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Supporting Information

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Light-Triggered Theranostics Based on Photosensitizer-Conjugated Carbon Dots for Simultaneous Enhanced-Fluorescence Imaging and Photodynamic Therapy

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Experimental Section

Synthesis of carbon dots

Carbon dots (C-dots) were prepared by the improved nitric acid oxidation method. In a typical experiment, 0.5 g of the raw soot (purchased from Jixi Kaiwen Hu Limited Co.) was washed with actone under ultrasonication. Then it was centrifugated and dried under vaccum at 80 ºC. Afterwards, the cleaned soot was refluxed in 25 mL 5 M HNO_3 at 120 ºC for 12~18 h. The obtained homogeneous black solution was cooled and centrifugated at 3000 rpm for 10 min to remove the unreacted precipitation. The light brown solution was collected, neutralized and dialyzed against water, followed by a centrifugation step to yield purified C-dots in the supernatant. Then the supernatant was precipitated by adding acetone and centrifugated at 14000 rpm for 10 min. Size separation was performed by high-speed stepwise centrifugation (4000~10000 rpm) in the water/ethanol/chloroform

solvent. The supernatant was collected at 10000 rpm and the precipitation was discarded. Finally, the yellow solution (including C-dots sized in the range of 1~2 nm) was obtained. C-dots were passivated with PEG 2000N at 120 ºC with the presention of nitrogen gas. The excess amount of PEG 2000N was removed by membrane dialysis (MWCO 3000).

Synthesis of chlorin e6-conjugated carbon dots (C-dots-Ce6)

PEG-coated C-dots (C-dots-NH_2) were terminated with amine groups. Covalent binding of Ce6 to the C-dots-NH_2 was performed using a modification of the standard EDC–NHS reaction. Typically, 1 mg of Ce6 was dissolved in 1 mL of DMSO, followed by the addition of 1 molar equivalent of NHS and EDC for activation for 30 min. Following activation of carboxyl groups, 2 mg of C-dots-NH_2 in phosphate buffered saline (PBS) (pH=7.4, 2 mL) were added and the mixed solution was allowed to react at room temperature for 12 h. The resultants were washed five times by distilled water and ethanol alternatively to remove unreacted chemicals by centrifugation at 14000 rpm for 10 min. The products were dispersed in PBS buffer (pH= 7.4) for further characterization and application.

Characterization of C-dots-Ce6

The morphology and size of the aqueous dispersion of the samples were taken on a MultiMode Nanoscope III A scanning probe microscopy (SPM) system (Veeco, USA).

The commercially available AFM cantilever tips with a force constant of ~ 48 N/m and resonance vibration frequency of ~ 330 kHz were used. The scanning rate was set at 1.5 Hz. The samples for AFM were prepared by dropping aqueous suspension (～0.01 mg/mL) of the material on freshly cleaved mica surface and dried under vacuum at 80 ºC. UV-vis spectra were measured at 20 ºC with a Shimadzu UV-2450 UV-visible spectrophotometer equipped with a 10-mm quartz cell, where the light path length was 1 cm. Fluorescence spectra were recorded on a Hitachi FL-4600 spectrofluorimeter. The Fourier transform infrared (FTIR) spectra were recorded on a Perkin-Elmer Paragon-1000 FTIR Spectrometer. The surface charge of the samples was measured by Zeta potential measurements in water (NICOMP 380ZLS Zeta potential/Particle sizer).

Conjugation Efficiency Measurements

The standard curve was established. UV-vis measurements of C-dots-Ce6 were carried out. The absorbance at 663 nm was used for validating the presence of Ce6 and estimating the Ce6 conjugation efficiency. Every experiment was repeated three times.

Single Oxygen Detection

Singlet oxygen sensor green (SOSG), which is highly selective for singlet oxygen, was applied to a range of biological systems that are known to generate singlet oxygen. To evaluate the SOG of C-dots-Ce6 (1 μM), the SOSG was introduced at the

concentration of 2.5 μM. The control groups include (1) SOSG alone irradiated with light; (2) free Ce6 in SOSG irradiated with light; (3) pure C-dots with light. The SOG was induced by irradiation at 671 nm. The SOSG fluorescence was read out with the excitation at 494 nm with maximum at 534 nm after the irradiation to determine the samples' SOG. The sample's SOG was evaluated by the SOSG fluorescence enhancement compared with the background or control sample.

