

## **Supplemental Information**

### **In Vivo Monitoring Therapeutic Response by Self-Reporting Upconverting Covalent Organic Framework Nanoplatform**

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## EXPERIMENTAL SECTION

**Materials and instruments.** All chemicals were obtained from commercial suppliers and used without purification. Ultrapure water was utilized from a Milli-Q reference system (Millipore). TEM images were recorded using JEM-2100F (JEOL). DLS measurements were made on a Zetasizer 3000Hs (Malvern). The UV-vis absorption spectra were collected on a UV-2450 UV-vis spectrometer (Shimadzu). Fluoromax-4 spectrofluorometer (HORIBA JobinYvon) was used to collect the UCL signal with the slits set at 5.0 nm. Cell viability was monitored by measuring the absorption at 490 nm using a Synergy 2 Multi-Mode Microplate Reader (Bio-Tek, Winooski, VT). Fluorescence bioimages of live cells were picked up through an inverted Nikon A1Si laser scanning confocal microscope (Nikon). UCL images of live mice were collected by an IVIS Lumina II in vivo imaging system (Caliper LifeScience, USA).

**Synthesis of OA-UCNPs.**  $Y(CH_3COO)_3$  (0.778 mmol),  $Yb(CH_3COO)_3$  (0.20 mmol),  $Er(CH_3COO)_3$  (0.02 mmol) and  $Tm(CH_3COO)_3$  (0.002 mmol) were added to a three-necked flask (50 mL) containing OA (6 mL) and 1-octadecene (15 mL). To acquire a homogeneous solution, the above mixture was heated to 120 °C for 30 min. After cooled down to 50 °C, methanol solution (10 mL) containing NaOH (2.5 mmol) and  $NH_4F$  (4 mmol) was slowly added into the three-necked flask. The mixture was stirred for another 1 h. And then, to remove methanol, the solution was slowly heated and degassed at 100 °C for 10 min. Subsequently, under argon atmosphere, the solution was heated to 300 °C and stirred for 1.5 h. After natural cooling, OA-UCNPs were precipitated with ethanol addition and washed with ethanol/cyclohexane (9:1, v/v) for three times. The resulting nanoparticles were dried by freezing vacuum dryer for further experiments.

**Synthesis of PAA-UCNPs.** PAA-1800 (300 mg) were added to a three-necked flask (100 mL) containing diethylene glycol (30 mL). To acquire a homogeneous solution, the above mixture was heated to 110 °C. After cooled down to 70 °C, cyclohexane solution containing OA-UCNPs (100 mg) was added slowly. To remove cyclohexane,

the solution was slowly heated and degassed at 110 °C for 10 min. Subsequently, under argon atmosphere, the solution was heated to 240 °C and stirred for 1.5 h. After natural cooling, PAA-UCNPs were precipitated with ethanol addition and washed with ethanol/water (1:1, v/v) for three times. The resulting nanoparticles were dried by freezing vacuum dryer for further experiments.

**Synthesis of UCCOFs.** The UCCOFs (45 nm) were prepared according to our optimized synthetic methodology with a two-step reaction. In the first step, the dispersion of PAA-UCNPs (10 mg) in THF (10 mL) was mixed with TAP (20  $\mu$ mol) under ultra-sonication for 30 min. The obtained solution was kept at 50 °C for 2 h under vigorous stirring, which allowed the interaction between carboxyl groups from PAA-UCNPs and amino groups from TAP. Subsequently, a THF solution of DHTA (40  $\mu$ mol) was added dropwise and the reaction kept in 50 °C for 12 h. The products were recovered *via* centrifugation and washed three times with ethanol. In this step, the core-shell nanoparticles were obtained, consisting of PAA-UCNPs in core and amorphous polyimine network in shell. And then, this core-shell nanoparticles were dispersed the mixed solvent of 1,2-dichlorobenzene/n-butanol/6 M acetic acid (1 mL/1 mL/0.2 mL) in a Pyrex tube under ultra-sonication 30 min. The tube was then flash frozen at 77 K (liquid N<sub>2</sub> bath) and degassed by three freeze-pump-thaw cycles. The tube was sealed off and then heated at 120 °C for 3 days. The products were collected *via* centrifugation, washed with THF, DMAc and acetone, and dried at 60 °C under vacuum for 12 h. By virtue of the flexible controllability in monomer concentrations and ratios, the different size of UCCOFs were acquired. PAA-UCNPs (10 mg), TAP (10  $\mu$ mol) and DHTA (20  $\mu$ mol) were used for synthesis UCCOFs (35 nm). And PAA-UCNPs (10 mg), TAP (40  $\mu$ mol) and DHTA (80  $\mu$ mol) were used for synthesis UCCOFs (65 nm). While substituting OA-UCNPs for PAA-UCNPs, irregular COFs were acquired.

