## **Supplementary Information**

## **Experimental Section:**

*Materials.* Methoxy-poly (ethylene glycol) (mPEG, MW=5000 Da) was purchased from Xi'an Ruixi Biological Technology Co. Ltd (Xi'an, China). Methacryloyl chloride, thioacetic acid, sodium methylate, 2,2-azobisisobutyronitrile (AIBN) and propylene sulfide were purchased from GL Biochem (Shanghai, China) Ltd. IR780 was obtained from Alfa Aesar (Ward Hill, MA, USA). Doxycycline (Doxy) was obtained from Calbiochem (Darmstadt, Germany). Tetrahydrofuran (THF), Dichloromethane (DCM), and triethylamine were used after water-removed. All other chemicals and solvents were used as received without further purification.

*Synthesis of mPEG*<sub>50</sub> *methacrylate.* 1.0 g of mPEG, 0.6 mL of methacryloyl chloride and 0.6 mL of trimethylamine were dissolved in DCM and the mixture was reacted for 24 h. After 24h, the reaction mixture was filtered and concentrated. Last, the precipitation was obtained in cold diethyl ether and stored for further use.

Synthesis of mPEG<sub>50</sub> thioacetate. 1.0 g of the resultant mPEG methacrylate was dispersed in 8 mL of THF. 0.0312 g of AIBN and 0.54 mL of thioacetic acid were added into the above solution. Subsequently, the mixture was evacuated by a vacuum pump under liquid nitrogen and argon flow. After repetition for three times, the mixture was stirred at 60 °C under argon flow. The obtained solution was filtered and concentrated. Last, the precipitation was obtained in cold diethyl ether and stored for further use.

Synthesis and characterization of  $mPEG_{50}$ -b-PPS<sub>45</sub>. 1.0 g of the resultant mPEG thioacetate and sodium methylate (0.6 mmol, 0.5 M in methanol) were added into a predried flask and dissolved in THF with nitrogen atmosphere. The mixture was reacted for 0.5 h at room temperature. Then 30 mmol of propylene sulfide was added into the above solution and reaction was sustained overnight at 60 °C. The solvent was removed by vacuum rotary evaporation and the resultant product was extracted with methanol. <sup>1</sup>H-nuclear magnetic resonance (<sup>1</sup>H NMR) was used to identify the chemical structure of obtained mPEG<sub>50</sub>-b-PPS<sub>45</sub>.

Preparation of nanoparticles. IR780 and Doxy were synchronously loaded into mPEG<sub>50</sub>-b-PPS<sub>45</sub> nanoparticles (NPs/ID) through the double emulsion (W/O/W) method with some changes. Briefly, IR780 and 20 mg of mPEG<sub>50</sub>-b-PPS<sub>45</sub> were dissolved in 1 mL of dichloromethane. Then, 200  $\mu$ L of Doxy aqueous solution was added to the above solution. The mixture was emulsified by sonication for 3 min in an ice bath to form primary W/O emulsion. Then, 4 mL of 1 % (w/v) emulsifier (PVA and F-68 mixtures) was added and for sonicated for another 5 min to form W/O/W emulsion. After evaporated with a rotary evaporator to remove oil phase, the nanoparticles were collected by centrifugation at 13,000 pm for 10 min.

*Characterization of nanoparticles.* The hydrodynamic diameter of nanoparticles was determined by dynamic light scanning with a Zeta Sizer Nano series Nano-ZS (Malvern Instruments Ltd, Malvern, UK). The morphology of nanoparticles was characterized by transmission electron microscope (TEM, FEI Company, Philips, Netherlands). The absorbance spectrum of samples were recorded using a UV-visible spectroscopy (Beckman DU 640, Fullerton, USA).

Singlet oxygen detection. Singlet oxygen sensor green (SOSG) was used as a singlet oxygen probe to detect the singlet oxygen generated from samples after laser irradiation. Briefly, 50  $\mu$ L of NPs/ID (IR780: 25  $\mu$ g/mL, Doxy<sub>1</sub>: 37.5  $\mu$ g/mL, Doxy<sub>2</sub>: 75  $\mu$ g/mL) were mixed with 6  $\mu$ L of SOSG (50 mM) and then irradiated with 808 nm laser (2 W/cm<sup>2</sup>). The fluorescence was measured by the microplate reader (Tecan, Männedorf, Switzerland).

*Cell culture.* Human breast adenocarcinoma MDA-MB-231 triple negative breast cancer cell line, mouse fibroblast 3T3 cell line, and human umbilical endothelial (HUVEC) cell line were kept in our laboratory. The cells were incubated in completed RPMI-1640 media under 5 % CO<sub>2</sub> atmosphere at 37 °C, supplemented with 10% fetal bovine serum (FBS), 0.1 mg/mL streptomycin, and 100 U/mL penicillin.

