SUPPLEMENTAL DATA

[^{18}F]Fluoroaminosuberic Acid ([^{18}F]FASu) Synthesis

Briefly, $^{18}F^-/H_2{}^{18}O$ produced on TRIUMF's TR13 cyclotron, azeotropically dried with t-butyl ammonium bicarbonate and combined with the precursor (di-tert-butyl 2-((bis-tert-butoxycarbonyl)amino)-5-(tosyloxy)octanedioate in DMSO. The reaction was kept at 95°C for 15 min after which di-tert-butyl 2-((bis-tert-butoxycarbonyl)amino)-5-fluorooctanedioate was purified by C18 SepPak. Acetonitrile was removed under vacuum followed by the addition of 2M HCl and heating at 100°C for 15 min. The final product was purified by SCX cation exchange column and formulated in PBS buffer. Decay corrected radiochemical yield (d.c. RCY) 18 ± 6% (n=6), radiochemical purity (RCP) > 98%, molar activity (MA) 17.5 ± 7 GBq/mmol (n=6).

(4S)-4-(3-[^{18}F]Fluoropropyl)-*L*-glutamate ([^{18}F]FSPG) Synthesis

Briefly, $^{18}F^-/H_2{}^{18}O$ was azeotropically dried with Kryptofix 2.2.2./K_2CO_3. The precursor di-tert-butyl (2S,4S)-2-tert-butoxycarbonylamino-4-nitrophenylsulfonyloxy-propyl)-pentanedioate was added and kept at 70°C for 5 min and the fluorinated product purified by C18 SepPak. After eluting in 2 mL acetonitrile, HCl (2 M, 2 mL) was added and the mixture kept at 100°C for 8 min. The final product was purified by SCX cation exchange column and taken up in PBS buffer. d.c. RCY 28 ± 7% (n=4), RCP > 98%, MA 15 ± 5 GBq/mmol (n=4).

In Vitro Uptake Specificity Studies

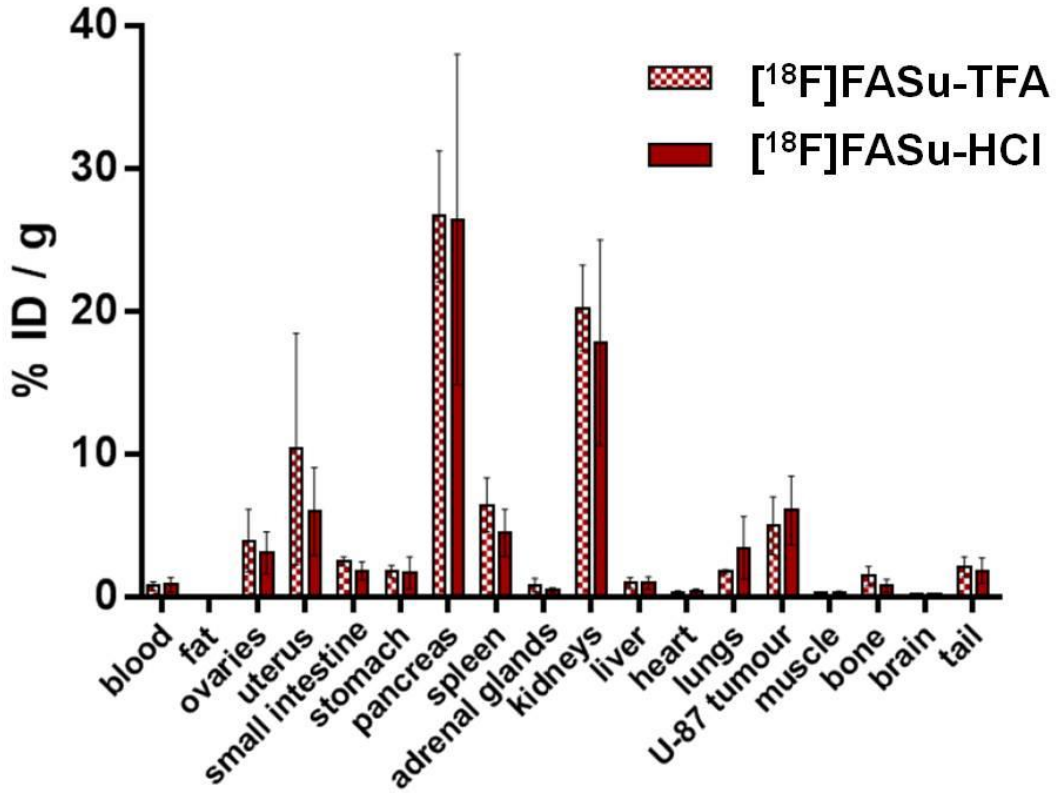
MDA-MB-231 triple-negative breast cancer cells were obtained as a gift from Dr. Connie Eaves (BC Cancer, Vancouver, Canada). Glioblastoma U-87 cell line and prostate cancer PC-3 cells were obtained from ATCC. HT-29 human colorectal cancer cells were obtained as a gift from Dr. Donald Yapp (BC Cancer, Vancouver, Canada). SKOV3 ovarian cancer cells and A549 non-small cell lung cancer (NSCLC) cell line were obtained as a gift from Dr. Poul Sorensen (BC Cancer, Vancouver, Canada).

