SUPPLEMENTAL MATERIAL

Metabolomic analysis of pressure-overloaded and infarcted mouse hearts

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A. Detailed Methods

Echocardiographic Assessment: Transthoracic echocardiography of the left ventricle was performed as previously described[1] with adjustments for the Vevo 770 echocardiography machine. Body temperature was maintained (36.5°C-37.5°C) using a rectal thermometer interfaced with a servo-controlled heat lamp. Mice (C57BL/6J) were anesthetized with 2% isoflurane, maintained under anesthesia with 1.5% isoflurane, and examined. The mouse was placed chest up on an examination board interfaced with the Vevo 770. Next, depilatory cream

was applied to the mouse's chest and wiped clean to remove all hair in the area of interest. The 707-B (30 MHz) scan head was used to obtain 2D images of the parasternal long axis. M-modes were taken from the same image windows. The probe was then rotated to acquire a short axis view of the heart. Beginning at the level of the papillary muscles and moving apically, serial 2D images were taken every millimeter. Diastolic and systolic volumes were acquired by applying Simpson's rule of discs to the serially acquired short axis images. Stroke volume (SV) was calculated as: Diastolic volume - Systolic Volume. Ejection Fraction was calculated as: (SV/Diastolic Volume)*100%. Cardiac output was determined by: SV*HR. All measurements were taken by utilizing the Vevo 770's rail system to maintain probe placement and allow for precise adjustments of position. Left ventricular diameters during diastole (LVIDd) and left ventricular diameter during systole (LVIDs) and heart rate (HR) were determined from long axis M-modes. Left ventricular fractional shortening (%FS) was calculated as: ((LVIDd-LVIDs)/LVIDd)*100%. Relative wall thickness was calculated as (diastolic posterior wall thickness + diastolic anterior wall thickness)/LVIDd. Velocity of circumferential shortening corrected for heart rate (Vcfc) was calculated as ((FS/ET)/√RR).

Transverse Aortic Constriction (TAC) surgery: The TAC surgery was conducted in 2.75–3.25-month-old, male C57BL/6J mice by constriction of the transverse aorta as described[2, 3] and in accordance with the University of Louisville Animal Care and Use Committee. Briefly, C57BL/6J mice were anesthetized with ketamine (50 mg/kg, intra-peritoneal) and pentobarbital (50 mg/kg, intra-peritoneal), orally intubated with polyethylene-60 tubing, and ventilated (Harvard Apparatus Rodent Ventilator, model 845) with oxygen supplementation. The aorta was visualized through an intercostal incision and a 7-0 nylon suture was looped around the aorta between the brachiocephalic and left common carotid arteries. The suture was tied around a 27-gauge needle (put adjacent to the aorta) to constrict the aorta to a reproducible diameter; the needle was removed, leaving a discrete region of stenosis (TAC mice), and the chest was

closed. Mice were extubated upon recovery of spontaneous breathing and were allowed to recover in warm, clean cages supplemented with oxygen. Analgesia (ketoprofen, 5 mg/kg, subcutaneous) was given before mice recovered from anesthesia (and by 24 and 48 hours later). Sham age-matched mice were subjected to the same procedure except the suture was only passed underneath the aorta and not tied off.

Myocardial Infarction Studies: Adult (4.25-month-old) male C57BL/6J mice were subjected to nonreperfused, *in vivo* coronary ligation to induce heart failure, as described previously[1, 4-6] and in accordance with the University of Louisville Animal Care and Use Committee. Using sterile technique, mice were subjected to a thoracotomy and the left coronary artery visualized and permanently occluded with 7-0 silk suture with the aid of a dissecting microscope. After ligation, the chest and skin were closed using 4-0 silk and polyester sutures respectively. Upon recovery of spontaneous respiration, the intubation tube was removed and mice were allowed to recover in a temperature-controlled area supplemented with 100% oxygen.

Sample preparation for metabolomic analysis: Upon sacrifice, hearts were washed for 5 s in ice-cold PBS to remove excess blood (and metabolites therein) and then snap-frozen in liquid nitrogen. At the time of analysis, sample metabolites were extracted with methanol. A recovery standard was introduced at the beginning of the extraction process. The extracted samples were split into equal parts for analysis on the GC/MS and LC/MS/MS platforms. Also included were several technical replicate samples created from a homogeneous pool containing a small amount of all study samples. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. Each sample was then frozen and dried under vacuum. Samples were then prepared for the appropriate instrument, either LC/MS or GC/MS.

LC/MS: The LC/MS portion of the platform was based on a Waters ACQUITY UPLC and a Thermo-Finnigan LTQ mass spectrometer, which consisted of an electrospray ionization (ESI)

source and linear ion-trap (LIT) mass analyzer. The sample extract was split into two aliquots, dried, then reconstituted in acidic or basic LC-compatible solvents, each of which contained 11 or more injection standards at fixed concentrations. One aliquot was analyzed using acidic positive ion optimized conditions and the other using basic negative ion optimized conditions in two independent injections using separate dedicated columns. Extracts reconstituted in acidic conditions were gradient eluted using water and methanol both containing 0.1% formic acid, while the basic extracts, which also used water/methanol, contained 6.5 mM ammonium bicarbonate. The MS analysis alternated between MS and data-dependent MS^2 scans using dynamic exclusion.

GC/MS: In addition to the LIT front end, the LC/MS portion of the platform had a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer backend. For ions with counts greater than 2 million, an accurate mass measurement could be performed. Accurate mass measurements could be made on the parent ion as well as fragments. The typical mass error was less than 5 ppm. Fragmentation spectra (MS/MS) were typically generated in data-dependent manner, but if necessary, targeted MS/MS was employed, such as in the case of lower level signals.

The samples destined for GC/MS analysis were re-dried under vacuum desiccation for a minimum of 24 h prior to being derivatized under dried nitrogen using bistrimethyl-silyl-triflouroacetamide (BSTFA). The GC column was 5% phenyl and the temperature ramp is from 40° to 300° C in a 16 min period. Samples were analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization. The instrument was tuned and calibrated for mass resolution and mass accuracy on a daily basis. The information output from the raw data files was automatically extracted as discussed below.

Absolute values of BCAAs, polyamines, and prolylhydroxyproline were measured in mouse hearts and plasma samples by GC/MS using EZ:Faast GCMS Physiological amino acid analysis kit (Phenomenex KG0-7166) according to manufacturer's instructions and the literature[7, 8]. Internal standards used for these assays include: 1,4-butane-d8-diamine (10 µM), spermidine-(butane-d8) (10 µM), 3,4-dehydro-L-proline (10 µM), and L-norvaline (100 µM). Mouse heart samples were pulverized under liquid nitrogen, and ~25 mg powder was homogenized in a 10× volume (w/v) of 0.2 M perchloric acid, along with internal standards. The suspension was sonicated in an ultrasonic bath for 30 min at 25°C, and centrifuged at 16,000×g at 4°C for 20 min. The supernatant was then transferred to a clean tube and centrifuged again under the same conditions. Then, 200 µl of the supernatant from heart was used for analysis. For plasma, 200 µl of each plasma sample was used, as suggested by the manufacturer (Phenomenex KG0-7166).

