Supplemental Information

NAD+-Dependent Activation
of Sirt1 Corrects the Phenotype
in a Mouse Model of Mitochondrial Disease

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**Supplemental Material Online**

Reagents and materials

Anti COI, COX5a, Ndufa9, SDH-HA; Core 2 were from Invitrogen; anti-GAPDH was from Millipore; anti-FOXO1 and anti-acetylated-FOXO1 were from Cell Signaling and Santa Cruz, respectively. Anti-mouse secondary antibodies were from Amersham. Chemicals were from Sigma. Nicotinamide Riboside was custom synthesized by Novalix, France. MRLB-45696 was kindly provided by Merck. PJ34 was from Tocris Bioscience. Oligonucleotides were from PRIMM, Italy. The sequences of all the primers used in this study are available on request.

NR was dissolved in water and added to a standard powder diet (Mucedola, Italy) at a concentration of 400 mg/Kg/day; MRLB-45696 was dissolved in DMSO and added to a standard powder diet at 50 mg/Kg/day were added to a standard diet; pellets containing the drugs or the vehicles were reconstituted by hand and kept frozen at -20°C until needed. The diet supply was changed every three days, and only the amount needed was thawed at each time and administered *ad libitum* for one month. PJ-34 was dissolved in DMSO, diluted in saline and administered for one month by ip injection 10 mg/Kg, b.i.d. Control animals were injected with solution containing the vehicle.

