Tissue macrophage numbers vary during health versus disease. Abundant inflammatory macrophages destruct tissues, leading to atherosclerosis, myocardial infarction and heart failure. Emerging therapeutic options create interest in monitoring macrophages in patients. Here we describe positron emission tomography (PET) imaging with $^{18}$F-Macroflor, a modified polyglucose nanoparticle with high avidity for macrophages. Due to its small size, Macroflor is excreted renally, a prerequisite for imaging with the isotope flourine-$^{18}$. The particle’s short blood half-life, measured in three species, including a primate, enables macrophage imaging in inflamed cardiovascular tissues. Macroflor enriches in cardiac and plaque macrophages, thereby increasing PET signal in murine infarcts and both mouse and rabbit atherosclerotic plaques. In PET/magnetic resonance imaging (MRI) experiments, Macroflor PET imaging detects changes in macrophage population size while molecular MRI reports on increasing or resolving inflammation. These data suggest that Macroflor PET/MRI could be a clinical tool to non-invasively monitor macrophage biology.
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schaemic heart disease leads worldwide mortality statistics. While conventional risk factors such as hyperlipidemia and hypertension are well understood and effectively treated, the atherosclerosis disease burden keeps growing. Macrophage function is increasingly seen as therapeutically interesting, as per genome-wide association studies showing inflammation-related mutations in patients with myocardial infarction (MI) and fundamental immunology discoveries. Data obtained from animals with cardiovascular disease suggest that surplus inflammatory macrophages in either the arterial wall or ischaemic heart muscle promote tissue destruction, morbidity and death. In patients, most available evidence focuses on myocardial infarction (MI) and fundamental immunology discoveries. Data obtained from animals with cardiovascular disease suggest that surplus inflammatory macrophages in either the arterial wall or ischaemic heart muscle promote tissue destruction, morbidity and death. In patients, most available evidence focuses on myocardial infarction (MI).

Macrophage numbers and phenotypes do not necessarily correlate with blood monocytes, we need to directly survey vascular and cardiac tissues. In preclinical research, intravital microscopy and flow cytometry enable tissue macrophage quantification. Yet it is not practical to biopsy atherosclerotic blood vessels and infarcted or failing hearts in patients. Quantitative macrophage imaging, currently unavailable for clinical applications, would overcome this barrier, provide better understanding of macrophages’ roles in human disease, identify patients at risk for complications and evaluate emerging macrophage-targeting therapeutics.

Nanoparticles, which can be efficiently internalized by phagocytic myeloid cells, are a promising strategy for quantitatively and specifically imaging macrophages in human cardiovascular organs. Previously developed nanoparticles with effective macrophage uptake also have long circulation times with high blood pool activity that limit target-to-background ratios in the vascular system. Further, the long circulation times precluded the use of the clinically facile PET isotope for nanoparticle tracking, because the radioisotope decays faster than nanoparticles exit from the blood pool adjacent to the imaging target, that is, diseased cardiovascular tissues.

To solve these problems, we shrank nanoparticles to a size below the renal excretion threshold and optimized biological behaviour through biocompatible chemistries. Here we describe a class of modified polyglucose nanoparticles that we named macrins. More specifically, macrins are a class of lysine-crosslinked low molecular weight carboxymethyl polyglucose polymers, each containing 22 glucose units. Macrins can be considered glycogen biomimetics that lack the central glycolytic core. For our imaging applications, we labelled a 5 nm macrin with either PET imaging agent (termed Macrolite) or a fluorochrome for correlative studies. In three animal species, we confirm rapid renal excretion of Macrolite and also show its high affinity for macrophages residing in cardiovascular organs. In a number of imaging experiments, this approach provides quantitative and specific PET data on inflammation in atherosclerotic plaque and ischaemic myocardium.

Results
Small nanoparticles are excreted renally. Macrolite uptake was assessed by flow cytometry, which enable tissue macrophage quantification. Yet it is not practical to biopsy atherosclerotic blood vessels and infarcted or failing hearts in patients. Quantitative macrophage imaging, currently unavailable for clinical applications, would overcome this barrier, provide better understanding of macrophages’ roles in human disease, identify patients at risk for complications and evaluate emerging macrophage-targeting therapeutics.

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Free amines were capped by reaction with excess succinic anhydride, which shifted the zeta potential from $-11.1$ to $-19.9 \pm 1.0 \text{mV}$, thereby indicating a change in the surface charge. Succinylated did not change particle size. Macrolite was labelled with $18\text{F}$ with high radiochemical purity (Fig. 1c). The average specific activity was $10.2 \pm 1.9 \text{mCi} \text{mg}^{-1}$.

Macrolite was first tested in healthy mice (Fig. 1d,e) and rabbits (Fig. 1f). In both species, the highest activity occurred in the kidneys, while uptake in liver and spleen was considerably lower, as measured 120 min after injection. Together with the rapid clearance from the blood pool (Fig. 1d, blood half-life of 6.5 min measured in five normal mice and 22.5 min measured in four normal rabbits), these data indicate that Macrolite is rapidly cleared from circulation by the renal system. Macrolite stability in mouse serum was tested by size exclusion radiochromatography. The data indicate that Macrolite is stable for 120 min with no appearance of additional peaks (Supplementary Fig. 1).

To test if similarly rapid renal excretion occurs in primates, we conducted a dynamic imaging experiment using a clinical PET/magnetic resonance imaging (MRI) scanner (Fig. 2a–b). Macrolite, 2.5 mCi, was injected intravenously, followed by 90 min of PET data acquisition. As anticipated, Macrolite rapidly departed the blood pool and accumulated in the kidneys and bladder (Fig. 2a,b). Fitting the blood pool activity yielded a blood half-life of 21.7 min (Fig. 2c). Thus, data obtained in all three species document rapid renal excretion of Macrolite.

