Supplementary information

Supplementary Materials and Methods

Generation of genome-edited cell line by CRISPR-Cas9

In order to disrupt Pyruvate Dehydrogenase E1 Subunit Alpha 1 (PDHA1), CRISPR-Cas9 mediated genome editing was applied as previously described [25]. In brief, Cas9 and single guide RNAs (caccGATGCAGACTGTACGCCGAA and aaacTTCGGCGTACAGTCTGCATC) were simultaneously expressed using the PX458 plasmid by transient transfection using Xtremegene [26]. Transfected cells were FACS sorted and single cells were expanded. Analysis of resulting clones was done by western blotting using the following antibodies: Pyruvate Dehydrogenase (3205S; Cell Signaling Technology), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (MAB374; Merck Millipore) (Figure S1).

Generation of GLS over-expressing A549 cells

cDNAs of glutaminase (GLS) or GLS^{S482C} (UniProt O94925, isoform 1, also known as GLS1 or KGA) were amplified by polymerase chain reaction (PCR) using the pcDNA5/FRT/TO vector as previously described and the primers aaaaaagcaggcttccggggatcccGCGGCATGATGCGGCTGCGAGGCTCGGGGA and aaagctgggtcgcggccgcATTACAACAATCCATCAAGATTCTT [18]. The cDNAs were cloned into pEntry and shuttled to pInducer20 using standard gateway cloning [27]. A549 cells were infected with lentiviruses made with the GLS-encoding pInducer20 constructs and selected with G418.

Organoid culture

The study was approved by the responsible local ethics committees (Institutional Review Board of the University Medical Center Utrecht (STEM: 10-402/K; TcBio 14-008; Metabolic Biobank: 19-489, active since 05-06-2020). All biopsies were used after written informed consent. Intrahepatic cholangiocyte organoids were established and cultured as described previously [28]. Liver biopsies were cut into small pieces and digested using 10 mg/ml Collagenase D (Sigma Aldrich) in Hanks' Balanced Salt Solution (HyClone, Thermo Fisher Scientific) for 20 min at 37 °C. Digested samples were then diluted and washed with cold GF-(without growth factors) (Advanced DMEM/F12 (Gibco) supplemented with 2 mM GlutaMAX (Gibco), 10 mM HEPES (Gibco), 100 U/ml Pen-Strep (Gibco) and centrifuged for 5 min, 4 °C at 300 g. The cell pellet was plated in basement membrane extract (BME) (Bio-techne) and culture medium was added. Culture medium was based on Advanced DMEM/F12 supplemented with 2 mM GlutaMAX, 10 mM HEPES, 100 U/ml PenStrep, 2 % B27 without vitamin A (Gibco), 10 mM Nicotinamide (Sigma Aldrich), 1.25 mM N-Acetylcysteine (Sigma Aldrich), 10 % R-Spondin 1 (RSPO1) conditioned media (homemade), 10 nM Gastrin (Tocris), 50 ng/ml epidermal growth factor (EGF) (Peprotech), 100 ng/ml FGF10 (Peprotech), 25 ng/ml hepatocyte growth factor (HGF) (Peprotech), 50 μg/ml Primocin (InvivoGen), 5 μM A83-01 (Tocris), and 10 μM Forskolin (Tocris). For the first 3 days in culture, the medium was supplemented with 30 % Wnt conditioned media (homemade), 25 ng/ml Noggin (Peprotech), and human embryonic stem (hES) cell cloning recovery solution (Stemgent). The medium was changed every 3–4 days and organoids were passaged 1:4–1:8 each week.

Supplementary tables

Table S1. Medium used for isotope tracing experiments

Cell line	**Medium**
HEK293T-WT HEK293T-PDHA1-/-	DMEM, no glucose and no sodium-pyruvate (ThermoFisher Scientific) 1 % P/S (v/v) and 25 mM D-Glucose ($^{13}C_6$glucose, 99%, Cambridge Isotope Laboratories, Inc.)

A549-WT A549::pIND_GLS A549::pIND_GLS^{S482C}	RPMI 1640 medium, no glutamine (ThermoFisher Scientific), 1 % P/S (v/v) and 2 mM ^{13C5}Glutamine (99%, Cambridge Isotope Laboratories, Inc.) RPMI 1640 medium, no glutamine (ThermoFisher Scientific), 1 % P/S (v/v) and 2 mM ^{15N2}Glutamine (98%, Cambridge Isotope Laboratories, Inc.)

