

Electronic Supplementary Information

ES-MIONS Based Dual-modality PET/MRI Probes for Acidic Tumor

Microenvironment Imaging

*Xiuyan Wei¹ §, Haitao Zhao² §, Gang Huang³, Jianhua Liu¹ *, Weina He¹ *, Qingqing Huang³ **

1 Medical Chemistry and Bioinformatics Center, College of Basic Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China.

2 Department of Nuclear Medicine, Institute of Clinical Nuclear Medicine, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China.

3 Shanghai Key Laboratory of Molecular Imaging, Shanghai University of Medicine and Health Sciences, Shanghai 201318, China

§These authors contributed equally to this work.

* E-mail: jhliu7912@sjtu.edu.cn (J. Liu); hewn0319@sjtu.edu.cn (W. He); qingqinghuang80@gmail.com (Q. Huang)

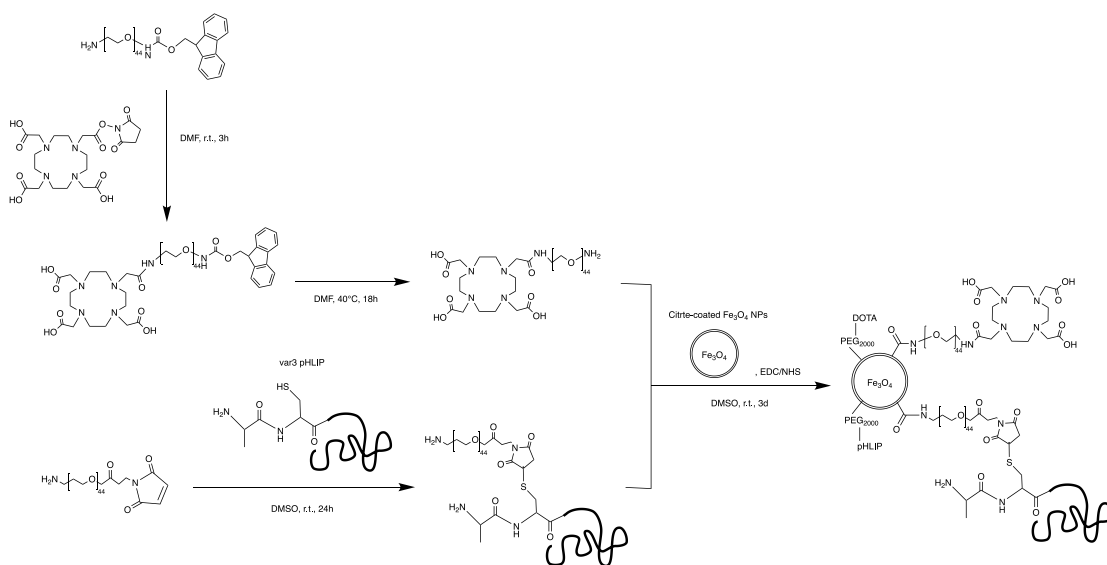


Figure S1. Synthesis route of Fe_3O_4 -PEG-DOTA/pHLIP NPs.

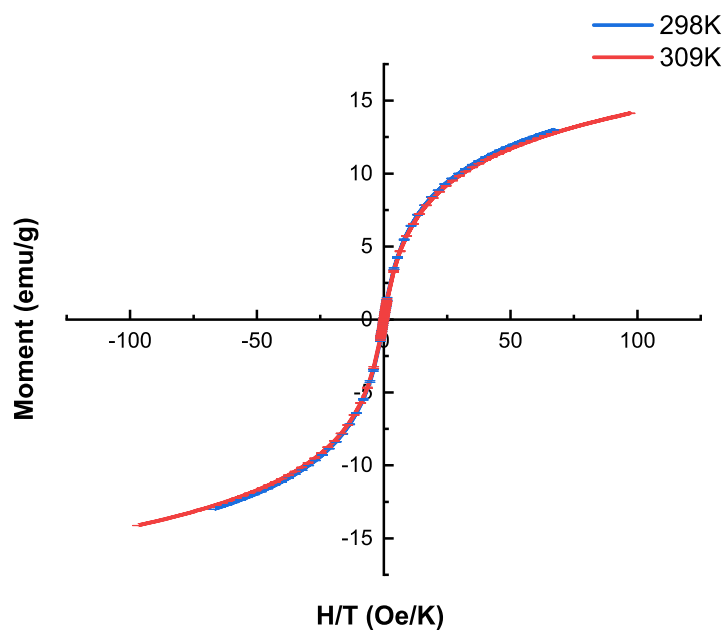


Figure S2. Magnetization curves of citrate-stabilized Fe_3O_4 NPs measured at 298K and 309K.

