Supporting Information

Gadolinium Oxysulfide-Coated Gold Nanorods with Improved Stability and Dual-modal Magnetic Resonance/Photoacoustic Imaging Contrast Enhancement for Cancer Theranostics

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Experimental Section

Chemicals and apparatus. Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.99%), silver nitrate ($AgNO_3$, >99%) and cetyltrimethylammonium bromide (CTAB) were purchased from Sigma-Aldrich. Ascorbic acid (AA), hexamethylenetetramine ($C_6H_{12}N_4$), sodium borohydride ($NaBH_4$), thioacetamide (TAA), polyacrylic acid (PAA), and gadolinium nitrate hexahydrate ($Gd(NO_3)_3 \cdot 6H_2O$) were purchased from Sinopharm Chemical Reagent Co. Ltd (China). All chemicals were used as received without further purification. Ultrapure water obtained from a Millipore water purification system (18.2 MΩ resistivity) was used in all runs.

Apparatus. Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images were captured on a Tecnai G2 F20 at an accelerating voltage of 200 kV. The X-ray diffraction (XRD) patterns were obtained on a Rigaku Ultima IV system. The X-ray photoelectron (XPS) data were collected on a Thermo escalab 250Xi XPS spectrometer. The X-ray spectroscopy (EDS) and energy-dispersive X-ray (EDX) element mapping analysis was performed on a Tecnai G2 F20 microscope at an accelerating voltage of 200 kV. The metal concentration of the samples was determined by an Agilent 7500ce inductively coupled plasma mass spectrometry (ICP-MS). Ultraviolet-visible-near-infrared light (UV-Vis-NIR) absorption spectra was recorded using a SH-1000 Lab microplate reader (Corona Electric, Hitachinaka, Japan) at room temperature. Photothermal irradiation was operated using a semiconductor laser unit (KS3-11312-110, BWT, Beijing Kaipulin Co. Ltd, China). IR thermographs were collected using a FLIR Ax5 infrared camera. The photoacoustic imaging experiments was carried on an Endra Nexus 128 small animal photoacoustic imaging system at 808 nm. The fluorescence images of cells were taken on a confocal fluorescence microscope (Nikon C2).

Synthesis of GNRs@Gd_2O_2S with diffierent shell thinkness. The CTAB capped GNRs were firstly synthesized according to the previous report with slight modification[1]. In brief, the

seed solution was firstly synthesized by adding $HAuCl_4$ (0.01 M, 0.25 mL) to the CTAB solution (0.1 M, 9.75 mL). A freshly prepared, ice-cold $NaBH_4$ solution (0.01 M, 0.6 mL) was then added into the mixture solution under stirring for 2 min. To further grow GNRs, $HAuCl_4$ (0.01 M, 2 mL) were firstly incubated with $AgNO_3$ (0.01 M, 0.35 mL), and CTAB solution (0.1 M, 40 mL), followed by the addition of AA (0.1 M, 0.32 mL). Finally, the seed solution (25 μL) was injected into the growth solution, and the solution was gently mixed for 30 s and left undisturbed at 27-30 ˚C for 6 h to obtain the desired GNRs. To synthesize GNRs@Gd_2O_2S with a shell thickness of 4 nm, the as-prepared uncentrifuged GNRs (10 mL) were mixed with 1 mL of 0.1 M hexamethylenetetramine, 0.5 mL of 0.1 M AA, and 40 μL of 0.1 M thioacetamide. After adding 40 μL of 0.1 M $Gd(NO_3)_3$, the reaction mixture was heated to 80 °C and mildly stirred for 8 h. Dark purple solution was obtained. The products were collected by centrifugation (10000 rpm, 10 min), followed by washing with ethanol and deionized water for several times, and then disperse in deionized water for further use. Finally, the mixture of GNRs@Gd_2O_2S and polyacrylic acid was sonicated for 6 h to obtain water-soluble PAA modified GNRs@Gd_2O_2S. The GNRs@Gd_2O_2S with 8 nm and 20 nm shell thickness were obtained using similar procedures except for the concentrations of thioacetamide and gadolinium nitrate hexahydrate were both 0.5 M and 0.8 M, respectively.

