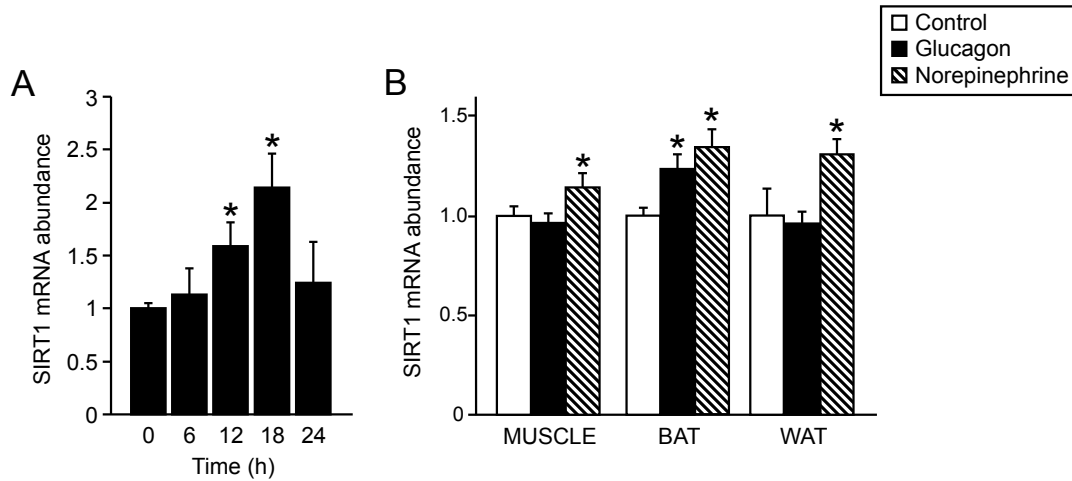


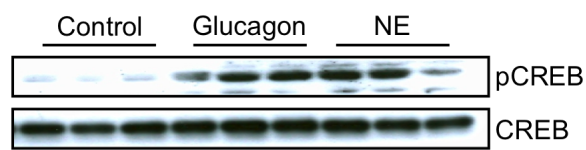
## Supplemental Information

### CREB and ChREBP oppositely regulate SIRT1 expression in response to energy availability

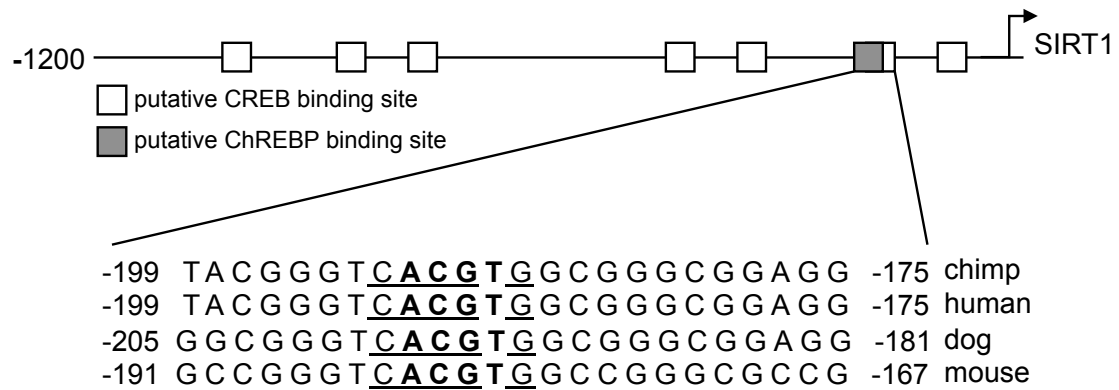
Lilia G. Noriega, Jérôme N. Feige, Carles Canto, Hiroyasu Yamamoto, Jiujiu Yu, Mark A. Herman, Chikage Mataka, Barbara B. Kahn and Johan Auwerx



