Supporting Information

An Intelligent T_1-T_2 Switchable MRI Contrast Agent for Non-invasive Identification of Vulnerable Atherosclerotic Plaques

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Experimental Section

Materials

Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), polyacrylic acid (PAA, Mw ≈ 3 kDa), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), and 2-(N-Morpholino)ethanesulfonic acid (MES) were obtained from Aladdin Industrial Inc. (Shanghai, China). Diethylene glycol (DEG) was obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Hyaluronic acid (HA, Mw ≈ 66-99 kDa) was obtained from Ruixi Biological Technology Co. Ltd (Xi'an, China).

Synthesis of iron oxide nanoparticles-oleic acid (IONP-OA)

First, 3.4 g of $FeCl_3 \cdot 6H_2O$ was dissolved in 12 mL distilled water to obtain a clear solution. Then, 10.5 g of sodium oleate was add to the above clear solution. A mixed solvent composed of 45 mL n-hexane and 25 mL ethanol was injected into the reaction mixture and the resulting solution was heated at 70℃ for 4 h. The upper organic layer (iron oleate) was separated and washed three time with distilled water. Then, n-hexane was evaporated from the iron oleate complex by slow heating. Second, 2 g of iron oleate complex was dissolved in a mixture of 20 mL oleyl alcohol and 0.3 mL oleic acid at room temperature. The mixture was refluxed at 260℃ for 30 min in an N_2 atmosphere. Thereafter, the resulting solution was cooled to room temperature, washed with 50 mL acetone and separated by centrifugation. Then the nanoparticles were washed with ethanol three times, and dispersed in cyclohexane.

Synthesis of iron oxide nanoparticles-polyacrylic acid (IONP-P)

1g of PAA was dissolved into 16 mL of DEG, and then the mixture was heated to 110℃

with vigorous stirring under N_2 flow. 1 mL cyclohexane solution of IONP-OA nanoparticles was injected to the hot solution. The mixture was heated to 240℃ and kept at this temperature for 1 h under stirring. After the solution was cooled to room temperature, it was centrifuged at 4000 rpm for 5 min. The supernatant was added with 50 mL distilled water, and ultrafiltration centrifugal filter (10 kDa, Millpore, Ireland) was used for centrifuging for 5 times. Finally, the IONP-P solution was collected and stored in a refrigerator.

Synthesis of amine-functionalized hyaluronic acid (HA-NH_2)

The synthesis of amine-functionalized HA (HA-NH_2) was performed according to a pervious report[1]. 200 mg of HA was dissolved in 10 mL of MES buffer to obtain the HA solution. 92 mg of EDC and 55 mg of NHS were dissolved in 200 μL of MES buffer and added sequentially to HA solution during stirring. Subsequently, the pH of solution was adjusted to 7 using 5 M NaOH. Then 128 μL of ethylenediamine was added to the solution, and the pH was immediately adjusted to 7 using 5 M HCl and kept stirring for 12 h. The HA-NH_2 was purified by dialysis against water using a dialysis bag (molecular weight cut-off = 14 kDa, Viskase, USA) and four series of ethanol precipitation. The ethanol precipitation includes the following steps: NaCl was added to the solution of HA to obtain a concentration of 5% NaCl. Subsequently, 3 parts of ethanol was added to the solution, and the mixture was shaken vigorously until a white precipitate appeared. Then, the white precipitate was collected by centrifugation at 4000 rpm for 20 min. After four times of ethanol precipitation, the white HA-NH_2 was dissolved in ultrapure water, dialyzed against water overnight and freeze-dried.

The dry lyophilizate was weighed and dissolved in 10 mL of ultrapure water, and stored in a refrigerator.

Synthesis of iron oxide nanoparticles-hyaluronic acid-polyacrylic acid (IONP-HP)

For the synthesis of IONP-HP, EDC (92 mg) and NHS (55 mg) were dissolved into IONP-P aqueous solution and kept stirring for 4 h at room temperature. Then, 1 mL of HA-NH_2 aqueous solution was added dropwise to the above solution under stirring, and keep stirring overnight. The final product was collected by centrifugation using ultrafiltration centrifugal tube (30 kDa, Millpore, Ireland).

