Supplemental Information

LC-MS determination of HSA complexation with Evans blue

The mass analysis was performed on a Waters LC-MS system (Acquity UPLC system and a Waters Q-Tof Premier high resolution mass spectrometer). An Acquity BEH Shield RP_{18} column (150 x 2.1 mm) was eluted with a two-solution gradient of solution A (2 mM ammonium formate, 0.1% formic acid, and 5% CH_3CN) and solution B (2 mM ammonium formate and 0.1% formic acid in CH_3CN). The elution profile, at 0.35 mL/min, was: 100% (v:v) A and 0% B initially; gradient 0 - 40% B over 5 min; isocratic elution at 40% B for an additional 5 min; washing with 100% B over 2 min; and re-equilibrium with A for an additional 4 min. Ion detection was achieved in positive ESI mode using a source capillary voltage of 3.5 kV, source temperature of 110 °C, desolvation temperature of 200 °C, cone gas flow of 50 L/h (N_2), and desolvation gas flow of 700 L/h (N_2). The spectra were transformed into a mass scale using MaxEnt 1 software (Waters).

Saturation binding assay

Both NEB and bovine serum albumin (BSA) were dissolved in distilled H_2O. In a 15 μL volume, 100 μg of BSA was mixed with different concentration of NEB (ranging from 0 to 10 μg). After incubation for 5 min under room temperature, 15 μL of 2x loading buffer was added to each vial. Then the samples were loaded to 1.5% agarose gel for electrophoresis (100v, 40 min). The gel was imaged with a Maestro II optical imaging system with a green filter set (523 nm excitation and 560-750 nm in 10 nm steps acquisition setting). The fluorescence signal was unmixed based on the different spectrum of NEB/BSA and NEB. The signal intensity of NEB/BSA in each lane was quantified with the software provided by the manufacturer. Non-linear regression was plotted using GraphPad Prism (GraphPad Software Inc.).

In vitro and *in vivo* serum stability

^{18}F-AlF-NEB or ^{64}Cu-NEB were mixed with 50 μL aliquots of mouse serum and incubated at 37 ºC. At 30, 60 and 120 min, the aliquots were mixed with an equal volume of CH_3CN, the layers were assayed to determine extraction efficiency, and a portion of the supernatant was subjected to radioHPLC analysis using an on-line radioactivity detector. For *in vivo* stability assay, 3.7 MBq of ^{18}F-AlF-NEB was injected into a normal mouse. At 60 min after injection, the blood and urine samples were collected. Equal volume of CH_3CN was added and the supernatant was subjected to radioHPLC analysis.

ECG gated PET imaging of Sprague-Dawley rats

For ECG gated PET studies, the rats were imaged in a prone position within the PET scanner (Inveon small animal PET, Siemens), and were kept at 37 °C using a heating pad with continuous rectal measurement of body temperature. ECG electrodes were placed on the forepaws and the left hindpaw. Respiration was measured using a small pressure detector lying under the thorax of the mice. The cardiac excitation and respiration were recorded with a Biovet system (Spin Systems Pty Ltd.) throughout the scan. A list mode PET scan of 30 min was acquired at 15 min after intravenous injection of 18.5 MBq of ^{18}F-AlF-NEB.

M-mode echocardiogram

At 2 days after MI, the mice received 2% isoflurane for general anesthesia and were placed on the scanning table. Echocardiographic images were obtained using a dedicated small-animal high resolution-imaging unit and a 30-MHz linear transducer (Vevo 770; Visualsonics**)**. Using the parasternal short-axis view, LV end-diastolic and LV end-systolic diameters (LVEDD and LVESD, respectively) were measured, and LV fractional shortening was calculated as =

(LVEDD – LVESD)/LVEDD x 100. All measurements were averaged based on 3 consecutive cardiac cycles.

Dosimetry

About 1.85 MBq of ^{18}F-AlF-NEB (n=5) and 3.7 MBq of ^{64}Cu-NEB (n=4) in a volume of 100 µl of PBS were injected into Balb/c mice *via* the tail vein. Multiple time-point PET images (up to 4 h for ^{18}F-AlF-NEB and 24 h for ^{64}Cu-NEB) were acquired. After image reconstruction, region of interests were outlined on major organs to calculate percent injected dose per gram of tissue (%ID/g). Standard organ weight was used to calculate number of disintegration in each organ. Determination of organ doses for a reference human male was made using the OLINDA/EXM program (Vanderbilt University, Nashville, TN).

