Supplementary Materials & Methods

Muscle or liver-specific Sirt3 deficiency induces hyperacetylation of mitochondrial proteins without affecting global metabolic homeostasis

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Animal Procedures. All mice were maintained in a temperature-controlled (23 °C) facility with a 12 hr light/dark cycle and were given free access to food and water. Regular chow diet and high-fat diet were obtained from Harlan (Madison, WI) or Research Diet (New Brunswick, NJ), respectively. The control diet (2016S) contained 22% calories from protein, 66 % carbohydrate and 12% fat, whereas the high-fat diet (D12492) contained 20 % protein, 20 % carbohydrate and 60 % fat (given in % of calories).

Indirect calorimetry to monitor O_2 consumption, CO_2 production, and measurement of activity was measured using Comprehensive Lab Animal Monitoring System (CLAMS) (Columbus Instruments, Columbus, OH)[1].

ipGTT ans iITT were performed in animals that were fasted overnight. Glucose was administered by intraperitoneal injection at a dose of 2 g/kg bodyweight. For iITT, Insulin was injected at a dose of 0.50 U/kg bodyweight. Glucose quantification was done with the Glucose RTU (bioMérieux Inc., Marcy l'Etoile, France). Plasma insulin concentrations were measured using ELISA for mouse (Cristal Chem Inc., Downers Grove, IL).

The systolic and diastolic blood pressure and heart rate was measured by a computerized tail-cuff system (BP-2000, Visitech Systems, Apex, NC) in conscious animals[2]. Following 10 preliminary measurements in pre-warmed tail cuff (36 °C) device to accustom mice to the procedure, 10 actual measurements cycles were collected on 5 consecutive days at fixed diurnal interval and averaged for each individual animal. As movement artifact could reduce the number of successful when 7 out of 10 measurements were valid with a standard deviation less than 10 mmHg.

HR was also monitored in the procedure. For each individual, the average value of BP and HR in the last 2 days was used for analysis.

For the cold test, mice were placed in individual pre-cooled cages in a room with a controlled temperature of 4 ± 1, and temperature was recorded every hour using a thermometer (BIO-TK9882, Bioseb, FRANCE) inserted with a rectal probe (RET-3, Physitemp Instruments Inc, USA).

For the endurance exercise protocol, 8-week-old non-fasted C57BL6/J male mice were subjected to a resistance running test, using a variable speed belt treadmill enclosed in a plexiglass chamber with a stimulus device consisting of a shock grid attached to the rear of the belt (Panlab). Animals were acclimatized to the chamber the day preceding the running test. For the habituation, mice run at 21 cm/s for 10 min with a 5º incline. For the actual test, we used a protocol at 5º incline where, beginning at 18 cm/s, speed increased gradually by 3 cm/s every 5 min. The distance run and the number of shocks were monitored during the test, and exhaustion was assumed when mice received more than 50 shocks in a 2.5 min interval. Mice were removed from the treadmill on exhaustion.

Primer sequences.

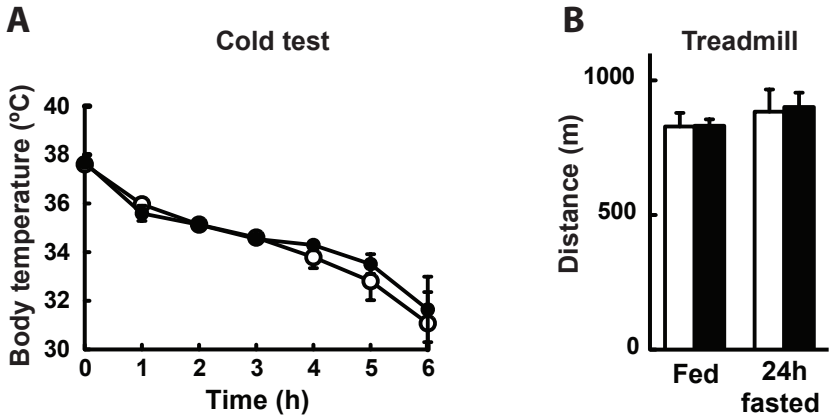
Gene	**Forward**	**Reverse**
Sirt3	AGGTGGAGGAAGCAGTGAGA	GCTTGGGGTTGTGAAAGAAA
PPARd	CTCTTCATCGCGGCCATCATTCT	TCTGCCATCTTCTGCAGCAGCTT
FoxO1	AAGGATAAGGGCGACAGCAA	TCCACCAAGAACTCTTTCCA
ERRa	ACTGCCACTGCAGGATGAG	CACAGCCTCAGCATCTTCAA
Glut1	ACTGGGCAAGTCCTTTGAGA	GTCTAAGCCAAACACCTGGGC