QA/QC: A data normalization step was performed to correct variation resulting from instrument inter-day tuning differences. Each compound was corrected in run-day blocks by registering the medians to equal one (1.00) and normalizing each data point proportionately (block correction). Instrument variability was determined by calculating the median relative standard deviation (RSD) for the internal standards that were added to each sample prior to injection into the mass spectrometers. Process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the samples, which are technical replicates of pooled samples. Values for instrument and total process variability were 5% for internal standards and 15% for endogenous biochemicals, respectively. For QA/QC purposes, additional samples were included with each day's analysis. Furthermore, a selection of QC compounds was added to every sample, including those under test. These compounds were carefully chosen so as not to interfere with measurement of the endogenous compounds.

Metabolite identification: The 288 metabolites measured included known compounds detectable by the Metabolon GC or LC/MS platforms. Compounds were identified by comparison to library entries of purified standards. The combination of chromatographic properties and mass spectra gave an indication of a match to the specific compound or an isobaric entity, and the Metabolon Laboratory Information Management System (LIMS) was used for determining such analytical characteristics of metabolites. At the time of analysis, more than 1000 commercially available purified standard compounds had been registered into LIMS.

A variety of curation procedures were carried out to ensure that a high quality data set was made available for statistical analysis and data interpretation. The QC and curation processes were designed to ensure accurate and consistent identification of true chemical entities, and to remove those representing system artifacts, mis-assignments, and background noise. Visualization and interpretation software were used to confirm the consistency of peak identification among the various samples. Library matches for each compound were checked for each sample and corrected if necessary.

The bioinformatics system consisted of four major components, the LIMS system, data extraction and peak-identification software, data processing tools for QC and compound identification, and a collection of information interpretation and visualization tools. The purpose of the LIMS system was to enable fully auditable laboratory automation through a secure, easy to use, and highly specialized system. The scope of the LIMS system encompasses sample accessioning, sample preparation and instrumental analysis and reporting and advanced data analysis. Some of the subsequent software systems were grounded in the LIMS data structures, which have been modified to leverage and interface with the Metabolon information extraction and data visualization systems, as well as other data analysis software such as Metaboanalyst (http://www.metaboanalyst.ca/)[9, 10].

Metabolomic analysis and additional statistical considerations: For screening for significant changes in metabolites, missing values were imputed by replacing missing values with half of the minimum value in the original data or by imputing the minimum value after rescaling the median to 1. The latter of these imputation methods was used only for constructing z-score plots. To identify and remove variables that were unlikely to be of use when modeling the data, data filtering by interquartile range was performed[11]. The data were then quantile-normalized within replicates after log transformation. This step was performed to transform the intensity values so that the distribution is more Gaussian. Further univariate and multivariate testing was performed exactly as outlined by Xia et al.[9, 10], using Metaboanalyst 2.0 software (http://www.metaboanalyst.ca/)[9, 10, 12]. Metabolic pathway analysis (MetPA) was performed as described previously[13]. Briefly, overrepresentation analysis was used for pathway enrichment analysis and relative between-ness centrality was used for topographical analysis. Data in Tables are expressed as mean ± SEM. Multiple groups (TAC and sham) were compared using ANOVA in Metaboanalyst, followed by Bonferroni or Tukey post-tests. Unpaired t test (Metaboanalyst) was used for direct comparisons between MI and corresponding sham groups. A p value less than 0.05 was considered significant.

