

Supporting Information

Extracellular vesicles with high dual drug loading for safe and efficient combination chemo-phototherapy

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Table S1. Size changes of EV(ICG/PTX) incubated in 5% FBS-containing PBS for various periods of time.

Incubation time (h)	Size (nm)
0	155.7±2.4
4	149.1±2.5
12	156.0±4.3
72	156.2±6.6
96	155.2±6.2
120	150.0±5.0

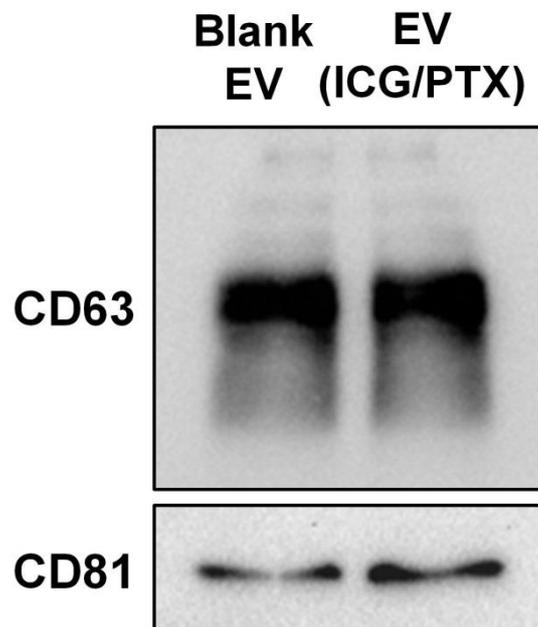


Figure S1. Western blot of marker proteins, CD63 and CD81 from blank EVs and EV(ICG/PTX).

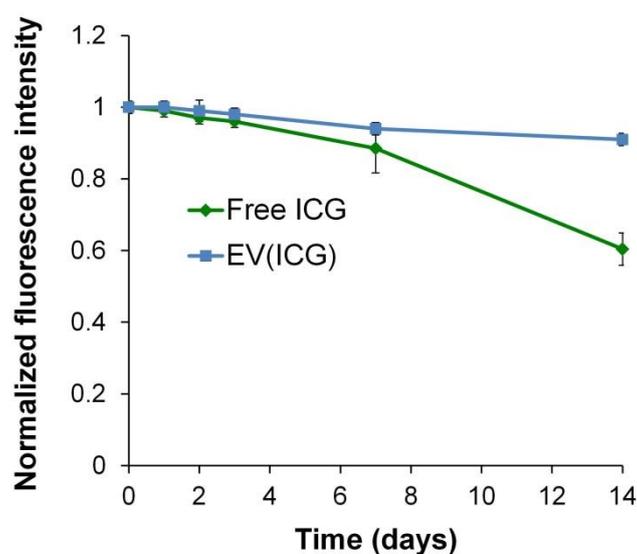


Figure S2. Fluorescence intensity changes of free ICG and EV(ICG) in PBS at 4 °C for 14 days.

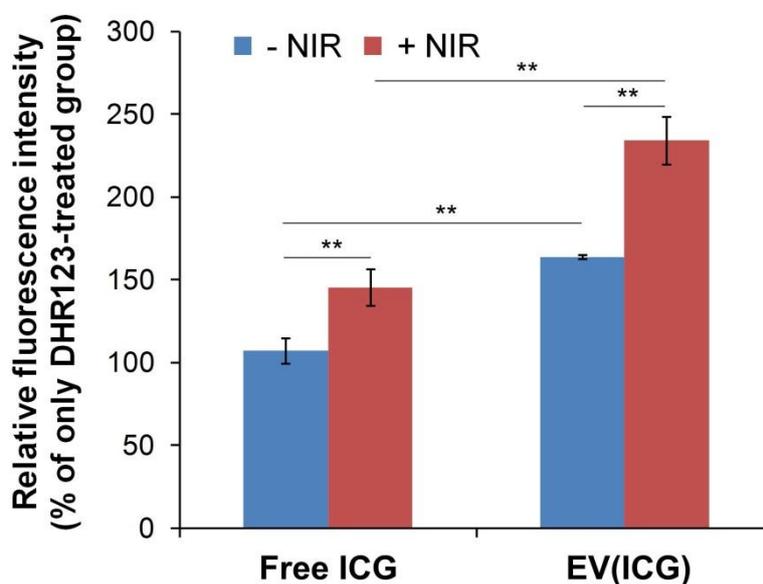


Figure S3. Relative ROS generation from free ICG and EV(ICG) in DI water before and after NIR light irradiation, determined by dihydrorohdamine 123 (DHR123) staining. The fluorescence intensities of free ICG and EV(ICG) groups were normalized by the fluorescence intensity of the sample treated with DHR123 only in DI water (** $p < 0.01$).

