Systematically evaluating DOTATATE and FDG as PET immuno-imaging tracers of cardiovascular inflammation

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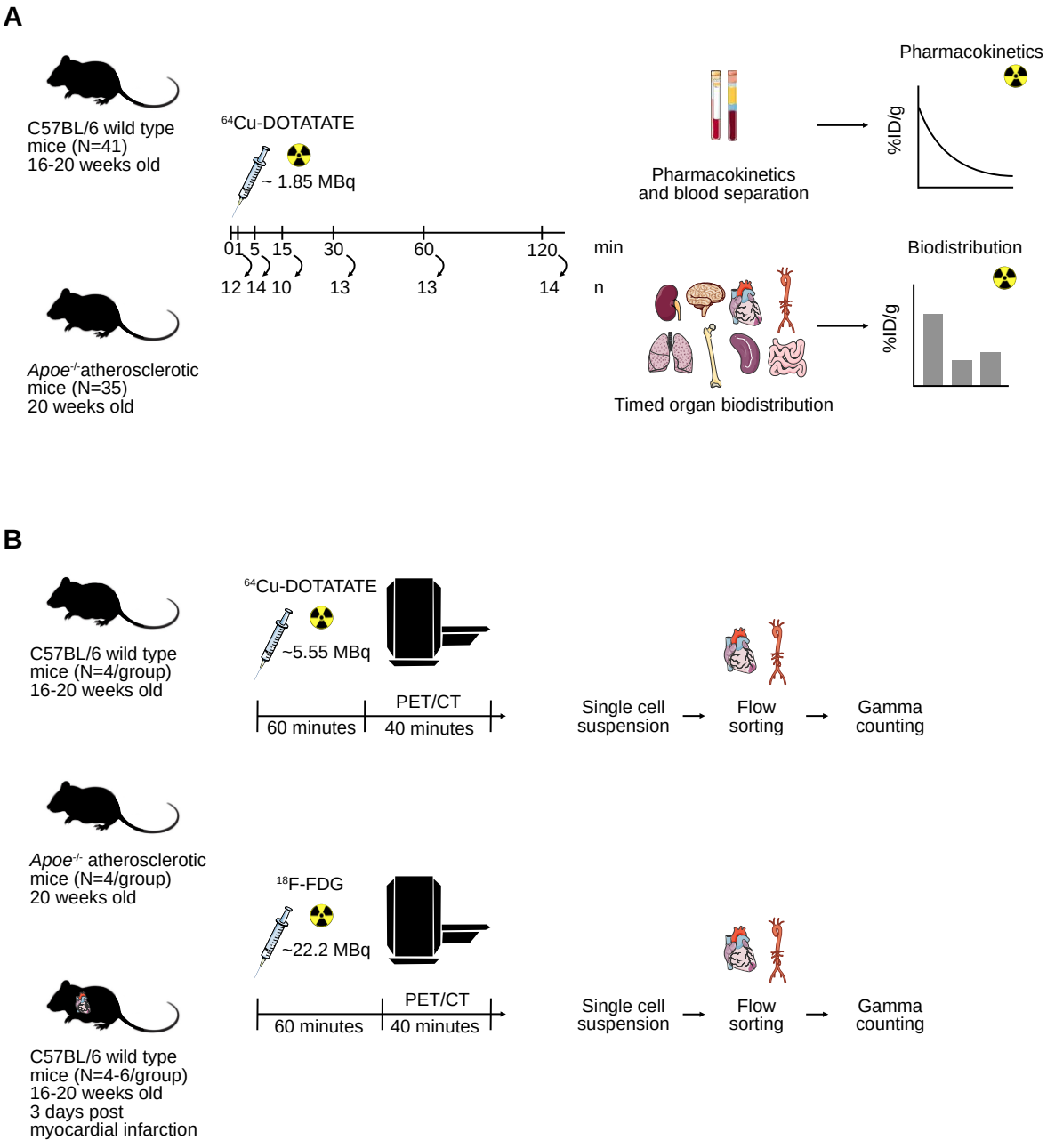
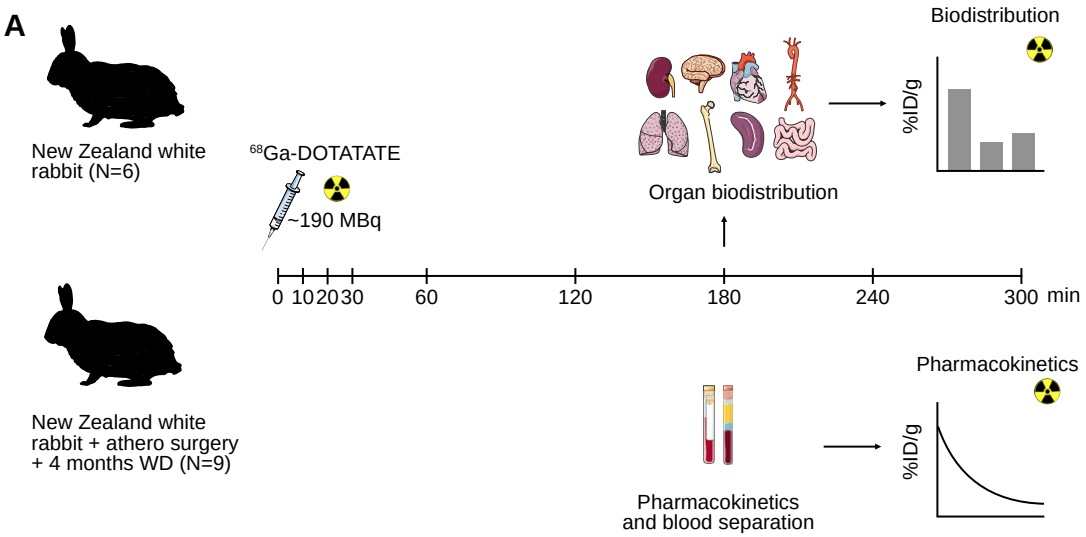
Supplemental material

