

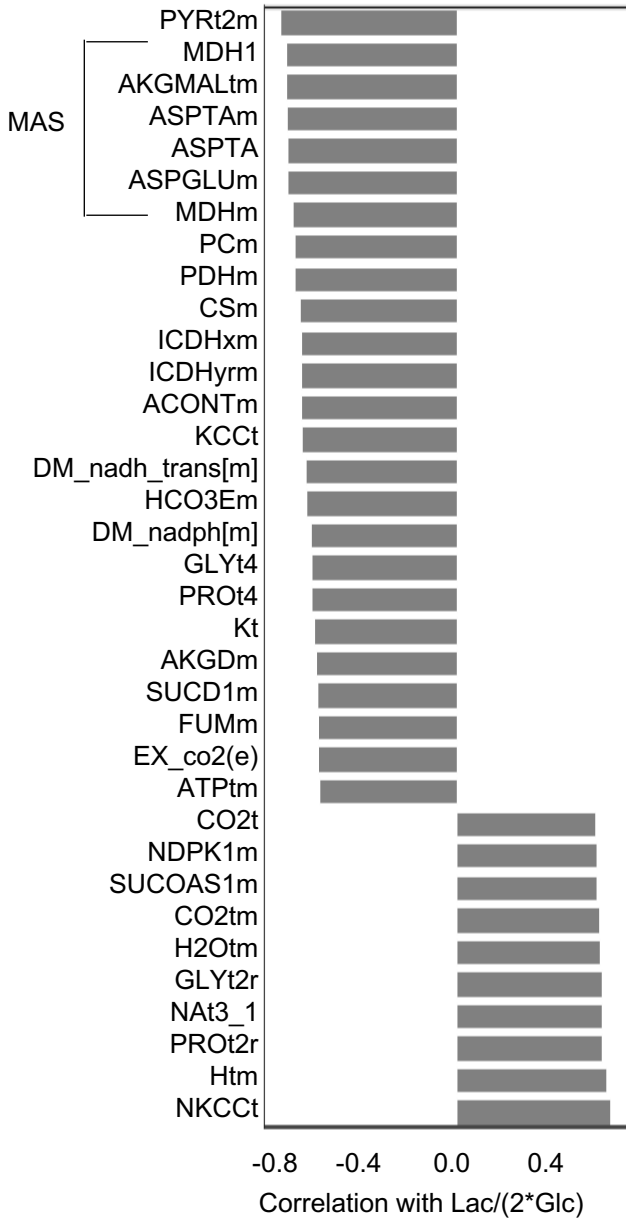
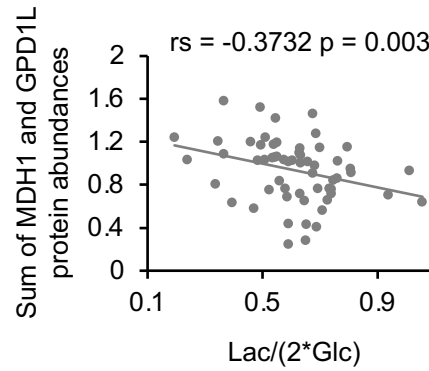
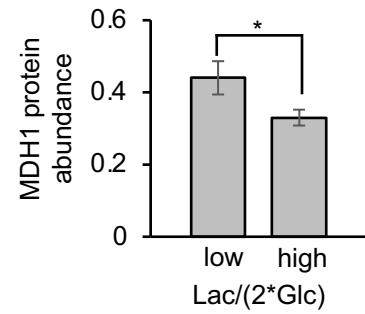
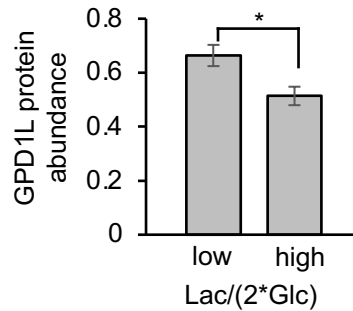
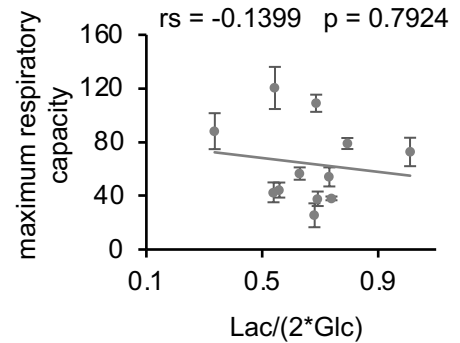
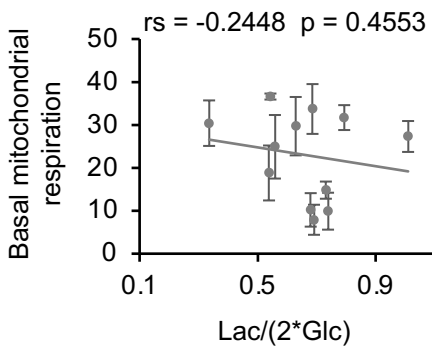
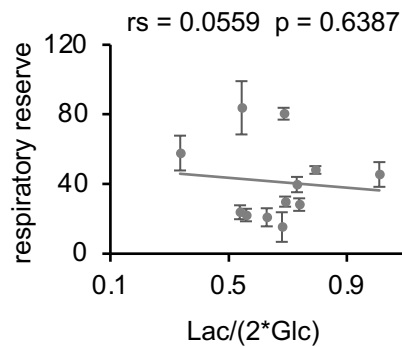
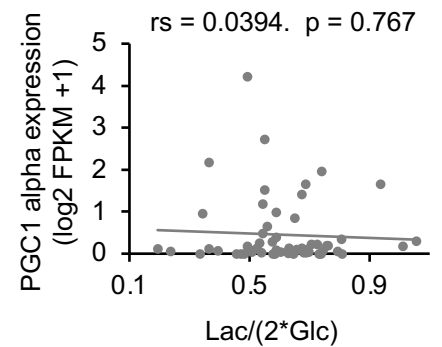
Figure S1**A****B****C****D****E****F****G****H**

Figure S1: Analysis of protein abundances and mitochondrial respiratory capacity across the NCI-60 cell lines. Related to Figure 1.

(A) Plot of the top 35 metabolic fluxes that correlate with lactate-excretion rate across the NCI-60 cell lines. Fluxes were calculated by flux-balance analysis. PYRt2m transports pyruvate from the cytosol to mitochondria. The next top 6 metabolic fluxes correspond to steps in the malate-aspartate shuttle. Full reaction names are provided in Table S1.

