HA targeted-biodegradable nanocomposites responsive to endogenous and exogenous stimulation for multimodal imaging and chemo-/photothermal therapy

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Experimental section

Materials and reagents. Trisodium citrate dehydrate (99%), diethylenetriamine pentaacetic acid (DTPA, 99%), N-(2-aminoethyl-3-aminopropy) trimethoxysilane (DAMO, 97%), tetraethoxysilicane (TEOS, 98%), indocyanine green (ICG, 90%), L-glutathione (GSH) and dioctyl sodium sulfosuccinate (AOT) were obtained from J&K Scientific Ltd (Beijing, China). Gadolinium (Ⅲ) chloride hexahydrate ($GdCl_3 \cdot 6H_2O$, 99%), cetyltrimethylammonium chloride (CTAC, 97%), polyoxyethylene lauryl ether (Brij 30) and triethanolamine (TEA，98%) were purchased from Aladdin Bio-chemical Technology Co., Ltd (Shanghai, China). Bis[3-(triethoxysilyl)propyl]tetrasulfide (BTES, 98%), and hyaluronic acid (HA) were purchased from Heowns Biochem Technologies LLC. (Tianjin, China). Doxorubicin hydrochloride (DOX·HCl, ⩾98%) was obtained from Huafeng United Technology Co., Ltd (Beijing, China). Fetal bovine serum (FBS), and DMEM medium were purchased from Beijing solarbio science &technology Co., Ltd (Beijing, China). The calcein-AM and propidium iodide (PI) kit was purchased from Meilun Biotechnology Co., Ltd (Dalian, China). The 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazole monosodium salt (CCK-8) kit was obtained from MedChemExpress LLC. (Shanghai, China). There was no additional purification of all reagents.

Apparatus. The transmission electron microscopy (TEM) (HITACHI, Japan), and the JEM-2800 high resolution transmission electron microscopy (HRTEM) (JEOL, Japan)

were employed to observe the morphology of materials. The contents of Gd elements in the Si_{Gd}NPs and HIDSi_{Gd}NPs@PDA-HA were measured on an inductively coupled plasma emission spectrometer (ICP-AES) (Thermo, USA). N_2 adsorption-desorption isotherms were obtained on an ASAP 2460 specific surface area and microporous physical adsorption analyzer (Micromeritics, USA). The Brookhaven Instruments Corporation Zeta PALS (USA) was used to measured zeta potential. Thermo gravimetric analysis (TGA) was performed on a DSC8231/TG8121 thermogravimetric analyzer (HITACHI, Japan). The UV-visible (UV-vis) absorption spectra were performed on a UV-2450 spectrophotometer (Shimadzu, Japan), and the photoluminescence (PL) spectra were obtained on a fluorescence spectrophotometer (F-4500, Hitachi, Japan). An FLS-920 spectrophotometer (Edinburgh, UK) was adopted to measure the fluorescence lifetime decay curve with exciting at 405 nm. A microplate reader (Sunrise, Austrla) was employed to detect the absorbance in the MTT assay. The confocal fluorescence images were gained on a confocal laser scanning microscopy (NIKON, A1+, JP). The imaging of mice was performed on a nuclear magnetic resonance imaging research system (HT/MRSI60-50A, China) and some samples were measured on a nuclear magnetic resonance imaging research system (HT/MRSI60-35A, China).

Cell culture and cytotoxicity assay. HeLa cells or MCF-7 cells were cultured in DMEM (high glucose) containing 10% fetal bovine serum (FBS) in a CO_2 incubator (5% CO_2, 37℃). HK-2 cells were cultured in RPMI-1640 culture media containing 10%

FBS. The cell toxicity of various therapeutic reagents was assessed by CCK-8 kit. Typically, HeLa cells, MCF-7 cells and HK-2 cells were seeded in 96-well plates with a density of 5×10^4 cell/well for 12 h. Then, different concentrations of HMONs (0, 50, 100, 150, 200, 300 and 500 mg mL^{-1}) were added to wells and co-cultured with cells for another 24 h. Subsequently, the medium was moved, and the cells were washed with PBS and treated with 10 μL of CCK-8 for 1.5 h at 37℃. Finally, the absorption intensity was measured by microplate reader at the wavelength of 450 nm.

