Supporting Information

Polyethyleneimine-Coated Manganese Oxide Nanoparticles for Targeted Tumor PET/MR Imaging

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Additional Experimental Details:

Synthesis of the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs. We used a solvothermal method to prepare the PEI−NH_2−Mn_3O_4 NPs. In detail, $Mn(acac)_2$ (253.0 mg) was dissolved in 10 mL DEG reacted with PEI (100 mg) by magnetic stirring. After 1 h continuous stir of mixture in air, the mixture was autoclaved (KH-50 Autoclave, Shanghai Yuying Instrument Co., Ltd., Shanghai) for 24 h in a sealed vessel (50 mL) under a 2 bar gauge pressure at 180ºC. Then the mixture was cooled down to room temperature. Black precipitate was collected through centrifugation at 8000 rmp for 5 min, followed by dialysis to purify the formed PEI−NH_2−Mn_3O_4 NPs. Briefly, the reaction mixture was extensively dialyzed against water (nine times, 8 L) through a 14,000 MWCO membrane for 3 days to remove the excess of reactants, followed by lyophilization to acquire the PEI−NH_2−Mn_3O_4 NPs.

FA with 2 molar equivalents of NH_2−PEG−COOH (26.484 mg, 0.060 mmol) dissolved in 3 mL DMSO was mixed with a 3 mL of DMSO solution containing EDC (11.502 mg, 0.060 mmol), and the reaction mixture was stirred for 3 h to activate the carboxylic acid group of FA. The activated FA was then added to a 5 mL of DMSO solution containing NH_2−PEG−COOH (60 mg, 0.030 mmol) under vigorous magnetic stirring at room temperature and reacted for 24 h. The reaction mixture was dialyzed against phosphate buffered saline (PBS, 3 times, 4 L) and water (3 times, 4 L) with a 1 000 MWCO membrane for 3 days for purification, followed by lyophilization to obtain the powder of FA−PEG−COOH.

Then the obtained PEI−NH_2−Mn_3O_4 NPs (50.53 mg, 0.00151 mmol) were dissolved in 5.0 mL DMSO to mix with a DMSO solution of 7 molar equivalents of FI (3.0 mL, 4.12 mg, 0.01058 mmol), and reacted for 24 h under magnetic stirring at room temperature. NOTA−NHS (11.99 mg,

0.01816 mmol, in 3.0 mL DMSO) with 12 molar equivalents of the PEI−NH_2−Mn_3O_4 NPs was then added into the mixture of the FI−PEI−NH_2−Mn_3O_4 NPs and stirred continuously for 24 h to form the NOTA−FI−PEI−NH_2−Mn_3O_4 NPs. PEGylated FA was modified on the surface of the NOTA−FI−PEI−NH_2−Mn_3O_4 NPs subsequently. Briefly, FA−PEG−COOH (74.96 mg, in 5.0 mL DMSO) with 22 molar equivalents of the PEI−NH_2−Mn_3O_4 NPs was activated with EDC (114.0 mg, in 5.0 mL DMSO) for 3 h, then mixed with the formed NOTA−FI−PEI−NH_2−Mn_3O_4 NPs through stirring for 3 days to provide the raw product of the NOTA−FA−FI−PEG−PEI−NH_2−Mn_3O_4 NPs. The raw product the NOTA−FA−FI−PEG−PEI−NH_2−Mn_3O_4 NPs were subsequently acetylated and purified *via* dialysis. In detail, triethylamine (742 μL) was added into the NOTA−FA−FI−PEG−PEI−NH_2−Mn_3O_4 NPs solution, then completely mixed and stirred for 30 min. Excess acetic anhydride (420 μL, 5 molar equivalents of PEI terminal amines) was then added dropwise into the above mixture solution. The reaction continued for 24 h. The final reaction mixture was purified and lyophilized to obtain the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs according to the procedure similar to the purification of FA−PEG−COOH, except that the dialysis membrane with MWCO of 14 000 was used. In order to compare with the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs, the NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs without FA conjugation was also synthesized as a control.

