Supplementary Information

Covalent organic framework-engineered polydopamine nanoplatform for multimodal imaging-guided tumor photothermal-chemotherapy

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1. Reagents and Materials. Ammonia aqueous solution (NH_4OH, 28-30%), ethanol, dimethyl sulfoxide (DMSO) and N, N-carbonyl diimidazole (CDI) were purchased from China National Pharmaceutical (Shanghai, China). Dopamine hydrochloride (99%), Doxorubicin hydrochloride, 1,3,5-Tris(4-aminophenyl)benzene and 2,4,6-Triformyl phloroglucinol were purchased from Energy Chemical Technology (Shanghai, China) Co., Ltd. Folic acid was purchased from Beijing Bailing Technology Co., Ltd. F127, IR808 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Company. Annexin V-Alexa Fluor 488/PI Apoptosis Detection Kit was purchased from Yeasen (Shanghai, China). The mouse breast cancer cell line (4T1) was purchased from Shanghai AOLU Biological Technology Co., Ltd, China. Female Balb/c mice (6-8 weeks, 20 g) were obtained from SiPeiFu Beijing Biotechnology Co., Ltd. Fetal bovine serum (FBS) in cell culture medium was purchased from Gibco**.** Confocal dish was purchased from Cellvis, Mountain View, CA. All aqueous solutions were prepared using sartorius ultrapure water (18.2 $M\Omega \cdot cm^{-1}$). All chemicals were of analytical grade and were used without further purification.

2. Instruments. Transmission electron microscopy (TEM, HT7700, Japan) was carried out to characterize the morphology of the nanoparticles. The Zeta potential was monitored with a Malvern Zeta Sizer Nano (Malvern Instruments). Fluorescence spectra were obtained with FLS-980 Edinburgh. UV-Vis absorption spectra were achieved with UV-1700 (Shimadzu, Japan). Fourier infrared spectrometer (Nicolet iS50 FT-IR) was used to characterize the FT-IR spectra. Confocal fluorescence imaging assay were performed by a TCS SP8 confocal laser scanning microscope (Leica, Germany). In vivo fluorescence imaging experiments were conducted using Bioluminescent Living Imager (IVIS Lumina III, USA). MTT assay was performed in a microplate reader (Synergy 2, Biotek, USA). All pH measurements were performed with a pH3c digital pH meter (Shanghai LeiCi Device Works, Shanghai, China) equipped with a combined glass-calomel electrode. Photoacoustic images were obtained at 808 nm using a PA tomography system (Endra Nexus 128, Ann Arbor, MI). The sections were observed through a Nikon Eclipse 80i microscope.

3. Experimental Section

Preparation of Polydopamine (PDA) Nanoparticles. 335 μL of ammonia aqueous solution (NH_4OH, 28-30%) was added into the mixture of 4 mL of ethanol and 9 mL of water and stir evenly in 30℃ for 30 min. Dopamine hydrochloride (50 mg) dissolved in 1 mL of water was added to the above mixture. The solution immediately turned light yellow and gradually turned dark brown, the mixture was stirred for 8 hours. Next, the solution was centrifuged(15000rpm,10min) and washed three times with water to collect the PDA NPs. Finally, it was re-dispersed in water and stored at 4℃ for further use.

Preparation of PDA@COF. 2,4,6-Triformylphloroglucinol (TP, 0.003 mmol) and 1,3,5-Tris(4-aminophenyl) benzene (Tab, 0.004mmol) were respectively dissolved in 1 mL of ethanol and then alternatively added into PDA (0.2 mg/mL, 10 mL) solution drop by drop. The mixture was stirred at room temperature for 8 hours. Finally, the PDA@COF NPs were separated by centrifugation and washed with ethanol for three times (15000rpm,10min), the final precipitation was re-dispersed in water and stored at 4℃ for further use.

Fluorescence curves of Dox. Dox solutions with a concentration gradient of 0.5-3 μg/mL were prepared, and the fluorescence spectra from 530-700 nm were recorded to draw the standard curve. λ_{ex} = 596 nm.

Preparation of PDA@COF@Dox and PDA@Dox. Dox (1 mg) was mixed with 1 mL of PDA@COF aqueous solution (1 mg/mL) and stirred at 30 °C for 8 h. The PDA@COF@Dox nanoparticle**s** were collected by centrifugation (15000rpm, 10min) and washed with water for three times. PDA@Dox was prepared via the same procedure, excepted that PDA@COF was replaced by PDA NPs.

