Supporting information

Active-target T_1-weighted MR Imaging of Tiny Hepatic Tumor *via* RGD Modified Ultra-small Fe_3O_4 Nanoprobes

Zhengyang Jia,[1,⊥] Lina Song,[1,⊥] Fengchao Zang,[2] Jiacheng Song,[3] Wei Zhang,[1] Changzhi Yan,[1] Jun Xie,[1] Zhanlong Ma,[3] Ming Ma,[1] Gaojun Teng,[2] Ning Gu[1,*] and Yu Zhang[1,*]

[1]State Key Laboratory of Bioelectronics, Jiangsu Key Laboratory for Biomaterials and Devices, School of Biological Science and Medical Engineering & Collaborative Innovation Center of Suzhou Nano Science and Technology, Southeast University, Nanjing 210096, China

[2]Jiangsu Key Laboratory of Molecular and Functional Imaging, Medical School, Southeast University, Nanjing 210009, China

[3]Department of Radiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

*Corresponding author: Yu Zhang

E-mail: zhangyu@seu.edu.cn. Tel: +86 25 8327 2496. Fax: +86 25 8327 2496.

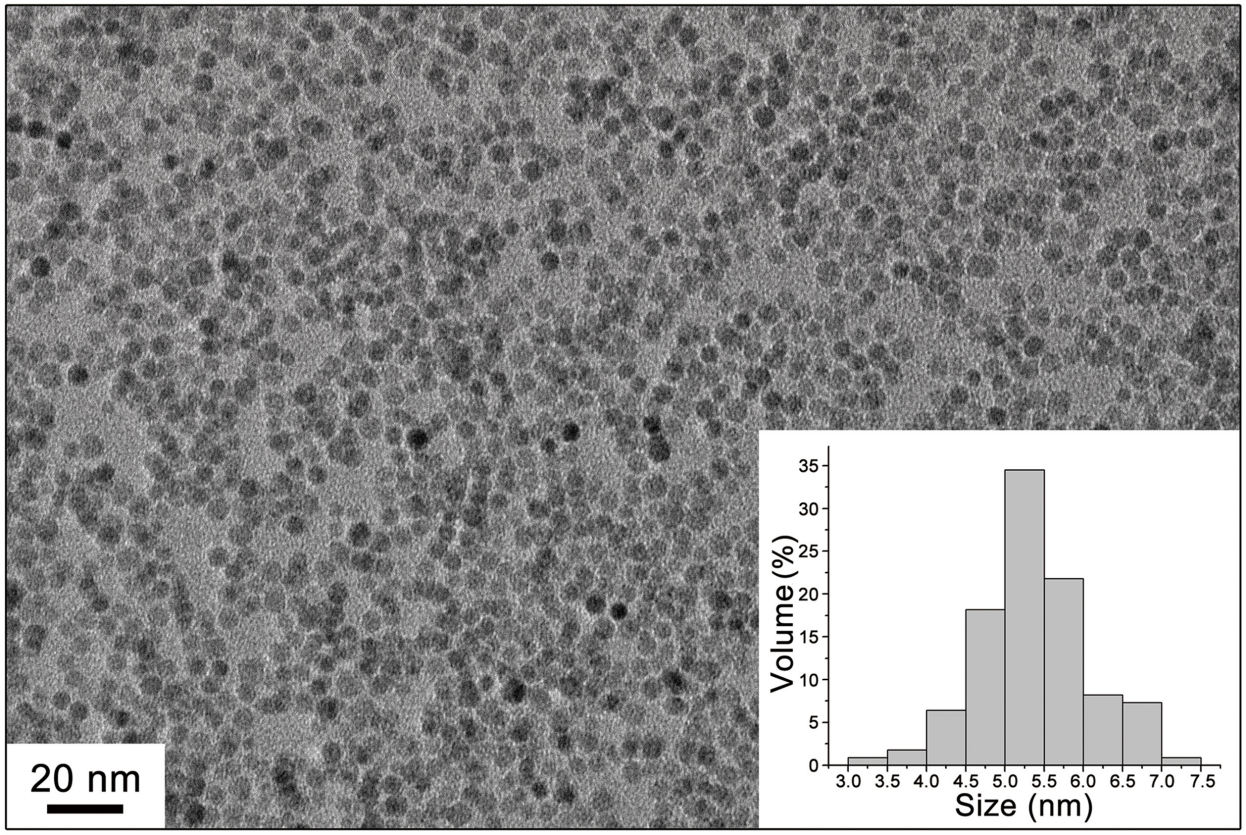

Figure S1. TEM image of oleylamine coated T_1-Fe_3O_4 dispersed in hexane. Inset was a size distribution histogram of 100 particles. D_{TEM} = 5.3±0.6 nm.

