Supplementary Information

SUPPLEMENTAL FIGURES

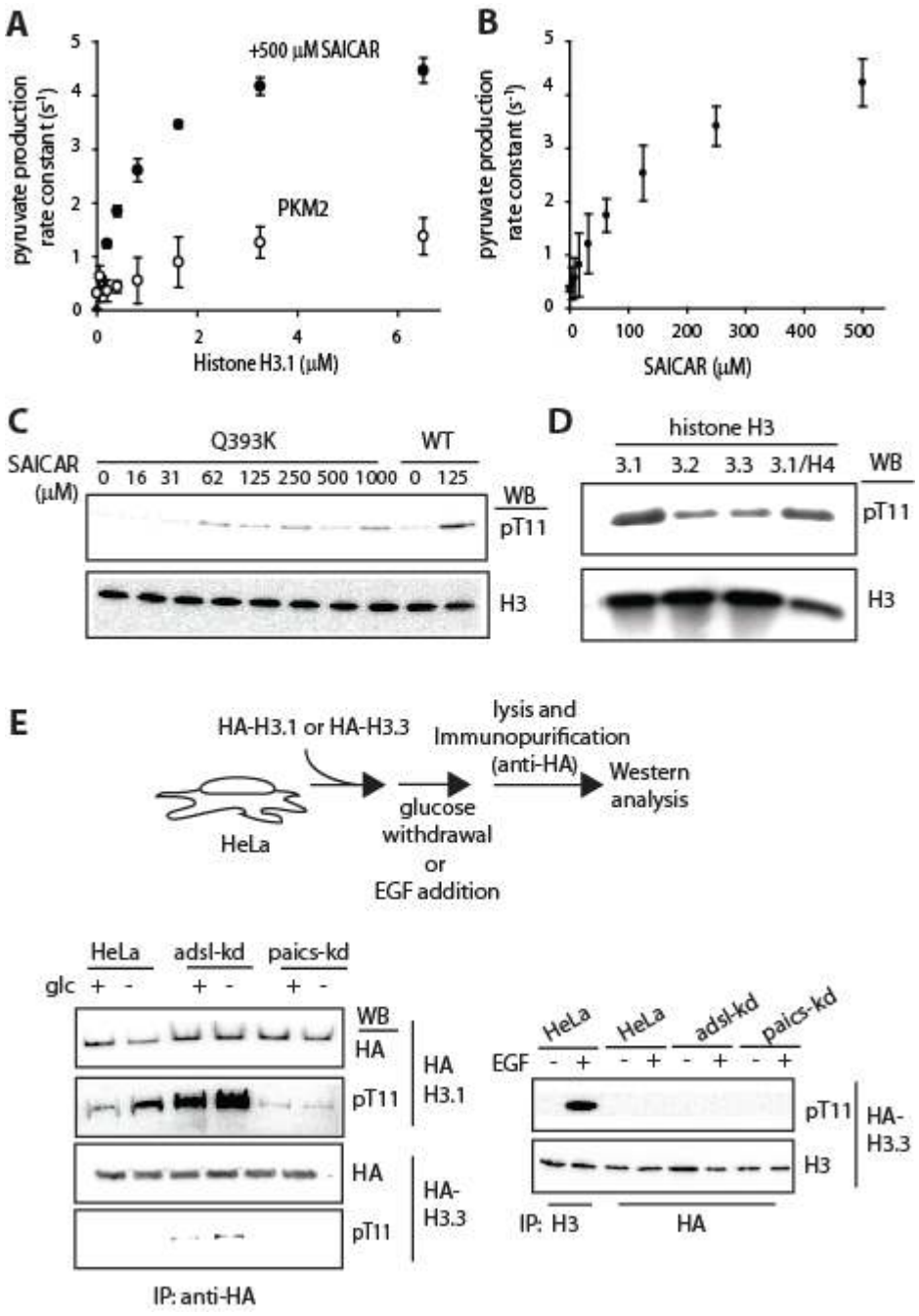

Figure S1. PKM2-SAICAR complex phosphorylates histone H3.1,related to **Figure 1**. (**A**) Protein kinase activity was measured by monitoring the production of pyruvate using a lactate dehydrogenase coupled assay while varying PEP and recombinant histone H3.1 concentrations. The experiment was carried out in the absence of ADP or other nucleotides. PKM2 pre-incubated (•) with 500 μM SAICAR or (○) without any ligand were used (avg. ± s.e.m.) (**B**) Protein kinase activity was carried out as in (A) varying SAICAR concentrations with histone H3.1 (1 μM) as the substrate (EC_{50}: ~250 μM SAICAR). (**C**) Phosphorylation of recombinant histone H3.1 (1 μM) by PKM2 Q393K (10 nM) in the presence of varying SAICAR concentrations and 0.5 mM PEP (10 minutes reaction time at ambient temperature). Wild type PKM2 (WT) phosphorylation of H3.1 in the absence or presence of 125 μM SAICAR was included for comparison. (**D**) Phosphorylation of different subtype of recombinant histone H3 monomers (1 μM) by PKM2 (10 nM)-SAICAR (0.5 mM). '3.1/H4' denotes H3.1/H4 heterotetramer. (**E**) Phosphorylation of hemaglutinin (HA)-tagged H3.1 and H3.3 in HeLa cells. HeLa cells and derivatives were transiently transfected with plasmids encoding either HA-H3.1 or HA-H3.3. Cells were then subjected to glucose-withdrawal (30 min in glucose-free medium) or in EGF (10 ng/mL)-containing medium (3 hours) followed by lysis, immunopurification using anti-HA (12CA5) or anti-H3 antibodies, and subjected to Western analysis. The results indicate that cells constitutively accumulating SAICAR ('adsl-kd') have constitutive H3.1 T11 phosphorylation while cells whose SAICAR synthase expression was knocked down ('paics-kd') were unable to induce H3.1 phosphorylation. H3.3 was not noticeably phosphorylated in any of conditions tested.