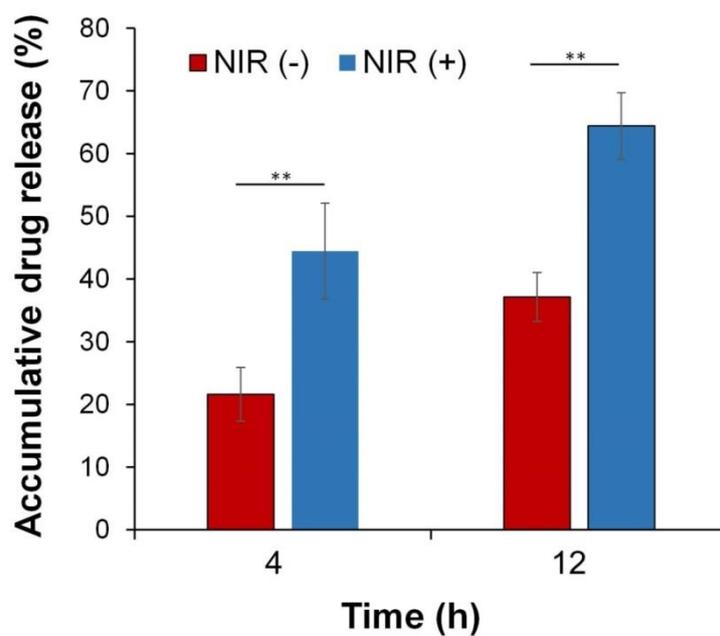


Figure S4. Drug release profiles of PTX from EV(ICG/PTX) in the absence or presence of NIR light irradiation (100 s) at different incubation time. The PTX release from the EV(ICG/PTX) was facilitated when exposed to NIR light (** $p < 0.01$).

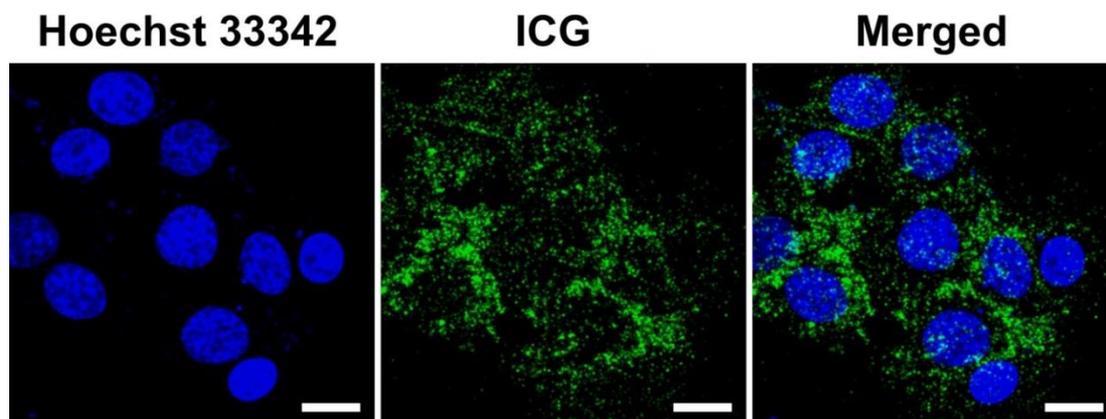


Figure S5. Live confocal microscopic images of MCF-7 cells treated with free ICG (green dots). Hoechst 33342 (blue dots) was used to stain cellular nuclei. Scale bars indicate 20 μm .

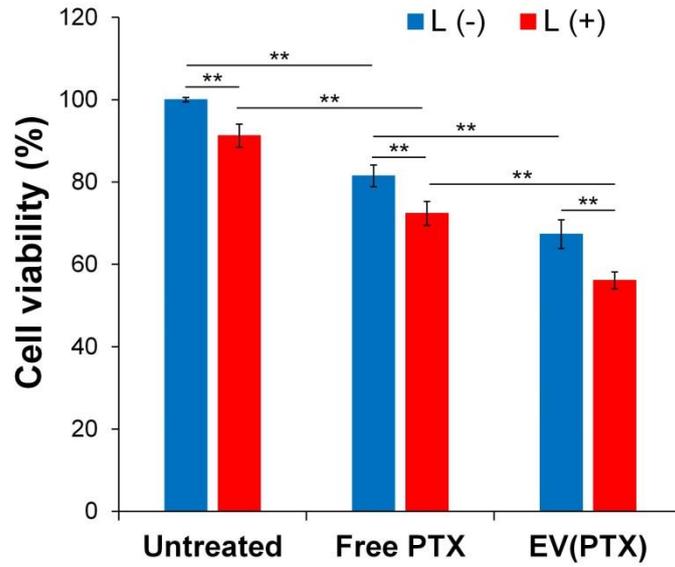


Figure S6. Cell viability of MCF-7 cells after treatments with free PTX and EV(PTX) (9 $\mu\text{g/mL}$ PTX) before and after NIR light irradiation (0.8 W/cm^2 , 100 s) (** $p < 0.01$).