**Synthesis of UCCOFs-1.** The UCCOFs-1 were prepared with a two-step reaction. In the first step, PEI-600 (20 mg), EDC·HCl (5 mg) and NHS (5 mg) were mixed in UCCOFs aqueous solution (2 mg mL<sup>-1</sup>, 2 mL) under vigorous stirring at room temperature for 12 h. The products UCCOFs-PEI were collected *via* centrifugation, washed three times with ethanol and deionized water. In the second step, ICG (10 mg)

was added into the UCCOFs-PEI solution ( $2 \text{ mg mL}^{-1}$ ,  $1 \text{ mL}$ ) under vigorous stirring at room temperature for 12 h. The precipitate was washed with ethanol and deionized water to remove free ICG and collected by centrifugation. The as-obtained UCCOFs-1 were re-dispersed by sonication to form a homogeneous solution.

**Synthesis of PEG Functionalized Poly(styrene-co-maleic anhydride) (PSMA-PEG<sub>5000</sub>) and Nanoparticles PEGylation.** PMSA (15 mg), PEG<sub>5000</sub>-NH<sub>2</sub> and TEA (6  $\mu\text{L}$ ) were mixed in dichloromethane (5 mL) under vigorous stirring at room temperature for 24 h. And then, EDC·HCl (11 mg) and TEA (6  $\mu\text{L}$ ) were added into the mixture solution and stirred at room temperature for another 24 h. The solvent was removed under vacuum and the products were dialyzed with an ultrafiltration device (3.5K MWCO) for 3 days. The PSMA-PEG<sub>5000</sub> was isolated *via* lyophilization as white powder. For nanoparticles PEGylation, PSMA-PEG<sub>5000</sub> (5 mg) were added into the UCCOFs or UCCOFs-1 solution ( $1 \text{ mg mL}^{-1}$ ) under ultra-sonication for 1h. The resultant nanoparticles were washed by ethanol and deionized water to remove the reactant residuals.

**Singlet Oxygen Generation.** Typically, 5  $\mu\text{L}$  of SOSG stock solution (5 mM) was added into 1 mL of UCCOFs solution ( $0.2 \text{ mg mL}^{-1}$ ). The solution was irradiated by a 980 nm laser ( $1.2 \text{ W cm}^{-2}$ ) for various times or kept in the dark as the control, and fluorescence intensity of SOSG was measured ( $E_x = 488 \text{ nm}$ ,  $E_m = 525 \text{ nm}$ ) to determine the  $^1\text{O}_2$  concentration.

**Cell Culture.** The HeLa cells were grown in Dulbecco's Modified Eagle's medium (DMEM), or 4T1 cells were cultured in RPMI-1640 culture medium, supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin-streptomycin) at 37 °C in a humidified incubator containing 5% CO<sub>2</sub>. The entire process of cell culture followed American Type Culture Collection (ATCC) instructions.

**Cellular Uptake and Live Cell Imaging.** The HeLa cells were first incubated with DMEM in a 30 mm optical culture dish for 24 h at 37 °C, then used DMEM was replaced and UCCOFs ( $100 \mu\text{g mL}^{-1}$ ) was added into the fresh DMEM for incubating another 4 h at 37 °C. After washing with DPBS for three times, UCL imaging was recorded with magnification at  $60\times$  by confocal laser scanning microscopy. The images

were collected at 500-550 nm upon excitation at 980 nm laser. To investigate the subcellular trafficking of UCCOFs, LysoTracker Red (50 nM) was applied to the cells for another 30 min incubation. Then the cells were washed with DPBS and visualized by fluorescence imaging. The images were collected at 570-620 nm upon excitation at 561 nm laser.

**Intracellular Singlet Oxygen Generation.** The HeLa cells were first incubated with DMEM in a 30 mm optical culture dish according to the above protocol. Then used DMEM was replaced and UCCOFs ( $100 \mu\text{g mL}^{-1}$ ) was added into the fresh DMEM for incubating another 4 h at  $37^\circ\text{C}$ . After washing with DPBS, the cells were incubated with  $\text{H}_2\text{DCFH-DA}$  ( $10 \mu\text{M}$ ) in serum-free cell culture medium for 20 min at  $37^\circ\text{C}$ . Then, the cells were washed with serum-free cell culture medium to fully remove  $\text{H}_2\text{DCFH-DA}$  and irradiated with 980 nm laser for 10 min ( $1.2 \text{ W cm}^{-2}$ , 5 min break after 1 min irradiation). The fluorescence imaging of the cells was recorded with magnification at  $60\times$  by confocal laser scanning microscopy. The images were collected at 500-550 nm upon excitation at 488 nm laser.

