SUPPLEMENTARY INFORMATION

Double genetic disruption of lactate dehydrogenases A and B is required to ablate the 'Warburg effect' restricting tumor growth to oxidative metabolism

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Figures Legends Figure S1 Figure S2 Figure S3

Supplementary figures legends

Figure S1: **Mitochondrial function is altered in** *LDHA/B***-DKO cells.** Basal respiration (**A**, **D**), maximal respiration (**B**, **E**) and oxygen consumption related to ATP production (**C**, **F**) were analyzed by means of an Oroboros oxygraph in LS174T (**A-C**) and B16 (**D-F**) WT and *LDH*-KO cells. Mitochondrial respiration was determined by high-resolution respirometry. Cells were suspended at a concentration of approximately 0.5×10^6 cells/ml in culture medium and a total of 2.1 ml was added to the each chamber. Basal respiration (**A**, **D**) was determined after a stabilization phase of 15 min. Maximum capacity of the electron transfer system (ETS, **B**, **E**) was determined after stepwise uncoupling with FCCP in the presence of oligomycin. Oxygen consumption related to ATP production (O₂-ATP, **C**, **F**) was calculated as the difference of basal respiration and LEAK respiration (oligomycin-inhibited respiration). Shown are single values and the median of at least three independent experiments.. * p < 0.002, *** p < 0.0008,

Figure S2: Impact of *LDHA/B*-knock-out on mitochondrial content. Median fluorescence intensity of LS174T (**A**) and B16 cells (**D**) labeled with Mitotracker probe was analyzed by flow cytometry. mtDNA/nDNA ratio content of WT and *LDH*-KO LS174T (**B**) and B16 (**E**) cells evaluated by qPCR. The mean \pm S.E.M. is representative of four independent experiments performed in duplicate. * p < 0.03, ** p < 0.003. COXIV and cytochrome c levels in LS174T (**C**) and COXIV, cytochrome c and ATP8 protein levels in B16 cells (**F**), as revealed by proteomic analysis.

Figure S3: *LDHA/B*-DKOs show an increase in the isotopologues of alanine. (A) Labeling pattern for ¹³C₆-glucose tracing. Blue circles represent a ¹³C atom, black a ¹²C. Flux with one round through the TCA is shown. For alanine (pyruvate) only fully labeled products are expected, but the *LDHA/B*-DKOs show an increase in the m+1 and m+2 isotopologues of alanine. (B) Labeling pattern for ¹³C₅-glutamine tracing. Blue circles represent a ¹³C atom, black a ¹²C. Flux with one round through the TCA is shown. For alanine (pyruvate) no labeled products are expected, but the *LDHA/B*-DKOs show an increase in the m+1 and m+2 isotopologues of alanine. (B) Labeling pattern for ¹³C₅-glutamine tracing. Blue circles represent a ¹³C atom, black a ¹²C. Flux with one round through the TCA is shown. For alanine (pyruvate) no labeled products are expected, but the *LDHA/B*-DKOs show an increase in the m+1 and m+2) isotopologues of alanine.







B16











С





Fig S3 A Glucose ●●●●●●● Glucose-6-P → PPP Glycerinaldehyd-3-P ↓ C NAD' NADH+H' 1,3-Bisphosphoglycerate NAD⁺ NADH+H⁺ Glu a-KG THF N', N"-methylene-THF 3-Phosphoglycerate $\xrightarrow{\checkmark}$ 3-Phosphonooxypyruvate $\xrightarrow{\checkmark}$ Phosphoserine \rightarrow Serine $\xrightarrow{\checkmark}$ Glycine 000 000 $\begin{array}{c} \text{NAD}^{\bullet} \text{ NADH+H}^{\bullet} & \text{Glu} & a \text{-KG} \\ \hline \end{array}$ 2.5 2 1.5 2.5 -CO2 Isotope f -CO2 TTC NAL NADH+H 1 Acetyl-CoA -----0.5 OAA Citrate Acetyl-CoA ---- Lipidsynthesis 00 0 m+1 m+2 IAC Isocitrate -Alanine NAD -CO2 B16_wt B16_LDHA_co B16_LDHB_co Fumarate ee NADH+H: B16_LDHA/B_co LS174T_wt LS174T_LDHA/B_co NAD a-KG ••••• NADH+H: /-CO2 Succinate Glu Succinyl-CoA **66**00 Gln Glucose ●●●●●● Glucose-6-P → PPP ●●● Glycerinaldehyd-3-P ↓ (NAD* NAD++++* ••• 1,3-Bisphosphoglycerate ◆ 3-Phosphoglycerate → 3-Phosphonoxypyruvate → Phosphoserine → Serine THF N',N"-methylene-THF Glycine 18 16 $\begin{array}{ccc} & & & \\ & & & \\ & & & \\ & \bullet \bullet \bullet & \\ & & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & & & \\ & &$ ≥ 14 fraction [NADPH+H* NADP* -CO2 000 Isotope f Pyruvate 🖌 Malate -CO₂ Malate NAD* NAD* NAD+ NAD+ NAD+ Acety 00000 2 Asp - OAA - OAA - NADH+H* Malate NAD* → Lipidsynthesis ⁰ OAA Acetyl-CoA Citrate + m+1 m+2 m+3 0000 Alanine 0000 Isocitrate 10000 B16_wt B16_LDHA_co B16_LDHB_co NAD' -CO2 B16_LDHA/B_co LS174T_wt LS174T_LDHA/B_co Fumarate NADH+H-0000 NAD a-KG Succinate NADH+H /-CO2 Glu Succinyl-CoA

Gin eeeee

B