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, v, assuming steady-state mass balances $S \cdot v = 0$. 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).

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 @ 12345	C14H28O5NSi2
Mal419	Mal @ 1 2 3 4	C18H39O5Si3
Asp302	Asp @ 1 2	C14H32O2NSi2
Asp390	Asp @ 234	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 @ 23	C11H30O5Si3P
GLP445	GLP.mnt @ 1 2 3	C14H38O6Si4P
P5P357	P5P @ 4 5	C11H30O5Si3P
P5P459	P5P @ 3 4 5	C15H40O6Si4P

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

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

Pire UB			[1,2]gluc/[U]gln		[1]gluc/[U]gluc		[U]gIn				
eff 32.3 3.7 3.8.6 23.7 3.8.7 3.8.6 23.7 3.2.2 3.2.2 1.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2		Flux	LB	UB	Flux	LB	UB	Flux	LB	UB	
Ed 0.00 6.57 0.4.1 4.5.2 0.5.0 0.5.4.4 0.4.2 0.4.2.5 0.5.0 0.5.0.5	R1	32.93	30.71	35.18	33.32	30.7	34.85	29.97	27.03	32.22	$Gluc.x (abcdef) \rightarrow G6P (abcdef)$
i = 1, 2 = 1, 2 = 1, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 < 3, 2 <	R2	60.06	55 71	64.31	45 97	42 26	51 16	39.48	34 7	45.32	$Lac (abc) \rightarrow Lac x (abc)$
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	D3	7 305	6 275	9 245	7 746	6 253	0 301	6 800	5 880	7.97	$G[n \times (abcde)] \rightarrow G[n (abcde)]$
$ \begin{array}{c} \mathbf{r} \\ \mathbf$	NJ D4	7.303	0.275	0.245	7.740	0.233	9.301	0.039	0.009	1.01	$Gin (abcde) \rightarrow Gin (abcde)$
Bay and the set of t	R4	4.02	3.339	4.704	3.497	2.833	4.194	3.673	2.989	4.349	$Giu (abcde) \rightarrow Giu.x (abcde)$
Her Alter State S	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
ele och i 30E-05 0 Inf 24E-04 0 Inf 0.88 0.00 0.87 0.00 0.87 0.00 0.87 0.00 0.87 0.00 0.87 0.00 0.87 0.00 0.87 0.00 0.87 0.00 0.87 0.00 0.87 0.00 0.87 0.00 <	R6 net	29.69	27.1	32.29	22.44	20.11	27.56	29.97	5.345	32.43	G6P (abcdef) \rightarrow F6P (abcdef)
R_{1} 31.85 29.14 34.14 28.64 24.55 29.25 29.27 21.71 32.81 DHP/Anc/Action/ - GAP (and) R8 bcr.01 0.00 br 100.30 10.41 1m 100.57 0 br DAP (anc) - GAP (anc) R8 bcr.01 0.00 br 1.03.8 10.41 1m 100.57 0 br DAP (anc) - GAP (anc) R8 bcr.01 0.00 br 1.02.87 0.00 br DAP (anc) - GAP (anc) R8 bcr.01 0.00 br.01 1.00 Br 2.00 Br	R6 exch	1.50E+05	0	Inf	2.40E+05	49.35	Inf	-1.24E-04	0	Inf	G6P (abcdef) \leftrightarrow F6P (abcdef)
Re de 2016 202.0 202.0 202.0 201.0 21.7 22.8 100 <	R7	31.85	29.14	34.14	28.64	25.95	32.55	29.97	21.71	32.43	F6P (abcdef) \rightarrow DHAP (cba) + GAP (def)
res top: res top: res top: res top: res re	R8 net	29.86	26.23	32.00	28.64	24.15	31 38	20.07	21.7	32.01	$DHAP (abc) \rightarrow GAP (abc)$
No. No. <td></td> <td>1 005 07</td> <td>20.25</td> <td>52.05</td> <td>20.04</td> <td>24.15</td> <td>01.00</td> <td>1 005 07</td> <td>21.7</td> <td>52.51</td> <td>$DHAP (abc) \rightarrow OAP (abc)$</td>		1 005 07	20.25	52.05	20.04	24.15	01.00	1 005 07	21.7	52.51	$DHAP (abc) \rightarrow OAP (abc)$
		1.00E-07			100.3	10.41		1.000-07			DHAP (abc) \leftrightarrow GAP (abc)
Pice Add Age: O Int CASE 0 Int Here Age D Int CASE 0 D Int CASE 0 D Int CASE 0 D <thd< th=""> D <thd< th=""> <th< td=""><td>R9 net</td><td>62.79</td><td>58.44</td><td>67.04</td><td>60.38</td><td>54.48</td><td>66.56</td><td>59.94</td><td>51.04</td><td>65.79</td><td>$GAP (abc) \rightarrow 3PG (abc)$</td></th<></thd<></thd<>	R9 net	62.79	58.44	67.04	60.38	54.48	66.56	59.94	51.04	65.79	$GAP (abc) \rightarrow 3PG (abc)$
R10 62.67 68.32 68.32 69.29 64.37 62.30 68.47 69.44 51.04 63.53 76 (abc) - hyrc (abc) R11 abc 51.71 2.064 51.81 51.84 53.84	R9 exch	4.96E+01	0	Inf	2.405	0	Inf	1E-07	0	Inf	$GAP (abc) \leftrightarrow 3PG (abc)$
R11 etc 65.71 65	R10	62.67	58.32	66.89	60.29	54.39	66.47	59.94	51.04	65.83	$3PG (abc) \rightarrow Pyr.c (abc)$
R1 etc. 5.0E-01 0 Inf 10.8.2 0 Inf 5.7.1 0 Inf Pric (abc) - Lac (abc) R12 etc. 2.6.4 10.8.1 4.4.2 0.8.2 0.4.20 10.8.2 2.6.4 10.8.7 Cabc Pric (abc) - Lac (abc) R13 etc. 3.7.16 0.3.248 0.0 4.502 10.8.2 2.7.16 Pric (abc) - Lac (abc) R14 1.980 0 6.228 10.95-07 0 2.4.8 0.9.7 0.9.248 R14 1.980 0 6.228 1.025-07 0 2.4.8 0.9.27 1.0.9.27 0.0.248 0.0.248 0 2.3.28 0.2.7 0.2.83 0.0.2.7 0.2.33 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.8 0.0.2.7 0.0.2.7 0.0.2.8 0.0.2.7 0.0.2.8 0.0.2.7 0.0.2.8 0.0.2.7 0.0.2.8 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.0.2.7 0.	R11 net	60.06	55.71	64.31	45.97	42.26	51.16	39.48	34.7	45.32	Pyr.c (abc) \rightarrow Lac (abc)
Pire 1 2if 4 2.077 3.288 14.32 8.883 20.2 20.46 10.8 28.49 Pire (acc) $\rightarrow Pire (abc) \rightarrow Pire (abc) here) R13 1.966 0 0.0165 0 0.027 1.006-07 0 0.209 DHAP (abc) \rightarrow CP(abc) \rightarrow CP(abc) R13 1.966 0 1.782 0.239 DHAP (abc) \rightarrow CP(abc) \rightarrow CP(abc) R14 1.966 0 1.782 0.239 DHAP (abc) \rightarrow CP(abc) \rightarrow CP(abc) R15 3.246 0.472 1.330 0.099 4.035 1.3452-05 0.6248 GP (abcc) \rightarrow PP(abcc) + PP(bcc) + O2(a) R16 0.030 0 6.313 0.099 1.342 1.4507 0 1.879 Per (abcc) + PP(bcc) + O2(a) PP(abcc) + PP(abcc) + SP(abcc) + $	R11 exch	5.30E+01	0	Inf	10.82	0	Inf	5.741	0	Inf	$Pvr.c (abc) \leftrightarrow Lac (abc)$
$ \begin{array}{c} rel 2 \ acc b \\ rel 3 $	R12 net	2 614	2 077	3 268	14.32	8 883	20.2	20.46	10.8	28.40	$P_{\rm VII} c (abc) \rightarrow P_{\rm VII} m (abc)$
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	R12 not	2.014	10 14	3.200 40 EG	0.9165	0.000	4 502	16 10	0 720	20.43	P_{M} (abc) \rightarrow P_{M} (abc)
Hall and Labor U Labor U Labor U Labor U Labor		20.02	10.14	40.30	0.0100	0	4.302	10.12	0.730	20.01	$Fyl.c (abc) \leftrightarrow Fyl.in (abc)$
