Supplementary information for Oncogenic K-Ras decouples glucose and glutamine metabolism to support cancer cell growth

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Contents

List of abbreviations

- Cell name abbreviations

N, NIH3T3 cells (normal); T, NIH3T3 K-Ras (transformed); R, NIH3T3-GEF-DN (reverted).

- Gene name abbreviations

Acly, ATP citrate lyase; Aco2, Aconitase 2; Ada, Adenosine deaminase; Adsl, Adenylosuccinate lyase; Adss, Adenylosuccinate synthetase; Anpep, Alanyl (membrane) aminopeptidase; Aprt, Adenine phosphoribosyl transferase; Atic, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase; Cad, Carbamoyl-phosphate synthetase (aspartate transcarbamylase); Cs, Citrate synthase; Enpp1 and Enpp3, Ectonucleotide pyrophosphatase 1 and *3; Fh, Fumarate hydratase; Gart, Phosphoribosylglycinamide formyltransferase; Gclc*, *Glutamate-cysteine ligase -catalytic subunit-*; *Gclm*, *Glutamate-cysteine ligase*; *Gfpt1*, *Glucosamine-fructose-6-phosphate aminotransferase 1*; *Ggta1*, *Glycoprotein galactosyltransferase α; Gmps, Guanine monphosphate synthetase; Got1* and *Got2*, *Glutamate oxaloacetate transaminase 1* and *2; Gpt*, *Glutamic pyruvic transaminase*; *Gls*, *Glutaminase*; *Glud1, Glutamate dehydrogenase 1*; *Gsr*, *Glutathione reductase*; *Gss*, *Glutathione synthetase*; *Gstm1,2,5,7*, *Glutathione S-transferase mu 1,2,5,7*; *Idh, Isocitrate dehydrogenase (Idh1, Idh2, Idh3a, Idh3b and Idh3g); Impdh1 and 2, Inosine 5'-phosphate dehydrogenase 1and 2; Mdh (Mdh2), Malate; Mgst1, 2, 3, Microsomal glutathione S-transferase 1,2,3; Nt5c, 5',3'-nucleotidase-cytosolic-; Ogdh, α-ketoglutarate dehydrogenase; Paics, Phosphoribosylaminoimidazole carboxylase; Pfas, Phosphoribosylformylglycinamidine synthase; Ppat, Phosphoribosyl pyrophosphate amidotransferase*; *Prps1 and 2, Phosphoribosyl pyrophosphate synthetase 1 and 2; Sdh (Sdha, Sdhc and Sdhd), Succinate dehydrogenase complex; Slc1a5*, *solute carrier family 1 (neutral amino acid transporter)*, *member 5*; *Srm, Spermidine synthase; Sucla2, succinate-coenzyme A ligase-ADP forming-2* and *Sucgl1, succinate-coenzyme A ligase-GDP forming-2; Xdh, Xanthine dehydrogenase*.

- Metabolites abbreviations

Glc, glucose; G6P, glucose 6-phosphate; GAP, glyceraldehyde 3-phosphate; Pyr, pyruvate; Lac, lactate; AcCoA, acetyl-CoA; Cit, citrate; Isocit, isocitrate; AKG, α-ketoglutarate; SucCoA, succinil-CoA; Suc, succinate; Fum, fumarate; Mal, malate; OAA, oxalacetate; Gln, glutamine; Glu, glutamate; 5-oxo, 5-oxoproline; Asp, aspartate; Ala, alanine

Supplementary Materials and Methods

- Metabolic flux analysis (MFA): description and assumptions

MFA was conducted using the elementary-metabolite unit (EMU-) based software package Metran as previously described using a [U-$^{13}C_5$]glutamine tracer (Antoniewicz et al., 2007; Metallo et al., 2009; Noguchi et al., 2009; Young et al., 2008). Flux estimations and confidence intervals are subject to the following assumptions:

1. Cellular metabolism and isotopic labeling are at steady state. Cells were cultured in the presence of [U-$^{13}C_5$]glutamine for 54 hours. Labeling of glycolytic and TCA cycle intermediates has been demonstrated to be constant after such time (Maier et al., 2008; Munger et al., 2008).
2. All CO_2 used in carboxylation reactions is unlabeled.
3. Protein turnover are negligible relative to glucose and glutamine consumption.
4. Two separate compartments of pyruvate are assumed to exist, with cytosolic pyruvate used to generate lactate and mitochondrial pyruvate used for alanine synthesis. These compartments are exchangeable and required to fit the differential labeling observed in lactate and alanine. The former being primarily glucose derived, and the latter containing more label from glutamine.
5. Fumarate and succinate are symmetric metabolites.
6. Dilution pools of unlabeled succinate and citrate are assumed to exist. These pools do not participate in central carbon metabolism and are accounted for using a dilution flux of unlabeled metabolite. Measurements are comprised of both pools. Isotopic enrichment of succinate pools from tracers is often observed to be decreased in tracer studies. Such effects are hypothesized to be due to intracellular compartmentalization (Chatham et al., 2003). Citrate labeling may be diluted due to turnover of unlabeled fatty acids; this dilution pool contributed to ~10% of the measured pool in normal and transformed cells.
7. The pentose phosphate pathway (PPP) is included in the network. The percentage of glycolytic flux that proceeds through the oxidative PPP branch was estimated using the M0 and M1 labeling of lactate in cells cultured with a 1:1 mixture of [1-^{13}C]glucose and [U-$^{13}C_6$]glucose.
8. Amino acid fluxes to biomass were estimated based on cell growth rate and published values of per cell amino acid abundances in mammalian cells cells, as previously described (Metallo et al., 2009).

Isotopic labeling was quantified in the metabolite ion fragments listed below. In the case of redundant fragment measurements, mass isotopomer distributions (MIDs) were highly reproducible (i.e. within 1-2%). The formulas listed below were used to correct for natural isotope abundance.

GC/MS metabolites and fragments used for isotope quantification are shown in the table below

Metabolite	Carbons	Formula	m/z range		
Ala	23	C10H26ONSi2	232	-	239
Ala	123	C11H26O2NSi2	260	-	268
Asp	1234	C18H40O4NSi3	418	-	428
Cit	123456	C20H39O6Si3	459	-	470
Cit	123456	C26H55O7Si4	591	-	602
Gln	12345	C19H43N2O3Si3	431	-	441
Glu	2345	C16H36O2NSi2	330	-	340
Glu	12345	C19H42O4NSi3	432	-	442
Lac	23	C10H25O2Si2	233	-	240
Lac	123	C11H25O3Si2	261	-	269
Pyr	123	C6H12O3NSi	174	-	182
Suc	1234	C12H25O4Si2	289	-	298
The following measurements were also included for MDA-MB-231 fits					
AKG	12345	C14H28O5NSi2	346	-	355
Asp	12	C14H32O2NSi2	302	-	310
Fum	1234	C12H23O4Si2	287	-	297
Mal	1234	C18H39O5Si3	419	-	428
Glu	12345	C17H36NO3Si2	358	-	368

Network and carbon atom transitions describing central carbon metabolism for MFA (Table below). Dot suffixes indicate localization to a specific compartment: .x, extracellular; .c, cytosolic; .m, mitochondrial; .d, dilution; .mnt, measurement. Forward arrows (→) indicate net fluxes and reversible arrows (←→) indicate exchange fluxes, or min(υ_F,υ_R). Metabolites lacking a suffix are assumed to be equilibrated between compartments.

Glycolysis
Glc.x (abcdef) → G6P (abcdef)
G6P (abcdef) ←→ F6P (abcdef)
F6P (abcdef) → DHAP (cba) + GAP(def)
DHAP (abc) ←→ GAP (abc)
GAP (abc) ←→ 3PG (abc)
3PG (abc) → Pyr.c (abc)
Pyr.c (abc) ←→ Lac (abc)
Lac (abc) → Lac.x (abc)
Pentose Phosphate Pathway
G6P (abcdef) → P5P (bcdef) + CO2 (a)
P5P (abcde) + P5P (fghij) ←→ S7P (abfghij) + GAP (cde)
S7P (abcdefg) + GAP (hij) ←→ F6P (abchij) + E4P (defg)
P5P (abcde) + E4P (fghi) ←→ F6P (abfghi) + GAP (cde)
Anaplerotic Fluxes
Pyr.m (abc) + CO2 (d) → Oac (abcd)
Mal (abcd) ←→ Pyr.m (abc) + CO2 (d)
Glu (abcde) ←→ Akg (abcde)
Oac (abcd) + Glu (efghi) → Asp (abcd) + Akg (efghi)

