Supporting Information

Oxygen Vacancies-Enhanced CeO_2:Gd Nanoparticles for Sensing Tumor Vascular Microenvironment by Magnetic Resonance Imaging

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Supporting Methods

***In Vitro* Cytotoxicity Assay.** A549 cells (adenocarcinomic human alveolar basal epithelial cells) and 4T1 cells (murine mammary carcinoma cells) line were cultured in high-glucose DMEM (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS), streptomycin (100 mg/ml) and penicillin (100 units/mL). All cell lines were obtained from Shanghai Institute of cells, Chinese Academy of Sciences, and cultured at 37°C in humidified atmosphere together with 5% CO_2. Cytotoxicities of PEG-CeO_2:Gd were evaluated using A549 cells and 4T1 cells. With different concentrations of Ce^{3+} (0.006, 0.013, 0.0250, 0.050, 0.100,0.250 and 0.500 mg/mL) were placed into the wells. The cells were then incubated for 24 h at 37 °C under 5% CO_2, and the cell viability was detected by MTT assay. The abovementioned cells were seeded into a 96-well cell culture plate at the density of 10^6/well and then were incubated for 24 h. We found that PEG-CeO_2:Gd showed no obvious toxicity to A549 and 4T1 cells, which conform to the previous discoveries that PEGylation can ameliorate nanoparticles' biocompatibility

Confocal Fluorescence Imaging. Confocal laser scanning microscopy (CLSM) images of A549 cells were obtained after co-incubation with 200 μg/mL PEG-CeO_2:Gd for 6 h. The cell nucleus is stained with DAPI (blue fluorescence). Under the excitation of a 490 nm laser, PEG-CeO_2:Gd uptaken by the A549 cells emit strong yellow fluorescence (merging of green and red fluorescence) in the cytoplasm of the cells (**Figure S8**), demonstrating the presence of PEG-CeO_2:Gd in the cytoplasm but not in the nucleus.

***In Vivo* Toxicity Assay.** All animal experiments were conducted according to the protocols authorized by Institutional Animal Care and the care regulations approved by the administrative committee of laboratory animals of Fudan University. All Balb/c Nude and Kunming mice with the average weight of 20 g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Fifteen healthy female Balb/c mice (6 weeks old) were randomly divided into 3 groups. After an intravenous (*i.v.*) injection of a 50 mg kg^{-1} Gd dose of PEG-CeO_2:Gd (in 150 μl saline) with the same volume of saline as the control, we measured the body weight of the mice every three days (each group containing 5 mice, **Figure S10**). Over a period of one month, we did not observe any remarkable behavioral changes compared to the control group.

Fifteen healthy female Balb/c mice (6 weeks old) were randomly divided into 3 groups, and the injection dose of the PEG-CeO_2:Gd of 50 mg kg^{-1} was administered and hematological, blood biochemical and histological analyses were performed at 1, 3 and 30 days after the intravenous injection. At 3 days and 30 days after the intravenous injection, the mice were sacrificed to gather their major visceral organs (heart, liver, spleen, lung and kidney), which were preserved in a 10% formalin solution for further histopathology analysis using a typical hematoxylin and eosin (H&E) staining assay. To further evaluate the potential *vivo* toxicity, H&E stained tissue sections including heart, liver, spleen, lung and kidney were monitored using an optical microscope (**Figure S12**). The standard hematology parameters including alanine aspartate aminotransferase (AST), nephric blood urea nitrogen (BUN), aminotransferase (ALT), alkaline phosphatase (ALP) and creatinine (CREA) indicators, together with the complete blood panel parameters including red blood cells (RBC), white blood cells (WBC), red blood cell distribution width-standard deviation (RDW-SD), hemoglobin (HGB), mean corpuscular volume(MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), hematocrit (HCT) and lymphocyte (LYM) of all groups were evaluated by authoritative standard biochemistry test (**Figure S11**).

MRA Imaging. *In vivo* MR angiography imaging was conducted applying a clinical MRI scanner (Magnetom Verio TIM; Siemens Healthcare, Erlangen, 3.0 T). For MRA imaging of mices, seven week female Balb/c mice of approximately 20 g were bought Beijing Vital River Laboratory Animal Technology Co., Ltd. MRA with time-resolved imaging with contrast kinetics was acquired in the sagittal or coronal after PEG-CeO_2:Gd injection or Magnevist at low concentrations (5 mg Gd^{3+}/kg). A precontrast phase was acquired and served as a mask previous to the contrast agents injection. The scanning of subsequent phases was then initiated simultaneously with injection of Magnevist or PEG-CeO_2:Gd. The mask's complex subtraction was performed automatically for the background signal's maximum suppression. For maximum suppression of the background signal, the complex subtraction of the mask was performed automatically. The scan parameters for the TRICKS-MRA are as follows: TE = 1.3 ms; TR = 3.41 ms; slice thickness = 1.0 mm; flip angle = 24°; voxel size = 0.6×0.6×1.0 mm; FOV = 164×164 mm; temporal resolution = 1.59 s; bandwidth = 650 Hz/Px.

Figures

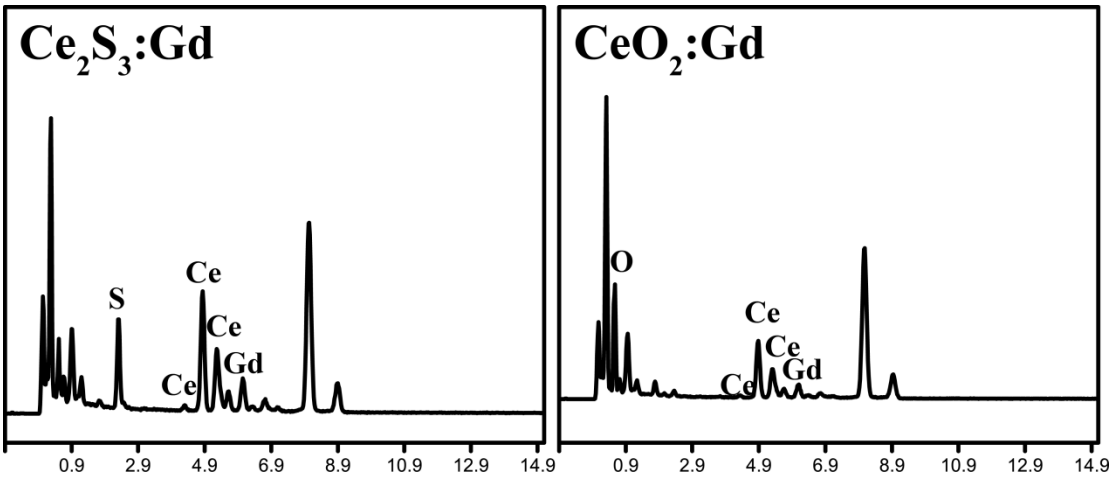

Figure S1. The elemental analysis of Ce_2S_3:Gd and oxidated PEG-CeO_2:Gd were measured by TEM-EDS.