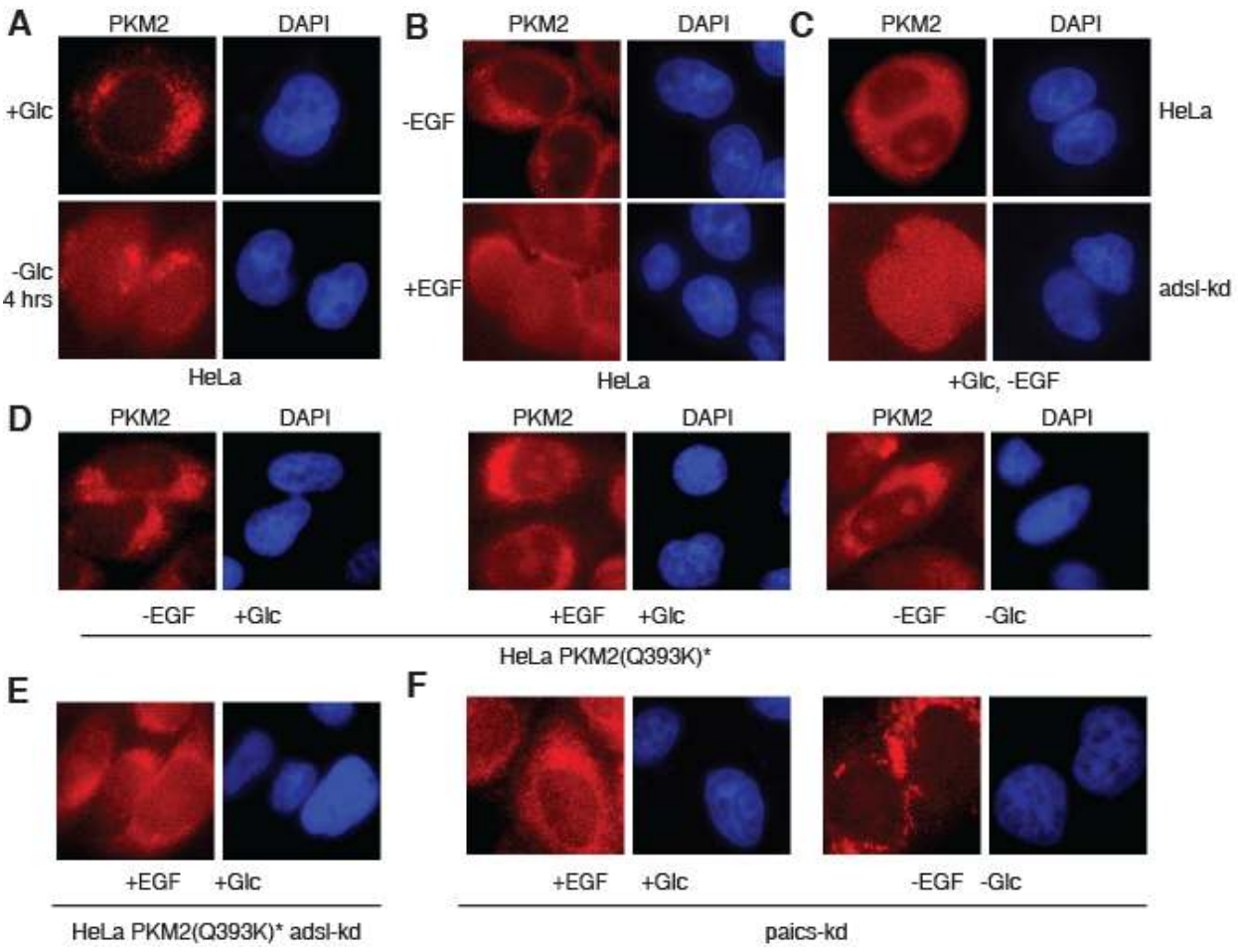

Figure S2. Effect of SAICAR on the subcellular localization of endogenous PKM2, related to **Figure 1**. Subcellular localization of PKM2 was detected as described in the Extended Experimental Procedures. (**A**) Effect of glucose withdrawal on the subcellular localization of PKM2 ('+Glc' and '-Glc' denote cells in DMEM with 25 mM glucose and cells incubated in glucose-free DMEM for 4 hours, respectively). (**B**) Effect of EGF on the subcellular localization of PKM2 ('-EGF' and '+EGF' denotes cells in medium without EGF and cells incubated for 3 hours in medium containing 10 ng/mL recombinant human EGF, respectively). (**C**) Effect of constitutive SAICAR accumulation on PKM2

subcellular localization. (**D**) Effect of replacing endogenous PKM1/2 with plasmid encoded SAICAR-insensitive PKM2 mutant (PKM2 Q393K). Little nuclear localization of PKM2, regardless of glucose or EGF conditions, was observed. (**E**) Effect of constitutive SAICAR accumulation on the localization of the SAICAR-insensitive PKM2 mutant. The SAICAR-insensitive PKM2 mutant was not efficiently localized to the nucleus even in *adsl-kd* cells. (**F**) Subcellular localization of PKM2 in cells whose SAICAR synthase expression was knocked down.