R18 R19 R19 <td>R13 net</td> <td>1.989</td> <td>0</td> <td>6.258</td> <td>1.00E-07</td> <td>0</td> <td>6.027</td> <td>1.00E-07</td> <td>0</td> <td>8.289</td> <td>DHAP (abc) \rightarrow GLP (abc)</td>	R13 net	1.989	0	6.258	1.00E-07	0	6.027	1.00E-07	0	8.289	DHAP (abc) \rightarrow GLP (abc)
R14 1.989 0 6.263 1.006-07 0 6.289 6.12P (abc) \rightarrow GLP (abc) \rightarrow GLP (abc) GLP (abc) \rightarrow GLP (abc) </td <td>R13 exch</td> <td>3.70E+00</td> <td>0</td> <td>Inf</td> <td>0.2449</td> <td>0</td> <td>Inf</td> <td>1.182</td> <td>0</td> <td>Inf</td> <td>DHAP (abc) \leftrightarrow GLP (abc)</td>	R13 exch	3.70E+00	0	Inf	0.2449	0	Inf	1.182	0	Inf	DHAP (abc) \leftrightarrow GLP (abc)
R16 a.2.45 1.782 6.2.29 10.8 8.778 12.8.5 1.322-05 0 2.4.80 OPP (bcdef) \rightarrow CPP (bcdef) \rightarrow CO2 (a) R16 a.2.42 4.4.2 1.722 1.6.77 0 1.4.21 1.6.07 0 Inf PSP (bcdef) \rightarrow PSP (bdf) \rightarrow CSP (adph) \rightarrow CAP (cde) R16 a.2.42 4.4.24 1.3.33 3.0.99 4.3.02 1.4.22 1.6.07 0 Inf PSP (bcdef) \rightarrow PSP (bdf) \rightarrow CSP (adph) \rightarrow CAP (cde) R16 u 1.0.02 0.4174 1.3.33 3.0.99 4.3.02 1.4.5.16 1.6.57 2.2.33 STP (bcdef) \rightarrow ESP (bdf) \rightarrow CSP (adph)	R14	1.989	0	6.258	1.00E-07	0	6.027	1.00E-07	0	8.289	$GLP (abc) \rightarrow GLP.x (abc)$
R16 10.82 0.4174 1.333 3.099 1.999 4.035 1.43E-05 1.637 8.233 PP (nabce) + PP (nb) - SP (nb) - SP (nb) - SP (nb) + PP (nb) - PP (nb) +	R15	3.245	1.762	6.229	10.88	8.778	12.85	1.32E-05	0	24.86	G6P (abcdef) \rightarrow P5P (bcdef) + CO2 (a)
R16 R245 4.42 17.26 16.07 0 1.342 1.607 0 10^{-1} PP (pac)(-) + PP (pb)(-) $PP (pb)(-)$ $PP $	R16 net	1 082	0 4 1 7 4	1 393	3 099	1 999	4 035	1 43E-05	-1 657	8 283	P5P (abcde) + P5P (fghii) \rightarrow S7P (abfghii) + GAP (cde)
Pir 7 met1.0320.41741.3031.0091.0394.0351.43E-031.237Pic 2033STP (abcdeff) - 6.04P(h)] - FP (abcdeff)-FP	R16 eych	8 245	4.42	17.26	1E-07	0	1 342	1 607	0	Inf	P5P (abcde) + P5P (fabii) \leftrightarrow S7P (abfabii) + GAP (cde)
$ \begin{array}{c} n,n \\ n,n $		1.000	4.42	1 202	2 000	1 000	1.042	1.007	1 057	0 000	$FJF (abcdefr) + GAP (hii) \rightarrow STF (abigning) + GAF (cdefr)$
R1 R2 R4 R3 R4	R17 net	1.062	0.4174	1.393	3.099	1.999	4.035	1.43E-05	-1.057	0.263	S7P (abcdelg) + GAP (hij) \rightarrow F6P (abchij) + E4P (delg)
R18 ct 1.082 0.4174 1.393 3.099 1.999 4.035 1.436-05 -1.657 8.283 PSP (abde) + E4P (gb) - FOP (abgh) + CAP (cde) R18 ct 0.2572 0.11646 0.3574 1.00E-07 0 0.2525 1.01E-07 0 0.6987 Pyr. (abc) + CO2 (d) - OAA (abcd) R20 ct 0.4377 0.3337 0.6381 1.222 2.672 1.411 0 2.211 Mat (abcd) - Pyr.m (abc) + CO2 (d) R21 ct 5.57 4.52 6.603 1.589 2.241 1.812 3.169 Pyr.m (abc) + CO2 (d) R23 ct 1.435 1.118 1.928 1.242 2.677 1.218 3.169 Cit (abcd) + ACCA.m (bc) - CO2 (d) R24 4.353 3.378 5.201 7.092 5.149 0.166 4.243 2.866 6.609 AKG (abcd) - SU (abcd) + CO2 (a) R25 ct 1.333 3.378 5.201 7.092 5.149 0.168 0.2878 3.811 Cit (abcd) + CO2 (a) R24 4.353 3.378 5.201 7.092 5	R17 exch	0.9363	0	6.812	9.27E+01	45.75	260.7	3.809	0	Inf	S/P (abcdetg) + GAP (hij) \leftrightarrow F6P (abchij) + E4P (detg)
R18 expl 1.00E-07 0 1.58 1.01E-07 0 Inf PSP (abdgh) + EAP (abgh) = CAP (abgch) CAP (abgch) R20 rt 3.149 2.188 3.964 3.848 2.342 5.439 2.824 1.882 3.857 Mat (abcc) = Pyrm (abc) + CO2 (a) CAP (abgch) R20 rt 4.52 6.803 1.9.06 1.1.3 2.5.41 2.3.18 1.2.63 3.199 Pyrm (abc) - CO2 (a) CACAM (abc) - CO2 (a) R21 rt 1.5.8 3.118 2.4.91 3.8.47 1.2.8.3 3.190 Pyrm (abc) - CO2 (a) CACAM (abc) - CO2 (a) R23 rt 1.3.33 3.118 2.4.91 3.8.47 1.2.8.2 0.8.16 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00 7.5.6 6.0.00	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)
R19 R20 net0.2672 0.18460.18460.38741.00E-0700.2627 0.26240.0897Pyrm (abc) + CO2 (a) \rightarrow OAA (abcd)R20 net0.4770.33370.63811.2921.2822.6721.41102.211Mal (abcd) \rightarrow Pyrm (abc) + CO2 (a)R210.5674.526.80318.0611.332.5412.3181.2633.199Pyrm (abc) + CO2 (a)R225.6574.526.80318.0611.332.5412.3181.2633.199Pyrm (abc) - ACCA (abcd) \rightarrow DX (abcd) + CO2 (b)R23 net1.5491.251.8281.1420.8022.3750.7240.5031.255Ci (abcde) \rightarrow AKC (abcde) + CO2 (b)R25 net4.3533.3785.2017.0925.1499.1660.42432.8666.609Ku (abcd) \rightarrow Su (abcd) + CO2 (b)R25 net4.3533.3785.2017.0925.1499.1660.4775401.515Su (abcd) \rightarrow Su (abcd) + CO2 (b)R25 net4.3533.3785.2017.0925.1499.160.42432.8666.609Fun (abcd) \rightarrow Su (abcd) + CO2 (b)R25 net4.3533.3785.2017.0925.1499.160.42432.8666.609Fun (abcd) \rightarrow Su (abcd) + CO2 (b)R25 net4.3333.3785.2017.0925.1499.160.42732.8666.609Fun (abcd) \rightarrow Su (abcd) + CO2 (b)R25 net4.3333.3785.2017.0925.1499.16	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)
R20R21R21R21R23R23R23R23R23R23R23R23R23R23R23R24R24R24R24R24R24R24R24R24R24R24R25R23R23R23R23R23R23R23R23R23R23R23R23R23R23R23R24R	R19	0.2672	0.1846	0.3574	1.00E-07	0	0.5225	1.00E-07	0	0.6987	Pyr.m (abc) + CO2 (d) \rightarrow OAA (abcd)
R20co.h0.4770.3370.63811.9291.2822.6721.41102.211Mal (abcd) - $PCru (m (bc) + CO2 (n)$ R215.6574.526.60318.0611.332.5412.31812.6331.99 $Pcru (m (bc) - ACCA (m (c) + CO2 (n))$ R23ext1.5491.251.8281.1322.5412.3181.26331.99 $Pcru (m (bc) - ACCA (m (c) + CO2 (n))$ R23ext1.5491.251.8281.5420.90252.3750.72490.5031.255Cit (abcde) - $ACG (abcde) + CO2 (n)$ R244.3533.3785.2017.0925.1499.1964.2432.8866.609Su (abcd) - Fur (abcd)R25ext0.3755.2017.0925.1499.1964.2432.8866.609Fur (abcd) - Mat (abcd)R26ext0.3765.2017.0925.1499.1964.2432.8866.609Fur (abcd) - Mat (abcd)R26ext1.00679.244.110.7953.5951.243.87719.7413.45Fur (abcd) - Mat (abcd)R27ext1.2230.98571.433.2842.8861.006Fur (abcd) - Mat (abcd)Abcd)R27ext1.2230.98571.433.2843.9831.4958.4712.2082.18911.723.39Cit (abcd) - AAC (abcd)R27ext1.0250.1710.12680.10260.10260.10260.10261.18<	R20 net	3 149	2 188	3 964	3 848	2 342	5 439	2 824	1 882	3 857	Mal (abcd) \rightarrow Pvr m (abc) + CO2 (d)