Cells were cultured in their respective media supplemented with 10% fetal bovine serum and 100 U/mL penicillin-streptomycin. MDA-MB-231 cells were maintained in Dulbecco's Modified Eagle's Medium; SKOV3 and HT-29 cells in McCoy's 5A Medium; U-87 cells in Roswell Park Memorial Institute 1640 Medium; A549 and PC-3 cells in F12K Medium.

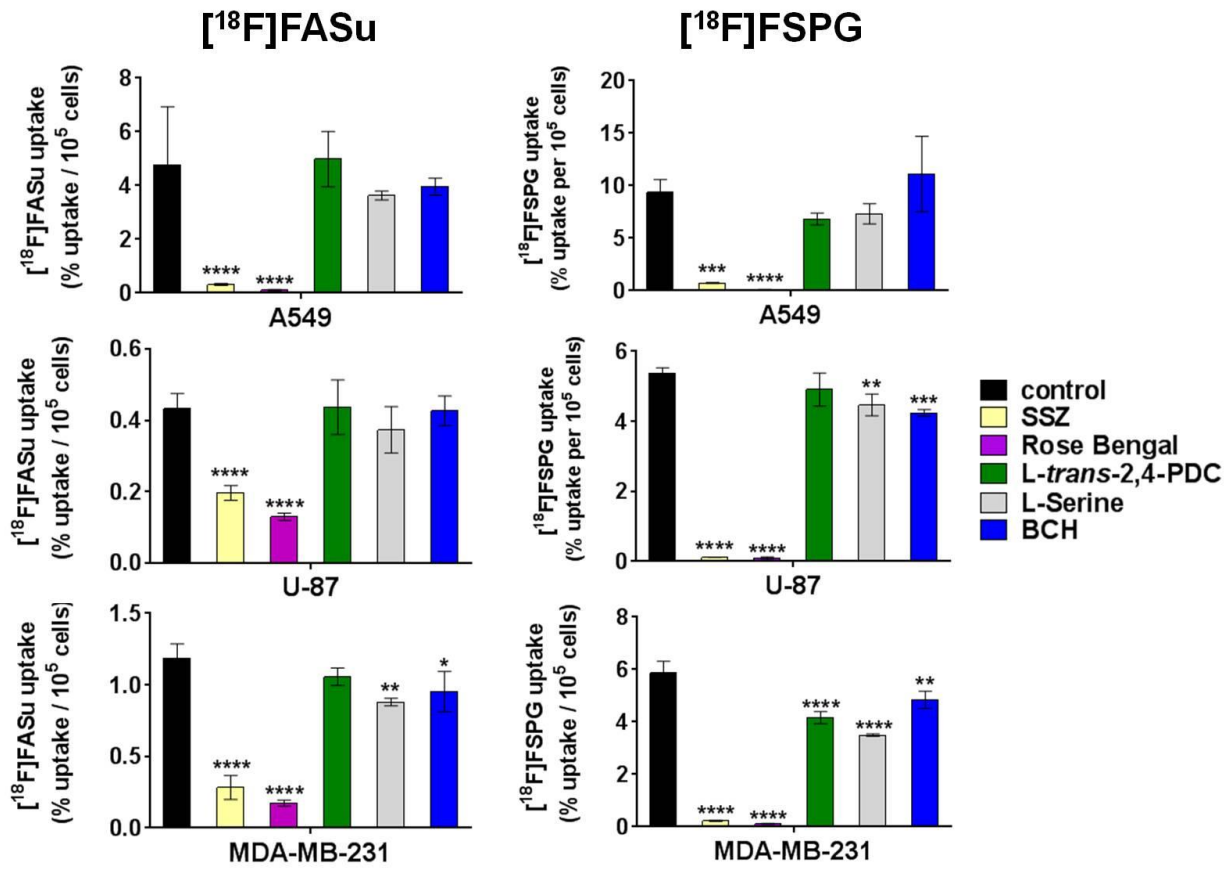
Western Blotting

Tumor tissue of euthanized Rag2M mice was dissected, snap-frozen, minced, homogenized in RIPA buffer supplemented with protease inhibitors (Roche complete mini tablets). The lysates were centrifuged (16,000 × g, 20 min, 4°C). Supernatant was collected and protein concentration determination was determined using Pierce BCA Protein Assay Kit (Thermofisher Scientific). Next, 10 µg of total protein per sample was electrophoresed onto an 8% SDS-PAGE gel. Proteins were transferred onto a nitrocellulose membrane (Amersham) using a semi-dry transfer apparatus (BioRad, 10 V for 25 min). After transfer the membrane was blocked for 1 h with 1 × TBS-tween (TBS-T) with 5% skim milk powder, and probed overnight at 4 ºC with anti-human xCT/*SCL7A11* rabbit monoclonal antibody (Cell Signaling Technologies (CST), cat.