Electronic Supplementary Material

Supplemental Methods

Blood Analysis

Before euthanasia, the blood sugar levels were measured on samples taken from the lateral tail vein in awake mice. The mice were sacrificed, their chest was cut open, and a heart puncture was performed for blood sampling. Next, the mice were perfused with 5 ml PBS via the left ventricle of the heart to clear the blood from circulation before the organs were dissected for analysis ex vivo. Blood samples were centrifuged at RT for 5 min at 6000 rcf, and plasma was collected in tubes containing EDTA for determination of plasma lipid levels. Commercially available kits were used for enzymatic determination of nonesterified fatty acids (NEFAs) (Wako Chemicals, Richmond, VA, USA), triglycerides (TG) (Sigma-Aldrich), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c)/very low-density lipoprotein cholesterol (VLDL-c) (HDL and LDL/VLDL Cholesterol Quantification Kit, BioVision, Milpitas, CA, USA).

Embedding and Lipid Staining

Dissected hearts were weighed and placed in PBS on ice for embedding in optimal cutting temperature compound (O.C.T. Compound, Tissue-Tek, Sakura Finetek Europe BV, Alphen aan den Rijn, The Netherlands). Serial 8-μm-thick cryostat sections starting at the aortic sinus were collected on Superfrost Plus microscope slides fixed in 4% FA for 10 min at RT and stored at −20°C until immunohistochemical staining or phosphor imaging (PI) plate exposure. Standardized portions of whole aortas were resected under a stereomicroscope using microdissection tools. Vessels were fixed for 24 h in 4% FA, washed with PBS, cut open, and then pinned on custom-made Parafilm pads to expose the intimal surface. Next, the aortas were rinsed with 70% ethanol for 5 min and stained with 0.5% Sudan IV (Sigma/Aldrich) for 6 min to visualize lipid-rich atherosclerotic plaques. After staining, the aortas were rinsed with 80% ethanol for 3 min. En face micrographs of all aortas were acquired using a Leica digital camera DC480 connected to a Leica MZ6 stereomicroscope, before

exposure on a PI plate for imaging by autoradiography (ARG). The en face images were used to determine the plaque burden by area calculations made using Fiji ImageJ software.[1]

Immunohistochemistry

For each mouse, immunohistochemical staining was performed on aortic root sections: CD68 for macrophages, SR-A1 for macrophage scavenger receptor A, Smooth muscle alpha-actin for vascular smooth muscle cells, and von Willebrand factor for endothelial cells. Slides were rinsed with Tris-buffered saline (TBS) (Biocare Medical, Pacheco, CA, USA) and blocked with Rodent block M (Biocare Medical) for 20 min and with Background Sniper (Biocare Medical) for 20 min at RT. Primary antibodies; rat anti-mouse CD68 (Dako, Agilent, Santa Clara, CA, USA) 1:50, rabbit anti-mouse SR-A1 (Novus, Bio-Techne, Abingdon, UK) 1:25, mouse anti-human/mouse Smooth muscle alpha-actin (Dako) 1:1500 or rabbit anti-human/mouse von Willebrand factor (Dako) 1:200, and isotype controls; rat IgG2a (BioRad) 1:50, polymer negative control serum mouse & rabbit IgG1 (Biocare Medical) ~0.33 µg/ml, diluted in Da Vinci Green (Biocare Medical) were incubated for 1 h at RT. The slides were washed in TBS, thereafter rabbit, mouse or rat MACH 3 alkaline phosphatase (AP) probe, and the polymer (Biocare Medical) was incubated for 15 min, respectively, and the slides were washed in between and after polymer incubation. After this, chromogen Warp Red or Deep Space Black (Biocare Medical) together with levamisole (Vector Laboratories, Burlingame, CA, USA) was incubated with the sections for approximately 10 min. The reaction was stopped with Millipore water. Sections were counterstained with hematoxylin QS (VECTOR Laboratories) and treated with 95% and 99% ethanol, xylene for 1 min, and mounted using Pertex (HistoLab, Västra Frölunda, Sweden).

