Doxorubicin-induced oxidative stress and endothelial dysfunction in conduit arteries is prevented by mitochondrial-specific antioxidant treatment

Zachary S. Clayton, PhD[1], Vienna E. Brunt, PhD[1], David A. Hutton, BA[1], Nicholas S. VanDongen, MS[1], Angelo D'Alessandro, PhD[2], Julie A. Reisz, PhD[2], Brian P. Ziemba, PhD[1] & Douglas R. Seals, PhD[1,2]

[1] Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO, USA
[2]Department of Medicine, Anschutz Medical Campus, University of Colorado Denver, Aurora, CO, USA

Supplemental Materials

Supplemental Materials and Methods

Animals

Male C57BL6/J mice were purchased from Jackson Laboratories at 3 months of age and allowed to acclimate to our facilities for 4 weeks prior to beginning the study. Mice were housed in standard cages on a 12:12 h light:dark cycle and body mass, energy intake and water consumption were monitored throughout the study. All mice were allowed access to normal rodent show (Harlan 7917) and water *ad libitum*. Mice were randomly assigned to receive Doxorubicin (DOXO; 10 mg/kg intraperitoneal injection from a 2 mg/ml stock; R & Systems 2252/50 Minneapolis, MN; suspended in sterile 1x PBS) or Sham (intraperitoneal injection of 150 μL sterile 1x PBS). DOXO and Sham were prepared fresh prior to injection. An additional cohort of mice (n = 5) was supplemented *in vivo* with the mitochondrial-targeted antioxidant MitoQ (250 μM in the drinking water) as previously described by our laboratory[1], immediately following DOXO administration. Bottles of MitoQ-enriched drinking water were protected from light using foil covering.

Vascular endothelial function.

Four weeks following injection of DOXO or sham, mice were anaesthetized with inhaled isoflurane and sacrificed by exsanguination via cardiac puncture. Endothelium-dependent dilation (EDD) and endothelium-independent dilation were measured in isolated carotid arteries as previously described (1-3). The carotid arteries were dissected free of surrounding tissue and cannulated onto glass micropipettes in warmed (37°C) physiological saline solution in pressure myograph chambers (Danish Myo Technology A/S, Aarhus, Denmark). Arteries were pressurized to 50 mmHg intraluminal pressure and allowed to equilibrate for 45 min prior to the beginning of experiments, as previously described by our laboratory (4,5). Following pre-constriction with phenylephrine (2 μM; Sigma-Aldrich Corp., St Louis, MO, USA), EDD was assessed by measuring the increase in luminal diameter in response to increasing concentrations of acetylcholine (ACh; 1 x 10^{-9} Mol/L to 1 x 10^{-4} Mol/L; Sigma-Aldrich Corp.). Endothelium-independent dilation as measured as dilation in response to increasing doses of the exogenous nitric oxide (NO) donor sodium nitroprusside (SNP; 1 x 10^{-10} Mol/L to 1 x 10^{-4} Mol/L; Sigma-Aldrich Corp). Following all dose responses, maximal carotid artery diameter was confirmed by 20-30 min incubation in Ca^{2+}-free physiological saline solution. To account for baseline differences in vessel diameter, all dose-response data are reported on a percentage basis.

NO-mediated EDD.

EDD was assessed in the presence of the endothelial NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 0.1 mMol/L, 30 min incubation; Sigma-Aldrich Corp.), and the contribution of NO (NO-mediated dilation) was calculated as the difference between peak dilation to ACh alone and peak dilation in the presence of L-NAME, as previously described (2).

Tonic ROS suppression of EDD.

Carotid arteries were incubated for 60 min with 1 mMol/L 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL), a superoxide dismutase (SOD) mimetic, to scavenge ROS prior to assessment of EDD to ACh (6). Tonic suppression was determined as the increase in peak EDD from ACh alone.

Tonic mitochondrial ROS suppression of EDD.

Carotid arteries were incubated for 60 min with 1 μMol/L mitoquinol mesylate (MitoQ) to scavenge mitochondrial ROS prior to assessment of EDD to ACh (1,7).

Aortic whole-cell and mitochondria-specific ROS.

Measurement of ROS in the thoracic aorta was performed using electron paramagnetic resonance (EPR) spectroscopy as previously described (1,2,6,7). Briefly, the aorta was removed and dissected free of surrounding tissue. Segments of 1 mm were incubated for 1 h at 37ºC in Krebs-HEPES buffer with the superoxide-specific spin probe 1-hydroxy-3methylcarbonyl-2,2,5,5-tetramethylpyrrolidine (1.0 mMol/L; Enzo Life Sciences, Inc., Farmington, NY, USA) or mitochondrial ROS-specific spin probe MitoTEMPO-H (0.5 mMol/L; Enzo Life Sciences, Inc.) for detection of whole-cell and mitochondria-specific ROS, respectively. The signal amplitude was analyzed using a MS300 X-band EPR spectrometer (Magnettech GmbH, Berlin, Germany) with the following settings: centerfield, 3350 G; sweep, 80 G; microwave modulation, 3000 mG, and microwave attenuation, 7 dB.

Aortic protein abundance.

Protein abundance was measured in segments of thoracic aorta following mechanical homogenization in radio-immunoprecipitation assay lysis buffer supplemented with protease and phosphatase inhibitors (1 mMol/L sodium orthovanadate, 1X complete mini protease inhibitor cocktail tablet [Roche, Mannheim, Germany], 1 mMol/L phenylmethylsulfonyl fluoride, 1:100 Phosphatase Inhibitor Cocktail [Sigma], 5 mMol/L sodium fluoride and 5 mMol/L sodium pyrophosphate). Aortic total protein content was

quantified using a bicinchoninic acid assay (Thermo Fisher Scientific, Eugene, OR). Next, protein abundance of SOD1 (anti-goat; 1:500; R&D Systems, Minneapolis, MN, cat no. AF3787), SOD2 (anti-goat; 1:50; R&D Systems, cat no. AF3419) and SOD3 (anti-rabbit; 1:200; R&D Systems, cat no. MAB34201) were determined by loading 20 ng/mL of aortic protein per capillary in a 25-lane (capillary) automated western blot quantitative analyzer (ProteinSimple, San Jose, California), according to the manufacturer's guidelines, as previously described (3). Anti-goat and anti-rabbit secondary antibodies were provided by the manufacturer and used according to the manufacturer's guidelines. A grayscale analysis of the band intensities to quantify protein abundance was then performed using Compass software (ProteinSimple), with target proteins expressed relative to loading control (GAPDH; 1:200; Cell Signaling, cat no. 2118).

Plasma exposure and ROS cell culture experiments.

Human umbilical vein endothelial cells (HUVECs; Lonza, Basel, Switzerland) were cultured and passaged (10 passages maximum [a passage number in which endothelial cells maintain their phenotype[8]]) at 37°C and 5% CO_2 to ~80% confluency in Endothelial Cell Growth Media (EGM)-2 media (Lonza) supplemented with an additional 2% fetal calf serum (FCS; Sigma-Aldrich Corp.), 100 μg/mL penicillin and 172 μg/mL streptomycin (Gibco, Gaithersburg, MD). Cells were washed with Hanks' balanced salt solution (HBSS), lifted with Trypsin-Like Enzyme (TrypLE) cell dissociation enzyme (Gibco), washed and resuspended in HBSS, counted and diluted to 9×10^3 cells/mL (designed to result in 60-80% confluency over 48-hour incubation) in FCS-supplemented EGM-2. 100 μL of HUVEC cell suspension was added to each well of a high content image plate with 0.2 mm glass bottom (Corning, Kennebunk, ME) coated with bovine gelatin substrate (Attachment Factor Solution; Cell Applications, Inc., San Diego, CA) to facilitate cell adhesion. After a 24-hour incubation, attached cells were washed once with HBSS and incubated in FCS-free EGM-2 media supplemented with 10% mouse

plasma for 24 hours (37°C, 5% CO_2). Attached cells were then washed twice with HBSS and incubated for the designated amount of time (37°C, 5% CO_2) with a fluorescent probe mixture ([2.9 μMol/L Hoechst 33342 nuclear probe, 9.2 μMol/L CellROX Deep Red ROS probe, 60 minute incubation]; or [2.9 μMol/L Hoechst 33342 nuclear probe, 5 μMol/L MitoSOX Deep Red mitochondrial ROS probe, 10 minute incubation]; or [2.9 μMol/L Hoechst 33342 nuclear probe, 0.2 μMol/L Mito Tracker Green mitochondrial volume probe, 45 minute incubation]). After dye removal and three washes with HBSS, cells were left in 100 μL of HBSS for subsequent imaging. Microscopy was carried out on an EVOS FL microscope (Invitrogen, Carlsbad, CA) set at 20X magnification. Cells were selected for imaging based on characteristic endothelial cell and nuclear morphology. Cell images of CellROX, MitoSOX and Mito Tracker fluorescent dyes were analyzed with Fiji, the open-source processing program distribution of ImageJ (National Institutes of Health) ([9]). For CellROX, MitoSOX and Mito Tracker, image intensity was averaged over the entire cell area.

Supplemental VEGF-A experiments.

HUVEC cells were cultured and passaged in conditions identical to those used for mouse plasma supplementation experiments. Recombinant mouse VEGF 164 (VEGF-A) protein (R&D, Minneapolis, MN, cat no. 493-MV-005) reconstituted in 0.1% w/v bovine serum albumin (BSA) in PBS was mixed with mouse plasma from DOXO-treated mouse plasma and added to FCS-free EGM-2 media for a final concentration of 90 pg/mL VEGF-A and 10% plasma. For control mouse plasma, a similar volume of 0.1% w/v BSA in PBS was mixed with mouse plasma and added to FCS-free EGM-2 media to achieve identical concentrations of plasma, media and vehicle. Both VEGF-A-supplemented mouse plasma derived from DOXO treated mice media solutions and Sham mouse media solutions were added to each well of 60-80% confluent HUVEC cells as in non-VEGF-A supplemented conditions. Fluorescent

probe concentrations were identical, and washes, imaging and analysis were performed identically to this description above.

Plasma metabolomics analysis.

Sample Preparation & UHPLC-MS – hydrophilic metabolite analysis. Prior to LC-MS analysis, samples were placed on ice, and 20 µL aliquots were diluted with 480 µL of ice-cold methanol/acetonitrile/water (5/3/2). Extractions were performed and resulting samples analyzed using a 5 min C18 gradient on a Thermo Vanquish-Q Exactive system (San Jose, CA, USA), as previously described ([10]).

Sample Preparation – lipid analysis. Prior to LC-MS analysis, samples were placed on Ice, and 10 uL aliquots were diluted with 90 uL of ice-cold methanol. Suspensions were vortexed to mix and placed at -20°C for 30 min. Insoluble material was removed by centrifugation at 12,000 g for 10 min at 4°C. Supernatants were isolated and diluted 1:1 (v/v) with 10 mMol/L ammonium acetate for analysis by ultra-high performance LC-MS (UHPLC-MS) ([11]).