TCA cycle
Pyr.m (abc) → AcCoA.m (bc) + CO2 (a)
AcCoA.m (ab) + Oac (cdef) → Cit (fedbac)
Cit (abcdef) ←→ Akg (abcde) + CO2 (f)
Akg (abcde) → Suc (bcde) + CO2 (a)
Suc (abcd) ←→ Fum (abcd)
Fum (abcd) ←→ Mal (abcd)
Mal (abcd) ←→ Oac (abcd)
Amino acids
Pyr.m (abc) + Glu (defgh) → Ala (abc) + Akg (defgh)
Gln.x (abcde) → Gln (abcde)
Gln (abcde) → Glu (abcde)
Glu (abcde) → Glu.x (abcde)
Biomass production
Cit (abcdef) → AcCoA.c (ed) + OAA (fcba)
AcCoA.c (ab) → Fatty acids (ab)
0.18 Asp + 0.28 Glu + 0.15 Ala + 0.22 Gln → Biomass
P5P (abcde) → NTP (abcde)
Dilution and Mixing Fluxes
Suc.d (abcd) → Suc.mnt (abcd)
0 Suc (abcd) → Suc.mnt (abcd)
Cit.d (abcdef) → Cit.mnt (abcdef)
0 Cit (abcdef) → Cit.mnt (abcdef)
Pyr.c (abc) ←→ Pyr.m (abc)
0 Pyr.c (abc) → Pyr.mnt (abc)
0 Pyr.m (abc) → Pyr.mnt (abc)

Abbreviations:

Acetyl coenzyme A, AcCoA; α-ketoglutarate, AKG; alanine, Ala; aspartate, Asp; citrate, Cit; fumarate, Fum; glutamine, Gln; glutamate, Glu; malate, Mal; oxaloacetate, OAA; lactate, Lac; pyruvate, Pyr, succinate, Suc; palmitate, Palm; glucose, Glc; glucose-6-phosphate, G6P, fructose-6-phosphate, F6P, dihydroxyacetone phosphate, DHAP; glyceraldehyde phosphate, GAP; 3-phosphoglycerate, 3PG; pentose-5-phosphate, P5P; erythrose-4-phosphate, E4P; sedoheptulose-7-phosphate, S7P.

Supplementary Figures

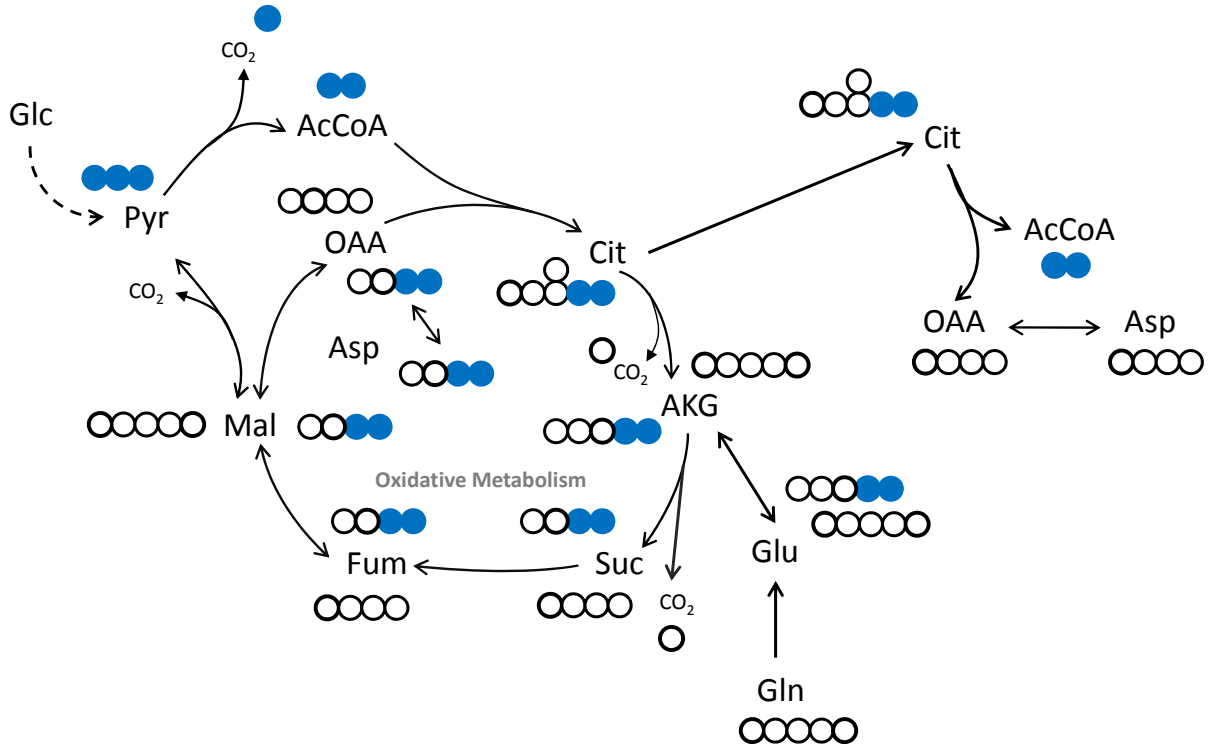

Figure S1. Atom transition map depicting labeling patterns of metabolites derived from [U-$^{13}C_6$]glucose in central carbon metabolism. Only one cycle is shown after oxidation of pyruvate. Addition of a labeled AcCoA molecule to unlabeled oxaloacetate generates M2 mass isotopomers, or isotopologues, within the TCA cycle as well as in the glutamate and aspartate pools. Labeling patterns arising from compound symmetry and some unlabeled intermediates are omitted for simplification.

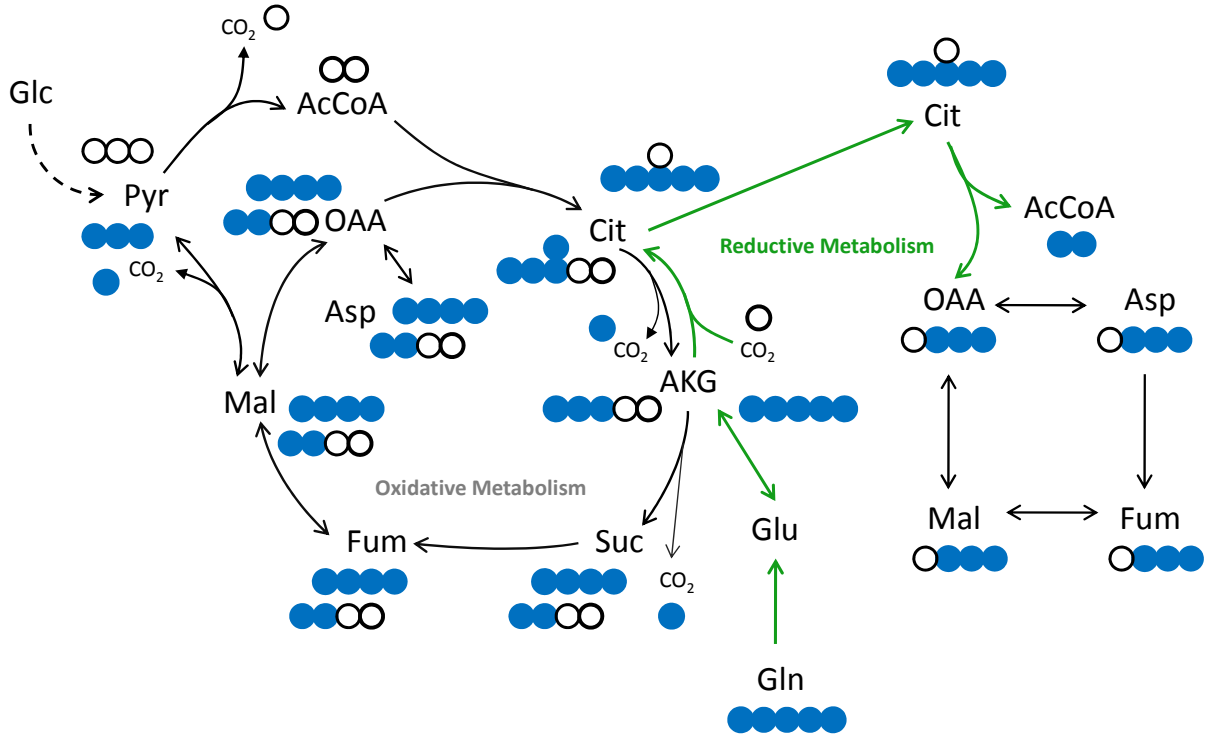

Figure S2. Atom transition map depicting labeling patterns of metabolites derived from [U-$^{13}C_5$]glutamine during oxidative and reductive metabolism. Mass isotopomers, or isotopologues, generated by reductive carboxylation include M5 citrate, M3 aspartate, M3 malate, and M3 fumarate; any mass isotopomers labeled therein provide evidence of reductive pathway activity. Labeling patterns arising from compound symmetry and some unlabeled intermediates are omitted for simplification. For oxidative metabolism, labeling patterns arising from two TCA cycles are shown.

