Supporting Information

Rational fabrication of two-photon responsive metal-organic framework for enhanced photodynamic therapy

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Materials. All starting materials were obtained from commercial supplies and used without further purification. The chemicals of trimesic acid (98%), Hexadecyl trimethyl ammonium bromide (99%), triethylamine (99%), copper nitrate trihydrate (99.99%) folic acid (99%) were purchased from Aladdin Co., Ltd. The chemicals of 2′,7′-dichlorofluorescein diacetate (DCFH-DA) were purchased from Macklin Co., Ltd. (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Beyotime Biotech Co., Ltd. (China). Annexin V-FITC/PI Apoptosis Detection Kit and Calcein AM /PI Kit was obtained from Shanghai Bestbio (China). Ethanol and N,N-Dimethylformamide (99.5%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Deionized (DI) water was used throughout.

Apparatus. UV-Vis absorption spectra were recorded on a UV-265 spectrophotometer. SEM and Mapping were detected by REGULUS8230*. PXRD patterns were recorded on SmartLab 9KW. Fluorescence measurements were carried out on a Hitachi F-7000 fluorescence spectrophotometer. IR spectra were recorded on a Nicolet FT-IR instrument (mid-IR: 4000 ~ 400 cm^{-1} range with KBr discs). One- and two-photon imaging data acquisition and processing were performed using Lecia TCS SP8 DIVE FALCON which equipped with single-wavelength laser (output wavelength: 405 nm, 456 nm, 488 nm, 514 nm, 561 nm, 633 nm) and femtosecond laser (adjustable output wavelength: 680 - 1080 nm, 80 MHz, 140 fs).

ZnL1 Loading efficiency calculating.

The amount of **ZnL1** encapsulated into **MOF-199** or **MOF-199@FA** was calculated according to the amount of Zn element upon calibrations by ICP-MS. The encapsulated amount of **ZnL1** was calculated through the following equations:

ZnL1 loading amount (%) = (mass of loaded **ZnL1** / mass of **ZnL1@MOF-199**) × 100%.

ZnL1 loading amount (%) = (mass of loaded **ZnL1** / mass of **ZnL1@MOF-199@FA**) × 100%.

Hydroxyl radical (·OH) detection by Terephthalic acid (TA).

Terephthalic acid (0.05 mmol) was dissolved into 100 mL NaOH solution (2 mM) and then the generation of ·OH at different pH values (6.5 and 7.4 in PBS buffer solution) was studied. Next, H_2O_2 (1 mM, 200 μL) and **MOF-199** (1 mg mL^{-1}, 100 μL) were added into 1mL acetate/acetic acid buffer solution and incubated for 10 minutes. 1 mL terephthalic acid was added into the above mixtures and incubated for another 10 minutes. The fluorescence of the mixture was measured at 435 nm in different pH (λ_{ex} = 315 nm, λ_{em} = 420-450 nm).

Electron spin resonance (ESR) assay.

The ESR measurements were carried out with a Bruker Nano X-band spectrometer at 298 K. The spin adducts of the complexes were detected using three settings as follows: 1 G field modulation, 20 mW microwave power, and 100 G scan range. The spin traps 2,2,6,6-tetramethylpiperidine (TEMP for trapping $^1O_2$, 20 mM) and 5,5- dimethyl-1-pyrroline-N-oxide (DMPO for trapping, 100 μM) were used to verify the formation of reactive oxygen species (ROS) generated by **ZnL1@MOF-199@FA** (50 μg mL^{-1}). The ESR signals of the **ZnL1@MOF-199@FA** (50 μg mL^{-1}) before and after the light irradiation (LED light, 400-700 nm, 40 mW cm^{-2}) were recorded.

Singlet oxygen ($^1O_2$) detection.

In this study, the amount of singlet oxygen was detected by a singlet oxygen sensor named 9,10-anthracenedipropanoic acid (ABDA), because the newly generated singlet oxygen could cause an absorbance decrease of the chemical probe at around 378 nm. ABDA (100 μM), **ZnL1@MOF-199@FA** (50 μg mL^{-1}) and H_2O_2 (100 μM) were incubated together in a hypoxia environment and exposed to white light irradiation for 0-6 min (the laser beam was purchased from Xian Midriver Optoelectronics Technology Co., Ltd, China.). The absorbance of the probe was measured at the same time-scale to evaluate the generation of singlet oxygen.

Cytotoxicity assays in cells.