In vitro photothermal effect measurement. To study the photothermal effect induced by the 808 nm laser irradiation, GNRs@Gd_2O_2S with different concentrations (equal Au concentration of 12.5, 25, 50 and 100 μg mL^{-1}) were suspended in a quartz cuvette (total volume of 1 mL) and irradiated by a 808 nm laser at with output power of 1 W cm^{-2} for 5 min. The temperatures of the solutions were monitored by a digital thermometer with a thermocouple micrprobe (φ = 0.5 mm) submerged in the solution in a 1 cm square cuvette. IR thermographs under irradiation of different power densities were collected using a FLIR Ax5 infrared camera.

Calculation of photothermal conversion efficiency (η). The photothermal conversion efficiency (η) was calculated according to equation 1 in previous methods[2].

$$\eta = \frac{hA \Delta T_{max} - Q_s}{I(1-10^{-A_{808}})} \quad (1)$$

Where ΔT_{max} is the temperature change at the maximum steady-state temperature. Q_S is the heat dissipation from the light absorbance of the solvent, which is measured independently to be 25.1 mW using pure water. I is the laser power (1 W cm^{-2}), A_{808} is the absorbance of the GNRs or GNRs@Gd_2O_2S at 808 nm. hA is determined by the following equation 2:

$$\tau_s = \frac{m_D C_D}{hA} \quad (2)$$

Where τ_s is sample system time constant that can be obtained from the slope of the plot of cooling time vs -Ln($\Delta T/\Delta T_{max}$), m_D and C_D are the mass (1 g for H_2O) and heat capacity (4.2 J g^{-1} for H_2O) of the used solvent. Using the above two equations, the photothermal conversion efficiency (η) of nanoparticles can be calculated.

Stability of the nanorods under the nanosecond laser irradiation. 250 μL of 25 μg mL^{-1} GNRs or GNRs@Gd_2O_2S solution was added into a 96-well plates. Then a tunable OPO laser system (OPOTEK, Inc.) was used to stimulate an 810 nm laser beam (7 ns, 10 Hz), and irradiated each well for 20 min with average fluence of 0, 10, 30, 50 mJ cm^{-2}, respectively. The spectrum and morphology changes of GNRs or GNRs@Gd_2O_2S before and after irradiation were characterized by UV-Vis-NIR absorption spectra and TEM, respectively.

Cytotoxicity assay. The cytotoxicity was measured using a standard MTT assay. HeLa (or Hep G2 and L02) cells were cultured in RPMI-1640 medium (Gibco) with 10% fetal bovine serum (FBS, Gibco) at 37 °C in a humidified atmosphere with 5% CO_2. In a typical experiment, HeLa (or Hep G2 and L02) cells were seeded in 96-well plates and then incubated with 100 μL of varying Au ion concentrations of GNRs or GNRs@Gd_2O_2S for 24 h at 37 °C in a humidified 5% CO_2 atmosphere. Then the culture medium was removed, and

each well was filled with 100 μL of a new culture medium containing MTT (0.5 mg mL^{-1}) and the plate was incubated for 4 h at 37 °C. The medium was discarded and each well was filled with 200 μL DMSO. The OD_{490} value (Abs.) of each well was measured by a SH-1000 Lab microplate reader immediately. Cell viability was calculated from the OD_{490} value of the experimental group by subtracting that of the blank group.

Finite-difference time-domain (FDTD) simulation. The electromagnetic finite-difference time-domain (FDTD) simulation (FDTD solutions, version 8.6, Lumerical Solutions, Inc.) was applied to simulate the plasmonic properties of individual and aggregate GNRs or GNRs@Gd_2O_2S. For simulation, the size of GNRs (longitudinal LSPR peak at ~ 815 nm) was set as 16 nm in diameter and 61 nm in length, and that of GNRs@Gd_2O_2S was set as 20 nm in diameter and 58 nm in length, the thickness of the Gd_2O_2S shell was chosen to be 20 nm. To model the complex permittivity of gold, the dielectric function of Au was used from Johnson and Christ. The refractive index of Gd_2O_2S coating and surrounding water is chosen to be 1.66 and 1.333, respectively. Since the GNRs mainly aggregated in side-by-side fashion, the simulation of the absorption cross section and near-field enhancement side by side oriented assembly with a constant gap distance (2 nm) were utilized to demonstrate the effect of GNRs coupling on the shift of longitudinal LSPR band. During the simulation, the propagation direction and polarization of incident light were perpendicular and parallel to the length axis of GNRs. Futhermore, the perfectly matched layer (PML) and the fine mesh (1nm) were adopted to accurately model GNRs assembly in a 3D-FDTD simulation while maintaining tractable simulation times .

