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Supplementary Information for:

Carbon dots-assisted luminescence of singlet oxygen: generation dynamics but not cumulative amount of singlet oxygen responsible for photodynamic therapy efficacy

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Experimental section

Materials. All chemicals used in the experiment were analytical grade without further purification. The deionized water was from a Millipore water purification system. $FeSO_4$, H_2O_2, KO_2, dimethyl sulfoxide, HNO_3, $NaNO_2$, NaOH, HCl and ascorbic acid were purchased from Beijing Chemical Reagent Company (Beijing, China). Nitro blue tetrazolium chloride was purchased from Nacalai Tesque Inc. (Tokyo, Japan). NaN_3 was supplied by Tianjin Fuchen Chemical Reagent Co., Ltd. (Tianjin, China). TBHP, 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), trypsin, pronase, and PMA were obtained from Sigma-Aldrich (St. Louis, USA). CCK-8, Annexin V-FITC apoptosis detection kit, proteinase K and Lyso-Tracker Red were obtained from Beyotime (Hangzhou, China). HB, HY, *m*THPC and Ce6 were obtained from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). MB was obtained from J&K Scientific Ltd. (Beijing, China). HeLa cells were supplied by the American Type Culture Collection (ATCC, Rockville, MD). H_2O_2 stock solution (0.1 M) was prepared daily from commercial H_2O_2 (30%, *v/v*). $NaIO_4$ stock solution (0.1 M) was prepared by dissolving $NaIO_4$ with deionized water. Working solutions of H_2O_2 and $NaIO_4$ were freshly obtained by diluting the stock solution with deionized water. $^1O_2$ was prepared from the reaction of $NaIO_4$ and H_2O_2 ($NaIO_4$: H_2O_2 = 1: 1). $^{\bullet}OH$ (100 μM) was prepared from the Fenton reaction (Fe^{2+}/H_2O_2 = 10: 1). $ONOO^-$ (100 μM) was generated from the reaction of H_2O_2-HCl and $NaNO_2$ solutions. The concentration of $ONOO^-$ was determined by the UV-vis absorbance at 302 nm (ε = 1670 M^{-1} cm^{-1}). $O_2^{\bullet-}$ (100 μM) was prepared by dissolving dry KO_2 into the anhydrous dimethyl sulfoxide solution. $ROO^{\bullet}$ (100 μM) was generated by thermolysis of AAPH in deionizer water at 37 ˚C for 30 min.

Apparatus and characterizations. The morphology of the Im-CDs was obtained using a HRTEM (JEM-3010, JEOL). Size distributions of the Im-CDs were determined by DLS using a Zetasizer from Nano ZS (Malvern Instruments Ltd, UK). The XRD measurements were performed on a D8 ADVANCE X-ray diffractometer (Bruker, Germany) equipped with graphite-monochromatized Cu Kα radiation (λ= 0.154 nm). And a 2θ angle of the diffractometer was ranging from 10° to 60° with a scan rate of 0.5°·min^{-1}. Raman spectrum was recorded in a Renishaw Micro-Raman Spectroscopy InVia Raman system (England) using a charge-coupled device (CCD) detector and a Renishaw laser which excited at 514 nm. The lifetime and the

quantum yield were obtained using Edinburgh Instruments' LifeSpec ps spectrometer and measured by exciting at 331 nm with a nanosecond flashlamp. The percentage contribution of each lifetime component to the total decay was calculated with the F900 Edinburgh instruments software. The UV-vis absorption spectrum was obtained by using a Shimadzu UV-3600 spectrophotometer (Tokyo, Japan). The deionized water was used as the corresponding reagent blank for the absorbance measurement. The functional groups in the Im-CDs were detected by FT-IR spectra collected with a Nicolet 6700 FT-IR spectrometer (Thermo, America). The FL spectra were obtained by using Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan). The CL spectrum was obtained using a Hitachi F-7000 fluorescence spectrophotometer without an excitation light. XPS spectra were performed on a Thermo-VG Scientific ESCALAB 250 spectrometer with a monochromatic Al Kα X-ray source (1486.6 eV). The absorbance spectra were detected by a Multimode Plate Readers (PerkinElmer Enspire, USA) in CCK-8 assay. The confocal fluorescence images were obtained by using Leica TCS SP8 Laser Scanning Confocal Microscope (Germany Leica Co., Ltd). The CL detection was performed on a BPCL analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China).

Synthesis of Im-CDs. The Im-CDs were synthesized by one-step hydrothermal carbonization method. Briefly, 3.1 g histidine was dissolved in 30 mL deionized water. The solution was stirred at room temperature for several minutes. Then the transparent solution was transferred to the Teflon-lined autoclave chamber and heated at 240 °C for 8 h. After cooling to room temperature, the obtained products were dialyzed against deionized water with a cellulose ester membrane bag (Mw = 200) for 48 h to remove the excess precursors. The purified Im-CDs were freeze-dried for further use.

Synthesis of serine-CDs. Serine-CDs were synthesized by carbonizing serine. Briefly, 3.1 g serine was dissolved in 30 mL deionized water. Then the solution was transferred into a Teflon-lined autoclave chamber and heated at 240 °C for 8 h. The resultant dark brown products were dialyzed against deionized water with a cellulose ester membrane bag (Mw = 200) for 48 h to remove the excess precursors. The purified serine-CDs were freeze-dried for further use.

Subcellular localization of Im-CDs. For cellular localization imaging, HeLa cells were plated on the confocal dishes for 12 h and incubated with 0.5 $mg \cdot mL^{-1}$ Im-CDs for 4 h. Then HeLa cells were stained with Lyso-Tracker Red (lysosome dye) for another 1 h. Subsequently, HeLa cells

were rinsed twice with PBS to remove the residual substance and cultured with 2.0 mL of PBS for the FL imaging. The emission of the Im-CDs was collected at 420–500 nm (excited at 405 nm) for blue channel, and the emission of lysosomes was collected at 580–650 nm (excited at 542 nm) for red channel.

***In vitro* hemolysis assay.** Erythrocytes were collected from 1 mL of human blood sample by centrifugation at 8000 rpm for 10 min and then washed five times with 5 mL of PBS solution. The stock erythrocyte suspension was prepared by mixing centrifuged erythrocytes into 10 mL of PBS. Then 0.2 mL of the stock erythrocyte suspension was mixed with (a) 0.8 mL of sterilized deionized water as a positive control; (b) 0.8 mL of PBS as a negative control; and (c) 0.8 mL of the Im-CDs at concentrations ranging from 0.06 to 1.00 mg·mL^{-1}. All the mixtures were incubated for 3 h at 37 °C. Finally, the mixtures were centrifuged for 5 min at 8000 rpm. 200 μL of supernatants was transferred to a 96-well plate for measurement of absorbance at 541 nm. The percentage of hemolysis was calculated as follows: Hemolysis % = ($A_{sample} - A_{negative}$) / ($A_{positive} - A_{negative}$) × 100.

Real-time *in vitro* monitoring of $^1O_2$ in PDT. A 600 nm LED (60 mW·cm^{-2}) was used as the light source for the $^1O_2$ generation test of HY, HB and MB. We used a 650 nm LED (60 mW·cm^{-2}) as the light source for the $^1O_2$ generation test of Ce6 and *m*THPC. The interval of BPCL analyzer was set at 1.0 s per spectrum, and the work voltage was -1000 V. First, HeLa cells were incubated with one type of photosensitizers (*i.e.,* HY, MB, HB, Ce6 and *m*THPC) for 4 h, washed with the DMEM medium, and then incubated with 0.5 mg·mL^{-1} Im-CDs for another 4 h. The concentrations of HY, HB, MB, Ce6 and *m*THPC were 1.0, 2.0, 6.0, 10.0 and 10.0 μg·mL^{-1}, respectively. Then HeLa cell were detached by trypsin (0.25%) for 2 min after washed with PBS. Thereafter HeLa cells were resuspended in 1.0 mL of PBS and added into the quartz vial, which was positioned in front of the PMT in the BPCL analyzer. A monochromatic filter (490 nm) was placed between the CL quartz vial and the PMT. When the light was turned on, the CL intensities were monitoring in real time by the BPCL analyzer.

CCK-8 assays. HeLa cells were seeded into six 96-well plates in the density of 1.0×10^4 cells per well. After 24 h, the HeLa cells in two 96-well plates were incubated with one type of photosensitizer (*i.e.,* HY, MB, HB, Ce6 and *m*THPC) for 12 h. One plate was irradiated with the LED light (600 nm or 650 nm), and the other plate was stored in the dark. After incubated under

37 °C for another 24 h, HeLa cells were washed with the DMEM medium, and 200.0 μL of DMEM containing CCK-8 (10.0 μL) was added to each well. After 1 h of incubation, the absorbance at 450 nm was measured on a Multimode Plate Readers.

Cell apoptosis assay. HeLa cells were incubated in the six-well plate for 24 h at 37 °C and then treated at 37 °C for 12 h with PBS buffer, HB, MB and HY, respectively. HeLa cells in each treatment were collected and divided into two groups, in which one group was treated with 600 nm irradiation (60 $mW \cdot cm^{-2}$) and the other group was not treated further. Afterwards, HeLa cells were incubated under 37 °C for 24 h. HeLa cells were detached by adding 0.5 mL trypsin (0.25%) for 2 min after washed with PBS. Then HeLa cells were resuspended in 1.0 mL of binding buffer, and incubated for an additional 30 min after 5.0 μL Annexin V-FITC and 10.0 μL PI were added. Finally, HeLa cells were analyzed by flow cytometry.

Colony forming assay. The colony forming assay was performed in a six-well culture plate containing 5 mL of culture medium. The number of cells seeded was determined in preliminary dose response studies. HeLa cells were treated at 37 °C for 12 h with various concentrations of *m*THPC, and then detached by adding 0.5 mL trypsin (0.25%) for 2 min after washed with PBS. The cells from a single-cell suspension were seeded in triplicate with 10^3, 10^4, or 10^5 $cells \cdot mL^{-1}$ and incubated at 37 °C, 5% CO_2/95% air. The colonies (>20 cells) were counted manually under a microscope after incubation for two weeks. The surviving fraction was calculated as the number of colonies at a specific time point divided by the number of colonies without light irradiation which was defined as time zero.

Animal model. Animals received care in accordance with Institutional Animal Care and Use Committee (IACUC) of Laboratory Animals and the animal procedures were approved by the Ethics Review Committee for Animal Experimentation of Beijing Vital River Laboratory Animal Technology Co., Ltd (P2018054). HeLa cells (~5.0×10^4 $cells \cdot site^{-1}$) were implanted subcutaneously into the right shoulder of the female Balb/c nude mice. *In vivo* PDT experiments were performed when the tumors reached ~70 mm^3.