B. Supplementary References

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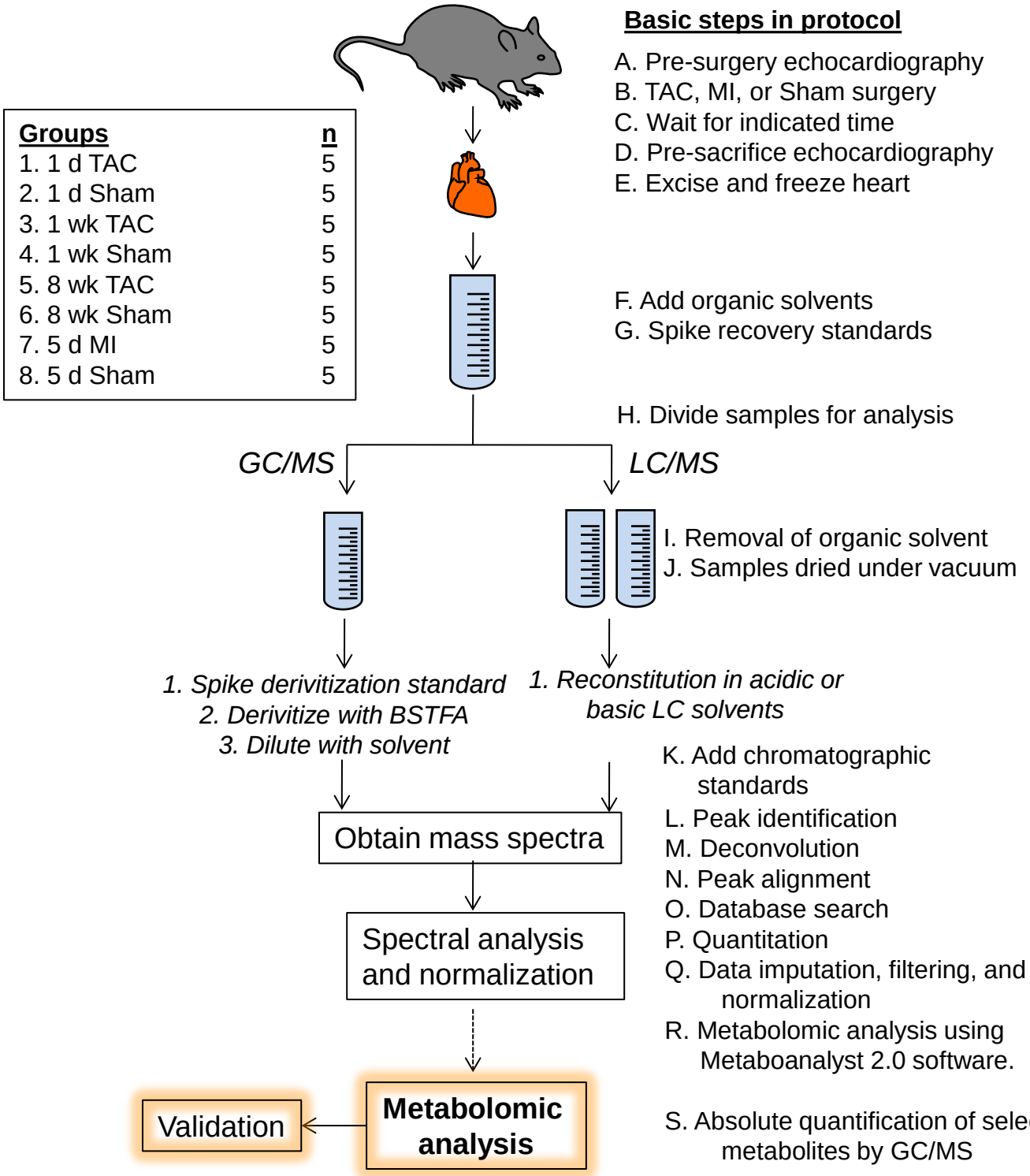
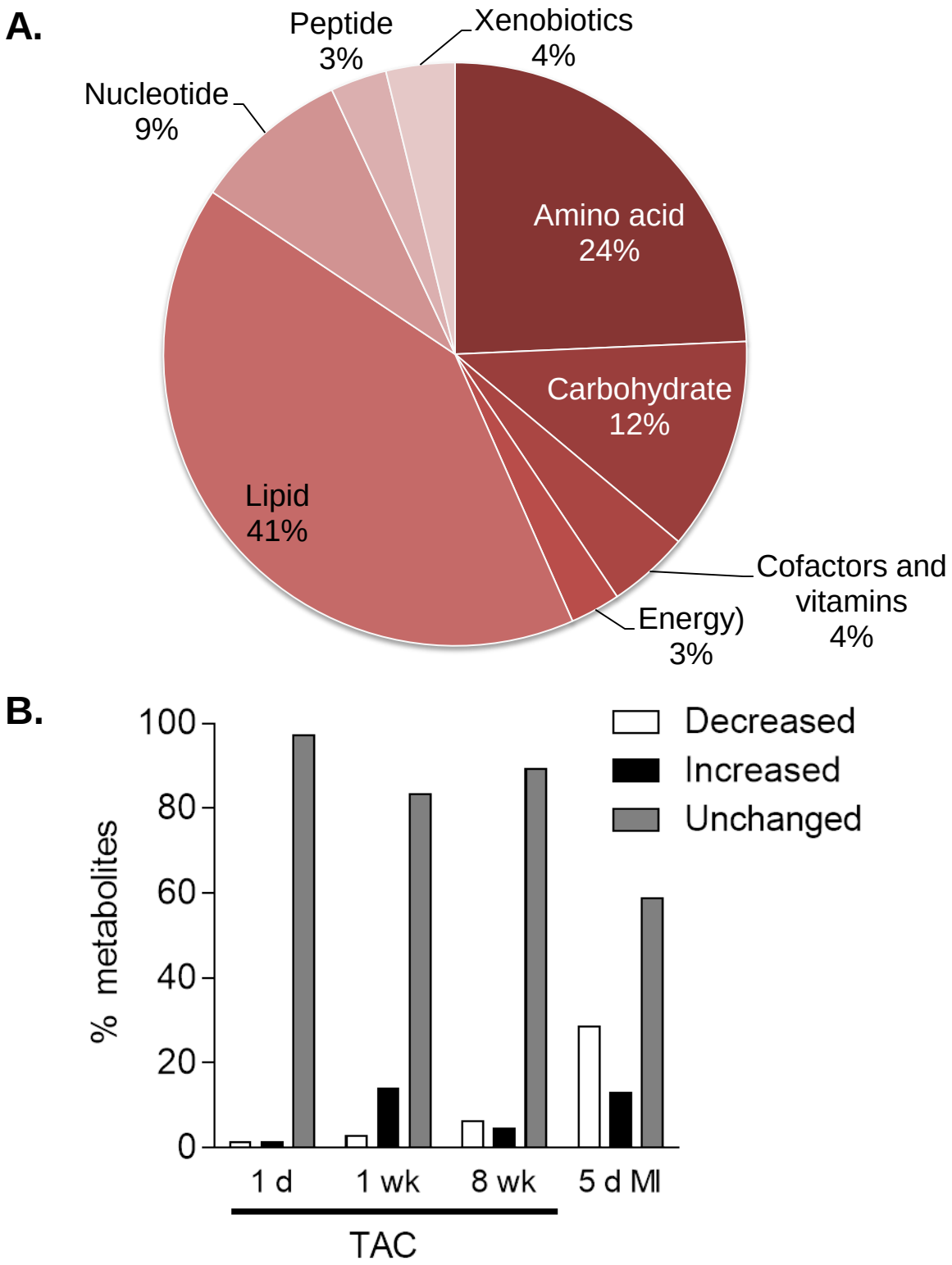
Supplemental Figures