Subcellular localization

MGC803 cells were obtained from the Chinese Academy of Science and were cultured in RPMI 1640 medium containing 10% fetal bovine serum and incubated at 37 °C in a humidified 5% CO_2 atmosphere. MGC803 cells (3×10^3 cells per well) were seeded in 96-well plates and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. After being rinsed with PBS (pH 7.4), the cells were incubated with C-dots-Ce6 at a concentration of 10 μM for 2 h at 37 ºC in the dark under the same conditions. The cells were rinsed with PBS again, and stained by DAPI (4',6-diamidino-2-phenylindole) and then detected by fluorescence imaging using an upright Olympus IX71 optical microscope integrated with a CRi Nuance multispectral imaging system.

Laser-induced *in vitro* PDT effect

Three plates were set up as one dark control and two experimental groups for the CCK-8 assay and these plates were seeded. The cells of experimental groups were then rinsed again with PBS and incubated with different concentrations of Ce6 or C-dots-Ce6 for 24 h at 37 ºC in the dark before being illuminated using a 671 nm laser with energy density of 30 mW/cm^2 for 3 min. After illumination, cells were incubated for 48 h in a 5% CO_2, 95% air humidified incubator at 37 °C. Dark control group is kept identical to that experimental group except for illumination. Trypan blue staining was carried out on MGC803 cells incubated with C-dots-Ce6 at a concentration of 10 μM for 24 h at 37 ºC prior to irradiation for 3 min with a 671 nm laser (30 mW/cm^2)

Gastric Cancer Model

All animal operations were in accordance with institutional animal use and care regulations, Shanghai Jiaotong University. Female nude mice weighing 20~22 g were purchased from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China). Animals were maintained in our animal care facility and housed eight per cage at room temperature (22 ± 2 ºC) with food and water ad libidum. Animals were i.p. injected with a 3.5 mg/10 g weight chloral hydrate, equivalent to 0.07 mL/10 g wt 10% chloral hydrate. Four-week-old female nude mice were injected s.c. on the right rear flank area with 5×10^6 MGC803 cancer cells in 100 μL of serum free RPMI-1640 medium, as described previously. Animals were maintained under pathogen-free conditions.

Fluorescence imaging monitored photodynamic therapy in vivo

In the subcutaneous injection experiments, 100 μL of C-dots-Ce6 (100 μM) was injected into the left rear flank area of mice. Then the fluorescence images of mice were recorded on a Carestream Molecular Imaging In-Vivo MS FX PRO system. The excitation wavelength is set at 430 nm.

In the tail vein injection experiments, when the xenografts reached about 25~30 mm^3, the fluorescence imaging and photodynamic therapy were conducted. After systemic tail vein injection of C-dots-Ce6, the mice were anesthetized and placed on an animal plate heated to 37 °C. The fluorescent scans were performed at various time points (2, 4, 6, 8 and 24 h) post injection using Cambridge Research & Instrumentation (CRi) in vivo imaging system (CRi, MA, USA). The tumor bearing mice were sacrificed by exsanguinations at 4 and 24 h post-injection and the tumor and major organs were harvested. The animals were randomized into five groups of five animals per group to determine tumor growth rate after the following treatments: (a) saline; (b) saline and laser; (c) C-dots-Ce6 administration only; (d) Ce6 administration and irradiation; and (e) C-dots-Ce6 administration and irradiation. For PDT treatment, C-dots-Ce6 (5 μmol Ce6 eq kg^{-1}) were delivered via tail vein injection, and after 8 h circulation, a 1.5 cm diameter area encompassing the tumor was irradiated for 10 min using a 671 nm laser (100 mW/cm^2) coupled to a 1 m quartz fiber optic cable terminating in a microlens to distribute light uniformly throughout the treatment field. Growth curves representing tumor

regrowth for the control and treated groups were estimated by measuring tumors in two dimensions using a vernier caliper. Tumor volume (V) was determined by the following equation: $V = AB^2*0.52$, where A was the longer and B was the shorter diameter (mm).