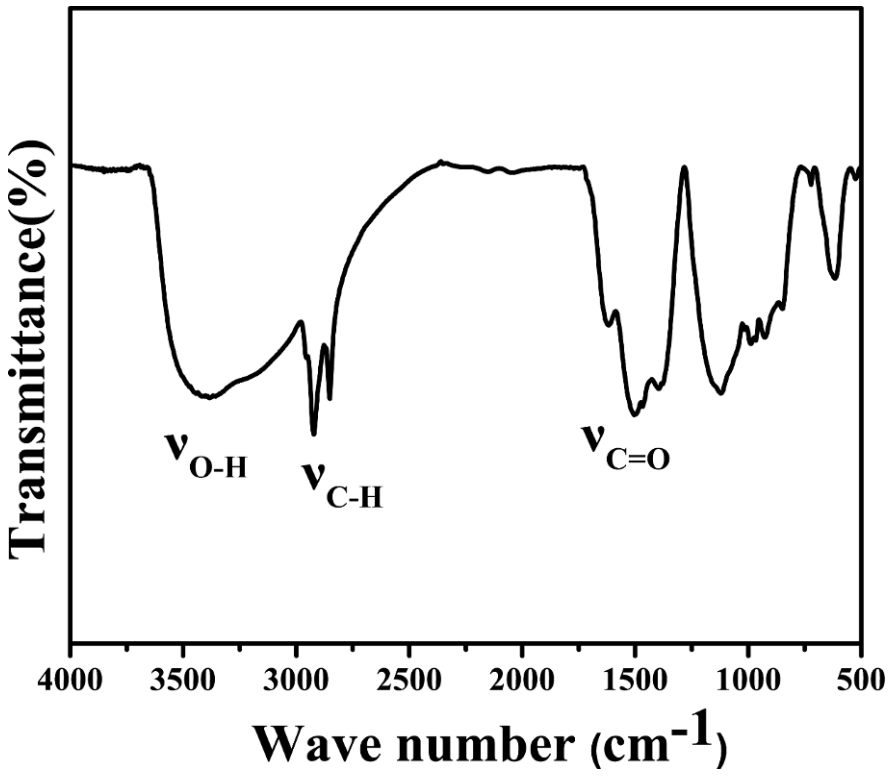

Figure S2. The FT-IR spectrum of PEG-modified PEG-CeO_2:Gd. The broad band at about 3450 cm^{-1} is attributed to the O–H stretching vibration of adsorbed water and/or

PEG. Other characteristic bands of PEG (stretching vibrations of C=O) are also emerged, indicating their successful common surface modification with DSPE-PEG.

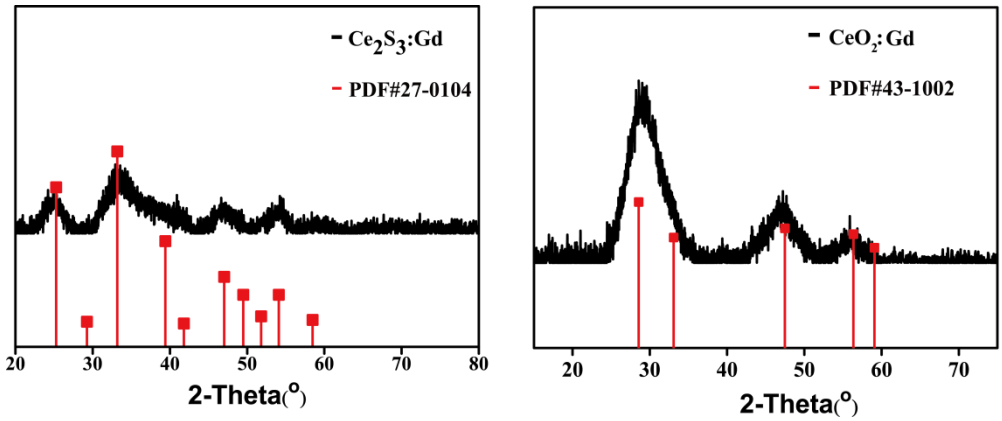

Figure S3. The XRD pattern shows that the as-synthesized Gd ions doping Ce_2S_3 (JCPDS No.27-0104) changed into ultra-small Gd ions doping CeO_2 (JCPDS No.43-1002) after hydrolysis reaction.

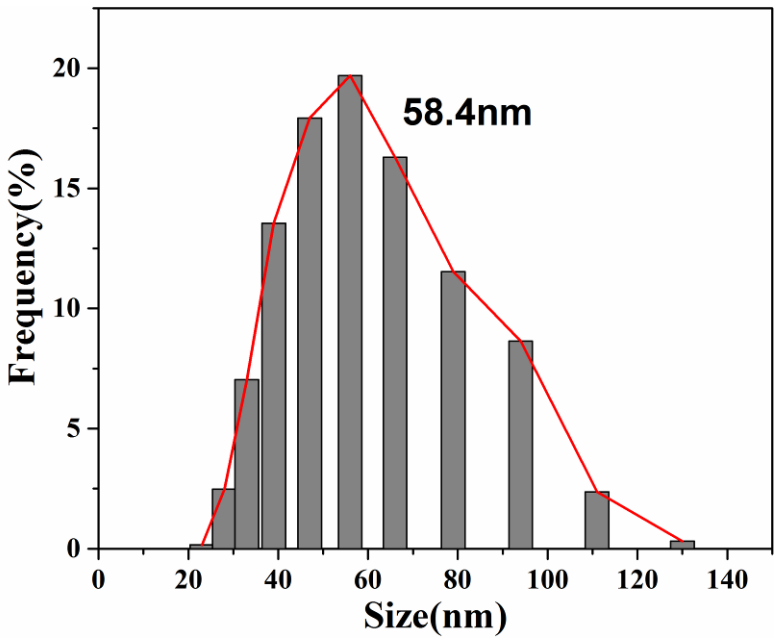

Figure S4. The hydrated particle size distribution of PEG-CeO_2:Gd in water by dynamic light scattering (DLS) measurement. DLS showed that the hydrated size of PEG-CeO_2:Gd is 58.4 nm (PDI = 0.165), and the zeta potential is +19.8 mV.

W (mM)	0.42	0.21	0.105	0.0525	0.2625	0.01313
T_1 (ms)	2296	2407	2252	2316	2360	2316

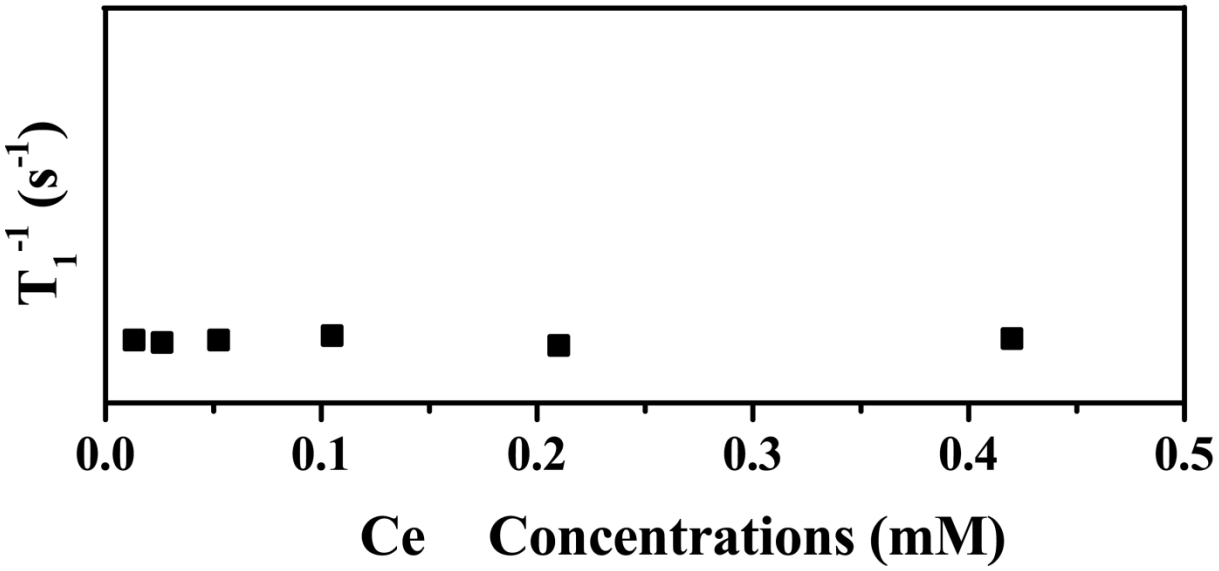

Figure S5. T_1-weighted MR images of PEG-CeO_2 with different cerium element concentrations.

