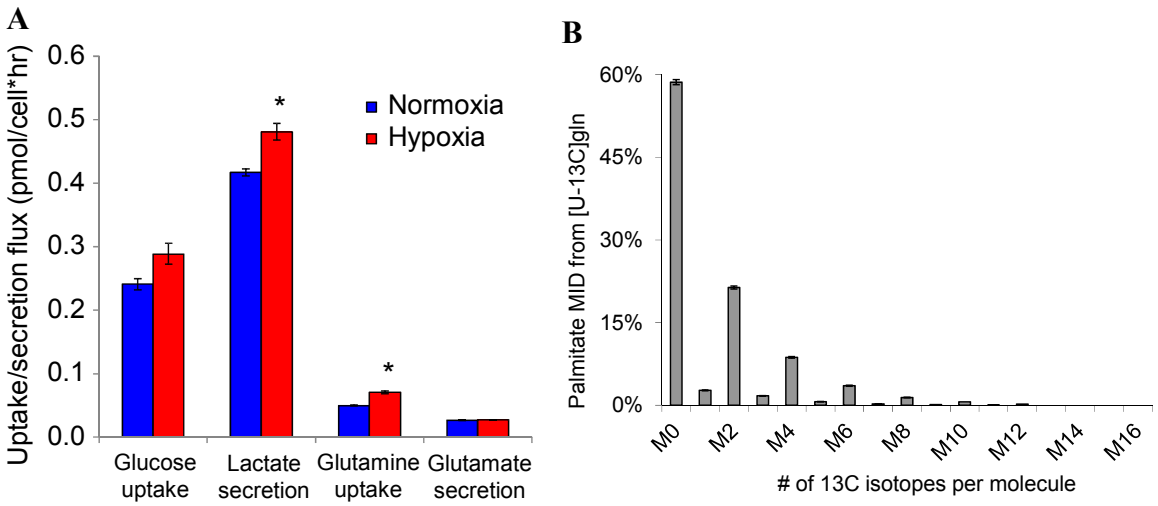


Figure S1. A) Cell-specific uptake and secretion of glucose/glutamine and lactate/glutamate. Metabolite levels were measured at the start and end of culture and normalized to the integral viable cell density to calculate fluxes. Error bars indicate s.e.m. (n=3). * denotes $p < 0.05$. B) Mass isotopomer distribution (MID) of palmitate extracted from total lipids from H460 cells cultured in the presence of [U-$^{13}C_5$]glutamine for 3 days. Significant levels of isotope were detected from glutamine in lipid biomass.

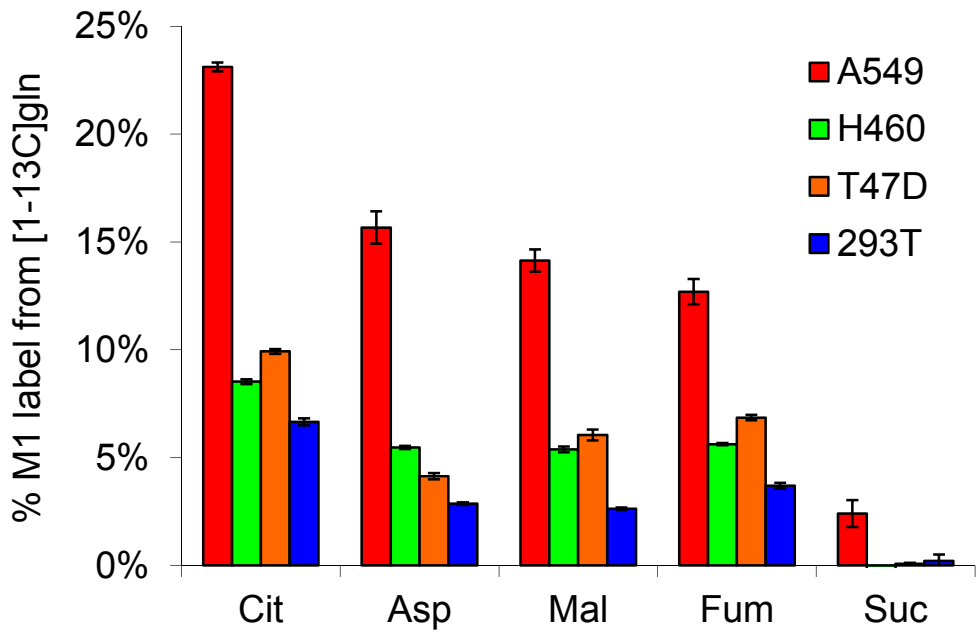


Figure S2. Evidence for reductive carboxylation in tumor cell lines using [1-$^{13}$C]glutamine tracer. Percentage of M1 label in metabolite pools detected using GC/MS in A549 and H460 lung carcinoma, T47D breast cancer, and human embryonic kidney 293T cells cultured for 24 hours in the presence of [1-$^{13}$C]glutamine.

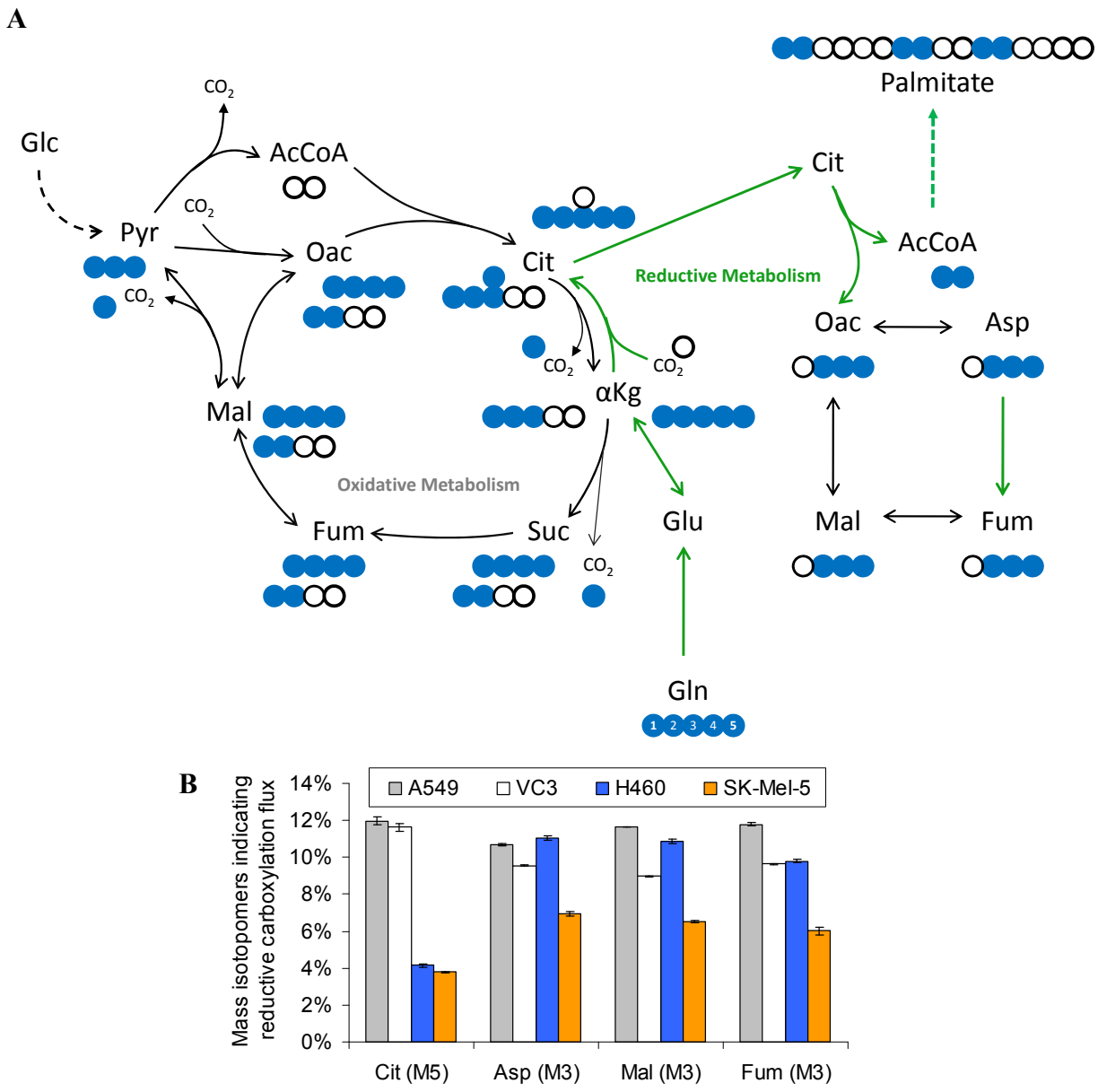


Figure S3. Evidence for reductive carboxylation in tumor cell lines using [U-$^{13}C_5$]glutamine tracer. A) Carbon atom transition map depicting labeling patterns of metabolites derived from [U-$^{13}C_5$]glutamine during oxidative and reductive metabolism. Mass isotopomers generated by reductive carboxylation 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. When two patterns are listed for a given metabolite, the lower pattern depicts that generated in the second turn of the TCA cycle. B) Cells were cultured for 24 hours in the presence of [U-$^{13}C_5$]glutamine before metabolite extraction and GC/MS analysis. Relative abundance of reductive carboxylation-specific mass isotopomers are depicted as measured in A549 lung carcinoma, VC3 glioblastoma, H460 lung carcinoma, and SK-Mel-5 melanoma cell lines. Error bars indicate s.e.m. (n=3).

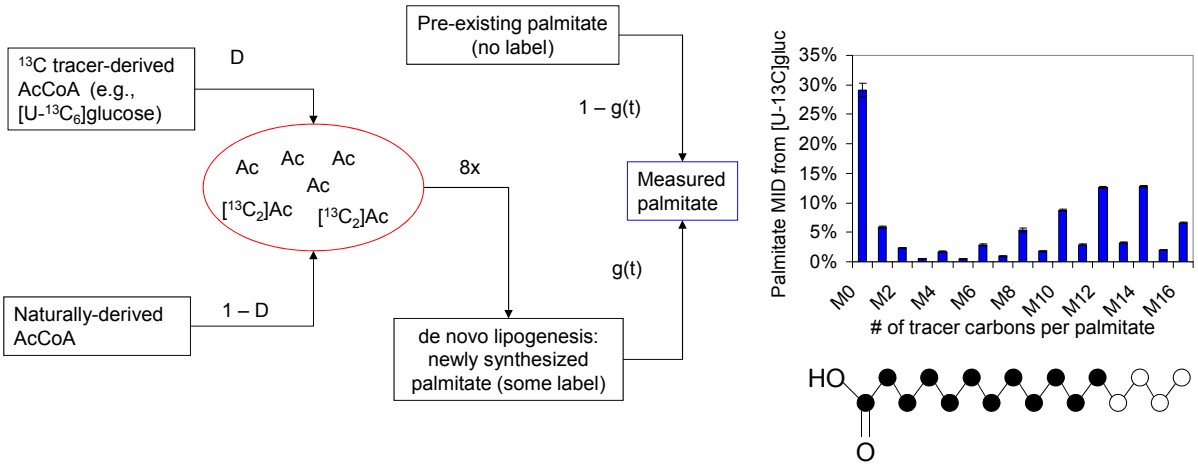


Figure S4. Overview of Isotopomer Spectral Analysis (ISA). ISA applied to fatty acid synthesis provides an estimate of the relative enrichment in the lipogenic AcCoA pool (red) from a given tracer (e.g. [U-$^{13}C_6$]glucose, [U-$^{13}C_5$]glutamine, or [5-$^{13}$C]glutamine). Cells are grown in the presence of tracer to generate labeled fatty acids, and the mass isotopomer distribution (MID) for palmitate is measured via GC/MS. MIDs represent the relative abundance of all mass isotopomers for a given metabolite pool and sum to 100%. The measured pool is comprised of pre-existing palmitate with no label and newly synthesized fatty acids, which may have more than one 13C label per molecule (depending on the level of enrichment in the precursor pool). The D parameter indicates the level of isotope enrichment in the AcCoA pool, and the g(t) parameter indicates the percentage of fatty acids that are newly synthesized, which depends on cell growth and time. These parameters are estimated for a given tracer and MID, and the 95% confidence interval is determined via parameter continuation/sensitivity analysis.

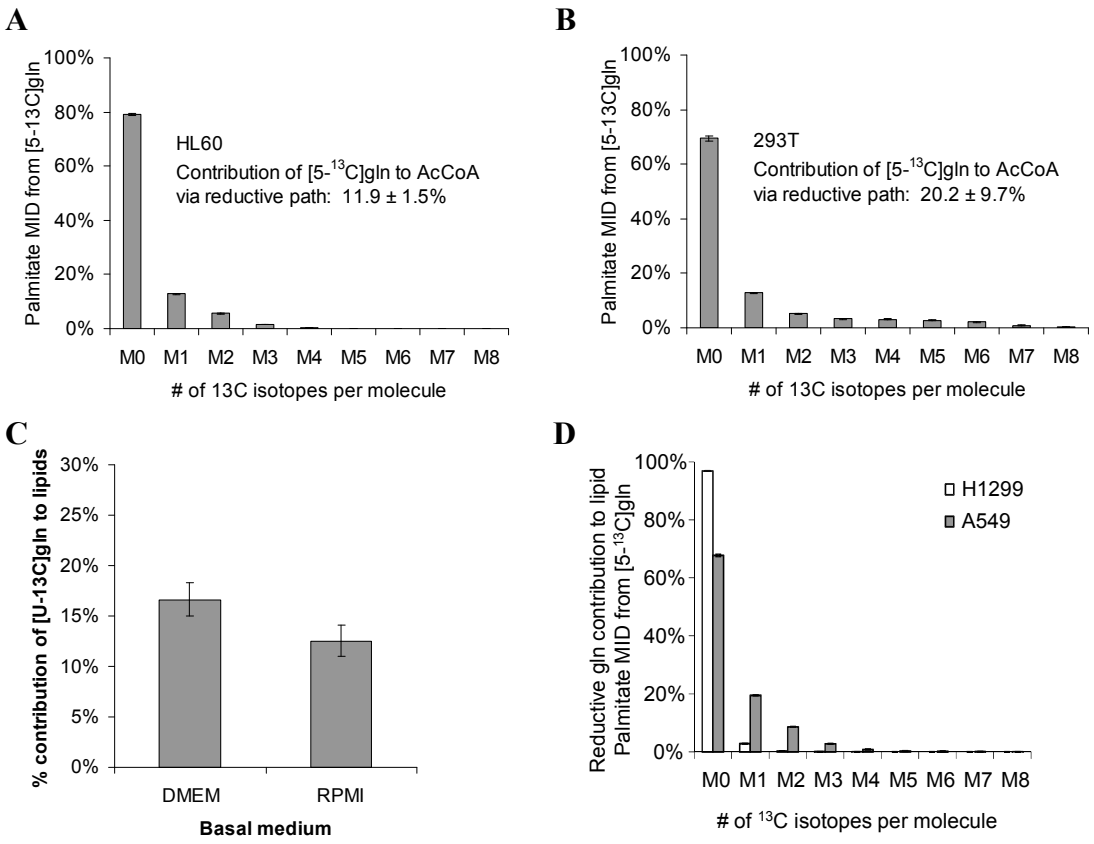


Figure S5. Evidence for use of reductive carboxylation for lipogenesis in other cell lines and culture medium. A,B) MID data of palmitate labeling from [5-$^{13}$C]glutamine in HL60 acute myeloid leukemia cells (A) and human embryonic kidney 293T cells (B). Percent contribution of the glutamine through reductive carboxylation to lipogenic AcCoA determined by ISA (95% confidence interval from model). C) Comparison of ISA data in H460 cells cultured in DMEM or RPMI 1640 medium with [U-$^{13}C_5$]glutamine. Note that basal RPMI medium contains unlabeled glutamate, which causes dilution of the [U-$^{13}C_5$]glutamine tracer upstream of reductive carboxylation. DMEM data obtained from Fig. 1B; RPMI data obtained from Fig. S1B. D) Palmitate MID from A549, and H1299 cells cultured with [5-$^{13}$C]glutamine and corrected for natural isotope abundance. H1299 cells were the only cell line tested that did not incorporate significant label from [5-$^{13}$C]glutamine under normoxia.

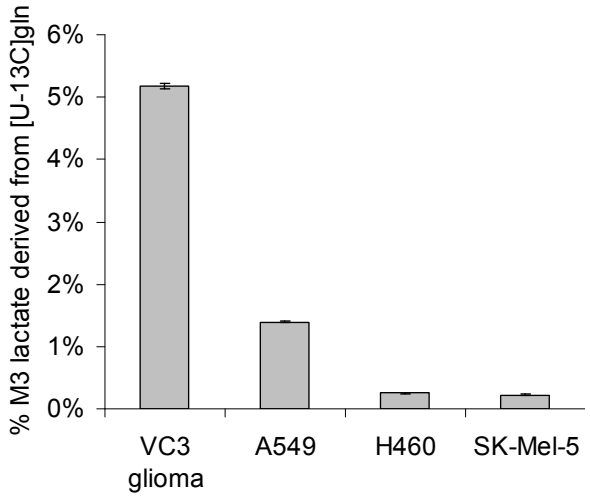


Figure S6. Cells derived from glioblastoma tumors exhibit elevated glutaminolysis compared to other cell types. Comparison of the relative abundance of M3 lactate mass isotopomers derived from [U-$^{13}C_5$]glutamine in glioblastoma (VC3), lung carcinoma (A549 and H460), and melanoma (SK-Mel-5) cell lines. Error bars indicate s.e.m. (n=3).

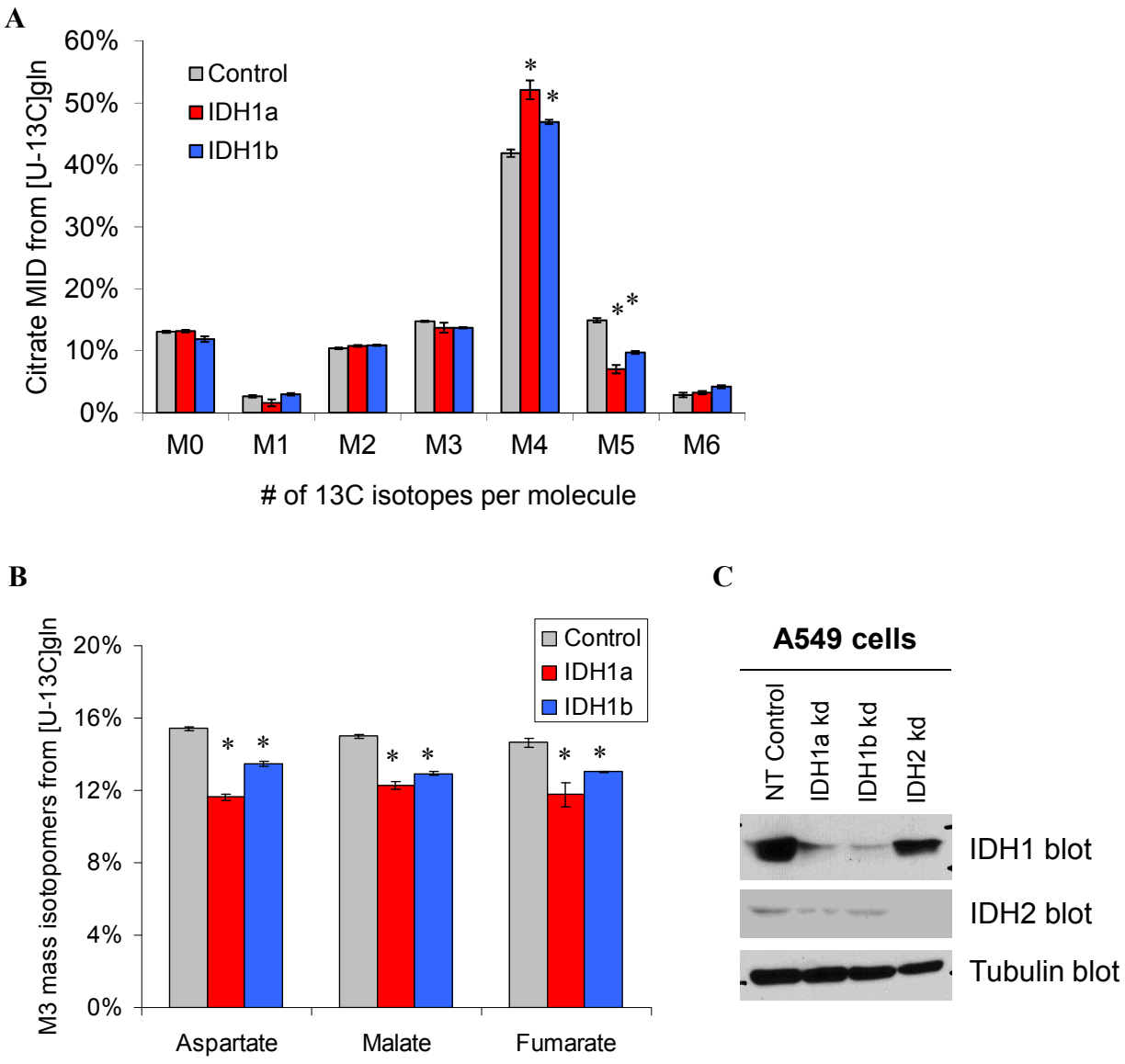


Figure S7. Evidence for decreased reductive carboxylation upon IDH1 knockdown in A549 cells using [U-$^{13}C_5$]glutamine. A) M5 mass isotopomers are decreased in IDH1 knockdown cells and M4 abundance increases, indicating a relative increase in oxidative glutamine metabolism. B) M3 mass isotopomers in aspartate, malate, and fumarate pools from [U-$^{13}C_5$]glutamine are decreased upon IDH1 knockdown. Error bars indicate s.e.m. (n=3). * indicates $p < 0.01$. Trends were observed in at least 3 independent knockdown experiments. C) No compensatory upregulation of IDH2 was observed in cells expressing IDH1-targeting shRNAs, nor did we observe increased IDH1 protein in response to IDH2-targeting shRNAs.

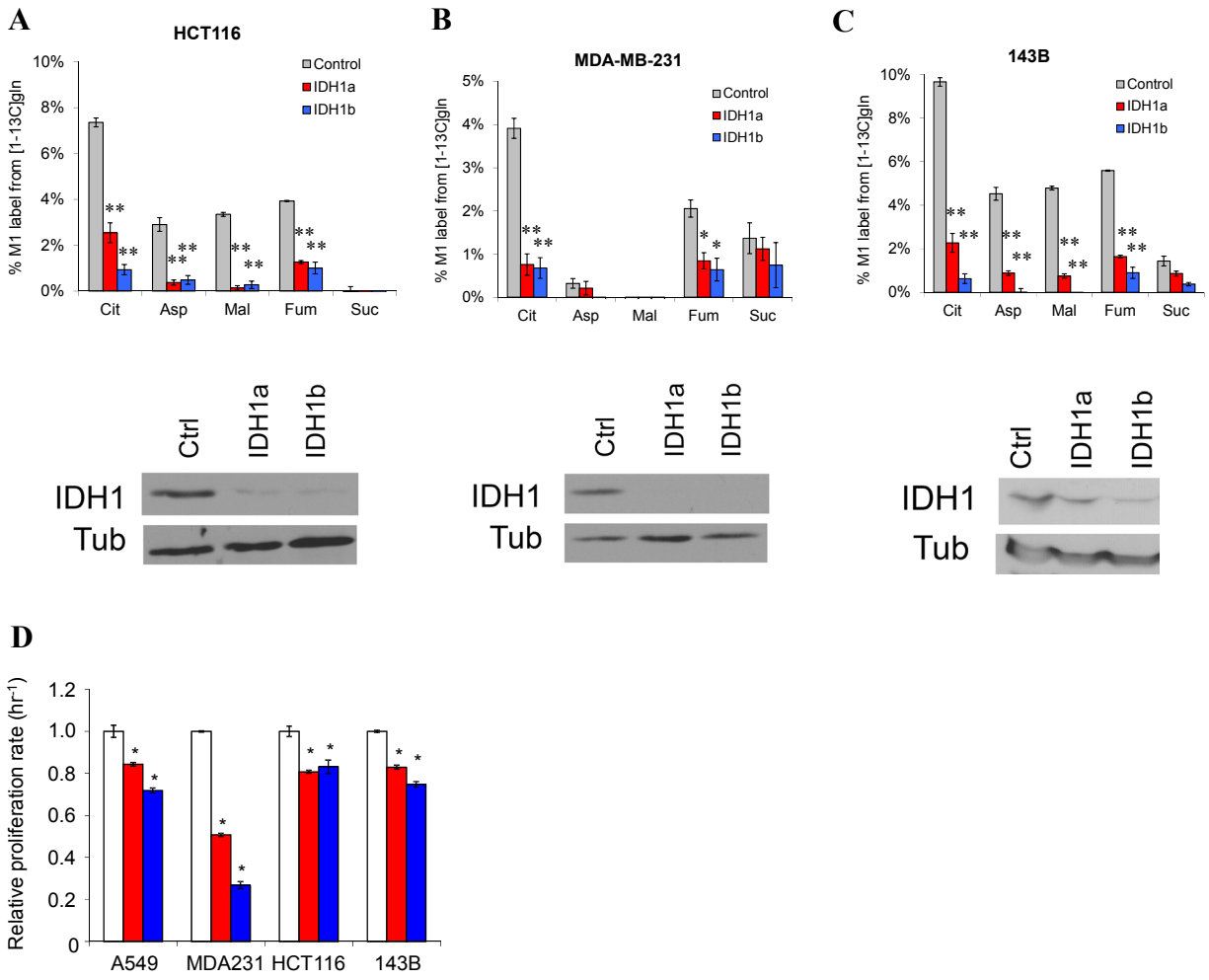


Figure S8. Targeting of IDH1 mRNA with shRNA's reduces reductive carboxylation flux in HCT116 colon carcinoma (A), MDA-MB-231 breast carcinoma (B), and 143B osteosarcoma (C) cell lines. A,B) Decreased M1 label was observed in citrate, asparatate, malate, and fumarate from the [1-$^{13}$C]glutamine tracer. Error bars indicate s.e.m. (n=3). Western blots indicate decreased protein levels of IDH1 upon shRNA expression. Knockdown of protein was not complete, as indicated by detection of IDH1 in blots at long exposures (not shown for B). D) Relative proliferation rates of cell lines stably expressing control or IDH1-targeting shRNAs. * indicates $p < 0.05$ and ** indicates $p < 0.001$ comparing control and knockdowns.

