Supplementary Materials for

Insulin Receptor Substrates (IRS) are Essential for the Bioenergetic and Hypertrophic Response of the Heart to Exercise Training

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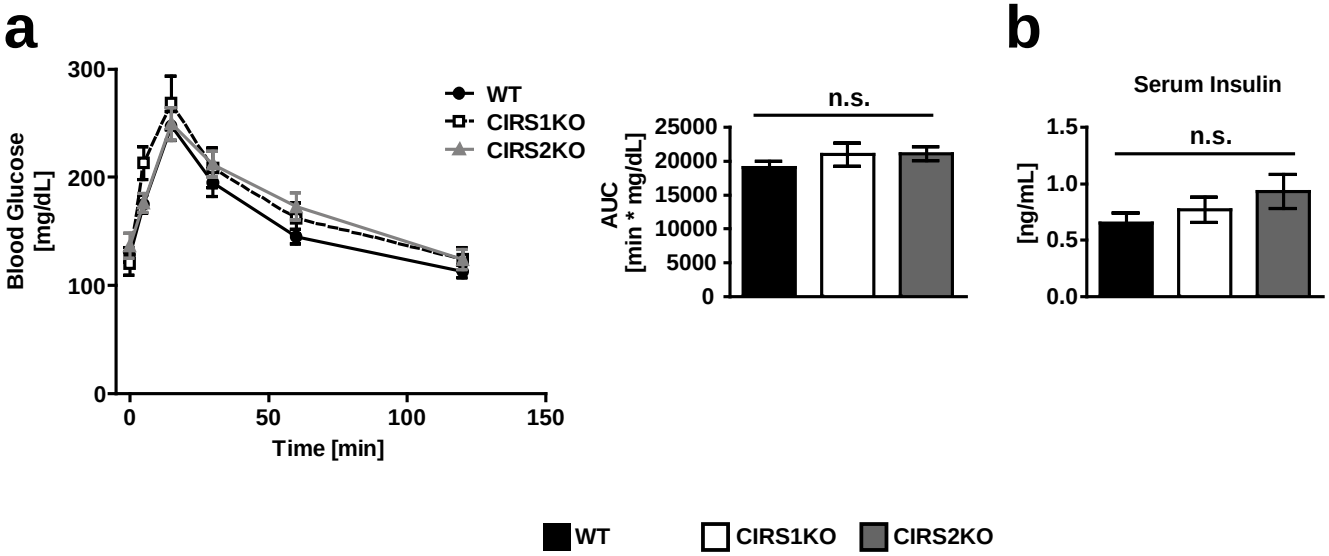
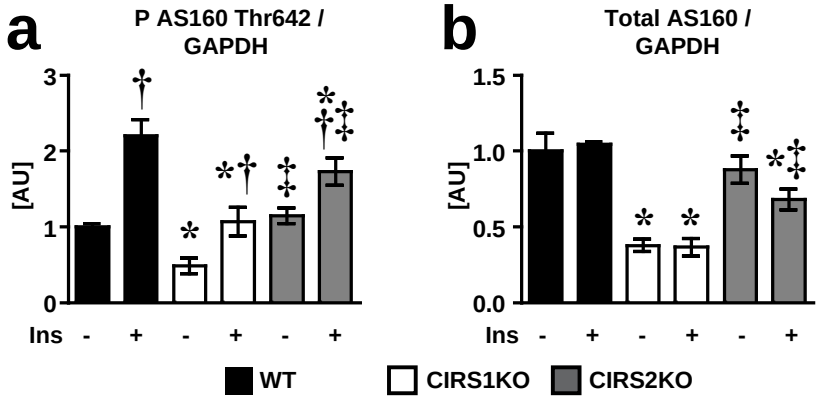
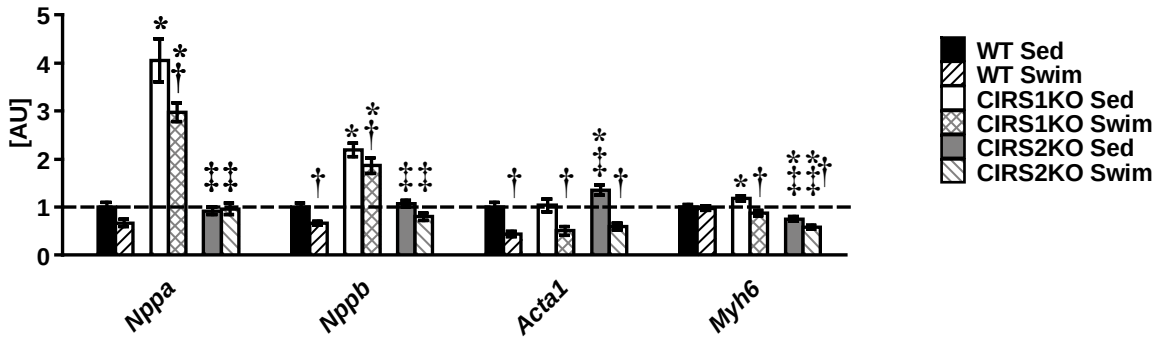