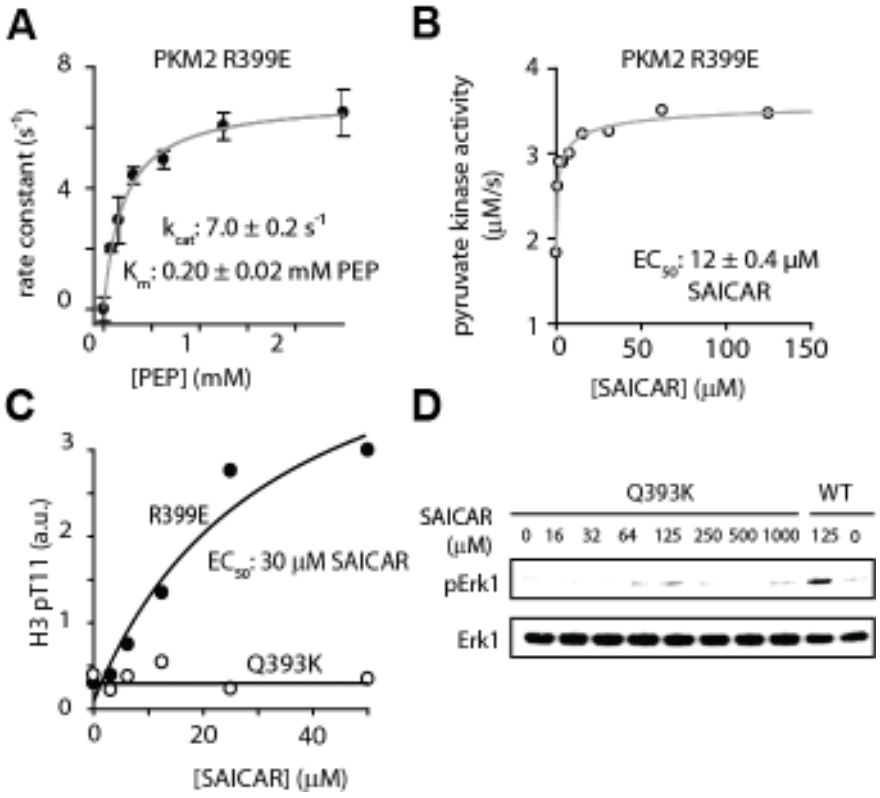

Figure S3. Constitutively nuclear PKM2 mutant is hypersensitive to SAICAR, related to **Figure 1**. (**A**) Pyruvate kinase activity assay with varying amount of PEP indicates that constitutively nuclear PKM2 mutant (R399E; Gao et al., 2012) is in its relaxed form (K_m in low μM PEP) instead of the tense form typically found in the wild-type PKM2. (**B** and **C**) PKM2 R399E mutant is also hypersensitive to SAICAR (EC_{50} 12 μM SAICAR, in comparison with EC_{50} value of 0.3 mM SAICAR for the wild-type PKM2) when its pyruvate kinase activity (**B**) and histone H3.1 T11 kinase activity (**C**) were measured in the presence of varying amount of SAICAR. (**D**) Protein kinase activity of PKM2 Q393K was measured as in Figure S1C using Erk1 (1 μM) as a substrate (0.5 mM PEP, 30 minutes at ambient temperature). Phosphorylation of Erk1 by Wild-type PKM2 (WT, 10 nM) in the presence or absence of 125 μM SAICAR is also included.

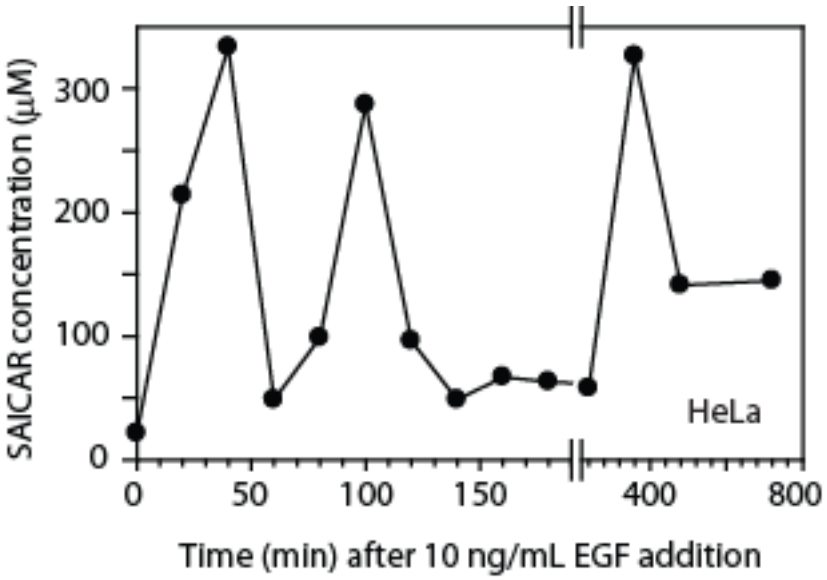

Figure S4. Effect of EGF addition to the cellular concentration of SAICAR in HeLa cells, related to **Figures 3** and **4**. HeLa cells were incubated in DMEM supplemented with dialyzed FBS (10%) for 24 hrs. The medium was replaced with the medium supplemented with dialyzed FBS and 10 ng/mL recombinant human EGF. Cells were harvested at each time point and cellular concentration of SAICAR was analyzed using LC-MS as previously described (Keller et al., 2012).