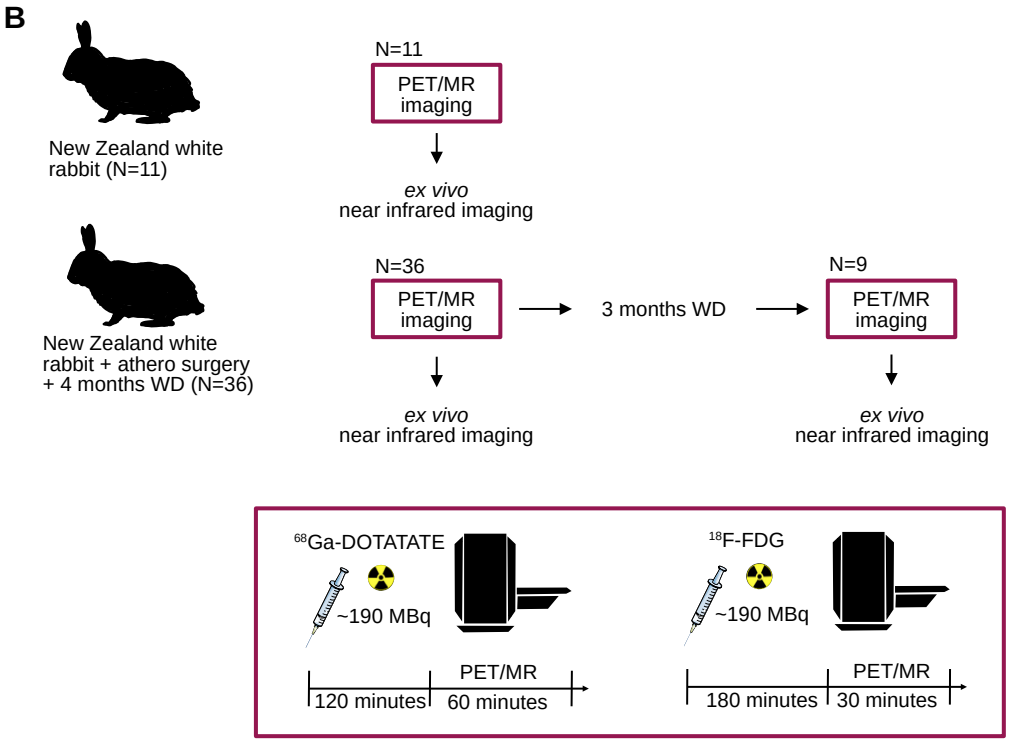
SUPPLEMENTAL FIGURES



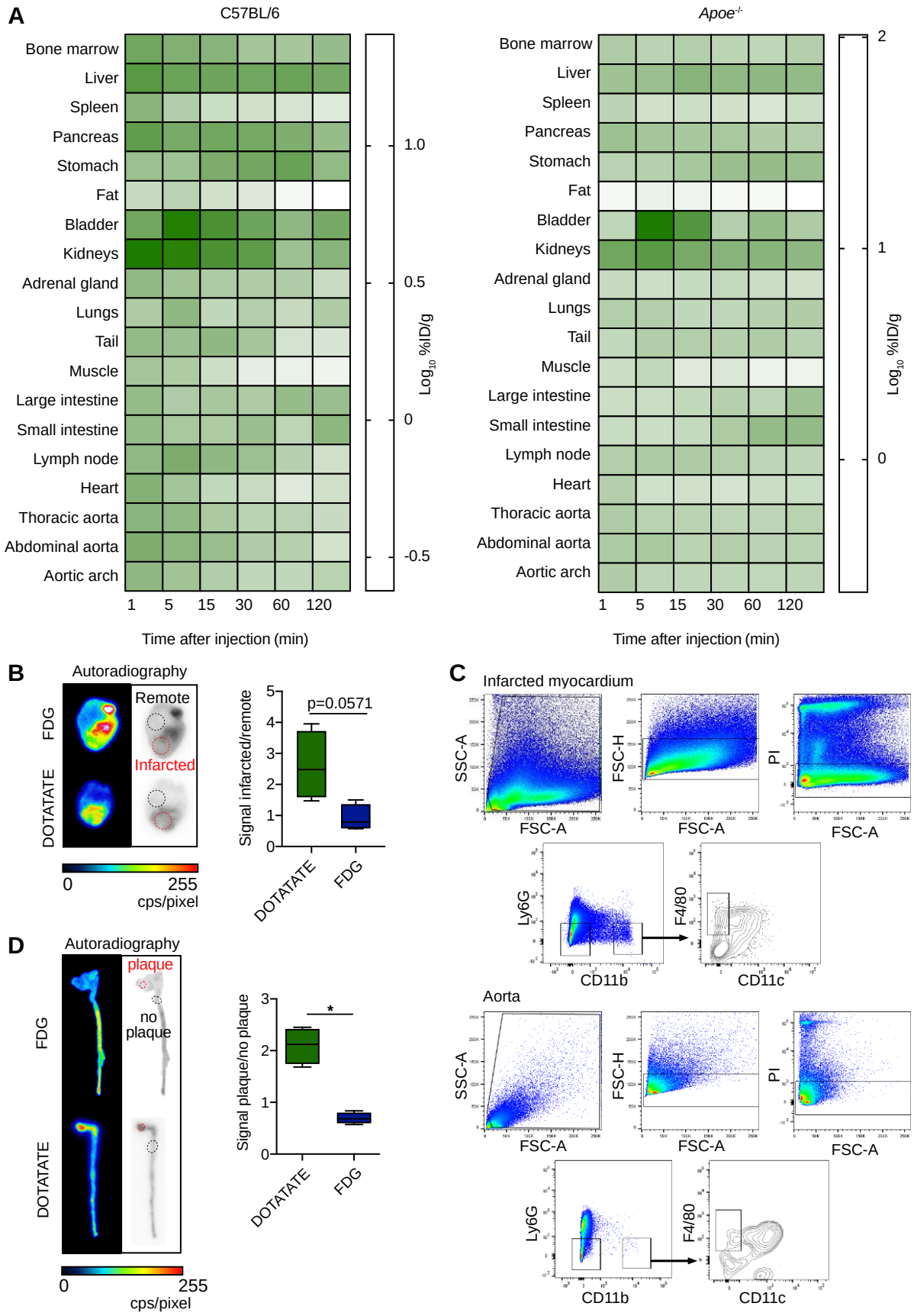
Supplementary Figure S1. Flowchart of the experimental design in mice. A) C57Bl/6 and *Apoe*$^{-/-}$ mice at 16-20 weeks of age were injected with [^{64}Cu]Cu-DOTATATE and euthanized at different time-points. Animals were perfused and organs of interest were

gamma-counted. Blood was processed for plasma, mononuclear and polynuclear cell separation. Results were plotted as %ID/g. B) Healthy and MI (3 days after infarct) C57Bl/6 mice, and *Apoe*$^{-/-}$ mice were injected with either [^{18}F]F-FDG or [^{64}Cu]Cu-DOTATATE. Tracer was allowed to circulate for 60 minutes. Animals were injected with eXIA™160 contrast agent (Binitio Biomedical Inc, Ontario, Canada). PET/CT acquisition was performed for 40 minutes. Mice were then sacrificed and perfused. Aorta and heart, the organs of interest, were collected and flow sorted. After sorting, cells were gamma-counted and activity per cell ratio was calculated. ID: injected dose.