Subcellular localization of NPs/ID.  $1\times10^5$  of MDA-MB-231 cells were seeded into the specific culture plates and incubated overnight. After incubated with NPs/ID (IR780: 5  $\mu$ g/mL, Doxy: 7.5  $\mu$ g/mL) for 2 h, the cells were irradiated by an 808 nm laser at a power of 2 W/cm<sup>2</sup> for 20 s. then cells were further incubated for another 4 h. To detect the intracellular localization of NPs, the endo/lysosomes were labeled with LysoTracker Green (Invitrogen, Carlsbad, California, USA). The cells were observed and analyzed by confocal laser scanning microscopy (CLSM, Carl Zeiss Inc., USA).

In vitro cellular cytotoxicity.  $8 \times 10^4$  of MDA-MB-231 were seeded overnight in the 96well plates followed by incubation with different drug formulations with the IR780 concentration ranging from 0.625 to 50 µg/mL, the Doxy concentration ranging from 0.938 to 75 µg/mL or 1.875 to 150 µg/mL for 24 or 48 h. The laser irradiation groups were treated with a 808 nm laser. Subsequently, the culture medium was replaced by 100 µL of MTT solution (5 mg/mL) and incubation for 4 h. Then the MTT solution was replaced by 150 µL of DMSO and the absorbance of samples were measured by a microplate reader (Tecan) at 570 nm.

Apoptosis assay.  $2 \times 10^6$  of MDA-MB-231 cells were seeded overnight in the 96-well plates. Then, the cells were pre-treated with free culture medium containing 100 mM of CoCl<sub>2</sub>. After incubated for 24 h, the cells were treated with Doxy<sub>1</sub>, Doxy<sub>2</sub>, NPs/I, NPs/ID<sub>1</sub> or NPs/ID<sub>2</sub> (IR780: 5 µg/mL, Doxy<sub>1</sub>: 7.5 µg/mL, Doxy<sub>1</sub>: 15 µg/mL). After incubated for 4 h, the cells were irradiated by a NIR laser (808 nm, 2 W/cm<sup>2</sup>) for 20 s. Then cells were further incubated for another 15 h. Subsequently, the cells were

harvested and stained with Annexin V-FITC/PI Cell Staining Kit. Finally, the cells were analyzed by flow cytometry.

*live-dead* assay. MDA-MB-231 cells were seeded into 6-well plates at a density of 200,000 cells per well and incubated for 24 h. After incubated for 24 h, the cells were treated with  $Doxy_1$ ,  $Doxy_2$ , NPs/I,  $NPs/ID_1$  or  $NPs/ID_2$  (IR780: 5 µg/mL,  $Doxy_1$ : 7.5 µg/mL,  $Doxy_2$ : 15 µg/mL). After incubated for 4 h, the cells were irradiated by an 808 nm laser (1 W/cm<sup>2</sup>) for 20 s. The cells were further incubated for another 15 h. Subsequently, the cells were harvested and stained with Live-Dead Cell Staining Kit. Finally, the cells were analyzed by inverted fluorescence microscope (Olympus IX 70; Olympus).

*Western-blot analysis.*  $2.0 \times 10^5$  of MDA-MB-231 cells were seeded into 6-well plates and incubated overnight. Then the cells were pre-treated with free culture medium with or without 100 mM of CoCl<sub>2</sub>. The cells were then treated with Doxy<sub>1</sub>, Doxy<sub>2</sub>, NPs/I, NPs/ID<sub>1</sub> or NPs/ID<sub>2</sub> (IR780: 5 µg/mL, Doxy<sub>1</sub>: 7.5 µg/mL, Doxy<sub>2</sub>: 15 µg/mL). After incubated for 4 h, the cells were irradiated by an 808 nm laser (0.5 W/cm<sup>2</sup>) for 20 s. The cells were further incubated for another 15 h. Subsequently, the cells were washed with PBS, scraped off the plates, and centrifuged at 2000 rpm for 5 min. A western-blot study was performed as described previously.

In vitro cellular ATP level analysis.  $2.0 \times 10^5$  of MDA-MB-231 cells were seeded into 6well plates and incubated overnight. Then the cells were pre-treated with free culture medium with or without 100 mM of CoCl<sub>2</sub>. After incubated for 24 h, the cells were treated with Doxy<sub>1</sub>, Doxy<sub>2</sub>, NPs/I, NPs/ID<sub>1</sub> or NPs/ID<sub>2</sub> (IR780: 5 µg/mL, Doxy<sub>1</sub>: 7.5 µg/mL, Doxy<sub>2</sub>: 15 µg/mL). After incubated for 4 h, the cells were irradiated by an 808 nm laser (0.5 W/cm<sup>2</sup>) for 20 s. The cells were further incubated for another 24 or 48 h. Subsequently, the cells were lysed in ATP Detection Kit and analyzed by microplate reader (Tecan).