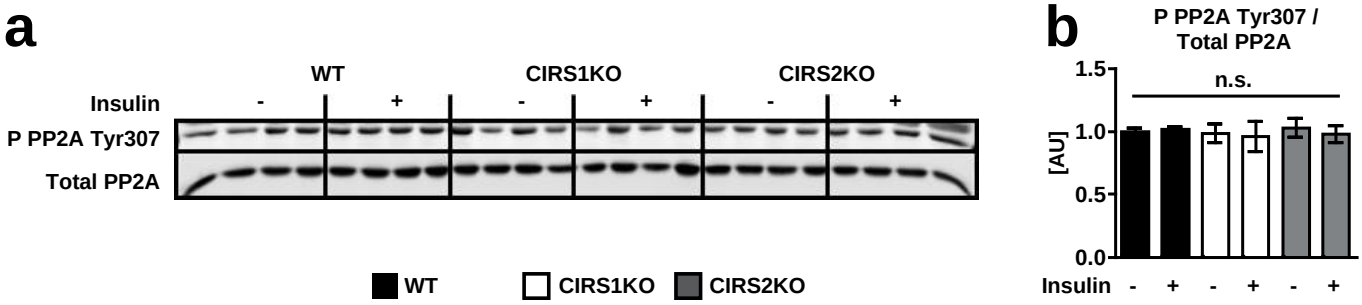
Supplementary Figure 1: (a) Glucose tolerance tests and (b) serum insulin levels at the age of 9 wk. Blood glucose was measured at the indicated time points post injection. n.s., no significant difference observed; AUC, area under the curve. Data are reported as mean values ± SEM, n≥7.



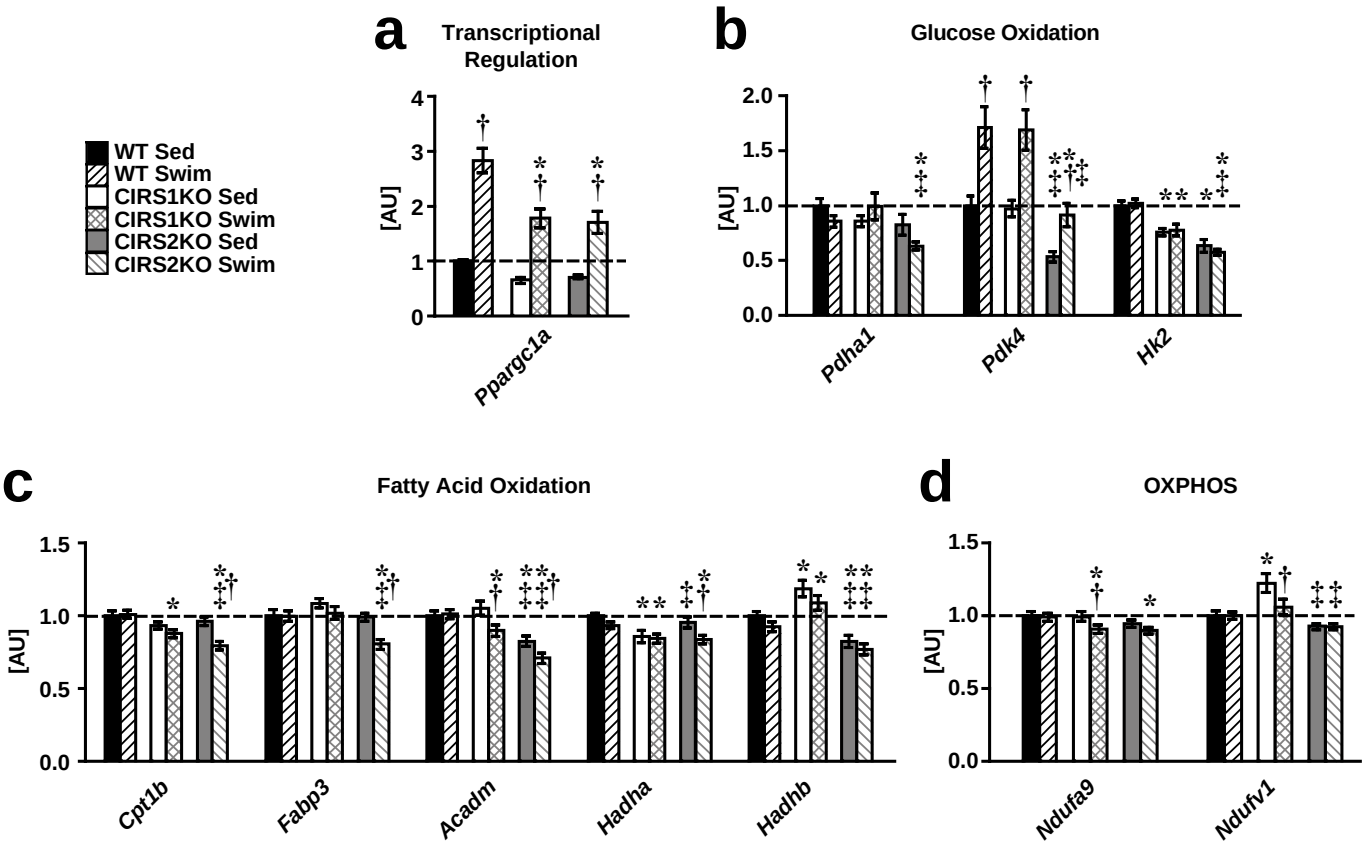
Supplementary Figure 2: Quantification of (a) insulin stimulated AS160 Thr642 phosphorylation and (b) total AS160 expression normalized to GAPDH each in genotypes as indicated (n=4). Two-way ANOVA was performed to analyze differences by insulin stimulation and genotype followed by Fisher's PLSD post hoc analysis. P AS160 Thr642 / GAPDH: $p<0.05$ for insulin stimulation and for genotype respectively, Total AS160 / GAPDH: $p<0.05$ for genotype. * $p<0.05$ vs. WT same insulin concentration, † $p<0.05$ vs. same genotype 0 nM insulin, ‡ $p<0.05$ vs. CIRS1KO same insulin concentration.



