Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/10/eaaz0575/DC1

Supplementary Materials for

MTH1 inhibitor amplifies the lethality of reactive oxygen species to tumor in photodynamic therapy

Lingzhi Zhao*, Junyao Li, Yaoquan Su, Liqiang Yang, Liu Chen, Lei Qiang, Yajing Wang, Huijing Xiang, Huijun Phoebe Tham, Juanjuan Peng*, Yanli Zhao*

*Corresponding author. Email: zlz@cpu.edu.cn (L.Z.); pjj@cpu.edu.cn (J.P.); zhaoyanli@ntu.edu.sg (Y.Z.)

Published 4 March 2020, *Sci. Adv.* **6**, eaaz0575 (2020)
DOI: 10.1126/sciadv.aaz0575

This PDF file includes:

Supplementary Materials and Methods
Fig. S1. Characterizations for the silica shell of calcined SCLMs.
Fig. S2. Determination of the $^1O_2$ production by the bleaching of DPBF.
Fig. S3. Cytotoxicity assessment of C@SCLMs in the dark.
Fig. S4. Cellular apoptosis of A431 cells treated by different approaches.
Fig. S5. In vitro cell viability and the calculated therapeutic effect for different cancerous cell lines.
Fig. S6. Cell viability assay for human dermal fibroblasts.
Fig. S7. Effect of the mitochondrial protector to the combined treatment.
Fig. S8. Results of flow cytometric apoptosis analysis for A431 cells subjected to the treatment after the protection of MitoQ.
Fig. S9. Cell cycle analysis of A431 cells treated by TH588.
Fig. S10. Supplementary data for the in vivo animal experiments.

Supplementary Materials

Supplementary Materials and Methods

Materials

All chemicals were used as received without further purifications. Aminopropyl triethoxysilane (APES), dimethyldimethoxysilane (DEDMS), pluronic surfactant F108 ($PEO_{132}PPO_{50}PEO_{132}$), and tetraethoxysilane (TEOS) were purchased from Sigma-Aldrich. MTH1 inhibitor was purchased from Selleck Corporation. 1,3-Diphenylisobenzofuran (DPBF) and tween 80 were purchased from Aladdin Chemical Company. Ce6 was purchased from J&K Chemical Company. Annexin V-FITC/PI apoptosis detection kit, ApopTag in situ apoptosis Detection Kit, Cell Cycle and Analysis Kit, and Mito Tracker Red were purchased from Beyotime Biotechnology. The rabbit antibodies against p53, p21 and Cleaved Caspase-3 were purchased from Cell Signaling Biotechnology, antibodies against BAX and Bcl-2 were purchased from Santa Cruz Biotechnology, antibody against Caspase-3 was purchased from Proteintech, antibody against α-tubulin was purchased from Bioworld Technology, peroxidase-conjugated affinipure goat anti-rabbit immunoglobulin G was purchased from Zsbio, and Alexa488-conjugated avidin was purchased from Invitrogen. Other chemicals were purchased from Sigma-Aldrich. The cell lines (A431, HeLa, MCF-7, HCT116, B16F10 and HDF) were provided by the Institute of Biochemistry and Cell Biology, SIBS, CAS (China).

Characterizations and Measurements

Transmission electron microscopy (TEM) images were captured on a JEOL JEM-1400 transmission electron microscope with operation voltage of 100 kV. Dynamic light scattering (DLS) and zeta potential experiments were performed at 25 °C using a Malvern Zetasizer NanoZS. Fluorescence spectra were recorded in an Edinburgh FLS-980 spectrometer. UV-vis absorption spectra were obtained by a Philes G9 ultraviolet and visible spectrophotometer. Confocal images of cells and tissues were recorded on a LSM700 Confocal Laser Scanning Microscope. For the nitrogen adsorption/desorption isotherms, the calcined SCLMs were degassed at 80 °C under vacuum, and analyzed by a Quantachrome Autosorb-iQ instrument under -196 °C in liquid nitrogen.