#12691), at 1:500 dilution in 1 × TBS-T 1% skim milk powder). For EAAT blots, 20 µg of protein was loaded per lane and the following antibodies were used at 1:1000 dilution: CST, anti-EAAT1 D44E2 rabbit IgG, anti-EAAT2 E3P5K rabbit IgG, anti-EAAT3 E1E6M rabbit IgG from CST and anti-EAAT4 antibody LS-C402250 from LSBio. An anti-β-actin monoclonal antibodies (Abcam, cat.#ab119716, for xCT blots and cat#ab115777 for EAAT blots) were used at 1:1000 dilution to monitor β-actin as a loading control. After the primary antibody, the membrane was washed 3 × with TBS-T. Next, the membrane was incubated with goat anti-rabbit IgG-HRP from CST (cat.#7074) at 1:2000 dilution in 1 × TBS-T 1% skim milk powder for 1 h at room temperature. After this incubation, the blot was washed nine times for 5-10 min each with ~2 mL 1 × TBS-T. The blot was developed using ECL Select Western Blotting Detection Reagent (GE Healthcare –

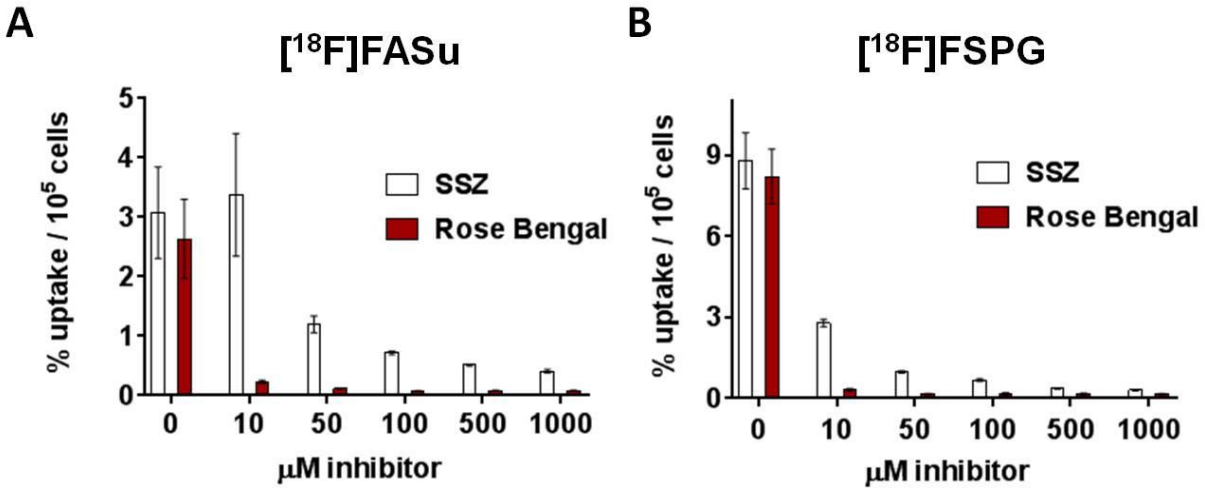
Amersham), according to manufacturer's instructions, and visualized with ImageQuant LAS 4000 (GE Healthcare).



