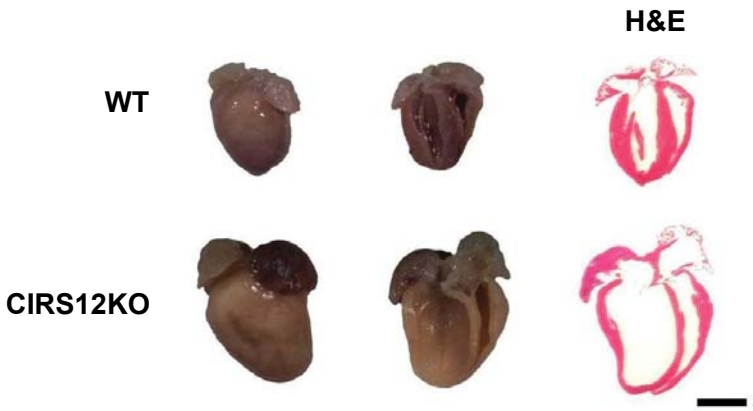
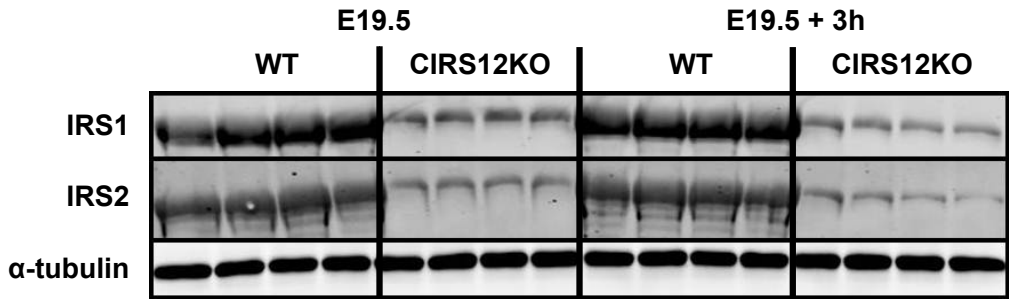
Supplemental Material for

Insulin receptor substrate signaling suppresses neonatal autophagy in the heart

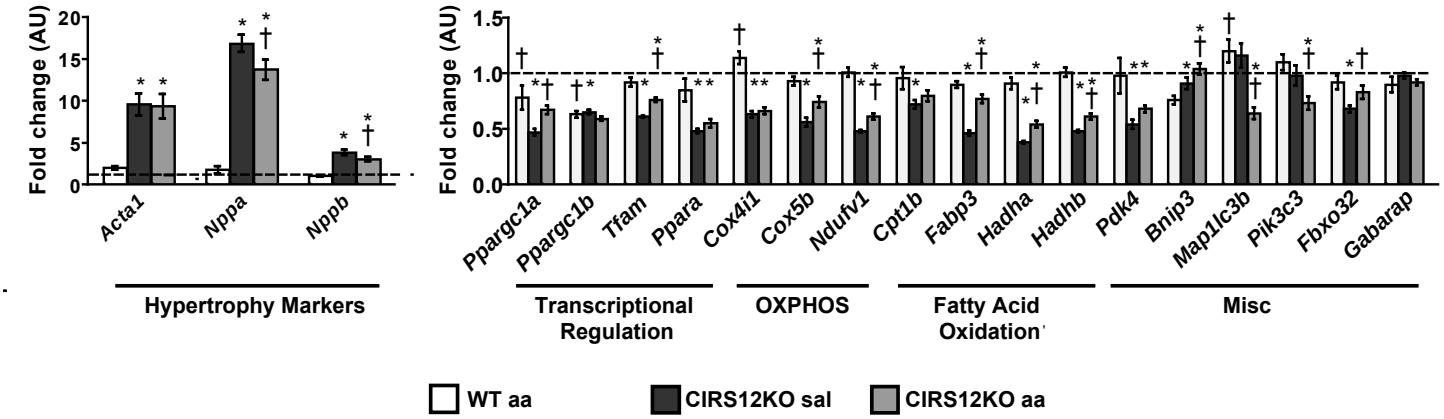
Christian Riehle, Adam R. Wende, Sandra Sena, Karla Maria Pires, Renata Oliveira Pereira, Yi Zhu, Heiko Bugger, Deborah Frank, Jack Bevins, Dong Chen, Cynthia N. Perry, Xiaocheng C. Dong, Steven Valdez, Monika Rech, Xiaoming Sheng, Bart C. Weimer, Roberta A. Gottlieb, Morris F. White and E. Dale Abel



