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SUPPLEMENTARY MATERIALS AND METHODS

Construction of targeting vector

See Materials and Methods for outline. The original PL451 vector carries a neomycin acetyltransferase resistance gene controlled by two promoters for expression, respectively, in eukaryotic and prokaryotic cells. The neomycin selection cassette is flanked by flippase recognition target (FRT) sites to allow genomic deletion at a later step. It was adapted for use as an AOX entry vector as described, and *Ciona* AOX (Hakkaart et al., 2006), amplified using primers Ciona Aox F *AscI*, GGCGCGCCACCATGTTGTCTACCGGAAGTAA and Ciona Aox R *BamHI*, GGATCCCTATTGTCCAGGTGGATAAGGAT (these and all primers shown 5' to 3'), was directionally cloned using these restriction sites (*AscI* and *BamHI*), upstream of the neomycin resistance cassette. The final plasmid, pRosa26-Aox, was produced by cotransformation of this construct with Rosa26 targeting plasmid pRosa26-DTA into recombineering competent *E. coli* (EL250). Following selection for kanamycin resistance and verification by PCR and sequencing, it was used for mouse ES cell electroporation. Correct integration was verified first by negative (DTA) and positive (G418) selection, followed by Southern blotting (Koetsier et al., 1993) using gene-specific restriction enzymes and probes to distinguish the wild-type and manipulated alleles, as shown in Fig. S1B, S1C. Briefly, PCR-amplified 5' and 3' probes were labelled with [α -³²P]dCTP using the Rediprime II DNA Labeling System (Amersham/GE, Cat. No. RPN1633) according to manufacturer's instructions. Radio-labeled probes were column purified using G50 Quick Spin Columns (Roche) and used to probe Southern blots of genomic DNA from putative positive clones, digested with *EcoRI* for probing with the 5' probe (manipulated allele 5.8 kb, wild-type allele 15.6 kb) and *BglII* for probing with the 3' probe (manipulated allele 7.5 kb, wild-type allele 6.7 kb). The exact probe sequences were as follows (5' to 3').

5' probe:

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CATTAATGGACGCTGCACTGCTGTCCTTCCCTGGAGACAGCAGCCAGCAC
TACTCAAGCTTCTCACGTAGCAACCAGAGCTCCAGAGCCAGCAGCTGCTG
CCGCCTTGTATACTCACTCCTGTGATCCAACACAGGAGCAACCTTTTCTTT
ACCCACCCCCACTTCTTAACACACTTTTTTTTGGGGGGGGGGGGGAACA
AGTGCTCCATGCTGGAAGGATTGGAAGTATGCTTTTAGAAAGGAACAATC
CTAAGGTCACCTTTTAAATTGAGGTCTTTGATTTGAAAATCAACAATAACCA
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AATTCCAAATATTCGTTTTAATTAACCAGCAATGTGGATATAAGCATTAA
GTTTTAGTTTTAAAAGGTCAATTTTCCAAACATTCAGCAATCATATTTAA
ATTTACAGCTAGGAACAAGAGCCTTGGGTCATG

3' probe:

ATATTGCTCGCACCAACACAAAAGTTAGTATGTTGCAAAAGACATGCTAT
ATTAAACTCTCTAAAGACAGTATACATTTAAGAAATGTTCTGGACAAACA
CTTCTACATGTCAGTTTTATATATATTATAACACAAACGGTGTTAATTGAA
TAATTAAGCATTTTAAAAGCATGAAACTACAACCATTGTTTCATAAATAAG
TTCTAACGTTGTGGTTTTATAATTTGGAACCTTGGGGAAATGTTCAAACA
TTTTATGACAAACACCTTTATCCTCACATACAAAGAAAGCTACAATACTTA
AAATGGTAATTGCATAACACATTGCATGGATTATACAAGGTGTA ACTTAA
GAGTTTAATTCATACAGAACACATATGTTCTACCTACACTACATTAGAAAA
ATCCAAACTGCATAGCAACATTTAACACAGTGACATTACTGTCACTGACC
ATCATGCCTCTGCTTGCTTCTGAGAACATAAATGGCAACATCTTGGGAT.

Full details of metabolomics methods

(i) *Metabolite extraction*

Approximately 20 mg of skeletal muscle and heart tissue samples were taken from -80 °C on the day of analysis and immediately transferred to Precellys homogenization tubes containing 2.8 mm ceramics (zirconium oxide) beads (Bertin Technology, Germany). A total of 20 µl of internal standard mix was added to the samples and equilibrated for 10 min on ice. The extraction was carried out in two steps. First, 0.5 ml of acetonitrile (ACN)+1% formic acid (FA) was added and homogenization was carried out with a Precellys homogenizer (3 cycles of 30 sec, 1 min standing time, 5,500 rpm.). The samples were placed on ice to cool during the standing times. After the first round of homogenization, the samples were centrifuged at 14,000 rpm at 4 °C for 15 min in a microcentrifuge and the supernatants were transferred to Eppendorf tubes. The second round of homogenization was carried out by adding 0.5 ml of 90:10 ACN:H₂O + 1% FA and then the same procedure was followed as above. The combined supernatants were centrifuged at 14,000 rpm at 4 °C for 15 min and 800 µl of the final supernatant was transferred to an Ostro 96-well plate (Waters Corporation, Milford, USA). The extracts were filtered by applying vacuum at a delta pressure of 400-500 mbar on a Hamilton StarLine robot vacuum station. The clean extract was collected into a 96-well collection plate, which was placed under the Ostro plate. The

collection plate was then sealed and centrifuged for 10 min, 4,000 rpm, 4 °C, and placed in the auto-sampler of the liquid chromatography system for injection.

(ii) Instrumentation and analytical conditions

Sample analysis was performed on an ACQUITY ultra pressure liquid chromatography tandem mass spectrometry (UPLC-MS/MS) system (Waters Corporation, Milford, USA) and the detection system, a Xevo® TQ-S tandem triple quadrupole mass spectrometer (Waters Corporation, Milford, USA), was operated in both positive and negative polarities for measurement of metabolites. A detailed description of the analytical conditions and instrument parameters has been published elsewhere (Roman-Garcia et al., 2014).

(iii) Metabolomics data analysis

Metabolomics data analysis was carried out using a web-based comprehensive metabolomics data processing tool, MetaboAnalyst 2.0 (<http://www.metaboanalyst.ca>, Xia et al., 2009, 2012). The non-transformed data was autoscaled i.e., mean-centered and divided by standard deviation. Dendrograms were plotted using Ward's linkage clustering algorithm and Pearson's correlation similarity measure. In order to explain the separation among groups, unsupervised multivariate regression technique, principal component analysis was performed.

Respirometry on tissues from *AOX^{Rosa26}* founder mouse

Mouse tissues were processed, mitochondrial pellets resuspended and mitochondrial protein assayed as described in Materials and Methods. Oxygen consumption was measured with a Hansatech Oxytherm unit. For each assay, mitochondria equivalent to 75 mg of protein were transferred into respiration buffer (225 mM sucrose, 75 mM mannitol, 10 mM Tris/HCl, 10 mM KCl, 10 mM KH₂PO₄, 5 mM MgCl₂, 1 mg/ml BSA), which was pre-equilibrated at 37 °C. Oxygen consumption was measured by the successive addition, to final concentrations, of complex II substrate mix (10 mM sodium succinate + 1 mM ADP in the presence of 150 nM rotenone), cIII inhibitor (30 ng/ml antimycin A) and AOX inhibitor (200 μM n-propyl-gallate).

Respirometric titration of antimycin inhibition

Heart mitochondria were prepared as for ROS measurement. Oxygen consumption of aliquots containing 100 μg mitochondrial protein was measured using an O2k oxygraph (Oroboros) in 5 mM MgCl₂, 120 mM mannitol, 20 mM MOPS, 5 mM

KH₂PO₄, 60 mM KCl, pH 7.4, using a multiple substrate inhibitor protocol as described previously (Gizatullina et al., 2011), using increasing amounts of antimycin A.