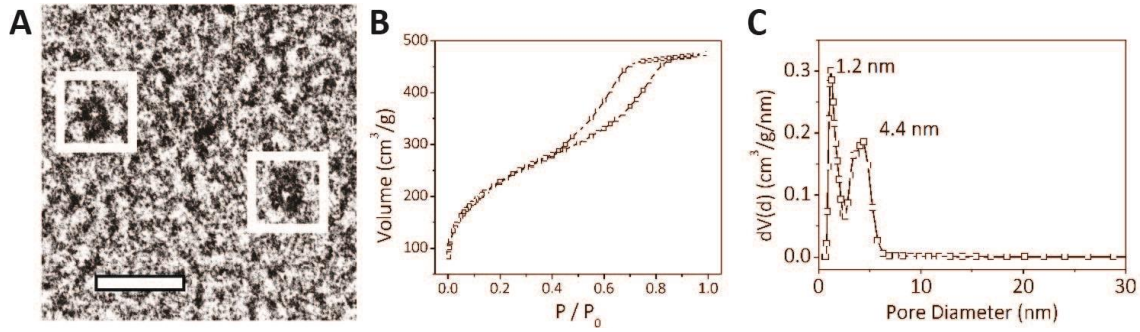

Fig. S1. Characterizations for the silica shell of calcined SCLMs. (**A**) High resolution TEM image of the calcined SCLMs. Scale bar = 10 nm. (**B**) Nitrogen adsorption-desorption isotherm of calcined SCLMs under -196 °C in liquid nitrogen. (**C**) Pore size distribution curve of calcined SCLMs calculated by non-localized density functional theory method. Data were derived from the adsorption branch of the isotherm.