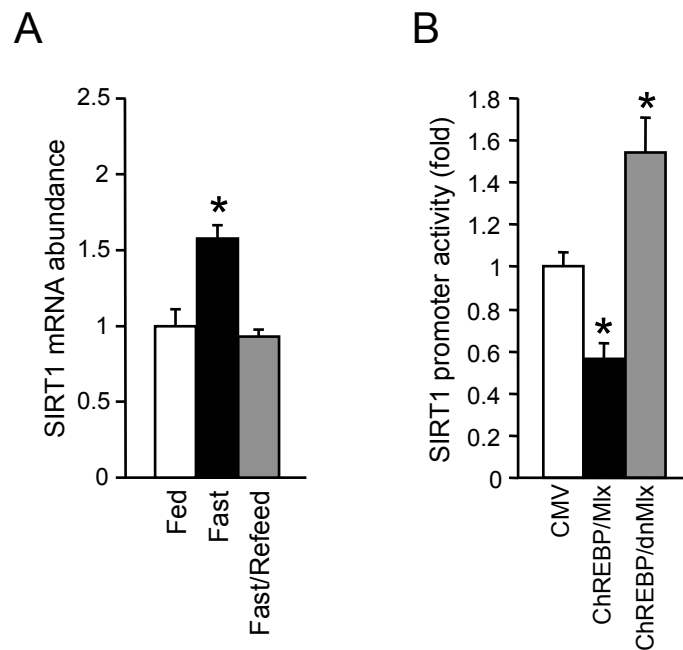
**Figure S1 (Supplemental related to Figure 1). (A) Hepatic SIRT1 expression upon fasting.** SIRT1 mRNA abundance was measured by qPCR in liver of mice after the indicated hours of fasting (n=5). Gene expression was normalized to the housekeeping genes  $\beta$ 2-microglobulin and 36B4. Values are presented as the average  $\pm$  SEM and \* indicates a statistical difference vs control (p-value <0.05). **(B) Humoral factors released during fasting induce SIRT1 expression.** SIRT1 mRNA abundance was measured by qPCR in muscle, brown (BAT) and white (WAT) adipose tissue of mice 1h after the intraperitoneal administration of PBS (control), glucagon (50  $\mu$ g/kg) or norepinephrine (1 mg/kg) (n=5). Gene expression was normalized to the housekeeping genes  $\beta$ 2-microglobulin and 36B4. Values are presented as the average  $\pm$  SEM and \* indicates a statistical difference vs control (p-value <0.05)



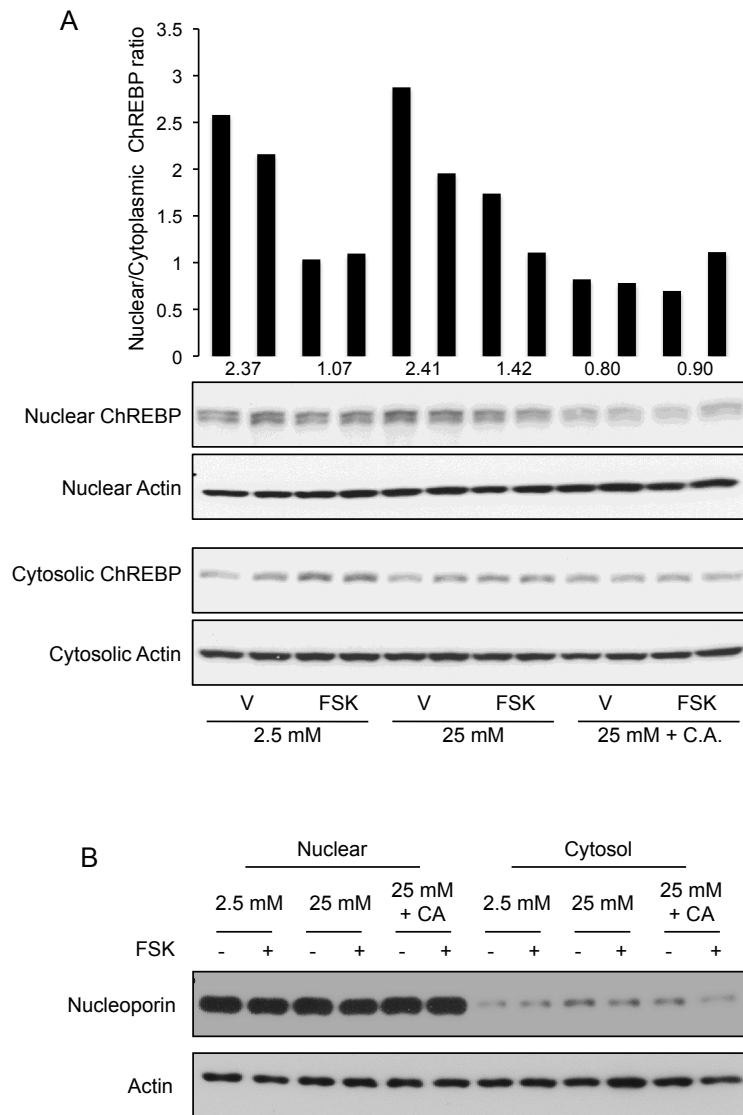
**Figure S2 (Supplemental related to Figure 2). Glucagon and norepinephrine induce hepatic CREB activation.** CREB phosphorylation (pCREB) and CREB protein levels were analyzed by western blot in liver of mice 1h after the intraperitoneal administration of PBS (control), glucagon (50  $\mu\text{g}/\text{kg}$ ) or norepinephrine (1  $\text{mg}/\text{kg}$ ).