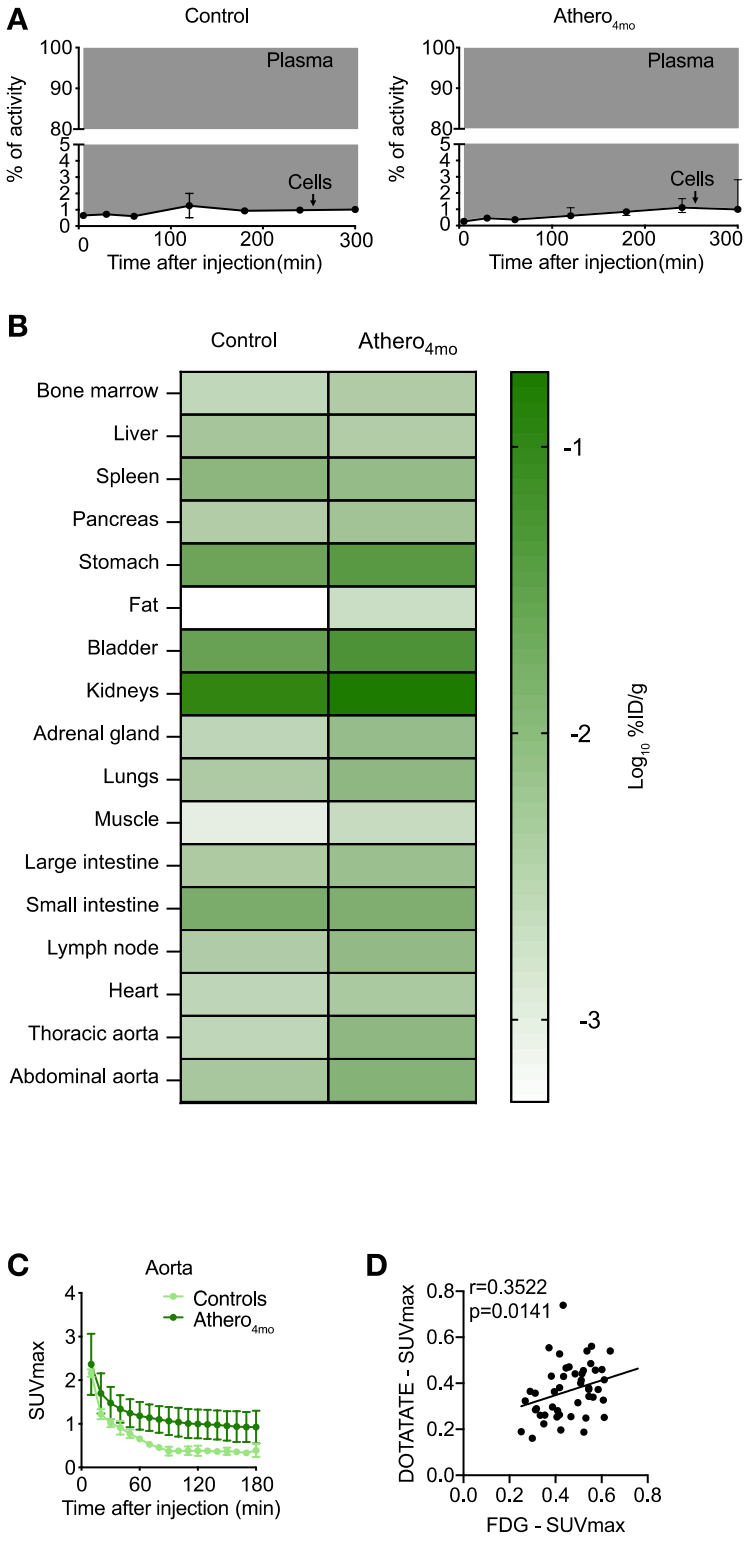

A
New Zealand white rabbit (N=6)
68Ga-DOTATATE
~190 MBq
Organ biodistribution
Biodistribution
%ID/g
0 10 20 30 60 120 180 240 300 min
New Zealand white rabbit + athero surgery + 4 months WD (N=9)
Pharmacokinetics and blood separation
Pharmacokinetics
%ID/g


B
New Zealand white rabbit (N=11)
N=11
PET/MR imaging
ex vivo near infrared imaging
New Zealand white rabbit + athero surgery + 4 months WD (N=36)
N=36
PET/MR imaging
3 months WD
N=9
PET/MR imaging
ex vivo near infrared imaging
ex vivo near infrared imaging
68Ga-DOTATATE
~190 MBq
120 minutes
PET/MR 60 minutes
18F-FDG
~190 MBq
180 minutes
PET/MR 30 minutes