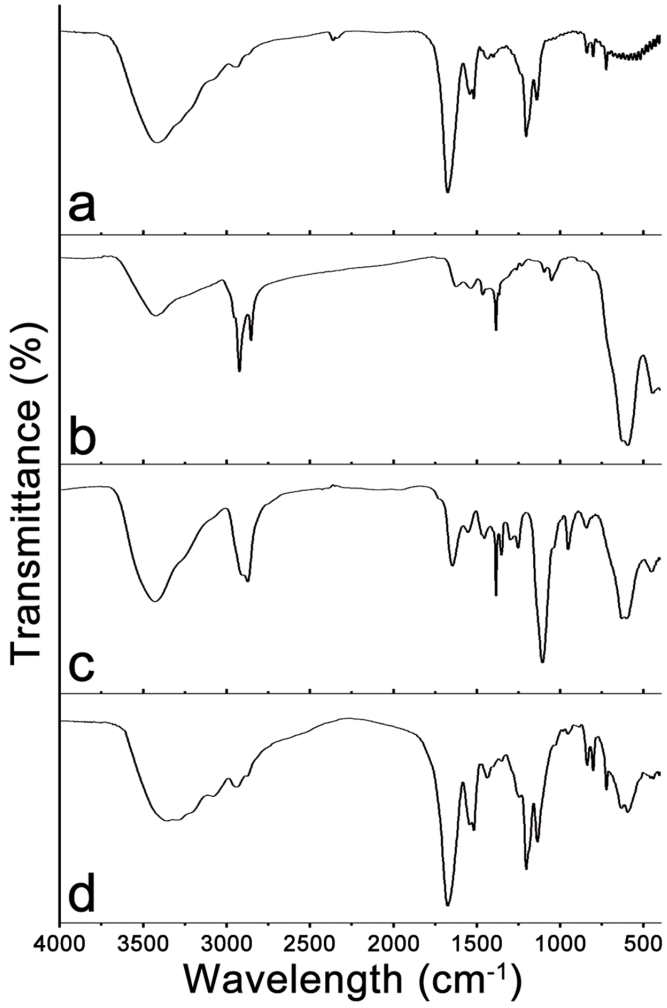

Figure S2. IR spectra of (a) free c(RGDyK) peptide, (b) hydrophobic T_1-Fe_3O_4, (c) PEGylated T_1-Fe_3O_4 and (d) RGD-modified T_1-Fe_3O_4.

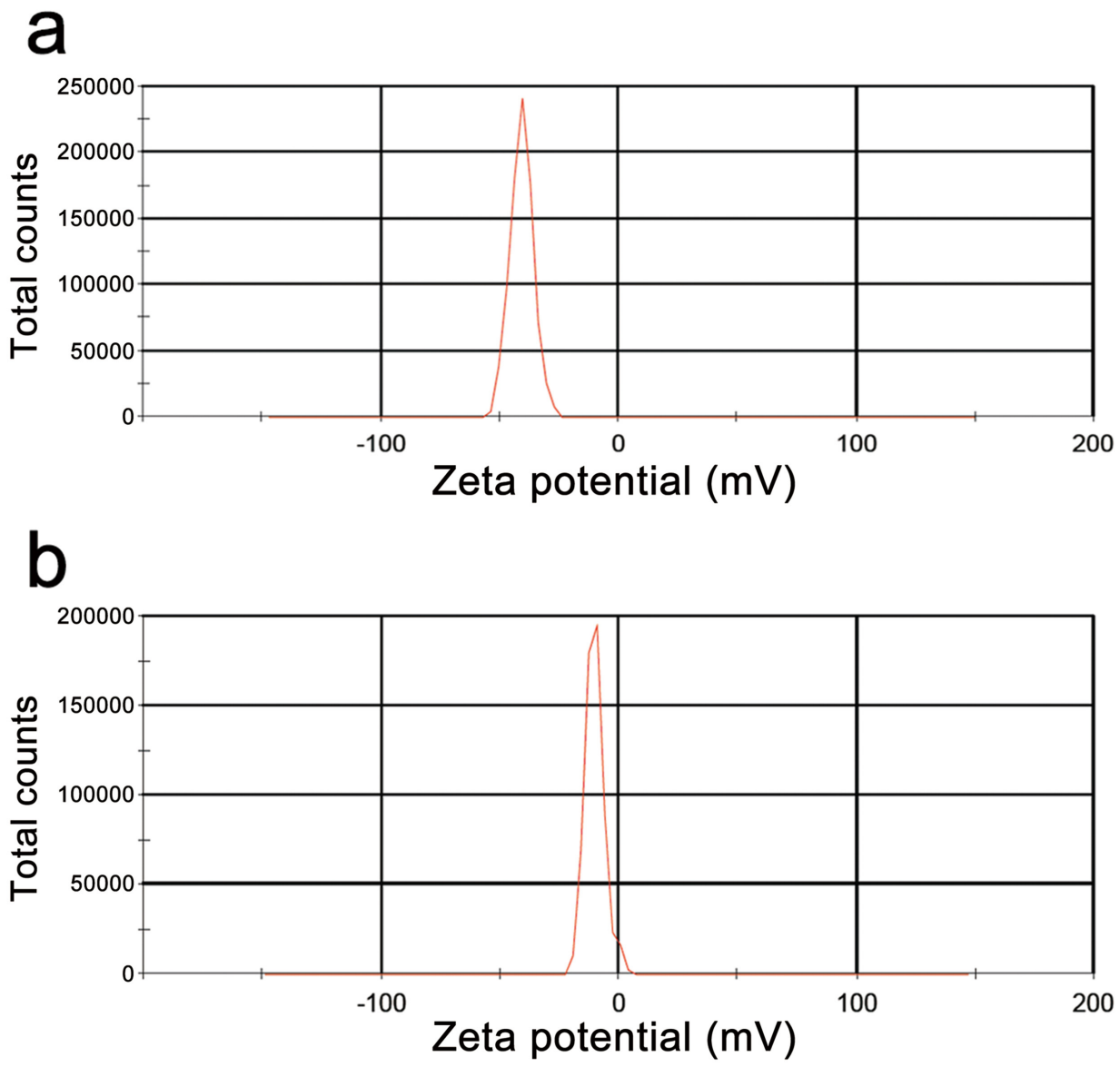

Figure S3. Zeta potentials of (a) PEGylated T_1-Fe_3O_4 and (b) RGD-modified T_1-Fe_3O_4 in neutral water solutions.

