Near-Infrared Light-Triggered Nanobomb for In Situ On-Demand Maximizing Photothermal / Photodynamic Efficacy for Cancer Therapy

- Supporting Information

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Table S1. Composition of the test liposomes

Liposomes	DPPC (mmol)	DSPE-PEG_{2000} (mmol)	DSPE-PEG_{2000}-FA (mmol)	Chol (mmol)	Cypate (mmol)	Ce6 (mmol)
Cy/Ce6/CO_2-Lip	60	5	-	20	14	5
Cy/Ce6-Lip-FA	60	4	1	20	14	5
Cy/Ce6/CO_2-Lip-FA	60	4	1	20	14	5

Synthesis of DSPE-PEG-FA

100 mg of folate were dissolved in 4 mL of DMSO, and then 2 mL of pyridine containing 400 mg of DSPE-PEG_{2000}-NH_2 was mixed into the above solution followed by the addition of 130 mg of DCC. After reacting at room temperature for 4 h, pyridine was removed using rotavapor. Subsequently, the solution was added 50 mL of distilled water and centrifuged. The supernatant was dialyzed against 50 mM $NaHCO_3$ for 3 d and distilled water for 2 d. Finally, the solution was lyophilized to obtain the DSPE-PEG-FA.

Figure S1. Synthesis route of DSPE-PEG-FA.