To investigate the synergistic therapy, the HeLa cells, MCF-7 cells and HK-2 cells were incubated with $HIDSi_{Gd}NPs$@PDA, HIDSiGdNPs@PDA-HA (0, 12.5, 25, 50, 75, 100 mg mL^{-1}), DOX (0, 0.75, 1.5, 3, 4.5, 6 μg mL^{-1}) and ICG (0, 0.5, 1, 2, 3, 4 μg mL^{-1}) for 24 h. For irradiation group, the cells were irradiated with 808 nm NIR laser (1.5 W cm^{-2}) for 5 min. After incubation for another 12 h, the cell viabilities were measured by CCK-8 kit.

Calcein-AM and propidium iodide (PI) staining tests were performed to further confirm the chemo-/photothermal collaborative therapy. HeLa cells were seeded into the 35 mm confocal dishes and incubated for 12 h. Then, HIDSiGdNPs@PDA (20 μg mL^{-1}), $HIDSi_{Gd}NPs$@PDA-HA (20 μg mL^{-1}), DOX (1.2 μg mL^{-1}, equivalent to the loaded amount of $HIDSi_{Gd}NPs$@PDA-HA) and ICG (0.8 μg mL^{-1}, equivalent to the loaded amount of $HIDSi_{Gd}NPs$@PDA-HA) were added into the dishes, respectively, and the cells were cultured for 24 h. For PTT group, the HeLa cells were irradiated with 808 nm NIR laser (1.5 W cm^{-2}) for 5 min. After 12 h, all cells were washed with

PBS, stained with Calcein-AM/PI, and imaged by confocal laser scanning microscope (CLSM).

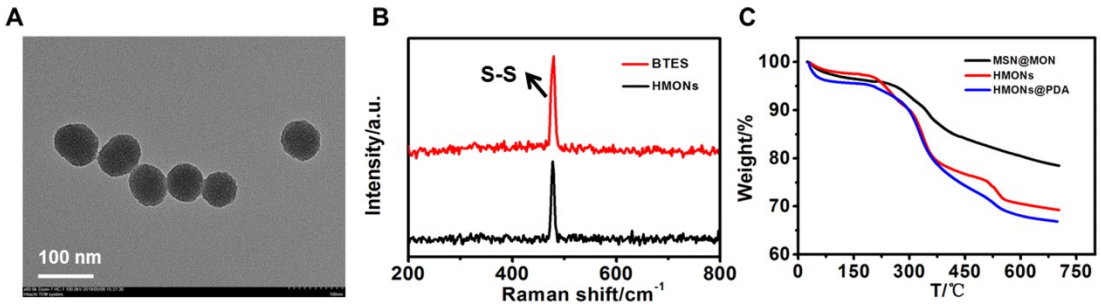

Fig. S1 (A) TEM of MSN@MON. (B) Raman spectra of BTES (red line) and HMONs (black line). (C) TGA curves of MSN@MON (black line), HMONs (red line), and HMONs@PDA (blue line).

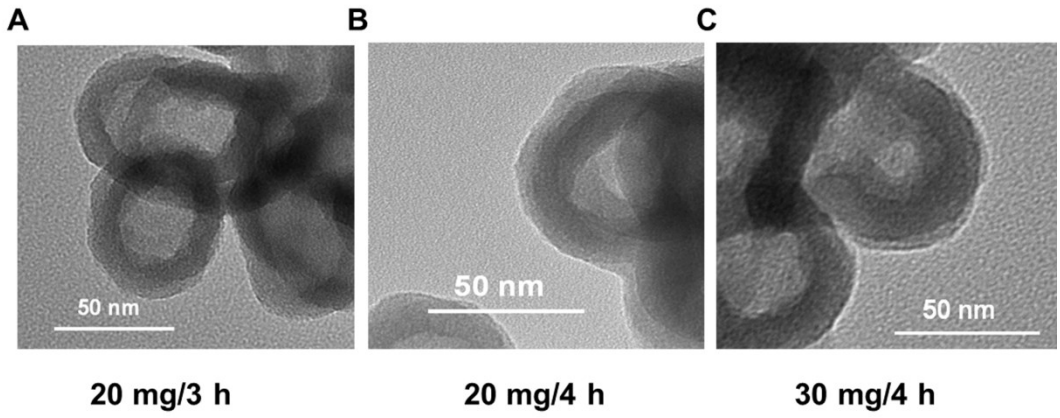

Fig. S2 EM of HIDSi$_{Gd}$NPs@PDA in different reaction time (t) and different reaction amount (Q) of DA. (A) t=3 h, Q=20 mg. (B) t=4 h, Q=20 mg. (C) t=4 h, Q=30 mg.

