Supplementary Information

Al^{18}F-Labeling Of Heat-Sensitive Biomolecules For Positron Emission Tomography Imaging

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Table of Contents

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    1          11         21         31         41         51
    |          |          |          |          |          |
  1                          DAHKSE VAHRFKDLGE ENFKALVLIA FAQYLQQCPF    60
 61 EDHVKLVNEV TEFAKTCVAD ESAENCDKSL HTLFGDKLCT VATLRETYGE MADCCAKQEP   120
121 ERNECFLQHK DDNPNLPRLV RPEVDVMCTA FHDNEETFLK KYLYEIARRH PYFYAPELLF   180
181 FAKRYKAAFT ECCQAADKAA CLLPKLDELR DEGKASSAKQ RLKCASLQKF GERAFKAWAV   240
241 ARLSQRFPKA EFAEVSKLVT DLTKVHTECC HGDLLECADD RADLAKYICE NQDSISSKLK   300
301 ECCEKPLLEK SHCIAEVEND EMPADLPSLA ADFVESKDVC KNYAEAKDVF LGMFLYEYAR   360
361 RHPDYSVVLL LRLAKTYETT LEKCCAAADP HECYAKVFDE FKPLVEEPQN LIKQNCELFE   420
421 QLGEYKFQNA LLVRYTKKVP QVSTPTLVEV SRNLGKVGSK CCKHPEAKRM PCAEDYLSVV   480
481 LNQLCVLHEK TPVSDRVTKC CTESLVNRRP CFSALEVDET YVPKEFNAET FTFHADICTL   540
541 SEKERQIKKQ TALVELVKHK PKATKEQLKA VMDDFAAFVE KCCKADDKET CFAEEGKKLV   600
601 AASQAALGL
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Figure S1: Amino acid sequence of human serum albumin (ALBU_HUMAN (P02768)). *Of the 609 amino acids encoded by the ALB gene and translated to form the precursor protein, only 585 amino acids are observed in the final product present in the blood; the first 24 amino acids, are cleaved after translation. HSA possesses 59 lysine residues (K) in its amino acid sequence. Reduced HSA contains 17 disulfide bonds and one free thiol group at Cys34. The theoretical average neutral molecular mass of reduced HSA is 66437.4 Da [M] (calculated for $C_{2936}H_{4590}N_{786}O_{889}S_{41}$) and the observed mass after deconvolution was 66438.3 ± 0.3 Da [M].*

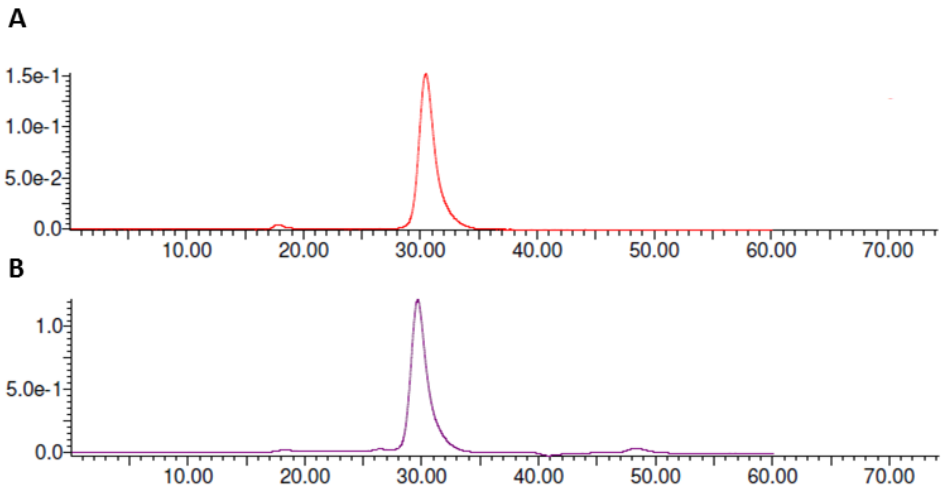

Figure S2: SEC-UV280 nm chromatogram of (A) HSA (Rt: 30.45 min) and (B) RESCA-HSA (Rt: 29.64 min). *No aggregates or degradation products were observed.*

