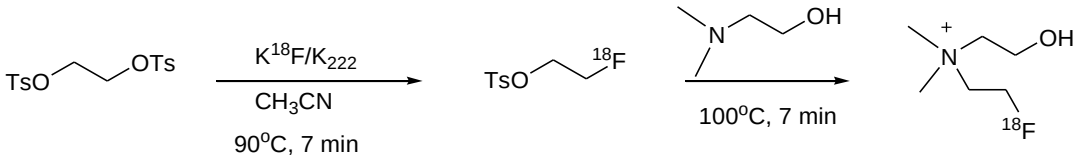
Supplementary Methods and References

Synthesis of [^{18}F]fluoroethylcholine (FCH) and [^{18}F]fluoroacetate (FACE)

[^{18}F]FCH was synthesized using the one-pot two step synthesis described previously [1].



[^{18}F]-Fluoride, produced by the ^{18}O(p, n)^{18}F nuclear reaction using >95% enriched ^{18}O-water, was trapped on a QMA-carbonate cartridge and ^{18}O-water was removed. A volume of 1 mL of a solution of potassium carbonate (2 mg) and Kryptofix 2.2.2. (6 mg) in water/acetonitrile (2:8) was used to elute [^{18}F]-fluoride from the cartridge into a sealed Wheaton 5-mL reaction vial. The contents then were dried by addition of 0.5 mL of acetonitrile followed by evaporation at 110°C of the solvent using a nitrogen stream. This process was repeated three times. The reaction vial was cooled to 60ºC and 1,2-bis(tosyloxy)ethane (10 mg) in acetonitrile (400 µL) was added and the reaction vial was heated at 90°C for 7 min. Subsequently, acetonitrile was removed from the reaction mixture at 90ºC for 3-5 min by a nitrogen stream. Dimethylaminoethanol (300 µL) was added to the reaction vial and the mixture was heat at 100ºC for 7 min. The mixture was cooled to 50°C, diluted with 96% aqueous EtOH (3 mL) [2] and this solution containing [^{18}F]FCH, was passed through a cation exchange cartridge (plus CM, Waters). The cartridge was washed first with EtOH (10 mL) and finally with water (20 mL) [3]. The [^{18}F]FCH was collected in a vial through a 0.22 um sterile filter by eluting with 1 mL of sterile 0.9% saline and released for quality control testing. Synthesis was complete within 60 min with an overall yield of 10-20%. Radiochemical purity was >95% as assessed by radio-TLC using 50/50 (v/v) saline/95% aqueous acetonitrile (R_f = 0.7) as eluent. The non-radioactive FECH was used as a standard. Chemical identification and purity

(>95%) were confirmed by radio-HPLC on an Agilent 1100 series HPLC system equipped with a Bioscan Flowcount FC-3400 Pin diode detector and an Agilent G1362A refractive index detector. The HPLC column was Phenomenex Luna C18 reversed-phase (150 x 4.6 mm) and the mobile phase consisted of 5 mmol/L heptanesulfonic acid (pH 2-2.5) adjusted using 85% phosphoric acid) and acetonitrile (90/10 v/v) at a flow rate of 1 mL/min.

[^{18}F]Fluoroacetate ([^{18}F]ACE) was synthesized using as described previously [4] with minor modification. Radioactivity was collected on a Chromafix 30-PS-HCO_3 cartridge, where [^{18}F]fluoride was trapped and ^{18}O-water was removed. A volume of 1 mL of a solution of potassium carbonate (2 mg) and Kryptofix 2.2.2. (6 mg) in water/acetonitrile (2:8) was used to elute [^{18}F]fluoride from the cartridge into a sealed Wheaton 5-mL reaction vial. The contents were then dried by addition of 0.5 mL of acetonitrile followed by evaporation at 110°C of the solvent using a nitrogen stream. This process was repeated three times. The reaction vial was cooled to 100ºC and ethyl (p-tosyloxy)acetate (10 mg, ABX) in acetonitrile (400 uL) was added and the reaction vial heated at 100°C for 5 min. The temperature of the reaction vial was lowered to 80ºC, and ethyl [^{18}F]fluoroacetate was then distilled into a vial containing 1 ml of ethanol and 200 uL of 1 N NaOH. After 5 min at 40ºC, hydrolysis was complete and ethanol was removed using a nitrogen stream. The residual mixture was neutralized with 1N HCl (180 uL), diluted with 5 mL of 0.9% saline solution and passed through a sterile 0.22-um filter. Radio-TLC (95% acetonitrile/water) showed only one radioactive peak (Rf = 0.2) corresponding to unlabeled fluoroacetate standard. Analytic HPLC (Bio-Rad 125-0100, 10% acetonitrile 7 mN H_2SO_4 solution) showed only one radioactive peak eluting at 4.3 min. The total preparation time was ~60 min with a yield of 20%.

References

[1] Asti M, Farioli D, Iori M, Guidotti C, Versari A, Salvo D: Efficient automated one-step synthesis of 2-[18F]fluoroethylcholine for clinical imaging: optimized reaction conditions and improved quality controls of different synthetic approaches. Nucl Med Biol 2010, 37:309-15.

