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Supplementary materials

Ion Channels-Targeting Near-Infrared Photothermal Switch with Synergistic Effect for Specific Cancer Therapy

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Experimental Section

Drug loading and the experiments of drug release *in vitro*: The solution of Dox (1 mg mL^{-1}) and hPBNCs (0.5 mg mL^{-1}) were mixed and stirred in dark for 24 h to obtain the Dox-loaded hPBNCs (Dox-hPBNCs). After that, the mixture was collected by centrifugation and washed with D.I. water repeatedly to remove the free Dox. All the supernatant solutions were collected together. The loading amount of Dox in hPBNCs was measured by UV-vis spectroscopy at 488 nm and calculated by the below equation:

Loading amount = $(m_0-m_1)/m$,

where the m_0 is the initial weight of Dox, m_1 is the weight of Dox in supernatants and m refers to the weight of hPBNCs@PDA.

In drug release experiment, the Dox-hPBNCs@PDA were dispersed in PBS solution (pH 7.4, 5.0 or 4.0) under magnetic stirring for 37 °C. And at 4 h time points, the samples dispersed in PBS (pH 5.0 and 7.4) were treated with 808 nm laser irradiation (0.5 W cm^{-2}) for 10 min to test the effect of laser on Dox release. All the supernatants of obtaining by centrifugation at different time points (0 h, 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h) were detected by UV-vis absorption spectra and calculated the amount of Dox release via the absorbance at 488 nm.

***In vitro* photothermal studies:** D.I. water, hPBNCs, hPBNCs@PDA-TRPV1 were placed in the centrifuge tubes and irradiated using 808 nm laser for 8 min individually, and the temperature was continuously monitored by a thermometer every 30 s for 8 min.

Detection of •OH *in vitro*: Electron spin resonance (ESR) measurement was used to detect the generation of •OH using DMPO. H_2O_2 (100 mM) and the DMPO (100 mM) were mixed in PBS, and then measured by the ESR spectrum after the addition of hPBNCs (10 μg mL^{-1}). The reaction system without hPBNCs or H_2O_2 in DMPO solution was measured as the blank ESR spectrum. TMB was applied for the monition of •OH. For detecting •OH, TMB and H_2O_2 were dispersed in 400 μL of PBS (pH 4.0 and 7.4) containing hPBNCs (5 μg mL^{-1}). After incubating for 10 min at room temperature, the solution was monitored by UV-vis-NIR spectroscopy. Then, different amount (0, 0.05, 0.1, 1, and 5 μg mL^{-1}) of hPBNCs and H_2O_2 were dispersed in 400 μL of PBS (pH 4.0) for a uniform solution, which was also detected by UV-vis spectroscopy. The concentration of H_2O_2 and TMB used in this experiment was 10 mM and 0.42 mM, respectively.

Cell Culture: U373 human astrocytoma-glioblastoma cell line and HeLa cervical adenocarcinoma epithelia cell line were purchased from American Type Culture Collection (ATCC). U373 cells and HeLa cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% pen/strep (100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin), a humidified environment at 37 °C under 5% CO_2.

Detection of intracellular reactive oxygen species (ROS): For ROS detection, U373 cells were seeded in 24-well plates (1×10^5 cells/well) and incubated with RPMI 1640 medium for 24 h. Subsequently, hPBNCs, hPBNCs@PDA-TRPV1, or Dox-hPBNCs@PDA-TRPV1 (different nanoparticles: 20 μg mL^{-1}) were added. After incubation for 2 h, the cells were irradiated by 808 nm laser (0.5 W cm^{-2}) for 5 min (20

s break after 40 s illumination) and further incubated for 12 h. Then the cells were washed with PBS three times and further incubated with DCFH-DA (10 μM) in serum-free RPMI 1640 medium for 40 min. Afterward, the cells were digested and collected by centrifugation. The fluorescence of DCF was measured by fluorescence spectrophotometer with the same live cell numbers for each sample (Ex: 488 nm, Em: 525 nm).