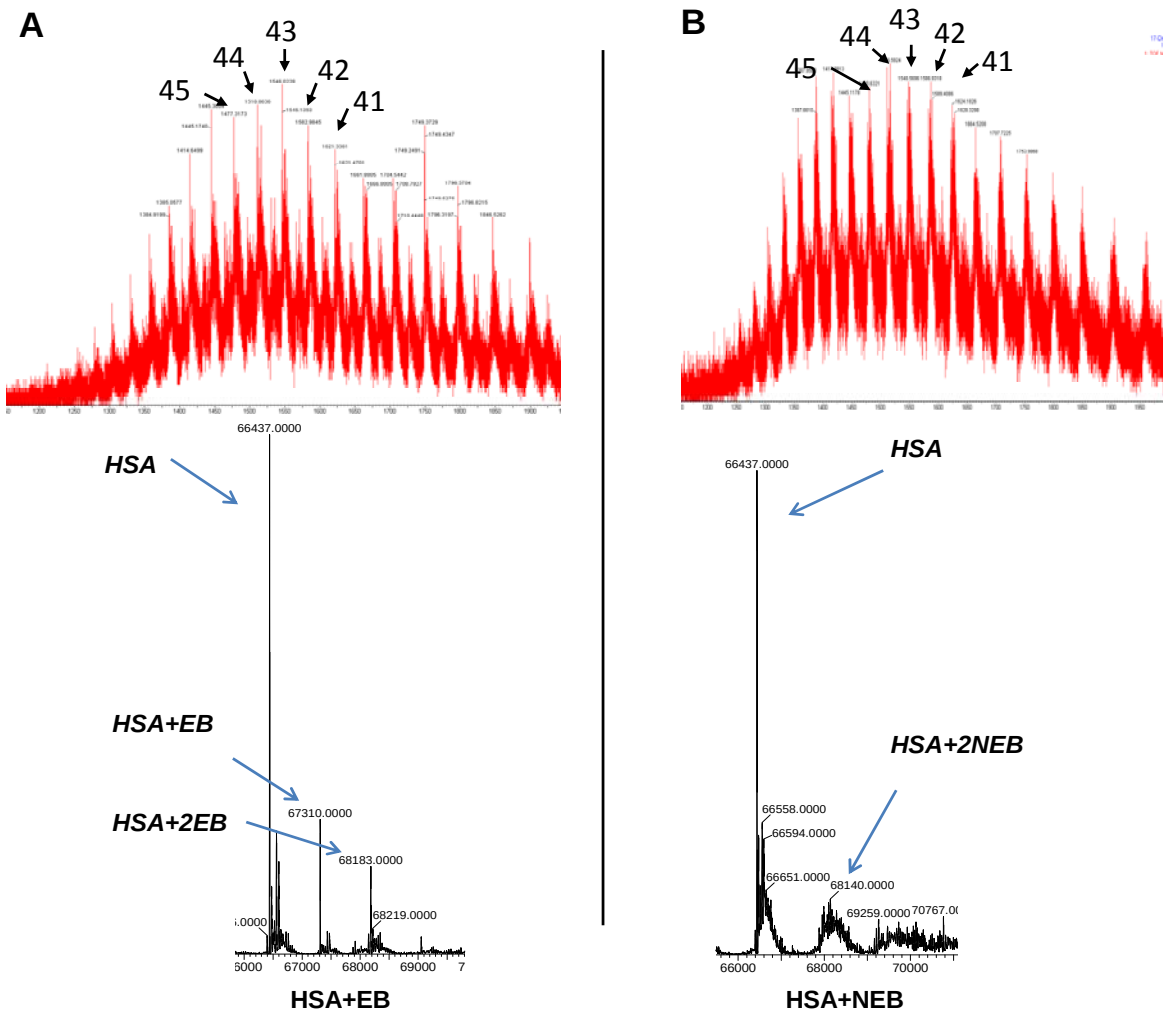
Supplemental Table 1. Number of disintegration in major organs of ^{18}F-FAl-NEB and ^{64}Cu-NEB obtained from PET imaging in Balb/c mice (MBq-h/MBq)