MRI phantom and relaxivity studies at 0.5 T. The MRI phantom and relaxivity studies were performed on a 0.5 T NMI20-Analyst NMR system (Niumag Corporation, Shanghai, China). A series of GNRs@Gd_2O_2S or Gd-DTPA aqueous solution with different Gd ion concentrations (0.05, 0.1, 0.2, and 0.4 mM) were prepared. T_1-weighted phantom images were acquired using a 2D multi-slice spin-echo (MSE) sequence with the following parameters:

TR/TE = 100/2 ms, 512 × 512 matrices, slices = 1, thickness = 1 mm, NS = 4. The T_1 times were measured by an inversion recovery (IR) sequence. The r_1 values were determined from the slope of the plot of $1/T_1$ against Gd concentration.

In vivo PAI and MRI. Animal experiments were executed according to the protocol approved by the Institutional Animal Care and Use Committee of Fuzhou University. BALB/c nude mice (weight ~28 g) were obtained from Shanghai SLAC laboratory Animal Co., Ltd. Tumor-bearing mice were prepared by subcutaneously injecting a suspension of 2×10^6 Hep G2 cells in PBS (100 μL) into the back of the hind leg. *In vivo* PAI was performed on an Endra Nexus 128 instrument,.which producted a 3D photoacoustic images by a hemispherical ultrasonic detector with 128 identical ultrasonic transducers spirally installed on the surface. PAI images were scanned before and after the intratumor injection of GNRs@Gd_2O_2S (100 μL, 50 μg mL^{-1}) dispersion. *In vivo* MRI was performed on an 7 T MRI scanner (Bruker Biospec 70/30 USR). Axial and coronal two-dimensional (2D) fast spin echo sequence images were first acquired to ensure the imaging position of the implanted tumor. T_1-weighted multislice spinecho images were collected before and after intratumor injection of GNRs@Gd_2O_2S (100 μL, 50 μg mL^{-1}) using the following parameters: TR/TE = 800/4 ms, matrix = 256 × 256, thickness = 2 mm, FOV = 60 × 60.

Photothermal therapy. For *in vitro* photothermal therapy, Hep G2 cells were seeded in 96-well plates and incubated with 100 μL of different Au concentrations of GNRs or GNRs@Gd_2O_2S for 12 h, then treated with 808 nm laser irradiation (1 W cm^{-2}) for 5 min. Subsequently, a standard MTT assay was performed to evaluate the cell viability. For fluorescence imaging, Hep G2 cells were incubated with 50 μg mL^{-1} GNRs@Gd_2O_2S for 4 h and treated with 1 W cm^{-2} laser irradiation for 5 min, then the cells were co-stained with Calcein AM and propidium iodide (PI), and imaged using a confocal laser scanning microscopy. For *in vivo* tumor therapy, about 100 μL of 50 μg mL^{-1} GNRs@Gd_2O_2S was

intratumorally injected into tumors, and exposed to 808 nm at 1 W cm^{-2} for 8 min (GNRs@Gd_2O_2S + laser group, five mice per group). The mice of the other three groups were used as controls: mice without any treatment (blank group), mice were only intratumorally injected with GNRs@Gd_2O_2S (GNRs@Gd_2O_2S only group), and mice intratumorally injected with PBS and then irradiated with laser (PBS + laser group). The tumor sizes were measured by caliper after treatment every two days. the tumor volume was calculated according to the equation: tumor volume = (tumor length)×(tumor width)^2/2. Relative tumor volumes were calculated as V/V_0 , where V is the tumor volume calculated after treatment, while V_0 is the initial tumor volume before treatment. IR thermographs was recorded by a FLIR Ax5 infrared camera.

Histology and immunohistochemistry. After various treatments, tumor-bearing mice were sacrificed and the tumors were collected for analysis. 16 days after treatments, mice in GNRs@Gd_2O_2S + laser group were also sacrificed and major organs were collected. The tissue sections were stained with H&E following the standard protocol.

[1] B. Nikoobakht, M. A. El-Sayed, *Chem. Mater.* **2003**, 15, 1957.

