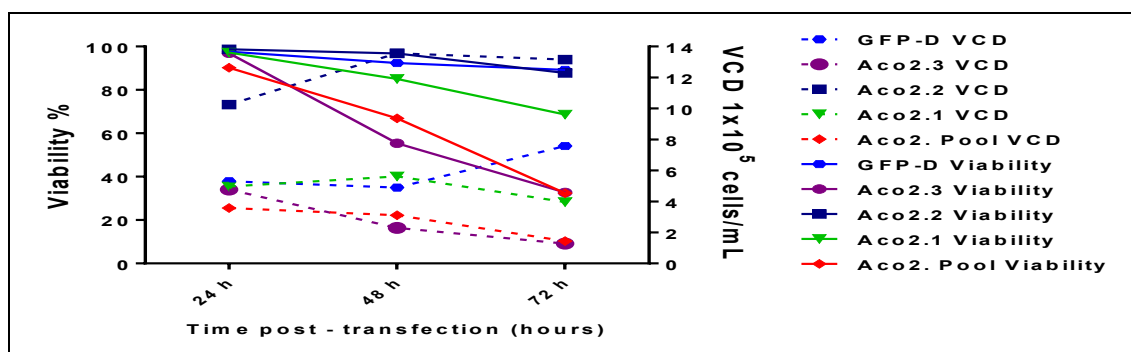


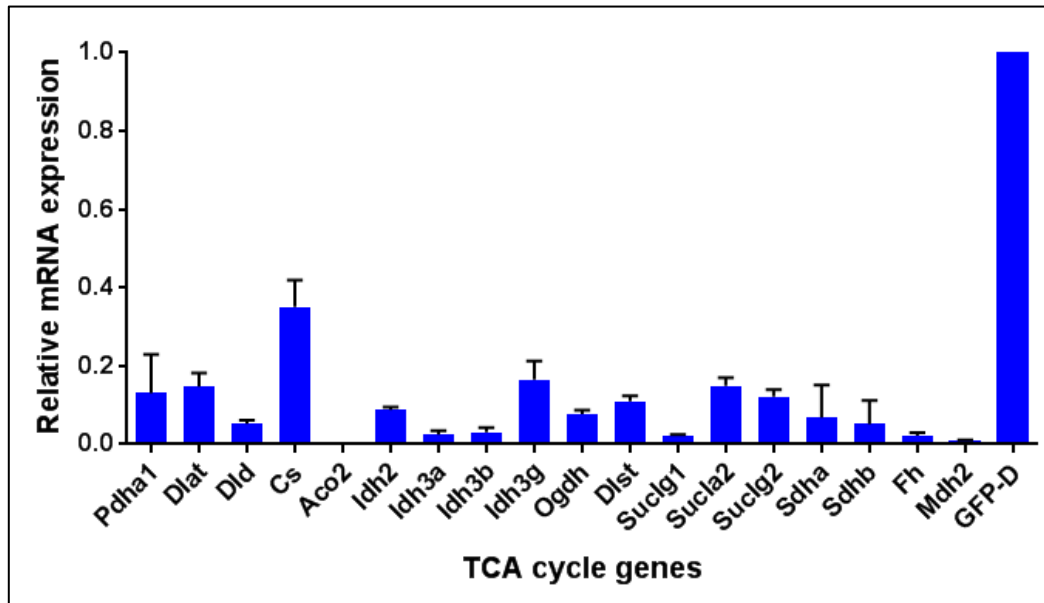
## Supplementary data

**Table S 1: Sequence of siRNA duplexes**

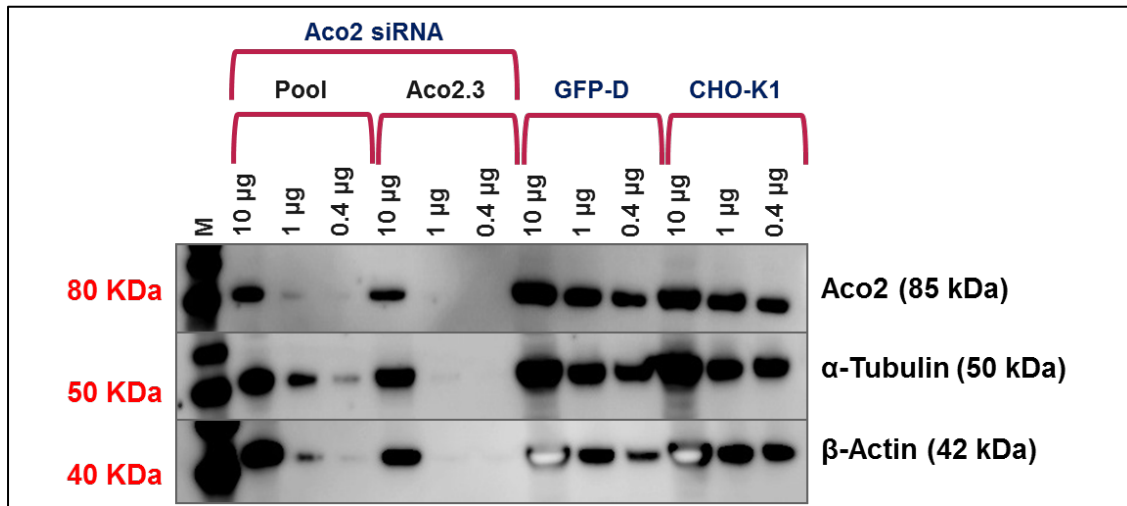
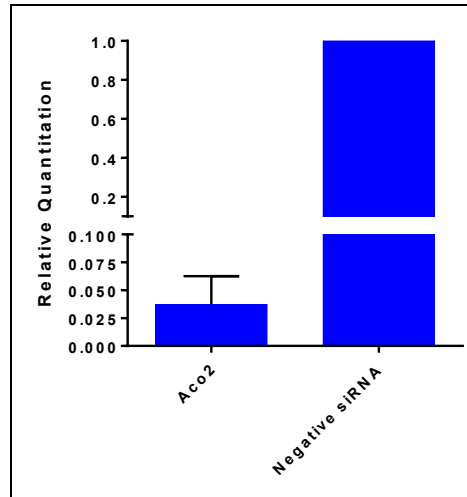
Gene	Accession number	Length of mRNA (bp)	siRNA name	siRNA sequence (sense)	siRNA sequence (antisense)	Gene Location (bp)
<i>Aco2</i>	XM_003514310	2915	Aco2.1	GUGUAUAAUUU CCUGGCAA	UUGCCAGGAAAU UAUACAC	624-642
			Aco2.2	CCAGUAUGACCA AGUGAUU	AAUCACUUGGUC AUACUGG	1172-1190
			Aco2.3	GCAAUGACGCAA ACCCUGA	UCAGGGUUUGCG UCAUUGC	1612-1630
