Supplementary Information

Mitochondria-responsive drug release along with heat shock mediated by multifunctional glycolipid micelles for precise cancer chemo-phototherapy

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The photothermal conversion efficiency (η) of IR780-CSOSA was calculated using the following equations (1−4):

$$\eta = \frac{hS(T_{max}-T_{surr})-Q_{dis}}{I(1-10^{-A808})} \quad (1)$$

$$hS = \frac{\sum m_i C_i}{\tau_s} \quad (2)$$

$$\tau_s = -\frac{t}{\ln(\theta)} \quad (3)$$

$$\theta = \frac{T-T_{surr}}{T_{max}-T_{surr}} \quad (4)$$

In equation 1, h and S are the heat transfer coefficient and surface area of the container, respectively. T_{max} and T_{surr} are the final and initial temperature of the solution, respectively. Q_{dis} represents the heat dissipation of solvent; I is the laser power (I = 1.0 W), and A_{808} is the sample absorbance at 808 nm. The A_{808} value for IR780-CSOSA was 2.663. In equation 2, m and C are the mass (0.5 g) and heat capacity (4.2 J g^{-1} $^{o}C^{-1}$) of water, respectively. In equation 3, τ_s is the time constant which was calculated by the linear fitting of t versus $-ln(\theta)$, wherein, t is the time after irradiation in second (s). In equation 4, θ is the dimensionless driving force, and T is the solution temperature. According to the experimental data, the (T_{max} $-T_{surr}$) for IR780-CSOSA was 24.7 °C; $\tau_{IR780\text{-}CSOSA}$ was calculated to be 151 s. (Figure S2) Therefore, the hS of IR780-CSOSA was 13.9 mW/°C. The Q_{dis} of IR780-CSOSA was measured to be 9.5 mW. Substituting these parameters into the above equations, the η value of IR780-CSOSA was calculated to be 33.5%.

Supplementary tables and figures

Table S1. Sizes and zeta potentials of different materials

Material	Size (nm)	PI	Zeta potential (mV)
CSOSA	85.0±3.8	0.40±0.01	37.4±1.5
IR780-CSOSA	149.7±2.5	0.18±0.02	39.4±0.6

Data represent the mean ± standard deviation (n = 3). PI: polydispersity index.

Table S2. Characteristics of DOX-loaded micelles

Material	Size (nm)	PI	Zeta potential (mV)	EE (%)	DL (%)
CSOSA/DOX	48.4±1.1	0.39±0.05	36.1±0.7	87.0%	11.5%
IR780-CSOSA/DOX	119.0±7.6	0.08±0.03	37.8±0.9	81.1%	10.9%

Data represent the mean ± standard deviation (n = 3). PI: polydispersity index.

Table S3. DOX solubility in different temperature at pH 6.8 (n=3)

DOX	37 °C	43 °C	56 °C
Solubility（μg/mL）	7.10±0.18	11.06±0.19	19.50±0.18

Data represent the mean ± standard deviation (n = 3).

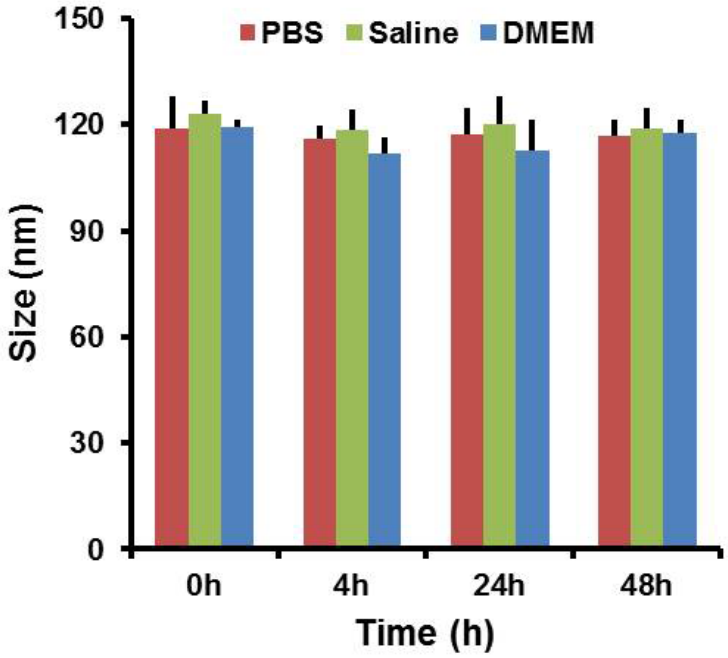

Figure S1. The size changes of IR780-CSOSA/DOX micelles during 48 h incubation within PBS, saline and DMEM.

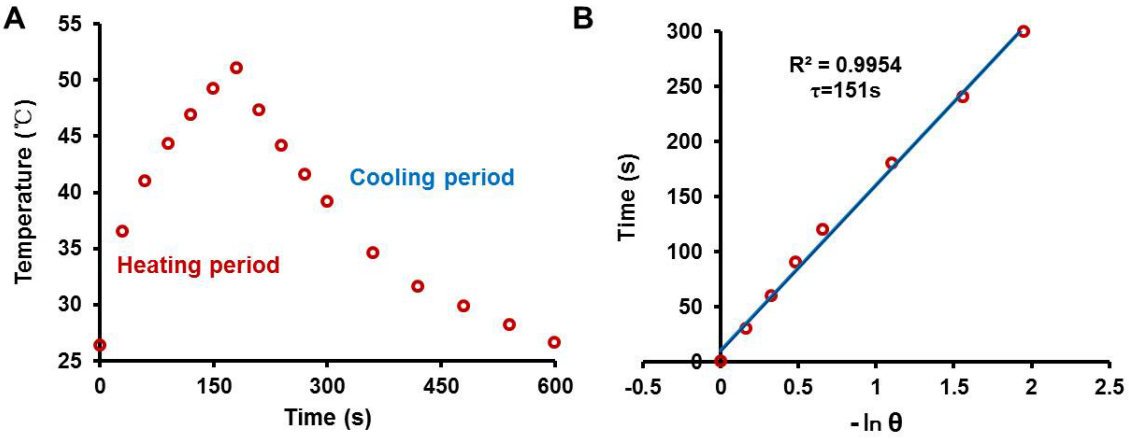

Figure S2. (A) Photothermal curve of IR780-CSOSA when illuminated with an 808 nm laser (1 W/cm^2) for 180s and then cooled naturally. (B) Plot of cooling time vs negative natural logarithm of the temperature driving force obtained from the cooling stage.

