Supplementary materials and methods

Reverse-phase protein array (RPPA) construction, processing, probing and analysis

Snap-frozen LuCaP tumors pieces (~100mg) were minced and homogenized in lysis buffer containing 2% sodium dodecyl sulfate (SDS), 50 mM Tris-HCl, 5% glycerol, 5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM NaF, 10 mM b-GP, 1 mM PMSF, 1 mM Na_3VO_4, and 1 mM ditheothreitol (DTT) and supplemented with protease and phosphatase inhibitor cocktail (Thermo Scientific). Protein lysates were filter cleared using AcroPrepTM Advance 96-Well Filter Plates (Pall Corporation,) by centrifuging at 1962 x g for 4 to 6 hours at room temperature. The total protein amount in tumor lysates was quantified using a BCA protein assay kit (Thermo Fisher Scientific) following the manufacturer's instructions.

Tumor protein lysates were printed onto 16-pad nitrocellulose-coated slides (Grace Biolabs) using Aushon 2470 microarrayer (Aushon BioSystems). Each sample was printed in duplicate generating 78 sample spots on each subarray. A total of 6 slides were printed, allowing probing with 96 validated antibodies. Slides were stored at - 20°C until processing. RPPA slides were washed with 1M Tris-HCl (pH 9.0) for 2-4 days to remove SDS. Slides were then washed 2 to 3 times with phosphate-buffered saline (PBS) for 5 min each and blocked with Odyssey Blocking Buffer (OBB, Licor, NE, USA) for one hour at RT. After blocking, arrays were incubated with primary antibodies in OBB at 4°C overnight. The next day, arrays were washed three times with PBS and incubated with IRDye labeled secondary antibodies in OBB for 1 hr at room temperature. Arrays were rewashed three times in PBS and once in ddH2O and spun dry. The RPPA slides treated with IR-labeled secondary antibodies were scanned using Licor Odyssey CLX Scanner (LiCOR). Each spot's total signal intensity was quantified using the Array-Pro analyzer software package (Media Cybernetics, MD, USA). The measurement of a specific protein from an individual sample was then normalized to total β-actin (Sigma, Cat #A1978).

Metabolomic analysis of glucose-derived metabolites in 2D cultured PC3 and C42B cells using mass spectrometry

The supernatant (50uL) was reacted with 50 μL of 250 mM 3-NPH in 50% methanol, 50 μL of 150 mM EDC in methanol and 50 μl of 7.5% pyridine in 75% aqueous methanol at 30C for 30 minutes. A 20 μl aliquot of this mixture was injected for UPLC/MS analysis. Chromatographic separation of malate, lactate, succinate and fumarate was conducted using ultra high-pressure liquid chromatography (Agilent 1290 Infinity II), Chromolith C18 reverse phase column (100 × 2 mm, 1.5 µm) and a mobile phase gradient from 30% methanol–water with 0.1% formic acid to 95% methanol–water with 0.1% formic acid. Derivatized carboxylic compounds were introduced into the electrospray ion source (Jet Stream) and analyzed in the negative ion mode. An Agilent 6495 triple quadrupole mass spectrometer with MassHunter Workstation software (Agilent) was used to quantify analytes. We used the two most

sensitive multiple reaction monitoring transitions for each analyte to quantify and verify analyte identity. Parent and product ion transitions for quantitation were m/z 403.3 to 137.0 (malate), m/z 406.3 to 140.0 (malate-d3, CDN Isotopes, Pointe-Claire, Quebec, Canada), m/z 385.3 to 152.1 (fumarate), m/z 387.3 to 152.1 (succinate), and m/z 224.2 to 137.1 (lactate). All tricarboxylic acid standards were purchased from Sigma. Data were normalized by cell number and basal cell culture medium metabolite content.

