**Supplementary Information**

**Materials and Methods**

**Animal studies**

All of the animal experiments were carried out in compliance with the Guide for the Care and Use of Laboratory Animal Resources, and experimental protocols were approved by the Animal Care and Use Committee, National Cancer Institute (NCI-CCR-ACUC).1 The human pancreatic tumor cell lines, Hs776t, and SU.86.86 cells were obtained from Threshold Pharmaceuticals (Redwood City, CA), and MiaPaCa-2 cells were purchased from the American Type Culture Collection (Manassas, VA). All cell lines were authenticated by IDEXX RADIL (Columbia, MO) utilizing a panel of microsatellite markers. Hs766t, MiaPaCa-2, and SU.86.86 pancreatic tumor bearing mice (n=9 each) were formed by injecting 3×105 cells subcutaneously into the right hind legs of mice. Other conditions for cell culture and xenograft tumor development were as described previously.2Athymic nude mice supplied by the Frederick Cancer Research Center, Animal Production (Frederick, MD) were all female of approximately three months of age when the study began. The tumors were imaged when the tumors grew to one cm3, which took approximately one month for all cell lines. Both respiration and temperature were monitored continuously through the experiment and the degree of anesthesia adjusted to keep respiration and body temperature within a normal physiological range of 35–37 °C and 60–90 breaths per min.

**Image Registration**

Prior to imaging, all animals were placed on a detachable bed that served to restrain the leg in a similar orientation for each imaging procedure. A multistep procedure in MATLAB was used to register the EPR images to the FDG-PET images. First, an approximate FOV of the FDG-PET image was isolated from the whole body FDG-PET image and downsampled from 72×72 to 64×64 to match the resolution of the EPR image using nearest neighbor interpolation. An intensity based, rigid body transformation was then applied to align the images approximately in the xy plane, using pyramid based registration by progressive alignment of successively higher resolution approximations to the true image.3 First, a coarse 16×16 representation of each image was created using the low pass Gaussian filter of Burt and Adelson with the shape factor *a* set to 0.375.4 The two low resolution images were then registered against each other using gradient descent to maximize the mutual information between the images.5 New 32×32 approximations were created from the aligned images and the process repeated. The images at the original 64x64 resolution were then aligned using the 32×32 alignment as a seed. The translation from this alignment was used to shift the FOV of the FDG-PET image to the correct position in the xy plane. With the FOV corrected in the xy plane, the same procedure was then applied in the xz plane to generate the final registered images. The hyperpolarized MRI images were registered in the xy plane to the other aligned images by a similar procedure using the anatomical 1H MRI as a reference and summing slices so a consistent 8 mm thickness is used throughout.

**Hyperpolarized 13C MRI**

Hyperpolarized 13C MRI experiments were performed on a 3T MRI scanner (MR solutions Inc.) using a 17 mm diameter home-built 13C solenoid coil with a saddle 1H coil. A solution of 30 µl of [1-13C] pyruvate, containing 15 mM of OX063 and 2.5 mM of chelated gadolinium, ProHance (Bracco Diagnostics), was polarized at 3.35T and 1.4 K in a Hypersense DNP polarizer (Oxford Instruments). Each hyperpolarized sample was rapidly dissolved in 4.5 ml of a superheated alkaline dissolution buffer, containing 40 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 30 mM of NaCl and 100 mg/L of ethylenediaminetetraacetic acid. 13C two dimensional spectroscopic images were acquired 30 seconds after the injection of a hyperpolarized [1-13C] pyruvate solution. A 32×32 mm2 field of view in a 8 mm axial slice through the tumor, a matrix size of 14×14, spectral width of 4000 Hz, repetition time of 85 ms, and 250 µs Gaussian excitation pulse with a flip angle of 10° were used. The lactate and pyruvate signals were calculated from the magnitude of the signal in a 1 ppm range from 184.8 ppm and 172.6 ppm.

**18F-FDG PET**

18F-FDG PET images were acquired using an energy window of between 250 and 700 keV. Computed tomography (CT) images were acquired using radiographic tube settings of 50 kV and a current of 0.18 mA with a nominal resolution of 0.375 mm x 0.375 mm x 0.375 mm. A two-dimensional ordered-subset expectation maximization algorithm was used to reconstruct PET images after the correction for radioactive tracer decay and random and scattered coincidences. MIM software version 6.5.6 (MIM software Inc., Cleveland, Ohio) was used for calculating 18F-FDG Standardized Uptake Values (SUV).

**EPR Imaging**

A single-point imaging pulse scheme was used to map the three-dimensional distribution of *T2\** relaxation time, which linearly correlates with the local concentration of oxygen, to a resolution of 0.4375 mm x 0.4375 mm x 0.4375 mm. Triarylmethyl EPR oxygen tracer, OX063 (1.125 mmol/kg bolus, GE healthcare) was injected intravenously by 0.12 mmol/min of continuous infusion.

**Western blot**

The mice (n=3 for each group) were euthanized and tumor biopsy samples were excised for western blot procedures were performed as described previously.6

**Immunohistochemistry**

Tumor were fixed with 4% paraformaldehyde, frozen using ultracold ethanol, and a 10 μm thick section cut from the center of the tumor. The frozen tumor sections were then thawed and fixed with ice cold acetone. Following blocking, the sections were incubated with rat anti-mouse CD31 antibody (1:250; BD) overnight at 4℃. After incubation. tumor sections were then washed with PBS and incubated with goat anti-rat IgG Alexa Flour 488 (H+L; 1:500; Invitrogen) and stained with Prolong Gold antifade reagent (Invitrogen). Fluorescence microscopy and imaging was performed using a BZ-9000 BIOREVO (KEYENCE) fluorescence microscope. Images were captured with the BZ-9000E viewer at 20x magnification and stitched to compose an image of the whole tumor using the BZ-II Analyzer. Corresponding H&E stained images were also acquired for histological evaluation following standard procedures.

**Statistical analysis**

All of the experimental results are expressed as means ± SDs. A student two-tailed t-test was used to determine statistical significance. The minimum level of significance was set at probability values of less than 0.05. For the comparisons of bulk tissues, corrections for multiple comparisons across the different cell types (n=3) were made using the two-stage linear step-up procedure of Holm *et al*7 with a confidence level of 5%.

**Supplementary Figure S1**

**Figure S1** H&E (Top) and CD31(Bottom) staining of 100 m thick slices from the center of the xenograft tumors indicated (four mice per cell line). Scale bars are 1 mm across.

Regional differences in CD31 were not evident in any of the cell lines, except where necrosis was visibly present by H&E. Despite the difference in pO2 values, the core and periphery of all cell lines had similar levels of the endothelial marker CD31 in all cell lines, suggesting differences in vascularity are not the primer driver for the rim/interior difference in FDG uptake and O2 levels. A switch between glycolysis and OXPHOS as one moves from the rim to the interior of the tumor is consistent with several previous experimental observations, including redox imaging of breast cancer xenografts which show an oxidized core and reduced rim as would be expected for enhanced glycolysis in the rim region,8 and direct imaging of glucose metabolism by 13C MRI which shows increased glucose utilization at the rim of MiaPaCa-2 and Hs766t tumors.9

**References**

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