Electronic Supporting Information for

Multifunctional nanoprobe based on europium (III) complex-Fe_3O_4 nanoparticle for bimodal time-gated luminescence/magnetic resonance imaging of cancer cells *in vitro* and *in vivo*

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1. Experimental section

Preparation of Fe_3O_4 nanoparticles

$Fe(acac)_3$ (0.706 g, 2.0 mmol), 1,2-dodecanediol (2.024 g, 10 mmol), oleic acid (2 mL), oleylamine (2 mL) and benzyl ether (20 mL) were added into a 100 mL three-necked flask. After stirring for 10 min at room temperature under an argon atmosphere, the mixture was heated at 200 °C for 1 h. Then the solution was further heated to 300 °C. After stirring for another 0.5 h at 300 °C, the solution was cooled to room temperature and 40 mL of ethanol was added. The solid nanoparticles were collected by centrifugation and washed three times with ethanol to get rid of the unreacted materials.

Synthesis of APTES-BHHBCB-Eu^{3+} conjugate

The APTES-BHHBCB-Eu^{3+} conjugate was prepared by adding BHHBCB (0.036 mmol, 30.0 mg) and europium(III) triflate (0.018 mmol, 11.1 mg) to 2.0 ml of acetone containing 24.0 mg of APTES (0.108 mmol). The mixture was stirred for 4 h at room temperature, and the obtained APTES-BHHBCB-Eu^{3+} solution was used in the next process without further purification.

Photobleaching experiments

To evaluate the photo-stability of Fe-Eu@SiO_2-FA nanoprobe, the Tris-HCl buffer (0.01 M, pH 7.4) solution containing Fe-Eu@SiO_2-FA NPs or pure BHHBCB-Eu^{3+} complex (0.05% Triton X-100 was added as a cosolvent) in a 1.0 cm quartz cell was irradiated from a distance of 2 cm by a 30 W deuterium lamp. The emission intensities were recorded at every 5 min interval for a period of 1 h.

MTT assay

The cytotoxicity of Fe-Eu@SiO_2-FA nanoprobe to HeLa cells was measured by the MTT test using the previously reported method.[1] HeLa cells cultured in Dulbecco's modified Eagle Medium (DMEM) were washed with an isotonic saline solution (140 mM NaCl, 10 mM glucose, and 3.5 mM KCl), and then incubated with different concentrations of Fe-Eu@SiO_2-FA NPs (0, 8, 15, 25, 35, 45, 60 and 100 mg L^{-1}) for 24 h at 37 °C in a 5% CO_2/95% air incubator. After the culture medium was removed, the cells were further incubated with the isotonic saline solution containing 250 μg mL^{-1} of MTT for 4 h in the incubator. After the supernatants were removed, the cells were dissolved in DMSO, and then the absorbance at 490 nm was measured.

In vivo distribution and toxicity evaluation

After three KM mice (female, ~20 g bodyweight) were anesthetized by 1.5% isoflurane in oxygen, they were injected with Fe-Eu@SiO_2-FA NPs (200 μL in physiological saline solution, 10 g L^{-1}) via tail vein. Then the mice were continuously monitored by sequential T_2-weighted MRI on a MesoMR23-060H-I Analyzing and Imaging system. In each experiment, the MR intensity analysis of ROIs was performed using the Horos V3.3.1 software for Mac.

To further examine the biocompatibility of Fe-Eu@SiO_2-FA nanoprobe, three KM mice (female, ~20 g bodyweight) were intravenously injected with 200 μL physiological saline solution containing 2.0 mg Fe-Eu@SiO_2-FA nanoprobe. After 24 h, they were sacrificed by dislocating cervical vertebra and the main organs (heart, liver, spleen, lung and kidney) were surgically dissected. The collected organs were fixed with 4% formaldehyde in PBS and embedded in paraffin. Then the standard hematoxylin and eosin (H&E) staining was carried out for histological analysis.

Statistical analysis

All the experiments were performed three times and the values were presented as the mean ± SD. Statistical comparison between two groups was determined by Student's test. All statistical analyses were conducted with Excel ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). A value of $P < 0.05$ was considered statistically significant.

