

## Electronic Supplementary Information

# Cytotoxicity, tumor targeting and PET imaging of sub-5 nm KGdF<sub>4</sub> multifunctional rare earth nanoparticles†

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## Table of Contents

### 1. Experimental section

#### 1.1 Materials

#### 1.2 Synthesis of OA-KGdF<sub>4</sub>:9%Eu REs

#### 1.3 Synthesis of OA-NaYF<sub>4</sub>: 20%Yb, 1%Tm REs

#### 1.4 Synthesis of PAA-coated REs

#### 1.5 Synthesis of RGD-conjugated REs

#### 1.6 Characterization of REs

#### 1.7 Preparation of [<sup>18</sup>F]KGdF<sub>4</sub> REs

#### 1.8 Cell culture and cytotoxicity assay

#### 1.9 Tumor xenografts and In vivo microPET/CT imaging

### 2.0 Biodistribution assays

#### 2.1 In vivo MRI

### 2. Supporting figures

Fig.S1 EDXA pattern of the OA-coated KGdF<sub>4</sub> REs

Fig.S2 XRD pattern of the OA-coated KGdF<sub>4</sub> REs and the cubic phase of NaGdF<sub>4</sub> (JCPDS No. 27-0697).

Fig.S3. FTIR spectra of the OA-capped KGdF<sub>4</sub> and PAA-coated KGdF<sub>4</sub> REs.

Fig.S4. Zeta potential of the PAA-coated KGdF<sub>4</sub> nanoparticles.

Fig.S5 TGA curve of the PAA-coated KGdF<sub>4</sub> REs.

Fig. S6 Size changes of the PAA-coated KGdF<sub>4</sub> REs over time.

Fig. S7 Fluorescence Z scanning imaging of live H1299 cells with the KGdF<sub>4</sub>:Eu<sup>3+</sup> REs (20 µg/mL, 2 h).

Fig. S8 TEM image of the NaYF<sub>4</sub> REs.

Fig. S9 MicroPET/CT images of the U87MG tumor bearing mice injected with the [ $^{18}\text{F}$ ]NaYF<sub>4</sub> REs.

Fig. S10 Time-dependent biodistribution of the PAA-coated KGdF<sub>4</sub> REs in mice.

Fig. S11 *In vivo* MR images of the mice before and after injection of the PAA-coated KGdF<sub>4</sub> REs.

Table S1. ROI analysis of T<sub>1</sub> relaxation time (ms) from the *in vivo* MR images.

## 1. Experimental section

### 1.1 Materials

GdCl<sub>3</sub>·6H<sub>2</sub>O, (99.999%), EuCl<sub>3</sub>·6H<sub>2</sub>O(99.99%), KOH (90%), NH<sub>4</sub>F (99.99+%), 1-octadecene (90%) and oleic acid (90%) were all purchased from Sigma-Aldrich. Diethylene glycol (DEG) and poly(acrylic acid) (PAA, Mw ≈ 2000) were obtained from Aladdin Industrial Inc. All chemicals were used as received without further purification.

### 1.2 Synthesis of OA-KGdF<sub>4</sub>:9%Eu REs

In a typical experiment, 0.91 mmol of GdCl<sub>3</sub>, and 0.09mmol of EuCl<sub>3</sub> were added to a 50-ml flask containing 6 mL of oleic acid and 15 mL of 1-octadecene. The solution was heated to 160 °C for 30 min and then cooled to room temperature. A 10 mL methanolic solution of NH<sub>4</sub>F (2.64mmol) and KOH (4 mmol) was slowly added to the flask using a syringe and pump system at a rate of 1.5 mL min<sup>-1</sup>, and the resulting solution was stirred for 30 min. The solution was slowly heated to remove methanol, degassed at 120 °C for 30 min, and finally heated at 300 °C for 120 min under argon. Nanoparticles were precipitated via addition of ethanol and were collected by centrifugation, washed with ethanol and hexane several times. For synthesis of KGdF<sub>4</sub>: 20%Yb, 1%Tm nanoparticles, 0.79mmol GdCl<sub>3</sub>, 0.2mmol YbCl<sub>3</sub>, 0.01mmol TmCl<sub>3</sub> were used.

### 1.3 Synthesis of OA-NaYF<sub>4</sub>: 20%Yb, 1%Tm REs

In a typical experiment, 0.79 mmol of YCl<sub>3</sub>, 0.2 mmol of YbCl<sub>3</sub> and 0.01mmol of TmCl<sub>3</sub> were added to a 50-ml flask containing 8 mL of oleic acid and 15 mL of 1-octadecene. The solution was heated to 160°C for 30 min and then cooled to room temperature. A 10 mL methanolic solution of NH<sub>4</sub>F (2.5mmol) and KOH (4 mmol) was slowly added to the flask and the resulting solution was stirred for 30 min. The solution was slowly heated to remove methanol, degassed at 120°C for 30 min, and finally heated at 300 °C for 60 min under argon. Nanoparticles were precipitated via addition of ethanol and were collected by centrifugation, washed with ethanol and hexane several times.