$ \begin{array}{c} \hline \begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	P20 ovch	0.477	0.3337	0.6391	1 020	1 292	2,672	1 411	0	2 211	Mal (abod) \rightarrow Pyrm (abo) + CO2 (d)
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		0.477	0.3337	0.0001	1.929	11.202	2.072	02.40	10.00	2.211	$\frac{1}{2} \frac{1}{2} \frac{1}$
R22 5.657 4.52 0.803 18.06 11.33 25.41 2.318 12.63 31.99 Actoal m(a) + UA (Gob) - Un (febrae) R23 ext 1.543 1.148 1.58 3.148 2.441 3.241 2.375 0.7249 0.8003 1.255 Cit (abcde) - AKG (abcde) + CO2 (i) R24 4.333 3.378 5.201 7.092 5.149 9.196 4.243 2.286 6.609 Suc (abcd) - Fun (abcd) ACG (abcde) + CO2 (i) R25 ext 0.357 5.201 7.092 5.149 9.196 4.243 2.286 6.609 Suc (abcd) - Fun (abcd) ACG (abcd) + CO2 (i) R26 ext 1.00470 9.176 5.149 9.196 4.243 2.286 6.609 Fun (abcd) - Mal (abcd) R27 ext 2.201 7.092 5.149 9.196 4.243 2.886 6.609 Fun (abcd) - Mal (abcd) R27 ext 2.203 0.01617 9.244 1.72 3.036 1.01 Mal (abcd) - OAA (abcd) R27 ext 2.208 5.104 1.17 2.208 2.189 11.72 3.03 ACCA (abcd)	RZI	5.057	4.52	0.803	18.06	11.33	25.41	23.18	12.03	31.99	$Pyr.m(abc) \rightarrow AcCoA.m(bc) + CO2(a)$
R23 net 1.343 1.118 1.58 3.118 2.491 3.847 1.292 0.8726 3.811 Cft (abcde) - 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 (bod) + CO2 (a) R25 net 4.353 3.378 5.201 7.092 5.149 9.196 4.243 2.886 6.609 Suc (abcd) - Furm (abcd) R26 net 4.333 3.378 5.201 7.092 5.149 9.196 4.243 2.886 6.609 Nucleoch - Mal (abcd) R26 net 4.333 3.378 5.201 7.092 5.19 0.246 1.07754 0 1.159 Suc (abcd) - FAMG (abcd) Demoty R27 net 1.038 0.867 1.42 2.868 1.016 Mal (abcd) - OAA (abcd) DA(abcd) R28 4.314 3.268 5.383 1.495 8.471 2.208 2.189 11.72 3.039 AcCoAA (cab) - GA (abcd) DA(abcd) R29 4.314 3.268 5.033 1.495	R22	5.657	4.52	6.803	18.06	11.33	25.41	23.18	12.63	31.99	AcCoA.m (ab) + OAA (cdet) \rightarrow Cit (fedbac)
R23R23R23R25R25R25R25R25R25R25R47R45R45R47R45R45R47R45R45R47R45R45R45R47R45R45R45R45R45R45R45R45R45R45R45R45R45R45R45R45R	R23 net	1.343	1.118	1.58	3.118	2.491	3.847	1.292	0.8726	3.611	Cit (abcdef) \rightarrow AKG (abcde) + CO2 (f)
R244.3533.3785.2017.0925.1499.1964.2432.8866.609AKC (abcde) \rightarrow Suc (bcde) + CO2 (a)R25 ext0.31501.7362.51E-0805.9160.0775401.159Suc (abcd) \rightarrow Fur (abcd)R26 ext1.0E+0792.46Inf6.7953.5591.3.2438.771.9.741.34Fur (abcd) \rightarrow Mal (abcd)R27 ext1.2030.98571.4.33.2452.5363.9631.4.180.10383.661Mal (abcd) \rightarrow OAA (abcd)R27 ext1.2030.98571.4.33.2452.5363.9631.4.180.10383.661Mal (abcd) \rightarrow OAA (abcd)R27 ext2.2885.104Inf0.683605.3861.0E+072.1.6InfMal (abcd) \rightarrow ACCAAC (ed) \rightarrow OAA (abcd)R284.3143.2685.38314.958.4712.2.0821.9911.7230.39Citacbef) \rightarrow AcCAAC (ed) \rightarrow OAA (abcd)R310.10550.084540.10550.084550.12550.10560.12560.12560.12560.1256R33 ext0.096980.0053450.01330.0072860.003150.01450.077500.235226 (abc) + Gly (ab) + AEG (abcd)R34 ext1.5671.7573.4233.6582.1235.2722.1791.5763.503Glu (abcde) \rightarrow AFG (abcde)R35 ext5.068-70.1380.037680.034540.01450.0775500.923Glu (abcd	R23 exch	1.549	1.25	1.828	1.542	0.9025	2.375	0.7249	0.5003	1.255	Cit (abcdef) \leftrightarrow AKG (abcde) + CO2 (f)
R25 exch0.31501.7362.51E/0805.9160.0775401.159Suc (abcd) \rightarrow Fum (abcd)R26 exch0.00E+0792.40inf6.7953.55913.243.87719.74134Fum (abcd) \rightarrow Mal (abcd)R27 net1.0230.98571.433.2452.5363.9631.4180.1033.661Mal (abcd) \rightarrow OAA (abcd)R27 exch229.861.04inf0.883605.3861.00E+0723.16InfMal (abcd) \rightarrow OAA (abcd)R284.3143.2685.38314.958.47122.0821.8911.7230.39Cit (abcd) \rightarrow OAA (abcd)R294.3143.2685.38314.958.47122.0821.8911.7230.39Cit (abcd) \rightarrow OAA (abcd)R300.10590.068410.12640.10580.12550.10880.083550.1256Pyrm (abcd) \rightarrow Cit (abc) $+$ AKG (defgh)R310.12710.10260.15170.12660.10190.15060.12690.10240.1507OAA (abcd) \rightarrow Git (abcd) \rightarrow AKG (defgh)R320.11910.096490.003450.003450.01330.0072860.0036150.175500.921Ser (abc) \rightarrow Oid (abcd) \rightarrow AKG (defgh)R33net0.0084960.0098980.003450.01330.0072860.01450.0775500.921Ser (abc) \rightarrow Oid (abcde)R347.1926.1588.137.6336.1219.1886.775.74	R24	4.353	3.378	5.201	7.092	5.149	9.196	4.243	2.886	6.609	AKG (abcde) \rightarrow Suc (bcde) + CO2 (a)
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	R25 net	4 353	3 378	5 201	7 092	5 149	9 196	4 243	2 886	6 609	Suc (abcd) \rightarrow Fum (abcd)
R26net4.3533.3785.2017.1925.1499.1964.2432.8866.609Fum (abcd) - Mal (abcd)R26net1.00E+0792.46Inf6.7953.55913.2438.7719.74134Fum (abcd) - Mal (abcd)R27net1.2030.98571.433.2452.5363.9631.4180.10383.61Mal (abcd) - OAA (abcd)R27exch22.9851.04Inf0.883605.3861.00E+0723.16InfMal (abcd) - OAA (abcd)R284.3143.2685.38314.958.47122.0821.8911.7230.39Ci (abcde) - ACA (cel) - OAA ((bch)R300.10590.085410.12640.10260.12550.10580.084550.1266Prm (abc) - MA (abc) + AKG (defph)R310.12710.10250.15170.12660.10190.15060.12890.01240.1507OAA (abcd) + Glu (defph) - Asp (abcd) + AKG (defph)R320.11910.096490.14250.084290.007760.1009-2.62E-0600.23523PG (abc) - Glu (defph) - Asp (abcd) + AKG (defph)R33 net0.0098980.00953450.01330.0072860.0036150.011450.0775500.0221Ser (abc) - Glu (abcd) - AKG (abcde)R347.1926.1588.137.6336.0216.7775.503Glu (abcde) - AKG (abcde)R35net2.6571.7553.4233.6582.1235.2	R25 exch	0.315	0	1 736	2 51E-08	0	5 916	0 07754	0	1 159	Suc (abcd) \leftrightarrow Eum (abcd)
