Supporting Information

Engineered extracellular vesicle-based sonotheranostics for dual stimuli-sensitive drug release and photoacoustic imaging-guided chemo-sonodynamic cancer therapy

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Table S1. IC_{50} values of ICG and PTX in various SBC-EV(ICG/PTX) samples against MCF-7 cells before and after US irradiation.

Samples	ICG (μg/mL)	PTX (μg/mL)
Free ICG	212.56±9.00	-
Free ICG + US	197.08±10.54	-
Free PTX	-	15.95±0.28
Free PTX + US	-	15.16±0.09
SBC-EV(ICG/PTX) (28:2)	185.58±3.82	13.26±0.27
SBC-EV(ICG/PTX) (28:2) + US	174.49±3.74	12.46±0.73
SBC-EV(ICG/PTX) (28:4)	91.71±0.54	12.01±0.25
SBC-EV(ICG/PTX) (28:4) + US	75.41±2.23	10.78±0.32
SBC-EV(ICG/PTX) (28:10)	27.04±0.16	9.66±0.06
SBC-EV(ICG/PTX) (28:10) + US	17.17±0.02	6.13±0.01
SBC-EV(ICG/PTX) (28:13)	14.22±0.39	6.49±0.07
SBC-EV(ICG/PTX) (28:13) + US	12.99±0.21	5.95±0.08
SBC-EV(ICG/PTX) (28:18)	9.77±0.07	6.27±0.05
SBC-EV(ICG/PTX) (28:18) + US	9.26±0.27	5.81±0.09
SBC-EV(ICG/PTX) (28:22)	7.95±0.11	6.24±0.09
SBC-EV(ICG/PTX) (28:22) + US	7.08±0.03	5.56±0.02

Table S2. The area under the plasma concentration-time curve (AUC) of ICG from tumor-bearing mice (n = 4) after intravenous injection of free ICG and EV(ICG/PTX) (%ID: percentage of an injected dose).

Samples	AUC (%ID·h/mL)
Free ICG	0.7±0.3
EV(ICG/PTX)	4.0±0.5

Table S3. qRT-PCR primer sequences.

Samples	**qRT-PCR primers**
NOX1	Forward: GGTTTTACCGCTCCCAGCAGAA Reverse: CTTCCATGCTGAAGCCACGCTT
β-actin	Forward: ATGAAGTGTGACGTTGACATCCG Reverse: GCTTGCTGATCCACATCT

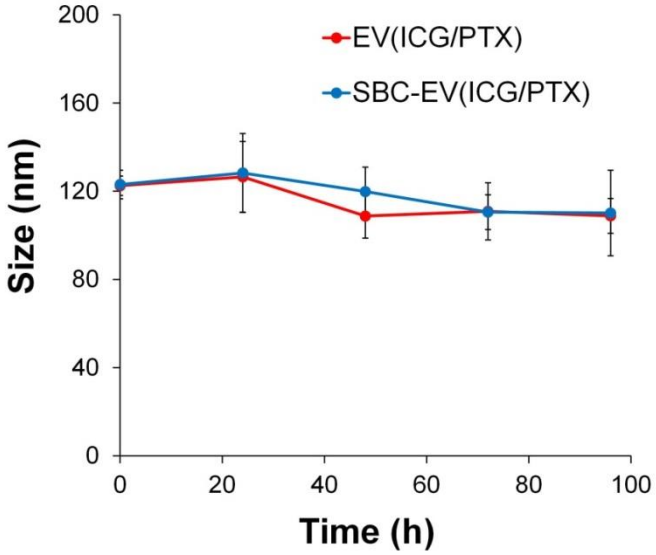

Figure S1. Size changes of EV(ICG/PTX) and SBC-EV(ICG/PTX) incubated in 10% FBS-containing PBS for 96 h.