Organs	**^{18}F-FAl-NEB**	**^{64}Cu-NEB**
Brain	0.0010475	0.013168
Heart	0.0012447	0.015647
Kidneys	0.0028363	0.035655
Liver	0.0137634	0.173017
Lung	0.0277512	0.348856
Muscle	0.0639366	0.803738
Spleen	0.0066572	0.083687
Blood	0.2040529	2.56512

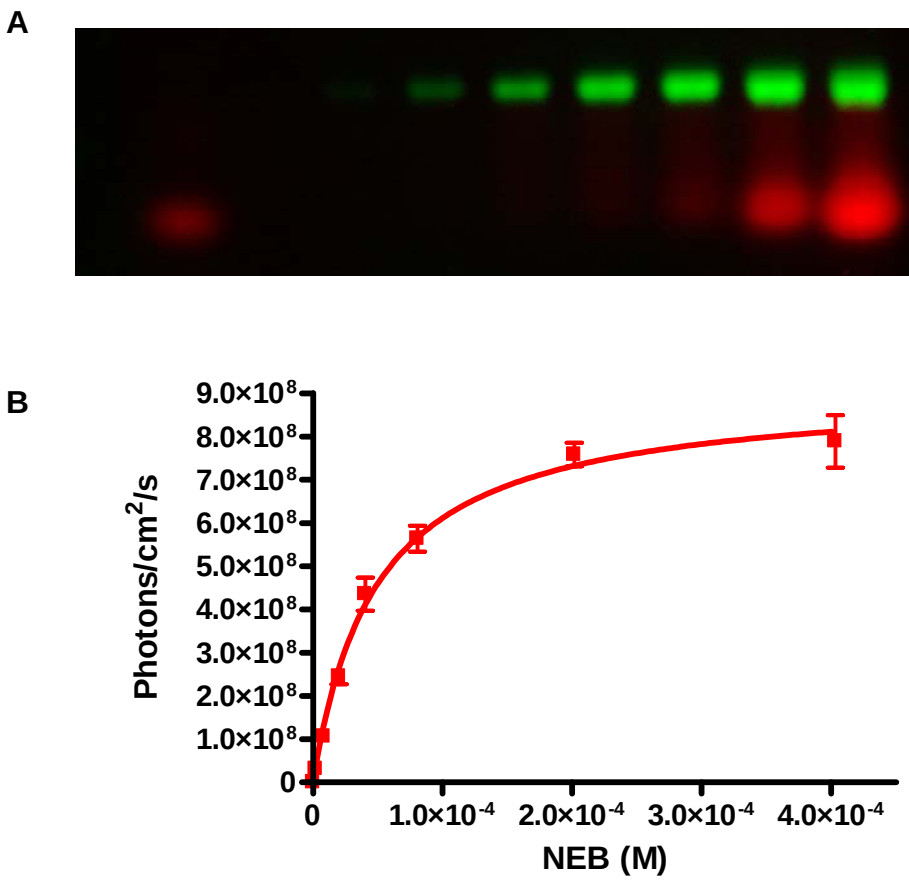
Supplemental Table 2. Human absorbed radiation doses resulting from PET imaging of Balb/c mice (n = 5 for ^{18}F-FAl-NEB and n = 4 for ^{64}Cu-NEB)

Organ	^{18}F-FAl-NEB	^{64}Cu-NEB
Brain	3.41E-04	1.32E-03
Intestine	5.04E-04	1.15E-03
Stomach	1.69E-03	3.87E-03
Heart	6.12E-02	3.06E-01
Kidneys	3.29E-03	1.44E-02
Liver	3.94E-03	1.42E-02
Lung	9.35E-03	4.15E-02
Muscle	1.41E-03	4.88E-03
Red marrow	1.25E-03	3.32E-02
Pancreas	2.57E-03	5.86E-03
Spleen	9.92E-03	4.91E-02
Total Body	1.57E-03	5.45E-03

