Metabolic flux analysis (MFA): description and assumptions

For experimental validation of optimized tracer combinations, MFA was conducted using the elementary-metabolite unit (EMU-) based software package Metran as previously described (Antoniewicz et al., 2007; Metallo et al., 2009; Noguchi et al., 2009; Young et al., 2008). Briefly, a stoichiometric matrix, *S,* is formulated based on a simple network describing central carbon metabolism (Table 2). Simulated experimental measurements are generated for a given flux vector, υ, assuming steady-state mass balances $S \cdot \upsilon = 0$. The simulated measurement values are compared with actual measurements of fluxes and mass isotopomer distributions (MIDs), and flux values are iteratively adjusted to minimize the squared residuals using an EMU-based algorithm (Antoniewicz et al., 2007; Young et al., 2008). Upon obtaining a global fit after performing at least 20 estimations, flux confidence intervals were determined by performing sensitivity analyses on each flux with respect to isotope measurements and the global fit (Antoniewicz et al., 2006). Finally, precision scores characterizing flux confidence intervals for each data set were calculated as previously described (Metallo et al., 2009). Flux estimations and confidence interval calculations are subject to the following assumptions:

1. Cellular metabolism and isotopic labeling are at steady state. Labeling of glycolytic and TCA cycle intermediates has been demonstrated to be constant after such time (Maier et al., 2008; Munger et al., 2008). Steady state labeling of organic and amino acids are demonstrated in Supplementary Figure 5 when using the combined [1,2-$^{13}C_2$]glucose + [U-$^{13}C_5$]glutamine.
2. CO_2 is not balanced within the system, and unlabeled CO_2 freely exchanges with CO_2 pools in cells such that labeled CO_2 is not necessarily reincorporated in carboxylation reactions.
3. 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. The combining of these pools for measurements is recapitulated within the model through the use of "mixing fluxes," which allow for the estimation of relative pool sizes for pyruvate measurements. All other measured metabolites are not compartmentalized between the mitochondria and cytosol and are therefore assumed to be isotopically equilibrated or predominantly in one compartment. Similarly, to better estimate fluxes and confidence intervals from the TCA cycle to pyruvate (i.e., pyruvate cycling), the primary enzyme assumed to catalyze this reaction was mitochondrial malic enzyme (converting malate to pyr.m). This assumption improved resolution of malic enzyme fluxes and allowed fits of pyruvate, lactate, and alanine data.

4. Fumarate and succinate are symmetric metabolites.

5. Dilution pools of several metabolites (glycine, serine, P5P, GLP, succinate) 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). Other dilution pools presumably arise from turnover of unlabeled biomass.

6. Amino acid fluxes to biomass were based on cell growth rate and published values of per cell amino acid abundances in mammalian cells, as previously described (Metallo et al., 2009).

Isotopic labeling was quantified in the metabolite ion fragments listed in Supplementary Table 1. In the case of redundant fragment measurements, mass isotopomer distributions (MIDs) were highly reproducible (i.e. within 1-2%). The formulas listed in Supplementary Table 1 were used to correct for natural isotope abundance using methods adapted from Fernandez et al (Fernandez et al., 1996).

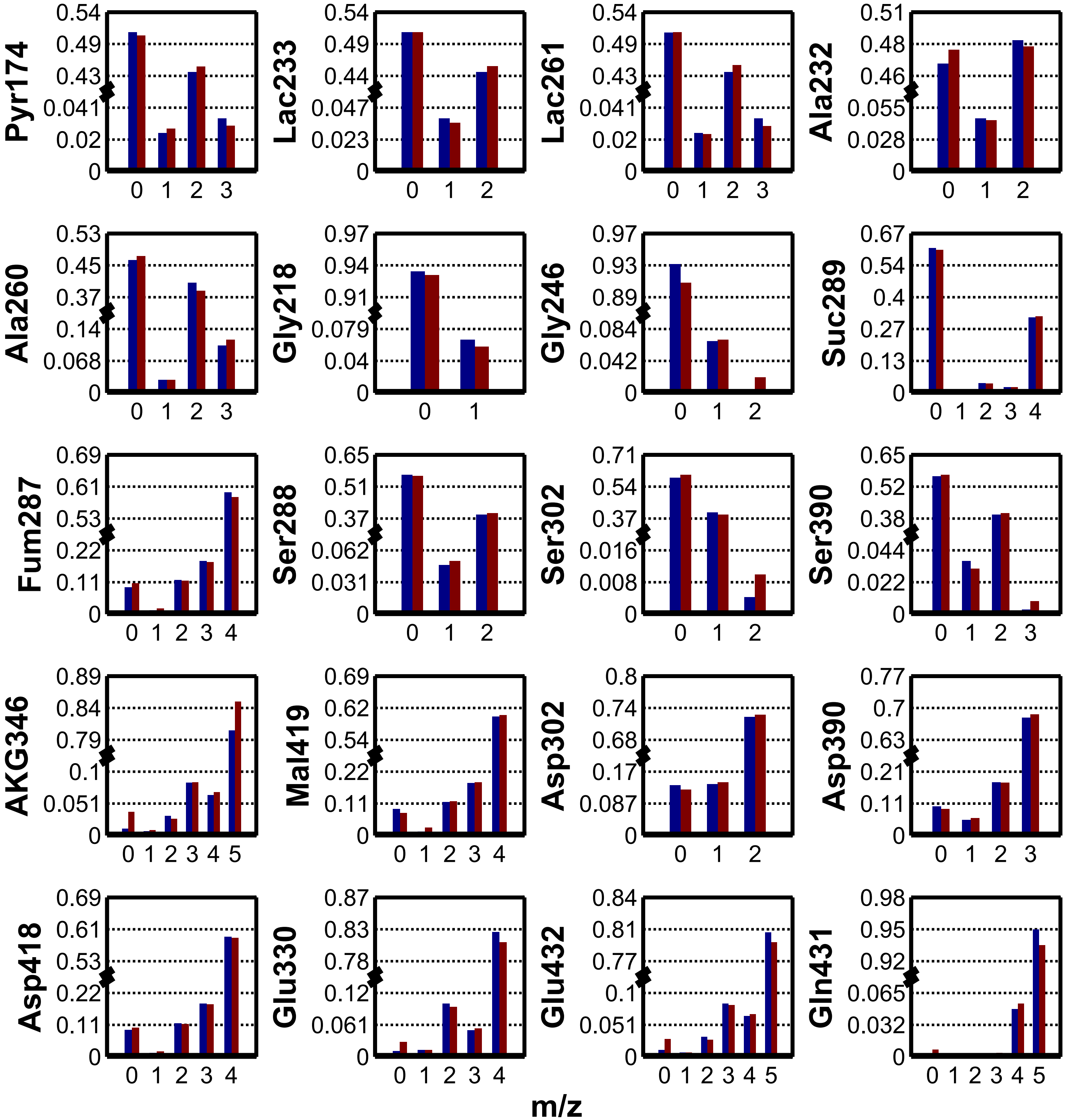
Supplementary Table 1: Metabolite ion fragments used in GC/MS analysis

Measurement	*Carbon atoms*	*Formula*
Pyr174	Pyr.mnt @ 1 2 3	C6H12O3NSi
Lac233	Lac @ 2 3	C10H25O2Si2
Lac261	Lac @ 1 2 3	C11H25O3Si2
Ala232	Ala @ 2 3	C10H26ONSi2
Ala260	Ala @ 1 2 3	C11H26O2NSi2
Gly218	Gly @ 2	C9H24ONSi2
Gly246	Gly @ 1 2	C10H24O2NSi2
Suc289	Suc.mnt @ 1 2 3 4	C12H25O4Si2
Fum287	Fum @ 1 2 3 4	C12H23O4Si2
Ser288	Ser @ 2 3	C14H34NOSi2
Ser302	Ser @ 1 2	C14H32O2NSi2
Ser390	Ser @ 1 2 3	C17H40O3NSi3
AKG346	AKG @ 1 2 3 4 5	C14H28O5NSi2
Mal419	Mal @ 1 2 3 4	C18H39O5Si3
Asp302	Asp @ 1 2	C14H32O2NSi2
Asp390	Asp @ 2 3 4	C17H40O3NSi3
Asp418	Asp @ 1 2 3 4	C18H40O4NSi3
Glu330	Glu @ 2 3 4 5	C16H36O2NSi2
Glu432	Glu @ 1 2 3 4 5	C19H42O4NSi3
Gln431	Gln @ 1 2 3 4 5	C19H43N2O3Si3
Cit459	Cit @ 1 2 3 4 5 6	C20H39O6Si3
Cit591	Cit @ 1 2 3 4 5 6	C26H55O7Si4
GLP357	GLP.mnt @ 2 3	C11H30O5Si3P
GLP445	GLP.mnt @ 1 2 3	C14H38O6Si4P
P5P357	P5P @ 4 5	C11H30O5Si3P
P5P459	P5P @ 3 4 5	C15H40O6Si4P