In vitro **biocompatibility test.** In order to assess the biocompatibility of the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs and the NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs, MTT assay was utilized to test the cytotoxicity of the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs and the NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs towards HeLa cells *in vitro*. Briefly, HeLa cells (1×10^4 cells/well) were seeded into a 96-well plate. After 24 h incubation to bring the cells to confluence, the medium was replaced with 200 μL fresh medium containing the

NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs or the NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs with different Mn concentrations (0, 5, 10, 25, 50, and 100 μg/mL, respectively). After 24 h incubation, an MTT solution (20 μL in PBS, 5 mg/mL) was added to each well and the cells were incubated for another 4 h at 37ºC. After that, the medium in each well was removed, and DMSO (200 μL) was added to dissolve the formed formazan crystals. The assays were carried out according to the manufacturer's instructions at 570 nm using a Thermo Scientific Multiskan MK3 ELISA reader (Thermo Scientific, Waltham, MA). For each sample, mean and standard deviation for the triplicate wells were analyzed.

Leica DM IL LED inverted phase contrast microscope was also utilized to observe the cell morphology after incubation with the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs or the NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs at different Mn concentrations (0, 5, 10, 25, 50, and 100 μg/mL, respectively) for 24 h. For all samples, 200× magnification was set.

Confocal microscopy. The targeting specificity of the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs by HeLa cells was further evaluated by confocal microscopy (Carl Zeiss LSM 700, Jena, Germany) according to our previous reports [1-2]. Briefly, cover slips with a diameter of 14 mm were pretreated with 5% HCl, 30% HNO_3, and 75% alcohol and then fixed in a 12-well culture plate. HeLa cells (8×10^4) were seeded into each well with 1 mL fresh medium and cultured at 37ºC and 5% CO_2 overnight to allow the cells to attach onto the cover slips. Then, the medium was replaced with 1 mL fresh medium containing PBS (control), the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs or the NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs at different Mn concentrations (5 and 10 μg/mL, respectively). After 4 h incubation at 37ºC and 5% CO_2, the cells were rinsed with PBS for 3 times, fixed with glutaraldehyde (2.5%) for 20 min at 4ºC, and counterstained with Hoechst 33342 (1 μg/mL) for 20 min at 37ºC using a standard procedure. The FI fluorescence was excited with a 488 nm argon blue laser, and the emission was collected through a 505-525 nm barrier filter. The optical section thickness was set at 5 mm. 63× oil-immersion objective lens was utilized to scan all samples.

Table S1. ζ potential and hydrodynamic size of the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs and the NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs.

Sample	ζ potential (mV)	Hydrodynamic size (nm)	Polydispersity index
NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs	17.6 ± 1.9	249.9 ± 11.5	0.429
NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs	14.1 ± 1.6	476.5 ± 13.5	0.659

Table S2. Decay-corrected biodistribution of the ^{64}Cu−NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs and the ^{64}Cu−NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs at 18 h post-injection in tumor-bearing mice quantified by microPET imaging.[a]

Tissue	^{64}Cu−NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs	^{64}Cu−NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs + FA blocking	^{64}Cu−NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs
	percent injected dose/gram (%ID/g)		
Tumor (T)	3.58 ± 0.28	1.58 ± 0.47	1.49 ± 0.19
Muscle (M)	0.67 ± 0.27	0.57 ± 0.07	0.41 ± 0.08
Liver (L)	12.10 ± 1.47	15.11 ± 0.53	14.92 ± 0.92
Kidneys (K)	13.21 ± 1.83	16.67 ± 0.65	15.66 ± 0.58
	tumor-to-normal tissue uptake ratio		
T/M	5.35 ± 0.31	2.78 ± 0.68	3.64 ± 0.16
T/L	0.30 ± 0.06	0.10 ± 0.03	0.10 ± 0.01
T/K	0.28 ± 0.05	0.09 ± 0.02	0.10 ± 0.01

[a] The results are presented as mean ± SD (n = 4/group).