The study of the PDT/CDT effect of **ZnL1@MOF-199@FA** was carried out using the methylthiazolyldiphenyltetrazolium bromide (MTT) assay. **ZnL1@MOF-199@FA** stock solutions were diluted by fresh medium in to desired concentration (0, 25, 50, 100 μg mL^{-1}). HeLa cells or QSG-7701 cells were cultured in a 96- well plate for 24 h before experiments. The cell medium was then exchanged by different concentrations of **ZnL1@MOF-199@FA** medium solutions. They were incubated at 37 °C in 5% CO_2 for 12 h, and then irradiated by light (830 nm, 100 mW cm^{-2}) for 15 min before cell viability was measured by the MTT assay. The cell medium solutions were exchanged by 100 μL of fresh medium, followed by the addition of 20 μL (5 mg mL^{-1}) MTT solution to each well. The cell plates were then incubated at 37 °C in 5% CO_2 for 4 h. After MTT medium removal, the formazan crystals were dissolved in DMSO (100 μL $well^{-1}$) and the absorbance was measured at 490 nm using a microplate reader. And duplicated experiments have been tested.

Intracellular ROS detection.

The intracellular ROS under two photon irradiations was measured using the fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). DCFH-DA diffuses through the cell membrane and is enzymatically hydrolyzed by intracellular esterases to form the non-fluorescent compound DCFH, which is then rapidly oxidized to form the highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of ROS. HeLa cells were incubated with 50 μg mL^{-1} **ZnL1@MOF-199@FA** for 4 hours, and 2′, 7′-dichlorofluorescein diacetate (DCFH-DA) was added following. Then, the confocal laser scanning microscopy (CLSM) images were collected after the resulting cells were exposed to 830 nm of light irradiation (0.1 W cm^{-2}). After that, confocal fluorescence imaging was performed to give the level of intracellular ROS with the excitation wavelength of 488 nm and emission wavelength from 500 nm to 540 nm.

Intracellular singlet oxygen detection.

HeLa cells were incubated with 50 μg mL^{-1} **ZnL1@MOF-199@FA** for 4 hours followed by incubation with 1 μM singlet oxygen sensor green (SOSG) for 10 min.

After that, cells were washed with PBS and then irradiated with 830 nm light at a power density of 0.1 W cm^{-2}. The green fluorescence was observed using CLSM with the excitation wavelength of 504 nm, and emission collection wavelength from 500 nm to 550 nm.

Flow Cytometry Study.

Cells seeded into the 6-well plates were cultured for 24 h. Next, the medium was replaced with medium (3 mL) containing **ZnL1@MOF-199@FA** (50 μg mL^{-1}), at 37 °C for 12 hours. After irradiated by laser (830 nm, 100 mW cm^{-2}, 10 min), the cells were collected by centrifugation and resuspended in binding buffer containing Propidium Iodide (PI, 10 μL) and Annexin-V FITC (5 μL) for 15 min in darkness. The signal was collected by a BD FACS Calibur flow cytometer (Beckaman/Gallios).

Live/Dead assay with calcein AM/PI.

HeLa cells with a density of 10^5 cells per well were cultured in a 6-well plate for 24 h to allow the attachment of cells. After cells were washed twice by PBS solution, **ZnL1@MOF-199@FA** (50 μg mL^{-1}) and H_2O_2 (100 μM) were added to above culture medium. The cells were incubated for 4 h and irradiated by laser (830 nm, 10 min), calcein AM and PI were used to confirm the viability of Hela cells. Fluorescence images were collected by CLSM.

Annexin V-FITC and PI assay.

HeLa cells are incubated with 50 μg mL^{-1} **ZnL1@MOF-199@FA** for 24 h at 35 °C with 5% CO_2, and then irradiated by light (830 nm, 100 mW cm^{-2}) for 15 min. Then, the cells are further stained with Annexin V-FITC (1 μM) and PI (1 μM). After 20 min, the cells are washed by PBS solution (3×1 mL per well) and 1 mL of PBS solution is added into each well. The fluorescent images of the cells are collected by confocal laser scanning microscopy. The green fluorescence of Annexin V-FITC is collected between 510-540 nm upon excitation at 488 nm. The red fluorescence of PI is collected between 590-620 nm upon excitation at 488 nm.