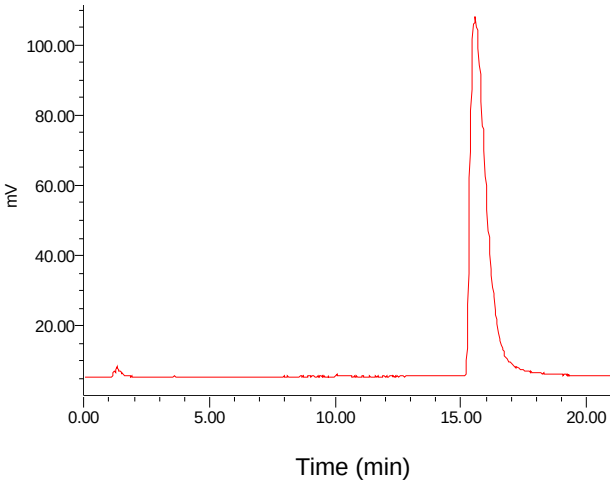
Doses are expressed as mSv/MBq



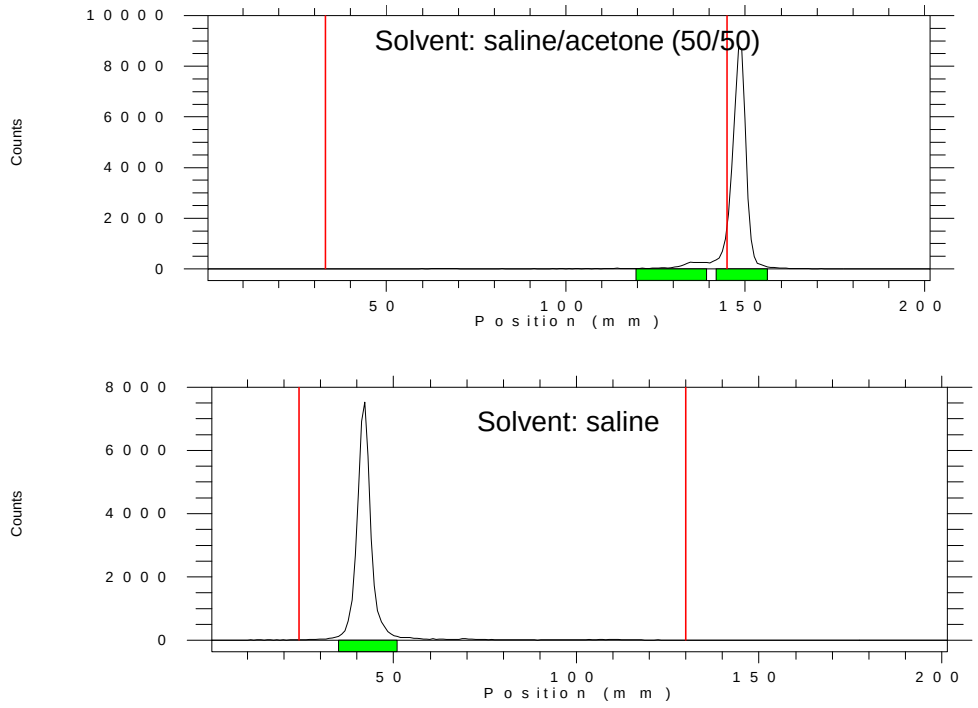
Supplemental Figure 1. Determination of complexation of Evans blue (A) and NOTA conjugated truncated Evans blue (B) with human serum albumin (HSA) by LC/MS. The signals detected by LC/MS were shown in upper panels with n+ labeled. The mass reconstructions were shown in the lower panels.