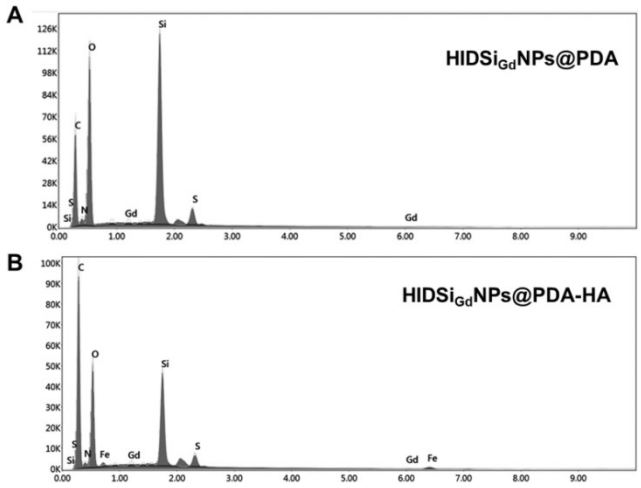

Fig. S3 nergy-dispersive X-ray spectroscopy spectrum (EDS) of HIDSi$_{Gd}$NPs@PDA and HIDSi$_{Gd}$NPs@PDA-HA.

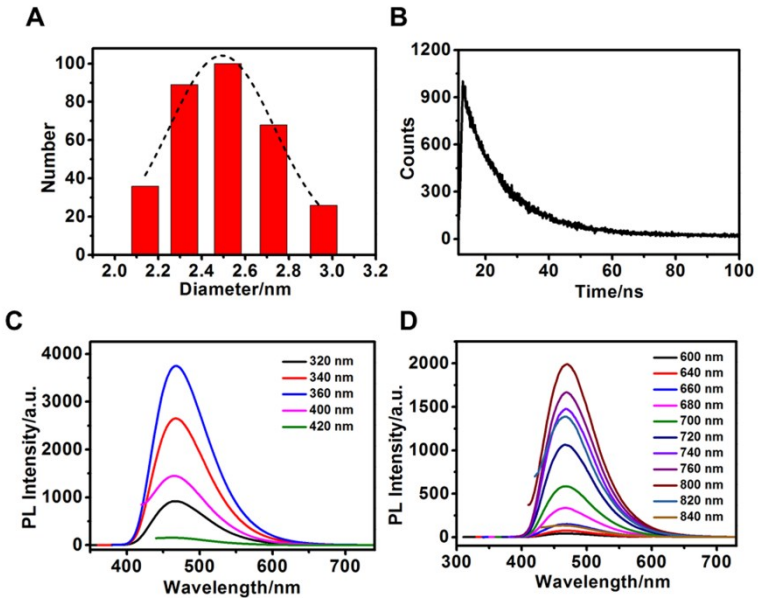

Fig. S4 Properties of the Si$_{Gd}$NPs aqueous solution. (A) Dynamic light scattering (DLS) characterization. (B) The fluorescence lifetime spectrum. (C) The excitation wavelength-independent emission spectra with the excitation wavelength changed from 320 to 420 nm. (D) Up-conversion fluorescence spectra with the excitation wavelength ranged from 600 to 840 nm.