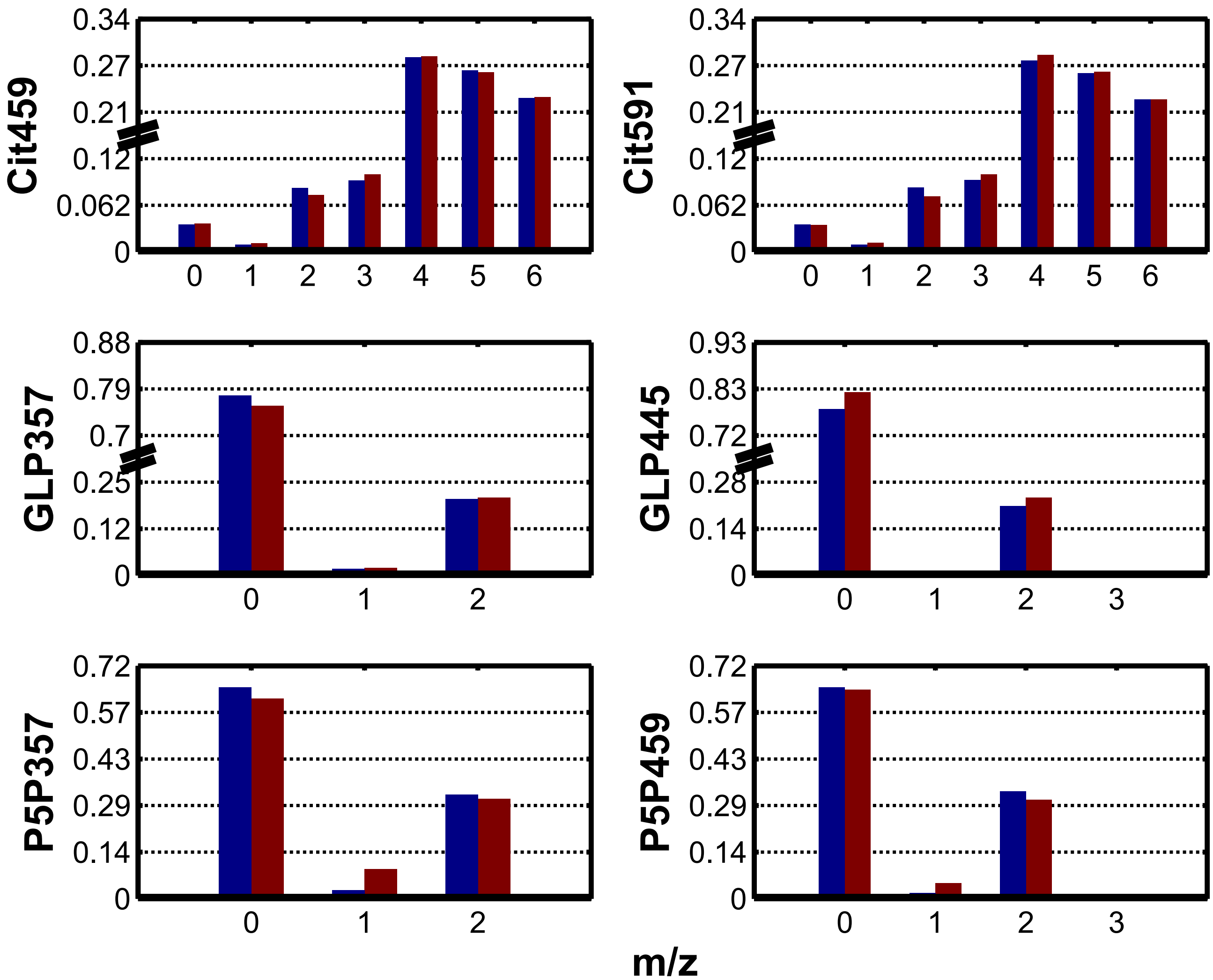
Supplementary Table 2: Fluxes and 95% confidence intervals determined using optimized and selected tracers