<i>GFP-D</i>	pTurbo GFP-dest1 plasmid		GFP2.1	GAAGAAAGAUC UCGAGAUA	UAUCUCGAGAUC UUUCUUC	



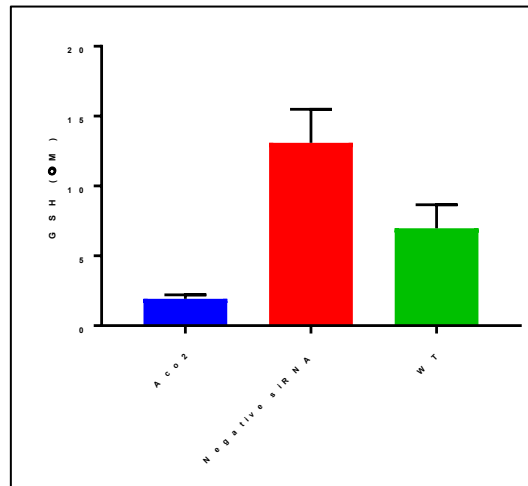
**Fig S 1: Selection of optimal siRNA duplex targeting *Aco2* gene.** The siRNA transfections targeting the TCA cycle genes were performed using Lipofectamine® RNAiMAX transfection reagent. The cell growth was measured 24-72 h post-transfection using the Vi-CELL XR Cell Viability Analyser. The GFP-D siRNA targeted the GFP-destabilised gene and acted as a negative control.