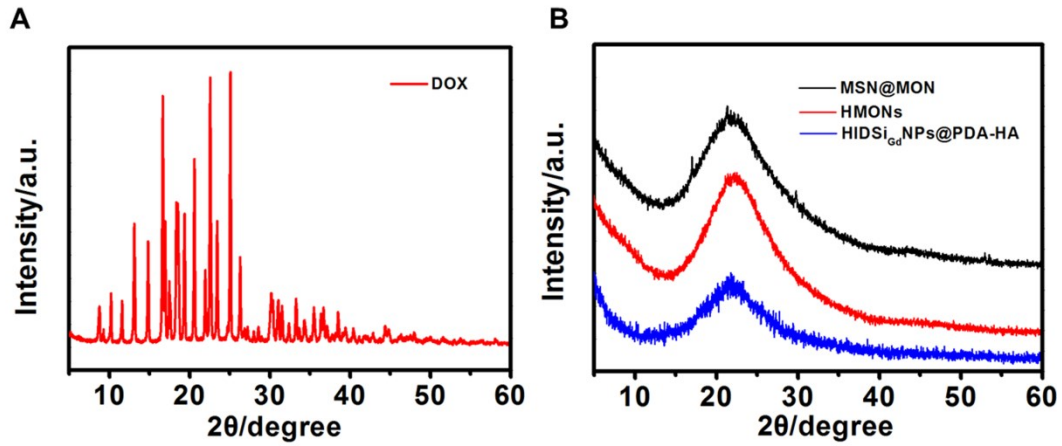

Fig. S5 XRD of (A) DOX, and (B) MSN@MON (black line), HMONs (red line), and HIDSi$_{Gd}$NPs@PDA-HA (blue line).

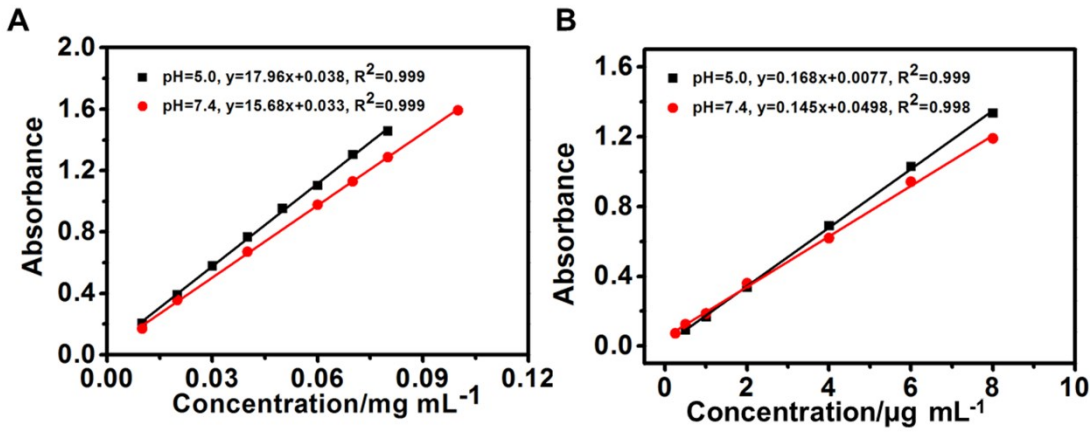

Fig. S6 Calibration curves of DOX (A) and ICG (B) at pH 5.5 and 7.4.

