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Supplementary Information

A hypoxia-activated photothermal agent inhibits multiple heat shock proteins for low-temperature photothermal therapy

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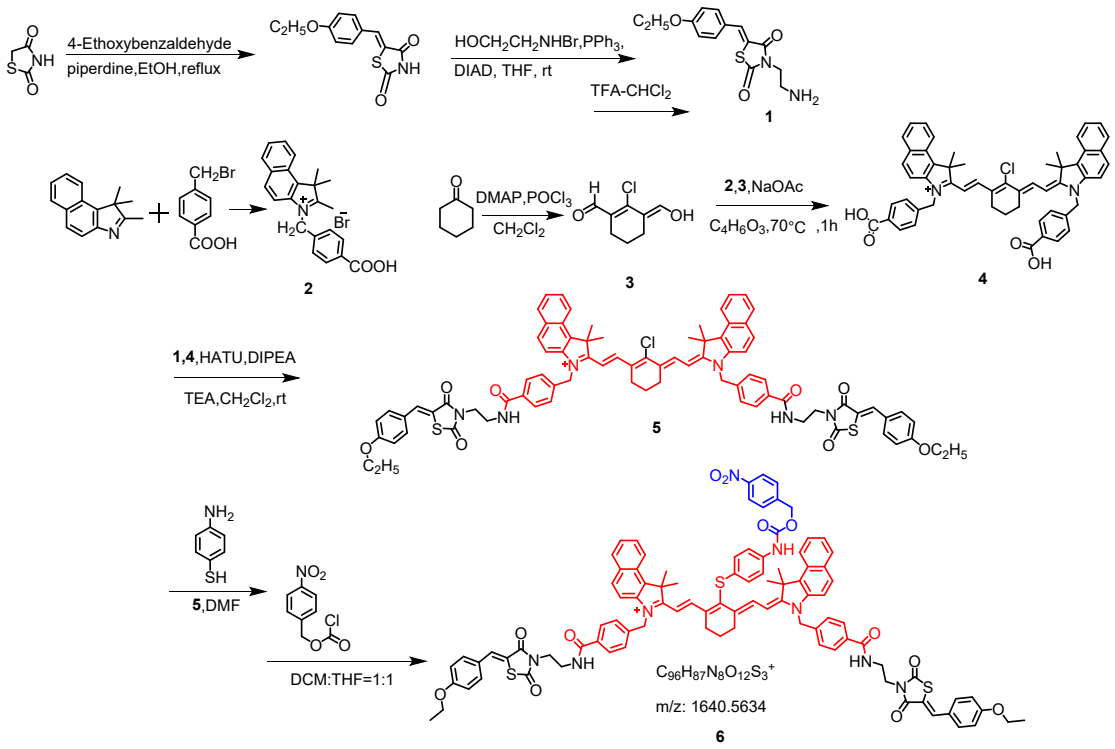
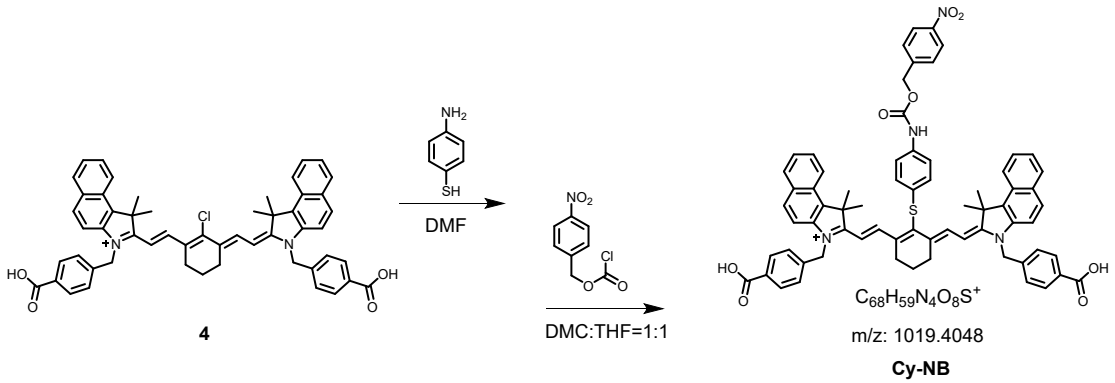
Experimental Procedures

Reagents. Alpha-bromo-*p*-toluic-acid, 2,3,3-trimethyl-4,5-benzo-3H-indole, 4-Aminothiophenol, 2,4-Thiazolidinedione, 4-Ethoxybenzaldehyde, tert-Butyl N-(2-bromoethyl) carbamate, 4-Nitrobenzyl chloroformate, dicumarin and nicotinamide adenine dinucleotide (NADH) were obtained from Tianjin Heowns Biochemical Technology Co., Ltd. Nitroreductase (NTR) was purchased from Sigma-Aldrich. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2h-tetrazoliubromide (MTT) was obtained from Beijing Solarbio Science & Technology Co., Ltd. Annexin V-Alexa Fluor 488/PI Apoptosis Detection Kit was purchased from Yeasen (Shanghai, China). Antibody against HSP70/27/90 was purchased from Boster Biological Technology Co., Ltd. Acetonitrile, anhydrous diethyl ether, dichloromethane (DCM), dichlorobenzene, toluene, acetic anhydride dimethyl sulfoxide (DMSO) and triethylamine (TEA) were purchased from China National Pharmaceutical (Shanghai, China).

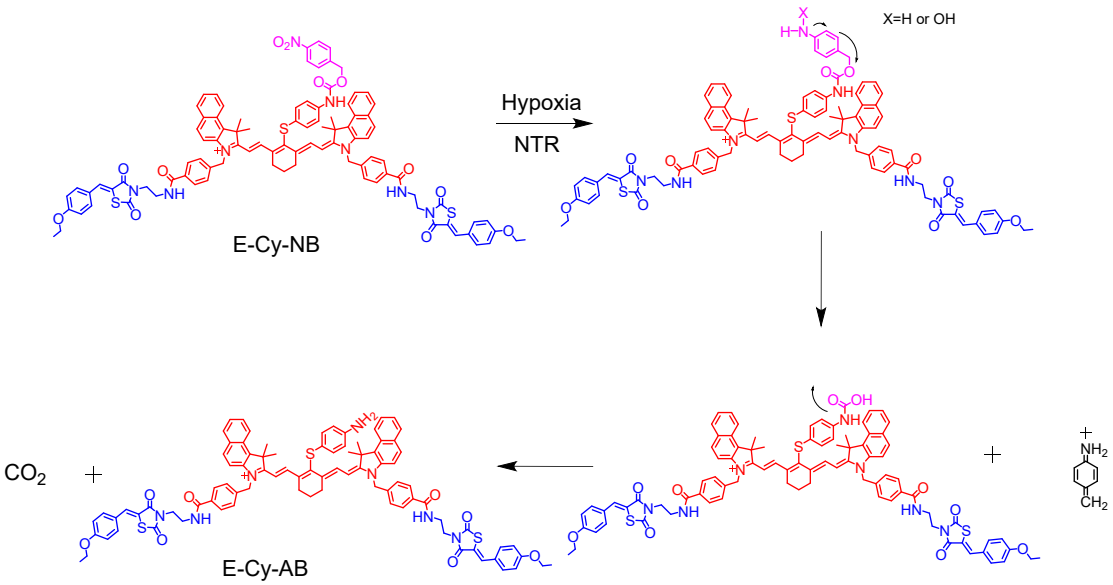
Instruments: Fluorescence spectra were acquired with fluorescence spectrometer (FLS-980, Edinburgh, UK). UV-vis absorption spectra were measured on a pharmaspec UV-1700 UV-Visible spectrophotometer (Shimadzu, Japan). Confocal fluorescence imaging studies were performed with a TCS SP8 confocal laser scanning microscopy (Leica, Germany). Absorbance was measured in a microplate reader (Synergy 2, Biotek, USA) in the MTT assay. All the nuclear magnetic resonance (NMR) spectra were recorded on a Bruker NMR spectrometer. High resolution mass spectra (HRMS) were recorded on a Bruker Daltonics maXis UHR-TOF mass spectrometer.



Scheme S1 Synthesis of E-Cy-NB.



Scheme S2 Synthesis of Cy-NB.



Scheme S3 From E-Cy-NB to E-Cy-AB.

Synthesis of compound 1 (ERK inhibitor): The compound 2 was carefully prepared according to the references.[1] 2,4-thiazolidinedione (1.9 g, 16.0 mmol) and 4-ethoxybenzaldehyde (1.4 mL, 10.0 mmol) were added in 100.0 mL anhydrous C_2H_5OH in 250 mL flask at room temperature. After that, piperidine (0.3 mL, 3.0 mmol) was put into the mixed solution. After stirred at 75 °C for 20 h, the solution was cool down to room temperature (r.t.). The tan crystalline precipitate ((Z)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione) was formed and collected after bring washed with water and dryed. (Z)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione (249.0 mg, 1.0 mol), K_2CO_3 (166.0 mg, 1.2 mmol), tetrabutylammonium iodide (TBAI, 37.5 mg, 0.1 mmol) and tert-butyl N-(2-bromoethyl) carbamate (336.0 mg, 1.5 mmol) were added into 50 mL anhydrous THF at 24 °C. The mixed solution was keep stirring for 10 h. After cooled down to r.t., the yellow crystalline precipitate was formed. The product was filtrated, washed using water, and finally dissolved in the mixed solution of DCM/TFA (4:1, v/v). Then, keep the solution stirred at r.t. for 1 h. The mixed DCM solvent was removed by rotary evaporator (60% yield). The compound **1** was collected after filtrating, washing with ethyl ether and drying. ^{1}H-NMR (400 MHz, d^6-DMSO) δ 8.00 (bs, J = 4.0 Hz, 2H), 7.88 (s, 1H), 7.59 (d, J = 4.0 Hz, 2H), 7.10 (d, J = 8.0 Hz, 2H), 4.14-4.11 (m, 2H), 3.93-3.90 (m, 2H), 3.11-3.08 (m,

2H), 1.37-1.32 (m, 3H); HRMS (ESI): *m/z* calcd for $C_{14}H_{17}N_2O_3S^+$: 293.0954, found: 293.0979.

