Supplementary Information

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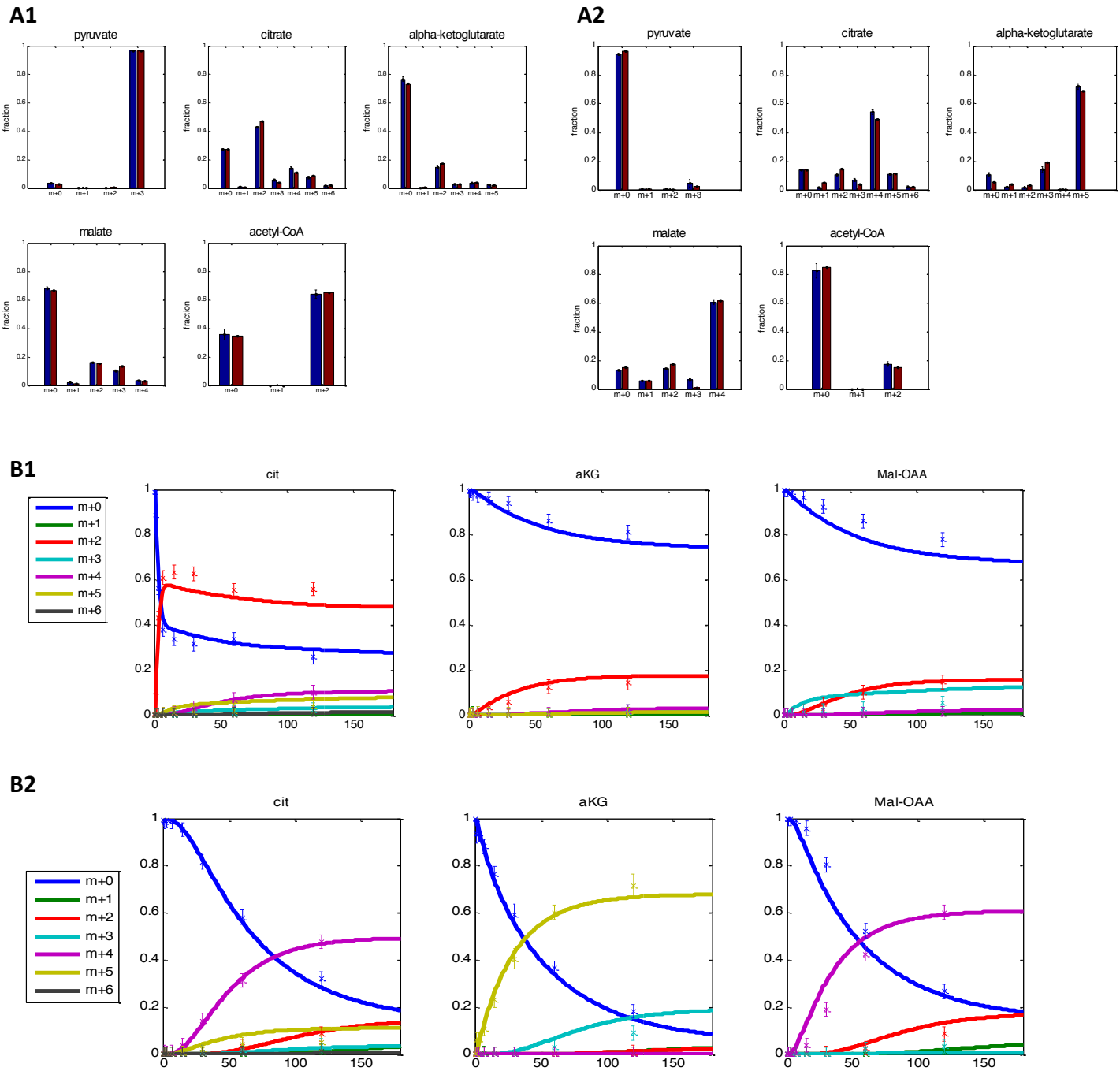