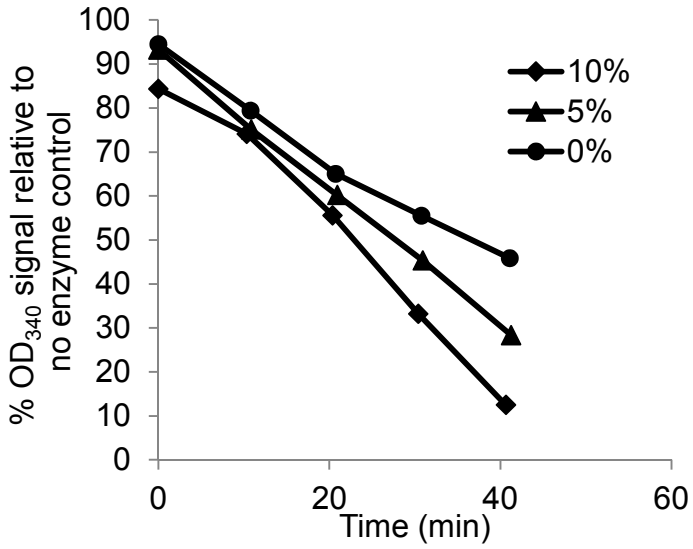


Figure S9. IDH1 can reductively utilize CO2 and consume NADPH. IDH1 was recombinantly produced in E.coli. After purification, NADPH consumption in the presence of aKG was quantified via measurement of NADPH fluorescence at 0%, 5%, and 10% $CO_2$.

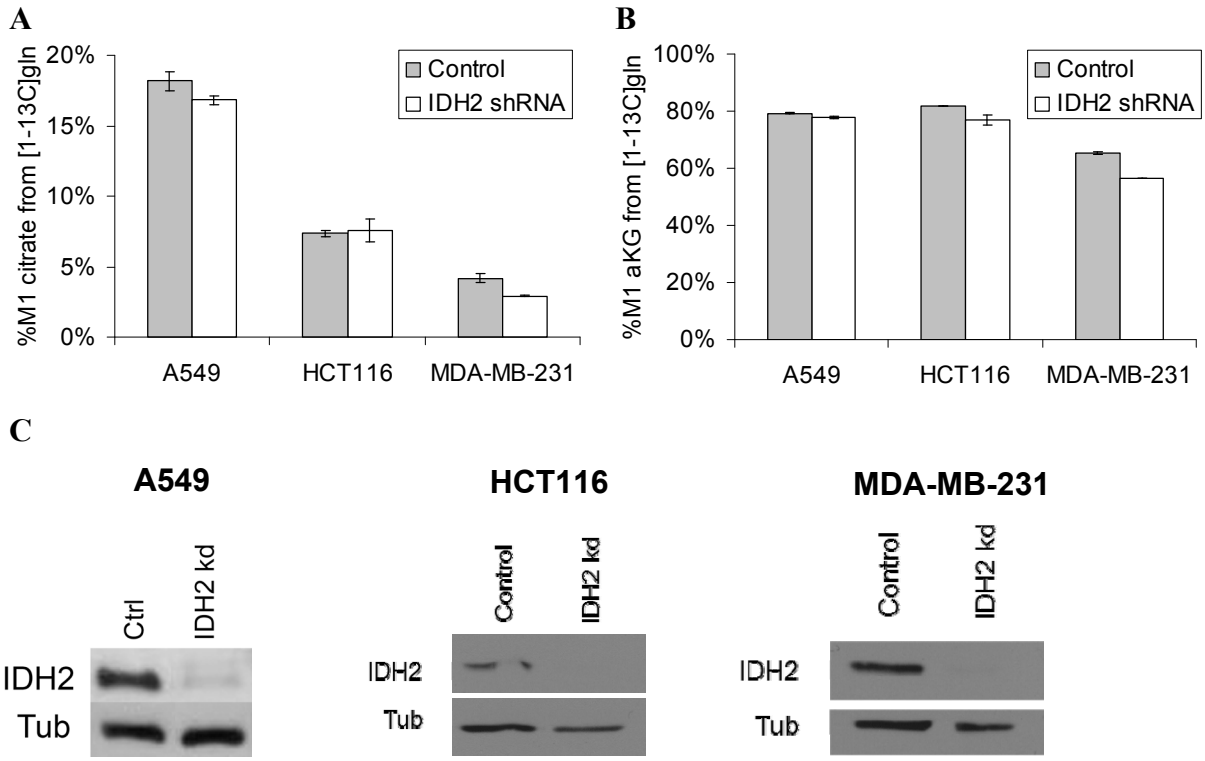


Figure S10. Knockdown of IDH2 protein with shRNA does not affect reductive carboxylation flux. A) Stable A549, HCT116, and MDA-MB-231 cell lines expressing decreased levels of IDH2 generated similar citrate labeling patterns from [1-$^{13}$C]glutamine to control cells expressing scrambled shRNAs. B) The slight decrease in M1 citrate levels in MDA-MB-231 cells arises from decreases in M1 label in the αKG pool. Error bars indicate s.e.m. (n=3). C) Validation of knockdown in each cell line by Western blotting.

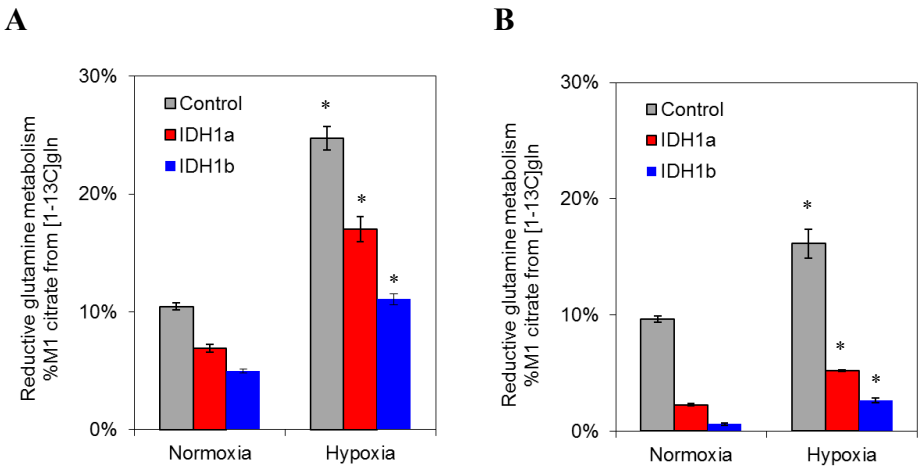


Figure S11. Hypoxia increases relative flux through reductive carboxylation, and IDH1 knockdown significantly attenuates this change. A,B) Relative flux through reductive carboxylation increases in A549 (A) and 143B (B) cells expressing control or IDH1-targeting shRNAs when cultured under hypoxia, as demonstrated by transfer of [1-$^{13}$C]glutamine to citrate. Error bars indicate s.e.m. (n=3). * denotes $p < 0.01$.

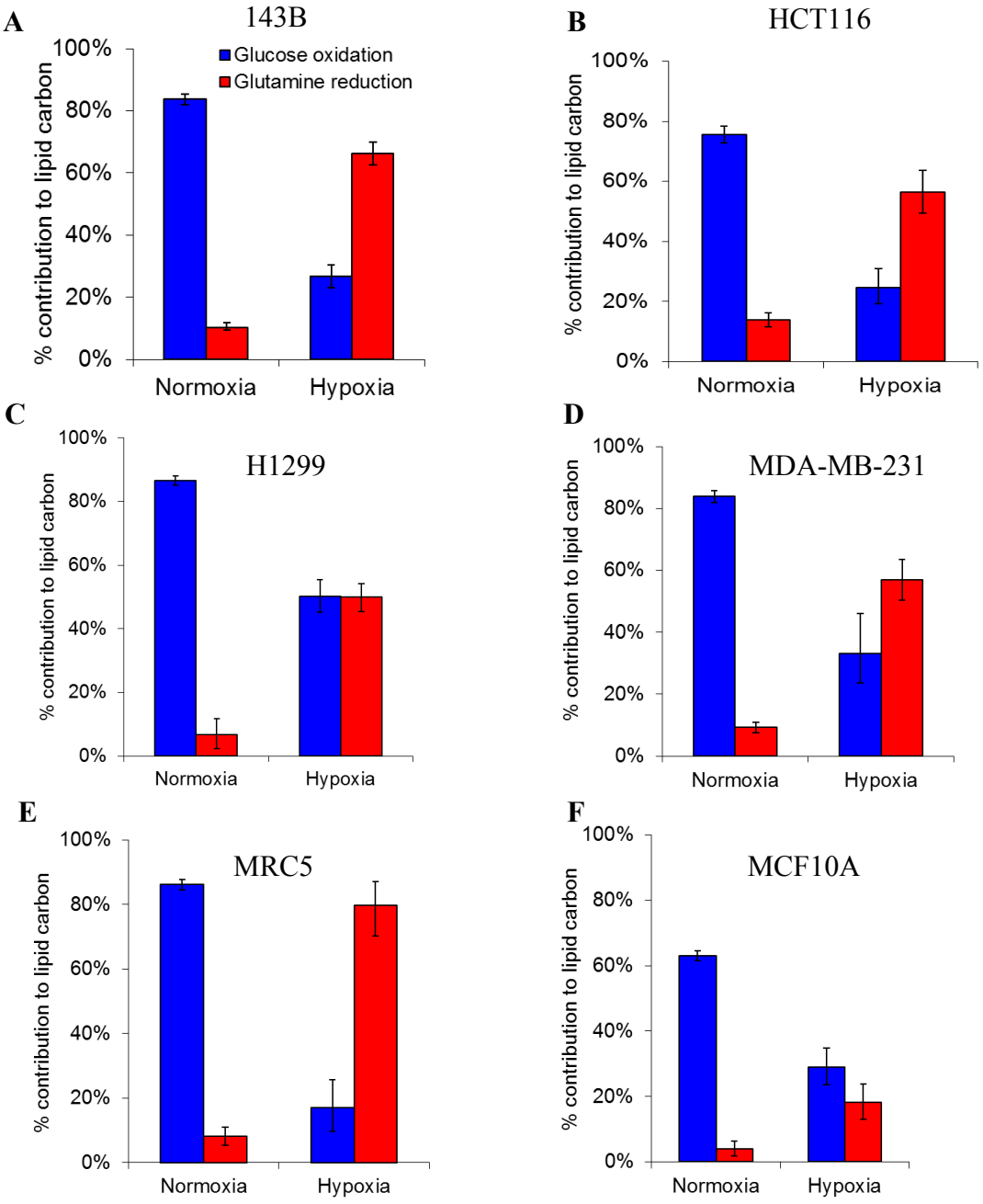


Figure S12. Human cells change their carbon source for lipid synthesis under hypoxia. A-F) ISA analysis to determine the contribution of glucose and glutamine (reductive pathway only) to AcCoA in 143B (A), HCT116 (B), H1299 (C), MDA-MB-231 (D), MRC5 (E), and MCF10A (F) cells. Cells were grown for 3 days under 21% or 1-2% $O_2$ in the presence of [U-$^{13}C_6$]glucose or [5-$^{13}$C]glutamine tracers before extraction. Spent medium was analyzed at the conclusion of culture to ensure that tracer substrates did not expire. Note that MCF10A cells were cultured in DMEM/F12 basal medium with 5% horse serum that was not dialyzed. Therefore unlabeled glutamine, glutamate, and proline were present an potentially dilute the contribution of [5-$^{13}$C]glutamine to lipids. Error bars indicate 95% confidence intervals from ISA model.

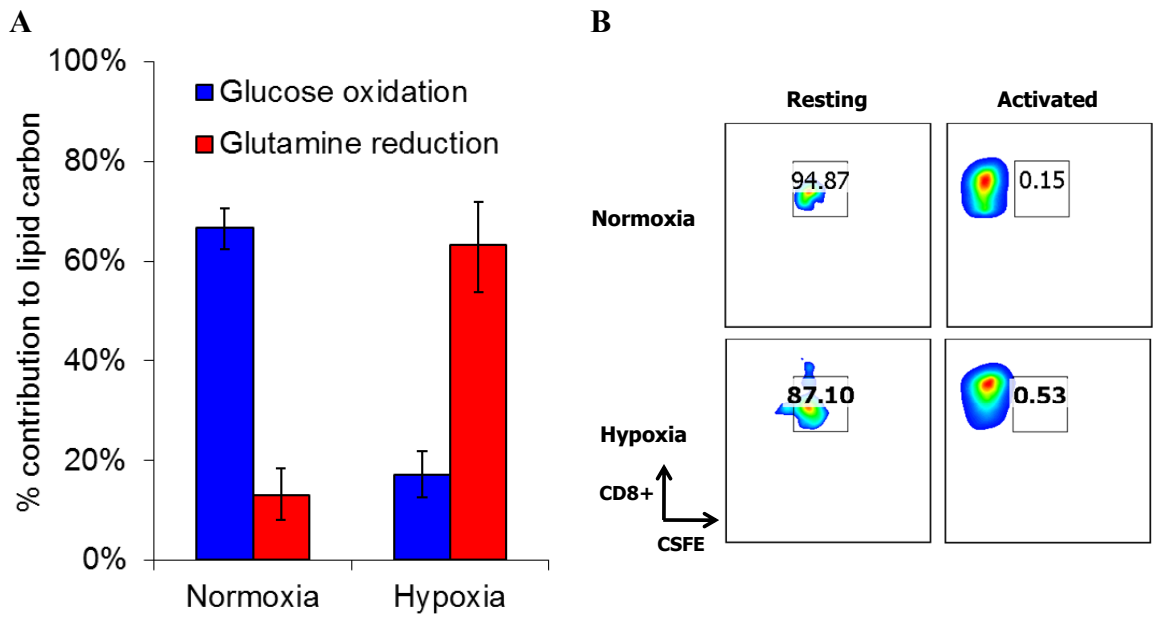


Figure S13. Activated T lymphocytes reductively metabolize glutamine to lipid. Primary CD8+ T cells isolated from spleens from OT-1 mice were expanded under normoxia or hypoxia in the presence of SIINFEKL peptide and IL-2 as described in the Supplemental Methods. A) The contribution of [U-$^{13}C_6$]glucose and [5-$^{13}$C]glutamine to palmitate in biomass was determined after 7 days of proliferation by ISA. Error bars indicate 95% confidence intervals from ISA model. B) CD8 expression and proliferation by dilution of the CSFE dye were quantified by flow cytometry.

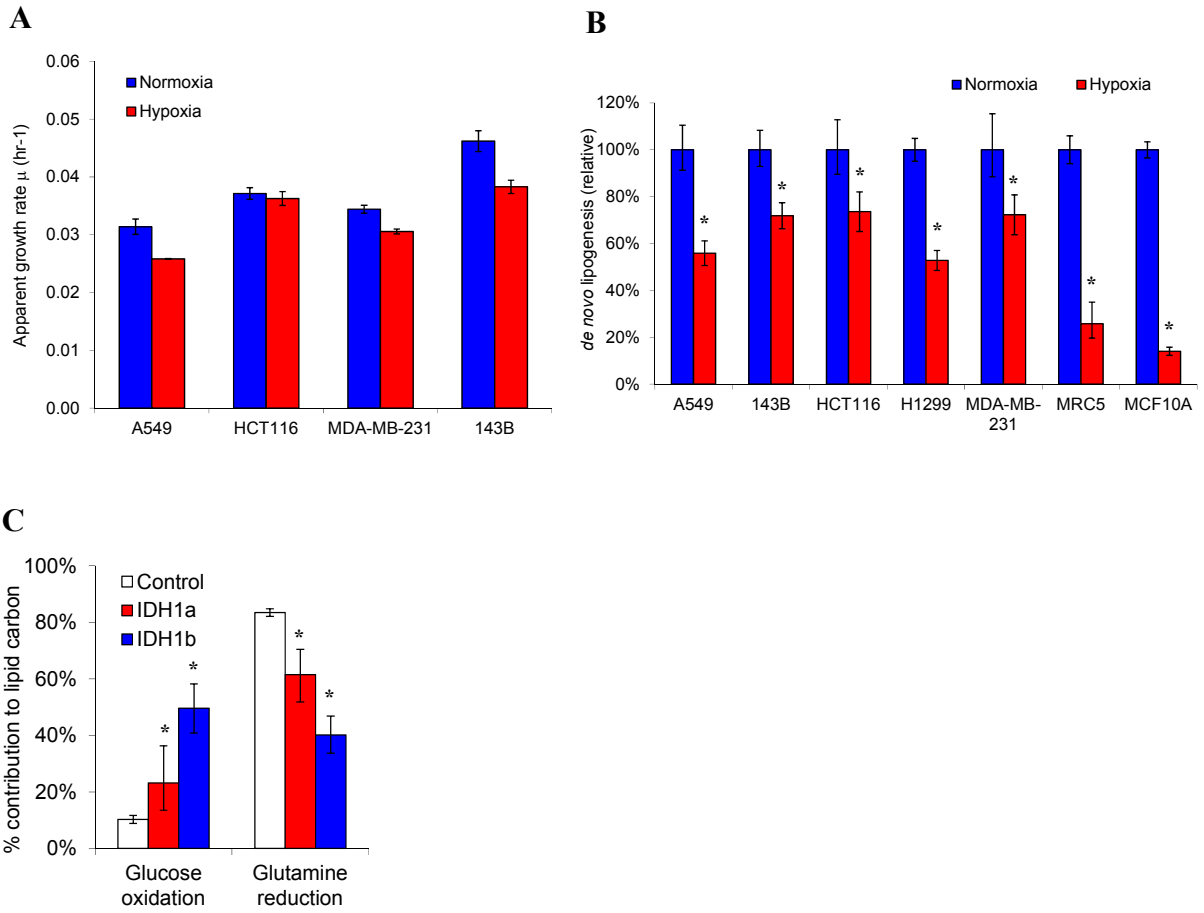


Figure S14. Rates of proliferation and *de novo* lipogenesis in cells cultured under hypoxia. A) Apparent cell growth rates were calculated assuming exponential growth from cells grown for 3 days under normoxia or hypoxia. Error bars for proliferation rates indicate s.e.m. (n=3). B) Relative rates of *de novo* lipogenesis were determined via ISA modeling as described in Fig. S4. g(t) values were normalized to that of normoxic culture and further scaled by palmitate abundances (measured by GC/MS) in order to account for differences in cell growth/number. Although all cells exhibited a decrease *de novo* lipogenesis, new fatty acid synthesis was decreased to a greater extent in non-transformed cells (MRC5, MCF10A) compared to transformed cell lines. C) Relative contribution of glucose oxidation and glutamine reduction to palmitate in A549 cells cultured under hypoxia and expressing control or IDH1-targeting (IDH1a, IDH1b) shRNAs, as determined by ISA. Error bars for (B) and (C) are 95% confidence intervals. * denotes $p < 0.05$

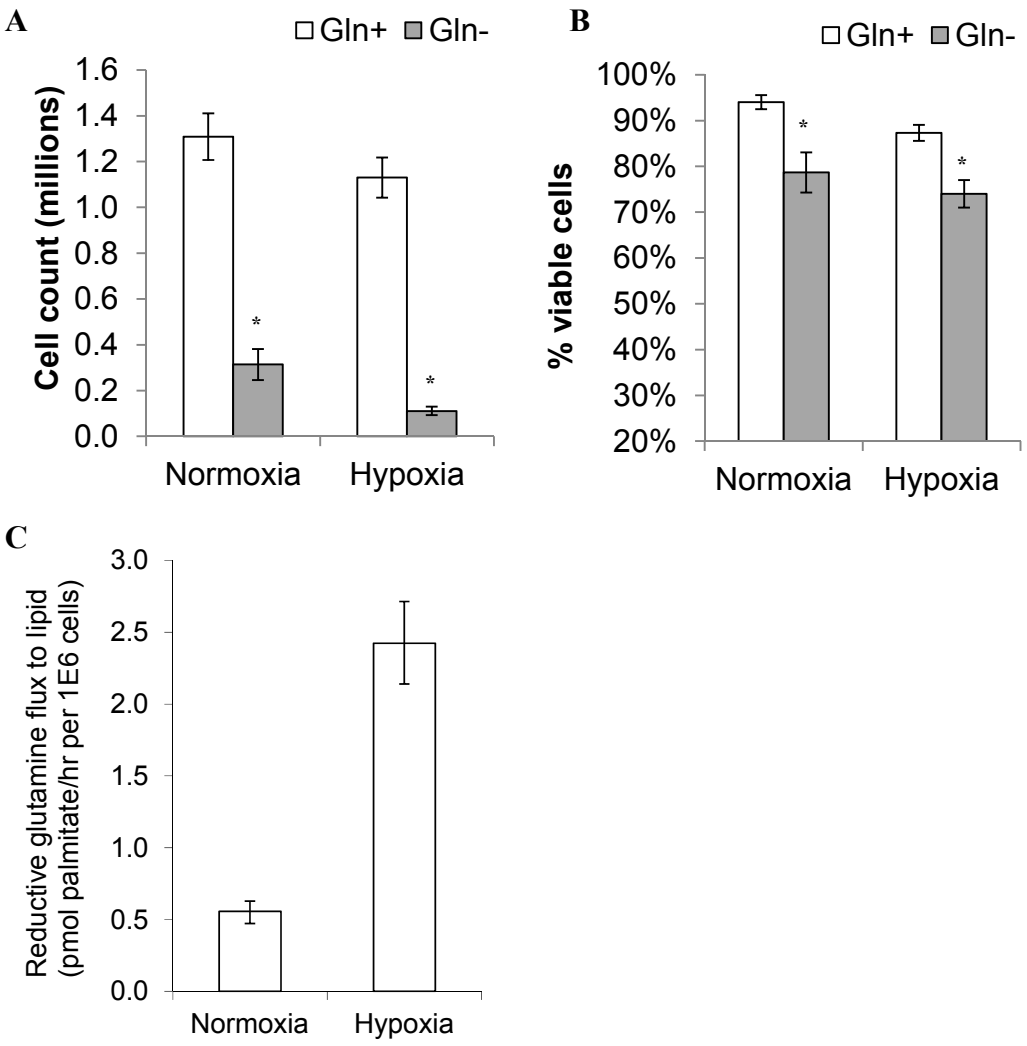


Figure S15. Net flux of glutamine to palmitate through the reductive pathway increases under hypoxia in cells capable of growing without glutamine. A,B) A549 cells require glutamine for proliferation at normoxia and hypoxia. Cell number (A) and viability (B) data for A549 cells grown for 3 days under normoxia or hypoxia in the presence and absence of glutamine. Error bars indicate s.e.m., and * denotes $p < 0.05$ comparing +/- glutamine samples. C) Huh7 cells, which can proliferate in the absence of glutamine, were cells were cultured for 4 days in the presence of [5-$^{13}$C]glutamine under normoxia or hypoxia. Cells were extracted and labeling was observed in palmitate methyl esters obtained from the total pool of fatty acids (free fatty acids and biomass). Absolute flux was calculated using the ISA fit parameters, quantifying measured fatty acids with a heptadeconoate internal standard, and dividing by the integral viable cell density. Error bars represent 95% confidence intervals obtained from the ISA fit. Results were reproduced in 3 replicates.