Supplementary Figure S2 Flowchart of the experimental design in rabbits. A) Healthy and atherosclerotic ($athero_{4mo}$) New Zealand White rabbits were injected with [^{68}Ga]Ga-DOTATATE and pharmacokinetics profile was determined by blood sampling at different time-points. Blood was processed for plasma and cell separation. At 180 minutes, animals were sacrificed and perfused. Organs of interest were gamma-counted. Results were plotted as %ID/g. B) Healthy and atherosclerotic ($athero_{4mo}$) New Zealand White rabbits were injected with either [^{68}Ga]Ga-DOTATATE or [^{18}F]F-FDG. Tracer was allowed to circulate for 120 ([^{68}Ga]Ga-DOTATATE) and 180 minutes ([^{18}F]F-FDG), after which, rabbits were imaged with PET/MR for 60 or 30 minutes, respectively. Control animals and $athero_{4mo}$ were then sacrificed and perfused. Organs of interest were gamma counted and aortas were imaged with near infrared. A group of 9 atherosclerotic animals ($athero_{7mo}$) was kept and fed with Western Diet for additional 3 months, after which animals were imaged again with [^{68}Ga]Ga-DOTATATE and [^{18}F]F-FDG, sacrificed and perfused for aorta harvesting. Aortas were imaged with near infrared. Athero surgery: balloon denudation surgery; ID: injected dose; WD: Western Diet.


A
C57BL/6
Apoe-/-
Bone marrow
Liver
Spleen
Pancreas
Stomach
Fat
Bladder
Kidneys
Adrenal gland
Lungs
Tail
Muscle
Large intestine
Small intestine
Lymph node
Heart
Thoracic aorta
Abdominal aorta
Aortic arch
1 5 15 30 60 120
Time after injection (min)
1.0
0.5
0
-0.5
2
1
0
Log10 %ID/g
B
Autoradiography
FDG
DOTATATE
Remote
Infarcted
0
255
cps/pixel
p=0.0571
Signal infarcted/remote
DOTATATE
FDG
C
Infarcted myocardium
SSC-A
FSC-A
FSC-H
PI
Ly6G
CD11b
F4/80
CD11c
Aorta
D
Autoradiography
plaque
no plaque
*
Signal plaque/no plaque

Supplementary Figure S3. [^{64}Cu]Cu-DOTATATE uptake in mice and autoradiography of [^{64}Cu]Cu-DOTATATE and [^{18}F]F-FDG in cardiovascular disease animal models. A) Heatmap visualization of [^{64}Cu]Cu-DOTATATE distribution at different timepoints in healthy C57Bl/6 (left) and atherosclerotic *Apoe*$^{-/-}$ (right) mice (N=5 per group). Data was obtained by *ex vivo* gamma counting of blotted organs. B) Representative autoradiography images showing radioactivity distribution in the myocardium of MI mice injected with [^{18}F]F-FDG or [^{64}Cu]Cu-DOTATATE. Regions of interest (ROI) represented in red (infarcted myocardium) and black (non-infarcted remote myocardium). Quantification of signal was plotted as ratio between infarcted and remote myocardium. (N=4 per group) C) Representative plot of gating strategy for flow sorting of heart (top) and aorta (bottom) tissues D) Representative autoradiography images showing radioactivity distribution in the aorta of C57Bl/6 mice and *Apoe*$^{-/-}$ mice injected with [^{18}F]F-FDG or [^{64}Cu]Cu-DOTATATE. Regions of interest (ROI) represented in red (plaque) and black (no plaque). Quantification of signal was plotted as ratio between plaque and no plaque signal. (N=4 per group). cps: counts per second; DOTATATE: [^{64}Cu]Cu-DOTATATE; FDG: [^{18}F]F-FDG; ID: injected dose. Data are presented as median (interquartile range).


A
Control
Athero4mo
Plasma
Cells
% of activity
Time after injection(min)
B
Control
Athero4mo
Bone marrow
Liver
Spleen
Pancreas
Stomach
Fat
Bladder
Kidneys
Adrenal gland
Lungs
Muscle
Large intestine
Small intestine
Lymph node
Heart
Thoracic aorta
Abdominal aorta
Log10 %ID/g
C
Aorta
Controls
Athero4mo
SUVmax
Time after injection (min)
D
r=0.3522
p=0.0141
DOTATATE - SUVmax
FDG - SUVmax