Table S2. Composition of the internal standard working solution

Standard	**Concentration**
$^{15N2-13C}$glycine	100 µM (Cambridge Isotope Laboratories, Buchem b.v., Apeldoorn, The Netherlands)
^{2H4}alanine, ^{2H3}leucine, ^{2H3}methionine, ^{13C6}phenylalanine, ^{13C6}tyrosine, ^{2H3}aspartate, ^{2H3}glutamate, ^{2H2}ornithine, ^{2H2}citrulline and $^{2H4-13C}$arginine	20 µM (Cambridge Isotope Laboratories, Buchem b.v., Apeldoorn, The Netherlands)
^{2H9}carnitine	6.08 µM (Cambridge Isotope Laboratories, Buchem b.v., Apeldoorn, The Netherlands)
^{2H3}acetylcarnitine	1.52 µM (Cambridge Isotope Laboratories, Buchem b.v., Apeldoorn, The Netherlands)
^{2H3}palmitoylcarnitine	0.608 µM (Cambridge Isotope Laboratories, Buchem b.v., Apeldoorn, The Netherlands)
^{2H3}propionylcarnitine, ^{2H3}butyrylcarnitine, ^{2H9}isovalerylcarnitine, ^{2H3}octanoylcarnitine, ^{2H9}myristoylcarnitine	0.304 µM (Cambridge Isotope Laboratories, Buchem b.v., Apeldoorn, The Netherlands)

Table S3. DI-HRMS-BIT library depicting the assigned HMDB names and corresponding adducts with m/z values used to annotate mass peaks derived from DI-HRMS in positive and negative ion modus.

Assigned HMDB name	**[Isotopologue]**	**theoretical m/z**
Glucose		
D-Glucose;D-Galactose;D-Mannose;Myoinositol;3-Deoxyarabinohexonic acid;Beta-D-Glucose;D-	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$	181.070665 203.052607 219.026547

Fructose;Allose;L-Sorbose;Alpha-D-Glucose;D-Tagatose;Beta-D-Galactose;Scyllitol;L-Gulose;L-Galactose	$[M-H]^-$ $[M+Cl]^-$	179.056112 215.032789
^{13C6}Glucose	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$ $[M-H]^-$ $[M+Cl]^-$	187.090794 209.072736 225.046676 185.076241 221.052918
Glucose 6-phosphate/ Fructose 6-phosphate		
Glucose 6-phosphate;Fructose 6-phosphate;Myo-inositol 1-phosphate;Galactose 1-phosphate;Dolichyl phosphate D-mannose;Fructose 1-phosphate;Mannose 6-phosphate;D-Myo-inositol 4-phosphate;Glucose 1-phosphate;Inositol phosphate;Beta-D-Glucose 6-phosphate;Beta-D-Fructose 6-phosphate;D-Tagatose 1-phosphate;D-Mannose 1-phosphate;Sorbose 1-phosphate;Beta-D-Fructose 2-phosphate;1D-myo-Inositol 3-phosphate;D-Tagatose 6-phosphate;D-fructose 1-phosphate	$[M-H]^-$	259.022442
^{13C6}Glucose 6-phosphate;^{13C6}Fructose 6-phosphate	$[M-H]^-$	265.042571
Fructose 1,6-bisphosphate		
Fructose 1,6-bisphosphate;"1D-Myo-inositol 1,4-bisphosphate";"D-Fructose 2,6-bisphosphate";"Alpha-D-Glucose 1,6-bisphosphate";"1D-Myo-inositol 1,3-bisphosphate";"1D-Myo-inositol 3,4-bisphosphate";"D-Tagatose 1,6-bisphosphate";"D-Mannose 1,6-bisphosphate";"beta-D-Fructose 1,6-bisphosphate"	$[M-H]^-$	338.988773
^{13C6}Fructose 1,6-bisphosphate	$[M-H]^-$	345.008902
Glyceraldehyde 3-phosphate/ Dihydroxyacetone phosphate		
D-Glyceraldehyde 3-phosphate;Dihydroxyacetone phosphate	$[M-H]^-$	168.990748
^{13C6}D-Glyceraldehyde 3-phosphate;Dihydroxyacetone phosphate	$[M-H]^-$	172.000812
Glyceric acid 1,3-biphosphate		
Glyceric acid 1,3-biphosphate;2,3-Diphosphoglyceric acid	$[M-H]^-$	264.951993
^{13C3}Glyceric acid 1,3-biphosphate	$[M-H]^-$	267.962058
2-phosphoglycerate/ 3-phosphoglycerate		
2-Phosphoglyceric acid;3-Phosphoglyceric acid;2-Phospho-D-glyceric acid;(2R)-2-Hydroxy-3-(phosphonatooxy)propanoate	$[M-H]^-$	184.985663
^{13C3}2-Phosphoglyceric acid;3-Phosphoglyceric acid	$[M-H]^-$	187.995727
Phosphoenolpyruvic acid		
Phosphoenolpyruvic acid	$[M-H]^-$	166.975098
^{13C3}Phosphoenolpyruvic acid	$[M-H]^-$	169.985163
Pyruvate		
Pyruvic acid;Malonic semialdehyde	$[M-H]^-$	87.008768
^{13C3}Pyruvic acid	$[M-H]^-$	90.018833
Lactate		