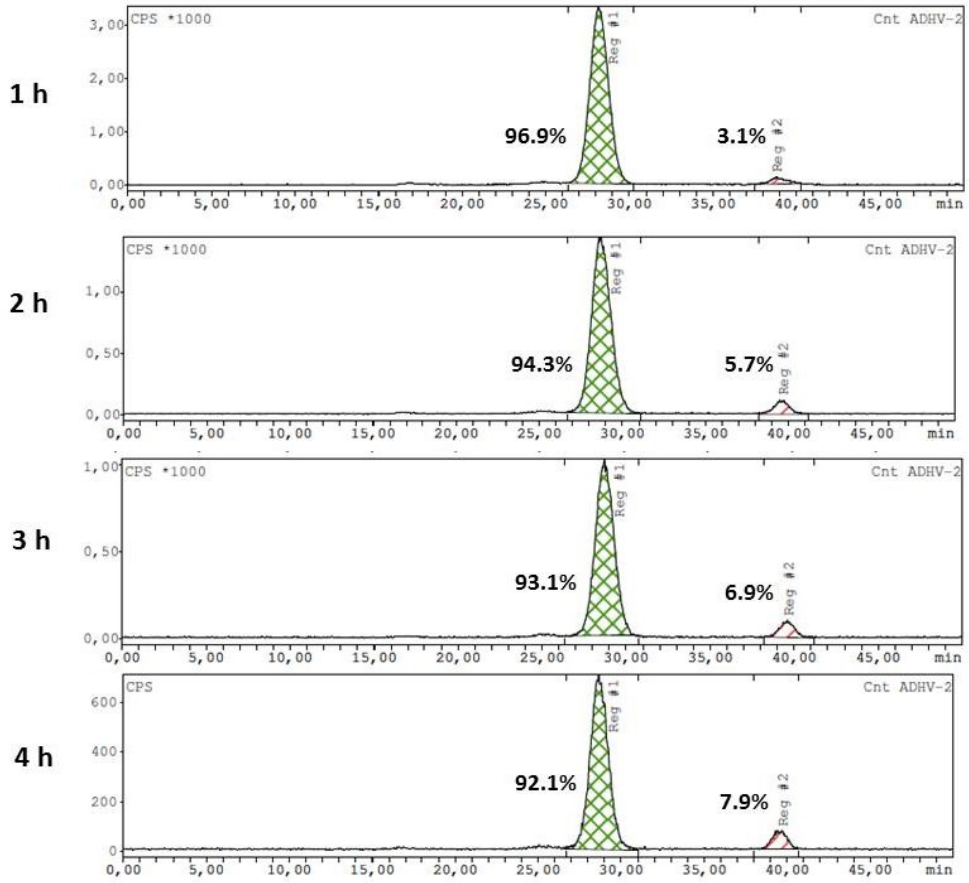

Figure S3: SEC-radio-chromatogram of (±)-[^{18}F]AlF(RESCA)-HSA (Rt 28.5 min) after 1, 2, 3 and 4 hours incubation in rat plasma at 37°C. *After 4 h incubation in rat plasma at 37 °C, 92.1% of (±)-[^{18}F]AlF(RESCA)-HSA was still intact.*

Table S1: Biodistribution of (±)-[^{18}F]AlF(RESCA)-HSA in healthy female Wistar rats at 1 h, 3 h and 6 h p.i. (n=4/ timepoint). *The results are presented as standard uptake value (SUV; tissue activity (MBq/g)/[injected dose (MBq)/body weight (g)]) and with standard deviation.*

	1 h p.i	STDEV	3 h p.i	STDEV	6 h p.i	STDEV
blood	11.76	0.55	10.10	0.73	7.94	0.13
bone	1.24	0.17	1.53	0.17	1.89	0.12
muscle	0.20	0.04	0.21	0.03	0.32	0.04
kidneys	2.74	0.09	3.07	0.22	3.85	0.17
liver	2.18	0.18	2.40	0.16	2.48	0.11
pancreas	0.71	0.09	0.65	0.12	0.99	0.11
lungs	3.55	1.42	3.95	0.85	3.08	0.76
spleen	1.85	0.17	1.97	0.22	2.52	0.07
heart	2.83	0.61	2.49	0.40	2.75	0.74

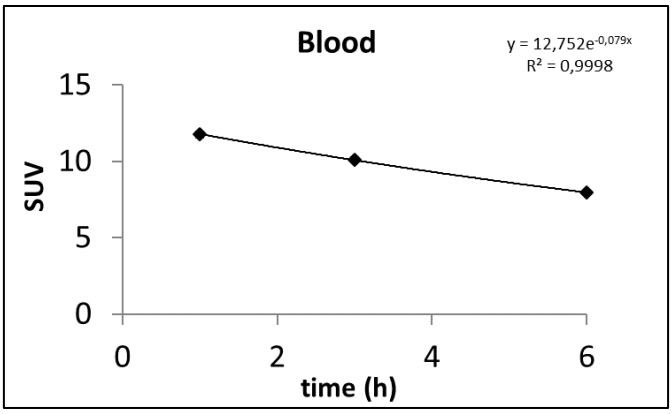

Figure S4: Blood data points of (±)-[^{18}F]AlF(RESCA)-HSA in healthy female Wistar rats. *Blood data points were fitted with a one- component exponential equation ($y = 12.75e^{(-0.08x)}$; $R^2 = 0.9998$), from which the blood biological half-life (Tb) was calculated to be 8.7 h.*

