Supporting Information for

**H-ferritin–nanocaged gadolinium nanocores for ultra-sensitive MR molecular imaging**

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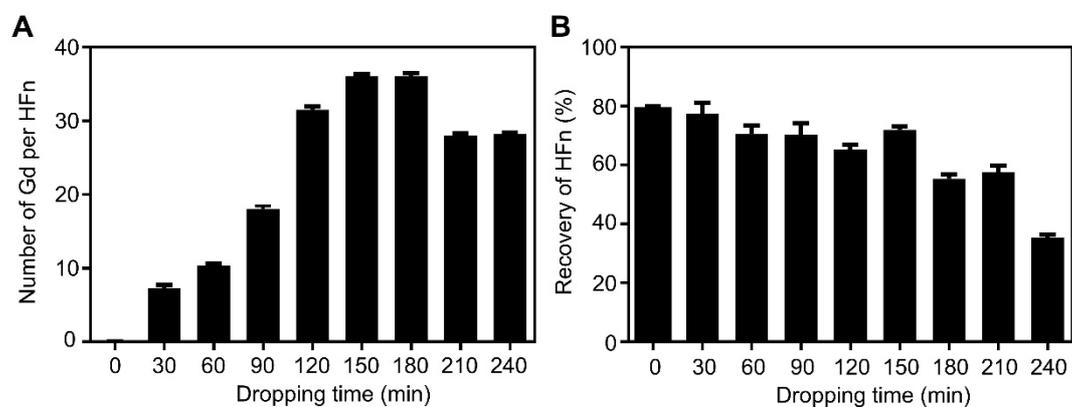## Contents

## 1. Supporting Figures



**Figure S1. (A)** Number of loaded Gd per HFn nanocage depends on the Gd dropping time. **(B)** HFn recovery yield (%) depends on the Gd dropping time. The encapsulated Gd was quantified using ICP-OES, and the HFn concentration was determined by using BCA protein assay kit.
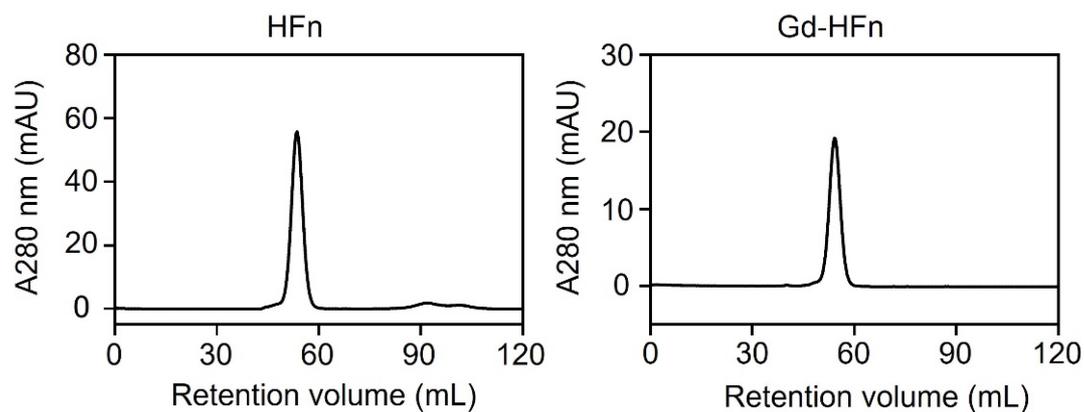
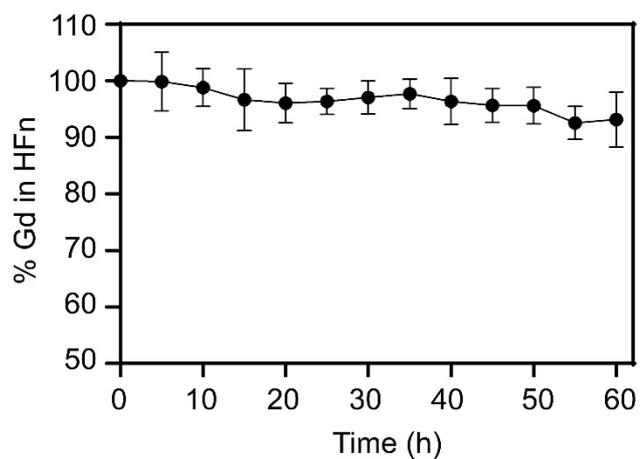**Figure S2.** Representative SEC analysis of the HFn and Gd-HFn NPs.