Real-time *in vivo* monitoring of $^1O_2$ in PDT. Similar with the process of monitoring the dynamics of $^1O_2$ generated in HeLa cells during PDT, the Im-CDs (0.5 mg/mL) and each photosensitizer (*i.e.,* HY, MB, HB, Ce6 and *m*THPC) were injected into mice tumor in turn. The concentrations of HY, HB, MB, Ce6 and *m*THPC were 10.0, 6.0, 2.0, 1.0, and 5.0 $mg \cdot kg^{-1}$

respectively. Then the mouse was fixed in front of a PMT. A low-pass interference filter was placed between the mouse and the PMT. When the light was turned on, the CL signals were monitored in real time using a BPCL analyzer.

***In vivo* PDT.** The mice were randomly divided into 7 groups for HY, HB and MB (n = 5): (1) PBS with irradiation, (2) HY with irradiation, (3) HB with irradiation, (4) MB with irradiation, (5) HY without irradiation, (6) HB without irradiation, and (7) MB without irradiation. The irradiation was performed with 600 nm (60 $mW \cdot cm^{-2}$). The mice were randomly divided into 5 groups (n = 5) for Ce6 and *m*THPC: (1) PBS with irradiation, (2) Ce6 with irradiation, (3) *m*THPC with irradiation, (4) Ce6 without irradiation, and (5) *m*THPC without irradiation. The irradiation was performed with 650 nm (60 $mW \cdot cm^{-2}$). The tumor size was measured by a caliper every other day and was calculated according to the formula: the volume = (tumor length) × $(\text{tumor width})^2 \times 0.5$. Relative tumor volume was calculated as V/V_0 (V_0 is the tumor volume measured before PDT).

Histological staining and blood analysis. H&E staining was used to stain the tumor slices and major organ (*i.e.,* heart, liver, spleen, lung, and kidney) slices. The tumors and major organs were collected from the mice of control and treatment groups after PDT. The final H&E stained slices were scanned with an optical microscope. Blood of mice was collected intravenously post-injection with 100 μL Im-CDs (0.5 $mg \cdot mL^{-1}$) at day 1, day 7 and day 14. Untreated healthy mice were used as the control. A standard biochemical procedure was carried out for the separation and analyzation of blood.

Statistical analysis. Statistical analyses were performed using Student's t-test. *$p < 0.05$ was considered to be significant.

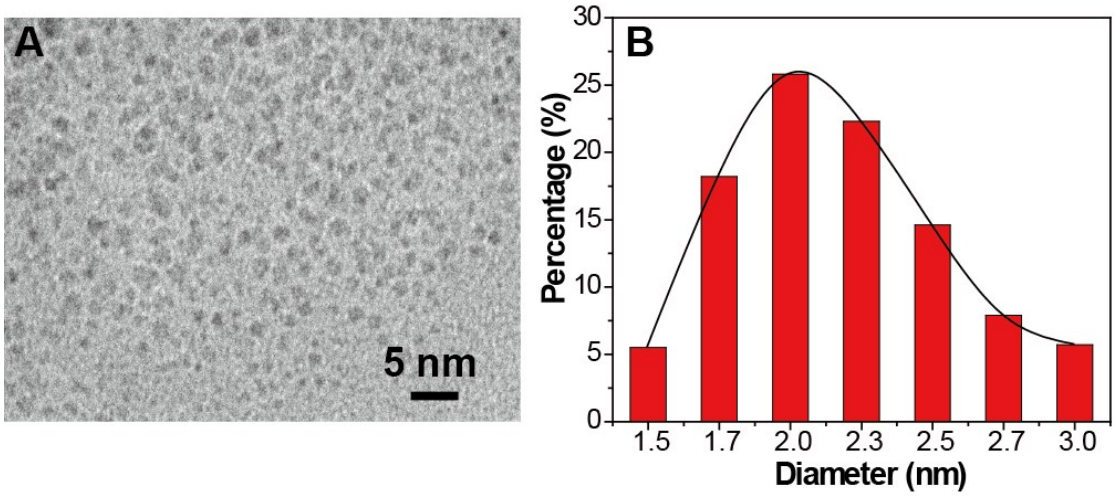

Fig. S1. (A) HR-TEM image of the Im-CDs, and (B) particle size distribution histograms of the Im-CDs.

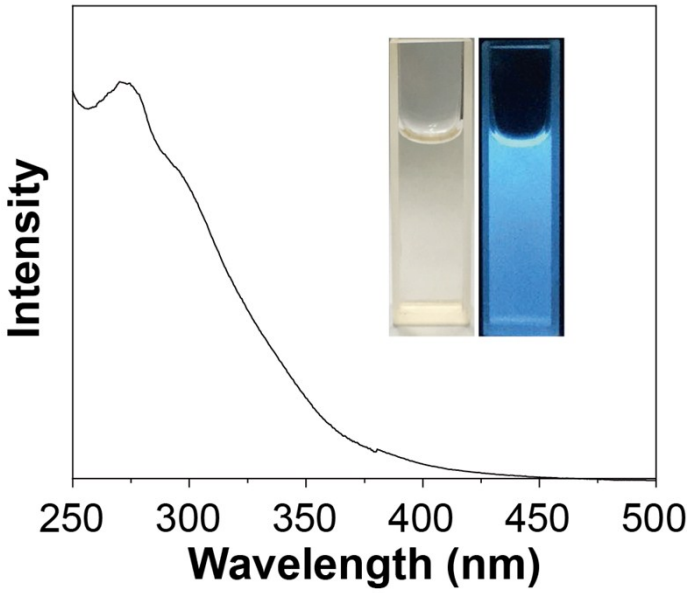

Fig. S2. UV absorption spectrum of the Im-CDs. Inset: optical images under (left) daylight and (right) UV light.

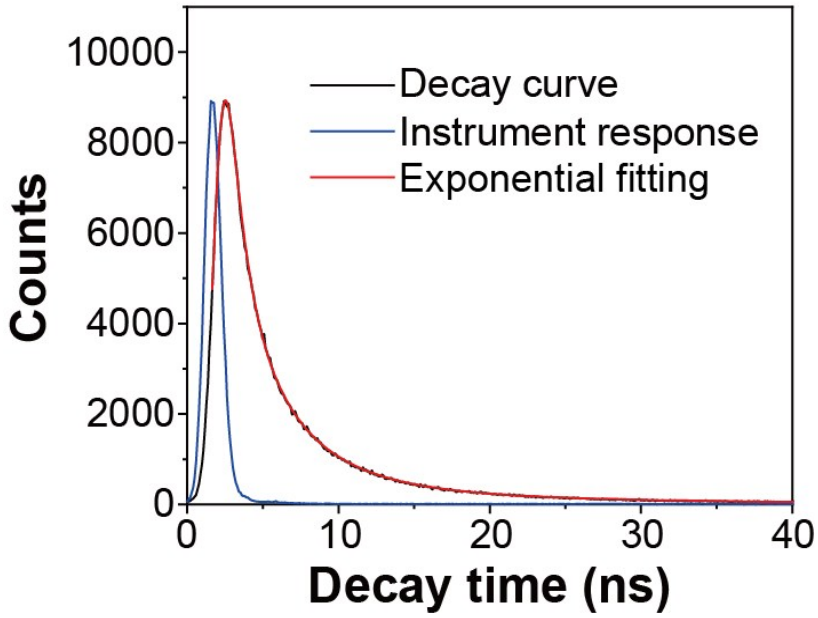

Fig. S3. Lifetime of the Im-CDs and the parameter generated by the exponential fitting (λ_{ex} = 330 nm and λ_{em} = 400 nm).

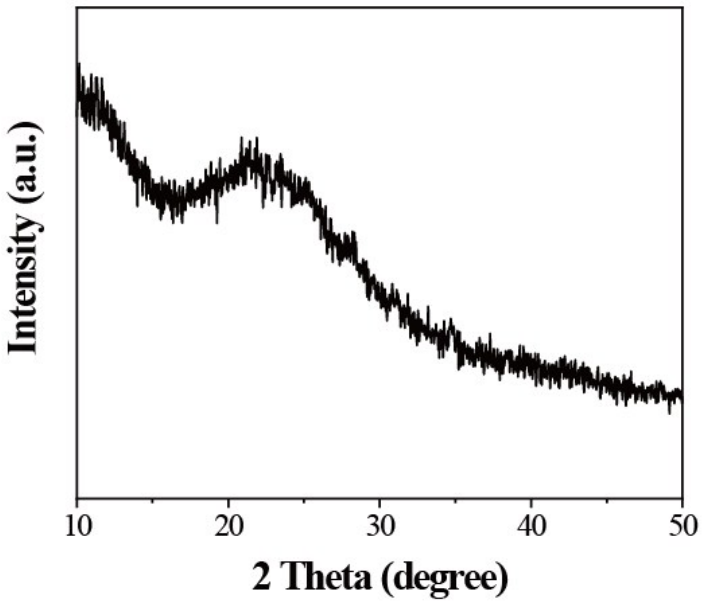

Fig. S4. XRD pattern of the Im-CDs.

Fig. S5. Schematic diagram of a static injection setup.

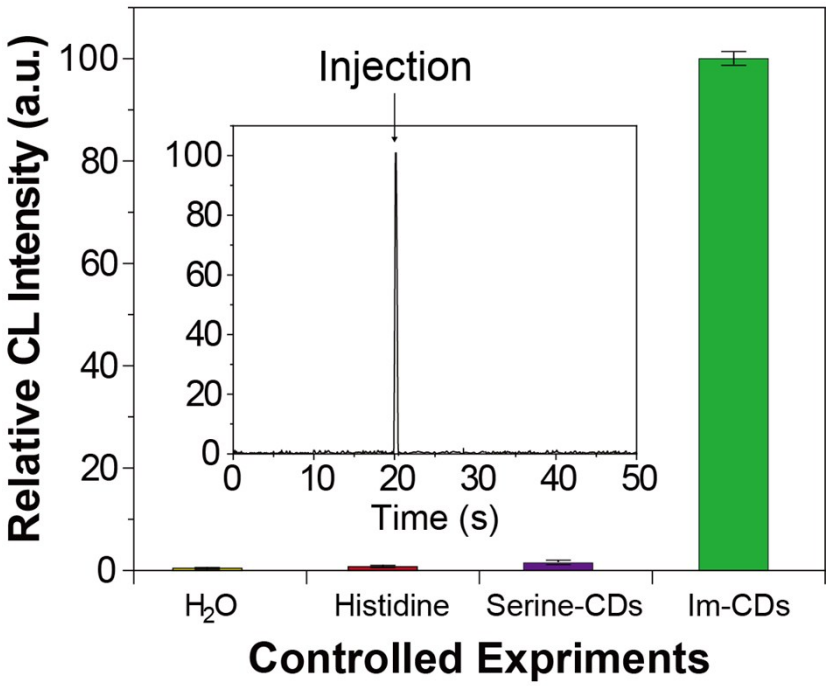

Fig. S6. CL emissions of H_2O, histidine, serine-CDs and the Im-CDs in the presence of $^1O_2$ from the IO_4^--H_2O_2 system. Inset: The CL kinetic curve of the IO_4^--H_2O_2 system with the Im-CDs. The concentrations of histidine, the Im-CDs, serine-CDs, IO_4^- and H_2O_2 were 10.0 mM, 0.5 mg·mL^{-1}, 0.5 mg·mL^{-1}, 10.0 μM and 10.0 μM, respectively. Error bars were defined as standard deviation, n = 5.