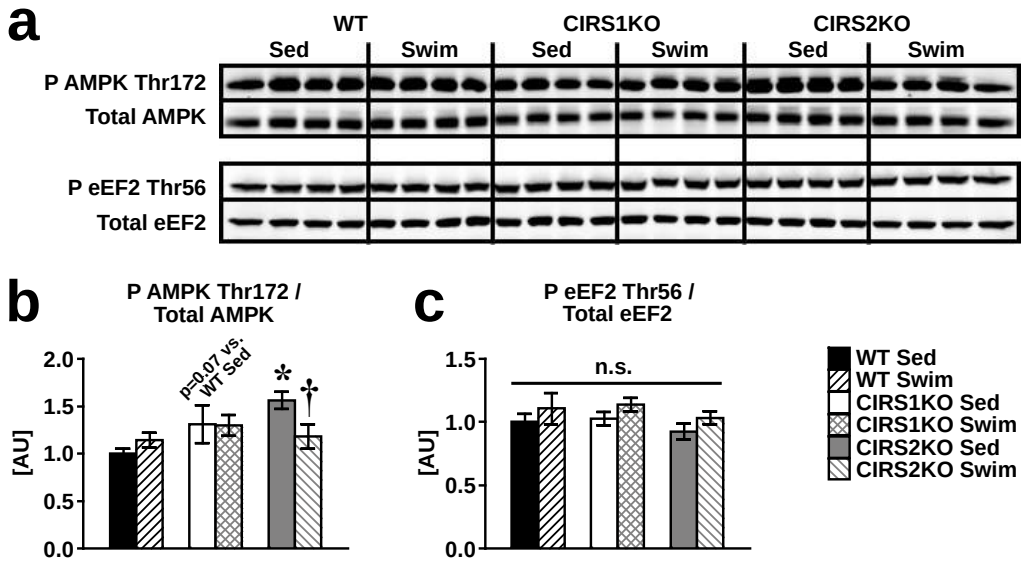
Supplementary Figure 3: mRNA expression of heart failure markers and genes involved in cardiac structure in WT, CIRS1KO and CIRS2KO hearts under sedentary conditions and following chronic exercise training as indicated (n=8). Data are presented as fold change vs. WT Sed. Gene expression was normalized to *Rps16*. Two-way ANOVA was performed to analyze differences by swim training and genotype followed by Fisher's PLSD post hoc analysis. *Nppa:* $p<0.05$ for swim training, genotype, and their interaction, *Nppb:* $p<0.05$ for swim training and for genotype respectively, *Acta1:* $p<0.05$ for swim training and for genotype respectively, and *Myh6:* $p<0.05$ for swim training, genotype, and their interaction. * $p<0.05$ vs. WT same treatment, † $p<0.05$ vs. sedentary same genotype, ‡ $p<0.05$ vs. CIRS1KO same treatment.



