Supplementary Information for

Inhibition of mitochondrial respiration impairs nutrient consumption and metabolite transport in human retinal pigment epithelium

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Supplemental Methods

LDH Activity Assay

The LDH activity assay was performed using lactate dehydrogenase-SL assay kit (Sekisui Diagnostic, # 327-30) according to the manual. Protein, which was extracted from the liver with 50mM K_2PO_4 (pH=7.2) served as the positive control. Twenty μl of medium or positive control was mixed with 80μl fresh-made reaction buffer, which contains 260 mM N-methyl-D-glucamine, 40mM lactate, and 2mM NAD. The absorbance at the excitation of 340nm was measured every 30s at 37°C for 20 minutes by the FilterMax F5 Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). The LDH activity is calculated as delta change, as we previously reported (1, 2) and normalized over the DMSO group.

References

1. Du, J.; Yanagida, A.; Knight, K.; Engel, A. L.; Vo, A. H.; Jankowski, C.; Sadilek, M.; Tran, V. T.; Manson, M. A.; Ramakrishnan, A.; Hurley, J. B.; Chao, J. R., Reductive carboxylation is a major metabolic pathway in the retinal pigment epithelium. *Proc Natl Acad Sci U S A* **2016,** 113, (51), 14710-14715.
2. Yam, M.; Engel, A. L.; Wang, Y.; Zhu, S.; Hauer, A.; Zhang, R.; Lohner, D.; Huang, J.; Dinterman, M.; Zhao, C.; Chao, J. R.; Du, J., Proline mediates metabolic communication between retinal pigment epithelial cells and the retina. *J Biol Chem* **2019,** 294, (26), 10278-10289.

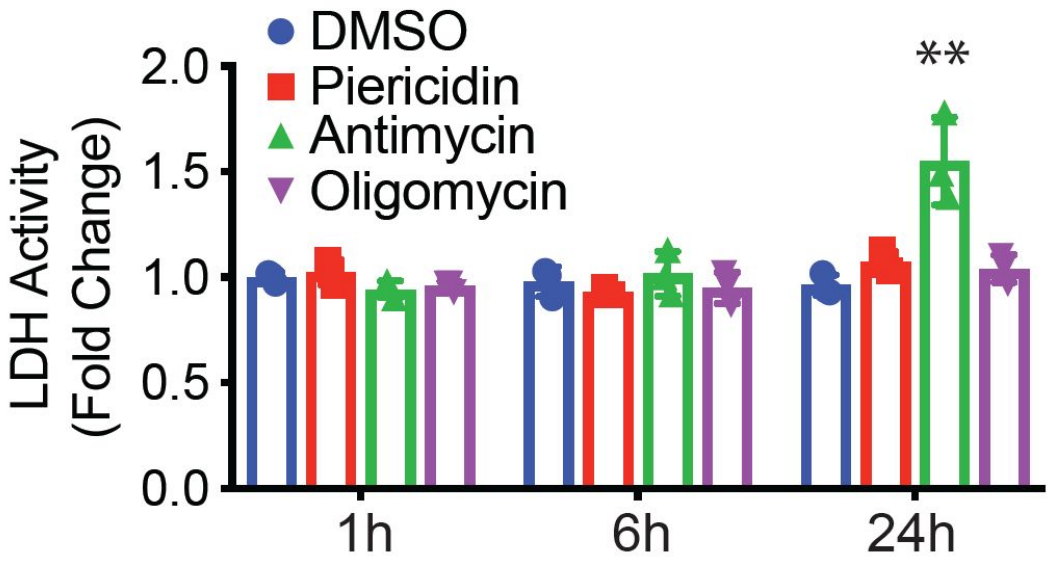

Fig. S1. The impact of mitochondrial inhibitors on LDH activity. The data were presented as fold changes of Δ absorbance density at 340nm over the groups with DMSO at different time points. N=3. **P<0.01 vs. the groups treated with DMSO.