Calculation of the drug loading efficiency. To determine the drug carrying capacity of the drug carriers, PDA@COF@Dox and PDA@Dox solutions were placed in a water bath (80 °C) and incubated for 30 min and then centrifuged (15000 rpm,10min). After the supernatant was sucked out, the precipitate was dissolved back in 1 mL of deionized water. The above release process was performed at least twice to ensure the complete release of Dox. The fluorescence intensity of supernatant was tested, and the

respective drug loading capacities were calculated according to Dox standard curve.

Preparation of PDA@COF@Dox/IR808. The PDA@COF@Dox NPs (1 mg) were mixed with IR808 (0.1 mg) in 1 mL of water under mild stirring at 30 °C for 8 h. The PDA@COF@Dox/IR808 was collected through centrifugation and washed with water for three times, and the NPs were finally re-dispersed in water for the further use.

Synthesis of FA-F127. Folic acid (87.58 mg, 0.2 mmol) was dissolved in the dried dimethyl sulfoxide (DMSO, 5mL) and then N, N-carbonyl diimidazole(CDI, 35.53 mg，0.2 mmol) was added into the solution and stirred at room temperature under dark for 24 h. Then, F127 (620 mg, 0.05 mmol) was added to the above reaction solution, further reacted for 24 h. Subsequently, the solution was transferred into dialysis bag (MWCO3500) and dialyzed with water for 3 days, the solution was changed every 4 h. The resulting product was freeze-dried and then vacuum-dried to obtain the yellow product.

Preparation of PDA@COF@Dox/IR808@FA-F127. The PDA@COF@Dox/IR808 nanosphere (1mg/mL, 5mL) was mixed with FA-F127 (1 mg) and stirred at 30 °C for 8 h. The final nanoprobe (PDA@COF@Dox/IR808@FA-F127) was collected by centrifugation (15000rpm, 10min), and washed with water for three times and the final solution was stored at 4℃ for further use.

Evaluating the photothermal performance of PDA@COF@Dox/IR808@FA-F127. For evaluating the photothermal conversion performance of PDA@COF@Dox/IR808@FA-F127, 1 mL of PDA@COF@Dox/IR808@FA-F127 solutions with different concentrations (0.05 mg/mL，0.1 mg/mL，0.2 mg/mL) in a quartz cuvette were irradiated with 808 nm NIR laser (2 W/cm^2) for 620 s, and the temperature was recorded every 20 seconds.

Test drug leakage effects of PDA@Dox and PDA@COF@Dox. For testing the drug leakage of PDA and PDA@COF, a series of the PDA@Dox and PDA@COF@Dox solutions (1 mg/mL) were prepared. The solutions were stored at room temperature. At 0,2,4,6,8,12,24 h, the supernatants were collected and the released Dox was detected and calculated by fluorescence standard curve.

The cumulative release curves of Dox from PDA@COF@Dox nanoparticles under different conditions. To test the Dox release effect of PDA@COF@Dox, a series of identical nanoprobes were dispersed in different PBS (pH=6.6, 7.4) and stirred at room temperature. The fluorescence intensity of the supernatants of each sample was measured at different time periods (0-8 h). The released Dox was calculated with the standard curves. To further stimulate the NIR controlled release of Dox, 808 nm laser (1 W/cm^2) was employed to irradiate the solutions of PDA@COF@Dox (pH= 6.6) for several periods. In each cycle, the solution was exposed to an 808 nm laser for 15 min, then the supernatants were detected. Then the samples were stored at room temperature and stirred under dark for 2 h, and then the fluorescence intensity was measured. The above experiments were repeat for three times.

Cell culture. 4T1 cells were incubated in cell culture dishes with a diameter of 10 cm containing RPMI 1640 supplemented with 10 % (v/v) fetal bovine serum (FBS), 1 % penicillin and streptomycin at 37 °C in a humidified atmosphere containing 5 % CO_2.

NIR-triggered Dox release in cancer cells. 4T1 cells were seeded in confocal dishes. When the density of the cells reached about 80%, they were incubated with PDA@COF@Dox. 3 h later, the cells were washed with PBS and imaged with CLSM. Then the cells were further irradiated with 808 nm laser (1 W/cm^2) for 5 min, and further analyzed with PBS. The fluorescence intensity of the images was analyzed with Image J, and the experiments were repeated for three times.

MTT assay. For the cell toxicity assay, 4T1 cells were seeded onto a 96-well plate (10^4 per well) and cultured for 24 h. The cells were divided into five groups: PBS, PDA @COF@F127-FA, PDA @COF@F127-FA+Light, Nanoprobe and Nanoprobe+Light. Each group was incubated in 1640 medium containing the material (25, 50, 75, 100 μg/mL) for 6 hours at 37℃ and 5% CO_2 on a 96-well plate. Subsequently，fresh medium was added to the 96-well plate. The control group was PBS group. For the laser irradiation groups, cells were irradiated with 808 nm near-infrared laser (1 W/cm^2) for 5min. All the groups were further incubated for 12 h. Then the culture medium was removed and 200 μL MTT (0.5 mg/mL) was added into

the wells and incubated at 37 °C for 4 h. After removing the MTT medium, 200 μL of DMSO was added to each well. The absorbance was measured at 490 nm by microplate reader.