UHPLC-MS lipid analysis. Samples were analyzed on a Thermo Vanquish UHPLC system (San Jose, CA, USA) coupled online to a Thermo Q Exactive mass spectrometer (Bremen, Germany). Lipids were resolved over a Waters ACQUITY HSS T3 column (2.1 x 150 mm, 1.8 µMol/L) using an aqueous phase (A) of 25% acetonitrile and 5 mM ammonium acetate and a mobile phase (B) of 50% isopropanol, 45% acetonitrile and 5 mMol/L ammonium acetate. Samples were eluted from the column using either the solvent gradient: 0-1 min 25% B and 0.3 mL/min; 1-2 min 25-50% B and 0.3 mL/min, 2-8 min 50-90% B and 0.3 mL/min, 8-10 min 90-99% B and 0.3 mL/min, 10-14 min hold at 99% B and 0.3 mL/min, 14-14.1 min 99-25% B and 0.3 mL/min, 14.1-16.9 min hold at 25% B and 0.4 mL/min, 16.9-17 min hold at 25% B and resume flow of 0.3 mL/min. The mass spectrometer was operated in negative ion mode,

scanning in Full MS mode (2 μscans) from 150 to 1500 m/z at 70,000 resolution, with 4 kV spray voltage, 45 sheath gas, 15 auxiliary gas. Samples were analyzed in randomized order with a technical mixture injected incrementally to qualify instrument performance. Acquired data was then converted from .raw to .mzXML file format using RawConverter. Metabolites were assigned using Maven (Princeton, NJ, USA) ([12,13]).

Plasma cytokine and chemokine analysis.

Plasma was diluted 1:2 with sample dilution buffer provided by the manufacturer, and proteins were analyzed using the Quantibody Mouse Cytokine Array 1 (RayBiotech, Peachtree Corners, GA) according to the manufacturer's instructions. The signal was visualized using an Amersham Typhoon fluorescence scanner (GE Healthcare Bio-Sciences, Pittsburgh, PA). Data were analyzed using ImageQuant TL software (General Electric).

Statistical analysis.

All statistical analyses were performed using GraphPad Prism version 7.0c (GraphPad Software, Inc., La Jolla, CA), and data were reported as means ± SEM. Carotid artery dose responses were assessed using a two-way analysis of variance (ANOVA) with a between factor of group and repeated factor of dose. VEGF-A supplementation cell culture experiments were assessed using a two-way ANOVA with a between factor of group and across conditions (Vehicle and VEGF-A). A Sidak's multiple comparisons *post hoc* test was performed when significant main effect differences were detected. Differences across groups in morphological and artery characteristics, water intake, food intake, NO-mediated dilation, ROS, immunoblots, plasma exposure cell culture experiments, plasma cytokines and plasma metabolomics were analyzed by independent samples *t* tests. The relationship between significantly altered metabolites and total and mitochondrial ROS were assessed using linear regressions. Significance was set to $\alpha = 0.05$.

SUPPLEMENTAL RESULTS.

Supplemental Table 1. Tissue mass for soleus and tibialis anterior muscles, subcutaneous WAT and liver.

	Sham (n = 4)	DOXO (n = 8)	*P* Value
Soleus (mg)	19.5 ± 3.9	17.8 ± 2.8	0.70
Tibialis anterior (mg)	144.0 ± 11.3	133.4 ± 8.6	0.50
Subcutaneous white adipose tissue (mg)	267.8 ± 25.5	255.6 ± 43.9	0.90
Liver (g)	1.5 ± 0.1	1.4 ± 0.4	0.30

Data are the mean ± SEM.

Supplemental Table 2. Individual metabolite abundance in plasma from DOXO and Sham treated mice.

