Supporting Online Material for

Histone Lysine Demethylase JARID1a Activates CLOCK-BMAL1 and Influences the Circadian Clock

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Supporting Online Materials.

Materials and Methods

Plasmids, cell lines and transient transfections

Full-length cDNAs encoding CLOCK, BMAL1, CRY1 and JARID1a are in pcDNA3.1 expression backbone, and *Per2:Luc* reporter plasmids have been described previously (*7, 10*). JARID1b and JARID1c expression constructs were a kind gift from Dr. Ralf Janknecht. Myc-tagged full-length or truncated JARID1a was generated by subcloning the JARID1a coding region from pcDNA3.1HF-Jarid1a into pCS3+6MT. *Jarid1a*$^{-/-}$ and *Jarid1a*$^{+/+}$ mouse embryonic fibroblasts were derived from littermate mice and were a kind gift from Dr. William Kaelin. U2OS cells stably expressing *Bmal1:Luc* reporter were previously described (*11*). End-point luciferase assays in HEK293T cells were carried out as previously described (*12*) with Transit LT-1 (Mirus) as a transfection reagent. For *Jarid1a*$^{-/-}$ luciferase assays, we performed reverse transfections in 96-well format with Transit LT-1 (Mirus) as described in (*12*) with some modification. Specifically, 40,000 cells/well, total 2 μg of DNA (200 ng Luciferase reporter construct, 200 ng CMV-BMAL1, 500 ng CMV-CLOCK and up to 1,100 ng of JARID1a expression vector) and 3 μl of transfection reagent were used per transfection. Luminescence was measured in a Tecan Infinite M200 reader 52 h post transfection. For generating cells stably expressing *Per2:Luc* reporter, both *Jarid1a*$^{-/-}$ and *Jarid1a*$^{+/+}$ cells were transfected with *Per2:Luc* reporter (*10*) subjected to selection by growth in cell culture media supplemented by 2 μg/ml Puromycin. Monoclonal lines were generated by serial dilution and antibiotics selection. Transient transfection in *Jarid1a*$^{-/-}$ and *Jarid1a*$^{+/+}$ mouse embryo fibroblasts was accomplished with XtremeGene 9 (Roche) according to the manufacturer's instructions. Briefly, 200,000 cells were seeded onto 35-mm dishes and transfected 24 hours later with 2.5 μg DNA and 5 μl reagent (in 200 μl serum-free media). 24–36 h posttransfections cells were shocked and subject to real-time luciferase measurements as described below.

Antibodies

Histone H3 (Active Motif 39159), H3K4me3 (Active Motif 39159), H3K9Ac (Upstate ab444-1 and Active Motif 39137), HDAC1 (Upstate ab7028), BMAL1 (Santa Cruz H170 sc8790), CLOCK (Santa Cruz H276 sc25361), JARID1a (Bethyl A300-897A), and Flag M2 (Sigma) were used in this study. Antibodies against JARID1a used for liver chromatin immunoprecipitation were a kind gift from Dr. William Kaelin and have been described before (*7*). Anti-Myc 9E10 antibody was purified from supernatant of 9E10 hybridoma cells (ATCC) by Protein A affinity

chromatography. Immunoblot and ChIP validation for anti-BMAL1 (Santa Cruz H170 sc8790) and anti-JARID1a (Bethyl A300-897A) antibodies is provided in fig. S17.

Chromatin Immunoprecipitations (ChIP)

From Liver- ChIP was performed as previously described (*13*) with minor modifications. 5 μg of antibodies were used with 50 μl of anti-Rabbit IgG magnetic Dynabeads (Invitrogen M-280) for immunoprecipitation. In addition, enriched DNA was desalted using PCR purification columns (Qiagen 28106) following phenol:chloroform extraction and ethanol precipitation.