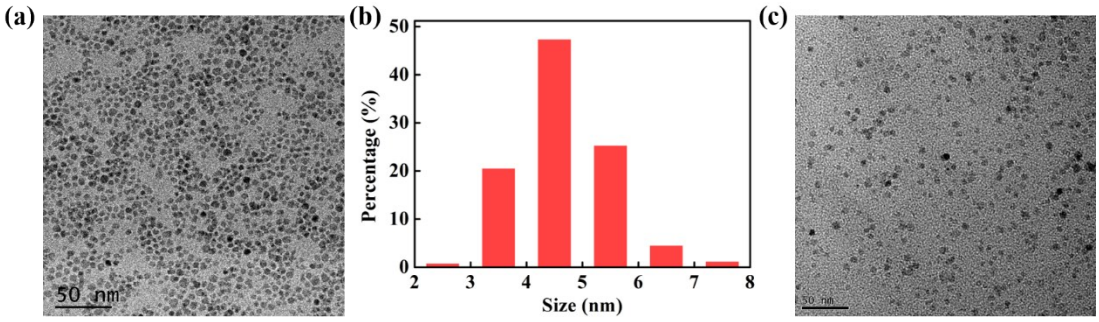

Fig. S1 (a) TEM image and (b) size distribution of IONP-OA. (c) TEM image of IONP-P.