**Figure S3 (Supplemental related to Figure 2, 3 and 4). Schematic representation of the human SIRT1 promoter depicting putative CREB and ChREBP binding sites.** Sequences show the alignment of chimpanzee, human, dog and mouse SIRT1 promoter region, with overlapping CREB and ChREBP binding sites, as determined by the Genomatix analysis software. Bold letters indicate the putative binding site for CREB with a core/matrix similarity of 1/0.931 and underlined nucleotides indicate the putative binding site for ChREBP with a core/matrix similarity of 1/0.835.



**Figure S4 (Supplemental related to Figure 3). ChREBP-mediated regulation of SIRT1 promoter activity.** (A) Hepatic SIRT1 mRNA abundance was measured by qPCR in liver of mice fed, fasted and fast/refed (n=5). Gene expression was normalized to the housekeeping genes  $\beta$ 2-microglobulin and 36B4. Values are presented as the average  $\pm$  SEM and \* indicates a statistical difference vs fed (p-value <0.05). (B) HepG2 cells were transfected with a SIRT1 promoter luciferase reporter and a ChREBP plasmid together with its partner Mlx or a dominant negative isoform of Mlx (dnMlx) that inhibits DNA binding of the dimer. Values are presented as the average  $\pm$  SEM and \* indicates a statistical difference vs CMV (p-value <0.05).



**Figure S5 (Supplemental related to Figure 4). ChREBP localization in HepG2 cells upon FSK treatment.** HepG2 cells were stimulated 4h with 10  $\mu$ M Fsk in 2.5 mM or 25 mM glucose. A third group was treated with cantharidic acid (CA) 1h before treatment of Fsk in 25 mM glucose. ChREBP (A) and Nucleoporin (B) protein levels were then measured by western blot in nuclear and cytosolic fractions.

## Supplemental Experimental Procedures

*Plasmids, adenoviruses & reagents.* Expression plasmids of CREB, and the inactive KCREB variant (Walton et al, 1992) were obtained from Clontech. ChREBP, Mlx and dnMlx expression plasmids were a kind gift from Howard Towle. A fragment of the human SIRT1 promoter beginning 1202 bp upstream of the starting ATG site was cloned into the pGL3-luciferase vector (Promega) using HindIII and SacI restriction sites, after PCR amplification of the clone RZPDB737C042021D6 (RZPD Deutsches Ressourcenzentrum für Genomforschung GmbH) with the following primers: Forward: 5'-agtcGAGCTCtccccatgctctcactactgacccaacaaac-3' Reverse: 5'-tcgtAAGCTTtcttccaactgcctctctggcctctccc-3. We isolated a series of nested PCR fragments using the 1202 bp construct. These fragments began either at position -668, -316, -160 or -96 relative to the start site. The ACREB expressing adenovirus was generated through LR recombination using the ViraPower adenoviral expression system (Invitrogen). The entry vector was generated from a plasmid encoding the CREB inhibitor ACREB (Ahn et al, 1998). An adenovirus coding for GFP only (pAD-GFP) was used as a control in all experiments. Viruses were purified using the Adeno-X Maxi purification kit (Clontech). Glucagon (Novo Nordisk) was dissolved in 0.9% NaCl, and forskolin (Sigma) was dissolved in DMSO. The shRNA plasmid against ChREBP was purchased from Origene.

*Correlation plot from BxD resource gene expression studies.* The BxD resource is a mouse genetic reference population (GRP) (Abiola et al, 2003; Chesler et al, 2005; Chesler et al, 2004). The BXD set of recombinant inbred (RI) mouse strains was derived by crossing F2 mice obtained from an intercross of C57BL/6J (B6) and DBA/2J (D2) mice and then inbreeding the resulting progeny for at least 20 generations to generate the BxD GRP (Argmann et al, 2005; Chesler et al, 2005; Chesler et al, 2004). This genetic reference panel is a remarkable resource because data for hundreds of interesting phenotypic traits, including clinical traits and molecular traits, i.e. microarray expression studies (in multiple tissues) have been acquired over a 25-year period. All these data are publically available on the [www.genenetwork.com](http://www.genenetwork.com) website. The web-based software on the Genenetwork site furthermore allows the identification of all genes whose expression is significantly correlated with a gene of interest (SIRT1 in our example) in a particular tissue (liver in our case). SIRT1 gene expression varies significantly in the liver of the different mice of the BxD RI panel (similar expression profiles can be generated for all probes contained in the microarrays). The software allows then the calculation of a correlation plot between SIRT1 and other genes (e.g ChREBP; Figure 3B).