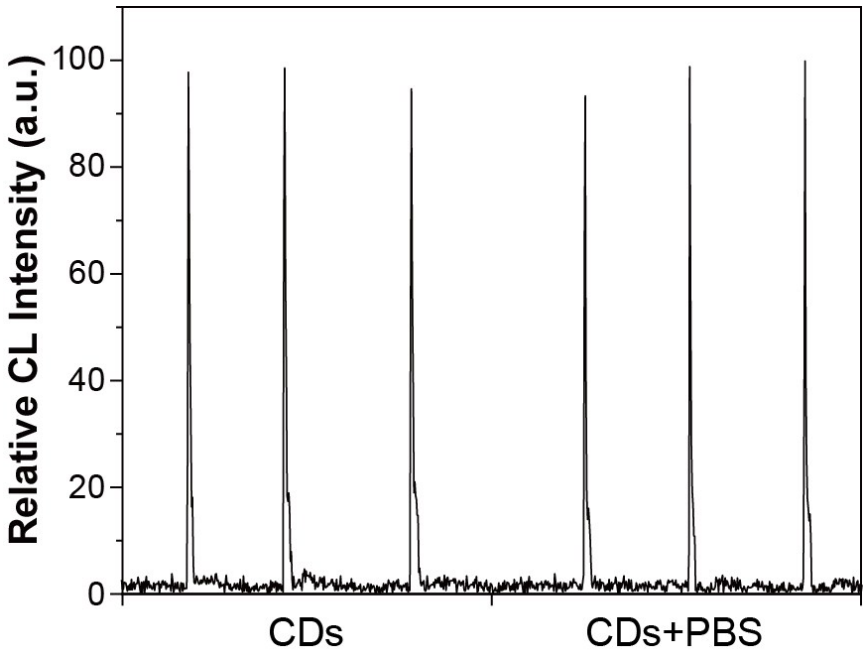

Fig. S7. CL response of Im-CDs with PBS and without PBS in the presence of $^1O_2$ from the IO_4^--H_2O_2 system. The concentrations of Im-CDs and PBS were 0.5 mg·mL^{-1} and 50.0 mM.

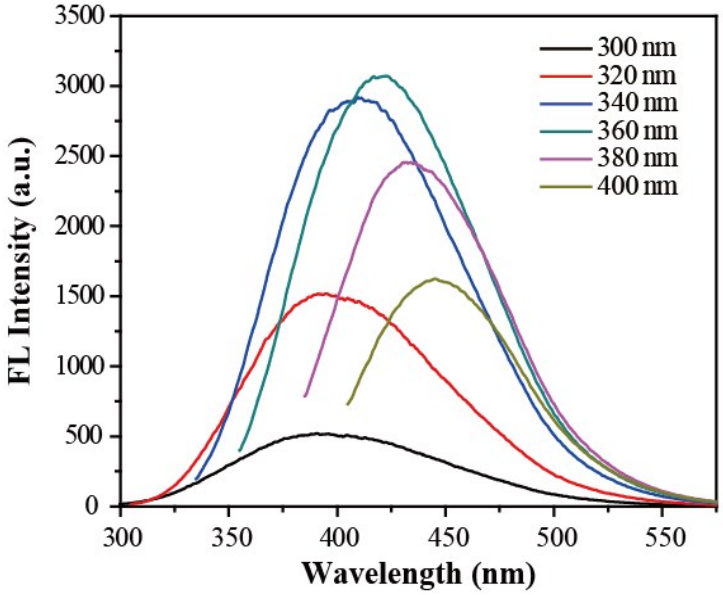

Fig. S8. FL emission spectra of the as-prepared serine-CDs under various excitation wavelengths from 300 to 400 nm.

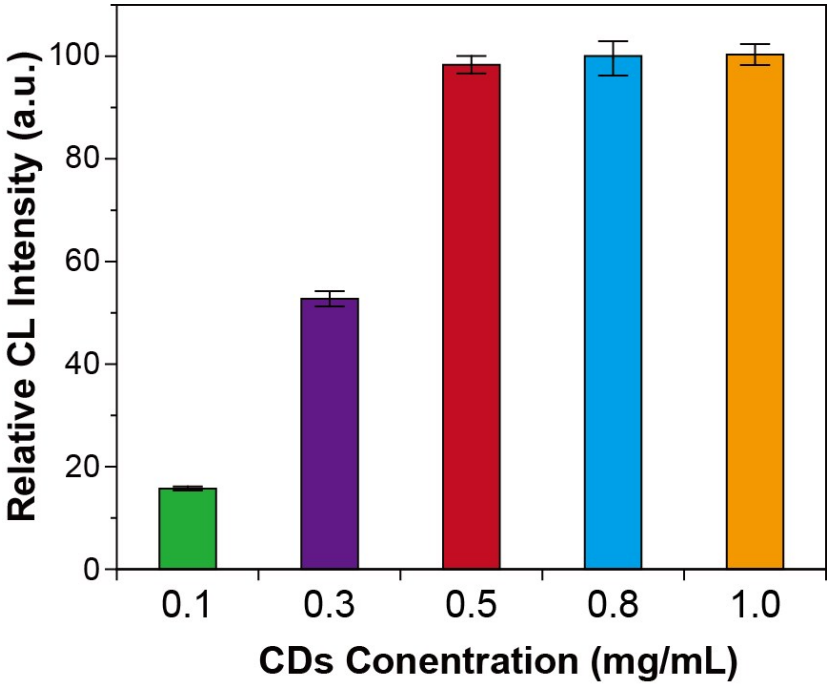

Fig. S9. Effects of the concentrations of Im-CDs on the CL emissions of the Im-CDs in the presence of $^1O_2$ from the IO_4^--H_2O_2 system. Error bars were defined as standard deviation, n = 5.

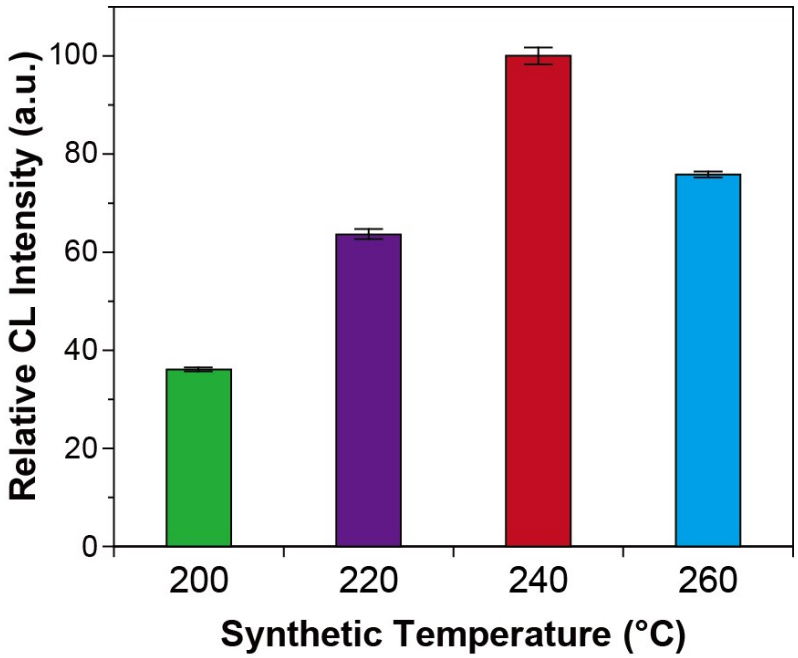

Fig. S10. Effects of the synthetic temperature of Im-CDs on the CL emissions of the Im-CDs in the presence of $^1O_2$ from the IO_4^--H_2O_2 system. Error bars were defined as standard deviation, n = 5.

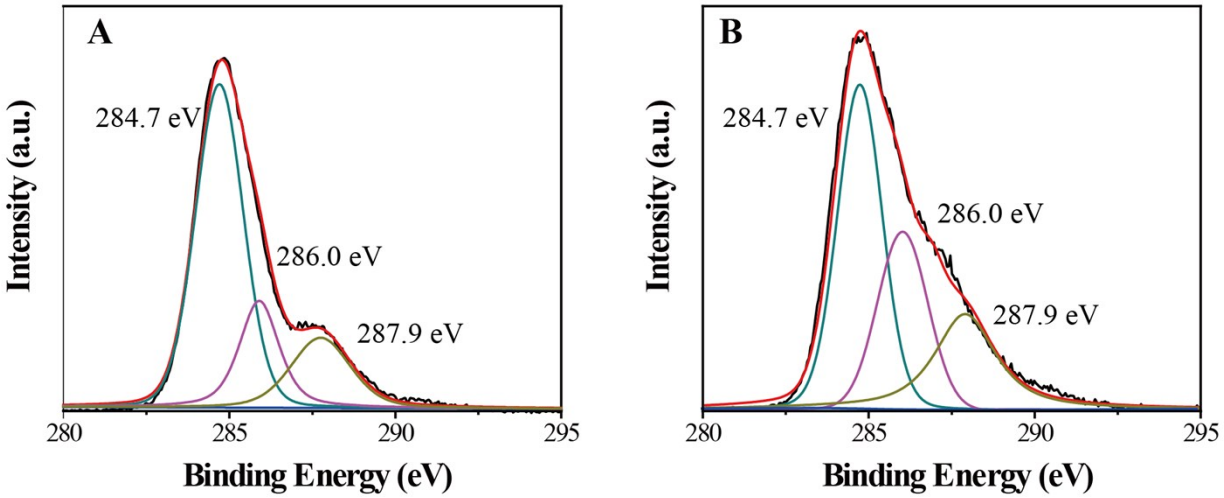

Fig. S11. XPS C 1s spectra of the Im-CDs before (A) and after (B) reacted with $^1O_2$ from the IO_4^--H_2O_2 system. The concentrations of the Im-CDs and $^1O_2$ were 0.5 mg·mL^{-1} and 10.0 mM, respectively.

