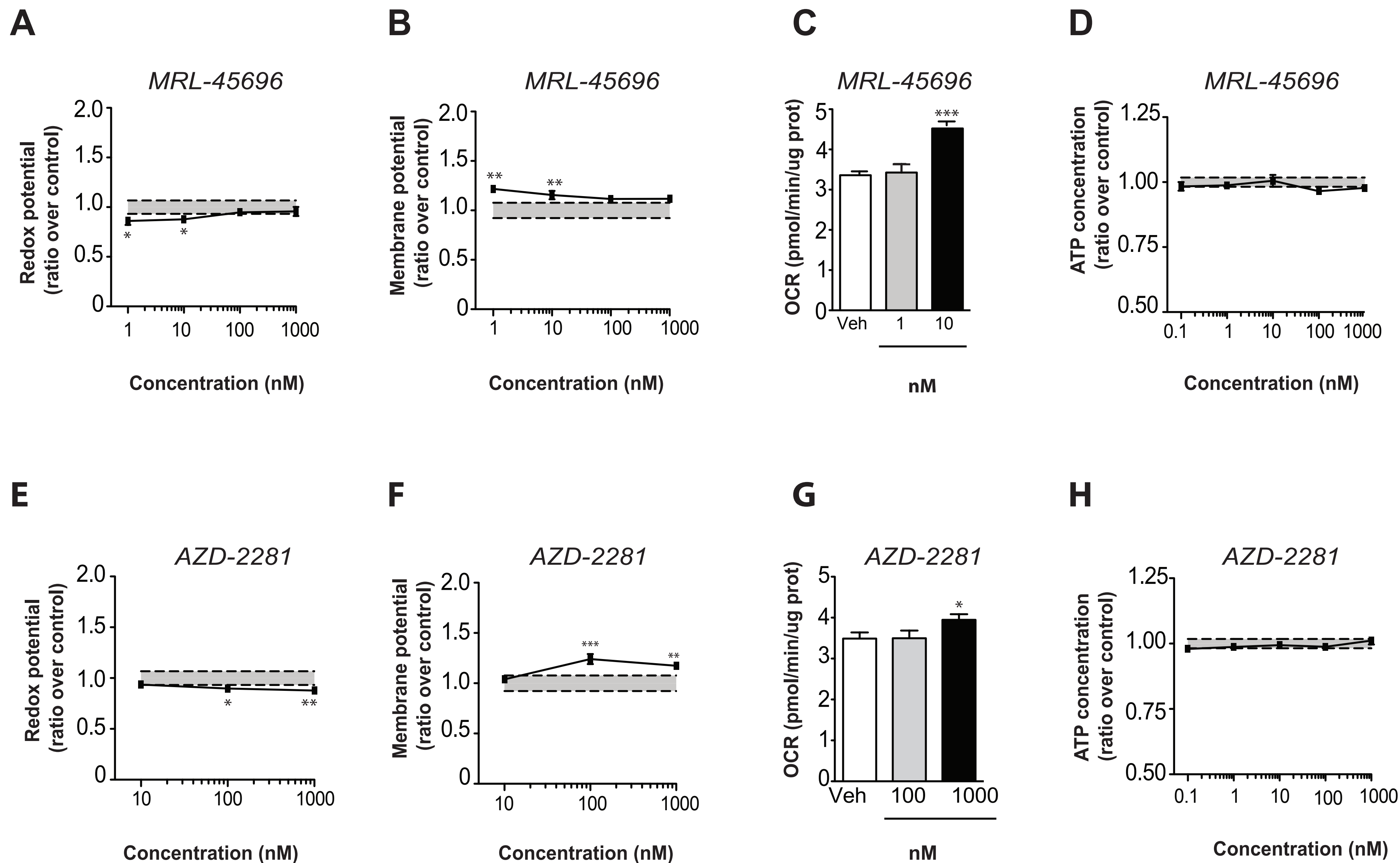


Figure S1 related to Figure 1. Mitochondrial functional tests in C2C12 myotubes using MRL-45696 and AZD-2281 PARP inhibitors. Myotubes treated with PARP inhibitors for 24 hours were tested for (A) redox potential, (B) membrane potential, (C) respiration and (D) ATP levels. Treatments were performedusing low glucose DMEM supplemented with 1% albumin-bound oleic acid. Results are presented as means +/- SEM from 10-12 samples per group.*, p<0.05; **, p<0.01; ***, p<0.001.