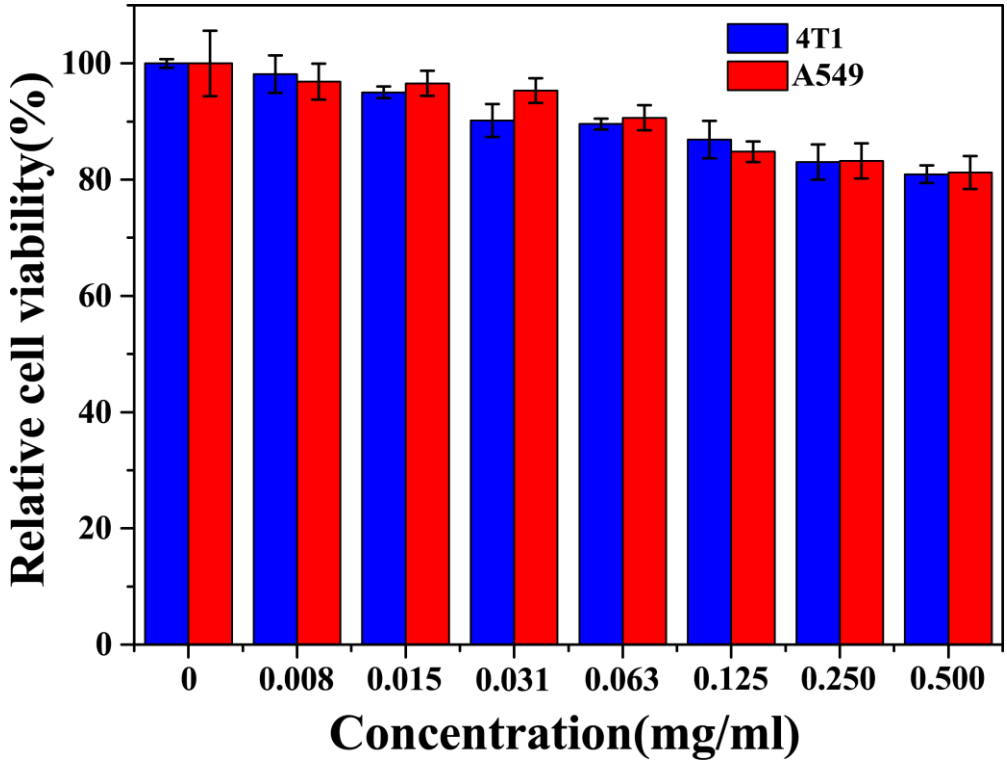

Figure S6. *In vitro* cell viabilities of 4T1 and A549 cells co-incubated with PEG-CeO_2:Gd of different concentrations (0, 8, 15, 31, 63, 125, 250, 500 μg/mL) for 24 h.

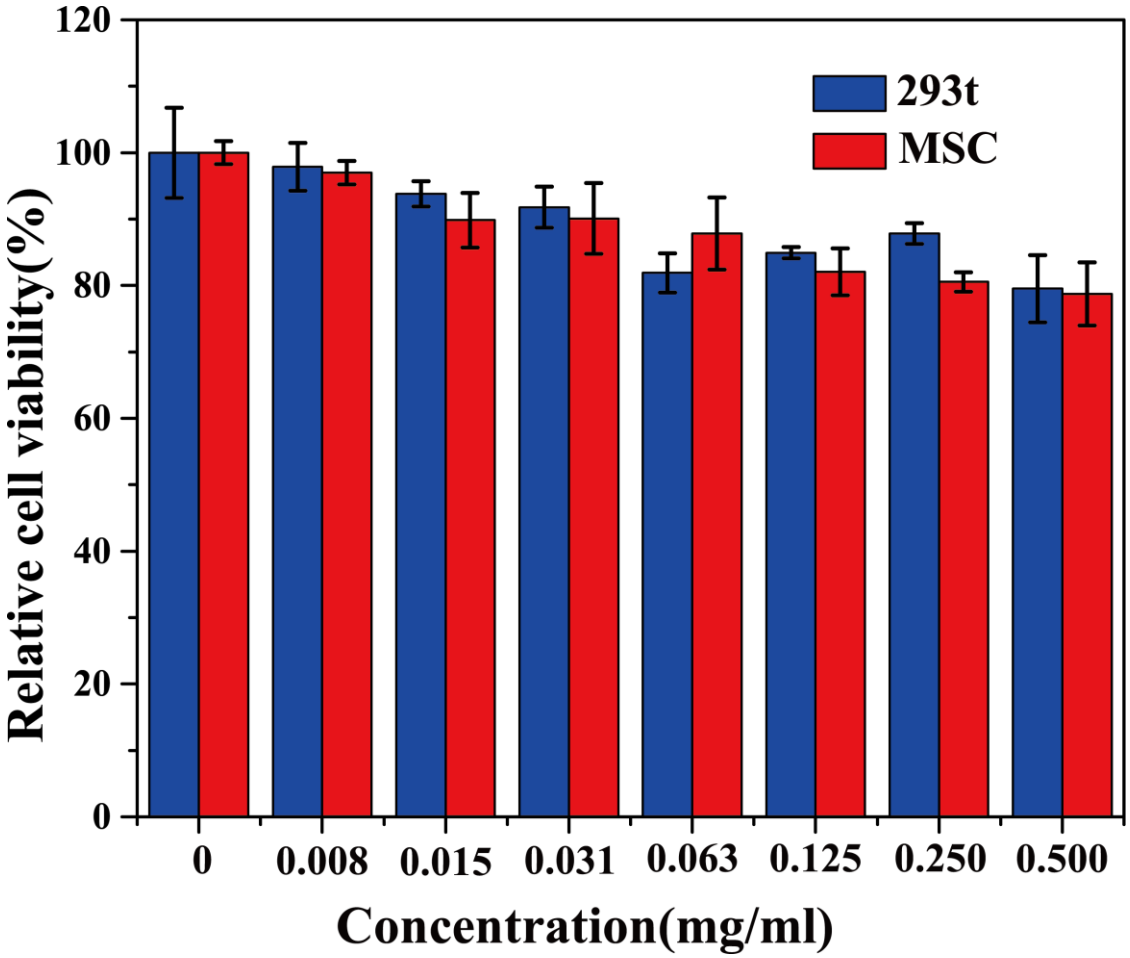

Figure S7. *In vitro* cell viabilities of 293t and MSC cells co-incubated with PEG-CeO_2:Gd of different concentrations (0, 8, 15, 31, 63, 125, 250, 500 μg/mL) for 24 h.