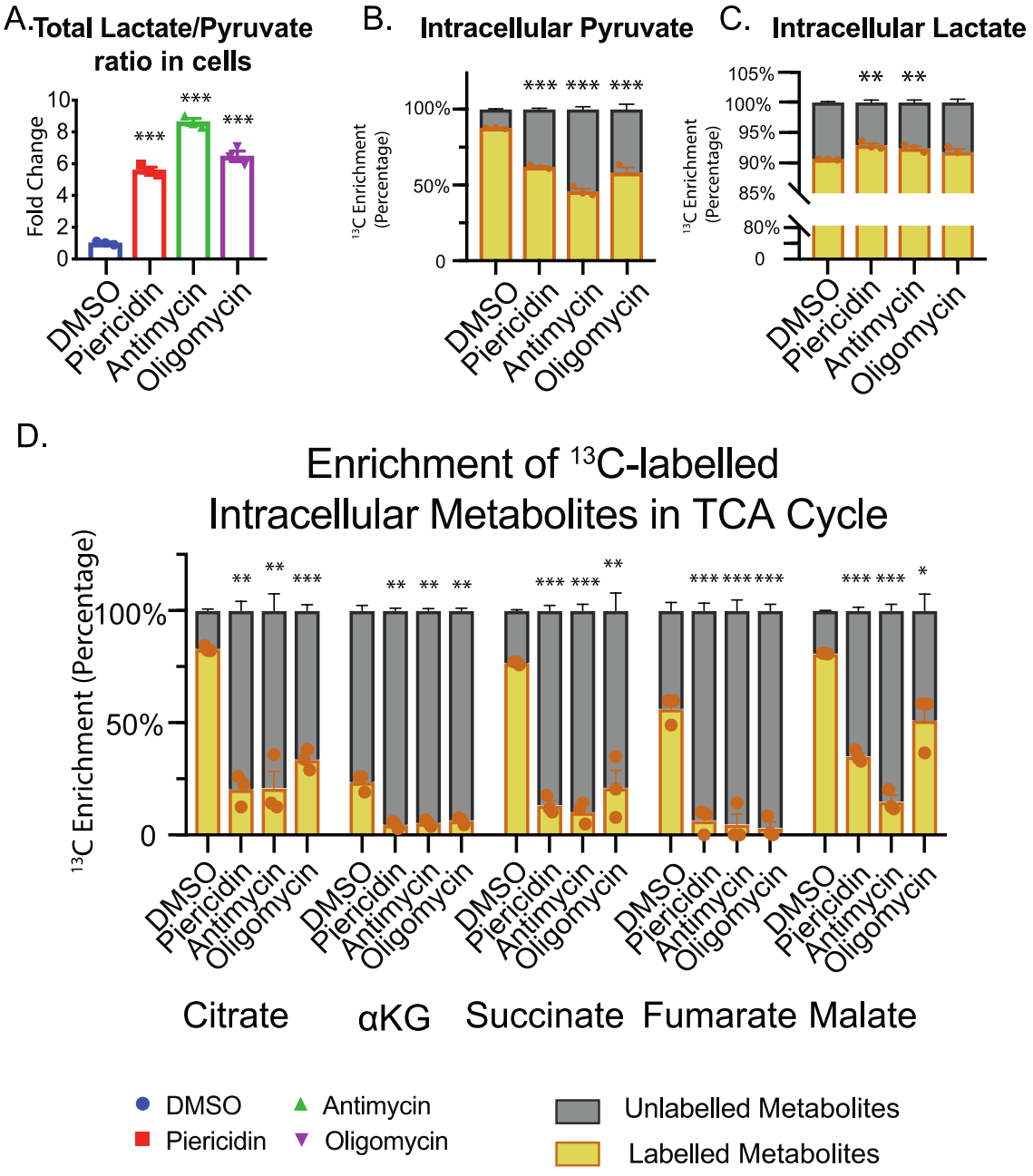

Fig. S2. The impact of the inhibition of mitochondrial respiration on glycolysis and TCA cycle intermediates. (A) Lactate/pyruvate ratio over the groups with DMSO. **(B-D)** Enrichment of ^{13}C-labeled intracellular metabolites in glycolysis and the TCA cycle. N=3. *P<0.05, **P<0.01, *P<0.001 vs. the groups treated with DMSO.

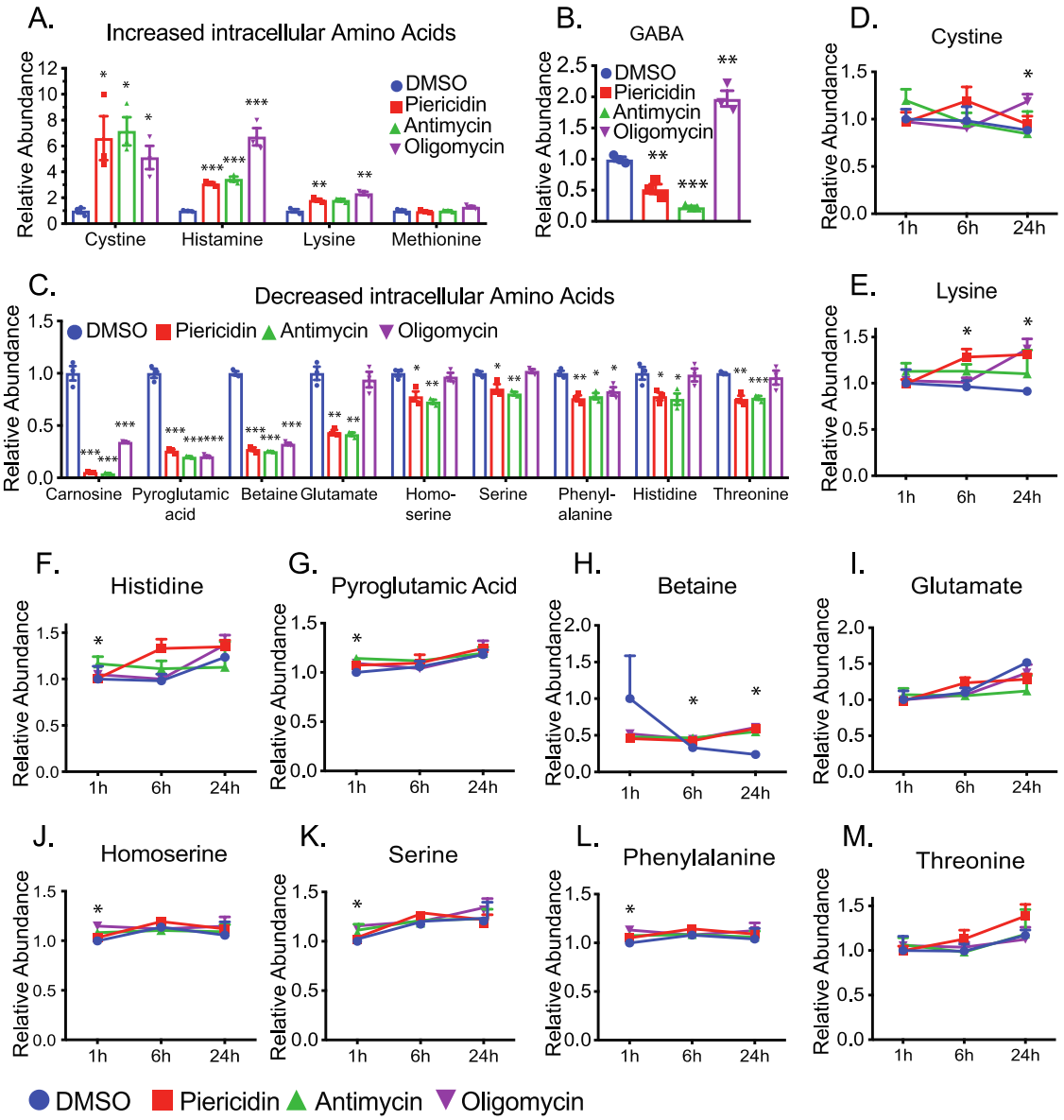

Fig. S3. The impact of mitochondrial inhibition on the metabolism of amino acids. (A-C) The relative abundance of intracellular amino acids. **(D-M)** The relative abundance of amino acids in media at different time points. N=3. *P<0.05, **P<0.01, *P<0.001 vs. the groups treated with DMSO.