**Figure S3.** Stability of Gd-HFn NPs in 10% mouse serum at 37 °C over 60 h of incubation (n = 3, bars represent means ± s.d.)
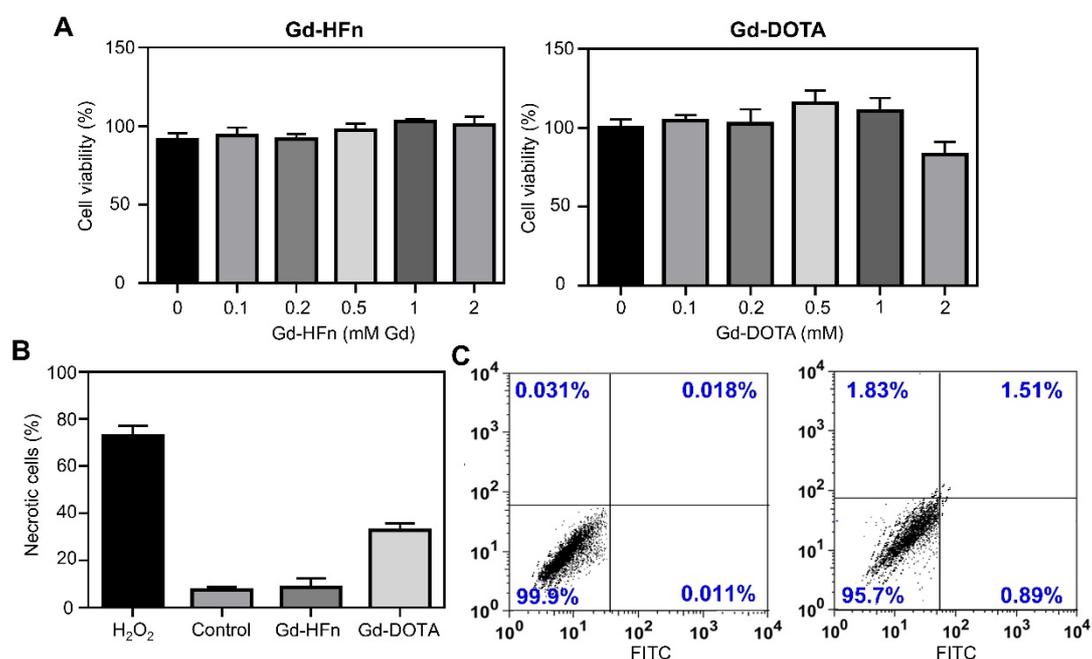
**Figure S4. (A)** Cell viability of MDA-MB-231 cells after treatment with Gd-HFn or Gd-DOTA for 24 h over a dose range of 0-2 mM Gd. CCK8 assay was used for cell viability assessment. (n = 5, bars represent means ± s.d.) **(B)** The percentage of necrotic cells measured by quantification of LDH release in the cell medium after incubation in the presence or absence (Negative control) of Gd-HFn or Gd-DOTA (2 mM Gd). Hydrogen peroxide ($H_2O_2$) was used as positive control. Results are expressed as percentage of dead cells with respect to the control. (n = 5, bars represent means ± s.d.) **(C)** Cell apoptosis analysis was performed using Annexin V-FITC assay using flow cytometry. Left, MDA-MB-231 cells untreated. Right, MDA-MB-231 cells treated with Gd-HFn for at concentration of 2 mM Gd.
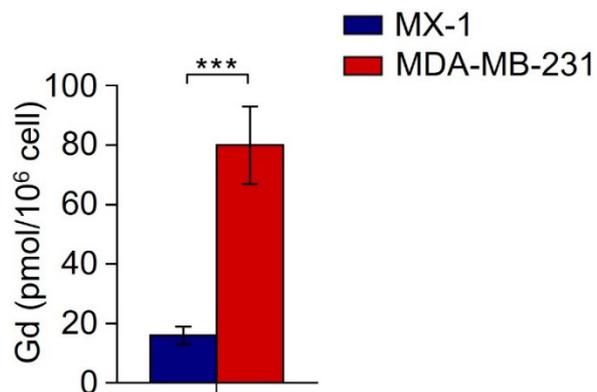
**Figure S5.** Cellular uptake of Gd-HFn measured by ICP-OES (n = 5, mean ± SD, unpaired Student's t-test, ***$P < 0.001$).
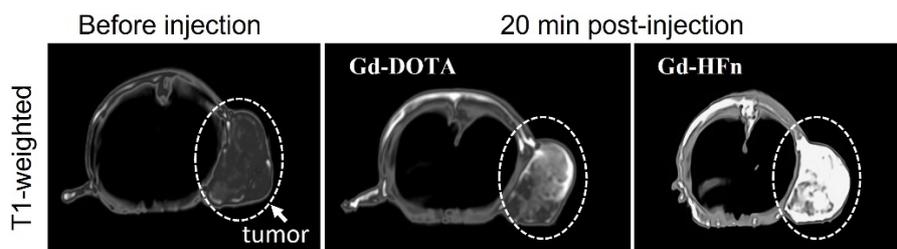