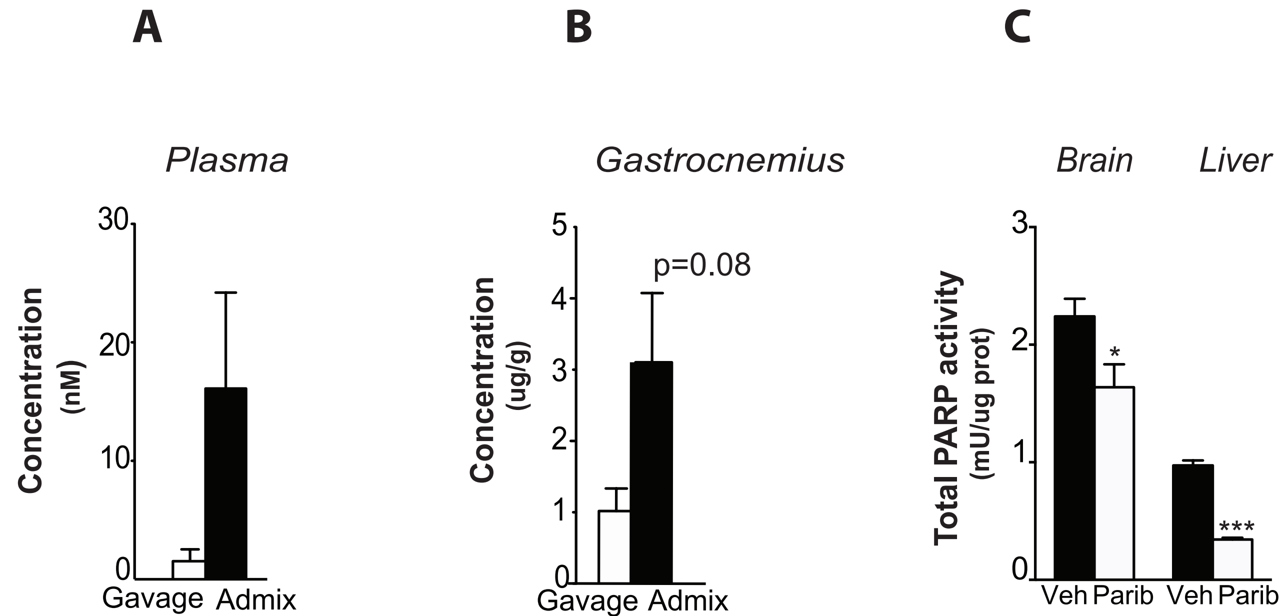


Figure S2 related to Figure 1. The comparison of oral gavage and dietary admix administration routes for MRL-45696 and total PARP activity in the brain and liver. MRL-45696 was administered via oral gavage twice per day with the dose of 25 mg/kg/day in 0.5% methylcellulosa or as a food admix 50 mg/kg/day for 5 days. (A) Plasma and (B) muscle samples were collected at 6hr after the last oral gavage or without restricting the food consumption of the food admix group. Results are presented as means +/- SEM from 5 mice per group. (C) Total poly(ADP-ribose) (PARP) activity was measured in the brain and livers of refed vehicle and MRL-45696-treated mice (n=7 per group) on chow diet after 18 weeks of the treatment. *, $p<0.05$; ** and ***, $p<0.001$.

