EXTENDED EXPERIMENTAL PROCEDURES

Animal Experiments and Treatments

C57BL/6J mice of 6, 22, and 30 months of age were obtained from the National Institutes of Aging mouse aging colony. Additionally 22-month-old caloric restricted mice were also obtained from the National Institutes of Aging mouse aging colony. Mice were acclimated for at least one-week prior to sacrifice. 6 and 22-month-old mice were given interperitoneal (IP) injections of 500 mg NMN/kg body weight per day or the equivalent volume of PBS for 7 consecutive days at 6:00 pm and 8:00 am on day 8 and sacrificed 4 hr after last injection. Whole body adult-inducible *SIRT1* knockout mice (SIRT1 iKO) (Price et al., 2012) were treated with tamoxifen for 5 weeks and the efficiency of deletion in DNA from tail samples was determined by PCR. Animals were then maintained on regular diet for 2-6 months. For fasting experiments, mice were fasted for 16 hr prior to sacrifice. For NMN experiments both WT and *SIRT1* iKO mice were given IP injections of 500 mg NMN/kg body weight per day or the equivalent volume of PBS for 7 consecutive days at 6:00 pm and 8:00 am on day 8 and sacrificed 4 hr after last injection. Whole body *SIRT1* overexpressor (SIRT1-Tg) mice (Price et al., 2012) of 6 months of age were given IP injections of 300 mg DMOG/kg body weight per day or the equivalent volume of PBS for 5 consecutive days, after which they were sacrificed. Whole body adult-inducible *EglN1* knockout mice (Minamishima et al., 2008) were treated with IP injection of tamoxifen for 3 days after which they were allowed to rest for an additional 3 days. The mice were then given IP injections of 500 mg NMN/kg body weight per day or the equivalent volume of PBS for 7 consecutive days at 6:00 pm and 8:00 am on day 8 and sacrificed 4 hr after last injection. All animal studies followed the guidelines of and were approved by the Harvard Institutional Animal Care and Use Committee.

Overexpression of *NMNAT1* In Vivo

The *NMNAT1* plasmid was a kind gift From Professor Toshiyuki Araki (National Center of Neurology and Psychiatry, Japan) and was generated by cloning mouse NMNAT1 cDNA with a C-terminal 6xHis-Tag into the BamH1 site of a pIRES-eGFP plasmid (Clonetech). The plasmid was purified using endotoxin-free Maxi-Prep Kit (QIAGEN) and resuspended in sterile 0.9% saline. Mice were anaesthetized, their hind limbs shaved and wiped with ethanol. Hyaluronidase (15 units in 50 μl saline) was injected into the tibialis cranialis muscle transcutaneously along the length of the muscle using an insulin syringe. Following a 1.5 hr waiting period, plasmid (50 μg in 50 μl saline) was injected along the complete length of one tibialis muscle. This was followed by eight pulses of 100 V/cm and 20 msec at a frequency of 1 Hz via tweezer electrodes attached to an ECM-830 electroporator (BTX). For each mouse, the contralateral muscle received an equal volume saline and underwent the electroporation protocol, to act as an internal control. Mice were sacrificed 7 days following electroporation and both tibialis muscles were collected for subsequent analyses. Animal experiments followed the guidelines and were approved by the Garvan Institute, Australia.

Ex Vivo Incubation of Soleus with Insulin and Determination of Insulin-Stimulated Glucose Uptake

Ex-vivo incubation of soleus with insulin was performed as described before (Wu et al., 2009) with slight modifications. Briefly, two strips of soleus muscle were isolated and incubated in Krebs Henseleit buffer, pH 7.4, containing 20 mM HEPES and 2% bovine serum albumin (KHB) at 37°C for 20 min. After pre-incubation in KHB, muscles were incubated in KHB supplemented with 5.5 mM glucose, and 2 mM pyruvate for another 30 min (control), or in the same buffer but containing human recombinant insulin (100 μU/mL) for 30 min. For insulin stimulated glucose uptake, the samples were incubated with 2-deoxyglucose (2-DG), an analog of glucose which is known to be transported by the same glucose carrier system as glucose and phosphorylated to 2-deoxyglucose-6-phosphate but not metabolized further. Samples were then washed with KHB, immediately frozen in liquid nitrogen, and stored at −80°C. 2-DG uptake was measured using a commercially available kit (BioVision) according to the manufacturer's instructions.

Adenovirus Cloning and Production

TFAM and control (*GFP*) adenoviruses were constructed by cloning the inserts into the pAd/CMV/V5-DEST Gateway Vector to generate adenoviral expression vectors. The adenoviral expression vectors were digested with PacI for 30 min. The linearized adenoviral expression vectors were then transfected using X-tremeGENE HP into 293 cells to produce adenoviruses. The media was changed the next day and the transfected 293 cells were maintained in culture to monitor for cytopathic effect. The adenoviruses were harvested when 80% of cytopathic effect was observed and used as crude adenoviral lysates.

C2C12 Cell Culture, Treatments, and Adenoviral Infections

C2C12 cell line (ATCC) was cultured in low glucose Dulbecco's modified eagle medium (DMEM) (Invitrogen) supplemented with 10% FBS (Invitrogen) and a mix of antibiotic and antimycotic (Invitrogen). To inhibit SIRT1, cells were treated the vehicle (0.001% DMSO) or 10 μM EX-527 (Tocris) for 24 hr. C2C12 myoblasts were infected with an empty or *SIRT1* adenovirus as described before (Gerhart-Hines et al., 2007) and the media was replaced with fresh DMEM for an additional 48 hr.

Generation of Primary Myoblasts from CreERT2 *SIRT1* Flox/Flox Mice, Induction of *SIRT1* Exon4 Excision, Adenoviral Infections, and Treatments

Primary myoblasts cells were isolated from CreERT2 *SIRT1* flox/flox (Price et al., 2012) as previously described (Hubbard et al., 2013). To induce the excision of exon 4 of SIRT1 the cells were treated with 250 nM 4-hydroxytamoxifen (Sigma-Aldrich), or vehicle (0.001%

ethanol) as previously described (Hubbard et al., 2013) for the time points indicated. For testing the effect of AMPK activation on the regulation of mitochondrial-encoded and nuclear encoded genes, CreERT2 *SIRT1 flox*/flox cells were infected with an *AMPKα2*-DN adenovirus (Eton Bioscience) for 48 hr during which 250 nM 4-hydroxytamoxifen was added to promote SIRT1 exon 4 excision. For *TFAM* addback experiments, *SIRT1* excision was induced with 250 nM 4-hydroxytamoxifen for either 24 or 48 hr after which the cells were infected with control or *TFAM* adenovirus for an additional 24 hr. To address the role of *SIRT1* on *NMNAT1*'s effects, CreERT2 *SIRT1* flox/flox myoblasts were infected with adenovirus expressing empty or *NMNAT1* (Applied Biological Materials) and simultaneously *SIRT1* excision was induced with with 250 nM 4-hydroxytamoxifen for 24 hr. To evaluate the effects of SIRT1 on HIF-1α hydroxylation, CreERT2 *SIRT1* flox/flox cells were treated for 6 hr with 20 μM MG132 (Cayman) after SIRT1 was excised for 24 hr. Un-induced CreERT2 *SIRT1* flox/flox cells were used as WT primary myoblasts for subsequent experiments and genetic manipulations.

