# Supplement Information

# GSH/pH Dual Response Drug Delivery System for Photothermal Enhanced Gene-Immunotherapy

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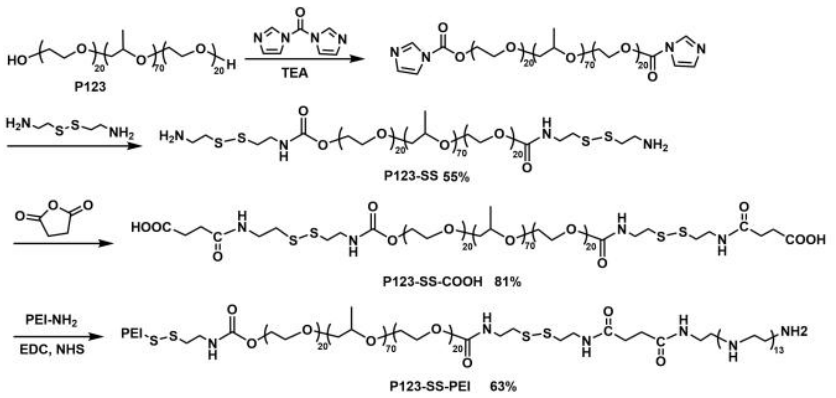
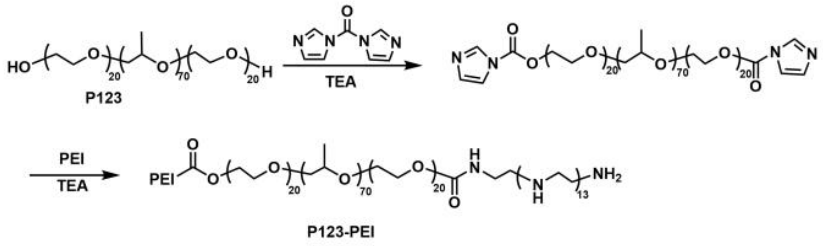
**Materials:** *N, N′*-carbonyldiimidazole (CDI), cystamine dihydrochloride, polyethyleneimine (molecular weight, $M_W$ = 600), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), Pluronic P123 ($M_W$ = 5800), *N*-hydroxysuccinimide (NHS), dimethyl sulfoxide (DMSO) and indocyanine green (ICG) were purchased from Energy Chemical (Shanghai, China). Succinic anhydride and triethylamine (TEA) were purchased from Sinopharm (Shanghai, China). Pyrene was obtained from Aladdin (Shanghai, China). NC siRNA and FAM-siRNA (sense: UUCUCCGAACGUGUCACGUTT; antisense: ACGUGACACGUUCGGAGAATT), siPD-L1 (sense: GAGGUAAUCUGGACAAACATT; antisense: UGUUUGUCCAGAUUACCUCTT) were purchased from GenePharma (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Biomics Biotechnologies Co. Ltd. (Nantong, China). Trypsine-EDTA was purchased from Cienry Biotechnology Co. Ltd. (Hangzhou, China). Fetal bovine serum (FBS) was purchased from Sijiqing Biological Engineering Technology Co. Ltd. (Hangzhou, China). BCA protein content detection kit and live/dead viability apoptosis detection kit were purchased from KeyGen BioTECH (Nanjing, China).

Anti-fluorescence quenching sealing tablet, ELISA kit and ATP detection kit were purchased from Beyotime Biotechnology (Shanghai, China). 4',6-Diamidino-2-phenylindole (DAPI), calreticulin (CALR), FITC-labeled Goat Anti-Rabbit IgG, enhanced radio immunoprecipitation assay (RIPA) lysate, red blood cell lysate and phenylmethanesulfonyl fluoride (PMSF) were purchased from Boster (Wuhan, China). Polyacrylamide gel electrophoresis (PAGE) rapid gel preparation kit (10%) was purchased from Yamei (Shanghai, China). 4% Paraformaldehyde was purchased from BBI Life Sciences Corporation (Shanghai, China). PE-HMGB1, FITC-CD11c, APC-CD80, PE-CD86, BV421-CD3, PerCP-CD4, PE-CD8a, APC-CD25, PE-Foxp3 and APC-PD-L1 antibodies were purchased from Dakewe Biotechnology Co. Ltd. (Beijing, China). PD-L1 antibody was purchased from Abcam (Cambridge, Britain). Dulbecco's modified eagle medium (DMEM), roswell park memorial institute 1640 (RPMI1640) medium and penicillin-streptomycin were purchased from Gibco-BRL (Burlington, Canada). True-uclear transcription factor buffer set was purchased from Biolegend (California, America). RiboGreen RNA assay kit and RiboGreen RNA reagent, RediPlate$^{TM}$ 96 RiboGreen RNA quantitation kit were purchased from Thermo Fisher Scientific (Waltham, MA, American).

**Methods:** $^{1}$H nuclear magnetic resonance ($^{1}$H-NMR) spectra were recorded on a Bruker Advance 500 MHz spectrometer with $CDCl_3$ as a deuterated solvent. Absorption spectra were collected by Hitachi U-5300. The sizes were conducted on dynamic light scattering (DLS) using a Malvern Zetasize Nano ZS90 instrument and morphologies were performed on transmission electron microscopy (TEM) using a Hitachi S-4800. Flow cytometry (FCM) data derived from CytoFLEX S. Cellular fluorescence images were captured by confocal laser scanning microscope (CLSM) using Laser Scanning Microscope LSM800 (Zeiss). Thermal images were performed on an IR thermal camera FLIR ONEPro.




**Scheme S1** The synthetic routes of compound **PSP**.




**Scheme S2** The synthetic routes of compound **PP**.

**Synthesis of P123-SS: PSP** was synthesized according to the literature method [1]. Pluronic P123 (5.80 g, 1.00 mmol) and CDI (1.46 g, 9.00 mmol) were dissolved in anhydrous DMSO (10 mL), respectively, then mixed in a round-bottomed flask. TEA (0.8 mL) was added into the above solution and stirred under dark condition for 4 h. Then cystamine dihydrochloride (2.03 g, 9.00 mmol) was added to the solution and stirred for another 24 h at room temperature. After the reaction, the solution was dialyzed in a dialysis bag ($M_W$ = 3500 Da) for two days and freeze-dried to obtain P123-SS (yield: 55%).

**Synthesis of P123-SS-PEI (PSP):** P123-SS (2.74 g, 0.45 mmol) and succinic anhydride (0.40 g, 4.04 mmol) were dissolved in anhydrous DMSO, and the reaction was stirred under nitrogen at room temperature for 48 h. Then the solution was dialyzed for two days, and freeze-dried to obtain P123-SS-COOH (yield: 81%).

P123-SS-COOH (1.75 g, 0.25 mmol) and EDC (0.96 g, 5.00 mmol) were dissolved in anhydrous DMSO, then NHS (0.58 g, 5.00 mmol) was added and the reaction was stirred at room temperature for 24 h. After the reaction, PEI solution (3.00 g, 5.00

mmol) was added, and the reaction was continued for 24 h. The solution was dialyzed for two days and freeze-dried to obtain P123-SS-PEI (**PSP**, yield: 63%).

**Synthesis of P123-PEI (PP):** Pluronic P123 (5.80 g, 1.00 mmol) and CDI (1.46 g, 9.00 mmol) were dissolved in anhydrous DMSO (10 mL), respectively, then mixed in a round-bottomed flask. TEA (0.8 mL) was added into the above solution and stirred under dark condition for 4 h. Then PEI (4.80 g, 8.00 mmol) was added to solution and stirred 24 h at room temperature. The solution was dialyzed for two days and freeze-dried to obtain **PP** (yield: 63%).