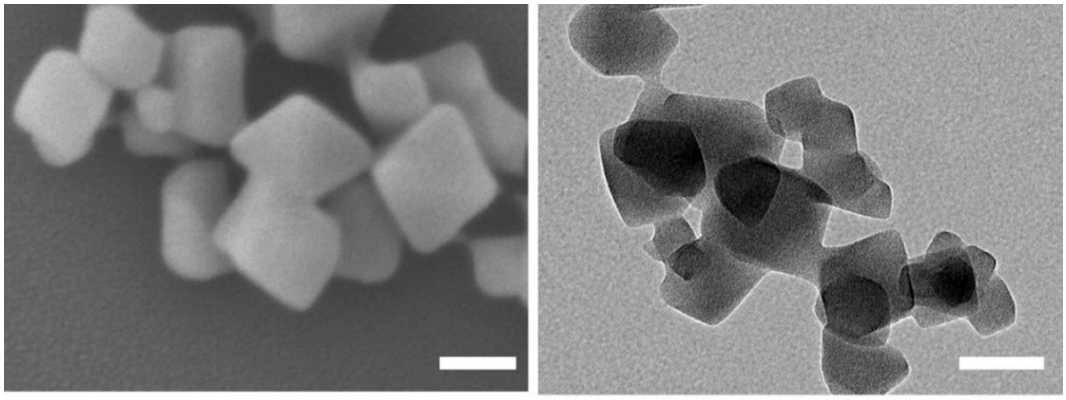

Fig. S1 SEM and TEM images of **MOF-199** (scale bar: 100 nm).

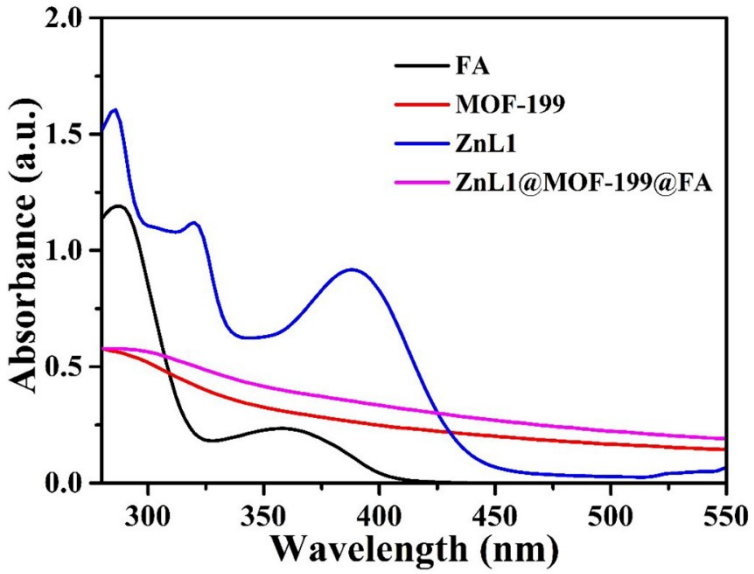

Fig. S2 UV−Vis absorption spectra of FA, **ZnL1**, **MOF-199** and **ZnL1@MOF-199@FA** in ethanol solution.

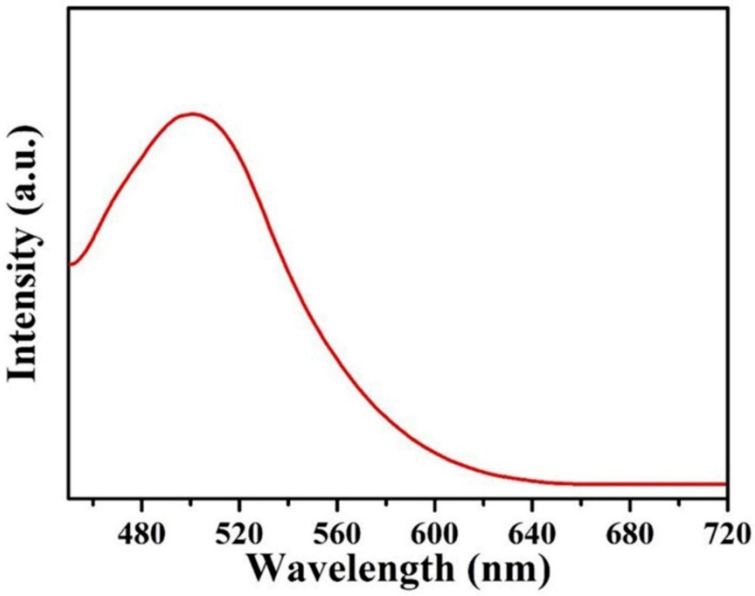

Fig. S3 Fluorescence spectra of **ZnL1@MOF-199@FA** (λ_{ex} =300 nm, c = 50 μg mL^{-1}).