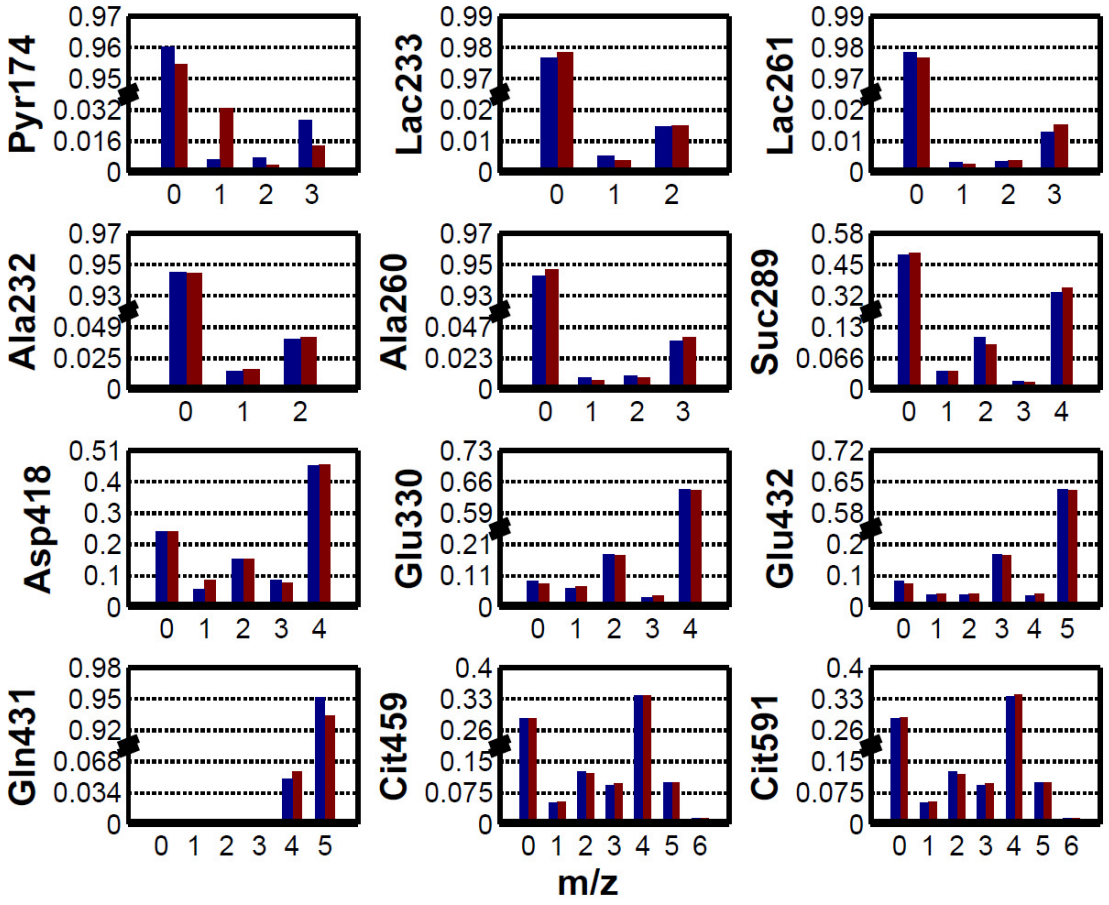

Figure S3. Simulated and measured MIDs from MFA in normal NIH3T3 cells. Simulated values were obtained using Metran and the model fit (fluxes) listed in Table 1 and Supplementary Table 2. Cells were cultured as described in Methods using [U-$^{13}C_5$]glutamine and metabolite labeling was quantified via GC/MS. MIDs are corrected for natural abundance.

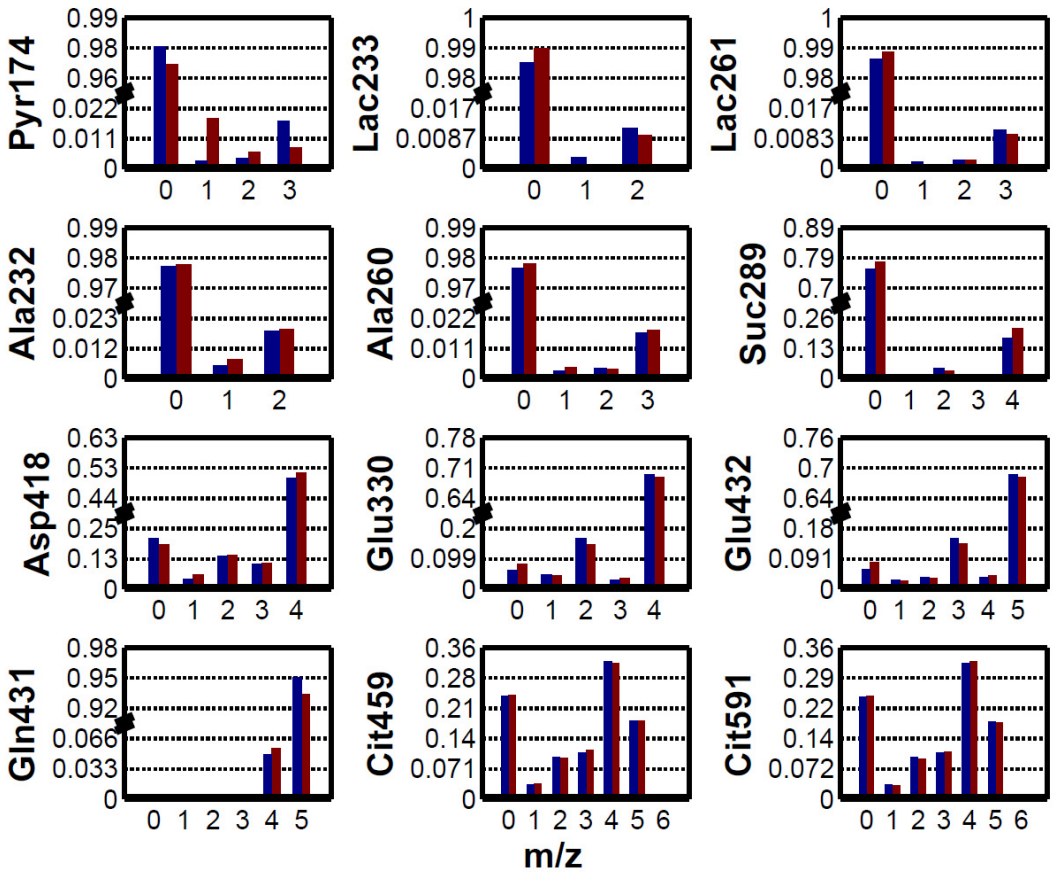

Figure S4. Simulated and measured MIDs from MFA in transformed NIH3T3 cells expressing oncogenic K-ras. Simulated values were obtained using Metran and the model fit (fluxes) listed in Table 1 and Supplementary Table 2. Cells were cultured as described in Methods using [U-$^{13}C_5$]glutamine and metabolite labeling was quantified via GC/MS. MIDs are corrected for natural abundance.

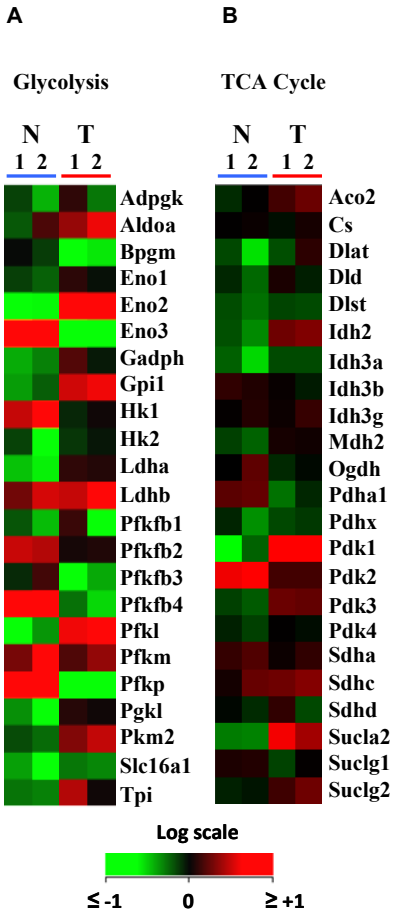

Figure S5. Gene expression profiles of relevant metabolic pathways in normal and transformed fibroblasts. Heat maps of glycolytic (**A**) and TCA cycle (**B**) related genes of normal (blue line) and transformed (red line) cell lines grown in 25mM glucose + 4mM glutamine along a time course of 72h are shown. Transcriptional analysis was performed at two different time points (1=48h and 2=72h), corresponding respectively to 48h and 72h after the replacement of initial growth medium with a new medium containing 25mM glucose + 4mM glutamine (time 0h). Color scale bar indicates the normalized expression intensity (log scale), in particular low expression in green, high expression in red and no changed expression in black. Glycolytic genes: Adpgk, ADP-dependent glucokinase; Aldo1a, -Aldolase A-; Bpgm, 2,3-Bisphosphoglycerate mutase; Eno1, 2, 3, Enolase 1, 2, 3; Gadph, Glyceraldehyde 3-phosphate dehydrogenase; Gpi, Glucose phosphate isomerase 1; Hk1 and Hk2, Hexokinase 1 and 2; Ldha and Ldhb, Lactate dehydrogenase A and B; Pfkb1, 2, 3, 4, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1, 2, 3, 4; Pfkl, Pfkm and Pfkp, Phosphofructokinase liver, muscle and platelet; Pgk1, Phosphoglycerate kinase 1; Pkm2, Pyruvate kinase muscle isoenzyme type 2; Slc16a1, Solute carrier family 16 (monocarboxylic acid transporters), member 1; Tpi1,