[2] Y. Liu, K. Ai, J. Liu, M. Deng, Y. He, L. Lu, *Adv. Mater.* **2013**, 25, 1353.

Supporting Figures

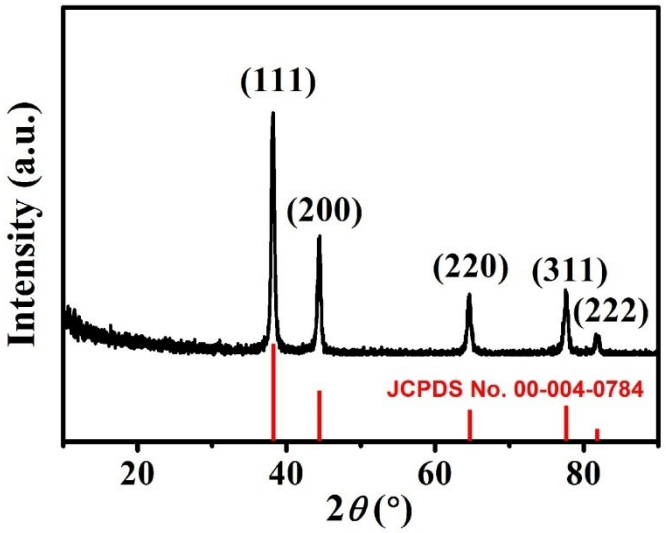

Figure S1. XRD pattern of GNRs@Gd_2O_2S

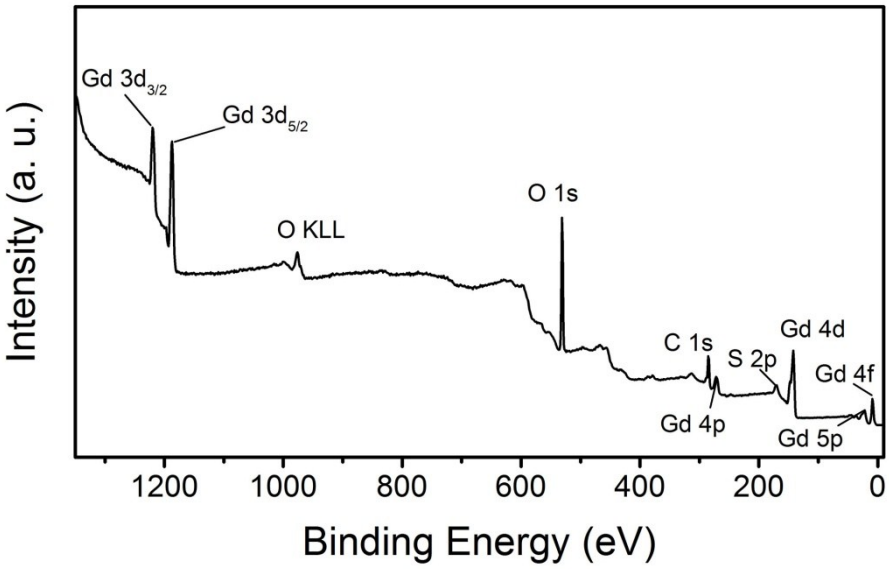

Figure S2. XPS spectrum of the GNRs@Gd_2O_2S.

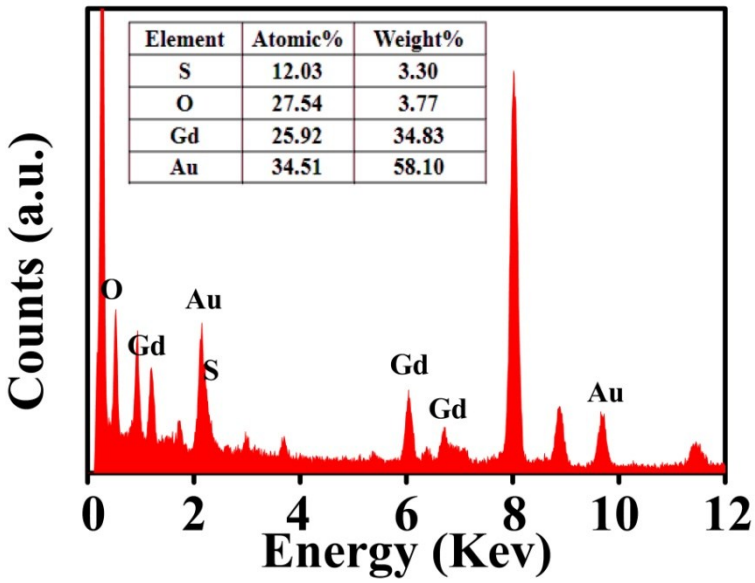

Figure S3. EDS analysis spectrum of GNRs@Gd_2O_2S.

