Supplementary Information for

Polypyrrole nanoparticles for high-performance *in vivo* near-infrared photothermal cancer therapy

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Experimental Details

Reagents: Pyrrole (98+%) were purchased form Alfa Aesar. Polyvinylpyrrolidone (PVP, MW=30, 000, AR) and Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, ⩾99%) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 4T1 murine breast cancer cells were purchased from cell storeroom of Chinese Academy of Science. RPMI-1640 cell culture medium, fetal calf serum and Penicillin-Streptomycin compound were purchased from Hyclone Laboratories Inc. The water used in all experiments was ultrapure. All the reagents were used as received without further purification except for pyrrole. Pyrrole was first vacuum reruned before the experiments and stored in the dark at -20 ^{o}C for use.

Synthesis of polypyrrole nanoparticles: Polypyrrole nanoparticles with narrow size distribution were readily achieved via chemical oxidation polymerization of the corresponding monomer in aqueous solution. Briefly, PVP (1 g) was dissolved in ultrapure water (25 mL) in a 50 mL sealed container by stirring at room temperature for 0.5 h. 130 μL pyrrole was added to the solution. After 10 min, 1 mL iron (III) chloride hexahydrate (0.75 g mL^{-1}) was quickly added to the reaction mixture. The polymerization was carried out for another 3 hours. Then, the products were purified by washing with copious amounts of ethanol-acetone mixture for several times, and finally redispersed in water.

Synthesis of SiO_2@PPy: SiO_2@PPy nanoparticles were fabricated by seeded polymerization. Firstly, ~50 nm silica nanoparticles were synthesized following a slightly modified stöber process. After washing for several times using water and ethanol, the products were then redispersed in water with a concentration of 27 mg mL^{-1}. 5 mL of SiO_2 solution and 100 mg PVP were mixed in 50 mL water and stirred for 3 h at room temperature. After that, 8 μL pyrrole was added to the solution and stirred overnight. Then 50 mg $FeCl_3 \cdot 6H_2O$ was added to the solution and the polymerization was carried out for 24 h.

Characterization: Transmission electron microscopy (TEM) studies were performed on a TECNAI F-30 high resolution transmission electron microscopy operating at 300 kV. Scanning electron microscopy (SEM) images were obtained on Hitachi S4800 scanning electron microscope with a field emission electron gun.

Xenograft tumor mouse model with tumor cells: Female Balb/c mice (weight ~20 g) were obtained from Shanghai SLAC laboratory Animal Co., Ltd. and all the experiments were carried out under protocols approved by Xiamen University Laboratory Animal Center. The 4T1 murine breast tumor models were generated by subcutaneous injection of 5×10^6 cells in ~100 μL PBS onto the right rear flanks of each mouse. The PPy NPs were injected to the mice when the tumor volumes approached 60~70 mm^3. The photothermal therapy *in vivo* was carried out by using an optical fiber coupled 808 nm high power laser diode (BWT Beijing Co., Ltd.). Experimentally, the tumor on each mouse was exposed to the 808 nm NIR laser with a power density of 1 W cm^{-2} for 5 minutes. Then, the tumor sizes were measured by a caliper every other day and calculated as the volume = (tumor length) × (tumor width)^2/2. Relative tumor volumes were defined as the tumor change during the experiment, which were calculated as V/V_0 (V_0 was the initiated tumor volume when the treatment started, while V was the tumor volume during the treatment).

***In vivo* NIR imaging:** To image the temperature of tumors when they were exposed to laser, an infrared thermography (HM-300, Guangzhou SAT Infrared Technology Co., Ltd.) was used to capture the temperature change on the site of the tumor.

Histology examination: Sixty days after the injection of PPy NPs, major organs from the treatment group and age-matched female Balb/c control mice (without any tumor and injection of PPy NPs) were harvested and fixed in 10% neutral buffered formalin, processed routinely into paraffin, sectioned at 10 microns, stained with hematoxylin & eosin (H&E) and examined by a digital microscope. Tissues which were examined included liver, kidneys, spleen, heart, and lung.

SiO_2@PPy in mouse organs for biodistribution: After injected with 500 μg SiO_2@PPy, the mice were sacrificed at 1, 6 and 24 h to obtain the major organs including liver, kidneys, spleen, heart, lung and tumor. At every point, there were 3 mice sacrificed. Then the organs were dissected and weighed for distribution studies. Inductively coupled plasma-atomic emission spectrometry (ICP-AES) was used to measure the quantity of silicon.