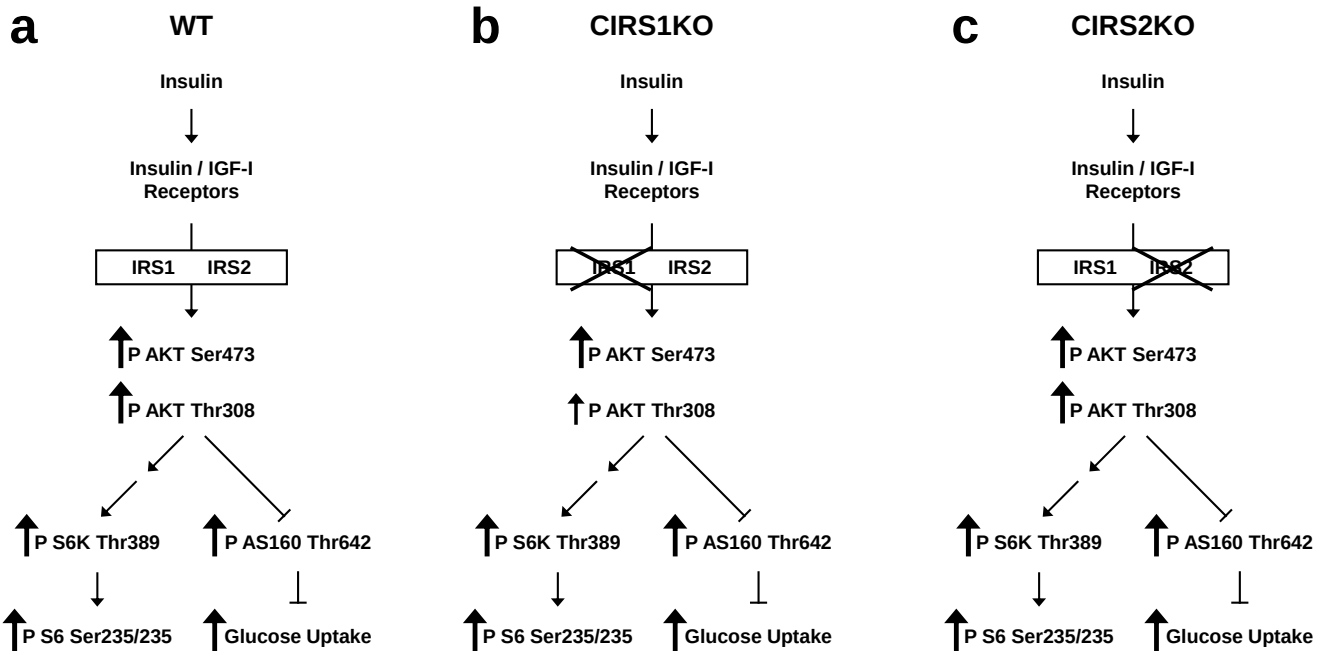
Supplementary Figure 4: (a) Representative immunoblots and (b) quantification of insulin stimulated PP2A phosphorylation in genotypes as indicated. Hearts were perfused in the isolated working heart mode for 1 hour with Krebs Henseleit buffer containing 5 mM glucose and 0.4 mM palmitate in the presence or absence of 1 nM insulin (n=4). Two-way ANOVA was performed to analyze differences by insulin stimulation and genotype followed by Fisher's PLSD post hoc analysis. n.s., no significant difference observed.



Supplementary Figure 5: (a-d) mRNA expression in WT, CIRS1KO and CIRS2KO hearts under sedentary conditions and following chronic exercise training as indicated (n=8). Data are presented as fold change vs. WT Sed. Gene expression was normalized to *Rps16*. Two-way ANOVA was performed to analyze differences by swim training and genotype followed by Fisher's PLSD post hoc analysis; *Ppargc1a:* $p<0.05$ for swim training, genotype, and their interaction, *Pdha1:* $p<0.05$ for genotype, *Pdk4:* $p<0.05$ for swim training and for genotype respectively, *Hk2:* $p<0.05$ for genotype, *Cpt1b* and *Fabp3:* $p<0.05$ for swim training, genotype, and their interaction, *Acadm, Hadha, Hadhb* and *Ndufa9:* $p<0.05$ for swim training and for genotype respectively, *Ndufv1:* $p<0.05$ for genotype. * $p<0.05$ vs. WT same treatment, † $p<0.05$ vs. sedentary same genotype, ‡ $p<0.05$ vs. CIRS1KO same treatment.



Supplementary Figure 6: (a) Representative immunoblots and (b/c) quantification from ventricle homogenates obtained from WT, CIRS1KO and CIRS2KO hearts under sedentary conditions and following chronic exercise training as indicated (n=8). Two-way ANOVA was performed to analyze differences by swim training and genotype followed by Fisher's PLSD post hoc analysis; P AMPK Thr172 / Total AMPK: $p<0.05$ for genotype. * $p<0.05$ vs. WT same treatment, † $p<0.05$ vs. sedentary same genotype; n.s., no significant difference observed.



Supplementary Figure 7: IRS isoforms play redundant roles in the regulation of myocardial insulin signaling and glucose uptake in isolated cardiomyocytes. Figure indicates changes following insulin stimulation in (a) WT, (b) CIRS1KO, and (c) CIRS2KO relative to basal conditions.