		[1,2]gluc/[U]gln			[1]gluc/[U]gluc			[U]gln			
		Flux	LB	UB	Flux	LB	UB	Flux	LB	UB	
R1		32.93	30.71	35.18	33.32	30.7	34.85	29.97	27.03	32.22	Gluc.x (abcdef) → G6P (abcdef)
R2		60.06	55.71	64.31	45.97	42.26	51.16	39.48	34.7	45.32	Lac (abc) → Lac.x (abc)
R3		7.305	6.275	8.245	7.746	6.253	9.301	6.899	5.889	7.87	Gln.x (abcde) → Gln (abcde)
R4		4.02	3.339	4.704	3.497	2.833	4.194	3.673	2.989	4.349	Glu (abcde) → Glu.x (abcde)
R5		0.7061	0.5694	0.8429	0.7035	0.5663	0.8367	0.705	0.569	0.8373	0.18 Asp + 0.23 Glu + 0.17 Ser + 0.11 Gly + 0.15 Ala + 0.16 Gln → Biomass
R6	net	29.69	27.1	32.29	22.44	20.11	27.56	29.97	5.345	32.43	G6P (abcdef) → F6P (abcdef)
R6	exch	1.50E+05	0	Inf	2.40E+05	49.35	Inf	-1.24E-04	0	Inf	G6P (abcdef) ↔ F6P (abcdef)
R7		31.85	29.14	34.14	28.64	25.95	32.55	29.97	21.71	32.43	F6P (abcdef) → DHAP (cba) + GAP (def)
R8	net	29.86	26.23	32.09	28.64	24.15	31.38	29.97	21.7	32.91	DHAP (abc) → GAP (abc)
R8	exch	1.00E-07	0	Inf	160.3	16.41	Inf	1.00E-07	0	Inf	DHAP (abc) ↔ GAP (abc)
R9	net	62.79	58.44	67.04	60.38	54.48	66.56	59.94	51.04	65.79	GAP (abc) → 3PG (abc)
R9	exch	4.96E+01	0	Inf	2.405	0	Inf	1E-07	0	Inf	GAP (abc) ↔ 3PG (abc)
R10		62.67	58.32	66.89	60.29	54.39	66.47	59.94	51.04	65.83	3PG (abc) → Pyr.c (abc)
R11	net	60.06	55.71	64.31	45.97	42.26	51.16	39.48	34.7	45.32	Pyr.c (abc) → Lac (abc)
R11	exch	5.30E+01	0	Inf	10.82	0	Inf	5.741	0	Inf	Pyr.c (abc) ↔ Lac (abc)
R12	net	2.614	2.077	3.268	14.32	8.883	20.2	20.46	10.8	28.49	Pyr.c (abc) → Pyr.m (abc)
R12	exch	28.62	18.14	40.56	0.8165	0	4.502	16.12	8.738	25.01	Pyr.c (abc) ↔ Pyr.m (abc)
R13	net	1.989	0	6.258	1.00E-07	0	6.027	1.00E-07	0	8.289	DHAP (abc) → GLP (abc)
R13	exch	3.70E+00	0	Inf	0.2449	0	Inf	1.182	0	Inf	DHAP (abc) ↔ GLP (abc)
R14		1.989	0	6.258	1.00E-07	0	6.027	1.00E-07	0	8.289	GLP (abc) → GLP.x (abc)
R15		3.245	1.762	6.229	10.88	8.778	12.85	1.32E-05	0	24.86	G6P (abcdef) → P5P (bcdef) + CO2 (a)
R16	net	1.082	0.4174	1.393	3.099	1.999	4.035	1.43E-05	-1.657	8.283	P5P (abcde) + P5P (fghij) → S7P (abfghij) + GAP (cde)
R16	exch	8.245	4.42	17.26	1E-07	0	1.342	1.607	0	Inf	P5P (abcde) + P5P (fghij) ↔ S7P (abfghij) + GAP (cde)
R17	net	1.082	0.4174	1.393	3.099	1.999	4.035	1.43E-05	-1.657	8.283	S7P (abcdefg) + GAP (hij) → F6P (abchij) + E4P (defg)
R17	exch	0.9363	0	6.812	9.27E+01	45.75	260.7	3.809	0	Inf	S7P (abcdefg) + GAP (hij) ↔ F6P (abchij) + E4P (defg)
R18	net	1.082	0.4174	1.393	3.099	1.999	4.035	1.43E-05	-1.657	8.283	P5P (abcde) + E4P (fghi) → F6P (abfghi) + GAP (cde)
R18	exch	1.00E-07	0	2.11	1.00E-07	0	1.589	1.01E-07	0	Inf	P5P (abcde) + E4P (fghi) ↔ F6P (abfghi) + GAP (cde)
R19		0.2672	0.1846	0.3574	1.00E-07	0	0.5225	1.00E-07	0	0.6987	Pyr.m (abc) + CO2 (d) → OAA (abcd)
R20	net	3.149	2.188	3.964	3.848	2.342	5.439	2.824	1.882	3.857	Mal (abcd) → Pyr.m (abc) + CO2 (d)
R20	exch	0.477	0.3337	0.6381	1.929	1.282	2.672	1.411	0	2.211	Mal (abcd) ↔ Pyr.m (abc) + CO2 (d)
R21		5.657	4.52	6.803	18.06	11.33	25.41	23.18	12.63	31.99	Pyr.m (abc) → AcCoA.m (bc) + CO2 (a)
R22		5.657	4.52	6.803	18.06	11.33	25.41	23.18	12.63	31.99	AcCoA.m (ab) + OAA (cdef) → Cit (fedbac)
R23	net	1.343	1.118	1.58	3.118	2.491	3.847	1.292	0.8726	3.611	Cit (abcdef) → AKG (abcde) + CO2 (f)
R23	exch	1.549	1.25	1.828	1.542	0.9025	2.375	0.7249	0.5003	1.255	Cit (abcdef) ↔ AKG (abcde) + CO2 (f)
R24		4.353	3.378	5.201	7.092	5.149	9.196	4.243	2.886	6.609	AKG (abcde) → Suc (bcde) + CO2 (a)
R25	net	4.353	3.378	5.201	7.092	5.149	9.196	4.243	2.886	6.609	Suc (abcd) → Fum (abcd)
R25	exch	0.315	0	1.736	2.51E-08	0	5.916	0.07754	0	1.159	Suc (abcd) ↔ Fum (abcd)
R26	net	4.353	3.378	5.201	7.092	5.149	9.196	4.243	2.886	6.609	Fum (abcd) → Mal (abcd)
R26	exch	1.00E+07	92.46	Inf	6.795	3.559	13.24	38.77	19.74	134	Fum (abcd) ↔ Mal (abcd)
R27	net	1.203	0.9857	1.43	3.245	2.536	3.963	1.418	0.1038	3.661	Mal (abcd) → OAA (abcd)
R27	exch	229.8	51.04	Inf	0.8836	0	5.386	1.00E+07	23.16	Inf	Mal (abcd) ↔ OAA (abcd)
R28		4.314	3.268	5.383	14.95	8.471	22.08	21.89	11.72	30.39	Cit (abcdef) → AcCoA.c (ed) + OAA (fcba)
R29		4.314	3.268	5.383	14.95	8.471	22.08	21.89	11.72	30.39	AcCoA.c (ab) → FA (ab)
R30		0.1059	0.08541	0.1264	0.1055	0.08495	0.1255	0.1058	0.08535	0.1256	Pyr.m (abc) + Glu (defgh) → Ala (abc) + AKG (defgh)
R31		0.1271	0.1025	0.1517	0.1266	0.1019	0.1506	0.1269	0.1024	0.1507	OAA (abcd) + Glu (efghi) → Asp (abcd) + AKG (efghi)
R32		0.1191	0.09649	0.1425	0.08429	0.06776	0.1009	-2.62E-06	0	0.2352	3PG (abc) + Glu (defgh) → Ser (abc) + AKG (defgh)
R33	net	0.008998	0.005345	0.0133	0.007286	0.003615	0.01145	0.07755	0	0.0921	Ser (abc) → Gly (ab) + MEETHF (c)
R33	exch	0.004846	0.000985	0.008932	0.005285	0.001489	0.009385	0.1784	0	Inf	Ser (abc) ↔ Gly (ab) + MEETHF (c)
R34		7.192	6.158	8.13	7.633	6.121	9.188	6.787	5.744	7.707	Gln (abcde) → Glu (abcde)
R35	net	2.657	1.755	3.423	3.658	2.123	5.272	2.719	1.576	3.503	Glu (abcde) → AKG (abcde)
R35	exch	5.00E+04	119.2	Inf	593.3	108.6	Inf	1.00E-07	0	Inf	Glu (abcde) ↔ AKG (abcde)
R36		1.00E-07	0	3.239	1.581	0	4.415	-2.97E-05	0	4.973	P5P (abcde) → NTP (abcde)
R37		0.008998	0.005345	0.0133	0.007286	0.003615	0.01145	0.07755	0	0.0921	MEETHF (a) → MEETHF.x (a)
R38		0.06867	0.05501	0.0826	0.0701	0.05615	0.08443	2.42E-06	0	0.0925	Gly.p (ab) → Gly (ab)
R39		0.009976	0.005906	0.01471	0.04259	0.03394	0.05166	0.1974	0	0.2344	Ser.p (abc) → Ser (abc)
R40		1	0	1	0.1413	0	1	1	0	1	P5P.dil (abcde) → P5P.mnt (abcde)
R41		1E-07	0	1	0.8587	0	1	1E-07	0	1	0 P5P (abcde) → P5P.mnt (abcde)
R43		0.7787	0.512	0.8027	0.5637	0.5151	0.6237	0.4361	0	1	GLP.dil (abc) → GLP.mnt (abc)
R44		0.2213	0.1973	0.488	0.4363	0.3763	0.4849	0.5639	0	1	0 GLP (abc) → GLP.mnt (abc)
R46		0.6061	0.577	0.6356	0.4807	0.3945	0.5654	0.187	0.1424	0.2232	Suc.dil (abcd) → Suc.mnt (abcd)
R47		0.3939	0.3644	0.423	0.5193	0.4346	0.6055	0.813	0.7768	0.8576	0 Suc (abcd) → Suc.mnt (abcd)
R49		1	0.8851	1	1	0.9287	1	0.9455	0.7868	1	0 Pyr.c (abc) → Pyr.mnt (abc)
R50		-8.16E-05	0	0.1149	1.00E-07	0	0.07129	0.05448	0	0.2132	0 Pyr.m (abc) → Pyr.mnt (abc)