Live/dead cell staining assay. 4T1 cells were seeded in confocal dishes and cultured for 24 h. The cells were divided into five groups: PBS, PDA@COF@F127-FA, PDA@COF@F127-FA+Light, Nanoprobe and Nanoprobe+Light. When the cell density approached about 80%, each group was incubated in 1640 culture medium containing the corresponding material (100 μg/mL) for 6 h, and then cultured in fresh culture medium. The control group was PBS group, the laser irradiation group was irradiated with 808 nm near-infrared laser (1 W/cm^2) for 5 min. All the groups were further incubated for 12 h after treatments. Subsequently, each group of cells were stained with live/dead cell staining assay kit and further imaged on a laser scanning confocal microscope.

Flow cytometry analysis of apoptosis. 4T1 cells were cultured in cell dishes for 24 h. The cells were divided into five groups: PBS, PDA@COF@F127-FA, PDA@COF@F127-FA+Light, Nanoprobe and Nanoprobe+Light. When the cell density approached about 80%, each group was incubated in 1640 culture medium containing the corresponding material (100 ug/mL) for 6 h, and then cultured in fresh culture medium. All the cells were further cultured for another 6 h. For laser irradiation groups, 808 nm laser (1 W/cm^2) was employed to irradiate the cells for 5 min. Subsequently, the cells were washed with PBS thrice and treated with Annexin V-FITC/PI for 20 min. Then the cells were analyzed by flow cytometry.

In vitro targeting capability of the nanoprobe. 4T1 cells were inoculated in confocal dishes. After cell density reached 80%, the cells were divided into four groups. Two of the groups were preincubated with 1640 medium containing folic acid (100 ug/mL) for 6 h, and the medium was sucked away. Subsequently the two groups were incubated respectively with 1640 medium containing PDA@COF@Dox/IR808 (100ug/mL) and PCDIF (100ug/mL) for 6 h. Finally, the cells were cultured in fresh medium. The other two groups were incubated directly with 1640 medium containing PDA@COF@Dox/IR808 (100ug/mL) and PCDIF (100ug/mL) for 6 h. Finally, it was

cultured with fresh medium and analyzed with laser confocal microscope.

In vivo experiments. All animal experiments were conducted and agreed with the Principles of Laboratory Animal Care (People's Republic of China) and the Guidelines of the Animal Investigation Committee, Biology Institute of Shandong Academy of Science, China. Balb/C female mice (4-6 weeks old, about 20 g) were fed with normal conditions of 12 h light and dark cycles and given access to food and water adlibitum. Take 4T1 breast cancer model as an example to evaluate the treatment effect. Subcutaneously injected 100 μL serum-free 1640 culture medium containing $1x10^7$ 4T1 cells into the right axillary region of Balb/ C mice. After the tumor size had reached approximately 75-100 mm^3, the mice were used in subsequent experiments.

In vivo fluorescence imaging. In vivo fluorescence imaging was performed to compare the tumor targeting effect of NPs w/wo F127-FA modification. When the tumor volume was approximately 100 mm^3, 50 μL of 4 mg/mL Nanoprobe or PDA@COF@Dox/IR808 were i.v. administrated into the 4T1 tumor bearing Balb/c mice. At different time points of 0,3, 9, 12 and 24 h post injection, the IR808 fluorescence in the mice was recorded with a live body imaging system.

In vivo distribution experiment. 4T1 tumor bearing mice were intravenously injected with Nanoprobe or PDA@COF@Dox/IR808 (50 μL,4 mg/mL). At the time points of 12 h the mice were sacrificed and dissected. Then, in vivo imaging systems were used to record IR808 fluorescence of tumors and major organs.

In vivo photothermal imaging. To further determine the tumor targeting effect of the nanoprobe, in vivo photothermal imaging was performed. The mice were intravenously injected with 50 μL of nanoprobe (4 mg/mL). At different time points (0, 3, 6, 9, 12 and 24 h) post injection, 808 nm NIR laser (1 W/cm^2) was used to irradiate the tumor area for 10 min, and the tumor temperature of the mice was recorded by infrared camera after the irradiation.

In vivo PA imaging assay. In vivo PA imaging was performed to further determine the in vivo tumor targeting effect of the nanoprobe. 4T1 tumor bearing mice were intravenously injected with 50 μL of nanoprobe (4 mg/mL). Subsequently, PA

imaging was conducted at different time points (0, 3, 6, 9, 12, 24 h) after injection.