**Figure S6.** MR imaging of large tumors with Gd-DOTA or Gd-HFn in living mice.
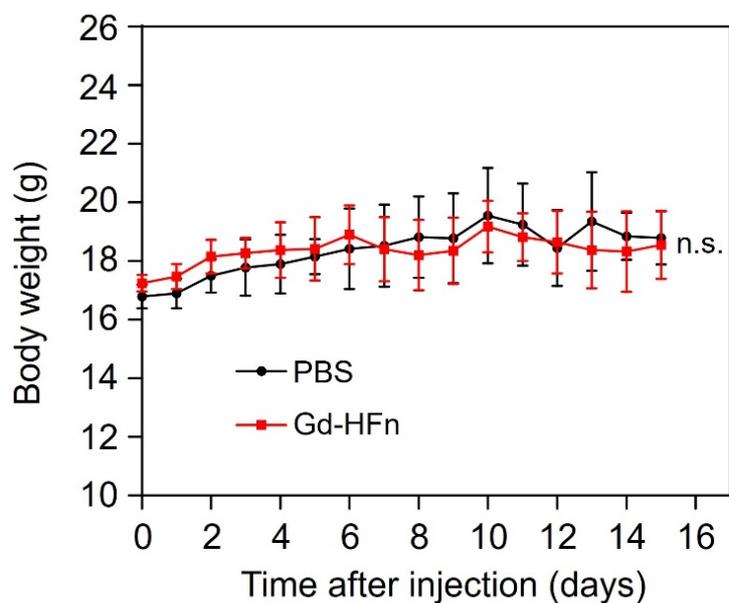
**Figure S7.** Toxicity evaluation of Gd-HFn *in vivo*. Healthy female BALB/c mice were administered intravenously on day 0 of Gd-HFn (0.016 mmol Gd/kg body weight) or PBS (n = 6 per group, bars represent means ± s.d.). n.s., not significant.



