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Supplemental Data

The NAD$^+$-Dependent Deacetylase SIRT1 Acts as a Rheostat of CLOCK-Mediated Chromatin Remodeling and Circadian Control

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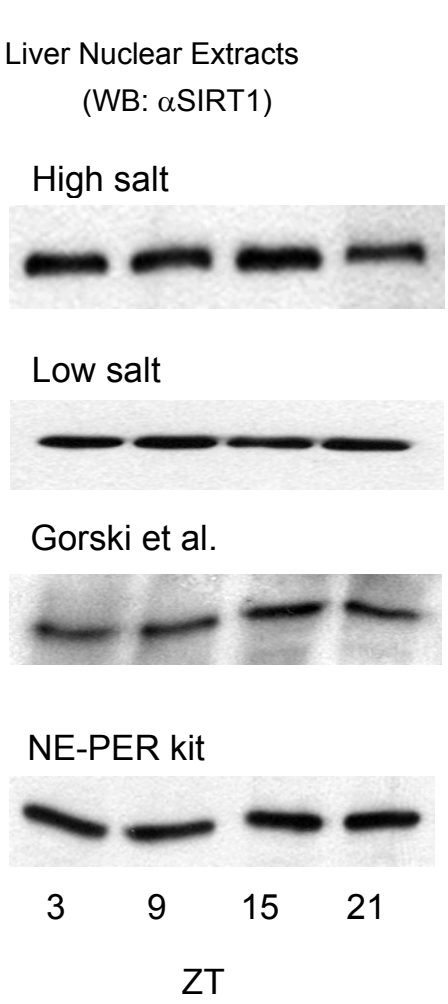
Figure S1.

To assess whether SIRT1 protein levels oscillate in a circadian manner, nuclear extracts were prepared following various methods from mouse liver. The protein levels were monitored using a Western analysis using specific anti-SIRT1 antibodies (see Experimental Procedures). Independently from the protocol used, SIRT1 levels displayed marginal or no oscillation.



Type of preparation

Nuclear extracts were prepared following the method described by Andrews and Faller (1991) Nucleic Acids Res. 19: 2499, using a nuclear lysis buffer containing 450 mM NaCl.

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Nuclear extracts prepared using the method of Gorski et al. Cell (1986) 47:767. Nuclei are purified over a sucrose cushion and the resulting nuclear pellet is extracted. Nuclei are lysed with ammonium sulfate and chromatin is centrifuged out. Nuclear proteins are precipitated by addition of 0.3g/ml solid ammonium sulfate.

Nuclear extracts were prepared using the commerically available kit NE-PER Nuclear and Cytoplasmic Extraction Reagents (PIERCE Biotechnology)

Supplemental Figure 2

Effect of different treatments on the CLOCK - SIRT1 interaction.
The effect of various agents on the CLOCK-SIRT1 interaction was analyzed. None of these treatments used significantly affected the efficiacy of the interaction. JEG3 cells were cultured in BME containing 10% FBS and transfected with Myc-CLOCK, Myc-BMAL1, and Flag-SIRT1 constructs. Negative control (NC) represents cells transfected only with Myc-CLOCK and Myc-BMAL1. 24h after transfection cells were replated, allowed to readhere for another 16 hours, and treated with indicated reagents for 1, 2 and 6 hours. Here the results with 6h treatment are shown, the same results were obtained at 1h and 2h. Nuclear extracts were processed as previously described (Hirayama et al. 2007). This experiment was performed 3 times and a representative result is shown.

Legend: Lane 1: control (C); lane 2: 50% horse serum (HS); lane 3: 1 mM NAD^+ (NAD^+); lane 4: 10 mM glucose (Glc); lane 5: 1 mM pyruvate (Pyr); 6: 100 μM DFO (DF); lane 7: 100 μM resveratrol; (Res) lane 8:120 μM splitomicin (Split). Lane 9: negative control (NC)

