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Supporting information for

Supercharged fluorescent protein functionalized water-soluble poly (*N*-phenylglycine) nanoparticles for highly effective imaging-guided photothermal therapy

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Materials and instruments. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. NPG were purchased from Aladdin Reagent Company (Shanghai, China). APS, H_2SO_4, were obtained from Xilong Chemical Corporation (Guangdong, China). DMEM medium (high glucose) was obtained from Gibco Company (USA). PBS, propidium iodide (PI) and MTT were purchased from Sigma Company (USA). Calcein-AM was obtained from Invitrogen (Shanghai, China). 4T1, HeLa, and B16 cells were supplied from Immunetech International (Guilin, China). The dialysis bags with 1000 and 8000-14000 Da molecular weight cutoff (MWCO) were obtained from Sinopharm Chemical Reagent Co. Ltd. Water was purified with a Milli-Q apparatus (Millipore, Bedford, MA). UV-vis-NIR spectra were collected from 250-900 nm by Lambda 45 UV-vis-NIR spectrophotometer (Perkin Elmer, USA). The FT-IR spectra were obtained on a Perkin Elmer FT-IR spectrometer. The AFM experiment was performed on a di NanoScope IVa Controller, Vecco, USA. The TEM image was carried out on JEM100C×II (JEOL, Japan). Zeta potential measurement was carried out on Malvern Zetasizer Nano ZS-90. Confocal microscopy (Carl Zeiss LSM 710, Jena, Germany) was used to observe the photothermal therapy effects of SPNPG/ ScGFP. The *in vivo* (living mice) imaging was carried out using an IVIS Lumina XR (IS1241N6071) *in vivo* imaging system.

Synthesis of PNPG

PNPG was synthesized referring to the synthetic method reported by Nabid *et al*,[1] as follows: NPG (1.06 g) was dissolved in 50 mL of 0.1 M H_2SO_4, and the mixture was well stirred for 10 min at room temperature. Subsequently, 1.60 g APS in 50 mL of 0.1 M H_2SO_4 was slowly added in 1 h under the ice-water bath. The reaction mixture was stirred for another 24 h under ice water bath, and the dark-green precipitate was formed. After warming up to room temperature, the resulting precipitate was filtered, and washed with the amount of water to remove inorganic salts and reactants. Finally, after dried in a vacuum, dark-green power was obtained.

Synthesis of SPNPG

PNPG (1.0 g) was dispersed in 30 mL of 1,2-dichloroethane (DCE) being heated at 80°C. The chlorosulfonic acid (0.3 g) was added dropwise during 30 min into the dispersion liquid, and then the reaction mixture was held for 5 h. The produced sulfonated poly (*N*-phenylglycine) was separated by filtration, immersed in 400 mL of

water, and heated for 4 h at 100°C to promote its hydrolysis. After concentrating the resulting greenish solution until almost dried by evaporation, the SPNPG was precipitated and washed by acetone, then collected by filtration and dried at 60°C under vacuum.

Synthesis of SPNPG/ScGFP

ScGFP was a gift from professor Yangzhong Liu, which was prepared referring to the reported method.[2] SPNPG was suspended in the buffer solution at the concentration of 1 mg/mL. Then, the suspension of ScGFP was gradually dripped into the suspension of SPNPG and then ultrasonicated for 30 min. The SPNPG/ScGFP was formed by electrostatic self-assembly between negatively charged SPNPG and positively charged ScGFP. Following centrifugation at 1000 rpm for 10 min until the supernatant was colorless, and unbinding proteins were removed. The resulting precipitate was dissolved in a buffer solution.

Photothermal imaging of SPNPG and SPNPG/ScGFP

The photothermal images of pure water, SPNPG and SPNPG/ScGFP were taken by the infrared camera (MAG30, Magnity Electronics, China) in conjunction with the 808 nm laser. The aqueous solution of SPNPG and SPNPG/ScGFP was placed in a specimen bottle and irradiated by 808 nm laser (1 W/cm^2). The temperature signals recorded at different time intervals (0-10 min) were analyzed with Magnity Electronics tools systems. Under the same condition, as the control, the photothermal efficacy of pure water was also tested.