**Fig S 2: mRNA expression of TCA cycle genes post-siRNA transfections.** Total RNA extraction was performed 48 h post-transfection followed by generation of cDNA for qPCR. Relative quantitation of mRNA post-siRNA mediated knock-down of TCA cycle genes measured by TaqMan® gene expression assay using the comparative  $C_T$  method ( $\Delta\Delta C_T$ ). The relative gene expression of TCA cycle genes was compared in transfected samples and GFP-D negative transfections. The GFP-D siRNA targeted the *GFP*-destabilised gene. Data represents mean  $\pm$  SD of three replicates.



**Fig S 3: Reduction of ACO2 (A) mRNA and (B) protein expression post-siRNA transfections.** (A) Total RNA extraction was performed 48 h post-transfection followed by generation of cDNA for qPCR. Relative quantitation of mRNA post-siRNA mediated knock-down of TCA cycle genes measured by TaqMan® gene expression assay using the comparative  $C_T$  method ( $2^{-\Delta\Delta C_T}$ ). The relative gene expression of *Aco2* gene was compared in transfected and negative controls. Data represents mean  $\pm$  SD of three replicates. (B) 72 h post-siRNA transfections, the lysates were generated using the CCLR method and quantified using the BCA assay. Dilutions of total protein lysates (10  $\mu$ g, 1  $\mu$ g and 0.4  $\mu$ g) were blotted on a western blot for the ACO2 protein (~85 kDa). The  $\alpha$ -TUBULIN (~50 kDa) and  $\beta$ -ACTIN (~42kDa) proteins were used as a loading control. The GFP-D lysates represented the sample from negative control transfection. The CHO-K1 lysates were generated from the non-transfected culture grown in identical conditions as siRNA transfections. 'M' represents the marker.



**Fig S 4: Intracellular levels of glutathione (GSH) measured in CHO cells post-siRNA mediated knock-down of *Aco2* gene.** The luminescence-based GSH-Glo™ Glutathione assay was used to measure levels of glutathione 72 h post-siRNA transfection as per manufacturer's recommended protocol using luciferin-NT substrate and glutathione S-transferase enzyme. Data represents mean  $\pm$  SD of three replicates.

**Table S 2: Summary of gene expression in glucose metabolism in *Aco2* knock-down samples**

Pathway	Genes	Fold regulation	
		<i>Up</i>	<i>Down</i>
Glycolysis	<i>Pgam2</i>	4.40	
Glycolysis	<i>Gck</i>	2.27	
Glycolysis	<i>Hk3</i>	6.85	
Glycolysis	<i>Gapdhs</i>	3.19	
Glycolysis	<i>Eno3</i>	2.32	
Glycolysis	<i>Pklr</i>	7.20	
Glycolysis/ Gluconeogenesis	<i>Adh1</i>	2.27	
Gluconeogenesis	<i>G6pc3</i>		-3.16
Gluconeogenesis	<i>Fbp2</i>		-9.86
Gluconeogenesis	<i>Pck2</i>		-3.66
Glucose regulation	<i>Pdk4</i>	3.14	
Glucose regulation	<i>Pdk2</i>		-2.85
TCA cycle	<i>Aco2</i>		-27.49
TCA cycle	<i>Idh1</i>		-2.57
TCA cycle	<i>Ogdh</i>		-3.77
TCA cycle	<i>Suc1g2</i>		-2.10
Glycogen synthesis	<i>Gys2</i>	2.27	
Glycogen degradation	<i>Pyg1</i>		-4.52
Glycogen degradation	<i>Ag1</i>	2.29	
Glycogen regulation	<i>Phka2</i>		-2.17
Glycogen regulation	<i>Phkg1</i>	5.25	
Fatty acid biosynthesis	<i>Acly</i>	2.28	