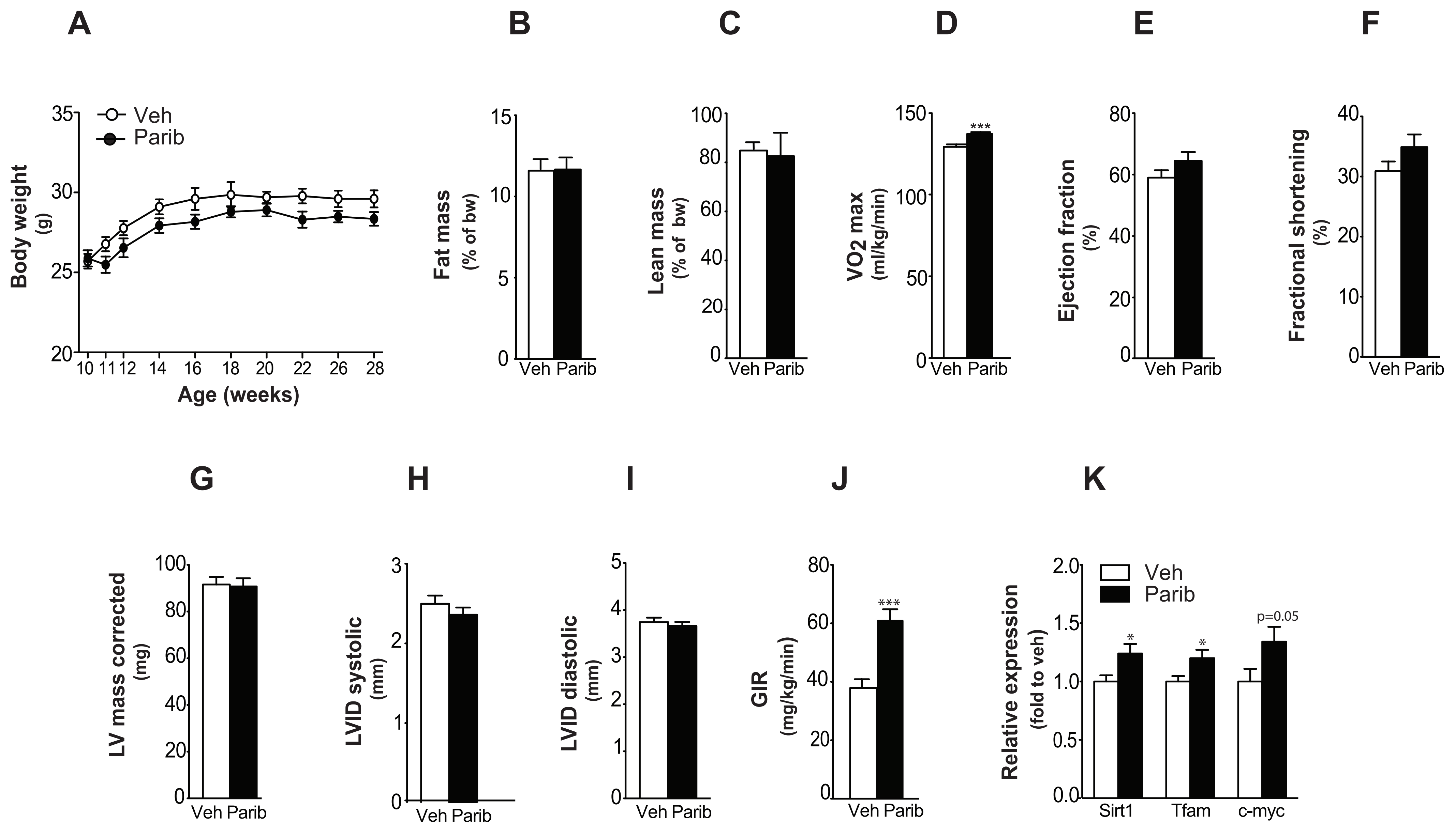
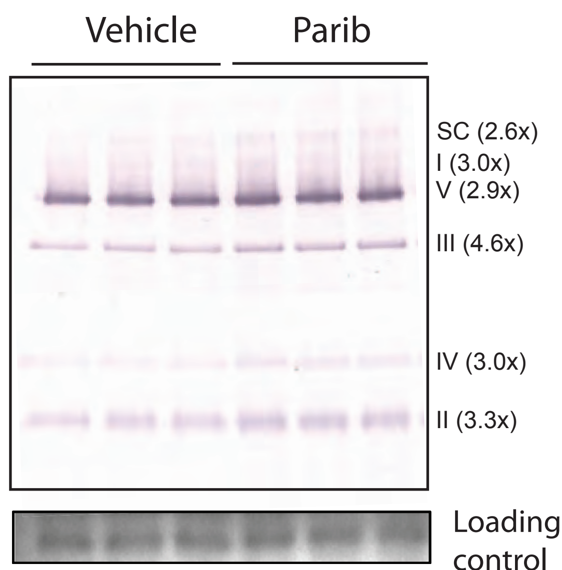
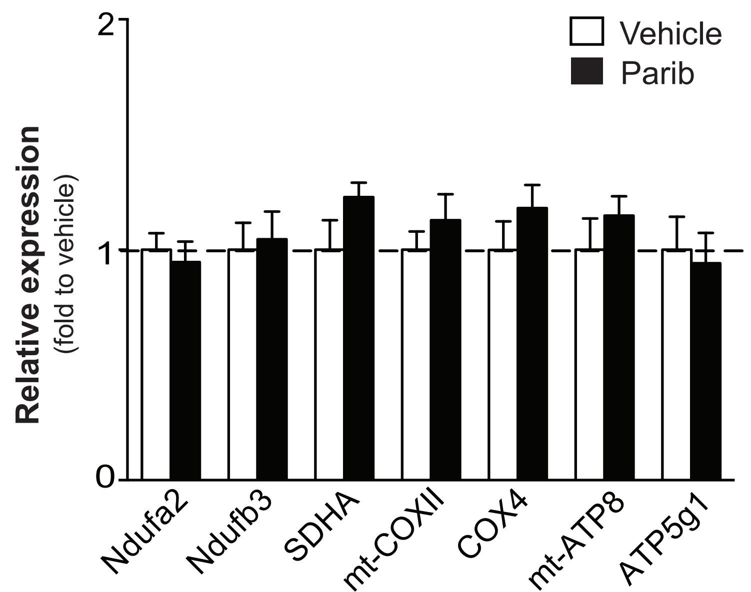


