Electronic Supplementary Information

Facile synthesis of folic acid-functionalized iron oxide nanoparticles with ultrahigh relaxivity for targeted tumor MR imaging

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Part of experimental details:

Preparation of PEI-coated Fe_3O_4 NPs. PEI-coated Fe_3O_4 NPs (Fe_3O_4@PEI NPs) were synthesized *via* a mild reduction route according to the literature[1, 2] with some modifications. Briefly, $FeCl_3 \cdot 6H_2O$ (1.3 g) was dissolved in 20 mL water and placed into a 250-mL three-necked flask. Under vigorous magnetic stirring, the solution was bubbled with N_2 atmosphere for 15 min, and then freshly prepared sodium sulfite solution (0.2 g, 10 mL) was added slowly into the flask. After 30 min, PEI (0.5 g, 5 mL) and ammonia (2 mL) were successively added into the flask. The reaction mixture was vigorously stirred for 30 min at 60~70 ^oC, and then for another 1.5 h at room temperature. The product of Fe_3O_4@PEI NPs was magnetically collected and washed 3 times with water. Finally, the product was centrifuged (8000 rpm, 10 min) to remove the aggregated larger particles.

Acetylation of the remaining PEI surface amines on the surfaces of PEI-coated Fe_3O_4 NPs. The remaining PEI surface amines on the particle surfaces were acetylated according to the literature.[3, 4] Briefly, triethylamine (493 μL) was added into an aqueous solution of the Fe_3O_4@PEI-FI-PEG-FA NPs under vigorous vibrating using a shaker at room temperature. After 30 min, acetic anhydride (402 μL) was dropwise added into the above mixture solution and the reaction was continued for 1 day. The mixture was subjected to 3 cycles of magnetic separation/washing/redispersion steps to remove excess reactants and by-products. The formed final product (FA-functionalized Fe_3O_4 NPs) was dispersed in water and stored under 4 ^oC before further use.

Hemolysis assay. Fresh human blood stabilized with EDTA was kindly provided by Shanghai First People's Hospital (Shanghai, China) and used with the permission by the ethical committee of Shanghai First People's Hospital. Human red blood cells (HRBCs) were obtained according to our previous work.[5-7] For hemolysis assay, the purified HRBCs were 10 times diluted with PBS. Then, 0.1 mL diluted HRBC suspension was mixed with 0.9 mL water as a positive control, 0.9 mL PBS as a negative control, and 0.9 mL FA-functionalized Fe_3O_4 NPs dispersed in PBS at different Fe

concentrations (0.5-8.0 mM), respectively. Each mixture solution was gently vortexed and then kept still for 2 h at room temperature. After centrifugation (10 000 rpm, 1 min), the photo of each sample was taken and the absorbance of the supernatants (hemoglobin) for each sample was recorded by Lambda 25 UV-vis spectrophotometer. The hemolysis percentage of each sample was calculated by dividing the difference in absorbances at 541 nm between the samples and the negative control by the difference in absorbances at 541 nm between the positive and negative controls.

Confocal microscopic observation. The cellular uptake of the FA-functionalized Fe_3O_4 NPs was also evaluated by confocal microscopy (Carl Zeiss LSM 700, Jena, Germany) according to our previous report.[8] Coverslips with a diameter of 14 mm were pretreated and fixed in a 12-well tissue culture plate according to our previous report.[8] HeLa-HFAR cells were seeded at a density of 5×10^4 cells per well with 1 mL DMEM medium and cultured at 37 °C and 5% CO_2 overnight to allow the cells to be well attached. After that, the medium of cells was removed carefully and 1 mL fresh medium containing PBS (control) or the FA-functionalized Fe_3O_4 NPs at an Fe concentration of 0.4 mM was added into each well. After incubation at 37 °C and 5% CO_2 for 4 h, the cells were washed 3 times with PBS, fixed with glutaraldehyde (2.5%), and counterstained with Hoechst 33342 (1 μg/mL) using a standard procedure. Finally, the cells on the coverslips were imaged using a 63 × oil-immersion objective lens. HeLa-LFAR cells were also treated and observed in a similar manner for comparison.

