Supporting Information

Size-Tunable Gd_2O_3@Albumin Nanoparticles Conjugating Chlorin e6 for Magnetic Resonance Imaging-Guided Photo-Induced Therapy

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1. Experimental Section

1.1. Synthesis

In the synthesis of GA-NPs, bovine serum albumin (BSA, 0.25 g) was dissolved in 9.0 mL of de-ionized water, and then 1.0 mL $Gd(NO_3)_3$ solution (100 mM) was slowly added under vigorous stirring. Next, NaOH (2.0 M) was used to adjust pH of the solution to 12, and the mixture was then allowed to react under vigorous stirring at the temperature of 37 °C for 1, 4, 8, or 12 h nanocrystal growth. The mixture was further dialyzed against distilled water for 24 h to remove excess precursors in order to obtain Gd_2O_3@albumin. Next, the carboxylic acid group on Ce6 was conjugated with free amine group in BSA through carbodiimide-catalyzed amide formation, in which EDC (9.6 mg) and NHS (10.9 mg) were added to 4.0 mL Ce6 solution (3.75 mg mL^{-1}) for 1 h. Then, this mixture was added to 10.0 mL solution of Gd_2O_3@albumin, and stirred in the dark for 12 h. Finally, GA-NPs were obtained after the centrifuge ultrafiltration (10k MW, 5500 rpm, 20 min/each) in the solution of citric acid (10.0 mg mL^{-1}) and PBS (pH 7.4, 10 mM) , respectively.

1. 2. Characterization

The morphology of GA-NPs was characterized using a JEM-2100 high-resolution transmission electron microscope (HRTEM, JEOL, Japan) at 200 kV. For scanning electron microscopy imaging, the sample was dried on silicon wafer, and observed using scanning electron microscope (FEI Quanta FEG250). The hydrodynamic size was measured using dynamic light scattering (DLS, Zetasizer ZS90, Malvern). Fourier transform infrared (FT-IR) spectra were recorded on a Magna-560 spectrometer (Nicolet, USA). CD spectrum was performed using a spectrometer (J-810, JASCON CO., LTD, Japan). The concentration of Gd

was measured using an X series inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Elemental, UK). The absorbance and fluorescence spectra were measured using UV-vis spectrophotometer (UV2600, Shimadzu) and fluorescence spectrophotometer (LS 55, PerkinElmer), respectively. NIRF and MR imaging were performed using IVIS Lumina II imaging system and 1.5 T MR imaging system (Philips, Achieva), respectively. For the photostability**,** GA-NPs and free Ce6 (10.0 μg mL^{-1} Ce6, each 0.5 mL) were irradiated at 0.8 W cm^{-2} (660 nm) for 0, 0.5, 1, 2, 4, 6, 8, and 15 min, respectively. The chemical stability of GA-NPs was evaluated in PBS containing 10% fetal bovine serum (FBS) at pH 7.4. The absorbances of Ce6 were measured using UV-vis Spectrophotometer (UV2600, Shimadzu).

1.3. Monitoring of Singlet Oxygen, Singlet Oxygen Quantum Yield, and Photothermal Effect

Free Ce6 and GA-NPs containing various concentrations of Ce6 were dispersed in PBS solutions containing 0.32 μg mL^{-1} DPBF (1.0 mL), and were further irradiated at 0.8 W cm^{-2} (660 nm) for singlet oxygen measurement. The fluorescent spectrum was scanned from 430 to 550 nm using an excitation wavelength of 403 nm. Then, the fluorescence intensity at 485 nm was recorded. The solution of DPBF without Ce6 in darkness was used as the control. To evaluate the photothermal effect of GA-NPs, the solutions (0.5 mL) were irradiated at the concentrations of 0.5, 1, 2, 5, and 10 μg mL^{-1} Ce6 using a 660 nm laser (0.8 W cm^{-2}, 3 min). Then, the thermometer was submerged in the solutions for monitoring the temperature at an interval of 30 s.

