

ADVANCED HEALTHCARE MATERIALS

Supporting Information

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Photo-Enhanced Synergistic Induction of Ferroptosis for Anti-Cancer Immunotherapy

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Name	Cat #	Fluorophore	Company
Anti-GPX4 antibody	A1933	\	Abclonal
Anti-LC3 antibody	Ab192890	\	Abcam
Anti-HMGB1 antibody	A19529	\	Abclonal
Anti-Calreticulin antibody	A1066	\	Abclonal
Anti-GAPDH antibody	AC002	\	Abclonal
Anti-FSP1 antibody	24972	\	Cell Signaling Technology (CST)
Anti-CD8 antibody	98941	\	CST
Anti-TNF α -antibody	11948	\	CST
Anti-granzyme B-antibody	44153	\	CST
Anti-mouse PD-L1	BE0101	\	Bio X Cell
Anti-rabbit IgG H&L (HRP)	ab6721	\	Abcam
Anti-IA/IE antibody	107605	FITC	Biologend
Anti-CD11c antibody	117321	Pacific Blue	Biologend
Anti-CD80 antibody	104714	APC	Biologend
Anti-CD86 antibody	105014	PE/Cy7	Biologend
Anti-CD45 antibody	157213	FITC	Biologend
Anti-CD3 antibody	100221	APC/Cy7	Biologend
Anti-CD4 antibody	100427	Pacific Blue	Biologend
Anti-CD8 antibody	100733	Percep/Cy5.5	Biologend
Anti-rabbit IgG H&L	ab175471	AF568	Abcam

Table S1. Antibodies used in this study for immunofluorescence, immunohistochemistry, flow cytometry test, and anti-PD-L1 immunotherapy.

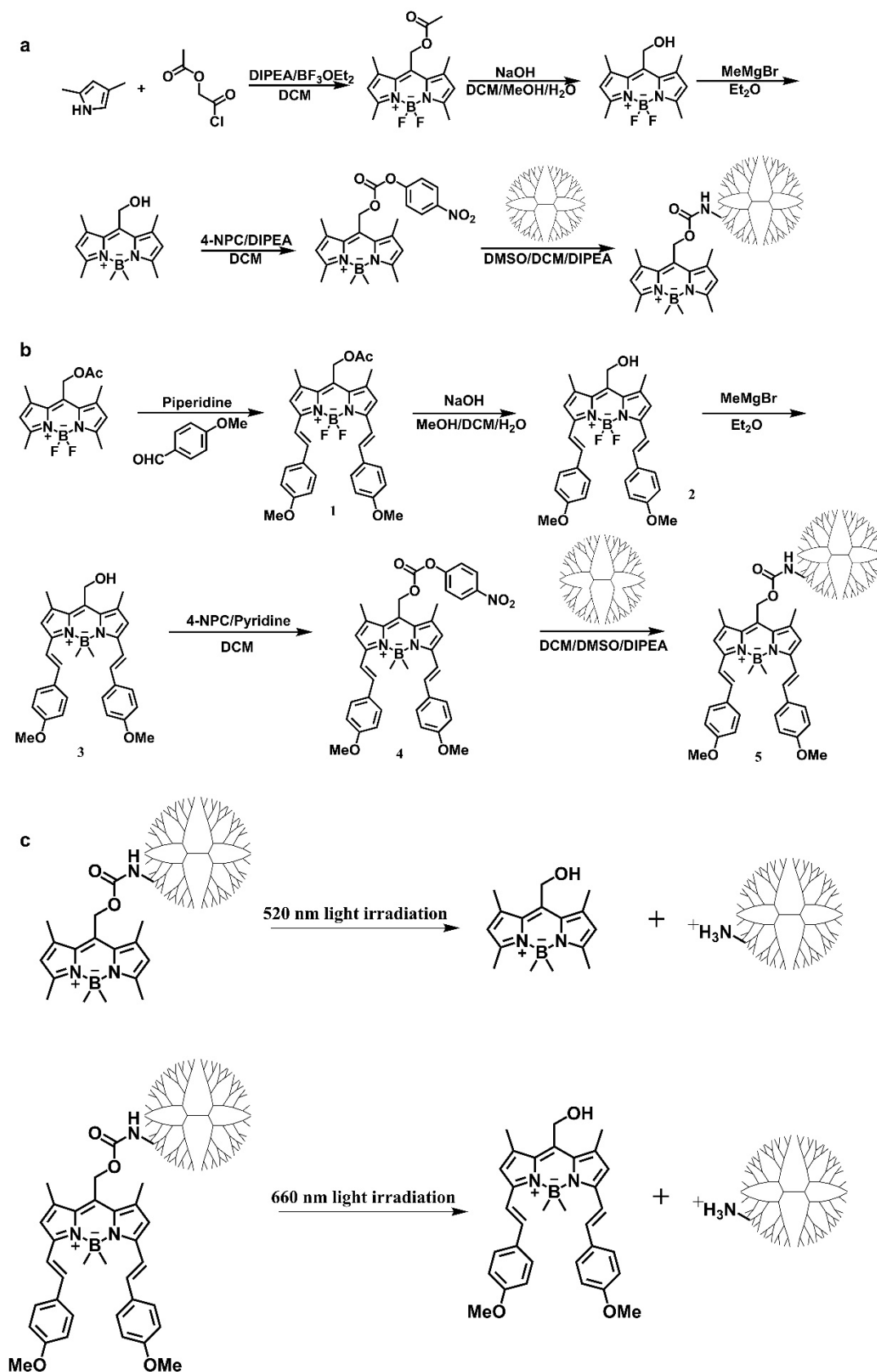


Figure S1. Synthesis route of green light-responsive BODIPY-modified PAMAM (BMP) (a) and NIR light-responsive BODIPY-modified PAMAM (NBMP) (b). (c) Photocleavage of BODIPY molecules from BMP.

