

## Supporting Information

### **Induction of Mitochondrial Apoptosis for Cancer Therapy via Dual-Targeted Cascade-Responsive Multifunctional Micelles**

Guoqing Wei, Yi Wang, Xuehui Huang, Guang Yang, Jingya Zhao and Shaobing Zhou\*

Key Laboratory of Advanced Technologies of Materials, Ministry of Education, and School of Materials Science and Engineering, Southwest Jiaotong University, Chengdu, Sichuan 610031, P.R. China

\*Corresponding Author:

E-mail: shaobingzhou@swjtu.edu.cn or shaobingzhou@hotmail.com

## Supplementary experimental section

**Materials.** Chlorin e6 (Ce6) was bought from J&K Chemical Co. Ltd. Cisplatin (cis-Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>) (purity 99%) was bought from Shandong Boyuan Chemical Company, China. Hyaluronic acid (HA, molecular weights 20 KDa) was purchased from Zhenjiang Dong Yuan Biotechnology Corporation, China. Triphenylphosphonium (TPP), Cystamine dihydrochloride, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC•HCl), N-hydroxysuccinimide (NHS), N,N-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino) pyridine (DMAP) were obtained from Adamas Reagent Co., Ltd. The dialysis bags (molecular weight cutoff, MWCO: 2, 3.5 and 14 kDa) were bought from Green Bird Inc, China. N-Boc-ethylenediamine (Boc-NH-NH<sub>2</sub>) was synthesized as our previous report.<sup>1</sup>

**Cell lines and culture conditions.** Highly metastatic mouse melanoma cells (B16-F10) and human umbilical vein endothelial cells (HUVEC) were obtained from Sichuan University (China). B16F10 cells were cultured in RPMI 1640, while HUVEC cells were cultured in F12 medium, both supplemented with 10% (v/v) fetal bovine serum (FBS) at 37 °C in a 5% CO<sub>2</sub> atmosphere under fully humidified conditions.

**Animals.** Female C57 mice (18 ± 2 g) were purchased from the Experimental Animal Center of Sichuan University and fed under conditions of 25 °C and 55% humidity. Animal experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University (P. R. China), and all protocols for this animal study conformed to the Guide for the Care and Use of Laboratory Animals.

**Synthesis of HA–CYS Conjugates.** The Cystamine-modified HA (HA–CYS) was synthesized by linking the carboxyl groups of HA with the amino groups of cystamine according to the protocols described in the literature.<sup>2</sup> The solutions of EDC•HCl (0.40 mmol) and NHS (0.40 mmol) were added to 4 mg/mL of HA (0.50 mmol) in PBS buffer (0.01 m, pH = 7.4) to activate the carboxyl groups of HA, followed by the addition of 1.12 g of cystamine dihydrochloride (1.12 g, 5.0 mmol) and its reaction for 8 h at 25 °C during stirring. The resulting solution was dialyzed

(MWCO 3500) with DI water for 3 d and collected by lyophilization. It was then stored at 4 °C until further use.

**Synthesis of Ce6–TPP Conjugates.** TPP modified Ce6 was synthesized by linking the carboxyl groups of Ce6 with the amino groups of TPP–NH<sub>2</sub>. Briefly, Ce6 (0.2 g, 0.335 mmol) was pre-activated with activated DCC (0.365 mM) and NHS (0.365 mM) in DMF (50 mL) for 3 h at 25 °C. Subsequently, TPP–Boc (0.23 g, 0.403 mM), which was prereacted with trifluoroacetic acid (TFA) until the Boc reacted completely, was added into the mixture rapidly, and was then stirred in dark at 25 °C until the Ce6 disappeared. The solution was concentrated under reduced pressure and the solid material was washed with cold ethyl ether three times. It was then dried under vacuum to obtain Ce6–TPP. All reactions were monitored by TLC.

**Synthesis of HA–ss–Ce6–TPP Conjugates.** Amphiphilic HA–ss–Ce6–TPP conjugates were prepared based on the chemical grafting of Ce6–TPP to HA–CYS. In brief, the carboxyl group of Ce6–TPP (0.3 g, 0.265 mM) was reactivated by equimolar amounts of DCC and NHS in DMF in dark at 25 °C for 3 h. Subsequently, 0.01 mM HA–CYS was added into the reaction mixture and stirred at 25 °C for 48 h. The final product (HA–ss–Ce6–TPP) was obtained by dialyzing (MWCO 35 kDa) exhaustively against DI water for 3 d to remove any extra reactant. To determine the amount of Ce6 in the conjugate, dried samples were suspended in DMSO, and the absorbance of Ce6 was determined at 406 nm using a UV/Vis spectrophotometer.

**Synthesis of Prodrug PCT.** PCT was synthesized by conjugating Ce6–TPP on one of the axes ends of cisplatin. The cisplatin (IV) derivative *c,c,t*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>(OOCCH<sub>2</sub>CH<sub>2</sub>COOH)(OH)] (Cisplatin–COOH) was obtained by referring to a previously published method.<sup>3</sup> Briefly, cisplatin (0.5 g, 1.66 mM) was dissolved in 3 mL of DI water with the addition of 30 mL of 30% H<sub>2</sub>O<sub>2</sub>. The reaction solution was condensed through rotary evaporation after a 6 h reaction at 50 °C. Recrystallization of *c,t,c*-[PtCl<sub>2</sub>(OH)<sub>2</sub>(NH<sub>3</sub>)<sub>2</sub>] was performed in situ, and the resulting compound was collected and washed with cold water, ethanol, and ether and then dried. The obtained yield of *c,t,c*-[PtCl<sub>2</sub>(OH)<sub>2</sub>(NH<sub>3</sub>)<sub>2</sub>] was 45.0%. A solution of *c,t,c*-[PtCl<sub>2</sub>(OH)<sub>2</sub>(NH<sub>3</sub>)<sub>2</sub>] (0.47 g, 1.41 mM) in DMSO (8 mL) was added to succinic

anhydride (0.565 g, 5.646 mM). The mixture was stirred in dark at 70 °C overnight, and the solution was concentrated under reduced pressure. Moreover, the solid material was washed with cold acetone three times and then dried. Finally, the pale yellow cisplatin–COOH was obtained at a yield of 50.7%.

N-Boc-ethylenediamine (Boc-NH-NH<sub>2</sub>, 0.05 g, 0.31 mM), which was synthesized as our previous report,<sup>1</sup> was added into the solution of Cisplatin–COOH (0.134 g, 0.31 mM) in 20 mL DMF, which was pre-activated by equimolar amounts of DCC and NHS in DMF in dark conditions at 25 °C for 3 h. The mixture was stirred in the dark at 25 °C until it became a light yellow solution. The reaction was terminated until the Boc–NH–NH<sub>2</sub> disappeared using TLC. The mixture was condensed by vacuum evaporation and the resulting yellow oily product was dissolved in methanol and precipitated with ethyl ether. The precipitate was washed twice with ethyl ether and dried under vacuum to obtain cisplatin–NH–NH–Boc, the Boc of which has prereacted with trifluoroacetic acid (TFA) completely in DMF to obtain cisplatin–NH–NH<sub>2</sub>.

