SUPPLEMENTAL INFORMATION

Figure S1, Characterization of the *PKM2* flox Allele, Related to Figure 1

(A) Southern blot screening of embryonic stem cell clones for homologous recombination of the targeting construct using a 5' probe. Insertion of a novel KpnI site creates a new band at ~6.0 kb. A clone with successful integration is marked with an arrowhead.

(B) Southern blot analysis of mouse genomic DNA using a probe on the 5' side of the inserted KpnI site; this probe is the same as was used in (A). Specific bands at 8.3 kb ($PKM2^{+}$) and 6.0 kb ($PKM2^{fl}$ allele) are marked with arrowheads; nonspecific bands are marked with an asterisk (*).

(C) Southern blot analysis of mouse genomic DNA using a probe on the 3' side of the inserted KpnI site. Specific bands at 8.3 kb ($PKM2^{+}$ allele) and 2.3 kb ($PKM2^{fl}$ allele) are marked with arrowheads; nonspecific bands are marked with an asterisk (*).

Figure S2, Analysis of Tumor Histology and PKM Splicing Following Exon 10 Excision, Related to Figure 2

(A) Staining of $PKM2^{+/+}$ and $PKM2^{\Delta/\Delta}$ tumors for Ki-67 and cleaved caspase 3 (CC3). Scale bars represent 200 μm.

(B) Histology showing livers from a $PKM2^{+/+}$ $BRCA^{fl/fl}$ *MMTV-Cre* $p53^{+/-}$ mouse with a breast tumor (left panels), and a $PKM2^{fl/fl}$ $BRCA^{fl/fl}$ *MMTV-Cre* $p53^{+/-}$ mouse with a breast tumor (right panels). Metastases are marked with arrowheads. Scale bars represent 500 μm and 20 μm in the low and high magnification images, respectively.

(C) Diagram showing normal alternative PKM splicing (top) and possible resulting amplicons used in RT-PCR analysis (bottom). Inclusion of exon 9 with NcoI sites yields the PKM1 transcript and inclusion of exon 10 with a PstI site yields the PKM2 transcript. RT-PCR amplification of spliced PKM transcripts using PCR primers in exons 8 and 11 (depicted as small arrows, top) produces amplicons of identical length that can be distinguished by different restriction sites (PstI & NcoI).

(D) Diagram showing PKM splicing following exon 10 deletion (top) and resulting amplicons (bottom). Inclusion of exon 9 creates PKM1 transcript, while exclusion of exon 9 in the absence of exon 10 results in PKM-skip transcript. RT-PCR amplification of these spliced transcripts using primers annealing to exons 8 and 11 (depicted as small arrows,

top) results in the amplicons depicted (bottom). These amplicons lack any PstI sites, and the location of NcoI restriction sites is shown.
(E) The amplicons and digestion fragments as numbered in Figure 2F. The PstI restriction site is marked with a "P".
(F) The amplicons and digestion fragments as numbered in Figure 2G. The NcoI and PstI restriction sites are marked with "N" and "P", respectively.
(G) Alignment of partial PKM2 and PKM-skip cDNA sequences in the region including parts of exons 8 to 11 (top). A diagram of the PKM-skip message showing the frameshift at the exon 8-11 junction, the location of the stop codon, and the distance to the following exon-exon junction is also shown.

Figure S3, Analysis of *PKM2*$^{\Delta/\Delta}$ Mouse Mammary Tumors, Related to Figure 3
(A) Schematic showing relative lengths and molecular masses of PKM1 and PKM2 protein (top), as well as the putative PKM-skip protein (bottom).
(B) Recombinant 6x-His-PKM2 and 6x-His-PKM-skip protein preparations were separated via SDS-PAGE and stained with Coomassie blue. 6x-His-PKM-skip protein and degradation fragments are indicated with a bracket; arrowheads indicate unrelated contaminating bacterial proteins.
(C) Western blots showing that two separate anti-PKM antibodies recognize recombinant PKM-skip protein. The location of 6x-His-PKM-skip protein and degradation fragments is indicated with a bracket. The monoclonal antibody recognizes a defined epitope around G200.
(D) Ribbon structure of a PKM2 tetramer (left) from the PDB (3BJF) (Christofk et al., 2008b), and the same structure shown when residues missing in the putative PKM-skip protein are omitted (right). The PKM-skip truncation eliminates the endogenous PKM dimer-dimer interface and the binding site of the allosteric activator fructose-1,6-bisphosphate (FBP). Ligands found at the active site and FBP binding site are shown in magenta.
(E) The ribbon structure of one subunit of a PKM2 tetramer is shown in gray, with residues retained in the putative PKM-skip protein highlighted in yellow. The A, B, and C domains