To explore Macrolite uptake by macrophages, we used dynamic intravital confocal microscopy to study the hearts of $C57BL/6\text{GFP}^+\text{mice}$. In naive hearts, no signal was seen in the 680 nm channel, but cardiac macrophages were brightly green fluorescent protein (GFP) positive (Fig. 2d). Shortly after the fluorescent nanoparticle was injected into the tail vein, intravascular signal appeared in cardiac vessels. While blood pool signal faded rapidly thereafter, we observed nanoparticle enrichment in cardiac macrophages (Fig. 2d,e). These data imply that the nanoparticles undergo rapid distribution to macrophages, and that these nanoparticles are cleared from the blood pool within a time frame that enables $18\text{F}$ PET imaging of macrophages residing in cardiovascular tissues.

To test the hypothesis that Macrolite enters macrophages via a biologically active process, we studied in vitro uptake into primary cells harvested from mouse spleens at different temperatures. Splenocytes were incubated with the fluorescently labelled nanoparticle Macrolite for 15 min and 12 h at either 4 or 37°C. Uptake of Macrolite was assessed by flow cytometry, which also identified macrophages by their typical surface marker profile. We found substantial Macrolite uptake only in the cells that were incubated at 37°C, indicating that nanoparticle uptake is inhibited if the cells are on ice (Supplementary Fig. 2). These data indicate that Macrolite uptake is a biologically active process.

Imaging inflammatory atherosclerosis. Macrophages enter arterial wall segments to remove cholesterol deposits. In patients and animals with atherosclerosis, the cells fail in this task and instead create sub-endothelial inflammatory lesions. Continued recruitment and local proliferation feed macrophage population growth, which perpetuates inflammation and swells lesions that impede blood flow. Macrophages also destabilize arterial tissue integrity by secreting proteolytic and pro-inflammatory enzymes, which can lead to atherosclerotic plaque disruption, thrombotic arterial occlusion and sudden downstream ischemia. This makes macrophages a potential therapeutic target and indicates the need to monitor them. In $ApoE^{-/-}$ mice on pro-atherogenic diet, we observed Macrolite enrichment in the aortic root and arch (Fig. 3a,b), vascular territories that are affected by inflammatory
atherosclerosis. Standard uptake values obtained by PET/computed tomography (CT) imaging 120 min after intravenous injection of $525 \pm 167 \mu$Ci Macroflor were significantly increased in mice with atherosclerosis (Fig. 3c). These in vivo data correlated well with activity measured in excised aortae (Fig. 3d,e). Using autoradiography exposure of aortae, we detected radioactivity co-localized with fatty atherosclerotic plaques (Fig. 3f).

The nanoparticle’s uptake profile into cells that reside in the aortic wall showed a significant predilection for macrophages, while other leukocytes such as lymphocytes and neutrophils showed negligible nanoparticle incorporation (Fig. 3g–i). Immunofluorescent labelling of the myeloid marker CD11b, which is highly expressed by macrophages, co-localized with nanoparticles in histological sections of aortic plaques (Fig. 3j).

We next explored Macroflor imaging of atherosclerosis in rabbits using a clinical PET/MRI scanner. Ninety minutes after Macroflor injection, we observed high PET signal in the rabbits’ kidneys (Fig. 4a,b), thereby confirming Macroflor’s renal excretion. Significantly increased PET signal localized into the infrarenal aortae of rabbits that developed atherosclerotic lesions therein after exposure to atherogenic diet and infrarenal aortic balloon injury (Fig. 4a,c). In a cohort of rabbits that received balloon injury with intermediate pressure, aortic standard uptake values were higher than in the control cohort but lower than in rabbits that received full-pressure endothelial denudation (Fig. 4c). Autoradiography on excised aortae documented radioactivity in a pattern reflecting atherosclerotic lesion distribution (Fig. 4d). Ex vivo scintillation counting of excised rabbit aortae revealed increased activity in rabbits with atherosclerotic disease (Fig. 4f). Two days before Macroflor imaging, rabbits underwent $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) PET to enable comparison. The rabbit cohorts had similar PET signal distributions (Fig. 4f). When aortic segments were studied, $^{18}$F-FDG and $^{18}$F-nanoparticle signal correlated with an $R^2 = 0.518$ ($P < 0.0001$, Pearson correlation coefficient, Fig. 4g). The difference in myocardial uptake between Macroflor and $^{18}$F-FDG, shown in Fig. 4h and Supplementary Fig. 3 may have implications for cardiac PET inflammation imaging.

**PET imaging of macrophages in acute MI.** Ischaemic organ injury profoundly expands the macrophage pool. The cells derive from blood monocytes made in the bone marrow and spleen but also proliferate locally in the infarct. Macrophage numbers peak around day 3 after ischemia, before tissues transition to resolution
of inflammation. During the resolution phase, macrophages assume less inflammatory phenotypes and promote tissue repair. However, their overabundance, or a delayed phenotype transition, promotes heart failure. Infarct macrophages, therefore, are a potential therapeutic target, but we currently lack tools for monitoring them quantitatively. To explore Macroflor PET imaging after MI, we imaged mice with permanent coronary ligation.

In vivo imaging showed Macroflor uptake into the ischaemic myocardium (Fig. 5a,b) to be significantly higher than myocardial standard uptake values in control mice without MI (Fig. 5c). These in vivo data were corroborated by scintillation counting (Fig. 5d) and autoradiography exposure (Fig. 5e) of excised mouse hearts. Flow cytometric evaluation of cardiac leukocytes showed significant enrichment in macrophages (Fig. 5f–h).