HK2	CCGCCGTGGTGGACAAGATA	AGCAGTGATGAGAGCCGCTC
Pdk1	GGTCCAGTGGATAAGCGAAA	TTTCTGCACCACTTGTGAGC
Sirt4	CGAGCAAAAGCTCCCAATAG	GTTCATTTCCAGCCTTTGGA
Sirt5	CCACCGACAGATTCAGGTTT	TTCCCGTTAGTGCCCTGCTTTA
Lcad	GTAGCTTATGAATGTGTGCAACTC	GTCTTGCGATCAGCTCTTTCATTA
Mcad	GATCGCAATGGGTGCTTTTGATAGAA	AGCTGATTGGCAATGTCTCCAGCAAA
Cpt1b	CCCATGTGCTCCTACCAGAT	CCTTGAAGAAGCGACCTTTG
Pfkfb3	TCATGGAATAGAGCGCC	GTGTGCTCACCGATTCTACA
Sdhb	GGACCTATGGTGTTGGATGC	GTGTGCACGCCAGAGTATTG
Ndufa2	GCACACATTTCCCCACACTG	CCCAACCTGCCCATTCTGAT
CytC	TCCATCAGGGTATCCTCTCC	GGAGGCAAGCATAAGACTGG
Ucp3	ACTCCAGCGTCGCCATCAGGATTCT	TAAACAGGTGAGACTCCAGCAACTT
Myhc I	CCAAGGGCCTGAATGAGGAG	GCAAAGGCTCCAGGTCTGAG
Myhc IIa	AAGCGAAGAGTAAGGCTGTC	GTGATTGCTTGCAAAGGAAC
Myhc IIb	ACAAGCTGCGGGTGAAGAGC	CAGGACAGTGACAAAGAACG
Pgc1α	AAGTGTGGAACTCTCTGGAACTG	GGGTTATCTTGGTTGGCTTTATG
Scd1	GAGGCCTGTACGGGATCATA	CCGAAGAGGCAGGTGTAGAG

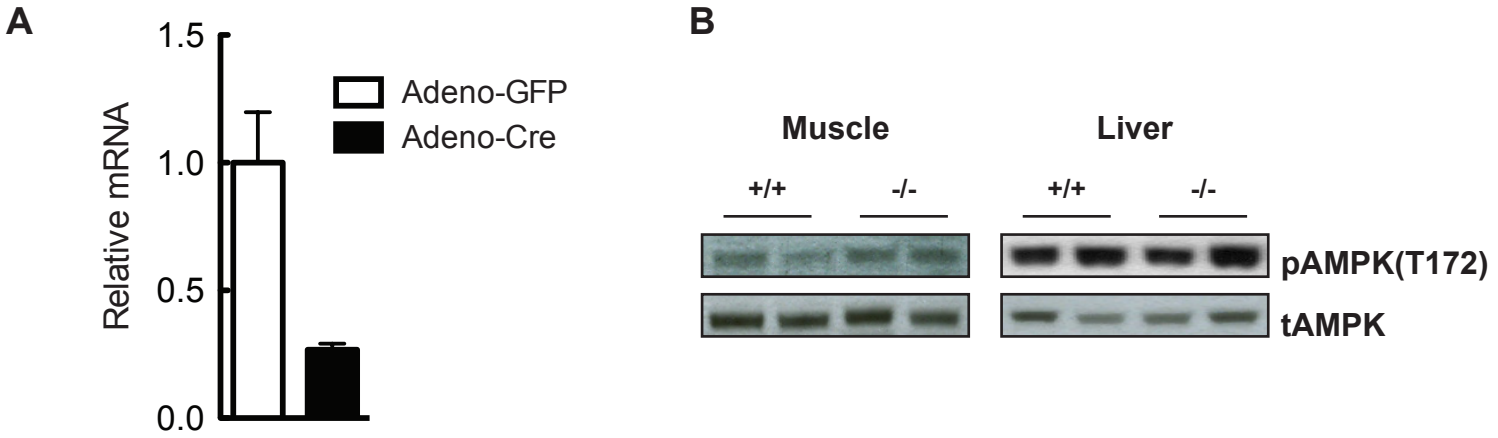
Supplemental References

1 Watanabe, M. *et al.* Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 439, 484-489,(2006).

2 Koutnikova, H. *et al.* Identification of the UBP1 locus as a critical blood pressure determinant using a combination of mouse and human genetics. *PLoS Genet* 5, e1000591,(2009).



Supplementary Figure 1. Comes from Fig.3. Absence of *Sirt3* specifically in muscle does not affect adaptive thermogenesis in fed state or muscle endurance after 24h fasting. (A) *Sirt3*skm+/+ and *Sirt3*skm-/- mice on HFD for 14 weeks were exposed to 4ºC for 6 h and rectal temperature was monitored. (B) Endurance exercise test on *Sirt3*skm+/+ and *Sirt3*skm-/- mice before and after a 24h fast. Results are presented as mean values (n=8-10 mice for each group)± SEM.



Supplementary Figure 2. (Comes from Figure 4). (A) Levels of *Sirt3* mRNA were quantified from *Sirt3*$^{L2/L2}$ primary hepatocytes after Adeno-Cre infection. **(B)** Muscle and liver lysates from *Sirt3*Skm or *Sirt3*Hep mice subjected to HFD were blotted against the indicated proteins.