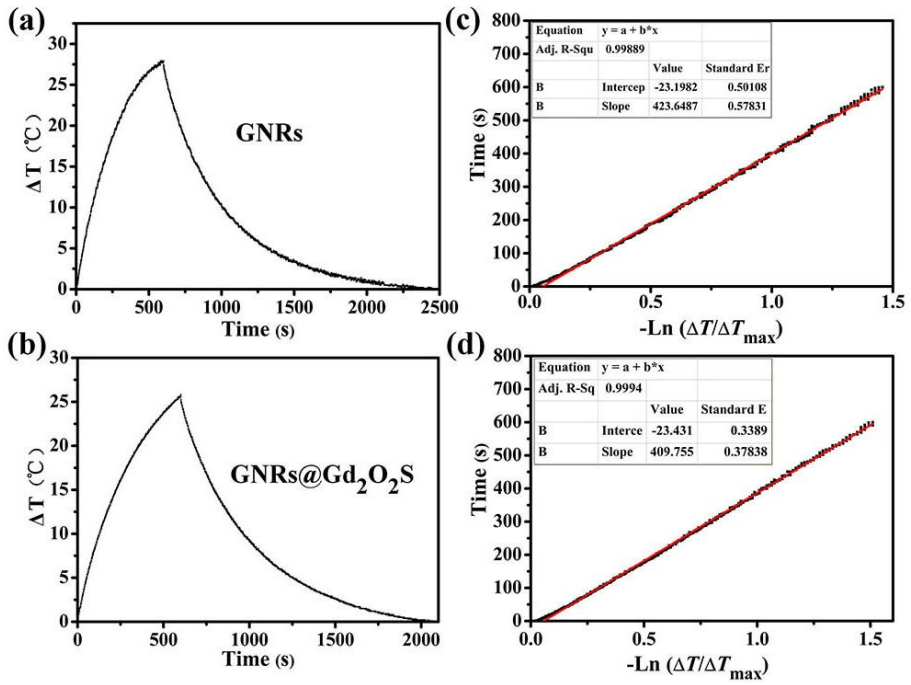

Figure S4. The temperature change curves of (a) GNRs and (b) GNRs@Gd_2O_2S aqueous solutions under 808 nm laser irradiation for 10 min, respectively. The linear fitting of laser irradiation times versus -Ln($\Delta T/\Delta T_{max}$) obtained from the cooling period for (c) GNRs and (d) GNRs@Gd_2O_2S, respectively.

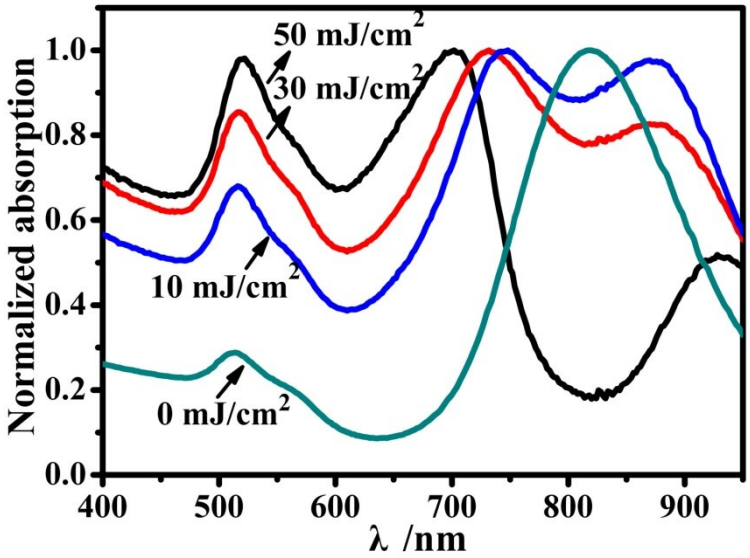

Figure S5. Normalized absorption spectra of GNRs under nanosecond laser irradiation (7 ns pulse duration, 10 Hz) with different laser fluence.