Statistical Analysis

Cell viability was calculated using the following formula: average A value of experimental group/average A value of control group × 100%. Results were expressed as means ± SD. Comparisons between two groups were made by unpaired two tailed Student's t test using SPSS 15.0 software. *P*-value of less than 0.05 was considered statistically significant.

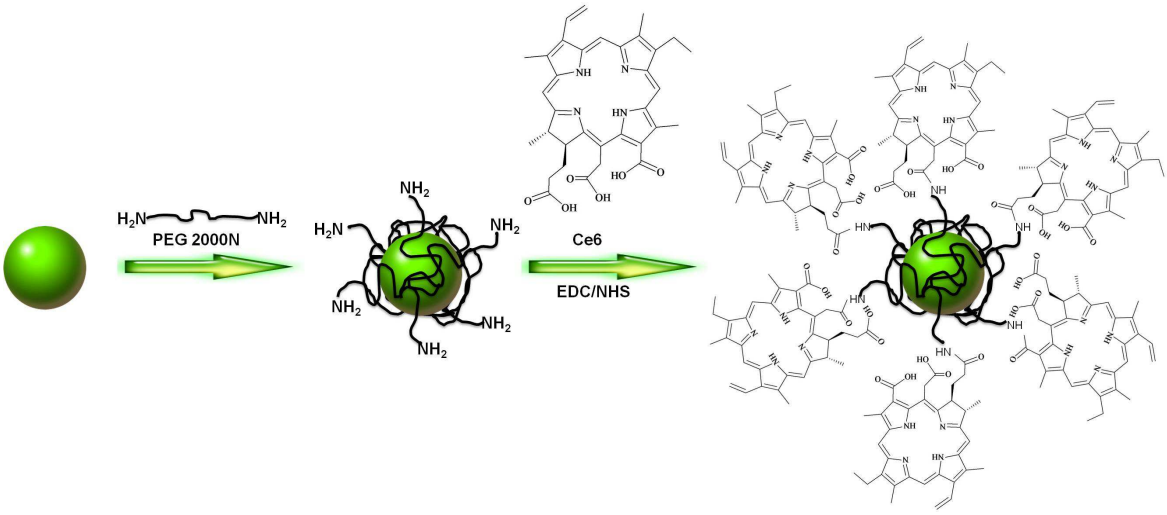

Figure S1. Synthetic scheme of chlorin e6-conjugated C-dots (C-dots-Ce6) using a modified EDC–NHS reaction.

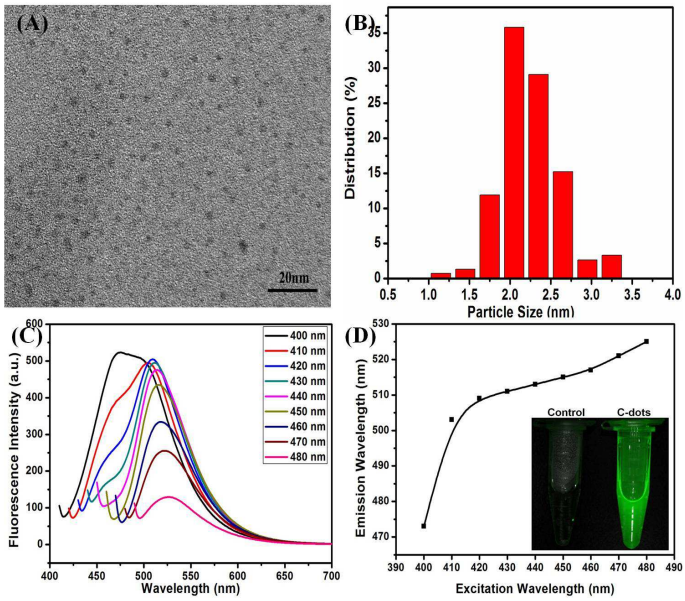

Figure S2. (A) TEM image of C-dots-NH_2; (B) The histogram showing the size distribution of C-dots-NH_2; (C) Fluorescence emission spectra of C-dots-NH_2 under varied excitation wavelengths; (D) The changes of fluorescence emission peaks of C-dots-NH_2 under varied excitation wavelengths. The inset is fluorescence images of C-dots-NH_2 in PBS buffer (pH= 7.4). Pure PBS buffer solution is used as a control.

