Supporting Information

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SI Methods

Metabolite Extraction. To each cell pellet suspended in 100 μL buffer was added 900 μL −40 °C MeOH containing an internal standard solution consisting of the following U-^{13}C-, U-^{15}N-labeled amino acids: Aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine alanine, arginine, tyrosine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, proline, and cysteine. The mixture was mixed by vortex for 30 s and then sonicated for 30 s using a bath sonicator (Model 2510; Branson). Cellular debris was removed by centrifugation (16,000 $\times$ g for 5 min at −20 °C). The supernatant was transferred to a fresh microfuge tube, and the solvent removed en vacuo overnight using a MiVac Duo Concentrator (Genevac).

Metabolite Derivitization. To ensure metabolite volatility for gas chromatographic analysis, each sample underwent a two-step derivatization process. The extracted metabolites were suspended in 50 μL pyridine containing *O*-methylhydroxylamine (20 mg/mL; Sigma-Aldrich) and incubated at 60 °C for 30 min. This was followed by the addition of 50 μL N-methyl-N-trimethylsilyltrifluoroacdtamide plus 1% trimethylchlorosilane (Pierce). This mixture was incubated at 37 °C for 30 min. After derivatization, the solution was immediately transferred to an auto sampler vial containing 10 μL of a fatty acid methyl ester solution used as a retention index standard.

GC-MS Analysis. An Agilent 7683 auto injector was used to add 1 μL of each sample into the injector set to a 2:1 split ratio. An Agilent 6890 gas chromatograph was used for analysis. A Restek 30-m Rtx-5MS with a 10-m guard column was used for separation. Helium was used as the carrier gas with the flow rate set to 1 mL/min. The oven was programmed for an initial temperature of 70 °C for 1 min followed by an 8 °C/min ramp to 250 °C. A second temperature ramp of 20 °C/min was used to a final temperature of 330 °C holding for 3 min. The transfer line into the mass spectrometer was held at 250 °C. A MicroMass GCT Premier (Waters) time-of-flight mass spectrometric was used for analysis. Normal −70 eV electron impact conditions were used for fragmentation. Data were recorded using MassLynx version 4.1 (Waters).

Data Analysis. Chromatographic peak detection and height analysis for each chromatogram was performed using MarkerLynx (Waters). To find possible variation in each sample group, the data were transferred to SIMCA-P+ ver.12.0 (Umetrics), where principle component analysis (PCA) and partial least squares-discriminate analysis (PLS-DA) were performed. This produced a possible list of metabolites that had changed because of treatment. Each metabolite was investigated further using Excel (Microsoft) for spreadsheet development. Significance testing was performed using JMP Pro version 7 (SAS).

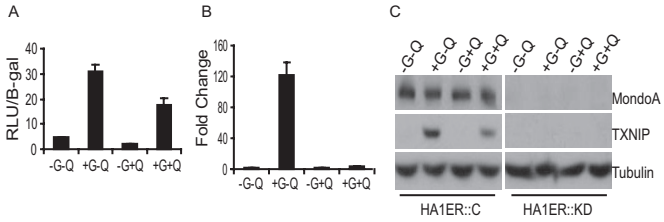

Fig. S1. Glutamine represses TXNIP at the transcriptional level. (*A*) Expression of TXNIP-Luciferase reporter gene was determined by Luciferase assay system in HA1ER cells cultured under the indicated growth conditions. (*B*) qRT-PCR was used to determine the relative expression of *TXNIP* in A549::C cells cultured under the indicated growth conditions. The normalized (*β-actin*) data are presented as fold change relative to the starved condition (-G-Q). (*C*) Expression of MondoA, TXNIP, and Tubulin in HA1ER::C and HA1ER::KD cells cultured under the indicated growth conditions was determined by western blotting.