References:

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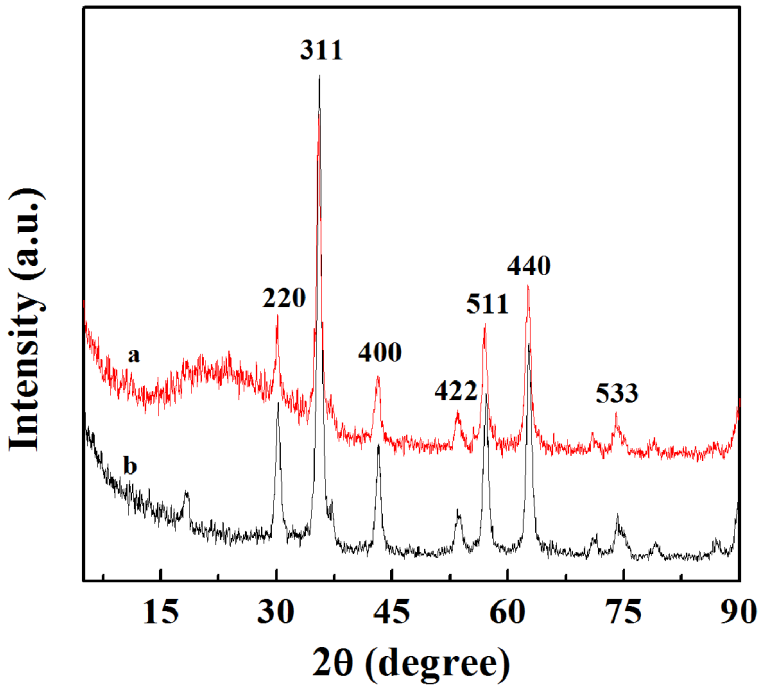

Fig. S1 XRD pattern of the formed Fe_3O_4@PEI NPs (a) and naked Fe_3O_4 NPs (b).

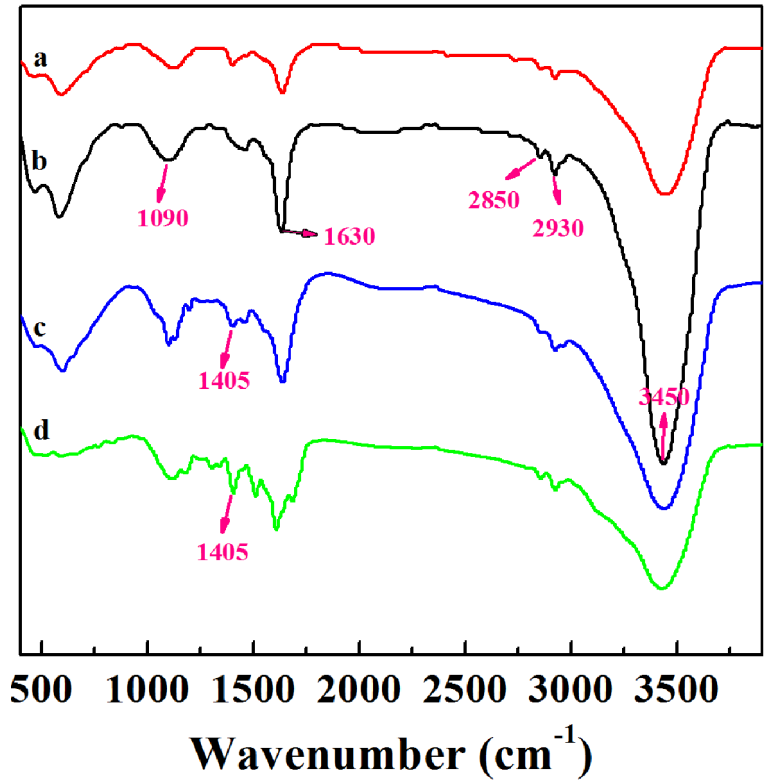

Fig. S2 FTIR spectra of the naked Fe_3O_4 NPs (a), Fe_3O_4@PEI NPs (b), Fe_3O_4@PEI-PEG-FA NPs (c), and FA (d).