**Dual targeted PET/MRI reports on phenotype and number.** Macrophages may not only change in number but also shift their phenotypes and inflammatory activities. A change in

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**Figure 2 | Macroflor PET/MRI in non-human primate and rapid uptake into mouse cardiac macrophages.** Macroflor is excreted rapidly via the kidneys and is taken up by cardiac macrophages. (a) PET maximum intensity projection images from dynamic scan. A single primate experiment was carried out. (b) Full-body PET/MR images 90 min into dynamic scan (Left: PET; Right: PET/MRI). (c) Macroflor time-activity curve derived from PET SUV data in non-human primate cardiac blood pool. (d) Intravital dynamic confocal microscopy of Macrolite uptake into cardiac macrophages carried out in a single Cx3cr1GFP/+ reporter mouse. The upper panel shows green GFP signal indicating cardiac macrophages and red VT680 signal reporting on Macrolite presence. The 0 min image was acquired before fluorescent probe injection into the tail vein. Intravascular signal is detected 2 min later, while the 30 min time point illustrates co-localized Macrolite and GFP+ macrophages. Scale bar, 50 μm. (e) Higher magnification images illustrate co-localization of Macrolite and macrophage signal. Scale bars, 50 μm (upper panel) and 25 μm. Mouse cartoon image is reproduced from the servier medical art image data bank (http://www.servier.com/Powerpoint-image-bank).
Figure 3 | Macroflor PET/CT in mice with atherosclerosis. Macroflor-enabled macrophage imaging in aortic plaques of mice with atherosclerosis. (a) Representative PET/CT images of several experiments in ApoE<sup>−/−</sup> and wild-type control mice after IV Macroflor injection. PET scale bar is in kBq/cc n = 14. (b) Three-dimensional rendering derived from PET/CT in ApoE<sup>−/−</sup> mouse shows PET signal in red (arrows). (c) In vivo standard uptake values (SUV) for aortic roots of wild-type and ApoE<sup>−/−</sup> mice (n = 5–7 per group, unpaired t-test). (d) Ex vivo gamma count reports percent injected dose per gram aortic tissue (%IDGT (percent injected dose per gram tissue), n = 5–7 per group, unpaired t-test). (e) Correlation of (c,d) for individual wild-type (black) and ApoE<sup>−/−</sup> mice (grey), counts per minute (CPM). (f) Ex vivo Oil-Red-O staining and corresponding autoradiography of representative aortae. (g) Flow cytometric gating on aortic cells after IV Macrolite injection. (h) Mean fluorescence intensity (MFI) of Macrolite in respective cells retrieved from ApoE<sup>−/−</sup> mouse aorta. (i) VT680 fluorescence indicating Macrolite uptake, obtained in three ApoE<sup>−/−</sup> mouse aortae (one-way analysis of variance). (j) Fluorescent microscopy of aortic root plaque after IV Macrolite injection. Scale bar, 100 μm. Data are shown as mean ± s.e.m., * indicates P<0.05. Mouse cartoon image is reproduced from the servier medical art image data bank (http://www.servier.com/Powerpoint-image-bank).
macrophage phenotype occurs when infarct inflammation transitions to resolution on day 3 in wild-type mice with permanent coronary ligation. In mice and patients with inflammatory co-morbidities such as pre-existing atherosclerosis, oversupply of inflammatory myeloid cells compromises resolution and leads to post-MI heart failure because inflammatory macrophage phenotypes impede tissue regeneration. Consequently, monitoring macrophage numbers and phenotypes is vital to furthering our understanding of ischaemic heart disease. Recently-developed PET/MRI systems can image more than one target simultaneously by combining two imaging agents detectable with either PET or MRI. We here combined Macroflor PET imaging with MRI sensing of myeloperoxidase (MPO), an enzyme expressed by inflammatory macrophages. We previously validated the gadolinium (Gd)-based, activatable enzyme reporter MPO-Gd for infarct imaging and found the signal to be specific for inflammatory myeloid cells, especially neutrophils and the inflammatory L6C\textsuperscript{high} monocyte subset.

We tested dual-target PET/MRI imaging at two time points after acute MI. The first imaging occurred on day 2 after coronary ligation, which coincides with the inflammatory phase characterized by an abundance of inflammatory neutrophils, monocytes and macrophage subsets (Fig. 6a). The second imaging session was on day 6 (Fig. 6b), which coincides with the resolution phase of infarct healing, when reparative macrophage phenotypes support tissue healing via crosstalk with fibroblasts and endothelial cells in sprouting neo-vessels. The PET standard uptake value for reporting macrophage numbers increased between day 2 and day 6 (Fig. 6c), a change that indicates an expanding macrophage pool in the healing infarct. The increase in Macroflor PET signal correlates well with previous flow cytometric studies documenting rising macrophage numbers between days 2 and 6 after MI\textsuperscript{7,10}. The data also imply that macrophages of different phenotypes readily incorporate Macroflor. Concomitantly, we observed declining MRI MPO signal (Fig. 6d). Thus, while the macrophage population expanded, the cells produced less MPO, a dynamic that is
consistent with infarct inflammation switching to resolution. This sensing strategy could identify at-risk individuals that do not undergo phenotype switching.

Finally, we tested dual-target PET/MRI in mouse atherosclerosis. Figure 6e illustrates the activation and retainment of MPO-Gd in the aortic root, a site of inflammatory atherosclerosis in ApoE−/− mice consuming a pro-atherogenic diet. After MPO-Gd injection, the contrast-to-noise ratio rose significantly (Fig. 6f). Immunostaining for MPO demonstrated its presence in the region of interest (Fig. 6g). To provide contrast with the macrophage retention phenotype observed in day 6 infarcts, we subjected ApoE−/− mice to coronary ligation, which promotes disease-exacerbating inflammation systemically and in plaques11. Ex vivo histology and flow cytometry previously documented that acute MI accelerates atherosclerosis in mice. Comparing litter mate ApoE−/− mice with and without MI, we detected not only increased Macroflor PET signal but also increased MPO-Gd retention by MRI. Increased signal in both imaging channels indicates that macrophage numbers expanded but without the resolution documented by lower MPO-Gd retention in the 6 day old infarct; indeed, plaque inflammation flared. Ex vivo scintillation counting confirmed a higher Macroflor uptake in aortic specimens harvested from mice after MI (Fig. 6k). In the clinic, such an integrated imaging strategy may identify disease-promoting inflammatory incidents.