R26 Ref 4.353 3.378 3.211 1.092 3.149 9.190 4.243 2.360 0.005 Full (abcd) Mal (abcd) R27 ret 1.203 0.9857 1.43 3.245 2.536 3.963 1.418 0.1038 3.661 Mal (abcd) $OAA (abcd)$ R27 ret 1.203 0.9857 1.43 1.845 5.383 1.495 8.471 22.08 21.88 11.72 30.39 Cit (abcde) $\rightarrow ACoA (abcd)$ R29 4.314 3.268 5.383 14.95 8.471 22.08 21.89 11.72 30.39 Cit (abcde) $\rightarrow ACoA (abcd)$ R30 0.1059 0.08541 0.1264 0.1056 0.1259 0.1054 0.1226 0.1270 DAA (abcd) AGG (abc) $+ AKG (defp)$ R31 0.1271 0.1026 0.119 0.1506 0.1289 0.1024 0.157 OAA (abcd) AGG (abcd) $+ AKG (defp)$ AIGG (abcd) R32 0.1191 0.009844 0.009845 0.01145 0.07755 0 0.0221 Ser (abc) $- Gly (ab) + METHF (c) R33$	D26 pot	4 252	2 270	F 201	7 002	E 140	0.0106	4 242	2 006	6 600	Euro (abod) · Mal (abod)
Rzb exch1.00E+0792.49IIIT0.7953.39313.2438.7719.741.94Fun (abcc) \rightarrow Mal (abcd)R27 net1.2030.98571.433.2422.5363.9631.4180.10383.661Mal (abcd) \rightarrow OAA (abcd)R27 exch229.851.04Inf0.883605.3661.00E+0723.16InfMal (abcd) \rightarrow OAA (abcd)R284.3143.2685.38314.958.47122.0821.8911.7230.39Ottabcdeh \rightarrow AcCoA. (ab) \rightarrow FA (ab)R300.10590.085410.12640.10550.084950.12550.10580.085350.1256Pyr.m (abc) + Glu (defh) \rightarrow As (abc) + AKG (defgh)R310.12710.10250.15170.12660.01092.62E-0600.23523PG (abc) + Glu (defh) \rightarrow Ser (abc) + AKG (defgh)R33 net0.008980.0095450.004260.0036150.011450.0775500.0921Ser (abc) \rightarrow Glu (abcde)R347.1926.1588.137.6336.1219.1886.7875.7447.707Glu (abcde) \rightarrow AKG (abcde)R35 net0.0094860.0093450.01330.0072660.0036150.011450.0775500.922Glu (abcde) \rightarrow AKG (abcde)R361.06E-0701.16Glu (abcde) \rightarrow AKG (abcde)AKG (abcde)AKG4.4152.97E-0504.973PSP (abcde) \rightarrow HETHF (c)R35 net5.00E+04119.21.165.933.310.86 </td <td>R20 Het</td> <td>4.303</td> <td>3.370</td> <td>5.201</td> <td>7.092</td> <td>0.149</td> <td>9.190</td> <td>4.243</td> <td>2.000</td> <td>0.009</td> <td>$Full (abcu) \rightarrow Mal (abcu)$</td>	R20 Het	4.303	3.370	5.201	7.092	0.149	9.190	4.243	2.000	0.009	$Full (abcu) \rightarrow Mal (abcu)$
R27 exch1.2030.98571.433.2452.5363.9631.4180.10383.661Mal (abcd) \rightarrow OAA (abcd)R27 exch229.851.04Inf0.883605.3861.00E+0723.16InfMal (abcd) \rightarrow OAA (abcd)R284.3143.2685.38314.958.47122.0821.8911.7230.39Cl (abcdef) \rightarrow ACCOA.c (ed) \rightarrow CAA (fcba)R300.10590.085410.12640.10550.084950.12550.10580.085350.1256Pyr.m (abc) + Glu (defgh) \rightarrow Ala (abc) + AKG (defgh)R310.12710.10250.15170.12660.10190.15060.12690.10240.1507OAA (abcd) + Glu (defgh) \rightarrow Ala (abc) + AKG (defgh)R320.11910.096490.007760.1009 $-2.62E-66$ 00.23253PG (abc) + Glu (defgh) \rightarrow AKG (defgh)R33 excl0.0089880.0034550.01330.0072860.001450.0175500.0221Ser (abc) \rightarrow Glu (abcde)R347.1926.1588.137.6336.1219.1886.7875.7447.707Gln (abcde) \rightarrow Glu (abcde)R35 excl0.054450.01330.0072860.0036150.011450.0775500.921Ser (abc) \rightarrow Glu (abcde)R361.00E-070Inf10.0E-070InfGlu (abcde) \rightarrow Glu (abcde)Glu (abcde)R35 excl0.05450.01430.0072860.0036150.01450.0775500.921MEETHF (c)	R26 excn	1.00E+07	92.46	Int	6.795	3.559	13.24	38.77	19.74	134	$Fum(abcd) \leftrightarrow Mai(abcd)$
R27R	R27 net	1.203	0.9857	1.43	3.245	2.536	3.963	1.418	0.1038	3.661	Mal (abcd) \rightarrow OAA (abcd)
R284.3143.2685.38314.958.47122.0821.8911.7230.39Cit (abcde) \rightarrow ACCoAc. (ab) \rightarrow CAC (ab)CAC(Ab)C	R27 exch	229.8	51.04	Inf	0.8836	0	5.386	1.00E+07	23.16	Inf	Mal (abcd) ↔ OAA (abcd)
R294.3143.2685.38314.958.47122.0821.8911.7230.39AcCoA.c (ab) \rightarrow FA (ab)R300.10590.085410.12640.10550.084950.12550.10580.085350.1266Pyr.m (abc) + Glu (deff) \rightarrow Ala (abc) + AKG (defgh)R310.12710.10260.10190.15060.12690.10240.507OAA (abcd) + AkG (defgh) \rightarrow Asp (abcd) + AKG (defgh)R320.11910.096490.14250.084290.067760.1009-2.62E-0600.23523PG (abc) \rightarrow Glu (defgh) \rightarrow Ser (abc) \rightarrow AKG (defgh)R33net0.0099860.0093520.0072860.0014890.0093550.17840InfSer (abc) \rightarrow Glu (abcde)R347.1926.1588.137.6336.1219.1886.7875.7447.707Gln (abcde) \rightarrow Glu (abcde)R35net2.6571.7553.4233.6582.1235.2722.7191.5763.503Glu (abcde) \rightarrow AKG (abcde)R361.00E-0703.2391.58104.415-2.97E-0504.973PSP (abcde) \rightarrow NE (abcde)R370.089980.0053450.01330.0072860.0036150.014430.0775500.921MEETHF (a) MEETHF (a)R380.068670.55010.08260.07100.056150.084432.24E-0600.9225Gly (ab)R380.0699760.0059060.014710.042590.033940.051660.1974	R28	4.314	3.268	5.383	14.95	8.471	22.08	21.89	11.72	30.39	Cit (abcdef) \rightarrow AcCoA.c (ed) + OAA (fcba)
R300.10590.085410.12640.10550.084950.12550.10580.085350.1256Pyr.m (abc) + Glu (defgn) \rightarrow Ala (abc) + AKG (defgn)R310.12710.10250.15170.12660.10190.15060.12690.10240.1507OA(abcd) + Glu (defgn) \rightarrow Asg (abcd) + AKG (defgn)R320.11910.096490.14250.084290.067760.1009-2.62E-0600.23523PG (abc) - Glu (defgn) \rightarrow Ser (abc) + AKG (defgn)R33nct0.0089880.0008550.01330.0072860.0038150.011450.0775500.0921R347.1926.1588.137.6336.1219.1886.7875.7447.707Glu (abcde) \rightarrow Glu (abcde)R35 exch5.06E+04119.21nf593.3108.61nf1.00E-070InfGlu (abcde) \rightarrow AKG (abcde)R361.00E+0703.2391.58104.415-2.97E-0504.973PSF (abcde) \rightarrow NFE (abcde)R380.068670.059060.014710.042590.033440.051660.197400.02344R390.0099760.0059060.014710.042590.033940.051660.197400.2344R441010.68370.484701PSF (abcde) \rightarrow PSF nut (abcde)R441010.68370.48470.4863010.612 (abcd) \rightarrow PSF nut (abcde)R440.2130.19730.	R29	4.314	3.268	5.383	14.95	8.471	22.08	21.89	11.72	30.39	AcCoA.c (ab) \rightarrow FA (ab)