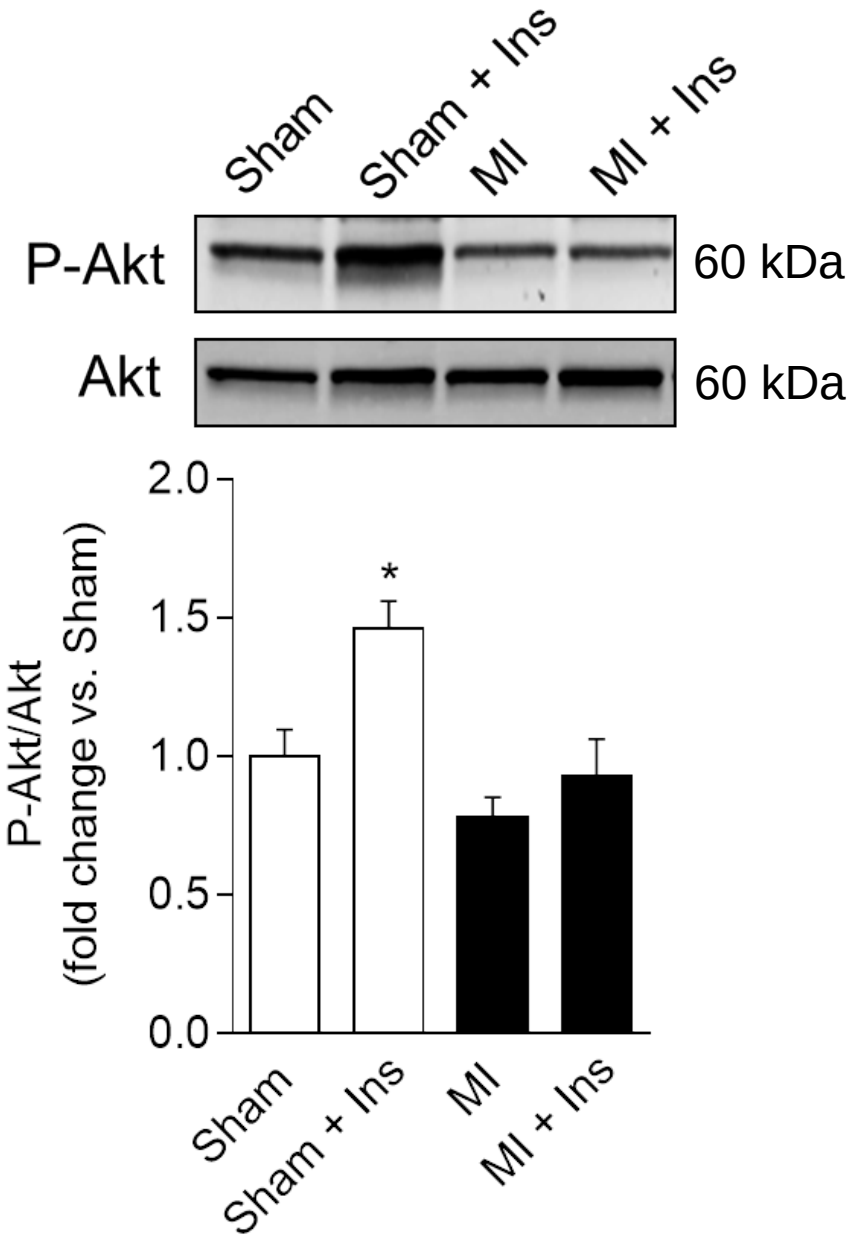
Supplemental Fig. I



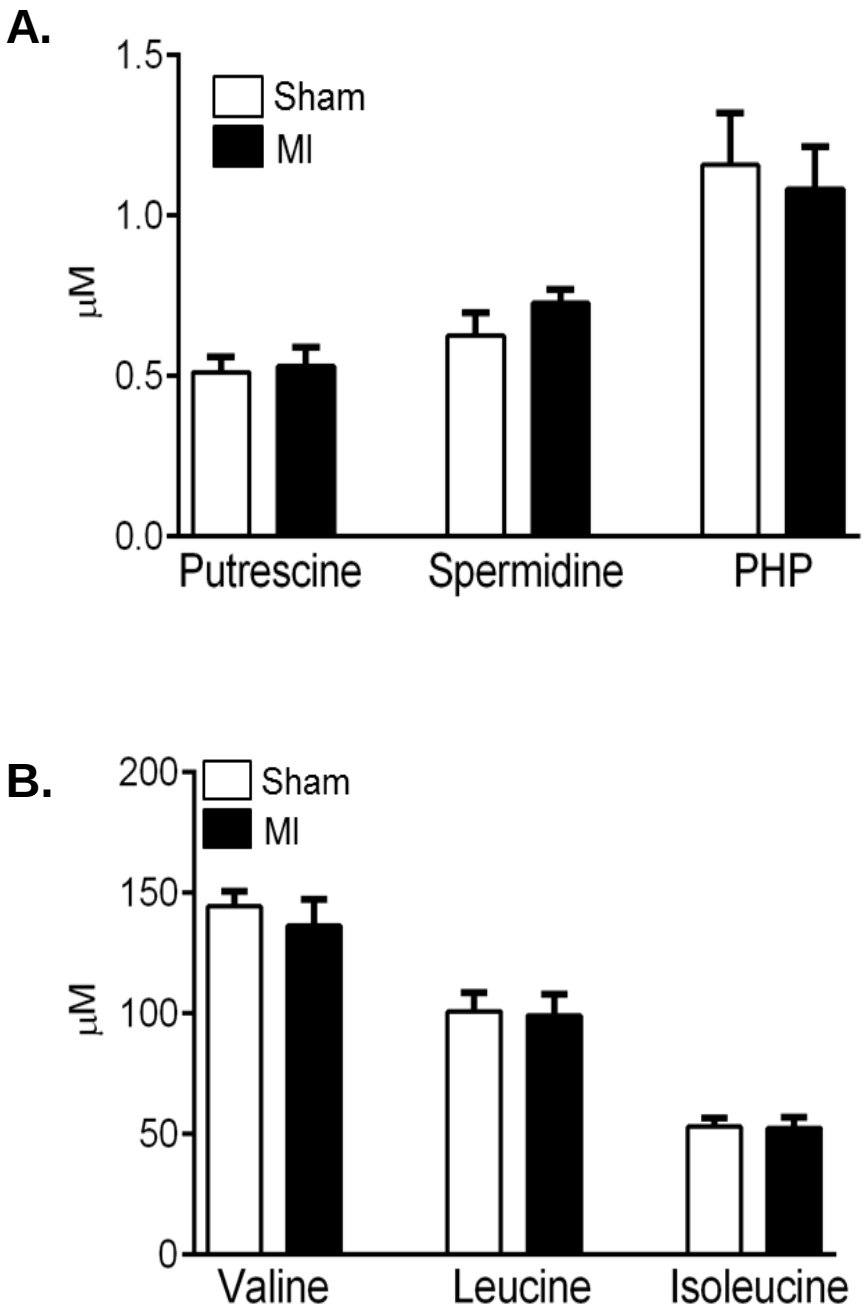
Supplemental Fig. I: Flow chart illustrating procedure for metabolomic profiling of heart tissue. Mice were subjected to TAC or MI for the respective time, after a pre-surgery echo. For each group, there was a respective sham group. The heart was then procured, and metabolites were extracted. The samples were divided for GC/MS or LC/MS analysis. Following spectral analysis, the data were imputed, normalized, and analyzed using Metaboanalyst 2.0 software. Steps F–P were performed by Metabolon, Inc. The absolute abundance of certain metabolites, such as BCAAs, polyamines, and pro-hydroxy-pro, were then measured by GC/MS in subsequent groups of animals (validation, n = 10 – 13 per group).



Supplemental Fig. II: Metabolite distribution and directional changes in hearts subjected to TAC and MI. (**A**) Class distribution of identified metabolites: Proportion of metabolites from each metabolite superfamily of those identified in the analysis. (**B**) Percentage of metabolites that changed at each time point after TAC or with MI compared to their respective Sham. n = 5 animals per group



Supplemental Fig. III. **Heart failure induces myocardial insulin resistance**. Western blot analysis of phosphorylation of S473 of Akt in mouse hearts: Mice subjected to 5 d of permanent coronary ligation (MI) or sham surgery were administered insulin (1.5 U/kg in 0.9% saline, i.p.) 15 min prior to sacrifice. n = 10-16 per group; *p<0.05 vs. Sham (One-way ANOVA with Tukey multiple comparison post test).



Supplemental Fig. IV. Plasma levels of polyamines, prolylhydroxyproline (pro-hydroxy-pro; PHP), and BCAAs. Mice were subjected to sham surgery or 5 d of MI and polyamines (putrescine and spermidine), PHP, and BCAAs were measured by GC/MS. n = 4-6 per group, $p > 0.05$ between Sham and MI.

Supplemental Table I. List of myocardial metabolites that were significantly different between TAC and Sham groups.