To measure singlet oxygen quantum yield (Φ_Δ), DPBF was used as a probe and ZnPc acted as a reference agent (Φ_Δ^{ZnPc}=0.56 in DMF). The solutions of GA-NPs and ZnPc containing DPBF at the concentration of 0.32 μg mL^{-1} were irradiated under 660 nm (0.8 W cm^{-2}) for 120 s. The absorbance of DPBF at 415 nm was observed in 120 s. Φ_Δ was obtained

according to the equation of $\Phi_{\Delta}= (\Phi_{\Delta}^{ZnPc} \cdot W \cdot I^{ZnPc}) / (W^{ZnPc} \cdot I)$, where I and I^{ZnPc} are the rates of light absorption of GA-NPs and ZnPc, respectively. W and W^{ZnPc} are the DPBF photo-bleaching rates in the presences of GA-NPs and ZnPc, respectively.

1.4. Cellular Uptakes, DHE Staining, and AO Staining

4T1 cells were seeded in the plates (3×10^5 cells/well) and incubated overnight in RPMI 1640 containing 10% FBS. GA-NPs and free Ce6 (4.0 μg mL^{-1} Ce6) were added to the wells, respectively. After 6 and 24 h incubation, the cells were washed 3 times, and incubated with 0.5 mL trypsin for 3 min, followed by the centrifuge for cell counting. The counted cells were disrupted under ultrasonication, and then Ce6 was extracted from the cells using methanol as solvent, and further measured using UV-vis analysis.

Reactive oxygen species (ROS) formation was monitored by fluorescence microscopy using dihydroethidium (DHE) as the indicator. Briefly, 4T1 cells were seeded on 24-well plates at the density of 8×10^4 each well and incubated with PBS and GA-NPs at the concentrations of 0.1, 0.2, and 0.4 μg mL^{-1} for 6 h. After the cells were washed 3 times using PBS, 0.2 mL DHE solution (5 μM, PBS) was added into the cells for further 30 min incubation at 37 ºC, followed by 5 min irradiation (660 nm, 0.8 W cm^{-2}) or not. Then, the cells were washed using PBS, and observed using an optical microscope with the excitation of green light.

To evaluate the disruption of lysosomal membranes, AO was utilized as an intracellular indicator of acidic organelle integrity in 4T1 cells. When normal cells were excited with blue light, AO in lysosomes emits an intense red fluorescence, and AO in nuclei and cytosol exhibits green fluorescence. When the acidic compartments such as the lysosomes are disrupted, the red fluorescence from AO disappears and only green fluorescence can be observed. In this study, 4T1 cells were seeded overnight on glass slides in 35-mm dishes, and

treated with PBS, free Ce6, and GA-NPs at the concentrations of 0.1, 0.2, and 0.4 μg mL^{-1} for 6 h, respectively. Then, the cells were incubated in fresh medium, followed by 5 min irradiation at 660 nm, 0.8 W cm^{-2}. After 1 h, the cells were washed using PBS and further incubated with 6 mM AO (1.0 mL) for 15 min. The cells were washed 3 times using PBS, followed by fluorescent microscopy observation (IX 51, Olympus) with the excitation wavelength of 488 nm, and emission wavelengths from 515 to 545 nm (green) and from 610 to 640 nm (red).

1.5. Photocytotoxicity

For photod-induced cytotoxicity, 4T1 cells were incubated with GA-NPs and free Ce6 at various concentrations including 0.1, 0.5, 1.0, 2.0, and 4.0 μg mL^{-1} Ce6 for 24 h treated with or without 1.0 mM Vc, respectively, and then the cells were washed using PBS, followed by 5 min irradiation (660 nm, 0.8 W cm^{-2}). After 24 h, the cell viability was evaluated using MTT assay. Moreover, to investigate the photo-induced cytoxicity under 0.15 W cm^{-2}, 4T1 cells were incubated with GA-NPs and free Ce6 at various concentrations including 0.1, 0.5, 1.0, 2.0, and 4.0 μg mL^{-1} Ce6 for 24 h, respectively, followed by 25 min irradiation (660 nm, 0.15 W cm^{-2}). After 24 h, the cell viability was evaluated using MTT assay.