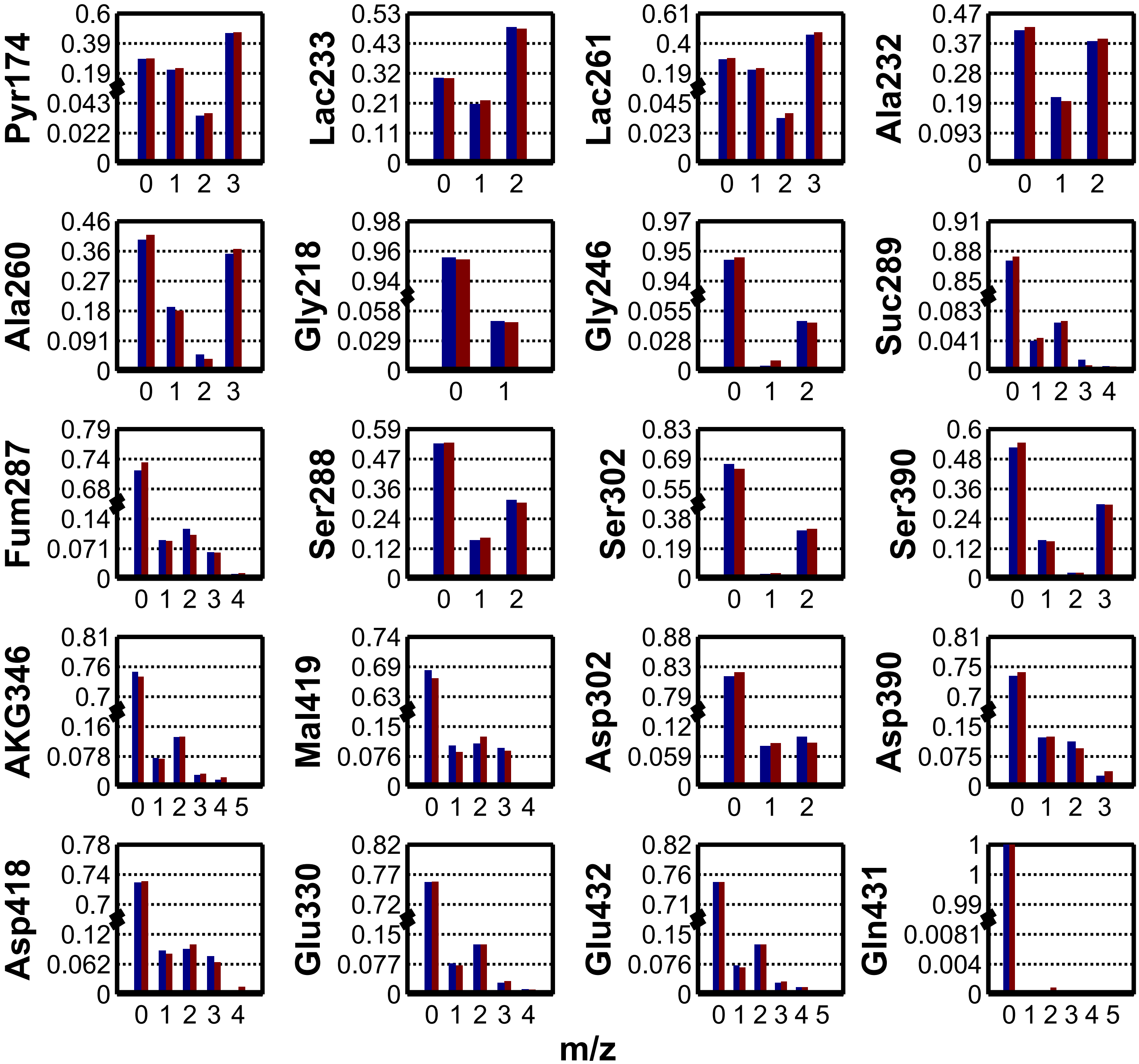
→ indicates net flux: $(\upsilon_F-\upsilon_R)$; ↔ indicates exchange flux: $\min(\upsilon_F,\upsilon_R)$.



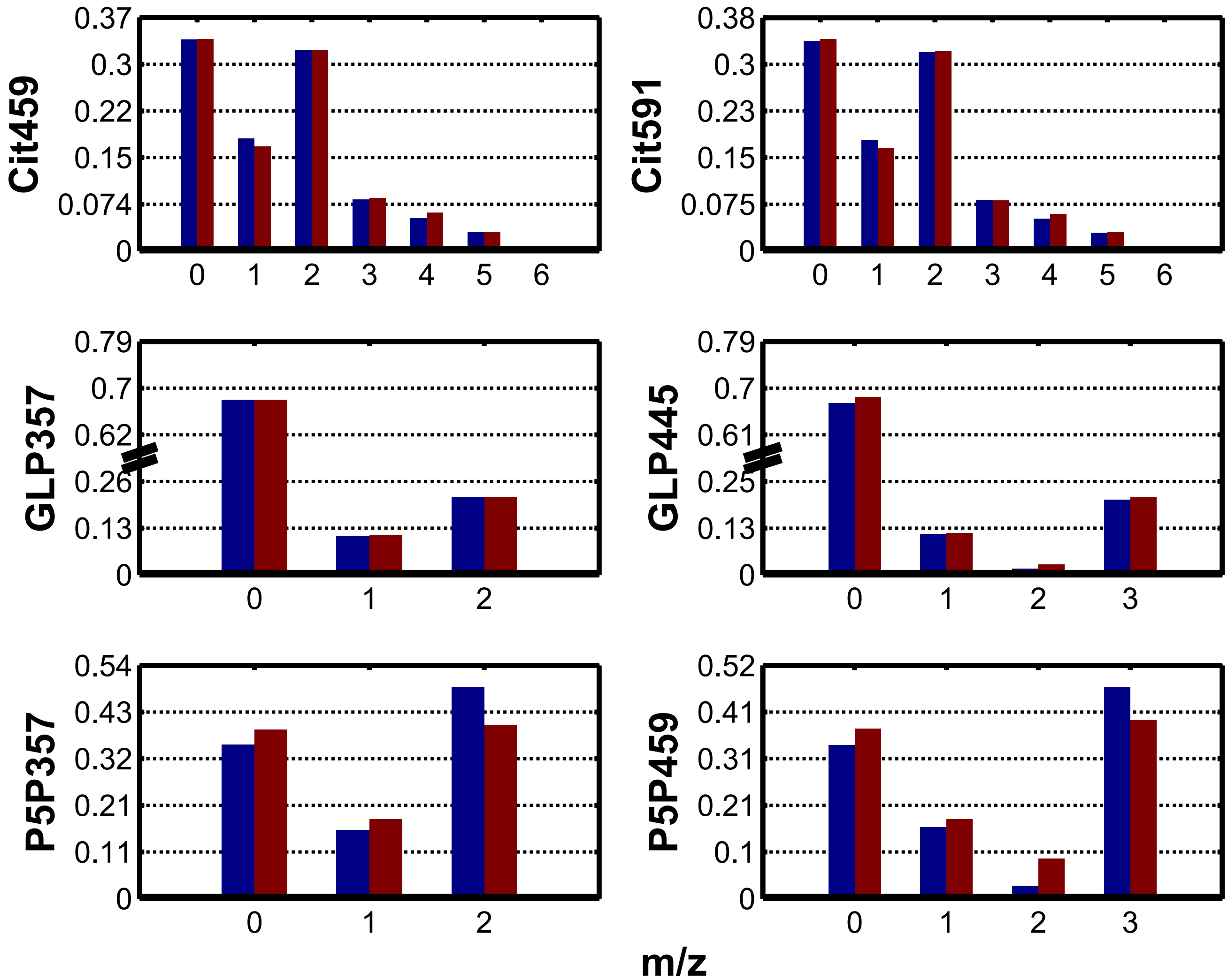
Supplementary Figure 1A: Simulated and measured MIDs from MFA model using A549 cells cultured with a mixture of [1,2-13C2]glucose and [U-13C5]glutamine. Simulated values (blue) were obtained using Metran and the model fit (fluxes) listed in Supplementary Table 3. For measured values (red), labeling was determined via GC/MS and corrected for natural isotope abundance.