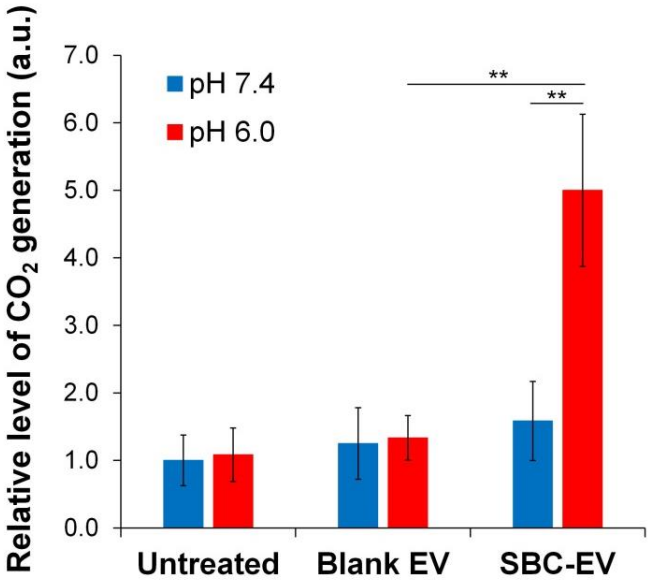

Figure S2. Relative levels of CO_2 generation by blank EV and SBC-EV under different pH conditions, determined by an acid-base titration method (**$p < 0.01$; $n = 3$).

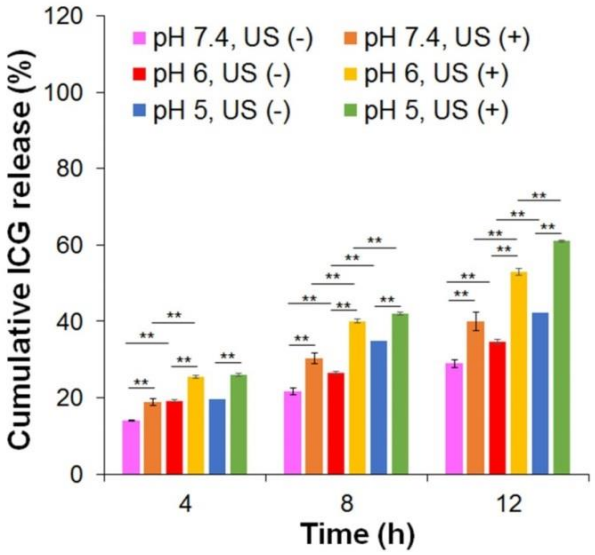

Figure S3. ICG release profiles from SBC-EV(ICG/PTX) at different pH and US (1 min of irradiation) conditions (**$p < 0.01$; $n = 3$).

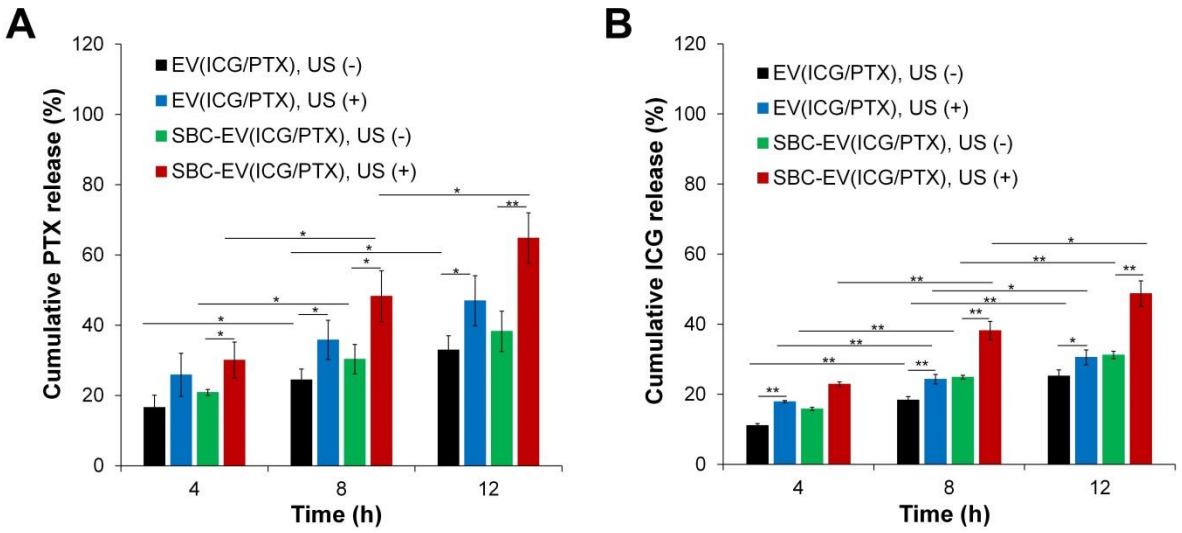

Figure S4. Release profiles of PTX and ICG from SBC-EV(ICG/PTX) at pH 6.6 and US (1 min of irradiation) conditions (*$p < 0.05$, **$p < 0.01$; $n = 3$).

