Supporting information

Reactive Oxygen Species Activatable Heterodimeric Prodrug as Tumor Selective Nanotheranostics

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Schemes, Figures and Tables

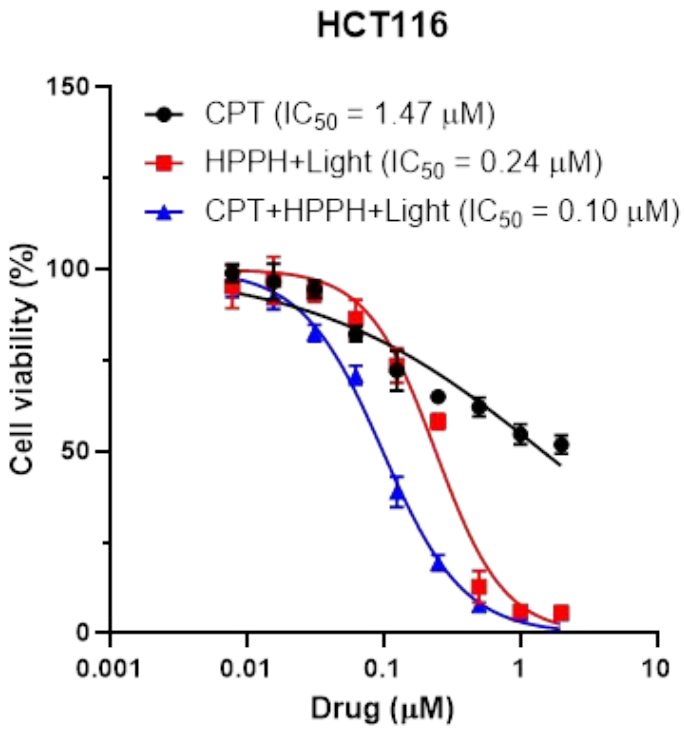
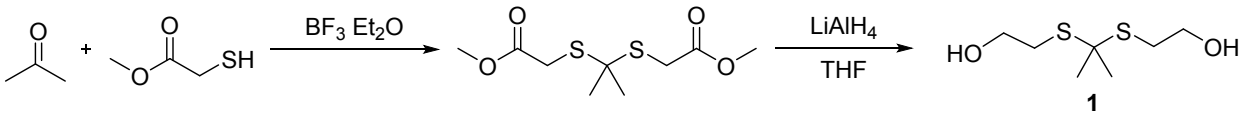


Figure S1. Synergistic therapy of HPPH and CPT. Cell viability of HCT116 cells evaluated by MTT assay. Cells were incubated with CPT, HPPH, CPT+HPPH for 24 h, followed by treatment with/without 671-nm light irradiation (light intensity = 15 mW/cm^2) for 5 min and incubated for another 24 h. The combination index was calculated to be 0.49, which is small than 1and suggests the synergistic effect.



Scheme S1. Synthetic route of **1**.

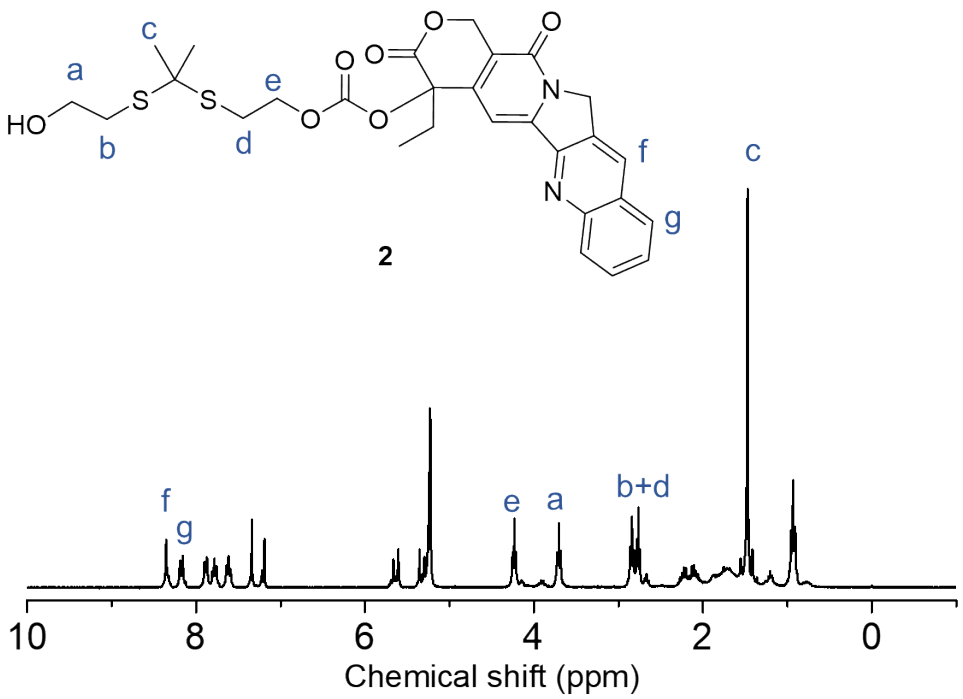


Figure S2. ^{1}H NMR spectra of CPT prodrug compound **2**.