Synthesis of compound 2: The compound 2 was carefully prepared according to the references.[2] The 2,3,3-trimethyl-4,5-benzo-3H-indole (0.5 g, 2.5 mmol) and alpha-bromo-*p*-toluic acid (0.8 g, 4 mmol) were mix up within 3.0 mL acetonitrile. After stirred at 80 °C for 5 h, the unpurified compound was then dissolved in 6.0 mL of DCM. Anhydrous diethyl ether was added. The desired product was gradually precipitated. The purified product **2** was dried under vacuum (50% yield).

Synthesis of compound 3: The compound 3 was carefully prepared according to the references.[2] Phosphoryl chloride (37 mL) and anhydrous DCM (35 mL) were put into a mixture solution of DMF/DCM (1/1 v/v, 80 mL) and stirred under ice/water bath. Cyclohexanone (10.0 g) was dropped into the above solution. Then, the ice/water bath was removed. And the mixed solution was heating reflux for 5 h. After that, *ca.* 500 g small ice was added into the solution and staid for one night. After filtrating, washing with iced diethyl ether and drying, the product was get. The orange product was denoted as **3** and used for the following steps (60% yield).

Synthesis of compound 4: The compound 4 was carefully prepared according to the references.[2] Under the protection of argon, 2 (0.5 g, 1.0 mmol), 3 (86.5 mg, 0.5 mmol), and CH_3COONa (82.0 mg, 1.0 mmol) were mixed with 10mL acetic anhydride in a 50 mL reaction flask. The mixed solution was kept heating at 70 °C for 1 h. The mixture was cooled to r.t.. The crude product was formed through removed the ethanol under reduced pressure. The crude product was purified through silica gel chromatography methods, using the DCM/CH_3OH (v/v, 10:1) as the eluent (50% yield). ^{1}H-NMR (400 MHz, d^6-DMSO) δ 8.35 (t, J = 16.0 Hz, 4H), 8.10 (d, J = 20.0 Hz, 4H), 7.99 (d, J = 32.0 Hz, 5H), 7.74-7.67 (m, 5H), 7.59-7.50 (m, 2H), 7.49 (d, J = 36.0 Hz, 4H), 6.63 (d, J = 24.0 Hz, 2H), 5.75 (s, 4H), 2.83-2.73 (m, 4H), 2.01 (s, 12H), 1.91 (s, 2H); HRMS (ESI): *m/z* calcd for $C_{54}H_{48}ClN_2O_4^+$: 823.3297, found: 823.3233.

Synthesis of compound 5: The **1** (438.0 mg, 1.5 mmol), **4** (450.0 mg, 0.5 mmol), *N,N*-diisopropylethylamine (185 μL, 1.0 mmol) and HATU (190.0 mg, 0.5 mmol) were mix up in DCM (10 mL). After stirred at 24 °C for 2 h, the DCM was removed

under vacuum. The crude product was purified by silica gel chromatography, in which the DCM/CH_3OH (v/v, 20:1) as the eluent (50% yield).

Synthesis of compound 6 (E-Cy-NB): Under the protection of argon, **5** (0.4 g 0.3 mmol) and 4-aminothiophenol (0.2 g 1.3 mmol) were added in 10 mL DMF and stirred under 24 °C for 5 h. Then, the DMF solvent was removed under vacuum. The purified product was obtained by silica gel chromatography, in which the DCM/CH_3OH (v/v, 20:1) as the eluent (80% yield). Then the product (0.4 g, 0.3 mmol) and triethylamine (0.2 mL, 2.5 mmol) were added slowly into a mixture solution of DMF/THF (1/1 v/v, 8 mL) and stirred under ice/water bath for 1 h. Next, 4-nitrobenzyl chloroformate (0.3 g, 1.5 mmol) was added into the mixed solution. After stirred at 0 °C for 7 h, the solvent was removed under vacuum. The purified product was obtained by silica gel chromatography, in which the DCM/CH_3OH (v/v, 10:1) as the eluent (40% yield). ^{1}H-NMR (400 MHz, d^6-DMSO) δ 9.92 (s, 1H), 8.77 (d, J = 10.0 Hz, 2H), 8.26 (s, 6H), 8.04 (d, J = 12.0 Hz, 5H), 7.82 (s, 2H), 7.72-7.65 (m, 12H), 7.52 (s, 12H), 7.35-7.28 (m, 6H), 7.06 (d, J = 12.0 Hz, 3H), 6.38 (d, J = 12.0 Hz, 2H), 5.66 (s, 4H), 5.32 (s, 1H), 5.23 (s, 1H), 4.22 (s, 1H), 4.08-4.06 (m, 3H), 3.82 (d, J = 8.0 Hz, 3H), 1.90 (s, 2H), 1.82 (s, 10H), 1.31-1.29 (m, 5H), 1.23 (s, 6H); HRMS (ESI): *m/z* calcd for $C_{96}H_{87}N_8O_{12}S_3^+$: 1640.5634, found: 1640.5865

Synthesis of Cy-NB: Under the protection of argon, 4 (450.0 mg, 0.5 mmol) and 4-aminothiophenol (0.2 g 1.3 mmol) were added in 5 mL DMF. After stirred at 24 °C for 3 h, the DMF solvent was removed under vacuum. The purified product was obtained by silica gel chromatography, in which the DCM/CH_3OH (v/v, 5:1) as the eluent (75% yield). Then the product (0.3 g, 0.3 mmol) and triethylamine (0.2 mL, 2.5 mmol) were added slowly into a mixture solution of DMF/THF (1/1 v/v, 15 mL) and stirred under ice/water bath for 1 h. Next, 4-nitrobenzyl chloroformate (0.3 g, 1.3 mmol) was added into the mixed solution. After stirred at 0 °C for 6 h, the solvent was removed under vacuum. The purified product was obtained by silica gel chromatography, in which DCM/CH_3OH (v/v, 5:1) as the eluent (45% yield). ^{1}H-NMR (400 MHz, d^6-DMSO) δ 10.04 (s, 1H), 8.26 (s 4H), 8.02 (s, 10H), 7.72 (s, 4H), 7.50 (s, 10H), 5.47 (d, J = 32.0 Hz, 4H), 3.82 (s, 2H), 3.16 (s, 2H), 2.53 (s, 2H), 2.50

(s, 6H), 1.94-1.91 (m, 2H)，1.84 (s, 12H); HRMS (ESI): *m/z* calcd for $C_{68}H_{59}N_4O_8S^+$: 1091.4048, found: 1091.4004.

UV-vis absorbance and fluorescence spectra. The UV-vis absorption spectra of E-Cy-NB in CH_3OH were recorded with the help of UV-vis spectrophotometer. Besides, the fluorescent spectra of E-Cy-NB in DMSO/water were obtained by using fluorescence spectrometer.

Calculation of singlet oxygen generation and quantum Yield. The $^1O_2$ quantum yield was detected according to the literature method.[4] The singlet oxygen ($^1O_2$) generation efficiency of E-Cy-NB/Cy-NB was evaluated by singlet oxygen capture agent, DPBF. Briefly, the absorbance of DPBF at 415 nm was adjusted to about 1.0, and then E-Cy-NB/Cy-NB or methylene blue (MB) was added to the cuvette and the absorbance was adjusted to 0.4 at *ca.* 800 or 660 nm, respectively. The relative quantum yields were calculated with reference to MB in DMSO for which the quantum yield is 0.52.[5] The mixture was then placed in a cuvette and irradiated with a 808 nm light source for different time (0, 2, 4, 6, 8, 10 min), and the corresponding absorption spectra was measured immediately. The slopes of absorbance of DPBF at 415 nm versus irradiation time were measured and used to compare the $^1O_2$ generation ability. The emission maxima of DPBF with different irradiation times were obtained, and the singlet oxygen quantum yields were determined using the following equation:

$$\Phi_{\Delta sam} = \Phi_{\Delta std}\left(\frac{m_{sam}}{m_{std}}\right)\left(\frac{F_{std}}{F_{sam}}\right)$$

where “sam” and “std” designate the “E-Cy-NB/Cy-NB” and “MB”, respectively. “m” is the slope of absorbance attenuation curve of DPBF at 415 nm, and “F” is the absorbance correction factor, which is obtained by $F = 1-10^{-O.D.}$. (O.D. is the absorbance of the solution at 808 nm).

Photothermal performance evaluation of PTA. To evaluate the photothermal ability, the E-Cy-AB solution with different concentrations (0, 25 μM, 50 μM, and 100 μM) was irradiated using 808 nm laser (0.33 W/cm^2). The temperature of solution was monitored with the help of thermometer. To measure the photothermal conversion efficiency, E-Cy-AB solution (50 μM) was exposed to 808 nm irradiation (0.33 W/cm^2) for 300 s. When irradiation was quit, the solution was cool down to

room temperature. Three off/on cycles of it were carried out.

Referring to a previously described method,[3] the photothermal conversion efficiency (η) was calculated.

$$\eta = \frac{hS(\Delta T_1 - \Delta T_2)}{I(1-10^{-A})} \qquad hS = \frac{mc}{\tau_s} \qquad t = -\tau_s \ln(\theta) \qquad \theta = \frac{T - T_{surr}}{T_{max} - T_{surr}}$$

ΔT_1 and ΔT_2 are the maximum temperature changes of sample and H_2O, respectively. I represent the laser power. As known, A is the absorbance of E-Cy-AB (10 μM) at 830 nm. And m and c are the mass and heat capacity of solvent, respectively. T is the temperature at moment t in the cooling process. T_{max} is the maximum temperature of sample. T_{surr} is the surrounding temperature.

Cell culture. 4T1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium. All cells were supplemented with 10% fetal bovine serum (BI) and 100 units/mL of 1% antibiotics penicillin/streptomycin (Gibco) to maintain at 37 °C in a 100% humidified atmosphere containing 5% CO_2. The hypoxia condition was built by anaerobic culture bag.