	Sub-pathway metabolism	*Metabolite*	*KEGG*	*p-value*	*–log10(p)*	*FDR*	*1 d TAC / 1 d Sham*	*1 wk TAC / 1 wk Sham*	*8 wk TAC / 8 wk Sham*
Amino Acid	Alanine and aspartate metabolism	aspartate	C00049	0.009381	2.0278	0.063876	1.16±0.12	**1.51±0.05**	0.83±0.15
	Alanine and aspartate metabolism	beta-alanine	C00099	0.014867	1.8278	0.076595	0.81±0.03	**1.83±0.25**	1.20±0.16
	Cysteine, methionine, SAM, taurine metabolism	methionine	C00073	8.28E-05	4.0819	0.006542	0.88±0.02	**1.62±0.19**	**1.31±0.04**
	Cysteine, methionine, SAM, taurine metabolism	hypotaurine	C00519	0.003038	2.5174	0.035934	0.96±0.10	2.25±0.71	1.15±0.14
	Glutathione metabolism	glutathione, oxidized (GSSG)	C00127	0.000928	3.0326	0.021989	0.90±0.04	**1.25±0.04**	1.13±0.05
	Glycine, serine and threonine metabolism	betaine	C00719	0.000605	3.2186	0.019538	0.76±0.02	1.34±0.18	**1.56±0.12**
	Glycine, serine and threonine metabolism	threonine	C00188	0.008613	2.0649	0.063876	1.05±0.07	**1.33±0.08**	0.85±0.10
	Glycine, serine and threonine metabolism	homoserine	C00263, C02926	0.00884	2.0535	0.063876	0.89±0.10	**1.30±0.06**	0.84±0.15
	Glycine, serine and threonine metabolism	glycine	C00037	0.01755	1.7557	0.081556	0.77±0.03	**1.41±0.10**	0.92±0.12
	Glycine, serine and threonine metabolism	serine	C00065	0.022693	1.6441	0.088542	0.94±0.07	**1.64±0.17**	0.96±0.15
	Lysine metabolism	lysine	C00047	0.009329	2.0302	0.063876	0.73±0.02	**1.44±0.14**	0.70±0.17
	Lysine metabolism	pipecolate	C00408	0.019775	1.7039	0.082927	1.46±0.24	1.33±0.21	**1.39±0.14**
	Phenylalanine & tyrosine	tyrosine	C00082	0.00309	2.5101	0.035934	0.92±0.06	**1.35±0.10**	0.74±0.10

metabolism									
Phenylalanine & tyrosine metabolism	phenylalanine	C00079	0.015291	1.8156	0.076703	0.85±0.04	**1.31±0.06**	0.88±0.12
Polyamine metabolism	putrescine	C00134	7.48E-05	4.1264	0.006542	1.62±0.26	3.61±1.04	**3.42±0.47**
Polyamine metabolism	spermidine	C00315	0.00066	3.1808	0.019538	0.94±0.05	**1.27±0.08**	**1.48±0.12**
Polyamine metabolism	5-methylthioadenosine (MTA)	C00170	0.042072	1.376	0.12124	1.03±0.17	1.39±0.23	1.45±0.21
Tryptophan metabolism	C-glycosyltryptophan	-	0.000161	3.7935	0.009533	1.36±0.06	**2.10±0.36**	**1.66±0.20**
Tryptophan metabolism	tryptophan	C00078	0.030268	1.519	0.10549	1.18±0.09	**1.43±0.16**	0.99±0.11
Urea cycle; arginine-, proline-, metabolism	proline	C00148	0.003296	2.482	0.035934	0.97±0.04	**1.54±0.19**	1.05±0.10
Urea cycle; arginine-, proline-, metabolism	assymetric dimethylarginine (ADMA)	C03626	0.003445	2.4628	0.035934	0.62±0.05	**1.89±0.28**	0.81±0.07
Urea cycle; arginine-, proline-, metabolism	ornithine	C00077	0.021918	1.6592	0.088043	0.95±0.07	2.44±0.68	1.17±0.22
Urea cycle; arginine-, proline-, metabolism	citrulline	C00327	0.035575	1.4489	0.1155	1.09±0.04	1.13±0.06	0.81±0.13
Urea cycle; arginine-, proline-, metabolism	arginine	C00062	0.048724	1.3123	0.12831	0.99±0.24	1.58±0.34	1.00±0.24
Valine, leucine and isoleucine metabolism	isobutyrylcarnitine	-	0.017118	1.7666	0.081556	1.11±0.16	0.72±0.16	0.86±0.13
Valine, leucine	leucine	C00123	0.029147	1.5354	0.10376	0.74±0.04	**1.25±0.03**	0.83±0.13

	and isoleucine metabolism								
	Valine, leucine and isoleucine metabolism	isoleucine	C00407	0.043397	1.3625	0.12124	0.92±0.05	**1.28±0.07**	1.02±0.14
Carbohydrate	Glycolysis, gluconeogenesis, pyruvate metabolism	glycerate	C00258	0.01729	1.7622	0.081556	0.93±0.03	**0.73±0.06**	0.75±0.13
	Glycolysis, gluconeogenesis, pyruvate metabolism	pyruvate	C00022	0.022789	1.6423	0.088542	1.05±0.12	0.74±0.07	**0.64±0.12**
	Nucleotide sugars, pentose metabolism	UDP-N-acetylglucosamine	C00043	0.043115	1.3654	0.12124	1.48±0.08	1.66±0.27	1.38±0.20
	Nucleotide sugars, pentose metabolism	xylulose	C00310	0.043646	1.3601	0.12124	0.91±0.06	1.02±0.15	0.76±0.38
	Nucleotide sugars, pentose metabolism	ribose	C00121	0.043677	1.3597	0.12124	0.96±0.05	1.10±0.11	0.67±0.32
Cofactors And Vitamins	Ascorbate and aldarate metabolism	glucarate (saccharate)	C00818	0.019614	1.7074	0.082927	1.24±0.37	2.82±0.78	1.60±0.15
	Pantothenate and CoA metabolism	pantothenate	C00864	0.005268	2.2784	0.048018	1.40±0.18	0.96±0.08	0.74±0.10
	Pantothenate and CoA metabolism	phosphopantetheine	C01134	0.02761	1.5589	0.10313	1.12±0.05	0.95±0.12	0.87±0.21
	Riboflavin metabolism	flavin adenine dinucleotide (FAD)	C00016	0.007649	2.1164	0.062509	1.14±0.04	0.90±0.09	**0.54±0.16**
Energy	Krebs cycle	succinylcarnitine		0.010815	1.966	0.067489	1.07±0.10	0.81±0.07	0.64±0.12

