Supporting Information

Core-shell structured carbon dots with up-conversion fluorescence and photo-triggered nitric oxide-releasing property

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1. Experimental

1.1 Materials. All the chemicals used in the processes were commercially available without further purification. Levofloxacin, triton X-100, 5,5-dimethyl-1-pyrroline N-oxide (DMPO), 2,2,6,6-tetramethylpiperidine

(TEMP), and 2-phenyl-4,4,5,5,-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) were purchased from Shanghai Aladdin Industrial Corporation. L-Arginine (Arg) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Phosphate buffer saline (PBS), Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, trypsin, penicillin/streptomycin, and fetal bovine serum (FBS) were purchased from Gibco (Shanghai, China). Regenerated cellulose dialysis bags were purchased from Shanghai Yuanye Biotechnology Co., Ltd. Calcein-AM/PI and commercial probes of the lysosome, mitochondria, endoplasmic reticulum, ROS, and RNS were purchased from Beyotime Biotechnology (Shanghai, China). CCK-8 and AO staining kits were purchased from Beijing Soleibao Technology Co., Ltd. The water used in the experiment is ultrapure, obtained from Kertone Water Treatment Co., Ltd.

1.2 Instrumentation and Characterization. The morphology of Arg-CDs was observed by JEM-2100 transmission electron microscope (TEM, JEOL, Tokyo, Japan). The high-resolution transmission electron microscopy (HRTEM) result was obtained by JEOL-F200 transmission electron microscopy (Tokyo, Japan) at 200 kV. Fourier transform infrared (FTIR) spectrum was obtained using a Nexus 870 infrared spectrum spectrometer. X-ray photoelectron spectroscopy (XPS) was measured using an ESCALAB250 system with Al Kα radiation (1486.6 eV). Raman

spectrum was recorded using a laser confocal micro-Raman spectroscopy (InVia-Reflex, Renishaw, London, Britain). Powder X-ray diffraction (XRD) was obtained on a PRO diffractometer with Cu Ka radiation on X'Pert. The ultraviolet-visible (UV-Vis) spectra were measured using a UV-1800PC spectrophotometer (Shanghai meipuda instrument co., LTD, China). The photoluminescence (PL) emission spectroscopy was measured by Hitachi F-7000 fluorescence spectrophotometer. Absolute quantum yields (QY) were obtained using a HORIBA FLSP920 system in the calibration sphere. The up-conversion fluorescence emission spectrum was measured by using a multi-photon test system (Astrella-1k-SUP, TOPAS-Prime) and femtosecond pulsed laser (fs-laser) (680-1080 nm) as the light source. The Zeta potential results were obtained with a Zeta size analyzer (Nano ZS90, Malvern Instruments Ltd.). The cytotoxicity data were acquired by a microplate reader (CMax Plus, Shanghai Meigu Molecular Devices Co., Ltd., Shanghai). Fluorescence microscopy images were recorded with Olympus IX-51 inverted fluorescence microscope (Olympus, Tokyo, Japan). Confocal laser scanning microscopy (CLSM) was recorded with a laser scanning microscope (Leica SP8 DIVE).

1.3 Synthesis of carbon dots (Arg-CDs). The Arg-CDs were prepared by the hydrothermal decomposition of L-Arginine and Levofloxacin. Briefly, the mixture of L-Arginine (0.5 g) and Levofloxacin (0.5 g) was dissolved in 20 mL deionized water and then transferred into 50 mL poly

(tetrafluoroethylene)-lined autoclaves. After heating at 180 ℃ for 4 h, the solution was allowed to cool to room temperature naturally. The yellow solution was filtered (0.22 μm) and dialyzed in regenerated cellulose dialysis tubing with MWCO 500-1000 for 120 h to remove unreacted L-Arginine and Levofloxacin. Finally, the dialyzed solution was vacuum-dried at -60 ℃to collect a powder.

1.4 Cytotoxicity assay. Typical CCK-8 assays were performed to detect cell viability. First, HepG2 cells were separately inoculated into 96-well cell culture plates at a density of 8000 cells per well and incubated at 37 ℃for 24 h. Then, various concentrations of Arg-CDs (0-300 μg/mL) were added to each well for 24 h to measure the viability of the cells. 10 μL of CCK-8 was added to each well and left for 4 h in the dark. Finally, the optical density (OD) was recorded at 450 nm using an enzyme marker (CMAX PLUS, SpectraMax® Absorbance Reader). The experiments were repeated three times.