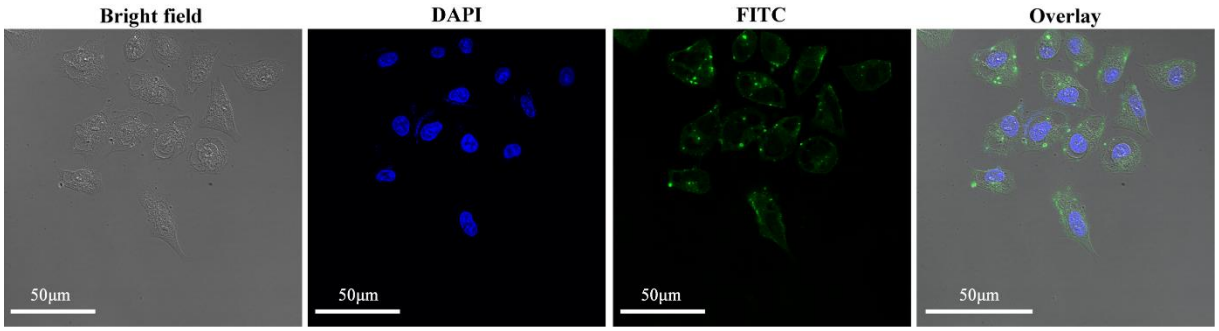

Figure S8. Confocal laser scanning microscopy (CLSM) images of A549 cells after co-incubation with 200 μg/mL PEG-CeO_2:Gd for 6 h.

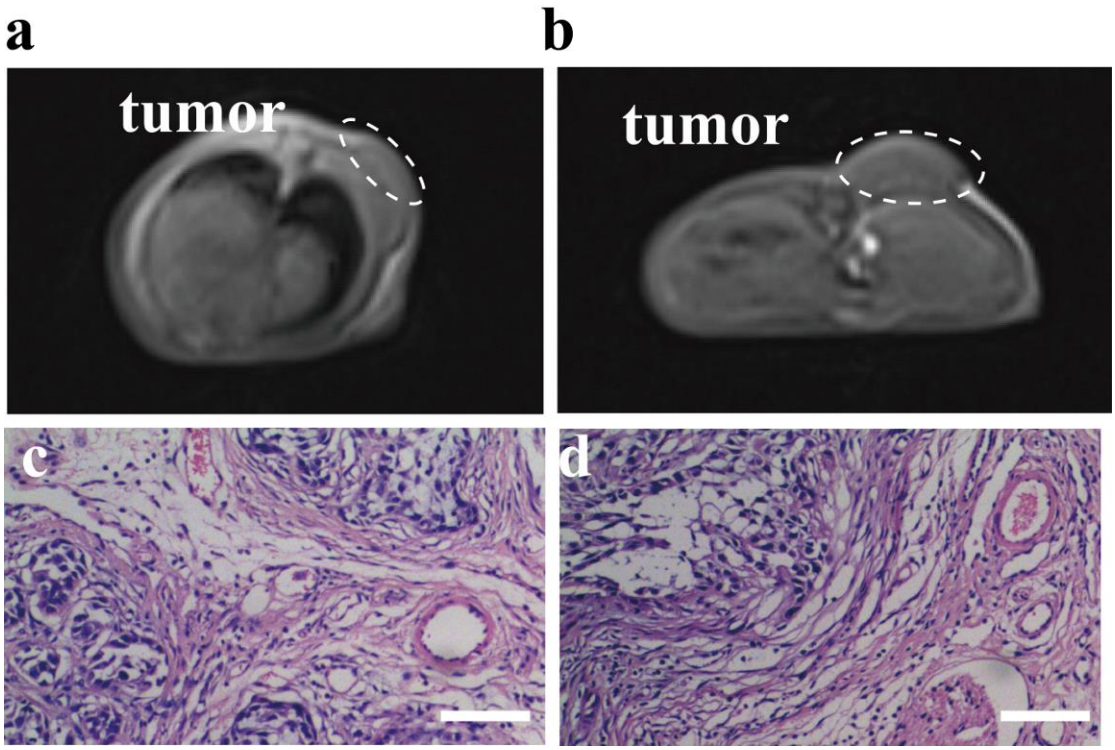

Figure S9. (a) The T_1 images of tissue before the injection with PEG-CeO_2:Gd group. (b) The T_1 images of tissue before the injection with Magnevist group shows that the size of A549 xenograft tumor is similar. (c) and (d) Images of hematoxylin and eosin (H&E) stained A549 xenograft tumour tissue sections of PEG-CeO_2:Gd group and Magnevist group. Images share the same scale bar (200 μm).

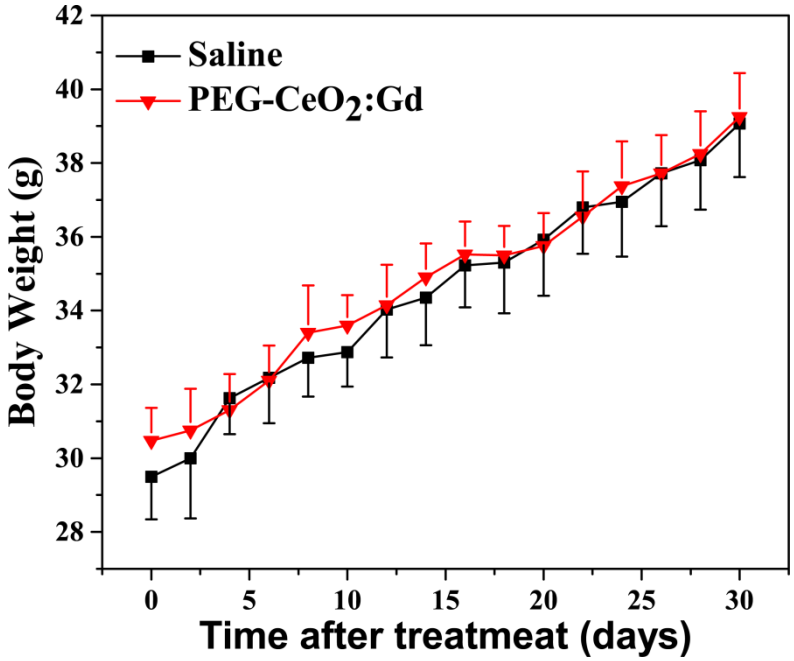

Figure S10. Weight measurement of the control group (intravenous injection of 150 μL saline) and the 30 days group (intravenous injection of 150μL PEG-CeO_2:Gd, 60 mg kg^{-1}).n = 4, mean ± s.d.

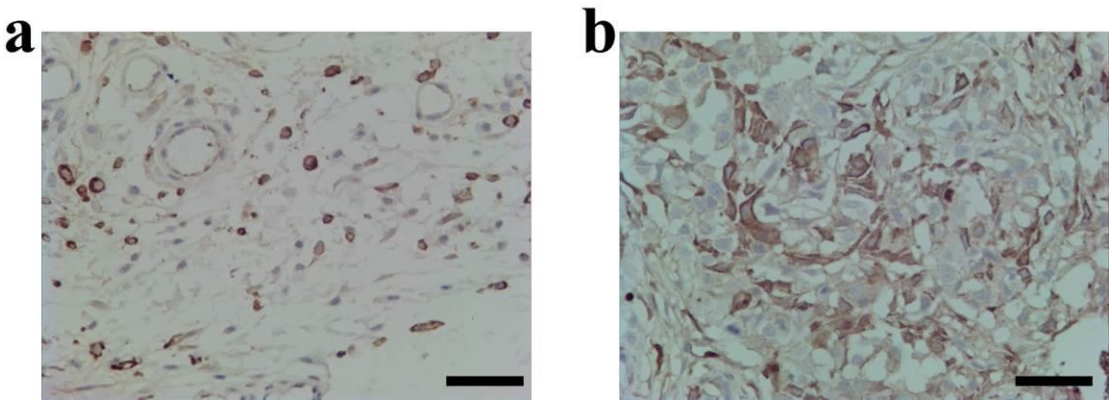

Figure S11. (a) Immunohistochemical staining of the A549 xenograft tumor tissue of PEG-CeO_2:Gd-injected group. (b) Immunohistochemical staining of the A549 xenograft tumor tissue of Magnevist-injected group. Images share the same scale bar (60 μm).

