Supporting Information

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SI Materials and Methods

Animals were maintained in a temperature-controlled (23° C) facility with a 12 h light/dark cycle. Mice had ad libitum access to water and either regular rodent chow (DO4, UAR, France) or HF diet (D12327, Research Diet). Body weight was recorded every week and food intake was measured every second day for 15 consecutive days.

RNA and DNA Analysis. RNA was extracted from tissues or cultured cells using the TRIzol reagent (Invitrogen), following the manufacturer's instructions(1). cDNA were synthesized according the instructions of the SuperScript II Reverse Transcriptase kit (Invitrogen). Quantitative RT-PCR were performed on a LightCycler 480, using LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics)(2). The sequences of primers used are available at www-igbmc.ustrasbg.fr/Departments/Dep_V/Dep_VA/Publi/Paper.html. 18s transcript was used as the invariant control. MtDNA content was measured exactly as described in ref. 3.

Immunoblotting and immunoprecipitations. Protein extracts were separated by SDS/PAGE and immunoblotted using antibodies against PGC-1 (H-300; Santa Cruz Biotechnology), HA (H 3663; Sigma, FR), SRC-3 (BD Biosciences), GCN5 (SC-20698; Santa Cruz Biotechnology), SIRT1 (anti-Sir2; Upstate) and α-Actin (SC-1616; Santa Cruz). PGC-1α acetylation was analyzed by immunoprecipitation of PGC-1 from nuclear lysates (50 αg) with anti-PGC-1 antibody (1 αg), or alternatively with a anti-HA antibody (1 αg), coupled to protein A Sepharose beads (CL-4B; Amersham). This was followed by an immunoblot using an anti-acetylated lysine antibody (Cell Signaling). ChIP analyses in C2C12 cells were performed using an anti-SRC-3 antibody (BD Biosciences). PCR amplification was carried out for 30 cycles with 2 αl of sample DNA solution and PCR products were separated on 1.5% agarose gel. Three sets of primers were used to amplify three different regions of the GCN5 mouse promoter (Fig. 5*D*): region A: forward 5′- GACTGGCCTGTTT-GAGCTTC -3′; reverse 5′- TTGCTGAACGGAATCAAGTG -3,' region B: forward 5′- TAGGGGTCTTCTCAGCTTGC -3′; reverse 5′- TCACAGCTACGGCACAACTC-3′;.and region C: forward 5′-TAGCAGGCTGACCTTTGCTT-3′; reverse 5′-CTGGACCAGGAGTCAGGAAA-3′

Cell Culture,Ttransient Transfections and Adenoviral Infections: *SRC-3 Impact on PGC-1 Acetylation.* SRC-3$^{-/-}$ vs. $^{+/+}$ MEFs, prepared from embryos at E13.5 and self-immortalized (4), were infected with either the Ad-Flag-HA-PGC-1α or the control Ad-Flag-HA-GFP adenovirus. After 36h MEFs were incubated in the presence of 20 mM nicotinamide 16h before harvesting and analysis. SRC-3 siRNA (siGenome Smart; Dharmacon) and control siRNA (siControl Non-Targetting; Dharmacon) were transfected into C2C12 myoblasts using Lipofectamine 2000 Reagent (Invitrogen). C2C12 myoblasts were then differentiated into myotubes by serum deprivation and 24 h after transfection, myotubes were infected overnight with the Ad-Flag-HA-PGC-1α or the Ad-Flag-HA-GFP adenovirus.

Impact of SRC-3 on GCN5-mediated PGC-1α Acetylation. C2C12 cells were infected with adenoviruses encoding PGC-1α, GCN5 and/or SRC-3. PGC-1α acetylation and protein levels were analyzed as described previously.

GCN5 Promoter Analysis. C2C12 myoblasts were cotransfected, using Transfectin Reagent (Bio-Rad), with either pCR3.1 control or pCR3.1-SRC-3 expression vectors and a luciferase reporter contruct that was under the control of a specific fragment of the mouse GCN5 promoter (−1760/−1690 bp). The cells were harvested 48 h after transfection and assayed for luciferase activity.

1. Rocchi S, *et al.* (2002) A Unique PPARgamma ligand with potent insulin-sensitizing yet weak adipogenic activity. *Mol Cell* 8(4):737–747.
2. Watanabe M, *et al.* (2004) Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *J Clin Invest* 113(10):1408–1418.
3. Lagouge M, *et al.* (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* 127:1109–1122.
4. Picard F, *et al*. (2002) SRC-1 and TIF2 control energy balance between white and brown adipose tissues. *Cell* 111:931–941.