*Gene expression analysis.* Total RNA was prepared from either hepatic cells or from homogenized mouse tissues using Trizol extraction (Invitrogen). cDNAs were prepared by reverse transcription of 1000 ng of total RNA using the Superscript II enzyme (Invitrogen). The resulting cDNAs were amplified in 384 well plates using a SYBR green PCR kit (Qiagen) in a LC480 cycler (Roche). All data were normalized to cyclophilin and 18s for human samples and to  $\beta$ 2-microglobulin and 36B4 for mouse samples, using the DDCT method.

*Protein analysis.* Western blots were performed on 20  $\mu$ g of total proteins directed against SIRT1 (Upstate), CREB and phospho-CREB (Cell signaling), ChREBP (Novus Biologicals), PGC1 $\alpha$

(Abcam), Acetyl-lysine (Cell signaling) and Tubulin (Santa Cruz) or Actin (Sigma) diluted at 1/1000. Detection was performed using ultra-sensitive horseradish peroxidase chemiluminescence (Pierce).

*Transfection assays.* Human hepatoma HepG2 cells were cultured in 24-well plates and transfected using Lipofectamine 2000 (Invitrogen), according to the manufacturer's instruction and using 50 ng pCMV- $\beta$ -galactosidase, 150 ng SIRT1 promoter vector and the indicated amounts of pCMV CREB, pCMV KCREB, ChREBP/Mlx or an empty expression plasmid per well. Cell extracts were prepared 48h posttransfection and luciferase levels were normalized to  $\beta$ -galactosidase activity.

*Adenoviral infection of cells.* HepG2 cells were washed with PBS and left for 1h in serum free DMEM containing the appropriate amount of viral particles (MOI=40 for each virus used). The medium was then replaced with fresh medium for another 48h and treatments were performed after this period.

*Genomatix analysis of the sirt1 promoter.* Extraction and transcription factor analysis of a 1200 bp fragment of the SIRT1 promoter from different organism was performed with the eldorado/gene2promoter module from Genomatix software.

*Electrophoretic mobility shift assay.* Nuclear extracts were obtained from a 10 cm dish of CHO cells transfected with 24  $\mu$ g of CREB or ChREBP/Mlx. A complementary pair of biotinylated or cold oligonucleotides with the sequence 5' ACTACGGGTCACGTGGCGGGCGGA 3' was obtained from SIGMA. A cold oligonucleotide carry on a mutation to alanine of the overlapped nucleotides was used for competition assays. The EMSA was performed according manufacturer's instructions using the LightShift Chemiluminescent EMSA Kit from Thermo Scientific. The Flag antibody used for supershift was from SIGMA. We only show the upper part of the EMSA due to space issues in the figure.

*Chromatin Immunoprecipitation assay.* HepG2 cells were grown to 90% confluency and, after 4h of stimulation with forskolin or high glucose treatment, the chromatin immunoprecipitation assay was performed as described elsewhere (Feige et al, 2007). Briefly, after the indicated treatment, cells were washed twice with PBS and cross-linked for 10 min at room temperature in 1% formaldehyde. Cross-linking was stopped by 5min incubation in 0.125 M glycine. Chromatin was extracted and sonicated on ice for 180s in 10s sonication periods spaced by 20s. Immunoprecipitation was performed with an anti-CREB antibody, an anti ChREBP antibody or an IgG control (Santa Cruz) and the precipitated DNA fragments were analyzed by SyBR green qPCR amplification using primers directed against a -200 bp (Forward: 5'-CTTCCAGCCCAGGCGGAGCG-3' and Reverse: 5'-GATTTAAACCCCATCACGTGACC CG-3') or a -3000 bp (Forward: 5'-GGATTACAGGCAGCAGCCACC-3' and Reverse: 5'-GGTTGCGTGGGGGTCAAGG-3') *SIRT1* promoter region. In vivo CHIP analysis was performed as previously described (Duggavathi et al, 2008) using liver samples from C57B6 mice in fed and fasted conditions, WT and ChREBP<sup>-/-</sup> mice and mice fed CD or HFD for 18 weeks. The proximal region of the SIRT1 promoter was amplified using the primers Forward: 5' AAATCTCCCGCAGCCGAGCC 3' and Reverse: 5' GCCACCTCGTCCGCCATCTT 3'.

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