Submitochondrial fractionation

To analyze membrane association of AOX, 500 µg of mouse liver mitochondria were suspended in 500 µl 10 mM Hepes/KOH, pH 7.4, and an equal volume of 200 mM sodium carbonate solution was added (final pH adjusted to 9.5 with HCl). After thorough mixing, samples were incubated on ice for 30 min and then centrifuged at 230,000 *g*_{max} in a Beckman TLA120.2 rotor for 1 h at 4 °C. The pellet containing the membrane fraction was dissolved in 60 µl SDS-PAGE sample buffer. The supernatant fraction was precipitated on ice for 30 min by the addition of 150 µl of 100% (w/v) trichloroacetic acid, pelleted at 20,000 *g*_{max} for 30 min at 4 °C, washed twice with ice-cold acetone, air dried and resuspended in 60 µl SDS-PAGE sample buffer. One-third of each fraction was electrophoresed on SDS-12% polyacrylamide gels, and processed for Western blotting and probing with AOX and OXPHOS subunit antibodies as described in Materials and Methods.

Western blotting of mitochondrial protein extracts from brain

To study the expression of AOX in brains of newborn or young mice, and according to gene dosage, a slightly variant procedure was used for protein extraction.

Washed tissue was snap-frozen in liquid nitrogen, then placed into 400 µl of RIPA buffer (Sigma) containing PierceTM protease inhibitor (ThermoFisher Scientific) in Eppendorf tubes and crushed on ice with a chilled mortar and pestle. Frozen tissue pieces were transferred to a fresh tube and a further 450 µl of the same buffer solution added. The tissue was further disaggregated using scissors and a plastic grinding rod for use with Eppendorf tubes, left on ice for 30 min, then centrifuged at 14,000 *g*_{max} for 5 min at 4 °C. The supernatants were collected, their protein concentration determined by the Bradford method, and 20 µg aliquots used for SDS-PAGE and Western blotting via standard methods. Blots were probed for AOX using the customized antibody (1:10,000) and were stripped and reprobed for GAPDH (rabbit antibody, Cell Signaling 2118, 1:1,500), in both cases detected using anti-rabbit secondary antibody (see main Materials and Methods).

SUPPLEMENTARY REFERENCES

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Xia, J., Mandal, R., Sinelnikov, I., Broadhurst, D. and Wishart, D.S. (2012) MetaboAnalyst 2.0 – a comprehensive server for metabolomics data analysis. *Nucleic Acids Res.* **40**, W127-133.

Xia, J., Psychogios, N., Young, N. and Wishart, D. (2009). MetabSoAnalyst: a web server for metabolomics data analysis and interpretation. *Nucleic Acids Res.* **37**, W652-660.

SUPPLEMENTARY FIGURES

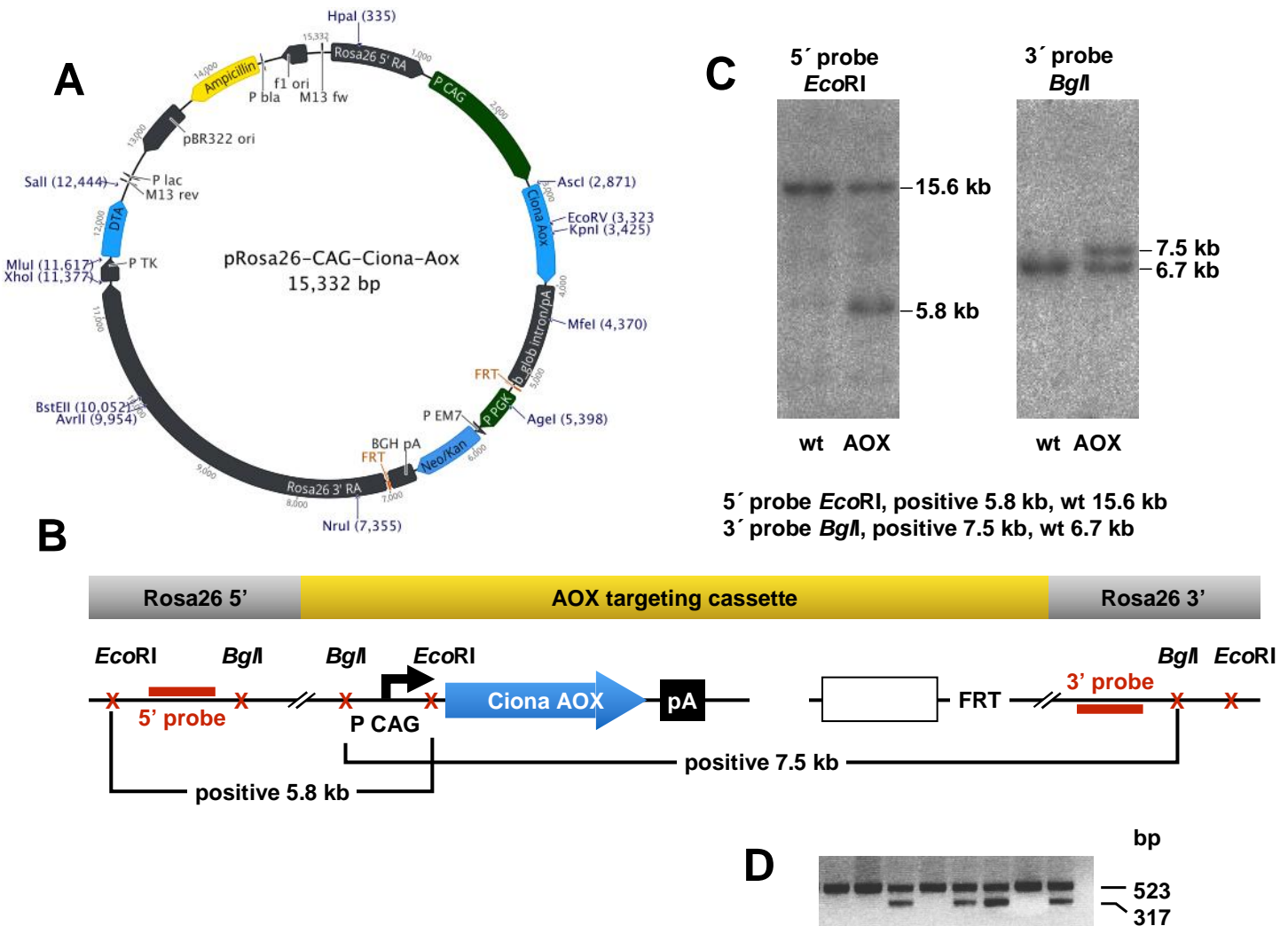
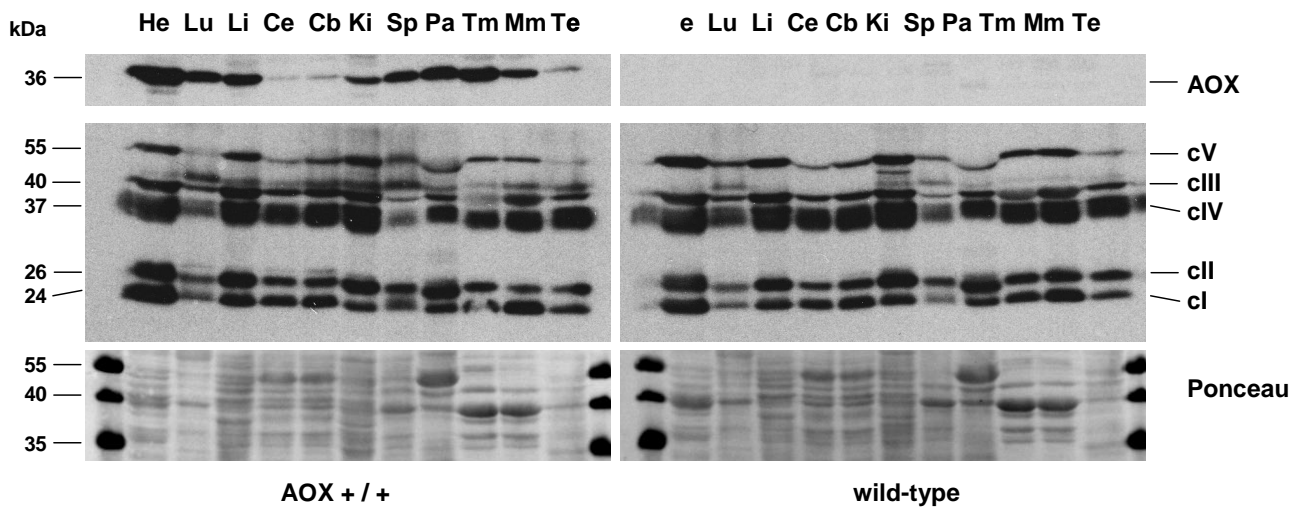