**Figure S8.** Representative images of organ histology examination from mice administered with PBS or Gd-HFn at a dose of 0.016 mmol Gd/kg animal body weight. Tissue slices were stained with hematoxylin and eosin (H&E). No noticeable abnormality was found in the heart, liver, spleen, lung, and kidney.
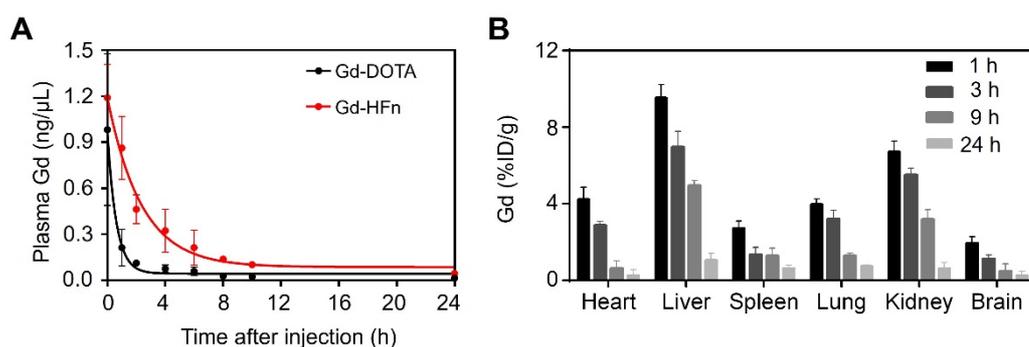
**Figure S9. Total body clearance of Gd-HFn. (A)** Plasma concentrations of Gd as a function of time after injection. Five healthy mice were intravenously injected with Gd-DOTA or Gd-HFn at a dose of 0.016 mmol Gd/kg animal body weight. Plasma samples at different time points were drawn and the plasma concent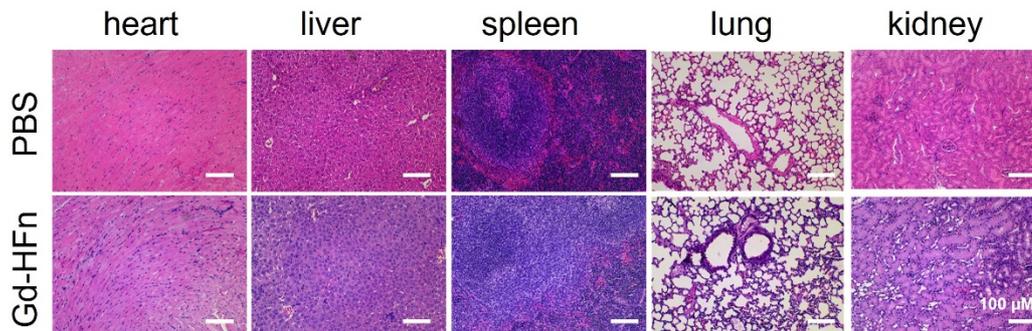rations of Gd were measured by ICP-OES. (n=5 independent measurements, error bars represent mean ± s.d.). **(B)** Biodistribution of Gd-HFn. Tissue samples at different time points were drawn and the tissue concentrations of Gd were measured by ICP-OES. Data are presented as percentage of injected dose (%ID) per gram of tissue. Values are expressed as means ± s.d. for a group of five animals.