R310.12710.10250.15170.12660.10190.15060.12830.010240.1507OAA (abcd) + Glu (defgh) \rightarrow Asp (abcd) + AKG (defgh)R320.11910.098490.14250.084290.067760.1009-2.62E-0600.23523PG (abc) + Glu (defgh) \rightarrow Asp (abcd) + AKG (defgh)R33net0.0089860.0098550.001330.0072860.0036150.011450.0775500.0921Ser (abc) \rightarrow Glu (abcd) \rightarrow AKG (defgh)R347.1926.1588.137.6336.1219.1886.7875.7447.077Glu (abcde) \rightarrow Glu (abcde)R35net2.6571.7553.4233.6582.1235.2722.7191.5763.503Glu (abcde) \rightarrow AKG (abcde)R35net5.00E+04119.2Inf593.3108.6Inf1.00E-070InfGlu (abcde) \rightarrow AKG (abcde)R361.00E-0703.2391.58104.4152.97E-0504.973P5P (abcde) \rightarrow AKG (abcde)R370.008980.0053450.01330.0072860.036150.011450.0775500.0925Gly, (ab) \rightarrow MEETHF (a)R380.068670.055010.08260.07010.056150.084432.42E-0600.0254Ser, (abc) \rightarrow Ser (abc)R40101101101P5P (abcde) \rightarrow P5P:nnt (abcde)R411E-07010.8587011E-07	R30	0 1059	0.08541	0 1264	0 1055	0.08495	0 1255	0 1058	0.08535	0 1256	Pvr m (abc) + Glu (defgh) \rightarrow Ala (abc) + AKG (defgh)
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	D31	0 1271	0 1025	0.1517	0.1266	0 1010	0.1506	0.1260	0 1024	0.1507	OAA (abcd) + Glu (afabi) + Aca (abcd) + AKG (afabi)
R320.11910.096490.14250.042490.007760.1009-2.62E-0600.23223PG (abc) + GB (abc) + GB (abc) + AFG (abc) + GB (abc)	D22	0.1271	0.1025	0.1405	0.1200	0.1013	0.1000	0.1200	0.1024	0.1307	2PC(abc) + Clu(defeb) = Car(abc) + A(C(defeb))
R33net0.0089980.0053450.017330.0072860.0038150.017450.0775500.0921Ser (abc) \rightarrow Gly (ab) + MEETHF (c)R347.1926.1588.137.6336.1219.1886.7875.7447.707Gln (abcde) \rightarrow Gly (ab) + MEETHF (c)R35net2.6571.7553.4233.6582.1235.2722.7191.5763.503Glu (abcde) \rightarrow AKG (abcde)R35net2.6571.7553.4233.6582.1235.2722.7191.5763.503Glu (abcde) \rightarrow AKG (abcde)R361.00E-0703.2391.58104.415-2.97E-0504.973P5P (abcde) \rightarrow MTC (abcde)R370.0089980.0053450.01330.0072860.0036150.014450.0775500.0921MEETHF (a) \rightarrow MEETHF.x (a)R380.068670.055010.08260.0710.056150.084432.42E-0600.0923Gly (abcde) \rightarrow NTC (abcd)R401010.411301101P5P.dli (abcde) \rightarrow P5P.mnt (abcde)R411E-07010.4858701101P5P.dli (abcde) \rightarrow P5P.mnt (abcde)R430.77870.5120.80270.55110.62370.4361010GLP.dli (abcd) \rightarrow SP.mnt (abcde)R440.22130.19730.4880.43630.37630.44840.5639010.GLP.dli (abcd) \rightarrow	RJZ	0.1191	0.09049	0.1425	0.06429	0.00770	0.1009	-2.02E-00	0	0.2352	$3FG(abc) + Giu(deigii) \rightarrow 3ei(abc) + ArG(deigii)$
R33ch. 0.0048460.0008950.00089520.0052850.0014890.0093850.17840InfSer (abc) \leftrightarrow (abc) \rightarrow (u (abc) \rightarrow (dbc) (abc)R347.1926.1588.137.6336.1219.1886.7875.7447.707Gln (abcde) \rightarrow Glu (abcde)R35net2.6571.7553.4233.6582.1235.2722.7191.5763.503Glu (abcde) \rightarrow AKG (abcde)R361.00E-0701.01593.3108.6Inf1.00E-070InfGlu (abcde) \rightarrow NTP (abcde)R370.008980.0053450.01330.0072860.0036150.014432.42E-0600.0921MEETHF (a) \rightarrow MEETHF x (a)R380.068670.055010.08260.07010.056150.084432.42E-0600.02344Ser, (abc) \rightarrow SP (abcde) \rightarrow PSP.mnt (abcde)R401010.411301101PSP (abcde) \rightarrow PSP.mnt (abcde)R411E-07010.85870.51510.62370.4361010 PSP (abcde) \rightarrow PSP.mnt (abcde)R440.22130.19730.4880.43630.37630.48490.5639010 GLP (abc) \rightarrow SU.mnt (abcc)R470.39390.36440.4230.51930.43460.60550.8130.778610 GLP (abc) \rightarrow SU.mnt (abcc)R460.60610.5770.63560.48070.39450.56540.1870.14240.2232<	R33 net	0.008998	0.005345	0.0133	0.007286	0.003615	0.01145	0.07755	0	0.0921	Ser (abc) \rightarrow Gly (ab) + MEETHF (c)
R347.1926.1588.137.6336.1219.1886.7875.7447.707Gln (abcde) \rightarrow Glu (abcde)R35net2.6571.7553.4233.6582.1235.2722.7191.5763.503Glu (abcde) \rightarrow AKG (abcde)R35exch5.00E+04119.2Inf593.3108.6Inf1.00E-070InfGlu (abcde) \rightarrow AKG (abcde)R361.00E-0703.2391.58104.415-2.97E-0504.973P5P (abcde) \rightarrow NTP (abcde)R370.008980.0053450.01330.0072860.0036150.011450.0775500.0921MEETHF (a) \rightarrow MEETHF x(a)R380.068670.055010.08260.07010.056150.084432.42E-0600.0925Gly.p (ab) \rightarrow Gly (ab)R390.0099760.0059060.014710.042590.033940.051660.197400.2344Ser.p (abc) \rightarrow Ser (abc)R411E-07010.1413011010P5P (abcde) \rightarrow P5P.mnt (abcde)R430.77870.5120.80270.56370.51510.62370.48490.5639010EP.dil (abcd) \rightarrow SU.mnt (abcd)R440.22130.19730.4880.43630.37630.48490.5639010EP.dil (abcd) \rightarrow SU.mnt (abcd)R440.22130.19730.4880.43630.37630.84890.5639010EP.dil (abcd) \rightarrow S	R33 exch	0.004846	0.000985	0.008932	0.005285	0.001489	0.009385	0.1784	0	Inf	Ser (abc) \leftrightarrow Gly (ab) + MEETHF (c)
R35 net2.6571.7553.4233.6582.1235.2722.7191.5763.503Glu (abcde) → AKG (abcde)R35 exch5.00E+04119.2Inf593.3108.6Inf1.00E+070InfGlu (abcde) → AKG (abcde)R361.00E+0703.2391.58104.415-2.97E+0504.973P5P (abcde) → NTP (abcde)R370.008980.0053450.01330.0072660.036150.011450.0775500.921MEETHF (a) → MEETHF (a)R380.068670.055010.08260.07010.056150.084432.42E+0600.02344Ser.p (abc) → Ser (abc)R401010.141301101P5P (abcde) → P5P:mnt (abcde)R411E+07010.8587011E+07010 GLP (abc) → GLP:mnt (abcde)R440.22130.19730.4880.43630.37630.48490.5639010 GLP (abc) → GLP:mnt (abcc)R470.39390.36440.4230.51930.43460.60550.8130.776810 Suc (abcd) → Suc.mnt (abcd)R4910110.928710.948500.21420.2232Suc (abcd) → Suc.mnt (abcd)R470.39390.36440.4230.51930.43460.60550.8130.776810 Suc (abcd) → Suc.mnt (abcd)R491010.9287 <td< td=""><td>R34</td><td>7.192</td><td>6.158</td><td>8.13</td><td>7.633</td><td>6.121</td><td>9.188</td><td>6.787</td><td>5.744</td><td>7.707</td><td>$Gin (abcde) \rightarrow Glu (abcde)$</td></td<>	R34	7.192	6.158	8.13	7.633	6.121	9.188	6.787	5.744	7.707	$Gin (abcde) \rightarrow Glu (abcde)$