In vivo drug release. To further determine the NIR responsive drug release effect of the nanoprobe, in vivo drug release experiments were carried out. The mice were divided into two groups, each group was intratumorally injected with 10 μL of PCDIF (4 mg/mL, PBS solution). 1 h later, the in vivo fluorescence signal of IR808 was collected by the live body imaging system. After 2 h post injection, the laser irradiation group was treated with 808 nm laser for 10 min, and the mice in the two groups were further imaged by the live body imaging system. The fluorescence signal changes were compared by fluorescence intensity quantification. The fluorescence signals for Dox in the tumor slices with the same treatments were also collected by CLSM.

Evaluation of in vivo antitumor efficacy. 4T1 tumor-bearing Balb/c mice were divided into five groups: PBS, PDA @COF@F127-FA, PDA @COF@F127-FA+Light, Nanoprobe and Nanoprobe+Light. The light group were irradiated with 808 nm (1 W/cm^2) laser for 10 min at 12 h after injection intravenously. Tumor volume and body weight (tumor volume =$W^2 \times L/2$, W= width, L= length) were measured every 2 days after treatment for 12 days.

4. Supplementary data

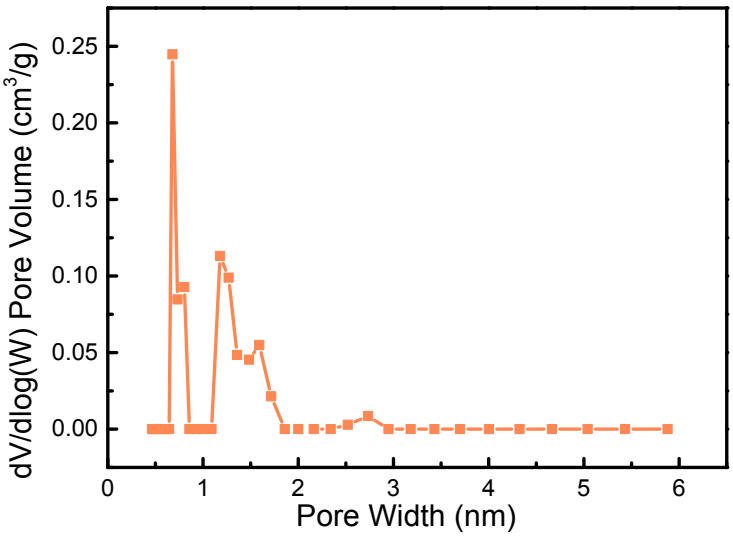


Figure S1. The pore width distribution of PDA@COF obtained by N_2 adsorption and desorption experiment and BET analysis.

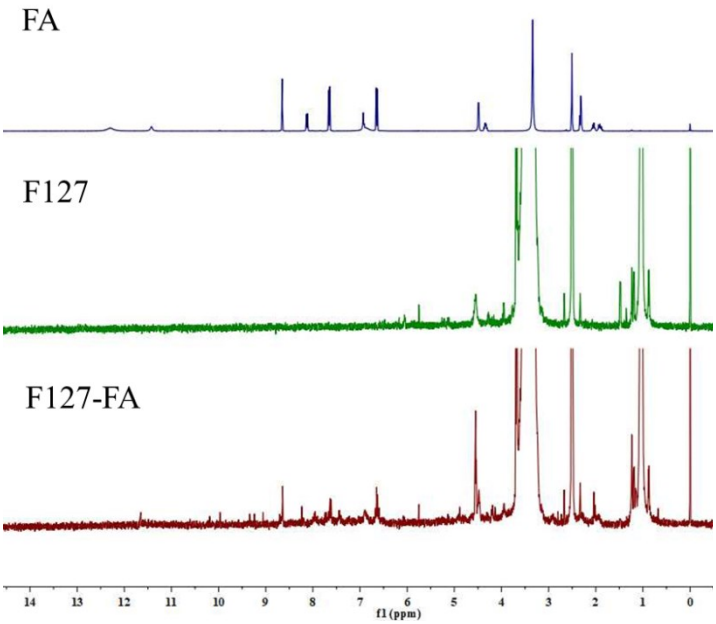


Figure S2. The ^1H NMR spectra of FA, F127 and F127-FA.