GLUT-1 immunofluorescence characterization in PCa cells cultured in 2D

PC3 and C4-2B cells were cultivated in a 96-well plate and treated after 24 hours with 0 or 30 nM IACS-10759. After additional 24 hours, cells were fixed in 100% ice-cold ethanol for 20 minutes and blocked for 1 hour with staining solution (0.5% IgePal630, 10% DMSO and 5% fetal bovine serum in PBS) at room temperature. Cells were incubated for 1 hour with the anti-GLUT-1 primary antibody (rabbit monoclonal, ab115730, RRID:AB_10903230, Abcam, 1:100 in staining solution) and rinsed 3 times in 0.5% PBS-IgePal. Cells were incubated with Alexafluor-secondary antibody (goat anti-rabbit AlexaFluor 488, A-11008, RRID:AB_143165, Invitrogen, 1:200 in staining solution), rinsed 3 times in 0.5% PBS-IgePal and imaged using an EVOS FL Cell Imaging System (AMG) equipped with 10X objective. Image analysis was performed with FIJI software: the background was removed and GLUT-1 fluorescence intensity (mean grey value) was measured in 30 cells/group.

Growth of cells treated with 2-deoxyglucose

PC3 or C4-2B cells were plated in a 96-well plate (n=3/group; 1000 cells/well). After 1 day, cells were incubated with 0.1-10 mM 2-deoxyglucose glucose in complete DMEM medium supplemented with 0.5 mM glucose. 3 days-post treatment, cells were fixed in 100% ice-cold ethanol and stained with crystal violet. Each well was automatically acquired at the EVOS microscope (1 image/well, 4x objective, equal to ~75% of the total area). The resulting image was thresholded in FIJI and area occupied by stained cells was computed.

