Supporting Information

Effects of Nanoprobe Morphology on Cellular Binding and Inflammatory Responses: Hyaluronan Conjugated Magnetic Nanoworms for Magnetic Resonance Imaging of Atherosclerosis Plaques

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Table of Contents

HA quantification on HA-NW

Each NW on average contains 15 iron oxide cores and each iron oxide core is about 6 nm in diameter. The lattice volume for magnetite is about 592 $Å^3$, which means each iron oxide core contains 191 lattices. Since each lattice has 8 molecules of Fe_3O_4 then each NW has 22920 molecules of Fe_3O_4 (15 x 191 x 8 = 22920). Based on TGA 9%, of HA-NW mass is iron oxide core while 70% is HA that results in an average of 1331 molecules of HA for each HA-NW particle.

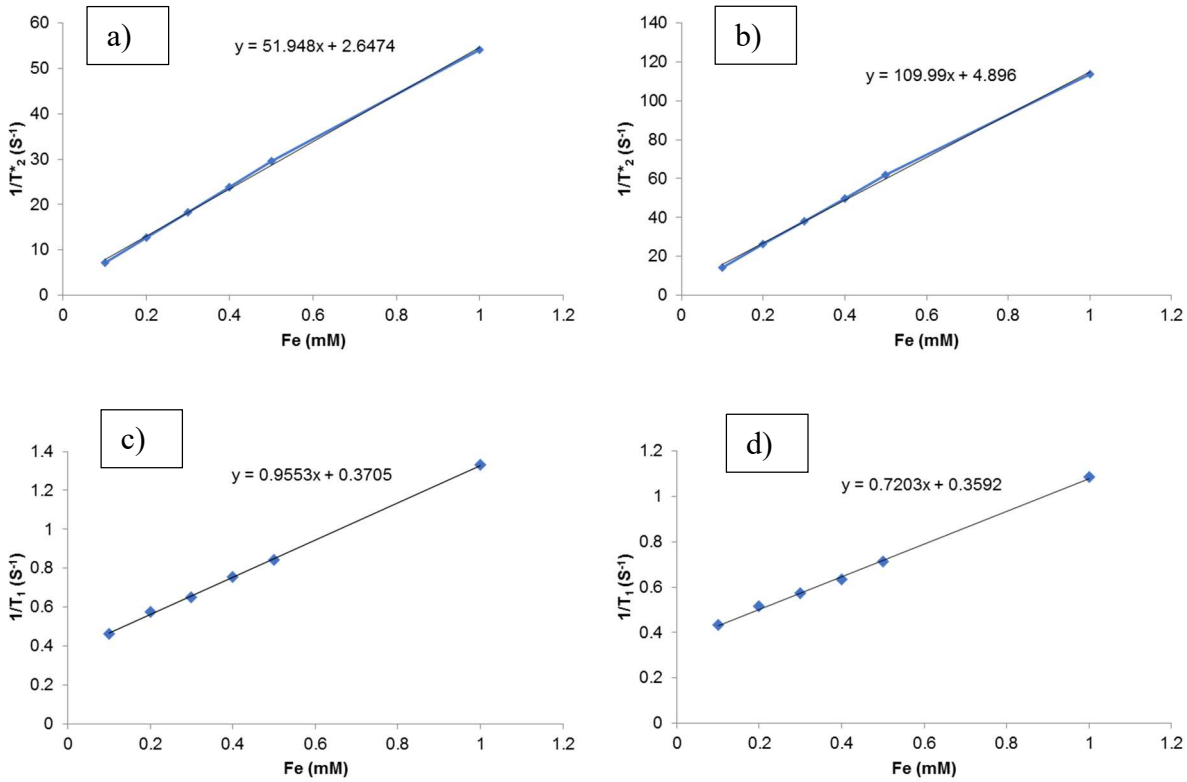

Figure S1. r_2* and r_1 measurements for prepared nanoparticles in a 7 T magnet. (a,c) are r_2* and r_1 for HA-SPIONs respectively. (b,d) are are r_2* and r_1 for HA-NWs respectively.

