SUPPLEMENTAL INFORMATION

Oxidation of alpha-ketoglutarate is required for reductive carboxylation in cancer cells with

mitochondrial defects

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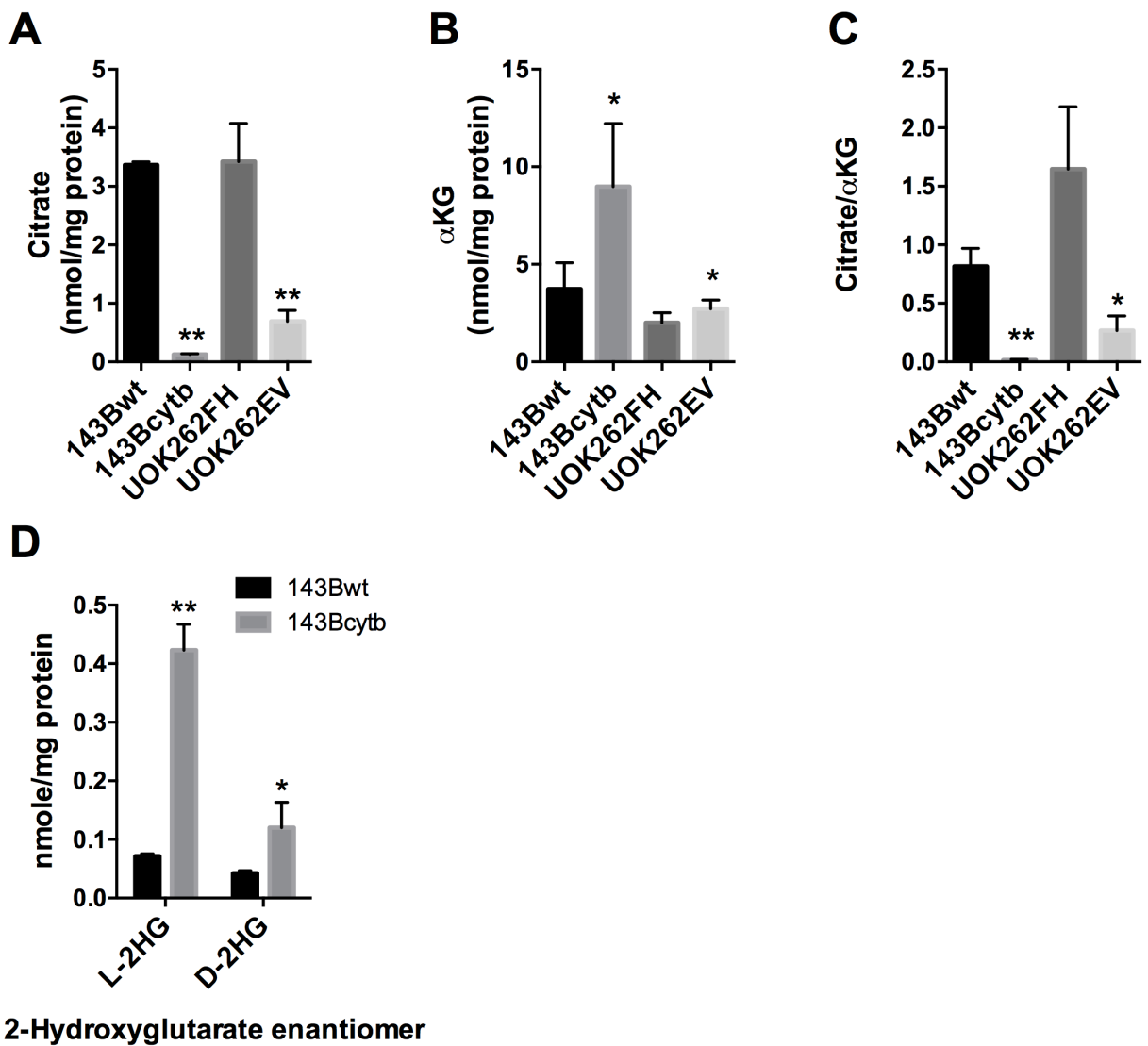
DeBerardinis[1,4*]

Figure S1, related to Figure1. Abundance of selected metabolites in cells using oxidative or reductive glutamine metabolism.

(A-C) Quantitative abundance of metabolites extracted from triplicate samples of 143B*wt*, 143B*cytb*, UOK262FH and UOK262EV cells. *$p < 0.05$; ** $p < 0.005$, Student's t-test.