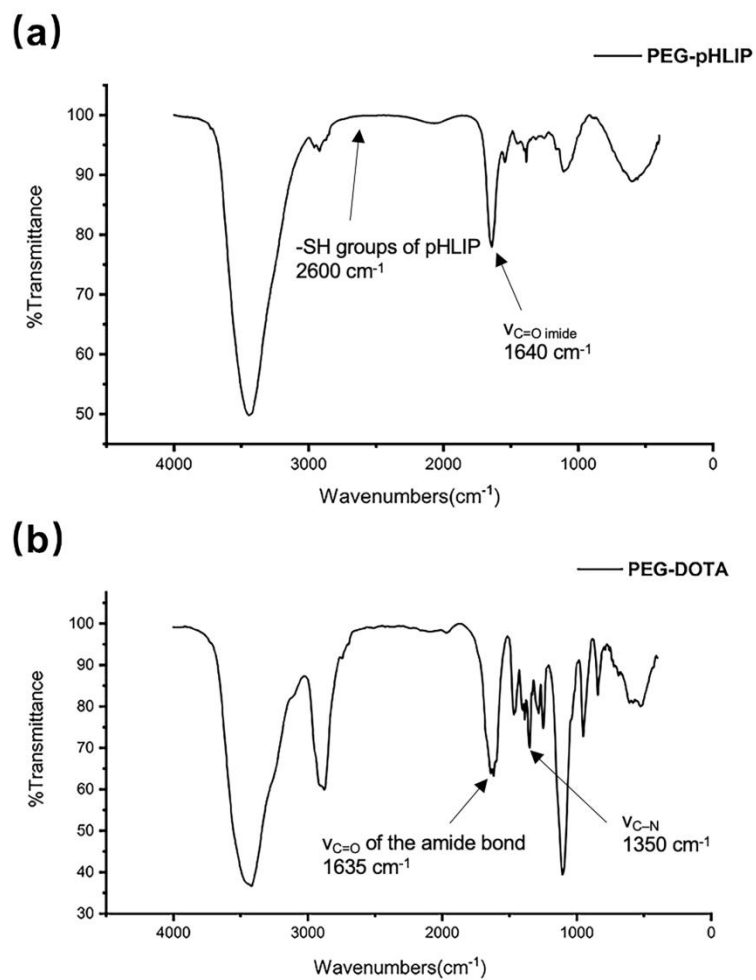


Figure S3. FTIR spectra of (a) NH_2 -PEG-pHLIP and (b) NH_2 -PEG-DOTA.