Triosephosphate isomerase 1. TCA cycle genes: Aco2, Aconitase 2; Cs, Citrate synthase; Dlat, Dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex); Dld, Dihydrolipoamide dehydrogenase; Dlst, Dihydrolipoamide S-succinyltransferase (component of 2-oxo-glutarate complex); Idh2, Isocitrate dehydrogenase 2 (NADP+); Idh3a, Idh3b and Idh3g, Isocitrate dehydrogenase 3 (NAD+) α, β and γ; Mdh2, Malate dehydrogenase 2-NAD-; Ogdh, α-ketoglutarate dehydrogenase; Sdha, Sdhc and Sdhd, Succinate dehydrogenase complex, subunit A, C and D; Sucla2, Succinate-Coenzyme A ligase-ADP-forming-β subunit; Suclg1 and Suclg2, Succinate-CoA ligase-GDP-forming-α and β subunits.

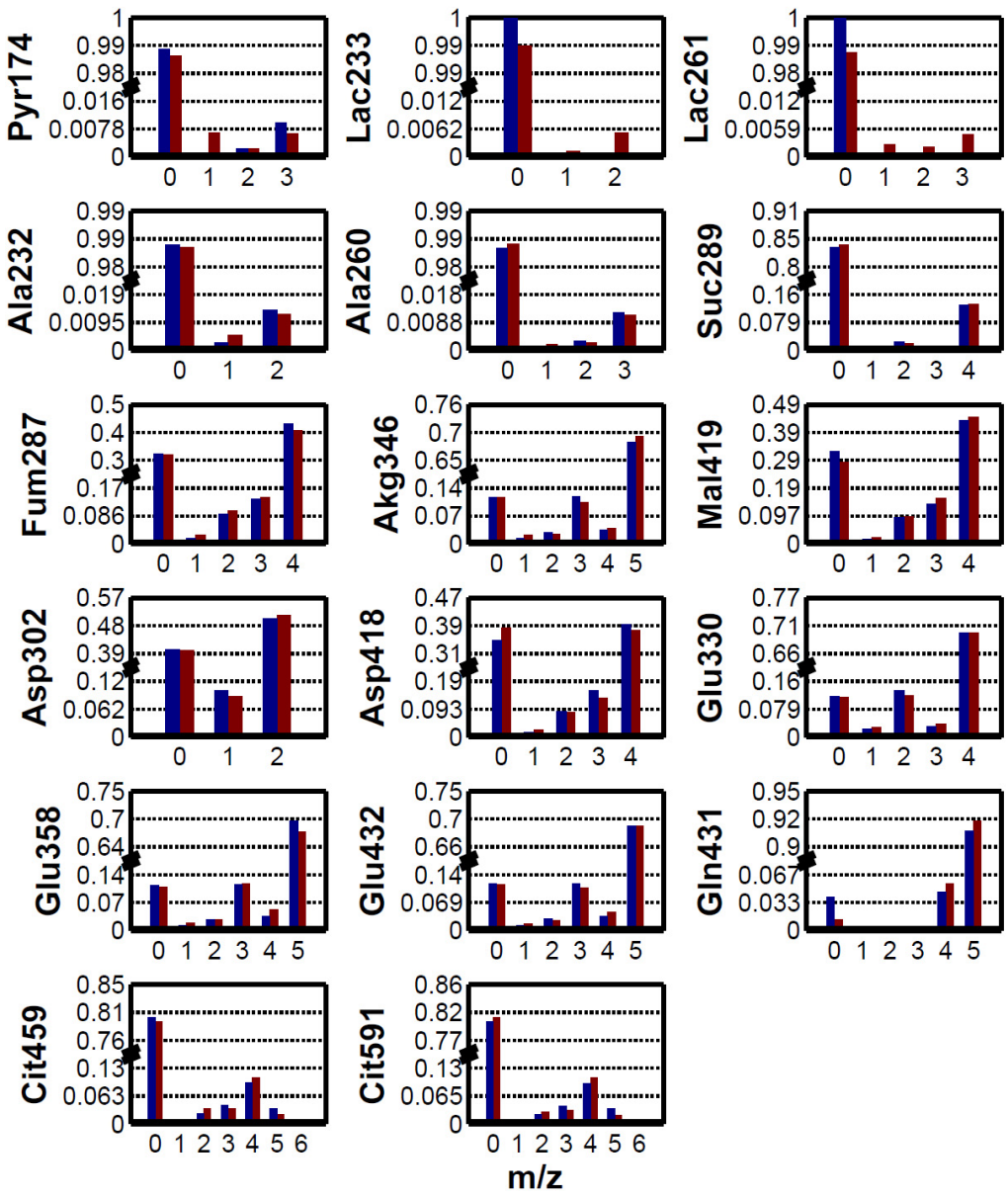

Figure S6. Simulated and measured MIDs from MFA in MDA-MB-231 cells. Simulated values were obtained using Metran and the model fit (fluxes) listed in Supplementary Table 4. Cells were cultured as described in Methods using [U-$^{13}C_5$]glutamine and metabolite labeling was quantified via GC/MS. MIDs are corrected for natural abundance.