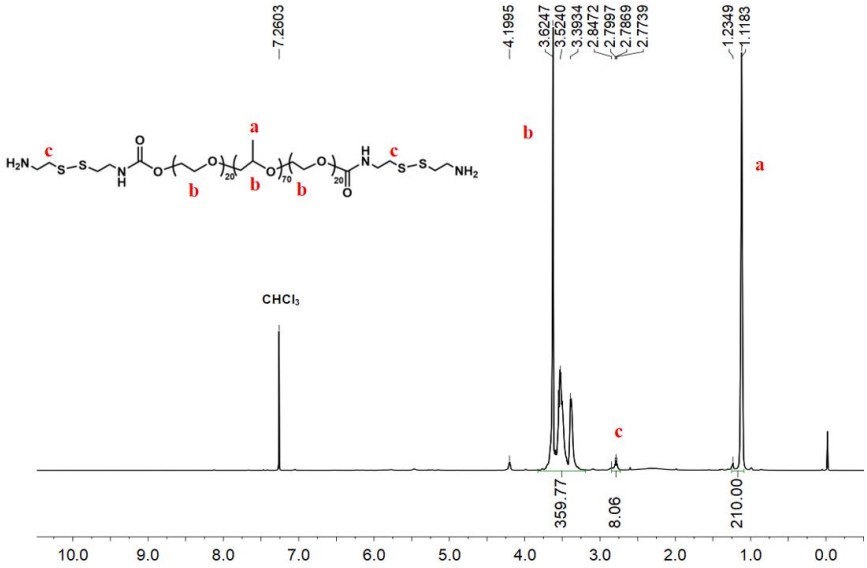



**Fig. S1** $^{1}$H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of P123-SS.

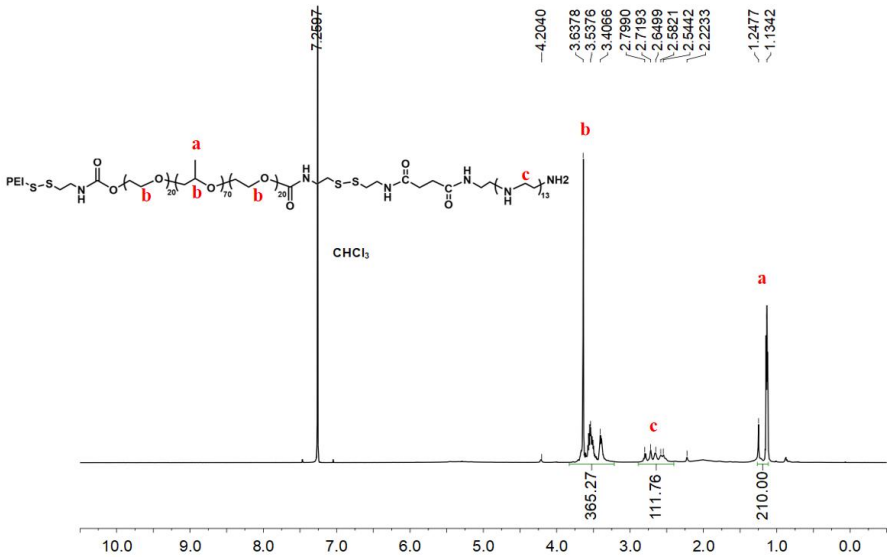



**Fig. S2** $^{1}$H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of **PSP**.

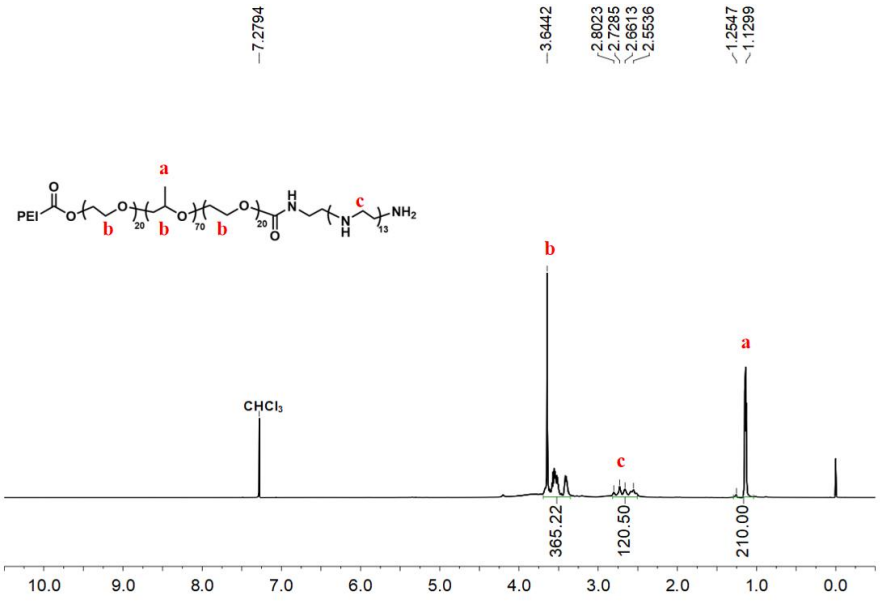



**Fig. S3** $^{1}$H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of **PP**.

**Critical micelle concentration (CMC) determination of PSP and PP micelles:** Pyrene acetone solution ($6\times10^{-6}$ mol·L$^{-1}$, 1 mL) was added to volumetric flasks (10 mL). After the acetone was evaporated, different concentrations of **PSP** and **PP** solution was added to the above volumetric flask, respectively. The volume was adjusted and fully dissolved by ultrasound for detection. The concentration of the pyrene solution was as follows: $1.0\times10^{0}$, $1.0\times10^{-1}$, $1.0\times10^{-2}$, $1.0\times10^{-3}$, $1.0\times10^{-4}$, $1.0\times10^{-5}$, $1.0\times10^{-6}$, $1.0\times10^{-7}$, $1.0\times10^{-8}$ mg/mL. The emission spectrum at 350-550 nm was measured by a fluorescence spectrophotometer with a fixed excitation wavelength of 335 nm, and the logarithmic relationship between the fluorescence intensity ratio of $I_{373}/I_{384}$ and the concentration of **PSP**, **PP** micelles was used to calculate CMC.

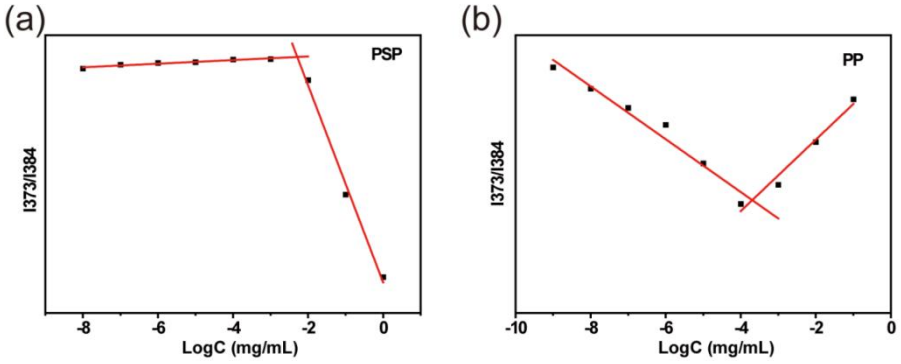



**Fig. S4** CMC of **PSP** and **PP** micelle.

**Particle size and zeta potential determination of PSP and PP micelles:** The **PSP** and **PP** micelles solution were taken at 25 °C to characterize their particle size and surface zeta potential by DLS. The solution (10 μL) was dropped onto a carbon-coated copper mesh for electron microscopy, and its morphology was observed and characterized by TEM.

**pH/Reduction response determination of PSP and PP micelles:** The particle size and zeta potential of **PSP** and **PP** micelles under different pH condition were determined by DLS to investigate whether they respond to pH. The micelles were dissolved in PBS solution (pH = 7.4, 6.5 and 5.7), respectively, and the particle size and zeta potential of the micelles were detected by DLS after 2 h. **PSP** and **PP** micelles were incubated in GSH solution (10 mM) for 2 h, respectively, then dialyzed for 2 h and freeze-dried. The PEI (-$CH_2CH_2N$-, proton signal) characteristic absorption peak of **PSP** was detected by $^1H$ NMR.

