Electronic Supplementary Information (ESI)

One-pot preparation of long-wavelength carbon dots with targeting ability toward different organelles

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1. Experimental materials, reagents and instruments

Materials and Reagents: All chemical agents were acquired commercially and utilized without subsequent purification processing. p-phenylenediamine from Macklin (Shanghai, China). Anhydrous ethanol and other reagents were supplied by Aladdin Reagent Co. Ltd (Shanghai, China). Human Liver cancer HepG2 cells, HL-7702 cells were obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Fetal bovine serum (FBS) and 1640 medium were supplied by Invitrogen Corp. A Cell Counting Kit-8 was purchased from Dojindo Molecular Technologies, Inc. (Rockville, MD).

Instrumentations: Fourier transform infrared spectroscopy (FT-IR) was performed on an FTIR Nicolet 380 spectrometer. Transmission electron microscopy (TEM) observation was performed on a Tecnai G2 F20 S-TWIN instrument. X-ray photoelectron spectroscopy (XPS) of the CDs was performed on an Axis Ultra DLD spectrograph with Al/Kα as the source. CLSM observation was performed using a confocal laser scanning microscope (CLSM, ZEISS LSM 780).

2. Synthesis of the carbon dots

Carbon dots were synthesized in anhydrous ethanol by solvothermal synthesis method. First, p-phenylenediamine (0.6 g) was mixed with 60 mL anhydrous ethanol and ultrasound was performed for 10 min, and then the mixture was transferred into a poly(tetrafluoroethylene)-lined autoclave (100 ml), and heated at constant temperature of 180 ºC for 12 h. After the reaction, the autoclave was cooled to room temperature. The obtained dark brown solution was purified by column chromatography (dichloromethane / methanol = 50:1) to obtain **Y-CDs** and **R-CDs**. Two CDs were dissolved in anhydrous ethanol and stored in a refrigerator at 4 ºC.

3. Optical properties

UV vis/fluorescence: **Y-CDs** and **R-CDs** with concentration of 0.02 mg/mL were employed for UV vis/Fluorescence spectra measurement at room temperature. Absolute PLQY: ethanol solution or aqueous solution of **Y-CDs** and **R-CDs** were diluted until the absorbance is 0.10 at the corresponding maximum absorption peaks. Then the absolute PLQY was acquired at the corresponding maximum excitation wavelength at room temperature with a blank quartz cell as the reference.

4. Cell culture

HeLa (human cervical cancer cells) and HepG2 (human liver cancer cells) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin, 10 kU/mL). 7702 (human normal liver cells) and B16 (mouse melanoma cells) were cultured in 1640 supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin, 10 kU/mL). All cells were cultured at 37 ºC with 5% CO_2 humidification atmosphere.

5. Cytotoxicity assay

The cytotoxicity of **R-CDs** and **Y-CDs** were investigated in 7702 and HepG2 cells. Briefly, 7702 and HepG2 cells were cultured at 1×10^4 per well in a 96-well plate for 24 h, then the old medium was replaced by fresh culture medium containing CDs of various concentration (5, 10, 20, 40, 60 μg/mL). The cells were further incubated for 24 h. The medium was removed and 100 μL fresh medium containing 10 μL CCK-8 was added and incubated for another 1-4 h. Finally, the absorbance at 450 nm was recorded by bio-RAD 680. The relative survival rate of cells was calculated as follows:

$$\text{Cell survival rate (\%)} = (OD_{450}\text{-sample}/OD_{450}\text{-control}) \times 100\%.$$

The untreated cell controls were taken as 100% cell viability.

6. Co-localization of CDs with the commercial probe

Firstly, HepG2 cells were stained in DMEM media with **Y-CDs**/**R-CDs** at a concentration of 20 μg/mL for 20 minutes, and then washed by PBS (10 mM, pH 7.4) three times. After that, the cells were incubated with commercial organelle probes (500 nM Lyso-Tracker Green/1 μM ER-Tracker Green) for 30 min. CLSM observation was performed using a confocal laser scanning microscope (Green fluorescence channel, $\lambda_{ex}/\lambda_{em}$ = 405/500-540 nm, Red fluorescence channel, $\lambda_{ex}/\lambda_{em}$ = 488/600-650 nm).

7. Photostability experiment

HepG2 cells were incubated with **Y-CDs**/**R-CDs** (20 μg/mL, 20 min), Lyso-Tracker Green (500 nM, 30 min) and ER Traker Green (1 μM, 30 min), respectively. The fluorescence image of CDs

($\lambda_{ex}/\lambda_{em}$ = 488/600-650 nm) and commercial organelle probes ($\lambda_{ex}/\lambda_{em}$ = 405/500-540 nm) were observed under a confocal microscope after 100 s of continuous laser irradiation.

