Supplemental Figures and Legends.

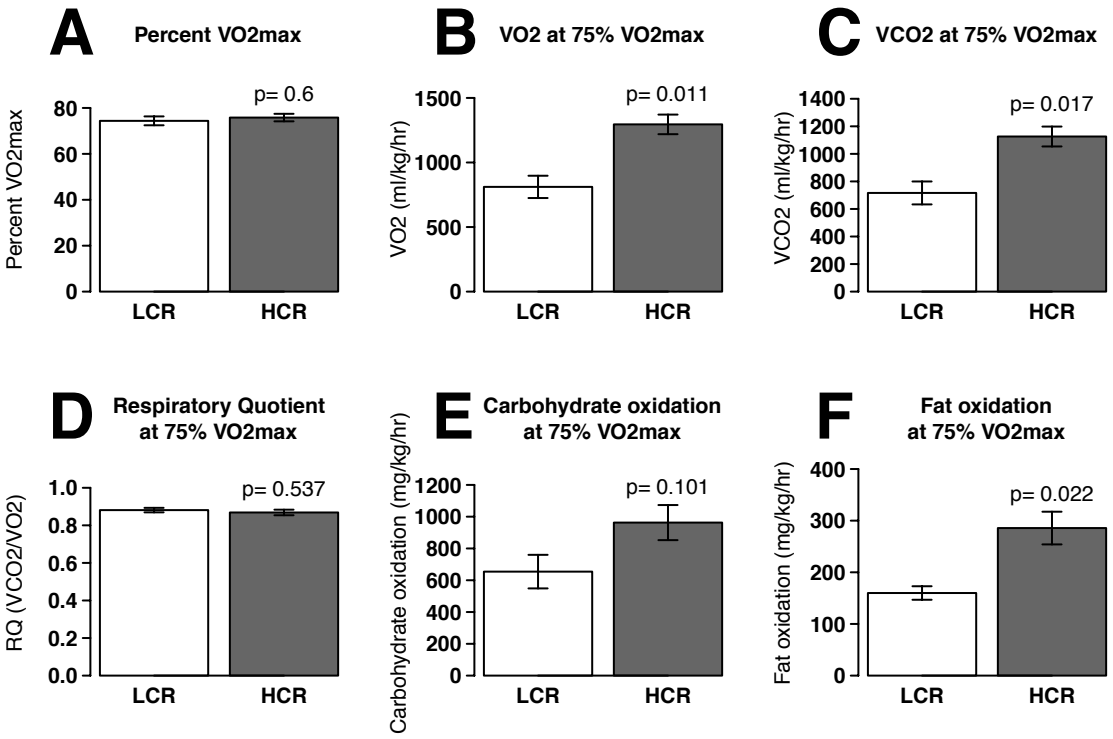

Figure S1. Capacity to oxidize fat is higher even at same relative exercise intensity, supporting fuel utilization differences presenting in Figure 1. HCR (n=4) and LCR (n=3) were exercised at 75% of their VO_2max (A) while sampling VO_2 and VCO_2. The above data show the average volume oxygen consumed (VO_2) (B) and volume carbon dioxide produced (VCO_2) (C) for the last 15 min of a 30 min exercise period. Values are mean ± SEM for HCR and LCR. Respiratory Quotient (RQ) (D), estimated carbohydrate oxidation (E) and fat oxidation (F) were calculated from the measured VO_2 and VCO_2. P-values were calculated with Student's t-test.

