

Supplemental experimental Procedures

Database searches and sequence analysis

Human exons 10 to 12 and intron 10 were conserved only in placentals (Euarchontoglires; Afrotheria; Laurasiatheria; Xenarthra) and marsupials (Diprotodontia : Wallaby [*Macropus eugenii*]; Didelphimorphia: Opossum [*Monodelphis domestica*]; Dasyuromorphia: Tasmanian devil [*Sarcophilus harrisii*]). Thus, we only examined species from these taxons.

The accession numbers for placentals are as follow. Euarchontoglires (Primates, *Homo sapiens*, ENSG00000160789; Glires, *Mus musculus*, ENSMUSG00000028063; Scandentia, *Tupaia belangeri* (tree shrew), ENSTBEG00000005069); Afrotheria (Proboscidae, *Loxodonta africana* (elephant), ENSLAFG00000002832; Hyracoidea, *Procavia capensis*, ENSPCAG00000012533); Laurasiatheria (Insectivora, *Sorex araneus* (shrew), ENSSARG00000000613; Chiroptera, *Myotis lucifugus* (bat), ENSMLUG00000026412); Xenarthra (Pilosa, *Choloepus hoffmani* (sloth), ENSCHOG00000007028; Cingulata, *Dasyus novemcinctus* (armadillo), ENSDNOG00000010869).

The accession numbers for marsupials are as follow. Diprotodontia (Macropodidae, *Macropus eugenii* (wallaby), ENSMEUG00000006464; Phalangeridae, *Trichosurus vulpecula* (common brush-tail possum), EC333811.1); Didelphimorphia, *Monodelphis domestica* (opossum), ENSMODG00000016946; Dasyuromorphia, *Sarcophilus harrisii* (Tasmanian devil), ENSSHAG00000013712; Sauropsids, *Gallus gallus* (chicken), ENSGALG00000006083; Amphibia, *Xenopus tropicalis*, ENSXETG00000024161; Actinopterygii, *Danio rerio* (zebrafish), ENSDARG00000013415).

Animal experiments

Transgenic mice (LMNA^{G609G/+} and LMNA^{LCS/LCS}) were generated as previously described [1]. We obtained *Lmna*^{LCS/LCS} mice by intercrossing *Lmna*^{LCS/+} mice. We then generated *Lmna*^{G609G/+} mice by crossing *Lmna*^{LCS/LCS} mice with mice that ubiquitously expressed the CRE recombinase under the control of the cytomegalovirus (CMV) promoter. In order to avoid “genetic background issues”, we crossed *Lmna*^{LCS/LCS} mice with C57/BL6 mice that expressed CRE recombinase under the control of the CMV promoter or with C57BL6 wild-type (WT) mice. The respective F1 mice (*Lmna*^{G609G/+} and *Lmna*^{LCS/+}) were then intercrossed to generate F2 mice. F2 mice were studied in comparison to WT control littermates (*Lmna*^{+/+}), which were obtained from both crosses.

For the determination of the presence of tumor, mice were observed for the appearance of macroscopically detectable tumors. When tumor size caused the loss of 30% of the animal's body weight tumor-bearing mice were euthanatized, and tumors were dissected and processed for histological analysis.

Micro-computed Tomography

Adipose tissue quantification was performed with a SkyScan-1178 X-ray micro-computed tomography system. Mice were anesthetized and scanned with the following parameters: 104- μ m voxel size, 49 kV, 0.5-mm-thick aluminum filter, 0.9° rotation step. Total adipose tissue volume was determined between the lumbar vertebra 1 (L1) and the caudal vertebra 4 (C4), whereas intra-abdominal and subcutaneous adipose tissues areas were measured on one section at the lumbar 5 (L5) level. 3-D reconstructions and analyses of bone parameters and adipose tissue areas or volumes were performed with NRecon and CTA software (Skyscan).