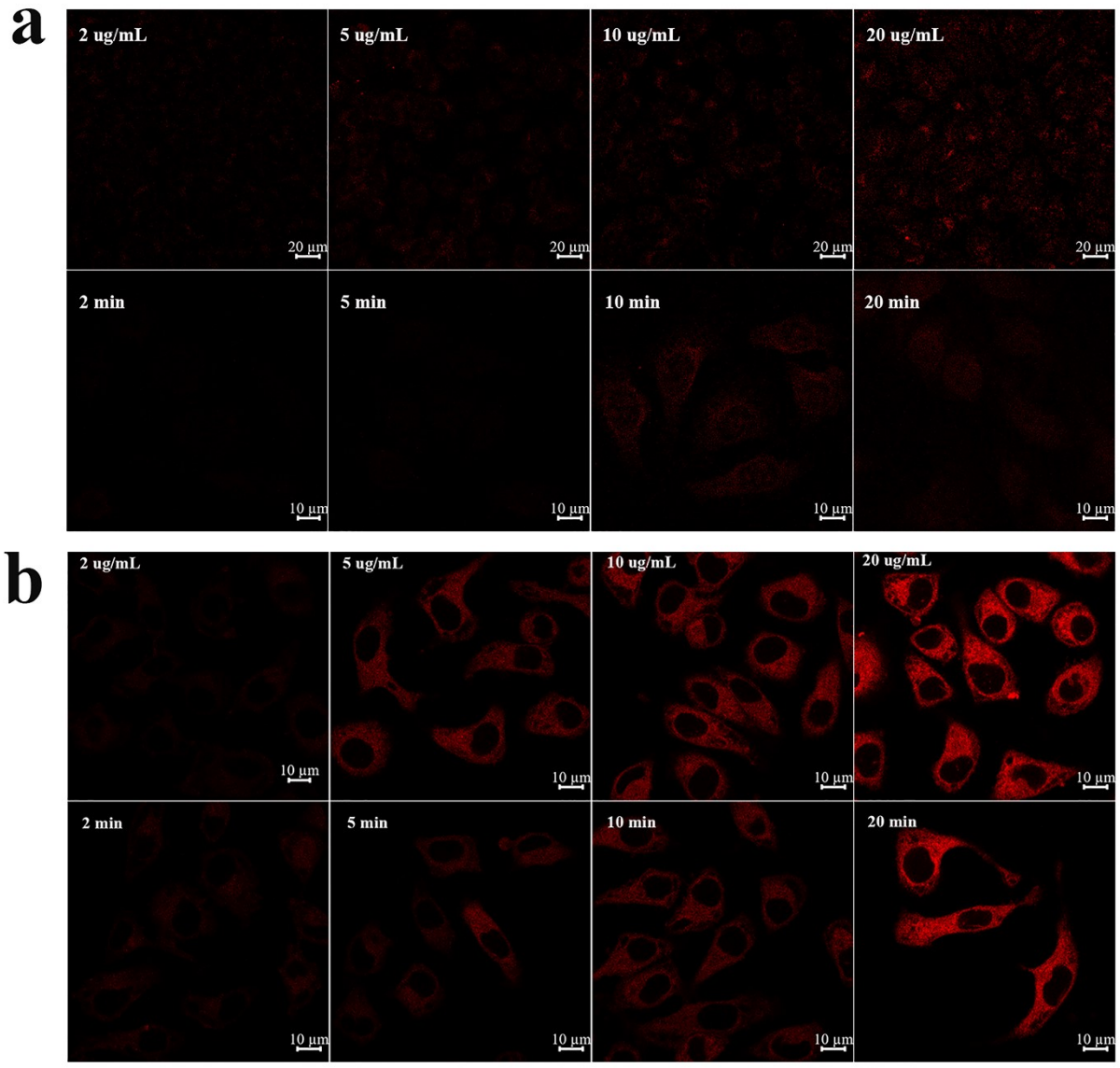

Fig. S1 Fluorescence images of HepG2 cells incubated with **Y-CDs** (a) and **R-CDs** (b) with different concentrations and times ($\lambda_{ex}/\lambda_{em}$ = 488/600-650 nm).