Finally, 1.2 equimolar amounts of Ce6–TPP (36 mg, 0.032 mM) were reactivated with 1.44 equimolar amounts of DCC and NHS in DMF in dark at 25 °C for 3 h. cisplatin–NH–NH–Boc (18.1 mg, 0.038 mM) was added to the above solution. The mixture was stirred under dark conditions for 24 h at 25 °C. Subsequently, the solvent was evaporated. The resulting oily residue was dissolved in methanol and precipitated in cold ethyl ether. The compound was purified by flash chromatography with eluent methanol/DCM = 1:10 to obtain 19.4 mg of the final product of PCT (yield ≈ 40%). The chemical structure of the final product was confirmed by <sup>1</sup>H NMR.

**Characterization of the Chemical Compounds.** The chemical structures of synthesized chemical compounds were confirmed by <sup>1</sup>H NMR spectra, which were recorded at room temperature on a Bruker AM 300 apparatus. Deuterated chloroform (CDCl<sub>3</sub>) or dimethyl sulfoxide DMSO-*d*<sub>6</sub> was used as a solvent, and tetramethylsilane (TMS) was used as the internal reference. Fourier transform infrared (FT-IR) spectra were obtained with a Nicolet 5700 spectrometer in the range of 4000 to 500 cm<sup>-1</sup>. KBr tablets were prepared by grinding the polymers with KBr and

compressing the powders into a transparent tablet.

**Preparation of the Micelles.** The PCT-loaded HCCT (PCT@HCCT) micelles were prepared by dialysis method. Briefly, 20 mg HCCT with PCT (whose amount was shown in **Table 1** in **SI**) were co-dissolved in 10 mL DMSO and added into 10 mL of deionized water under high-speed stirring. Then the mixture was stirred under a moderate speed. After 12 h, the mixture was transferred into a dialysis bag (MWCO 3500 Da) and dialyzed against deionized water to remove DMSO, and the micelles could be formed. Finally, the prodrug micelles were collected by lyophilization for the following experiments. The drug-loading content (DLC) and encapsulation efficiency (EE) of the PCT-loaded HCCT micelles were lyophilized, redissolved in DMSO and measured by a UV-vis spectrophotometer (UV-2550, Shimadzu, Japan). The Ce6 concentration was quantified by absorbance at 406 nm through standard curve. Moreover, the DLC and EE of PCT-loaded micelles could be calculated as determined platinum measurement via inductively coupled plasma mass spectrometry (ICP-MS) analysis. DLC was calculated as  $\text{DLC (\%)} = \frac{\text{weight of Ce6 or platinum in micelles}}{\text{weight of PCT-loaded HCCT micelles}}$ , and EE was calculated as  $\text{EE (\%)} = \frac{\text{weight of Ce6 or platinum in micelles}}{\text{weight of Ce6 or platinum in PCT in feed}}$ . Blank micelles were prepared through the same method.

**Characterization of Micelles.** The micelles size and zeta potential were measured at 25 °C by dynamic light scattering (DLS) (Zeta-Sizer, Malvern Nano-ZS90, Malvern, U.K.). The morphologies of micelles were observed by transmission electron microscopy (TEM) (Hitachi H-600; Hitachi Ltd., Japan).

**Stability Analysis.** The stability of PCT@HCCT micelles were evaluated by size change under H<sub>2</sub>O, PBS (pH 7.4) and RPMI 1640 cell culture medium containing 10% fetal bovine serum (FBS). DLS was used to determine the size of the two micelles at different time points. Each measurement was carried out in triplicate at 25 °C and an average value was reported.

**In vitro Drug Release.** The lyophilized powder of PCT@HCCT (10 mg) were redissolved in 10 mL PBS (pH 7.4 or 5.0) with or without HAase (0.5 mg/mL). Then the micelles were dialyzed against the corresponding PBS solutions in a shaking

incubator at 37 °C. The amount of platinum in the media at selected time intervals from 0.5 to 24 h were measured using ICP-MS. Herein, the pH 7.4 buffer without HAase was used to simulate the blood circulation, while pH 5.0 buffers with 0.5 mg/mL of HAase was applied to simulate conditions within intracellular endo/lysosomes.

**Singlet Oxygen Generation and Quantum Yield *in vitro*.** Singlet oxygen generation and singlet oxygen quantum yield ( $\Phi\Delta$ ) were measured using 1,3-Diphenylisobenzofuran (DPBF) according to the reference.<sup>4</sup> In brief, the ethanol solution of DPBF (6  $\mu$ L, 5 mM) was added to a PBS solution of free Ce6, PCI, HCCT and PCI@HCCT (3 mL, 5  $\mu$ M Ce6 concentration). The system was then irradiated with 650 nm light within 60 s (250 mW/cm<sup>2</sup>). The absorption spectra of the mixture after the designated time intervals of laser irradiation at 407 nm were obtained on a UV-Vis spectrophotometer (UV-2550, Shimadzu, Japan). The value of singlet oxygen quantum yields ( $\Phi\Delta$ ) was calculated according to the equation of  $\Phi\Delta(x) = \Phi\Delta(\text{std}) (S_x/F_{\text{std}}) (F_{\text{std}}/F_x)$ , where superscripts x and std represent the sample and Ce6, respectively. In addition, S is the slope of a plot of the difference in change in absorbance of DPBF (at 407 nm) with the irradiation time, and F is the absorption correction factor, which is calculated by  $F = 1 - 10^{-OD}$  (for OD at the irradiation wavelength). Ce6 in PBS as the standard ( $\Phi\Delta$  (Ce6) = 0.64).<sup>5</sup>

**Quartz Crystal Microbalance (QCM) Assays.** Gold electrodes were immersed in a micellar solution of 1 mg·mL<sup>-1</sup> PCT@HCCT. At 48 h, the electrodes were rinsed with deionized water three times and placed in the QCM chamber. Prior to the measurements, PBS was used to equilibrate until stabilization was achieved within  $\pm$  2 Hz in 30 min. Subsequently,  $5 \times 10^5$  B16F10 cells in PBS were injected into the chamber. The B16F10 cells, which were pretreated by molecular HA for 6 h, were used as the control group. The mass change on the electrode surface was proportional to the output oscillation frequency shift ( $\Delta f$ ), thus making the QCM sensor convenient in evaluating the interactions between PCT@HCCT and cells.

**Cellular Uptake Observed and Colocalization Imaging.** B16F10 cells were seeded in a 6-well plate at a density of  $1 \times 10^5$  cells per well. After incubated for 24 h

at 37 °C, the PCT@HCCT was added at a concentration of 5  $\mu$ M Ce6. Then cells were exposed to light irradiation (band pass: 400-700 nm, 5 mW/cm<sup>2</sup>) for 10 min after 6 h and were further incubated for other 3 h. The cells were washed with PBS, collected, and re-suspended in PBS. The samples were analyzed by flow cytometer (BD Biosciences, USA). Additionally, the cellular uptake was also measured via CLSM analysis (Leica TCS SP8, Leica, Germany). After the cell were exposed to light irradiation (band pass: 400-700 nm, 5 mW/cm<sup>2</sup>) for 10 min after 1 or 6 h and were further incubated for other 3 h. the medium was removed and cells were washed with PBS. After that, the MitoTracker Green was added for 30 min at 37 °C. The cells were imaged directly via CLSM (Leica TCS SP8, Leica, Germany) after repeated washing with PBS. To investigate the HA-receptor mediated internalization of PCT@HCCT, 10 mg/mL of free HA was incubated with B16F10 cells for 2 h before the addition of PCT@HCCT.