are boxed with a dashed line. Ligands found at the active site and FBP binding site are shown in magenta.

(F) A 6xHis-PKM-skip protein preparation was subjected to size exclusion chromatography, and collected fractions were analyzed by SDS-PAGE and Coomassie blue staining. The full-length 6xHis-PKM-skip protein is marked by an arrowhead on the right side of the panel; arrowheads at the top of the panel indicate fractions corresponding to the eluent volume of the indicated molecular weight standards. Marker lanes are indicated with an "M".

(G) Pyruvate kinase activity of recombinant 6xHis-PKM2 and 6xHis-PKM-skip, as determined using an LDH coupled assay. Values are reported as means ± s.e.m., n=3.

(H) The protein kinase activity of PKM-skip protein with nuclear lysate as a substrate was investigated using the conditions indicated. Reacted lysate was analyzed by SDS-PAGE and autoradiography. Two autoradiographs of the same experiment are shown to allow visualization of proteins phosphorylated in the presence of radioactive ATP and PEP. Similar labeling patterns are observed with each substrate, and the labeling is the same with and without PKM-skip protein. In addition, cold ATP effectively competes for all phosphorylation events observed, while cold PEP is unable to compete for any of these events. This suggests transfer of phosphate from radioactive PEP to ATP is responsible for any phosphorylation events observed.

Figure S4, Effects of PKM2 Deletion on Cells, Related to Figure 4

(A) Genotyping PCR of two *PKM2*$^{fl/\Delta}$ *Cre-ER*^{T2} tumor cell lines showing PKM2 exon 10 excision following two treatments with 1 μm 4-hydroxytamoxifen (4-OHT).

(B) The oxygen consumption rate of tumor cells with or without PKM2 expression was measured using a Clark electrode. An inhibitor of fatty acid β-oxidation, *R*-etomoxir was applied at 50 μM where indicated. Values are reported as means ± s.e.m., n=4.

(C) Effect of PKM2 deletion in Tumor Cell Line 1 on response to hypoxia. Data are reported as HRE-luciferase induction normalized to control renilla luciferase expression. Values are reported as means ± s.e.m., n=3.

(D) Effect of PKM2 deletion in Tumor Cell Line 2 on response to hypoxia. Data are reported as HRE-luciferase induction normalized to control Renilla luciferase expression. Values are reported as means ± s.e.m., n=3.

(E) Effect of PKM2 deletion in Cell Lines 1 and 2 on Glut1 induction in response to hypoxia. Glut1 transcript levels were determined by qPCR and are reported relative to β-actin. Values are reported as means ± s.e.m., n=3.
(F) Effect of PKM2 deletion in Cell Lines 1 and 2 on PDK1 induction in response to hypoxia. Glut1 transcript levels were determined by qPCR and are reported relative to β-actin. Values are reported as means ± s.e.m., n=3.
(G) Effect of PKM2 deletion in Cell Lines 1 and 2 on LDHA induction in response to hypoxia. Glut1 transcript levels were determined by qPCR and are reported relative to β-actin. Values are reported as means ± s.e.m., n=3.
(H) Effect of PKM2 deletion on β-catenin nuclear function in Cell Line 1. Cells were treated with 10 mM LiCl to stabilize β-catenin, or with 10 mM NaCl as control. The TOPFLASH and β-catenin-insensitive FOPFLASH luciferase reporters were co-transfected with a control Renilla luciferase construct. Reporter luciferase induction was determined relative to Renilla luciferase control, and data are presented as fold induction due to lithium treatment. Values are reported as means ± s.e.m., n=3.
(I) Effect of PKM2 deletion on β-catenin nuclear function in Cell Line 2. Cells were treated with 10 mM LiCl to stabilize β-catenin, or with 10 mM NaCl as control. The TOPFLASH and luciferase reporter was co-transfected with a control Renilla luciferase construct. Reporter luciferase induction was determined relative to Renilla luciferase control, and data are presented as fold induction due to lithium treatment. Values are reported as means ± s.e.m., n=3.
(J) Effect of PKM2 deletion on induction of Axin2, a β-catenin target gene, in response to 10 mM LiCl treatment, using Cell Line 2. 10 mM NaCl was used as control. Axin2 transcript levels were determined via qPCR and are reported relative to β-actin. Values are reported as means ± s.e.m., n=3.