(D) Measurement of 2-hydroxyglutarate enantiomers in 143B*wt* and 143B*cytb* cells. Data are average and S.D. of three independent cultures. *$p < 0.05$; ** $p < 0.005$, Student's t-test.
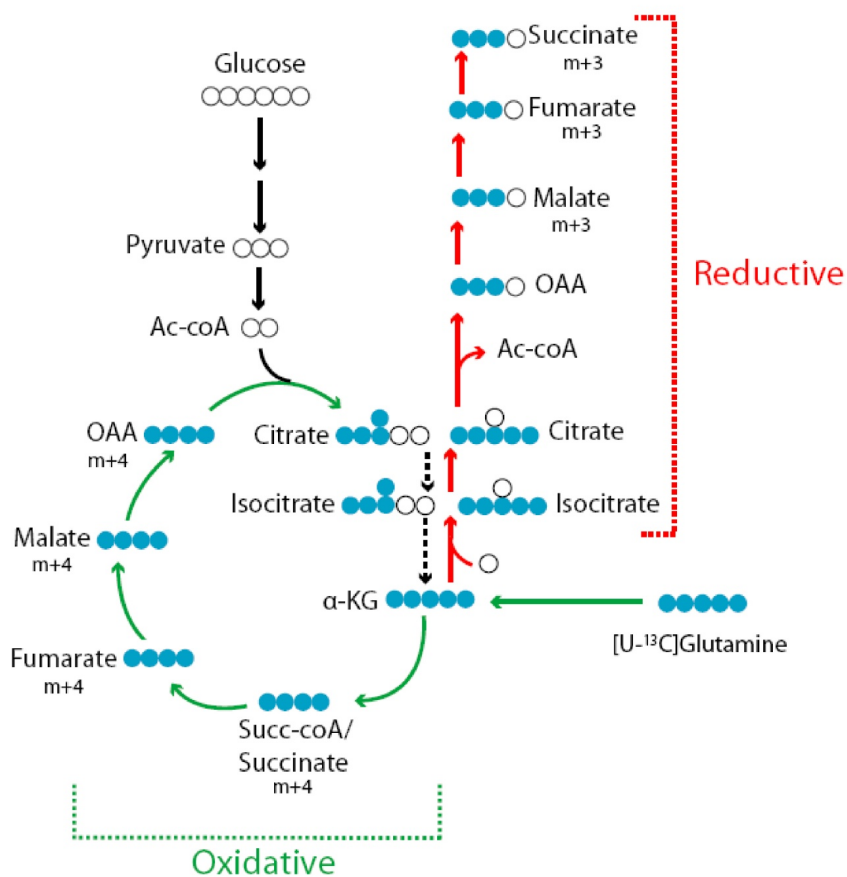
Figure S2, related to Figure 2. Schematic for oxidative and reductive glutamine metabolism. Metabolism of [U-^{13}C]glutamine produces fully labeled AKG. Oxidation of AKG in the TCA cycle (green arrows) produces succinyl-coA, succinate, fumarate, malate and OAA with four ^{13}C-carbons (m+4). Condensation of labeled OAA with unlabeled acetyl-coA generates citrate with four ^{13}C-carbons. Reduction of AKG through reductive carboxylation pathway (red arrows) produces citrate with five ^{13}C carbons. Cleavage of this citrate produces OAA with three ^{13}C-carbons. Further metabolism of this yields malate, fumarate and succinate with three ^{13}C-carbons (m+3). Blue circles, ^{13}C; white circles, [12]C.
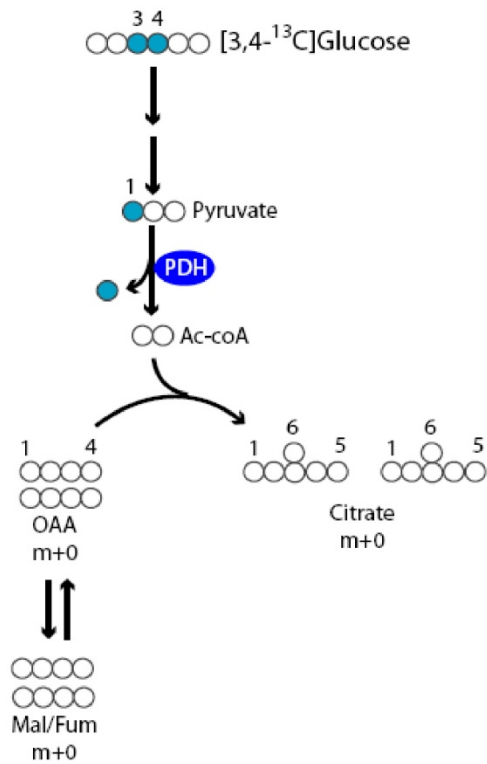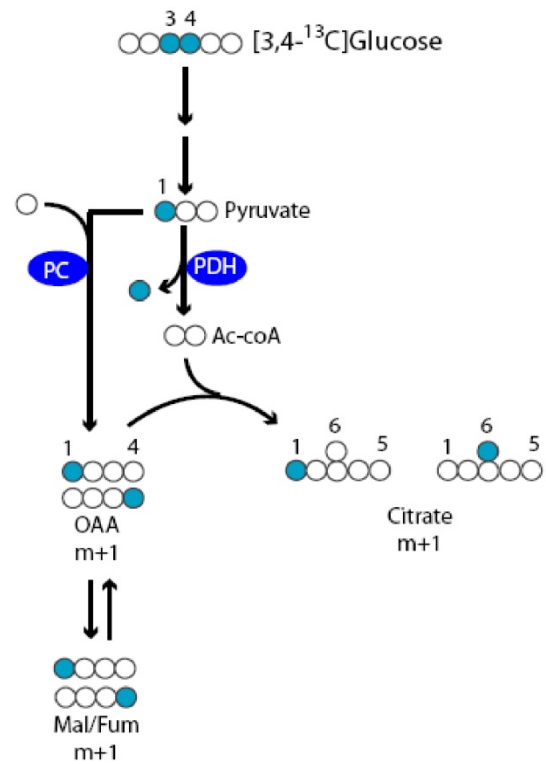
Figure S3, related to Figure 3. Schematic for detection of pyruvate carboxylase activity.

