Supplemental Materials and Methods

Human Sample Collection

Human lumbar IVD tissues were obtained at surgery or post-mortem (PM) examination with informed consent of the patient or relatives (Sheffield Research Ethics Committee #09/H1308/70). Two PM IVDs were recovered from two donors, comprising intact IVDs within the complete motion segment from which the IVDs were removed. Twenty-eight surgical IVD tissue samples were obtained from 28 patients undergoing micro-discectomy procedures for treatment of low back/neck pain and root pain as caused by prolapse of the IVD (Supplemental Table 1 for sample details). NP tissue was carefully separated from the AF and endplate fragments under a stereomicroscope and fixed in 10% neutral buffered formalin and processed for histological and immunohistochemical examination (Le Maitre et al., 2005). H&E stained sections, used to score degree of morphological degeneration, were scored numerically (0-3) on four degenerative features as reported previously. Samples were classified as non-degenerate (≤ 4), moderately-degenerate (4.1-6.9) and severely-degenerate (≥ 7) based on histological examination. AF tissues were not included in any of the analyses due to chances of cross contamination from extraneous connective tissues, endplate fragments and blood. Grading was performed independently by two researchers and grades were averaged.

Human Immunohistochemical Analysis

Immunohistochemistry confirmed and localized expression of MCT1 (1:10, Santa Cruz, sc-50324) MCT4 (1:10, Santa Cruz, sc-50329), and EMMPRIN/CD147 (C-19) (1:10, Santa Cruz, sc-9754) in 30 human IVDs: 2 PM and 28 surgical samples (Supplementary Table 1). Tissue sections were dewaxed, rehydrated, endogenous peroxidases quenched. Samples were blocked in goat (MCT1, MCT4) or donkey (CD147) serum, then incubated overnight at 4°C with rabbit anti-human MCT1, MCT4 (1:10), or goat anti-human CD147 (1:10), with pre-immune rabbit and goat IgG (Abcam) used as negative control at equal IgG concentrations. After washing, MCT1 and MCT4 sections were incubated with biotinylated goat anti-rabbit secondary antibody (1:500; Abcam, ab6720) and CD147 incubated with biotinylated donkey anti-goat secondary antibody (1:500; Abcam, ab6884) and binding detected by formation of streptavadin- biotin complex (Vector Laboratories, Peterborough, UK) with 3,3'-diaminobenzidine tetrahydrochloride solution (Sigma-Aldrich). Sections were counterstained with Mayers Haematoxylin (Leica Microsystems), dehydrated, cleared and mounted in Pertex (Leica Microsystems). Sections were visualized, and images captured using an Olympus BX60 microscope and CellSens software (Olympus). Random fields of view were assessed for immunopositive cells until a total of 200 NP cells were counted, the number of immunopositive cells was expressed as a percentage of the total count.

Tissue RNA isolation

NP tissue was dissected from lumbar and caudal discs of 14-month WT and MCT4 KO mice and immediately placed in RNAlater® Reagent (INVITROGEN). 3-4 mice per genotype were sacrificed for RNA isolation and NP tissue pooled from single animal served as an individual sample. NP tissue was collected in RNAlater® Reagent and homogenized with a Pellet Pestle Motor (Sigma Aldrich, Z359971). Total RNA was extracted from the tissue lysates using RNeasy® Mini kit (Qiagen). RNA samples were analyzed using Clariom S Mouse array (see below).

Cell Metabolomics

NP cells were plated in 10cm plates at a density of 1×10^6 cells and cultured in DMEM with 10% dialyzed FBS (Sigma), 5mM D-glucose, and 4mM L-glutamine in hypoxic conditions until treated for All procedures regarding collection of animal tissues was performed as per approved protocols by