**TEM Analysis of Intracellular Transport and Tracking.** B16F10 cells were seeded in a culture bottle. When adhered to the dish, the cells were treated with PCT@HCCT solution and then incubated using 5% CO<sub>2</sub> at 37 °C. After the cells were exposed to light irradiation (band pass: 400–700 nm, 5 mW/cm<sup>2</sup>) for 10 min and were further incubated for another 6 h, they were harvested and washed three times with PBS, then further fixed at 4 °C with 2.5% glutaraldehyde for 4 h and postfixed with 1% aqueous osmium tetroxide. The cells were then dehydrated with increasing concentrations of ethanol (50, 70, 80, 90, 95, and 100%) for 15 min each, and embedded in Epon 812. The embedded samples were sectioned into slices (60–80 nm) using a sliding ultramicrotome (Leica UC7, Leica, Germany) and stained with uranyl acetate and lead citrate. The thin sections, supported by copper grids, were observed using a TEM system (HT7700, HITACHI, Japan). The platinum was confirmed based on EDS analyses (FEI Tecnai G<sup>2</sup> F20, FEI, USA).

**Intracellular ROS Detection.** The generation of ROS inside cells was detected using DCFH-DA as the sensor. The B16F10 cells were seeded in confocal dish or a 6-well plate at a density of  $10 \times 10^4$  cells per well. After incubation for 24 h, the cells were incubated with PCT@HCCT in the dark for 3 h. And then the cells were

exposed to light irradiation (band pass: 400-700 nm, 5 mW/cm<sup>2</sup>) for 10 min. After light, the cells were washed twice with PBS, and incubated with DCFH-DA (10 mM) for 20 min. The cells were repeatedly washed with PBS and then observed via Confocal Laser Scanning Microscope (CLSM, Leica TCS SP8, Leica, Germany) or were harvested by trypsinization, centrifuged (1500 rpm, 5 min), and re-suspended in PBS for detecting the fluorescence intensity of ROS via a flow cytometer (BD Biosciences, USA).

**Lysosomal Disruption.** B16F10 cells ( $1 \times 10^5$  cells per well) were seeded in a glass-bottom dish and incubated for 24 h, after that, the PCT@HCCT was added. Then cells were exposed to light irradiation (band pass: 400-700 nm, 5 mW/cm<sup>2</sup>) for 10 min after 3 h incubated. Afterwards, the cells were washed with PBS, and LysoTracker Green (10 nM) was further added for 10 min incubation at 37 °C. Finally, the cells were washed with PBS and observed via CLSM (Leica TCS SP8, Leica, Germany).

**Cytocompatibility Assay.** Cytocompatibility of blank micelles was evaluated by alamarBlue assay and live/dead staining. For alamarBlue assay, B16 or HUVEC cells were seeded onto 48- well plates at a density of  $1 \times 10^4$  cells per well. After 24 h preincubation, the cells were incubated with HCCT blank micelles for 24 h at a concentration of 10 µg/mL to 250 µg/mL, then the cells were washed by PBS and treated with 300 µL of alamarBlue solution (10% alamarBlue, 80% media 199 (Gibcos), and 10% FBS, v/v) for further 3 h incubation. The plate was read on an automated microplate spectrophotometer (ELX800 Biotek, USA) at an adsorption wavelength of 570 nm. Each experiment was performed in quintuplicate. Cell viability was calculated as cell viability (%) =  $(OD_{570}(\text{sample}) - OD_{570}(\text{blank}) / OD_{570}(\text{control}) - OD_{570}(\text{blank})) \times 100$ . The cytotoxicity study of PCT@HCCT with B16F10 cells under preset conditions were also evaluated through the similar method as described above. To determine PCT@HCCT micelles-induced cellular apoptosis, after the B16F10 cells were induced with PCT@HCCT under preset conditions, the cells were washed with PBS, costained with PI and Annexin V-FITC for 15 min at 37 °C, and then examined using flow cytometric measurement (BD Biosciences, USA) to



determine the apoptosis and necrosis ratio.

**Mitochondrion Membrane Potential Measurement.** The change of mitochondrion membrane potential was measured by JC-1 assay using CLSM and flow cytometry. For CLSM, the B16F10 cells were seeded in confocal dish at a density of  $1 \times 10^5$  cells per well. After incubated for 24 h at 37 °C, the PCT@HCCT was added. After 3 h, the medium was removed and cells were washed with PBS for three times. Then cells were exposed to light irradiation (band pass: 400-700 nm, 5 mW/cm<sup>2</sup>) for 10 min. After light, the cells were incubated for 30 min. Then the mitochondria were stained with JC-1 (10 μM) for 20 min at 37 °C. Cells were washed twice with PBS three times again and were examined by CLSM (TCS SP5, Germany). For flow cytometry, after PCT@HCCT was internalized by B16F10 cells for 3 h, the cells were rinsed with PBS thoroughly. Then light irradiation was performed for 10 min. 30 min later, the cells were collected and re-suspended in 0.6 mL JC-1 solution (10 μM) for 20 min. The cells were washed with PBS and analyzed by flow cytometer (BD Biosciences, USA). To investigate the influence of ROS generation, B16F10 cells were pre-incubated with 0.5 mM Vitamin C (Vc) for 2 h before the addition of PCT@HCCT.

***In vivo* Target Ability.** Balb/c nude mice were i.v. injected with  $1 \times 10^6$  B16F10 cells. One week later, PBS and various micelles (including PCT@HCCT and PCT@mPEG-PCL) at an equal dose of 5.0 mg Ce6/kg body weight were intravenously injected into B16 tumor bearing nude mice. Among them, PBS and PCT@mPEG-PCL treated as blank control group and non-target control group respectively. Then mice were anesthetized and imaged via IVIS imaging system (Xenogen, Alameda, CA) at 1 h, 6 h, and 12 h post injection, then mice were sacrificed to separate the organs and tumors for ex vivo fluorescence imaging.

***In vivo* Antitumor Efficacy.** C57 mice bearing B16F10 tumor of  $110 \pm 7$  mm<sup>3</sup> were randomly divided into five groups (eight mice per group). PBS, HCCT, PCT@HCCT, and PCT@mPEG-PCL were intravenously injected into mice every 2 days for 3 times. PCT dosage was 3.63 mg/kg body weight. Six hours post injection, the mouse grouped injected with PCT@HCCT and PCT@mPEG-PCL were light by

650 nm laser for 20 min at photodensity of 250 mW/cm<sup>2</sup>. The first day of treatment was defined as day 0. The body weight and tumor volume were recorded every 2 days, and the survival of mice were monitored throughout the experiment. The tumor volume was calculated by formula  $V = X \times Y \times Y/2$  (X, the longest dimension; Y, the shortest dimension).

**Western Blotting Analysis.** B16F10 cells with density of  $1 \times 10^6$  were seeded in 6-well plate. After 24 h preincubation, PCT@HCCT was added. After 3 h incubation, the cells were exposed to light irradiation (band pass: 400-700 nm, 5 mW/cm<sup>2</sup>) for 10 min or not. After 4 h incubation, cells were washed in PBS and the pellets lysed in a buffer containing Triton X-100 and protease inhibitors. Proteins were recovered and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Then, proteins were transferred to a PVDF membrane and blocked with 5% fat-free milk. The primary antibodies (dilution) were incubated overnight at 4 °C, as follows: cytochrome C (abcam, 1/1000), caspase 3 (CST, 1/1000) or GAPDH (Mouse, Sigma) (1/1000), followed by secondary antibody incubation. Finally the proteins were detected using ECL reagent according to the manufacturer's instructions.

