Supporting Information

PET Imaging of Extracellular pH in Tumors with ^{64}Cu- and ^{18}F-Labeled pHLIP® Peptides: A Structure-Activity Optimization Study

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Methods & Materials

Radiolabeling

Labeling methods for ^{64}Cu-NOTA-pHLIP. Following a preparation similar to Zeglis *et al.*,[1] 3.13-11.26 mCi (116 – 417 MBq) of ^{64}Cu in 0.1 M HCl (2.5-6.0 µL, 1.86×10^4-1.25×10^4 mCi/µmol, Washington University, St. Louis, MO) was added to 150 µL of 100 mM NH_4OAc buffer (pH ~5.5). An aliquot (25-37 µL) of NOTA-pHLIP derivative (0.46-0.63 mM) in DMSO was added to the pH adjusted ^{64}Cu solution. The solution was placed on a thermomixer (Eppendorf, Hamberg, Germany) at 80 °C for 15 min at 1100 rpm. An aliquot of the crude product mixture was HPLC-analyzed (5-95 % acetonitrile in water (with 0.1 % trifluoroacetic acid) over 15 min, Jupiter C-18 column, Phenomenex, 250 mm × 4.6 mm, 5 µm, 300 Å) to ensure that product was present (^{64}Cu-NOTA-pHLIP eluted at 10.3 min; ^{64}Cu-NO2A-cyspHLIP

eluted at 13.1 min). The remainder of the reaction mixture was separated using a pretreated Oasis HLB Plus Light or C18 Sep-Pak Light cartridge (Waters, Milford, MA). Free and unbound ^{64}Cu was removed by washing the cartridge with 5 mL of water. The pure ^{64}Cu-labeled NOTA-pHLIP derivatives were then eluted with 1 mL ethanol in 0.1 mL fractions. The ethanolic fractions with the highest activities were combined and diluted with sterile phosphate buffered saline (PBS) to provide < 10 % ethanol in the solution before HPLC analysis (Jupiter C-18, 5 µm, 300 Å, 250 × 4.6 mm, Phenomenex; 5-95 % AcN in water with 0.1 % TFA over 15 min at 1 mL/min) to ensure that no free ^{64}Cu was present. The radiochemical purity of the species was always > 93 % and the specific activity (using the original amount of peptide added to the reaction mixture) was estimated to be 56.0-318.4 µCi/nmol (3.05-11.8 GBq/µmol). The reaction yielded 1.29-9.54 mCi (47.7-353 MBq) of product with an isolated yield of 40.7-84.7 %.

Labeling methods for ^{18}F-AlF-NOTA-pHLIP. Following a modified labeling method described by Dijkgraaf *et al.*,[2] 52.0-60.8 mCi (1.92-2.25 GBq) of ^{18}F target water (produced from ^{18}O enriched target water using a GEMS PETtrace-800 cyclotron, Memorial Sloan Kettering Cancer Center, New York, NY) was obtained. The target water was loaded onto a preconditioned chromafix cartridge (30-PS-HCO_3, Advanced Biochemical Compounds (ABX), Germany). [All of the preconditioning solvents and the elution solution were prepared with metal-free water and trace-metal free reagents.] The ^{18}F was eluted from the cartridge with approximately 100 µL of 0.4 M $KHCO_3$ in metal-free water into a 1.5 mL tube (ThermoFisher Scientific, Waltham, MA). The pH was adjusted to ~ 4.1 with metal-free acetic acid (Sigma Aldrich). To the pH adjusted solution, 100 µL of acetonitrile was added. If the total activity exceeded 50 mCi (1.85 GBq), the mixture was split between multiple 1.5 mL tubes prior to continuing (~ 10 mCi/40 nmol reaction). To the aliquotted mixture, 20 µL (40 nmol) of 2 mM

$AlCl_3$ in 0.1 M NH_4OAc buffer (pH ~ 4.1) was added to each tube. The solution was allowed to react for 5 min at room temperature before 14-17 µL of 4-5 mM NOTA-peptide in DMSO (68-75 nmol) was added, the tube capped, and the reaction mixture reacted at 75 °C for 15 minutes in a thermomixer. After reaction, the mixture was diluted with 2 mL of metal-free water. An aliquot of the reaction mixture was HPLC-analyzed (20-95 % acetonitrile in water (with 0.1 % trifluoroacetic acid) over 15 min with an Atlantis T3, Waters, 250 mm × 4.6 mm, 5 µm; Jupiter, Phenomenex, 250 mm x 4.6 mm, 5 µm, 300 Å, column) to ensure that product was formed (^{18}F-AlF-NOTA-WT eluted between 10.1 and 12.1 min; ^{18}F-AlF-NOTA-var3 eluted between 10.2 and 11.4 min; ^{18}F-AlF-NOTA-var7 eluted between 8.2 and 10.3 min; ^{18}F-AlF-NOTA-cysWT eluted between 13 and 16 min; ^{18}F-AlF-NOTA-cysVar3 eluted between 12 and 15 min; ^{18}F-AlF-NOTA-cysVar7 eluted between 12 and 14 min). While the HPLC analysis was being performed, the remainder of the reaction mixture was separated using a pretreated Oasis HLB Plus Light or C18 Sep-Pak Light cartridge (Waters, Milford, MA) eluting first with water (5 mL) to remove any unbound ^{18}F species and then ethanol (0.1 mL fractions for 4 fractions and then 0.6 mL). The ^{18}F-AlF-NOTA-pHLIP derivatives eluted within the first three fractions in ethanol. The ethanolic solution was diluted with sterile PBS to provide < 10 % ethanol in the solution before HPLC analysis (Jupiter C-18, 5 µm, 300 Å, 250 × 4.6 mm, Phenomenex; 5-95 % AcN in water with 0.1 % TFA over 15 min at 1 mL/min). The radiochemical purity of the species was always ≥ 95% and the specific activity (using the original amount of peptide added to the reaction mixture) was estimated to be 79.9-178.1 µCi/µmol (2.96-6.60 GBq/µmol). The reaction yielded 6.02-9.24 mCi (223-342 MBq) with an overall, decay-corrected (to beginning of labeling procedure) isolated yield of 2.33-48.6 %.

Nonradioactive standards

General labeling methods for Cu-NOTA-pHLIP: Approximately 200 µL of 36.4 M $Cu(OAc)_2$ in 0.1 M NH_4OAc (pH ~ 5) in metal-free water was added to 5-6 mg of peptide in approximately 200 µL of DMSO. The mixtures were reacted in a thermomixer at 50 °C for 1 h, and then filtered through a 0.2 µm filter. The filter was washed with an additional 1 mL of 50% AcN in water and 2 mL of water, sequentially. The combined filtrate was HPLC purified (10-95% AcN in water over 30 min with a Jupiter C-18 column, 5 µm, 300 Å, 250 × 4.6 mm, Phenomenex). The collected fraction (14.5-15.5 min for Cu-NOTA-WT, 13.9-15 min for Cu-NOTA-Var3, 13.2-14.1 min for Cu-NOTA-Var7, 12.2-13.5 min for Cu-NO2A-cysWT, 13-15.3 min for Cu-NO2A-cysVar3, and 12.5-16 min Cu-NO2A-cysVar7) was lyophilized.

General labeling methods for AlF-NOTA-pHLIP: Sodium fluoride (19.8 mg, 0.47 mmol) was dissolved in 150 µL of 0.1 M NH_4OAc in metal-free water (pH ~ 4.1). The resulting solution was added to 13.7 mg $AlCl_3$ (0.057 mmol) with 50 µL of 0.1 M NH_4OAc buffer (pH ~ 4.1). The AlF_3 solution (60 µL) was added to approximately 7 mg of peptide dissolved in 60 µL of EtOH. The reaction mixtures were reacted at 50 °C on a thermomixer for 1 h before being HPLC purified (10-95% AcN in water over 30 min with a Jupiter column, 5 µm, 300 Å, 250 × 4.6 mm, Phenomenex). The collected fraction (14.7-16.5 min for AlF-NOTA-WT, 13.8-15.4 min for AlF-NOTA-Var3, 13-14.6 min for AlF-NOTA-Var7, 12.5-17.5 min for AlF-NO2A-cysWT and AlF-NO2A-cysVar3, and 12-16 min AlF-NO2A-cysVar7) was lyophilized.

Cell culture methods and in vivo models

4T1 cell culture. The 4T1 cells, derived from spontaneous breast tumor in a BALB/c mouse, were provided by Fred Miller (Karmanos Cancer Institute, Detroit, MI) and cultured in Dulbecco's modified Eagle's high glucose media with 10 % FCS, 2 mM L-glutamine, penicillin, and streptomycin or from ATCC (Manassas, VA) and cultured in RPMI-1640 medium modified

to contain 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose, 1.5 g/L $NaHCO_3$, penicillin, and streptomycin.

4T1 orthotopic BALB/c mouse model. The cells were removed from the flasks, concentrated, and resuspended in minimal media for cell counting. The cells were then diluted to contain approximately 1 million cells in 30 µL of media (for orthotopic allografts). Following an MSKCC Institutional Animal Care and Use Committee approved protocol, 8-10 week old BALB/c mice (Charles River Laboratories, Wilmington, MA) were surgically implanted with one million 4T1 cancer cells into the first or the last mammary fat pad of the right side of the animal using aseptic surgical techniques and sterile staple closures while the mice were under anesthetic. Additionally, mice were given injections of meloxicam (24 h pain killer) in the scruff and bupivacaine intradermally prior to surgical incisions. One day post surgery, the mice were again given meloxicam and checked to ensure that the closure was healing. Two and three days post surgery, the mice were checked to ensure that the animals were healthy and recovering well from the surgery. Five to seven days post surgery, the staples were removed. Five to nine days post surgery, the mice were injected with 50-75 µCi (0.9-0.4 nmol) of the respective radiopharmaceutical for *in vivo* biodistribution or 500-600 µCi (9-11 nmol) for *in vivo* imaging. The mice receiving ^{64}Cu-NOTA-pHLIP were sacrificed at 1, 2, 4, 12, 24, 36, and 48 h; the mice receiving ^{18}F-AlF-NOTA-pHLIP were sacrificed at 0.5, 1, 2, 4, 6 and 8 h.

PC3 cell culture. The PC3 cells, prostate cancer derived from a human bone metastasis, were purchased from ATCC (Manassas, VA) and cultured in F-12K Medium (Kaighn's Modification of Ham's F-12 Medium) with, 10% FCS, 2 mM L-glutamine, 1.5 g/L $NaHCO_3$, penicillin, and streptomycin.

PC3 shoulder nude mouse xenografts. The cells were removed from the flasks, concentrated, and resuspended in minimal media for cell counting. The cells were then diluted to contain approximately 3 million cells in 150 µL of 1:1 media and matrigel (for shoulder xenografts). Following an MSKCC Institutional Animal Care and Use Committee approved protocol, 150 µL of the cell suspension was injected subcutaneously in 6-9 week old nude male mice (CRL). Mice were used in studies at 3 weeks post inoculation. The mice were injected with 50-75 µCi (0.9-0.4 nmol) of the respective radiopharmaceutical for *in vivo* biodistribution or 500-600 µCi (9-11 nmol) for *in vivo* imaging. The mice receiving ^{64}Cu-NOTA-cysVar3 were sacrificed at 1, 4, 12, 24, and 48 h; the mice receiving ^{18}F-AlF-NOTA-cysVar3 were sacrificed at 1, 4, 6 and 8 h.

LNCaP cell culture. The LNCaP cells, prostate cancer derived from a human supraclavicular lymph node metastasis, were purchased from ATCC (Manassas, VA) and cultured in RPMI-1640 medium modified to contain 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose, and 1.5 g/L $NaHCO_3$, penicillin, and streptomycin.

LNCaP shoulder nude mouse xenografts. The cells were removed from the flasks, concentrated, and resuspended in minimal media for cell counting. The cells were then diluted to contain approximately 3-6 million cells in 150 µL of 1:1 media and matrigel (or shoulder xenografts). Following an MSKCC Institutional Animal Care and Use Committee approved protocol, 150 µL of the cell suspension was injected subcutaneously in 6-8 week old nude male mice (CRL). Mice were used in studies at 3-8 weeks post inoculation. The mice were injected with 50-75 µCi (0.9-0.4 nmol) of the respective radiopharmaceutical for *in vivo* biodistribution or 500-600 µCi (9-11 nmol) for *in vivo* imaging. The mice receiving ^{64}Cu-NOTA-cysVar3 were

sacrificed at 1, 4, 12, 24, and 48 h; the mice receiving ^{18}F-AlF-NOTA-cysVar3 were sacrificed at 1, 4, 6 and 8 h.

B16-F10 cell culture. The B16-F10 cells, derived from spontaneous melanoma in a C57Bl/6 mouse, were purchased from ATCC (Manassas, VA) and cultured in Dubelco's modified Eagle's high glucose media with 10 % FCS, 4 mM L-glutamine, 1 mM sodium pyruvate, 1.5 g/L $NaHCO_3$, penicillin, and streptomycin.

Orthotopic B16-F10 shoulder allografts. The cells were removed from the flasks, concentrated, and resuspended in minimal media for cell counting. The cells were then diluted to contain approximately 100,000 cells in 100 µL of media (for shoulder allografts). Following an MSKCC Institutional Animal Care and Use Committee approved protocol, C57Bl/6 female mice (CRL) and BALB/c female mice (CRL) were injected with 100 µL of the cell suspension intradermally as per a media substituted protocol from Overwijk & Restifo (Current Protocols in Immunology, 2000: 20.1.1-20.1.29). The mice were used in studies at approximately 9-14 days post inoculation. The mice were injected with 50-75 µCi (0.9-0.4 nmol) of the respective radiopharmaceutical for *in vivo* biodistribution or 500-600 µCi (9-11 nmol) for *in vivo* imaging. The mice receiving ^{64}Cu-NOTA-cysVar3 were sacrificed at 1, 4, 12, 14.5, and 24 h; the mice receiving ^{18}F-AlF-NOTA-cysVar3 were sacrificed at 1, 4, 6 and 8 h.

U-87 MG cell culture. The human glioblastoma cell line, U-87 MG, was purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum, 2mM L-Glutamine, 1500mg/L Sodium Bicarbonate, 100 units/mL penicillin G and 100 µg/mL streptomycin (prepared by the MSKCC Media Preparation Core).

U-87 MG shoulder xenografts: The cells were removed from the flasks, concentrated, and resuspended in minimal media for cell counting. The cells were then diluted to contain approximately 1 million cells in 150 µL of 1:1 media and matrigel (or shoulder xenografts). Following an MSKCC Institutional Animal Care and Use Committee approved protocol, 150 µL of the cell suspension was injected subcutaneously in 12 week old nude male mice (CRL). Mice were used in studies at 2-3 weeks post inoculation. The mice were injected with 400-500 µCi (7-10 nmol) for in vivo imaging. The mice were sacrificed at 6 h after imaging (n=6).

Supporting Information Figures

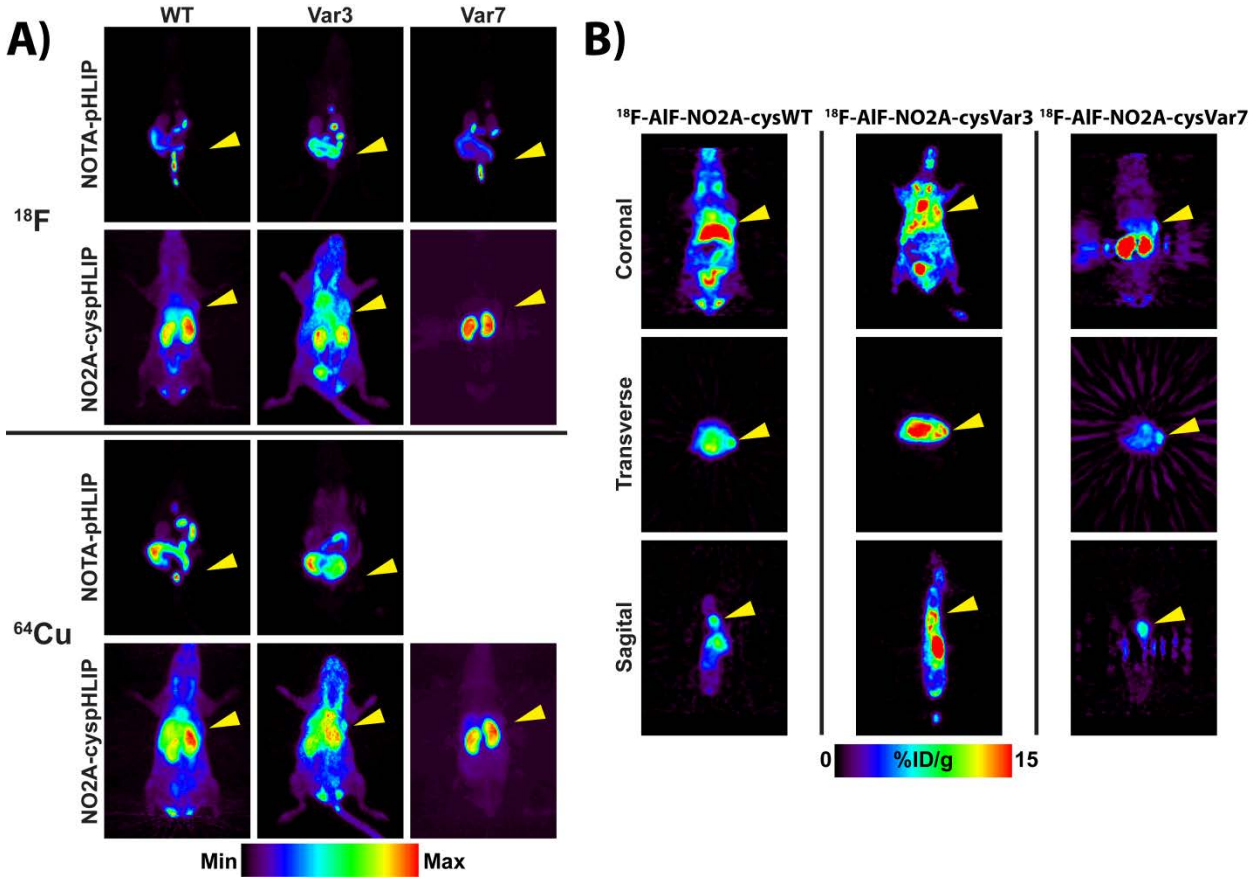


Fig. S1. PET Maximum intensity projection (MIP) showing differences in the tracer distribution at 4 h p.i. for all 12 tracers (A) and the PET imaging slices at 15 %ID/g at 4 h p.i. for the ^{18}F-AlF-cyspHLIP constructs (V) in BALB/c female mice bearing 4T1 tumor allografts. The yellow arrowheads indicate where the tumor is located in the mouse. The MIPs correspond to the same images shown in **Fig. 3**. The slices are the same as those shown in **Fig. 3**, just at 15 %ID/g. Only *ex vivo* biodistribution data was obtained for ^{64}Cu-NOTA-Var7.

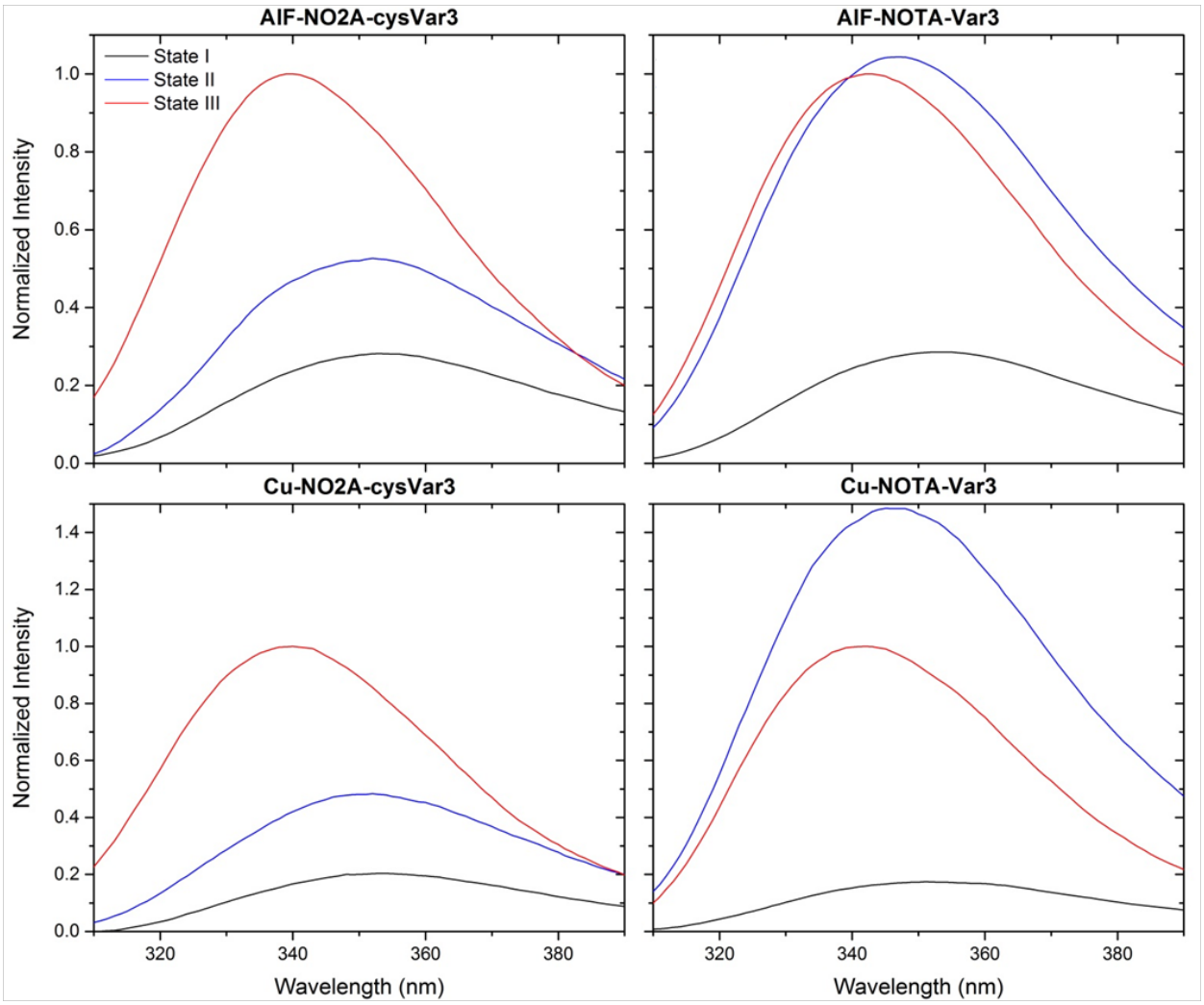


Fig. S2. Three states of NO2A-cysVar3 and NOTA-Var3 constructs. The constructs were examined under varying conditions for the presence of the three states of pHLIP® peptide: State I is the construct in solution at pH 8 (black lines), State II is the construct in the presence of POPC liposomes at pH 8 (blue lines), and State III is the construct folded and inserted in the lipid membrane when the pH is dropped from pH 8 to pH 4 by the addition of an aliquot of HCl (red lines). The concentrations of construct and lipids were 5 μM and 1 mM, respectively.

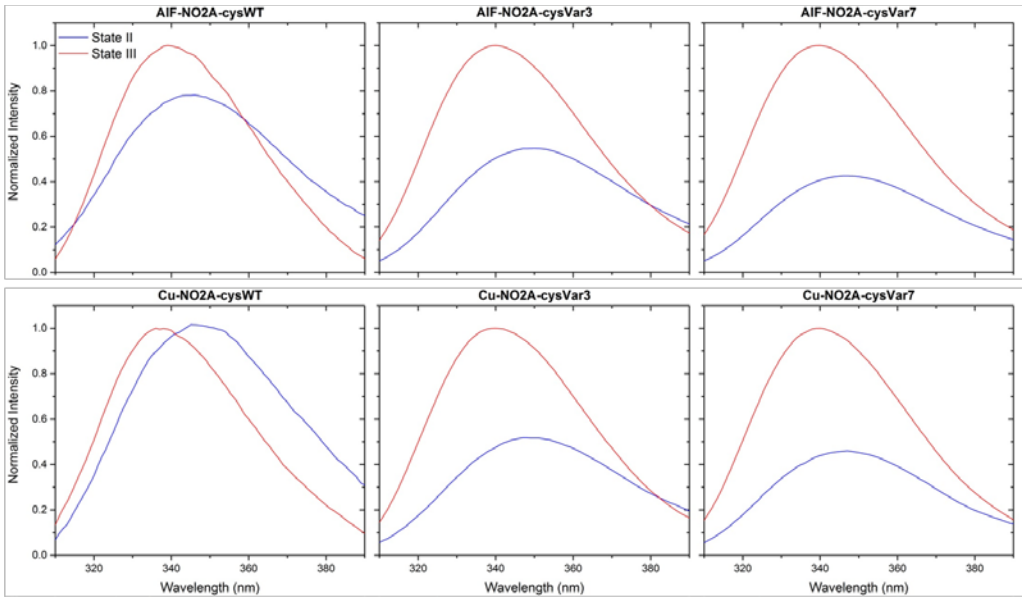


Fig. S3. State II and State III of NO2A-cyspHLIP constructs. The constructs were examined in the presence of liposomes under varying pH levels: State II is the construct in the presence of POPC liposomes at pH 8 (blue lines), State III is the construct folded and inserted in the lipid membrane when the pH is dropped from pH 8 to pH 4 by the addition of an aliquot of HCl (red lines). The concentrations of construct and lipids were 5 μM and 1 mM, respectively, with physiological levels of free magnesium and calcium ions (0.65 mM and 1.25 mM, respectively).