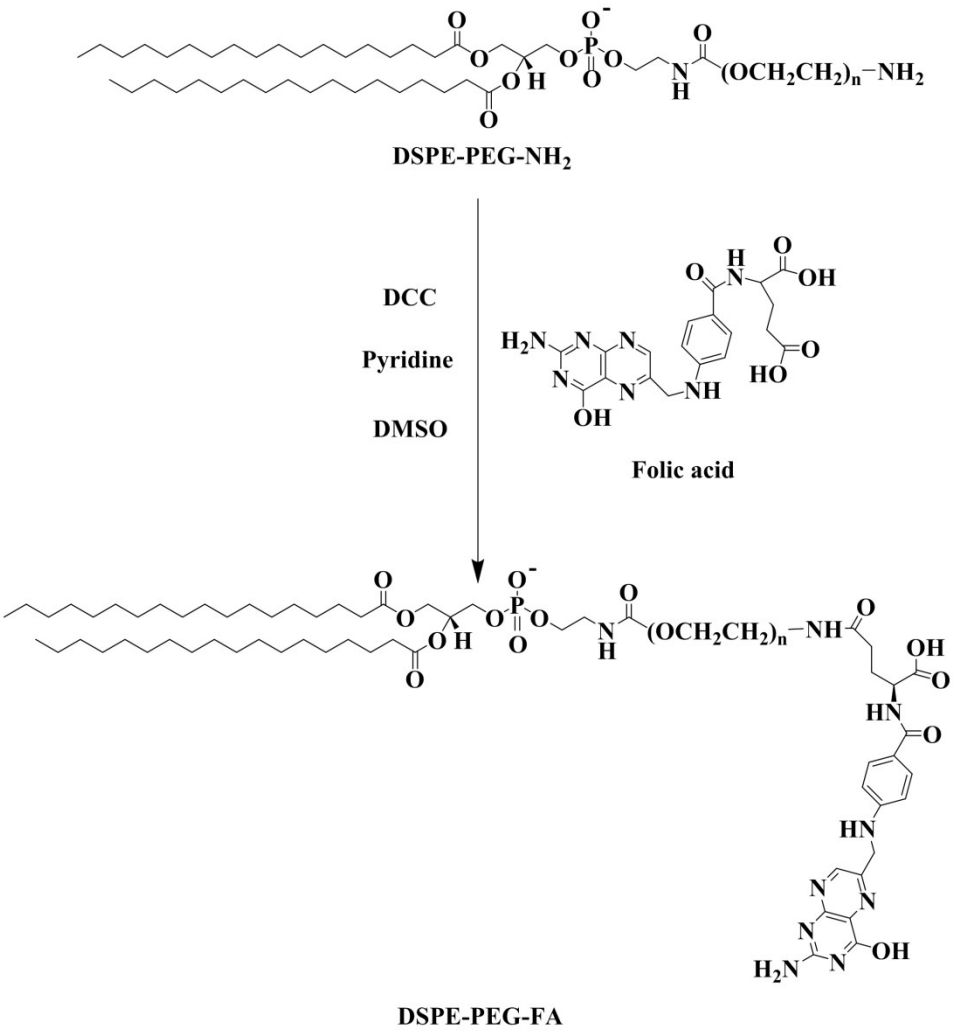

Figure S2. ^{1}H-NMR spectra of a) DSPE-PEG-NH_2 and b) DSPE-PEG-FA. After FA linkage, the proton peaks of PEG (-CH_2CH_2O-) of DSPE-PEG-FA were located at 3.3-3.6 ppm. The new amide linkage between DSPE-PEG-NH_2 and FA appeared at 8.04 ppm. A series of peaks of DSPE were observed at 0.85 ppm (-CH_3-), 1.19 ppm (-CH_2-), 1.51 ppm (-CH_2CH_2C=O), 2.28 ppm (-CH_2C=O) and 4.19 ppm (CH_2-O-C=O). The characteristic peaks of arene groups of FA can be found at 6.6-8.7 ppm. The ^{1}H NMR results verified the successful conjugation between FA and DSPE-PEG-NH_2.

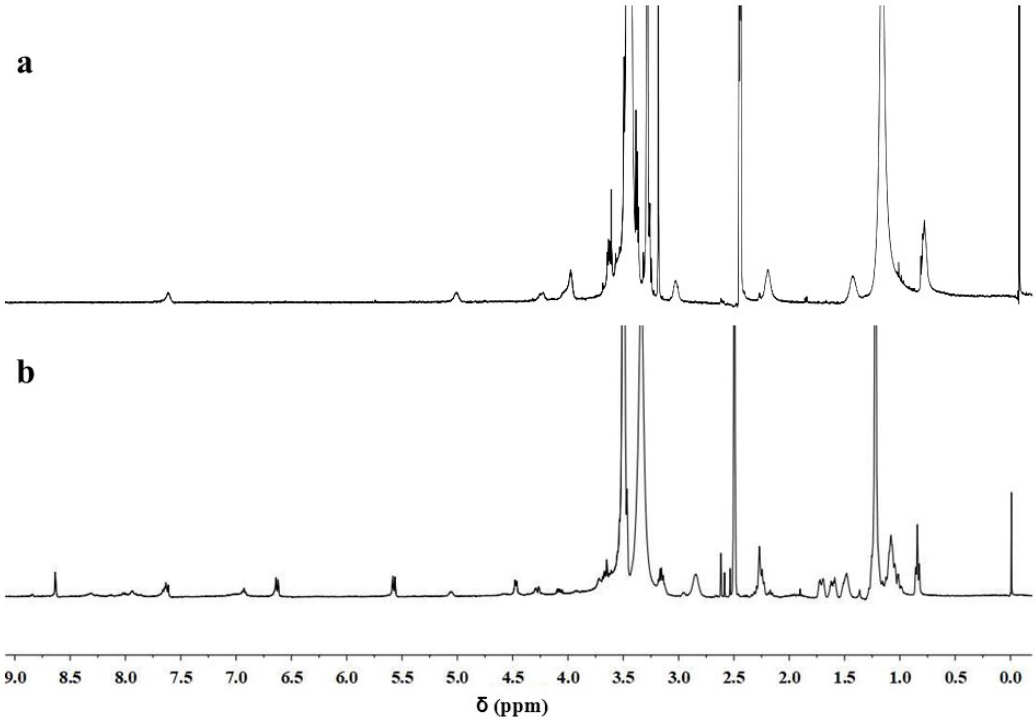

Figure S3. FT-IR and UV spectra of DSPE-PEG-NH_2, FA and DSPE-PEG-FA. In the FT-IR spectra, the characteristic peaks of DSPE-PEG-FA at 2851 cm^{-1} and 1626 cm^{-1} (O=C-NH) appeared. UV results of DSPE-PEG-FA exhibited two peaks at 286 and 355 nm from folate and 294 nm of DSPE-PEG-NH_2. These data confirmed that DSPE-PEG-FA was successfully synthesized.

