Supporting Information

Conjugation of a Scintillator Complex and Gold Nanorods for Dual-Modal Image-Guided Photothermal and X-Ray-Induced Photodynamic Therapy of Tumors

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Supporting information for experimental section

Materials

Tetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$, 99.9%), sodium borohydride ($NaBH_4$, 99%), cetyltrimethyl ammonium bromide (CTAB, 99%), tetraethyl orthosilicate (TEOS), ascorbic acid (99%), silver nitrate ($AgNO_3$, 99%), 3-aminopropyl-triethoxysilane (APTES, 99%), NHS-PEG_{3400}-COOH, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, 99%), N-hydroxysuccinimide (NHS, 98%), 9,10-anthracenediylbis (methylene) dimalonic acid (ABDA, 99%), singlet oxygen sensor green (SOSG), and 4-nitrophenyl chloroformate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (MO, USA). Methanol, ethanol, dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) were supplied by Sinopharm Chemical Reagent Co., Ltd. Ultrapure water was obtained from a Millipore autopure system with resistivity of 18.2 MΩ. All chemicals were analytical grade and used without further purification unless noted. Fetal bovine serum (FBS) was purchased from HyClone Inc. Breast cancer cell line (4T1) was obtained from Institute of Biochemistry and Cell Biology (Shanghai, China). Male BALB/c mice were purchased from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China).

Characterization

Transmission electron microscopy (TEM) images were carried out on a JEM-2100 transmission electron microscope operating at 200 kV (JEOL Ltd., Tokyo, Japan). The crystal structure had been proved by powder X-ray diffractometer (XRD) using Ultima IV X-ray powder diffractometer (Rigaku Co., Japan). Hydrodynamic size and ζ-potential were measured using ZetaSizer Nano-ZS size analyzer (Malvern Ltd., UK). Absorption spectra were recorded using a UV-Vis spectrometer (Agilent Cary60, USA). The mini-X X-ray tube (Amptek Inc.) was used as the X-ray source (50 kV, 70 μA). And X-ray excited optical luminescence (XEOL) was measured using an Ocean Optics QE Pro optical bench. Photothermal irradiation was carried out using an 808 nm semiconductor laser unit (KS3-11312-110, BWT, Beijing Kaipulin Co., Ltd., China).

The Cellular fluorescence images were examined using a laser scanning confocal microscopy (Olympus FV1200, Japan). CT images were recorded using a CT system (Siemens Inveon micro-CT instrument). Photoacoustic imaging was performed using a Vevo LAZR-X system (FUJIFILM VisualSonics Inc., Toronto, Canada).

Singlet oxygen ($^1O_2$) production in solutions

0.1 mL HP or GSE-HP solution (0, 20, 40, 60, 80, 100 100 μg·mL^{-1}) was added into an enzyme strip containing 0.1 mL ABDA (100 μg·mL^{-1}). The solution was irradiated by X-ray (1, 2, 3, 4, 5 Gy). The UV-Vis absorption was measured after the irradiation.

Photothermal effect and photothermal conversion efficiency

Photothermal effect was monitored with a FLIR A×5 camera (FLIR Systems Inc., Wilsonville, OR, USA) when 0.1 mL samples of GSE-HP (100 μg·mL^{-1}) under laser irradiation (808 nm, 0.8 W·cm^{-2}) for 600 s. The photothermal conversion efficiency (η) was calculated using the following reported equation:[1]

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A_{808}})} \qquad (2)$$

Where h (mW·(m^2·°C)$^{-1}$) is heat-transfer coefficient, S (m^2) is the surface area of the container, T_{max} is the equilibrium temperature, and T_{surr} is the surrounding temperature. In this experiment, $T_{max}-T_{surr}$ was 23.4 °C. The Q_{Dis} (mW) is the heat dissipation from light absorbed by a cuvette sample wall itself and it was measure to be 11.2 mW. I is the laser power (0.8 W) and A_{808} is the absorbance (0.798) of GSE-HP at 808 nm.