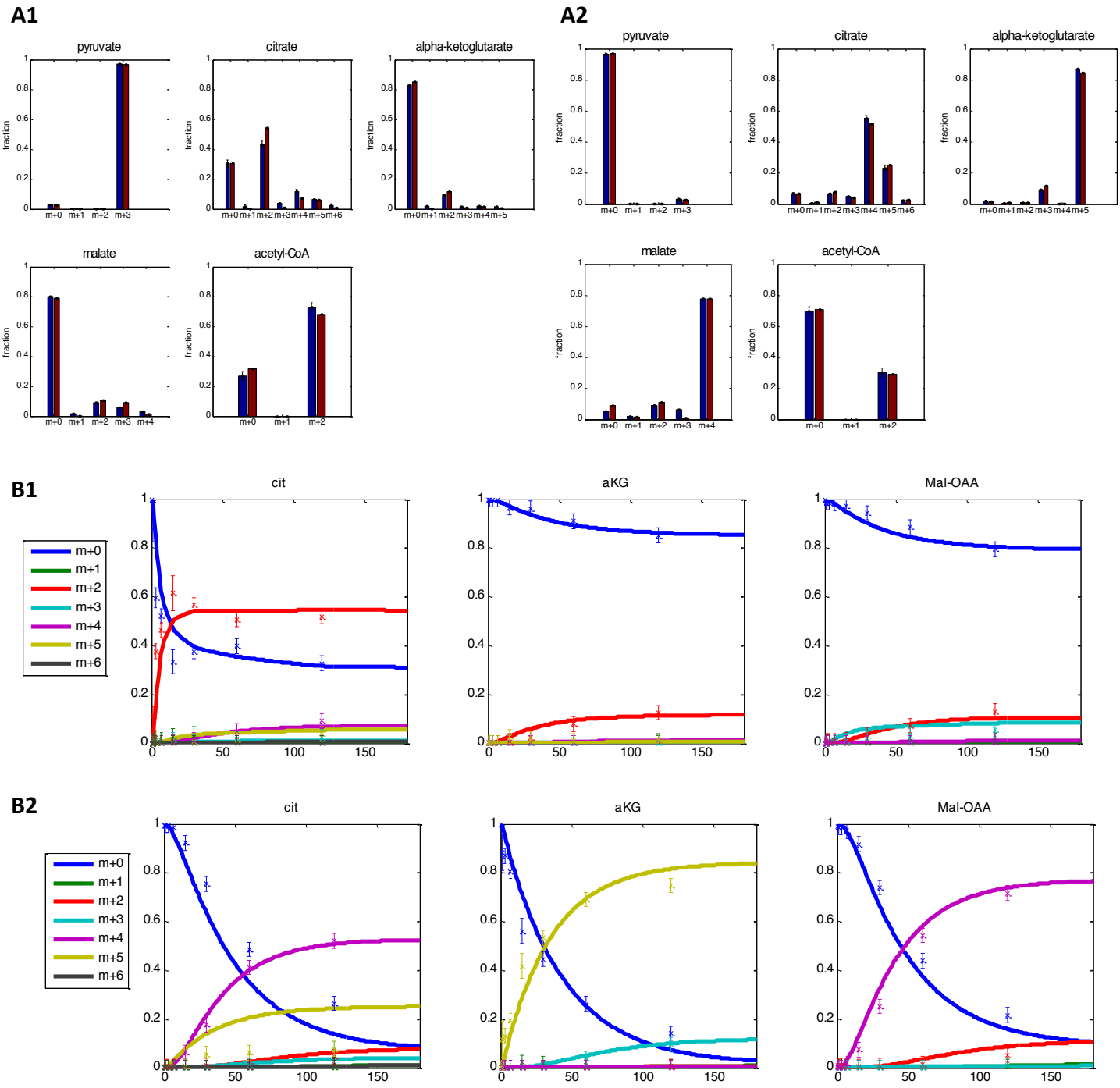
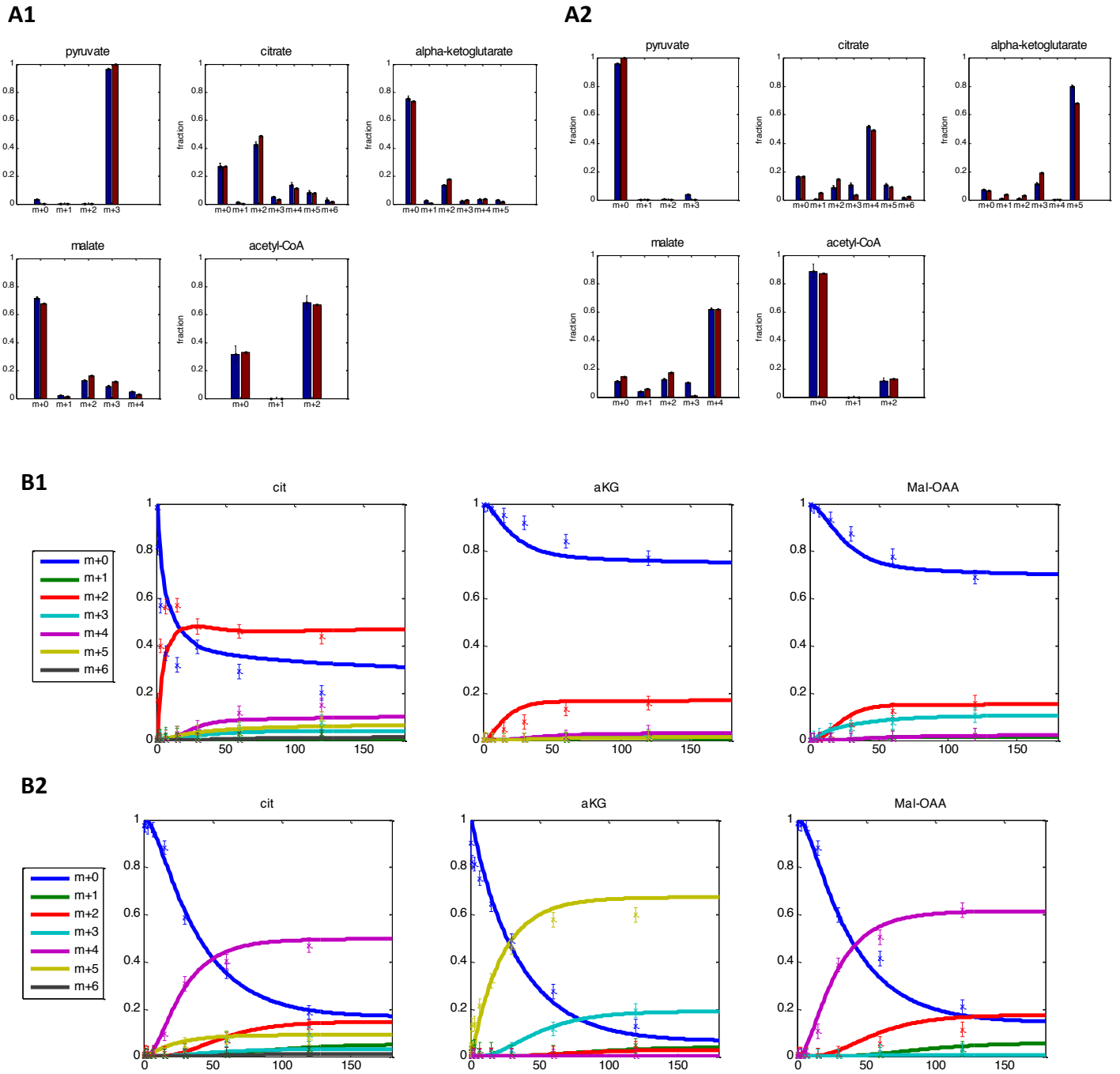