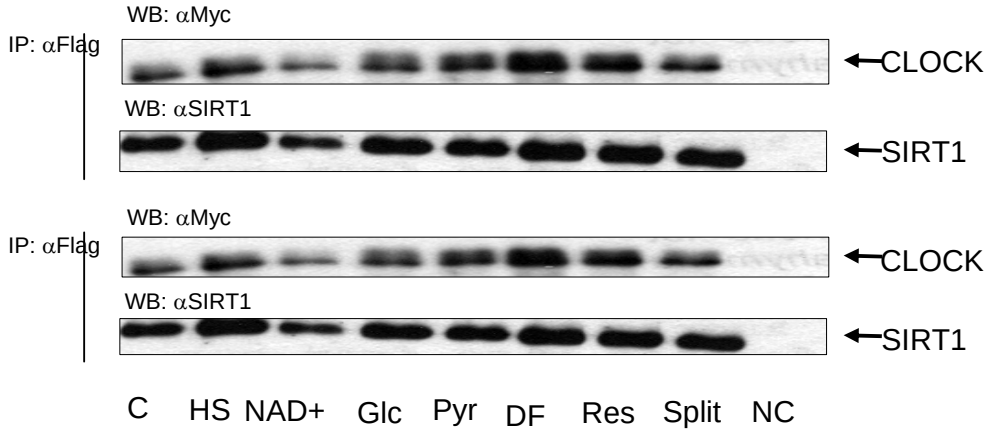

Figure S3.

A Specific Antibody that Recognizes BMAL1 only when it is Acetylated at K537.
We have raised a polyclonal antibody against a 11 aa peptide in which K537 was uniquely acetylated. The antibody has been successfully tested in Western analysis, immunoprecipitations and immunohistochemistry. Here we show results of a transfection in 293 cells in which CLOCK, BMAL1 or a BMAL1(K537R) mutant are ectopically expressed. Western analysis shows that the antibody specifically recognizes BMAL1 when it is acetylated and that CLOCK elicits this event (as indeed recently demonstrated in our laboratory (Hirayama et al 2007).

Hirayama, J., Sahar S., Grimaldi B., Tamaru, T., Takamatsu, K., Nakahata, Y. and Sassone-Corsi. P. (2007) CLOCK-mediated acetylation of BMAL1 controls circadian function. *Nature* 450: 1086-90