R35exch5.00E+04119.2Inf593.3108.6Inf1.00E-070InfGlu (abcde) → AKG (abcde)R361.00E-0703.2391.58104.415-2.97E-0504.973P5P (abcde) → NTP (abcde)R370.0089980.0053450.01330.0072860.0036150.011450.0775500.0921MEETHF (a) → MEETHF.x (a)R380.068670.055010.08260.07010.056150.084432.42E-0600.02344Ser.p (abc) → Sfr (abc)R401010.141301101P5P-dil (abcde) → P5P.mnt (abcde)R411E-07010.8587011E-07010.P5P (abcde) → P5P.mnt (abcde)R430.77870.5120.80270.56370.51510.62370.4361010 GLP (abc) → GLP.mnt (abcc)R440.22130.19730.4880.43630.37630.48490.5639010 GLP (abc) → GLP.mnt (abcc)R460.60610.5770.63560.48070.39450.56540.8130.776810 Suc (abcd) → Suc mnt (abcd)R470.30390.36440.4230.51930.43660.60550.8130.776810 Suc (abcd) → Suc mnt (abcd)R4910.8581110.928710.94550.786810 Pyr. (abc) → Pyr.mnt (abcc)R50-8.16E-0500.1149 </td <td>R35 net</td> <td>2.657</td> <td>1.755</td> <td>3.423</td> <td>3.658</td> <td>2.123</td> <td>5.272</td> <td>2.719</td> <td>1.576</td> <td>3.503</td> <td>Glu (abcde) \rightarrow AKG (abcde)</td>	R35 net	2.657	1.755	3.423	3.658	2.123	5.272	2.719	1.576	3.503	Glu (abcde) \rightarrow AKG (abcde)
R361.00E-0703.2391.58104.415-2.97E-0504.973P5P (abcde) → NTP (abcde)R370.0089980.0053450.01330.0072860.0036150.011450.0775500.0921MEETHF (a) → MEETHF.x (a)R380.068670.055010.08260.07010.056150.084432.42E-0600.0925Gly.p (ab) → Gly (ab)R390.0099760.0059060.014710.042590.033940.051660.197400.2344Ser. (abc)R401010.141301101P5P.dil (abcde) → P5P.mnt (abcde)R411E-07010.8587011E-07010P5P (abcde) → P5P.mnt (abcde)R430.77870.5120.80270.56370.51510.62370.4361010EP.(abc) → GLP.mnt (abcd)R440.22130.19730.4880.43630.37630.48490.5639010 GLP.dit (abcd) → Su.mnt (abcd)R460.60610.5770.63560.48070.39450.56540.1870.14240.2232Suc.dit (abcd) → Su.mnt (abcd)R470.39390.36440.4230.51930.43660.60550.8130.77680.5020.502R4910.8851110.928710.94550.786810Pyr.mnt (abcc)R50-8.16E-0500.11491.00E-070 <td< td=""><td>R35 exch</td><td>5.00E+04</td><td>119.2</td><td>Inf</td><td>593.3</td><td>108.6</td><td>Inf</td><td>1.00E-07</td><td>0</td><td>Inf</td><td>Glu (abcde) ↔ AKG (abcde)</td></td<>	R35 exch	5.00E+04	119.2	Inf	593.3	108.6	Inf	1.00E-07	0	Inf	Glu (abcde) ↔ AKG (abcde)
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	R36	1 00E-07	0	3 239	1 581	0	4 4 1 5	-2 97E-05	0	4 973	P5P (abcde) \rightarrow NTP (abcde)
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	D37	0.008008	0.005345	0.0133	0.007286	0.003615	0.01145	0.07755	0	0.0021	
R380.086670.05010.08260.07010.050150.084432.42E-0600.0926Gly, f(ab) - Gly (ab)R390.0099760.0059060.014710.042590.033940.051660.197400.2344Ser (abc)R401010.141301101P5P.dil (abcde) \rightarrow P5P.mnt (abcde)R411E-07010.8587011E-07010P5P (abcde) \rightarrow P5P.mnt (abcde)R430.77870.5120.80270.56370.51510.62370.436101GLP.dil (abcd) \rightarrow GLP.mnt (abcd)R440.22130.19730.4880.43630.37630.48490.5639010 GLP (abc) \rightarrow GLP.mnt (abcd)R460.60610.5770.63560.48070.39450.56540.1870.14240.2232Suc.dil (abcd) \rightarrow Suc.mnt (abcd)R470.39390.36440.4230.51930.43460.60550.8130.77680.85760 Suc (abcd) \rightarrow Suc.mnt (abcd)R4910.8851110.928710.94550.786810 Pyr.c (abc) \rightarrow Pyr.mnt (abc)R50-8.16E-0500.11491.00E-0700.071290.0544800.21320 Pyr.m (abc) \rightarrow Pyr.mnt (abc)	R37	0.006996	0.005345	0.0133	0.007260	0.003015	0.01143	0.07755	0	0.0921	$MEETHF(a) \rightarrow MEETHF.X(a)$
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	R38	0.06867	0.05501	0.0826	0.0701	0.05615	0.08443	2.42E-06	0	0.0925	$Giy.p(ab) \rightarrow Giy(ab)$
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	R39	0.009976	0.005906	0.01471	0.04259	0.03394	0.05166	0.1974	0	0.2344	Ser.p (abc) \rightarrow Ser (abc)
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	R40	1	0	1	0.1413	0	1	1	0	1	P5P.dil (abcde) \rightarrow P5P.mnt (abcde)
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	R41	1E-07	0	1	0.8587	0	1	1E-07	0	1	0 P5P (abcde) → P5P.mnt (abcde)
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	R43	0.7787	0.512	0.8027	0.5637	0.5151	0.6237	0.4361	0	1	$GLP.dil(abc) \rightarrow GLP.mnt(abc)$
R460.60610.5770.63560.48070.39450.56540.1870.14240.2232Suc.dil (abcd) \rightarrow Suc.mnt (abcd)R470.39390.36440.4230.51930.43460.60550.8130.77680.85760 Suc (abcd) \rightarrow Suc.mnt (abcd)R4910.8851110.928710.94550.786810 Pyr.c (abc) \rightarrow Pyr.mnt (abc)R50-8.16E-0500.11491.00E-0700.071290.0544800.21320 Pyr.m (abc) \rightarrow Pyr.mnt (abc)	R44	0 2213	0 1973	0 488	0 4363	0.3763	0 4849	0.5639	0	1	$0 \text{ GLP} (abc) \rightarrow \text{GLPmnt} (abc)$
R47 0.3939 0.3644 0.423 0.5193 0.4346 0.6055 0.813 0.7768 0.8276 0 Suc (abcd) \rightarrow Suc mnt (abcd) R49 1 0.8851 1 1 0.9287 1 0.9455 0.7868 1 0 Pyr.mt (abcd) R50 -8.16E-05 0 0.1149 1.00E-07 0 0.07129 0.05448 0 0.2132 0 Pyr.m (abc) \rightarrow Pyr.mt (abc)	D/6	0.6061	0.577	0.6356	0.4907	0.3045	0.5654	0.197	0 1424	0 2222	Suc dil (abcd) Suc mat (abcd)
rx47 0.3959 0.3044 0.423 0.5193 0.4346 0.0055 0.813 0.768 0.8576 0 SUc (abcd) \rightarrow Suc mnt (abcd) R49 1 0.8851 1 1 0.9287 1 0.9455 0.7868 1 0 Pyr.c (abc) \rightarrow Syr.mnt (abc) R50 -8.16E-05 0 0.1149 1.00E-07 0 0.07129 0.05448 0 0.2132 0 Pyr.m (abc) \rightarrow Pyr.mnt (abc)	D47	0.0001	0.077	0.0000	0.4007	0.3943	0.0004	0.107	0.1424	0.2232	Suctor (abod) \rightarrow Sucting (abod)
R4910.8851110.928710.94550.786810Pyr.m((abc) \rightarrow Pyr.mnt (abc)R50-8.16E-0500.11491.00E-0700.071290.0544800.21320 Pyr.m (abc) \rightarrow Pyr.mnt (abc)	K4/	0.3939	0.3644	0.423	0.5193	0.4346	0.6055	0.813	0.7768	0.8576	$v \operatorname{Suc}(\operatorname{abcu}) \rightarrow \operatorname{Suc.mnt}(\operatorname{abcu})$
<u>R50</u> -8.16E-05 0 0.1149 1.00E-07 0 0.07129 0.05448 0 0.2132 0 Pyr.m (abc) → Pyr.mnt (abc)	R49	1	0.8851	1	1	0.9287	1	0.9455	0.7868	1	$U Pyr.c (abc) \rightarrow Pyr.mnt (abc)$
	R50	-8.16E-05	0	0.1149	1.00E-07	0	0.07129	0.05448	0	0.2132	$0 \text{ Pyr.m (abc)} \rightarrow \text{Pyr.mnt (abc)}$