**Gel blocking experiment:** Agarose, *tris* acetate-EDTA buffer (TAE buffer) (1×) electrophoresis buffer and SYBR(R) green I nucleic acid gel stain (SYBR Green) were mixed to prepare a gel. NC siRNA (1.6 μg/μL) was added into the **PSP** and **PP** micelles solutions (5 μL) with different nitrogen and phosphorus mass ratios (*N*/*P*), and then incubated for 30 min. *N*/*P* of **PSP** micelle solution and NC siRNA: 0.08:1, 0.09:1, 0.1:1, 0.2:1, 0.3:1, 0.4:1, 0.5:1. *N*/*P* of **PP** micelle solution and NC siRNA: 0.08:1, 0.09:1, 0.1:1, 0.2:1, 0.3:1, 0.5:1 (electrophoresis conditions: 1×TAE electrophoresis buffer, voltage 120 mV, electrophoresis time 20 min, photographed under UV light). The most stable proportion was determined by measuring potential and particle size by DLS (*N*/*P*: 1:1, 5:1, 10:1, 15:1, 20:1, 25:1, 30:1).

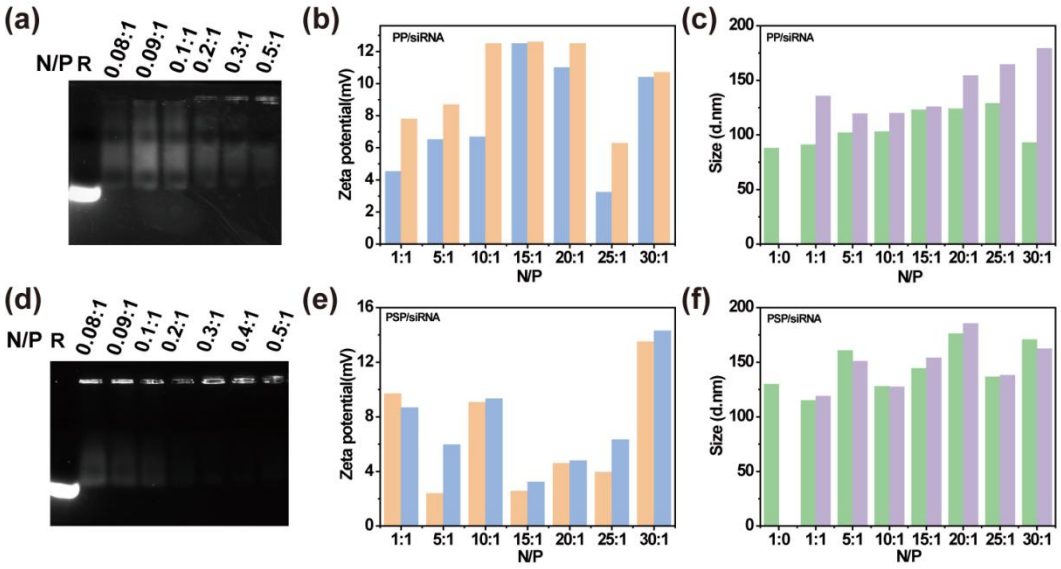



**Fig. S5** (a & d) Gel retardation assay of **PP**/siRNA and **PSP**/siRNA NPs with different *N*/*P* ratios. (b & e) Zeta potential and (c & f) Size of **PP**/siRNA and **PSP**/siRNA NPs determined by DLS measurement.

**Preparation of PSP/ICG and PP/ICG nanoparticles:** **PSP**/ICG and **PP**/ICG nanoparticles were prepared by nano-precipitation. Initially, ICG (5.60 mg) and tetrabutylammonium iodide (TBAI, 20.80 mg) were dissolved in DMSO and stirred overnight at room temperature as the organic phase. **PSP** (10.00 mg) and **PP** (10.00 mg) were dissolved in deionized water (20 mL), respectively, and the solutions were stirred overnight at room temperature. Then the above organic phase solution (3 mL) was added dropwise to the aqueous phase, protected from light and stirred overnight. Finally, the solutions were transferred into a dialysis bag ($M_W$ = 3500 Da), respectively, which was dialyzed for 24 h and freeze-dry. **PSP**/ICG and **PP**/ICG NPs were obtained for use.

**Drug-loading capacity of PSP and PP micelles:** Concentration-dependent absorption of ICG was depicted, and a standard curve of ICG was obtained by measuring the absorbance at a wavelength of 780 nm with a UV spectrophotometer. The drug loading capacity of **PSP** and **PP** micelles was determined by UV spectrophotometry. The absorption curves of ICG, **PP**/ICG and **PSP**/ICG NPs

aqueous solutions were plotted by UV spectrophotometer. The drug loading (DL) and encapsulation efficiency (EE) was calculated and determined according to the standard curve. Calculated as follows:

$$DL = \frac{mass\ of\ ICG\ in\ nanoparticles}{mass\ of\ nanoparticles} \times 100\%$$

$$EE = \frac{mass\ of\ ICG\ in\ nanoparticles}{total\ mass\ of\ ICG} \times 100\%$$

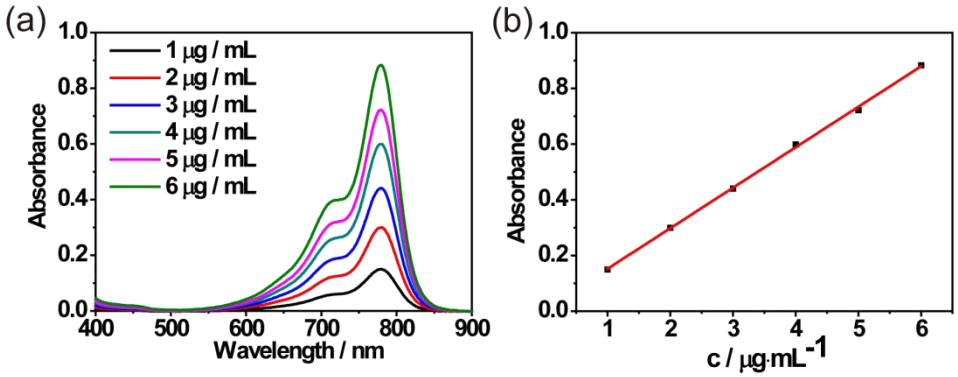



**Fig. S6** (a) Concentration-dependent absorbance of ICG in water. (b) Standard curves of ICG at various concentrations (A = 0.1454c + 0.00693, $R^2$ = 0.999).

**Preparation of PSP/ICG/siRNA and PP/ICG/siRNA NPs:** **PSP**/ICG, **PP**/ICG and siRNA were incubated for 30 min to obtain **PSP**/ICG/siRNA and **PP**/ICG/siRNA complexes through electrostatic interaction. The particle size and surface zeta potential were characterized by DLS and TEM.