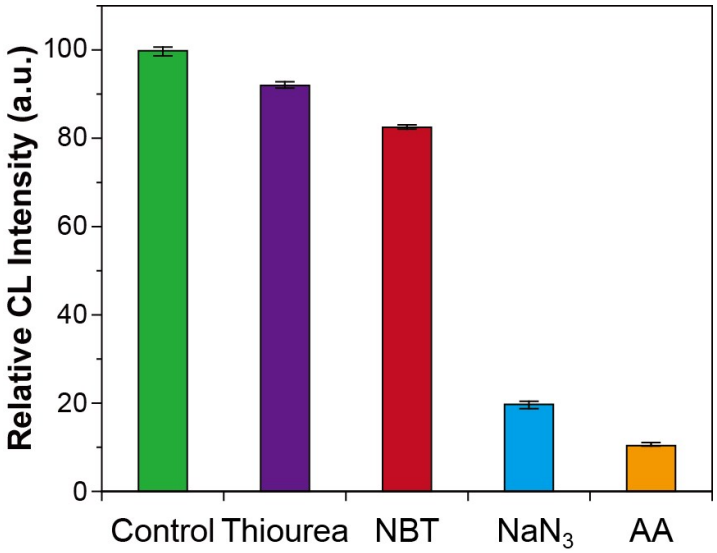

Fig. S12. Effects of scavengers on the CL emissions of the Im-CDs in the presence of $^1O_2$ from the IO_4^--H_2O_2 system. The concentrations of the Im-CDs, $^1O_2$ and scavengers (thiourea, NBT, NaN_3, AA) were 0.5 mg·mL^{-1}, 10.0 μM and 1.0 mM. Error bars were defined as standard deviation, n = 5.

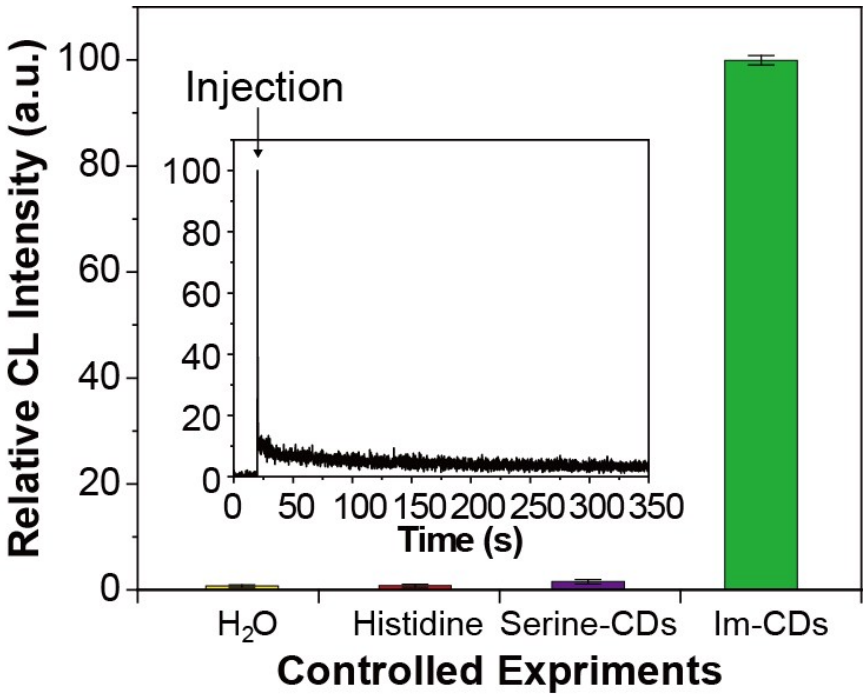

Fig. S13. CL emissions of H_2O, histidine, serine-CDs and the Im-CDs in the presence of $^1O_2$ from the Fe^{2+}-catalyzed TBHP system. Inset: The CL kinetic curve of the Fe^{2+}-catalyzed TBHP system with the Im-CDs. The concentrations of histidine, the Im-CDs, serine-CDs, Fe^{2+} and TBHP were 10.0 mM, 0.5 mg·mL^{-1}, 0.5 mg·mL^{-1}, 1.0 mM and 1.0 mM, respectively. Error bars were defined as standard deviation, n = 5.

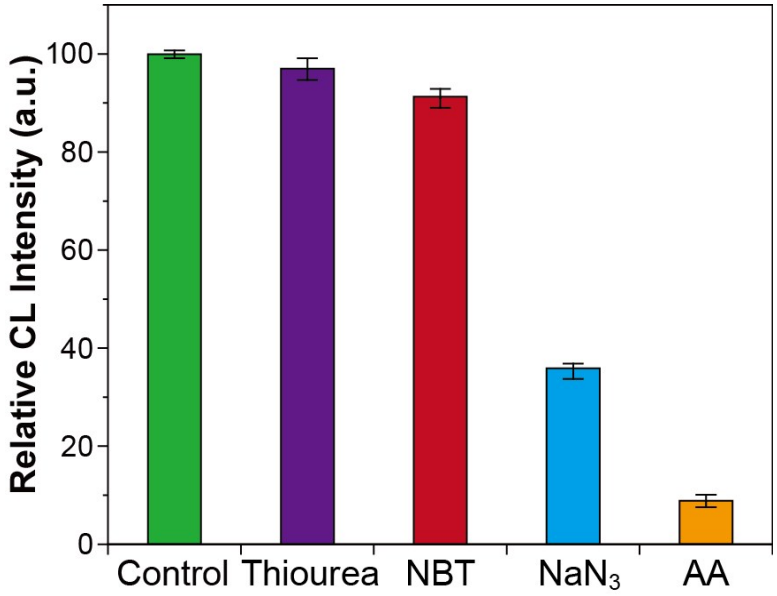

Fig. S14. Effects of scavengers on the CL emissions of the Im-CDs in the presence of $^1O_2$ from the Fe^{2+}-catalyzed TBHP system. The concentrations of the Im-CDs, Fe^{2+}, TBHP and scavengers (thiourea, NBT, NaN_3, AA) were 0.5 mg·mL^{-1}, 1.0 mM, 1.0 mM and 1.0 mM, respectively. Error bars were defined as standard deviation, n = 5.

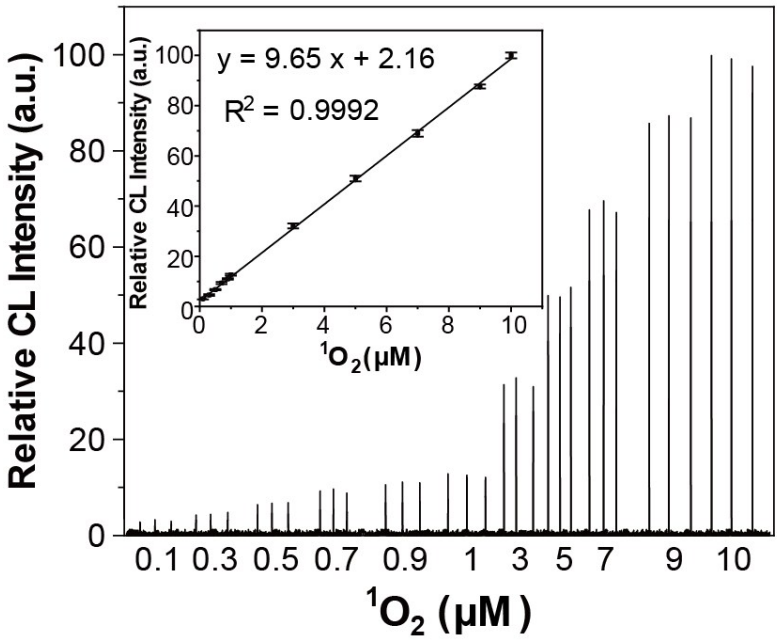

Fig. S15. CL intensities from the reactions of the Im-CDs with various concentrations of $^1O_2$ from the IO_4^--H_2O_2 system. Inset: The calibration curves for $^1O_2$ in the range of 0.1−10.0 μM. The detection limit for $^1O_2$ (S/N = 3) was 90.0 nM. The concentration of the Im-CDs was 0.5 mg·mL^{-1}. Error bars were defined as standard deviation, n = 5.

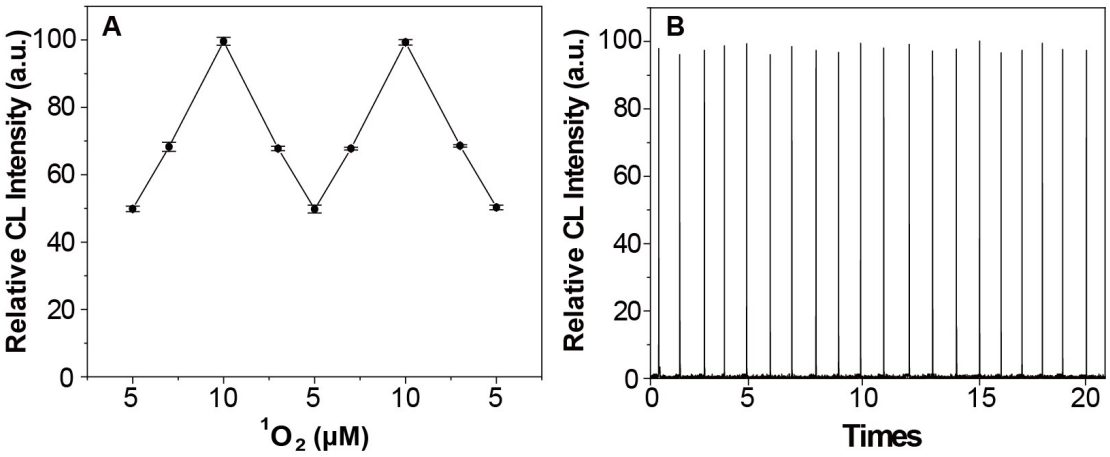

Fig. S16. (A) Reversibility of the Im-CDs for the measurement of $^1O_2$ from the IO_4^--H_2O_2 system. Error bars were defined as standard deviation, n = 5. (B) Stability of the Im-CDs for 20 repeated measurements of 10.0 μM $^1O_2$ from the IO_4^--H_2O_2 system. The relative standard deviation was 2.8%. The concentration of the Im-CDs was 0.5 mg·mL^{-1}.