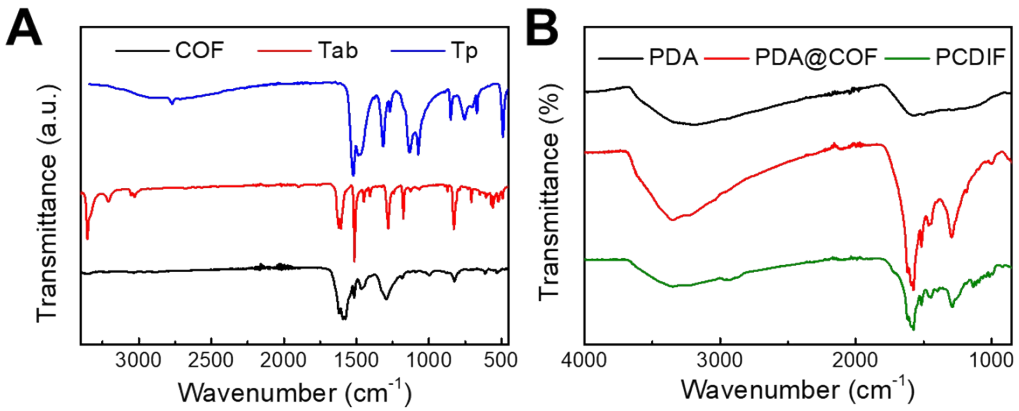
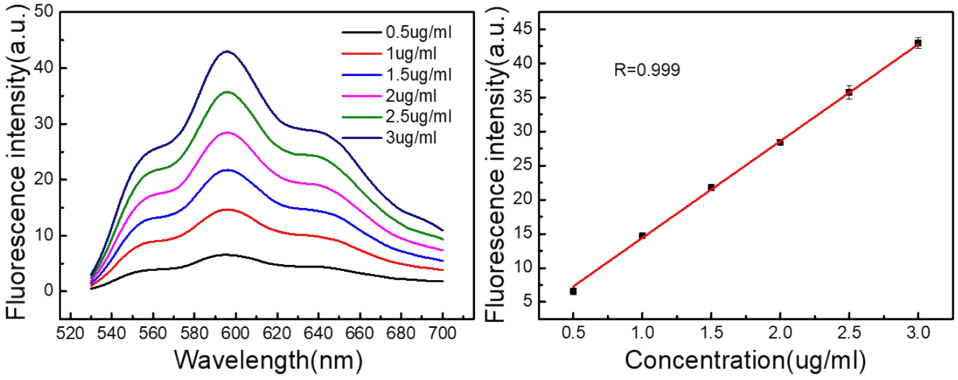


Figure S3. The FT-IR spectra of (A) COF, Tab, Tp and (B) PDA, PDA@COF, PCDIF.



	PDA	PDA@COF
Loading (mg/mg)	0.307	0.927
Loading efficiency (%)	30.7	92.7

Figure S4. The fluorescence spectra of Dox with different concentrations, the related standard curves of Dox and the Dox loading capacity of PDA and PDA@COF.

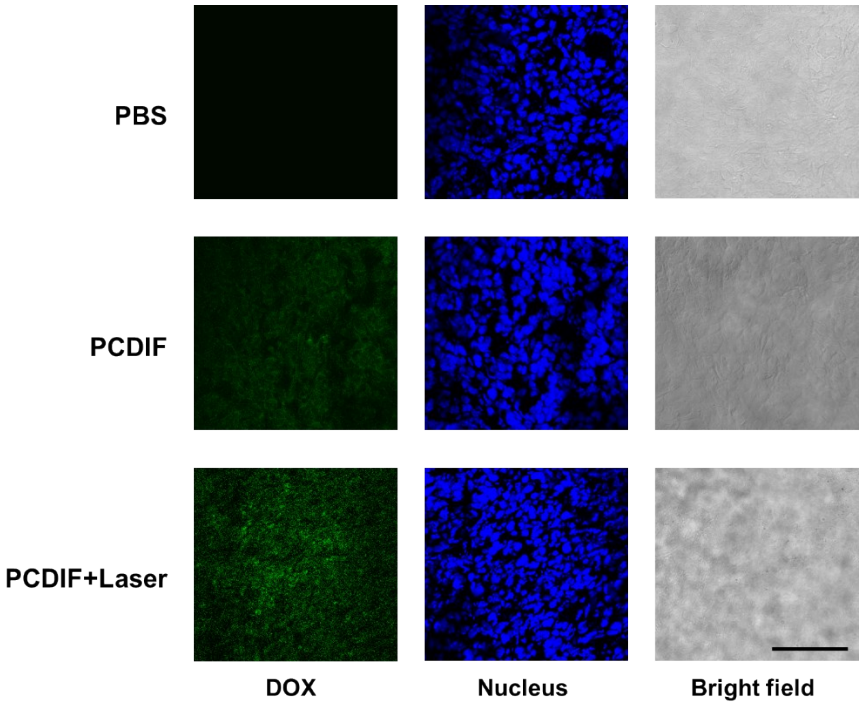


Figure S5. The confocal images Dox in tumor slices with different treatments.