Generation of RhoO Cells

CreERT2 *SIRT1* flox/flox primary myoblasts were mantained in normal growth media (F-10 nutrient mix supplemented with 2.5 ng/mL bFGF, 10 ng/mL EGF, 1 μg/mL insulin, 0.5 mg/mL fetuin. 0.4 μg/mL dexamethasone, antibiotic and antimycotic mix and 20% FBS) supplemented with 4 g/L glucose and 2 mM pyruvate, 50 ng/ml ethidium bromide (Alfa aesar) and 100 μg/mL uridine (Sigma-Aldrich) for 4 weeks to deplete mitochondrial DNA (rho0 cells). Parental cells were cultured in similar media without ethidium bromide and uridine for the same period of time and used as control cells. After 4 weeks of treatment the amount of mtDNA was checked by qPCR and mitochondrial-encoded subunits tested by Western blot to validate the cell model.

Generation, Culture, and Treatments of Primary Myoblasts from *PGC-1α/β* KO and Muscle-Specific *PGC-1α* Null Mice

Primary myoblasts were isolated from *PGC-1α/β* KO (Zechner et al., 2010) and muscle specific *PGC-1α* null mice (Handschin et al., 2007) as previously described (Hubbard et al., 2013). WT, *PGC-1α/β* KO and *PGC-1α* null primary myoblasts were plated and allowed to differentiate into myotubes by replacing the media with low glucose DMEM supplemented with 2% horse serum (Sigma-Aldrich) for 5 days. After the differentiation the cells were infected with empty vector or flag-*SIRT1* adenovirus as described before (Gerhart-Hines et al., 2007). Media was replaced with fresh DMEM supplemented with 2% horse serum (Sigma-Aldrich) for an additional 48 hr and, after that the cells were harvested for the different assays as described. To investigate the role of HIF-1α in SIRT1-mediated induction of mitochondrial-encoded genes, *PGC-1α/β* KO and *PGC-1α* null primary myotubes were treated for 12 hr with vehicle (0.001% DMSO) or 1mM DMOG (Sigma-Aldrich), 24 hr after infection with empty vector or flag-SIRT1 adenovirus. To study how nuclear energetics influence *PGC-1α/β* independent pathways, *PGC-1α/β* KO primary myotubes were infected with empty vector or *NMNAT1* adenovirus (Applied Biological Materials) for 24 hr.

NMNAT1, NMNAT2, NMNAT3, VHL, c-Myc, and HF1α Gene Silencing in Primary Myoblasts

sh*NMNAT1*#1 (TRCN0000035471; Open Biosystems), sh*NMNAT1*#2 (TRCN0000035473; Open Biosystems), sh*NMNAT2*#1 (TRCN0000111479; Open Biosystems), sh*NMNAT2*#2 (TRCN0000111476; Open Biosystems), sh*NMNAT3*#1 (TRCN0000035402; Open Biosystems), sh*NMNAT3*#2 (TRCN0000035400; Open Biosystems), sh*Myc*#1 (TRCN0000042517; Open Biosystems) Sh*Myc*#2 (TRCN0000054885; Open Biosystems), sh*HIF-1α* (TRCN0000054450; Open Biosystems), sh*VHL*#1 (TRCN0000009736; Open Biosystems), sh*VHL*#2 (TRCN0000009734; Open Biosystems) and control sh*GFP* lentivirus were produced by co-transfection of 293T cells with plasmids encoding psPAX2 (Addgene plasmid 12260), and pMD2.G (Addgene plasmid 12259) using X-tremeGENE HP (Roche) in accordance with the manufacturer's protocol. Media was changed 24 hr post-transfection and the virus harvested after 48 hr, filtered and used to infect CreERT2 *SIRT1* flox/flox primary myoblasts in the presence of 5 μg/mL polybrene (Sigma-Aldrich) via spin infection (2500 rpm, 30 min). Selection of resistant colonies was initiated 24 hr later using 2 μg/mL puromycin (Invivogen).

To evaluate the requirement of NMNAT1 for NMN's effects, *NMNAT1* knockdown, primary myoblasts were treated with vehicle (PBS) or 500 μM NMN (Sigma-Aldrich) for 24 hr.

ARNT and c-Myc Gene Silencing in C2C12 Cells

sh*Myc*#1 (TRCN0000042517; Open Biosystems), sh*Myc*#2 (TRCN0000054885; Open Biosystems), sh*ARNT*#1 and sh*ANRT*#2 (TRCN0000079930 and TRCN0000079931, respectively; Open Biosystems) and control sh*GFP* lentivirus were produced and C2C12 cells were transduced as described above.

HIF-1α and HIF-2α DPA in C2C12 Cells

pBabe empty (Addgene plasmid 1764), HIF-1α DPA (Addgene plasmid 19005), and HIF-2α DPA (Addgene plasmid 19006) retrovirus were produced by co-transfection of 293T cells with plasmids encoding *gagpol* (Addgene plasmid 14887) and *vsvg* (Addgene plasmid 8454) using X-tremeGENE HP (Roche) in accordance with the manufacturer's protocol. Media was changed 24 hr post-transfection and the virus harvested after 48 hr, filtered and used to infect C2C12 cells in the presence of 5 μg/mL polybrene (Sigma-Aldrich) via spin infection (2500 rpm, 30 min). Selection of resistant colonies was initiated 24 hr later using 2 μg/mL puromycin (Invivogen).

c-Myc Overexpression in C2C12 Cells and Primary Myoblasts

pMXsc-Myc (Addgene plasmid 13375) and empty retrovirus were produced as described above and C2C12 cells or CreERT2 *SIRT1* flox/flox primary myoblasts were transduced as described above.

HIF-1α Overexpression and Mutagenesis

Mutagenesis of *HIF-1α* (Addgene plasmid 19365) was performed with QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene) and using the following primers:

K709Q sense: 5′-cctgaggaagaactaaatccacagatactagctttgcag-3′, antisense: 3′-ggactccttcttgatttaggtgtctatgatcgaaacgtc-5′;
K709R sense: 5′-ctgaggaagaactaaatccaaggatactagctttgcagaatgc-3′, antisense: 3′-gactccttcttgatttaggttcctatgatcgaaacgtcttacg-5′;
K674Q sense: 5′-cctcaccaaacagagcaggacagggagtcatagaacagaca-3′, antisense: 3′-ggagtggtttgtctcgtcctgtccctcagtatcttgtctgt-5′

HIF-1α WT and mutant overexpressing cells were made using pBabe puromycin resistant vectors by retroviral transduction as described above.

Mitochondrial Membrane Potential, Reactive Oxygen Species, and Mitochondrial Mass Measurements

Mitochondrial membrane potential was evaluated by fluorescence of the potential dependent TMRM probe. Briefly, cells were incubated with 100 nM TMRM for 15 min in the dark, after which the media was replaced and the fluorescence was measure by flow cytometry. Reactive oxygen species and mitochondrial mass were also evaluated by flow cytometry using the fluorescent probes DHE and NAO respectively as described before (Bell et al., 2011; Gomes et al., 2012)

Succinate Dehydrogenase Activity

Succinate dehydrogenase activity was polarographically determined based on the O_2 consumption using phenazine metasulphate (PMS) as an artificial electron acceptor, as previously described (Singer, 1974). The reaction was carried out at 25°C in 1.3 ml of standard respiratory medium (as in mitochondrial respiration) supplemented with 5 mM succinate, 2 μM rotenone, 0.1 μg antimycin A, 1 mM KCN and 0.3 mg Triton X- 100. After the addition of the sample, the reaction was initiated with 1 mM PMS.