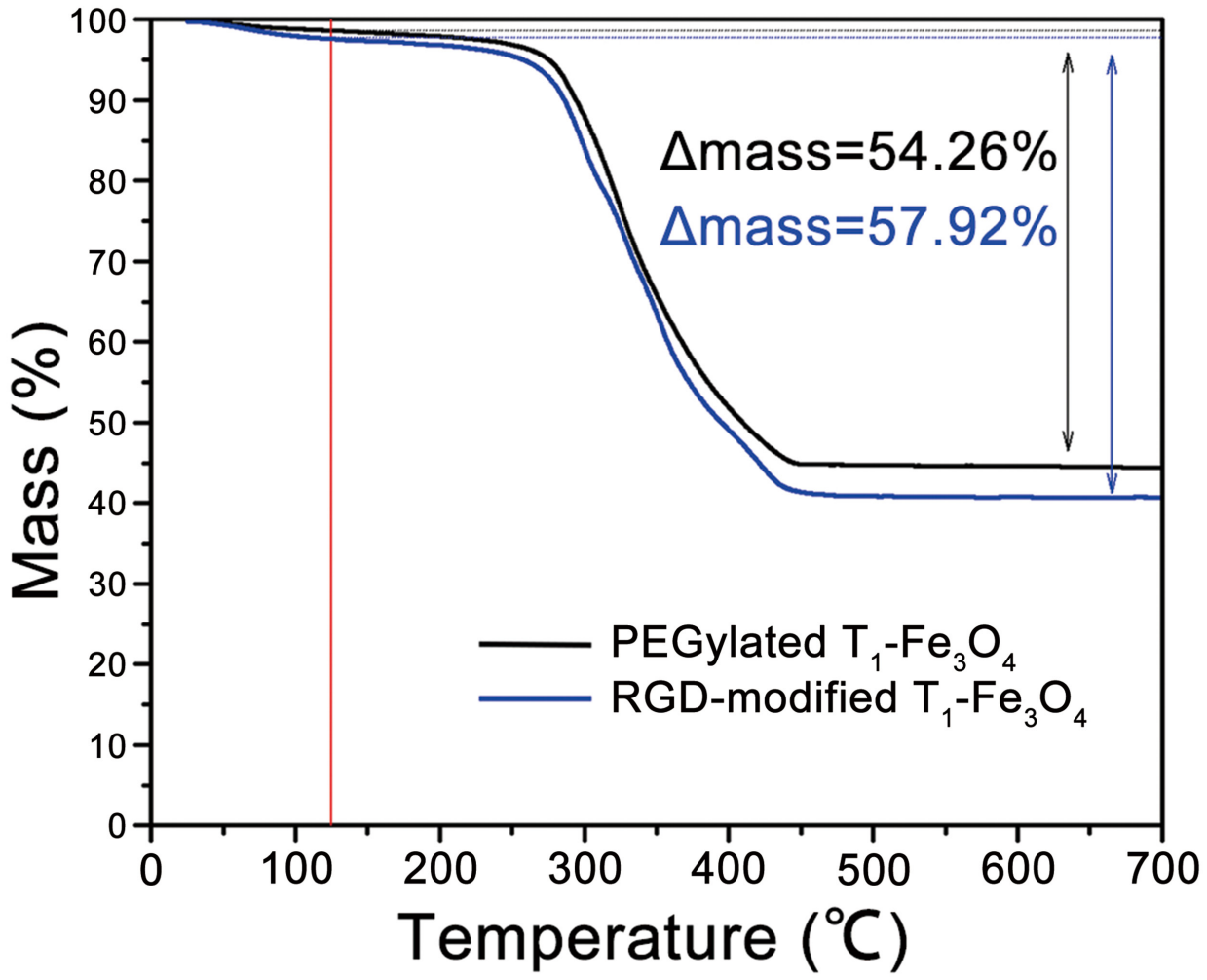

Figure S4. TGA of PEGylated T_1-Fe_3O_4 and RGD-modified T_1-Fe_3O_4. Heating rate was 20 °C/min under N_2 flow. The red vertical line indicated the temperature when most of the absorbed water has evaporated (125 °C).