### 1.4 Synthesis of PAA-coated REs

PAA-coated nanoparticles were synthesized according to the previous literature with a modified procedure. To a flask containing 10 mL diethylene glycol (DEG), PAA-2000 (50 mg) was added. The mixture was heated to 110°C to form a clear solution. The toluene solution containing 100 mg as-prepared OA-coated nanocrystals was injected slowly and maintained this temperature for 30 min under argon protection. Then the solution was heated to 240°C for 2 h. The resultant solution was cooled down to room temperature, and ethanol was added. The PAA-coated nanoparticles was recovered via centrifugation and washed three times with ethanol/water (1:1, v/v).

### 1.5 Synthesis of RGD-conjugated REs

Bioconjugation was carried out by EDC-mediated coupling reaction between the carboxyl groups on the REs and the amine-containing RGD peptide. In a typical conjugation reaction, 100  $\mu\text{L}$  of RGD peptide (5mg/mL in HEPES) was added to 1mL of KGdF<sub>4</sub> (2 mg/mL in water), followed by vortex mixing. Then, 50  $\mu\text{L}$  of freshly prepared aqueous EDC solution (10 mg) was added and above mixture was stirred for 1 h at room temperature. The uncoupled free RGD and excess EDC was removed by two washes using a 10 K OMEGA NANOSEP (Pall) under centrifugation at 8000 r.p.m. for 2 min.

### *1.6 Characterization of REs*

The size and morphology of REs were determined at 120 kV using a Tecnai G2 Spirit Biotwin low to high resolution transmission electron microscope (HR-TEM). TEM samples were prepared by dripping the NP solution onto a carbon-supported copper grid and drying at room temperature before observation. Energy-dispersive X-ray analysis (EDXA) of the samples was also performed during HR-TEM measurements. Powder X-ray diffraction (XRD) measurements were performed on a Bruker D8 diffractometer at a scanning rate of 4°/min in the 2 $\theta$  range from 10 to 70°(Cu Ka radiation,  $\lambda=1.54 \text{ \AA}$ ). The hydrodynamic diameter distribution and the zeta potential of REs were performed on a Zetasizer NanoZSP instrument (Malvern, UK) at 298 K. The upconversion luminescence emission spectra were recorded with an Edinburgh LFS-920 luminescence spectrophotometer, but the excitation source was an external 0e1Wadjustable 980 nm continuous-wave semiconductor laser (Beijing Hi-Tech Optoelectronic Co., China), instead of the Xeon source. The downconversion luminescence emission spectra were recorded on a Photo Technology International (PTI) QM 40 luminescence spectro-photometer. TGA was performed using a TG 209 F1 (NETZSCH). Determination of Gd<sup>3+</sup> concentration was performed by ICP-MS analysis (Thermo Fisher iCAPQ series QC 450). Samples were dissolved in deionized water (100 $\mu\text{L}$ ) and HNO<sub>3</sub> (100 $\mu\text{L}$ ), and diluted with deionized water to 5 mL. T<sub>1</sub> relaxation times were determined using a Bruker mq60 MR relaxometer operating at 1.5T. Excel was used to plot the relaxation rates over the concentration of Gd<sup>3+</sup> and to fit a linear function to determine the relaxivity values (i.e. gradient of linear fit). Fourier transform infrared (FT-IR) spectra of KGdF<sub>4</sub>:9%Eu powders before and after ligand exchange procedures were collected with a Bruker Equinox 55 spectrophotometer scanning from 500-4000 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup> for 64 scans. Measurements were performed with pressed pellets which were made using KBr powder as diluent.

### *1.7 Preparation of [<sup>18</sup>F]KGdF<sub>4</sub> REs*

In a typical synthesis, 2 mg KGdF<sub>4</sub>-PAA Was mixed with <sup>18</sup>F<sup>-</sup> (2.86 mCi) in distilled water (1mL), and then were incubated for 10 min at room temperature. After purification from unlabeled free <sup>18</sup>F<sup>-</sup> by centrifugation and three times water washing, the radioactivity of the solid (namely the materials) and the liquid (including the incubation solution from the materials and the washing water) was determined using an RM-905a Standard Grade Radioactivity Meter. The <sup>18</sup>F-labeling yield of the material was calculated by using the relation activity of the solid/total activity.

### *1.8 Cell culture and cytotoxicity assay*

Human glioblastoma U87MG cell line and human non-small cell lung carcinoma H1299 cell line were grown in DMEM supplemented with 10% FBS. Cultures were maintained at 37 °C under a humidified atmosphere containing 5% CO<sub>2</sub>.

The *in vitro* cytotoxicity was measured using the CCK-8 assay in U87MG and H1299 cell lines. Cells growing in log phase were seeded into a 96-well cell-culture plate at  $1 \times 10^4$ /well and then incubated for 24 h at 37 °C under 5% CO<sub>2</sub>. REs (100 μL/well) at different concentrations (10, 100, and 1000 μg/mL) were added to the wells of the treatment group, and 100 μL/well DMEM to the negative control group, respectively. The cells were incubated for 24 h at 37 °C under 5% CO<sub>2</sub>. Subsequently, 10 μL of CCK-8 was added to each well of the 96 well plate and incubated for an additional 2 h at 37 °C under 5% CO<sub>2</sub>. A Tecan microplate reader was used to measure the OD450 (A value) of each well. The following formula was used to calculate the viability of cell growth:

cell viability (%) = (mean of Absorbance value of treatment group / mean of Absorbance value of control) × 100.