Supplementary Table 1: Characteristics of mice pre and post exercise training

Parameter	WT Sed	WT Swim	CIRS1KO Sed	CIRS1KO Swim	CIRS2KO Sed	CIRS2KO Swim
BW pre exercise [g] $	22.1 ± 0.7	21.4 ± 0.9	15.8 ± 0.5 *	16.7 ± 0.7 *	23.0 ± 0.5 ‡	20.8 ± 0.7 †‡
BW post exercise [g] # $ &	26.9 ± 0.6	22.0 ± 0.6 †	18.8 ± 0.5 *	17.1 ± 0.7 *†	29.0 ± 0.5 *	22.7 ± 0.7 †‡
Δ BW pre-post exercise [g] # $	4.8 ± 0.3	0.6 ± 0.4 †	3.1 ± 0.4 *	0.4 ± 0.4 †	6.0 ± 0.5 *‡	1.8 ± 0.5 *†‡
Δ BW pre-post exercise [%] # $	18.0 ± 1.3	3.1 ± 1.7 †	16.2 ± 1.9	2.3 ± 1.9 †	20.6 ± 1.5	7.8 ± 2.3 †‡
HW [mg] $ &	110.7 ± 0.9	117.7 ± 2.1 †	90.6 ± 2.3 *	87.6 ± 3.4 *	123.2 ± 2.4 *‡	117.3 ± 2.3 ‡
TL [mm] # $	17.3 ± 0.1	16.7 ± 0.2 †	16.2 ± 0.1 *	15.9 ± 0.2 *	17.8 ± 0.1 *‡	16.9 ± 0.1 †‡
HW/TL [mg/mm] # $ &	6.39 ± 0.05	7.04 ± 0.10 †	5.57 ± 0.11 *	5.50 ± 0.16 *	6.93 ± 0.10 *‡	6.92 ± 0.10 ‡

Data are reported as mean values ± SEM, n=10. Two-way ANOVA was performed to analyze differences by swim training and genotype followed by Fisher's PLSD post hoc analysis; # $p<0.05$ for swim training, $ $p<0.05$ for genotype, and & $p<0.05$ for the interaction between swim training and genotype. * $p<0.05$ vs. WT same treatment, † $p<0.05$ vs. Sedentary same genotype, ‡ $p<0.05$ vs. CIRS1KO same treatment. BW, body weight; HW, heart weight; TL, tibia length.

Supplementary Table 2: Left ventricular hemodynamic parameters in WT, CIRS1KO and CIRS2KO hearts following chronic exercise training

Parameter	WT Sed	WT Swim	CIRS1KO Sed	CIRS1KO Swim	CIRS2KO Sed	CIRS2KO Swim
LVSP [mmHg]	103.6 ± 2.8	108.5 ± 4.6	105.1 ± 2.1	98.7 ± 2.4 *	107.1 ± 2.5	102.2 ± 2.2
LVMP [mmHg] $	1.5 ± 0.7	4.1 ± 1.1	6.8 ± 1.4 *	7.5 ± 1.3 *	2.8 ± 1.0 ‡	2.5 ± 0.7 ‡
LVEDP [mmHg] $	15.8 ± 2.1	21.3 ± 3.9	30.0 ± 2.9 *	28.0 ± 3.5	20.8 ± 3.6 ‡	16.1 ± 2.0 ‡
LV Dev P [mmHg] $	102.2 ± 2.8	104.4 ± 4.5	98.3 ± 2.0	91.2 ± 2.3 *	104.3 ± 2.7	99.7 ± 2.0 ‡
Max dP/dt [mmHg/s] $	8449.2 ± 537.7	7544.7 ± 429.3	7085.7 ± 478.3	6268.8 ± 344.5	7971.1 ± 512.4	8759.1 ± 534.3 ‡
Min dP/dt [mmHg/s]	-8548.3 ± 404.2	-7558.5 ± 629.9	-7776.5 ± 454.0	-7075.5 ± 496.6	-8114.9 ± 559.0	-8323.8 ± 457.2
Heart Rate [bpm] # $	498.9 ± 15.4	472.3 ± 24.0	545.7 ± 14.4	520.7 ± 17.1	508.5 ± 16.5	466.0 ± 12.5 ‡

Data are reported as mean values ± SEM, n=9-12. Two-way ANOVA was performed to analyze differences by swim training and genotype followed by Fisher's PLSD post hoc analysis; # $p<0.05$ for swim training and $ $p<0.05$ for genotype. * $p<0.05$ vs. WT same treatment, ‡ $p<0.05$ vs. CIRS1KO same treatment. LVSP, left ventricular systolic pressure; LVMP, left ventricular minimum pressure; LVEDP, left ventricular end-diastolic pressure; LV Dev P, left ventricular developed pressure; Max dP/dt, maximal rate of increase in left ventricular pressure; Min dP/dt, maximal rate of decrease in left ventricular pressure; bpm, beats per minute.

Supplementary Table 3: Cardiac substrate metabolism and function in isolated working WT, CIRS1KO and CIRS2KO hearts following chronic exercise training