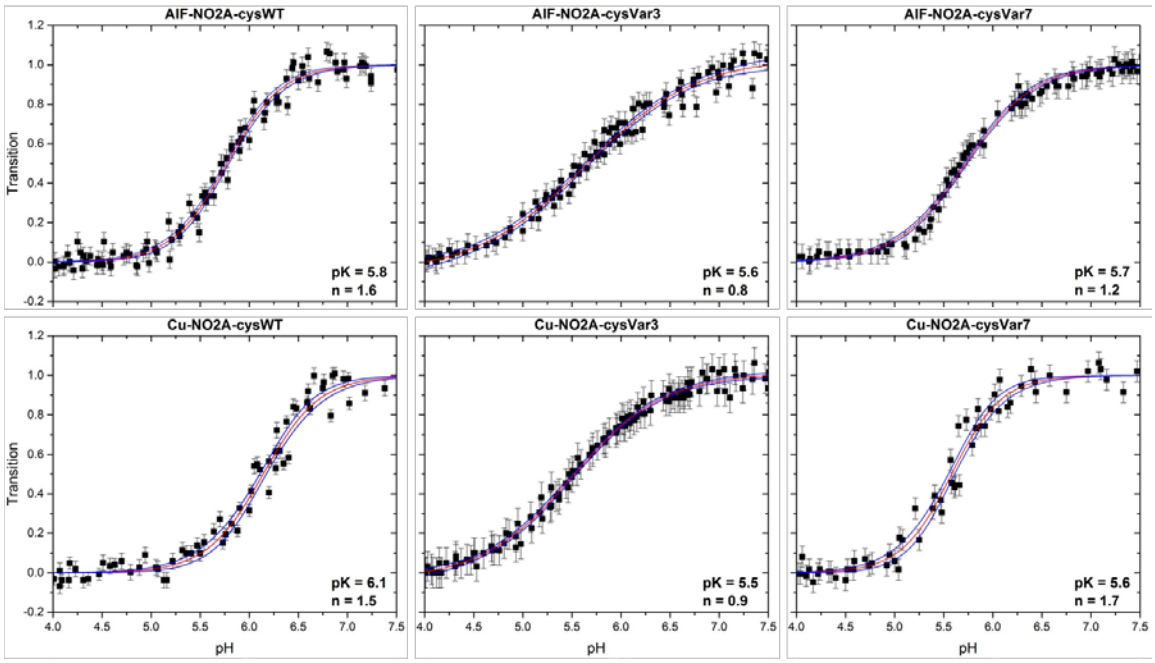


Fig. S4. pH dependent bilayer insertion of constructs. Changes in intrinsic construct fluorescence are used to measure the insertion of the construct population as a function of pH (transition from State II, at high pH, to State III, at low pH). Amount of construct population in State II is measured on the y-axis. The red and blue lines are fitting curves and 95% confidence intervals, respectively. These experiments were carried out with physiological levels of free magnesium and calcium ions (0.65 mM and 1.25 mM, respectively).

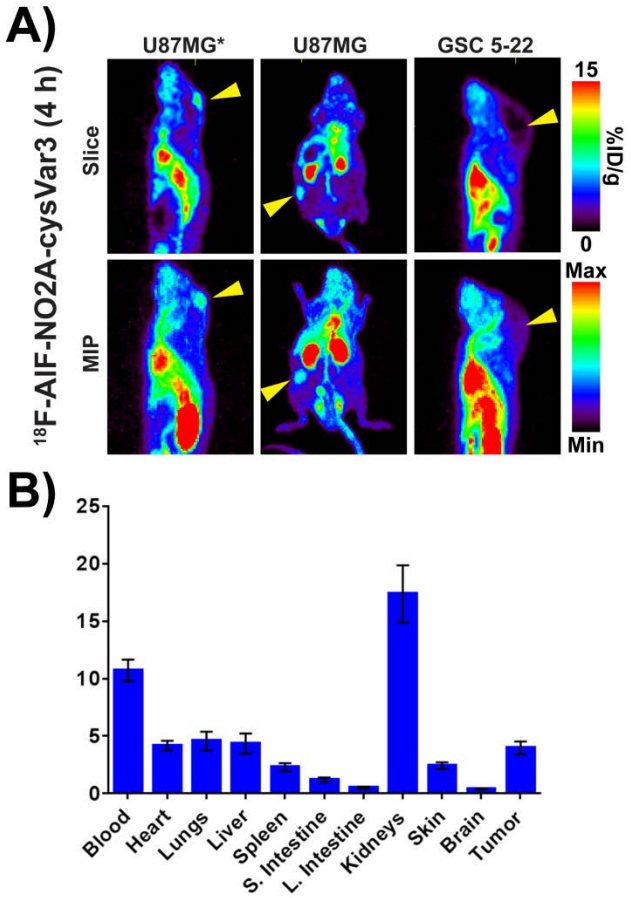


Fig. S5. Initial results from brain tumor uptake study. Images of the uptake of U87MG tumors xenografted in the skull (U87MG*) or flank (U87MG) on male nude mice and GSC 5-22 tumors orthotopically xenografted behind an intact BBB in IRC-SCID male mice at 4 h post injection (A). The U87MG* tumors did not infiltrate the brain and only the portion of the tumor that was in the skull bone and protruding from the skull showed uptake. The construct biodistribution of six male nude mice with flank U87MG xenografted tumors (B). The yellow arrowheads in (A) indicate where the tumor is located in the mouse.

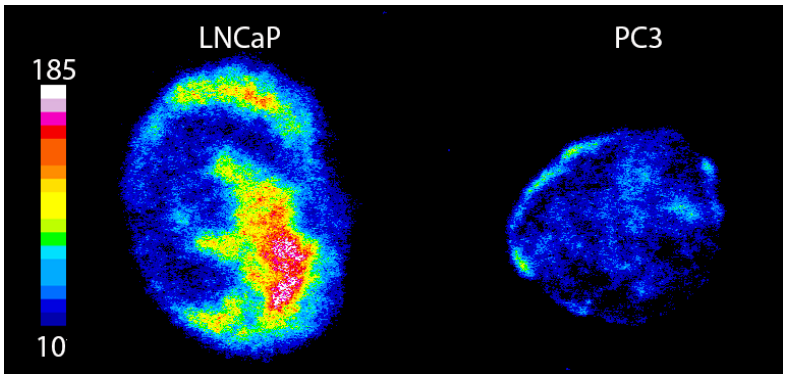


Fig. S6. Relative autoradiography of tumor slices from text **Fig. 6**. The slices show the overall difference in uptake of the tracer at 24 h p.i. Both of the slices were on the same autoradiography plate and, therefore, are relative to each other.

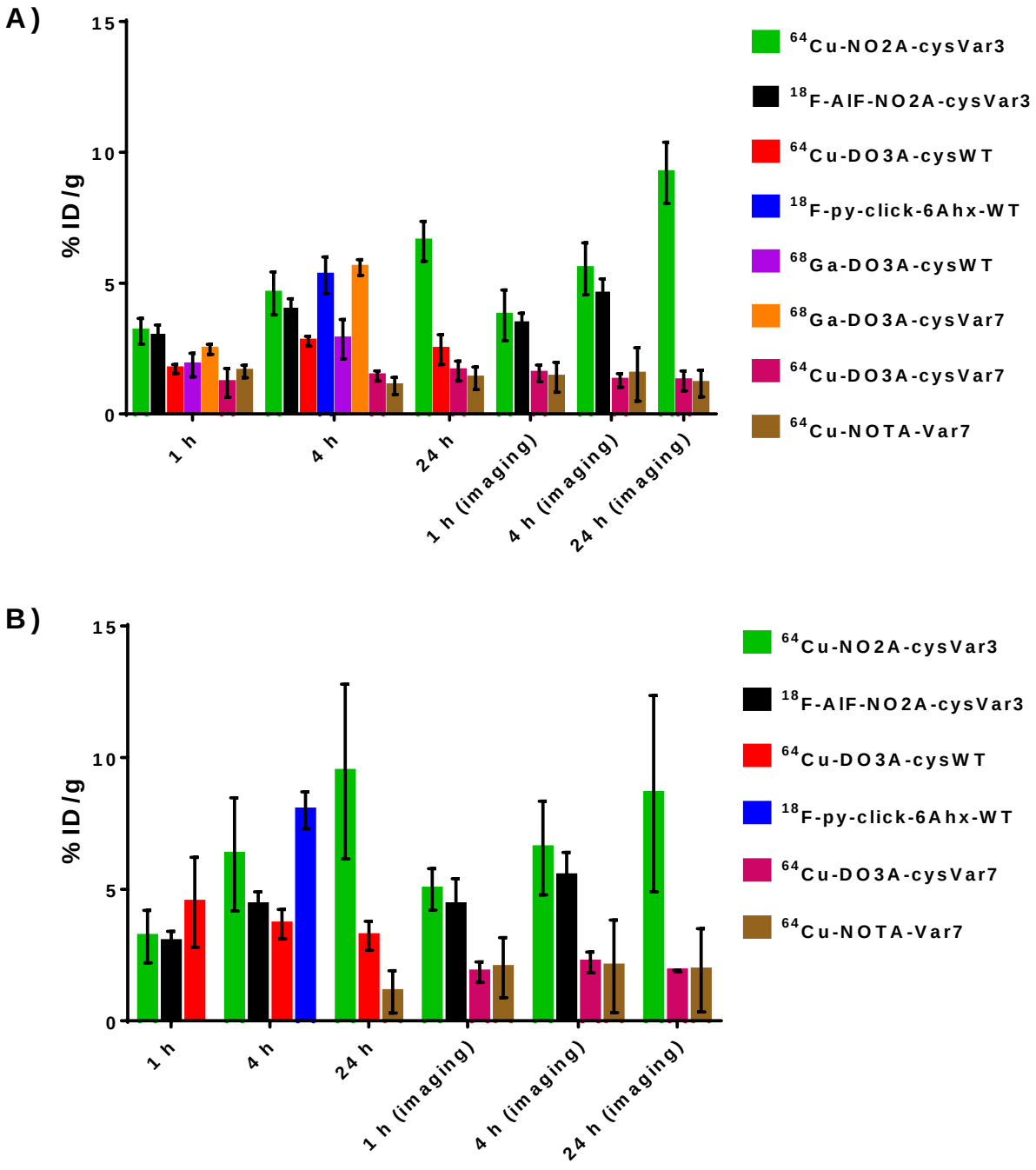


Fig. S7. Comparison of tumor uptake to previously reported studies for A) PC3 and B) LNCaP tumor bearing nude male mice at 1, 4, and 24 h with *ex vivo* biodistribution and *in vivo* imaging values. For imaging time points, ROIs were drawn on the coronal, sagittal, and transverse slices and the middle median value was tabulated for each mouse, the average of the four mice are shown with the standard deviation. Values from previous studies were used as reported.[3]

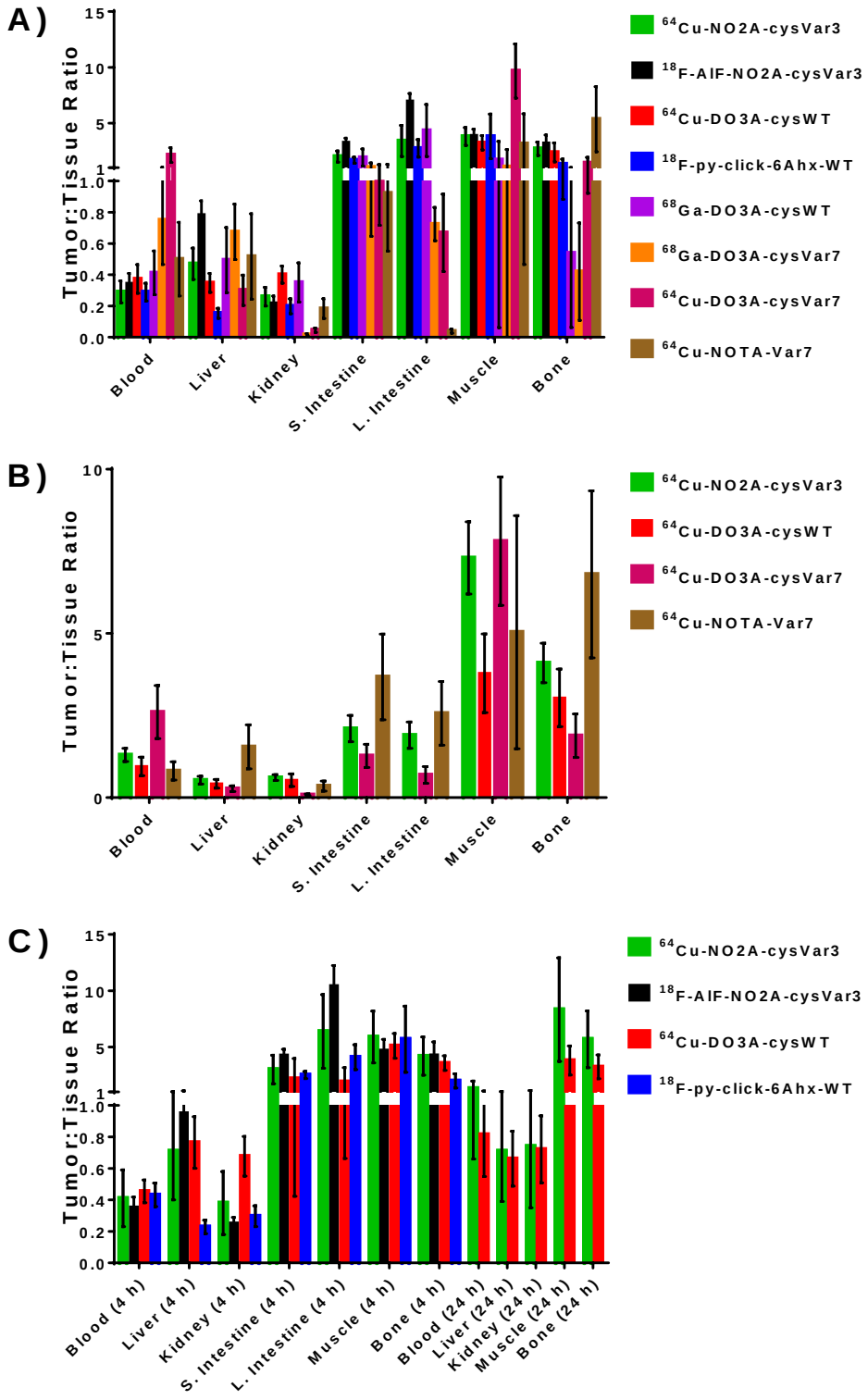


Fig. S8. Comparison of tumor:tissue ratios at 4 h and 24 h to previous studies: A) PC3 at 4 h; B) PC3 at 24 h; C) LNCaP at 4 and 24 h.

Supporting Information Tables

Table S1. General specific activities of tracers produced for this study.

Imaging Agent	Specific Activity (μCi/nmol)[a]	Specific Activity (μCi/nmol)[b]
^{64}Cu-NOTA-Var7	82.4	N/A
^{18}F-AlF-NOTA-Var7	79.9	N/A
^{64}Cu-NOTA-Var3	132.8[c]	N/A
^{18}F-AlF-NOTA-Var3	101.3	N/A
^{64}Cu-NOTA-WT	116.4	N/A
^{18}F-AlF-NOTA-WT	135.9	N/A
^{64}Cu-NO2A-cysVar7	113.7	219.6
^{18}F-AlF-NO2A-cysVar7	55.9	178.1
^{64}Cu-NO2A-cysVar3	45.5-523.3	47.5-340.2
^{18}F-AlF-NO2A-cysVar3	31.2-84.2	38.9-351.4
^{64}Cu-NO2A-cysWT	215.6	99.1
^{18}F-AlF-NO2A-cysWT	19.6	N/A

[a]Approximated from the amount of total peptide added.

[b]Calculated from the UV/vis standard concentration curve.

[c]In a separate experiment, a specific activity of 1656 μCi/nmol was attained, but the lower specific activity material was used in the studies in order to be comparable throughout.

Data for each tracer from this study in female, BALB/c mice with orthotopic 4T1 breast cancer allografts:

^{64}Cu-NOTA-WT

Table S1-1. Tissue uptake (mean %ID ± SD) of ^{64}Cu-NOTA-WT administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n = 4)	2 h (n = 4)	4 h (n = 4)	12 h (n = 4)	24 h (n = 3)
Blood	0.585 ± 0.291	0.413 ± 0.265	0.172 ± 0.047	0.533 ± 0.118	0.288 ± 0.119
Heart	0.057 ± 0.006	0.046 ± 0.006	0.048 ± 0.009	0.059 ± 0.009	0.040 ± 0.005
Lungs	0.359 ± 0.043	0.364 ± 0.049	0.314 ± 0.041	0.340 ± 0.074	0.236 ± 0.006
Liver	1.185 ± 0.298	0.991 ± 0.219	0.822 ± 0.150	0.858 ± 0.166	0.645 ± 0.024
Spleen	0.049 ± 0.009	0.039 ± 0.005	0.047 ± 0.007	0.052 ± 0.008	0.038 ± 0.004
Pancreas	0.041 ± 0.015	0.032 ± 0.005	0.044 ± 0.019	0.037 ± 0.006	0.031 ± 0.005
Stomach	0.282 ± 0.244	0.104 ± 0.028	0.121 ± 0.043	0.109 ± 0.019	0.074 ± 0.003
S. intestine	12.043 ± 2.341	1.684 ± 0.580	0.444 ± 0.050	0.376 ± 0.021	0.280 ± 0.010
L. intestine	2.486 ± 2.911	14.250 ± 1.086	8.510 ± 3.356	0.540 ± 0.138	0.291 ± 0.054
Kidneys	2.213 ± 0.110	1.849 ± 0.136	1.739 ± 0.183	1.434 ± 0.050	1.107 ± 0.094
Muscle	0.020 ± 0.005	0.017 ± 0.004	0.017 ± 0.004	0.018 ± 0.004	0.012 ± 0.004
Bone	0.012 ± 0.008	0.012 ± 0.004	0.014 ± 0.004	0.026 ± 0.015	0.012 ± 0.003
Skin	0.070 ± 0.023	0.080 ± 0.022	0.054 ± 0.007	0.095 ± 0.013	0.074 ± 0.012
Brain	0.033 ± 0.008	0.026 ± 0.005	0.022 ± 0.003	0.023 ± 0.001	0.017 ± 0.001
Tumor	0.101 ± 0.019	0.101 ± 0.012	0.136 ± 0.027	0.192 ± 0.071	0.198 ± 0.025

Table S1-2. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NOTA-WT administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n = 4)	2 h (n = 4)	4 h (n = 4)	12 h (n = 4)	24 h (n = 3)
Blood	1.529 ± 0.164	1.223 ± 0.255	1.192 ± 0.263	1.241 ± 0.225	0.862 ± 0.097
Heart	0.589 ± 0.061	0.476 ± 0.051	0.495 ± 0.042	0.519 ± 0.099	0.364 ± 0.036
Lungs	1.773 ± 0.161	1.470 ± 0.090	1.414 ± 0.083	1.252 ± 0.231	0.886 ± 0.106
Liver	1.496 ± 0.379	1.161 ± 0.231	0.904 ± 0.097	0.816 ± 0.180	0.637 ± 0.063
Spleen	0.348 ± 0.045	0.308 ± 0.021	0.318 ± 0.023	0.342 ± 0.050	0.272 ± 0.017
Pancreas	0.267 ± 0.082	0.223 ± 0.027	0.261 ± 0.066	0.266 ± 0.091	0.188 ± 0.031
Stomach	0.551 ± 0.264	0.352 ± 0.085	0.282 ± 0.080	0.302 ± 0.055	0.170 ± 0.023
S. intestine	11.075 ± 3.605	1.410 ± 0.447	0.391 ± 0.082	0.301 ± 0.026	0.198 ± 0.023
L. intestine	4.140 ± 4.791	20.733 ± 1.782	9.956 ± 3.415	0.648 ± 0.192	0.291 ± 0.063
Kidneys	8.326 ± 1.158	7.156 ± 0.725	6.232 ± 0.613	4.974 ± 0.766	3.527 ± 0.943
Muscle	0.191 ± 0.026	0.164 ± 0.026	0.166 ± 0.008	0.160 ± 0.019	0.124 ± 0.028
Bone	0.251 ± 0.063	0.234 ± 0.089	0.274 ± 0.051	0.348 ± 0.063	0.269 ± 0.042
Skin	0.700 ± 0.072	0.679 ± 0.102	0.715 ± 0.109	0.766 ± 0.116	0.512 ± 0.062
Brain	0.092 ± 0.012	0.069 ± 0.008	0.054 ± 0.002	0.057 ± 0.007	0.046 ± 0.003
Tumor	0.577 ± 0.074	0.574 ± 0.108	0.679 ± 0.025	0.904 ± 0.144	0.845 ± 0.127
Tumor-to-tissue ratios (rel. u.)					
Tumor/blood	0.377 ± 0.063	0.470 ± 0.132	0.569 ± 0.127	0.729 ± 0.176	0.980 ± 0.184
Tumor/liver	0.386 ± 0.110	0.494 ± 0.135	0.751 ± 0.085	1.108 ± 0.301	1.327 ± 0.239
Tumor/S. I.	0.052 ± 0.018	0.407 ± 0.150	1.737 ± 0.370	3.008 ± 0.543	4.264 ± 0.804
Tumor/L. I.	0.139 ± 0.162	0.028 ± 0.006	0.068 ± 0.024	1.395 ± 0.469	2.904 ± 0.767
Tumor/kidney	0.069 ± 0.013	0.080 ± 0.017	0.109 ± 0.011	0.182 ± 0.040	0.240 ± 0.073
Tumor/muscle	3.029 ± 0.565	3.509 ± 0.869	4.099 ± 0.255	5.665 ± 1.130	6.794 ± 1.832
Tumor/bone	2.301 ± 0.651	2.457 ± 1.048	2.473 ± 0.471	2.601 ± 0.626	3.146 ± 0.682

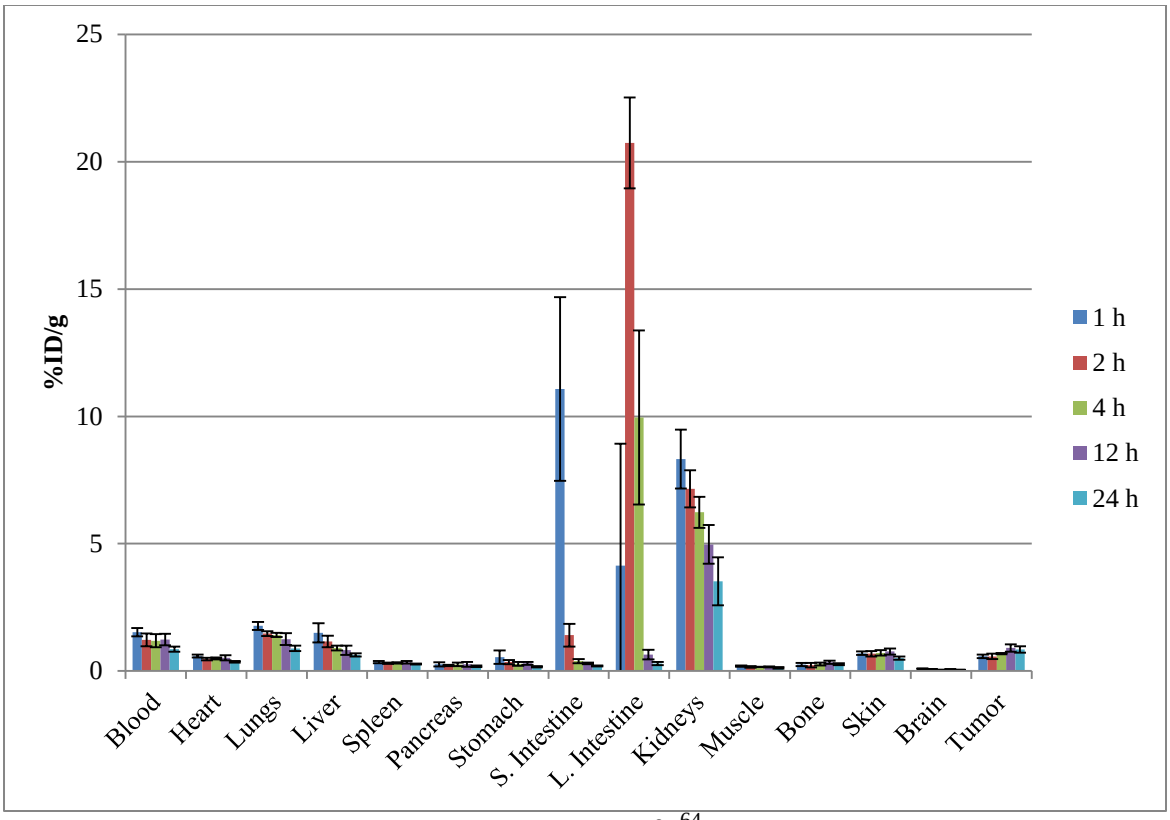
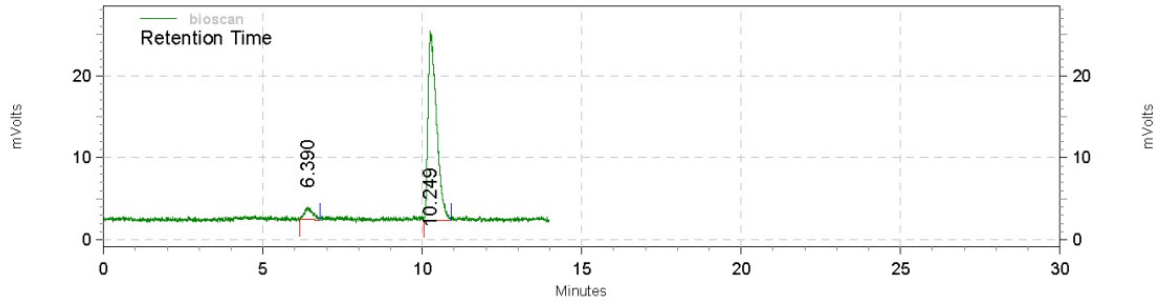


Fig. S1-1. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NOTA-WT (n = 4 (3 at 24 h)) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text.