L-Lactic acid;Hydroxypropionic acid;Glyceraldehyde;D-Lactic acid;Dihydroxyacetone;Methoxyacetic acid	[M-H]$^-$	89.024418
^{13C3}L-Lactic acid	[M-H]$^-$	92.034483
Glycerol 3-phosphate		
Glycerol 3-phosphate;Beta-Glycerophosphoric acid	[M-H]$^-$	171.006398
^{13C3}Glycerol 3-phosphate	[M-H]$^-$	174.016463
Phosphohydroxypyruvate		
Phosphohydroxypyruvic acid;3-Phosphonatooxypyruvate	[M-H]$^-$	182.970013
^{13C3}Phosphohydroxypyruvic acid	[M-H]$^-$	185.980077
Phosphoserine		
Phosphoserine	[M+H]$^+$ [M-H]$^-$	186.016110 184.001647
^{13C3}Phosphoserine	[M+H]$^+$ [M-H]$^-$	189.026264 187.011712
Serine		
Serine	[M+H]$^+$ [M+Na]$^+$ [M+K]$^+$ [M-H]$^-$	106.049870 128.031812 144.005752 104.035317
^{13C3}Serine	[M+H]$^+$ [M+Na]$^+$ [M+K]$^+$ [M-H]$^-$	109.059934 131.041877 147.015817 107.045381
L-Acetylcarnitine		
L-Acetylcarnitine	[M+H]$^+$	204.123034
^{13C2}L-Acetylcarnitine	[M+H]$^+$	206.129744
Citrate		
Citric acid;Isocitric acid;D-threo-Isocitric acid;Diketogulonic acid;"2,3-Diketo-L-gulonate";"(1R,2R)-Isocitric acid";"D-Glucaro-1,4-lactone"	[M-H]$^-$	191.019726
^{13C2}Citrate	[M-H]$^-$	193.026436
^{13C3}Citrate	[M-H]$^-$	194.029791
^{13C4}Citrate	[M-H]$^-$	195.033146
^{13C5}Citrate	[M-H]$^-$	196.036500
^{13C6}Citrate	[M-H]$^-$	197.039855
2-oxoglutarate		
2-oxoglutarate	[M-H]$^-$	145.014248
^{13C2}2-oxoglutarate	[M-H]$^-$	147.020957
^{13C3}2-oxoglutarate	[M-H]$^-$	148.024311
^{13C4}2-oxoglutarate	[M-H]$^-$	149.027666
^{13C5}2-oxoglutarate	[M-H]$^-$	150.031021
Succinate		
Succinate;Methylmalonic acid;Erythrono-1,4-lactone;Threonolactone	[M-H]$^-$	117.019332
^{13C2}Succinate	[M-H]$^-$	119.026042
^{13C3}Succinate	[M-H]$^-$	120.029397
^{13C4}Succinate	[M-H]$^-$	121.032752
Fumarate		

