SUPPLEMENTARY INFORMATION

MATERIALS AND METHODS

Materials

((2*S*)-2-[[(1*S*)-1-carboxy-5-[(6-fluoranylpyridine-3-carbonyl)-amino]-pentyl]-carbamoyl-amino]pentanedioic acid (DCFPyL; ABX GmbH, Radeberg, Germany) and all other chemicals (Sigma-Aldrich, St. Louis, MO, USA) were used without further purification. Fluorine-18 was obtained from the National Institutes of Health cyclotron facility (Bethesda, MD, USA). Chromafix 30-PS-HCO3 anion-exchange cartridge was procured from Macherey-Nagel (Düren, Germany). Phenomenex Luna C18 (2) column (10 × 250 mm, 5 µm) was purchased from Phenomenex (Torrance, CA, USA) whereas all other columns and Sep-Pak cartridges were obtained from Agilent Technologies (Santa Clara, CA, USA) and Waters (Milford, MA, USA), respectively. Semi-prep purification and analytical HPLC analyses for radioactive compounds were performed on an Agilent 1200 Series instrument outfitted with multi-wavelength detectors.

In vitro Binding Studies

In vitro saturation studies were performed to determine the binding affinity (K_d) and PSMA expression levels (B_{max}) in tumors from three LuCaP PDXs (LuCaP73, LuCaP167, and LuCaP136) and PC3 positive PSMA xenografts (human prostate cancer cell line transfected with human PSMA; PC3(+)). The PDX tumors were obtained from mouse xenograft models and stored at -70^{o}C until use. Membrane preparations from these PDXs were obtained by homogenization of the minced tissue at 20x (weight:volume) in homogenization buffer (50 mM tris + 1.5 mM EDTA + 0.25 M sucrose) followed by

centrifugation (500 x g; 8 min) and then removal of the supernatant for the assay. To the same amount of tumor membrane preparations increasing concentration of [^{18}F]DCFPyL (0.5-70 nM) representing total bound activity (B_t) were added to duplicate tubes. Non-specific binding (B_{nsp}) was determined by incubating the membrane preparations with the same concentration of [^{18}F]DCFPyL in the presence of the non-radioactive PSMA ligand DCFPyL (10^{-6}M). After incubation (2 h at RT) separation of bound [^{18}F]DCFPyL from free was accomplished by filtration of the membrane preparations using GF/C filter papers and 2 washes with saline. Filter papers were collected, and the radioactive content was counted on a gamma counter (PerkinElmer 2480 Wizard3) and specific binding (B_{sp}; B_t-B_{nsp}=B_{sp}) was determined. The data were analyzed using a non-linear regression curve fits including one-site hyperbola (GraphPad Prism 7). Aliquots of each membrane preparation were taken for the determination of the protein concentration (Bradford method).

H&E IHC staining

Hematoxylin and eosin (H&E) and immunohistochemistry (IHC) staining was performed on fast-frozen or formalin-fixed LuCaP73, LuCaP136, and LuCaP167 tumor sections (5mm) to determine PSMA, AR, and CD31 levels. Isotype control reagents were used in place of primary antibodies for negative controls. Slides were digitized with an Aperio ScanScope XT (Leica) at 200X in a single z-plane. Aperio whole-slide images were evaluated and graded for staining intensity by a board-certified veterinary pathologist. Machine learning was utilized on whole slide images to classify detected cells as tumors and to exclude regions of intratumoral necrosis and stroma. An automated algorithm was

utilized to quantify IHC staining, the result was reported as positive cells as % total cells and H-score. Microvessel density was quantified using anti-CD31 IHC and Aperio image analysis algorithms on manually annotated regions of interest within tumor tissue (regions of necrosis were avoided).