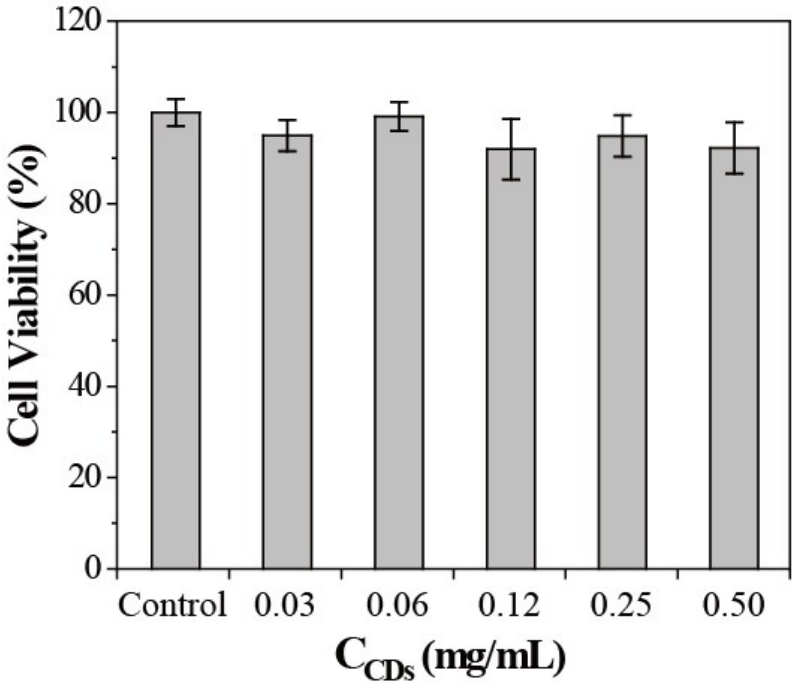

Fig. S17. Cells viability of HeLa cells incubated with various concentrations of the Im-CDs for 12 h. Error bars were defined as standard deviation, n = 5.

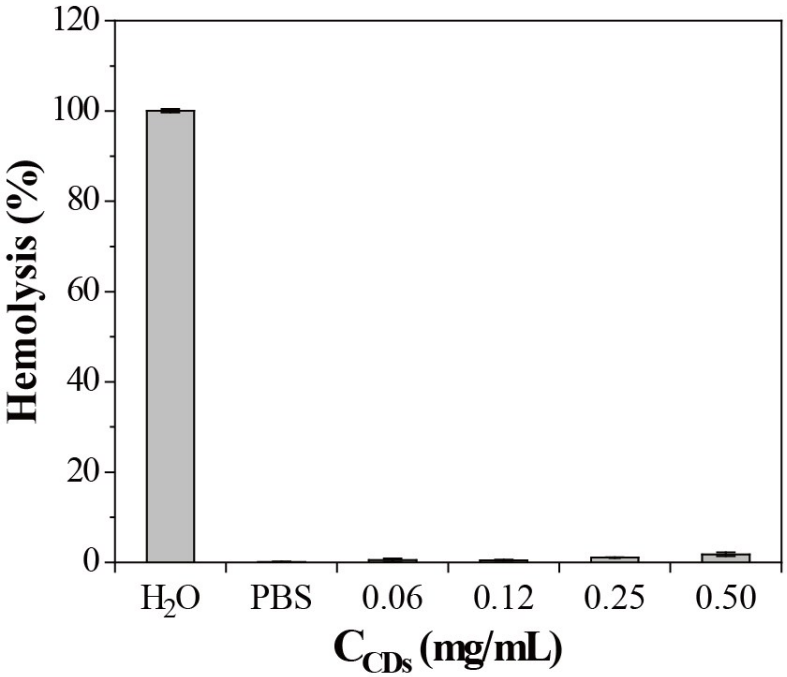

Fig. S18. Hemolytic activities of the Im-CDs after 3 h of incubation with purified erythrocytes at 37 ℃. Error bars were defined as standard deviation, n = 5.

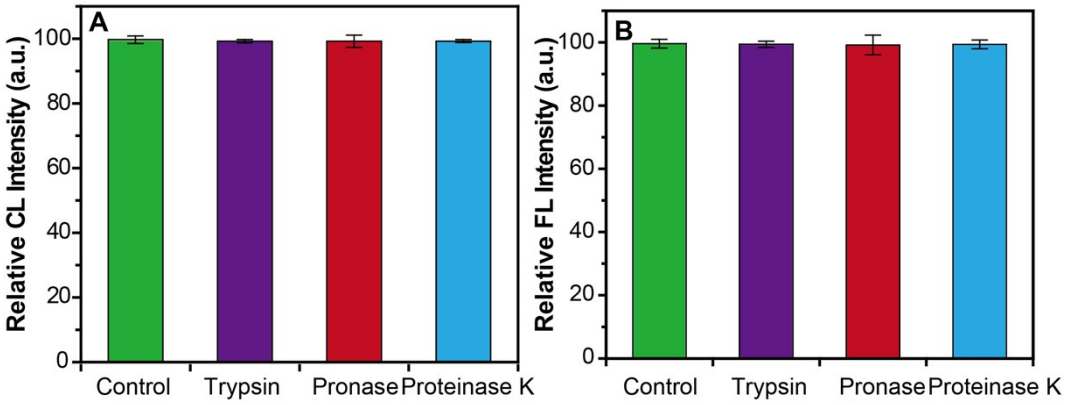

Fig. S19. Effects of various proteases (0.05% trypsin, 0.2 units·mL^{-1} pronase, 2.0 units·mL^{-1} proteinase K) on (A) the CL emissions of the Im-CDs and (B) the FL emissions of the imidazole-functionalized CD-$^1O_2$ system. The Im-CDs were incubated with proteases for 6 h at 37 °C. The concentrations of $^1O_2$ from the IO_4^--H_2O_2 system and the Im-CDs were 0.5 mg·mL^{-1} and 10.0 μM, respectively. Error bars were defined as standard deviation, n = 5.

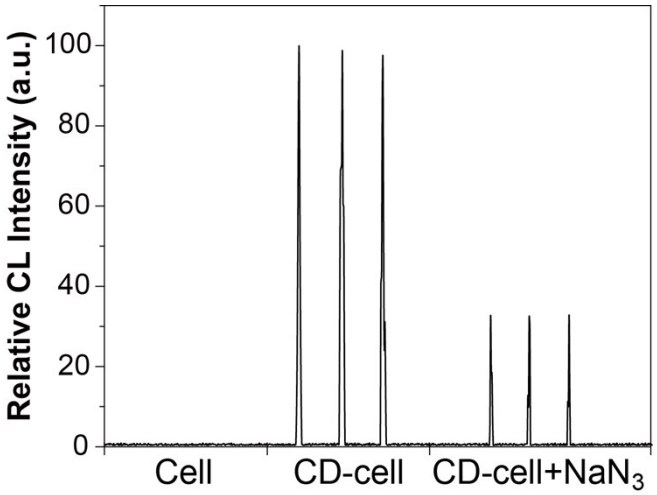

Fig. S20. CL response of the HeLa cells, HeLa cells loaded with the Im-CDs, and cells loaded with the Im-CDs and treated with 1.0 mM NaN_3 towards 70.0 μM $^1O_2$ from the IO_4^--H_2O_2 system. The concentration of the Im-CDs was 0.5 mg·mL^{-1}.

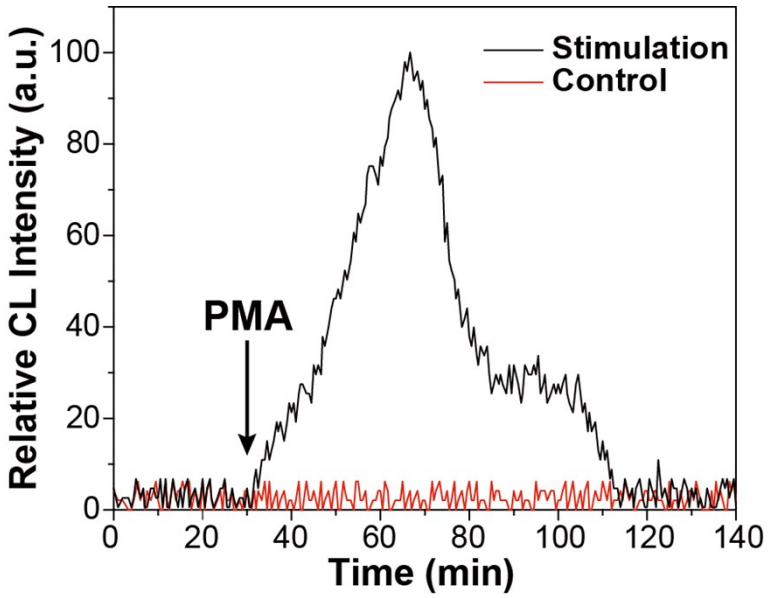

Fig. S21. Real-time monitoring the CL intensities of $^1O_2$ generated by 5.0 μg·mL^{-1} PMA in HeLa cells. The control signal referred to the CL response without PMA. The concentration of the Im-CDs was 0.5 mg·mL^{-1}.

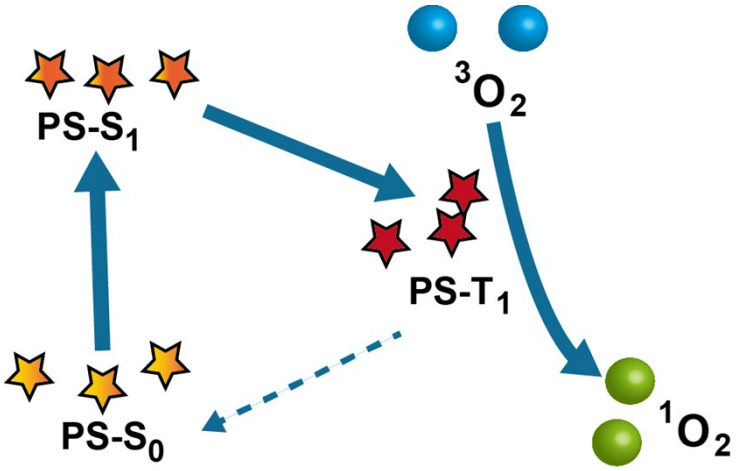

Fig. S22. The schematic representation of $^1O_2$ generation in PDT.

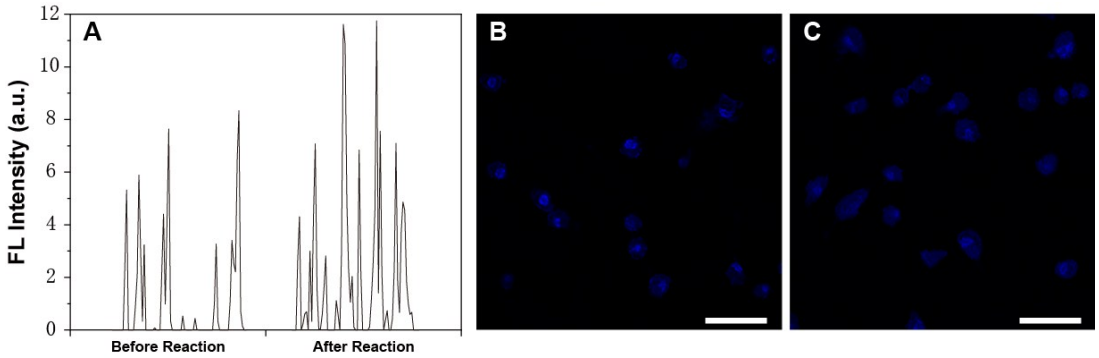

Fig. S23. (A) Intensity profiles of 0.5 mg·mL^{-1} Im-CDs in HeLa cells before and after the photosensitized reaction with 2.0 μg·mL^{-1}L HB. (B) and (C) Confocal fluorescence microscopy images of HeLa cells incubated with 0.5 mg·mL^{-1} Im-CDs before (B) and after (C) monitoring generation dynamics of $^1O_2$ during PDT. Scale bar: 50 μm.