The photothermal conversion efficiency of SPNPG/ScGFP

The photothermal conversion efficiency of the SPNPG/ScGFP was determined according to the reported method. To measure the photothermal conversion efficiency (η), the SPNPG/ScGFP aqueous dispersion samples were exposed to 808 nm NIR laser (1 W/cm^2) for 15 min, and then the laser was shut off. The heating and cooling temperature trends of the samples were recorded by a temperature gauge. The photothermal conversion efficiency was calculated according to the equation:

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_0}{I(1 - 10^{-A808})}$$

Where h is the heat transfer coefficient, S is the sample container surface area, T_{max} is the steady-state maximum temperature, T_{surr} is the ambient room temperature. Q_0 is

the baseline energy input by the solvent and the sample container without materials, I is the laser power, and A_{808} is the absorbance of the materials at 808 nm. In order to get hS, θ is herein introduced, which is defined as the ratio of ($T–T_{surr}$) to ($T_{max}–T_{surr}$):

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}}$$

Thus, the value of hS is calculated by the equation:

$$\tau_s = \frac{C_d m_d}{hS}$$

Where τs is the characteristic thermal time constant, the mass of the SPNPG/ScGFP solution (m_d) is g, and its heat capacity (C_d) is approximately 4.2 J g^{-1} k^{-1} (the heat capacity of water). The heat energy (Q_0) of the sample container and solvent without SPNPG/ScGFP is measured independently, using the following equation:

$$Q_0 = hS(T_{max} - T_{surr})$$

Cellular experiments

Cell culture

The 4T1, HeLa, and B16 cells were cultured in the RPMIDMEM medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, incubated at 37 ℃ under a 5% CO_2 atmosphere, respectively.

The cytotoxicity of SPNPG/ScGFP

The cytotoxicity of SPNPG/ScGFP was measured by MTT assay on 4T1, HeLa, and B16 cell lines. The cells were seeded in a 96-well cell culture plate at a density of 1104 cells per 100 mL per well at 37 ℃ under a 5% CO_2 for 24 h. 100 mL SPNPG/ScGFP (0, 25, 50, 75, 100 mg/mL) was added per well for 24 h. Thereafter, the MTT reagent (10 mL in PBS, 5 mg/mL) was added to each well for another 4 h to test the cytotoxicity through Infinite M1000. The cytotoxicity was expressed as the percentage of cell viability compared to that of untreated control cells.

The photothermal therapy effect of SPNPG/ScGFP

The photothermal therapy effects of SPNPG/ScGFP were also measured by MTT assay on B16 cell lines. The cells were also incubated with SPNPG/ScGFP (0, 25, 50, 75, 100 mg/mL) on 96-well plates for 24 h, containing 1104 cells per 200 mL in each

well. After that, an 808 nm laser (1 W/cm^2) was used to irradiate cells for 10 min. The resulting cells were incubated for additional 24 h. Then, the MTT reagent (10 mL in PBS, 5 mg/mL) was added into the cells for another 4 h to test the photothermal therapy effect by Infinite M1000.