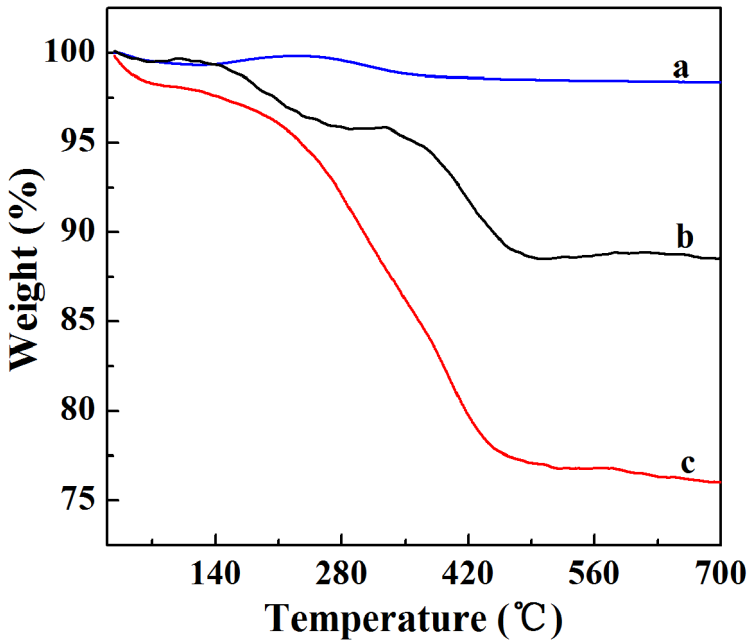

Fig. S3 TGA curves of naked Fe_3O_4 (a), Fe_3O_4@PEI (b), and Fe_3O_4@PEI-PEG-FA (c) NPs.

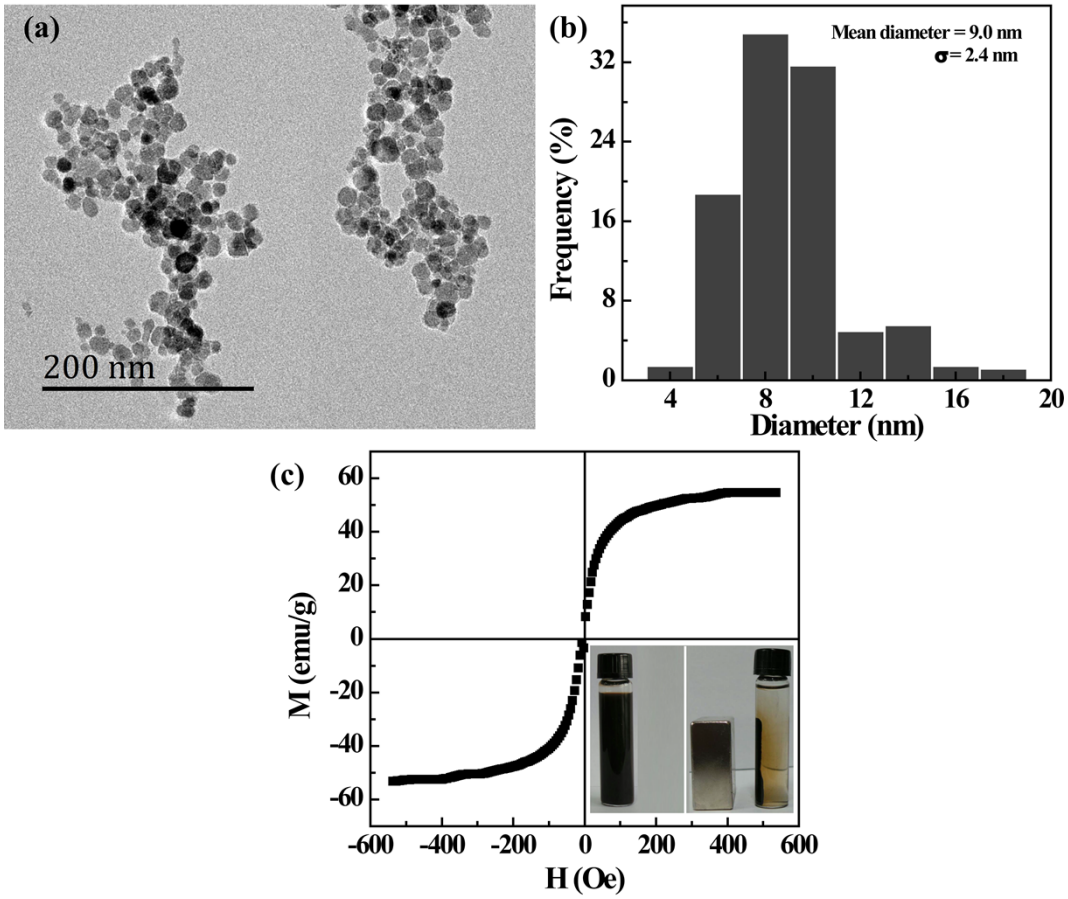

Fig. S4 TEM image (a) and size distribution histogram (b) of the Fe_3O_4@PEI NPs and room temperature magnetization curve of Fe_3O_4@PEI NPs (c). Inset of (c) is the digital picture of the aqueous suspension of Fe_3O_4@PEI NPs before and after exposed to a magnetic field.