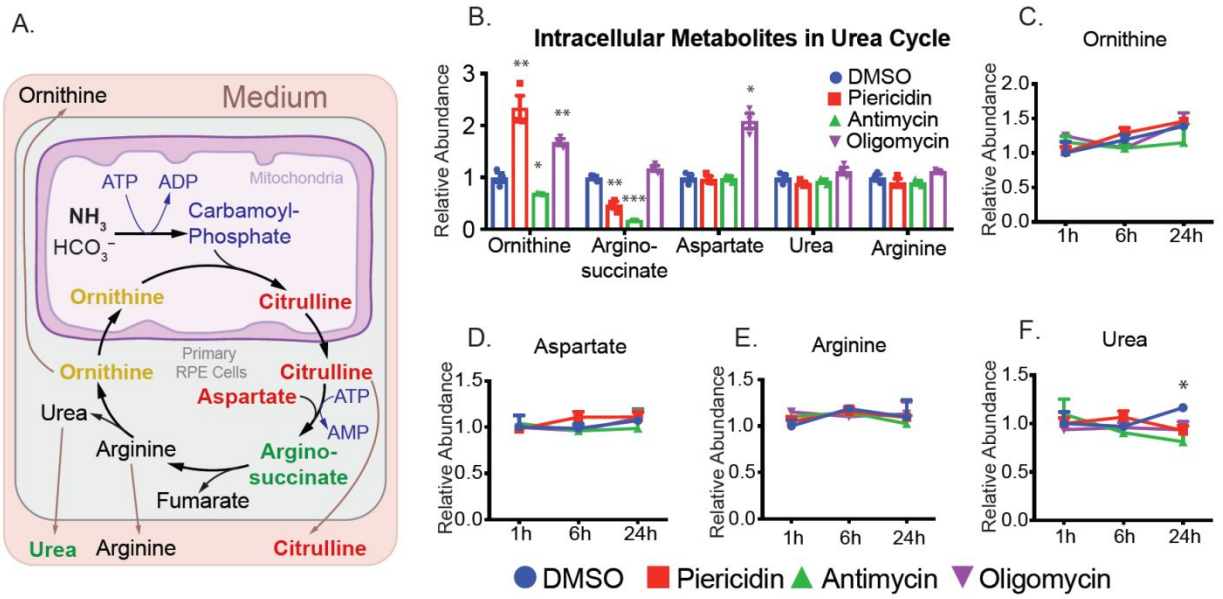

Fig. S4. The impact of mitochondrial inhibition on the urea cycle. (A) Schematic for the urea cycle. The color of metabolites represents the changes in relative abundance with mitochondrial inhibitors: red for the increased metabolites, green for decreased metabolites, orange for metabolites with mixed changes, and black for no change or not detected.) **(B)** Relative abundance of intracellular metabolites in the urea cycle. **(C-F)** The relative abundance of metabolites in the urea cycle in media at different time points. N=3. *P<0.05, **P<0.01, *P<0.001 vs the groups treated with DMSO.

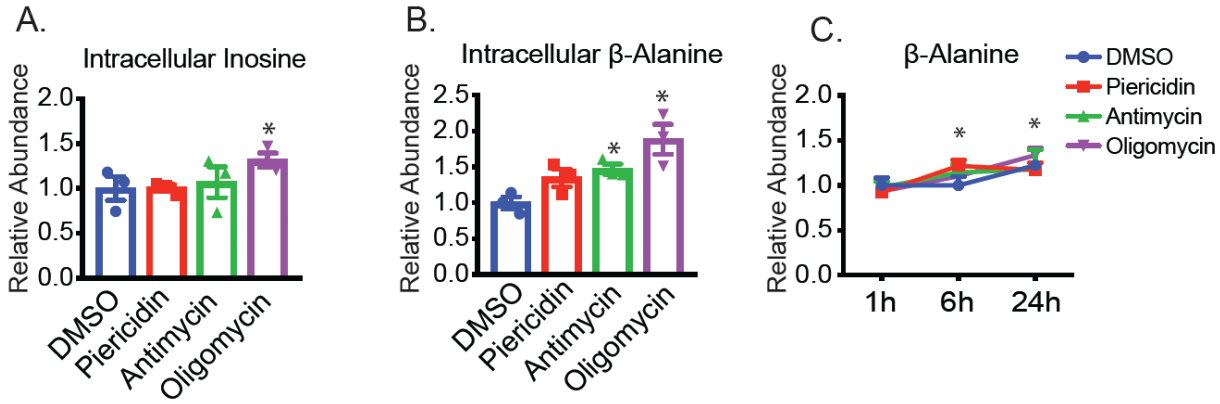

Fig. S5. The impact of mitochondrial inhibition on the metabolism of nucleotides. (A-B) The relative abundance of intracellular inosine and β-alanine. **(C)** The relative abundance of β-alanine in media at different time points. N=3. *P<0.05 vs. the groups treated with DMSO.

Table S1. Significantly changed metabolites in 1h media after inhibition of mitochondrial respiration.