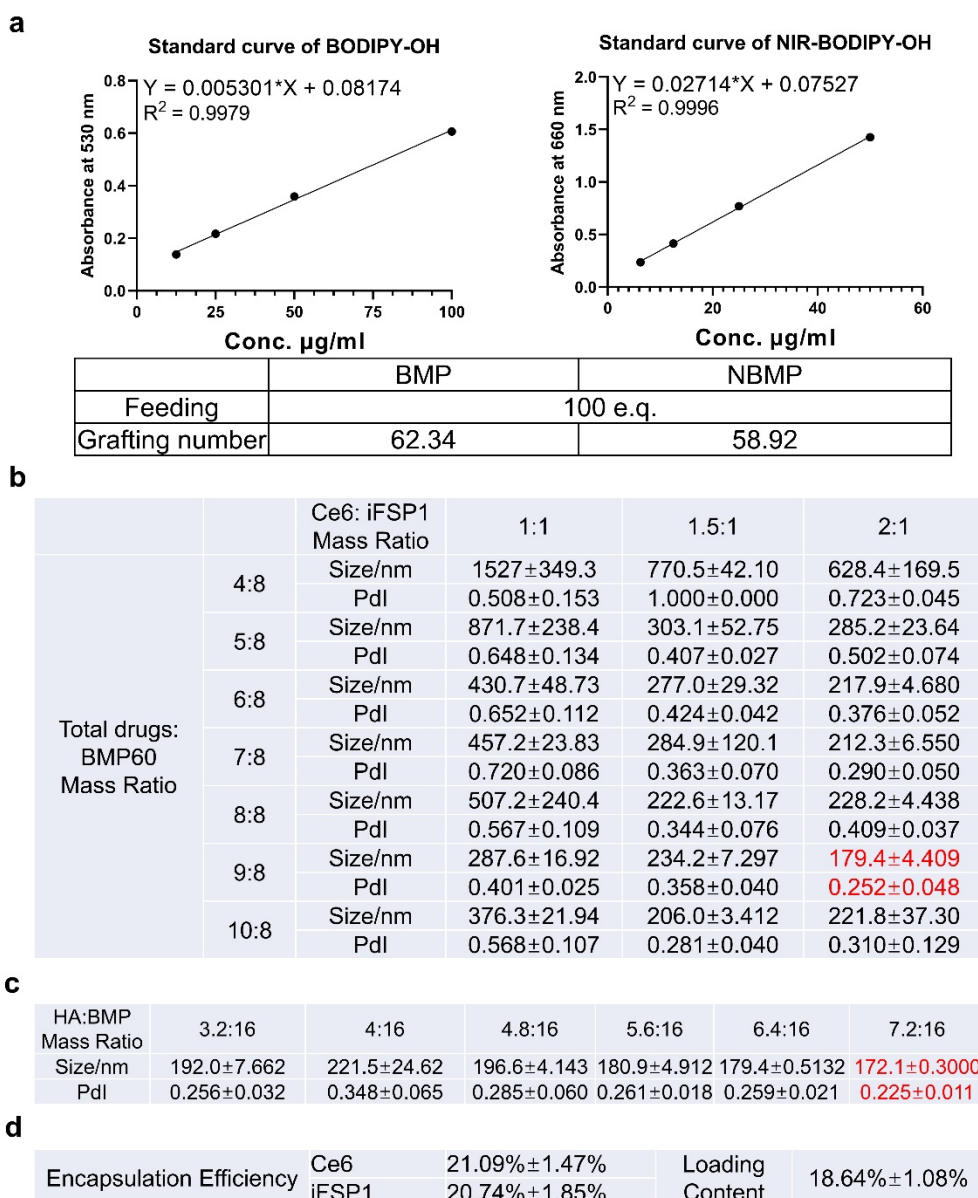


Figure S2. (a) Standard curves and grafting number calculation of green light-responsive and NIR light-responsive BODIPY. Formulation optimization of BMP-Ce6&iFSP1 nanoparticles (b) and HA-coated BMP-Ce6&iFSP1 nanoparticles (c). (d) Encapsulation efficiency and loading content evaluation of HBCiF-NPs.

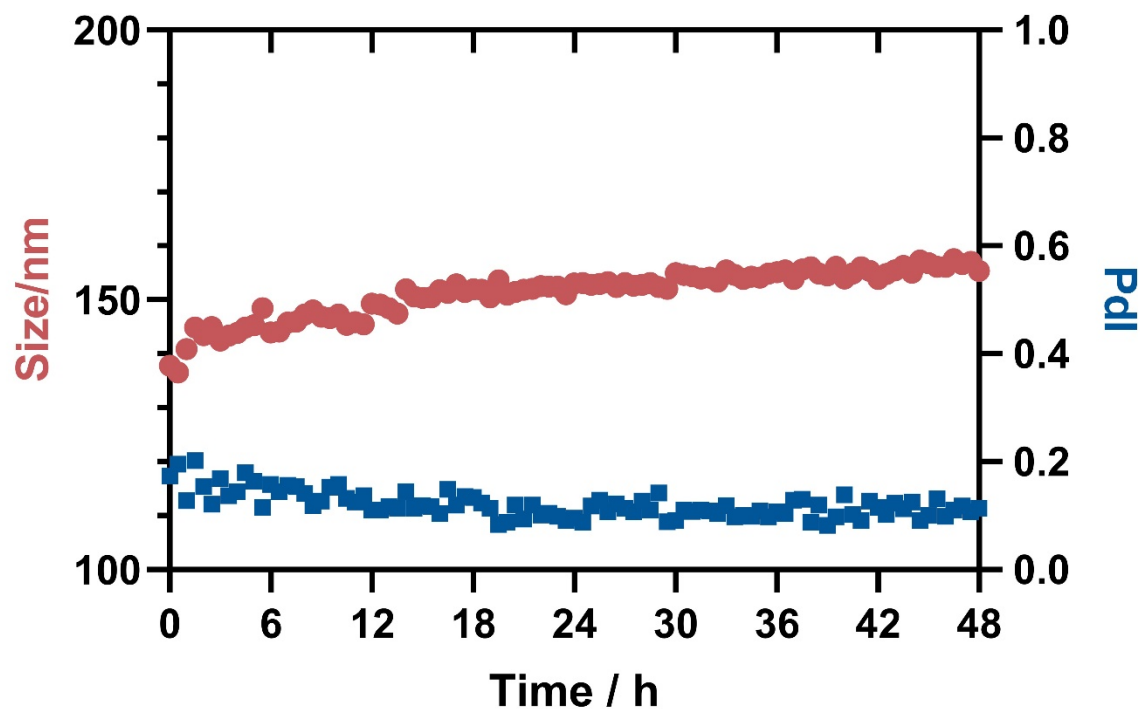


Figure S3. Stability measurement of HBCiF-NPs in DMEM complete medium containing fetal bovine serum for 48 h at 37 °C.

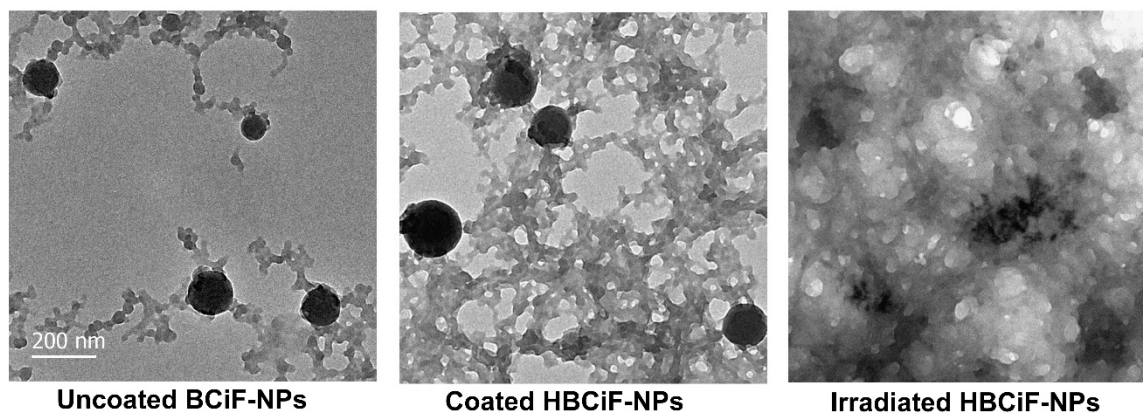


Figure S4. TEM images of BCiF-NPs, HBCiF-NPs, and 520 nm light-irradiated HBCiF-NPs. Scale bar: 200 nm. Light irradiation: Xe lamp, 520 nm, 25 mW/cm², 3 min.