Figure S3 related to Figure 2. Phenotype of chow diet -fed vehicle and MRL-45696-treated mice. (A) Body weight evolution during chow diet. (B) Fat and (C) lean body masses after 18 weeks of the treatments. (D) VO_2max test after 10 weeks treatment. (E) Ejection fraction, (F) fractional shortening, (G) corrected left ventricular mass, (H) left ventricular internal diameter during systole and (I) left ventricular internal diameter during diastole on chow diet-fed mice after 7 weeks of the treatments. (J) Glucose infusion rates during hyperinsulinemic-euglycemic clamp after 4 weeks of the treatments. (K) *Sirt1*, *Tfam* and *c-myc* gene expression in quadriceps of vehicle and MRL-45696-treated mice analyzed by quantitative RT-PCR. Sirt1, sirtuin 1 and Tfam, mitochondrial transcription factor A. Results are presented as means +/- SEM from 6-12 mice per group. *, $p<0.05$ and ***, $p<0.001$.





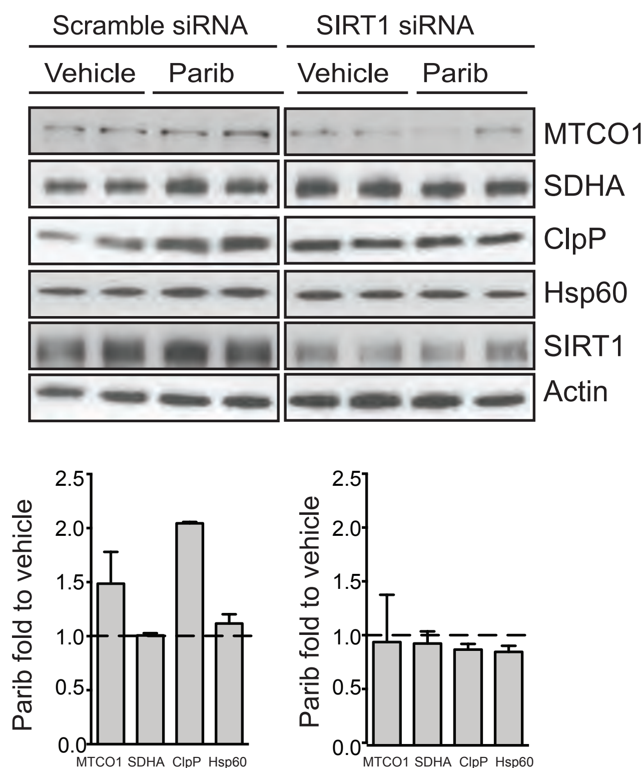


Figure S4 related to Figure 3. PARP inhibitor treatment increases abundance of mitochondrial respiratory complexes (MRC) in mouse embryonic fibroblasts without having an impact on mRNA levels of MRC subunit genes but induces UPRmt via a SIRT1 dependent mechanism. (A) Blue-Native Page using isolated mitochondria from mouse embryonic fibroblasts after treatments with vehicle or 100nM MRL-45696 for 48hr. The bottom panel showing a non-specific band served as a loading control. (B) Selected gene expression profiles in quadriceps muscle of vehicle and MRL-45696-treated mice on chow diet analyzed by quantitative RT-PCR. Results are presented as means +/- SEM from 6-10 mice per group. Ndufa2, NADH Dehydrogenase 1 alpha; Ndufb3, NADH Dehydrogenase 1 beta subcomplex subunit 3; SDHA, succinate dehydrogenase; mt-COXII, cytochrome C Oxidase subunit 2, COX4, cytochrome C Oxidase subunit 4; mt-ATP8, ATP synthase subunit 8 and ATP5g1, ATP synthase subunit C. (C) Mouse C2C12 myotubes were transfected with 40nM scramble and SIRT1 siRNA at the differentiation day 4. The treatment of cells with 10nM PARP inhibitor for 72hr induced UPRmt but SIRT1 knock-down attenuated the effect. SIRT1 protein expression was analyzed after 48h of transfection. Actin served as a loading control.

Table S1 related to Figure 1. Characteristics of PARP inhibitors tested. Ability of different PARP inhibitors to rescue NAD^+ decline upon H_2O_2-induced PARP-1 activation in C2C12 myotubes.