Intracellular mitochondrial membrane potential test: Briefly, U373 cells and HeLa cells were seeded and incubated in 24-well plates (1×10^5 cells/well) for 24 h. Then RPMI 1640 medium was replaced by fresh medium containing anhydrous $CaCl_2$ (150 mg L^{-1}). The hPBNCs@PDA-TRPV1 or Dox-hPBNCs@PDA-TRPV1 (20 μg mL^{-1}) were directly added into the medium. After 2 h, the well was exposed to the 808 nm laser (0.5 W cm^{-2}) for 5 min (20 s break after 40 s illumination), and then incubated at 37 °C for another 12 h. After that, the collected cells incubated with JC-1 in the dark at 37 °C for 20 min following the standard protocol. The cells were detected by fluorescence microscope (LEICA DMI4000B, Leica, Germany).

The detection of caspase-3 and cytochrome c (Cyt c): Briefly, U373 cells or HeLa cells were seeded into 6-well plates for 24 h. Then the cells were washed twice and the plates were added with RPMI 1640 medium with anhydrous $CaCl_2$ (150 mg L^{-1}). The Dox-hPBNCs@PDA-TRPV1 (20 μg mL^{-1}) were directly added into medium. After incubation for 2 h, the well was exposed to the 808 nm laser (0.5 W cm^{-2}) for 5 min (20 s break after 40 s illumination), and incubated at 37 °C for another 24 h. Then, the cells

were washed with PBS for three times and the protein was extracted through lysate. Changes in caspase-3 and Cyt c expression were analyzed by Western blot.

Hemolysis experiment: The red blood cells (RBCs) were gathered from the heparin-stabilized rat blood samples via centrifugation at 3500 rpm for 10 min to remove the plasma and buffy coat. After that, the remaining packed RBCs were washed with PBS (10 mM, pH 7.4) for several times until the traces of plasma were no longer visible. Then the packed RBCs were diluted with PBS in the ratio of 1:10 and put side. In hemolysis experiment, the diluted RBCs suspension (200 μL) were added to 600 μL of PBS containing different concentrations hPBNCs or hPBNCs@PDA nanoparticles. PBS and water (600 μL) were mixed with 200 μL diluted RBC suspension, which were considered as negative and positive controls, respectively. All the mixtures were incubated for 2 h at room temperature. After 2 h, the supernatant obtained by centrifuging the mixtures and the absorbance (Abs) at 570 nm was determined by UV-vis-NIR absorption spectra.

Sample hemolysis rate was calculated using the equation:

$$\text{Hemolysis rate } \% = (Abs_{(Sample)} - Abs_{(Negative\ control)}) / (Abs_{(Positive\ sample)} - Abs_{(Negative\ control)}) \times 100.$$

In vivo **toxicity test:** Ten-week-old female C57BL6/J mice of weighting between 20-25 g were supplied by the Experimental Animal Center of the Chinese Academy of Medical Sciences. All the animal handing protocols and procedures were performed following the guidelines of the Hebei committee for care and use of laboratory animals, and were approved by the Animal Experimentation Ethics Committee of the Hebei

Medical University (IACUC-Hebmu-2021002). Mice were kept in a sterile environment with free access to food and water. To test the toxicity of nanoparticles system *in vivo*, the mice were randomized divided into 3 groups, 6 mice per group. The mice were intravenously injected with PBS or PBS containing hPBNCs or hPBNCs@PDA (2.5 mg/kg body weight (B.W.)), respectively. All mice were weighted every day. After 21 days, the hearts, livers, spleens, lungs, kidneys, and brains of all mice were collected and fixed with 4% paraformaldehyde more than 24 h, then embedded in paraffin and prepared into 4 μm sections to carry out the Hematoxylin-eosin (H&E) staining. H&E-staining was performed according to standard procedures. Images were obtained by an optical microscope.

***In vivo* biodistribution:** The mice bearing subcutaneous U373 tumors were intravenously injected with 100 μL of hPBNCs@PDA-TRPV1 (2.0 mg/kg B.W.) via the tail vein. The photothermal imaging was recorded using FLIR infrared camera at 0, 2, 4, 8 and 24 h with an 808 nm laser at a power density of 0.4 W cm^{-2} for 5 min.

To study the biodistribution of hPBNCs@PDA-TRPV1, the tumor-bearing mice were randomly divided into two groups (4 mice per group) and treated with saline (control group) and hPBNCs@PDA-TRPV1 (20 mg/kg B.W.) through intravenous injection, respectively. After 4 h, mice were sacrificed, and the organs (heart, liver, spleen, lung, kidney and tumor) were extracted. The Fe content of the samples was detected by inductively coupled plasma mass spectrometry (ICP-MS).