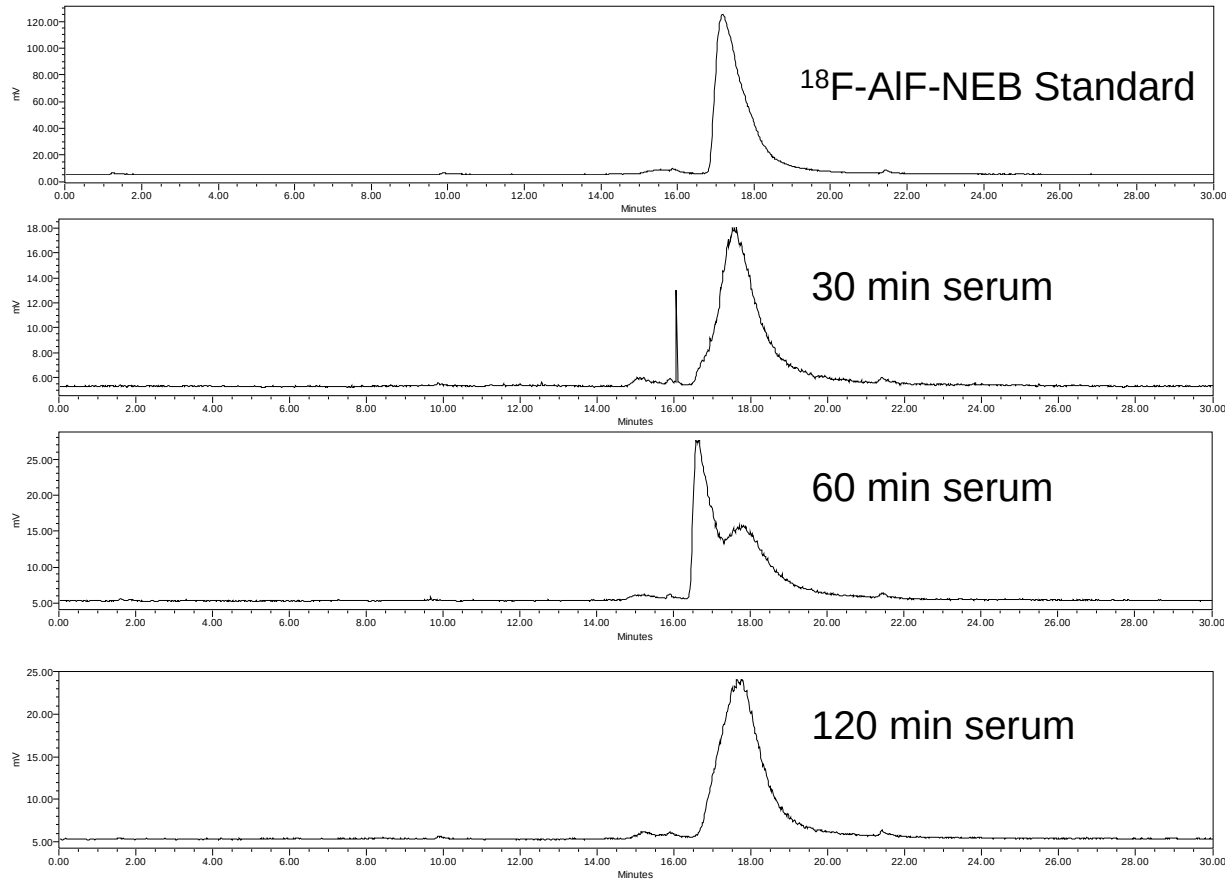
Supplemental Figure 2. Saturation binding assay of NOTA conjugated truncated Evans blue (NEB) and bovine serum albumin (BSA). (A) Optical imaging of agarose gel electrophoresis of BSA and different concentrations of NEB. After fluorescence signal unmixing by Maestro Imaging System, the NEB/BSA complex was presented as green color and unbound NEB was presented as red color. The first lane was NEB only without BSA. (B) Graphpad plot of quantification of optical images. The dissociation constant is 48.9 ± 3.81 μM.