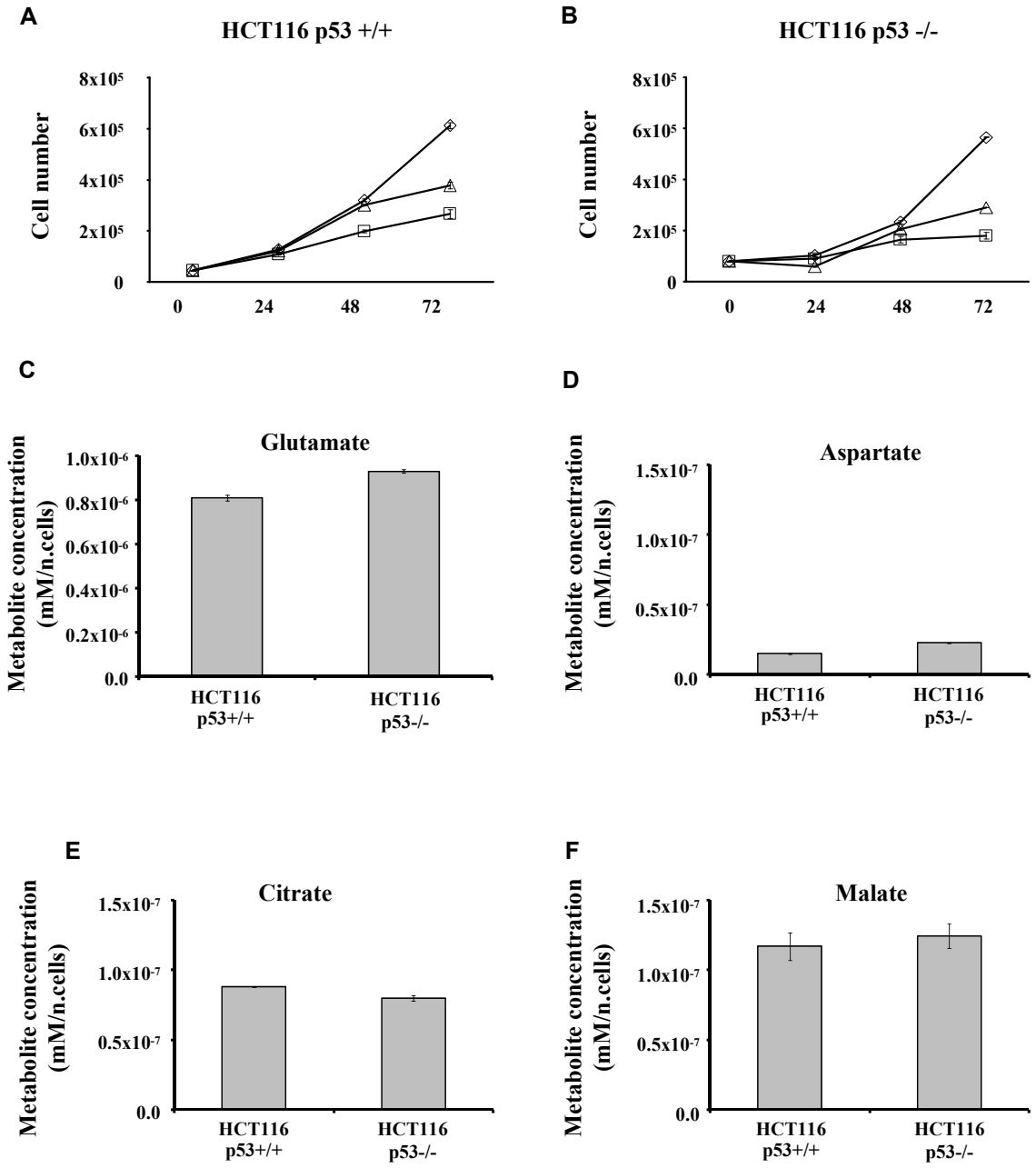

Figure S7. Proliferation and metabolic analyses in HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ human colon cancer cells. HCT116 $p53^{+/+}$ (**A**) and HCT116 $p53^{-/-}$ (**B**) cells were plated at 3000 cells/cm^2 in 6-well plates in normal medium. Culture medium was replaced after 18 hours with normal medium (25mM glucose + 4mM glutamine, ◇), or 1mM glucose + 4mM glutamine (□) or 25mM glucose + 0.5mM glutamine (△) then the cells were collected and counted at indicate time points. Intracellular levels of glutamate (**C**), aspartate (**D**), citrate (**E**) and malate (**F**) in the two human colon cancer cell lines, HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$, were measured by appropriate enzymatic assays. The cells for metabolic analyses were collected over 54 hours of growth in normal medium (25mM glucose + 4mM glutamine). Error bars indicate s.e.m. (n=3).

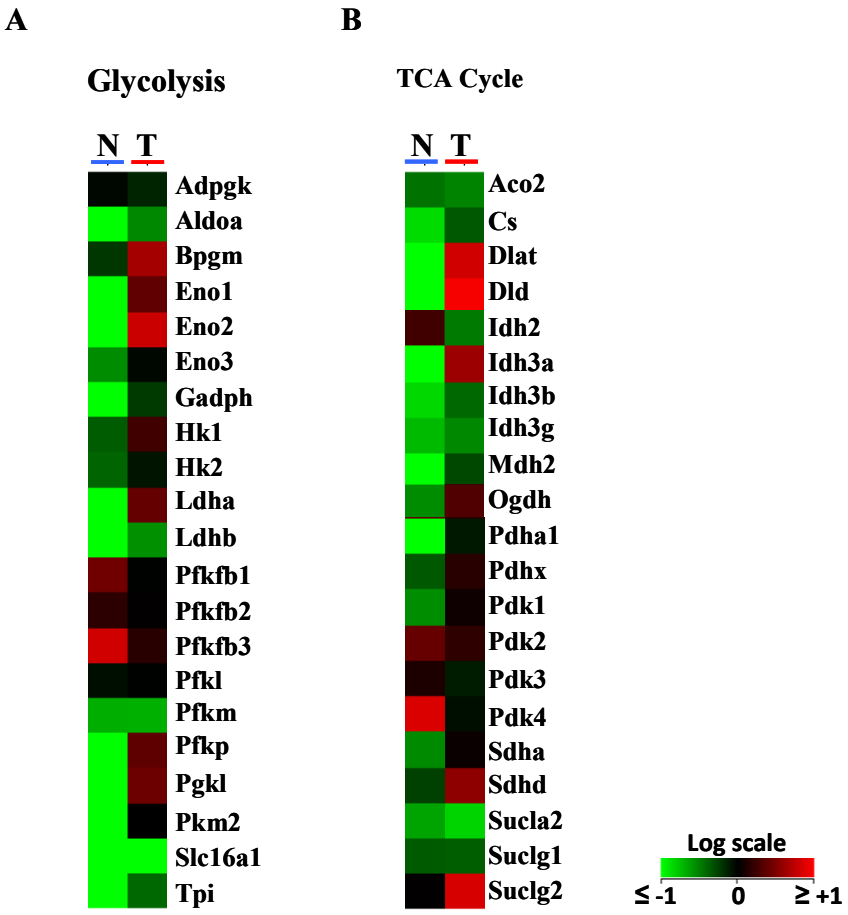

Figure S8. Gene expression profiles of relevant metabolic pathways in normal breast tissue and human transformed breast cell line, MDA-MB-231. Heat maps of glycolytic (**A**) and TCA cycle (**B**) related genes of breast normal tissue -N- (blue line) and MDA-MB-231 -T- (red line) are shown. Color scale bar indicates the normalized expression intensity (log scale), in particular low expression in green, high expression in red and no changed expression in black.

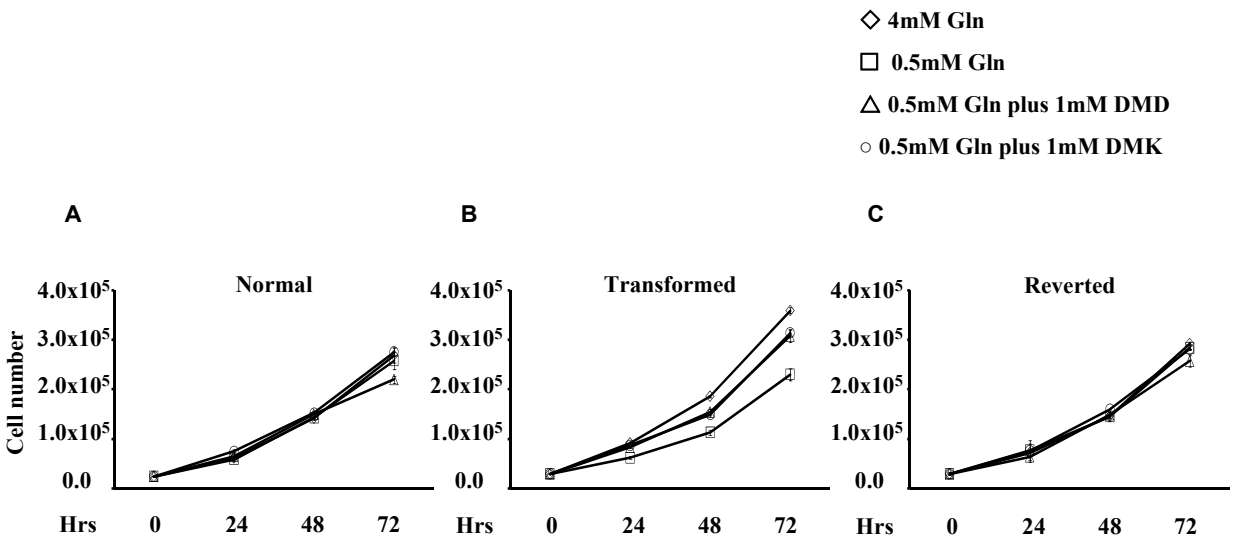

Figure S9. Dimethyl aspartate (DMD) and dimethyl α-ketoglutarate (DMK) sustain proliferation in K-ras transformed fibroblasts. Normal (**A**), transformed (**B**) and reverted (**C**) cell lines were plated at 3000 cells/cm2 in 6-well plates in normal medium. Culture medium was replaced after 18 hours with a normal medium, 25mM Glc + 4mM Gln (◇), or 25mM Glc + 0.5mM Gln (□), or 25mM Glc + 0.5mM Gln + 1mM DMD (△) and 25mM Glc + 0.5mM Gln + 0.5mM Gln + 2mM DMK (○). The cells were collected and counted at indicate time points Error bars indicate s.e.m. (n=3).