N.	compound	CmpdID	Pathway	DX1 DOXO F40-01-	DX2 DOXO F40-02-	DX3 DOXO F40-03-	DX4 DOXO F40-04-	DX5 DOXO F40-05-	DX15 DOXO F40-06-	DX17 DOXO F40-07-	DX19 DOXO F40-08-	Doxo7 Sham F40-18-	Doxo8 Sham F40-19-	Doxo9 Sham F40-20-	Doxo10 Sham F40-21-	medians		doxo treat/sham	
																DOXO	Sham	FC	p value
1	Alanine	C00041	Amino acids	1.88E+07	1.28E+07	2.91E+07	1.67E+07	1.89E+07	1.33E+07	1.59E+07	2.05E+07	1.59E+07	2.12E+07	1.77E+07	1.90E+07	1.77E+07	1.84E+07	0.96	9.39E-01
2	Arginine	C00062	Amino acids	4.40E+06	2.55E+04	1.82E+05	2.47E+05	1.16E+05	3.78E+06	4.35E+04	2.86E+05	3.47E+06	1.51E+05	9.55E+05	1.71E+05	2.14E+05	5.63E+05	0.38	9.63E-01
3	Asparagine	C00152	Amino acids	6.03E+05	3.98E+05	7.77E+05	8.83E+05	5.35E+05	4.97E+05	4.15E+05	9.14E+05	4.50E+05	3.05E+05	9.68E+05	4.61E+05	5.69E+05	4.55E+05	1.25	5.79E-01
4	Aspartate	C00049	Amino acids	2.09E+05	7.70E+05	1.86E+05	4.58E+05	5.70E+05	7.54E+04	1.87E+05	2.78E+05	7.57E+04	6.96E+05	1.96E+05	8.67E+05	2.44E+05	4.46E+05	0.55	5.22E-01
5	Glutamate	C00025	Amino acids	1.05E+06	3.23E+06	1.01E+06	1.77E+06	4.22E+06	8.83E+05	1.69E+06	1.48E+06	7.30E+05	3.56E+06	1.45E+06	3.36E+06	1.59E+06	2.40E+06	0.66	6.53E-01
6	Glutamine	C00064	Amino acids	9.62E+06	1.25E+07	9.55E+06	1.37E+07	9.24E+06	1.30E+07	6.16E+06	1.55E+07	9.03E+06	1.02E+07	1.40E+07	1.09E+07	1.10E+07	1.06E+07	1.05	9.47E-01
7	Glycine	C00037	Amino acids	2.36E+06	1.89E+06	2.83E+06	2.23E+06	1.98E+06	2.06E+06	1.83E+06	1.82E+06	2.28E+06	2.28E+06	1.98E+06	2.54E+06	2.02E+06	2.28E+06	0.88	4.60E-01
8	Histidine	C00135	Amino acids	1.12E+06	1.00E+06	1.12E+06	1.72E+06	8.19E+05	1.58E+06	4.30E+05	2.22E+06	1.46E+06	1.57E+06	1.82E+06	1.67E+06	1.12E+06	1.62E+06	0.69	2.26E-01
9	Leucine/isoleucine	C00123	Amino acids	1.14E+08	1.37E+08	1.23E+08	1.84E+08	1.25E+08	1.73E+08	1.20E+08	1.82E+08	1.30E+08	1.74E+08	1.79E+08	1.75E+08	1.31E+08	1.75E+08	0.75	2.66E-01
10	Lysine	C00047	Amino acids	1.03E+07	6.77E+06	1.46E+07	1.06E+07	5.75E+06	1.02E+07	3.83E+06	1.81E+07	8.26E+06	1.00E+07	1.48E+07	7.95E+06	1.03E+07	9.15E+06	1.12	9.26E-01
11	Methionine	C00073	Amino acids	1.57E+07	1.03E+07	2.21E+07	1.63E+07	1.22E+07	1.46E+07	1.22E+07	1.40E+07	1.69E+07	1.76E+07	1.77E+07	1.90E+07	1.43E+07	1.77E+07	0.81	1.24E-01
12	Phenylalanine	C00079	Amino acids	1.96E+07	2.27E+07	3.04E+07	3.10E+07	2.33E+07	2.81E+07	2.35E+07	3.94E+07	2.48E+07	3.41E+07	2.64E+07	3.30E+07	2.58E+07	2.97E+07	0.87	5.31E-01
13	Proline	C00148	Amino acids	2.83E+07	2.07E+07	5.23E+07	3.38E+07	2.44E+07	2.35E+07	2.66E+07	3.22E+07	2.49E+07	3.33E+07	4.02E+07	2.91E+07	2.75E+07	3.12E+07	0.88	7.79E-01
14	Serine	C00065	Amino acids	1.03E+06	6.88E+05	1.21E+06	1.05E+06	5.14E+05	7.18E+05	6.53E+05	9.03E+05	7.08E+05	8.16E+05	9.62E+05	9.67E+05	8.10E+05	8.89E+05	0.91	8.89E-01
15	Threonine	C00188	Amino acids	3.29E+06	2.30E+06	5.72E+06	3.49E+06	2.27E+06	2.94E+06	2.64E+06	4.24E+06	3.16E+06	3.31E+06	3.67E+06	3.39E+06	3.11E+06	3.35E+06	0.93	9.70E-01
16	Tryptophan	C00078	Amino acids	5.02E+07	5.43E+07	5.82E+07	6.33E+07	3.54E+07	5.13E+07	4.08E+07	9.27E+07	8.41E+07	5.35E+07	5.77E+07	6.27E+07	5.28E+07	6.02E+07	0.88	4.04E-01
17	Tyrosine	C00082	Amino acids	1.01E+07	8.45E+06	9.58E+06	1.27E+07	7.07E+06	8.83E+06	8.35E+06	1.01E+07	9.90E+06	8.54E+06	8.50E+06	9.79E+06	9.20E+06	9.17E+06	1.00	8.15E-01
18	Valine	C00183	Amino acids	1.00E+08	9.00E+07	1.30E+08	1.11E+08	8.58E+07	1.24E+08	1.09E+08	1.39E+08	1.02E+08	1.36E+08	1.40E+08	1.42E+08	1.10E+08	1.38E+08	0.80	1.32E-01
19	Cystine	C00491	Amino acids	6.97E+04	1.24E+05	8.67E+04	1.99E+05	1.85E+05	6.38E+04	1.19E+05	1.16E+05	1.67E+05	2.32E+05	1.80E+05	1.37E+05	1.17E+05	1.73E+05	0.68	6.84E-02
20	Adenosine	C00212	Nucleotides	6.31E+03	1.76E+04	5.43E+03	1.93E+04	9.39E+04	4.59E+03	3.90E+04	1.22E+04	1.44E+04	5.98E+04	2.00E+05	8.16E+04	1.49E+04	7.07E+04	0.21	6.33E-02
21	Guanine	C00242	Nucleotides	6.78E+05	4.06E+05	7.78E+05	7.16E+05	5.13E+05	6.38E+05	5.11E+05	5.95E+05	7.61E+05	7.39E+05	7.19E+05	7.76E+05	6.16E+05	7.50E+05	0.82	4.64E-02
22	Cytidine	C00475	Nucleotides	2.09E+05	2.17E+05	3.70E+05	2.46E+05	2.64E+05	1.77E+05	2.34E+05	2.85E+05	1.69E+05	2.58E+05	2.11E+05	2.23E+05	2.40E+05	2.17E+05	1.11	3.09E-01
23	Thymidine	C00214	Nucleotides	4.05E+05	3.90E+05	4.03E+05	6.23E+05	4.48E+05	5.76E+05	3.98E+05	4.54E+05	5.24E+05	4.17E+05	6.60E+05	4.47E+05	4.27E+05	4.86E+05	0.88	4.12E-01
24	Uracil	C00106	Nucleotides	4.45E+05	2.47E+06	5.77E+05	1.07E+06	1.77E+06	4.38E+05	1.70E+06	2.61E+06	2.87E+05	2.08E+06	7.74E+05	1.78E+06	1.38E+06	1.27E+06	1.09	7.78E-01
25	Inosine	C00294	Nucleotides	7.02E+05	4.91E+05	5.10E+05	8.45E+05	6.46E+05	4.76E+05	5.82E+05	6.40E+05	8.62E+05	9.86E+05	5.32E+05	5.18E+05	6.11E+05	6.97E+05	0.88	2.92E-01
26	Hypoxanthine	C00262	Nucleotides	3.20E+06	4.71E+06	3.80E+06	4.72E+06	5.23E+06	3.74E+06	4.49E+06	5.06E+06	3.25E+06	3.31E+06	5.05E+06	3.35E+06	4.60E+06	3.33E+06	1.38	2.10E-01
27	Xanthine	C00385	Nucleotides	4.48E+05	2.78E+05	6.46E+05	3.05E+05	1.59E+05	3.89E+05	1.17E+05	1.03E+06	2.19E+05	2.64E+05	8.62E+05	2.07E+05	3.47E+05	2.42E+05	1.44	8.60E-01
28	Allantoate	C00499	Nucleotides	3.12E+05	4.72E+05	8.91E+05	1.11E+06	5.55E+05	3.82E+05	3.64E+05	1.04E+06	2.57E+05	7.94E+05	7.94E+05	7.54E+05	5.13E+05	7.74E+05	0.66	9.62E-01
29	(S)(+)-Allantoin	C02350	Nucleotides	1.92E+05	2.69E+05	2.15E+05	2.43E+05	2.52E+05	1.97E+05	1.84E+05	2.59E+05	1.92E+05	2.57E+05	2.08E+05	2.53E+05	2.29E+05	2.30E+05	0.99	9.58E-01
30	5-Hydroxyisourate	C11821	Nucleotides	1.02E+05	2.30E+05	1.38E+05	1.44E+05	1.41E+05	1.46E+05	8.59E+04	1.89E+05	1.06E+05	1.63E+05	1.78E+05	1.64E+05	1.42E+05	1.64E+05	0.87	8.22E-01
31	Urate	C00366	Nucleotides	1.56E+05	5.85E+05	2.60E+05	5.54E+05	5.30E+05	3.10E+05	5.56E+05	1.48E+06	3.26E+05	4.27E+05	1.16E+06	5.33E+05	5.42E+05	4.80E+05	1.13	8.18E-01
32	4-Pyridoxate	C00847	Nucleotides	5.09E+05	3.00E+05	1.04E+06	7.30E+05	5.17E+05	4.10E+05	5.18E+05	4.24E+05	5.05E+05	6.23E+05	4.28E+05	4.34E+05	5.13E+05	4.69E+05	1.09	6.41E-01
33	Pyridoxamine 5'-phospha	C00647	Nucleotides	4.70E+05	3.43E+05	3.30E+05	3.33E+05	4.11E+05	3.77E+05	5.46E+05	4.90E+05	4.87E+05	4.08E+05	2.87E+05	3.38E+05	3.94E+05	3.73E+05	1.06	5.39E-01
34	Nicotinamide	C00153	Nucleotides	6.14E+05	4.48E+06	6.63E+05	2.30E+06	5.99E+06	1.11E+06	6.00E+06	1.78E+06	1.45E+06	6.22E+06	1.71E+06	5.68E+06	2.04E+06	3.70E+06	0.55	5.49E-01
35	Phosphate	C00009	Phosphates	2.52E+07	3.35E+07	1.88E+07	3.16E+07	2.77E+07	3.54E+07	2.15E+07	2.87E+07	3.79E+07	2.84E+07	3.74E+07	3.34E+07	2.82E+07	3.54E+07	0.80	7.92E-02
36	Diphosphate	C00013	Phosphates	2.13E+05	4.57E+05	1.68E+05	3.87E+05	2.84E+05	6.46E+05	2.27E+05	4.57E+05	5.77E+05	4.98E+05	4.85E+05	6.30E+05	3.36E+05	5.37E+05	0.63	4.87E-02
37	D-Glucose	C00031	Glycolysis	9.02E+07	1.08E+08	6.98E+07	1.10E+08	1.49E+08	1.23E+08	9.27E+07	1.22E+08	9.36E+07	1.26E+08	1.18E+08	1.46E+08	1.09E+08	1.22E+08	0.89	3.85E-01
38	Hexose phosphate	C02965	Glycolysis	1.74E+05	6.27E+06	3.59E+05	1.64E+06	9.07E+06	1.84E+05	7.71E+06	2.02E+06	2.75E+05	6.77E+06	1.01E+06	4.60E+06	1.83E+06	2.80E+06	0.65	9.04E-01
39	D-Glyceraldehyde 3-phosp	C00118	Glycolysis	6.84E+07	1.11E+08	1.00E+08	7.58E+07	5.54E+07	7.77E+07	7.55E+07	8.84E+07	7.86E+07	7.46E+07	1.24E+08	6.06E+07	7.67E+07	7.66E+07	1.00	8.22E-01
40	1-3-Bisphosphoglycerate	C00236	Glycolysis	3.81E+04	1.00E+06	0.00E+00	1.14E+06	8.05E+06	6.30E+04	5.34E+06	1.71E+03	1.11E+06	9.97E+05	4.28E+04	2.78E+06	5.34E+05	1.05E+06	0.51	6.63E-01
41	2/3-Phospho-D-glycerate	C00631	Glycolysis	2.04E+05	1.33E+06	1.73E+05	1.02E+06	3.70E+06	2.43E+05	2.52E+06	1.92E+05	4.89E+05	2.19E+06	7.33E+05	1.93E+06	6.31E+05	1.33E+06	0.47	8.27E-01
42	Phosphoenolpyruvate	C00074	Glycolysis	3.28E+04	4.70E+05	1.23E+04	3.54E+05	1.23E+06	3.06E+04	9.98E+05	1.61E+04	1.25E+05	6.87E+05	1.56E+05	6.65E+05	1.93E+05	4.11E+05	0.47	9.55E-01
43	Pyruvate	C00022	Glycolysis	9.56E+06	6.11E+06	9.17E+06	1.12E+07	6.74E+06	7.11E+06	7.63E+06	7.88E+06	1.50E+07	1.23E+07	8.16E+06	6.74E+06	7.76E+06	1.02E+07	0.76	1.55E-01
44	Lactate	C01432	Glycolysis	1.11E+08	2.17E+08	2.49E+08	2.49E+08	2.60E+08	1.90E+08	1.78E+08	2.69E+08	2.84E+08	3.23E+08	2.73E+08	2.70E+08	2.33E+08	2.78E+08	0.84	3.10E-02
45	Mannitol/sorbitol	C00392	Other sugars	3.95E+05	4.32E+05	3.24E+05	5.54E+05	3.17E+05	2.99E+05	2.25E+05	3.18E+05	4.78E+05	2.96E+05	2.77E+05	4.75E+05	3.21E+05	3.86E+05	0.83	7.21E-01
46	D-Ribose	C00121	Other sugars	2.03E+06	2.62E+06	1.73E+06	2.72E+06	3.27E+06	2.72E+06	2.64E+06	3.52E+06	2.63E+06	3.47E+06	3.22E+06	3.08E+06	2.68E+06	3.15E+06	0.85	1.95E-01
47	D-Arabitol	C01904	Other sugars	4.25E+05	5.95E+05	4.60E+05	7.53E+05	6.18E+05	5.16E+05	4.69E+05	6.99E+05	5.74E+05	5.10E+05	5.03E+05	6.43E+05	5.55E+05	5.42E+05	1.02	8.85E-01
48	Citrate	C00158	TCA cycle	3.50E+07	5.85E+07	1.20E+07	5.97E+07	5.00E+07	5.56E+07	4.48E+07	3.45E+07	7.34E+07	7.52E+07	3.28E+07	6.56E+07	4.74E+07	6.95E+07	0.68	1.20E-01
49	2-Oxoglutarate	C00026	TCA cycle	7.85E+06	8.61E+06	8.42E+06	1.31E+07	6.00E+06	1.44E+07	7.12E+06	7.73E+06	1.75E+07	7.77E+06	7.68E+06	6.03E+06	8.13E+06	7.72E+06	1.05	8.09E-01
50	2-Oxoglutaramate	C00940	TCA cycle	1.30E+05	6.99E+05	5.46E+05	6.52E+05	6.73E+05	2.12E+05	1.35E+06	4.49E+05	3.20E+05	1.36E+06	4.64E+05	9.52E+05	5.99E+05	7.08E+05	0.85	4.72E-01
51	Succinate	C00042	TCA cycle	2.79E+06	2.86E+07	7.12E+06	3.14E+07	3.35E+07	1.88E+07	2.52E+07	5.15E+07	1.72E+07	2.41E+07	2.87E+07	3.14E+07	2.69E+07	2.64E+07	1.02	9.52E-01
52	Fumarate	C00122	TCA cycle	1.21E+06	2.78E+06	1.77E+06	3.21E+06	3.50E+06	1.61E+06	3.12E+06	2.85E+06	2.54E+06	3.82E+06	2.67E+06	3.23E+06	2.82E+06	2.95E+06	0.96	2.72E-01
53	Malate	C00149	TCA cycle	2.61E+07	6.39E+07	3.77E+07	6.91E+07	7.94E+07	3.38E+07	6.78E+07	6.50E+07	5.21E+07	8.37E+07	5.82E+07	7.24E+07	6.45E+07	6.53E+07	0.99	3.37E-01
54	2-Hydroxyglutarate/Citran	C02630	Alternative Carboxylic acids	1.66E+06	2.98E+06	1.62E+06	3.24E+06	3.83E+06	2.54E+06	2.67E+06	2.65E+06	2.65E+06	2.69E+06	3.32E+06	2.56E+06	2.66E+06	2.67E+06	1.00	7.06E-01
55	6-Phospho-D-gluconate	C00345	Pentose Phosphate Pathway	1.63E+04	2.05E+05	2.78E+04	2.97E+04	3.64E+05	3.05E+04	3.79E+05	2.21E+04	1.47E+04	1.63E+05	3.04E+04	2.49E+05	3.01E+04	9.67E+04	0.31	8.27E-01
56	Sedoheptulose 1-phospha	C06222	Pentose Phosphate Pathway	3.00E+05	5.94E+05	2.76E+04	2.41E+05	4.09E+05	4.60E+05	6.32E+05	2.02E+05	6.14E+05	6.87E+05	1.14E+05	4.71E+05	3.55E+05	5.42E+05	0.65	4.23E-01
57	alpha-D-Ribose 1-phosph	C00620	Pentose Phosphate Pathway	3.26E+05	1.53E+06	3.37E+05	1.58E+06	2.59E+06	2.70E+05	2.03E+06	1.59E+06	8.57E+05	2.79E+06	3.54E+06	3.70E+06	1.55E+06	3.17E+06	0.49	4.41E-02
58	Glutathione	C00051	GSH homeostasis	6.37E+04	1.12E+05	2.72E+04	1.52E+05	1.71E+05	4.68E+04	1.37E+05	2.95E+05	1.26E+05	1.51E+05	8.78E+04	1.35E+05	1.24E+05	1.31E+05	0.95	9.88E-01
59	Glutathione disulfide	C00127	GSH homeostasis	6.38E+03	2.76E+05	1.12E+04	3.60E+05	4.26E+05	3.25E+04	1.09E+06	2.18E+05	1.76E+05	1.05E+06	2.68E+05	1.31E+06	2.47E+05	6.60E+05	0.37	1.62E-01
60	5-Oxoproline	C01879	GSH homeostasis	3.56E+06	4.49E+06	3.47E+06	4.65E+06	3.85E+06	4.54E+06	2.54E+06	5.41E+06	3.29E+06	3.78E+06	5.02E+06	4.44E+06	4.17E+06	4.11E+06	1.01	8.98E-01
61	Ascorbate	C00072	GSH homeostasis	3.45E+06	4.64E+06	1.58E+06	5.28E+06	2.89E+05	6.59E+06	4.59E+05	8.12E+05	4.97E+06	1.13E+07	4.90E+06	4.38E+06	2.51E+06	4.94E+06	0.51	6.17E-02
62	Dehydroascorbate	C05422	GSH homeostasis	2.29E+06	3.03E+06	1.20E+06	3.73E+06	3.32E+06	2.63E+06	3.05E+06	2.25E+06	3.98E+06	4.02E+06	2.54E+06	2.96E+06	2.83E+06	3.47E+06	0.81	1.73E-01
63	gamma-Glutamyl-Se-meth	C05695	Gamma-glutamyls	1.23E+05	2.49E+05	3.53E+05	4.40E+05	2.24E+05	1.40E+05	9.62E+04	4.13E+05	1.16E+05	2.55E+05	2.32E+05	1.94E+05	2.36E+05	2.13E+05	1.11	4.56E-01
64	Dimethylglycine	C01026	ine biosynthesis and one-carbon metabol	1.47E+06	1.37E+06	2.30E+06	1.76E+06	1.90E+06	1.25E+06	2.01E+06	2.05E+06	1.46E+06	2.44E+06	2.03E+06	2.31E+06	1.83E+06	2.17E+06	0.84	2.43E-01
65	Ornithine	C01602	Urea cycle	1.55E+06	2.15E+06	7.46E+06	4.15E+06	2.37E+06	1.68E+06	1.19E+06	7.69E+06	1.01E+06	3.69E+06	5.56E+06	2.96E+06	2.26E+06	3.33E+06	0.68	8.84E-01
66	L-Citrulline	C00327	Urea cycle	9.09E+05	1.08E+06	2.06E+06	1.22E+06	8.11E+05	8.56E+05	7.60E+05	1.32E+06	1.02E+06	1.40E+06	1.07E+06	1.26E+06	9.96E+05	1.17E+06	0.85	7.91E-01
67	Putrescine	C00134	Polyamines	1.33E+05	5.92E+04	1.55E+05	8.75E+04	2.65E+04	1.31E+05	3.56E+04	2.61E+05	7.84E+04	6.30E+04	1.95E+05	5.75E+04	1.09E+05	7.07E+04	1.54	7.84E-01
68	Spermidine	C00315	Polyamines	8.31E+05	1.47E+06	1.67E+06	1.10E+06	1.69E+06	1.14E+06	7.30E+05	2.94E+06	1.05E+06	1.89E+06	3.15E+06	1.53E+06	1.31E+06	1.71E+06	0.76	3.52E-01
69	Spermine	C00750	Polyamines	5.19E+04	8.49E+04	9.11E+04	7.25E+04	8.51E+04	7.65E+04	6.56E+04	1.19E+05	7.70E+04	9.48E+04	1.22E+05	6.60E+04	8.07E+04	8.59E+04	0.94	5.02E-01
70	N-Acetylneuraminate	C00270	Aminosugars	5.40E+04	6.83E+04	7.70E+04	1.90E+05	4.64E+04	2.13E+04	5.59E+04	1.30E+05	4.35E+04	6.56E+04	3.94E+04	1.40E+05	6.21E+04	5.45E+04	1.14	7.97E-01
71	N-Glycoloyl-neuraminate	C03410	Aminosugars	2.62E+04	1.00E+05	2.47E+04	4.31E+04	1.36E+05	1.03E+04	1.35E+05	3.41E+05	2.54E+04	6.69E+04	8.98E+04	9.01E+04	7.18E+04	7.83E+04	0.92	5.60E-01
72	Creatine	C00300	Arginine and proline metabolism	7.35E+07	6.54E+07	6.62E+07	7.41E+07	7.34E+07	5.01E+07	4.94E+07	1.07E+08	6.55E+07	7.64E+07	8.22E+07	5.88E+07	6.98E+07	7.09E+07	0.98	9.30E-01
73	Creatinine	C00791	Arginine and proline metabolism	4.22E+06	4.38E+06	9.05E+06	6.03E+06	6.68E+06	4.34E+06	4.11E+06	6.45E+06	5.62E+06	6.95E+06	5.20E+06	6.51E+06	5.20E+06	6.06E+06	0.86	6.67E-01
74	4-Acetamidobutanoate	C02946	Arginine and proline metabolism	5.21E+05	1.72E+06	7.46E+05	1.07E+06	1.02E+06	8.47E+05	9.38E+05	7.72E+05	9.86E+05	9.92E+05	1.55E+06	1.61E+06	8.92E+05	1.27E+06	0.70	1.53E-01
75	N-Acetylornithine	C00437	Arginine and proline metabolism	3.32E+05	2.61E+05	6.59E+05	3.95E+05	3.34E+05	2.72E+05	3.21E+05	3.77E+05	2.75E+05	3.87E+05	3.75E+05	4.00E+05	3.33E+05	3.81E+05	0.87	8.88E-01
76	Guanidinoacetate	C00581	Arginine and proline metabolism	3.33E+05	2.79E+05	2.14E+05	3.12E+05	2.43E+05	3.51E+05	2.42E+05	3.52E+05	2.70E+05	2.45E+05	3.52E+05	2.17E+05	2.95E+05	2.57E+05	1.15	5.72E-01
77	Pantothenate	C00864	Panthothenate metabolism	5.58E+05	5.62E+05	9.91E+05	4.27E+05	4.78E+05	4.26E+05	6.67E+05	7.88E+05	5.37E+05	5.86E+05	7.04E+05	4.55E+05	5.60E+05	5.61E+05	1.00	7.05E-01
78	Taurine	C00245	Sulfur metabolism	1.71E+06	2.84E+06	1.77E+06	2.08E+06	3.34E+06	1.59E+06	2.16E+06	2.43E+06	1.82E+06	2.37E+06	1.94E+06	2.96E+06	2.12E+06	2.15E+06	0.98	9.28E-01
79	L-Methionine S-oxide	C02989	Sulfur metabolism	1.00E+05	2.08E+04	2.01E+05	9.17E+04	7.35E+04	4.48E+04	6.06E+04	3.81E+04	9.63E+04	7.01E+04	1.12E+05	8.86E+04	6.71E+04	9.24E+04	0.73	6.68E-01
80	5-Hydroxyindoleacetate	C05635	Indole and Tryptophan	1.63E+05	9.61E+04	1.76E+05	3.45E+05	1.44E+05	1.50E+05	1.13E+05	1.47E+05	5.02E+05	2.49E+05	3.75E+05	3.43E+05	1.48E+05	3.59E+05	0.41	3.39E-03
81	3-Methyleneoxindole	C02796	Indole and Tryptophan	5.47E+05	5.62E+05	6.45E+05	6.44E+05	3.69E+05	5.40E+05	4.81E+05	8.93E+05	7.70E+05	6.27E+05	5.90E+05	5.59E+05	5.54E+05	6.08E+05	0.91	5.55E-01