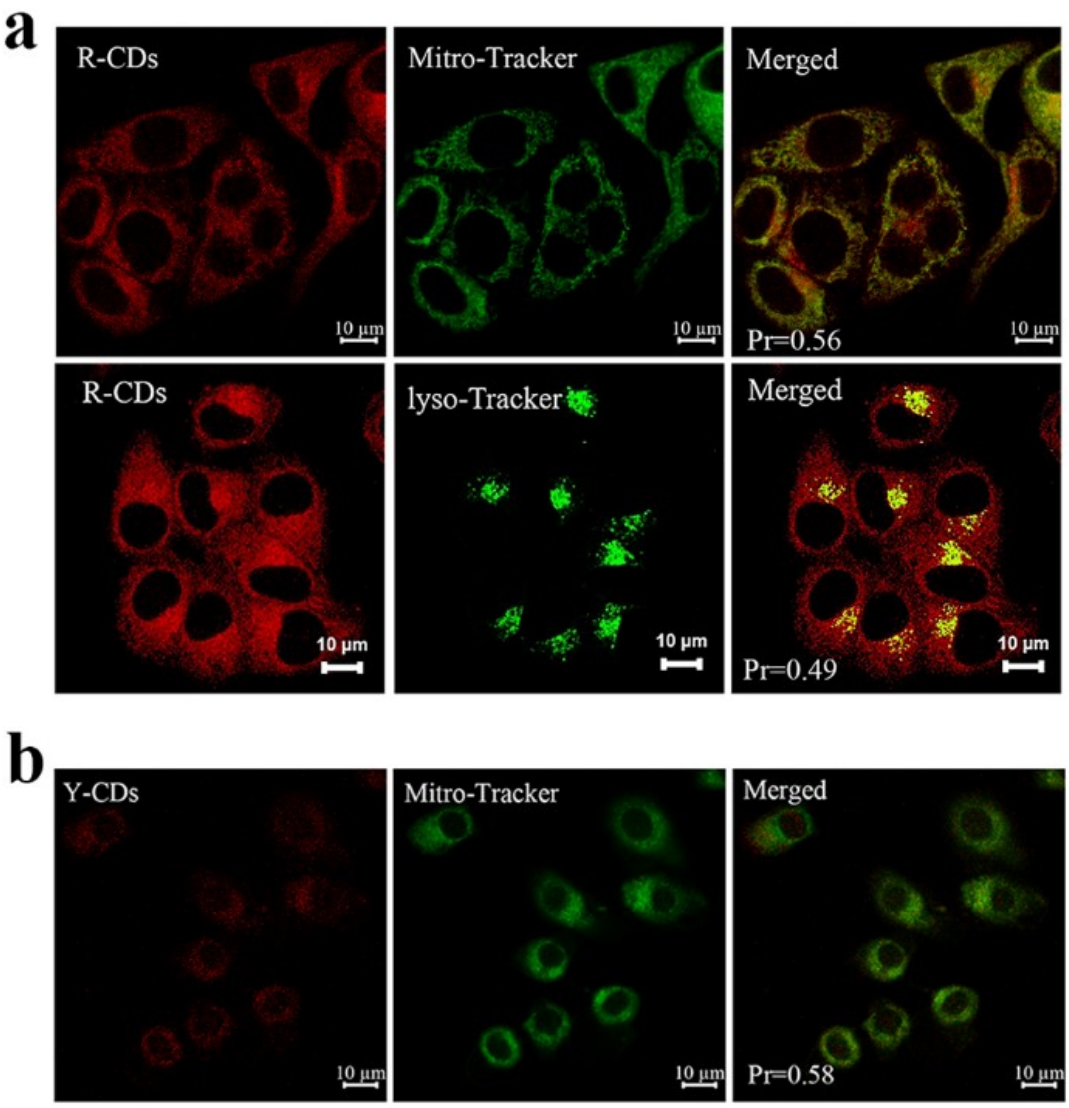

Fig. S2 (a) Co-localization images of HepG2 cells incubated with **R-CDs** and co-stained with Mitro-Tracker and Lyso-Tracker respectively. (b) Co-localization images of HepG2 cells incubated with **Y-CDs** and co-stained with Mitro-Tracker.