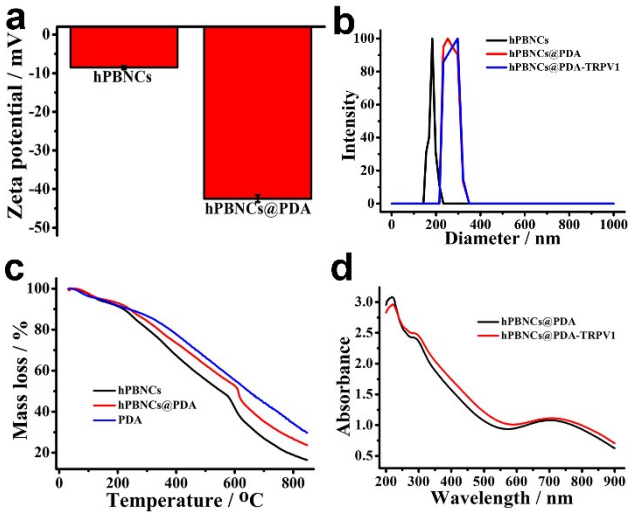

Figure S1. Characterizations of hPBNCs@PDA-TRPV1. (a) Zeta potential of hPBNCs and hPBNCs@PDA. (b) DLS analysis of hPBNCs, hPBNCs@PDA and hPBNCs@PDA-TRPV1. (c) The TGA curves of hPBNCs, hPBNCs@PDA, and PDA. (d) The UV-vis spectra of hPBNCs@PDA and hPBNCs@PDA-TRPV1.

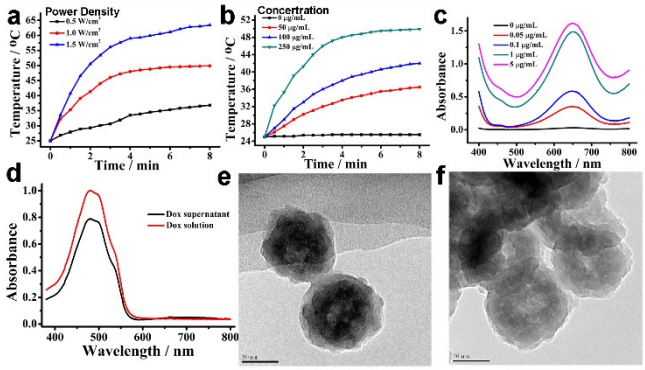

Figure S2. (a) The temperature of hPBNCs@PDA-TRPV1 as a function of laser irradiating time at different power density. (b) The temperature of hPBNCs@PDA-TRPV1 with different concentration as a function of laser irradiating time at 1.0 W cm^{-2}. (c) UV-vis spectra of hPBNCs + TMB + H_2O_2 at different concentration of hPBNCs. (d) The UV-vis spectra of Dox before and after loading with hPBNCs. The TEM images of Dox-hPBNCs@PDA dispersed in PBS at (e) pH 7.4 and (f) pH 5.0 after 24 h, respectively.

