Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Nucleus-selective Self-Augmenting Cascade Nanoassemblies for Targeted Synergistic Photo-Chemo Therapy of Tumor

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1. General

1.1 Materials and Reagents

All chemical reagents were used as supplied without further purification unless otherwise specified. Chlorin e6 (Ce6) was purchased from Frontier Scientific, Inc. (Salt Lake City, UT, USA). Human Serum Albumin (HSA) and 4, 6-diamidino-2-phenylindole (DAPI) were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Cell Counting Kit-8 (CCK-8) was purchased from Chongqing Bioground Biotechnology Co., Ltd. (Chongqing, China). Hydrogen peroxide (H_2O_2) was purchased from Chengdu Jinshan Chemical Reagent Co., Ltd. (Chengdu, China). Catalase (CAT) were purchased from Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). 1-ethyl-3(3-dimethylpropylamine) carbodiimide (EDCI), cisplatin ($PtCl_2(NH_3)_2$) and 1-Adamantanecarboxylic acid were purchased from Meryer Chemical Technology Co., Ltd. (Shanghai, China). N-hydroxysuccinimide (NHS), sodium azide (NaN_3), methyl-β-cyclodextrin (M-β-CD), genistein were purchased from Bide Pharmatech Ltd. (Shanghai, China). Chlorpromazine (CHP) was obtained from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Ivermectin was obtained from Aladdin Reagent Co., Ltd. (Shanghai, China).

1.2 Instruments

NMR data were recorded on Agilent 400MR-DD2. DLS, Zeta was measured by NanoBrook Omni. Tissue sections of H&E were imaged by Leica DMi8. Absorbance was recorded by Agilent Cary60. Cell flow cytometry was performed on CytoFLEX. In vivo fluorescence was analyzed with an IVIS Lumina imaging system (PerkinElmer). Mass spectra were performed on Waters Acquity SQD. Transmission electron microscope (TEM) was conducted by Hitachi H-7500 electron microscope.

2 Experimental Section

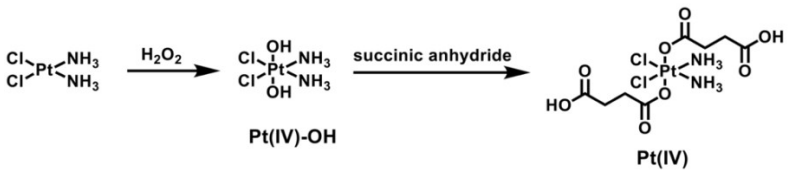

Fig. S1. Synthetic routes of Pt(IV) prodrug.

Synthesis of Pt(IV) prodrug

Cisplatin (200 mg, 0.6 mmol) was suspended in water (6 mL). Then, excess of H_2O_2 (30%, 10 mL) was added and stirred at 50 °C for 1 h. Recrystallization of the obtained **Pt (IV)-OH** was performed in situ, collected, and washed with cold water, ethanol, and ether, and then dried. A solution of **Pt (IV)-OH** (100 mg, 0.3 mmol) in dry DMF (2 mL) was added to

succinic anhydride (75 mg, 0.75 mmol). The mixture was stirred at 70 °C in the dark for overnight, and the cisplatin prodrug **Pt(IV)** was obtained.

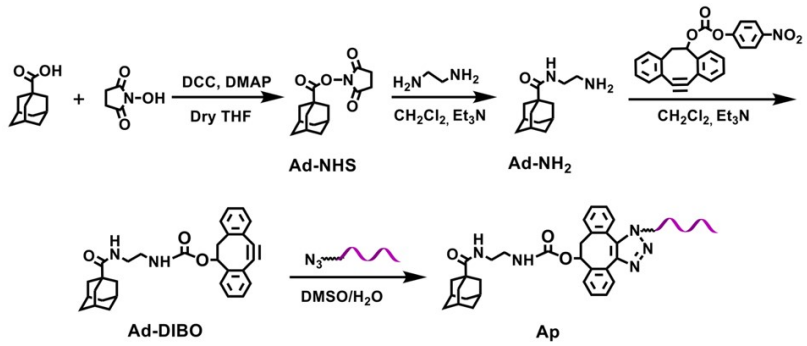

Fig. S2. Synthetic routes of adamantane modified AS1411 aptamer (Ap).