Figure S5, Proliferative and Metabolic Phenotypes of Wild-Type and PKM2-deficient Tumors, Related to Figure 6

(A) Additional images showing PKM1/PCNA dual staining of allograft tumors. Quantification of the PCNA+ and PCNA-, as well as PKM1 high and low expressing cells is shown below. Four fields were scored for each of 3 tumors. Scale bars represent 20 μm.

(B) Additional images showing PKM1/PCNA dual staining of autochthonous tumors. Quantification of the PCNA+ and PCNA-, as well as PKM1 high and low expressing cells is shown below. Four fields were scored for each of 4 tumors. Scale bars represent 20 μm.

(C) PAS staining for glycogen content without and with diastase treatment of *PKM2*$^{+/+}$ and *PKM2*$^{\Delta/\Delta}$ tumors is shown. Scale bars represent 500 μm.

(D) Oil red O staining for neutral lipid stores in *PKM2*$^{+/+}$ and *PKM2*$^{\Delta/\Delta}$ tumors is shown. Scale bars represent 100 μm and 20 μm in the low and high magnification images, respectively.

(E) Percent enrichment of fully-labeled ^{13}C-glucose in the serum of mice at the time of tumor harvest. Cohorts of mice harboring allograft tumors derived from Tumor Cell Lines 1 and 2 were infused to different glucose serum enrichments. Percentages are calculated as (M+6 Glucose)/(Total Glucose)*100 and reported as means ± s.e.m.; n=8 mice for each genotype of Line 1; n=6 for Line 2 *PKM2*$^{fl/\Delta}$ mice and n=4 for Line 2 *PKM2*$^{\Delta/\Delta}$ mice. All calculations have been corrected for natural ^{13}C-isotope abundance.

(F) Absolute lactate concentrations were determined in Cell Line 2 allograft tumors via mass spectrometry. Values are reported as means ± s.e.m.; n=5 *PKM2*$^{fl/\Delta}$ tumors and n=3 *PKM2*$^{\Delta/\Delta}$ tumors.

(G) Relative abundance of fully-labeled ^{13}C-lactate in serum and tumors formed from cell lines that were or were not deleted for PKM2 as shown from mice that were infused with fully-labeled ^{13}C-glucose. Relative enrichment percent is calculated as (M+3 Lactate)/(Total Lactate)*100 and reported as means ± s.e.m.; n=8. All calculations have been corrected for natural ^{13}C-isotope abundance.

Table S1. PKM2 Mutations in Human Cancers, Related to Figure 7

PKM2 mutations were found in TCGA data from 8 different human cancer types. PKM2 mutations are reported by amino acid alteration and mutation type: Nonsense, Missense, or frameshift due to deletion (FS Deletion). The center reporting the mutation, number of mutations found, and the number of cases for each cancer type sequenced are also reported. The location of each point mutation in the PKM2 structure is provided when applicable.

