Supporting Information

Direct Water-phase Synthesis of Lead Sulphide Quantum Dots Encapsulated by β-Lactoglobulin for *in vivo* Second Near Infrared Window Imaging with Reduced Toxicity

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Experimental Section

Chemical Reagents

Bovine milk β-lactoglobulin (LG), $Pb(CH_3COO)_2$, sodium hydroxide (NaOH) and Na_2S were purchased from Sigma-Aldrich. All chemicals were used as received without further purification. Ultrapure water with a resistivity of ~18.2 $m\Omega \cdot cm^{-1}$ was used as the solvent throughout the experiments.

LG-PbS QDs synthesis

All the solutions were freshly prepared prior to the synthesis. For a typical synthesis, 500 μL of 50 mg mL^{-1} LG protein was mixed with 500 μL of 10 mM $Pb(CH_3COO)_2$ and then incubated at room temperature for 5 min. The pH of the solution was then adjusted to 7.5 using 1 M NaOH, and 300 μL of 10 mM Na_2S was quickly injected into the solution, which was heated to 100°C for 30s under microwave irradiation in the microwave system (Discover, CEM, USA).

Characterizations of LG-PbS QDs

The absorption spectrum of LG-PbS QDs was recorded on an Agilent 8453 UV-Vis spectrometer. The emission spectrum of LG-PbS QDs was measured using a FluoroLog-3 fluorometer conjugated to an InGaAs infrared detector with corrections. IR-26 was used as the standard for the QY determination. The QY was calculated according to previous reports[1]. TEM and HR-TEM imaging was conducted on a JEOL JEM-2010F field emission TEM with an accelerating voltage of 200 Kv. High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) test was performed in a FEI Tecnai F20 field emission gun TEM/STEM microscope operated at 200 kV and fitted with a Fischione HAADF detector, an 80 mm^2 Oxford Instruments X-Max Silicon Drift Detector (SDD) and a Gatan Orius SC600A CCD camera. Nanocluster aqueous dispersions were drop-cast onto TEM grids coated with ultrathin carbon support (Agar Scientific); samples were dried under an ultraviolet lamp. Dynamic light scattering (DLS) was performed at a fixed scattering angle of 90° on a Brookhaven light scattering system (BI-200SM Laser Light Scattering Goniometer) with a BI-APD detector using a He-Ne laser at 633 nm.

Cell culture

Human embryonic kidney cell line (293T) was cultured in DMEM contained 10% FBS (fetal bovine serum) and antibiotics (50 units/mL penicillin and 50 units/mL streptomycin). The cells were incubated at 37 ºC under a humidified atmosphere containing 5% CO_2.

Cell Proliferation and Viability Measurement

The 293T cells were seeded into 96-well plates at 8×10^3 cells per well in 200 mL of culture medium. After 24 h of incubation, the medium was removed and replaced with another 200 mL of culture medium containing serial dilutions of LG-PbS QDs from 0.25 to 250 μg mL^{-1} for 24 h incubation. 239T cells without the treatment were used as control. Then, 20 μL of 5 mg mL^{-1} MTT assay stock solution in PBS was added to each well. After the cells were incubated for 4 h, the medium containing unreacted MTT was carefully removed. Then, the obtained blue formazan crystals were dissolved in 200 293T L $well^{-1}$ DMSO, and the absorbance was measured in a BioTek Synergy H4 hybrid reader at a wavelength of 490 nm. The blank was subtracted to the measured optical density (OD) values, and the cell viability was expressed as % of the values obtained for the untreated control cells.