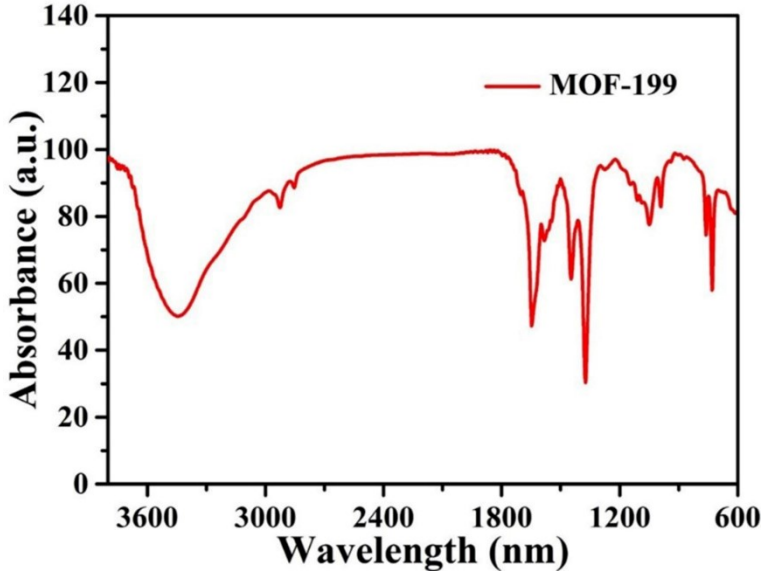

Fig. S4 FTIR of **MOF-199**.

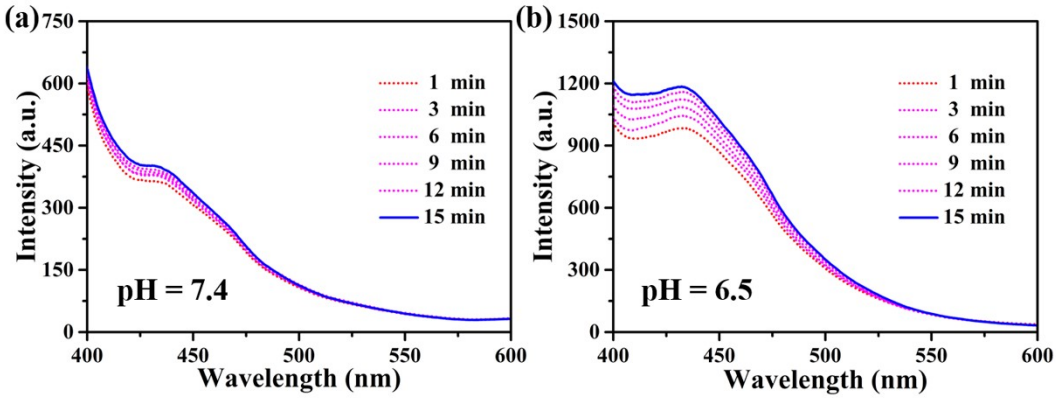

Fig. S5 Determination of the formation of ·OH by terephthalic acid as a fluorescent probe. (a) pH = 7.4. (b) pH = 6.5. Reaction conditions: **MOF-199** (50 μg mL^{-1}), H_2O_2 (100 μM), TA (0.05 mM), PBS buffer.

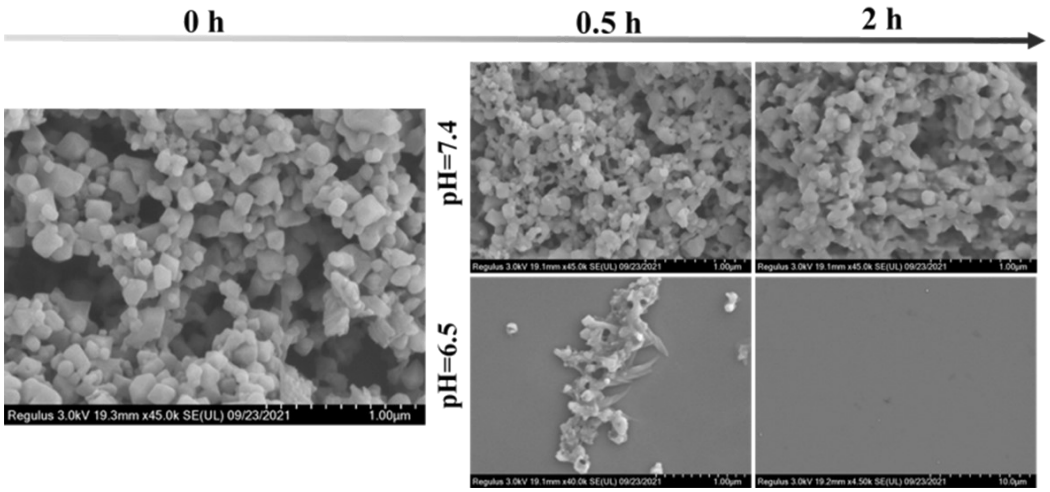

Fig. S6 SEM images of **MOF-199** before and after incubation in neutral or acidic environment for 2 h.