### *1.9 Tumor xenografts and In vivo microPET/CT imaging*

Animal procedures were reviewed and approved by the Institutional Animal Care Use Committee of Shanghai Jiao Tong University. Tumor cells were harvested when they reached near confluence by incubation with 0.05% trypsin-EDTA. Cells were pelleted by centrifugation and resuspended in sterile PBS. U87MG cells ( $5 \times 10^6$  cells/site) or H1299 cell ( $5 \times 10^6$  cells/site) were implanted subcutaneously into the left shoulder (stomach position) of five-week-old female athymic nude mice, respectively. At 20 days after implant, the tumor-bearing mice were subjected to imaging studies.

PET/CT imaging was performed on an Inveon system (Siemens Preclinical Solutions). PET scans were terminated after 600 million events were recorded, with a typical acquisition time of 60 min. Images were reconstructed using 2D filtered back projection (FBP). The CT X-ray source was used with a power of 80kVp and 500 μA and an exposure time of 370-400 ms. Typically, 360 projections were acquired. 1 h after intravenously injection of <sup>18</sup>F-labeled nanoparticles (~60 μCi/2.22 MBq) via tail vein, mice were anesthetized and then imaged by MiroPET/CT imaging to verify the *in vivo* behavior of <sup>18</sup>F-labeled REs. Reconstructed CT and PET images were fused and analyzed using Inveon Research Workplace (IRW) software.

### *2.0 Biodistribution assays*

To study the tissue distribution for KGdF<sub>4</sub>-PAA in the healthy Kunming mice, the organs (heart, liver, spleen, lung, kidneys, bone, urine and blood, n=3) were surgically removed at various times (post-1 h, 24 h,) after delivery the KGdF<sub>4</sub>-PAA by injection through the tail vein. The Gd ions were quantified through ICP-MS method. Samples and standards were analyzed on a ThermoFisher iCAPQ series QC 450. The organs were surgically removed from the animal, placed into a centrifuge tube containing 1 mL of concentrated HNO<sub>3</sub>, and incubated for 2 h under heat treatment (80 °C) for dissolution of the tissues. The samples were then diluted by addition to 5 mL of water. Finally the obtained liquid was subjected to ICP-MS analysis.

### *2.1 In vivo MRI*

The Kunming mice was anesthetized and scanned before and after the administration of KGdF<sub>4</sub>-PAA nanoparticles. MRI was conducted on a 7T MRI scanner (Bruker bioSpect 70/20 USR, Germany) using a T1-mapping sequence (TR =1000 ms, TE =6.0 ms, FA=90 deg, matrix =256×256, FOV =4.5×3.5 cm<sup>2</sup>, slice thickness =1 mm). The PBS solution (400 uL) of the KGdF<sub>4</sub>-PAA (1.936 mg Gd/kg) nanoparticles was injected intravenously and MRI images were obtained at 1h post injection.