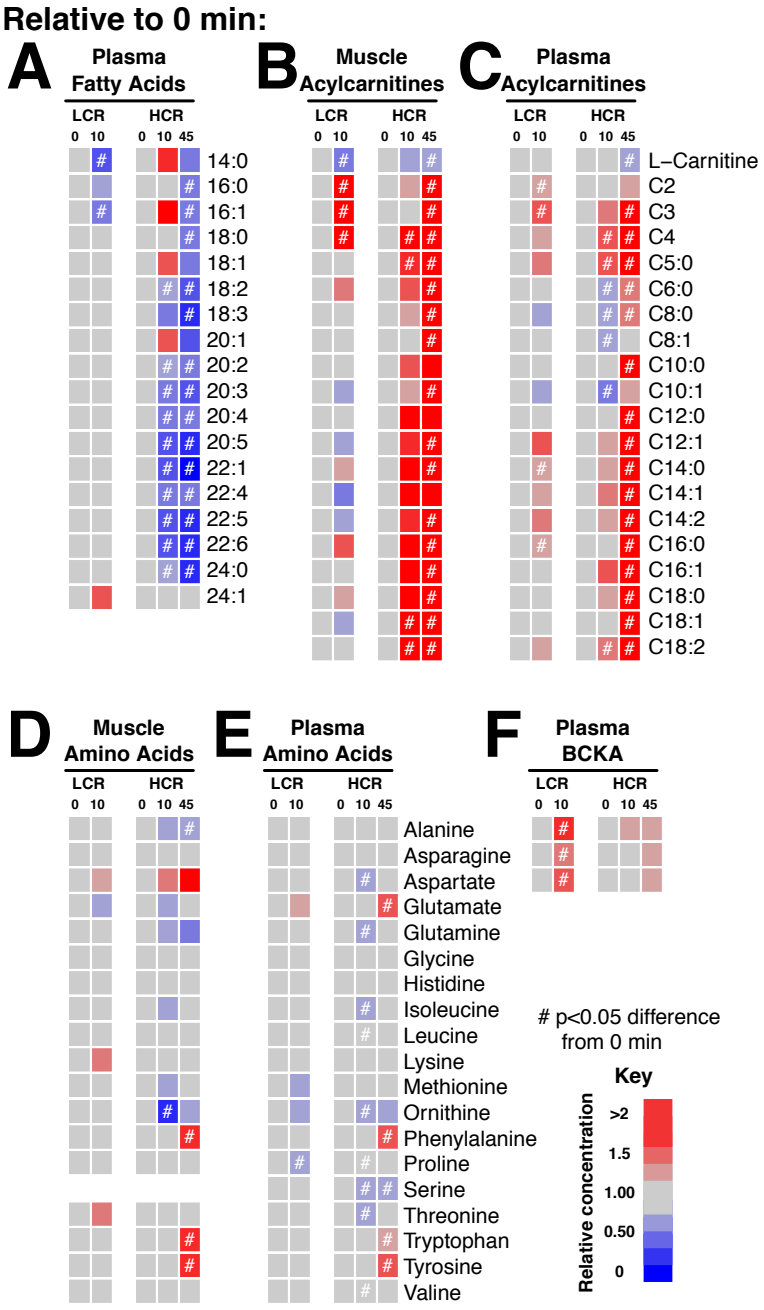

Figure S2. Muscle and plasma lipids, acylcarnitines, and amino acids; same data as presented in Figure 2 with values relative to 0 min for both HCR and LCR. Plasma fatty acids (A) and muscle and plasma acylcarnitines (B and C) are listed by carbon chain-length. Muscle and plasma amino acids (D and E) and branched chain keto acids (F) α-ketoisocaproic acid (KIC), α-ketoisovaleric acid (KIV), and α-ketomethylvaleric acid (KMV) values are presented in heat colors relative to resting values (0 min). # p<0.05 difference from 0 min.

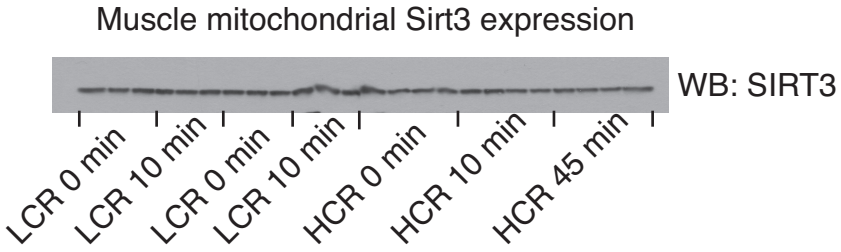

Figure S3. SIRT3 expression in mitochondria does not differ between HCR and LCR, confirmation of proteomic data presented in Table S3A.
Mitochondrial proteins isolated from gastrocnemius tissue of HCR and LCR were separated by SDS-PAGE. Anti-SirT3 antibody (Cell Signaling) was used to detect SIRT3 expression.