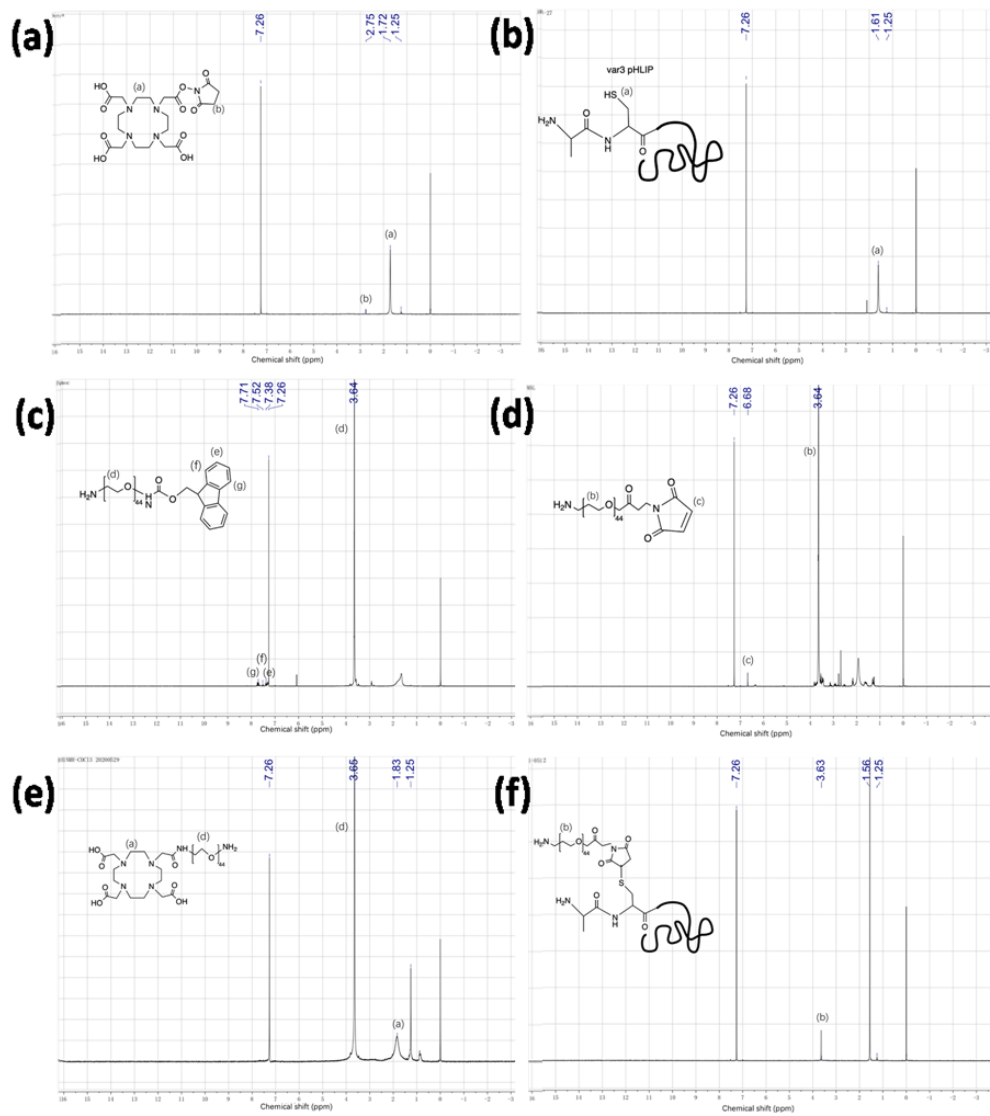


Figure S4. ^1H NMR spectra of (a) DOTA-NHS, (b) Var3 pHLIP, (c) NH_2 -PEG-Fmoc, (d) NH_2 -PEG-Mal, (e) NH_2 -PEG-DOTA and (f) NH_2 -PEG-pHLIP.

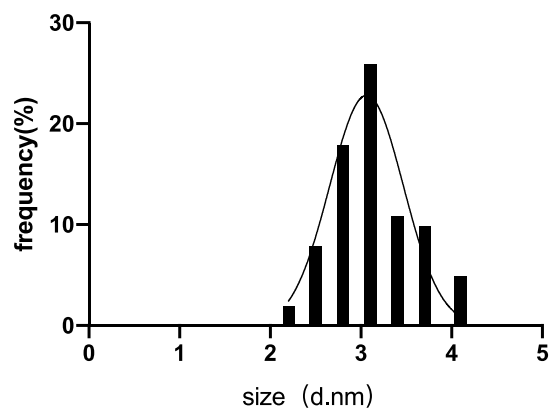


Figure S5. Size distribution of citrate-stabilized Fe₃O₄ NPs with Gaussian fitting.