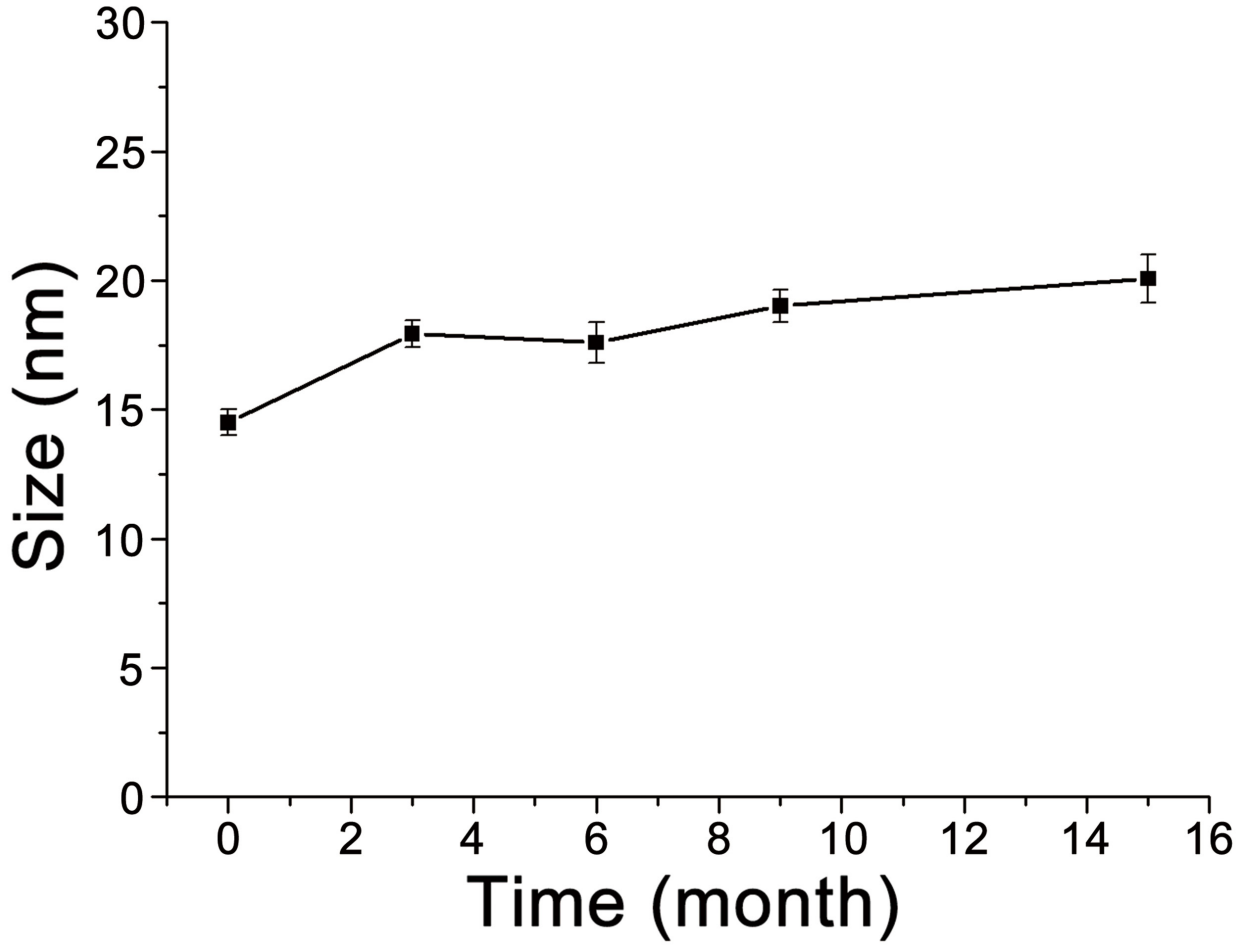

Figure S5. D_H of RGD-modified T_1-Fe_3O_4 was tracked by using DLS measurement. It showed an approximate 5 nm increase and no further aggregation formed in its aqueous solution during the 15-month storage. The error bars represented ±s.d. of three independent experiments.

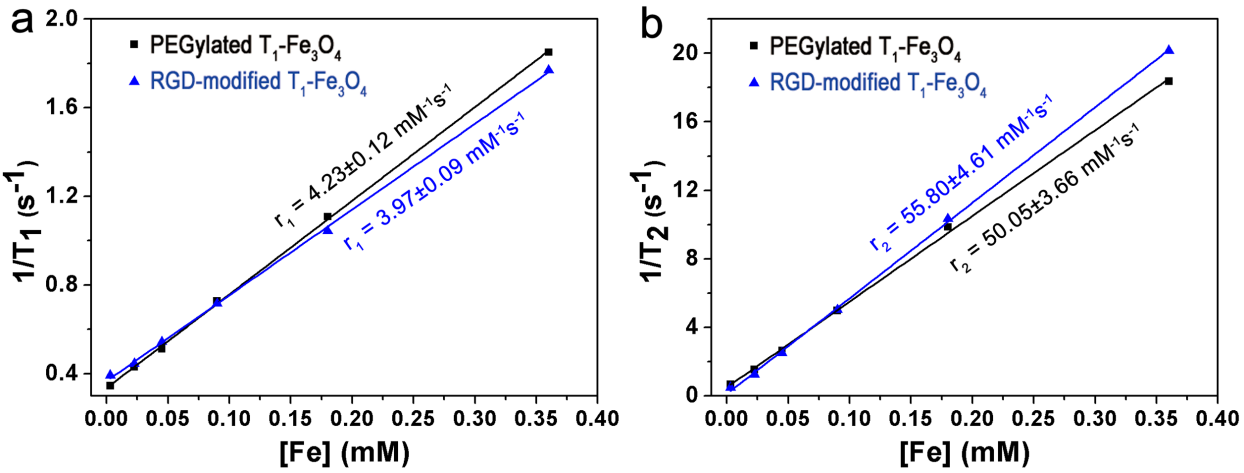

Figure S6. Plots of (a) $1/T_1$ and (b) $1/T_2$ against Fe concentration of PEGylated T_1-Fe_3O_4 and RGD-modified T_1-Fe_3O_4 in water at 7.0 T. r_1 and r_2 were calculated from the slopes of the corresponding linear fits of the experimental data.