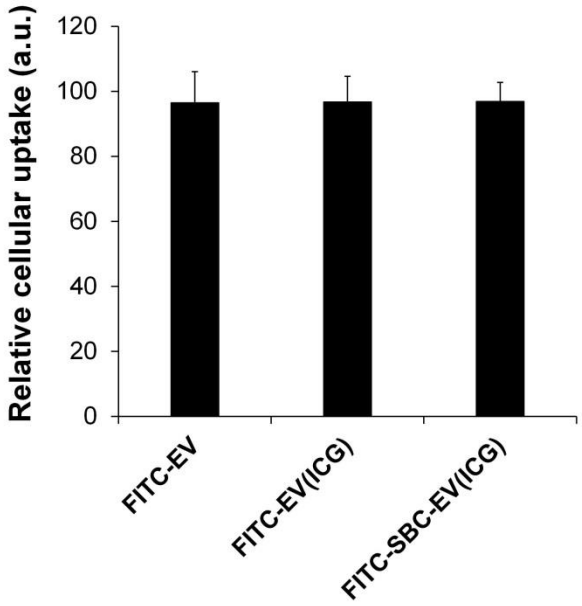

Figure S5. Relative cellular uptake of various FITC-labeled EVs by MCF-7 cells after 4 h of incubation.

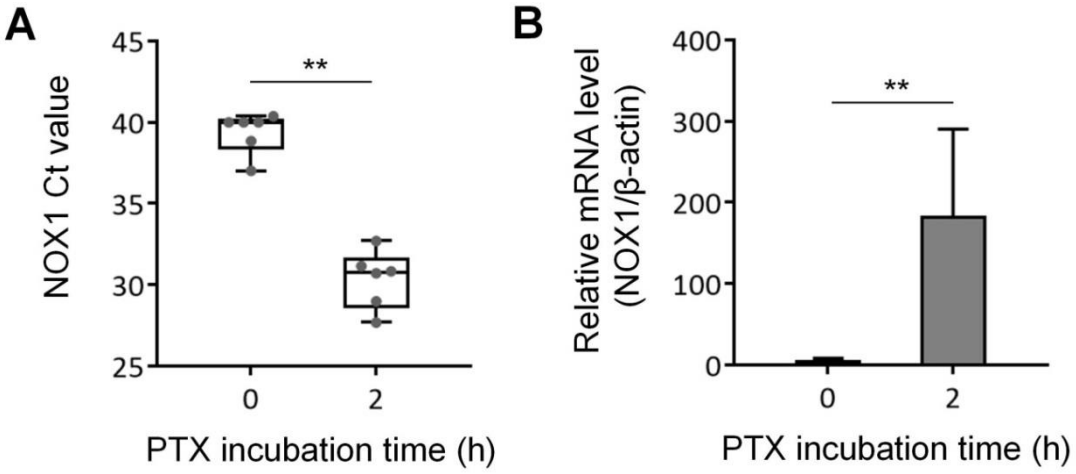

Figure S6. The effect of PTX on NOX1 expression in MCF-7 cells. Cycle threshold (Ct) values of NOX1 mRNA (A) and relative NOX1 mRNA expression level (B) in MCF-7 cells after treatment with free PTX were analyzed. $^{**}p < 0.01$ ($n = 6$).