Supplementary Figure S4. Analysis of [^{68}Ga]Ga-DOTATATE biodistribution in rabbits and [^{18}F]F-FDG and [^{68}Ga]Ga-DOTATATE aortic uptake comparison. A) [^{68}Ga]Ga-DOTATATE radioactivity distribution in blood fractions of control (left) and athero$_{4mo}$ (right) rabbits as determined by gamma counting. Graphs show the percentage of activity associated with cells or plasma over time. Controls N=2 and athero$_{4mo}$ N=3 B) Heatmap visualization of [^{68}Ga]Ga-DOTATATE distribution at different timepoints in healthy (left) and athero$_{4mo}$ rabbits (right) Controls N=6 and athero$_{4mo}$ N=9. Data was obtained by *ex vivo* gamma counting of blotted organs. C) [^{68}Ga]Ga-DOTATATE SUV$_{max}$ of abdominal aorta in control and athero$_{4mo}$ animals over the first 3 hours after injection, acquired by dynamic PET/MR imaging. N=3 per group. Data are presented as mean ± standard error of the mean. D) Correlation between [^{18}F]F-FDG and [^{64}Cu]Cu-DOTATATE aortic uptake in control, athero$_{4mo}$ and athero$_{7mo}$ animals. N=57. DOTATATE: [^{64}Cu]Cu-DOTATATE; FDG: [^{18}F]F-FDG; ID: injected dose; SUV$_{max}$: maximum standardized uptake value.

SUPPLEMENTARY TABLE S1. Blood circulation half-lives of DOTATATE-based radiotracers in mice and rabbits.

	Mice		Rabbits	
	C57Bl/6	*Apoe*$^{-/-}$	Control	Athero$_{4mo}$
%fast	79.14	11.27	39.7	53.17
$t_{1/2}$ fast (minutes)	1.939	0.557	3.419	19.17
%slow	20.86	88.73	60.3	46.83
$t_{1/2}$ slow (minutes)	15.81	7.627	32.86	92.82

Weighed $t_{1/2}$ (minutes)	4.83	6.83	21.17	53.66

SUPPLEMENTARY TABLE S2. Tissue radioactivity distribution of [^{64}Cu]Cu-DOTATATE in female C57BL/6 mice in %ID/g.

WT	1 min	5 min	15 min	30 min	60 min	120 min
Bone marrow	6.5 (5.4-7.1)	4.3 (3.6-5.6)	3.5 (3-5.6)	1.9 (1.7-2.9)	2.1 (1.6-2.5)	2.5 (2.1-4.1)
Liver	10 (8.5-11.1)	7.1 (6.1-7.7)	6.5 (6.1-7)	6.4 (6-6.4)	6.9 (6.7-7.8)	5.4 (4.8-6.4)
Spleen	3.7 (3.2-4.2)	1.5 (1.2-2)	0.9 (0.8-0.9)	0.7 (0.7-0.8)	0.6 (0.6-0.8)	0.5 (0.5-0.7)
Pancreas	8.7 (7.5-9.3)	5.3 (4.7-6.3)	6.1 (5.7-6.4)	5.8 (5.2-6.2)	4.8 (4.5-5)	2.6 (2.5-3.4)
Stomach	2.3 (2.2-3.1)	2.4 (1.7-3.2)	5 (3.5-7)	7.8 (3.6-8.7)	8 (4.8-10.8)	3.2 (2.7-3.6)
Fat	1.1 (0.5-1.5)	1.3 (1.1-1.5)	0.8 (0.6-0.9)	0.5 (0.4-0.8)	0.3 (0.2-0.4)	0.2 (0.2-0.5)
Bladder	4.6 (2.5-24)	36.4 (8.2-78.9)	7.9 (6.1-34.9)	6.8 (4-12.6)	3.3 (1.9-6.6)	10.4 (1.5-15.2)
Kidneys	26.8 (19.1-33.4)	20.5 (18.7-24.2)	11.5 (11.3-13.6)	8.2 (7.3-10.9)	6.1 (3-6.7)	3.7 (3.3-4.6)
Adrenal gland	3.3 (2.9-3.5)	2.2 (2.1-2.5)	1.7 (1.4-2.2)	1.6 (1.5-1.7)	1.6 (1.5-1.8)	0.6 (0.6-1.7)
Lungs	1.8 (1.2-2.1)	3.2 (2.7-4)	0.9 (0.7-2)	1.3 (1.1-2.2)	1.1 (0.8-1.1)	1.8 (1.2-2.6)
Tail	3.5 (2-4.9)	2.7 (1.6-4)	3.6 (2.9-3.7)	1.8 (1.6-2.9)	1.6 (0.6-1.7)	0.6 (0.5-0.7)
Muscle	2 (1.7-2.5)	1.6 (1.5-2.1)	0.9 (0.7-0.9)	0.5 (0.3-0.6)	0.3 (0.3-0.6)	0.3 (0.2-0.7)
Large intestine	2.7 (2.3-3.7)	1.6 (1.4-1.9)	1.9 (1.7-2.2)	1.7 (1.3-2.1)	3 (2.5-3.3)	3.1 (1.8-3.5)
Small intestine	3 (2.3-3.6)	2 (1.6-2.1)	1.7 (1.3-2.4)	2.6 (1.6-4.4)	2.4 (0.9-3.7)	3.3 (3.1-3.8)
Lymph node	3 (2.8-3.8)	4.7 (4.5-5.5)	3.2 (2.8-3.9)	2.2 (1.5-4.7)	1.3 (1.2-1.7)	0.6 (0.4-1.8)
Heart	3.8 (3.6-4.9)	2.1 (1.9-2.5)	1.1 (0.9-1.2)	0.9 (0.8-1)	0.8 (0.4-0.9)	0.7 (0.7-0.8)
Thoracic aorta	3.2 (2.9-4.6)	3.1 (2.9-3.5)	1.6 (1.4-2.4)	1.3 (1.2-1.4)	1.2 (1.2-1.4)	1 (0.7-1.1)
Abdominal aorta	5.1 (4.1-5.3)	3.8 (3-4)	2.4 (2.4-3.2)	1.7 (1.3-2.1)	1.3 (1.1-1.8)	0.7 (0.3-1.9)
Aortic arch	2.8 (2.7-4.5)	2.2 (1.8-2.9)	1.3 (1.2-2.1)	1.1 (1-1.2)	1.1 (1-1.1)	1.2 (1.2-1.6)