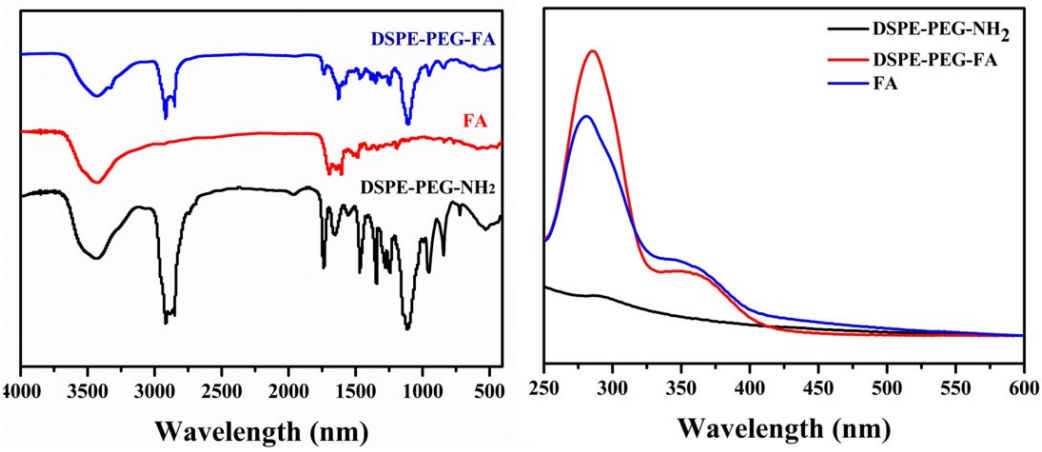

Figure S4. Photographs of Cy/Ce6/CO_2-Lip, Cy/Ce6-Lip-FA, and Cy/Ce6/CO_2-Lip-FA in PBS (pH 7.4) for 7 d. All of the liposomes exhibited a good stability without aggregation and precipitation.

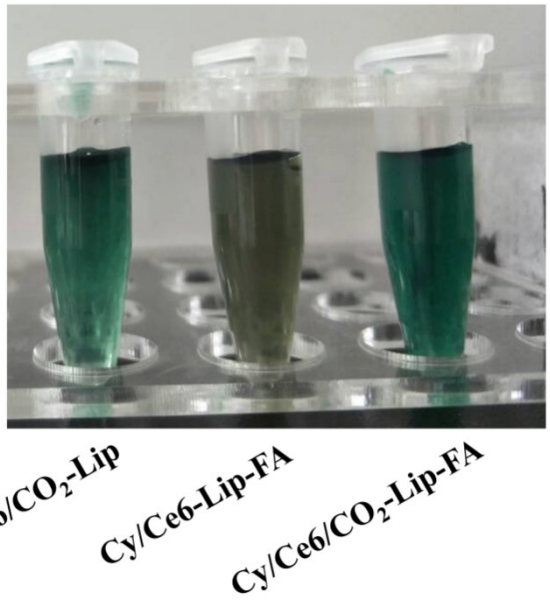

Figure S5. UV absorbance of free Cypate/Ce6 and Cy/Ce6/CO_2-Lip-FA without irradiation.

