Supplemental Methods:

^{18}F-DHMT Synthesis:

^{18}F-DHMT was synthesized by an optimized and fully automated process developed at the Yale University PET Center as recently described (1). Briefly, reaction of 2-azidoethyl 4-toluenesulfonate with K^{18}F/Kryptofix-222 (K-222) provided 2-[^{18}F]fluoroethyl azide (2), which after purification using a solid phase extraction method, underwent a Cu(I)-catalyzed click chemistry reaction with the alkyne precursor to give the radioligand ^{18}F-DHMT with a high radiochemical yield. Purification by HPLC and formulation with ethanol-saline-sodium ascorbate provided an ^{18}F-DHMT solution suitable for intravenous injection (pH 7.0) after filtration of the formulated solution through a sterile, 0.22 μm membrane filter for terminal sterilization. The formulated ^{18}F-DHMT was shown by HPLC analysis to be stable for at least 8 hours after synthesis (maintaining > 97% radiochemical purity). The formulated ^{18}F-DHMT product had a specific activity of 2.19 ± 0.9 mCi/nmol for the PET imaging studies.

MicroPET/CT Image Reconstruction, Data Correction and Analysis

All PET images were reconstructed using a 3D ordered subset expectation maximization / maximum *a posteriori* (OSEM3D/MAP) algorithm with 2 OSEM3D iterations and 18 MAP iterations on the Siemens Inveon Acquisition Workplace. This algorithm yielded a 256*256*159 image dimension with a voxel size of 0.388*0.388*0.796 mm^3. CT images were reconstructed using the Feldkamp filtered back-projection algorithm with a voxel size of 0.445x0.445x0.445 mm^3 and an image dimension of 192x192x298. PET images were corrected for attenuation, scatter, randoms, decay, normalization, and dead time.

3D Gaussian filtering with 2 mm full-width-at-half-maximum (FWHM) was applied on the reconstructed images using AMIDE software (3). In this study, the filtered PET images were only used for volumes of interest (VOIs) definition and image display, while the image quantification was performed on the unfiltered PET images. VOIs were drawn on the left ventricular (LV) myocardium and within the LV cavity using the Seg3D software (4). CT images were used to localize the heart and confirm the epicardial surfaces for VOI edge placement. The myocardial VOI was drawn on the co-registered PET image based on activity thresholding together with a priori information of LV myocardial thickness. Standard uptake values (SUVs) were then calculated for the LV myocardium, liver and LV blood pool. Differences in blood pool SUV were observed between groups (see below), therefore the ROS activity ratio was determined as the ratio between LV myocardial SUV and LV blood pool SUV to account for differences in tissue tracer bioavailability.

Gamma Well Counting of Myocardial ^{99m}Tc-RP805 Activity

^{99m}Tc-RP805 is a radiolabeled peptidomimetic that structurally resembles MMP inhibitors and binds at nanomolar concentrations to the active catalytic site of several MMPs (MMP 2, -3, -7, -9, -12, and -13) known to play an active role in myocardial injury and repair (5). ^{99m}Tc-RP805 was used to quantify myocardial MMP activity in control (n=5) and in DOX treated rats at 4 (n=5) and control (n=5) and DOX treated rats at 8 weeks (n=4) following chemotherapy initiation with gamma well counting, as previously described (5,6). Briefly, rats were injected with ~ 5 mCi of ^{99m}Tc- RP805 via the tail vein and were euthanized with saturated KCl 4 hours following tracer injection. Following euthanasia, animal hearts were rapidly excised, trimmed, washed in 0.9% NaCl, and cut into 3 mm-thick short-axis slices. A basal short-axis slice was further subdivided into 6 radial segments to measure regional differences in myocardial ^{99m}Tc-RP805 retention, a

marker of *in vivo* MMP activity. ^{99m}Tc-RP805 radioactivity in each tissue segment was measured by gamma well counting (Cobra Auto-Gamma, Perkin Elmer, Waltham, MA) using an energy window (120 to 160 keV) centered on the peak gamma emission of ^{99m}Tc. Raw counts were corrected for spill-up/spill-down, background activity, decay, and tissue weight. Corrected counts were converted to mCi/g of tissue with the use of previously determined counter efficiency. Activity in each myocardial segment was then calculated as percentage of injected dose (%ID) by correcting for decay to the time of radiotracer injection. The calculated %ID was then computed by dividing corrected tissue counts (mCi/g) by the corrected injected dose (mCi) and expressing it as %ID per gram tissue (%ID/g). There were no regional differences in ^{99m}Tc-RP805 uptake, thus only global left ventricular ^{99m}Tc-RP805 uptake values are reported.

Histopathological Analyses of Myocardium

A mid-ventricular short-axis slice was fixed in 10% buffered formalin for at least 24 hours, processed, embedded in paraffin blocks, serially sectioned at 3 to 5 µm, transferred to glass slides, stained with hematoxylin and eosin (H&E) or Masson's trichrome (MT), and mounted with glass coverslips by routine methods. H&E stained sections were evaluated for the presence and severity of myocardial toxicity (cardiomyocyte vacuolation, degeneration) or necrosis, inflammation (histiocytic myocarditis), and MT-stained sections were evaluated for the presence and severity of myocardial fibrosis by a veterinarian (C.J.B.) trained in veterinary pathology with extensive expertise in rodent pathology blinded to both treatment group and time point. The tissue parameters were assessed and scored using a semi-quantitative criterion-based analysis adapted from prior published methods (7) as follows: 0 - within normal limits; 1 - minimal changes; 2 - mild changes; 3 - moderate changes; 4 - marked changes; and 5 - severe changes. Myocardial degeneration,

inflammation, and fibrosis were independently scored, and a total severity score determined by summing the values for the three variables.