Metabolite	**f value**	**p value**	**FDR**
Lactate	84.056	2.17E-06	0.000187
Cis-aconitic acid	18.863	0.00055	0.018053
Glycerol	15.177	0.001152	0.018053
oxalic acid	14.905	0.001223	0.018053
3-Aminoisobutanoic acid	14.088	0.001475	0.018053
N1-Methylnicotinamide	13.916	0.001536	0.018053
Glucose	13.744	0.0016	0.018053
Uracil	13.543	0.001679	0.018053
Succinate	10.919	0.003354	0.032047
4-hydroxyproline	10.388	0.003918	0.033697
Proline	9.3191	0.005466	0.042732

Data were analyzed with One Way-Anova using Fisher's FSD. False discovery rate (FDR).

Table S2. Significantly changed metabolites in 6h media after inhibition of mitochondrial respiration.

Metabolite	f value	p value	FDR
Lactate	1183.4	6.28E-11	5.53E-09
Glucose	957.35	1.46E-10	6.44E-09
N-Aetyl-Glycine	658.48	6.50E-10	1.91E-08
Proline	143.75	2.69E-07	5.91E-06
Succinate	118.25	5.77E-07	1.02E-05
Choline	76.951	3.06E-06	4.48E-05
3-Aminoisobutanoic acid	56.382	1.00E-05	0.000126
3-Hydroxybutyric acid	53.695	1.21E-05	0.000127
Isoleucine	52.669	1.30E-05	0.000127
Leucine	44.399	2.48E-05	0.000201
N-Asp	44.257	2.51E-05	0.000201
N1-Methylnicotinamide	40.625	3.45E-05	0.000253
a-Ketoglutarate	28.659	0.000125	0.000806
Hypoxanthine	28.441	0.000128	0.000806
Pyruvate	27.466	0.000146	0.000854
Cis-aconitic acid	25.106	0.000201	0.001105
Uracil	21.365	0.000356	0.001844
biotin	20.956	0.000381	0.001863
Cytidine	15.79	0.001008	0.00467
Valine	13.315	0.001776	0.007813
Citrate	12.257	0.002322	0.009563
Isocitrate	12.147	0.002391	0.009563
Erythritol	11.679	0.00271	0.01037
Glycine	11.105	0.00318	0.011659
Betaine	10.725	0.003547	0.012486
Glutamine	10.39	0.003916	0.013253
GAP	9.5493	0.005076	0.016543
hypotaurine	9.3035	0.005494	0.017265
Inosine	8.7804	0.006536	0.019833
Methionine	8.6768	0.006771	0.019834
Phenylpyruvate	8.5853	0.006987	0.019834
Uridine	7.534	0.010218	0.028099

Table S3. Significantly changed metabolites in 24h media after inhibition of mitochondrial respiration.

Metabolite	f value	p value	FDR
N-Aetyl-Glycine	396.83	4.87E-09	4.34E-07
Citrate	194.91	8.12E-08	2.72E-06
Isocitrate	189.02	9.17E-08	2.72E-06
Guanine	81.15	2.49E-06	3.75E-05
Glucose	80.943	2.51E-06	3.75E-05
Proline	80.841	2.53E-06	3.75E-05
Butyrylcarnitine	51.732	1.39E-05	0.000177
3-Aminoisobutanoic acid	47.674	1.90E-05	0.000206
Phenylpyruvate	45.577	2.25E-05	0.000206
Propionylcarnitine	44.972	2.36E-05	0.000206
Choline	44.091	2.54E-05	0.000206
Hypoxanthine	42.447	2.93E-05	0.000217
Erythritol	38.493	4.22E-05	0.000279
3-Hydroxybutyric acid	38.096	4.39E-05	0.000279
hypotaurine	35.926	5.45E-05	0.000324
Guanosine	35.298	5.82E-05	0.000324
Nicotinamide	32.8	7.62E-05	0.000399
Succinate	32.115	8.24E-05	0.000407
Uracil	30.207	0.000103	0.000483
N1-Methylnicotinamide	26.107	0.000175	0.000777
Citrulline	24.348	0.000224	0.00095
Cytidine	22.645	0.00029	0.001126
Isobutyrylcarnitine	22.627	0.000291	0.001126
Betaine	20.094	0.000441	0.001637
Valine	15.654	0.001038	0.003696
Creatine	14.603	0.00131	0.004483
Alanine	13.944	0.001526	0.00503
Cis-aconitic acid	13.213	0.001821	0.005788
N-Asp	12.666	0.002089	0.006411
myo inositol	12.411	0.002231	0.006618
Pyruvate	12.14	0.002395	0.006877
Ethanolamine	10.564	0.003718	0.010341
Leucine	9.4832	0.005184	0.013981
PEP	9.2877	0.005522	0.014313
Isoleucine	9.229	0.005629	0.014313
Glutamine	8.9763	0.006119	0.014874
IPP	8.9447	0.006184	0.014874

Xanthine	7.5011	0.010346	0.024232
Acetyl L Carnitine	7.3605	0.010918	0.024916
Urea	7.2707	0.011304	0.025152
Lactate	6.8862	0.013164	0.028575
a-Ketoglutarate	6.2399	0.017237	0.036525
Inosine	5.6898	0.02201	0.045556

Table S4. Significantly changed metabolites in cells at 24h after inhibition of mitochondrial respiration.