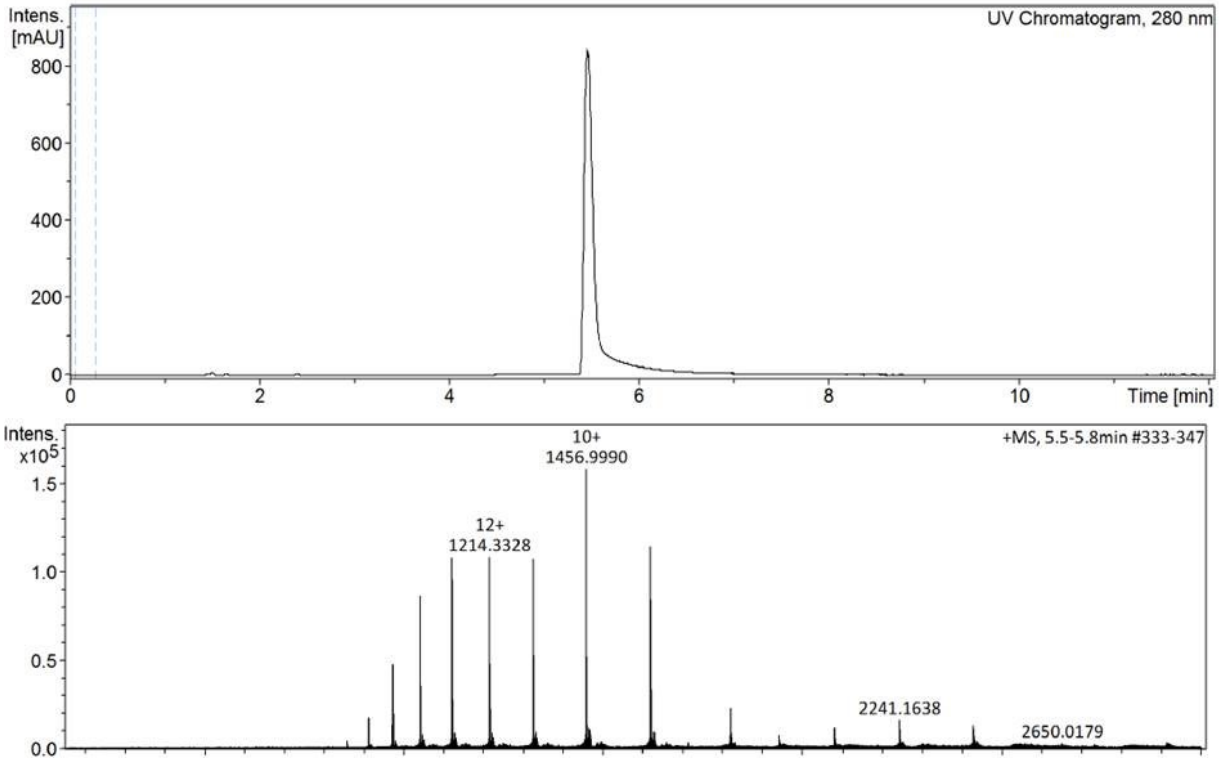

Figure S5: *(*A) RPLC-UV280 nm chromatogram of unlabeled NbV4m119 (Rt: 5.5 min) and (B) MS spectrum of unlabeled NbV4m119. *The theoretical average neutral molecular mass of the unlabeled NbV4m119 was 14577.85 Da [M] (Calculated for $C_{643}H_{950}N_{188}O_{196}S_4$) and the observed mass after deconvolution was 14577.61 ± 0.02 Da [M].*

qvqlvesggglvqaggslrlscaasgrtfssygmgwfrqapgkerefvaairwnggst

<----------FR1---------><-CDR1--><-------FR2------><-CDR2->

yyadsvkgrftisrdnakntvylqmnslkpedtavyycaagrwdkygssfqdeydy

<----------------FR3-----------------><-------CDR3----->

wgqgtqvtvssHHHHHH

<---FR4---><-HIS->

Fig S6: Deduced amino-acid sequence of NbV4m119, consisting of complementarity determining regions (CDR) sequences alternated with structural framework (FR) sequences and hexahistidine tag (HIS). *The CDR_1, CDR_2 and CDR_3 regions are indicated in boxes. The cysteines (structural cysteine 23(1st-CYS) and cysteine 104(2nd-CYS)) are highlighted in red. NbV4m119 possesses 5 lysine residues in its amino acid sequence (highlighted) in blue. The hexahistidine tag was inserted genetically for the purpose of immobilized metal ion affinity chromatography purification .*

