Supporting Information

Tumor-activated targetable photothermal-chemotherapy of IR780/zoledronic acid-containing hybrid polymeric nanoassemblies with folate modification to treat aggressive breast cancer

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Preparation of PEG non-detachable ZA/IR780@HPNs

First, ZA (2.0 mg) dissolved in pH 8.5 Tris buffer (0.85 mL) was added into 0.85 mL of pH 6.0 Tris buffer containing PEI (5.7 mg) and then stirred for 30 min to attain the ZA/PEI ionic complexes. Then PLGA (6.0 mg), TPGS (0.8 mg), PEG-stearic acid (PEG-C18) (2.0 mg) and IR780 (0.6 mg) dissolved in DMSO (0.3 mL) were dropwise added to the ZA/PEI-containing aqueous solution (1.7 mL) under stirring. The mixed solution was gently stirred at 25 ^{o}C for 30 min, followed by equilibrium for 1 h. The obtained solution containing PEG non-detachable ZA/IR780@HPNs was dialyzed (Cellu Sep MWCO 6000~8000) against pH 8.0 phosphate buffer (10 mM) at 4 ^{o}C to remove DMSO, unloaded ZA and IR780 molecules.

ZA Loading Efficiency and Content. To quantify ZA loaded within hybrid nanoparticles, a prescribed volume of ZA-carrying nanoparticle suspension was freeze-dried and then dispersed in DMSO to disrupt the colloidal structure and allow ZA precipitation. The ZA precipitate was collected by centrifugation and then freeze-dried. After complete dissolution of ZA pellets in deionized water, the ZA concentration was measured by high performance liquid chromatography (LC-2050C 3D, Shimadzu) with a reversed-phase C18 column (4.6 × 250 mm and 5 mm, Sigma-Aldrich (USA)). The mobile phase was consisting of aqueous orthophosphoric acid solution (pH 6.0) and acetonitrile (88:12, v/v) with a gradient elution pumped at a flow rate of 1.0 mL/min, and the temperature of column was kept at 27 °C. The absorbance of ZA at 215 nm was determined. The data presented herein represent an average of triplicate measurements. The drug loading efficiency (DLE) and drug loading content (DLC) were calculated by following formulas:

DLE (%) = (weight of ZA loaded/weight of ZA in feed) × 100%.

DLC (%) = (weight of ZA loaded/total weight of ZA-containing hybrid nanoparticles) × 100%.

***In Vivo* TRAMP-C1 Tumor Growth Inhibition.** Male C57BL/6J mice (6~8 weeks old), purchased

from National Laboratory Animal Center (Taiwan), were cared according to the Guidance Suggestions for the Care and Use of Laboratory Animals, approved by the Administrative Committee on Animal Research in the Chung Shan Medical University (Taiwan) (IACUC Approval No: 2514). To establish xenograft tumor model, a total of 8×10^6 TRAMP-C1 cells were subcutaneously injected into the right thigh of each mouse. Tumor volume (V) was calculated as follows: $V = L \times W^2/2$, where W is the tumor measurement at the widest point and L the tumor dimension at the longest point.

When tumor volume of mice reached 80~110 mm^3, mice were randomly divided into 5 groups (n=3 per group): (i) PBS; (ii) free ZA/IR780 mixtures; (iii) free ZA/IR780 mixtures + NIR laser; (iv) ZA/IR780@HPNs and (v) ZA/IR780@HPNs + NIR laser. Mice in different groups were intravenously injected with the corresponding reagents at a ZA dosage of 3.2 mg/kg and an IR780 dosage of 1.0 mg/kg. At 24 h post-injection, the tumor site of mice in the prescribed groups was irradiated with 808 nm laser (1.25 W/cm^2) for 5 min. The tumor volume and body weight of different groups were measured every two days until 18 days post-treatment. After all mice were euthanized, the tumors were gathered and weighted.

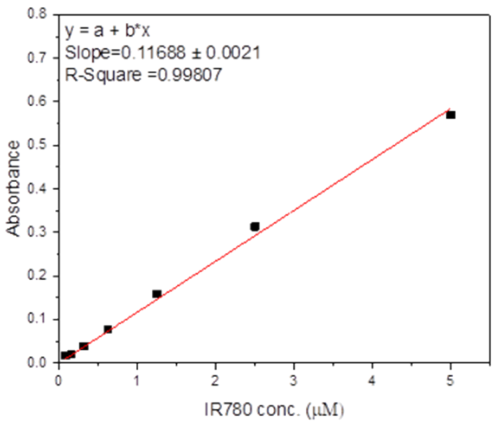

Fig. S1. Calibration curve of IR780 with various concentrations in pH 7.4 phosphate buffer used for drug loading characterization.