Cell viability %=OD_{490nm} Value (sample)/OD_{490nm} Value (control)×100%

Cell Apoptosis and Cycle Assay

Apoptosis and necrosis processes in the 293T cells induced by various concentration of LG-PbS QDs from 0.25 to 250 μg mL^{-1} for 24 h were measured using an apoptosis and necrosis assay kit according to the manufacturer's instructions. In brief, the 293T cells were plated

onto a six-well plate at a density of 2×10^5 cells per well overnight and then treated with various concentration of LG-PbS QDs for 24 h. The cells were harvested, washed twice with PBS, re-suspended in 500 μL of PBS, and finally incubated in anti-annexin V-Fluorescein isothiocyanate (FITC) and propidium iodide (PI).

Tail DNA Assay (Single Cell Gel Electrophoresis)

The comet assay was developed following Guidi et al.'s [2] approach with slight modifications. Eppendorf tubes containing cells were spun at 3,000 rpm for 2 min, and the pellets were re-suspended in low-melting-point (LMP) agarose. Two drops were placed on each 1% normal-melting-point (NMP) agarose-coated slide, a coverslip was placed on top of each gel, and the slides were chilled to allow for cover-slip removal. After storing at 4°C for a few minutes, the slides without coverslips were placed in a chilled lysing solution for 1 h and then immersed in an electrophoresis buffer for 20 min at 4°C. The electrophoresis period lasted 20 min (25 V and 300 mA; 0.75 V/cm), and then the slides were washed in a neutralization buffer (0.4 M Tris; pH 7.5).

Fifty cells for each half slide were scored, and one hundred cells were scored for each replicate treatment (n=4). The means were analyzed through an analysis of variance (ANOVA). Before and after the experiments, an aliquot the exposed and control cells was used to assess the cell viability of the trypan blue dye exclusion technique, which involves mixing 0.4% trypan blue solution with the cell pellet, smearing the mixture on a Bürker chamber, and scoring the white (live) and blue (dead) cells.

In Vivo Imaging

Four to six-week-old nude female mice were obtained from the Experimental Animal Center of the Chinese Academy of Sciences in Shanghai, China. The mice were raised in an animal facility under filtered air conditions (22±2 °C), fed a standard pellet diet and provided with purified water. The study was performed within the Guidelines for the Care and Use of Research Animals. We intravenously injected 200 μL of the LG-PbS QDs at a concentration of 80 nM. During injection and imaging, the mice were anesthetized with isoflurane at various time points after the injection period. NIR-II fluorescence images were collected using a two-dimensional InGaAs array (Photonic Science) for collecting photons in NIR-II. An 808 nm diode laser was used as an excitation light source and filtered by 850 and 1,000 nm short-pass filters. The excitation power density level along the imaging plane was 6 mW cm^{-2}, which is much lower than the safe exposure limit of 329 mW cm^{-2} at 808 nm outlined by the International Commission on Nonionizing Radiation Protection. The light emitted from the animal was filtered through a 1,000 nm long-pass filter coupled with an InGaAs camera for

NIR-II imaging. These *in vivo* images are further analyzed through a surface plot analysis using ImageJ software (National Institutes of Health). After that, the mice were sacrificed to harvest their major organs for *ex vivo* NIR-II imaging. Then, they were seperated into two groups and weighed. One group were fixed in fixed in 4% paraformaldehyde, embedded in paraffin and sectioned at 8-micron thickness and stained with hematoxylin & eosin (H&E). The slices were analyzed with a digital microscope (Leica).

The inductively coupled plasma-mass spectrometry (ICP-MS) analysis

After in vivo imaging, these mice were sacrificed to harvest their major organs for measuring Pb concentrations. These main organs of mice were dissolved in digest solution (HNO_3:$HClO_4$ = 4:1) with the volume of 5 mL. Blood sample were collected from the mice fundus artery. These samples were heated to 220 ^oC for 1 h and then added to 2 ml $HClO_4$ for continue heating. The reaction was stopped when it became clear and the solution was cooled down to room temperature. Each of the resulting solutions was then diluted by deionized water to 10 mL, and subsequently analyzed by ICP-MS (Thermo Fisher X II) to determine the concentration of Pb in each sample.