Figure S1

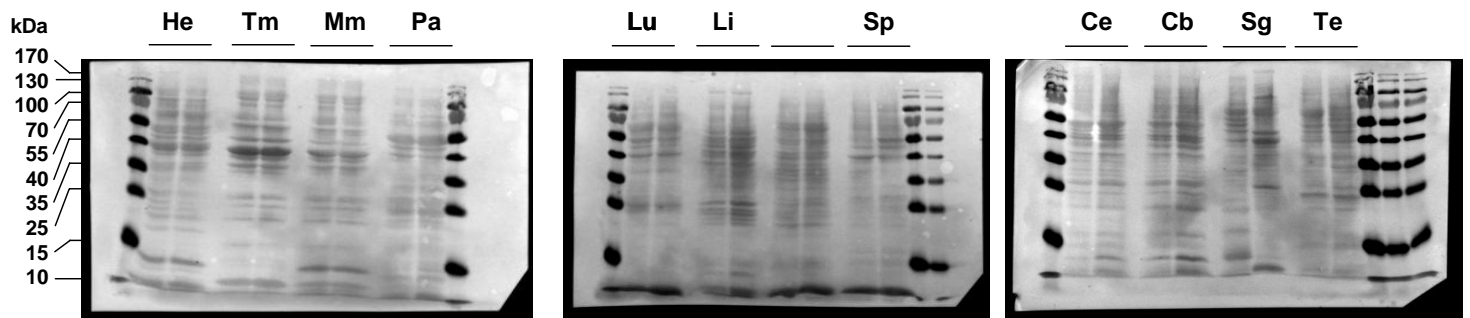
Supplementary Information on Rosa26-AOX construct and expression

(A) Full map of Rosa26-AOX targeting construct. (B) Schematic map of mouse *Rosa26* locus, showing relevant restriction sites for verifying insertion of AOX targeting cassette (other non-relevant sites in insert omitted for clarity). Note that the FRT-flanked PGK-neo selection cassette was excised *in vivo*, following first germline transmission of the modified allele. (C) Southern blot of DNA from selected ES cells positive for targeted AOX insertion, alongside parental (wt) cells, digested and probed as indicated. Fragment sizes, estimated from markers, correspond with those predicted for the insertion as shown in (B). (D) Sample multiplex PCR for genotyping of mice according to the scheme described in Materials and Methods: 523 bp product is from the wild-type *Rosa26* allele, 317 bp product is from AOX.