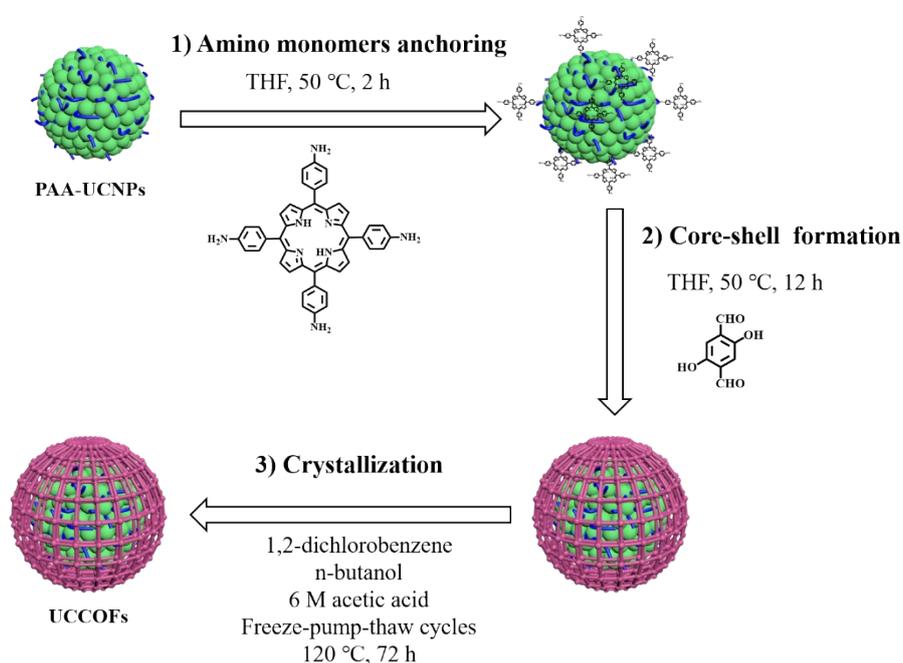
**Cell Viability Test.** The HeLa cells were seeded in a 96-well plate ( $1 \times 10^4$  cells per well) and incubated with different concentrations of UCCOFs-1 (5, 25, 50, 100 and  $200 \mu\text{g mL}^{-1}$ ) for 4 h at  $37^\circ\text{C}$ , respectively. The cells were irradiated with 980 nm laser for 10 min ( $1.2 \text{ W cm}^{-2}$ , 5 min break after 1 min irradiation) and then incubated for another 24 h. The relative cellular viabilities were measured by a standard MTS assay. To investigate the influence of irradiation time, the HeLa cells were seeded in a 96-well plate ( $1 \times 10^4$  cells per well) and incubated with UCCOFs-1 ( $100 \mu\text{g mL}^{-1}$ ) for 4 h at  $37^\circ\text{C}$ . The cells were irradiated with 980 nm laser ( $1.2 \text{ W cm}^{-2}$ , 5 min break after 1 min irradiation) for different irradiation time period (0, 5, 10 and 20 min) and then incubated for another 24 h. The relative cellular viabilities were measured by a standard MTS assay.

**UCL Imaging.** The HeLa cells were seeded in a 96-well plate ( $1 \times 10^4$  cells per well) and incubated with UCCOFs-1 ( $100 \mu\text{g mL}^{-1}$ ) for 4 h at  $37^\circ\text{C}$ . The cells were irradiated with 980 nm laser ( $1.2 \text{ W cm}^{-2}$ , 5 min break after 1 min irradiation) for different irradiation time period (0, 5, 10 and 20 min). After irradiation, the lysates of HeLa cells

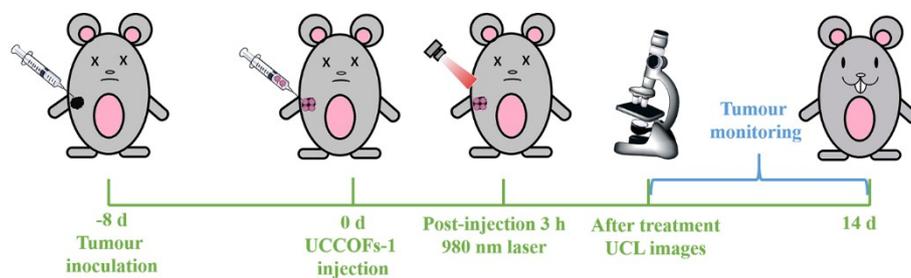
were immediately acquired and the UCL images of lysates in tubes were collected by an IVIS Lumina II in vivo imaging system (Caliper LifeScience, USA) with 980 nm laser ( $0.1 \text{ W cm}^{-2}$ ).