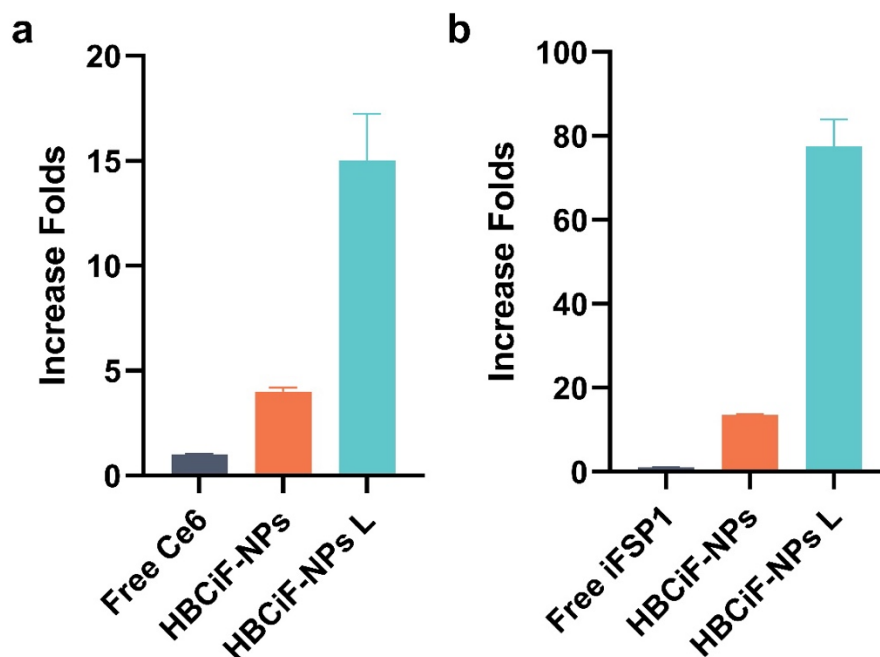


Figure S5. Fluorescence increase folds analyzed by flow cytometry of A549 cells treated with free Ce6 or iFSP1, HBCiF-NPs, and 520 nm light-irradiated HBCiF-NPs for 4 h. Fluorescence of iFSP1 and Ce6 was detected by DAPI and Qdot655 channel, respectively. L: light irradiation. Light irradiation: Xe lamp, 520 nm, 25 mW/cm², 3 min. n=3, means \pm SD.

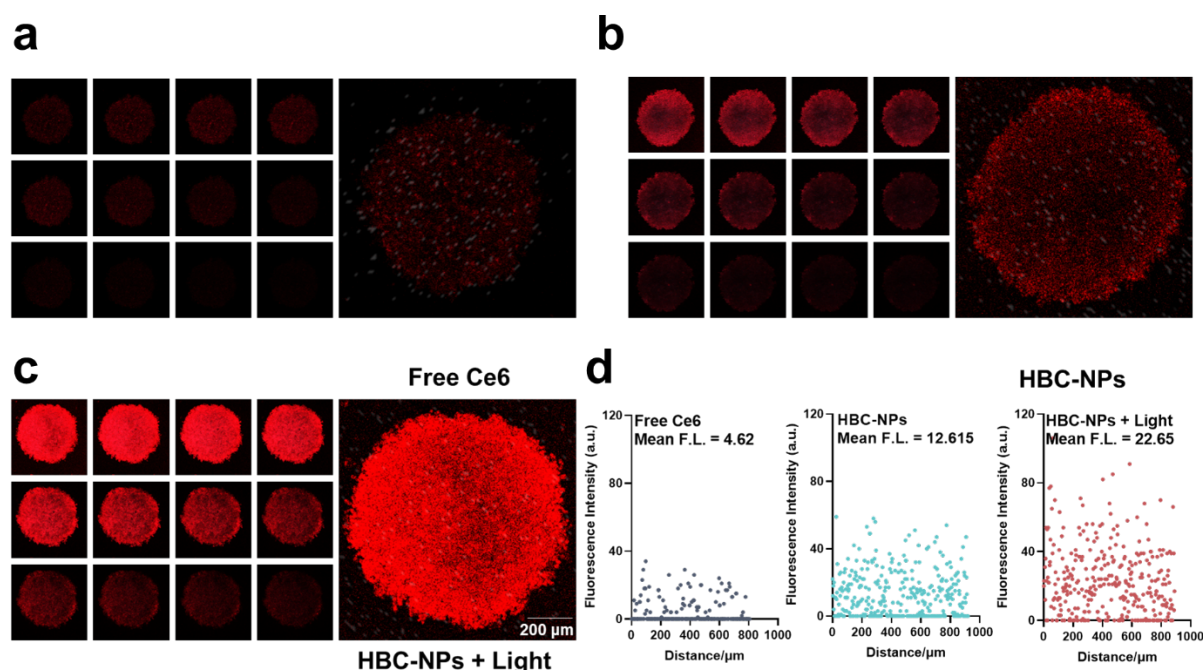


Figure S6. CLSM images of A549 tumor spheroids treated with free Ce6 (a), HBC-NPs (b), and HBC-NPs + light (c). Ce6 was visualized by AF647 channel (scale bar: 200 μ m). (d) Fluorescence intensities of 324, 369, 357 points along spheroid diameters in the Free Ce6, HBC-NPs, and HBC-NPs + Light groups, respectively, analyzed by ZEN3.4 (blue edition). Light irradiation: Xe lamp, 520 nm, 25 mW/cm², 3 min.

