# Assessment of PET Tracer Uptake in Hormone-Independent and Hormone-Dependent Xenograft Prostate Cancer Mouse Models

## Supplemental Data

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## Material and Methods

### Preparation of Radiopharmaceuticals

#### $^{18}$F-FDG

The $^{18}$F-FDG was synthesized in a FDG MicroLab module (GE Healthcare, Münster, Germany) as already been published (1), using mannose triflate (ABX, Radeberg, Germany) as the precursor. Radiochemical purity, as determined by thin layer chromatography (TLC), was >95%.

#### $^{18}$F-FLT

$^{18}$F-FLT was synthesized using 5'-benzoyl-2, 3'-anhydrothymidine (2), synthesized in our laboratory, as the precursor. Radiosynthesis was performed on a modified TracerLAB FX FN PET tracer Synthesizer (GE Healthcare). After purification and sterile filtration, $^{18}$F-FLT was obtained in a 12 3% radiochemical yield (9.5±2.3 GBq, 65 min from end of beam (EOB)). Specific activity was 165±58 GBq/µmol at the end of the synthesis. Radiochemical purity was >99%, as determined by high performance liquid chromatography (HPLC).

#### $^{11}$C-choline

$^{11}$C-choline was synthesized from N,N-dimethylethanolamine (DMAE; >99.5%; Sigma-Aldrich, Munich, Germany) by alkylation using $^{11}$C-CH3I (3). Briefly, high specific activity $^{11}$C-CH3I was prepared in an automated module (PETtrace MeI Microlab, GE Medical Systems (GEMS), Uppsala, Sweden). The radiolabeling was performed in our custom-made automated synthesizer. After purification and formulation, the product was obtained in >80% radiochemical yield. The total synthesis time was 20 min from EOB. The radiochemical purity of the final formulated, sterile filtered radiotracer was >97%, as determined by HPLC. Residual content of DMAE was always far below 50 mg/L.

#### $^{18}$F-FEC

$^{18}$F-FEC was synthesized using an improvement on the method published by Hara et al. (4). The synthesis was performed in a modified TRACERlab FX FN (GE Healthcare). Azeotropically dried $^{18}$F-fluoride was reacted with 1,2-bis(tosyloxy)ethane (Sigma-Aldrich) in acetonitrile to yield 2-18F-fluoroethyltosylate, which was purified by HPLC and solid phase extraction (SPE) and then reacted with DMAE to yield $^{18}$F-FEC, which was purified using a cation exchange SPE cartridge (Waters, USA). The total synthesis time was 55 min from EOB, and specific activities were 50-90 GBq/µmol at the end of

the synthesis. The radiochemical purity of the final, sterile filtered product was >98%, as determined by HPLC. The residual DMAE content was between 50 and 100 mg/L.

**Laboratory animals**

BALB/c-nude male mice were obtained from Charles River (Sulzfeld, Germany) at the age of six weeks and fed *ad libitum*.

All animal experiments were approved and performed according to current guidelines for the care and use of research animals of the German Animal Protection Law.

**Cell Culture and Tumor Grafting**

The PC-3 and DU145 cells were cultured in RPMI-1640 medium (Biochrom AG, Berlin, Germany), supplemented with 10% fetal calf serum (HyClone, PERBIO-Thermo Fisher Scientific, Bonn, Germany) and penicillin/streptomycin (1%(v/v), Biochrom). The cells were maintained at 37°C in a humid atmosphere with 5% CO2.

PC-3 ($6x10^6$ cells in 200 µL NaCl) and DU145 ($8x10^6$ cells in 200 µL NaCl) cells were injected subcutaneously into 7-8 weeks old BALB/c-nude male mice, dorsal below the right upper flank.

Tumor cultivation and implantation of the CWR22 and PAC120 model were performed at OncoDesign Biotechnologies (Dijon, France). For the CWR22 and PAC120 tumor model, the mice were irradiated with 2.5 Gy before a piece of viable tumor zone was implanted through a small subcutaneous incision, below the upper right flank. After one *in vivo* passage, the tumor was harvested, dissected and cleaned before a 2x2x1 $mm^3$ viable tumor outgrowth was grafted subcutaneously into 6-8 weeks old BALB/c-nude male mice.