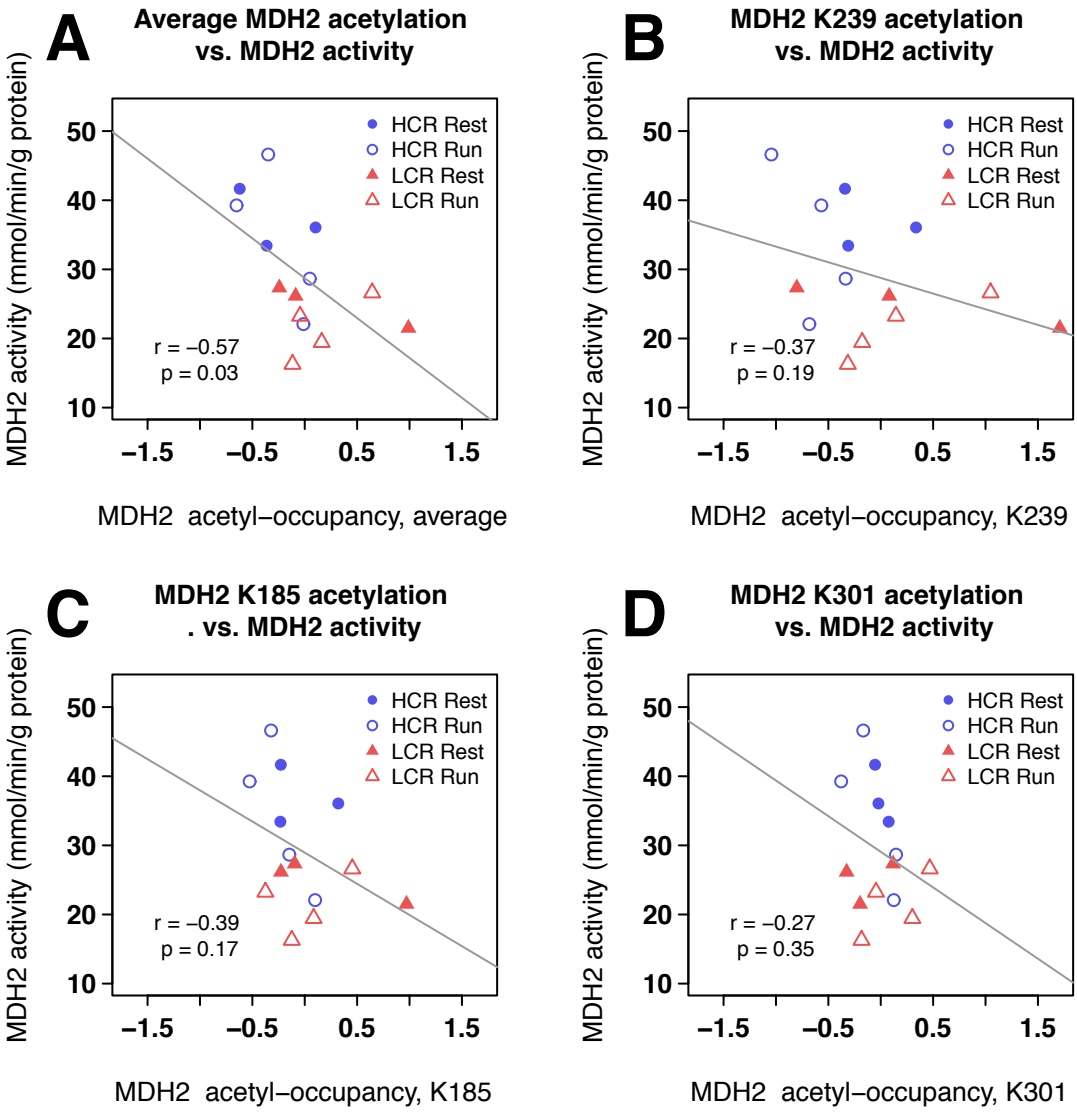

Figure S4. Correlations between MDH2 acetylation state and MDH2 activity in isolated mitochondria from skeletal muscle, related to Figure 5. Using mitochondria isolated from gastrocnemius tissue of the same animals used for the proteomics study (HCR and LCR at 0 min or 10 min of exercise), we determined mitochondrial MDH activity (monitoring change in absorbance at 350 nm, in the presence of NADH and oxaloacetate). Average acetylation state (A) was more strongly correlated to MDH activity than acetyl-occupancy of individual acetyl-sites K239 (B), K185 (C), or K301 (D).