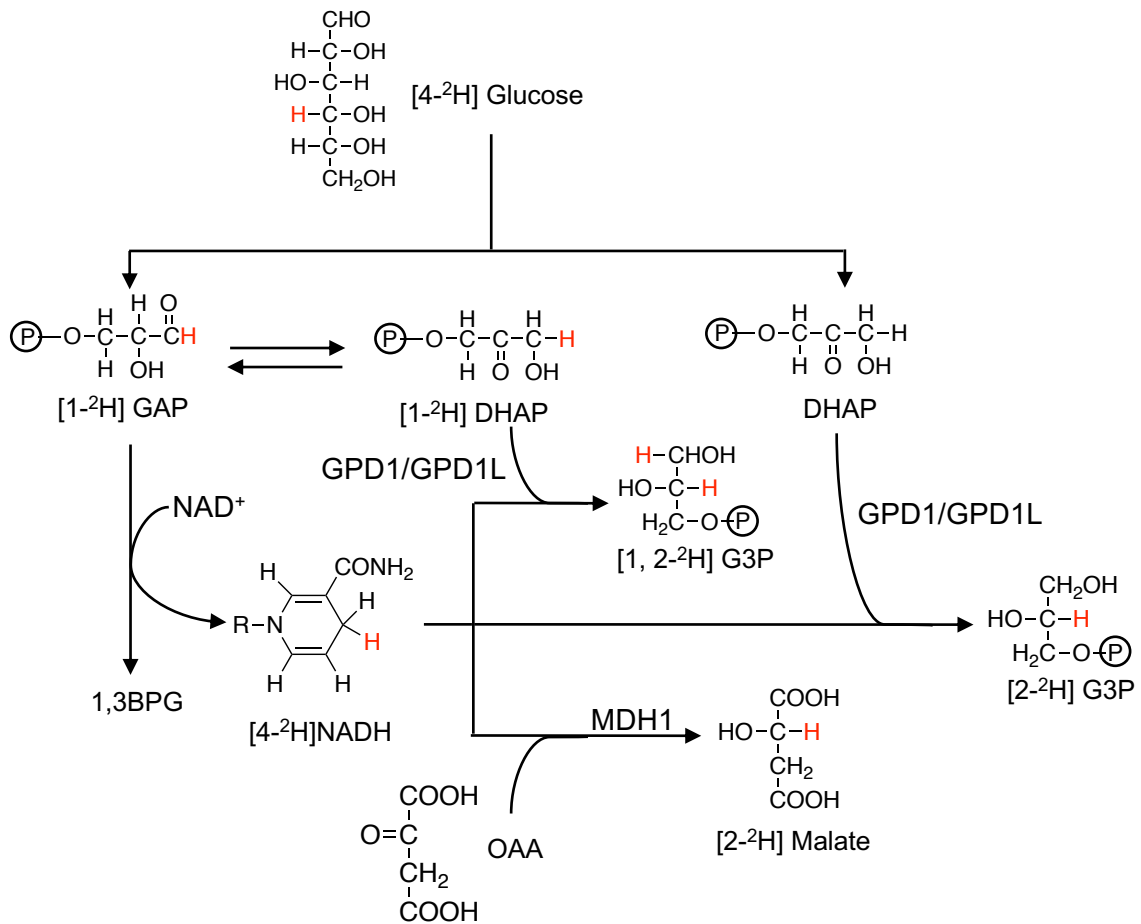
(B-D) Protein abundances are consistent with flux-balance analysis data. (B) Lactate-excretion rate is significantly correlated with the sum of MDH1 and GPD1L protein abundances across the NCI-60 cell lines. A correlation coefficient (r_s) and a p value were determined by using a Spearman's rank correlation.

(C-D) Cancer cell lines from the NCI-60 panel with low lactate-excretion rates have higher protein abundances of both MDH1 (C) and GPD1L (D) relative to cancer cell lines with high lactate-excretion rates. The maximum lactate-excretion rate among the NCI-60 cell lines was 1.1. We grouped cell lines by using half of this maximum value as a threshold (i.e., cell lines below the threshold were classified as “low” and cell lines above it were classified as “high”). Error bars denote standard error. p values were determined by using a two-tailed Student's t test. * $p \leq 0.05$. MDH1 protein abundances and GPD1L protein abundances shown in panels B-D were derived from Guo et al., 2019. Each MDH1 or GPD1L protein abundance was normalized by using the maximum MDH1 or GPD1L protein abundance from all of the NCI-60 cell lines, respectively.

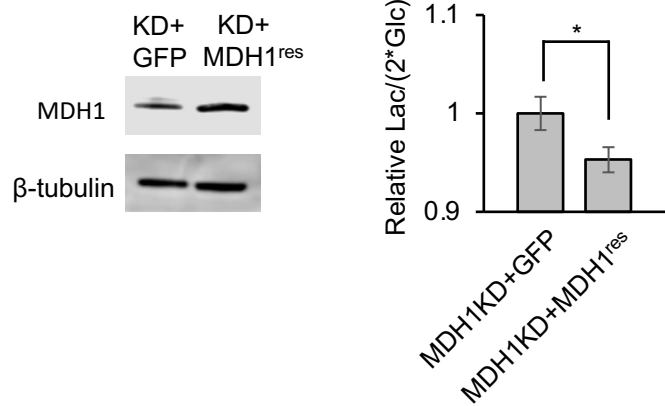
(E-H) Lactate-excretion rate is not linked with mitochondrial respiratory capacity. Lactate-excretion rate is not correlated with mitochondrial maximum respiratory capacity (E), basal mitochondrial respiration (F), or respiratory reserve (G) for 12 cancer cell lines from the NCI-60 panel. Moreover, no correlation was observed between lactate-excretion rate and expression of peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1 α), a master regulator for mitochondrial biogenesis (H). Thus, although mitochondrial respiratory capacity might be regulated by additional mechanisms independent of PGC1 α , the results shown here indicate that the lactate-excretion rate is not tightly linked with mitochondrial respiration capacity. The mitochondrial respiration data shown in panels E, F, and G were obtained from Teh et al., 2019. The PGC1 α expression data shown in H were obtained from Luna et al., 2021. Error bars denote standard error. Correlation coefficients (r_s) and p values were determined by using a Spearman's rank correlation.

Figure S2

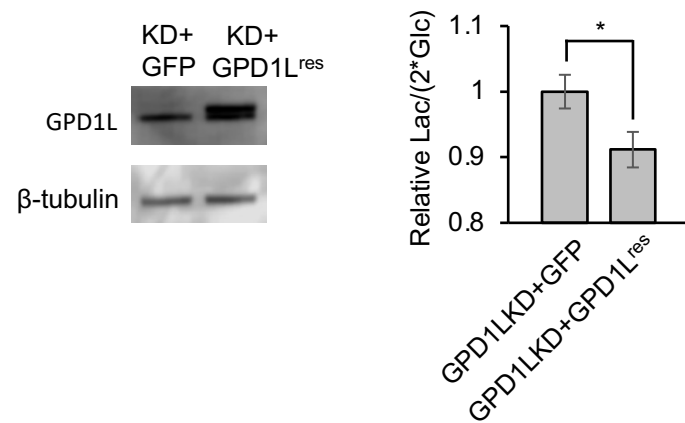
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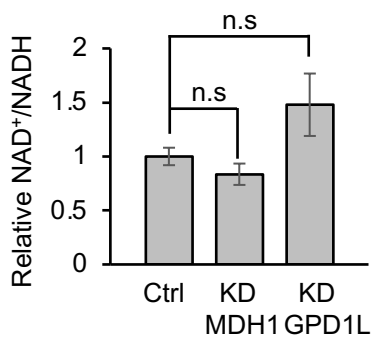
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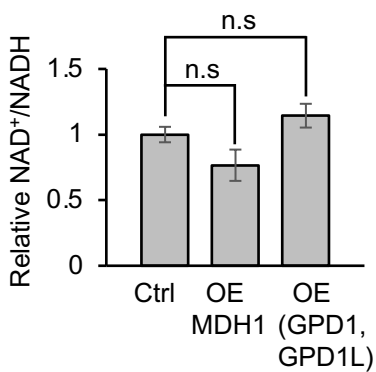
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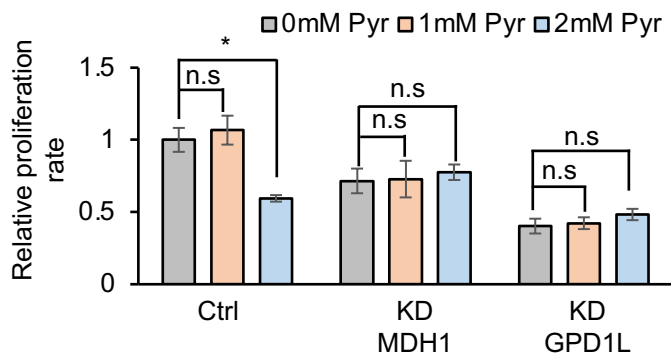


Figure S2: Influence of MDH1 and GPD1/GPD1L expression on lactate-excretion rate and proliferation in PC3 cells. Related to Figure 2.

(A) Schematic to show the fates of deuterium derived from [4-²H] glucose. Deuterium labels are shown in red color.

(B-C) Lactate-excretion rates decrease when siRNA-resistant human MDH1 (MDH1^{res}) (B) or human GPD1L (GPD1L^{res}) (C) are expressed in MDH1 or GPD1L knockdown cells. Control cells overexpressed GFP instead of a dehydrogenase. n=9 replicates per group. p values were determined by using a two-tailed Student's t test.

(D) Knocking down MDH1 or GPD1L does not change the ratio of NAD⁺ to NADH in whole cells. n=6 replicates per group. p values were determined by using a one-way ANOVA followed by Dunnett's test.

(E) Overexpressing MDH1 or GPD1/GPD1L does not change the ratio of NAD⁺ to NADH in whole cells. n=6 replicates per group. p values were determined by using a one-way ANOVA followed by Dunnett's test.