Synthesis of Ad-DIBO

1-Adamantanecarboxylic acid (2.5 g, 13 mmol), N-hydroxysuccinimide (1.9 g, 13 mmol) and dicyclohexylcarbodiimide (2.6 g, 13 mmol) were dissolved in dry THF. The catalytic amount of 4-dimethylamino-pyridine was added to the mixed solution and stirred at room temperature for 12 h. The reaction mixture was filtered to remove DCU byproducts, and THF was evaporated to obtain crude products. NHS ester (Ad-NHS) was obtained by column chromatography with petroleum ether/ethyl acetate (v/v = 5:1) in 60% yield. Add a solution of **Ad-NHS** (500 mg, 1.8 mmol) in DCM dropwise to the mixture solution ethylenediamine (1.1 g, 18 mmol) and triethylamine (912 mg, 9 mmol) in DCM. After stirring 2 h at room temperature, the mixture was washed with saturated solution of sodium chloride (5×50 mL) and the organic layer was collected and dried to obtain compound **Ad-NH_2** in 50% yield. **Ad-NH_2** (200 mg, 8.9 mmol) and DIBO (519 mg, 13.3 mmol) were dissolved in DCM. Triethylamine (136 mg, 13 mmol) was added into the mixture solution and stirred at room temperature for 12 h. The mixture solution was washed with saturated sodium carbonate solution (2×50 mL) and solvent removal to afford the crude product, which was further purified by column chromatography with petroleum ether/ethyl acetate (v/v = 1:1) as eluent to get compound **Ad-DIBO** as white solid in 50% yield.

Preparation of Ap

100 μL Ad-DIBO (200 μM) in DMSO was mixed with 50 μL AS1411 aptamer (5'-N_3-TTTTTTTTGGTGGTGGTGGTTGTGGTGGTGGTGG-3') (50 μM) in distilled water. After incubating at 4 °C overnight, DMSO was removed through dialysis in deionized water. The resulting product was stored at 4 °C for further use.

Preparation of HPC

To prepare Ce6 active Ester and Pt (IV) active Ester. Ce6 (1 mg, 0.16 μmol), EDC (0.32 mg, 0.16 μmol) and NHS (0.21 mg, 0.18 μmol) were mixed in DMSO (100 μL) under room temperature for 2 h. Similarly, Pt (IV) (1 mg, 0.18 μmol), EDC (0.35 mg, 0.18 μmol), and NHS (0.23 mg, 0.2 μmol) were mixed in DMSO (100 μL) under room temperature for 2 h. Then, to prepare HPC. Ce6 active Ester and Pt (IV) active Ester ware added into 2 mL phosphate buffered saline (PBS, 10 mM, pH=7.4) containing HSA (12 mg) under magnetic stirring in the dark for overnight. The solution was ultrafiltered by a centrifugal filter device (molecular weight cut-off MWCO = 10 kDa) three times to remove free Ce6 and Pt (IV).

Agarose gel electrophoresis

For study the loading Efficiency of proteins, the different samples including free HPC, free CAT and the mass ratios of CL: HPC: CAT: was 1:1:1, 2:1:1, and 3:1:1, 4:1:1, 5:1:1, respectively. The whole preparation process was simple and easy to operate without additional separation and purification steps. The resulting complexes were electrophoresed on the 1.5% (W/V) agarose gel for 40 min, following stained by Coomassie Blue to verify the encapsulation of proteins. The loading efficiency of proteins was calculated by the following equation: Loading Efficiency = (weight of protein in the nanoformulations/weight of nanoformulations) x 100%

Dye Labeling of CAT and HPC

For the cellular uptake and biodistribution studies, CAT and HPC were labeled with fluorescein isothiocyanate (FITC) and Cy7 respectively. Briefly, CAT (0.5 mg) and HPC (1 mg) were dissolved in sodium bicarbonate solution (0.1 M, pH=9), and mixed with 10 times excessive FITC-NHS and Cy7-NHS solution respectively. The reaction mixture was sheltered from light and stirred overnight at 4 °C. The resulting dye labeled CAT-FITC and HPC-Cy7 were purified by dialysis and kept at 4 °C for further use.