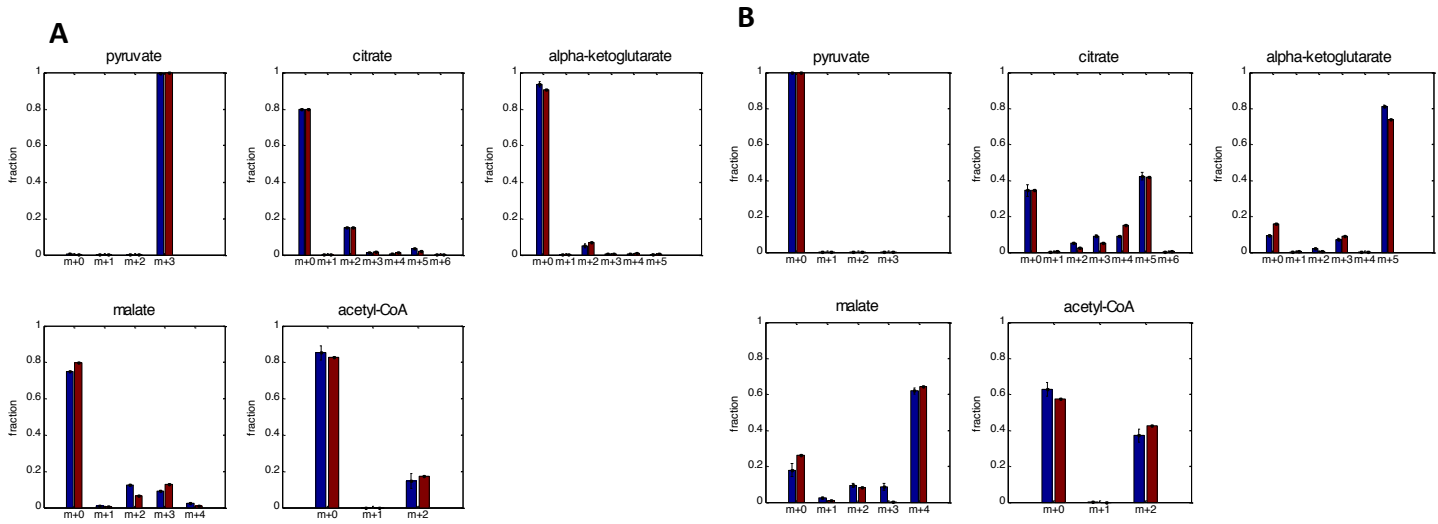
Supplementary Figure 1. Metabolite labeling patterns in parental cell line in normoxia. (A) Measured (blue bars) and simulated (red bars) steady-state labeling patterns for indicated metabolites after 72 h of cell growth in U-^{13}C-glucose (A1) or U-^{13}C-glutamine (A2). (**B**) Kinetic flux profiling after switch to U-^{13}C-glucose (B1) or U-^{13}C-glutamine (B2). Points are experimental data (mean ± 2 SD, N=2-4) and lines are simulation output.