The value of hS was calculated according to these following equations：

$$\tau_s = \frac{\Sigma_i m_i C_{p,i}}{hS} \qquad (3)$$

$$t = -\tau_s \ln(\theta) \qquad (4)$$

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \qquad (5)$$

τ_s was determined to be 345.56 s, thus hS was deduced to be 11.55 mW·°C^{-1} (substituted m = 1 g, C = 4.2 J·g^{-1}·K^{-1} in Equation (3)). Finally, the photothermal conversion efficiency (η) was calculated to be 38.4% from Equation (2).

In vitro CT and Photoacoustic (PA) imaging

For *in vitro* imaging experiments, 200 μL of GSE-HP of different concentrations (0, 6.25, 12.5, 25, 50, 100, and 200 $\mu g \cdot mL^{-1}$) were added into PCR tubes to perform CT and photoacoustic imaging. CT imaging parameters were as follows: effective pixel size, 112.93 μm; field of view, 57.82 mm × 86.73 mm; binning, 4 × 4; rotation steps, 180; exposure time, 300 $ms \cdot rotation^{-1}$; 80 kV, 500 μA. Images of phantom CT images were analyzed with Kodak Molecular Imaging Software. HU values were measured by the Siemens Inveon micro-CT software. PA imaging was performed using a Vevo LAZR-X system (FUJIFILM VisualSonics Inc., Toronto, Canada) equipped with a 40 MHz, 256-element linear array transducer on tumors. A 780-nm excitation filter was used.

Cell toxicity assay

4T1 breast cancer cell line was incubated in DMEM medium under 37 °C within 5% CO_2 atmosphere. The cells were pipetted into 96-well plates (5000 cells per well) and incubated for 24 h. Then, the as-prepared samples of indicated concentrations (0, 6.25, 12.5, 25, 50, 100 $\mu g \cdot mL^{-1}$) were added to the cell culture medium and incubated with the cells for another 24 h. The cytotoxicity was evaluated according to the standard MTT assays protocol.

Cellular uptake

Typically, 4T1 cells (1×10^5 cells) were seeded on glass-bottomed microwell dishes (Ø = 10 mm). After 24 h, 2 mL GSE-HP (100 $\mu g \cdot mL^{-1}$) was added and incubated with cells for another 24 h. Cells were washed with PBS and pre-stained with 5 μM DAPI for 30 min. And then washed again before replenishing with fresh medium. The fluorescence images were imaged under a confocal laser scanning microscope (Olympus FV1200).

In vitro X-PDT and PTT efficacy assay

4T1 cells (1×10^4 cells) were seeded into 96-well plates and incubated for 24 h at 37 °C with 5% CO_2. Then, the cells were incubated for another 24 h with 100 μL various composites including GSE-HP suspension at different concentration (0, 6.25, 12.5, 25, 50, 100 $\mu g \cdot mL^{-1}$) in fresh medium and washed three times with PBS. The cells were received different treatments: 1) no treatment, 2) X-PDT (X-ray: 2 Gy), 3) PTT (808 nm laser: 0.8 $W \cdot cm^{-2}$, 10 min), 4) X-PDT + PTT (X-ray: 2 Gy + 808 nm laser: 0.8 $W \cdot cm^{-2}$, 10 min). Then the cells were further incubated for 24 h in DMEM, and cell viability was measured by the MTT assay.

To stain live and dead cells, the cells of different groups were incubated with calcein-AM (4 μM) and propidium iodide (4 μM) for 30 min at 37 °C, respectively. Cellular fluorescence images were then examined using an Olympus FV1200 laser scanning confocal microscope.

In vitro $^1O_2$ production

4T1 cells (1×10^5 cells) were seeded in glass-bottomed microwell dishes (Ø = 10 mm) and incubated for 24 h at 37 °C. The cells were then incubated with 100 $\mu g \cdot mL^{-1}$ of GSE-HP for 24 h before being washed with PBS for three times and pre-stained with 5 μM SOSG for 30 min. Next, the cells were washed with PBS and irradiated by X-ray (2 Gy). Then the cells were incubated with 5 μM DAPI for 30 min and the images were acquired on an Olympus FV1200 confocal laser scanning microscope using 473 nm laser with a FITC filter.