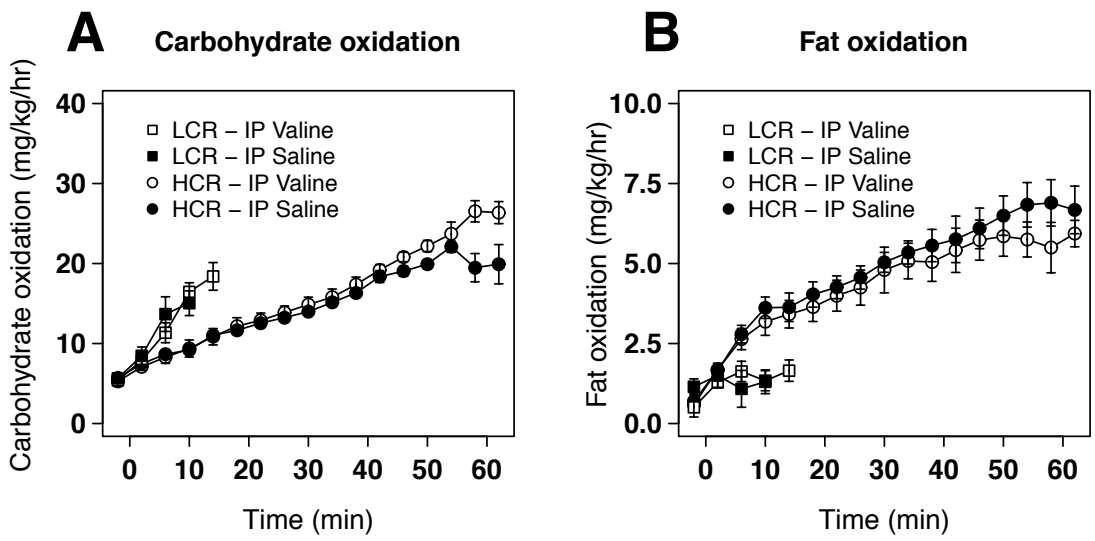

Figure S5. Intraperitoneal (IP) injection of valine does not affect running time or fuel selection, relevant to experimental procedures. HCR and LCR were injected with valine (100 mg/kg) and run to exhaustion while sampling VO_2 and VCO_2. Estimated carbohydrate oxidation (A) or fat oxidation (B) were not different between valine injection and control saline injection (n=6). Values are mean ± SEM.

Supplemental Tables.

Table S1. Body weight and running times for LCR and HCR at generation 27 (mean ± SEM), related to Figure 1.

	LCR (n=16)	HCR (n=23)
Body weight (g)	439.0 ± 12.5	294.2 ± 7.4[a]
Percent fat mass (%)	15.6 ± 0.6	3.9 ± 0.4[a]
Percent lean mass (%)	72.1± 0.6	78.9 ± 0.3[a]
Time to exhaustion (min)	10.51 ± 0.79	45.11 ± 1.13[a]
Distance (m)	129.7 ± 12.0	944.1 ± 37.8[a]
Total Work (J)	144.9 ± 14.4	701.8 ± 27.8[a]
VO_2max (mL/kg/min)	26.5 ± 0.8	39.4 ± 0.5[a]
VO_2max (mL/kg_{lean}/min)	36.8 ± 1.0	50.0 ± 0.6[a]

[a]p value <0.0001

Table S2. Metabolomics data set, related to Figures 1-3. Microsoft Excel file contains muscle and plasma metabolite values for HCR and LCR at 0, 10, and 45 min (HCR only) of exercise (A, first tab); correlation coefficients (Pearson *r*) of these metabolite values with fat oxidation, carbohydrate oxidation and percent exhaustion (B, second tab); and muscle and serum metabolite values from an independent, confirmation data set where HCR and LCR were run to quarter (HCR only), half, and full exhaustion (C, third tab). Metabolite values are presented as mean ± SEM for each group, and permutation p-values are given for each comparison.

Table S3. Proteomics data set, related to Figures 4-6. Microsoft Excel file contains log2 fold change values for quantified proteins (A, first tab), phospho-sites (B, second tab), and acetyl-sites (C, third tab). Quantitative proteomics was performed on mitochondria isolated from skeletal muscle of HCR and LCR collected at rest and after 10 min of exercise.

Table S4. Fluxomics data set, related to Figure 6. Microsoft Excel file contains serum and muscle metabolite values obtained from U-$C^{13}N^{15}$ valine injected HCR and LCR; data are presented as mean ± SEM for each group, with permutation p-values for comparisons. This table includes relative peak area for each downstream metabolite (sum of all isotopes) and percent enrichment (relative incorporation of C^{13} and N^{15}) in the various metabolite pools (A, first tab). Nitrogen flux of N^{15} is presented as relative isotope enrichment (M+1) within amino acid pools (B, second tab).

Supplemental Experimental Procedures.

Fuel use at same relative intensity.
HCR (n=4) and LCR (n=3), independent cohort, were run at 75% of their VO_2max by adjusting incline and then speed. Animals were exercise for 30 min, and all animals reached and maintained a steady 75%VO_2max for the last 15 min of exercise. Volume of oxygen consumed (VO_2) and carbon dioxide produced (VCO_2) were sampled every 2 minutes.

Independent data set for confirming metabolomics analysis.
For confirming metabolite values that are coincident with exhaustion, we exercised male HCR and LCR (Generation 31 at 4.5 months of age) to quarter exhaustion (HCR only, ~15 min), half exhaustion (~6 min for LCR, ~30 min for HCR), and full exhaustion. At specified time points, animals were anesthetized with continuous inhalation of isoflurane. Tissues were collected under anesthesia and frozen immediately in liquid nitrogen; removal of heart ensured euthanasia. Tissues were stored at -80^{o}C.

Estimating fuel use.
For estimating fat and carbohydrate oxidation from VO_2 and VCO_2, we applied the parameters described previously (Peronnet and Massicotte, 1991), with modifications to account for increased glycogen utilization as exercise intensity increases (Jeukendrup and Wallis, 2005), see equations 1-4.

Equation 1. Fat oxidation.

Fat oxidation (g/kg/min) = $1.695 \times VO_2 - 1.701 \times VCO_2$

Equation 2. Carbohydrate oxidation at 0-40% VO_2max.

Carbohydrate oxidation (g/kg/min) = $4.585 \times VCO_2 - 3.226 \times VO_2$

Equation 3. Carbohydrate oxidation at 40-50% VO_2max.