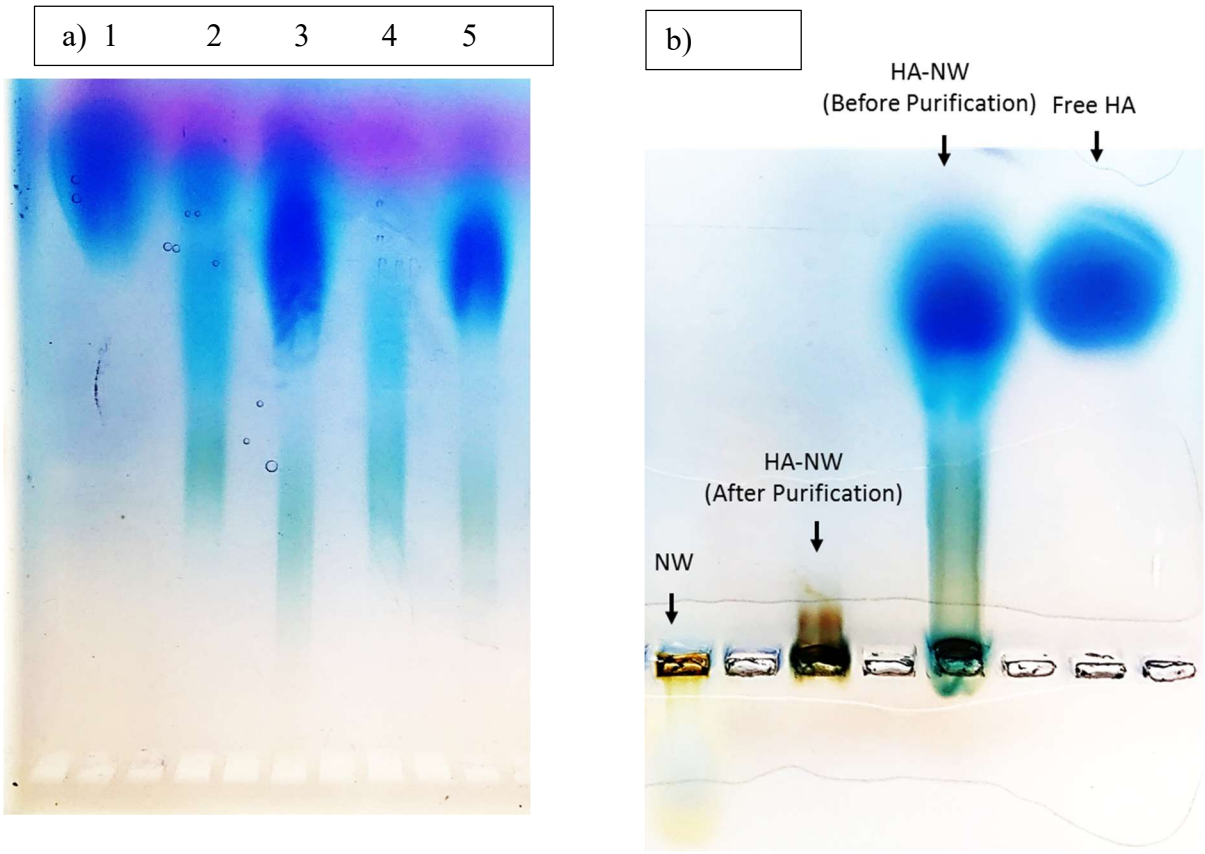

Figure S2. Removal of free HA from HA-SPIONs and HA-NWs. a) Lane 1 contains free HA. HA polymer showed blue color following staining with Stains-All dye. HA-SPION before and after washing with 10% $(NH_4)_2SO_4$ were placed in lanes 2 and 4 respectively. HA-NWs before and after washing with 10% $(NH_4)_2SO_4$ were in lanes 3and 5 respectively. b) Complete removal of free HA from HA-NWs by washing with 35% $(NH_4)_2SO_4$ was shown by gel electrophoresis. Purified HA-NW not only had no free HA but also moved towards the cathode on the gel while un-conjugated NWs did not move towards the cathode.

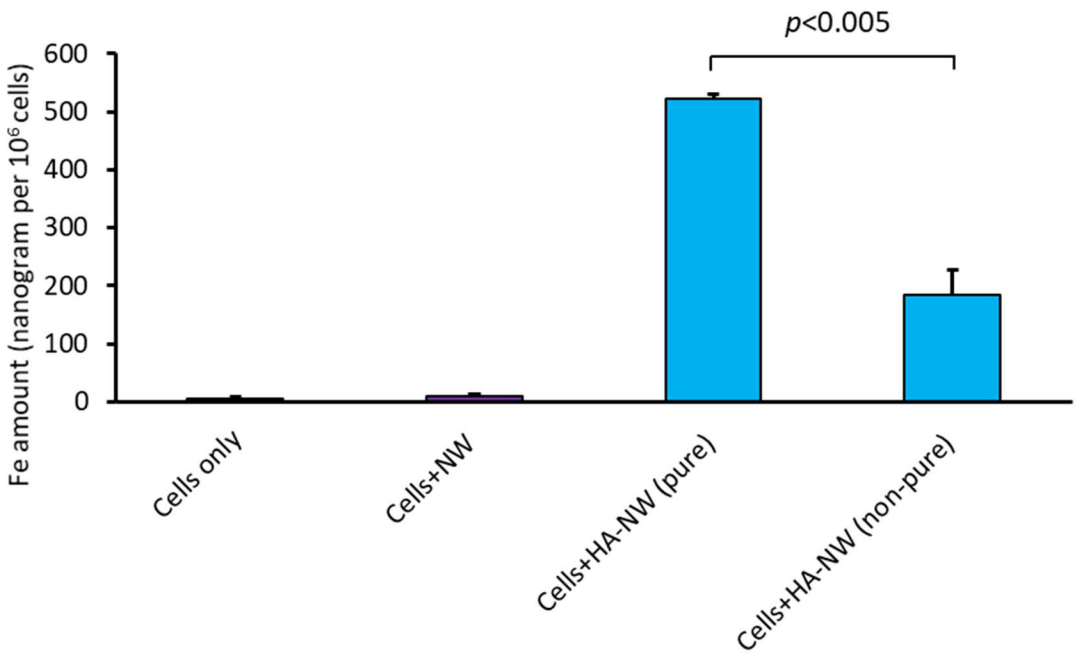

Figure S3. Free HA containing HA-NWs have reduced interactions with CD44 expressing cells compared to pure HA-NWs highlighting the importance of completely removing free HA following synthesis. CD44 expressing SKOV-3 cells were incubated with NWs, pure HA-NWs and non-pure HA-NWs. Intracellular Fe levels were quantified by ICP following removing unbound HA-NWs.