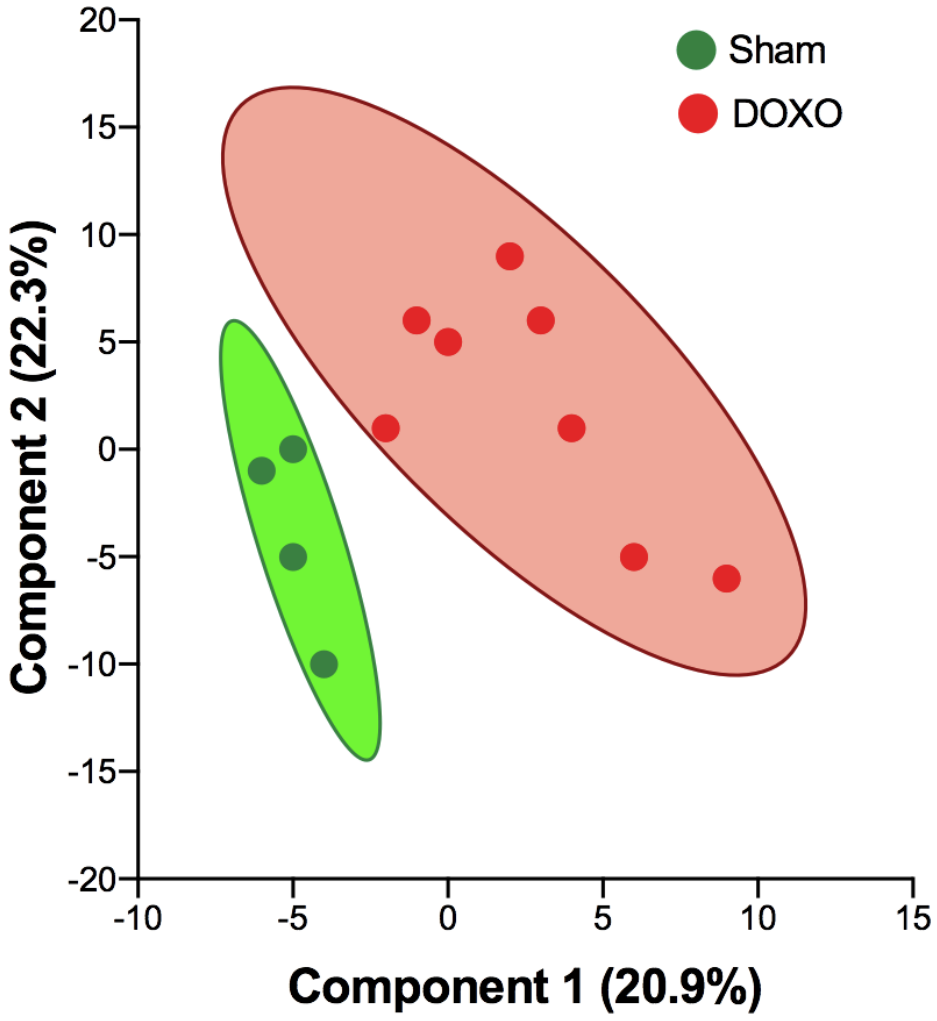
82	Indole	C00463	Indole and Tryptophan	2.21E+05	2.33E+05	2.79E+05	2.53E+05	1.45E+05	2.50E+05	2.28E+05	3.97E+05	3.10E+05	2.20E+05	2.50E+05	2.38E+05	2.42E+05	2.44E+05	0.99	9.24E-01
83	Indole-3-acetaldehyde	C00637	Indole and Tryptophan	1.31E+05	6.64E+05	2.45E+05	1.37E+05	1.30E+06	2.71E+05	9.67E+05	2.56E+06	1.66E+05	2.13E+05	2.17E+06	7.71E+05	4.67E+05	4.92E+05	0.95	9.33E-01
84	Indolepyruvate	C00331	Indole and Tryptophan	1.25E+05	1.04E+05	1.28E+05	1.62E+05	3.56E+04	8.79E+04	1.05E+05	1.45E+05	1.24E+05	1.19E+05	1.40E+05	1.14E+05	1.15E+05	1.21E+05	0.95	5.46E-01
85	Indoxyl	C05658	Indole and Tryptophan	2.99E+05	3.36E+05	6.83E+05	2.11E+05	9.93E+04	2.33E+05	1.28E+05	5.25E+05	2.55E+05	2.88E+05	4.50E+05	1.95E+05	2.66E+05	2.71E+05	0.98	8.75E-01
86	kynurenine	C00328	Indole and Tryptophan	4.83E+04	2.74E+05	2.06E+05	1.78E+05	3.61E+05	9.99E+04	3.86E+05	2.20E+05	6.62E+04	4.61E+05	8.23E+04	2.22E+05	2.13E+05	1.52E+05	1.40	8.73E-01
87	Anthranilate	C00108	Indole and Tryptophan	1.21E+06	7.40E+05	2.46E+06	1.21E+06	1.25E+06	8.37E+05	1.16E+06	8.71E+05	1.05E+06	1.93E+06	1.36E+06	1.44E+06	1.18E+06	1.40E+06	0.85	4.71E-01
88	Picolinic acid	C10164	Indole and Tryptophan	1.17E+06	5.94E+05	1.92E+06	7.58E+05	3.20E+05	1.08E+06	2.82E+05	2.53E+06	5.94E+05	6.05E+05	2.18E+06	4.92E+05	9.19E+05	5.99E+05	1.53	8.19E-01
89	L-Adrenaline	C00788	Signaling	1.36E+07	5.79E+06	6.89E+06	8.60E+06	6.73E+06	1.10E+07	6.80E+06	7.23E+06	9.20E+06	5.46E+06	1.12E+07	6.24E+06	7.06E+06	7.72E+06	0.91	8.53E-01
90	Dopamine	C03758	Signaling	4.68E+05	5.43E+05	7.27E+05	7.52E+05	4.55E+05	5.48E+05	4.16E+05	7.29E+05	4.66E+05	7.35E+05	7.37E+05	6.87E+05	5.46E+05	7.11E+05	0.77	3.72E-01
91	Serotonin	C00780	Signaling	8.16E+04	6.72E+05	9.86E+04	9.17E+04	1.10E+06	2.29E+05	7.76E+05	1.94E+06	1.27E+05	2.36E+05	1.85E+06	6.58E+05	4.50E+05	4.47E+05	1.01	8.30E-01
92	Glycerol 3-phosphate	C00093	Glycerophospholipid biosynthesis	7.16E+05	5.63E+06	6.18E+05	2.33E+06	7.92E+06	1.11E+06	6.09E+06	2.79E+06	1.20E+06	5.20E+06	2.91E+06	5.84E+06	2.56E+06	4.06E+06	0.63	8.11E-01
93	Ethanolamine phosphate	C00346	Glycerophospholipid biosynthesis	1.89E+05	1.80E+05	1.84E+05	2.48E+05	1.94E+05	1.73E+05	2.30E+05	1.34E+05	1.42E+05	1.95E+05	2.22E+05	3.10E+05	1.87E+05	2.09E+05	0.90	4.01E-01
94	Sphingosine	C00319	Glycerophospholipid biosynthesis	6.35E+04	7.73E+04	6.43E+04	7.21E+04	3.97E+04	8.74E+04	5.19E+04	7.99E+04	6.84E+04	5.84E+04	5.68E+04	5.27E+04	6.82E+04	5.76E+04	1.18	3.63E-01
95	Sphingosine 1-phosphate	C06124	Glycerophospholipid biosynthesis	2.48E+05	1.82E+05	1.56E+05	1.65E+05	1.15E+05	2.76E+05	6.16E+04	3.38E+05	2.69E+05	2.38E+05	3.04E+05	1.24E+05	1.73E+05	2.54E+05	0.68	4.54E-01
96	Choline	C00114	Glycerophospholipid biosynthesis	3.27E+07	6.97E+07	4.30E+07	3.65E+07	6.98E+07	4.06E+07	8.87E+07	5.84E+07	2.48E+07	4.38E+07	6.45E+07	5.23E+07	5.07E+07	4.81E+07	1.05	4.79E-01
97	Acetylcholine	C01996	Glycerophospholipid biosynthesis	3.26E+06	3.04E+06	2.56E+06	2.52E+06	2.32E+06	3.96E+06	2.53E+06	3.23E+06	2.77E+06	2.93E+06	4.09E+06	2.61E+06	2.80E+06	2.85E+06	0.98	6.48E-01
98	L-Carnitine	C00318	Carnitine and fatty acid metabolism	3.99E+07	2.84E+07	3.13E+07	3.48E+07	2.77E+07	4.64E+07	2.20E+07	3.30E+07	3.39E+07	2.92E+07	4.47E+07	3.06E+07	3.21E+07	3.23E+07	1.00	7.22E-01
99	acetyl-carnitine	C02571	Carnitine and fatty acid metabolism	9.38E+07	6.48E+07	6.26E+07	7.39E+07	6.43E+07	1.09E+08	5.22E+07	8.97E+07	6.06E+07	7.30E+07	8.57E+07	7.04E+07	6.93E+07	7.17E+07	0.97	7.18E-01
100	propionyl-carnitine	C03017	Carnitine and fatty acid metabolism	2.72E+06	3.26E+06	3.38E+06	3.39E+06	3.34E+06	3.58E+06	2.98E+06	5.81E+06	2.53E+06	3.72E+06	3.81E+06	4.21E+06	3.36E+06	3.76E+06	0.89	9.83E-01
101	butanoyl-l-carnitine	C02862	Carnitine and fatty acid metabolism	2.02E+06	9.51E+06	7.51E+06	1.13E+07	6.00E+06	6.18E+06	4.77E+06	7.84E+06	5.49E+06	6.72E+06	7.80E+06	1.37E+07	6.85E+06	7.26E+06	0.94	4.41E-01
102	acyl-C4-OH	HMDB13127	Carnitine and fatty acid metabolism	3.04E+05	4.49E+05	2.76E+05	5.17E+05	6.10E+05	5.31E+05	5.17E+05	9.40E+05	4.38E+05	6.70E+05	6.60E+05	6.74E+05	5.17E+05	6.65E+05	0.78	4.29E-01
103	acyl-C5	C20826	Carnitine and fatty acid metabolism	1.03E+06	2.07E+06	1.31E+06	2.61E+06	1.44E+06	1.23E+06	1.38E+06	3.46E+06	1.50E+06	1.48E+06	1.84E+06	2.36E+06	1.41E+06	1.67E+06	0.84	9.60E-01
104	hexanoyl-L-carnitine	HMDB00756	Carnitine and fatty acid metabolism	5.01E+05	2.90E+06	6.59E+05	3.79E+06	1.94E+06	1.65E+06	1.89E+06	2.54E+06	1.72E+06	1.16E+06	1.27E+06	5.81E+06	1.92E+06	1.49E+06	1.28	6.00E-01
105	L-octanoylcarnitine	HMDB00791	Carnitine and fatty acid metabolism	5.00E+04	4.16E+05	7.94E+04	3.21E+05	2.30E+05	2.20E+05	1.36E+05	2.40E+05	2.41E+05	1.49E+05	1.65E+05	4.79E+05	2.25E+05	2.03E+05	1.11	5.74E-01
106	O-Decanoyl-L-carnitine	HMDB00651	Carnitine and fatty acid metabolism	6.06E+04	1.80E+05	8.41E+04	2.23E+05	9.23E+04	1.96E+05	9.18E+04	3.88E+05	1.59E+05	7.53E+04	9.96E+04	1.34E+05	1.36E+05	1.17E+05	1.16	4.25E-01
107	O-Decenoyl-L-carnitine	CID57357170	Carnitine and fatty acid metabolism	9.56E+04	9.63E+04	7.52E+04	2.53E+05	1.61E+05	1.66E+05	1.09E+05	3.54E+05	2.01E+05	1.05E+05	4.53E+05	1.80E+05	1.35E+05	1.91E+05	0.71	3.36E-01
108	O-dodecanoyl-carnitine	HMDB02250	Carnitine and fatty acid metabolism	4.99E+04	1.19E+05	4.84E+04	1.71E+05	5.83E+04	1.12E+05	8.40E+04	2.10E+05	9.21E+04	6.54E+04	6.64E+04	1.01E+05	9.79E+04	7.93E+04	1.23	4.32E-01
109	O-tetradecanoyl-L-carnitin	HMDB05066	Carnitine and fatty acid metabolism	9.18E+04	1.49E+05	7.85E+04	1.42E+05	1.10E+05	1.63E+05	1.08E+05	2.39E+05	1.54E+05	1.37E+05	9.74E+04	1.34E+05	1.26E+05	1.35E+05	0.93	8.68E-01
110	Tetradecenoyl Carnitine	HMDB13329	Carnitine and fatty acid metabolism	9.01E+04	2.14E+05	1.15E+05	2.86E+05	6.65E+04	2.77E+05	1.69E+05	6.08E+05	2.22E+05	1.02E+05	9.51E+04	1.25E+05	1.92E+05	1.14E+05	1.69	3.35E-01
111	L-Palmitoylcarnitine	C02990	Carnitine and fatty acid metabolism	9.03E+05	7.54E+05	9.66E+05	4.00E+05	6.80E+05	4.91E+05	6.20E+05	3.57E+05	3.60E+05	8.28E+05	5.86E+05	8.35E+05	6.50E+05	7.07E+05	0.92	9.68E-01
112	Hexadecenoyl-carnitine	HMDB06317	Carnitine and fatty acid metabolism	8.09E+04	2.02E+05	5.15E+04	1.39E+05	1.52E+05	1.57E+05	1.52E+05	2.31E+05	1.18E+05	1.87E+05	9.34E+04	1.44E+05	1.52E+05	1.31E+05	1.16	7.62E-01
113	O-octadecenoyl-L-carnitin	HMDB06351	Carnitine and fatty acid metabolism	1.65E+06	8.94E+05	1.73E+06	6.91E+05	4.87E+05	5.39E+05	4.95E+05	4.28E+05	4.14E+05	6.39E+05	4.80E+05	6.44E+05	6.15E+05	5.60E+05	1.10	2.71E-01
114	acyl-C18:2	HMDB01064	Carnitine and fatty acid metabolism	1.52E+05	4.53E+05	1.36E+05	1.77E+05	2.85E+05	1.93E+05	1.67E+05	9.58E+05	2.43E+05	4.92E+05	1.73E+05	2.89E+05	1.85E+05	2.66E+05	0.69	9.18E-01
115	Pentanoate (valerate)	C00803	Saturated Fatty acids	4.75E+04	2.57E+04	5.45E+04	5.49E+04	4.80E+04	6.20E+04	6.52E+04	4.59E+04	1.83E+04	2.53E+04	6.04E+04	1.04E+05	5.12E+04	4.29E+04	1.20	9.14E-01
116	Hexanoic acid (caproate)	C01585	Saturated Fatty acids	4.91E+05	5.40E+05	5.47E+05	5.02E+05	3.03E+05	2.73E+05	3.27E+05	3.73E+05	3.99E+05	3.66E+05	5.73E+05	3.27E+05	4.32E+05	3.82E+05	1.13	9.63E-01
117	Heptanoic acid	C17714	Saturated Fatty acids	4.10E+05	4.09E+05	4.02E+05	4.37E+05	3.29E+05	3.45E+05	3.32E+05	5.22E+05	4.04E+05	2.55E+05	5.81E+05	3.36E+05	4.05E+05	3.70E+05	1.09	9.45E-01
118	Octanoic acid (caprylate)	C06423	Saturated Fatty acids	1.54E+06	1.77E+06	1.00E+06	1.62E+06	1.08E+06	1.34E+06	1.00E+06	1.43E+06	1.14E+06	1.09E+06	1.84E+06	1.47E+06	1.39E+06	1.31E+06	1.06	8.48E-01
119	Nonanoic acid (pelargona	C01601	Saturated Fatty acids	7.65E+06	8.46E+06	5.71E+06	6.21E+06	4.21E+06	6.59E+06	5.97E+06	6.67E+06	6.76E+06	5.71E+06	6.81E+06	5.81E+06	6.40E+06	6.28E+06	1.02	8.14E-01
120	Decanoic acid (caprate)	C01571	Saturated Fatty acids	3.39E+07	6.78E+07	3.33E+07	3.44E+07	2.24E+07	4.07E+07	3.13E+07	2.95E+07	3.49E+07	2.96E+07	5.78E+07	2.83E+07	3.36E+07	3.22E+07	1.04	9.07E-01
121	Dodecanoic acid	C02679	Saturated Fatty acids	1.21E+07	1.22E+07	1.18E+07	2.00E+07	6.37E+06	2.82E+07	5.48E+06	7.22E+06	8.40E+06	6.56E+06	8.58E+06	1.18E+07	1.19E+07	8.49E+06	1.40	3.36E-01
122	Tetradecanoic acid	C06424	Saturated Fatty acids	3.71E+07	4.25E+07	3.22E+07	6.57E+07	3.06E+07	7.52E+07	4.20E+07	4.31E+07	3.92E+07	3.28E+07	3.30E+07	5.59E+07	4.22E+07	3.61E+07	1.17	5.30E-01
