Description of in vitro and in vivo studies:

***In vivo* blood pool PET imaging**

0.57 mCi (21MBq) of ^{68}Ga-labeled rat serum albumin were intravenously administered to healthy rats (n=31; 8 of these subsequently received [^{18}F]FDG for landmarking purposes) and the biodistribution of the tracer was measured for 30 minutes from the time-point of injection using a small animal PET scanner (Inveon P 120, Siemens Preclinical Imaging, Knoxville, TN) and additional ECG-gated acquisition for cardiac imaging. Subsequently, a 7 minute transmission scan was performed for attenuation correction using a sealed ^{57}Co rotating point source. For myocardial landmarking, 0.95 mCi (35MBq) of [^{18}F]FDG were injected and ECG-gated [^{18}F]FDG image data were acquired over 30 minutes (acquisition started about one hour after the beginning of the experiment). Data processing was conducted on an Inveon Research Workplace (V 1.4.3.6, Siemens Medical Solutions) with a dedicated reconstruction algorithm (OSEM3D 4 iterations, MAP 32 iterations, zoom factor 1.42, matrix size 128x128, scatter correction). To allow assessment of temporal changes in the tracer-uptake, images were framed dynamically.

***In vivo* PET imaging of apoptosis**

After intravenous application of 0.27-0.32 mCi (10-12 MBq) ^{68}Ga-labeled annexin V into mice with a myocardial infarction (n=11), a µ-PET scan was performed and dynamic ECG-gated images were acquired over the course of 90 minutes. Subsequently, a 7 minute transmission scan was performed for attenuation correction. For myocardial landmarking, 0.95 mCi (35MBq) of [^{18}F]FDG were then injected and dynamic ECG-gated PET data were acquired over 30 minutes, followed by another 7 minute transmission scan for attenuation correction. Data processing was conducted on an Inveon Research Workplace with a dedicated reconstruction algorithm (OSEM3D 4 iterations, MAP3D 32 iterations,

zoom factor 1.00, matrix size 256x256, scatter correction). To allow assessment of temporal changes in the tracer-uptake, images were framed dynamically. Images were then analyzed visually and with a standardized multiple VOI technique. VOIs were placed over defined regions of the heart (infarction, healthy myocardium) and the liver and time-activity-curves were created reflecting the dynamic uptake of the tracer in these regions (**Supplemental Figure S3**).

PET data processing

List mode data were acquired using a µ-PET scanner and divided into temporal frames of increasing length varying from 20s to 10min for the assessment of temporal changes in regional tracer accumulation. Tomographic volumes were created using scatter and attenuation correction, 4 iterations of 3D OSEM, followed by 32 iterations of 3D MAP iterative reconstruction, yielding a transaxial spatial resolution of approximately 1.4 mm.

3D rendering of PET images

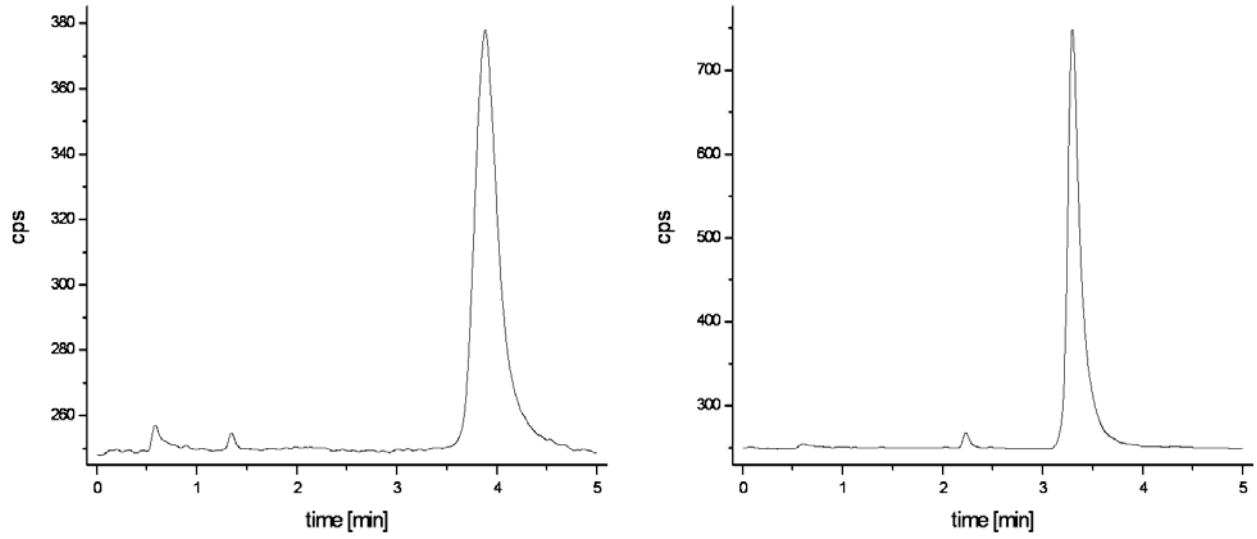
The 3D images were obtained using a dedicated software package (Hybrid Viewer, research version, Hermes Medical Solutions, Stockholm, Sweden). The software supports real-time direct 3D volume rendering with multidimensional classification to assign specific visual attributes (color and opacity) to the features of interest. By using a classification based on a combination of properties, different features can be visualized separately from each other.