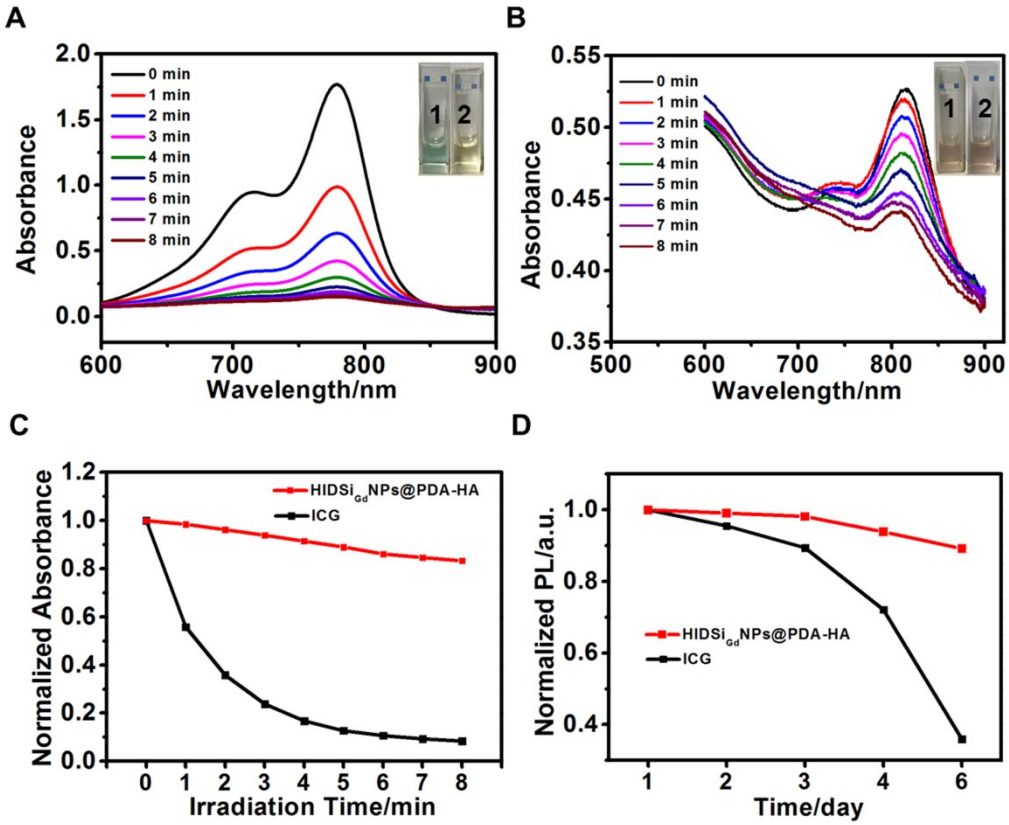

Fig. S7 (A) The UV-vis-NIR absorption spectra of free ICG aqueous solutions irradiated with 808 nm laser for different times. Insets: photographs of ICG solutions before and after the NIR irradiation of 8 min. (B) The UV-vis-NIR absorption spectra of $HIDSi_{Gd}$NPs@PDA-HA aqueous solutions irradiated with 808 nm laser for different times. Insets: photographs of $HIDSi_{Gd}$NPs@PDA-HA solutions before and after the NIR irradiation of 8 min. (C) Normalized maximum absorbance of $HIDSi_{Gd}$NPs@PDA-HA and free ICG solutions irradiated with 808 nm laser for different times based on (A) and (B). (D) FL stability of ICG from $HIDSi_{Gd}$NPs@PDA-HA and free ICG.

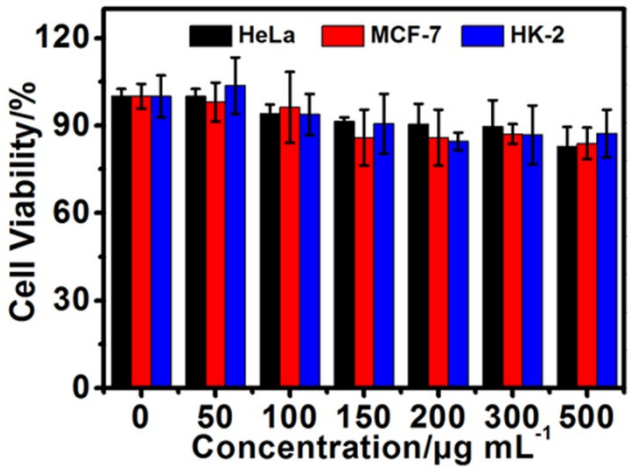

Fig. S8 Cell viability of HeLa, MCF-7 and HK-2 cells incubated with different concentrations of HMONs.

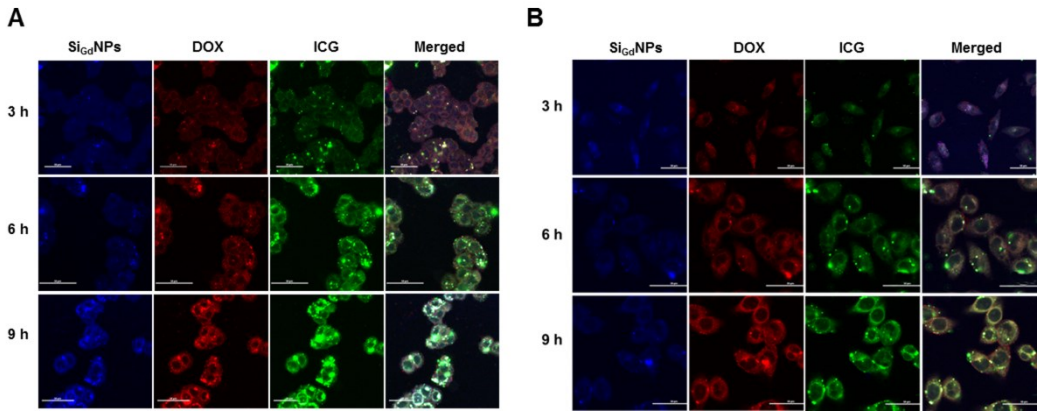

Fig. S9 Fluorescence images of (A) HeLa cells, and (B) MCF-7 cells after incubation with HIDSi$_{Gd}$NPs@PDA-HA for 3 h, 6 h, and 9 h.