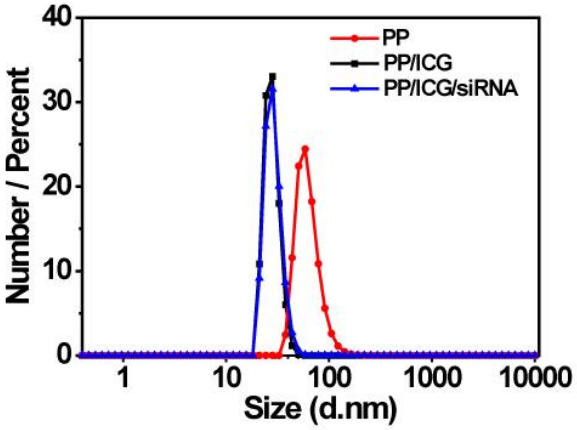



**Fig. S7** Size distribution of **PP**, **PP**/ICG, **PP**/ICG/siRNA by DLS.

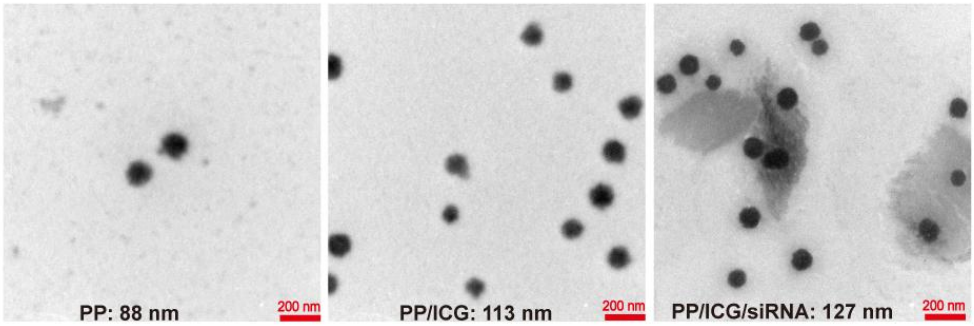



**Fig. S8** The morphology of **PP**, **PP**/ICG and **PP**/ICG/siRNA by TEM (Scale bar represents 200 nm).

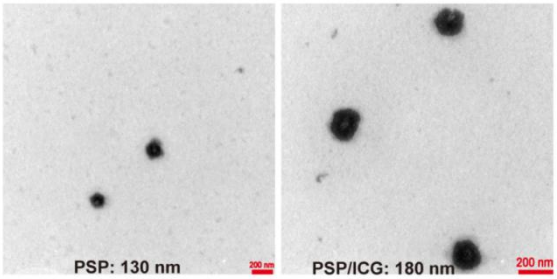



**Fig. S9** The morphology of **PSP** and **PSP**/ICG by TEM (Scale bar represents 200 nm).

**Release of siRNA and ICG:** siRNA release was determined under different acidic (pH = 5.7 and 7.4) and reducing conditions (GSH = 0 and 10 mM). **PSP**/siRNA and **PP**/siRNA solutions were placed in dialysis bags ($M_W$ = 3500 Da), respectively. Then the dialysis bag was placed in a solution containing 0 or 10 mM GSH and a solution at pH = 5.7 or 7.4, respectively, and stirred. External dialysis fluid (20 μL) was taken out at intervals, and external dialysis fluid (20 μL) was added for the next measurement within 8 hours. According to the operation of the Rediplate™ 96 RiboGreen® RNA quantification kit, the release of siRNA was measured by a fluorescence-based microplate reader (excitation: 485 nm, emission: 530 nm), and the release kinetics were plotted. The siRNA content at different time points was calculated by the standard curve of the obtained siRNA. The experimental operation of ICG release was the same as the above procedure, and the release of ICG in different periods was measured by the UV-vis spectrophotometer.

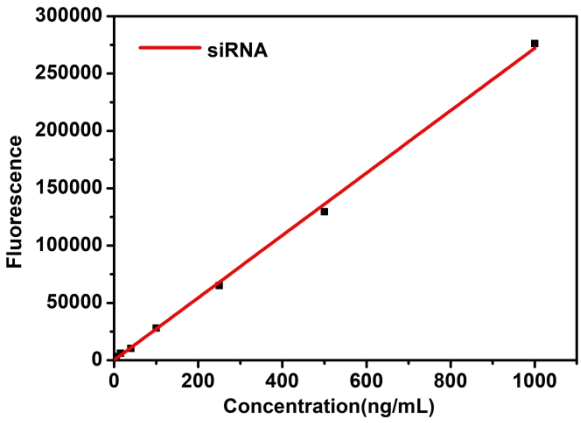



**Fig. S10** Concentration-dependent absorbance of siRNA (A = 120.519 c + 271.988, $R^2 = 0.999$).

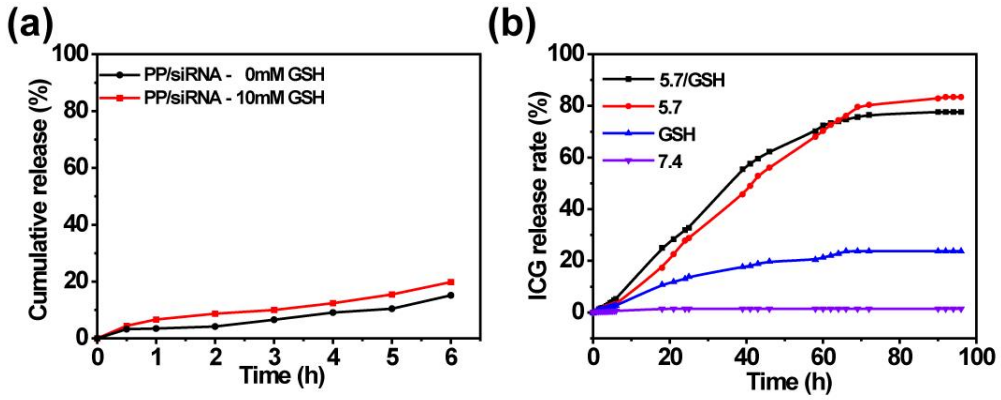



**Fig. S11** (a) *In vitro* siRNA release profiles of **PP**/siRNA in different environments (GSH: 0 or 10 mM) over time, which was determined by Microplate. (b) ICG release rate of **PP**/ICG NPs in different environments (pH: 7.4 or 5.7, GSH: 0 or 10 mM) over time, which was determined by UV-vis spectroscopy.

**Concentration-dependent photothermal property:** The aqueous solutions of free ICG, **PSP**/ICG and **PSP**/ICG NPs with different ICG concentrations (0, 10, 20, 30, 40 μg/mL) were irradiated under NIR laser (808 nm, 1.0 W/cm$^2$, 10 min). Meanwhile, the temperature changes were recorded every minute.

**Power density-dependent photothermal property:** The aqueous solutions of free ICG, **PSP**/ICG and **PSP**/ICG NPs (equivalent ICG dose: 40 μg/mL) under the 808 nm

NIR laser of different power densities (0.3, 0.7, 1.0, 1.5 W/cm$^2$ ) were irradiated for 10 min, and the temperature changes were recorded every minute.

**Photothermal stability:** ICG, **PP**/ICG and **PSP**/ICG NPs (equivalent ICG dose: 40 μg/mL) were irradiated under 808 nm laser irradiation (0.7 W/cm$^2$, within 10 min), respectivley. This process was repeated 3 cycles.

**Western blot (WB):** The expression of PD-L1 protein in 4T1 cells co-cultured with NC siRNA, siPD-L1 and **PSP**/siPD-L1 NPs was identified by WB analysis. After incubation for 24 h, the protein of 4T1 cells was extracted with RIPA buffer. Protein extracts (1 mg/mL, 10 μL) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membrane (PVDF) at 4 ℃. After 1 h, cell membranes were blocked with 5% nonfat dehydrated milk for 2 h at 37 ℃. Western blot detection was performed with anti-PD-L1 antibody, and monoclonal antibody β-Tubulin, respectively.