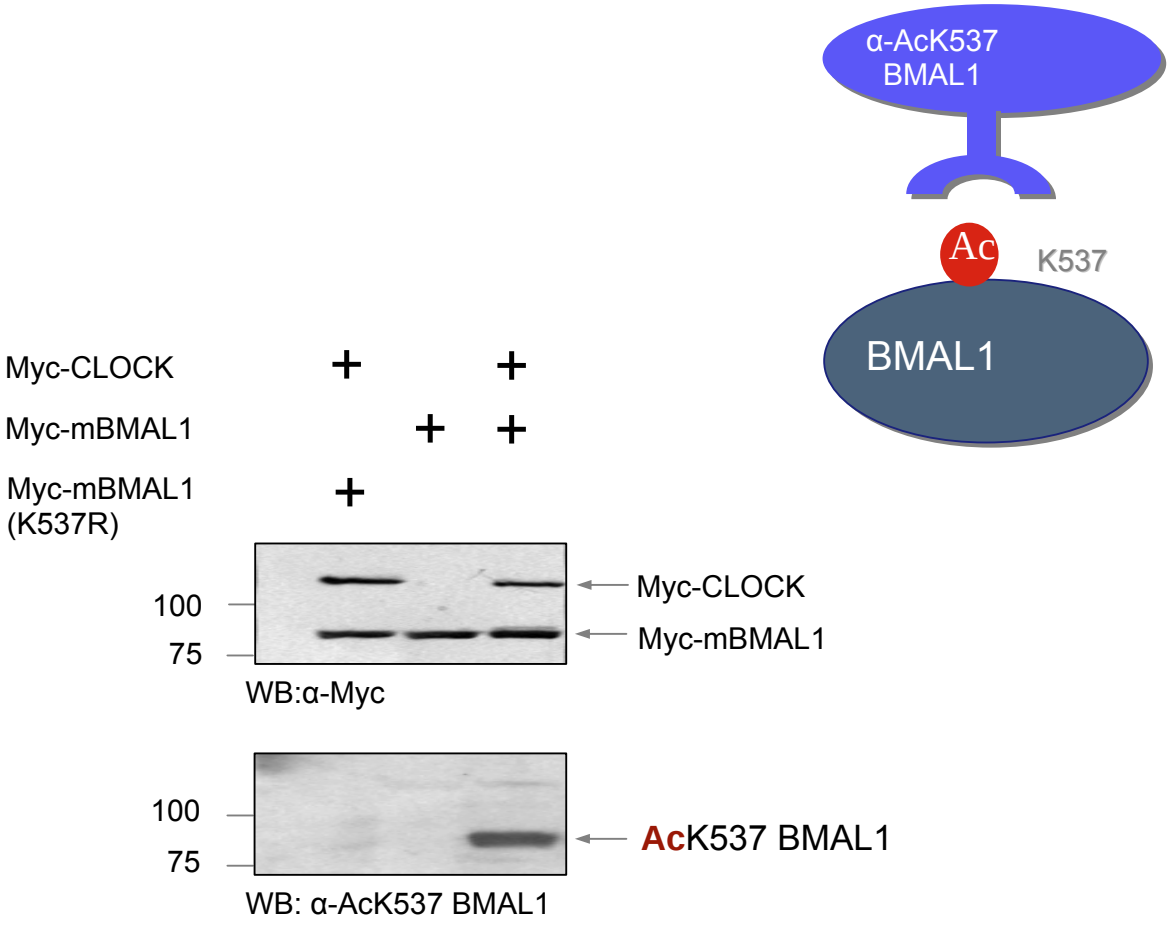

Figure S4.

SIRT1 deacetylates BMAL1(K537) *in vitro*. For this experiment we used an anti-acetyl-BMAL1 antibody developed in our laboratory (see Supplementary Figure 3). Acetylated BMAL1 was prepared from HDAC inhibitors-treated JEG3 cultured cells transfected with Flag-Myc-BMAL1 and Myc-CLOCK. Recombinant SIRT1 and deacetylation buffer were used from SIRT1 Fluorimetric Activity Assay/Drug Discovery Kit (AK-555; BIOMOL International). Immunoprecipitated Ac-BMAL1 and recombinant SIRT1 were incubated in deacetylation buffer with 5 mM NAD^+ or 10 mM nicotinamide (NAM) for 90 min at 37°C. Reactions were stopped by adding SDS-PAGE denaturing buffer. Samples were subjected to SDS-PAGE and probed with Ac-BMAL1 or Myc antibodies.

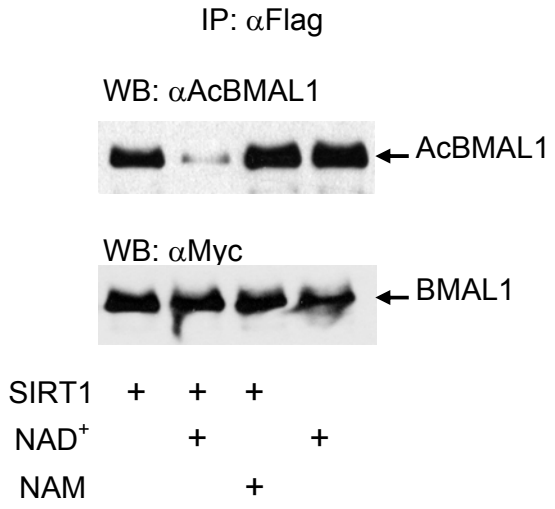

Figure S5.

This is a lower exposure of the panel presented in Fig. 7B. This allows to better visualize BMAL1 phosphorylated and non-phosphorylated bands. While BMAL1 protein appears always to be more abundant - possibly indicating increased stability - in SIRT1-null cells, overall the pattern of phosphorylation doesn't change in the absence of SIRT1. The only time point where there may be a significant difference is at ZT21. In livers from WT mice the non-phosphorylated band appears to be prominent, whereas the bands corresponding to the phosphorylated the non-phosphorylated BMAL1 appears to equally represented in the livers from the SIRT1Δex4. mice