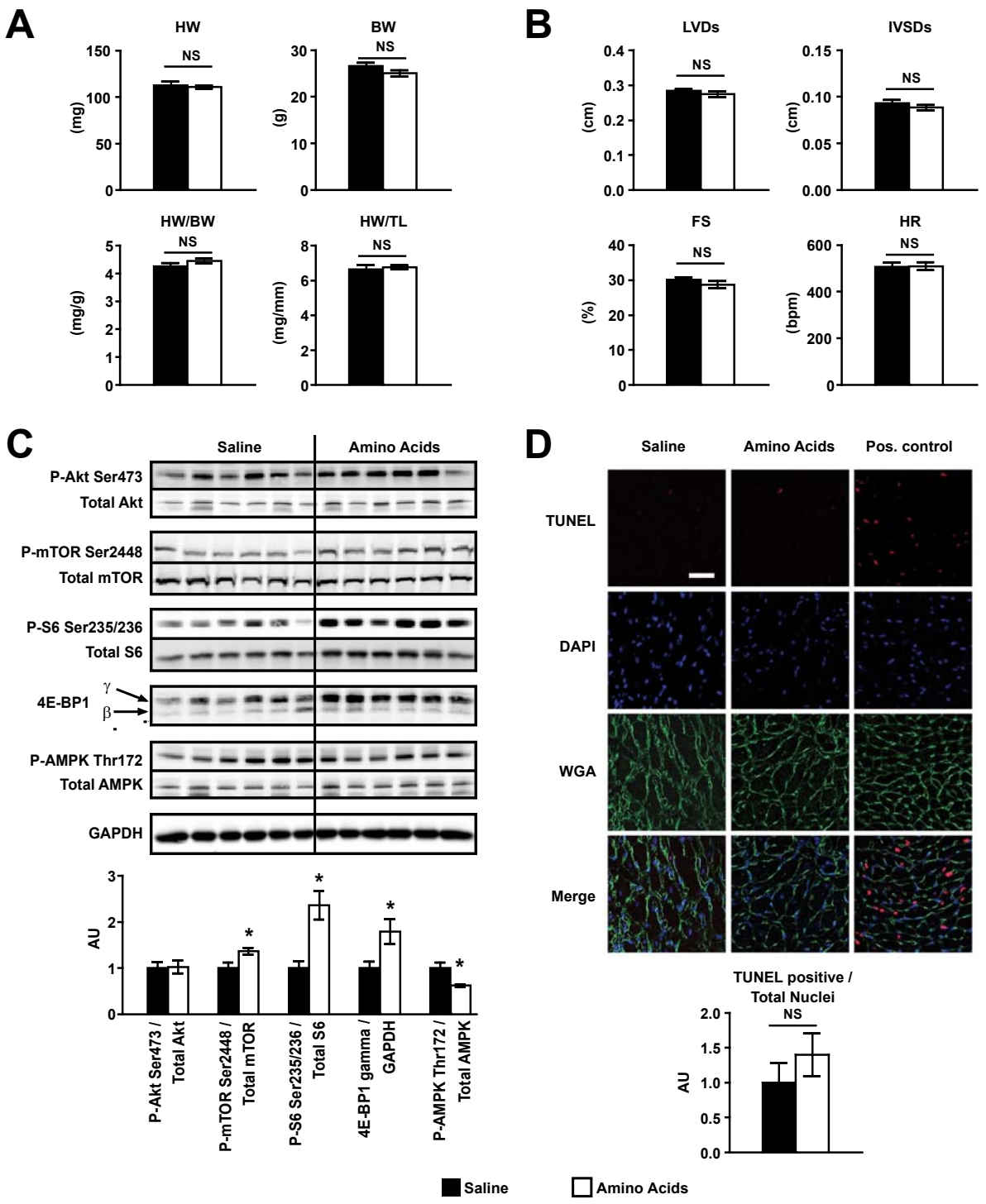
Supplemental Figure 1: Representative photographs and H&E stainings from WT and CIRS12KO hearts at the age of 8 weeks. Scale bar: 3 mm.



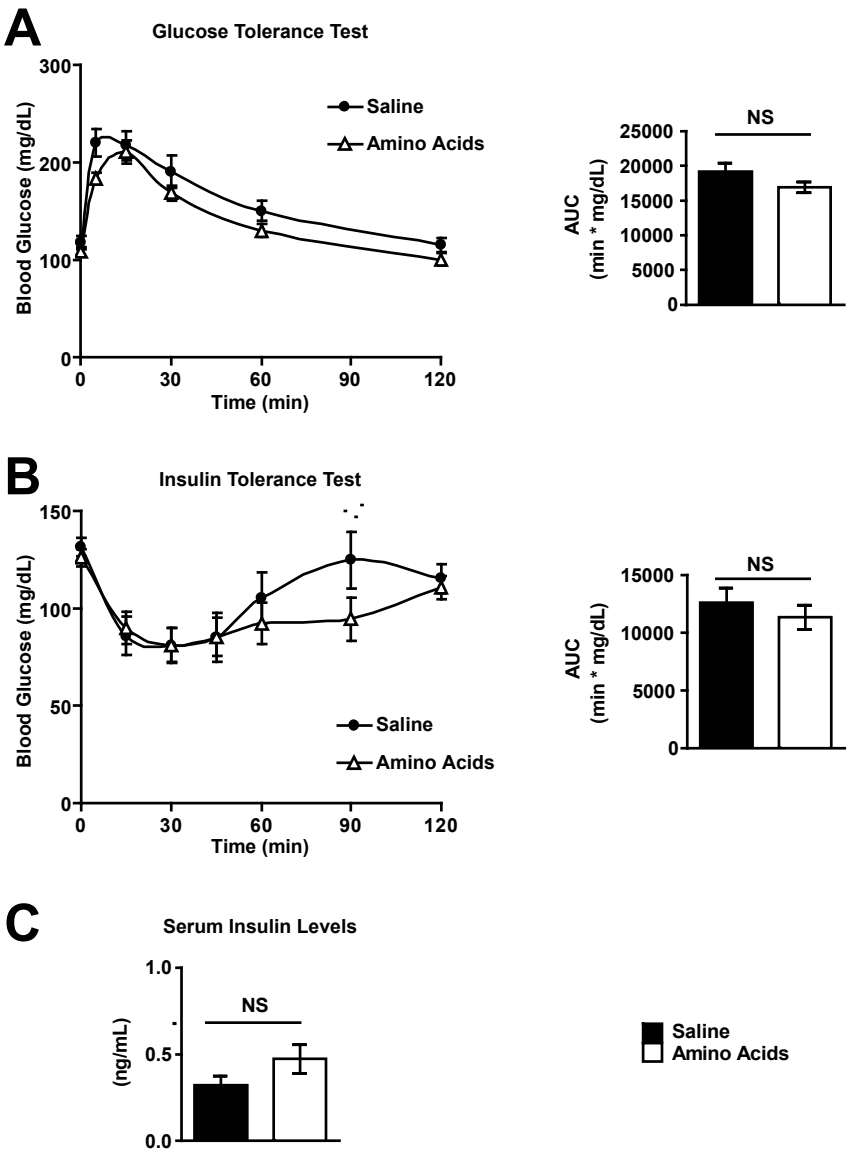
Supplemental Figure 2: Representative immunoblots for IRS1 and IRS2 in ventricle homogenate proteins from WT and CIRS12KO mice. Mice were obtained by Caesarean delivery at E19.5 and either immediately sacrificed or placed in a humidified, temperature-controlled chamber for 3 hours before sacrifice (E19.5 + 3h).



Supplemental Figure 3: Gene expression in ventricle homogenates obtained from CIRS12KO and WT controls following Saline (sal) or Amino Acid (aa) treatment at the age of 6 wk. Data are presented as fold change vs. WT Saline and reported as mean values ± SEM (n=6). Gene expression was normalized to *Rps16.* Gene names are shown in Supplemental Table 11. * $P < 0.05$ vs. WT same treatment, † $P < 0.05$ vs. Saline treatment same genotype (ANOVA / Fisher's PLSD).