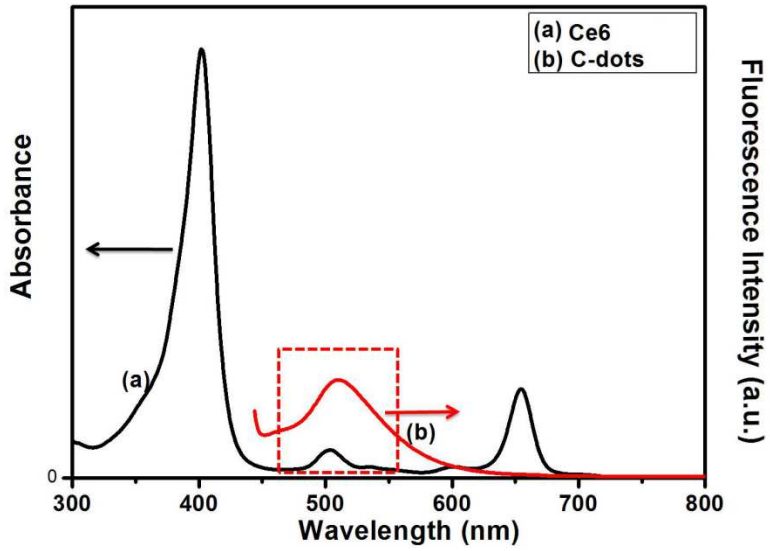

Figure S3. (a) UV-vis absorbance spectrum of Ce6, (b) Fluorescence emission spectrum of C-dots (The excitation wavelength is 430 nm).

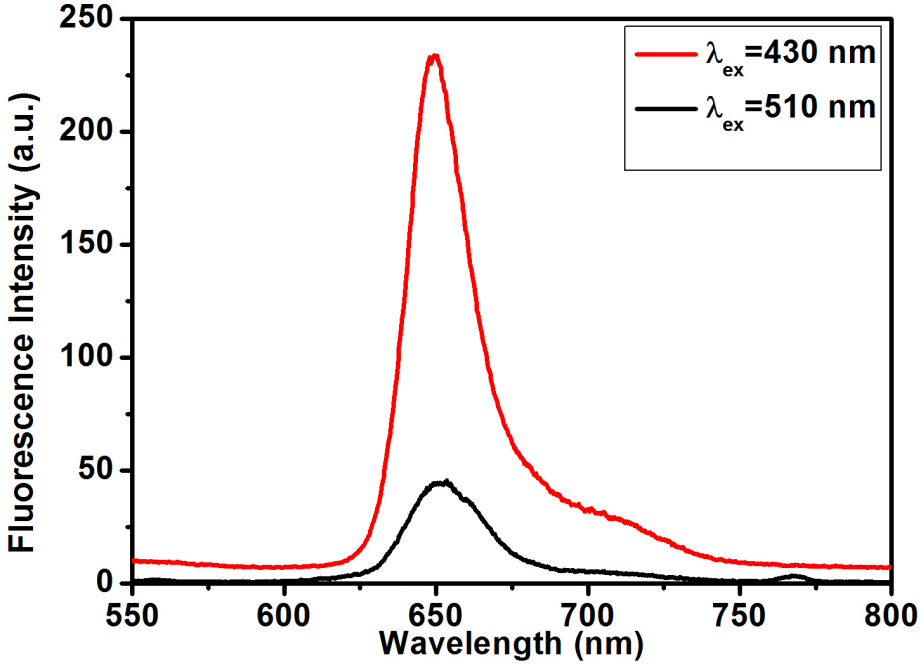

Figure S4 Fluorescence emission spectra of C-dots-Ce6 by excitation at wavelengths of 430 nm and 510 nm, respectively.