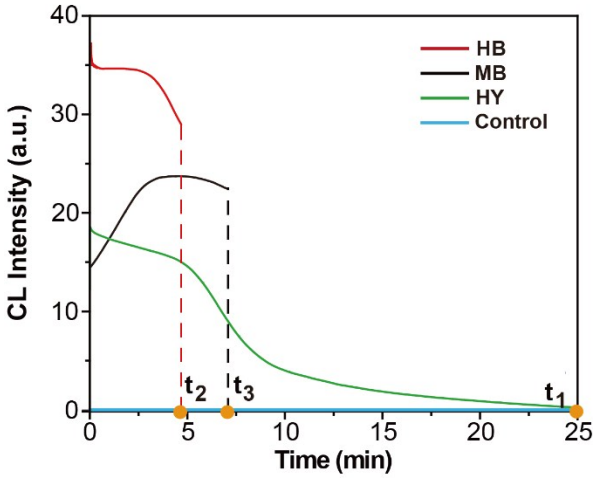

Fig. S24. CL intensities from the photosensitized reaction with HB, MB, and HY in HeLa cells during PDT. Control meant the photosensitized reaction without photosensitizer. The concentration of the Im-CDs was 0.5 mg·mL^{-1}, and the concentrations of HB, MB and HY were 2.0, 6.0 and 1.0 μg·mL^{-1}, respectively. The treatment time (t_1, t_2 and t_3) for HY, HB and MB was 25.0, 4.5 and 6.9 min, respectively.

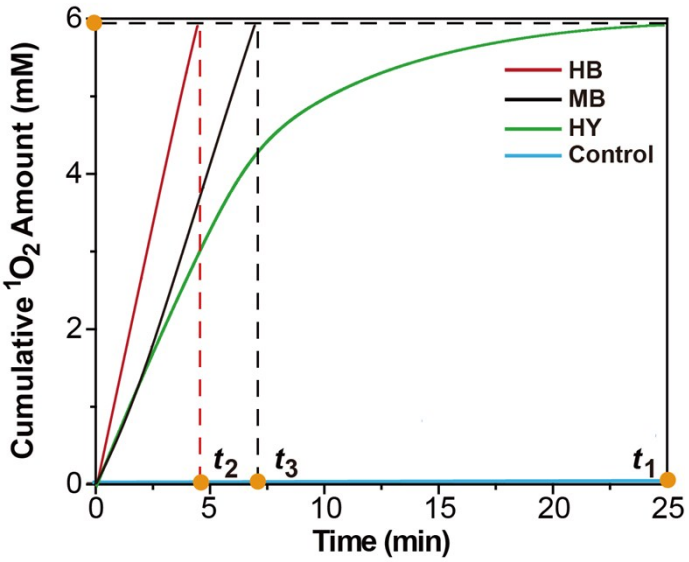

Fig. S25. Cumulative amount of $^1O_2$ generation from the photosensitized reaction with HB, MB, and HY in HeLa cells during PDT. Control in A and B meant the photosensitized reaction without photosensitizer. The concentration of the Im-CDs was 0.5 mg·mL^{-1}, and the concentrations of HB, MB, HY were 2.0, 6.0 and 1.0 μg·mL^{-1}, respectively. The treatment time (t_1, t_2 and t_3) for HY, HB, MB, was 25.0, 4.5 and 6.9 min, respectively.

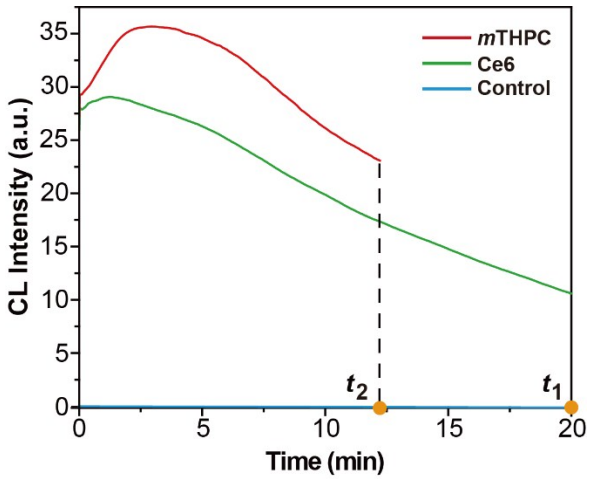

Fig. S26. CL intensities from the photosensitized reaction with *m*THPC and Ce6 in HeLa cells during PDT. Control meant the photosensitized reaction without photosensitizer. The concentrations of Im-CDs, *m*THPC and Ce6 were 0.5 mg·mL^{-1}, 10.0 μg·mL^{-1} and 10.0 μg·mL^{-1}, respectively. The treatment time (t_1 and t_2) for Ce6 and *m*THPC was 20.0 and 12.2 min, respectively.

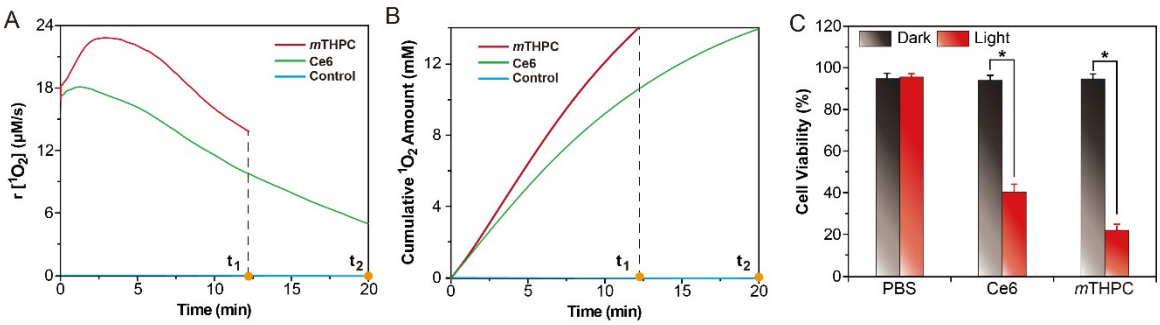

Fig. S27. (A) The generation dynamics of $^1O_2$ from the photosensitized reaction with *m*THPC and Ce6 in HeLa cells during PDT. (B) Cumulative amount of $^1O_2$ generation from the photosensitized reaction with *m*THPC and Ce6 in HeLa cells during PDT. (C) Relative viabilities of HeLa cells after incubation with PBS, *m*THPC and Ce6 with or without 650 nm irradiation (60 mW·cm^{-2}). After irradiation, HeLa cells were incubated under 37 °C for 24 h. Error bars were defined as standard deviation, n = 9. Control meant the photosensitized reaction without photosensitizer. The concentration of the Im-CDs was 0.5 mg·mL^{-1}, and the concentrations of *m*THPC and Ce6 were 10.0 and 10.0 μg·mL^{-1}, respectively. The treatment time (t_1 and t_2) for Ce6 and *m*THPC was 20.0 and 12.2 min, respectively.

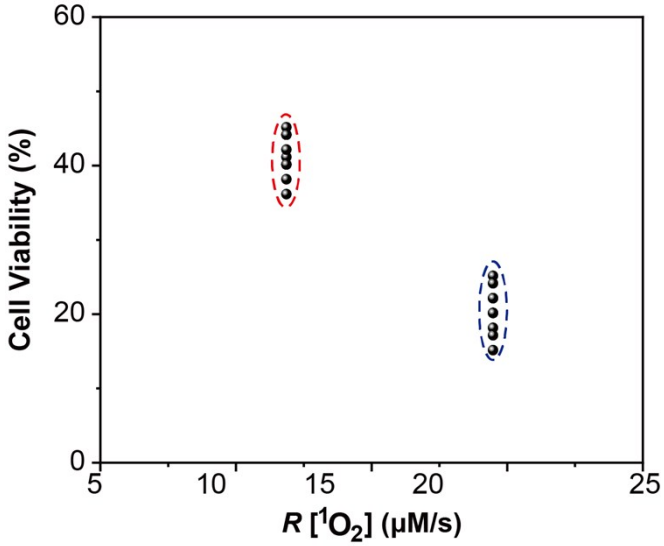

Fig. S28. Relative viabilities of HeLa cells as a function of the average generation dynamics of $^1O_2$ from the photosensitized reaction with *m*THPC (blue circle) and Ce6 (red circle) in HeLa cells under the same cumulative amount of $^1O_2$.

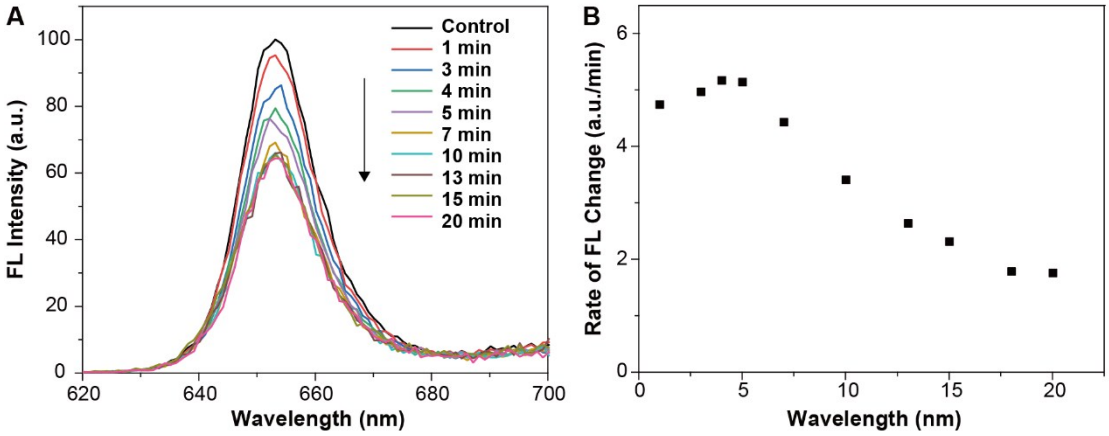

Fig. S29. (A) The fluorescence intensities of *m*THPC after various radiation time. (B) The rate of the change of *m*THPC fluorescence intensities after various radiation time. The concentration of *m*THPC was 10.0 μg·mL^{-1}.