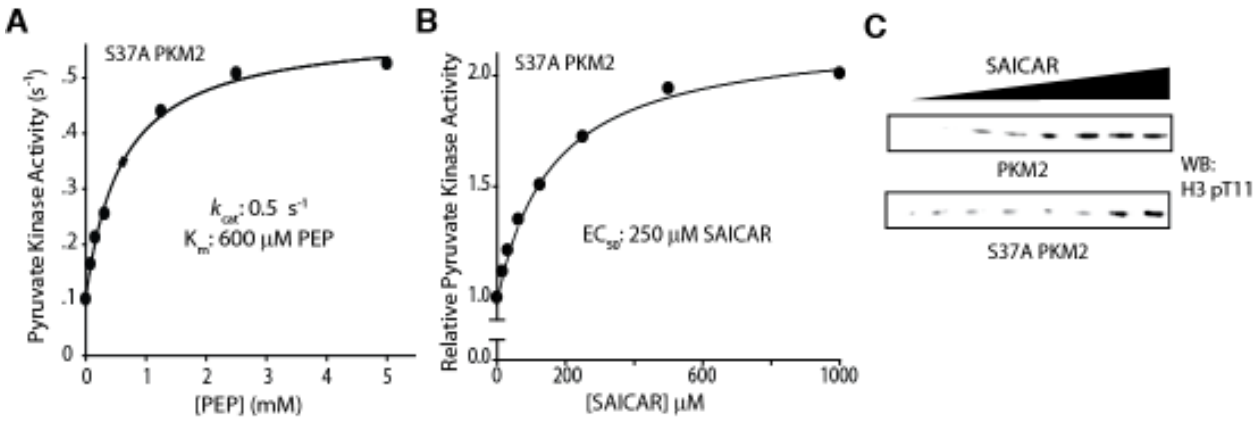

Figure S5. Characterization of PKM2 S37A mutant's enzyme activities, related to **Figure 5**. (**A**) Pyruvate kinase activity assay (rate constants) with varying amount of PEP indicates that PKM2 S37A mutant has pyruvate kinase activity similar to the wild-type PKM2 although kinetic parameters are slightly different (k: 0.5 s^{-1}; K_m: 600 μM PEP). (**B** and **C**) PKM2 S37A mutant is as sensitive to SAICAR as wild type PKM2 in both pyruvate kinase activity (**B**) and histone H3.1 T11 kinase activity (**C**).

SUPPLEMENTAL TABLE

Serine/Threonine Kinase	Receptor Tyrosine Kinase	Non-Receptor Tyrosine Kinase	Transcription Factor	Other Proteins
AKT3	ALK	ABL1	ALS2CR8	ADD1
BRSK1	CSF1R	ABL2	CARF	ALDOC
CAM2KB	EGFR	BLK	CASZ1	BEAN1
CAMKK2	EPHA1	BMX	HMBOX1	BIN1
CHEK1	EPHA2	DYRK1B	HSF1	BMI1
CHEK2	EPHA5	DYRK3	MEF2A	C10orf47
CHUK	EPHA8	FER	MEF2D	C19orf26
DCAMKL2	EPHB3	JAK2	MEF2D	C20orf67
DCLK2	EPHB4	JAK3	NFIC	CASS4
DYRK1B	ERBB2	PTK2	NFYA	CCDC6
DYRK3	FGFR1	SYK	NR1D1	CDC25A
GSK3A	FGFR2	TTN	NR4A1	CYPF12
HIPK1	FGFR3		RP5-860F19.3	DIXDC1
IKBKB	FGFR4		RXRA	EIF4G3
IKBKE	FLT1		RXRA	EP400NL
MAP2K1	FLT4		SOX5	EPB49
MAP2K2	KDR		TOX2	FAM122A
MAP3K11	KIT		TSC22D4	FSD1
MAP3K2	LTK		WDR62	GRB7
MAP3K3	MERTK		ZXDC	PM_2148
MAP4K2	MET			IL1B
MAPK12	MST1R			KANK4
MAPK13	NTRK2			KBTBD7
MAPKAPK2	NTRK3			KIAA1509
MAPKAPK3	PDGFRA			KIF26A
MAPKAPK5	PDGFRA alpha			NUP35
MARK2	RET			PAPOLA
NEK1	ROS1			PCM1
NEK6	TEK			PEPD
NLK	TYRO3			PHACTR4
PAK2				PSCD1
PAK6				R3HDM2
PAK7				RAG1AP1
PBK				SC4MOL
PDPK1				SDCCAG3
PRKAA2				SNAP91
PRKCE				SOCS3
PRKCQ				TFF2
PRKD1				WAC
PRKD3				ZFAND5
PRKG2				
RPS6KA2				
RPS6KA3				
RPS6KA5				
STK25				
STK33				
STK4				
TAOK2				
TBK1				
TSSK2				
TTN				
WNK2				