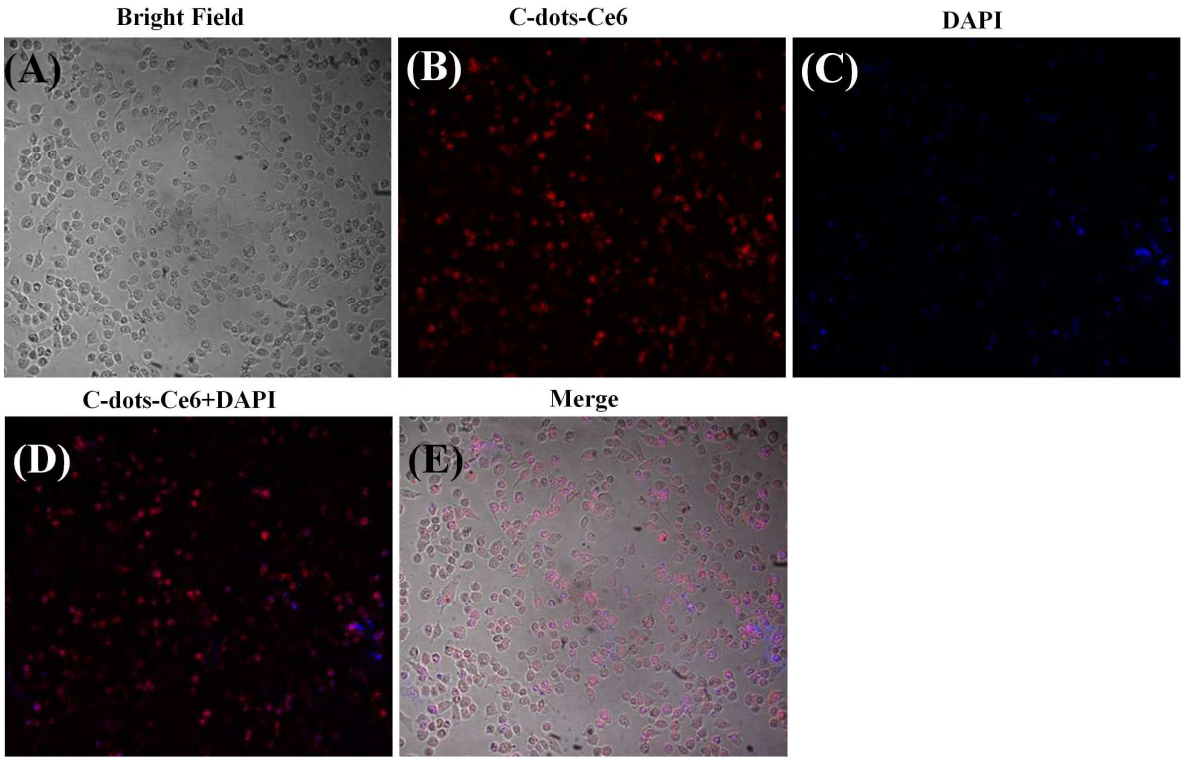

Figure S5. Subcellular localization of C-dots-Ce6, monitored by fluorescence imaging using an upright Olympus IX71 optical microscope. (A) Bright field; (B) fluorescence image of C-dots-Ce6; (C) fluorescence image of DAPI; (D) fluorescence image of C-dots-Ce6 and DAPI; (E) Merged image. MGC803 cells were incubated with C-dots-Ce6 at a concentration of 10 μM for 2 h at 37 ºC. The nuclei were stained by DAPI.