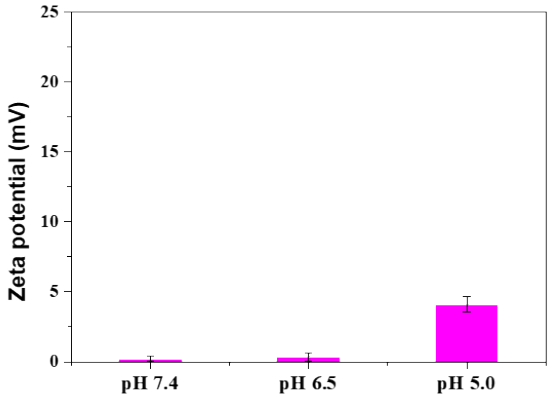

Fig. S2 Zeta potential of PEG non-detachable ZA/IR780@HPNs in aqueous solutions of different pH (n = 3).

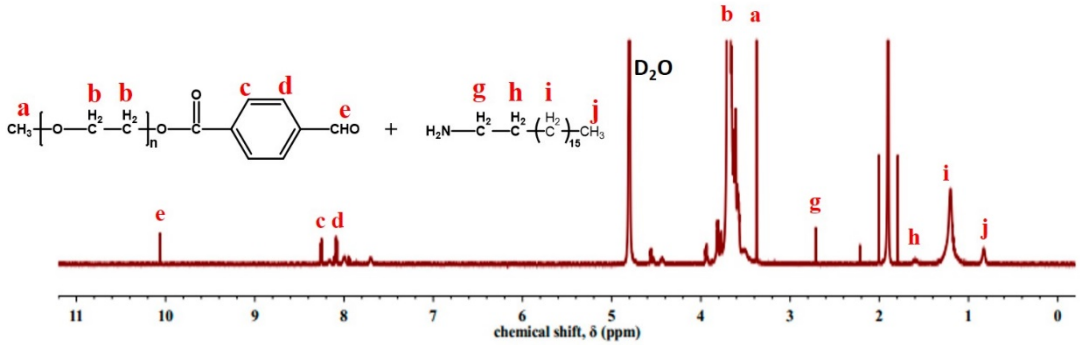

Fig. S3 ^{1}H-NMR spectrum of acid-pretreated PEG-b-C18 conjugates in D_2O.

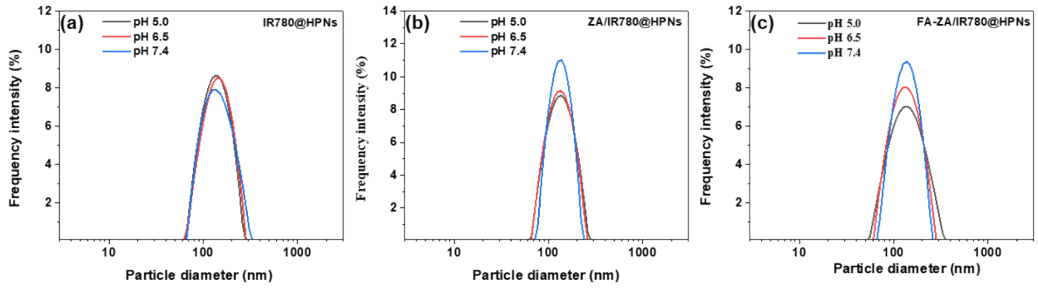

Fig. S4 DLS size distribution profiles of (a) IR780@HPNs, (b) ZA/IR780@HPNs and (c) FA-ZA/IR780@HPNs in aqueous solutions of different pH.

