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Electronic Supplementary Information

Conjugation of photosensitizer to near infrared light renewable persistent luminescence nanoparticles for photodynamic therapy

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Table of the Contents

1. Experimental details

Materials and reagents: $Zn(NO_3)_2.6H_2O$ (99.99%), Ga_2O_3 (99.99%), GeO_2 (99.999%), $Cr(NO_3)_3 \cdot 9H_2O$ (99.99%), 3-amino propyl-triethoxysilane (APTES, 98%) were purchased from Aladdin (Shanghai, China). Silicon phthalocyanine (Si-Pc), thiazolyl blue tetrazolium bromide (MTT) was obtained from Sigma-Aldrich (St Louis, USA). 1, 3-Diphenylisobenzofuran (DPBF) was purchased from J&K Scientific (Beijing, China). $NH_3 \bullet H_2O$, NaOH, ethanol absolute, DMSO and N,N-dimethylformamide (DMF) were purchased from Concord Chemical Research Institute (Tianjin, China).

Instrumentation: X-ray (XRD) patterns were collected on a D/max-2500 diffractometer (Rigaku, Tokyo, Japan) equipped with Cu Kα radiation (λ = 1.5418 Å) over the angular range of 10 to 80 degrees. Transmission electron microscopy (TEM) were performed on JEOL-100CX II microscopy (JEOL, Japan) operating at an accelerate voltage of 100 kV, and high-resolution TEM (HRTEM) on Philips Tecnai G^2 F20 microscopy (Philips, Eindhoven, Netherlands) operating at an acceleration voltage of 200 kV. Photoluminescence spectra and time decay curves were recorded on an F-4500 spectrofluorometer (Hitachi, Tokyo, Japan). Fourier transform infrared spectra (FT-IR) were obtained on a Nicolet 6700 spectrometer (Thermo Scientific, Madison, USA) in KBr plates. The absorption spectra were recorded on a UV-3600 spectrophotometer (Shimadzu, Japan). Thermo gravimetric analysis (TGA) was conducted with a PTC-10A TG-DTA thermo-analyzer (Rigaku, Tokyo, Japan). NIR afterglow decay images were acquired on an IVIS Lumina II Imaging System (Xenogen, USA) equipped with CCD camera (detection wavelength: 515-875 nm) under luminescence imaging mode without any excitation/emission filters. The absorbance for the MTT experiment was recorded on a microplate reader (Molecular Devices Co., Menlo Park, CA).

Preparation of the PLNPs: $Zn_3Ga_4Ge_2O_{13}$:0.3%Cr^{3+} nanoparticles were synthesized via a solvo-thermal approach in combination with a subsequent sintering in air. The chemical composition of the PLNPs modified from a previously reported work.[1] $Zn(NO_3)_2{\cdot}6H_2O$ and $Cr(NO_3)_3{\cdot}9H_2O$ were dissolved in ultrapure water. Ga_2O_3 was dissolved in concentrated nitric acid under hydrothermal condition at 150°C overnight. GeO_2 was dissolved in dilute ammonium solution (30 wt%). The starting materials were mixed according to the chemical formula of $Zn_3Ga_4Ge_2O_{13}$: 0.3%Cr^{3+} under vigorous stirring, ethanol (20 mL) and ethylene glycol (10 mL) were then added to the reaction mixture. The resulting solution was adjusted to pH 8.0 with ammonia solution (30 wt%). The turbid solution was kept stirring at room temperature for 3 h, then heated in a 30 mL teflon-line stainless steel autoclave at 120 °C for 24 h. The resulting mixture was cooled down to room temperature, centrifugally washed with ultrapure water and ethanol absolute sequentially, then dried at 60°C for 2 h. Finally, the dry white compound was ground and annealed in air at 1000 °C for 3 h. Thereafter, the resulting powder was wet-grounded with minimum ethanol for 30 min, dispersed in 5 mM NaOH solution for overnight stirring, and centrifuged to obtain hydroxylated long-persistent luminescence nanoparticles (PLNP-OH).