Discussion
 Imaging cardiovascular targets has specific challenges because the tissues are in direct contact with the blood pool in which the imaging probe circulates. Macrophages, cells with high pathological relevance, internalize nanoparticles that can deliver isotopes, fluorochromes or rare earth metal to be detected by nuclear, optical and MRI. Among those modalities, PET is uniquely quantitative, and in the clinic, 18F is the most commonly used isotope. Until now, slow hepatic nanoparticle elimination impeded PET macrophage imaging with 18F labelled particles, as the isotope decay usually outpaces nanoparticle elimination from the blood pool. Here we describe how Macroflor, a polyglucose nanoparticle shrunk to a size below the renal excretion threshold,
solves this problem. Though it is excreted within minutes, Macroflor’s high avidity for macrophages makes it a suitable PET agent for imaging these cells. Because Macroflor’s synthesis relies on sugar molecules and uses facile click labelling for PET isotope attachment, it is highly suitable for clinical translation.

Sensitive imaging tools, including optical detection of fluorescent proteins expressed under control of macrophage-specific promoters, recently revealed macrophage presence and density in cardiovascular and other organs. In the steady state, these cells pursue surveillance and defense. One of their prime activities, phagocytosis of dying cells, infectious agents and other foreign material, also results in avid nanoparticle uptake. Macrophages expand by orders of magnitude in injured and diseased tissue, and depending on their numbers and inflammatory properties, macrophages may promote healing or disease. In cardiovascular organs, overabundance of inflammatory macrophages damages vascular and cardiac structures, ultimately leading to ischemia, stroke, heart failure and death. Thus, macrophages are being explored as therapeutic targets in heart disease and many other pathologies. But because macrophages also pursue salutary functions in tissue repair and defense, broadly targeting macrophages may have deleterious effects. As a consequence, imaging tools for monitoring macrophages or their functions will likely be companion strategies during drug development. Different imaging strategies for monitoring macrophages have been proposed, comprising imaging of adhesion molecules, cell surface receptors and secreted factors executing inflammatory functions. These strategies likely differ in their sensitivity and specificity for macrophage presence, phenotype target range and pharmacokinetics. Some approaches may be sensitive for early

Figure 6 | Dual channel macrophage PET/MRI in ischaemic heart disease. Imaging macrophages and myeloperoxidase detects different inflammatory phenotypes. (a) PET/MRI on day 2 and (b) day 6 post MI in wild-type mice. White dotted line on PET/MRI outlines myocardium. Yellow dashed line on MRI outlines the infarct identified by gadolinium enhancement and wall motion abnormality in cine loops. (c) In vivo PET standard uptake value (SUV) in infarct zone on days 2 and 6 post MI (n = 4 per group, unpaired t-test). (d) In vivo MRI contrast to noise ratio (CNR) in infarct 90 min after IV MPO-Gd injection (n = 4 per group, unpaired t-test). (e) MRI of aortic root in ApoE−/− mouse before and 90 min after IV MPO-Gd. Arrows indicate enhancement in the aortic root. (f) Quantified MRI contrast to noise ratio (CNR) before and after MPO-Gd administration (n = 8 per group, unpaired t-test). (g) Immunofluorescent staining for the MR imaging target myeloperoxidase (MPO) in aortic root of ApoE−/− mouse. Control stain: omission of primary antibody on an adjacent slide. Scale bar, 500 μm. Representative image of n = 4 ApoE−/− mice. (h) PET/MRI following systemic administration of Macroflor and MPO-Gd in ApoE−/− mice without and 3 weeks after MI (n = 4–5). (i) MPO-Gd MRI contrast to noise ratio (CNR) in aortic roots of ApoE−/− mice with and without MI. (j) In vivo PET SUV of aortic root after IV Macroflor injection (n = 4–5 per group, unpaired t-test). (k) Ex vivo scintillation counting of aortae harvested from ApoE−/− mice after IV Macroflor injection (n = 4–5 per group, unpaired t-test). Data are shown as mean ± s.e.m., * indicates P < 0.05. Mouse cartoon image is reproduced from the servier medical art image data bank (http://www.servier.com/Powerpoint-image-bank).
cell activation, such as imaging of alarmins\textsuperscript{12}, while others, including PET imaging of Macrophor enrichment, report on macrophages in all inflammatory stages, even on non-inflammatory tissue resident cells. We envision that Macroflor will enable detection of increased macrophage numbers in cardiovascular organs and monitor the cell population size as a function of therapy. Large animal innate imaging is required next, since PET imaging in mouse thoracotomy innate model reaches limitations in terms of spatially distinguishing inflammation in the body wall from myocardial injury. When combined with an MR imaging agent, dual-target data may additionally report on orthogonal biomarkers that reflect macrophage phenotypes.

Cardiovascular \textsuperscript{18}F-FDG PET detected arterial and myocardial inflammation in several preclinical studies\textsuperscript{13–15} and clinical trials\textsuperscript{16–22}. We, therefore, correlated \textsuperscript{18}F-FDG and Macroflor uptake in rabbits with atherosclerosis. The data show imaging agent overlap. In rabbits with atherosclerosis\textsuperscript{17} and in human endarterectomy specimens\textsuperscript{17}, \textsuperscript{18}F-FDG associates with macrophages detected by histology, which supports the correlation observed in vivo between Macroflor and \textsuperscript{18}F-FDG. However, the 0.518 correlation coefficient suggests that both agents behave distinctly. For example, if not sufficiently suppressed with appropriate measures, \textsuperscript{18}F-FDG enriches in cardiomyocytes, especially if these are distressed\textsuperscript{23}. This consideration may be relevant for imaging the myocardium and coronary arteries. We observed negligible myocyte uptake of Macroflor, perhaps dictated by the different distribution and tissue penetration of small molecule and nanoparticle based PET imaging agents. The favourable Macroflor uptake profile for macrophages and its rapid pharmacokinetics motivate our next translational steps, which include imaging in large animals and toxicity in preparation for first-in-human studies.