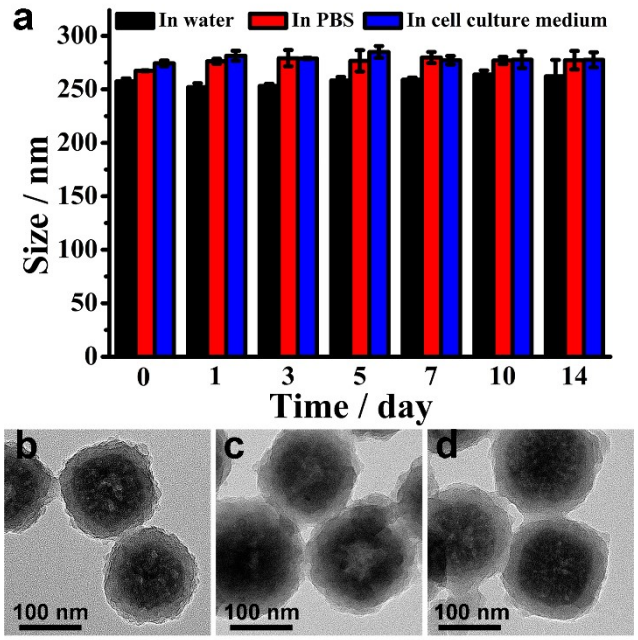

Figure S3. The stability of hPBNCs@PDA-TRPV1 in different solutions. (a) DLS studies of hPBNCs@PDA-TRPV1 in different solutions. The TEM images of hPBNCs@PDA-TRPV1 incubated in (b) water, (c) PBS (pH 7.4) and (d) cell culture medium (RPMI 1640 medium with 10% fetal bovine serum) for 14 days.

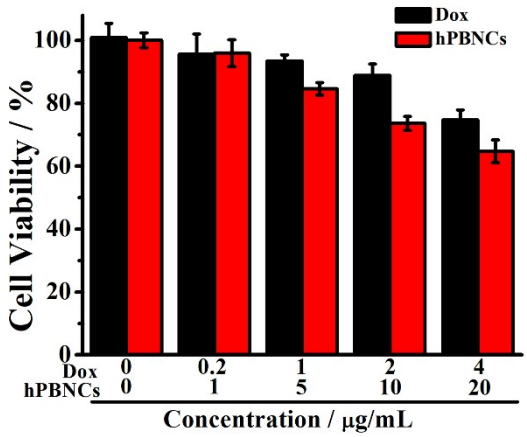

Figure S4. The cell viabilities of U373 cells treated with free Dox and hPBNCs.

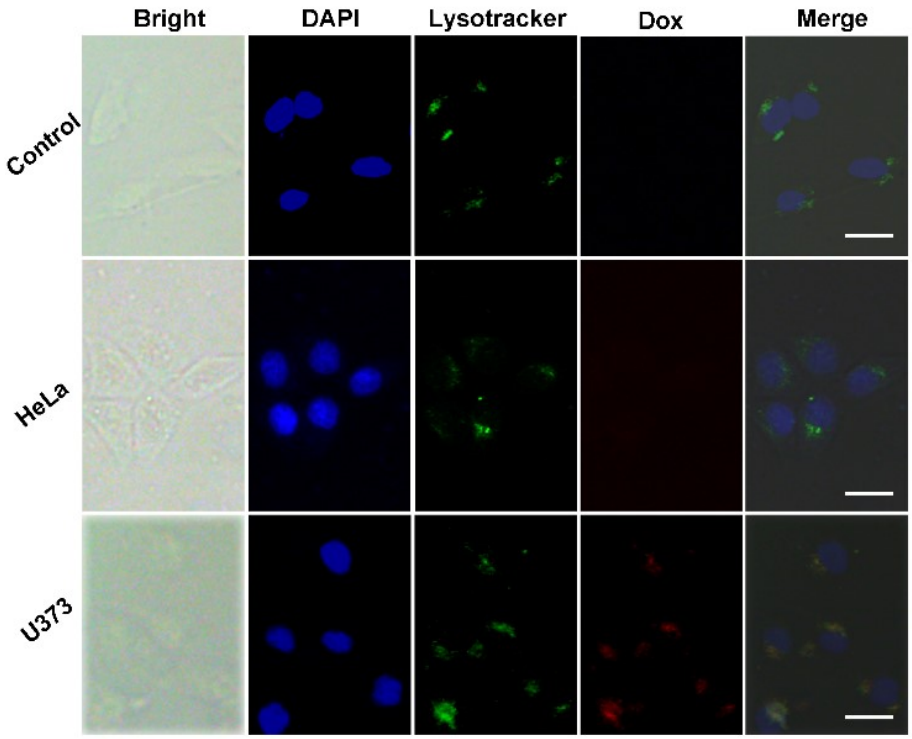

Figure S5. Inverted fluorescence microscope images of U373 or HeLa cells incubated at 37 °C for 6 h after treated with Dox-hPBNCs@PDA-TRPV1. Cell nucleus was stained by DAPI. Lysosome was stained by Lyso-Tracker green DND-26. All images were visualized via 20 × lens, Scale Bar = 25 μm.