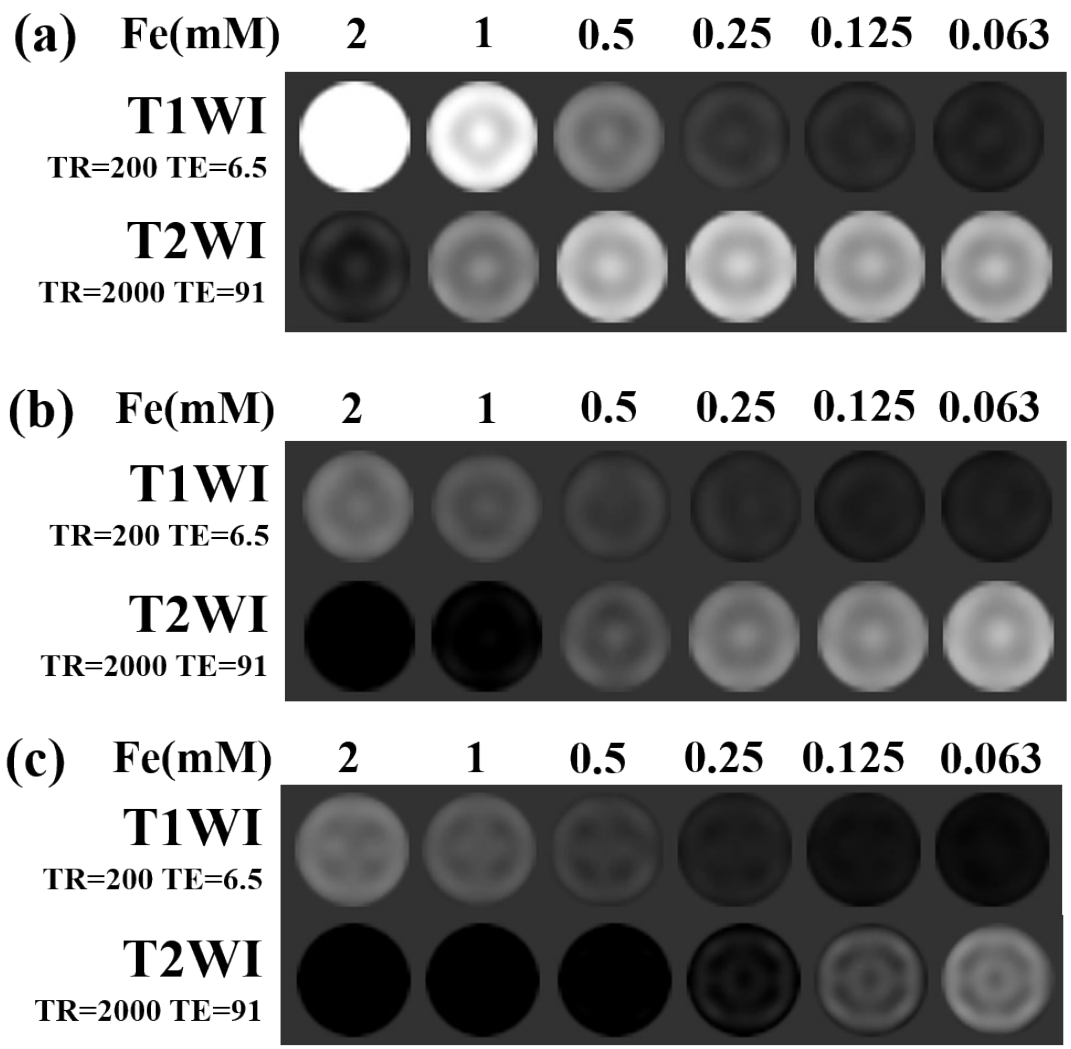

Fig. S2 T_1-weighted images (T1WI) and T_1-weighted images (T2WI) of IONP-HP at the acid environment of (a) pH = 6, (b) pH = 5, (c) pH = 4 with different Fe concentration on a 1.5 T MRI scanner.

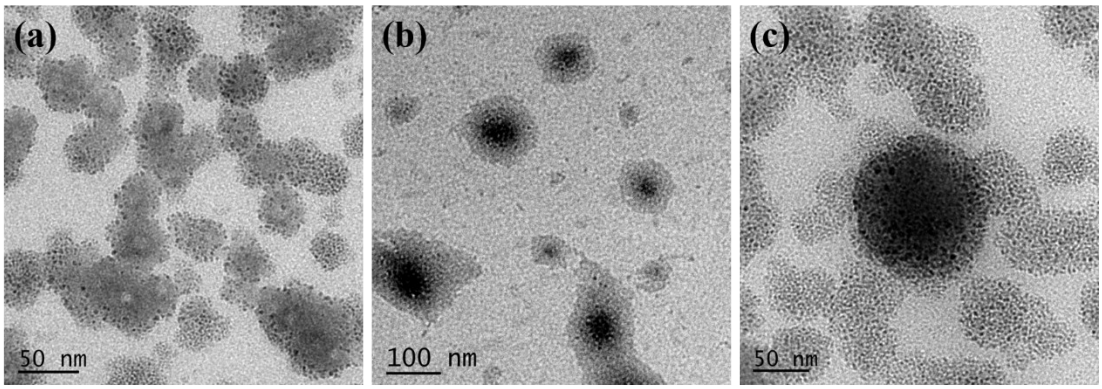

Fig. S3 TEM images of the formed IONP-HP clusters at (a) pH = 6, (b) pH = 5 and (c) pH = 4.