## 2. Supporting Figures

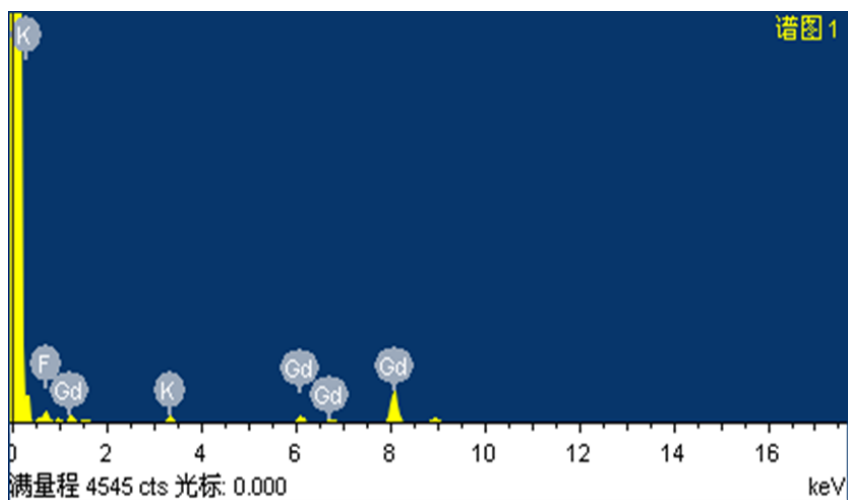


Fig.S1 EDXA pattern of the OA-coated  $\text{KGdF}_4$  REs

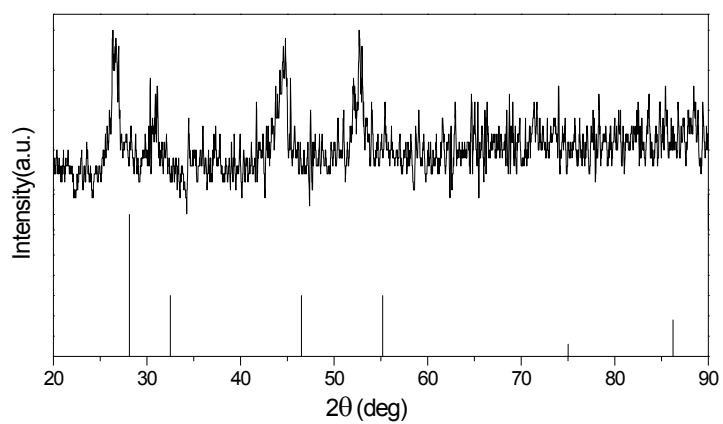


Fig.S2 XRD patterns of the OA-coated  $\text{KGdF}_4$  REs and the cubic phase of  $\text{NaGdF}_4$  (JCPDS No. 27-0697).

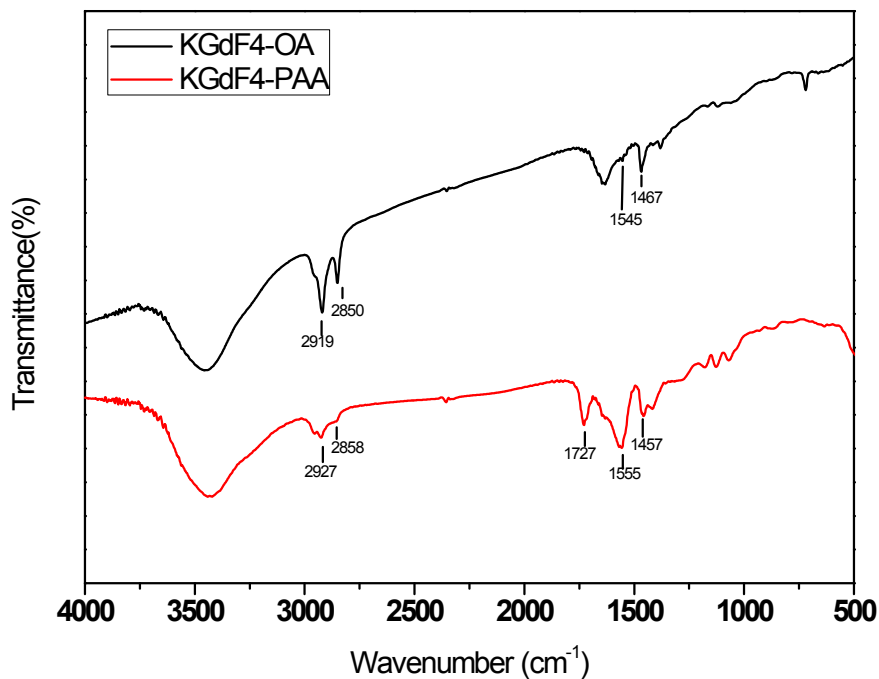


Fig.S3. FTIR spectra of the OA-capped KGdF<sub>4</sub> and PAA-coated KGdF<sub>4</sub> REs.

	Mean (mV)	Area (%)	St Dev (mV)
<b>Zeta Potential (mV): -13.8</b>	<b>Peak 1: -13.8</b>	100.0	8.57
<b>Zeta Deviation (mV): 8.57</b>	<b>Peak 2: 0.00</b>	0.0	0.00
<b>Conductivity (mS/cm): 0.0282</b>	<b>Peak 3: 0.00</b>	0.0	0.00

**Result quality : See result quality report**

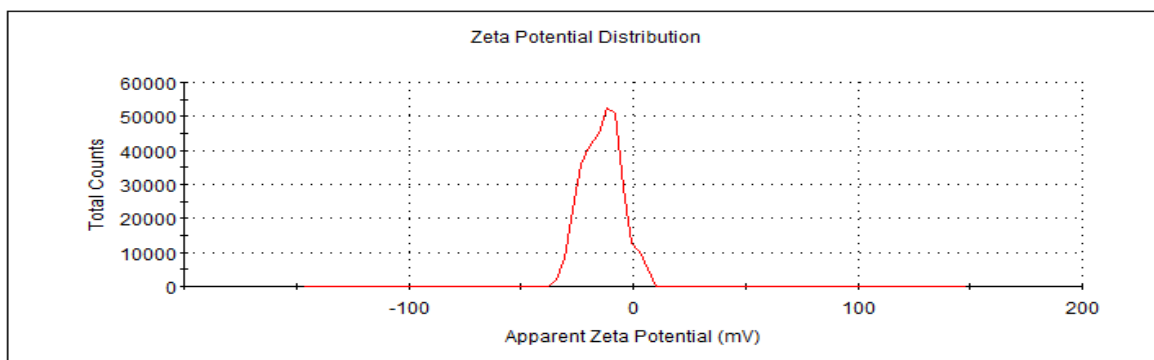


Fig.S4. Zeta potential of the PAA-coated KGdF<sub>4</sub> nanoparticles.