**Histology and Immunohistochemistry**

The tumor samples were fixed in 4.5% buffered formalin for at least 24 hours, embedded in paraffin, cut in 2-3 µm thick sections and stained by H&E. Immunohistochemistry was performed on an automated immunostainer (Ventana Medical System, Inc., Tucson, AZ) (5). The following primary antibodies were used: Ki67 (SP6 clone, Thermo Fisher Scientific), TK-1, GLUT1 and GLUT3 (Abcam, Cambridge, UK). The Ki67 and TK-1 indices were evaluated by counting 100 cells in three different areas per slide and tumor. The results correspond to the mean value in % of positive cells found in each group. The analysis was performed by a pathologist (LQM).

## References


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2. Reischl, G., Blocher, A., Wei, R., Ehrlichmann, W., Kuntzsch, M., Solbach, C., Dohmen, B.M., Machulla, H.-J. Simplified, automated synthesis of 3`[18F]fluoro-3`-deoxy-thymidine ([18F]FLT) and simple method for metabolite analysis in plasma. Radiochim. Acta. 2006; 94:447-451.
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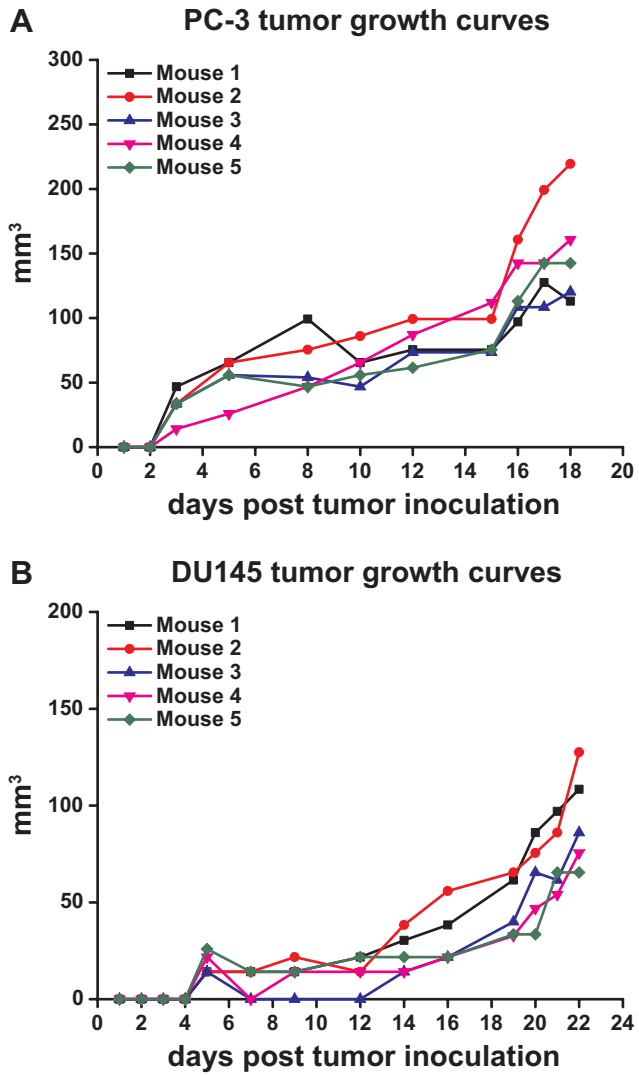
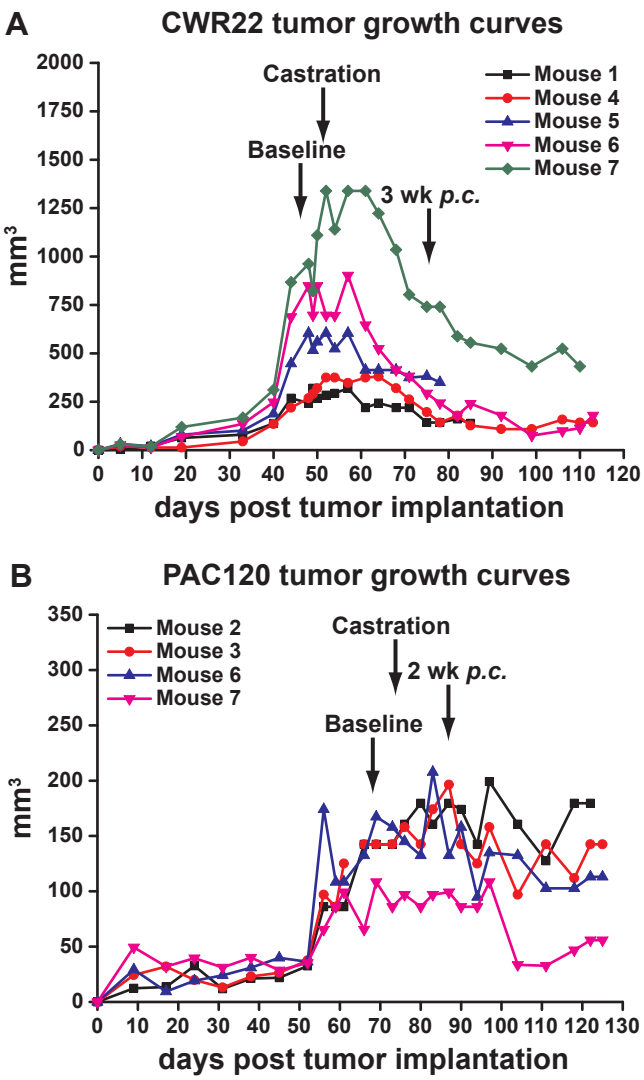
4. Hara, T., N. Kosaka, and H. Kishi. Development of (18)F-fluoroethylcholine for cancer imaging with PET: synthesis, biochemistry, and prostate cancer imaging. J Nucl Med. 2002; 43(2):187-199.
5. Kunder, S., J. Calzada-Wack, G. Holzlwimmer, et al. A comprehensive antibody panel for immunohistochemical analysis of formalin-fixed, paraffin-embedded hematopoietic neoplasms of mice: analysis of mouse specific and human antibodies cross-reactive with murine tissue. Toxicol Pathol. 2007; 35(3):366-375.