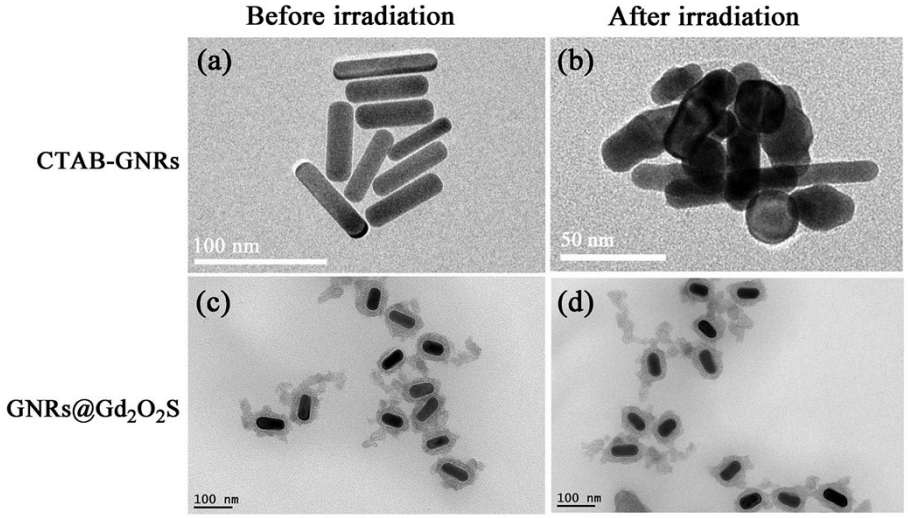

Figure S6. TEM images of the GNRs and GNRs@Gd_2O_2S before and after 50 mJ cm^{-2} laser irradiation.

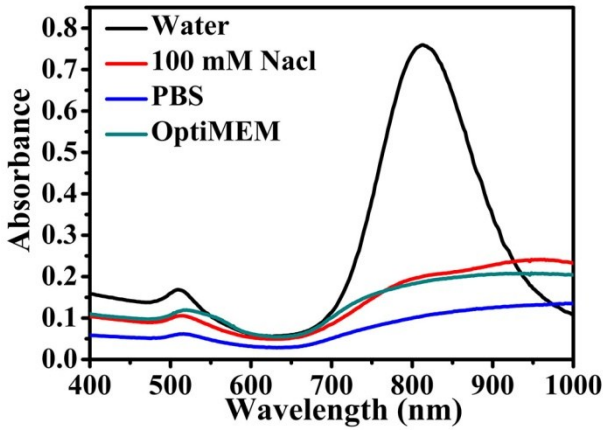

Figure S7. Absorption spectra of GNRs dispersed in water, 100 mM NaCl, PBS, and OptiMEM, respectively.

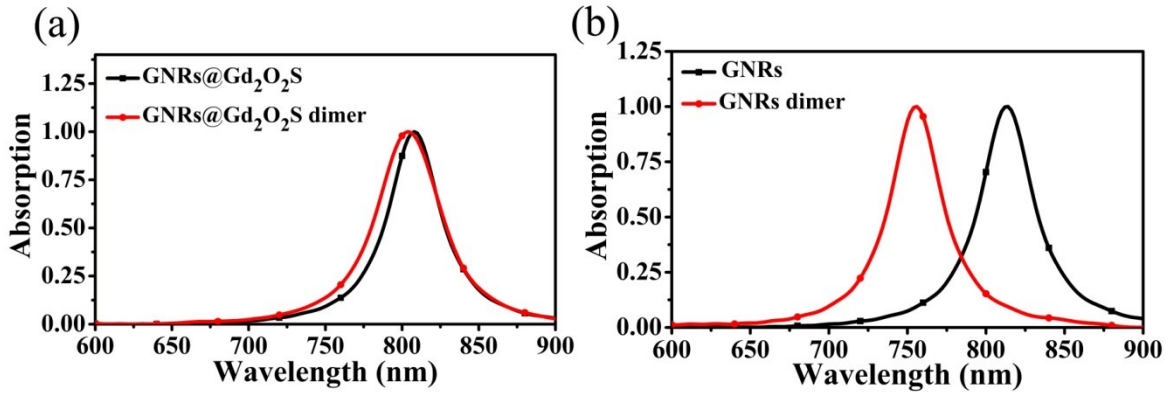

Figure S8. FDTD simulated absorption spectra of (a) GNRs and their SS dimer, (b) GNRs@Gd_2O_2S and their SS dimer, respectively.