Figure S1 related to Figure 1. *Metabolic features of Sco2^{KOKI}-Parp1^{-/-} double mutants*
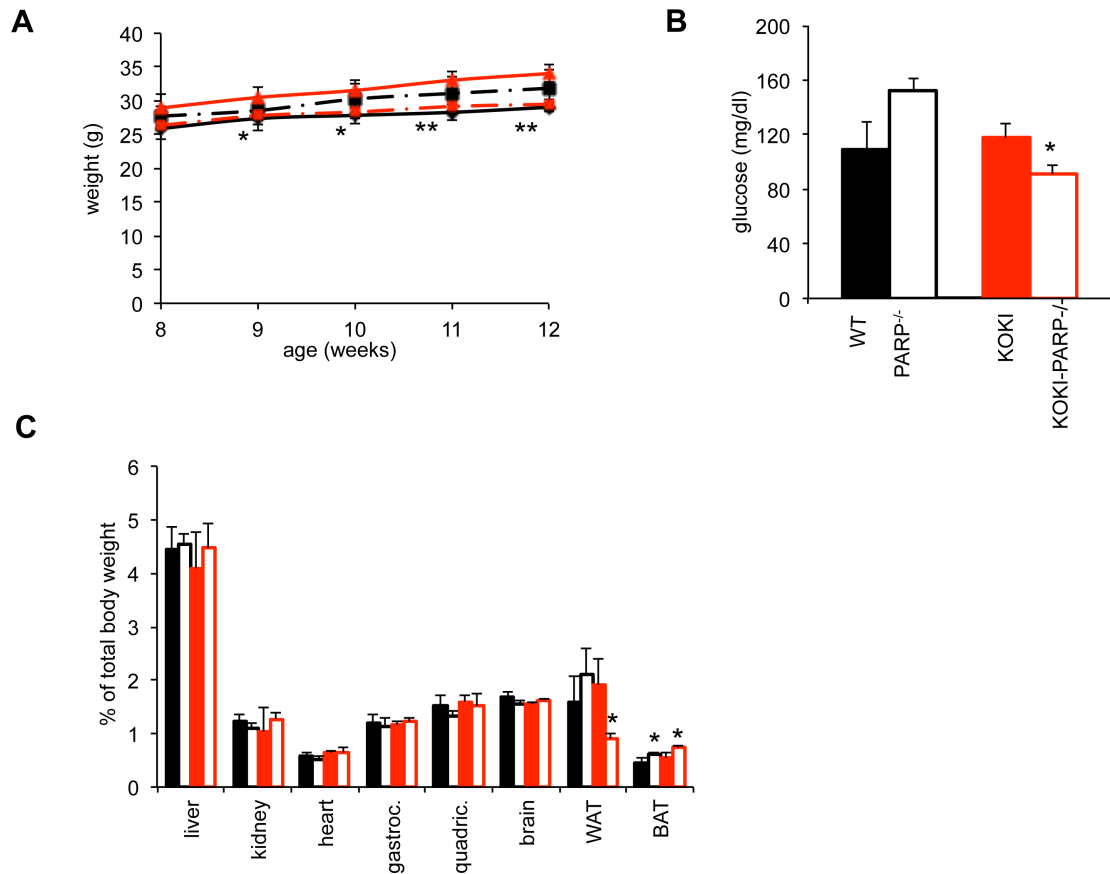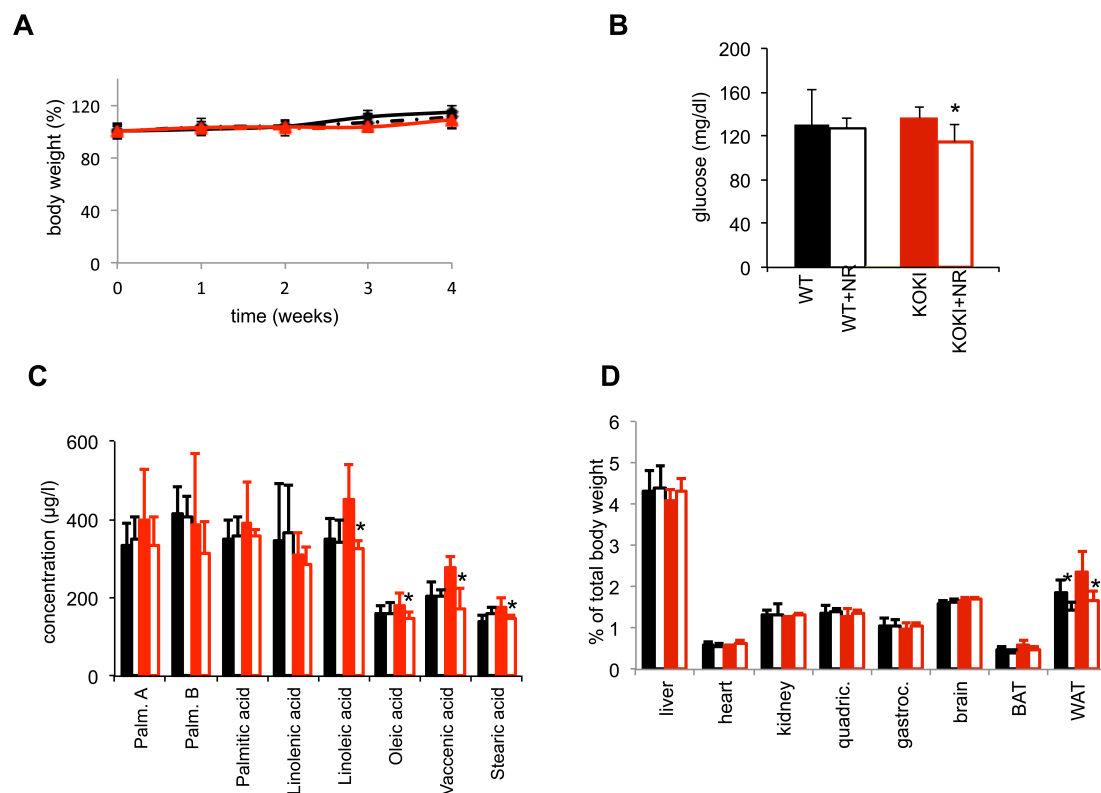

A) Body weight. Solid black: WT; dashed black: Parp1$^{-/-}$; dashed red: Parp1$^{-/-}$-Sco2^{KOKI}, solid red: Sco2^{KOKI}. Error bars represent the standard deviation (SD). The asterisks represent the significance levels calculated by unpaired, two-tail Student's t test: *p<0.05; **p<0.01.