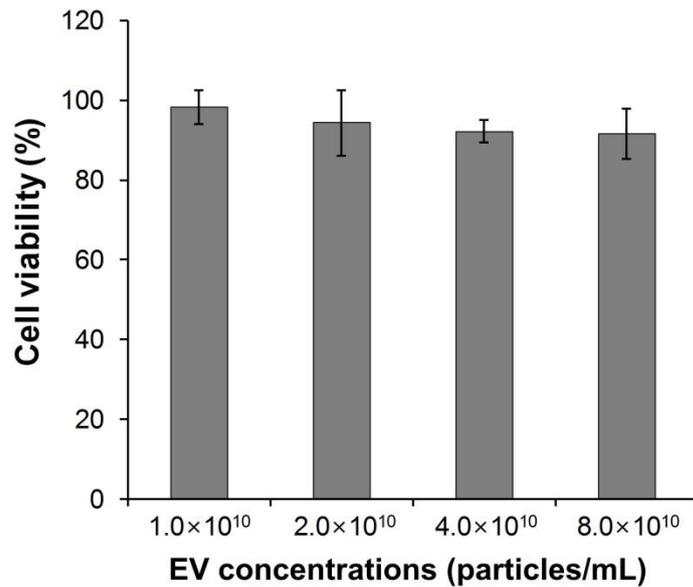


Figure S7. Cell viability of MCF-7 cells after treatment with blank EVs at different concentrations.

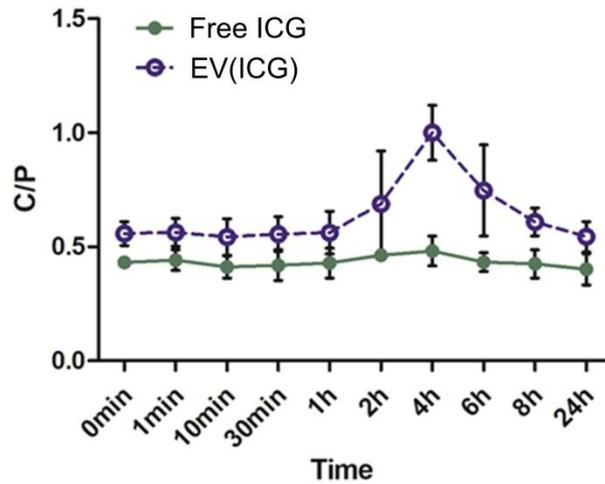


Figure S8. Time course graph of fluorescence intensity ratio of tumor core area/peritumoral tissue area (C/P) in the free ICG or EV(ICG)-treated mice ($n = 4$).