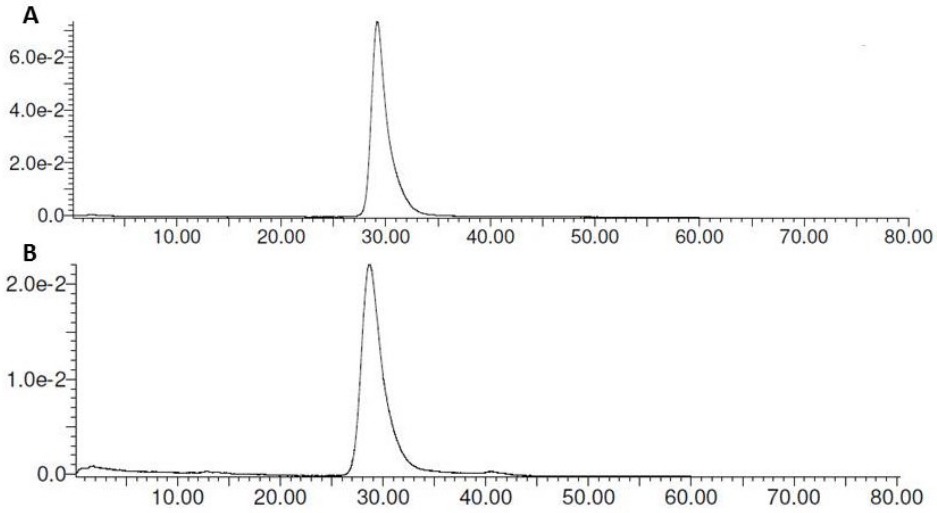

Figure S7: SEC-UV280 nm chromatogram of (A) NbV4m119 (Rt: 29.19 min) and (B) purified RESCA-NbV4m119 (Rt 28.68 min). *No aggregates or degradation products were observed.*

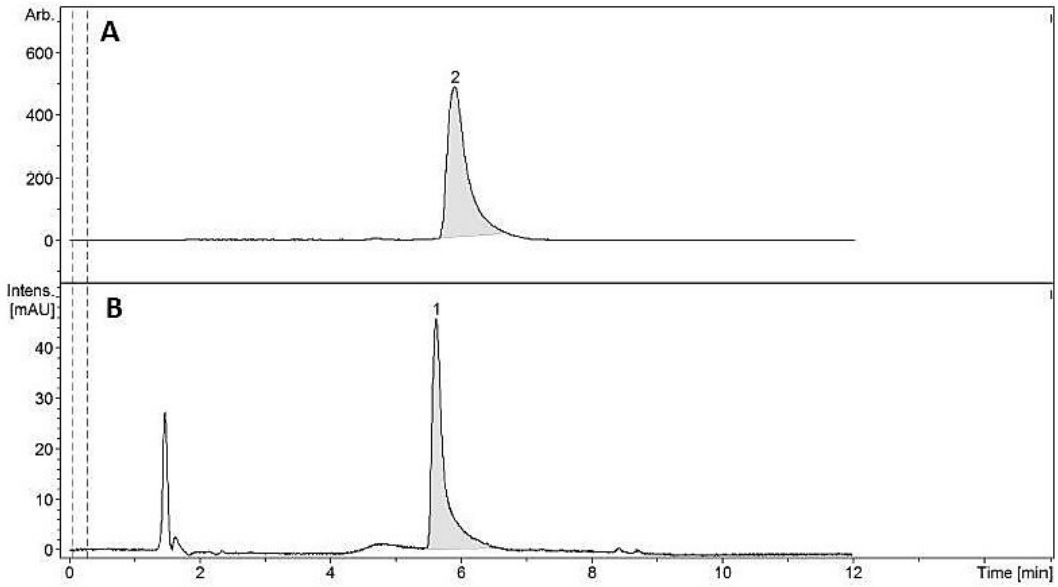

Figure S8: (A) radio-chromatogram of (±)-[^{18}F]AlF(RESCA)-NbV4m119 (Rt 5.9 min) and (B) RPLC-UV280 nm chromatogram of (±)-[^{18}F]AlF(RESCA)-NbV4m119 (Rt: 5.6 min).