1.6. Biodistribution

GA-NPs with various size were administrated into the mice bearing 4T1 tumors *via* tail vein, which were sacrificed at 6, 12, and 24 h post-injection for extracting heart, liver, spleen, lung, kidney and tumor, respectively,. Then, each tissue was further treated with 2.0 mL nitric acid overnight. After all the tissues were digested in the solutions, the concentrations of Gd

were measured using ICP-MS. Then, the distributions of Gd from GA-NPs at various tissues were calculated.

1.7. *In Vivo* MRI.

For *in vivo* MR imaging, GA-NPs with different size and Gd-DPTA (0.5 mmol Gd kg^{-1}, which is equal to 5 mg kg^{-1} Ce6) were injected into the mice bearing 4T1 tumor *via* tail vein. The T_1-weight images were obtained at different time using a 1.5 T MR imaging system (Philips, Achieva) with a fat-saturated 3D gradient echo imaging sequence (TR/TE = 400/10 ms, 256×256 matrices, slices = 3, thickness = 2 mm, averages = 4, FOV = 60×60). Also, MRI signals at the tumor and muscle were calculated, respectively.

1.8. *In Vivo* Infrared Thermography, and *In Vivo* Anticancer Efficacy.

For *in vivo* infrared thermography, GA-NPs were injected into the mice bearing 4T1 tumor at the doses of 5 mg kg^{-1} Ce6. The tumors were irradiated for 5 min using a 660 nm laser (0.8 W cm^{-2}) at 24 h post-injection, and simultaneously the imaging was performed using an infrared camera (Fotric 225) during the irradiation. To evaluate the photo-induced efficacy of GA-NPs, 4T1 cells (2×10^6 cells/mouse) were subcutaneously transplanted into the flanks of female mice. When the tumors reached a size of ~60 mm^3, PBS, free Ce6 and GA-NPs were injected intravenously into the mice at a single dose of 5 mg kg^{-1} Ce6, respectively. Subsequently, the tumors suffered from 660 nm irradiation (5 min, 0.8 W cm^{-2}) at 24 h post-injection. For further evaluate the anticancer efficacy from the sole PTT, Vc was administrated into the tumor *via* intratumor injection before irradiation, followed by the same irradiation. Then, the tumor volumes were monitored everyday. The tumor volume (V) was calculated as follows: $V= L \times W^2/2$ (W is the size at the widest point, and L is the size at the

longest point). Finally, the tumors were detached from the mice at 12 days post-irradiation under anesthetic status for taking photos.

1.9. *Ex Vivo* Haematoxylin and Eosin (H&E) Staining.

PBS, free Ce6 and GA-NPs were intravenously injected into the mice bearing 4T1 tumor (~60 mm^3) at a single dose of 5 mg kg^{-1} Ce6, respectively. Then, the tumors suffered from 660 nm irradiation (5 min, 0.8 W cm^{-2}) at 24 h post-injection. The tumor, heart, liver, spleen, lung, and kidney were dissected from the mice at 6 h post-irradiation, fixed in a 4% formaldehyde solution for 24 h at room temperature, and frozen. Finally, the sections of 10 μm thickness were made in a cryostat, followed by the H&E staining (BBC Biochemical, WA). Finally, the sections were observed using an IX73 bright field microscopy (Olympus).

