EXTENDED EXPERIMENTAL PROCEDURES

Reagents, Antibodies, and Plasmids

Reagents were obtained from the following sources: PKCζ, phospho-PKCζ, PKCλ/ι, ULK, phospho-ULK LC3B, phospho-JNK, ACC, phospho-ACC, phospho-S6K, 4EBP1, phospho-4EBP1, phospho-AMPK, phospho-Thr, ATG5 antibodies and JNK siRNA were from Cell Signaling Technology; actin, JNK, S6K, AMPK, c-Jun antibodies and AMPK shRNA lentiviral particles were from Santa Cruz Biotechnology; caspase-3 antibody was from R&D System; p62 antibody was from BD Transduction Laboratories; anti-Ki67 (clone sp6) antibody was from Lab Vision Corporation; anti-PHGDH antibody was from Sigma; and anti-PSAT1 antibody was from Novus Biologicals. Affinity-purified phospho-Ser311-RelA polyclonal antibody was from Alpha Diagnostic International. All antibodies were used according to manufacturers' instructions. DMEM no glucose, glutamine and antibiotics were from Invitrogen; RPMI1640 was from US Biological, and labeled glutamine was from Cambridge Isotope Laboratories. Anti-Flag M2 Affinity gel, Flag peptide and all reagents used to derivatize metabolites for GC/MS analysis were from Sigma. The colon tissue microarray was from BioChain Institute, Inc (Cat. number Z7020032). Cobalt (II) chloride hexahydrate, SP600125, pepstatin A and E64d were from Sigma; rapamycin was from Cell Signaling Technology; AICAR and compound C were from EMD. The Luminescence ATP Detection Assay System (PerkinElmer) was used to determine intracellular ATP levels and the PE Active Caspase-3 Apoptosis Kit (550914, BD Pharmingen) for active caspase 3 analyses. TRC lentiviral shRNAs were from Open Biosystems, the TRC number for each shRNA is as follow: human PKCζ #1, TRCN000001219; human PKCζ #2, TRCN0000010114; human PKCζ #3, TRCN0000010121; human ATG5, TRCN0000099431; human PHGDH, TRCN0000028532; human PSAT1, TRCN0000035268. Retroviral pWZLHygro-HA-PKCζ and pWZL-Hygro-HA-PKCζK281R have been previously described (Galvez et al., 2009). Retroviral pBabe-Puro-Flag-Myc-PHGDH, pBabe-Puro-Flag-Myc-PHGDH-S55/T57/T78-7 AAA, pBabe-Puro-Flag-Myc-PHGDH-S55/T57/78-EEE were used to generate the cell lines stably overexpressed PHGDH WT, S55/T57/T78 AAA and EEE mutations. Plasmids of pCMV6-Flag-Myc-empty, pCMV6-Flag-Myc-PHGDH, pCMV6-Flag-Myc-PHGDH S55A, pCMV6-Flag-Myc-PHGDH S55E, pCMV6-Flag-Myc-PHGDH T57A, pCMV6-Flag-Myc-PHGDH T57E, pCMV6-Flag-Myc-PHGDH T78A, pCMV6-Flag-Myc-PHGDH T78E, pCMV6-Flag-Myc-PHGDH S55/T57/T78 AAA, pCMV6-Flag-Myc-PHGDH S55/T57/T78 EEE were transfected to 293T cells to express the PHGDH in vitro.

Cell Lysis, Western Blot, and Immunoprecipitations

Cells were rinsed once with ice-cold PBS and lysed in ice-cold lysis buffer (25 mM Tris [pH 8.0], 100 mM NaCl, 1% TritonX-100, 10% Glycerol) and one tablet of EDTA-free protease inhibitors (Roche; per 25 ml). The soluble fractions of cell lysates were isolated by centrifugation at 13,000 rpm for 15 minutes. For immunoprecipitations, primary antibodies were added to 0.5 ~1 mg cell the lysates and incubated with rotation overnight at 4°C. 40 μl of 50% slurry of protein G-sepharose or protein A-sepharose was then added and the incubation continued for an additional 1 hour. Immunoprecipitates were washed three times with lysis buffer and once with high-salt buffer (HEPES 40 mM, 500 mM NaCl and 0.5% Tx-100). Cell extracts or immunoprecipitated proteins were denatured by the addition of 20 μl of sample buffer followed by boiling for 5 minutes, resolved by 8% to 14% SDS-PAGE, and then transferred to nitrocellulose-ECL membranes (GE Healthcare). The immune complex was detected by chemiluminescence (Thermo Scientific).

Stable Isotope Labeling

Cultures of the SW480 cell lines described above were initiated at a density of approximately 106 cells per 10 cm2 dish. Labeling was started with cell cultures at ~40% confluency. At that point, (t) = 0 h, the medium was replaced with either "glucose-free" or "glutamine-only" medium. "Glucose-free" medium consists of DMEM supplemented with 10% v/v dialyzed fetal bovine serum, 1% v/v penicillin/streptomycin solution, and 4 mM total glutamine. The glutamine was isotopically labeled at all five carbon positions ([U-13C5] glutamine) or at the alpha nitrogen (α-[15N] glutamine). "Glutamine-only" medium consists of RPMI supplemented with 10% v/v dialyzed fetal bovine serum, 1% v/v penicillin/streptomycin solution and 2 mM total glutamine, isotopically labeled as above. Samples of each medium were collected at the beginning of labeling for t = 0 h analysis. A sample of the labeling medium was taken at t = 0 h and stored as a reference for analysis. For each dish, additional media samples were collected at the time of cell collection (24h). Labeled cells were rinsed with phosphate-buffered saline, detached with trypsin and subjected to centrifugation at 500 X g for 5 min. Cell pellets were stored at -80°C for subsequent analysis.