***Ex vivo* autoradiography and apoptosis staining (TUNEL assay)**

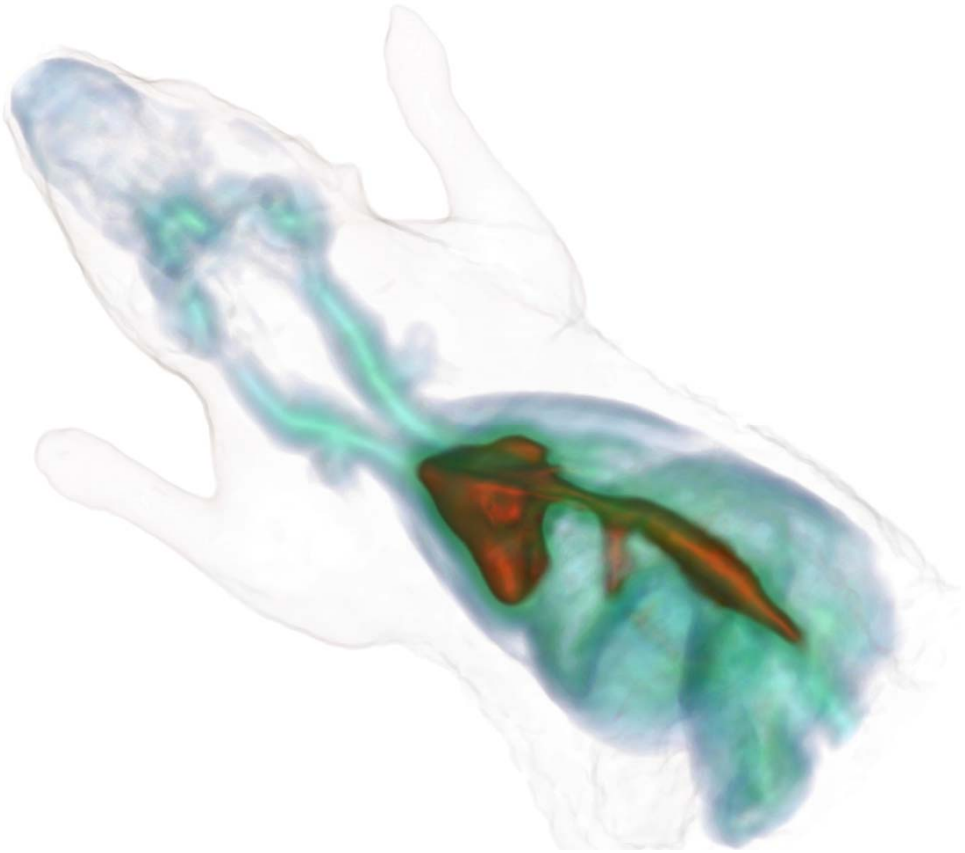
For *ex vivo* autoradiography, mice with left-ventricular infarction, affecting the anterior wall of the left ventricle and the apex were injected with 0.27-0.32 mCi (10-12 MBq) [^{68}Ga]annexin V and measured