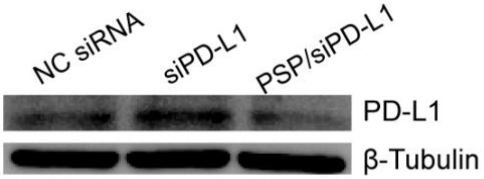



**Fig. S12** PD-L1 protein expression level.

**MTT assay:** MTT assay was used to assess the therapeutic effect of photothermal therapy. 4T1 cells and B16F10 cells were seeded into a 96-well plate (1×10$^4$ cells/well), and cultured in an environment with a carbon dioxide ($CO_2$) content of 5% at 37 °C incubation for 18 h. The medium was replaced with different concentrations of free ICG and **PSP**/ICG (equivalent ICG dose: 1, 2, 3, 4, 5, and 6 μg/mL). After 4 h, the samples were irradiated with 808 nm laser irradiation (1.0 W/cm$^2$) for 1 min, and at the same time set a dark treatment group without laser irradiation. After 24 h of incubation, serum-free DMEM medium containing 0.5 mg/mL MTT (100 μL) was

added to each well for another 4 h. Then the solution of each well was replaced with DMSO (100 μL). The absorbance of each well was measured with a microplate reader at a wavelength of 570 nm. Cell viability values were calculated by the following formula:

$$V\% = \frac{[A]sample - [A]blank}{[A]positive - [A]blank} \times 100\%$$

$V$% is the cell viability, [$A$]sample is the absorbance *per* well treated with the sample, [$A$]positive is the absorbance *per* well without sample treatment, [$A$]blank is the absorbance *per* well of the blank control value.

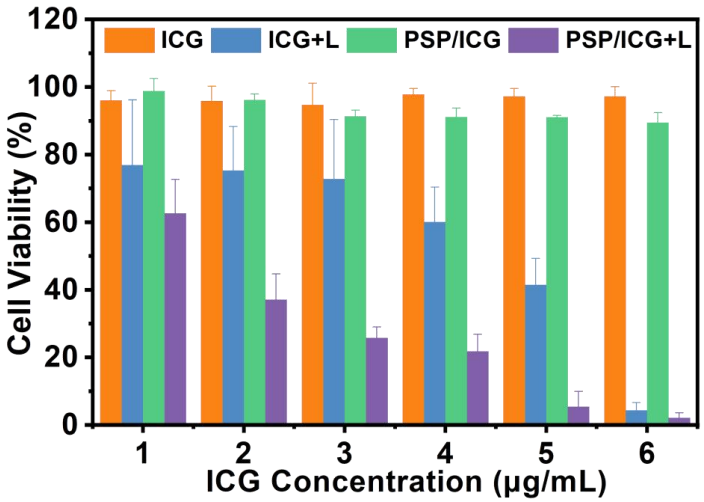



**Fig. S13** B16F10 cells viability was evaluated by MTT assay after co-incubation with free ICG or **PSP**/ICG NPs at different ICG concentrations in the absence or presence of 808 nm laser irradiation (1.0 W/cm$^2$, 1 min).

**Cell dead/alive experiment:** The cell dead/alive experiment was detected by calcein acetoxymethyl ester (Calcein-AM) and propidium iodide (PI) through an inverted fluorescence microscope. 4T1 cells and B16F10 cells were seeded into 24-well plate ($5\times10^4$ cells/well). After overnight incubation for 18 h, the medium was replaced with 4 μg/mL ICG content of free ICG, **PSP**/ICG NPs serum-free medium for 4 h. Then the cells were irradiated with 808 nm near-infrared light at a power of 1.0 W/cm$^2$ for 5 min. After overnight incubation for 24 h, the medium was removed and washed with PBS. Next, cells were stained with Calcein-AM and PI for 30 min. The green

fluorescence of Calcein-AM and the red fluorescence of PI could be observed by fluorescence microscope, which indicated live and dead cells, respectively.

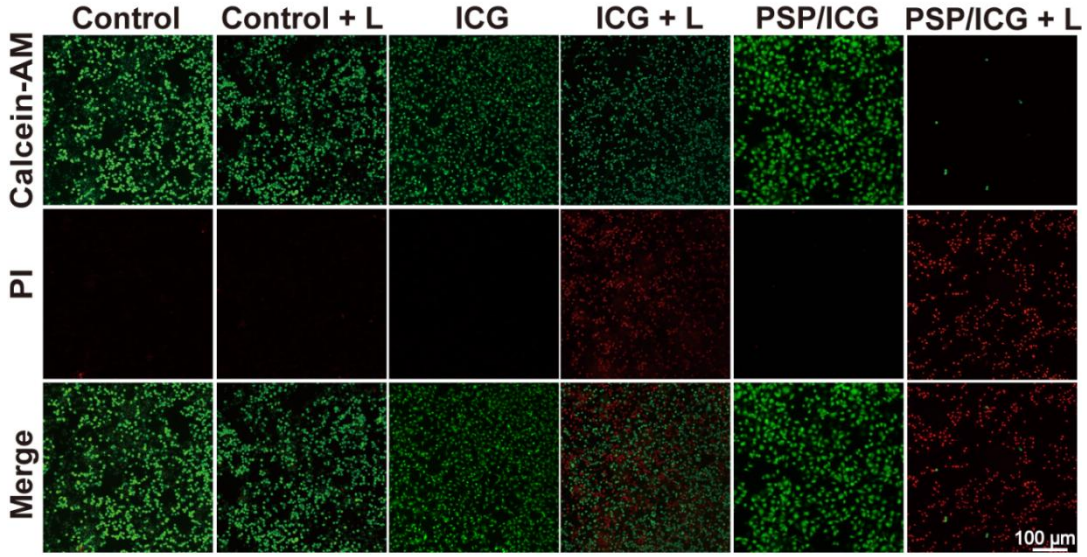



**Fig. S14** Fluorescence images of Calcein-AM/PI stained B16F10 cells after different treatments. The scale bar was 100 μm.

**Cell uptake experiment:** Cell uptake experiment was assessed by confocal laser scanning microscopy (CLSM). 4T1 and B16F10 cells were seeded in 24-well plate ($5\times10^4$ cells/well) and incubated overnight. **PSP**/ICG/FAM-siRNA was then added to the well (FAM-siRNA was green fluorophore, excitation: 480 nm, emission: 520 nm). After fixation with 4% paraformaldehyde, DAPI (excitation: 405 nm, emission: 440-480 nm) stained nucleus was blue fluorophore. ICG was red fluorophore (excitation: 633 nm, emission: 650-700 nm). Cell uptake capacity was detected by flow cytometry. 4T1 and B16F10 cells were seeded on a 12-well plate ($1\times10^5$ cells/well) and cultured for 18 h. Subsequently, the medium was replaced with free ICG and **PSP**/ICG (equivalent ICG dose: 1 μg/mL), free FAM-siRNA and **PSP**/FAM-siRNA (equivalent FAM-siRNA dose: 2 μg/mL) were added to each well, respectively. After incubation of 1, 2, 4, 6 and 8 h, the uptake efficiency of cells was quantitatively evaluated by collecting the fluorescence signal of flow cytometry.

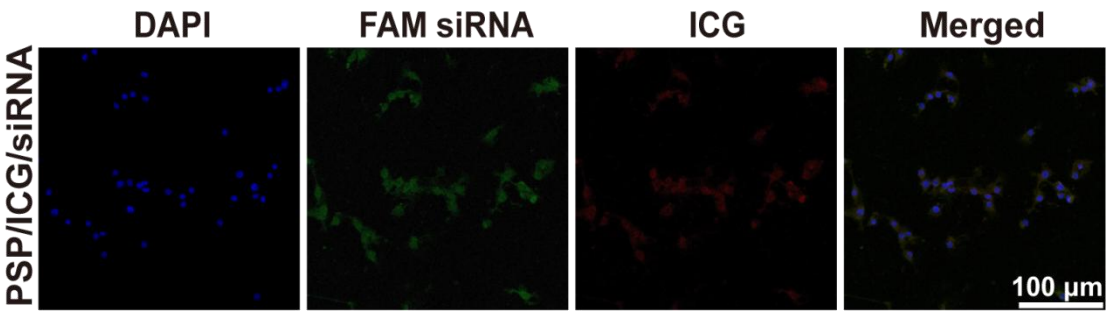



**Fig. S15** CLSM images of B16F10 cells co-culture with **PSP**/ICG/siRNA NPs (equivalent ICG dose: 1 μg/mL) for 4 h. The cell nucleus was stained as blue channel by DAPI (405/440-480 nm), ICG was recorded as red channel (633/650-700 nm), and FAM-siRNA was recorded as green channel (480-520 nm). The scale bar was 100 μm.