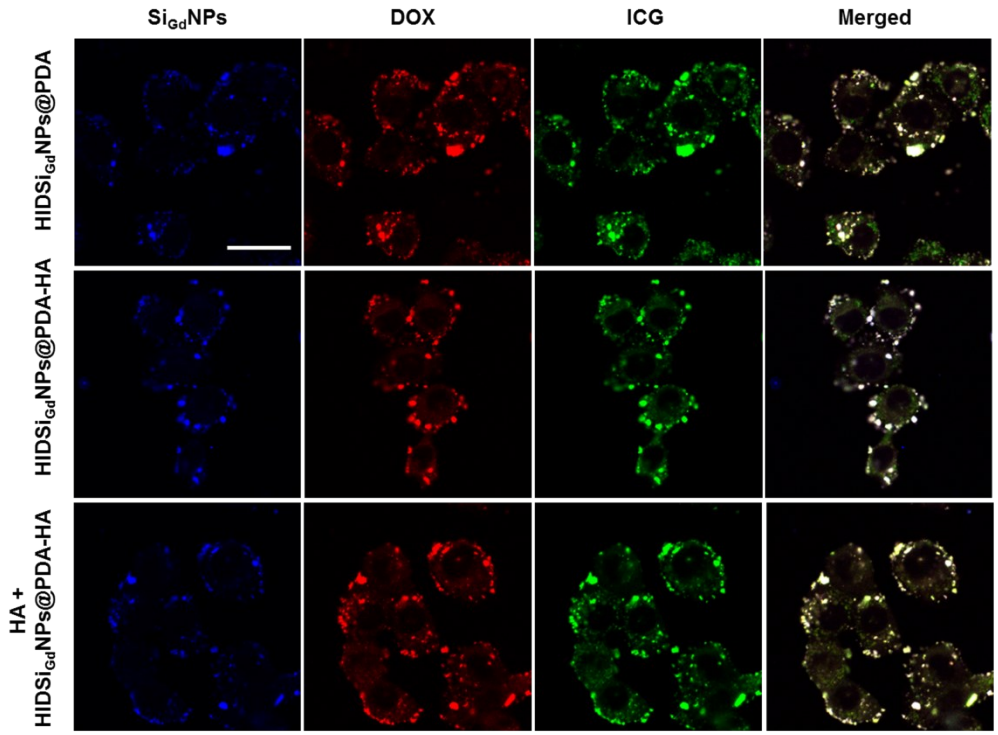

Fig. S10 Fluorescence images of HK-2 cells after incubation with HIDSi$_{Gd}$NPs@PDA, HIDSi$_{Gd}$NPs@PDA-HA, and free HA+HIDSi$_{Gd}$NPs@PDA-HA, respectively. Scale bar: 50 μm.

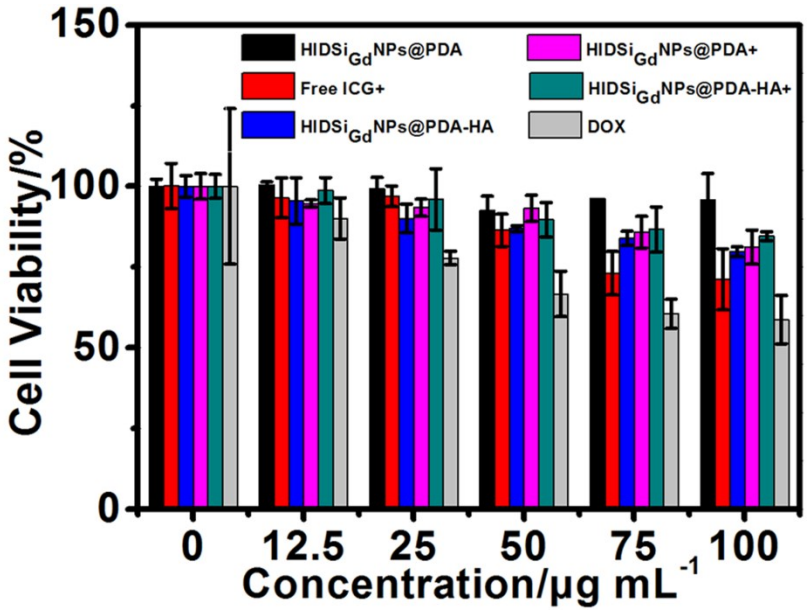

Fig. S11 Cell viability of HK-2 cells with different treatments (1.5 W cm^{-2} of 808 nm NIR laser, 5min).