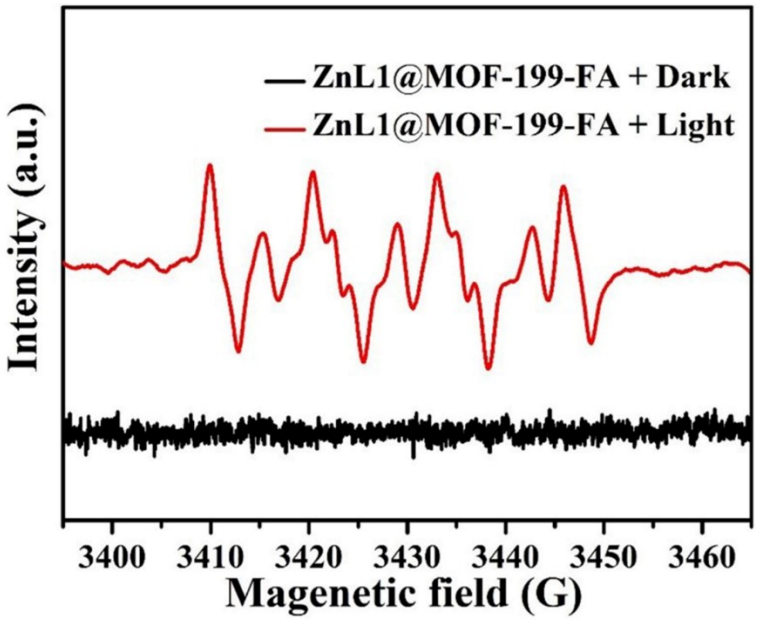

Fig. S7 ESR signals of **ZnL1@MOF-199@FA** trapped by 5,5-dimethyl-1-pyrroline-N-oxide.

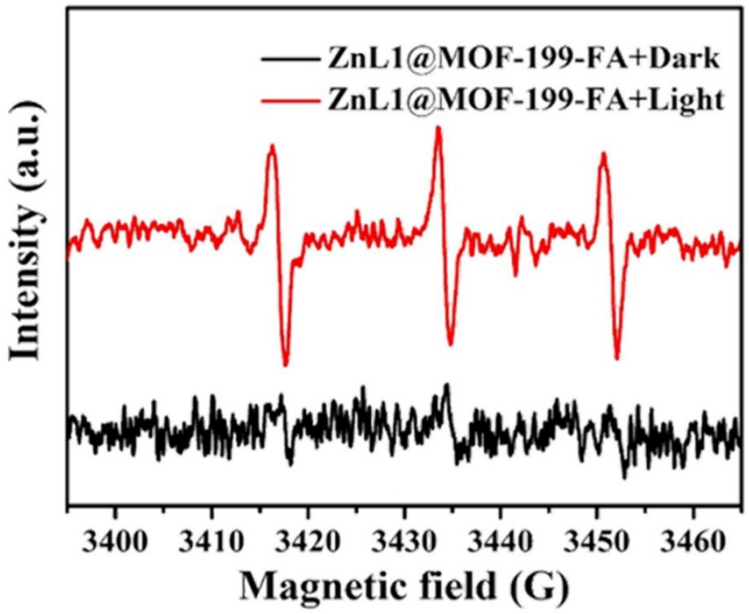

Fig. S8 ESR signals of **ZnL1@MOF-199@FA** trapped by 2,2,6,6-tetramethylpiperidine.