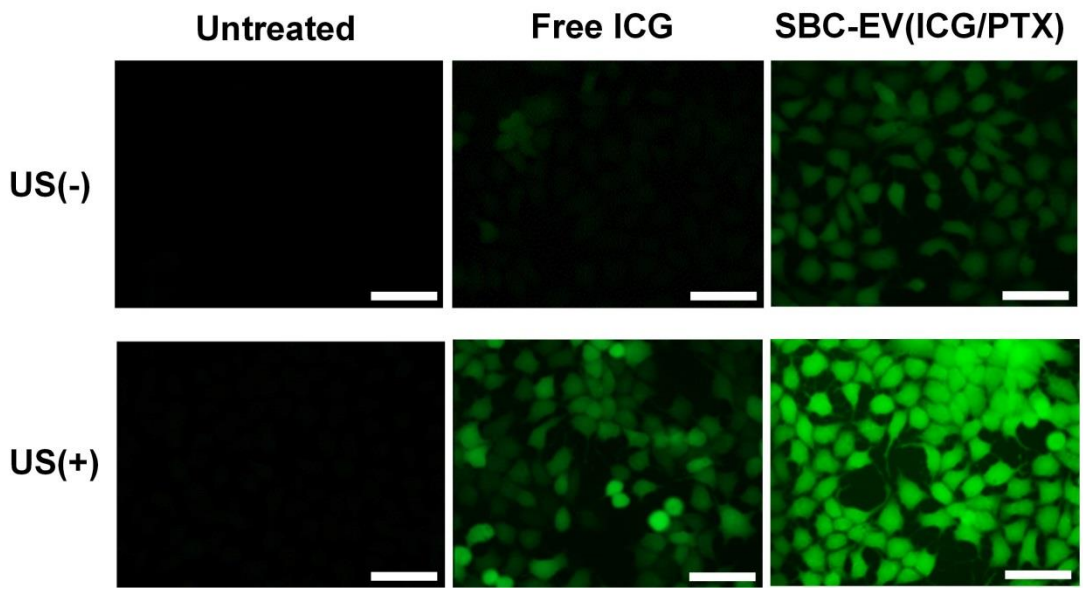

Figure S7. Fluorescence images of MCF-7 cells generating intracellular ROS after treatment with free ICG and SBC-EV(ICG/PTX) under US exposure (1 MHz, 0.3 W/cm^2, 1 min). Scale bars indicate 25 μm.

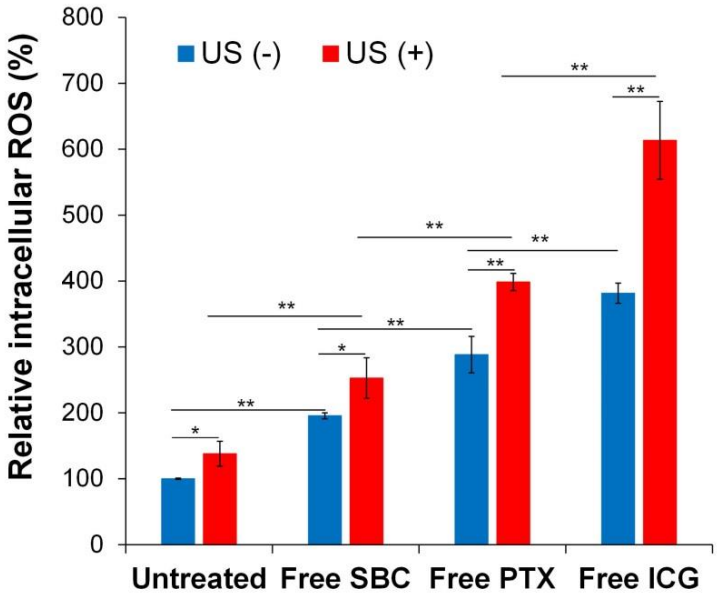

Figure S8. Relative intracellular ROS levels of MCF-7 cells treated with free SBC (4 nM), free PTX (10 μg/mL), and free ICG (28 μg/mL) before and after US irradiation (1 MHz, 0.3 W/cm^2, 1 min) (*p < 0.05, **p < 0.01, n = 3).