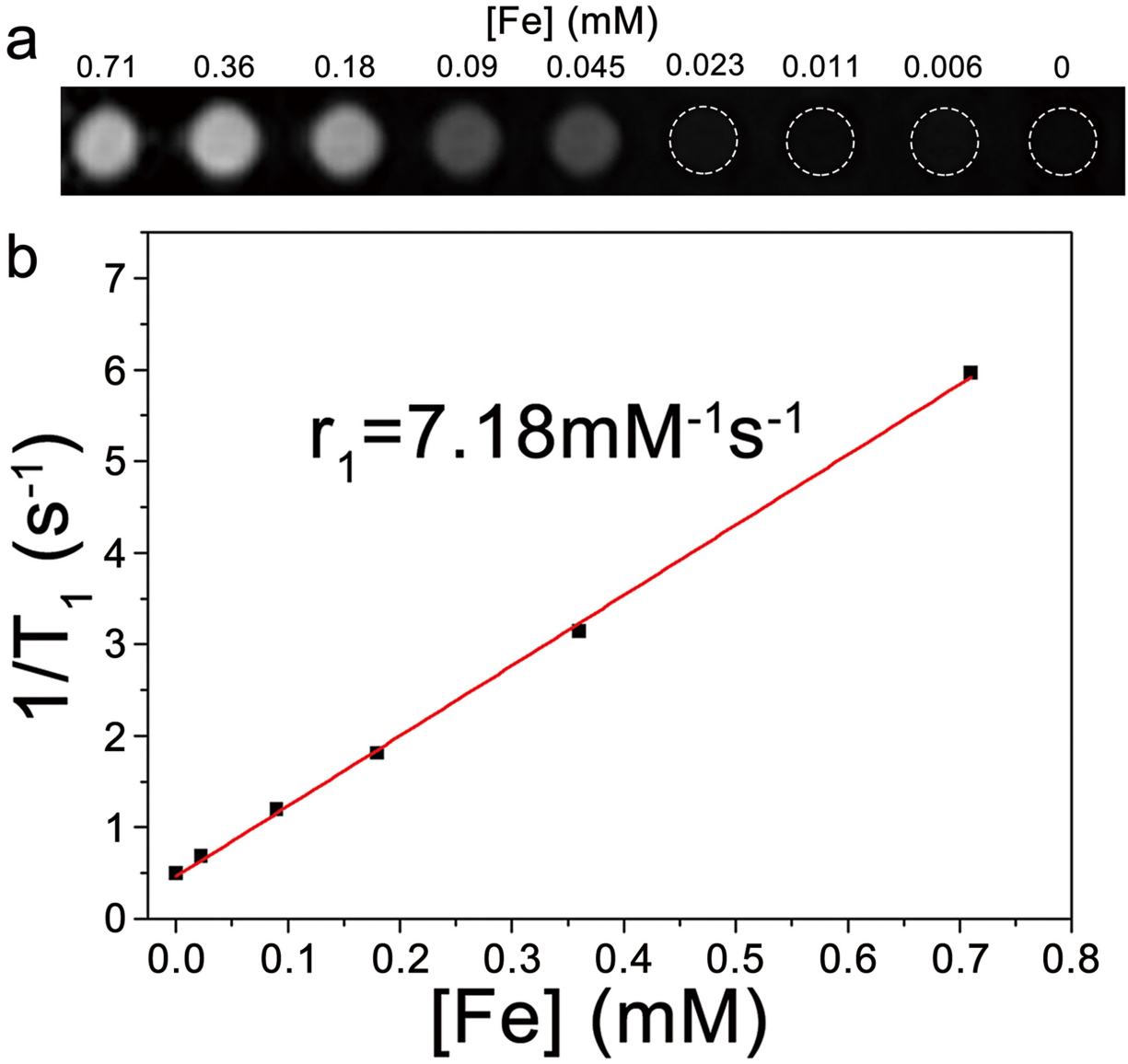

Figure S7. (a) T_1-weighted MR image of RGD-modified T_1-Fe_3O_4 at different Fe concentrations in water solution after 15-month storage. (b) Plots of $1/T_1$ against Fe concentration of RGD-modified T_1-Fe_3O_4 after 15-month storage at 3.0 T. r_1 was obtained as 7.18±0.17 $mM^{-1}s^{-1}$ from the slope of the corresponding linear fit of the experimental data.