Fumarate;Maleic acid	$[M-H]^-$	115.003682
^{13C2}Fumarate	$[M-H]^-$	117.009858
^{13C3}Fumarate	$[M-H]^-$	118.013918
^{13C4}Fumarate	$[M-H]^-$	119.017375
Malate		
Malate	$[M-H]^-$	133.014247
^{13C2}Malate	$[M-H]^-$	135.020957
^{13C3}Malate	$[M-H]^-$	136.024311
^{13C4}Malate	$[M-H]^-$	137.027666
Aspartate		
L-Aspartate;D-Aspartate;Iminodiacetic acid	$[M+Na]^+$ $[M-H]^-$ $[M+Cl]^-$	156.026727 132.030231 168.006909
^{13C2}L-Aspartate	$[M+Na]^+$ $[M-H]^-$ $[M+Cl]^-$	158.033436 134.036941 170.013618
^{13C3}L-Aspartate	$[M+Na]^+$ $[M-H]^-$ $[M+Cl]^-$	159.036791 135.040296 171.016973
^{13C4}L-Aspartate	$[M+Na]^+$ $[M-H]^-$ $[M+Cl]^-$	160.040146 136.043651 172.020328
^{15N1}L-Aspartate	$[M+Na]^+$ $[M-H]^-$ $[M+Cl]^-$	157.023762 133.027266 169.003944
Glutamine		
L-Glutamine;Ureidoisobutyric acid;D-Glutamine;Alanylglycine	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$ $[M-H]^-$ $[M+Cl]^-$	147.076419 169.058361 185.032301 145.0618657 181.0385432
^{13C2}L-Glutamine	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$ $[M-H]^-$ $[M+Cl]^-$	149.0831287 171.0650707 187.0390107 147.0685754 183.0452529
^{13C3}L-Glutamine	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$ $[M-H]^-$ $[M+Cl]^-$	150.0864835 172.0684255 188.0423655 148.0719302 184.0486077
^{13C4}L-Glutamine	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$ $[M-H]^-$ $[M+Cl]^-$	151.0898384 173.0717804 189.0457204 149.0752851 185.0519626
^{13C5}L-Glutamine	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$ $[M-H]^-$	152.0931932 174.0751352 190.0490752 150.0786399

	[M+Cl]-	186.0553174
	[M+H]+	148.0734539
	[M+Na]+	170.0553959
^{15N1}L-Glutamine	[M+K]+	186.0293359
	[M-H]-	146.0589006
	[M+Cl]-	182.0355781
	[M+H]+	149.0704888
	[M+Na]+	171.0524308
^{15N2}L-Glutamine	[M+K]+	187.0263708
	[M-H]-	147.0559355
	[M+Cl]-	183.0326130
Glutamate		
	[M+H]+	148.060434
L-Glutamic acid;N-Methyl-D-aspartic acid;N-	[M+Na]+	170.042377
Acetylserine;D-Glutamic acid;L-4-Hydroxyglutamate	[M+K]+	186.016317
semialdehyde;DL-Glutamate	[M-H]-	146.045881
	[M+Cl]-	182.022559
	[M+H]+	150.067144
	[M+Na]+	172.049086
^{13C2}L-Glutamic acid	[M+K]+	188.023026
	[M-H]-	148.052591
	[M+Cl]-	184.029268
	[M+H]+	151.070499
	[M+Na]+	173.052441
^{13C3}L-Glutamic acid	[M+K]+	189.026381
	[M-H]-	149.055946
	[M+Cl]-	185.032623
	[M+H]+	152.073854
	[M+Na]+	174.055796
^{13C4}L-Glutamic acid	[M+K]+	190.029736
	[M-H]-	150.059301
	[M+Cl]-	186.035978
	[M+H]+	153.077208
	[M+Na]+	175.059151
^{13C5}L-Glutamic acid	[M+K]+	191.033091
	[M-H]-	151.062656
	[M+Cl]-	187.039333
	[M+H]+	149.057469
	[M+Na]+	171.039412
^{15N1}L-Glutamic acid	[M+K]+	187.013352
	[M-H]-	147.042916
	[M+Cl]-	183.019594
Beta-alanine/ L-Alanine		
Beta-alanine;L-Alanine;Sarcosine;D-Alanine;Ethyl carbamate	[M-H]-	88.040402
^{13C2}Beta-Alanine/^{13C2}L-Alanine	[M-H]-	90.047112
^{13C3}Beta-Alanine/^{13C3}L-Alanine	[M-H]-	91.050467
^{15N1}Beta-Alanine/^{15N1}L-Alanine	[M-H]-	89.037437
L-Asparagine		
L-Asparagine;Ureidopropionic acid; Glycyl-glycine;N-	[M+H]+	133.060769
Carbamoylsarcosine;D-Asparagine	[M+Na]+	155.042711