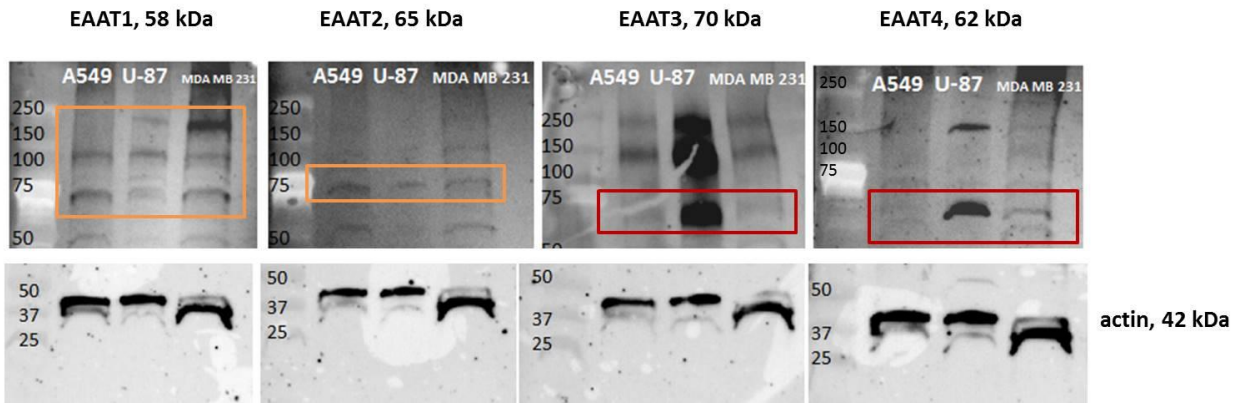
Supplemental Figure 1. Validation study: biodistribution of [^{18}F]FASu in two different formulations. Statistical analysis revealed no significant differences in the uptake of two tracer formulations (n>3).



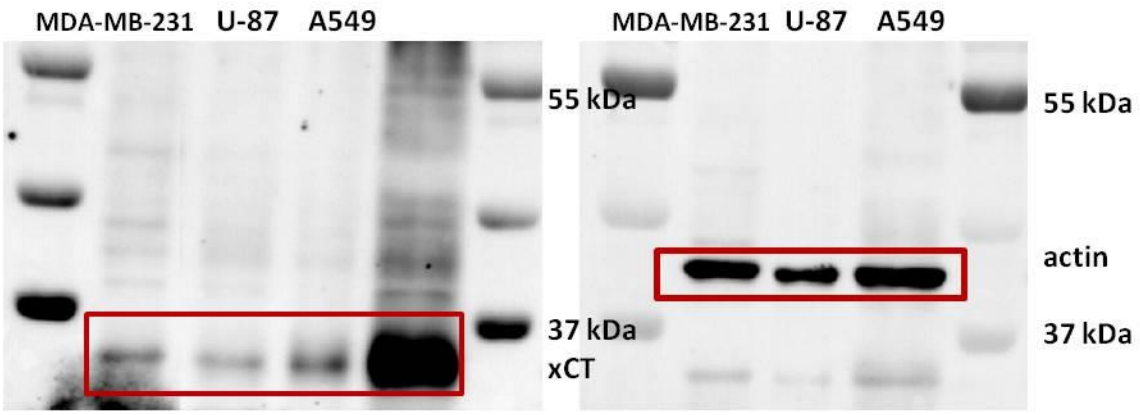
Supplemental Figure 2. Two-way ANOVA analysis of 1 h uptake of [^{18}F]FASu (**A**, left panel) and [^{18}F]FSPG (**B**, right panel) in A549, U-87 and MDA-MB-231 cells, expressed as percent uptake per 100.000 cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