Confocal microscopy measurement

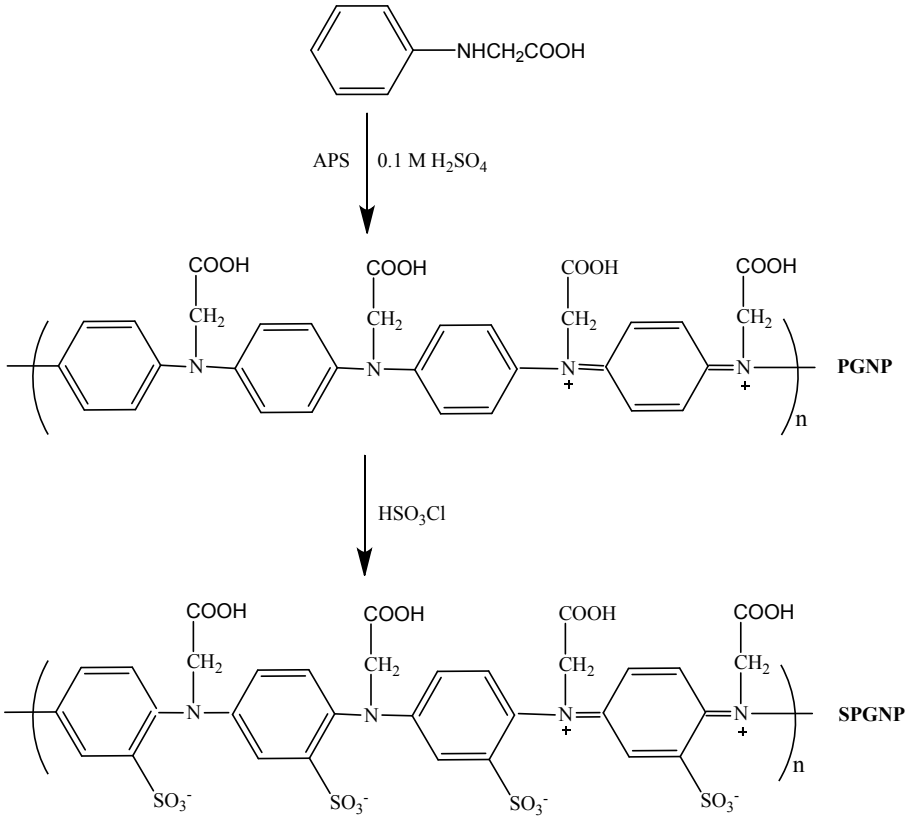
Confocal microscopy (Carl Zeiss LSM 710, Jena, Germany) was used to observe the photothermal therapy effects of SPNPG/ScGFP for B16 cells. Briefly, B16 cells were seeded into 24-well microplates at a density of 2104 cells per well and cultured at 37 ℃ under a 5% CO_2 atmosphere for 24 h, respectively. 1 mL fresh medium containing 100 mg/mL SPNPG/ScGFP was added into each well for another 24 h. The medium was discarded, and the cells were washed with PBS 3 times to remove the unbound SPNPG/ScGFP. 1 mL fresh medium was added again. An 808 nm laser (1 W/cm^2) was used to irradiate cells for 10 min, and the cells were stained with calcein-AM (1.6%) and PI (2%) for 10 minutes before and after irradiation. Then, confocal images were taken before and after illumination using a 20 objective lens.

Flow Cytometry

B16 cell suspensions incubate with SPNPG/ScGFP, and the rhodamine-modified SPNPG, were analyzed in a BD FACSVersTM flow cytometer equipped with a 488 nm laser. For each sample, 10000 events were collected. The data were processed with FlowJo software.

References

1. M. R. Nabid, S. S. Taheri, R. Sedghi, A. A. Entezami, *Iran. Polym. J.* 2008, **17**, 1.
2. Q. Wu, Q. Cheng, S. Yuan, J. Qian, K. Zhong, Y. Qian and Y. Liu, *Chem. Sci.*, 2015, **6**, 6607.



Scheme S1. Schematic illustration of the preparation of sulfonated poly(*N*-phenylglycine) (SPNPG).