**Suppl. Figure 1**




**Suppl. Figure 2**



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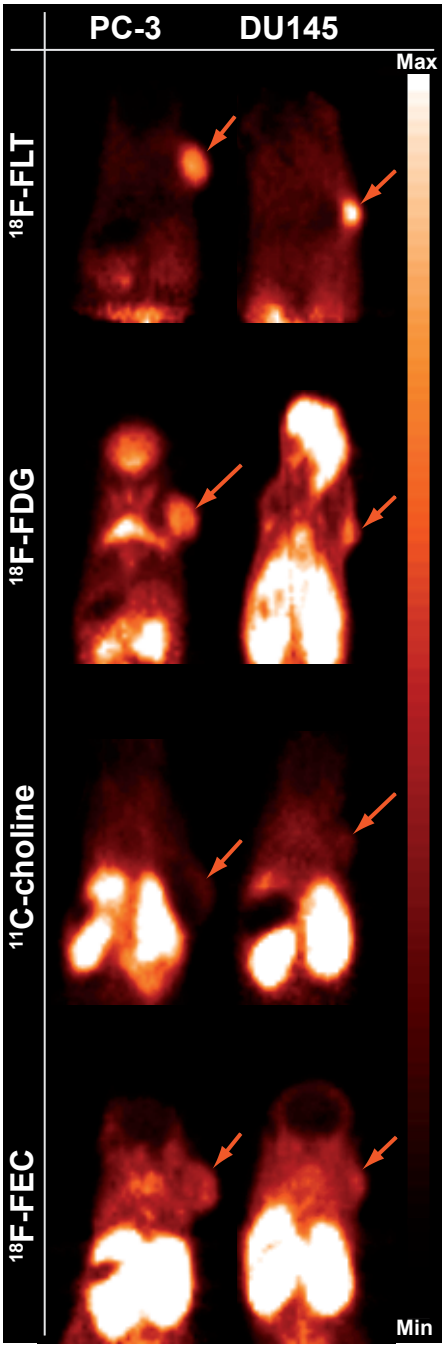