Whole Blood Flow Cytometry

WT (n=6) or *Apoe*$^{-/-}$ (n=6) mice were injected intravenously with either 150 µl (0.1 mg/ml) HSA-FITC or Mal-HSA-FITC. One additional WT mouse was used as an unstained control. The FITC-labeled probes circulated for 5 min. After, the animals were anesthetized and euthanized, and blood was withdrawn via heart puncture. Whole blood was placed directly in an RBC Lysis/Fixation solution (BD, Franklin Lakes, NJ, USA) and incubated for 15 min at RT. After this, the blood was centrifuged at 400 ×*g* for 5 min and washed twice with PBS. Cells were incubated with FC-block (1:10) (BD Biosciences, San Jose, CA, USA) for 10 min at RT, and subsequently were stained with commercial antibodies, PE

anti-mouse F4/80 (eBioscience, Thermo Fisher Scientific), APC anti-mouse CD11b (LSbio, Seattle, WA, USA), Brilliant Violet 510 anti-mouse CD3, PerCP anti-mouse CD11c, PE-Cy7 anti-mouse CD19, PE/Cy7 anti-mouse CD115, Pacific Blue anti-mouse Ly-6G, Brilliant Violet 510 anti-mouse Ly-6C (BioLegend, San Diego, CA, USA) and incubated for 45 min at 4°C. Fluorescence minus one and single staining were used as the control. After incubation, the cells were washed three times with PBS and analyzed using a Verse BD flow cytometer. Data was analyzed in FlowJo. Macrophages were classified as $(F4/80/CD11b)^{hi}$, monocytes as $(CD11b/CD115)^{hi}$ or $Ly\text{-}6C^{hi}$, neutrophils as $(CD11b/Ly\text{-}6G)^{hi}$ and $Ly\text{-}6C^{lo}$, dendritic cells as $CD11c^{hi}$, B-cells as $CD19^{+}$, and T-cells as $CD3^{+}$. The probe uptake was considered in the FITC-positive cells.