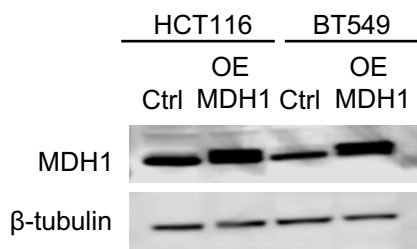
(F) Treatment with 2 mM pyruvate significantly impairs the proliferation of control cells but not the proliferation of MDH1 and GPD1L knockdowns. The results suggest that MDH1 and GPD1L knockdown influences cytosolic NAD⁺ and NADH levels. These data may reflect differences in cytosolic redox state that cannot be measured by assessing NAD⁺/NADH from whole cells (D-E). n=3 replicates per group. p values were determined by using a one-way ANOVA followed by Dunnett's test.

Error bars denote standard error. *p≤0.05, n.s.>0.05.

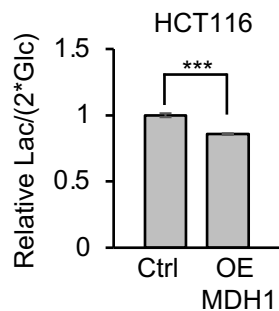
Ctrl, control; OE, overexpression; KD, knockdown; GAP, glyceraldehyde 3-phosphate; 1,3BPG, 1,3-bisphosphoglycerate; G3P, glycerol 3-phosphate; DHAP, Dihydroxyacetone phosphate; OAA, Oxaloacetate; Pyr, pyruvate.

Figure S3

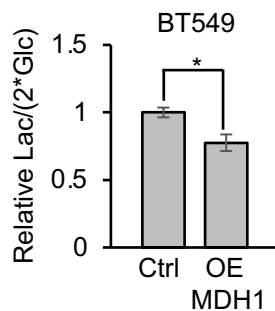
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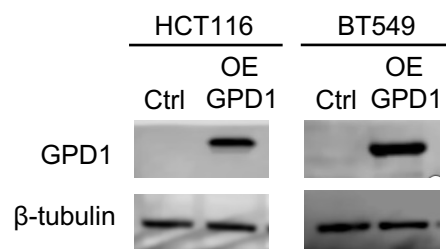
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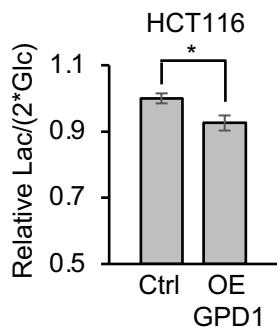
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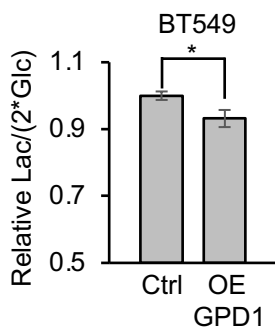
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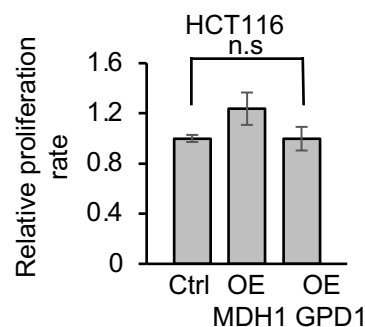
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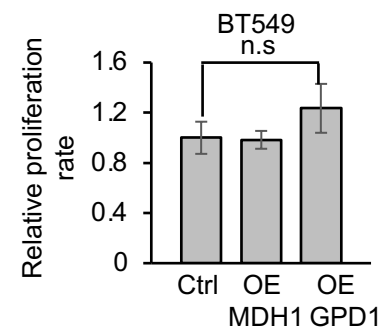
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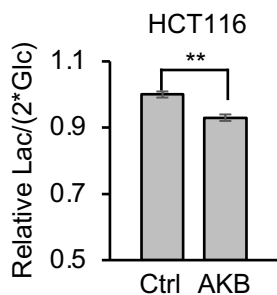
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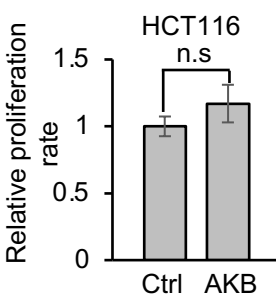
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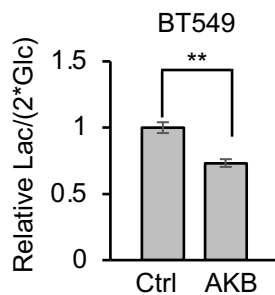
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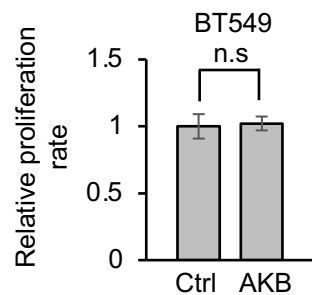
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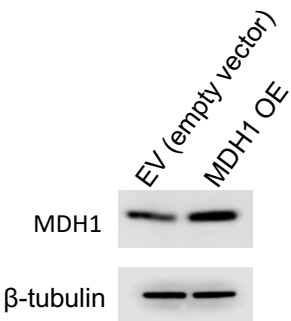
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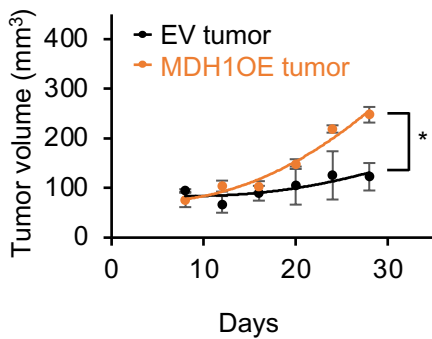
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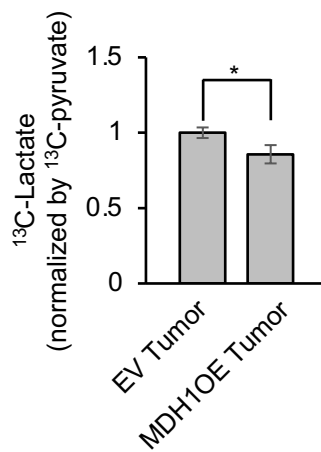
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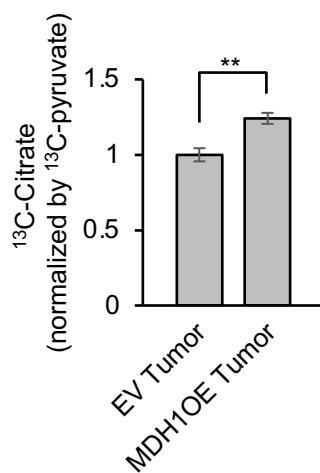


Figure S3: Influence of MDH1 and GPD1/GPD1L expression on lactate-excretion rate and proliferation in multiple cell lines and mouse tumors. Related to Figure 2.