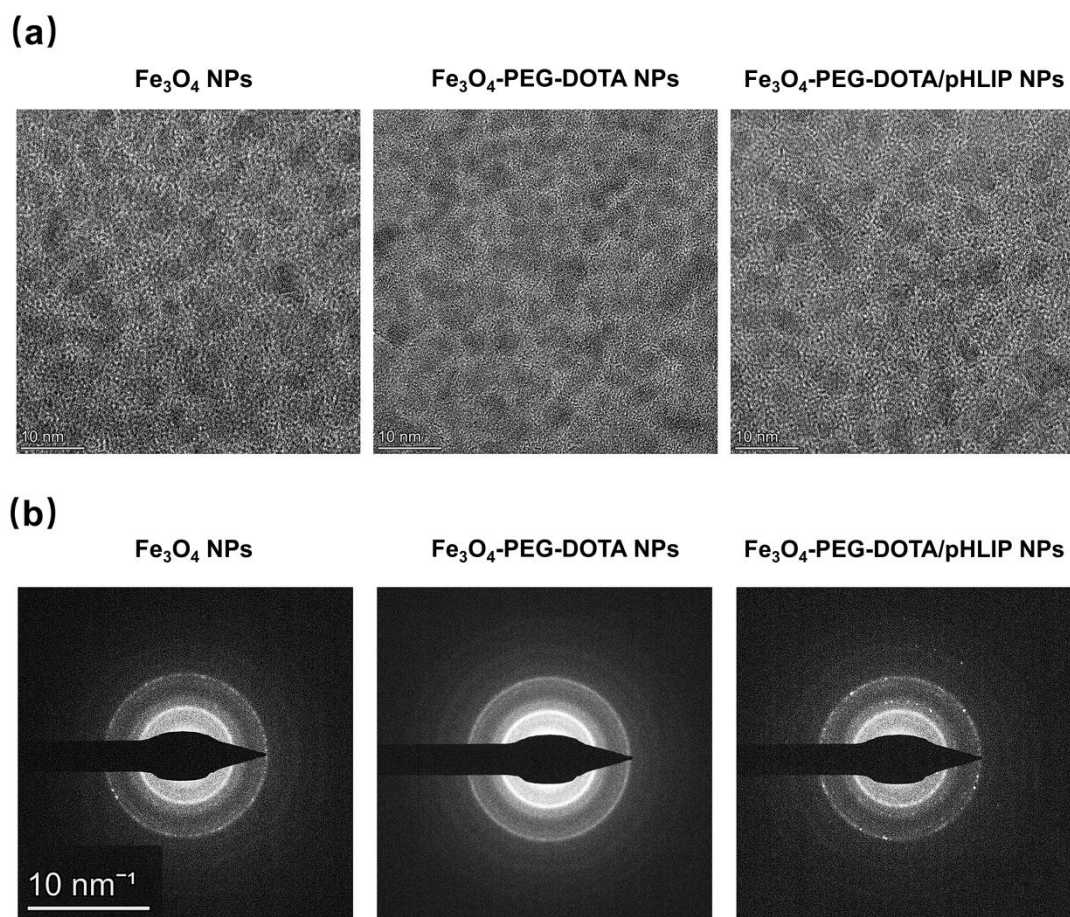


Figure S6. (a) TEM images and (b) SAED patterns of citrate-stabilized Fe₃O₄ NPs, Fe₃O₄-PEG-DOTA NPs and Fe₃O₄-PEG-DOTA/pHLIP NPs.