**Immunohistochemical Analyses.** At day 15, one mouse in each group was sacrificed to separate the tumors, which were then fixed in 4% formaldehyde solution in PBS. For immunohistochemical staining, the fixed tumors were dehydrated by gradient ethanol and embedded in paraffin block. Haematoxylin and eosin (H&E) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) were performed by the Pathology core (Yale University, New Haven, CT).

**Statistical analysis.** Results were expressed as means  $\pm$  S. D., the single factorial analysis of variance (ANOVA) was performed was used to determine the significance of the difference data. The statistical significance were considered significant for  $p$  values  $**p < 0.01$ ,  $^{\#}p > 0.05$ .

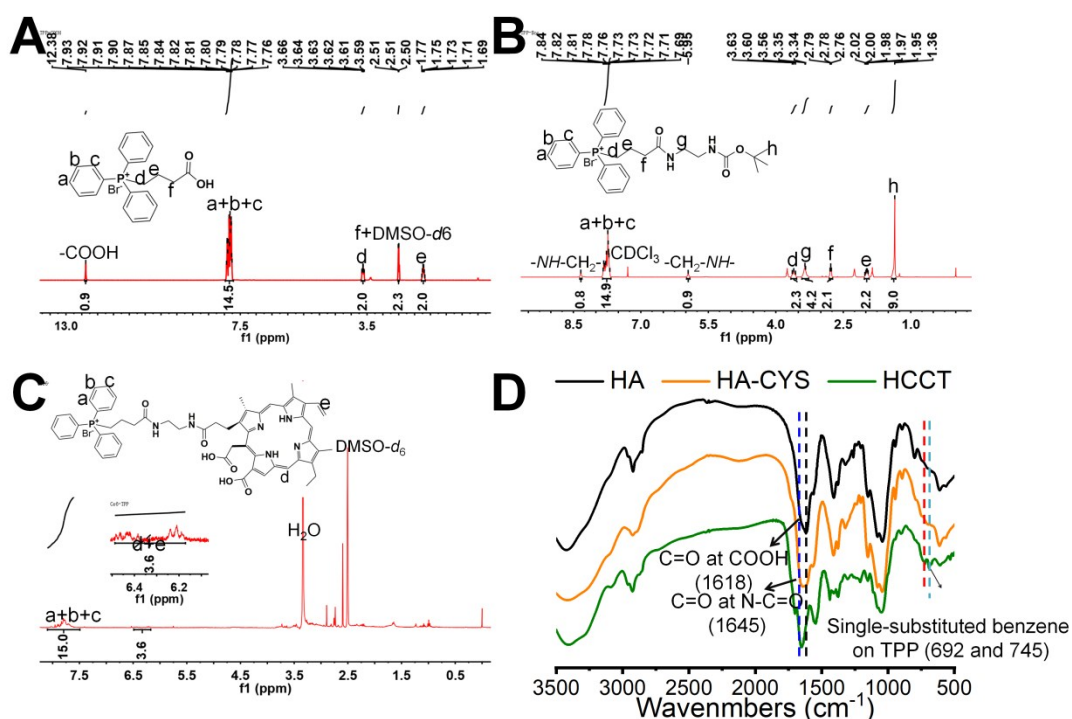
## Reference

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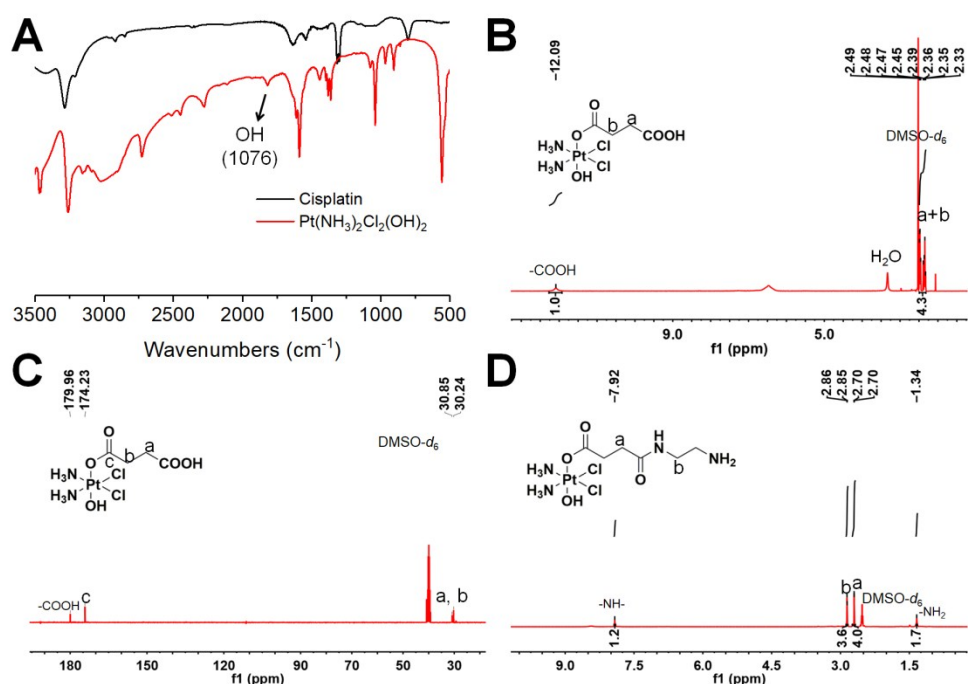
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**Table S1.** Characterization of PCT@HCCT.

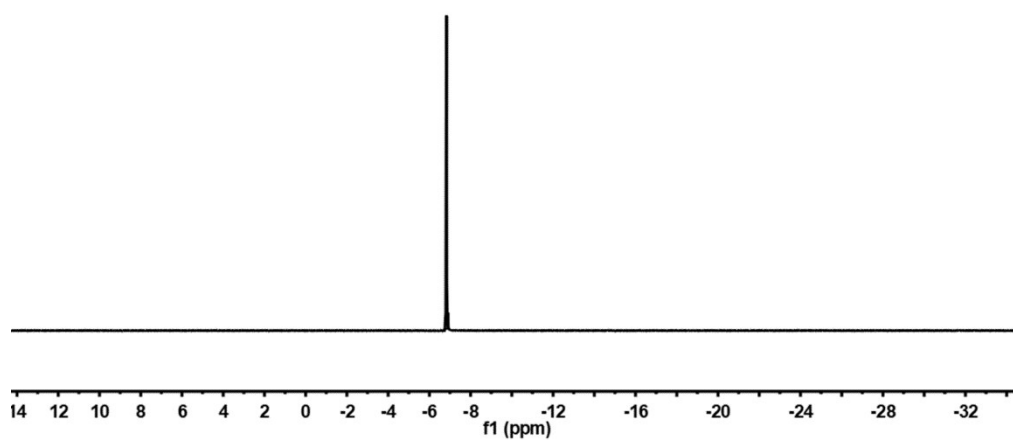
Entry	PCT / HCCT (w/w)	Size (nm) / PDI <sup>a</sup>	DLC <sup>b</sup> (%)	EE <sup>c</sup> (%)
1	0.15/1	122/0.236	10.6	79.3
2	0.25/1	124/0.213	15.3	72.0
3	0.35/1	136/0.206	17.6	61.2

<sup>a</sup> Polydispersity index (PDI).<sup>b</sup> Drug loading content (DLC).<sup>c</sup> Encapsulation efficiency (EE).