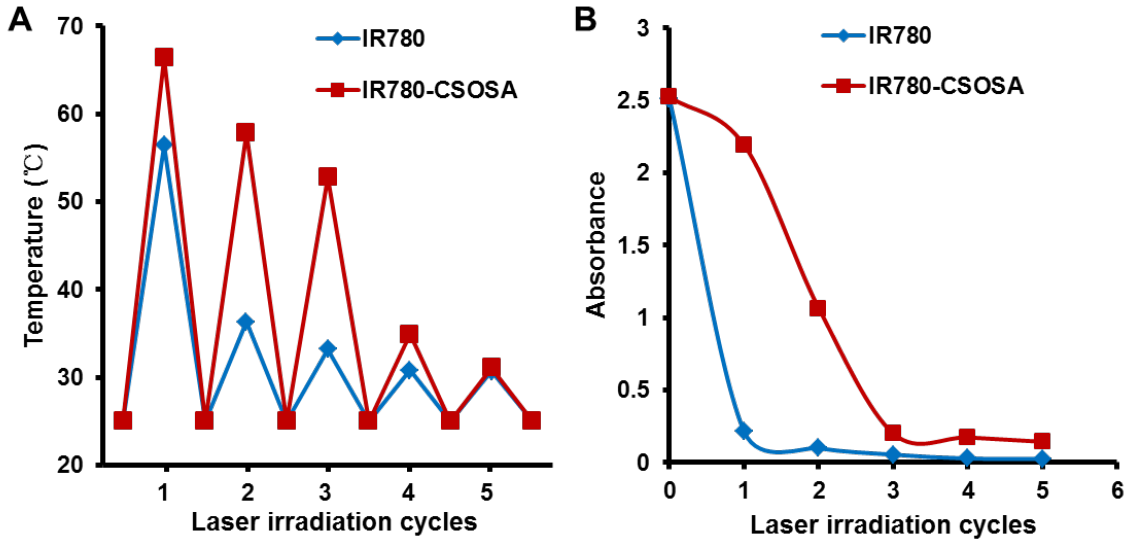

Figure S3. The photothermal curves and UV-vis absorbance curves of free IR780 and IR780-CSOSA micelles during five cycles of irradiations.

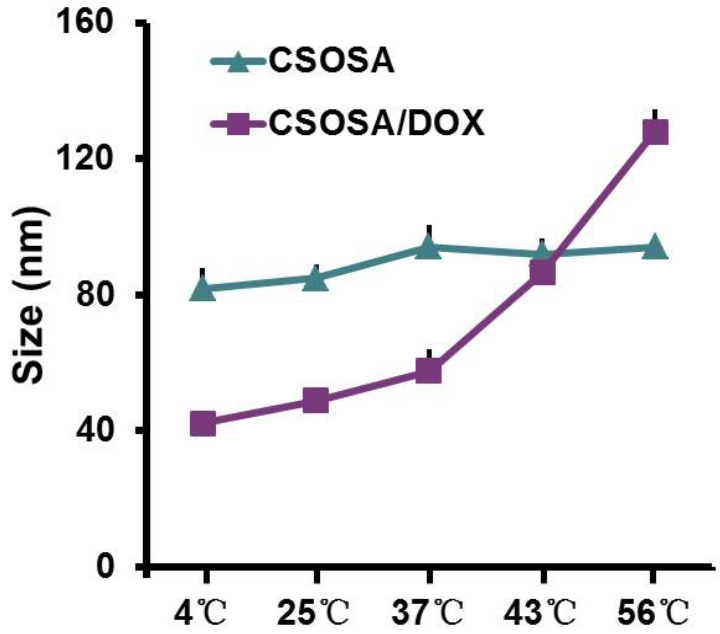

Figure S4. Size changes of CSOSA and CSOSA/DOX micelles at different temperatures (4 °C, 25 °C,37 °C,43 °C,56 °C) by Zetasizer.

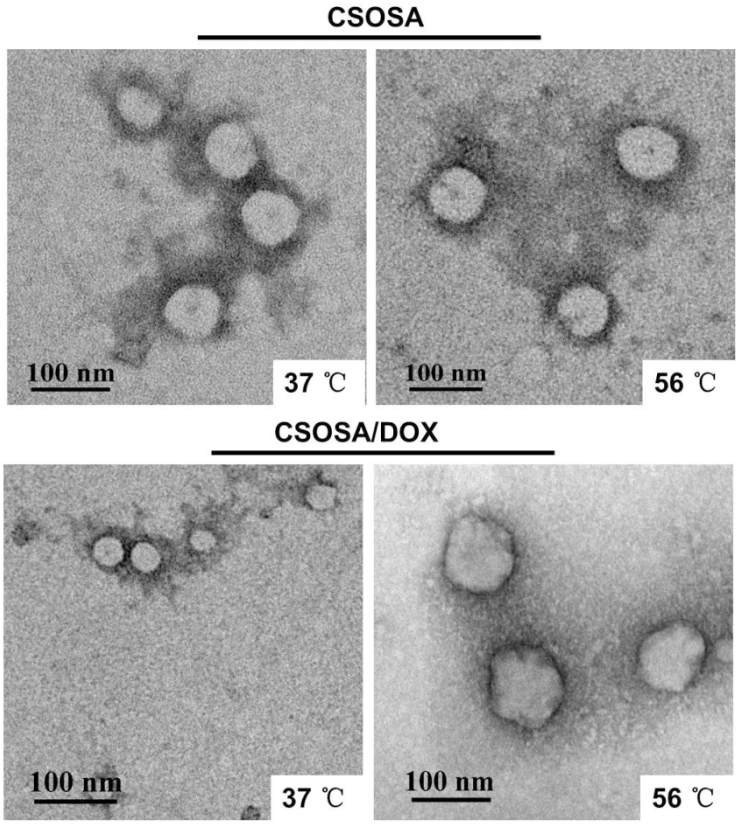

Figure S5. TEM images of CSOSA and CSOSA/DOX micelles at 37 °C and 56 °C.