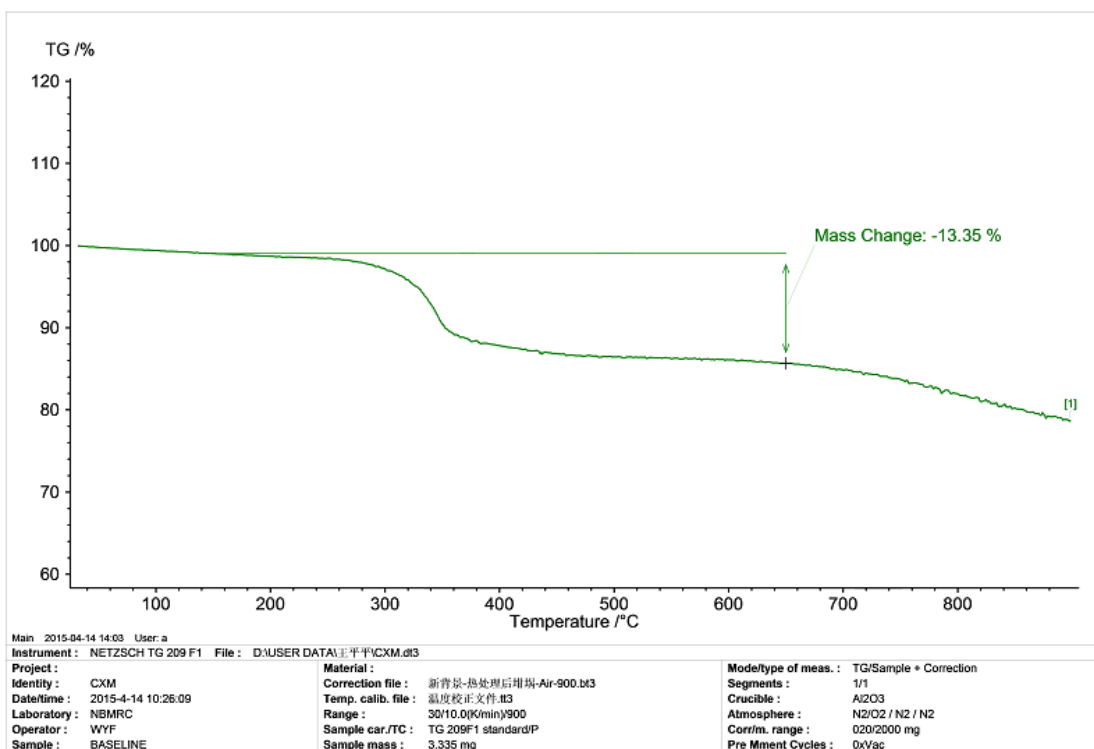


Fig.S5 TGA curve of the PAA-coated KGdF<sub>4</sub> REs.

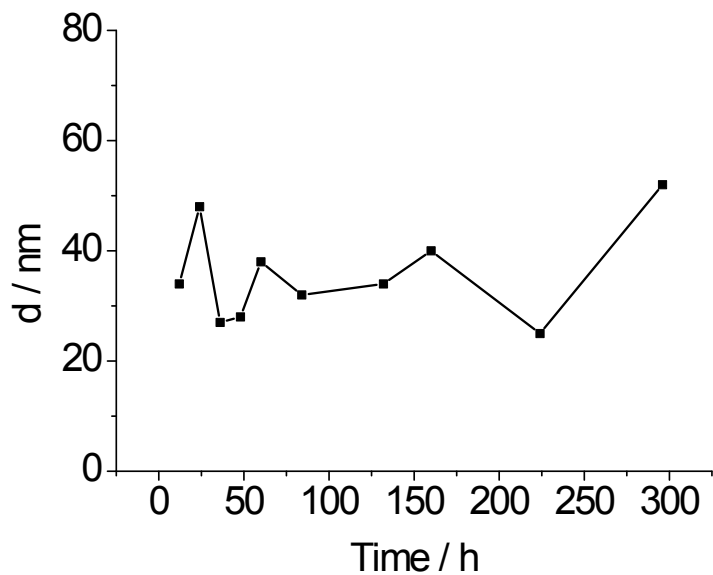


Fig. S6 Size changes of the PAA-coated KGdF<sub>4</sub> REs over time.