- (A) Overexpression of MDH1 increases MDH1 protein levels in HCT116 cells and BT549 cells. Control cells overexpressed GFP instead of a dehydrogenase.
- (B-C) Lactate-excretion rates decrease when MDH1 is overexpressed in HCT116 cells (B) and BT549 cells (C). n=5 replicates per experimental group. p values were determined by using a two-tailed Student's t test. * $p \leq 0.05$, *** $p \leq 0.001$.
- (D) Overexpression of GPD1 increases GPD1 protein levels in HCT116 cells and BT549 cells. Control cells overexpressed GFP instead of a dehydrogenase.
- (E-F) Lactate-excretion rates decrease when GPD1 is overexpressed in HCT116 cells (E) and BT549 cells (F). n=4 replicates per experimental group. p values were determined by using a two-tailed Student's t test. * $p \leq 0.05$.
- (G-H) Proliferation rate is not significantly different in HCT116 cells (G) and BT549 cells (H) when MDH1 or GPD1 is overexpressed. n=4 replicates per experimental group. p values were determined by using a one-way ANOVA followed by Dunnett's test. n.s. >0.05 .
- (I-J) Treatment of HCT116 cells with 1 mM AKB decreases the lactate-excretion rate without impairing cellular proliferation. n=3 replicates per experimental group. p values were determined by using a two-tailed Student's t test. ** $p \leq 0.01$, n.s. >0.05 .
- (K-L) Treatment of BT549 cells with 2 mM AKB decreases the lactate-excretion rate without impairing cellular proliferation. n=3 replicates per experimental group. p values were determined by using a two-tailed Student's t test. ** $p \leq 0.01$, n.s. >0.05 .
- (M) PC3 cells overexpressing MDH1 have higher MDH1 expression levels compared to EV controls.
- (N) Tumors formed from PC3 cells overexpressing MDH1 grow faster than tumors formed from control PC3 cells. Tumor volume was calculated by using the formula $V = (1/2) \times (L \times W^2)$, where L and W are tumor length and tumor width, respectively. n=4 replicates per experimental group. p values were determined by using a two-tailed Student's t test. * $p \leq 0.05$.
- (O) Tumors overexpressing MDH1 have decreased labeling of lactate relative to labeling of pyruvate. n=4 replicates per experimental group. p values were determined by using a two-tailed Student's t test. * $p \leq 0.1$.
- (P) Tumors overexpressing MDH1 have increased labeling of citrate relative to labeling of pyruvate. n=4 replicates per experimental group. p values were determined by using a two-tailed Student's t test. ** $p \leq 0.05$.

Error bars denote standard error.

Ctrl, control; OE, overexpression; EV, empty vector; Lac, lactate; Glc, glucose; AKB, alpha-ketobutyrate.

Figure S4

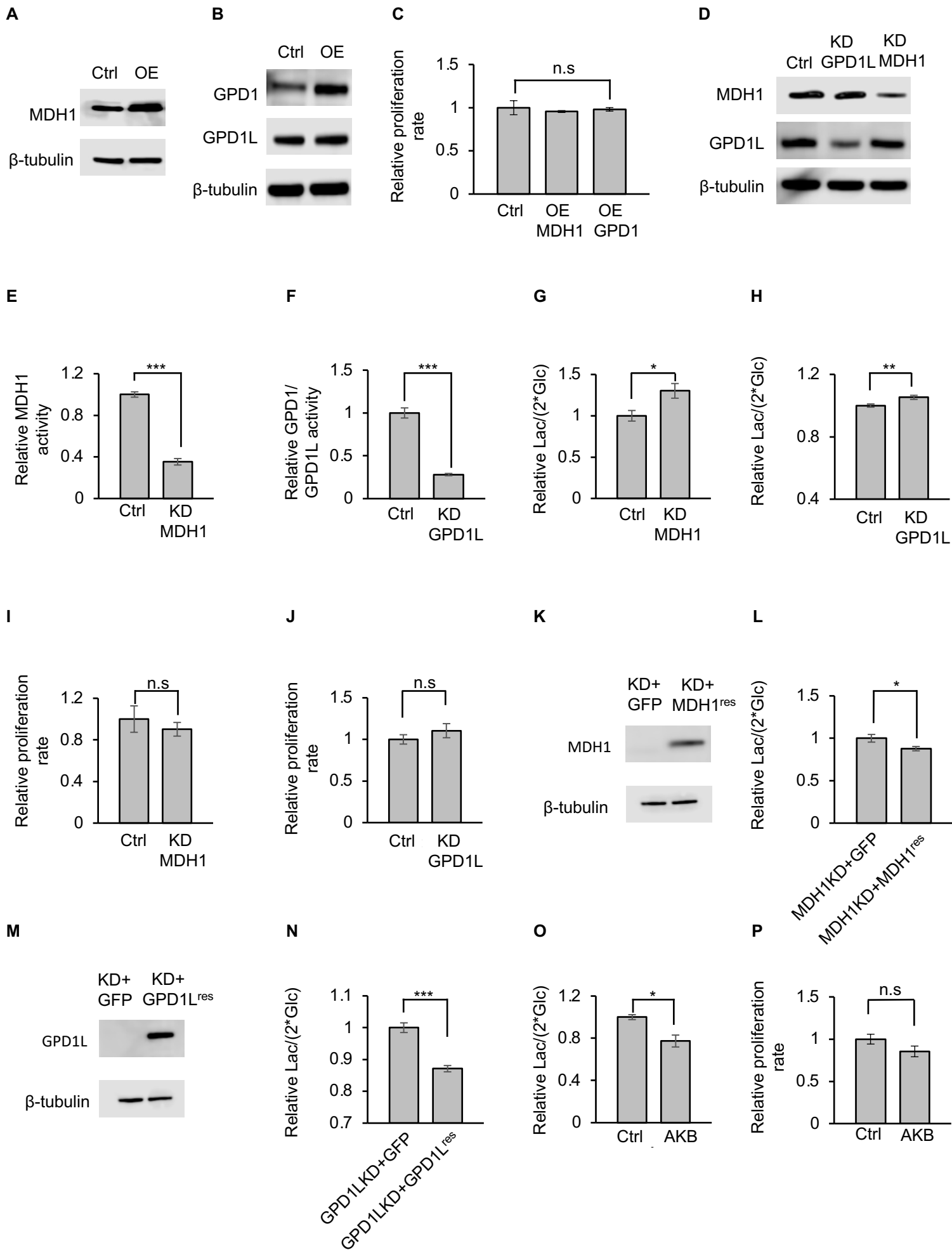


Figure S4: The effect of MDH1 or GPD1/GPD1L knockdown or overexpression on lactate production in H-Ras 3T3 cells. Related to Figure 3.

(A-B) Western blots for MDH1 (A) and GPD1/GPD1L (B) in H-Ras 3T3 cells. Overexpression of MDH1 and GPD1 in H-Ras 3T3 cells leads to more detected MDH1 protein and GPD1 protein, respectively. Control cells overexpressed GFP instead of a dehydrogenase.

(C) Proliferation rate of H-Ras 3T3 cells before and after overexpression of MDH1 or GPD1. n=3 replicates per experimental group. p values were determined by using a one-way ANOVA followed by Dunnett's test.

(D) Knocking down MDH1 and GPD1L in H-Ras 3T3 cells decreases detected MDH1 protein and GPD1L protein, respectively. Control cells were treated with scrambled siRNA.

(E-F) Knocking down MDH1 (E) and GPD1L (F) in H-Ras 3T3 cells decreases MDH1 and GPD1/GPD1L activity, respectively. MDH1 and GPD1/GPD1L activity was determined as in Figure 3C and 3D. p values were determined by using a two-tailed Student's t-test.

(G-H) Lactate-excretion rates in H-Ras 3T3 cells are increased after MDH1 (G) or GPD1L (H) knockdown. Control cells were administered scrambled siRNA. n=3 replicates per experimental group in panel G. n=9 replicates per experimental group in panel H. p values were determined by using a two-tailed Student's t test.

(I-J) Proliferation rate of H-Ras 3T3 cells before and after MDH1 (I) or GPD1L (J) knockdown. n=3 replicates per experimental group. p values were determined by using a two-tailed Student's t test.

(K-N) Lactate-excretion rates decrease when siRNA-resistant mouse MDH1 (MDH1^{res}) (K-L) or mouse GPD1L (GPD1L^{res}) (M-N) are expressed in MDH1 or GPD1L knockdown cells. Control cells overexpressed GFP instead of a dehydrogenase. n=9 replicates per group. p values were determined by using a two-tailed Student's t test.

(O) Lactate-excretion rate decreases when H-Ras 3T3 cells are given 1 mM AKB. n=3 replicates per experimental group. p values were determined by using a two-tailed Student's t test.

(P) Proliferation rate is not significantly different when H-Ras 3T3 cells are given 1 mM AKB. n=3 replicates per experimental group. p values were determined by using a two-tailed Student's t test.

Error bars denote standard error. *p≤0.05, **p≤0.01, ***p≤0.001, n.s.>0.05.

Ctrl, control; KD, knockdown; Lac, lactate; Glc, glucose; AKB, alpha-ketobutyrate.