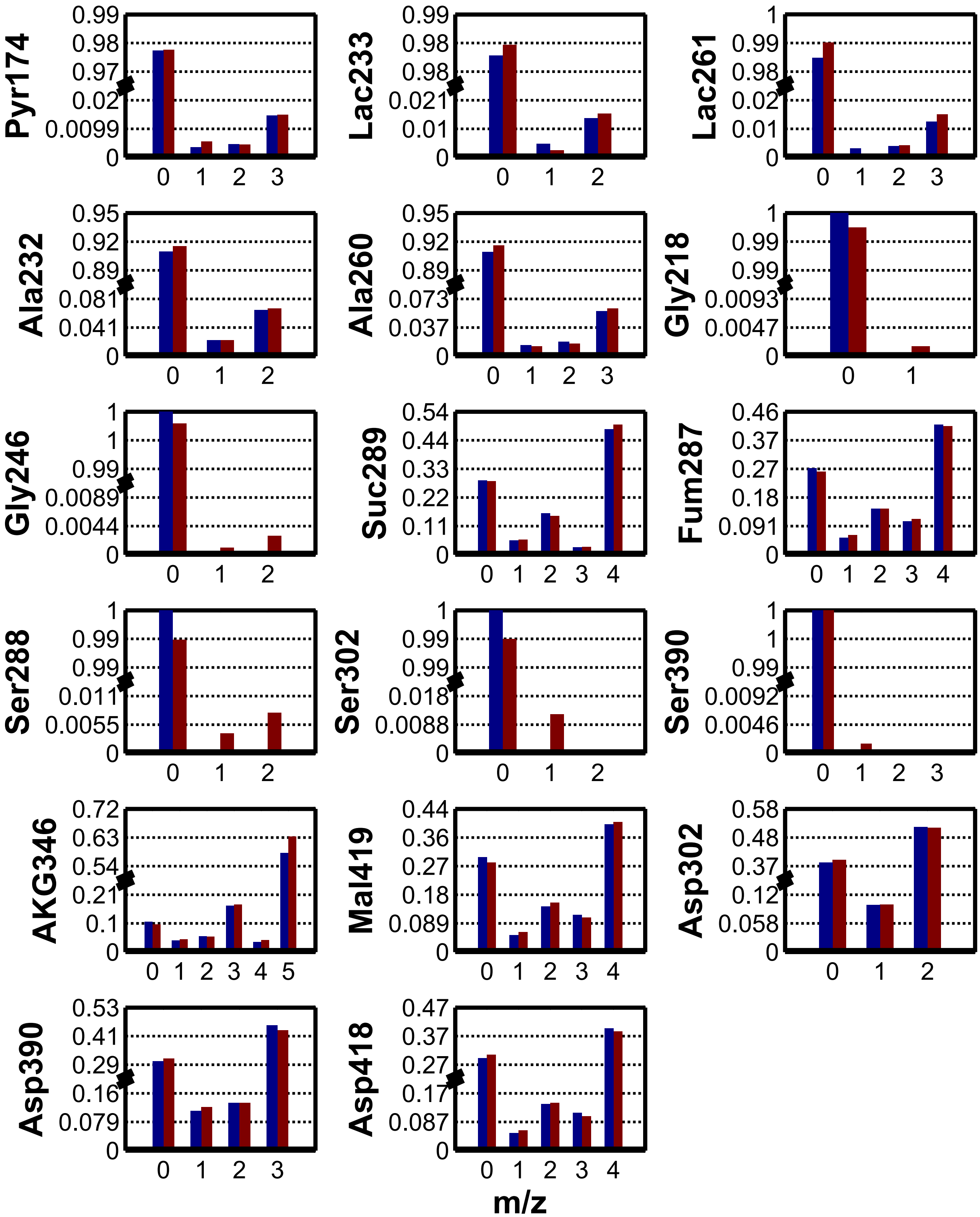
Supplementary Figure 1B: Simulated and measured MIDs from MFA model using A549 cells cultured with a mixture of [1,2-13C2]glucose and [U-13C5]glutamine. Simulated values (blue) were obtained using Metran and the model fit (fluxes) listed in Supplementary Table 3. For measured values (red), labeling was determined via GC/MS and corrected for natural isotope abundance.



Supplementary Figure 2A: Simulated and measured MIDs from MFA model using A549 cells cultured with a 1:1 mixture of [U-13C6]glucose and [1-13C]glucose. Simulated values (blue) were obtained using Metran and the model fit (fluxes) listed in Supplementary Table 3. For measured values (red), labeling was determined via GC/MS and corrected for natural isotope abundance.



Supplementary Figure 2B: Simulated and measured MIDs from MFA model using A549 cells cultured with a 1:1 mixture of [U-13C6]glucose and [1-13C]glucose. Simulated values (blue) were obtained using Metran and the model fit (fluxes) listed in Supplementary Table 3. For measured values (red), labeling was determined via GC/MS and corrected for natural isotope abundance.



Supplementary Figure 3: Simulated and measured MIDs from MFA model using A549 cells cultured with unlabeled glucose and [U-13C5]glutamine. Simulated values (blue) were obtained using Metran and the model fit (fluxes) listed in Supplementary Table 3. For measured values (red), labeling was determined via GC/MS and corrected for natural isotope abundance.

Supplementary Table 3: Mass isotopomer distribution measurements and standard errors used for experimental validatior

Mass isotopomer abundances	[1,2-13C2]gluc+[U-13C5]gln		[1-13C]gluc+[U-13C6]gluc		[U-13C5]gln	
Ion	**Average**	**SEM**	**Average**	**SEM**	**Average**	**SEM**
Pyr_174 (M0)	42.66%	0.50%	24.31%	0.50%	83.21%	0.50%
Pyr_175 (M1)	7.61%	0.50%	21.96%	0.50%	10.72%	0.50%
Pyr_176 (M2)	40.85%	0.50%	6.32%	0.50%	4.23%	0.50%
Pyr_177 (M3)	6.74%	0.50%	41.81%	0.50%	1.63%	0.50%
Pyr_178 (M4)	1.93%	0.50%	3.77%	0.50%	0.15%	0.50%
Lac_233 (M0)	38.41%	0.50%	22.77%	0.50%	74.21%	0.50%
Lac_234 (M1)	11.00%	0.50%	21.96%	0.50%	16.21%	0.50%
Lac_235 (M2)	39.38%	0.50%	42.38%	0.50%	8.04%	0.50%
Lac_236 (M3)	7.54%	0.50%	8.92%	0.50%	1.23%	0.50%
Lac_237 (M4)	3.18%	0.50%	3.44%	0.50%		
Lac_261 (M0)	37.62%	0.50%	21.26%	0.50%	73.13%	0.50%
Lac_262 (M1)	10.34%	0.50%	21.13%	0.50%	16.67%	0.50%
Lac_263 (M2)	37.78%	0.50%	8.32%	0.50%	7.33%	0.50%
Lac_264 (M3)	9.88%	0.50%	38.11%	0.50%	2.28%	0.50%
Lac_265 (M4)	3.63%	0.50%	7.42%	0.50%		
Ala_232 (M0)	36.00%	0.50%	31.89%	0.50%	68.83%	0.50%
Ala_233 (M1)	11.27%	0.50%	21.67%	0.50%	16.78%	0.50%
Ala_234 (M2)	40.94%	0.50%	35.75%	0.50%	11.72%	0.50%
Ala_235 (M3)	8.12%	0.50%	7.51%	0.50%	2.04%	0.50%
Ala_236 (M4)	3.25%	0.50%	2.77%	0.50%		
Ala_260 (M0)	35.29%	0.50%	30.77%	0.50%	67.97%	0.50%
Ala_261 (M1)	10.22%	0.50%	20.85%	0.50%	16.59%	0.50%
Ala_262 (M2)	33.22%	0.50%	8.64%	0.50%	7.83%	0.50%
Ala_263 (M3)	15.55%	0.50%	30.77%	0.50%	5.98%	0.50%
Ala_264 (M4)	4.46%	0.50%	6.03%	0.50%	1.17%	0.50%
Ala_265 (M5)	1.13%	0.50%	2.57%	0.50%		
Gly_218 (M0)	71.02%	0.50%	72.43%	0.50%	76.06%	0.50%
Gly_219 (M1)	19.21%	0.50%	18.69%	0.50%	15.94%	0.50%
Gly_220 (M2)	7.32%	0.50%	7.17%	0.50%	6.68%	0.50%
Gly_221 (M3)	2.37%	0.50%	1.51%	0.50%	1.18%	0.50%
Gly_246 (M0)	68.45%	1.70%	71.33%	0.50%	75.11%	0.50%
Gly_247 (M1)	20.35%	0.59%	16.36%	0.50%	16.55%	0.50%
Gly_248 (M2)	8.99%	0.50%	10.12%	0.50%	7.15%	0.50%
Gly_249 (M3)	2.09%	0.61%	1.73%	0.50%	1.06%	0.50%
Suc_289 (M0)	44.52%	1.66%	64.37%	0.50%	20.58%	0.50%
Suc_290 (M1)	11.65%	0.60%	18.71%	0.50%	9.12%	0.50%
Suc_291 (M2)	7.68%	0.50%	12.42%	0.50%	14.13%	0.50%
Suc_292 (M3)	3.24%	0.50%	3.07%	0.50%	5.37%	0.50%
Suc_293 (M4)	25.66%	1.74%	1.29%	0.50%	39.67%	0.50%
Suc_294 (M5)	4.95%	0.50%			7.52%	0.50%
Suc_295 (M6)	2.30%	0.50%			3.57%	0.50%
Fum_287 (M0)	7.72%	0.50%	53.81%	0.50%	19.52%	0.68%
Fum_288 (M1)	3.32%	0.50%	19.48%	0.50%	9.32%	0.50%
Fum_289 (M2)	9.72%	0.50%	14.77%	0.50%	14.09%	0.50%
Fum_290 (M3)	15.62%	0.50%	7.97%	0.50%	11.81%	0.50%
Fum_291 (M4)	48.38%	0.50%	2.98%	0.50%	34.49%	0.76%
Fum_292 (M5)	10.32%	0.50%	0.83%	0.50%	7.28%	0.50%
Fum_293 (M6)	4.39%	0.50%			3.07%	0.50%
Ser_288 (M0)	40.43%	1.08%	38.86%	0.50%	71.70%	0.50%
Ser_289 (M1)	14.45%	0.50%	22.07%	0.50%	19.22%	0.50%
Ser_290 (M2)	33.97%	0.70%	29.05%	0.50%	7.86%	0.50%
Ser_291 (M3)	8.23%	0.50%	7.15%	0.50%	1.22%	0.50%
Ser_292 (M4)	2.93%	0.50%	2.65%	0.50%		