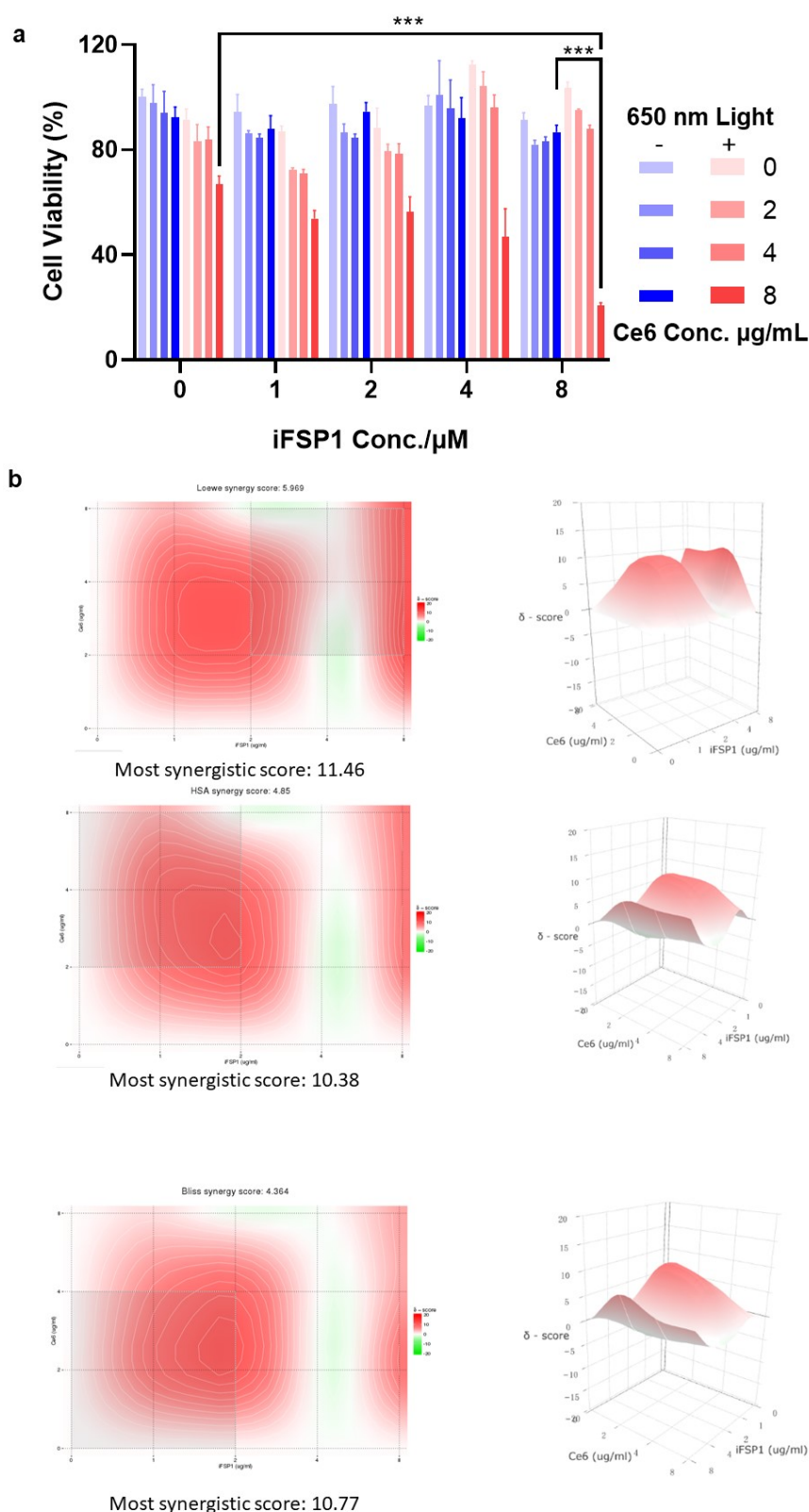


Figure S7. (a) MTT assay of Ce6 and iFSP1 dose-dependent cytotoxicity on A549 cells (means \pm SD, n=3). Light irradiation: Xe lamp, 650 nm, 5 mW/cm², 30 min (b) Synergism analysis of iFSP1 and Ce6 (analysis was completed by SynergyFinder).

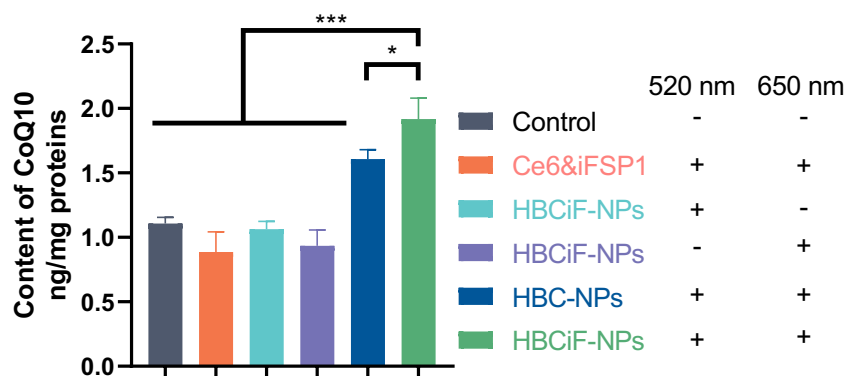


Figure S8. Measurement of intracellular CoQ10 in 4T1 cells by ELISA. Light irradiation: Xe lamp, 520 nm, 25 mW/cm², 3 min; 650 nm, 5 mW/cm², 30 min. n=4. *p<0.05, ***p<0.001

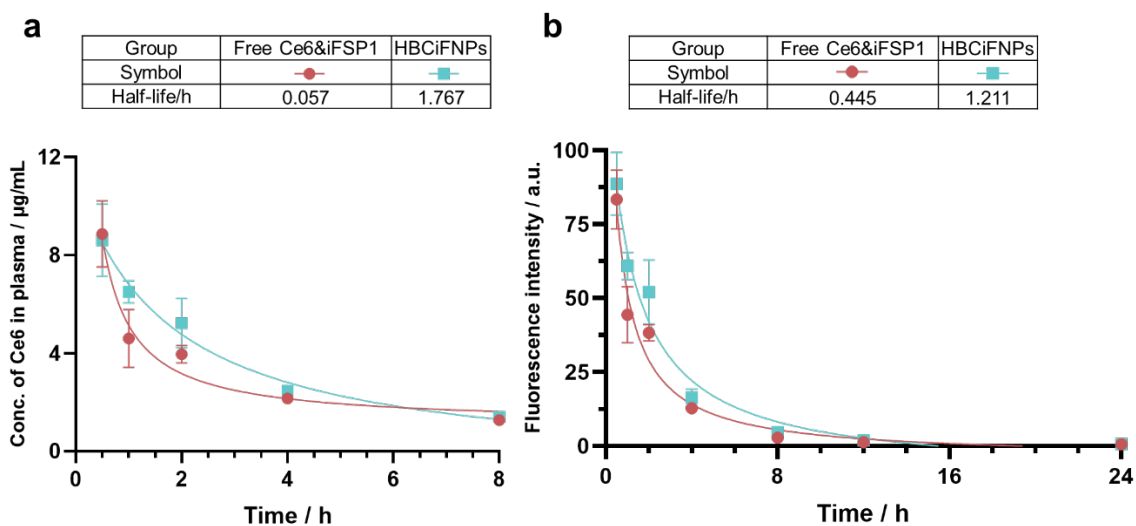


Figure S9. Pharmacokinetic study of Ce6 in the plasma of BALB/c mice by HPLC (a) and fluorescence intensity (b) administered with free Ce6&iFSP1 and HBCiF-NPs, separately. Ce6 dosage: 3 mg/kg. n=5, means ± SD.