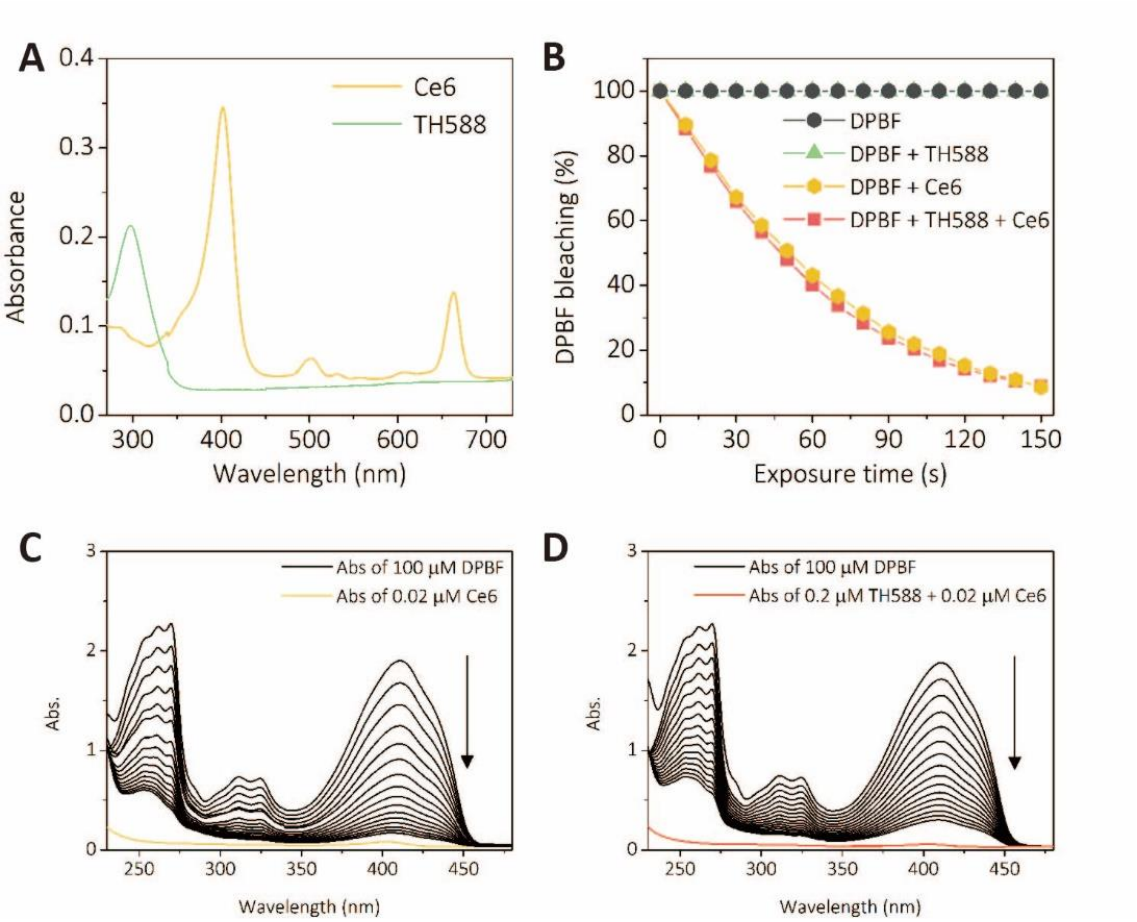

Fig. S2. Determination of the $^1O_2$ production by the bleaching of DPBF. (**A**) UV-vis absorption spectra of TH588 and Ce6. (**B**) Production of $^1O_2$ by TH588 and Ce6 under 660 nm irradiation. Absorption spectral changes of 100 μM DPBF with the addition of (**C**) 0.02 μM Ce6 and (**D**) 0.2 μM TH588 together with 0.02 μM Ce6.