B) Fasting glucose. Fasting glucose analysis. Solid black: WT; black outline: Parp1$^{-/-}$; solid red: Sco2^{KOKI}, red outline: Parp1$^{-/-}$-Sco2^{KOKI}. Unpaired Student's t test *p<0.05

C) Organ weights/Body weight. Note that white adipose tissue is significantly reduced in Sco2^{KOKI} mice upon NR-treatment. Color codes as in B. Unpaired, two-tail Student's t test: *p<0.05

Figure S2 related to Figure 2. *Metabolic effects of a one-month treatment with NR*

A) Body weight. Solid black: vehicle-treated WT; black outline: NR-treated WT; solid red: vehicle-treated Sco2^{KOKI}, red outline: Sco2^{KOKI}. Error bars represent the standard deviation (SD).

B) Fasting glucose. Solid black: vehicle-treated WT; black outline: NR-treated WT; solid red: vehicle-treated Sco2^{KOKI}, red outline: NR-treated Sco2^{KOKI}.

C) Total fatty acids analysis. Color codes as in B. The asterisks represent the significance levels calculated by unpaired, two-tail Student's t test: *p<0.05. Note that
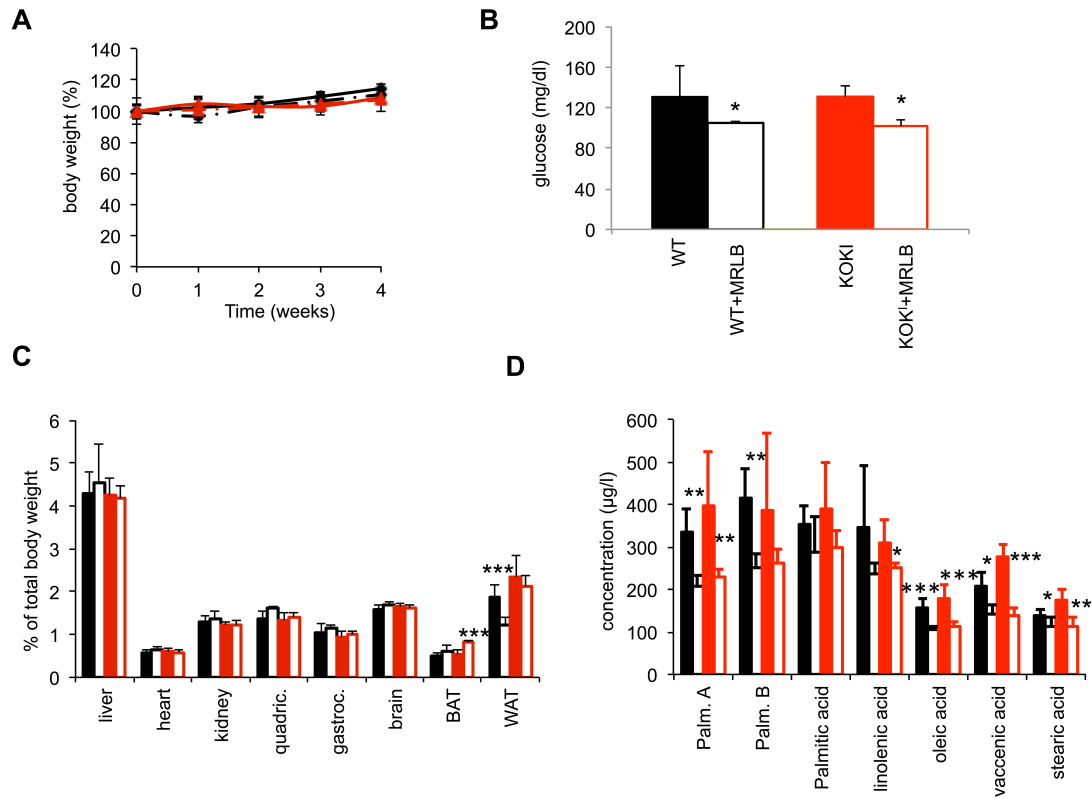
the values of pal. A has been multiplied by 100, and that of palm B and vaccenic acid by 10 for visualization clarity.