Metabolite	f value	p value	FDR
Phosphocreatine	7112.1	4.86E-14	6.12E-12
2-Methylbutyroylcarnitine	2075.3	6.67E-12	3.11E-10
AMP	2021.9	7.40E-12	3.11E-10
Isobutyrylcarnitine	720.15	4.55E-10	1.34E-08
Oxidized glutathione	671.69	6.01E-10	1.34E-08
Butyrylcarnitine	661.07	6.40E-10	1.34E-08
Myristoylcarnitine	567.3	1.18E-09	2.12E-08
Glucose	543.96	1.39E-09	2.19E-08
IMP	501.9	1.92E-09	2.55E-08
Aconitate	495.14	2.02E-09	2.55E-08
NADH	446.09	3.06E-09	3.51E-08
Citraconic Acid	436.14	3.35E-09	3.52E-08
Succinate	282.87	1.87E-08	1.81E-07
3-HB	267.34	2.33E-08	2.10E-07
IPP	258.01	2.68E-08	2.25E-07
2-hydroxyglutarate	233.05	4.01E-08	3.16E-07
Myo inositol	201.47	7.13E-08	5.28E-07
4-hydroxyproline	184.59	1.01E-07	7.04E-07
a-Ketoglutarate	161.55	1.70E-07	1.13E-06
Propionylcarnitine	153.46	2.08E-07	1.25E-06
CAMP	153.31	2.09E-07	1.25E-06
Hypotaurine	151.49	2.19E-07	1.25E-06
Citrulline	144.62	2.62E-07	1.38E-06
N-Aetyl-Glycine	144.57	2.63E-07	1.38E-06
NADPH	115.9	6.24E-07	3.01E-06
Isocitrate	115.06	6.42E-07	3.01E-06
NADP	114.97	6.44E-07	3.01E-06
Betaine	110.98	7.39E-07	3.22E-06
Citrate	110.85	7.42E-07	3.22E-06
NAD	109.62	7.75E-07	3.26E-06
Cytosine	92.405	1.51E-06	6.12E-06
1-Methyladenosine	89.12	1.73E-06	6.82E-06
Adenine	86.282	1.96E-06	7.50E-06
Uracil	81.54	2.44E-06	9.06E-06
Argininosuccinate	74.84	3.40E-06	1.19E-05
Hypoxanthine	74.826	3.40E-06	1.19E-05
Carnosine	73.718	3.60E-06	1.21E-05
Lysine	73.558	3.63E-06	1.21E-05

PEP	68.663	4.73E-06	1.53E-05
Choline	60.793	7.54E-06	2.38E-05
UDP	55.99	1.03E-05	3.17E-05
Glucose 1-phosphate	54.489	1.14E-05	3.43E-05
Aminoadipic acid	53.834	1.20E-05	3.46E-05
Cytidine	53.728	1.21E-05	3.46E-05
3PG	53.143	1.26E-05	3.52E-05
Taurine	51.152	1.45E-05	3.98E-05
3-Aminoisobutanoic acid	50.463	1.53E-05	4.10E-05
Proline	50.08	1.57E-05	4.11E-05
Ornithine	49.898	1.60E-05	4.11E-05
N-Asp	48.631	1.76E-05	4.35E-05
Gamma-Aminobutyric acid	48.615	1.76E-05	4.35E-05
Guanine	47.679	1.90E-05	4.51E-05
Hexanoylcarnitine	47.666	1.90E-05	4.51E-05
Leucine	47.091	1.99E-05	4.57E-05
ADP	47.05	1.99E-05	4.57E-05
Isoleucine	44.614	2.43E-05	5.48E-05
Guanosine	43.517	2.67E-05	5.91E-05
Histamine	42.402	2.94E-05	6.40E-05
Malate	41.407	3.22E-05	6.87E-05
Pyroglutamic acid	39.511	3.83E-05	8.05E-05
Glycerol	39.216	3.94E-05	8.14E-05
Thiamine	38.175	4.35E-05	8.85E-05
Pantothenic acid	37.817	4.51E-05	8.94E-05
Glucose 6-phosphate	37.75	4.54E-05	8.94E-05
Lactate	36.004	5.41E-05	0.000105
Valine	33.06	7.41E-05	0.000141
ATP	30.693	9.72E-05	0.000183
Glycerate	29.992	0.000106	0.000196
Aspartate	28.177	0.000133	0.000242
GAP	27.301	0.000149	0.000268
Acetylglycine	25.91	0.00018	0.000319
Xanthine	24.614	0.000216	0.000377
Glutamine	23.262	0.000264	0.000452
Acetyl L Carnitine	23.216	0.000266	0.000452
biotin	22.588	0.000293	0.000492
Glutaric Acid	21.46	0.000351	0.000581
Erythritol	20.339	0.000423	0.000692
Glycine	20.238	0.000431	0.000695

Methionine	19.516	0.000489	0.000779
L-Arginine	18.662	0.00057	0.000899
Trigonelline	17.496	0.000712	0.001108
Tryptophan	16.487	0.000872	0.001339
Nicotinamide	16.214	0.000922	0.001386
Urea	16.205	0.000924	0.001386
N1-Methylnicotinamide	14.959	0.001209	0.001792
Palmitate	14.851	0.001238	0.001815
Acetoacetate	14.43	0.001363	0.001973
Phenyalanine	14.363	0.001384	0.001981
Stearic acid	13.634	0.001643	0.002326
Creatine	13.554	0.001675	0.002345
Xanthurenic acid	13.446	0.00172	0.002381
Histidine	13.343	0.001764	0.002415
Cystathionine	13.198	0.001827	0.002461
Erythrono 1,4 Lactone	13.179	0.001836	0.002461
Maleic Acid	12.136	0.002398	0.00318
Asparagene	11.676	0.002713	0.00356
Serine	11.552	0.002806	0.003645
Hippurate	11.456	0.002882	0.003706
Adipic Acid	11.38	0.002943	0.003746
Fumarate	11.056	0.003225	0.004063
Cis-aconitic acid	10.878	0.003394	0.004234
Creatinine	10.382	0.003925	0.004849
Oxalic acid	10.345	0.003969	0.004856
L-Asparagine	10.013	0.00439	0.005319
D-Ribulose 5-phosphate	9.9053	0.004538	0.005446
N-alpha-Acetyl-L-lysine	9.628	0.00495	0.005884
Threonine	9.2331	0.005621	0.006619
Succinic semiadehyde	8.9561	0.00616	0.007187
L-Homoserine	8.8324	0.006422	0.007423
Riboflavin	8.3701	0.007531	0.008626
Glutathione	7.8015	0.009244	0.010493
Tyrosine	7.2492	0.0114	0.012824
Urate	6.9685	0.012735	0.014201
Alanine	6.8475	0.013371	0.014779
Beta-Alanine	6.5583	0.015059	0.0165
Glutamate	6.5207	0.015298	0.016617
Cystine	4.4229	0.04115	0.044315
carbonate	4.3858	0.041974	0.044819

Table S5. The reagents and key resources.