Table S1. PKM2 Mutations in Human Cancers, Related to Figure 7

AA Change	Type	Center	Number	Total Cases	Cancer Type	Location in Structure
E154*	Nonsense	Broad	3	170	Lung Adenocarcinoma	
E304*	Nonsense	Broad	3	170	Lung Adenocarcinoma	
V375fs	FS Deletion	WashU	5	240	Uterine	
L394*	Nonsense	MDAnderson	2	192	Endometrial	
K266N	Missense	WashU	5	240	Uterine	A domain
P117L	Missense	WashU	5	240	Uterine	Hinge between A and B domains, near active site
R455Q	Missense	WashU	5	240	Uterine	C domain, near FBP binding pocket
R516C	Missense	WashU	5	240	Uterine	C domain, on loop FBP binding pocket loop
R246S	Missense	Broad	3	170	Lung Adenocarcinoma	A domain
V417L	Missense	Broad	3	170	Lung Adenocarcinoma	C domain, near dimer-dimer interface
L144P	Missense	Broad	2	132	Stomach	B domain
R92H	Missense	Broad	2	132	Stomach	A domain
E282K	Missense	Broad	4	286	Head and Neck	A domain
R319L	Missense	Broad	4	286	Head and Neck	C domain, forms intersubunit salt bridge with E28
S222L	Missense	Broad	4	286	Head and Neck	A domain
T60M	Missense	Broad	4	286	Head and Neck	A domain
D354N	Missense	Broad	1	88	Bladder	A domain
L465M	Missense	Broad	1	88	Bladder	A domain
S287I	Missense	Broad	2	220	Colon	A domain
G415R	Missense	Baylor	1	418	Kidney	C domain, at dimer-dimer interface

EXTENDED EXPERIMENTAL PROCEDURES

Generation of PKM2 Conditional Mice and Embryonic Fibroblasts (MEFs)

The relevant genomic DNA for the *PKM2* locus from 129S6/SvEvTac-derived TC1 embryonic stem (ES) cells was amplified using PCR and cloned into the pKOII targeting vector using standard molecular biology techniques (Bardeesy et al., 2002). The vector was introduced into TC1 ES cells by electroporation, selected with neomycin, and clones with homologous recombination at the *PKM2* locus were identified by Southern blot. The relevant genomic sequence encompassing *PKM2* exons 8 through 12 from the targeted ES cells was verified by DNA sequencing to confirm proper placement of the loxP sites and lack of any coding sequence mutations. Appropriately targeted ES cells were injected into blastocysts derived from C57/B6 mice and implanted into pseudo-pregnant females to generate chimeric animals. Germline transmission of the targeted allele was determined through breeding and confirmed with Southern blot. These mice were then crossed with FlpO-expressing transgenic mice to delete the neomycin resistance cassette and the FlpO transgene eliminated from the colony through breeding (Raymond and Soriano, 2007). MEFs were prepared from E13.5 *PKM2* $^{+/+}$ *Cre-ER* or *PKM2* $^{fl/fl}$ *Cre-ER* embyros using standard protocols. Where indicated, MEFs were treated with 500 nM 4-hydroxytamoxifen or mock treated and analyzed 4 days later.

Southern Blotting

Genomic DNA was prepared from embryonic stem cells or mouse tail tissue, digested with Asp718 (cuts at same site as KpnI, Roche), separated by agarose gel electrophoresis, neutral transferred to a membrane (Hybond XL, GE Healthcare), and UV crosslinked prior to radioactive probe hybridization using QuickHyb (Stratagene) according to the

manufacturers instructions. The DNA probes were prepared using a Rediprime II Kit (GE Healthcare), and probe binding was visualized by autoradiography.

PCR Genotyping

PCR genotyping was performed using forward (5′-TAGGGCAGGACCAAAGGATTCCCT-3′) and reverse (5′-CTGGCCCAGAGCCACTCACTCTTG-3′) primers to amplify the genomic locus including *PKM2* exon 10.