Metabolic Analysis

For glucose tolerance test, 18h-fasted mice were injected i.p. with glucose (1.7g/kg) and glycemia were measured at time 0, 30, 60 and 90 min after injection. For insulin tolerance test, 8h-fasted mice were injected i.p. with insulin (0.75U/kg) and glucose level was measured at time 0, 20 and 40 min after injection.

Energy expenditure, oxygen consumption, carbon dioxide production, and RER were measured at 22 °C on a Comprehensive Lab Animal Monitoring System (Columbus Instruments, Columbus, OH). Male mice were acclimatized individually in metabolic cages with *ad libitum* access to standard chow and water for 24 hours prior to a 24-hour period of automated recordings. Air samples from individual cages were passed through sensors to determine O₂ and CO₂ content. Experiments were performed in five animals per genotype.

Serum profile

For serum lipid profile analysis, we use serum collected by cardiac puncture. The analysis was performed by the Genotoul Anexplor platform (Toulouse, France) on a COBAS-MIRA+ biochemical analyser.

Western blot Analysis

Mouse perigonadal white adipose tissue samples were snap-frozen in liquid nitrogen immediately after collection. Samples were then reduced into powder form with a tissue grinder, homogenized, and dissolved at 150 mg/ml in a buffer consisting of 63 mM Tris-HCl (pH 7.5), 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, and 8 M urea. Samples were stored at -80 °C. Protein extracts were size-fractionated on 4–12% gradient SDS–polyacrylamide Bis-Tris gels (Invitrogen) and transferred to nitrocellulose membranes for Western blotting. Western blots were probed with a 1:1000 dilution of anti-lamin A/C (sc-20681, Santa Cruz Biotechnology, Santa Cruz, CA, USA), 1:500 dilution of anti-UCP1 (ab10983, Abcam,

Cambridge, CB4 0FL, UK), 1:100 dilution of anti-PGC1a (H-300) (sc-13067, Santa Cruz Biotechnology), or 1:1000 dilution of anti- γ -tubulin (T5326, SIGMA, St Louis, MO 63103, USA). Antibody binding was detected with a 1:5000 dilution of rabbit IgG/mouse IgG HRP-linked whole antibody (NA934/ NA931, GE Healthcare, GE Healthcare Europe GmbH, Freiburg, Germany) followed by Super signal West Pico ECL (Perbio Science, France) and exposure to X-ray film.

Microarray data analysis

All the data are deposited in GEO (NCBI) under accession number [GSE51204](#). Affymetrix Mouse Exon 1.0 ST arrays were hybridized by GenoSplice technology (www.genosplice.com) according to the Ambion WT protocol (Life Technologies, France) and the labeling and hybridization recommendations of Affymetrix (Santa Clara, USA). Briefly, 100 ng of total RNA was mixed with bacterial transcripts, and the mixture was reverse transcribed into cDNA. After synthesizing double-stranded cDNA, an overnight *in vitro* transcription reaction was performed. Amplified cRNA samples were then reverse transcribed into sense cDNA with incorporation of dUTP. Single-stranded DNA was treated with a combination of uracil DNA glycosylase and apurinic/apyrimidinic endonuclease 1. DNA fragments were biotin-labeled with terminal deoxynucleotidyl transferase. Targets were finally prepared according to the Affymetrix recommendations for hybridizing exon arrays. Microarrays were hybridized, washed, and scanned with Affymetrix instruments. The RIN values for the total RNA samples were between 7.7 and 8.8 (average: 8.43). Raw data were controlled with Expression console (Affymetrix).