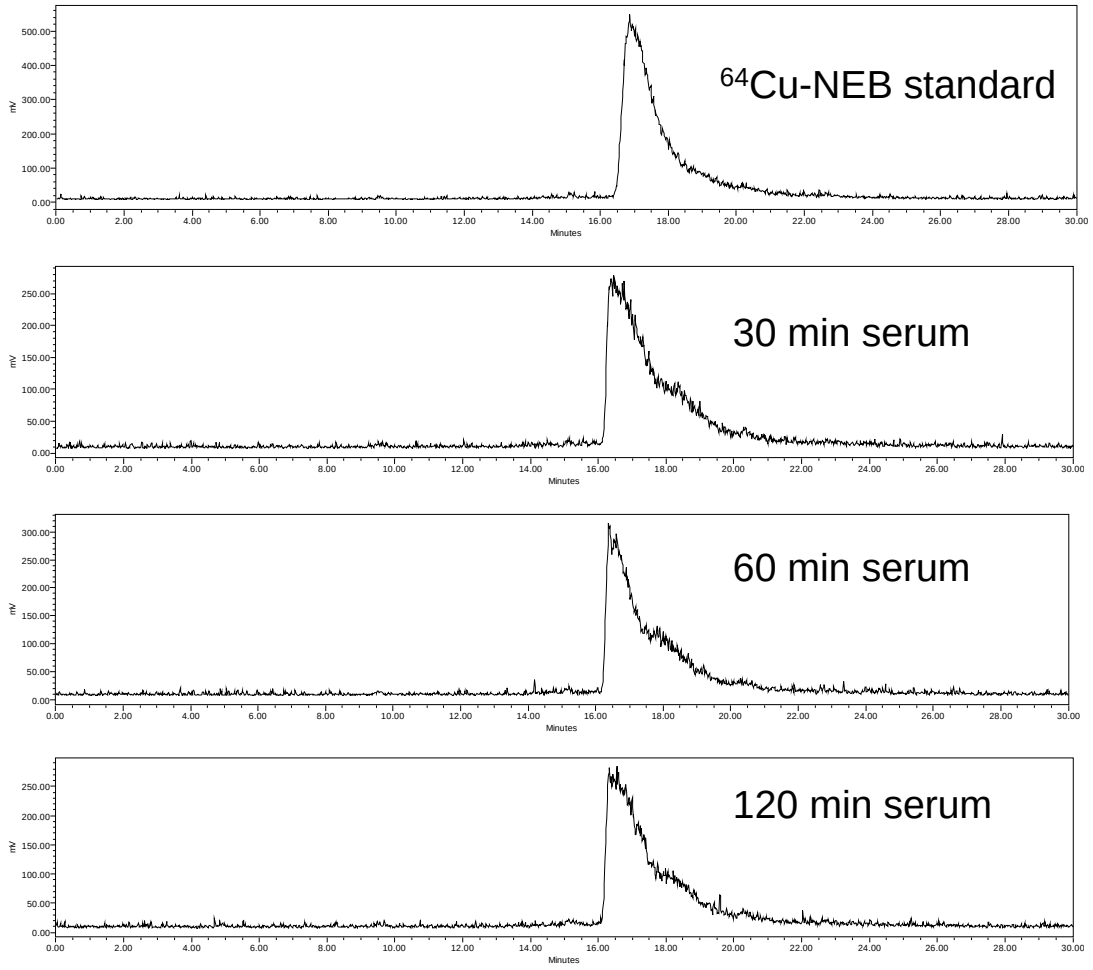
Supplemental Figure 3. ^{18}F-labeling of NEB was achieved by the formation of ^{18}F-aluminum fluoride complex ^{18}F-AlF-NEB. The radiochemical purity was >95% based on radioHPLC analysis.