Blood circulation of SiO_2@PPy NPs: Sprague-dawley rats (weight ~200 g) was introduced to test the blood circulation of SiO_2@PPy NPs. After injected with 800 μg SiO_2@PPy NPs, 0.4 mL blood was collected form jugular veins of the rats at different time. Three rats were used in the experiment. Then, ICP-AES was used to measure the quantity of silicon.

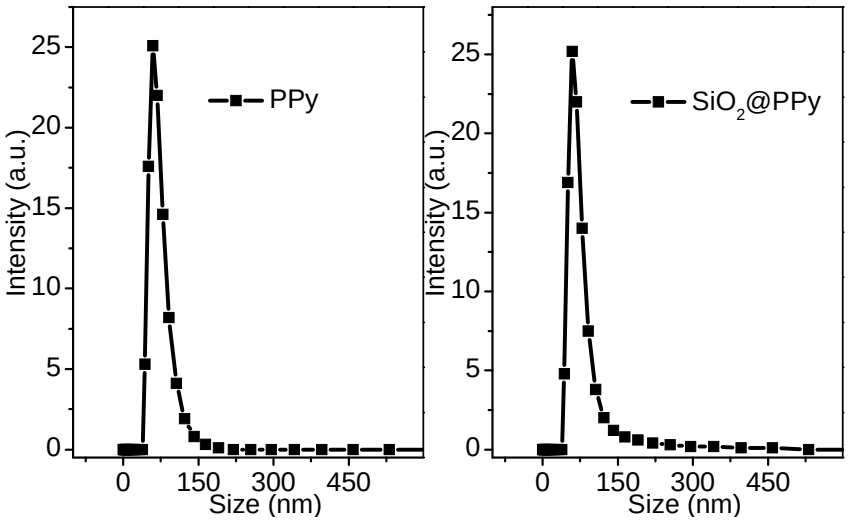

Figure S1. Sizes of PPy NPs and SiO_2@PPy NPs characterized by dynamic light scattering (DLS).

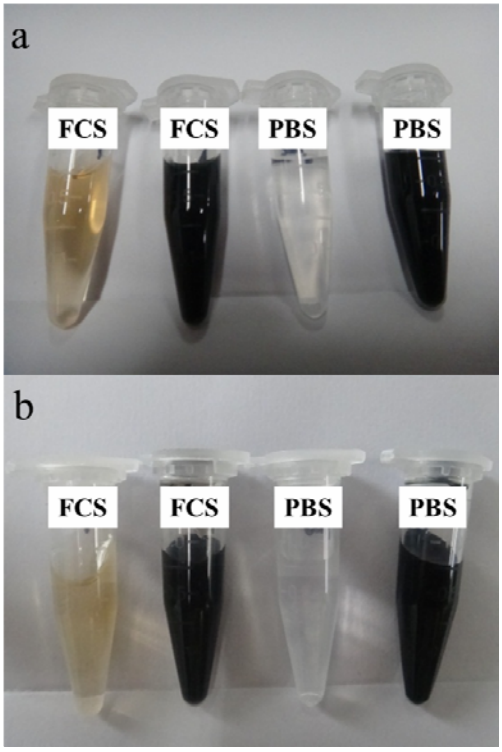

Figure S2. Photos of the PPy NPs in phosphate buffered saline (PBS) and fetal calf serum (FCS). (a) one day, (b) five days after PPy NPs were mixed with PBS or FCS.