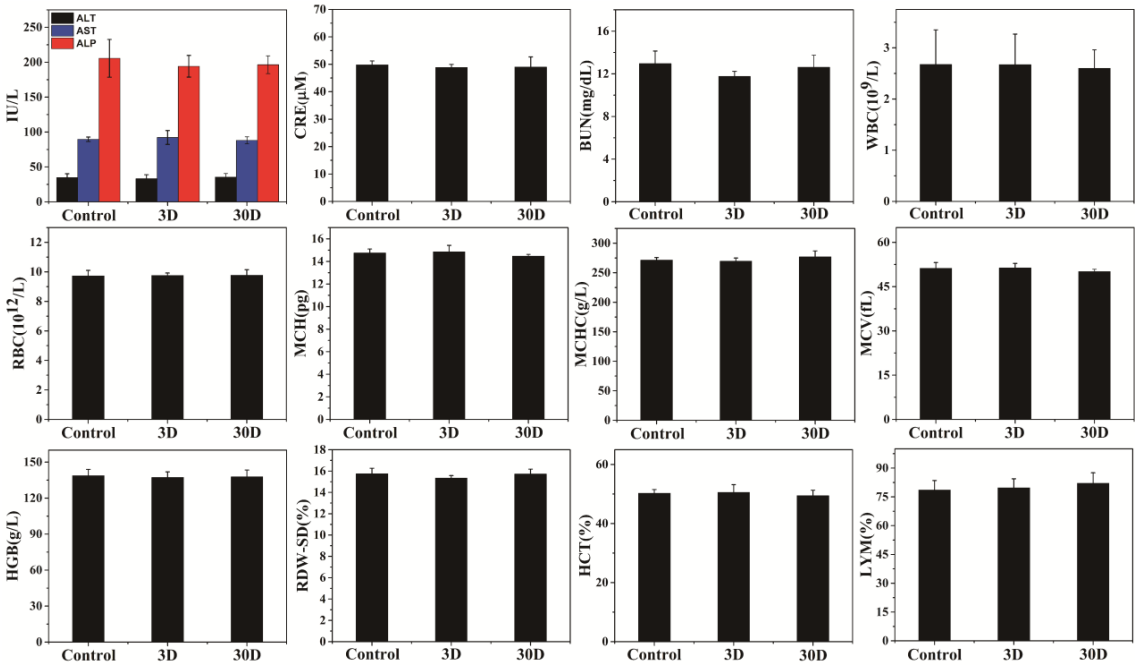

Figure S12. Blood biochemical parameters and hematology data obtained from the mice after the intravenous injection of PEG-CeO_2:Gd (60 mg kg^{-1}, 150 μL, n = 5, mean ± s.d.) in 3 and 30 days with 150 μL saline injection as control.

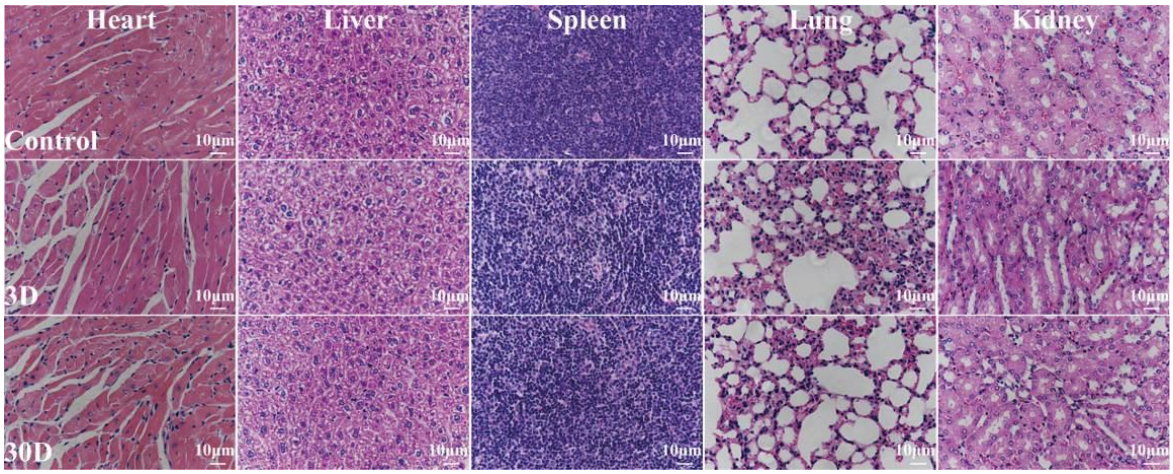

Figure S13. Pathological images of H&E stained tissue sections from heart, liver, spleen, lung and kidney of Kunming mice, harvested in 3th and 30th day after i.v. injection of a 50 mg kg^{-1} Gd dosage of PEG-CeO_2:Gd, showing no significant change of H&E tissue sections of the main organ.

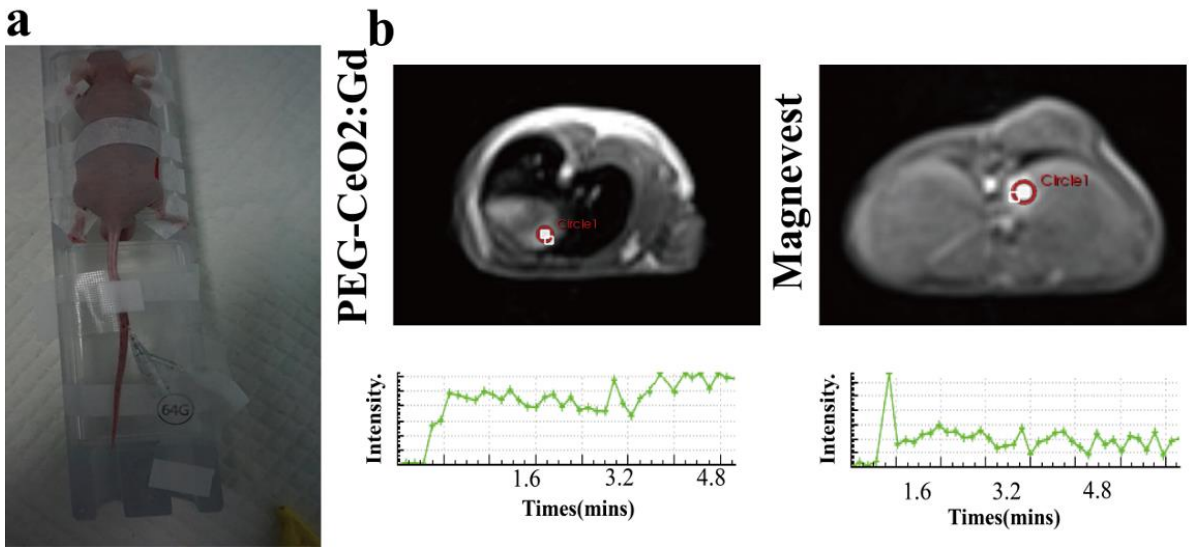

Figure S14. (a) Digital photos of nude mouse treat with 64G venous indwelling needle for MRA or DWI/DCE-PWI. (b) Blood circulation characteristic curve of PEG-CeO_2:Gd and Magnevest by MR, showing PEG-CeO_2:Gd can keep high T_1 signal time longer than Magnevest at same concentraion of Gd^{3+}.