D) Organ weights/Body weight. Note that white adipose tissue is significantly reduced in Sco2^{KOKI} mice upon NR-treatment. Color codes as in A.

Error bars represent the standard deviation (SD); unpaired, two-tail Student's t test: *$p<0.05$.

Figure S3 related to Figure 3. *Metabolic effects of one-month treatment with MRLB-45696*

A) Body weight. Solid black: vehicle-treated WT; dashed black: MRLB-45696 treated WT; solid red: vehicle-treated $Sco2^{KOKI}$, red outline: MRLB-45696-treated $Sco2^{KOKI}$. Error bars represent the standard deviation (SD).

B) Fasting glucose analysis. Color codes as in A. Unpaired, two-tail Student's t test: *$p<0.05$.

C) Organ weights/Body weight. Note that white adipose tissue is significantly reduced in Sco2^{KOKI} mice upon MRLB-45696 treatment. Color codes as in A. Unpaired, two-tail Student's t test: ***$p<0.001$.

D) Total fatty acids analysis. Color codes as in B. Note that the values of pal. A has been multiplied by 100, and that of palm B and vaccenic acid by 10 for visualization

clarity. The asterisks represent the significance levels calculated by unpaired, two-tail

Student's t test: *p<0.05; **p<0.01; ***p<0.001.

Figure S4 related to Figure 4. *Effect of PJ34 on skeletal muscle*
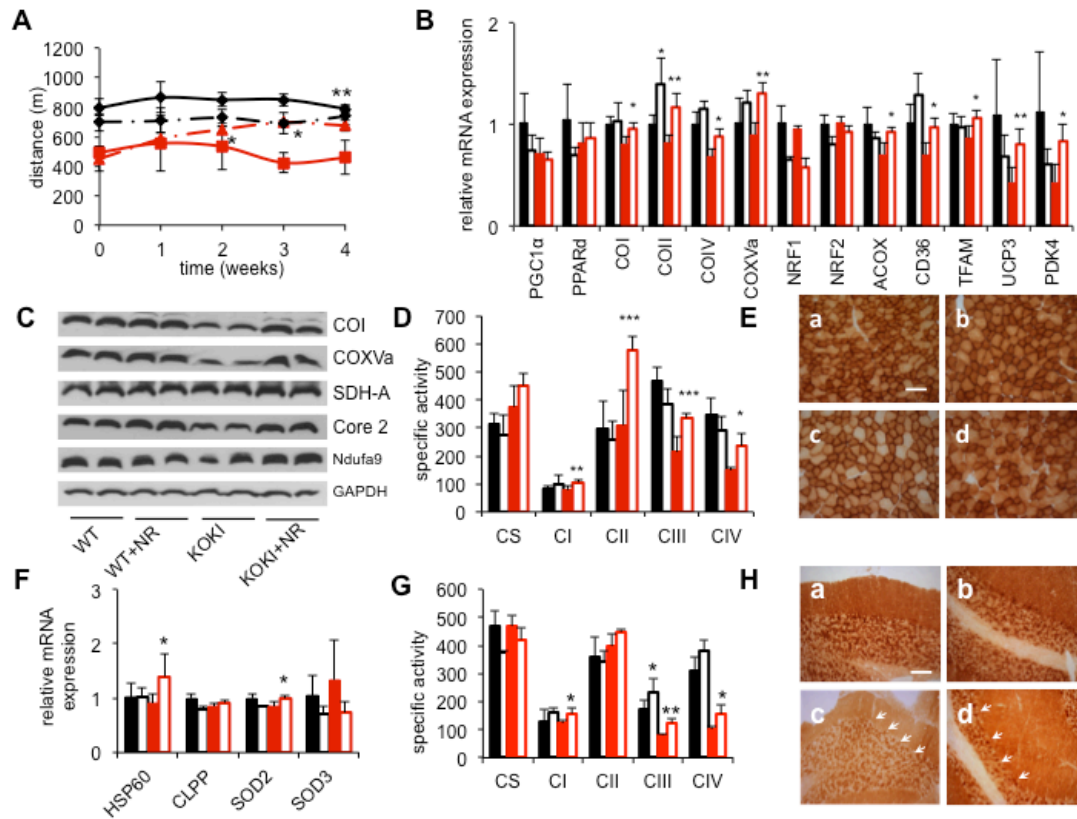
A) Treadmill analysis of motor performance. Solid black: vehicle-treated WT; dashed black: PJ34-treated WT; dashed red: vehicle-treated $Sco2^{KOKI}$, solid red: vehicle-treated $Sco2^{KOKI}$; dashed red: PJ34-treated $Sco2^{KOKI}$.