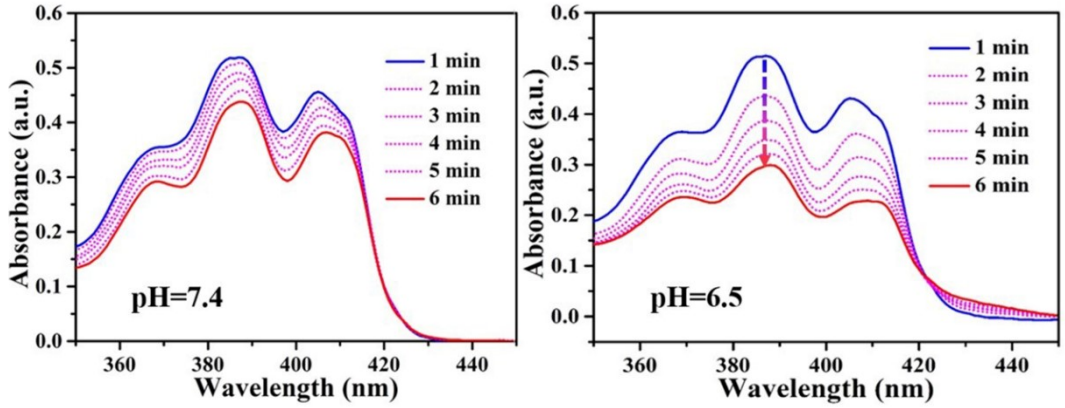

Fig. S9 The UV–Vis spectra of 9,10-anthracenedipropanoic acid (ABDA) probe treated with **ZnL1@MOF-199@FA** (50 μg mL^{-1}) and H_2O_2 (100 μM) under light irradiation within 6 min in N_2 atmosphere.

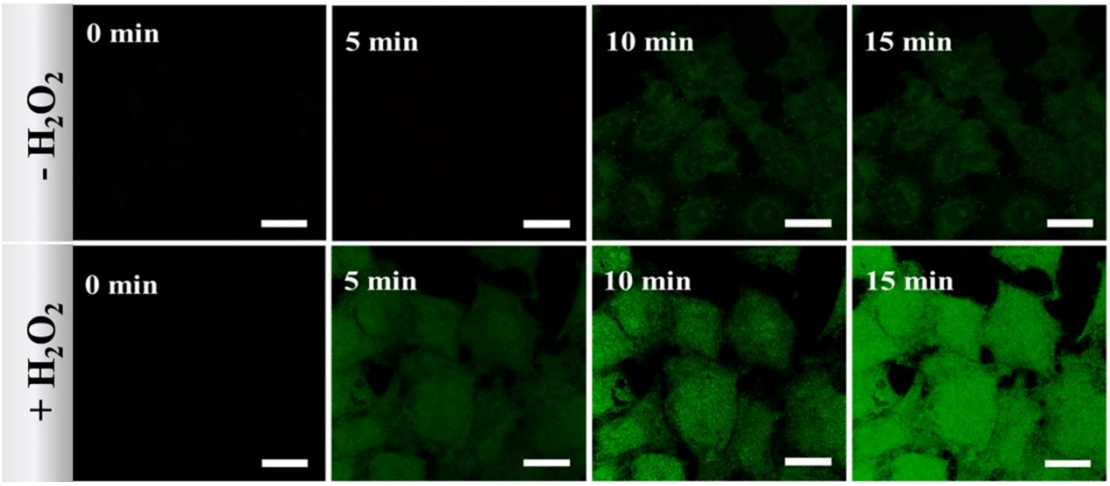

Fig. S10 Two-photon CLSM images (830 nm, 0.1 W cm^{-2}) of Hela cells treated with **ZnL1@MOF-199@FA** and DCFH-DA (fluorescence indicator as the generation of ·OH). scale bar: 20 μm.