2. Characterization of Fe-Eu@SiO_2-FA nanoprobe

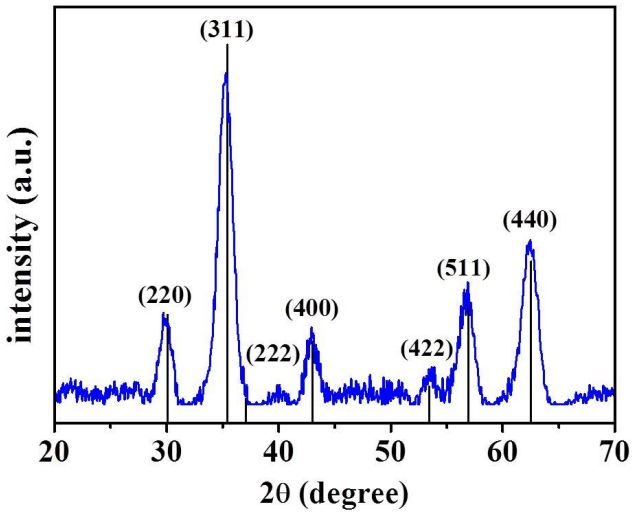

Fig. S1 The PXRD patterns of the synthesized Fe_3O_4 NPs.

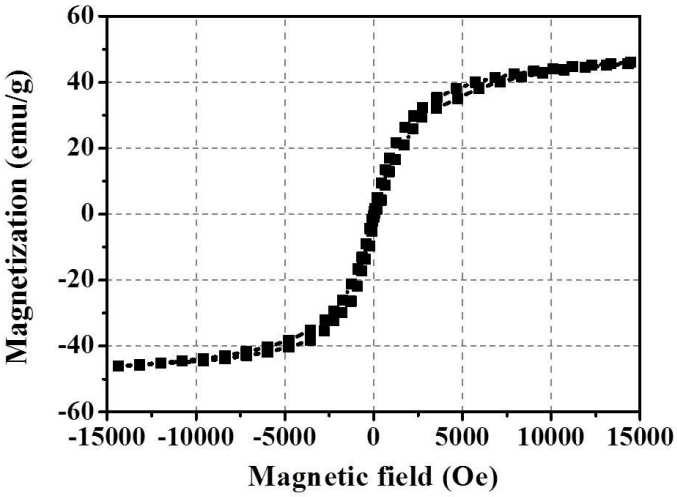

Fig. S2 Magnetic hysteresis loops of the synthesized Fe_3O_4 NPs at room temperature.

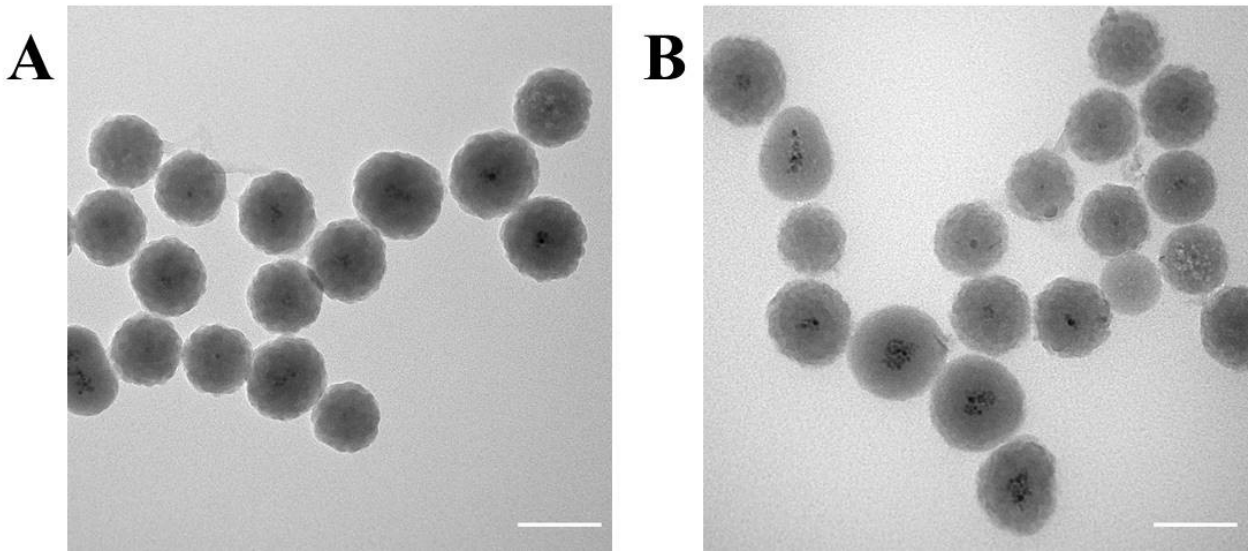

Fig. S3 TEM images of Fe-Eu@SiO_2 (A) and Fe-Eu@SiO_2-FA NPs (B). Scale bar: 100 nm