Carbohydrate oxidation (g/kg/min) = $4.344 \times VCO_2 - 3.061 \times VO_2$

Equation 4. Carbohydrate oxidation at 50-100% VO_2max.

Carbohydrate oxidation (g/kg/min) = $4.210 \times VCO_2 - 2.962 \times VO_2$

Muscle and plasma Metabolites.
For glycolytic and citric acid cycle analysis, frozen gastrocnemius muscle samples were pulverized in a liquid nitrogen-chilled mortar. Portions of this frozen tissue were then extracted with 1 mL of a solvent mixture consisting of 8:1:1 HPLC grade methanol:chloroform:water, using a ratio of 1 mL solvent per 30 mg of wet tissue mass. Samples were sonicated using a probe sonicator (Branson 450, 40% duty cycle, output power 4) for 20 sec and then centrifuged at 16,000 x *g* to pellet proteins and residual cell debris. Supernatants were transferred to autosampler vials and analyzed by hydrophilic interaction chromatography – electrospray – time of flight mass spectrometry (HILIC-ESI-TOF-MS) on an Agilent 6220 (Santa Clara, CA) as described previously (Lorenz et al., 2011).

For acylcarnitine analysis, weighed samples and plasma were extracted using a mixture of 8:2 of HPLC grade acetonitrile:water, probe sonicated for 20 sec (muscle) or vortexed (plasma) and then centrifuged. Supernatants were collected and then dried by vacuum centrifugation at 45°C. Dried samples were reconstituted in a 9:1 mixture of mobile phases A/B used for the acyl-carnitine analysis. The free carnitine and acylcarnitine species were then analyzed without derivatization using reversed phase liquid chromatography (RPLC) coupled to an Agilent 6410 tandem quadrupole mass spectrometer. The column used was a Waters Xbridge C18 2.5 μ, 2.1 x 50 mm (Milford, MA), mobile phase A was 5mM ammonium acetate in water adjusted to pH 9.9 with ammonium hydroxide and mobile phase B was acetonitrile. Elution was performed at a flow rate of 0.25 mL/min, and the gradient consisted of a 7 min linear ramp from 0 to 80% B, a 3 minute wash at 100%B, and a 6-minute re-equilibration period at 0%B. Detection was performed using multiple reaction monitoring (MRM) in positive ion mode, using precursor/product ion transitions specified elsewhere (Ghoshal et al., 2005). Mass spectrometer parameters were as follows: capillary voltage 4000V, gas temperature 325ºC, gas flow 10L/min, nebulizer pressure 40 psi.

For amino acid analysis, weighed pulverized gastrocnemius and plasma were extracted in 8:1:1 methanol:chloroform:water, probe sonicated for 20 sec (muscle only) and then centrifuged. Supernatants were collected and then dried by vacuum centrifugation at 45°C. The dried samples were then derivatized and analyzed using the EZ-faast kit from Phenomenex (Torrance, CA) and analyzed using an Agilent 6890 GC with a 5973 mass selective detector (Badawy et al., 2007). For mass isotopomer analysis of stable-isotope labeled amino acids, samples were derivatized using methoxyamine hydrochloride and N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) and then analyzed by GC-MS on a DB-5MS performed using a 30 m × 250 μm DB 5-MS column (J&W Scientific, Folsom, CA) as described previously (Fiehn et al., 2000).

For branched-chain keto-acid and β-hydroxyisobutryric acid analysis, weighed muscle and plasma were extracted as above and 200 μL of the resulting

supernatant were dried by vacuum centrifugation at 45°C. The dried samples were reconstituted in 200 μL of water and analyzed by reversed phase liquid chromatography coupled to ESI-TOF-MS in negative ion mode using a Waters Acquity HSS T3 1.8 µ column, with chromatographic conditions as described previously (Evans et al., 2013).

Agilent Masshunter Quantitative Analysis software was used to quantitate peak area. Accurate mass and retention times determined using authentic standards were used to identify metabolites. Natural isotope abundance correction of data from stable-isotope labeled samples was performed using an in-house written script running in MATLAB (2012a, The MathWorks, Natick, MA).