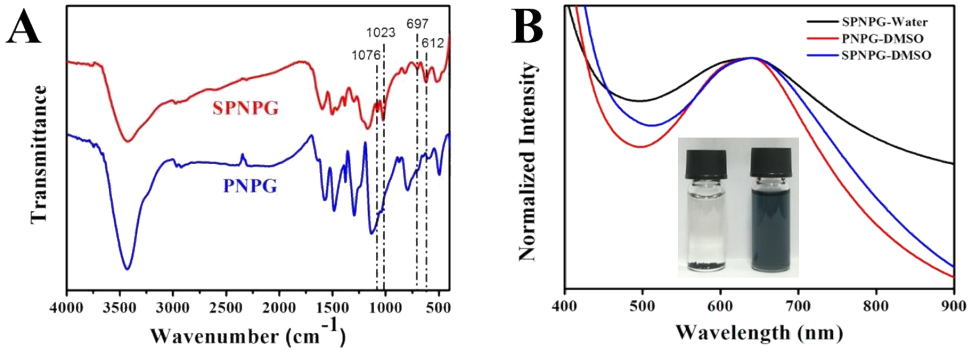


Figure S1. A) FT-IR spectra of PNPG and SPNPG; B) UV-vis-NIR spectra of PNPG in DMSO，SPNPG in DMSO and SPNPG in H_2O at 25℃, inset, water solubility test of PNPG and SPNPG, left: PNPG, right: SPNPG.

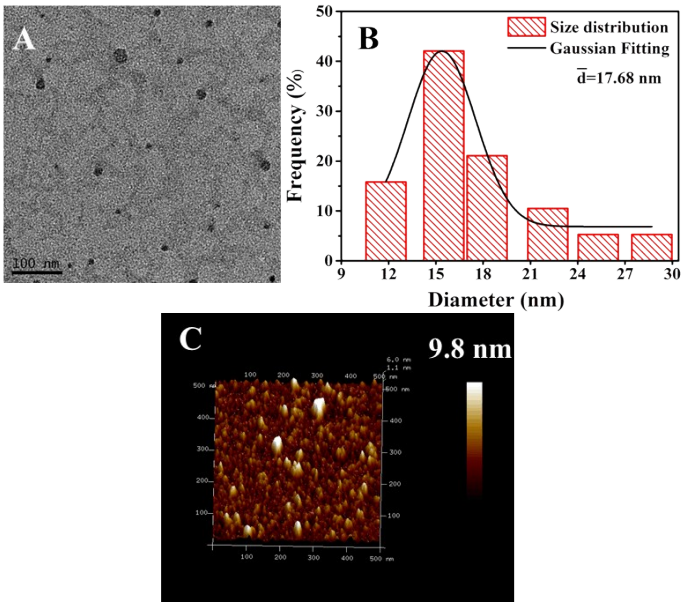


Figure S2. Characterization of the SPNPG. A) TEM image of SPNPG; B) Size distribution; (C) AFM image of SPNPG.

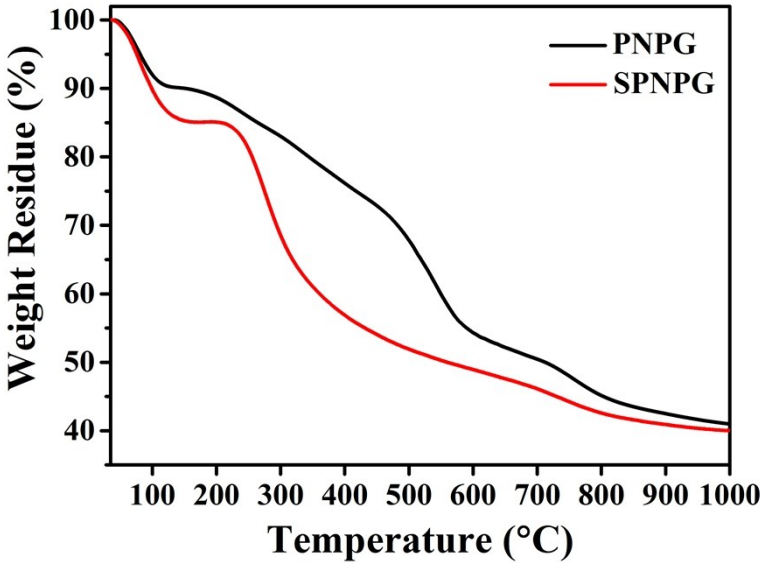


Figure S3. TGA curves of PNPG and SPNPG recorded at a rate of 10 ℃/min under an N_2 atmosphere.