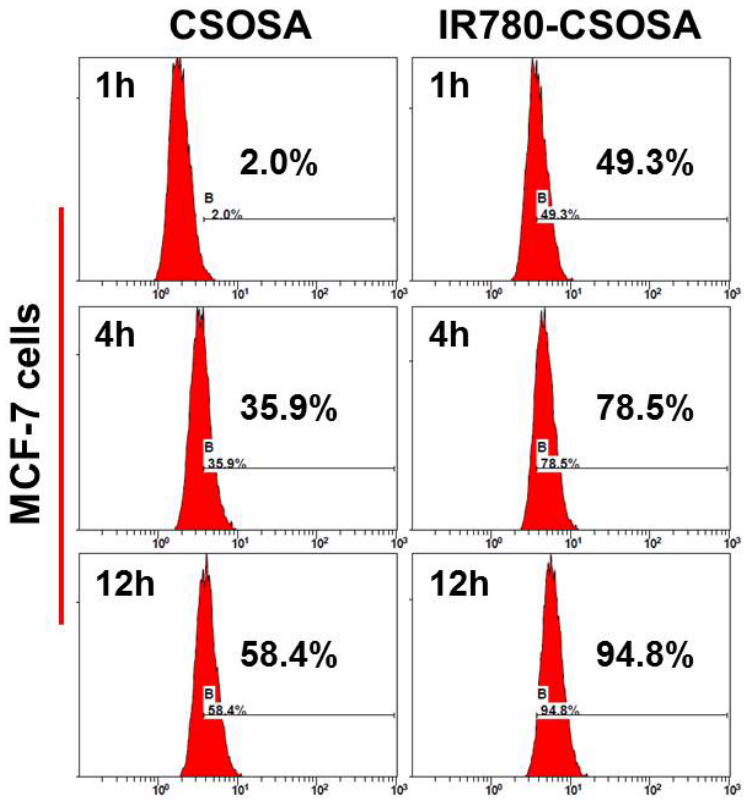

Figure S6. MCF-7 cells were incubated with FITC-labeled CSOSA or IR780-CSOSA micelles for 1, 4, and 12 h, respectively. Quantitative uptake assays were determined by flow cytometry.

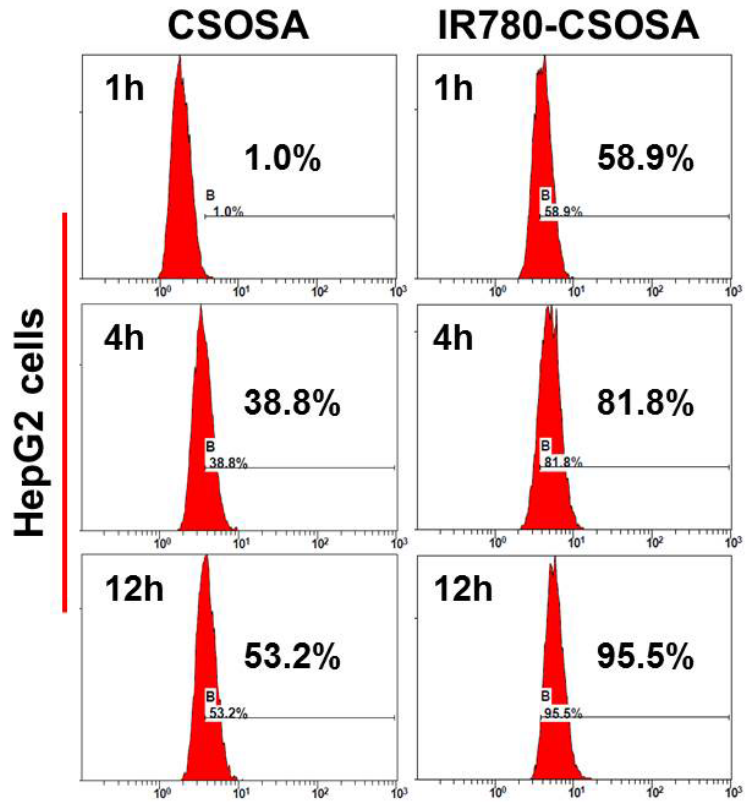

Figure S7. HepG2 cells were incubated with FITC-labeled CSOSA or IR780-CSOSA micelles for 1, 4, and 12 h, respectively. Quantitative uptake assays were determined by flow cytometry.

