

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 (O_2 -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.. * $p < 0.05$, ** $p < 0.002$, *** $p < 0.0008$,

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 $^{13}C_6$ -glucose tracing. Blue circles represent a ^{13}C atom, black a ^{12}C . 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 $^{13}C_5$ -glutamine tracing. Blue circles represent a ^{13}C atom, black a ^{12}C . 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+3 (and m+1 and m+2) isotopologues of alanine.

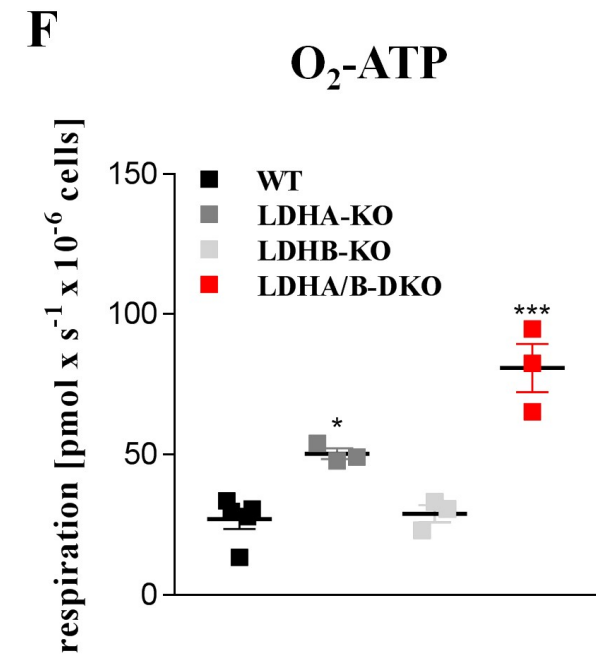
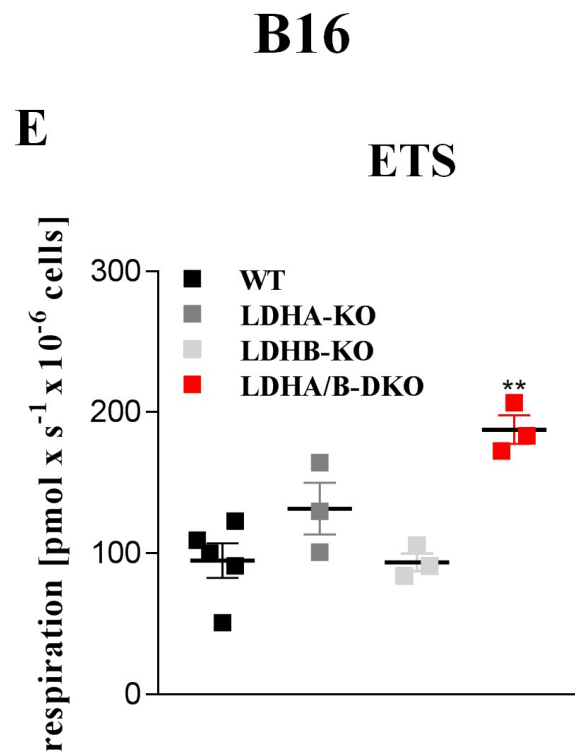
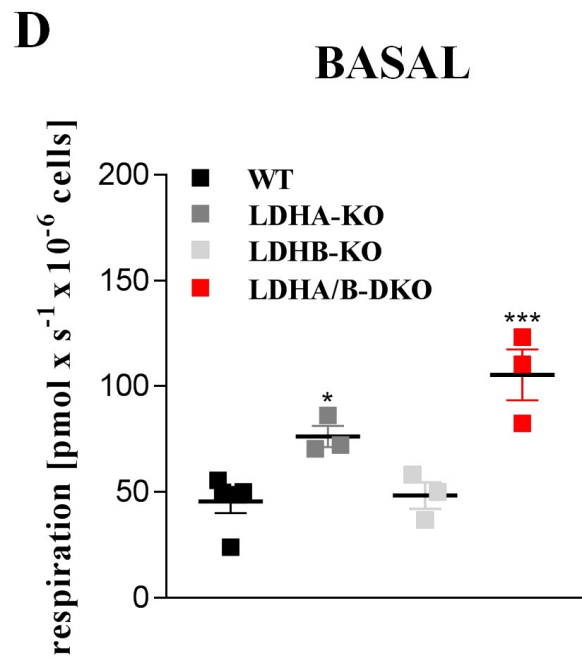
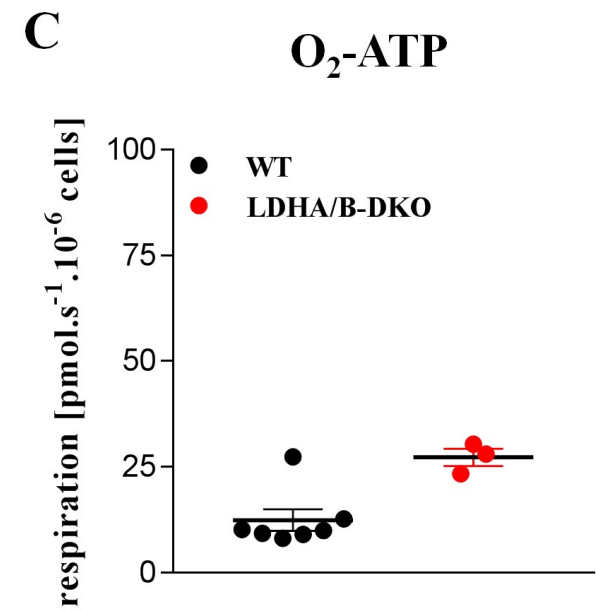
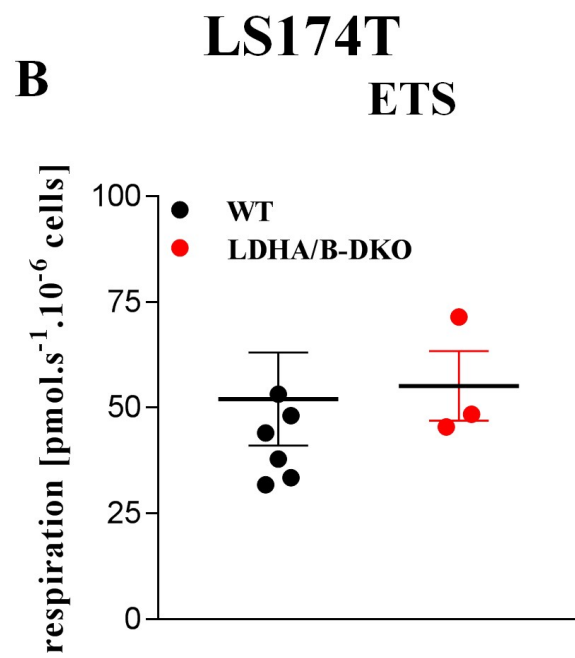
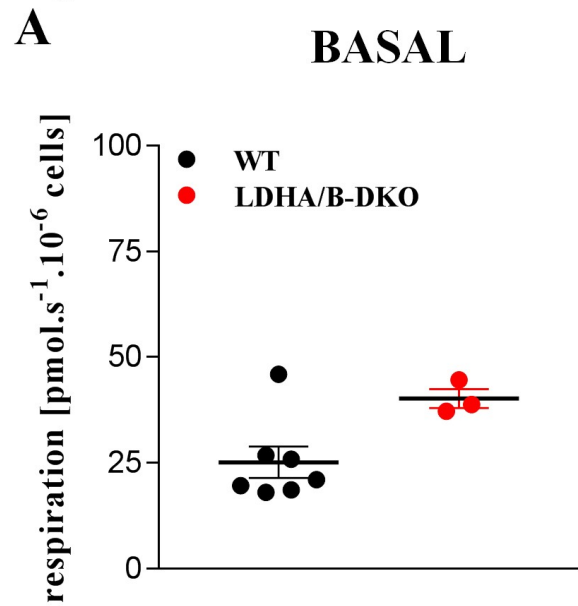
Fig.S1

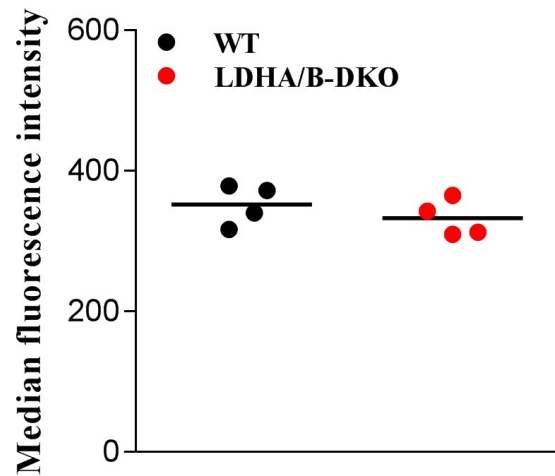