**Animal Experiment.** Female Babl/c nude mice (4-6 weeks) were purchased from Hunan SJA Laboratory Animal Co. Ltd. (Changsha, China). The tumor model was established by the subcutaneous injection of 4T1 cells ( $1.0 \times 10^7$  cells) at the right axilla. Tumors were allowed to grow for one week before use. The mice were randomly divided into six groups ( $n = 5$ , each group) for the treatment with saline (50  $\mu\text{L}$ ), NIR (20 min), UCCOFs-1, UCCOFs-1 + NIR 5 min, UCCOFs-1 + NIR 10 min and UCCOFs-1 + NIR 20 min (UCCOFs-1 dose: 50  $\mu\text{L}$ , 10  $\text{mg kg}^{-1}$ ). NIR treatment was performed 3 h post-injection by irradiating the tumour regions with a 980 nm laser for predetermined time ( $1.2 \text{ W cm}^{-2}$ , 5 min break after 1 min irradiation). After NIR treatment, the mice were anesthetized to be motionless and UCL images were collected by an IVIS Lumina II in vivo imaging system (Caliper LifeScience, USA) with 980 nm laser ( $0.1 \text{ W cm}^{-2}$ ). The tumor sizes and body weight were measured every other day and the tumor volume calculated as the volume = (tumor length)  $\times$  (tumor width)<sup>2</sup>/2. Relative tumor volumes were calculated as  $V/V_0$  ( $V_0$  was the tumor volume when the treatment was initiated). After two weeks of treatment, the mice were sacrificed and their major organs (heart, liver, spleen, lung, and kidneys) were removed for hematoxylin and eosin (H&E) staining. All animal operations were performed according to the guidelines and regulations for animal use and care.

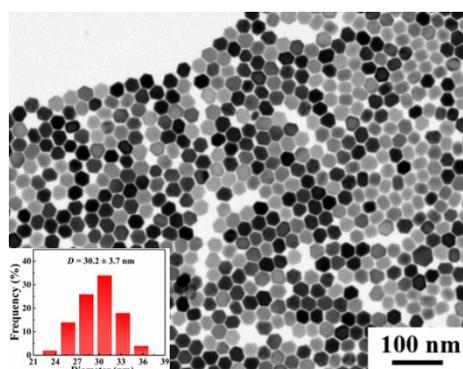
## Results and Discussion



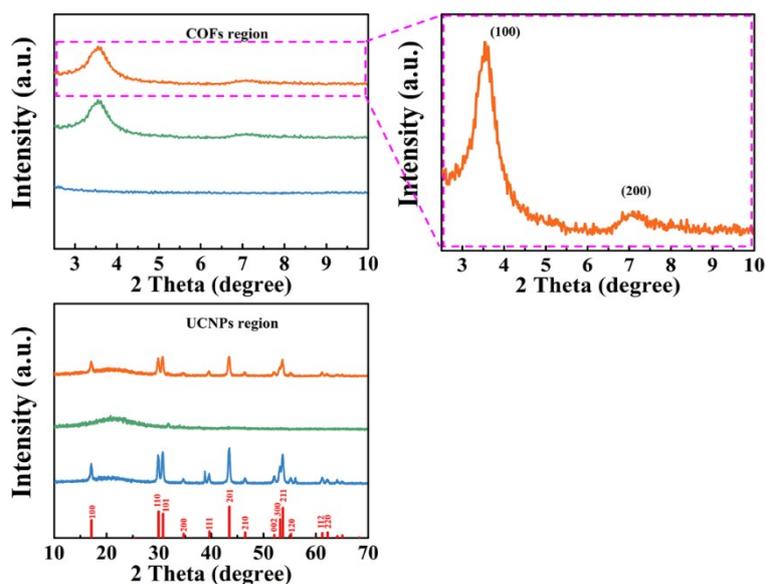
**Scheme S1.** Schematic illustration of core-mediated COF synthetic strategy.



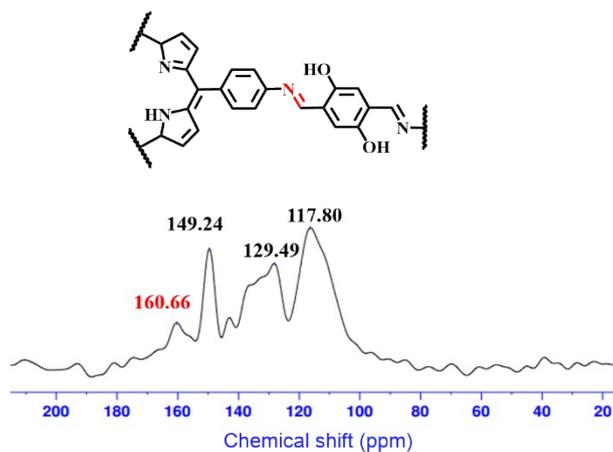
**Scheme S2.** Schematic illustration of the time points for animal experiment.



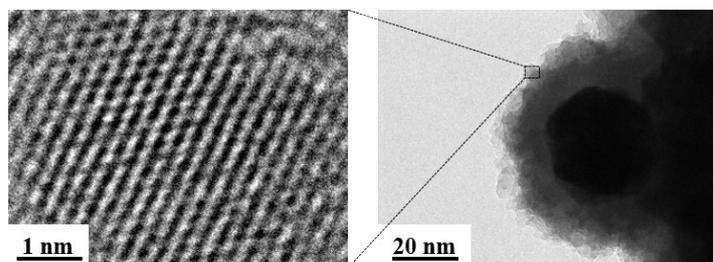
**Fig. S1.** TEM image of OA-UCNPs.