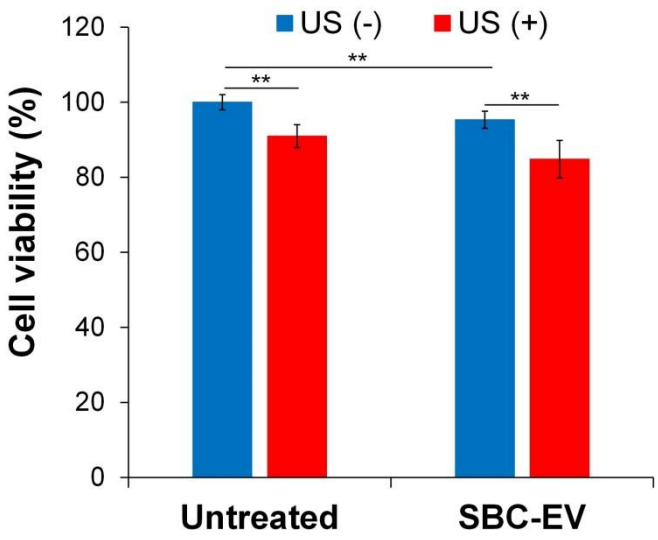

Figure S9. Viabilities of MCF-7 cells treated with SBC-EV at 4 nM of SBC concentration before and after US irradiatiion (0.3 W/cm^2, 1 min) (**p < 0.01, n = 3).

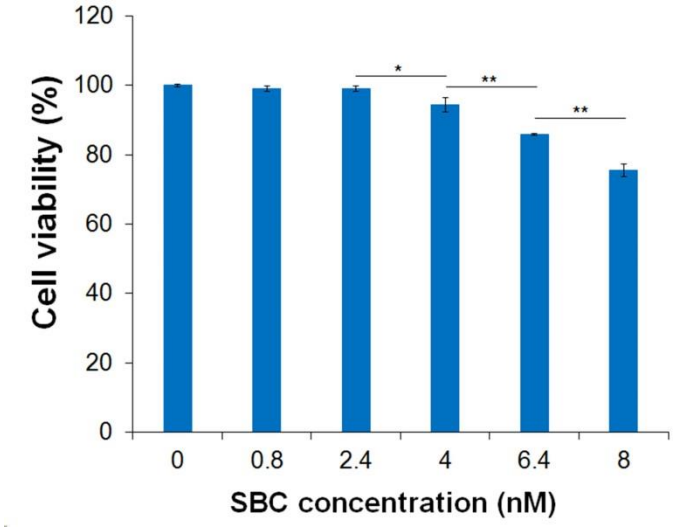

Figure S10. Viabilities of MCF-7 cells treated with SBC-EV at different concentrations of SBC (*p < 0.05, **p < 0.01, n = 3).