2. Supporting figures

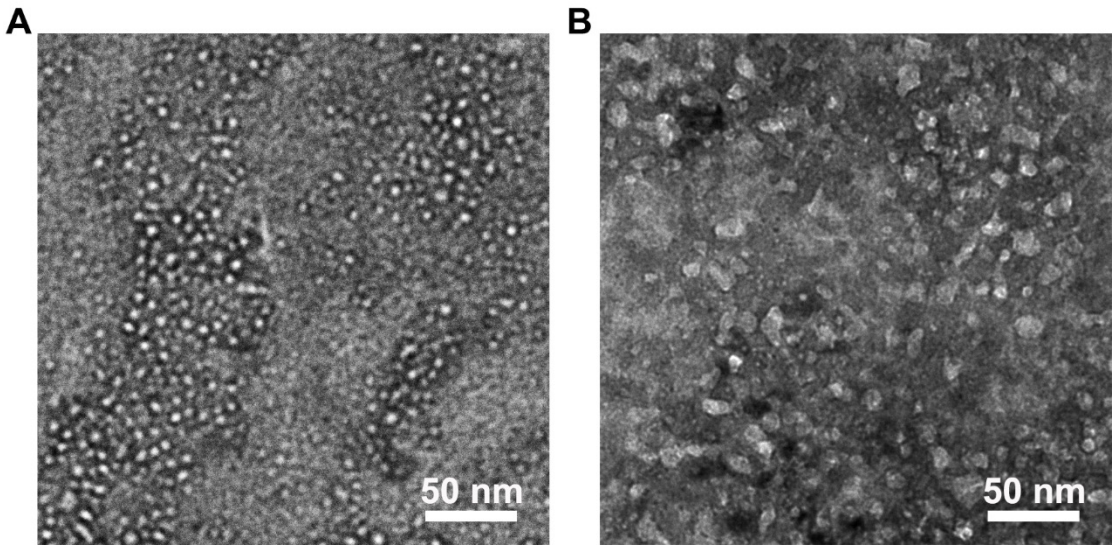

Figure S1. TEM images of free BSA at pH 7.4 (A, 6.5 ± 0.8 nm) and pH 12 (B, 12.0 ± 2.1 nm) negatively stained with 3% phosphomolybdic acid, respectively.

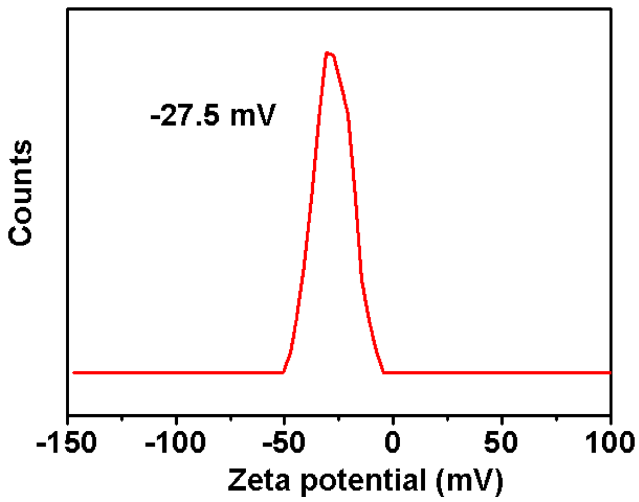

Figure S2. Zeta potential of 10.1 nm GA-NPs.

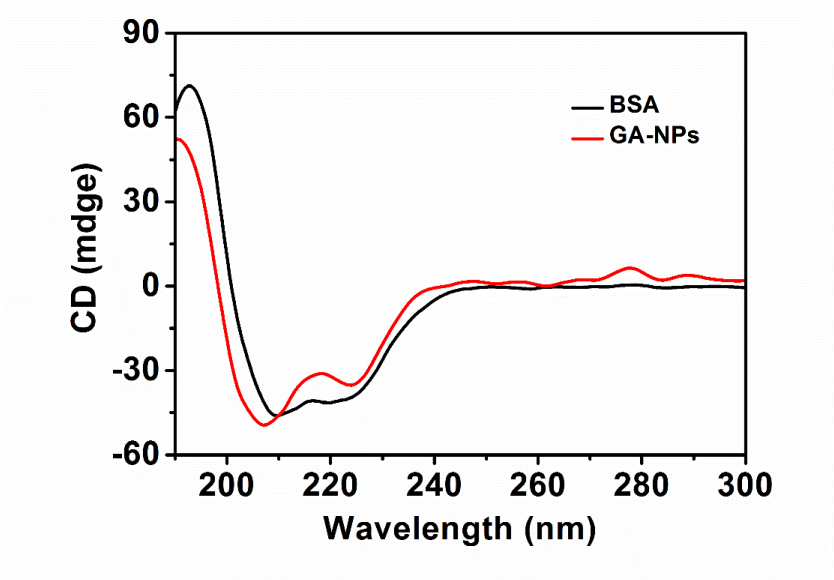

Figure S3. Circular dichroism spectra of BSA and 10.1 nm GA-NPs.