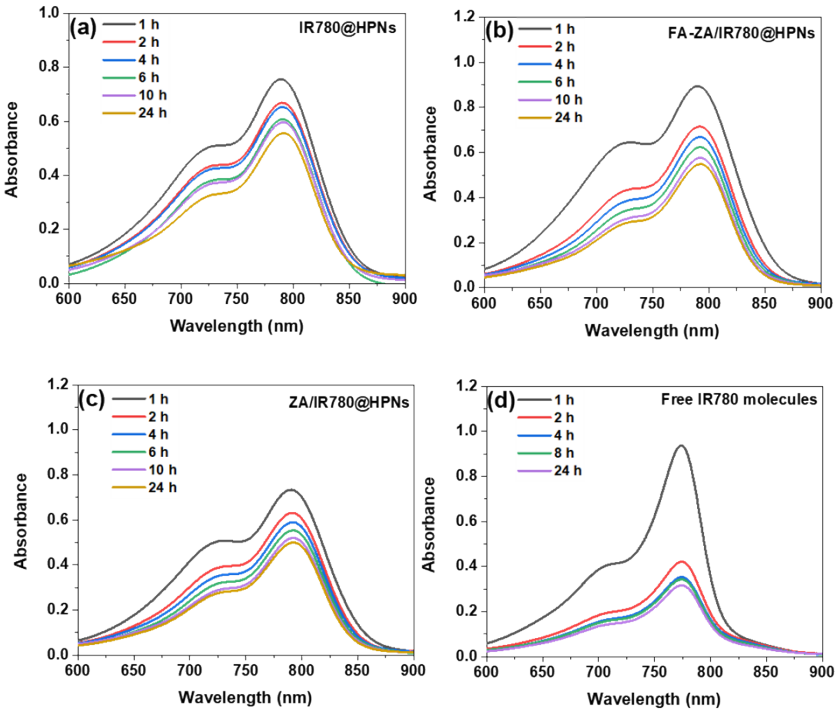

Fig. S5 UV/Vis spectra of (a) IR780@HPNs, (b) FA-ZA/IR780@HPNs, (c) ZA/IR780@HPNs and (d) free IR780 molecules dispersed in pH 7.4 PBS exposed to white light at different time intervals.

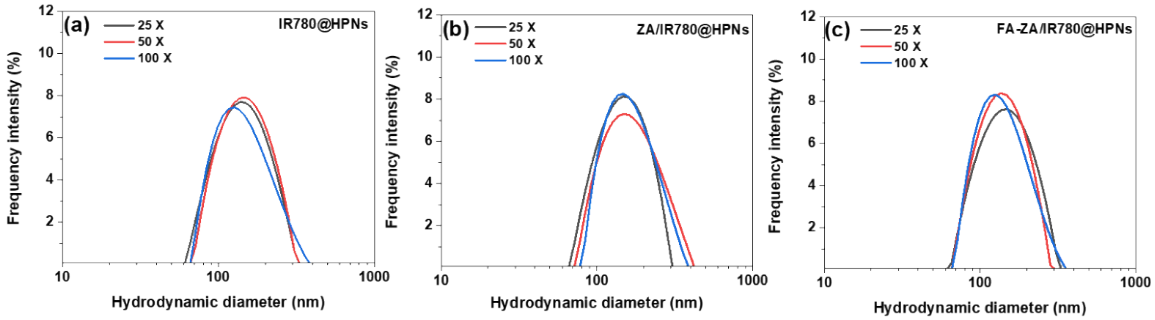

Fig. S6 DLS size distribution profiles of (a) IR780@HPNs, (b) ZA/IR780@HPNs and (c) FA-ZA/IR780@HPNs diluted with pH 7.4 PBS.

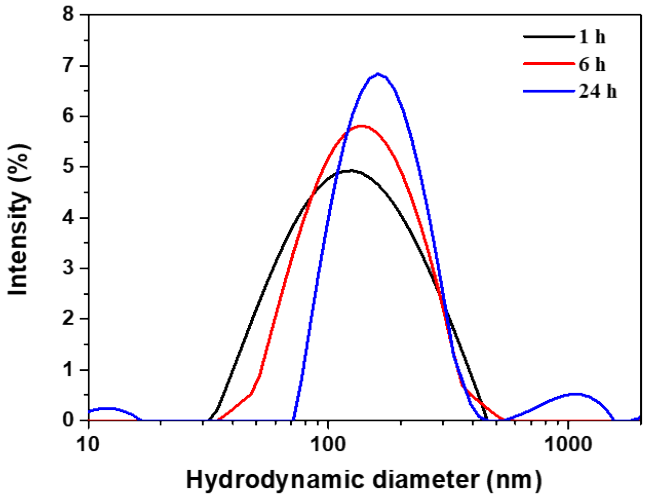

Fig. S7 DLS size distribution profiles of ZA/IR780@HPNs without TPGS decoration in 10% FBS-containing PBS at different time intervals.