	$[M+K]^+$	171.016651
	$[M-H]^-$	131.046216
	$[M+Cl]^-$	167.022893
	$[M+H]^+$	135.067478
	$[M+Na]^+$	157.049421
^{13C2}L-Asparagine	$[M+K]^+$	173.023361
	$[M-H]^-$	133.052925
	$[M+Cl]^-$	169.029603
	$[M+H]^+$	136.070833
	$[M+Na]^+$	158.052776
^{13C3}L-Asparagine	$[M+K]^+$	174.026716
	$[M-H]^-$	134.056280
	$[M+Cl]^-$	170.032958
	$[M+H]^+$	137.074188
	$[M+Na]^+$	159.056130
^{13C4}L-Asparagine	$[M+K]^+$	175.030070
	$[M-H]^-$	135.059635
	$[M+Cl]^-$	171.036312
	$[M+H]^+$	134.057803
	$[M+Na]^+$	156.039746
^{15N1}L-Asparagine	$[M+K]^+$	172.013686
	$[M-H]^-$	132.043251
	$[M+Cl]^-$	168.019928
	$[M+H]^+$	135.054838
	$[M+Na]^+$	157.036781
^{15N2}L-Asparagine	$[M+K]^+$	173.010721
	$[M-H]^-$	133.040286
	$[M+Cl]^-$	169.016963
Proline		
	$[M+H]^+$	116.070605
L-Proline;D-Proline;Acetamidopropanal;	$[M+Na]^+$	138.052548
4-Amino-2-methylenebutanoic acid;Pterolactam;	$[M+K]^+$	154.026488
	$[M+H]^+$	121.087379
^{13C5}L-Proline	$[M+Na]^+$	143.069322
	$[M+K]^+$	159.043262
	$[M+H]^+$	117.067640
^{15N1}L-Proline	$[M+Na]^+$	139.049582
	$[M+K]^+$	155.023522
Glycine		
	$[M+H]^+$	76.039305
Glycine;Acetohydroxamic Acid;Ethyl nitrite	$[M+Na]^+$	98.021247
	$[M+H]^+$	78.046015
^{13C2}Glycine	$[M+Na]^+$	100.027957
Ornithine		
Ornithine;D-Ornithine	$[M+H]^+$	133.097154
^{13C1}Ornithine	$[M+H]^+$	134.100509
^{13C5}Ornithine	$[M+H]^+$	138.113928
Leucine		
	$[M+H]^+$	132.101905
L-Leucine;L-Isoleucine;L-Alloisoleucine; L-	$[M+Na]^+$	154.083848
Norleucine;Aminocaproic acid;Beta-Leucine;D-Leucine;3-	$[M+K]^+$	170.057788