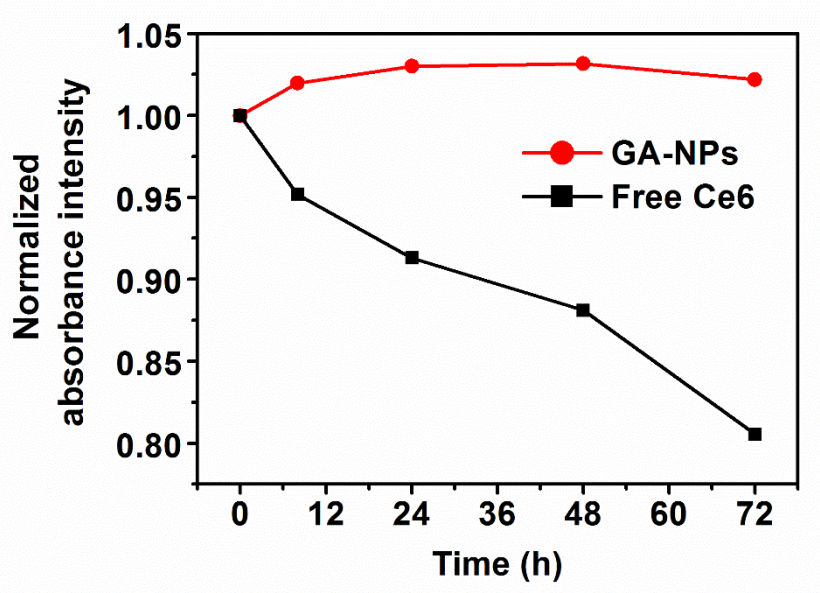

Figure S4. Chemical stability of 10.1 nm GA-NPs and free Ce6 in PBS containing 10% fetal bovine serum (FBS) at pH 7.4.

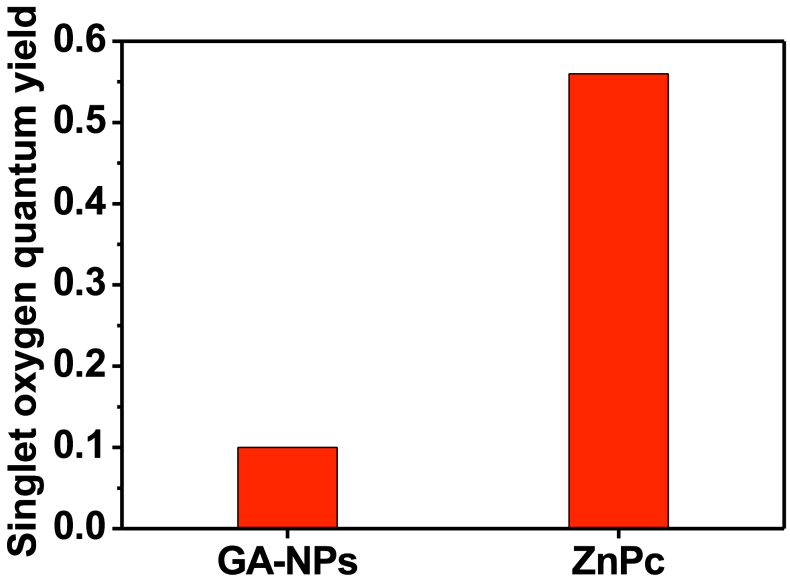

Figure S5. Singlet oxygen quantum yield of GA-NPs in aqueous solution using ZnPc in dimethyl formamide as a reference.