(N=3-7 per time-point). Data are presented as median (interquartile range).

SUPPLEMENTARY TABLE S3. Tissue radioactivity distribution of [^{64}Cu]Cu-DOTATATE in female *Apoe*$^{-/-}$ mice 12 weeks on Western Diet represented in %ID/g.

Apoe-/-	1 min	5 min	15 min	30 min	60 min	120 min
Bone marrow	3.1 (2.2-3.5)	2.3 (1-4)	2.6 (2.1-3.2)	1.8 (1.5-3.1)	1.9 (1.2-2.4)	2.7 (2-3)
Liver	4.4 (4.1-4.9)	4.9 (4.1-6.5)	8.6 (6.4-12.6)	6.9 (6.6-7.4)	6.9 (5.9-8.7)	6.3 (5.6-7.5)
Spleen	2 (1.6-2.5)	1.1 (0.8-1.3)	1.2 (0.8-1.6)	1.1 (0.8-1.2)	0.8 (0.6-1.2)	1.2 (1.1-1.3)
Pancreas	4.7 (3.9-6)	4.1 (3-4.4)	3.5 (2.9-4.2)	3.4 (3.1-3.8)	2.7 (2.3-3.5)	2.6 (1.9-3.2)
Stomach	2.1 (1.6-2.6)	2.4 (1.8-3.2)	3.3 (3.1-4.7)	5.4 (4.1-6.7)	5.4 (4.1-9.4)	5 (4-6.8)
Fat	0.3 (0.3-0.3)	0.4 (0.3-0.7)	0.4 (0.3-0.5)	0.3 (0.2-0.6)	0.2 (0.2-0.7)	0.2 (0.2-0.4)
Bladder	1.7 (1.6-2.1)	98.7 (69.1-165)	38.9 (16-63.1)	2.9 (1.8-3.6)	4.7 (2.5-17.5)	2.4 (1.4-10.2)
Kidneys	15.6 (15.1-17.5)	22.9 (18.7-48.4)	13.2 (11.9-16)	8.8 (7.3-10.5)	11.2 (6-11.6)	7.1 (7-8.1)
Adrenal gland	1.4 (1.2-1.5)	1.2 (1-1.5)	1.4 (1.2-1.7)	1.5 (1.4-1.9)	1.4 (1.1-1.6)	1.1 (0.7-1.5)
Lungs	2.1 (2.1-3.3)	2.6 (2.2-3)	1.9 (0.9-2.7)	1.6 (1.3-2.5)	2.5 (1.6-3.1)	2.8 (2.2-3.5)
Tail	1.8 (1-2.7)	2.5 (1.7-4.5)	2.5 (1.6-4.7)	3.1 (1.6-3.3)	2 (1.5-5.9)	2.7 (1.3-3.4)
Muscle	1.2 (1.1-1.5)	1.4 (1.3-2.2)	0.6 (0.5-0.9)	0.7 (0.5-1.4)	0.4 (0.3-0.6)	0.3 (0.3-0.7)
Large intestine	1.3 (1-1.5)	1.3 (1.1-1.6)	1.8 (1.1-2.3)	2.8 (1.4-3.9)	2.2 (1.1-3.1)	3.8 (3.6-6.5)
Small intestine	1.4 (0.7-3)	1.2 (1-1.7)	1.8 (1-2)	3.1 (1.8-5.6)	7.1 (4.7-7.8)	7.2 (4.4-9.5)
Lymph node	2.6 (1.8-3.8)	3.2 (2.5-4)	3 (2.2-4.1)	2.4 (1.6-4.6)	2.1 (1.3-2.7)	1.8 (1.4-2.6)
Heart	2.3 (2.1-2.8)	1.4 (0.7-1.6)	1.1 (0.8-1.3)	0.9 (0.8-1.2)	1.3 (1.1-1.5)	1.4 (1.2-1.4)
Thoracic aorta	2.9 (2.5-3.2)	2.1 (1.9-2.4)	2 (1.8-2.3)	1.8 (1.6-2.3)	2.1 (1.6-2.2)	2.1 (2-2.4)
Abdominal aorta	3.1 (2.9-3.6)	3.2 (2.6-4.6)	2.1 (1.7-3)	2.5 (1.9-3.6)	2.2 (1.7-2.3)	2.1 (1.9-2.5)
Aortic arch	2.9 (2.4-3)	1.9 (1.9-1.9)	1.7 (1.6-2)	1.7 (1.5-2.1)	1.8 (1.3-2)	1.9 (1.9-1.9)