The minimum values used for s.e.m. was 0.5% to account for disagreement between theoretical and measured MIDs.

Supplementary Table 3 (cont): Mass isotopomer distribution measurements and standard errors used for experimental validatior

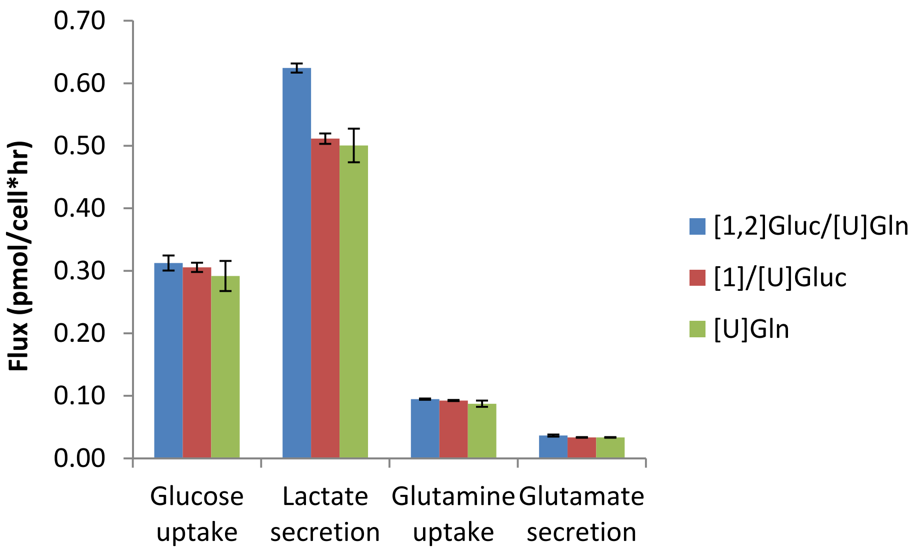
Mass isotopomer abundances	[1,2-13C2]gluc+[U-13C5]gln		[1-13C]gluc+[U-13C6]gluc		[U-13C5]gln	
Ion	**Average**	**SEM**	**Average**	**SEM**	**Average**	**SEM**
Ser_302 (M0)	43.63%	1.07%	46.43%	0.50%	71.33%	0.50%
Ser_303 (M1)	40.33%	0.85%	14.80%	0.50%	19.72%	0.50%
Ser_304 (M2)	12.50%	0.50%	28.62%	0.50%	7.60%	0.50%
Ser_305 (M3)	3.37%	0.50%	7.02%	0.50%	1.01%	0.50%
Ser_306 (M4)			3.13%	0.50%		
Ser_390 (M0)	36.47%	0.69%	34.76%	0.50%	64.38%	0.50%
Ser_391 (M1)	14.78%	0.50%	21.73%	0.50%	22.60%	0.50%
Ser_392 (M2)	33.02%	0.67%	10.36%	0.50%	10.46%	0.50%
Ser_393 (M3)	10.94%	0.50%	22.89%	0.50%	2.36%	0.50%
Ser_394 (M4)	4.27%	0.50%	6.93%	0.50%		
Ser_395 (M5)			2.94%	0.50%		
Akg_346 (M0)	2.53%	0.50%	52.94%	0.51%	6.87%	0.50%
Akg_347 (M1)	1.25%	0.50%	19.16%	0.50%	4.95%	0.50%
Akg_348 (M2)	2.25%	0.50%	16.56%	0.50%	5.46%	0.50%
Akg_349 (M3)	6.45%	0.50%	6.30%	0.50%	13.62%	0.50%
Akg_350 (M4)	6.42%	0.50%	3.57%	0.50%	6.42%	0.50%
Akg_351 (M5)	61.73%	0.50%	1.21%	0.50%	47.49%	0.92%
Akg_352 (M6)	12.80%	0.50%			10.00%	0.50%
Akg_353 (M7)	5.80%	0.50%			4.53%	0.50%
Mal_419 (M0)	4.90%	0.50%	42.11%	0.50%	17.60%	0.50%
Mal_420 (M1)	3.46%	0.50%	20.60%	0.50%	10.23%	0.50%
Mal_421 (M2)	9.05%	0.50%	17.26%	0.50%	14.28%	0.50%
Mal_422 (M3)	15.11%	0.50%	11.27%	0.50%	11.71%	0.50%
Mal_423 (M4)	44.78%	0.50%	4.47%	0.50%	30.86%	0.58%
Mal_424 (M5)	14.36%	0.50%	3.18%	0.50%	9.76%	0.50%
Mal_425 (M6)	6.64%	0.50%	0.84%	0.50%	4.43%	0.50%
Mal_426 (M7)	1.39%	0.50%			0.94%	0.50%
Asp_302 (M0)	9.05%	0.50%	59.53%	0.50%	28.44%	0.92%
Asp_303 (M1)	12.97%	0.50%	21.97%	0.50%	14.51%	0.50%
Asp_304 (M2)	56.92%	0.50%	14.08%	0.50%	42.14%	0.88%
Asp_305 (M3)	14.28%	0.50%	3.31%	0.50%	10.21%	0.50%
Asp_306 (M4)	5.46%	0.50%	0.95%	0.50%	3.86%	0.50%
Asp_307 (M5)	1.06%	0.50%				
Asp_390 (M0)	5.61%	0.50%	47.26%	0.50%	20.21%	0.63%
Asp_391 (M1)	5.66%	0.50%	24.51%	0.50%	14.79%	0.50%
Asp_392 (M2)	13.52%	0.50%	16.57%	0.50%	14.50%	0.50%
Asp_393 (M3)	49.83%	0.50%	7.52%	0.50%	33.39%	0.86%
Asp_394 (M4)	16.34%	0.50%	2.47%	0.50%	10.89%	0.50%
Asp_395 (M5)	7.34%	0.50%	0.67%	0.50%	4.75%	0.50%
Asp_396 (M6)	1.48%	0.50%				
Asp_418 (M0)	6.36%	0.50%	46.31%	0.50%	19.27%	0.59%
Asp_419 (M1)	3.43%	0.50%	22.06%	0.50%	10.90%	0.50%
Asp_420 (M2)	8.79%	0.50%	16.36%	0.50%	14.03%	0.50%
Asp_421 (M3)	14.83%	0.50%	9.30%	0.50%	11.48%	0.50%
Asp_422 (M4)	44.03%	0.50%	4.13%	0.50%	29.54%	0.74%
Asp_423 (M5)	14.49%	0.50%	1.40%	0.50%	9.45%	0.50%
Asp_424 (M6)	6.42%	0.50%	0.43%	0.50%	4.21%	0.50%
Asp_425 (M7)	1.37%	0.50%			0.94%	0.50%
Glu_330 (M0)	2.02%	0.50%	53.13%	0.50%	8.37%	0.50%
Glu_331 (M1)	1.50%	0.50%	20.44%	0.50%	6.93%	0.50%
Glu_332 (M2)	7.40%	0.50%	16.40%	0.50%	15.13%	0.50%
Glu_333 (M3)	5.91%	0.50%	6.46%	0.50%	6.75%	0.50%
Glu_334 (M4)	61.09%	0.50%	2.67%	0.50%	46.20%	0.87%
Glu_335 (M5)	15.02%	0.50%	0.65%	0.50%	11.28%	0.50%
Glu_336 (M6)	5.92%	0.50%			4.43%	0.50%
Glu_337 (M7)	0.95%	0.50%			0.72%	0.50%