Supplementary Figures

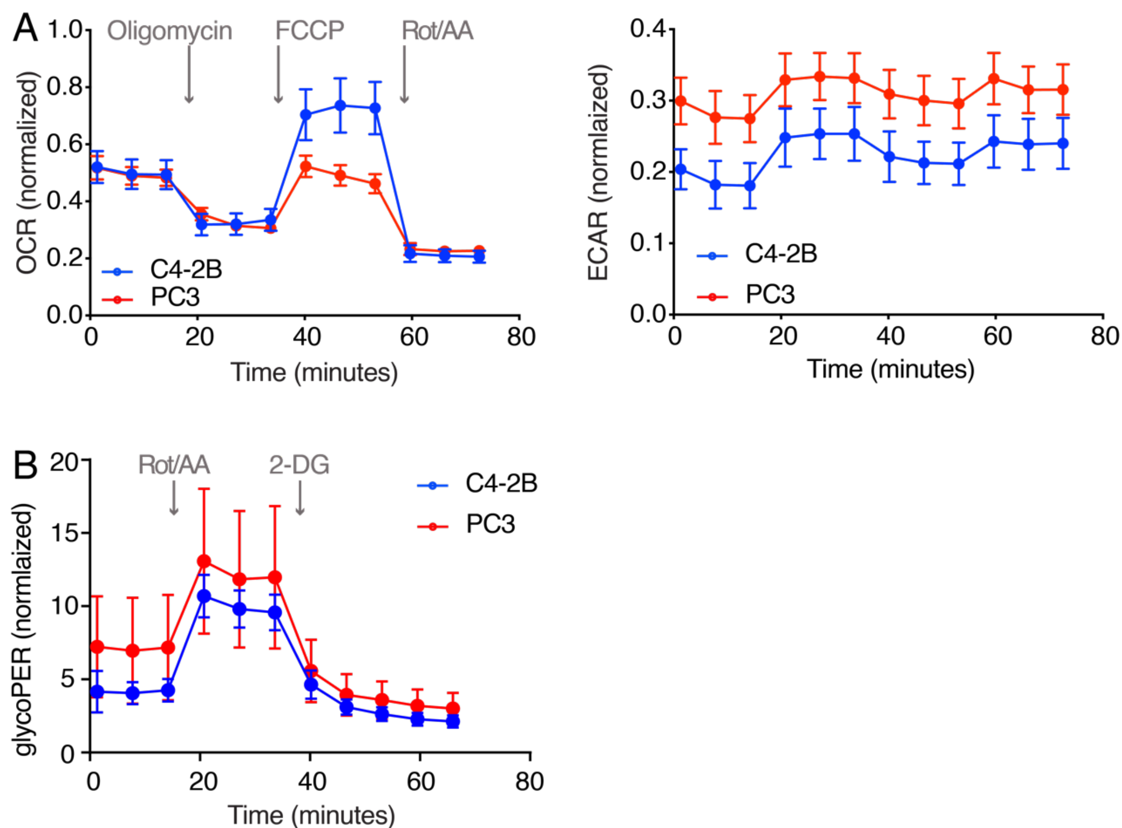

Fig. S1. Seahorse assays on C4-2B and PC3 cells cultured in 2D
A) Oxygen Consumtion Rate (OCR) and EstraCellular Acidification Rate (ECAR) curves from Seahorse Mito Stress test run on C4-2B and PC3 cells cultured in 2D. B) glycolytic Proton Efflux Rate (glycoPER) from Seahorse Glycolytic Rate assay run on C4-2B and PC3 cells cultured in 2D. Means ± SD, the experiments were performed 2 times, 1 representative experiment is shown, n=8 wells/group, OCR and glycoPER values were normalized over live cell area/well.