HPLC Chromatogram of the Reconstituted ^{64}Cu-NOTA-WT Injectate

BIOSCAN



bioscan Results

Retention Time	Area	Area %	Height	Height %
6.390	25927	5.77	1475	6.01
10.249	423382	94.23	23073	93.99
Totals	449309	100.00	24548	100.00

^{64}Cu-NOTA-Var3

Table S2-1. Tissue uptake (mean %ID ± SD) of ^{64}Cu-NOTA-var3 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n = 5)	2 h (n = 5)	4 h (n = 5)	12 h (n = 5)	24 h (n = 4)
Blood	0.288 ± 0.059	0.271 ± 0.185	0.225 ± 0.122	0.232 ± 0.048	0.479 ± 0.214
Heart	0.057 ± 0.014	0.052 ± 0.007	0.047 ± 0.009	0.039 ± 0.004	0.041 ± 0.012
Lungs	0.420 ± 0.047	0.458 ± 0.058	0.388 ± 0.073	0.303 ± 0.067	0.276 ± 0.044
Liver	1.093 ± 0.224	0.892 ± 0.188	0.808 ± 0.081	0.713 ± 0.042	0.647 ± 0.140
Spleen	0.047 ± 0.008	0.048 ± 0.005	0.045 ± 0.002	0.050 ± 0.010	0.045 ± 0.006
Pancreas	0.052 ± 0.015	0.044 ± 0.021	0.045 ± 0.011	0.037 ± 0.009	0.041 ± 0.023
Stomach	0.092 ± 0.018	0.119 ± 0.041	0.111 ± 0.013	0.115 ± 0.025	0.096 ± 0.046
S. intestine	17.226 ± 2.329	1.364 ± 0.327	0.394 ± 0.075	0.335 ± 0.029	0.322 ± 0.063
L. intestine	1.859 ± 0.941	18.364 ± 3.419	5.350 ± 3.021	0.514 ± 0.128	0.342 ± 0.026
Kidneys	2.591 ± 0.170	2.141 ± 0.203	1.955 ± 0.280	1.454 ± 0.097	1.266 ± 0.178
Muscle	0.017 ± 0.007	0.020 ± 0.004	0.018 ± 0.001	0.014 ± 0.003	0.016 ± 0.006
Bone	0.016 ± 0.015	0.011 ± 0.006	0.022 ± 0.009	0.020 ± 0.008	0.008 ± 0.006
Skin	0.055 ± 0.016	0.062 ± 0.009	0.060 ± 0.013	0.056 ± 0.018	0.062 ± 0.016
Brain	0.033 ± 0.004	0.030 ± 0.007	0.022 ± 0.004	0.018 ± 0.002	0.017 ± 0.002
Tumor	0.110 ± 0.029	0.124 ± 0.024	0.145 ± 0.048	0.165 ± 0.029	0.196 ± 0.026

Table S2-2. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NOTA-Var3 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n = 5)	2 h (n = 5)	4 h (n = 5)	12 h (n = 5)	24 h (n = 4)
Blood	1.215 ± 0.223	1.226 ± 0.361	1.371 ± 0.244	1.113 ± 0.136	1.073 ± 0.223
Heart	0.509 ± 0.103	0.490 ± 0.067	0.489 ± 0.047	0.401 ± 0.052	0.404 ± 0.104
Lungs	1.516 ± 0.212	1.499 ± 0.231	1.420 ± 0.117	1.048 ± 0.180	1.079 ± 0.179
Liver	1.273 ± 0.109	1.073 ± 0.197	1.034 ± 0.150	0.631 ± 0.067	0.719 ± 0.125
Spleen	0.305 ± 0.047	0.333 ± 0.029	0.340 ± 0.032	0.289 ± 0.019	0.308 ± 0.055
Pancreas	0.291 ± 0.070	0.261 ± 0.057	0.279 ± 0.041	0.219 ± 0.029	0.203 ± 0.045
Stomach	0.267 ± 0.091	0.240 ± 0.054	0.194 ± 0.050	0.227 ± 0.074	0.250 ± 0.038
S. intestine	13.092 ± 2.311	1.034 ± 0.352	0.320 ± 0.042	0.263 ± 0.024	0.259 ± 0.043
L. intestine	1.988 ± 1.539	25.768 ± 5.740	7.449 ± 2.726	0.507 ± 0.065	0.395 ± 0.058
Kidneys	9.335 ± 0.826	7.715 ± 0.750	7.165 ± 1.126	4.735 ± 0.413	4.397 ± 0.874
Muscle	0.187 ± 0.019	0.198 ± 0.052	0.171 ± 0.017	0.143 ± 0.031	0.152 ± 0.051
Bone	0.216 ± 0.113	0.269 ± 0.078	0.339 ± 0.018	0.320 ± 0.070	0.220 ± 0.136
Skin	0.728 ± 0.205	0.720 ± 0.099	0.775 ± 0.102	0.699 ± 0.192	0.672 ± 0.187
Brain	0.087 ± 0.013	0.076 ± 0.018	0.063 ± 0.011	0.046 ± 0.004	0.042 ± 0.010
Tumor	0.515 ± 0.083	0.663 ± 0.167	0.649 ± 0.091	0.755 ± 0.056	0.926 ± 0.175
Tumor-to-tissue ratios (rel. u.)					
Tumor/Blood	0.42 ± 0.10	0.54 ± 0.21	0.47 ± 0.11	0.68 ± 0.10	0.86 ± 0.24
Tumor/Liver	0.40 ± 0.07	0.62 ± 0.19	0.63 ± 0.13	1.20 ± 0.15	1.29 ± 0.33
Tumor/S. I.	0.04 ± 0.01	0.64 ± 0.27	2.03 ± 0.39	2.87 ± 0.34	3.57 ± 0.90
Tumor/L. I.	0.26 ± 0.20	0.03 ± 0.01	0.09 ± 0.03	1.49 ± 0.22	2.35 ± 0.56
Tumor/Kidney	0.06 ± 0.01	0.09 ± 0.02	0.09 ± 0.02	0.16 ± 0.02	0.21 ± 0.06
Tumor/Muscle	2.75 ± 0.52	3.34 ± 1.22	3.80 ± 0.65	5.27 ± 1.22	6.11 ± 2.35
Tumor/Bone	2.39 ± 1.31	2.46 ± 0.94	1.92 ± 0.29	2.36 ± 0.55	4.21 ± 2.72

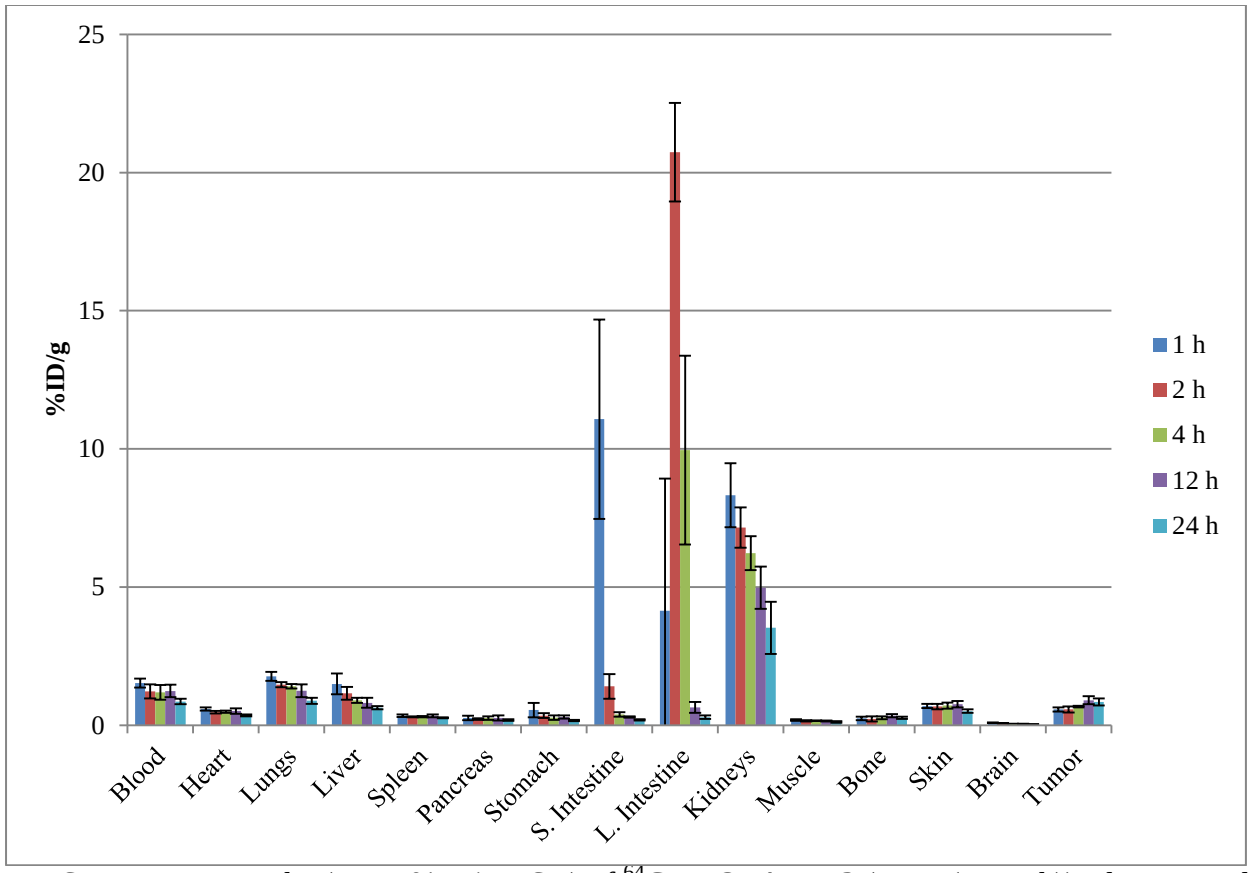
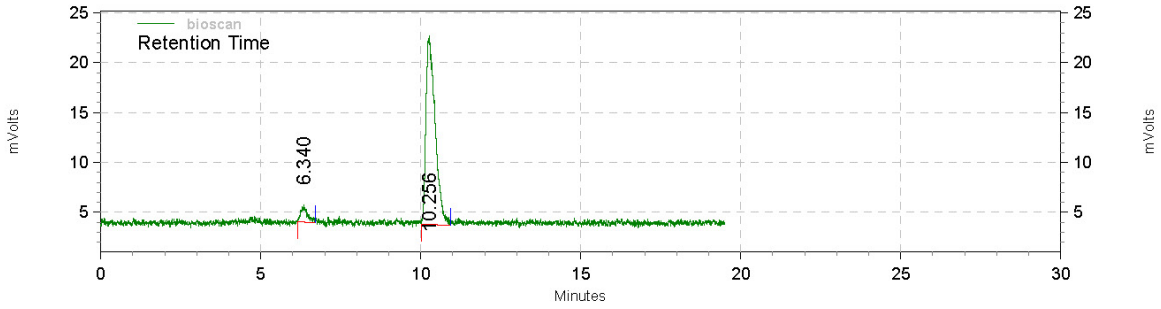


Fig. S2-1. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NOTA-Var3 (n = 5 (4 at 4 h)) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text.

HPLC Chromatogram of the Reconstituted ^{64}Cu-NOTA-Var3 Injectate

BIOSCAN



bioscan Results

Retention Time	Area	Area %	Height	Height %
6.340	23906	5.96	1735	8.39
10.256	377018	94.04	18950	91.61
Totals	400924	100.00	20685	100.00

^{64}Cu-NOTA-Var7

Table S3-1. Tissue uptake (mean %ID ± SD) of ^{64}Cu-NOTA-var7 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n = 4)	2 h (n = 4)	4 h (n = 4)	12 h (n = 4)	24 h (n = 4)
Blood	0.258 ± 0.212	0.228 ± 0.118	0.070 ± 0.033	0.074 ± 0.028	0.163 ± 0.116
Heart	0.037 ± 0.006	0.030 ± 0.004	0.035 ± 0.004	0.031 ± 0.009	0.028 ± 0.005
Lungs	0.252 ± 0.010	0.289 ± 0.074	0.267 ± 0.059	0.206 ± 0.059	0.142 ± 0.020
Liver	0.923 ± 0.229	0.640 ± 0.246	0.673 ± 0.038	0.396 ± 0.028	0.298 ± 0.050
Spleen	0.032 ± 0.004	0.024 ± 0.001	0.034 ± 0.004	0.034 ± 0.005	0.025 ± 0.002
Pancreas	0.023 ± 0.005	0.018 ± 0.006	0.032 ± 0.014	0.026 ± 0.009	0.018 ± 0.004
Stomach	0.081 ± 0.017	0.070 ± 0.024	0.133 ± 0.026	0.085 ± 0.006	0.052 ± 0.016
S. intestine	9.532 ± 1.902	0.832 ± 0.367	0.374 ± 0.103	0.233 ± 0.030	0.153 ± 0.026
L. intestine	0.300 ± 0.072	12.452 ± 1.188	5.608 ± 0.658	0.201 ± 0.026	0.265 ± 0.103
Kidneys	1.875 ± 0.173	1.679 ± 0.078	1.764 ± 0.059	1.067 ± 0.094	0.867 ± 0.124
Muscle	0.010 ± 0.005	0.015 ± 0.001	0.012 ± 0.006	0.008 ± 0.003	0.007 ± 0.002
Bone	0.003 ± 0.002	0.002 ± 0.001	0.011 ± 0.002	0.008 ± 0.003	0.004 ± 0.002
Skin	0.028 ± 0.014	0.041 ± 0.011	0.039 ± 0.004	0.043 ± 0.014	0.038 ± 0.005
Brain	0.030 ± 0.007	0.019 ± 0.002	0.016 ± 0.002	0.010 ± 0.004	0.011 ± 0.003
Tumor	0.074 ± 0.029	0.063 ± 0.017	0.094 ± 0.017	0.158 ± 0.066	0.161 ± 0.036

Table S3-2. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NOTA-Var7 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n = 4)	2 h (n = 4)	4 h (n = 4)	12 h (n = 4)	24 h (n = 4)
Blood	0.988 ± 0.172	0.804 ± 0.078	0.833 ± 0.068	0.759 ± 0.113	0.615 ± 0.083
Heart	0.333 ± 0.055	0.316 ± 0.042	0.353 ± 0.041	0.307 ± 0.036	0.282 ± 0.075
Lungs	1.094 ± 0.153	0.979 ± 0.104	0.996 ± 0.066	0.904 ± 0.088	0.666 ± 0.107
Liver	1.011 ± 0.090	0.915 ± 0.152	0.739 ± 0.041	0.479 ± 0.038	0.449 ± 0.091
Spleen	0.217 ± 0.036	0.215 ± 0.020	0.279 ± 0.016	0.213 ± 0.031	0.175 ± 0.030
Pancreas	0.182 ± 0.040	0.150 ± 0.032	0.198 ± 0.043	0.182 ± 0.029	0.122 ± 0.019
Stomach	0.324 ± 0.062	0.299 ± 0.107	0.300 ± 0.076	0.078 ± 0.015	0.200 ± 0.070
S. intestine	9.167 ± 2.676	0.935 ± 0.329	0.307 ± 0.077	0.158 ± 0.012	0.179 ± 0.044
L. intestine	0.461 ± 0.081	18.811 ± 2.211	7.782 ± 0.609	0.255 ± 0.043	0.368 ± 0.098
Kidneys	6.760 ± 0.950	6.065 ± 0.619	6.790 ± 0.461	4.143 ± 0.512	3.410 ± 0.525
Muscle	0.123 ± 0.018	0.141 ± 0.018	0.118 ± 0.025	0.104 ± 0.016	0.101 ± 0.020
Bone	0.141 ± 0.055	0.099 ± 0.053	0.229 ± 0.093	0.247 ± 0.039	0.136 ± 0.064
Skin	0.490 ± 0.053	0.497 ± 0.060	0.563 ± 0.145	0.566 ± 0.092	0.510 ± 0.120
Brain	0.071 ± 0.016	0.053 ± 0.003	0.042 ± 0.004	0.032 ± 0.007	0.032 ± 0.005
Tumor	0.366 ± 0.034	0.414 ± 0.037	0.546 ± 0.075	0.673 ± 0.078	0.693 ± 0.040
Tumor-to-tissue ratios (rel. u.)					
Tumor/Blood	0.371 ± 0.073	0.515 ± 0.068	0.655 ± 0.105	0.886 ± 0.168	1.126 ± 0.165
Tumor/Liver	0.362 ± 0.047	0.452 ± 0.086	0.738 ± 0.110	1.403 ± 0.198	1.542 ± 0.325
Tumor/S. I.	0.040 ± 0.012	0.443 ± 0.161	1.780 ± 0.510	4.259 ± 0.599	3.878 ± 0.972
Tumor/L. I.	0.794 ± 0.158	0.022 ± 0.003	0.070 ± 0.011	2.642 ± 0.540	1.883 ± 0.514
Tumor/Kidney	0.054 ± 0.009	0.068 ± 0.009	0.080 ± 0.012	0.162 ± 0.028	0.203 ± 0.033
Tumor/Muscle	2.966 ± 0.518	2.947 ± 0.465	4.618 ± 1.167	6.444 ± 1.231	6.878 ± 1.430
Tumor/Bone	2.598 ± 1.040	4.193 ± 2.268	2.388 ± 1.030	2.727 ± 0.536	5.087 ± 2.404

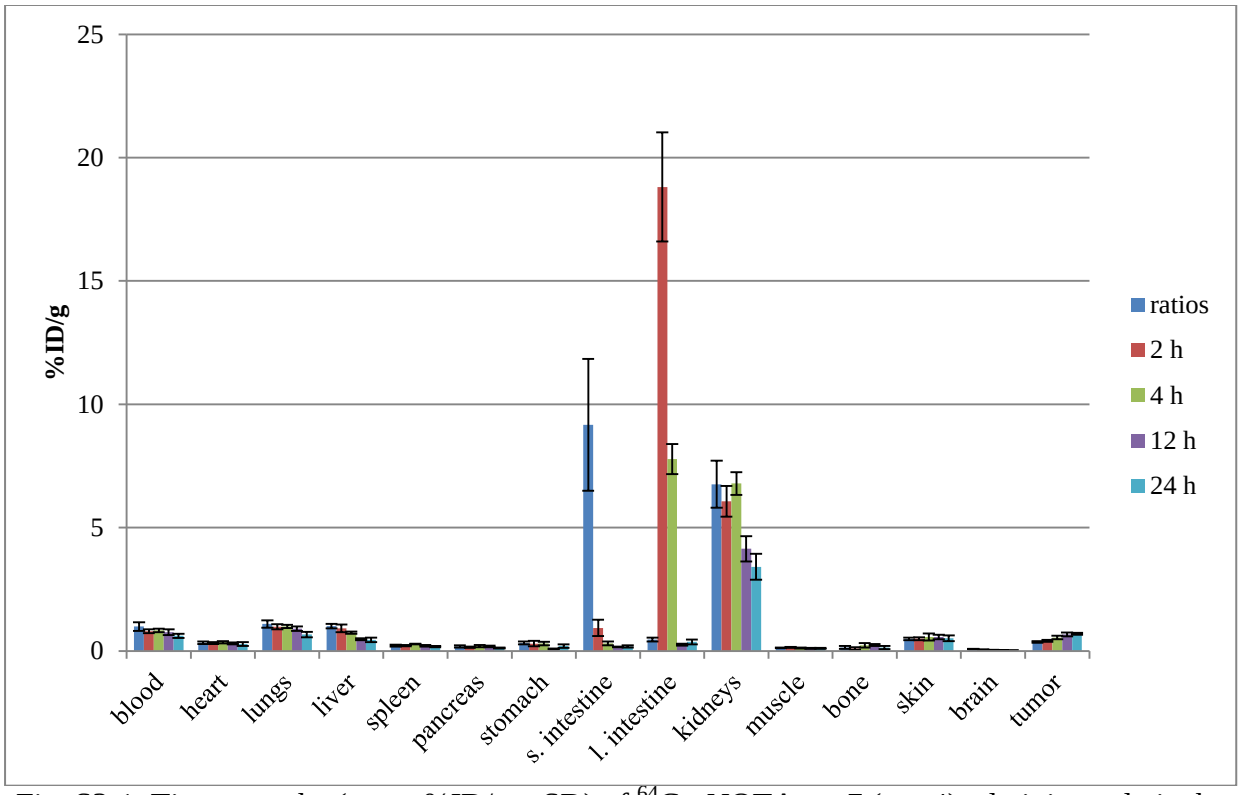
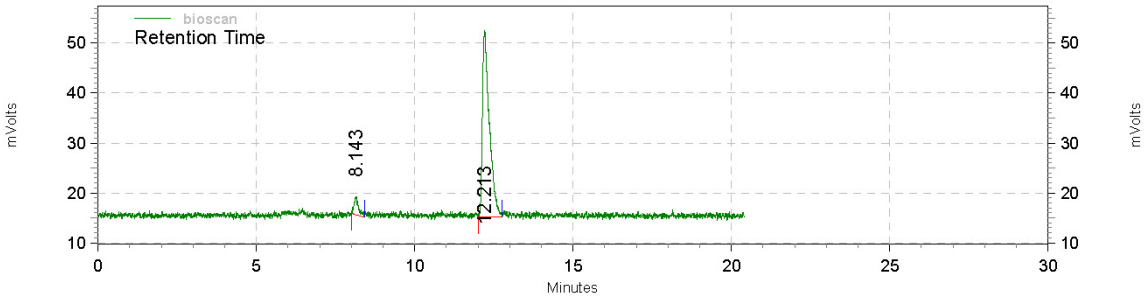


Fig. S3-1. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NOTA-var7 (n = 4) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text.

HPLC Chromatogram of the Reconstituted ^{64}Cu-NOTA-Var7 Injectate

BIOSCAN



bioscan Results

Retention Time	Area	Area %	Height	Height %
8.143	36720	6.07	3580	8.72
12.213	567879	93.93	37462	91.28
Totals	604599	100.00	41042	100.00

^{18}F-AlF-NOTA-WT

Table S4-1. Tissue uptake (mean %ID ± SD) of ^{18}F-AlF-NOTA-WT administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n = 5)	1 h (n = 5)	2 h (n = 5)	4 h (n = 5)
Blood	1.319 ± 0.188	1.112 ± 0.131	0.625 ± 0.423	0.637 ± 0.462
Heart	0.093 ± 0.015	0.103 ± 0.031	0.092 ± 0.025	0.061 ± 0.014
Lungs	0.525 ± 0.082	0.533 ± 0.125	0.468 ± 0.114	0.485 ± 0.062
Liver	1.681 ± 0.216	1.218 ± 0.260	1.238 ± 0.281	0.794 ± 0.142
Spleen	0.069 ± 0.009	0.074 ± 0.010	0.078 ± 0.006	0.068 ± 0.014
Pancreas	0.049 ± 0.018	0.043 ± 0.008	0.049 ± 0.009	0.044 ± 0.009
Stomach	0.108 ± 0.006	0.129 ± 0.033	0.118 ± 0.009	0.141 ± 0.027
S. intestine	12.837 ± 0.988	13.737 ± 1.399	1.120 ± 0.148	0.738 ± 0.243
L. intestine	0.197 ± 0.039	0.266 ± 0.079	16.243 ± 1.989	16.258 ± 1.370
Kidneys	5.518 ± 0.849	3.471 ± 0.123	2.861 ± 0.052	2.289 ± 0.250
Muscle	0.040 ± 0.007	0.038 ± 0.005	0.036 ± 0.008	0.031 ± 0.011
Bone	0.025 ± 0.011	0.013 ± 0.003	0.034 ± 0.018	0.046 ± 0.004
Skin	0.114 ± 0.051	0.129 ± 0.035	0.099 ± 0.029	0.105 ± 0.003
Brain	0.087 ± 0.007	0.063 ± 0.009	0.052 ± 0.006	0.039 ± 0.008
Tumor	0.159 ± 0.045	0.163 ± 0.042	0.175 ± 0.028	0.187 ± 0.060

Table S4-2. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NOTA-WT administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n = 5)	1 h (n = 5)	2 h (n = 5)	4 h (n = 5)
Blood	2.305 ± 0.308	2.034 ± 0.288	2.096 ± 0.250	1.978 ± 0.192
Heart	0.919 ± 0.105	0.884 ± 0.167	0.856 ± 0.091	0.664 ± 0.119
Lungs	2.353 ± 0.250	2.288 ± 0.072	2.186 ± 0.273	2.049 ± 0.211
Liver	1.964 ± 0.473	1.163 ± 0.166	1.158 ± 0.189	0.973 ± 0.213
Spleen	0.482 ± 0.080	0.423 ± 0.042	0.418 ± 0.046	0.434 ± 0.058
Pancreas	0.359 ± 0.089	0.318 ± 0.018	0.355 ± 0.062	0.324 ± 0.059
Stomach	0.406 ± 0.114	0.364 ± 0.087	0.442 ± 0.077	0.518 ± 0.152
S. intestine	12.969 ± 1.568	13.510 ± 0.999	1.080 ± 0.198	0.820 ± 0.245
L. intestine	0.264 ± 0.037	0.368 ± 0.126	24.375 ± 2.182	24.064 ± 2.848
Kidneys	18.931 ± 3.456	12.308 ± 0.760	10.247 ± 0.914	8.550 ± 1.138
Muscle	0.360 ± 0.070	0.315 ± 0.034	0.349 ± 0.073	0.283 ± 0.041
Bone	0.635 ± 0.181	0.627 ± 0.185	0.702 ± 0.166	0.671 ± 0.305
Skin	1.256 ± 0.240	1.138 ± 0.093	1.027 ± 0.178	1.032 ± 0.133
Brain	0.218 ± 0.018	0.165 ± 0.015	0.135 ± 0.009	0.100 ± 0.015
Tumor	0.995 ± 0.242	0.930 ± 0.129	0.944 ± 0.142	1.005 ± 0.232
Tumor-to-tissue ratios (rel. u.)				
Tumor/Blood	0.43 ± 0.12	0.46 ± 0.09	0.45 ± 0.09	0.51 ± 0.13
Tumor/Liver	0.51 ± 0.17	0.80 ± 0.16	0.82 ± 0.18	1.03 ± 0.33
Tumor/S. I.	0.08 ± 0.02	0.07 ± 0.01	0.87 ± 0.21	1.22 ± 0.46
Tumor/L. I.	3.77 ± 1.06	2.52 ± 0.93	0.04 ± 0.01	0.04 ± 0.01
Tumor/Kidney	0.05 ± 0.02	0.08 ± 0.01	0.092 ± 0.016	0.12 ± 0.03
Tumor/Muscle	2.76 ± 0.86	2.96 ± 0.52	2.70 ± 0.70	3.54 ± 0.97
Tumor/Bone	1.57 ± 0.59	1.48 ± 0.48	1.345 ± 0.377	1.50 ± 0.76

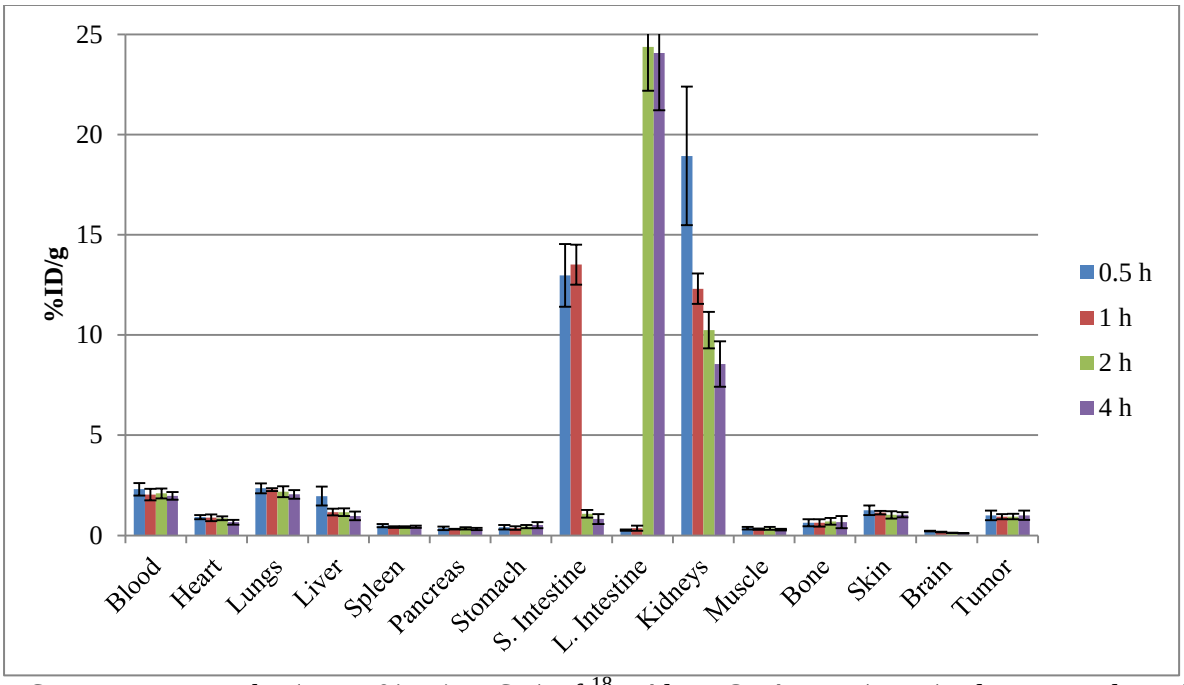
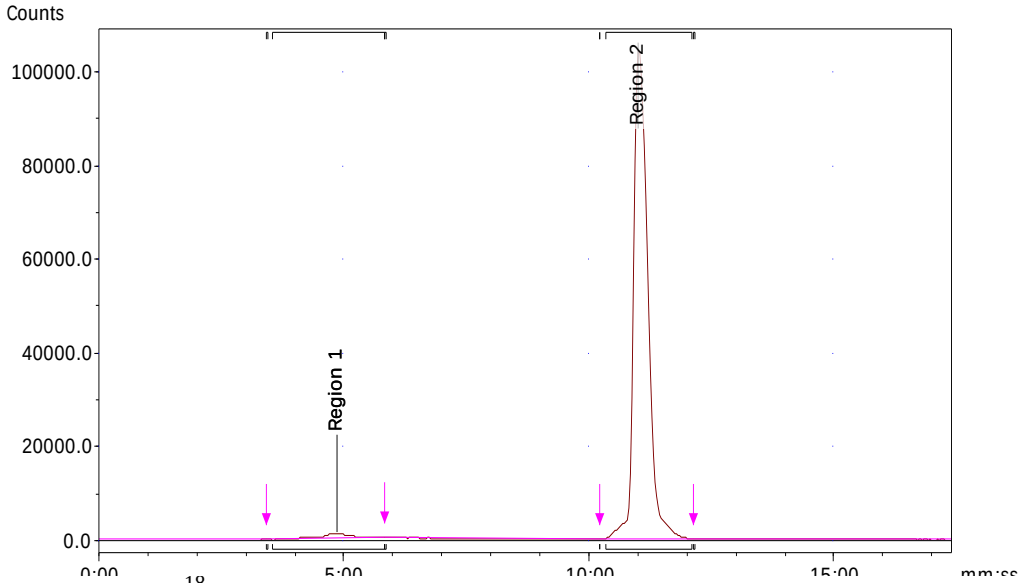


Fig. S4-1. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NOTA-WT (n = 5) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text.