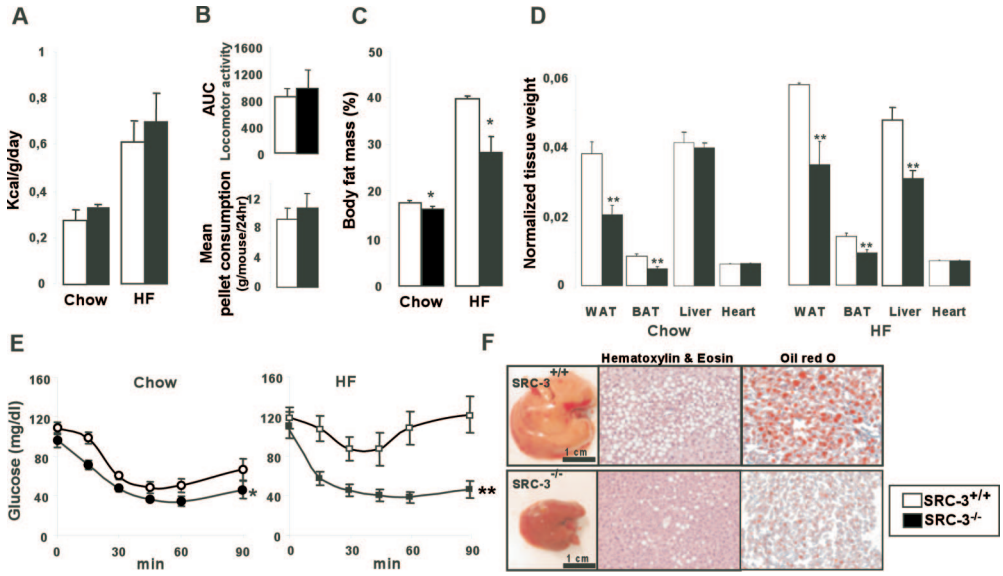

Fig. S1. (*A*) Caloric intake expressed as kcal of food per gram of body weight per day. The graph represents the mean of 15 measurements (*B*) Average area under the curve (AUC) calculated from the individual monitorings of spontaneous locomotor activity in a cohort of SRC-$3^{+/+}$ and SRC-$3^{-/-}$ mice fed a high fat diet, during the last 24 h of the experiment (top graph) and average food consumption in gram per mouse, also over the last 24 h (bottom graph) ($n = 6$). (*C*) Body fat mass, evaluated in anesthetized 22 week old male SRC-$3^{+/+}$ and SRC-$3^{-/-}$ mice either fed a regular chow or high fat diet by dual energy X-ray absorptiometry ($n = 8$). (*D*) Epididymal white adipose tissue (WAT), brown adipose tissue (BAT), liver and heart weights, measured at sacrifice, in male SRC-$3^{+/+}$ and SRC-$3^{-/-}$ mice fed a regular chow diet or high fat diet and normalized to body weight ($n = 8$). (*E*) Serum glucose levels evolution during an intraperitoneal insulin tolerance test performed after an acute injection of insulin (0.75 UI/kg) in overnight fasted chow or high fat (HF) fed SRC-$3^{+/+}$ and SRC-$3^{-/-}$ mice ($n = 8$). (*F*) Representative macroscopic and microscopic liver images (hematoxylin and eosin and oil red O stainings) of 22-week-old male SRC-$3^{+/+}$ and SRC-$3^{-/-}$ mice, fed a high fat diet for 12 weeks. Note the more extensive vacuolization in SRC-$3^{+/+}$ mice reflecting lipid accumulation. *, $P < 0.05$ and **, $P < 0.01$ indicate significant differences as compared to SRC-$3^{+/+}$ mice.