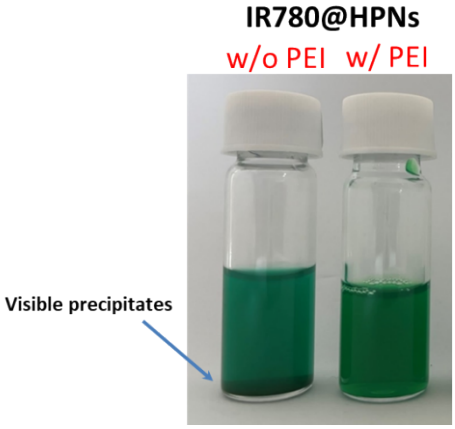

Fig. S8 Photographs of IR780@HPNs with or without PEI addition in pH 7.4 PBS.

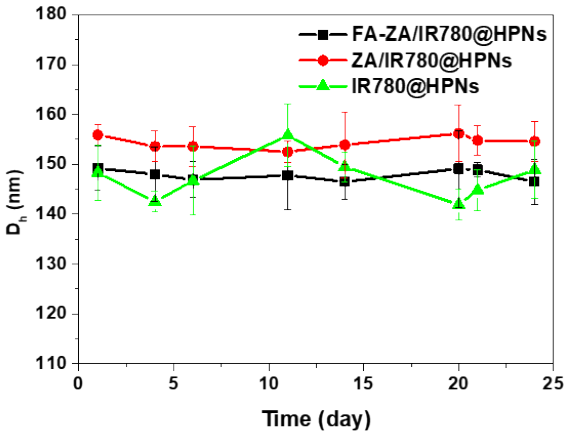

Fig. S9 Colloidal stability of various PEI-containing cargo-loaded HPNs stored in PBS at 37 °C (n = 3).

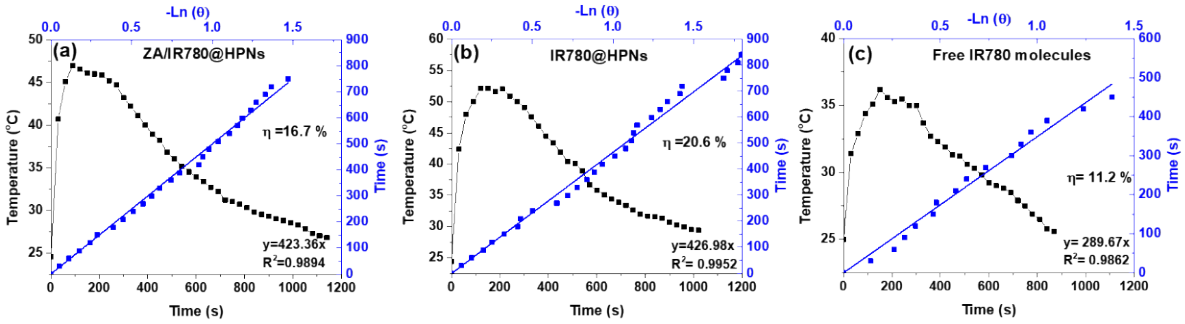

Fig. S10 Temperature profile of (a) ZA/IR780@HPNs, (b) IR780@HPNs and (c) free IR780 molecules (IR780 concentration: 80 μM) in pH 7.4 PBS after exposure to 808 nm laser irradiation (1.0 W/cm^2) for single on/off cycle, and plot of cooling time versus negative logarithm of the temperature driving force.