(A)   Metabolism of [3,4-^{13}C]glucose produces pyruvate m+1 (labeled on the 1-carbon). During oxidative metabolism of pyruvate through the pyruvate dehydrogenase (PDH) reaction this labeled carbon is released in the form of CO_2 and no label is present in TCA cycle metabolites.

(B)   If pyruvate carboxylase (PC) is active, labeled carbon is retained to produce OAA and malate m+1. Condensation of an unlabeled acetyl-coA with a labeled OAA produces citrate m+1, labeled on either the 1- or 6-position.
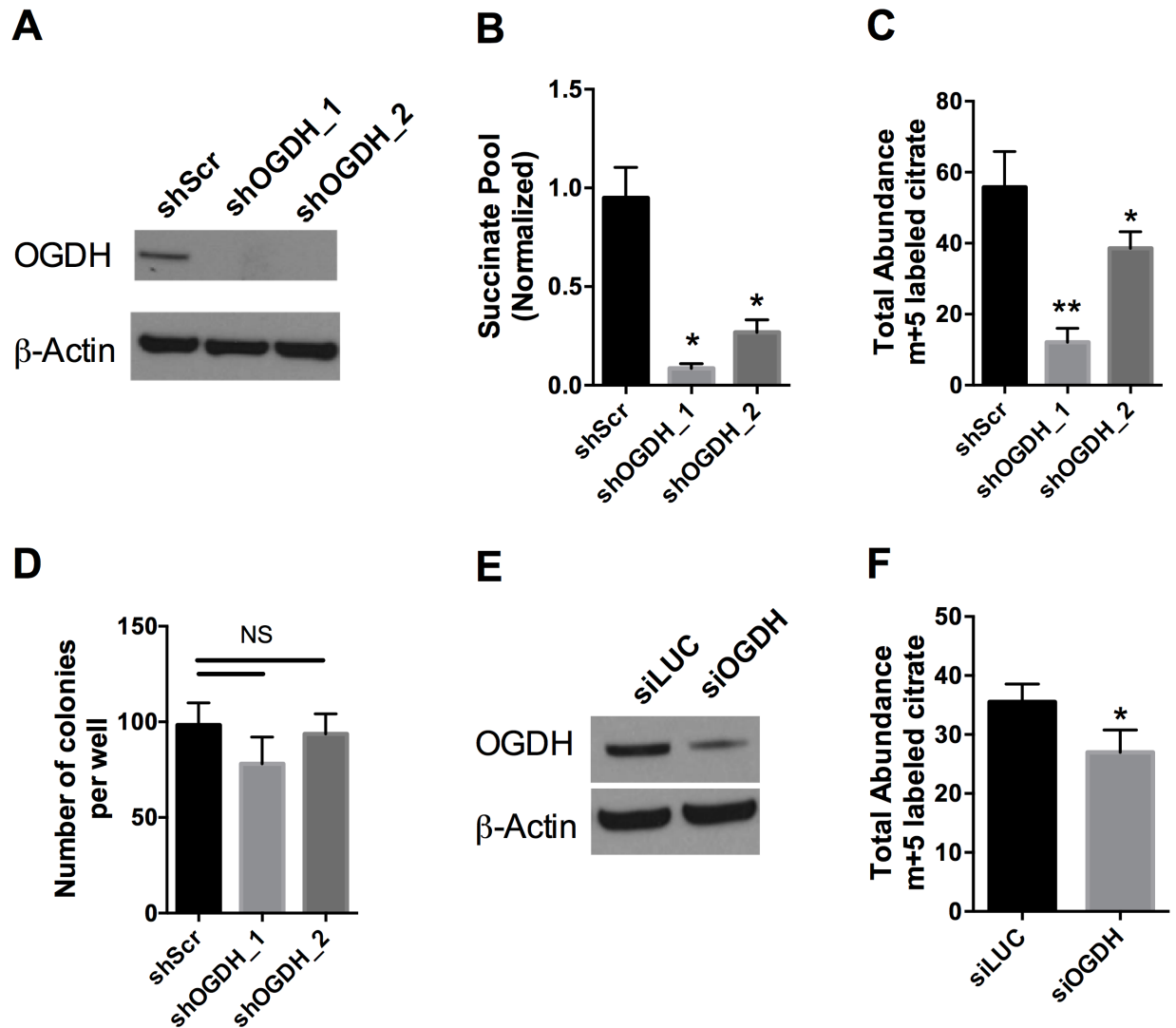
Figure S4, Related to Figure 5. AKG dehydrogenase is required for maximal reductive carboxylation in 143B*cytb* and UOK262EV cells.