Singlet oxygen ($^1O_2$) detection by SOSG

To measure $^1O_2$ generation efficiency, samples including HPC, HPC/CL, HPC-CAT/CL and HPC-CAT/CL-Ap+H_2O_2 were incubated in water solution, respectively. Singlet oxygen sensor green reagent SOSG (1 μM) was added to each sample and the fluorescence was recorded at 530 nm under 494 nm excitation after irradiation (660 nm, 100 mW cm^{-2}).

Cellular uptake

To investigate the cellular internalization of nanoparticles, breast cancer cells 4T1 cells were seeded in 96-well plates and incubated 12 h at 37 °C under 5% CO_2. Discard the original culture medium and wash 2-3 times with PBS. Then, 100 μL fresh medium containing HPC, HPC/CL, HPC-CAT/CL or HPC-CAT/CL-Ap (7 μg/mL for Ce6 and 5 μg/mL for CAT-FITC)

was then added. After incubation for 2 hours, discard the culture medium and fix the cells with 4% paraformaldehyde. After 10 minutes, the nucleus was stained with DAPI and imaged using a fluorescence microscope. For flow cytometry, the cells were trypsinsized, washed, and dispersed in PBS and then analyzed on a BD FACS Calibur.

Endocytosis mechanism

4T1 cells (density of 1×10^4 cells/well) were seeded in 96-well plates and incubated 12 h at 37 °C under 5% CO_2. The cells were incubated with different endocytosis inhibitor (10 mM NaN_3, 25 μM chloropromazine (CHP), 10 mM M-β-CD or 200 μM genistein) or placed at 4 °C for 1 h, and then HPC-CAT/CL-Ap was added and incubated at 37 °C or 4 °C for another 2 h. After that cells were trypsinsized and collected for flow cytometry analysis.

Time-lapsed cell imaging

4T1 cells were seeded in 96-well plates and incubated overnight. HPC-CAT/CL-Ap was added into the plate and observed at 0, 5, 10, 20, 40 min by fluorescent microscopy.

Nucleus transport mechanism

To explore the nuclear transport mechanism of HPC-CAT/CL-Ap nanoparticles, 4T1 cells were preincubated with ivermectin (15 μM) for 2 h and then HPC-CAT/CL-Ap was added for further incubating another 2 h. After that, the cells were stained with DAPI and imaged with fluorescent microscopy.

In vitro ROS Detected

2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) was utilized to detect intracellular ROS. Briefly, 4T1 cell was seeded in 48-well plate and cultured overnight. Afterward, the cells were incubated with HPC, HPC/CL, HPC-CAT/CL and HPC-CAT/CL-Ap for 6 h, then removing the medium and washing 3 times with PBS. DCFH-DA was added and coincubated for 30 min. After irradiation by a 660 nm laser (100 mW cm^{-2}) for 2 min, the cells were collected for flow cytometry.

Detection of cisplatin in the cells by using ICP-MS

4T1 cells were seeded in 6-well culture plates and incubated overnight at 37 °C. Then, the cells were incubated with HPC, HPC/CL, HPC-CAT/CL and HPC-CAT/CL-Ap for 2 h. After that, cells were washed with PBS three times, and then lysed by using cell lysis buffer. Thereafter, it was subjected to centrifugation at 2×10^4 rpm for 15 min. The supernatant was collected for ICP-MS testing of cisplatin contents.

