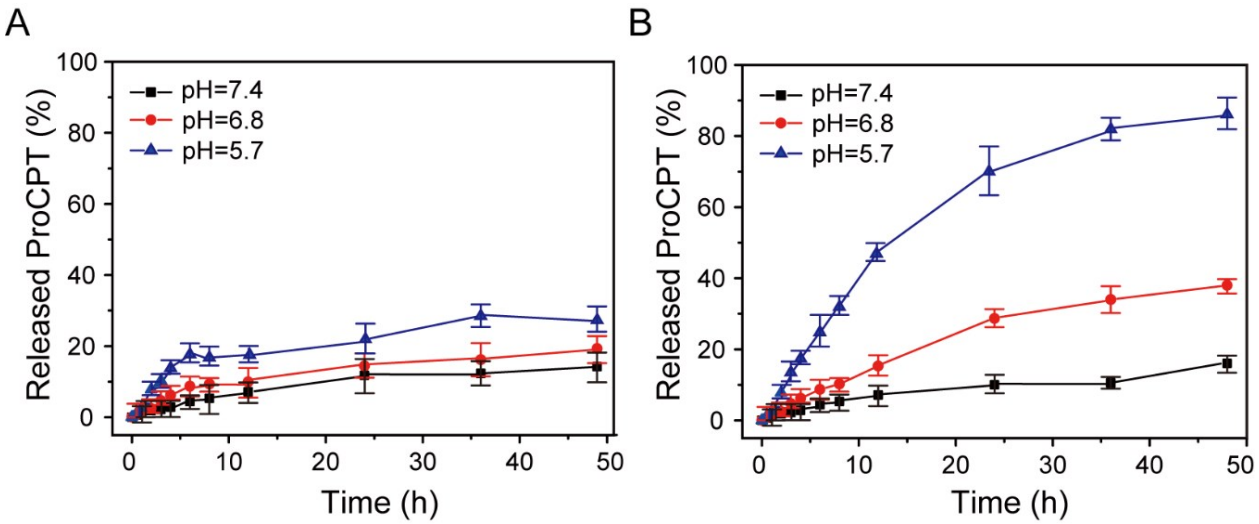

Fig. S6. Qualitative analysis of prodrug ProCPT release. ProCPT release profiles of (A) ProCPT@P1 and (B) ProCPT@P2 by RP-HPLC at pH 5.7, 6.8 and 7.4. Data are expressed as mean ± s.d. (n = 3).

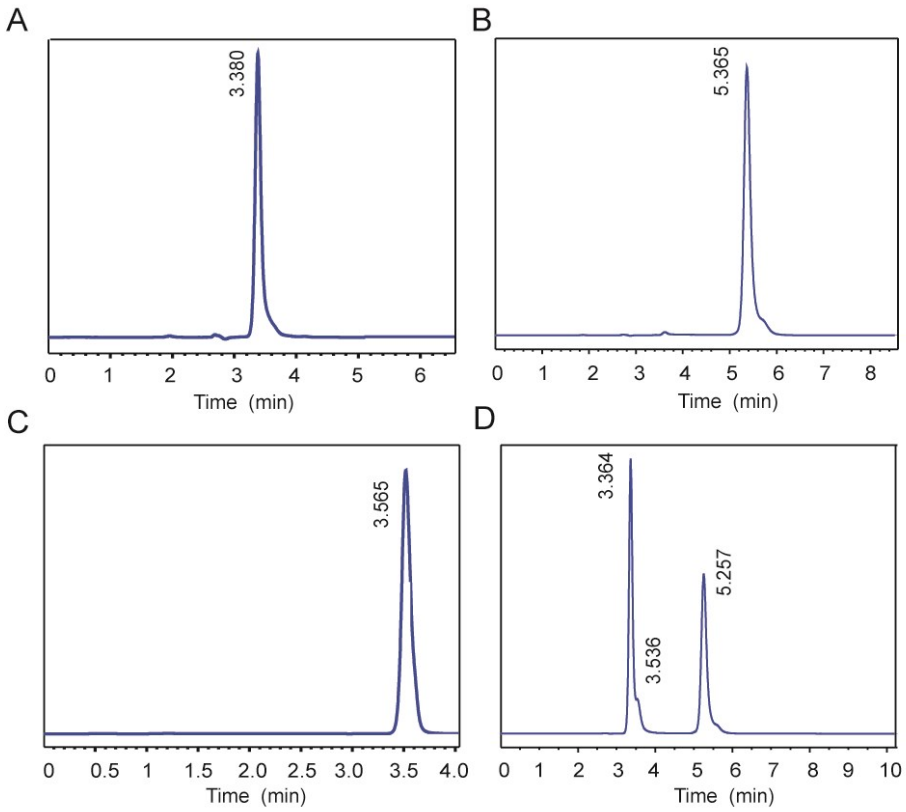

Fig. S7. RP-HPLC spectra recorded for (A) CPT, (B) p-hydroxybenzyl alcohol, (C) ProCPT, and (D) ProCPT + H_2O_2.