**Table S1.** Blood routine parameters of healthy mice treated with PBS, Gd-DOTA or Gd-HFn.

| Blood routine parameters | PBS (n=5) | | Gd-DOTA (n=5) | | Gd-HFn (n=5) | | Normal arrange |
|---|---|---|---|---|---|---|---|
| | 1 Week | 2 Weeks | 1 Week | 2 Weeks | 1 Week | 2 Weeks | |
| WBC | 3.96±0.76 | 8.94±0.96 | 4.55±0.64 | 8.78±0.70 | 4.34±0.87 | 8.82±1.27 | 0.80-10.60 |
| NEU | 0.42±0.18 | 1.08±0.17 | 0.68±0.13 | 1.04±0.08 | 0.83±0.24 | 1.27±0.24 | 0.23-3.60 |
| LYM | 3.41±0.75 | 7.52±0.89 | 3.71±0.61 | 7.09±0.63 | 3.28±0.67 | 6.77±1.23 | 0.60-8.90 |
| MON | 0.06±0.01 | 0.25±0.05 | 0.09±0.01 | 0.36±0.04 | 0.10±0.02 | 0.31±0.07 | 0.04-1.40 |
| EOS | 0.05±0.02 | 0.24±0.09 | 0.06±0.01 | 0.23±0.04 | 0.11±0.06 | 0.21±0.07 | 0-0.51 |
| BAS | 0.01±0.00 | 0.05±0.01 | 0.02±0.01 | 0.05±0.02 | 0.02±0.01 | 0.06±0.02 | 0-0.12 |
| NEU% | 10.84±5.25 | 9.66±1.95 | 15.00±3.37 | 11.88±0.64 | 19.30±4.50 | 14.70±5.62 | 6.5-50.0 |
| LYM% | 85.72±5.24 | 85.38±3.44 | 81.30±3.64 | 80.70±1.29 | 75.40±4.99 | 78.84±6.38 | 40.0-92.0 |
| MON% | 1.58±0.33 | 2.30±0.71 | 1.92±0.23 | 4.14±0.26 | 2.38±0.47 | 3.46±1.08 | 0.9-18.0 |
| EOS% | 1.42±0.29 | 2.24±1.10 | 1.40±0.21 | 2.66±0.55 | 2.36±0.78 | 2.24±0.29 | 0-7.5 |
| BAS% | 0.44±0.08 | 0.42±0.07 | 0.38±0.13 | 0.62±0.20 | 0.56±0.16 | 0.76±0.27 | 0-1.5 |
| RBC | 9.62±0.35 | 9.17±0.43 | 9.57±0.23 | 8.86±0.51 | 9.07±0.61 | 8.97±0.43 | 6.50-11.50 |
| HGB | 152.20±3.60 | 148.60±6.41 | 150.80±5.78 | 144.80±7.55 | 142.40±7.66 | 145.20±4.79 | 110-165 |
| HCT | 46.24±1.76 | 44.30±1.73 | 46.30±1.36 | 43.78±2.29 | 43.52±2.42 | 43.78±1.39 | 35.0-55.0 |
| MCV | 48.06±0.62 | 48.32±0.54 | 48.38±0.47 | 49.46±0.57 | 48.04±0.86 | 48.82±1.03 | 41.0-55.0 |
| MCH | 15.84±0.22 | 16.22±0.25 | 15.74±0.31 | 16.38±0.40 | 15.76±0.27 | 16.24±0.27 | 13.0-18.0 |
| MCHC | 329.60±5.46 | 335.20±2.79 | 325.40±6.15 | 331.20±4.49 | 327.60±2.73 | 332.40±2.33 | 300-360 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **PLT** | 1051.40±129.56 | 1028.40±373.36 | 1055.00±118.87 | 940.00±169.35 | 1526.40±322.21 | 1190.80±168.04 | 400-1600 |
| **MPV** | 4.68±0.22 | 4.82±0.34 | 4.56±0.05 | 4.78±0.04 | 4.58±0.10 | 4.78±0.07 | 4.0-6.2 |
| **PDW** | 15.18±0.07 | 15.28±0.07 | 15.30±0.11 | 15.22±0.07 | 15.14±0.12 | 15.18±0.12 | 12.0-17.5 |
| **PCT** | 0.49±0.07 | 0.48±0.17 | 0.48±0.05 | 0.45±0.08 | 0.70±0.13 | 0.57±0.08 | 0.100-0.780 |

Plasma samples were obtained 1 week and 2 weeks after treatment. WBC, white blood cell; NEU, neutrophil; LYM, lymphocyte; MON, monocyte; EOS, eosinophilic cell; BAS, basophil; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, Platelets; MPV, mean platelet volume; PDW, platelet distribution width; PCT, thrombocytocrit.