HPLC Chromatogram of the Reconstituted ^{18}F-AlF-NOTA-WT Injectate

Chromatogram: ^{18}F



Regions: ^{18}F Detector: PMT

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	3:32	5:49	4:51	51254.9	2.36	2.43
Region 2	10:21	12:06	11:01	2119006.0	97.64	100.50
2 Peaks				2170261.0	100.00	102.93

^{18}F-AlF-NOTA-Var3

Table S5-1. Tissue uptake (mean %ID ± SD) of ^{18}F-AlF-NOTA-Var3 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n = 5)	1 h (n = 5)	2 h (n = 5)	4 h (n = 4)
Blood	0.866 ± 0.319	0.481 ± 0.473	0.337 ± 0.229	1.083 ± 0.234
Heart	0.117 ± 0.020	0.095 ± 0.019	0.093 ± 0.022	0.078 ± 0.006
Lungs	0.815 ± 0.339	0.573 ± 0.157	0.588 ± 0.197	0.543 ± 0.107
Liver	2.398 ± 1.056	1.627 ± 0.382	1.091 ± 0.215	0.779 ± 0.050
Spleen	0.096 ± 0.009	0.076 ± 0.007	0.055 ± 0.014	0.070 ± 0.008
Pancreas	0.116 ± 0.115	0.047 ± 0.008	0.047 ± 0.014	0.061 ± 0.030
Stomach	0.235 ± 0.049	0.149 ± 0.029	0.132 ± 0.015	0.130 ± 0.018
S. intestine	12.015 ± 1.355	13.855 ± 1.696	2.964 ± 0.687	1.078 ± 0.208
L. intestine	0.326 ± 0.101	1.350 ± 1.253	13.535 ± 0.993	14.054 ± 1.192
Kidneys	6.242 ± 0.722	3.442 ± 0.594	2.748 ± 0.105	2.236 ± 0.141
Muscle	0.041 ± 0.014	0.044 ± 0.012	0.033 ± 0.011	0.028 ± 0.004
Bone	0.027 ± 0.019	0.022 ± 0.014	0.017 ± 0.014	0.016 ± 0.009
Skin	0.171 ± 0.030	0.180 ± 0.018	0.161 ± 0.040	0.106 ± 0.041
Brain	0.080 ± 0.014	0.063 ± 0.006	0.054 ± 0.007	0.041 ± 0.011
Tumor	0.188 ± 0.033	0.164 ± 0.025	0.134 ± 0.035	0.216 ± 0.047

Table S5-2. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NOTA-Var3 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n = 5)	1 h (n = 5)	2 h (n = 5)	4 h (n = 4)
Blood	5.778 ± 2.950	2.851 ± 0.208	1.998 ± 0.319	2.201 ± 0.374
Heart	1.128 ± 0.154	1.024 ± 0.112	0.879 ± 0.098	0.775 ± 0.079
Lungs	3.470 ± 0.434	2.567 ± 0.497	2.375 ± 0.279	2.217 ± 0.380
Liver	2.960 ± 0.390	1.605 ± 0.396	1.302 ± 0.332	0.923 ± 0.153
Spleen	0.706 ± 0.106	0.554 ± 0.053	0.440 ± 0.148	0.469 ± 0.093
Pancreas	0.754 ± 0.414	0.405 ± 0.070	0.380 ± 0.140	0.332 ± 0.020
Stomach	1.034 ± 0.138	0.648 ± 0.300	0.586 ± 0.096	0.482 ± 0.223
S. intestine	10.125 ± 1.224	12.675 ± 2.221	2.671 ± 0.830	0.927 ± 0.152
L. intestine	0.434 ± 0.077	1.879 ± 1.637	19.737 ± 2.432	22.047 ± 2.461
Kidneys	21.180 ± 3.755	11.398 ± 1.054	10.166 ± 1.290	8.237 ± 1.106
Muscle	0.413 ± 0.040	0.391 ± 0.105	0.316 ± 0.040	0.282 ± 0.055
Bone	0.626 ± 0.177	0.547 ± 0.127	0.516 ± 0.063	0.540 ± 0.119
Skin	1.926 ± 0.399	1.557 ± 0.270	1.411 ± 0.163	1.126 ± 0.115
Brain	0.222 ± 0.033	0.162 ± 0.012	0.133 ± 0.017	0.107 ± 0.023
Tumor	1.142 ± 0.244	0.913 ± 0.046	0.802 ± 0.129	0.923 ± 0.119
Tumor-to-tissue ratios (rel. u.)				
Tumor/Blood	0.198 ± 0.109	0.320 ± 0.028	0.401 ± 0.091	0.419 ± 0.090
Tumor/Liver	0.386 ± 0.097	0.569 ± 0.143	0.615 ± 0.186	1.000 ± 0.211
Tumor/S. I.	0.113 ± 0.028	0.072 ± 0.013	0.300 ± 0.105	0.996 ± 0.208
Tumor/L. I.	2.631 ± 0.733	0.486 ± 0.424	0.041 ± 0.008	0.042 ± 0.007
Tumor/Kidney	0.054 ± 0.015	0.080 ± 0.008	0.079 ± 0.016	0.112 ± 0.021
Tumor/Muscle	2.763 ± 0.648	2.334 ± 0.635	2.535 ± 0.520	3.277 ± 0.765
Tumor/Bone	1.823 ± 0.646	1.668 ± 0.397	1.554 ± 0.315	1.709 ± 0.436

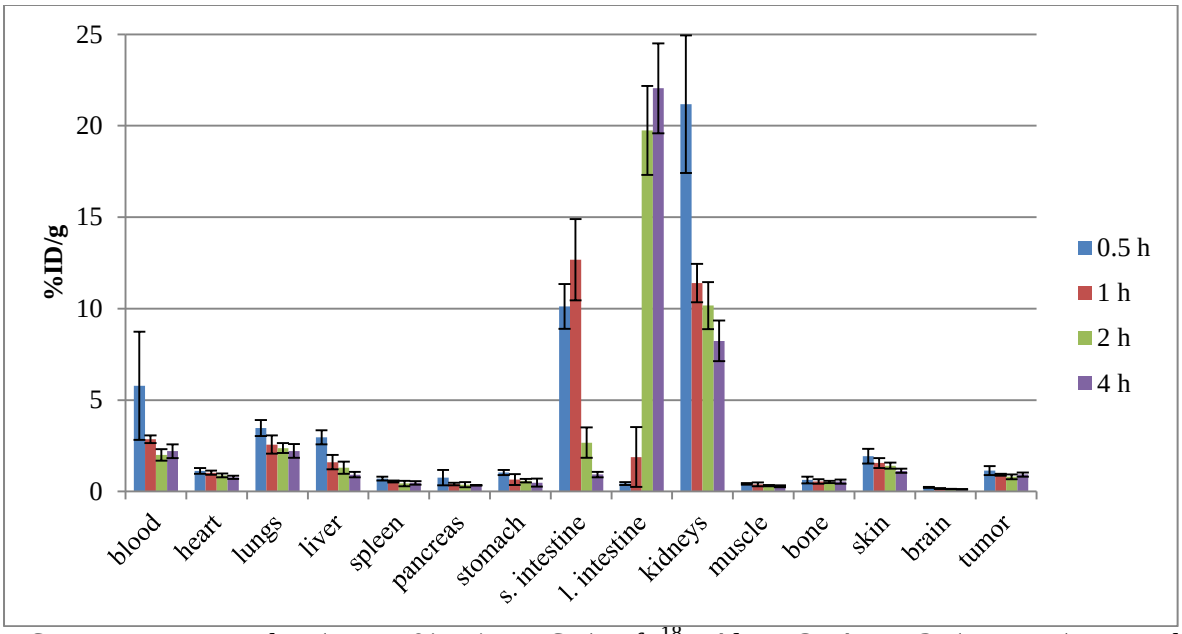
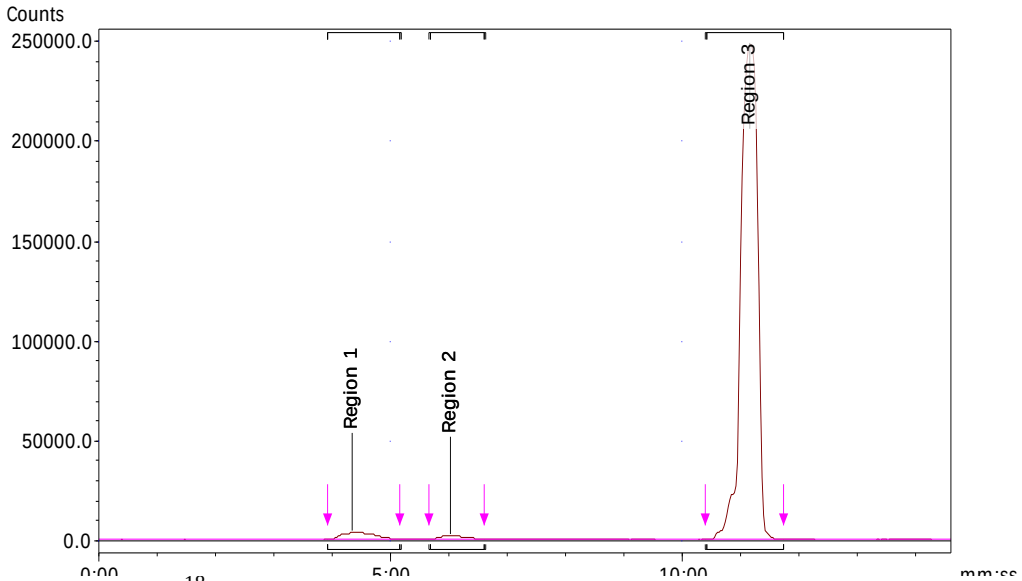


Fig. S5-1. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NOTA-Var3 (n = 5 (4 at 4 h)) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text.

HPLC Chromatogram of the Reconstituted ^{18}F-AlF-NOTA-Var3 Injectate

Chromatogram: ^{18}F



Regions: ^{18}F Detector: PMT

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	3:56	5:10	4:20	125455.0	2.43	2.46
Region 2	5:41	6:36	6:02	43790.0	0.85	0.86
Region 3	10:25	11:44	11:10	4995537.0	96.72	98.12
3 Peaks				5164782.0	100.00	101.45

^{18}F-AlF-NOTA-Var7

Table S6-1. Tissue uptake (mean %ID ± SD) of ^{18}F-AlF-NOTA-Var7 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n = 5)	1 h (n = 5)	2 h (n = 5)	4 h (n = 5)
Blood	0.840 ± 0.323	0.523 ± 0.262	0.206 ± 0.126	0.149 ± 0.111
Heart	0.105 ± 0.015	0.101 ± 0.038	0.091 ± 0.022	0.076 ± 0.012
Lungs	0.625 ± 0.122	0.555 ± 0.304	0.485 ± 0.196	0.593 ± 0.054
Liver	1.258 ± 0.785	1.083 ± 0.384	1.043 ± 0.440	0.863 ± 0.132
Spleen	0.078 ± 0.015	0.070 ± 0.014	0.070 ± 0.025	0.077 ± 0.014
Pancreas	0.053 ± 0.013	0.042 ± 0.008	0.055 ± 0.014	0.054 ± 0.015
Stomach	0.163 ± 0.036	0.127 ± 0.039	0.136 ± 0.013	0.167 ± 0.052
S. intestine	13.273 ± 1.269	11.429 ± 5.124	4.863 ± 2.873	0.905 ± 0.293
L. intestine	0.207 ± 0.050	1.006 ± 1.208	10.930 ± 2.777	13.783 ± 4.870
Kidneys	4.639 ± 0.460	2.934 ± 0.361	2.360 ± 0.178	2.147 ± 0.138
Muscle	0.035 ± 0.008	0.024 ± 0.004	0.031 ± 0.012	0.031 ± 0.007
Bone	0.011 ± 0.004	0.011 ± 0.006	0.014 ± 0.007	0.022 ± 0.021
Skin	0.106 ± 0.024	0.091 ± 0.026	0.121 ± 0.037	0.128 ± 0.027
Brain	0.122 ± 0.098	0.066 ± 0.021	0.053 ± 0.006	0.047 ± 0.006
Tumor	0.174 ± 0.057	0.163 ± 0.055	0.163 ± 0.036	0.209 ± 0.070

Table S6-2. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NOTA-Var7 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n = 5)	1 h (n = 5)	2 h (n = 5)	4 h (n = 5)
Blood	2.961 ± 0.684	2.263 ± 0.412	2.547 ± 0.642	2.292 ± 0.277
Heart	0.994 ± 0.167	0.885 ± 0.247	0.918 ± 0.195	0.775 ± 0.056
Lungs	2.440 ± 0.351	2.508 ± 0.683	2.532 ± 0.420	2.325 ± 0.212
Liver	1.946 ± 0.408	1.433 ± 0.605	1.545 ± 0.635	1.089 ± 0.066
Spleen	0.578 ± 0.156	0.419 ± 0.048	0.489 ± 0.096	0.485 ± 0.029
Pancreas	0.406 ± 0.047	0.340 ± 0.054	0.422 ± 0.083	0.373 ± 0.051
Stomach	0.727 ± 0.373	0.444 ± 0.097	0.603 ± 0.154	0.593 ± 0.153
S. intestine	13.049 ± 3.020	12.437 ± 6.386	4.931 ± 2.811	0.776 ± 0.196
L. intestine	0.332 ± 0.078	4.535 ± 6.803	18.246 ± 4.799	22.081 ± 6.021
Kidneys	17.053 ± 2.372	10.443 ± 0.757	9.250 ± 1.538	7.759 ± 0.658
Muscle	0.351 ± 0.046	0.296 ± 0.117	0.327 ± 0.088	0.325 ± 0.073
Bone	0.441 ± 0.086	0.355 ± 0.093	0.402 ± 0.063	0.571 ± 0.202
Skin	1.439 ± 0.207	1.217 ± 0.184	1.482 ± 0.329	1.377 ± 0.148
Brain	0.434 ± 0.495	0.170 ± 0.034	0.128 ± 0.020	0.118 ± 0.011
Tumor	0.864 ± 0.070	0.794 ± 0.230	0.915 ± 0.096	1.105 ± 0.125
Tumor-to-tissue ratios (rel. u.)				
Tumor/Blood	0.29 ± 0.07	0.35 ± 0.12	0.36 ± 0.10	0.48 ± 0.08
Tumor/Liver	0.44 ± 0.10	0.6 ± 0.3	0.6 ± 0.3	1.01 ± 0.13
Tumor/S. I.	0.066 ± 0.016	0.06 ± 0.04	0.19 ± 0.11	1.4 ± 0.4
Tumor/L. I.	2.60 ± 0.64	0.17 ± 0.27	0.050 ± 0.014	0.050 ± 0.015
Tumor/Kidney	0.051 ± 0.008	0.08 ± 0.02	0.099 ± 0.019	0.14 ± 0.02
Tumor/Muscle	2.5 ± 0.4	2.7 ± 1.3	2.8 ± 0.8	3.4 ± 0.9
Tumor/Bone	2.0 ± 0.4	2.2 ± 0.9	2.3 ± 0.4	1.9 ± 0.7

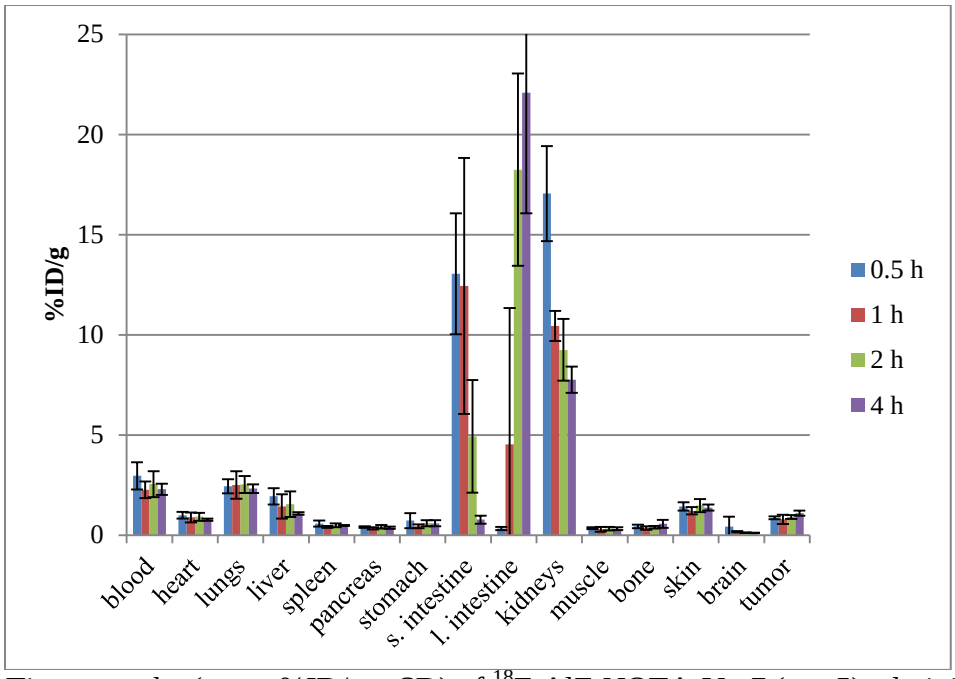
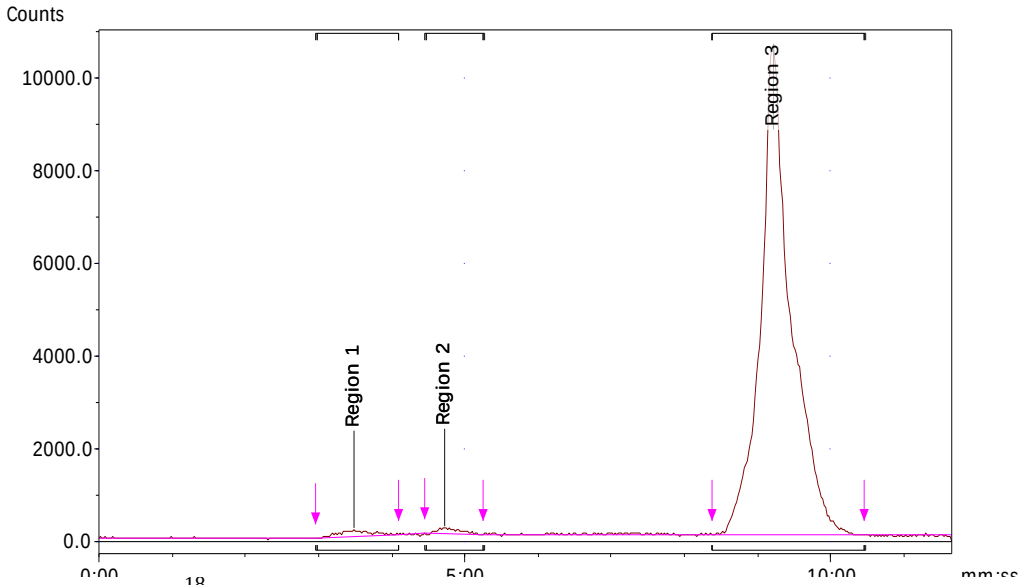


Fig. S6-1. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NOTA-Var7 (n = 5) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text.

HPLC Chromatogram of the Reconstituted ^{18}F-AlF-NOTA-Var7 Injectate

Chromatogram: ^{18}F



Regions: ^{18}F Detector: PMT

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	2:59	4:05	3:29	2153.0	0.70	0.70
Region 2	4:28	5:15	4:44	3042.0	0.98	0.99
Region 3	8:23	10:27	9:13	304179.0	98.32	99.27
3 Peaks				309374.0	100.00	100.96

^{64}Cu-NO2A-cysWT

Table S7-1. Tissue uptake (mean %ID ± SD) of ^{64}Cu-NO2A-cysWT administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n=4)	2 h (n=4)	4 h (n=4)	4 h + glucose (n=3)	12 h (n=4)	24 h (n=5)	48 h (n=5)
Blood	2.92 ± 0.76	5.56 ± 1.52	2.72 ± 0.44	2.85 ± 0.87	1.46 ± 0.47	0.67 ± 0.18	0.56 ± 0.15
Heart	0.70 ± 0.12	0.73 ± 0.17	0.51 ± 0.04	0.45 ± 0.03	0.45 ± 0.07	0.47 ± 0.04	0.48 ± 0.07
Lungs	5.27 ± 0.71	3.06 ± 0.52	2.88 ± 0.46	2.57 ± 0.09	1.79 ± 0.34	1.72 ± 0.10	1.08 ± 0.19
Liver	16.7 ± 3.19	17.3 ± 1.58	22.9 ± 1.00	21.9 ± 1.04	23.3 ± 3.93	23.5 ± 1.99	17.3 ± 1.13
Spleen	0.51 ± 0.01	0.62 ± 0.08	0.72 ± 0.02	0.68 ± 0.09	1.01 ± 0.16	1.09 ± 0.11	0.63 ± 0.05
Pancreas	0.26 ± 0.05	0.44 ± 0.18	0.40 ± 0.04	0.28 ± 0.00	0.40 ± 0.05	0.46 ± 0.06	0.41 ± 0.04
Stomach	0.65 ± 0.07	0.69 ± 0.04	1.06 ± 0.02	0.93 ± 0.27	1.33 ± 0.24	1.65 ± 0.28	1.16 ± 0.10
S. intestine	3.54 ± 0.26	4.33 ± 0.59	6.76 ± 0.61	5.03 ± 0.37	8.92 ± 0.65	8.94 ± 0.76	6.69 ± 0.65
L. intestine	0.78 ± 0.12	1.73 ± 0.13	3.19 ± 0.29	2.49 ± 0.17	6.17 ± 0.50	6.26 ± 0.78	5.28 ± 0.90
Kidneys	7.95 ± 0.84	9.93 ± 1.21	12.1 ± 1.16	8.30 ± 0.24	6.01 ± 0.65	4.09 ± 0.24	2.92 ± 0.18
Muscle	0.15 ± 0.04	0.15 ± 0.03	0.13 ± 0.04	0.08 ± 0.01	0.20 ± 0.06	0.14 ± 0.05	0.12 ± 0.03
Bone	0.14 ± 0.07	0.10 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.13 ± 0.01	0.14 ± 0.03	0.10 ± 0.01
Skin	0.31 ± 0.12	0.30 ± 0.13	0.34 ± 0.05	0.32 ± 0.15	0.35 ± 0.08	0.32 ± 0.06	0.21 ± 0.06
Brain	0.30 ± 0.03	0.22 ± 0.05	0.25 ± 0.04	0.16 ± 0.05	0.13 ± 0.02	0.19 ± 0.00	0.20 ± 0.01
Tumor	0.36 ± 0.10	0.48 ± 0.15	0.80 ± 0.13	0.49 ± 0.10	1.12 ± 0.28	1.29 ± 0.45	0.82 ± 0.22

Table S7-2. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NO2A-cysWT administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n=4)	2 h (n=4)	4 h (n=4)	4 h + glucose (n=3)	12 h (n=4)	24 h (n=5)	48 h (n=5)
Blood	26.5 ± 1.12	24.6 ± 2.61	17.6 ± 0.86	13.8 ± 0.82	6.40 ± 0.65	3.64 ± 0.15	1.90 ± 0.11
Heart	6.87 ± 1.05	7.08 ± 1.66	5.52 ± 0.15	4.11 ± 0.35	4.01 ± 0.67	4.36 ± 0.21	5.01 ± 0.49
Lungs	19.2 ± 2.29	15.4 ± 1.78	12.3 ± 1.55	10.1 ± 1.23	8.18 ± 1.63	6.73 ± 0.40	6.38 ± 0.25
Liver	17.5 ± 1.14	20.8 ± 2.62	21.9 ± 0.64	19.5 ± 2.13	19.2 ± 3.33	23.3 ± 1.79	17.9 ± 1.74
Spleen	4.64 ± 0.23	5.65 ± 0.80	7.07 ± 0.44	6.50 ± 0.90	8.79 ± 1.60	9.80 ± 0.56	6.07 ± 0.72
Pancreas	2.27 ± 0.14	2.83 ± 0.51	3.00 ± 0.08	2.15 ± 0.25	2.71 ± 0.38	3.34 ± 0.40	2.87 ± 0.08
Stomach	1.77 ± 0.19	1.83 ± 0.24	1.82 ± 0.13	2.32 ± 0.15	2.81 ± 0.45	4.30 ± 0.90	3.93 ± 0.89
S. intestine	2.74 ± 0.15	3.53 ± 0.44	4.56 ± 0.26	3.43 ± 0.33	6.47 ± 0.83	6.33 ± 0.45	5.36 ± 0.65
L. intestine	1.03 ± 0.08	2.16 ± 0.27	4.09 ± 0.34	3.07 ± 0.13	6.52 ± 0.86	7.21 ± 1.05	5.97 ± 0.98
Kidneys	27.3 ± 2.15	34.3 ± 3.96	40.6 ± 4.04	26.3 ± 0.92	18.2 ± 3.39	13.3 ± 0.58	9.86 ± 0.77
Muscle	1.04 ± 0.12	1.37 ± 0.46	1.15 ± 0.08	0.82 ± 0.07	1.25 ± 0.13	1.06 ± 0.11	0.94 ± 0.12
Bone	2.31 ± 0.19	2.58 ± 0.29	3.04 ± 0.11	2.13 ± 0.38	3.04 ± 0.28	3.11 ± 0.36	1.99 ± 0.15
Skin	2.53 ± 0.53	2.92 ± 0.59	3.23 ± 0.12	2.36 ± 0.79	3.30 ± 0.51	3.22 ± 0.15	2.14 ± 0.13
Brain	0.75 ± 0.05	0.58 ± 0.12	0.62 ± 0.06	0.39 ± 0.09	0.38 ± 0.06	0.48 ± 0.03	0.51 ± 0.02