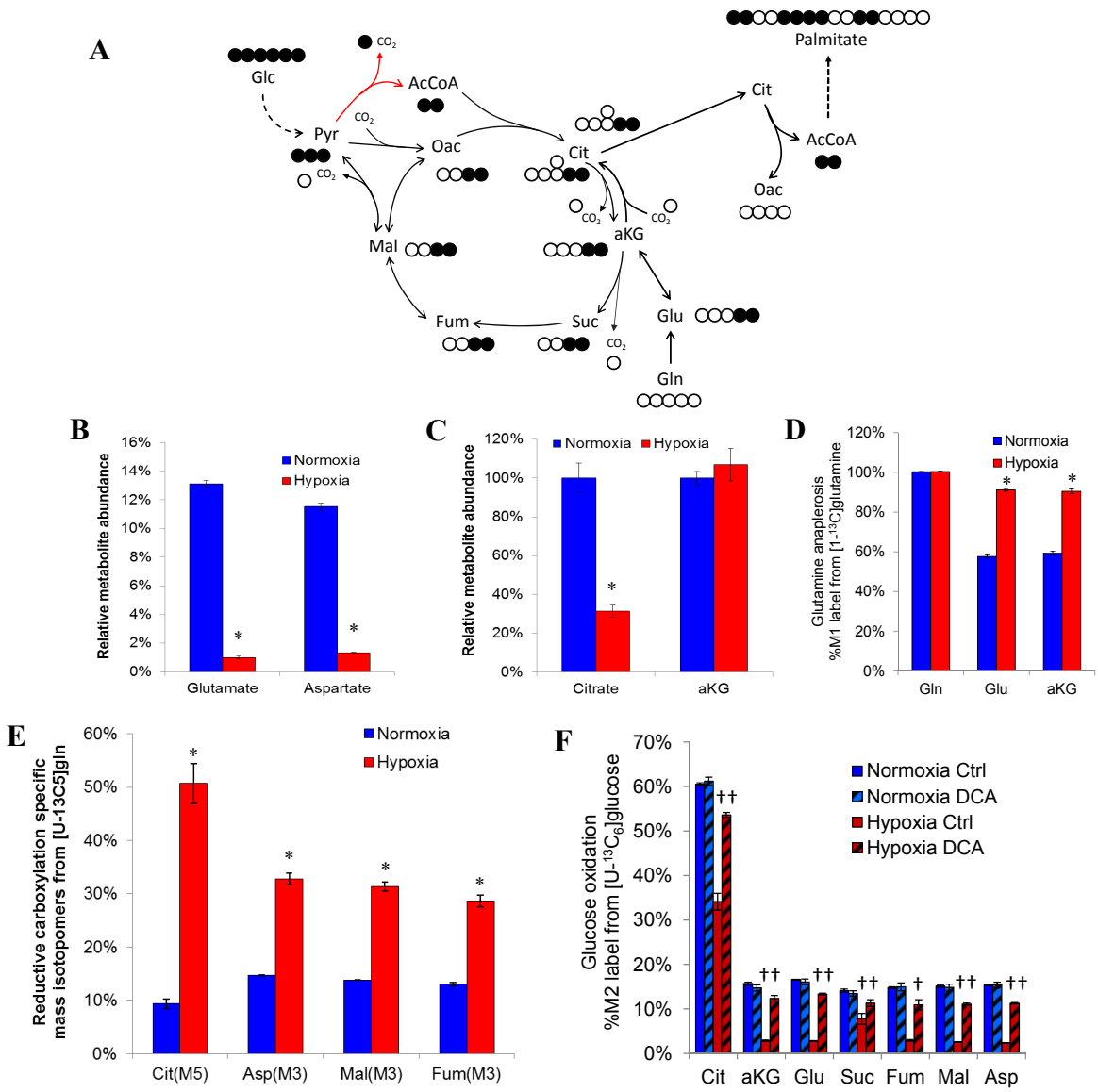


Figure S16. Hypoxia affects PDH flux and citrate levels in A549 cells. A) Atom transition map depicting oxidation of [U-$^{13}C_6$]glucose. M2 labeled TCA cycle metabolites (or aspartate) arise from PDH activity (red), generating dually labeled AcCoA that enters the TCA cycle. Although some recycling occurs, the most abundant isotopologues observed were M2, as anaplerosis of unlabeled carbons from glutamine/glutamine is significant in cultured cells. B) Transfer of label from [U-$^{13}C_6$]glucose oxidation to TCA cycle metabolites in A549 cells cultured under normoxia and hypoxia indicates a relative decrease in PDH flux. C) Relative citrate and aKG levels in A549 cells cultured under hypoxia. Sum integration of all potentially labeled ions is shown, with abundances normalized to cell number and internal standard signal. D) Anaplerosis of glutamine in MRC5 cells, determined by labeling from [1-$^{13}$C]glutamine. E) Reductive carboxylation specific isotopomers from [U-$^{13}C_5$]glutamine in A549 cells. F) Relative level of glucose oxidation in cells in A549 cells cultured with or without dichloroacetate (DCA) under normoxia and hypoxia, as determined by M2 labeling from [U-$^{13}C_6$]glucose. Error bars indicate s.e.m. (n=3). * indicates $p < 0.001$ comparing normoxia to hypoxia. † denotes $p < 0.05$, and †† denotes $p < 0.001$ comparing control to DCA in hypoxia.

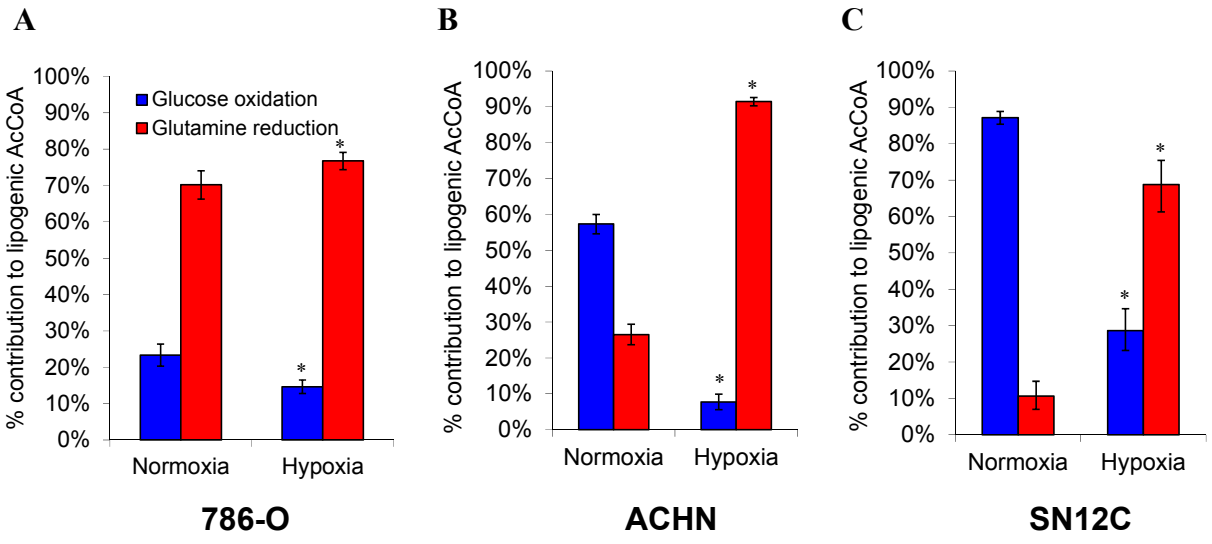


Figure S17. ISA of lipogenesis in renal clear cell carcinoma (RCC) cell lines under normoxia and hypoxia. A) VHL-deficient 786-O cells slightly increase the utilization of reductive glutamine metabolism for lipogenesis under hypoxia, as this pathway is used at high levels even under normoxia. B,C) RCC cell lines that express wild-type VHL behave normally, preferentially using glucose oxidation under normoxia and reductive carboxylation under hypoxia. Error bars indicate 95% confidence intervals from ISA model. * indicates $p < 0.05$ comparing normoxia to hypoxia.

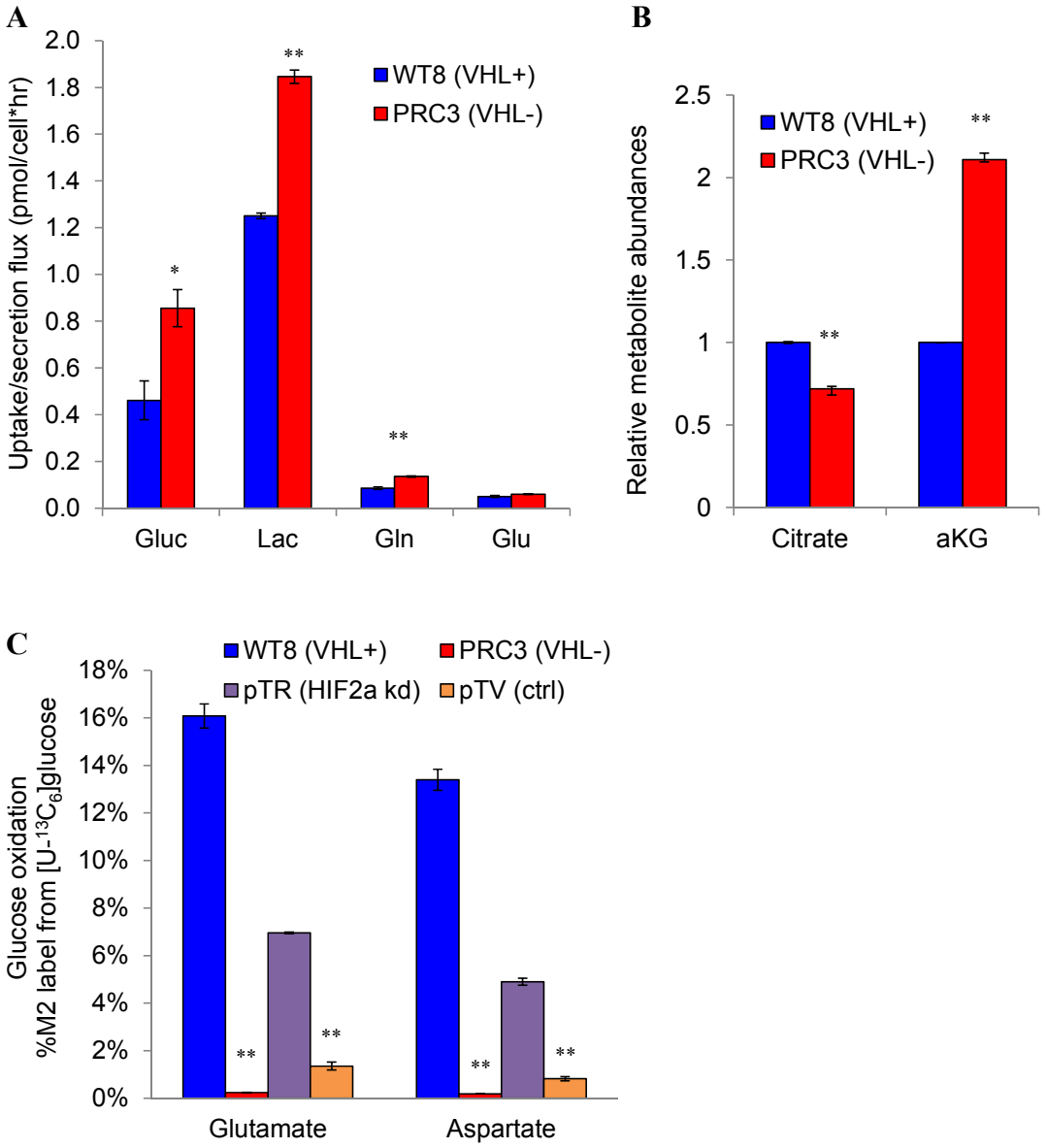


Figure S18. Metabolic effects of VHL expression and HIF2α knockdown in 786-O cells. A) Cell-specific uptake and secretion of glucose/glutamine and lactate/glutamate, respectively. Metabolite levels were measured at the start and end of culture and normalized to the integral viable cell density to calculate fluxes. B) Relative metabolite abundances in extracts of PRC3 cells normalized to WT8 cells (and a norvaline internal standard). Equal numbers of cells were plated, and cells were extracted 18 hours later. * denotes $p < 0.05$ comparing WT8 to PRC3 cells. ** denotes $p < 0.01$ comparing WT8 to PRC3 cells. C) Relative level of glucose oxidation in PRC3, WT8, vector control (pTV), or HIF2α shRNA (pTR) cells, as measured by the relative abundance of M2 isotopomers in glutamate and asparate pools in cells cultured with [U-$^{13}C_6$]glucose. ** denotes $p < 0.01$ comparing WT8 to PRC3 or pTV to pTR cells. Error bars indicate s.e.m. (n=3).

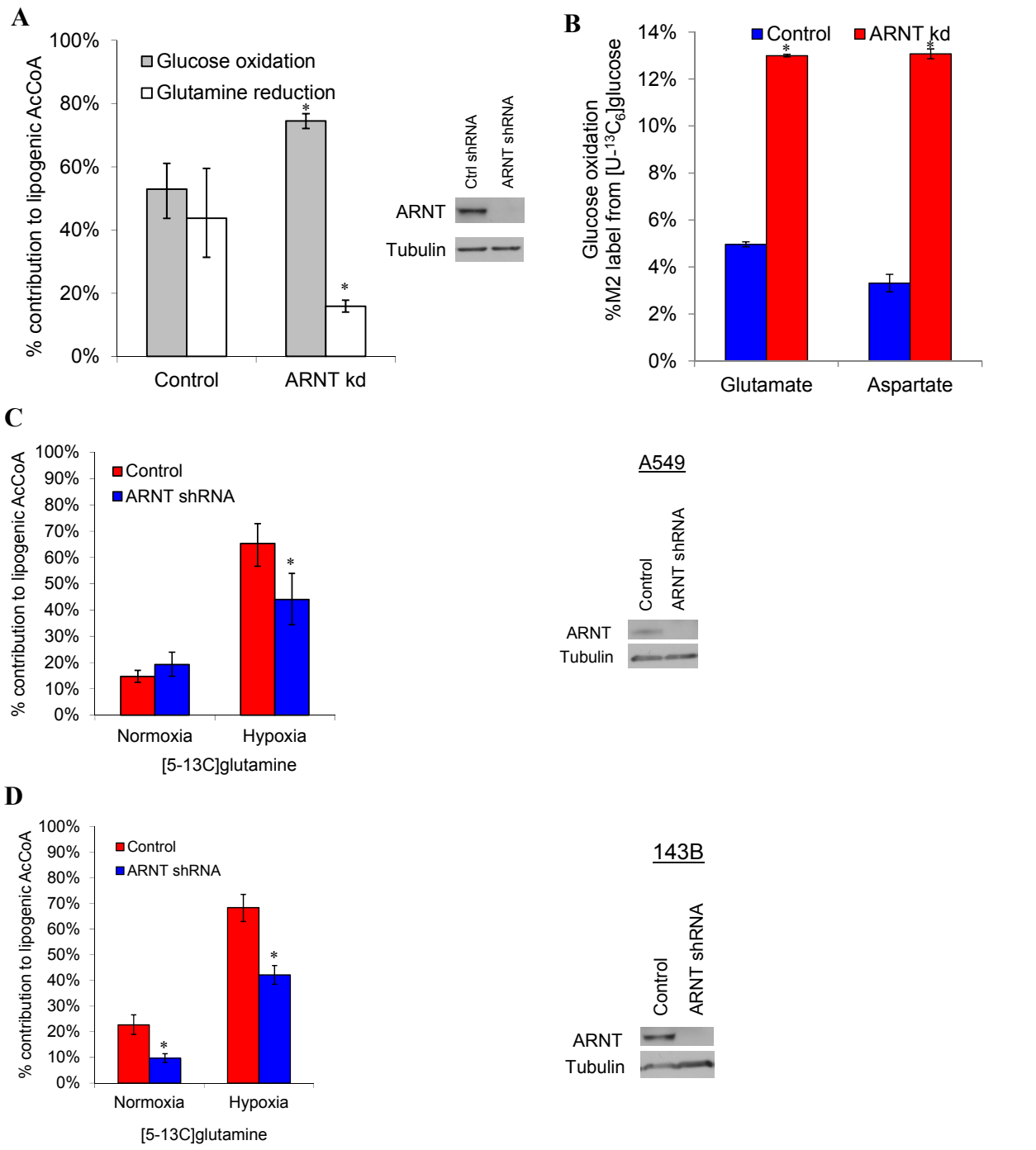


Figure S19. ARNT knockdown modulates glucose oxidation and reductive glutamine metabolism. A) ISA of lipogenesis in UMRC2 cells expressing control or ARNT-targeting shRNAs. B) Relative glucose oxidation in cells in (A), as measured by M2 labeling from [U-13C6]glucose. Error bars indicate s.e.m. (n=3). C,D) ISA of lipogenesis using [5-$^{13}$C]glutamine with A549 (C) and 143B (D) cells expressing shRNAs targeting ARNT. Error bars indicate 95% confidence intervals from ISA model. * indicates $p < 0.05$ comparing control to knockdown.

**Metabolic flux analysis (MFA): description and assumptions**

MFA was conducted using the elementary-metabolite unit (EMU-) based software package Metran as previously described[1-4]. Flux estimations and confidence intervals are subject to the following assumptions:

1. Cellular metabolism and isotopic labeling are at steady state. Cells were selected in the presence of puromycin for at least 2 (but no more than 4) passages and labeled for 24 hours with [U-$^{13}C_5$]glutamine. Metabolite labeling does not significantly change over the course of 12 to 24 hours, allowing for an acceptable assumption of pseudo steady state (Fig S20).

2. Dissolved $CO_2$ exchanges freely with gaseous $CO_2$ such that unlabeled $CO_2$ is available for use in carboxylation reactions. Labeled $CO_2$ from metabolism may be incorporated if required.

3. Fatty acid oxidation and protein turnover are negligible relative to glucose and glutamine consumption.

4. Two separate compartments of pyruvate are assumed to exist, with cytosolic pyruvate (primarily glucose derived) used to generate lactate and mitochondrial pyruvate (derived from TCA cycle metabolites) 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 isotopic label from glutamine.

5. Fumarate and succinate are symmetric metabolites, and a dilution pool of unlabeled succinate is assumed to exist. Isotopic enrichment of succinate pools from tracers is often observed to be decreased in tracer studies. Such effects have been hypothesized to be caused by intracellular compartmentalization[5]. This pool is modeled by inclusion of a dilution flux and does not participate in central carbon metabolism. Measured succinate is comprised of both pools (metabolically active and dilution compartment).

6. The pentose phosphate pathway (PPP) is included in the network. The percentage of glycolytic flux that proceeds through the oxidative PPP branch was determined via the M1/M2 ratio of lactate in control or IDH1 knockdown A549 cells cultured with [1,2-$^{13}C_2$]glucose[6]. No significant change was observed between control and knockdown cells.

7. Amino acid and fatty acid fluxes to biomass were determined by quantifying per cell metabolites of A549 protein hydrolysates (aspartate, glutamate, alanine) or total fatty acids from chloroform extracts (palmitate, oleate, stearate) that were evaporated, hydrolyzed, and transesterified. These values were multiplied by the observed growth rate, μ, to obtain fluxes.

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.

Table S1. GC/MS metabolites and fragments used for isotope quantification

| **Metabolite** | **Carbons** | **Formula** | **m/z range** |
|---|---|---|---|
| αKG | 12345 | C14H28O5NSi2 | 346 - 355 |
| Ala | 23 | C10H26ONSi2 | 232 - 239 |
| Ala | 123 | C11H26O2NSi2 | 260 - 268 |
| Asp | 12 | C14H32O2NSi2 | 302 - 310 |
| Asp | 1234 | C18H40O4NSi3 | 418 - 428 |
| Cit | 123456 | C20H39O6Si3 | 459 - 470 |
| Cit | 123456 | C26H55O7Si4 | 591 - 602 |
| Fum | 1234 | C12H23O4Si2 | 287 - 297 |
| Gln | 12345 | C19H43N2O3Si3 | 431 - 441 |
| Glu | 2345 | C16H36O2NSi2 | 330 - 340 |
| Glu | 12345 | C19H42O4NSi3 | 432 - 442 |
| Mal | 1234 | C18H39O5Si3 | 419 - 428 |
| Lac | 23 | C10H25O2Si2 | 233 - 240 |
| Lac | 123 | C11H25O3Si2 | 261 - 269 |
| Pyr | 123 | C6H12O3NSi | 174 - 182 |
| Suc | 1234 | C12H25O4Si2 | 289 - 298 |
| Palmitate | 1-16 | C17H34O2 | 270 - 276 |
| Stearate | 1-18 | C19H38O2 | 298 - 316 |

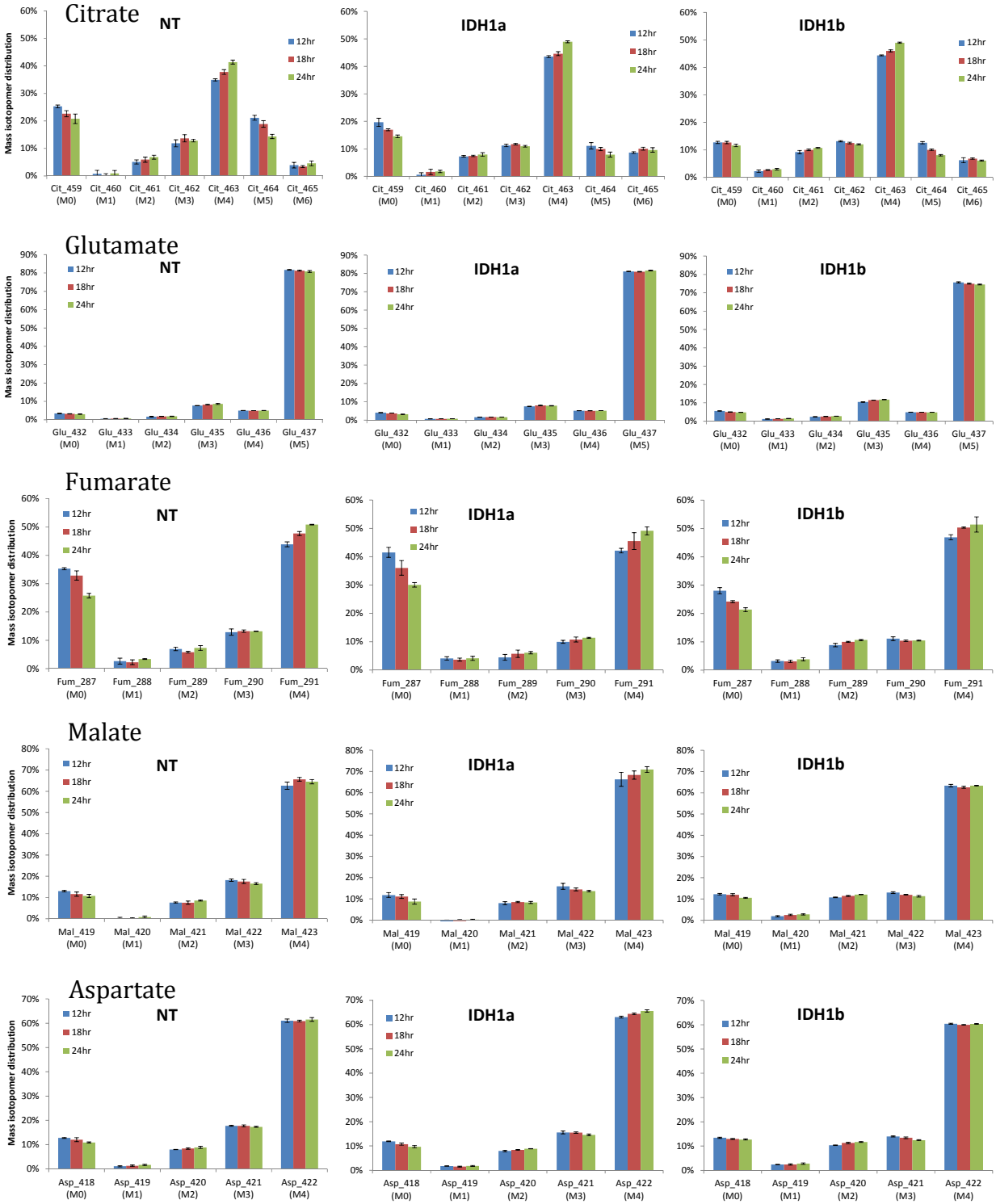


Figure S20. MIDs of TCA metabolites extracted from control or A549-IDH1 knockdown cells at 12, 18, and 24 hours after addition of [U-$^{13}C_5$]glutamine. Results indicate that cells have achieved metabolic and isotopic steady state at the time of extraction. Although some minor labeling changes were observed, such trends occur over a long time period and do not invalidate the pseudo-steady state assumption. In addition, similar trends were observed in all three cell types, indicating this was independent of IDH knockdown.