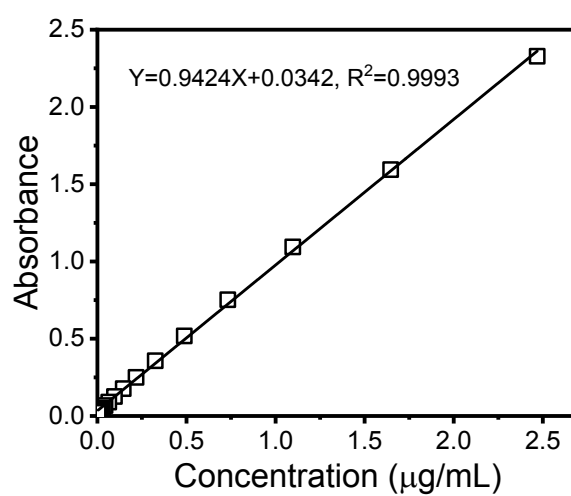
**Fig. S1.** (A) <sup>1</sup>H NMR spectrum of TPP-COOH in CDCl<sub>3</sub>: δ 12.38 (s, -COOH), 7.93-7.76 (m, 3 × -C<sub>6</sub>H<sub>5</sub>), 3.66-3.59 (m, -CH<sub>2</sub>-CH<sub>2</sub>-), 2.51-2.48 (m, -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-), 1.77-1.68 (m, -CH<sub>2</sub>-COOH). (B) <sup>1</sup>H NMR spectrum of TPP-Boc in DMSO-*d*<sub>6</sub>: δ 8.33 (s, -NH-CH<sub>2</sub>-), 7.84-7.69 (m, 3 × -C<sub>6</sub>H<sub>5</sub>), 5.95 (s, -CH<sub>2</sub>-NH-), 3.63-3.56 (m, -CH<sub>2</sub>-CH<sub>2</sub>-), 3.35-3.34 (m, -NH-CH<sub>2</sub>-CH<sub>2</sub>-NH-), 2.79-2.76 (m, -CH<sub>2</sub>-CO-), 2.02-1.95 (m, -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-), 1.36 (s, 3 × -CH<sub>3</sub>). (C) <sup>1</sup>H NMR spectrum of Ce6-TPP in DMSO-*d*<sub>6</sub>: δ 8.03-7.67 (m, 3 × -C<sub>6</sub>H<sub>5</sub>), 6.18-6.54 (m, -CH=CH<sub>2</sub>, 3 × -CH=C<sub>2</sub>NH-). (D) FT-IR spectra of HA, HA-CYS and HCCT, respectively.



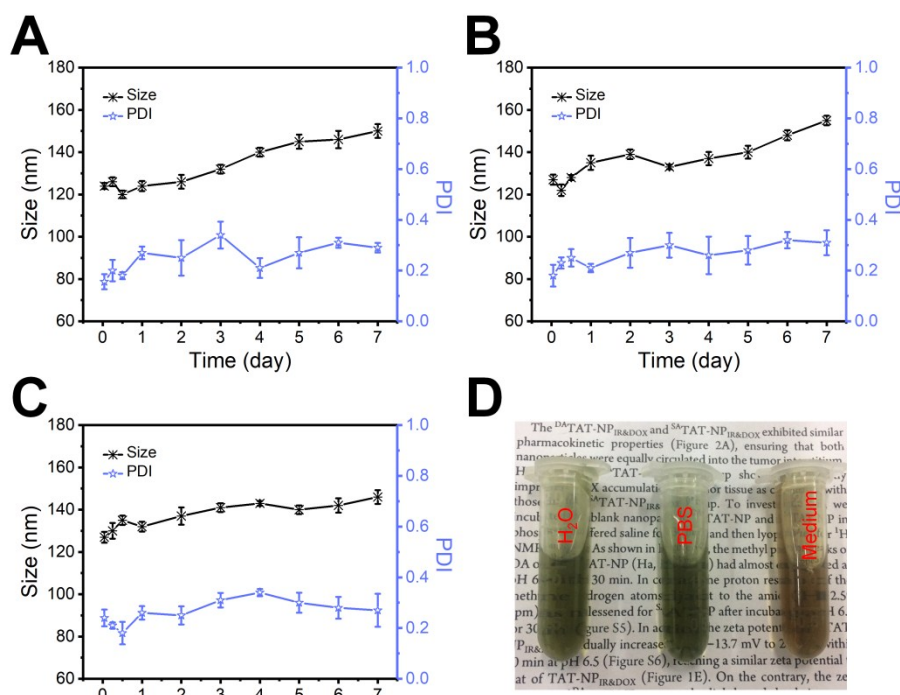
**Fig. S2.** (A) IR spectra of cisplatin and  $\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{OH})_2$ . Clear appearance of hydroxyl ( $1076\text{ cm}^{-1}$ ) was observed in  $\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{OH})_2$ . (B)  $^1\text{H}$  NMR spectrum and (C)  $^{13}\text{C}$  NMR spectrum of  $\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{OH})(\text{OCOCH}_2\text{CH}_2\text{COOH})$  (Cisplatin-COOH) in  $\text{DMSO}-d_6$ :  $\delta_{\text{H}}$  12.09 (s, -COOH), 2.49-2.33 (m,  $-\text{CH}_2-\text{CH}_2-\text{COOH}$ );  $\delta_{\text{C}}$  179.96 (-COOH), 174.23 (-COO-), 30.85 ( $-\text{CH}_2-\text{COOH}$ ), 30.24 ( $-\text{OCO}-\text{CH}_2-$ ). (B)  $^1\text{H}$  NMR spectrum of cisplatin- $\text{NH}-\text{NH}_2$  in  $\text{DMSO}-d_6$ :  $\delta_{\text{H}}$  7.92 (s, -NH-), 2.85 (d,  $-\text{NH}-\text{CH}_2-\text{CH}_2-\text{NH}_2$ ), 2.70 (d,  $-\text{CO}-\text{CH}_2-\text{CH}_2-\text{CO}-$ ), 1.34 (s,  $-\text{NH}_2$ ).



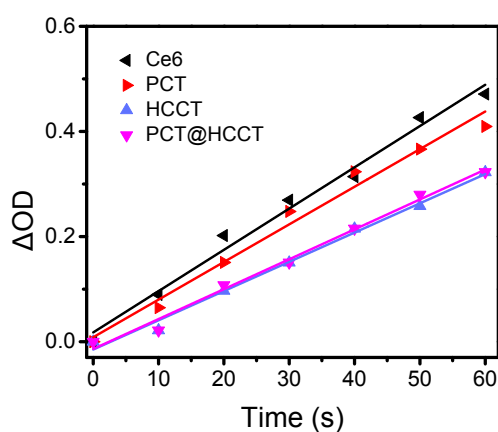
**Fig. S3.**  $^{31}\text{P}$  NMR spectrum of TPP-Ce6 in  $\text{DMSO-}d_6$ .