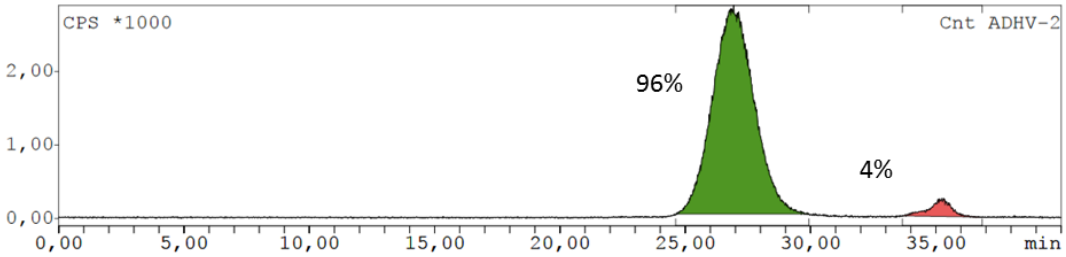

Figure S9: SEC-radio-chromatogram of (±)-[^{18}F]AlF(RESCA)-NbV4m119 (Rt 26.9 min) after 6 hours incubation in storage buffer. *468 MBq (±)-[^{18}F]AlF(RESCA)-NbV4m119 in 4 ml sodium phosphate buffer (0.01 M, pH 7.4, 0.14 M NaCl).*

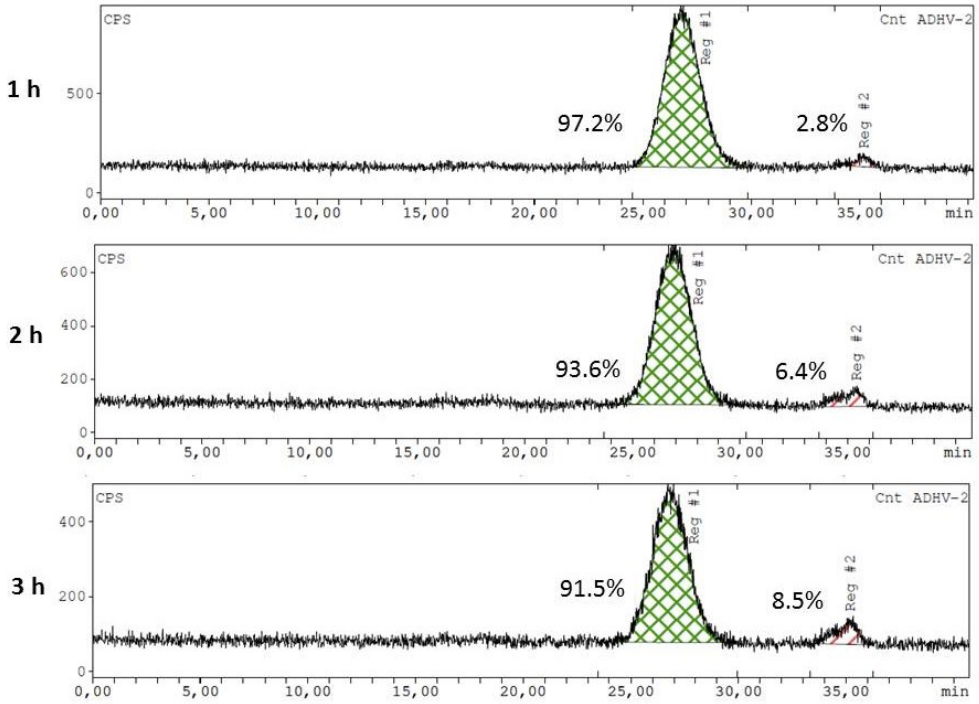

Figure S10: SEC-radio-chromatogram of (±)-[^{18}F]AlF(RESCA)-NbV4m119 (Rt 26.9 min) after 1, 2 and 3 hours incubation in rat plasma at 37°C. *After 3 h incubation in rat plasma at 37 °C, 91.5% of* (±)-[^{18}F]AlF(RESCA)-NbV4m119 *was still intact.*

Table S2: Biodistribution of (±)-[^{18}F]AlF(RESCA)-NbV4m119 in wild-type (WT) mice at 1 h and 3 h p.i. and in CRIg$^{-/-}$ mice at 3 h p.i.. *The results are presented as average standard uptake value (SUV; tissue activity (MBq/g)/[injected dose (MBq)/body weight (g)]) and with standard deviation, (n=3/group). **P < 0.005, ***P < 0.001*

	1 h p.i WT	STDEV	3 h p.i WT	STDEV	3 h p.i CRIg$^{-/-}$	STDEV
blood	0.20	0.02	0.13	0.02	0.05	0.01
bone	0.57	0.07	1.01	0.30	0.93	0.09
muscle	0.10	0.03	0.07	0.00	0.03	0.00
kidneys	37.63	4.76	8.89**	2.25	19.69**	2.37
liver	2.38	0.36	2.31***	0.15	0.27***	0.10
pancreas	0.21	0.08	0.12	0.04	0.04	0.02
lungs	2.23	0.26	0.83	0.90	0.69	0.47
spleen	0.29	0.06	0.22	0.13	0.28	0.20

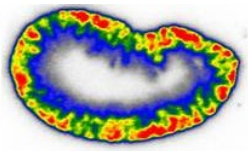

Figure S11: Ex vivo autoradiography on renal tissue of naïve WT mouse injected with (±)-[^{18}F]AlF(RESCA)-NbV4m119 and sacrificed at 1 h after i.v. injection