Table S2. Network and carbon atom transitions describing central carbon metabolism for MFA. Suffixes indicate localization to a specific compartment: .x, extracellular; .c, cytosolic; .m, mitochondrial; .d, dilution; .mnt, measurement. Dilution and measurement compartments do not partake in central metabolism. Metabolites lacking a suffix are assumed to be equilibrated between compartments. → indicates net flux: ($\upsilon_F$-$\upsilon_R$); ←→ indicates exchange flux: min($\upsilon_F$,$\upsilon_R$).

| |
|---|
| **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) ←→ Asp (abcd) |
| **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) ←→ Ala (abc) |
| Gln.x (abcde) → Gln (abcde) |
| Gln (abcde) → Glu (abcde) |
| Glu (abcde) → Glu.x (abcde) |
| **Biomass production** |
| Cit (abcdef) → AcCoA.c (ed) + Oac (fcba) |
| AcCoA.c (ab) → Fatty acids (ab) |
| 0.6 Asp + 0.5 Glu + 0.42 Ala + 0.5 Gln → Biomass |
| P5P (abcde) → NTP (abcde) |
| **Dilution and Mixing Fluxes** |
| Suc.d (abcd) → Suc.mnt (abcd) |
| 0 Suc (abcd) → Suc.mnt (abcd) |
| Pyr.c (abc) ←→ Pyr.m (abc) |
| 0 Pyr.c (abc) → Pyr.mnt (abc) |
| 0 Pyr.m (abc) → Pyr.mnt (abc) |

Table S3. Estimated fluxes for A549 cells expressing non-targeting control shRNAs

| **Pathway/Reaction** | Flux | 95% confidence interval | |
|---|---|---|---|
| **Glycolysis** | fmol/cell*hr | Lower bound | Upper bound |
| Glc.x -> G6P | 98.23 | 87.81 | 108.90 |
| G6P -> F6P | 90.22 | 79.77 | 100.90 |
| G6P <-> F6P | 648.20 | 0.00 | Inf |
| F6P -> DHAP + GAP | 95.23 | 84.80 | 106.00 |
| DHAP -> GAP | 95.23 | 84.80 | 106.00 |
| DHAP <-> GAP | 168.90 | 0.00 | Inf |
| GAP -> 3PG | 193.00 | 172.10 | 214.50 |
| GAP <-> 3PG | 497.60 | 0.00 | Inf |
| 3PG -> Pyr.c | 193.00 | 172.10 | 214.50 |
| Pyr.c -> Lac | 183.90 | 163.10 | 205.50 |
| Pyr.c <-> Lac | 3.0E+03 | 0.00 | Inf |
| Lac -> Lac.x | 183.90 | 163.10 | 205.50 |
| **Pentose Phosphate Pathway** | | | |
| G6P -> P5P + CO2 | 8.00 | 6.43 | 9.57 |
| P5P + P5P -> S7P + GAP | 2.50 | 2.18 | 2.83 |
| P5P + P5P <-> S7P + GAP | 4.1E+08 | 0.00 | Inf |
| S7P + GAP -> F6P + E4P | 2.50 | 2.18 | 2.83 |
| S7P + GAP <-> F6P + E4P | 283.60 | 0.00 | Inf |
| P5P + E4P -> F6P + GAP | 2.50 | 2.18 | 2.83 |
| P5P + E4P <-> F6P + GAP | 397.50 | 0.00 | Inf |
| **Anaplerotic reactions** | | | |
| Pyr.m + CO2 -> Oac | 0.00 | 0.00 | 0.27 |
| Mal -> Pyr.m + CO2 | 4.42 | 3.75 | 5.06 |
| Mal <-> Pyr.m + CO2 | 1.52 | 0.99 | 1.60 |
| Glu -> Akg | 5.04 | 4.76 | 5.48 |
| Glu <-> Akg | 477.60 | 198.40 | Inf |
| Oac -> Asp | 0.62 | 0.50 | 0.73 |
| Oac <-> Asp | 942.60 | 0.00 | Inf |
| **TCA Cycle** | | | |
| Pyr.m -> AcCoA.m + CO2 | 13.01 | 12.22 | 13.90 |
| AcCoA.m + Oac -> Cit | 13.01 | 12.22 | 13.90 |
| Cit -> Akg + CO2 | 3.52 | 3.35 | 3.69 |
| Cit <-> Akg + CO2 | 2.93 | 2.80 | 3.07 |
| Akg -> Suc + CO2 | 8.56 | 8.29 | 8.91 |
| Suc -> Fum | 8.56 | 8.29 | 8.91 |
| Suc <-> Fum | 0.00 | 0.00 | 1.61 |
| Fum -> Mal | 8.56 | 8.29 | 8.91 |
| Fum <-> Mal | 9.9E+05 | 311.30 | Inf |
| Mal -> Oac | 4.14 | 3.86 | 4.36 |
| Mal <-> Oac | 134.60 | 64.70 | 1768.00 |
| **Amino acids** | | | |
| Pyr.m -> Ala | 0.43 | 0.35 | 0.51 |
| Pyr.m <-> Ala | 0.03 | 0.00 | Inf |
| Gln.x -> Gln | 19.68 | 18.49 | 21.19 |
| Gln -> Glu | 19.16 | 18.03 | 20.70 |
| Glu -> Glu.x | 13.61 | 11.90 | 15.27 |
| **Biomass** | | | |
| Cit -> AcCoA.c + Oac | 9.49 | 8.88 | 10.03 |
| AcCoA.c -> Fatty acids | 9.49 | 8.88 | 10.03 |
| 0.6*Asp + 0.5*Glu + 0.42*Ala + 0.5*Gln -> Biomass | 1.03 | 0.84 | 1.22 |
| P5P -> NTP | 0.50 | 0.40 | 0.60 |
| **Dilution/Mixing** | | | |
| Suc.d -> Suc.mnt | 0.55 | 0.53 | 0.56 |
| 0*Suc -> Suc.mnt | 0.45 | 0.44 | 0.47 |
| Pyr.c -> Pyr.m | 9.02 | 8.15 | 9.79 |
| Pyr.c <-> Pyr.m | 98.59 | 70.22 | 140.20 |
| 0*Pyr.c -> Pyr.mnt | 1.00 | 0.84 | 1.00 |
| 0*Pyr.m -> Pyr.mnt | 0.00 | 0.00 | 0.16 |

SSE = 83.6

Expected SSE = [72.5 127.3] (95% conf., 98 DOF)

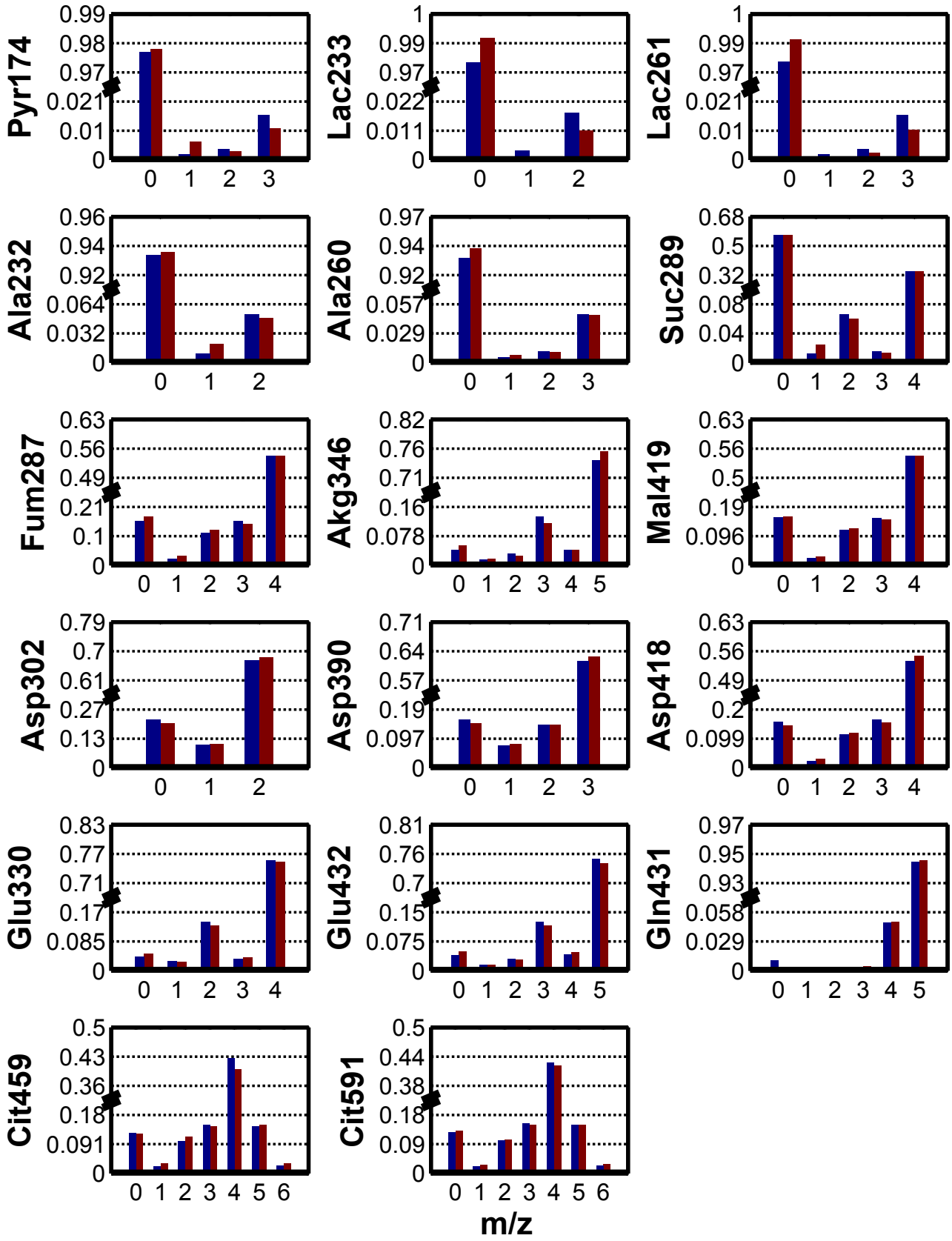


Figure S21. Simulated and measured MIDs from MFA in A549 cells expressing non-targeting control shRNAs. Simulated values were obtained using Metran and the model fit listed in Table S3. Cells were cultured as described in Methods using [U-13C5]glutamine and metabolite labeling was quantified via GC/MS. MIDs are corrected for natural abundance.

Table S4. Estimated fluxes for A549 cells expressing IDH1-targeting shRNAs (IDH1a)

| **Pathway/Reaction** | Flux | 95% confidence interval | |
|---|---|---|---|
| **Glycolysis** | fmol/cell*hr | Lower bound | Upper bound |
| Glc.x -> G6P | 94.96 | 82.80 | 107.10 |
| G6P -> F6P | 86.95 | 74.71 | 99.19 |
| G6P <-> F6P | 3.2E+04 | 0.00 | Inf |
| F6P -> DHAP + GAP | 91.96 | 79.79 | 104.10 |
| DHAP -> GAP | 91.96 | 79.79 | 104.10 |
| DHAP <-> GAP | 566.90 | 0.00 | Inf |
| GAP -> 3PG | 186.40 | 162.10 | 210.80 |
| GAP <-> 3PG | 1.7E+08 | 0.00 | Inf |
| 3PG -> Pyr.c | 186.40 | 162.10 | 210.80 |
| Pyr.c -> Lac | 174.60 | 149.90 | 199.30 |
| Pyr.c <-> Lac | 706.50 | 0.00 | Inf |
| Lac -> Lac.x | 174.60 | 149.90 | 199.30 |
| **Pentose Phosphate Pathway** | | | |
| G6P -> P5P + CO2 | 8.01 | 6.45 | 9.58 |
| P5P + P5P -> S7P + GAP | 2.50 | 1.98 | 3.03 |
| P5P + P5P <-> S7P + GAP | 3.0E+04 | 0.00 | Inf |
| S7P + GAP -> F6P + E4P | 2.50 | 1.98 | 3.03 |
| S7P + GAP <-> F6P + E4P | 1.9E+05 | 0.00 | Inf |
| P5P + E4P -> F6P + GAP | 2.50 | 1.98 | 3.03 |
| P5P + E4P <-> F6P + GAP | 1.76 | 0.00 | Inf |
| **Anaplerotic reactions** | | | |
| Pyr.m + CO2 -> Oac | 2.14 | 0.52 | 2.57 |
| Mal -> Pyr.m + CO2 | 5.44 | 3.47 | 7.22 |
| Mal <-> Pyr.m + CO2 | 0.00 | 0.00 | 1.69 |
| Glu -> Akg | 3.90 | 2.59 | 5.28 |
| Glu <-> Akg | 499.30 | 145.90 | Inf |
| Oac -> Asp | 0.61 | 0.49 | 0.72 |
| Oac <-> Asp | 0.01 | 0.00 | Inf |
| **TCA Cycle** | | | |
| Pyr.m -> AcCoA.m + CO2 | 14.66 | 13.30 | 16.02 |
| AcCoA.m + Oac -> Cit | 14.66 | 13.30 | 16.02 |
| Cit -> Akg + CO2 | 4.01 | 3.38 | 4.67 |
| Cit <-> Akg + CO2 | 1.88 | 1.50 | 2.34 |
| Akg -> Suc + CO2 | 7.91 | 6.48 | 9.41 |
| Suc -> Fum | 7.91 | 6.48 | 9.41 |
| Suc <-> Fum | 0.00 | 0.00 | 4.32 |
| Fum -> Mal | 7.91 | 6.48 | 9.41 |
| Fum <-> Mal | 1.1E+06 | 46.26 | Inf |
| Mal -> Oac | 2.48 | 1.82 | 3.32 |
| Mal <-> Oac | 98.66 | 41.31 | Inf |
| **Amino acids** | | | |
| Pyr.m -> Ala | 0.42 | 0.34 | 0.50 |
| Pyr.m <-> Ala | 0.00 | 0.00 | Inf |
| Gln.x -> Gln | 23.47 | 20.64 | 26.29 |
| Gln -> Glu | 22.97 | 20.44 | 25.78 |
| Glu -> Glu.x | 18.56 | 15.70 | 21.42 |
| **Biomass** | | | |
| Cit -> AcCoA.c + Oac | 10.65 | 9.48 | 11.84 |
| AcCoA.c -> Fatty acids | 10.65 | 9.48 | 11.84 |
| 0.6*Asp + 0.5*Glu + 0.42*Ala + 0.5*Gln -> Biomass | 1.01 | 0.81 | 1.20 |
| P5P -> NTP | 0.50 | 0.40 | 0.60 |
| **Dilution/Mixing** | | | |
| Suc.d -> Suc.mnt | 0.76 | 0.74 | 0.78 |
| 0*Suc -> Suc.mnt | 0.24 | 0.22 | 0.26 |
| Pyr.c -> Pyr.m | 11.79 | 9.98 | 13.61 |
| Pyr.c <-> Pyr.m | 82.77 | 42.18 | 154.10 |
| 0*Pyr.c -> Pyr.mnt | 1.00 | 0.68 | 1.00 |
| 0*Pyr.m -> Pyr.mnt | 0.00 | 0.00 | 0.32 |

SSE = 93.4

Expected SSE = [58.0 107.8] (95% conf., 81 DOF)

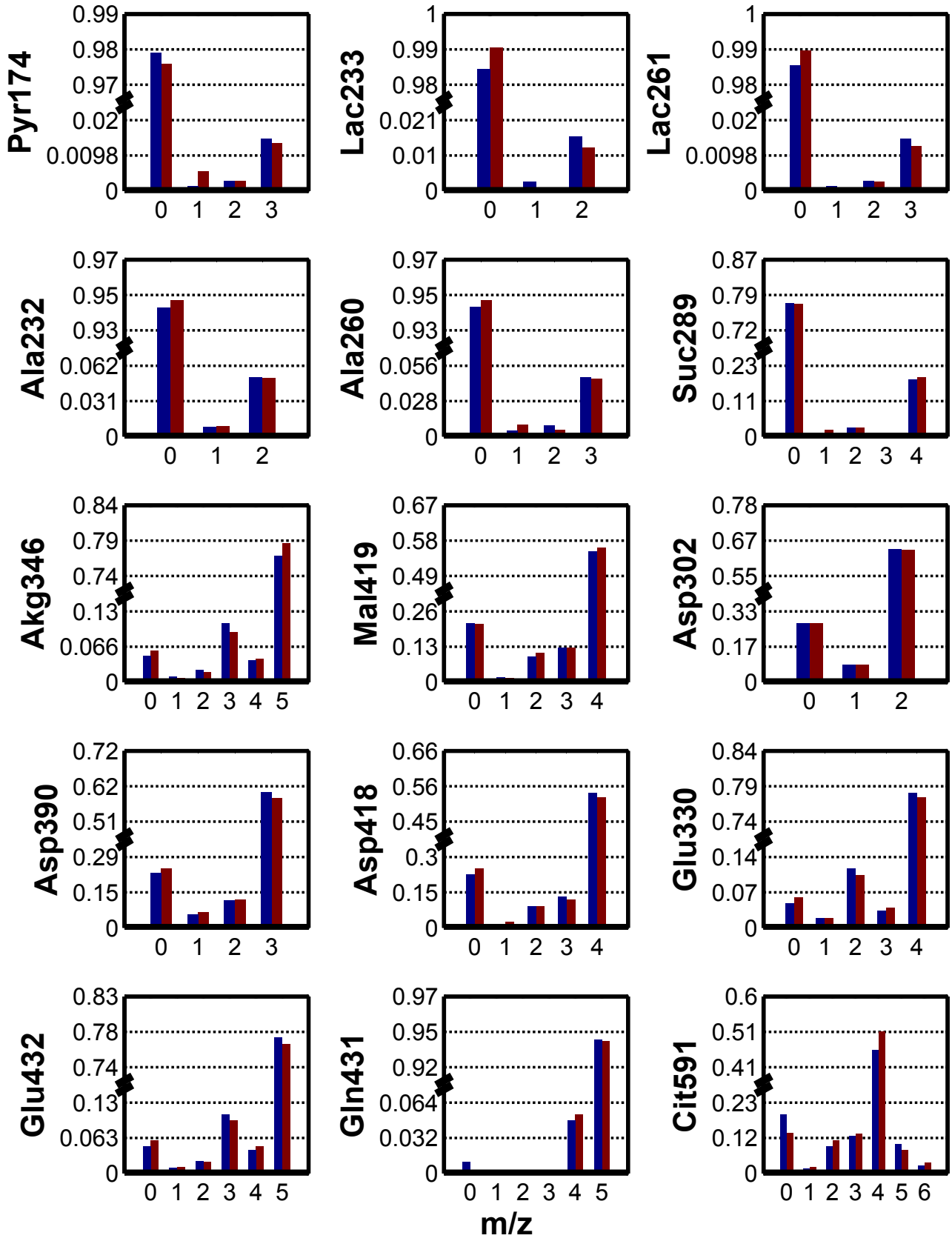


Figure S22. Simulated and measured MIDs from MFA in A549 cells expressing IDH1a shRNAs. Simulated values were obtained using Metran and the model fit listed in Table S4. Cells were cultured as described in Methods using [U-13C5]glutamine and metabolite labeling was quantified via GC/MS. MIDs are corrected for natural abundance.

Table S5. Estimated fluxes for A549 cells expressing IDH1-targeting shRNAs (IDH1b)

| **Pathway/Reaction** | Flux | 95% confidence interval | |
|---|---|---|---|
| **Glycolysis** | fmol/cell*hr | Lower bound | Upper bound |
| Glc.x -> G6P | 110.60 | 95.97 | 125.50 |
| G6P -> F6P | 102.60 | 87.90 | 117.60 |
| G6P <-> F6P | 0.00 | 0.00 | Inf |
| F6P -> DHAP + GAP | 107.60 | 92.96 | 122.50 |
| DHAP -> GAP | 107.60 | 92.96 | 122.50 |
| DHAP <-> GAP | 550.40 | 0.00 | Inf |
| GAP -> 3PG | 217.80 | 188.40 | 247.50 |
| GAP <-> 3PG | 160.00 | 0.00 | Inf |
| 3PG -> Pyr.c | 217.80 | 188.40 | 247.50 |
| Pyr.c -> Lac | 204.60 | 175.20 | 234.30 |
| Pyr.c <-> Lac | 695.70 | 0.00 | Inf |
| Lac -> Lac.x | 204.60 | 175.20 | 234.30 |
| **Pentose Phosphate Pathway** | | | |
| G6P -> P5P + CO2 | 8.01 | 6.45 | 9.57 |
| P5P + P5P -> S7P + GAP | 2.50 | 1.99 | 3.02 |
| P5P + P5P <-> S7P + GAP | 6.3E+07 | 0.00 | Inf |
| S7P + GAP -> F6P + E4P | 2.50 | 1.99 | 3.02 |
| S7P + GAP <-> F6P + E4P | 1.1E+06 | 0.00 | Inf |
| P5P + E4P -> F6P + GAP | 2.50 | 1.99 | 3.02 |
| P5P + E4P <-> F6P + GAP | 1.9E+06 | 0.00 | Inf |
| **Anaplerotic reactions** | | | |
| Pyr.m + CO2 -> Oac | 0.00 | 0.00 | 1.30 |
| Mal -> Pyr.m + CO2 | 3.56 | 2.40 | 5.03 |
| Mal <-> Pyr.m + CO2 | 1.64 | 0.27 | 1.81 |
| Glu -> Akg | 4.16 | 3.36 | 5.04 |
| Glu <-> Akg | 442.50 | 216.10 | 7234.00 |
| Oac -> Asp | 0.60 | 0.48 | 0.71 |
| Oac <-> Asp | 372.30 | 0.00 | Inf |
| **TCA Cycle** | | | |
| Pyr.m -> AcCoA.m + CO2 | 16.33 | 15.07 | 17.43 |
| AcCoA.m + Oac -> Cit | 16.33 | 15.07 | 17.43 |
| Cit -> Akg + CO2 | 5.05 | 4.43 | 5.69 |
| Cit <-> Akg + CO2 | 2.39 | 2.06 | 2.63 |
| Akg -> Suc + CO2 | 9.21 | 8.33 | 10.21 |
| Suc -> Fum | 9.21 | 8.33 | 10.21 |
| Suc <-> Fum | 0.29 | 0.00 | 1.74 |
| Fum -> Mal | 9.21 | 8.33 | 10.21 |
| Fum <-> Mal | 1.6E+07 | 310.60 | Inf |
| Mal -> Oac | 5.65 | 4.31 | 6.30 |
| Mal <-> Oac | 124.70 | 55.82 | Inf |
| **Amino acids** | | | |
| Pyr.m -> Ala | 0.42 | 0.34 | 0.50 |
| Pyr.m <-> Ala | 0.00 | 0.00 | Inf |
| Gln.x -> Gln | 25.06 | 22.28 | 27.84 |
| Gln -> Glu | 24.57 | 21.78 | 27.33 |
| Glu -> Glu.x | 19.91 | 16.95 | 22.85 |
| **Biomass** | | | |
| Cit -> AcCoA.c + Oac | 11.28 | 10.40 | 12.17 |
| AcCoA.c -> Fatty acids | 11.28 | 10.40 | 12.17 |
| 0.6*Asp + 0.5*Glu + 0.42*Ala + 0.5*Gln -> Biomass | 1.00 | 0.80 | 1.19 |
| P5P -> NTP | 0.50 | 0.40 | 0.60 |
| **Dilution/Mixing** | | | |
| Suc.d -> Suc.mnt | 0.15 | 0.13 | 0.17 |
| 0*Suc -> Suc.mnt | 0.85 | 0.83 | 0.87 |
| Pyr.c -> Pyr.m | 13.19 | 11.76 | 14.42 |
| Pyr.c <-> Pyr.m | 49.63 | 30.59 | 75.51 |
| 0*Pyr.c -> Pyr.mnt | 0.82 | 0.66 | 0.98 |
| 0*Pyr.m -> Pyr.mnt | 0.18 | 0.02 | 0.34 |