Compound	MW	IC50 PARP-1 (nM)*	IC50 PARP-2 (nM)*	IC50 NAD+ rescue (nM)
BSI-201	292	No effect	No effect	No effect
PJ-34	332	20-110	NA	640
ABT-888	244	5	3	48
AZD-2281	435	5	1	34
MRL-45696	440	<1	<1	1-10

* based on the literature. NA, not available.

SUPPLEMENTAL PROCEDURES

Materials and methods

The half maximal inhibitory concentration (IC50) tests in C2C12 myotubes Murine C2C12 myoblasts were maintained in high glucose DMEM supplemented with 10% fecal calf serum (FCS) and 1% pen-strep. The cells were differentiated into myotubes as described using 2% horse serum instead of 10% FCS in regular high glucose DMEM medium (Canto et al., 2009). C2C12 myotubes were pretreated with several concentrations of PARP inhibitors for one hr followed by treatment with 500μM H_2O_2 for one additional hr. After treatments, NAD^+ was extracted and measured using HPLC as described (Ramsey et al., 2009). IC50 values were calculated using GraphPad Prism software.

Cell culture conditions in human cells

Primary myoblasts obtained from obese subjects were cultured in low glucose DMEM supplemented with 10% FCS, dexamethazone, human epidermal growth factor, fetuin and gentamycin and differentiated into myotubes using 2% horse serum in alpha-MEM supplemented with fetuin and 1% pen-strep for 7 days. Human *NDUFS1* mutant fibroblasts were cultured in high glucose DMEM supplemented with 10% FCS and 1% pen-strep.

Mitochondrial functional tests in C2C12 cells

C2C12 myoblasts were differentiated into myotubes as described (Canto et al., 2009) in 96-well plates. Four-day-differentiated myotubes were treated with different concentrations of MRL-45696 and AZD-2881 in low glucose DMEM containing 1% albumin-bound oleic acid (Sigma), 10% FCS, 2% hepes, 1% non-essential amino acids and 1% pensterp for 24hr. The Alamar Blue (Life Technologies, Paisley, UK) assay was used as a marker for global metabolic redox activity. Mitochondrial

membrane potential and ATP levels were measured using the fluorescent tetramethylrhodamine methyl ester (TMRM) probe (T-668, Invitrogen, Paisley, UK) and Cell Titer Glo (Promega, Madison, WI, USA), respectively. Basal oxygen consumption (OCR) was measured using the Seahorse XF96 equipment (Seahorse Bioscience Inc.). All results were normalized to protein amount assessed by Bradford kit (Bio-Rad).