Fatty Acid Oxidation

Fatty acid oxidation was measured by using a 3H-palmitate protocol adapted from a previous publication (Djouadi et al., 2003). Briefly, cells were gently rinsed with PBS and cultured in 400 μl of [9,10-3H] palmitate:albumin for 4 hours at 37oC. After incubation, cells were dislodged and added to 10% TCA for centrifugation at 1,100 *g* at 4oC for 10 minutes. The supernatant was then mixed with 6N NaOH and applied to ion-exchange columns. The columns were washed with 1ml of water and the eluates containing 3H liberated from palmitate were counted using a scintillation counter. Background CPM was subtracted from all values.

cDNA Transfection-Based Experiments

293T cells were plated in 10 cm culture dishes (2 million cells/dish). 24 hours later, cells were transfected with 20 μg of the Flag plasmids for PHGDH and the different mutants described above. Two days after transfection, the cells were lysed as above. The Flag constructs were immunoprecipitated with the Anti-Flag M2 affinity gel and eluted with Flag peptide.

Enzyme Assays

PHGDH elutes, obtained as described above, were assayed in the direction of β-NAD reduction in a mixture containing 333 mM Tris/HCl pH 9.0, 333 mM hydrazine pH 9.0, 1.7 mM EDTA pH 9.0, 3.3 mM glutathione, 0.97 mM β-NAD, and 7.2 mM D(-)3 phosphoglycerate dehydrogenase. 2 μg of PHGDH WT or of the different mutants was added to each reaction well in 96-well-plate. The reduction of 3-PG was measured at 25 C by the increase of β-NADH at 340 nm for 30 min in a spectrophotometer (DTX880, BECKMAN). For kinetic measurements, the D(-)3 phosphoglycerate dehydrogenase (3-PG) concentrations were varied from 0.25 to 1mM. The enzymatic activities were expressed in μmol/min.mg protein. To evaluate the enzyme activity, the nonlinear, least-squares curve fitting was carried out with GraphPad Prism 5.00.

Histological Analysis

The total number of polyps was counted under a dissecting microscope. After processing, tissue samples were stained for H&E to determine polyp area using ImageJ software (http://rsbweb.nih.gov/ij/) and polyp diameter using a 25-point Chalkley eyepiece graticule. After being embedded in paraffin, the intestines were cut into 4 μm sections that were then stained with H&E for further analysis. For immunohistochemistry, sections were deparaffinized and rehydrated, and antigen retrieval was performed in 10 mM citric acid-2 mM EDTA (pH 6.2). Tissues were then incubated with primary antibody overnight followed by incubation with biotinylated secondary antibody. Antibodies were visualized with avidin-biotin complex (Vectastain Elite kit; Vector Laboratories) using diaminobenzidine as the chromogen. Slides were then counterstained with hematoxylin, dehydrated, and mounted. Colon tissue microarray samples were stained using the same protocol.

Immunofluorescence Assays

SW480 cells were plated on glass coverslips in 24-well tissue culture plates. 24 hours later cells were starved for glucose, rinsed with PBS once, fixed with warmed 4% formaldehyde and permeabilized with 0.3% Triton X-100. After rinsing twice with PBS, the coverslips were blocked for one hour in blocking buffer (0.3% BSA in PBS) and incubated with primary antibody in blocking buffer overnight at 4°C, rinsed twice with blocking buffer and incubated with secondary antibodies for one hour at room temperature in dark, following by tyramide signal amplification. Glass cover slips were mounted on Mowiol and examined with a Fluoview 1000 Olympus Laser Point Scanning Confocal Microscope.

Phosphorylation In Vitro

For *in vitro* phosphorylation assays, 1 μg of recombinant PHGDH (Creative Biomart) was incubated at 30°C for 60 min in kinase assay buffer containing 25 mM Tris–HCl pH 7.5, 5 mM MgCl2, 0.5 mM EGTA, 1 mM dithiothreitol (DTT), 100 μM ATP and 50 μCi of [γ-32P] ATP in the presence of recombinant PKCζ (Upstate).