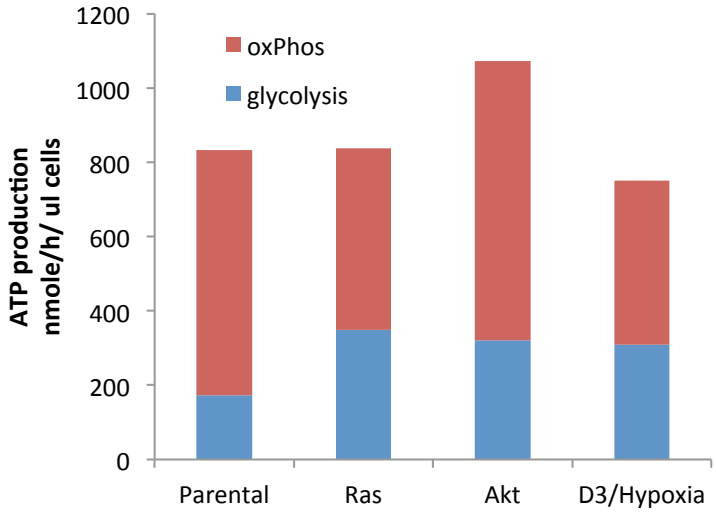
Supplementary Figure 2. Metabolite labeling patterns in Ras cell line in normoxia. (A) Measured (blue bars) and simulated (red bars) steady-state labeling patterns for indicated metabolites after 72 h of cell growth in U-^{13}C-glucose (A1) or U-^{13}C-glutamine (A2). (**B**) Kinetic flux profiling after switch to U-^{13}C-glucose (B1) or U-^{13}C-glutamine (B2). Points are experimental data (mean ± 2 SD, N=2-4) and lines are simulation output.