Mitochondrial and cytosolic protein translation assays in mouse embryonic fibroblasts (MEFs)

For mitochondrial protein translation assay, pulse labeling was performed as described (Weraarpachai et al., 2009) after 40hr 100nM MRL-45696 treatment. Shortly, MEFs were pulse-labeled with 200uCi/ml of an [^{35}S] EasyTag methionine/cysteine mix (PerkinElmer) for 1–4hr at 37°C in a CO_2 incubator in methionine/cysteine-free DMEM containing 100 ug/ml of emetine. After labeling, cells were chased for 10 min in normal DMEM. Cytosolic protein translation was measured using puromycin incorporation into newly synthesized proteins, as published (Schmidt et al., 2009) after 48hr 100nM MRL-45696 treatment. Newly synthesized proteins were detected using a monoclonal anti-puromycin antibody (#3RH11, KeraFast, Boston, MA). Then proteins were separated using western blot technique and detected by either Typhoon scanner (GE Healthcare Life Sciences, Cleveland Ohio) or x-ray films.

Transfection of C2C12 myotubes

C2C12 myoblasts were differentiated into myotubes as described (Canto et al., 2009) in 6-well plates. Four-day-differentiated myotubes were transfected with 40nM scramble or SIRT1 siRNA (Microsynth) using lipofectamine in serum- and antibiotic-free DMEM medium following manufacturer’s protocols. After 5h of transfection, regular antibiotic-free differentiation medium was changed. Next day, cells were

treated with 10nM MRL-45696 in antibiotic-free low glucose DMEM supplemented with 1% oleic acid for 72hr. Cells were harvested with lysis buffer (50 mM Tris, 150 mM KCl, EDTA 1 mM, NP40 1%, nicotinamide 5mM, Na-butyrate 1mM, protease inhibitors pH7.4) and proteins were subjected to western blotting. See the western blotting protocol below.

Generation of *Sirt1*$^{skm-/-}$ mice

For the generation of *Sirt1* floxed (*Sirt1*$^{L2/L2}$) mice, genomic DNA covering the *Sirt1* locus was amplified from the 129Sv strain by high-fidelity PCR. The resulting DNA fragments were assembled into the targeting vector of the Institut Clinique de la Souris (Strasbourg, France). The construct in which exons 5, 6 and 7 were flanked by LoxP sites was then electroporated into 129Sv embryonic stem (ES) cells. G418-resistant colonies were selected and analyzed for homologous recombination by PCR and positive clones were verified by Southern blot hybridization. Correctly targeted ES cell clones were injected into blastocysts and transferred into pseudopregnant females, resulting in chimeric offspring that were mated to female C57BL/6J mice that express the Flp recombinase under the control of the ubiquitous cytomegalovirus promoter (Rodriguez et al., 2000). Offspring that transmitted the mutated allele and that lost the Flp transgene (*Sirt1*$^{L2/WT}$ mice) were then selected, and backcrossed to C57BL/6J mice for ten generations. In order to obtain *Sirt1*$^{skm-/-}$ mice, *Sirt1*$^{L2/L2}$ mice were crossed with human skeletal actin HSA-CRE mice that were on a C57BL/6J background (Miniou et al., 1999).

Long-term animal experiments

Ten weeks old male C57BL/6J mice, purchased from Charles River, were fed with pellets containing vehicle or PARP inhibitor (50 mg/g/kg) for 18 weeks. Additionally, *Sirt1*$^{skm-/-}$ mice were treated with PARP inhibitor (50 mg/g/kg) for 14 weeks. The pellets were prepared by mixing the powder food and water with DMSO (vehicle) or

with PARP inhibitor dissolved in DMSO. Pellets were dried under the hood at least over night. Powder chow (D12450B) and high fat (D12492) diets were from Research Diets Inc. Most clinical tests were carried out according to standard operational procedures (SOPs) established and validated within the Eumorphia program (Champy et al., 2008). Body composition was determined by Echo-MRI (Echo Medical Systems) and oxygen consumption (VO_2), food intake and activity levels were monitored by indirect calorimetry using the comprehensive laboratory animal monitoring system (Columbus Instruments). Endurance exercise capacity test, VO_{2max} test, echocardiography and hyperinsulinemic-euglycemic clamp were performed as published previously (Yamamoto et al., 2011) (Lagouge et al., 2006). All animals were sacrificed after a 2hr, overnight fast or 4 to 6hr refed condition using isoflurane inhalation. Tissues were collected upon sacrifice and flash-frozen in liquid nitrogen.