Protein Digestion, TiO2-Based Phosphopeptide Enrichment

Proteins from *in vitro* phosphorylation reactions (above) were reduced with dithiothreitol, alkylated with indoleacetamide (both from Sigma) and digested with modified sequencing grade trypsin (Promega) using standard procedures. The trypsin:substrate ratio (w:w) was 1:50. Peptides were desalted using a peptide microtrap (Bruker-Michrom). Desalting was with 200 μl 95:5 solvents A:B (solvent A = 0.1% formic acid, solvent B = 100% acetonitrile) at 100 μl/min. Desalted peptides were eluted from the microtrap with 200 μl 70:30 solvents A:B at 100 μl/min and dried in a speed vac. Volumes of each solvent for phosphopeptide enrichment were 200 μl. Forty μl of TiO2 beads (GL Sciences, 5020-75000) as a 10 mg/ml slurry, in loading solvent, plus 160 μl loading solvent was added to the micro-centrifuge tube containing desalted peptides. Loading solvent was 65% ACN/2.0% trifluoroacetic acid (TFA) containing 10 mg/ml glutamic acid (Sigma-Aldrich). Tubes were vortexed for 60.0 min, centrifuged for 60 s and supernatants (nonbinding fractions) were saved. Loading solvent was added to the tubes, which were vortexed for 30.0 min. Tubes were centrifuged again and supernatants (wash fraction 1) were pooled with nonbinding fractions. Wash solvent 1 (65% ACN/0.5% TFA) was added to the tubes, vortexed, and centrifuged as in the previous step. The supernatant (second wash fraction) was pooled with the nonbinding and first wash fraction. Wash solvent 2 (50% ACN/0.1% TFA) was added to the tubes, which were vortexed for 30.0 min. Tubes were centrifuged, the supernatant (third wash fraction) was pooled with the nonbinding and wash fractions 1 plus 2. Elution solvent 1 (50% ACN/0.3 M NH4OH) was added to the sample tube, and incubated at 45°C, 1,400 rpm, 60.0 min. Tubes were centrifuged and the supernatant (first elution fraction) saved. Elution solvent 2 (5.0% ACN/0.3 M NH4OH) was added to the sample tube, and incubated at 45°C, 14,000 rpm, 60.0 min. Tubes with the TiO2 resin were centrifuged and supernatant (second elution fraction) was pooled with the first elution fraction. Nonbinding plus wash fractions were desalted as described above, dried in a speed vac, and re-suspended in 100 μl of 95:5 solvents A:B prior to electrospray ionization (ESI)- liquid chromatography (LC)- tandem mass spectrometry (MS/MS). Elution fractions were dried in a speed vac until the volumes were <50 μl. One hundred μl of 95:5/solvents A: B was added to each fraction, which was analyzed by ESI-LC-MS/MS as described below.

Electrospray Ionization-Liquid Chromatography Tandem Mass Spectrometry

Twenty five percent of each TiO2 elution fraction was run twice, and 10% of each nonbinding plus wash fraction was also run twice. Seven and 12 μl of 0.32% H3PO4 was added to the remaining elution and nonbinding plus wash fraction, respectively. Each was run again twice as described above. ESI-LC-MS/MS used a Paradigm MS2 HPLC, a 0.2 ID X 150 mm Magic C18 column, a captive spray

source operated at 1.40 kV (Bruker-Michrom), a column heater (Phoenix S & T) operated at 30C and an LTQ Orbitrap Velos mass spectrometer with electron transfer dissociation (ETD; Thermo Fisher). Instruments were controlled by Xcalibur v. 2.6.0 build software (Thermo Fisher) with custom plug-ins (Bruker-Michrom). The flow rate was 2.0 μl/min with an HPLC gradient of 10.0%-30.0% solvent B from 0.0 to 18.0 min, 80.0% B from 18.1 to 24.0 min, and 10.0% B from 24.1 to 31.0 min. The decision tree MS/MS method (Swaney et al., 2008) was top 20 data-dependent. Precursor ions were scanned in the Orbitrap at a resolution of 60,000, and MS/MS scans were in the dual pressure cell linear ion trap. Dynamic exclusion was enabled with a repeat count of 1, repeat duration and exclusion duration of 30.0 s. The signal threshold to trigger MS/MS scans was 500 counts. Collision-induced dissociation (CID) activation used relative collision energy of 35.0 and an activation Q of 0.250 for 10.0 ms. ETD activation was for a maximum of 100.0 ms and was automatically controlled by the Xcalibur instrument software.

Searches of MS/MS Spectra against a Protein Database

MS/MS data were searched against an ipi.HUMAN.v.3.73 protein database (89,652 entries) using semi-tryptic specificity, with Sorcerer-SEQUEST on Sorcerer Enterprise hardware/software (SageN Research, Inc., Milpitas, CA). Precursor ion mass tolerance was 5.0 ppm and product ion mass tolerance was 0.5 atomic mass units (amu). Static carbamidomethylation of Cys residues (+57.021465 amu), differential oxidation of Met residues (+15.99492 amu), and differential phosphorylation of S, T, and Y residues (+79.966331 amu) were specified. For ETD spectra, the Versasearch script (SageN) specified modification of peptide N-termini (b- to c-ions, +17.02655 amu) and C-termini (y- to z-radical ions, -16.018724 amu) to account for the predominance of c- and z-type, rather than b- and y-type product ions in ETD spectra (Syka et al., 2004). Postsearch filtering was with ProteinProphet (trans-proteomic pipeline (TPP), Institute for Systems Biology, Seattle, WA) at protein FDRs of 0.005-0.009. There was no ambiguity of the identity of peptides derived from PKCζ and PHGDH.