Cytochrome c Oxidase Activity

Cytochrome *c* oxidase activity was polarographically determined based on the O_2 consumption upon cytochrome c oxidation, as previously described (Brautigan et al., 1978). The reaction was carried out at 25°C in 1.3 ml of standard respiratory medium (as in mitochondrial respiration) supplemented with 2 μM rotenone, 10 μM oxidized cytochrome c, 0.3 mg Triton X-100. Following addition of the sample, the reaction was initiated by adding 5 mM ascorbate plus 0.25 mM tetramethylphenylene-diamine (TMPD). Determination of COX activity by spectrophotometry in protein extracts from the skeletal muscle was performed using a commercially available kit (Sigma-Aldrich) according to the manufacturer's instructions.

Electron Microscopy

Skeletal muscle from mice were fixed in 2.5% glutaraldehyde and 2.5% paraformaldehyde in cacodylate buffer (Electron Microscopy Sciences) then were removed, put directly into fixative, then were embedded and photographed with an electron microscope (Tecnai G^2 Spirit BioTWIN). Mitochondrial area was quantified with Image J software.

Analysis of mtDNA Integrity

Total DNA was extracted with DNeasy blood and tissue kit (QIAGEN). Integrity of mtDNA was assessed using the long range PCR mediated detection method as described previously (Santos et al., 2006), using the following primer sequences: Fwd: GCCAGCCT GACCCATAGCCATAATAT; Rev: GAGAGATTTTATGGGTGTAATGCGG.

Gene Expression and mtDNA Analysis

RNA from skeletal muscle tissue was extracted using the RNeasy for fibrous tissue mini kit (QIAGEN) according to the instructions. RNA from C2C12 cells and primary myoblasts were extracted with RNeasy mini kit (QIAGEN) also according to the instructions. RNA from brain, liver, heart and white adipose tissue was extracted using Trizol (Invitrogen) according to the manufacture's instructions. RNA samples were quantified using the NanoDrop 1000 spectrophotometer (Thermo Scientific). cDNA was synthesized with the iSCRIP cDNA synthesis kit (BioRad) using 600 ng of RNA. Quantitative RT-PCR reactions were performed using 1 μM of primers and LightCycler® 480 SYBR Green Master (Roche) on a LightCycler® 480 detection system (Roche). Calculations were performed by a comparative method ($2^{-\Delta CT}$) using 18S as an internal control. For mtDNA analysis, total DNA was extracted with DNeasy blood and tissue kit (QIAGEN) according to the manufacturer's instructions. mtDNA was amplified using primers specific for the mitochondrial cytochrome c oxidase subunit 2 (COX2) gene and normalized to genomic DNA by amplification of the ribosomal protein s18 (rps18) nuclear gene. Primers were designed using the IDT software (IDT) or obtained from primer bank (http://pga.mgh.harvard.edu/primerbank/citation.html) and the primer sequences can be found in the Table S1.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation was performed using a commercial available kit (Millipore) according to the manufacturer's instructions and using anti-HIF1α (Cayman) and anti-c-Myc (Abcam) antibodies and utilizing the following primer sequences: *TFAM* promoter Fw: TTGGCTGGCTAAGCTCATCT, Rv: AAGGCTGAGAAGCGATAGCA (Ahuja et al., 2010); *LDHA* Fw: ATCGATG CATTTGGGCTC, Rv: CAACCCGACATGCTCCTCA (Hu et al., 2007).

Coimmunoprecipitation

Proteins from CreERT2 *SIRT1* flox/flox primary myoblasts were crosslinked using 1 mM DSP (Pierce) after which the cells were lysed in a low-stringency IP buffer (0.05% NP-40, 50 mM NaCl, 0.5 mM EDTA, 50 mM Tris-HCl, pH 7.4) supplemented with protease Inhibitor cocktail (Roche) and 25 U/ml endonuclease (Pierce). Endogenous HIF-1α protein was imunoprecipitated using anti-HIF-1α antibody (Cayman) coated A/G magnetic beads (Pierce). Anti-rabbit IgG antibody (Milipore) coated A/G magnetic beads were used as a control. Immunoprecipitated material was washed ten times for 25 min each in low stringency lysis buffer, after which it was eluted in SDS-PAGE buffer supplemented with 50 mM DTT and boiled for 10 min. Imunoprecipitated proteins and input were run on SDS-PAGE under reducing conditions. The separated proteins were then electrophoretically transferred to a polyvinylidene difluoride membrane (Perkin-Elmer). Proteins of interest were revealed with anti-HIF-1α (Cayman) and anti-c-Myc (Abcam) antibodies overnight at 4°C. The immunostaining was detected using Clean-Blot IP detection reagent (Pierce) according to the manufacturer's instructions. Bands were revealed using Amersham ECL detection system (GE Healthcare).

Immunoblot

Protein extracts from tissue or C2C12 cells were obtained by lysis in ice-cold lysis buffer (150 mM NaCl,10 mM Tris HCl (pH 7.4),1 mM EDTA,1 mM EGTA, 1% Triton X-100, 0.5% NP-40) supplemented with a cocktail of protease and phosphatase inhibitors (Roche). Protein content was determined by the Bradford protein assay (Biorad), and 50 μg proteins were run on SDS-PAGE under reducing conditions. The separated proteins were then electrophoretically transferred to a polyvinylidene difluoride membrane (Perkin-Elmer). Proteins of interest were revealed with specific antibodies: anti-TFAM (Aviva biosciences), anti-COX2, anti-COX4 (Mitosciences), anti-SIRT1, anti-β-tubulin, anti-Flag (Sigma-Aldrich), anti-HIF1α (Cayman), anti-HA (Covance), anti-HIF-2α, anti-NMNAT1 (Novus Biologicals), Anti-NMNAT2, anti-c-Myc, anti-MuRF1, anti-Atrogin1 (Abcam), Anti-NMNAT3 (Santa Cruz Biotecnologies), anti-HIF-1α-OH, anti-VHL, anti-pAMPK (Thr172), anti-AMPK, anti-pACC (Ser79), anti-ACC, anti-pATK (Ser473), anti-AKT (Cell Signaling), anti-pIRS1 (Tyr608), anti-IRS1 (Milipore), anti-MyHCIIa and anti-MyHCIIb (Developmental Studies Hybridoma bank, University of Iowa) overnight at 4°C. The immunostaining was detected using horseradish peroxidase–conjugated anti-rabbit, anti-mouse or anti-goat immunoglobulin for 1 hr at room temperature. Bands were revealed using Amersham ECL detection system (GE Healthcare).