Institutional Animal Care and Use Committee (IACUC) of the Thomas Jefferson University, in accordance with the IACUC's relevant guidelines and regulations. MCT inhibitors for 2 hours. Cells were harvested in ammonium acetate (150 mM) on ice and immediately frozen in liquid nitrogen. Cell pellet samples were extracted with 200uL of methanol: H_2O= 4:1(v:v) containing 2.5µM U13C succinate, and 5µM citrate. The samples were put into liquid nitrogen for 1 min, and then sonication (with ice in water) for 5 min (frozen-thaw cycle). The frozen-thaw cycle was repeated 3 times. The samples were centrifuged at 12,000 rpm for 10 minutes, and the supernatant was pipetted into a high recovery GC sampling vial. The leftover supernatant was pipetted to make pooled QC samples. The samples were dried under gentle nitrogen flow and derivatized with a two-step derivatization procedure. First, the samples were methoximized with 50 µl of methoxyamine hydrochloride (MOA, 15 mg/mL in piridine) at 30 ^{0}C for 90 minutes. The silylation step was done with 50 µl of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA, containing 1% TMCS) at 70 ^{0}C for 60 minutes. QC sample was run multiple times during the analysis. The samples were analyzed by gas chromatography time-of-flight mass spectrometry (GC-TOFMS premier, Waters, USA).

Metabolomics Data Analysis

The metabolomics dataset comprised of 84 known metabolites that were standardized, i.e. subtracted the mean and divided by the standard deviation per metabolite, and was subsequently analyzed with multivariate statistics [PMID: 29654587] using R version 3.3.0. Specifically, the metabolic profiles were visualized with Principal Component Analysis (PCA) and clustered with hierarchical clustering. Metabolites with significant concentration differences were identified using SAM with 5,000 permutations and 5% FDR threshold. To determine the most enriched KEGG pathways, the P-value of each represented mouse metabolic pathway was calculated using hypergeometric test.

Seahorse XF Analyzer Respiratory Assay

The Seahorse XF24 instrument was used to measure extracellular acidification rate (ECAR) and O_2 consumption rate (OCR), as reported by Csordás et al (Csordás et al., 2013) . Rat NP cells transduced with either shCtrl or sh*Car3* plasmids were seeded on 24-well XF Analyzer plate at 15,000 cells per well in DMEM (5mM glucose, 4 mM glutamine, pH 7.4 @ 37C). The microplate was incubated for 1 h in no CO_2 at 37oC. OCR was calculated using the Akos algorithm, a standard algorithm which we determined was appropriate even for the low OCR readings that we recorded in NP cells; appropriateness was based on the approximate linearity of the pO_2 vs time traces. ECAR was measured from readings of H^+ concentration. After each measurement, the probe array rises, after which the solution in each well was mixed for 2 min (by gently moving the probe array up and down) to remove O_2 and metabolite gradients, followed by a 2-min waiting period before the next measurement phase (i.e., lowering of the probe). Our experiments included 8 OCR and ECAR measurements to create a baseline, followed by the injection of Antimycin A (Sigma Aldrich). 3 OCR and ECAR measurements were then made. All measurements were normalized to total protein concentration using a standard BCA assay. Mitochondrial OCR was calculated by subtracting the final OCR value (pMoles O_2/min) after Antimycin A treatment from the average of the 3 OCR values before Antimycin A treatment. All quantitative data is represented as mean ± SE, n=3 independent experiments; 4-6 technical replicates per experiment.

Plasmids and Reagents

LV-shHIF-1α (#232222 designated clone 1, #54450 designated clone 2) and control pLKO.1 were purchased from Sigma-Aldrich. siHIF-1α was a gift from Dr. Connie Cepko (Addgene). The following plasmids were obtained from the Addgene repository: HRE-Luc (#26731) developed by Navdeep Chandel, HA-HIF1α (#18949) developed by Dr. William Kaelin, psPAX2 (catalogue no. 12260) and

pMD2G (catalogue no. 12259) developed by Dr. Didier Trono. *SLC16A3*-luc-P was a gift from Dr. Andrew Halestrap (Ullah et al., 2006). *SLC16A3*-luc-PI was cloned *de novo* as explained in the method section below.