Real-Time PCR

Total RNA was extracted by using TRIzol reagent (Invitrogen), and purified by using RNeasy mini kits (QIAGEN) following the manufacturer's protocol. The RNA was treated with DNAase (Ambion) before reverse transcription (Applied Biosciences). Reverse transcription was performed with 1 μg of total RNA. Quantitative real-time PCR was utilized to evaluate expression levels from the cDNA by using Absolute SYBR Green real-time PCR Mastermix (Thermo Fisher Scientific) and a real-time PCR thermocycler (Eppendorf). Expression levels of each gene were measured and then normalized with 18S. Primers are used as follows: Human PHGDH F, 5'-TGGTGG AAAAGCACCTT-3'; Human PHGDH R, 5'-AACAATAAG GCCACA GTCC -3'; Mouse PHGDH F, 5'-AAAGGGACCAT CCAGGTGGTTACA -3'; Mouse PHGDH R, 5'-ATGCTTCTCTCAGAAGGCCGACAA -3'; Human PSAT1 F, 5'- GGTGCAGGCTGG AAACAGCTCC-3'; Human PSAT1 R, 5'- TGCCTCCCACAGACACGTAGAAT-3'; Mouse PSAT1 F, 5'-AAACTTCTTATCCAGG CCGGTGGA -3'; Mouse PSAT1 R, 5'-TCCCAGCCTGCACTTTGTAGTCAA -3'; Human PSPH F, 5'-GAGCGGACTCCCTTTTAAGC -3'; Human PSPH R, 5'-CAGGGAGGTGAGCTGTGC -3'; Human SN2-F, 5'- GGAACTGCAGGATCCAAAGA-3'; Human SN2-R, 5'-AGGTCAGCAGGAGGTGGATG -3'; Human ASCT2 F, 5'-GCCATCAACGCCTCCGTGGGA -3'; Human ASCT2 R, 5'-ACGGGCACC TTCACCCTGGTTC -3'; Human ALT2 F, 5'-CTTGGCCGACCAGCCACTTCTG -3'; Human ALT2 R, 5'-GCATAGTGCCATCACC TGCCGGA -3'; Human GOT1 F, 5'-GCCCAGCACTCAAGGAGAAGCG -3'; Human GOT1 R, 5'-ACACAGCATTGTGATTCTCCCAGG -3'; Human GOT2 F, 5'-ACGTTCTGCCTAGCGTCCGCA-3'; Human GOT2 R, 5'-TGCACAGTGACAAACCGGCCACT-3'; Human GLS1 F, 5'-TCCATGTAGCTGCTGCAGAGG -3'; Human GLS1 R, 5'-CCGTTGTCAGAATCTCCTTGAGGT -3'; Human GLS2 F, 5'-TGGCCTGCGCTACAACAAGCTC -3'; Human GLS2 R, 5'-TGACTGGAATGTGGCATTGCTGAA -3'; Human GLUD1 F, 5'- ACCC ACGGCAGAGTTCCAAGACA -3'; Human GLUD1 R, 5'- CGCATAATTTGCCTGGCAGAACGC -3'; Human GLUD2 F, 5'- ACCAC TTGCTCCTGTCTGTTCA -3'; Human GLUD2 R, 5'- TTGCCTGGCAGAACGCTCCA -3'; Human PEPCK1 F, 5'- TTGGAGGCCGTA GACCTGC -3'; Human PEPCK1 R, 5'- GGCCATGCTAAGCCAGTGG -3'; Human PEPCK2 F, 5'- TCCAGCGAGCTGTGGATGAG-3; Human PEPCK2 R, 5'- TGCCACCACATAGGCTGAGT-3'; Human ME1 F, 5'- TGGTGTTTCGGAAGCCAAGAGGTC -3'; Human ME1 R, 5'- ACCCACAGGGATGCCCATTCCA -3'; Human ME2 F, 5'- GAGCAGGAGAGGCTGCTCTTGGAA -3'; Human ME2 R, 5'-ATCAGGTATGCTCTCTGGGGCTG -3'; Human ME3 F, 5'- CAGGCCCCGTGGACTGTTCAT -3'; Human ME3 R, 5'- ACCCC TCCGCATGCCGTGTA-3'.

Gas Chromatography/Mass Spectrometry

Cell pellets were resuspended in 0.6 ml cold (−20°C) 50% aqueous methanol containing 100 μM norvaline as an internal standard, frozen on dry ice for 30 min, then thawed on ice for 10 min before centrifugation. The supernatant was then partitioned with 0.3 ml chloroform to reduce the fatty acid content. The methanol fraction was dried by centrifugal evaporation and stored at −80 before analysis. Samples of culture medium (0.1 ml) were processed similarly, except that 0.5 ml methanol-water mixture was added initially. Metabolites were derivatized for GC/MS analysis as follows. First, 50 μl of 20 mg/ml ethoxyamine-hydrochloride was added to the dried pellet and incubated for 20 min at 80°C. After cooling, 50 μl N-tert-butyldimethylsilyl-Nmethyltrifluoroacetamide was added and samples were re-incubated for 60 min at 80°C before centrifugation for 5 min at 14,000 rpm (4°C). The supernatant was transferred to an autosampler vial for GC/MS analysis. A Shimadzu QP2010 Plus GC-MS was programmed with an injection temperature of 250°C, injection split ratio 1/10 to 1/50 (depending upon sample concentration) and injected with 0.5-3 μl of sample. GC oven temperature started at 130°C for 4 min, rising to 230°C at 4°C/min and to 280°C at 20°C/min with a final hold at this temperature for 2 min. GC flow rate with helium carrier gas was 50 cm/s. The GC column used was a 15 m x 0.25 mm x 0.25 μm SHRXI-5ms (Shimadzu). GC-MS