The minimum values used for s.e.m. was 0.5% to account for disagreement between theoretical and measured MIDs.

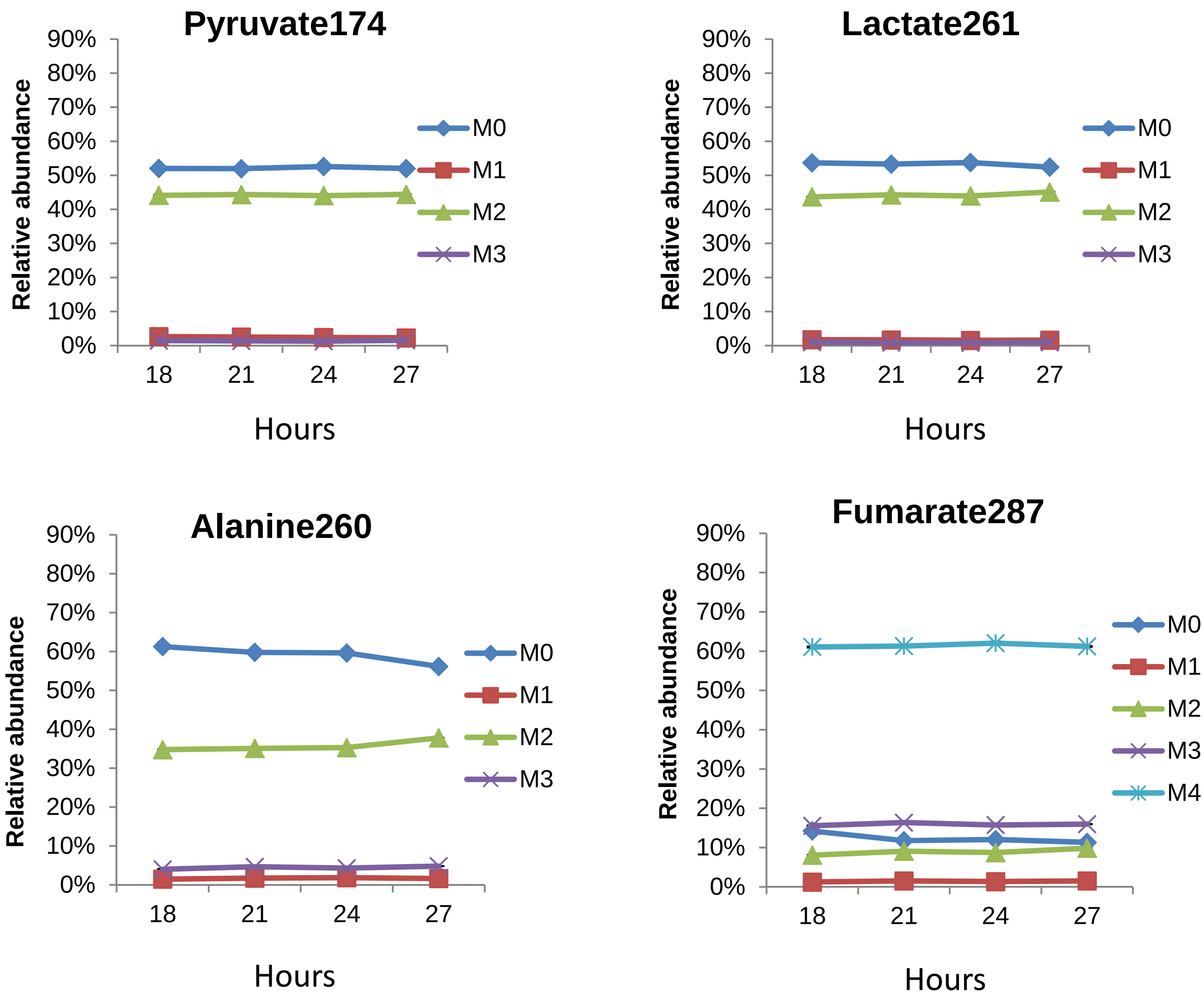
Supplementary Table 3 (cont): Mass isotopomer distribution measurements and standard errors used for experimental validatior