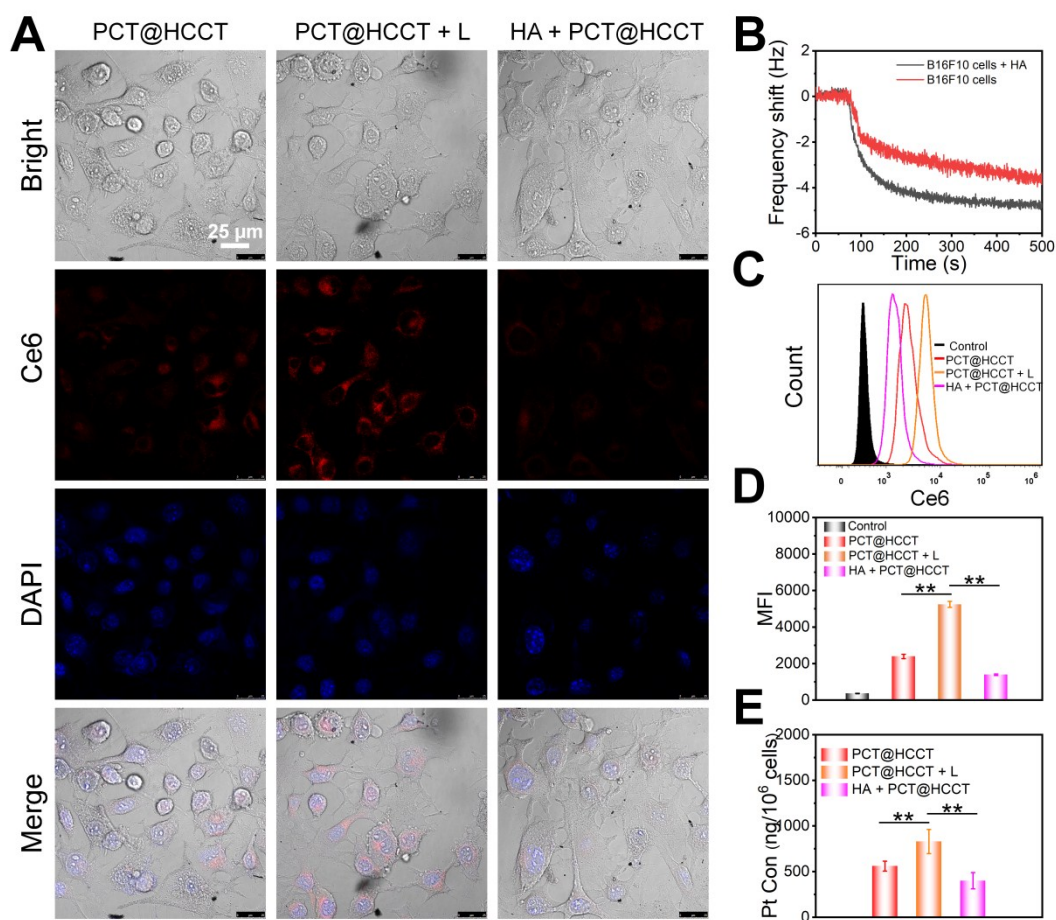
**Fig. S4.** Grafting rate at  $\lambda = 405$  nm of Ce6 in DMSO by UV-vis spectrophotometer.



**Fig. S5.** Stability assay of PCT@HCCT (A) in H<sub>2</sub>O solution, (B) in PBS (pH = 7.4) and (C) in 10% FBS-containing solution after 7-day storage at room temperature. (D) The photographs of PCT@HCCT suspended in corresponding conditions after 7-day storage at room temperature.



**Fig. S6.** <sup>1</sup>O<sub>2</sub> production from a plot of changes in absorbance by DPBF at 407 nm against different irradiation time (with 650 nm laser) in the presence of PCT (red) in DMSO and HCCT (blue) and PCT@HCCT (purple) in PBS solution with Ce6 (black) as a standard.



**Fig. S7.** In vitro evaluation of active targeting capability. **(A)** Cell internalization of PCT@HCCT towards B16F10 cells under various conditions. **(B)** Adsorption behavior of B16F10 cells, which was pre-incubated with or without HA, to PCT@HCCT. **(C)** Flow cytometry analysis of PCT@HCCT after incubation with B16F10 cells under various conditions for 6 h. **(D)** Mean fluorescence intensity (MFI) of B16F10 cells treated with PCT@HCCT under various conditions for 6 h. **(E)** Quantitative analysis of the Pt in cells by ICP-MS. Data are shown as mean  $\pm$  SD ( $n = 3$ ), \*\* $p < 0.01$  denote statistically significant differences.