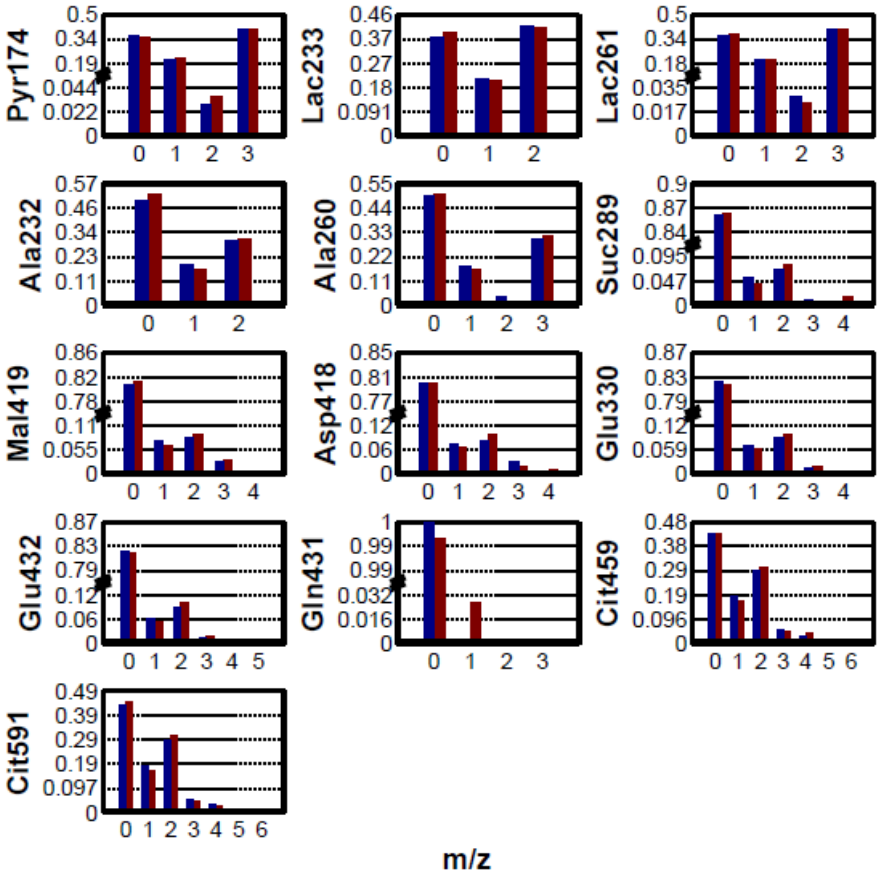

Figure S10. Simulated and measured MIDs from MFA in normal NIH3T3 cells. Simulated values were obtained using Metran and the model fit (fluxes) listed in Supplementary Table 3. Cells were cultured as described in Methods using a 1:1 mixture of [U-$^{13}C_6$]glucose and [1-^{13}C]glucose. Metabolite labeling was quantified via GC/MS. MIDs are corrected for natural abundance.

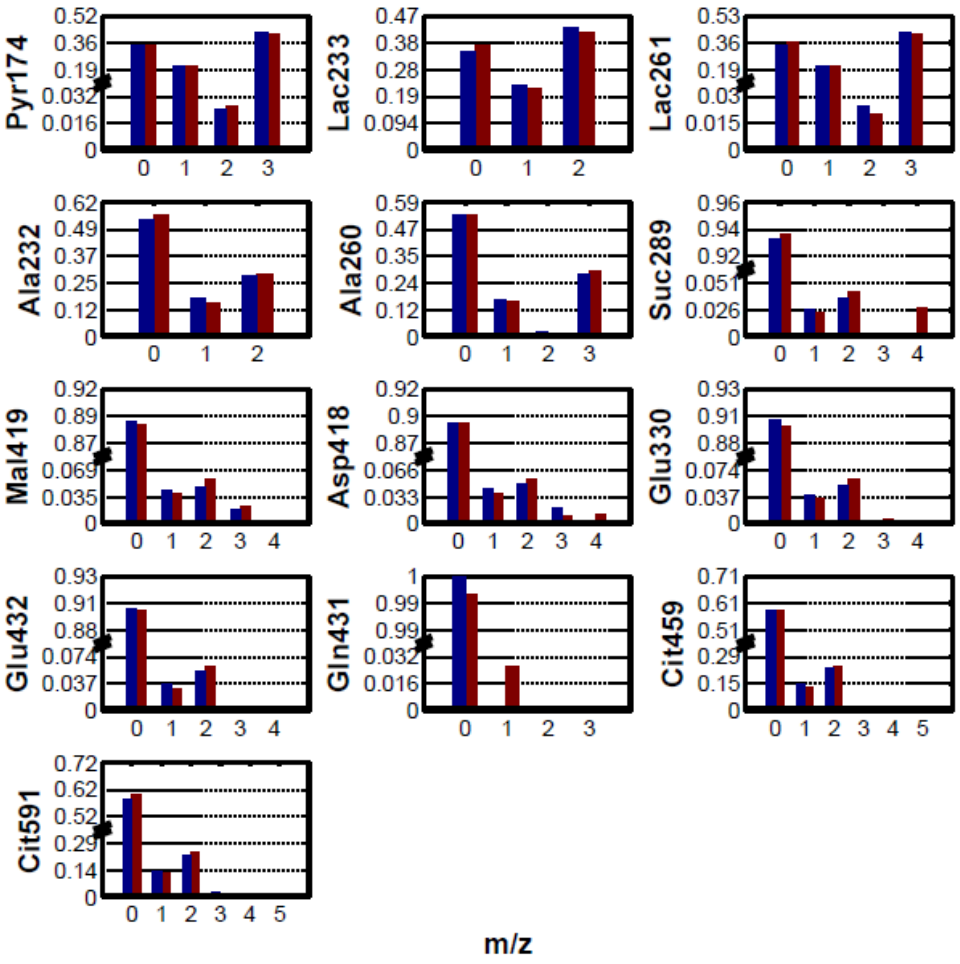

Figure S11. Simulated and measured MIDs from MFA in transformed NIH3T3 cells expressing oncogenic K-ras. Simulated values were obtained using Metran and the model fit (fluxes) listed in Table 1 and Supplementary Table 2. Cells were cultured as described in Methods using a 1:1 mixture of [U-$^{13}C_6$]glucose and [1-^{13}C]glucose. Metabolite labeling was quantified via GC/MS. MIDs are corrected for natural abundance.

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Supplementary Tables

Supplementary Table S1. Summary of genes involved in glutamine metabolism and their correlation to specific pathways.

Glutathione Metabolism	Nucleotides Metabolism	Aspartate and Glutamate Metabolism	Amino sugar Metabolism	Nicotinate and nicotinamide metabolism
Anpep	Adsl	Adsl	Gfpt1	Enpep1
Gclc	Adss	Adss		Enpe3
Gclm	Cad	Cad		
Ggta1	Ppat	Gfpt1		
Gsr	Enpep1			
Gss	Enpep3			
Gstm1				
Gstm2				
Gstm5				
Gstm7				
Idh1				
Mgst1				
Mgst2				
Mgst3				
Srm				

Supplementary Table S2. MFA results in N and T cells using [U-13C5]glutamine tracer