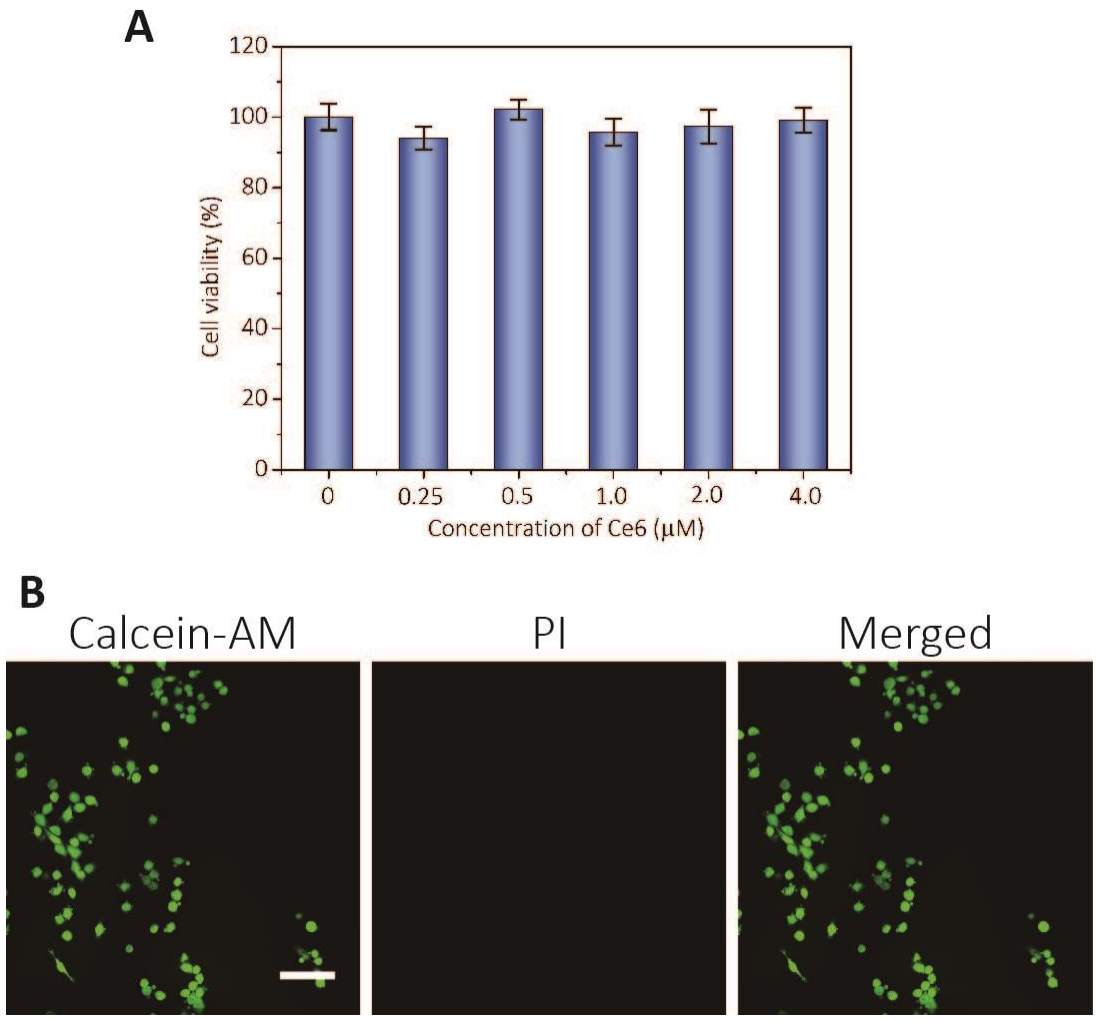

Fig. S3. Cytotoxicity assessment of C@SCLMs in the dark. (**A**) Cell viability of A431 cells incubated with C@SCLMs containing different concentrations of Ce6 in the dark conditions for 24 h. (**B**) Live/dead cell imaging of A431 cells incubated with C@SCLMs and co-stained by calcein-AM/PI. Scale bar: 100 μm.

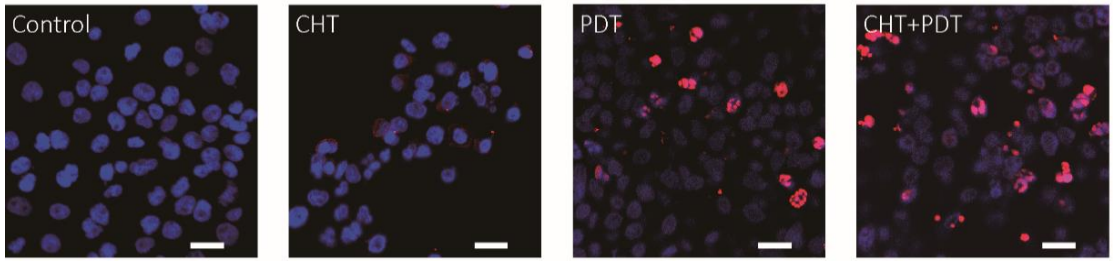

Fig. S4. Cellular apoptosis of A431 cells treated by different approaches. Confocal microscopy of TUNEL staining assay for A431 cells. Scale bar: 30 μm.

