Supporting Online Material

Materials and methods

Animals Male BALB/c mice and *clock/clock* mutant mice were housed under 12-h light/12-h dark (LD) cycles over 2 weeks. All protocols using animals in this study were approved and reviewed by the Institutional Animal Care and Use Committee of the University of California, Irvine.

Plasmids FLAG-tagged *mClock*/pSG5 and Myc-tagged *mBmal1*/pCS2 have been described (*S1, S2*). Human *Nampt* promoter, referred as "-1637/pGL4.10" in this study, was kind gift of Dr. Kazuya Yamagata (*S3*). -1637/pGL4.10 was digested by *Acc65I* and *AatII*, *Tth111I*, or *SmaI* followed by blunting by Klenow enzyme to construct -1081/pGL4.10, -734/pGL4.10, or -170/pGL4.10, respectively. pRL-CMV vector was purchased from Promega.

Antibodies and reagents Antibodies against acetyl-Histone H3 (06-599), SIRT1 (07-131) and GAPDH (MAB374) were purchased from Millipore and antibodies against Clock (sc-6927) and normal rabbit IgG (sc-2027) were purchased from Santa Cruz Biotechnology. Antibodies against BMAL1 and acetyl-lysine 537 BMAL1 were described (*S4, S5*). FK866 was purchased from Axon Medchem. NAD (N8535), nicotinamide (N0636), and 2-chloroadenosine (C5134) were purchased from SIGMA.

Cell culture, Transient transfections and Luciferase assays All cells used in this study were grown at 37°C and 5% CO_2. Wild type, *clock/clock* mutant and *Cry1*$^{-/-}$/*Cry2*$^{-/-}$ double KO MEF cells were grown in Dulbecco's Modified Eagle Medium (4.5 g/L glucose) supplemented with 10% fetal bovine serum (FBS) and antibiotics. MEF cells established from homozygous *Sirt1* deficient mice and counterpart wild type MEF cells were cultured in Dulbecco's Modified Eagle Medium (4.5 g/L glucose) supplemented with 7.5% newborn bovine serum, 2.5% FBS and antibiotics. JEG3 cells were grown in Basal Medium Eagle supplemented with 10% FBS and antibiotics. For transient transfection luciferase assays, JEG3 were transfected with various combinations of expression and reporter plasmids using FuGENE HD (Roche Molecular Biochemicals), according to the manufacturer's protocol. Luciferase activities were assayed with the dual-luciferase reporter assay system (Promega) in a Berthold luminometer.

NAD^+ and nicotinamide analysis by liquid chromatography/ tandem mass spectrometry (LC/MS^n) 50 % Serum-treated MEFs were washed and harvested with ice-cold PBS. Cells were centrifuged (1,000 g, 5min at 4 °C) and kept at -80 °C until sample preparation. NAD^+ and nicotinamide (NAM) were extracted as described (*S6*). Cells were resuspended with 200 μl of extraction buffer (99% 5 mM ammonium formate/1% methanol containing 1 μM 2-chloroadenosine (*S7*)) and sonicated. After centrifugation (18,000 g, 10 min, 4 °C), 20 μl of resultant supernatant was kept for protein measurement, the rest of supernatant was filtered with a 0.22 μm filter followed by a regenerated cellulose 3000 molecular weight cut-off Microcon YM-3 filter (Millipore) to remove the cellular debris and large molecules. NAD^+ and NAM were identified and quantified by LC/MS^n, using a 1100-LC system equipped with a ion-trap XCT and electrospray as ionization source (Agilent Technologies, Palo Alto, CA).

Analytes were separated using a ZORBAX SB-CN column (2.1x150 mm i.d., 5 μm, Agilent Technologies, Wilmington, DE) maintained at 30°C. Mobile phase was water containing 5 mM ammonium acetate and 0.25 % acetic acid (A) and methanol containing 5 mM ammonium acetate and 0.25 % acetic acid (B). A gradient from 0% to 50 % B in 10 min and then to 70 % B from 10 to 15 min was applied at a flow rate of 0.15 ml/min. Total run time was 19 min and post-time was 15 min (100 % A). Injection volume was 10 μl. Detection was set in the positive mode, capillary voltage was 4.0 kV, skim1 40 V, and capillary exit 140 V. N_2 was used as drying gas at a flow rate of 10 liters/min, temperature of 350 °C and nebulizer pressure of 60 PSI. Helium was used as collision gas. Cell-derived NAD^+ and NAM were identified by comparison of their LC retention times and MS^2 fragmentation patterns with those of authentic standards. Full-scan MS^2 spectra of NAD^+ and 2-chloroadenosine was acquired using multiple reaction monitoring with isolation width of 2 and fragmentation voltage of 1.1 V. Ion charge control was on, smart target set at 100,000 and max accumulation time at 200 ms at 26,000 *m/z* per sec. Extracted ion chromatograms were used to quantify NAM (*m/z* 123.3) and NAD^+ (*m/z* 664.3>523.8) using 2-chloroadenosine (*m/z* 302.3>170.5) as an internal standard (*S7*). Limits of quantification were 1 pmol for both NAM and NAD^+. Detection and analysis were controlled by Agilent/Bruker Daltonics software version 5.2.