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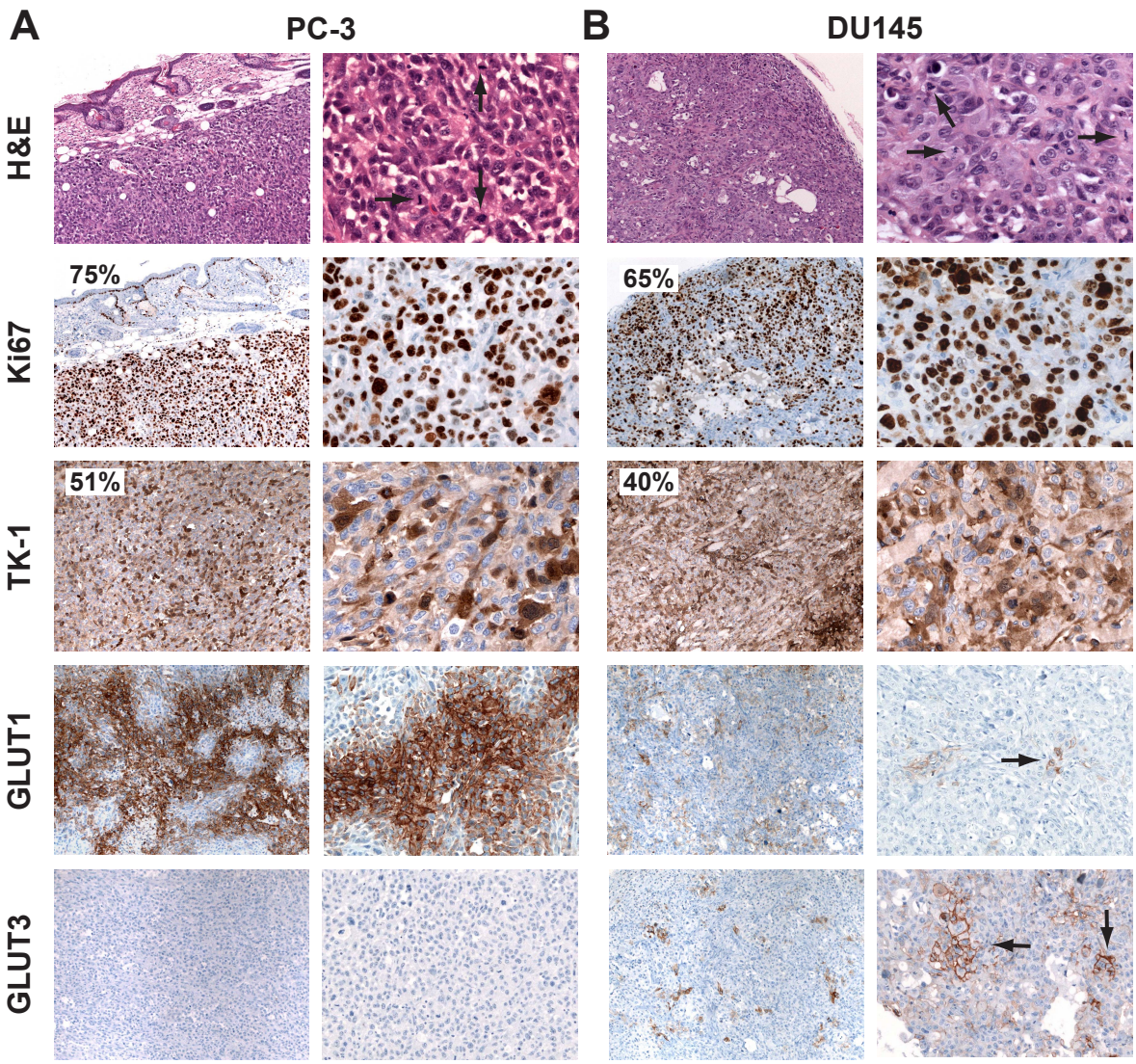