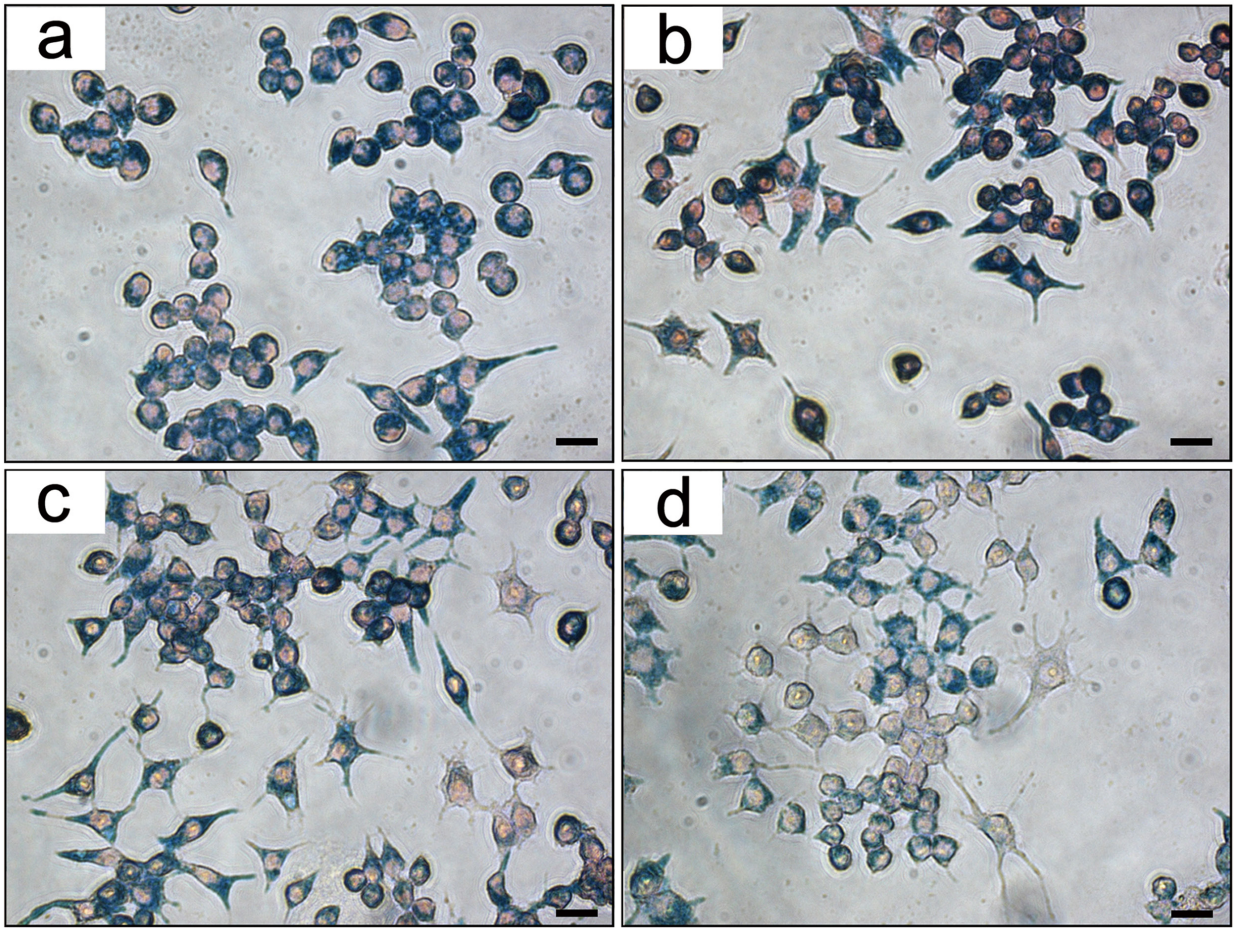

Figure S8. Prussian blue staining of RAW 264.7 macrophages incubated with DMSA-modified T_1-Fe_3O_4 for 24 h at Fe concentration of (a) 100 μg/mL, (b) 50 μg/mL, (c) 25 μg/mL and (d) 12.5 μg/mL. High-level non-specific uptake was clearly observed for all groups despite Fe concentration was very low. Scale bar: 20 μm for all images.