Table S1. List of proteins identified as potential PKM2-SAICAR substrates from the protein microarray experiment (see also **Figure 2**).

EXTENDED EXPERIMENTAL PROCEDURES

Materials

The preparation of SAICAR, analysis of cellular SAICAR concentration, recombinant PKM2, and pyruvate kinase assays were performed as previously described (Keller et al., 2012). Plasmids for bacterial expression of human Erk1 (Addgene plasmid 29578) and active Mek2 (Addgene plasmid 29580), which were originally constructed by D. Maly (U. Washington), were obtained from Addgene. Expression plasmid for GST-PAK2 (Addgene plasmid 31671) was originally constructed by A. Brunet lab (Banko et al, 2011). Plasmids encoding mutant Erk1 and PKM2 were constructed using QuikChange Lightening site directed mutagenesis kit (Agilent). Plasmids constructed in our lab were sequenced from both ends of inserts or mutation sites.

Proteins

Recombinant Erk1 (and its mutants), constitutively active MEK2, MAPK11, and GST-PAK2 were expressed in *E. coli* BL21 Codonplus RIL (DE3) cells harboring appropriate plasmids. Cells were grown in 0.50 liter Terrific Broth supplemented with chloramphenicol (25 μg/mL) and ampicillin (50 μg/mL) or kanamycin (50 μg/mL) at 37°C until optical density at 600 nm (OD_{600}) reached 0.6-0.8. Protein expression was induced by the addition of isopropylthio-β-D-galactopyranoside to 0.5 mM followed by incubation at 22°C for 12-18 hours. All subsequent steps were carried out at 4°C or on ice unless specifically noted otherwise. Cells were collected by centrifugation (5,000 g 20 min), and the cell pellet was resuspended in lysis buffer (20 mM HEPES, 500 mM NaCl, 5% glycerol, 1 mM DTT, 10 mM imidazole, pH 7.4 for His-tag proteins; 50 mM Tris-HCl, pH

8.0, 150 mM NaCl, 1 mM DTT for GST-tagged proteins) supplemented with phenylmethylsulfonyl fluoride (0.3 mM). Cells were lysed by sonication, and cell debris was removed by centrifugation (15,000g for 20 min). Cleared lysate was applied to 5 mL HiTrap Chelating column (for His-tagged proteins; charged with zinc chloride) or 5 mL GST Capture Column (for GST-tagged proteins). After washing with lysis buffer until A_{280} reached baseline, bound proteins were eluted with linear gradient of the lysis buffer supplemented with 500 mM imidazole (for His-tagged proteins) or 10 mM reduced glutathione (for GST-tagged proteins). SDS-PAGE and Commassie Blue staining determined the fractions containing protein. Desired fractions were combined and dialyzed against 10 mM sodium phosphate, 1.8 mM potassium phosphate, pH 7.4, 137 mM NaCl, 2.7 mM KCl, 5% glycerol, 0.5 mM EDTA, 2 mM DTT buffer. Proteins were concentrated, if desired, using Amicon Ultracel centrifugal filters. Protein concentrations were determined by measuring absorbance at 280 nm in denaturing conditions as in literature (Edelhoch, 1969), and stored at -80°C or at 4°C. Recombinant Erk1 was stable only for about a week after its purification in our hands, and was thus used within a week of lysis. Recombinant histone H3, H3/H4 tetramer, and H2A/H2B tetramer were purchased from New England Biolabs. Purified nucleosomes were gifts from G. Bowman's lab (Johns Hopkins University). The purified nucleosomes were found to contain H3 pT11, according to our Western analysis. Thus, the nucleosome was treated with biotinylated alkaline phosphatase (NEB). The alkaline phosphatase was removed from the reaction using 25 µL magnetic avidin beads (Pierce) and then the remaining dephosphorylated nucleosome was used for **Figure 1**. Recombinant human EGF was

purchased from Sigma-Aldrich. Recombinant Grb2, Mek2, B-Raf, and EGFR expressed and purified from insect cells were purchased from Invitrogen.