Animal experiments

Male BALB/c mice were purchased from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China). All the animal experiments were carried out according to the guidelines of the Regional Ethics Committee for Animal Experiments and the Care Regulations approved by the Institutional Animal Care and Use Committee of Xiamen University.

In vivo bio-distribution and blood clearance

First, tumor-bearing mice were constructed by subcutaneously injecting of 4×10^6 4T1 cells into the back of the hind leg. BALB/c mice (4~6 weeks old) were used for the tumor model establishment and intravenously (*i.v.*) injected with GSE-HP (8 $mg \cdot kg^{-1}$). To confirm the *in vivo* distribution and blood clearance of GSE-HP nanoparticles, mice were sacrificed at 2, 4, 6, 8, 24, and 48 h post-injection (n = 3). The main organs (livers, heart, lungs, spleen, kidneys, stomach, intestine, brain, muscle, and tumor) and blood were weighed and digested using HNO_3-H_2O_2 mixture. The Au concentration in all samples were determined by ICP-MS and deduced as the percentage of injected dose per gram of tissue (%ID/g). The *in vivo* blood circulation half-life of GSE-HP was calculated using the double-component pharmacokinetic model.

Serum-chemistry and complete blood analysis

To verify the biosafety, BALB/c mice were intravenously injected with GSE-HP (20 $mg \cdot kg^{-1}$). Blood samples were collected from the mice eye socket vein for serum chemistry and complete blood analysis at day 3 and 7 after injection (n = 3). The results were compared with bloods from the same mice before injection. The blood serum samples were analyzed using an Auto Biochemistry Analyzer (Mindray, BS-220) for some liver and renal function markers: alanine transaminase (ALT), aspartate transaminase (AST), and blood urea nitrogen (UREA). For complete blood analysis, a series of indicators like white blood cell, red blood cell, hemoglobin, *etc.*, were measured using an Auto Hematology Analyzer (Mindray, BC-2600).

Statistical Analysis

All data were calculated and presented as mean ± standard deviation. Comparison between two groups were analyzed with a Student's t-test (*: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$).

Supporting information for figures

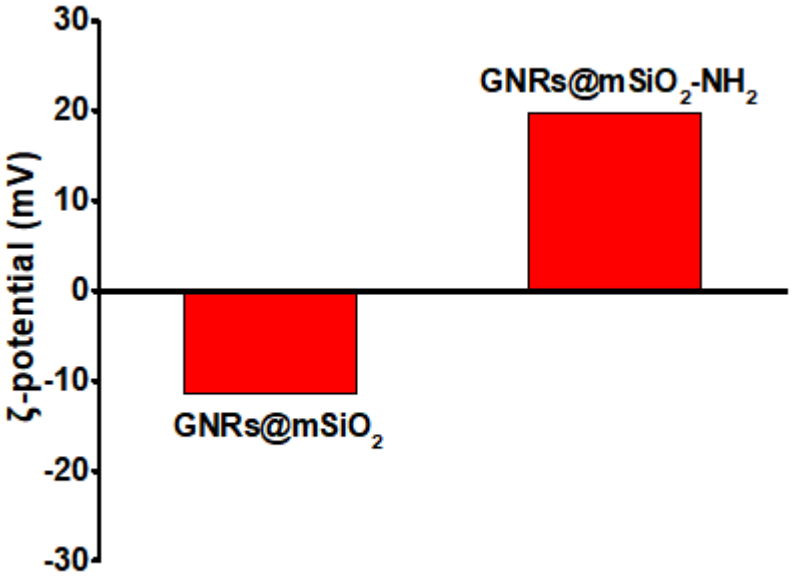

Figure S1. Zeta potential of GNRs@$mSiO_2$ and GNRs@$mSiO_2$-NH_2.