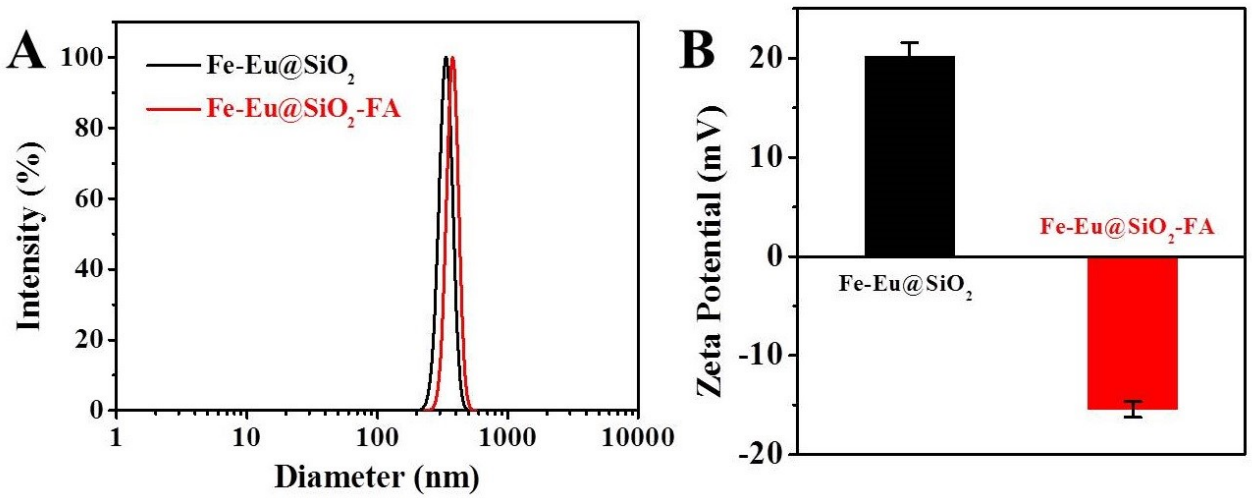

Fig. S4 (A) Hydrated particle size distributions of Fe-Eu@SiO_2 NPs (black) and Fe-Eu@SiO_2-FA NPs (red) in water by dynamic light scattering (DLS) measurement. (B) Zeta potential distributions of Fe-Eu@SiO_2 NPs (black) and Fe-Eu@SiO_2-FA NPs (red) in 0.01M Tris-HCl of pH = 7.4.

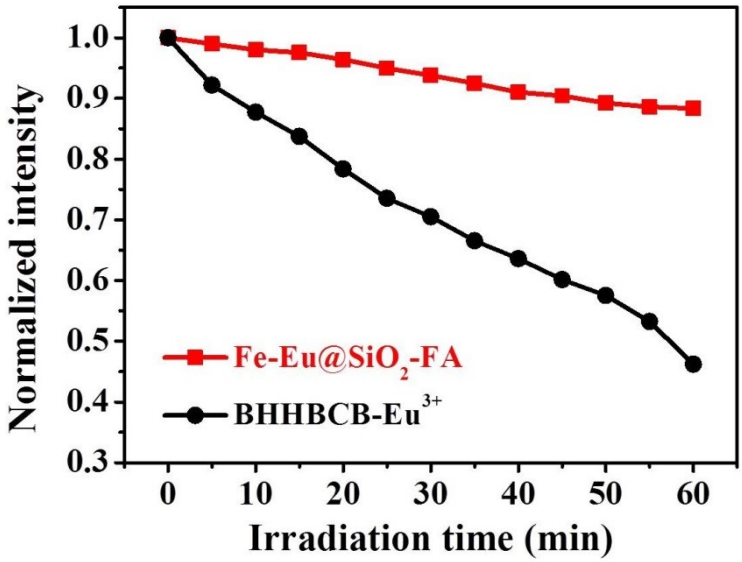

Fig. S5 Luminescence intensity changes of Fe-Eu@SiO_2-FA NPs (red) and free BHHBCB-Eu^{3+} complex (black) in 0.01M Tris-HCl of pH = 7.4 under continuous irradiation of 30 W deuterium lamp.