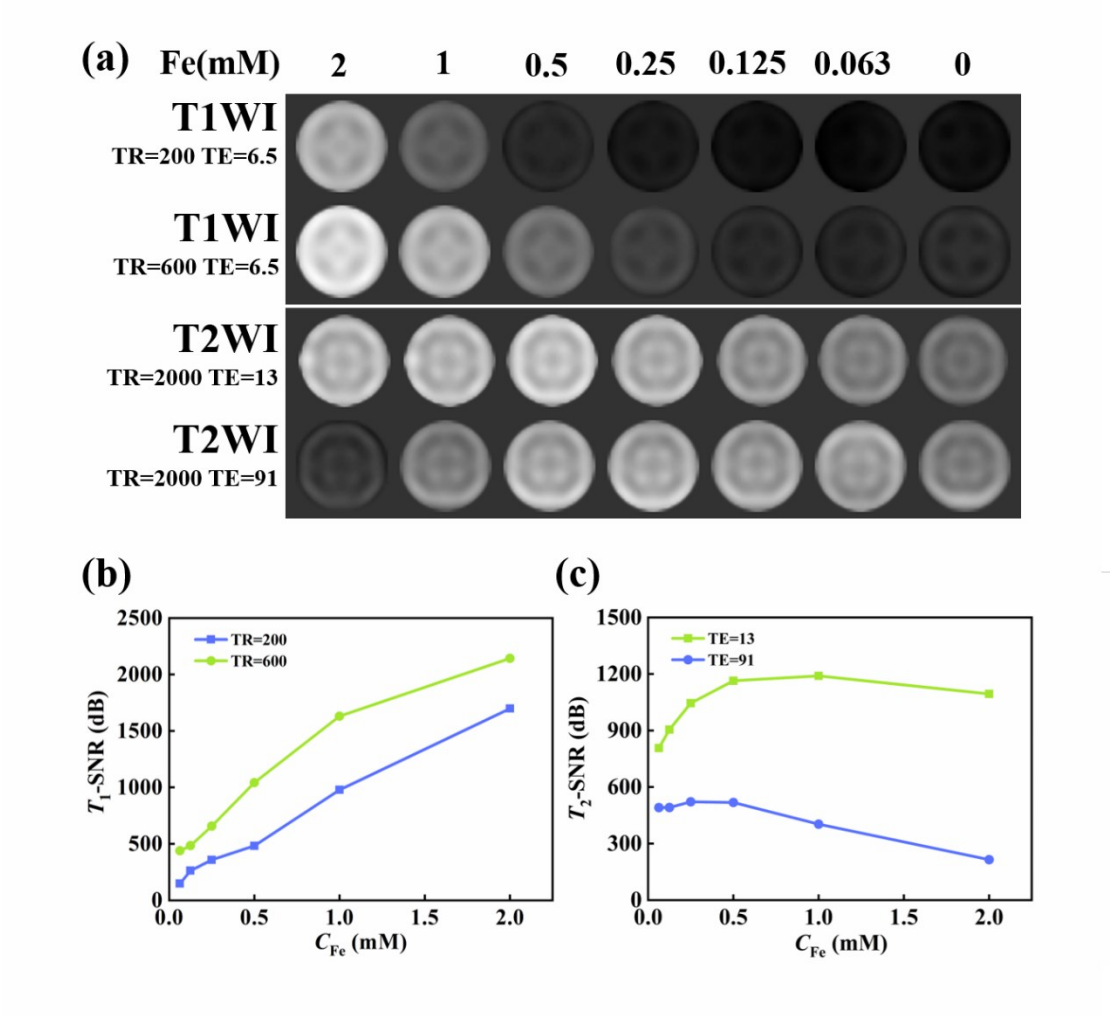

Fig. S4 (a) T_1-weighted images (TR = 200 and 600 ms; TE = 6.5 ms) and T_2-weighted images (TR = 2000 ms; TE = 13, 91 ms) of IONP-P at different Fe concentrations on a 1.5 T MRI scanner. The SNRs of IONP-P from the (b) T_1- and (c) T_2-weighted images at different Fe concentrations.