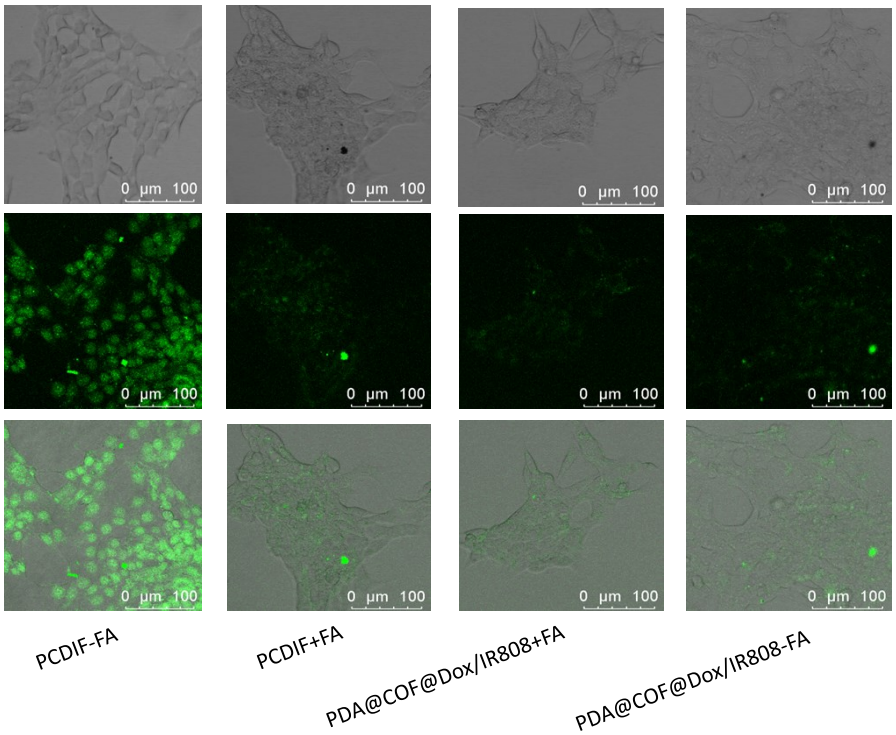


Figure S6. Confocal images of 4T1 cancer cells co-incubated with PCDIF and PDA@COF@Dox/IR808 with (+) or without (-) FA pretreatment.

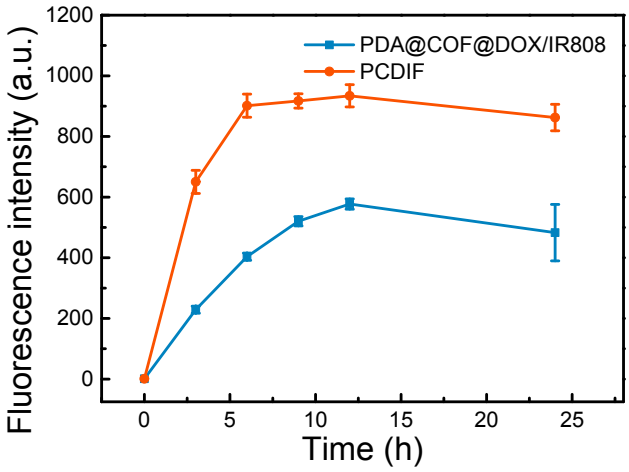


Figure S7. The fluorescence intensities of the tumor region of mice at different timepoints post i.v. injection of PDA@COF@Dox/IR808 or PCDIF.