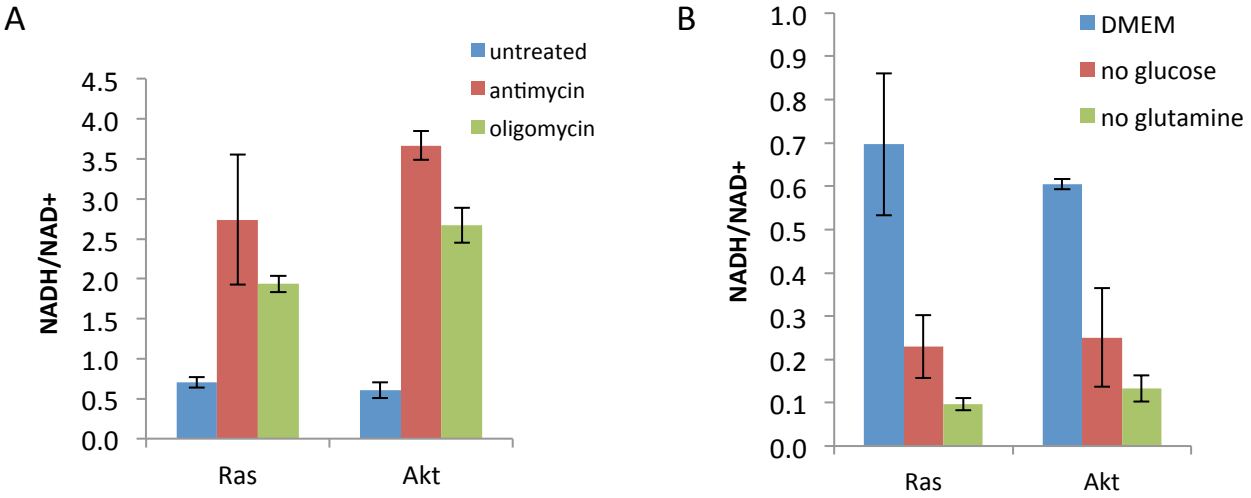
Supplementary Figure 3. Metabolite labeling patterns in Akt cell line in normoxia. (A) Measured (blue bars) and simulated (red bars) steady-state labeling patterns for indicated metabolites after 72 h of cell growth in U-^{13}C-glucose (A1) or U-^{13}C-glutamine (A2). (**B**) Kinetic flux profiling after switch to U-^{13}C-glucose (B1) or U-^{13}C-glutamine (B2). Points are experimental data (mean ± 2 SD, N=2-4) and lines are simulation output.



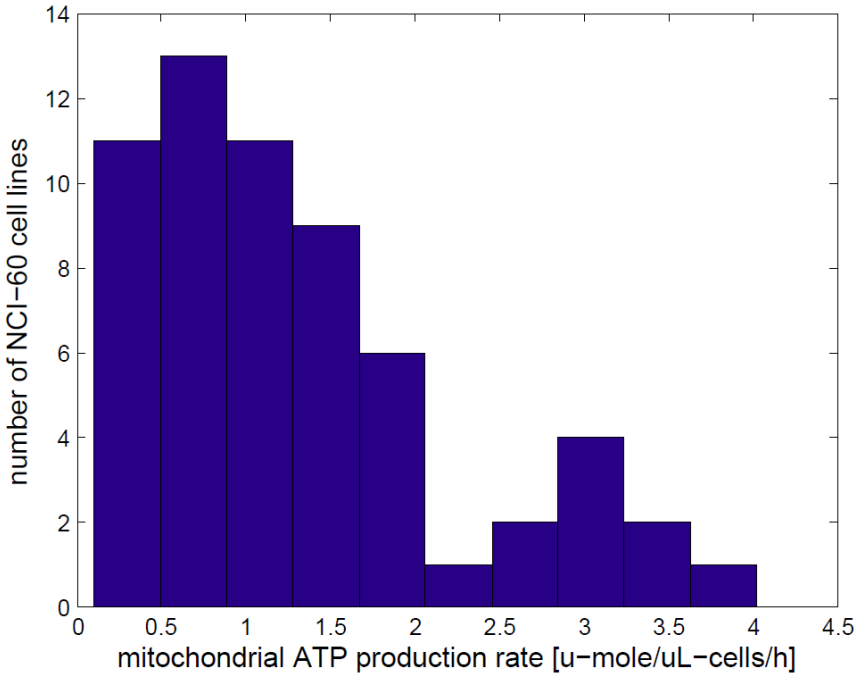
Supplementary Figure 4. Metabolite labeling patterns in parental cell line in hypoxia. Measured (blue bars) and simulated (red bars) steady-state labeling patterns for indicated metabolites after 72 h of cell growth in U-^{13}C-glucose (A) or U-^{13}C-glutamine (B).