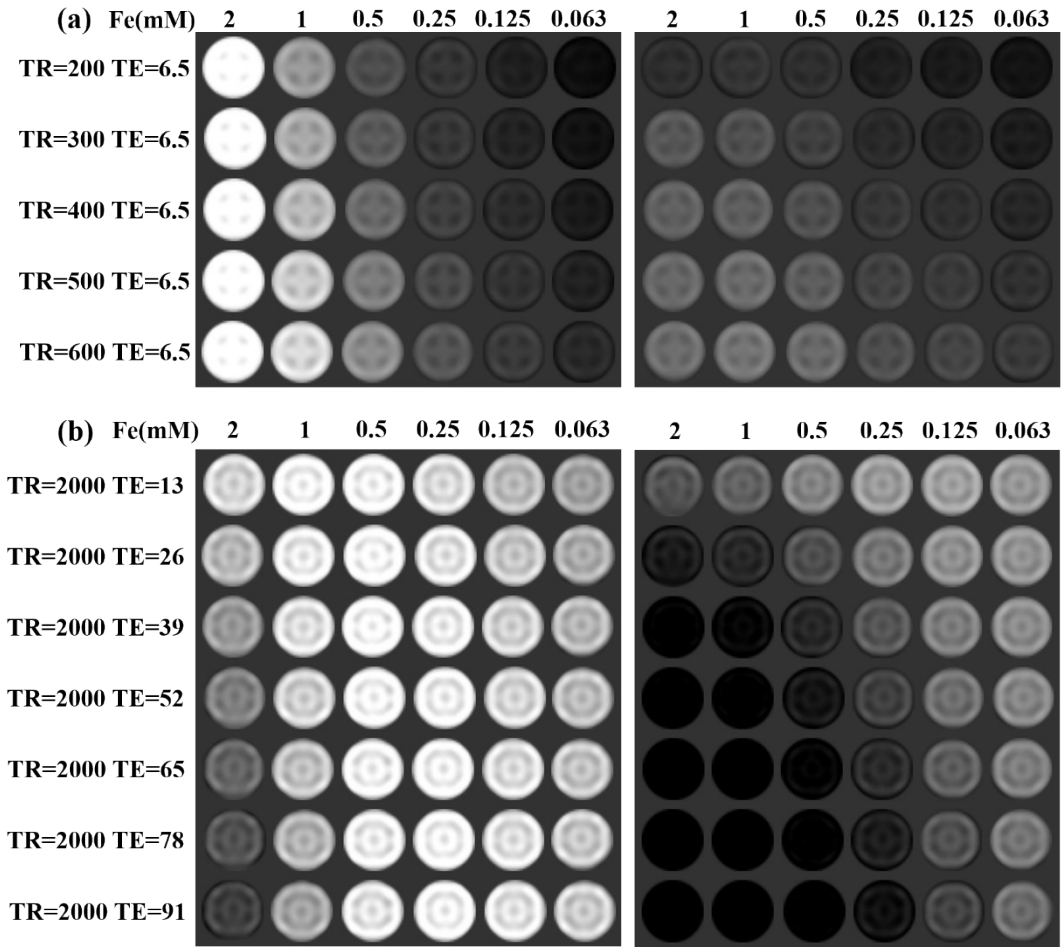

Fig. S5 (a) T_1-weighted images (TR = 200, 300, 400, 500, 600 ms and TE = 6.5 ms) of (left) dispersed and (right) clustered IONP-HP *versus* different Fe concentrations ([Fe] = 0, 0.063, 0.25, 0.5, 1, and 2 mM) at 1.5 T. (b) T_2-weighted images (TE = 13, 26, 39, 52, 65, 78, and 91 ms; TR = 2000 ms) of (left) dispersed and (right) clustered IONP-HP versus different Fe concentrations at 1.5 T.