SSE = 107.4

Expected SSE = [72.5 127.3] (95% conf., 98 DOF)

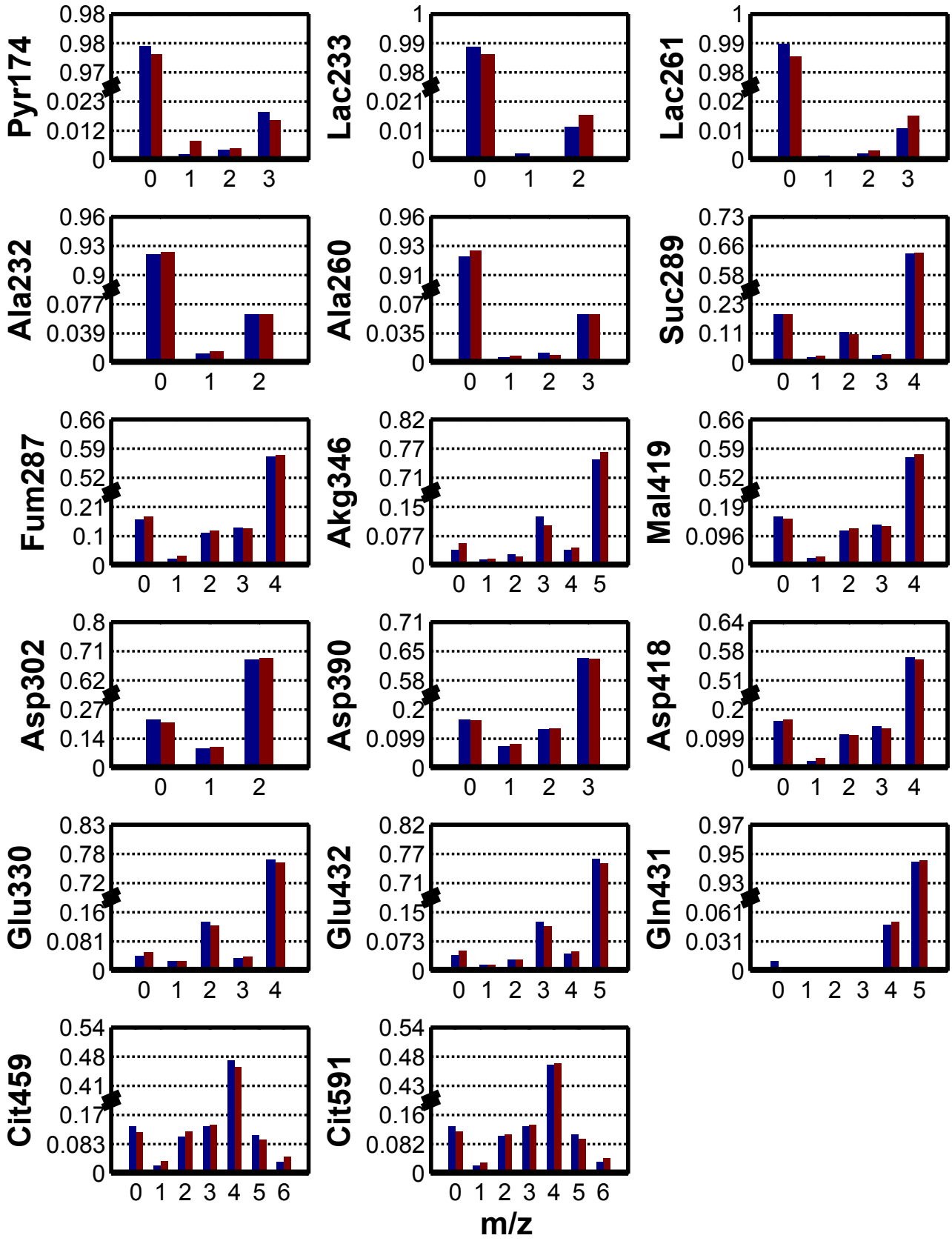


Figure S23. Simulated and measured MIDs from MFA in A549 cells expressing IDH1b shRNAs. Simulated values were obtained using Metran and the model fit listed in Table S5. Cells were cultured as described in Methods using [U-13C5]glutamine and metabolite labeling was quantified via GC/MS. MIDs are corrected for natural abundance.

Table S6. Simplified network for Isotopomer Spectral Analysis

| **% Enrichment of AcCoA (D parameter)** | |
|---|---|
| Ac.l (ab) → Ac (ab) | (AcCoA containing tracer label) |
| Ac.d (ab) → Ac (ab) | (unlabeled AcCoA) |
| 8*Ac (ab) → Palm.s (abababababababab) | |
| **de novo lipogenesis (g(t) parameter)** | |
| Palm.s → Palm | Newly synthesized palmitate |
| Palm.d → Palm | Pre-existing (unlabeled) palmitate |
| 0*Palm.s + 0*Palm.d → Palm.mnt | Mixing of pools for measurement |

**Abbreviations:**

Acetyl coenzyme A, AcCoA; α-ketoglutarate, aKG; alanine, Ala; aspartate, Asp; citrate, Cit; fumarate, Fum; glutamine, Gln; glutamate, Glu; malate, Mal; oxaloacetate, Oac; 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 Information References**


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Table S7. Palmitate mass isotopomer distributions (MIDs) used for Isotopomer Spectral Analysis in cancer cell lines with [U-13C5]gln and [5-13C]gln tracers

| Metabolite/ion | A549 [U-13C5]gln | | | H460 [U-13C5]gln | | | MDA-MB-231 [U-13C5]gln | | | Sk-Mel-5 [U-13C5]gln | | | HCT116 [U-13C5]gln | | | A431 [U-13C5]gln | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Palmitate270 (M0) | 66.0% | 59.1% | 72.8% | 52.4% | 46.2% | 48.1% | 48.7% | 49.2% | 55.8% | 66.8% | 61.3% | 61.7% | 68.3% | 70.7% | 69.0% | 66.6% | 65.5% | 67.2% |
| Palmitate271 (M1) | 13.3% | 12.5% | 14.7% | 11.4% | 10.2% | 10.4% | 11.1% | 11.2% | 12.1% | 13.8% | 13.0% | 13.0% | 13.7% | 14.2% | 14.1% | 13.5% | 13.0% | 13.3% |
| Palmitate272 (M2) | 8.3% | 11.0% | 5.4% | 14.8% | 17.1% | 16.3% | 18.6% | 19.1% | 16.3% | 10.9% | 13.8% | 13.8% | 7.1% | 6.8% | 8.2% | 6.8% | 7.0% | 6.5% |
| Palmitate273 (M3) | 2.1% | 3.1% | 1.3% | 3.6% | 4.1% | 3.9% | 4.2% | 4.3% | 3.6% | 2.3% | 3.0% | 3.0% | 1.6% | 1.5% | 1.8% | 1.3% | 1.4% | 1.3% |
| Palmitate274 (M4) | 4.8% | 6.8% | 2.7% | 7.9% | 9.4% | 9.1% | 8.6% | 8.7% | 6.8% | 3.3% | 4.9% | 4.7% | 3.6% | 3.0% | 3.4% | 4.5% | 4.8% | 4.4% |
| Palmitate275 (M5) | 1.3% | 1.8% | 0.7% | 1.7% | 2.1% | 1.9% | 1.8% | 1.7% | 1.3% | 0.7% | 1.0% | 1.0% | 0.8% | 0.6% | 0.8% | 0.9% | 1.0% | 0.8% |
| Palmitate276 (M6) | 2.3% | 3.2% | 1.2% | 3.8% | 4.6% | 4.4% | 3.0% | 2.9% | 2.1% | 1.3% | 1.7% | 1.7% | 2.3% | 1.8% | 1.8% | 2.9% | 3.1% | 2.8% |
| Palmitate277 (M7) | 0.5% | 0.7% | 0.3% | 0.7% | 0.9% | 0.9% | 0.5% | 0.5% | 0.3% | 0.2% | 0.3% | 0.3% | 0.4% | 0.3% | 0.3% | 0.5% | 0.6% | 0.5% |
| Palmitate278 (M8) | 0.8% | 1.1% | 0.4% | 1.6% | 2.1% | 2.0% | 1.0% | 0.9% | 0.6% | 0.4% | 0.6% | 0.6% | 1.0% | 0.7% | 0.5% | 1.6% | 1.8% | 1.7% |
| Palmitate279 (M9) | 0.1% | 0.2% | 0.1% | 0.3% | 0.4% | 0.4% | 0.2% | 0.1% | 0.1% | 0.1% | 0.1% | 0.0% | 0.1% | 0.0% | 0.0% | 0.3% | 0.3% | 0.2% |
| Palmitate280 (M10) | 0.5% | 0.6% | 0.2% | 0.9% | 1.4% | 1.2% | 0.6% | 0.5% | 0.3% | 0.2% | 0.2% | 0.2% | 0.6% | 0.3% | 0.2% | 0.7% | 0.8% | 0.7% |
| Palmitate281 (M11) | 0.0% | 0.0% | 0.0% | 0.2% | 0.3% | 0.3% | 0.1% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.1% | 0.1% | 0.2% |
| Palmitate282 (M12) | 0.0% | 0.0% | 0.0% | 0.5% | 0.8% | 0.7% | 0.6% | 0.4% | 0.3% | 0.0% | 0.1% | 0.1% | 0.3% | 0.1% | 0.0% | 0.2% | 0.3% | 0.2% |
| Palmitate283 (M13) | 0.0% | 0.0% | 0.0% | 0.2% | 0.2% | 0.1% | 0.2% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% |
| Palmitate284 (M14) | 0.0% | 0.0% | 0.0% | 0.2% | 0.4% | 0.3% | 0.5% | 0.2% | 0.2% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.2% | 0.3% | 0.2% |
| Palmitate285 (M15) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% |
| Palmitate286 (M16) | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.3% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **Enrichment in AcCoA pool (D value %):** | **18.2%** | **17.8%** | **17.9%** | **16.3%** | **16.8%** | **16.8%** | **13.2%** | **12.7%** | **11.8%** | **10.2%** | **10.9%** | **10.6%** | **19.2%** | **17.0%** | **14.6%** | **22.8%** | **23.4%** | **23.5%** |
| Lower bound: | 15.8% | 15.7% | 16.3% | 14.5% | 15.2% | 15.2% | 11.7% | 11.2% | 10.1% | 7.9% | 9.0% | 8.7% | 15.9% | 13.3% | 11.6% | 19.7% | 20.4% | 20.2% |
| Upper bound: | 20.8% | 20.0% | 19.5% | 18.1% | 18.4% | 18.5% | 14.7% | 14.2% | 13.5% | 12.7% | 12.7% | 12.5% | 22.7% | 20.9% | 17.9% | 26.1% | 26.5% | 26.9% |
| **de novo lipogenesis g(t)*:** | **23.5%** | **34.2%** | **13.2%** | **45.5%** | **54.1%** | **51.4%** | **57.4%** | **58.5%** | **48.9%** | **31.1%** | **40.8%** | **40.8%** | **19.7%** | **17.4%** | **21.5%** | **21.0%** | **22.3%** | **20.1%** |
| Lower bound: | 20.9% | 31.6% | 12.0% | 42.5% | 51.1% | 48.4% | 53.4% | 54.2% | 44.5% | 27.0% | 36.7% | 36.6% | 17.7% | 15.2% | 19.0% | 19.2% | 20.6% | 18.3% |
| Upper bound: | 26.3% | 36.9% | 14.3% | 48.8% | 57.5% | 54.6% | 62.0% | 63.3% | 54.1% | 36.8% | 45.9% | 46.1% | 21.8% | 19.9% | 24.6% | 22.8% | 24.2% | 21.9% |

| Metabolite/ion | A549 [5-13C]gln | | | H460 [5-13C]gln | | | MDA-MB-231 [5-13C]gln | | | Sk-Mel-5 [5-13C]gln | | | HCT116 [5-13C]gln | | | A431 [5-13C]gln | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Palmitate270 (M0) | 62.1% | 62.5% | 74.0% | 60.2% | 59.5% | 50.3% | 54.1% | 55.0% | 54.5% | 63.3% | 63.4% | 63.9% | 62.7% | 62.7% | 63.4% | 71.0% | 70.9% | 70.4% |
| Palmitate271 (M1) | 24.3% | 24.2% | 19.2% | 22.3% | 21.6% | 24.1% | 27.9% | 27.8% | 28.0% | 24.4% | 24.5% | 24.6% | 23.2% | 24.2% | 24.6% | 17.9% | 17.7% | 18.0% |
| Palmitate272 (M2) | 9.2% | 9.0% | 4.8% | 8.9% | 8.6% | 11.7% | 11.9% | 11.5% | 11.7% | 7.9% | 7.9% | 7.8% | 8.0% | 7.9% | 7.9% | 5.4% | 5.6% | 5.5% |
| Palmitate273 (M3) | 3.1% | 3.1% | 1.4% | 4.2% | 4.2% | 6.0% | 4.0% | 3.8% | 4.0% | 2.5% | 2.3% | 2.2% | 2.9% | 2.7% | 2.5% | 2.6% | 3.0% | 2.9% |
| Palmitate274 (M4) | 0.9% | 0.8% | 0.4% | 2.2% | 2.5% | 3.5% | 1.3% | 1.1% | 1.1% | 0.9% | 0.8% | 0.7% | 1.4% | 1.1% | 0.9% | 1.6% | 1.6% | 1.6% |
| Palmitate275 (M5) | 0.2% | 0.2% | 0.1% | 1.2% | 1.7% | 2.3% | 0.4% | 0.3% | 0.3% | 0.4% | 0.4% | 0.3% | 0.8% | 0.5% | 0.3% | 0.6% | 0.7% | 0.7% |
| Palmitate276 (M6) | 0.0% | 0.0% | 0.0% | 0.8% | 1.3% | 1.5% | 0.0% | 0.1% | 0.0% | 0.5% | 0.5% | 0.4% | 0.8% | 0.6% | 0.4% | 0.3% | 0.1% | 0.3% |
| Palmitate277 (M7) | 0.0% | 0.0% | 0.0% | 0.3% | 0.5% | 0.5% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 0.0% | 0.2% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate278 (M8) | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.1% | 0.2% | 0.1% | 0.1% | 0.1% | 0.1% | 0.3% | 0.3% | 0.5% |
| Palmitate279 (M9) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% |
| Palmitate280 (M10) | 0.3% | 0.2% | 0.1% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% |
| Palmitate281 (M11) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% |
| Palmitate282 (M12) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate283 (M13) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate284 (M14) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 0.1% | 0.1% |
| Palmitate285 (M15) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 0.1% |
| Palmitate286 (M16) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **Enrichment in AcCoA pool (D value %):** | **13.8%** | **13.7%** | **14.1%** | **17.3%** | **19.0%** | **18.4%** | **11.9%** | **11.5%** | **11.6%** | **10.5%** | **10.2%** | **9.7%** | **12.9%** | **11.1%** | **10.3%** | **22.4%** | **23.7%** | **23.0%** |
| Lower bound: | 12.5% | 12.4% | 11.9% | 14.5% | 14.7% | 15.1% | 11.0% | 10.6% | 10.6% | 9.1% | 8.8% | 8.4% | 10.5% | 8.9% | 8.1% | 17.7% | 19.1% | 18.3% |
| Upper bound: | 15.1% | 14.9% | 16.5% | 20.2% | 23.7% | 22.0% | 12.9% | 12.5% | 12.6% | 11.9% | 11.6% | 11.1% | 15.3% | 13.5% | 12.6% | 27.5% | 28.5% | 27.8% |
| **de novo lipogenesis g(t)*:** | **34.0%** | **33.6%** | **13.2%** | **32.8%** | **31.4%** | **46.2%** | **53.7%** | **53.0%** | **53.9%** | **38.7%** | **39.2%** | **39.6%** | **34.4%** | **38.0%** | **39.2%** | **14.9%** | **15.1%** | **15.7%** |
| Lower bound: | 29.5% | 29.1% | 10.2% | 29.4% | 26.9% | 41.2% | 50.8% | 0.5% | 50.9% | 35.3% | 35.7% | 35.8% | 30.5% | 33.1% | 33.8% | 12.9% | 13.1% | 13.6% |
| Upper bound: | 39.1% | 38.7% | 17.0% | 36.8% | 37.1% | 52.2% | 56.9% | 56.4% | 57.3% | 42.8% | 43.5% | 44.2% | 39.5% | 44.5% | 46.6% | 17.3% | 17.2% | 17.9% |

* de novo lipogenesis, g(t), varies from well to well due to differences in cell growth and time cultured in the presence of tracer

ISA modeling deconvolutes enrichment constant (D value) from de novo lipogenesis to obtain independent values.

The consistency of D values across replicates reflects the similarity of metabolic pathways across replicates.

Here, mass isotopomer distributions (MIDs) %'s are not corrected for natural isotope abundance, as this is accounted for within Metran

Table S8. Palmitate mass isotopomer distributions (MIDs) used for ISA in A549 and H1299 under normoxia and hypoxia using [U-13C6]gluc and [5-13C]gln tracers

**[U-13C6]glucose**

| **Metabolite/ion** | **A549 normoxia** | | | **A549 hypoxia** | | | **H1299 normoxia** | | | **H1299 hypoxia** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Palmitate270 (M0) | 38.4% | 37.2% | 39.5% | 65.4% | 64.6% | 65.4% | 30.9% | 30.8% | 31.0% | 52.0% | 46.8% | 51.0% |
| Palmitate271 (M1) | 8.0% | 7.4% | 7.9% | 13.2% | 12.6% | 13.2% | 6.6% | 6.3% | 6.4% | 10.9% | 9.6% | 10.3% |
| Palmitate272 (M2) | 1.7% | 1.6% | 1.7% | 8.5% | 8.2% | 8.3% | 2.0% | 1.9% | 1.9% | 3.7% | 3.9% | 4.0% |
| Palmitate273 (M3) | 0.4% | 0.3% | 0.3% | 1.5% | 1.4% | 1.4% | 0.6% | 0.3% | 0.3% | 0.8% | 0.7% | 0.7% |
| Palmitate274 (M4) | 0.7% | 0.6% | 0.7% | 2.8% | 2.6% | 2.7% | 0.6% | 0.4% | 0.4% | 3.5% | 4.6% | 4.0% |
| Palmitate275 (M5) | 0.5% | 0.4% | 0.5% | 0.6% | 0.6% | 0.6% | 0.5% | 0.2% | 0.2% | 0.9% | 1.1% | 0.9% |
| Palmitate276 (M6) | 1.6% | 1.6% | 1.6% | 1.4% | 1.3% | 1.4% | 0.6% | 0.6% | 0.7% | 4.9% | 6.5% | 5.4% |
| Palmitate277 (M7) | 0.9% | 0.9% | 1.0% | 0.4% | 0.5% | 0.4% | 0.4% | 0.3% | 0.4% | 1.0% | 1.4% | 1.1% |
| Palmitate278 (M8) | 3.5% | 3.4% | 3.4% | 1.3% | 1.6% | 1.4% | 1.3% | 1.5% | 1.6% | 5.4% | 7.2% | 5.7% |
| Palmitate279 (M9) | 1.3% | 1.3% | 1.3% | 0.3% | 0.4% | 0.4% | 1.0% | 1.0% | 1.0% | 1.1% | 1.4% | 1.1% |
| Palmitate280 (M10) | 6.5% | 6.9% | 6.4% | 1.1% | 1.3% | 1.2% | 3.5% | 3.6% | 3.8% | 4.9% | 6.2% | 5.0% |
| Palmitate281 (M11) | 2.2% | 2.3% | 2.3% | 0.2% | 0.2% | 0.3% | 2.5% | 2.5% | 2.5% | 0.9% | 1.0% | 0.9% |
| Palmitate282 (M12) | 10.9% | 11.2% | 10.6% | 1.2% | 1.6% | 1.3% | 8.6% | 8.8% | 9.0% | 4.0% | 4.4% | 3.9% |
| Palmitate283 (M13) | 2.7% | 2.9% | 2.8% | 0.3% | 0.3% | 0.3% | 5.0% | 5.0% | 4.9% | 0.8% | 0.8% | 0.8% |
| Palmitate284 (M14) | 12.2% | 12.9% | 11.9% | 1.1% | 1.7% | 1.2% | 15.9% | 16.2% | 16.1% | 3.1% | 2.7% | 3.0% |
| Palmitate285 (M15) | 1.9% | 2.0% | 1.8% | 0.2% | 0.2% | 0.2% | 5.2% | 5.3% | 5.2% | 0.5% | 0.4% | 0.5% |
| Palmitate286 (M16) | 6.8% | 7.2% | 6.7% | 0.6% | 0.9% | 0.6% | 14.9% | 15.4% | 14.7% | 1.7% | 1.3% | 1.6% |
| **Enrichment in AcCoA pool (D value %):** | **78.4%** | **78.7%** | **78.4%** | **14.8%** | **15.9%** | **15.5%** | **86.7%** | **86.8%** | **86.4%** | **51.7%** | **49.4%** | **49.8%** |
| Lower bound: | 76.1% | 76.4% | 76.0% | 9.3% | 10.2% | 9.8% | 85.2% | 85.3% | 84.8% | 46.1% | 45.1% | 44.4% |
| Upper bound: | 80.6% | 80.8% | 80.7% | 21.1% | 22.7% | 22.1% | 88.1% | 88.2% | 87.8% | 57.5% | 53.6% | 55.1% |
| **de novo lipogenesis g(t)*:** | **51.2%** | **52.9%** | **50.0%** | **24.6%** | **24.4%** | **23.9%** | **59.2%** | **59.8%** | **59.4%** | **34.1%** | **41.0%** | **35.6%** |
| Lower bound: | 48.2% | 50.0% | 47.0% | 19.8% | 19.8% | 19.3% | 56.3% | 56.8% | 56.5% | 31.2% | 38.1% | 32.7% |
| Upper bound: | 54.1% | 55.9% | 52.9% | 31.8% | 30.9% | 30.6% | 62.1% | 62.7% | 62.3% | 37.1% | 44.0% | 38.6% |

**[5-13C]gln**

| **Metabolite/ion** | **A549 normoxia** | | | **A549 hypoxia** | | | **H1299 normoxia** | | | **H1299 hypoxia** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Palmitate270 (M0) | 58.6% | 59.0% | 58.4% | 55.2% | 56.1% | 55.1% | 74.3% | 73.8% | | 57.7% | 54.4% | 48.6% |
| Palmitate271 (M1) | 24.4% | 24.4% | 25.6% | 12.5% | 12.4% | 12.7% | 20.0% | 20.0% | | 15.4% | 14.5% | 13.4% |
| Palmitate272 (M2) | 9.9% | 9.5% | 9.8% | 3.0% | 2.8% | 3.0% | 3.8% | 3.8% | | 5.8% | 5.8% | 6.9% |
| Palmitate273 (M3) | 3.5% | 3.0% | 3.1% | 1.8% | 1.5% | 1.8% | 0.7% | 0.9% | | 5.2% | 6.0% | 7.7% |
| Palmitate274 (M4) | 1.3% | 1.2% | 1.1% | 2.6% | 2.3% | 2.6% | 0.4% | 0.6% | | 5.8% | 6.7% | 8.7% |
| Palmitate275 (M5) | 0.4% | 0.4% | 0.3% | 4.6% | 4.3% | 4.5% | 0.1% | 0.3% | | 4.6% | 5.8% | 7.1% |
| Palmitate276 (M6) | 0.3% | 0.3% | 0.2% | 7.0% | 6.9% | 7.1% | 0.2% | 0.2% | | 3.2% | 4.0% | 4.6% |
| Palmitate277 (M7) | 0.1% | 0.2% | 0.1% | 7.6% | 7.6% | 7.3% | 0.1% | 0.2% | | 1.6% | 2.0% | 2.1% |
| Palmitate278 (M8) | 0.4% | 0.3% | 0.3% | 4.5% | 4.7% | 4.4% | 0.2% | 0.1% | | 0.6% | 0.7% | 0.7% |
| Palmitate279 (M9) | 0.1% | 0.1% | 0.1% | 0.5% | 0.6% | 0.5% | 0.0% | 0.0% | | 0.1% | 0.1% | 0.1% |
| Palmitate280 (M10) | 0.2% | 0.3% | 0.2% | 0.2% | 0.2% | 0.2% | 0.1% | 0.1% | | 0.1% | 0.0% | 0.1% |
| Palmitate281 (M11) | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | | 0.0% | 0.0% | 0.0% |
| Palmitate282 (M12) | 0.2% | 0.4% | 0.3% | 0.2% | 0.2% | 0.2% | 0.0% | 0.0% | | 0.0% | 0.0% | 0.0% |
| Palmitate283 (M13) | 0.0% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.0% | 0.0% | | 0.0% | 0.0% | 0.0% |
| Palmitate284 (M14) | 0.3% | 0.5% | 0.3% | 0.2% | 0.2% | 0.2% | 0.0% | 0.0% | | 0.0% | 0.0% | 0.0% |
| Palmitate285 (M15) | 0.0% | 0.1% | 0.1% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | | 0.0% | 0.0% | 0.0% |
| Palmitate286 (M16) | 0.2% | 0.3% | 0.2% | 0.1% | 0.2% | 0.2% | 0.0% | 0.0% | | 0.0% | 0.0% | 0.0% |
| **Enrichment in AcCoA pool (D value %):** | **13.3%** | **12.5%** | **11.7%** | **77.6%** | **78.7%** | **77.2%** | **6.4%** | **7.1%** | | **54.6%** | **47.9%** | **47.2%** |
| Lower bound: | 12.2% | 10.6% | 9.9% | 73.6% | 74.7% | 73.1% | 1.9% | 2.7% | | 49.4% | 43.4% | 43.7% |
| Upper bound: | 14.6% | 14.5% | 13.6% | 81.3% | 82.3% | 80.9% | 11.4% | 12.2% | | 59.7% | 52.3% | 50.7% |
| **de novo lipogenesis g(t)*:** | **41.0%** | **41.5%** | **45.2%** | **30.1%** | **29.2%** | **30.0%** | **22.7%** | **21.9%** | | **27.7%** | **31.8%** | **39.1%** |
| Lower bound: | 38.6% | 37.4% | 40.6% | 27.3% | 26.4% | 27.2% | 14.4% | 14.4% | | 25.2% | 29.2% | 36.5% |
| Upper bound: | 43.8% | 46.6% | 50.8% | 32.9% | 32.0% | 32.8% | 62.2% | 48.7% | | 30.3% | 34.3% | 41.6% |

* de novo lipogenesis, g(t), varies from well to well due to differences in cell growth and time cultured in the presence of tracer

ISA modeling deconvolutes enrichment constant (D value) from de novo lipogenesis to obtain independent values.