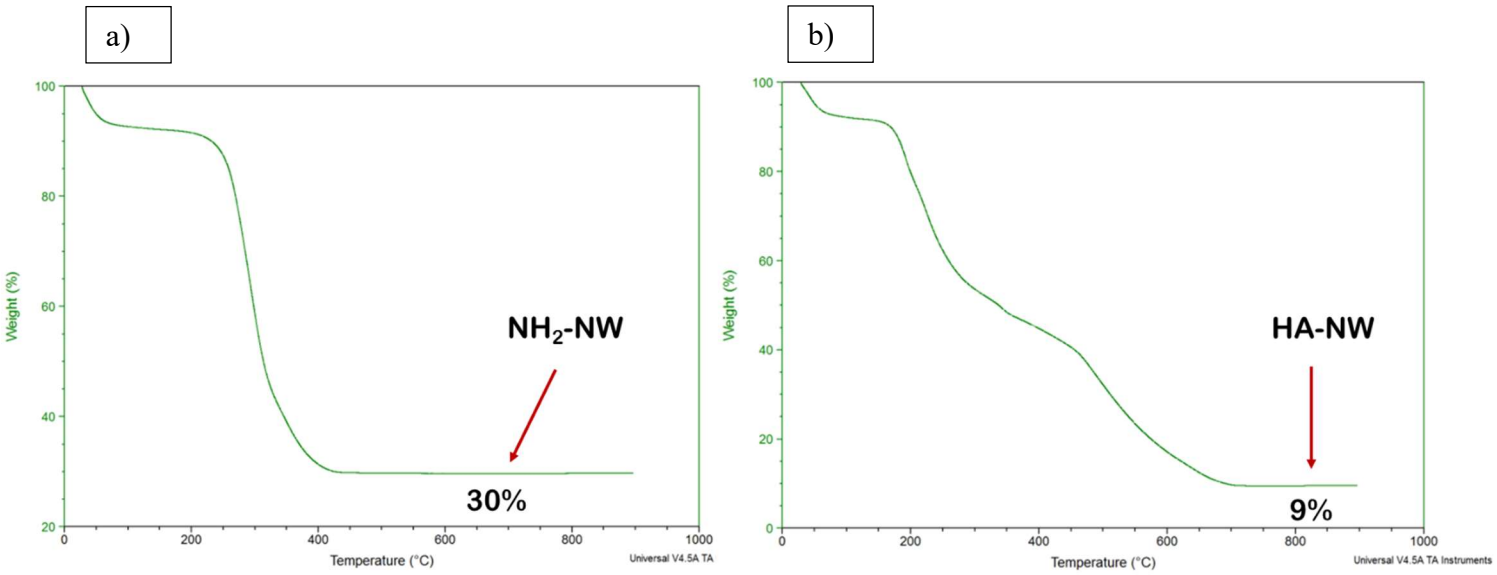

Figure S4. Thermogravimetric analysis (TGA) for (a) amine functionalized NWs and (b) HA conjugated NW.

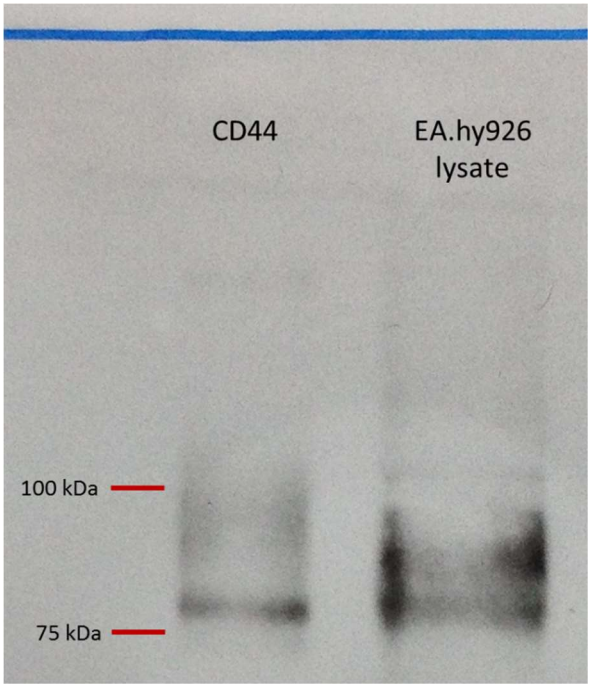

Figure S5. Western blotting showed the presence of CD44 in EA.hy926 cells.

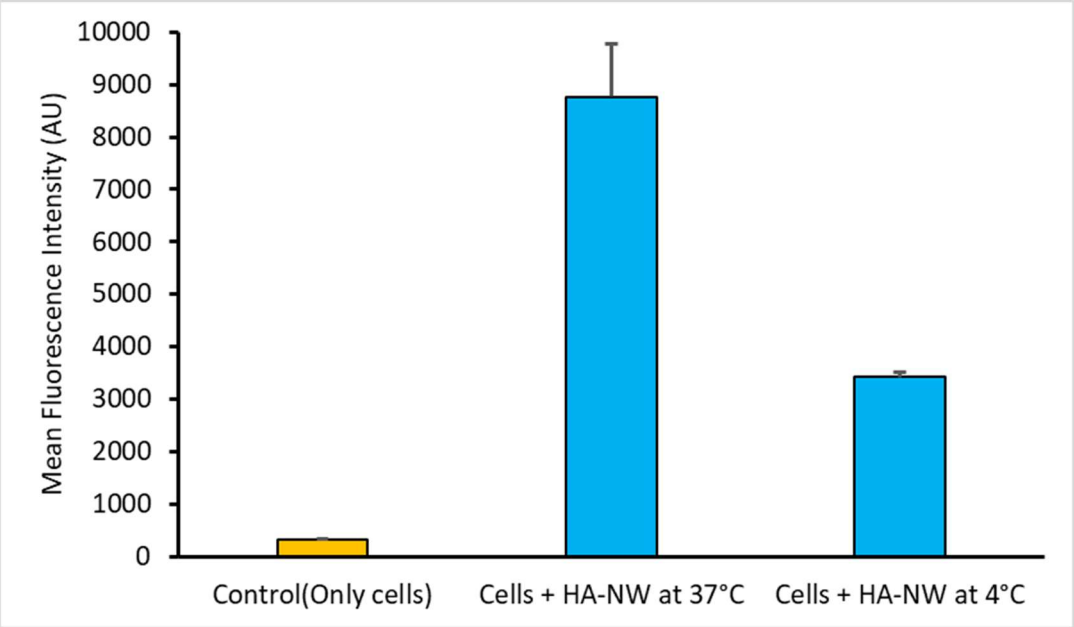

Figure S6. FACS study showed mean fluorescence intensities of SKOV-3 cells after incubation with HA-NWs in 37 ˚C and 4 ˚C respectively.