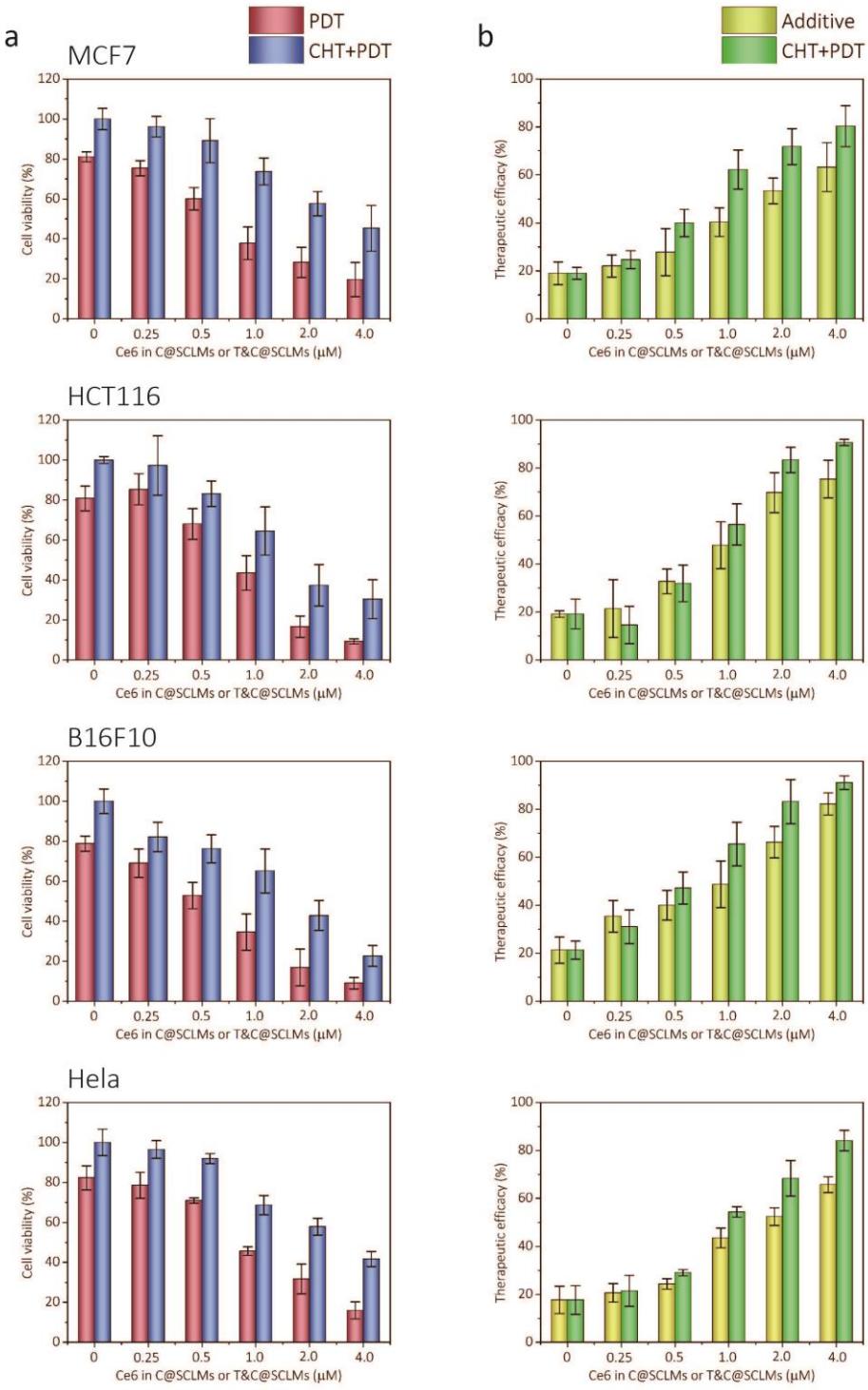

Fig. S5. In vitro cell viability and the calculated therapeutic effect for different cancerous cell lines. (**A**) Hela cells, (**B**) MCF-7 cells, (**C**) HCT116 cells and (**D**) B16F10 cells. The cells were incubated with C@SCLMs containing 0.25-4.0 μM Ce6 (PDT group) or C&T@SCLMs with 0.25-4.0 μM of Ce6 and 10 μM TH588 (combined therapy group) for 24 h, and irradiated using a 660 nm LED light with a power density of 20 mW cm^{-2} for 30 s. The viability was tested by MTT assay.

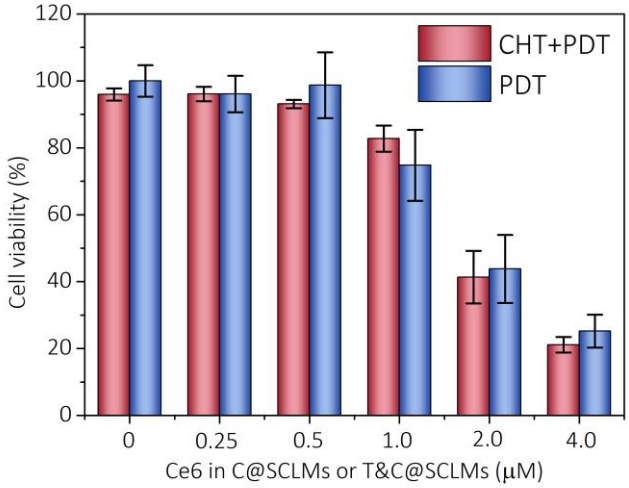

Fig. S6. Cell viability assay for human dermal fibroblasts. The cells were incubated with C@SCLMs containing 0.25-4.0 μM Ce6 (PDT group) or C&T@SCLMs with 0.25-4.0 μM of Ce6 and 10 μM TH588 (combined therapy group) for 24 h, and irradiated using a 660 nm LED light with a power density of 20 mW cm^{-2} for 30 s.