Uptake measurements of PTAs by fluorescence activated cell sorting (FACS). For each experiment, 4T1 cells were seeded onto 4 cm tissue culture plates and allowed to grow overnight to ~80% confluency. E-Cy-NB/Cy-NB/Cy (10 μM) were added to each dish and incubated at 37 °C / 8% CO_2 for 4 h. Cells were then washed once in PBS. Samples were obtained by using the cell spatula to gently scrape down. Pelleted in a fixed angle centrifuge for 5 minutes at 1500 rpm, and then brought up in 500 μL PBS. Each sample was quickly (within 5 minutes) analyzed on a FACS cell sorter (Becton-Dickinson) using the 640 nm laser line band pass emission filter. 10,000 cells were counted per sample. Three parallel experiments were conducted.

Cytotoxicity Assays. 4T1 cells were seeded in 96-well plates with an amount of 5 × 10^3 for 24 h and incubated with E-Cy-NB (0, 5 μM, 10 μM, 15 μM and 20 μM) for another 24 h. During which, the cells were cultured with fresh complete medium, and with or without irradiation using 808 nm laser (0.33 W/cm^2, 300 s). After that, 150 μL MTT solution (0.5 mg/mL) was added to each well. The remaining MTT solution was

removed 4 h later, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 490 nm with a RT 6000 microplate reader. The half-maximum inhibitory concentration (IC_{50}) value was calculated according to the MTT results. For Calcein-AM and PI co-staining Assay, 4T1 cells (2.0×10^5 per dish) were seeded on 35 mm confocal dishes and allowed to stabilize for 24 h. Then, 4T1 cells incubated with 10 μM E-Cy-NB were or not exposed to an 808 nm laser with the power density of 0.33 W/cm^2 for 300 s. After another 24 h of incubation, the cells were stained with calcein-AM and PI for 30 min to evaluate the PTT efficacy using TCS SP8 confocal laser scanning microscopy.

Immunofluorescence imaging experiment. For fluorescence imaging, 4T1 cells (4×10^3/well) were passed on confocal dishes and incubated for 24 h. Cells were washed three times with PBS (10 mM, pH = 7.4), and incubated with different treatment (PBS, NIR, E-Cy-NB, Cy-NB, E-Cy-NB + NIR and Cy-NB + NIR) (10 μM). Firstly, cells were incubated with materials for 4 h and washed three times with PBS (10 mM, pH = 7.4), and irradiated with NIR for 3 min. After 8 h, the cells were treated with 4% (w/v) paraformaldehyde for 20 min at 4 °C. Cells were washed three times with PBS (10 mM, pH = 7.4) and permeabilized with 0.25% (v/v) Triton X-100 for 5 min at 25 °C. Next, cells were treated with 5% bovine serum albumin (BSA) for 60 min at 25 °C. Then cells incubated with antibody against HSP27/70/90 for 60 min at 25 °C. Cells were washed three times with PBS (10 mM, pH = 7.4), and incubated with FITC-labeled secondary antibody for 60 min at 25 °C. Cells were washed three times with PBS (10 mM, pH = 7.4) and taken under a confocal microscope.

Animal tumor xenograft models. All animal experiments were carried out in accordance with the principles of laboratory animal care (ROC). Balb/c mice (4-6 weeks old, female, weighing about 18 g) were fed under normal conditions with an average of 12 h of light and dark cycles per day, and given enough food and water. The establishment of mouse tumor model: 4T1 cells were trypsin zed and redispersed in 50 μL RPMI 1640 serum-free medium (about 2×10^5 cells), and then subcutaneously inoculated into the right armpit of the mouse. By measuring the length (L) and width (W) of mouse tumors, the tumor volume (V) of tumor-bearing mice

were calculated ($V = L \times W^2/2$). The calculation method of the relative tumor volume of mice is V/V_0 (V_0 is the tumor volume of mice before treatment). When the mouse tumor volume reached about 20 – 40 mm^3, the Balb/c mice were treated.

Living tumor treatment experiment. Seven groups of tumor-bearing mice were divided at random (five in each group): (1) 50 μL PBS buffer solution, (2) 50 μL PBS buffer solution with laser irradiated, (3) 50 μL E-Cy-NB solution (0.2 mM), (4) 50 μL E-Cy-NB solution (0.2 mM) with laser irradiated, (5) 50 μL Cy-NB solution (0.2 mM) with laser irradiated, (6) 50 μL ICG solution (2.0 mM) and (7) 50 μL Cy-NB + ERK inhibitor solution (0.2 mM) with laser irradiated. The mice with laser groups were treated with 808 nm irradiation (0.33 W/cm^2) for 1 h. Remarkably, the irradiation was performed 8 h after intratumoral injection in the group (4/5/7). During the treatment (20 days), the mouse weight was measured with a vernier caliper with a scale and the change of tumor volume every other day.

In vivo biological safety experiment. The mice were divided into 6 groups and treated with different materials, and five major organs (liver, lung, spleen, kidney, and heart) were dissected after 7 days for H&E staining. The mice were subjected to eyeball blood collection and divided into two batches. One aliquot was immediately stored at 4 °C for routine blood testing. The other was stored at room temperature for 1 h, centrifuged at 4500 rpm at 4 °C, and the supernatant was stored at –80 °C for biochemical analysis.

Supplementary Figures

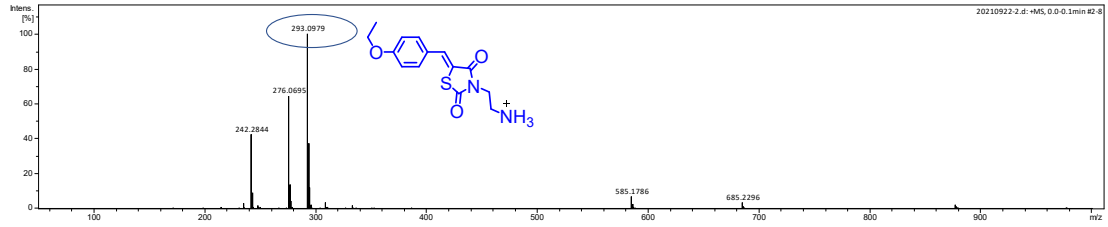


Fig. S1 HRMS spectrum of compound **1** (ERK inhibitor).

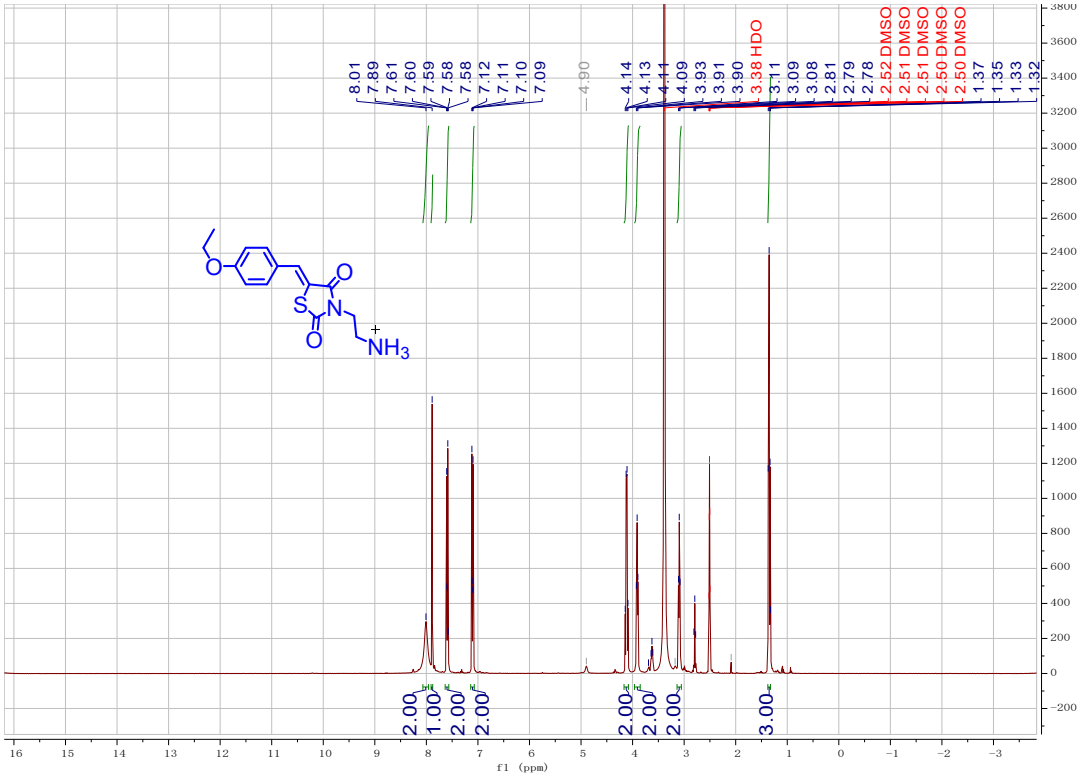


Fig. S2 ^{1}H-NMR spectrum of compound **1** (ERK inhibitor) (*d*^{6}-DMSO).

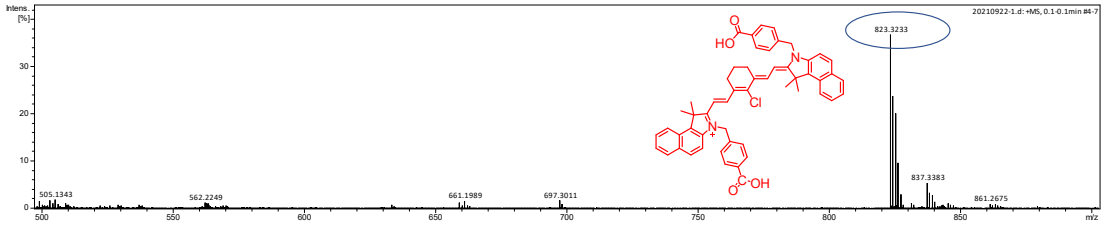


Fig. S3 HRMS spectrum of compound **4**.