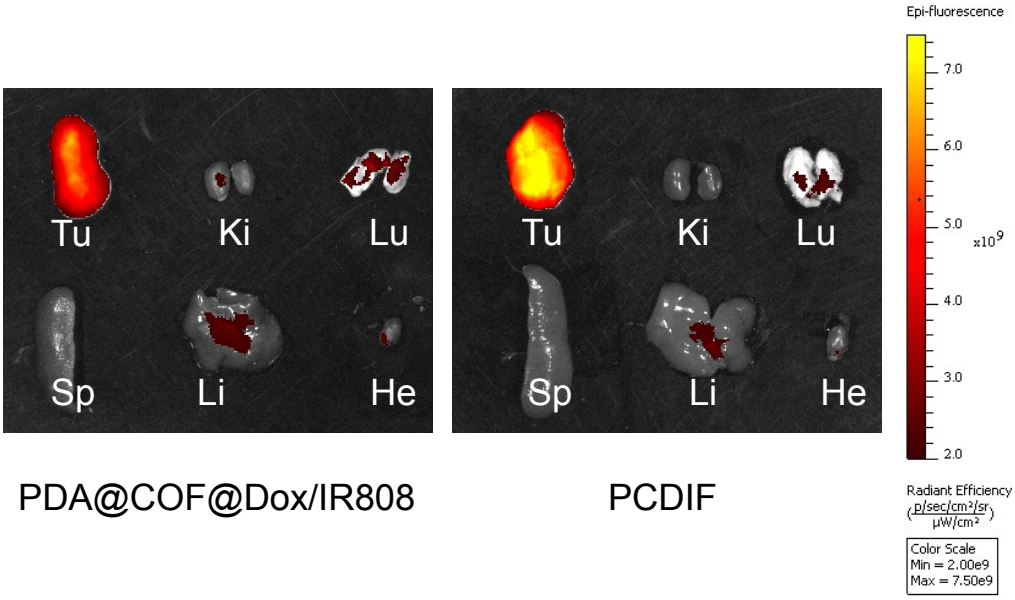


Figure S8. The drug distribution of PDA@COF@Dox/IR808 and PCDIF after intravenous injection at 12 h.

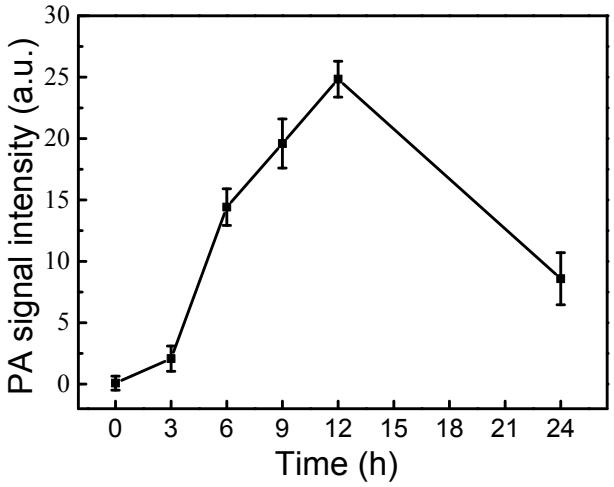


Figure S9. The PA signal intensities of the tumor region of mice at different timepoints post i.v. injection of PCDIF.

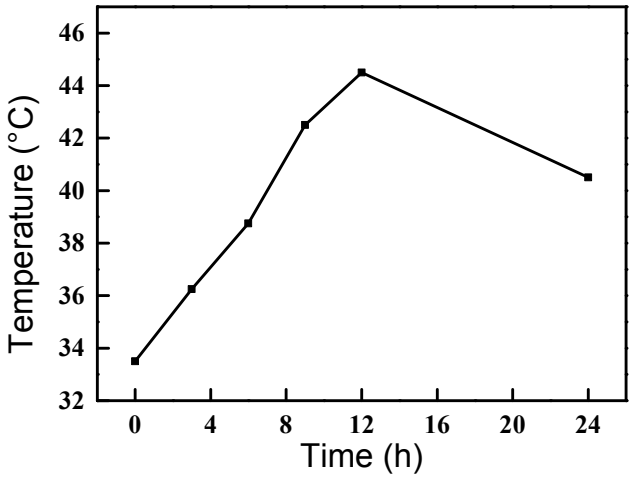


Figure S10. The tumor temperatures of the mice at different timepoints post i.v. injection of PCDIF under the exposure of 808 nm laser (1 W/cm^2, 10 min).