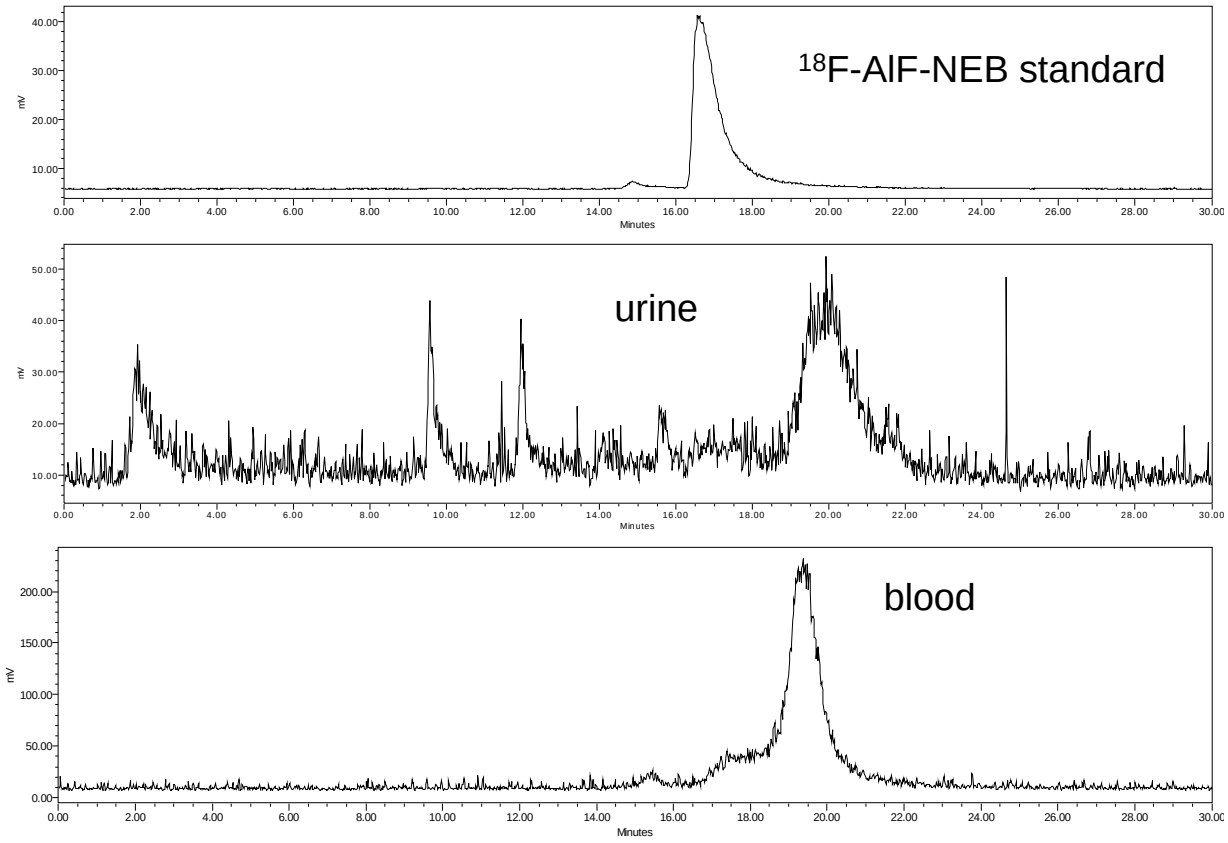
Supplemental Figure 4. Radioactive thin layer chromatography (radioTLC) on silica gel plate with saline/acetone (50/50) (upper panel) or saline only (lower panel) as solvent. Only one peak was identified, indicating the purity of the product.



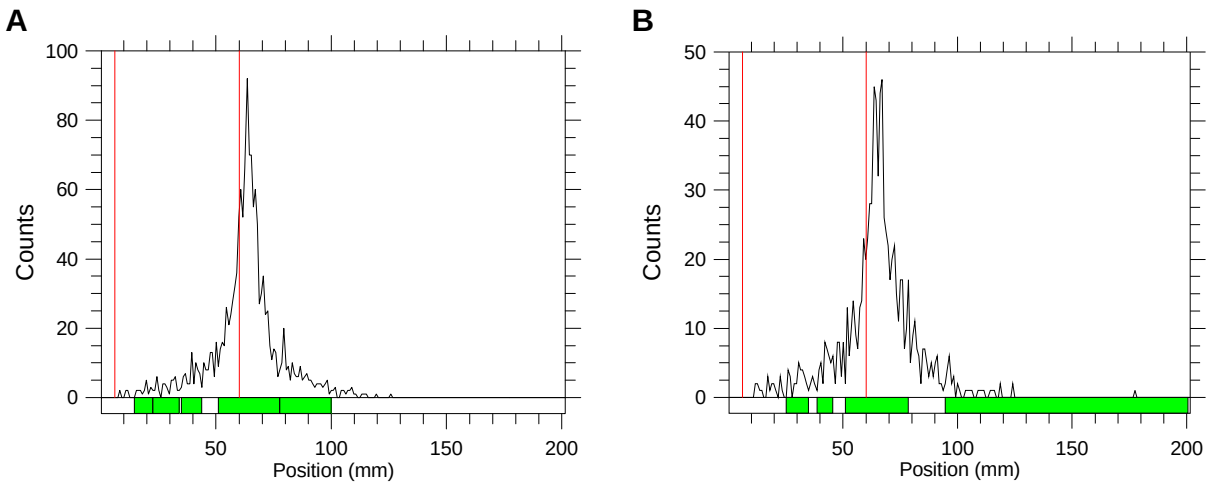
Supplemental Figure 5. In vitro serum stability of ^{18}F-AlF-NEB determined by radio-HPLC.