B) Expression analysis of FAO- and OXPHOS-related genes in $Sco2^{KOKI}$ and WT muscles of PJ34-treated and vehicle-treated mice. Solid black: vehicle-treated WT; dashed black: PJ34-treated WT; solid red: vehicle-treated $Sco2^{KOKI}$, red outline: PJ34-treated $Sco2^{KOKI}$.

C) Western-blot immunovisualization of COX1, COXVa, SDH 70KDa, Core 2, 39 Ka complex I subunit proteins in skeletal muscle of PJ34-treated and vehicle-treated mice of the different genotypes. GAPDH was taken as loading control.

D) MRC activities (nmoles/min/mg of protein). Color codes as in B. CS: citrate synthase; CI-IV, MRC complexes I-IV. Note that the activity of cII has been

multiplied by 10 for visualization clarity. Error bars represent the SD. Unpaired, two-tail Student's t test: **p<0.01; ***p<0.001.

E) COX staining in muscles from PJ34-treated and vehicle-treated $Sco2^{KOKI}$ and WT muscles. Scale bar: 100 μm.

F) COX staining in skeletal muscles of PJ34-treated and vehicle-treated $Sco2^{KOKI}$ and WT mice. (a) vehicle-treated WT; (b) PJ34-treated WT; (c) vehicle-treated $Sco2^{KOKI}$; (d) PJ34-treated $Sco2^{KOKI}$. Scale bar: 100 μm.

G) mRNA expression analysis of mtUPR genes *Hsp60*, *Clpp* and Sod2 in *Sco2KOKI* and WT muscles of PJ34-treated and vehicle-treated mice. *Sod3* was taken as a non mtUPR related stress protein. Color codes as in D. The levels of the gene transcripts, retro-transcribed into cDNA, were normalized to that of the Hprt gene transcript, taken as a standard, and expressed as time-fold variation relative to the WT. Error bars represent SD. Unpaired, two-tail Student's t test: **p<0.01.

F) MRC activities (nmoles/min/mg of protein). Solid black: vehicle-treated WT; black outline: PJ34-treated WT, solid red: vehicle-treated Sco2^{KOKI}, red outline: PJ34-treated Sco2^{KOKI}. CS: citrate *synthase*; CI-IV, MRC complexes I-IV. Note that the activity of cII has been multiplied by 10 for visualization clarity. Error bars represent the SD. The asterisks represent the significance levels calculated by unpaired, two-tail Student's t test: *p<0.05; **p<0.01.

G) COX staining. The arrows indicate Purkinje cells showing increased COX activity. Scale bar: 100 μm.