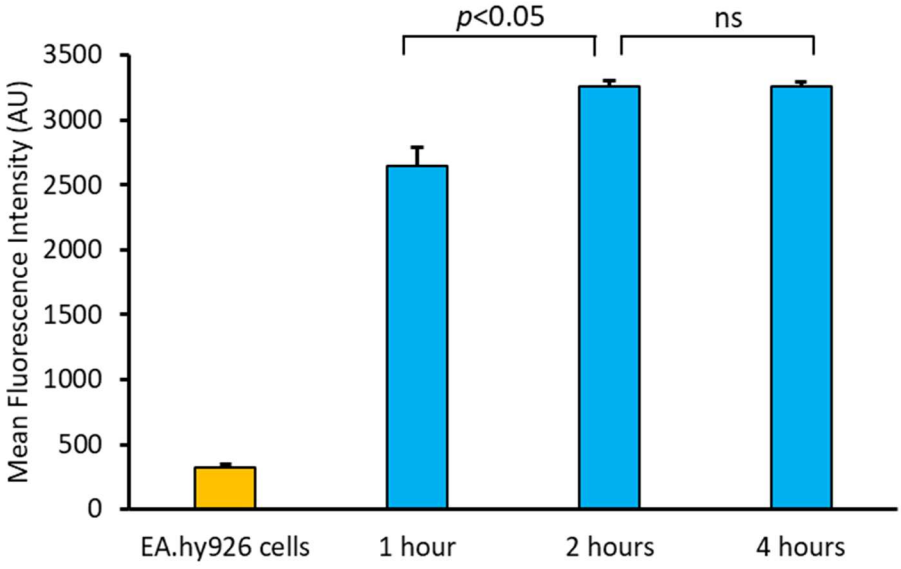

Figure S7. Monitoring EA.hy926 cellular uptake of HA-NW at different time intervals. 50 µg/ml HA-NWs were added to each well and MFI were measured by FACS. The *p*-values were obtained from student's *t*-test.

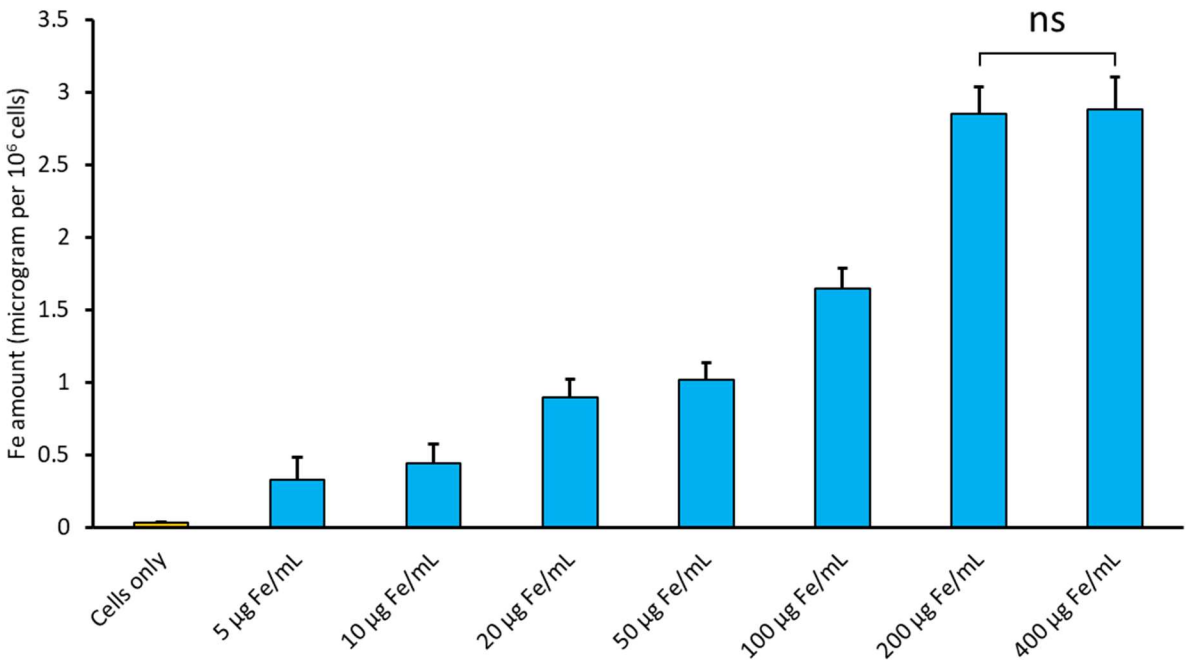

Figure S8. Incubation of SKOV-3 cells with different amounts of HA-NWs.

Table S1. Results for blood chemistry panel analysis 24 h after injection of HA-NWs.

	Values	Reference Values
Total Protein	5.6 g/dL	4.5-6.5 g/dL
Albumin	2.7 g/dL	2.4-3 g/dL
Globulin	2.9 g/dL	1.8-3 g/dL
Total bilirubin	0.9 mg/dL	0-1.0 mg/dL
Direct bilirubin	0.0 mg/dL	
Indirect bilirubin	0.9 mg/dL	
alkaline phosphatase U/L	27	15-45
gamma-glutamyl transferase U/L	<3	0-9
alanine transaminase U/L	30	10-35
aspartate aminotransferase U/L	278	43-397