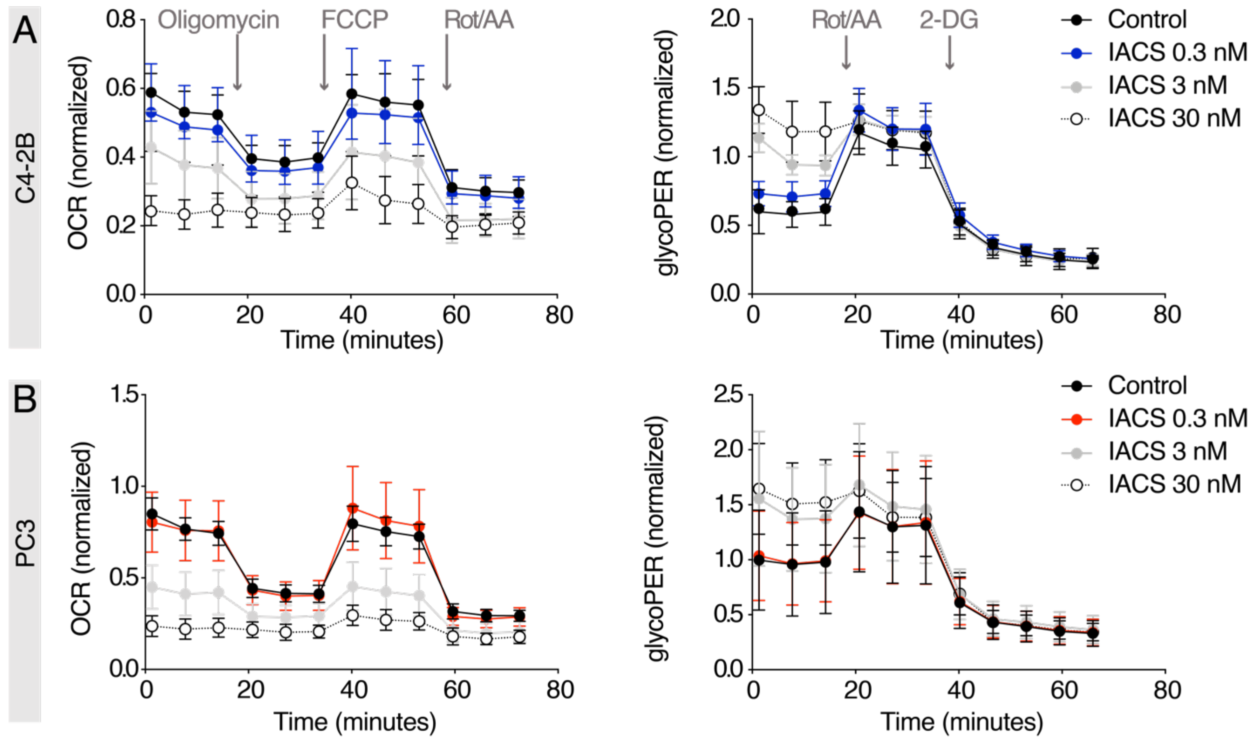

Fig. S2. Seahorse assays on C4-2B and PC3 cells cultured in 2D and treated with IACS-10759
Oxygen Consumption Rate (OCR) and glycolytic Proton Efflux Rate (glycoPER) curves from Seahorse Mito Stress test and Seahorse Glycolytic Rate assay run on **A**) C4-2B and **B**) PC3 cells cultured in 2D and treated with 0.3 nM, 3 nM, 30 nM IACS-10759. Means ± SD, the experiments were performed 2 times, 1 representative experiment is shown, n=8 wells/group, OCR and glycoPER values were normalized over live cell area/well.