1.5 Hemolysis Assay. Red blood cells (RBCs) were obtained from the mouse blood. The 500 μL fresh mouse blood was discarded from the supernatant by centrifugation (2000 rpm, 5 min) and resuspended the sediment into 1 mL saline. Repeat the above operation 3-5 times until the supernatant is colorless. The obtained red blood cells were suspended in 1 mL saline for use. Take 50 μL of the above red blood cell suspension and add different concentrations of Arg-CDs in PBS. At the same time, the

negative control group (physiological saline) and positive control group (1% Triton X-100 solution) were set up. After incubation at 37 °C for 2 h, the system was centrifuged at 2000 rpm for 5 min, the supernatant was transferred into a 96-well plate, and their absorbance at 450 nm was measured with a microplate reader.

1.6 Cell culture and subcellular localization. HepG2, SY5Y, and HUVEC cells were purchased from the Cell Culture Center of Peking Union Medical College. The cells were cultured in a DMEM medium containing 1% penicillin/streptomycin and 10% FBS in a 37°C, 5% CO_2 incubator.

The cells were seeded in a confocal laser dish at 37 °C overnight, and then the Arg-CDs were added and co-incubated for 6 h. After washing with PBS 3 times, the cells were incubated with commercial organelle probes containing Lyso-Tracker Green, Mito-Tracker Green, and ER-Tracker Green for 30 min. The fluorescence images were obtained on a widefield fluorescence microscope with a 63×oil lens.

1.7 Detection of the ROS and NO. The intracellular ROS generation was detected by using the ROS probe DCFH-DA. The intracellular NO generation was detected by using NO probe DAF-FM DA. Briefly, the HepG2 cells were planted in 12-well plates at 37 °C overnight, and then the Arg-CDs were added and co-incubated for 6 h. After 12 min light irradiation (400-500 nm, 100 mW cm^{-2}), the cells were stained with DCFH-

DA and DAF-FM DA for 20 min. After washing with PBS 3 times, the cells were observed in an inverted fluorescence microscope at 488 nm or 561 nm excitation wavelength. Griess Reagent from Beyotime Biotechnology (Shanghai, China) was adopted for NO quantitative determination.

1.8 Live/Dead Cell Staining Assay. The calcein-AM and propidium iodide (PI) were used to evaluate the live and dead cells. HepG2 cells were seeded in a six-well plate with a density of 1.0×10^5 cells per well and incubated with Arg-CDs at the same concentration (200 μg mL^{-1}). As for laser irradiation groups, all treatment was similar to that above and irradiated by an LED light for 12 min. After all the treatment, HepG2 cells were stained with calcein-AM (2 μM) and PI (4 μM) and then analyzed by fluorescence microscopy.

1.9 AO staining test. The operation was the same as the calcein-AM/PI test. The cells were stained with AO for 15 min and imaged in a widefield fluorescence microscope with a 63ß oil lens.

Growth Inhibition Effect of Arg-CDs on MCs. Briefly, 45 μL 1% hot agarose solution was added into each well of 96-well plates beforehand. After cooling the agarose solution, 5×10^4 cells were planted into the well and incubated at 37 °C overnight to form 3D MCs. A fluorescence microscope observed the cytotoxicity of Arg-CDs to HepG2 MCs. Then, the Arg-CDs were added to treat 3D MCs when the disaster reached 200

μm and accepted 12 min light irradiation (400-500 nm, 100 mWcm^{-2}) daily. The daily morphology of 3D MCs was recorded. The above experiments were repeated three times.

1.10 Statistical analysis. Statistical analysis was performed using two-tailed Student's t-tests, and all data were given as mean ± standard deviation (SD).

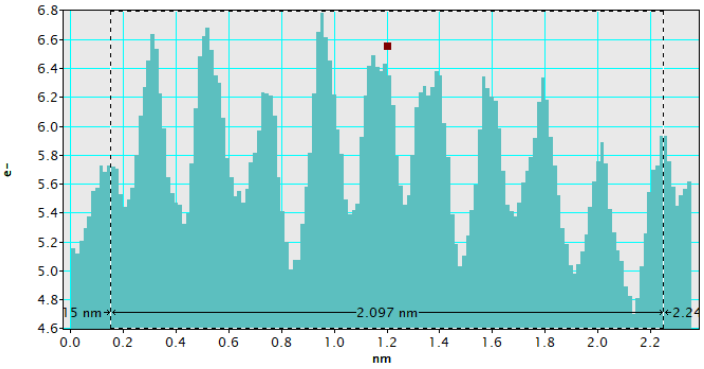

Fig. S1. Line profile of diffraction fringes for the Arg-CDs.