Tumor	5.03	±	1.48	7.55	±	2.15	11.7	±	1.71	7.10	±	0.81	14.3	±	2.72	12.8	±	1.55	6.16	±	0.48
Tumor-to-tissue ratios (rel. u.)																					
Tumor/Blood	0.19	±	0.06	0.31	±	0.09	0.66	±	0.10	0.52	±	0.07	2.2	±	0.5	3.5	±	0.5	3.2	±	0.3
Tumor/Liver	0.29	±	0.09	0.36	±	0.11	0.53	±	0.08	0.36	±	0.06	0.75	±	0.19	0.55	±	0.08	0.34	±	0.04
Tumor/S. I.	1.8	±	0.5	2.1	±	0.7	2.6	±	0.4	2.1	±	0.3	2.2	±	0.5	2.0	±	0.3	1.15	±	0.17
Tumor/L. I.	4.9	±	1.5	3.5	±	1.1	2.9	±	0.5	2.3	±	0.3	2.2	±	0.5	1.8	±	0.3	1.03	±	0.19
Tumor/Kidney	0.18	±	0.06	0.22	±	0.07	0.29	±	0.05	0.27	±	0.03	0.8	±	0.2	0.96	±	0.12	0.62	±	0.07
Tumor/Muscle	4.8	±	1.5	5.5	±	2.4	10.1	±	1.7	8.6	±	1.2	11	±	2	12	±	2	6.6	±	1.0
Tumor/Bone	2.2	±	0.7	2.9	±	0.9	3.8	±	0.6	3.3	±	0.7	4.7	±	1.0	4.1	±	0.7	3.1	±	0.3

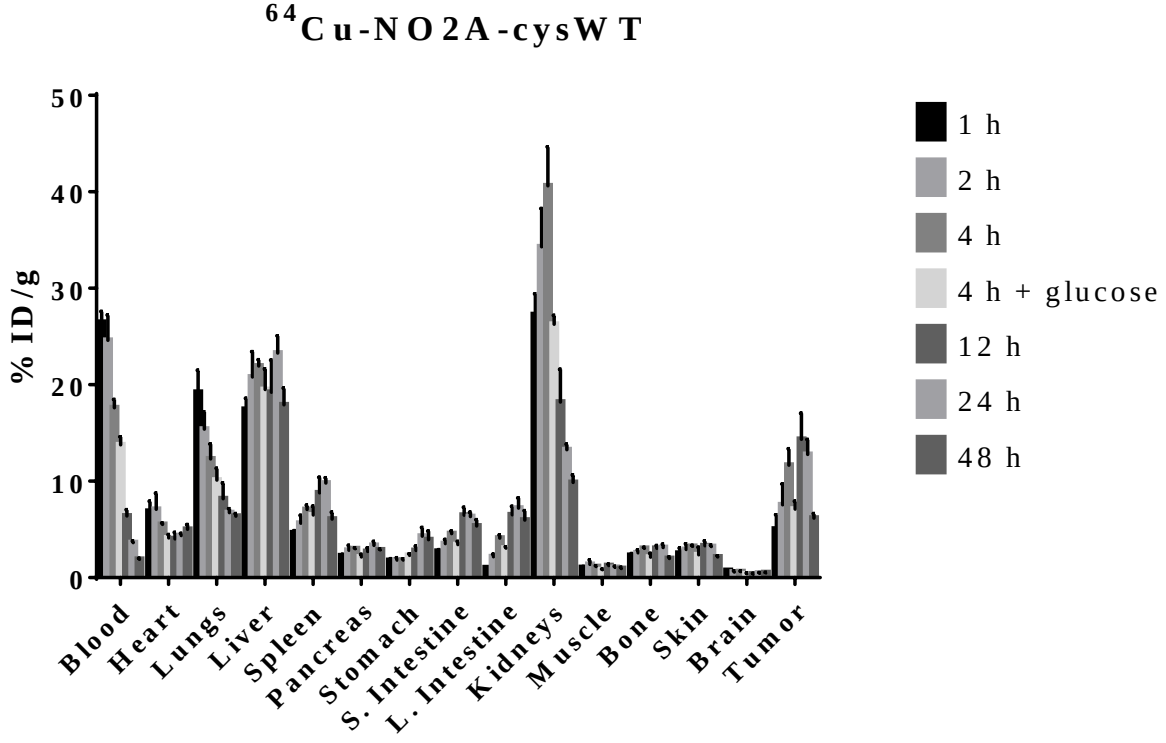
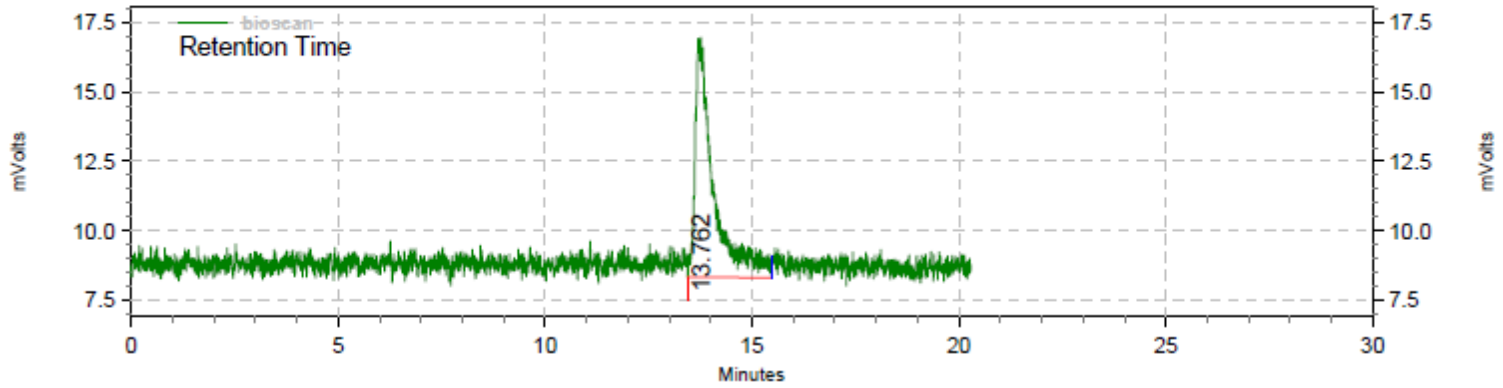


Fig. S7-1. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NO2A-cysWT (n = 3-5) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text. Glucose was administered i.p. 30 min prior to tracer injection.

HPLC Chromatogram of the Reconstituted ^{64}Cu-NO2A-cysWT Injectate

BIOSCAN



bioscan Results

Retention Time	Area	Area %	Height	Height %
13.762	264129	100.00	8650	100.00
Totals	264129	100.00	8650	100.00

^{64}Cu-NO2A-cysVar7

Table S8-1. Tissue uptake (mean %ID ± SD) of ^{64}Cu-NO2A-cysVar7 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n=4)	2 h (n=4)	4 h (n=4)	4 h + glucose (n=4)	12 h (n=4)	24 h (n=5)	48 h (n=5)
Blood	1.96 ± 1.25	0.87 ± 0.41	0.45 ± 0.09	0.39 ± 0.15	0.74 ± 0.38	0.47 ± 0.10	0.28 ± 0.12
Heart	0.24 ± 0.04	0.21 ± 0.01	0.28 ± 0.03	0.25 ± 0.02	0.49 ± 0.11	0.46 ± 0.08	0.36 ± 0.04
Lungs	1.52 ± 0.27	1.02 ± 0.19	1.04 ± 0.06	0.86 ± 0.12	1.56 ± 0.13	1.36 ± 0.16	0.79 ± 0.07
Liver	6.14 ± 0.70	9.31 ± 0.68	15.7 ± 1.3	13.7 ± 0.9	18.8 ± 2.8	16.4 ± 1.7	10.3 ± 1.1
Spleen	0.22 ± 0.02	0.26 ± 0.02	0.35 ± 0.04	0.35 ± 0.02	0.68 ± 0.20	0.55 ± 0.11	0.23 ± 0.02
Pancreas	0.14 ± 0.01	0.18 ± 0.02	0.28 ± 0.05	0.24 ± 0.03	0.38 ± 0.05	0.40 ± 0.04	0.24 ± 0.07
Stomach	0.44 ± 0.10	0.65 ± 0.04	1.48 ± 0.03	1.10 ± 0.36	1.83 ± 0.30	1.33 ± 0.16	1.02 ± 0.20
S. intestine	2.66 ± 0.29	4.74 ± 0.92	9.04 ± 0.95	5.91 ± 0.44	9.73 ± 0.76	8.21 ± 0.34	4.70 ± 0.27
L. intestine	0.85 ± 0.17	2.07 ± 0.26	6.39 ± 0.68	4.30 ± 0.87	8.64 ± 1.32	5.79 ± 0.27	4.52 ± 0.51
Kidneys	35.5 ± 3.92	40.5 ± 1.57	29.7 ± 3.4	25.1 ± 2.0	8.14 ± 0.52	5.23 ± 0.47	2.97 ± 0.19
Muscle	0.10 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.09 ± 0.03	0.10 ± 0.02	0.11 ± 0.03	0.08 ± 0.01
Bone	0.03 ± 0.01	0.03 ± 0.02	0.14 ± 0.05	0.07 ± 0.00	0.10 ± 0.02	0.08 ± 0.01	0.05 ± 0.01
Skin	0.39 ± 0.04	0.32 ± 0.10	0.29 ± 0.02	0.31 ± 0.04	0.33 ± 0.03	0.24 ± 0.05	0.14 ± 0.01
Brain	0.08 ± 0.02	0.06 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.18 ± 0.04	0.25 ± 0.02	0.21 ± 0.03
Tumor	0.38 ± 0.10	0.48 ± 0.09	0.71 ± 0.13	0.63 ± 0.11	0.78 ± 0.24	0.57 ± 0.24	0.37 ± 0.03

Table S8-2. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NO2A-cysVar7 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n=4)	2 h (n=4)	4 h (n=4)	4 h + glucose (n=4)	12 h (n=4)	24 h (n=5)	48 h (n=5)
Blood	7.21 ± 0.72	3.78 ± 1.22	2.31 ± 0.18	2.36 ± 0.23	2.62 ± 0.61	2.46 ± 0.35	1.43 ± 0.07
Heart	2.65 ± 0.33	2.14 ± 0.28	2.68 ± 0.33	2.46 ± 0.25	4.41 ± 0.88	4.46 ± 0.60	4.01 ± 0.18
Lungs	6.26 ± 0.72	4.01 ± 0.46	4.21 ± 0.23	3.85 ± 0.23	5.79 ± 0.57	6.04 ± 0.81	5.08 ± 0.30
Liver	7.52 ± 0.37	10.2 ± 0.70	15.6 ± 1.58	16.7 ± 2.00	17.1 ± 2.93	18.4 ± 2.35	11.9 ± 0.68
Spleen	2.00 ± 0.12	2.29 ± 0.25	3.31 ± 0.24	3.47 ± 0.28	5.59 ± 0.88	5.43 ± 0.72	2.54 ± 0.18
Pancreas	1.12 ± 0.08	1.23 ± 0.14	1.84 ± 0.10	1.98 ± 0.14	2.52 ± 0.24	2.83 ± 0.29	2.33 ± 0.26
Stomach	1.74 ± 0.62	2.02 ± 0.42	3.33 ± 0.31	3.27 ± 0.83	4.09 ± 1.39	4.68 ± 0.76	2.67 ± 0.81
S. intestine	2.32 ± 0.30	4.02 ± 0.55	6.37 ± 0.11	4.93 ± 0.57	6.96 ± 0.73	6.52 ± 0.56	4.08 ± 0.29
L. intestine	1.24 ± 0.21	2.82 ± 0.21	8.19 ± 0.85	6.63 ± 1.37	9.88 ± 1.28	7.42 ± 0.89	6.20 ± 0.96
Kidneys	145.5 ± 12.80	139.0 ± 13.4	95.5 ± 11.9	93.8 ± 5.16	27.6 ± 4.46	19.2 ± 3.08	9.73 ± 0.33
Muscle	0.98 ± 0.29	0.60 ± 0.06	0.64 ± 0.07	0.73 ± 0.08	0.82 ± 0.16	0.90 ± 0.15	0.71 ± 0.05
Bone	0.93 ± 0.19	0.95 ± 0.24	1.57 ± 0.18	1.40 ± 0.14	2.12 ± 0.26	1.91 ± 0.22	1.11 ± 0.05
Skin	2.53 ± 0.28	2.12 ± 0.11	2.59 ± 0.05	2.63 ± 0.13	2.79 ± 0.46	2.47 ± 0.20	1.51 ± 0.15
Brain	0.20 ± 0.03	0.16 ± 0.02	0.25 ± 0.02	0.22 ± 0.03	0.48 ± 0.06	0.62 ± 0.07	0.53 ± 0.04
Tumor	6.54 ± 0.35	7.02 ± 0.82	9.07 ± 1.81	11.2 ± 0.55	9.60 ± 0.90	7.28 ± 1.21	3.90 ± 0.58

Tumor-to-tissue ratios (rel. u.)																		
Tumor/Blood	0.91	±	0.10	1.9	±	0.6	3.9	±	0.8	4.7	±	0.5	3.7	±	0.9	3.0	±	0.6	2.7	±	0.4
Tumor/Liver	0.87	±	0.06	0.69	±	0.09	0.58	±	0.13	0.67	±	0.09	0.56	±	0.11	0.40	±	0.08	0.33	±	0.05
Tumor/S. I.	2.8	±	0.4	1.7	±	0.3	1.4	±	0.3	2.3	±	0.3	1.4	±	0.2	1.1	±	0.2	0.96	±	0.16
Tumor/L. I.	5.3	±	0.9	2.5	±	0.3	1.1	±	0.2	1.7	±	0.4	1.0	±	0.2	1.0	±	0.2	0.63	±	0.14
Tumor/Kidney	0.045	±	0.005	0.050	±	0.008	0.09	±	0.02	0.119	±	0.009	0.35	±	0.06	0.38	±	0.09	0.40	±	0.06
Tumor/Muscle	7	±	2	11.7	±	1.8	14	±	3	15.4	±	1.9	12	±	2	8	±	2	5.5	±	0.9
Tumor/Bone	7.1	±	1.5	7	±	2	5.8	±	1.3	7.9	±	0.9	4.5	±	0.7	3.8	±	0.8	3.5	±	0.5

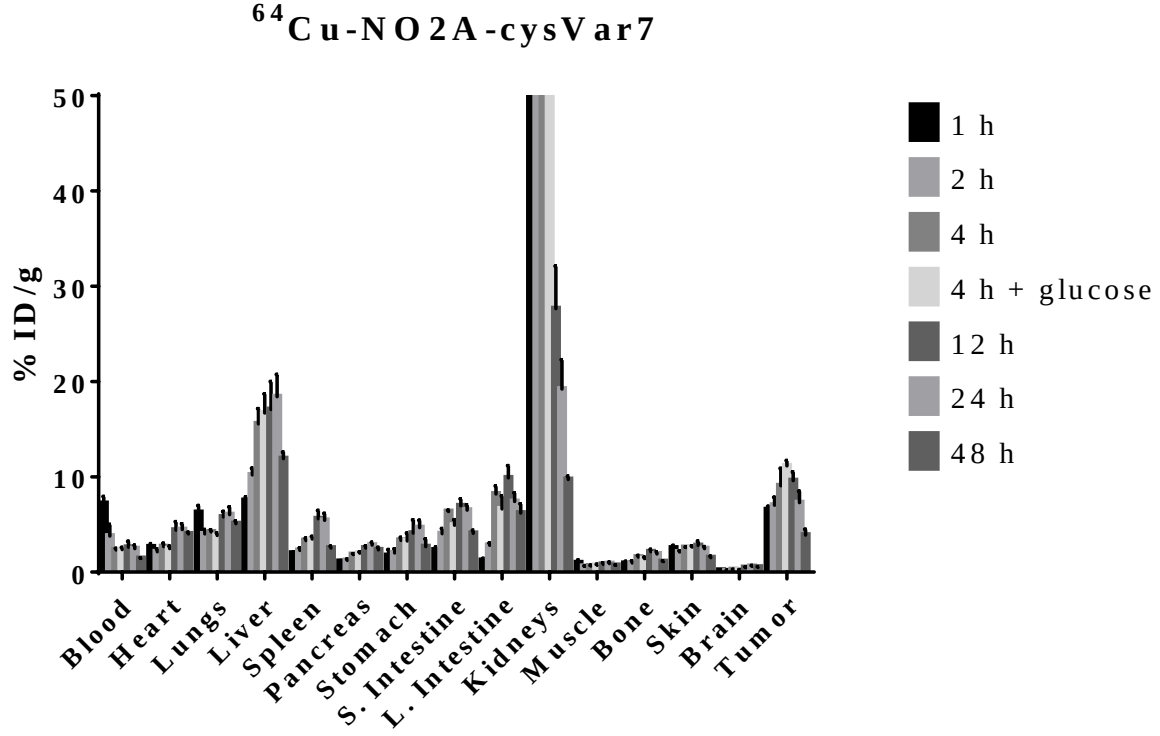
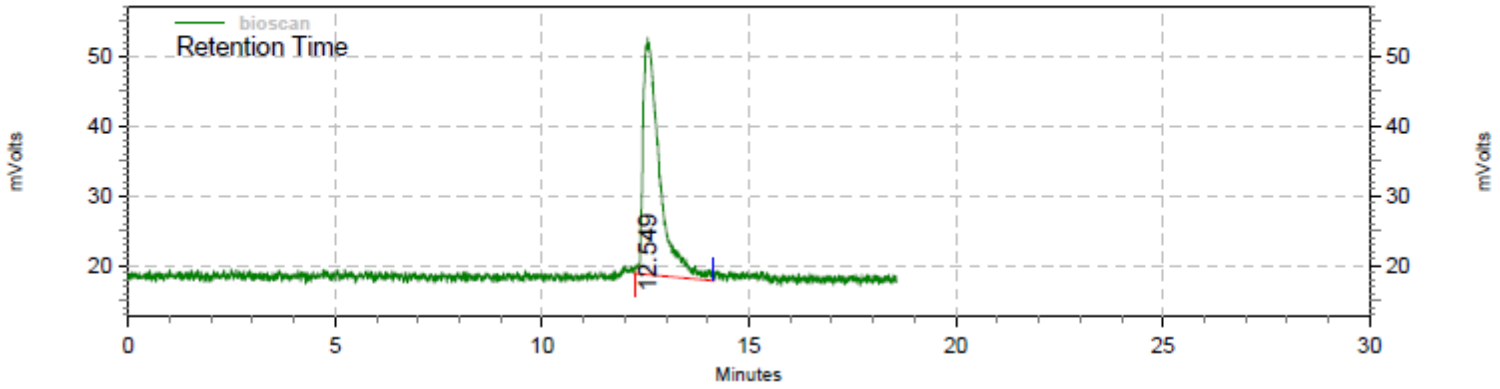


Fig. S8-1. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NO2A-cysVar7 (n = 4-5) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text. Glucose was administered i.p. 30 min prior to tracer injection.

HPLC Chromatogram of the Reconstituted ^{64}Cu-NO2A-cysVar7 Injectate

BIOSCAN



bioscan Results

Retention Time	Area	Area %	Height	Height %
12.549	936112	100.00	33902	100.00

Totals	936112	100.00	33902	100.00

^{18}F-AlF-NO2A-cysWT

Table S9-1. Tissue uptake (mean %ID ± SD) of ^{18}F-AlF-NO2A-cysWT administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n=4)	1 h (n=4)	2 h (n=4)	4 h (n=4)	4 h + glucose (n=4)	6 h (n=4)
Blood	12.3 ± 5.05	5.71 ± 0.45	6.72 ± 3.43	6.20 ± 3.06	4.16 ± 1.89	2.39 ± 0.99
Heart	0.59 ± 0.07	0.66 ± 0.25	0.51 ± 0.08	0.48 ± 0.15	0.35 ± 0.05	0.66 ± 0.18
Lungs	3.72 ± 2.15	4.09 ± 0.32	2.84 ± 0.29	1.74 ± 0.23	1.99 ± 0.13	0.98 ± 0.21
Liver	13.2 ± 0.74	12.9 ± 0.61	12.2 ± 0.79	14.4 ± 1.53	17.9 ± 1.03	13.1 ± 1.32
Spleen	0.43 ± 0.04	0.40 ± 0.08	0.43 ± 0.02	0.60 ± 0.03	0.63 ± 0.08	0.93 ± 0.26
Pancreas	0.29 ± 0.04	0.33 ± 0.08	0.27 ± 0.04	0.27 ± 0.07	0.26 ± 0.08	0.57 ± 0.25
Stomach	0.50 ± 0.04	0.42 ± 0.05	0.50 ± 0.12	0.38 ± 0.01	0.60 ± 0.15	0.83 ± 0.23
S. intestine	2.62 ± 0.29	2.55 ± 0.38	3.14 ± 0.53	3.16 ± 0.18	2.82 ± 0.35	3.58 ± 0.30
L. intestine	0.60 ± 0.21	0.68 ± 0.24	0.78 ± 0.03	1.17 ± 0.32	1.09 ± 0.26	1.64 ± 0.28
Kidneys	4.95 ± 0.53	6.85 ± 0.74	9.95 ± 1.17	15.0 ± 0.72	11.4 ± 1.58	17.7 ± 2.34
Muscle	0.12 ± 0.02	0.12 ± 0.01	0.14 ± 0.02	0.15 ± 0.03	0.10 ± 0.02	0.45 ± 0.31
Bone	0.07 ± 0.02	0.05 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.16 ± 0.07	0.47 ± 0.26
Skin	0.28 ± 0.08	0.35 ± 0.06	0.51 ± 0.07	0.50 ± 0.08	0.20 ± 0.09	0.64 ± 0.24
Brain	0.26 ± 0.04	0.18 ± 0.03	0.14 ± 0.02	0.13 ± 0.03	0.13 ± 0.02	0.47 ± 0.24
Tumor	0.22 ± 0.06	0.25 ± 0.08	0.35 ± 0.10	0.55 ± 0.12	0.48 ± 0.15	0.89 ± 0.32

Table S9-2. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NO2A-cysWT administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n=4)	1 h (n=4)	2 h (n=4)	4 h (n=4)	4 h + glucose (n=4)	6 h (n=4)
Blood	35.8 ± 3.22	24.3 ± 1.66	21.6 ± 1.75	15.9 ± 0.63	14.0 ± 0.73	10.0 ± 1.91
Heart	6.33 ± 0.75	6.18 ± 1.69	5.40 ± 0.59	4.78 ± 0.53	3.79 ± 0.48	7.48 ± 2.47
Lungs	17.0 ± 5.66	15.1 ± 1.58	12.4 ± 0.44	8.73 ± 0.84	7.27 ± 0.26	6.34 ± 1.53
Liver	16.3 ± 1.39	13.7 ± 1.52	15.4 ± 1.23	12.6 ± 11.4	18.4 ± 1.31	14.5 ± 1.60
Spleen	4.06 ± 0.43	3.64 ± 0.31	4.12 ± 0.15	5.31 ± 0.34	5.89 ± 0.45	8.25 ± 2.44
Pancreas	2.09 ± 0.09	1.97 ± 0.29	1.86 ± 0.18	2.10 ± 0.43	2.01 ± 0.58	5.00 ± 2.53
Stomach	2.47 ± 0.70	1.35 ± 0.15	2.01 ± 0.34	1.31 ± 0.96	1.96 ± 0.16	2.05 ± 1.11
S. intestine	2.58 ± 0.39	2.12 ± 0.19	2.83 ± 0.27	2.85 ± 0.31	2.34 ± 0.20	3.15 ± 0.54
L. intestine	0.93 ± 0.21	0.92 ± 0.21	1.19 ± 0.08	1.47 ± 0.14	1.61 ± 0.26	2.54 ± 0.34
Kidneys	18.8 ± 1.96	23.4 ± 2.15	36.1 ± 6.63	59.1 ± 3.63	42.6 ± 10.9	64.8 ± 14.3
Muscle	0.96 ± 0.10	0.93 ± 0.05	1.18 ± 0.04	1.23 ± 0.17	0.81 ± 0.12	3.71 ± 2.52
Bone	2.43 ± 0.39	1.82 ± 0.19	1.99 ± 0.30	1.94 ± 0.45	2.70 ± 0.15	11.2 ± 6.44
Skin	1.97 ± 0.11	1.93 ± 0.11	2.87 ± 0.13	2.85 ± 0.22	1.95 ± 0.34	6.39 ± 2.36
Brain	0.70 ± 0.12	0.49 ± 0.08	0.41 ± 0.03	0.34 ± 0.06	0.32 ± 0.05	1.39 ± 0.35
Tumor	3.56 ± 0.92	4.20 ± 1.02	6.52 ± 0.92	8.16 ± 0.53	6.66 ± 0.91	15.6 ± 3.49

Tumor-to-tissue ratios (rel. u.)						
Tumor/Blood	0.10 ± 0.03	0.17 ± 0.04	0.30 ± 0.05	0.51 ± 0.04	0.48 ± 0.07	1.6 ± 0.5
Tumor/Liver	0.22 ± 0.06	0.31 ± 0.08	0.42 ± 0.07	0.6 ± 0.6	0.36 ± 0.06	1.1 ± 0.3
Tumor/S. I.	1.4 ± 0.4	2.0 ± 0.5	2.3 ± 0.4	2.9 ± 0.4	2.8 ± 0.5	4.9 ± 1.4
Tumor/L. I.	3.8 ± 1.3	4.6 ± 1.5	5.5 ± 0.9	5.5 ± 0.6	4.1 ± 0.9	6.1 ± 1.6
Tumor/Kidney	0.19 ± 0.05	0.18 ± 0.05	0.18 ± 0.04	0.138 ± 0.012	0.16 ± 0.05	0.24 ± 0.08
Tumor/Muscle	3.7 ± 1.0	4.5 ± 1.1	5.5 ± 0.8	6.6 ± 1.0	8 ± 2	4.2 ± 3.0
Tumor/Bone	1.5 ± 0.4	2.3 ± 0.6	3.3 ± 0.7	4.2 ± 1.0	2.5 ± 0.4	1.4 ± 0.9