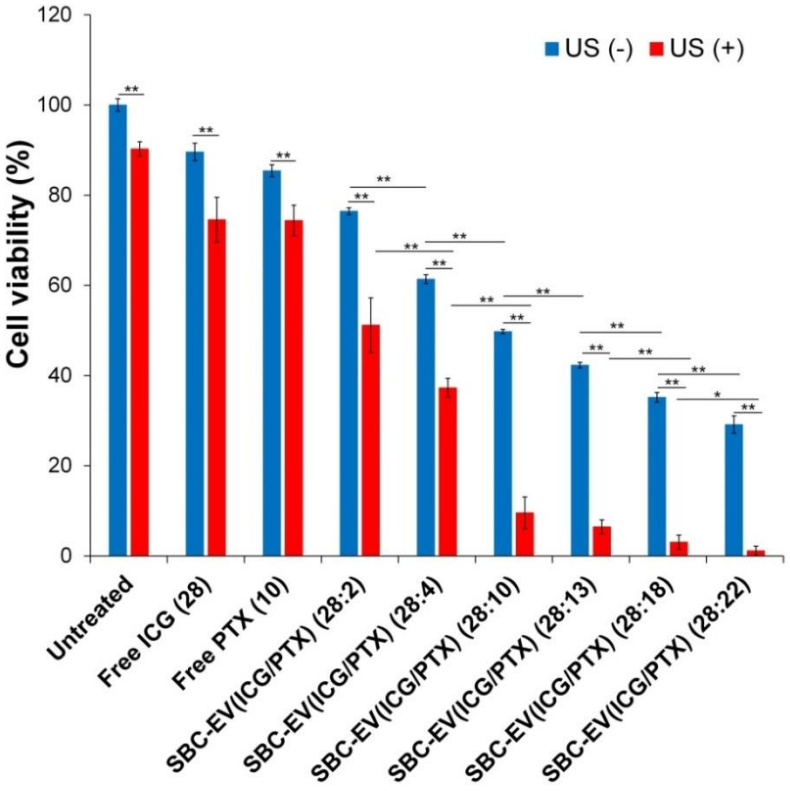

Figure S11. Viabilities of MCF-7 cells treated with various SBC-EV(ICG/PTX) at different concentrations of ICG and PTX before and after US irradiation (0.3 W/cm^2, 1 min). The number indicates concentrations (μg/mL) of ICG and PTX in the SBC-EV(ICG/PTX) (*p < 0.05, **p < 0.01, n = 3).

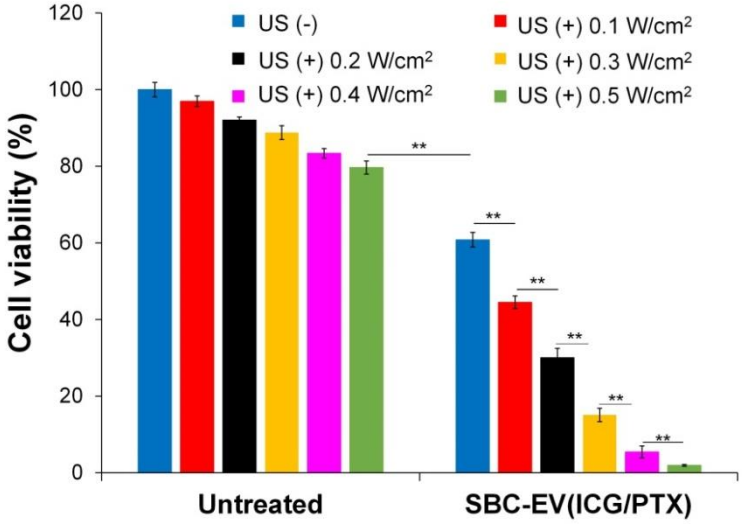

Figure S12. Viabilities of MCF-7 cells treated with SBC-EV(ICG/PTX) after irraidation with various intensities of US (**p < 0.01, n = 3).

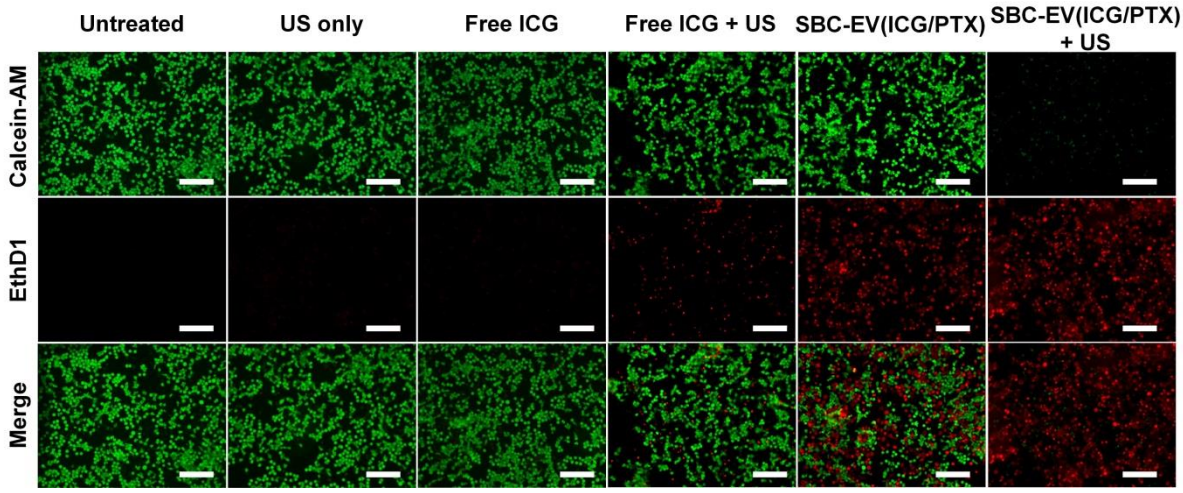

Figure S13. Live (green color) and dead (red color) cell staining of MCF-7 cells treated with free ICG and SBC-EV(ICG/PTX) before and after after US irradiation (0.3 W/cm^2, 1 min). Scale bars indicate 200 μm.