In vitro cytotoxicity

To explore the synergistic effect of chemotherapy and photodynamic therapy, 4T1 cells (density of 1×10^4 cells/well) were seeded in 96-well plates and incubated for overnight. Then

the medium was replaced with 100 uL fresh medium containing with various concentrations cisplatin, HPC/CL and HPC/CL with laser (L+). After 24 h incubation, the cell viability was measured by performing CCK-8 assays. To study the effect of CAT on photodynamic therapy, 4T1 cells were seeded into 96-well plates and incubated for overnight with normoxic (21% O_2) and hypoxic (1% O_2) conditions. Then, 100 uL fresh medium containing HPC, HPC/CL, HPC-CAT/CL and HPC-CAT/CL-Ap was added into the wells for 24 h before without laser or with laser (660 nm, 100 mW cm^{-2}, 2 min). The cell viability was measured by CCK-8 assays.

Animal Model

All animal experiments were carried out in compliance with the requirements of the National Act on the use of Experimental Animals (People's Republic of China) and were approved by the Experimental Animal Ethical Committee of Chongqing University Cancer Hospital. Female BALB/c-nude mice (6-8 weeks) and female BALB/c mice (6-8 weeks) were supplied by the Animal Center of Chongqing Medical University (Chongqing, China).

Biodistribution

Breast cancer 4T1 cells (2×10^6) were injected subcutaneously into female BALB/c-nude mice. When the tumor volume grew to approximately 100 mm^3. 4T1 tumor-bearing mice were intravenously injected with HPC-Cy7, HPC-Cy7-CAT/CL or HPC-Cy7-CAT/CL-Ap (0.9 mg/kg for Ce6, 1 mg/kg for CAT). The mice were imaged at different time points (2, 4, 6, 12, 24 h postinjection) by small animal imaging system (IVIS Lumina III, USA). 24 h after administration, the mice were sacrificed. Major organs (heart, liver, spleen, lung and kidney) and tumors were imaged and analyzed by an IVIS Lumina imaging system. The excitation wavelength for imaging is 740 nm and the emission wavelength is 790 nm.

In vivo antitumor activity prevents lung metastasis

For anti-tumor therapy studies, breast cancer 4T1 cells (2×10^6) were injected subcutaneously into female BALB/c-nude mice. When the tumor volume grew to approximately 50 mm^3, the mice were randomly divided into 6 groups (n=6): (1) Control group, (2) free Pt group, (3) HPC group with laser, (4) HPC/CL group with laser, (5) HPC-CAT/CL-Ap group without laser (L-) and (6) HPC-CAT/CL-Ap group with laser. 150 μL of different samples with equal amount of Pt (0.8 mg/kg), Ce6 (0.9 mg/kg) were intravenously injected into the mice every three days for a total of four times. The tumor sizes of the mice were measured every other day during the experiment. The tumor volumes value was calculated as the following equation: tumor volumes = width $(mm)^2$ × length (mm) ×1/2. At the end of experiment, the tumor was excised and weighed. For histological examination,

tumor tissues and major organs (heart, liver, spleen, lung and kidney) were collected for hematoxylin and eosin (H&E) staining. The lung metastasis was imaged by bioluminescence, analyzed via an IVIS Lumina imaging system after intraperitoneal injection of D-luciferin solutionon the 25st day. At the end of experiment, the lungs were harvested, photographed and hematoxylin and eosin (H&E) staining for evaluate the anti-lung metastasis efficacy.

Statistical analysis

Experimental data were presented as the mean ± standard deviation (SD) of at least three independent experiments. Analyses were performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA). Statistical analysis was conducted using one-way ANOVA or Student's t-test. Statistical significance is indicated as *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

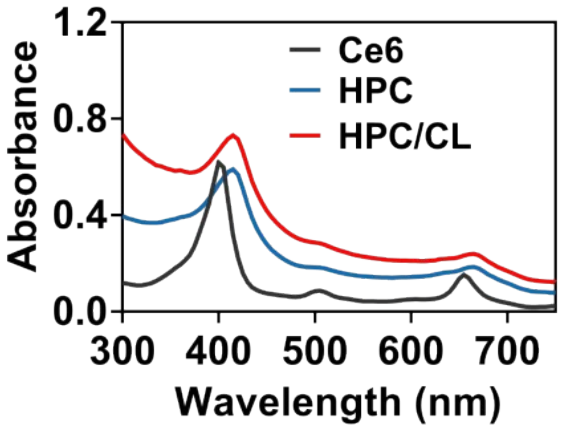

Fig. S3. UV-Vis absorption of Ce6, HPC and HPC/CL nanocomplex.