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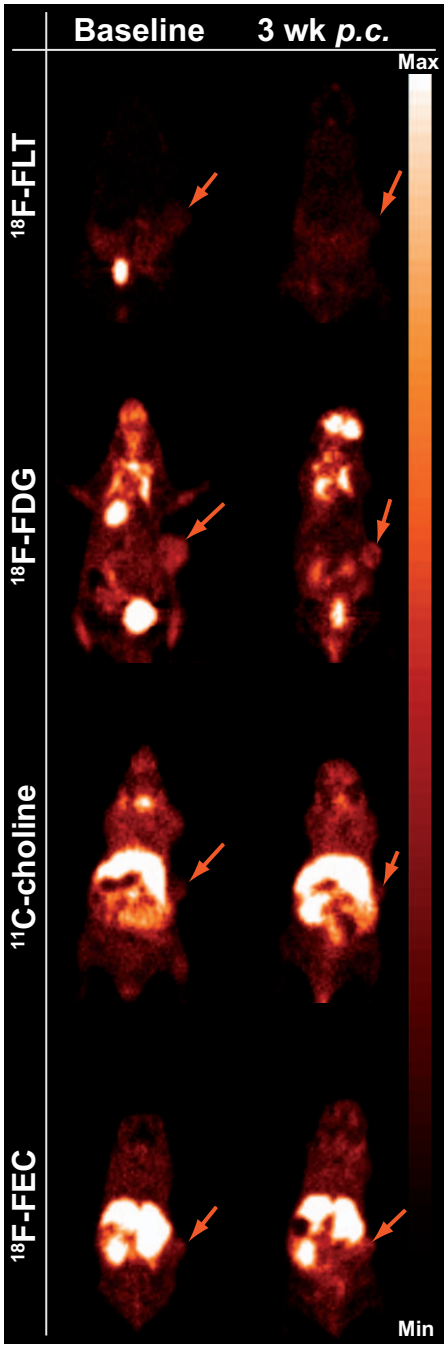


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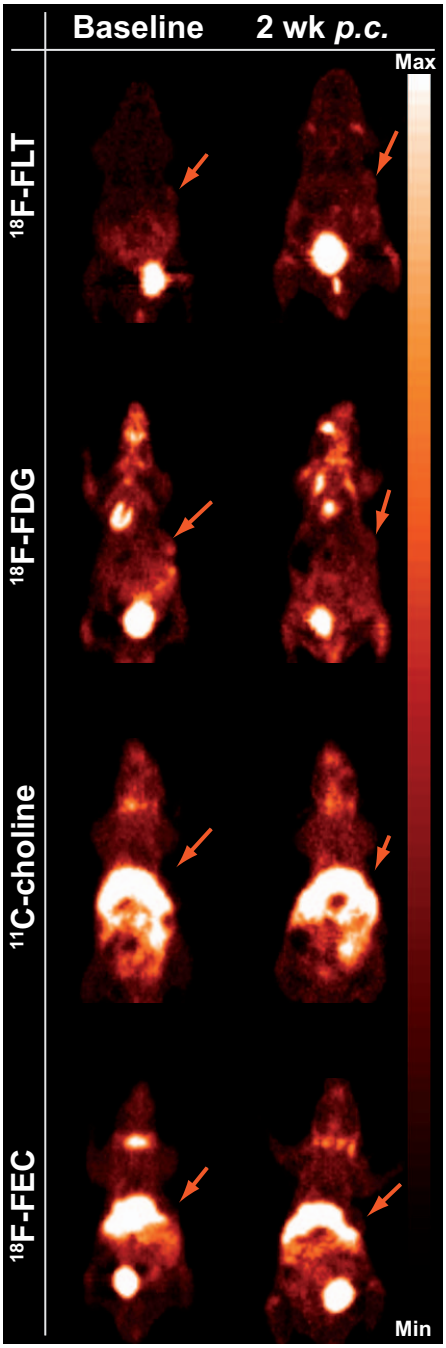