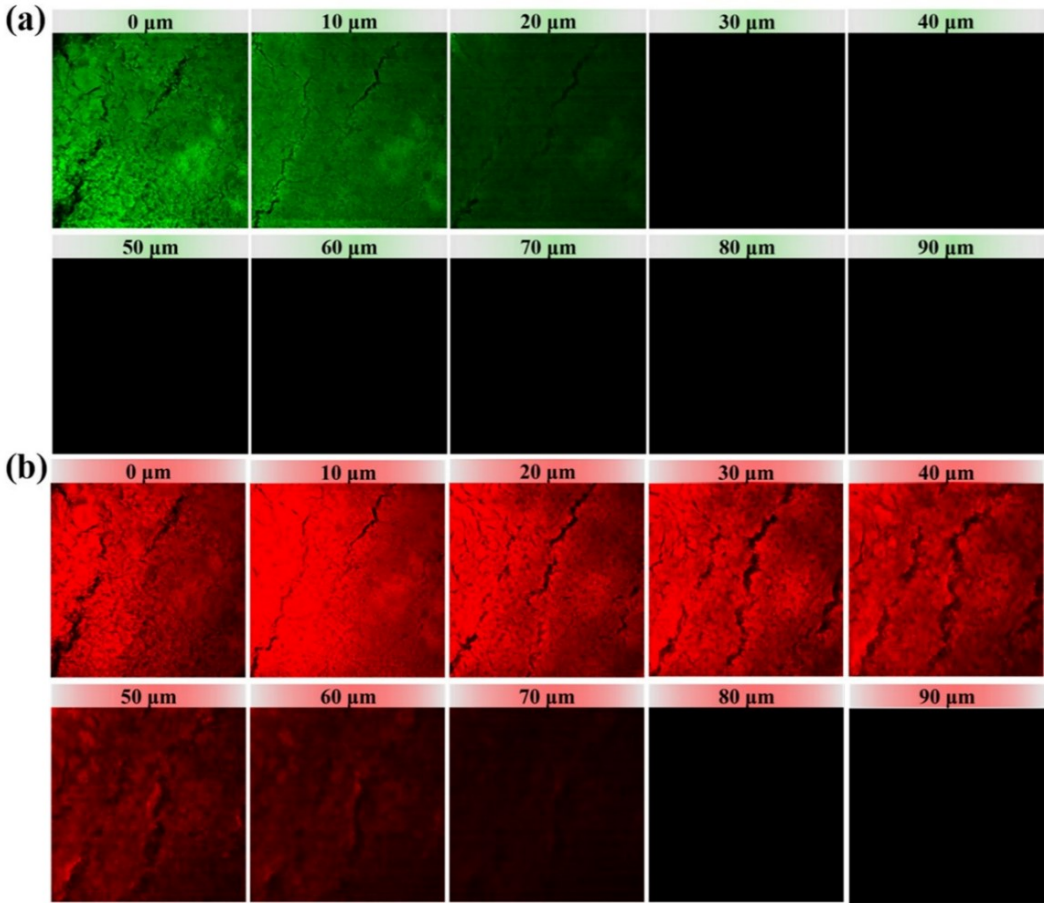

Fig. S11 (a) One-photon (458 nm, 0.5 W cm^{-2}) and (b) two-photon (830 nm, 0.5 W cm^{-2}) fluorescence images of tissue section incubated with **ZnL1@MOF-199@FA** with different penetration depth along the z axis.

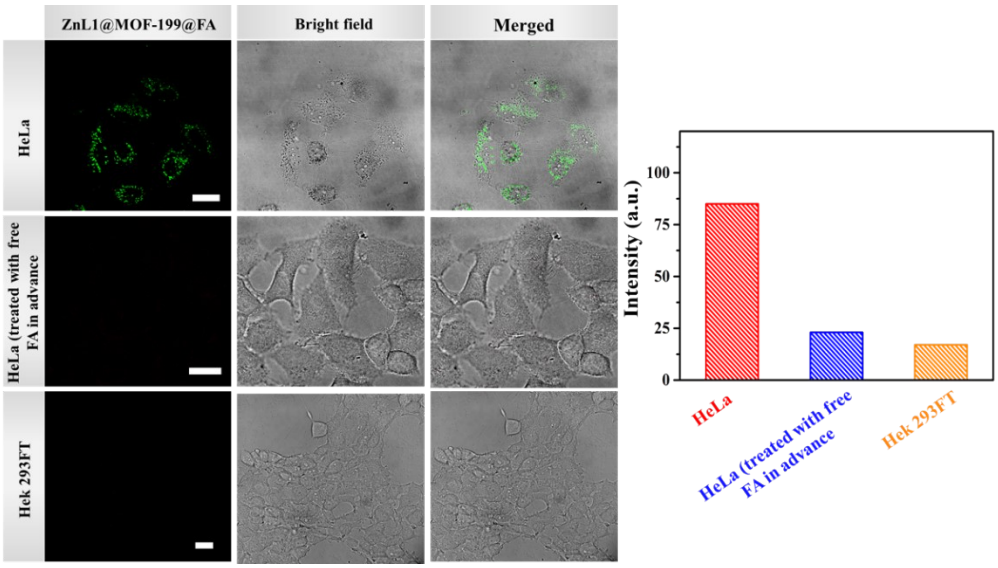

Fig. S12: Confocal images to check the cell uptake of **ZnL1@MOF-199@FA**.