(A)   Abundance of OGDH protein in 143B*cytb* cells stably expressing either a scrambled shRNA (shScr) or shRNAs targeting *OGDH* (shOGDH_1; shOGDH_2).

(B)   Relative abundance of succinate in 143B*cytb* cells stably expressing *OGDH* shRNAs. Data are the average and S.D. of three independent cultures. *$p < 0.05$, Student's t-test.

(C)   Abundance of m+5 labeled citrate in 143B*cytb* cells stably expressing *OGDH* shRNAs and cultured with [U-^{13}C]glutamine. Data are the average and S.D. of three independent cultures. *$p < 0.05$; **$p < 0.005$, Student's t-test.

(D)   Colony formation in soft agar in 143Bcytb cells stably expressing shScr, shOGDH_1 or shOGDH_2. Data are the average and S.D. of three independent cultures.

(E)   Abundance of OGDH protein in UOK262EV cells transfected with siLUC or siOGDH.

(F)   Abundance of m+5 labeled citrate in UOK262EV cells transfected with siLUC or siOGDH. Data are the average and S.D. of three independent cultures. *$p < 0.05$, Student's t-test.
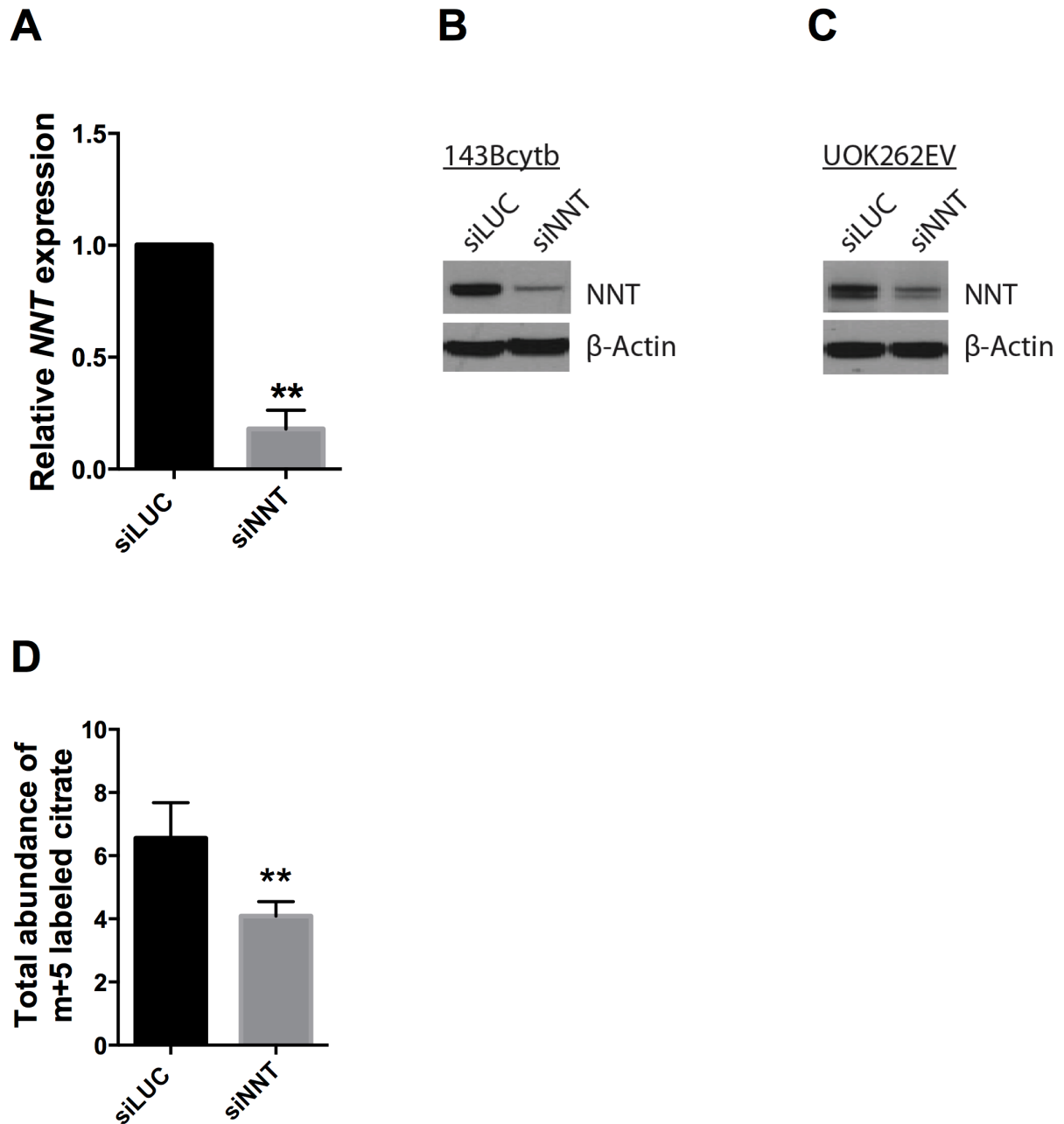
Figure S5, related to Figure 6. Nicotinamide nucleotide transhydrogenase (NNT) is required for maximal reductive carboxylation in 143B*cytb* and UOK262EV cells.

(A) Quantitative PCR analysis of *NNT* mRNA following transient transfection with siRNA pools against luciferase (siLuc) or *NNT* (siNNT) in 143B*cytb* cells. Abundance of *NNT* was normalized to the housekeeping transcript *GAPDH*. Data are the average and S.D. of technical quadruplicates. **$p<0.005$, Student's t-test.