Methods

Mice. Female C57Bl/6 (B6) and apolipoprotein E knockout (Apoe\textsuperscript{−/−}) mice were purchased from the Jackson Laboratory (Jackson). ApoE\textsuperscript{−/−} mice were on average 8–12 weeks old when they began a high-cholesterol diet (Harlan Teklad, Madison, Wisconsin) for at least 12 weeks. Mice were induced in both B6 and ApoE\textsuperscript{−/−} mice by permanent coronary artery ligation. Anesthetized with isoflurane, mice were intubated and ventilated. Left thoracotomy was performed in the fourth intercostal space to allow permanent ligation of the left anterior descending coronary artery with monofilament 8–0 suture (Ethicon, Somerville, NJ). The chest wall was closed with 7–0 nylon sutures and the skin was sealed with glue. Mice received isoflurane (2% v/v) and oxygen (21%) anaesthesia in all procedures. The fractions were analysed for glucose content by spotting on ITLC plates. The fractions were analysed for glucose content by spotting on ITLC plates. All animal experiments were approved by the New England Research Institute Subcommittee on Research Animal Care.

Rabbits. Male New Zealand White rabbits (2.5–3 months old), purchased from Charles River Laboratories (Wilmington, MA) underwent double balloon injury of the thoracic and abdominal aorta to induce atherosclerosis. Denudation was performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the Icahn School of Medicine at Mount Sinai, NYC.

Chemistry. Unless otherwise noted, solvents and reagents were purchased from Sigma-Aldrich (St Louis, MO, USA) and used without further purification. Unless otherwise noted, water used for experiments and high-performance liquid chromatography (HPLC) was purified using a MilliQ filtration system (Waters). [\textsuperscript{18}F]-Fluoride ion (n.c.a.) in \textsuperscript{18}F-enriched water was purchased from PETNET (Woburn, MA, USA). Analytical HPLC of radiolabelled compounds was performed with an Agilent 1,200 Series HPLC and a Poroshell 120 EC-C18 (4.6 × 50 mm, 3.0 μm particle size) with 0.5% formic acid in H₂O (A) and MeCN (B); gradient: 0–0.3 min, 5% B; 0.3–7.5 min, 100% B; 7.5–10 min, 100% B; 2.5 min min\textsuperscript{-1} with a multichannel-wavelength ultraviolets/Vis detector, fluorescence detector and a flow-through χ-detector connected in series.

Solid-phase extraction cartridges were Lichrolut C18 3-cc cartridge (200 mg, 2.5 ml of concentrate). The fractions were analysed for glucose content by spotting on ITLC plates. Fractions staining positive for nanoparticles were transferred to 10-kDa mwco filters and concentrated by centrifugation. Ultraviolet absorption measurements were performed in concentrated 10-kDa mwco filters. The contents of the filters was washed with water (3 × 400 l) resulting in 135 μl of concentrate.

\textsuperscript{18}F nanoparticle labelling. Nanoparticle \textsuperscript{18}F labelling was performed using a biorthogonal click reaction\textsuperscript{26,27}. Briefly, [\textsuperscript{18}F]-fluoride, n.c.a. (3.030 MBq, 82 ± 9 μg) in H₂O (> 300 μl), K₂CO₃ (350 μl, 33 mM) in water and Kryptofix 2.2.2 (350 μl, 33 mM, K₂22) in MeCN were added to a 10-ml microwave tube. The [\textsuperscript{18}F]-fluoride/K₂CO₃/MeCN mixture was stirred at room temperature for 0.5 min. After cooling to room temperature, the reaction was loaded onto a PD-10 cartridge (conditioned with MilliQ water) and eluted with MilliQ water. Fractions staining positive for nanoparticles were transferred to 10-kDa mwco filters and concentrated by centrifugation. Ultraviolet absorption measurements were performed in concentrated 10-kDa mwco filters. The contents of the filters was washed with water (3 × 400 l) resulting in 135 μl of concentrate.
evaporation of organic solvent resulted in 30 ± 4 mCi of 18F-P3-C#C, a 52 ± 8% average decay-corrected radiochemical yield (dcRCY) in 56 ± 8 min (n = 13). Analytical HPLC demonstrated >99% radiochemical purity of 18F-P3-C#C. To the concentrated 18F-P3-C#C solution (100–200 µL of H2O), tetraakis(acetonitrile) copper(I) hexafluorophosphate ([Cu(CH3CN)4PF6], 40 µL, 80 mCi in MeCN), bathophenanthroline disulfonate (40 µL, 80 mCi in 1 × PBS) and Macrin-N3 (20 µL, 0.9 ± 0.1 mg, 5.5 mCi azide in H2O) were added in a 1.5-ml centrifuge tube with a magnetic stir bar. The tube was flushed with argon for 30 s and closed. The sealed centrifuge tube was inserted into a 10-mL microwave test tube containing 1 mL H2O and heated to 60 °C (30 Watts) for 5 min. The centrifuge tube was removed, and the reaction mixture was analysed by radio-TLC (ITLC, 100% MeCN mobile phase). The reaction mixture was loaded into a PD-10 (GE Healthcare, preconditioned with H2O) and eluted with H2O (2 × 1,000 µL followed by 8 × 500 µL fractions). Fractions 4–7 were combined and concentrated using 10-kDa mwco filters resulting in 12 ± 3 mCi, a 37 ± 7% average dcRCY over the two-step synthesis in 107 ± 8 min (n = 13).

**Hot cell 18F nanoparticle labelling.** [18F]Fluoride, n.c.a. (−37 GBq, ± 0.3 Ci) in H2O (± 5,000 mCi) was loaded onto a quaternary ammonium methyl (QMA) cartridge and eluted with a solution containing K2CO3 (800 µL, 130 mCi) and Kryptofix 2.2.2 (9 mg, K222) in MeCN (1.92 mL) into a 10 mL sealed conical reaction vessel. Water was removed by azoetric distillation at 120 °C in a heating block (10 min incubation) under vacuum and nitrogen-controlled stream flow. The dried [18F]K2CO3/K222 was cooled to room temperature and 2-(2-prop-2-ynoxy)ethylxothien-2-ylmethanol (5 mCi, 5.6 mg) in MeCN (400 µL) was added. Following the reaction, the vessel was heated to 90 °C for 10 min. After cooling to room temperature (3 min), the mixture was diluted with water (900 µL) and purified by semi-preparative HPLC equipped with a reversed-phase C18 Luna 20 × 10 mm column (Phenomenex, Torrance, CA) in isocratic conditions (5.5 mL/min, 30/70 MeCN/ H2O, 200 mM in 1% C18 Lichrolut EN SPE (Millipore, Billerica, MA). The trapped material was further eluted with acetonitrile (1,750 µL) and the organic solvent dried at 65 °C resulting in a 23% average dcRCY [18F]P3-C#C in 45 ± 5 min (n = 6). Analytical HPLC demonstrated >98% mixture was loaded onto a PD-10 (GE Healthcare, preconditioned with H2O) and eluted with H2O (2 × 1,000 µL followed by 8 × 1,000 µL fractions). Fractions were combined to produce a final 70 ± 10 mCi, a 13 ± 4% dcRCY over the two-step synthesis in 102 ± 7 min.