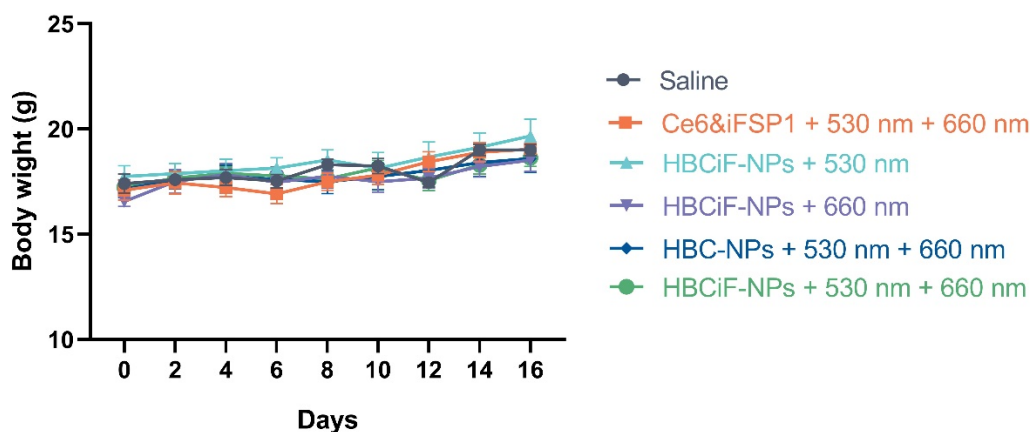


Figure S10. Mouse body weight in response to different treatments as shown in the figure. Light irradiation: Laser, 530 nm, 80 mW/cm², 5 min; 660 nm, 20 mW/cm², 1 min. Data are means \pm SD, n=5.

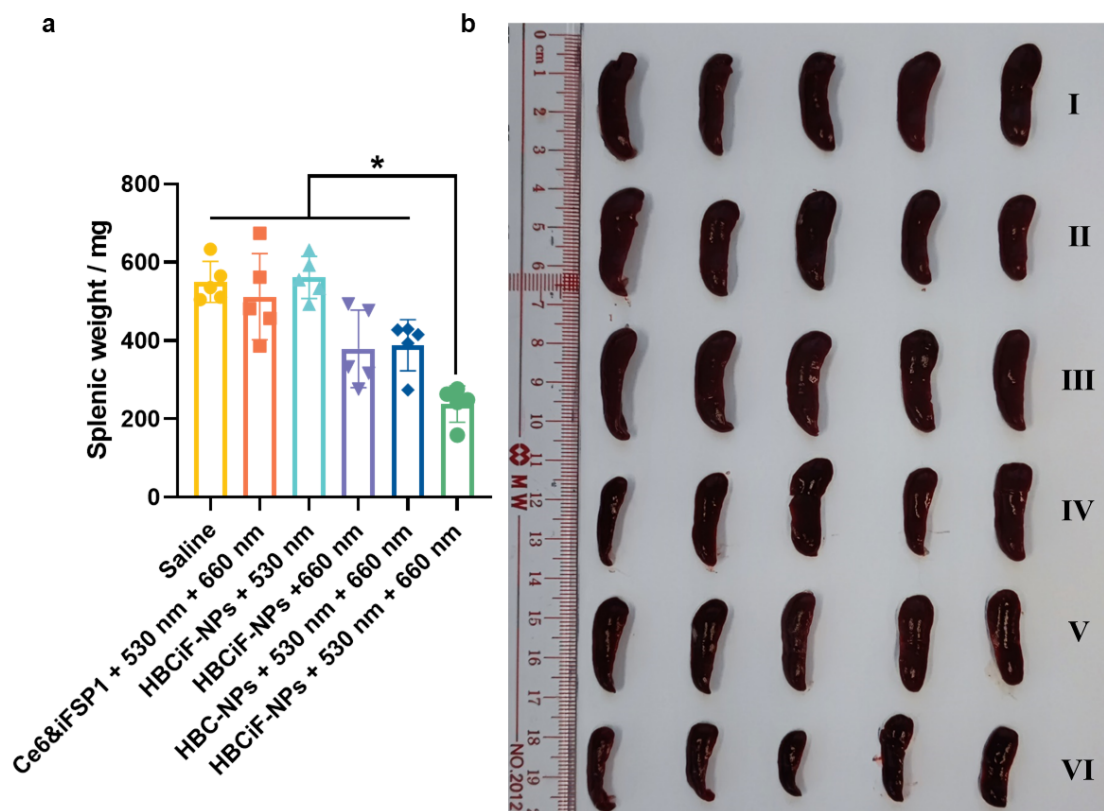


Figure S11. Splenic weight (a) and spleen photo (b) of 4T1 tumor-bearing mice after different treatments as shown in the figure. Light irradiation: Laser, 530 nm, 80 mW/cm², 5 min; 660 nm, 20 mW/cm², 1 min. Data are means \pm SD. *p<0.05.

Ivan-ChenKang-NIR-BODIPY-CDC13-28Jun2022. 1. fid
Dark greenish blue, long, light sensitive

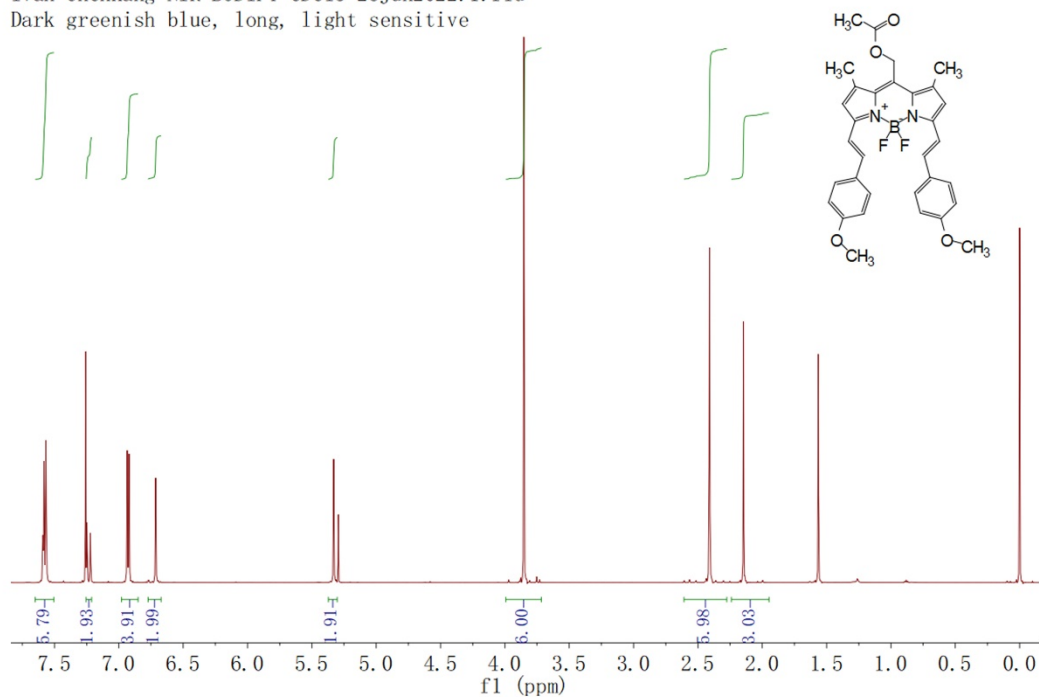


Figure S12. ¹H-NMR spectrum of NIR-BODIPY-Me₂-OAc.