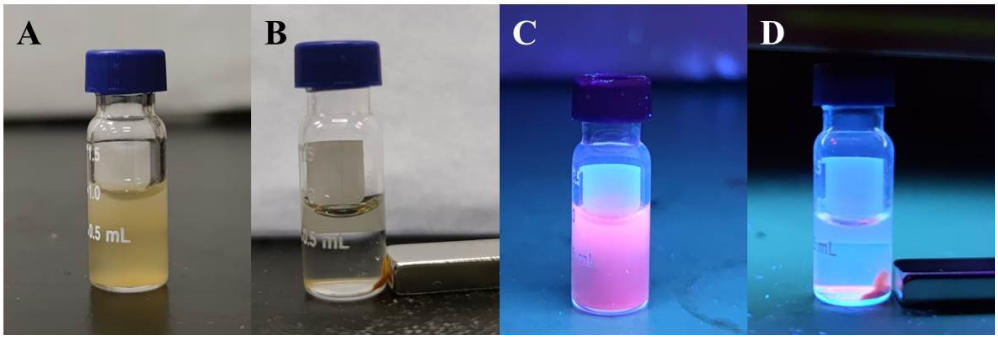

Fig. S6 Photographs of Fe-Eu@SiO_2-FA NPs suspension in water before (A) and after (B) being treated with a magnet. Photographs of Fe-Eu@SiO_2-FA NPs suspension under UV lamp excitation before (C) and after (D) treated with a magnet.

3. Cytotoxicity and biocompatibility of Fe-Eu@SiO_2-FA nanoprobe

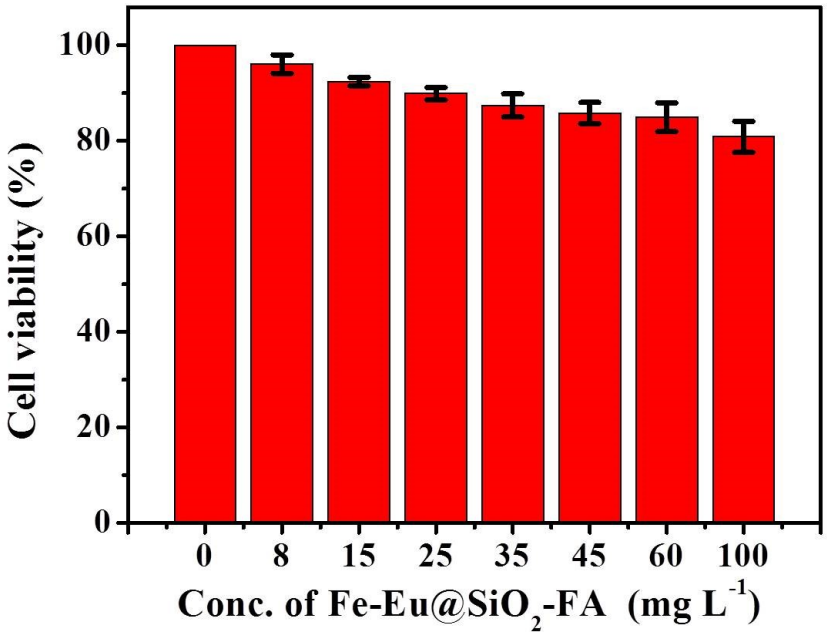

Fig. S7 Viabilities of HeLa cells after being incubated with different concentrations of Fe-Eu@SiO_2-FA NPs for 24 h.

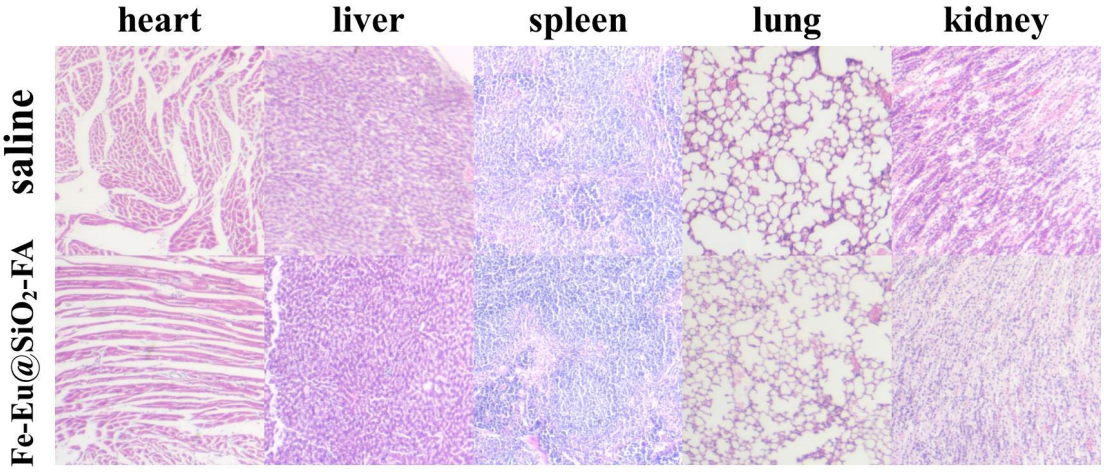

Fig. S8 Images of H&E stained main organs of the KM mice after intravenous injection of physiological saline and Fe-Eu@SiO_2-FA nanoprobe (200 μL, 10 mg mL^{-1} in PSS) for 24 h.