	Normal	Lower bound 95% conf	Upper bound 95% conf	Transformed	Lower bound 95% conf	Upper bound 95% conf	
R1	**65.54**	61.19	69.89	**83.08**	77.60	88.63	Gluc.x -> G6P
R2 net	**62.54**	58.18	66.90	**82.08**	76.60	87.63	G6P -> F6P
R2 exch	**4.08**	0.00	Inf	**18.58**	0.00	Inf	G6P <-> F6P
R3	**64.21**	59.86	68.54	**82.41**	76.94	87.97	F6P -> DHAP + GAP
R4 net	**64.21**	59.86	68.54	**82.41**	76.94	87.97	DHAP -> GAP
R4 exch	**36.65**	0.00	Inf	**13.11**	0.00	Inf	DHAP <-> GAP
R5 net	**129.20**	120.60	137.90	**165.00**	154.00	176.10	GAP -> 3PG
R5 exch	**0.00**	0.00	Inf	**0.00**	0.00	Inf	GAP <-> 3PG
R6	**129.20**	120.60	137.90	**165.00**	154.00	176.10	3PG -> Pyr.c
R7 net	**114.90**	105.90	123.40	**158.10**	146.90	169.20	Pyr.c -> Lac
R7 exch	**300.50**	0.00	Inf	**9.23**	0.00	Inf	Pyr.c <-> Lac
R8	**114.90**	105.90	123.40	**158.10**	146.90	169.20	Lac -> Lac.x
R9 net	**0.00**	0.00	0.00	**0.00**	0.00	0.00	DHAP -> GLP
R9 exch	**75.86**	0.00	Inf	**123.80**	0.00	Inf	DHAP <-> GLP
R10 net	**0.00**	0.00	0.00	**0.00**	0.00	0.00	GLP -> GLP.x
R10 exch	**23.88**	0.00	Inf	**30.63**	0.00	Inf	GLP <-> GLP.x
R11	**3.00**	2.71	3.29	**1.00**	0.90	1.10	G6P -> 6PG
R12	**3.00**	2.71	3.29	**1.00**	0.90	1.10	6PG -> P5P + CO2
R13 net	**0.83**	0.73	0.93	**0.17**	0.13	0.20	P5P + P5P -> S7P + GAP
R13 exch	**25.51**	0.00	Inf	**47.93**	0.00	Inf	P5P + P5P <-> S7P + GAP
R14 net	**0.83**	0.73	0.93	**0.17**	0.13	0.20	S7P + GAP -> F6P + E4P
R14 exch	**375.40**	0.00	Inf	**184.30**	0.00	Inf	S7P + GAP <-> F6P + E4P
R15 net	**0.83**	0.73	0.93	**0.17**	0.13	0.20	P5P + E4P -> F6P + GAP
R15 exch	**40.29**	0.00	Inf	**153.40**	0.00	Inf	P5P + E4P <-> F6P + GAP
R16	**0.00**	0.00	3.15	**0.00**	0.00	1.90	Pyr.m + CO2 -> Oac
R17 net	**1.85**	0.90	5.86	**1.81**	0.75	4.72	Mal -> Pyr.m + CO2
R17 exch	**2.77**	0.00	3.19	**1.58**	0.00	1.89	Mal <-> Pyr.m + CO2
R18	**16.09**	13.50	19.08	**8.58**	7.65	9.57	Pyr.m -> AcCoA.m + CO2
R19	**16.09**	13.50	19.08	**8.58**	7.65	9.57	AcCoA.m + Oac -> Cit
R20 net	**10.10**	9.09	11.15	**5.42**	4.91	5.97	Cit -> Akg + CO2
R20 exch	**3.29**	2.67	3.90	**3.67**	3.22	4.15	Cit <-> Akg + CO2
R21	**12.13**	10.57	13.70	**7.41**	6.12	8.75	Akg -> Suc + CO2
R22 net	**12.13**	10.57	13.70	**7.41**	6.12	8.75	Suc -> Fum
R22 exch	**37.19**	0.00	Inf	**0.00**	0.00	1.50	Suc <-> Fum
R23 net	**12.13**	10.57	13.70	**7.41**	6.12	8.75	Fum -> Mal
R23 exch	**0.00**	0.00	Inf	**42250**	1.49	Inf	Fum <-> Mal
R24 net	**10.28**	6.68	11.33	**5.60**	3.52	6.15	Mal -> Oac
R24 exch	**36.20**	0.00	Inf	**57630**	3.24	Inf	Mal <-> Oac
R25	**5.99**	3.38	9.08	**3.16**	2.37	4.08	Cit -> AcCoA.c + Oac
R26	**5.99**	3.38	9.08	**3.16**	2.37	4.08	AcCoA.c -> FA
R27	**0.15**	0.13	0.16	**0.15**	0.14	0.17	Pyr.m + Glu -> Ala + Akg
R28	**0.18**	0.16	0.20	**0.18**	0.16	0.20	Oac + Glu -> Asp + Akg
R29	**20.32**	18.94	21.70	**16.05**	14.85	17.24	Gln.x -> Gln
R30	**20.10**	18.72	21.49	**15.83**	14.63	17.01	Gln -> Glu
R31	**17.79**	16.39	19.19	**13.55**	12.46	14.65	Glu -> Glu.x
R32 net	**1.70**	0.75	2.71	**1.66**	0.60	2.72	Glu -> Akg
R32 exch	**168.10**	115.20	340.70	**202.20**	105.90	1895	Glu <-> Akg
R33	**1.00**	0.90	1.10	**1.00**	0.90	1.10	0.18*Asp + 0.28*Glu + 0.17*Ser + 0.11*Gly + 0.15*Ala + 0.22*Gln -> Biomass
R34	**0.50**	0.45	0.55	**0.50**	0.45	0.55	P5P -> NTP
R35 net	**14.39**	11.84	17.10	**6.91**	6.08	7.74	Pyr.c -> Pyr.m
R35 exch	**74.00**	39.37	133.40	**275.80**	86.53	4566	Pyr.c <-> Pyr.m
R36	**0.44**	0.01	0.86	**0.00**	0.00	1.00	0*Pyr.c -> Pyr.mnt
R37	**0.56**	0.14	0.99	**1.00**	0.00	1.00	0*Pyr.m -> Pyr.mnt
R38	**1.00**	1.00	1.00	**1.00**	1.00	1.00	Pyr.mnt -> Pyr.fix
R39	**0.44**	0.30	0.46	**0.74**	0.73	0.76	Suc.dil -> Suc.mnt
R40	**0.56**	0.54	0.70	**0.26**	0.24	0.27	0*Suc -> Suc.mnt
R41	**1.00**	1.00	1.00	**1.00**	1.00	1.00	Suc.mnt -> Suc.fix
R42	**0.10**	0.08	0.12	**0.09**	0.08	0.11	Cit.dil -> Cit.mnt
R43	**0.90**	0.88	0.92	**0.91**	0.89	0.92	0*Cit -> Cit.mnt
R44	**1.00**	1.00	1.00	**1.00**	1.00	1.00	Cit.mnt -> Cit.fix

Net flux: $(\upsilon_F-\upsilon_R)$ (→) and Exchange flux: min (υ_F,υ_R) (↔)

Fluxes are listed x 10^{14} mol/(cell*hr)

Supplementary Table S3. MFA results in N and T cells using a 1:1 mixture of [U-13C6]glucose and [1-13C]glucose

	Normal	Lower bound 95% conf	Upper bound 95% conf	Transformed	Lower bound 95% conf	Upper bound 95% conf	
R1	**56.87**	50.37	65.06	**80.32**	74.81	85.45	Gluc.x -> G6P
R2 net	**53.68**	47.23	61.89	**79.32**	73.81	84.44	G6P -> F6P
R2 exch	**0.00**	0.00	Inf	**0.00**	0.00	Inf	G6P <-> F6P
R3	**55.47**	49.54	63.66	**79.66**	74.15	84.78	F6P -> DHAP + GAP
R4 net	**55.47**	49.54	63.66	**79.66**	74.15	84.78	DHAP -> GAP
R4 exch	**80.61**	0.00	Inf	**29.67**	0.00	Inf	DHAP <-> GAP
R5 net	**111.80**	98.87	128.20	**159.50**	148.50	169.70	GAP -> 3PG
R5 exch	**17.32**	0.00	Inf	**0.00**	0.00	Inf	GAP <-> 3PG
R6	**111.80**	98.87	128.20	**159.50**	148.50	169.70	3PG -> Pyr.c
R7 net	**108.50**	95.45	125.80	**158.00**	148.00	169.20	Pyr.c -> Lac
R7 exch	**268.60**	0.00	Inf	**15.71**	0.00	Inf	Pyr.c <-> Lac
R8	**108.50**	95.45	125.80	**158.00**	148.00	169.20	Lac -> Lac.x
R9 net	**0.00**	0.00	0.00	**0.00**	0.00	0.00	DHAP -> GLP
R9 exch	**58.12**	0.00	Inf	**112.70**	0.00	Inf	DHAP <-> GLP
R10 net	**0.00**	0.00	0.00	**0.00**	0.00	0.00	GLP -> GLP.x
R10 exch	**289.80**	0.00	Inf	**123.00**	0.00	Inf	GLP <-> GLP.x
R11	**3.20**	2.62	3.66	**1.00**	0.91	1.10	G6P -> 6PG
R12	**3.20**	2.62	3.66	**1.00**	0.91	1.10	6PG -> P5P + CO2
R13 net	**0.90**	0.70	1.06	**0.17**	0.13	0.20	P5P + P5P -> S7P + GAP
R13 exch	**29630**	0.00	Inf	**3738**	0.00	Inf	P5P + P5P <-> S7P + GAP
R14 net	**0.90**	0.70	1.06	**0.17**	0.13	0.20	S7P + GAP -> F6P + E4P
R14 exch	**0.00**	0.00	3.64	**4.78**	0.00	Inf	S7P + GAP <-> F6P + E4P
R15 net	**0.90**	0.70	1.06	**0.17**	0.13	0.20	P5P + E4P -> F6P + GAP
R15 exch	**3.28**	0.08	5.84	**0.00**	0.00	7.85	P5P + E4P <-> F6P + GAP
R16	**1.29**	0.00	1.97	**0.00**	0.00	0.92	Pyr.m + CO2 -> Oac
R17 net	**11.75**	8.84	13.59	**5.50**	3.73	7.87	Mal -> Pyr.m + CO2
R17 exch	**0.00**	0.00	2.02	**0.55**	0.00	0.93	Mal <-> Pyr.m + CO2
R18	**13.64**	12.23	15.55	**6.87**	6.30	7.99	Pyr.m -> AcCoA.m + CO2
R19	**13.64**	12.23	15.55	**6.87**	6.30	7.99	AcCoA.m + Oac -> Cit
R20 net	**10.18**	8.91	11.96	**3.38**	2.83	4.42	Cit -> Akg + CO2
R20 exch	**1.17**	0.00	1.75	**2.37**	0.00	2.93	Cit <-> Akg + CO2
R21	**20.82**	18.21	24.13	**9.05**	7.00	10.78	Akg -> Suc + CO2
R22 net	**20.82**	18.21	24.13	**9.05**	7.00	10.78	Suc -> Fum
R22 exch	**11.38**	0.00	Inf	**9.57**	0.00	Inf	Suc <-> Fum
R23 net	**20.82**	18.21	24.13	**9.05**	7.00	10.78	Fum -> Mal
R23 exch	**0.00**	0.00	Inf	**0**	0.00	Inf	Fum <-> Mal
R24 net	**9.07**	7.66	12.12	**3.56**	2.27	4.60	Mal -> Oac
R24 exch	**130.80**	0.00	Inf	**34460**	0.00	Inf	Mal <-> Oac
R25	**3.46**	2.78	4.15	**3.49**	3.16	3.83	Cit -> AcCoA.c + Oac
R26	**3.46**	2.78	4.15	**3.49**	3.16	3.83	AcCoA.c -> FA
R27	**0.15**	0.12	0.18	**0.15**	0.14	0.16	Pyr.m + Glu -> Ala + Akg
R28	**0.18**	0.14	0.21	**0.18**	0.16	0.20	Oac + Glu -> Asp + Akg
R29	**24.79**	21.99	27.39	**18.33**	16.82	19.64	Gln.x -> Gln
R30	**24.57**	21.77	27.17	**18.11**	16.60	19.42	Gln -> Glu
R31	**13.66**	11.38	16.18	**12.16**	11.10	13.35	Glu -> Glu.x
R32 net	**10.64**	8.88	12.60	**5.68**	3.92	7.09	Glu -> Akg
R32 exch	**40110000**	500.60	Inf	**3408000**	155.50	Inf	Glu <-> Akg
R33	**0.99**	0.80	1.19	**1.00**	0.90	1.09	0.18*Asp + 0.28*Glu + 0.17*Ser + 0.11*Gly + 0.15*Ala + 0.22*Gln -> Biomass
R34	**0.50**	0.40	0.60	**0.50**	0.45	0.55	P5P -> NTP
R35 net	**3.33**	1.73	5.25	**1.52**	0.01	2.74	Pyr.c -> Pyr.m
R35 exch	**27.30**	19.56	35.00	**9.45**	5.85	14	Pyr.c <-> Pyr.m
R36	**1.00**	0.93	1.00	**0.98**	0.92	1.00	0*Pyr.c -> Pyr.mnt
R37	**0.00**	0.00	0.07	**0.02**	0.00	0.08	0*Pyr.m -> Pyr.mnt
R38	**1.00**	1.00	1.00	**1.00**	1.00	1.00	Pyr.mnt -> Pyr.fix
R39	**0.21**	0.12	0.31	**0.32**	0.14	0.49	Suc.dil -> Suc.mnt
R40	**0.79**	0.69	0.88	**0.68**	0.51	0.86	0*Suc -> Suc.mnt
R41	**1.00**	1.00	1.00	**1.00**	1.00	1.00	Suc.mnt -> Suc.fix
R42	**0.00**	0.00	0.08	**0.00**	0.00	0.22	Cit.dil -> Cit.mnt
R43	**1.00**	0.92	1.00	**1.00**	0.78	1.00	0*Cit -> Cit.mnt
R44	**1.00**	1.00	1.00	**1.00**	1.00	1.00	Cit.mnt -> Cit.fix

