Supporting Information

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SI Materials and Methods

RNA Interference and Immunoblotting. IDH2 oligonucleotides from Sigma-Proligo were transfected using Lipofectamine RNAiMAX (Invitrogen). Manufacturer protocols were used to transfect 50 pmol of each siRNA into 1.2×10^6 cells at a final siRNA concentration of 16.7 nM. For protein analysis, cells were lysed in RIPA buffer [50 mM Tris·HCl, pH 7.4/150 mM NaCl/1% sodium deoxycholate/0.1% SDS/1× complete EDTA-free protease inhibitor mixture tablets (Roche Applied Science)] and cleared lysates were separated on 4-12% Bis-Tris gels (Invitrogen) followed by transfer onto nitrocellulose. After blocking in 3% skim milk, blots were probed using mouse monoclonal IDH2 antibody (Abcam, ab55271), goat polyclonal β-actin antibody (Santa Cruz, sc1616), rabbit polyclonal HIF1α antibody (Cayman Chemicals, 10006421), and rabbit monoclonal GAPDH antibody (Cell Signaling, 2118S).

GC-MS Analysis. 1 μL of MTBSTFA-derivatized extracts was injected into an Agilent 7890A GC equipped with an HP-5MS (30 m × 0.25 mm × 0.25 μM) capillary column (Agilent J&W GC Columns) connected to an Agilent 5975C Mass Selective Detector using electron impact ionization (EI) with an ionizing voltage of −70 eV and an electron multiplier set to 1,060 V. For analysis of t-butyl dimethyl silyl (TBDMS) derivatives of organic and amino acids, the GC temperature was started at 100 °C and held for 3 min. The temperature was then ramped to 230 °C at 4 °C/min and held for 4 min. The temperature was then ramped to 300 °C at 50 °C/min and held for 5 min. Mass range of 50–500 amu was recorded in scan mode at 2.71 scans/sec. Isotopic enrichment in citrate was monitored using ions at *m/z* 459, 460, 461, 462, 463, 464, and 465 for M+0, M+1, M+2, M+3, M+4, M+5, and M+6 (containing 0–6 ^{13}C-enriched atoms), respectively, formed through the loss of a t-butyl (−57 amu) and t-butyldimethylsilanol (−132 amu) from the molecular ion, tetra-TBDMS-citrate (648 amu). Isotopic enrichment in malate was monitored using ions at *m/z* 419, 420, 421, 422, 423, for M+0, M+1, M+2, M+3, M+4 (containing 0–4 ^{13}C-enriched atoms), respectively, formed through the loss of a t-butyl (−57 amu) from the molecular ion, tri-TBDMS-malate (476 amu). Isotopic enrichment in aspartate was monitored using ions at *m/z* 418, 419, 420, 421, 422 for M+0, M+1, M+2, M+3, M+4 (containing 0–4 ^{13}C-enriched atoms), respectively, formed through the loss of a t-butyl (−57 amu) from the molecular ion, tri-TBDMS-aspartic acid (475 amu). Isotopic enrichment in fumarate was monitored using ions at *m/z* 287, 288, 289, 290, 291 for M+0, M+1, M+2, M+3, M+4 (containing 0–4 ^{13}C-enriched atoms), respectively, formed through the loss of a t-butyl (−57 amu) from the molecular ion, di-TBDMS-fumaric acid (344 amu). Isotopic enrichment in 2-hydroxyglutarate (2HG) was monitored using ions at *m/z* 433, 434, 435, 436, 437, 438 for M+0, M+1, M+2, M+3, M+4, M+5 (containing 0–5 ^{13}C-enriched atoms), respectively, formed through the loss of a t-butyl (−57 amu) from the molecular ion, tri-TBDMS-2HG (490 amu).

Isotopomer distributions for citrate, malate, aspartate, and fumarate were simultaneously corrected for naturally-occurring heavy isotopes of all elements in each mass fragment using a correction matrix as described (1, 2). Briefly, each EI spectral fragment (isotopomer distribution) is used to build a mass distribution vector (MDV). The MDV is simultaneously corrected for the effects of natural isotopic abundance in the carbon skeleton and derivatization chains using a correction matrix. This matrix is itself the product of the individual correction matrices for all of the different atomic species of the fragment ion set, constructed using the number of atoms of each element in the fragment and the natural abundance of stable isotope of each element. All calculations were performed using the statistics package, R V. 2.9.1 (The R Foundation for Statistical Computing).

Statistical Analysis. Student *t* test was used to calculate final *P* values.

1. Nanchen A, Fuhrer T, Sauer U (2007) *Metabolomics: Methods and Protocols*, ed Weckwerth W (Humana Press, Totowa, NJ), pp 177−197.

2. van Winden WA, Wittmann C, Heinzle E, Heijnen JJ (2002) Correcting mass isotopomer distributions for naturally occurring isotopes. *Biotechnol Bioeng* 80:477−479.