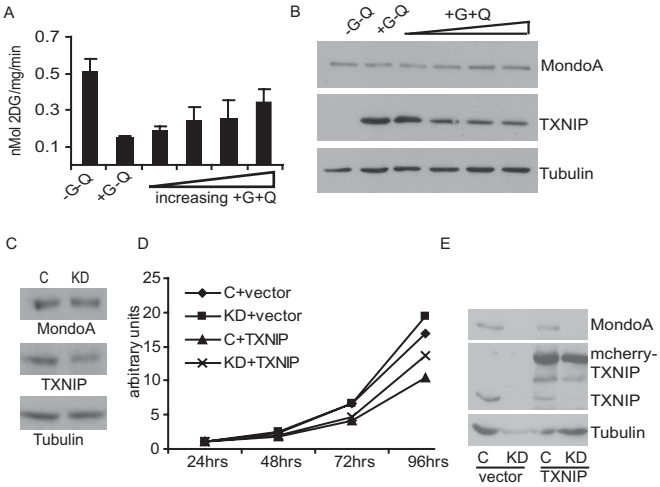

Fig. S2. TXNIP levels dictate glucose uptake and cell proliferation. (*A*) Glucose uptake was determined in BxPC-3 cells cultured in different concentrations of glutamine. (*B*) Expression of MondoA, TXNIP, and Tubulin in BxPC-3 cells cultured under the indicated growth conditions was determined by western blotting. Glutamine was used at 0.0, 0.1, 0.5, 1.0, 2.0, and 5.0 mM concentration. (*C*) Expression of MondoA, TXNIP, and Tubulin in control ''C'' and TXNIP knockdown ''KD'' BxPC-3 cells was determined by western blotting. (*D*) Cell proliferation was performed as described in Fig. 1*A* in control ''C'' and MondoA knockdown ''KD'' HA1ER cells expressing mCherry vector or mCherry-TXNIP. (*E*) Expression of MondoA, TXNIP, and Tubulin in control ''C'' and MondoA knockdown ''KD'' HA1ER cells expressing mCherry vector or mCherry-TXNIP was determined by western blotting.

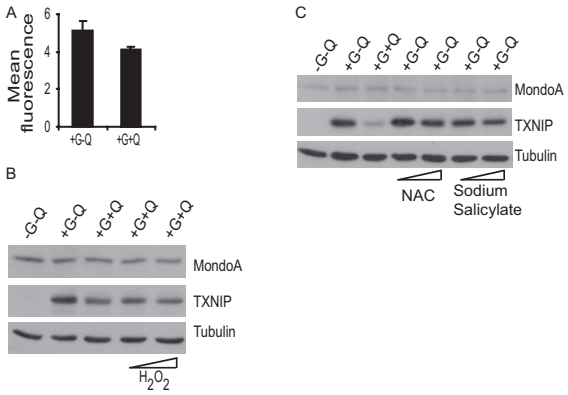

Fig. S3. TXNIP expression is not significantly affected by reactive oxygen species. (*A*) ROS levels in BxPC-3 cells were measured as mean fluorescence of CM-H_2DCFDA (Invitrogen) under the indicated growth conditions. (*B* and *C*) expression MondoA, TXNIP, and Tubulin in BxPC-3 cells grown under the indicated conditions were determined by western blotting. All treatments were 16 h. In panel *B*, H_2O_2, an ROS mimic, was used at 0.1 and 0.5 mM. In panel *C*, N-acetyl cysteine (NAC) and sodium salicylate, both anti-oxidants, were used 0.5 and 1 mM.

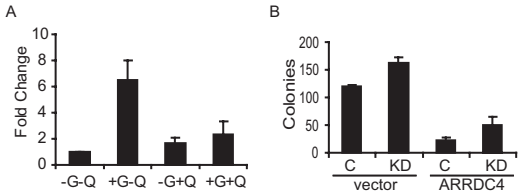

Fig. S4. ARRDC4 is a MondoA effector. (*A*) qRT-PCR was used to determine the relative expression of *ARRDC4*, normalized to *β-actin*, in BxPC-3 cells cultured under the indicated growth conditions. (*B*) Anchorage-independent growth of control ''C'' and MondoA knockdown ''KD'' HA1ER cells expressing mCherry vector or mCherry-ARRDC4.