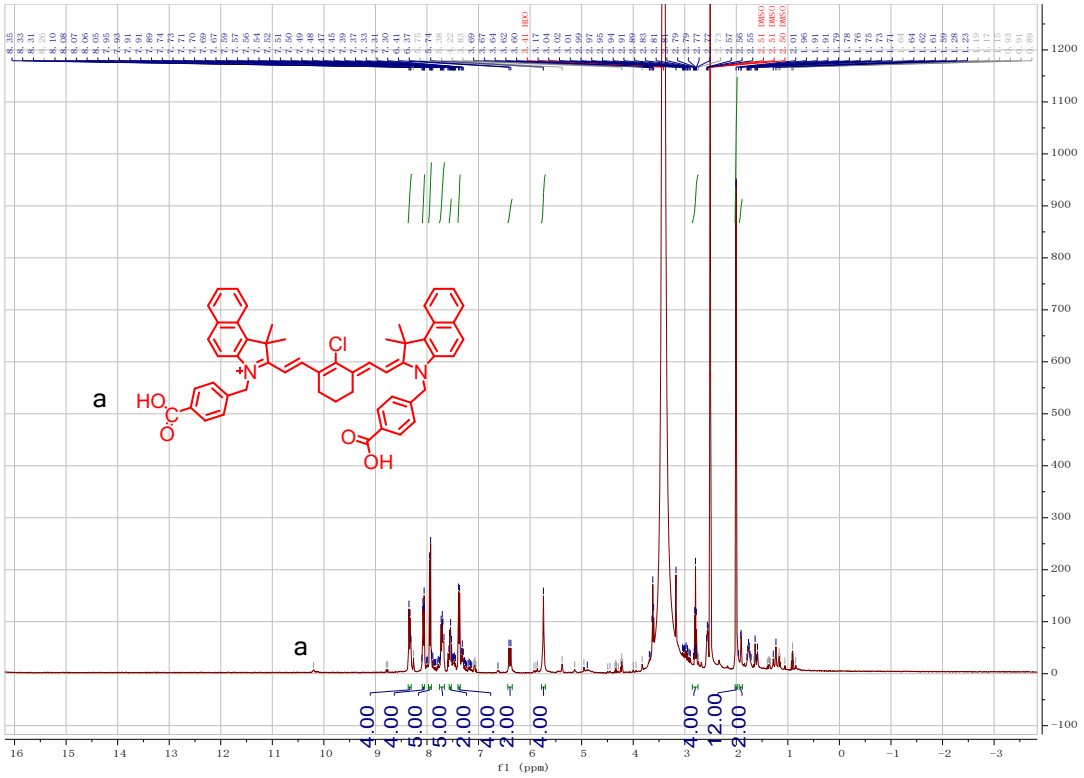


Fig. S4 ^{1}H-NMR spectrum of compound **4** (*d*^{6}-DMSO).

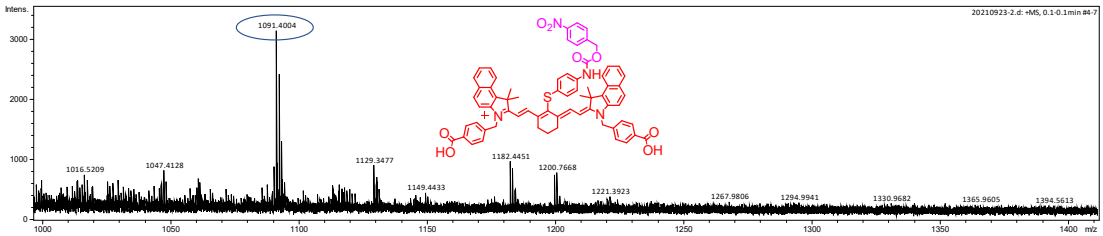


Fig. S5 HRMS spectrum of Cy-NB.

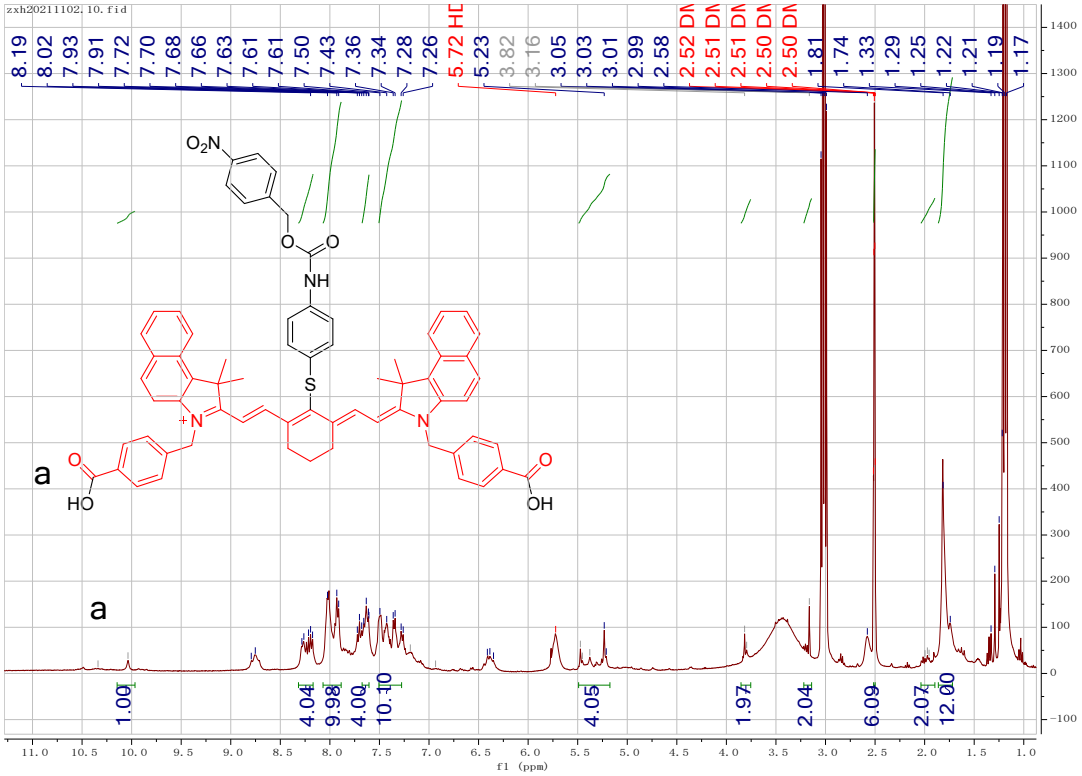


Fig. S6 ^{1}H-NMR spectrum of Cy-NB (*d*^{6}-DMSO).

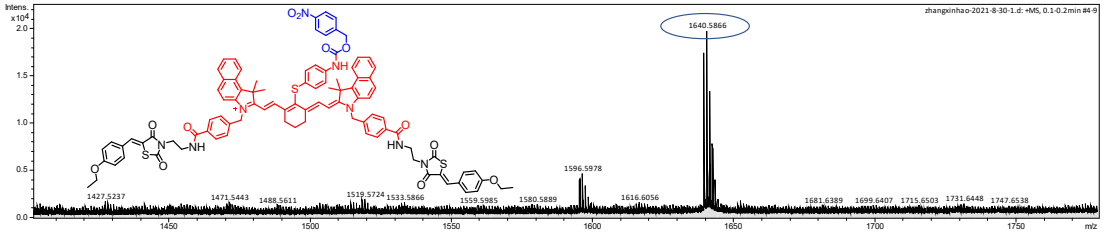


Fig. S7 HRMS spectrum of E-Cy-NB.

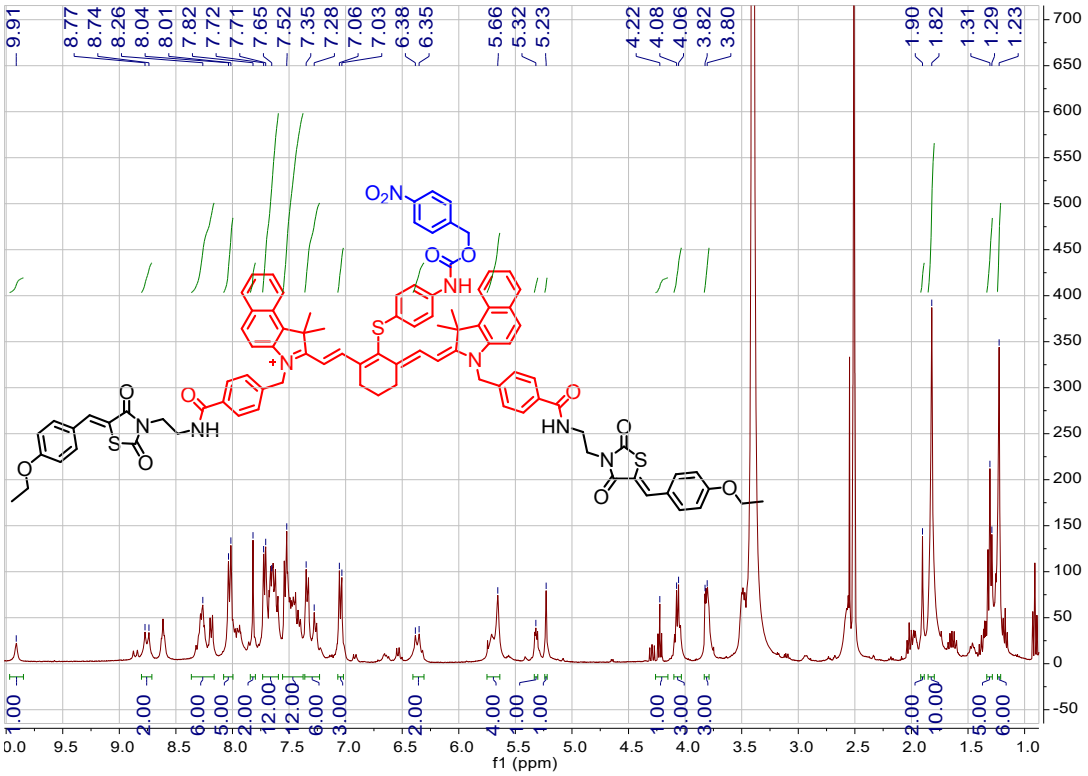


Fig. S8 ^{1}H-NMR spectrum of E-Cy-NB (d^{6}-DMSO).