Affymetrix Mouse Exon 1.0 ST Array dataset analysis and visualization were performed with EASANA[®] (GenoSplice technology), which is based on the GenoSplice FAST

DB[®] annotation [2]. Exon Array data were normalized by quantile normalization. Background correction was performed with antigenomic probes, and probe selection was conducted as previously described [2-4]. Only probes that targeted exons annotated from FAST DB[®] transcripts were selected in order to focus on well-annotated genes whose mRNA sequences were available in public databases [2-4]. We did not perform analysis with poor-quality probes (e.g., probes labeled by Affymetrix as “cross-hybridizing”) or probes that had low signals comparable to antigenomic background probes that had the same GC content. Only probes that had DABG p-values ≤ 0.05 on at least half of the arrays were considered for statistical analysis [2-4]. In addition, we only analyzed genes that were expressed in at least one of the compared conditions. To be considered as expressed, the DABG p-value had to be ≤ 0.05 for at least half of the gene probes. We performed an unpaired Student’s t-test to compare gene intensities in the different biological replicates. Genes were considered significantly regulated when the fold change was ≥ 1.5 , and the p-value was ≤ 0.05 .

Ultrastructural transmission electron microscopy (TEM)

After collecting mouse perigonadal WAT, small pieces of tissue were immersed in a solution of 2.5% glutaraldehyde in Sorensen’s buffer (0.1 M, pH 7.4) overnight at 4 °C. They were then rinsed in Sorensen’s buffer and post-fixed in a 0.5% osmic acid for 2 hours (h) at room temperature in the dark. After two rinses in Sorensen’s buffer, tissues were dehydrated in a graded series of ethanol solutions (30-100%). Tissues were embedded in EmBed 812 with an Automated Microwave Tissue Processor for Electronic Microscopy, Leica EM AMW. Thin sections (70 nm; Leica-Reichert Ultracut E) of each block were collected at different levels. These sections were counterstained with uranyl acetate and observed on a

Hitachi 7100 transmission electron microscope (TEM) in the Centre de Ressources en Imagerie Cellulaire de Montpellier (France).

Histological Studies

For paraffin treatment, tissues were fixed with 4% paraformaldehyde, dehydrated, embedded in paraffin, sectioned, and stained with Hematoxylin and eosin (HE) following standard procedures for morphological evaluation. HE staining of tissue sections was visualized with a Nanozoomer slide scanner (Hamamatsu). Scanned images were analyzed with NDP.view software.

RT-qPCR analysis

Mouse perigonadal white adipose tissue samples were snap-frozen in liquid nitrogen immediately after collection, reduced into powder in a tissue grinder, and dissolved in TRIreagent (Sigma). After precipitation in alcohol, RNA pellets were washed in cold 75% ethanol and resuspended in nuclease-free water. Samples were quantified and evaluated for purity (260-nm/280-nm and 260-nm/230-nm ratio) with a NanoDrop ND-1000 spectrophotometer. Total RNA (1.5 µg) was then converted to cDNA with the Maxima First Strand cDNA Synthesis Kit (#K1642, Thermo Scientific, US). Gene expression was quantified by SYBR green real-time PCR on the LightCycler[®] 480 System (Roche Applied Science). Reactions were run in triplicate. The mean expression value for the housekeeping genes Hprt1 and B2M was used as an internal control to normalize variability in expression. Primer sequences are provided in Supplemental Table 1.

Mitochondrial DNA quantification

Tissues were digested overnight at 55 °C in a lysis buffer containing 100 mM Tris (pH 8), 5 mM EDTA, 0.2% SDS, 200 mM NaCl, and 0.1mg/ml proteinase K for DNA extraction.

DNA was then precipitated with isopropanol, and pellets were resuspended in 200 μ l nuclease-free water. DNA samples were sonicated (five times, 30 seconds each time) with a bioruptor apparatus (Diagenode) and purified with a 25/24/1 mixture of phenol, chloroform, and isoamyl alcohol prior to ethanol precipitation. The pellet was then resuspended in 200 μ l nuclease-free water. Quantitative PCR (qPCR) analysis of DNA samples diluted at either 1 ng/ μ l (for MEFs) or 5 ng/ μ l (for WAT samples) was performed with primers specific for mitochondria (mND5, m16s) and nuclei (LN11, mHK2). qPCR was performed with a LightCycler[®] 480 and LightCycler[®] 480 SYBR Green I Master (Roche).