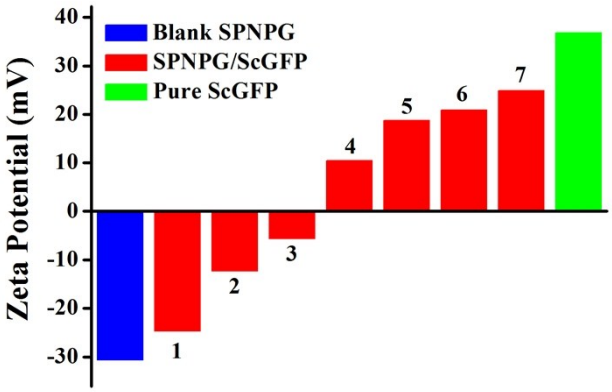


Figure S4. Zeta potential value of SPNPG, pure ScGFP and SPNPG/ScGFP when adding different concentrations of ScGFP.

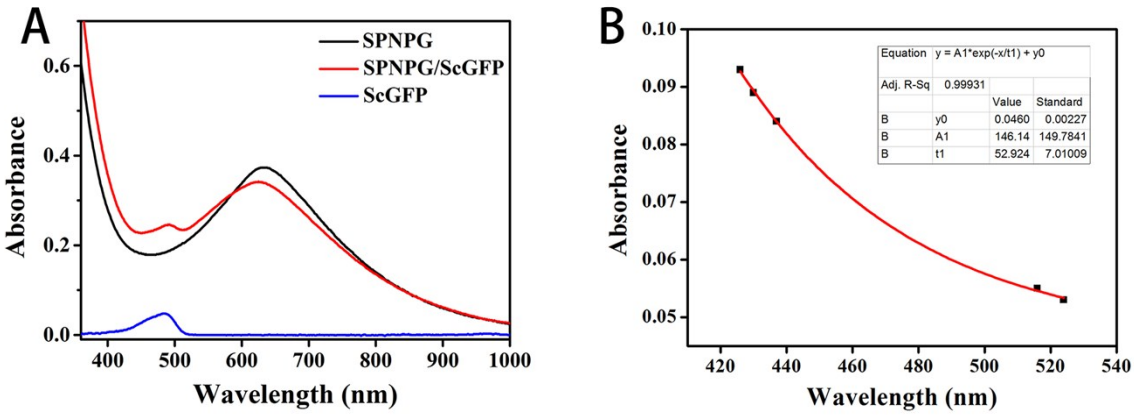


Figure S5. A) UV-Vis spectra of ScGFP (1000 nM), SPNPG (0.1 mg/mL) and SPNPG/ScGFP (0.1 mg/mL); B) measuring the ScGFP binding capacity in SPNPG by fitting method.

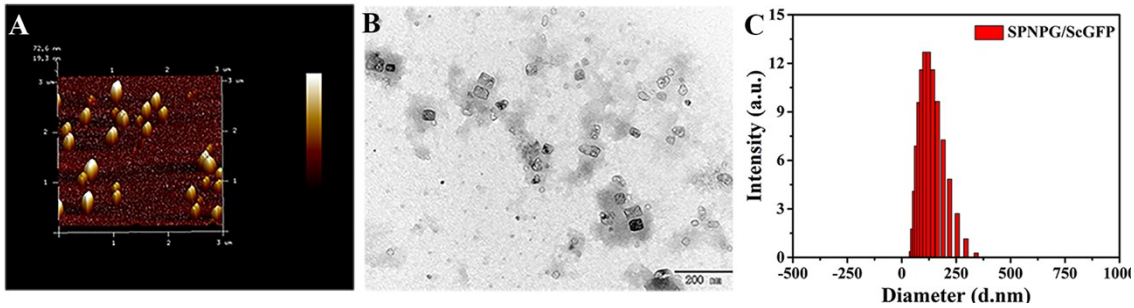


Figure S6. A) AFM image of SPNPG/ScGFP; B) TEM image of SPNPG/ScGFP; C) hydrodynamic diameter distribution of SPNPG/ScGFP.