Supplemental Figure 4: (A) Heart weights and (B) cardiac function of WT hearts at the age of 20 wk. (n=6-9); HW, heart weight; BW, body weight; HW/BW, heart weight to body weight ratio; HW/TL, heart weight to tibia length ratio; LVDs, Left ventricular cavity diameter at systole; IVSDs, Interventricular septum diameter at systole; FS, Fractional shortening; HR, heart rate. (C) Immunoblots and quantification from ventricle homogenates obtained from WT hearts at the age of 6 wk. (D) Representative TUNEL, DAPI and wheat germ agglutinin (WGA) stains, and stereological quantification are shown in the panels as labeled from 6 wk. old mice (n=4, Scale bar: 20μm). Saline or Amino Acid injections were performed daily starting the day of birth. NS, no significant difference observed; * $P < 0.05$ vs. Saline treatment (Unpaired Student's t-test). Data are reported as mean values ± SEM.



Supplemental Figure 5: (A) Glucose tolerance tests, (B) insulin tolerance tests, and (C) plasma insulin levels at the age of 20 wk following Saline or Amino Acid treatment starting the day of birth. Blood glucose was measured at the indicated time points post injection. No significant difference was observed between animals subjected to the different treatments (n=9-13). NS, no significant difference observed; AUC, area under the curve. Data are reported as mean values ± SEM.

Supplemental Table 1: Cardiac function of CIRS12KO mice

Group (n)	Age	LVDd (mm)	LVDs (mm)	IVSDd (mm)	IVSDs (mm)	LVPWd (mm)	LVPWs (mm)	FS (%)	EF (%)
WT (15)	1 d	1.34 ± 0.03	0.73 ± 0.03	n.d.	n.d.	0.39 ± 0.02	0.56 ± 0.02	45.35 ± 2.10	82.6 ± 2.1
CIRS12KO (12)	1 d	1.40 ± 0.03	0.78 ± 0.04			0.31 ± 0.02 *	0.51 ± 0.02	44.29 ± 2.39	81.7 ± 2.1
WT (10)	2 wk	2.55 ± 0.07	1.78 ± 0.08	n.d.	n.d.	0.49 ± 0.03	0.65 ± 0.04	30.34 ± 1.86	65.6 ± 2.6
CIRS12KO (6)	2 wk	2.46 ± 0.11	1.77 ± 0.13			0.41 ± 0.02 *	0.57 ± 0.06	28.39 ± 3.16	62.2 ± 4.3
WT, Male (4)	4 wk	3.44 ± 0.18	2.38 ± 0.13	0.56 ± 0.03	0.98 ± 0.04	0.58 ± 0.01	0.89 ± 0.03	30.65 ± 0.16	66.7 ± 0.2
CIRS12KO, Male (5)	4 wk	3.58 ± 0.15	2.97 ± 0.18 *	0.43 ± 0.03 *	0.63 ± 0.05 *	0.52 ± 0.01 *	0.69 ± 0.03 *	17.22 ± 2.63 *	42.6 ± 5.5 *
WT, Female (12)	4 wk	3.40 ± 0.09	2.46 ± 0.09	0.53 ± 0.01	0.85 ± 0.03	0.53 ± 0.01	0.82 ± 0.03	27.80 ± 1.23	57.5 ± 5.0
CIRS12KO, Female (10)	4 wk	3.48 ± 0.07	2.87 ± 0.12 *	0.42 ± 0.02 *	0.61 ± 0.03 *	0.46 ± 0.02 *	0.65 ± 0.03 *	18.47 ± 2.04 *	44.9 ± 4.1 (p=0.067 vs. WT Female same age)

Data are reported as mean values ± SEM. * $P < 0.05$ vs. WT same age (unpaired Student's t-test)
LVDd, Left ventricular cavity diameter at diastole; LVDs, Left ventricular cavity diameter at systole; IVSDd, Interventricular septum diameter at diastole; IVSDs, Interventricular septum diameter at systole; LVPWd, Left ventricular posterior wall thickness at diastole; LVPWs, Left ventricular posterior wall thickness at systole; FS, Fractional shortening; EF, Ejection fraction; n.d., not determined

Supplemental Table 2: Heart weights of CIRS12KO mice

Group (n)	Age	BW (g)	HW (mg)	TL (mm)	HW/BW (mg/g)	HW/TL (mg/mm)
WT (15)	1 d	1.23 ± 0.05	7.6 ± 0.4	n.d.	6.16 ± 0.20	n.d.
CIRS12KO (15)	1 d	1.26 ± 0.06	7.2 ± 0.4		5.80 ± 0.26	
WT (8)	2 wk	6.67 ± 0.38	40.9 ± 2.1	n.d.	6.16 ± 0.21	n.d.
CIRS12KO (8)	2 wk	6.46 ± 0.45	30.8 ± 1.5 *		4.85 ± 0.21 *	
WT, Male (10)	4 wk	14.89 ± 0.41	69.8 ± 2.4	13.53 ± 0.20	4.70 ± 0.13	5.16 ± 0.16
CIRS12KO, Male (10)	4 wk	12.79 ± 0.92	66.4 ± 4.7	13.38 ± 0.45	5.53 ± 0.77	5.05 ± 0.50
WT, Female (10)	4 wk	13.19 ± 0.57	67.2 ± 2.3	13.20 ± 0.34	5.15 ± 0.20	5.13 ± 0.24
CIRS12KO, Female (10)	4 wk	11.69 ± 0.58	68.9 ± 6.5	12.63 ± 0.35	6.13 ± 0.84	5.51 ± 0.58

Data are reported as mean values ± SEM. * $P < 0.05$ vs. WT same age (unpaired Student's t-test)
BW, Body weight; HW, Heart weight; TL, Tibia length; n.d.; not determined

Supplemental Table 3: Summary of changes in mitochondrial protein abundance in mitochondria obtained from 4 week old CIRS12KO hearts compared with age matched WT controls ($IRS1^{lox/lox}$: $IRS2^{lox/lox}$)

	Decreased	**Increased**	**Total detected**
Citrate cycle (matrix fraction)	5	2	10
Fatty acid oxidation (membrane fraction)	6	0	15
Oxidative phosphorylation (membrane fraction)	2	5	42

Supplemental Table 4: Mitochondrial abundance of proteins involved in citrate cycle, pyruvate decarboxylation, fatty acid oxidation, and oxidative phosphorylation in CIRS12KO hearts (4 wk)