interface temperature was 300°C and (electron impact) ion source temperature was 200°C, with 70 V/150 μA ionization voltage/current. The mass spectrometer was set to scan m/z range 50-600, with ~1 kV detector. GC/MS data were analyzed to determine isotope labeling and quantities of metabolites. Metabolites with baseline separated peaks were quantified on the basis of total ion count peak area, using standard curves generated from running standards in the same batch of samples. To determine 13C or 15N labeling, the mass distribution for known fragments of metabolites was extracted from the appropriate chromatographic peak. These fragments contained either the whole carbon skeleton of the metabolite, or lacked the alpha carboxyl carbon, or (for some amino acids) contained only the backbone minus the side-chain (Nanchen et al., 2007). For each fragment, the retrieved data comprised mass intensities for the lightest isotopomer (without any heavy isotopes, M0), and isotopomers with increasing unit mass (up to M6) relative to M0. These mass distributions were normalized by dividing by the sum of M0 to M6, and corrected for the natural abundance of heavy isotopes of the elements H, N, O, Si, and C, using matrix-based probabilistic methods as described (Nanchen et al., 2007; van Winden et al., 2002) and implemented in Microsoft Excel (Portnoy et al., 2010). Labeling results are expressed as average fraction the particular compound that contains isotopic label from the particular precursor.

Quantification of Glucose and Glutamine Consumption and Lactate Production

The amount of glucose present in the media samples was determined using the D-Glucose Enzymatic Bioanalysis Kit from R-Biopharm (No. 10716251035). The assay was performed generally according to the manufacturer's protocol but scaled down to microplate format. Briefly, 4 μl of medium was added to 50 μl of reconstituted reagent solution and 96 μl of water. After reading the absorbance at 340 nm, 1 μl of enzyme suspension was added. The plate was incubated at 37°C for 30 min at room temperature and the final absorbance was read at 340 nm. The total amount of glutamine present in the media samples was determined using the L glutamine/ammonia assay kit from Megazyme (K-GLNAM). Glutamate dehydrogenase is used to first measure the total amount of ammonium in the media, followed by the amount of ammonium present after all glutamine has been deaminated by glutaminase. The assay was performed according to the manufacturer's protocol for microplate format. The amount of lactate present in media samples was determined by generally following the Sigma Diagnostics Procedure No. 826-UV. All components were purchased from Sigma. Nicotinamide adenine dinucleotide (10 mg) was dissolved in 2 ml glycine buffer, 4 ml water and 100 μl lactate dehydrogenase (1,000 U/ml). A sample of medium (2.5 μl) was added to 145 μl of this enzyme mixture and incubated at room temperature for 30 min. The level of lactate was calculated from the observed decrease in absorbance at 340 nm.

Microarray Data Analysis

Microarray studies were performed in the Genomics and Microarray Laboratory at the Department of Environmental Health, University of Cincinnati Medical Center. Briefly, total RNA was extracted from three independent cultures of SW480 shNT and shPKCζ cell lines and hybridized on Affymetrics human ST 1.0 microarrays. Scanning of the images and the first pass processing of probe-level fluorescence intensities will be performed using the Microarray Suite 5.0 software (MAS 5.0; Affymterix, Santa Clara, CA). The data were be normalized, and the calculation of the gene-specific summary measures was be performed by the robust multi-array average (RMA) procedure (Irizarry et al., 2003) based on the Entrez gene-centric probeset definitions provided by the University of Michigan "brainarray" group (Dai et al., 2005). Statistical significance of genes differentially expressed between SW480 shPKCζ and shNT cell lines were assessed using Empirical Bayes linear model (Sartor et al., 2006). The functional enrichment analysis was performed using LRpath methodology (Sartor et al., 2009) and gene lists in the Molecular Signature Database (MSigDB) (Subramanian et al., 2005) separately for genes upregulated and down-regulated after PKCζ knockdown.

For the human colorectal cancer data set [GEO GSE14333], raw data (CEL files) were downloaded for all 290 samples and normalized using RMA procedure (Irizarry et al., 2003) and the "brainarray" (Dai et al., 2005) Entrez gene-centric probeset definitions for the Affymetrix HG U133+ 2.0 microarrays. Samples from patients with stage B and stage C carcinomas were stratified into low and high PKCζ groups based on the median expression level of PKCζ gene (i.e. the half of the samples with greater than median expression level were assigned to high expression group and the other half of the samples to the low expression group). The statistical significance in the differences in survival times was assessed using the nonparametric log rank test. All analyses were performed using R software and Bioconductor packages (Gentleman et al., 2004).