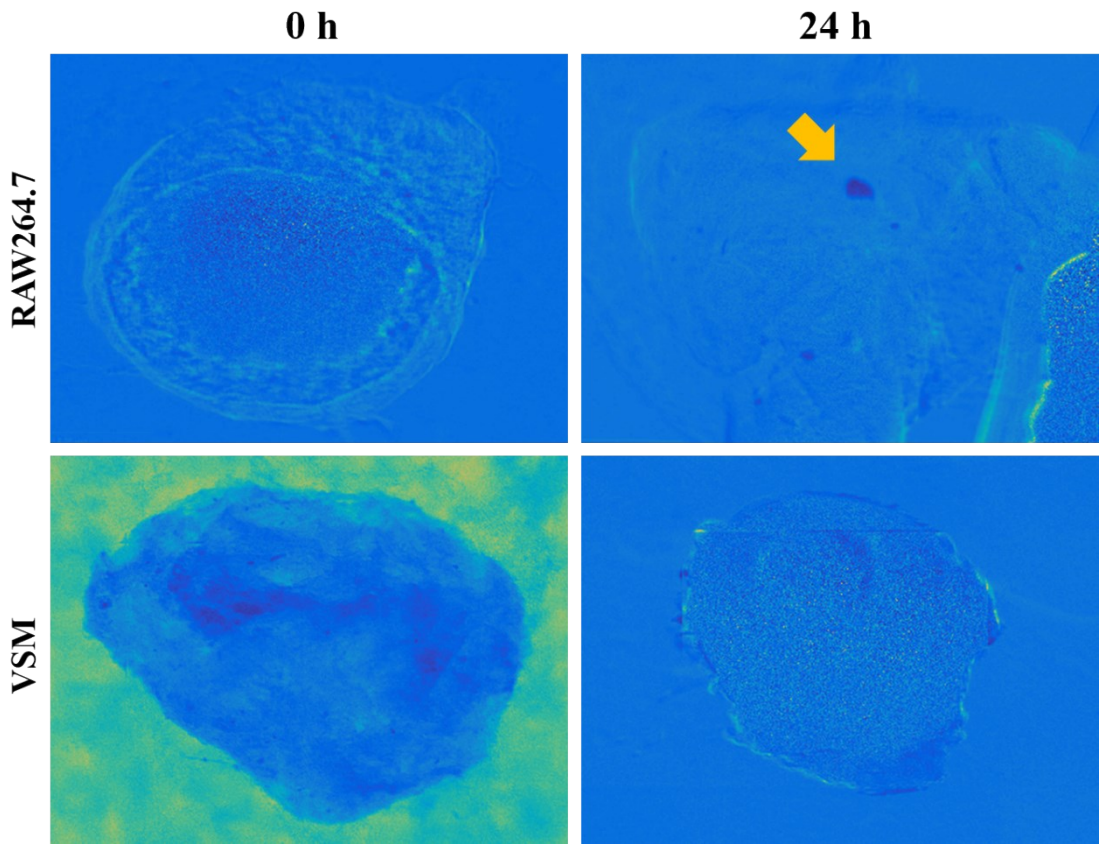

Fig. S6 2-D soft X-ray microscopy images of RAW264.7 and VSM cells incubated with IONP-HP for 24 h.

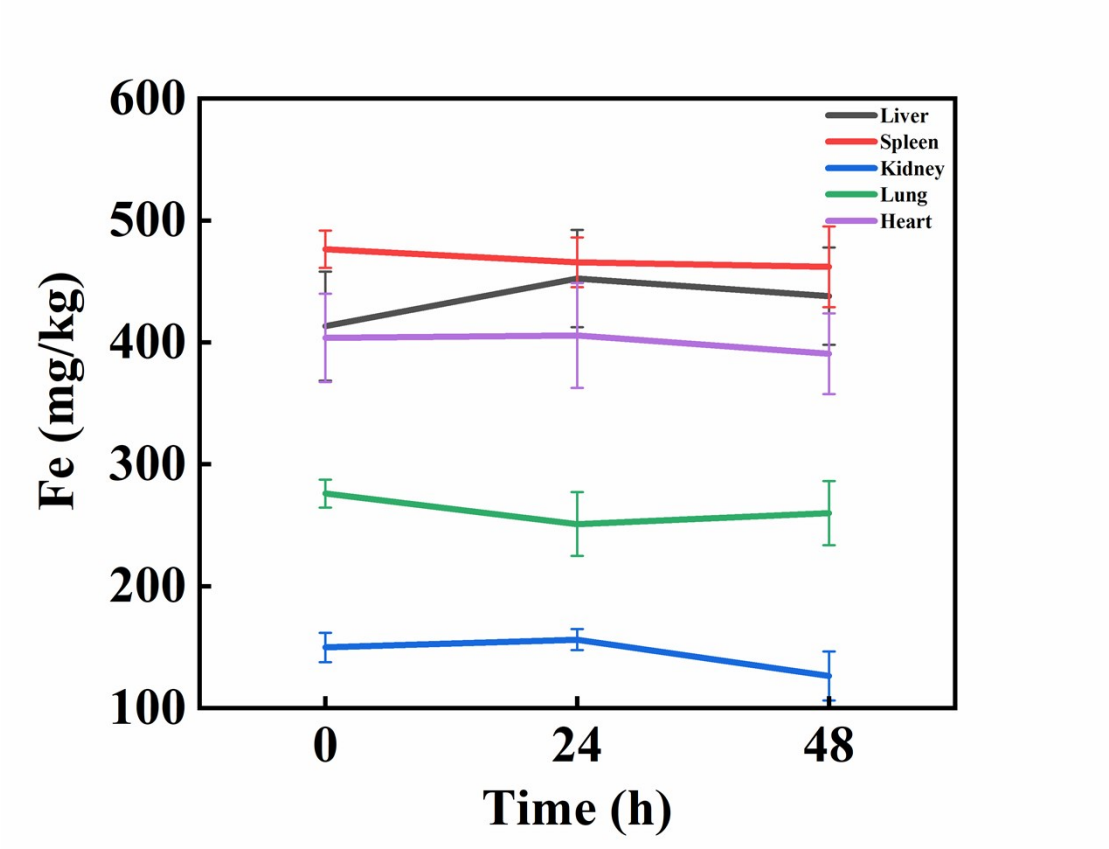

Fig. S7 Biodistribution of Fe in mice major organs after intravenous injection of IONP-HP.

