Supporting information (Figures S1, S2, S3, and S4)

Figure S1. Example MS¹ and MS² spectra of citrate in A549 spheroid extracts.

The two UHR-FTMS spectra were acquired from a control A549 spheroid extract in Fig. 1A. The isotopologue species of citrate were assigned based on the chromatographic retention time and accurate mass. ¹²C represent the all ¹²C (0) isotopologue. A: MS¹ spectrum of the intact citrate molecule; B: MS² spectrum of the ¹³C⁵-3,4,5-citrate fragment.
Figure S2. \(^{13}\)C atom tracing for \(^{13}\)C\(_6\)-glucose and \(^{13}\)C\(_5\),\(^{15}\)N\(_2\)-Gln transformations in the Krebs cycle

The pathway scheme in A tracks the transformations of \(^{13}\)C\(_6\)-glucose into various Krebs cycle metabolites in terms of the number and position of the \(^{13}\)C atom (●/●/●). For example, the condensation of \(^{13}\)C\(_2\)-1,2-acetyl CoA (AcCoA, ●●●) with preexisting unlabeled oxaloacetate (OAA, ●●●) produces \(^{13}\)C\(_2\)-1,2-citrate (●●●●), which with the loss of CO\(_2\) at C6 and inversion of the molecular structure at the aconitase (ACO) step (1) generates \(^{13}\)C\(_4\)-5,α-ketoglutarate (αKG, ●●●●). Subsequent loss of another CO\(_2\) from αKG at C1 via the oxoglutarate dehydrogenase (OGDH) reaction generates \(^{13}\)C\(_2\)-1,2- (●●●●) or \(^{13}\)C\(_2\)-3,4-succinate (●●●●) and subsequently \(^{13}\)C\(_2\)-3,4-\(^{15}\)N\(_2\)-1,2-fumarate and malate. Such label scrambling occurs due to the structural symmetry of succinate. Carboxylation of \(^{13}\)C\(_2\)-1,2,3-pyruvate (●●●) at C3 by pyruvate carboxylase (PC) leads to the synthesis of \(^{13}\)C\(_2\)-1,2,3-Asp (●●●) while loss of CO\(_2\) at C4 from \(^{13}\)C\(_2\)-3,4-malate via the malic enzyme (ME) reaction produces \(^{13}\)C\(_1\)-3-pyruvate (●●●). Likewise, the scheme in B traces the transformations of \(^{13}\)C\(_6\),\(^{15}\)N\(_2\)-Gln into various Krebs cycle metabolites in terms of the number and position of the \(^{13}\)C atom (●/●/●). Deamidation of labeled Gln produces \(^{13}\)C\(_5\),\(^{15}\)N\(_2\)-Glu, which is deaminated to \(^{13}\)C\(_4\)-αKG (●●●●) to enter the Krebs cycle. Subsequent Krebs cycle reactions generate \(^{13}\)C\(_2\)-malate (●●●), which undergoes ME and pyruvate dehydrogenase (PDH) reactions to produce \(^{13}\)C\(_2\)-pyruvate (●●●), \(^{13}\)C\(_2\)-AcCoA, respectively. \(^{13}\)C\(_2\)-pyruvate (●●●) is then transformed via the Krebs cycle reactions to produce \(^{13}\)C\(_2\)-1,2-citrate (●●●●), \(^{13}\)C\(_2\)-4,5-αKG (●●●●), and \(^{13}\)C\(_2\)-3,4-malate (●●●●). \(^{13}\)C\(_2\)-pyruvate can also be carboxylated by PC to produce \(^{13}\)C\(_2\)-1,2,3-malate/Asp (●●●●) and \(^{13}\)C\(_2\)-3,4,6-citrate (●●●). Red arrows depict cleavage of carbon-carbon bonds. Not all reaction products are shown. ●: \(^{12}\)C; ◦: \(^{14}\)N; ○: \(^{15}\)N. See Fig. 1 for all other abbreviations.
Figure S3. $^{13}$C atom tracing for $[^{13}C_6]$-glucose transformations via glycolysis, Ser→Gly synthesis, and gluconeogenesis pathways.

$[^{13}C_6]$-glucose is converted to fructose-1,6-bisphosphate (F1,6BP, ••••••) via phosphorylation and isomerization, which is cleaved between C3 and C4 to generate $^{13}$C$_3$-dihydroxyacetone-3-phosphate (DHAP) and $^{13}$C$_3$-glyceraldehyde-3-phosphate (GAP) (●●●). Subsequent glycolytic reactions generate $^{13}$C$_2$-pyruvate, which in turn produce $^{13}$C$_2$-1,2-oxaloacetate (OAA, •●•●•) and $^{13}$C$_3$-1,2,3-OAA (●●●●) via PDH- or PC-initiated Krebs cycle reactions. The two labeled OAA species then undergo the first step of gluconeogenic (PCK) reaction to produce $^{13}$C$_3$-PEP (●●●) and $^{13}$C$_1$-1,2-PEP (●●●), which lead to the synthesis of $^{13}$C$_2$-4,5,6-F1,6BP (●●●●●) and $^{13}$C$_1$-1,2-F1,6BP (●•●•●•) via subsequent gluconeogenic reactions. The glycolytic product 3-phosphoglycerate (3-PG ●●●) is the precursor to Ser (●●●) and in turn Gly (●●●) synthesis. Not all reaction products are shown. ● represents $^{12}$C and ●/● refer to $^{13}$C derived from glycolysis/gluconeogenesis respectively. Solid and dash arrows indicate single- and multi-step reactions. Red arrows depict cleavage of carbon-carbon bonds. HK: hexokinase; PGI: phosphoglucose isomerase; PFK: phosphofructose kinase; FBP1: fructose-bisphosphatase 1; ALDO: fructose-bisphosphate aldolase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; PGK1: phosphoglycerate kinase 1; PGAM: phosphoglycerate mutase; ENO: enolase; PK: pyruvate
kinase; LDH: Lactate Dehydrogenase; PCK: phosphoenolpyruvate carboxykinase; PC: pyruvate carboxylase; SHMT: serine hydroxymethyltransferase.

Figure S4. Sources of carbon and nitrogen atoms for the synthesis of UDP-GlcNAc.
The diagram is a copy of Fig. 1 in (2). Atoms are numbered according to the international convention in individual subunits. G, A, R, and U represent the glucose, acetyl, ribose, and uracil subunits, respectively. UDP-GlcNAc: uridine diphosphate-N-acetylglucosamine; PRPP: phosphoribosyl pyrophosphate.

References