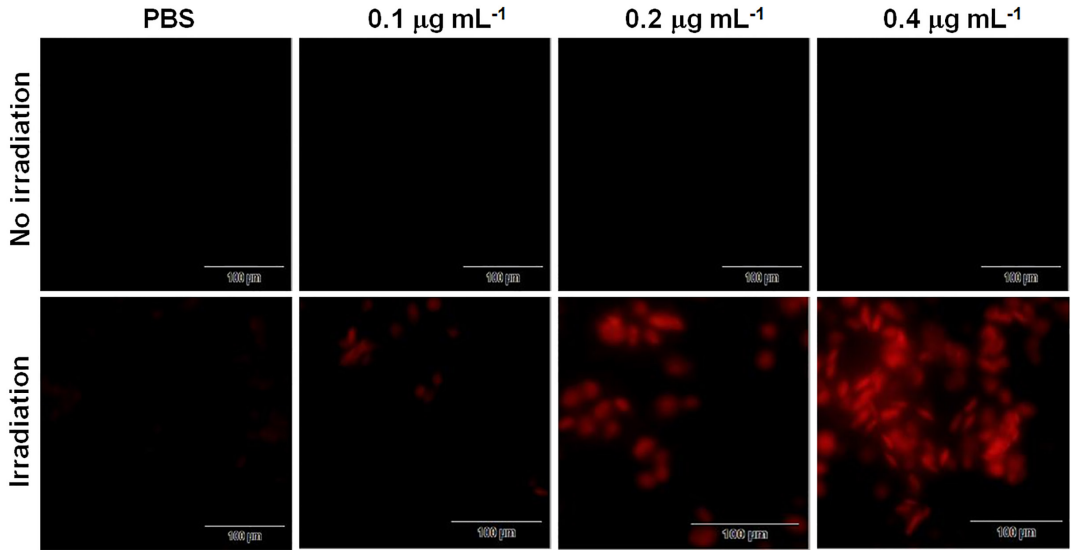

Figure S6. Observation of intracellular singlet oxygen level of 4T1 cells treated with 10.1 nm GA-NPs at various concentrations of Ce6 using DHE staining in the absence and presence of 5 min irradiation (660 nm, 0.8 W cm^{-2}).

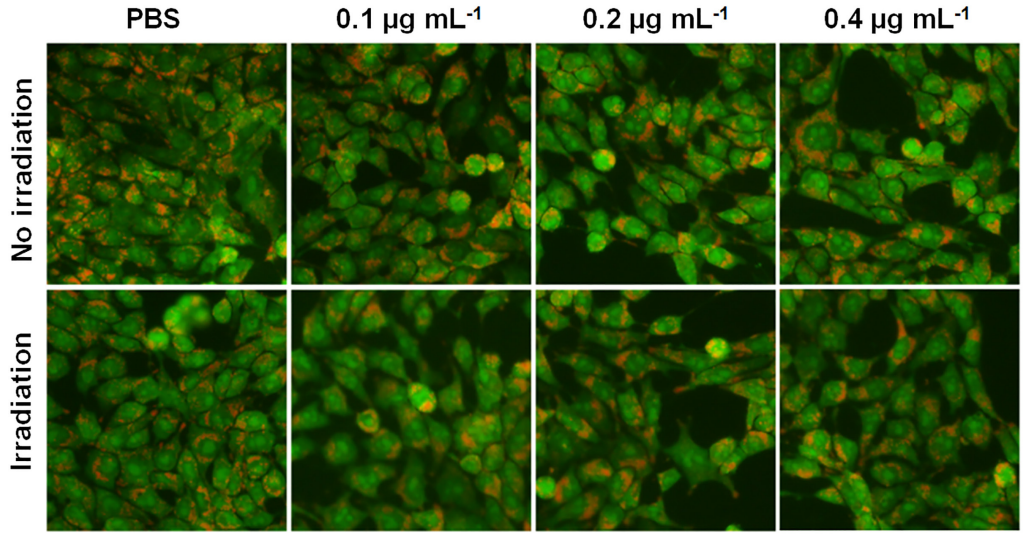

Figure S7. Observation of lysosomal disruption of 4T1 cells treated with free Ce6 at various concentrations using AO staining with or without 5 min irradiation (660 nm, 0.8 W cm^{-2}).