Surface functionalization of the PLNPs: PLNP-OH was coated with 3-aminopropyltriethoxysilane (APTES) as follows: 50 mg of dry precipitate of PLNP-OH was dispersed in 20 mL of DMF by sonication, and 200 µL of APTES was dropped under vigorous stirring and stirred at 80℃ for 24 h. Thereafter, the excess APTES was centrifugally washed off several times with DMF and ethanol absolute sequentially. Finally, the resulting APTES-PLNPs were dried under vacuum overnight. To conjugate silicon phthalocyanine (Si-Pc) to APTES-PLNPs, 200 µg mL^{-1} of Si-Pc (6 mL) was mixed with APTES-PLNPs dry sample by sonication. Subsequently, the mixture was gently stirred at

room temperature in the dark for 30 h. Finally, the resulting complex Si-Pc-PLNPs were precipitated and the excess Si-Pc was centrifugally washed off with ethanol absolute several times, and dried under vacuum overnight. The Si-Pc content in the Si-Pc-PLNPs samples was determined by measuring the absorbance at 672 nm.

Singlet oxygen ($^1O_2$) detection. The persistent singlet oxygen ($^1O_2$) generation capability of Si-Pc-PLNPs was examined by recording the fluorescence quenching of 1,4-diphenyl-2, 3-benzofuran (DPBF), as the fluorescence probe. In a typical experiment, as the solvent of DPBF, acetonitrile was bubbled with air for 1 h to ensure the oxygen during $^1O_2$ detection. Then, the DPBF (100 μL, 34 μM in acetonitrile) was added to the dispersion of pre-excited (254 nm, 6W) Si-Pc-PLNPs (2 mL, 0.75 mg mL^{-1}) in the dark. The fluorescence of DPBF at 455 nm was recorded every 2 min (ex. 410 nm). After 22 min, the mixture was irradiated with the 808 nm NIR laser to re-activate the NIR persistent luminescence for further generation of $^1O_2$, the fluorescence of DPBF was recorded every 2 min again. The control experiments were carried out with three parallel groups: (1) Si-Pc solution (2 mL) and DPBF (100 μL). (2) DPBF and 808 nm NIR light. (3) DPBF only.

Cell culture. HepG2 cells were originally cultured in DMEM (Roswell Park Memorial Institute's medium, high glucose) medium supplemented with 10% fetal bovine serum (FBS) and 100 units mL^{-1} of penicillin − streptomycin. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

Intracellular PDT analysis by MTT. HepG2 and 293T cells were respectively plated in 96-well microtiter plate with 5×10^4 cells/well, and cultured in 100 μL growth medium under a humidified 5% CO_2 at 37 °C for 24 h. The cells were subsequently exposed to various concentrations of Si-Pc-PLNPs (0-300 μg mL^{-1}), APTES-PLNPs (0-300 μg mL^{-1}) and Si-Pc (0-20 μg mL^{-1}) for 24 h. Finally, the

standard MTT assay was carried out to determine the cell viability. The cells were labeled with 0.5 mg mL^{-1} solution of MTT in full culture medium for 4 h, then the supernatant was removed and replaced with 100 μL of DMSO and the formazan absorbance was analyzed by a microplate reader at 570 nm.

We further investigated intracellular PDT effect of nanoparticles on cancer cells. HepG2 cells were plated in 96-well microtiter plate with 5×10^4 cells/well, and incubated in 100 μL growth medium under a humidified 5% CO_2 atmosphere at 37 °C for 24 h. Thereafter, cells were exposed to pre-excited (with a UV lamp at 254 nm for 10 min) Si-Pc-PLNPs (200 μg mL^{-1}), APTES-PLNPs (200 μg mL^{-1}) or Si-Pc (10 μg mL^{-1}) separately, and incubated for another 8 h, washed three times with PBS solution, and fresh cell culture medium was added before irradiation with 808 nm NIR light at 1.0 W cm^{-2} for 0, 3, 5 or 10 min. After overnight incubation, the cells were labeled with 0.5 mg mL^{-1} solution of MTT in full culture medium. Finally, the supernatant was removed and replaced with 100 μL of DMSO and the formazan absorbance was analyzed by a microplate reader at 570 nm.

To assess the effect of 808 nm NIR light irradiation on the therapeutic efficiencies, adherent HepG2 cells without any treatment were irradiated with different exposure powers of 808 nm NIR light at 0.5, 1.0, 1.5 W cm^{-2} and were further incubated for 24 h. Cell viabilities were determined by a standard MTT assay. Both irradiation power (0.5, 1.0 W cm^{-2}) and concentration dependent therapeutic efficiencies of high and low concentration of Si-Pc-PLNPs (80 or 200 μg mL^{-1}), APTES-PLNPs (80 or 200 μg mL^{-1}) and Si-Pc (4 or 10 μg mL^{-1}) were also investigated.