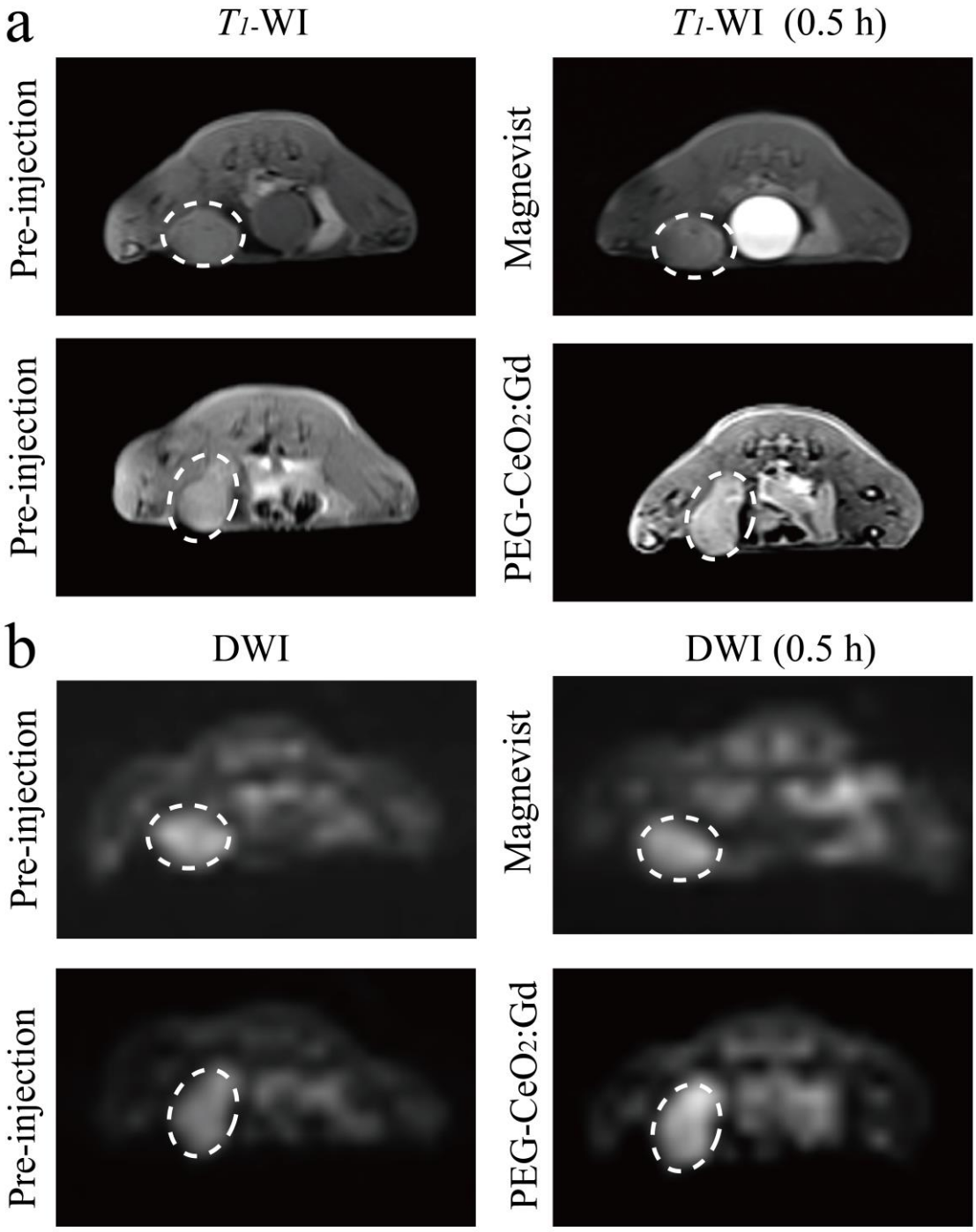

Figure S15. a) *In vivo* T_1-weighted MRI about tumor of mice (the white dotted circles) before and after the intravenous administration of PEG-CeO_2:Gd or Magnevist. (b) *In vivo* DWI about tumor of mice (the white dotted circles) before and after the intravenous administration of PEG-CeO_2:Gd or Magnevist, verifying the DWI signal enhancement of PEG-CeO_2:Gd behavior *in vivo.*

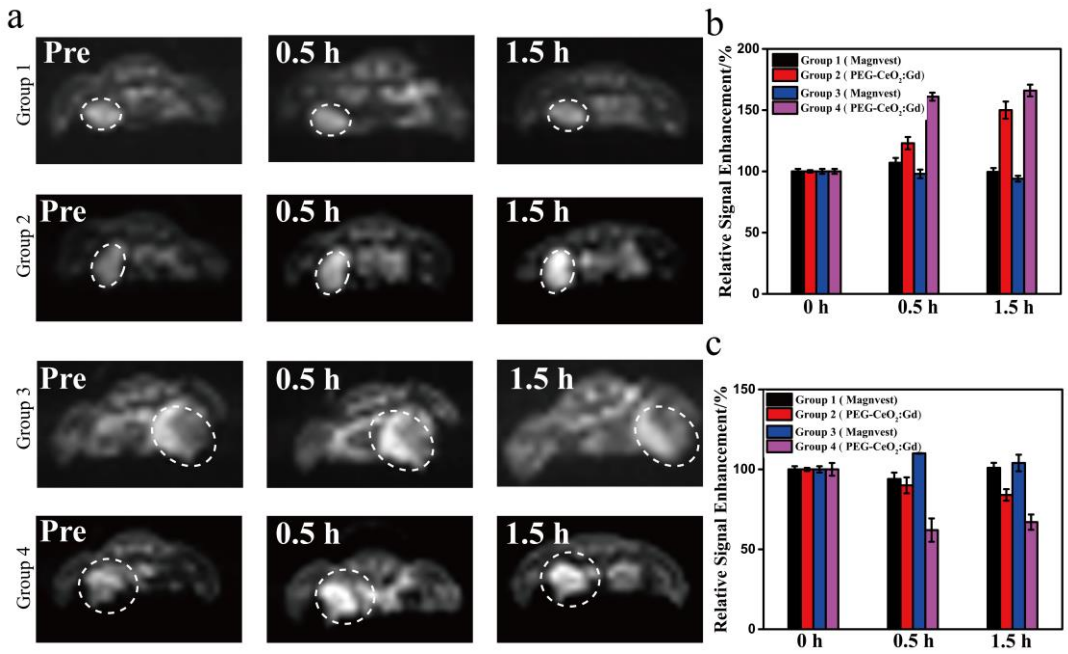

Figure S16. We devided mice into 4 groups: tumor-bearing mice (82 mm^3) injected with Magnevst is Group 1; tumor-bearing mice (84 mm^3) injected with Magnevst is Group 2; tumor-bearing mice (478 mm^3) injected with PEG-CeO_2:Gd is Group 3; tumor-bearing mice (289 mm^3) injected with PEG-CeO_2:Gd is Group 4. (a) Intensity change of four groups' DWI signal at different times. (b) The signal enhancement of tumors tissue obtained from four groups with different times. (b=1000) (c) Relative ADC value enhancement of four mice groups with different times.