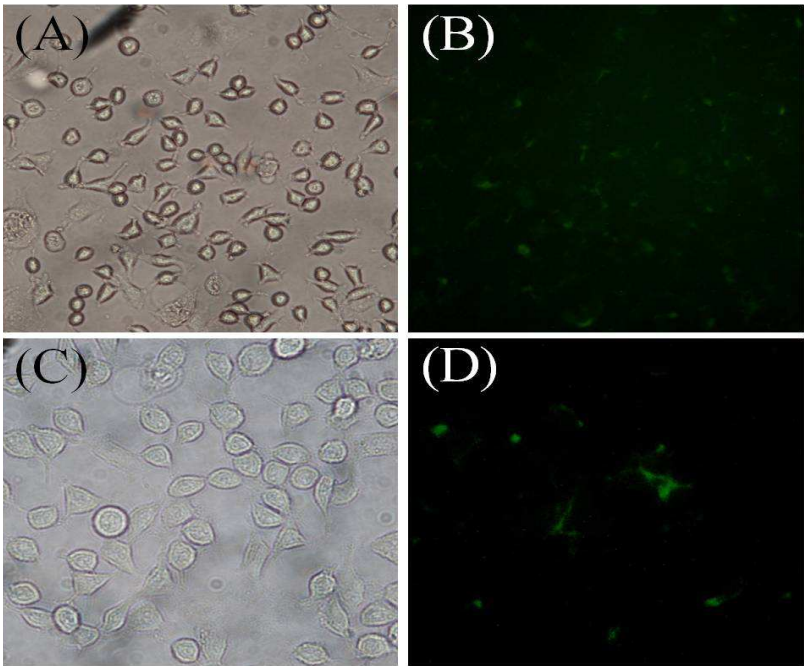

Figure S6 Subcellular localization of C-dots-NH_2, monitored by fluorescence imaging using an upright Olympus IX71 optical microscope. MGC803 cells were incubated with C-dots-NH_2 at a concentration of 10 μM for 2 h at 37 ºC. (A-B) Low magnification (10 X); (C-D) High magnification (40 X).

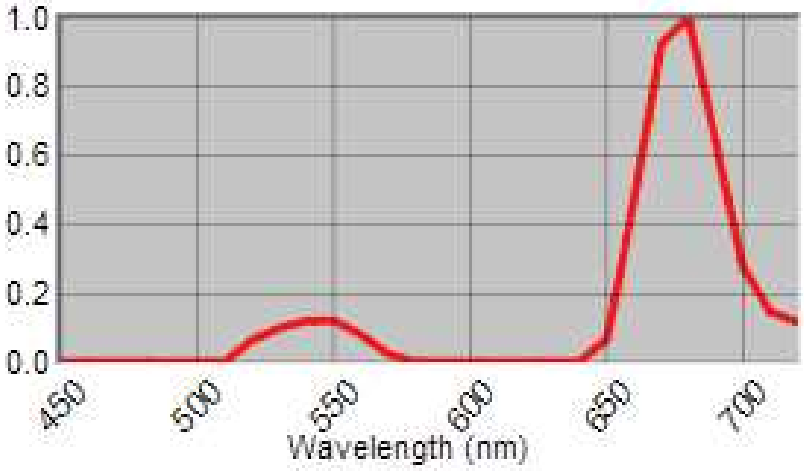

Figure S7. Fluorescence emission spectrum of C-dots-Ce6 in MGC803 tumor cells. The excitation wavelength is 430 nm.