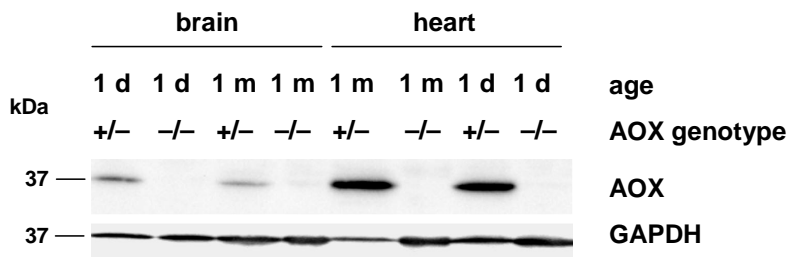
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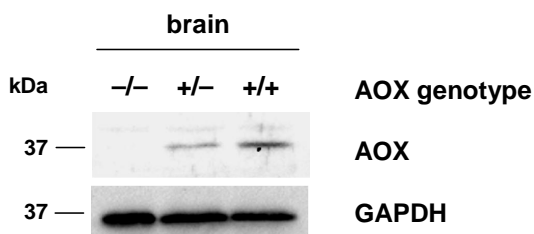
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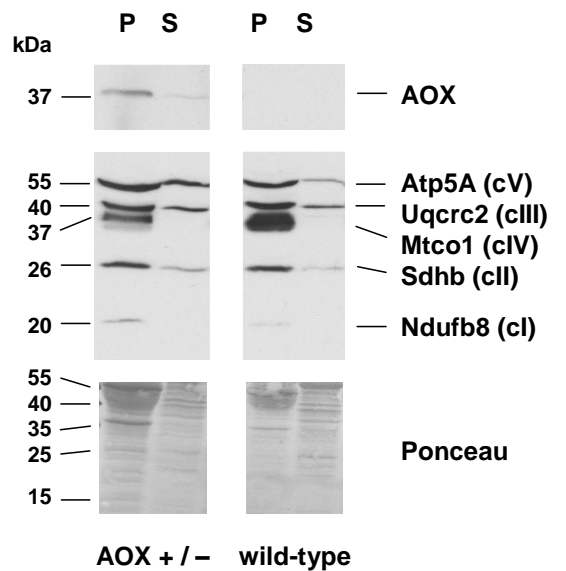
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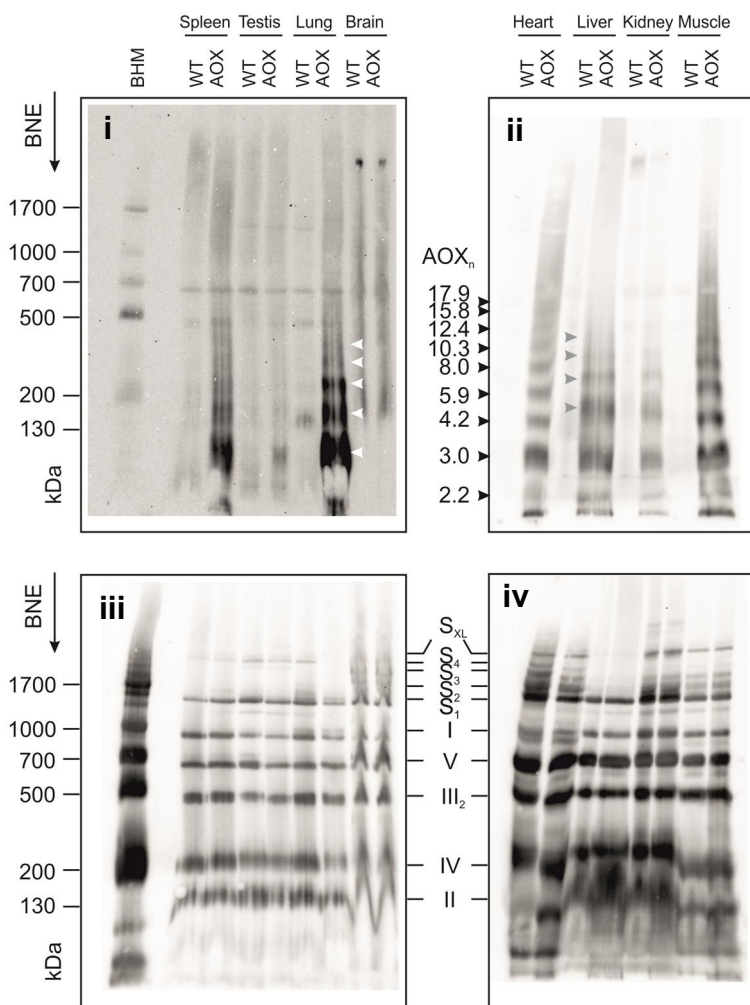
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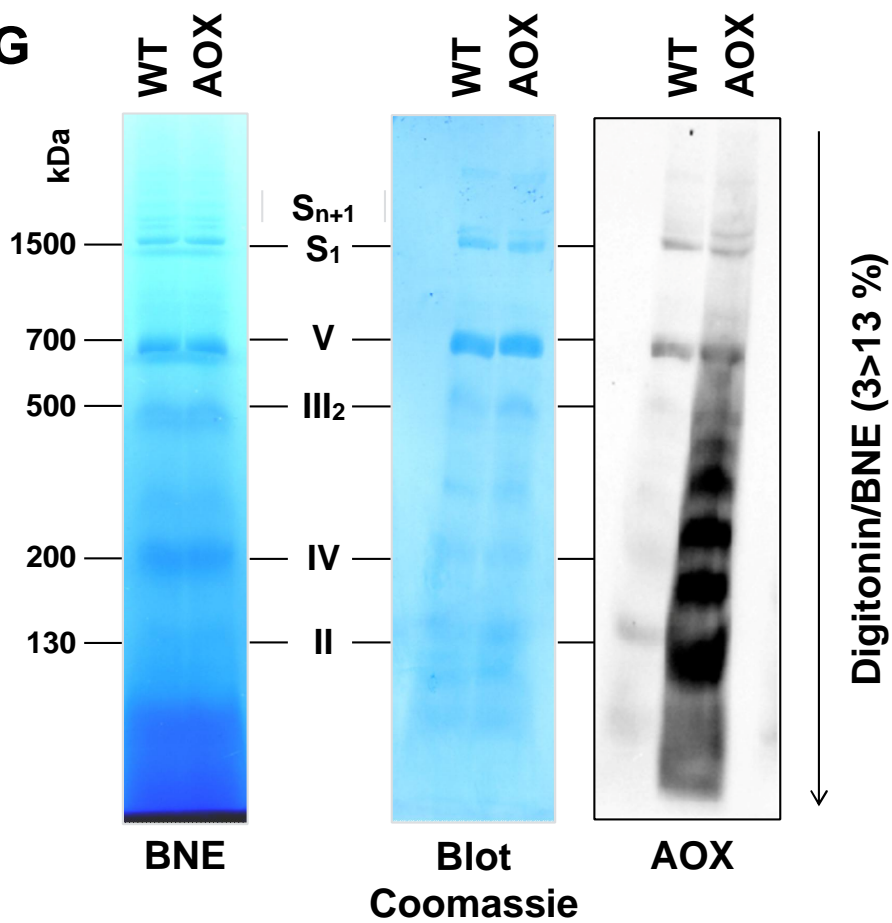
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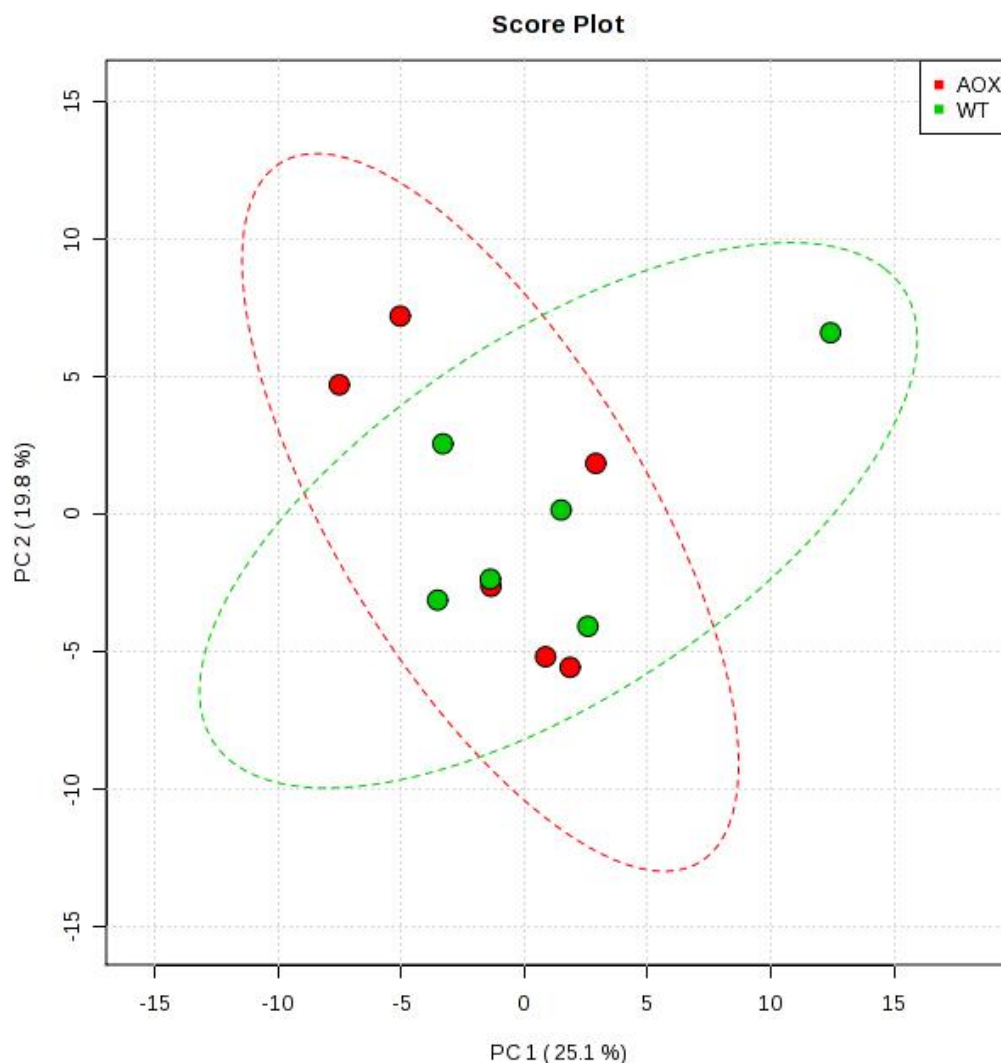
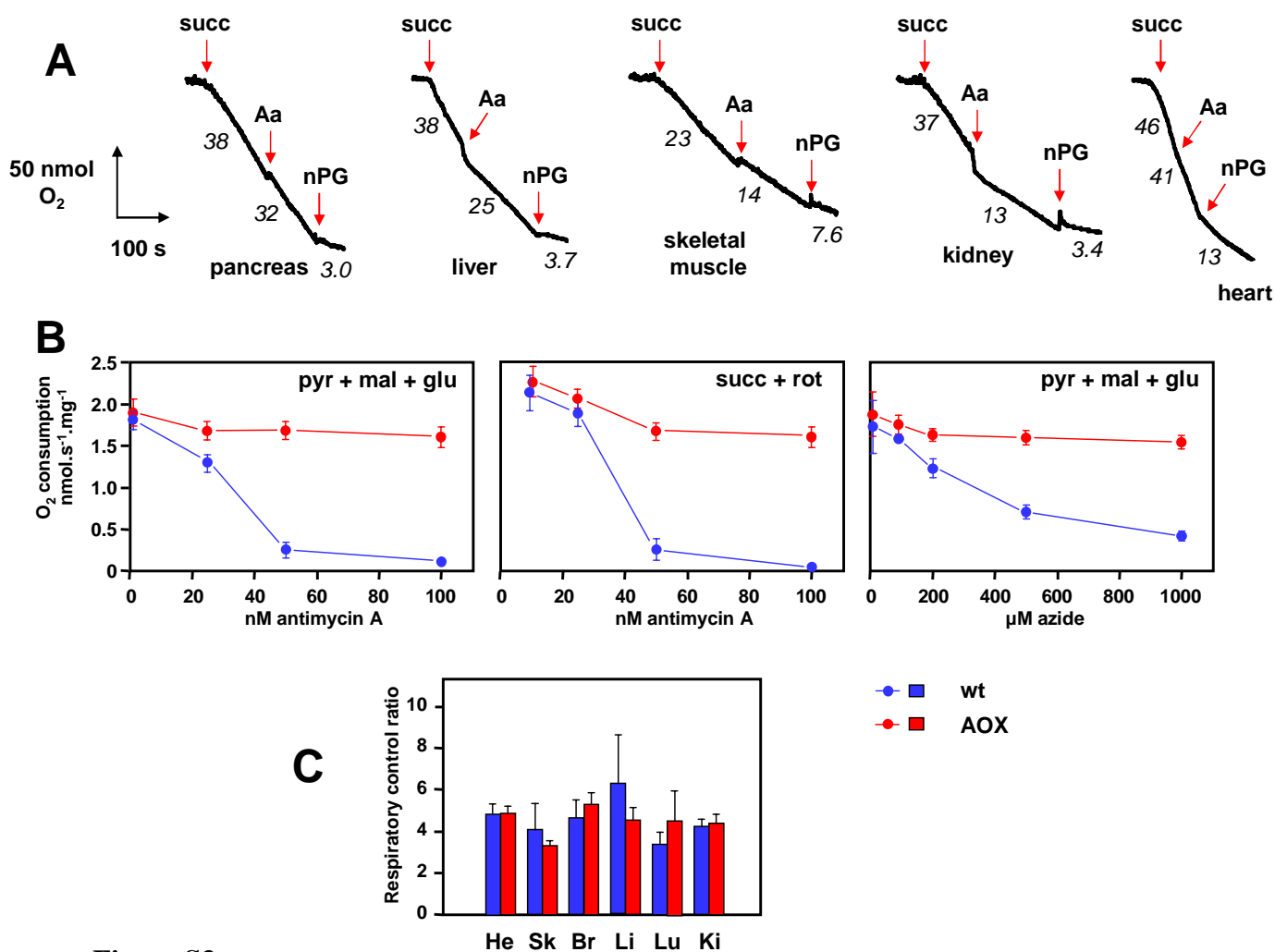