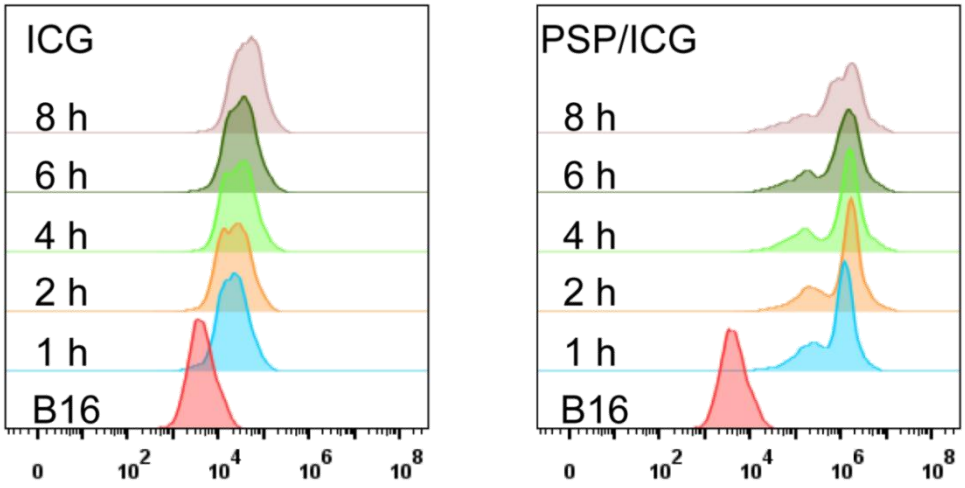



**Fig. S16** The flow cytometry of ICG and **PSP**/ICG at 1, 2, 4, 6, and 8 h, respectively.

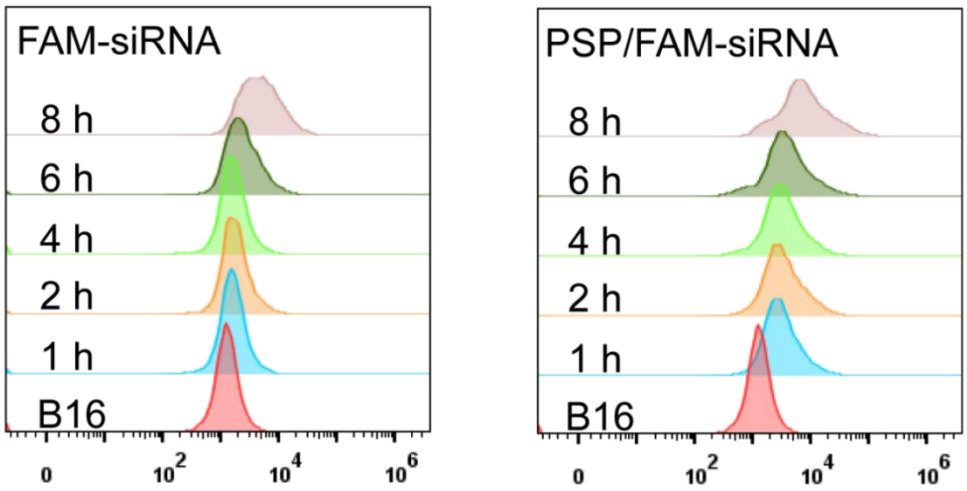



**Fig. S17** The flow cytometry of FAM-siRNA and **PSP**/FAM-siRNA at 1, 2, 4, 6, and

8 h, respectively.

**Cell apoptosis:** 4T1 cells were seeded in 12-well plate ($1\times10^5$ cells/well) and cultured for 18 h. Free ICG and **PSP/**ICG (equivalent ICG dose: 4 μg/mL) were added to each well, respectively. After 4 h, light groups were irradiation under 808 nm laser (1.0 W/cm$^2$, 5 min), and then the cells were incubated for 24 h. Next, 4T1 cells were collected and washed with PBS. 4T1 cells were stained with AnnexinV-FITC/PI and analyzed by flow cytometry.

**Determination of ICD:** 4T1 cells were seeded in 12-well plate ($1\times10^5$ cells/well) and incubated for 18 hours. Free ICG, siPD-L1, **PSP/**ICG, **PSP/**ICG/siPD-L1 (siPD-L1: 2 μg/mL, ICG: 4 μg/mL) were added to 4T1 cells and incubated for 4 h. The cells incubated with different materials were irradiated under 808 nm laser at different laser intensity (0, 1.4 W/cm$^2$) for 5 min and cultured for 24 h. Next, the cells were incubated with the primary antibody anti-CRT for 30 min and washed with PBS. Then the cells were incubated with the secondary antibody FITC-IgG for 30 min and washed with PBS. Finally, intracellular calreticulin (CRT) was detected by flow cytometry and CLSM.

The high mobility group protein B1 (HMGB1) release was the same as the CRT expression experiment. After incubation for 24 h, 4T1 cells were stained by PE-anti-HMGB1 antibody for 1 h. Finally, the release of HMGB1 was detected by CLSM. The content of HMGB1 in the supernatant of the cells was measured via ELISA kit.

Besides, ATP levels in 4T1 cells were measured by ATP determination kit.

**Maturation of DCs:** 4T1 cells were seeded in 12-well plates ($1\times10^5$ cells/well) and incubated for 18 hours. Free ICG, siPD-L1, **PSP/**ICG, **PSP/**ICG/siPD-L1 (siPD-L1: 2 μg/mL, ICG: 4 μg/mL) were added to 4T1 cells and incubated for 4 h. The cells incubated with different materials were irradiated under 808 nm laser at different laser intensity (0, 1.4 W/cm$^2$) for 5 min and cultured for 24 h. The supernatant was

discarded and washed three times with PBS, and the cells were gently scraped and transferred to a 1.5 mL centrifuge tube. Then 4T1 cells were stained with FITC-CD11c, APC-CD80 and PE-CD86 antibodies. The maturation of dendritic cells was detected by flow cytometry.

**Construction of animal tumor models:** Female BALB/c nude mice (6 weeks old, weight about 16-18 g) were purchased from Charles River Laboratory China Branch (Zhejiang, China) with production license number SCXK (Zhejiang) 2019-0001 and certificate number 20211228Abzz0619072327. The mice were resided at the Laboratory Animal Center of Hangzhou Normal University with use license number SYXK (Zhejiang) 2020-0026, and cultivated in a pathogen-free environment with appropriate humidity and temperature. All animal procedures were performed by the animal care and use guidelines of the Organizational Animal Care and Use Committee. 4T1 cells ($6\times10^5$) were dispersed in PBS (100 μL) and then injected subcutaneously into the right back dorsum of the mice. About a week, until the tumor volume grows to about 100 $mm^3$, the mouse tumor model was established and 4T1 tumor-bearing mice were treated by different treatments (PBS, **PSP**/ICG, **PSP**/siPD-L1, **PSP**/ICG/siPD-L1 NPs with or without irradiation) intratumor injection to investigate the therapeutic effect.

***In vivo* distribution studies:** 4T1 tumor-bearing mice were injected with 100 μL of the dispersion of **PSP**/ICG, **PSP**/ICG/siPD-L1 (ICG: 3 mg/kg, siPD-L1: 0.5 mg/kg), respectively. Fluorescence images of the mice were taken by fluorescence image (excitation: 787 nm, emission: 902 nm) within a predetermined period. Mice were sacrificed at 48 h from each group and major livers including heart, liver, spleen, lung, kidney and tumor were removed for further analysis *in vivo*.