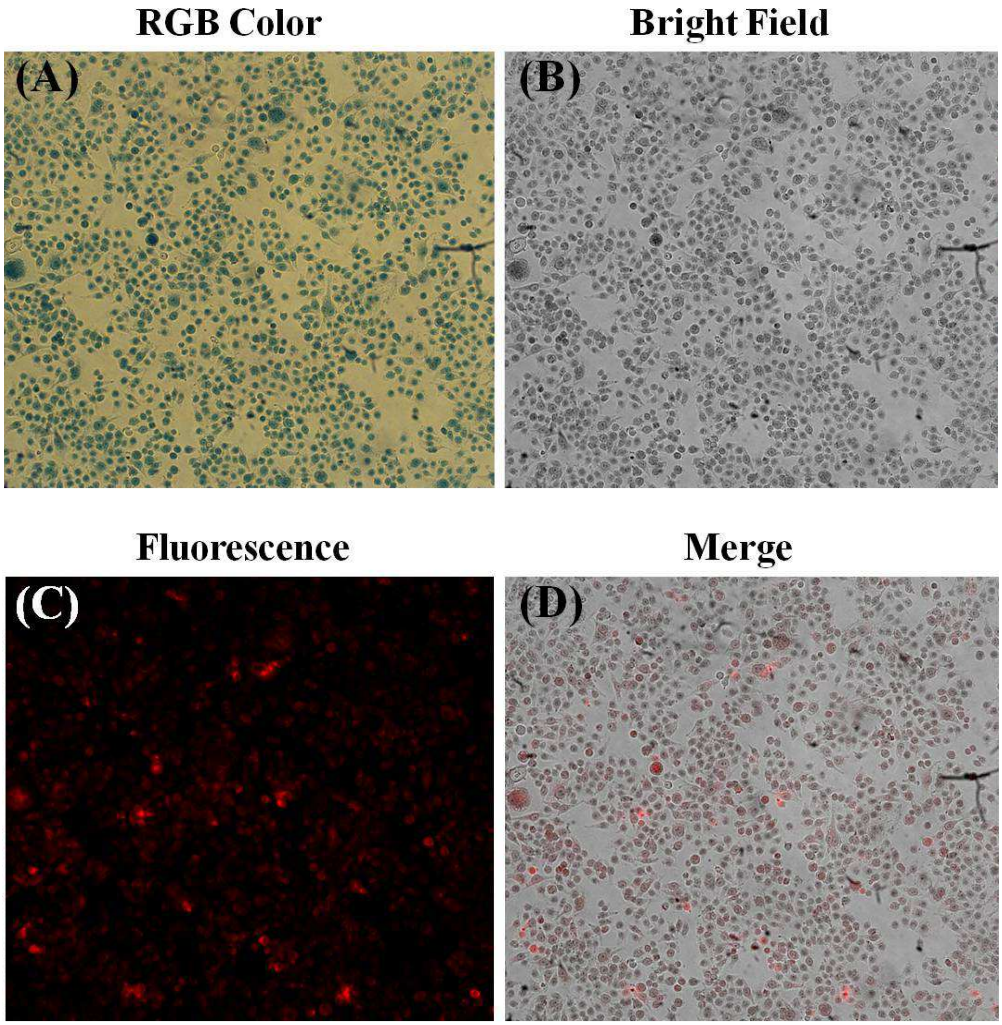

Figure S8. Trypan blue staining images of MGC803 cells incubated with C-dots-Ce6 at a concentration of 10 μM for 2 h at 37 ºC prior to irradiation for 2 min with a 671 nm laser (30 mW/cm^2) under various imaging conditions. (A) RGB color; (B) Bright field; (C) Fluorescence field; (D) Merge.