NAD^+ concentration measured in this study are in complete agreement with previous studies; *K*m for NAD^+ is 23 μM for human SIRT1 and 70 μM for yeast Sir2 (*S8, S9*). In vitro deacetylation assays indicate that Yeast Sir2 has deacetylation activity even within 8.75 μM and 10 μM NAD^+ concentration range (*S9, S10*). Furthermore, *K*m for NAD^+ is 15 μM for human CD38, another enzyme that utilizes NAD^+ as a substrate (*S11*). These notions indicate that the NAD^+ levels in *c/c* MEFs (1 pmol/μg protein or 4 μM), although remarkably low,

would still be within the physiological range for various NAD^+-dependent enzymes (Sirt1, CD38, etc.).

Preparation of cDNA and protein extracts from cultured cell lines and mice livers Total RNA extraction was done using Trizol (Invitrogen) according to the manufacturer's instructions. Total RNA was then reverse-transcribed into cDNA by using Superscript II Reverse Transcriptase (Invitrogen) with oligo random hexamers. For preparation of protein extracts, MEFs were washed twice with phosphate buffered saline (PBS) and lysed in RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl, 5 mM EDTA, 15 mM MgCl2, 1% NP40, 1 x protease inhibitor cocktail (Roche), 1mM DTT, 1 μM trichostatin A (TSA), 10 mM nicotinamide (NAM), 10 mM NaF, 1 mM PMSF).

In silico circadian elements search The sequences were downloaded from the NCBI Gene database. Each Nampt gene sequence spanning from 2 kbp upstream of the translation start codon was examined. Multiple sequence alignments of these sequences were obtained by ClustalW 2.0.5 with default parameters. The circadian promoter elements (*S12, S13*), the E-box, the ROR binding element (RORE) and the DBP binding element (DBPE), were then searched from these alignments using a pattern finding tool, fuzznuc, with the following consensus sequences allowing for a 2-base mismatch:

RORE: [AT]A[AT]NT[AG]GGTCA

DBPE: [GA]T[GT]A[TC]GTAA[TC]

E-Box: CACGTG

A search for circadian clock elements by in silico analyses revealed the presence of three

putative E-boxes highly conserved in the human, rat, and mouse *Nampt* genes (Fig. 3B) and partially conserved in the corresponding zebrafish and chicken genes (not shown). Other circadian promoter elements are not present within 2 kb upstream from the transcription start site of human, rat and mouse *Nampt* genes.

Chromatin Immunoprecipitation (ChIP) Assays and Quantitative Real-time Reverse Transcription (RT)-PCR We performed the conventional and dual cross-linking ChIP assays as described (*S4, S13*). Each quantitative real-time RT-PCR (q-PCR) was performed using the Chromo4 real time detection system (BIO-RAD). The PCR primers for m*Dbp* mRNA and 18S rRNA were described (*S13*). The PCR primers for m*Nampt* mRNA, m*Nmnat1~3* mRNAs, m*Nampt* TSS, and m*Nampt* 3'R were designed with a real-time PCR primer design tool, Real-Time PCR Primer Design (available at https://www.genscript.com/ssl-bin/app/primer), and the sequences of the primers were as follows:

m*Nampt* mRNA FW: GGTCATCTCCCGATTGAAGT

m*Nampt* mRNA RV: TCAATCCAATTGGTAAGCCA

m*Nmnat1* mRNA FW: TGGAGACTGTGAAGGTGCTC

m*Nmnat1* mRNA RV: TGAGCTTTGTGGGTAACTGC

m*Nmnat2* mRNA FW: AGAATTCCGACTGGATCAGG

m*Nmnat2* mRNA RV: GGTCACCCTCTTCATCAGGT

m*Nmnat3* mRNA FW: CCGTCATCACCTACATCAGG

m*Nmnat3* mRNA RV: AGCCAGTCTTTCCTTTCCCT

m*Nampt* TSS FW: GTGACGGTCGGCTTTAGG

m*Nampt* TSS RV: GGACTGAGGAGGACGTGAG

m*Nampt* 3'R FW: CTTSCGCGAATGTTTAGGCA

m*Nampt* 3'R RV: GCATATTAGAGCCACAGGCA

For a 20 µl PCR, 50 ng of cDNA template was mixed with the primers (final concentrations of 200 nM), and 10 µl of iQ^{TM} SYBR Green Supermix (BIO-RAD). The reaction was first incubated at 95 °C for 3 min, followed by 40 cycles at 95 °C for 30 s and 60 °C for 1 min.

NAMPT inhibitor "FK866" treatment experiments MEFs in growing phase (60~80% confluent) were pre-treated with 10 nM FK866 dissolved in ethanol/serum-free DMEM 16 hr prior to high serum treatment. After 2 hr serum treatment, medium was changed to serum-free medium. During and after serum treatment, MEFs were treated with 10 nM FK866.

References

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