TFAM Promoter Activity and TFAM Promoter Mutagenesis

TFAM promoter activity was evaluated using a *TFAM* promoter-luciferase plasmid. A fragment of the mouse *TFAM* promoter (1.4kb upstream of the coding sequence) was cloned into a pGL4.15 vector (Promega). c-Myc binding site was mutated using the QuickChange lightning kit (Stratagene) according to the manufacturer's instructions and using the following primer sequences: sense: 5′-ggtttggctggctaagctcatctagtcaaaaaaatctgcaaagtgggaaacaatattcag-3′ antisense: 5′-ctgaatattgtttcccactttgcagatttttttgactagatgagcttagccagccaaacc-3′. Primary myoblasts were transfected with either with *TFAM*-luciferase full length promoter or c-*myc* binding site mutant using X-tremeGENE HP (Roche) in accordance with the manufacturer's protocol. For *SIRT1* and *PGC-1α* overexpression experiments cells were infected with the respective adenovirus or empty vector 24 hr after transfection with the luciferase plasmid. For *c-Myc* overexpression or *c-Myc* knockdown primary cells were produced as described above and transfected with either with *TFAM*-luciferase full length promoter or c-*myc* binding site mutant using X-tremeGENE HP (Roche) in accordance with the manufacturer's protocol. To access *TFAM* promoter in SIRT1 iKO, CreERT2 SIRT1flox/flox primary myoblasts were transfected with *TFAM*-luciferase full length promoter or c-*myc* binding site mutant using X-tremeGENE HP (Roche) in accordance with the manufacturer's protocol and 24 hr after treated either with 250 nM 4-hydroxytamoxifen (Sigma-Aldrich), or vehicle (0.001% ethanol) for additional 24 hr. To assess *TFAM* promoter in response to HIF-1α stabilization, primary myoblasts were transfected with *TFAM*-luciferase full length promoter or c-*myc* binding site mutant using X-tremeGENE HP (Roche) in accordance with the manufacturer's protocol and 24 hr after treated either with 1mM DMOG (Sigma-Aldrich), or vehicle (0.001% DMSO) for an additional 12 hr. Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega) with Renilla as the reference.

HRE, VHL Promoter, and c-Myc Activity

HIF-mediated transcriptional activity was measured using an HRE-luciferase plasmid (Bell et al., 2011). *VHL* promoter activity was measured using a commercially available luciferase plasmid (Affymetrix). The plasmids were transfected using X-tremeGENE HP (Roche) in accordance with the manufacturer's protocol. Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega) with Renilla as the reference 48 hr after transfection. c-Myc-mediated transcriptional activity was measured using a luciferase plasmid containing *CDK4* Myc binding sites (Addgene plasmid 16564) and a mutated version as an internal negative control (Addgene plasmid 16565). The plasmids were transfected into CreERT2 *SIRT1* flox/flox primary myoblasts using X-tremeGENE HP (Roche) in accordance with the manufacturer's protocol, *SIRT1* excision was induced with 250 nM 4-hydroxytamoxifen

(Sigma-Aldrich) for the time points showed 24 hr after transfection. Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega) with Renilla as the reference.

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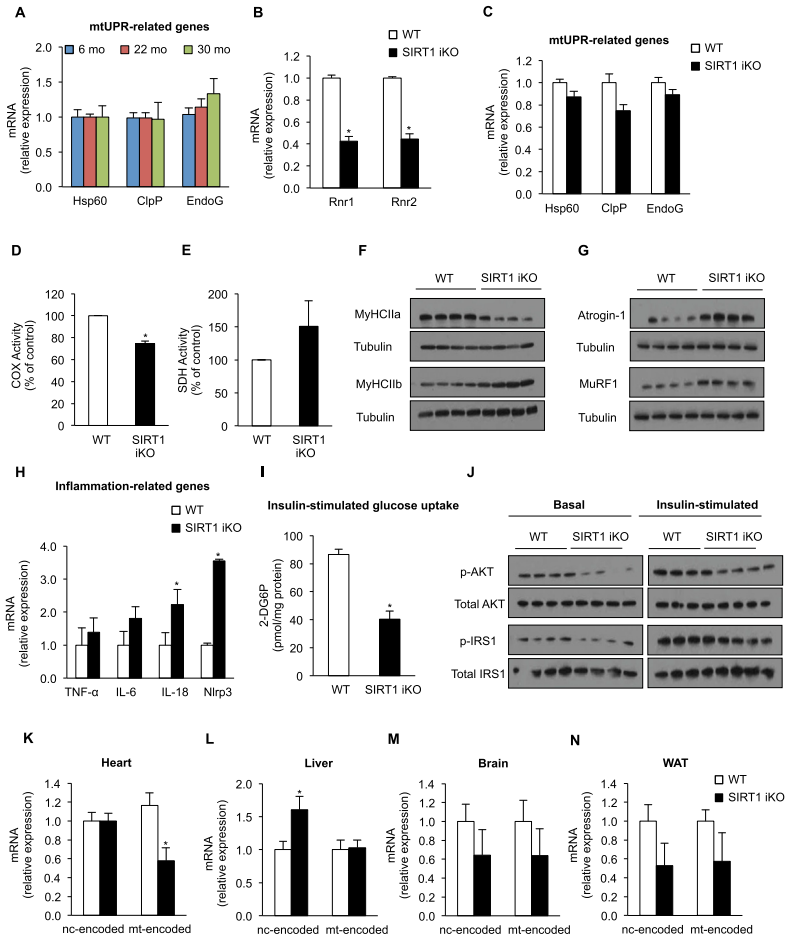

Figure S1. SIRT1 Deletion Results in Impaired Skeletal Muscle Function and a Specific Decline in Mitochondrially Encoded Genes in the Heart, but Not in Liver, WAT, and Brain, Related to Figure 1

(A) Expression of mitochondrial unfolded protein response (mtUPR) markers (*Hsp60*, *ClpP* and *EndoG*) in gastrocnemius of 6-, 22-, and 30-month-old mice. Relative expression values were normalized to 6-month-old mice (n = 5).

(B) Mitochondrial ribosomal RNA (*Rnr1* and *Rnr2*) expression in gastrocnemius of WT and *SIRT1* iKO mice. Relative expression values were normalized to WT mice (n = 5, *p < 0.05 versus WT).

(C) Expression of mitochondrial unfolding protein response (mtUPR) markers (*Hsp60*, *ClpP* and *EndoG*) in gastrocnemius of WT and SIRT1 iKO mice. Relative expression values were normalized to WT mice (n = 5).

(D) Cytochrome c Oxidase (COX) activity evaluated polarographically in gastrocnemius of WT and *SIRT1* iKO mice (n = 5, *p < 0.05 versus WT).

(E) Succinate dehydrogenase (SDH) activity, evaluated polarographically, in gastrocnemius of WT and *SIRT1* iKO mice (n = 5).

(F) Immunoblot for MyHCHIIa, MyHCIIb and Tubulin in gastrocnemius of WT and SIRT1 iKO mice.

(G) Immunoblot for Atrogin-1, MuRF1 and Tubulin in gastrocnemius of WT and SIRT1 iKO mice.

(H) Expression of inflammatory markers (*TNF-α*, *IL-6*, *IL-18* and *Nlrp3*) in gastrocnemius of WT and *SIRT1* iKO mice. Relative expression values were normalized to WT mice (n = 5, *p < 0.05 versus WT).

(I) Insulin sensitivity analyzed by 2-DG uptake in soleus of WT and *SIRT1* iKO mice upon insulin stimulation (n = 5, *p < 0.05 versus WT).

(J) Immunoblot for p-AKT, Total AKT, p-IRS-1 and Total IRS-1 in soleus of WT and *SIRT1* iKO mice under basal conditions and upon insulin stimulation.

(K) Expression of nuclear (*NDUFS8*, *SDHb*, *Uqcrc1*, *COX5b*, *ATP5a1*) versus mitochondrial-encoded genes (*ND1*, *Cytb*, *COX1*, *ATP6*) in heart of WT and SIRT1 iKO mice. Relative expression values were normalized to WT mice stimulation (n = 4, *p < 0.05 versus WT).