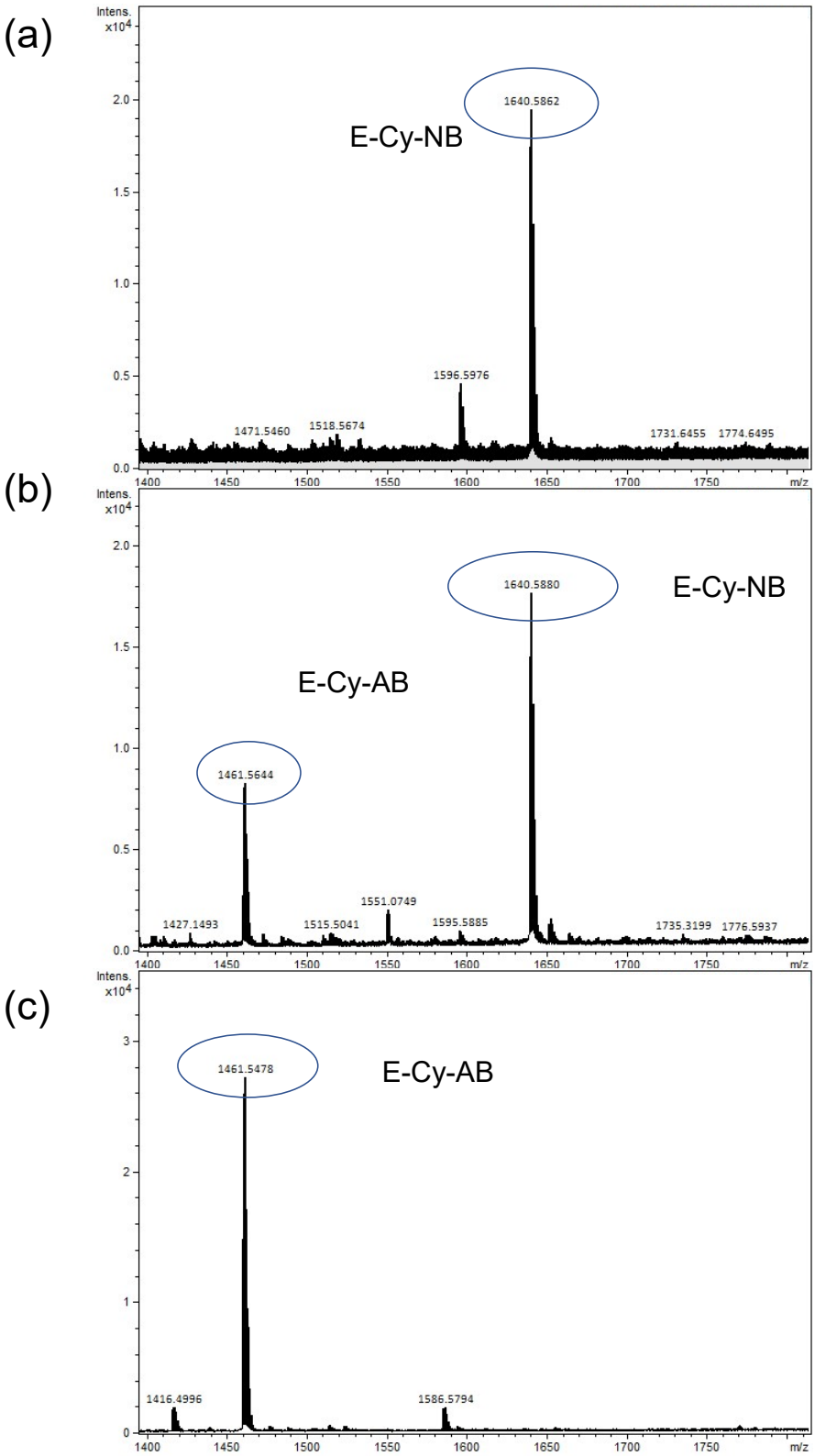


Fig. S9 (a) HRMS spectrum of E-Cy-NB without NTR added; (b) HRMS spectrum of E-Cy-NB with NTR (5 μg/mL) added after 30 min, and (c) HRMS spectrum of E-Cy-AB.

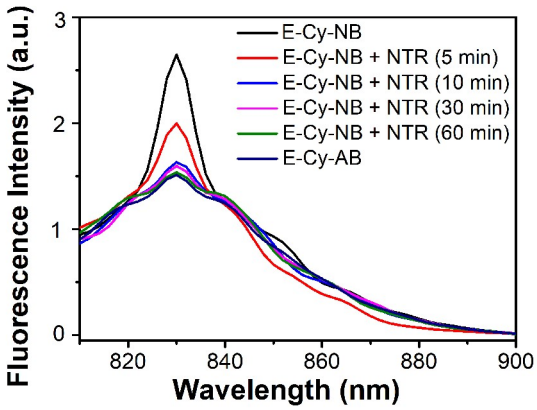


Fig. S10 The fluorescence spectrum of E-Cy-NB (2.0 μM) solution and E-Cy-AB (2.0 μM) solution (DMSO/water, v/v=1:9). Change of fluorescence with E-Cy-NB (2.0 μM) solution after keeping them with NTR (5 μg/mL) for different times.

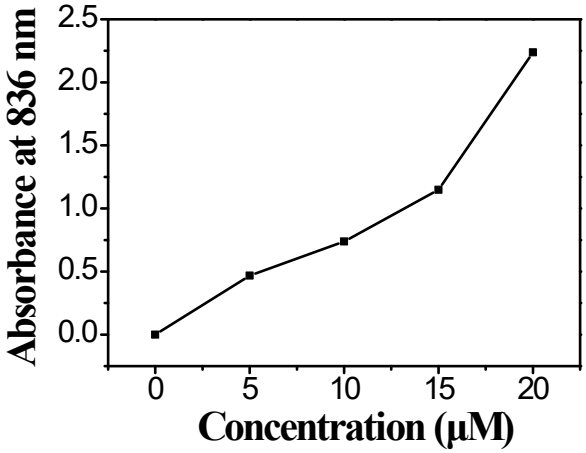


Fig. S11 The UV-vis absorption spectra of E-Cy-NB, which shows concentration versus absorbance at 836 nm.

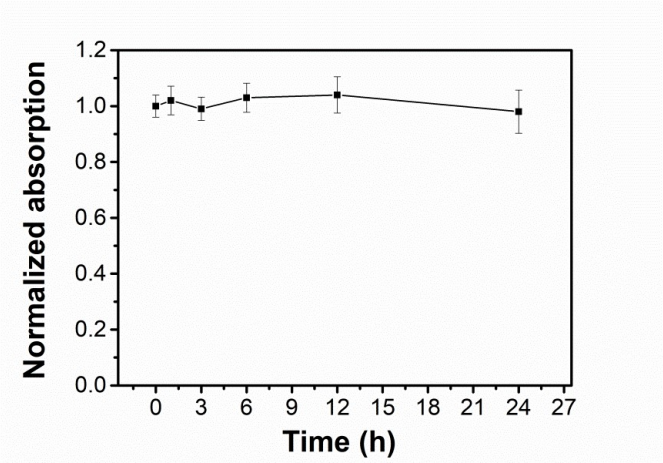


Fig. S12 Photostability of E-Cy-NB in DMSO/water (v/v = 1:9).

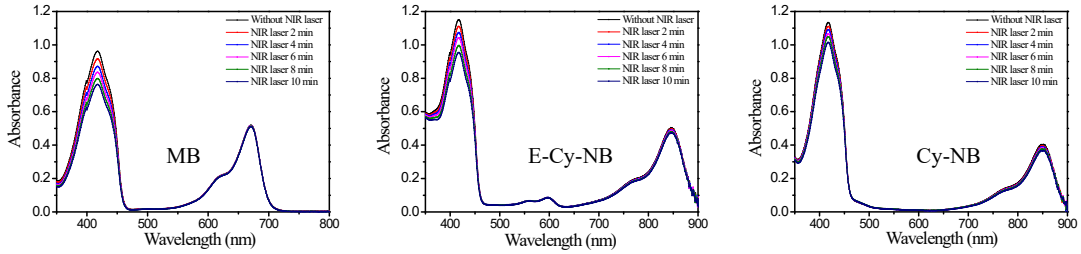


Fig. S13 The absorbance of DPBF and MB/E-Cy-NB/Cy-NB in DMSO. Then solution irradiated with 808 nm laser (0.33 W/cm^2) for different time.

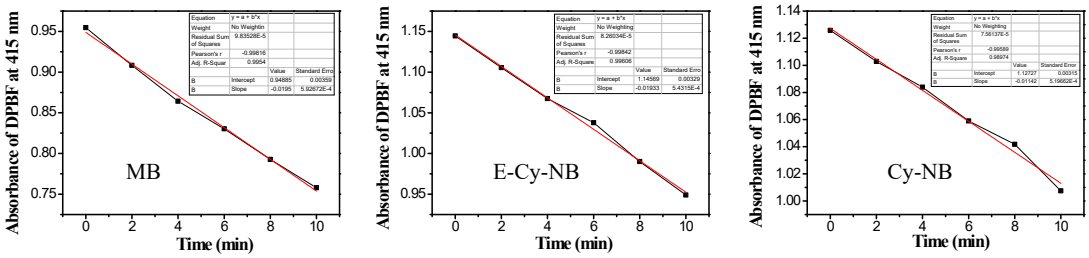


Fig. S14 The slope of absorbance attenuation curve of DPBF at 415 nm.