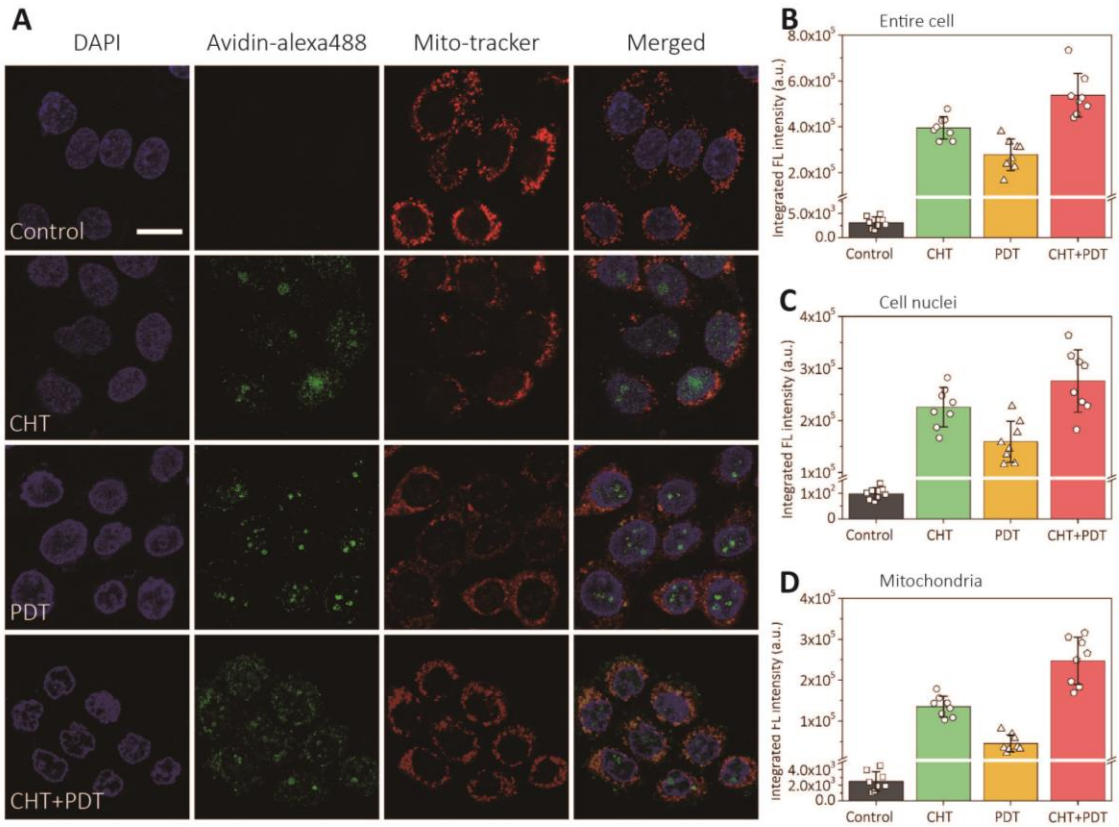

Fig. S7. Effect of the mitochondrial protector to the combined treatment. A431 cells were pretreated by a mitochondria-targeted antioxidant MitoQ, subjected to different treatments, and co-stained by DAPI, avidin-alexa488 and Mito Tracker Red. Scale bar: 20 μm. (**A**) Co-localization of 8-oxo-dG and mitochondria. Integrated fluorescence intensity from (**B**) entire cell, (**C**) nuclei region, and (**D**) mitochondria region. Data show the mean value and standard deviation of 8 individual cells.