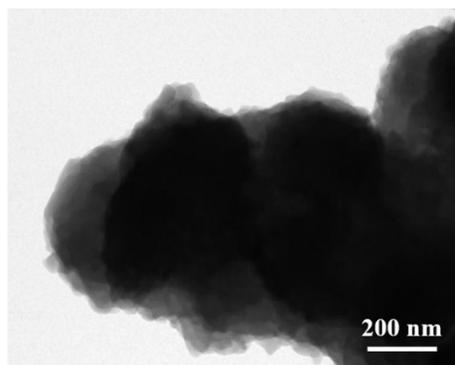
**Fig. S2.** The magnification of PXRD patterns in COFs region (top) and UCNPs region (bottom). Blue: UCNPs; Green: porphyrin-based COFs; Orange: UCCOFs; Red: Standard pattern of NaYF<sub>4</sub> (JCPDS No. 28-1192).



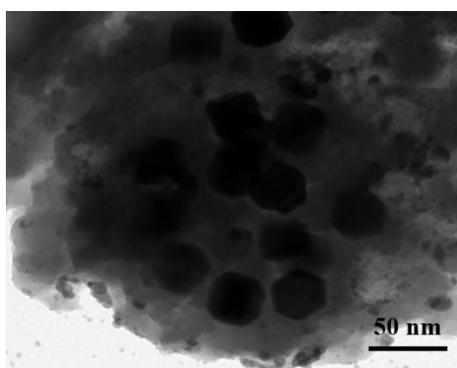
**Fig. S3.** Solid-state <sup>13</sup>C CP-MAS NMR spectrum of UCCOFs.



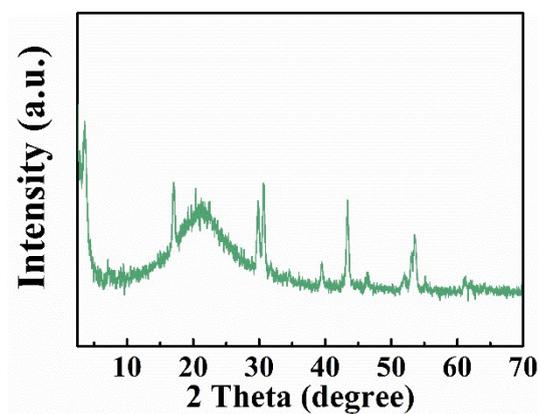
**Fig. S4.** HRTEM image of COF shell in UCCOFs.



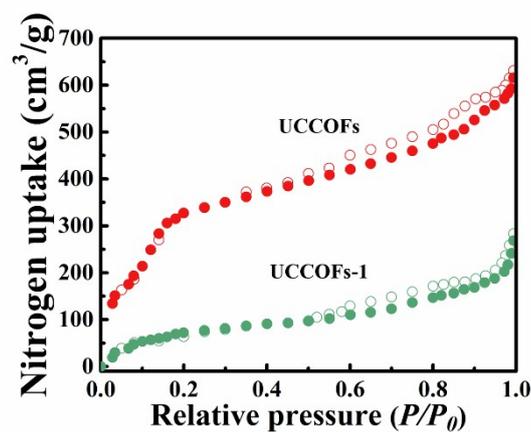
**Fig. S5.** TEM image of COFs without PAA-UCNPs core.



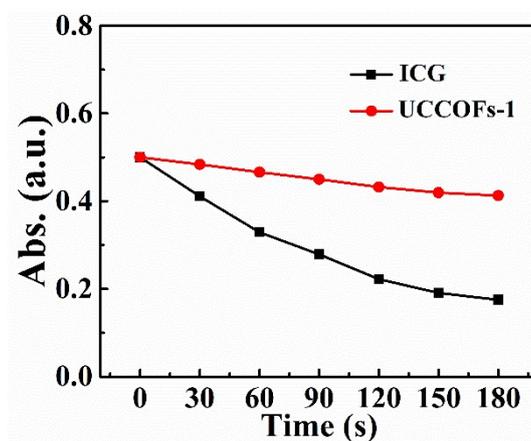
**Fig. S6.** TEM image of imine polymerization with OA-UCNPs (irregular COFs).



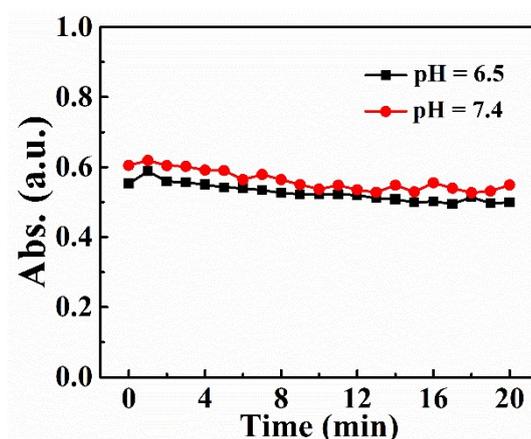
**Fig. S7.** PXRD patterns of UCCOFs-1.