Figure S2

Supplementary data on protein expression and biochemistry of AOX^{Rosa26} mice (A) Western blots of 20 μ g total protein extracts from the indicated tissues (He - heart, Lu - lung, Li - liver, Ce - cerebrum, Cb - cerebellum, Ki - kidney, Sp - spleen, Pa - pancreas, Tm - thigh muscle, Mm - masseter muscle, Te - testis) of 24 week-old male hemizygous AOX^{Rosa26} (+) and wild-type littermate control (-) mice, probed for AOX and for representative subunits of the five OXPHOS complexes (see Materials and Methods; protein molecular weights extrapolated from markers). Ponceau S staining of the membranes is shown below, including actual molecular weights of markers. (B) Ponceau S staining of the blot membranes from the experiment shown in Fig. 2A. Molecular weights of markers as indicated. (C, D) Western blots of 20 μ g aliquots of total protein extracts brain and heart of one-day (1d) and one-month (1m) old hemizygous (+/-) and homozygous (+/+) AOX^{Rosa26} and wild-type littermate control (-/-) mice, probed for AOX and for GAPDH as a loading control. Protein molecular weights extrapolated from markers. (E) Western blot of carbonate-extracted proteins from liver mitochondria of hemizygous AOX^{Rosa26} (+/-) and wild-type littermate control mice, probed for AOX and representative subunits of the OXPHOS complexes as indicated. Ponceau S stained membranes are shown below. The cropped images are from nonadjacent tracks of the same gel. AOX co-fractionates with the

more tightly membrane-associated pellet (P) than supernatant (S) fraction. Protein molecular weights extrapolated from markers and as shown for Ponceau S-stained image. (F) Complexes of OXPHOS and AOX in mouse tissues, as indicated. Mitochondrial complexes from mouse tissue were isolated by BNE (3 to 13% acrylamide gradient gels) and PVDF blots were probed with an antibody against (panels i and ii), AOX and (panels iii and iv), an antibody cocktail against rodent OXPHOS complexes (Mitosciences) and complex IV (rabbit antiserum). Assignment of mitochondrial complexes I, cI; III₂, dimeric cIII; IV, cIV; V, cV; S₀₋₁, respiratory supercomplexes containing cI, dimeric cIII and 0 or 1 copy of cIV; S_{XL}, larger supercomplexes; wt, wild-type; AOX, male hemizygous *AOX^{Rosa26}* mouse. Black arrows indicate AOX oligomers of heart and muscle tissue, grey arrows indicate AOX oligomers of liver and kidney, white arrows are the markers for lung. Bovine heart mitochondria (BHM) as molecular weight ladder. Note: AOX appears in high molecular complexes (panels i and ii). When reprobed for OXPHOS complexes (panels iii and iv), AOX-containing tissues exhibit a similar migration pattern as controls, which also differs from the ladder of AOX multimers. Thus, it can be inferred that AOX does not form heteromers with endogenous complexes. Furthermore, the molecular weights of each OXPHOS complex from mouse were used for exponential regression. Apparent molecular weights of AOX complexes were divided by that of the AOX monomer to infer the stoichiometry of AOX homo-oligomers. AOX oligomers from tissues other than heart and muscle seem to have a slightly decreased mobility, which may reflect different solubilization behaviour determined by lipid/protein content rather than additional or altered polypeptides. (G) Western blot of BNE gel of extracts from heart mitochondria of hemizygous *AOX^{Rosa26}* (AOX) and wild-type littermate control (WT) mice, probed for AOX, alongside Commassie Blue stained blot (CB); protein molecular weights extrapolated from bovine heart mitochondrial extract, used as marker. Complexes and supercomplexes denoted as in part (F). Note the faint bands in the control track, which are non-specific cross-reaction to complexes with high protein content. (H) Principal component analysis of metabolome data from heart of hemizygous *AOX^{Rosa26}* (red circles) and wild-type littermate control mice (green circles). The two sets of analyzed data largely overlap. Both for heart (here) and skeletal muscle (Fig. 2C), none of 100 individual metabolites analysed showed a significant difference between *AOX^{Rosa26}* and wild-type samples, nor was any significant alteration seen in any specific metabolic pathway.

**Figure S3****Supplementary data on functionality of AOX in AOX^{Rosa26} mice**

(A) Oxygraph traces for mitochondria isolated from indicated tissues of hemizygous AOX^{Rosa26} founder mouse (i.e. in the generation after removal of neomycin-resistance cassette), according to the scale arrows shown left. Red arrows indicate times at which substrate mix (succinate), and inhibitors (Aa – antimycin A, nPG – n-propyl gallate) were added. Rates of oxygen consumption estimated from slope (2 significant figures, nmol O₂/min), shown in italics. (B) Respirometry of isolated heart mitochondria from wild-type and hemizygous AOX^{Rosa26} mice as indicated, using the substrates and inhibitors shown (pyr + mal + glu – pyruvate, malate and glutamate mix, succ + rot – succinate plus rotenone). Means + SD for at least three biological replicates in each case. (C) Respiratory control ratios (means + SD) for the mitochondrial preparations analysed in Fig. 3A, computed from the raw data of the same experiment. RCR is here defined as the ratio between (oxygen consumption in the presence of the cI-linked substrate mix and ADP, minus the residual value after addition of rotenone, i.e. state 3) and the oxygen consumption in the presence of the cI-linked substrate mix alone (i.e. state 2). RCR values for AOX^{Rosa26} and control mouse mitochondria showed no significant difference for any tissue tested (Student's *t* test, $p > 0.05$).