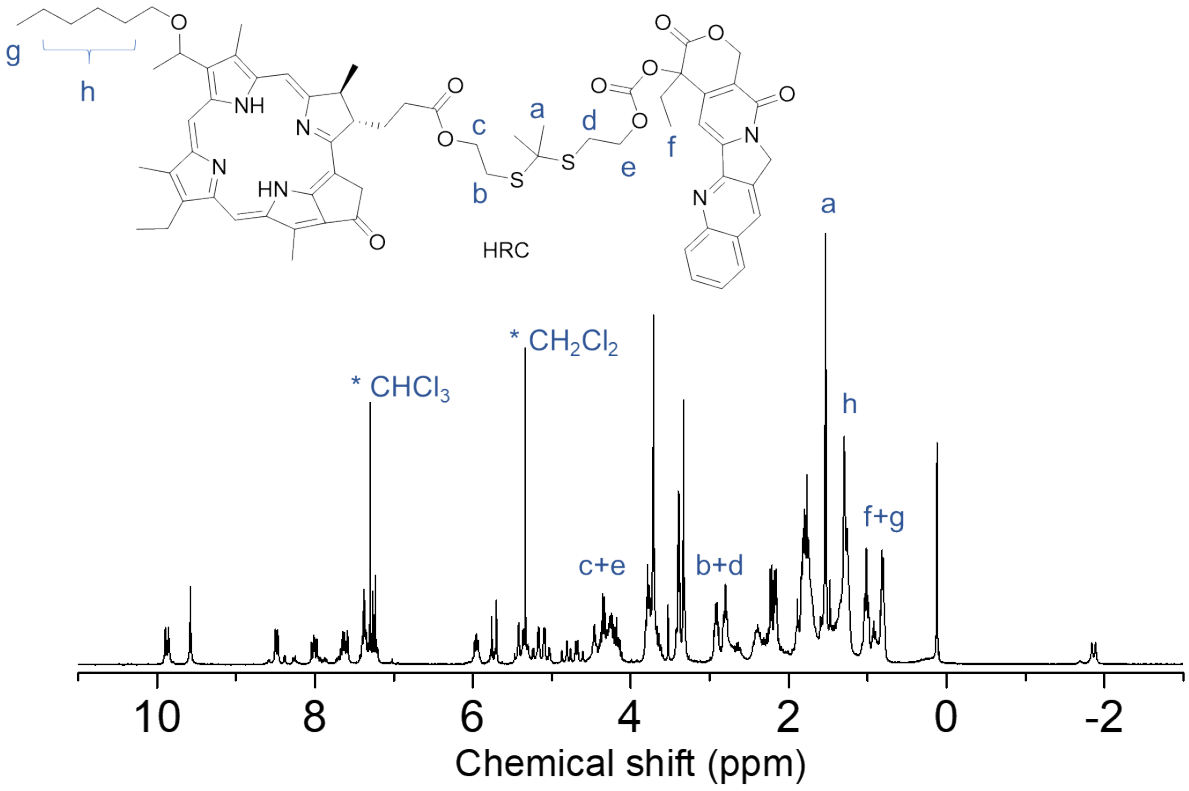


Figure S3. ^{1}H NMR spectra of HRC. Solvent peaks of $CHCl_3$, DCM and TMS were noted.

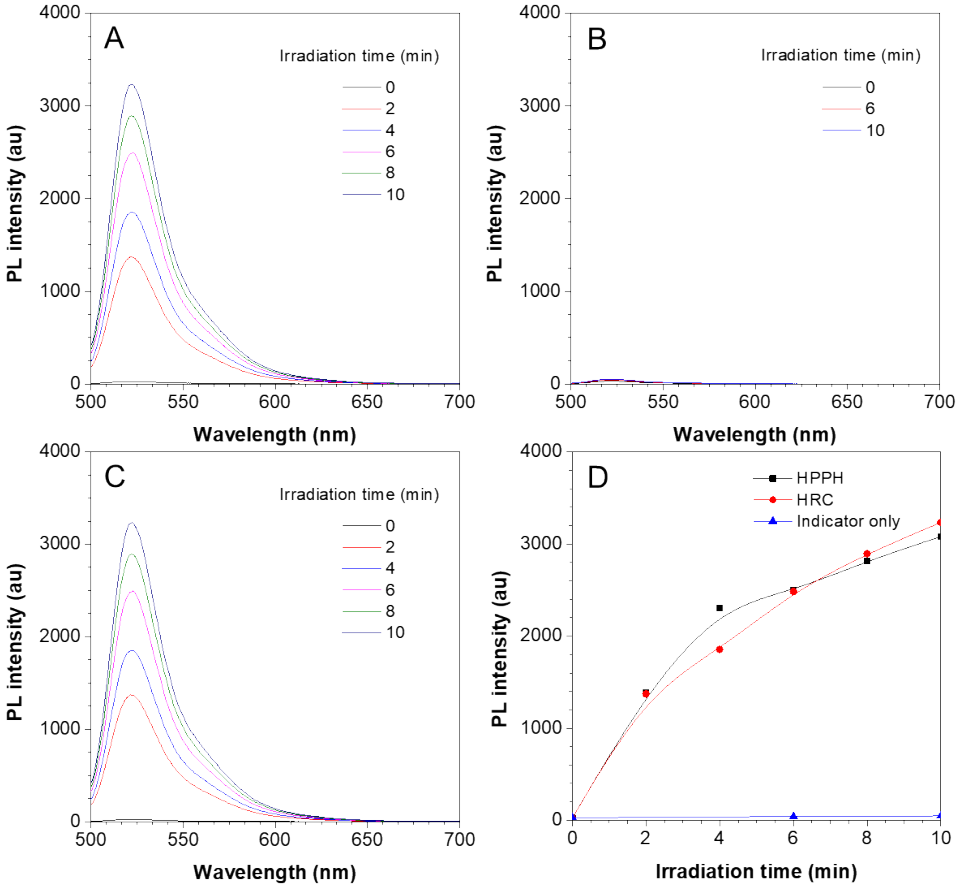


Figure S4. *In vitro* ROS generation using DCFH as ROS indicator. PL spectra of (A) DCFH and HPPH, (B) DCFH only, and DCFH and HRC in PBS under the 671-nm laser irradiation for various time. Concentration of HPPH, HRC or DCFH = 2 μM. Laser intensity: 100 mW/cm^2.