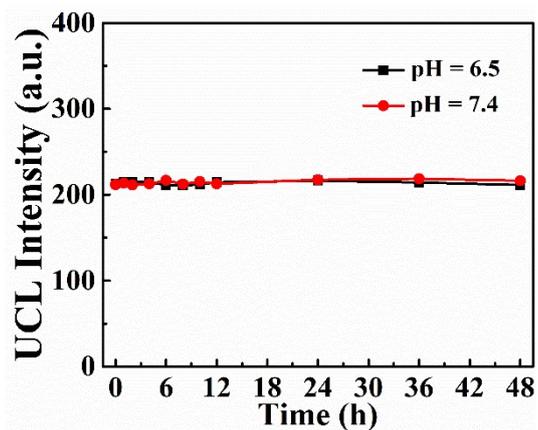
**Fig. S8.** Gas sorption measurements for UCCOFs (red) and UCCOFs-1 (green).



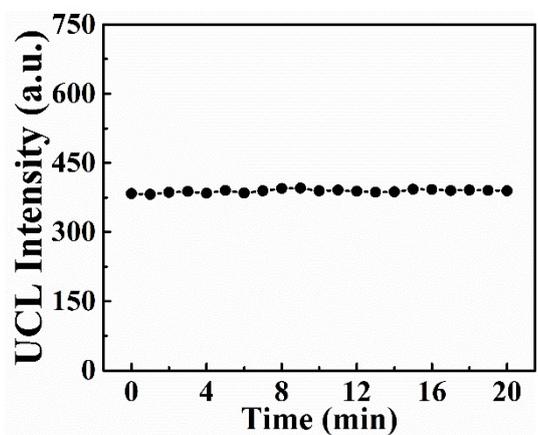
**Fig. S9.** Comparison of the photostability of free ICG and UCCOFs-1 by the change of absorption at 780 nm under 808 nm laser irradiation ( $0.65 \text{ W cm}^{-2}$ ).



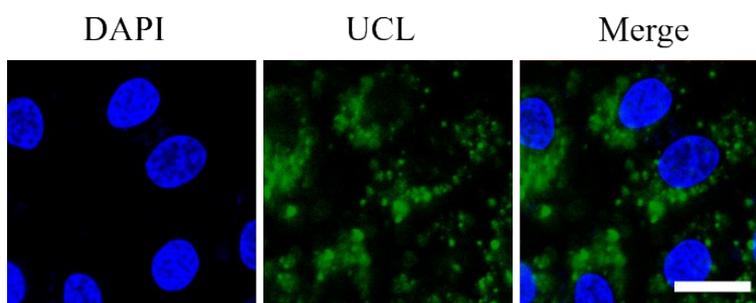
**Fig. S10.** Comparison of the photostability of free ICG at the physiological pH (pH = 7.4) and acidic tumor microenvironment (pH = 6.5) under 980 nm laser irradiation ( $1.2 \text{ W cm}^{-2}$ ).



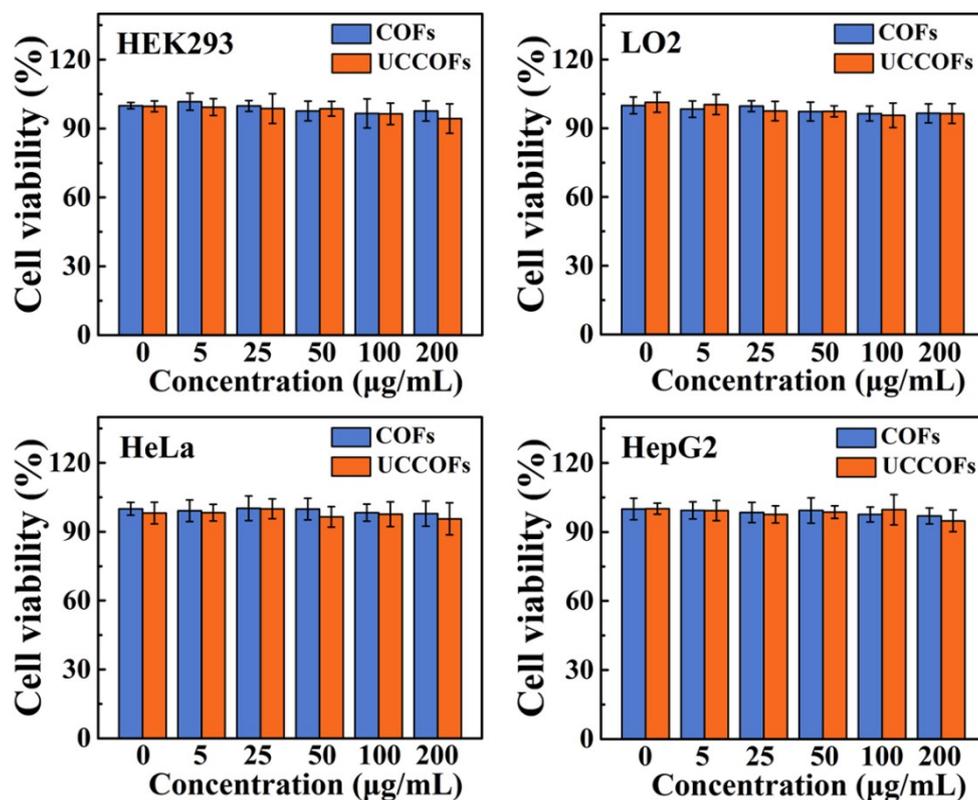
**Fig. S11.** The UCL intensity at 808 nm of UCCOFs-1 under the physiological pH (pH = 7.4) and acidic tumor microenvironment (pH = 6.5).