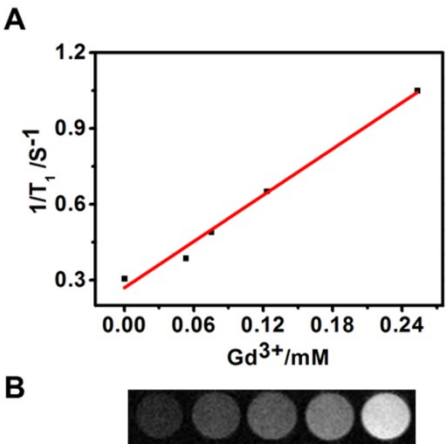

Fig. S12 The r_1 relaxivity curve of Si_{Gd}NPs. (B) T_1-weighted MR images of Si_{Gd}NPs containing different concentrations of Gd^{3+} (0 mM, 0.053 mM, 0.075 mM, 0.123 mM, 0.253 mM).

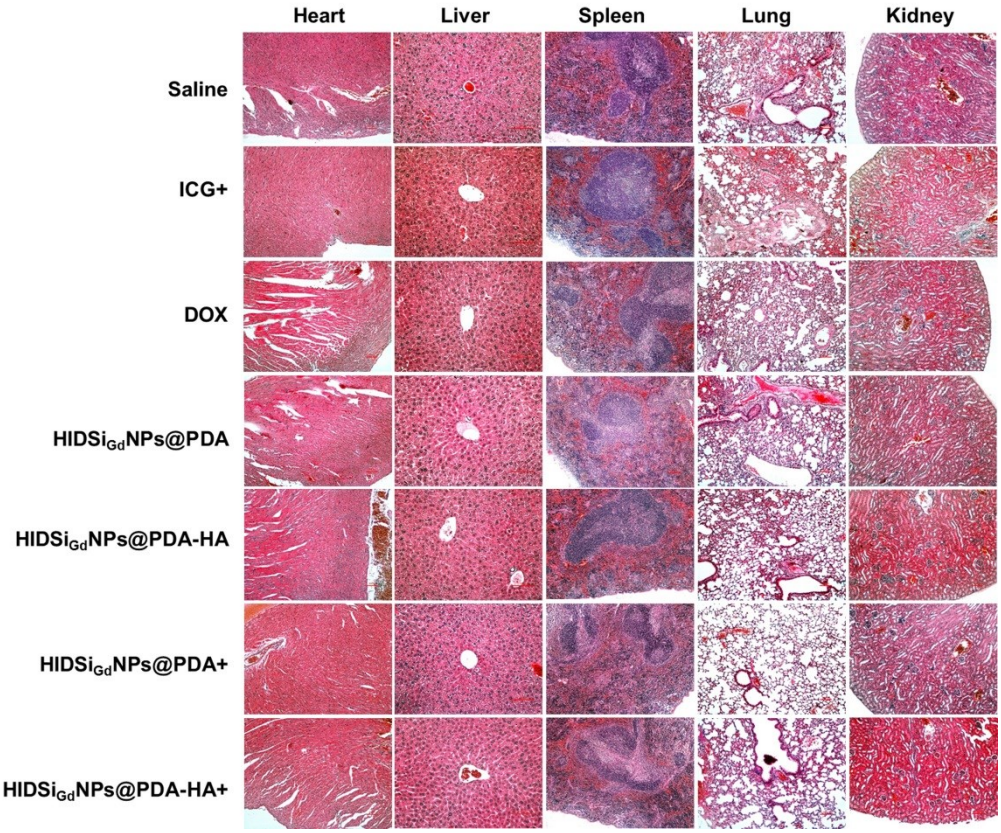

Fig. S13 Images of H&E-stained sections of the heart, liver, spleen, lung and kidney from the different groups after treatment.