Quantitative RT-PCR and Analysis of PKM Splicing

Quantitative RT–PCR was performed on Trizol-extracted RNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems). qPCR reactions were performed using Fast SYBR Green Master Mix (Applied Biosystems) and a Roche Light Cycler 480II thermocycler (Roche). *PKM2*, *PKM1*, and *PKLR* expression were reported relative to *GAPDH* expression. qPCR primers were: *PKM2* forward 5′- GTCTGGAGAAACAGCCAAGG-3′, *PKM2* reverse 5′-CGGAGTTCCTCGAATAGCTG-3′; *PKM1* forward 5′-GTCTGGAGAAACAGCCAAGG-3′, *PKM1* reverse 5′-TCTTCAAACAGCAGACGGTG-3′; *PKLR* forward 5′-AAGGGTCCCGAGATACGCA-3′, *PKLR* reverse 5′-CTGCAACGACCTGGGTGATA-3′; *GAPDH* forward 5′-AGCTTGTCATCAACGGGAAG-3′, *GAPDH* reverse 5′-TTTGATGTTAGTGGGGTCTCG-3′; *PDK1* forward 5′-GGACTTCGGGTCAGTGAATGC-3′, *PDK1* reverse 5′-TCCTGAGAAGATTGTCGGGGA-3′; *GLUT1* forward 5′-CAGTTCGGCTATAACACTGGTG-3′, *GLUT1* reverse 5′-GCCCCCGACAGAGAAGATG-3′; beta-actin forward 5′-GGCATAGAGGTCTTTACGGATGTC-3′, beta-actin reverse 5′-TATTGGCAACGAGCGGTTCC-3′. TaqMan probes were used for LDHa and Axin2. Quantification of mouse PKM splicing was performed as reported previously (Clower et al., 2010).

Western Blot Analysis and Immunohistochemistry

Western blots were performed using standard techniques with primary antibodies against PKM1 (Sigma, SAB4200094), PKM2 (Cell Signaling Technology, #4053), PKM (Cell Signaling Technology, #3190; abcam, ab6191), FLAG (Cell Signaling Technology, #2368), beta-actin (abcam, ab1801), or GAPDH (Cell Signaling Technology, #2118) and detected using HRP-conjugated secondary antibodies, and chemiluminescence. The anti-PKM antibody #3190 from Cell Signaling Technologies recognizes an epitope that includes G200. For immunohistochemistry, formalin-fixed, paraffin-embedded tissue sections were stained with the following primary antibodies following antigen retrieval: PKM1 (Cell Signaling Technology, #7067), PKM2 (Cell Signaling Technology, #4053 at 1:800 dilution), PCNA (Cell Signaling Technology, #2586 at 1:2000 dilution), Ki-67 (BD Pharmingen, 556003 at 1:100 dilution), Cleaved Caspase 3 (Cell Signaling Technology, #9661 at 1:400 dilution), and/or FLAG antigen (Cell Signaling Technology, #2368 at 1:250 dilution). Staining was performed on an automated stainer or using a DAB peroxidase substrate kit and/or a Vector Red alkaline phosphatase substrate kit (Vector Laboratories) according to the manufacturer's protocol. Stained sections were counterstained with haematoxylin. Quantification of PKM1/PCNA dual-stained tumor sections was performed using ImageJ image processing software (National Institutes of Health). All cells in each field were scored as PCNA-positive or -negative and PKM1-high or -low; four fields were scored for each of 3 allograft and 4 spontaneous tumors.

Histology

Haematoxylin and eosin staining was used for routine histology. Glycogen stores were visualized in formalin-fixed, paraffin-embedded tumor sections using periodic acid-Schiff

stain with or without pre-digestion with diastase. Neutral lipid stores were stained in fresh-frozen tumor sections with Oil Red O.

Tumor-Derived Cell Lines

Freshly dissected tumors were minced, disaggregated with warm trypsin and propagated in Delbucco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and penicillin/streptomycin. Once established, tumor cell lines were retrovirally infected with an MSCV-CreER^{T2}-puro vector and a pLHCX expression vector containing no insert, FLAG-PKM1 cDNA, or FLAG-PKM2 cDNA as described previously (Christofk et al., 2008a). Puromycin (2 μg/mL) and hygromycin (100 μg/mL) were used to select for cells with stable integration of the constructs, and 1 μM 4-hydroxytamoxifen was used to induce recombination of PKM2^{fl} alleles where indicated.