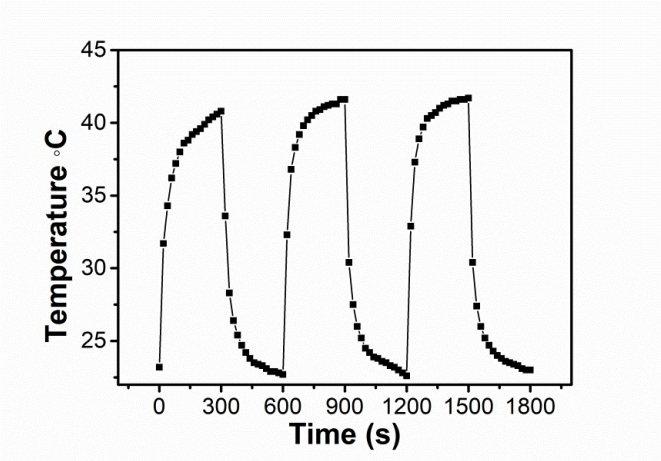


Fig. S15 On/off cycles of E-Cy-AB (10 μM) with NIR irradiation.

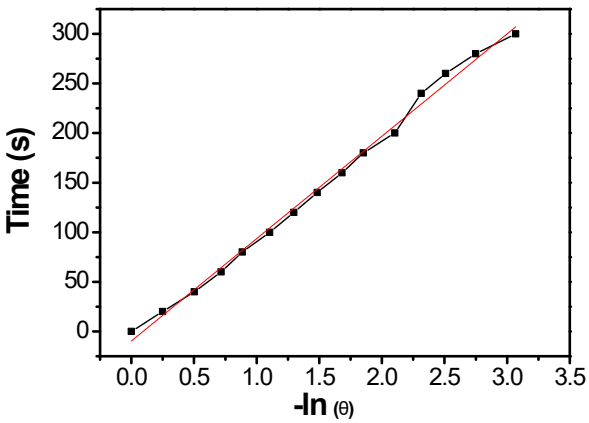


Fig. S16 Plot of time versus -ln (θ). τs of E-Cy-AB equal 103.2324.

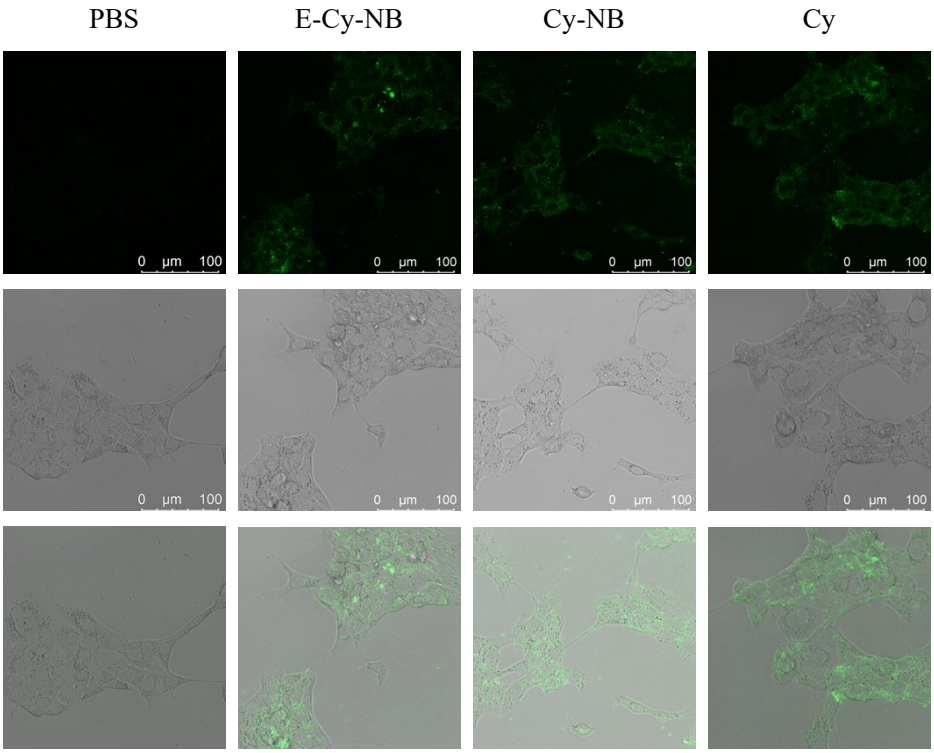


Fig. S17 Confocal luminescence images of 4T1 cells co-incubated with E-Cy-NB/Cy-NB/Cy (10 μM).

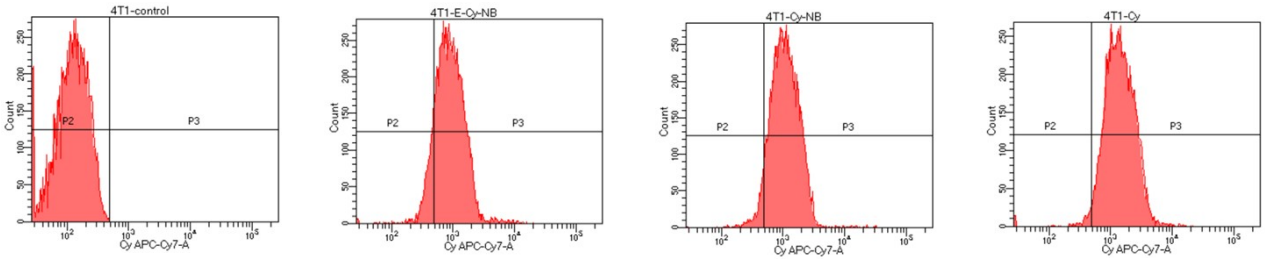


Fig. S18 Cellular uptake of E-Cy-NB, Cy-NB, and Cy by FACS.

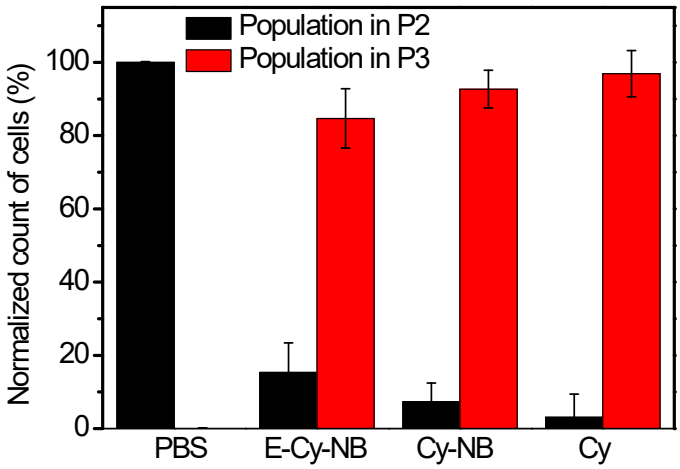


Fig. S19 Quantification of Fig. S18.

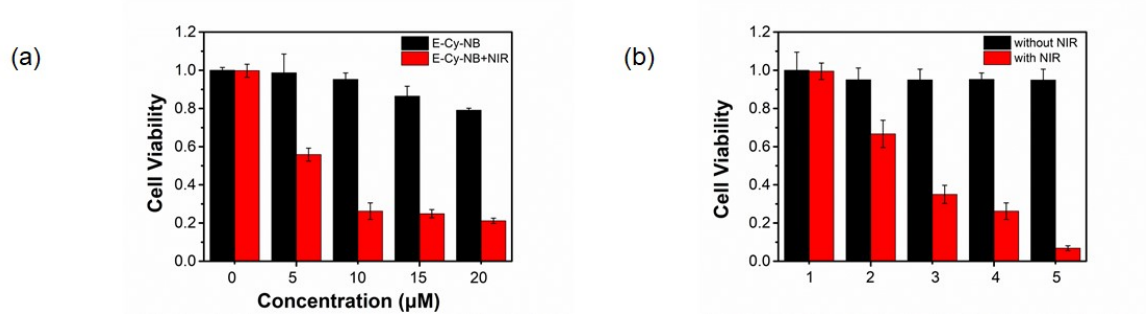


Fig. S20 Viabilities of 4T1 cells treated with different concentrations of (a) E-Cy-NB (0 – 20 μM) with or without laser (808 nm, 0.33 W/cm^2, 300 s). (b) Viabilities of different 4T1 cells groups (1: PBS, 2: 50 μM ICG, 3: 100 μM ICG, 4: 10 μM E-Cy-NB and 5: 10 μM E-Cy-NB under hypoxia condition with or without laser (808 nm, 0.33 W/cm^2, 300 s)).

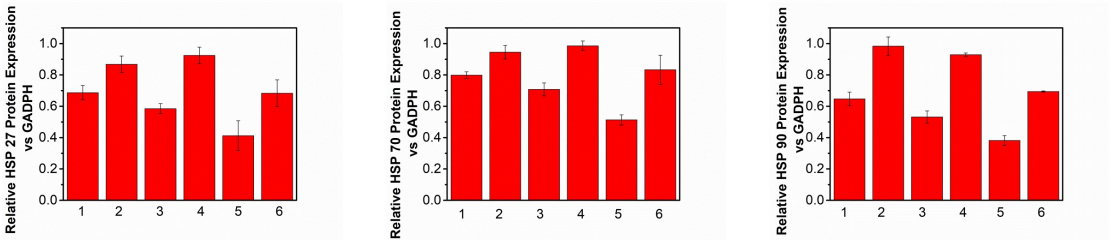


Fig. S21 Quantification of HSPs levels in different 4T1 cells groups (1: PBS, 2: NIR, 3: 10 μM E-Cy-NB + NIR, 4: 10 μM Cy-NB + NIR, 5: 10 μM E-Cy-NB and 6: 10 μM Cy-NB) for 8 h (corresponding to Fig. 3a).