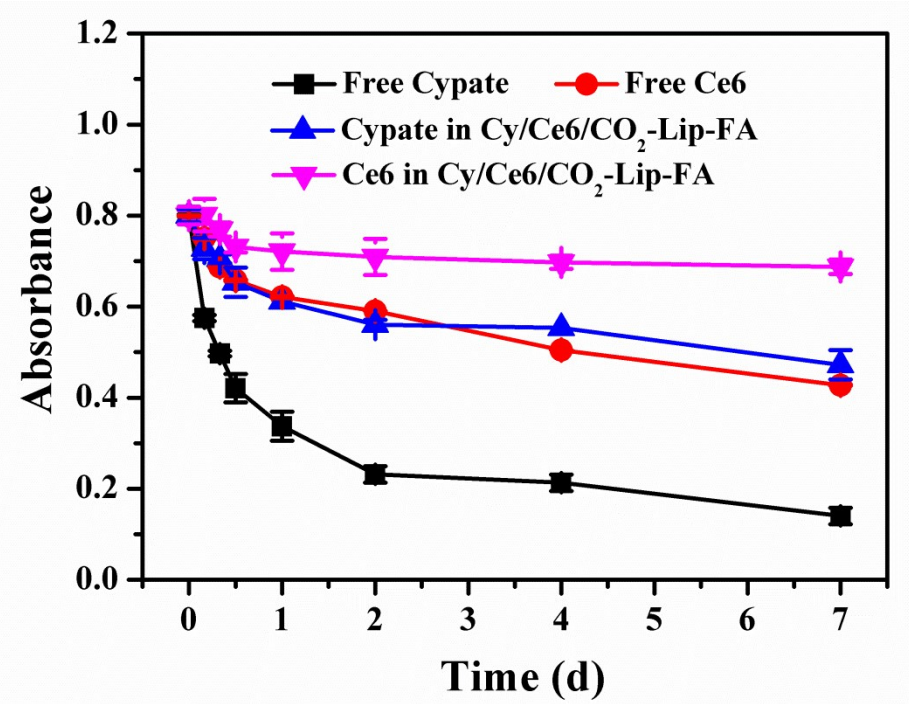

Figure S6. The leakage ratios of Ce6 and Cypate from Cy/Ce6/CO_2-Lip-FA in 50% FBS at 37 °C for 24 h.

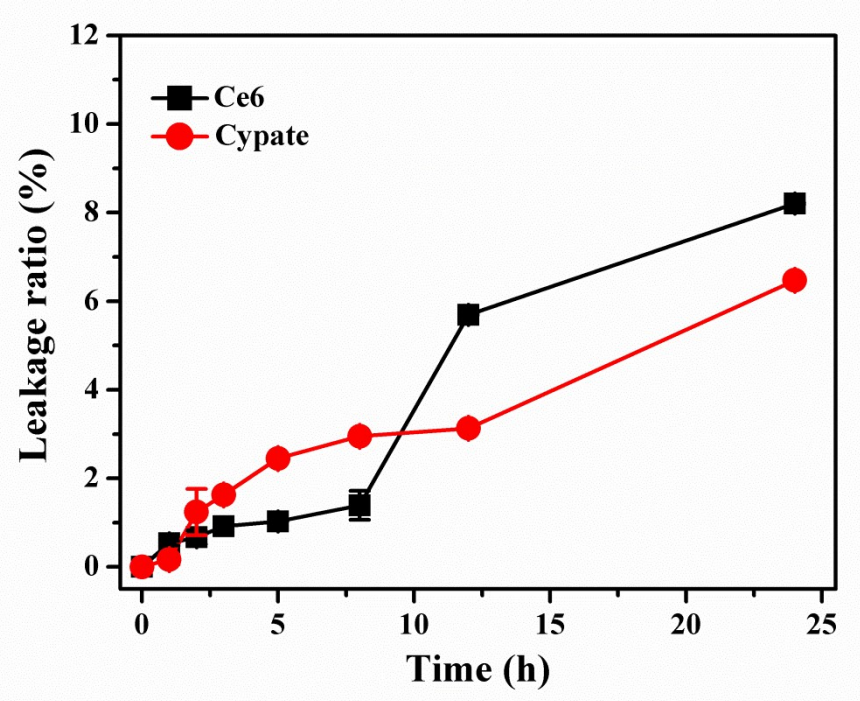

Figure S7. UV absorbance of free Cypate/Ce6 after irradiation at a) 785 nm and b) 660 nm. After 785 nm laser irradiation, the absorbance of Cypate at 785 nm decreased gradually, while the peak of Ce6 at 660 and 404 nm was almost unaffected. It indicated that 785 nm irradiation may induce PTT but cannot produce Ce6 photobleaching before PDT effect. On the contrary, upon 660 nm laser irradiation, the peaks at 404, 660 and 785 nm gradually decreased and disappeared. These results demonstrated that 660 nm irradiation could lead to the photobleaching of Cypate and Ce6 at the same time. Therefore, after co-encapsuling in the liposomes, Cypate could actively inhibit the phototoxicity of Ce6 (PDT effect) prior to PDT.