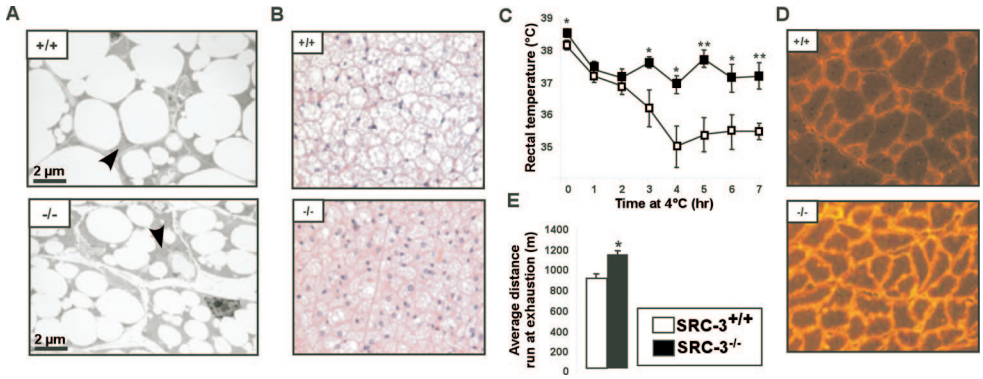

Fig. S2. (*A*) Representative pictures (magnification 5000X) of the brown adipose tissue of SRC-$3^{+/+}$ and SRC-$3^{-/-}$ mice fed a high fat (HF) diet, analyzed by electron microscopy. Mitochondria are indicated by arrows. (*B*) Hematoxylin and eosin staining of brown adipose tissue sections (magnification 20X) of representative 22-week-old SRC $3^{+/+}$ and SRC-$3^{-/-}$ mice fed a high fat diet. (*C*) Evolution of rectal temperaturein mice fed a HF diet during 7h at 4°C ($n = 8$) (*D*) Immunohistochemical detection of the specific mitochondrial GTPase protein (OPA-1), using an anti-OPA1 antibody on gastrocnemius muscle sections of SRC-$3^{+/+}$ and SRC-$3^{-/-}$ mice fed a high fat diet. (*E*) Average distance run until exhaustion of SRC-$3^{+/+}$ and SRC-$3^{-/-}$ mice fed a regular chow.

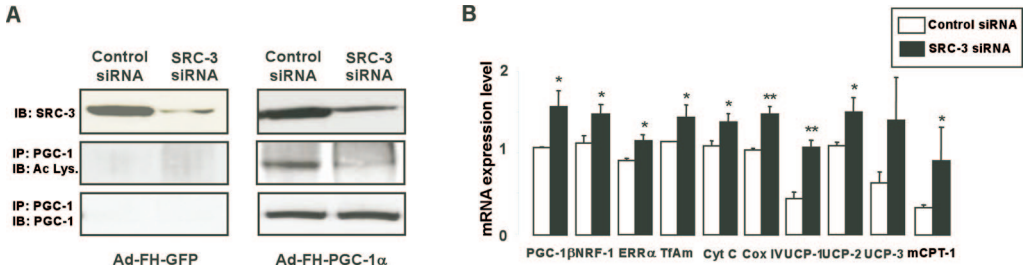

Fig. S3. (*A*) PGC-1 acetylation status in C2C12 cells infected with either a control adenovirus expressing GFP (*Left*) or an adenovirus expressing PGC-1α (*Right*). Prior to adenoviral infection, cells were transfected with control or SRC-3 specific siRNA. PGC-1 was first immunoprecipitated (IP) using an anti-PGC-1 antibody and its acetylation level was then detected through an immunoblot (IB) using an anti-acetylated lysine antibody (Ac Lys.). Total immunoprecipitated PGC-1 was detected using an anti-PGC-1 antibody. (*B*) mRNA expression levels PGC-1β, NRF-1, ERRα, Tfam, Cyt C, Cox IV, UCP-1, UCP-2, UCP-3 and mCPT-1 in C2C12 cells infected with Ad-FH-PGC-1α adenovirus and transfected as described in A with a control siRNA or a SRC-3 siRNA ($n = 6$). *, $P < 0.05$ and **, $P < 0.01$ indicate significant differences as compared to SRC-$3^{+/+}$ mice.

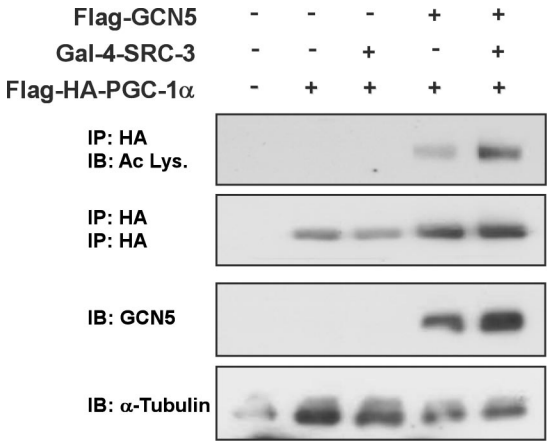

Fig. S4. PGC-1α acetylation levels determined in extracts of HEK293 cells transfected with PGC-1α, GCN5 and/or SRC-3 expression vectors. Tubulin is used as a loading control. PGC-1α was immunoprecipitated (IP) using an anti-HA antibody. Its acetylation level was detected through an immunoblot (IB) using an anti-acetylated lysine antibody (Ac Lys.). Total immunoprecipitated PGC-1α was detected using an anti-HA antibody.