Figure S5

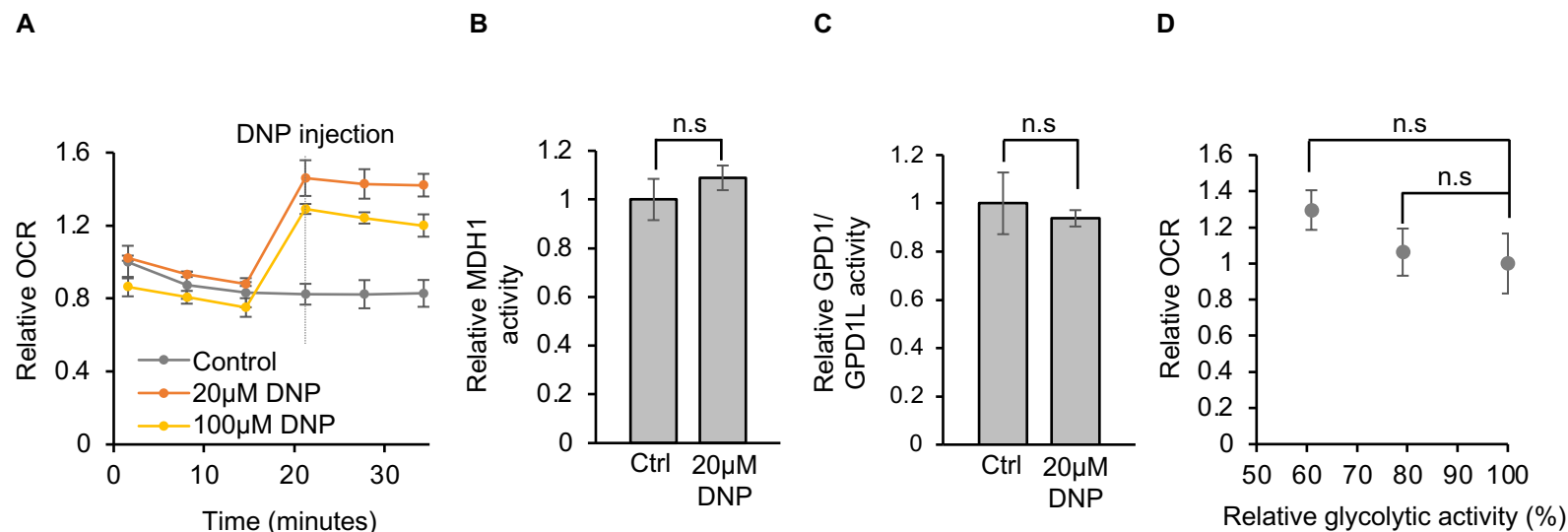


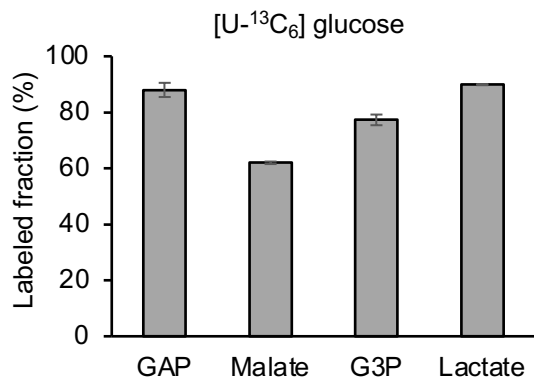
Figure S5: Analysis of MDH1 or GPD1/GPD1L activities after increasing oxygen consumption rate with a mitochondrial uncoupler 2,4-dinitrophenol (DNP). Related to Figure 3.

(A-C) Increasing oxygen consumption rate (OCR) with a mitochondrial uncoupler DNP does not significantly increase MDH1 or GPD1/GPD1L activity. Even though 20 µM DNP is sufficient to increase mitochondrial respiration in H-Ras 3T3 cells (A), it does not result in increased MDH1 (B) or GPD1/GPD1L (C) activity beyond their saturation values. We note, however, that the aspartate/glutamate antiporter (a component of the MAS) translocates a proton. The results shown here may therefore be conflated by disrupting the mitochondrial proton gradient with DNP. MDH1 activity was determined by the fraction of [2-²H] malate normalized to the fraction of [1-²H] GAP. GPD1/GPD1L activity was determined by the fraction of [1,2-²H] G3P normalized to the fraction of [1-²H] DHAP and [4-²H] NADH. n=3 replicates per group. p values were determined by using an unpaired two-tailed Student's t test. (D) OCR as a function of glycolytic activity in H-Ras 3T3 cells. Glycolytic activities were derived from Figure 3F. OCR is not significantly different when MDH1 and GPD1/GPD1L activities are saturated (which occurs when glycolysis is greater than 60% of its unattenuated flux). n=3 replicates per experimental group. p values were determined by using a one-way ANOVA followed by Dunnett's test. Error bars denote standard error. n.s.>0.05.

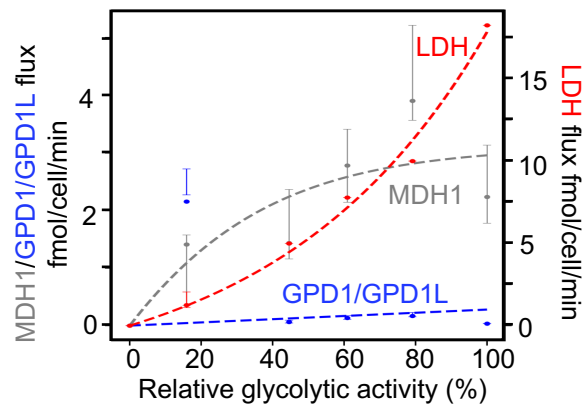
GAP, glyceraldehyde 3-phosphate; G3P, glycerol 3-phosphate; DHAP, dihydroxyacetone phosphate.

Figure S6

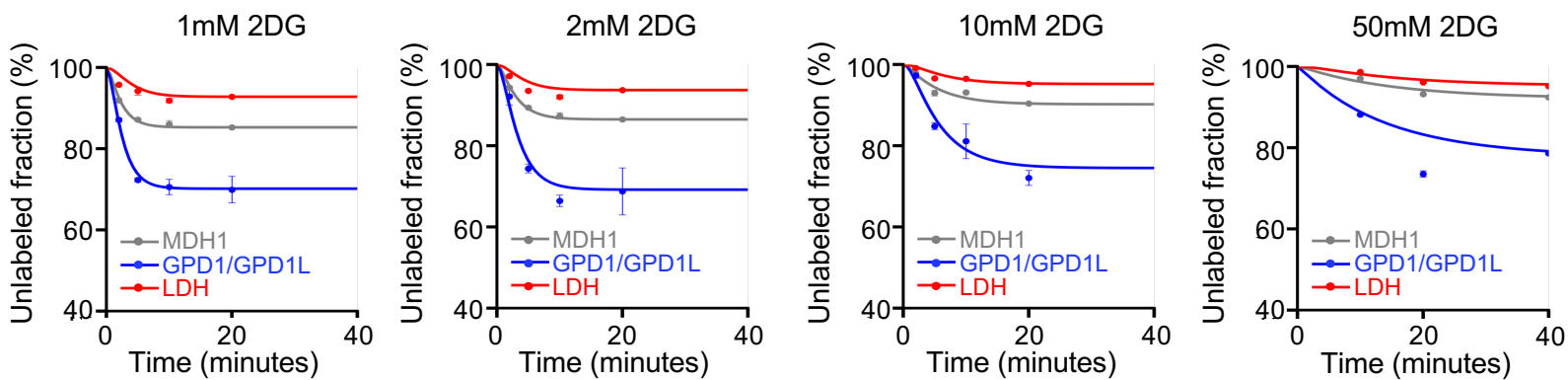
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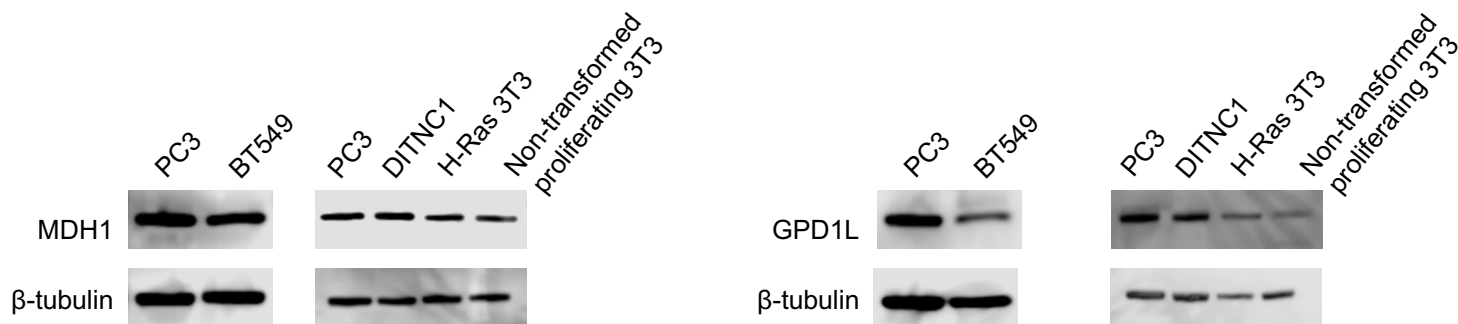


Figure S6: Absolute quantitation of NAD⁺ regeneration fluxes under various conditions. Related to Figure 5.