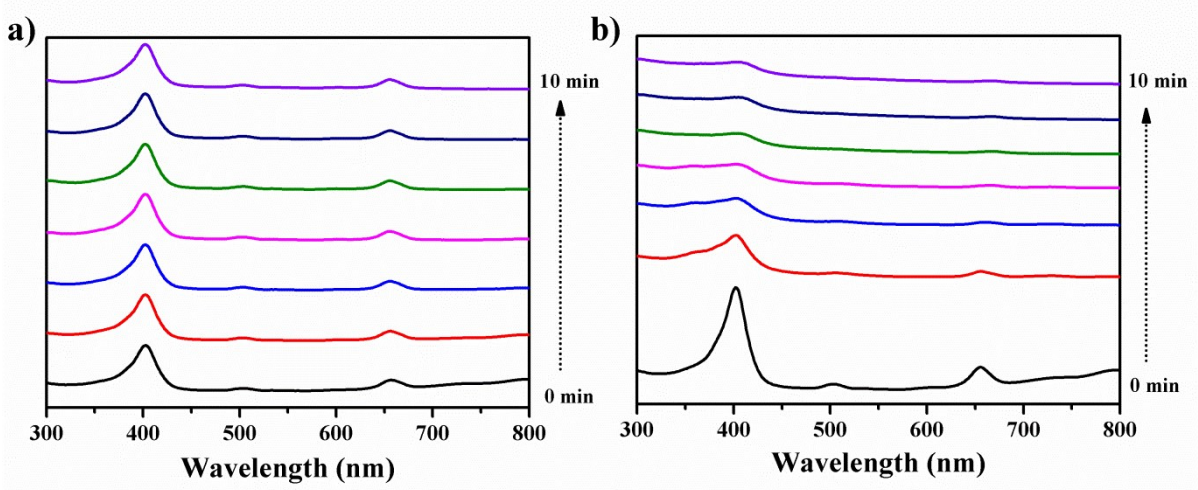

Figure S8. a) Schematic diagram of experimental system for temperature monitoring of simulated *in vivo* environment under NIR laser irradiation; b) Thermal infrared images of simulated *in vivo* conditions with or without the slice of pork tissue blocking between laser and test Cy/Ce6/CO_2-Lip-FA NPs solution.

