

Supplemental Figure Legends

Supplemental Figure 1. Dynamic hyperpolarized [1-¹³C] pyruvate MRSI of DU145 and PC3 xenografts. PC3 and DU145 xenografts were grown in the hind leg of male athymic nu/nu mice. Dynamic ¹³C-CSI was performed with images acquired every 6 s up to 240 s after the following the injection of hyperpolarized [1-¹³C] pyruvate. Representative time intensity curves are shown for A) PC3 and B) DU145 xenografts. AUC: area under the curve measured between 10 and 240 seconds.

Supplemental Figure 2. ¹H MRS of DU145 and PC3 xenografts.

PC3 and DU145 xenografts were grown in the hind leg of male athymic nu/nu mice. At approximately 1000 mm³, the ratio of lactate to creatine in DU145 and PC3 xenografts as measured by ¹H-MRS. Representative spectra for (A) PC3 and B) DU145 at TE 14 ms and 140 ms (inset). The lactate peak inverts at 140 ms. ppm: parts per million.

Supplemental Figure 3. Densitometry of Western blotting of DU145 and PC3 xenografts.

A) PC3 and DU145 xenografts were grown in the hind leg of male athymic nu/nu mice. At approximately 1000 mm³ volume, tumors were harvested and probed with western blot analysis of the indicated proteins using β-actin as the loading control. The optical density (OD) of the indicated bands was normalized to actin and expressed a mean. B) PC3 and DU145 cells were grown in culture in exponential phase of growth. Protein concentrations in cell lysates were probed by western blotting. The OD of the indicated bands was normalized to actin and expressed as a mean. Bars: mean, error bars: standard deviation. p values noted for p < 0.05

Supplemental Figure 4. In vitro metabolic phenotype of DU145 and PC3 tumor cells.

Exponentially growing DU145 and PC3 tumor cells were utilized. A) Lactate content of exponentially growing tumor cells was measured and normalized to total protein in the cell pellet and conditioned media. B) Plated cells were incubated with XF assay medium, treated with varying concentrations of FX-11, and ECAR was measured. Bars and points: mean, error bars: standard deviation, *ns*: not significant.

Supplemental Figure 5. LDH Activity in tumor xenografts and cell lines.

LDH activity was measured in PC3 (n=3) and DU145 (n=3) xenograft tumors (A) and cells grown *in vitro* (B). LDH activity was normalized to tissue weight for tumor and protein concentration for cells grown *in vitro*. Columns: mean, error bars: standard deviation, **p*<0.05.

Supplemental Figure 6. Dynamic hyperpolarized [1-¹³C] pyruvate NMR of DU145 and PC3

cells in vitro. A and B) Exponentially growing PC3 and DU145 tumor cells were resuspended in high glucose DMEM lacking sodium pyruvate and containing either DMSO or FX-11. Hyperpolarized [1-¹³C] pyruvate was added to a final concentration of 25.8mM with a pH of 7.5 and 150 scans of one dimensional ¹³C NMR spectra were immediately acquired. C) Graphical representation of the NMR data from (A) and (B). Columns: mean, error bars: standard deviation, * *p*<0.05, *ns*: not significant.