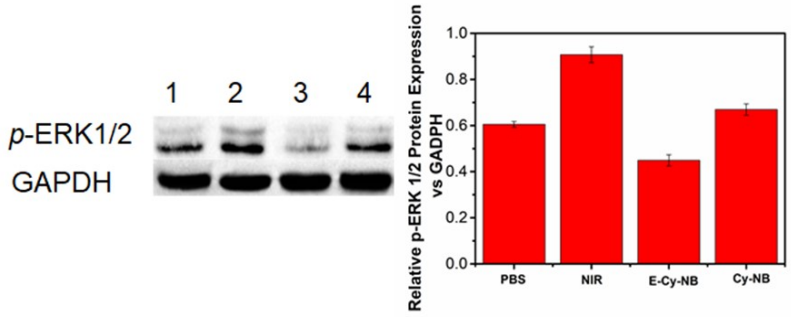


Fig. S22 Western blotting assay of *p*-ERK 1/2 protein in 4T1 cells treated with different groups (1: PBS; 2: NIR; 3: 10 μM E-Cy-NB and 4: 10 μM Cy-NB).

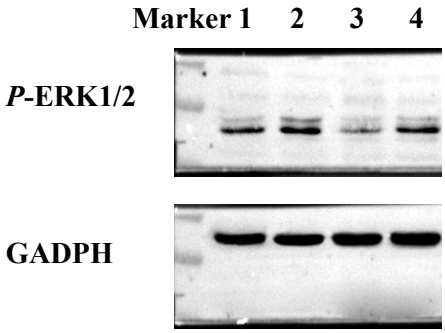


Fig. S23 The original pictures of the western blot corresponding to Fig. S14.

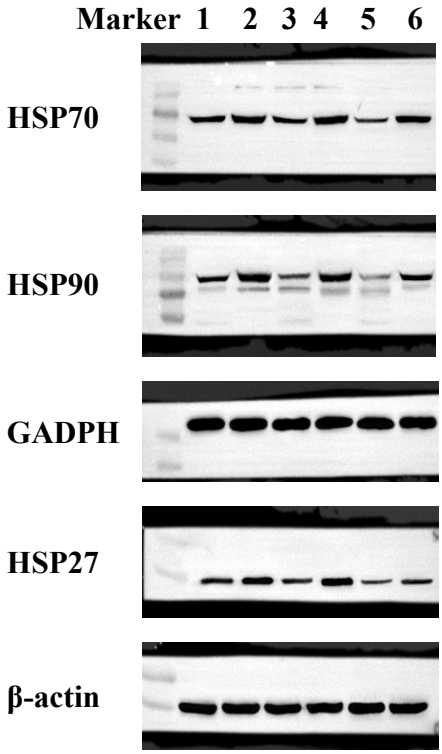


Fig. S24 The original pictures of the western blot corresponding to Fig. 3a. (1: PBS, 2: NIR, 3: E-Cy-NB + NIR, 4: Cy-NB + NIR, 5: E-Cy-NB and 6: Cy-NB).

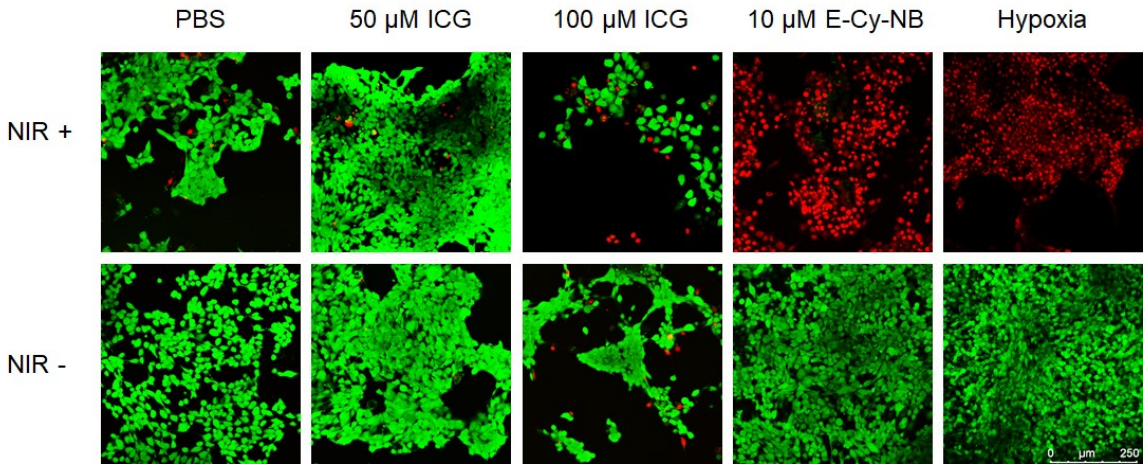


Fig. S25 AM-PI costained images of 4T1 cells incubated with different groups, with or without laser (PBS, 50 μM ICG, 100 μM ICG, 10 μM E-Cy-NB under normal condition, 10 μM E-Cy-NB under hypoxia condition); scale bar = 250 μm.

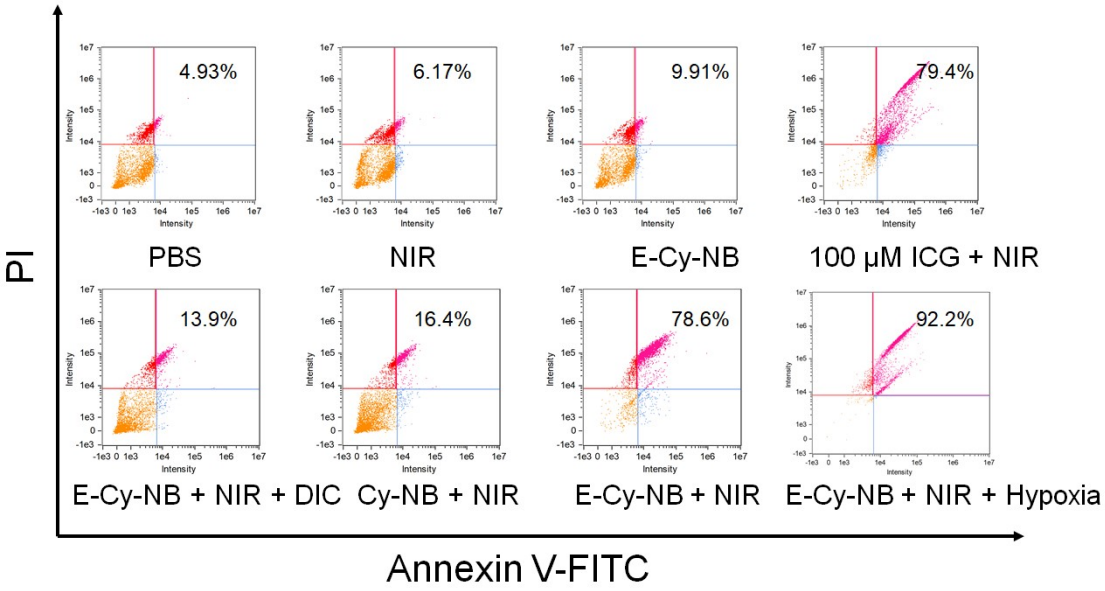


Fig. S26 Flow cytometry analysis of 4T1 cells after co-incubated with different treatments (PBS; 10 μM Cy-NB; 10 μM E-Cy-NB; 10 μM E-Cy-NB + 10 μM DIC; 10 μM E-Cy-NB under hypoxia, 100 μM ICG) for 8h, and irradiated with NIR or not. DIC: (dicumarin, a known NTR inhibitor).

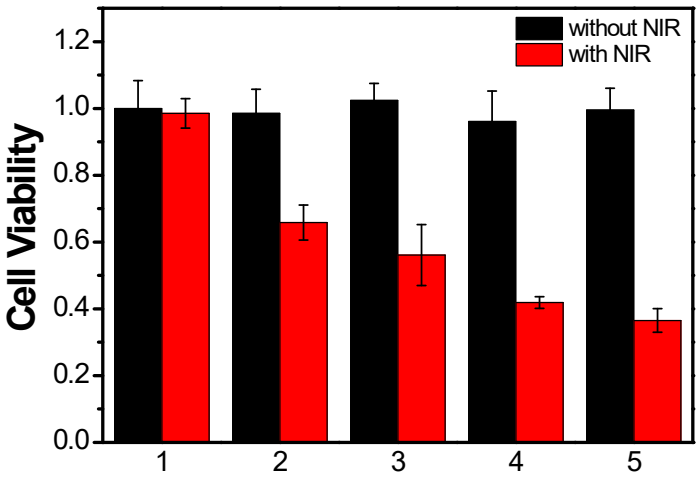


Fig. S27 Cell viabilities of 4T1 cells treated with different groups (1: PBS; 2: 10 μM IR825; 3: 10 μM IR825 + 10 μM PES; 4: 10 μM IR825 + 10 μM GA and 5: 10 μM IR825 + 10 μM PES+ 10 μM GA), with or without NIR laser (808 nm, 0.33 W/cm^2, 300 s).