Supplementary Figure 5. ATP production rates from oxidative phosphorylation and glycolysis in parental, Ras, Akt iBMK cells in normoxia and parental cells in hypoxia. Oxidative ATP production rate is calculated based on the assumption that all cytosolic NADH are shuttled into mitochondrial through glycerol-phosphate shuttle, thus it is a lower bond of oxidative ATP production.

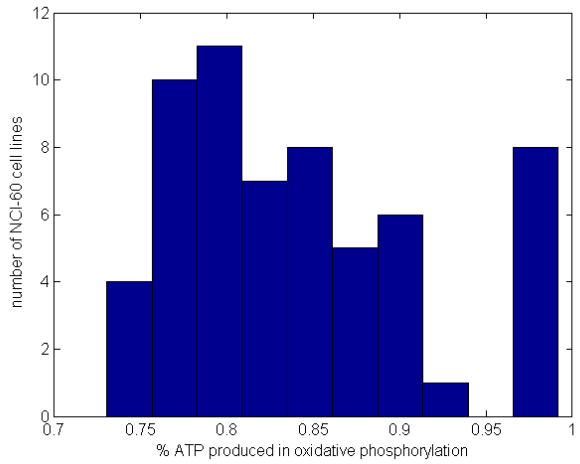


Supplementary Figure 6. (A) Effect of oxidative phosphorylation inhibitors on the NADH/NAD$^+$ ratio in Ras and Akt-drvien cells in normoxia. NADH and NAD$^+$ levels were measured 5 min after addition of vehicle (DMSO), the complex III inhibitor antimycin A (4µg/ml) or the ATP synthase inhibitor oligomycin (8 µg/ml) (mean ± SD of N = 3). (B) NADH/NAD$^+$ ratio in Ras and Akt in normoxia measured after switching cells to complete media or media lacking glucose or glutamine for 8 hours (mean ± SD of N = 3).



Supplementary Figure 7. Rates of ATP production by oxidative phosphorylation across the NCI-60 cell lines predicted based on flux balance analysis using the glycolysis and TCA cycle reaction network shown in Supplementary Table 4 and constrained by experimental data on metabolite uptake and excretion rates from Jain et al.

A



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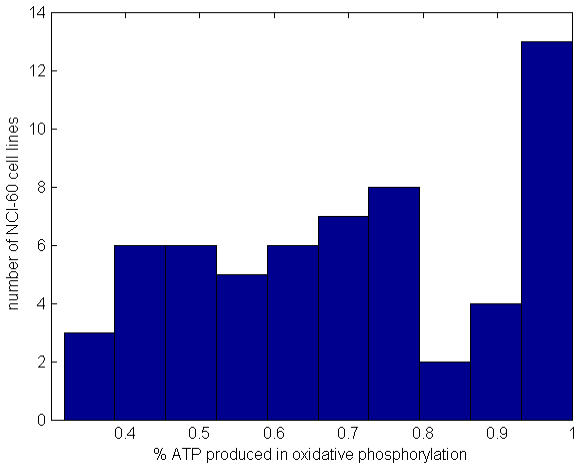
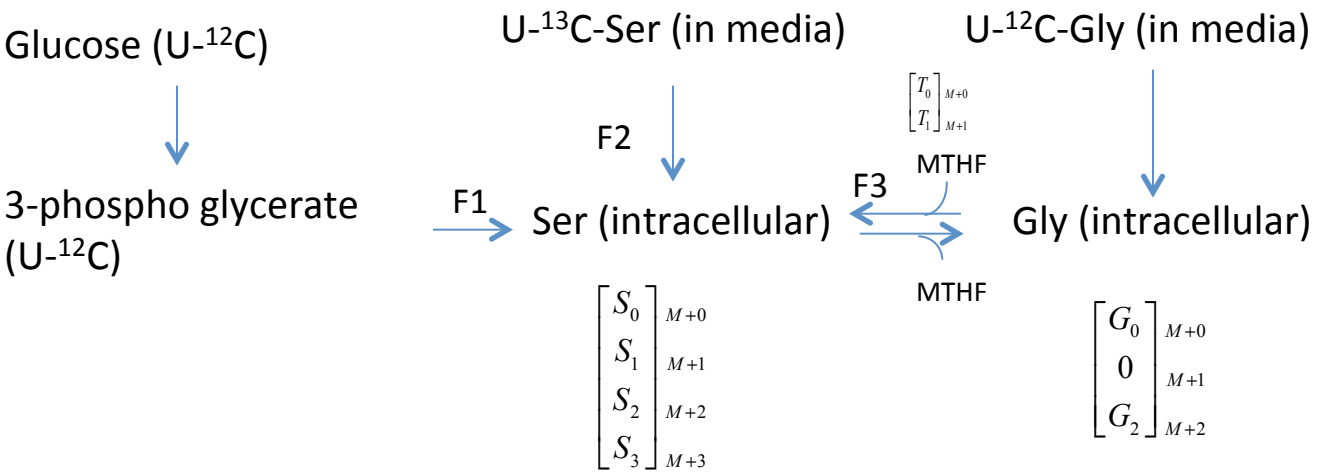