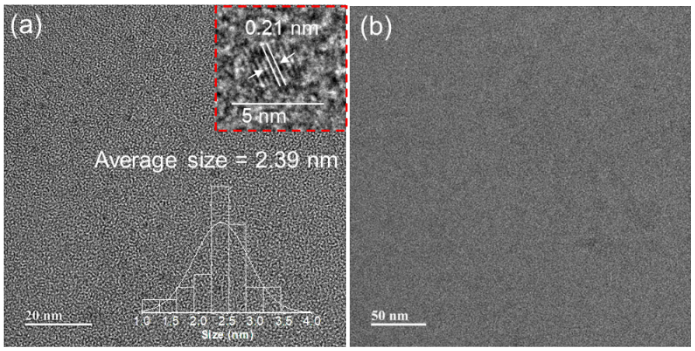

Fig. S2 (a) TEM image of single precursor levofloxacin-derived (named as N-CDs), the inset shows the high-resolution TEM image of an individual N-CD and size distribution of N-CDs. (b) TEM image of the product synthesized from a single precursor of arginine.

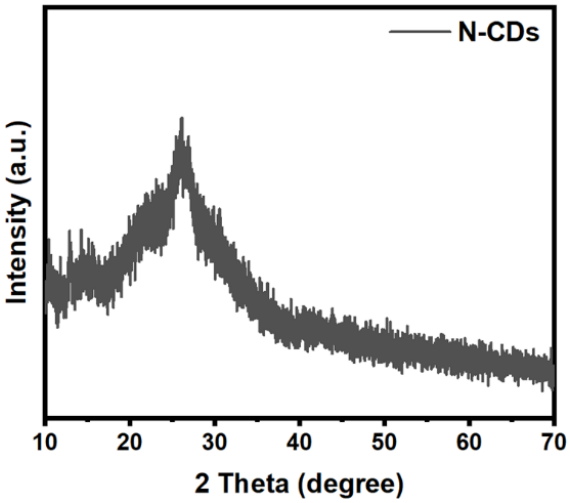

Fig. S3 XRD pattern of the levofloxacin-derived N-CDs.

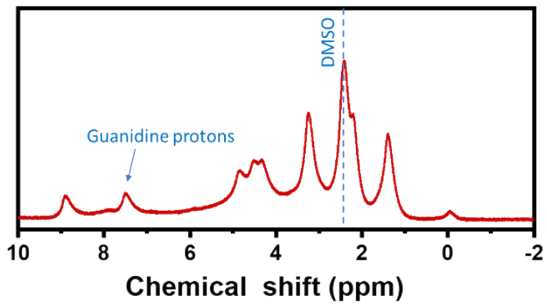

Fig. S4 ^{1}H NMR spectrum of the Arg-CDs (10 mg mL^{-1} in DMSO-D6).

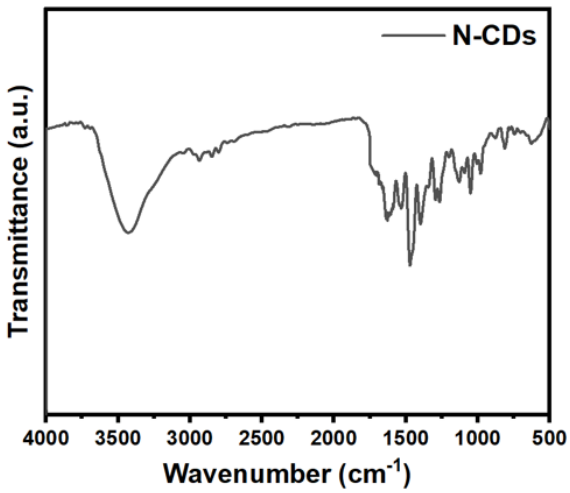

Fig. S5 FT-IR spectra of the levofloxacin-derived N-CDs.