Ivan-ChenKang-CK-2-CDC13-14Sep2022. 1. fid
Dark blue, long, light sensitive

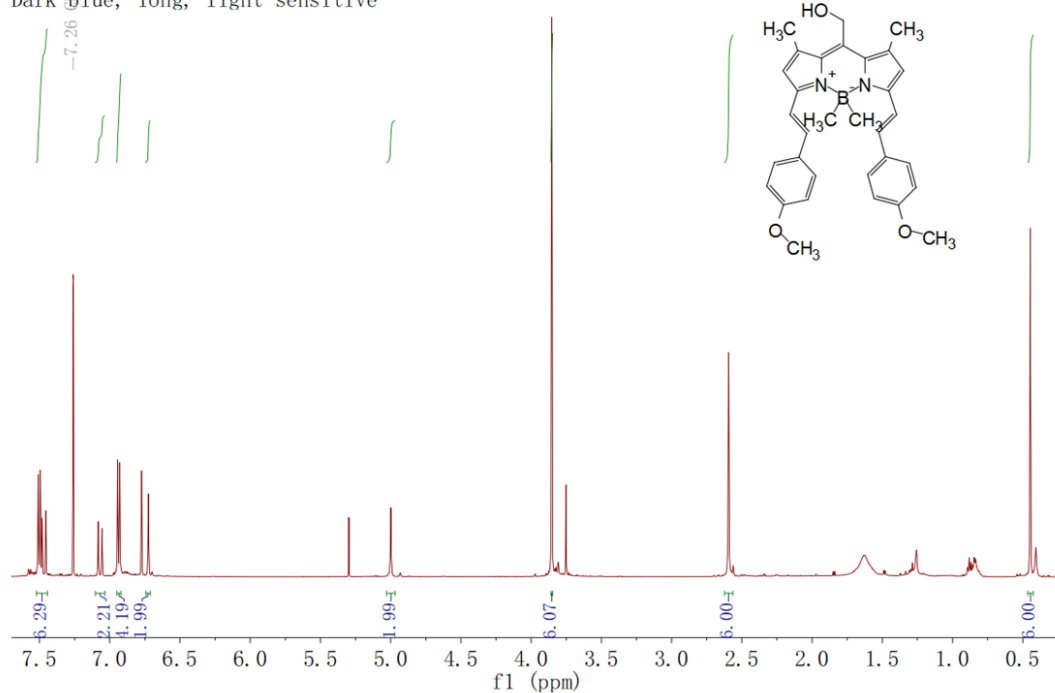


Figure S13. ¹H-NMR spectrum of NIR-BODIPY-Me₂-OH.

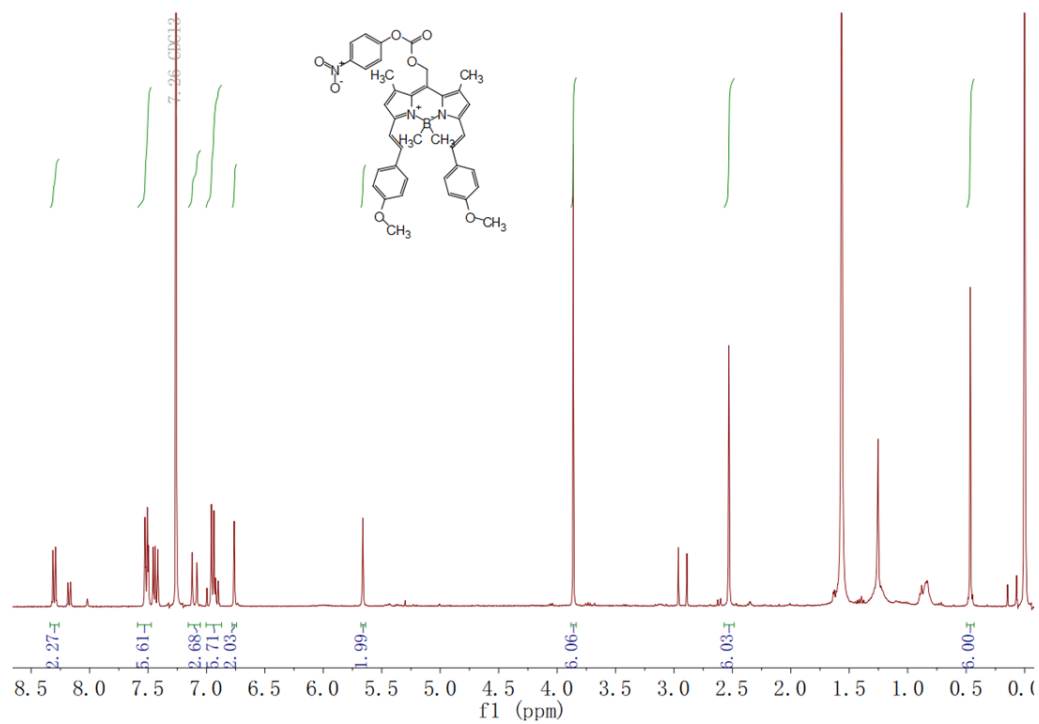


Figure S14. $^1\text{H-NMR}$ spectrum of NIR-BODIPY-Me₂-4NPC.