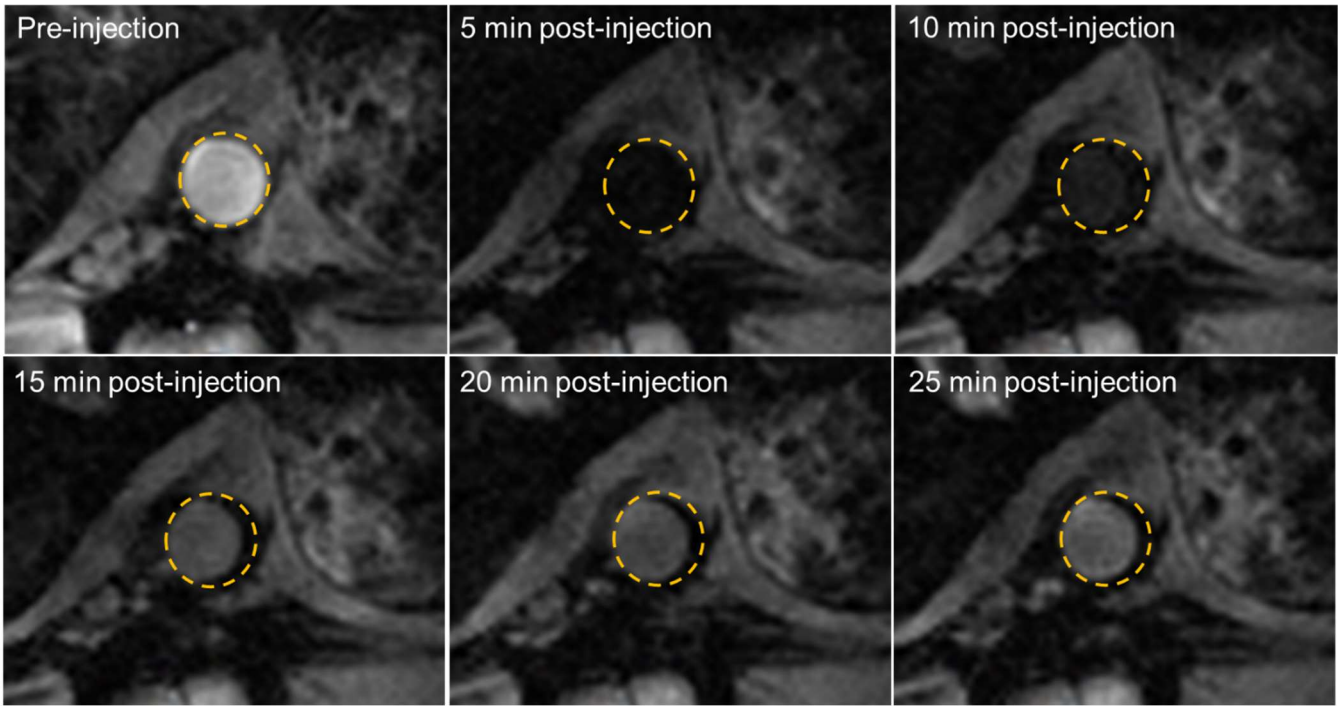

Figure S9. MRI contrast changes of the aorta of ApoE knockout mice upon injection of HA-NWs (4 mg/Kg).

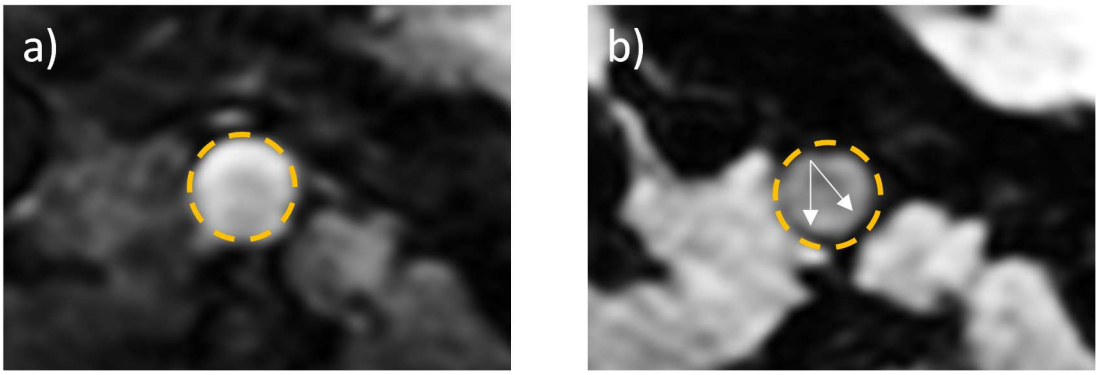

Figure S10. MRI contrast changes of the aorta of ApoE knockout mice a) before and b) 20 minutes after injection of HA-NWs (2.8 mg/Kg).