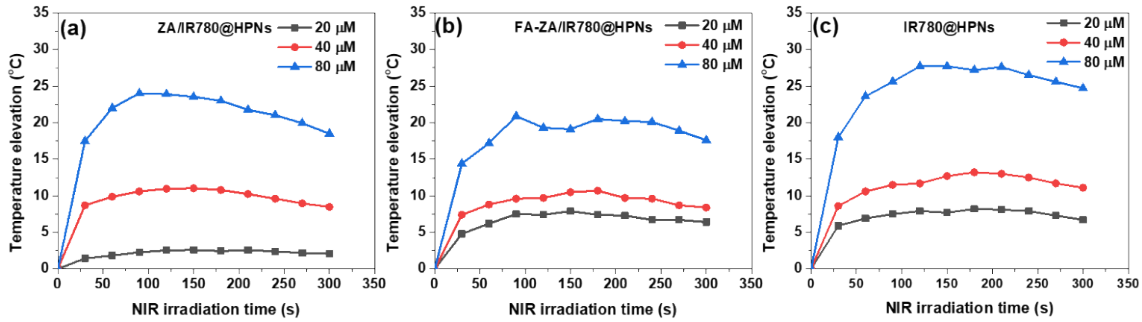

Fig. S11 Temperature change profiles of (a) ZA/IR780@HPNs, (b) FA-ZA/IR780@HPNs and (c) IR780@HPNs with different IR780 concentrations in PBS during irradiation of 808 nm NIR laser (1.25 W/cm^2).

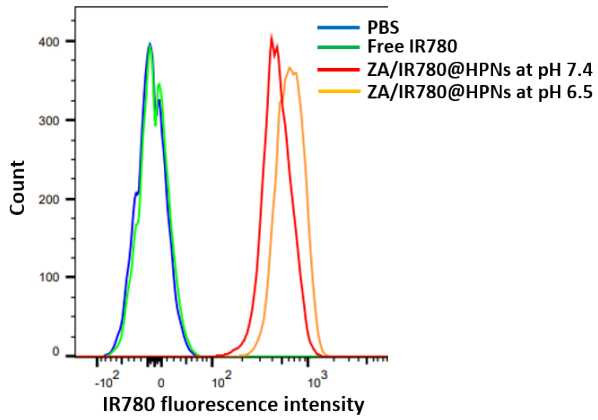

Fig. S12 Flow cytometric histograms of 4T1 cells incubated with ZA/IR780@HPNs at pH 7.4 and 6.5 for 1 h (IR780 concentration =1.5 μM).

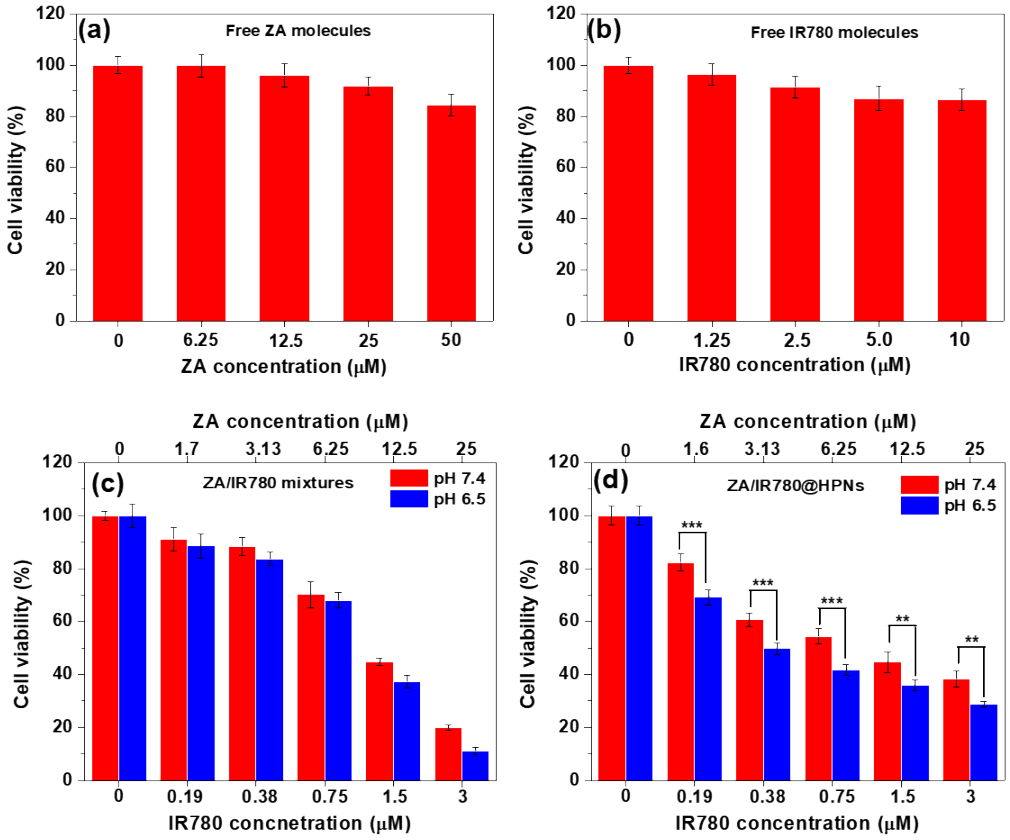

Fig. S13 Cell viability of 4T1 cells incubated with (a) free ZA molecules, (b) free IR780 molecules, (c) ZA/IR780 mixtures and (d) ZA/IR780@HPNs, respectively, at 37 °C for 24 h in the lack of NIR laser irradiation.