(L) Expression of nuclear (*NDUFS8*, *SDHb*, *Uqcrc1*, *COX5b*, *ATP5a1*) versus mitochondrial-encoded genes (*ND1*, *Cytb*, *COX1*, *ATP6*) in liver of WT and *SIRT1* iKO mice. Relative expression values were normalized to WT mice (n = 5).

(M) Expression of nuclear (*NDUFS8*, *SDHb*, *Uqcrc1*, *COX5b*, *ATP5a1*) versus mitochondrial-encoded genes (*ND1*, *Cytb*, *COX1*, *ATP6*) in brain of WT and *SIRT1* iKO mice. Relative expression values were normalized to WT mice (n = 5).

(N) Expression of nuclear (*NDUFS8*, *SDHb*, *Uqcrc1*, *COX5b*, *ATP5a1*) versus mitochondrial-encoded genes (*ND1*, *Cytb*, *COX1*, *ATP6*) in white adipose tissue (WAT) of WT and *SIRT1* iKO mice. Relative expression values were normalized to WT mice (n = 5).

Values are expressed as mean ± SEM.

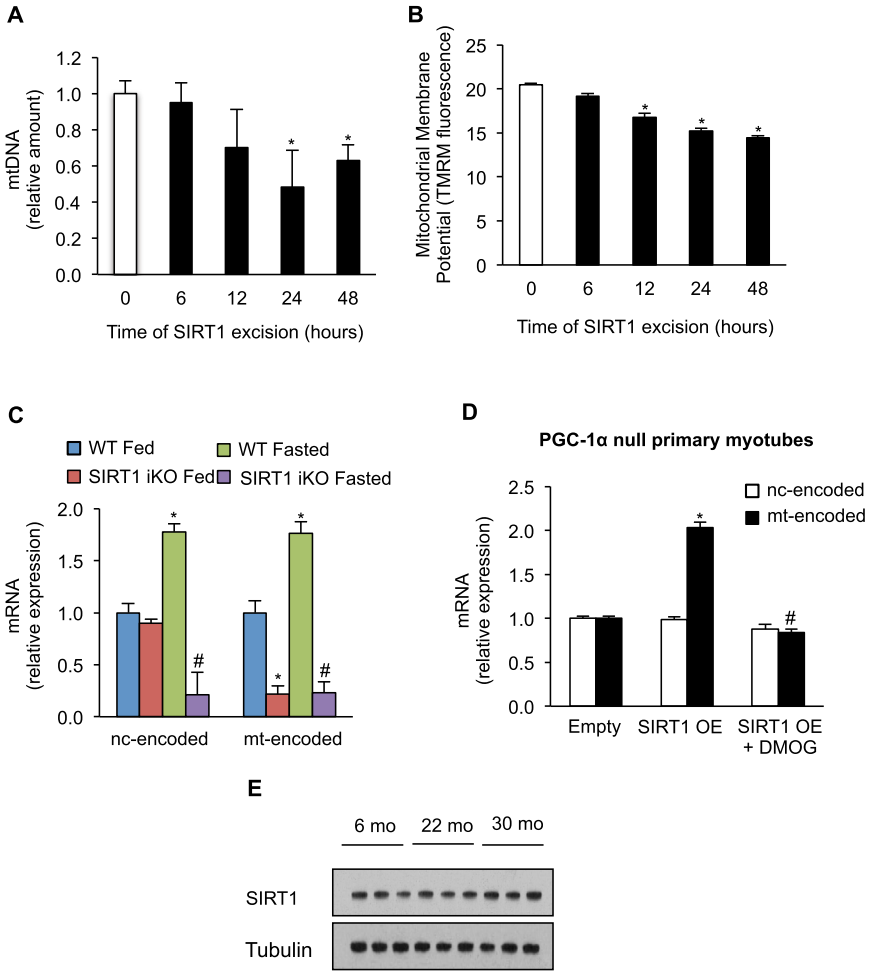

Figure S2. SIRT1 Regulates Mitochondrial Metabolism through a Byphasic Response Dependent on the Energetic State of the Cell, Related to Figure 2

(A) Mitochondrial DNA content in *SIRT1* flox/flox Cre-ERT2 primary myoblasts treated with vehicle (0 h) or OHT to induce *SIRT1* excision for 6, 12, 24 and 48 hr. Relative amount was normalized to vehicle cells (n = 4, *p < 0.05 versus vehicle).

(B) Mitochondrial membrane potential, analyzed by TMRM fluorescence, in *SIRT1* flox/flox Cre-ERT2 primary myoblasts treated with vehicle (0h) or OHT to induce *SIRT1* excision for 6, 12, 24 and 48 hr (n = 4, *p < 0.05 versus vehicle).

(C) Expression of nuclear (*NDUFS8*, *SDHb*, *Uqcrc1*, *COX5b*, *ATP5a1*) versus mitochondrial-encoded genes (*ND1*, *Cytb*, *COX1*, *ATP6*) in gastrocnemius of WT and *SIRT1* iKO mice under fed and fasted conditions. Relative expression values were normalized to WT fed mice (n = 5, *p < 0.05 versus WT Fed; #p < 0.05 versus *SIRT1* iKO Fed).

(D) Expression of mitochondrial-encoded genes (*ND1*, *Cytb*, *COX1* and *ATP6*) in PGC-1α null myotubes infected with an adenovirus for overexpression of *SIRT1* or empty vector and treated with the HIF-1α stabilizing compound DMOG. Relative expression was normalized to empty vector (n = 5, *p < 0.05 versus empty vector; #p < 0.05 versus *SIRT1* OE).

(E) Immunoblot for SIRT1 and tubulin in gastrocnemius of 6-, 22-, and 30-month-old mice.

Values are expressed as mean ± SEM (*p < 0.05 versus WT).