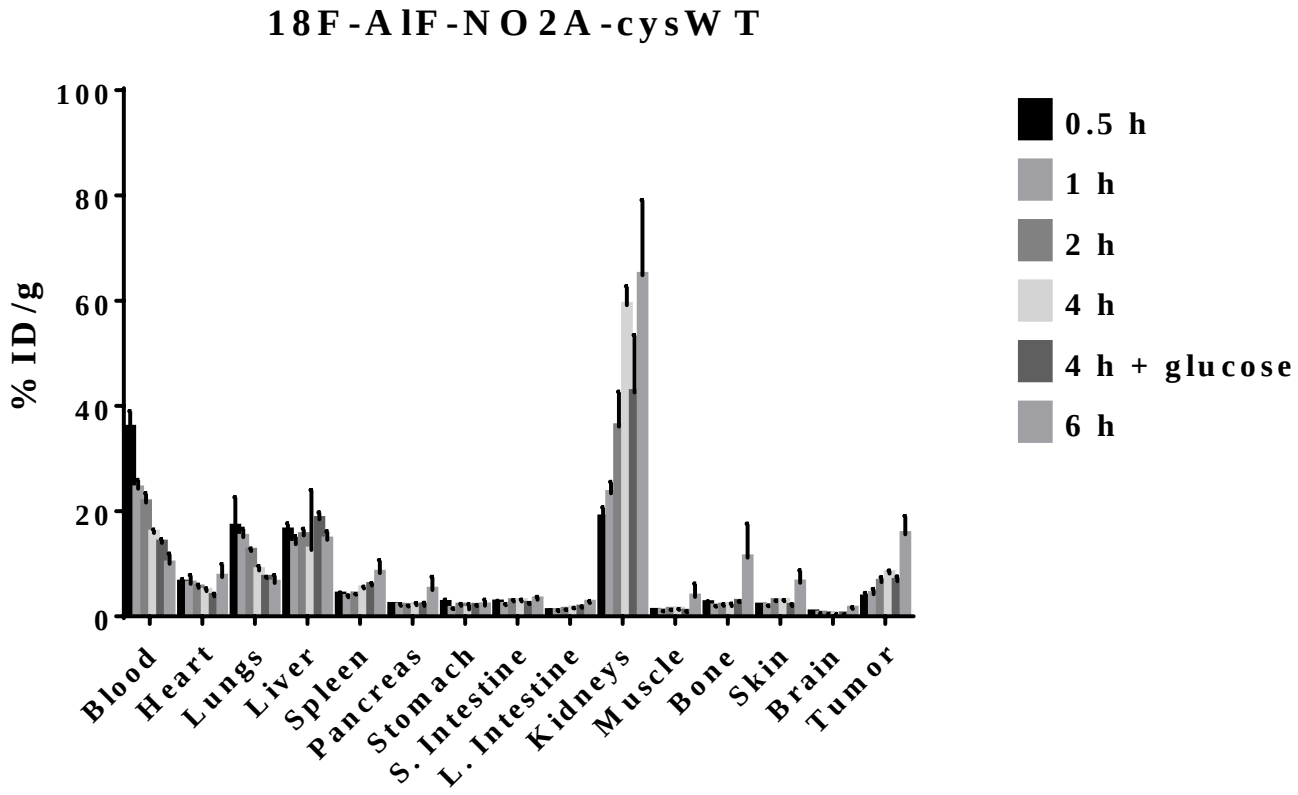
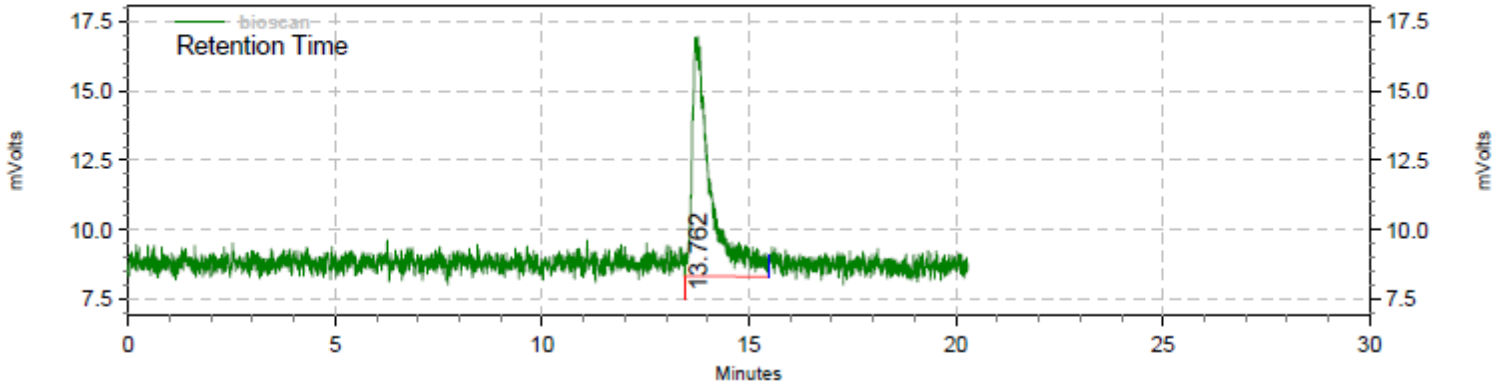


Fig. S9-1. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NO2A-cysWT (n = 4) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text. Glucose was administered i.p. 30 min prior to tracer injection.

HPLC Chromatogram of the Reconstituted ^{18}F-AlF-NO2A-cysWT Injectate

BIOSCAN



bioscan Results

Retention Time	Area	Area %	Height	Height %
13.762	264129	100.00	8650	100.00
Totals	264129	100.00	8650	100.00

^{18}F-AlF-NO2A-cysVar7

Table S10-1. Tissue uptake (mean %ID ± SD) of ^{18}F-AlF-NO2A-cysVar7 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n=4)	1 h (n=4)	2 h (n=3)	4 h (n=4)	4 h + glucose (n=3)	6 h (n=5)
Blood	3.89 ± 2.41	1.92 ± 1.54	3.21 ± 2.40	0.71 ± 0.37	3.12 ± 0.52	0.12 ± 0.09
Heart	0.36 ± 0.05	0.24 ± 0.04	0.33 ± 0.31	0.08 ± 0.02	0.13 ± 0.01	0.063 ± 0.005
Lungs	1.83 ± 0.54	1.40 ± 0.32	1.05 ± 0.44	0.40 ± 0.09	0.08 ± 0.04	0.17 ± 0.03
Liver	4.97 ± 0.36	3.55 ± 0.18	4.26 ± 2.00	2.15 ± 0.35	0.18 ± 0.03	2.17 ± 0.22
Spleen	0.19 ± 0.02	0.15 ± 0.01	0.18 ± 0.12	0.12 ± 0.01	1.04 ± 0.12	0.11 ± 0.02
Pancreas	0.17 ± 0.03	0.14 ± 0.03	0.14 ± 0.08	0.07 ± 0.01	0.61 ± 0.05	0.05 ± 0.01
Stomach	0.40 ± 0.10	0.22 ± 0.01	0.25 ± 0.12	0.15 ± 0.01	52.4 ± 9.57	0.17 ± 0.02
S. intestine	1.63 ± 0.34	1.34 ± 0.26	1.96 ± 1.54	0.96 ± 0.08	0.04 ± 0.01	0.67 ± 0.08
L. intestine	0.70 ± 0.05	0.51 ± 0.07	0.69 ± 0.36	0.59 ± 0.06	0.02 ± 0.00	0.53 ± 0.03
Kidneys	23.8 ± 4.50	40.1 ± 4.20	42.2 ± 21.6	62.9 ± 3.68	0.30 ± 0.04	70.5 ± 5.72
Muscle	0.13 ± 0.03	0.06 ± 0.01	0.07 ± 0.03	0.03 ± 0.01	0.03 ± 0.00	0.043 ± 0.003
Bone	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.40 ± 0.11	0.05 ± 0.02
Skin	0.54 ± 0.06	0.35 ± 0.05	0.38 ± 0.16	0.27 ± 0.03	0.81 ± 0.23	0.15 ± 0.02
Brain	0.10 ± 0.03	0.04 ± 0.01	0.09 ± 0.11	0.020 ± 0.003	1.04 ± 0.91	0.012 ± 0.004
Tumor	0.21 ± 0.05	0.30 ± 0.09	0.27 ± 0.09	0.53 ± 0.11	0.11 ± 0.03	0.51 ± 0.10

Table S10-2. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NO2A-cysVar7 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n=4)	1 h (n=4)	2 h (n=3)	4 h (n=4)	4 h + glucose (n=3)	6 h (n=5)
Blood	14.9 ± 2.24	8.51 ± 2.65	4.87 ± 0.42	1.64 ± 0.18	2.37 ± 0.35	0.52 ± 0.17
Heart	3.91 ± 0.56	2.34 ± 0.19	1.85 ± 0.26	0.89 ± 0.12	1.21 ± 0.19	0.70 ± 0.07
Lungs	9.45 ± 1.91	5.72 ± 0.87	3.96 ± 0.21	1.66 ± 0.07	2.22 ± 0.27	0.97 ± 0.20
Liver	5.89 ± 0.65	3.84 ± 0.29	3.86 ± 0.33	2.90 ± 0.18	4.02 ± 0.14	2.49 ± 0.13
Spleen	2.06 ± 0.14	1.40 ± 0.17	1.38 ± 0.07	1.19 ± 0.10	1.61 ± 0.21	1.05 ± 0.06
Pancreas	1.29 ± 0.13	0.90 ± 0.19	0.71 ± 0.03	0.49 ± 0.03	0.64 ± 0.24	0.41 ± 0.05
Stomach	1.90 ± 0.61	0.87 ± 0.09	0.94 ± 0.12	0.68 ± 0.10	0.80 ± 0.09	0.39 ± 0.11
S. intestine	1.73 ± 0.30	1.26 ± 0.11	1.11 ± 0.09	0.91 ± 0.03	1.03 ± 0.12	0.62 ± 0.08
L. intestine	1.11 ± 0.06	0.77 ± 0.11	0.79 ± 0.06	0.91 ± 0.12	1.05 ± 0.17	0.75 ± 0.15
Kidneys	91.0 ± 20.4	139 ± 11.9	196 ± 20.3	246 ± 25.6	198 ± 23.7	254 ± 24.4
Muscle	1.22 ± 0.06	0.63 ± 0.06	0.59 ± 0.12	0.35 ± 0.06	0.44 ± 0.06	0.32 ± 0.05
Bone	1.03 ± 0.13	0.79 ± 0.17	0.78 ± 0.37	0.60 ± 0.12	0.86 ± 0.14	0.79 ± 0.08
Skin	3.23 ± 0.40	2.19 ± 0.23	1.93 ± 0.13	1.59 ± 0.10	1.82 ± 0.12	1.45 ± 0.16
Brain	0.29 ± 0.04	0.14 ± 0.03	0.10 ± 0.01	0.05 ± 0.01	0.08 ± 0.00	0.03 ± 0.01
Tumor	4.52 ± 0.54	5.04 ± 0.94	6.60 ± 1.22	8.61 ± 1.21	6.20 ± 1.55	7.86 ± 1.40

Tumor-to-tissue ratios (rel. u.)												
Tumor/Blood	0.30	±	0.06	0.6	±	0.2	1.4	±	0.3	5.3	±	0.9	2.6	±	0.8	15	±	6
Tumor/Liver	0.77	±	0.12	1.3	±	0.3	1.7	±	0.3	3.0	±	0.5	1.5	±	0.4	3.2	±	0.6
Tumor/S. I.	2.6	±	0.6	4.0	±	0.8	6.0	±	1.2	9.5	±	1.4	6.0	±	1.7	13	±	3
Tumor/L. I.	4.1	±	0.5	6.5	±	1.5	8.3	±	1.6	9.5	±	1.8	5.9	±	1.8	10	±	3
Tumor/Kidney	0.050	±	0.013	0.036	±	0.007	0.034	±	0.007	0.035	±	0.006	0.031	±	0.009	0.031	±	0.006
Tumor/Muscle	3.7	±	0.5	8.0	±	1.7	11	±	3	24	±	5	14	±	4	24	±	6
Tumor/Bone	4.4	±	0.8	6.4	±	1.8	8	±	4	14	±	4	7	±	2	10	±	2

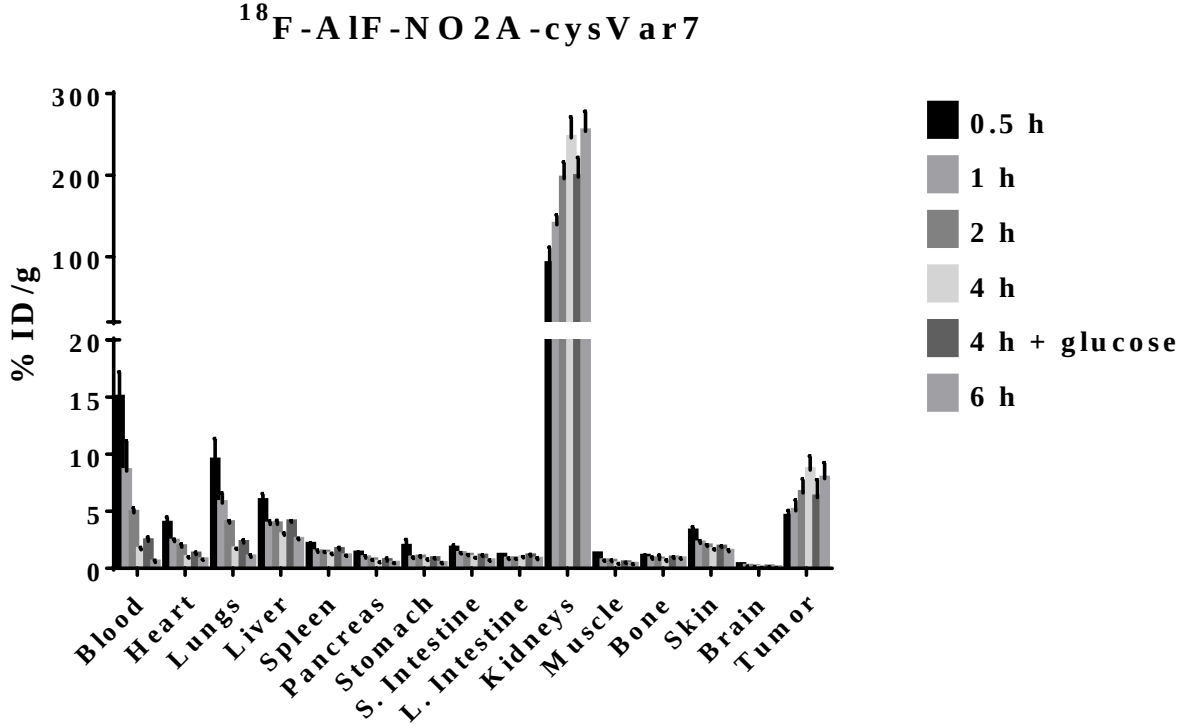
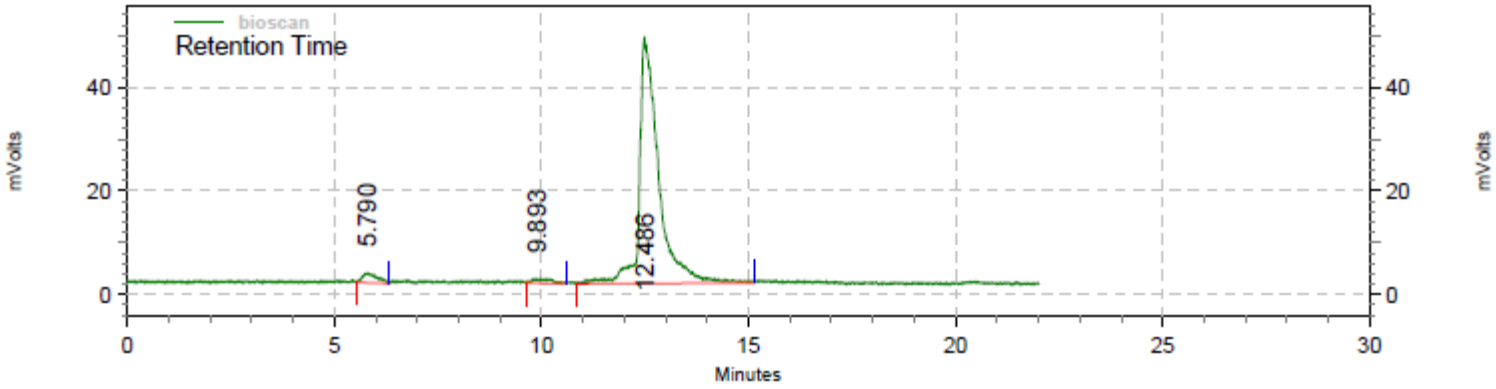


Fig. S10-1. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NO2A-cysVar7 (n = 3-5) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text. Glucose was administered i.p. 30 min prior to tracer injection.

HPLC Chromatogram of the Reconstituted ^{18}F-AlF-NO2A-cysVar7 Injectate

BIOSCAN



bioscan Results

Retention Time	Area	Area %	Height	Height %
5.790	47058	2.92	1956	3.88
9.893	26421	1.64	887	1.76
12.486	1540366	95.45	47597	94.36
Totals	1613845	100.00	50440	100.00

^{64}Cu –NO2A-cysVar3

Table S11-1. Tissue uptake (mean %ID ± SD) of ^{64}Cu-NO2A-cysVar3 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n=5)	2 h (n=5)	4 h (n=5)	4 h + glucose (n=5)	12 h (n=5)	24 h (n=8)	36 h (n=3)	48 h (n=4)
Blood	9.25 ± 4.66	4.14 ± 3.47	3.61 ± 3.04	2.67 ± 1.31	1.56 ± 0.41	2.82 ± 0.85	1.10 ± 0.24	0.64 ± 0.07
Heart	0.76 ± 0.11	0.70 ± 0.13	0.59 ± 0.07	0.70 ± 0.09	0.48 ± 0.06	0.49 ± 0.06	0.45 ± 0.02	0.48 ± 0.04
Lungs	6.41 ± 1.03	4.28 ± 1.18	4.44 ± 0.18	3.74 ± 1.28	1.90 ± 0.50	1.39 ± 0.17	1.51 ± 0.11	1.12 ± 0.10
Liver	11.9 ± 0.47	10.3 ± 1.55	10.5 ± 1.03	12.5 ± 2.43	10.6 ± 0.66	12.3 ± 1.75	14.5 ± 0.14	13.5 ± 0.35
Spleen	0.37 ± 0.02	0.40 ± 0.03	0.41 ± 0.05	0.45 ± 0.08	0.42 ± 0.05	0.55 ± 0.08	0.73 ± 0.17	0.68 ± 0.13
Pancreas	0.46 ± 0.04	0.38 ± 0.05	0.37 ± 0.07	0.36 ± 0.03	0.29 ± 0.01	0.40 ± 0.07	0.42 ± 0.03	0.43 ± 0.06
Stomach	0.56 ± 0.07	0.55 ± 0.07	0.61 ± 0.08	0.54 ± 0.11	0.86 ± 0.15	0.97 ± 0.20	1.01 ± 0.02	1.17 ± 0.13
S. intestine	4.32 ± 0.35	3.65 ± 0.52	4.31 ± 0.40	3.78 ± 0.79	5.44 ± 0.38	5.47 ± 0.75	5.26 ± 0.21	4.90 ± 0.31
L. intestine	1.32 ± 0.19	1.77 ± 0.16	2.45 ± 0.16	2.29 ± 0.36	3.66 ± 0.20	5.44 ± 0.87	4.11 ± 0.04	5.50 ± 0.43
Kidneys	6.92 ± 0.17	7.45 ± 0.44	8.52 ± 0.69	8.13 ± 0.80	6.05 ± 0.60	3.98 ± 0.52	3.94 ± 0.20	3.15 ± 0.21
Muscle	0.11 ± 0.04	0.13 ± 0.02	0.15 ± 0.01	0.14 ± 0.03	0.12 ± 0.04	0.11 ± 0.04	0.12 ± 0.03	0.09 ± 0.03
Bone	0.07 ± 0.03	0.05 ± 0.02	0.04 ± 0.01	0.05 ± 0.03	0.13 ± 0.06	0.08 ± 0.05	0.09 ± 0.00	0.09 ± 0.04
Skin	0.37 ± 0.04	0.46 ± 0.11	0.64 ± 0.21	0.44 ± 0.13	0.43 ± 0.08	0.33 ± 0.12	0.28 ± 0.05	0.30 ± 0.06
Brain	0.20 ± 0.04	0.18 ± 0.04	0.13 ± 0.03	0.17 ± 0.02	0.17 ± 0.03	0.16 ± 0.02	0.20 ± 0.01	0.22 ± 0.02
Tumor	0.19 ± 0.05	0.32 ± 0.09	0.53 ± 0.15	0.56 ± 0.12	1.12 ± 0.41	1.67 ± 0.87	2.37 ± 0.49	2.18 ± 0.33

Table S11-2. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NO2A-cysVar3 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	1 h (n=5)	2 h (n=5)	4 h (n=5)	4 h + glucose (n=5)	12 h (n=5)	24 h (n=8)	36 h (n=3)	48 h (n=4)
Blood	27.8 ± 2.54	20.0 ± 2.25	15.4 ± 1.76	23.2 ± 4.10	9.88 ± 1.30	6.77 ± 0.41	4.13 ± 0.51	3.34 ± 0.80
Heart	8.24 ± 1.29	6.48 ± 1.03	5.12 ± 0.63	7.26 ± 1.26	4.42 ± 0.54	4.80 ± 0.50	4.37 ± 0.29	5.10 ± 0.72
Lungs	24.6 ± 3.62	16.0 ± 2.56	14.7 ± 0.93	17.7 ± 4.09	8.17 ± 1.08	7.74 ± 0.95	6.19 ± 0.41	7.51 ± 1.45
Liver	14.1 ± 0.86	11.4 ± 1.57	10.4 ± 0.63	14.3 ± 2.63	10.3 ± 1.66	13.6 ± 1.58	13.0 ± 0.40	16.2 ± 2.35
Spleen	4.09 ± 0.45	3.39 ± 0.22	3.45 ± 0.35	5.19 ± 0.86	4.32 ± 0.62	5.07 ± 0.40	5.85 ± 0.37	4.90 ± 0.29
Pancreas	3.27 ± 0.39	2.36 ± 0.15	2.31 ± 0.25	3.04 ± 0.28	2.46 ± 0.34	2.68 ± 0.25	2.92 ± 0.11	3.24 ± 0.26
Stomach	2.24 ± 0.41	2.04 ± 0.35	1.27 ± 0.35	1.84 ± 0.35	1.09 ± 0.25	3.09 ± 1.19	1.56 ± 0.06	3.39 ± 1.03
S. intestine	3.48 ± 0.29	2.98 ± 0.38	2.68 ± 0.24	3.49 ± 0.61	3.92 ± 0.36	4.91 ± 0.64	4.14 ± 0.17	5.37 ± 0.15
L. intestine	2.02 ± 0.29	2.08 ± 0.12	2.99 ± 0.20	3.68 ± 0.62	4.31 ± 0.62	6.69 ± 1.33	4.77 ± 0.07	7.52 ± 0.67
Kidneys	25.3 ± 1.99	23.1 ± 1.42	24.0 ± 2.78	33.1 ± 6.29	20.5 ± 2.44	13.3 ± 1.14	12.4 ± 0.63	10.6 ± 1.84
Muscle	1.19 ± 0.10	1.03 ± 0.10	1.10 ± 0.15	1.39 ± 0.28	1.30 ± 0.18	1.20 ± 0.13	1.06 ± 0.18	1.01 ± 0.23
Bone	2.26 ± 0.29	1.18 ± 0.22	1.10 ± 0.14	1.88 ± 0.42	2.36 ± 0.31	1.78 ± 0.23	2.10 ± 0.10	1.88 ± 0.30
Skin	2.62 ± 0.14	2.63 ± 0.49	3.43 ± 0.34	3.27 ± 0.62	3.45 ± 0.54	3.17 ± 0.25	2.83 ± 0.11	2.93 ± 0.58

Brain	0.63	±	0.09	0.48	±	0.09	0.35	±	0.07	0.54	±	0.07	0.42	±	0.08	0.42	±	0.03	0.49	±	0.03	0.57	±	0.08
Tumor	4.19	±	0.65	5.43	±	0.93	8.21	±	0.86	8.81	±	1.78	14.5	±	2.45	19.6	±	2.34	19.2	±	1.88	16.2	±	1.90
Tumor-to-tissue ratios (rel. u.)																								
Tumor/Blood	0.15	±	0.03	0.27	±	0.06	0.53	±	0.08	0.38	±	0.10	1.5	±	0.3	2.9	±	0.4	4.7	±	0.7	4.8	±	1.3
Tumor/Liver	0.30	±	0.05	0.47	±	0.10	0.79	±	0.10	0.61	±	0.17	1.4	±	0.3	1.4	±	0.2	1.47	±	0.15	1.00	±	0.19
Tumor/S. I.	1.2	±	0.2	1.8	±	0.4	3.1	±	0.4	2.5	±	0.7	3.7	±	0.7	4.0	±	0.7	4.6	±	0.5	3.0	±	0.4
Tumor/L. I.	2.1	±	0.4	2.6	±	0.5	2.7	±	0.3	2.4	±	0.6	3.4	±	0.7	2.9	±	0.7	4.0	±	0.4	2.2	±	0.3
Tumor/Kidney	0.17	±	0.03	0.23	±	0.04	0.34	±	0.05	0.27	±	0.07	0.71	±	0.15	1.5	±	0.2	1.55	±	0.17	1.5	±	0.3
Tumor/Muscle	3.5	±	0.6	5.3	±	1.0	7.4	±	1.3	6.3	±	1.8	11	±	2	16	±	3	18	±	3	16	±	4
Tumor/Bone	1.9	±	0.4	4.6	±	1.2	7.4	±	1.2	4.7	±	1.4	6.2	±	1.3	11.0	±	1.9	9.1	±	1.0	8.6	±	1.7