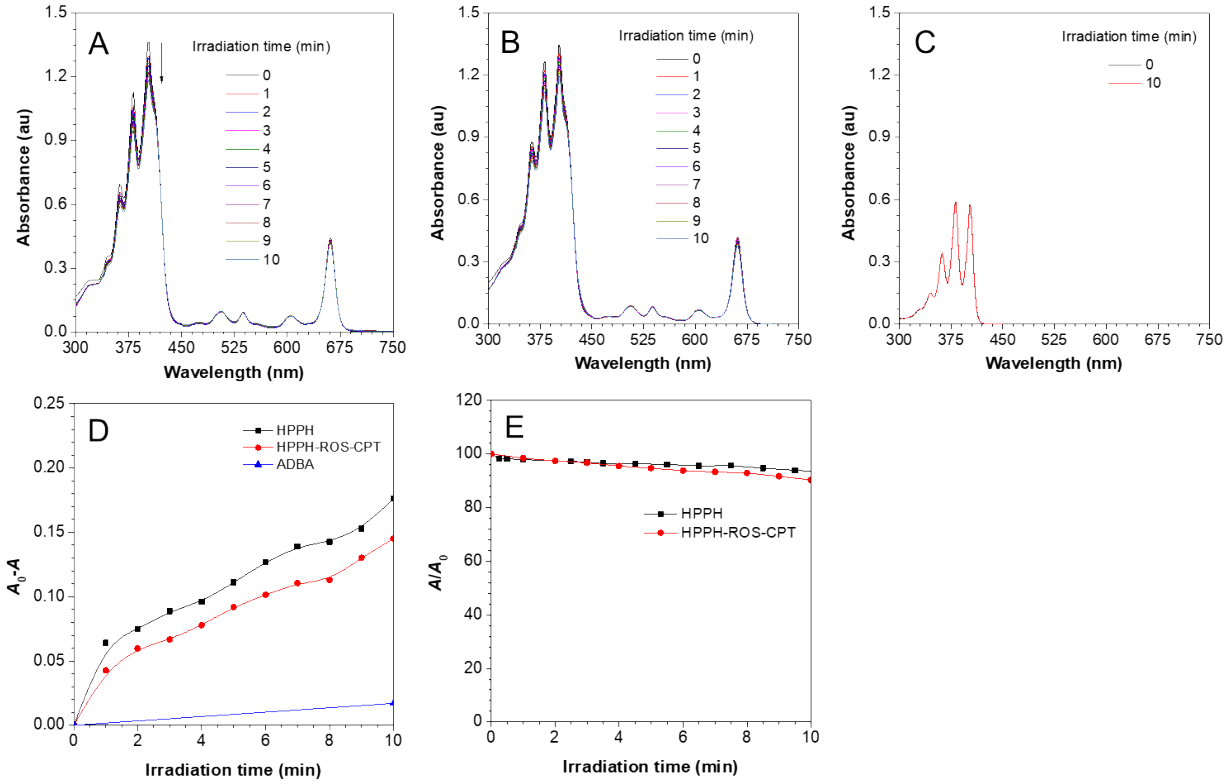


Figure S5. *In vitro* ROS generation determined by 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ADBA). UV-Vis spectra of (A) ADBA and HPPH, (B) ADBA and HRC, (C) ABDA only in DMSO under the 671-nm laser irradiation. Concentration of ABDA = 50 μM. Concentration of HPPH or HRC = 10 μM. (D) Absorption change of ABDA at 382 nm upon the laser irradiation in (A), (B) and (C). (D) Photostability of HPPH or HRC determined by the UV-Vis absorption at 660 nm. Laser intensity = 100 mW/cm^2.

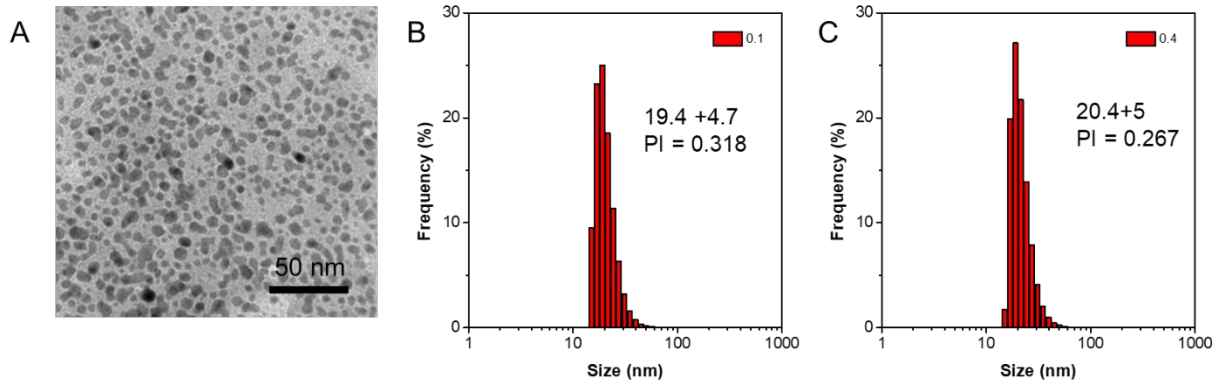


Figure S6. (A) TEM image of HRC@F127 at a drug feeding ratio of 0.2. (B & C) Size distribution analyzed by dynamic laser scattering (DLS) of HRC@F127 with different drug feeding ratios (w/w): (B) 0.1 and (C) 0.4.