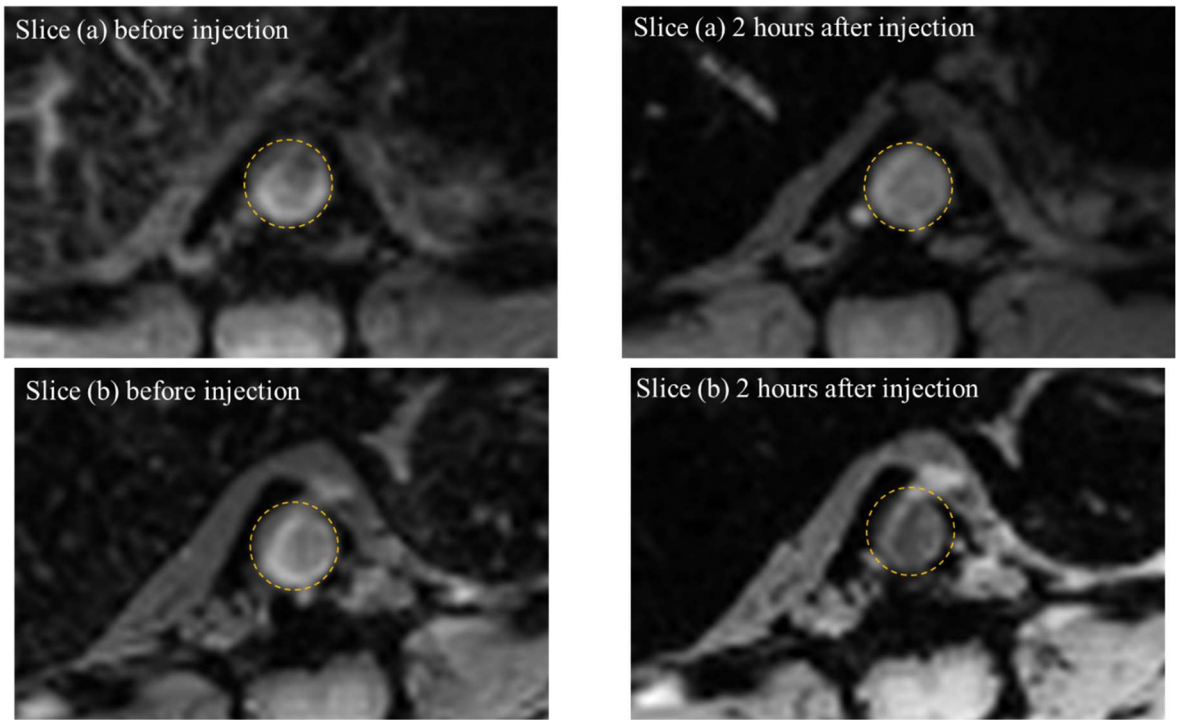

Figure S11. Analysis of MRI of different slices of the aorta of ApoE knockout mice showed signal loss on the aorta wall in some locations but not all of them; indicating HA-NWs only interacted with selective areas of aorta wall that were presumably atherosclerosis plaques.

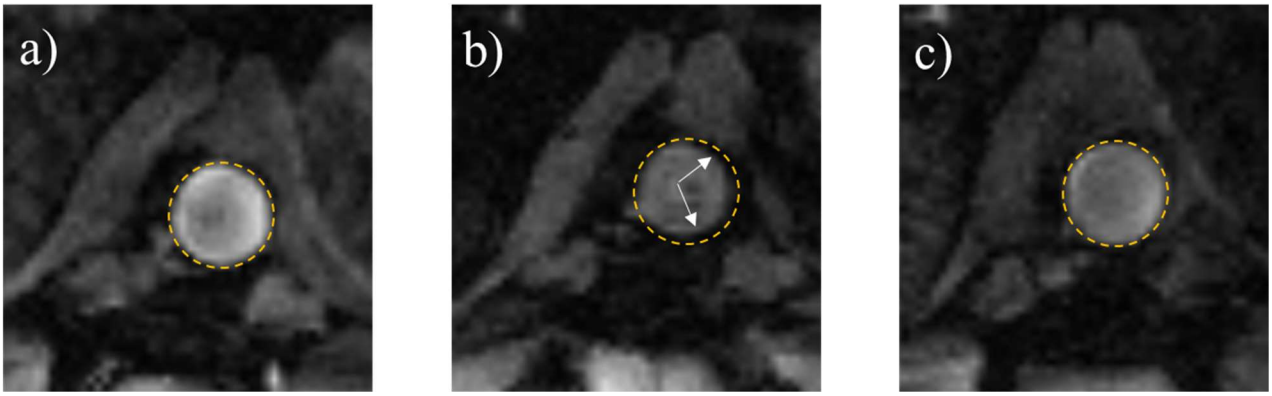

Figure S12. The signal intensity changes of the aorta lumen disappeared when ApoE knockout mouse was scanned one week after HA-NW administration. (a) ApoE knockout mouse aorta before injection, (b) ApoE knockout mouse aorta 0.5 hour after injection and (c) ApoE mouse aorta image one week after injection of HA-NWs.

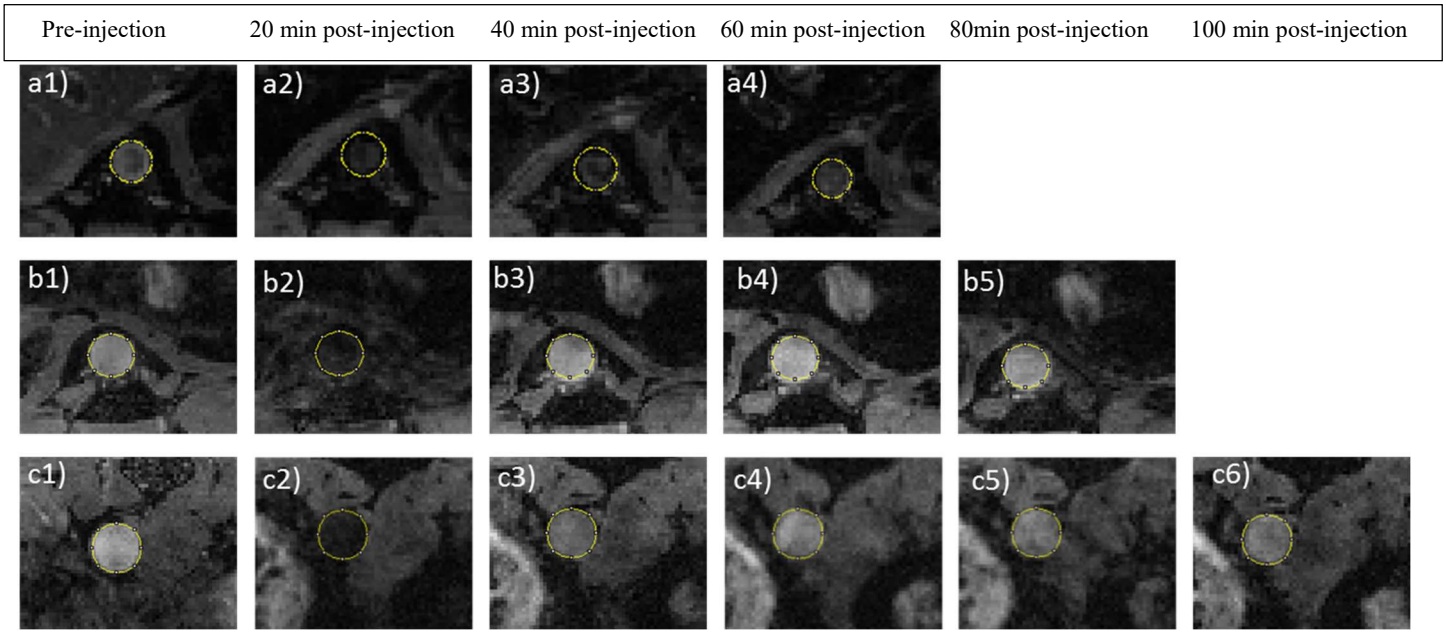

Figure S13. a) HA-NWs were injected to the wild type mouse and the abdominal aorta was scanned before (a1) and different time intervals after injection (a2-4). b) NWs were injected to the ApoE knockout mouse and the abdominal aorta was scanned before (b1) and different time intervals after injection (b2-5). c) HA-NWs were mixed with free HA and injected to ApoE knockout mouse and the abdominal aorta was imaged before (c1) and different time intervals after injection (c2-6).