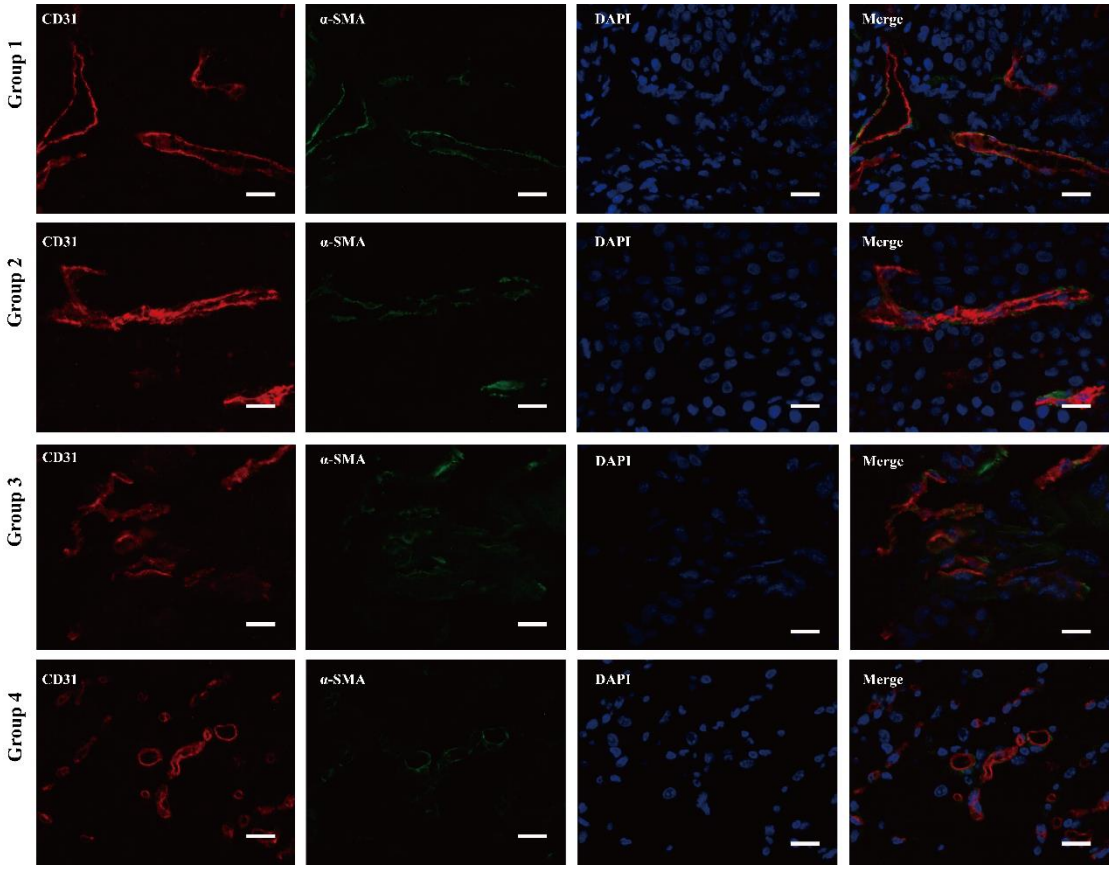

Figure S17. Immunofluorescence images of tumor slices in four groups stained by CD31 (red), α-SMA (green), DAPI (blue). Images share the same scale bar (15 μm).

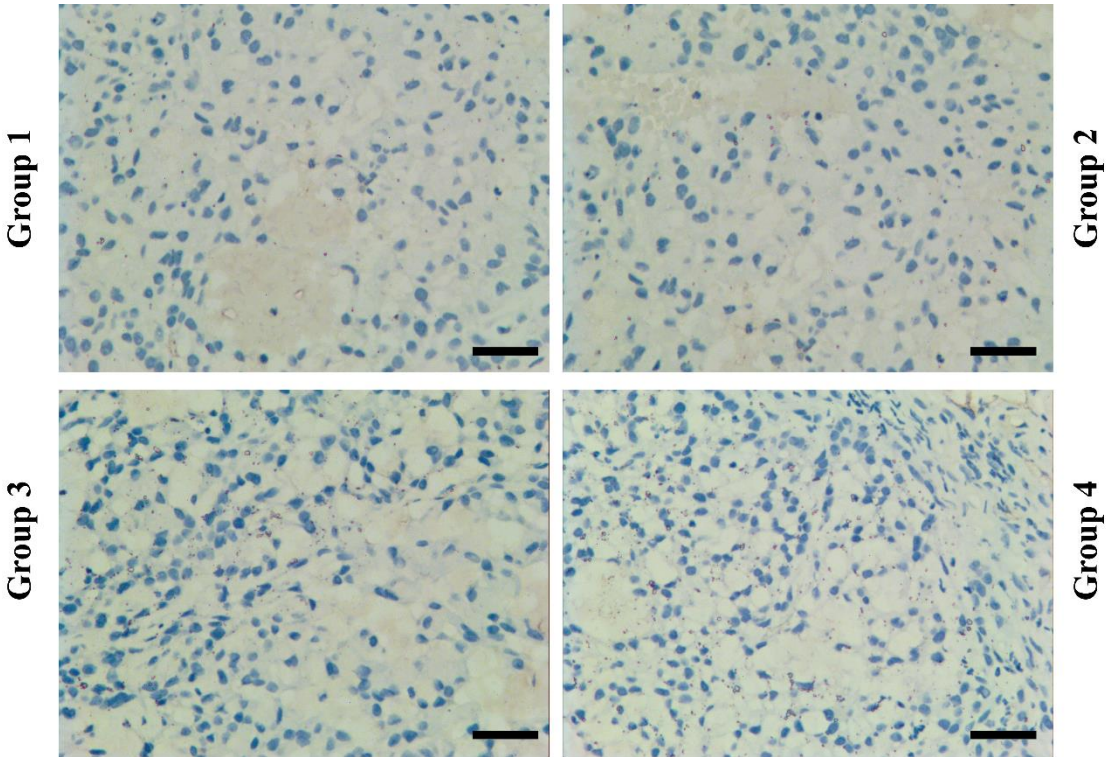

Figure S18. Immunocytochemistry images of tumor slices in four groups stained by CD31 (brownish-red). Images share the same scale bar (50 μm).