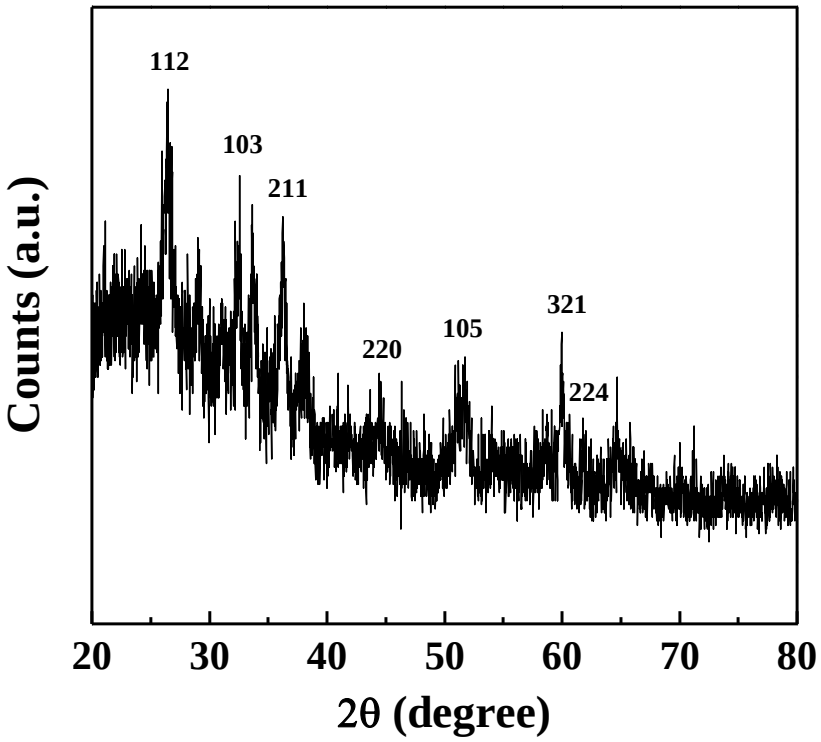

Figure S1. XRD pattern of the PEI−NH_2−Mn_3O_4 NPs.

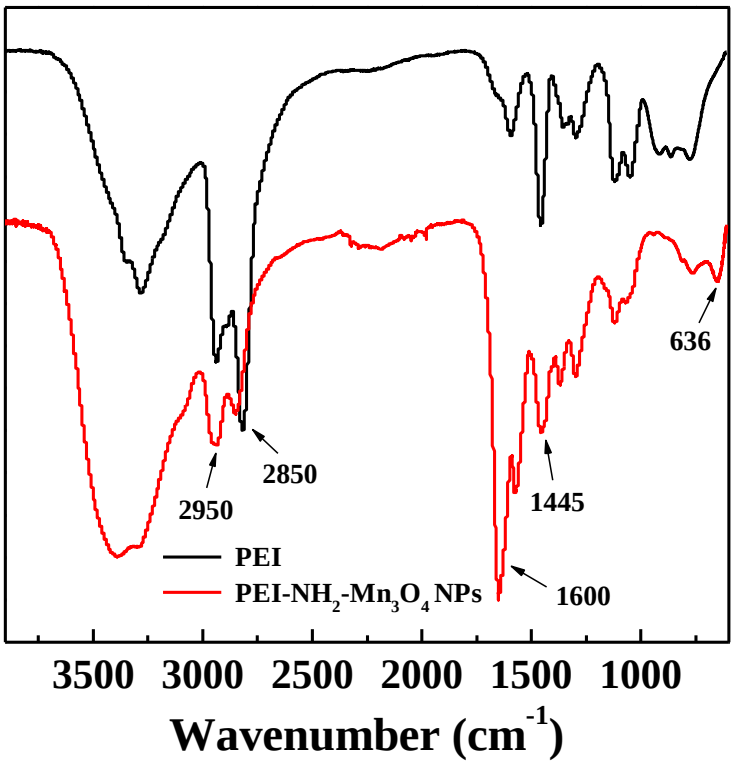

Figure S2. FTIR spectra of PEI and the PEI−NH_2−Mn_3O_4 NPs.