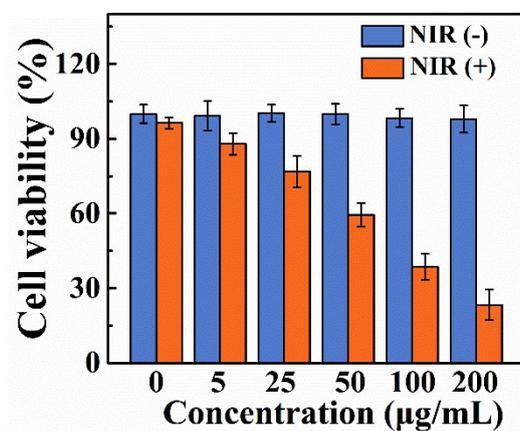
**Fig. S12.** The UCL intensity at 541 nm of UCCOFs-1 under 980 nm laser irradiation ( $1.2 \text{ W cm}^{-2}$ ).



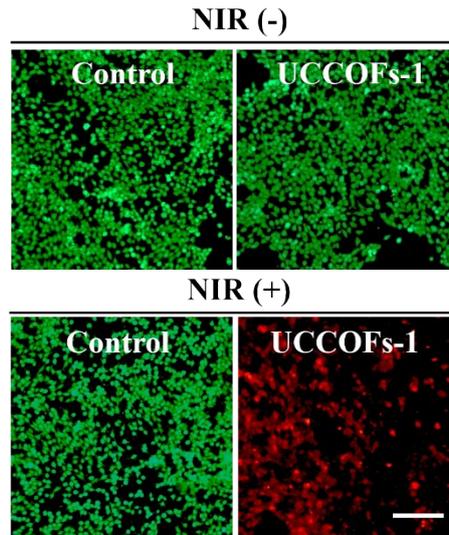
**Fig. S13.** Confocal fluorescence images of HeLa cells treated with UCCOFs. Scale bar, 20  $\mu\text{m}$ .



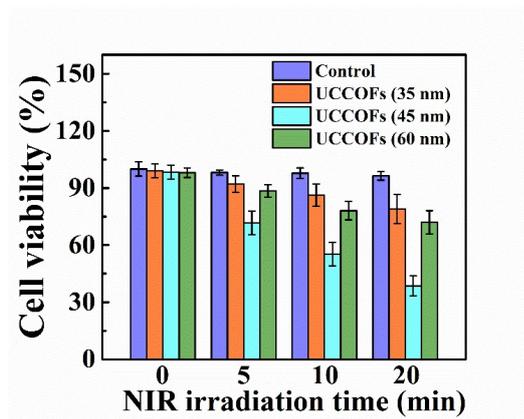
**Fig. S14.** Cell viability of different cells with different concentration COFs and UCCOFs (0, 5, 25, 50, 100 and 200 µg mL<sup>-1</sup>).



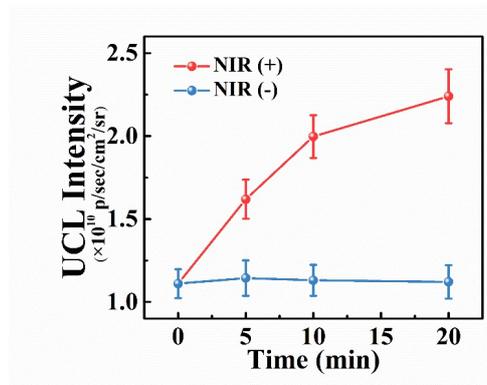
**Fig. S15.** Cell viability of HeLa cells with different concentration UCCOFs-1 (0, 5, 25, 50, 100 and 200 µg mL<sup>-1</sup>) with or without NIR irradiation.