Units: μ moles	Metabolite name	Comments	AOX						WT					
			1_MS 213	2_MS 214	3_MS 216	4_MS 223	5_MS 240	6_MS 250	7_MS 215	8_MS 224	9_MS 230	10_MS 241	11_MS 267	12_MS 234
	L-Kynurenine		0.32477145	0.235384813	0.378403433	0.333710114	0.241343922	0.25326214	0.294975904	0.29199635	0.309873677	0.381382987	0.286037241	0.36350566
	Octanoylcarnitine		0.304376953	0.38424892	0.654086644	0.58932559	0.38424892	0.356185796	0.567738572	0.684308469	0.248250706	0.310853059	0.371296709	0.453327377
	Cystathionine		0.301530037	0.245691141	0.329449484	0.351785043	0.318281705	0.184268356	0.337825319	0.382496435	0.209395859	0.435543386	0.178684466	0.234523362
	Hexanoylcarnitine		0.29662972	0.528670711	0.641102944	0.586082915	0.418630654	0.339688873	0.543023762	0.590867266	0.349257574	0.43537588	0.387532377	0.385140201
	Palmitoyl Carnitine		0.157378994	0.19860159	0.163659187	0.185320807	0.133532892	0.176865447	0.220555095	0.379187023	0.192770093	0.503697863	0.09010563	0.19522162
	Folic Acid	interfering peak and folic acid could be under that; integrated as a shoulder, unreliable values	0.120868746	0.299360965	0.158815911	0.244548394	0.189735823	0.12930145	0.198168526	0.122274197	0.243142943	0.112436043	0.167248614	0.18130312
	Taurochenodeoxycholic Acid		0.094372735	0.064570818	0.086922256	0.052153353	0.04966986	0.068296058	0.110515439	0.055878593	0.08195527	0.093130988	0.151493074	0.054636846
	Decanoylcarnitine		0.086536976	0.078669978	0.151439708	0.151439708	0.165206954	0.194708196	0.155373207	0.237976684	0.084570227	0.098337473	0.114071468	0.241910183
	Normetanephrine		0.077885389	0.098203317	0.094816996	0.067726425	0.067726425	0.060953783	0.08127171	0.064340104	0.077885389	0.104975959	0.067726425	0.067726425
	Isovaleryl Carnitine		0.073339051	0.025289328	0.080925849	0.030347193	0.025289328	0.01770253	0.053107589	0.030347193	0.032876126	0.09104158	0.06322332	0.048049723
	2-deoxyuridine	ND and minor values	0.070678624	0.127765205	0.372421981		0.106017936				0.065241807	0.067960216		
	Isobutyryl Carnitine		0.059010051	0.053645501	0.061692326	0.034869576	0.037551851	0.0321873	0.037551851	0.050963226	0.061692326	0.050963226	0.059010051	0.029505025
	Myristoyl Carnitine		0.030490416	0.038952926	0.04585084	0.044296263	0.033713116	0.039542363	0.043139096	0.068675237	0.036526717	0.073692965	0.027017247	0.047076468
	Cotinine	ND	0.024645794	0.035208277	1.922371938	0.056333244	1.707601447	0.014083311	0.028166622	1.545643372	0.007041655	0.014083311	0.014083311	0.024645794
	Arachidyl Carnitine	RT shift	0.02094588	0.043063963	0.017932679	0.020750029	0.017696207	0.032264605	0.022376316	0.035744946	0.028254741	0.03649063	0.012701287	0.023657325
	Dodecanoyl Carnitine		0.010253494	0.01032574	0.017044605	0.018196927	0.014198118	0.015124672	0.014983792	0.02619635	0.011965721	0.02198261	0.011745371	0.019251717
	4-Pyridoxic Acid		0.010160983	0.003386994	0.003386994	0.003386994	0.003386994	0.003386994	0.020321965	0.003386994		0.013547977	0.010160983	0.003386994
	5-Deoxy-5Methylthio Adenosine	<LLOQ but quantify since the resp. is good	0.003403755	0.002959242	0.004455559	0.002130738	0.002817332	0.004215564	0.002750551	0.003355756	0.006857595	0.006396388	0.004904245	0.003652097
	Kynurenic Acid	mostly ND	0.003279684		0.009839053			0.003279684	0.019678106	0.006559369		0.009839053	0.013118737	0.003279684
	Stearyl Carnitine		0.001293376	0.00529331	0.001311075	0.000841375	0.001036062	0.001813449	0.001556135	0.00279233	0.002396149	0.003263391	0.000352615	0.001611955
	Orotic acid	minor concentrations		0.278174627	0.484818636				0.290096397			0.413288017		0.544427484
	Homogentisic acid	ND												
	D-Glucuronic acid	ND												
	Gamma-Glutamylcysteine			0.441329938					0.10413403		0.10413403		0.10413403	
	cGMP	ND												
	Spermidine	ND												
	Adenine	ND												
	Pyridoxine	ND												
	5-Hydroxyindole-3-acetic acid	ND												
	L-5-Hydroxytryptophan	ND												
	Nicotinic Acid	ND												

ULOQ = Upper limit of quantification
 LLOQ=Lower limit of quantification
 ND=Not detected
 RT=Retention time

TABLE S2. Heart		AOX						WT					
Units: μmoles													
Metabolite name	Comments	1_MS 213	2_MS 214	3_MS 216	4_MS 223	5_MS 240	6_MS 250	7_MS 215	8_MS 224	9_MS 230	10_MS 241	11_MS 267	12_MS 234
Glutamine		75825.93431	57284.67332	65654.56736	42218.23423	59416.85686	79586.1489	66430.15259	64585.53283	61683.40254	52893.54611	90361.74882	60735.42981
Taurine		46296.51904	43392.96665	41854.48966	35973.24357	42462.36753	54187.79352	46113.82002	47806.2438	38373.14472	39624.51008	44551.98417	44331.82618
Alanine		24567.58807	27069.97198	20811.47156	20861.28577	22502.20715	24372.24544	20971.55131	25721.3872	24477.63551	22200.05666	21287.83559	23473.76889
Cysteine	>ULOQ	5874.963696	5875.961221	6002.108476	5991.638436	5728.41675	5988.856679	5655.458247	5859.386971	5532.170366	5287.459678	6732.340556	6009.395924
Hydroxyproline		4620.287322	4989.901781	3788.642962	4050.210353	4202.529828	4463.087209	3811.11403	4625.109015	4337.73767	4678.069485	3979.726534	4195.477695
Creatine		4370.923915	4328.274441	4286.581438	3435.448146	4292.294713	4975.578898	4375.430959	4723.199993	3860.274024	4410.217229	4509.05292	4529.253734
Acetoacetic acid		1651.305502	2206.819748	1678.984582	1697.745423	1588.034224	2128.072365	1758.054386	1884.055643	1571.903189	1567.134865	2525.569262	1874.24549
Hypoxanthine	dil. also >ULOQ	1284.848284	1592.444839	1535.00215	966.9480583	1186.214157	1639.452601	1471.149747	1513.469175	1185.541465	1084.950789	1360.703862	1219.408492
Succinate		1153.082665	1215.158566	995.6500437	731.5437872	847.0907209	671.7676728	844.6941732	861.5760661	1065.436848	1057.552541	816.2904974	1073.775596
Adenosine		976.1713865	1113.865294	1555.140532	1078.772077	1199.811435	1250.61004	1217.40679	1602.78664	966.2347846	489.3879181	343.2852593	629.4300087
Myoinositol		788.9179869	706.5085342	685.4700771	669.810086	653.0190621	612.9110224	522.5719443	587.2085699	437.8337995	530.1094889	668.8234279	848.3347072
Glycine		672.8043603	741.9611236	459.8442068	530.1305411	404.0357992	571.9074793	410.9461789	455.7965087	618.2739296	621.204782	570.5963672	374.8279202
Inosine		647.6025473	727.9636384	798.91097	493.5438191	640.8516716	793.2112328	651.6135248	770.2221771	550.9470765	490.5652468	607.292951	
Lysine		547.4171703	543.4053578	396.6758383	413.9880783	551.0362462	566.9165568	406.508471	397.5211876	381.8066708	675.6512748	452.4725382	455.4991779
Carnitine		537.2615547	466.3481948	540.9070449	448.359038	369.0248795	357.2537643	355.9039319	420.6286521	417.1753904	508.4479163	389.6827597	404.8321695
Aspartate		531.1613103	418.9412571	387.9103837	350.4216773	333.7507094	503.6340805	260.2133416	505.0755236	313.865505	445.9525872	234.0940005	257.5569479
L-Glutamic Acid		525.9784757	421.0478929	566.9013904	399.128065	428.3346862	584.8432691	585.3479438	522.3825109	303.0526878	440.0560391	454.76117	564.5171506
Serine		525.3274712	562.2104214	452.1858052	392.6647376	385.5435312	344.591902	402.7489325	576.1365263	413.6453522	531.053594	265.1944347	445.5565021
Threonine		418.250486	473.260337	326.2805701	287.3948106	311.7698251	358.4567328	336.5689413	342.1699579	379.2305804	376.8636515	258.9507322	421.3926087
AMP		351.8229931	339.3245464	283.3118147	416.4977658	406.9842105	580.2210808	266.2025854	348.7560515	365.5309346	442.0819443	620.4910865	396.0622248
Asparagine		337.6734824	339.7248511	244.0262888	181.6746739	158.6636816	219.4971936	239.6091699	209.5636528	198.0195163	271.3284314	319.6446134	203.0405617
Citrulline		314.3037983	308.3282108	282.5626991	281.8029237	275.632019	280.3683195	225.4760414	257.0914733	211.2307746	273.6011592	318.5968109	278.9231634
Acetylcarnitine		288.2094793	358.3018654	258.6582854	251.2419084	273.5726618	232.1987315	241.2679473	345.9307876	276.2099146	231.5016748	172.9543744	247.0849339
Arginine		254.1208818	276.3308118	211.0840169	212.7028882	289.3218783	314.6105552	195.8941812	230.8830529	217.4539561	345.2914816	328.4077101	259.4007482
Niacinamide		246.2204628	287.4428794	271.0767464	189.8526295	224.3070047	328.837507	255.0458913	249.6738251	203.3582325	229.1685341	310.3743632	281.5587525
Proline		229.3821493	165.0077959	132.9471912	114.2604388	107.6201218	114.1632332	133.4597837	121.1851522	172.7603734	191.7037723	142.9034907	113.8181641
Histidine		228.6186102	211.624947	173.3164373	152.8977093	110.9988489	196.6107165	199.5983774	161.4222085	201.7843976	222.5904721	182.424475	158.3732151
Leucine		208.7907305	174.0459463	164.1421405	134.5559107	104.072323	126.8752104	106.3182242	116.0024909	153.1714276	219.5434176	134.1475736	144.5154762
IMP		205.5363951	155.1607288	130.2625462	138.6116406	136.9816887	195.9089909	227.968808	161.7468032	126.8214773	188.7543039	336.2072309	227.4279846
Tyrosine		193.5359883	138.1371488	134.9390833	131.6580538	133.7546475	132.6862517	109.9026067	106.9781885	129.0214927	160.4715628	201.7823894	122.1141558
Valine		186.3127898	175.3715584	114.7767799	118.056009	123.5250625	175.7923974	119.8185867	128.9276566	139.1704552	162.7437407	99.61868561	136.5367031
Glutathione		158.9653822	2715.967117	387.7132145	571.5241814	108.5039468	365.9580012	1349.571445	1497.926898	681.7792815	227.3007595	80.95737701	240.8464854
Creatinine		156.8824003	168.3715943	143.0888307	120.16402	91.45427843	102.1151344	86.07767628	113.5419769	108.5603776	209.5308098	102.2783343	110.2349311
L-Methionine		152.380112	134.6859656	112.5379172	89.40095399	94.30497158	82.32035874	107.8630131	101.360193	127.209247	128.085061	104.7158646	100.0594709
Glyceraldehyde		142.8482612	101.3387919	107.9989665	87.69291943	66.79635803	97.22950117	91.51049953	87.64133981	116.7051785	141.8151539	141.6523579	104.302175
Choline		125.6293493	165.4958581	179.1527368	155.5390572	129.8904241	133.3457359	135.8166233	170.5180305	99.14815389	114.7971075	152.2248879	83.74753966
D-Ribose 5-phosphate	bad chromatography and unreliable values	123.2469783	145.7364685	87.06832714	125.3497354	129.8990514	156.7489081	90.43009158	125.2473092	127.3225729	141.9741904	223.2039785	108.8415612
Betaine		111.8521951	74.66044951	21.92286798	108.3474805	64.06565435	65.72004356	30.05506394	78.11141401	138.6307836	72.78512909	77.4965453	79.35153073
Isoleucine		92.16972762	91.47762607	60.30699731	68.62361377	70.04896272	89.33099512	55.58648071	69.21592482	74.92659025	90.05289194	52.63272902	79.10573152
Phenylalanine		91.51982216	77.37004116	69.75215906	63.00787092	61.63389465	72.65762893	62.23346705	64.30196179	76.53160128	78.55547738	108.1306518	70.49306138
Xanthine		72.63953891	108.5617392	81.45167571	71.09049147	80.3202614	99.76791289	116.8543324	83.47108549	132.2423663	71.92722585	96.02055158	97.47360173
Phosphoethanolamine		63.9958708	66.46537902	38.6998217	41.89348862	47.49023162	49.58159104	40.18990649	56.04340275	46.00718134	46.38112737	33.81404769	44.43980488
Pantothenic Acid		63.93577945	74.93029732	53.39332171	42.48493747	51.71753569	55.4821474	52.72159815	55.87393662	65.78957688	64.65832999	44.57367827	51.49108118
Xanthosine		61.51707796	78.77594198	74.17473788	53.04753715	61.67378268	75.89918819	47.38246516	92.33993572	57.04771972	42.16926148	38.69674604	44.8892669
Uracil		51.33324184	64.59452486	48.58249565	45.24233942	49.61526378	46.27389	45.25069631	49.65084976	48.76955724	48.41491503	29.22488653	31.88758095
deoxycytidine		47.33793415	39.23790384	33.27995156	55.66781897	39.71664227	59.98681222	42.23616876	38.92275826	35.2739715	31.90977952	68.98414605	55.52650445
Ornithine		46.80701717	48.96169407	21.6722805	25.22964478	19.97105204	28.59043381	17.69740503	23.98729671	33.05900042	37.79258705	32.04570852	27.44515299
Adenine		38.58291745	44.8486803	49.66780926	31.8882877	30.79316675	28.55609931	32.38316943	42.46016436	42.65490353	27.63620689	19.41951741	30.04611568