123	Hexadecanoic acid	C00249	Saturated Fatty acids	3.79E+08	4.63E+08	2.95E+08	3.52E+08	3.70E+08	3.78E+08	3.19E+08	3.42E+08	3.31E+08	3.60E+08	3.18E+08	3.57E+08	3.61E+08	3.44E+08	1.05	4.59E-01
124	Octadecanoic acid (steari	C01530	Saturated Fatty acids	1.19E+08	1.42E+08	1.01E+08	1.34E+08	1.04E+08	1.35E+08	1.39E+08	1.15E+08	1.17E+08	1.39E+08	1.13E+08	1.29E+08	1.27E+08	1.23E+08	1.03	8.99E-01
125	Tetradecenoic acid	C08322	Monounsaturated Fatty Acids	5.29E+06	5.27E+06	5.30E+06	9.78E+06	3.97E+06	1.20E+07	4.86E+06	4.82E+06	4.27E+06	3.43E+06	3.27E+06	5.79E+06	5.28E+06	3.85E+06	1.37	1.74E-01
126	Hexadecenoic acid	C08362	Monounsaturated Fatty Acids	1.14E+08	1.61E+08	6.83E+07	1.45E+08	1.17E+08	1.97E+08	1.32E+08	1.01E+08	1.18E+08	9.75E+07	9.87E+07	8.24E+07	1.25E+08	9.81E+07	1.27	1.73E-01
127	Octadecenoic acid (oleic a	C00712	Monounsaturated Fatty Acids	2.45E+08	3.23E+08	2.19E+08	2.53E+08	3.06E+08	2.81E+08	2.27E+08	3.31E+08	1.97E+08	3.23E+08	1.89E+08	1.97E+08	2.67E+08	1.97E+08	1.35	1.64E-01
128	Linoleate	C01595	Poly-unsaturated Fatty Acids	2.28E+08	3.70E+08	1.90E+08	2.94E+08	3.04E+08	2.79E+08	3.29E+08	5.29E+08	2.36E+08	4.00E+08	2.11E+08	3.07E+08	2.99E+08	2.72E+08	1.10	6.63E-01
129	Octadecatrienoic acid (lin	C06427	Poly-unsaturated Fatty Acids	3.65E+07	4.47E+07	2.89E+07	3.68E+07	4.25E+07	4.33E+07	3.08E+07	4.86E+07	4.50E+07	5.18E+07	3.78E+07	2.98E+07	3.97E+07	4.14E+07	0.96	6.70E-01
130	Eicosatetraenoic acid	C00219	Poly-unsaturated Fatty Acids	3.17E+07	7.35E+07	2.92E+07	4.68E+07	6.32E+07	5.21E+07	9.01E+07	8.76E+07	3.30E+07	5.79E+07	2.49E+07	5.73E+07	5.76E+07	4.52E+07	1.28	2.56E-01
131	Eicosapentaenoic acid	C06428	Poly-unsaturated Fatty Acids	3.20E+07	5.53E+07	2.95E+07	4.36E+07	4.09E+07	4.73E+07	4.71E+07	5.41E+07	4.17E+07	5.77E+07	3.24E+07	3.66E+07	4.54E+07	3.91E+07	1.16	7.92E-01
132	Docosahexaenoic acid	C06429	Poly-unsaturated Fatty Acids	6.69E+07	1.21E+08	6.25E+07	8.73E+07	7.39E+07	1.07E+08	9.45E+07	1.19E+08	9.90E+07	1.13E+08	6.79E+07	6.08E+07	9.09E+07	8.34E+07	1.09	6.73E-01
133	Dodecanedioic acid	C02678	Poly-unsaturated Fatty Acids	2.28E+05	2.02E+05	3.28E+05	2.31E+05	2.76E+05	1.44E+05	7.41E+04	2.54E+05	2.18E+05	2.97E+05	2.90E+05	2.07E+05	2.29E+05	2.54E+05	0.90	4.29E-01
134	(8Z-11Z-14Z)-Icosatrienoic	C03242	Essential fatty acids	8.42E+06	1.77E+07	5.98E+06	9.11E+06	1.50E+07	1.29E+07	1.47E+07	9.02E+06	9.17E+06	1.83E+07	5.72E+06	8.27E+06	1.10E+07	8.72E+06	1.26	6.66E-01
135	(5Z-8Z-11Z-14Z-17Z)-Icosa	C06428	Essential fatty acids	3.20E+07	5.53E+07	2.95E+07	4.36E+07	4.09E+07	4.73E+07	4.71E+07	5.41E+07	4.17E+07	5.77E+07	3.24E+07	3.66E+07	4.54E+07	3.91E+07	1.16	7.92E-01
136	(7Z-10Z-13Z-16Z-19Z)-Doc	C16513	Essential fatty acids	1.30E+07	1.99E+07	1.40E+07	1.73E+07	1.43E+07	1.93E+07	1.13E+07	2.25E+07	1.62E+07	1.60E+07	1.21E+07	9.57E+06	1.58E+07	1.40E+07	1.13	2.18E-01
137	3alpha-7alpha-12alpha-Tr	C00695	Bile acids	2.60E+05	1.51E+06	9.65E+05	4.82E+06	2.57E+05	4.28E+05	2.88E+07	4.18E+07	9.20E+05	4.46E+05	4.91E+05	8.75E+06	1.24E+06	7.06E+05	1.75	4.11E-01
138	Taurocholate	C05122	Bile acids	6.55E+05	1.10E+08	2.34E+07	3.36E+08	6.77E+06	4.14E+05	5.56E+08	5.65E+08	2.35E+05	9.58E+06	5.51E+05	4.83E+08	6.67E+07	5.07E+06	13.16	6.24E-01
139	Taurochenodeoxycholate	C05465	Bile acids	1.48E+05	1.77E+07	3.36E+06	3.24E+07	7.41E+05	3.93E+04	1.19E+08	9.52E+07	5.56E+04	1.57E+06	1.49E+05	5.68E+07	1.05E+07	8.60E+05	12.24	4.83E-01
140	3alpha-12alpha-Dihydrox	C04483	Bile acids	3.28E+05	1.40E+06	1.14E+06	3.76E+05	2.26E+05	2.53E+05	7.83E+05	1.04E+06	2.93E+05	1.93E+05	2.72E+05	3.28E+05	5.80E+05	2.82E+05	2.05	1.04E-01
141	13(S)-HODE	C14762	Arachidonate metabolism	4.23E+05	8.24E+05	1.38E+06	8.37E+05	7.92E+05	1.12E+06	6.39E+05	1.88E+06	6.89E+05	9.51E+05	9.42E+05	8.34E+05	8.31E+05	8.88E+05	0.94	5.92E-01
142	9(S)-HODE	C14767	Arachidonate metabolism	2.15E+05	3.33E+05	4.44E+05	3.85E+05	2.80E+05	2.93E+05	2.54E+05	4.33E+05	2.91E+05	3.04E+05	1.81E+05	2.49E+05	3.13E+05	2.70E+05	1.16	1.50E-01
143	11(R)-HPETE/Leukotriene	C14820	Arachidonate metabolism	3.89E+04	5.15E+04	7.51E+04	4.38E+04	1.44E+05	3.74E+04	6.35E+04	1.19E+05	4.33E+04	4.27E+04	7.55E+04	4.77E+04	5.75E+04	4.55E+04	1.26	3.77E-01
144	Prostaglandin A2	C05953	Arachidonate metabolism	4.61E+04	9.14E+04	1.42E+05	5.26E+04	1.31E+05	1.25E+05	1.04E+05	1.31E+05	5.84E+04	7.50E+04	6.77E+04	9.18E+04	1.14E+05	7.13E+04	1.60	1.59E-01
145	Prostaglandin D2/Thromb	C00696	Arachidonate metabolism	1.53E+05	3.52E+04	8.79E+04	5.02E+05	1.09E+05	8.69E+04	7.84E+04	7.03E+04	2.33E+05	8.24E+04	1.04E+05	1.40E+05	8.74E+04	1.22E+05	0.71	9.97E-01
146	Prostaglandin F2alpha/be	C00639	Arachidonate metabolism	3.06E+03	5.46E+03	7.31E+03	5.58E+03	1.83E+04	5.19E+03	2.21E+04	1.50E+04	3.84E+03	4.98E+03	7.18E+03	1.36E+04	6.45E+03	6.08E+03	1.06	4.85E-01
147	Prostaglandin D3 (isobars	C13802	Arachidonate metabolism	5.97E+04	5.08E+04	5.20E+04	1.17E+05	5.51E+04	6.91E+04	1.05E+05	4.88E+04	2.26E+05	1.12E+05	6.22E+04	4.69E+04	5.74E+04	8.72E+04	0.66	1.95E-01
148	Prostaglandin G2	C05956	Arachidonate metabolism	8.94E+03	1.04E+04	1.22E+04	1.35E+04	1.77E+04	1.61E+04	3.51E+04	2.99E+04	8.34E+03	1.75E+04	1.82E+04	1.86E+04	1.48E+04	1.79E+04	0.83	6.64E-01
149	Triacanthine	C08435	Other	9.38E+07	6.48E+07	6.26E+07	7.39E+07	6.43E+07	1.09E+08	5.22E+07	8.97E+07	6.06E+07	7.30E+07	8.57E+07	7.04E+07	6.93E+07	7.17E+07	0.97	7.18E-01
150	2-Methyleneglutarate	C02930	Other	4.45E+06	5.47E+06	3.69E+06	6.04E+06	8.18E+06	6.56E+06	5.94E+06	6.65E+06	6.02E+06	8.07E+06	6.56E+06	8.58E+06	5.99E+06	7.31E+06	0.82	1.10E-01
151	Peptide tryptophan	C02732	Other	1.93E+05	2.72E+05	3.22E+04	2.06E+05	4.34E+05	3.39E+05	4.43E+05	2.47E+05	1.11E+05	3.23E+05	1.78E+05	3.59E+05	2.60E+05	2.51E+05	1.04	7.35E-01
152	Methylenediurea	C06381	Other	8.04E+05	6.93E+05	7.49E+05	7.72E+05	6.07E+05	5.23E+05	6.40E+05	1.15E+06	7.01E+05	6.53E+05	8.14E+05	6.08E+05	7.21E+05	6.77E+05	1.07	6.43E-01
153	beta-Butoxyethyl nicotinat	C13138	Other	1.44E+05	9.64E+04	1.03E+05	1.34E+05	1.67E+05	1.13E+05	7.41E+04	9.74E+04	2.00E+05	2.44E+05	1.75E+05	1.16E+05	1.08E+05	1.87E+05	0.57	1.69E-02
154	2-Deoxy-alpha-D-glucosid	C03573	Other	1.11E+05	8.04E+04	9.82E+04	8.84E+04	1.77E+05	7.61E+04	2.01E+05	9.11E+04	9.71E+04	5.58E+04	8.02E+04	1.51E+05	9.46E+04	8.87E+04	1.07	5.03E-01
155	cis-p-Coumarate	C06738	Other	3.78E+05	3.88E+05	3.52E+05	5.49E+05	2.95E+05	3.33E+05	4.00E+05	4.15E+05	5.57E+05	3.37E+05	4.30E+05	4.96E+05	3.83E+05	4.63E+05	0.83	2.12E-01
156	N-Acyl-D-mannosaminolac	C03792	Other	7.25E+05	8.25E+05	7.05E+05	9.61E+05	1.00E+06	1.06E+06	9.66E+05	1.04E+06	9.50E+05	1.08E+06	1.09E+06	8.09E+05	9.63E+05	1.02E+06	0.95	4.15E-01
157	N6-Methyl-L-lysine	C02728	Other	4.18E+05	3.08E+05	2.47E+05	5.08E+05	3.35E+05	4.24E+05	1.96E+05	3.11E+05	4.25E+05	4.59E+05	5.32E+05	4.53E+05	3.23E+05	4.56E+05	0.71	4.59E-02
158	Catechin	C17590	Other	2.66E+05	1.12E+06	2.79E+05	9.06E+05	1.19E+06	4.53E+05	8.76E+05	1.31E+06	3.44E+05	1.12E+06	8.60E+05	1.08E+06	8.91E+05	9.70E+05	0.92	8.41E-01
159	D-Ribitol 5-phosphate	C01068	Other	9.88E+04	9.60E+05	2.13E+05	7.70E+05	3.67E+05	3.16E+05	2.75E+05	5.62E+05	1.04E+06	1.08E+06	5.96E+05	9.17E+05	3.42E+05	9.78E+05	0.35	2.03E-02
160	Riboflavin	C00255	Other	9.05E+04	9.18E+04	6.18E+04	1.82E+05	8.01E+04	6.32E+04	6.71E+05	2.13E+05	6.95E+04	8.80E+04	5.77E+04	2.12E+05	9.11E+04	7.88E+04	1.16	5.04E-01
161	Glucosinolate	C17260	Other	1.42E+06	9.74E+05	9.66E+05	9.79E+05	9.30E+05	9.08E+05	9.06E+05	6.45E+05	1.31E+06	1.06E+06	1.01E+06	8.23E+05	9.48E+05	1.04E+06	0.91	5.15E-01
162	S-Acylglutathione	C02589	Other	1.83E+04	3.90E+04	1.65E+04	5.15E+04	7.42E+04	2.28E+04	5.51E+04	1.99E+04	3.28E+04	4.56E+04	4.11E+04	4.11E+04	3.09E+04	4.11E+04	0.75	7.94E-01
163	Pyridoxamine	C00534	Other	1.34E+06	9.09E+05	1.31E+06	1.29E+06	8.73E+05	1.26E+06	6.10E+05	1.75E+06	1.13E+06	1.42E+06	1.26E+06	1.17E+06	1.27E+06	1.21E+06	1.05	6.81E-01
164	9-Oxononanoic acid	C16322	Other	3.96E+05	3.24E+05	4.57E+05	5.19E+05	4.37E+05	3.22E+05	2.84E+05	3.38E+05	3.79E+05	3.77E+05	4.02E+05	3.45E+05	3.67E+05	3.78E+05	0.97	8.35E-01
165	N-Amidino-L-aspartate	C03139	Other	2.07E+06	2.74E+06	2.29E+06	3.42E+06	2.73E+06	3.82E+06	2.65E+06	2.54E+06	3.04E+06	3.61E+06	3.78E+06	3.04E+06	2.69E+06	3.32E+06	0.81	1.00E-01