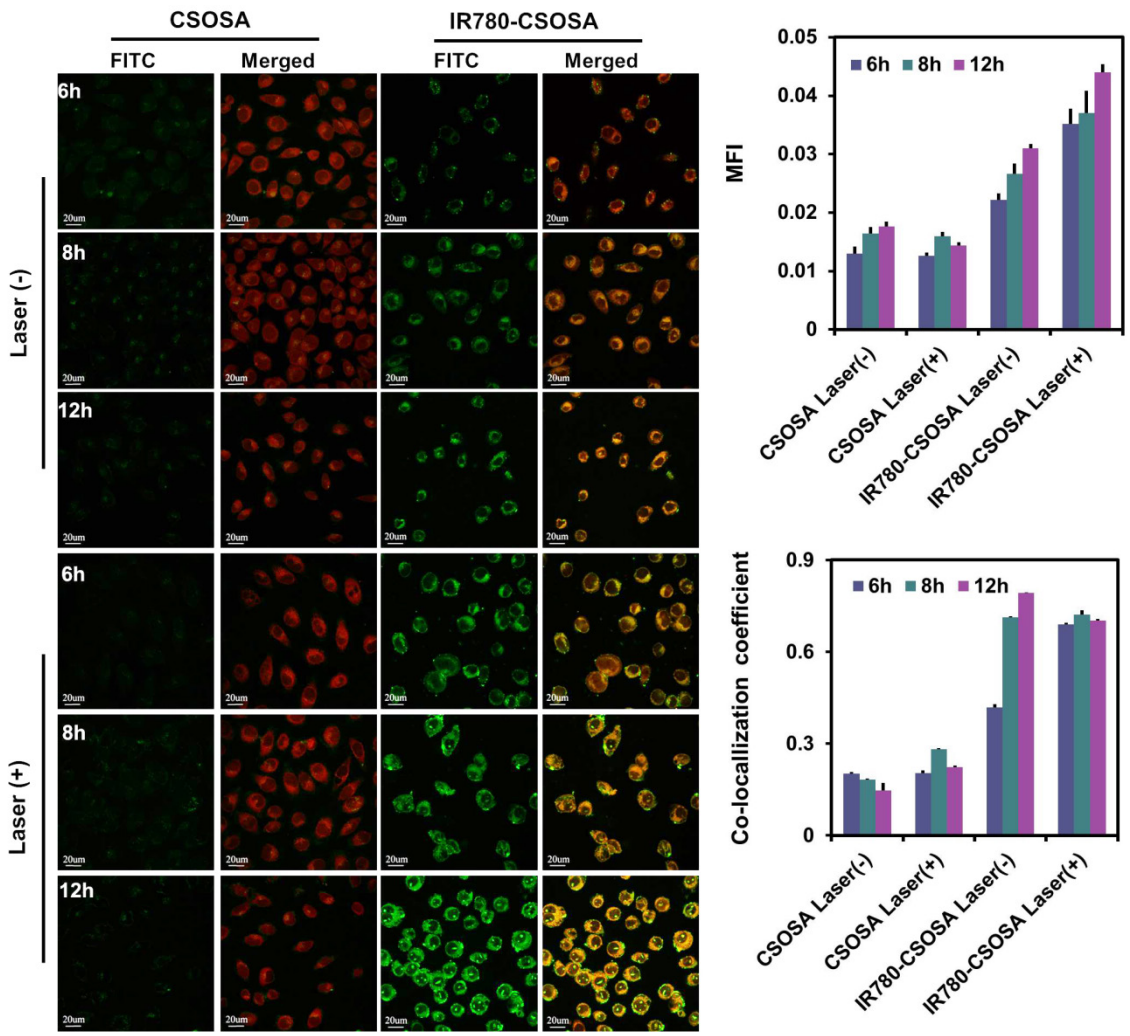

Figure S8. NIR-triggered internalization of micelles inside mitochondria *in vitro*. MCF-7 cells were treated with the FITC-CSOSA or FITC-IR780-CSOSA for 4 h. Then, the cells were irradiated with or without laser (808 nm, 1 W/cm^2) for 3 min and continuously incubated for 2, 4 h and 8 h to observe the cellular uptake and mitochondrial co-localization of micelles by CLSM. Green: FITC-labeled micelles. Red: Mitotracker red. Yellow spots in the merged pictures denoted the co-localization of the micelles within mitochondrial compartments. The mean fluorensence intensity (MFI) analysis of micelles. The co-localization coefficient of micelles and mitochondria.

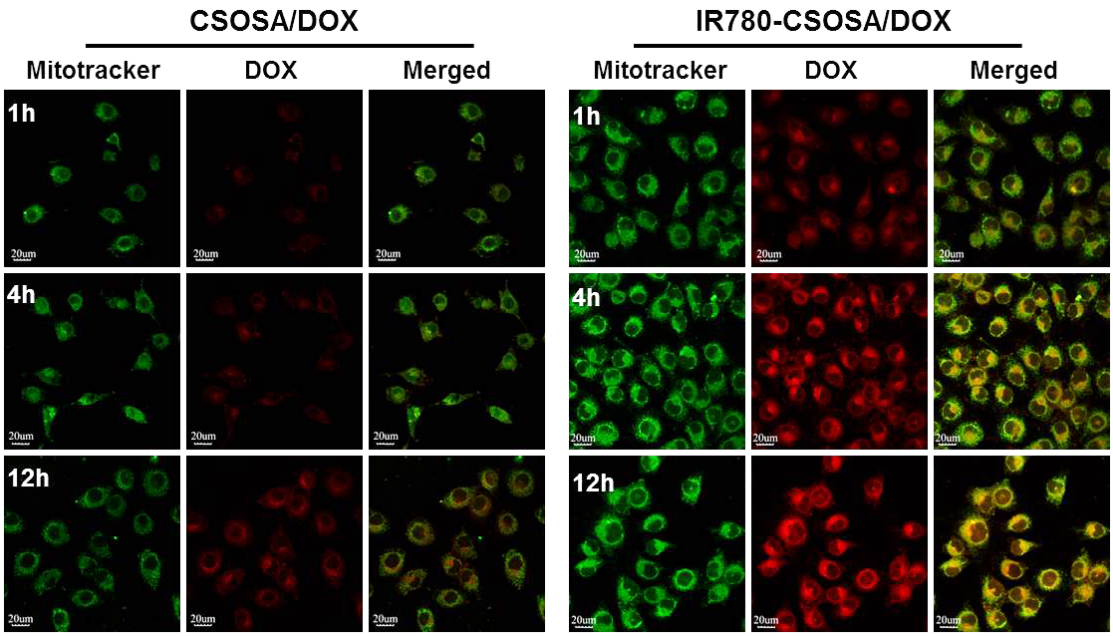

Figure S9. *In vitro* cellular uptake and mitochondrial co-localization of CSOSA/DOX or IR780-CSOSA/DOX micelles on MCF-7 cells for 1, 4, and 12 h, respectively. Green: Mitotracker green. Red: DOX. Yellow spots in the merged pictures denoted the co-localization of DOX-loaded micelles within mitochondrial compartments.