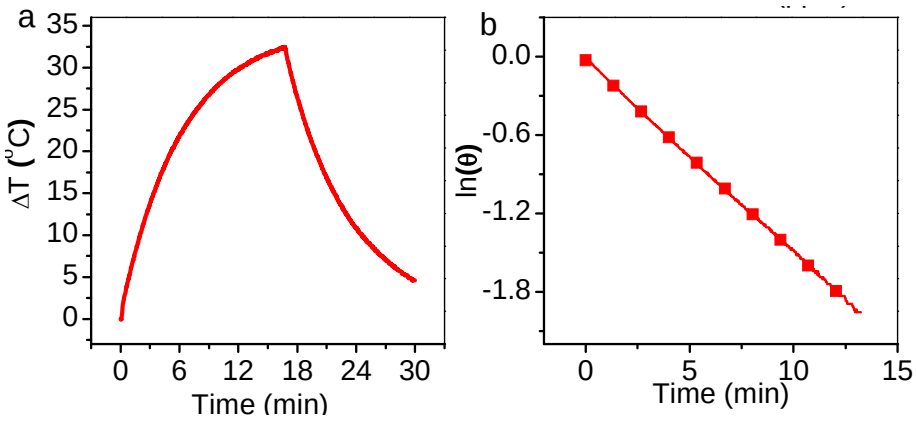

Figure S3. (a) Photothermal effect of 20 ppm PPy NPs after being irradiated for 16 min the laser was shut off. (b) Time constant for heat transfer from the system is determined to be τ_s = 401.4 s by applying the linear time data from the cooling period of Figure S3a.

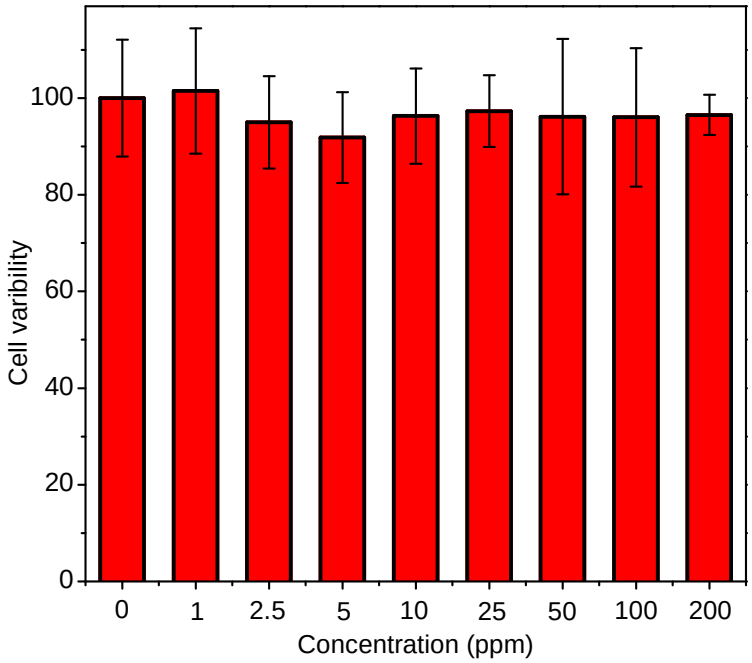

Figure S4. QSG-7701 cell viability under different concentrations of PPy NPs.

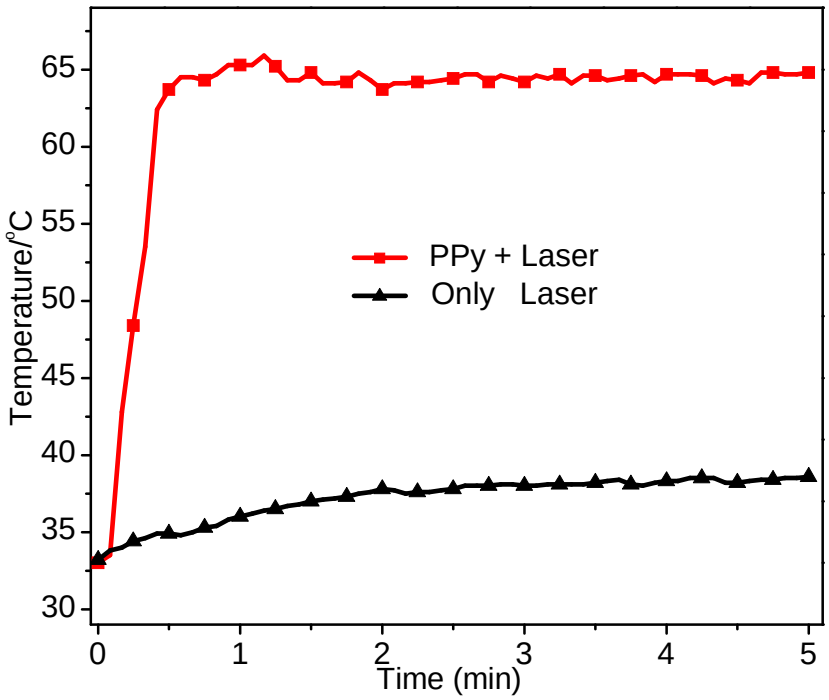

Figure S5. Temperature change of the tumor site with irradiation by 1 W cm^{-2} laser, red line is with PPy + Laser while the black one is only laser.