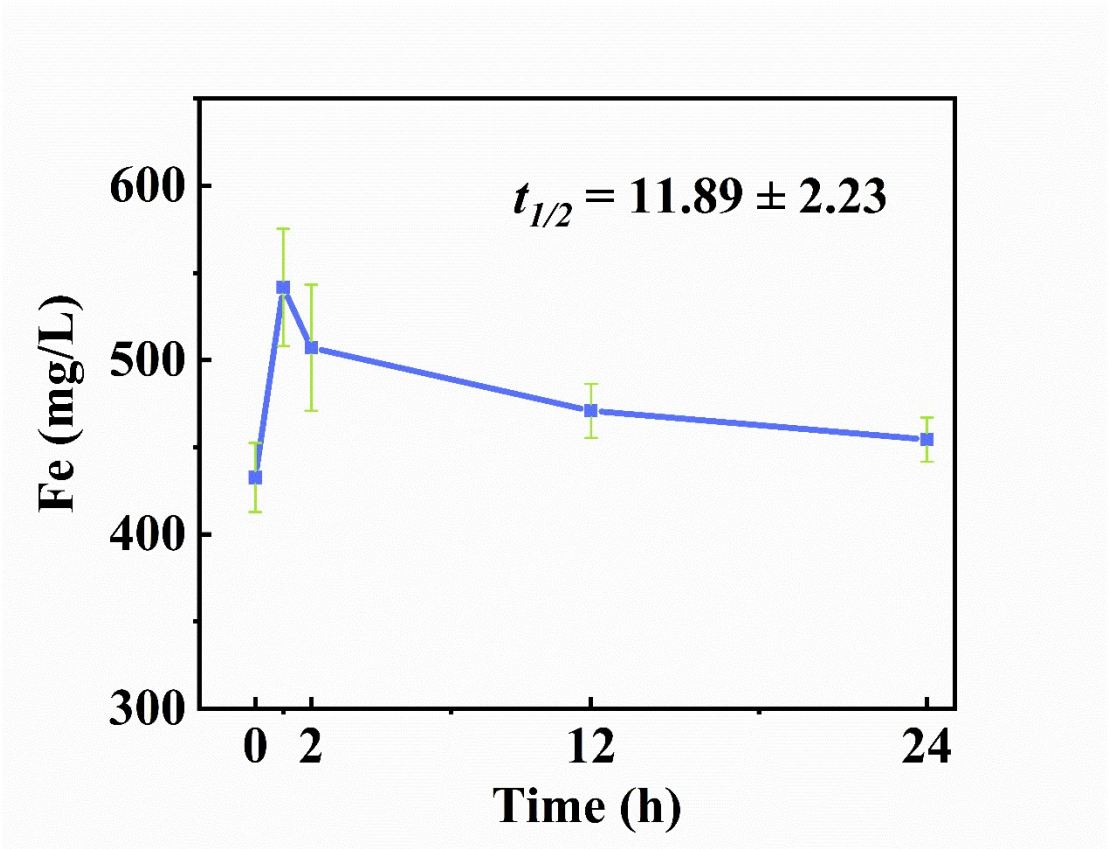

Fig. S8 Concentration curve of Fe in blood before and after intravenous injection of IONP-HP in mice.

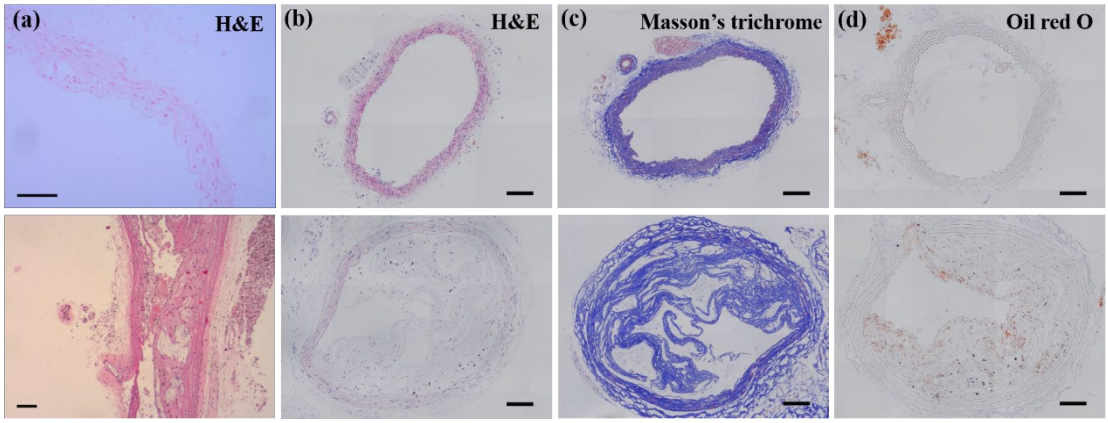

Fig. S9 (a) Micrographs of (up) stable and (down) vulnerable plaques. (b-d) Histological analysis of (up) healthy aortas and (down) vulnerable plaques by different dye-staining. Scale bar is 100 μm.