	Krebs cycle	malate	C00149	0.028575	1.544	0.10376	1.08±0.03	1.00±0.05	0.75±0.10
Lipid	Carnitine metabolism	2-methylmalonyl carnitine	-	0.0003	3.5229	0.014219	1.11±0.05	0.95±0.12	**0.51±0.09**
	Carnitine metabolism	oleoylcarnitine	-	0.008229	2.0847	0.063876	1.60±0.41	1.07±0.28	0.55±0.27
	Carnitine metabolism	3-dehydrocarnitine	C02636	0.01428	1.8453	0.07521	0.77±0.07	0.90±0.11	**0.57±0.10**
	Carnitine metabolism	deoxycarnitine	C01181	0.019147	1.7179	0.082927	0.97±0.07	1.37±0.21	1.12±0.05
	Carnitine metabolism	carnitine	C00487	0.043996	1.3566	0.12124	0.87±0.05	0.83±0.07	0.77±0.11
	Fatty acid, dicarboxylate	2-hydroxyglutarate	C02630	0.002326	2.6335	0.030838	1.33±0.04	1.09±0.12	**1.54±0.16**
	Fatty acid, monohydroxy	13-HODE + 9-HODE	-	0.038989	1.4091	0.12124	0.76±0.10	0.82±0.11	0.74±0.30
	Glycerolipid metabolism	phosphoethanolamine	C00346	0.012316	1.9095	0.0695	0.77±0.18	**1.96±0.34**	**0.47±0.13**
	Glycerolipid metabolism	choline	C00114	0.018509	1.7326	0.082768	0.89±0.03	1.07±0.04	0.82±0.07
	Glycerolipid metabolism	glycerol 3-phosphate (G3P)	C00093	0.042412	1.3725	0.12124	0.87±0.06	1.42±0.18	1.76±0.29
	Inositol metabolism	inositol 1-phosphate (I1P)	-	0.002273	2.6435	0.030838	0.70±0.03	1.26±0.11	**0.48±0.11**
	Inositol metabolism	myo-inositol	C00137	0.047891	1.3197	0.12753	**0.73±0.04**	0.97±0.09	0.73±0.12
	Long chain fatty acid	nonadecanoate (19:0)	C16535	0.021374	1.6701	0.087337	0.86±0.13	0.99±0.13	**0.50±0.16**
	Long chain fatty acid	margarate (17:0)	-	0.027851	1.5552	0.10313	0.74±0.07	0.93±0.10	0.67±0.21
	Long chain fatty acid	10-heptadecenoate (17:1n7)	-	0.029333	1.5326	0.10376	0.91±0.03	0.92±0.03	0.71±0.22
	Long chain fatty acid	arachidate (20:0)	C06425	0.034772	1.4588	0.1155	0.80±0.16	0.95±0.08	**0.55±0.16**
	Long chain fatty acid	arachidonate (20:4n6)	C00219	0.040091	1.397	0.12124	0.83±0.07	1.26±0.17	0.66±0.24

	Long chain fatty acid	palmitate (16:0)	C00249	0.040488	1.3927	0.12124	0.89±0.05	1.07±0.06	0.72±0.17
	Lysolipid	1-heptadecanoylglycerophosphocholine	-	0.000914	3.0391	0.021989	0.81±0.06	0.79±0.10	**0.48±0.17**
	Lysolipid	1-palmitoylglycerophosphocholine	-	0.001118	2.9515	0.024092	0.92±0.02	0.93±0.06	**0.50±0.19**
	Lysolipid	1-palmitoylglycerophosphocholine	-	0.001358	2.8672	0.024752	0.92±0.02	0.93±0.06	0.50±0.19
	Lysolipid	1-oleoylglycerophosphocholine	-	0.003639	2.439	0.035934	0.82±0.02	0.95±0.10	0.52±0.25
	Lysolipid	1-pentadecanoylglycerophosphocholine	-	0.006899	2.1612	0.058391	0.87±0.06	**0.73±0.03**	0.69±0.22
	Lysolipid	1-palmitoleoylglycerophosphocholine	-	0.010759	1.9682	0.067489	0.75±0.04	0.78±0.09	0.85±0.35
	Lysolipid	2-oleoylglycerophosphocholine	-	0.01362	1.8658	0.075066	0.90±0.05	1.09±0.12	0.56±0.26
	Lysolipid	2-docosahexaenoylglycerophosphocholine	-	0.01422	1.8471	0.07521	1.03±0.06	1.06±0.06	0.59±0.17
	Lysolipid	1-stearoylglycerophosphoglycerol	-	0.015535	1.8087	0.076703	1.38±0.23	1.02±0.12	1.09±0.43
	Lysolipid	1-docosapentaenoylglycerophosphocholine	-	0.031127	1.5069	0.10691	0.69±0.12	1.05±0.17	0.85±0.26
	Lysolipid	2-docosahexaenoylglycerophosphoethanolamine	-	0.039019	1.4087	0.12124	1.02±0.06	1.06±0.06	0.59±0.17
	Lysolipid	2-docosapentaenoylglycerophosphocholine	-	0.046267	1.3347	0.12461	0.84±0.10	1.08±0.17	0.50±0.20
	Sphingolipid	erythro-	-	0.000534	3.2728	0.019538	1.04±0.07	0.68±0.12	0.69±0.13

		sphingosylphosphorylcholine							
	Sphingolipid	sphingosine	C00319	0.002153	2.6669	0.030838	0.94±0.11	1.06±0.10	**0.52±0.16**
	Sphingolipid	stearoyl sphingomyelin	C00550	0.002342	2.6304	0.030838	0.90±0.04	0.83±0.13	**0.46±0.14**
	Sphingolipid	sphinganine	C00836	0.041374	1.3833	0.12124	1.41±0.29	0.88±0.14	0.89±0.18
	Sterol/Steroid	7-beta-hydroxycholesterol	C03594	0.00354	2.451	0.035934	0.85±0.10	0.65±0.07	0.60±0.14
Nucleotide	Purine metabolism, (hypo)xanthine/inosine containing	inosine	C00294	0.011753	1.9299	0.0695	0.96±0.04	0.99±0.06	0.59±0.17
	Purine metabolism, (hypo)xanthine/inosine containing	inosine 5'-monophosphate (IMP)	C00130	0.03397	1.4689	0.11501	1.29±0.13	0.85±0.08	**0.64±0.09**
	Purine metabolism, (hypo)xanthine/inosine containing	hypoxanthine	C00262	0.044582	1.3508	0.12145	1.06±0.03	1.19±0.06	0.85±0.08
	Purine metabolism, adenine containing	adenosine 3'-monophosphate (3'-AMP)	C01367	1.41E-05	4.8523	0.00333	0.97±0.05	1.04±0.05	**0.44±0.11**
	Purine metabolism, adenine containing	adenosine 3',5'-diphosphate	C00054	0.001609	2.7936	0.027231	1.24±0.10	**0.60±0.10**	**0.53±0.14**
	Purine metabolism, adenine containing	adenosine 2'-monophosphate (2'-AMP)	C00946	0.009433	2.0253	0.063876	0.67±0.10	1.23±0.10	0.70±0.20
	Purine metabolism, adenine containing	adenosine 5'-diphosphate (ADP)	C00008	0.018337	1.7367	0.082768	1.09±0.08	0.84±0.12	0.52±0.25