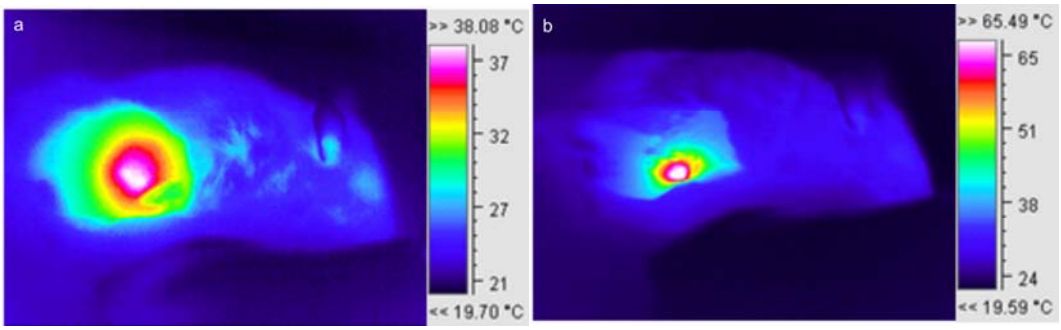

Figure S6. IR images of the tumor sites with irradiation by 1 W cm^{-2}: (a) with no PPy NPs, (b) with PPy NPs.

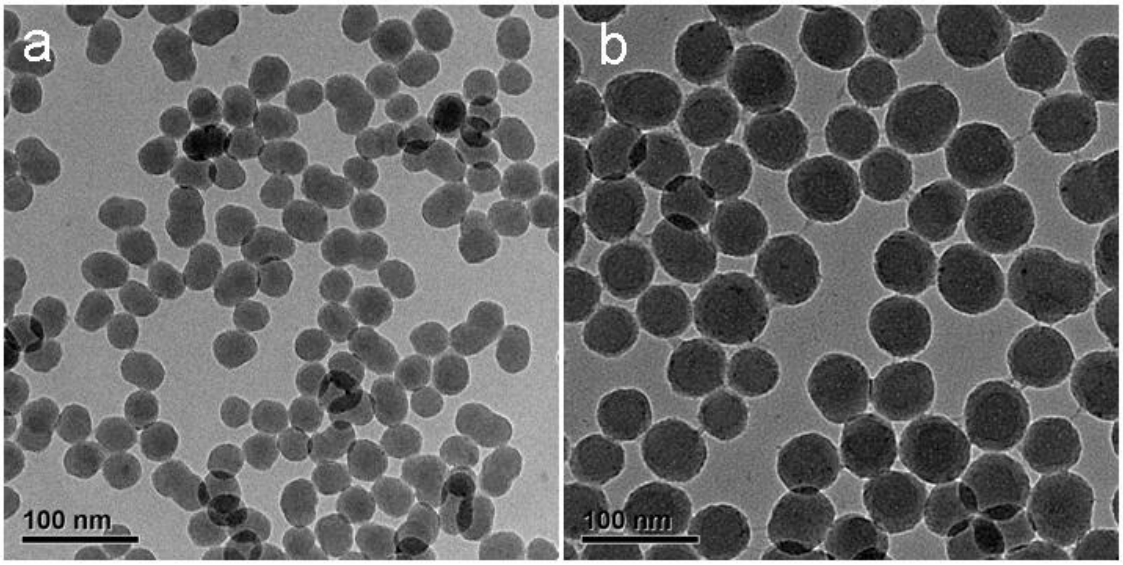

Figure S7. TEM images of SiO_2 nanoparticles (a) used for PPy coating and the as-prepared SiO_2@PPy nanoparticles (b).