Respirometry on permeabilized muscle fibers

Mitochondrial function in permeabilized EDL muscle fibers was evaluated using high-resolution respirometry (Oroboros Oxygraph-2k; Oroboros Instruments, Austria), as described (Boushel et al., 2007), with minor modifications. Briefly, basal O_2 flux from Complex I was measured by adding malate (1.6 mM), glutamate (20 mM) and pyruvate (9.8 mM). Oxidative phosphorylation (Complex I) was quantified by the addition of ADP (4.8 mM), followed by the addition of succinate (9.6 mM) to stimulate complex I + complex II driven coupled respiration. Then, the protonophore agent FCCP (0.2 mM) was titrated to achieve maximum electron transfer flux. Finally, electron transport through complexes I and III was inhibited by sequential addition of rotenone (0.1 mM) (ETS Complex II) and antimycin (2.4 mM), respectively. O_2 flux obtained in each step of the protocol was normalized by the weight of the muscle sample used for the analysis.

***C. elegans* studies**

Western blotting of PAR content was performed in pooled (approximately n=500) *Mev-1(kn1)* worms using antibody described below (see western blotting). Oxygen consumption was measured using the Seahorse XF96 equipment (Seahorse Bioscience Inc.). Typically, 100 worms per conditions were recovered from NGM plates with M9 medium, washed three times in 2 mL M9 to eliminate residual bacteria, and resuspended in 500 µL M9 medium. Worms were transferred in 96-well standard Seahorse plates (#100777-004) (10 worms per well) and oxygen consumption was measured 6 times. Respiration rates were normalized to the number of worms in each individual well. *C. elegans* movement was recorded for 45 seconds at days 1, 3, and 5 of adulthood using a Nikon DS-L2 / DS-Fi1 camera and controller setup, attached to both a computer and a standard bright field microscope. Seven plates of worms, with 10 worms per plate, were measured in each condition. The movement of worms during aging was calculated by following the worm centroids using a modified version of the freely-available for the Parallel Worm Tracker for MATLAB (Ramot et al., 2008).

DNA and cellular damage

Liver DNA isolation, liquid chromatography tandem mass spectrometry (LC/MS-MS) detection of 8-oxo-dG lesions and the quantification were performed using materials and method described previously (Taghizadeh et al., 2008). Lipid peroxidation was evaluated by assessing 4-hydroxy-2-nonenal content in gastrocnemius muscles using the kit from Cayman chemicals.

Enzyme activity measurements

Total Poly(ADP-ribose) activity was analyzed in tissue lysates using the Universal PARP Colorimetric Assay (Trevigen). CS activity was determined in tissue or cell homogenates using the CS Assay (Sigma).

NAD^+ determination

NAD^+ was extracted using acidic extraction method (Yang and Sauve, 2006) and analyzed with mass-spectrometry as described (Yang and Sauve, 2006).

Fatty acid oxidation measurement in tissue homogenates and cells

Oleic acid oxidation in muscle homogenates and cells was determined as published (Hirschey et al., 2010; Manning et al., 1990), respectively.

Western blotting

Tissues were lysed in lysis buffer (50 mM Tris, 150 mM KCl, EDTA 1 mM, NP40 1%, nicotinamide 5mM, sodiumbutyrate 1mM, protease inhibitors pH 7,4). Proteins were separated by SDS-PAGE and transferred onto nitrocellulose membranes. Antibody incubations were performed in 5% milk. SIRT1, MTCOI and SDHA antibodies were from Abcam; Anti-FOXO1 antibody was from Cell Signaling; PAR antibody was from Millipore; Tubulin and ClpP antibodies were from Sigma Inc, and Hsp90, actin, anti-acetyl-FRKH(FOXO) and Hsp60 antibodies were from Santa Cruz Inc. Antibody cocktail (the Mitoprofile Total OXPHOS Rodent WB Antibody Cocktail) for mitochondrial subunits was purchased from Mitosciences. The MHCI and MHCIIb antibodies were produced using hybridomas from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242. Antibody detection reactions were developed by enhanced chemiluminescence (*Advansta,* CA, USA) using x-ray films.