Lentiviral Particle Production and Viral Transduction

HEK293T cells were seeded in 15-cm plates (3×10^6 cells/plate) in Opti-MEM (Life Technologies) with 2% FBS 2 days before transfection. The cells were transfected with 18 μg of control shRNA (pLKO.1) and shRNA against HIF-1α (LV-shHIF-1α) along with 12 μg of psPAX2 and 6 μg of pMD2.G. After 16 h, the transfection medium was removed and replaced with DMEM with 10% heat-inactivated FBS and penicillin-streptomycin. Lentiviral particles were harvested at 48 h and 60 h post-transfection. NP cells were plated in DMEM with 10% heat-inactivated FBS 1 day before transduction. Cells in 10-cm plates were transduced with 5 ml of conditioned media containing viral particles along with 8 μg/ml Polybrene. After 24-48 h, media was removed and replaced with DMEM with 10% FBS and continued for 3 days. The cells were cultured in hypoxia or normoxia for additional 24 h and harvested for protein and mRNA extraction 5 days after transduction.

Chromatin Immunoprecipitation

Rat NP cells were plated in 15-cm plates and cultured under normoxic or hypoxic conditions for 24 hours. ChIP assay was performed using ChIP-IT® high sensitivity kit (Active Motif, Carlsbad, CA) according to the manufacturer's recommendations. Cells were lysed and chromatin sheared by sonication. Input DNA was generated by treating aliquots with RNase, proteinase K, and heat, followed by ethanol precipitation. DNA complexes were immunoprecipitated by incubation with anti-HIF-1α antibody (Cell Signaling) overnight at 4°C followed by binding to protein G-agarose beads for 3 h at 4°C. Cross-links were reversed by treatment with proteinase K and heat for 2.5 h, and DNA was purified using DNA purification elution buffer. Real time PCR analysis was performed using ChIP-IT® quantitative PCR analysis kit (Active Motif) using the primer pairs for putative HRE sites as shown in Supplemental Table S2. Negative control primers and standard curve primers used were provided with kit. Real time PCR was performed with Power SYBR® Green PCR Master Mix (Applied Biosystems). The C_t values were recorded, and the data were normalized based on primer efficiency, input DNA C_t values, amount of chromatin, and re-suspension volume, based on manufacturer's recommendations. Criteria for positive binding include ≥ 5 HIF-1 binding events/ 1,000 cells and binding efficiency 5-fold greater than negative control. Data is represented as mean ± SE, n ≥ 3 independent experiments.

Plasmid Cloning

Insert DNA was amplified from Human Mixed Genomic DNA (204ng) (Promega) by incubating with Q5 High-Fidelity DNA Polymerase (0.02 U/μl) (NEB), dNTPs (200 μM) (NEB) and the following primers (0.5 μM) (IDTDNA):

FWD: 5'-TAAATT**GCTAGC**ccagtgaggtgccaaatgtg-3' (Nhe1 Restriction Site)

REV: 5'-TGCTTA**CTCGAG**gaacccaaaactccaccctc -3' (Xho1 Restriction Site)

The leader sequence is underlined, restriction site is bolded, and hybridization sequence follows. PCR product was isolated from the rest of the sample using the QIAquick PCR Purification Kit (Qiagen) and product size confirmed on agarose gel. PCR product and recipient plasmid were restriction digested with Nhe1 and Xho1 enzymes (NEB) overnight. Products were run on an agarose gel and gel purification was performed to isolate DNA using QIAquick Gel Extraction Kit (NEB). DNA ligation was conducted to

fuse the insert (0.06 pmol) and recipient plasmid (0.02 pmol) at a ratio of 1:3 recipient to donor using T4 DNA Ligase (NEB). Ligation reaction was transformed into DH5alpha cells and purified with Wizard Plus SV MiniPrep DNA Purification System (Promega). A diagnostic restriction digest was performed with Xho1 and Nhe1 enzymes to confirm the vector and insert bands were ligated. Final plasmid construct was verified by sequencing and analyzed using FinchTV software from Geospiza, Inc. (http://www.geospiza.com/finchtv/).