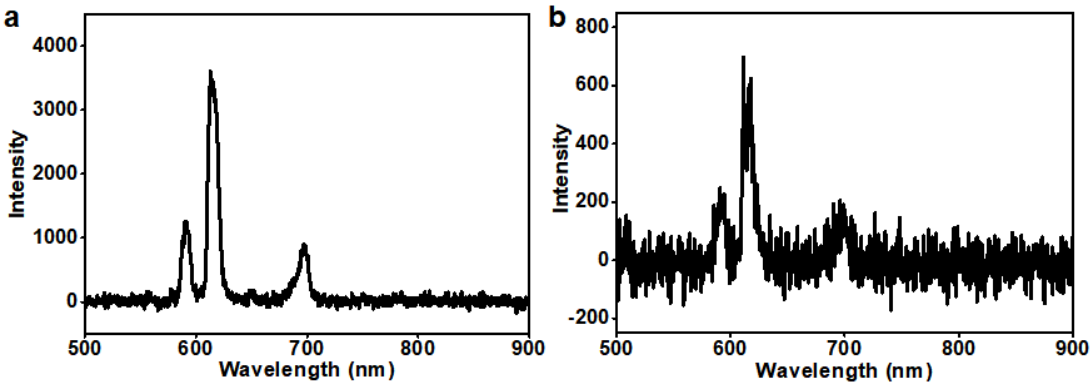

Figure S2. The XEOL spectrum of (a) EuBA and (b) APTES-EuBA.

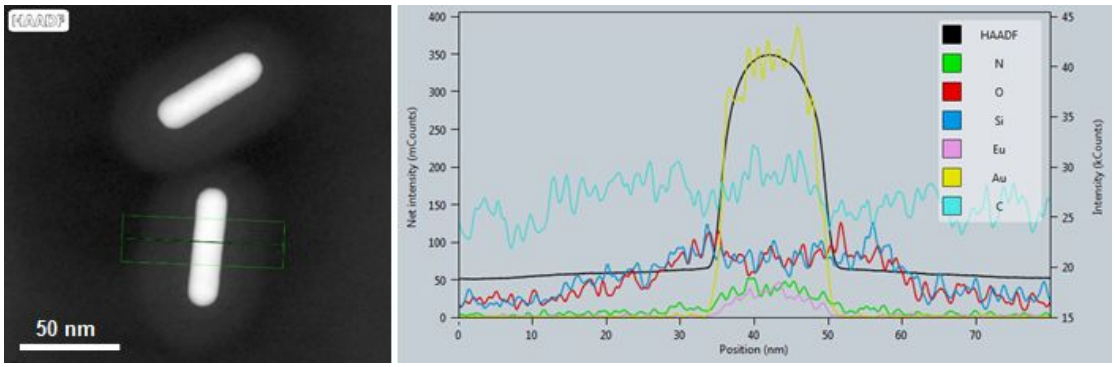

Figure S3. EDS analysis of GSE-HP.

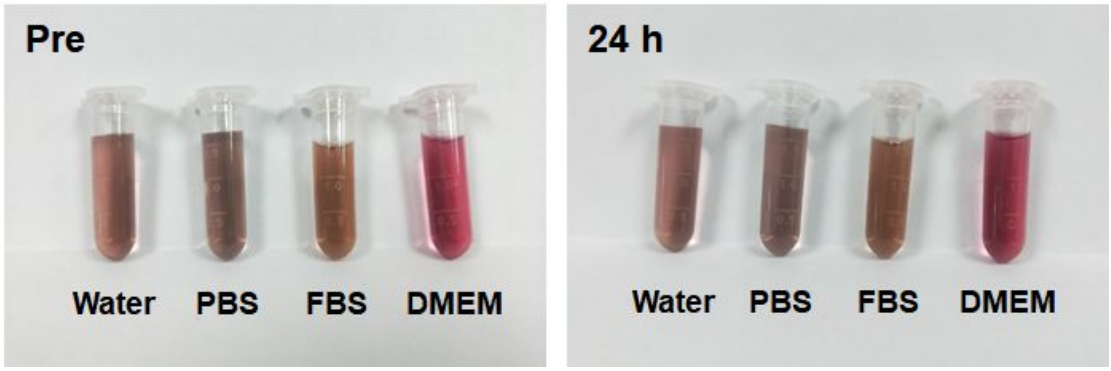

Figure S4. *In vitro* stability of GSE-HP dispersed in different medium (water, PBS, FBS, and DMEM).