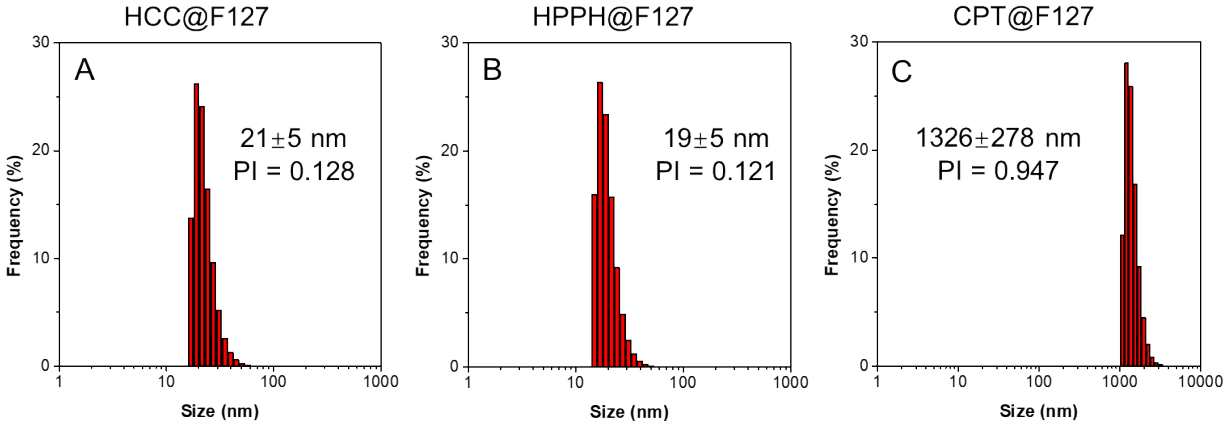


Figure S7. Size distribution analyzed by dynamic laser scattering (DLS) of (A) HCC@F127, (B) HPPH@F127, and (C) CPT@F127.

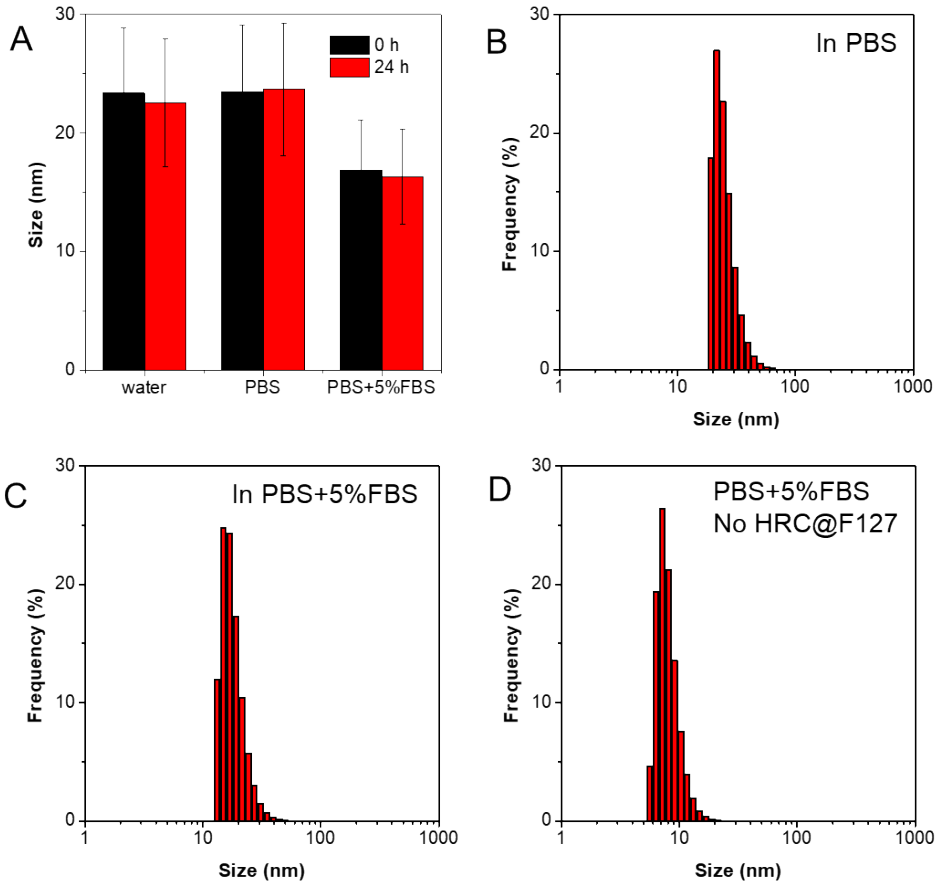


Figure S8. (A) Mean hydrodynamic size distribution of HRC@F127 in water, in PBS and in PBS+5%FBS. (B-D) DLS analysis of hydrodynamic size distribution of (B) HRC@F127 in PBS and (C) HRC@F127 in PBS+5% FBS and (D) no HRC@F127 in PBS+5% FBS. (The smaller hydrodynamic size of HRC@F127 in PBS+5%FBS might be due to the interference of proteins in FBS, which has a mean size of 8 ± 2 nm.)