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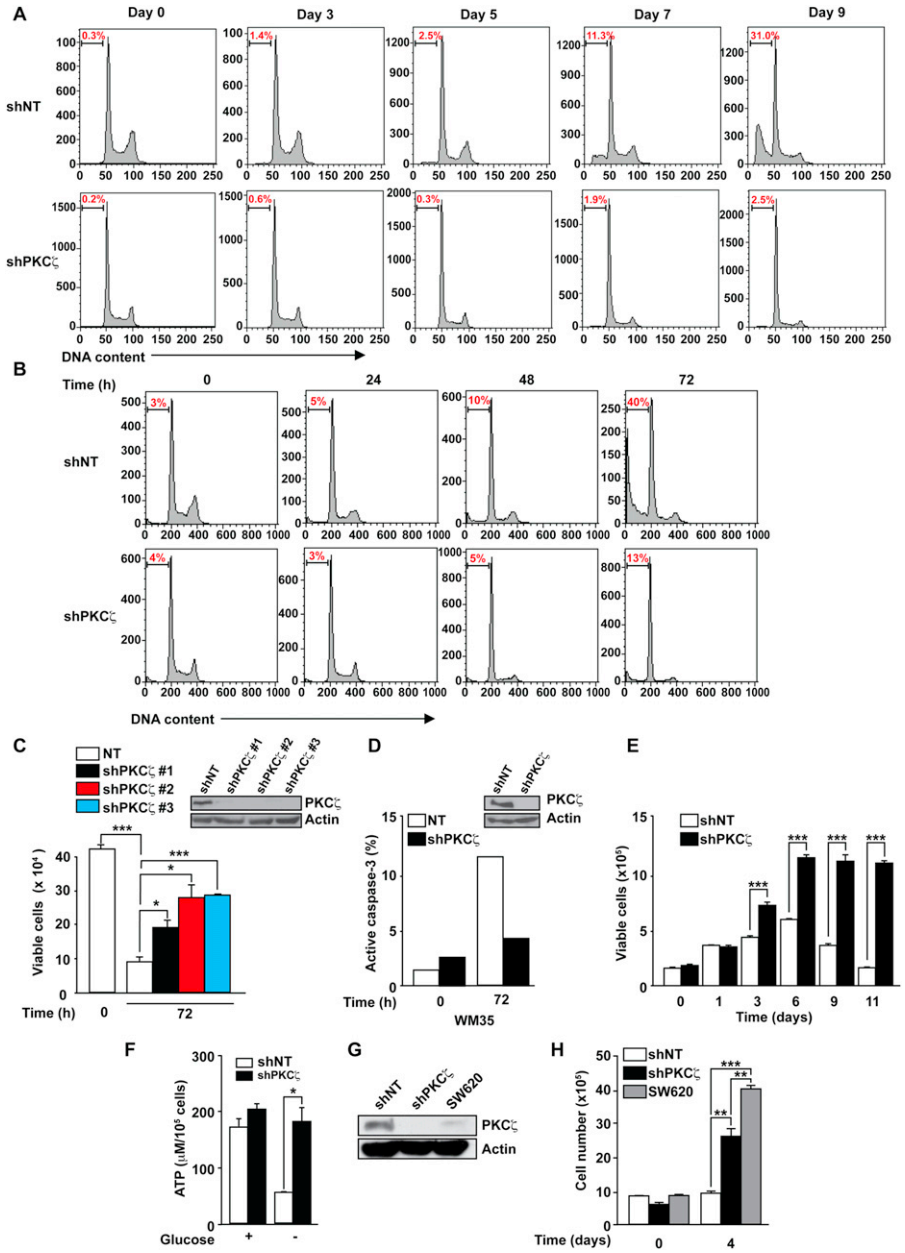

Figure S1. Cell Growth and Survival by PKCζ Depletion, Related to Figure 1

(A) Cell cycle analysis of cells in experiment of Figure 1D.

(B) Cell cycle analysis of cells in experiment of Figure 1G.

(C) WM35 cells infected with nontargeting lentiviral shRNA (shNT) or with different shRNAs specific for PKCζ (#1, #2, #3) were cultured under glucose-deprived conditions for 72 hr and cell viability was determined by trypan blue exclusion assay. Results are the mean ± SEM (n = 3). *p < 0.05, ***p < 0.001. Knockdown efficiency was determined by immunoblotting in cell lysates.

(D) Caspase-3 activity was determined by FACs in WM35 shNT or shPKCζ cells cultured as above for indicated time. PKCζ and actin protein levels were analyzed by western blot (inset).

(E) Viability of SW480 shNT and shPKCζ cells cultured without glucose with media change every other day. Number of cells was determined by trypan blue exclusion assay. Results are the mean ± SEM (n = 3). ***p < 0.001.

(F) Intracellular ATP levels in SW480 shNT or shPKCζ cells cultured under normal or glucose-deprived conditions for 48 hr. Results are the mean ± SEM (n = 3). *p < 0.05.

(G) Cell lysates from SW480 shNT or shPKCζ, and SW620 cells were analyzed by immunoblotting.

(H) Viability of SW480 shNT or shPKCζ and SW620 cells cultured under nutrient stress conditions for 4 days. Number of cells was determined by trypan blue exclusion assay. Results are the mean ± SEM (n = 3). **p < 0.01, ***p < 0.001.