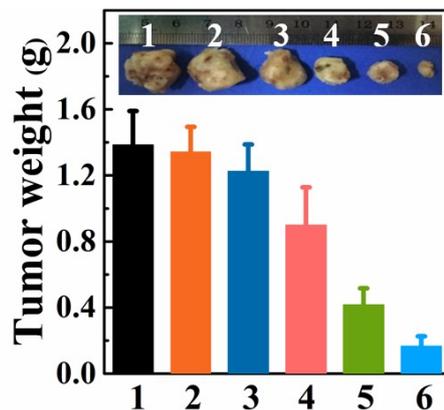
**Fig. S16.** Fluorescence images of HeLa cells costained with calcein-AM (green, live cells) and PI (red, dead cells).



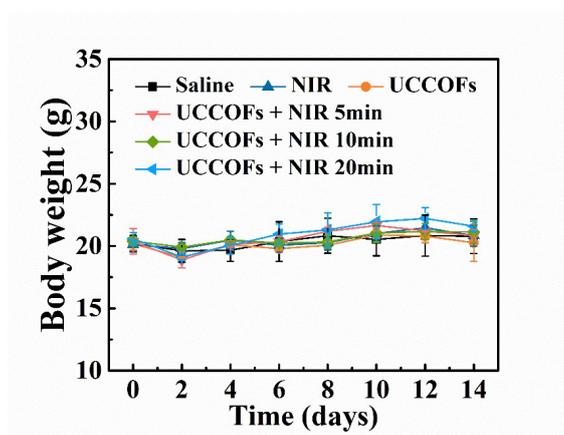
**Fig. S17.** Cell viability of HeLa cells with various sizes of UCCOFs.



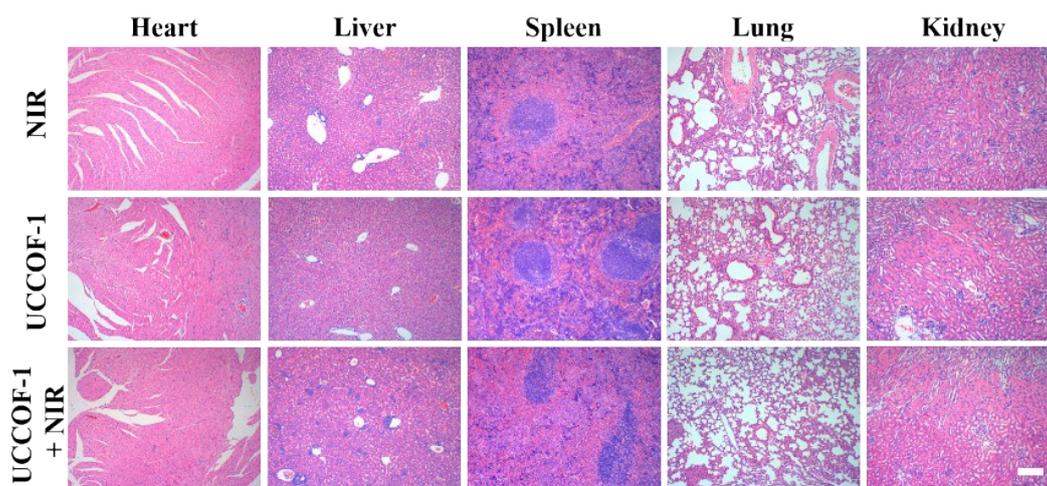
**Fig. S18.** UCL intensity of 4T1 tumor-bearing live mice after injection of UCCOFs-1 at different NIR irradiation times.



**Fig. S19.** Tumor weights after different treatment 14 days. Inset: representative images of the tumors after different treatment 14 days (1: saline, 2: UCCOFs-1, 3: NIR, 4: UCCOFs-1 + NIR 5 min, 5: UCCOFs-1 + NIR 10 min, 6: UCCOFs-1 + NIR 20 min).

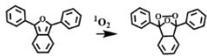
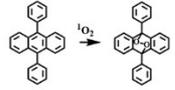
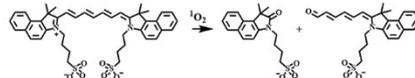
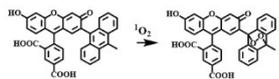


**Fig. S20.** The body weight data after different treatment 14 days.



**Fig. S21.** H&E stained images of major organs after 14 days after different treatment. Scale bar, 100  $\mu$ m.

**Table S1.** Conventional methods for  $^1\text{O}_2$  detection *in vitro*.

Reagent	Test method	Spectral properties	Mechanism
9,10-Diphenylanthracene (DPA)	Ultraviolet-visible spectrophotometry	Absorption at 355 nm	
1,3-Diphenylisobenzofuran (DPBF)	Ultraviolet-visible spectrophotometry	Absorption at 418 nm	
Indocyanine green (ICG)	Ultraviolet-visible spectrophotometry	Absorption at 780 nm	
Singlet Oxygen Sensor Green® (SOSG)	Fluorescence detection	Excitation/Emission 504 nm/525 nm	
2',7'-Dichlorodihydrofluorescein diacetate ( $\text{H}_2\text{DCFH-DA}$ )	Fluorescence detection	Excitation/Emission 502 nm/530 nm	