Canonical Pathway	Accession	CIRS12KO
Citrate cycle (matrix fraction)		
aconitase 2, mitochondrial	NP_542364.1	0.88
citrate synthase	NP_080720.1	0.92
dihydrolipoamide dehydrogenase	NP_031887.2	0.94
dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex)	NP_084501.1	1.09
fumarate hydratase 1	NP_034339.1	1.01
isocitrate dehydrogenase 2 (NADP+), mitochondrial	NP_766599.1	0.93
isocitrate dehydrogenase 3 (NAD+) alpha	NP_083849.1	0.88
malate dehydrogenase 2, NAD (mitochondrial)	NP_032643.2	1.10
oxoglutarate dehydrogenase (lipoamide)	NP_035086.2	1.11
succinate-Coenzyme A ligase, ADP-forming, beta subunit	NP_035636.1	0.88
Pyruvate decarboxylation (matrix fraction)		
pyruvate dehydrogenase (lipoamide) beta	NP_077183.1	0.99
pyruvate dehydrogenase E1 alpha 1	NP_032836.1	0.95
Fatty acid oxidation (membrane fraction)		
acetyl-Coenzyme A acetyltransferase 1 precursor	NP_659033.1	0.84
acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	NP_803421.1	0.76
acetyl-Coenzyme A dehydrogenase, long-chain	NP_031407.2	1.03
acetyl-Coenzyme A dehydrogenase, medium chain	NP_031408.1	0.76
acyl-CoA synthetase long-chain family member 1	NP_032007.2	0.93
acyl-Coenzyme A dehydrogenase, very long chain	NP_059062.1	0.93
carnitine palmitoyltransferase 1b, muscle	NP_034078.1	1.04
carnitine palmitoyltransferase 2	NP_034079.1	0.80
hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit	NP_849209.1	0.82
hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit	NP_663533.1	0.83
L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain	NP_032238.1	0.55
PREDICTED: similar to acyl-CoA synthetase long-chain family member 1 isoform 2	XP_996295.1	0.90
PREDICTED: similar to acyl-CoA synthetase long-chain family member 1 isoform 3	XP_996322.1	0.88
PREDICTED: similar to acyl-CoA synthetase long-chain family member 1 isoform 4	XP_996347.1	0.92
PREDICTED: similar to acyl-CoA synthetase long-chain family member 1 isoform 5	XP_996374.1	0.93
Oxidative phosphorylation (membrane fraction)		
Complex I		
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10	NP_077159.1	1.02
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13	NP_075801.1	1.10
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2	NP_035015.2	1.16
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4	NP_035016.1	1.39
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9	NP_079634.1	0.94
NADH dehydrogenase (ubiquinone) 1 beta subcomplex 8	NP_080337.1	1.12
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	NP_080960.1	1.14
NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 2	NP_077182.1	1.06
NADH dehydrogenase (ubiquinone) Fe-S protein 1	NP_663493.1	1.05

Canonical Pathway	Accession	CIRS12KO
NADH dehydrogenase (ubiquinone) Fe-S protein 2	NP_694704.1	1.16
NADH dehydrogenase (ubiquinone) Fe-S protein 3	NP_080964.1	0.98
NADH dehydrogenase (ubiquinone) Fe-S protein 5	NP_001025445.1	1.14
NADH dehydrogenase (ubiquinone) Fe-S protein 6	NP_035018.1	1.04
NADH dehydrogenase (ubiquinone) Fe-S protein 8	NP_659119.2	1.16
NADH dehydrogenase (ubiquinone) flavoprotein 1	NP_598427.1	1.12
Complex II		
succinate dehydrogenase Fp subunit	NP_075770.1	0.97
succinate dehydrogenase Ip subunit	NP_075863.2	0.99
Complex III		
ubiquinol cytochrome c reductase core protein 2	NP_080175.1	0.97
ubiquinol-cytochrome c reductase binding protein	NP_080495.1	1.04
ubiquinol-cytochrome c reductase core protein 1	NP_079683.2	1.00
ubiquinol-cytochrome c reductase hinge protein	NP_079917.1	0.95
ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	NP_079986.1	1.16
neuronal protein 15.6	NP_062308.1	1.16
Complex IV		
cytochrome c oxidase subunit II	NP_904331.1	0.97
cytochrome c oxidase subunit IV isoform 1	NP_034071.1	0.87
cytochrome c oxidase, subunit Va	NP_031773.1	1.09
cytochrome c oxidase, subunit VIb polypeptide 1	NP_079904.1	0.92
cytochrome c oxidase, subunit VIc	NP_444301.1	0.90
cytochrome c oxidase, subunit VIIa 1	NP_034074.1	5.56
cytochrome c oxidase, subunit VIIa 2	NP_034075.2	1.11
Complex V		
ATP synthase, H+ transporting mitochondrial F1 complex, beta subunit	NP_058054.2	1.00
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1	NP_033855.2	1.06
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	NP_082138.1	0.99
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F	NP_058035.1	0.93
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f, isoform 2	NP_065607.1	0.92
ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	NP_031531.1	0.92
ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit precursor	NP_079589.1	1.14
ATP synthase, H+ transporting, mitochondrial F1 complex, gamma subunit	NP_065640.1	1.08
ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit	NP_613063.1	1.05
Other		
cytochrome c-1	NP_079843.1	1.16
hypothetical protein LOC66152	NP_932096.1	1.08
low molecular mass ubiquinone-binding protein	NP_079628.1	0.98

Data are presented as fold change compared to age matched WT hearts ($IRS1^{lox/lox}$: $IRS2^{lox/lox}$). Highlighted cells indicate a significant difference compared to WT (red = increased, yellow = decreased).

Supplemental Table 5: Heart weights and lung weights at 6 weeks of age following Amino Acid or Saline treatment

Group	BW (g)	HW (mg)	TL (mm)	HW/BW (mg/g)	HW/TL (mg/mm)	WLW (mg)	WLW / BW (mg/g)	WLW / TL (mg/mm)
WT sal	18.31 ± 0.79	87.69 ± 3.90	15.10 ± 0.13	4.79 ± 0.05	5.80 ± 0.23	125.86 ± 5.62	6.89 ± 0.21	8.33 ± 0.35
WT aa	18.13 ± 0.95	92.47 ± 5.58	14.73 ± 0.23	5.10 ± 0.15	6.27 ± 0.35	130.49 ± 3.93	7.27 ± 0.26	8.85 ± 0.20
CIRS12KO sal	15.66 ± 0.42	110.20 ± 3.30 *	14.88 ± 0.16	7.09 ± 0.38 *	7.40 ± 0.21 *	238.39 ± 12.31 *	15.33 ± 1.02 *	16.01 ± 0.79 *
CIRS12KO aa	17.07 ± 0.60 †	83.26 ± 3.56 †	14.84 ± 0.25	4.89 ± 0.17 †	5.60 ± 0.17 †	122.11 ± 5.75 †	7.18 ± 0.32 †	8.23 ± 0.36 †