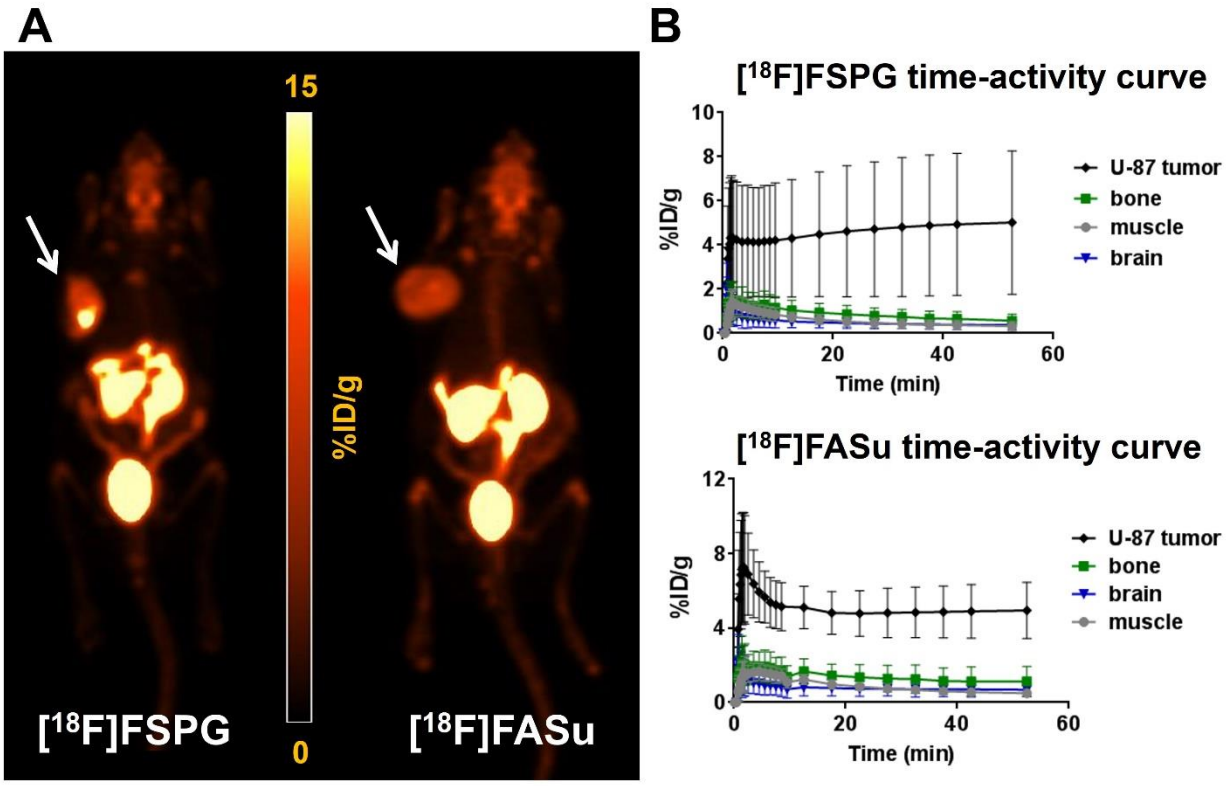
Supplemental Figure 3. 60 min *in vitro* uptake of [^{18}F]FASu and [^{18}F]FSPG in A549 cells. Dose-dependent competition cell uptake assays were carried out in A549 cells using 148 kBq/well of either [^{18}F]FASu (**A**) or [^{18}F]FSPG (**B**) and increasing concentration of sulfasalazine (SSZ, white bars) or Rose Bengal (red bars).



Supplemental Figure 4. Western blot image of cell lysates indicating EAAT3 70 kDa and EAAT4 62 kDa protein expression in U-87 and MDA-MB-231 whole cell lysates. EAAT1 and EAAT2 bands detected correspond to predicted molecular weights of the respective glycoproteins and homodimers (*1*) in all three cell lysates and in the case of EAAT1 homotrimers as well in U-87 and MDA-MB-231 lysates. Corresponding actin blots are shown below. 20 µg of protein was loaded per lane.



Supplemental Figure 5. Western blot image of tumour lysates indicating 35 kDa xCT protein expression in MDA-MB-231, U-87 and A549 tumour lysates. Corresponding actin blot is shown on the right. 10 µg of protein was loaded per lane.



Supplemental Figure 6. Comparative study: uptake of [^{18}F]FSPG and [^{18}F]FASu in U-87 xenograft-bearing mice at 1 h post – injection. (**A**) MIP PET images of [^{18}F]FSPG and [^{18}F]FASu at 45 – 60 min after injection. White arrows indicate location of U-87 tumors. (**B**) Time-activity curves of selected ROIs for [^{18}F]FSPG and [^{18}F]FASu from 0 – 55 min after injection.

REFERENCES

1. Parkin GM, Udawela M, Gibbons A, Dean B. Glutamate transporters, EAAT1 and EAAT2, are potentially important in the pathophysiology and treatment of schizophrenia and affective disorders. *World J Psychiatry*. 2018;8:51-63.