Parameter	WT Sed	WT Swim	CIRS1KO Sed	CIRS1KO Swim	CIRS2KO Sed	CIRS2KO Swim
Palmitate Oxidation [nmol/min/g dhw] # $ &	338.5 ± 37.6	660.1 ± 122.1 †	380.7 ± 35.3	399.8 ± 36.8 *	304.8 ± 31.8	388.1 ± 28.2 *
Glucose Oxidation [nmol/min/g dhw] # $	147.2 ± 20.0	300.8 ± 50.2 †	266.0 ± 37.4	417.1 ± 57.8 *†	205.1 ± 37.7	247.5 ± 32.3 ‡
Glycolysis [nmol/min/g dhw] #	620.1 ± 98.2	1083.2 ± 109.6 †	912.5 ± 111.6	1343.9 ± 220.0 †	791.1 ± 88.1	960.6 ± 123.2
O_2 Consumption [µL/min/ g whw] $	128.1 ± 6.2	107.2 ± 7.3	168.9 ± 9.8 *	167.1 ± 9.5 *	146.6 ± 9.4	143.6 ± 7.1 *‡
Cardiac Efficiency [%] # $	17.1 ± 1.1	22.8 ± 1.7 †	13.3 ± 0.9 *	16.2 ± 1.2 *	13.8 ± 0.8 *	18.0 ± 1.4 *†
Cardiac Power [mW/g dhw] $ &	26.8 ± 1.5	32.2 ± 1.7 †	30.8 ± 1.3	36.2 ± 1.5 †	30.6 ± 1.5	20.6 ± 1.3 *†‡
Heart Rate [bpm] $ &	262.4 ± 5.9	279.2 ± 12.1	240.8 ± 5.2 *	261.6 ± 5.9 †	259.2 ± 5.5	213.6 ± 9.9 *†‡
Developed Pressure [mmHg] &	32.7 ± 1.5	33.6 ± 1.0	31.0 ± 0.7	36.3 ± 1.0 †	36.7 ± 1.5 *‡	29.4 ± 1.4 *†‡
Cardiac Output [mL/min] # $ &	11.3 ± 0.3	12.1 ± 0.3 †	9.9 ± 0.2 *	12.0 ± 0.3 †	10.9 ± 0.2 ‡	10.5 ± 0.5 *‡

Data are reported as mean values ± SEM. Two-way ANOVA was performed to analyze differences by swim training and genotype followed by Fisher's PLSD post hoc analysis; # $p<0.05$ for swim training, $ $p<0.05$ for genotype, and & $p<0.05$ for the interaction between swim training and genotype. * $p<0.05$ vs. WT same treatment, † $p<0.05$ vs. Sedentary same genotype, ‡ $p<0.05$ vs. CIRS1KO same treatment. n=6-10 hearts per group for metabolism, oxygen consumption and efficiency; n=14-16 for cardiac function (pooled data from glucose and palmitate perfusions).

Supplementary Table 4: Tissue glycogen content in WT, CIRS1KO and CIRS2KO hearts following chronic exercise training

	WT Sed	WT Swim	CIRS1KO Sed	CIRS1KO Swim	CIRS2KO Sed	CIRS2KO Swim
Glycogen [µmol / g tissue] $ &	6.97 ± 0.82	10.89 ± 1.34 †	15.27 ± 0.67 *	9.74 ± 1.15 †	11.67 ± 1.48 *‡	13.65 ± 1.33 ‡

Data are reported as mean values ± SEM, n=7-8. Two-way ANOVA was performed to analyze differences by swim training and genotype followed by Fisher's PLSD post hoc analysis; $ $p<0.05$ for genotype, and & $p<0.05$ for the interaction between swim training and genotype. * $p<0.05$ vs. WT same treatment, † $p<0.05$ vs. Sedentary same genotype, ‡ $p<0.05$ vs. CIRS1KO same treatment.

Supplementary Methods

Glucose tolerance tests

For Glucose tolerance tests, mice were fasted for 6 h (fast starting at 6 am) and were then injected intraperitoneally with 1 g glucose / kg body weight. Blood glucose concentrations were measured using a glucometer (Glucometer Elite; Bayer, Tarrytown, NY). Area under the curve (AUC) was calculated using the GraphPad Prism Software (GraphPad, San Diego, CA).

Measurement of serum insulin levels

Blood samples were collected by submandibular bleed after a 6 h fast (starting at 6 am) and serum insulin concentrations were measured using a rat insulin RIA kit (Millipore, Billerica, MA).

Protein extraction and immunoblotting analysis

For protein extraction, 50 µg of frozen tissue was homogenized in 500 µL lysis buffer (50 mmol/L Hepes, 150 mmol/L NaCl, 10% Glycerol, 1% Triton X-100, 1.5 mmol/L $MgCl_2$, 1 mmol/L EGTA, 10 mmol/L Sodium Pyrophosphate, 100 mmol/L Sodium Fluoride and 100 µmol/L Sodium Vanadate, 1 mmol/L PMSF, 10 µg/mL Aprotinin, and 10 µg/mL Leupeptin) using a tissue lyser (Qiagen, Valencia, CA). Proteins were resolved by SDS-PAGE and electro-transferred to nitrocellulose (IRS immunoblotting) or PVDF membranes (other targets). The following primary antibodies were used:

Antigen	**Company**
4E-BP1	Abcam, Cambridge, MA
α-Tubulin	Sigma-Aldrich, St. Louis, MO
Phospho-AMPK (Thr172)	Cell Signaling, Danvers, MA
AMPK	Cell Signaling, Danvers, MA
Phospho-Akt (Ser473)	Cell Signaling, Danvers, MA
Phospho-Akt (Ser473)	Cell Signaling, Danvers, MA
Phospho-Akt (Thr308)	Cell Signaling, Danvers, MA
Akt	Cell Signaling, Danvers, MA
Phospho-AS160 (Thr642)	Cell Signaling, Danvers, MA
AS160	Millipore, Billerica, MA
CPT1b	Santa Cruz Biotechnology, Santa Cruz, CA