Supplemental Figures and Legends

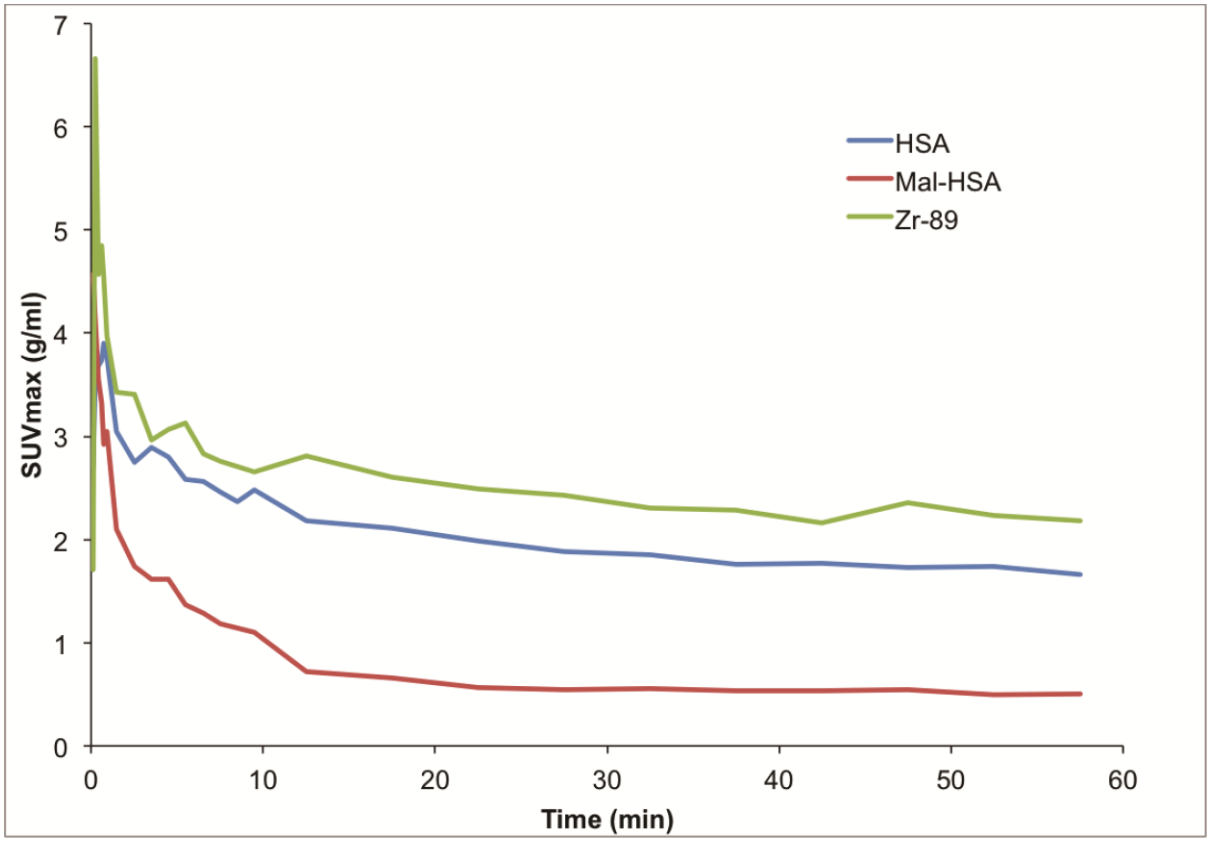
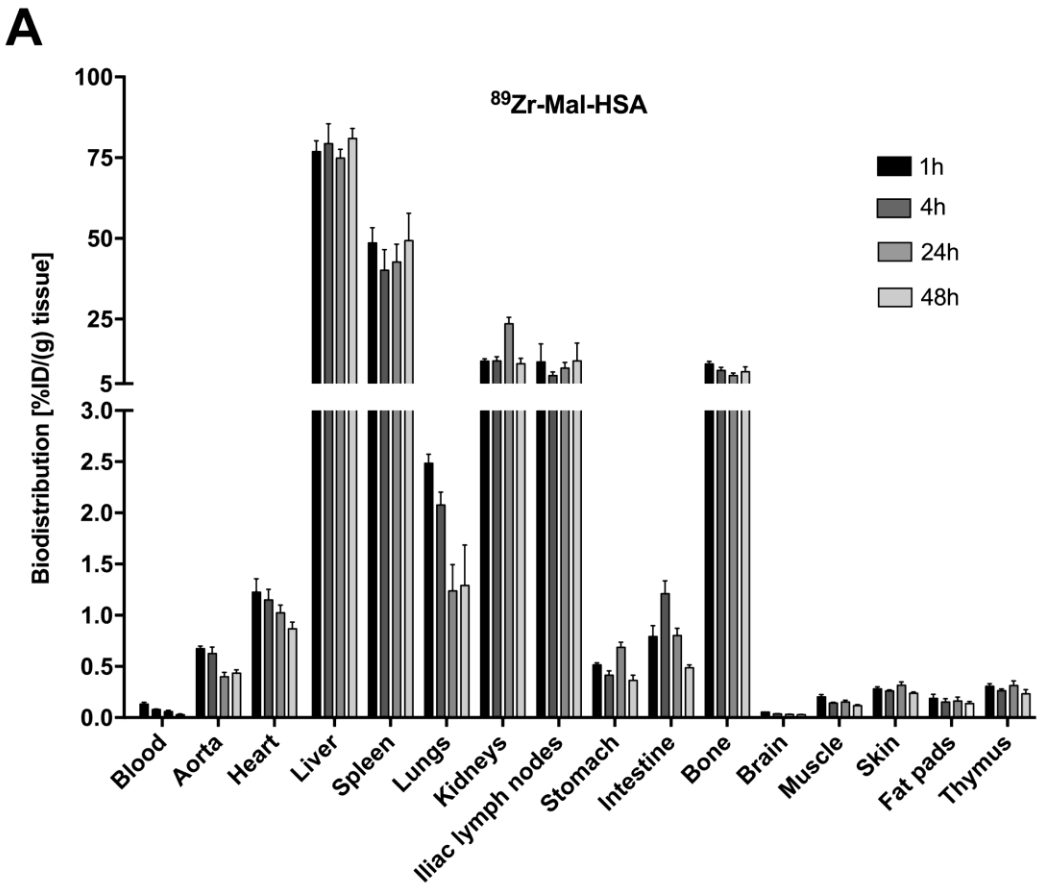


Figure S1 In vivo TRCs showing blood pool clearance from dynamic PET scans: comparison of pure ^{89}Zr (n=2), ^{89}Zr-Mal-HSA (n=23), and ^{89}Zr-HSA (n=20).