**Table S 3: Summary of gene expression in amino acid metabolism in *Aco2* knock-down samples**

Pathway	Genes	Fold regulation	
		<i>Up</i>	<i>Down</i>
Alanine, Asparagine, & Aspartate / Aspartic Acid	<i>Asns</i>	3.2304	
Alanine, Asparagine, & Aspartate / Aspartic Acid	<i>Gad2</i>	2.0851	
Alanine, Asparagine, & Aspartate / Aspartic Acid/Cofactor	<i>Aldh5a1</i>		-9.1041
Alanine, Asparagine, & Aspartate / Aspartic Acid/Isoleucine/Valine	<i>Abat</i>	2.7846	
Alanine, Asparagine, & Aspartate / Aspartic Acid/Phenylalanine	<i>Got1</i>		-6.5435
Alanine, Asparagine, & Aspartate / Aspartic Acid/Serine, Glycine, & Threonine	<i>Agxt</i>	9.4873	
Arginine	<i>Agmat</i>	13.4302	
Arginine	<i>Gamt</i>		-6.9133
Arginine	<i>Oat</i>		-5.582
Arginine	<i>Gatm</i>	2.9989	
Arginine	<i>Otc</i>	2.8917	
Arginine	<i>Arg2</i>		-2.5459
Arginine	<i>Nos2</i>	4.0552	
Arginine	<i>Nags</i>		-12.0562
Arginine/Glutamine & Glutamate / Glutamic Acid	<i>Aldh4a1</i>		-2.4529
Arginine/Glutamine & Glutamate / Glutamic Acid	<i>Ass1</i>	4.0552	
Arginine/Glutamine & Glutamate / Glutamic Acid	<i>Cps1</i>	4.0552	
Arginine/Glutamine & Glutamate / Glutamic Acid	<i>Glud1</i>		-3.5571
Arginine/Glutamine & Glutamate / Glutamic Acid	<i>Asl</i>		-4.5101
Arginine/Methionine	<i>Srm</i>		-4.5575
Arginine/Tryptophan/Histidine	<i>Aoc1</i>	4.0552	
Cysteine/Cofactor/Sulfur	<i>Cth</i>		-9.0325
Cysteine/Sulfur	<i>Cdo1</i>	2.5319	
Glutamine & Glutamate / Glutamic Acid	<i>Nit2</i>		-2.3987
Glutamine & Glutamate / Glutamic Acid	<i>Cad</i>		-4.2865
Glutamine & Glutamate / Glutamic Acid	<i>Gls</i>	2.4821	
Proline	<i>Lap3</i>		-2.7831
Proline	<i>Prodh2</i>	4.4425	
Proline	<i>Pycr1</i>		-6.4977
Proline/ Serine, Glycine, & Threonine	<i>Dao</i>	4.0552	
Serine, Glycine, & Threonine	<i>Dmgdh</i>	4.0552	
Serine, Glycine, & Threonine	<i>Phgdh</i>		-3.4575
Serine, Glycine, & Threonine	<i>Shmt2</i>		-4.835
Serine, Glycine, & Threonine	<i>Srr</i>		-4.9477
Serine, Glycine, & Threonine	<i>Chdh</i>	4.0552	
Serine, Glycine, & Threonine	<i>Sardh</i>		-12.0048
Serine, Glycine, & Threonine/Arginine	<i>Amt</i>		-2.4313
Serine, Glycine, & Threonine/Cofactor	<i>Alas1</i>	2.1602	

Pathway	Genes	Fold regulation	
		<i>Up</i>	<i>Down</i>
Serine, Glycine, & Threonine/Methionine/Sulfur	<i>Bhmt</i>	4.0552	
Tryptophan	<i>Cyp1b1</i>	4.0552	
Tryptophan	<i>Ogdhl</i>	4.0552	
Tryptophan	<i>Tph2</i>	3.1263	
Tryptophan	<i>Cat</i>		-3.0808
Tryptophan	<i>Wars</i>		-4.0764
Tryptophan/Cofactor/Vitamin	<i>Haa0</i>	4.0552	
Tryptophan/Cofactor/Vitamin	<i>Ido1</i>	4.0552	
Tryptophan/Cofactor/Vitamin	<i>Kynu</i>	4.0552	
Tryptophan/Cofactor/Vitamin	<i>Kmo</i>	4.0552	
Tryptophan/Histidine/Phenylalanine	<i>Ddc</i>	5.7669	
Tryptophan/Leucine/Fatty acid degradation	<i>Ehhadh</i>		-8.8264
Tryptophan/Lysine	<i>Aadat</i>		-4.679