Net flux: $(\upsilon_F-\upsilon_R)$ (→) and Exchange flux: min (υ_F,υ_R) (↔)

Fluxes are listed x 10^{14} mol/(cell*hr)

Supplementary Table S4. MFA results in MDA-MB-231 cells using [U-13C5]glutamine

	Normal	Lower bound 95% conf	Upper bound 95% conf	
R1	**232.00**	216.70	247.30	Gluc.x -> G6P
R2 net	**172.10**	156.00	188.30	G6P -> F6P
R2 exch	**99.07**	0.00	Inf	G6P <-> F6P
R3	**211.40**	196.00	226.70	F6P -> DHAP + GAP
R4 net	**211.40**	196.00	226.70	DHAP -> GAP
R4 exch	**0.00**	0.00	Inf	DHAP <-> GAP
R5 net	**442.40**	411.80	473.00	GAP -> 3PG
R5 exch	**0.00**	0.00	Inf	GAP <-> 3PG
R6	**442.40**	411.80	473.00	3PG -> Pyr.c
R7 net	**420.70**	390.20	451.30	Pyr.c -> Lac
R7 exch	**0.00**	0.00	Inf	Pyr.c <-> Lac
R8	**420.70**	390.20	451.30	Lac -> Lac.x
R9 net	**0.00**	0.00	0.00	DHAP -> GLP
R9 exch	**66.89**	0.00	Inf	DHAP <-> GLP
R10 net	**0.00**	0.00	0.00	GLP -> GLP.x
R10 exch	**116.40**	0.00	Inf	GLP <-> GLP.x
R11	**59.97**	54.09	65.84	G6P -> 6PG
R12	**59.97**	54.09	65.84	6PG -> P5P + CO2
R13 net	**19.66**	17.74	21.60	P5P + P5P -> S7P + GAP
R13 exch	**49**	0.00	Inf	P5P + P5P <-> S7P + GAP
R14 net	**19.66**	17.74	21.60	S7P + GAP -> F6P + E4P
R14 exch	**20.35**	0.00	Inf	S7P + GAP <-> F6P + E4P
R15 net	**19.66**	17.74	21.60	P5P + E4P -> F6P + GAP
R15 exch	**306.70**	0.00	Inf	P5P + E4P <-> F6P + GAP
R16	**2.92**	2.61	3.22	Pyr.m + CO2 -> Oac
R17 net	**0.54**	0.20	2.02	Mal -> Pyr.m + CO2
R17 exch	**0.00**	0.00	0.30	Mal <-> Pyr.m + CO2
R18	**17.97**	16.93	18.98	Pyr.m -> AcCoA.m + CO2
R19	**17.97**	16.93	18.98	AcCoA.m + Oac -> Cit
R20 net	**7.70**	7.10	8.07	Cit -> Akg + CO2
R20 exch	**3.81**	3.33	4.56	Cit <-> Akg + CO2
R21	**6.28**	5.37	6.88	Akg -> Suc + CO2
R22 net	**6.28**	5.37	6.88	Suc -> Fum
R22 exch	**0.15**	0.00	6.78	Suc <-> Fum
R23 net	**6.28**	5.37	6.88	Fum -> Mal
R23 exch	**1.00E+09**	5.60E+06	Inf	Fum <-> Mal
R24 net	**5.73**	5.11	6.30	Mal -> Oac
R24 exch	**46.08**	34.42	71.56	Mal <-> Oac
R25	**10.27**	9.46	11.21	Cit -> AcCoA.c + Oac
R26	**10.27**	9.46	11.21	AcCoA.c -> FA
R27	**1.32**	0.08	2.48	Pyr.m + Glu -> Ala + Akg
R28	**0.95**	0.10	1.79	Oac + Glu -> Asp + Akg
R29	**30.61**	28.82	32.91	Gln.x -> Gln
R30	**29.45**	28.17	30.98	Gln -> Glu
R31	**29.39**	26.69	32.08	Glu -> Glu.x
R32 net	**-1.42**	-2.00	-1.10	Glu -> Akg
R32 exch	**995**	322.90	Inf	Glu <-> Akg
R33	**5.30**	0.32	9.93	0.18*Asp + 0.28*Glu + 0.17*Ser + 0.11*Gly + 0.15*Ala + 0.22*Gln -> Biomass
R34	**1.00**	0.90	1.10	P5P -> NTP
R35 net	**21.67**	19.66	23.99	Pyr.c -> Pyr.m
R35 exch	**0.00**	0.00	18.92	Pyr.c <-> Pyr.m
R36	**0.21**	0.00	0.86	0*Pyr.c -> Pyr.mnt
R37	**0.79**	0.14	1.00	0*Pyr.m -> Pyr.mnt
R38	**1.00**	1.00	1.00	Pyr.mnt -> Pyr.fix
R39	**0.82**	0.80	0.83	Suc.dil -> Suc.mnt
R40	**0.19**	0.17	0.20	0*Suc -> Suc.mnt
R41	**1.00**	1.00	1.00	Suc.mnt -> Suc.fix
R42	**0.71**	0.70	0.73	Cit.dil -> Cit.mnt
R43	**0.29**	0.27	0.30	0*Cit -> Cit.mnt
R44	**1.00**	1.00	1.00	Cit.mnt -> Cit.fix

Net flux: $(\upsilon_F-\upsilon_R)$ $(\rightarrow)$ and Exchange flux: min (υ_F,υ_R) $(\leftrightarrow)$

Fluxes are listed x 10^{14} mol/(cell*hr)