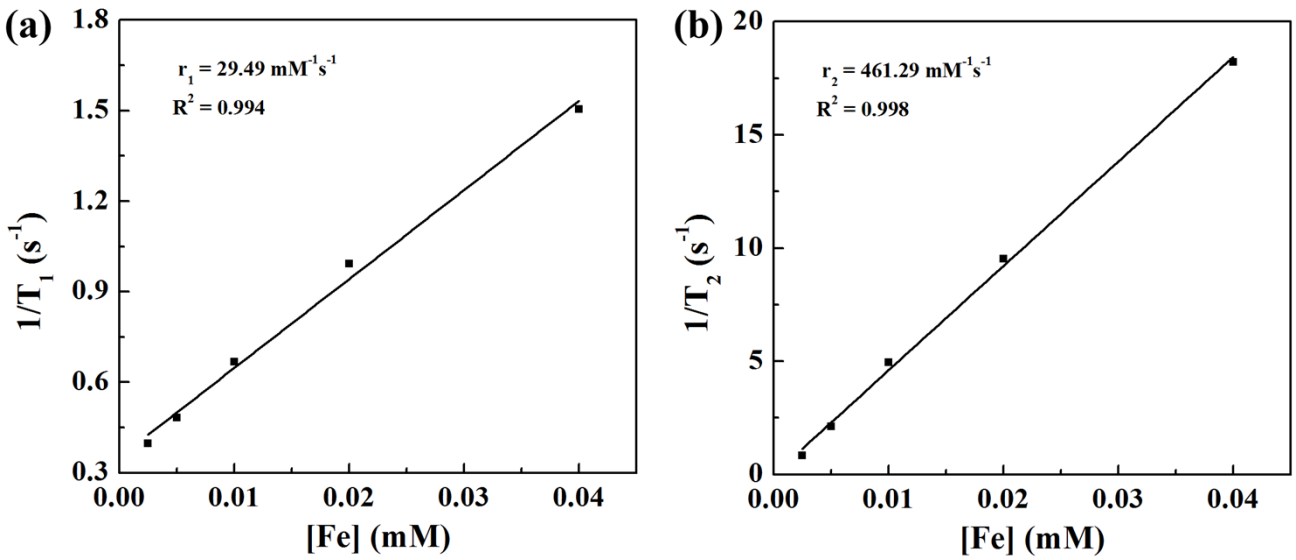

Fig. S5 Linear fitting of $1/T_1$ (a) and $1/T_2$ (b) of the Fe_3O_4@PEI NPs as a function of Fe concentration.