Ivan-ChenKang-CK-1-DMSO-d6-14Sep2022. 1. fid
Dark blue, long, light sensitive

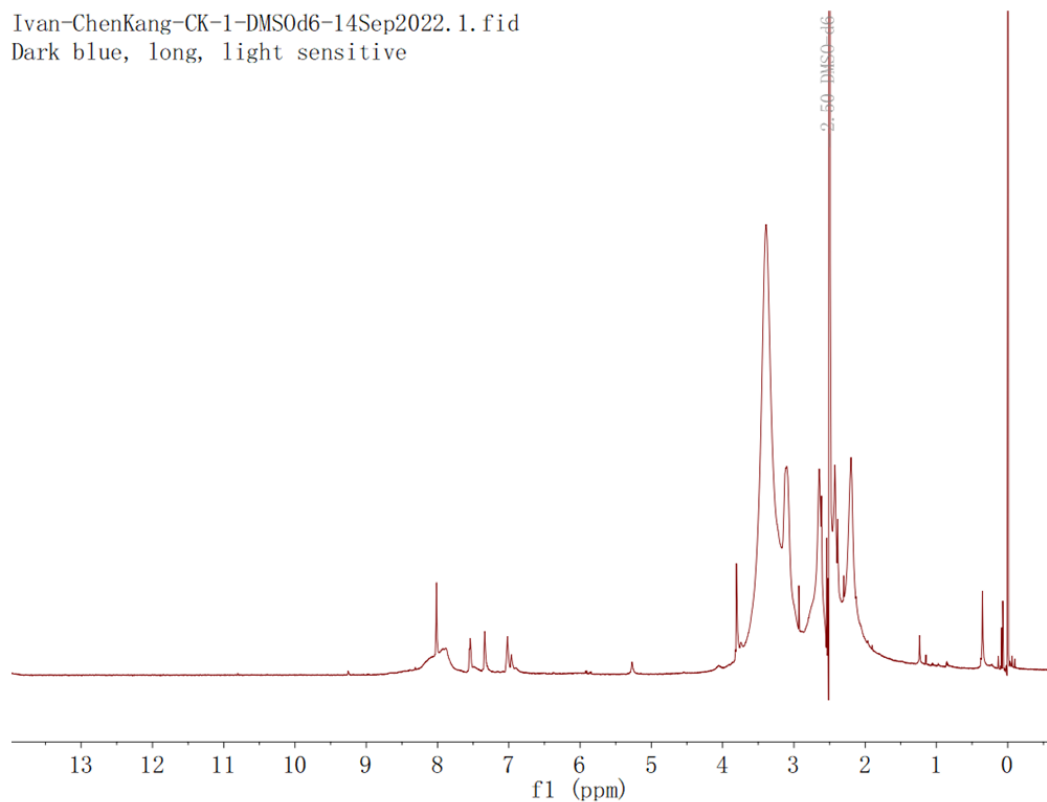


Figure S15. $^1\text{H-NMR}$ spectrum of NBMP.

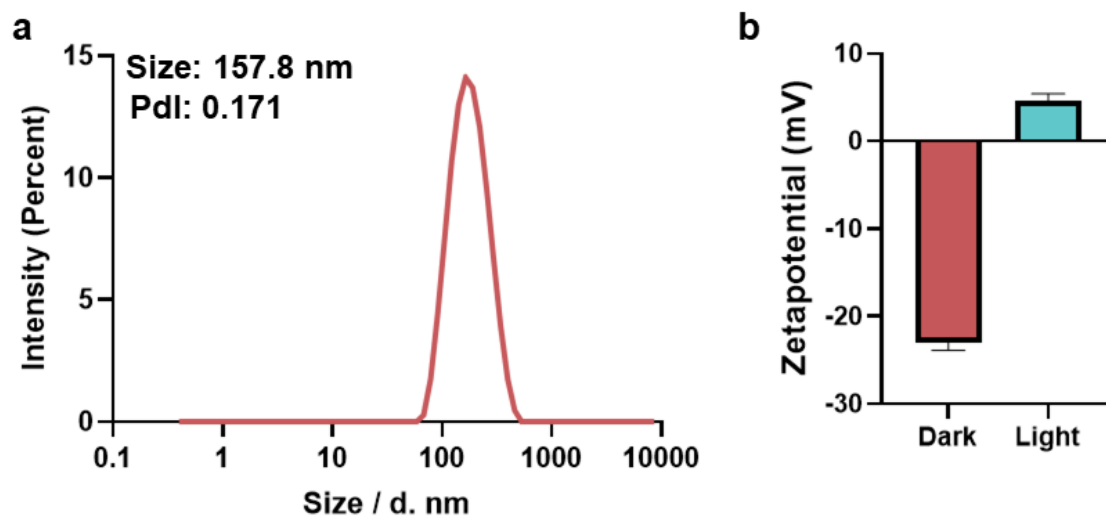


Figure S16. (a) Size distribution of HNCiF-NPs. (b) Zeta potential of HNCiF-NPs with or without light irradiation. Light irradiation: Xe lamp, 650 nm, 50 mW/cm², 5 min (means \pm SD, n=3).

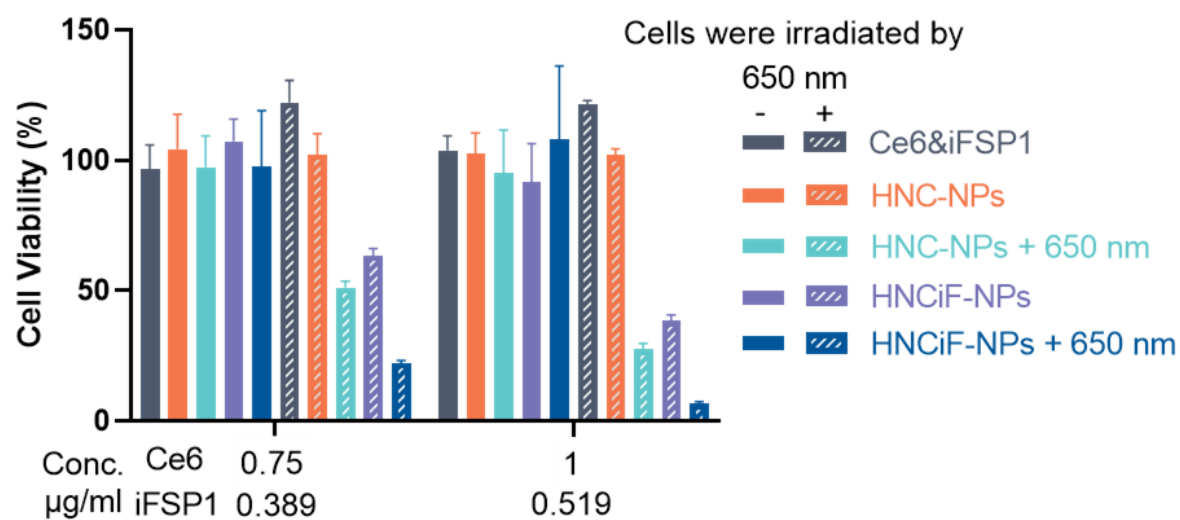


Figure S17. MTT assay of Ce6/iFSP1 dose-dependent cytotoxicity on 4T1 cells with different treatments as mentioned in the figure. Light irradiation: 650 nm light, 50 mW/cm², 5 min for nanoparticle pre-irradiation, and 5 mW/cm², 15 min for cell irradiation (means \pm SD, n=3).