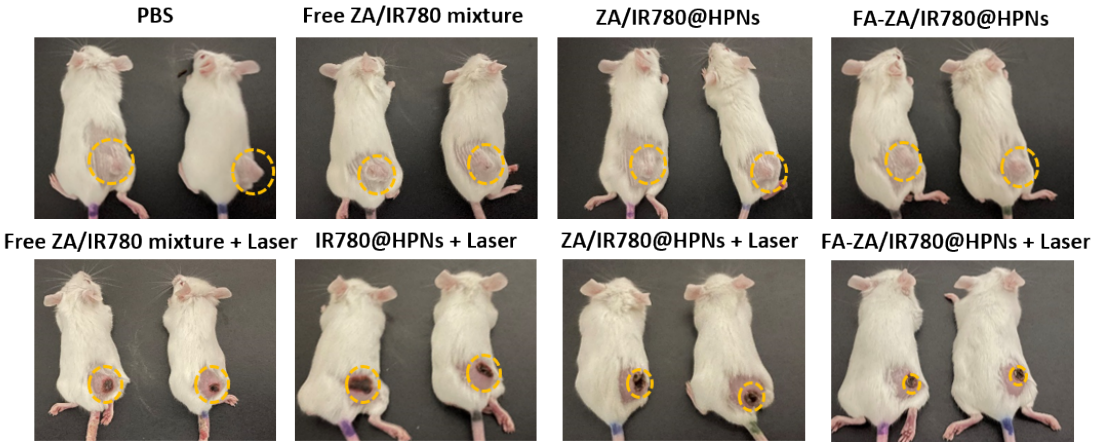

Fig. S14 Photographs of 4T1 tumor-bearing mice receiving different treatments for 20 days. The circled regions are the tumor sites.