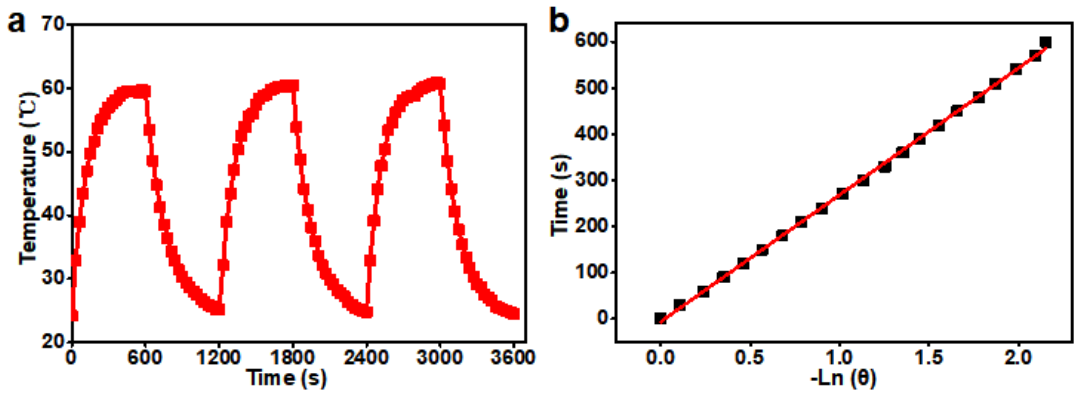

Figure S5. (a) Temperature variations of GSE-HP under continuous irradiation using 808 nm laser for several cycles. (b) The time constant for heat transfer from the system was determined to be τ_s = 274.94 s by applying the linear time data from the cooling period (from 600 s to 1200 s) versus negative natural logarithm of the driving force temperature obtained from the cooling stage.

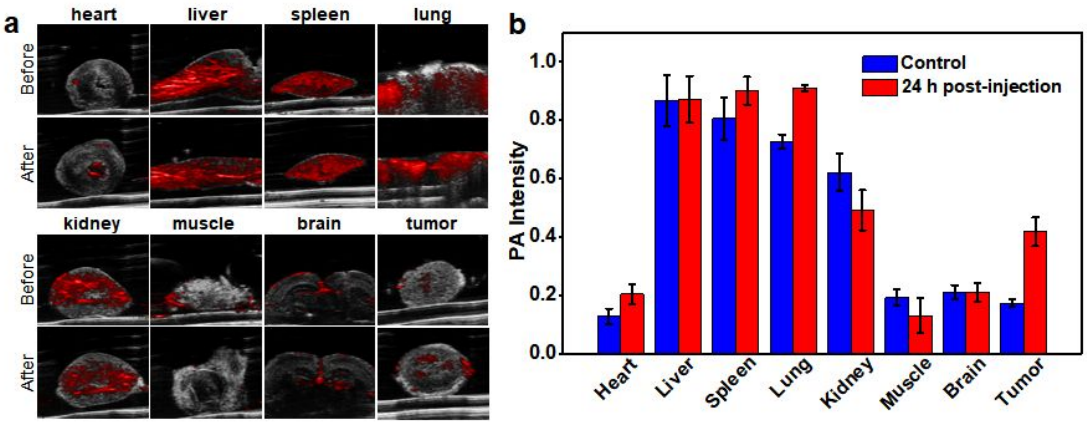

Figure S6. Photoacoustic (PA) images (a) and corresponding PA signals (b) of main tissues and tumor before and after injection with GSE-HP.