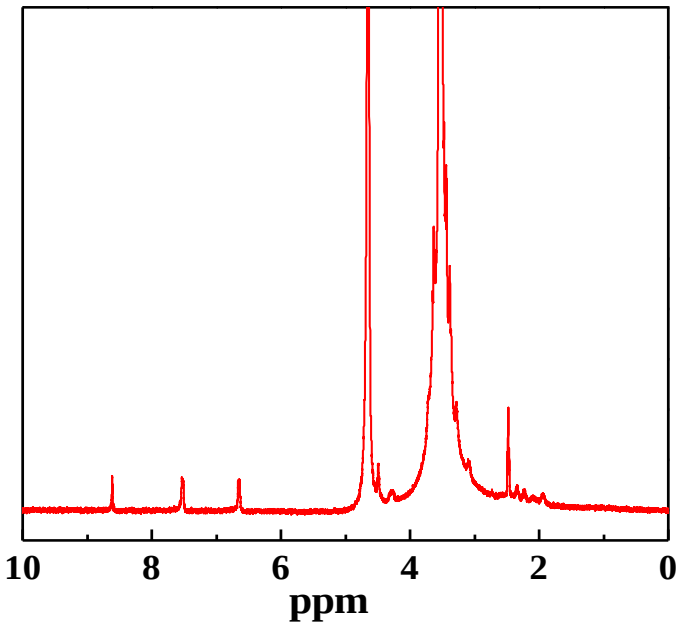

Figure S3. ^{1}H NMR spectrum of FA−PEG−COOH.

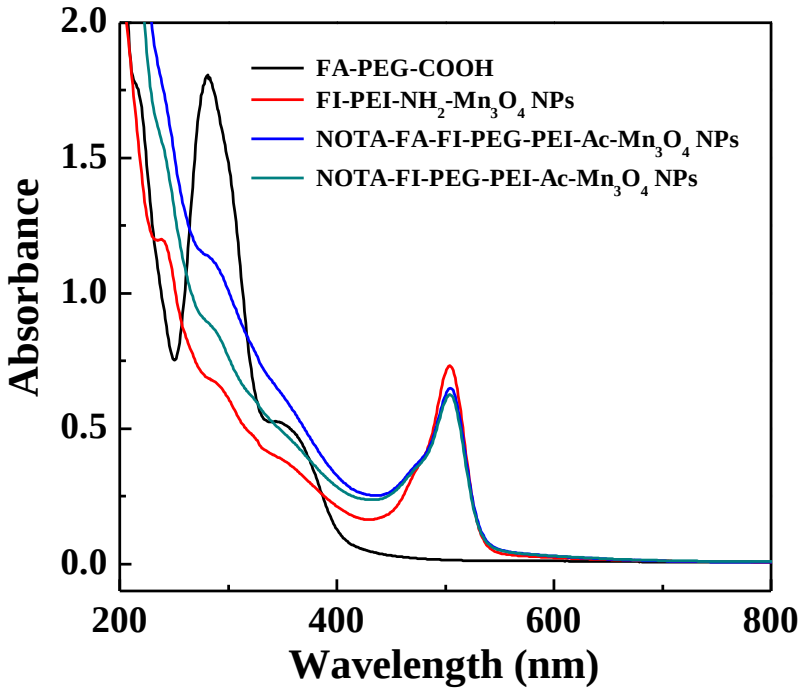

Figure S4. UV-Vis spectra of FA−PEG−COOH, the FI−PEI−NH_2−Mn_3O_4 NPs, the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs, and the NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs dispersed in water.