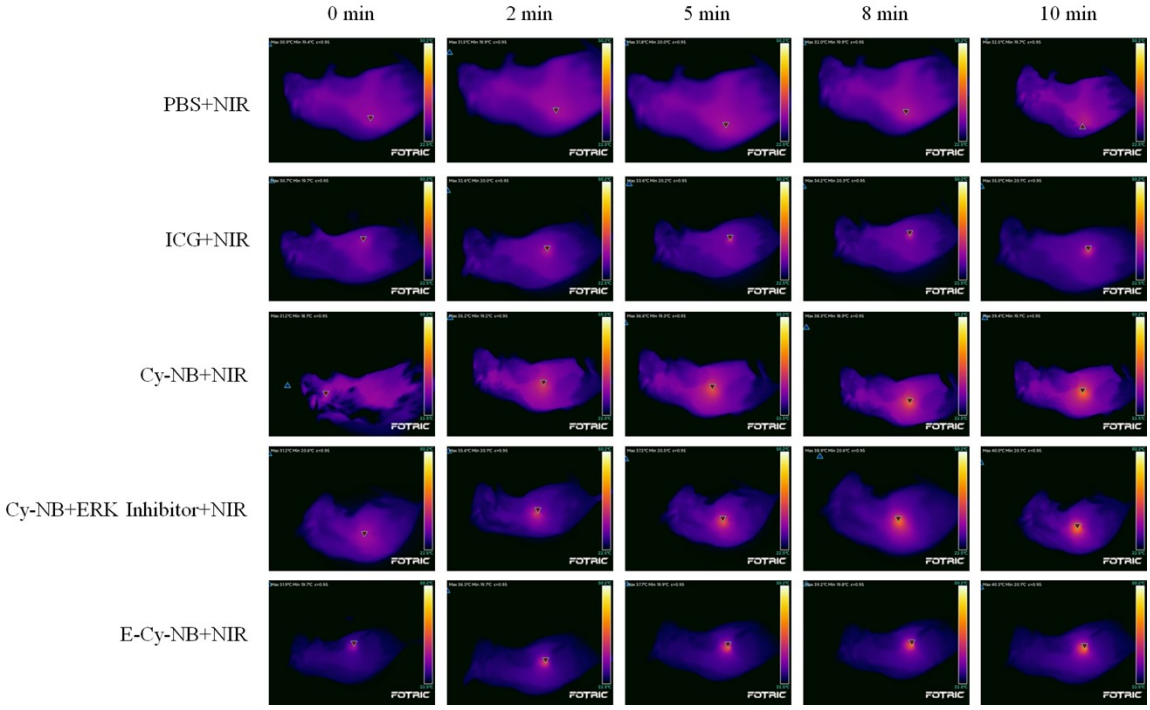


Fig. S28 IR thermal images of 4T1 tumor-bear mice under NIR irradiation (0.33 W/cm^2) with different treatments.

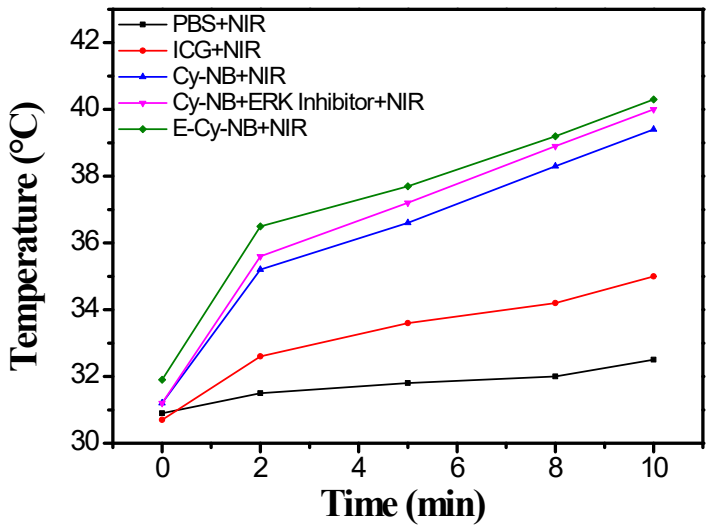


Fig. S29 Quantitative treatment of IR thermal images of 4T1 tumor-bear mice under NIR irradiation (0.33 W/cm^2) with different treatments.

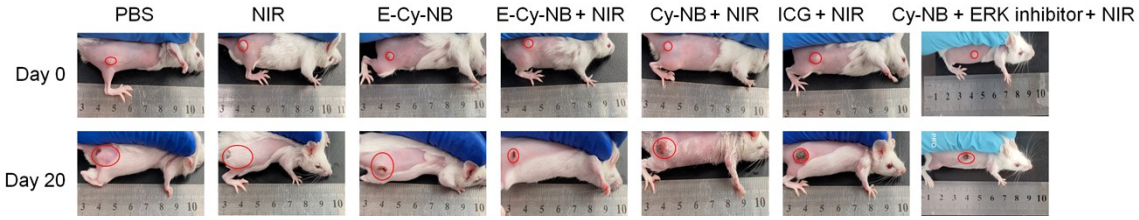


Fig. S30 Photograph of mice in day 0 and day 20 with different treatments.

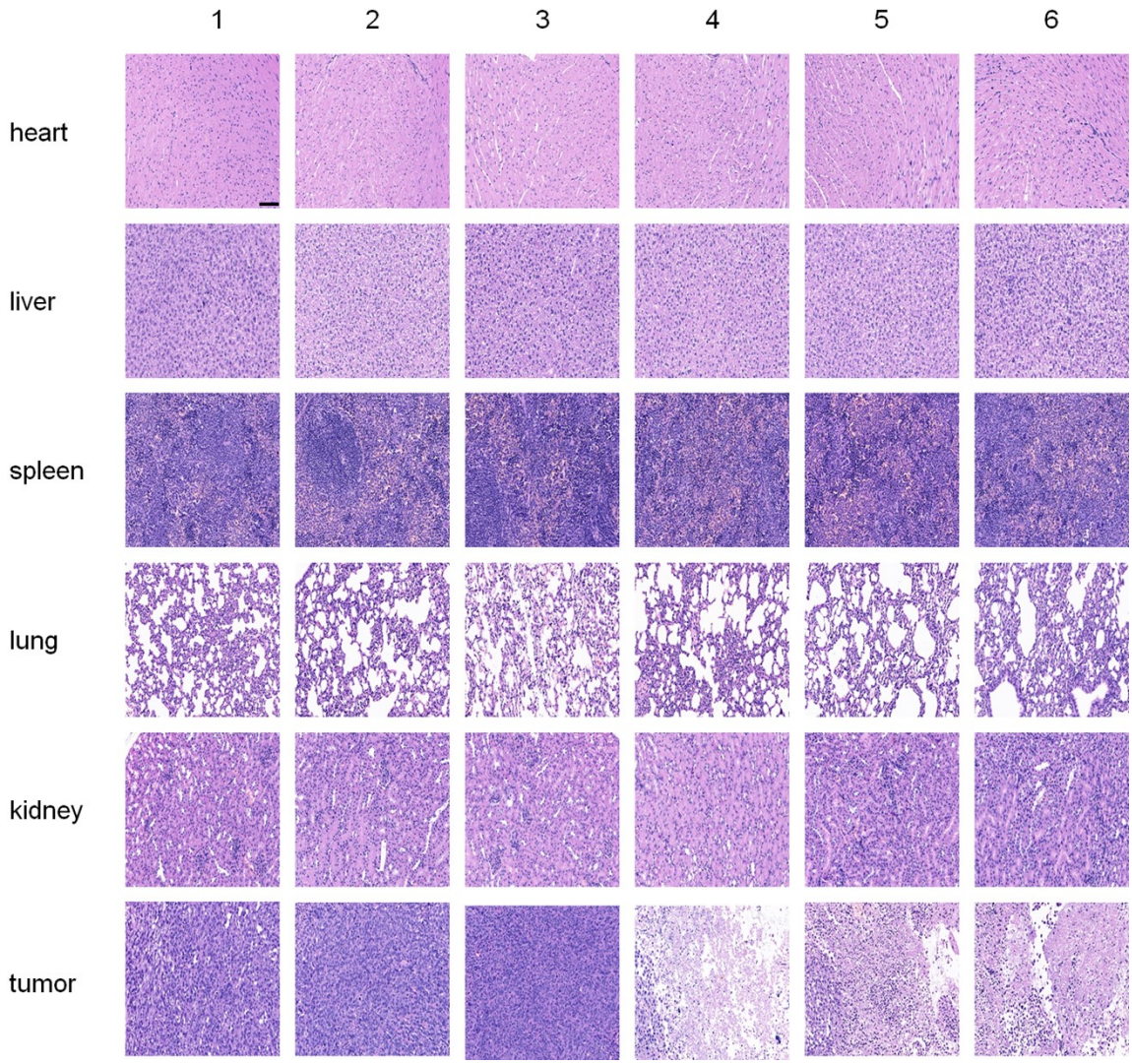


Fig. S31 H&E staining of major organs and tumor with different treatments (1: PBS, 2: NIR, 3: E-Cy-NB, 4: E-Cy-NB + NIR, 5: Cy-NB + NIR, and 6: ICG + NIR). Scale bar = 100 μm.

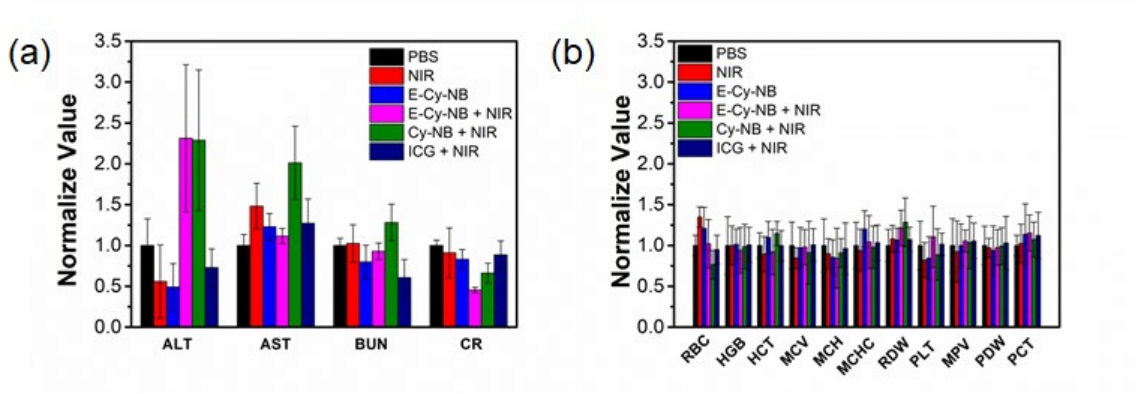


Fig. S32 Blood routine (a) and blood biochemistry (b) of analysis with different groups. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen and CR: creatinine. RBC: red blood cell count; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concerntration; RDW: red cell distribution width; PLT: platelets; MPV: mean platelet volume; PDW: platelet distribution width and PCT: platelet cubic thrombocytocrit.

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