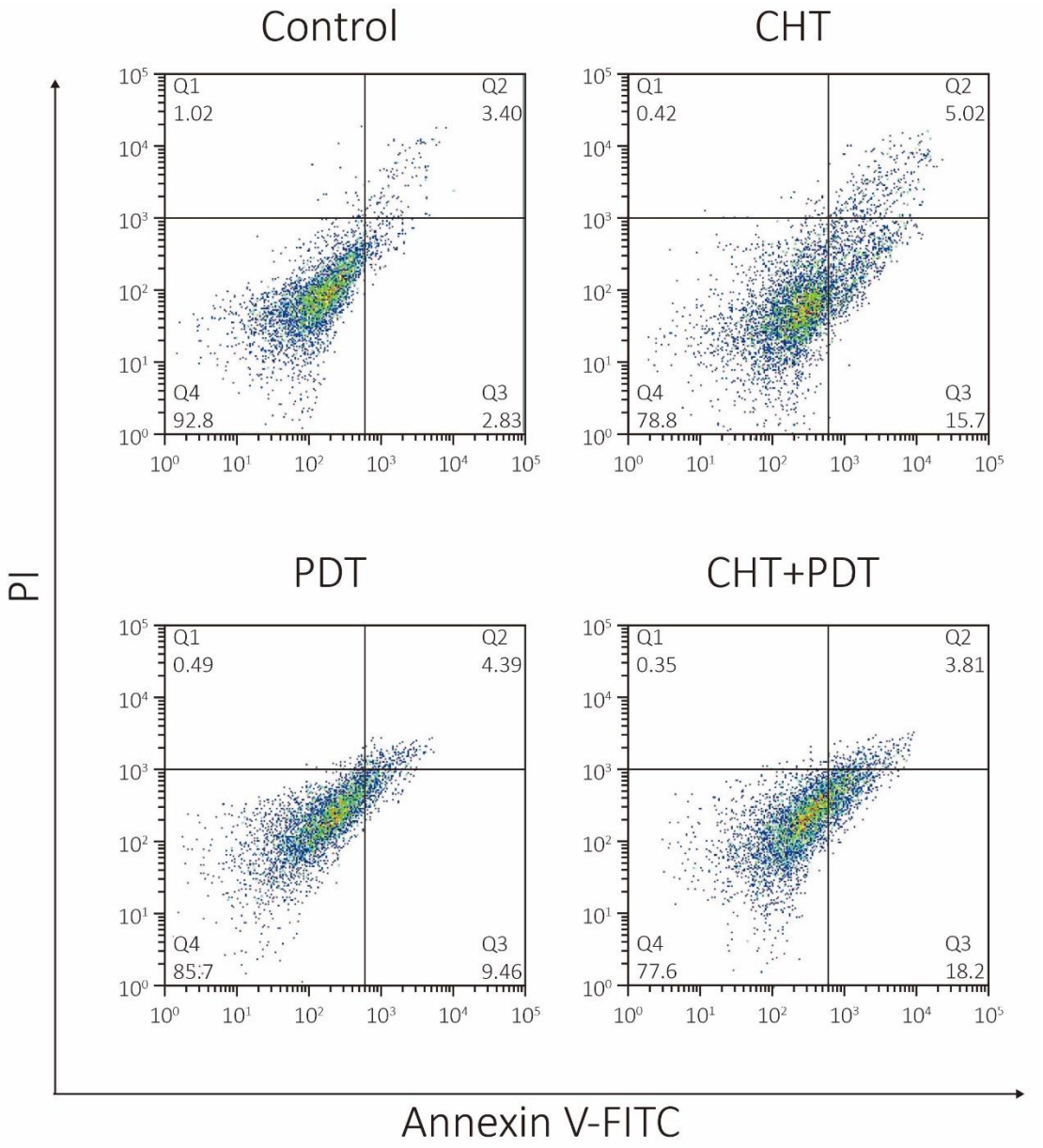

Fig. S8. Results of flow cytometric apoptosis analysis for A431 cells subjected to the treatment after the protection of MitoQ. A431 cells were incubated with 0.2 μM MitoQ for 24 h, respectively treated by CHT, PDT and combined therapy, and co-stained by Annexin V-FITC/PI.