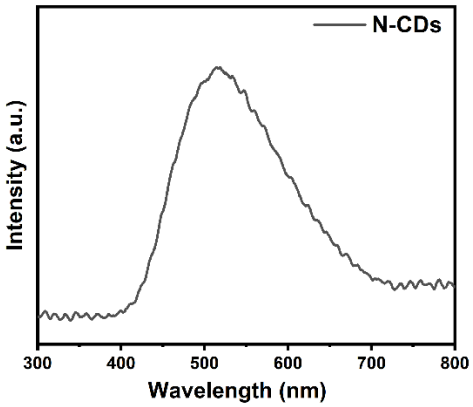

Fig. S6 PL spectra of N-CDs under excitation of 740 nm laser.

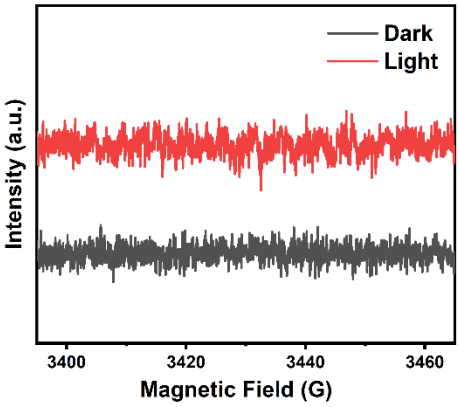

Fig. S7 EPR spectrums of DMPO trapping the •OH produced by Arg-CDs under LED irradiation (400-500 nm, 100 mW cm^{-2}, 2 min).

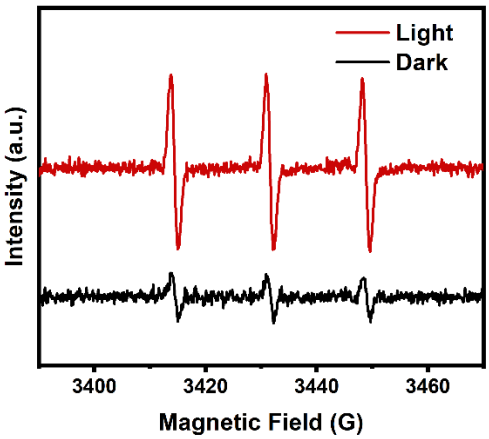

Fig. S8 EPR spectra of N-CDs that TEMP trapping the $^1O_2$.

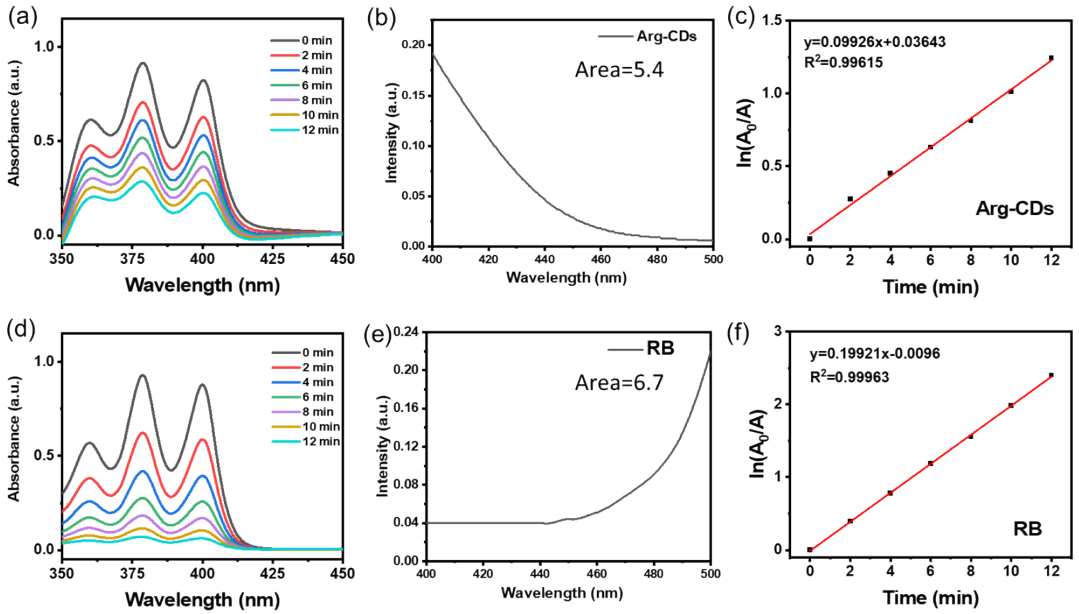

Fig. S9 Calculations of the $^1O_2$ QY of Arg-CDs in H_2O: (a) UV-vis absorption spectra of the ABDA $^1O_2$ trap in the presence of Arg-CDs at different irradiation times. (b) The absorption spectrum of Arg-CDs (c) Decomposition rate constants of ABDA by Arg-CDs. (d) UV-vis absorption spectra of ABDA photobleaching by RB at different irradiation times. (e) The absorption spectrum of RB. (f) Decomposition rate constants of ABDA by RB.