Reagent or Media components	**SOURCE**	**IDENTIFIER**
Piericidin	Cayman Chemical	15379
Antimycin A	Sigma-Aldrich	A8674
Oligomycin	Sigma-Aldrich	75351
FCCP	Cayman Chemical	15218
rotenone	Sigma Aldrich	R8875
DMSO	Sigma-Aldrich	D2650
[$^{13}C_6$]-Glucose	Cambridge-Isotope Laboratories	CLM-1396
MEM a	Gibco Life Technologies (Invitrogen)	12561056
Non-essential amino acids	Life Technologies (Invitrogen)	1140-050
N1 medium supplement	Sigma-Aldrich	N6530-5mL
FBS	Atlanta Biologicals(R&D Systems)	S11550
Taurine	Sigma-Aldrich	T0625-10G
Hydrocortisone	Sigma-Aldrich	H0396-100MG
3, 3', 5-Triiodo-L-Thyronine	Sigma-Aldrich	T6397-100MG
Penicillin-Streptomycin (5,000 U/mL)	Life Technologies (Invitrogen)	15070-063
DMEM, no glucose, no glutamine, no phenol red	ThermoFisher	A1443001
Y-27632 dihydrochloride	Tocris/R&D Systems	1254
XF Calibrant Buffer (100mL)	Agilent-Seahorse	103059-000
XF 24 well culture plate	Agilent- Seahorse	100777-004
XFe24 Extracellular Flux Assay Kit (Sensor cartridge)	Agilent-Seahorse	102340-100
XF Base Media (100mL)	Agilent- Seahorse	103193-100
Sodium pyruvate (100mL)	Gibco	11360-070
L-glutamine (100mL)	Gibco	25030-081
Cytotoxicity experiments		
Lactate Dehydrogenase-SL (kit)	Sekisui Diagnostics	327-30
Mass Spectrometry (LC MS and GC MS)		
Methanol, Optima™ LC/MS Grade	Fisher Chemical	A456-500
Acquity UPLC BEH Amide 1.7 µm Vanguard pre-column 2.1 x 5mm column	Waters	186004799
Acquity UPLC BEH Amide 1.7 µm 2.1 x 50mm column	Waters	186004800
Water, Optima™ LC/MS Grade	Fisher Chemical	7732-18-5
Acetonitrile, Optima™ LC/MS Grade	Fisher Chemical	75-05-8
Ammonium Acetate	Sigma-Aldrich	431311

Ammonium hydroxide 28 - 30% in water ACS	VWR	AC42330-5000
Methoxyamine hydrochloride	Sigma-Aldrich	226904
Pyridine	Sigma-Aldrich	270970
N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide	Sigma-Aldrich	394882
DB-5ms GC Column, 30 m, 0.25 mm, 0.25 µm,	Agilent Technologies	122-5532

Table S6. The list of metabolites and parameters for LC MS and GC MS

Names	Nutrients	Precursor (Da)	Product (Da)	Declustering potential	Collision Energy	HMDB	Platform	Polarity
Acetylglycine	Amino acid	116.0	74.0	-37.0	-14.0	HMDB00532	LCMS	-
Aconitate	Glucose	173.0	85.0	-37.0	-18.0	HMDB00072	LCMS	-
Adenine	Nucleotide	134.0	107.1	-92.0	-25.0	HMDB00034	LCMS	-
Adenosine 5'-diphosphate	Energy	426.0	79.1	-65.0	-84.0	HMDB01341	LCMS	-
Aminoadipic acid	Amino acid	160.1	116.1	-47.0	-20.0	HMDB00510	LCMS	-
Adenosine monophosphate	Energy	346.0	134.0	-82.0	-40.0	HMDB00045	LCMS	-
Cyclic AMP	Nucleotide	328.0	134.0	-87.0	-32.0	HMDB00058	LCMS	-
Citraconic Acid	Glucose	129.0	85.0	-21.0	-12.0	HMDB00634	LCMS	-
Citrulline	Amino acid	174.1	131.1	-32.0	-20.0	HMDB00904	LCMS	-
Creatinine	Amino acid	112.0	41.0	-67.0	-36.0	HMDB00562	LCMS	-
D-Ribulose 5-phosphate	Nucleotide	229.0	79.0	-70.0	-45.0	HMDB0000618	LCMS	-
Glucose	Glucose	179.0	89.0	-50.0	-15.0	HMDB00122	LCMS	-
Glucose 1-phosphate	Glucose	259.0	79.0	-85.0	-60.0	HMDB0001586	LCMS	-
Glucose 6-phosphate	Glucose	259.0	79.0	-46.0	-62.0	HMDB01401	LCMS	-
Glutaric Acid	Amino acid	131.0	87.0	-45.0	-16.0	HMDB00661	LCMS	-
Glutathione	Energy	306.0	143.1	-61.0	-26.0	HMDB00125	LCMS	-
Guanosine	Nucleotide	282.1	150.0	-67.0	-33.0	HMDB00133	LCMS	-
Hippurate	Amino acid	178.0	77.1	-61.0	-23.0	HMDB00714	LCMS	-
Hypotaurine	Amino acid	108.1	64.0	-40.0	-17.0	HMDB0000965	LCMS	-
Hypoxanthine	Nucleotide	135.0	65.0	-108.0	-37.0	HMDB00157	LCMS	-
Inosinic acid	Nucleotide	347.0	79.0	-118.0	-86.0	HMDB00175	LCMS	-
Inosine	Nucleotide	267.0	135.0	-123.0	-30.0	HMDB0000195	LCMS	-
Myo inositol	Amino acid	179.0	87.0	-105.0	-24.0	HMDB0000211	LCMS	-
NADH	NAD metabolism	664.0	397.0	-7.0	-46.0	HMDB01487	LCMS	-
Oxalic acid	Nucleotide	89.0	61.0	-45.0	-10.0	HMDB0002329	LCMS	-
Pantothenic acid	Vitamin	218.1	71.0	-70.0	-41.0	HMDB0000210	LCMS	-
Phosphocreatine	Amino acid	210.0	79.0	-42.0	-49.0	HMDB0041624	LCMS	-
Uridine 5'-diphosphate	Nucleotide	403.0	79.0	-30.0	-73.0	HMDB00295	LCMS	-
Urate	Nucleotide	167.0	124.0	-86.0	-19.0	HMDB0000289	LCMS	-
Xanthine	Nucleotide	151.0	108.0	-70.0	-23.0	HMDB00292	LCMS	-
Xanthurenic acid	Nucleotide	204.0	160.0	-67.0	-19.0	HMDB00881	LCMS	-
Adipic Acid	Dicarboxylic acids	145.0	83.0	-50.0	-17.0	HMDB0000448	LCMS	-
Isopentenyl pyrophosphate	Lipid	245.1	79.0	-60.0	-50.0	HMDB0001347	LCMS	-
1-Methyladenosine	Nucleotide	282.1	150.1	48.0	35.0	HMDB03331	LCMS	+
3-Aminoisobutanoic acid	Nucleotide	104.1	58.0	55.0	38.0	HMDB03911	LCMS	+
Acetyl L Carnitine	Carnitine	204.1	85.0	71.0	30.0	HMDB00201	LCMS	+