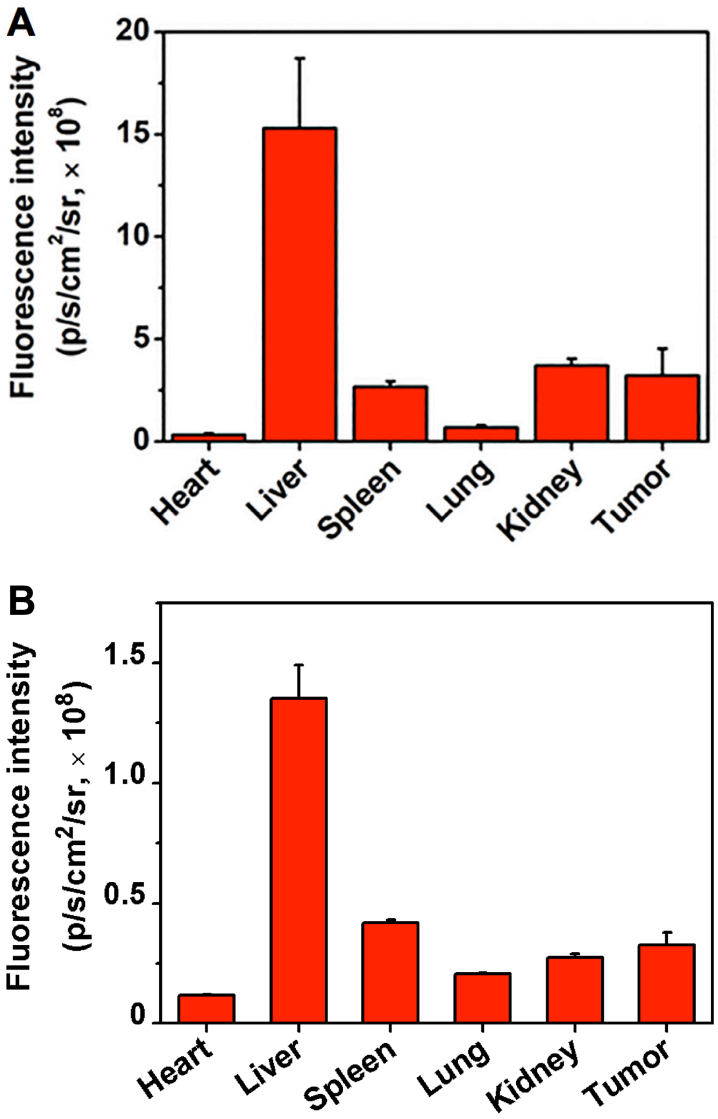

Figure S8. *Ex vivo* distribution of Ce6 at various tissues of the mice treated with 10.1 nm GA-NPs (A) and free Ce6 (B) at the dose of 5 mg kg^{-1} at 24 h post-injection.

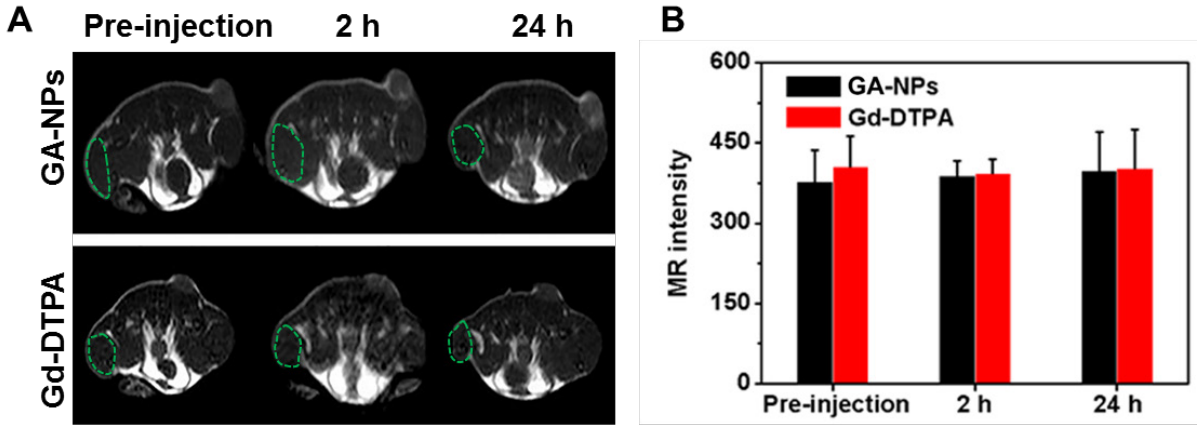

Figure S9. A) *In vivo* MRI and B) signal intensities at the muscles of the mice bearing 4T1 tumor injected with Gd-DTPA and 10.1 nm GA-NPs (0.5 mmol Gd kg^{-1}) at pre-injection, 2 h, and 24 h post-injection, respectively. The muscle is highlighted using green circle.