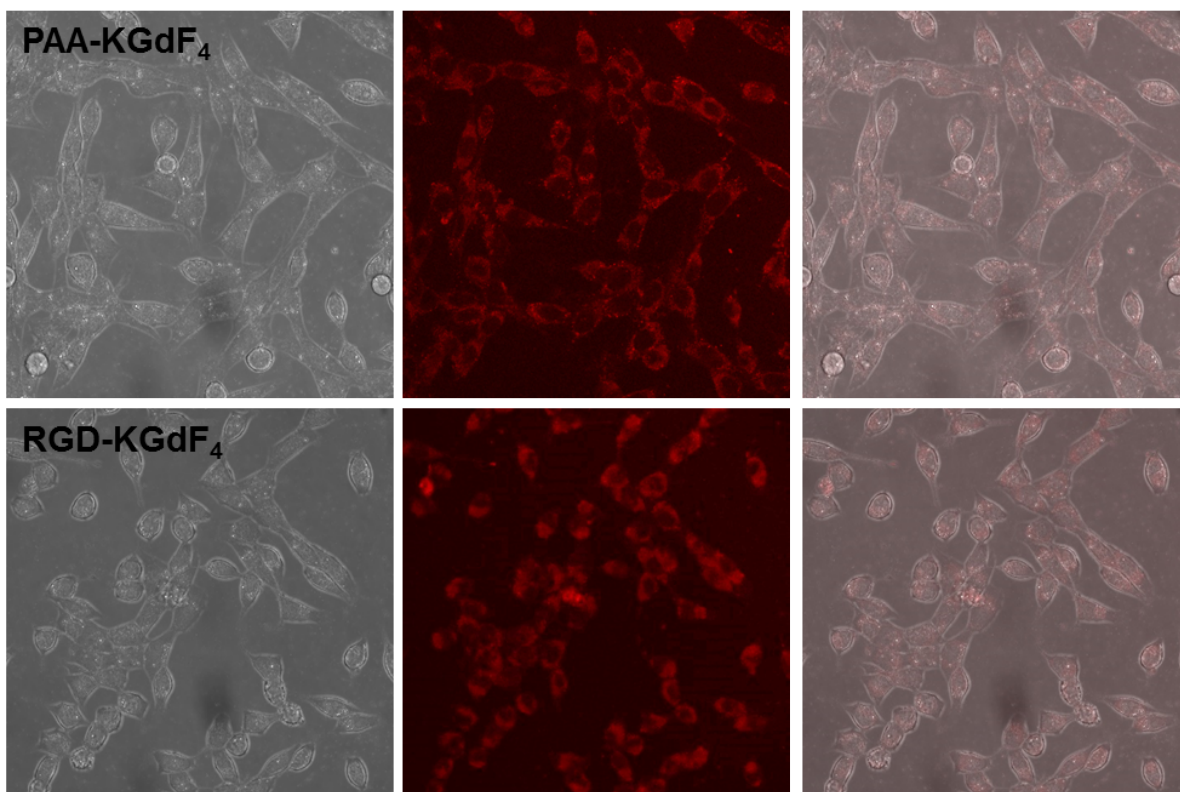


Fig. S7 Fluorescence Z scanning imaging of live H1299 cells with the KGdF<sub>4</sub>:Eu<sup>3+</sup> REs (20  $\mu\text{g}/\text{mL}$ , 2 h).

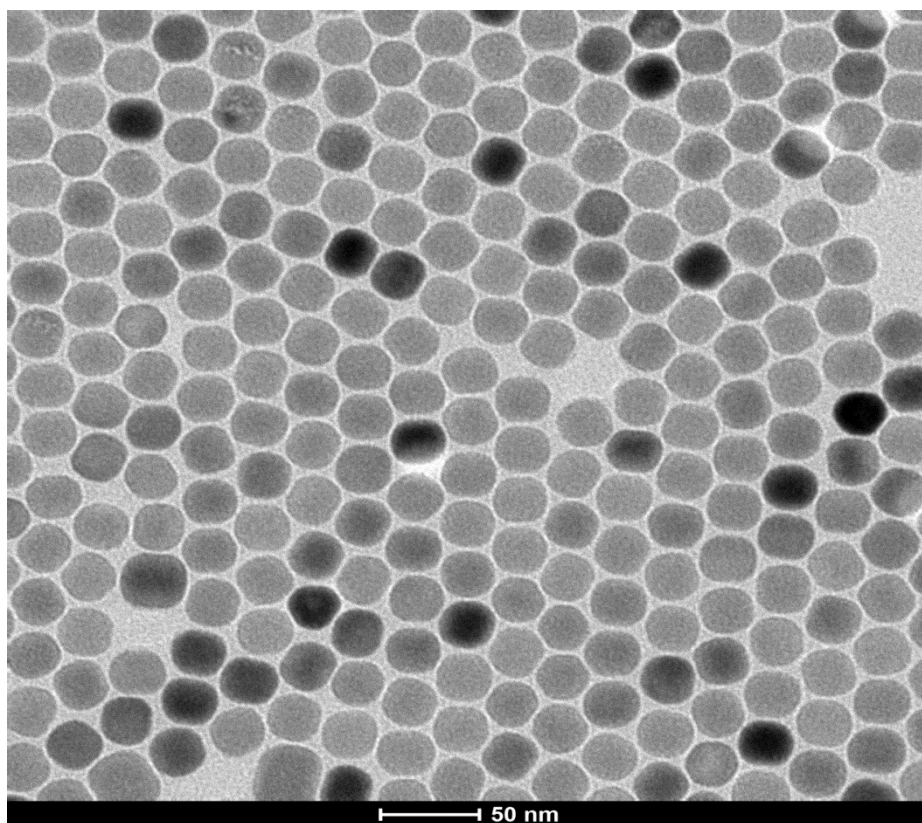


Fig. S8 TEM image of the NaYF<sub>4</sub> REs.