Antigen	**Company**
CPT2	Abcam, Cambridge, MA
Phospho-eEF2 (Thr56)	Cell Signaling, Danvers, MA
eEF2	Cell Signaling, Danvers, MA
GAPDH	Cell Signaling, Danvers, MA
Phospho-GSK-3β (Ser9)	Cell Signaling, Danvers, MA
GSK-3α/β	Santa Cruz Biotechnology, Santa Cruz, CA
IRS1	Millipore, Billerica, MA
IRS2	Millipore, Billerica, MA
Phospho-JAK2 (Tyr221)	Cell Signaling, Danvers, MA
JAK2	Cell Signaling, Danvers, MA
OXPHOS Complex I, NDUFA9	Invitrogen Corporation, Carlsbad, CA
OXPHOS Complex II, 30 kDa subunit	Invitrogen Corporation, Carlsbad, CA
OPXHOS Complex V, subunit α	Invitrogen Corporation, Carlsbad, CA
Phospho-p70 S6 Kinase (Thr389)	Cell Signaling, Danvers, MA
p70 S6 Kinase	Cell Signaling, Danvers, MA
PGC-1α	Santa Cruz Biotechnology, Santa Cruz, CA
PI3 kinase, p85	Millipore, Billerica, MA
Phospho-PP2A-Cα/β (Tyr 307)	Santa Cruz Biotechnology, Santa Cruz, CA
PP2A-Cα/β	Santa Cruz Biotechnology, Santa Cruz, CA
Phospho-S6 (Ser235/236)	Cell Signaling, Danvers, MA
S6	Cell Signaling, Danvers, MA
Phospho-Stat3 (Tyr705)	Cell Signaling, Danvers, MA
STAT3	Cell Signaling, Danvers, MA

Protein detection was carried out with the appropriate horseradish peroxidase-conjugated secondary antibody and ECL detection systems (Amersham Biosciences, Piscataway, NJ) or Alexa fluor anti-Rabbit 680 (Invitrogen) and anti-Mouse 800 (VWR International, West Chester, PA) as secondary antibodies and fluorescence was quantified using the LI-COR Odyssey imager (Lincoln, NE) (1).

Mitochondrial function in saponin-permeabilized cardiac fibers

Saponin-permeabilized cardiac fibers

Mitochondrial function was measured in saponin-permeabilized subendocardial cardiac fibers (2). Small pieces (2 to 5 mg wet weight) of left ventricular cardiac muscle were dissected from freshly excised hearts and permeabilized for 30 minutes at 4 °C in buffer A (50 µg/mL saponin, 7.23 mmol/L K_2EGTA, 2.77 mmol/L $K_2CaEGTA$, 6.56 mmol/L $MgCl_2$, 20 mmol/L imidazole, 0.5 mmol/L dithiothreitol, 53.3 mmol/L K-methanS, 20 mmol/L taurine, 5.3 mmol/L Na_2ATP, 15 mmol/L PCr, and 3 mmol/L KH_2PO_4, pH 7.1 adjusted at 25 °C). Next, fibers were washed twice for 10 minutes at 4 °C in buffer B (7.23 mmol/L K_2EGTA, 2.77 mmol/L $K_2CaEGTA$, 1.38 mmol/L $MgCl_2$, 20 mmol/L imidazole, 0.5 mmol/L dithiothreitol, 100 mmol/L K-methanS, 20 mmol/L taurine, 3 mmol/L KH_2PO_4, 2 mg/mL BSA, pH 7.1 adjusted at 25 °C) using palmitoyl-carnitine (20 µmol/L) or pyruvate (10 mmol/L) as substrates, each combined with malate (5 mmol/L).

Mitochondrial oxygen consumption

Mitochondrial oxygen consumption rates of cardiac fibers were measured using an oxygen sensor probe (Ocean Optics, Dunedin, FL) in 2 mL of KCl buffer at 25 °C (125 mmol/L KCl, 20 mmol/L HEPES, 3 mmol/L Mg-Acetate, 0.4 mmol/L EGTA, 2 mg/mL BSA, 5 mmol/L KH_2PO_4 and 0.3 mmol/L Dithiothreitol, pH 7.1 adjusted at 25 °C) with palmitoyl-carnitine (20 µmol/L) or pyruvate (10 mmol/L) as substrates, each combined with malate (5 mmol/L). V_{ADP} was determined as maximal mitochondrial oxygen consumption obtained by incubating muscle fibers with 1 mmol/L ADP. The solubility of oxygen in KCl buffer was 246.87 nmol of O_2 / mL. Oxygen consumption rates were expressed as nmol O_2 * min^{-1} * mg dry fiber $weight^{-1}$.

Mitochondrial ATP production

For measurement of ATP production, ADP was added to 2 mL of buffer B to a final concentration of 1 mmol/L. Next, 10 µL Buffer B obtained from the respiration chamber were added to 190 µL frozen DMSO every 10 seconds for a 1-min time period. ATP production was determined using a bioluminescence assay based on the luciferin/luciferase reaction with the ATP assay kit (Promega Corporation, Madison, WI).