 \rightarrow indicates net flux: $(\upsilon_{F} \cdot \upsilon_{R})$; \leftrightarrow 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.

Mass isotopomer abundances	[1,2-13C2]gluc	:+[U-13C5]gIn	[1-13C]gluc+	[U-13C6]gluc	[U-13C5]gln		
lon	Average	SEM	Average	SEM	Average	SEM	
Pvr 174 (M0)	42 66%	0.50%	24 31%	0.50%	83 21%	0.50%	
$P_{\rm Vr} = 175 (M1)$	7 61%	0.50%	21.01%	0.50%	10 72%	0.50%	
P yr_173 (M1)	1.0170	0.30%	21.9070	0.50%	10.7270	0.50%	
Pyr_176 (IVI2)	40.85%	0.50%	0.32%	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%	
$Lac_{235} (M2)$	30 38%	0.50%	12 38%	0.50%	8 0/1%	0.50%	
$Lac_{235}(M2)$	7 5 4 0/	0.50%	42.0070 9.000/	0.50%	1 220/	0.50%	
	7.54%	0.50%	0.92%	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%			
	26.00%	0.50%	21.80%	0.50%	69.920/	0.50%	
	30.00%	0.50%	31.09%	0.50%	00.03%	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)	10.220/0	0.50%	20.95%	0.50%	16 50%	0.50%	
	10.22%	0.50%	20.0076	0.50%	7.000/	0.50%	
	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%			
Glv 218 (M0)	71.02%	0.50%	72.43%	0.50%	76.06%	0.50%	
Glv 219 (M1)	19 21%	0.50%	18 69%	0.50%	15 94%	0.50%	
$G_{\rm M} = 220 ({\rm M}2)$	7 32%	0.50%	7 17%	0.50%	6 68%	0.50%	
G_{1y}_{220} (M_{2})	7.32 /0	0.00%	1.11/0	0.50%	0.0076	0.50%	
GIY_221 (1013)	2.31%	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%	
Glv 247 (M1)	20.35%	0.59%	16.36%	0.50%	16.55%	0.50%	
Glv 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%	
0	44.500/	1.000/	04.070/	0.50%	00 50%	0.50%	
Suc_289 (MU)	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 (MO)	7 700/	0.50%	52 010/	0.50%	10 520/	0.600/	
Fure 200 (M4)	1.1270	0.50%	00.01%	0.50%	19.02%	0.00%	
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%	
Sor 280 (M1)	1/ /50/	0.50%	20.00/0	0.50%	10.000/	0.50%	
Ser 200 (M2)	14.40%	0.30%	22.01%	0.50%	13.2270	0.00%	
	33.91%	0.70%	29.05%	0.50%	1.00%	0.50%	
	0.23%	0.50%	7.15%	0.50%	1.22%	0.50%	
Ser 292 (M4)	2.93%	0.50%	2.65%	0.50%			