Mitochondrial Preparation. Crude mitochondria were enriched using previously described methods (Pagliarini et al. 2008), with some modifications for the current study. Rat EDL tissue was initially homogenized by crushing with a mortar and pestle (Coors) under liquid nitrogen, and all subsequent steps were carried out at 4ºC. The homogenate was then transferred into a Potter-Elvehjem glass/teflon homogenizer along with 8 mL of MSHE buffer (220mM mannitol, 70mM sucrose, 5mM HEPES pH 7.4, 1mM EGTA), supplemented with protease inhibitors, deacetylase inhibitors and 0.5% BSA, for further homogenization. After homogenization with 5 strokes at 1000 rpm, the resulting homogenate was decanted into a new 15 mL tube. The homogenizer was rinsed with an additional 2 mL of MSHE, which was then added to the homogenate. The sample was centrifuged at 800 x g for 10 min in bench-top conical centrifuge. Any lipid that formed at the top of the supernatant was carefully aspirated. The supernatant, containing the mitochondria, was gently drawn off with a pipet-aid and transferred to an ultra-clear 12 mL centrifuge tube. The samples were centrifuged at 8000 x g for 10 min, and the resulting supernatant and any loose material was aspirated and discarded, leaving a dark brown pellet with a light brown halo around the center. An additional 10 mL of MSHE buffer was added to the pellet, which was resuspended by washing from the side of the tube until homogenous using as few pipetting strokes as possible. The sample was centrifuged an additional time at 8000 x g for 10 min and the crude mitochondria were resuspended in 1 mL of MSHE using the same method as before. Crude mitochondria were transferred to a 1.5 mL microfuge tube and centrifuged at 8,000 x g for 10 min in bench-top microfuge. The supernatant was aspirated and discarded. The pellet was re-suspended in MSHE (without BSA) and centrifuged at 8,000 x g for 10 min in bench-top centrifuge. The supernatant was aspirated and discarded, and the crude mitochondrial pellet was flash frozen in liquid N2 and stored at -80°C until ready for use.

Sample Preparation for proteomic analysis. Mitochondrial cell pellets were re-suspended in approximately 500µL of lysis buffer (50mM Tris (pH 8), 8M urea, 40mM NaCl, 2mM $MgCl_2$, 50mM NaF, 50mM β-glycerophosphate, 1mM sodium

orthovanadate, 10mM sodium pyrophosphate, mini EDTA-free protease inhibitor (Roche Diagnostics, Indianapolis, IN), phosSTOP phosphatase inhibitor (Roche Diagnostics, Indianapolis, IN), and deacetylase inhibitors) and lysed on ice with a probe sonicator. Protein content was evaluated using a BCA assay (Thermo Fisher Scientific, San Jose, CA).

Proteins were reduced with 5mM dithiothreitol (incubation at 58°C for 30 minutes) and alkylated with 15mM iodoacetamide (incubation in the dark, at ambient temperature, for 30 minutes). Alkylation was quenched by adding an additional 5mM dithiothreitol (incubation at ambient temperature for 15 minutes). Proteins were enzymatically digested in a two-step process. First, proteinase LysC (Wako Chemicals, Richmond, VA) was added to each sample at a ratio of 1:100 (enzyme:protein) and the resulting mixtures were incubated at 37ºC for 3 hours. Next, samples were diluted to a final concentration of 1.5 M urea (pH 8) with a solution of 50mM Tris and 5mM $CaCl_2$. Sequencing-grade trypsin (Promega, Madison, WI) was added to each sample at a ratio of 1:50 (enzyme:protein) and the resulting mixtures were incubated at ambient temperature overnight. Digests were quenched by bringing the pH to ~2 with trifluoroacetic acid and immediately desalted using C18 solid-phase extraction columns (SepPak, Waters, Milford, MA).

Desalted material was labeled with TMT 10-plex isobaric labels (Thermo-Pierce, Rockford, IL). Only eight of the ten labels were used per experiment for the comparison of two biological replicates across each the four conditions. Prior to quenching the TMT reactions, ~5µg of material from each TMT channel was combined into a test mix and analyzed by LC-MS/MS to evaluate labeling efficiency and obtain optimal ratios for sample recombination. Following quenching, tagged peptides were combined in equal amounts by mass (~500µg per channel) and desalted. All experiments had ≥ 99% labeling efficiency, calculated by the number labeled peptides divided by the total number of peptide identifications.

Protein fractionation and enrichment. Labeled peptides were fractionated by strong cation exchange (SCX) using a polysulfoethylaspartamide column (9.4×200mm; PolyLC) on a Surveyor LC quaternary pump (Thermo Scientific). Each dried and mixed TMT sample was resuspended in buffer A, injected onto the column, and subjected to the following gradient for separation: 100% buffer A from 0-2 min, 0-15% buffer from 2-5 min, and 15-100% buffer B from 5-35 min. Buffer B was held at 100% for 10 minutes and then the column was washed extensively with buffer C and water prior to recalibration. Flow rate was held at 3.0 mL/min throughout the separation. Buffer compositions were as follows: buffer A [5mM KH2PO4, 30% acetonitrile (pH 2.65)], buffer B [5mM KH2PO4, 350mM KCl, 30% acetonitrile (pH 2.65)], buffer C [50mM KH2PO4, 500mM KCl (pH 7.5)]. Twelve fractions were collected over the first 50-minute elution period

and were immediately frozen, lyophilized, and desalted. A small portion of each, 5%, was extracted and used for protein analysis. The remaining material was retained for phospho and acetyl lysine enrichment.