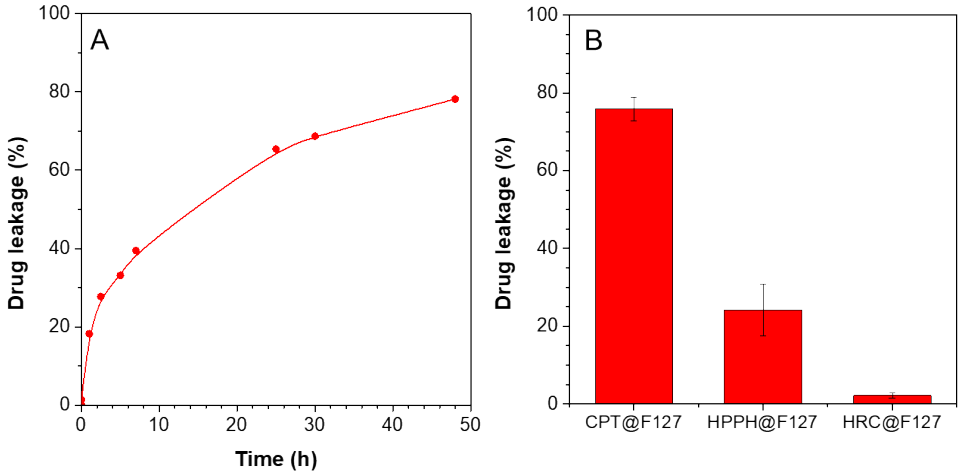


Figure S9. (A) Drug leakage curve of CPT@F127 in PBS *versus* time *via* a dialysis method. (B) Percentage of drug leakage of CPT@F127, HPPH@F127 and HRC@F127 in PBS after incubation for 48 h.

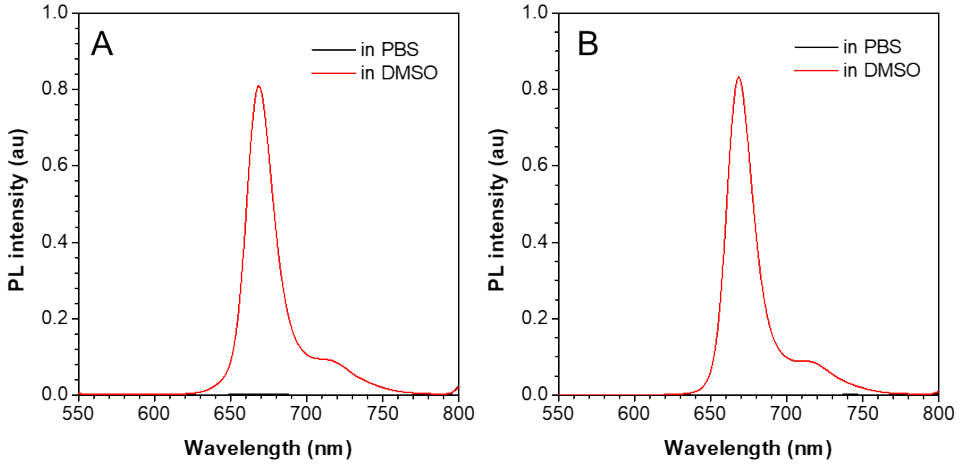


Figure S10. PL spectra of (A) HPPH@F127 and (B) HCC@F127 in PBS and in DMSO. Concentration = 20 μM.

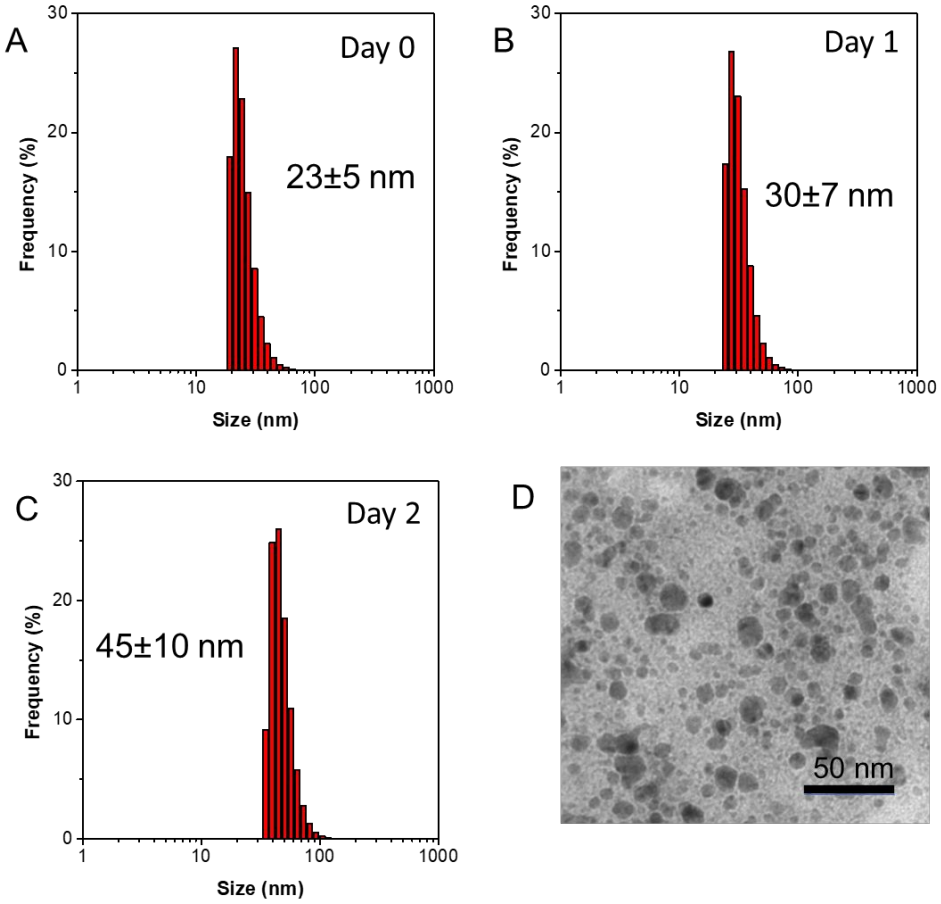


Figure S11. (A–C) DLS analysis of hydrodynamic size distribution of HRC@F127 in water and treated with H_2O_2 for (A) 0 h, (B) 24 h and (C) 48 h. (D) TEM image of HRC@F127 in water and treated with H_2O_2 for 48 h. Scale bar = 50 nm.