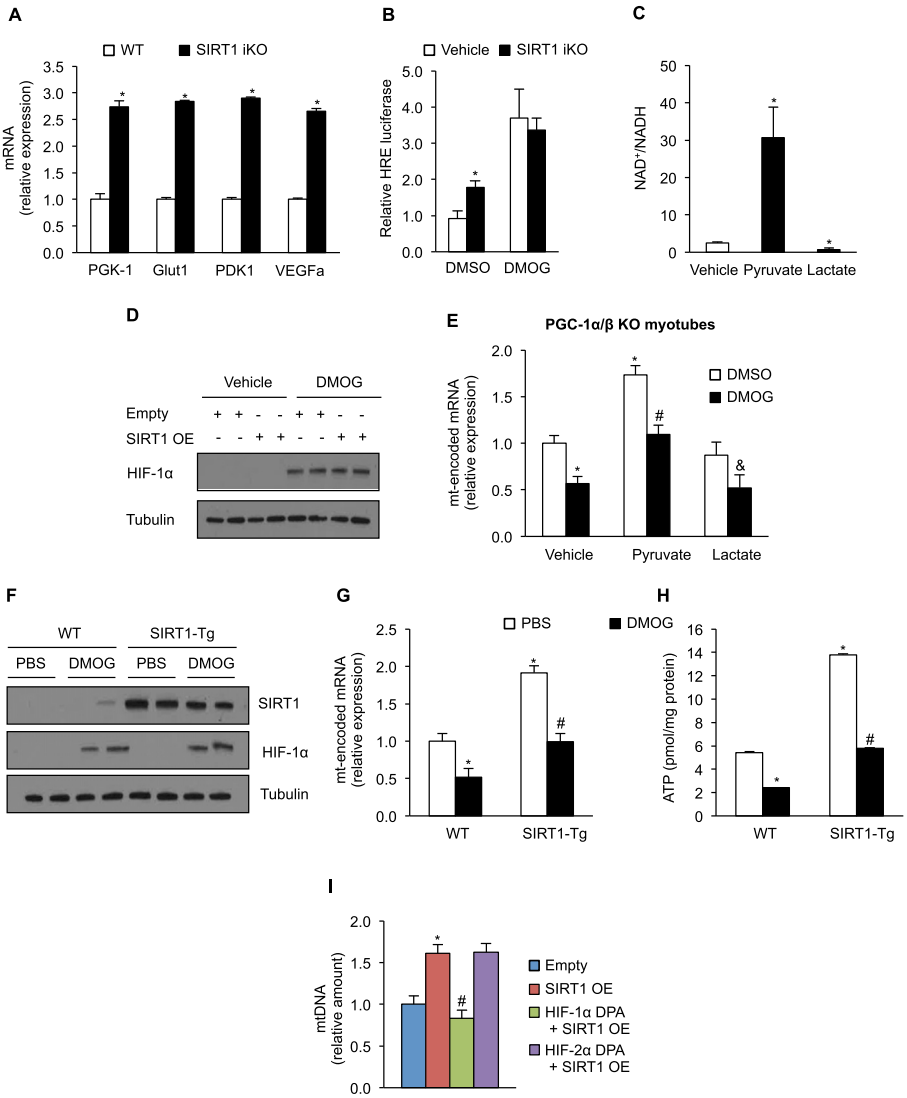

Figure S3. SIRT1 Regulates OXPHOS through HIF-1α, Related to Figure 3

(A) HIF-1α target genes (*PGK-1*, *Glut1*, *PDK1* and *Vegfa*) expression in gastrocnemius of WT and *SIRT1* iKO mice. Relative expression values were normalized to WT mice (n = 5, *p < 0.05 versus WT).

(B) Hypoxia response element activity in *SIRT1* flox/flox Cre-ERT2 primary myoblasts treated with vehicle or OHT to induce *SIRT1* excision for *SIRT1* iKO and treated with or without DMOG. Relative luciferase activity was normalized to vehicle cells (n = 6, *p < 0.05 versus vehicle).

(C) NAD^+/NADH ratio measured in primary myoblasts treated with either 10 mM pyruvate, 10 mM lactate or vehicle for 24h (n = 4, *p < 0.05 versus vehicle).

(D) Immunoblot of HIF-1α and Tubulin in *PGC-1α/β* KO myotubes treated with adenovirus overexpressing *SIRT1* or empty vector as well as treatment with DMSO or DMOG.

(E) Expression of mitochondrial-encoded genes (*ND1*, *Cytb*, *COX1* and *ATP6*) in *PGC-1α/β* KO myotubes treated with 10 mM pyruvate, 10 mM lactate or vehicle for 24h in the presence or absence of DMOG. Relative expression values were normalized to vehicle cells (n = 4, *p < 0.05 versus vehicle DMSO; #p < 0.05 versus pyruvate DMSO; & versus lactate DMSO).

(F) Immunoblot for SIRT1, HIF-1α and Tubulin in gastrocnemius of WT and *SIRT1*-Tg mice treated with vehicle (PBS) or DMOG.

(G) Expression of mitochondrial-encoded genes (*ND1*, *Cytb*, *COX1* and *ATP6*) in gastrocnemius of WT and *SIRT1*-Tg mice treated with vehicle (PBS) or DMOG. Relative expression values were normalized to WT PBS mice (n = 5, *p < 0.05 versus WT PBS; #p < 0.05 versus *SIRT1*-Tg PBS).

(H) ATP content in gastrocnemius of WT and *SIRT1*-Tg mice treated with vehicle (PBS) or DMOG (n = 5, *p < 0.05 versus WT PBS; #p < 0.05 versus *SIRT1*-Tg PBS).

(I) Mitochondrial DNA content analyzed by qPCR in empty vector, HIF-1α DPA or HIF-2α DPA C2C12 cells treated with adenovirus overexpressing *SIRT1* or empty vector. Relative amount was normalized to control cells (n = 5, *p < 0.05 versus empty vector, #p < 0.05 versus SIRT1 OE).

Values are expressed as mean ± SEM.