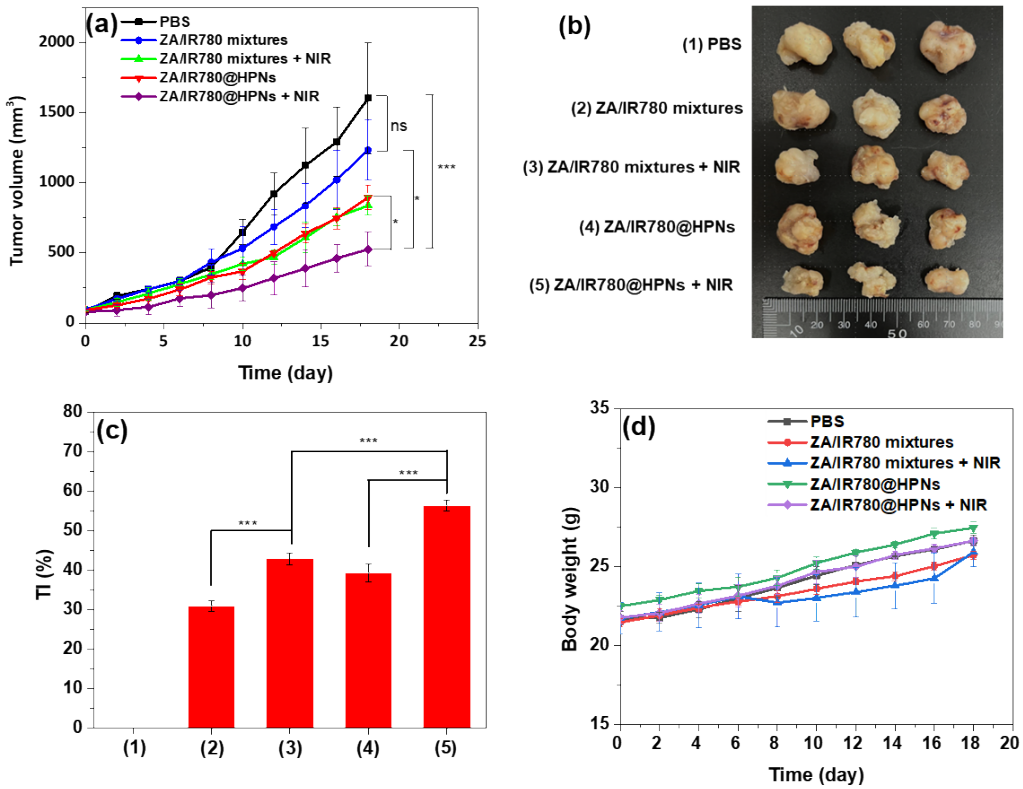

Fig. S15 (a) Tumor growth suppression profiles of TRAMP-C1 tumor-bearing mice treated with ZA/IR780 mixtures and ZA/IR780@HPNs, respectively, followed by NIR laser irradiation (5 min, 1.25 W/cm^2) at 24 h post-injection or without any laser treatment. (b) Morphology and size of the tumors isolated from the euthanized mice at day 18 after the treatment. (c) TI value of various treatments. (d) Body weight of TRAMP-C1 tumor-bearing mice receiving different treatments.

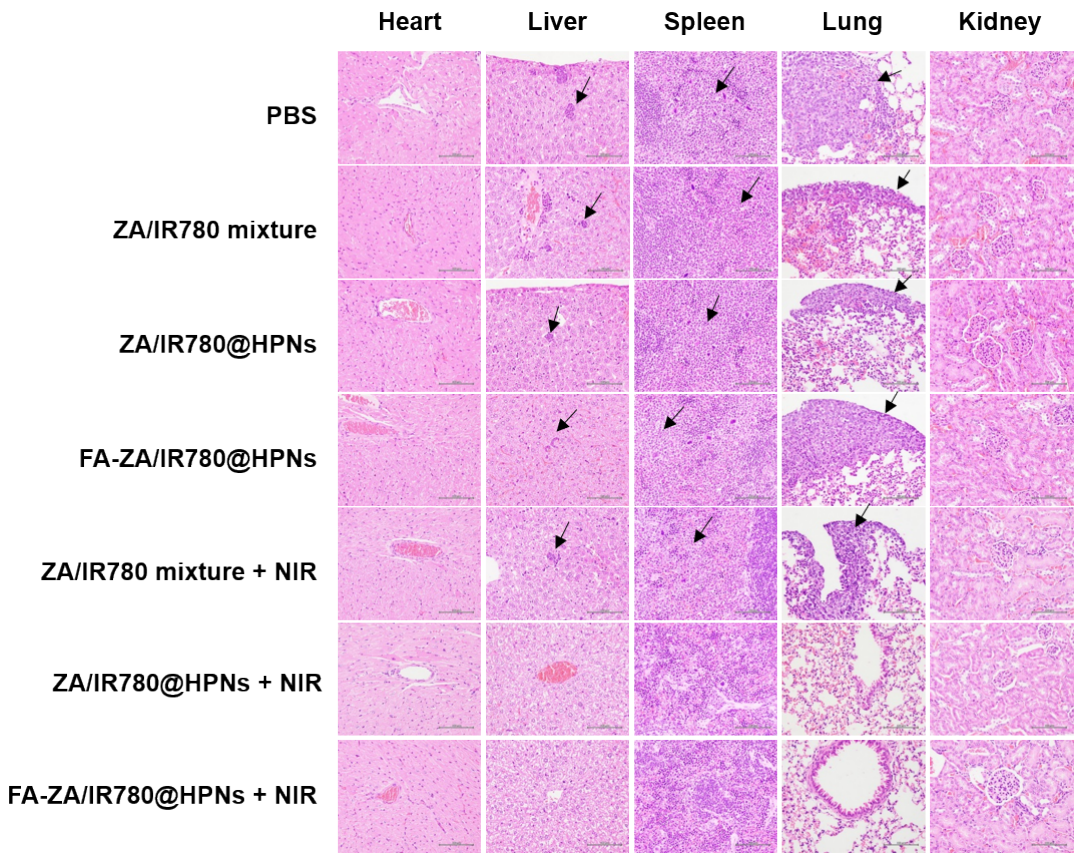

Fig. S16 H&E-stained images of major organs from the 4T1 tumor-bearing mice receiving various treatments and sacrificed at 20 days post treatment. Scale bars are 100 μm. Black arrows symbolize the cancer metastasis.