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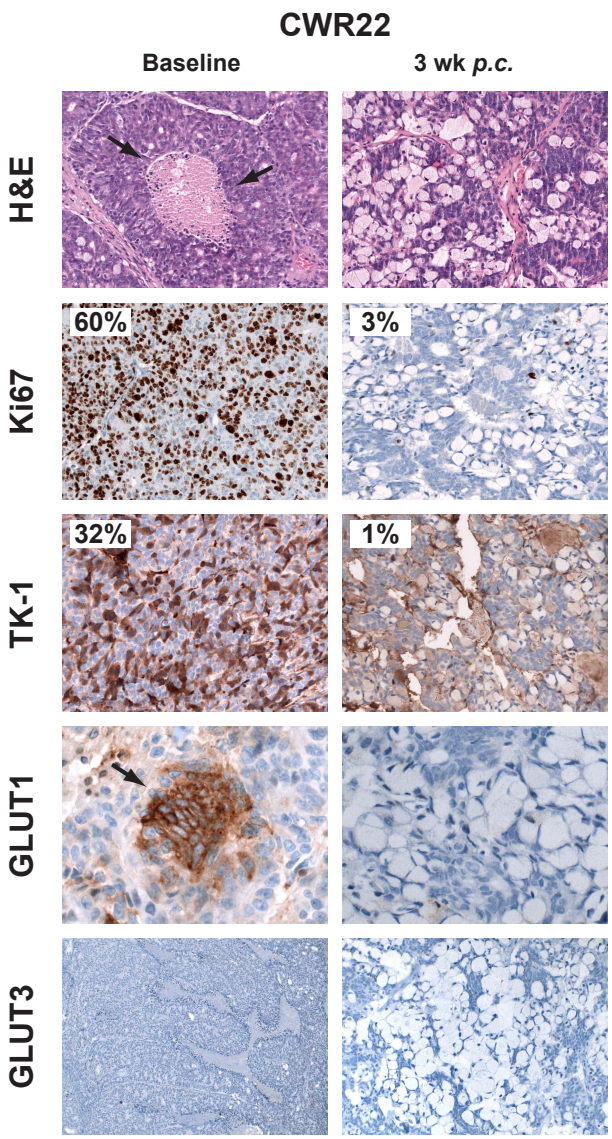


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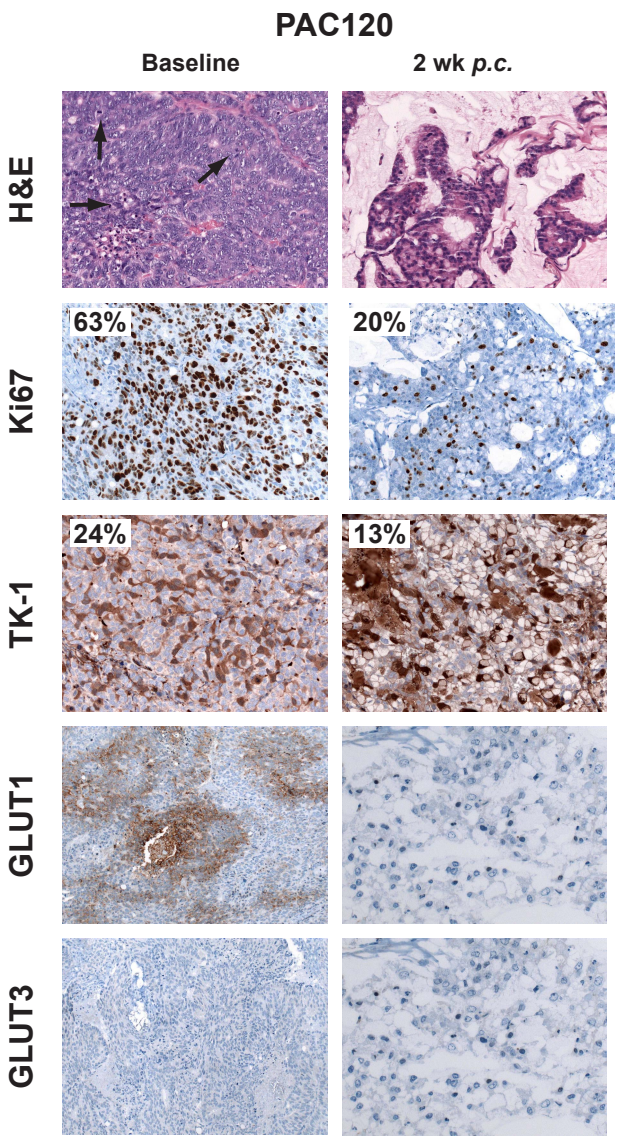

**Suppl. Table 1.** Summarized results of the study with overview of the investigated xenografts by PET imaging

| Tumor model | [$^{18}$F]FLT | [$^{18}$F]FDG | [$^{11}$C]choline | [$^{18}$F]FEC | Therapy response |
|---|---|---|---|---|---|
| PC-3 | +++ | +++ | 0 | 0 | n.a. |
| DU145 | +++ | + | 0 | 0 | n.a. |
| CWR22 | + | +++ | 0 | 0 | Yes |
| PAC120 | 0 | ++ | 0 | + | unclear |

# Supplementary Figure Legends

**Suppl. Figure 1:** Tumor growth curves (mm3): PC-3 (A) and DU145 (B) tumor volumes in representative xenografts are shown. Tumor volumes were assessed by external measurement with a caliper along the long axis (length, a) and the short axis (width, b). Volume was calculated by the formula: (radical(a*b))*(a*b)*0.5236 (approximated to the volume calculation of an ellipsoid).