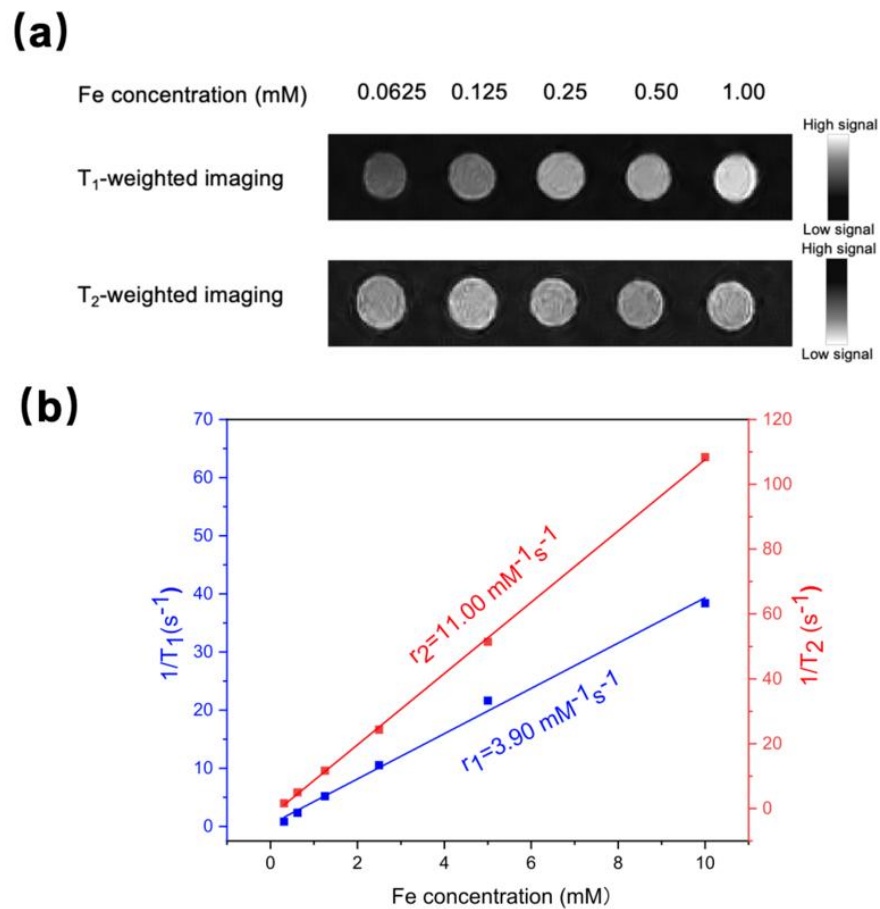


Figure S7. (a) Phantom images acquired from T₁-weighted and T₂-weighted MRI scans for Fe₃O₄-PEG-DOTA NPs at different Fe concentrations at the room temperature 32-34 °C and pH 6.8. (b) Plot of the relaxation rates of Fe₃O₄-PEG-DOTA NPs as a function of Fe concentration at the room temperature 32-34 °C and pH 6.8.

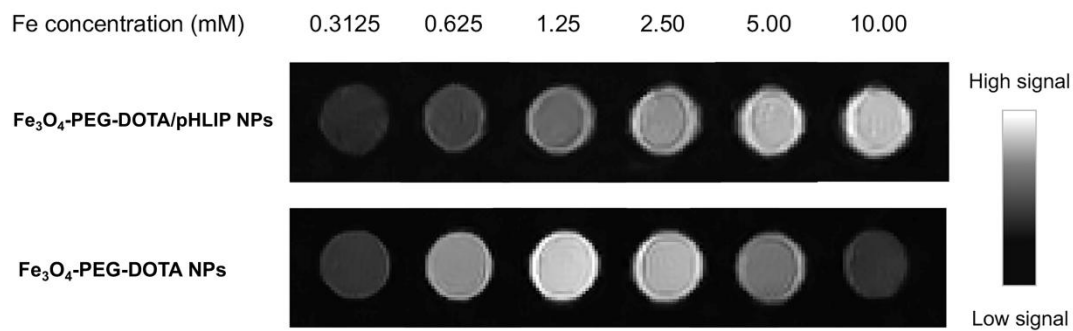


Figure S8. T₁-weighted MR imaging of Fe₃O₄-PEG-DOTA and Fe₃O₄-PEG-DOTA/pHLIP NPs at the Fe concentrations of 0.3125, 0.625, 1.25, 2.50, 5.00, 10.00 mM, respectively.

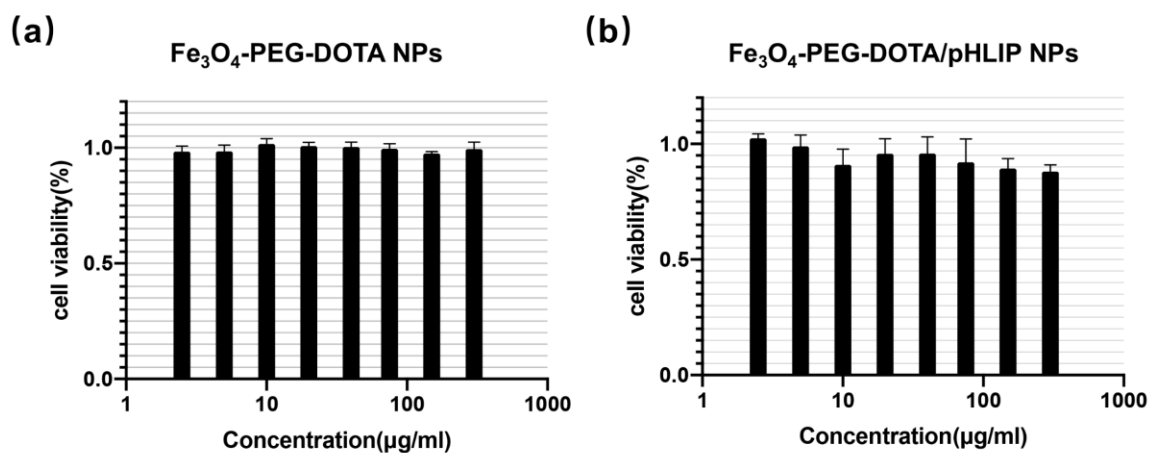


Figure S9. Cytotoxicity of 4T1 cells after treated with the (a) Fe₃O₄-PEG-DOTA NPs and (b) Fe₃O₄-PEG-DOTA/pHLIP NPs at different Fe concentrations for 24 h. 4T1 cells treated with PBS were used as control.