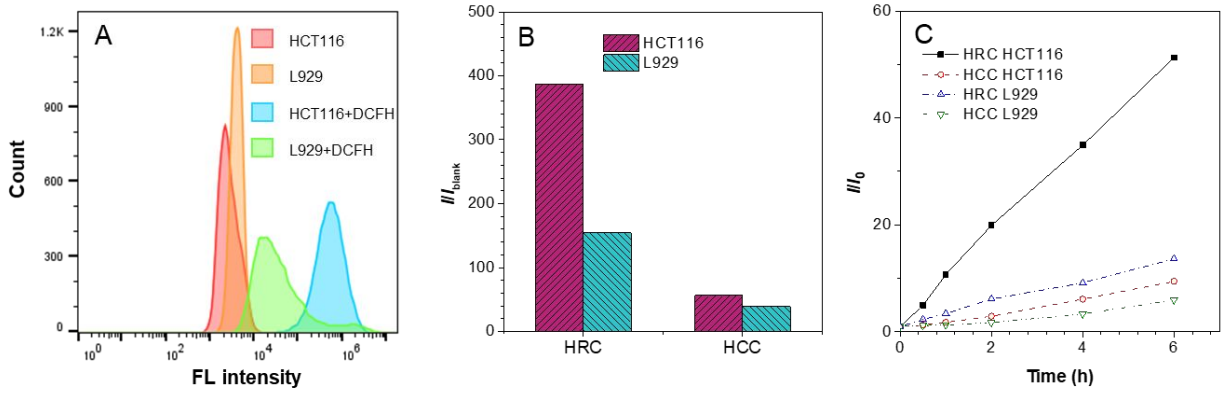


Figure S12. (A) Flow cytometry results of HCT116 cells and L929 cells incubated with/without 5 μM DCFH-DA for 1 h. (B) Mean fluorescence intensity of HCT116 and L929 cells incubated with 5 μM HRC@F127 or HCC@F127 for 24 hours analyzed by flow cytometry. (C) Mean fluorescence intensity of

HCT116 and L929 cells incubated with 5 μM HRC@F127 or HCC@F127 for various hours analyzed by flow cytometry.

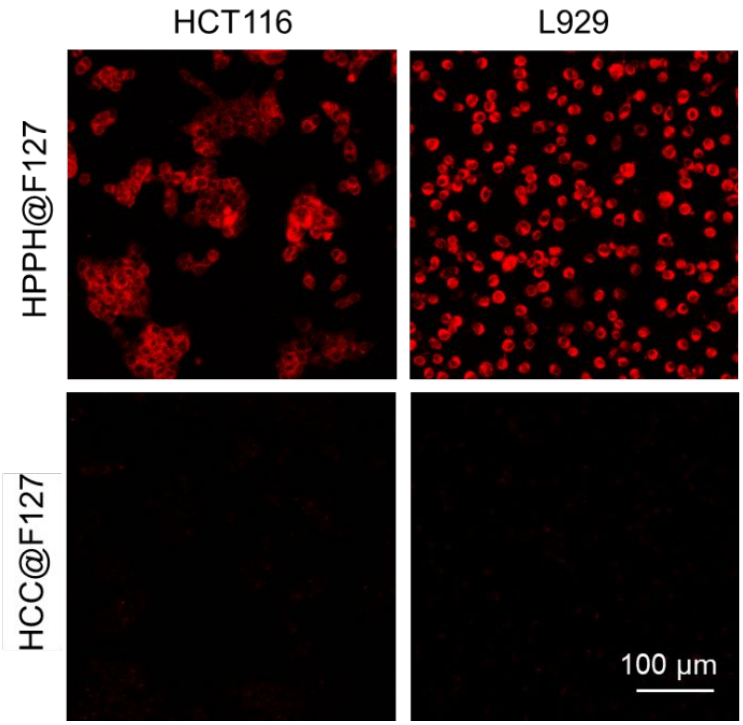


Figure S13. Fluorescence images of HCT116 cells and L929 cells incubated with HPPH@F127 and HCC@F127 for 24 h. Concentration = 5 μM.