Data - mean values ± SEM are from male mice, n=7; sal, Saline treatment; aa, Amino Acid treatment; * $P < 0.05$ vs. WT same treatment, † $P < 0.05$ vs. Saline treatment same genotype (ANOVA / Fisher's PLSD); BW, Body weight; HW, Heart weight; TL, Tibia length; WLW, Wet lung weight

Supplemental Table 6: Contractile function assessed by transthoracic echocardiography following Amino Acid or Saline treatment

Group	Age (Wk)	LVDd (cm)	LVDs (cm)	IVSDd (cm)	IVSDs (cm)	LVPWd (cm)	LVPWs (cm)	FS (%)	EF (%)
WT sal	4	0.351 ± 0.011	0.255 ± 0.008	0.062 ± 0.002	0.090 ± 0.003	0.056 ± 0.002	0.080 ± 0.004	27.35 ± 0.68	61.60 ± 1.07
WT aa	4	0.329 ± 0.009	0.239 ± 0.010	0.063 ± 0.002	0.089 ± 0.004	0.056 ± 0.001	0.073 ± 0.002	27.43 ± 1.46	61.42 ± 2.14
CIRS12KO sal	4	0.384 ± 0.008	0.310 ± 0.008	0.051 ± 0.002	0.070 ± 0.003	0.053 ± 0.002	0.069 ± 0.004	19.20 ± 1.08	47.10 ± 2.09
CIRS12KO aa	4	0.334 ± 0.005	0.256 ± 0.007	0.058 ± 0.002	0.082 ± 0.002	0.055 ± 0.003	0.071 ± 0.005	23.38 ± 1.38	54.77 ± 2.28
WT sal	5	0.361 ± 0.008	0.263 ± 0.008	0.069 ± 0.002	0.100 ± 0.004	0.056 ± 0.001	0.075 ± 0.002	27.23 ± 1.11	61.30 ± 1.74
WT aa	5	0.365 ± 0.007	0.278 ± 0.007	0.062 ± 0.002	0.090 ± 0.003	0.059 ± 0.002	0.078 ± 0.003	23.72 ± 1.17	55.35 ± 2.03
CIRS12KO sal	5	0.426 ± 0.004	0.361 ± 0.006	0.052 ± 0.001	0.069 ± 0.003	0.050 ± 0.001	0.065 ± 0.004	15.38 ± 0.99	39.28 ± 2.09
CIRS12KO aa	5	0.376 ± 0.005	0.304 ± 0.006	0.054 ± 0.002	0.074 ± 0.002	0.050 ± 0.002	0.063 ± 0.003	19.29 ± 0.82	47.32 ± 1.63
WT sal	6	0.371 ± 0.007	0.273 ± 0.005	0.066 ± 0.002	0.095 ± 0.003	0.058 ± 0.001	0.079 ± 0.002	26.27 ± 0.39	59.89 ± 0.63
WT aa	6	0.373 ± 0.011	0.267 ± 0.011	0.067 ± 0.003	0.102 ± 0.004	0.060 ± 0.003	0.085 ± 0.004	28.48 ± 1.09	63.22 ± 1.66
CIRS12KO sal	6	0.441 ± 0.009	0.370 ± 0.008	0.057 ± 0.004	0.075 ± 0.004	0.055 ± 0.005	0.071 ± 0.006	15.92 ± 1.15	40.38 ± 2.39
CIRS12KO aa	6	0.398 ± 0.011	0.321 ± 0.016	0.055 ± 0.002	0.079 ± 0.005	0.055 ± 0.003	0.070 ± 0.004	19.62 ± 1.93	47.52 ± 3.76

Data - mean values ± SEM are from male mice, n=6-9; sal, Saline treatment; aa, Amino Acid treatment;
LVDd, Left ventricular cavity diameter at diastole; LVDs, Left ventricular cavity diameter at systole; IVSDd, Interventricular septum diameter at diastole; IVSDs, Interventricular septum diameter at systole; LVPWd, Left ventricular posterior wall thickness at diastole;LVPWs, Left ventricular posterior wall thickness at systole; FS, Fractional shortening; EF, Ejection fraction

Supplemental Table 7: Invasive measurement of left ventricular pressures at six weeks of age following Amino Acid or Saline treatment as assessed by catheterization

Group	LVSP (mmHg)	LVMP (mmHg)	LV Dev P (mmHg)	Heart Rate (bpm)	Max *dp/dt* (mmHg/s)	Min *dp/dt* (mmHg/s)
WT sal	92.11 ± 2.29	4.04 ± 2.37	88.07 ± 3.09	553.06 ± 18.15	7036.03 ± 546.65	-6362.95 ± 474.97
WT aa	94.97 ± 4.40	2.99 ± 2.45	91.98 ± 5.97	600.24 ± 12.98	8038.32 ± 613.12	-7602.14 ± 646.50
CIRS12KO sal	62.07 ± 3.20 *	15.10 ± 1.59 *	46.97 ± 2.70 *	583.82 ± 25.66	2596.50 ± 152.57 *	-2628.74 ± 145.02 *
CIRS12KO aa	77.35 ± 3.96 * †	5.53 ± 2.64 †	71.82 ± 5.72 * †	639.73 ± 12.47 †	4760.06 ± 677.12 * †	-5328.42 ± 1115.04 * †

Data - mean values ± SEM are from male mice, n=6; sal, Saline treatment; aa, Amino Acid treatment; * $P < 0.05$ vs. WT same treatment, † $P < 0.05$ vs. Saline treatment same genotype (ANOVA / Fisher's PLSD). LVSP, left ventricular systolic pressure; LVMP, left ventricular minimum pressure; LV Dev P, left ventricular developed pressure; bpm, beats per minute; Max *dp/dt*, maximal rate of increase in left ventricular pressure; Min *dp/dt*, maximal rate of decrease in left ventricular pressure

Supplemental Table 8: Heart weights and lung weights at 2 and 6 weeks of age in *Becn1*$^{+/-}$ CIRS12KO Cross