***In vivo* thermal imaging:** 4T1 tumor-bearing mice were injected with PBS, **PSP**/ICG, **PSP**/ICG/siPD-L1 (ICG: 3 mg/kg, siPD-L1: 0.5 mg/kg), respectively. Then the mice with **PSP**/ICG, **PSP**/ICG/siPD-L1 were irradiated (808 nm, 1.0 $W/cm^2$). Tumor

temperature and photothermal image were recorded every minute for 10 minutes with a FLIR infrared thermal imager.

**Therapy effect in mice:** 4T1 tumor-bearing mice were randomly divided into eight groups (n = 6) with different treatments: 1. PBS, 2. PBS + Laser, 3. **PSP**/ICG, 4. **PSP**/ICG + Laser, 5. **PSP**/siPD-L1, 6. **PSP**/siPD-L1 + Laser, 7. **PSP**/ICG/siPD-L1, 8. **PSP**/ICG/siPD-L1 + Laser (ICG: 3 mg/kg, siPD-L1: 0.5 mg/kg). The mice were injected every two days for a total of 6 times. After 4 h of injection, the mice in the laser group were irradiated with 808 nm near-infrared light at a power of 1.0 W/cm$^2$ for 5 min. During irradiation, the spot of near-infrared light should be sufficient to cover the entire tumor site. During treatments, the body weight and tumor volume of the mice were recorded every two days, and the maximum and minimum diameters of the tumors were indirectly measured with a vernier caliper. Tumor volume in mice was calculated as $V = 1/2\ ab^2$ ($a$ represents the largest diameter, $b$ represents the smallest diameter).

**Detection of immune indicators *in vivo*:** To evaluate the immune response *in vivo*, mice were sacrificed on day 16 after PTT. The expression of CRT, HMGB1 and PD-L1 on the tumor slices derived from the 4T1 tumor-bearing mice with different treatments via the immunofluorescence staining. Tumors were collected for T cell proliferation, Treg cell inhibition, DCs maturation, and PD-L1 expression by flow cytometry.

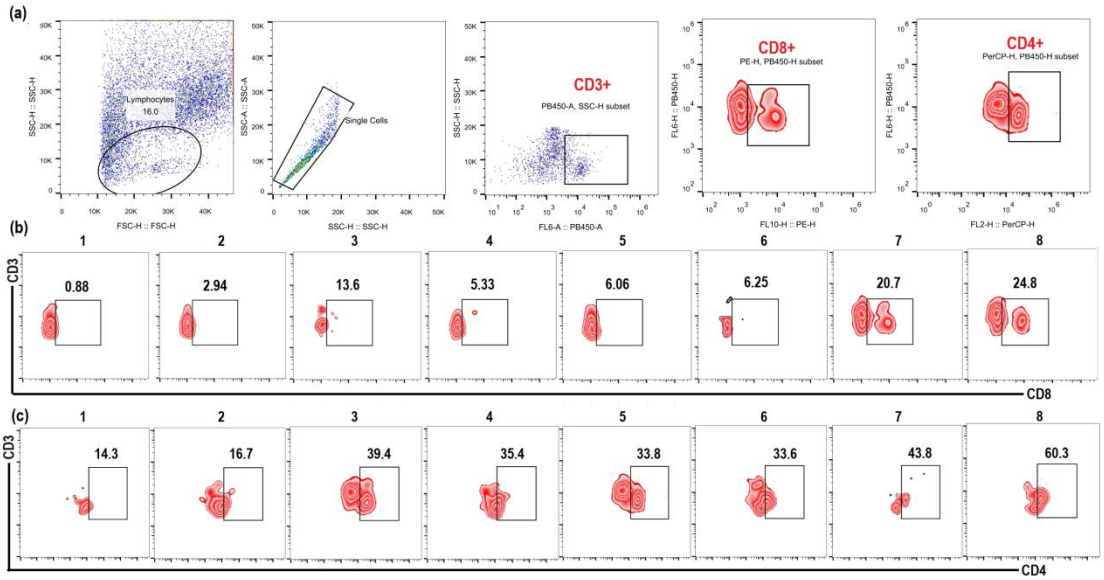



**Fig. S18** Gating strategies for flow cytometry analysis of (a) $CD3^+CD8^+$ T cells and $CD3^+CD4^+$ T cells. (b) Flow cytometric analysis of $CD8^+$ T cells in $CD3^+$ T cells inside the tumors of the mice with different treatments. (c) Flow cytometric analysis of $CD4^+$ T cells in $CD3^+$ T cells inside the tumors of the mice with different treatments. (1: PBS, 2: PBS + Laser, 3: **PSP**/ICG, 4: **PSP**/ICG + Laser, 5: **PSP**/siPD-L1, 6: **PSP**/siPD-L1 + Laser, 7: **PSP**/ICG/siPD-L1, 8: **PSP**/ICG/siPD-L1 + Laser).

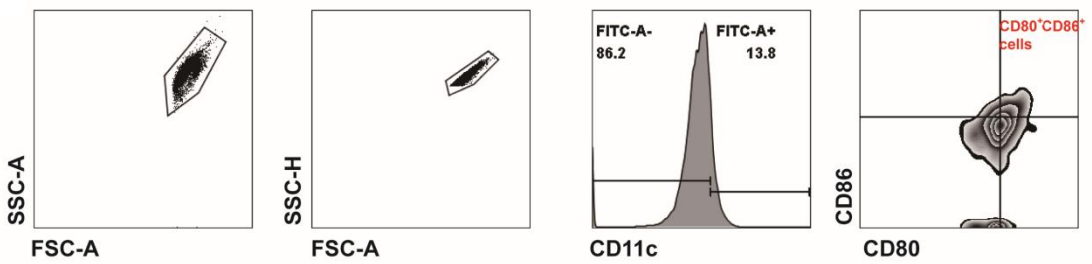



**Fig. S19** Gating strategy to analyze matured DCs ($CD80^+CD86^+$) in tumors.

***In vivo*** **biological safe experiment:** After 15 days of treatments, the 4T1 tumor-bearing mice were sacrificed. The main organs (heart, lung, liver, spleen, and kidney) and tumors were removed and stained with H&E and Ki67 for

histopathological analysis. At the same time, blood was collected for further blood biochemistry and haematology analysis. The observed indexes were haemoglobin (HGB), mean corpuscular haemoglobin (MCH), platelet distributionwidth (PDW), total protein (TP), mean corpuscular haemoglobin concentration (MCHC), alkaline phosphatase (ALP), mean platelet volume (MPV), UREA, red blood cells (RBC), blood routine levers of haematocrit (HCT), mean corpuscular volume (MCV), red blood cell distribution width-standard deviation (RDW-SD).