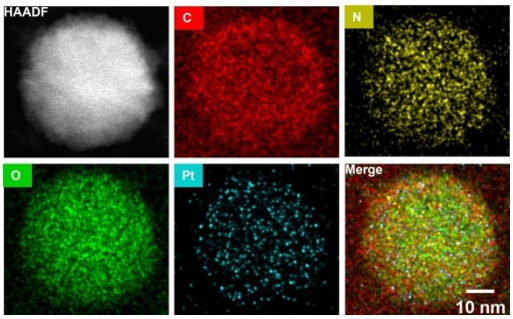

Fig. S4. EDS element analysis diagram of HPC-CAT/CL-Ap nanoparticles. Scale bars: 10 nm.

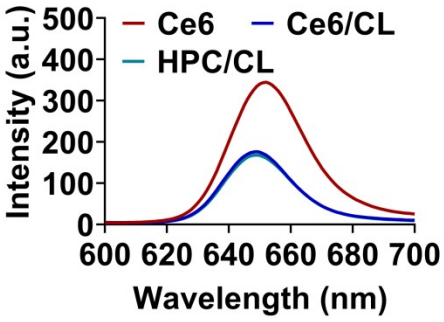

Fig. S5. The fluorescence intensity of Ce6 in different states, including free Ce6, Ce6/CL, and HPC/CL.

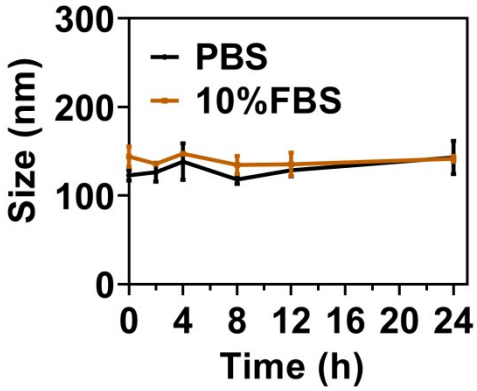

Fig. S6. Serum stability of HPC-CAT/CL-Ap nanoparticles in PBS or PBS containing 10% (w/w) fetal bovine serum (FBS). The nanoparticles exhibited good stability in 10%FBS within 24h.

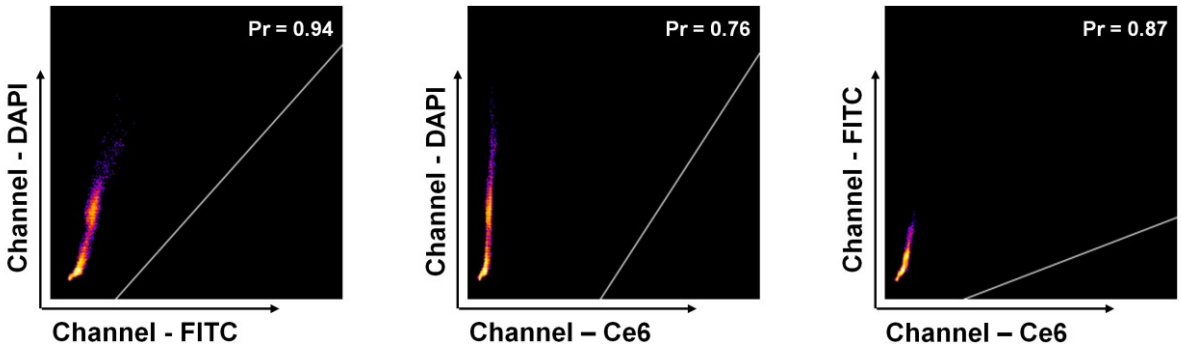

Fig. S7. The Pearson's correlation coefficient (Pr) illustrating the co-localization of catalase and Ce6 in the nucleus.