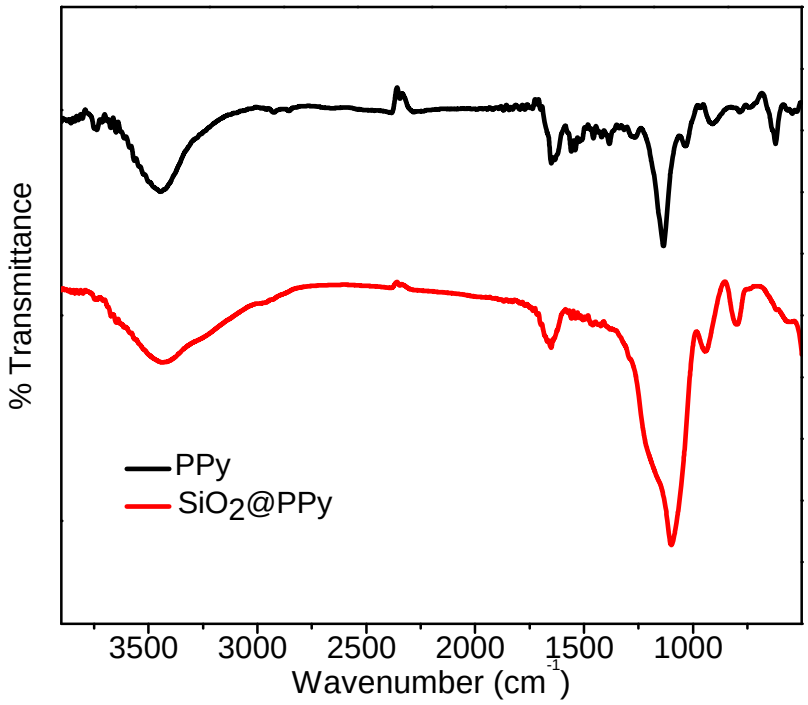

Figure S8. FT-IR spectra of PPy NPs and SiO_2@PPy NPs.

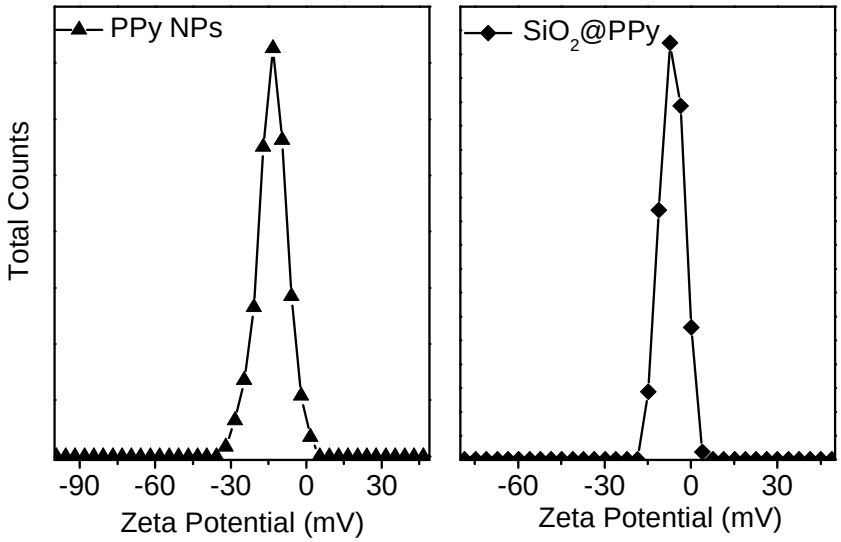

Figure S9. Zeta potentials of PPy NPs and SiO_2@PPy NPs in aqueous solution.

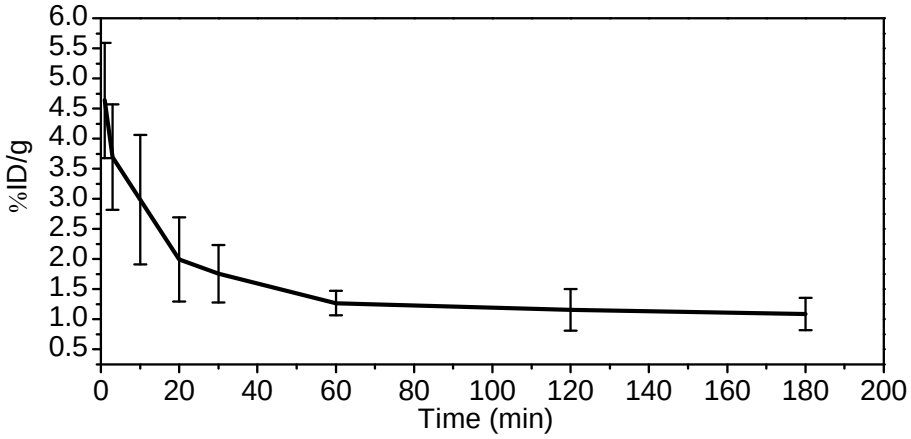

Figure S10. The blood circulation curve of SiO_2@PPy NPs in the blood at different time points after injection.