Units: μmoles	Metabolite name	Comments	AOX						WT					
			1_MS 213	2_MS 214	3_MS 216	4_MS 223	5_MS 240	6_MS 250	7_MS 215	8_MS 224	9_MS 230	10_MS 241	11_MS 267	12_MS 234
	Allantoin		38.12664412	46.75217041	37.93806751	48.4581081	38.5387509	27.89105724	30.30822728	59.09411334	41.60791662	38.11530677	11.86977365	39.00126769
	S-5-Adenosyl-L-Methionine		35.94597564	25.75399971	28.67269638	34.34541638	29.69579041	86.80537191	45.6922704	25.09975791	19.93774698	16.96891336	142.5954194	28.7080122
	Aminodipic Acid		34.31323466	36.42749389	33.88838889	27.30006522	29.17786276	28.429138	26.78464573	30.28387831	22.09136441	28.30711561	29.25665766	39.81309552
	Guanosine		32.9196611	52.32145735	41.83933844	30.05984612	31.54959882	39.1659889	26.42081439	51.29407991	27.27869974	23.9225198	19.3246743	20.60766711
	GABA		31.56182841	29.41138949	17.27906876	17.0726093	10.16193228	10.21986365	23.09638893	18.00414332	15.36877733	18.13727117	7.497690837	42.70684942
	Tryptophan		31.2465125	33.89274668	25.00215567	25.71833525	27.23119815	27.87796224	22.65089938	23.22800493	24.06615529	30.54476284	32.65970266	26.7084706
	Homoserine		20.41416318	20.28126688	16.10466736	17.31495063	16.29078467	20.22846247	11.42309315	19.13139101	23.93013477	18.13565819	24.46697958	19.08030509
	3-Hydroxyanthranilic acid		18.89695971	16.11150366	17.15317842	17.58572076	14.7142991	12.10519004	0.33328893	14.68440154	15.98859146	10.67257835	15.4997785	9.207233278
	Homocysteine		13.93165052	15.02917605	13.47241982	10.25284629	11.27608316	11.96653618	14.82981796	12.87361092	12.84468517	12.66511596	9.547654911	11.9450944
	Taurocholic Acid		11.71288292	11.78228361	10.22875729	8.447428728	8.371193848	7.215434081	8.204911328	9.777315891	9.73395249	9.271405537	5.91820182	7.907359468
	Carnosine		11.2949688	11.97423429	8.004375529	11.13814983	8.745200488	11.21320028	11.72391075	9.890645245	6.208676375	11.63432734	14.63733234	12.34444112
	Trimethylamine-N-Oxide		8.050259435	4.663519395	6.524549813	3.419281023	2.894165353	6.790246119	4.085958231	6.277519148	1.879778649	4.802850967	3.346600653	3.229320966
	S-Adenosyl-L-Homocysteine		5.664102371	5.238406953	6.315154557	6.463084279	5.484214017	7.516282569	5.433241615	5.981380554	3.922486122	5.47399694	7.634400377	5.493559496
	Guanidinoacetic Acid		5.345862043	5.931148341	3.777612564	4.538378818	4.497647129	5.472241509	3.776818058	3.823535028	4.623073189	8.37256023	7.489598909	3.935984151
	Asymmetric dimethylarginine		5.3197634	7.591315009	5.526519039	5.868228663	4.277182524	6.465522732	6.201534335	6.442856597	5.090679011	5.360096239	5.348594479	4.477803891
	Nicotinic Acid		5.294177545	6.093970913	7.820448184	7.41316887	6.548569613	6.736788895	6.813941163	7.765418611	6.053908174	3.115419669	2.911729619	4.188849102
	Hexanoylcarnitine		4.983833815	6.550134426	5.344454221	4.441240645	4.824132201	4.451526998	3.708302096	5.661991553	4.612855291	5.493343203	2.239171641	6.055552211
	cGMP		4.611698825	4.221979063	6.300202954	4.14597212	6.156867874	0.180529967	4.863671725	4.96916505	4.698594707	4.290618903	13.08153166	5.685678732
	DimethylGlycine		3.688237054	4.829418866	2.559387465	2.798692749	1.542249769	2.056593707	2.914675803	3.411874943	2.850728715	2.006783557	1.985548071	2.909321814
	Propionylcarnitine		3.230358073	2.172627176	2.438066434	2.845448306	1.775410576	2.537948805	1.902533594	2.109551088	1.75873496	2.181164863	2.63620359	2.754817476
	Sorbitol	bad chromatography and unreliable values	2.114916675	2.740163817	4.784832978	1.828546538	8.040292787	47.8859563	5.028468927	5.488363705	15.7307441	13.67602985	11.95256515	
	Cytidine		1.961647852	1.974298544	2.339459734	1.713531064	1.594318701	1.599572819	1.828356494	1.885590671	1.521143632	1.772703653	1.349160542	1.412873097
	Symmetric dimethylarginine		1.743544142	2.248241375	1.477899492	1.5448244	1.152077632	2.166931599	1.857396232	1.510717847	1.114842601	1.516238692	1.112542249	1.436769198
	D-Glucuronic acid		1.329703561			2.831499187					1.827064327	1.718747932		
	1-methylhistamine		1.036643271	0.941880318	0.665174476	0.930233198	0.879332804	0.803205243	0.771782799	0.953031816	0.743334088	1.230976713	0.61377846	0.784222915
	Glycocholic Acid		0.932446936	0.705064728	0.611140486	0.665690897	0.438788369	0.704611695	0.57722969	0.501693227	0.561666701	0.53044745	0.836164262	0.715271277
	Sucrose	bad chromatography and unreliable values	0.904602559	0.82310569	0.856360472	0.740920166	0.723540692	0.64470783	0.691989425	0.878379306	0.648350452	0.707357302	0.692659957	0.927944335
	NAD		0.866558828	1.432573299	1.450367153	3.198132531	1.926824204	4.049110068	1.538832296	0.226148123	1.079599621	1.727516234	4.709788589	2.320594914
	Cytosine		0.808198542	0.845104879	0.785362396	0.956996408	0.829694669	0.80462516	0.591227244	1.043874262	0.890498	0.789549954	0.530033075	0.77709895
	2-deoxyuridine		0.712087139	0.827864162	1.162826472	0.729512138	0.574046349	0.882286703	0.743212917	0.850997819	0.457644092	1.10266809	0.771022238	0.77822602
	3-OH-DL-KYNURENINE		0.636491357	0.684911626	0.47797723	0.667203642	0.640032954	0.693654944	0.521140441	0.808148129	0.569920404	0.640171298	0.549473216	0.528527991
	L-5-Hydroxytryptophan		0.631564762	0.865158366	1.067510375	1.09638561	1.173602212	0.743403475	1.012689686	1.33454291	1.218027817	0.865580931	1.179067379	0.890850282
	Taurochenodeoxycholic Acid		0.62795121	0.718635957	0.780065157	0.57695268	0.479537667	1.392792554	0.706392337	0.652997237	0.657504777	0.375019863	0.451374856	0.340933922
	2-Aminoisobutyric acid		0.579073069	0.656916468	0.494672538	0.618235397	0.519457298	0.643982673	0.514945509	0.625815203	0.534737224	0.449975749	0.738670083	0.560424342
	Hippuric acid		0.576931831	0.979038982	0.663461218	0.64351687	0.541821468	0.727726341	0.772601125	0.83269328	0.84269328	0.501690391	0.242967871	0.678419479
	Octanoylcarnitine		0.465264998	0.621835639	0.457342562	0.69726068	0.717897869	0.472323953	0.370497989	0.725107933	0.460170462	0.570091557	0.18864895	0.776506623
	Cystathionine		0.336429346	0.201382977	0.094228136	0.273191797	0.189768487	0.299240642	0.102939004	0.016584152	0.116898728	0.293377558	0.140909453	0.270148577
	Decanoylcarnitine		0.301542026	0.356788019	0.361232872	0.63952792	0.439135818	0.31682367	0.229047642	0.370810942	0.265452174	0.449736598	0.088916743	0.474124291
	Normetanephrine		0.274359749	0.401651566	0.322919596	0.263049436	0.179542754	0.264505554	0.210527593	0.231759828	0.190141939	0.339817339	0.17402305	0.11120679
	Gamma-Glutamylcysteine		0.251979559	0.209235019	0.252896931	0.247541466	0.289517398	0.313542606	0.170209552	0.218185587	0.282996624	0.218755845	0.197086049	0.228822135
	L-Kynurenine		0.232345667	0.20195421	0.157767416	0.186281753	0.107055396	0.316637266	0.111852479	0.202579917	0.486412287	0.23854314	0.230230183	0.251831954
	cAMP		0.206168911	0.281480293	0.172837454	0.186761675	0.209805413	0.212537499	0.200855474	0.241252672	0.216871154	0.236711756	0.225067414	0.225067414
	Orotic acid	minor conc	0.132967472	0.097281641	0.077332546	0.056906581		0.066722171	0.054959358	0.087942921	0.103202787	0.077014632	0.074471322	0.136305567
	Isobutyrylcarnitine		0.123438297	0.127890874	0.140578035	0.113352943	0.099244176	0.082050793	0.090365846	0.121372945	0.111636287	0.126254686	0.061692326	0.106405851
	Kynurenic Acid	minor peaks at shifted RT	0.065036139	0.040832069	0.032698452	0.059690254	0.027910113	0.060182206	0.036437292	0.033485576	0.0296306105	0.036240511	0.047948984	0.050802309