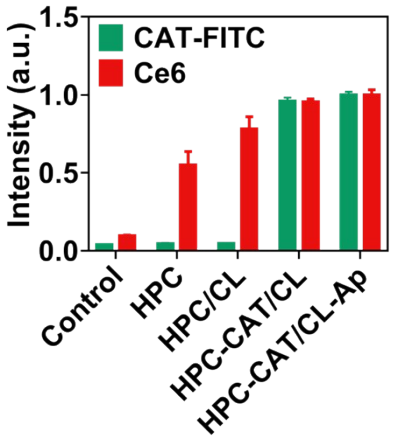

Fig. S8. Quantitative analysis of the uptake efficiency by flow cytometry.

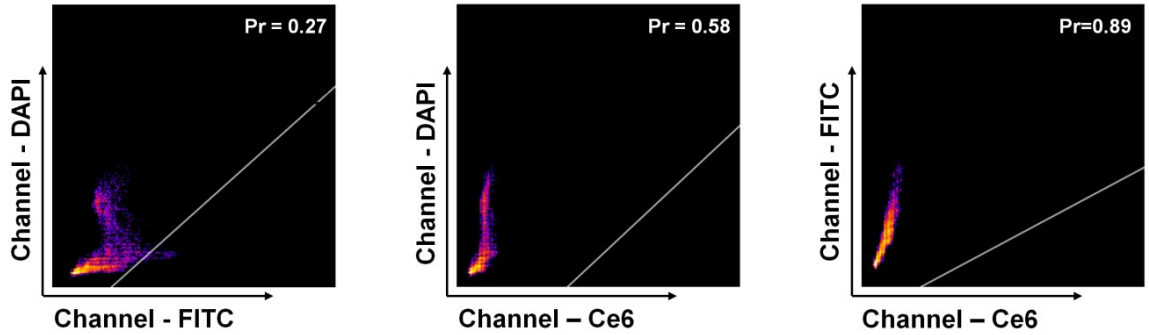

Fig. S9. Pearson's correlation coefficient (Pr) illustrating that ivermectin could block the nuclear entry of HPC-CAT/CL-Ap nanoparticles.

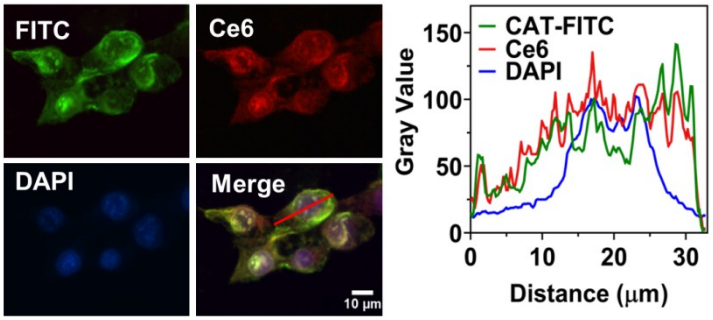

Fig. S10. Fluorescent images (left) and intensity profiles (right) in 4T1 cells after treated with HPC-CAT/CL-Ap in the presence of Ivermectin (15 μM). Red: Ce6, Green: FITC (CAT), Blue: DAPI (nuclei). Scale bars: 10 μm.

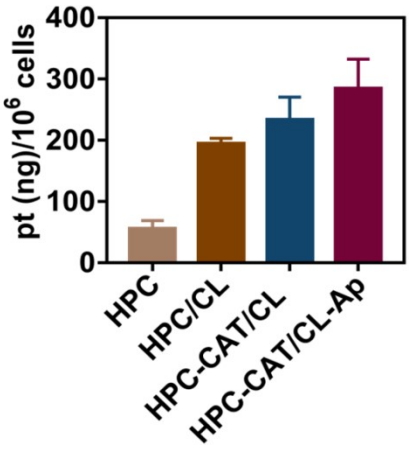

Fig. S11. The maximized uptake of Pt drugs.