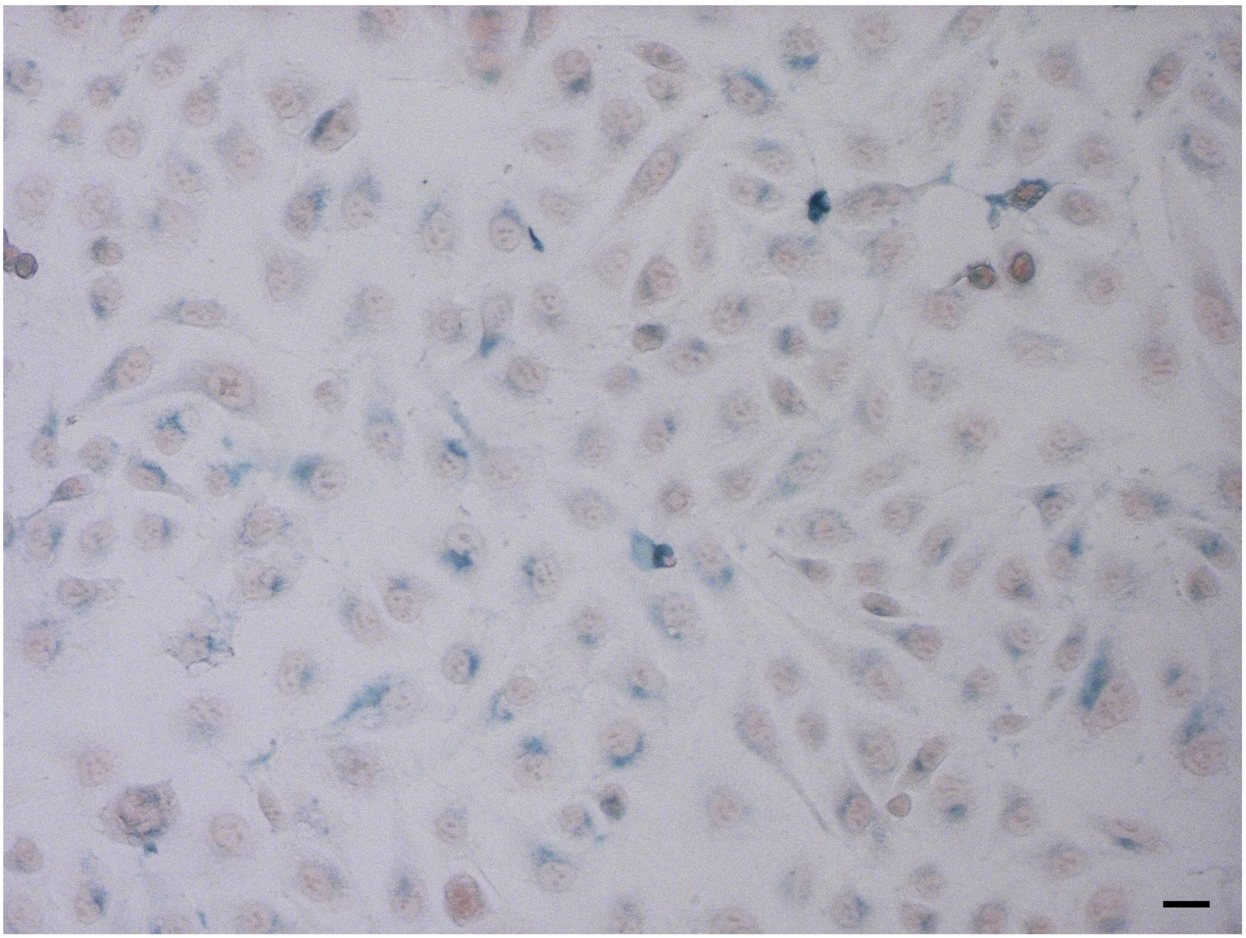

Figure S9. Prussian blue staining of HUVECs incubated with RGD-modified T_1-Fe_3O_4 which had been stored for 15 months. Comparatively high-level targeting specificity was observed with the incubating concentration of 100 μg Fe/mL. Scale bar, 20 μm.

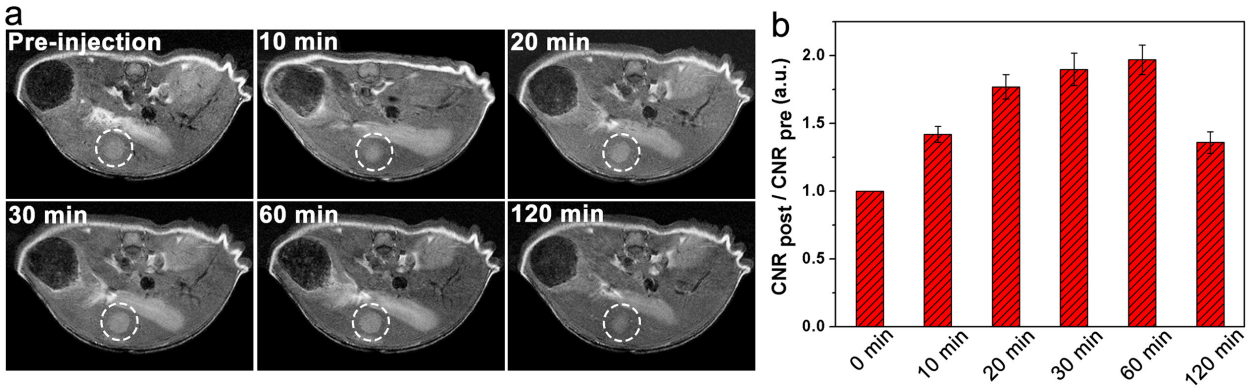

Figure S10. (a) T_1-weighted MR images of mice bearing orthotopic hepatic tumor (white dashed circles) before and at the time points of 10, 20, 30, 60 and 120 min after the administration of PEGylated T_1-Fe_3O_4. (b) Quantification of T_1 signal changes of tumor-to-liver CNR at the corresponding time points. The error bars represented ±s.d. of three independent experiments.

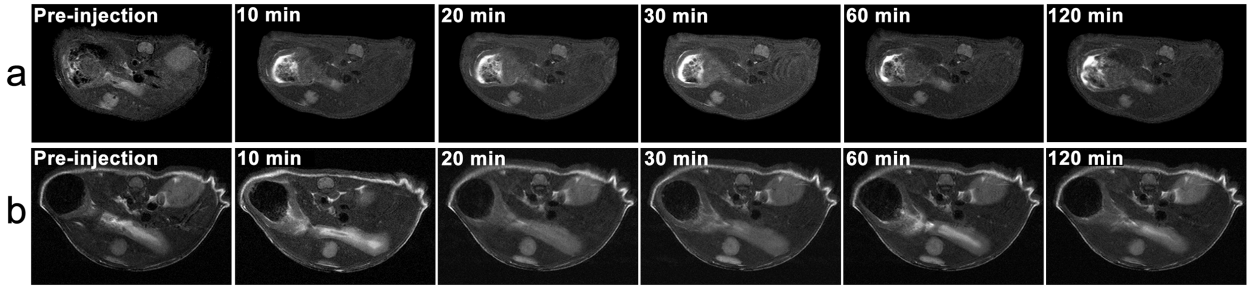

Figure S11. T_2-weighted MR images of tumor-bearing mice before and at the time points of 10, 20, 30, 60 and 120 min after the administration of (a) RGD-modified T_1-Fe_3O_4 and (b) PEGylated T_1-Fe_3O_4.