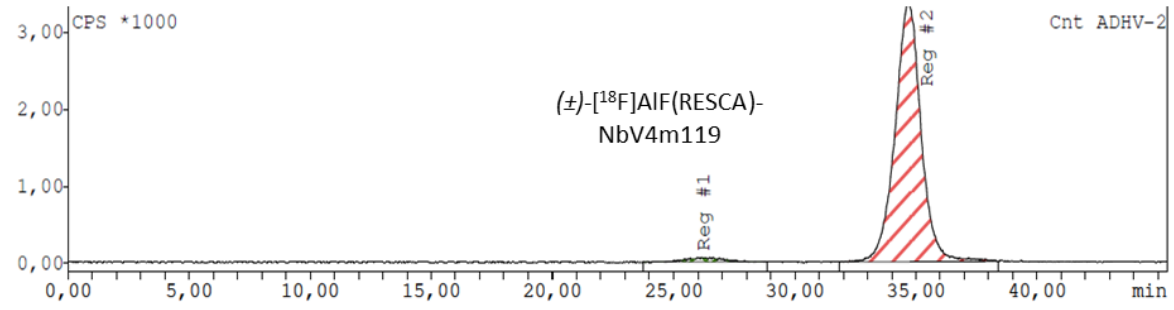

Figure S12: SEC-radio-chromatogram of urine sample of naïve WT mouse 3 h after i.v. injection of (±)-[^{18}F]AlF(RESCA)-NbV4m119. *Only 2.2% of (±)-[^{18}F]AlF(RESCA)-NbV4m119 (Rt 26.2 min) is still intact, the rest of activity (97.9%) elutes under the form of small ^{18}F-labeled degradation products,[^{18}F]F$^-$ and/or different Al^{18}F-species (Rt 34.7 min)*