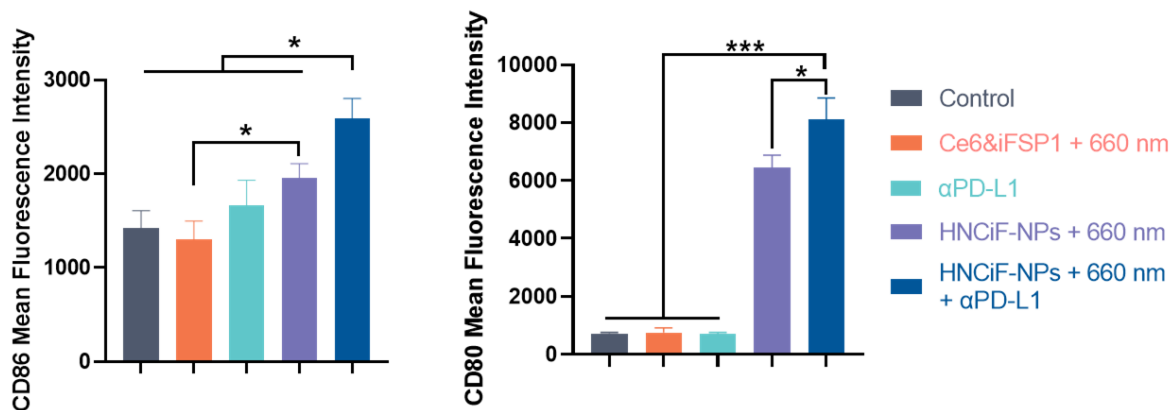


Figure S18. Mean fluorescence intensity analysis on CD80/CD86 fluorescent antibody-treated splenic DCs from the mice with different treatments as shown in the figure (means \pm SD, n=3). Light irradiation: Laser, 660 nm, 60 mW/cm², 1 min.

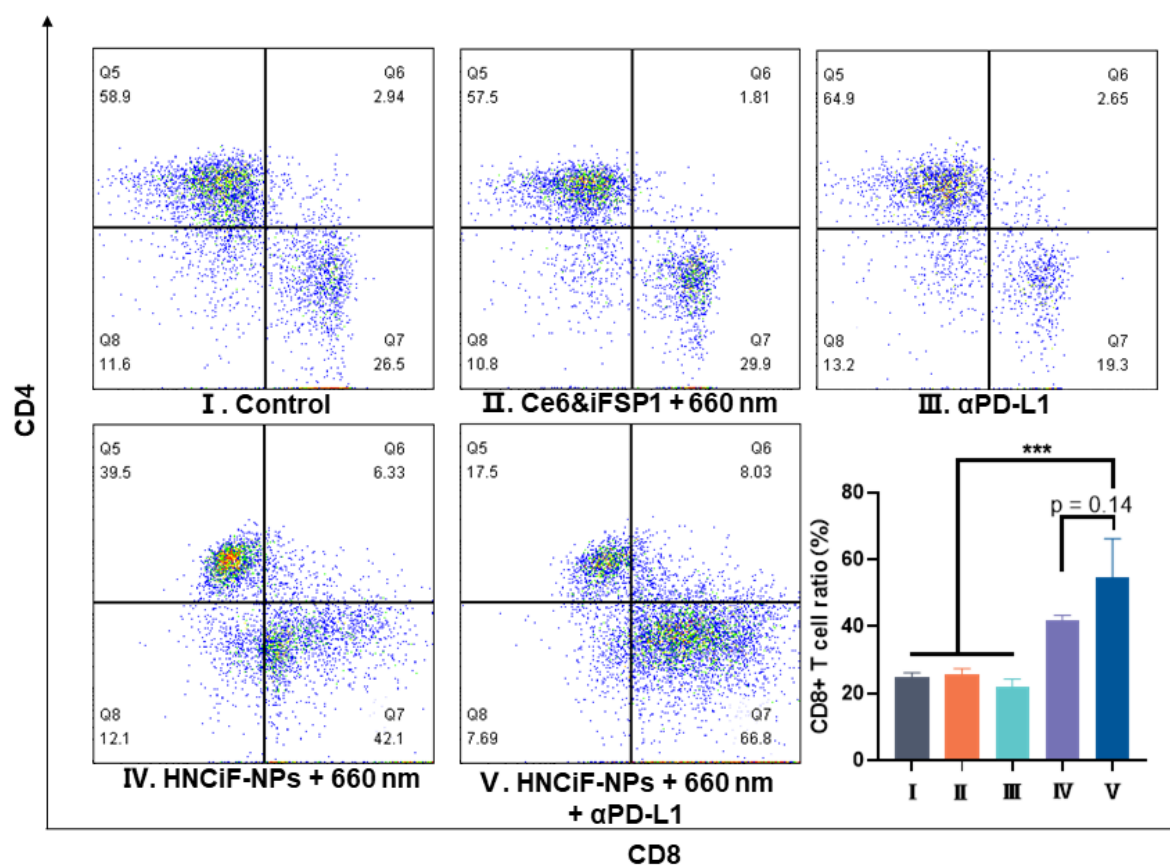


Figure S19. Percentage splenic CD8⁺ T cells within the CD3⁺ cells of the mice with different treatments (means \pm SD, n=3). Light irradiation: Laser, 660 nm, 60 mW/cm², 1 min.

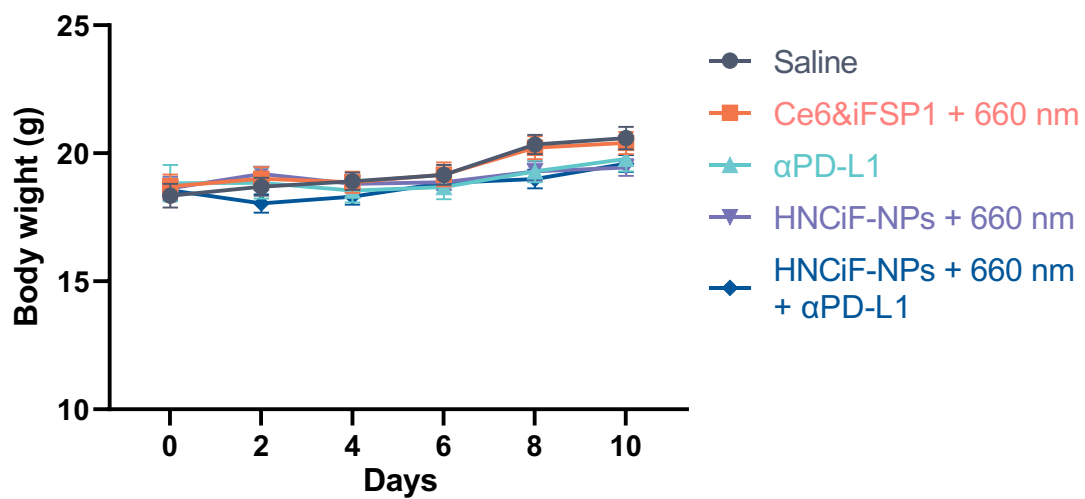


Figure S20. Mouse body weight in response to different treatments as shown in the figure (means \pm SD, n=5). Light irradiation: Laser, 660 nm, 60 mW/cm², 1 min.