	Purine metabolism, guanine containing	guanosine 5'-monophosphate (GMP)	-	0.001319	2.8797	0.024752	1.23±0.05	1.08±0.06	1.10±0.08
	Purine metabolism, guanine containing	guanosine	C00387	0.025676	1.5905	0.09815	1.09±0.12	1.37±0.24	0.55±0.24
	Pyrimidine metabolism, cytidine containing	cytidine	C00475	0.011	1.9586	0.067489	1.08±0.07	**1.37±0.07**	0.95±0.12
	Pyrimidine metabolism, cytidine containing	cytidine 5'-monophosphate (5'-CMP)	C00055	0.035162	1.4539	0.1155	0.82±0.05	1.07±0.10	0.72±0.10
	Pyrimidine metabolism, uracil containing	uracil	C00106	0.011106	1.9544	0.067489	1.11±0.19	**1.77±0.25**	1.25±0.16
	Pyrimidine metabolism, uracil containing	uridine	C00299	0.012204	1.9135	0.0695	0.90±0.05	0.96±0.06	**0.59±0.12**
Xenobiotics	Benzoate metabolism	4-hydroxybenzoate	C00156	0.019944	1.7002	0.082927	0.82±0.11	1.45±0.31	0.85±0.20

Wild-type C57BL/6J mice were subjected to sham surgery or transverse aortic constriction (TAC) for 1 day (1 d), 1 week (1 wk), or 8 weeks (8 wk). Metabolites extracted from the hearts were then subjected to LC or GC mass spectrometric analysis. To eliminate potential bias imposed by data imputation, only those metabolites that had 3 or more samples represented in each sham and TAC group are listed above. Metabolites found to be significantly different by one-way ANOVA are shown; and in bold are those values found to be significantly different (i.e., $p<0.05$) from the respective sham group via post-hoc *t*-tests. The –log10(p) value is the p value converted to the negative base 10 logarithm of the p value. The (-) indicates no current KEGG identification number.

Supplemental Table II. List of myocardial metabolites that were significantly different between the MI and Sham groups.

Super pathway	*Sub-pathway metabolism*	*Metabolite*	*KEGG*	*p-value*	*–log10(p)*	*FDR*	*Fold change vs. Sham*
Amino Acid	Butanoate metabolism	2-aminobutyrate	C02261	0.006302	2.2005	0.0384	1.97±0.29
	Creatine metabolism	creatine	C00300	6.27E-05	4.2029	0.003792	0.56±0.03
	Cysteine, methionine, SAM, taurine metabolism	methionine	C00073	0.000424	3.3727	0.008764	1.68±0.12
	Cysteine, methionine, SAM, taurine metabolism	N-acetylmethionine	C02712	0.005245	2.2803	0.036264	2.02±0.23
	Cysteine, methionine, SAM, taurine metabolism	taurine	C00245	0.024526	1.6104	0.077118	0.87±0.04
	Glutamate metabolism	glutamine	C00064	0.000471	3.3272	0.008764	0.68±0.03
	Glutathione metabolism	glutathione, oxidized (GSSG)	C00127	0.003743	2.4268	0.031233	1.23±0.04
	Glutathione metabolism	cysteine-glutathione disulfide	-	0.033147	1.4796	0.094372	1.59±0.17
	Glycine, serine and threonine metabolism	betaine	C00719	0.00013	3.8846	0.006314	1.71±0.10
	Glycine, serine and threonine metabolism	glycine	C00037	0.019671	1.7062	0.07286	1.51±0.15
	Lysine metabolism	pipecolate	C00408	0.003961	2.4022	0.031593	1.58±0.13
	Phenylalanine & tyrosine metabolism	phenylalanine	C00079	0.011641	1.934	0.054175	1.37±0.10
	Phenylalanine & tyrosine metabolism	tyrosine	C00082	0.032599	1.4868	0.094372	1.41±0.13
	Polyamine metabolism	putrescine	C00134	0.000227	3.6448	0.008764	4.49±0.74
	Polyamine metabolism	spermidine	C00315	0.000537	3.2698	0.009288	1.76±0.13
	Tryptophan metabolism	C-glycosyltryptophan*	-	0.005634	2.2492	0.037172	1.53±0.03
	Tryptophan metabolism	tryptophan	C00078	0.011448	1.9413	0.054175	1.57±0.18
	Tryptophan metabolism	kynurenine	C00328	0.013499	1.8697	0.061636	2.45±0.53
	Urea cycle; arginine-, proline-, metabolism	proline	C00148	0.002061	2.6858	0.024611	1.83±0.18
	Urea cycle; arginine-, proline-, metabolism	citrulline	C00327	0.004047	2.3929	0.031593	0.81±0.03
	Urea cycle; arginine-, proline-, metabolism	assymetric dimethylarginine (ADMA)	C03626	0.009392	2.0272	0.050211	2.40±0.20
	Valine, leucine and isoleucine metabolism	isoleucine	C00407	0.008556	2.0678	0.047409	1.40±0.11
	Valine, leucine and isoleucine metabolism	valine	C00183	0.011632	1.9343	0.054175	1.30±0.08
	Valine, leucine and isoleucine metabolism	leucine	C00123	0.02372	1.6249	0.077118	1.30±0.08
	Valine, leucine and isoleucine metabolism	hydroxyisovaleroyl carnitine	-	0.049576	1.3047	0.1348	0.78±0.08