RNA extraction and quantitative RT-PCR

Total RNA was extracted from hearts using TRIzol reagent (Invitrogen Corporation, Carlsbad, CA) and purified with the RNeasy kit (Qiagen Inc., Valencia, CA). RNA concentration was determined by measuring the absorbance at 260 nm and 280 nm using a spectrophotometer (NanoDrop 1000, NanoDrop products, Wilmington, DE). Total RNA (~ 3 μg) was reverse transcribed (SuperScriptTM III Reverse Transcriptase Kit, Invitrogen, Carlsbad, CA).

Platinum Taq DNA polymerase (Invitrogen), primers, SYBR-green (Invitrogen) fluorescent dye and cDNA samples were transferred to a 384-well plate in triplicate and real-time polymerase chain reaction was performed with an ABI Prism 7900HT instrument (Applied Biosystems, Foster City, CA). The following cycle profile was used: 1 cycle at 95 °C for 5 min, 40 cycles of 95 °C for 15 sec, 55 °C for 15 sec, 72 °C for 30 sec, and 1 cycle of 72 °C for 7 min. Data were normalized to *Rps16* and expressed as fold change compared to WT sedentary controls. The following primers were used:

Gene Name **Gene Sequence of forward and reverse primers (5'→ 3')** **GenBank Accession Number**
Actin, alpha 1, skeletal muscle *(Acta1)* CCTGTATGCCAACAACGTCA CTCGTCGTACTCCTGCTTGG NM_001272041.1
Acyl-Coenzyme A dehydrogenase, medium chain *(Acadm)* ACTGACGCCGTTCAGATTTT GCTTAGTTACACGAGGGTGATG NM_007382.5
Carnitine palmitoyltransferase 1b, muscle *(Cpt1b)* TGCCTTTACATCGTCTCCAA AGACCCCGTAGCCATCATC NM_009948.2
Fatty acid binding protein 3 *(Fabp3)* GACGGGAAACTCATCCTGAC TCTCCAGAAAAATCCCAACC NM_010174.1
Hexokinase 2 *(Hk2)* CCTTGGATCTTGGAGGAACA CAAGGGAAGGAGAAGGTGAA NM_013820.3

Hydroxyacyl-CoA Dehydrogenase - alpha subunit *(Hadha)* TCAGGAGGGCTCAAAGAATAA GAAAGCCAAGCCCAAAGAC NM_178878.2
Hydroxyacyl-Coenzyme A dehydrogenase - beta subunit *(Hadhb)* GCCAACAGACTGAGGAAGGA ACACTGGCAAGGCTGGATT NM_001289798.1
Myosin, heavy polypeptide 6, cardiac muscle, alpha *(Myh6)* GGGACCGTAGCAAGAAGGAA CCAGCTTTCCAGTAGCTCCA NM_001164171.1
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9 *(Ndufa9)* ATCCCTTACCCTTTGCCACT CCGTAGCACCTCAATGGACT NM_025358.3
NADH dehydrogenase (ubiquinone) flavoprotein 1 *(Ndufv1)* TGTGAGACCGTGCTAATGGA CATCTCCCTTCACAAATCGG NM_133666.3
Natriuretic peptide precursor type A *(Nppa)* ATGGGCTCCTTCTCCATCA CCTGCTTCCTCAGTCTGCTC NM_008725.2
Natriuretic peptide precursor type B *(Nppb)* GGATCTCCTGAAGGTGCTGT TTCTTTTGTGAGGCCTTGGT NM_008726.5
Peroxisome proliferator activated receptor, gamma, coactivator 1 alpha *(Ppargc1a)* GTAAATCTGCGGGATGATGG AGCAGGGTCAAAATCGTCTG NM_008904.2
Pyruvate dehydrogenase E1 alpha 1 *(Pdha1)* GGGACGTCTGTTGAGAGAGC TGTGTCCATGGTAGCGGTAA NM_008810.2

Pyruvate dehydrogenase kinase, isoenzyme 4 *(Pdk4)* GCTTGCCAATTTCTCGTCTC CTTCTCCTTCGCCAGGTTCT NM_013743.2
Ribosomal protein S16 *(Rps16)* TGCTGGTGTGGATATTCGGG CCTTGAGATGGGCTTATCGG NM_013647.2

Primer pairs were designed based on GenBank reference sequence using 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.

References

1. **Riehle C, Wende AR, Zaha VG, Pires KM, Wayment B, Olsen C, Bugger H, Buchanan J, Wang X, Moreira AB, Doenst T, Medina-Gomez G, Litwin SE, Lelliott CJ, Vidal-Puig A, Abel ED.** 2011. PGC-1beta deficiency accelerates the transition to heart failure in pressure overload hypertrophy. Circulation research **109:**783-793.
2. **Veksler VI, Kuznetsov AV, Sharov VG, Kapelko VI, Saks VA.** 1987. Mitochondrial respiratory parameters in cardiac tissue: a novel method of assessment by using saponin-skinned fibers. Biochimica et biophysica acta **892:**191-196.