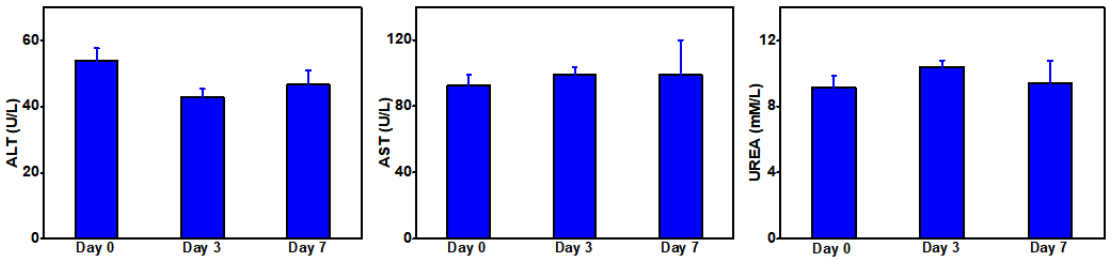

Figure S7. Serum chemistry of mice treated with GSE-HP. Data are mean ± s.d. ALT, alanine transaminase; AST, aspartate transaminase; UREA, blood urea nitrogen. Mice were intravenously treated with GSE-HP (8 mg·kg^{-1}). Blood samples were collected for serum chemistry analysis on day 0, 3 and 7 after treatment.