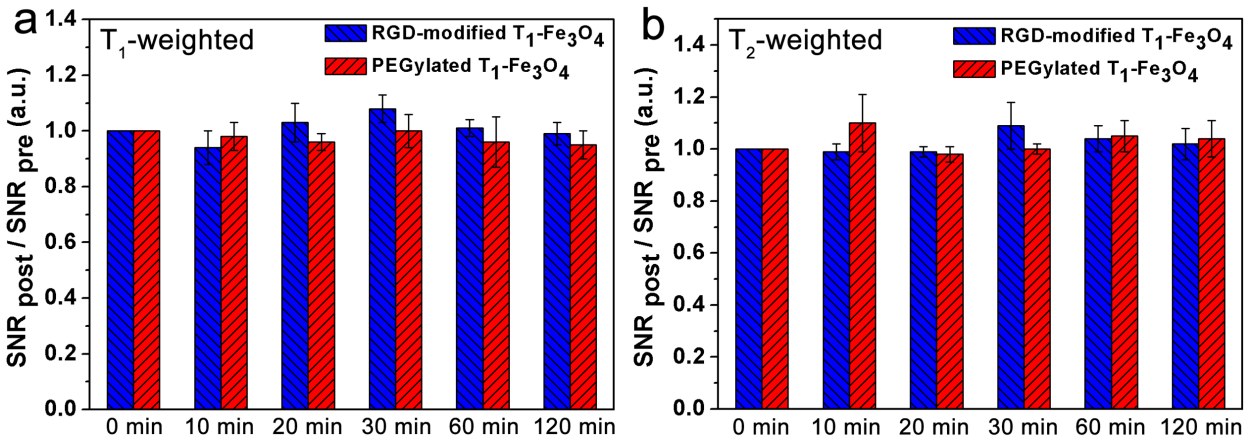

Figure S12. Quantification of (a) T_1 and (b) T_2 signal changes (SNR) in normal liver tissue at the corresponding time points after administration of RGD-modified T_1-Fe_3O_4 or PEGylated T_1-Fe_3O_4. The error bars represented ±s.d. of three independent experiments.

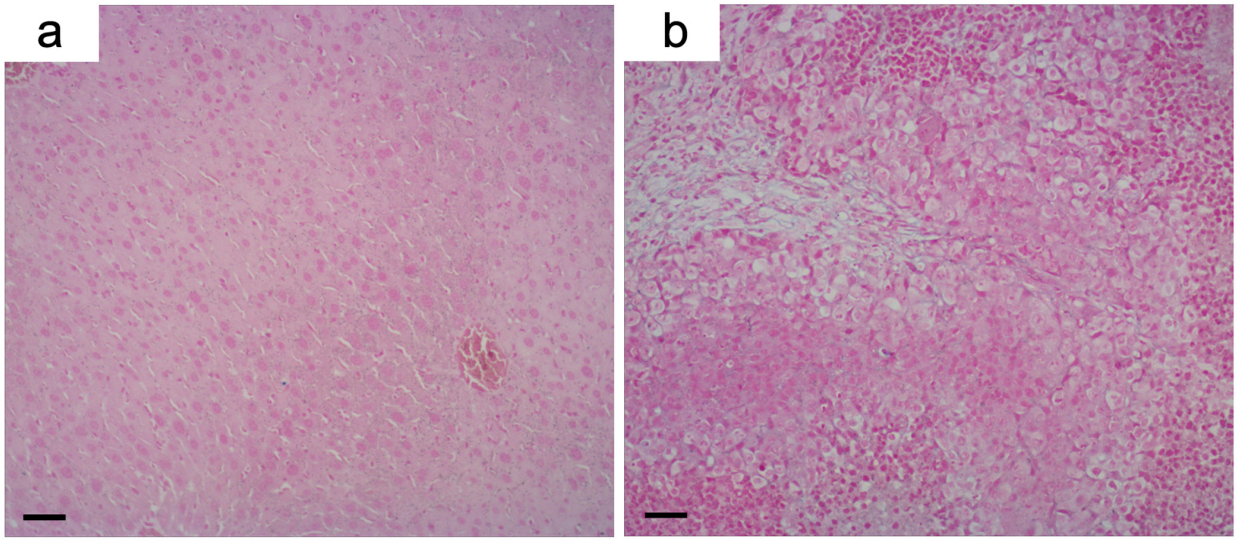

Figure S13. Prussian blue stained images of (a) normal liver tissue and (b) orthotopic hepatic tumor after the administration of PEGylated T_1-Fe_3O_4. Scale bar: 20 μm for all images.