Phospho enrichment. Phosphopeptides were enriched using immobilized metal affinity chromatography (IMAC) with magnetic beads (Qiagen, Valencia, CA). Following equilibration with water, the magnetic beads were incubated with 40mM EDTA (pH 8.0) for 1 hour, with shaking. Next, the beads were washed four times with water and incubated with 30mM $FeCl_3$ for 1 hour, with shaking. Beads were then washed four times with 80% acetonitrile/0.15% TFA. Each of the 12 fractions were re-suspended in 80% acetonitrile/0.15% TFA and incubated with the magnetic beads for 45 minutes, with shaking. Following this incubation, all unbound peptides were collected for subsequent acetyl lysine enrichment. Bound peptides were washed three times with 80% acetonitrile/0.15% TFA and eluted with 50% acetonitrile, 0.7% NH_4OH. Eluted peptides were immediately acidified with 4% FA, frozen, and lyophilized. Each phospho peptide fraction was re-suspended in 20µL 0.2% FA for LC-MS/MS analysis.

Acetyl lysine enrichment. Peptides unbound by IMAC enrichment were pooled into 6 fractions. Each fraction was dissolved in 50mM HEPES (pH 7.5)/100mM KCl buffer, combined with approximately 50µL pan-acetyl lysine antibody-agarose conjugate (Immunechem), and rotated overnight at 4°C. Samples were rinsed eight times with 50mM HEPES (pH 7.5)/100mM KCl buffer prior to elution with 0.1% TFA. Eluted peptides were then desalted, frozen, and lyophilized. Each acetyl lysine fraction was re-suspended in 20µL 0.2% FA for LC-MS/MS analysis.

Proteomic LC-MS/MS analysis. All experiments were performed using a NanoAcquity UPLC system (Waters, Milford, MA) coupled to an Orbitrap Fusion (Q-OT-qIT) mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Reverse-phase columns were made in-house by packing a fused silica capillary (75µm i.d., 360 µm o.d, with a laser-pulled electrospray tip) with 1.7µm diameter, 130 Å pore size Bridged Ethylene Hybrid C18 particles (Waters) to a final length of 30cm. The column was heated to 60°C for all experiments. Samples were loaded onto the column for 12 minutes in 95:5 buffer A [water, 0.2% formic acid, and 5% DMSO]:buffer B [acetonitrile, 0.2% formic acid, and 5% DMSO] at a flow-rate of 0.30µL/min. Peptides were eluted using the following gradient: an increase to 7% B over 1 min, followed by a 42 min linear gradient from 7% to 18% B, followed by a 28 min linear gradient from 18% to 27% B, followed by a final 1 min ramp to 75% B which was held for 3 minutes. The column was equilibrated with 5% buffer B for an additional 25 min. Precursor peptide cations were generated from the eluent through the utilization of a nanoESI source. Phospho and acetyl enriched fractions were each analyzed in duplicate. Mass spectrometry instrument methods consisted of MS^1 survey scans (8e5 target value; 60,000 resolution; 350Th – 1400Th) that were used to guide ten

subsequent data-dependent MS/MS scans (0.7Th isolation window, HCD fragmentation; normalized collision energy of 37; 5e4 target value, 60,000 resolution). Dynamic exclusion duration was set to 60s, with a maximum exclusion list of 500 and an exclusion width of 0.55Th below and 2.55Th above the selected average mass. Maximum injection times were set to 100ms for all MS^1 scans, 120ms for MS/MS scans in whole protein analyses, and 200ms for MS/MS scans in phospho and acetyl enrichment analyses.

Proteomic data analysis. Data was processed using the in-house software suite COMPASS (Wenger et al., 2011). OMSSA (Geer et al., 2004) (version 2.1.8) searches were performed against a target-decoy database (Uniprot (rat), www.uniprot.org, August 19, 2013). Searches were conducted using a 3Da precursor mass tolerance and a 0.01Da product mass tolerance. A maximum of 3 missed tryptic cleavages were allowed. The fixed modifications specified were carbamidomethylation of cysteine residues, TMT 10-plex on peptide N-termini, and TMT 10-plex on lysine residues. The variable modifications specified were oxidation of methionine and TMT 10-plex on tyrosine residues. Additional variable modifications were specified for phospho-peptide analyses (phosphorylation of threonine, serine, and tyrosine residues) and acetyl-peptide analyses (acetylation of lysine residues). Note that the acetylation modification mass shift was set to -187.1523 Da to account for the difference between an acetyl group and a TMT 10-plex tag, which enables the use of TMT 10-plex as a fixed modification on lysine residues, even for acetylated peptides. TMT quantification of identified peptides was performed within COMPASS as described previously (Phanstiel et al., 2011). Peptides identified within each of 12 fractions were grouped into proteins according to previously reported rules (Nesvizhskii and Aebersold, 2005) using COMPASS. Protein quantification was performed by summing all of the reporter ion intensities within each channel for all peptides uniquely mapping back to a given protein. Protein, phospho-peptide, and acetyl-peptide experiment sets were processed separately.