In vitro cellular ROS detection.  $1.0 \times 10^3$  of MDA-MB-231 cells were seeded into a specific plate and incubated overnight. The RPMI-1640 medium were pre-treated with or without 100 mM of CoCl<sub>2</sub>. After 24 h incubation, the cells were treated with NPs/I, NPs/ID<sub>1</sub> or NPs/ID<sub>2</sub> (IR780: 5 µg/mL, Doxy<sub>1</sub>: 7.5 µg/mL, Doxy<sub>2</sub>: 15 µg/mL). After incubation for 1.5 h, the cells were irradiated by an 808 nm laser (0.5 W/cm<sup>2</sup>) for 20 s and further incubated for another 3 h. Later, the cells were stained with DCFH-DA and imaged by confocal laser scanning microscopy (CLSM, Carl Zeiss Inc., USA) and quantitatively analyzed.

In vivo biodistribution of NPs/ID. Female BALB/c nude mice of 18-20 g and 4-6 weeks old were purchased from Charles River (Beijing, China). All animal experiments were performed in compliance with the Experimental Ethics Committee in Beijing. A total of  $1.0 \times 10^7$  of MDA-MB-231 cells suspended in 0.1 mL of saline were subcutaneously injected into the armpit of the mice to establish MDA-MB-231 tumor-bearing mice.

After tumors reached ~100 mm<sup>3</sup> in volume, 200  $\mu$ L of free IR780 and NPs/ID (IR780: 100  $\mu$ g/mL) were intravenously administrated. In vivo fluorescence images of mice were recorded at 3, 5, 8, 12 and 24 h after injection. After in vivo imaging, the mice were sacrificed the major organs were exfoliated and collected for further ex vivo fluorescence imaging.

Immunohistochemical staining. 200  $\mu$ L of free IR780 or NPs/ID (IR780: 100  $\mu$ g/mL, Doxy<sub>1</sub>: 150  $\mu$ g/mL, Doxy<sub>2</sub>: 300  $\mu$ g/mL) were intravenously injected into the tumorbearing mice. The NIR 808 nm laser irradiation (2 W/cm<sup>2</sup>) was conducted in the tumor sites for 20 s at 24 h after intravenous injection. After another 24 h, the mice were sacrificed. The tumor tissues were collected, embedded in an optimal cutting temperature (OCT) compound and cut into 7  $\mu$ m slides for histological analysis.

In vivo analysis of ATP level. 200  $\mu$ L of free IR780 or NPs/ID (IR780: 100  $\mu$ g/mL, Doxy<sub>1</sub>: 150  $\mu$ g/mL, Doxy<sub>2</sub>: 300  $\mu$ g/mL) were intravenously injected into the tumor-bearing mice. The NIR 808 nm laser irradiation (2 W/cm<sup>2</sup>) was conducted in the tumor sites for 20 s at 24 h after intravenous injection. After another 24 h, the mice were sacrificed. The tumor tissues were collected, lysed with ATP Detection Kit and analyzed by the microplate reader (Tecan).

In vivo ROS detection. 200  $\mu$ L of free IR780 or NPs/ID (IR780: 100  $\mu$ g/mL, Doxy<sub>1</sub>: 150  $\mu$ g/mL, Doxy<sub>2</sub>: 300  $\mu$ g/mL) were intravenously injected into the tumor-bearing mice. The NIR 808 nm laser irradiation (2 W/cm<sup>2</sup>) was conducted in the tumor sites for 20 s at 24 h after intravenous injection. After another 24 h, the mice were sacrificed. The tumors were collected and immersed into 0.1 mg/mL of collagenase in Krebs-Ringer buffer. Then the tumor tissues were cut into pieces, shaken for 20 min, and filtered through 35 mm nylon mesh. The filtrate was collected. The mono-dispersed cells were obtained by centrifugation of the filtrate at 1500 rpm for 5 min and further washing with Krebs-Ringer buffer. Finally, the cells were stained with of DCFH-DA and analyzed by the flow cytometer.