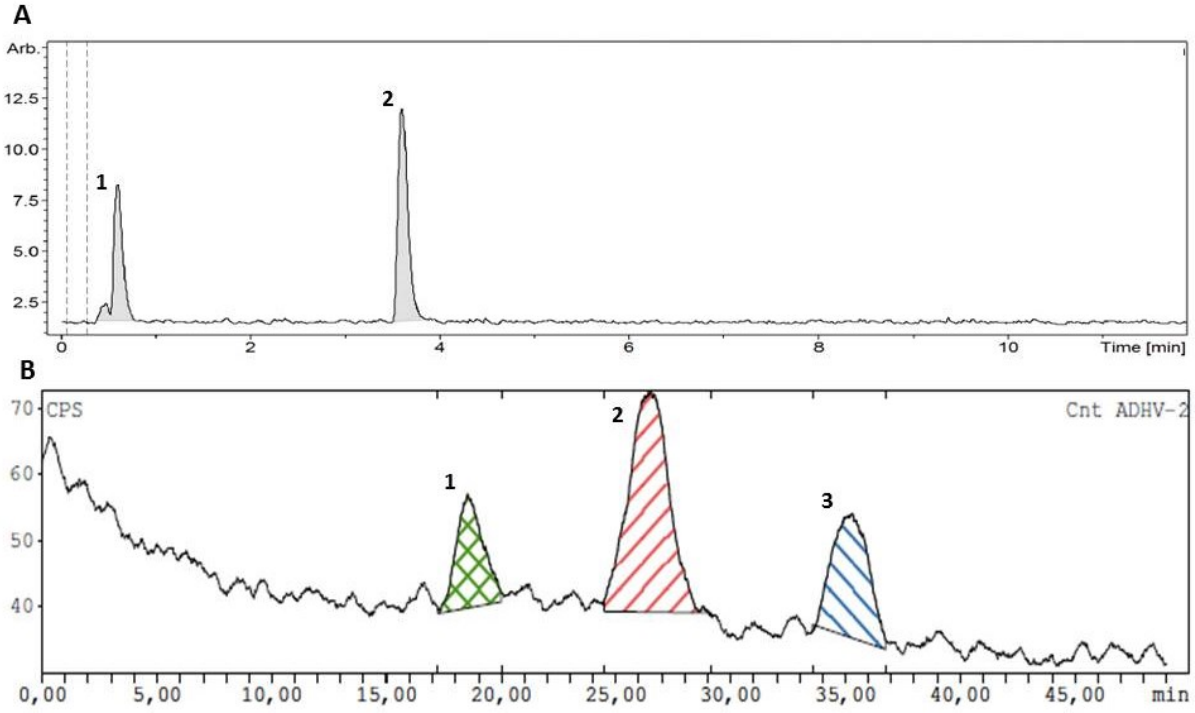

Figure S13: Analysis of metabolites in urine and plasma ***A:*** *RP-HPLC analysis of the radiometabolite fraction eluting at 34.7 min on size exclusion HPLC of urine obtained from naïve WT mouse 1 h after i.v. injection of (±)-[^{18}F]AlF(RESCA)-NbV4m119 (**Figure S12**). An Acquity UPLC BEH C18 column (1.7 µm, 2.1 mm x 150 mm, Waters) was used with following method: Solvent A (H_2O) and solvent B (acetonitrile), flow rate 0.3 ml/min. The elution gradient was: 0-1 min: 99% A; 1-7 min: from 99% A to 1% A; 7-10 min: 1% A; 10.1-12 min: 99% A. Two major peaks are observed: peak 1, (Rt 0.6 min, 40 %) and peak 2 (Rt 3.6 min, 60%). The radiometabolites eluting at the void volume (peak 1, 40%) are probably [^{18}F]{AlF}$^{2+}$,[^{18}F]F$^-$ and/or very polar fluorine-18 containing degradation fragments of (±)-[^{18}F]AlF(RESCA)-NbV4m119. The remaining fraction (60%) elutes later (peak 2) which indicates that this fraction consist of a somewhat more lipophilic radiometabolite of [^{18}F]AlF-RESCA-NbV4m119.* ***B:*** *SEC-radio-chromatogram of a plasma sample of naïve WT mouse 1 h after i.v. injection of [^{18}F]AlF-RESCA-NbV4m119. The tracer was partially bound to other proteins present in plasma (peak 1, Rt 18.4 min 19%), 54% of tracer was observed as intact (±)-[^{18}F]AlF(RESCA)-NbV4m119 (peak 2, Rt 26.8 min) and 27% of tracer was degraded in low molecular weight radiometabolites (peak 3, Rt 35.1 min). Studies with (±)-[^{18}F]AlF(RESCA)-HSA, which is not excreted by the kidneys due to the high molecular weight of albumin, confirms the high in vivo stability in blood of the Al^{18}F-RESCA complex because only minor increase in bone uptake was observed over time.*