4. *In vivo* distribution of Fe-Eu@SiO_2-FA nanoprobe

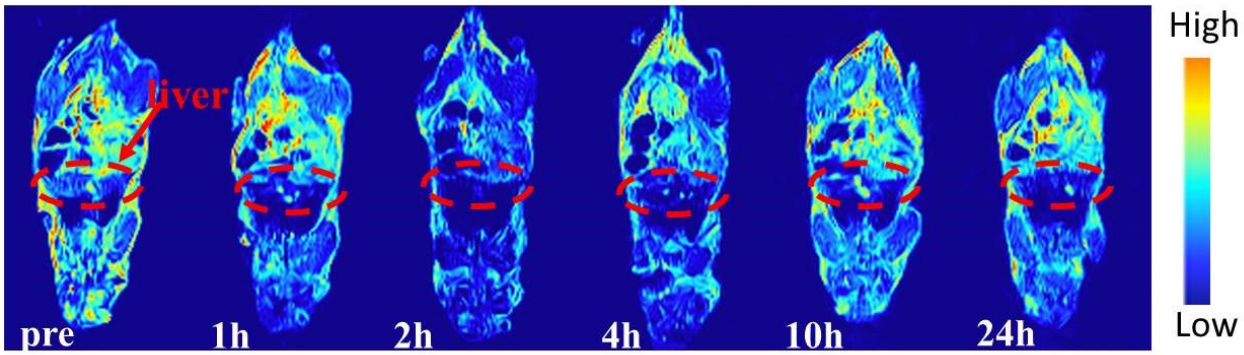

Fig. S9 *In vivo* T_2-weighted MR images of KM mice at different time intervals after intravenous injection of Fe-Eu@SiO_2-FA nanoprobe in longitudinal plane (TR = 2000, TE = 40, recorded at 310 K under 0.5 T magnetic field).

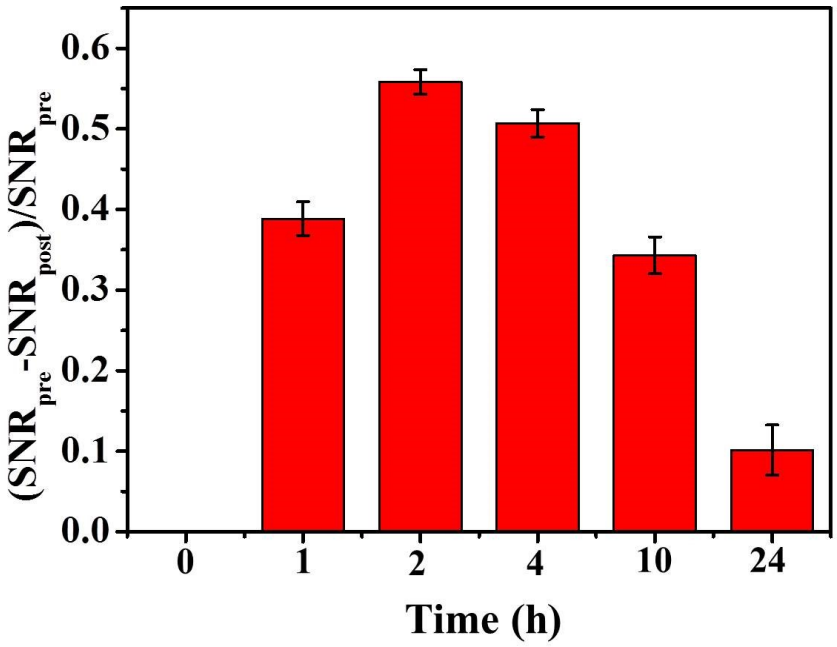

Fig. S10 Quantification results of liver contrast values in KM mice at different time intervals after injection of Fe-Eu@SiO_2-FA nanoprobe.

5. References

1. U. Schatzschneider, J. Niesel, I. Ott, R. Gust, H. Alborzinia and S. Wölfl, *ChemMedChem*, 2008, **3**, 1104-1109..