(B) Abundance of NNT protein in 143B*cytb* cells transfected with siLUC or siNNT.

(C) Abundance of NNT protein in UOK262EV cells transfected with siLUC or siNNT.

(D) Abundance of m+5 labeled citrate in UOK262EV cells transfected with siLUC or siNNT. Data are the average and S.D. of three independent cultures. **$p< 0.005$, Student's t-test.

Table S1, Related to Figure 1. The list of 92 endogenous metabolites and their MRM transitions (*m/z*), declustering potentials (DP, in Volts) and collision energies (CE, in Volts) for the corresponding ionization modes on an AB QTRAP 5500 performed in positive/negative switching mode. Chromatographically co-eluted metabolites with shared MRM transitions were counted as one metabolite and shown in a grouped format (e.g. alanine/sarcosine).

Table S2, Related to Figure 1. The relative abundance and statistical analysis of metabolites detected from the two groups of isogenic cell lines. Relative abundance was calculated by normalizing the peak areas integrated using MuliQuant to the protein content (mg) of each sample. Fold changes were calculated by comparing the mean values of three biological replicates. The three metabolites highlighted in yellow (proline, 2-hydroxyglutarate and succinate) displayed highly significant ($p<0.005$, Student's t-test) differences in abundance between the two members of both pairs, and in all three cases the direction of the difference (i.e. higher or lower) was shared in the two cell lines using reductive carboxylation.