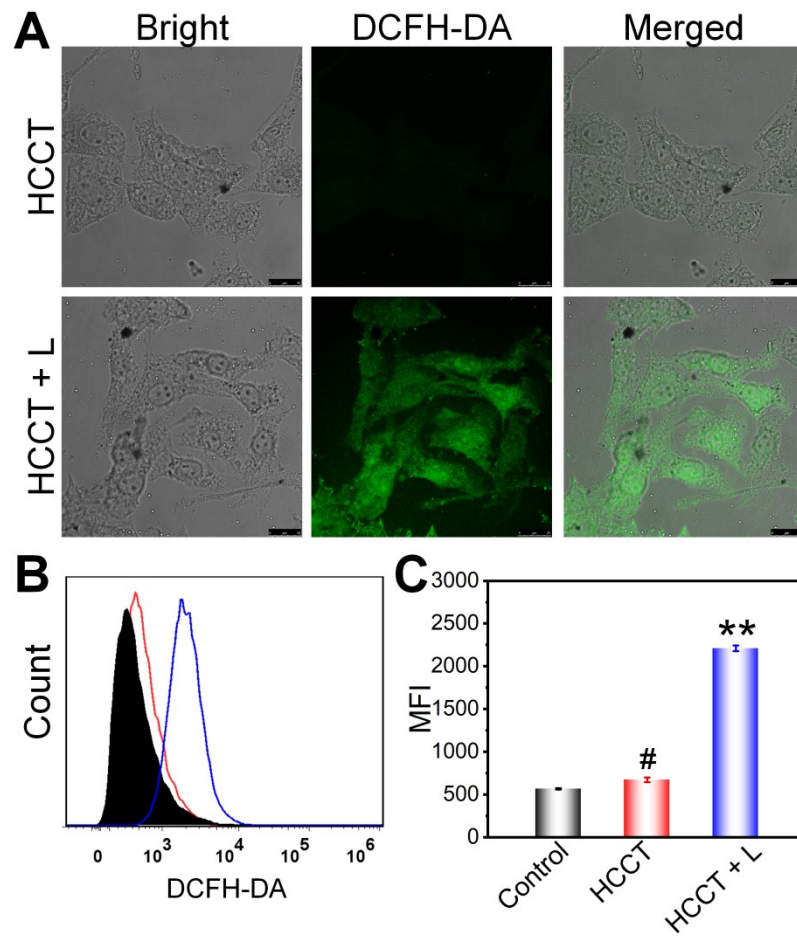
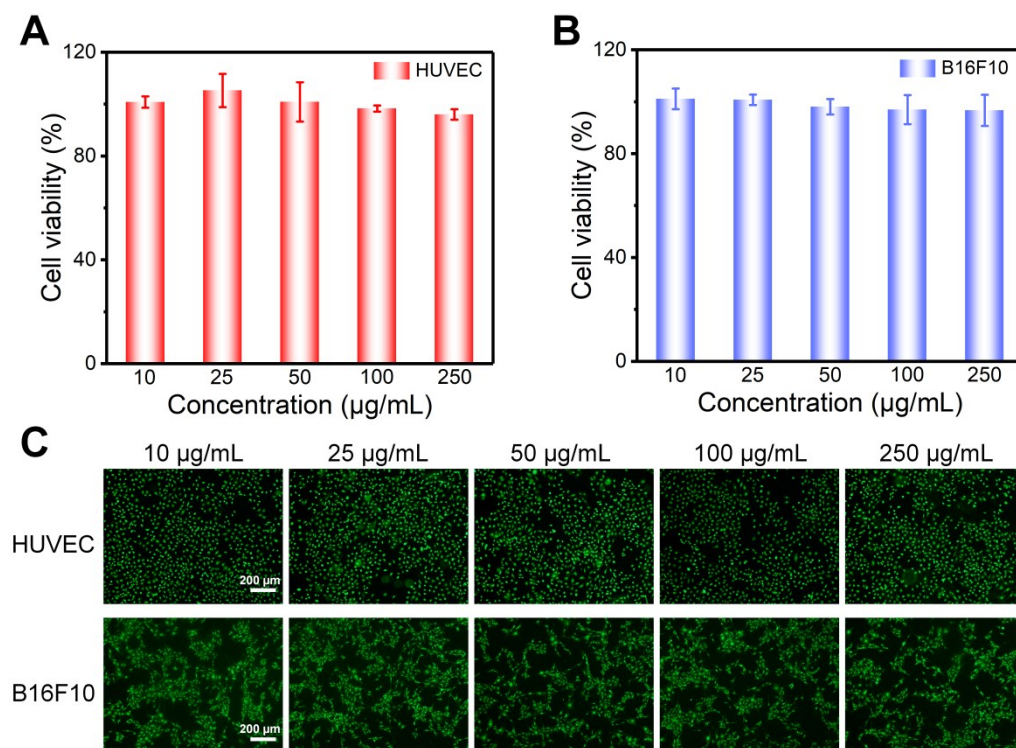
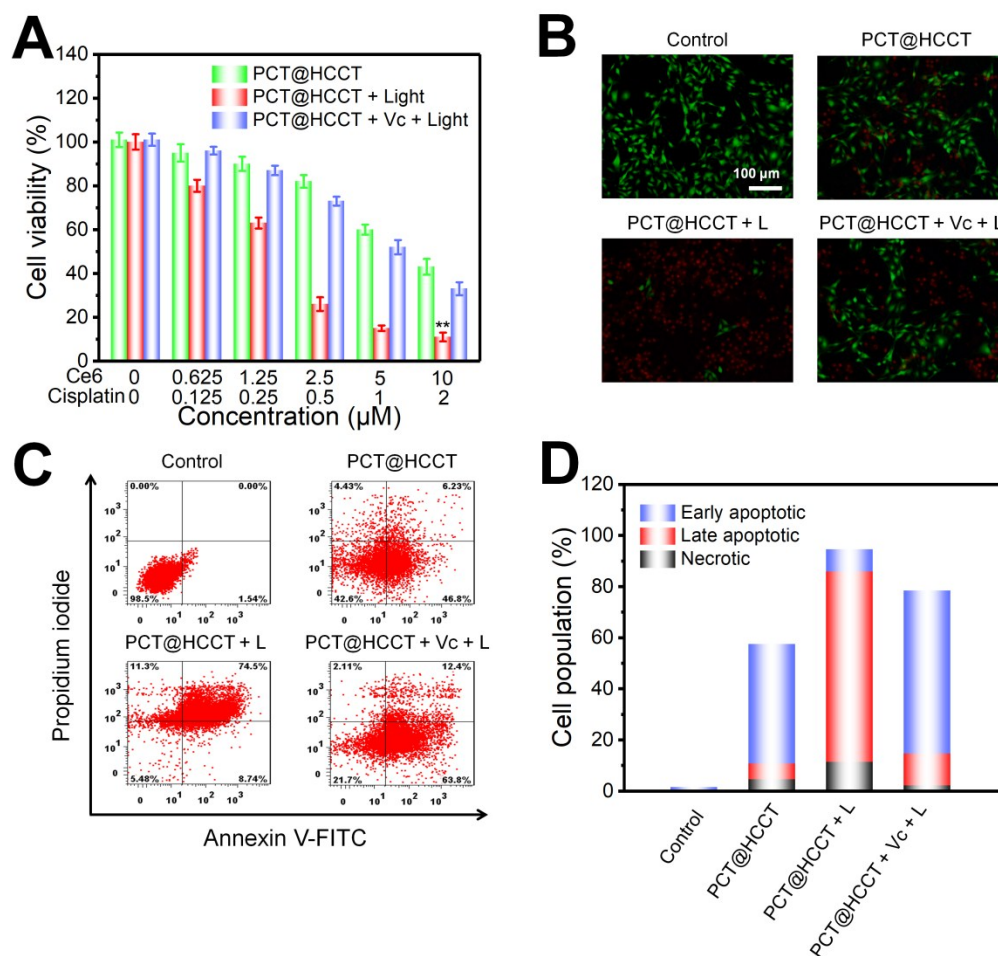


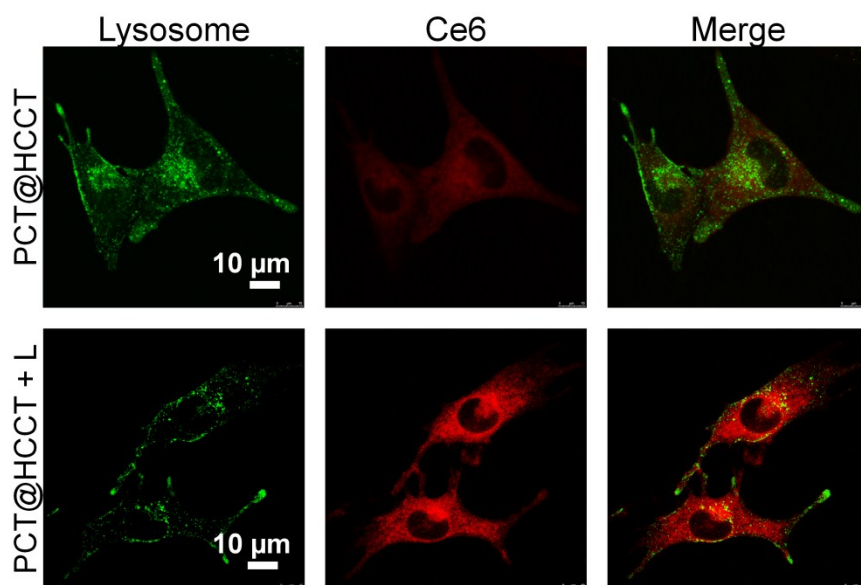
Fig. S8. CLSM images of HCCT micelles-induced ROS generation in B16F10 cells under various conditions. \*\* $p < 0.01$  and # $p > 0.05$  vs control (n = 3).