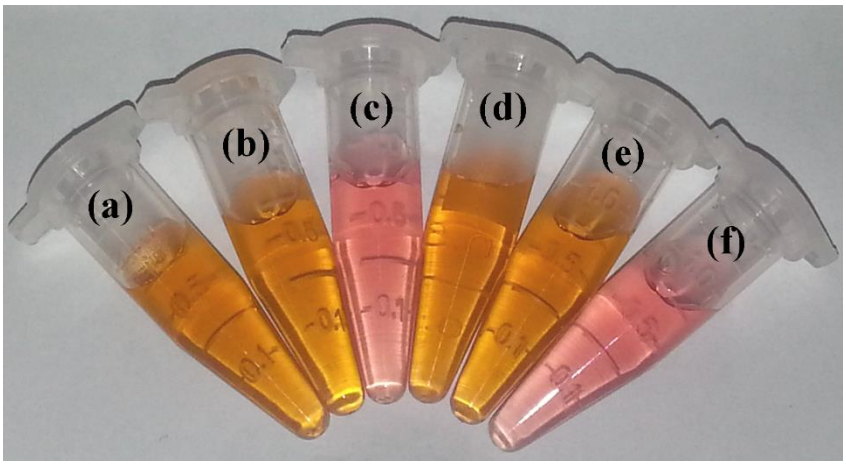

Figure S5. Photographs of the NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs (a-c) and the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs (d-f) dispersed in water (a, d), PBS (b, e), and cell culture medium (c, f) for three weeks.

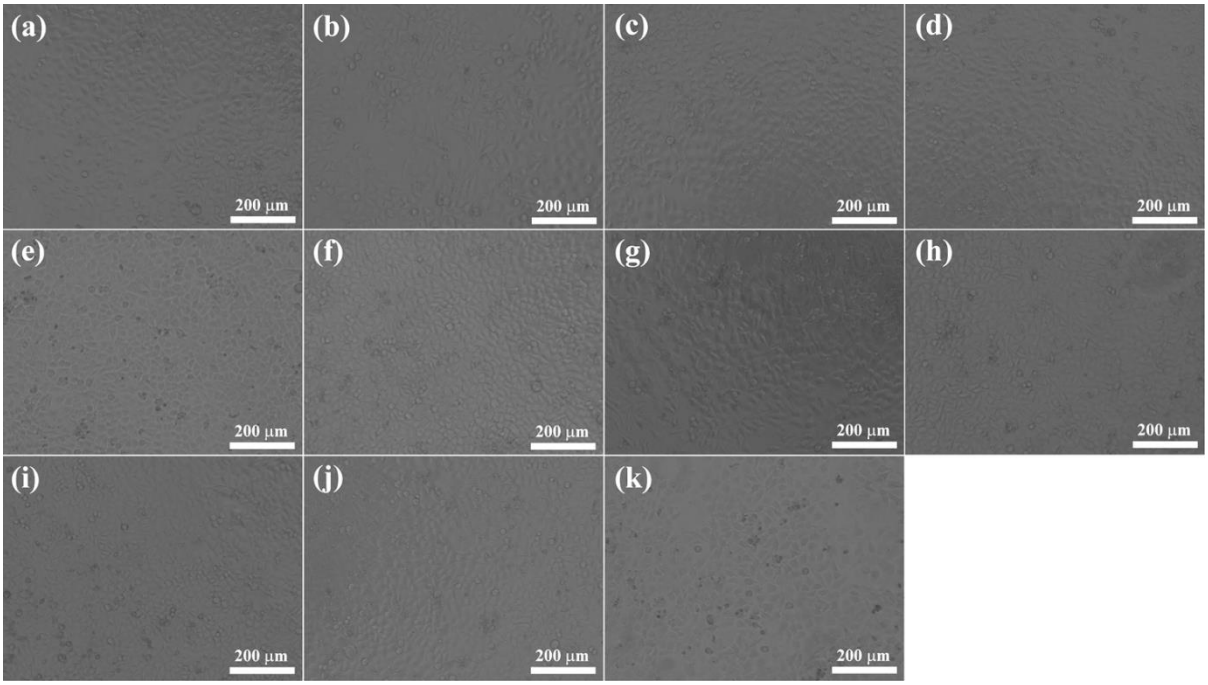

Figure S6. Micrographs of HeLa cells treated with PBS (a), the NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs at the Mn concentration of 5 (b), 10 (c), 25 (d), 50 (e), and 100 (f) μg/mL, the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs at the Mn concentration of 5 (g), 10 (h), 25 (i), 50 (j), and 100 (k) μg/mL for 24 h, respectively.

