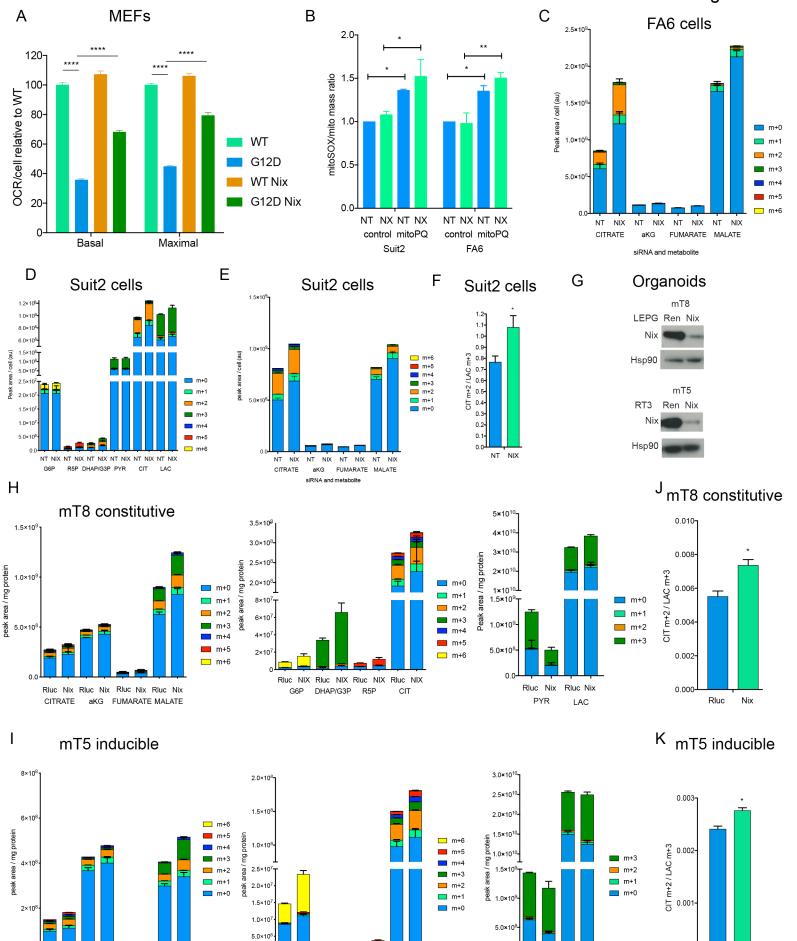
Figure S5



0.0

G6P

Rluc Nix Rluc Nix

aKG FUMARATE MALATE

Rluc Nix

CITRATE

Rluc Nix

Rluc NIX Rluc NIX Rluc NIX Rluc NIX

R5P

CIT

DHAP/G3P

0.000

0.0

Rluc Nix Rluc Nix

LAC shRNA and metabolite

PYR

NIX Rluc shRNA

Supplementary Figure 5. Nix limits glucose flux into the TCA cycle in human PDAC cell lines and organoids. A) Basal and maximal OCR per cell of LSL-KrasG12D MEFs infected with Adeno-Empty (WT) or Adeno-Cre (G12D) and then transfected with control (NT) or Nix siRNA. B) Relative mitochondrial ROS (MitoSox Red fluorescence) level normalized to mitochondrial mass (MitoTracker Green fluorescence) for siNT and siNIX (NX) for Suit2 and FA6 cells cultured in low glucose conditions and treated with control or mitoPQ (5uM) for 20 minutes prior to analysis. n=3 independent experiments. C) Isotopologue analysis of TCA cycle intermediates: citrate, alpha ketoglutarate (aKG), fumarate, and malate for siNT and siNIX FA6 cells cultured with 2mM 13C6-glucose for 45'. n=3 independent experiments. D/E) Isotopologue analysis of glycolytic (D) and TCA cycle (E) intermediates for siNT and siNIX Suit2 cells cultured with 2mM 13C6-glucose for 45'. n=3 independent experiments. F) Ratio of citrate (m+2) to lactate (m+3) for siNT and siNIX Suit2 cells (from D). n=3 independent experiments. G) Immunoblot for Nix protein in mT5 organoids with RT3GEPIR.shRluc713 and shNix447 (RT3) hairpins at 72 hours after doxycycline addition or of mT8 organoids infected with LEPG-shNix447 or shRluc713 hairpins. H) From left to right: Normalized isotopologue pool sizes for TCA cycle (left panel), glycolytic (middle), and (m+0 to m+3) pyruvate and lactate, in mT8 organoids infected with LEPG.shRluc713 or LEPG.shNix447 cultured with 2mM 13C6-glucose for 45'. n=3 technical replicates. I) From left to right: Normalized isotopologue pool sizes for TCA cycle (left panel), glycolytic (middle), and (m+0 to m+3) pyruvate and lactate, in mT5 organoids infected with RT3.shRluc713 or RT3.shNix447 cultured with 2mM 13C6-glucose for 45', 72 hours after addition of doxycycline, n=3 technical replicates. J/K) Ratio of citrate (m+2) to lactate (m+3) for (J) mT8 LEPG.shRluc713 or LEPG.shNix447 organoids or (K) mT5 RT3.shRluc713 or RT3.shNix447 organoids as in (H/I), n=3 technical replicates.