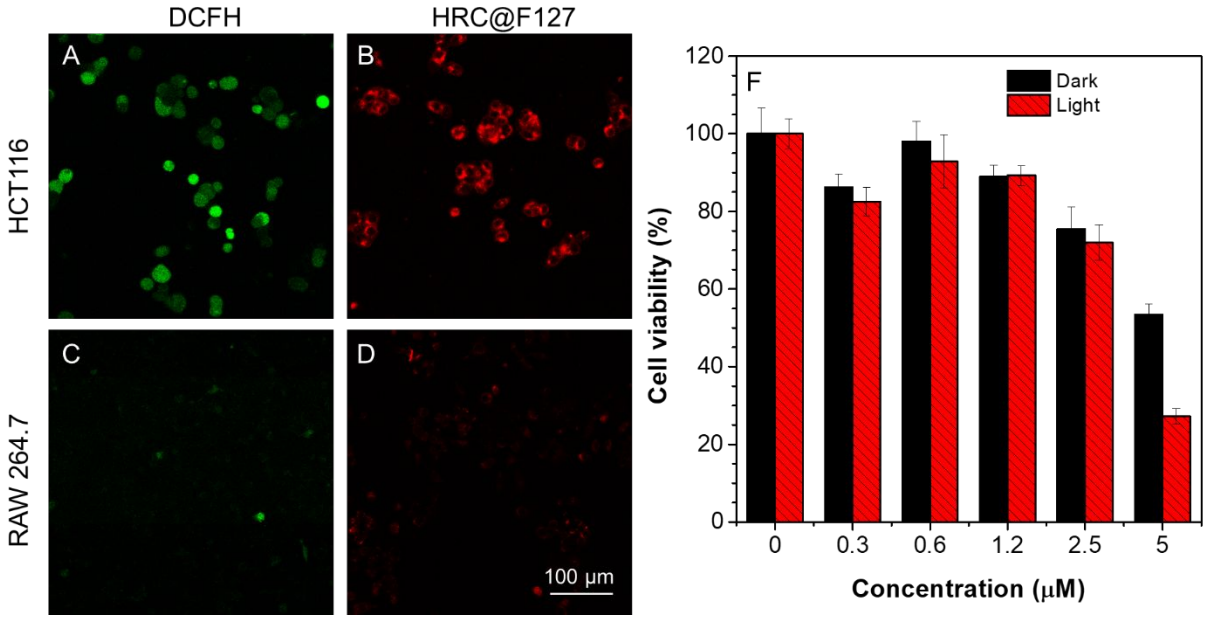


Figure S14. (A–D) Fluorescence images of HCT116 cells and RAW 264.7 cells incubated with 5 μM DCFH for 1 h or 2 μM HCC@F127 for 24 h. (F) Cell viability of RAW 264.7 incubated with different concentrations of HRC for 48 h without or with a treatment with 671-nm light irradiation (light intensity = 15 mW/cm^2) for 5 min at the time point of 24 h.

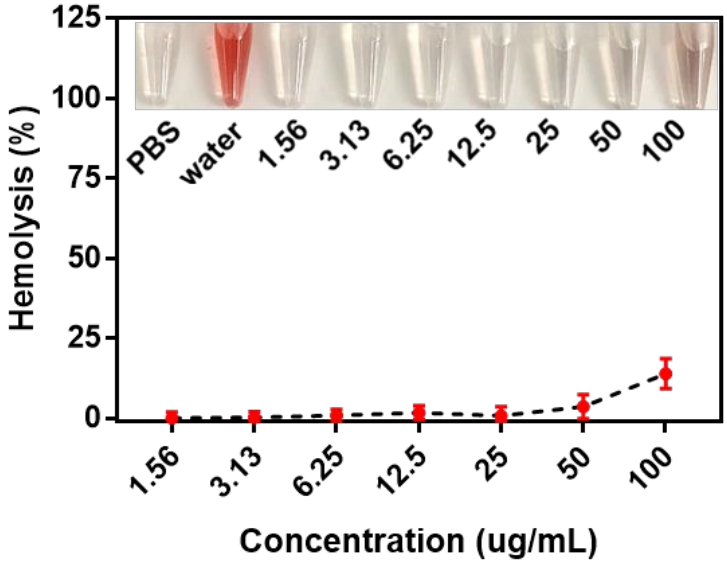


Figure S15. Hemolysis test of HRC@F127 at various concentrations.

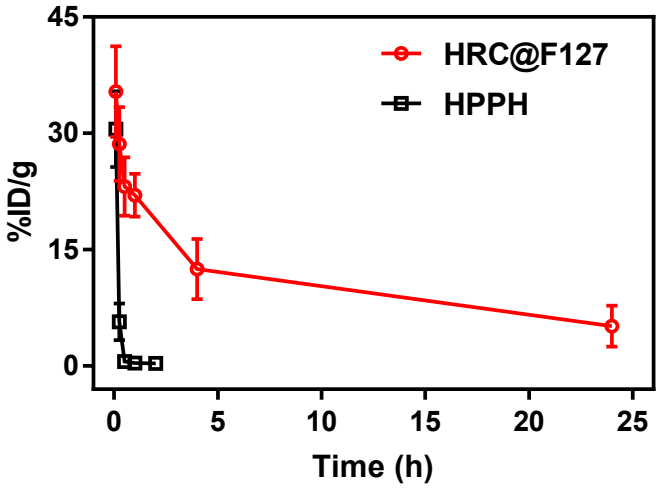


Figure S16. Pharmacokinetics of HRC@F127 and HPPH in mice after intravenous injection. HRC@F127 NPs have a much-prolonged circulation half-life ($t_{1/2}$ = 1.6 h) than HPPH molecules ($t_{1/2}$ = 0.1 h).