Supplemental Tables

Table S1 related to Figure 1. Specific activities of citrate synthase (CS) and respiratory chain complexes (CI-IV) in skeletal muscle from WT, Parp1$^{-/-}$, Sco2^{KOKI} and Sco2^{KOKI}-Parp1$^{-/-}$ mice (n. 4). Values are expressed as nanomoles/min/mg protein.

	CS	CI	CII	CIII	CIV
WT	296±39	75±6	375±47	446±78	325±72
PARP$^{-/-}$	374±29	74±18	265±92	453±98	419±52
SCO2^{KOKI}	437±30	86±4	350±87	286±41	130±24
SCO2^{KOKI} – PARP$^{-/-}$	492±113	108±3***	530±20*	433±24**	238±37**

* Student's *t* test p<0.05
** Student's *t* test p<0.01
*** Student's *t* test p<0.005

Table S2 related to Figure 1. Specific activities of citrate synthase (CS) and respiratory chain complexes (CI-IV) in brain from WT, Parp1$^{-/-}$, Sco2^{KOKI} and Sco2^{KOKI}-Parp1$^{-/-}$ mice (n. 4). Values are expressed as nanomoles/min/mg protein.

	CS	CI	CII	CIII	CIV
WT	420±41	97±11	340±18	134±13	296±19
PARP$^{-/-}$	488±42	102±3	430±10***	132±22	303±37
SCO2^{KOKI}	477±47	106±4	318±26	70±10	93±21
SCO2^{KOKI} – PARP$^{-/-}$	452±65	132±11**	417±29**	107±5**	156±3**

** Student's *t* test p<0.01
*** Student's *t* test p<0.005

Table S3 related to Figure 2 and 3: Specific activities of citrate synthase (CS) and respiratory chain complexes (CI-IV) in skeletal muscle from WT and Sco2^{KOKI} mice treated with NR, MRLB-45696 or PJ-34 (n. 4). Values are expressed as nanomoles/min/mg protein.

	CS	CI	CII	CIII	CIV
WT	316±34	80±14	294±103	469±49	346±62
WT - NR	306±44	77±8	343±78	422±67	324±52
WT - MRL	300±69	91±25	345±93	522±76	371±56
WT - PJ34	273±74	96±34	260±85	386±57	291±51
SCO2^{KOKI}	375±76	84±4	342±99	229±41	153±6
SCO2^{KOKI}-NR	400±36	93±4**	540±55***	394±22***	231±11***
SCO2^{KOKI} - MRL	331±13	132±11***	542±79**	554±35***	206±18***
SCO2^{KOKI} - PJ34	452±45	106±9**	580±49***	336±19***	235±47*

* Student's *t* test $p < 0.05$
** Student's *t* test $p < 0.01$
*** Student's *t* test $p < 0.005$

Table S4 related to Figure 4: Specific activities of citrate synthase (CS) and respiratory chain complexes (CI-IV) in brain from WT and Sco2^{KOKI} mice treated with NR, MRLB-45696 or PJ-34 (n. 4). Values are expressed as nanomoles/min/mg protein.

	CS	CI	CII	CIII	CIV
WT	468±53	126±47	360±70	171±33	311±50
WT - NR	501±32	105±5	385±64	138±12	268±31
WT - MRL	383±40	139±28	302±53	187±26	309±19
WT - PJ34	374±74	164±7	345±68	233±31*	380±48
SCO2^{KOKI}	435±83	120±11	410±48	75±10	102±11
SCO2^{KOKI}-NR	455±32	143±14	443±25	89±18	108±25
SCO2^{KOKI} - MRL	340±17	124±7*	473±25	124±8***	128±14*
SCO2^{KOKI} - PJ34	421±40	157±23*	448±10	125±14**	156±32

* Student's *t* test p<0.05
** Student's *t* test p<0.01
*** Student's *t* test p<0.005