for 90 minutes using a μ-PET scanner. Subsequently, the mice were sacrificed, the heart was excised, rinsed with isotonic saline and slowly frozen in liquid nitrogen. The heart was sliced (slice thickness 25 μm) and the slices were placed on an autoradiography film (Fujifilm MS Imaging Plates in a Fujifilm BAS Cassette 2 2025) for 24 hours. Readout and interpretation of the films was accomplished by using a Fujifilm FLA-5100 together with the Image Reader FLA-5000 2.1 software.

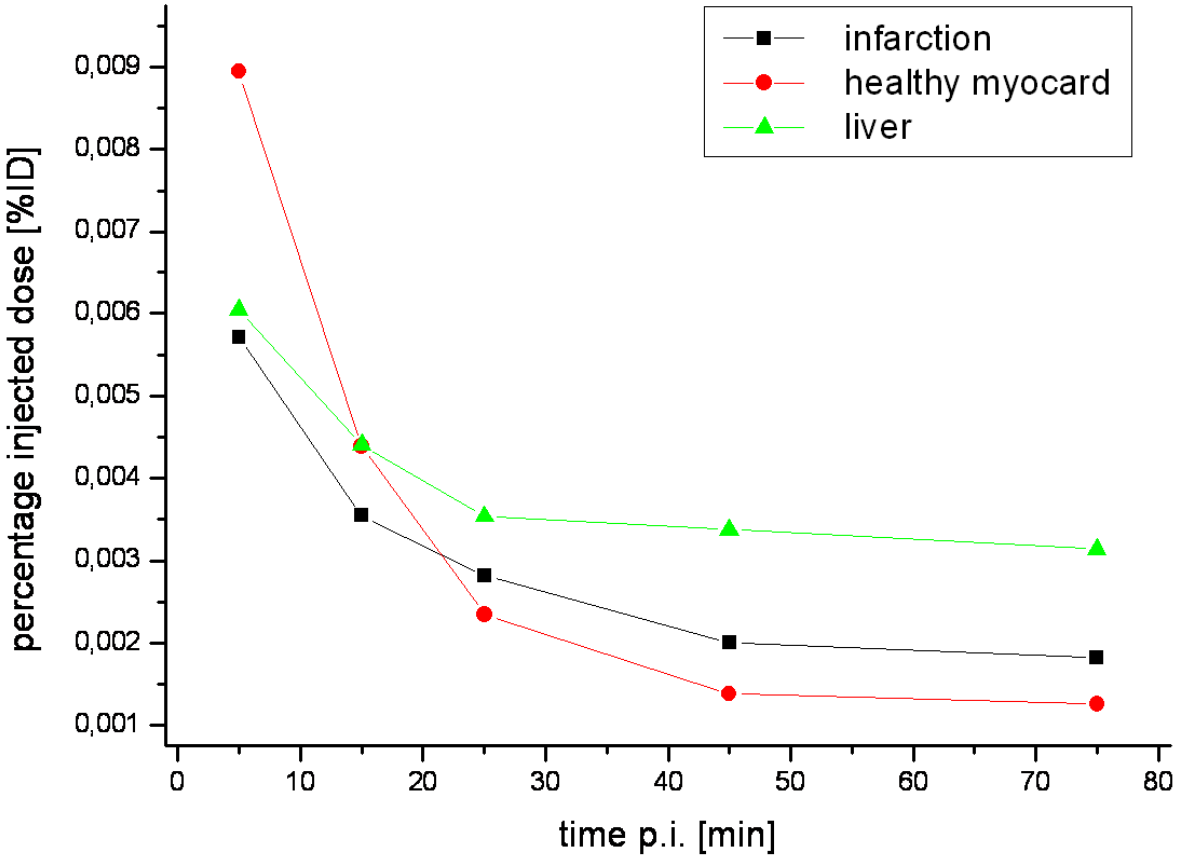
To detect DNA fragments associated with apoptosis using TUNEL staining, the heart was fixed in 10% neutral buffered formalin and subsequently embedded in paraffin and then cut into slices. For further processing of the slices, the standardized ApopTag® Apoptosis Detection Kit staining procedure was used. Slices were deparaffinized, pretreated with Proteinase K (20μL/mL) for 15 minutes, washed and equilibration buffer was added. Subsequently Working Strength TdT Enzyme was added (55 μL/5 cm^2) and the slices were incubated for 1 hour at 37°C. After further steps of buffering and washing, color was developed in peroxidase substrate and counterstaining was performed in 0.5% methyl green. Finally, the specimen was mounted on silanized glass slides.