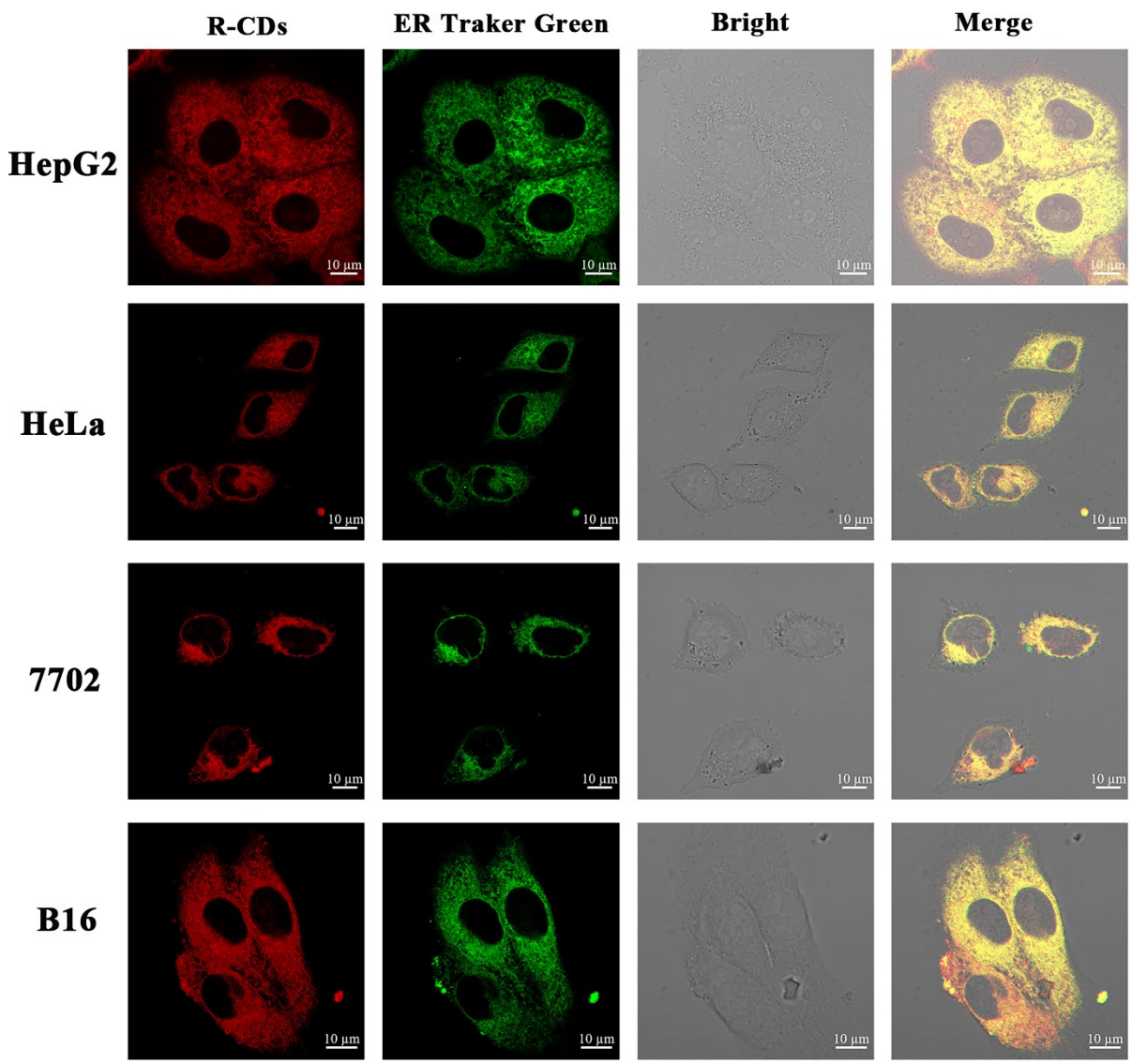

Fig. S3 Co-localization images of various types of cells stained with **R-CDs** (20 μg/mL) for 20 min and co-stained with ER-Tracker Green (1 μM) for 30 min.

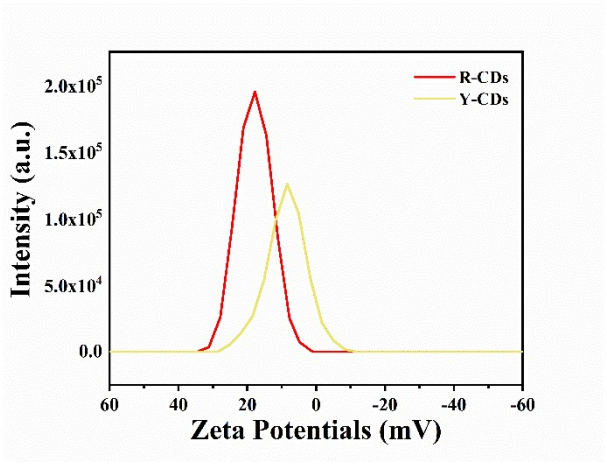

Fig. S4 Zeta potentials of **Y-CDs** and **R-CDs**.