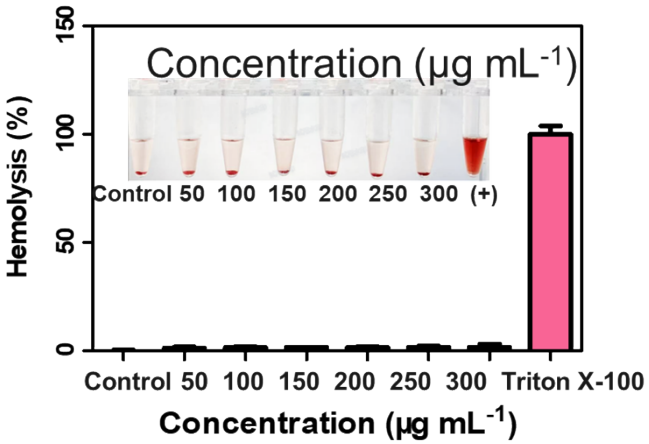

Fig. S10 Photographs and hematological evaluation of RBCs treated with

different Arg-CDs concentrations, respectively (n = 6).

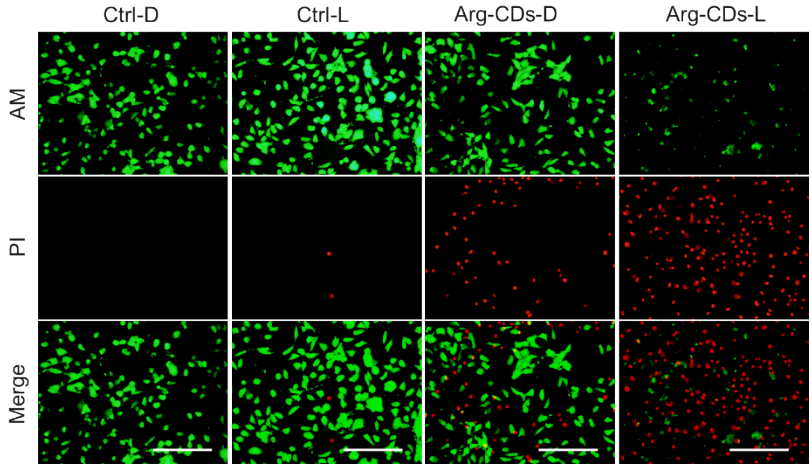

Fig. S11 Microscopy images of the HepG2 cells labeled with Calcein-AM and PI after incubation with Arg-CDs under dark or LED light irradiation (400-500 nm, 100 mW cm^{-2}) for 12 min, scar bar = 100 μm.

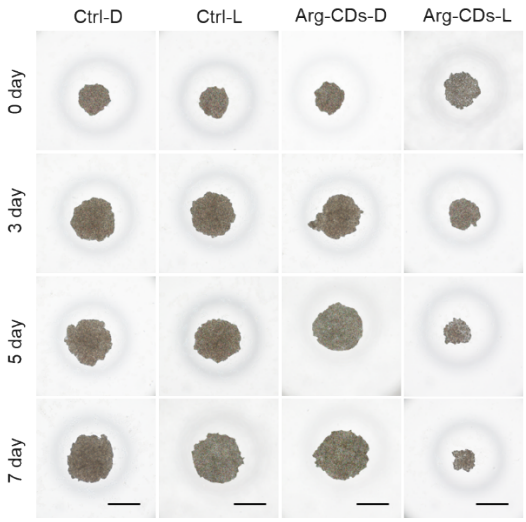

Fig. S12 Fluorescence microscopy bright-field images of HepG2 3D MCs co-incubated with PBS and Arg-CDs (200 μg mL^{-1}) under dark or light irradiation (10 min/day), respectively. These images were captured every day. Scale bar = 200 μm.