A
89Zr-Mal-HSA
Biodistribution [%ID/(g) tissue]
1h
4h
24h
48h
Blood
Aorta
Heart
Liver
Spleen
Lungs
Kidneys
Iliac lymph nodes
Stomach
Intestine
Bone
Brain
Muscle
Skin
Fat pads
Thymus

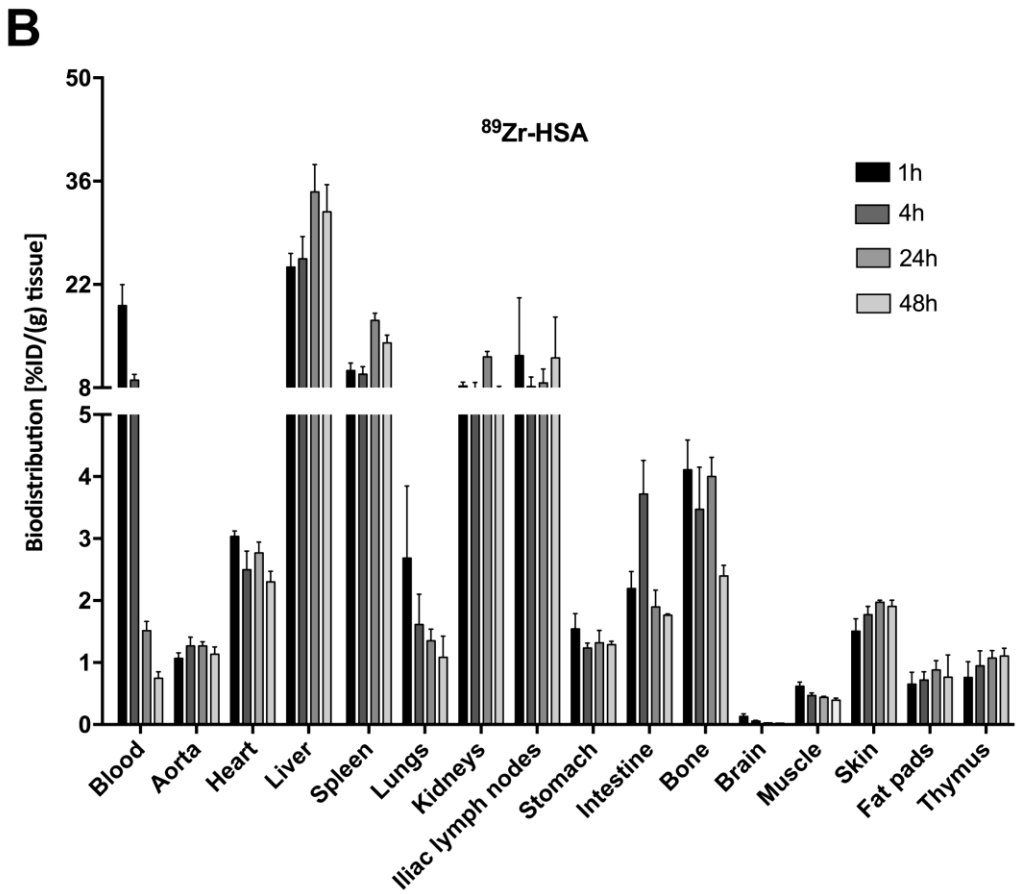

B
89Zr-HSA
Biodistribution [%ID/(g) tissue]
1h
4h
24h
48h
Blood
Aorta
Heart
Liver
Spleen
Lungs
Kidneys
Iliac lymph nodes
Stomach
Intestine
Bone
Brain
Muscle
Skin
Fat pads
Thymus

Figure S2 Biodistribution of (**A**) ^{89}Zr-Mal-HSA and (**B**) ^{89}Zr-HSA was studied in *Apoe*$^{-/-}$ mice at different time points; (**A**) 1 h (n=4), 4 h (n=4), 24 h (n=4), and 48 h (n=3), and (**B**) 1 h (n=3), 4 h (n=3), 24 h (n=3), and 48 h (n=3) postinjection, and measured using a gamma counter. The radiotracers were mainly distributed to the liver, spleen, kidneys, bone, and iliac lymph nodes. Data are presented as mean ± SEM.