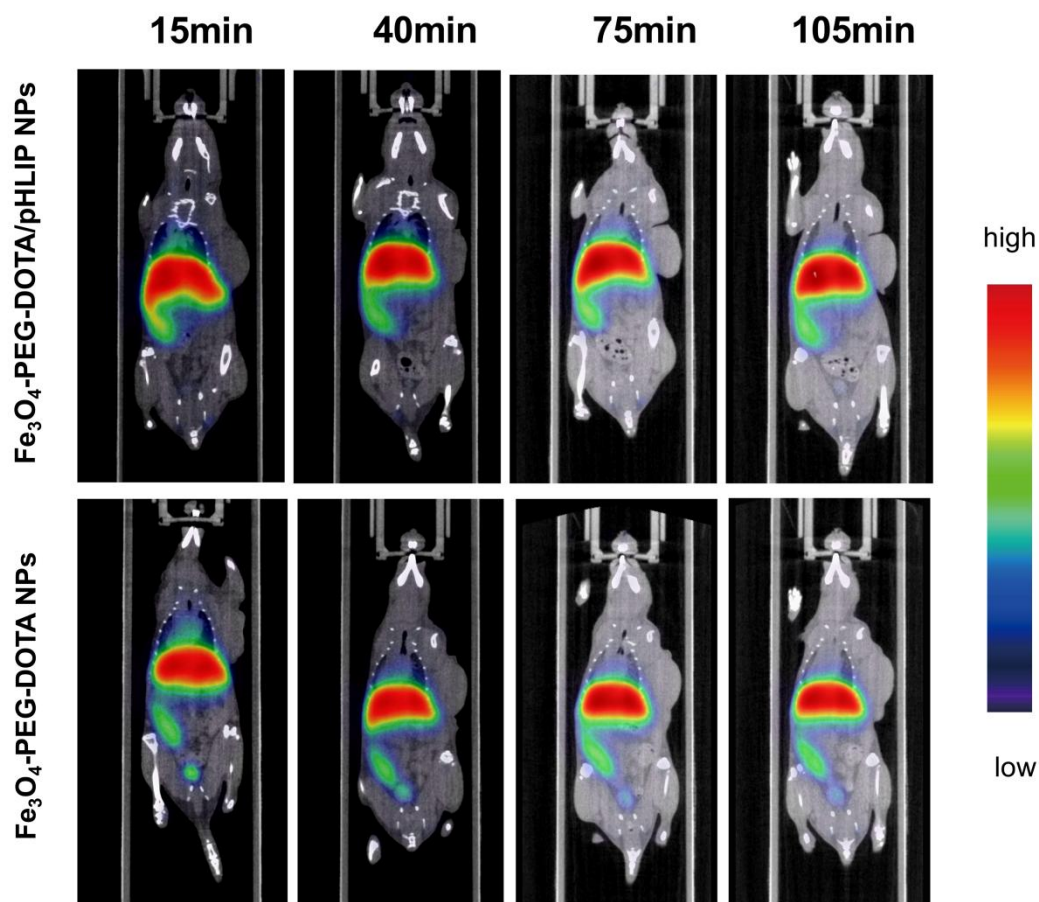


Figure S10. Whole-body PET/CT imaging on mice at 15 min, 40 min, 75 min, 105 min after the intravenous injection of $\text{Fe}_3\text{O}_4\text{-PEG-}^{68}\text{Ga]DOTA/pHLIP NPs}$ or $\text{Fe}_3\text{O}_4\text{-PEG-}^{68}\text{Ga]DOTA NPs}$.

Table S1. Mean signal strength of in vitro tumor cellular uptake of $\text{Fe}_3\text{O}_4\text{-PEG-}^{68}\text{Ga]DOTA/pHLIP NPs}$ and $\text{Fe}_3\text{O}_4\text{-PEG-}^{68}\text{Ga]DOTA NPs}$ of 4T1 cells in pH 6.0, 6.4, 6.9, 7.4 respectively.

| pH | $\text{Fe}_3\text{O}_4\text{-PEG-DOTA/pHLIP NPs}$ (cpm) | $\text{Fe}_3\text{O}_4\text{-PEG-DOTA NPs}$ (cpm) |
|-----|---|---|
| 6.0 | 9130 | 3172 |
| 6.4 | 6507 | 3390 |
| 6.9 | 6603 | 3133 |
| 7.4 | 4493 | 3196 |