Allograft Tumor Experiments

PKM2$^{\Delta/\Delta}$ mouse tumor cell lines with stably integrated empty vector, pLHCX-FLAG-PKM1, or pLHCX-FLAG-PKM2 were suspended in sterile PBS, and 5×10^6 cells injected into the flanks of nu/nu mice. Tumor growth was monitored by caliper measurement in two dimensions, tumor volume was estimated by the equation $V = (\pi/6)(L*W^2)$, and tumors harvested for analysis at the time indicated.

Tissue Microarray Analysis

PKM2 and PKM1 expression in human breast cancers was determined using IHC as described above. We analyzed three tissue microarrays: US BioMax BR1504, a multi-tissue TMA containing tissue obtained from the archives of the Institute of Pathology at the University of Basel (Baumhoer et al., 2008; Schraml et al., 1999), and two breast cancer TMAs from Beth-Israel Deaconess Medical Center. Each TMA core was scored for PKM2

IHC intensity independently by both a pathologist (D.D.V.) and another member of the team (T.D.). Tumors that showed no positive staining for PKM2 were given a score of 0, those with weak staining were given a score of 1, and tumors with strong PKM2 staining were given a score of 2. PKM2 scores from one TMA (US BioMax BR1504) were quantified relative to tumor subtype as determined by IHC staining results provided by US BioMax. Statistical significance was determined by the Chi-square test.

Preparation of Recombinant Proteins

Human PKM2 cDNA or cDNA corresponding to the predicted mouse PKM-skip protein product was cloned in pET28a with an in-frame N-terminal 6x-HIS tag. Proteins were expressed in *E. coli* strain BL21(DE3) and purified using nickel–nitrilotriacetic acid (Ni-NTA) affinity chromatography as previously described (Anastasiou et al., 2012).

Size Exclusion Chromatography

Recombinant PKM-skip protein was separated on a HiPrep 16/60 Sephacryl S-200 HR column (GE Healthcare) in 150 mM sodium chloride and 50 mM sodium phosphate, pH 7.2 as previously described (Anastasiou et al., 2012). Fractions were analyzed by SDS-PAGE and proteins visualized by staining with Coomassie blue.

Pyruvate Kinase Assay

Pyruvate kinase activity of recombinant proteins was determined using a lactate dehydrogenase-linked assay as previously described (Anastasiou et al., 2012). Assays were performed at 25° C in buffer containing 50 mM Tris pH 7.5, 100 mM KCl, 5 mM $MgCl_2$, and 100 U/ml lactate dehydrogenase with the following substrates: 500 μM PEP, 600 μM ADP, 180 μM NADH.

Protein Kinase Assay

Protein kinase activity was assayed as described previously (Gao et al., 2012). Briefly, purified recombinant His-tagged PKM-skip (10 μg/mL) was incubated with nuclear lysate (100 μg/mL) prepared from H1299 cells, ~1 μCi [γ-^{32}P]-ATP (Perkin Elmer) or ~0.1 μCi [^{32}P]-PEP (prepared as described in Vander Heiden et al. (2010)), and kinase buffer (50 mM Tris-HCl [pH 7.5], 10 mM KCl, 50 mM $MgCl_2$, and 1 mM DTT) at 30° C for 1 hour. Nuclear lysate was prepared using a protocol modified from Wang et al. (1994). Where indicated, 1 mM cold competitor ATP or PEP was added to the reaction. Reactions were stopped by addition of SDS-PAGE loading buffer and heated to 100° C. Reaction products were analyzed by SDS-PAGE and autoradiography.

Oxygen Consumption Assay

Oxygen consumption rates were measured at 37° C in standard growth medium using an Oxytherm electrode unit and Oxygraph software (Hansatech) in the presence or absence of 50 μM *R*-etomoxir.