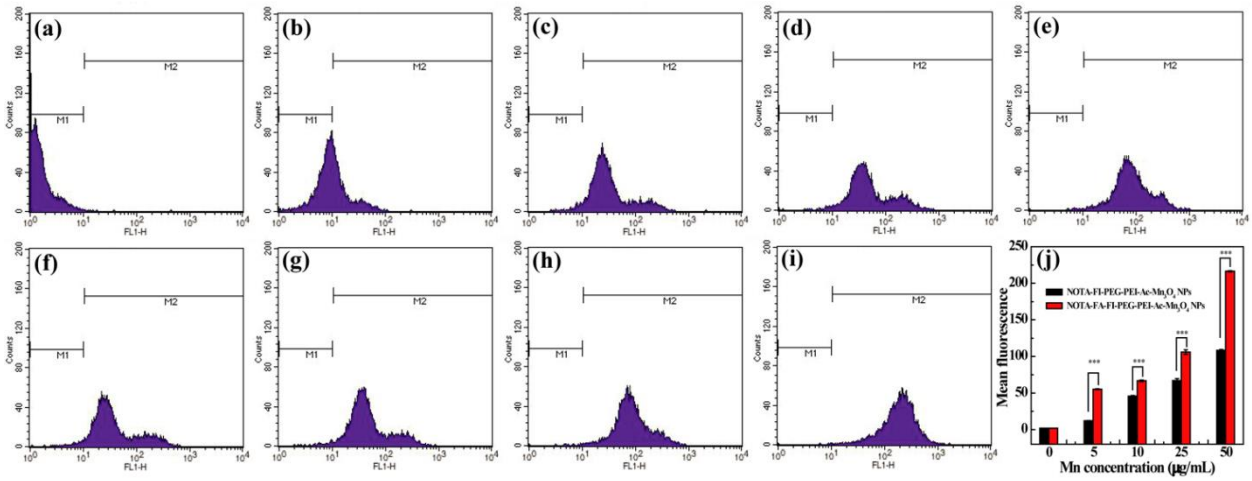

Figure S7. Flow cytometric analysis of HeLa cells treated with PBS (a), the NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs at the Mn concentration of 5 (b), 10 (c), 25 (d) and 50 (e) μg/mL and the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs at the Mn concentration of 5 (f), 10 (g), 25 (h) and 50 (i) μg/mL, respectively for 4 h. (j) The graph summarizes the binding of functionalized PEI nanomaterials with HeLa cells.

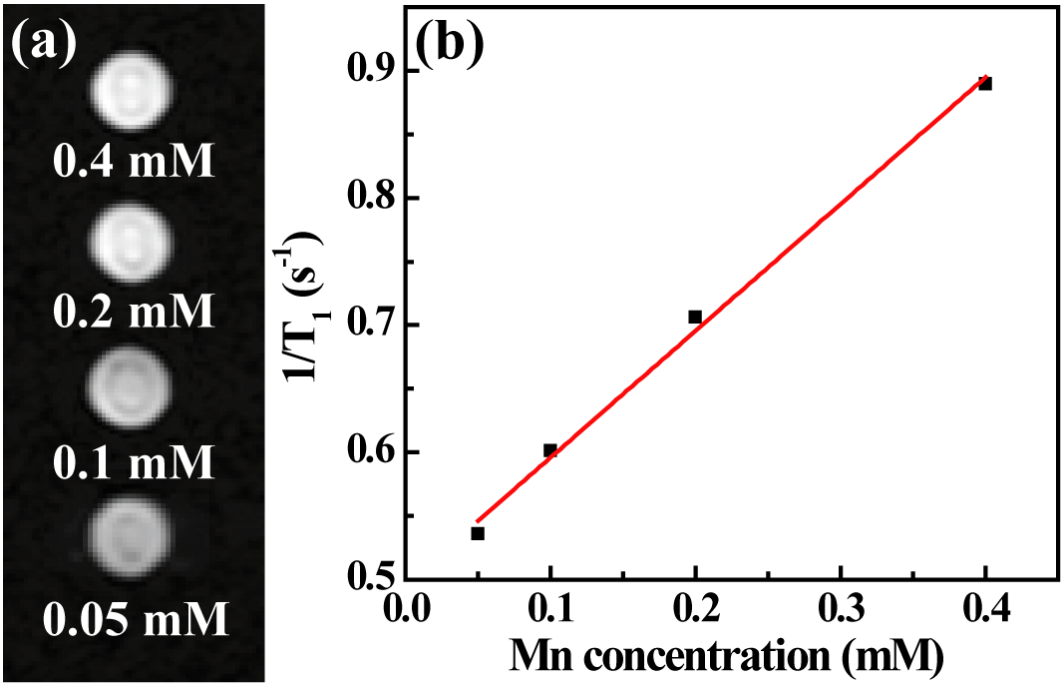

Figure S8. T_1 MR images (a) and linear fitting of inverse T_1 ($1/T_1$) (b) of the NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs as a function of Mn concentration.