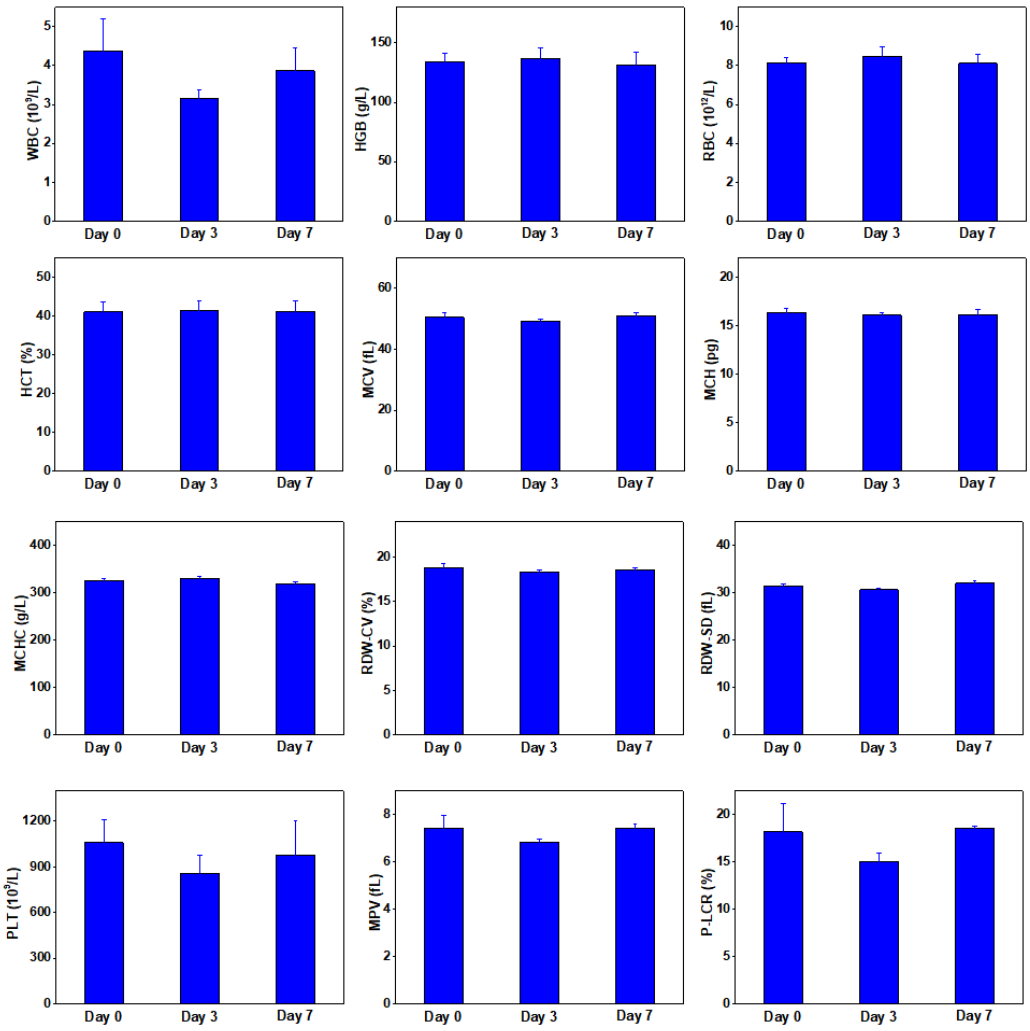

Figure S8. Hematological analysis of mice treated with GSE-HP. Data are mean ± s.d. WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-SD, standard deviation of RBC distribution width; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; HCT, hematocrit; PLT, platelets; MPV, mean platelet volume. Mice were intravenously treated with GSE-HP (8 mg·kg^{-1}). Blood samples were collected for hematological analysis on day 0, 3 and 7 after treatment.

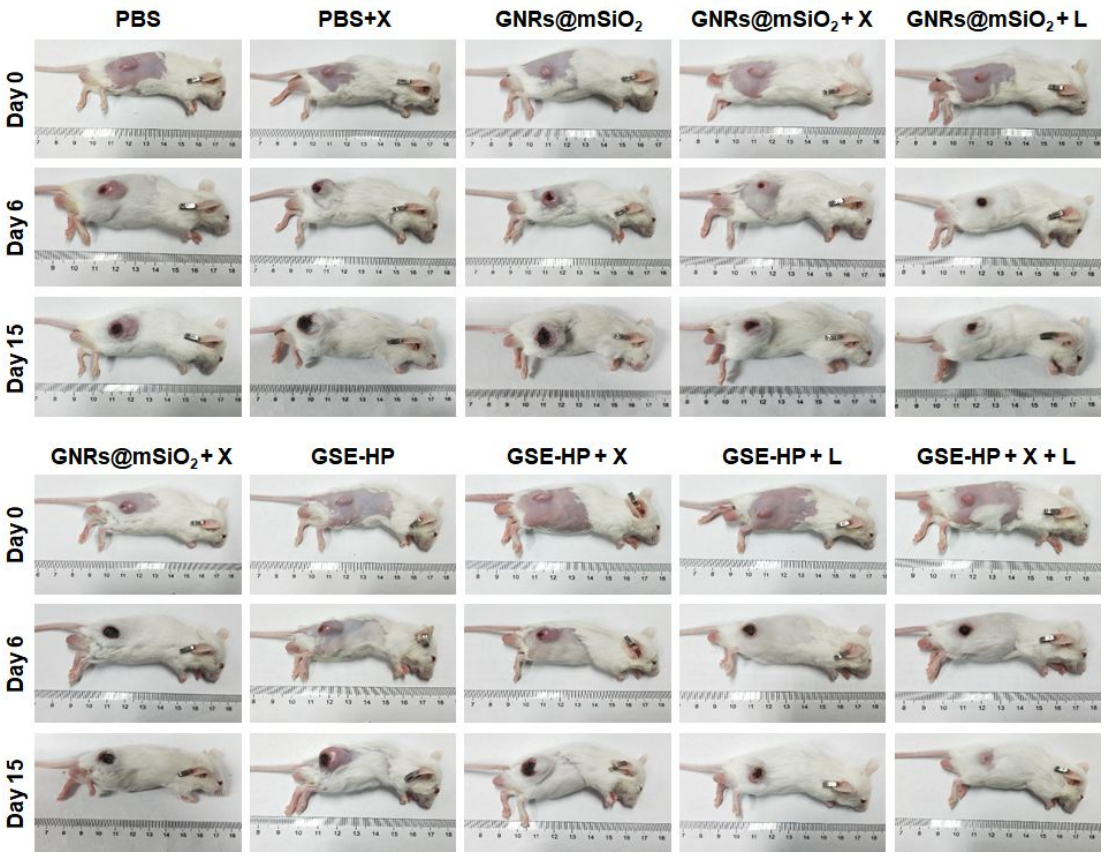

Figure S9. Representative photos of different groups of tumor-bearing mice after various treatments on day 0, 6, and 15.