166	N-Acyl-D-glutamate	C06379	Other	3.37E+05	2.49E+05	1.46E+05	5.31E+05	3.18E+05	4.91E+05	1.72E+05	3.07E+05	5.51E+05	3.56E+05	2.70E+05	3.27E+05	3.13E+05	3.41E+05	0.92	4.98E-01
167	Hydroxyacetone phospha	C03505	Other	2.30E+05	2.62E+05	8.14E+04	2.57E+05	1.97E+05	3.47E+05	2.72E+05	2.24E+05	2.80E+05	3.21E+05	2.93E+05	2.71E+05	2.44E+05	2.86E+05	0.85	1.78E-01
168	N-Acetylmethionine	C02712	Other	1.63E+05	9.61E+04	1.76E+05	3.45E+05	1.44E+05	1.50E+05	1.13E+05	4.87E+05	5.02E+05	2.49E+05	3.75E+05	3.43E+05	1.56E+05	3.59E+05	0.44	6.96E-02
169	10-Hydroxydecanoic acid	C02774	Other	3.97E+05	1.46E+06	4.35E+05	8.77E+05	9.09E+05	8.84E+05	7.20E+05	1.79E+06	5.46E+05	7.11E+05	6.49E+05	6.89E+05	8.80E+05	6.69E+05	1.32	2.73E-01
170	2-Oxo-7-methylthioheptan	C17220	Other	1.35E+06	8.76E+05	1.10E+06	1.53E+06	9.20E+05	1.50E+06	7.29E+05	1.73E+06	1.46E+06	1.37E+06	1.88E+06	1.27E+06	1.23E+06	1.41E+06	0.87	2.16E-01
171	L-Homocitrulline	C02427	Other	8.71E+04	6.77E+04	1.37E+05	1.18E+05	8.20E+04	1.04E+05	1.32E+05	1.12E+05	5.30E+04	1.73E+05	5.10E+04	6.66E+04	1.08E+05	5.98E+04	1.81	4.30E-01
172	TMAO (trimethylamine N-c	C01104	Bacterial metabolite	2.13E+06	2.15E+06	8.77E+06	1.78E+06	1.32E+06	2.91E+06	2.16E+06	4.05E+06	3.38E+06	2.76E+06	2.52E+06	2.29E+06	2.15E+06	2.64E+06	0.82	7.43E-01