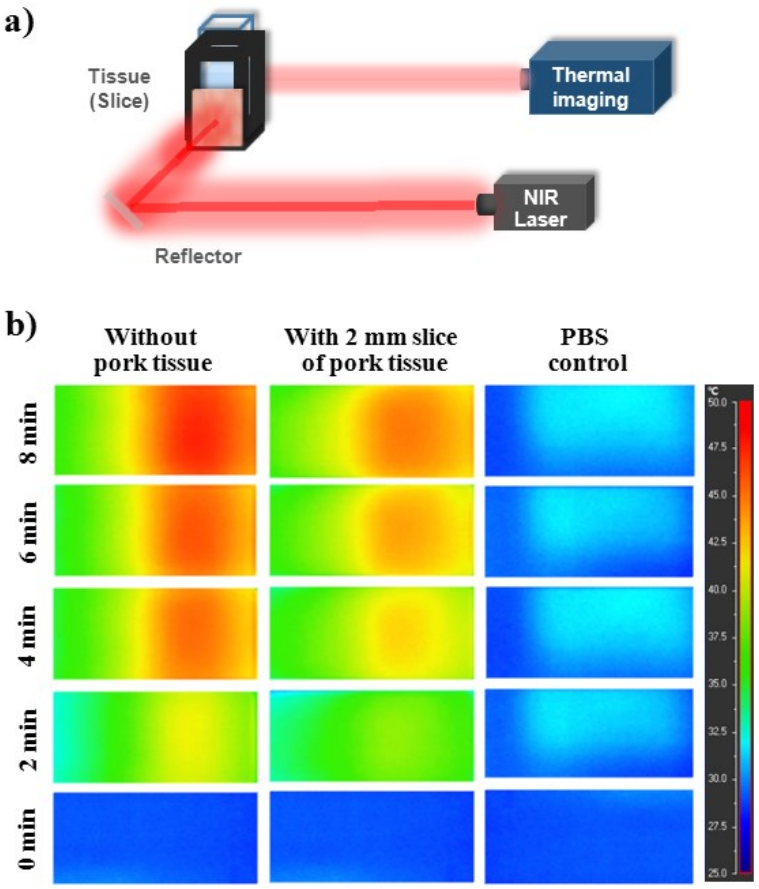

Figure S9. Free folate competition assay. Briefly, the U14 cells were pre-incubated overnight. After medium removal, the cells were treated with the fresh medium with Cy/Ce6/CO_2-Lip-FA (containing 1, 2, and 5 μg/mL of Ce6) for 4 h. Then, free folate (4 mM) was added to medium and incubated for another 2 h. The liposomes without free folate treatment were chosen as a control. The cellular uptake of Cy/Ce6/CO_2-Lip-FA with and without free folate treatment was observed using fluorescence microscope. The scale bars represent 50 μm. When free folate was added, the cellular uptake of Cy/Ce6/CO_2-Lip-FA reduced significantly. It was because free folate could compete the folate receptor on the tumor cell surface with Cy/Ce6/CO_2-Lip-FA. The results demonstrated Cy/Ce6/CO_2-Lip-FA was uptake by U14 cells via folate receptor-mediated endocytosis, which implied the folate was modified on the surface of liposomes.

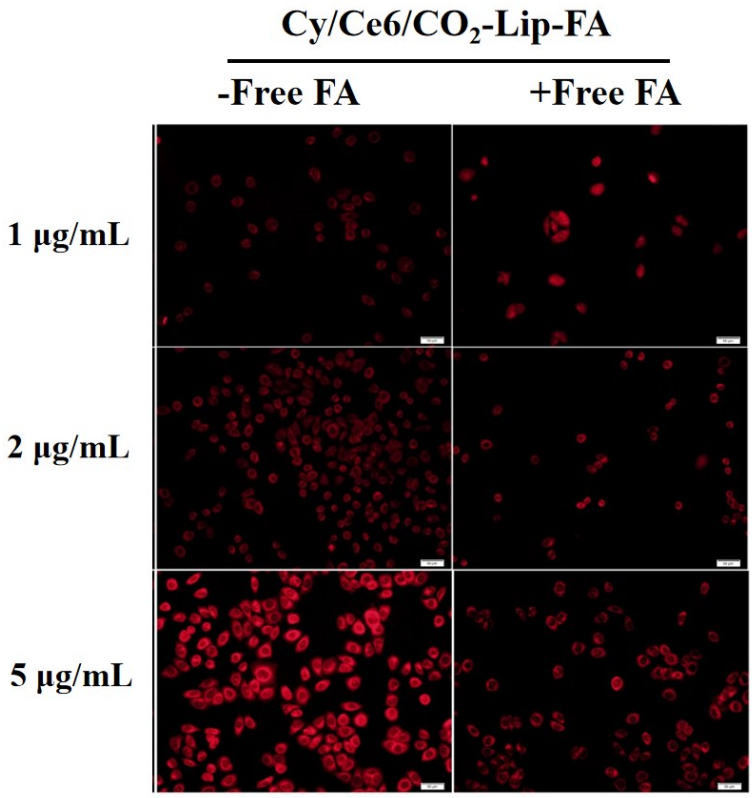

Figure S10. Trypan blue staining images of U14 cells treated with a) Cy/Ce6/CO_2-Lip, b) Cy/Ce6-Lip-FA and c) Cy/Ce6/CO_2-Lip-FA at the Cypate concentrations of 2, 5, and 10 μg/mL with or without irradiation. PBS is as a control. The dead and damaged cells can be stained by trypan blue due to the membrane incompleteness, while normal cells can exclude trypan blue.