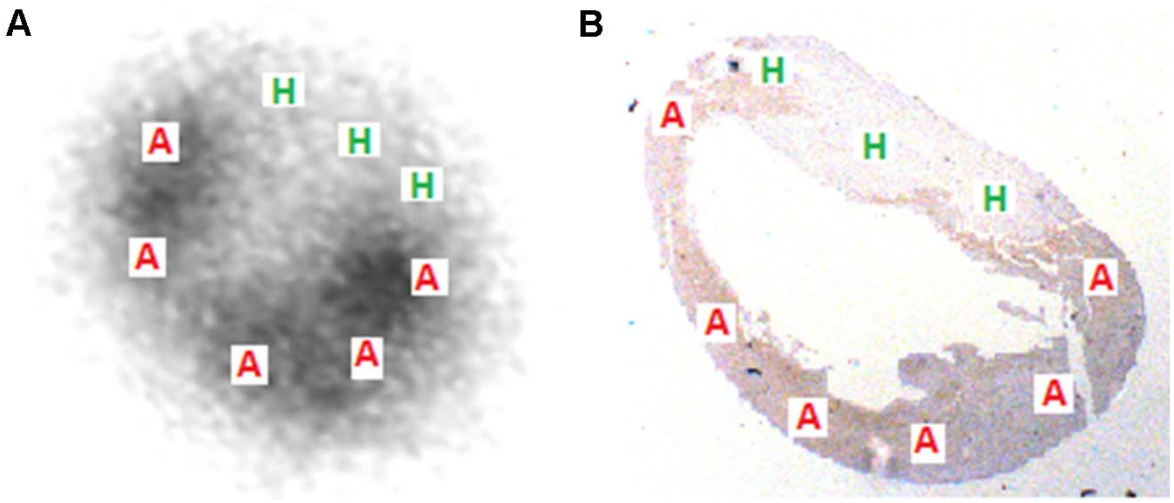
SUPPLEMENTAL FIGURE 1. Analytical radio-HPLC chromatograms of ^{68}Ga-labeled annexin V (left) and RSA (right) after 7 minutes labeling reaction of the chelator-derivatized precursor proteins with $^{68}Ga^{3+}$.