Pathology analysis. After treatment, pathology analysis was carried out by hematoxylin and eosin (H&E). The mice in the different groups were sacrificed, and major tissues including liver,

kidney, lung, heart and spleen were collected. The organs were fixed in 4% paraformaldehyde solution, dehydrated, and embedded in liquid paraffin. The slice organs were stained with hematoxylin and eosin (H&E) and imaged on an optical microscopy.

All experiments for animals were performed in compliance with the guidelines of the Tianjin Committee of Use and Care of Laboratory Animals, and approved by the Animal Ethics Committee of Nankai University.

2. Supplementary figures

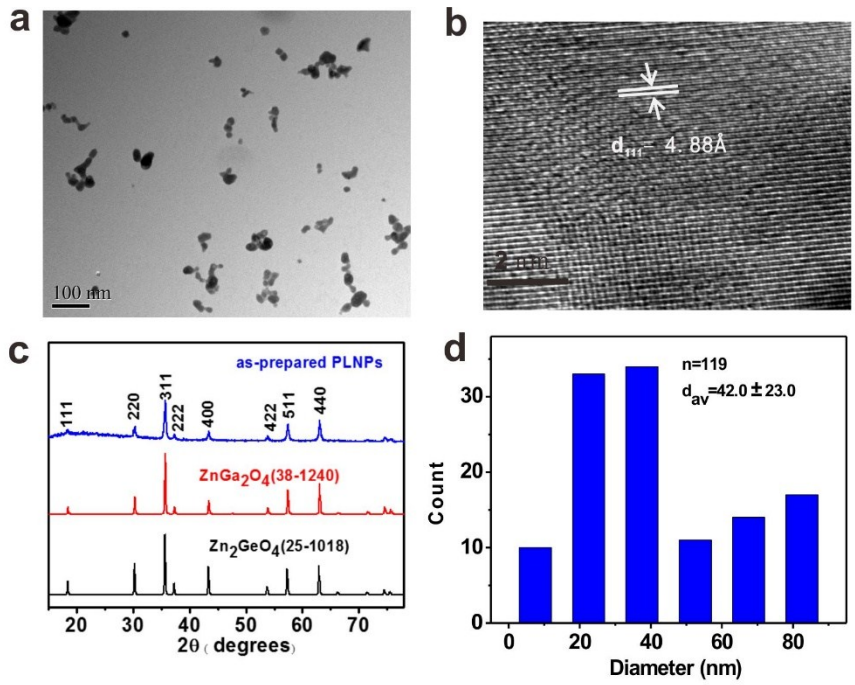

Fig. S1 Characterization of PLNPs: (a) TEM images of the PLNPs. (b) High-resolution TEM (HRTEM) images of the PLNPs, showing the distance between the lattice fringes was 4.81 Å. (c) XRD patterns of the PLNPs powder in contrast to the standard card of spinel phase zinc gallate and zinc germanate structure. Lattice fringes distance (4.81 Å) in HRTEM images corresponds to the spacing for the (111) lattice planes in XRD patterns. (d) TEM size distribution of the PLNPs.

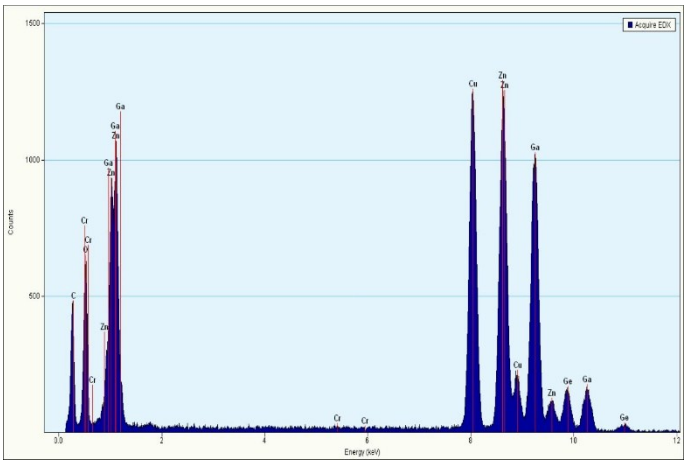

Fig. S2 EDX of PLNPs showing the presence of the Zn, Ga, Ge and Cr elements.