Group (n)	Age (Wk)	BW (g)	HW (mg)	TL (mm)	HW/BW (mg/g)	HW/TL (mg/mm)	WLW (mg)	WLW / BW (mg/g)	WLW / TL (mg/mm)
WT (6)	2	7.68 ± 0.93	43.73 ± 4.96	n.d.	5.79 ± 0.40	n.d.	n.d.	n.d.	n.d.
Becn1$^{+/-}$ (6)	2	7.10 ± 0.47	42.05 ± 1.89		6.01 ± 0.35				
CIRS12KO (6)	2	8.82 ± 0.68	48.55 ± 2.86		5.59 ± 0.27				
CIRS12KO x *Becn1*$^{+/-}$ (6)	2	7.59 ± 0.47	39.78 ± 2.75		5.28 ± 0.34				
WT (6)	6	18.78 ± 0.58	91.17 ± 3.34	15.38 ± 0.13	4.86 ± 0.12	5.92 ± 0.19	134.17 ± 1.80	7.17 ± 0.16	8.72 ± 0.08
Becn1$^{+/-}$ (7)	6	19.07 ± 0.89	89.71 ± 4.68	15.31 ± 0.26	4.70 ± 0.11	5.84 ± 0.23	135.57 ± 7.19	7.11 ± 0.18	8.83 ± 0.38
CIRS12KO (6)	6	19.82 ± 0.48	97.83 ± 5.02	15.50 ± 0.15	4.93 ± 0.18	6.32 ± 0.36	182.17 ± 9.21 *	9.20 ± 0.44 *	11.77 ± 0.66 *
CIRS12KO x *Becn1*$^{+/-}$ (10)	6	17.91 ± 0.58	85.80 ± 2.24 †	15.07 ± 0.19	4.83 ± 0.19	5.69 ± 0.13 †	137.20 ± 7.47 †	7.79 ± 0.66	9.10 ± 0.49 †

Data - mean values ± SEM are from male mice; * $P < 0.05$ vs. same *Becn1* genotype, † $P < 0.05$ vs. same IRS genotype (ANOVA / Fisher's PLSD). BW, Body weight; HW, Heart weight; TL, Tibia length; WLW, Wet lung weight; n.d.; not determined

S**upplemental Table 9:** Invasive measurement of left ventricular pressures at six weeks of age as assessed by catheterization in *Becn1*$^{+/-}$ CIRS12KO Cross

Group (n)	LVSP (mmHg)	LVMP (mmHg)	LV Dev P (mmHg)	Heart Rate (bpm)	Max *dp/dt* (mmHg/s)	Min *dp/dt* (mmHg/s)
WT (6)	94.63 ± 2.37	5.43 ± 1.10	89.19 ± 3.12	513.23 ± 7.77	7192.17 ± 359.39	-6949.82 ± 581.30
Becn1$^{+/-}$ (6)	102.78 ± 3.17	3.45 ± 1.06	99.33 ± 3.04	529.91 ± 13.04	7978.80 ± 381.46	-8249.27 ± 389.16
CIRS12KO (7)	75.71 ± 1.97 *	13.48 ± 1.48 *	62.23 ± 2.39 *	547.91 ± 21.55	3890.94 ± 283.27 *	-3682.20 ± 287.24 *
CIRS12KO x *Becn1*$^{+/-}$ (10)	90.48 ± 4.57 * †	6.68 ± 1.23 †	83.80 ± 5.50 * †	508.43 ± 13.90	6438.89 ± 684.55 †	-6375.12 ± 722.66 * †

Data - mean values ± SEM are from male mice; * $P < 0.05$ vs. same *Becn1*genotype, † $P < 0.05$ vs. same IRS genotype (ANOVA / Fisher's PLSD). LVSP, left ventricular systolic pressure; LVMP, left ventricular minimum pressure; LV Dev P, left ventricular developed pressure; bpm, beats per minute; Max *dp/dt*, maximal rate of increase in left ventricular pressure; Min *dp/dt*, maximal rate of decrease in left ventricular pressure

Supplemental Table 10: Contractile function assessed by transthoracic echocardiography in *Becn1*$^{+/-}$ CIRS12KO Cross

Group	Age (Wk)	LVDd (cm)	LVDs (cm)	IVSDd (cm)	IVSDs (cm)	LVPWd (cm)	LVPWs (cm)	FS (%)	EF (%)
WT	4	0.329 ± 0.009	0.229 ± 0.009	0.072 ± 0.001	0.099 ± 0.002	0.058 ± 0.001	0.082 ± 0.002	30.66 ± 0.77	66.57 ± 1.14
Becn1$^{+/-}$	4	0.341 ± 0.011	0.250 ± 0.010	0.069 ± 0.002	0.089 ± 0.003	0.062 ± 0.002	0.081 ± 0.005	26.88 ± 1.95	60.48 ± 3.09
CIRS12KO	4	0.356 ± 0.013	0.282 ± 0.018	0.060 ± 0.003	0.079 ± 0.005	0.053 ± 0.003	0.066 ± 0.004	21.18 ± 2.13	50.60 ± 3.93
CIRS12KO x *Becn1*$^{+/-}$	4	0.342 ± 0.005	0.253 ± 0.008	0.063 ± 0.002	0.084 ± 0.004	0.057 ± 0.002	0.078 ± 0.003	26.00 ± 1.48	59.03 ± 2.52
WT	6	0.363 ± 0.008	0.262 ± 0.010	0.075 ± 0.004	0.102 ± 0.007	0.065 ± 0.002	0.089 ± 0.003	28.04 ± 1.75	62.14 ± 2.80
Becn1$^{+/-}$	6	0.359 ± 0.008	0.250 ± 0.008	0.073 ± 0.002	0.105 ± 0.004	0.065 ± 0.003	0.092 ± 0.002	30.32 ± 1.13	66.01 ± 1.64
CIRS12KO	6	0.425 ± 0.013	0.352 ± 0.013	0.059 ± 0.002	0.074 ± 0.001	0.053 ± 0.001	0.069 ± 0.002	17.46 ± 1.07	43.50 ± 2.14
CIRS12KO x *Becn1*$^{+/-}$	6	0.386 ± 0.011	0.296 ± 0.013	0.067 ± 0.002	0.089 ± 0.004	0.059 ± 0.002	0.078 ± 0.004	23.58 ± 1.71	54.85 ± 2.95
WT	12	0.384 ± 0.013	0.274 ± 0.014	0.071 ± 0.003	0.097 ± 0.004	0.064 ± 0.003	0.088 ± 0.003	28.79 ± 1.65	63.65 ± 2.61
Becn1$^{+/-}$	12	0.363 ± 0.009	0.257 ± 0.012	0.074 ± 0.003	0.104 ± 0.006	0.069 ± 0.003	0.088 ± 0.006	29.21 ± 1.90	64.22 ± 2.82
CIRS12KO x *Becn1*$^{+/-}$	12	0.476 ± 0.014	0.401 ± 0.016	0.055 ± 0.005	0.069 ± 0.006	0.050 ± 0.004	0.066 ± 0.006	15.78 ± 1.47	40.05 ± 3.11