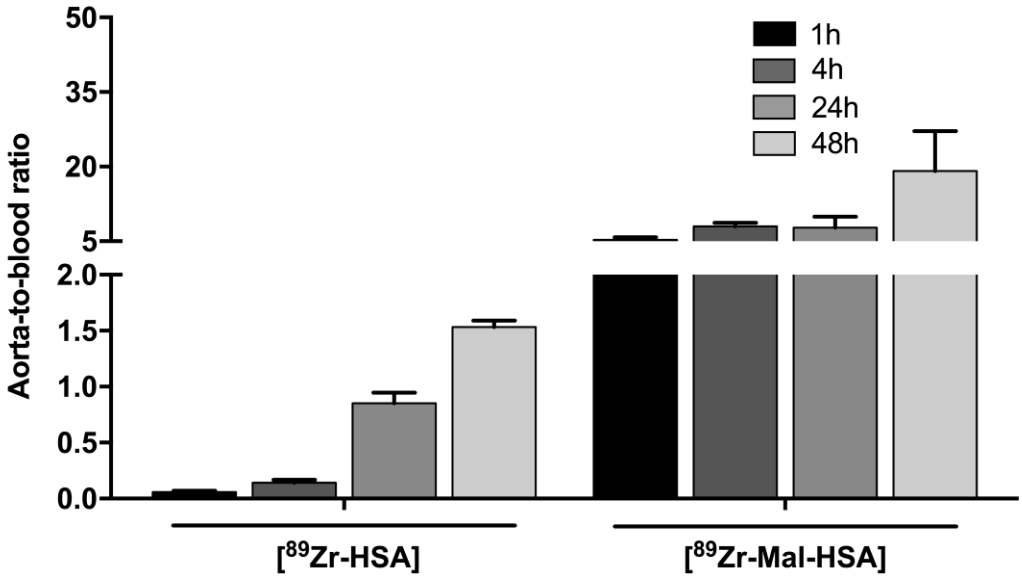


Figure S3 Aorta-to-blood ratio ((%ID/g aorta)/(%ID/g blood)) at different time-points postinjection of ^{89}Zr-HSA and ^{89}Zr-Mal-HSA (biodistribution animals from Figure S2). Data are presented as mean ± SEM.