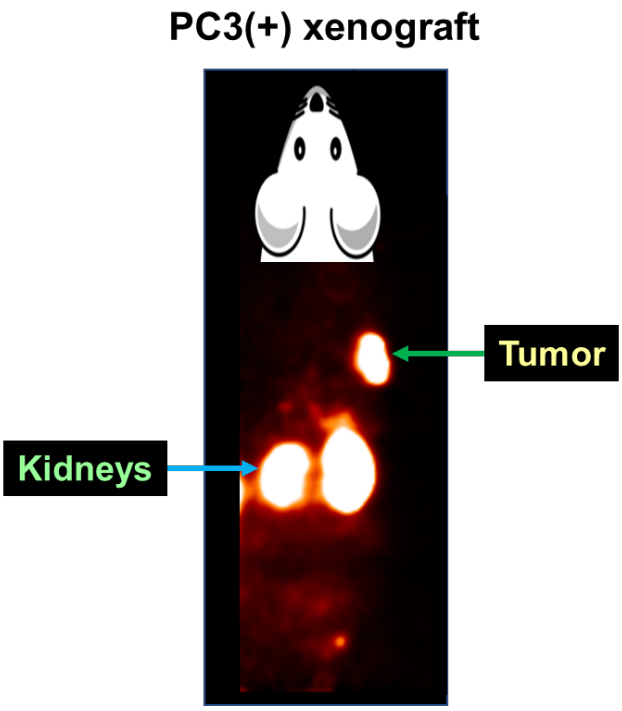
Real-time RT-PCR

Total RNA from control and treated tumor samples was isolated using a RNeasy RNA Isolation Kit (Qiagen). Reverse transcription (RT) of cDNA was performed using SuperScript IV VILO Master Mix (Invitrogen) and qPCR was done using FastStart Universal SYBR Green Master (ROX) (Roche) and the Stepone Plus RT-PCR System (Applied Biosystems). All reactions were normalized to hypoxanthine-guanine phosphoribosyltransferase 1 (*HPRT1*) and run in triplicate using the primers listed in SI Figure 1.