Data - mean values ± SEM are from male mice, n=5-10;
LVDd, Left ventricular cavity diameter at diastole; LVDs, Left ventricular cavity diameter at systole; IVSDd, Interventricular septum diameter at diastole; IVSDs, Interventricular septum diameter at systole; LVPWd, Left ventricular posterior wall thickness at diastole; LVPWs, Left ventricular posterior wall thickness at systole; FS, Fractional shortening; EF, Ejection fraction

Supplemental Table 11: Primer sequences used for quantification of mRNA levels by RT-PCR

Gene Name
Gene Sequence of forward and reverse primers (5'→ 3')
GenBank Accession Number

Actin, alpha 1, skeletal muscle *(Acta1)*
CCTGTATGCCAACAACGTCA
CTCGTCGTACTCCTGCTTGG
XM_134551

Acyl-Coenzyme A dehydrogenase, long-chain *(Acadl)*
ATGGCAAAATACTGGGCATC
TCTTGCGATCAGCTCTTTCA
NM_007381

Acyl-Coenzyme A dehydrogenase, medium chain *(Acadm)*
ACTGACGCCGTTCAGATTTT
GCTTAGTTACACGAGGGTGATG
NM_007382

BCL2/adenovirus E1B interacting protein 3 *(Bnip3)*
TTGGCGAGAAAAACAGCAC
GCTGAGAAAATTCCCCCTTT
NM_009760

Carnitine palmitoyltransferase 1b, muscle *(Cpt1b)*
TGCCTTTACATCGTCTCCAA
AGACCCCGTAGCCATCATC
NM_009948

Cyclophilin A *(Cphn)*
AGCACTGGAGAGAAAGGATTTGG
TCTTCTTGCTGGTCTTGCCATT
NM_008907

Cytochrome c oxidase subunit IV isoform 1 *(Cox4i1)*
CGCTGAAGGAGAAGGAGAAG
GCAGTGAAGCCAATGAAGAA
NM_009941

Cytochrome c oxidase, subunit Vb *(Cox5b)*
TGGAGGTGGTGTCCCTACTG
CTCTTGTTGCTGATGGATGG
M_009942

Estrogen related receptor alpha *(Esrra)*
GGAGGACGGCAGAAGTACAA
CAGGTTCAACAACCAGCAGA
NM_007953

Eukaryotic translation initiation factor 4E *(Eif4e)*
AGGTGGGCACTCTGGTTTTT
ATAGGCTCAATCCCGTCCTT
NM_007917

Eukaryotic translation initiation factor 4E binding protein 1 *(Eif4ebp1)*
CGTAGGACGCAATGATGCT
TGTTCACAAAATTCAAGGCAGA
NM_007918

Fatty acid binding protein 3 *(Fabp3)*
GACGGGAAACTCATCCTGAC
TCTCCAGAAAAATCCCAACC
NM_010174.1

F-box protein 32 *(Fbxo32)*
GCTGGATTGGAAGAAGATGTATT
TTGAGGGGAAAGTGAGACG
NM_026346

Gamma-aminobutyric acid (GABA) A receptor-associated protein-like 1 *(Gabarapl1)*
CTTCCACCCAGGCTTCATAG
TATGGGATGAGGAGCAGGAC
NM_020590

Gamma-aminobutyric acid receptor associated protein *(Gabarap)*
CGGATAGGAGACCTGGACAA
ACTGGTGGGTGGAATGACA
NM_019749

Hydroxyacyl-CoA Dehydrogenase - alpha subunit *(Hadha)*
TCAGGAGGGCTCAAAGAATAA
GAAAGCCAAGCCCAAAGAC
XM_131963

Hydroxyacyl-Coenzyme A dehydrogenase - beta subunit *(Hadhb)*
GCCAACAGACTGAGGAAGGA
ACACTGGCAAGGCTGGATT
NM_145558

Microtubule-associated protein 1 light chain 3 beta *(Map1lc3b)*
CGTCCTGGACAAGACCAAGT
ATTGCTGTCCCGAATGTCTC
NM_026160

NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9 *(Ndufa9)*
ATCCCTTACCCTTTGCCACT
CCGTAGCACCTCAATGGACT
NM_025358

NADH dehydrogenase (ubiquinone) flavoprotein 1 *(Ndufv1)*
TGTGAGACCGTGCTAATGGA
CATCTCCCTTCACAAATCGG
NM_133666

Natriuretic peptide precursor type A *(Nppa)*
ATGGGCTCCTTCTCCATCA
CCTGCTTCCTCAGTCTGCTC
K02781

Natriuretic peptide precursor type B *(Nppb)*
GGATCTCCTGAAGGTGCTGT
TTCTTTTGTGAGGCCTTGGT
D16497

Nuclear respiratory factor 1 *(Nrf1)*
CTTCAGAACTGCCAACCACA
GCTTCTGCCAGTGATGCTAC
NM_010938

Nuclear respiratory factor 2 *(Nrf2)*
AGTCTTCACTGCCCCTCATC
TCTGTCAGTGTGGCTTCTGG
NM_010902

Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha *(Ppargc1a)*
GTAAATCTGCGGGATGATGG
AGCAGGGTCAAAATCGTCTG
NM_008904

Peroxisome proliferative activated receptor, gamma, coactivator 1 beta *(Ppargc1b)*
TGAGGTGTTCGGTGAGATTG
CCATAGCTCAGGTGGAAGGA
NM_133249

Peroxisome proliferator activated receptor alpha *(Ppara)*
GAGAATCCACGAAGCCTACC
AATCGGACCTCTGCCTCTTT
NM_011144

Phosphoinositide-3-kinase, class 3 *(Pik3c3)*
TGTCAGATGAGGAGGCTGTG
CCAGGCACGACGTAACTTCT
NM_181414

Pyruvate dehydrogenase E1 alpha 1 *(Pdha1)*
GGGACGTCTGTTGAGAGAGC
TGTGTCCATGGTAGCGGTAA
NM_008810

Pyruvate dehydrogenase kinase, isoenzyme 4 *(Pdk4)*
GCTTGCCAATTTCTCGTCTC
CTTCTCCTTCGCCAGGTTCT
NM_013743

Ribosomal protein S16 *(Rps16)*
TGCTGGTGTGGATATTCGGG
CCTTGAGATGGGCTTATCGG
XM_003085753.1