**Fluorochrome labelling.** In order to perform correlative fluorescence measurements, we also prepared a fluorescent version (Macrolite). In a 1.5-ml centrifuge tube, 100 µL macrin was diluted with MES buffer (200 µL, 50 mM, pH 6.0) and treated with L(Na1.5 µL) and cetyltrimethylammonium bromide (CTAB) (2-µL 0.5% MiliQ water). The radioactive solution was centrifuged at 13,000 rpm for 2 min, and the nanoparticles were removed by aspiration. The aqueous phase was further purified by using a column of Zeba Spin Columns (ThermoFisher scientific) and used to prepare [18F]fluorochrome labelled nanoparticles. A bright-blood, three-dimensional (3D) time-of-flight non-contrast enhanced angiography sequence was acquired to localize arterial anatomical landmarks (renal arteries and iliac bifurcation). Imaging parameters were: TR, 23 ms; TE, 2.8 ms; flip angle, 20 degrees; spatial resolution, 0.7 × 0.7 × 0.7 mm. Images were reconstructed offline using the Siemens eTools software package, interfaced with custom built Matlab software (http://www.mathworks.com). Attenuation correction was performed by segmenting images into 2 compartments (soft tissue and air). For pharmacokinetic analysis, blood was sampled via both central ear arteries at 1, 5, 10, 15, 30, 60, 120, 180 and 210 min post injection. Blood was weighted and its radioactivity content measured on a Wizard 2,480 automatic gamma counter (Perkin Elmer). Radioactivity distribution measurements were performed 20 min after PET scan completion (210 min after injection of the tracer). Rabbits were killed and perfused with 500 mL saline. Organs were excised, blotted and weighed immediately before digital autoradiography was performed by placing the aortic samples in a film cassette against a phosphor imaging plate ( BASM-2325, FujiFilm), for 13 h at −20 °C. Phosphor imaging plates were read at 25 µm pixel resolution with a Typhoon 7000IQ plate reader (GE Healthcare). Image analysis was conducted, using OsiriX Imaging Software, by drawing ROIs on the selected vessels (liver, spleen, and abdominal aorta from left renal artery to iliac bifurcation). Blood activity was quantified in the cardiac chambers. Standardized uptake values (SUVs, defined as (Pixel value (Bq per ml)*Weight of the subject (kg)) per Dose (Bq))/1000 kg = 1) were obtained by averaging SUV max values in each ROI drawn on all slices over the whole organ or over at least 10 slices of the tissue of interest.

**PET/MRI baboon.** A male baboon (Papio anubis, weight = 22.6 kg) was deprived of food for 12 h before the study. Anaesthesia was induced with intramuscular ketamine (10 mg kg−1) and xylazine (0.5 mg kg−1). After endotracheal intubation, the baboon was catheterized antecubitally for radiotracer injection. Anaesthesia was maintained using isoflurane (1.5%–100% oxygen, 1 l/min) during the scan, and ketamine/xylazine effects were reversed with yobine (0.11 mg kg−1) before image acquisition. Vital signs were monitored using a 12-channel monitor (BASMS-2325, Fujifilm), for 13 h at −20 °C. Phosphor imaging plates were read at 25 µm pixel resolution with a Typhoon 7000IQ plate reader (GE Healthcare). Image analysis was conducted, using OsiriX Imaging Software, by drawing ROIs on the selected vessels (liver, spleen, and abdominal aorta from left renal artery to iliac bifurcation). Blood activity was quantified in the cardiac chambers. Standardized uptake values (SUVs, defined as (Pixel value (Bq per ml)*Weight of the subject (kg)) per Dose (Bq))/1000 kg = 1) were obtained by averaging SUV max values in each ROI drawn on all slices over the whole organ or over at least 10 slices of the tissue of interest.

**PET/CT mice.** Two mice after tail-vein injection of Macroflor (525 ± 167 µCi in 120 ± 10 µL), PET/CT imaging was initiated in conjunction with high-resolution contrast-enhanced vascular CT (Inveon, Siemens). The PET data reconstruction was done using ordered subsets expectation maximization and filtered back projection algorithms to achieve a spatial resolution approaching approximately 1 mm. To quantitate PET, anatomic CT data provided the basis for placing regions of interest (ROIs). The CT images were acquired with 80 kVp and 500 µA X-ray power, 370 to 400 ms exposure time and 90 µm isotropic resolution.