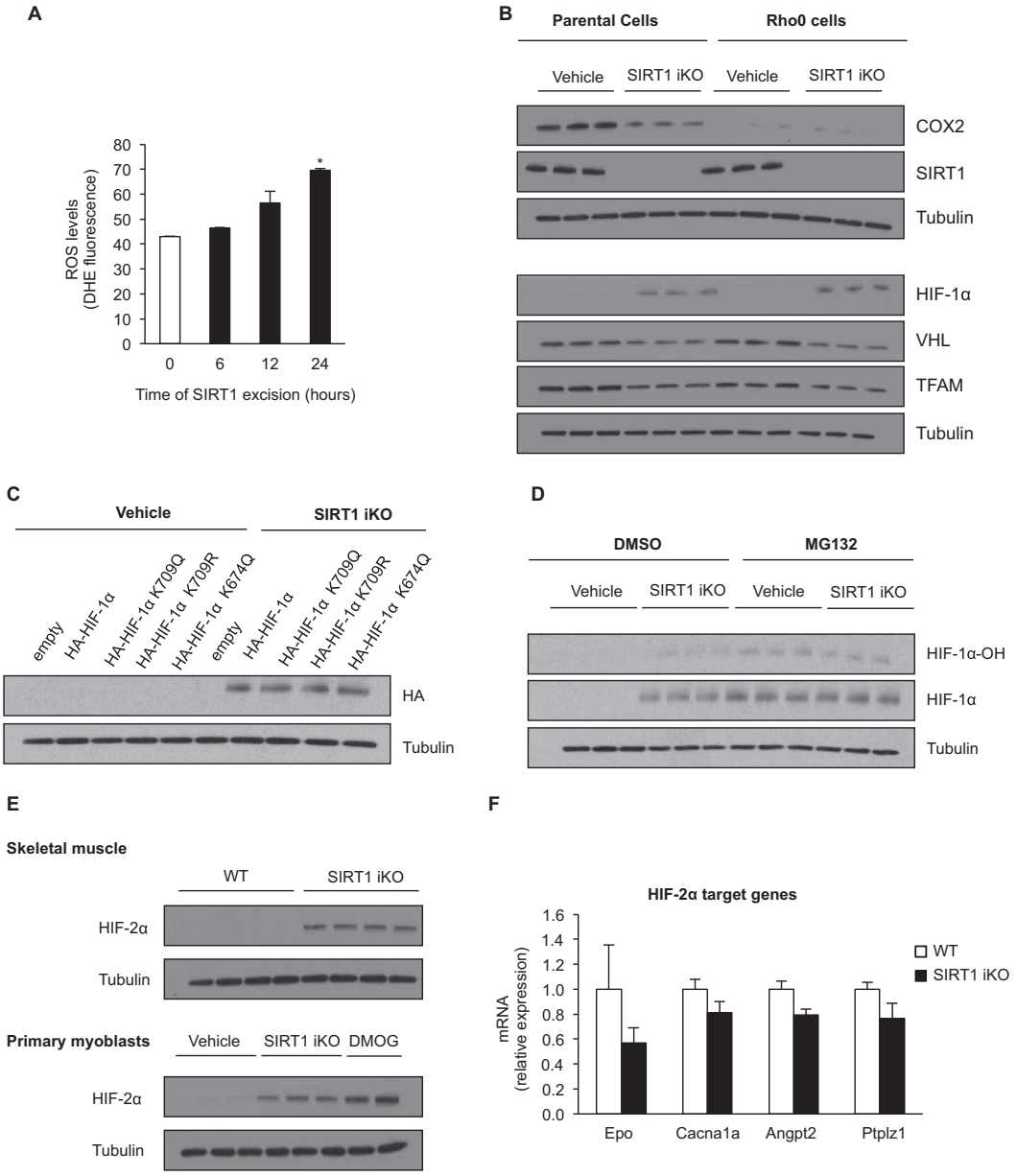

Figure S4. SIRT1 Regulates HIF-1α Protein Stability Independently of Retrograde Signaling, Acetylation, and Hydroxylation, Related to Figure 4

(A) ROS levels, measured by DHE fluorescence intensity, in *SIRT1* flox/flox Cre-ERT2 primary myoblasts treated with vehicle (0h) or OHT for 6, 12, 24 and 48 hr to induce *SIRT1* excision (n = 4, *p < 0.05 versus vehicle).

(B) Immunoblot for COX2, SIRT1, HIF1-α, VHL, TFAM and Tubulin in parental or rho0 cells derived from *SIRT1* flox/flox Cre-ERT2 primary myoblasts treated with vehicle, or OHT for 24h to induce *SIRT1* excision (*SIRT1* iKO).

(C) Representative immunoblot for HA and Tubulin in *SIRT1* flox/flox Cre-ERT2 primary myoblasts treated with vehicle or OHT for 24h to induce *SIRT1* excision (*SIRT1* iKO) and transduced with HA-HIF-1α, or the Q and R mutants of K709 and Q mutant of K674.

(D) Immunoblot for HIF-1α-OH, HIF-1α and Tubulin in SIRT1 flox/flox Cre-ERT2 primary myoblasts treated with vehicle or OHT for 24h to induce *SIRT1* excision (*SIRT1* iKO) in the presence and absence of the proteasome inhibitor, MG-132.

(E) Immunoblot for HIF-2α and Tubulin in gastrocnemius of WT and *SIRT1* iKO mice and in *SIRT1* flox/flox Cre-ERT2 primary myoblasts treated with vehicle or OHT for 24h to induce SIRT1 excision (*SIRT1* iKO) and treated with DMOG to stabilize HIFα.

(F) Expression of HIF-2α target genes (*Epo*, *Cacna1a*, *Angpt2* and *Ptplz1*), analyzed by qPCR, in gastrocnemius of WT and *SIRT1* iKO mice. Relative expression values were normalized to WT mice (n = 5).

Values are expressed as mean ± SEM.

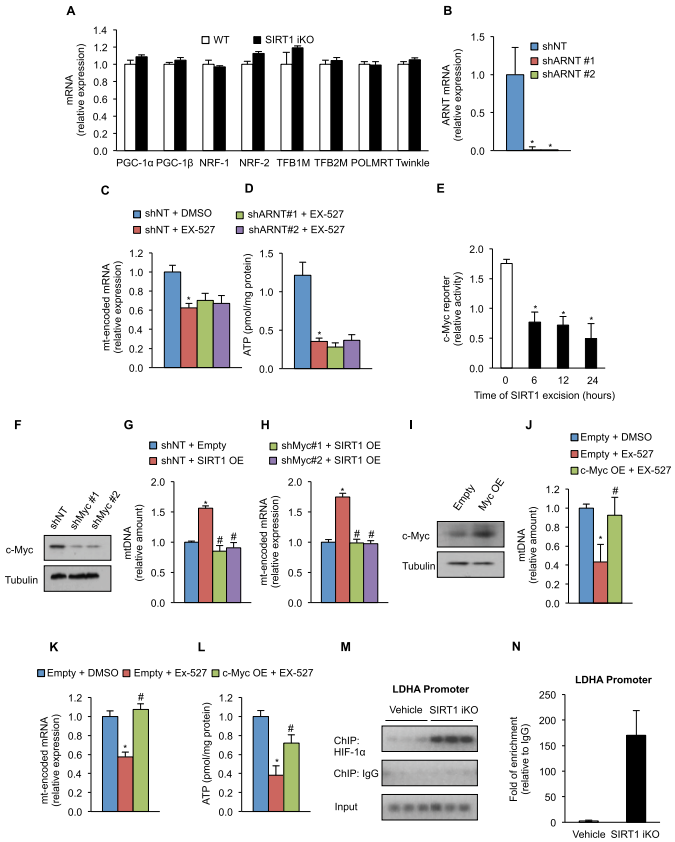

Figure S5. HIF-1α Suppresses Mitochondrially Encoded Genes via SIRT1 by Downregulating c-Myc and TFAM Independent of Its Transcriptional Partner ARNT, Related to Figure 5

(A) *PGC-1α*, *PGC-1β*, *NRF-1*, *NRF-2*, *TFB1M*, *TFB2M*, *POLMRT* and *Twinkle* expression in gastrocnemius of WT and *SIRT1* iKO mice. Relative expression values were normalized to WT mice (n = 5, *p < 0.05 versus WT).

(B) *ARNT* mRNA analyzed by qPCR in C2C12 cells transduced with *ARNT* or nontargeting shRNA. Relative expression values were normalized to shNT cells (n = 4, *p < 0.05 versus shNT).

(C) Expression of mitochondrial-encoded genes (*ND1*, *Cytb*, *COX1* and *ATP6*) analyzed by qPCR in C2C12 cells transduced with *ARNT* or nontargeting shRNA and treated with the specific SIRT1 inhibitor, EX-527, or DMSO for 24h. Relative expression was normalized to shNT DMSO cells (n = 6, *p < 0.05 versus shNT DMSO).

(D) ATP content in C2C12 cells transduced with *ARNT* or nontargeting shRNA and treated with the specific SIRT1 inhibitor, EX-527, or DMSO for 24h. (n = 4, *p < 0.05 versus shNT DMSO).

(E) c-Myc activity in *SIRT1* flox/flox Cre-ERT2 primary myoblasts and treated with vehicle (0 h) or OHT to induce *SIRT1* excision for 6, 12, 24 hr. Relative luciferase values were normalized to vehicle cells (n = 4, *p < 0.05 versus vehicle).

(F) Immunoblot for c-Myc and Tubulin in C2C12 cells transduced with c-Myc or nontargeting shRNA.

(G) Mitochondrial DNA content in C2C12 cells transduced with *c-Myc* or nontargeting shRNA and treated with adenovirus overexpressing *SIRT1* or empty vector. Relative amount was normalized to shNT empty cells (n = 5, *p < 0.05 versus shNT empty vector, #p < 0.05 versus shNT *SIRT1* OE).

(H) Expression of mitochondrial-encoded genes (*ND1*, *Cytb*, *COX1* and *ATP6*) in C2C12 cells transduced with *c-Myc* or nontargeting shRNA and treated with adenovirus overexpressing *SIRT1* or empty vector. Relative expression values were normalized to shNT empty cells (n = 6, *p < 0.05 versus shNT empty, #p < 0.05 versus shNT *SIRT1* OE).