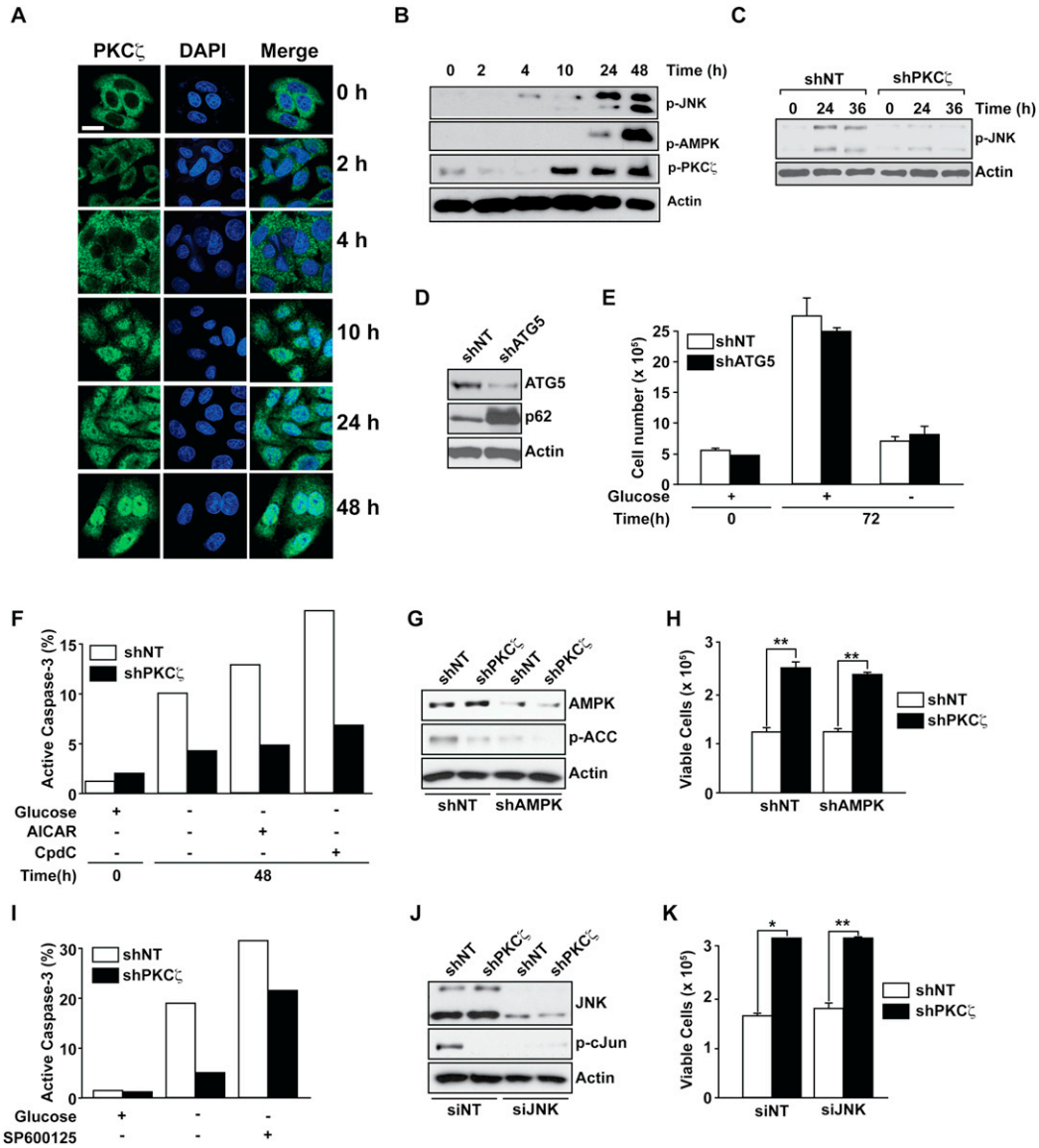

Figure S2. Stress-Signaling Activation by Glucose Deprivation, Related to Figure 2

(A) Normal or glucose-deprived for different times SW480 cells were coimmunostained for PKCζ and DAPI. Scale bar = 20 μM. Images are representative of two experiments.

(B) Cell lysates from SW480 cells cultured under glucose-deprived conditions were analyzed by immunoblotting with the specified antibodies. Results are representative of two experiments.

(C) Cell lysates from WM35 shNT or shPKCζ cells cultured under glucose deprivation for the indicated times were analyzed by western blot. These results are representative of three experiments.

(D) Cell lysates from SW480 shNT or shATG5 cells were analyzed by immunoblotting.

(E) SW480 shNT or shATG5 cells were cultured under glucose-deprived conditions and cell viability was determined after 72 hr. Results are the mean ± SEM (n = 3).

(F) Caspase-3 activity was determined by FACs in SW480 shNT or shPKCζ cells cultured under glucose-deprived conditions in the absence or presence of AICAR (0.2 mM) or Compound C (10 nM) for 48 hr.

(G and H) Western blot (G), and cell viability (H) analysis of SW480 shNT or shPKCζ cells in which AMPK has been knockdown or not cultured under glucose-deprived conditions for 72 hr. Results are the mean ± SEM (n = 3). **$p < 0.01$.

(I) Caspase-3 activity was determined by FACs in SW480 shNT or shPKCζ cells cultured under normal or glucose-deprived condition with or without SP600125 (10 μM) treatment for 48 hr.

(J and K) Western blot (J), and cell viability (K) analysis of SW480 shNT or shPKCζ cells in which JNK has been knockdown or not and cultured under glucose-deprived conditions for 72 hr. Results are the mean ± SEM (n = 3). *$p < 0.05$, **$p < 0.01$.