In vivo antitumor assays. Tumor-bearing mice were randomly divided into nine groups (five mice per group). After tumors reached ~100 mm<sup>3</sup> in volume, the mice were administrated with 200 µL different formulations (saline, Doxy<sub>1</sub>, Doxy<sub>2</sub>, NPs/I, NPs/ID<sub>1</sub>, NPs/ID<sub>2</sub>, NPs/I+NIR laser, NPs/ID<sub>1</sub>+NIR laser and NPs/ID<sub>2</sub>+NIR laser). For the laser-treated group, the tumor sites were irradiated by an 808 nm laser (2 W/cm<sup>2</sup>) for 20 s at 4 h post-injection. Tumor volume was measured by a caliper and calculated via the formula: volume =  $1/2 \times (\text{tumor length}) \times (\text{tumor width})^2$ . During the therapeutic process, body weights of all mice were recorded. After 14 days treatment, the mice were euthanized, and the major organs and tumors were exfoliated and collected for further hematoxylin-eosin-safran (H&E) histological staining and analysis.

Statistical analysis. All data were shown as mean  $\pm$  standard deviation (SD). Statistical significance was valuated using Student's t-test. The data differences were considered significant for \* p < 0.01, \*\* p < 0.005, \*\*\* p < 0.001



Figure S1. <sup>1</sup>H NMR spectra results of mPEG<sub>50</sub>-b-PPS<sub>45</sub>

partiere size.				
Emulsifier	Copolymer :	Size	PDI	EE(%)
	Doxy			
1%PVA	5:2	$257.1 \pm 3.2$	$0.169 \pm 0.009$	$71.6 \pm 2.5$
	10:2	$263.8 \pm 4.4$	$0.084 \pm 0.038$	$76.6 \pm 3.4$
	20:2	$279.6 \pm 2.8$	$0.079 \pm 0.021$	$80.3 \pm 1.6$
	5:5			$10.5 \pm 2.6$
	10:5	$278.3 \pm 1.4$	$0.084 \pm 0.030$	$56.7 \pm 4.6$
	20:5	$275.4 \pm 4.2$	$0.114 \pm 0.019$	$72.3 \pm 2.8$
1%PVA:1%F68=	10:2	$225.6 \pm 4.6$	$0.081 \pm 0.019$	$72.6 \pm 1.3$
(7:3)	20:2	$237.6 \pm 5.8$	$0.081 \pm 0.016$	$78.4 \pm 2.6$
	10:5	$233.4 \pm 4.3$	$0.149 \pm 0.025$	$62.4 \pm 3.6$
	20:5	$243.8 \pm 4.2$	$0.156 \pm 0.028$	$71.7 \pm 4.4$

Table S1. The effects of formulation parameters on encapsulation efficiency and particle size.



Figure S2. UV changes of NPs/I, NPs/ID<sub>1</sub> and NPs/ID<sub>2</sub>.



Figure S3. Fluorescence spectra of (A) free IR780, (B) NPs/I and (C) NPs/ID<sub>1</sub> after different storage times.



Figure S4. In vitro cytotoxicity of 3T3 cells or HUVEC cells treated with blank NPs for 24 h or 48 h. (A) 3T3 cells at 24 h. (B) HUVEC cells at 24 h. (C) 3T3 cells at 48 h. (D) HUVEC cells at 48 h.



Figure S5. (A) In vitro cytotoxicity of MDA-MB-231 cells treated with NPs/I, NPs/I+Doxy<sub>1</sub>, and NPs/I+Doxy<sub>2</sub> for 24 h. NIR laser irradiation was 808 nm, 0.5 W/cm<sup>2</sup>. (B) In vitro cytotoxicity of MDA-MB-231 cells treated with NPs/I, NPs/I+Doxy<sub>1</sub> and NPs/I+Doxy<sub>2</sub> for 24 h. IR780 concentration was 5  $\mu$ g/mL. NC represented normal condition; HC represented hypoxic condition.



Figure S6. ROS generation in MDA-MB-231 cells treatment with Doxy<sub>1</sub>, Doxy<sub>2</sub> NPs/I+NIR laser, NPs/ID<sub>1</sub>+NIR laser, or NPs/ID<sub>2</sub>+NIR laser analyzed by flow cytometry. NIR laser was 808 nm, 0.5 W/cm<sup>2</sup>. The values are expressed as mean  $\pm$  SD, (n = 3, \* p < 0.01, \*\* p < 0.005, \*\*\* p < 0.001, Student's *t*-test).



Figure S7. Ex vivo fluorescence images of main organs and tumor after 24 h intravenous injection of free IR780 and NPs/ID<sub>1</sub>.



Figure S8. Figure S4. Representative H&E sections of normal organs of tumor-bearing mice after treatment with saline, Doxy<sub>1</sub>, Doxy<sub>2</sub>, NPs/I, NPs/ID<sub>1</sub>, NPs/ID<sub>2</sub>, NPs/I+NIR, NPs/ID<sub>1</sub>+NIR and NPs/ID<sub>2</sub>+NIR. NIR laser was 808 nm, 2 W/cm<sup>2</sup>.