Cell culture

Mammalian cells were maintained and propagated in Dulbecco's Modified Eagle Medium (DMEM; 4 g/L glucose, 4 mM glutamine, without pyruvate) supplemented with 10% undialyzed fetal bovine serum (FBS), penicillin, and streptomycin at 37°C, 5% carbon dioxide, and humid conditions unless specifically noted otherwise. For glucose depletion or controlled EGF experiments, dialyzed FBS (Invitrogen) was used instead of normal FBS. For glucose depletion experiments, glucose-free DMEM (Invitrogen) was used. For controlled EGF experiments, recombinant EGF (10 ng/mL; Sigma-Aldrich) was added to medium containing dialyzed FBS. Cells were transfected with plasmids using the Neon Nucleotransfector (Invitrogen) using cell-type specific parameters provided by the vendor. Cell viability was determined using tryphan blue cell staining. To count cell numbers, cells were resuspended in trypsin/EDTA solution, diluted immediately with isotonic buffer, and then counted using a Moxi-Z cell counter.

Subcellular localization of PKM2

HeLa cells grown on coverslips were washed with PBS, fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for 5 min at room temperature, permeabilized with 0.01% Triton-X-100 in PBS for 5 min at room temperature, incubated in 5% goat serum in PBS for 1 hour, and stained with rabbit anti-PKM2 antibody (Sigma) for 45 min. Following incubation with solution containing DAPI and TRITC-

conjugated secondary antibody (Sigma), microscopic images were obtained using a Zeiss AxioVert 200 fluorescence microscope (Integrated Imaging Center, Johns Hopkins University, 63x optical zoom).

Protein Microarray

Human Protein Microarray ProtoArray v5.0 slide (Invitrogen) was blocked in 5% protease-free BSA (Roche) diluted in HPLC grade water (Fisher) for 30 minutes at room temperature without rocking and subsequently washed three times with HPLC grade water. The slide was then immersed into a 5-mL solution containing 10 nM rPKM2, 5% glycerol, 6.2 mM $MgCl_2$, 150 mM KCl, 10 mM HEPES, pH 7.4, and 0 or 0.5 mM SAICAR at 37°C for 30 minutes, followed by the addition of 0.5 mM PEP and incubated at 37°C for another 30 minutes. The slide was washed three times with HPLC grade water. A Pro-Q Diamond Phosphoprotein Stain Kit (Invitrogen) was used to stain phosphorylated proteins using the protocol provided by the vendor for staining of nitrocellulose blots. Fluorescence signals were detected using a Typhoon 9410 Variable Mode Imager (excitation 550 nm, excitation 580 nm, 600 PMT, 10 μm particle size; Integrated Imaging Center, Johns Hopkins University). Images were analyzed using ImageJ software with a microarray plugin. Sub-arrays were aligned using AlexaFluor-conjugated protein spots in each subarray.

Spectroscopic analysis of PKM2's protein kinase activity

Assay performed under similar condition to typical pyruvate kinase assays. The experiment was carried out in the absence of ADP or other nucleotides. In short, PKM2

(10 nM) was incubated for 30 minutes at 37°C with varying recombinant histone H3.1 concentrations in buffer (10 mM HEPES pH 7,4, 100 mM KCl, 6.2 mM $MgSO_4$) in the presence or absence of 500 µM SAICAR. After incubation, NADH (0.2 mM) and LDH (50 U/mL) were added, and immediately before detection, PEP (200 µM) was added. Change in fluorescence was measured for 30 minutes (excitation: 540 nm/emission: 590 nm), using the Tecan Infinite Series 200 Pro.