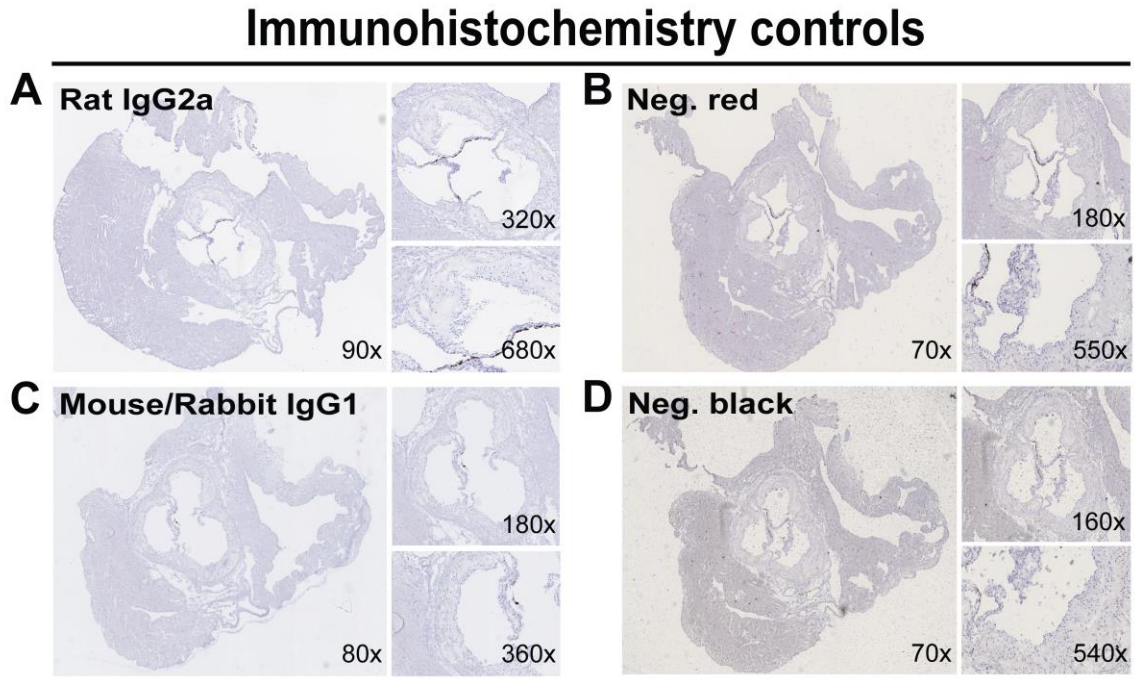


Figure S4 Negative controls from immunohistochemistry stainings of aortic root sections from *Apoe*$^{-/-}$ mice including zoomed areas. Isotype controls; (**A**) rat IgG2a and (**C**) Mouse/rabbit IgG1, or Chromogen controls; (**B**) Warp Red (Neg. Red) and (**D**) Deep Space Black (Neg. Black).

Supplemental Table

Table S1 Summary of the characteristics of all imaged mice

	HSA		Mal-HSA	
	WT	*Apoe*$^{-/-}$	WT	*Apoe*$^{-/-}$
Lesion % WA	-	8.1 ± 4.7	-	7.6 ± 3.8
Lesion % Arch	-	15.3 ± 6.6	-	15.0 ± 5.2
Lesion % TA	-	3.8 ± 3.0	-	3.0 ± 1.9
Lesion % AA	-	5.5 ± 5.6	-	3.0 ± 1.7
VLDL-c/LDL-c (mg/dl)	53.7 ± 19.8	515.4 ± 79.5	42.3 ± 19.5	523.3 ± 152
HDL-c (mg/dl)	49.7 ± 10.3	2.7 ± 0.9	44.1 ± 9.0	8.5 ± 12.7
TG (mg/dl)	14.5 ± 4.8	16.4 ± 5.0	15.5 ± 2.9	16.0 ± 5.0
NEFAs mM	0.37 ± 0.09	0.4 ± 0.09	0.36 ± 0.08	0.33 ± 0.14
Glucose mM	7.6 ± 1.7	8.6 ± 1.5	8.4 ± 1.9	8.4 ± 2.4
Body weight 16w (g)	21.5 ± 1.8	20.9 ± 1.7	21.1 ± 1.4	21.9 ± 2.0
Body weight 20w (g)	23.3 ± 2.3	22.8 ± 1.4	21.9 ± 1.4	22.8 ± 1.4

Notes: The data represent the lesion percentage determined by en face Sudan IV staining and analyzed using Fiji ImageJ, and blood lipid levels, plasma glucose measurements, and mouse weights at 16 and 20 weeks of age. Data are represented as mean ± SD: HSA WT (n=10), HSA *Apoe*$^{-/-}$ (n=10), Mal-HSA WT (n=11), and Mal-HSA *Apoe*$^{-/-}$ (n=12). The plaque burden did not differ significantly between *Apoe*$^{-/-}$ mouse groups (Mal-HSA vs. HSA); however, significant differences were observed when comparing the plaque burden within different parts of the aorta within each *Apoe*$^{-/-}$ mouse group: arch vs. TA (P<0.0001) and arch vs. abdominal aorta (P<0.0001). Additionally, the levels of HDL and VLDL/LDL differed significantly between WT and *Apoe*$^{-/-}$ mice (P<0.0001 and

100 P<0.0001, respectively). No significant differences were seen for TG, NEFAs, or blood glucose levels
101 between WT and *Apoe*$^{-/-}$ mice. The mouse weights did not differ significantly when comparing the
102 weight between groups at 16 or 20 weeks of age (one-way ANOVA with Tukey post hoc test).
103 **Abbreviations:** whole aortas (WA), aortic arch (arch), thoracic aorta (TA), abdominal aorta (AA), very
104 low-density lipoprotein cholesterol (VLDL-c), low-density lipoprotein cholesterol (LDL-c), high-density
105 lipoprotein cholesterol (HDL-c), triglycerides (TG), nonesterified fatty acids (NEFAs).
106

SUPPLEMENTAL REFERENCE

1. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nature Methods.* 2012;9(7):676-682.