(A) Fraction of labeled GAP, malate, G3P, and lactate in H-Ras 3T3 cells after introducing [U-¹³C₆] glucose for 48 hr. Error bars denote standard error. n=3 replicates per experimental group.

(B) Fluxes of MDH1, GPD1/GPD1L, and LDH as a function of glycolytic activity in H-Ras 3T3 cells. Glycolytic activities were derived from Figure 3F. Flux values of MDH1, GPD1/GPD1L, and LDH were determined by the measured metabolite concentrations, the rate of glucose consumption, the rate of lactate excretion, and equations (1)-(4). Flux curves were fit with a logistic function. Error bars show the interquartile range of the Monte Carlo simulated fluxes.

(C) Fraction of unlabeled malate, glycerol 3-phosphate, and lactate in H-Ras 3T3 cells as a function of time after introducing [4-²H] glucose. The measured metabolite concentrations, the rate of glucose consumption, the rate of lactate excretion, and equations (1)-(4) were used to find the best model fit from which the activities of MDH1, GPD1/GLPD1L, and LDH were determined. 2DG was added at the indicated concentration to decrease glycolytic activity. n=3 replicates per experimental time point for each 2DG concentration tested.

(D) Comparison of MDH1 and GPD1L protein levels in multiple cell lines. PC3 and BT549 cells were selected as representative cell lines from the NCI-60 panel. 3T3 cells were selected to enable direct comparisons between proliferating and non-proliferating cells. DITNC1 cells were chosen given the potential role of the glycerol-3 phosphate shuttle in the central nervous system.

GAP, glyceraldehyde 3-phosphate; G3P, glycerol 3-phosphate.

Table S1: Top 35 metabolic fluxes that correlate with lactate-excretion rate in the NCI-60 cell lines. Related to Figure 1.

Abbreviation	Description	Formula
PYRt2m	Pyruvate Mitochondrial Transport via Proton Symport (mitochondrial)	$h[c] + pyr[c] \rightleftharpoons h[m] + pyr[m]$
MDH1	Malate Dehydrogenase1 (cytosolic)	$h[c] + nadh[c] + oaa[c] \rightleftharpoons mal_L[c] + nad[c]$
AKGMALtm	Alpha-Ketoglutarate/Malate Transporter (mitochondrial)	$akg[m] + mal_L[c] \rightleftharpoons akg[c] + mal_L[m]$
ASPTAm	Aspartate Transaminase (mitochondrial)	$glu_L[m] + oaa[m] \rightleftharpoons akg[m] + asp_L[m]$
ASPTA	Aspartate Transaminase (cytosolic)	$akg[c] + asp_L[c] \rightleftharpoons glu_L[c] + oaa[c]$
ASPGLUm	Aspartate-Glutamate Mitochondrial Shuttle (mitochondrial)	$asp_L[m] + glu_L[c] + h[c] \rightarrow asp_L[c] + glu_L[m] + h[m]$
MDHm	Malate Dehydrogenase (mitochondrial)	$mal_L[m] + nad[m] \rightleftharpoons h[m] + nadh[m] + oaa[m]$
PCm	Pyruvate Carboxylase	$atp[m] + hco3[m] + pyr[m] \rightarrow adp[m] + h[m] + oaa[m] + pi[m]$
PDHm	Pyruvate Dehydrogenase	$coa[m] + nad[m] + pyr[m] \rightarrow accoa[m] + co2[m] + nadh[m]$
CSm	Citrate Synthase	$accoa[m] + h2o[m] + oaa[m] \rightarrow cit[m] + coa[m] + h[m]$
ICDHxm	Isocitrate Dehydrogenase (NAD ⁺)	$icit[m] + nad[m] \rightarrow akg[m] + co2[m] + nadh[m]$
ICDHym	Isocitrate Dehydrogenase (NADP ⁺)	$icit[m] + nadp[m] \rightleftharpoons akg[m] + co2[m] + nadph[m]$
ACONTm	Aconitate Hydratase	$cit[m] \rightleftharpoons icit[m]$
KCCt	K ⁺ -Cl ⁻ Symport	$cl[e] + k[e] \rightleftharpoons cl[c] + k[c]$
DM_nadh_trans[m]	NAD (P) Transhydrogenase	$nadh[m] + nadp[m] \rightleftharpoons nad[m] + nadph[m]$
HCO3Em	Carboxylic Acid Dissociation	$co2[m] + h2o[m] \rightleftharpoons h[m] + hco3[m]$
DM_nadph[m]	N.A.	$nadph[m] \rightleftharpoons h[m] + nadp[m]$
GLYt4	Transport of Glycine via Sodium Symport	$gly[e] + na1[e] \rightleftharpoons gly[c] + na1[c]$
PROt4	Na ⁺ /Proline-L Symporter	$na1[e] + pro_L[e] \rightleftharpoons na1[c] + pro_L[c]$
Kt	Transport of Potassium	$k[c] \rightleftharpoons k[e]$
AKGDm	2-Oxoglutarate Dehydrogenase	$akg[m] + coa[m] + nad[m] \rightleftharpoons co2[m] + nadh[m] + succoa[m]$
SUCD1m	Succinate Dehydrogenase	$q10[m] + succ[m] \rightleftharpoons fum[m] + q10h2[m]$
FUMm	Fumarase, Mitochondrial	$fum[m] + h2o[m] \rightleftharpoons mal_L[m]$
EX_co2(e)	Exchange of Carbon Dioxide	$co2_e \rightleftharpoons$
ATPtm	ADP/ATP Transporter, Mitochondrial	$adp[c] + atp[m] \rightleftharpoons atp[c] + adp[m]$
CO2t	CO ₂ Transporter via Diffusion	$co2[e] \rightleftharpoons co2[c]$
NDPK1m	Nucleoside-Diphosphate Kinase (ATP:GDP), Mitochondrial	$atp[m] + gdp[m] \rightleftharpoons adp[m] + gtp[m]$
SUCOAS1m	Succinate- Coenzyme A Ligase (GDP-Forming)	$coa[m] + gtp[m] + succ[m] \rightleftharpoons gdp[m] + pi[m] + succoa[m]$
CO2tm	CO ₂ Transport (Diffusion), Mitochondrial	$co2[c] \rightleftharpoons co2[m]$
H2Otm	H ₂ O Transport, Mitochondrial	$h2o[c] \rightleftharpoons h2o[m]$
GLYt2r	Glycine Reversible Transport via Proton Symport	$gly[e] + h[e] \rightleftharpoons gly[c] + h[c]$
Na3_1	Sodium Proton Antiporter	$na1[c] + h[e] \rightleftharpoons h[c] + na1[e]$
PROt2r	L-Proline Reversible Transport via Proton Symport	$h[e] + pro_L[e] \rightleftharpoons h[c] + pro_L[c]$
Htm	Uncoupling Protein	$h[c] \rightleftharpoons h[m]$
NKCCt	Na ⁺ -K ⁺ -Cl ⁻ Symport	$2.0 * cl[e] + k[e] + na1[e] \rightleftharpoons 2.0 * cl[c] + k[c] + na1[c]$

Table S2: Malate, G3P, and lactate concentrations in H-Ras 3T3 cells cultured with the indicated concentration of 2DG. Related to Figure 5.