**Suppl. Figure 2:** Tumor growth curves (mm3) of CWR22 xenografts pre and post castration are shown in A. Arrows indicate baseline PET imaging, castration and imaging after castration (p.c.). Tumor growth curves of PAC120 xenografts pre and post castration are shown in B. Arrows indicate baseline PET imaging, castration and imaging p.c. Tumor volumes were assessed by external measurement with a caliper along the long axis (length, a) and the short axis (width, b). Volume was calculated by the formula: (radical(a*b))*(a*b)*0.5236 (approximated to the volume calculation of an ellipsoid).

**Suppl. Figure 3:** Static PET images of the PC-3 and DU145 tumor bearing xenografts measured with $^{18}$F-FLT, $^{18}$F-FDG, $^{11}$C-choline and $^{18}$F-FEC. Arrows indicate the position of the tumor.

**Suppl. Figure 4:** Histology (H&E) and immunohistochemistry (Ki67, TK-1, GLUT1, GLUT3) of the hormone-independent tumors. A: PC-3. The tumor shows a diffuse, solid growth pattern with large pleomorphic cells and abundant mitoses (arrows). The Ki67 and TK-1 stains corroborate the high proliferation rate (brown staining). The tumor cells are positive for GLUT1 and negative for GLUT3 (left 100x magnification and right 400x magnification), and B: DU145 tumors. The tumor shows a solid growth pattern with large pleomorphic cells and numerous mitoses (arrows). The Ki67 and TK-1 antibodies are positive in many cells (brown staining). In contrast to PC-3 tumor, GLUT1 positive cells are rarely found (arrow), whereas GLUT3 is positive in group of cells (arrows) (left 100x magnification and right 400x magnification).

**Suppl. Figure 5:** Static PET images of CWR22 tumor bearing xenografts measured with $^{18}$F-FLT, $^{18}$F-FDG, $^{11}$C-choline and $^{18}$F-FEC pre and 3 weeks (3 wk) post castration. Arrows indicate the position of the tumor.

**Suppl. Figure 6:** Static PET images of PAC120 tumor bearing xenografts measured with $^{18}$F-FLT, $^{18}$F-FDG, $^{11}$C-choline and $^{18}$F-FEC pre and 2 weeks (2 wk) post castration. Arrows indicate the position of the tumor.

**Suppl. Figure 7:** Histology (H&E) and immunohistochemistry (Ki67, TK-1, GLUT1, GLUT3) of the hormone-dependent CWR22 tumors pre- and post-castration (*p.c.*). The baseline tumor shows a diffuse, solid growth with areas of necrosis (arrows) and abundant mitoses as demonstrated with the Ki67 and TK-1 stains (brown staining). GLUT1 is partially expressed in the tumor cells, whereas GLUT3 remains negative. After 3 weeks *p.c.* note the important tumor regression with striking reduction on cell proliferation, as shown with the Ki67 and TK-1 antibodies. GLUT1 is no longer expressed (all pictures 200x magnification).

**Suppl. Figure 8:** Histology (H&E) and immunohistochemistry (Ki67, TK-1, GLUT1, GLUT3) of the hormone-dependent PAC120 tumors pre- and post-castration (*p.c.*). The baseline tumor shows a diffuse, solid growth with pleomorphic cells and abundant mitoses (arrows). The proliferation rate of the tumor cells is highlighted with the Ki67 and TK-1 antibodies. GLUT1 is partially expressed by the tumor cells, whereas GLUT3 is negative. After 2 weeks *p.c.* there is a considerable regression of the tumor accompanied by a reduction in proliferation, as demonstrated by Ki67 and TK-1 stains. Post-castration, GLUT1 is no longer expressed by the tumor cells (all pictures 200x magnification).