Results

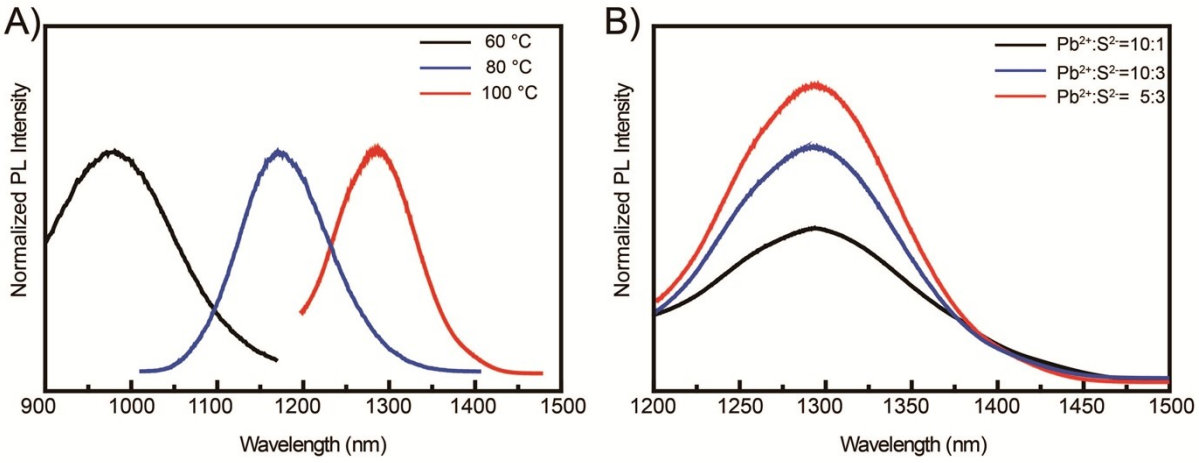

Fig. S1 A) Photoluminescence (PL) spectra of water solubleβ-lactoglobulin (LG)-PbS QDs with different emission peaks under different reaction temperature (60 °C, 80 °C and 100 °C),(excitation wavelength λex = 785 nm). (**b**) PL spectra of the given LG-PbS QDs prepared under different ratios of Pb^{2+} : S^{2-} . Reaction temperature is 100 °C.

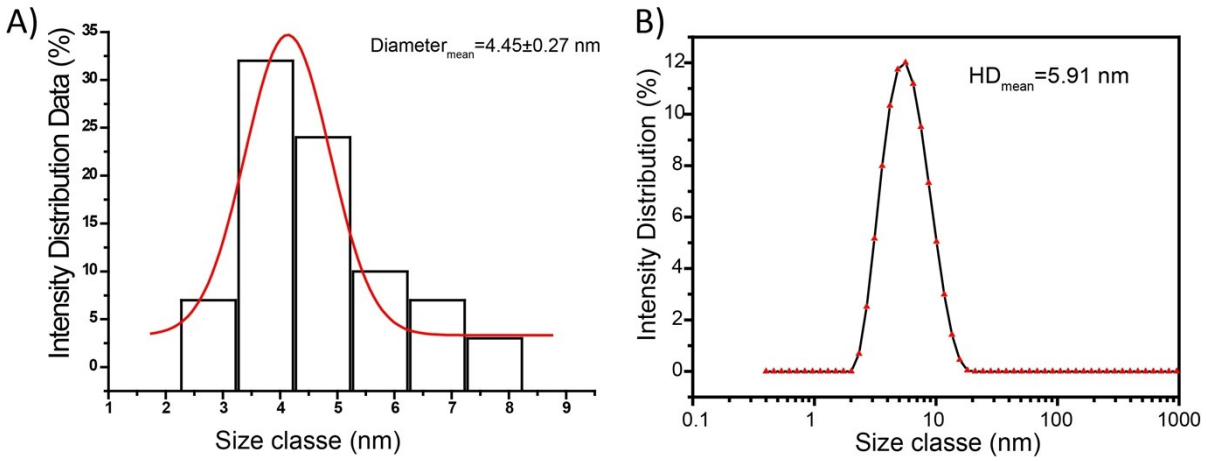

Fig.S2 A) The size distribution analysis of freshly prepared LG-PbS QDs in the corresponding high angle annular dark field scanning TEM (HAADF-STEM) images. B) Hydrodynamic diameter (HD) analysis of an LG-PbS QD sample using dynamic light scattering.