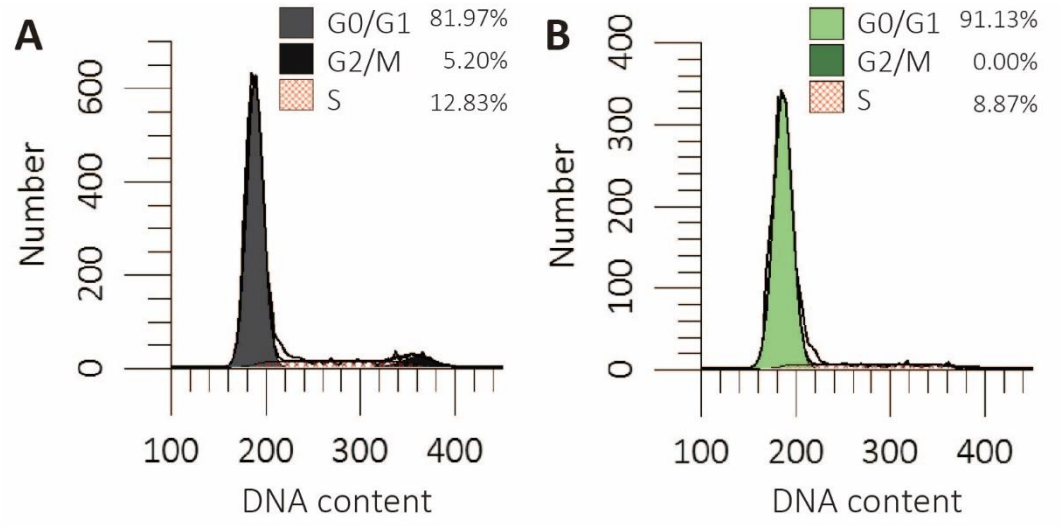

Fig. S9. Cell cycle analysis of A431 cells treated by TH588. The cell cycle of (**A**) A431 cells and (**B**) A431 cells treated by 20 μM TH588 after 24 h. The data were analyzed by flow cytometry.

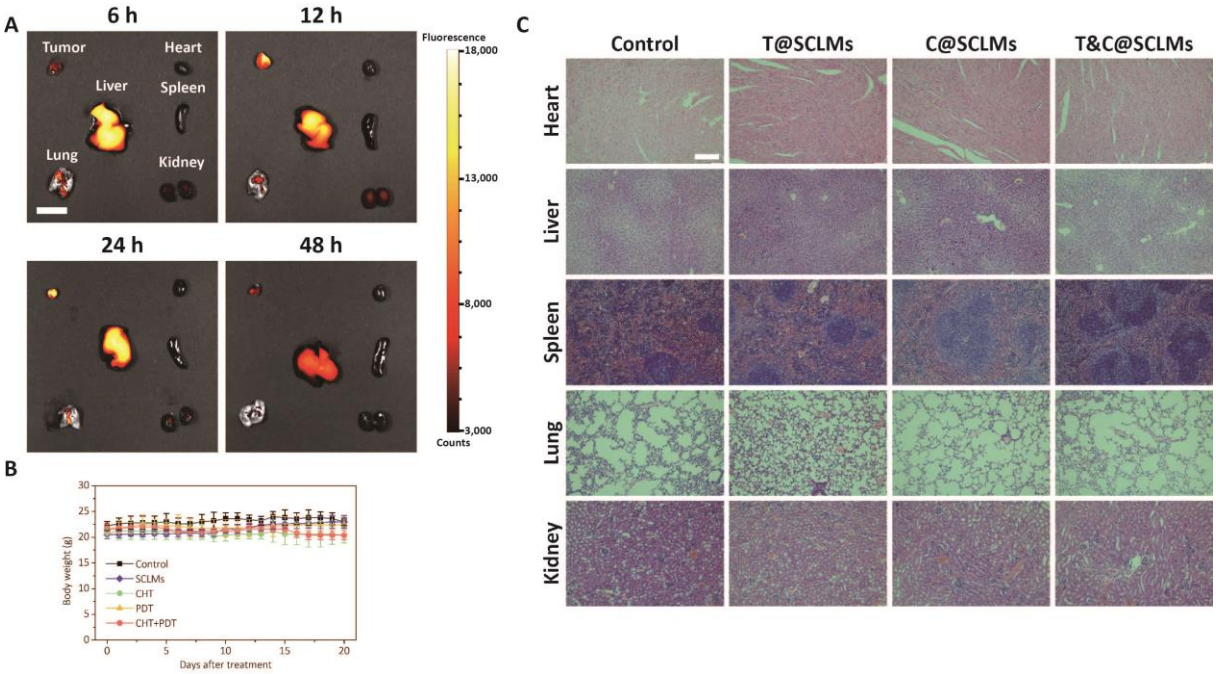

Fig. S10. Supplementary data for the in vivo animal experiments. (**A**) Ex vivo imaging of the main organs of mice injected with SCLMs labelled with Cy5.5. Scale bar = 1 cm. (**B**) Variation of body weights for mice subjected to different treatment approaches. (**C**) H&E staining of the main organs (heart, liver, spleen, lung and kidney) of nude mice injected with PBS, T@SCLMs, C@SCLMs, and T&C@SCLMs, respectively. Scale bar = 200 μM.