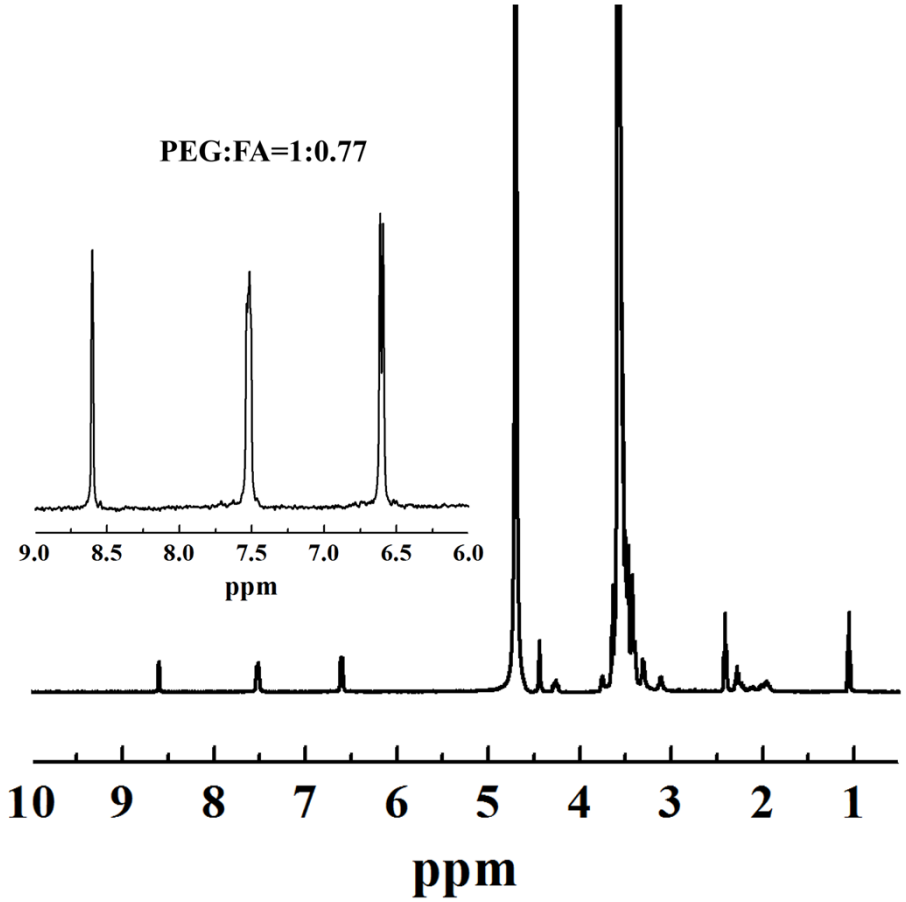

Fig. S6 ^1H NMR spectrum of PEGylated FA dissolved in D_2O.

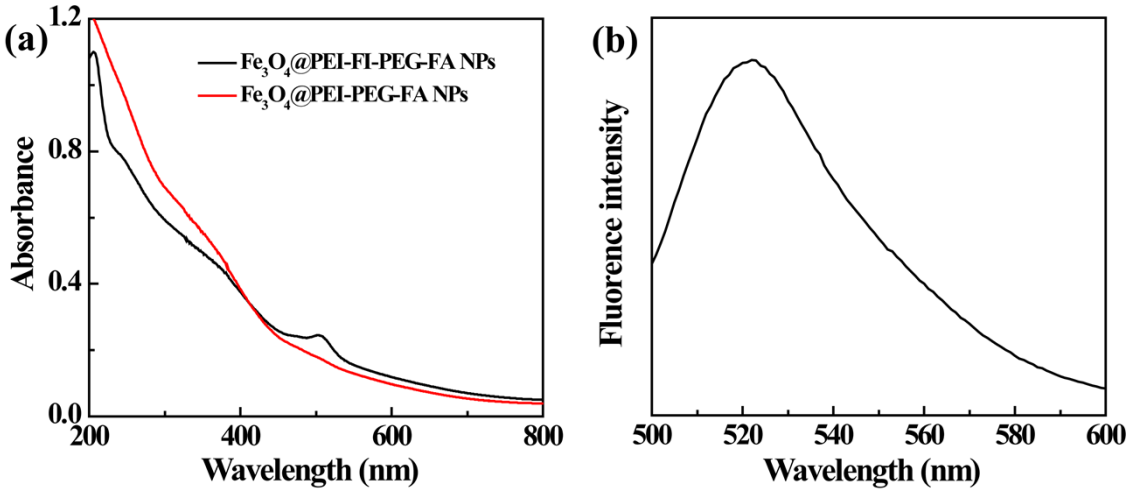

Fig. S7 (a) UV-vis spectra of the Fe_3O_4@PEI-PEG-FA NPs and Fe_3O_4@PEI-FI-PEG-FA NPs dispersed in water; (b) fluorescent emission spectrum of the Fe_3O_4@PEI-FI-PEG-FA NPs dispersed in water.