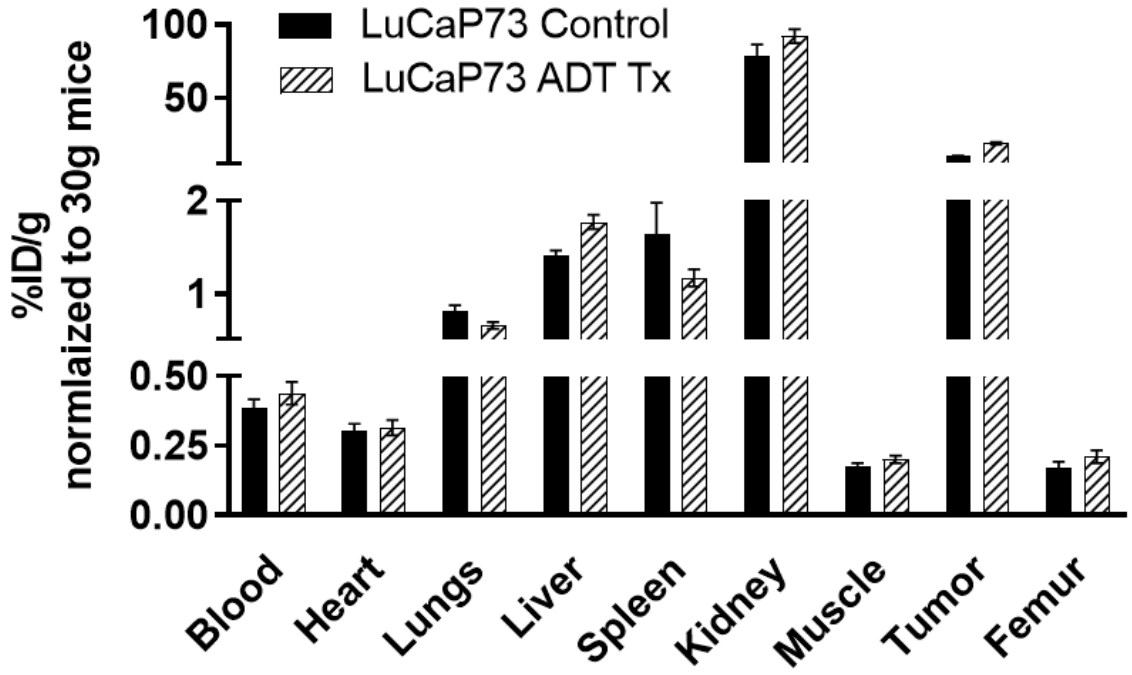
SI Figures

Gene	Primer
FOLH1 F	TCCACAGGAAATGAAGACATACA
FOLH1 R	CTGGAGTCTCTCACTGAACTTG
AR F	GGAAGCTGCAAGGTCTTCTT
AR R	CGAAGACGACAAGATGGACAA
KLK3 F	CACACCCGCTCTACGATATG
KLK3 R	GAGGTCCACACACTGAAGTT
FKBP5 F	AAACGAAGGAGCAACAGTAGAA
FKBP5 R	TTGGAATGTCGTGGTCTTCTC
HPRT1 F	CTTTCCTTGGTCAGGCAGTATAA
HPRT1 R	AGTCTGGCTTATATCCAACACTTC

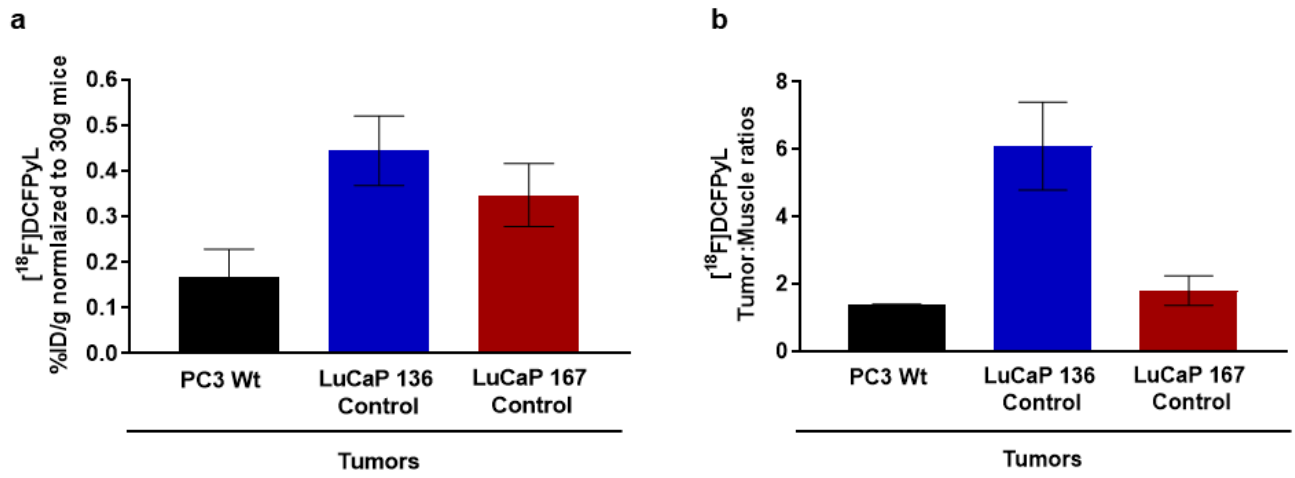
SI Figure 1. List of *FOLH1*, *AR*, *KLK3*, *FKBP5*, and *HPRT1* primers used for RT-qPCR.



SI Figure 2. Representative PET image of [^{18}F]DCFPyL in PC3(+) tumor bearing mice xenograft 1 h post-injection of [^{18}F]DCFPyL.



SI Figure 3. Biodistribution of [^{18}F]DCFPyL in ADT(degarelix) treated and control LuCaP73 tumor bearing mice xenografts at 1h post-injection of [^{18}F]DCFPyL. Each bar represents %ID/g ± SD, n=5-7 for each group.



SI Figure 4. [^{18}F]DCFPyL biodistribution (a) and tumor to muscle ratios (b; T:M) in PC3 Wt, LuCaP136 control, and LuCaP167 control tumor-bearing mice. Biodistribution was performed at 60 min post [^{18}F]DCFPyL injection. Each bar represents mean %ID/g ± SD (a), mean T:M ± SD (b); n=5-7.

Cell Lines	PSMA expression level (femtomoles/µg of protein)
LuCaP73	1.87±0.53
LuCaP136	0.038±0.0017
LuCaP167	0.013±0.0054
PC3(+)	13.95±1.60

SI Table 1. PSMA concentration (femtomoles/µg of protein) in different tumor types determined from in vitro saturation plots. Each value represents mean ± SD.