Supplementary Figure 8. Rates of ATP production by oxidative phosphorylation across the NCI-60 cell lines predicted based on flux balance analysis using a genome-scale metabolic model and constrained by experimental data on metabolite uptake and excretion rates from Jain et al. and biomass production rates. (A) With optimization of total ATP production. (B) With constraining oxygen consumption rates to a reasonable range without maximizing ATP production.



Supplementary Figure 9. Model to quantify *de novo* serine synthesis flux.

Supplementary Table 1. Doubling time of iBMK cells

	Parental	Ras	Akt	Parental-hypoxia
doubling time / h	24	22	21	48

Supplementary Table 2. Fatty acid contents in iBMK cells. Fatty acid contents in units of nmole/ µl cells.

	Parental	Ras	Akt	Parental-hypoxia
C16:0	5.5	7.8	8.1	10.2
C16:1	1.6	3.1	2.2	1.3
C18:0	2.8	3.0	4.4	7.8
C18:1	6.9	8.8	8.1	10.7

Supplementary Table 3. Metabolic flux distributions of parental, Ras, Akt cells in normoxia and parental iBMK cells in hypoxia. Fluxes are obtained as described in the Methods. The table shows the best estimate and standard deviation. Fluxes are in units of nmole/ (h * µl cells).

				Fluxes				Fluxes STD			
Reaction	Reaction description	Atom mapping	Constrained	Parental	Ras	Akt	Parental - hypoxia	Parental	Ras	Akt	Parental - hypoxia
F1	1/2 glucose.ext -> pyruvate	abc->abc	1	172.6	349.7	320.2	309.7	7.8	25.2	32.7	0.1
F2	pyruvate + NAD^+ -> acetyl-CoA (m) + CO_2 + NADH	abc->bc + a	0	20.5	10.8	24.5	4.7	<0.1	0.5	0.1	0.2
F3	acetyl-CoA (m) + malate + NAD^+ -> citrate + NADH	abcd+ef->dcbfea	0	26.4	11.3	30.4	8.4	<0.1	0.5	0.1	0.5
F4	citrate + NAD^+ -> alpha-ketoglutarate + CO_2 + NADH	abcdef>-abcde+f	0	30.0	14.8	32.6	35.2	0.1	0.9	0.1	1.1
F5	alpha-ketoglutarate + CO_2 + NADH -> citrate + NAD^+	abcde+f->abcdef	0	5.3	4.9	5.0	20.1	0.6	0.4	0.7	1.3
F6	alpha-ketoglutarate + NAD^+ + FAD -> fumarate + CO_2 + NADH + $FADH_2$	abcde->1/2 bcde + 1/2 edcb	0	77.2	64.6	84.6	57.1	<0.1	1.7	<0.1	<0.1
F7	malate/OAA + NAD^+ -> pyruvate + CO_2 + NADH	abcd -> abc	0	7.2	12.0	<0.1	<0.1	3.8	8.0	<0.1	<0.1
F8	pyruvate + CO_2 + NADH -> malate/OAA + NAD^+	abc + d -> abcd	0	8.5	5.4	7.2	8.0	1.2	1.0	1.3	0.6
F9	citrate -> malate + acetyl-CoA (c).ext	abcdef -> fcba + ed	0	2.1	1.5	4.3	0.2	0.2	0.2	0.4	0.1
F10	other sources (e.g. fatty acid) -> acetyl-CoA (m)	ab -> ab	0	5.9	0.5	5.9	3.7	0.8	0.7	0.9	0.7
F11	glutamine -> alpha-ketoglutarate	abcde -> abcde	1	52.5	54.7	57.0	42.0	<0.1	1.6	<0.1	1.0
F12	pyruvate + NADH -> lactate.ext + NAD^+	abc -> abc	1	150.8	345.6	288.5	297.0	8.8	26.5	33.2	0.3
F13	malate+ NAD^+ -> OAA + NADH	abcd -> abcd	0	54.2	48.1	65.8	56.9	1.0	3.7	1.6	0.7
F14	acetyl-CoA (c) -> biomass		1	2.1	1.5	4.3	0.2	0.2	0.1	0.4	0.1
F15	citrate.ext -> citrate	abcdef -> abcdef	0	0.4	0.1	1.6	6.9	0.8	0.2	1.0	0.5
F16	fumarate <-> malate/OAA	abcd -> 1/2 abcd + 1/2 dcba	0	77.2	64.6	84.6	57.1	<0.1	1.7	<0.1	<0.1
F17	1/2 glucose + NAD -> serine + NADH		1	5.6	4.0	9.6	0.4	0.6	1.2	2.3	<0.1
F18	pyruvate -> pyruvate.ext	abc -> abc	1	17.0	16.0	20.0	12.0	6.1	8.8	7.7	4.6
F19	NADH + 1/2 O2 => NAD	abc -> abc	1	306.2	224.1	351.5	204.5	0.0	6.3	0.0	0.0