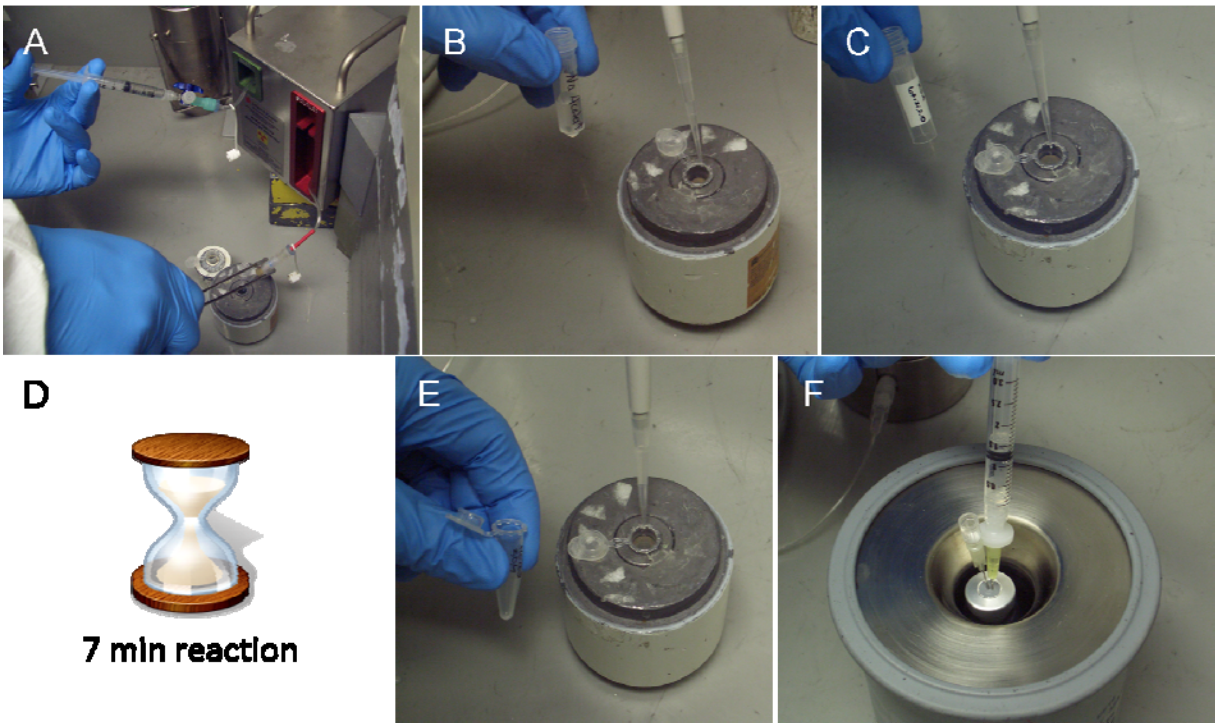
SUPPLEMENTAL FIGURE 2. *In vivo* blood pool imaging in a healthy rat with [^{68}Ga]albumin (30min p.i.). Volumes with similar count rates are merged to yield a combined surface. Areas with uptake higher than 670,000 counts per second (cps) (heart and aorta) are rendered in red and demonstrate the contrast the tracer provides to separate heart and aorta from other tissue. Areas between 300,000 and 670,000 cps are represented by a semi-transparent color scale from blue over green to yellow (blue indicates low and yellow high count rates). This allows to differentiate blood filled vessels and areas with high uptake from other tissues. The rat's skin is visualized as a transparent brown silhouette for orientation purposes.



SUPPLEMENTAL FIGURE 3. Time-activity curves ([%ID]/time p.i. [min]) derived from the image data obtained from a mouse with left-ventricular infarction after application of [^{68}Ga]annexin V. PET image data were collected over 90 minutes and analyzed using the volume of interest (VOI) technique (graph points are set in the middle of the time interval). Curves are reflecting the [^{68}Ga]annexin V biodistribution in the liver (green), the healthy myocard (red) and the infarct region of the myocard (black) and show an increasing accumulation of the [^{68}Ga]annexin V in the infarcted area compared to healthy myocard tissue.



SUPPLEMENTAL FIGURE 4. (A) Autoradiography image obtained from an axial heart slice of a mouse with left-ventricular infarction injected with [^{68}Ga]annexin V. The slice is oriented with the anteroapical wall to the right. (B) TUNEL assay image obtained from an axial heart slice of the same mouse with left-ventricular infarction indicating apoptosis (darker tissue regions). The dark regions in the autoradiographic image (A) correspond to the infarct region highlighted in the [^{68}Ga]annexin V *in vivo* PET image and the apoptotic regions delineated by TUNEL assay (B) (A: apoptosis; H: healthy tissue).



SUPPLEMENTAL FIGURE 5. Steps of protein labeling in pictures:

A) fractioned elution of the ^{68}Ge/^{68}Ga-generator using 2×1mL 0.1M HCl (the first mL is discarded, the second mL is used for the radiolabeling reaction),

B) addition of sodium acetate solution to adjust pH to 3.5-4.0,

C) addition of a solution of chelator-derivatized protein (precursor),

D) reaction at room temperature for 7 minutes,

E) neutralization of the reaction mixture to pH 7.0,

F) sterile filtration.

The ^{68}Ga-labeled protein is ready for *in vivo* application.