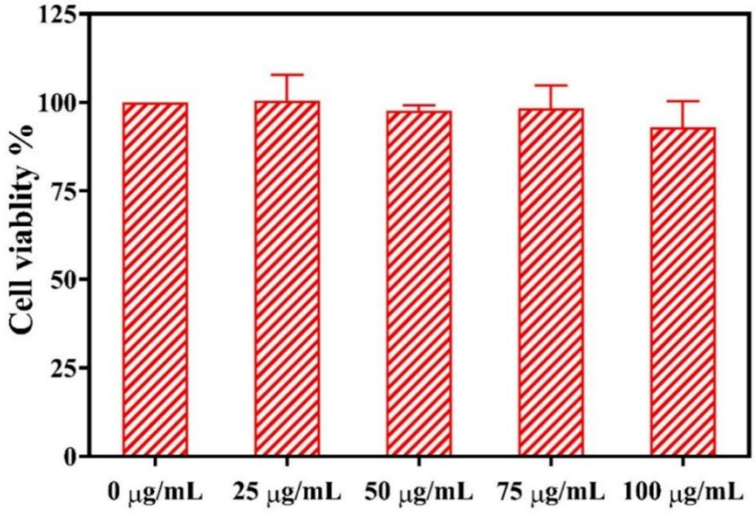

Fig. S13 Cell viability of the **ZnL1@MOF-199@FA** nanoparticles in QSG-7701 cells after 12 h of incubation with different treatments.

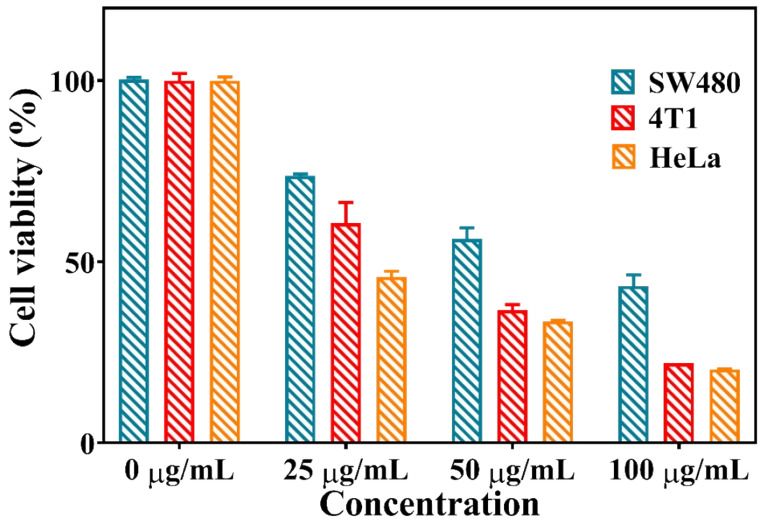

Fig. S14 Cell viability of the **ZnL1@MOF-199@FA** nanoparticles in HeLa, 4T1 and SW480 cancer cells after 12 h of incubation with different treatments.

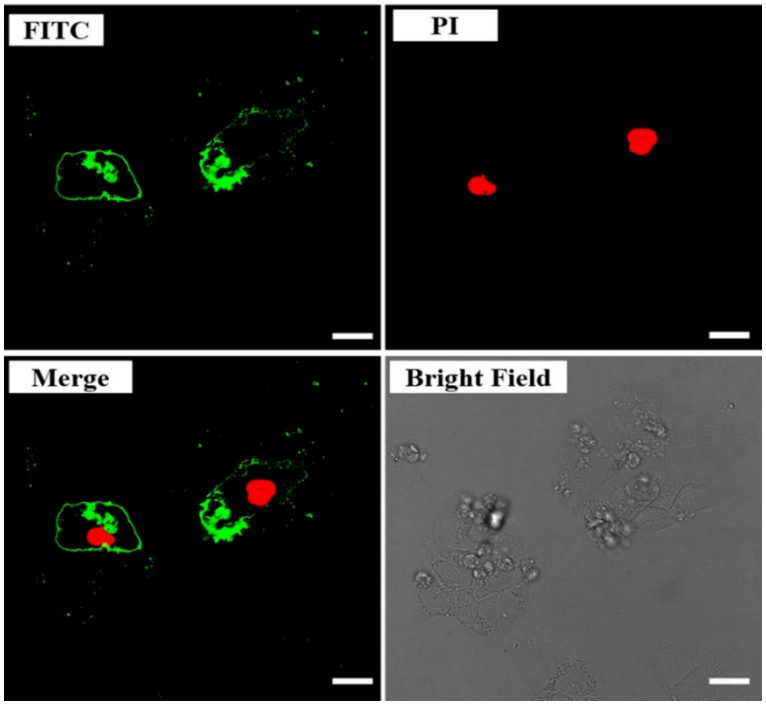

Fig. S15 CLSM images of HeLa cells incubated with **ZnL1@MOF-199@FA** for 12h (Annexin V-FITC / PI were indicators of apoptosis. scale bar: 20 μm.).