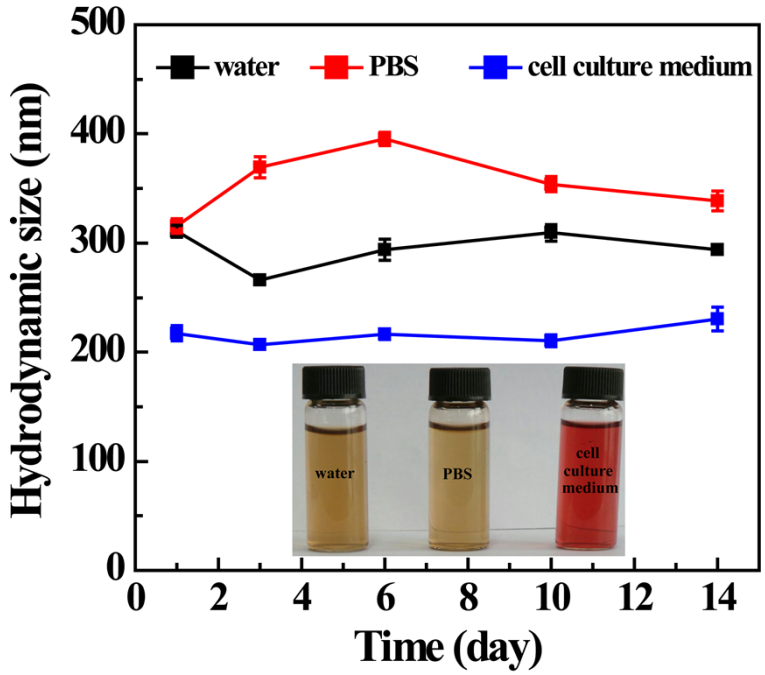

Fig. S8 Hydrodynamic size of the FA-functionalized Fe_3O_4 NPs dispersed in water, PBS buffer, or cell culture medium at different storage time periods. Shown in the inset is the photo of the particles dispersed in water, PBS, and cell culture medium for a period of one month.

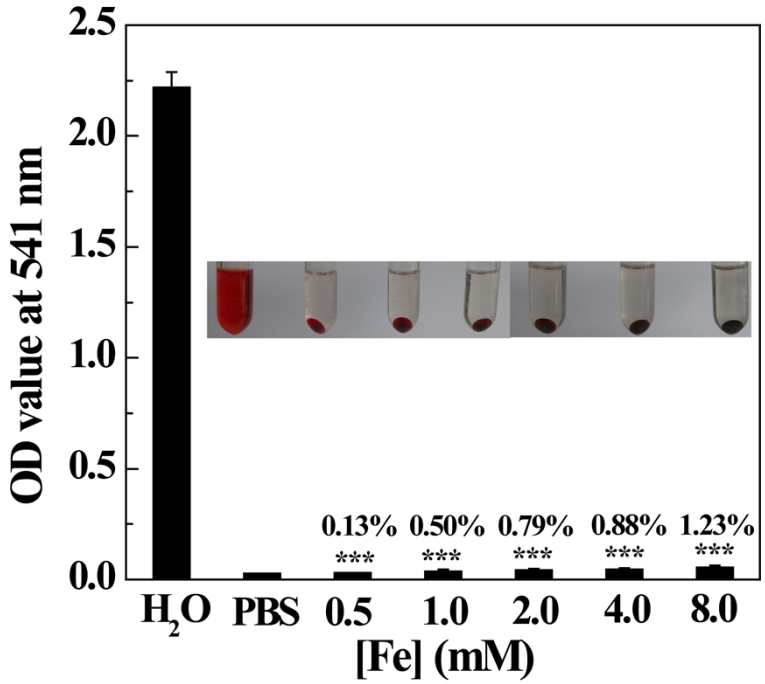

Fig. S9 Hemolytical activity of the FA-functionalized Fe_3O_4 NPs at different Fe concentrations. PBS and water were used as negative and positive control, respectively. The insets show the photograph of the HRBCs exposed to water, PBS, and PBS containing the particles at different Fe concentrations for 2 h, followed by centrifugation. The percentage values shown in the figure are the hemolysis percentages.