Glyceraldehyde-3-phosphate dehydrogenase *(Gapdh)*
AACGACCCCTTCATTGAC
TCCACGACATACTCAGCAC
NM_008084.2

TNF receptor superfamily member 6 *(Fas)*
CGATTCTCCTGGCTGTGAAC
TGGAATTAACAAAACAAGGATGG
NM_007987

Laminin, alpha 1 *(Lama1)*
ACCAAGGACTTCCTATCCAT
AGGCGATTTTATACCAGGTT
NM_008480.2

Transcription factor A, mitochondrial *(Tfam)*
CAAAAAGACCTCGTTCAGCA
CTTCAGCCATCTGCTCTTCC
NM_009360

Ubiquinol-cytochrome c reductase core protein 1 *(Uqcrc1)*
TGCCAGAGTTTCCAGACCTT
CCAAATGAGACACCAAAGCA
NM_025407

Uncoupling protein 2 *(Ucp2)*
TCTCCTGAAAGCCAACCTCA
CTACGTTCCAGGATCCCAAG
NM_011671.4

Primer pairs were designed based on GenBank reference sequences. We used the WWW interface Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) with default settings. To avoid non-specific amplifications, primer sequences were blasted against mouse genes. Dissociation curves were analyzed for all primer-pairs to ensure single product amplification.

Extended Experimental Procedures

Glucose Tolerance Tests and Insulin Tolerance Tests (ITT)

For Glucose Tolerance Tests, mice were fasted for 6 h fast starting at 6 am and were injected intraperitoneally with 1 g glucose / kg body weight. Insulin Tolerance Tests were performed on random fed animals by intraperitoneal injection of 0.75 U insulin / kg body weight. Blood glucose concentrations were measured using a glucometer (Bayer Glucometer Elite).

Metabolomic analysis and measurement of Amino Acids levels

Extraction of metabolites and Amino Acids for gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS)

Serum Amino Acids levels were determined using gas chromatography-mass spectrometry (GC-MS). Extraction was performed as previously described to remove proteins by precipitation. Briefly, 360 µL of -20°C 90% methanol (aq.) was added to 40 µL of the individual tubes containing serum to give a final concentration of 80% methanol. The samples were incubated for one hour at -20°C followed by centrifugation at 30,000 x g for 10 minutes using a rotor chilled to -20°C. The supernatant containing the extracted Amino Acids was then transferred to fresh disposable tubes and completely dried en vacuo.

For measurement of tissue metabolites and Amino Acid levels, about 40 mg of heart tissue were placed into a bead mill tube containing 1.4 mm ceramic beads (MoBio Laboratories). Weights were recorded for normalization purposes. MeOH (aq) was added to the tubes that had been chilled to -20°C to a final concentration of 80% MeOH (aq) and 20% tissue homogenate. Samples were homogenized for 30 seconds at 6.5 m/sec using an Omni Bead Ruptor 24 bead mill (Omni-Inc.) and incubated for one hour at -20°C to precipitate protein. Following incubation, cell debris was pelleted by centrifugation (14,000 g for 5 min at 4°C) and the supernatant reserved. A second extraction of the pellet was performed by the addition of -20°C MeHO (aq) to a final concentration of 50% MeOH (aq) and 50% tissue homogenate. Each sample was mixed by vortex, incubated for one hour at -20°C, and centrifuged (14,000 g for 5 min at 4°C) to remove cell debris. The two extracts were combined, mixed, and then split in half to new tubes. Samples were dried *en vacuo* (1).

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was performed with a Waters GCT Premier mass spectrometer fitted with an Agilent 6890 gas chromatograph and a Gerstel MPS2 autosampler. Dried samples were suspended in 40 µL of 40 mg/mL O-methoxylamine hydrochloride (MOX) in pyridine and incubated for one hour at 30°C. 25 µL of this solution was added to autosampler vials, then 20 µL of N-methyl-N-trimethylsilyltrifluoracetamide (MSTFA) was added using the autosampler and incubated for 60 minutes at 37°C with shaking. 1 µL of the sample was injected to the gas chromatograph inlet in the split mode at a 10:1 split ratio with the inlet temperature held at 250°C. The gas

chromatograph had an initial temperature of 95°C for one minute followed by a 40°C/min ramp to 110°C and a hold time of 2 minutes. This was followed by a second 5°C/min ramp to 250°C, a third ramp to 350°C, then a final hold time of 3 minutes. A 30 m Phenomenex-ZB5MSi column with a 5 m long guard column was employed for chromatographic separation. Data was collected using MassLynx 4.1 software (Waters). A two-step process was employed for data analysis, a targeted followed by non-targeted analysis. For the targeted approach, known metabolites and Amino Acids were identified and their peak area was recorded using QuanLynx. For the non-targeted approach, peak picking and analysis was performed using MarkerLynx. Principle component analysis (PCA) and partial least squares-discriminate analysis (PLS-DA) was performed using SIMCA-P 12.0 (Umetrics). Potential metabolite biomarkers were further investigated by manually recording the peak area. Metabolite identity was established using a combination of an in house metabolite library developed using pure purchased standards and the commercially available NIST library.

Liquid chromatography-mass spectrometry (LC-MS) analysis

LC-MS was performed using an Agilent 6520 QTOF-MS fitted with an Agilent 1100 LC. Each sample was suspended in 10 µL of 10 mM ammonium acetate (pH 6.8) and 90 µL of acetonitrile and placed into a chilled auto sampler tray (CTC Analytics). Each sample was analysed using two separate column chemistries, a SeQuant ZIC-HILIC at pH 3.2 in the positive mode and a SeQuant ZIC-pHILIC at pH 9.2 in the negative mode (Merck KGaA). 3 µL of sample were injected into each column with initial conditions set to 90% acetonitrile/10% buffer (10 mM ammonium formate pH 3.2 for HILIC chromatography or 10 mM ammonium formatate pH 9.2 for pHILIC chromatography) for one minute followed by a 20 minute ramp to 40% acetonitrile/60% buffer. The flow rate was set to 0.2 mL/min. Detection was performed in the positive mode for the HILIC analysis and the negative mode for the pHILIC analysis. Data analysis was performed using Mass Hunter Qual and Mass Profiler Professional (Agilent).

Supplemental References

1. A, J., Trygg, J., Gullberg, J., Johansson, A.I., Jonsson, P., Antti, H., Marklund, S.L., and Moritz, T. 2005. Extraction and GC/MS analysis of the human blood plasma metabolome. *Anal Chem* 77:8086-8094.