Adenosine	Nucleotide	268.1	136.0	95.0	38.0	HMDB00050	LCMS	+
Alanine	Amino acid	90.0	44.0	50.0	20.0	HMDB0000161	LCMS	+
ATP	Energy	507.9	136.1	14.0	60.0	HMDB00538	LCMS	+
Betaine	Amino acid	118.1	58.0	166.0	56.0	HMDB00043	LCMS	+
Carnosine	Amino acid	227.1	110.1	157.0	32.0	HMDB00033	LCMS	+
Choline	Amino acid	104.1	60.1	95.0	37.0	HMDB00097	LCMS	+
Creatine	Amino acid	132.1	90.0	170.0	17.0	HMDB00064	LCMS	+
Cytidine	Nucleotide	244.0	112.1	50.0	30.0	HMDB00089	LCMS	+
Cytosine	Nucleotide	112.0	95.0	97.0	23.0	HMDB0000630	LCMS	+
Flavin adenine diNucleotide	Energy	786.1	136.1	52.0	43.0	HMDB01248	LCMS	+
Glutamine	Amino acid	147.1	84.1	45.0	23.0	HMDB00641	LCMS	+
L-Arginine	Amino acid	175.1	70.1	45.0	33.0	HMDB00517	LCMS	+
L-Asparagine	Amino acid	133.1	70.1	55.0	24.0	HMDB0033780	LCMS	+
L-Homoserine	Amino acid	120.1	74.0	37.0	16.0	HMDB00719	LCMS	+
Lysine	Amino acid	147.1	84.0	38.0	22.0	HMDB0000182	LCMS	+
1-Methylnicotinamide	Nucleotide	137.0	78.0	110.0	37.0	HMDB00699	LCMS	+
NAD	NAD metabolism	664.0	136.0	27.0	48.0	HMDB00902	LCMS	+
NADP	NAD metabolism	744.0	136.0	9.0	78.0	HMDB00217	LCMS	+
NADPH	NAD metabolism	746.0	302.0	20.0	44.0	HMDB00221	LCMS	+
Nicotinamide	NAD metabolism	123.0	80.0	111.0	20.0	HMDB01406	LCMS	+
Ornithine	Amino acid	133.0	70.0	47.0	23.0	HMDB0000214	LCMS	+
Oxidized glutathione	Energy	613.0	231.0	16.0	42.0	HMDB03337	LCMS	+
Proline	Amino acid	116.1	70.1	70.0	44.0	HMDB00162	LCMS	+
Pyroglutamic acid	Amino acid	130.1	84.0	100.0	20.0	HMDB0000267	LCMS	+
Riboflavin	Nucleotide	377.1	243.1	14.0	29.0	HMDB00244	LCMS	+
Serine	Amino acid	106.0	60.0	40.0	22.0	HMDB0000187	LCMS	+
Thiamine	Vitamin	265.0	122.1	67.0	40.0	HMDB00235	LCMS	+
Threonine	Amino acid	120.1	102.0	50.0	10.0	HMDB0000167	LCMS	+
Trigonelline	NAD metabolism	138.0	92.0	60.0	27.0	HMDB00875	LCMS	+
Tryptophan	Amino acid	205.0	146.0	75.0	35.0	HMDB00929	LCMS	+
Tyrosine	Amino acid	182.1	136.0	40.0	17.0	HMDB0000158	LCMS	+
Valine	Amino acid	118.1	72.0	60.0	14.0	HMDB0000883	LCMS	+
2-MethylbutyroylCarnitine	Carnitine	246.2	85.1	100.0	43.0	HMDB0000378	LCMS	+
ButyrylCarnitine	Carnitine	232.2	85.1	72.0	30.0	HMDB0002013		+
Gamma-Aminobutyric acid	Lipid	104.1	87.0	57.0	13.0	HMDB0000112	LCMS	+
Guanine	Nucleotide	152.0	110.0	20.0	28.0	HMDB0000132	LCMS	+
HexanoylCarnitine	Carnitine	260.2	85.1	80.0	25.0	HMDB0000705	LCMS	+
Histamine	Amino acid	112.0	95.0	65.0	18.0	HMDB0000870	LCMS	+