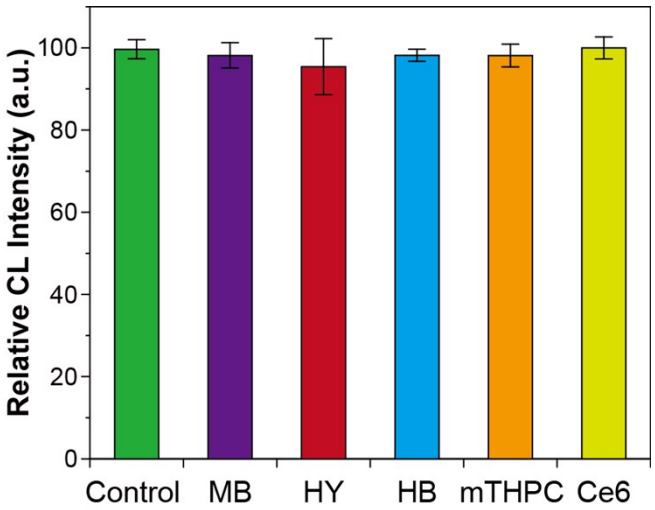

Fig. S30. Effects of the addition of photosensitizers on the CL emissions of the Im-CDs in the presence of $^1O_2$ from the IO_4^--H_2O_2 system. The concentration of the Im-CDs was 0.5 mg·mL^{-1}, and the concentrations of HB, MB, HY, *m*THPC and Ce6 were 2.0, 6.0, 1.0, 10.0 and 10.0 μg·mL^{-1}, respectively. Error bars were defined as standard deviation, n = 5.

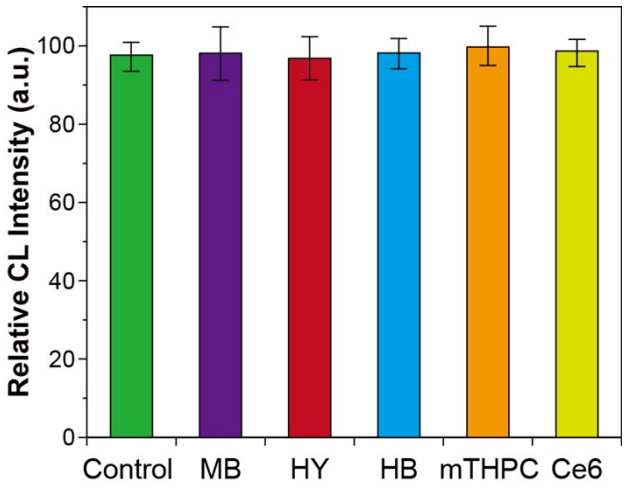

Fig. S31. Effects of the addition of photosensitizers on the CL emissions of the Im-CDs in the presence of $^1O_2$ from the Fe^{2+}-catalyzed TBHP system. The concentration of the Im-CDs was 0.5 mg·mL^{-1}, and the concentrations of, HB, MB, HY, *m*THPC and Ce6 were 2.0, 6.0, 1.0, 10.0 and 10.0 μg·mL^{-1}, respectively. Error bars were defined as standard deviation, n = 5.

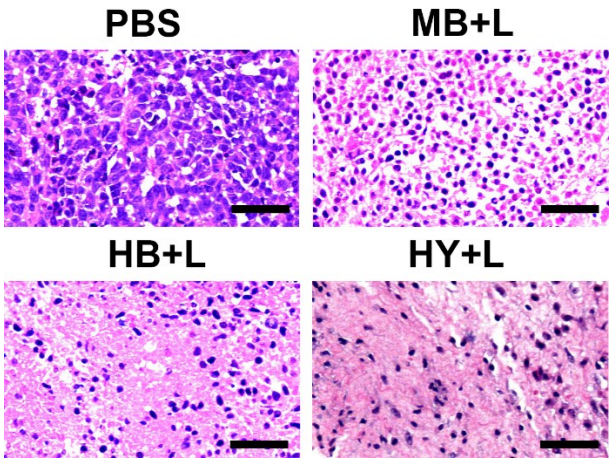

Fig. S32. H&E images of tumors treated with PBS, MB with irradiation, HB with irradiation, HY with irradiation and collected on day 16. Scale bar, 50 μm.

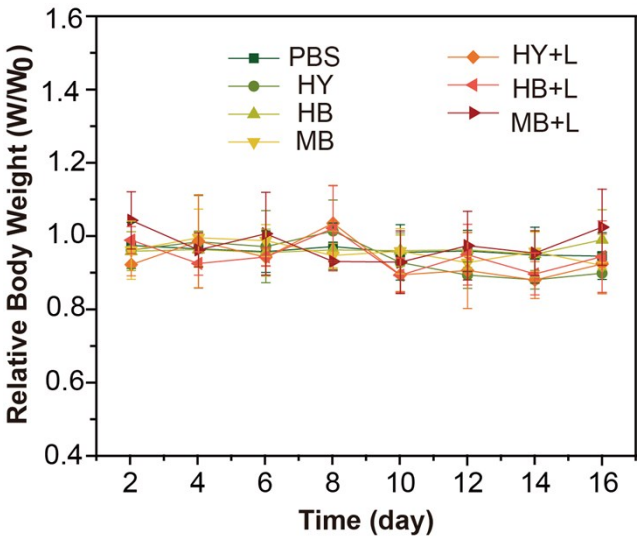

Fig. S33. Body weight curves of mice with the various treatments. Error bars were defined as standard deviation, n = 5 mice per group.

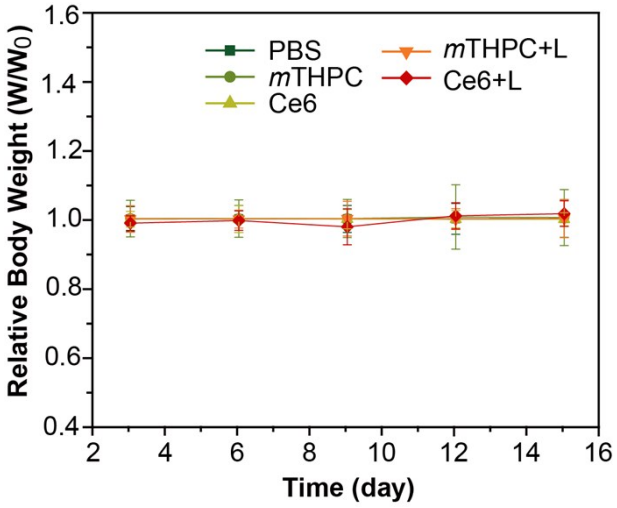

Fig. S34. Body weight curves of mice with the various treatments. Error bars were defined as standard deviation, n = 5 mice per group.

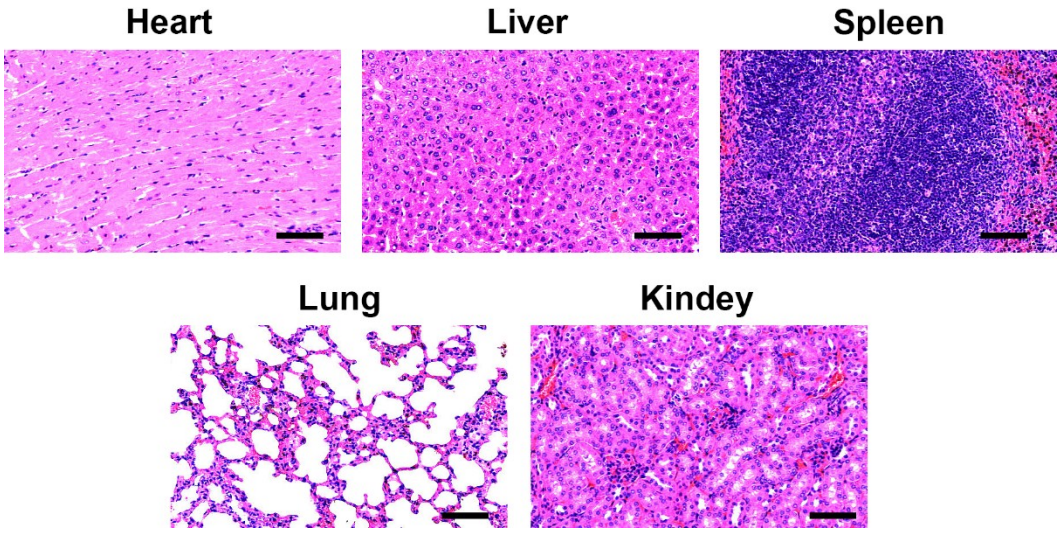

Fig. S35. H&E images of major organs treated with the Im-CDs and collected on day 16. Scale bar, 100 μm. The concentration of the Im-CDs was 0.5 mg·mL^{-1}.