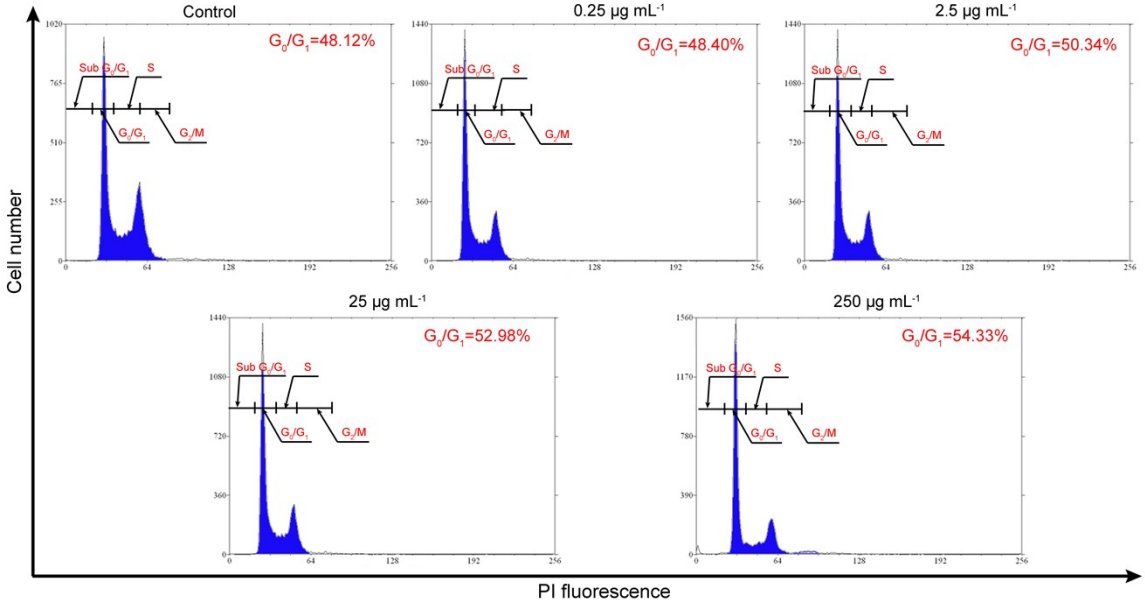

Fig.S3 Cell cycle of 293T cells after incubation with different concentrations of LG-PbS QDs for 24 h.