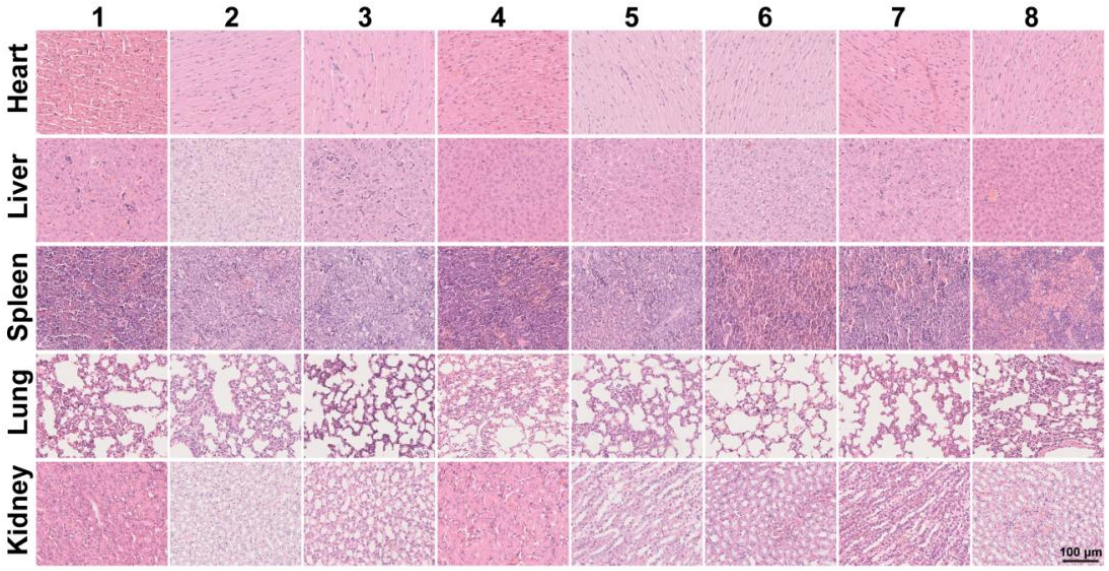



**Fig. S20** H&E-stained tissue sections of major organs (heart, liver, spleen, lung, and kidney) with different treatments (1: PBS, 2: PBS + Laser, 3: **PSP**/ICG, 4: **PSP**/ICG + Laser, 5: **PSP**/siPD-L1, 6: **PSP**/siPD-L1 + Laser, 7: **PSP**/ICG/siPD-L1, 8: **PSP**/ICG/siPD-L1 + Laser). The scale bar was 100 μm.

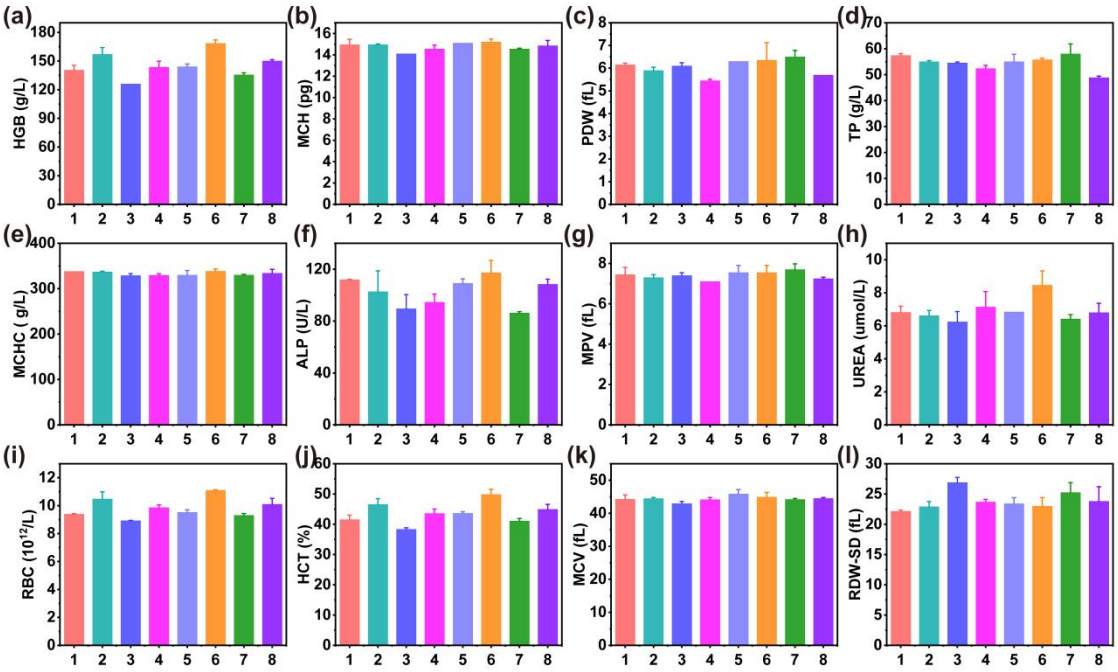



**Fig. S21** Blood biochemical and blood routine indexes in mice with different treatments (1: PBS, 2: PBS + Laser, 3: **PSP**/ICG, 4: **PSP**/ICG + Laser, 5: **PSP**/siPD-L1, 6: **PSP**/siPD-L1 + Laser, 7: **PSP**/ICG/siPD-L1, 8: **PSP**/ICG/siPD-L1 + Laser).

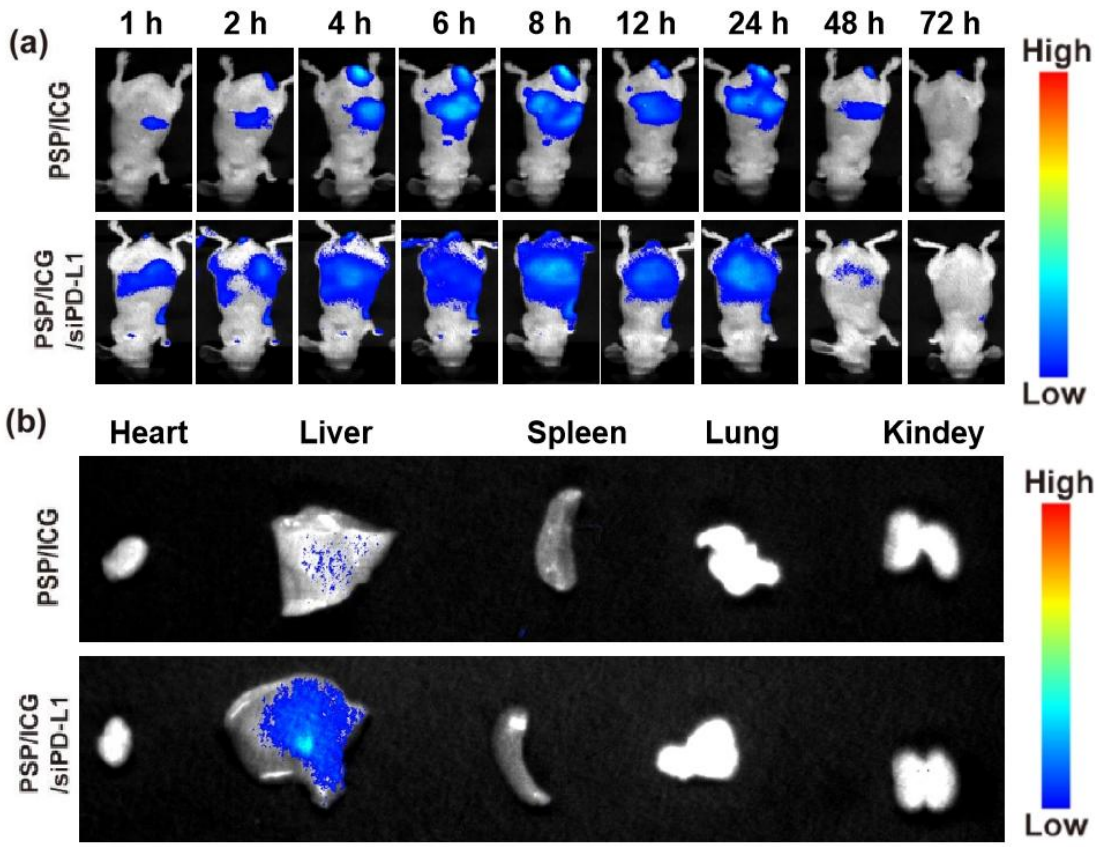



**Fig. S22** *In vivo* biodistribution on the nontumor model. (a) *In vivo* fluorescence images of mice at different time points after injection of **PSP**/ICG and **PSP**/ICG/siPD-L1, (b) *Ex vitro* fluorescence imaging of major organs (heart, liver, spleen, lung, kidney) of mice at 72 h after injection.

**Reference**

[1] H. B.Wang, Y. Li, M. Z. Zhang, D. Wu, Y. Q. Shen, G. P. Tang and Y. Ping, *Adv. Healthc. Mater.*, **2017**, *6*, 1601293.