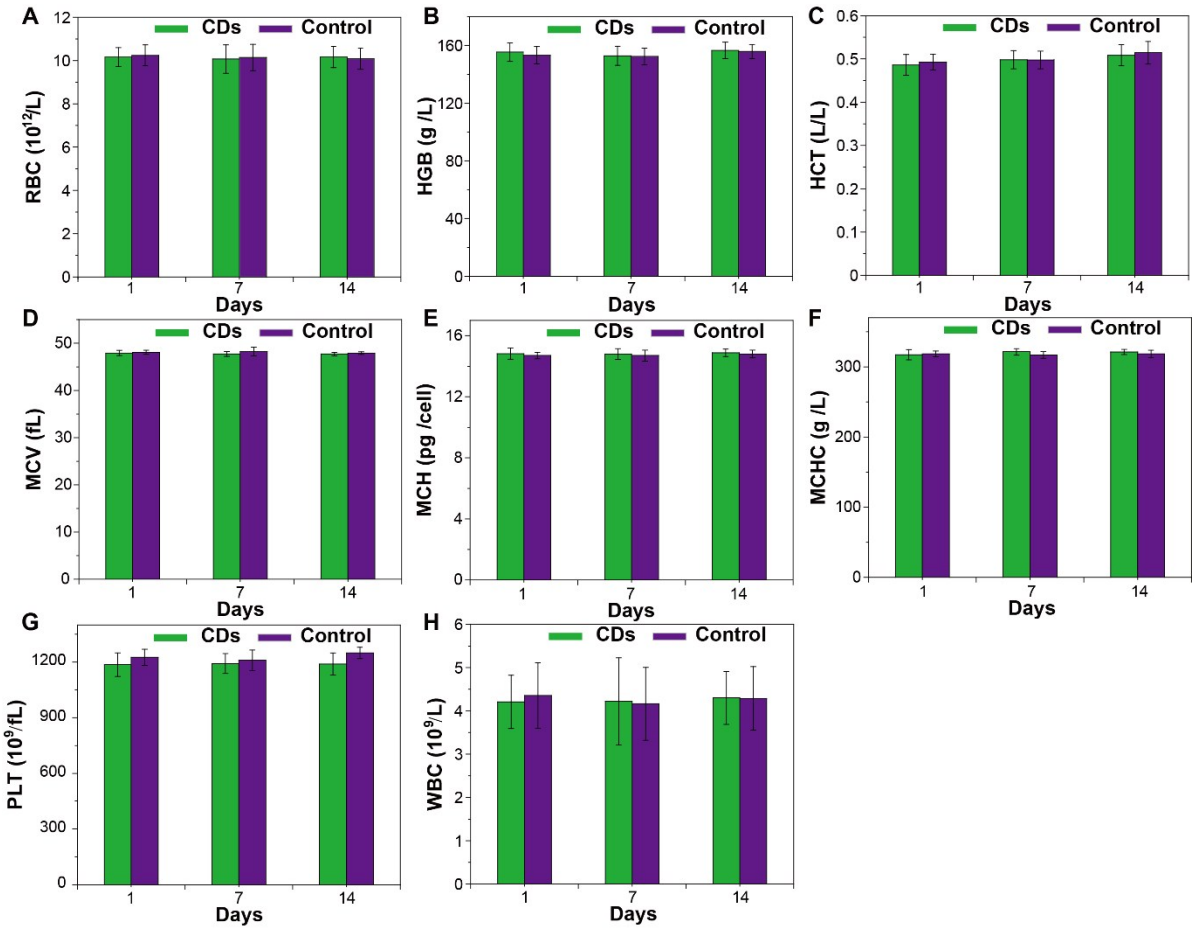

Fig. S36. Blood biochemistry results from mice treated with the Im-CDs including (A) red blood cell (RBC) counts, (B) hemoglobin (HGB), (C) hematocrit (HCT), (D) mean corpuscular volume (MCV), (E) mean corpuscular hemoglobin (MCH), (F) mean corpuscular hemoglobin concentration (MCHC), (G) platelets (PLT) and (H) white blood cell (WBC) counts. Error bars were defined as standard deviation, n = 5.

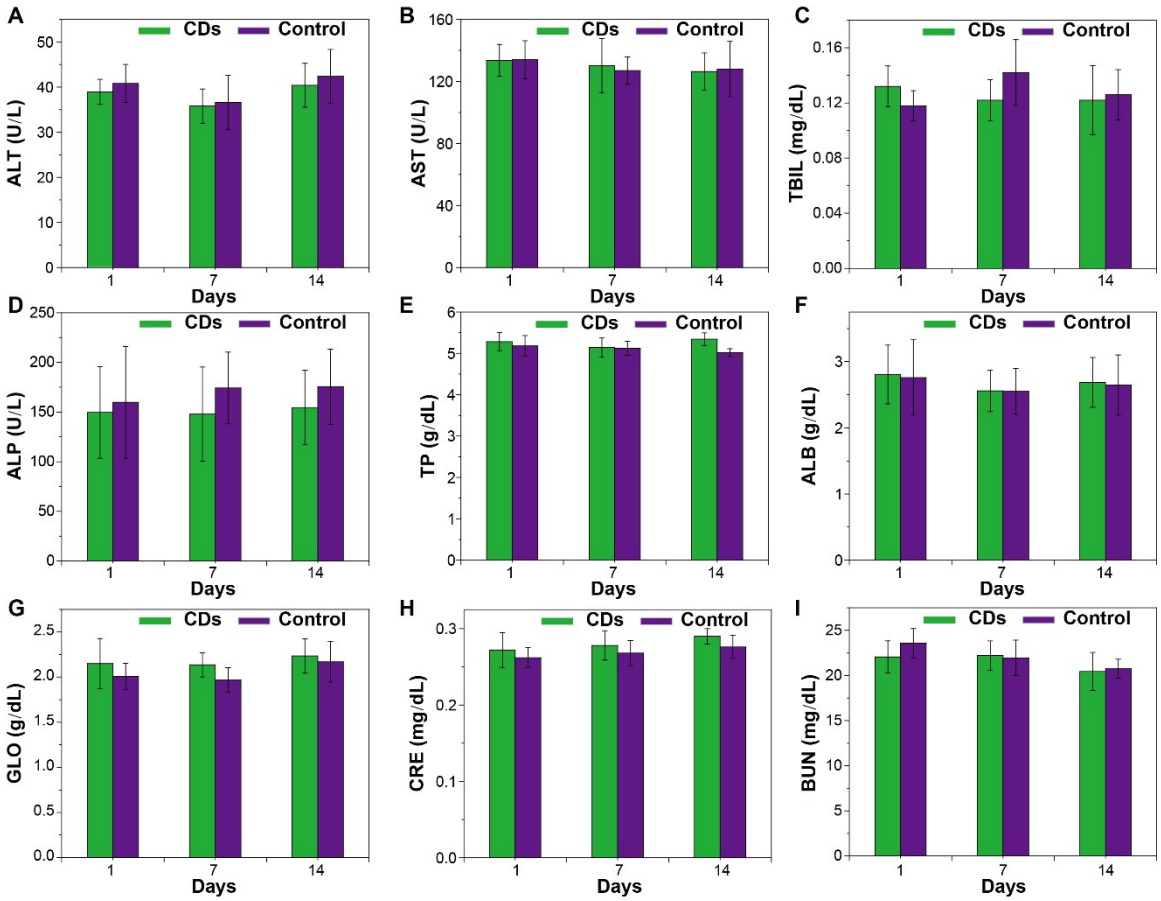

Fig. S37. Serum biochemistry results from mice treated with the Im-CDs including (A) alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST), (C) total bilirubin (TBIL), (D) alkaline phosphatase (ALP), (E) total protein (TP), (F) albumin (ALB), (G) globulin (GLO), (H) creatinine (CRE) and (I) blood urea nitrogen (BUN). Error bars were defined as standard deviation, n = 5.

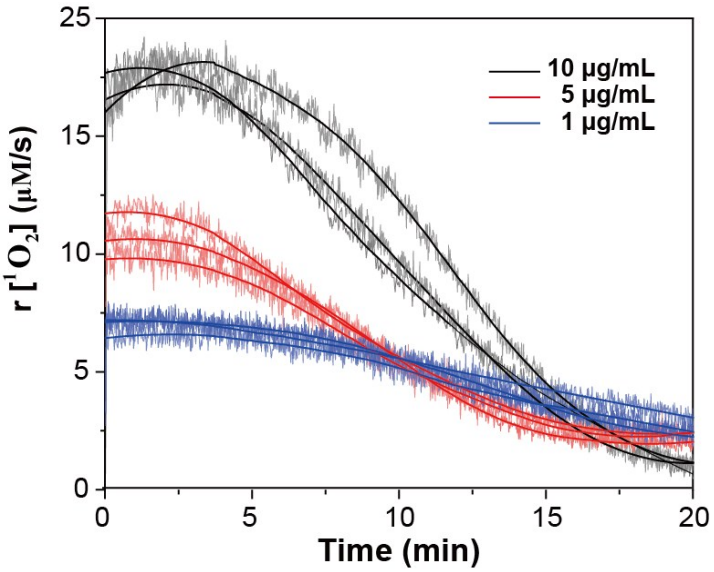

Fig. S38. The various generation dynamics of $^1O_2$ from the photosensitized reaction with various concentrations of *m*THPC in HeLa cells during PDT.

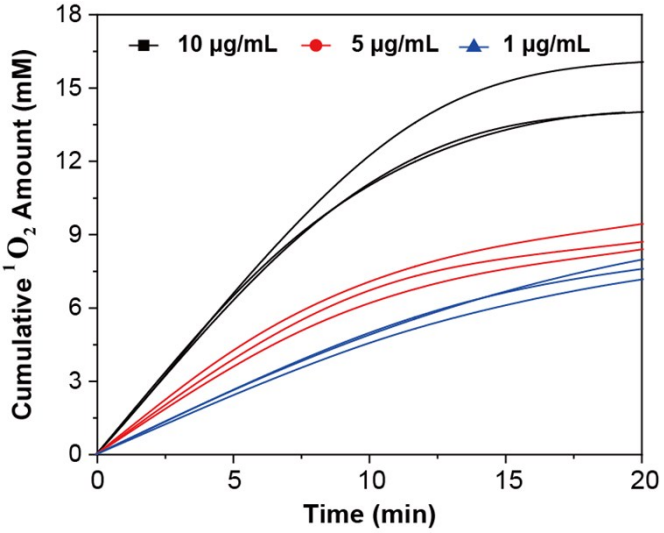

Fig. S39. Cumulative amount of $^1O_2$ from the photosensitized reaction with various concentrations of *m*THPC in HeLa cells during PDT.

Table S1. Fluorescence lifetime of Im-CDs.

Parameter	Value	Proportion
τ_1 (ns)	1.09	27.54 %
τ_2 (ns)	3.53	46.59 %
τ_3 (ns)	10.04	25.87 %
χ^2	1.012	—

Table S2. Peak positions and relative contents of XPS C1s for pristine CDs and $^1O_2$-treated CDs.

Structure	Peak position (eV)	Relative contents (%)	
		pristine CDs	$^1O_2$-treated CDs
C=C	284.7	62.0	47.0
C−O/C−N	286.0	19.3	21.2
C=O	287.9	18.1	31.8

Table S3. Tolerance limits of various coexistent substances on the determination of 10 μM $^1O_2$ from the IO_4^--H_2O_2 system.

Coexistent substances	Content in living cell	Tolerance (mM)
K^+	140.0−152.0 mM	200.0
Mg^{2+}	0.1−1.0 mM	10.0
Al^{3+}	0.5 μM	0.05
Zn^{2+}	<0.05 mM	0.05
Ca^{2+}	0.1 μM	0.025
Ba^{2+}	<2.0 mM	2.0
Fe^{3+}	<5.0 μM	0.01
Cr^{3+}	<0.4 μM	0.001
Pb^{2+}	<0.1 μM	0.0025
Ni^{2+}	<6.0 μM	0.02
Mn^{2+}	<5.0 μM	0.005
Cu^{2+}	10^{-18} M	0.002
NO_3^-	1.0−10.0 μM	40.0
S^{2-}	0.01−0.10 μM	0.01
I^-	<0.25 μM	0.05
Glutathione	1.0−10.0 mM	10.0
Cysteine	0.8±4.0 μM	0.02
Glycine	< 100.0 μM	1.0
Lysine	<20.0 μM	5.0
Ascorbic acid	<5.0 μM	0.01

An error of ±5.0% in the relative CL emissions are considered to be tolerable.