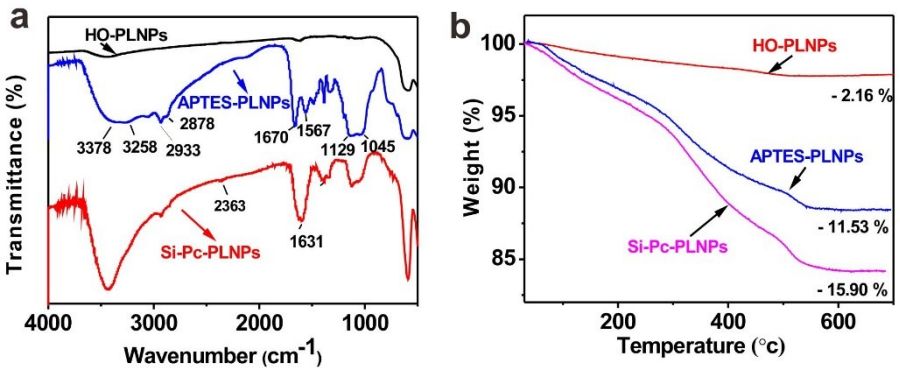

Fig. S3 Surface functionalization of the PLNPs: (a) FT-IR spectra of HO-PLNPs, APTES-PLNPs, and Si-Pc-PLNPs. (b) TGA curves of HO-PLNPs, APTES-PLNPs, and Si-Pc-PLNPs. TGA shows increased weight loss with gradual modification.

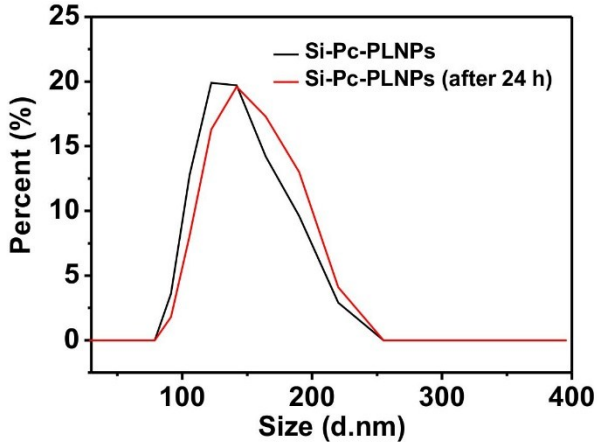

Fig. S4 Hydrodynamic size distribution of Si-Pc-PLNPs in aqueous solution prepared freshly and after 24 h setting.

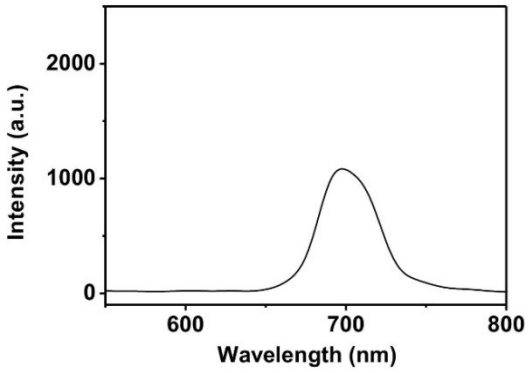

Fig. S5 Fluorescence spectrum of Si-Pc-PLNPs aqueous solution.

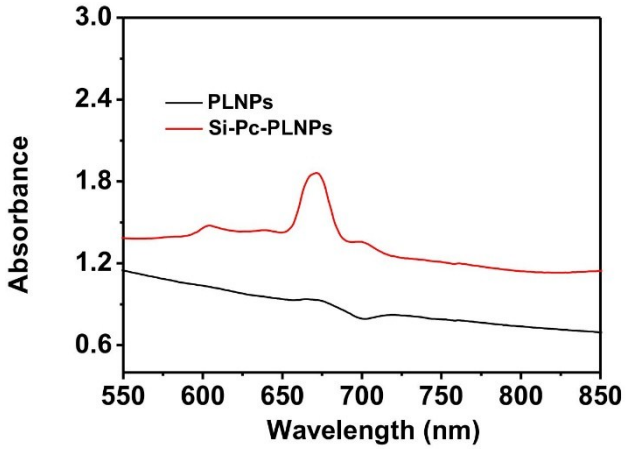

Fig. S6 Absorption spectra of PLNPs and Si-Pc-PLNPs aqueous solution.