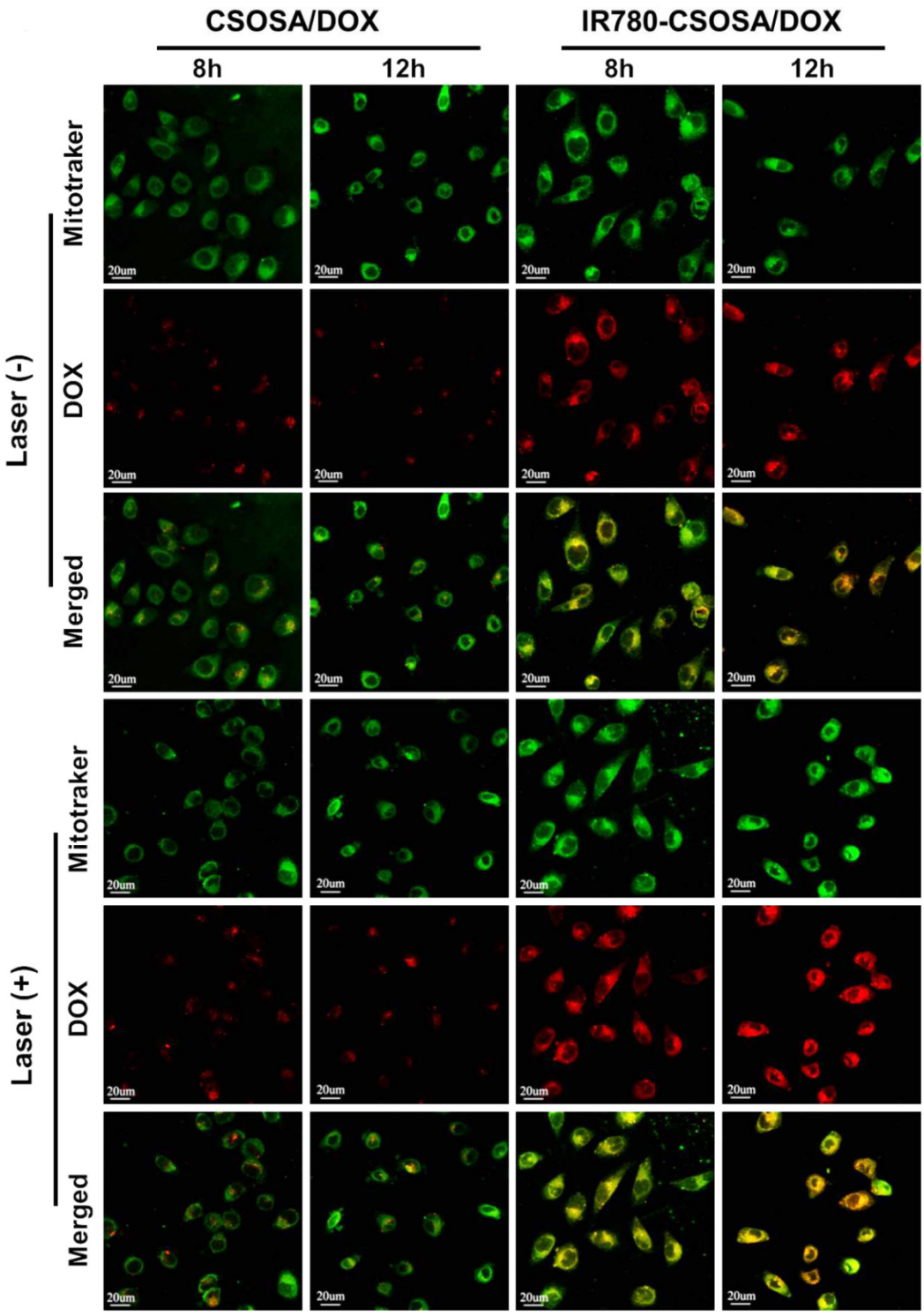

Figure S10. NIR-triggered drug release inside mitochondria in MCF-7 cells. MCF-7 cells were treated with CSOSA/DOX or IR780-CSOSA/DOX (equivalent DOX: 1.5 μg/mL) for 4 h. Next, the culture medium were replaced with fresh medium to remove the uninternalized nanoparticles and irradiated with or without laser (808 nm, 1 W/cm2) for 3 min, then continuously incubated for 4 h or 8 h to observe the NIR-triggered DOX release in mitochondria by CLSM. Green: Mitotracker green. Red: DOX. Yellow spots in the merged pictures denoted the co-localization of released DOX within mitochondrial compartments.

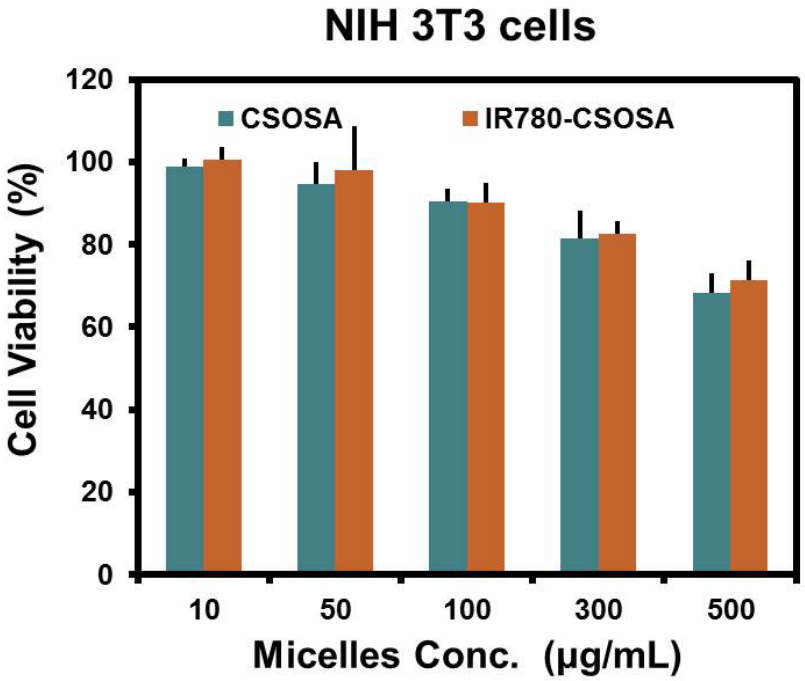

Figure S11. In vitro cytotoxicity of CSOSA and IR780-CSOSA against NIH 3T3 cells with different micelles concentrations for 48 h. (n = 3).