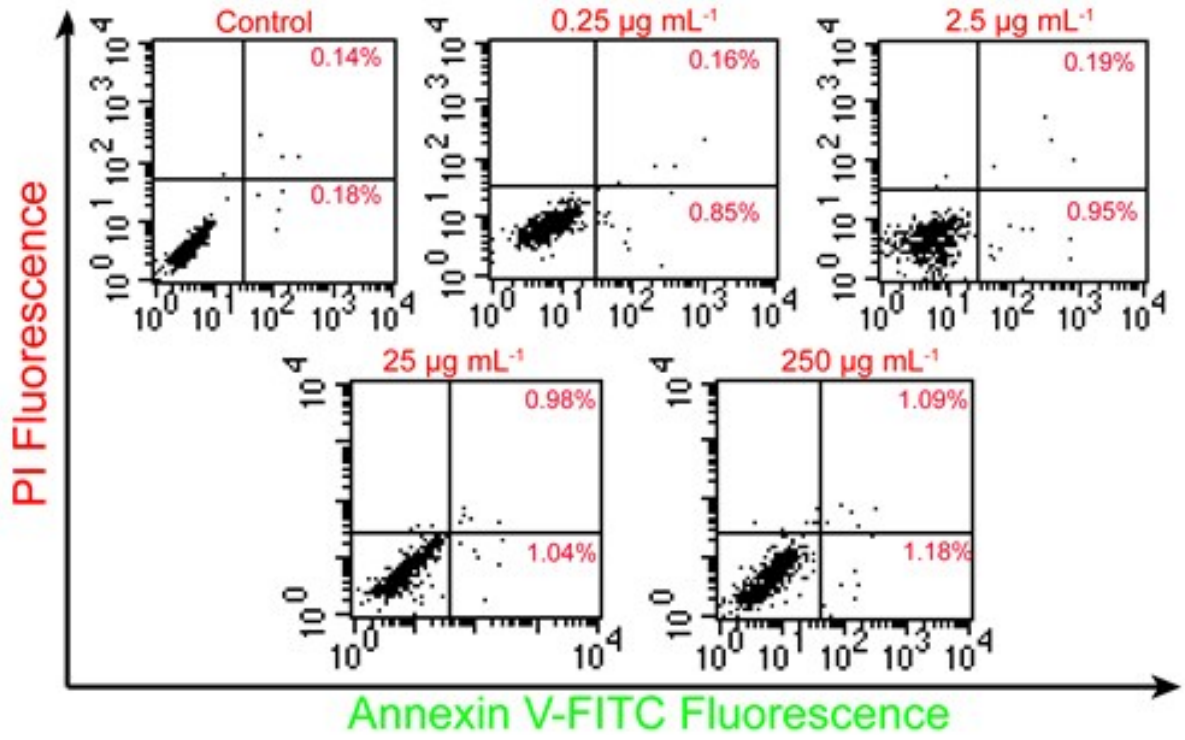

Fig.S4 Annexin V-APC/PI dual-stain analysis for apoptosis/necrosis of 293T cells after 24 h treatment with different concentrations of LG-PbS QDs.