The consistency of D values across replicates reflects the similarity of metabolic pathways across replicates.

Here, mass isotopomer distributions (MIDs) %'s are not corrected for natural isotope abundance, as this is accounted for within Metran

Table S9. Palmitate mass isotopomer distributions (MIDs) used for ISA in HCT116, MDA-MB-231, and 143B cell lines under normoxia and hypoxia using [U-13C6]gluc and [5-13C]gln tracers

| **[U-13C6]glucose** | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Metabolite/ion** | **HCT116 normoxia** | | | **HCT116 hypoxia** | | | **MDA-MB-231 normoxia** | | | **MDA-MB-231 hypoxia** | | | **143B normoxia** | | | **143B hypoxia** | | |
| Palmitate270 (M0) | 41.0% | 42.3% | 44.6% | 60.6% | 60.7% | 62.2% | 39.7% | 41.2% | 42.2% | 54.1% | 56.4% | 56.7% | 29.2% | 30.4% | | 42.5% | 48.2% | 44.8% |
| Palmitate271 (M1) | 9.3% | 8.7% | 8.8% | 12.7% | 12.0% | 12.4% | 8.1% | 8.5% | 8.7% | 11.0% | 11.3% | 11.2% | 6.1% | 6.4% | | 8.9% | 9.8% | 9.1% |
| Palmitate272 (M2) | 2.6% | 2.2% | 2.3% | 7.7% | 8.1% | 7.3% | 1.8% | 1.9% | 1.9% | 6.8% | 8.4% | 7.3% | 1.4% | 1.5% | | 9.6% | 13.6% | 11.4% |
| Palmitate273 (M3) | 0.5% | 0.6% | 0.6% | 1.4% | 1.5% | 1.4% | 0.4% | 0.5% | 0.5% | 1.2% | 1.5% | 1.3% | 0.3% | 0.4% | | 1.9% | 2.5% | 2.2% |
| Palmitate274 (M4) | 1.1% | 1.4% | 1.4% | 4.7% | 5.0% | 4.7% | 0.4% | 0.6% | 0.6% | 4.3% | 4.5% | 4.3% | 0.4% | 0.6% | | 9.9% | 9.0% | 9.6% |
| Palmitate275 (M5) | 0.4% | 0.6% | 0.4% | 1.0% | 1.1% | 0.9% | 0.2% | 0.5% | 0.4% | 0.9% | 1.0% | 0.9% | 0.4% | 0.5% | | 1.9% | 1.7% | 1.9% |
| Palmitate276 (M6) | 1.8% | 2.1% | 2.1% | 3.2% | 3.3% | 3.1% | 0.6% | 0.7% | 0.7% | 3.7% | 3.3% | 3.3% | 0.8% | 0.9% | | 9.0% | 5.3% | 6.9% |
| Palmitate277 (M7) | 0.7% | 0.8% | 0.8% | 0.7% | 0.6% | 0.7% | 0.3% | 0.4% | 0.4% | 0.8% | 0.7% | 0.7% | 0.5% | 0.5% | | 1.6% | 1.0% | 1.3% |
| Palmitate278 (M8) | 3.9% | 3.8% | 3.9% | 2.5% | 2.4% | 2.5% | 1.6% | 1.5% | 1.3% | 4.0% | 3.2% | 3.3% | 1.9% | 2.0% | | 6.7% | 3.1% | 4.4% |
| Palmitate279 (M9) | 1.3% | 1.4% | 1.3% | 0.6% | 0.5% | 0.5% | 0.6% | 0.6% | 0.6% | 0.8% | 0.6% | 0.6% | 0.8% | 0.8% | | 1.1% | 0.5% | 0.7% |
| Palmitate280 (M10) | 6.5% | 6.3% | 6.3% | 1.7% | 1.7% | 1.6% | 4.3% | 3.9% | 4.0% | 3.6% | 2.6% | 3.1% | 5.2% | 5.1% | | 3.8% | 1.7% | 2.5% |
| Palmitate281 (M11) | 2.0% | 2.0% | 1.9% | 0.2% | 0.2% | 0.2% | 1.5% | 1.5% | 1.5% | 0.6% | 0.5% | 0.5% | 2.0% | 2.0% | | 0.5% | 0.2% | 0.3% |
| Palmitate282 (M12) | 9.8% | 9.4% | 9.0% | 1.3% | 1.2% | 1.0% | 9.8% | 9.2% | 9.2% | 3.4% | 2.4% | 2.8% | 12.5% | 12.1% | | 1.7% | 1.2% | 1.8% |
| Palmitate283 (M13) | 2.3% | 2.4% | 2.1% | 0.2% | 0.2% | 0.2% | 2.9% | 2.6% | 2.6% | 0.5% | 0.4% | 0.5% | 3.7% | 3.6% | | 0.2% | 0.2% | 0.3% |
| Palmitate284 (M14) | 10.2% | 9.8% | 8.9% | 1.0% | 0.8% | 0.8% | 14.8% | 14.1% | 13.8% | 2.7% | 2.0% | 2.2% | 18.8% | 17.9% | | 0.6% | 1.2% | 1.6% |
| Palmitate285 (M15) | 1.5% | 1.5% | 1.2% | 0.1% | 0.1% | 0.1% | 2.3% | 2.2% | 2.2% | 0.3% | 0.3% | 0.3% | 3.0% | 3.0% | | 0.1% | 0.2% | 0.3% |
| Palmitate286 (M16) | 5.3% | 5.0% | 4.3% | 0.5% | 0.4% | 0.4% | 10.6% | 10.0% | 9.7% | 1.3% | 1.1% | 1.1% | 12.9% | 12.2% | | 0.2% | 0.7% | 1.0% |
| **Enrichment in AcCoA pool (D value %):** | **76.3%** | **75.9%** | **74.7%** | **24.9%** | **23.9%** | **25.3%** | **84.0%** | **84.0%** | **83.9%** | **40.4%** | **26.7%** | **32.6%** | **83.8%** | **83.6%** | | **32.3%** | **21.1%** | **26.5%** |
| Lower bound: | 73.5% | 73.1% | 71.6% | 19.4% | 18.7% | 19.6% | 82.1% | 82.1% | 81.9% | 29.1% | 19.1% | 22.7% | 82.0% | 81.8% | | 28.7% | 17.5% | 22.9% |
| Upper bound: | 78.9% | 78.6% | 77.6% | 31.2% | 29.6% | 31.7% | 85.7% | 85.8% | 85.7% | 54.5% | 37.2% | 46.5% | 85.5% | 85.4% | | 36.0% | 24.8% | 30.2% |
| **de novo lipogenesis g(t)*:** | **47.3%** | **46.0%** | **43.5%** | **26.5%** | **27.3%** | **25.0%** | **50.4%** | **48.2%** | **47.2%** | **31.6%** | **31.0%** | **29.4%** | **62.9%** | **61.1%** | | **48.6%** | **45.7%** | **46.6%** |
| Lower bound: | 44.3% | 43.1% | 40.6% | 23.2% | 23.9% | 21.7% | 47.4% | 45.3% | 44.2% | 27.5% | 26.4% | 25.2% | 59.2% | 57.4% | | 44.7% | 40.9% | 42.5% |
| Upper bound: | 50.2% | 49.0% | 46.4% | 30.1% | 31.0% | 28.5% | 53.3% | 51.2% | 50.1% | 35.7% | 35.8% | 33.9% | 66.7% | 64.9% | | 52.6% | 50.8% | 50.9% |

| **[5-13C]gln** | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Metabolite/ion** | **HCT116 normoxia** | | | **HCT116 hypoxia** | | | **MDA-MB-231 normoxia** | | | **MDA-MB-231 hypoxia** | | | **143B normoxia** | | | **143B hypoxia** | | |
| Palmitate270 (M0) | 60.6% | 62.1% | 61.5% | 59.1% | 59.5% | | 66.9% | 65.4% | 66.0% | 54.6% | 52.3% | 54.0% | 58.7% | 57.4% | 58.2% | 39.4% | 41.4% | |
| Palmitate271 (M1) | 22.6% | 22.6% | 23.0% | 14.0% | 14.1% | | 23.2% | 23.1% | 22.9% | 14.4% | 13.0% | 13.9% | 27.8% | 27.6% | 24.6% | 9.9% | 10.4% | |
| Palmitate272 (M2) | 8.3% | 8.1% | 8.3% | 4.3% | 4.8% | | 6.3% | 6.6% | 6.4% | 6.0% | 4.7% | 5.1% | 9.6% | 9.9% | 10.1% | 3.5% | 4.6% | |
| Palmitate273 (M3) | 3.0% | 2.8% | 3.0% | 3.4% | 3.7% | | 1.4% | 1.5% | 1.6% | 5.0% | 4.2% | 4.4% | 2.7% | 2.8% | 3.2% | 4.0% | 5.3% | |
| Palmitate274 (M4) | 1.6% | 1.4% | 1.5% | 4.1% | 4.5% | | 0.5% | 0.6% | 0.6% | 5.2% | 5.4% | 5.1% | 0.7% | 0.9% | 1.3% | 7.1% | 8.1% | |
| Palmitate275 (M5) | 0.7% | 0.6% | 0.6% | 4.5% | 4.6% | | 0.2% | 0.2% | 0.2% | 5.3% | 6.2% | 5.6% | 0.2% | 0.2% | 0.4% | 9.8% | 9.6% | |
| Palmitate276 (M6) | 0.6% | 0.4% | 0.4% | 4.5% | 3.8% | | 0.2% | 0.3% | 0.3% | 4.4% | 6.4% | 5.4% | 0.0% | 0.3% | 0.3% | 11.6% | 9.5% | |
| Palmitate277 (M7) | 0.3% | 0.2% | 0.2% | 3.3% | 2.5% | | 0.1% | 0.1% | 0.1% | 3.0% | 4.5% | 3.6% | 0.1% | 0.1% | 0.1% | 9.4% | 6.8% | |
| Palmitate278 (M8) | 0.5% | 0.5% | 0.4% | 1.8% | 1.3% | | 0.4% | 0.4% | 0.4% | 1.3% | 2.2% | 1.9% | 0.1% | 0.4% | 0.4% | 4.6% | 3.1% | |
| Palmitate279 (M9) | 0.1% | 0.1% | 0.1% | 0.3% | 0.2% | | 0.1% | 0.1% | 0.1% | 0.2% | 0.3% | 0.2% | 0.1% | 0.1% | 0.1% | 0.5% | 0.5% | |
| Palmitate280 (M10) | 0.3% | 0.3% | 0.3% | 0.3% | 0.3% | | 0.2% | 0.4% | 0.3% | 0.2% | 0.2% | 0.2% | 0.1% | 0.2% | 0.2% | 0.1% | 0.2% | |
| Palmitate281 (M11) | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | |
| Palmitate282 (M12) | 0.4% | 0.3% | 0.2% | 0.1% | 0.2% | | 0.2% | 0.4% | 0.3% | 0.1% | 0.2% | 0.2% | 0.0% | 0.1% | 0.2% | 0.0% | 0.2% | |
| Palmitate283 (M13) | 0.1% | 0.1% | 0.1% | 0.0% | 0.1% | | 0.0% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | |
| Palmitate284 (M14) | 0.5% | 0.3% | 0.3% | 0.3% | 0.2% | | 0.2% | 0.4% | 0.4% | 0.1% | 0.3% | 0.2% | 0.0% | 0.0% | 0.4% | 0.0% | 0.2% | |
| Palmitate285 (M15) | 0.1% | 0.1% | 0.1% | 0.0% | 0.0% | | 0.0% | 0.1% | 0.1% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | |
| Palmitate286 (M16) | 0.4% | 0.2% | 0.1% | 0.2% | 0.1% | | 0.1% | 0.3% | 0.3% | 0.1% | 0.2% | 0.1% | 0.0% | 0.0% | 0.3% | 0.0% | 0.1% | |
| **Enrichment in AcCoA pool (D value %):** | **14.3%** | **13.7%** | **13.7%** | **59.2%** | **53.7%** | | **8.5%** | **10.0%** | **9.3%** | **51.4%** | **61.9%** | **57.8%** | **9.1%** | **9.1%** | **12.9%** | **69.4%** | **63.2%** | |
| Lower bound: | 12.0% | 11.3% | 11.4% | 52.2% | 46.6% | | 6.9% | 8.2% | 7.8% | 44.4% | 56.2% | 50.3% | 8.0% | 8.7% | 11.8% | 66.1% | 59.4% | |
| Upper bound: | 16.6% | 16.1% | 16.1% | 66.5% | 60.8% | | 10.1% | 11.9% | 10.9% | 58.3% | 67.4% | 64.9% | 10.1% | 10.8% | 14.1% | 72.7% | 66.9% | |
| **de novo lipogenesis g(t)*:** | **34.9%** | **33.8%** | **34.9%** | **25.0%** | **24.8%** | | **35.6%** | **34.9%** | **34.7%** | **29.3%** | **32.1%** | **30.3%** | **53.9%** | **53.3%** | **42.6%** | **48.6%** | **45.9%** | |
| Lower bound: | 31.4% | 30.1% | 31.2% | 22.1% | 22.0% | | 31.1% | 31.1% | 30.8% | 25.7% | 28.6% | 26.6% | 49.6% | 49.5% | 40.1% | 45.0% | 42.2% | |
| Upper bound: | 39.2% | 38.3% | 39.4% | 27.8% | 27.7% | | 41.6% | 40.0% | 39.8% | 32.8% | 35.6% | 34.0% | 59.0% | 57.8% | 45.5% | 52.3% | 49.5% | |

* de novo lipogenesis, g(t), varies from well to well due to differences in cell growth and time cultured in the presence of tracer

ISA modeling deconvolutes enrichment constant (D value) from de novo lipogenesis to obtain independent values.

The consistency of D values across replicates reflects the similarity of metabolic pathways across replicates.

Here, mass isotopomer distributions (MIDs) %'s are not corrected for natural isotope abundance, as this is accounted for within Metran

Table S10. Palmitate mass isotopomer distributions (MIDs) used for ISA

| **[U-13C6]glucose** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Metabolite/ion** | **MRC5 normoxia** | | | **MRC5 hypoxia** | | | **MCF10A normoxia** | | | **MCF10A hypoxia** | | |
| Palmitate270 (M0) | 48.5% | 49.0% | 49.1% | 72.9% | 70.7% | 72.2% | 46.2% | 42.8% | 41.3% | 75.6% | 75.7% | 74.9% |
| Palmitate271 (M1) | 10.6% | 10.0% | 10.2% | 16.4% | 14.5% | 14.5% | 9.4% | 8.7% | 8.3% | 15.0% | 15.0% | 15.0% |
| Palmitate272 (M2) | 2.7% | 2.5% | 2.5% | 5.6% | 5.1% | 4.7% | 1.5% | 1.5% | 1.5% | 3.3% | 3.3% | 3.5% |
| Palmitate273 (M3) | 0.6% | 0.5% | 0.5% | 1.2% | 0.9% | 0.8% | 0.3% | 0.3% | 0.3% | 0.5% | 0.5% | 0.6% |
| Palmitate274 (M4) | 0.5% | 0.4% | 0.5% | 1.9% | 2.0% | 1.7% | 1.3% | 1.3% | 1.4% | 1.5% | 1.4% | 1.7% |
| Palmitate275 (M5) | 0.1% | 0.2% | 0.2% | 0.4% | 0.4% | 0.3% | 0.6% | 0.6% | 0.6% | 0.3% | 0.3% | 0.4% |
| Palmitate276 (M6) | 0.3% | 0.4% | 0.4% | 0.7% | 0.8% | 0.7% | 3.4% | 3.7% | 3.8% | 1.2% | 1.2% | 1.3% |
| Palmitate277 (M7) | 0.2% | 0.2% | 0.2% | 0.2% | 0.2% | 0.1% | 1.6% | 1.7% | 1.7% | 0.6% | 0.6% | 0.6% |
| Palmitate278 (M8) | 0.9% | 1.0% | 1.0% | 0.4% | 0.5% | 0.4% | 7.0% | 7.5% | 7.7% | 1.0% | 1.0% | 1.0% |
| Palmitate279 (M9) | 0.5% | 0.5% | 0.5% | 0.1% | 0.2% | 0.1% | 2.3% | 2.5% | 2.6% | 0.2% | 0.2% | 0.2% |
| Palmitate280 (M10) | 2.4% | 2.4% | 2.4% | 0.2% | 0.5% | 0.5% | 9.1% | 10.0% | 10.3% | 0.5% | 0.5% | 0.5% |
| Palmitate281 (M11) | 1.1% | 1.2% | 1.2% | 0.0% | 0.1% | 0.0% | 2.4% | 2.6% | 2.7% | 0.0% | 0.0% | 0.0% |
| Palmitate282 (M12) | 6.1% | 6.1% | 6.2% | 0.0% | 0.8% | 0.7% | 7.9% | 8.8% | 9.2% | 0.2% | 0.2% | 0.2% |
| Palmitate283 (M13) | 2.4% | 2.5% | 2.5% | 0.0% | 0.3% | 0.3% | 1.5% | 1.7% | 1.8% | 0.0% | 0.0% | 0.1% |
| Palmitate284 (M14) | 11.1% | 11.1% | 11.0% | 0.1% | 1.5% | 1.4% | 4.2% | 4.6% | 5.0% | 0.1% | 0.1% | 0.1% |
| Palmitate285 (M15) | 2.6% | 2.5% | 2.5% | 0.0% | 0.4% | 0.3% | 0.5% | 0.5% | 0.6% | 0.1% | 0.0% | 0.1% |
| Palmitate286 (M16) | 9.5% | 9.5% | 9.4% | 0.0% | 1.3% | 1.3% | 1.0% | 1.2% | 1.2% | 0.0% | 0.0% | 0.0% |
| **Enrichment in AcCoA pool (D value %):** | **86.21** | **86.23** | **86.05** | **13.95** | **18.61** | **18.24** | **62.74** | **63.09** | **63.31** | **29.48** | **29.25** | **28.38** |
| Lower bound: | 84.47 | 84.48 | 84.28 | 6.9 | 11.63 | 10.46 | 61.1 | 61.58 | 61.84 | 23.99 | 23.5 | 23.57 |
| Upper bound: | 87.82 | 87.86 | 87.7 | 22.04 | 26.94 | 27.81 | 64.36 | 64.57 | 64.74 | 35.3 | 35.37 | 33.46 |
| **de novo lipogenesis g(t)*:** | **38.74** | **38.48** | **38.36** | **13.84** | **14.34** | **12.63** | **42.65** | **46.8** | **48.61** | **7.805** | **7.674** | **8.767** |
| Lower bound: | 36.45 | 36.18 | 36.07 | 10.18 | 11.34 | 9.627 | 41.09 | 45.23 | 47.03 | 6.823 | 6.69 | 7.775 |
| Upper bound: | 41.03 | 40.77 | 40.65 | 20.64 | 18.12 | 16.65 | 44.22 | 48.37 | 50.18 | 8.799 | 8.671 | 9.773 |

| **[5-13C]gln** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Metabolite/ion** | **MRC5 normoxia** | | | **MRC5 hypoxia** | | | **MCF10A normoxia** | | | **MCF10A hypoxia** | | |
| Palmitate270 (M0) | 67.4% | 67.3% | 68.8% | 70.2% | 70.8% | 71.9% | 73.8% | 73.6% | 73.4% | 76.8% | 76.5% | 76.3% |
| Palmitate271 (M1) | 22.7% | 23.4% | 22.4% | 14.6% | 16.2% | 16.3% | 21.1% | 21.4% | 21.5% | 17.1% | 17.1% | 17.3% |
| Palmitate272 (M2) | 5.8% | 6.0% | 5.6% | 2.2% | 2.6% | 2.7% | 3.8% | 3.9% | 3.9% | 3.5% | 3.6% | 3.6% |
| Palmitate273 (M3) | 1.4% | 1.5% | 1.4% | 0.6% | 0.7% | 0.7% | 0.5% | 0.5% | 0.5% | 1.2% | 1.2% | 1.2% |
| Palmitate274 (M4) | 0.4% | 0.4% | 0.4% | 0.8% | 0.7% | 0.6% | 0.0% | 0.1% | 0.1% | 0.4% | 0.5% | 0.5% |
| Palmitate275 (M5) | 0.2% | 0.2% | 0.2% | 1.1% | 1.3% | 1.1% | 0.0% | 0.0% | 0.0% | 0.2% | 0.2% | 0.2% |
| Palmitate276 (M6) | 0.2% | 0.2% | 0.3% | 1.8% | 2.1% | 2.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% |
| Palmitate277 (M7) | 0.2% | 0.3% | 0.3% | 2.1% | 2.7% | 2.5% | 0.2% | 0.2% | 0.3% | 0.4% | 0.4% | 0.4% |
| Palmitate278 (M8) | 0.4% | 0.2% | 0.3% | 1.6% | 1.8% | 1.8% | 0.2% | 0.1% | 0.2% | 0.2% | 0.2% | 0.2% |
| Palmitate279 (M9) | 0.1% | 0.0% | 0.1% | 0.3% | 0.2% | 0.2% | 0.0% | 0.1% | 0.0% | 0.0% | 0.1% | 0.0% |
| Palmitate280 (M10) | 0.1% | 0.1% | 0.1% | 0.5% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% |
| Palmitate281 (M11) | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate282 (M12) | 0.2% | 0.1% | 0.0% | 0.8% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% |
| Palmitate283 (M13) | 0.1% | 0.0% | 0.0% | 0.3% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% |
| Palmitate284 (M14) | 0.4% | 0.2% | 0.1% | 1.5% | 0.4% | 0.1% | 0.1% | 0.1% | 0.0% | 0.1% | 0.1% | 0.1% |
| Palmitate285 (M15) | 0.1% | 0.1% | 0.0% | 0.4% | 0.1% | 0.1% | 0.1% | 0.0% | 0.0% | 0.1% | 0.1% | 0.0% |
| Palmitate286 (M16) | 0.4% | 0.1% | 0.1% | 1.3% | 0.3% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **Enrichment in AcCoA pool (D value %):** | **8.3** | **7.851** | **8.305** | **78.3** | **80.13** | **80.73** | **4.16** | **3.932** | **3.9** | **18.07** | **18.67** | **17.71** |
| Lower bound: | 5.624 | 5.286 | 5.423 | 68.06 | 71.27 | 71.06 | 1.817 | 1.668 | 1.683 | 12.72 | 13.4 | 12.74 |
| Upper bound: | 11.15 | 10.57 | 11.39 | 86.19 | 86.97 | 88.13 | 6.618 | 6.303 | 6.223 | 23.97 | 24.48 | 23.2 |
| **de novo lipogenesis g(t)*:** | **34.24** | **36.9** | **31.7** | **11.38** | **11.24** | **10.2** | **35.29** | **38.17** | **39.24** | **7.811** | **7.984** | **8.499** |
| Lower bound: | 27.38 | 29.4 | 24.96 | 9.216 | 9.082 | 8.049 | 23.93 | 25.65 | 26.46 | 6.343 | 6.548 | 6.982 |
| Upper bound: | 46.08 | 50.11 | 43.9 | 13.55 | 13.41 | 12.36 | 73.28 | 81.84 | 82.92 | 9.744 | 9.822 | 10.47 |