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

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

Mass isotopomer abundances	[1.2-13C2]aluc	:+[U-13C5]aln	[1-13C]aluc+l	U-13C6]aluc	[U-13C5]gin		
lon	Δverage	SEM	Δverage	SFM	Δverage	SEM	
Sor 202 (M0)	12 620/	1.07%	AVCIUGC 46.420/	0.50%	71 220/	0.50%	
Ser_302 (M1)	40.00%	0.959/	40.4376	0.50%	10 720/	0.50%	
$Set_{303}(NT)$	40.33%	0.00%	14.00%	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)	1.21 /0	0.0070	2 9/%	0.50%			
			2.3470	0.30 /8			
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%	0.00%	
Akg 252 (M6)	12 000/	0.50%	1.2170	0.0070	10 00%	0.5270	
Akg_352 (100)	12.00%	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_125 (M6)	6.64%	0.50%	0.84%	0.50%	1 13%	0.50%	
$M_{01} = 425 (M_{0})$	1 200/	0.50%	0.0476	0.3078	4.43%	0.50%	
Wai_420 (W7)	1.5976	0.3078			0.9478	0.3078	
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%	
$A_{cp} = 305 (M3)$	1/ 28%	0.50%	3 310/	0.50%	10 21%	0.00%	
$Asp_305 (M3)$	F 460/	0.50%	0.05%	0.50%	2 960/	0.50%	
Asp_300 (M4)	5.40%	0.50%	0.95%	0.50%	3.00%	0.30%	
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%		0.0070		010070	
App. (119 (MO)	6.26%	0.50%	46 210/	0 50%	10.070/	0 500/	
ASp_418 (INU)	0.30%	0.50%	40.31%	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 1/1%	0.50%	6 03%	0.50%	
Glu 232 (M2)	7 /00/	0.50%	16 100/	0.50%	15 100/	0.50%	
Giu_{332} (IVIZ)	7.4U%	0.00%	10.40%	0.50%	13.13%	0.00%	
Giu_333 (IVI3)	5.91%	0.50%	0.40%	0.50%	0.75%	0.50%	
	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%	

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

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

Mass isotopomer abundances	[1.2-13C2]alua	:+[U-13C5]aln	[1-13C]aluc+	[U-13C6]aluc	[U-13C5]aln		
lon	Average	SEM	Average	SEM	Average	SEM	
Glu 432 (M0)	1 75%	0.50%	47 02%	0.50%	7 18%	0.50%	
$G_{\rm H}$ (M0)	1.70%	0.50%	21 75%	0.50%	5.26%	0.50%	
$Glu_{433}(M1)$	2.16%	0.50%	21.73/0	0.50%	5.20%	0.50%	
GIU_434 (M2)	2.16%	0.50%	17.09%	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%	
	1.0170	0.0070			1.57 /0	0.3070	
Clp 421 (M0)	0 45%	0 50%	62 55%	0 50%	0 020/	0 50%	
$G_{11} = 432$ (M0)	0.43%	0.00%	02.0070	0.50%	0.0376	0.50%	
GIN_432 (MT)	0.17%	0.50%	23.37%	0.50%	0.34%	0.50%	
GIn_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 (M8)	2.09%	0.50%			2.09%	0.50%	
$C_{10} = 440 (M0)$	2.0370	0.50%			2.0370	0.50%	
GIN_440 (M9)	0.50%	0.50%			0.40%	0.50%	
Cit 450 (MC)	0.000/	0 500/	00.000/	0 500/	40.000/	0 500/	
	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 05%	0.50%	8 03%	0.50%	
	23.00 /0	0.00%	1.3376	0.50%	0.03 %	0.50%	
	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)	10.83%	0.50%	11 00%	0.50%	25.04%	0.50%	
$Cit_{595}(MF)$	19.00%	0.50%	6.020/	0.50%	23.04 /0	0.50%	
	23.90%	0.50%	0.02%	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%	
Glv3P 358 (M1)	15.93%	1.50%	20.60%	1.50%	19.29%	1.50%	
Glv3P_359 (M2)	22 31%	1 50%	23 68%	1 50%	10 30%	1 50%	
Gly3P 360 (M3)	6 28%	1.50%	£ 330%	1.50%	2 250/	1 50%	
Chop 204 (M4)	0.20%	1.00%	0.3376	1.50%	2.23 /6	1.50%	
Giyər_ədi (1V14)	3.38%	1.0U%	2.74%	1.50%	0.75%	1.50%	
	40.0407	0 500/	44.000/	1 500/		1 500/	
GIV3P_445 (INU)	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)	20 25%	0.70%	20 /20/	1 50%	Q 57%	1 50%	
DED 260 (M2)	23.00%	0.73/0	10 000/	1.50%	J.J1 /0	1.50%	
	8.03%	0.59%	10.63%	1.50%	2.47%	1.50%	
K5P_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%	

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

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-6phosphate, F6P, dihydroxyacetone phosphate, DHAP; glyceraldehyde phosphate, GAP; glycerol-3phosphate, GLP; 3-phosphoglycerate, 3PG; pentose-5-phosphate, P5P; erythrose-4-phosphate, E4P; sedoheptulose-7-phosphate, S7P

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