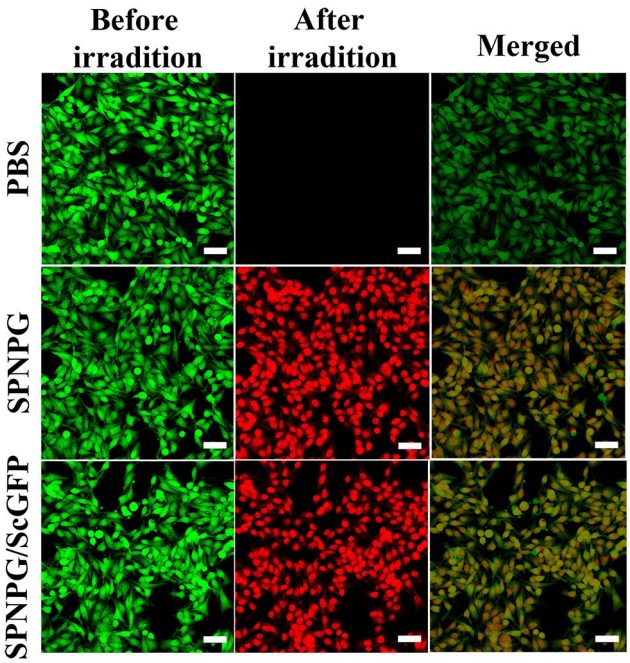


Figure S7. Fluorescence images of B16 cells stained with calcein-AM (green) and propidium iodide (red) after treatment with PBS, SPNPG, and SPNPG/ScGFP for 6 h, respectively. These images were taken before and after irradiation with the NIR laser (808 nm, 1 W /cm^2). Scale bar: 50 μm.

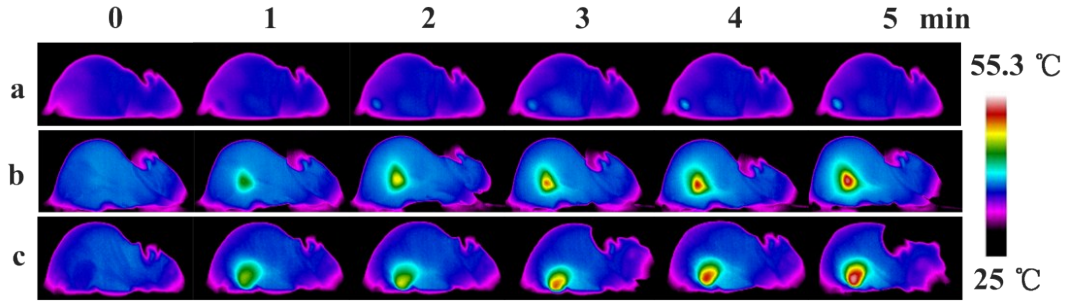


Figure S8. Infrared thermal phase diagram of mice injected with 0.9% NaCl, SPNPG and SPNPG/ScGFP solution upon exposure to 808 nm (1 W/cm^2) NIR laser for different time periods.