* de novo lipogenesis, g(t), varies from well to well due to differences in cell growth and time cultured in the presence of tracer
ISA modeling deconvolutes enrichment constant (D value) from de novo lipogenesis to obtain independent values.
The consistency of D values across replicates reflects the similarity of metabolic pathways across replicates.
Here, mass isotopomer distributions (MIDs) %'s are not corrected for natural isotope abundance, as this is accounted for within Metran

Table S11. Palmitate mass isotopomer distributions (MIDs) used for ISA

**[U-13C6]glucose**

| **Metabolite/ion** | **SN12C normoxia** | | | **SN12C hypoxia** | | | **ACHN normoxia** | | **ACHN hypoxia** | | | **786-O normoxia** | | | **786-O hypoxia** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Palmitate270 (M0) | 33.4% | 34.0% | 34.4% | 52.0% | 49.1% | 50.9% | 21.6% | 22.5% | 67.8% | 68.4% | 68.4% | 44.7% | 45.4% | 45.2% | 53.0% | 54.9% | 55.3% |
| Palmitate271 (M1) | 7.3% | 6.9% | 7.0% | 11.6% | 10.5% | 10.5% | 4.5% | 4.8% | 13.8% | 14.0% | 13.9% | 9.3% | 9.5% | 9.6% | 11.2% | 11.4% | 11.7% |
| Palmitate272 (M2) | 2.5% | 2.2% | 2.1% | 10.3% | 8.7% | 9.1% | 3.5% | 3.5% | 11.5% | 10.1% | 10.7% | 12.9% | 14.2% | 14.6% | 15.1% | 15.6% | 15.9% |
| Palmitate273 (M3) | 0.7% | 0.5% | 0.5% | 2.7% | 1.9% | 2.0% | 0.9% | 1.0% | 2.1% | 1.8% | 1.9% | 2.6% | 2.7% | 2.7% | 2.8% | 2.9% | 2.8% |
| Palmitate274 (M4) | 0.8% | 0.8% | 0.7% | 6.1% | 6.1% | 6.1% | 4.7% | 4.6% | 2.7% | 2.1% | 2.4% | 9.5% | 9.8% | 10.0% | 7.4% | 7.1% | 6.8% |
| Palmitate275 (M5) | 0.3% | 0.3% | 0.2% | 1.5% | 1.3% | 1.4% | 1.7% | 1.7% | 0.5% | 0.4% | 0.4% | 2.4% | 1.9% | 1.9% | 1.6% | 1.3% | 1.2% |
| Palmitate276 (M6) | 0.9% | 0.9% | 0.8% | 4.4% | 5.1% | 4.8% | 8.2% | 8.1% | 0.8% | 0.8% | 0.8% | 7.2% | 6.7% | 6.6% | 4.0% | 3.4% | 3.1% |
| Palmitate277 (M7) | 0.6% | 0.5% | 0.4% | 1.4% | 1.3% | 1.2% | 3.0% | 2.9% | 0.1% | 0.2% | 0.2% | 2.2% | 1.5% | 1.3% | 1.2% | 0.7% | 0.7% |
| Palmitate278 (M8) | 1.7% | 1.7% | 1.5% | 3.7% | 4.7% | 4.0% | 11.7% | 11.4% | 0.3% | 0.6% | 0.5% | 4.6% | 4.3% | 4.1% | 2.1% | 1.5% | 1.5% |
| Palmitate279 (M9) | 0.7% | 0.7% | 0.7% | 0.7% | 0.9% | 0.8% | 4.1% | 4.0% | 0.1% | 0.2% | 0.1% | 0.7% | 0.7% | 0.7% | 0.3% | 0.3% | 0.3% |
| Palmitate280 (M10) | 3.2% | 3.4% | 3.4% | 2.3% | 3.4% | 3.0% | 12.6% | 12.4% | 0.1% | 0.6% | 0.3% | 2.2% | 2.1% | 2.1% | 0.8% | 0.7% | 0.6% |
| Palmitate281 (M11) | 1.7% | 1.8% | 1.8% | 0.4% | 0.6% | 0.5% | 3.8% | 3.8% | 0.0% | 0.1% | 0.0% | 0.3% | 0.3% | 0.3% | 0.1% | 0.1% | 0.1% |
| Palmitate282 (M12) | 7.9% | 8.2% | 8.3% | 1.4% | 2.6% | 2.2% | 10.1% | 9.8% | 0.1% | 0.3% | 0.2% | 0.8% | 0.8% | 0.8% | 0.2% | 0.2% | 0.2% |
| Palmitate283 (M13) | 3.8% | 3.7% | 3.9% | 0.2% | 0.5% | 0.5% | 2.3% | 2.3% | 0.1% | 0.1% | 0.0% | 0.1% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% |
| Palmitate284 (M14) | 15.2% | 15.2% | 15.2% | 0.9% | 1.9% | 1.8% | 5.2% | 5.1% | 0.0% | 0.2% | 0.1% | 0.3% | 0.2% | 0.2% | 0.1% | 0.1% | 0.0% |
| Palmitate285 (M15) | 4.4% | 4.2% | 4.4% | 0.1% | 0.4% | 0.4% | 0.7% | 0.8% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate286 (M16) | 15.0% | 15.0% | 14.7% | 0.3% | 1.2% | 1.1% | 1.4% | 1.3% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **Enrichment in AcCoA pool (D value %):** | **87.31** | **87.13** | **87.04** | **24.38** | **32.17** | **29.22** | **57.39** | **57.26** | **7.459** | **7.856** | **7.817** | **25.07** | **22.73** | **22.14** | **16.17** | **14.3** | **13.4** |
| Lower bound: | 85.49 | 85.27 | 85.18 | 19.53 | 26.03 | 23.82 | 54.67 | 54.54 | 6.08 | 5.294 | 5.355 | 21.98 | 19.73 | 19.22 | 14.32 | 12.48 | 11.6 |
| Upper bound: | 88.99 | 88.84 | 88.75 | 29.97 | 38.88 | 35.09 | 60.02 | 59.98 | 8.882 | 10.54 | 10.37 | 28.23 | 25.84 | 25.17 | 18.08 | 16.16 | 15.25 |
| **de novo lipogenesis g(t)*:** | **55.74** | **55.54** | **55.19** | **36.31** | **37.86** | **35.96** | **70.75** | **69.44** | **36.16** | **61.56** | **32.85** | **48.55** | **49.24** | **50.04** | **44.43** | **44.79** | **45.58** |
| Lower bound: | 52.04 | 51.84 | 51.49 | 32.03 | 33.82 | 32.02 | 67.09 | 65.73 | 31.97 | 25.78 | 27.02 | 45.11 | 45.58 | 46.31 | 41.38 | 41.33 | 41.84 |
| Upper bound: | 59.43 | 59.24 | 58.89 | 40.77 | 41.98 | 40 | 74.41 | 73.14 | 41.85 | 41.74 | 42.98 | 52.13 | 53.11 | 54 | 47.79 | 48.72 | 49.91 |

**[5-13C]gln**

| **Metabolite/ion** | **SN12C normoxia** | | | **SN12C hypoxia** | | | **ACHN normoxia** | | | **ACHN hypoxia** | | | **786-O normoxia** | | | **786-O hypoxia** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Palmitate270 (M0) | 60.4% | 61.0% | 61.0% | 43.8% | 44.8% | 45.5% | 30.6% | 29.2% | 29.1% | 29.2% | 29.7% | 28.6% | 53.9% | 41.3% | 34.7% | 44.4% | 35.7% | 31.9% |
| Palmitate271 (M1) | 24.2% | 24.3% | 24.3% | 10.9% | 12.0% | 12.1% | 22.4% | 21.5% | 21.3% | 6.6% | 7.4% | 7.4% | 11.8% | 9.7% | 8.5% | 9.9% | 8.1% | 7.4% |
| Palmitate272 (M2) | 7.5% | 7.6% | 7.5% | 4.2% | 5.0% | 4.5% | 17.4% | 17.9% | 17.9% | 1.3% | 1.8% | 2.0% | 2.7% | 3.3% | 3.6% | 2.3% | 2.0% | 2.1% |
| Palmitate273 (M3) | 2.6% | 2.5% | 2.6% | 4.2% | 5.2% | 4.4% | 12.4% | 13.3% | 13.2% | 0.6% | 0.9% | 1.4% | 2.8% | 4.4% | 5.1% | 2.7% | 2.4% | 3.1% |
| Palmitate274 (M4) | 1.3% | 1.3% | 1.3% | 5.3% | 6.1% | 5.5% | 7.1% | 7.8% | 7.9% | 1.2% | 1.0% | 1.8% | 4.4% | 6.9% | 7.9% | 4.8% | 5.0% | 6.1% |
| Palmitate275 (M5) | 0.8% | 0.8% | 0.9% | 6.5% | 6.9% | 6.5% | 3.8% | 4.1% | 4.2% | 2.8% | 2.3% | 3.8% | 6.2% | 9.0% | 10.5% | 7.7% | 8.7% | 10.0% |
| Palmitate276 (M6) | 0.7% | 0.7% | 0.8% | 8.3% | 7.7% | 8.0% | 2.4% | 2.4% | 2.6% | 8.7% | 7.6% | 9.3% | 7.6% | 10.7% | 12.6% | 10.7% | 13.4% | 14.4% |
| Palmitate277 (M7) | 0.5% | 0.6% | 0.6% | 8.0% | 6.7% | 7.5% | 1.7% | 1.9% | 1.9% | 19.6% | 19.2% | 19.0% | 6.6% | 9.3% | 10.8% | 10.5% | 14.6% | 15.0% |
| Palmitate278 (M8) | 0.4% | 0.4% | 0.3% | 5.2% | 4.0% | 4.8% | 1.4% | 1.4% | 1.5% | 25.5% | 26.5% | 23.8% | 3.5% | 4.7% | 5.6% | 6.1% | 8.9% | 8.8% |
| Palmitate279 (M9) | 0.1% | 0.1% | 0.1% | 0.6% | 0.5% | 0.6% | 0.2% | 0.2% | 0.2% | 2.8% | 2.9% | 2.5% | 0.4% | 0.6% | 0.6% | 0.7% | 1.0% | 1.0% |
| Palmitate280 (M10) | 0.2% | 0.1% | 0.1% | 0.3% | 0.2% | 0.2% | 0.3% | 0.1% | 0.1% | 0.8% | 0.4% | 0.4% | 0.1% | 0.2% | 0.2% | 0.1% | 0.2% | 0.2% |
| Palmitate281 (M11) | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate282 (M12) | 0.2% | 0.1% | 0.1% | 0.4% | 0.1% | 0.0% | 0.2% | 0.1% | 0.0% | 0.4% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate283 (M13) | 0.1% | 0.1% | 0.0% | 0.2% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate284 (M14) | 0.4% | 0.3% | 0.2% | 0.9% | 0.3% | 0.2% | 0.1% | 0.1% | 0.0% | 0.2% | 0.1% | 0.1% | 0.1% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% |
| Palmitate285 (M15) | 0.2% | 0.1% | 0.1% | 0.3% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate286 (M16) | 0.4% | 0.2% | 0.1% | 0.8% | 0.3% | 0.2% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **Enrichment in AcCoA pool (D value %):** | **10.62** | **10.56** | **10.67** | **71.44** | **64.99** | **69.91** | **25.63** | **26.82** | **27.06** | **91.47** | **92.32** | **90.62** | **71.15** | **69.71** | **69.86** | **75.41** | **78.2** | **76.74** |
| Lower bound: | 7.022 | 6.931 | 7.006 | 64.84 | 56.83 | 62.1 | 22.76 | 24.03 | 24.25 | 90.35 | 91.25 | 89.28 | 66.77 | 66.06 | 65.84 | 72.76 | 75.95 | 74.39 |
| Upper bound: | 14.63 | 14.61 | 14.77 | 77.17 | 72.43 | 76.61 | 28.58 | 29.68 | 29.93 | 92.54 | 93.33 | 91.87 | 75.2 | 73.23 | 73.69 | 77.91 | 80.36 | 78.95 |
| **de novo lipogenesis g(t)*:** | **41.35** | **40.78** | **40.48** | **41.22** | **39.5** | **38.82** | **66.01** | **67.24** | **67.21** | **62.13** | **61.15** | **62.08** | **32.27** | **46.27** | **54.79** | **46.68** | **54.26** | **58.79** |
| Lower bound: | 32.35 | 31.77 | 31.56 | 36.61 | 34.17 | 33.52 | 61.01 | 62.38 | 62.39 | 59.38 | 58.41 | 59.23 | 29.45 | 42.65 | 50.85 | 41.19 | 51.39 | 55.85 |
| Upper bound: | 56.36 | 55.92 | 55.46 | 45.84 | 44.83 | 44.13 | 71.26 | 72.28 | 72.23 | 64.88 | 63.89 | 64.93 | 35.11 | 49.89 | 58.73 | 46.17 | 57.14 | 61.72 |

* de novo lipogenesis, g(t), varies from well to well due to differences in cell growth and time cultured in the presence of tracer

ISA modeling deconvolutes enrichment constant (D value) from de novo lipogenesis to obtain independent values.

The consistency of D values across replicates reflects the similarity of metabolic pathways across replicates.

Here, mass isotopomer distributions (MIDs) %'s are not corrected for natural isotope abundance, as this is accounted for within Metran

Table S12. Palmitate mass isotopomer distributions (MIDs) used for ISA

| [U-13C6]glucose | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Metabolite/ion** | | **UMRC2** | | | **PRC3** | | | **WT8** | | | **pTV** | | | **pTR** | |
| Palmitate270 (M0) | 70.3% | 69.0% | 68.0% | 72.6% | 73.0% | | 31.3% | 31.2% | 30.9% | 75.4% | 72.7% | 75.1% | | 56.0% | 55.9% |
| Palmitate271 (M1) | 13.8% | 13.6% | 13.5% | 15.3% | 14.5% | | 6.3% | 6.3% | 6.2% | 14.7% | 14.4% | 14.7% | | 10.8% | 10.9% |
| Palmitate272 (M2) | 4.8% | 5.0% | 5.2% | 6.9% | 6.3% | | 2.8% | 2.8% | 2.7% | 5.0% | 5.8% | 4.5% | | 4.2% | 4.2% |
| Palmitate273 (M3) | 0.8% | 0.9% | 0.8% | 1.4% | 1.0% | | 0.6% | 0.5% | 0.5% | 0.8% | 1.0% | 0.7% | | 0.8% | 0.8% |
| Palmitate274 (M4) | 1.9% | 2.1% | 2.3% | 1.3% | 1.1% | | 2.3% | 2.4% | 2.3% | 1.2% | 1.8% | 1.3% | | 3.6% | 3.7% |
| Palmitate275 (M5) | 0.5% | 0.5% | 0.5% | 0.4% | 0.2% | | 0.6% | 0.6% | 0.6% | 0.1% | 0.3% | 0.2% | | 0.8% | 0.9% |
| Palmitate276 (M6) | 1.8% | 2.0% | 2.1% | 0.3% | 0.4% | | 3.7% | 3.9% | 3.8% | 0.4% | 0.8% | 0.7% | | 4.6% | 4.5% |
| Palmitate277 (M7) | 0.5% | 0.6% | 0.6% | 0.3% | 0.3% | | 1.1% | 1.2% | 1.1% | 0.4% | 0.5% | 0.5% | | 1.3% | 1.3% |
| Palmitate278 (M8) | 2.0% | 2.2% | 2.3% | 0.6% | 0.7% | | 6.6% | 6.8% | 6.6% | 0.7% | 1.0% | 0.8% | | 5.5% | 5.4% |
| Palmitate279 (M9) | 0.4% | 0.5% | 0.5% | 0.1% | 0.2% | | 1.7% | 1.7% | 1.7% | 0.3% | 0.3% | 0.2% | | 1.1% | 1.1% |
| Palmitate280 (M10) | 1.5% | 1.6% | 1.8% | 0.2% | 0.6% | | 10.0% | 10.2% | 10.3% | 0.2% | 0.6% | 0.5% | | 4.7% | 4.7% |
| Palmitate281 (M11) | 0.2% | 0.2% | 0.3% | 0.0% | 0.0% | | 2.3% | 2.3% | 2.3% | 0.3% | 0.2% | 0.1% | | 0.7% | 0.7% |
| Palmitate282 (M12) | 0.9% | 1.1% | 1.2% | 0.1% | 0.6% | | 12.5% | 12.5% | 12.6% | 0.2% | 0.3% | 0.3% | | 3.4% | 3.3% |
| Palmitate283 (M13) | 0.1% | 0.2% | 0.2% | 0.0% | 0.1% | | 2.2% | 2.2% | 2.2% | 0.1% | 0.0% | 0.0% | | 0.4% | 0.5% |
| Palmitate284 (M14) | 0.4% | 0.5% | 0.6% | 0.2% | 0.6% | | 10.5% | 10.3% | 10.7% | 0.2% | 0.3% | 0.3% | | 1.7% | 1.6% |
| Palmitate285 (M15) | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | | 1.2% | 1.1% | 1.2% | 0.0% | 0.1% | 0.1% | | 0.1% | 0.1% |
| Palmitate286 (M16) | 0.1% | 0.1% | 0.1% | 0.2% | 0.4% | | 4.4% | 4.3% | 4.5% | 0.0% | 0.0% | 0.1% | | 0.4% | 0.4% |
| **Enrichment in AcCoA pool (D value %):** | **32.37** | **32.64** | **33.07** | **8.587** | **9.677** | | **78.75** | **78.45** | **77.58** | **11.77** | **14.38** | **15.77** | | **49.04** | **48.58** |
| Lower bound: | 25.04 | 23.65 | 24.88 | 4.734 | 5.349 | | 76.61 | 75.72 | 74.35 | 5.406 | 9.21 | 8.736 | | 43.27 | 42.82 |
| Upper bound: | 40.88 | 42.82 | 42.92 | 12.64 | 14.35 | | 80.77 | 81.04 | 80.63 | 18.9 | 20.1 | 24.52 | | 54.68 | 54.36 |
| **de novo lipogenesis g(t)*:** | **13.66** | **15.3** | **16.5** | **19.62** | **16.9** | | **33.63** | **32.81** | **28.26** | **11.04** | **13.8** | **9.709** | | **30.56** | **30.57** |
| Lower bound: | 12.06 | 13.24 | 14.43 | 15.13 | 13.09 | | 31.29 | 30.25 | 25.74 | 7.992 | 11.1 | 7.293 | | 27.6 | 27.61 |
| Upper bound: | 15.27 | 17.42 | 18.59 | 29.62 | 0.25 | | 35.97 | 35.37 | 30.78 | 17.7 | 17.63 | 13.18 | | 33.53 | 33.53 |

| [5-13C]gln | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Metabolite/ion** | | **UMRC2** | | | **PRC3** | | | **WT8** | | | **pTV** | | | **pTR** | |
| Palmitate270 (M0) | 57.0% | 58.4% | 57.2% | 51.3% | 52.7% | 56.4% | 44.0% | 43.8% | 44.8% | 68.6% | 69.8% | 71.5% | 64.3% | 63.0% | 66.8% |
| Palmitate271 (M1) | 13.4% | 13.6% | 13.3% | 10.7% | 11.4% | 12.4% | 23.4% | 24.0% | 23.8% | 14.3% | 14.5% | 14.8% | 15.8% | 15.8% | 15.9% |
| Palmitate272 (M2) | 3.9% | 3.6% | 3.9% | 1.8% | 2.4% | 2.6% | 13.7% | 14.0% | 14.0% | 2.4% | 2.4% | 2.4% | 5.6% | 6.0% | 5.0% |
| Palmitate273 (M3) | 3.2% | 2.8% | 3.1% | 1.3% | 1.5% | 1.6% | 7.6% | 7.8% | 7.8% | 1.0% | 1.1% | 0.9% | 4.1% | 4.6% | 3.5% |
| Palmitate274 (M4) | 3.4% | 3.2% | 3.5% | 2.7% | 2.8% | 2.6% | 4.3% | 4.3% | 4.3% | 1.7% | 1.5% | 1.3% | 3.5% | 3.7% | 2.9% |
| Palmitate275 (M5) | 4.1% | 3.7% | 4.0% | 5.1% | 5.1% | 4.6% | 2.4% | 2.4% | 2.4% | 2.4% | 2.4% | 2.0% | 2.6% | 2.6% | 2.2% |
| Palmitate276 (M6) | 5.2% | 4.9% | 5.1% | 8.0% | 7.9% | 6.7% | 1.4% | 1.4% | 1.3% | 3.5% | 3.1% | 2.6% | 1.7% | 1.7% | 1.4% |
| Palmitate277 (M7) | 5.7% | 5.6% | 5.6% | 9.0% | 8.9% | 7.3% | 0.8% | 0.9% | 0.8% | 3.2% | 3.0% | 2.6% | 1.2% | 1.2% | 1.1% |
| Palmitate278 (M8) | 3.6% | 3.7% | 3.6% | 5.5% | 5.2% | 4.5% | 0.5% | 0.5% | 0.3% | 1.9% | 1.7% | 1.3% | 0.6% | 0.8% | 0.8% |
| Palmitate279 (M9) | 0.4% | 0.4% | 0.4% | 0.7% | 0.6% | 0.5% | 0.1% | 0.1% | 0.1% | 0.3% | 0.2% | 0.2% | 0.1% | 0.2% | 0.2% |
| Palmitate280 (M10) | 0.1% | 0.1% | 0.1% | 0.4% | 0.2% | 0.2% | 0.2% | 0.2% | 0.1% | 0.3% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% |
| Palmitate281 (M11) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate282 (M12) | 0.0% | 0.0% | 0.0% | 0.6% | 0.2% | 0.1% | 0.3% | 0.1% | 0.1% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate283 (M13) | 0.0% | 0.0% | 0.0% | 0.2% | 0.1% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate284 (M14) | 0.1% | 0.0% | 0.0% | 1.3% | 0.5% | 0.3% | 0.6% | 0.2% | 0.2% | 0.2% | 0.2% | 0.2% | 0.2% | 0.2% | 0.2% |
| Palmitate285 (M15) | 0.1% | 0.1% | 0.0% | 0.3% | 0.1% | 0.1% | 0.1% | 0.1% | 0.0% | 0.0% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% |
| Palmitate286 (M16) | 0.0% | 0.0% | 0.0% | 1.3% | 0.5% | 0.3% | 0.6% | 0.3% | 0.2% | 0.1% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% |
| **Enrichment in AcCoA pool (D value %):** | **72.26** | **74.04** | **72.28** | **70.85** | **70.36** | **70.89** | **20.89** | **20.45** | **20.61** | **74.47** | **73.77** | **74.07** | **39.78** | **38.6** | **39.36** |
| Lower bound: | 62.28 | 63.8 | 61.93 | 68.34 | 67.8 | 68.41 | 17.98 | 17.57 | 17.46 | 69.96 | 68.73 | 67.8 | 34.42 | 33.7 | 33.05 |
| Upper bound: | 80.23 | 82.02 | 80.48 | 73.23 | 72.78 | 73.3 | 23.94 | 23.44 | 23.69 | 78.69 | 78.43 | 79.74 | 45.01 | 43.4 | 45.58 |
| **de novo lipogenesis g(t)*:** | **27.3** | **25.72** | **27.01** | **59.5** | **59.72** | **60.06** | **52.23** | **53.34** | **52.13** | **14.37** | **13.2** | **10.77** | **18.85** | **20.51** | **15.84** |
| Lower bound: | 23.45 | 21.88 | 23.15 | 56.52 | 56.74 | 57.08 | 47.78 | 48.78 | 47.59 | 12.72 | 11.56 | 8.77 | 16.52 | 18.12 | 13.55 |
| Upper bound: | 31.15 | 29.56 | 30.86 | 62.48 | 62.71 | 63.04 | 57.16 | 58.41 | 57.06 | 16.01 | 14.85 | 12.76 | 21.23 | 22.94 | 18.17 |