Carbohydrate	Aminosugars metabolism	erythronate*		0.010644	1.9729	0.054175	1.93±0.27
	Fructose, mannose, galactose, starch, and sucrose metabolism	mannitol	C00392	0.015945	1.7974	0.065401	0.62±0.06
	Glycolysis, gluconeogenesis, pyruvate metabolism	glycerate	C00258	0.020673	1.6846	0.07286	0.76±0.06
	Nucleotide sugars, pentose metabolism	ribose	C00121	0.019995	1.6991	0.07286	0.49±0.10
	Nucleotide sugars, pentose metabolism	ribulose	C00309	0.024108	1.6178	0.077118	0.47±0.10
	Nucleotide sugars, pentose metabolism	Isobar: ribulose 5-phosphate, xylulose 5-phosphate	-	0.025771	1.5889	0.078943	0.50±0.10
Cofactors and Vitamins	Ascorbate and aldarate metabolism	ascorbate (Vitamin C)	C00072	0.002267	2.6446	0.024933	56.5±7.94
	Ascorbate and aldarate metabolism	glucarate (saccharate)	C00818	0.021075	1.6762	0.07286	1.81±0.27
	Nicotinate and nicotinamide metabolism	nicotinamide	C00153	0.01152	1.9386	0.054175	0.77±0.03
	Pantothenate and CoA metabolism	pantothenate	C00864	5.18E-05	4.2857	0.003792	0.65±0.02
	Riboflavin metabolism	flavin adenine dinucleotide (FAD)	C00016	0.002136	2.6705	0.024611	0.36±0.06
Energy	Krebs cycle	succinylcarnitine	-	0.02041	1.6902	0.07286	0.71±0.05
	Krebs cycle	malate	C00149	0.027648	1.5583	0.083634	0.73±0.06
	Oxidative phosphorylation	acetylphosphate	C00227	0.002605	2.5843	0.027405	0.63±0.04
	Oxidative phosphorylation	phosphate	C00009	0.018947	1.7224	0.072782	0.83±0.04
Lipid	Carnitine metabolism	carnitine	C00487	1.05E-05	4.98	0.002534	0.75±0.01
	Carnitine metabolism	2-methylmalonyl carnitine	-	0.00045	3.3472	0.008764	0.64±0.04
	Carnitine metabolism	acetylcarnitine	C02571	0.000602	3.2201	0.009719	0.59±0.04
	Carnitine metabolism	oleoylcarnitine	-	0.021467	1.6682	0.073168	0.14±0.02
	Carnitine metabolism	palmitoylcarnitine	C02990	0.049172	1.3083	0.1348	0.19±0.03
	Fatty acid metabolism (also BCAA metabolism)	propionylcarnitine	C03017	0.018501	1.7328	0.072215	0.58±0.09
	Fatty acid synthesis	malonylcarnitine	-	0.000811	3.091	0.012265	0.60±0.05
	Fatty acid, monohydroxy	13-HODE + 9-HODE	-	0.000424	3.3728	0.008764	0.42±0.05
	Glycerolipid metabolism	choline phosphate	C00588	0.003631	2.44	0.031233	0.72±0.05
	Glycerolipid metabolism	glycerophosphorylcholine (GPC)	C00670	0.007965	2.0988	0.045893	3.25±0.62
	Glycerolipid metabolism	glycerol	C00116	0.035986	1.4439	0.10126	0.81±0.03
	Inositol metabolism	scyllo-inositol	C06153	0.006347	2.1974	0.0384	1.20±0.04
	Long chain fatty acid	eicosenoate (20:1n9 or 11)	-	0.00337	2.4724	0.031233	0.49±0.07
	Long chain fatty acid	myristoleate (14:1n5)	C08322	0.006136	2.2121	0.0384	0.59±0.07
	Long chain fatty acid	pentadecanoate (15:0)	C16537	0.014018	1.8533	0.062822	0.68±0.06
	Long chain fatty acid	arachidate (20:0)	C06425	0.014839	1.8286	0.064127	0.56±0.09
	Long chain fatty acid	nonadecanoate (19:0)	C16535	0.01981	1.7031	0.07286	0.54±0.11

	Long chain fatty acid	dihomo-linoleate (20:2n6)	C16525	0.020904	1.6798	0.07286	0.64±0.07
	Long chain fatty acid	10-nonadecenoate (19:1n9)	-	0.022333	1.651	0.075065	0.66±0.07
	Long chain fatty acid	margarate (17:0)	-	0.024677	1.6077	0.077118	0.60±0.10
	Long chain fatty acid	10-heptadecenoate (17:1n7)	-	0.024856	1.6046	0.077118	0.72±0.06
	Long chain fatty acid	stearidonate (18:4n3)	C16300	0.032957	1.4821	0.094372	0.62±0.09
	Long chain fatty acid	linoleate (18:2n6)	C01595	0.039169	1.4071	0.10895	0.76±0.06
	Lysolipid	1-oleoylglycerophosphoethanolamine	-	0.004674	2.3304	0.034272	0.54±0.03
	Lysolipid	1-stearoylglycerophosphocholine	-	0.005683	2.2454	0.037172	0.15±0.06
	Lysolipid	1-stearoylglycerophosphoinositol		0.011635	1.9342	0.054175	0.62±0.08
	Lysolipid	1-palmitoylglycerophosphocholine	-	0.015653	1.8054	0.065312	0.37±0.09
	Lysolipid	1-oleoylglycerophosphocholine	-	0.031837	1.4971	0.093957	0.33±0.09
	Medium chain fatty acid	caproate (6:0)	C01585	5.59E-05	4.2525	0.003792	0.20±0.03
	Medium chain fatty acid	pelargonate (9:0)	C01601	0.006852	2.1642	0.040445	0.76±0.04
	Monoacylglycerol	1-palmitoylglycerol (1-monopalmitin)	-	0.024479	1.6112	0.077118	0.63±0.09
	Sphingolipid	stearoyl sphingomyelin	C00550	0.003605	2.4431	0.031233	0.40±0.07
	Sterol/Steroid	7-beta-hydroxycholesterol	C03594	0.000336	3.4743	0.008764	0.43±0.02
Nucleotide	Purine and pyrimidine metabolism	methylphosphate	-	0.00862	2.0645	0.047409	0.41±0.08
	Purine metabolism, (hypo)xanthine/inosine containing	inosine	C00294	0.004462	2.3505	0.033742	0.53±0.07
	Purine metabolism, (hypo)xanthine/inosine containing	inosine 5'-monophosphate (IMP)	C00130	0.031247	1.5052	0.093354	0.49±0.12
	Purine metabolism, adenine containing	adenosine 3',5'-diphosphate	C00054	0.001872	2.7278	0.023837	0.39±0.05
	Purine metabolism, adenine containing	adenosine	C00212	0.009544	2.0203	0.050211	0.32±0.03
	Purine metabolism, adenine containing	adenosine 5'-diphosphate (ADP)	C00008	0.015578	1.8075	0.065312	0.19±0.02
	Purine metabolism, adenine containing	adenosine 5'-monophosphate (AMP)	C00020	0.017356	1.7606	0.070001	0.57±0.09
	Pyrimidine metabolism, cytidine containing	cytidine	C00475	0.000366	3.436	0.008764	1.35±0.05
	Pyrimidine metabolism, uracil containing	uracil	C00106	0.003286	2.4834	0.031233	1.73±0.18
	Pyrimidine metabolism, uracil containing	uridine 5'-monophosphate (UMP)	C00105	0.005036	2.2979	0.035845	1.98±0.28
Peptide	Dipeptide	pro-hydroxy-pro	-	0.000318	3.4973	0.008764	2.52±0.22
	gamma-glutamyl	gamma-glutamylglutamate	-	0.003368	2.4727	0.031233	2.23±0.14

Xenobiotics	Food component/Plant	ergothioneine	C05570	0.001799	2.7449	0.023837	5.29±1.19
	Food component/Plant	N-glycolylneuraminate	C03410	0.018037	1.7438	0.071558	2.16±0.40

Wild-type C57BL/6J mice were subjected to sham surgery or myocardial infarction (MI) for 5 d. Metabolites extracted from the hearts were then subjected to LC or GC mass spectrometric analysis. To eliminate potential bias imposed by data imputation, only those metabolites that had 3 or more samples represented in each sham and MI group are listed above. Metabolites found to be significantly different (i.e., $p<0.05$) by unpaired Student's *t*-test are shown. The (-) indicates no KEGG identification number.