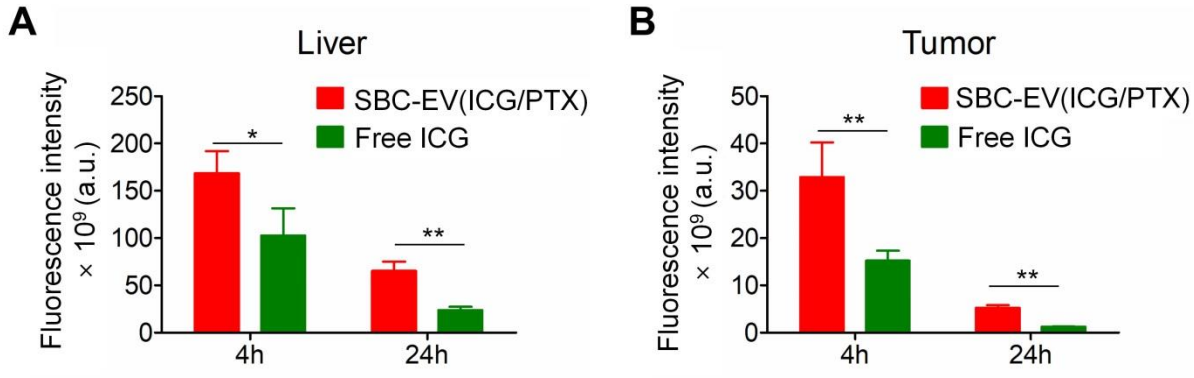

Figure S14. Quantitation of ICG from *ex vivo* imaging of liver (A) and tumor (B) at 4 h and 24 h-post i.v. administration of free ICG and SBC-EV(ICG/PTX) (*p < 0.05, **p < 0.01).

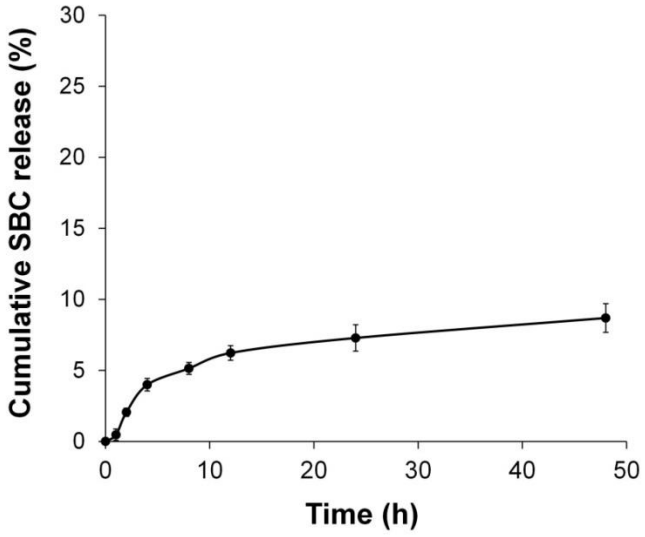

Figure S15. Release profiles of SBC from SBC-EV(ICG/PTX) at high salt concentrations (i.e., 260 mM KCl aqueous solution) mimicking in vivo conditions.