[2] Pascali G, D'Antonio L, Bovone P, Gerundini P, August T: Optimization of automated large-scale production of [(18)F]fluoroethylcholine for PET prostate cancer imaging. Nucl Med Biol 2009, 36:569-74.

[3] DeGrado TR, Coleman RE, Wang S, Baldwin SW, Orr MD, Robertson CN, Polascik TJ, Price DT: Synthesis and evaluation of 18F-labeled choline as an oncologic tracer for positron emission tomography: initial findings in prostate cancer. Cancer Res 2001, 61:110-7.

[4] Ponde DE, Dence CS, Oyama N, Kim J, Tai YC, Laforest R, Siegel BA, Welch MJ: 18F-fluoroacetate: a potential acetate analog for prostate tumor imaging--in vivo evaluation of 18F-fluoroacetate versus 11C-acetate. J Nucl Med 2007, 48:420-8.

Supplementary Figure legends

Figure S1. Suppression of [^{18}F]FCH uptake by treatment with rapamycin in TSC2-deficient cells *in vivo* (representative mouse 2). 30-40 min post-injection (p.i.) maximum intensity projection (MIP) [^{18}F]FCH-PET and CT images pre (**A, B**) and post (**C, D**) rapamycin treatment (72 hr). Left panels show MIP tumor VOI PET images superimposed on MIP CT. Right panels show fused MIP PET and CT images. Physiological liver (L) uptake is noted. Color bars show intensity per voxel in SUV for PET or Hounsfield units (HU) for CT. (**E**) shows corresponding time activity curves (TACs) for tumor (SUV_{mean} and SUV_{peak}, suppressed by rapamycin treatment) and muscle (unchanged with rapamycin treatment). Field of view (FOV) was used as a quality control for tracer injection.

Figure S2. [^{18}F]FCH uptake in TSC2-deficient cells *in vivo* after 72-hr exposure to vehicle control (two representative mice). 30-40 min p.i. MIP [^{18}F]FCH-PET and CT images pre (**A, B, E, F**) and post (**C, D, G, H**) vehicle (72 hr). Left panels (A, C, E, G) show MIP tumor VOI PET images superimposed on MIP CT. Right panels (B, D, F, H) show fused MIP PET and CT images. Physiological liver (L) uptake is noted. Color bars show intensity per voxel in SUV for PET or Hounsfield units (HU) for CT. (**I, J**) show corresponding TACs for tumor (SUV_{mean} and SUV_{peak}), muscle, and blood. FOV was used as a quality control for tracer injection.

* high uptake areas within the posterior wall of the chest, outside the lung field, of aspecific nature

Figure S3. [^{18}F]FCH and [^{18}F]FACE-PET images of LAM patient renal angiomyolipoma-derived tumor xenografts. 30-40 min p.i. MIP [^{18}F]FCH (top panels) and [^{18}F]FACE-PET (bottom panels) and CT images. Left panels show MIP tumor VOI PET image superimposed on

MIP CT. Right panels show fused MIP PET and CT images. Color bars show intensity per voxel in SUV for PET or Hounsfield units (HU) for CT.

Figure S4. [^{18}F]FACE-PET of TSC2-deficient tumors after 72-hr exposure to vehicle control (a representative mouse). 30-40 min and 80-90 min p.i. MIP [^{18}F]FACE-PET and CT images pre (**A, B, E, F**) and post (**C, D, G, H**) vehicle treatment (72 hr). Left panels (A, C, E, G) show MIP tumor VOI PET images superimposed on MIP CT. Right panels (B, D, F, H) show fused MIP PET and CT images. Color bars show intensity per voxel in SUV for PET or Hounsfield units (HU) for CT. (**I**) shows corresponding TACs for blood and (**J**) for tumor (SUV_{mean} and SUV_{peak}) and muscle (unchanged with rapamycin treatment). FOV was used as a quality control for tracer injection.

Figure S5. [^{18}F]FACE-PET of TSC2-deficient tumors after 48-hr exposure to rapamycin or vehicle control. 30-40 min p.i. MIP [^{18}F]FACE-PET and CT images pre (**A, B, E, F**) and post (**C, D, G, H**) rapamycin or vehicle treatment (48 hr). Left panels (A, C, E, G) show MIP tumor VOI PET images superimposed on MIP CT. Right panels (B, D, F, H) show fused MIP PET and CT images. Color bars show intensity per voxel in SUV for PET or Hounsfield units (HU) for CT. (**I, J**) show corresponding TACs for tumor (SUV_{mean} and SUV_{peak}) and muscle (unchanged with rapamycin treatment). FOV was used as a quality control for tracer injection.

Figure S6. Increased mitochondrial acetate oxidation induced by treatment with rapamycin. Seahorse assay was performed on ELT3 cells treated with rapamycin (rapa, 20 nM, 20 hr), fluoroacetate (FACE, 40 mM, 20 hr), or both (Rapa + FACE). Results are presented as ATP production (**A**), spare respiratory capacity (**B**), and proton leak (**C**) relative to DMSO. ANOVA with Tukey's multiple comparison test was applied. *$p<0.05$, **$p<0.01$, ***$p<0.001$. ELT3 cells

were treated with rapamycin (20 nM, 24 hr) and incubated with 1 uCi/ml of [1-^{14}C]acetate before ^{14}C-CO2 collection. One-sample T-test was applied, *$p<0.05$.