Aminocaproic acid;erythro-Isoleucine;6-Deoxyfagomine;N-(2-Hydroxyethyl)-morpholine;	$[M-H]^-$ $[M+Cl]^-$	130.087352 166.064030
^{13C6}L-Leucine	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$ $[M-H]^-$ $[M+Cl]^-$	138.122034 160.103977 176.077917 136.107481 172.084159
^{15N1}L-Leucine	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$ $[M-H]^-$ $[M+Cl]^-$	138.122034 160.103977 176.077917 136.107481 172.084159
Ketoleucine		
Ketoleucine;2-Methyl-3-ketovaleric acid;3-Methyl-2-oxovaleric acid; 2-Ketohexanoic acid;Mevalonolactone;3-Oxohexanoic acid;Adipate semialdehyde;"5-Ethoxy-4,5-dihydro-2(3H)furanone";Ethyl acetoacetate;Ethyl 3-oxobutanoate;Sherry lactone;"(4S,6S)-3,4,5,6-Tetrahydro-4-hydroxy-6-methyl-2H-pyran-2-one";Acetoin acetate;Methyl levulinate;Pantolactone;	$[M-H]^-$	129.055718
^{13C6}Ketoleucine	$[M-H]^-$	135.075847
Pyroglutamic acid		
Pyroglutamic acid;Pyrrolidonecarboxylic acid;Pyrroline hydroxycarboxylic acid;N-Acryloylglycine;1-Pyrroline-4-hydroxy-2-carboxylate;5-Oxoprolinate;dimethadione	$[M-H]^-$	128.035317
^{13C5}Pyroglutamic acid	$[M-H]^-$	133.052091
Glutathione		
Glutathione	$[M+H]^+$ $[M+Na]^+$	308.091082 330.073025
^{13C5}Glutathione	$[M+H]^+$ $[M+Na]^+$	313.107857 335.089800
^{15N1}Glutathione	$[M+H]^+$ $[M+Na]^+$	309.088118 331.070060
N-Acetyl-L-aspartic acid		
N-Acetyl-L-aspartic acid;N-Formyl-L-glutamic acid;D-N-(Carboxyacetyl)alanine;2-Amino-3-oxoadipate;Berteroin	$[M-H]^-$	174.040796
^{13C3}N-Acetyl-L-aspartic acid	$[M-H]^-$	177.050861
^{13C4}N-Acetyl-L-aspartic acid	$[M-H]^-$	178.054215
^{15N1}N-Acetyl-L-aspartic acid	$[M-H]^-$	175.037831
N-Formyl-L-glutamic acid		
N-Acetyl-L-aspartic acid;**N-Formyl-L-glutamic acid**;D-N-(Carboxyacetyl)alanine;2-Amino-3-oxoadipate;Berteroin	$[M-H]^-$	174.040796
^{13C5}N-Formyl-L-glutamic acid	$[M-H]^-$	179.057570
^{15N1}N-Formyl-L-glutamic acid	$[M-H]^-$	175.037831
D-Ribulose 5-phosphate/D-Ribose 5-phosphate		
D-Ribulose 5-phosphate;Xylulose 5-phosphate;Ribose 1-phosphate;**D-Ribose 5-phosphate**;D-Xylulose 1-phosphate;D-Arabinose 5-phosphate;Beta-L-arabinose 1-phosphate	$[M-H]^-$	231.026430
^{13C5}D-Ribulose 5-phosphate/^{13C5}D-Ribose 5-phosphate	$[M-H]^-$	236.043205
Sedoheptulose 7-phosphate		

D-Sedoheptulose 7-phosphate;Sedoheptulose 1-phosphate	[M-H]$^-$	289.033007
^{13C5}D-Sedoheptulose 7-phosphate	[M-H]$^-$	294.049781
Sedoheptulose 1,7-bisphosphate		
Sedoheptulose 1,7-bisphosphate	[M-H]$^-$	368.999337
^{13C5}Sedoheptulose 1,7-bisphosphate	[M-H]$^-$	374.016111
Valine		
L-Valine;Betaine; Vaporole;N-Methyl-a-aminoisobutyric acid;5-Aminopentanoic acid;Norvaline;Amyl Nitrite	[M+H]$^+$ [M-H]$^-$	118.086255 116.071702
^{13C5}L-Valine	[M+H]$^+$ [M-H]$^-$	123.103029 121.088476
alpha-Ketoisovaleric acid		
Alpha-ketoisovaleric acid;Methylacetoacetic acid;Levulinic acid;2-Oxovaleric acid;2-Methylacetoacetic acid;Glutarate semialdehyde;Ethyl pyruvate;Acetoxyacetone	[M-H]$^-$	115.040068
^{13C5}Alpha-ketoisovaleric acid	[M-H]$^-$	120.056841
(S)-3-Hydroxybutyric acid		
(S)-3-Hydroxybutyric acid;2-Hydroxybutyric acid;(R)-3-Hydroxybutyric acid;(S)-3-Hydroxyisobutyric acid;(R)-3-Hydroxyisobutyric acid;3-Hydroxybutyric acid; 4-Hydroxybutyric acid;Alpha-Hydroxyisobutyric acid;Ethoxyacetic acid;2-Methyl-3-hydroxypropanoate]	[M-H]$^-$	103.040068
^{13C4}(S)-3-Hydroxybutyric acid	[M-H]$^-$	108.056842
Propionylcarnitine		
Propionylcarnitine	[M+H]$^+$	218.138685
^{13C3}Propionylcarnitine	[M+H]$^+$	221.148749
Methylmalonic acid		
Methylmalonic acid; Succinate;"Erythrono-1,4-lactone";Threonolactone	[M-H]$^-$	117.019332
^{13C3}Methylmalonic acid	[M-H]$^-$	120.029397
^{13C4}Methylmalonic acid	[M-H]$^-$	121.032752

Supplementary Figures

Figure S1. Western blot analysis of total lysates from wild type and PDHA1-/- HEK293T cells.