Table S1. Genes and pathways regulated by glucose and/or glutamine

(Glucose + Glutamine upregulated, 1014 genes)	List	Array	z-score
Cell cycle	20	113	7.89
CD45 cell division cycle 45-like (S.cerevisiae)			
Cell division cycle 25 homology (S.pombe)			
Cell division cycle 6 homology (S.cerevisiae)			
Cell division cycle 7 homology (S.cerevisiae)			
Cyclin E1			
Cyclin E2			
Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)			
E2F transcription factor 1			
Minichromosome maintenance complex component 2			
Minichromosome maintenance complex component 3			
Minichromosome maintenance complex component 4			
Minichromosome maintenance complex component 5			
Minichromosome maintenance complex component 6			
Minichromosome maintenance complex component 7			
Origin recognition complex, subunit 1-like (yeast)			
Proliferating cell nuclear antigen			
Protein kinase, membrane associated tyrosine/threonine 1			
Retinoblastoma-like 1 (p107)			
S-phase kinase-associated protein 2 (p45)			
Structural maintenance of chromosomes 1A			
DNA polymerase	7	25	6.38
Polymerase (DNA directed), alpha 1			
Polymerase (DNA directed), alpha 2 (70kD subunit)			
Polymerase (DNA directed), epsilon 2 (p59 subunit)			
Polymerase (DNA directed), theta			
Polymerase (DNA directed), delta 3, accessory subunit			
Primase, DNA, polypeptide 1 (49kDa)			
Replication factor C (activator 1) 5, 36.5kDa			
Pathogenic Escherichia coli infection - EHEC	10	49	6.15
Toll-like receptor 4			
Tubulin, alpha 1b			
Tubulin, alpha 3c			
Tubulin, alpha 4a			
Tubulin, beta			
Tubulin, beta 2A			
Tubulin, beta 2C			
Tubulin, beta 3			
Tubulin, beta 6			
Tubulin, beta 8			
1, 4-Dichlorobenzene degradation	1	1	5.05
Carboxymethylenebutenolidase homolog (Pseudomonas)			
Pyrimidine metabolism	11	85	4.49
Deoxyuridine triphosphatase			
Dihydropyrimidine dehydrogenase			
Polymerase (DNA directed), alpha 1			
Polymerase (DNA directed), alpha 2 (70kD subunit)			
Polymerase (DNA directed), epsilon 2 (p59 subunit)			
Polymerase (DNA directed), delta 3, accessory subunit			
Primase, DNA, polypeptide 1 (49kDa)			
Replication factor C (activator 1) 5, 36.5kDa			
Ribonucleotide reductase M2 polypeptide			
Thymidine kinase 1, soluble			
Thymidylate synthetase			
Gap Junction	10	91	3.66
Platelet-derived growth factor receptor, alpha polypeptide			
Tubulin, alpha 1b			
Tubulin, alpha 3c			
Tubulin, alpha 4a			
Tubulin, beta			
Tubulin, beta 2A			
Tubulin, beta 2C			
Tubulin, beta 3			
Tubulin, beta 6			

(Glucose + Glutamine upregulated, 1014 genes)	List	Array	z-score
Tubulin, beta 8			
Synthesis and degradation of ketone bodies	2	8	3.16
3-hydroxy-3-methylglutaryl-Coenzyme A synthase (soluble)			
Acetyl-Coenzyme A acetyltransferase 2			
Tetrachloroethene degradation	2	8	3.16
Aldo-keto reductase family 1, member B10 (aldose reductase)			
Epoxide hydrolase 2, cytoplasmic			
One carbon pool by folate	3	16	3.15
Dihydrofolate reductase			
Serine hydroxymethyltransferase 1 (soluble)			
Thymidylate synthetase			
Glycosphingolipid biosynthesis - lactoseries	2	10	2.7
ABO blood group (transferase A, alpha 1–3-N-acetylgalactosaminyltransferase)			
ST3 beta-galactoside alpha-2,3-sialytransferase 4			
Methane metabolism	2	10	2.7
Catalase			
Serine hydroxymethyltransferase 1 (soluble)			
Glycine, serine and threonine metabolism	5	46	2.54
Aldo-keto reductase family 1, member B10 (aldose reductase)			
Choline dehydrogenase			
Guanidinoacetate N-methyltransferase			
Monoamine oxidase B			
Serine hydroxymethyltransferase 1 (soluble)			
1- and 2-Methylnaphthalene degradation	3	22	2.44
Alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide			
Establishment of cohesion 1 homolog 2 (S. cerevisiae)			
Biosynthesis of steroids	3	22	2.44
24-dehydrocholesterol reductase			
7-dehydrocholesterol reductase			
Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)			
Glycosylphoshatidylinositol (GPI)-anchor biosynthesis	3	23	2.34
Phosphatidylinositol glycan anchor biosynthesis, class M			
Phosphatidylinositol glycan anchor biosynthesis, class W			
Phosphatidylinositol glycan anchor biosynthesis, class Z			
Nitrogen metabolism	3	23	2.34
Carbonic anhyrase II			
Carbonic anhydrase IX			
Histidine ammonia-lyase			
MAPK signaling pathway	3	255	−2.25
Arrestin, beta 1			
Mitogen-activated protein kinase kinase 6			
Platelet-derived growth factor receptor, alpha polypeptide			
Retinol metabolism	1	4	2.23
Aldehyde dehydrogenase 1 family, member A1			
Ethylbenzene degradation	2	13	2.2
Establishment of cohesion 1 homolog 2 (S. cerevisiae)			
Patatin-like phospholipase domain containing 3			
Folate biosynthesis	4	39	2.14
Dihydrofolate reductase			
Nudix (nucleoside diphosphate linked moiety X)-type motif 8			
RAD54 homolog B (S. cerevisiae)			
RAD54-like (S. cerevisiae)			
Benzoate degradation via CoA ligation	3	26	2.09
Acetyl-Coenzyme A acetyltransferase 2			
Establishment of cohesion 1 homolog 2 (S. cerevisiae)			
Patatin-like phospholipase domain containing 3			
(Glutamine upregulated, 662 genes)			
One carbon pool by folate	4	16	4.99
Aldehyde dehydrogenase 1 family, member L1			
Dihydrofolate reductase			
Serine hydroxymethyltransferase 1 (soluble)			
Thymidylate synthetase			
Biosynthesis of steroids	4	22	4.02
24-dehydrocholesterol reductase			
7-dehydrocholesterol reductase			