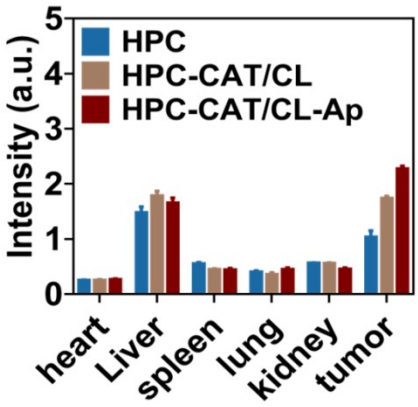

Fig. S12. Quantification of fluorescence intensities of the excised tumors and major organs of mice after 24 h injection.

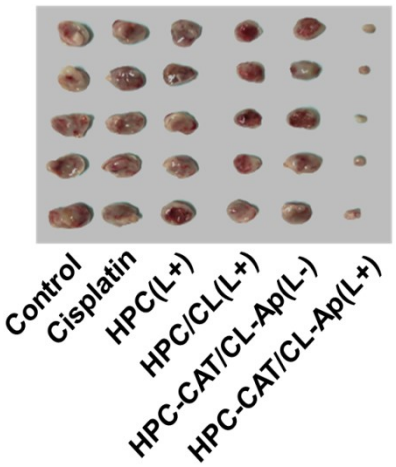

Fig. S13. Photographs of tumors taken from mice at the end of different treatments.

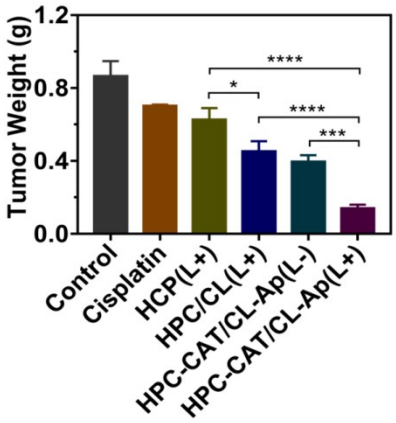

Fig. S14. Average tumor weights of the mice on day 18.

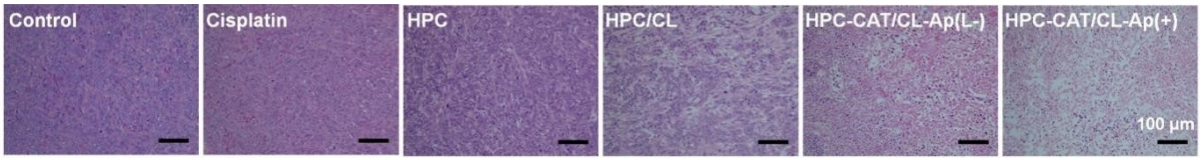

Fig. S15. H&E stained images of tumor slices taken from different treatment groups on day

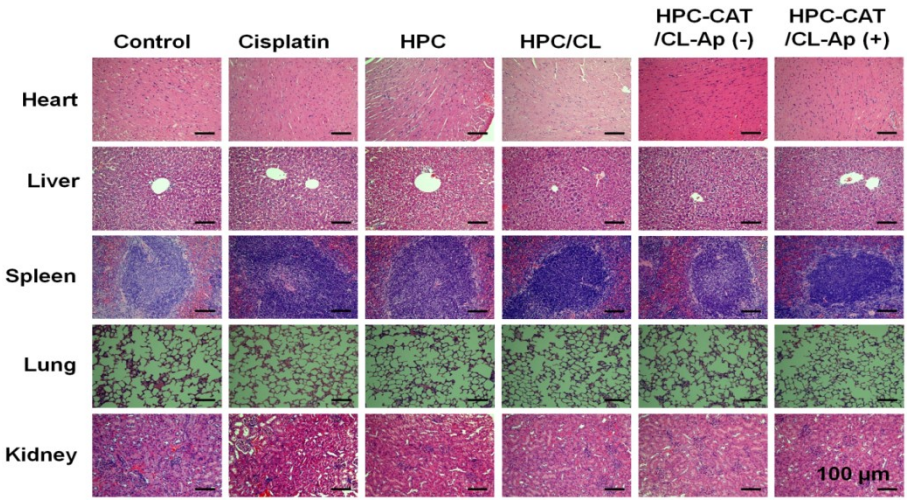

Fig. S16. H&E staining of the main organs after different treatments. Scale bars: 100 μm.