Statistical analysis

The survival curves were performed using the Kaplan Meier curve. The median survival is representative of the survival curves. We use the Log-rank (Mantel Cox) test to perform the statistical analyses of the survival curves.

All the other results were expressed as means \pm standard error of the means (S.E.M.). The significance of differences was determined with the Student *t* test, with significance defined as $p < 0.05$.

Cell culture and extracellular flux analysis.

MEFs were derived from embryos that were dissected 13.5 days after vaginal plugs were detected as previously described [5].

Animals were euthanized by cervical dislocation for preparation of stromal vascular fractions. Perigonadal and subcutaneous fat depots were rapidly excised, washed in phosphate-buffered saline, and minced. Adipose tissue samples were then digested for 45

minutes (min) at 37 °C in Dulbecco's Modified Eagle's medium (DMEM) containing 2 mg/ml collagenase A (Roche) and 20 mg/ml bovine serum albumin (Sigma) with mild agitation. Digestion was stopped by adding fetal bovine serum (FBS). Adipocytes were separated from stromal-vascular cells by size filtration (250-, 100-, and 37- μ m nylon filters) and centrifugation as previously described [6]. Incubation in red-blood-cell lysis buffer (Sigma) eliminated contaminating erythrocytes from the stromal-vascular fraction. Stromal-vascular fraction cells were then maintained in DMEM containing 10% FBS until confluence. The day of confluence was considered as day 0. Adipogenic differentiation was initiated in the same media supplemented with 0.5 M dexamethasone, 0.5 mM isobutylmethylxanthine, 170 nM insulin, and 1 μ M rosiglitazone. Dexamethasone and isobutylmethylxanthine were removed two days later, and cells were maintained for eight days in the presence of 170 nM insulin and 1 μ M rosiglitazone. The cell culture media was changed every other day. Oil Red O staining for lipid droplets was performed to evaluate adipocyte differentiation. Triglycerides and RNA were extracted at day 8 in order to further quantify the rate of differentiation.

Triglycerides measurement

Differentiated adipocytes were scraped in isopropanol (100 μ l for a 12-well plate) and centrifuged for 10 min at 13,000 rpm. We added 200 μ l of reagent (Triglycerides FS, ref: 5710 1 99 10 021, Diasys France, Condom, FRANCE) to 10 μ l of isopropanol extract in a 96-well plate. After a 20-min incubation at room temperature, absorbance was measured at 492 nm.

Seahorse Experiments

Mitochondrial function was determined with an XF-24 extracellular flux analyzer (XF24, Seahorse Bioscience, <http://www.seahorsebio.com/company/about.php>). OCR was

measured in adherent MEFs. Control and mutant fibroblast cells were seeded in an XF 24-well cell culture microplate (Seahorse Bioscience) at a density of 7×10^5 cells/well in 100 μ L DMEM: F12 media. Cells were incubated for 18 h at 37 °C in 5% CO₂. Growth media was replaced with 675 μ L XF media containing 2.5 mM glucose and 1 mM sodium pyruvate. (The pH of the media was adjusted to 7.4.) Cells were then pre-incubated for 1 h at 37 °C without CO₂ to allow cells to pre-equilibrate with the assay media before starting the Mito Stress Test procedure. After measuring baseline OCR as an indication of basal respiration, OCR was measured after sequentially adding the following to each well: oligomycin (10 μ g/ml – SIGMA O4871) to measure ATP Production, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP, 3 μ M, SIGMA C2920) to measure maximal respiration and spare capacity, and rotenone (1 μ M, SIGMA R8875) to measure proton leak. Data were expressed as pmol of O₂ per minute and were normalized by protein content as determined with the Bradford reagent (SIGMA B6916).

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