(Glucose + Glutamine upregulated, 1014 genes)	List	Array	z-score
Isopentenyl-diphosphate delta isomerase 1			
Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase			
Synthesis and degradation of ketone bodies	2	8	3.52
3-hydroxy-3-methylglutaryl-Coezyme A synthase 1 (soluble)			
Acetyl-Coenzyme A acetyltransferase 2			
Alanine and aspartate metabolism	4	32	3.02
Adenylosuccinate synthase like 1			
Argininosuccinate lyase			
Argininosuccinate synthetase 1			
Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)			
Novobiocin biosynthesis	1	3	2.98
Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)			
Cytokine-cytokine receptor interaction	14	247	2.3
Chemokine (C-C motif) ligand 20			
Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activitym alpha)			
Chemokine (C-X-C motif) ligand 14			
Chemokine (C-X-C motif) ligand 2			
Chemokine (C-X-C motif) ligand 3			
Chemokine (C-X-C motif) receptor 3			
Chemokine (C-X-C motif) receptor 4			
Colony stimulating factor 3 (granulocyte)			
Inteleukin 10 receptor, alpha			
Interleukin 17 receptor B			
Interleukin 8			
Platelet-derived growth factor receptor, alpha polypeptide			
Tumor necrosis factor (ligand) superfamily, member 9			
Tumor necrosis factor receptor superfamily, member 6b, decoy			
Type II diabetes mellitus	4	43	2.3
Adiponectin, C1Q and collagen domain containing			
Calcium channel, voltage-dependent, R type, alpha 1E subunit			
Protein kinase C, zeta			
Suppressor of cytokine signaling 1			
Alkaloid biosynthesis I	1	5	2.15
Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)			
Terpenoid biosynthesis	1	5	2.15
Isopentenyl-diphosphate delta isomerase 1			
Glutamate metabolism	3	30	2.14
Glutamate-cysteine ligase, modifier subunit			
Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)			
Glutamic-fructose-6-phosphate transaminase 1			
Complement and coagulation cascades	5	67	2.02
Complement component 1, r subcomponent			
Complement component 1, s subcomponent			
Complement component 3			
Complement factor B			
Plasminogen activator, tissue			
(Glucose upregulated, 73 genes)			
Keratan sulfate biosynthesis	1	16	4.11
Carbohydrate (N-acetylglucosamine 6-O) sulfotransferase			
Renin-angiotensin system	1	17	3.98
Alanyl (membrane) aminopeptidase			
B cell receptor signaling pathway	2	63	3.94
Inositol polyphosphate-5-phosphatase			
Vav 3 guanine nucleotide exchange factor			
Fc epsilon RI signaling pathway	2	74	3.57
Inositol polyphosphate-5-phosphatase			
Vav 3 guanine nucleotide exchange factor			
Neuroactive ligand-receptor interaction	4	300	3.13
Dopamine receptor D5			
Gonadotropin-releasing hormone receptor			
Growth hormone receptor			
Opioid receptor, kappa 1			
Natural killer cell mediated cytotoxicity	2	119	2.59
Tumor necrosis factor (ligand) superfamily, member 10			
Vav 3 guanine nucleotide exchange factor			

(Glucose + Glutamine upregulated, 1014 genes)	List	Array	z-score
Cytokine-cytokine receptor interaction	3	247	2.48
Growth hormone receptor			
Platelet-derived growth factor receptor, alpha polypeptide			
Tumor necrosis factor (ligand) superfamily, member 10			
Glutathione metabolism	1	39	2.43
Alanyl (membrane) aminopeptidase			

Genome-wide expression analysis in BxPC-3 cells. Genes up-regulated >2-fold in glucose only, glutamine only, or glucose plus glutamine media are presented. See text for details.