**PET/MRI mice.** Mouse PET/CT and MRI were performed sequentially using a custom-designed mouse bed and PET/CT gantry adapter24. To detect the presence of MPO in MI and atherosclerotic lesions, MPO-Gd, a small-molecule gd-gadolinium activatable sensor for MR imaging of MPO activity25, was injected IV (0.3 mmol kg−1) 90 min before the MRI scan. Two to three cine image slices were obtained using a 7-ventricular cardiac angiography sequence with a 3 Tesla Magnetom Verio scanner. Heart volume coil (Rapid Biomedical), electrocardiogram and respiratory gating (SA instruments) and a fast gradient echo FLASH sequence with the following parameters: echo time: 2.9 ms; 16 frames per R-R interval (repetition time 14 ms); resolution 156 µm × 156 µm × 1 mm; number of excitations 4; flip angle 60°. Contrast to noise ratio was calculated with the following formula: Contrast-to-noise ratio = (ROI − Background)/Noise standard deviation. The automated analysis software (SA Instruments, Telford, PA) was used to define ROIs in the heart and aorta. 13C-labeled diacetyl was administered as an intravenous bolus injection 15 min after tracer injection. 13C-labeled diacetyl uptake was quantified in the heart. The late phase 13C MRI acquisition started 20 min after PET scan completion (210 min after the tracer injection). At 30, 60, 90 and 120 min after tracer injection the 13C-MRI scans were acquired. The data were reconstructed offline using the Siemens eTools software platform, interfaced with custom built Matlab software (http://www.mathworks.com). Attenuation correction was performed by segmenting images into 2 compartments (soft tissue and air). For pharmacokinetic analysis, blood was sampled via both central ear arteries at 1, 5, 10, 15, 30, 60, 120, 180 and 210 min post injection. Blood was weighed and its radioactivity content measured on a Wizard 2,480 automatic gamma counter (Perkin Elmer). Radioactivity distribution measurements were performed 20 min after PET scan completion (210 min after injection of the tracer). Rabbits were killed and perfused with 500 mL saline. Organs were excised, blotted and weighed immediately before digital autoradiography was performed by placing the aortic samples in a film cassette against a phosphor imaging plate ( BASM-2325, FujiFilm), for 13 h at −20 °C. Phosphor imaging plates were read at 25 µm pixel resolution with a Typhoon 7000IQ plate reader (GE Healthcare). Image analysis was conducted, using OsiriX Imaging Software, by drawing ROIs on the selected vessels (liver, spleen, and abdominal aorta from left renal artery to iliac bifurcation). Blood activity was quantified in the cardiac chambers. Standardized uptake values (SUVs, defined as (Pixel value (Bq per ml)*Weight of the subject (kg)) per Dose (Bq))/1000 kg = 1) were obtained by averaging SUV max values in each ROI drawn on all slices over the whole organ or over at least 10 slices of the tissue of interest.
after 2.73 mCi Macroflor. PET data were stored in list mode, and reconstruction was performed using a 3D-OSEM method with detector efficiency, decay, dead time, and beam-hardening corrections applied. Dynamic PET data were reconstructed into the following time-frame: 4 × 15 s, 2 × 30 s, 3 × 1 min, 5 × 5 min and 6 × 10 min. Dynamic whole-body PET images at different time points (30 s, 1 min, 2 min, 10 min and 30 min post-radiotracer injection) and a sum image over 90 min were shown as maximum image projection. ROIs were manually delineated from the T1-weighted anatomical image of the cardiac blood pool using PMOD 3.4 (PMOD Technologies Ltd., Zurich, Switzerland) to plot time-activity curves of the cardiac blood pool.

**Intravital microscopy.** Intravital microscopy was performed using a custom-designed system and imaging protocol developed and validated previously29,30. Cx3cr1GFP/+;Ccr7−/−Ly6C+Ly6G− mice were used in this study. Mice were anesthetized using a 10% isoflurane in air intubation using a 17-gauge angiocath as a tracheal tube. Volume mode ventilation was performed with a tidal volume of 0.25 cc and a rate of 130 breaths per minute, and animal body temperature was maintained at 37 °C using a heat plate. A left thoracotomy was performed in the fourth intercostal space, and the ventricular surface was exposed by gently separating the parietal pericardium. A custom-designed tissue stabilizer was affixed to the heart to minimize motion artifacts. Images were acquired using prospective real-time cardiac gated achieving through cardiac pacing24. Macrolite was administered via tail vein injection during confocal microscopy that continued to acquire data at serial time points after injection. Simultaneous excitation with 488 and 633 nm and two-channel detection for GFP and VT680 were performed, and composite two-colour images were created by merging the detection channels in RGB space.

**Flow cytometry.** Two days after they received infarcts, C57BL/6 mice (age 12 weeks, n = 3) and ApoE−/− mice (HCD diet 12–16 weeks, n = 3) received IV injection of Macrolite (5 nmol VT680) and were euthanized 2 h later for flow cytometry analysis. All animals were perfused from the left ventricle with 20 ml PBS at 4 °C. Infarcted heart segments from C57BL/6 mice were harvested. ApoE−/− mouse aorta were excised using a dissecting microscope. Infarcted myocardial and aorta were minced and digested at 37 °C for 1 h with agitation at 750 r.p.m in medium containing collagenase I (450 U ml−1), collagenase XI (125 U ml−1), DNase I (30 U ml−1) and hyaluronidase (60 U ml−1) (all from Sigma-Aldrich). The tissues were then passed through 40 μm cell strainers and re-suspended in PBS with 0.5% bovine serum albumin (FACS buffer). The processed single cell suspensions (300 μl) were stained with a biotin-conjugated anti-mouse lineuge monoclonal antibody cocktail, containing antibodies directed against CD90 (30-H12), B220 (RA3-6B2), NK1.1 (PK136) and Ter-119 (TER-119) (from Biolegend or BD Biosciences/Pharmigen), used in combination with 488-nm excitation with F-18 fluorodeoxyglucose positron emission tomography. LSR II flow cytometer (BD Biosciences). Data were analysed with use of FlowJo software (Tree Star).

**Oil-Red-O staining and autoradiography.** Oil-Red-O staining depicted the distribution of plaques in ApoE−/− aortae that were subsequently photographed by a digital flatbed scanner and analysed by digital autoradiography. Aortae were exposed to a phosphor imager plate and read with a Typhoon FLA9000 system (Amersham Biosciences). For histological examination, myocardial rings were incubated with 1% triphenyltetrazolium (Sigma Aldrich) in PBS at 4 °C for 1 h, collagenase XI (1), DNase I (50 U ml−1), collagenase XI (1), DNase I (30 U ml−1) and hyaluronidase (60 U ml−1) (all from Sigma-Aldrich) for 1 h with agitation at 750 r.p.m in medium containing collagenase I (450 U ml−1), collagenase XI (125 U ml−1), DNase I (30 U ml−1) and hyaluronidase (60 U ml−1) (all from Sigma-Aldrich). The sections were counterstained with 4,6-diamidino-2-phenylindole, dihydrochloride for counterstaining. Images were exposed to a phosphor imager plate and read with a Typhoon FLA9000 system (Amersham Biosciences). Two-colour images were created by merging the detection channels in RGB space, and two-channel detection for GFP and VT680 was performed, and composite two-colour images were created by merging the detection channels in RGB space.