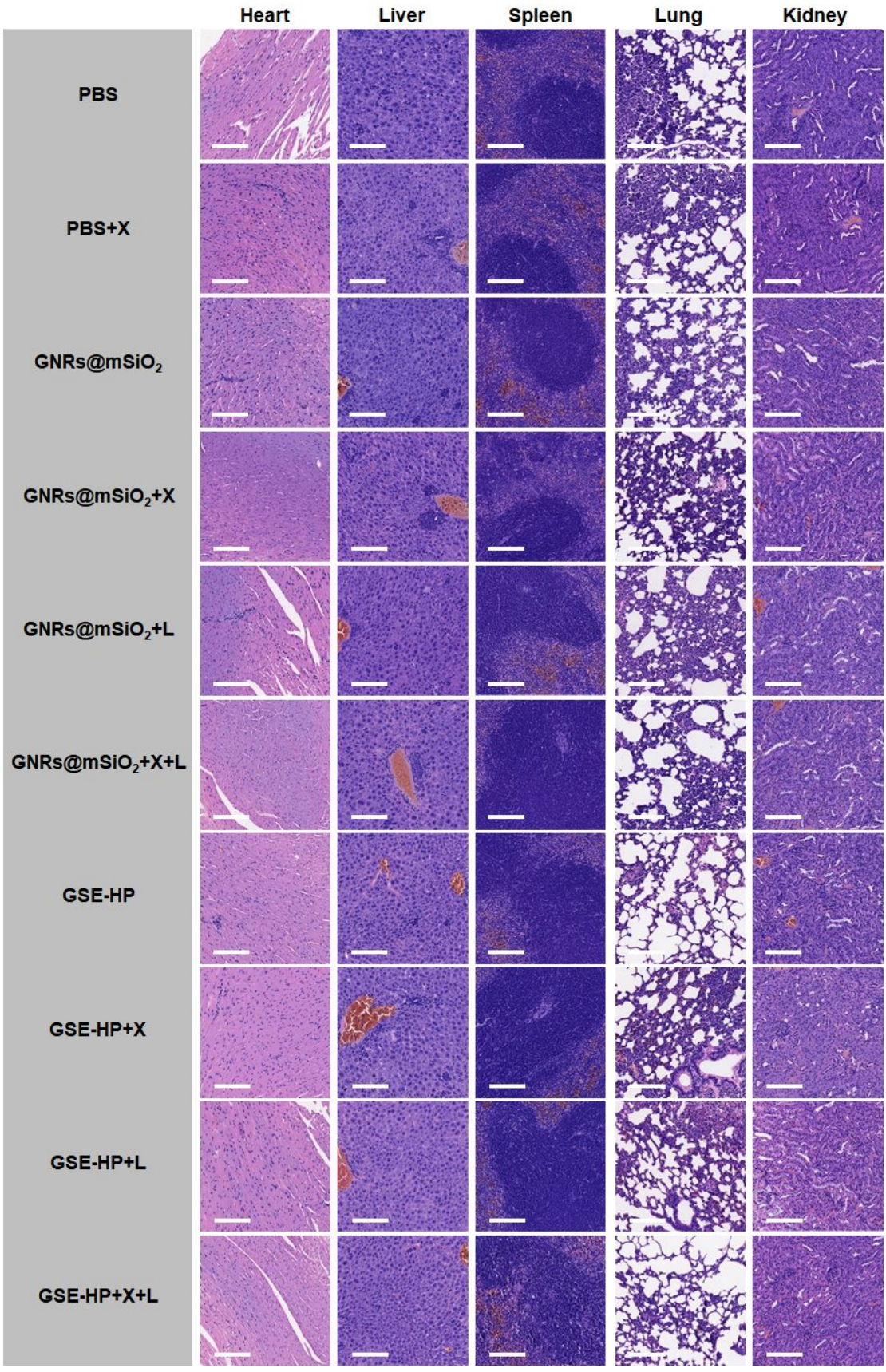

Figure S10. H&E-staining of normal tissue slices after different treatments.

Reference

1. Roper, D. K.; Ahn, W.; Hoepfner, M., Microscale Heat Transfer Transduced by Surface Plasmon Resonant Gold Nanoparticles. *J. Phys. Chem. C* **2007,** *111* (9), 3636-3641.