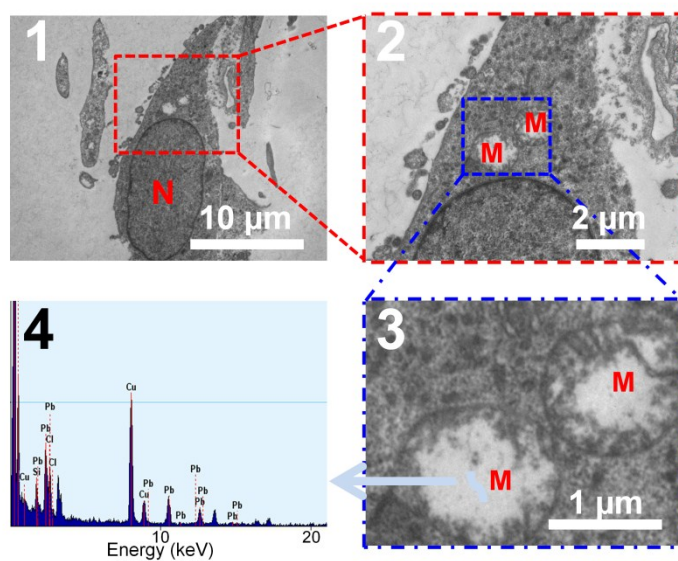
**Fig. S9.** (A) Cell viability of HUVEC cells and (B) B16F10 cells after incubation with blank micelles HCCT at different concentrations for 24 h. (C) Fluorescence images of HUVEC cells and B16F10 cells after treating with blank micelles HCCT at different concentrations for 24 h. The live cells were stained green. Data are shown as mean  $\pm$  SD (n = 3).



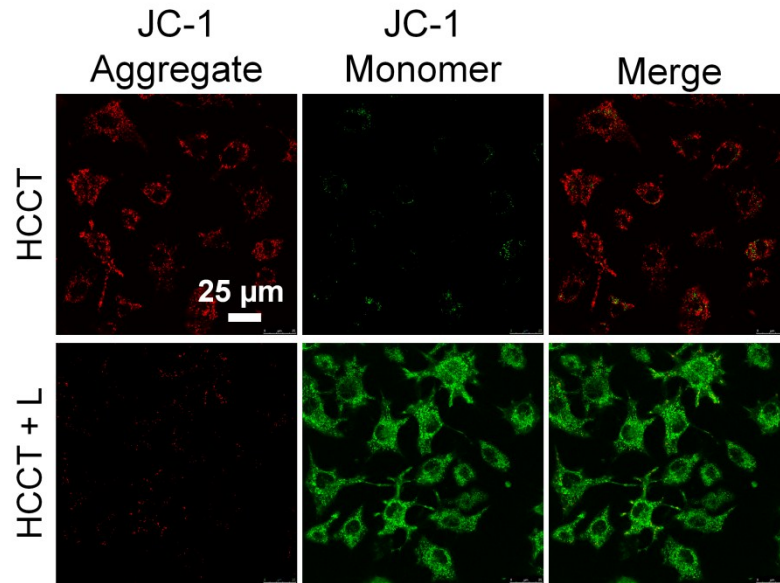
**Fig. S10.** In vitro cell viability. **(A)** Relative viabilities of B16F10 cells treated with PCT@HCCT at various doses after 24 h incubation. **(B)** Relative viability of B16F10 cells treated with PCT@HCCT after 24 h incubation with/without light irradiation (10 min) or with light irradiation (10 min) in the presence of 0.5 mM Vc. **(C)** Flow cytometry analysis in B16F10 showing the cell apoptosis by Annexin V-FITC/PI double-staining assay. Cells were treated with PCT@HCCT after 24 h incubation with/without light irradiation (10 min) or with light irradiation (10 min) in the presence of 0.5 mM Vc. Green FITC fluorescence and red PI fluorescence indicate live and dead cells, respectively. **(D)** Population of early apoptotic, late apoptotic, and necrotic B16F10 cells summarized from flow cytometry analysis. Data are shown as mean  $\pm$  SD ( $n = 3$ ),  $**p < 0.01$  denote statistically significant differences.



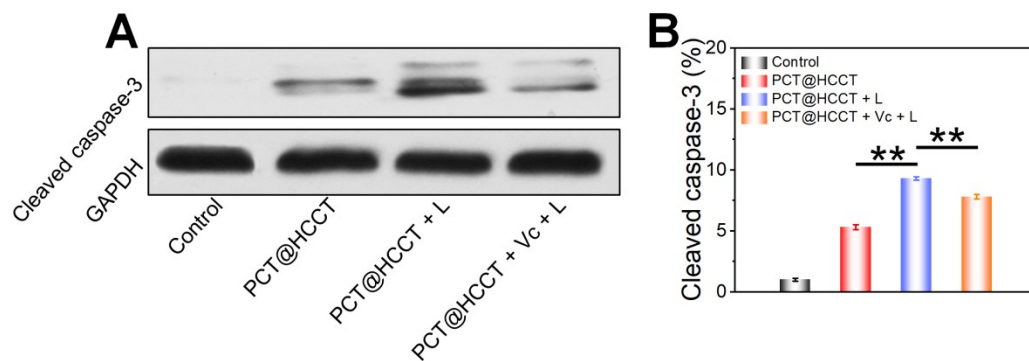
**Fig. S11.** Lysosomal escape of PCT@HCCT micelles. CLSM images of B16F10 cells incubated with PCT@HCCT with or without light irradiation. The lysosomes of the cells were stained with LysoTracker (green).



**Fig. S12.** (1-3) Mitochondria ultrastructure by TEM. (1-3) B16F10 cells were incubated PBS. (4) Elemental analysis of mitochondria. N: Nucleus; M: Mitochondria.

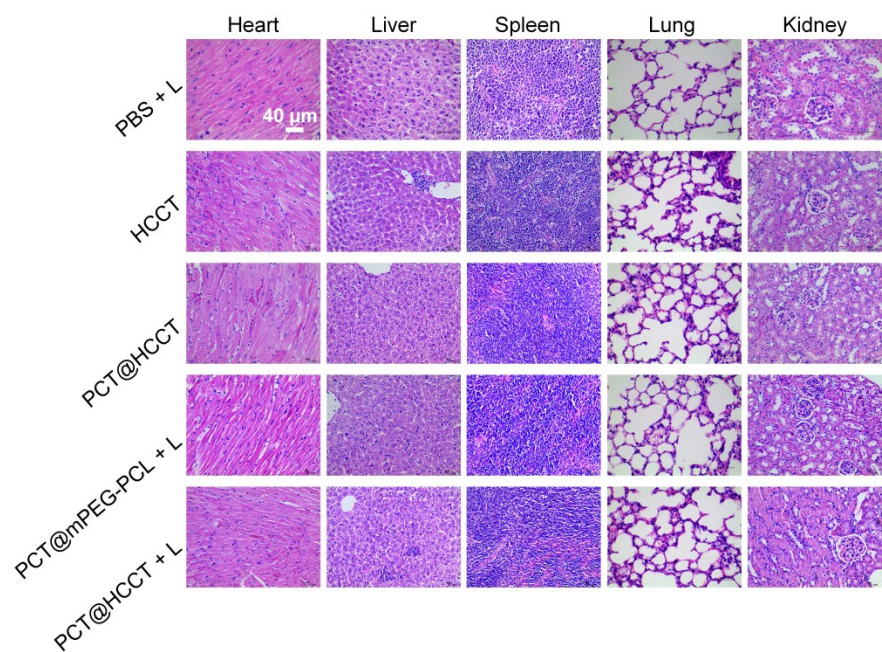


**Fig. S13.** CLSM images of  $\Delta\psi_m$  shift as determined by JC-1 assay of B16F10 cells.



**Fig. S14. (A)** Representative cleaved caspase-3 protein was analyzed by western blot. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. **(B)** Cleaved caspase-3 protein relative to GAPDH was determined by Image J, respectively.  $**p < 0.01$  denote statistically significant differences.





**Fig. S15.** H&E staining assay of heart, liver, spleen, lung, and kidney collected at day 15 post-intravenous injection.