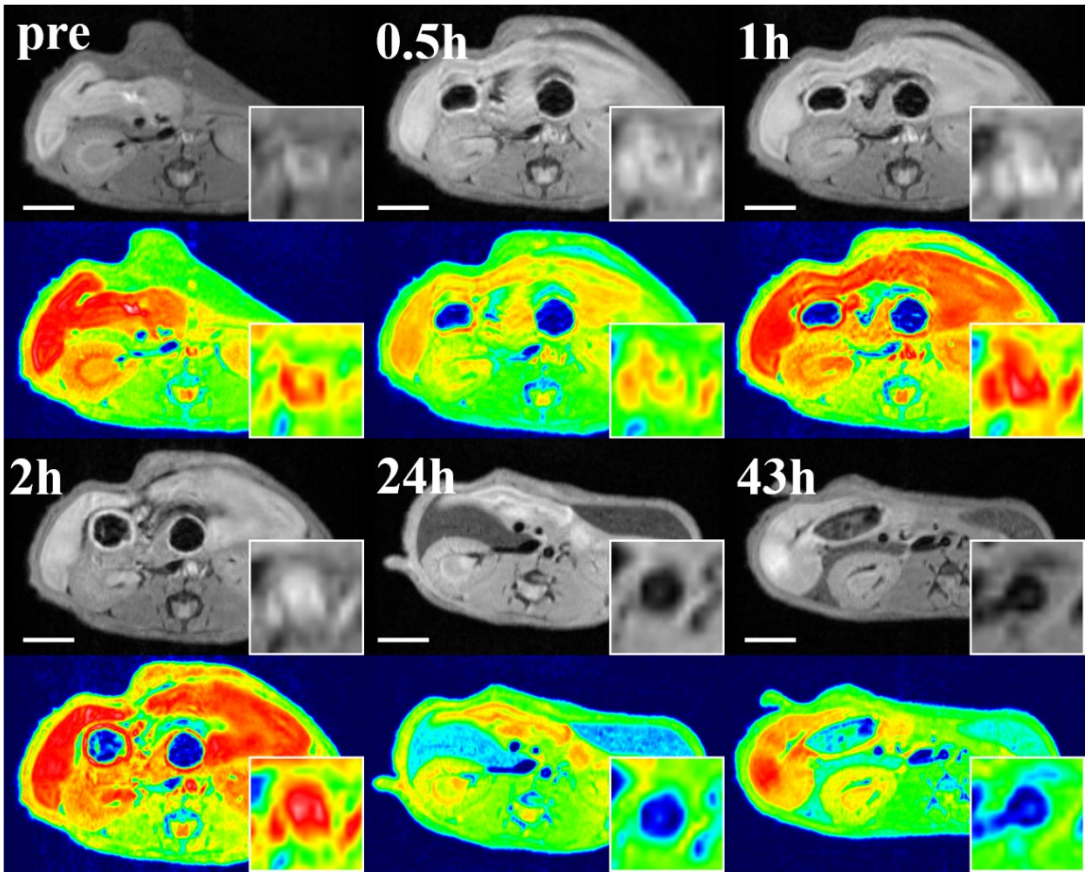

Fig. S10 T1-weighted images and corresponding color-coded images of ApoE-/- mouse in vulnerable plaque group at different time points before and after intravenous injection of IONP-HP. Scale bars is 5 mm.

Reference

1. T. J. Beldman, M. L. Senders, A. Alaarg, C. Perez-Medina, J. Tang, Y. Zhao, F. Fay, J. Deichmoller, B. Born, E. Desclos, N. N. van der Wel, R. A. Hoebe, F. Kohen, E. Kartvelishvily, M. Neeman, T. Reiner, C. Calcagno, Z. A. Fayad, M. P. J. de Winther, E. Lutgens, W. J. M. Mulder and E. Kluza, *ACS Nano*, 2017, **11**, 5785-5799.