**Table S 4: Summary of slope and  $R^2$  values for the extracellular electrolytes measured by the BioProfile FLEX Analyzer**

	$\text{NH}_4^+$		$\text{Na}^+$		$\text{K}^+$		$\text{Ca}^{++}$	
Sample	Slope ( $\pm$ SD)	$R^2(\pm$ SD)	Slope ( $\pm$ SD)	$R^2(\pm$ SD)	Slope ( $\pm$ SD)	$R^2(\pm$ SD)	Slope ( $\pm$ SD)	$R^2(\pm$ SD)
<i>Aco2</i>	0.01 $\pm$ 0.001	0.991 $\pm$ 0.008	- 0.413 $\pm$ 0.844	0.694 $\pm$ 0.402	- 0.008 $\pm$ 0.018	0.7 $\pm$ 0.256	0.000126195 $\pm$ 0.000	0.554 $\pm$ - 0.399
Negative siRNA	0.005 $\pm$ 0.001	0.994 $\pm$ 0.005	0.001 $\pm$ 0.001	0.54 $\pm$ 0.37	0.001 $\pm$ 0.001	0.9 $\pm$ 0.051	0.00006825 $\pm$ 0.000	0.901 $\pm$ 0.055

**Table S 5: Summary of slope and  $R^2$  values for the extracellular nutrient metabolites measured by the BioProfile FLEX Analyzer**

	Glutamine		Glutamate		Glucose		Lactate	
Sample	Slope ( $\pm$ SD)	$R^2(\pm$ SD)	Slope ( $\pm$ SD)	$R^2(\pm$ SD)	Slope ( $\pm$ SD)	$R^2(\pm$ SD)	Slope ( $\pm$ SD)	$R^2(\pm$ SD)
<i>Aco2</i>	- 0.009 $\pm$ 0.006	0.821 $\pm$ 0.223	0.007 $\pm$ 0.007	0.947 $\pm$ 0.039	- 0.002 $\pm$ 0.002	0.686 $\pm$ 0.324	0.003 $\pm$ 0.002	0.726 $\pm$ 0.484
Negative siRNA	- 0.005 $\pm$ 0.001	0.792 $\pm$ 0.247	0.001 $\pm$ 0	0.74 $\pm$ 0.288	-0.003 $\pm$ 0	0.938 $\pm$ 0.079	0.003 $\pm$ 0.001	0.932 $\pm$ 0.118

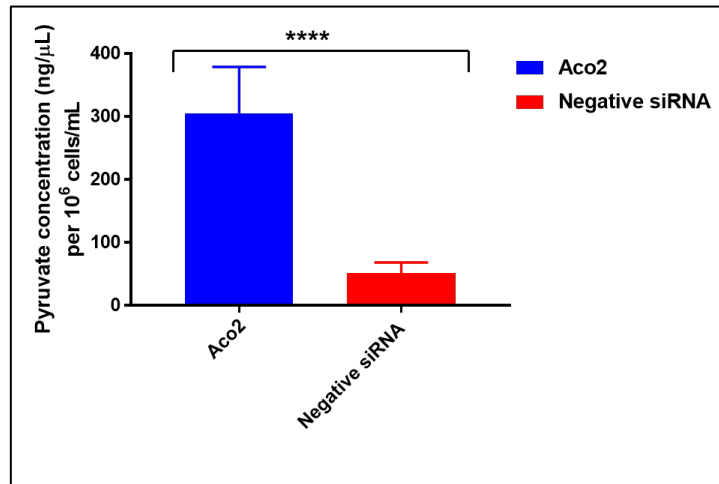
**Table S 6: Metabolites list from GC-MS analysis of *Aco2* knock-down and negative control samples.** The Peak Idx number represents the order of peak elution (1-100). The in-house library refers to University of Manchester Yeast Metabolomic Mass Spectral Library.