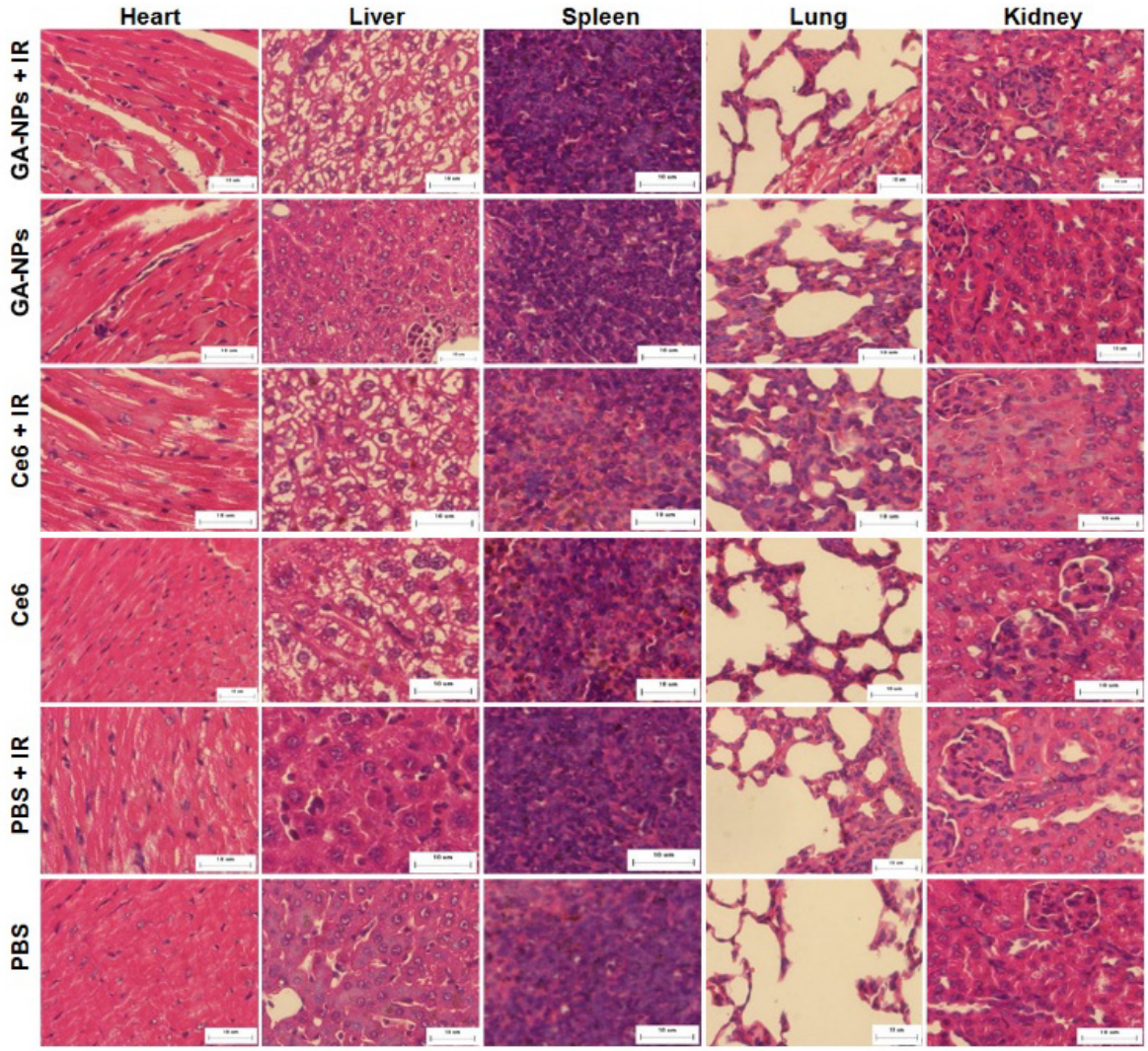

Figure S10. Hematoxylin and Eosin (H&E) staining of normal tissues such as heart, liver, spleen, lung, and kidney from the tumors of the mice treated with 10.1 nm GA-NPs at a single dose of 5 mg kg^{-1} Ce6, followed by 660 nm irradiation (IR) at tumor or not (The bar is 10 μm).