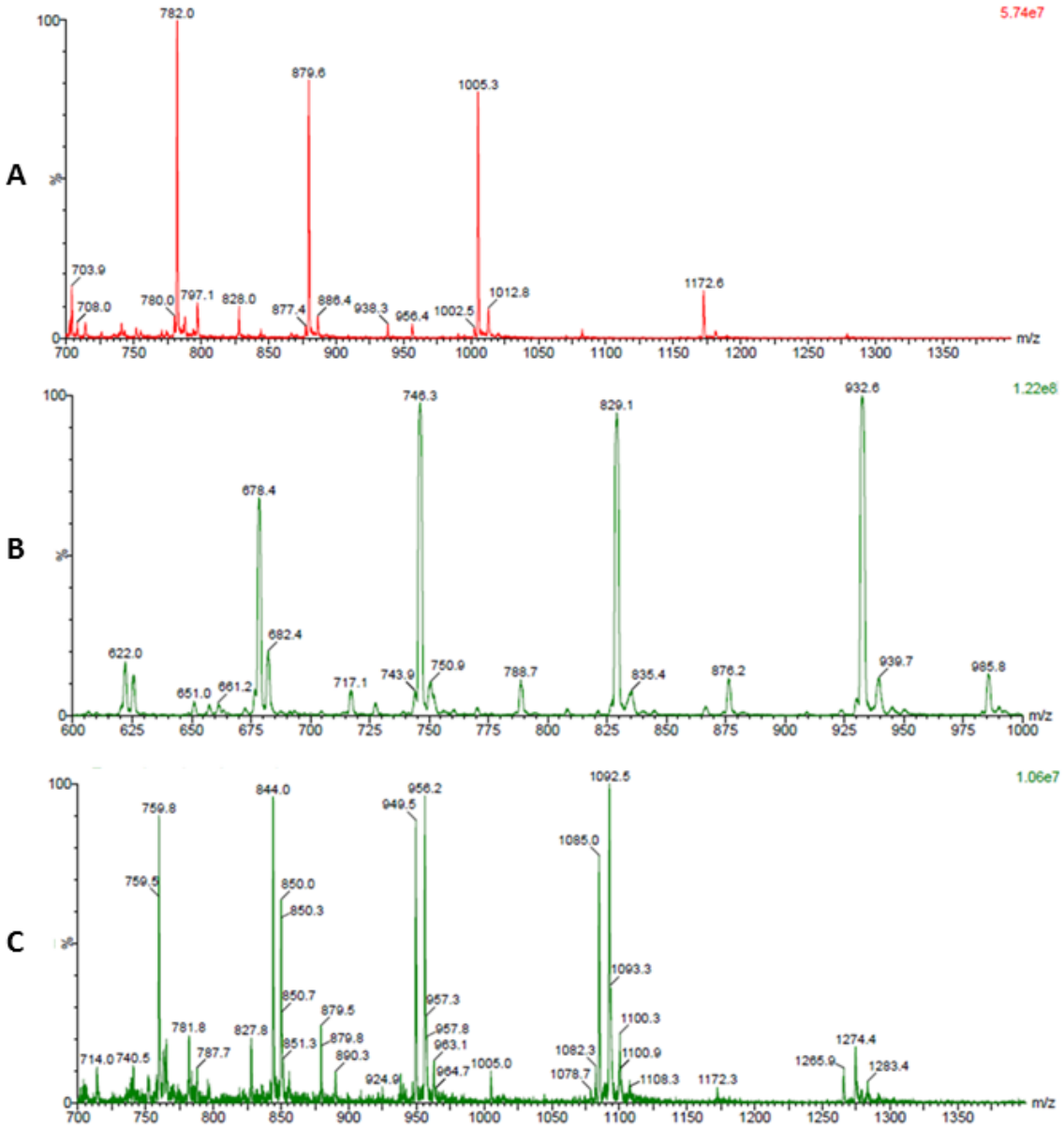

Figure S14: Mass Spectra of PEP04314. *MS spectra before **(A)** and after conjugation with maleimide-mono-amide-NOTA **(B)** and* (±)-H$_3$*RESCA-maleimide **(C)**. The observed molecular weight of PEP04314 was 7029.9 Da. The observed molecular weight of H$_2$NOTA-PEP04314 and* (±)-H$_3$*RESCA-PEP04314 was 7453 Da and 7588 Da, respectively. For compound* (±)-H$_3$*RESCA-PEP04314 we also observed a species with a molecular weight of 7640 Da, which is assumed to be the iron complex of* (±)-H$_3$*RESCA-PEP04314 (*(±)-Fe(*RESCA)-PEP04314).*

Table S3: $SUV_{120\text{-}180\ min}$ of (±)-[^{18}F]AlF(RESCA)-PEP04314 and [^{18}F]AlF(NOTA)-PEP04314. *Standardized uptake values 120-180 min after tracer administration ($SUV_{120\text{-}180\ min}$; tissue activity (MBq/cm^3)/[injected dose (MBq)/body weight (g)]) of (±)-[^{18}F]AlF(RESCA)-PEP04314 (n=3; n=1 in rhesus monkey A, n=1 in rhesus monkey B, n=1 in rhesus monkey C) and [^{18}F]AlF(NOTA)-PEP04314 (n=6; n=2 in rhesus monkey A, n=2 in rhesus monkey B, n=2 in rhesus monkey D). Regions of Interest (ROIs) were drawn for liver, kidney cortex, heart blood pool, salivary glands, bladder, lung, and muscle using both the PET and CT images to guide ROI placement. Bone ROIs were drawn on the lumbar spine.*

Rhesus monkey A

[^{18}F]AlF(NOTA)-PEP04314	Liver	Kidney Cortex	Heart Blood Pool	Bladder	Salivary Glands	Lung	Muscle	Bone
	8.0	70.1	1.0	4.6	3.7	0.3	0.1	1.6
	12.7	66.0	1.1	4.5	3.6	0.3	0.2	0.9

(±)-[^{18}F]AlF(RESCA)-PEP04314	Liver	Kidney Cortex	Heart Blood Pool	Bladder	Salivary Glands	Lung	Muscle	Bone
	10.6	36.5	2.6	21.0	4.9	0.5	0.3	2.4

Rhesus monkey B

[^{18}F]AlF(NOTA)-PEP04314	Liver	Kidney Cortex	Heart Blood Pool	Bladder	Salivary Glands	Lung	Muscle	Bone
	8.2	47.0	0.8	4.6	2.8	0.5	0.2	0.7
	8.0	63.6	0.9	6.9	3.1	0.4	0.2	0.8

(±)-[^{18}F]AlF(RESCA)-PEP04314	Liver	Kidney Cortex	Heart Blood Pool	Bladder	Salivary Glands	Lung	Muscle	Bone
	9.6	22.3	1.7	13.3	4.1	0.6	0.3	1.5

Rhesus monkey C

(±)-[^{18}F]AlF(RESCA)-PEP04314	Liver	Kidney Cortex	Heart Blood Pool	Bladder	Salivary Glands	Lung	Muscle	Bone
	8.2	38.5	2.0	9.2	4.8	0.7	0.2	2.9

Rhesus monkey D

[^{18}F]AlF(NOTA)-PEP04314	Liver	Kidney Cortex	Heart Blood Pool	Bladder	Salivary Glands	Lung	Muscle	Bone
	5.2	48.2	0.9	2.5	2.4	0.3	0.2	1.1
	7.2	45.6	0.8	3.6	3.5	0.3	0.2	0.7