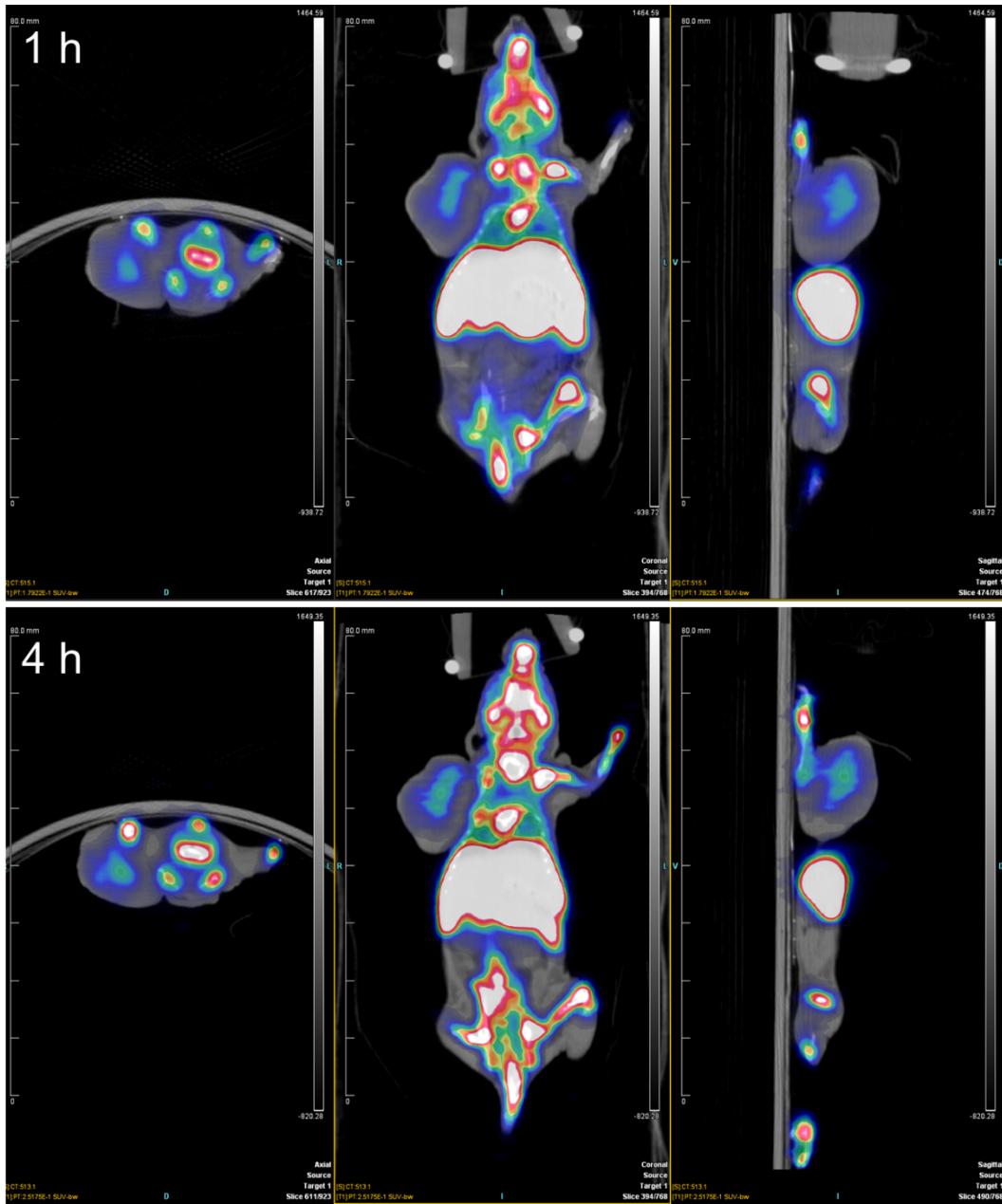


Fig. S9 MicroPET/CT images of the U87MG tumor bearing mice injected with the  $[^{18}\text{F}]\text{NaYF}_4$  REs.

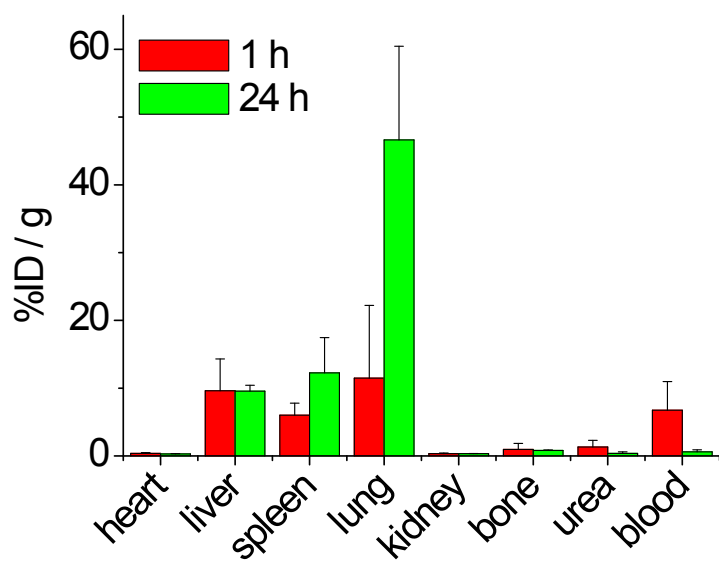


Fig. S10 Time-dependent biodistribution of the PAA-coated KGdF<sub>4</sub> REs in mice (n = 3).