* de novo lipogenesis, g(t), varies from well to well due to differences in cell growth and time cultured in the presence of tracer
ISA modeling deconvolutes enrichment constant (D value) from de novo lipogenesis to obtain independent values.
The consistency of D values across replicates reflects the similarity of metabolic pathways across replicates.
Here, mass isotopomer distributions (MIDs) %'s are not corrected for natural isotope abundance, as this is accounted for within Metran

Table S13. Palmitate mass isotopomer distributions (MIDs) used for ISA

**[U-13C6]glucose**

| **Metabolite/ion** | **A549 control normoxia** | | | **A549 5mM DCA normoxia** | | | **A549 control hypoxia** | | | **A549 5mM DCA hypoxia** | | | **UMRC2 Control** | | | **UMRC2 ARNT kd** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Palmitate270 (M0) | 31.2% | 31.8% | 33.0% | 38.9% | 45.2% | 46.5% | 53.1% | 58.6% | 55.7% | 63.8% | 59.4% | 64.4% | 64.6% | 65.6% | 64.1% | 52.4% | 46.0% | 50.4% |
| Palmitate271 (M1) | 6.2% | 6.3% | 6.2% | 7.6% | 8.8% | 9.2% | 11.0% | 11.9% | 11.3% | 12.6% | 12.1% | 12.6% | 13.2% | 13.4% | 12.9% | 10.7% | 9.4% | 10.0% |
| Palmitate272 (M2) | 1.6% | 1.7% | 1.8% | 1.7% | 2.0% | 2.1% | 13.4% | 12.5% | 12.3% | 2.7% | 2.8% | 2.8% | 4.9% | 4.6% | 4.7% | 2.7% | 2.4% | 2.6% |
| Palmitate273 (M3) | 0.3% | 0.3% | 0.3% | 0.3% | 0.3% | 0.4% | 2.6% | 2.4% | 2.4% | 0.5% | 0.6% | 0.5% | 0.8% | 0.8% | 0.8% | 0.4% | 0.4% | 0.4% |
| Palmitate274 (M4) | 0.8% | 0.9% | 0.9% | 0.5% | 0.7% | 0.9% | 7.0% | 5.4% | 6.1% | 1.6% | 1.8% | 1.6% | 1.9% | 1.9% | 1.9% | 0.8% | 0.8% | 0.9% |
| Palmitate275 (M5) | 0.3% | 0.3% | 0.4% | 0.2% | 0.2% | 0.3% | 1.4% | 1.0% | 1.2% | 0.5% | 0.6% | 0.5% | 0.5% | 0.5% | 0.4% | 0.2% | 0.2% | 0.3% |
| Palmitate276 (M6) | 1.7% | 1.7% | 1.8% | 1.2% | 1.2% | 1.1% | 3.7% | 2.3% | 3.2% | 2.7% | 3.2% | 2.4% | 2.1% | 2.0% | 2.1% | 1.5% | 1.4% | 1.7% |
| Palmitate277 (M7) | 0.7% | 0.7% | 0.8% | 0.6% | 0.6% | 0.6% | 0.8% | 0.5% | 0.7% | 0.8% | 0.9% | 0.7% | 0.8% | 0.5% | 0.6% | 0.6% | 0.6% | 0.6% |
| Palmitate278 (M8) | 4.0% | 3.7% | 3.9% | 2.9% | 3.0% | 2.8% | 2.4% | 1.5% | 2.1% | 3.6% | 4.4% | 3.3% | 2.7% | 2.7% | 2.8% | 3.1% | 3.7% | 3.3% |
| Palmitate279 (M9) | 1.6% | 1.5% | 1.6% | 1.3% | 1.2% | 1.2% | 0.4% | 0.3% | 0.4% | 0.9% | 1.1% | 0.9% | 0.8% | 0.6% | 0.7% | 1.1% | 1.4% | 1.2% |
| Palmitate280 (M10) | 7.8% | 7.6% | 7.5% | 6.3% | 6.0% | 5.8% | 1.6% | 1.1% | 1.5% | 3.8% | 4.7% | 3.6% | 2.8% | 2.4% | 2.9% | 5.3% | 6.3% | 5.3% |
| Palmitate281 (M11) | 3.0% | 2.8% | 2.8% | 2.6% | 2.3% | 2.2% | 0.4% | 0.3% | 0.4% | 0.9% | 1.2% | 0.8% | 0.5% | 0.3% | 0.6% | 1.6% | 2.1% | 1.7% |
| Palmitate282 (M12) | 12.4% | 12.4% | 11.9% | 10.9% | 9.2% | 8.9% | 1.1% | 0.9% | 1.2% | 3.0% | 3.7% | 3.0% | 2.4% | 2.3% | 2.6% | 6.9% | 8.8% | 7.5% |
| Palmitate283 (M13) | 3.7% | 3.6% | 3.6% | 3.4% | 2.5% | 2.4% | 0.2% | 0.2% | 0.3% | 0.5% | 0.7% | 0.5% | 0.5% | 0.4% | 0.5% | 1.8% | 2.5% | 2.1% |
| Palmitate284 (M14) | 14.3% | 14.1% | 13.6% | 12.4% | 9.7% | 9.4% | 0.6% | 0.7% | 0.9% | 1.6% | 2.1% | 1.8% | 1.3% | 1.3% | 1.6% | 6.6% | 8.5% | 7.1% |
| Palmitate285 (M15) | 2.5% | 2.5% | 2.3% | 2.2% | 1.7% | 1.5% | 0.1% | 0.1% | 0.2% | 0.2% | 0.3% | 0.2% | 0.1% | 0.2% | 0.2% | 1.1% | 1.4% | 1.2% |
| Palmitate286 (M16) | 8.1% | 8.2% | 7.7% | 7.0% | 5.3% | 4.8% | 0.2% | 0.3% | 0.4% | 0.5% | 0.6% | 0.6% | 0.3% | 0.5% | 0.6% | 3.4% | 4.2% | 3.6% |
| | | | | | | | | | | | | | | | | | | |
| **Enrichment in AcCoA pool (D value %):** | **78.57** | **78.72** | **78.3** | **79.05** | **77.26** | **76.97** | **17.65** | **14.48** | **17.35** | **56.46** | **57.08** | **57.22** | **52.46** | **51.48** | **54.8** | **74.04** | **74.96** | **74.46** |
| Lower bound: | 76.95 | 77.1 | 76.61 | 77.23 | 75 | 74.64 | 14.97 | 11.57 | 14.42 | 52.69 | 53.97 | 53.15 | 42.84 | 41.58 | 46.57 | 71.63 | 73 | 71.62 |
| Upper bound: | 80.14 | 80.3 | 79.94 | 80.75 | 79.38 | 79.15 | 20.44 | 17.56 | 20.43 | 60.21 | 60.09 | 61.24 | 61.01 | 60.16 | 62.08 | 76.38 | 76.86 | 77.17 |
| **de novo lipogenesis g(t)*:** | **59.95** | **59.24** | **57.81** | **50.92** | **43.22** | **41.67** | **41.96** | **37.26** | **38.06** | **21.13** | **26.12** | **20.37** | **18.69** | **17.2** | **18.98** | **33.01** | **41.09** | **35.95** |
| Lower bound: | 57.58 | 56.87 | 55.45 | 45.57 | 40.88 | 39.33 | 38.44 | 33.07 | 34.52 | 19.59 | 24.58 | 18.84 | 15.96 | 14.6 | 16.37 | 30.59 | 38.59 | 33.27 |
| Upper bound: | 62.31 | 61.61 | 60.18 | 53.27 | 45.56 | 44.01 | 45.92 | 42.4 | 42.09 | 22.66 | 27.66 | 21.91 | 21.42 | 19.79 | 21.58 | 35.43 | 43.59 | 38.63 |

**[5-13C]gln**

| **Metabolite/ion** | **A549 control normoxia** | | | **A549 5mM DCA normoxia** | | | **A549 control hypoxia** | | | **A549 5mM DCA hypoxia** | | | **UMRC2 Control** | | | **UMRC2 ARNT kd** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Palmitate270 (M0) | 51.4% | 52.0% | 52.1% | 59.1% | 59.1% | 59.8% | 43.1% | 43.7% | 44.1% | 63.0% | 61.6% | | 50.1% | 47.2% | 45.8% | 53.5% | 53.2% | 52.7% |
| Palmitate271 (M1) | 27.1% | 27.0% | 26.9% | 25.9% | 26.4% | 25.8% | 10.0% | 10.6% | 10.7% | 17.6% | 17.4% | | 14.6% | 15.5% | 15.4% | 25.2% | 24.9% | 25.0% |
| Palmitate272 (M2) | 12.5% | 12.3% | 12.4% | 9.6% | 9.6% | 9.5% | 2.9% | 3.1% | 3.4% | 7.5% | 7.9% | | 6.9% | 8.2% | 8.5% | 11.4% | 11.5% | 11.5% |
| Palmitate273 (M3) | 5.1% | 5.1% | 5.1% | 3.1% | 3.1% | 3.1% | 2.9% | 2.8% | 3.3% | 4.8% | 5.4% | | 5.5% | 6.6% | 6.8% | 5.2% | 5.4% | 5.3% |
| Palmitate274 (M4) | 2.0% | 1.9% | 1.9% | 0.9% | 0.9% | 0.9% | 4.7% | 4.3% | 4.9% | 2.8% | 3.5% | | 4.5% | 5.2% | 5.4% | 2.2% | 2.4% | 2.4% |
| Palmitate275 (M5) | 0.8% | 0.8% | 0.7% | 0.3% | 0.2% | 0.3% | 7.5% | 7.0% | 7.5% | 1.5% | 1.8% | | 4.2% | 4.5% | 4.8% | 1.0% | 1.1% | 1.1% |
| Palmitate276 (M6) | 0.3% | 0.3% | 0.3% | 0.1% | 0.1% | 0.1% | 10.7% | 10.4% | 10.2% | 0.8% | 0.9% | | 4.8% | 4.8% | 5.2% | 0.5% | 0.6% | 0.8% |
| Palmitate277 (M7) | 0.2% | 0.2% | 0.2% | 0.1% | 0.1% | 0.1% | 10.5% | 10.7% | 9.7% | 0.5% | 0.5% | | 4.4% | 4.4% | 4.7% | 0.2% | 0.4% | 0.4% |
| Palmitate278 (M8) | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 5.9% | 6.2% | 5.2% | 0.3% | 0.2% | | 2.8% | 2.8% | 2.9% | 0.3% | 0.4% | 0.4% |
| Palmitate279 (M9) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.7% | 0.6% | 0.5% | 0.1% | 0.1% | | 0.3% | 0.3% | 0.3% | 0.0% | 0.0% | 0.0% |
| Palmitate280 (M10) | 0.1% | 0.1% | 0.0% | 0.1% | 0.1% | 0.1% | 0.2% | 0.1% | 0.1% | 0.2% | 0.1% | | 0.4% | 0.1% | 0.0% | 0.2% | 0.0% | 0.0% |
| Palmitate281 (M11) | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.2% | 0.2% | | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate282 (M12) | 0.1% | 0.1% | 0.1% | 0.2% | 0.1% | 0.1% | 0.3% | 0.1% | 0.1% | 0.2% | 0.1% | | 0.5% | 0.2% | 0.2% | 0.2% | 0.2% | 0.2% |
| Palmitate283 (M13) | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 0.0% | 0.1% | 0.1% | 0.0% | 0.1% | 0.1% | | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% |
| Palmitate284 (M14) | 0.1% | 0.1% | 0.0% | 0.1% | 0.1% | 0.0% | 0.3% | 0.1% | 0.1% | 0.2% | 0.1% | | 0.5% | 0.1% | 0.1% | 0.1% | 0.0% | 0.0% |
| Palmitate285 (M15) | 0.1% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.1% | 0.0% | 0.1% | 0.1% | 0.1% | | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate286 (M16) | 0.1% | 0.0% | 0.0% | 0.1% | 0.1% | 0.0% | 0.2% | 0.1% | 0.0% | 0.1% | 0.1% | | 0.3% | 0.1% | 0.0% | 0.1% | 0.0% | 0.0% |
| | | | | | | | | | | | | | | | | | | |
| **Enrichment in AcCoA pool (D value %):** | **14** | **13.93** | **14.03** | **11.2** | **10.62** | **11.12** | **75.3** | **76.34** | **73.86** | **27** | **28.66** | | **47.7** | **41.65** | **41.88** | **15.45** | **16.07** | **16.13** |
| Lower bound: | 12.48 | 12.39 | 12.49 | 9.358 | 9.503 | 9.965 | 72.35 | 73.02 | 70.29 | 23.99 | 25.76 | | 33.36 | 30.18 | 30.56 | 13.73 | 14.33 | 14.06 |
| Upper bound: | 15.56 | 15.52 | 15.62 | 13.1 | 11.76 | 12.31 | 78.07 | 79.45 | 77.23 | 30.17 | 31.55 | | 66.56 | 55.78 | 56.06 | 17.24 | 17.88 | 18.23 |
| **de novo lipogenesis g(t)*:** | **52.78** | **51.9** | **51.7** | **45.37** | **47.25** | **44.45** | **44.43** | **43.03** | **42.46** | **23.97** | **25.51** | | **30.37** | **34.86** | **36.36** | **46.15** | **45.59** | **45.76** |
| Lower bound: | 49.07 | 48.18 | 48.01 | 40.53 | 43.87 | 41.31 | 41.58 | 39.46 | 38.88 | 22.12 | 23.73 | | 23.8 | 27.93 | 29.39 | 42.84 | 42.44 | 41.94 |
| Upper bound: | 56.98 | 56.14 | 55.87 | 51.5 | 51.19 | 48.08 | 47.29 | 46.61 | 46.04 | 25.87 | 27.35 | | 37.02 | 42.04 | 43.58 | 49.85 | 49.1 | 50.17 |

* de novo lipogenesis, g(t), varies from well to well due to differences in cell growth and time cultured in the presence of tracer

ISA modeling deconvolutes enrichment constant (D value) from de novo lipogenesis to obtain independent values.

The consistency of D values across replicates reflects the similarity of metabolic pathways across replicates.

Here, mass isotopomer distributions (MIDs) %'s are not corrected for natural isotope abundance, as this is accounted for within Metran

Table S14. Palmitate mass isotopomer distributions (MIDs) used for ISA

| | [5-13C]gln | | | [5-13C]gln | | | [5-13C]gln | | | [5-13C]gln | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Metabolite/ion** | **A549 Control Normoxia** | | | **A549 ARNT kd Normoxia** | | | **143B Control Normoxia** | | | **143B ARNT kd Normoxia** | | |
| Palmitate270 (M0) | 48.9% | 49.5% | 49.2% | 64.2% | 63.8% | 63.8% | 58.4% | 58.3% | 59.3% | 57.5% | 57.2% | 58.1% |
| Palmitate271 (M1) | 27.1% | 27.3% | 27.3% | 20.1% | 20.4% | 20.3% | 18.9% | 20.5% | 21.4% | 27.4% | 28.4% | 27.4% |
| Palmitate272 (M2) | 12.7% | 12.6% | 12.7% | 7.0% | 7.4% | 7.4% | 8.8% | 9.5% | 9.0% | 9.5% | 9.8% | 9.6% |
| Palmitate273 (M3) | 5.7% | 5.5% | 5.8% | 3.5% | 3.7% | 3.8% | 5.7% | 5.7% | 5.1% | 3.1% | 3.0% | 3.2% |
| Palmitate274 (M4) | 2.5% | 2.4% | 2.4% | 1.9% | 2.0% | 2.0% | 3.4% | 3.1% | 2.6% | 1.0% | 0.9% | 1.0% |
| Palmitate275 (M5) | 1.1% | 1.1% | 1.1% | 1.0% | 1.0% | 1.2% | 2.1% | 1.5% | 1.3% | 0.5% | 0.3% | 0.3% |
| Palmitate276 (M6) | 0.6% | 0.6% | 0.6% | 0.7% | 0.6% | 0.6% | 1.3% | 0.7% | 0.4% | 0.4% | 0.1% | 0.1% |
| Palmitate277 (M7) | 0.3% | 0.4% | 0.4% | 0.4% | 0.4% | 0.4% | 0.6% | 0.3% | 0.2% | 0.2% | 0.1% | 0.1% |
| Palmitate278 (M8) | 0.3% | 0.3% | 0.3% | 0.5% | 0.3% | 0.4% | 0.6% | 0.3% | 0.4% | 0.3% | 0.2% | 0.2% |
| Palmitate279 (M9) | 0.1% | 0.0% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.2% | 0.1% | 0.1% | 0.1% |
| Palmitate280 (M10) | 0.2% | 0.1% | 0.1% | 0.2% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 0.1% |
| Palmitate281 (M11) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate282 (M12) | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate283 (M13) | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate284 (M14) | 0.2% | 0.1% | 0.0% | 0.3% | 0.1% | 0.1% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% |
| Palmitate285 (M15) | 0.1% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate286 (M16) | 0.1% | 0.0% | 0.0% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **Enrichment in AcCoA pool (D value %):** | **14.86** | **14.51** | **14.78** | **19.27** | **18.99** | **19.43** | **25.85** | **22.13** | **19.97** | **9.87** | **9.227** | **9.961** |
| Lower bound: | 12.68 | 12.32 | 12.58 | 14.7 | 14.7 | 15.08 | 21.91 | 18.65 | 16.32 | 8.168 | 7.606 | 8.242 |
| Upper bound: | 17.15 | 16.82 | 17.07 | 24.17 | 23.57 | 24.06 | 29.96 | 25.96 | 23.83 | 11.64 | 10.9 | 11.74 |
| **de novo lipogenesis g(t)*:** | **54.4** | **54.37** | **54.33** | **24.76** | **25.96** | **25.71** | **30.13** | **32.45** | **32.27** | **52.48** | **56.38** | **51.46** |
| Lower bound: | 49.29 | 49.1 | 49.2 | 21.18 | 22.34 | 22.16 | 27.23 | 29.15 | 28.68 | 46.55 | 49.87 | 45.64 |
| Upper bound: | 60.42 | 60.6 | 60.41 | 29.2 | 30.41 | 30.03 | 33.24 | 35.99 | 36.42 | 60.19 | 64.98 | 59.01 |

| | [5-13C]gln | | | [5-13C]gln | | | [5-13C]gln | | | [5-13C]gln | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Metabolite/ion** | **A549 Control Hypoxia** | | | **A549 ARNT kd Hypoxia** | | | **143B Control Hypoxia** | | | **143B ARNT kd Hypoxia** | | |
| Palmitate270 (M0) | 37.0% | 38.2% | 39.7% | 64.4% | 62.4% | 63.9% | 70.4% | 59.4% | 64.5% | 39.2% | 37.4% | 40.3% |
| Palmitate271 (M1) | 10.8% | 12.1% | 11.9% | 15.4% | 15.9% | 16.0% | 14.1% | 13.1% | 14.1% | 14.8% | 12.1% | 16.1% |
| Palmitate272 (M2) | 5.8% | 6.3% | 6.0% | 4.4% | 5.3% | 4.9% | 2.5% | 2.7% | 3.2% | 10.9% | 8.1% | 11.8% |
| Palmitate273 (M3) | 6.1% | 6.2% | 6.0% | 3.0% | 3.9% | 3.6% | 1.3% | 2.1% | 2.6% | 11.0% | 9.8% | 10.9% |
| Palmitate274 (M4) | 7.3% | 6.8% | 6.7% | 2.8% | 3.3% | 3.0% | 1.9% | 3.2% | 3.3% | 9.9% | 10.8% | 8.8% |
| Palmitate275 (M5) | 8.1% | 8.0% | 7.8% | 2.5% | 3.1% | 2.8% | 2.5% | 4.3% | 3.9% | 7.2% | 9.7% | 6.1% |
| Palmitate276 (M6) | 9.4% | 8.9% | 8.9% | 2.4% | 2.6% | 2.6% | 3.0% | 5.7% | 3.8% | 4.3% | 7.2% | 3.6% |
| Palmitate277 (M7) | 7.9% | 7.9% | 7.9% | 1.7% | 1.9% | 1.8% | 2.5% | 5.4% | 2.9% | 1.9% | 3.6% | 1.6% |
| Palmitate278 (M8) | 4.6% | 4.6% | 4.3% | 1.2% | 1.1% | 1.1% | 1.2% | 3.6% | 1.4% | 0.7% | 1.2% | 0.6% |
| Palmitate279 (M9) | 0.6% | 0.5% | 0.5% | 0.2% | 0.2% | 0.2% | 0.3% | 0.4% | 0.2% | 0.1% | 0.2% | 0.1% |
| Palmitate280 (M10) | 0.5% | 0.2% | 0.1% | 0.6% | 0.1% | 0.1% | 0.1% | 0.2% | 0.0% | 0.1% | 0.0% | 0.1% |
| Palmitate281 (M11) | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate282 (M12) | 0.5% | 0.1% | 0.0% | 0.4% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate283 (M13) | 0.2% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate284 (M14) | 0.6% | 0.1% | 0.0% | 0.5% | 0.2% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate285 (M15) | 0.2% | 0.0% | 0.1% | 0.1% | 0.0% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Palmitate286 (M16) | 0.3% | 0.1% | 0.0% | 0.3% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **Enrichment in AcCoA pool (D value %):** | **65.09** | **65.3** | **65.6** | **46.35** | **42.67** | **42.99** | **69.46** | **73.52** | **62.23** | **39.98** | **49.71** | **36.77** |
| Lower bound: | 56.92 | 56.3 | 56.77 | 35.7 | 34.09 | 33.37 | 64.1 | 69.02 | 55.67 | 36.54 | 45.99 | 32.94 |
| Upper bound: | 72.45 | 72.91 | 73.25 | 57.2 | 51.58 | 53.1 | 74.38 | 77.61 | 68.51 | 43.41 | 53.35 | 40.62 |
| **de novo lipogenesis g(t)*:** | **46.94** | **45.3** | **44.11** | **18.18** | **21.13** | **19.41** | **13.14** | **25.31** | **19.61** | **50.29** | **51.17** | **49.16** |
| Lower bound: | 40.16 | 38.56 | 37.38 | 15.41 | 18.3 | 16.58 | 11.69 | 23.08 | 17.36 | 46.87 | 47.4 | 45.14 |
| Upper bound: | 53.7 | 52.05 | 50.84 | 20.99 | 24.01 | 22.28 | 14.58 | 27.55 | 21.86 | 53.73 | 54.94 | 53.22 |

* de novo lipogenesis, g(t), varies from well to well due to differences in cell growth and time cultured in the presence of tracer
ISA modeling deconvolutes enrichment constant (D value) from de novo lipogenesis to obtain independent values.
The consistency of D values across replicates reflects the similarity of metabolic pathways across replicates.
Here, mass isotopomer distributions (MIDs) %'s are not corrected for natural isotope abundance, as this is accounted for within Metran