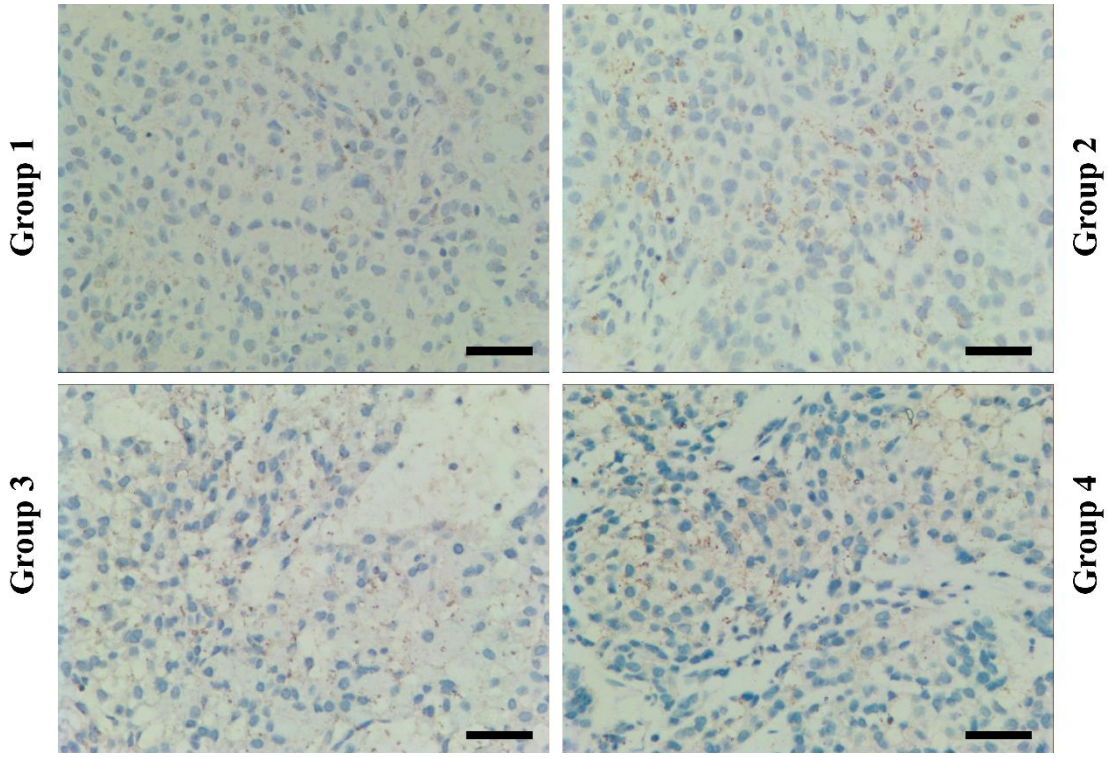

Figure S19. Immunocytochemistry images of tumor slices in four groups stained by Ang-1 (brown). Images share the same scale bar (50 μm).

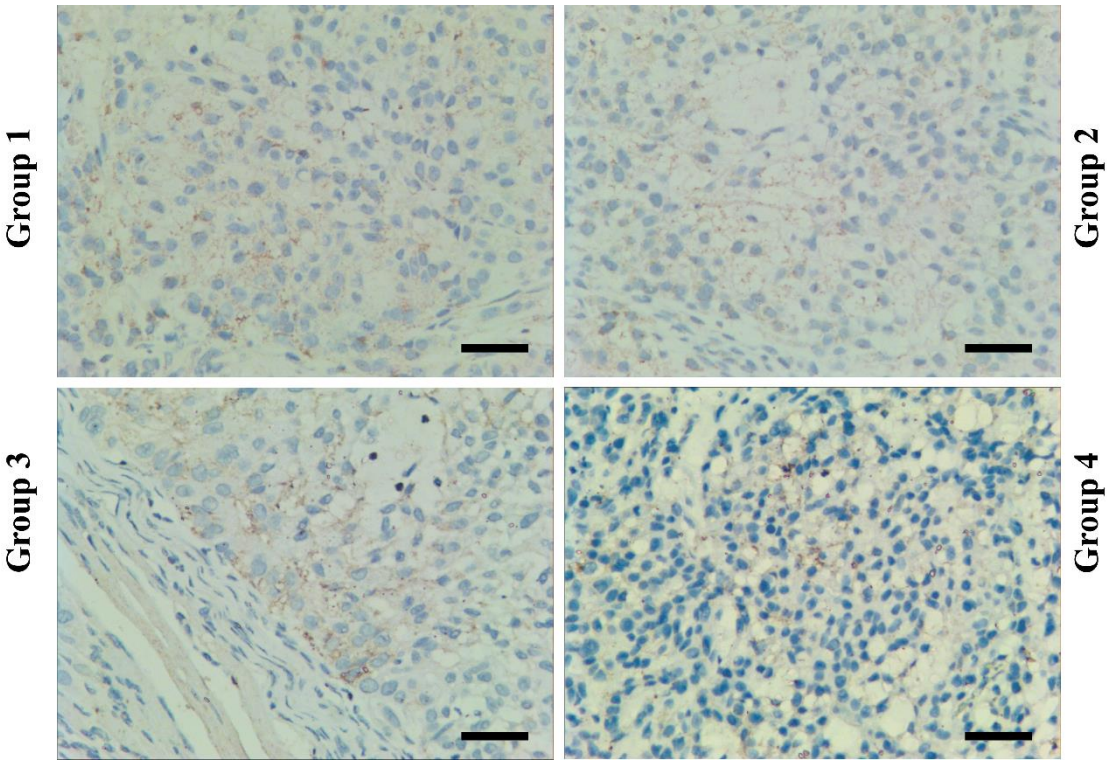

Figure S20 . Immunocytochemistry images of tumor slices in four groups stained by Ang-2 (brown). Images share the same scale bar (50 μm).

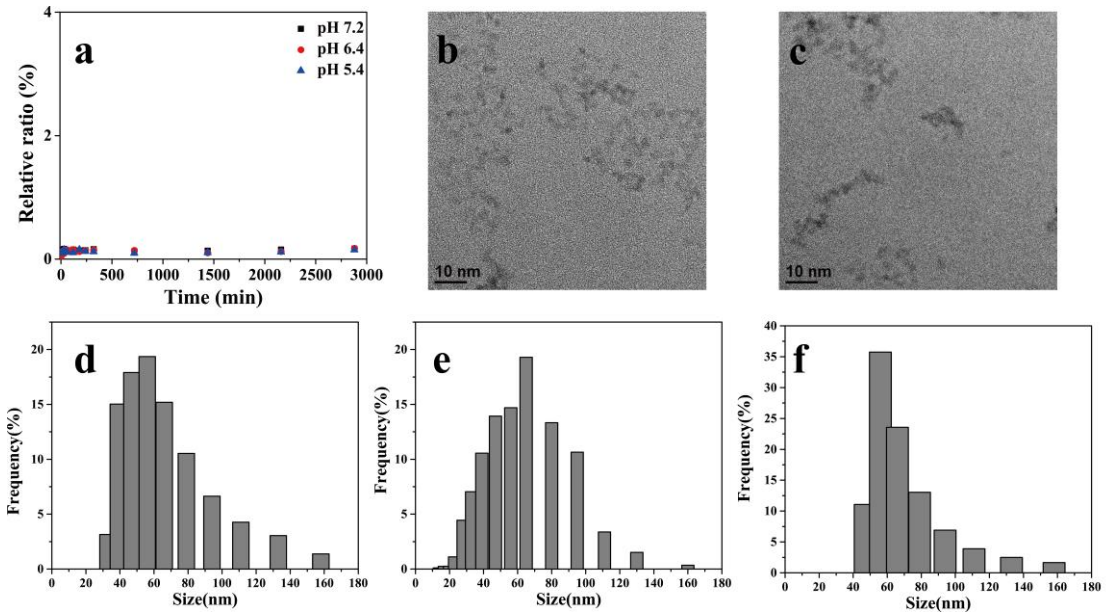

Figure S21. (a) The released curve of Ce amount from PEG-CeO_2:Gd at different time points (5 min,10 min, 20 min, 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, 12 h, 24 h, 36 h and 48 h) at different pH solutions, which revealed no potential Ce species leakage. (b) TEM image of PEG-CeO_2:Gd at pH 6.5 solutions. (c) TEM image of PEG-CeO_2:Gd at pH 5.4 solutions. (d)-(f) After 48 h treatment, the hydrated particle size distribution of PEG-CeO_2:Gd at different pH solutions (7.2, 6.5, 5.4) by dynamic light scattering measurement.