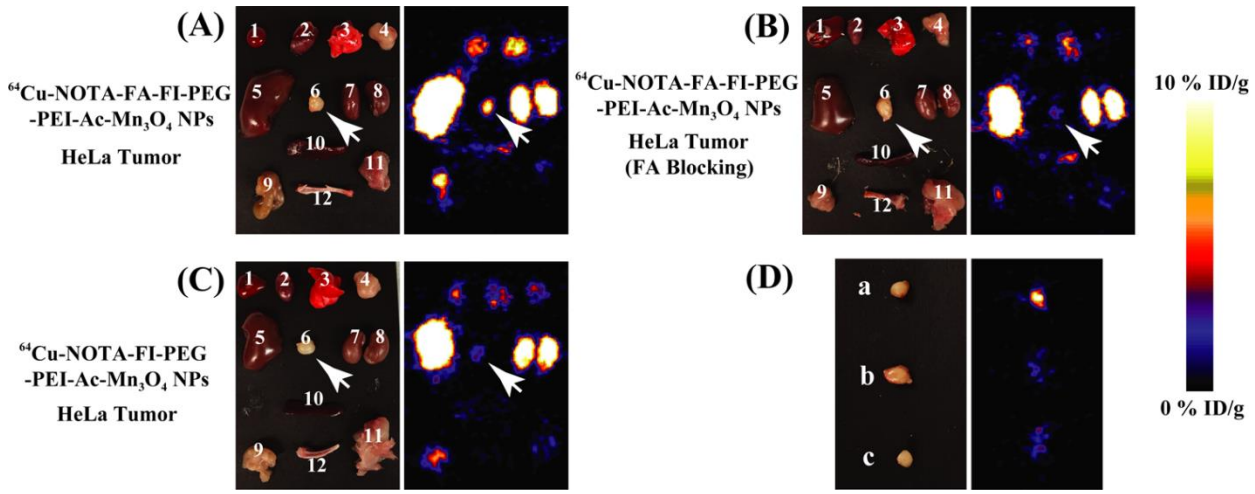

Figure S9. *Ex vivo* microPET imaging of tumor and normal tissues with the ^{64}Cu−NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs in (A) HeLa tumor model, (B) FR-blocked HeLa tumor model, and (C) with the ^{64}Cu−NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs at 24 h post-injection: *1* blood, *2* heart, *3* lung, *4* pancreas, *5* liver, *6* tumor, *7*,*8* kidneys, *9* intestine, *10* spleen, *11muscle*, and *12* bone. (D) microPET imaging of HeLa tumors: (a) the ^{64}Cu−NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs, (b) the ^{64}Cu−NOTA−FA−FI−PEG−PEI−Ac−Mn_3O_4 NPs with FA-blocking, and (c) the ^{64}Cu−NOTA−FI−PEG−PEI−Ac−Mn_3O_4 NPs, respectively. Tumors are indicated by arrows.

References

(1) Li, J.; He, Y.; Sun, W.; Luo, Y.; Cai, H.; Pan, Y.; Shen, M.; Xia, J.; Shi, X. Hyaluronic acid-Modified Hydrothermally Synthesized Iron Oxide Nanoparticles for Targeted Tumor MR Imaging. *Biomaterials* **2014**, *35*, 3666−3677.

(2) Li, J.; Zheng, L.; Cai, H.; Sun, W.; Shen, M.; Zhang, G.; Shi, X. Polyethyleneimine-Mediated Synthesis of Folic Acid-Targeted Iron Oxide Nanoparticles for *In Vivo* Tumor MR Imaging. *Biomaterials* **2013**, *34*, 8382−8392.