Immunoprecipitation

Muscle acetylated proteins were immunoprecipitated with anti-acetyl lysine agarose (ImmuneChem Pharmaceuticals Inc) following manufacturer's protocol. The acetylation level of SIRT3 target, Ndufa9, was detected from immunoprecipitates via

immunoblotting using antibody from Abcam. Input samples were probed with Ndufa9 and tubulin (Sigma Inc) antibodies and used as loading controls.

Histology

Succinate dehydrogenase and cytochrome c oxidase stainings were performed as described (Yamamoto et al., 2011). Gastrocnemius muscle cryosections were immunostained to detect MHCI using MHCI antibody described above.

Mitochondria DNA and gene expression analyses

Muscle total DNA and RNA were extracted either using proteinase K chloroform/phenol/isoamyl alcohol or TRIzol extraction, respectively. Mitochondrial DNA (mtDNA) was quantitated by measuring the ratio of a mitochondrial gene (16S RNA) to a nuclear gene (UCP2). Total RNA was transcribed to cDNA using QuantiTect Reverse Transcription Kit. Expression of selected genes was analyzed using The LightCycler480 System and SYBR Green chemistry. Data was normalized using 36B4. Primer sets for both quantitative real-time PCR analyses are shown below:

Gene	**Forward primer**	**Reverse primer**
16S	CCGCAAGGGAAAGATGAAAGAC	TCGTTTGGTTTCGGGGTTTC
UCP2	CTACAGATGTGGTAAAGGTCCGC	GCAATGGTCTTGTAGGCTTCG
36B4	AGATTCGGGATATGCTGTTGG	AAAGCCTGGAAGAAGGAGGTC
Sirt1	GTCTCCTGTGGGATTCCTGA	ACACAGAGACGGCTGGAACT
Tfam	AAGTGTTTTTCCAGCATGGG	GGCTGCAATTTTCCTAACCA
c-myc	TGAGCCCCTAGTGCTGCAT	AGCCCGACTCCGACCTCTT
Ndufa2	GCACACATTTCCCCACACTG	CCCAACCTGCCCATTCTGAT
Ndufb3	TACCACAAACGCAGCAAACC	AAGGGACGCCATTAGAAACG
SDHA	GGAACACTCCAAAAACAGACCT	CCACCACTGGGTATTGAGTAGAA

mt-COXII	CACTCATGAGCAGTCCCCTC	ACCCTGGTCGGTTTGATGTT
COX4	GGAACACTCCAAAAACAGACCT	GCAGTGAAGCCGATGAAGAAC
mt-ATP8	CAAACATTCCCACTGGCACC	TTGTTGGGGTAATGAATGAGGCA
ATP5g1	GCTGCTTGAGAGATGGGTTC	AGTTGGTGTGGCTGGATCA

Blue-Native Page

Blue-Native Page was performed using the Native-PAGE™ Novex® Bis-Tris Gel system (Invitrogen). Briefly, 50ug of isolated mitochondria (Frezza et al., 2007) was solubilized using Native-PAGE sample buffer with 0.5% n-dodecyl-β-D-maltoside (Invitrogen) and complexes were separated using a Native-PAGE Novex 3–12% Bis-Tris gel (Invitrogen). After running, the gel was transferred to a PVDF membrane using the iBlot TM Gel Transfer System (Invitrogen). The membrane was fixed with 8% acetic acid. After overnight drying, the membrane was destained with methanol. To detect OXPHOS complex, the Mitoprofile Total OXPHOS Rodent WB Antibody Cocktail (Mitosciences) was used. After incubation in the primary antibody dilution, the membrane was washed and detected using the Western Breeze® Chromogenic Western Blot Immunodetection Kit (Invitrogen).

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