Immunofluorescence

The terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) assay was performed to assess *in situ* cell death according to the manufacturer's directions (Sigma-Aldrich, St. Louis, MO) using paraffin embedded tissue. Briefly, tissue slices were dewaxed, rehydrated and protease treated (proteinase K, 20 μg/mL), followed by permeabilization (0.1% Triton-X-100, 0.1% Sodium Citrate) and staining with the TUNEL reaction mixture. To ensure that nuclei were only considered for quantification, the nuclear stain, 4,6-Diamidino-2-phenylindole, dihydrochloride (1:20,000 dilution) (DAPI, ThermoFisher Scientific, Waltham, MA) was used as a counterstain according to the manufacturer directions. In addition, the extracellular matrix antibody, anti-laminin (1:50 dilution) (Sigma-Aldrich, St. Louis, MO), was used as a counterstain according to the manufacturer directions to avoid counting cells within pericardial fat and blood vessel lumens.

Tissue sections were imaged on a fluorescent microscope (Nikon 80i) and subendocardial and subepicardial fields for the anterior, septal, posterior and lateral walls of the LV were imaged at 40X magnification for each tissue section. The number of cardiomyocytes with TUNEL positive and DAPI co-staining were counted manually per field using Image J software (analysis grid and cell counter). The number of DAPI stained nuclei was counted semi-automatically with a custom-developed algorithm for thresholding and segmentation (8,9) implemented in MATLAB 2017a (The MathWorks Inc., Natick, MA). The number of TUNEL positive cells were corrected for the number of DAPI stained cells and multiplied by 100 to obtain a TUNEL-positive index per field. The TUNEL-positive index was then averaged over the 8 fields for

subsequent statistical analysis. A biological positive control (infarct) and negative control were used to facilitate accurate TUNEL scoring.

References:

1. Zhang W, Cai Z, Li L et al. Optimized and Automated Radiosynthesis of [18F] DHMT for Translational Imaging of Reactive Oxygen Species with Positron Emission Tomography. Molecules (Basel, Switzerland) 2016;21:1696.
2. Zhou D, Chu W, Dence CS, Mach RH, Welch MJ. Highly efficient click labeling using 2-[(1)(8)F]fluoroethyl azide and synthesis of an (1)(8)FN-hydroxysuccinimide ester as conjugation agent. Nuclear medicine and biology 2012;39:1175-81.
3. Loening AM, Gambhir SS. AMIDE: A Free Software Tool for Multimodality Medical Image Analysis. Molecular Imaging 2003;2:15353500200303133.
4. Institute SCaI. "Seg3D" Volumetric Image Segmentation and Visualization. Scientific Computing and Imaging Institute (SCI).
5. Su H, Spinale FG, Dobrucki LW et al. Noninvasive targeted imaging of matrix metalloproteinase activation in a murine model of postinfarction remodeling. Circulation 2005;112:3157-3167.
6. Sahul ZH, Mukherjee R, Song J et al. Targeted Imaging of the Spatial and Temporal Variation of Matrix Metalloproteinase Activity in a Porcine Model of Postinfarct Remodeling Relationship to Myocardial Dysfunction. Circulation: Cardiovascular Imaging 2011;4:381-391.
7. Montgomery RR, Booth CJ, Wang X, Blaho VA, Malawista SE, Brown CR. Recruitment of macrophages and polymorphonuclear leukocytes in Lyme carditis. Infection and immunity 2007;75:613-620.
8. Otsu N. A threshold selection method from gray-level histograms. IEEE transactions on systems, man, and cybernetics 1979;9:62-66.
9. Meyer F. Topographic distance and watershed lines. Signal processing 1994;38:113-125.

Supplementary Table 1: PET image derived ^{18}F-DHMT SUVs

Variable	Control (4- week) (N=5)	DOX (4-Week) (N=5)	Control (6- week) (N=4)	DOX (6-Week) (N=4)
LV SUV, g/mL	0.42 (0.37, 0.49)	0.35 (0.31, 0.39) #	0.44 (0.36, 0.47)	0.35 (0.27, 0.43)
Liver SUV, g/mL	7.4 (6.99, 8.49)	3.67 (3.36, 6.09) **	7.35 (6.18, 9.08)	3.15 (2.98, 3.57) *
Blood SUV, g/mL	0.32 (0.28, 0.35)	0.18 (0.15, 0.26) *	0.37 (0.27, 0.42)	0.14 (0.10, 0.23) #

Values expressed as medians (1st quartile, 3rd quartile). LV = Left ventricle, SUV = Standardized Uptake Value.
#P = 0.06 vs. Time-matched control
*P< 0.05 vs. Time-matched control
**P< 0.01 vs. Time-matched control