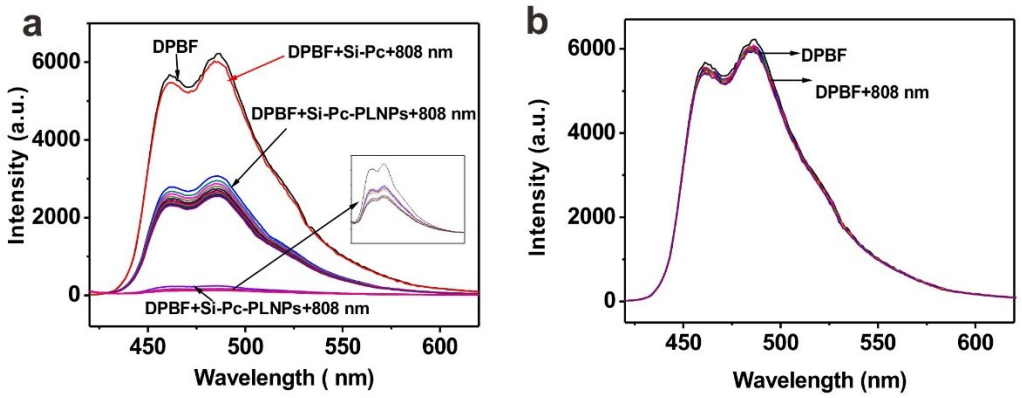

Fig. S7 Monitoring of persistent luminescence sensitized generation of $^1O_2$: (a) Time dependent persistent quenching and 808 nm light renewed persistent fluorescence quenching of DPBF. The samples were excited with a UV lamp at 254 nm for 10 min before adding DPBF. The persistent quenching of DPBF was recorded for 22 min without further excitation. Then, the samples were re-activated with 808 nm NIR laser for 100 s. Inset shows persistent quenching of DPBF florescence after 808 nm NIR laser irradiation. No obvious change in DPBF fluorescence by irradiating Si-Pc solution with 808 nm laser indicates the decrease in DPBF fluorescence in the presence of Si-Pc-PLNPs was due to re-activation of Si-Pc-PLNPs. (b) Effect of 808 nm NIR light irradiation on the fluorescence of DPBF, showing negligible effect of the excitation with 808 nm NIR light. All experiments were carried out under excitation at 410 nm.