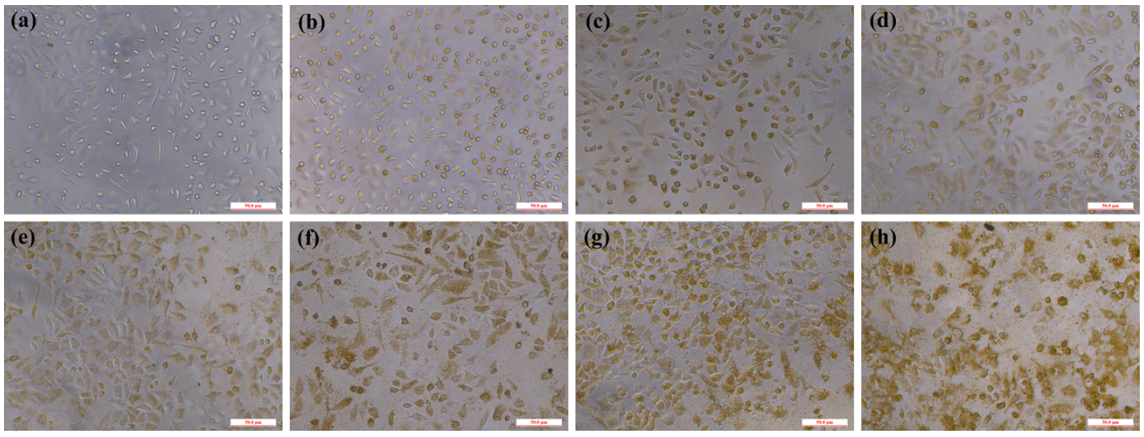

Fig. S10 Phase contrast microscopic images of HeLa cells treated with PBS (a) and FA-functionalized Fe_3O_4 NPs at the Fe concentrations of 0.2 mM (b), 0.4 mM (c), 0.6 mM (d), 0.8 mM (e), 1.0 mM (f), 1.5 mM (g), and 2.0 mM (h), respectively for 24 h. The scale bar in each panel represents 50 μm.