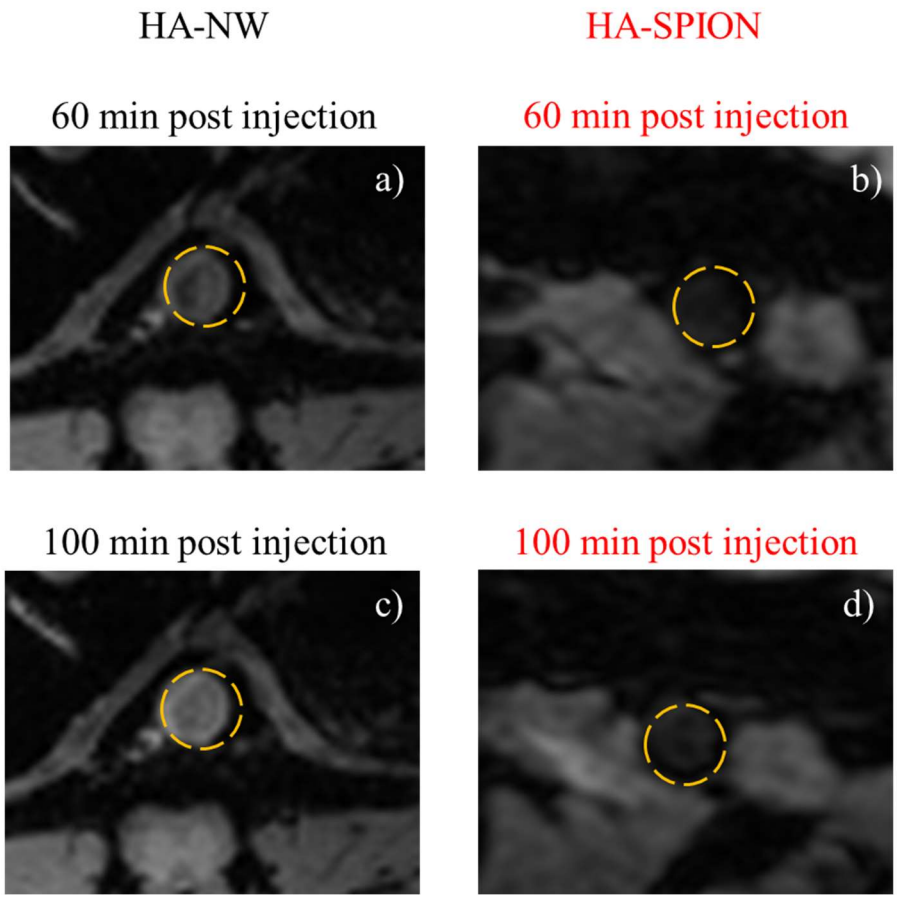

Figure S14. Comparison of HA-SPIONs (8 mg Fe/kg) and HA-NWs (8 mg Fe/kg) for MRI detection of atherosclerotic plaques in ApoE knockout mice. Aorta lumen MR signals recovered partially (panel a) and completely (panel c) 60 minutes and 100 minutes after injection of HA-NW enabling the detection of plaques on aorta walls. In contrast, aorta lumen signals did not recover 60 and 100 minutes after injection in mice receiving HA-SPIONs (panels b and d), preventing plaque detection at these time points.

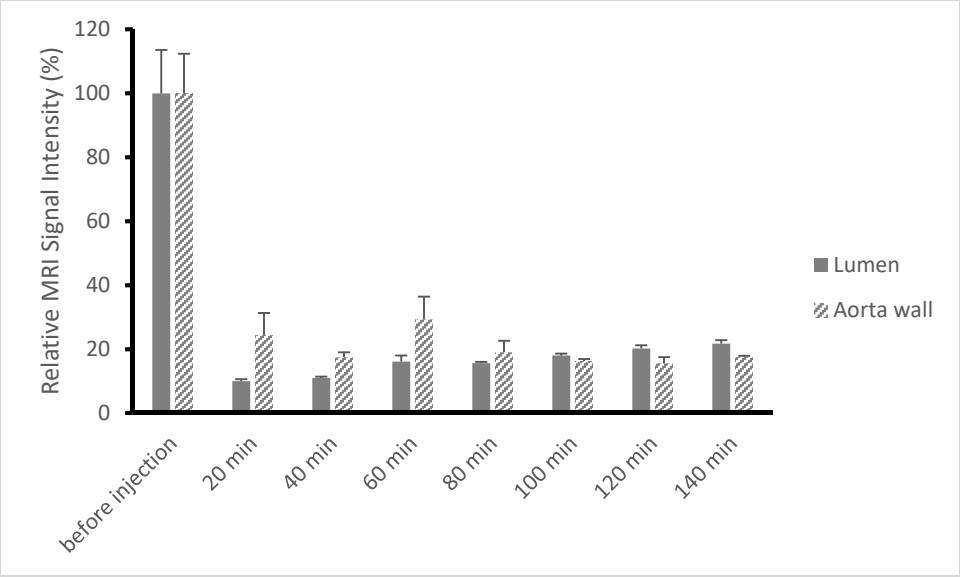

Figure S15. Quantification of signal intensity change for lumen and aorta wall before injection and at different time intervals after injection of HA-SPIONs (8 mg Fe/kg). Analysis was performed using ImageJ software. Lumen signals did not recover much 140 minutes after HA-SPION injection.