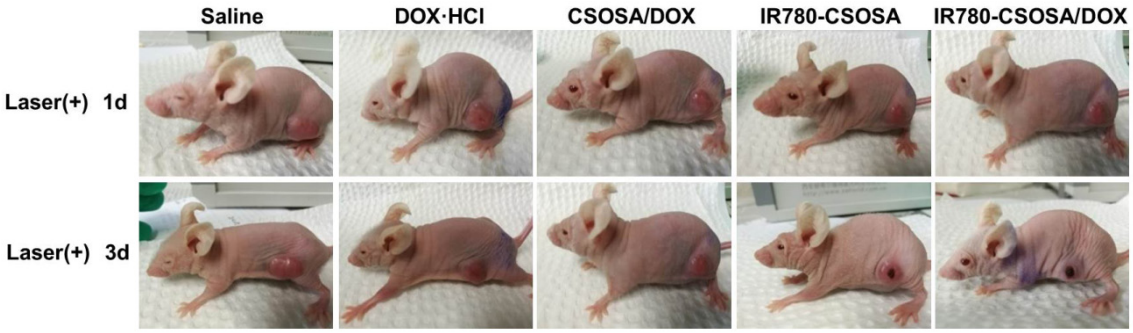

Figure S12. Representative images of MCF-7 tumor xenografted mice treated with different formulations (equivalent DOX: 2 mg/kg). After the day of injection, the tumors were exposed to the laser (808 nm, 0.5 W/cm^2) for 3 min.

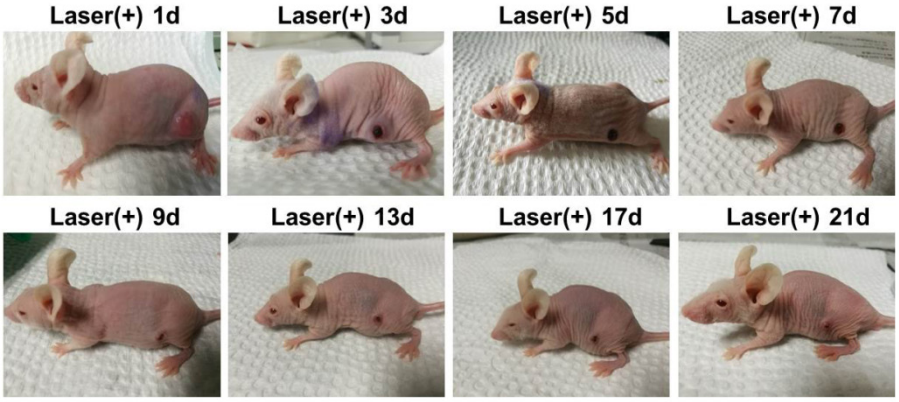

Figure S13. Representative images of MCF-7 tumor-xenografted mice treated with combined chemo-photothermal therapy IR780–CSOSA/DOX (equivalent DOX: 2mg/kg) under laser irradiation (808 nm, 0.5 W/cm^2, 3 min) in 21 days.

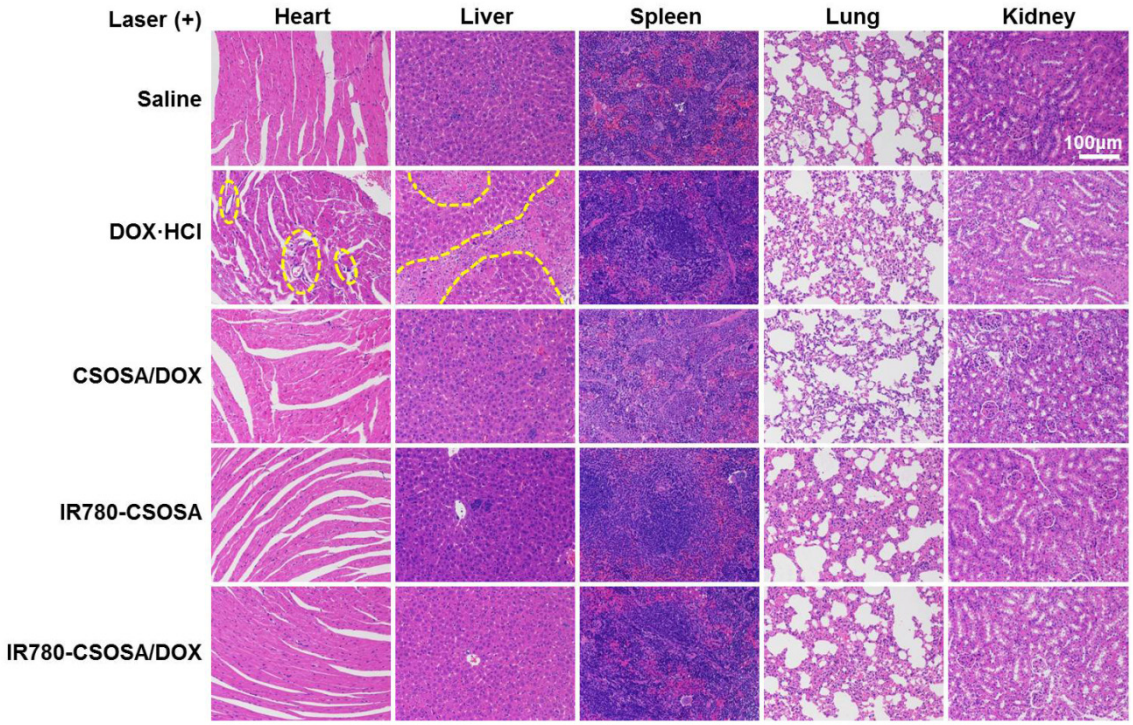

Figure S14. Representative HE staining of various organ tissues of MCF-7 tumor-xenografted mice in five treatment groups with laser irradiation (808 nm, 0.5 W/cm^2, 3 min). Yellow lines denoted pathological changes area. Scale bar: 100 μm.