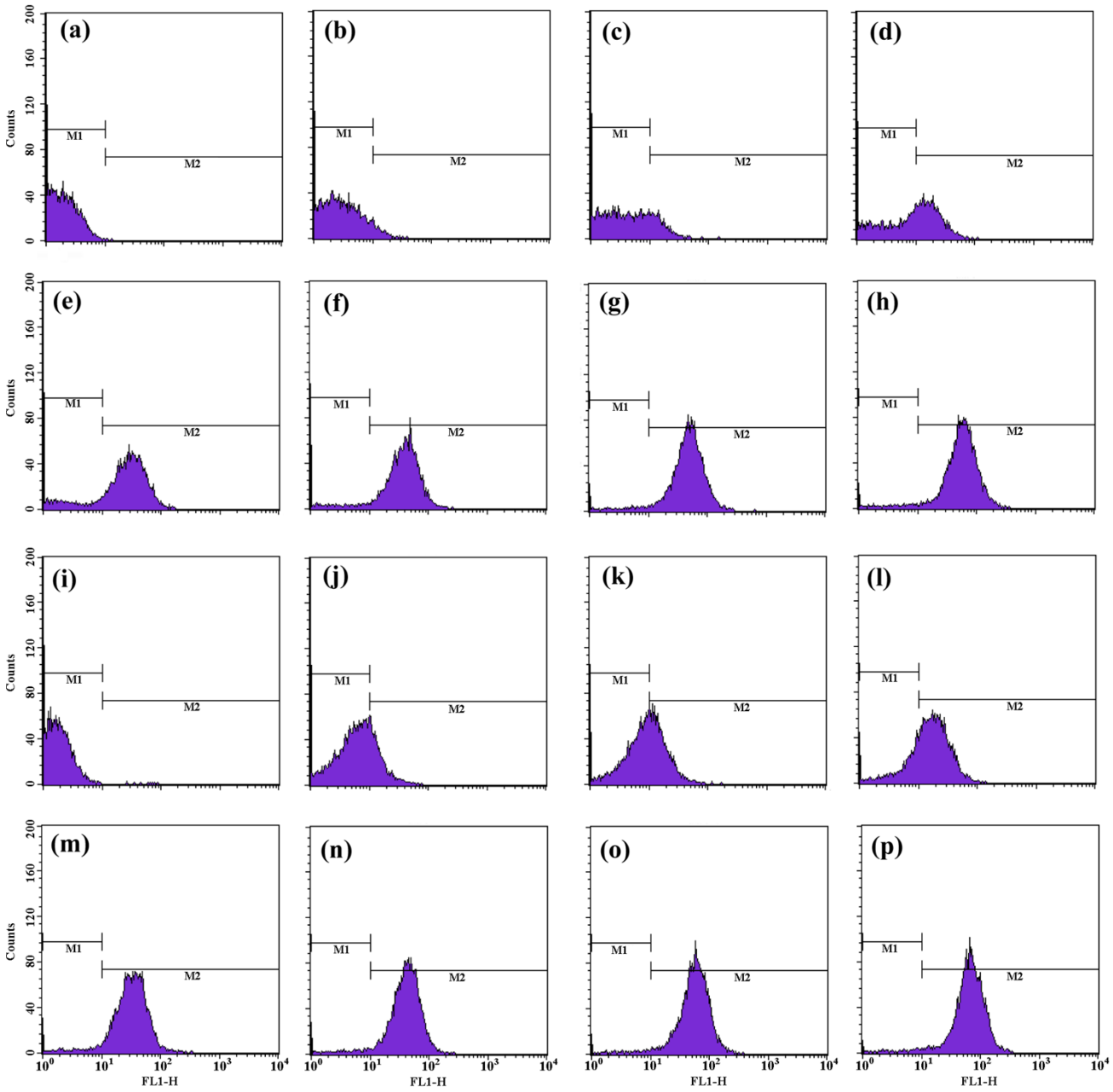

Fig. S11 Flow cytometric analysis of HeLa-HFAR cells treated with PBS (a), FA-functionalized Fe_3O_4 NPs at the Fe concentrations of 0.05 mM (b), 0.1 mM (c), 0.2 mM (d), 0.4 mM (e), 0.6 mM (f), 0.8 mM (g), 1.0 mM (h) and HeLa-LFAR cells treated with PBS (i), FA-functionalized Fe_3O_4 NPs at the Fe concentrations of 0.05 mM (j), 0.1 mM (k), 0.2 mM (l), 0.4 mM (m), 0.6 mM (n), 0.8 mM (o), 1.0 mM (p), respectively for 4 h.

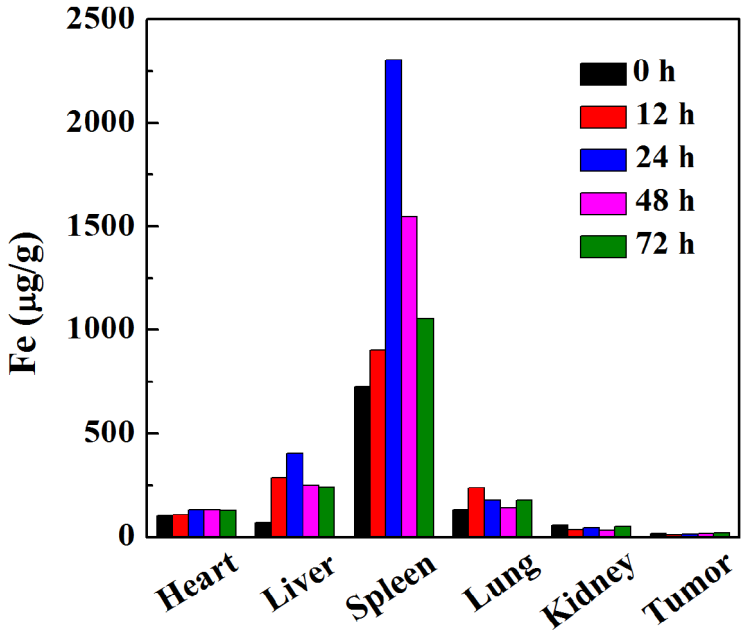

Fig. S12 *In vivo* biodistribution of Fe element in the major organs of the tumor-bearing mice including heart, liver, spleen, lung, kidney, and tumor at different time points post intravenous injection of the FA-functionalized Fe_3O_4 NPs ([Fe] = 80 mM, in 0.1 mL PBS).

Table S1 Zeta potentials and hydrodynamic sizes of the naked Fe_3O_4, Fe_3O_4@PEI, Fe_3O_4@PEI-FI-PEG-FA, and Fe_3O_4@PEI.Ac-FI-PEG-FA NPs. Data are provided as mean ± SD (n = 3).

Materials	Zeta potential (mV)	Hydrodynamic size (d. nm)	Polydispersity index (PDI)
Naked Fe_3O_4 NPs	-9.0 ± 0.4	1177.6 ± 60.9	0.42 ± 0.15
Fe_3O_4@PEI NPs	52.8 ± 1.9	164.2 ± 4.3	0.11 ± 0.04
Fe_3O_4@PEI-FI-PEG-FA NPs	39.4 ± 1.6	284.7 ± 5.9	0.19 ± 0.01
Fe_3O_4@PEI.Ac-FI-PEG-FA NPs	24.2 ± 2.3	310.5 ± 8.7	0.14 ± 0.03