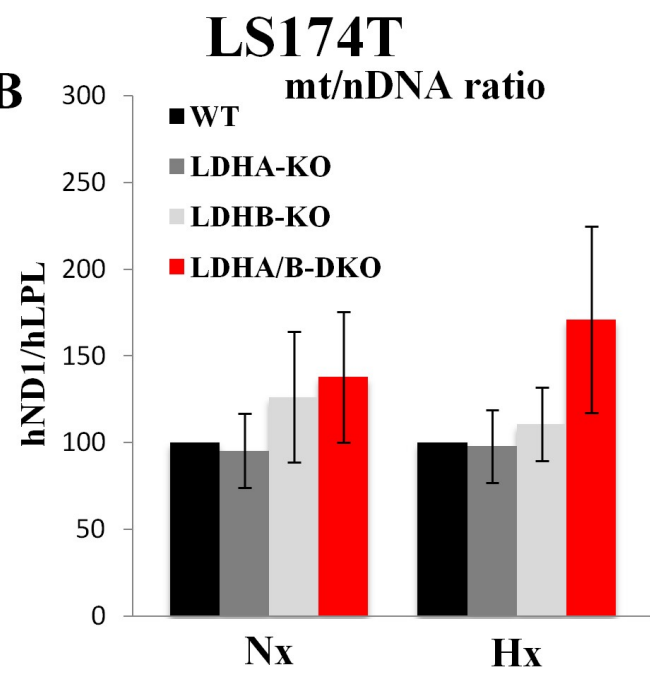
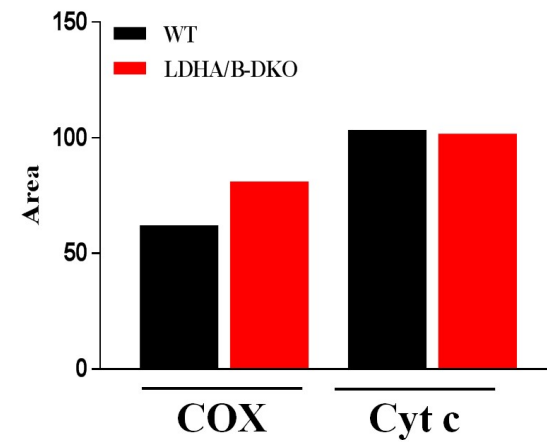
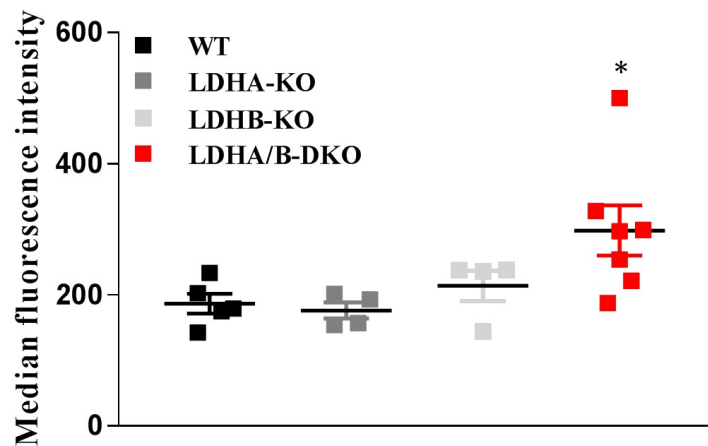
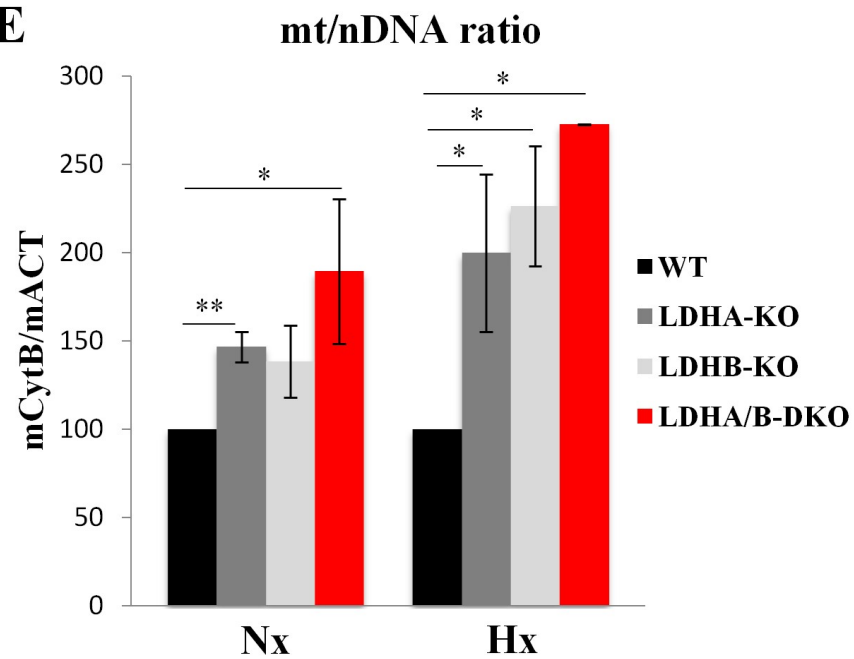
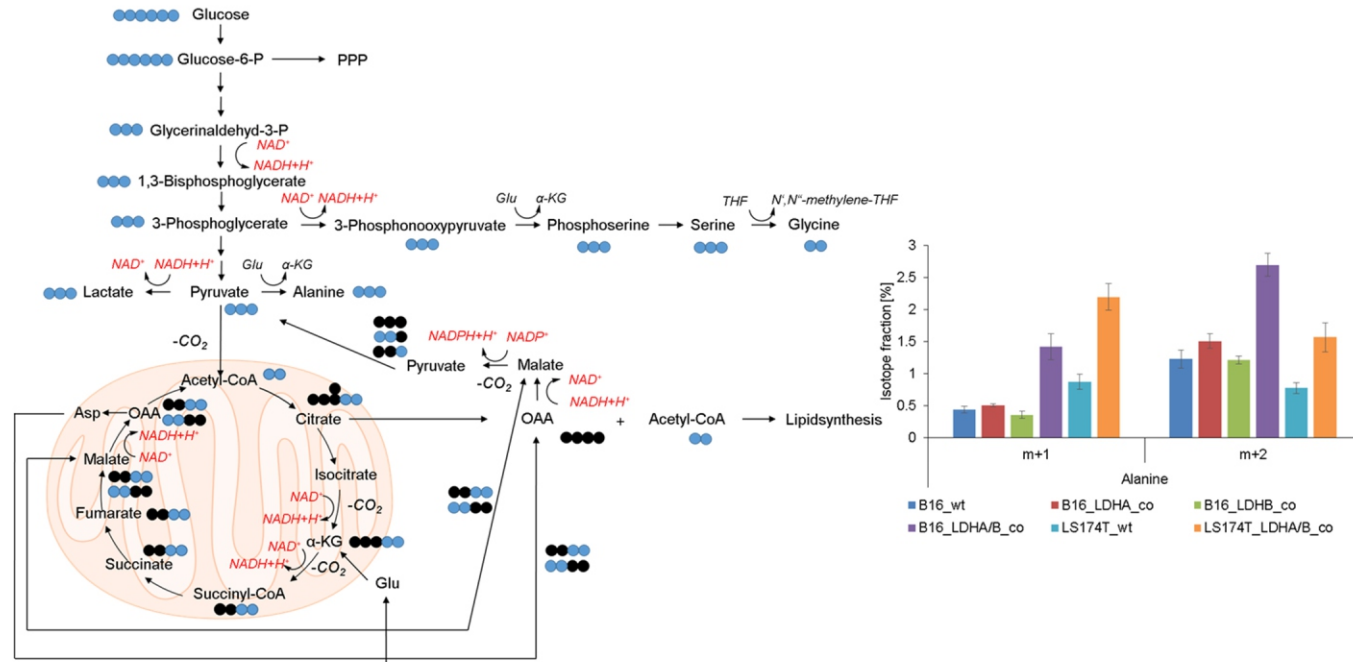
Fig S2**A****B****C****B16****D****E**

Fig S3 A



B

