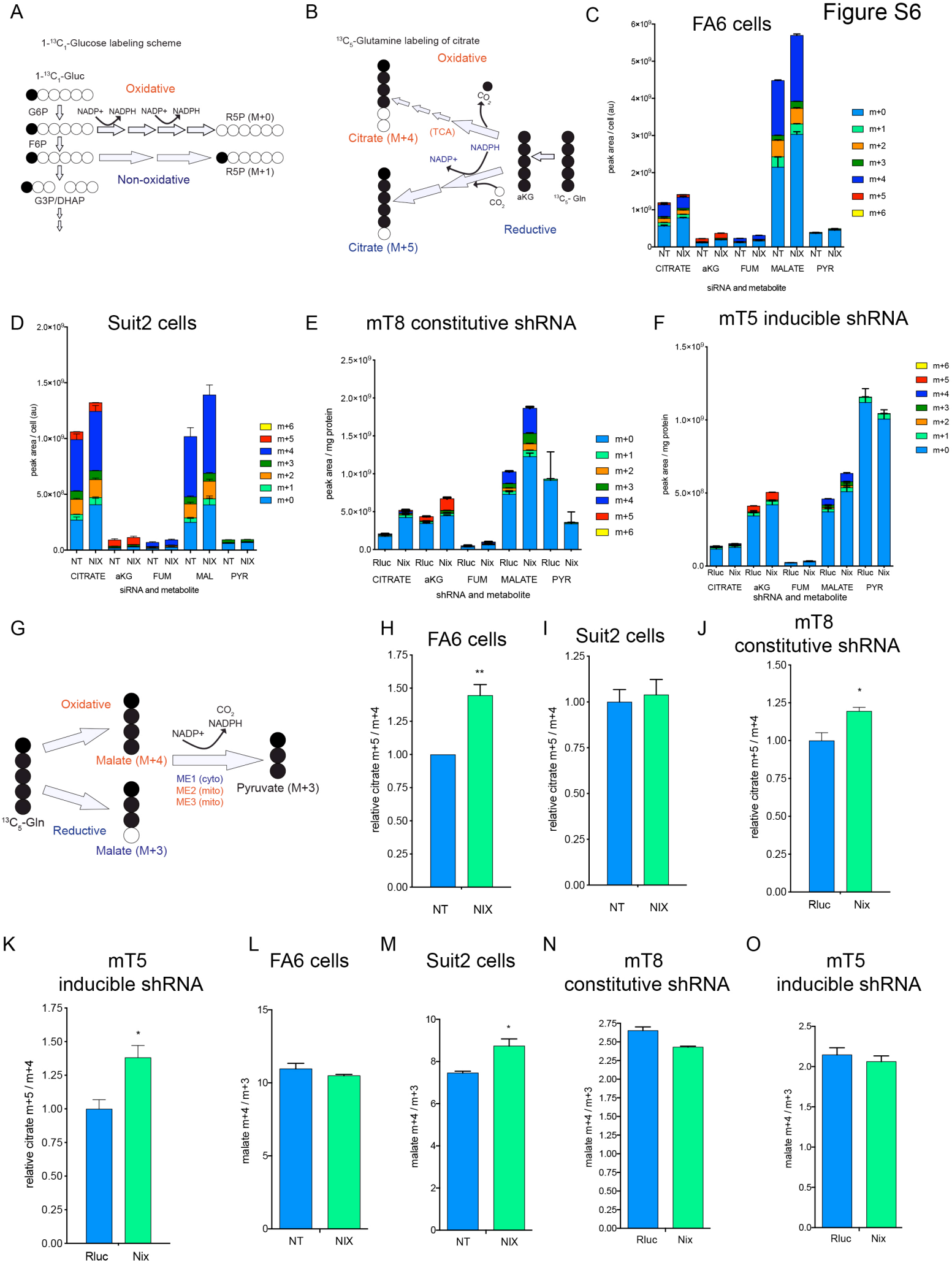
Figure S6


A
1-13C1-Glucose labeling scheme
1-13C1-Gluc
Oxidative
G6P
NADP+ NADPH NADP+ NADPH
R5P (M+0)
F6P
R5P (M+1)
Non-oxidative
G3P/DHAP
B
13C5-Glutamine labeling of citrate
Oxidative
Citrate (M+4)
CO2
(TCA)
NADPH
NADP+
CO2
aKG
13C5- Gln
Reductive
Citrate (M+5)
C
FA6 cells
peak area / cell (au)
m+0
m+1
m+2
m+3
m+4
m+5
m+6
NT NIX NT NIX NT NIX NT NIX NT NIX
CITRATE aKG FUM MALATE PYR
siRNA and metabolite
D
Suit2 cells
peak area / cell (au)
NT NIX NT NIX NT NIX NT NIX NT NIX
CITRATE aKG FUM MAL PYR
siRNA and metabolite
E
mT8 constitutive shRNA
peak area / mg protein
Rluc Nix Rluc Nix Rluc Nix Rluc Nix Rluc Nix
CITRATE aKG FUM MALATE PYR
shRNA and metabolite
F
mT5 inducible shRNA
peak area / mg protein
Rluc Nix Rluc Nix Rluc Nix Rluc Nix Rluc Nix
CITRATE aKG FUM MALATE PYR
shRNA and metabolite
G
Oxidative
Malate (M+4)
CO2
NADPH
NADP+
ME1 (cyto)
ME2 (mito)
ME3 (mito)
Pyruvate (M+3)
13C5-Gln
Reductive
Malate (M+3)
H
FA6 cells
relative citrate m+5 / m+4
NT NIX
I
Suit2 cells
relative citrate m+5 / m+4
NT NIX
J
mT8
constitutive shRNA
relative citrate m+5 / m+4
Rluc Nix
K
mT5
inducible shRNA
relative citrate m+5 / m+4
Rluc Nix
L
FA6 cells
malate m+4 / m+3
NT NIX
M
Suit2 cells
malate m+4 / m+3
NT NIX
N
mT8
constitutive shRNA
malate m+4 / m+3
Rluc Nix
O
mT5
inducible shRNA
malate m+4 / m+3
Rluc Nix

Supplementary Figure 6. Nix-depleted cells induce the flux of glucose to the oxPPP and the reductive carboxylation of glutamine to support NADPH production *in vitro.*