Units: μ moles	Metabolite name	Comments	AOX						WT					
			1_MS 213	2_MS 214	3_MS 216	4_MS 223	5_MS 240	6_MS 250	7_MS 215	8_MS 224	9_MS 230	10_MS 241	11_MS 267	12_MS 234
	Stearoyl Carnitine		0.063673269	0.084825155	0.144726483	0.289728609	0.176584879	0.154896217	0.138575318	0.125460568	0.121848209	0.17509061	0.005367763	0.106934534
	Palmitoyl Carnitine		0.031191366	0.040553433	0.04724506	0.267975597	0.115449973	0.118198043	0.048161084	0.066171031	0.044931714	0.130820532	0.002903328	0.034544942
	Isovalerylcarnitine		0.021318903	0.016033434	0.019093443	0.016438063	0.015173597	0.016134591	0.010191599	0.016109302	0.014516074	0.020029148	0.012973425	0.015021861
	Dodecanoyl Carnitine		0.019903735	0.027345059	0.025105438	0.069590828	0.054148275	0.050897211	0.02808558	0.036520284	0.027345059	0.041938724	0.007152341	0.040259008
	Myristoyl Carnitine		0.018868658	0.025748205	0.02771856	0.109471615	0.069012536	0.079298459	0.03279473	0.038972963	0.028970905	0.05914406	0.003172606	0.030089666
	5-Deoxy-5Methylthio Adenosine	>ULOQ	0.011519759	0.013836232	0.018990906	0.018448309	0.0172379	0.025335121	0.019408289	0.017843104	0.013564933	0.01291799	0.024709047	0.018197879
	Pyridoxine		0.009644304	0.007407412	0.008140819	0.009130919	0.008727545	0.009130919	0.008837556	0.009717644	0.008654204	0.008067478	0.009937666	0.00825083
	Chenodeoxycholic Acid		0.008408008	0.027247005	14.30153229	4.223522773	10.78415578	0.126926156	20.54828739	3.107470294	1.505618265	0.116100055	47.57584608	5.283105676
	4-Pyridoxic Acid		0.004132133	0.002269286	0.001016098	0.001625757	0.001219318	0.001998327	0.002574116	0.002167676	0.002337026	0.002946685	0.001117708	0.001862847
	Arachidyl Carnitine		0.003458078	0.004819526	0.007365434	0.012879299	0.009189775	0.006357963	0.011912671	0.005064587	0.004520008	0.006671096	0.000694339	0.005881456
	5-Hydroxyindole-3-acetic acid						0.031282607					0.088396081		
	Neopterin	ND												
	Folic Acid	ND												
	Homogentisic acid	ND												
	Cholic Acid	ND												
	UDP-Glucose	ND												

ULOQ = Upper limit of quantification
 LLOQ=Lower limit of quantification
 ND=Not detected
 RT=Retention time