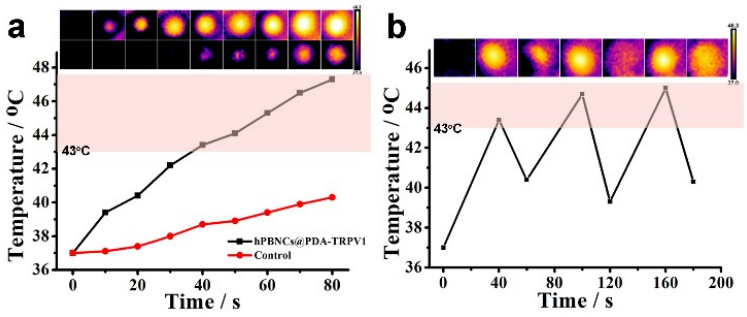

Figure S6. The local temperature on the U373 cells (a) upon NIR irradiation with different time, and (b) upon 40 s NIR irradiation followed by a 20 s cooling phase.

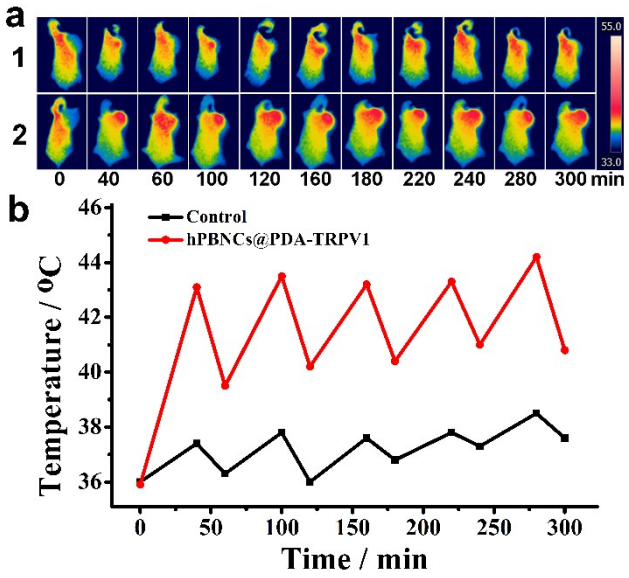

Figure S7. (a) IR thermal images and (b) the *in vivo* temperature variations of tumor-bearing mice under 808 nm laser irradiation for 5 min (0.4 W cm^{-2}, 20 s break after 40 s illumination) to control temperature after systemic administration of hPBNCs@PDA-TRPV1. 1 and 2 refer to control group and hPBNCs@PDA-TRPV1 group, respectively.

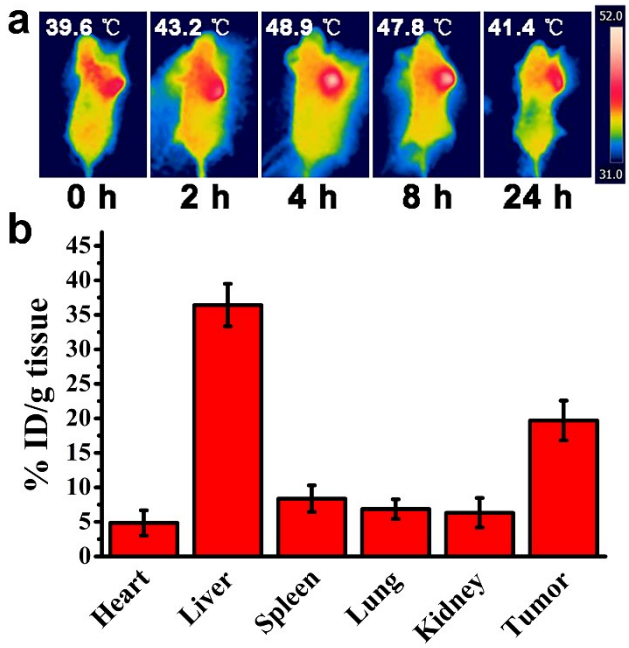

Figure S8. (a) IR thermal images of tumor-bearing mice under 808 nm laser irradiation at different times after intravenous injection of hPBNCs@PDA-TRPV1. (b) The biodistribution of hPBNCs@PDA-TRPV1 after intravenous injection by ICP-MS assay (n = 4).