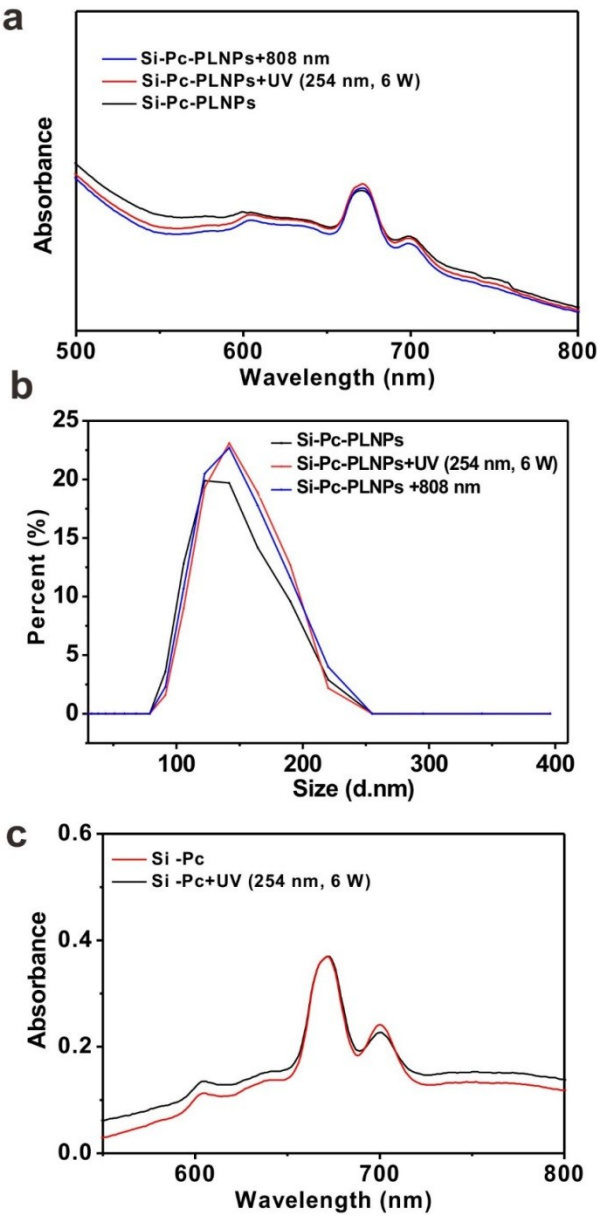

Fig. S8 Stability of Si-Pc and Si-Pc-PLNPs in aqueous solution: (a) Effect of 10 min UV and 808 nm NIR irradiation on the absorption spectra of Si-Pc-PLNPs. (b) Effect of 10 min UV and 808 nm NIR irradiation on the hydrodynamic size distribution. (c) Effect of 10 min UV irradiation on the absorption spectra of Si-Pc. No obvious changes in the spectra of Si-Pc-PLNPs before and after light irradiation indicate the Si-Pc and Si-Pc-PLNPs are stable during and after light irradiation.

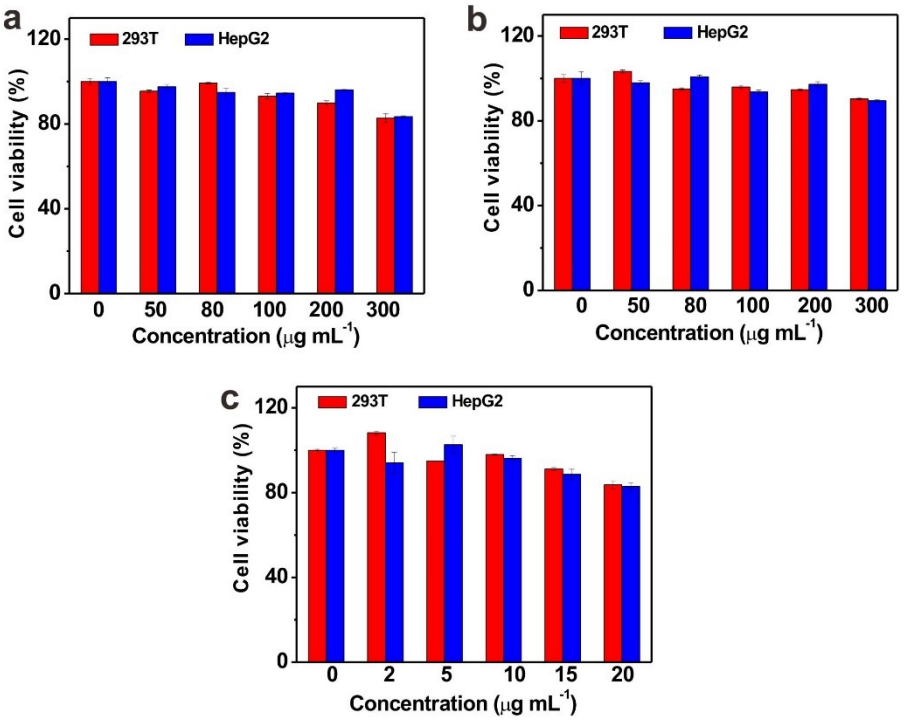

Fig. S9 Cell viability of 293T and HepG2 cells incubated with different concentration of the materials: (a) Si-Pc-PLNPs (0-300 μg mL^{-1}); (b) APTES- PLNPs (0-300 μg mL^{-1}); (c) Si-Pc (0-20 μg mL^{-1}). All the experiments were carried out without any irradiation. Error bar indicates one standard deviation (n=3).

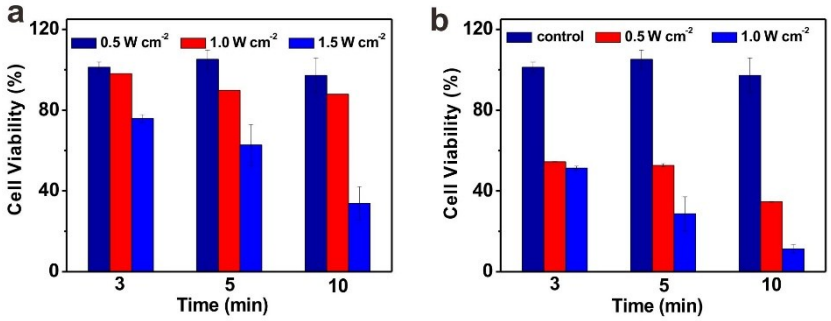

Fig. S10 Effect of light irradiation power and time on therapeutic efficiency: (a) Cell viability of HepG2 cells under 808 nm NIR light irradiation with various powers (0.5, 1.0, 1.5 W cm^{-2}) without any treatment. (b) Cell viability of HepG2 cells incubated with pre-excited Si-Pc-PLNPs (200 μg

mL^{-1}) for 8 h, then irradiated with 808 nm NIR light at various powers (0.5, 1.0 W cm^{-2}). Si-Pc-PLNPs were excited with UV lamp at 254 nm for 10 min before exposition to the cells. Error bar indicates one standard deviation (n=3).