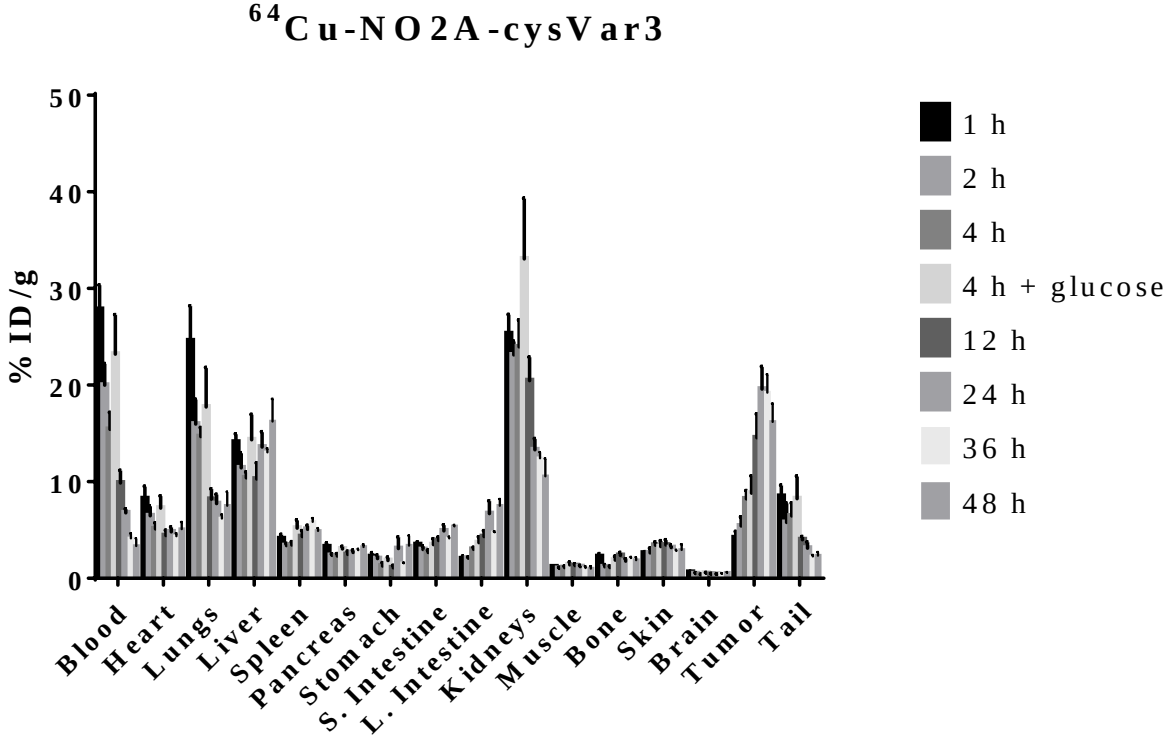
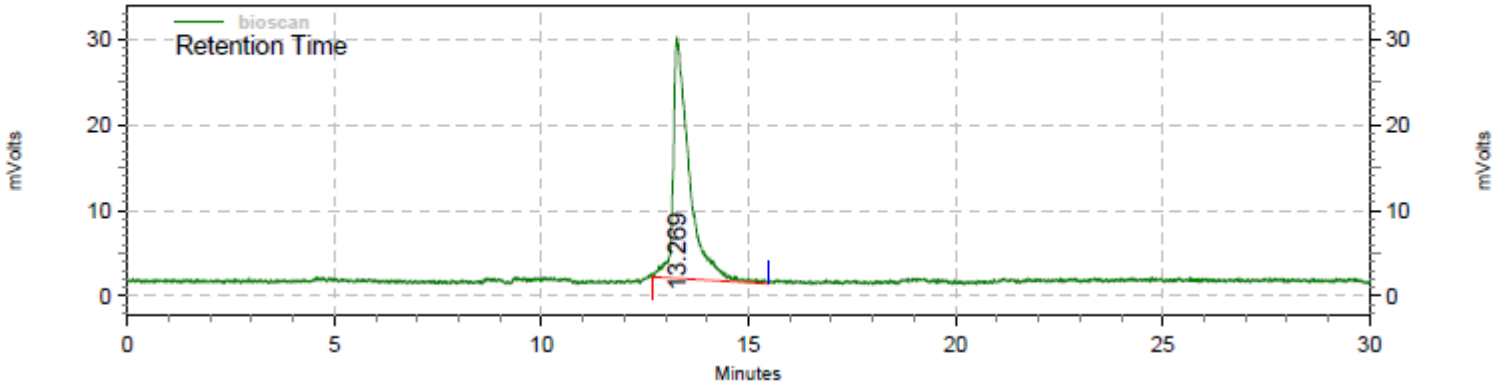


Fig. S11-1. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NO2A-cysVar3 (n = 3-8) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text. Glucose was administered i.p. 30 min prior to tracer injection.

HPLC Chromatogram of the Reconstituted ^{64}Cu –NO2A-cysVar3 Injectate

BIOSCAN



bioscan Results

Retention Time	Area	Area %	Height	Height %
13.269	763889	100.00	28222	100.00

Totals	763889	100.00	28222	100.00

^{18}F-AlF-NO2A-cysVar3

Table S12-1. Tissue uptake (mean %ID ± SD) of ^{18}F-AlF-NO2A-cysVar3 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n=5)	1 h (n=5)	2 h (n=5)	4 h (n=10)	4 h + glucose (n=4)	6 h (n=5)	8 h (n=5)
Blood	5.50 ± 3.19	9.36 ± 8.75	2.96 ± 1.05	5.81 ± 2.46	3.71 ± 1.51	2.46 ± 0.99	4.71 ± 2.12
Heart	1.26 ± 0.16	1.08 ± 0.23	0.82 ± 0.16	0.63 ± 0.12	0.66 ± 0.26	0.54 ± 0.08	0.55 ± 0.06
Lungs	11.2 ± 0.83	8.53 ± 2.35	7.02 ± 0.94	2.27 ± 1.04	3.98 ± 1.98	3.55 ± 0.64	1.06 ± 0.18
Liver	11.7 ± 1.93	12.1 ± 0.61	9.57 ± 1.32	8.00 ± 0.96	6.79 ± 2.07	7.91 ± 0.46	7.14 ± 0.67
Spleen	0.58 ± 0.03	0.49 ± 0.03	0.41 ± 0.02	0.45 ± 0.12	0.31 ± 0.09	0.36 ± 0.03	0.51 ± 0.05
Pancreas	0.70 ± 0.11	0.49 ± 0.18	0.41 ± 0.05	0.38 ± 0.08	0.26 ± 0.05	0.32 ± 0.02	0.26 ± 0.03
Stomach	0.91 ± 0.25	0.55 ± 0.08	0.53 ± 0.08	0.52 ± 0.06	0.38 ± 0.13	0.49 ± 0.09	0.44 ± 0.06
S. intestine	3.33 ± 0.44	3.91 ± 0.71	3.47 ± 0.69	2.09 ± 0.30	2.29 ± 0.66	1.90 ± 0.12	2.28 ± 0.12
L. intestine	1.57 ± 0.17	1.48 ± 0.08	1.50 ± 0.28	1.67 ± 0.61	1.54 ± 0.58	1.48 ± 0.31	1.10 ± 0.11
Kidneys	6.69 ± 0.67	8.56 ± 0.50	11.2 ± 0.61	10.1 ± 1.35	12.2 ± 2.88	10.6 ± 0.51	15.3 ± 1.74
Muscle	0.13 ± 0.05	0.16 ± 0.03	0.11 ± 0.03	0.16 ± 0.04	0.09 ± 0.05	0.15 ± 0.02	0.19 ± 0.07
Bone	0.14 ± 0.04	0.06 ± 0.03	0.12 ± 0.08	0.08 ± 0.06	0.07 ± 0.01	0.11 ± 0.01	0.11 ± 0.00
Skin	0.28 ± 0.09	0.42 ± 0.08	0.61 ± 0.16	0.29 ± 0.16	0.37 ± 0.12	0.36 ± 0.11	0.31 ± 0.07
Brain	0.34 ± 0.05	0.22 ± 0.02	0.21 ± 0.04	0.17 ± 0.05	0.12 ± 0.03	0.17 ± 0.03	0.19 ± 0.05
Tumor	0.29 ± 0.09	0.33 ± 0.06	0.47 ± 0.11	0.79 ± 0.22	0.56 ± 0.20	1.13 ± 0.21	1.97 ± 0.99

Table S12-2. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NO2A-cysVar3 administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad.

Tissue	0.5 h (n=5)	1 h (n=5)	2 h (n=5)	4 h (n=10)	4 h + glucose (n=4)	6 h (n=5)	8 h (n=5)
Blood	35.0 ± 2.37	32.3 ± 2.37	22.3 ± 2.67	20.8 ± 1.88	15.5 ± 2.06	16.5 ± 1.68	16.1 ± 1.87
Heart	10.7 ± 0.82	9.41 ± 1.13	7.02 ± 0.87	6.29 ± 1.03	5.60 ± 0.89	5.37 ± 0.62	5.91 ± 0.44
Lungs	36.3 ± 4.37	28.3 ± 3.61	22.2 ± 2.94	11.8 ± 3.79	16.6 ± 1.35	13.7 ± 1.97	7.03 ± 0.53
Liver	12.6 ± 0.55	12.9 ± 0.55	9.58 ± 1.52	9.06 ± 1.24	8.01 ± 1.18	8.85 ± 0.69	8.80 ± 0.25
Spleen	4.99 ± 0.30	4.00 ± 0.12	3.56 ± 0.35	3.73 ± 0.66	2.91 ± 0.35	3.36 ± 0.23	4.47 ± 0.60
Pancreas	3.90 ± 0.35	3.35 ± 0.30	2.49 ± 0.25	2.44 ± 0.20	1.87 ± 0.15	2.17 ± 0.20	2.09 ± 0.27
Stomach	1.92 ± 0.44	1.68 ± 0.23	1.31 ± 0.16	1.29 ± 0.62	1.11 ± 0.37	1.36 ± 0.43	0.86 ± 0.23
S. intestine	2.89 ± 0.22	3.24 ± 0.23	2.39 ± 0.25	2.06 ± 0.28	1.78 ± 0.23	1.79 ± 0.12	2.19 ± 0.29
L. intestine	1.76 ± 0.17	2.14 ± 0.10	2.05 ± 0.21	2.07 ± 0.63	2.05 ± 0.40	2.04 ± 0.41	1.57 ± 0.26
Kidneys	18.7 ± 1.45	26.2 ± 2.11	33.7 ± 3.00	34.3 ± 6.91	41.0 ± 1.54	35.2 ± 1.25	53.0 ± 6.26
Muscle	1.01 ± 0.15	1.23 ± 0.09	1.07 ± 0.14	1.55 ± 0.27	0.99 ± 0.39	1.47 ± 0.17	1.61 ± 0.39
Bone	2.14 ± 0.21	1.65 ± 0.31	1.81 ± 0.54	1.66 ± 0.61	1.44 ± 0.15	2.32 ± 0.12	2.37 ± 0.17
Skin	2.17 ± 0.21	2.63 ± 0.23	3.06 ± 0.39	2.92 ± 0.71	2.70 ± 0.45	3.73 ± 0.34	3.37 ± 0.20
Brain	0.85 ± 0.05	0.60 ± 0.05	0.51 ± 0.07	0.52 ± 0.14	0.34 ± 0.06	0.41 ± 0.07	0.47 ± 0.11
Tumor	4.35 ± 0.25	6.05 ± 0.63	7.42 ± 0.69	10.6 ± 2.26	9.09 ± 1.69	13.9 ± 1.37	13.9 ± 2.17

Tumor-to-tissue ratios (rel. u.)																								
Tumor/Blood	0.124	±	0.011	0.19	±	0.02	0.33	±	0.05	0.51	±	0.12	0.59	±	0.13	0.85	±	0.12	0.86	±	0.17
Tumor/Liver	0.35	±	0.02	0.47	±	0.05	0.78	±	0.14	1.2	±	0.3	1.1	±	0.3	1.6	±	0.2	1.6	±	0.3
Tumor/S. I.	1.51	±	0.14	1.9	±	0.2	3.1	±	0.4	5.1	±	1.3	5.1	±	1.2	7.8	±	0.9	6.3	±	1.3
Tumor/L. I.	2.5	±	0.3	2.8	±	0.3	3.6	±	0.5	5.1	±	1.9	4.4	±	1.2	6.8	±	1.5	9	±	2
Tumor/Kidney	0.23	±	0.02	0.23	±	0.03	0.22	±	0.03	0.31	±	0.09	0.22	±	0.04	0.40	±	0.04	0.26	±	0.05
Tumor/Muscle	4.3	±	0.7	4.9	±	0.6	6.9	±	1.1	6.9	±	1.9	9	±	4	9.5	±	1.4	9	±	2
Tumor/Bone	2.0	±	0.2	3.7	±	0.8	4.1	±	1.3	6	±	3	6.3	±	1.4	6.0	±	0.7	5.9	±	1.0

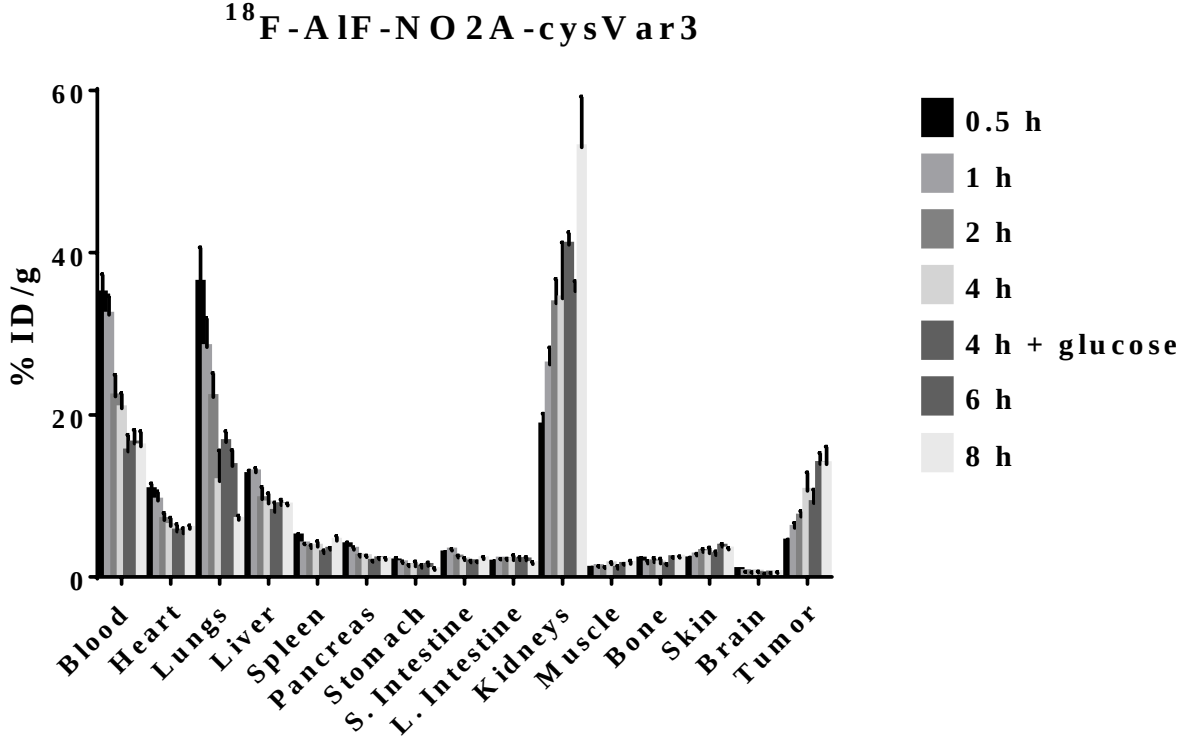
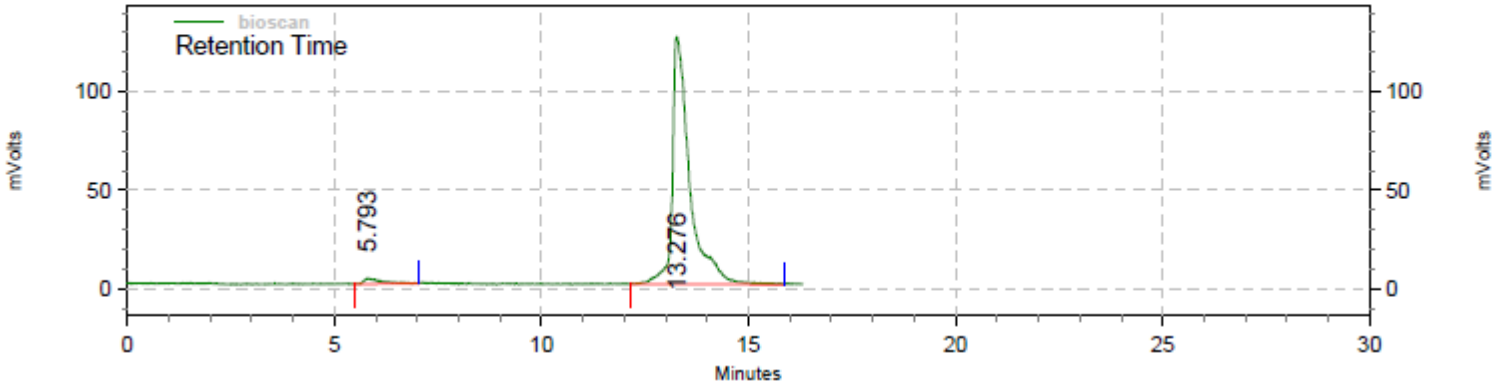


Fig. S12-1. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NO2A-cysVar3 (n = 4-10) administered via the lateral tail vein in female, BALB/c mice with orthotopic 4T1 breast cancer allografts implanted into the mammary fat pad full graph from text. Glucose was administered i.p. 30 min prior to tracer injection.

HPLC Chromatogram of the Reconstituted ^{18}F-AlF-NO2A-cysVar3 Injectate

BIOSCAN



bioscan Results

Retention Time	Area	Area %	Height	Height %
5.793	90241	2.40	3006	2.33
13.276	3669746	97.60	125746	97.67
Totals	3759987	100.00	128752	100.00

Data from lead tracers in other tumor models:

^{64}Cu-NO2A-cysVar3 in PC3 tumor-bearing male nude mice

Table S13-1. Tissue uptake (mean %ID ± SD) of ^{64}Cu-NO2A-cysVar3 administered via the lateral tail vein in male, nude mice with shoulder PC3 (prostate cancer) xenografts.

Tissue	1 h (n=8)	4 h (n=8)	12 h (n=8)	24 h (n=8)	48 h (n=8)
Blood	18.3 ± 3.05	10.1 ± 3.52	3.98 ± 1.12	2.30 ± 0.82	0.55 ± 0.21
Heart	0.80 ± 0.12	0.81 ± 0.28	0.66 ± 0.10	0.49 ± 0.08	0.41 ± 0.04
Lungs	2.09 ± 0.34	1.68 ± 0.36	1.29 ± 0.44	1.20 ± 0.18	0.90 ± 0.10
Liver	10.1 ± 1.67	11.6 ± 0.97	14.0 ± 2.35	16.7 ± 3.53	12.9 ± 1.90
Spleen	0.33 ± 0.05	0.33 ± 0.04	0.26 ± 0.04	0.29 ± 0.07	0.22 ± 0.03
Pancreas	0.37 ± 0.05	0.30 ± 0.03	0.27 ± 0.02	0.31 ± 0.04	0.25 ± 0.05
Stomach	0.56 ± 0.12	0.72 ± 0.06	0.93 ± 0.17	1.09 ± 0.29	1.12 ± 0.26
S. intestine	3.20 ± 1.01	3.69 ± 0.28	4.98 ± 0.75	6.06 ± 0.90	4.52 ± 0.67
L. intestine	1.38 ± 0.24	2.43 ± 0.66	3.96 ± 0.31	7.69 ± 1.23	7.83 ± 1.28
Kidneys	7.74 ± 2.26	8.61 ± 1.42	7.29 ± 0.98	5.43 ± 0.62	4.05 ± 0.48
Muscle	0.16 ± 0.03	0.15 ± 0.02	0.13 ± 0.04	0.12 ± 0.03	0.10 ± 0.03
Bone	0.07 ± 0.02	0.10 ± 0.07	0.10 ± 0.01	0.10 ± 0.04	0.08 ± 0.01
Skin	0.68 ± 0.37	0.85 ± 0.28	0.63 ± 0.20	0.66 ± 0.22	0.42 ± 0.11
Tumor	0.78 ± 0.30	1.04 ± 0.49	1.55 ± 0.71	2.34 ± 0.86	2.42 ± 0.78

Table S13-2. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NO2A-cysVar3 administered via the lateral tail vein in male, nude mice with shoulder PC3 (prostate cancer) xenografts.

Tissue	1 h (n=8)	4 h (n=8)	12 h (n=8)	24 h (n=8)	48 h (n=8)
Blood	22.0 ± 2.88	15.6 ± 2.21	7.51 ± 1.77	5.12 ± 0.77	2.48 ± 0.24
Heart	6.50 ± 0.80	5.71 ± 1.21	3.79 ± 0.82	3.32 ± 0.46	3.44 ± 0.26
Lungs	10.7 ± 1.66	8.63 ± 1.01	5.85 ± 0.89	5.69 ± 0.51	4.37 ± 0.57
Liver	8.38 ± 1.34	9.74 ± 1.10	9.66 ± 1.35	12.4 ± 2.39	11.1 ± 1.84
Spleen	3.42 ± 0.25	3.17 ± 0.27	2.96 ± 0.54	3.39 ± 0.25	3.06 ± 0.31
Pancreas	2.38 ± 0.40	2.02 ± 0.19	1.68 ± 0.31	1.74 ± 0.17	1.62 ± 0.21
Stomach	1.46 ± 0.44	1.41 ± 0.50	1.30 ± 0.39	1.56 ± 0.41	2.18 ± 0.35
S. intestine	2.02 ± 0.55	2.34 ± 0.38	2.22 ± 0.29	2.94 ± 0.36	3.08 ± 0.17
L. intestine	0.72 ± 0.17	1.34 ± 0.48	1.80 ± 0.23	3.13 ± 0.48	3.26 ± 0.18
Kidneys	16.1 ± 4.73	17.5 ± 2.72	13.9 ± 1.20	10.9 ± 1.11	8.88 ± 0.68
Muscle	1.13 ± 0.15	1.21 ± 0.11	1.05 ± 0.20	0.90 ± 0.09	0.81 ± 0.15
Bone	1.86 ± 0.31	1.71 ± 0.26	1.54 ± 0.26	1.61 ± 0.16	1.41 ± 0.15
Skin	2.80 ± 0.73	4.39 ± 0.84	3.67 ± 0.69	3.37 ± 0.37	2.60 ± 0.42
Tumor	3.16 ± 0.49	4.61 ± 0.82	5.58 ± 1.12	6.60 ± 0.76	6.32 ± 0.81
Tumor-to-tissue ratios (rel. u.)					
Tumor/Blood	0.14 ± 0.03	0.29 ± 0.07	0.7 ± 0.2	1.3 ± 0.2	2.6 ± 0.4
Tumor/Liver	0.38 ± 0.08	0.47 ± 0.10	0.58 ± 0.14	0.53 ± 0.12	0.57 ± 0.12
Tumor/S. I.	1.6 ± 0.5	2.0 ± 0.5	2.5 ± 0.6	2.2 ± 0.4	2.1 ± 0.3
Tumor/L. I.	4.4 ± 1.2	3.4 ± 1.4	3.1 ± 0.7	2.1 ± 0.4	1.9 ± 0.3
Tumor/Kidney	0.20 ± 0.07	0.26 ± 0.06	0.40 ± 0.09	0.61 ± 0.09	0.71 ± 0.11
Tumor/Muscle	2.8 ± 0.6	3.8 ± 0.8	5.3 ± 1.5	7.3 ± 1.1	7.8 ± 1.8
Tumor/Bone	1.7 ± 0.4	2.7 ± 0.6	3.6 ± 0.9	4.1 ± 0.6	4.5 ± 0.7

^{64}Cu-NO2A-cysVar3 in LNCaP tumor-bearing male nude mice

Table S13-3. Tissue uptake (mean %ID ± SD) of ^{64}Cu-NO2A-cysVar3 administered via the lateral tail vein in male, nude mice with shoulder LNCaP (prostate cancer) xenografts.

Tissue	1 h (n=8)	4 h (n=8)	12 h (n=8)	24 h (n=8)	48 h (n=11)
Blood	12.3 ± 5.56	11.1 ± 3.10	3.77 ± 1.21	4.29 ± 1.48	1.42 ± 0.55
Heart	0.94 ± 0.27	0.84 ± 0.29	0.56 ± 0.12	0.54 ± 0.11	0.38 ± 0.06
Lungs	2.50 ± 0.60	1.94 ± 0.49	1.33 ± 0.32	1.25 ± 0.40	0.71 ± 0.12
Liver	10.6 ± 1.85	10.1 ± 2.02	13.0 ± 2.07	14.5 ± 2.77	10.2 ± 3.13
Spleen	0.34 ± 0.07	0.26 ± 0.04	0.30 ± 0.06	0.35 ± 0.09	0.25 ± 0.09
Pancreas	0.42 ± 0.05	0.32 ± 0.06	0.25 ± 0.04	0.33 ± 0.05	0.26 ± 0.05
Stomach	0.62 ± 0.21	0.77 ± 0.13	0.86 ± 0.30	1.34 ± 0.28	0.95 ± 0.26
S. intestine	3.29 ± 0.54	3.99 ± 1.00	4.15 ± 0.64	6.92 ± 1.91	4.17 ± 0.55
L. intestine	1.37 ± 0.30	1.94 ± 0.69	3.91 ± 0.71	7.40 ± 2.22	5.95 ± 0.98
Kidneys	7.67 ± 2.14	8.46 ± 2.83	7.11 ± 0.65	5.94 ± 1.57	3.44 ± 0.62
Muscle	0.16 ± 0.04	0.13 ± 0.03	0.15 ± 0.04	0.13 ± 0.05	0.08 ± 0.02
Bone	0.07 ± 0.03	0.08 ± 0.03	0.09 ± 0.02	0.08 ± 0.02	0.04 ± 0.03
Skin	0.49 ± 0.17	0.78 ± 0.34	0.58 ± 0.10	0.85 ± 0.31	0.41 ± 0.22
Tumor	0.43 ± 0.48	1.13 ± 0.99	0.50 ± 0.48	2.24 ± 1.05	1.53 ± 1.67

Table S13-4. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NO2A-cysVar3 administered via the lateral tail vein in male, nude mice with shoulder LNCaP (prostate cancer) xenografts.