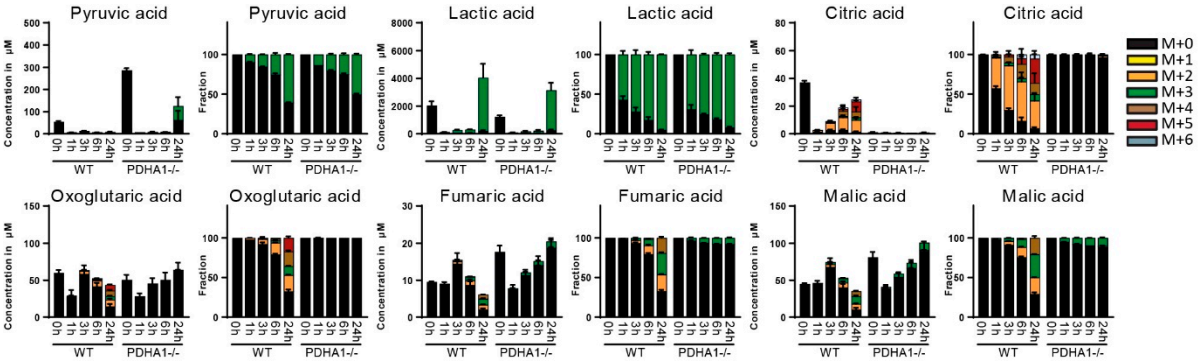

Figure S2. Targeted profiling of indicated metabolites in wild type and PDHA1 deficient HEK293T cells cultured in the presence of ^{13C6}glucose for 0 h, 1 h, 3 h, 6 h and 24 hours.

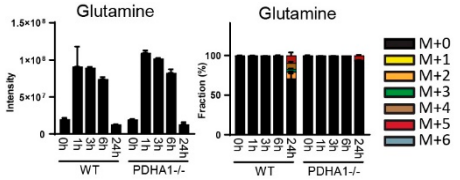

Figure S3. Total (labeled and unlabeled) intensity and fractional enrichment of glutamine in medium after 0 h, 1 h, 3 h, 6 h and 24 h incubation with ^{13C6}glucose in wild type and PDHA1 deficient HEK293T cells. Isomeric compounds for each component are listed in Table S3.

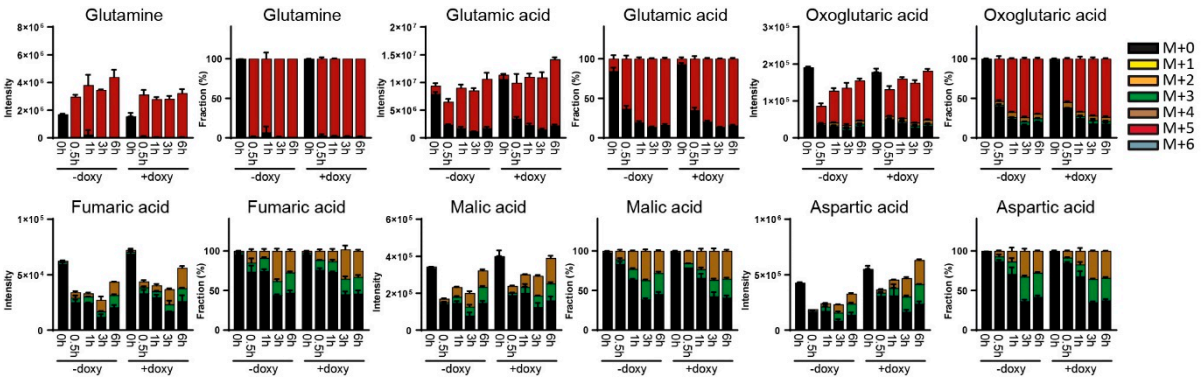

Figure S4. Metabolic consequences of overexpression of wild type GLS. Total (labeled and unlabeled) intensity and fraction of TCA cycle intermediates and aspartic acid after 0 h, 0.5 h, 1 h, 3 h and 6 h incubation with ^{13C5}glutamine and with/without doxycycline in A549::pIND_GLS cells. Isomeric compounds for each component are listed in Table S3.