Metabolite	Peak Idx	Name	MSI	Library
1	5	Pyridine, 3-trimethylsiloxy-	2	NIST
2	10	Dodecane	2	NIST
3	11	Unknown 6	4	UNKNOWN
4	13	Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-N-[2-[(trimethylsilyl)oxy]ethyl]-	2	NIST
5	14	Leucine_1252_1TMS	1	in-house
6	15	Unknown 7	4	UNKNOWN
7	16	Trimethylsilyl ether of glycerol	2	NIST
8	17	Nonadecane	2	NIST
9	18	Isoleucine_1285_1TMS	1	in-house
10	19	Leucine_1306_2TMS	1	in-house
11	20	L-Isoleucine, N-(trimethylsilyl)-, trimethylsilyl ester	2	NIST
12	21	Glycine d5_1343_3TMS	1	in-house
13	23	Benzene, 1-bromo-3-fluoro-	2	NIST
14	24	Proline	2	NIST
15	25	Unknown 9	4	UNKNOWN
16	26	Serine_1361_2TMS	1	in-house

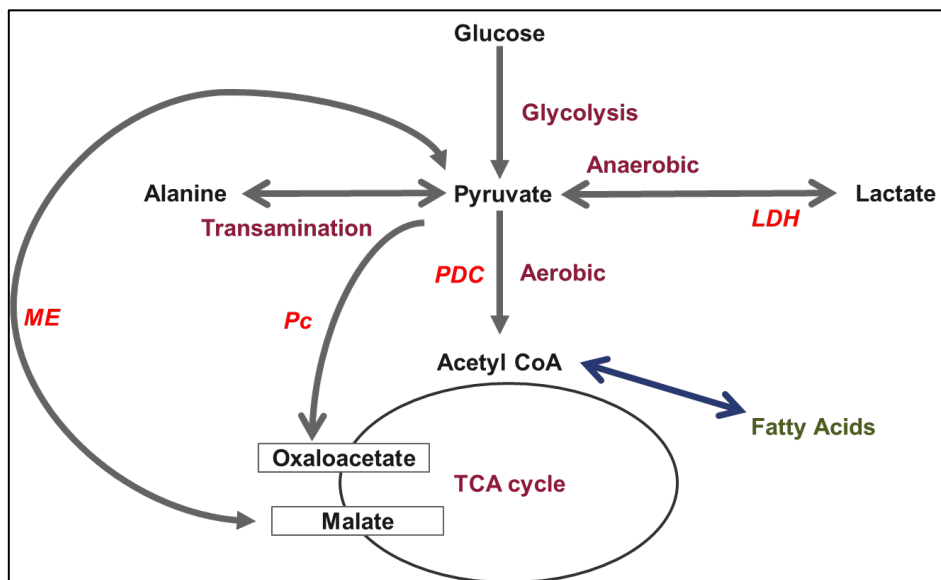


17	27	Phosphate_1372_4TMS	1	in-house
18	28	Proline_1381_2TMS	1	in-house
19	29	Benzene, 1,4-dimethyl-2,5-bis(1-methylethyl)-	2	NIST
20	30	Threonine_1384_2TMS	1	in-house
21	31	Serine_1408_3TMS	1	in-house
22	32	Threonine_1411_3TMS	1	in-house
23	33	Acetic acid, bis[(trimethylsilyl)oxyl]-, trimethylsilyl ester	2	NIST
24	34	Succinic acid d4_1419_2TMS	1	in-house
25	35	Unknown 10	4	UNKNOWN
26	36	4-hydroxyproline_1556_3TMS	1	in-house
27	37	Unknown 11	4	UNKNOWN
28	38	L-Aspartic acid, N-(trimethylsilyl)-, bis(trimethylsilyl) ester	2	NIST
29	39	Unknown 12	4	UNKNOWN
30	40	Gluconic acid, 2-methoxime, tetra(trimethylsilyl)-, trimethylsilyl ester	2	NIST
31	41	Glutamine_1689_3TMS	1	in-house
32	42	N-Acetyl-L-glutamic acid_2001_2TMS	1	in-house
33	43	L-Proline, 5-oxo-1-(trimethylsilyl)-, trimethylsilyl ester	2	NIST
34	44	Benzoic acid, 4-(4-propylcyclohexyl)-, 4'-cyano[1,1'-biphenyl]-4-yl ester	2	NIST
35	45	Phosphoric acid, 2-(trimethylsiloxy)-1-[(trimethylsiloxy)methyl]ethyl bis(trimethylsilyl) ester	2	NIST
36	46	Unknown 13	4	UNKNOWN
37	47	Unknown 14	4	UNKNOWN
38	48	Allose_1831_5TMS	1	in-house
39	49	Phosphoric acid, bis(trimethylsilyl) 2,3-bis[(trimethylsilyl)oxy]propyl ester	2	NIST
40	50	Glucose_1875_5TMS	1	in-house
41	51	Unknown 15	4	UNKNOWN
42	53	Galactose_1872_5TMS	1	in-house
43	54	Allose_1831_5TMS:2	1	in-house
44	55	Unknown 17	4	UNKNOWN
45	57	Unknown 19	4	UNKNOWN
46	60	Allose_1848_5TMS	1	in-house
47	61	Unknown 22	4	UNKNOWN
48	62	Unknown 23	4	UNKNOWN