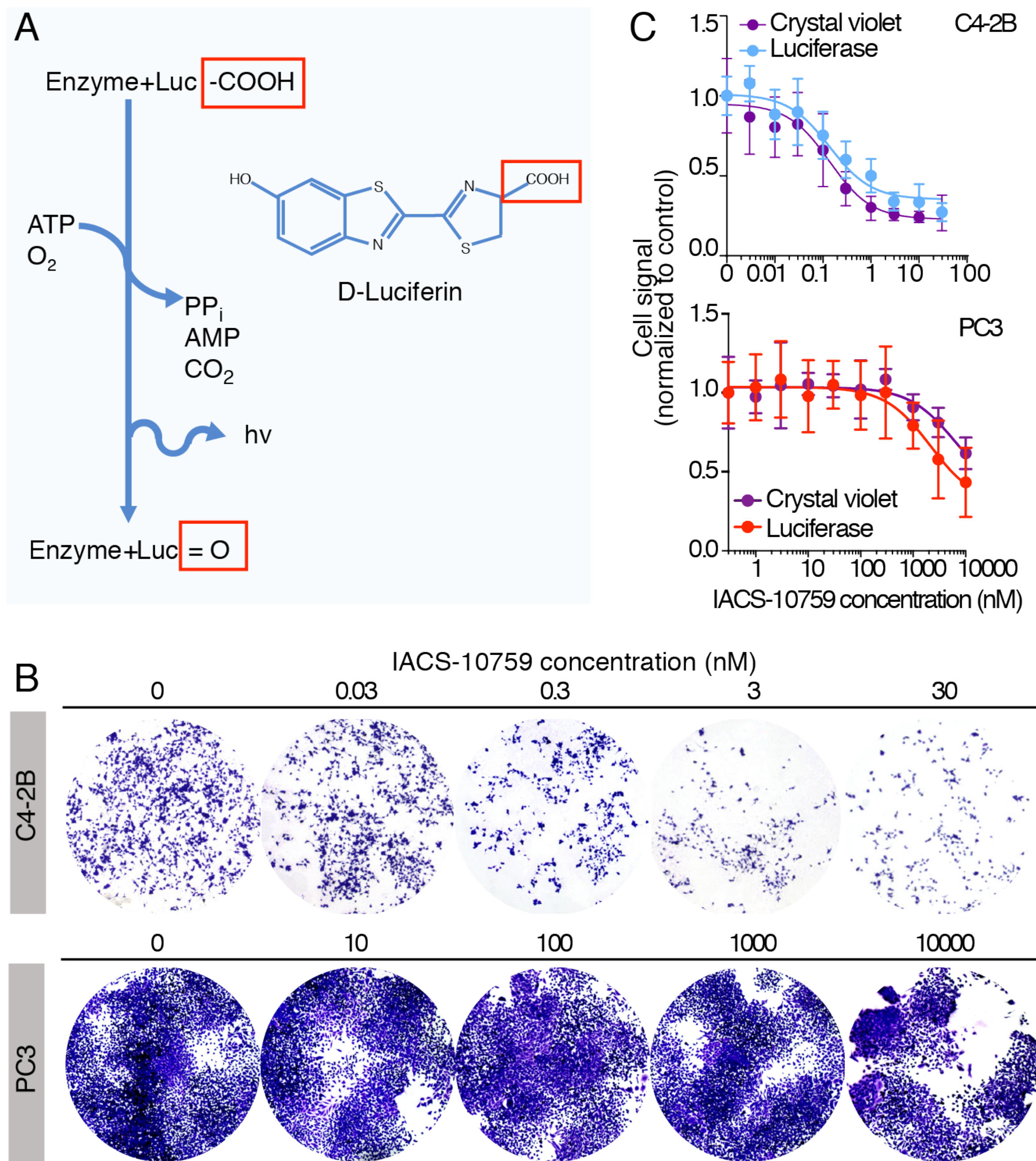

Fig. S3. Luciferase assay and crystal violet staining are comparable methods for monitoring PCa cells growth.
A) Schematic reaction chain of luciferase assay. **B**) Representative images of C4-2B and PC3 cells treated with different concentration of IACS-10759 and stained with crystal violet. **C**) Comparison between bioluminescence and crystal violet staining read out for C4-2B and PC3 cell dose-response curve to IACS-10759 5 days post-treatment.

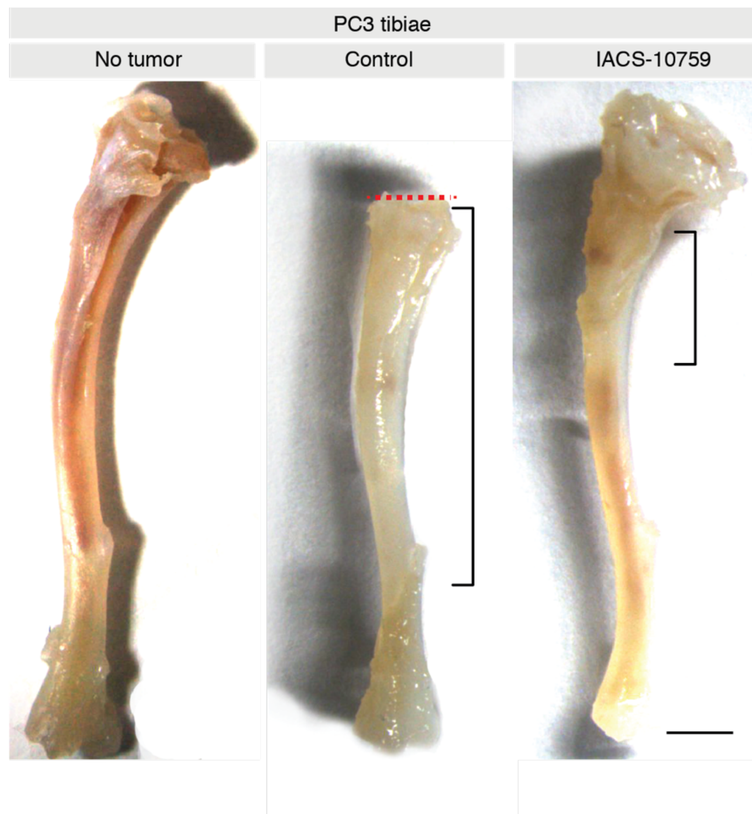

Fig. S4. Representative pictures of tumor-free, PC3 tumor-bearing control and PC3 tumor-bearing IACS-10759 treated tibiae.
Brackets highlight the area occupied by the tumor, red dotted line indicates bone rupture due to increased fragility. Bar, 200 μm.