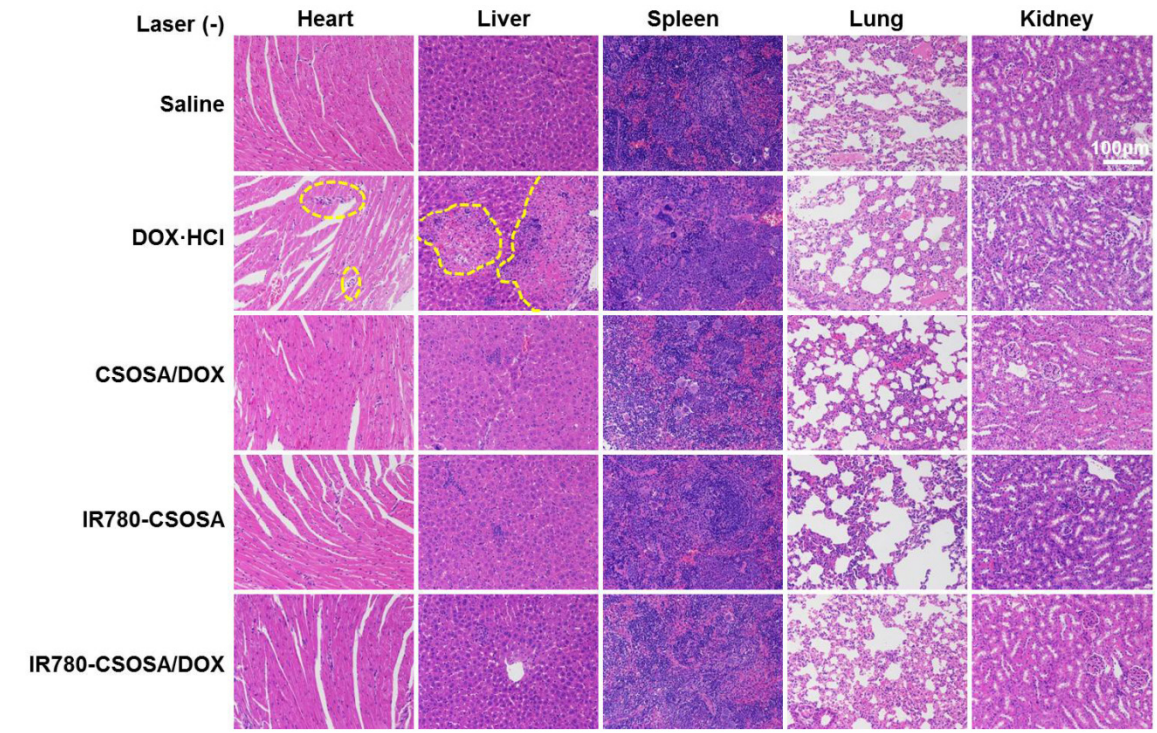

Figure S15. Representative HE staining of various organ tissues of MCF-7 tumor-xenografted mice in five treatment groups without laser irradiation. Yellow lines denoted pathological changes area. Scale bar: 100 μm.

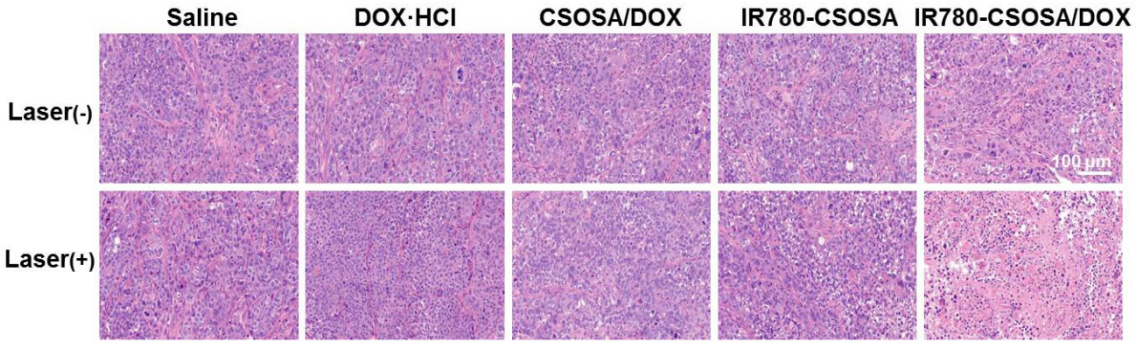

Figure S16. Representative HE images of tumor tissues in MCF-7 tumor-xenografted mice treated with different formulations with or without laser irradiation (808 nm, 0.5 W/cm^2, 3 min). Scale bar: 100 μm.