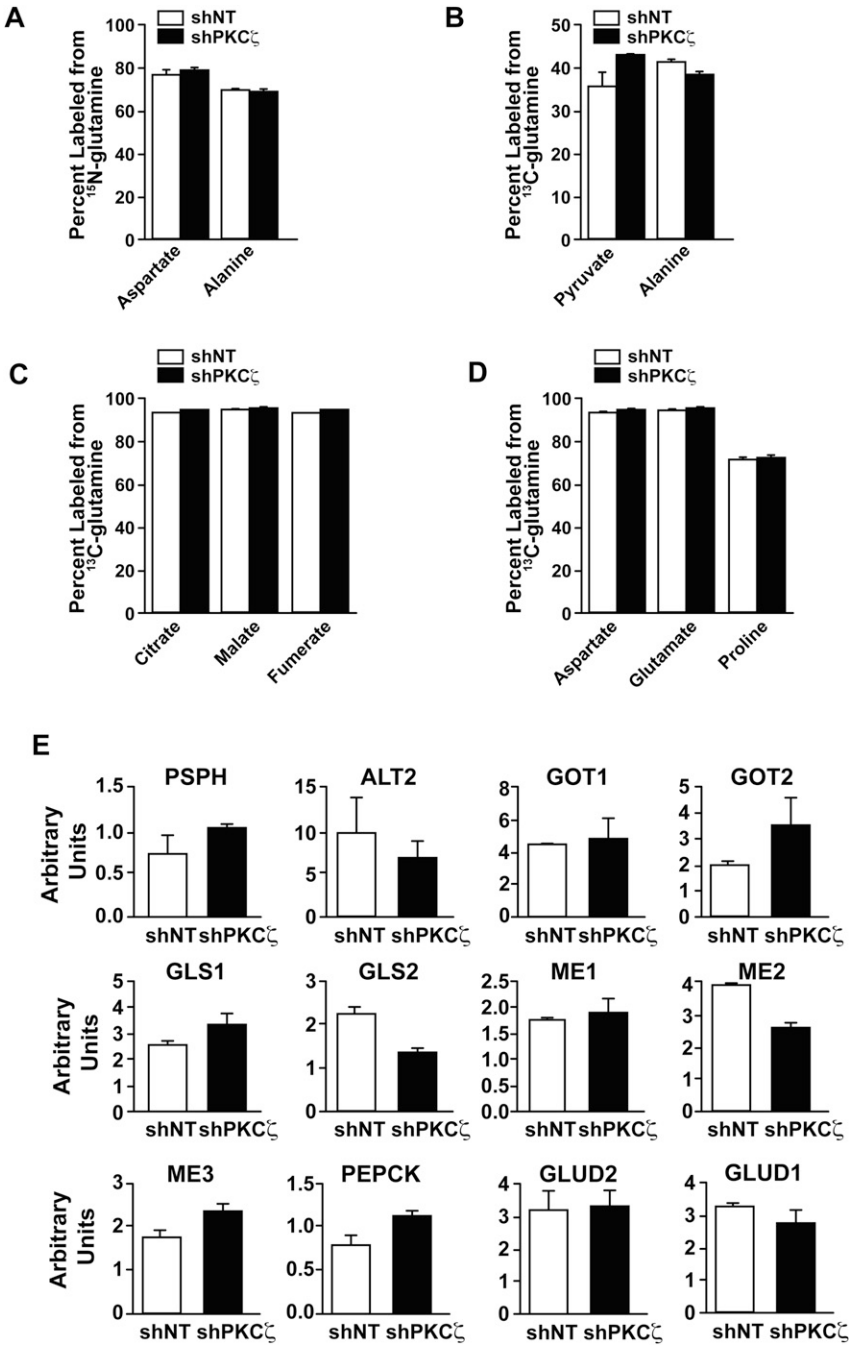

Figure S3. Glutamine Metabolism, Related to Figure 3

(A) The percent of aspartate, and alanine containing ^{15}N from glutamine in control (shNT) or PKCζ-deficient (shPKCζ) SW480 cells after 24 hr of growth. Glutamine was isotopically labeled only at the alpha position. Results are the mean ± SEM (n = 2-3).

(B) The percent of pyruvate and alanine containing ^{13}C from glutamine following 24 hr of growth in the SW480 control (shNT) or PKCζ-deficient (shPKCζ). Glutamine was isotopically labeled at all five carbons. Results are the mean ± SEM (n = 3-4).

(C) The percent of citrate, malate and fumarate containing ^{13}C from glutamine following 24 hr of growth in the SW480 control (shNT) or PKCζ-deficient (shPKCζ). Glutamine was isotopically labeled at all five carbons. Results are the mean ± SEM (n = 3-4).

(D) The percent of aspartate, glutamate and proline containing ^{13}C from glutamine following 24 hr of growth in the SW480 control (shNT) or PKCζ-deficient (shPKCζ). Glutamine was isotopically labeled at all five carbons. Results are the mean ± SEM (n = 3-4).

(E) mRNA expression of metabolic enzymes was determined by Q-PCR in SW480 shNT or shPKCζ cells cultured under glucose-deprived conditions for 48 hr. Results are the mean ± SEM (n = 3).

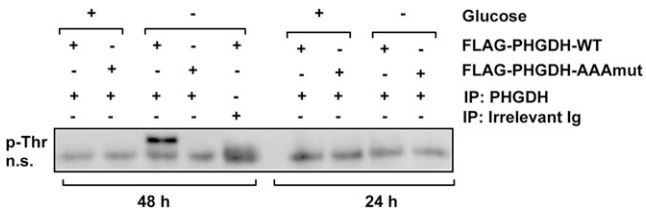

Figure S4. PKCζ-Dependent Threonine Phosphorylation of PHGDH, Related to Figure 4

Immunoprecipitates from SW480 cells stably expressing Flag-PHGDH or Flag-PHGDH AAA mutant cultured under normal or glucose-deprived conditions for 24 and 48h were analyzed by immunoblotting to determine the level of phosphorylated Thr. Results are representative of three experiments. Note that the panel corresponding to 48h time point is shown in Figure 4L.