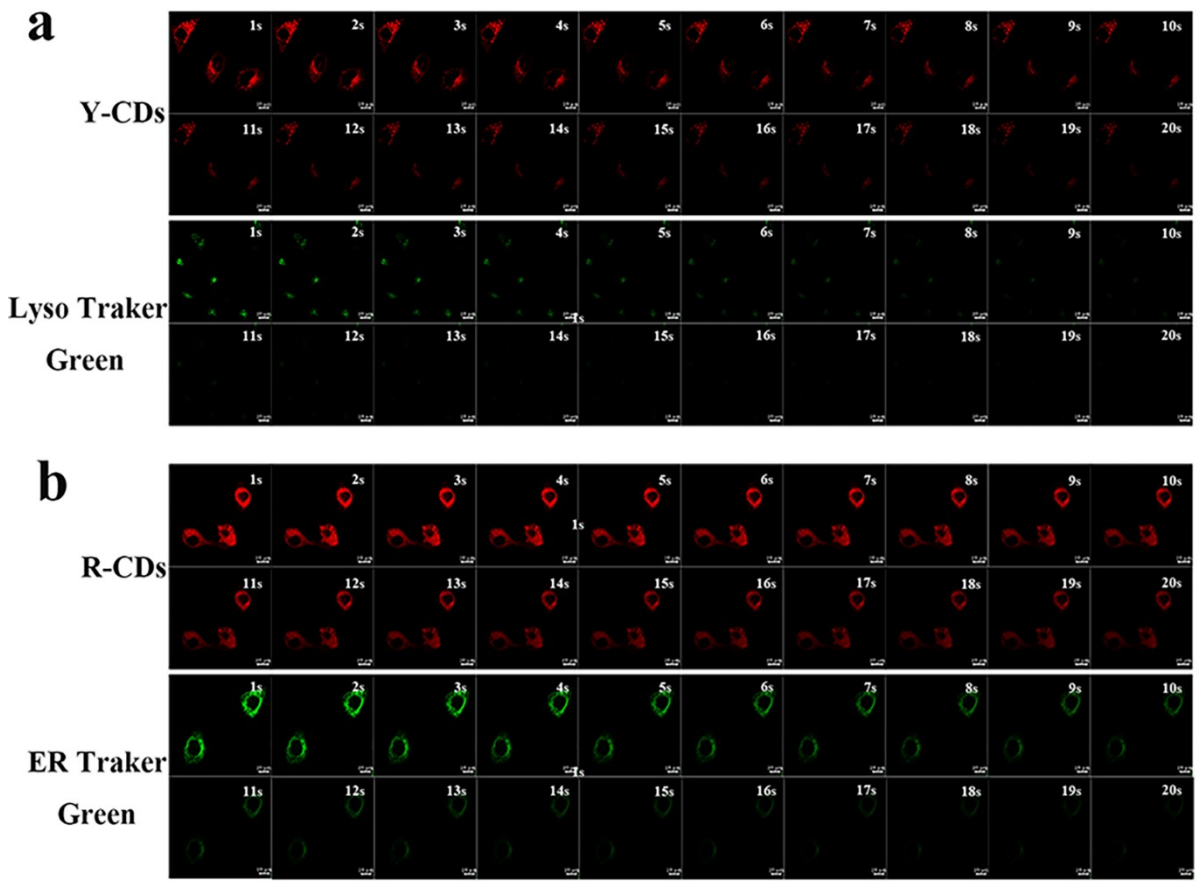

Fig. S5 Fluorescent images of HepG2 cells stained by Y-CDs / R-CDs (Red) and commercial dyes (green) with increasing time of scans.

Table S1 The C, N and O contents of the **Y-CDs** and **R-CDs**.

	C	N	O
Y-CDs	84.19%	1.72%	14.09%
R-CDs	90.45%	2.34%	7.12%

Table S2 The relative area ratio of the covalent bond of C, N and O content of the **Y-CDs** and **R-CDs**.

	Y-CDs	**R-CDs**
C-C/C=C	82.07%	77.95%
C-O/C-N	17.93%	12.47%
C=O		9.58%
C=N		14.71%
N-H	93.44%	75.99%
C-N	6.54%	9.29%
C=O	75.72%	83.365
C-O	24.28%	16.64%