(N=3-8 per time-point). Data are presented as median (interquartile range).

SUPPLEMENTARY TABLE S4. Number of cells analyzed by flow cytometry.

Tissue	Cells	Mouse 1	Mouse 2	Mouse 3	Mouse 4
Infarcted myocardium C57	Macrophages	13644	3871	3591	1400

	Other CD11b positive	55751	12479	7031	18511
	CD11b negative	403562	182839	264546	145387
	Total	472957	199189	275168	165298
Infarcted myocardium C57Bl/6 (DOTA)	Macrophages	22882	5168	7279	6314
	Other CD11b positive	65733	39530	35666	30890
	CD11b negative	602928	225498	466014	255403
	Total	691543	270196	508959	292607
Aorta *Apoe*$^{-/-}$ (FDG)	Macrophages	6414	3288	2275	1429
	Other CD11b positive	24293	15900	22842	9988
	CD11b negative	435993	855828	405410	191276
	Total	466700	875016	430527	202693
Aorta *Apoe*$^{-/-}$ (DOTA)	Macrophages	4281	3370	7270	6917
	Other CD11b positive	22812	21362	44521	40724
	CD11b negative	513480	179121	392296	321097
	Total	540573	203853	444087	368738

SUPPLEMENTARY TABLE S5. Tissue radioactivity distribution of [^{68}Ga]Ga-DOTATATE in male control rabbits and athero$_{4mo}$ in %ID/g.

	Controls Median (interquartile range)	Athero$_{4mo}$ Median (interquartile range)
Bone marrow	0.003 (0.002-0.009)	0.006 (0.003-0.012)
Liver	0.009 (0.005-0.013)	0.006 (0.004-0.007)
Spleen	0.015 (0.007-0.035)	0.012 (0.011-0.013)
Pancreas	0.005 (0.004-0.009)	0.009 (0.007-0.011)
Stomach	0.036 (0.022-0.071)	0.053 (0.041-0.079)
Fat	0 (0-0.001)	0.002 (0.002-0.005)
Bladder	0.053 (0.014-0.1)	0.075 (0.038-0.155)
Kidneys	0.128 (0.108-0.148)	0.157 (0.141-0.263)
Adrenal gland	0.003 (0.003-0.005)	0.012 (0.009-0.017)
Lungs	0.005 (0.003-0.012)	0.015 (0.012-0.02)
Muscle	0.001 (0.001-0.002)	0.003 (0.002-0.006)
Large intestine	0.005 (0.004-0.012)	0.01 (0.007-0.015)

Small intestine	0.025 (0.016-0.033)	0.021 (0.016-0.029)
Lymph node	0.005 (0.005-0.007)	0.013 (0.01-0.019)
Heart	0.003 (0.003-0.005)	0.006 (0.005-0.008)
Thoracic aorta	0.004 (0.002-0.006)	0.015 (0.011-0.018)
Abdominal aorta	0.006 (0.003-0.02)	0.017 (0.014-0.022)

(N=6-9 per group). Data are presented as median (interquartile range).