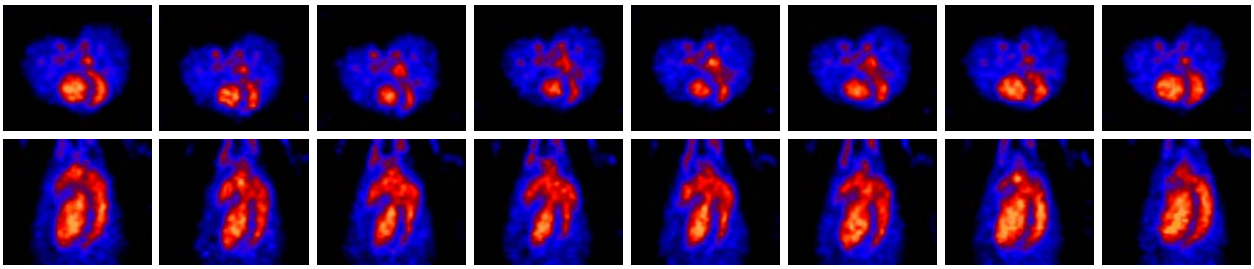
Supplemental Figure 6. In vitro serum stability of ^{64}Cu-NEB determined by radio-HPLC.



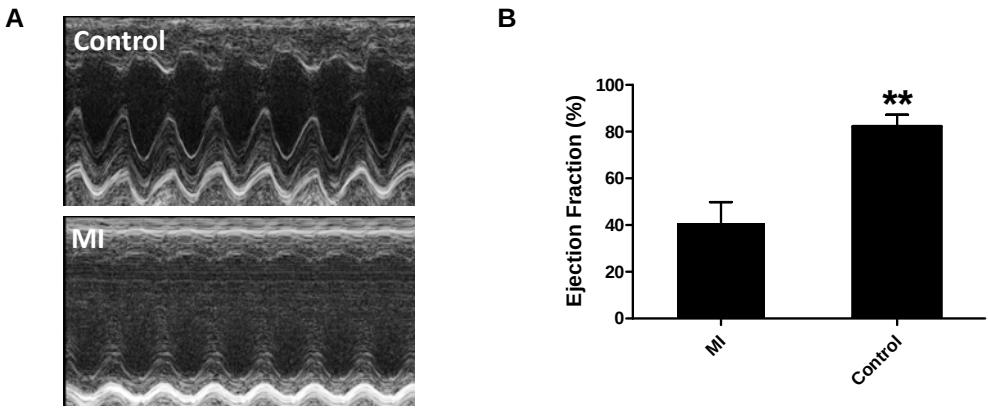
Supplemental Figure 7. In vivo stability of ^{18}F-AlF-NEB determined by radio-HPLC.



Supplemental Figure 8. Agarose gel electrophoresis of ^{18}F-AlF-NEB plus albumin (A) and mouse serum (B) at 30 min after injection of ^{18}F-AlF-NEB. Five microcurie in 10 μL of each sample was loaded on 0.8% agarose gel. The gel was run at room temperature for 40 min with a voltage of 100 volt. After that, the gel was scanned with a Bioscan AR-2000 RadioTLC scanner.



Supplemental Figure 9. Electrocardiography (ECG) gated PET images of healthy Sprague-Dawley rats using ^{18}F-AlF-NEB. Eight intervals of one cardiac cycle with transaxial (upper panel) and coronal (lower panel) sections are displayed.



Supplemental Figure 10. (A) Representative M-mode echocardiograms of control (upper panel) and myocardial infarcted (MI) (lower panel) mice. (B**)** Left ventricular ejection fraction (LVEF) calculated from echocardiograms.