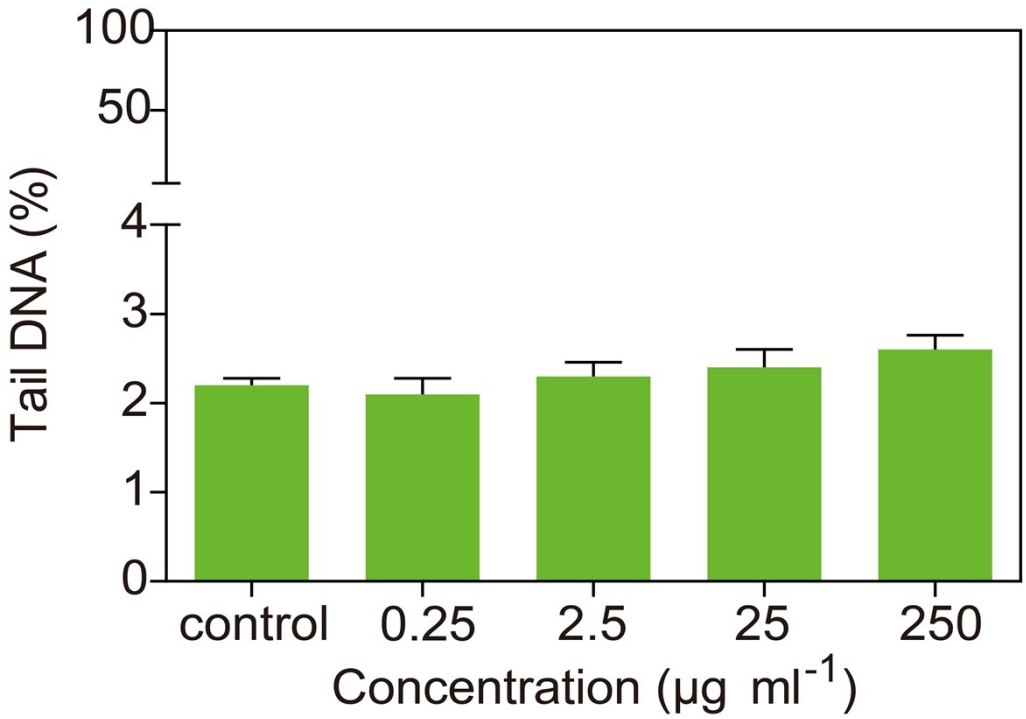

Fig.S5 Tail DNA analysis of 293T cells after incubation with different concentrations of LG-PbS QDs for 24 h.

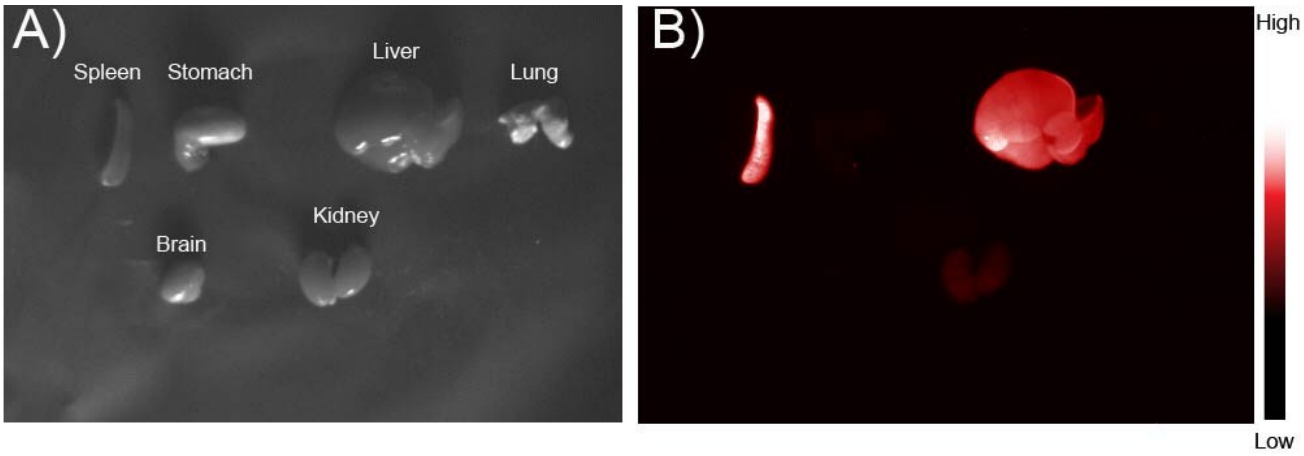

Fig.S6 The *ex vivo* A) Bright and B) fluorescence images of the key tissues in nude mice after the LG-PbS QD 24 h post-injection.