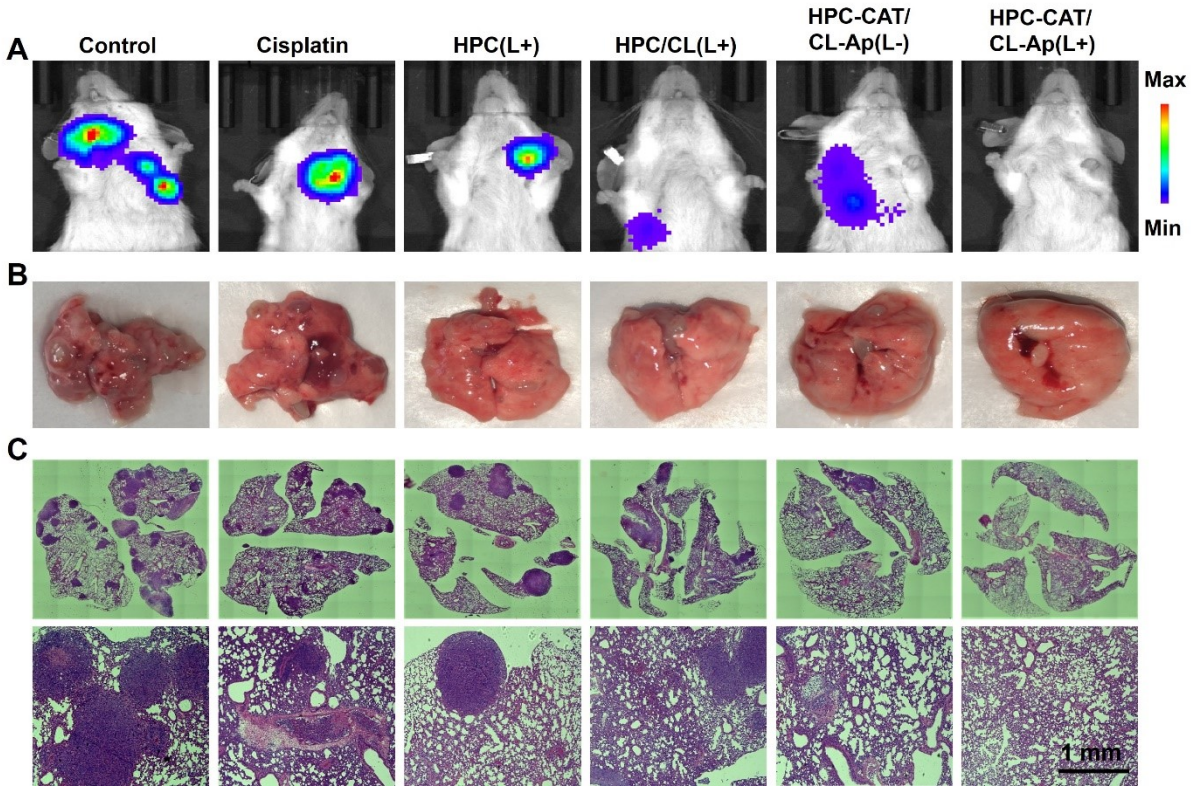

Fig. S17. Lung metastasis inhibition efficacy of different nanoparticles. (A) Luminescence imaging of 4T1 tumor-bearing mice with different treatments. (B) Images of each group's lung after different treatments. (C) H&E staining of lung tissues from the mice after different treatments. Scale bars: 1 mm.