Luciferase Reporter Assays

Cells were co-transfected with either a TOPFLASH, FOPFLASH, or HRE-luciferase reporter construct and a control *Renilla* luciferase construct (pRL-TK) at a 20:1 ratio using X-tremeGENE transfection reagent (Roche). Following treatment, cells were harvested and assayed using Dual-Luciferase Assay System reagents (Promega) and a Tecan Infinite M200 Pro plate reader. For experiments involving hypoxia, cells were incubated at 1% oxygen in a ProOx C21 C-chamber (BioSpherix).

Human Tumor DNA Sequence Analysis

Whole exome sequencing of 13 endometrioid endometrial tumors demonstrated a stop codon in exon 10 of PKM (Liang et al., 2012). Based on this observation, the PKM2

mutation was confirmed by Sequenom mass spectrometry of 234 human endometrial cancers. Additional mutations in PKM were identified through the cBIO portal (Cerami et al., 2012).

Mouse Glucose Infusion Studies

Venous catheters were implanted into the jugular vein of animals with allograft tumors 5-7 days before performing basal glucose turnover experiments as reported previously (Ayala et al., 2010; Jurczak et al., 2012). After surgical recovery, mice were fasted overnight before U-^{13}C-glucose was reconstituted in saline and infused into conscious mice for 120 min to achieve steady-state enrichments without perturbing endogenous glucose homeostasis. Next, mice were anesthetized with sodium pentobarbital injection and tissue harvested within 5 minutes, snap frozen in liquid nitrogen using Biosqueezer (BioSpec Products), and stored at -80 C for subsequent analysis.

Mass Spectrometry Metabolite Measurement

Metabolites were extracted and resuspended for analysis with isotope labeled internal standards. The samples from Line 1 were analyzed as described previously (Jain et al., 2012). Briefly, metabolites were separated by isocratic elution (40 % acetonitrile with 10 mM ammonium formate) from a C18 column (2.5 µm particle size, 2x50 mm, Shimazu) at 300 ul/min using a Shimatzu Prominince UHPLC. For MS analysis an ABSCIEX 5500 QTRAP equipped with a SelexION was used with lactate isotopologues measured in negative ion mode. For GC/MS, dried metabolites from plasma were resuspended in 20 µl of 2% methoxyamine hydrochloride in pyridine (Thermo) and held at 37 °C for 1.5 h. After dissolution and reaction, tert-butyldimethylsilyl derivatization was initiated by adding 25 µl *N*-methyl-*N*-(tert-butyldimethylsilyl)trifluoroacetamide + 1% tert-

butyldimethylchlorosilane (Sigma) and incubating at 37 °C for 1 h. GC/MS analysis was performed using an Agilent 7890A GC equipped with a 30m DB-35MS capillary column connected to an Agilent 5975C MS operating under electron impact ionization at 70 eV. One microliter of sample was injected in splitless mode at 270 °C, using helium as the carrier gas at a flow rate of 1 ml min^{-1}. For measurement of pyruvate and lactate, the GC oven temperature was held at 100 °C for 3 min and increased to 300 °C at 3.5 °C min^{-1}. The MS source and quadrupole were held at 230 °C and 150 °C, respectively, and the detector was run in scanning mode, recording ion abundance in the range of 100–605 m/z. MIDs were determined by integrating the appropriate ion fragments as described previously (Commisso et al., 2013). The samples from Line 2 were analyzed by hydrophilic interaction liquid chromatography (HILIC)/negative ion mode MS analyses using an Open Accela 1250 U-HPLC and a Q Exactive hybrid quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific; Waltham, MA). Briefly, chromatography used a Luna NH2 column (Phenomenex; Torrance, CA) eluted at a flow rate of 400 µL/min with initial conditions of 10% mobile phase A (20 mM ammonium acetate and 20 mM ammonium hydroxide in water) and 90% mobile phase B (10 mM ammonium hydroxide in 75:25 v/v acetonitrile/methanol) followed by a 10 min linear gradient to 100% mobile phase A. Analyses were carried out using negative ion mode and raw LC-MS data were processed using TraceFinder (v 3.0, Thermo Scientific) software.

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