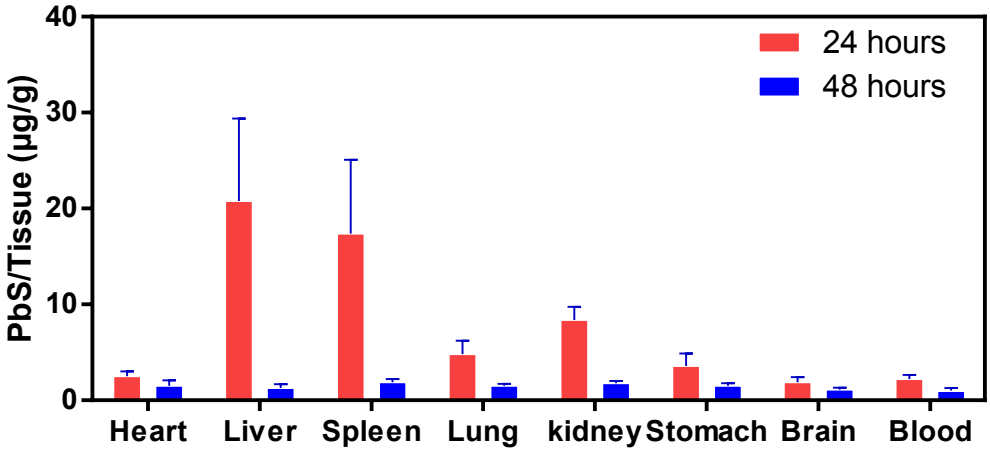

Fig. S7 Inductively coupled plasmon-mass spectrometry (ICP-MS) analysis of the the amount of Pb ion in main tissues of nude mice after both 24 h (red) and 48 h (Blue) post-injection.

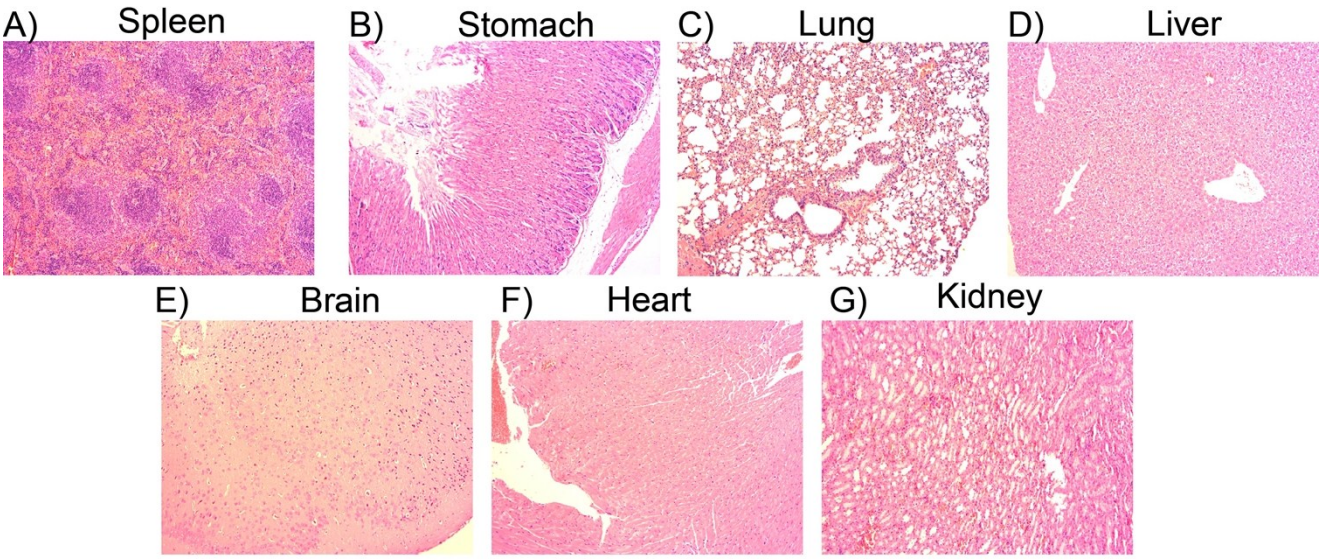

Fig.S8 hematoxylin and eosin (H&E) stained tissue sections of the nude mice, which were used to monitor histological changes in the spleen, stomach, lung, liver, brain, heart and kidney of the nude mice injected with LG-PbS QD after 48 h.

References:

1. Y. Zhang, G. Hong, Y. Zhang, G. Chen, F. Li, H. Dai and Q. Wang, *ACS Nano*, 2012, **6**, 3695-3702.
2. P. Guidi, M. Nigro, M. Bernardeschi, V. Scarcelli, P. Lucchesi, B. Onida, R. Mortera and G. Frenzilli, *Mutagenesis*, 2013, **28**, 171-180.