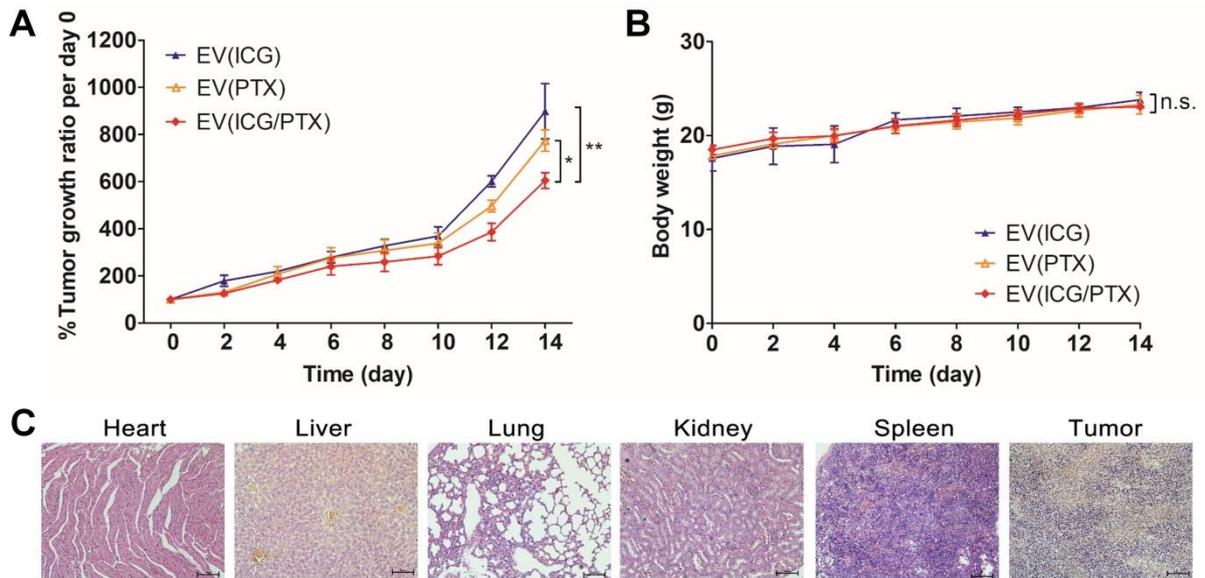


Figure S9. (A) Normalized tumor growth ratio of MCF-7 tumor-bearing mice treated with EV(PTX) compared to that with EV(ICG) and EV(ICG/PTX). The mice were irradiated with an 808 nm laser at 0.8 W/cm^2 for 5 min at 4 h after i.v. injection of each sample. (B) Body weights of MCF-7 tumor-bearing mice ($n = 4$) as a function of time after treatment with each sample and NIR laser irradiation (0.8 W/cm^2 , 5 min) ($*p < 0.05$; $**p < 0.01$; n.s.: not significant). (C) H&E staining of major organ and MCF-7 tumor of mice at day 14 after treatment with EV(PTX) and NIR light irradiation. Scale bars indicate $100 \mu\text{m}$.

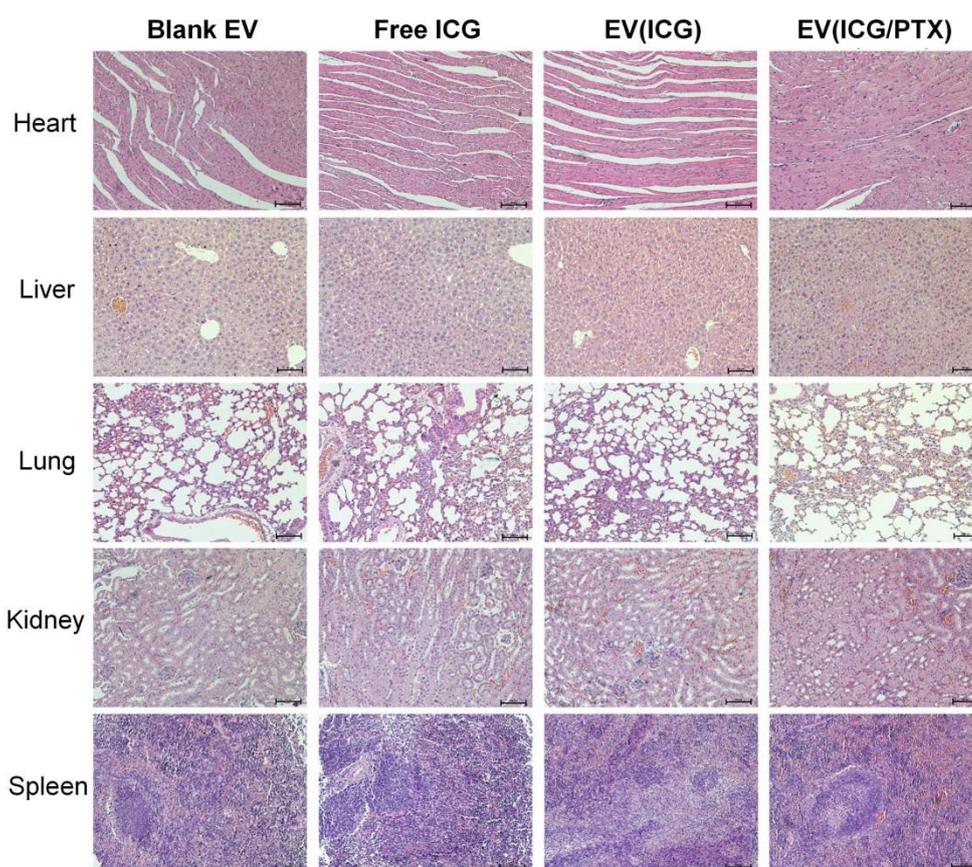


Figure S10. H&E staining of major organ tissues of the mice after 14 days post injection with various samples [e.g., blank EV, free ICG, EV(ICG), and EV(ICG/PTX)] and NIR light irradiation (0.8 W/cm^2 for 5 min). Scale bars indicate $100 \mu\text{m}$.