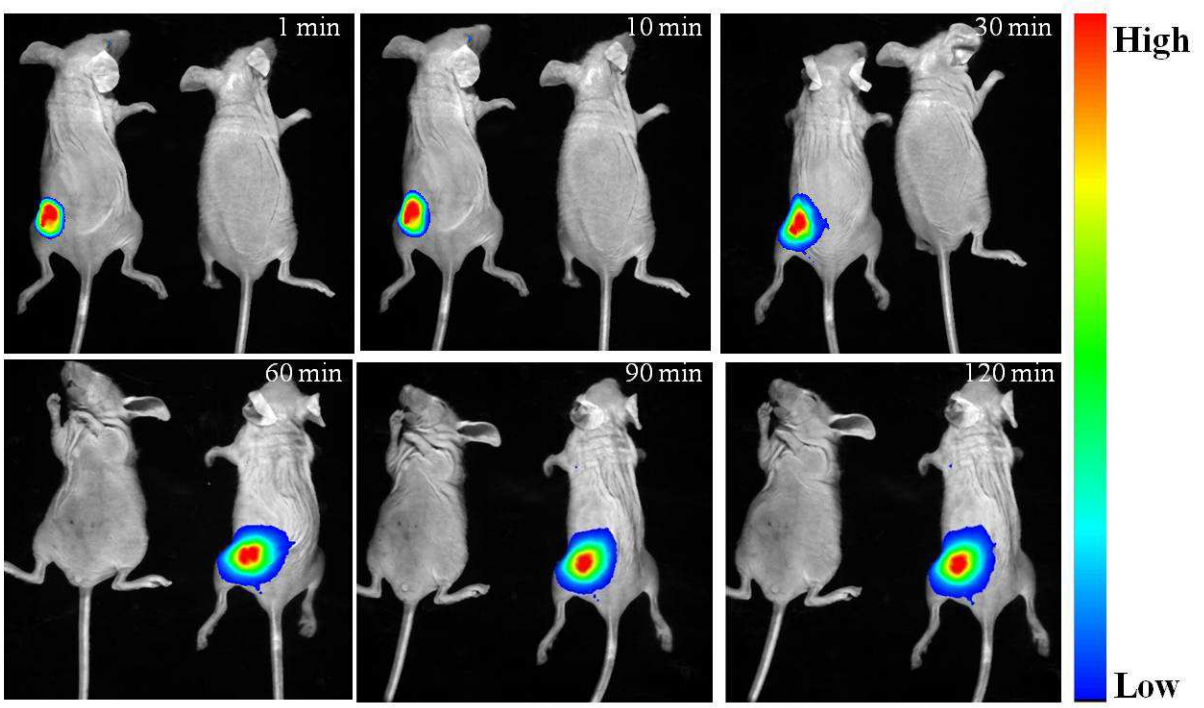

Figure S9 In vivo fluorescence imaging of mice after subcutaneous injection with and without C-dots-Ce6 at different time points. 100 μL of C-dots-Ce6 100 μM was injected into the left rear flank area of mice.

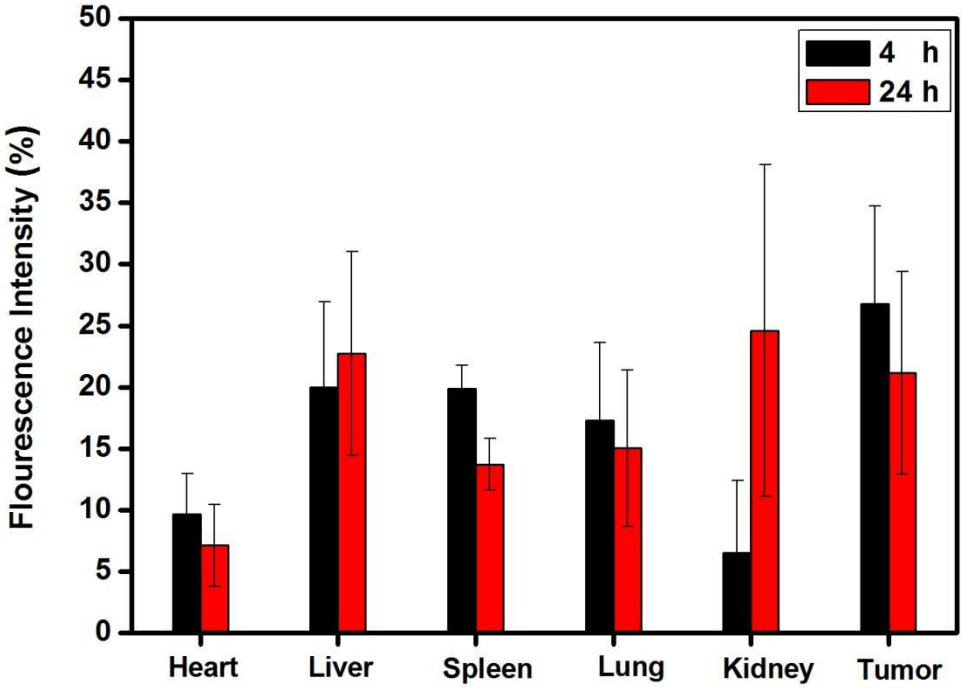

Figure S10. The fluorescence intensity of organs harvested at 4 and 24 h time points post-injection of C-dots-Ce6 to subcutaneous MGC803 gastric cancer model.