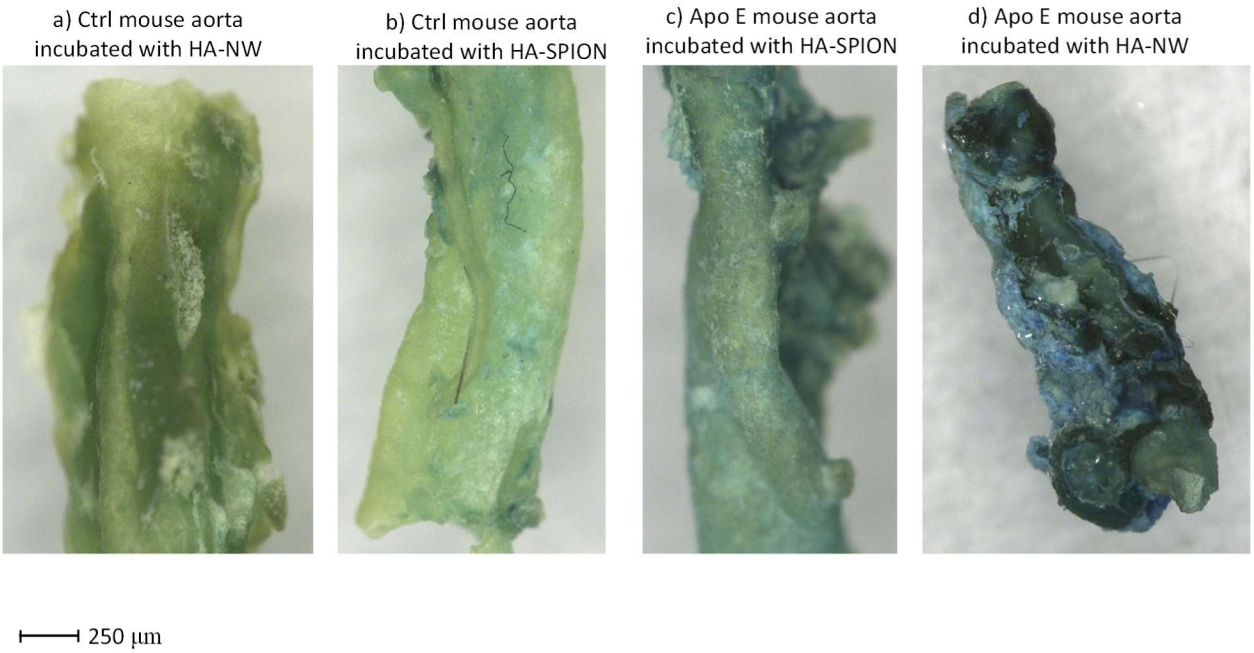

Figure S16. Comparison of Prussian blue staining of aortae after incubations with identical concentrations of HA-NWs or HA-SPIONs (0.4 mg Fe/kg) for 1 hour at 37 °C. a) aorta from wild type control mice stained with HA-NWs; b) aorta from wild type contrl mice stained with HA-SPIONs; c) aorta from ApoE knockout atherosclerotic mice stained with HA-SPIONs; d) aorta from atherosclerotic mice stained with HA-NWs. The much higher intensities of Prussian blue staining in panel d) compared to all other panels suggest the stronger binding of plaque tissues by HA-NWs.

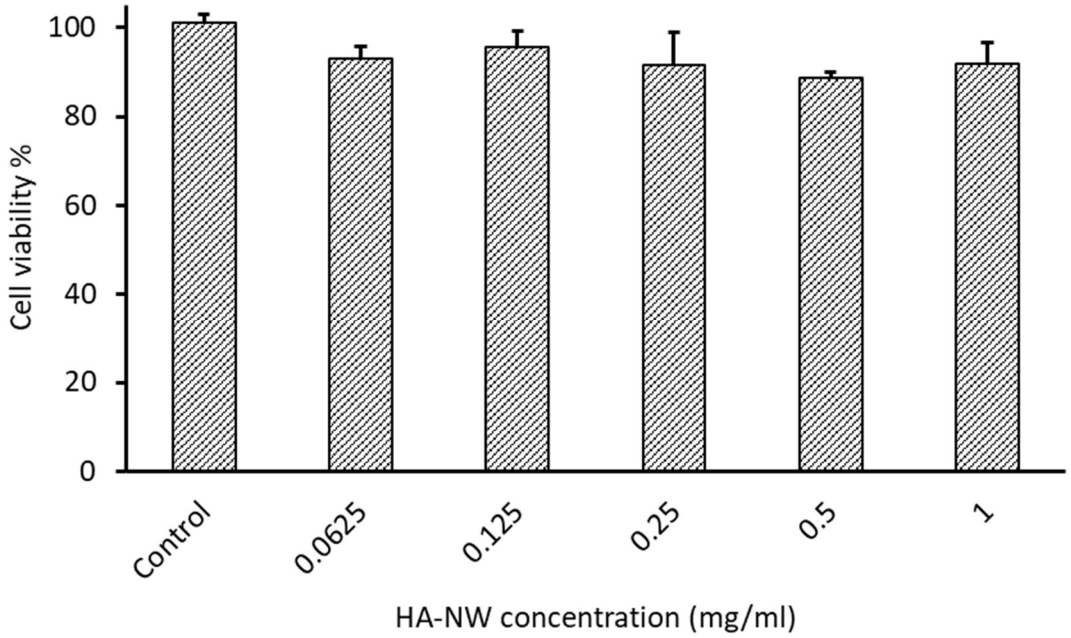

Figure S17. EA.hy926 cell viabilities following incubation with different concentrations of HA-NWs were measured by the MTS assay. No reductions of cell viability were observed.