Supplemental Table 3. Morphological characteristics of mice treated with DOXO with and without oral MitoQ supplementation

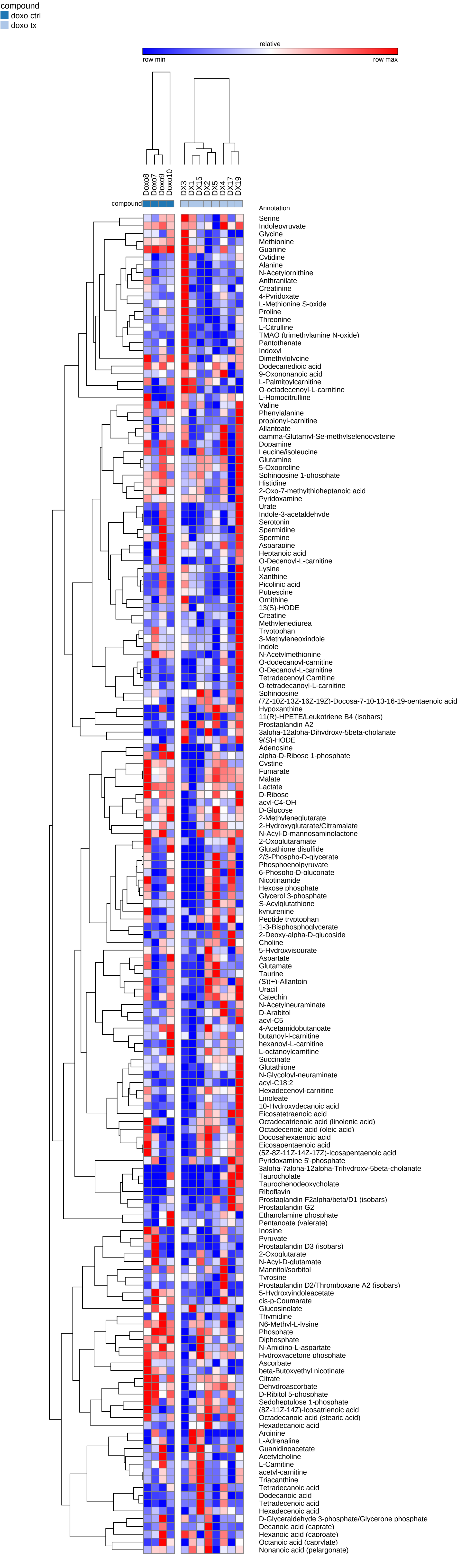
	DOXO (n = 4)	DOXO + MitoQ (n = 5)	*P* Value
Heart (mg)	120.0 ± 7.1	130.0 ± 7.8	0.355
Quadriceps (mg)	285.2 ± 20.2	300.0 ± 9.1	0.561
Gastrocnemius (mg)	226.8 ± 23.5	235.0 ± 6.5	0.772
Epididymal white adipose tissue (mg)	296.0 ± 32.1	342.5 ± 78.8	0.083

Data are the mean ± standard error of the mean.