(I) Immunoblot for c-Myc and Tubulin in C2C12 cells overexpressing *c-Myc*.

(J) Mitochondrial *DNA content, analyzed by qPCR, in C2C12 cells overexpressing* c-Myc and treated with EX-527 or DMSO. Relative amount was normalized to empty DMSO cells (n = 5, *p < 0.05 versus empty DMSO, #p < 0.05 versus empty EX-527).

(K) Expression of mitochondrial-encoded genes (*ND1*, *Cytb*, *COX1* and *ATP6*) analyzed by qPCR, in C2C12 cells overexpressing c-Myc and treated with EX-527 or DMSO. Relative expression values were normalized to empty DMSO cells (n = 6, *p < 0.05 versus empty DMSO, #p < 0.05 versus empty EX-527).

(L) ATP content in C2C12 cells overexpressing *c-Myc* and treated with EX-527 or DMSO. Relative expression values were normalized to empty DMSO cells (n = 6, *p < 0.05 versus empty DMSO, #p < 0.05 versus empty EX-527).

(M and N) Chromatin immunoprecipitation (M) and respective quantification by qPCR (N) of HIF-1α to the *LDHA* promoter in *SIRT1* flox/flox Cre-ERT2 primary myoblasts treated with vehicle, or OHT to induce *SIRT1* excision for 24 hr (SIRT1 iKO).

Values are expressed as mean ± SEM.

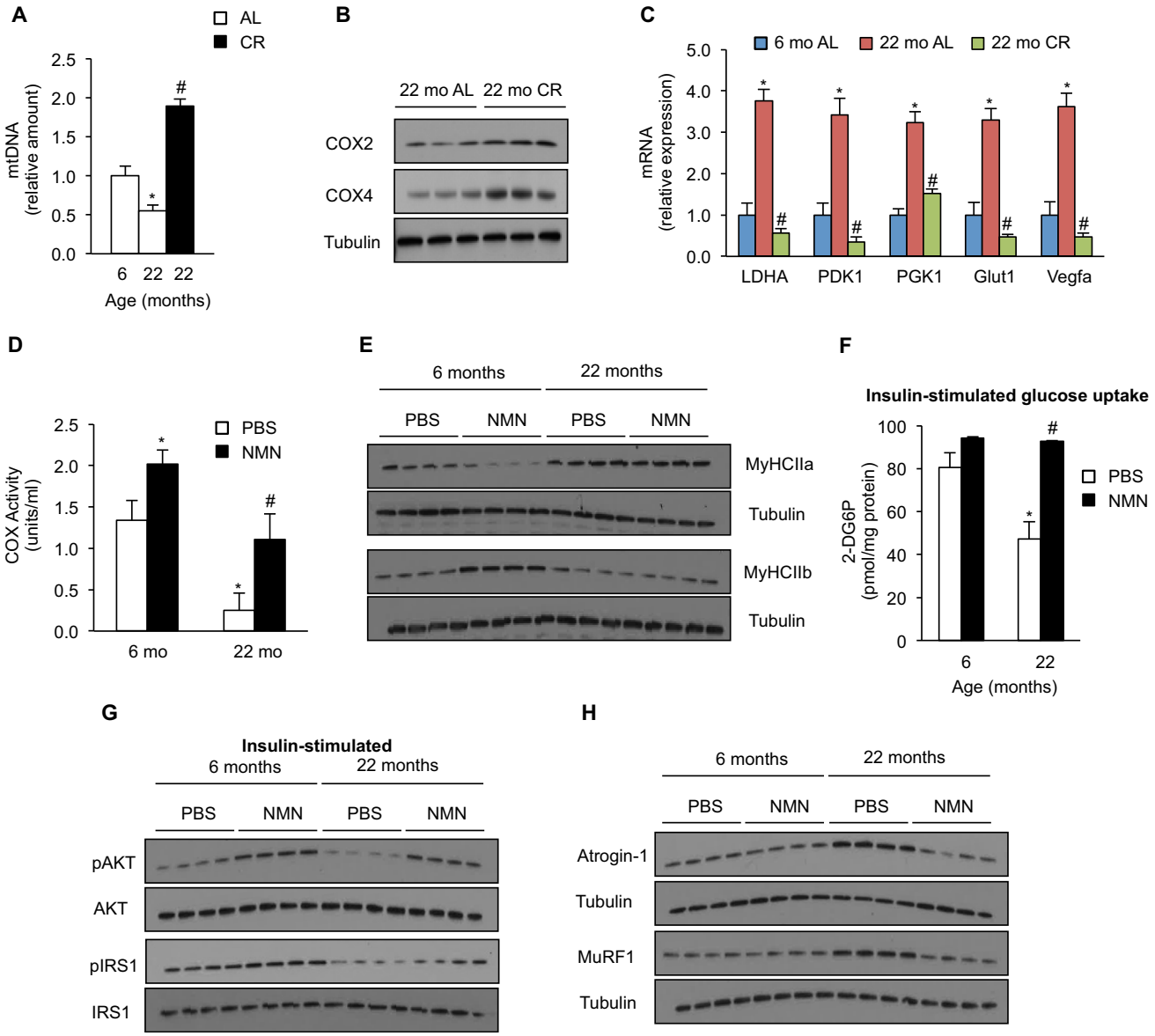

Figure S6. Increasing NAD$^+$ Levels Improves Mitochondrial Function and Skeletal Muscle Health in Old Mice, Related to Figure 7

(A) Mitochondrial DNA content analyzed by qPCR in gastrocnemius of 6- and 22-month AL and 22-month old CR mice. Relative amount was normalized to 6-month-old mice (n = 5, *p < 0.05 versus 6-month-old; #p < 0.05 versus 22-month-old AL).

(B) Immunoblot for COX2, COX4, and tubulin in gastrocnemius of 22-month-old AL and CR mice.

(C) Expression of HIF-1α target genes (*LDHA*, *PDK1*, *PGK1*, *Glut1* and *Vegfa*) in gastrocnemius of 6- and 22-month AL and 22-month old CR mice. Relative expression values were normalized to 6-month-old mice (n = 5, *p < 0.05 versus 6-month-old; #p < 0.05 versus 22-month-old AL).

(D) Cytochrome c Oxidase Activity (COX) activity, measured spectrophotometrically, in gastrocnemius of 6- and 22-month-old mice treated with vehicle (PBS) or NMN (n = 5, *p < 0.05 versus 6-month-old PBS; #p < 0.05 versus 22-month-old PBS).

(E) Immunoblot for MyHCIIa, MyHCIIb and tubulin in gastrocnemius of 6- and 22-month-old mice treated with vehicle (PBS) or NMN.

(F) Insulin sensitivity analyzed by 2-DG uptake in soleus of 6- and 22-month-old mice treated with vehicle (PBS) or NMN after insulin treatment (n = 6, *p < 0.05 versus 6-months-old PBS).

(G) Immunoblot for p-AKT, AKT, p-IRS-1, IRS-1in soleus of 6- and 22-month-old mice treated with vehicle (PBS) or NMN after insulin treatment.

(H) Immunoblot for Atrogin-2, MuRF1 and Tubulin in gastrocnemius of 6- and 22-month-old mice treated with vehicle (PBS) or NMN.

Values are expressed as mean ± SEM.