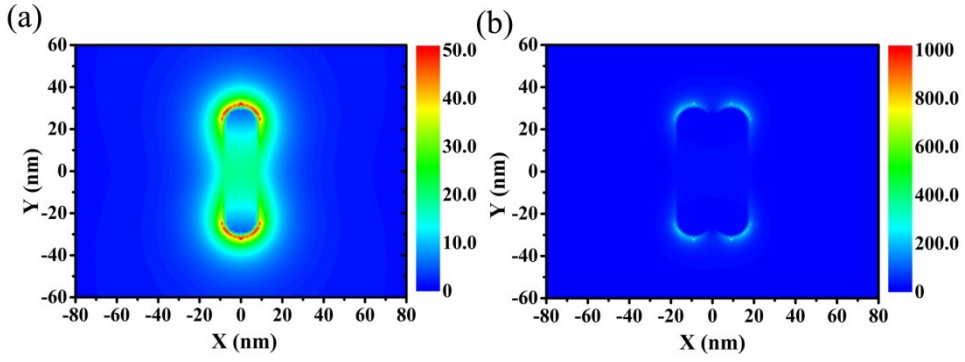

Figure S9. FDTD simulated localized electric field distributions of GNRs and GNRs SS dimer, respectively.

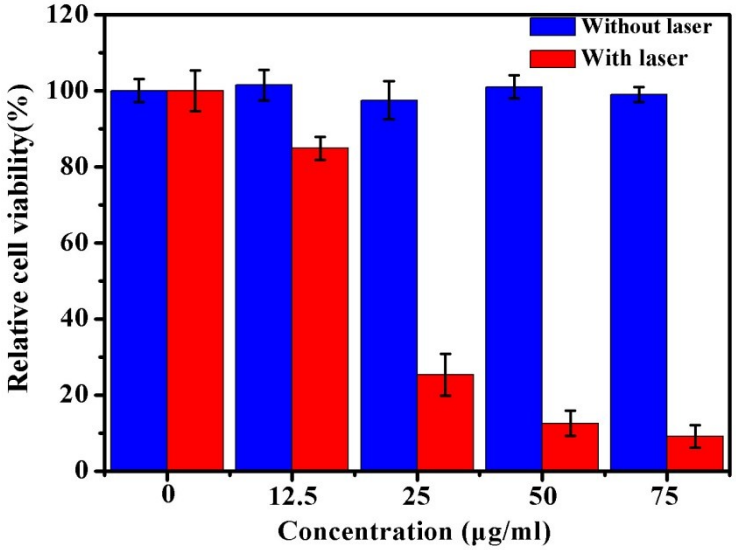

Figure S10. Cell viability of Hep G2 treated with different concentrations of GNRs@Gd_2O_2S with and without laser irradiation (1 W cm^{-2}).