**Table S2** Comparison of the relaxivity values of Gd-HFn with the major Gd-based contrast agents reported in the literature.

| Contrast agent | Structure | $r_1$ (mM$^{-1}$ s$^{-1}$) | $r_2$ (mM$^{-1}$ s$^{-1}$) | Refs |
|---|---|---|---|---|
| **Magnevist®** | Gd-DTPA | 3.9-4.3 (1.5 T) <br> 3.5-3.9 (3 T) <br> 3.5 ± 0.1 (1.4 T) | 3.8-5.4 (1.5 T) <br> 4.3-6.1 (3 T) <br> 5.1 ± 0.2 (1.4 T) | [1,10] |
| **Omniscan®** | Gd-DTPA-BMA | 4.0-4.6 (1.5T) <br> 3.8-4.2 (3T) | 4.2-6.2 (1.5 T) <br> 4.7-6.5 (3 T) | [2] |
| **ProHance®** | Gd-DO3A-HP | 3.9-4.3 (1.5 T) <br> 3.5-3.9 (3 T) | 4.2-5.8 (1.5 T) <br> 4.8-6.6 (3 T) | [2,9] |
| **Dotarem®** | Gd-DOTA | 3.94 (0.25T) <br> 2.96-3.8 (1.5 T) <br> 3.3-3.7 (3 T) <br> 2.85 (9.4) | 3.4-5.2 (1.5 T) <br> 4.0-5.8 (3 T) | [3,5,8,9] |
| **Multihance®** | Gd-BOPTA | 6.0-6.6 (1.5 T) <br> 5.2-5.8 (3 T) | 7.8-9.6 (1.5 T) <br> 10.0-12.0 (3 T) | [3] |
| **OptiMARK®** | Gd-DTPA-BMEA | 4.4-5.0 (1.5 T) <br> 4.2-4.8 (3 T) | 4.3-6.1 (1.5 T) <br> 5.0-6.8 (3 T) | [3] |
| **C-Cha-DOTA** | (Gd$^{3+}$)-chelating 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) | 19.5 (sphere), 17.2 (fiber) | / | [4] |
| **Ppdf-Gd** | piX-PEG8-SSSPLGLAK (DOTA)-PEG6-F4 | 28.2 (sphere), 51.5 (fiber) (0.25 T) | / | [5] |
| **SMNs-Gd, FMNs-Gd** | spherical micellar nanoparticles (Gd$^{3+}$), fibril-shaped micellar nanoparticles (Gd$^{3+}$) | 15.6, 18.5 (0.5 T) | / | [6] |
| **ultrasmall gadolinium oxide** | poly(acrylic acid-co-maleic acid) (PAAMA)-coated ultrasmall Gd$_2$O$_3$ | 40.6 (3 T) | 63.34 (3 T) | [7] |
| **PEG-P(Lys-DOTA-Gd)** | the micelle-forming poly(ethylene glycol)-b-poly(lysine) | 13.31 (1.5 T) <br> 5.54 (9.4 T) | / | [8] |
| **Elucirem® (FDA approved in 2022)** | Gadopiclenol C$_{35}$H$_{54}$GdN$_7$O$_{15}$ | 12.8 (1.4 T) <br> 11.6 (3 T) | / | [9] |

| | | | | |
|---|---|---|---|---|
| **GONP-12** | Gd$_2$O$_3$ -PAMPS-LA | 63.0±4.4 (9.4 T) | 73.5±2.4 (9.4 T) | [10] |
| **Gadolinium Oxide Nanoparticles** | PASA-coated Gd$_2$O$_3$ nanoparticles | 19.1 (3 T) | 53.7 (3 T) | [11] |
| **RGD2** | ES-GON5-PAA@RGD2 | 68.7±2.3 (1.5 T)<br>19.9±0.8 (7 T) | 70.5±1.6 (1.5 T)<br>54.8±2.7 (7 T) | [12] |
| **AFt–C4 NPs** | Gd(III) compound (C4) based on Apoferritin | 3.3 (0.5 T) | / | [13] |
| **Magnetoferritin** | Protein-coated | 8 (1.5 T) | 218 (1.5 T) | [6] |
| **The proposed MRI contrast agent** | HFn-Gd | 549 (1.5 T)<br>428 (3 T) | 1555 (1.5 T)<br>1286 (3 T) | This work |

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