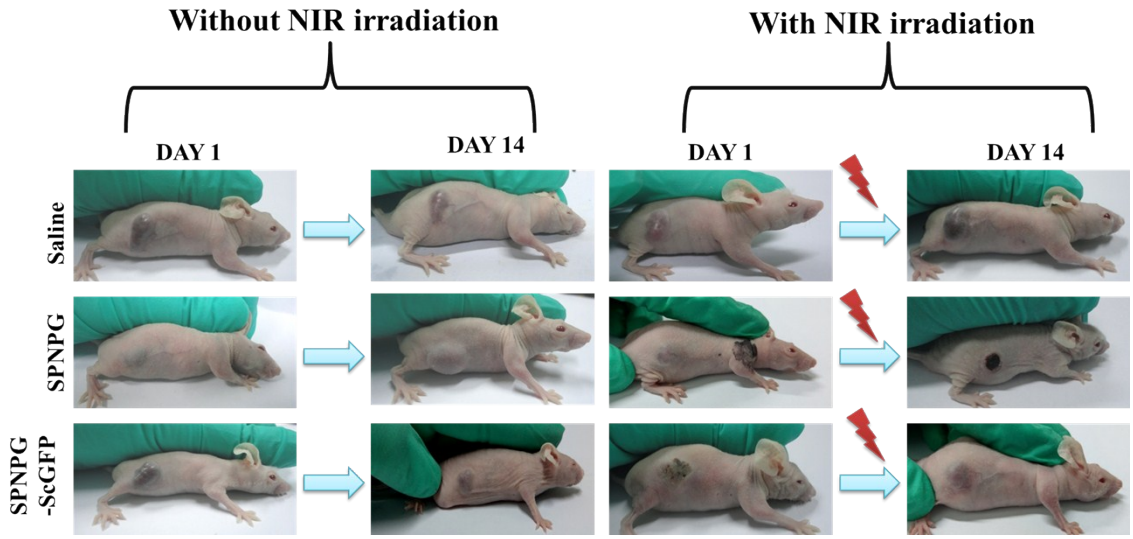


Figure S9. Representative photographs of B16 tumor-bearing mice after different treatments.

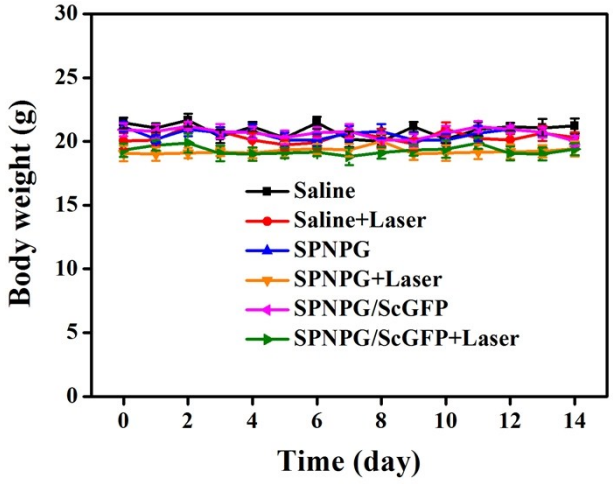


Figure S10. Body weight change curves of mice after various treatments (saline, SPNPG, and SPNPG/ScGFP).

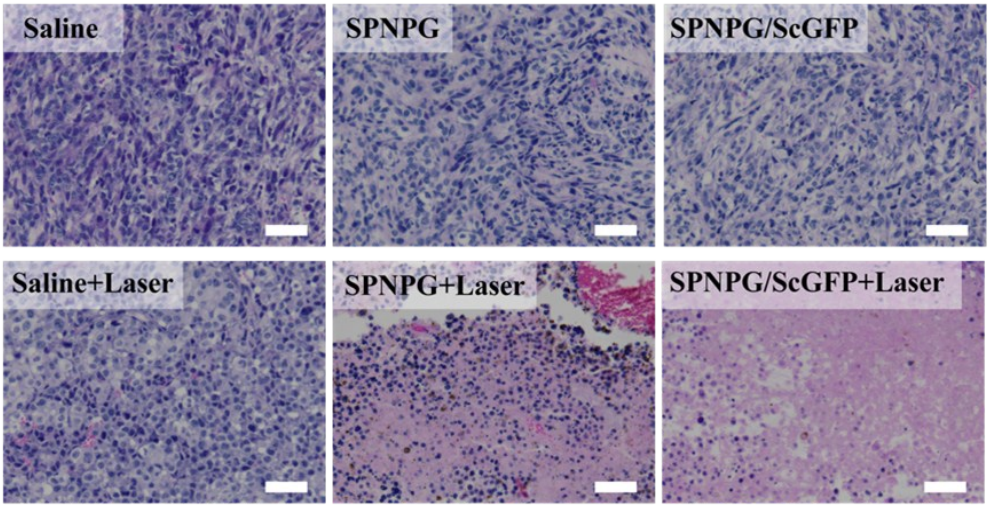


Figure S11. H&E stained different groups of B16 tumor-bearing mice. Scale bar: 50 μm.