Supplemental Figure S1. Principal components analysis based on partial least squares-discriminant analysis of metabolites in plasma from DOXO and Sham treated mice.

Supplemental Figure S2. Heat map analysis of metabolites in plasma from DOXO and Sham treated mice.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doxo ctrl
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relative
row min
row max
Doxo8
Doxo7
Doxo9
Doxo10
DX3
DX1
DX15
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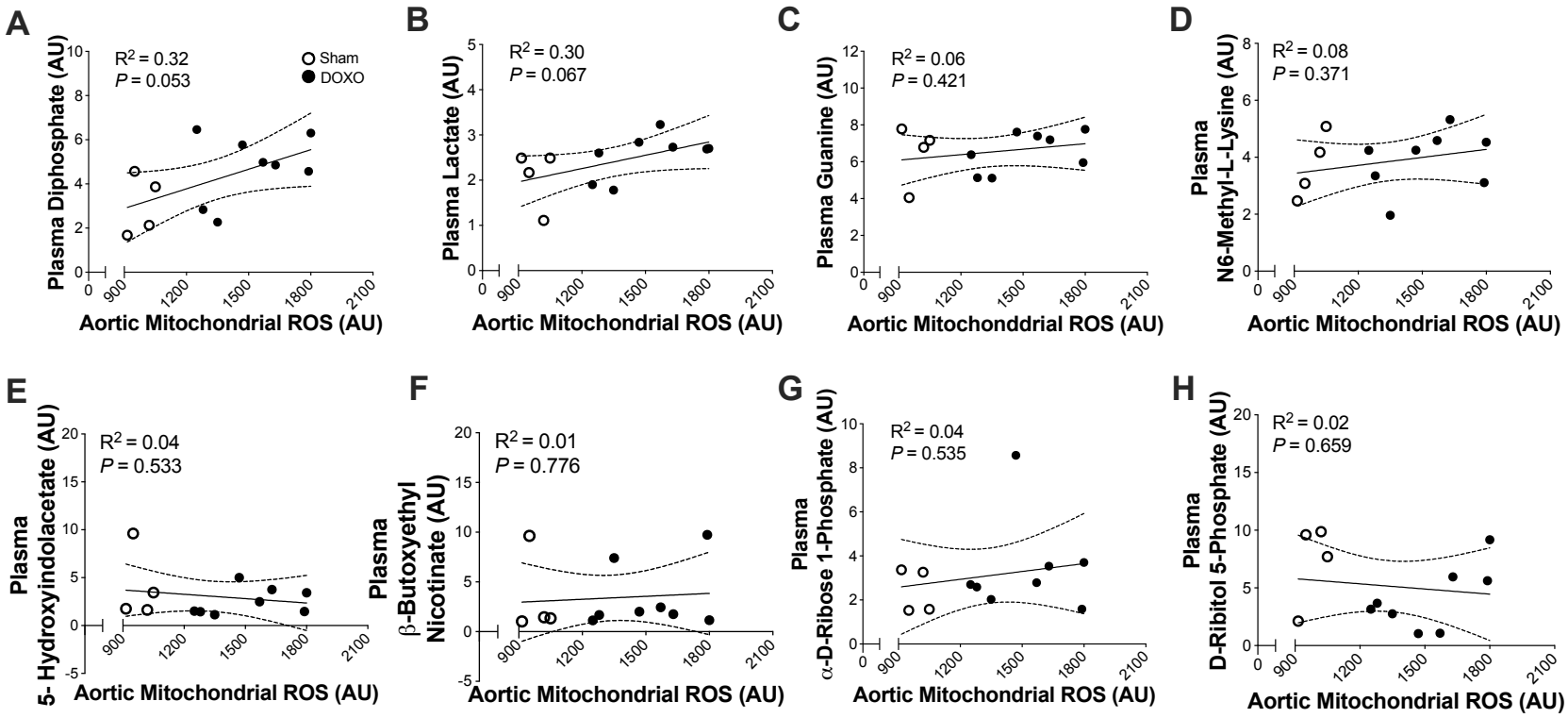
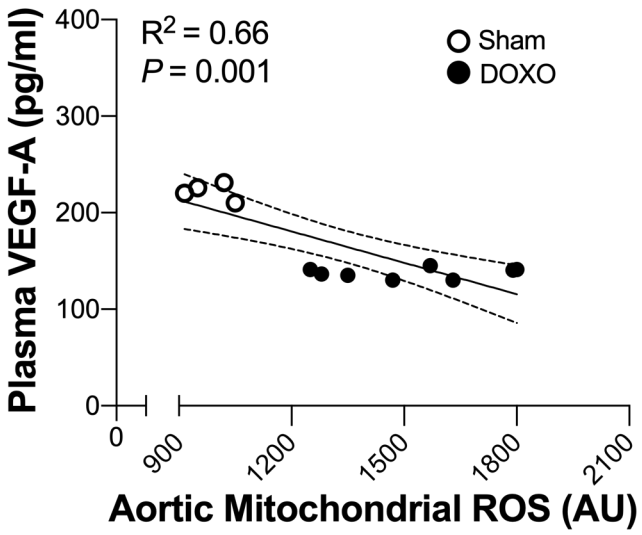
DX4
DX17
DX19
compound
Annotation
Serine
Indolepyruvate
Glycine
Methionine
Guanine
Cytidine
Alanine
N-Acetylornithine
Anthranilate
Creatinine
4-Pyridoxate
L-Methionine S-oxide
Proline
Threonine
L-Citrulline
TMAO (trimethylamine N-oxide)
Pantothenate
Indoxyl
Dimethylglycine
Dodecanedioic acid
9-Oxononanoic acid
L-Palmitoylcarnitine
O-octadecenoyl-L-carnitine
L-Homocitrulline
Valine
Phenylalanine
propionyl-carnitine
Allantoate
gamma-Glutamyl-Se-methylselenocysteine
Dopamine
Leucine/isoleucine
Glutamine
5-Oxoproline
Sphingosine 1-phosphate
Histidine
2-Oxo-7-methylthioheptanoic acid
Pyridoxamine
Urate
Indole-3-acetaldehyde
Serotonin
Spermidine
Spermine
Asparagine
Heptanoic acid
O-Decenoyl-L-carnitine
Lysine
Xanthine
Picolinic acid
Putrescine
Ornithine
13(S)-HODE
Creatine
Methylenediurea
Tryptophan
3-Methyleneoxindole
Indole
N-Acetylmethionine
O-dodecanoyl-carnitine
O-Decanoyl-L-carnitine
Tetradecenoyl Carnitine
O-tetradecanoyl-L-carnitine
Sphingosine
(7Z-10Z-13Z-16Z-19Z)-Docosa-7-10-13-16-19-pentaenoic acid
Hypoxanthine
11(R)-HPETE/Leukotriene B4 (isobars)
Prostaglandin A2
3alpha-12alpha-Dihydroxy-5beta-cholanate
9(S)-HODE
Adenosine
alpha-D-Ribose 1-phosphate
Cystine
Fumarate
Malate
Lactate
D-Ribose
acyl-C4-OH
D-Glucose
2-Methyleneglutarate
2-Hydroxyglutarate/Citramalate
N-Acyl-D-mannosaminolactone
2-Oxoglutaramate
Glutathione disulfide
2/3-Phospho-D-glycerate
Phosphoenolpyruvate
6-Phospho-D-gluconate
Nicotinamide
Hexose phosphate
Glycerol 3-phosphate
S-Acylglutathione
kynurenine
Peptide tryptophan
1-3-Bisphosphoglycerate
2-Deoxy-alpha-D-glucoside
Choline
5-Hydroxyisourate
Aspartate
Glutamate
Taurine
(S)(+)-Allantoin
Uracil
Catechin
N-Acetylneuraminate
D-Arabitol
acyl-C5
4-Acetamidobutanoate
butanoyl-l-carnitine
hexanoyl-L-carnitine
L-octanoylcarnitine
Succinate
Glutathione
N-Glycoloyl-neuraminate
acyl-C18:2
Hexadecenoyl-carnitine
Linoleate
10-Hydroxydecanoic acid
Eicosatetraenoic acid
Octadecatrienoic acid (linolenic acid)
Octadecenoic acid (oleic acid)
Docosahexaenoic acid
Eicosapentaenoic acid
(5Z-8Z-11Z-14Z-17Z)-Icosapentaenoic acid
Pyridoxamine 5'-phosphate
3alpha-7alpha-12alpha-Trihydroxy-5beta-cholanate
Taurocholate
Taurochenodeoxycholate
Riboflavin
Prostaglandin F2alpha/beta/D1 (isobars)
Prostaglandin G2
Ethanolamine phosphate
Pentanoate (valerate)
Inosine
Pyruvate
Prostaglandin D3 (isobars)
2-Oxoglutarate
N-Acyl-D-glutamate
Mannitol/sorbitol
Tyrosine
Prostaglandin D2/Thromboxane A2 (isobars)
5-Hydroxyindoleacetate
cis-p-Coumarate
Glucosinolate
Thymidine
N6-Methyl-L-lysine
Phosphate
Diphosphate
N-Amidino-L-aspartate
Hydroxyacetone phosphate
Ascorbate
beta-Butoxyethyl nicotinate
Citrate
Dehydroascorbate
D-Ribitol 5-phosphate
Sedoheptulose 1-phosphate
(8Z-11Z-14Z)-Icosatrienoic acid
Octadecanoic acid (stearic acid)
Hexadecanoic acid
Arginine
L-Adrenaline
Guanidinoacetate
Acetylcholine
L-Carnitine
acetyl-carnitine
Triacanthine
Tetradecanoic acid
Dodecanoic acid
Tetradecenoic acid
Hexadecenoic acid
D-Glyceraldehyde 3-phosphate/Glycerone phosphate
Decanoic acid (caprate)
Hexanoic acid (caproate)
Octanoic acid (caprylate)
Nonanoic acid (pelargonate)

Supplemental Figure 3.



Supplemental Figure S3. Linear regression comparing plasma metabolites significantly altered by doxorubicin (DOXO) and aortic mitochondrial reactive oxygen species (ROS). Linear regression of plasma concentrations of **A)** diphosphate, **B)** lactate, **C)** guanine, **D)** N6-Methyl-L-Lysine, **E)** 5-hydroxyindolacetate, **F)** beta-Butoxyethyl Nicotinate, **G)** alpha-D-Ribose 1 Phosphate, and **H)** D-Ribitol 5-Phosphate (derived from plasma metabolomics analyses), relative to aortic mitochondrial ROS (derived by electron paramagnetic resonance spectroscopy analyses). Dashed lines represent 95% confidence intervals. AU, amplitude units.



Supplemental Figure S4. Linear regression comparing plasma concentration of VEGF-A in Doxorubicin (DOXO) and Sham treated mice with aortic mitochondrial reactive oxygen species (ROS). Linear regression of plasma concentration of VEGF-A (derived from dot blot array analyses) relative to aortic mitochondrial ROS (derived by electron paramagnetic resonance spectroscopy analyses). Dashed lines represent the 95% confidence interval. AU, amplitude units.

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