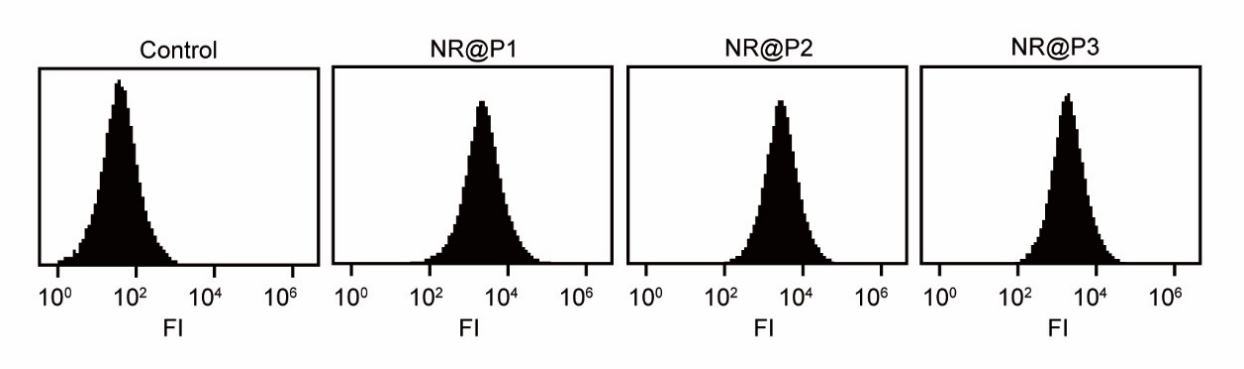

Fig. S8. Flow cytometry analysis of cellular internalization of various nanoparticles after incubation with tumor cells for 4 h. Nile red (NR) was encapsulated in the micelles. FI: fluorescence intensity.

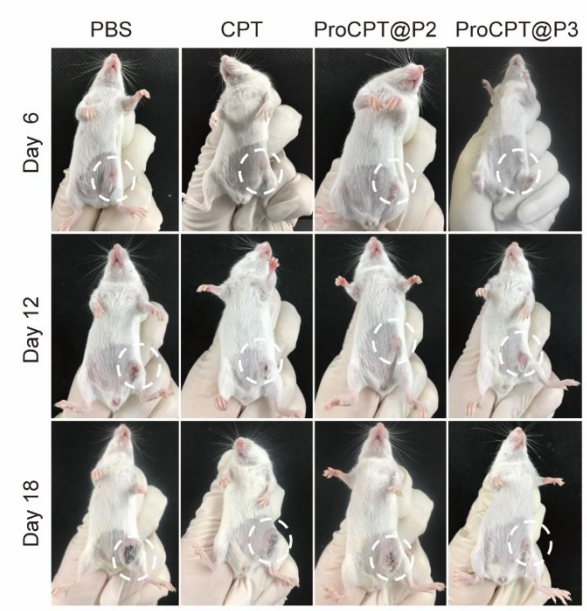

Fig. S9. Typical images of 4T1 tumor-bearing mice at predetermined time intervals after treatment with various nanoparticles.

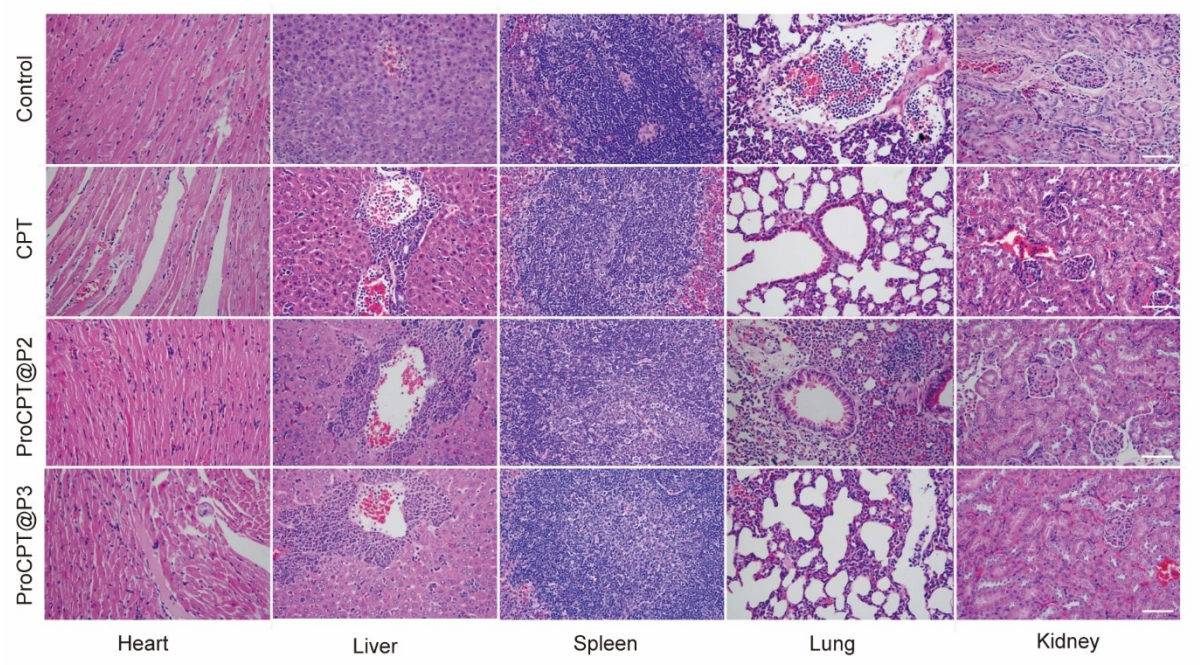

Fig. S10. H&E staining images of major organs in 4T1 tumor-bearing mice after 18 days post treatments with PBS, CPT, Procpt@P2, and Procpt@P3 at a CPT equivalent dose of 10 mg kg^{-1}. Scale bar represents 200 μm.

References:

1. M. Semsarilar, V. Ladmiral, A. Blanazs and S. P. Armes, *Langmuir*, 2012, **28**, 914-922.

2. J. F. Mukerabigwi, W. Yin, Z. Zha, W. Ke, Y. Wang, W. Chen, A. Japir, Y. Wang and Z. Ge, *J. Controlled Release*, 2019, **303**, 209-222.

3. Z. S. Zha, J. J. Li and Z. S. Ge, *Acs Macro Lett.*, 2015, **4**, 1123-1127.