Tissue	1 h (n=8)	4 h (n=8)	12 h (n=8)	24 h (n=8)	48 h (n=11)
Blood	20.8 ± 5.81	15.3 ± 3.85	6.60 ± 1.47	7.21 ± 2.52	2.56 ± 0.78
Heart	6.30 ± 1.68	5.49 ± 1.25	3.09 ± 0.56	4.71 ± 2.50	2.94 ± 0.48
Lungs	11.8 ± 3.01	9.27 ± 2.16	5.57 ± 0.76	7.45 ± 5.42	4.17 ± 0.78
Liver	8.49 ± 2.99	8.94 ± 2.51	8.22 ± 1.01	13.4 ± 3.93	8.78 ± 1.16
Spleen	3.63 ± 1.58	2.99 ± 0.79	2.65 ± 0.23	4.17 ± 1.31	3.39 ± 0.87
Pancreas	2.30 ± 0.55	1.86 ± 0.34	1.43 ± 0.13	1.89 ± 0.36	1.54 ± 0.23
Stomach	1.70 ± 0.81	1.32 ± 0.44	0.96 ± 0.36	1.94 ± 0.64	1.61 ± 0.56
S. intestine	2.07 ± 0.42	2.11 ± 0.53	1.99 ± 0.37	3.08 ± 0.81	2.40 ± 0.46
L. intestine	0.72 ± 0.17	0.99 ± 0.38	2.11 ± 0.58	3.65 ± 0.67	3.13 ± 0.66
Kidneys	14.9 ± 6.43	16.7 ± 6.94	11.6 ± 0.66	12.9 ± 5.07	7.49 ± 1.47
Muscle	1.00 ± 0.31	1.07 ± 0.21	0.87 ± 0.14	1.14 ± 0.49	0.74 ± 0.19
Bone	1.87 ± 1.16	1.51 ± 0.33	1.32 ± 0.14	1.67 ± 0.46	1.12 ± 0.27
Skin	2.33 ± 0.59	3.72 ± 0.91	3.36 ± 0.52	4.32 ± 0.87	2.42 ± 0.38
Tumor	4.09 ± 1.43	6.32 ± 2.15	5.14 ± 2.65	9.47 ± 3.32	6.12 ± 1.92
Tumor-to-tissue ratios (rel. u.)					
Tumor/Blood	0.20 ± 0.09	0.41 ± 0.18	0.78 ± 0.44	1.31 ± 0.65	2.40 ± 1.05
Tumor/Liver	0.48 ± 0.24	0.71 ± 0.31	0.62 ± 0.33	0.71 ± 0.32	0.70 ± 0.24
Tumor/S. I.	1.98 ± 0.80	3.00 ± 1.27	2.59 ± 1.42	3.08 ± 1.35	2.55 ± 0.94
Tumor/L. I.	5.65 ± 2.36	6.38 ± 3.27	2.43 ± 1.42	2.60 ± 1.03	1.96 ± 0.74
Tumor/Kidney	0.28 ± 0.15	0.38 ± 0.20	0.44 ± 0.23	0.74 ± 0.39	0.82 ± 0.30
Tumor/Muscle	4.08 ± 1.91	5.89 ± 2.30	5.92 ± 3.21	8.32 ± 4.60	8.28 ± 3.33
Tumor/Bone	2.19 ± 1.56	4.19 ± 1.70	3.90 ± 2.05	5.68 ± 2.52	5.48 ± 2.17

^{64}Cu-NO2A-cysVar3 in B16-F10 tumor-bearing female C57Bl/6 mice

Table S13-5. Tissue uptake (mean %ID ± SD) of ^{64}Cu-NO2A-cysVar3 administered via the lateral tail vein in female, C57Bl/6 mice with orthotopic B16-F10 (melanoma) allografts.

Tissue	1 h (n=4)	4 h (n=3)	12 h (n=4)	14.5 h (n=3)	24 h (n=4)
Blood	18.6 ± 1.60	6.70 ± 1.64	3.95 ± 0.65	1.05 ± 0.36	1.37 ± 0.67
Heart	1.28 ± 0.67	0.76 ± 0.07	0.58 ± 0.08	0.63 ± 0.13	0.45 ± 0.03
Lungs	2.55 ± 0.25	2.26 ± 0.43	2.58 ± 0.53	1.93 ± 0.03	1.09 ± 0.17
Liver	9.24 ± 1.45	9.54 ± 0.50	11.1 ± 1.04	9.77 ± 0.63	11.3 ± 0.74
Spleen	0.37 ± 0.09	0.32 ± 0.04	0.34 ± 0.02	0.37 ± 0.09	0.35 ± 0.05
Stomach	0.77 ± 0.20	0.76 ± 0.13	1.36 ± 0.95	0.78 ± 0.17	1.27 ± 0.17
S. intestine	4.38 ± 0.94	3.94 ± 0.90	5.64 ± 0.68	4.52 ± 0.66	5.16 ± 0.36
L. intestine	1.35 ± 0.33	2.72 ± 0.42	3.44 ± 0.38	3.89 ± 0.25	6.11 ± 0.27
Kidneys	4.54 ± 0.78	8.92 ± 0.99	8.08 ± 0.88	6.61 ± 1.23	4.82 ± 0.43
Muscle	0.10 ± 0.03	0.15 ± 0.05	0.15 ± 0.03	0.18 ± 0.07	0.13 ± 0.05
Bone	0.07 ± 0.02	0.17 ± 0.02	0.13 ± 0.04	0.35 ± 0.05	0.21 ± 0.08
Skin (ears)	0.30 ± 0.19	0.33 ± 0.08	0.28 ± 0.10	0.14 ± 0.01	0.36 ± 0.10
Skin (shoulder)	0.26 ± 0.09	0.22 ± 0.03	0.26 ± 0.10	0.21 ± 0.04	0.15 ± 0.03
Tumor	2.15 ± 1.37	1.45 ± 2.02	3.58 ± 2.10	1.27 ± 1.95	1.61 ± 0.93

Table S13-6. Tissue uptake (mean %ID/g ± SD) of ^{64}Cu-NO2A-cysVar3 administered via the lateral tail vein in female, C57Bl/6 mice with orthotopic B16-F10 (melanoma) allografts.

Tissue	1 h (n=4)	4 h (n=3)	12 h (n=4)	14.5 h (n=3)	24 h (n=4)
Blood	28.4 ± 0.74	19.5 ± 1.06	11.6 ± 1.52	12.0 ± 2.14	6.37 ± 0.39
Heart	8.24 ± 0.28	6.86 ± 0.52	4.55 ± 0.64	4.75 ± 0.72	4.32 ± 0.41
Lungs	12.6 ± 1.32	11.8 ± 3.38	9.94 ± 1.74	8.97 ± 1.30	5.81 ± 0.59
Liver	11.1 ± 1.47	10.3 ± 0.38	8.97 ± 0.68	10.1 ± 1.35	11.7 ± 1.42
Spleen	4.24 ± 0.34	4.33 ± 0.10	4.22 ± 0.41	4.54 ± 0.63	4.96 ± 0.29
Stomach	2.53 ± 0.45	1.55 ± 0.07	1.55 ± 0.73	2.00 ± 0.37	3.25 ± 0.54
S. intestine	3.41 ± 0.30	3.14 ± 0.30	3.43 ± 0.30	4.00 ± 0.29	5.44 ± 0.87
L. intestine	1.94 ± 0.40	2.98 ± 0.13	3.27 ± 0.37	4.35 ± 0.28	7.24 ± 0.51
Kidneys	13.5 ± 2.19	28.5 ± 5.17	26.0 ± 2.38	21.0 ± 1.61	16.2 ± 1.31
Muscle	0.75 ± 0.12	1.48 ± 0.31	1.23 ± 0.13	1.31 ± 0.30	1.28 ± 0.28
Bone	1.86 ± 0.49	2.53 ± 0.17	2.03 ± 0.39	2.66 ± 0.39	2.28 ± 0.19
Skin (ears)	1.80 ± 0.50	3.90 ± 0.71	3.35 ± 0.30	3.55 ± 0.30	3.73 ± 0.56
Skin (shoulder)	1.98 ± 0.43	5.68 ± 0.65	3.93 ± 0.73	5.49 ± 0.41	5.09 ± 0.88
Tumor	5.42 ± 0.76	9.29 ± 0.43	11.6 ± 0.51	11.2 ± 0.94	11.4 ± 2.92
Tumor-to-tissue ratios (rel. u.)					
Tumor/Blood	0.19 ± 0.03	0.48 ± 0.03	1.00 ± 0.14	0.94 ± 0.19	1.8 ± 0.5
Tumor/Liver	0.49 ± 0.09	0.90 ± 0.05	1.29 ± 0.11	1.11 ± 0.17	1.0 ± 0.3
Tumor/S. I.	1.6 ± 0.3	3.0 ± 0.3	3.4 ± 0.3	2.8 ± 0.3	2.1 ± 0.6
Tumor/L. I.	2.8 ± 0.7	3.1 ± 0.2	3.5 ± 0.4	2.6 ± 0.3	1.6 ± 0.4
Tumor/Kidney	0.40 ± 0.09	0.33 ± 0.06	0.45 ± 0.05	0.53 ± 0.06	0.70 ± 0.19
Tumor/Muscle	7.2 ± 1.5	6.3 ± 1.4	9.4 ± 1.1	9 ± 2	9 ± 3
Tumor/Bone	2.9 ± 0.9	3.7 ± 0.3	5.7 ± 1.1	4.2 ± 0.7	5.0 ± 1.3

^{18}F-AlF-NO2A-cysVar3 in PC3 tumor-bearing male nude mice

Table S13-7. Tissue uptake (mean %ID ± SD) of ^{18}F-AlF-NO2A-cysVar3 administered via the lateral tail vein in male, nude mice with shoulder PC3 (prostate cancer) xenografts.

Tissue	1 h (n=3)			4 h (n=4)			6 h (n=4)			8 h (n=4)		
Blood	10.7	±	5.84	6.88	±	1.21	6.37	±	3.17	4.62	±	2.24
Heart	0.84	±	0.22	0.61	±	0.07	0.82	±	0.19	0.68	±	0.09
Lungs	2.25	±	0.84	2.06	±	0.77	0.77	±	0.12	0.84	±	0.17
Liver	9.87	±	0.55	7.09	±	1.13	6.97	±	1.87	6.02	±	0.51
Spleen	0.24	±	0.03	0.29	±	0.06	0.21	±	0.05	0.35	±	0.03
Pancreas	0.37	±	0.06	0.27	±	0.03	0.19	±	0.02	0.30	±	0.09
Stomach	0.46	±	0.07	0.41	±	0.09	0.35	±	0.04	0.42	±	0.05
S. intestine	2.66	±	0.10	2.47	±	0.58	1.73	±	0.29	2.20	±	0.22
L. intestine	1.16	±	0.06	1.32	±	0.27	1.15	±	0.24	1.19	±	0.18
Kidneys	5.82	±	0.64	10.6	±	1.97	12.3	±	1.50	13.7	±	0.50
Muscle	0.10	±	0.03	0.17	±	0.02	0.12	±	0.03	0.15	±	0.01
Bone	0.05	±	0.01	0.06	±	0.03	0.10	±	0.02	0.09	±	0.01
Skin	0.59	±	0.22	1.10	±	0.26	0.29	±	0.09	0.42	±	0.09
Tumor	0.96	±	0.45	1.04	±	0.55	1.11	±	0.53	1.79	±	0.98

Table S13-8. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NO2A-cysVar3 administered via the lateral tail vein in male, nude mice with shoulder PC3 (prostate cancer) xenografts.

Tissue	1 h (n=3)			4 h (n=4)			6 h (n=4)			8 h (n=4)		
Blood	16.2	±	2.29	11.6	±	1.83	8.66	±	0.91	9.08	±	0.70
Heart	5.41	±	0.99	4.04	±	0.28	3.79	±	1.14	4.11	±	0.14
Lungs	9.39	±	3.35	7.69	±	1.72	3.93	±	0.86	4.27	±	0.45
Liver	6.03	±	0.68	5.12	±	0.35	4.16	±	0.40	4.19	±	0.45
Spleen	2.23	±	0.36	2.04	±	0.11	1.83	±	0.20	2.47	±	0.11
Pancreas	1.78	±	0.31	1.56	±	0.20	1.13	±	0.15	1.37	±	0.30
Stomach	0.65	±	0.02	0.53	±	0.03	0.63	±	0.10	0.44	±	0.11
S. intestine	1.28	±	0.15	1.22	±	0.08	0.86	±	0.14	1.38	±	0.09
L. intestine	0.58	±	0.06	0.58	±	0.02	0.72	±	0.19	0.74	±	0.13
Kidneys	10.7	±	1.10	18.2	±	3.15	19.2	±	4.25	25.8	±	2.51
Muscle	0.68	±	0.13	1.03	±	0.11	0.90	±	0.28	0.91	±	0.23
Bone	1.21	±	0.29	1.24	±	0.25	1.31	±	0.09	1.28	±	0.26
Skin	1.96	±	0.29	3.73	±	0.08	2.32	±	0.46	2.53	±	0.15
Tumor	3.05	±	0.36	4.02	±	0.35	4.19	±	0.55	3.99	±	0.27
Tumor-to-tissue ratios (rel. u.)												
Tumor/Blood	0.19	±	0.03	0.35	±	0.06	0.48	±	0.08	0.44	±	0.04
Tumor/Liver	0.51	±	0.08	0.79	±	0.09	1.01	±	0.16	0.95	±	0.12
Tumor/S. I.	2.4	±	0.4	3.3	±	0.4	4.8	±	1.0	2.9	±	0.3
Tumor/L. I.	5.2	±	0.8	7.0	±	0.7	5.8	±	1.7	5.4	±	1.0
Tumor/Kidney	0.29	±	0.04	0.22	±	0.04	0.22	±	0.06	0.15	±	0.02
Tumor/Muscle	4.5	±	1.0	3.9	±	0.5	4.7	±	1.6	4.4	±	1.1
Tumor/Bone	2.5	±	0.7	3.2	±	0.7	3.2	±	0.5	3.1	±	0.7

^{18}F-AlF-NO2A-cysVar3 in LNCaP tumor-bearing male nude mice

Table S13-9. Tissue uptake (mean %ID ± SD) of ^{18}F-AlF-NO2A-cysVar3 administered via the lateral tail vein in male, nude mice with shoulder LNCaP (prostate cancer) xenografts.

Tissue	1 h (n=4)	4 h (n=4)	6 h (n=4)	8 h (n=4)
Blood	17.7 ± 4.60	11.4 ± 0.78	5.43 ± 1.31	5.49 ± 1.77
Heart	0.66 ± 0.12	0.62 ± 0.10	0.73 ± 0.16	0.53 ± 0.08
Lungs	1.98 ± 0.24	1.15 ± 0.14	1.33 ± 0.30	0.83 ± 0.08
Liver	7.33 ± 1.08	5.00 ± 0.70	5.84 ± 0.23	5.42 ± 0.28
Spleen	0.30 ± 0.08	0.25 ± 0.03	0.25 ± 0.02	0.27 ± 0.08
Pancreas	0.33 ± 0.09	0.25 ± 0.05	0.23 ± 0.03	0.20 ± 0.04
Stomach	0.47 ± 0.08	0.53 ± 0.32	0.47 ± 0.03	0.43 ± 0.09
S. intestine	2.16 ± 0.31	1.98 ± 0.36	1.72 ± 0.08	2.57 ± 0.29
L. intestine	0.97 ± 0.23	1.08 ± 0.18	1.19 ± 0.09	1.38 ± 0.36
Kidneys	5.61 ± 0.62	8.87 ± 1.78	11.0 ± 1.50	13.9 ± 0.79
Muscle	0.14 ± 0.03	0.16 ± 0.03	0.16 ± 0.05	0.13 ± 0.02
Bone	0.05 ± 0.02	0.05 ± 0.03	0.08 ± 0.01	0.08 ± 0.03
Skin	0.71 ± 0.27	0.89 ± 0.17	0.42 ± 0.08	0.37 ± 0.05
Tumor	0.39 ± 0.26	0.78 ± 0.47	3.73 ± 3.95	1.81 ± 2.24

Table S13-10. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NO2A-cysVar3 administered via the lateral tail vein in male, nude mice with shoulder LNCaP (prostate cancer) xenografts.

Tissue	1 h (n=4)	4 h (n=4)	6 h (n=4)	8 h (n=4)
Blood	20.0 ± 2.48	12.4 ± 1.74	12.1 ± 2.16	10.2 ± 2.15
Heart	5.28 ± 0.92	4.41 ± 1.11	4.39 ± 0.69	3.69 ± 0.28
Lungs	9.19 ± 1.18	5.82 ± 0.49	6.48 ± 1.05	4.42 ± 0.51
Liver	5.87 ± 0.39	4.62 ± 0.51	4.56 ± 0.43	4.33 ± 0.49
Spleen	2.79 ± 0.52	1.96 ± 0.14	1.93 ± 0.25	2.60 ± 0.27
Pancreas	2.01 ± 0.30	1.37 ± 0.26	1.42 ± 0.24	1.42 ± 0.36
Stomach	1.07 ± 0.21	0.70 ± 0.14	0.63 ± 0.20	0.67 ± 0.13
S. intestine	1.38 ± 0.26	1.03 ± 0.05	0.97 ± 0.05	1.32 ± 0.29
L. intestine	0.47 ± 0.14	0.42 ± 0.06	0.47 ± 0.03	0.68 ± 0.34
Kidneys	10.7 ± 0.68	17.3 ± 1.51	23.9 ± 3.74	28.6 ± 2.36
Muscle	0.75 ± 0.09	0.93 ± 0.16	1.14 ± 0.24	1.06 ± 0.33
Bone	1.23 ± 0.22	1.02 ± 0.25	1.33 ± 0.14	1.24 ± 0.27
Skin	2.59 ± 0.45	3.03 ± 0.42	2.91 ± 0.48	2.64 ± 0.18
Tumor	3.04 ± 0.37	4.41 ± 0.47	4.79 ± 1.08	4.57 ± 0.75
Tumor-to-tissue ratios (rel. u.)				
Tumor/Blood	0.15 ± 0.03	0.36 ± 0.06	0.40 ± 0.11	0.45 ± 0.12
Tumor/Liver	0.52 ± 0.07	0.95 ± 0.15	1.1 ± 0.3	1.1 ± 0.2
Tumor/S. I.	2.2 ± 0.5	4.3 ± 0.5	4.9 ± 1.1	3.5 ± 0.9
Tumor/L. I.	7 ± 2	10.5 ± 1.8	10 ± 2	6.7 ± 3.6
Tumor/Kidney	0.28 ± 0.04	0.25 ± 0.03	0.20 ± 0.06	0.16 ± 0.03
Tumor/Muscle	4.1 ± 0.7	4.7 ± 0.9	4.2 ± 1.3	4.3 ± 1.5
Tumor/Bone	2.5 ± 0.5	4.3 ± 1.1	3.6 ± 0.9	3.7 ± 1.0

^{18}F-AlF-NO2A-cysVar3 in B16-F10 tumor-bearing female C57Bl/6 mice

Table S13-11. Tissue uptake (mean %ID ± SD) of ^{18}F-AlF-NO2A-cysVar3 administered via the lateral tail vein in female, C57Bl/6 mice with orthotopic B16-F10 (melanoma) allografts and one group of female, BALB/c mice with orthotopic B16-F10 (melanoma) allografts at 6 h.

Tissue	1 h (n=4)	4 h (n=3)	6 h (n=4)	8 h (n=3)	6 h (n=3)*
Blood	12.7 ± 4.93	8.16 ± 1.53	4.17 ± 0.88	4.17 ± 2.37	6.25 ± 2.32
Heart	0.92 ± 0.13	0.58 ± 0.07	0.55 ± 0.13	0.54 ± 0.08	0.67 ± 0.14
Lungs	2.70 ± 0.89	1.80 ± 0.65	2.06 ± 0.64	1.00 ± 0.10	1.53 ± 0.07
Liver	10.2 ± 0.90	6.59 ± 1.19	5.94 ± 0.45	5.88 ± 0.38	6.65 ± 0.57
Spleen	0.30 ± 0.02	0.29 ± 0.07	0.28 ± 0.03	0.26 ± 0.05	0.51 ± 0.08
Stomach	0.63 ± 0.12	0.51 ± 0.05	0.42 ± 0.06	0.45 ± 0.08	0.62 ± 0.09
S. intestine	3.04 ± 0.45	2.20 ± 0.39	2.40 ± 0.29	2.14 ± 0.19	2.79 ± 0.23
L. intestine	1.04 ± 0.13	1.14 ± 0.10	1.23 ± 0.05	1.28 ± 0.36	1.72 ± 0.04
Kidneys	6.11 ± 0.70	8.10 ± 0.58	11.8 ± 0.73	13.0 ± 1.76	10.20 ± 0.11
Muscle	0.14 ± 0.04	0.18 ± 0.06	0.15 ± 0.05	0.17 ± 0.03	0.17 ± 0.04
Bone	0.07 ± 0.03	0.09 ± 0.03	0.12 ± 0.04	0.14 ± 0.04	0.12 ± 0.00
Skin (ears)	0.33 ± 0.09	0.44 ± 0.15	0.34 ± 0.06	0.24 ± 0.05	0.26 ± 0.04
Skin (shoulder)	0.38 ± 0.11	0.58 ± 0.21	0.32 ± 0.17	0.20 ± 0.08	0.16 ± 0.05
Tumor	0.67 ± 0.16	1.56 ± 1.89	2.29 ± 1.97	1.96 ± 2.18	0.11 ± 0.07

*BALB/c time point.

Table S13-12. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NO2A-cysVar3 administered via the lateral tail vein in female, C57Bl/6 mice with orthotopic B16-F10 (melanoma) allografts and one group of female, BALB/c mice with orthotopic B16-F10 (melanoma) allografts at 6 h.

Tissue	1 h (n=5)	4 h (n=5)	6 h (n=5)	8 h (n=5)	6 h (n=3)*
Blood	29.3 ± 3.06	17.2 ± 1.77	15.3 ± 0.70	14.3 ± 0.80	21.1 ± 0.27
Heart	8.86 ± 1.02	5.79 ± 0.63	4.86 ± 0.53	5.36 ± 0.66	6.98 ± 0.81
Lungs	15.2 ± 1.14	10.1 ± 3.12	9.70 ± 1.84	5.82 ± 0.57	8.76 ± 0.47
Liver	10.1 ± 1.60	7.22 ± 0.64	7.41 ± 0.20	7.01 ± 0.35	8.02 ± 0.11
Spleen	3.79 ± 0.36	3.18 ± 0.41	3.22 ± 0.19	4.00 ± 0.14	4.89 ± 0.28
Stomach	1.90 ± 0.91	1.70 ± 0.35	1.18 ± 0.27	0.82 ± 0.12	0.86 ± 0.05
S. intestine	2.77 ± 0.52	2.08 ± 0.28	1.89 ± 0.16	1.93 ± 0.05	2.73 ± 0.09
L. intestine	1.48 ± 0.30	1.84 ± 0.14	1.59 ± 0.12	1.72 ± 0.23	2.05 ± 0.08
Kidneys	20.1 ± 3.35	28.9 ± 3.19	40.7 ± 3.41	46.0 ± 2.98	34.4 ± 0.71
Muscle	1.09 ± 0.15	1.46 ± 0.14	1.51 ± 0.23	1.46 ± 0.12	1.52 ± 0.16
Bone	2.33 ± 0.34	2.22 ± 0.50	2.64 ± 0.45	2.42 ± 0.13	2.75 ± 0.20
Skin (ears)	2.35 ± 0.34	3.61 ± 0.61	3.91 ± 0.21	2.78 ± 0.17	3.00 ± 0.28
Skin (shoulder)	2.77 ± 0.57	4.86 ± 0.74	4.93 ± 0.68	4.77 ± 0.35	5.24 ± 0.44
Tumor	6.12 ± 0.60	8.23 ± 0.84	8.02 ± 2.00	10.5 ± 1.73	8.62 ± 1.53
Tumor-to-tissue ratios (rel. u.)					
Tumor/Blood	0.21 ± 0.03	0.48 ± 0.07	0.52 ± 0.13	0.73 ± 0.13	0.41 ± 0.07
Tumor/Liver	0.61 ± 0.11	1.14 ± 0.15	1.1 ± 0.3	1.5 ± 0.3	1.07 ± 0.19
Tumor/S. I.	2.2 ± 0.5	4.0 ± 0.7	4.2 ± 1.1	5.4 ± 0.9	3.2 ± 0.6
Tumor/L. I.	4.1 ± 0.9	4.5 ± 0.6	5.0 ± 1.3	6.1 ± 1.3	4.2 ± 0.8
Tumor/Kidney	0.30 ± 0.06	0.28 ± 0.04	0.20 ± 0.05	0.23 ± 0.04	0.25 ± 0.04
Tumor/Muscle	5.6 ± 0.9	5.6 ± 0.8	5.3 ± 1.6	7.2 ± 1.3	5.7 ± 1.2
Tumor/Bone	2.6 ± 0.5	3.7 ± 0.9	3.0 ± 0.9	4.3 ± 0.8	3.1 ± 0.6

*BALB/c time point.

^{18}F-AlF-NO2A-cysVar3 in U87MG tumor-bearing male nude mice

Table S13-13. Tissue uptake (mean %ID ± SD) of ^{18}F-AlF-NO2A-cysVar3 administered via the lateral tail vein in male, nude mice with U87MG xenografted flank tumors at 6 h.

Tissue	6 h (n=6)
Blood	5.34 ± 2.40
Heart	0.73 ± 0.15
Lungs	0.91 ± 0.15
Liver	5.59 ± 0.72
Spleen	0.24 ± 0.06
Pancreas	0.21 ± 0.04
Stomach	0.46 ± 0.08
S. Intestine	2.13 ± 0.20
L. Intestine	1.15 ± 0.18
Kidneys	10.1 ± 1.1
Muscle	0.15 ± 0.03
Bone	0.079 ± 0.014
Skin	0.39 ± 0.07
Brain	0.151 ± 0.018
U87MG Tumor	0.49 ± 0.23

*BALB/c time point.

Table S13-14. Tissue uptake (mean %ID/g ± SD) of ^{18}F-AlF-NO2A-cysVar3 administered via the lateral tail vein in male, nude mice with U87MG xenografted flank tumors at 6 h.

Tissue	6 h (n=6)
Blood	10.7 ± 0.9
Heart	4.17 ± 0.42
Lungs	4.57 ± 0.81
Liver	4.34 ± 0.88
Spleen	2.30 ± 0.34
Pancreas	1.30 ± 0.24
Stomach	0.70 ± 0.24
S. Intestine	1.20 ± 0.21
L. Intestine	0.50 ± 0.09
Kidneys	17.4 ± 2.5
Muscle	0.88 ± 0.08
Bone	1.27 ± 0.11
Skin	2.43 ± 0.30
Brain	0.38 ± 0.04
U87MG Tumor	3.97 ± 0.56
Tumor-to-tissue ratios (rel. u.)	
Tumor/Blood	0.37 ± 0.06
Tumor/Liver	0.9 ± 0.2
Tumor/S. I.	3.3 ± 0.7
Tumor/L. I.	7.9 ± 1.8
Tumor/Kidney	0.23 ± 0.05
Tumor/Muscle	4.5 ± 0.8
Tumor/Bone	3.1 ± 0.5
Tumor/Brain	10.5 ± 1.9

*BALB/c time point.

Supporting Information References

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