Supplementary Table 4. Metabolite concentrations in iBMK cells. In units of nmole/ µl cells.

	Parental		Ras		Akt	
	mean	stdev	mean	stdev	mean	stdev
hexose-phosphate	0.8	0.2	1.2	0.2	0.8	0.03
ribose-phosphate	0.04	0.03	0.05	0.01	0.05	0.01
fructose-1,6-bisphosphate	1.1	0.07	1.5	0.14	1.5	0.33
3-phosphate-glycerate	0.2	0.05	0.5	0.1	0.3	0.06
pyruvate	4.6	0.3	4.4	0.2	5.9	0.2
acetyl-CoA	0.02	0.004	0.02	0.006	0.03	0.01
fumarate	0.3	0.06	0.4	0.06	0.3	0.01
succinate	0.2	0.04	0.2	0.1	0.3	0.03
citrate	0.9	0.02	0.4	0.07	0.9	0.11
malate	0.9	0.2	1.0	0.06	0.9	0.17
aspartate	10.5	0.7	10.3	4.3	10.7	4.9
glutamate	36.0	12	37.0	8	30.0	7
alpha-ketoglutarate	0.4	0.01	0.4	0.13	0.4	0.07

Supplementary Table 5. Constraints for MFA model

	Parental - normoxia		RAS		AKT		Parental - hypoxia	
	Average	Std	Average	Std	Average	Std	Average	Std
Glucose uptake (μmole/μL cells/h)	0.098	0.008	0.183	0.020	0.170	0.020	0.157	0.006
Glutamine uptake (μmole/μL cells/h)	0.040	0.008	0.054	0.005	0.045	0.008	0.037	0.003
Lactate secretion (F12) (μmole/μL cells/h)	0.143	0.014	0.340	0.040	0.270	0.050	0.295	0.011
Pyruvate + alanine secretion (F18) (μmole/μL cells/h)	0.017	0.003	0.016	0.005	0.020	0.005	0.012	0.003
O2 consumption by OxPhos (F19) (nmole/μL cells/h)	170.100	16.200	124.500	16.600	195.300	27.900	113.581	19.567
Demand flux: glucose to ribose (nmole/μL cells/h)	2.900	0.580	3.163	0.633	3.103	0.621	1.450	0.290
Demand flux: proteomic gln/glu/pro (nmole/μL cells/h)	2.846	0.569	3.105	0.621	3.252	0.650	1.423	0.285
Demand flux: acetyl-CoA (F14) (nmole/μL cells/h)	2.077	0.415	1.958	0.392	4.806	0.961	0.200	0.040
Demand flux: serine biosynthesis (F17) (nmole/μL cells/h)	5.430	0.600	3.980	0.840	6.480	1.780	0.430	0.030