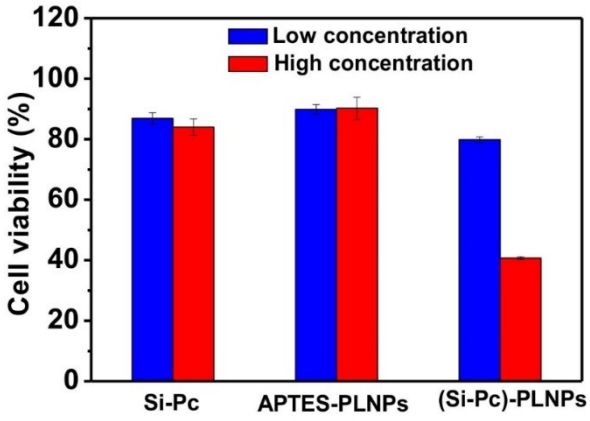

Fig. S11 Viability of HepG2 cells incubated with high and low concentrations of pre-excited Si-Pc-PLNPs (80 or 200 μg mL^{-1}, equivalently containing 4 or 10 μg mL^{-1} of Si-Pc), APTES-PLNPs (80 or 200 μg mL^{-1}) and Si-Pc (4 or 10 μg mL^{-1}) for 8 h, followed by 808 nm NIR light irradiation for 3 min. Si-Pc-PLNPs, APTES-PLNPs and Si-Pc were pre-excited with 254 nm for 10 min before exposition to the cells. Error bar indicates one standard deviation (n=3).

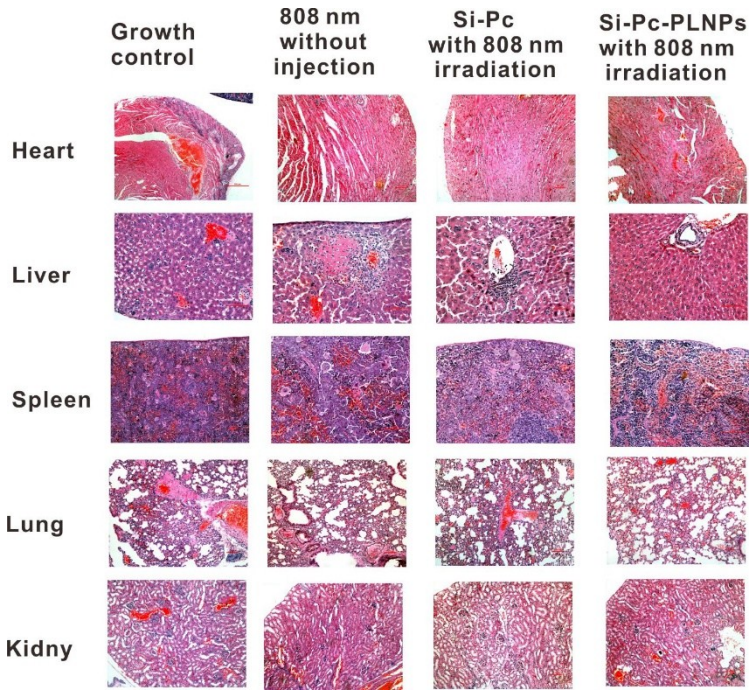

Fig. S12 H&E stained images of major organs from different control (groups of mice: without any injection (growth control), injected with Si-Pc-PLNPs but without 808 nm NIR laser irradiation, irradiated with 808 nm NIR laser but without any injection, injected with Si-Pc-PLNPs and 4 h later irradiated with 808 nm NIR laser for 10 min, injected with Si-Pc and 4 h later irradiated with 808 nm NIR laser.)

3. Supplementary reference

1. Z. Pan, Y. Y. Lu, F. Liu, *Nat. Mater.* , 2012, **11**, 58-63.