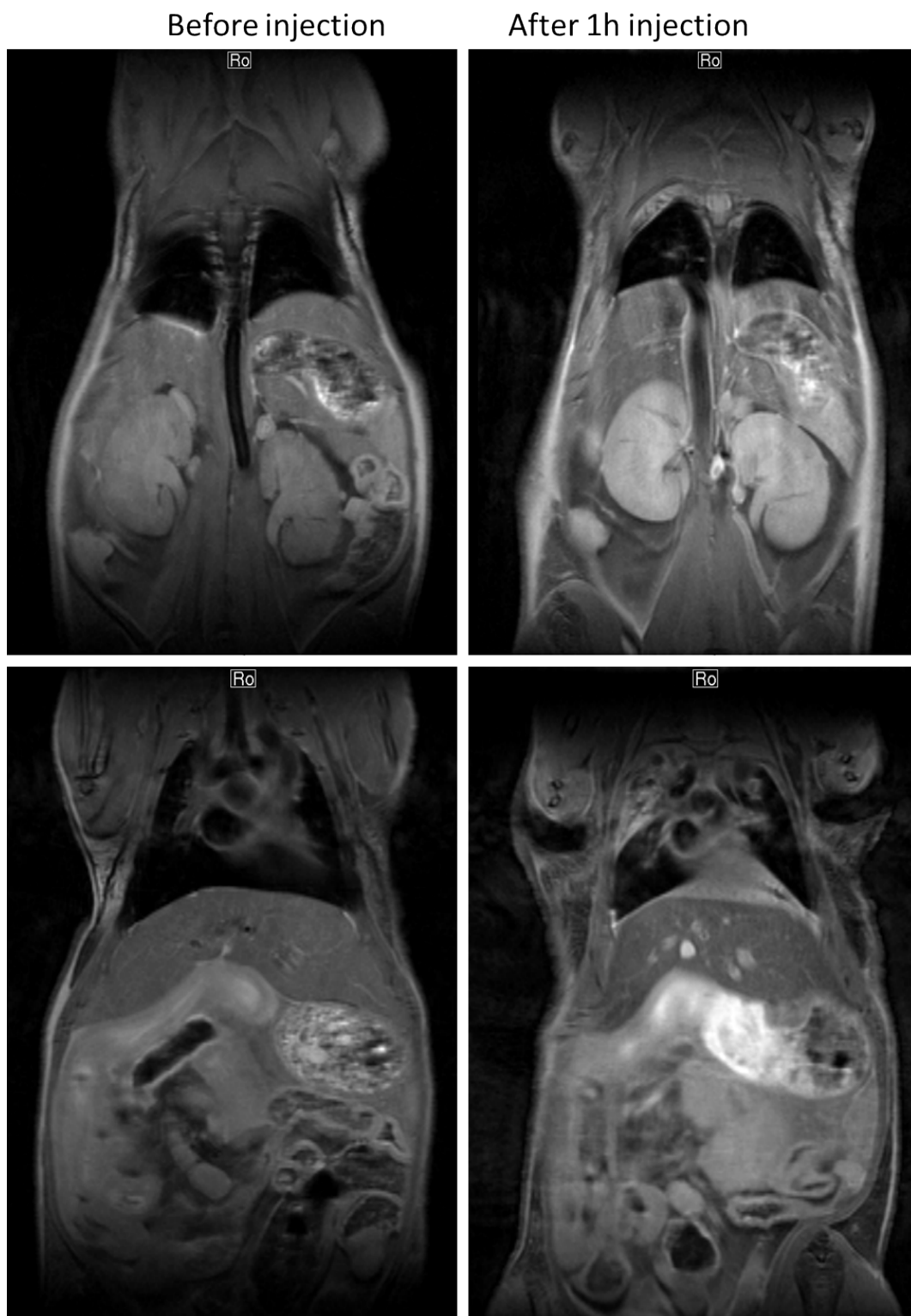


Fig. S11 *In vivo* MR images of the mice before and after injection of the PAA-coated KGdF<sub>4</sub> REs.

T <sub>1</sub> relaxation time (ms)	liver	lung	spleen	heart	kidney
Before injection	1053.24	863.29	1145	1181	1265
After 1h injection	427.90	584.64	912	754	959.58

Table S1. ROI analysis of T<sub>1</sub> relaxation time (ms) from the *in vivo* MR images.