2DG concentration (mM)	Concentration (fmol/cell)*		
	Malate	G3P	Lactate
0	4.26±0.06	0.26±0.01	93.72±4.29
1	3.13±0.07	0.21±0.01	39.11±3.96
2	2.18±0.09	0.18±0.01	23.68±1.94
10	1.25±0.08	0.14±0.01	17.46±1.97
50	0.63±0.03	0.09±0.002	9.22±1.35

*Data are presented as mean +/- SE

Table S3: Rate of glucose consumption and lactate excretion from H-Ras 3T3 cells cultured with the indicated concentration of 2DG. Related to Figure 5.

2DG concentration (mM)	rate (fmol/cell/min)*	
	Glucose uptake	Lactate excretion
0	15.66±0.11	18.23±0.16
1	9.28±0.15	9.99±0.11
2	7.63±0.10	7.77±0.12
10	7.19±0.89	4.99±0.06
50	4.88±0.24	2.62±0.02

*Data are presented as mean +/- SE

Table S4: Sequences for DsiRNA. Related to Figure 2, S2 and S4.

	Trifecta Kit ID (Genscript)	Ref# (Genscript)	DsiRNA Design ID (Genscript)	Strand	Sequence (5' --> 3')
Human MDH1 siRNA	hs.Ri.MDH1.13	295259761	hs.Ri.MDH1.13.1	+	rCrUrUrArGrArUrArArArUrArCrGrCrCrArArGrArArGrUCA
				-	rUrGrArCrUrUrCrUrUrGrGrCrGrUrArUrUrUrArUrCrUrArArGrGrC
		295259762	hs.Ri.MDH1.13.2	+	rGrGrArUrCrArCrArArCrCrGrArGrCrUrArArArGrCrUrCAA
				-	rUrUrGrArGrCrUrUrUrArGrCrUrCrGrGrUrUrGrUrGrArUrCrCrArA
		295259763	hs.Ri.MDH1.13.3	+	rGrCrUrGrUrUrArGrUrGrUrGrCrArUrUrCrUrArArArUrAAA
				-	rUrUrUrArUrUrUrArGrArArUrGrCrArCrArCrUrArArCrArGrCrArU
Mouse MDH1 siRNA	mm.Ri.Mdh1.13	210245448	mm.Ri.Mdh1.13.1	+	rCrArArGrArArArUrCrArGrUrUrArArGrGrUrCrArUrUrGTT
				-	rArArCrArArUrGrArCrCrUrUrArArCrUrGrArUrUrUrCrUrUrGrGrC
		210245451	mm.Ri.Mdh1.13.2	+	rGrUrArArUrArArUrGrCrUrArCrArUrUrCrArArArUrUrGTG
				-	rCrArCrArArUrUrUrGrArArUrGrUrArGrCrArUrUrArUrUrArCrUrG
		210245454	mm.Ri.Mdh1.13.3	+	rArGrArArUrGrUrCrArUrUrArUrCrUrGrGrGrGrArArArUCA
				-	rUrGrArUrUrUrCrCrCrArGrArUrArArUrGrArCrArUrUrCrUrUrU
Human GPD1L siRNA	hs.Ri.GPD1L.13	299201254	hs.Ri.GPD1L.13.1	+	rUrUrCrUrGrArUrGrCrUrUrArCrUrArCrArArUrArUrGrUGA
				-	rUrCrArCrArUrArUrUrGrUrArGrUrArArGrCrArUrCrArGrArArArC
		299201257	hs.Ri.GPD1L.13.2	+	rGrUrGrArGrArArGrArUrGrGrGrUrArUrUrGrArCrArUrCAG
				-	rCrUrGrArUrGrUrCrArArUrArCrCrCrArUrCrUrUrCrUrCrArCrGrG
		299201260	hs.Ri.GPD1L.13.3	+	rGrArArUrUrArCrCrGrUrGrGrUrUrGrArUrGrArUrGrCrAGA
				-	rUrCrUrGrCrArUrCrArUrCrArArCrCrArCrGrGrUrArArUrUrCrGrA
Mouse GPD1L siRNA	mm.Ri.Gpd1l.13	298541815	mm.Ri.Gpd1l.13.1	+	rGrArArUrUrUrUrArArArArArCrUrGrUrCrCrArArGrArAGA
				-	rUrCrUrUrCrUrUrGrGrArCrArGrUrUrUrUrUrArArArArUrUrCrUrC
		298541818	mm.Ri.Gpd1l.13.2	+	rArGrArArUrCrArCrUrGrUrGrGrUrArGrArCrGrArUrGrCAG
				-	rCrUrGrCrArUrCrGrUrCrUrArCrCrArCrArGrUrGrArUrUrCrUrGrA
		298541821	mm.Ri.Gpd1l.13.3	+	rGrArGrArArGrArUrGrGrGrGrArUrCrGrArCrArUrCrArGTG
				-	rCrArCrUrGrArUrGrUrCrGrArUrCrCrCrCrArUrCrUrUrCrUrCrUrC

Table S5: MRM transitions with precursor ions, product ions, and corresponding collision energies. Related to STAR Methods.

Compound name	Polarity	Precursor (<i>m/z</i>)	RF Lens (V)	Product (<i>m/z</i>)		Collision energy (V)
				Quantifier ion	Qualifier ion	
Malate	Negative	133	35	114.9		10.00
Malate	Negative	133	35		70.9	15.00
[U- ¹³ C ₄] Malate	Negative	137	35	118.9		10.00
[U- ¹³ C ₄] Malate	Negative	137	35		73.9	15.00
G3P	Negative	171	44	79.0		17.05
G3P	Negative	171	44		63.0	55.00
[U- ¹³ C ₃] G3P	Negative	174	44	79.0		17.05
[U- ¹³ C ₃] G3P	Negative	174	44		63.0	55.00
Lactate	Negative	89	36	43.1		11.19
Lactate	Negative	89	36		45.1	11.70
[U- ¹³ C ₃] Lactate	Negative	92	36	45.1		11.19
[U- ¹³ C ₃] Lactate	Negative	92	36		46.1	11.70

Data S1: Sequence for human MDH1^{res}, human GPD1L^{res}. Related to Figure 2 and S2.