A) Schematic of 1-13C1-glucose labeling in oxidative and non-oxidative branches of PPP in FA6 cells. B) Schematic of reductive carboxylation vs. oxidative metabolism of 13C5, 15N2-glutamine to citrate. C/D) Isotopologue analysis of TCA cycle intermediates for siNT and siNIX FA6 (C) and Suit2 (D) cells cultured for 45' with 2mM 13C5, 15N2-glutamine. n=4 (FA6) or n=3 (Suit2) independent experiments. E/F) isotopologue analysis (peak area normalized by mg protein) of TCA cycle intermediates for mT8 LEPG.shRluc713 or LEPG.shNix447 organoids (E) and mT5 RT3.shRluc713 or shNix447 organoids (F) cultured for 45' with 2mM 13C5, 15N2-glutamine, n=3 technical replicates. G) Schematic of reductive carboxylation vs. oxidative metabolism of 13C5, 15N2-glutamine to malate. H/I) Ratio of citrate (m+5) to citrate (m+4) from siNT and siNIX FA6 (H) and Suit2 (I) cells cultured with 13C5, 15N2-glutamine. n=4 (FA6) or n=3 (Suit2) independent experiments. J/K) Ratio of citrate (m+5) to citrate (m+4) from mT8 LEPG.shRluc713 or LEPG.shNix447 organoids (J) and mT5 RT3.shRluc713 or shNix447 organoids (K) cultured with 13C5, 15N2-glutamine, n=3 technical replicates. L/M) Ratio of malate (m+4) to malate (m+3) from siNT and siNIX FA6 (L) and Suit2 (M) cells cultured with 13C5, 15N2-glutamine. n=4 (FA6) or n=3 (Suit2) independent experiments. N/O) Ratio of malate (m+4) to malate (m+3) from mT8 LEPG.shRluc713 or LEPG.shNix447 organoids (N) and mT5 RT3.shRluc713 or shNix447 organoids (O) cultured with 13C5, 15N2-glutamine, n=3 technical replicates.