PTM localization. Phosphopeptide localization was performed using *Phosphinator* software within COMPASS, as described previously (Phanstiel et al., 2011). This program both localized phosphorylation sites and combined quantitative data for phospho-isoforms across all 12 fractions. All phosphosites had to be localized for a given phospho-peptide to be included in subsequent quantitative analysis.

Acetylpeptide localization was performed using *LoToR* software within COMPASS. Briefly, for each peptide spectral match (PSM) that contained an acetyl modification, theoretical fragmentation spectra were generated by performing *in silico* fragmentation of every possible peptide isoform. Each theoretical spectrum was then compared to the experimental spectrum using mass tolerances of 0.025Th. A peptide was declared localized if one isoform

matched at least one peak more than any other isoform matched. Localized acetylated peptides were grouped together on acetylated positions and reporter ion intensities were summed.

Mitochondrial Assignment. To generate a list of rat mitochondrial proteins, Entrez GeneIDs from the mouse MitoCarta compendium of mitochondrial proteins (Pagliarini et al., 2008) were mapped to NCBI HomoloGene group ID according to build 67. All rat genes corresponding to these groups were then mapped to UniProt identifiers using the UniProtKB ID mapping table (July 2013).

Protein Normalization. To account for variation in mitochondrial preparation, all quantitative data was normalized at the mitochondrial protein level. Only mitochondrial proteins were included in our analysis. All quantitative values were log2 transformed and mean normalized. To normalize phosphorylation and acetylation data for protein differences, the value for each phospho and acetyl isoform reporter ion channel had subtracted from it the quantitative value of that channel from the corresponding protein.

Western blot for SIRT3. Frozen gastrocnemius tissues collected from female HCR and LCR rats (generation 33, 6 month of age) at 0, 10, and 45 min of treadmill exercise were used for mitochondrial isolations. Isolated mitochondria were extracted in buffer (IB) containing 1% Triton X-100, 20mM HEPES, 20mM beta-glycerophosphate, 150mM NaCl, 10mM NaF, 10mM Na-Pyrophosphate, 1mM EDTA, protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Thermo Scientific). After 20s sonication, samples were centrifuged at 8,000 x g for 10 min. Protein concentration was determined with Pierce BCA kit (Rockford, IL) and was used to normalize sample concentrations prior to loading. Sample extracts were separated by SDS-PAGE (Criterion, BioRad) and transferred to a nitrocellulose membrane. After blocking with 5% non-fat dry milk in TBS-T, the membrane was incubated overnight at 4°C with anti-SirtT3 primary antibody (1:1000, Cell Signaling #D22A3, generously provided by David Lombard). The membrane was washed and incubated for 1 hr at 20°C with appropriate secondary antibodies conjugated to horseradish peroxidase (1:2000). After final washes, target proteins were detected with Pierce ECL solution (Rockford, IL).

MDH activity assay. Mitochondria were isolated, using method described above, from gastrocnemius muscle of male HCR and LCR at rest or at 10 min of exercise. Mitochondria were resuspended (0.02 ug protein/uL) in 100 mM potassium phosphate buffer (pH 7.4) containing deacetylase inhibitors (10 mM nicotinamide, 5 uM MS-275, 2 uM SAHA, 10 uM Trichostatin A, 4 mM sodium butryate; purchased from Caymen Chemical) and freeze-thawed 3 times to disrupt the mitochondrial membrane. In a 96 well plate, 0.5 ug of mitochondrial protein were reacted with buffer containing NADH (250 uM) and oxaloacetate

(200 uM). After initiation of the reaction, we monitored absorbance at 340 nm for 1.5 min. The change in absorbance per min and the mM extinction coefficient of NADH at 340 nm were used to calculate MDH activity.

Statistics and Figures. Statistical analysis and figures were made with the R statistical and graphing environment (R Core Team, 2013). Comparisons of metabolite levels were made between HCR and LCR at 0 min, 10 min and within HCR and LCR between 0 min and other time points. Protein and modification site fold-changes were calculated by averaging protein-normalized values for each condition and calculating the difference of averages. Comparisons were made between HCR and LCR at rest and 10 min exercise and within strain between rest and 10 min exercise. Isotope flux comparisons were made between HCR and LCR for Rest and Run and within HCR and LCR between Rest and Run. For all comparisons, p-values were determined by permutation t-test using R perm package (Fay, 2010). Heatmaps were plotted using the *gplots* package "heatmap.2" function (Gregory R. Warnes, 2013), and pathway enrichment analysis was performed using Enrichr (Chen et al., 2013).

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