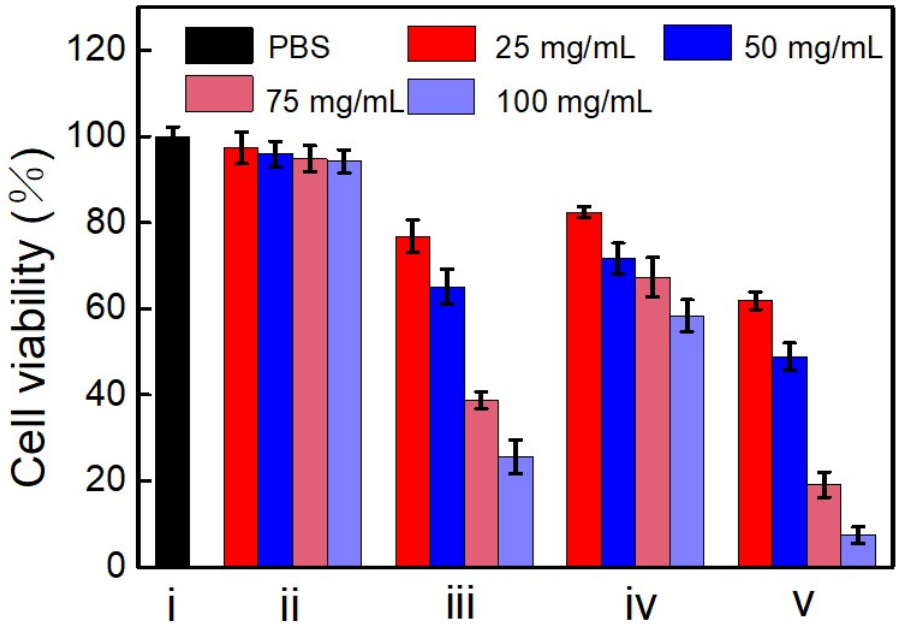


Figure S11. Cell viabilities of 4T1 cancer cells with different treatments characterized by the MTT assay. i: PBS, ii: PDA@COF@F127-FA, iii: PDA@COF@F127-FA+laser, iv: PCDIF, v: PCDIF+laser.

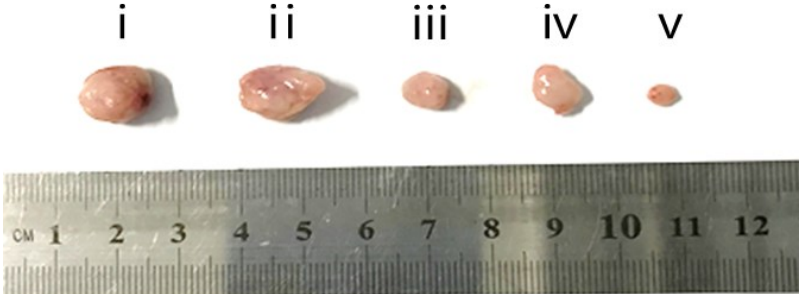


Figure S12. Photographs of representative tumor tissue dissected form mice with different treatments at day 12. i: PBS, ii: PDA@COF@F127-FA, iii: PDA@COF@F127-FA+laser, iv: PCDIF, v: PCDIF+laser.

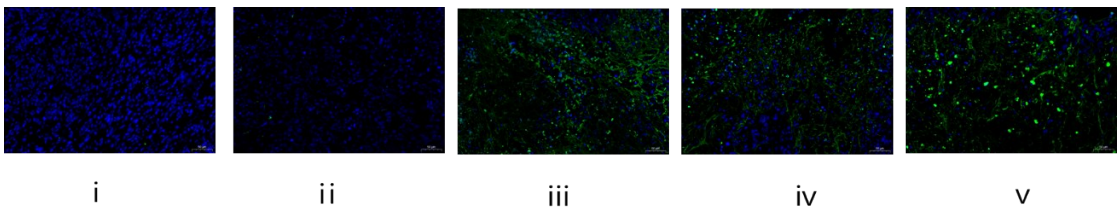


Figure S13. TUNEL staining of the tumor tissues of mice with different treatments. i: PBS, ii: PDA@COF@F127-FA, iii: PDA@COF@F127-FA+laser, iv: PCDIF, v: PCDIF+laser.

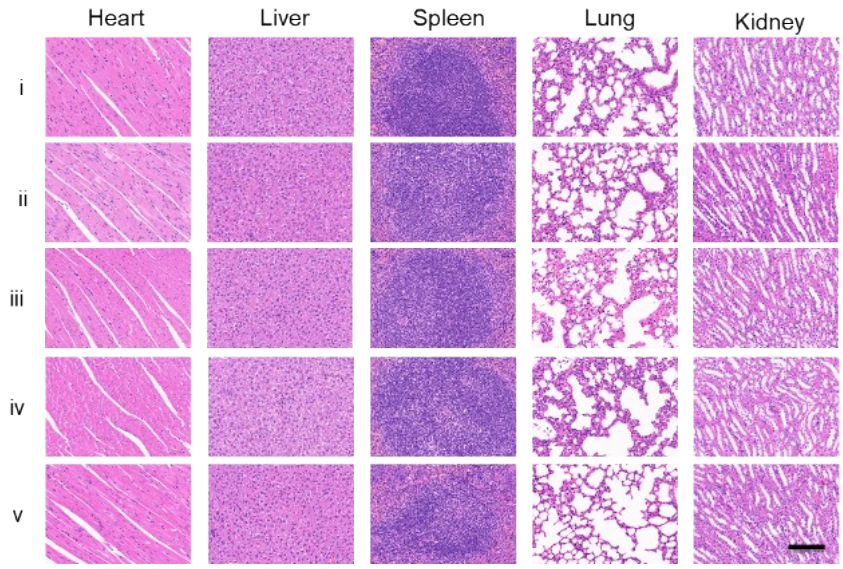


Figure S14. H&E staining of the main organs of mice with different treatments. i: PBS, ii: PDA@COF@F127-FA, iii: PDA@COF@F127-FA+laser, iv: PCDIF, v: PCDIF+laser. Scale bars are 100 μm