49	63	Lysine_1893_4TMS	1	in-house
50	64	Propanoic acid, 3-[bis(trimethylsilyl)amino]-2-methyl-, trimethylsilyl ester	2	NIST
51	65	Lysine_1916_3TMS	1	in-house
52	66	Unknown 24	4	UNKNOW N
53	67	Heneicosane	2	NIST
54	69	Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	2	NIST
55	70	Fructose_1763_5TMS	1	in-house
56	71	Ribitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-	2	NIST
57	72	Gluconic acid_1942_6TMS	1	in-house
58	73	Fructose_1763_5TMS:2	1	in-house
59	74	Silamine, 1,1,1-trimethyl-N-(trimethylsilyl)-N-[2-[(trimethylsilyl)oxy]ethyl]-:2	2	NIST
60	75	Hexadecanoic acid, trimethylsilyl ester	2	NIST
61	76	Xylitol_1663_5TMS	1	in-house
62	77	Sorbitol_1835_6TMS	1	in-house
63	78	Xylitol_1663_5TMS:2	1	in-house
64	79	Gluconic acid_1942_6TMS:2	1	in-house
65	80	Unknown 26	4	UNKNOW N
66	81	Benzo[b]naphtho[2,3-d]thiophene	2	NIST
67	82	Malonic acid, bis(2-trimethylsilylethyl ester	2	NIST
68	84	Ribitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-:2	2	NIST
69	85	Silane, [(1-methyl-1,3-propanediyl)bis(oxy)]bis[trimethyl-	2	NIST
70	86	Threitol_1474_4TMS	1	in-house
71	87	Unknown 27	4	UNKNOW N
72	89	Xylitol_1663_5TMS:3	1	in-house
73	91	Sorbitol_1835_6TMS:2	1	in-house
74	92	Unknown 29	4	UNKNOW N
75	93	Unknown 30	4	UNKNOW N
76	94	Ribitol_1662_5TMS	1	in-house
77	95	Ribitol_1660_5TMS	1	in-house
78	96	Unknown 31	4	UNKNOW N
79	97	Ribitol_1660_5TMS:2	1	in-house
80	100	Unknown 33	4	UNKNOW N



**Fig S 5: Pyruvate concentration in CHO cells post-siRNA mediated knock-down of *Aco2* gene.** The pyruvate assay kit (Sigma-Aldrich) was used to measure levels of pyruvate 72 h post-siRNA transfection as per manufacturer's recommended protocol for a colorimetric assay. Data represents mean  $\pm$  SD of four independent transfections. The asterisks represent  $p$  value calculated by two-ANOVA analysis -  $p \leq 0.0001$  (\*\*\*\*).



**Fig S 6: The fate of pyruvate in *Cricetulus griseus* (Chinese hamster).** Graphic illustration of pyruvate metabolism in CHO cells. The representation was adapted from KEGG pathway (Kanehisa and Goto, 2000). PDC: Pyruvate dehydrogenase complex; LDH: Lactate dehydrogenase, Pc: Pyruvate carboxylase.