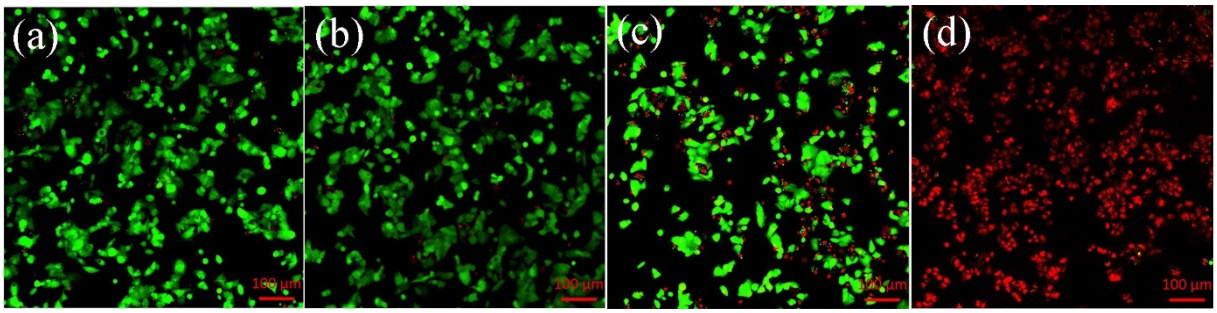

Figure S11. Confocal fluorescence images of calcein-AM/PI co-stained Hep G2 cells with different treatments: (a) 5 min of laser irradiation only; (b) incubated with GNRs@Gd_2O_2S (equal Au concentration 50 μg mL^{-1}) only; incubated with GNRs@Gd_2O_2S (50 μg mL^{-1}) and then treated with (c) 2 min, and (d) 5 min of laser irradiation, respectively.

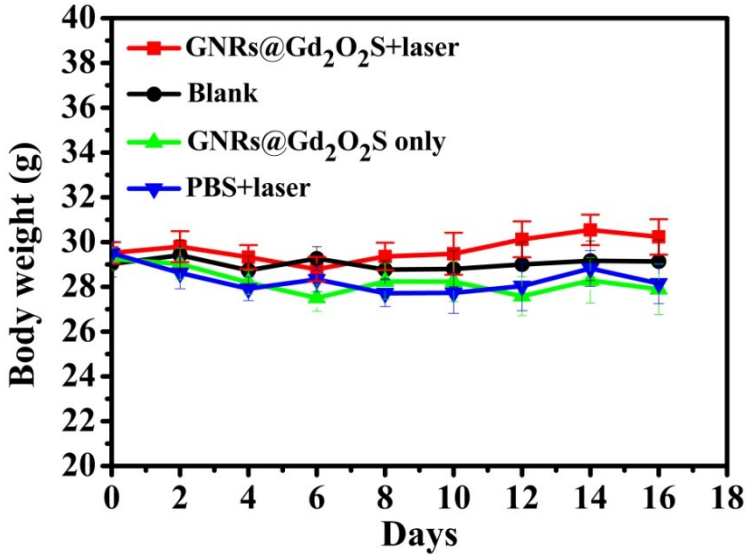

Figure S12. Body weight changes of mice after different treatments.

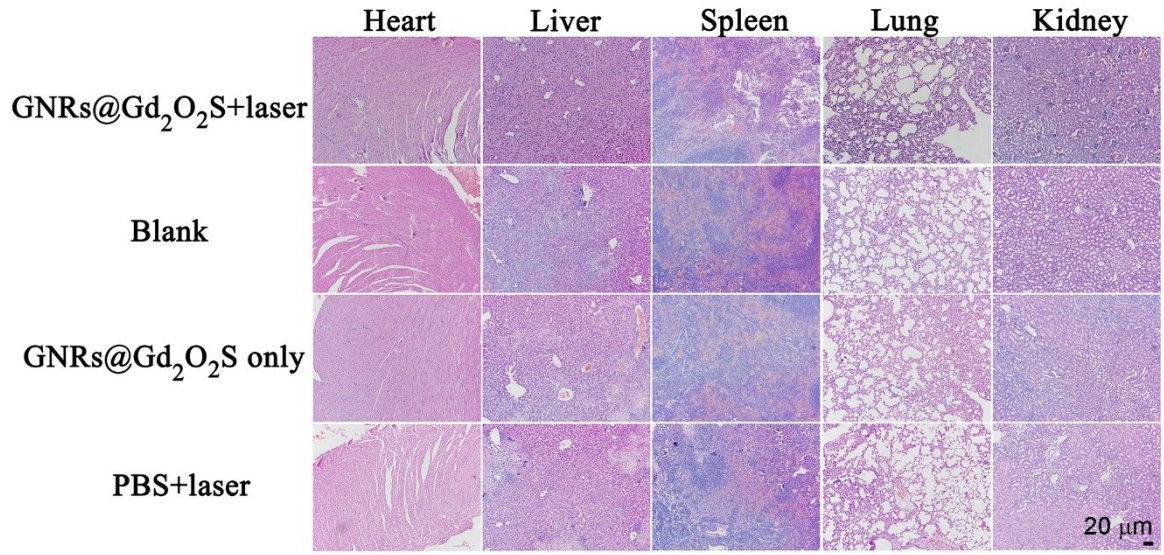

Figure S13. H&E stained images of major organs of mice 16 days after different treatments. All images share the same scale bar.