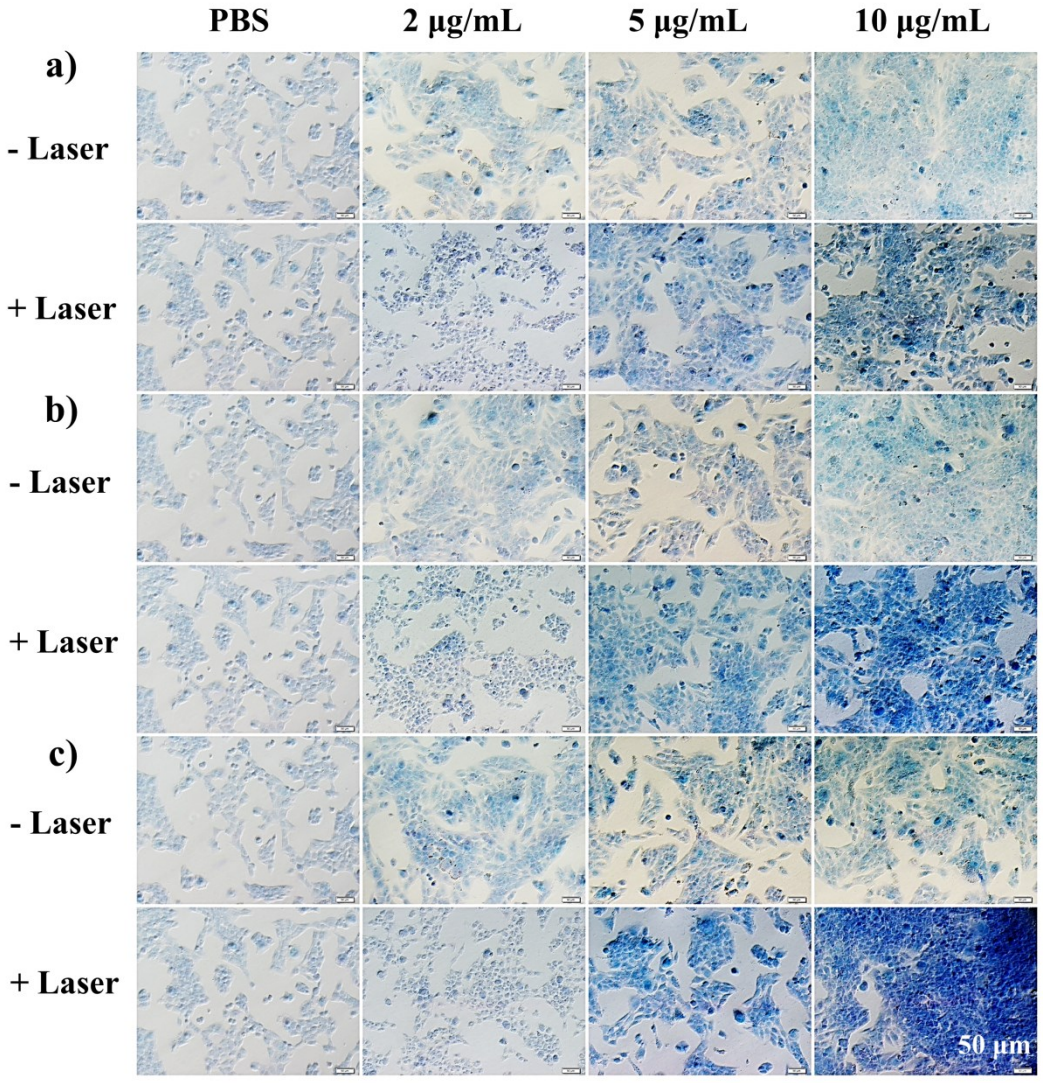

Figure S11. Images of H&E stained primary organ obtained from U14-bearing mice treated with saline, free Cypate/Ce6 and various liposomes upon 785/660 nm irradiation. The scale bars represent 100 μm. It seemed no obvious pathological abnormalities in the major organs of the mice after various treatments.