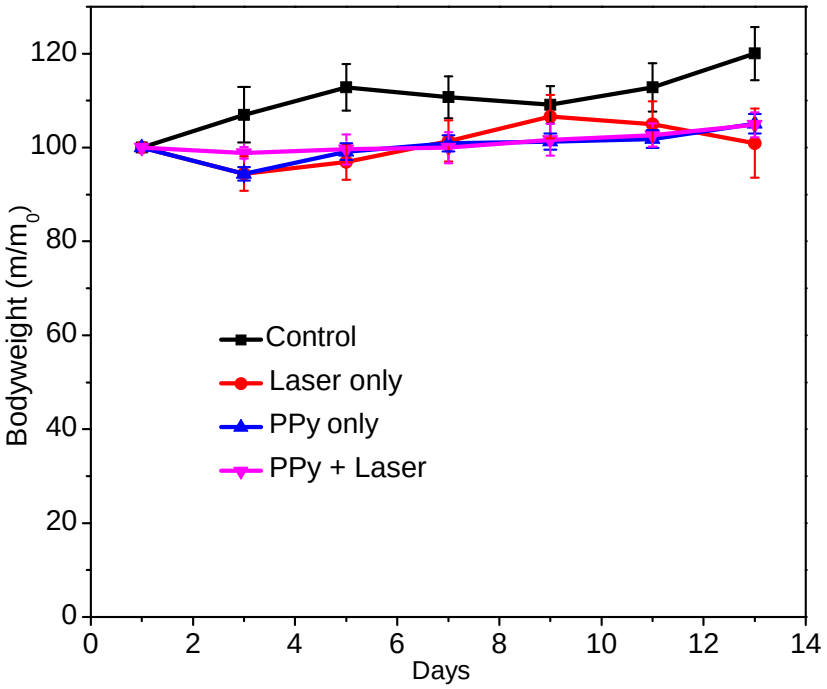

Figure S11. Body weight curves of four groups during the *in vivo* test.

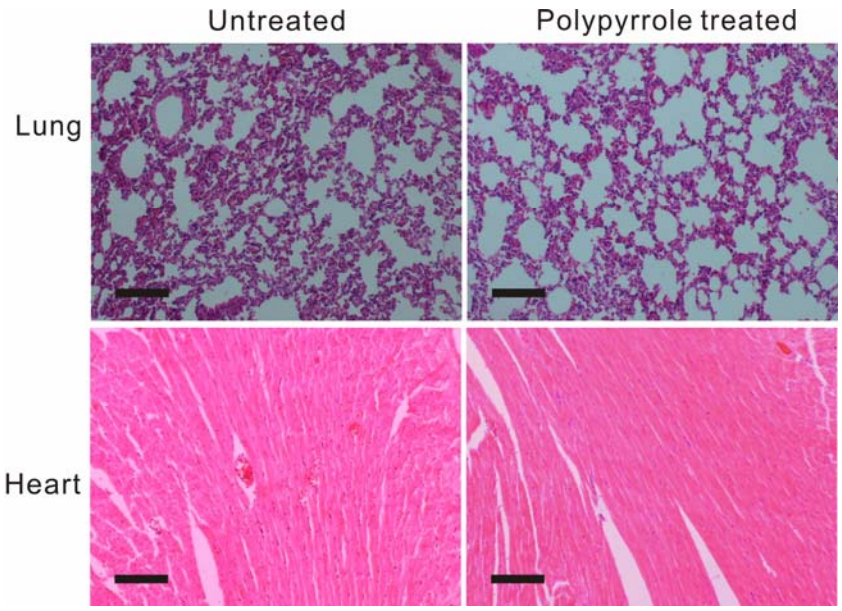

Figure S12. H&E stained images of heart and lung from mice untreated and survived after photothermal therapy. Scale bar: 100 μm.