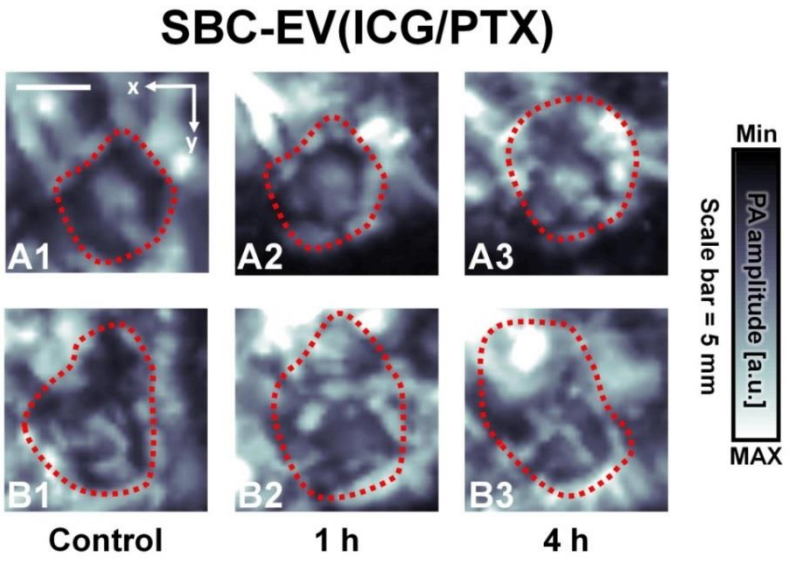

Figure S16. *In vivo* PA MAP images of tumors of MCF-7-bearing mice (A and B) at different time intervals after i.v. injection of SBC-EV(ICG/PTX). (MAP: maximum amplitude projection). Scale bar indicates 5 mm. The red circles in the images indicate tumor regions.

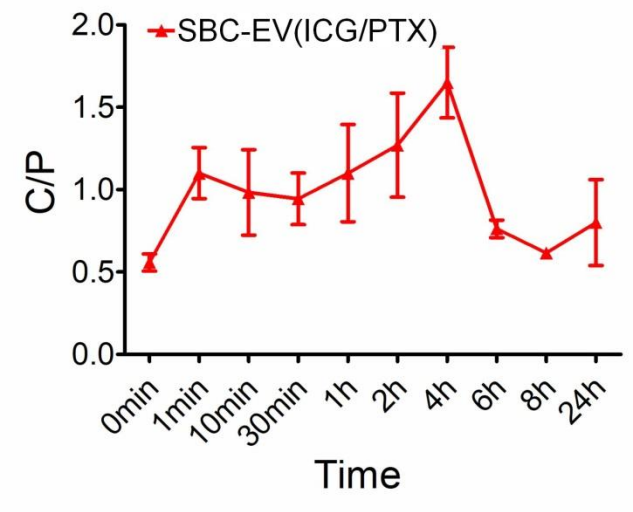

Figure S17. The time course ratios of the fluorescence intensity in the tumor core area to the peritumoral tissue area (C/P) in the SBC-EV(ICG/PTX)-treated mice as a function of time, determined by using IVIS.

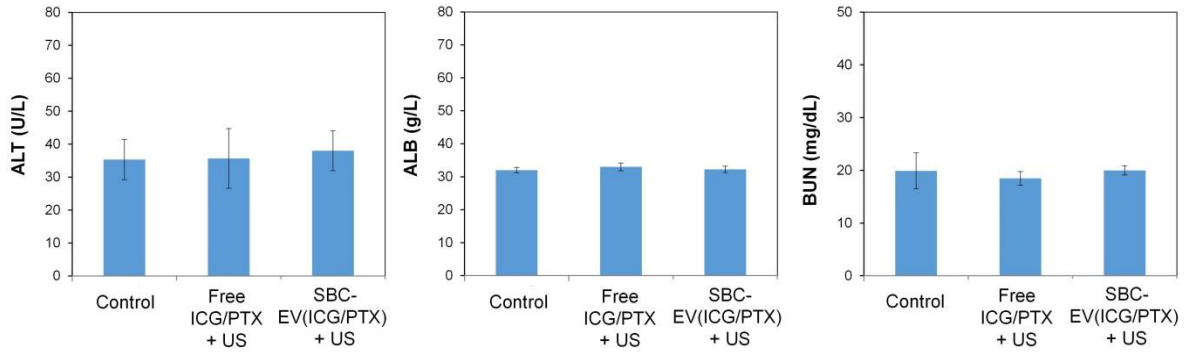

Figure S18. Serum biochemistry results obtained from mice injected with free ICG/PTX and SBC-EV(ICG/PTX) at day 14 after i.v. injection. The tumors of mice were irradiated with 3 min of US (1 MHz, 0.5 W/cm^2) at 4 h post-injection. These results show mean and standard deviations of alanine aminotransferase (ALT), albumin (ALB), and blood urea nitrogen (BUN).