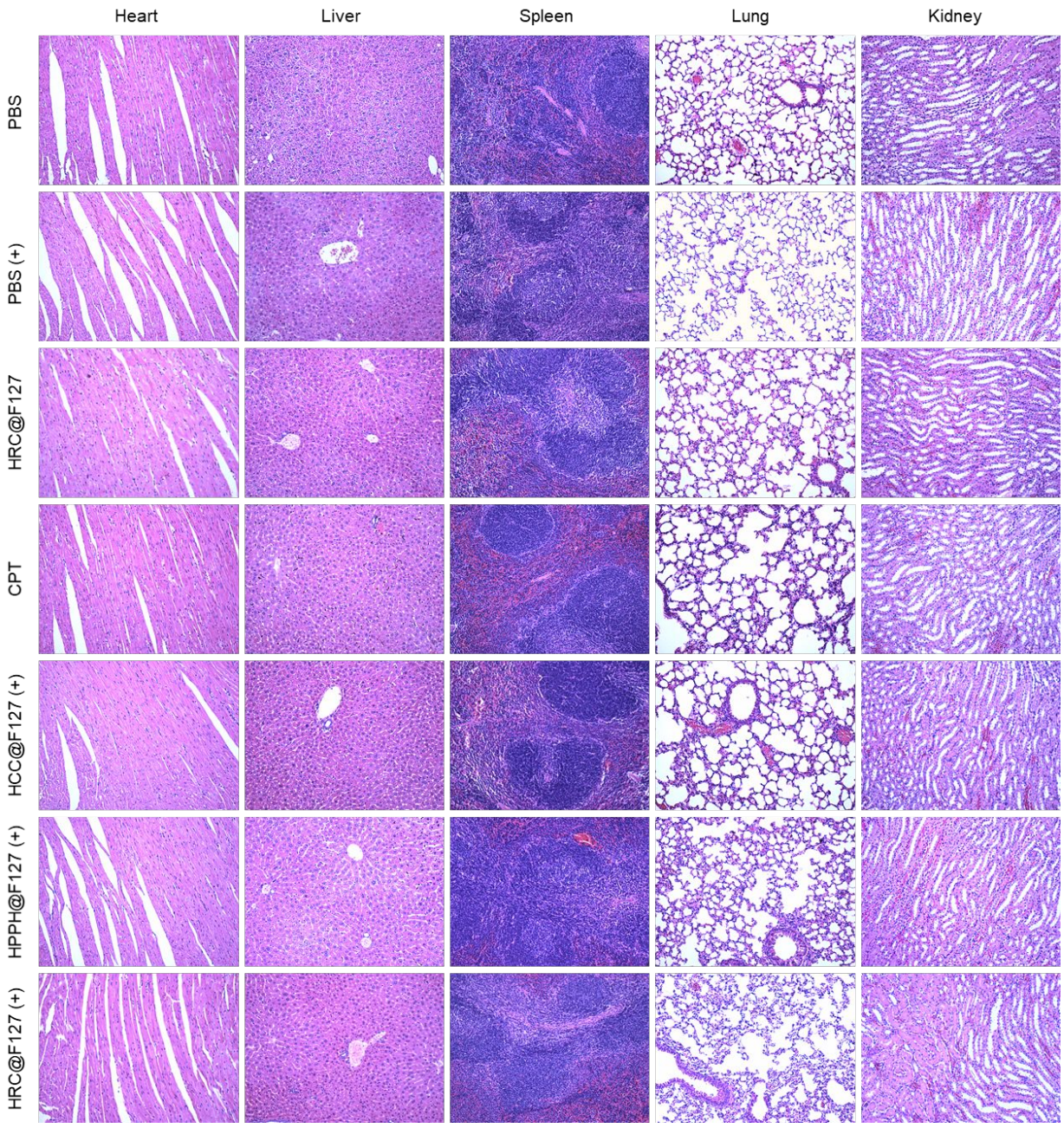


Figure S17. Hemotoxylin and Eosin (H&E) staining images of major organs in HCT116 tumor-bearing mice treated with specified regimens: G1 (PBS), G2 (PBS + Laser), G3 (HRC@F127), G4 (CPT), G5 (HCC@F127 + Laser), G6 (HPPH@F127 + Laser), and G7 (HRC@F127 + Laser).

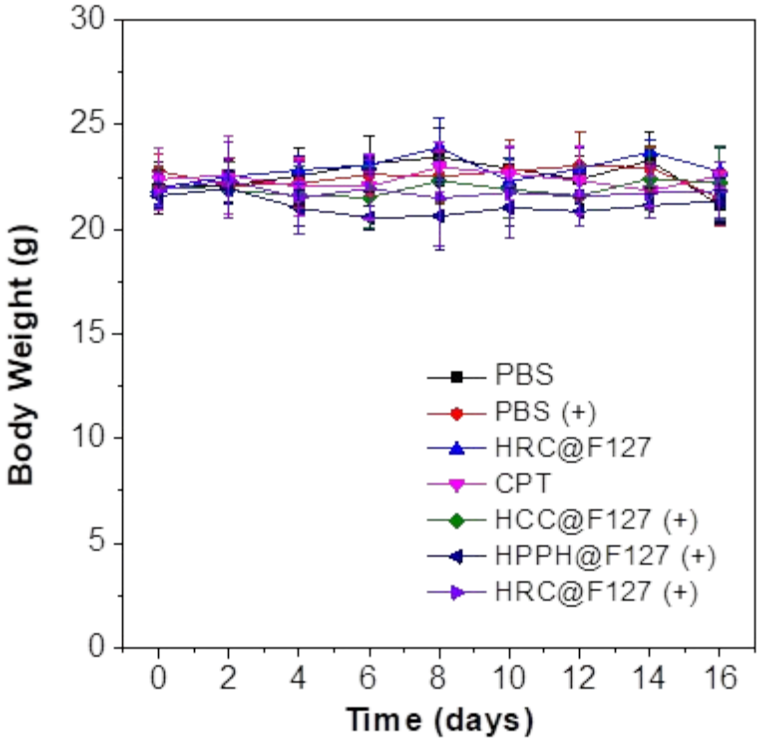


Figure S18. Change of body weight of HCT116 tumor-bearing mice with time after treated with specified regimens: G1 (PBS), G2 (PBS + Laser), G3 (HRC@F127), G4 (CPT NPs), G5 (HCC@F127 + Laser), G6 (HPPH NP + Laser), and G7 (HRC@F127 + Laser).