Human MDH1^{res}

ORF nucleotide sequence:

```

1   ATGTCTGAAC CAATCAGAGT CTTTGTGACT GGAGCAGCTG GTCAAATTGC ATATTCAGTG
61  CTGTACAGTA TTGGAAATGG ATCTGTCTTT GGTAAGATC AGCCTATAAT TCTTGTGCTG
121 TTGGATATCA CCCCATGAT GGGTGTCTTG GACGGTGTCC TAATGGAAC GCAAGACTGT
181 GCCCTTCCCC TCCTGAAAGA TGCATCGCA ACAGATAAAG AAGACGTTGC CTTCAAAGAC
241 CTGGATGTGG CCATTCTTGT GGGCTCCATG CCAAGAAGGG AAGGCATGGA GAGAAAAGAT
301 TTAGTAAAAG CAAATGTGAA AATCTTCAAA TCCCAGGGTG CAGCTTTGGA CAAGTATGCT
361 AAAAAATCGG TTAAGGTTAT TGTTGTGGGT AATCCAGCCA ATACCAACTG CCTGACTGCT
421 TCCAAGTCAG CTCCATCCAT CCCCAGGAG AACTTCAGTT GCTTGACTCG TTTAGACCAT
481 AATCGGGCCA AGGCCAGAT TGCTCTTAAA CTTGGTGTGA CTGCTAATGA TGTAAGAAT
541 GTCATTATCT GGGGAAACCA TTCCTCGACT CAGTATCCAG ATGTCAACCA TGCCAAGGTG
601 AAATTGCAAG GAAAGGAAGT TGGTGTTTAT GAAGCTCTGA AAGATGACAG CTGGCTCAAG
661 GGAGAATTTG TCACGACTGT GCAGCAGCGT GGCCTGCTG TCATCAAGGC TCGAAAACTA
721 TCCAGTGCCA TGTCTGCTGC AAAAGCCATC TGTGACCACG TCAGGGACAT CTGGTTTGGA
781 ACCCCAGAGG GAGAGTTTGT GTCCATGGGT GTTATCTCTG ATGGCAACTC CTATGGTGT
841 CCTGATGATC TGCTCTACTC ATTCCCTGTT GTAATCAAGA ATAAGACCTG GAAGTTTGT
901 GAAGGTCTCC CTATTAATGA TTTCTCACGT GAGAAGATGG ATCTTACTGC AAAGGAAGT
961 ACAGAAGAAA AAGAAAGTGC TTTTGAATTT CTTTCCTCTG CCTGA

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Human GPD1L^{res}

ORF nucleotide sequence:

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1   ATGGCAGCGG CGCCCCTGAA AGTGTGCATC GTGGGCTCGG GGAAGTGGGG TTCAGCTGTT
61  GCAAAAATAA TTGGTAATAA TGTCAAGAAA CTTCAGAAAT TTGCCTCCAC AGTCAAGATG
121 TGGGTCTTTG AAGAAACAGT GAATGGCAGA AACTGACAG ACATCATAAA TAATGACCAT
181 GAAAATGTAA AATATCTTCC TGGACACAAG CTGCCAGAAA ATGTGGTTGC CATGTCAAAT
241 CTTAGCGAGG CTGTGCAGGA TGCAGACCTG CTGGTGTTTG TCATTCCCCA CCAGTTCATT
301 CACAGAACTT GTGATGAGAT CACTGGGAGA GTGCCAAGA AAGCGTGGG AATCACCTC
361 ATCAAGGGCA TAGACGAGGG CCCCAGGGG CTGAAGCTCA TTTCTGACAT CATTCTGGAA
421 AAAATGGGCA TCGATATTAG CGTGCTGATG GGAGCCAACA TTGCCAATGA GGTGGCTGCA
481 GAGAAGTTCT GTGAGACCAC CATCGGCAGC AAAGTAATGG AGAACGGCCT TCTCTTAAA
541 GAACTTCTGC AGACTCCAAA TTTCCGGATC ACTGTCGTCTG ACGACGCCGA TACTGTTGAA
601 CTCTGTGGTG CGCTTAAGAA CATCGTAGCT GTGGGAGCTG GGTCTGCGA CGGCCTCCGC
661 TGTGGAGACA ACACCAAAGC GGCCGTCATC CGCCTGGGAC TCATGAAAT GATTGCTTTT
721 GCCAGGATCT TCTGCAAAGG CCAAGTGTCT ACAGCCACCT TCCTAGAGAG CTGCGGGGTG
781 GCCGACCTGA TCACCACCTG TTACGGAGGG CGGAACCGCA GGGTGGCCGA GGCCTTCGCC
841 AGAACTGGGA AGACCATTGA AGAGTTGGAG AAGGAGATGC TGAATGGGCA AAAGCTCCAA
901 GGACCGCAGA CTTCTGCTGA AGTGTACCGC ATCCTCAAC AGAAGGGACT ACTGGACAAG
961 TTTCCATTGT TTAGTGCAGT GTATCAGATC TGCTACGAAA GCAGACCAGT TCAAGAGATG
1021 TTGTCTTGTC TTCAGAGCCA TCCAGAGCAT ACATAA

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Data S2: Sequence for mouse MDH1^{res}, mouse GPD1L^{res}. Related to Figure 3 and S4.

Mouse MDH1^{res}

ORF nucleotide sequence:

```
1   ATGTCTGAAC CAATCAGAGT CCTTGTGACT GGAGCAGCTG GTCAAATTGC ATATTCACTG
61  TTGTACAGTA TTGGAATGG  ATCTGTCTTT GGGAAAGACC AGCCCATCAT TCTTGTGCTG
121 TTGGACATCA CCCCATGAT  GGGTGTCTG  GACGGTGTCC TGATGGAAGT GCAAGACTGT
181 GCCCTTCCCC TTCTGCAGGA TGTCATTGCA ACGGACAAAG AAGAGATTGC CTTCAAAGAC
241 CTGGATGTGG CTGTCCTAGT GGGCTCCATG CCAAGAAGGG AAGGCATGGA GAGGAAGGAC
301 CTAAGTAAAG CCAATGTGAA AATCTTCAA  TCCCAGGGCA CAGCCTTGG  GAAATACGCT
361 AAAAAAGTCCG TAAAAGTGAT CGTGGTGGGA AACCCAGCCA ATACGAACTG CCTGACAGCC
421 TCCAAGTCAG CGCCATCGAT CCCCAAGGAG AATTTCACTT GCCTGACTCG CTTGGACCAC
481 AACCGAGCAA AATCTCAAAT TGCTCTTAAA CTCGGTGTAA CCGCTGATGA TGTGAAAAAC
541 GTGATCATTT GGGGCAACCA CTCATCGACC CAGTATCCAG ATGTCAATCA TGCCAAGGTG
601 AAAGTGAAG  GAAAGGAAGT CGGTGTGTAT GAAGCCCTGA AAGACGACAG CTGGTGAAG
661 GGAGAGTTCA TCACGACTGT GCAACAGCGT GGTGCTGCTG TCATCAAGGC TCGGAAGCTG
721 TCCAGTGCAA TGTCTGCTGC GAAAGCCATC GCAGACCACA TCAGAGACAT CTGGTTTGG  A
781 ACCCCAGAGG GAGAGTTCGT GTCGATGGGT GTTATCTCTG ATGGCAACTC CTATGGTGT  C
841 CCTGATGACC TGCTCTACTC ATTCCCTGTC GTGATCAAGA ATAAGACCTG GAAGTTTGT  T
901 GAAGGCCTCC CCATTAATGA CTCTCCCGT  GAAAAGATGG ACCTGACAGC AAAGGAGCTG
961 ACCGAGGAAA AGGAGACCCG TTTTGAGTTT CTCTCTCTG  CGTGA
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Mouse GPD1L^{res}

ORF nucleotide sequence:

```
1   ATGGCAGCGG CGCCTCTGAA AGTGTGCATC GTGGGCTCGG GGAAGTGGGG ATCAGCTGTT
61  GCAAAAATCA TCGGCAGCAA CGTGAAGACC CTGCAGAAAT TCTCCTCCAC CGTCAAGATG
121 TGGGTCTTTG AGGAGACCGT GAACGGGAGG AAGCTGACAG ACATAATCAA CAATGACCAC
181 GAAAACGTGA AATATCTCCC AGGACACAAG CTGCCAGAGA ATGTGGTTCGC TGTCCCAAAC
241 CTCAGCGAAG CCGTGCAGGA CGCGGACCTG CTGGTGTTCG TCATCCCTCA CCAGTTCATC
301 CACAAGATCT GCGATGAGAT CACGGGCAGG GTGCCCGAGA AGGCCCTGGG GATCACCCCT
361 ATCAAGGGCA TAGATGAGGG CCCCACGGGG CTGAAGCTAA TCTCCGACAT CATCCGGGAA
421 AAAATGGGCA TTGATATTAG CGTCCTGATG GGGGCCAACA TCGCCAGTGA GGTGCTGCG
481 GAGAAGTTCT GCGAGACCAC CATTGGCAGC AAAGTGATGC AGAACGGCCT TCTCTTCAA
541 GAGCTGCTGC AGACGCCCAA CTTTAGGATT ACCGTGCTCG ATGACGCCGA TACCGTGGAG
601 CTTTGGGGTG CTCTTAAGAA CATTGTTGCT GTGGGAGCTG GCTTCTGCGA CGGCCTCCGC
661 TGTGGGGACA ACACCAAGGC GGCCGTCATA CGCCTGGGCC TCATGAAAT  GATCGCTTTC
721 GCCAAGATCT TCTGTAAGGG CCAGGTGTCC ACGGCCACCT TCTTGAGAG  CTGCGGGGTG
781 GCTGACCTCA TCACCACCTG CTACGGAGGG CGGAACCGCA GGGTGGCAGA GGCCTTCGCC
841 AGGACTGGGA AGACCATCGA AGAGCTGGAG AAGGAGCTGC TGAACGGGCA GAAGCTGCAG
901 GGACCTCAGA CCTCTGCGGA GGTGTACCGC ATCCTCAGGC AGAAGGGGCT GCTGGACAAG
961 TTTCCCTCT  TCACTGCAGT GTATCAGATC TGCTATGAAG GCAGGCCTGT CACGCAGATG
1021 CTGTCCTGCC TGCAGAGCCA CCCAGAGCAC ATCTGA
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