IsobutyrylCarnitine	Carnitine	232.1	85.1	70.0	30.0	HMDB0000736	LCMS	+
L-PalmitoylCarnitine	Carnitine	400.4	85.1	30.0	40.0	HMDB0000222	LCMS	+
PropionylCarnitine	Carnitine	218.1	85.1	60.0	24.0	HMDB0000824	LCMS	+
Argininosuccinate	Amino acid	291.1	69.9	40.0	32.0	HMDB0000052	LCMS	+
Cystathionine	Amino acid	221.1	79.1	-87.0	-30.0	HMDB0000099	LCMS	-
Erythritol	Sugar	123.0	80.0	40.0	33.0	HMDB0002994	LCMS	+
MyristoylCarnitine	Carnitine	372.4	85.1	70.0	25.0	HMDB0005066	LCMS	+
N-alpha-Acetyl-L-lysine	Amino acid	189.1	84.1	50.0	32.0	HMDB0000446	LCMS	+
2-hydroxyglutarate	Glucose	433.2	NA	NA	NA	HMDB0059655	GCMS	+
3-Hydroxybutyric acid	Ketone body	275.1	NA	NA	NA	HMDB0000357	GCMS	+
3-Phosphoglyceric acid	Glucose	585.3	NA	NA	NA	HMDB0000807	GCMS	+
4-hydroxyproline	Amino acid	314.2	NA	NA	NA	HMDB00725	GCMS	+
Acetoacetate	Ketone body	188.1	NA	NA	NA	HMDB0000060	GCMS	+
a-Ketoglutarate	Glucose	346.0	NA	NA	NA	HMDB0000208	GCMS	+
Asparagene	Amino acid	417.0	NA	NA	NA	HMDB0000168	GCMS	+
Aspartate	Amino acid	418.0	NA	NA	NA	HMDB0000191	GCMS	+
Cholesterol	Lipid	443.4	NA	NA	NA	HMDB0000067	GCMS	+
Citrate	Glucose	459.0	NA	NA	NA	HMDB0000094	GCMS	+
Cysteine	Amino acid	304.2	NA	NA	NA	HMDB0000574	GCMS	+
Cystine	Amino acid	302.2	NA	NA	NA	HMDB0000192	GCMS	+
Fumarate	Glucose	287.0	NA	NA	NA	HMDB0000134	GCMS	+
Glutamate	Amino acid	432.0	NA	NA	NA	HMDB0003339	GCMS	+
Glycerate	Lipid	391.2	NA	NA	NA	HMDB0000139	GCMS	+
Glycine	Amino acid	218.0	NA	NA	NA	HMDB0000123	GCMS	+
Histidine	Amino acid	196.1	NA	NA	NA	HMDB0000177	GCMS	+
Isocitrate	Glucose	591.3	NA	NA	NA	HMDB0000193	GCMS	+
Isoleucine	Amino acid	200.0	NA	NA	NA	HMDB0000172	GCMS	+
Lactate	Glucose	261.0	NA	NA	NA	HMDB0000190	GCMS	+
Leucine	Amino acid	200.0	NA	NA	NA	HMDB0000687	GCMS	+
Malate	Glucose	419.2	NA	NA	NA	HMDB0031518	GCMS	+
Methionine	Amino acid	218.0	NA	NA	NA	HMDB0000696	GCMS	+
Palmitate	Lipid	313.2	NA	NA	NA	HMDB0000220	GCMS	+
Phosphoenolpyruvic acid	Glucose	453.1	NA	NA	NA	HMDB0000263	GCMS	+
Phenylalanine	Amino acid	302.0	NA	NA	NA	HMDB0000159	GCMS	+
Pyruvate	Glucose	174.0	NA	NA	NA	HMDB0000243	GCMS	+
Stearic acid	Lipid	341.3	NA	NA	NA	HMDB0000827	GCMS	+
Succinate	Glucose	289.0	NA	NA	NA	HMDB0000254	GCMS	+
Succinic semiadehyde	Amino acid	159.1	NA	NA	NA	HMDB0001259	GCMS	+
Taurine	Amino acid	296.0	NA	NA	NA	HMDB0000251	GCMS	+
Uracil	Nucleotide	283.3	NA	NA	NA	HMDB00300	GCMS	+
Urea	Nucleotide	231.1	NA	NA	NA	HMDB0000294	GCMS	+

Beta-Alanine	Amino acid	260.2	NA	NA	NA	HMDB0000056	GCMS	+
Biotin	Vitamin	529.4	NA	NA	NA	HMDB0000030	GCMS	+
Carbonate	Glucose	233.1	NA	NA	NA	HMDB0031453	GCMS	+
Cis-aconitic acid	Glucose	459.2	NA	NA	NA	HMDB0000072	GCMS	+
Glyceraldehyde 3-phosphate	Glucose	484.2	NA	NA	NA	HMDB0001112	GCMS	+
Glycerol	Lipid	377.2	NA	NA	NA	HMDB0000131	GCMS	+
Maleic Acid	Glucose	287.1	NA	NA	NA	HMDB0000176	GCMS	+
N-Acetylglycine	Amino acid	288.1	NA	NA	NA	HMDB0000532	GCMS	+
N-Acetyl-L-aspartic acid	Amino acid	460.2	NA	NA	NA	HMDB0000812	GCMS	+