**References.**

1. Mozaffarian, D. et al. Executive summary: heart disease and stroke statistics–2016 update: a report from the American heart association. Circulation 133, 47–54 (2016).

2. Kathiresan, S. et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat. Genet. 41, 334–341 (2009).

3. Weissleder, R., Nahrendorf, M. & Pittet, M. J. Imaging macrophages with nanoparticles. Nat. Mater. 13, 125–138 (2014).

4. Nel, A. E. et al. Understanding biophysicschemical interactions at the nano-bio interface. Nat. Mater. 8, 543–557 (2009).

5. Hilgenfeld, L. et al. Ly-6C/6G monocytes depend on Nr4a1 to balance both inflammatory and reparative phases in the infarcted myocardium. Circ. Res. 114, 1611–1622 (2014).

6. Nahrendorf, M. et al. Activatable magnetic resonance imaging agent reports myeloperoxidase activity in healing infarcts and noninvasively detects the anti-inflammatory effects of atorvastatin on ischemia-reperfusion injury. Circulation 117, 1153–1160 (2008).

7. Swirski, F. K. et al. Myeloperoxidase-rich Ly-6C+ myeloid cells infiltrate allografts and contribute to an imaging signature of organ rejection in mice. J. Clin. Invest. 120, 2627–2634 (2010).

8. Nahrendorf, M. et al. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. J. Exp. Med. 204, 3037–3047 (2007).

9. Dutta, P. et al. Myocardial infarction accelerates atherosclerosis. Nature 487, 325–329 (2012).

10. Vogl, T. et al. Alarmin S100A8/S100A9 as a biomarker for molecular imaging of local inflammatory activity. Nat. Commun. 5, 4933 (2014).

11. Tawakol, A. et al. Noninvasive in vivo measurement of vascular inflammation with F-18 fluorodeoxyglucose positron emission tomography. J. Nucl. Cardiol. 12, 294–301 (2005).

12. Lee, W. W. et al. PET/MRI of inflammation in myocardial infarction. J. Am. Coll. Cardiol. 59, 153–163 (2012).

13. Silvola, J. M. et al. Effects of age, diet, and type 2 diabetes on the development and FDG uptake of atherosclerotic plaques. JACC Cardiovasc. Imaging 4, 1294–1301 (2011).

14. Tahara, N. et al. Simvastatin attenuates plaque inflammation: evaluation by fluorodeoxyglucose positron emission tomography. J. Am. Coll. Cardiol. 48, 1825–1831 (2006).

15. Tawakol, A. et al. In vivo 18F-fluorodeoxyglucose positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. J. Am. Coll. Cardiol. 48, 1818–1824 (2006).

16. Emami, H. et al. Splenic metabolic activity predicts risk of future cardiovascular events: demonstration of a cardioplexic axis in humans. JACC Cardiovasc. Imaging 8, 121–130 (2015).

17. Broekema, M. et al. Validation of 18F-FDG uptake in the arterial wall as an imaging biomarker of atherosclerotic plaques with 18F-fluorodeoxyglucose positron emission tomography–computed tomography (FDG-PET/CT). J. Neuroimaging 24, 117–124 (2014).

18. Rischpler, C. et al. PET/MRI early after myocardial infarction: evaluation of viability with late gadolinium enhancement transcardiac versus 18F-FDG uptake. Eur. Heart J. Cardiovasc. Imaging 16, 661–669 (2015).

19. Tarkin, J. M., Joshi, F. R. & Rudd, J. H. PET imaging of inflammation in atherosclerosis. Nat. Rev. Cardiol. 11, 443–457 (2014).
22. Chen, W. & Dilsizian, V. 18F-fluorodeoxyglucose PET imaging of coronary atherosclerosis and plaque inflammation. Curr. Cardiol. Rep. 12, 179–184 (2010).
23. Herrero, P. & Gropler, R. J. Imaging of myocardial metabolism. J. Nucl. Cardiol. 12, 345–358 (2005).
24. DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356 (1956).
25. Snyder, S. L. & Sobocinski, P. Z. An improved 2,4,6-trinitrobenzenesulfonyl acid method for the determination of amines. Anal. Biochem. 64, 284–288 (1975).
26. Devaraj, N. K., Keliher, E. J., Thurber, G. M., Nahrendorf, M. & Weissleder, R. 18F labeled nanoparticles for in vivo PET-CT imaging. Bioconjug. Chem. 20, 397–401 (2009).
27. Gill, H. S. & Marik, J. Preparation of 18F-labeled peptides using the copper(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition. Nat. Protoc. 6, 1718–1725 (2011).
28. Rodriguez, E., Nilges, M., Weissleder, R. & Chen, J. W. Activatable magnetic resonance imaging agents for myeloperoxidase sensing: mechanism of activation, stability, and toxicity. J. Am. Chem. Soc. 132, 168–177 (2010).
29. Aguirre, A. D., Vinegoni, C., Seba, M. & Weissleder, R. Intravital imaging of cardiac function at the single-cell level. Proc. Natl Acad. Sci. USA. 111, 11257–11262 (2014).
30. Vinegoni, C., Aguirre, A. D., Lee, S. & Weissleder, R. Imaging the beating heart in the mouse using intravital microscopy techniques. Nat. Protoc. 10, 1802–1819 (2015).

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Author contributions
E.J.K. and Y.-X.Y. performed experiments, analysed data and wrote parts of the manuscript. G.R.W., A.D.A., B.T., M.L.S., H.G., F.F., C.P., M., C.C., G.C., Y.S., G.C., Y.J., C.W. and H.-Y.W. performed experiments, imaging and analysed data. T.R., J.W.C., F.K.S., J.H., Z.A.F., W.J.M.M. supervised experiments, discussed strategy and data and revised the manuscript. R.W. and M.N. developed the concept, strategy and funding and wrote the manuscript.

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