Mass isotopomer abundances	**[1,2-13C2]gluc+[U-13C5]gln**		**[1-13C]gluc+[U-13C6]gluc**		**[U-13C5]gln**	
Ion	**Average**	**SEM**	**Average**	**SEM**	**Average**	**SEM**
Glu_432 (M0)	1.75%	0.50%	47.02%	0.50%	7.18%	0.50%
Glu_433 (M1)	1.07%	0.50%	21.75%	0.50%	5.26%	0.50%
Glu_434 (M2)	2.16%	0.50%	17.69%	0.50%	5.53%	0.50%
Glu_435 (M3)	6.05%	0.50%	7.57%	0.50%	12.28%	0.50%
Glu_436 (M4)	6.51%	0.50%	3.83%	0.50%	6.91%	0.50%
Glu_437 (M5)	54.24%	0.50%	1.48%	0.50%	41.60%	0.77%
Glu_438 (M6)	17.56%	0.50%			13.24%	0.50%
Glu_439 (M7)	8.35%	0.50%			6.24%	0.50%
Glu_440 (M8)	1.81%	0.50%			1.37%	0.50%
Gln_431 (M0)	0.45%	0.50%	62.55%	0.50%	0.83%	0.50%
Gln_432 (M1)	0.17%	0.50%	23.37%	0.50%	0.34%	0.50%
Gln_433 (M2)	0.15%	0.50%	10.78%	0.50%	0.22%	0.50%
Gln_434 (M3)	0.29%	0.50%	2.59%	0.50%	0.38%	0.50%
Gln_435 (M4)	3.60%	0.50%	0.59%	0.50%	3.60%	0.50%
Gln_436 (M5)	62.63%	0.50%			62.29%	0.50%
Gln_437 (M6)	20.43%	0.50%			20.22%	0.50%
Gln_438 (M7)	9.61%	0.50%			9.51%	0.50%
Gln_439 (M8)	2.09%	0.50%			2.09%	0.50%
Gln_440 (M9)	0.50%	0.50%			0.48%	0.50%
Cit_459 (M0)	2.30%	0.50%	20.86%	0.50%	10.86%	0.56%
Cit_460 (M1)	1.58%	0.50%	18.28%	0.50%	8.20%	0.50%
Cit_461 (M2)	5.44%	0.50%	27.73%	0.50%	12.70%	0.50%
Cit_462 (M3)	8.51%	0.50%	15.32%	0.50%	12.93%	0.50%
Cit_463 (M4)	21.41%	0.50%	9.94%	0.50%	26.91%	0.55%
Cit_464 (M5)	24.47%	0.50%	5.10%	0.50%	16.77%	0.50%
Cit_465 (M6)	23.80%	0.50%	1.95%	0.50%	8.03%	0.50%
Cit_466 (M7)	8.18%	0.50%	0.63%	0.50%	2.67%	0.50%
Cit_467 (M8)	3.30%	0.50%			0.77%	0.50%
Cit_468 (M9)	0.71%	0.50%				
Cit_591 (M0)	1.89%	0.50%	18.13%	0.50%	9.50%	0.52%
Cit_592 (M1)	1.60%	0.50%	17.90%	0.50%	8.10%	0.50%
Cit_593 (M2)	4.80%	0.50%	26.61%	0.50%	12.20%	0.50%
Cit_594 (M3)	7.87%	0.50%	16.62%	0.50%	12.71%	0.50%
Cit_595 (M4)	19.83%	0.50%	11.00%	0.50%	25.04%	0.50%
Cit_596 (M5)	23.96%	0.50%	6.02%	0.50%	17.73%	0.50%
Cit_597 (M6)	23.87%	0.50%	2.52%	0.50%	9.45%	0.50%
Cit_598 (M7)	10.33%	0.50%	0.90%	0.50%	3.69%	0.50%
Cit_599 (M8)	4.38%	0.50%			1.19%	0.50%
Cit_600 (M9)	1.21%	0.50%				
Gly3P_357 (M0)	52.10%	1.50%	46.65%	1.50%	67.42%	1.50%
Gly3P_358 (M1)	15.93%	1.50%	20.60%	1.50%	19.29%	1.50%
Gly3P_359 (M2)	22.31%	1.50%	23.68%	1.50%	10.30%	1.50%
Gly3P_360 (M3)	6.28%	1.50%	6.33%	1.50%	2.25%	1.50%
Gly3P_361 (M4)	3.38%	1.50%	2.74%	1.50%	0.75%	1.50%
Gly3P_445 (M0)	49.84%	0.50%	41.62%	1.50%	58.67%	1.50%
Gly3P_446 (M1)	18.88%	1.50%	22.09%	1.50%	21.55%	1.50%
Gly3P_447 (M2)	24.98%	1.50%	12.76%	1.50%	13.11%	1.50%
Gly3P_448 (M3)	5.73%	1.50%	17.32%	1.50%	4.53%	1.50%
Gly3P_449 (M4)	0.57%	1.50%	6.21%	1.50%	2.13%	1.50%
R5P_357 (M0)	42.19%	2.21%	24.56%	1.50%	59.85%	1.50%
R5P_358 (M1)	18.09%	1.68%	18.54%	1.50%	17.96%	1.50%
R5P_359 (M2)	29.35%	0.79%	32.43%	1.50%	9.57%	1.50%
R5P_360 (M3)	8.03%	0.59%	10.63%	1.50%	2.47%	1.50%
R5P_361 (M4)	2.35%	0.60%	6.04%	1.50%		
R5P_459 (M0)	38.62%	1.50%	19.83%	1.50%	46.35%	0.75%
R5P_460 (M1)	17.44%	1.50%	16.81%	1.50%	19.64%	1.50%
R5P_461 (M2)	27.95%	1.50%	12.36%	1.50%	14.14%	1.50%
R5P_462 (M3)	9.82%	1.50%	26.21%	1.50%	6.41%	1.50%
R5P_463 (M4)	6.17%	0.78%	11.96%	1.50%	5.29%	1.50%

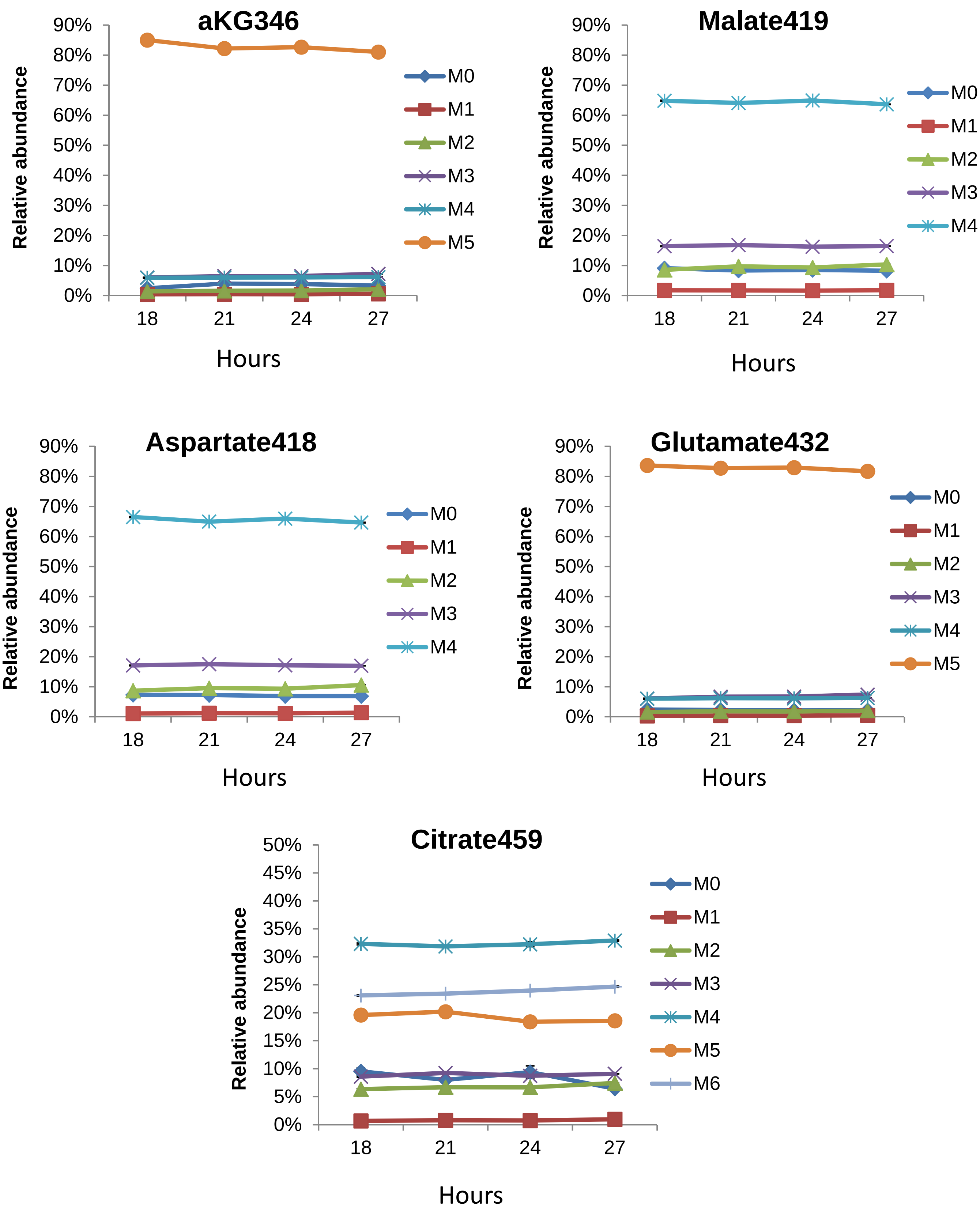
The minimum values used for s.e.m. was 0.5% to account for disagreement between theoretical and measured MIDs.



Supplementary Figure 4: Uptake and secretion fluxes of glucose, lactate, glutamine, and glutamate measured for each MFA experiment (n=3)



Supplementary Figure 5: Validation of isotopic steady state assumption when using combined [1,2-13C2]glucose + [U-13C]glutamine. Cells were cultured in the presence of tracers for 18 – 27 hours, metabolites were extracted at the specified times, and MIDs were determined via GC/MS analysis.



Supplementary Figure 5 (cont): Validation of isotopic steady state assumption when using combined [1,2-13C2]glucose + [U-13C]glutamine. Cells were cultured in the presence of tracers for 18 – 27 hours, metabolites were extracted at the specified times, and MIDs were determined via GC/MS analysis.

Abbreviations:

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

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