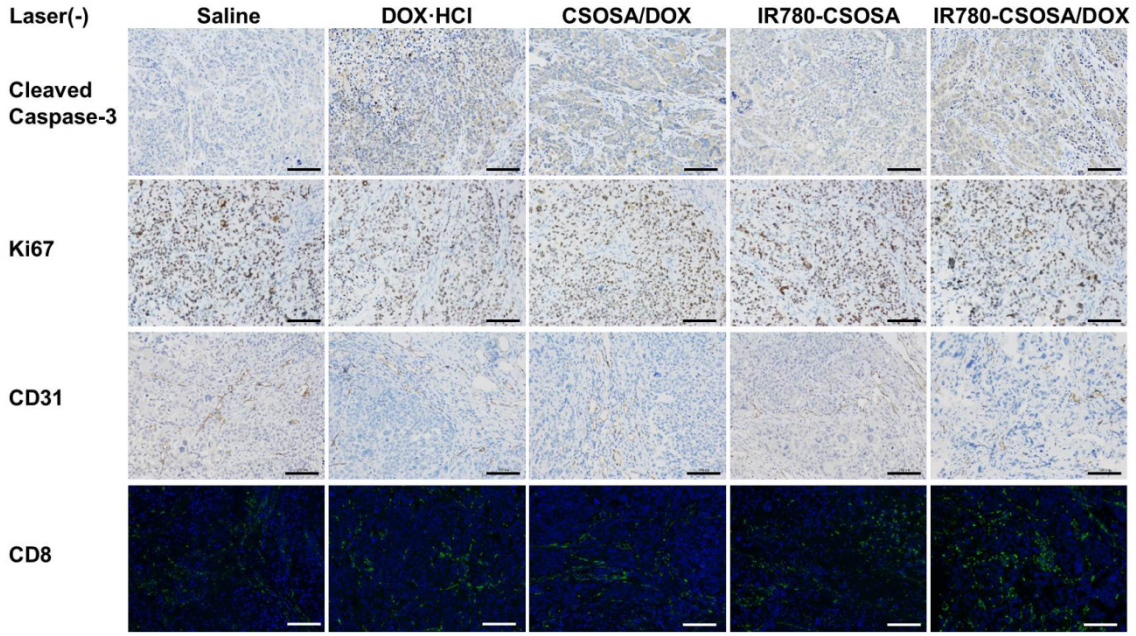

Figure S17. Under no laser irradiation, the induction of apoptosis on MCF-7 tumor tissues stained with cleaved caspase-3 antibody (brown). Cell proliferation evaluation by Ki67 staining (brown). Tumor blood vessel staining with CD 31 antibody (brown). CD 8$^+$ T cells in MCF-7 tumor tissues were detected by immunofluorescent staining (green). Scale bar: 100 μm.

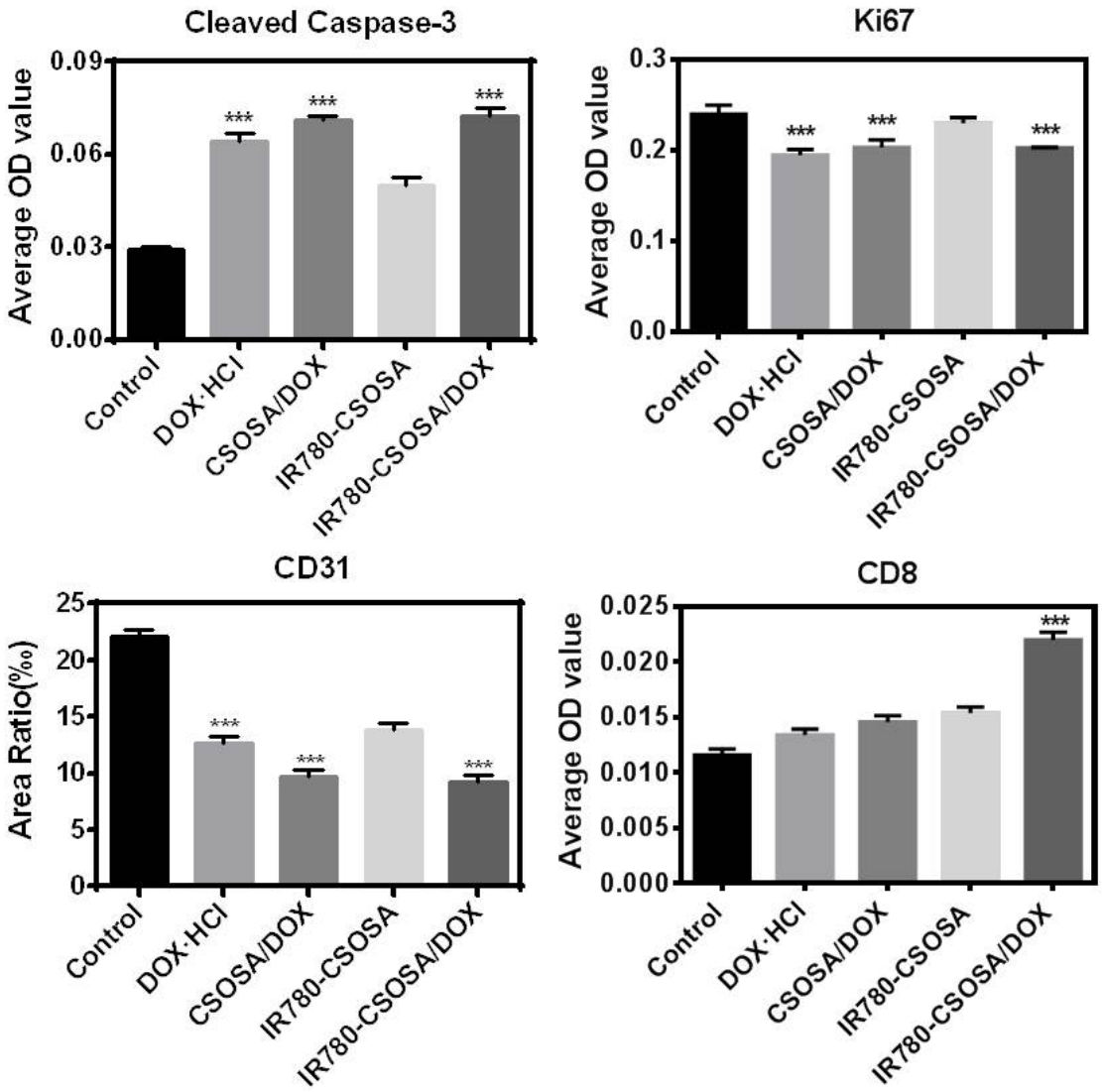

Figure S18. Under no laser irradiation, the quantitative analysis of cleaved caspase-3, Ki67, CD31 and CD8 levels in MCF-7 tumor-xenografted mice treated with different formulations. (n = 3), ***P < 0.001.