From Cells- 10^7 cells were seeded onto 15-cm dishes and incubated for at least 3 days for desynchronization of cellular oscillators. The cells were removed from the incubator, crosslinked for 5 minutes with 1% formaldehyde at room temperature and then quenched in PBS supplemented with 125 mM Glycine for 15 minutes. The cells were then collected, centrifuged, lysed on ice with ChIP lysis buffer (50 mM Tris HCl pH 8.0, 10 mM EDTA, 1% SDS, and 25% glycerol) supplemented with protease inhibitor (EDTA Complete, Roche) and the chromatin sheared to an average fragment size of less than 500 bp in a Bioruptor XL (Diagenode). ChIPs were then performed as described in (*13*) with the exception that Protein G Dynabeads (Invitrogen) were used. Additionally, for transcription factor chromatin immunoprecipitation cells were crosslinked with 1.5 mM Ethylene glycol-bis (succinimidylsuccinate) (EGS, Sigma) prior to formaldehyde crosslinking. Similarly, for HEK293T-based chromatin immunoprecipitation, 2 x 10^6 cells were seeded on 10-cm plates 24 h before transfection with a total of 16 μg DNA and 48 μl Transit LT-1 according to the manufacturer's protocol. 36–48 h post transfection the cells were processed as described above.

Co-Immunoprecipitation (Co-IP)

For epitope-tagged JARID1a and CLOCK-BMAL1 co-IP, HEK293T cells were seeded in 6-well plates at a density of 250,000 cells/well 24 h before transfection with a total of 2 μg DNA and 6 μl Transit LT-1 (Mirus). 36 hours posttransfections cells were harvested with 1 ml Detachment buffer (10 mM Tris HCl pH 8.0, 150 mM NaCl and 10 mM EDTA), lysed on ice with 400 μl protease and phosphatase inhibitor (PhosSTOP and cOmplete EDTA-free protease inhibitors, Roche Cat #04906845001 and #11873580001) supplemented lysis buffer (40 mM Tris HCl pH 8.0, 250 mM NaCl, 0.5 mM Na_3VO_4, 50 mM NaF and 0.5% Nonidet P40). After centrifugation (20,000 *g*, 4 ºC), the extracts were precleared by incubation with 20 μl Dynabead protein G (Invitrogen) at 4ºC for 2 h. Concurrently, antibody-Dynabead complexes were prepared by binding (per IP) 20 μl Dynabead protein G with 10 μg anti-Myc antibody in a final volume of 100 μl of PBS-0.1% Tween-20, which were subsequently added to 300 μl of the

precleared extract. The immunoprecipitation reactions were then incubated overnight with tumbling, washed twice in lysis buffer, boiled in Laemmli buffer and subjected to SDS-PAGE and immunoblotting analysis.

For JARID1a and HDAC1 immunoprecipitation, we followed an identical protocol with the following modifications: (a) following lysis, 300 μl of centrifugation precleared extracts were diluted in 1,200 μl of Dilution buffer (40 mM Tris HCl pH 8.0, 93.75 mM NaCl, 0.5 mM Na_3VO_4 and 50 mM NaF) and (b) complexes were washed four times in Wash Buffer (40 mM Tris HCl pH 8.0, 125 mM NaCl, 0.5 mM Na_3VO_4, 50 mM NaF and 0.1% Nonidet P40).

Native JARID1a and CLOCK-BMAL1 Co-IP

U2OS cells grown to confluence in 15-cm tissue culture plates were washed twice in PBS and lysed on ice with 3 ml Buffer I (10 mM HEPES pH 7.5, 10 mM KCl and 0.1% Triton X-100) supplemented with phosphatase and protease inhibitors (PhosSTOP and cOmplete EDTA-free protease inhibitors, Roche Cat#04906845001 and #11873580001) for five minutes. A crude nuclear pellet was collected by centrifugation at 4ºC, 800 *g* for 3 min and resuspended in Buffer II (10 mM HEPES pH 7.4, 50 mM NaF, 400 mM NaCl and 2 mM EDTA) supplemented with phosphatase and protease inhibitors. Following a 45 minute incubation on ice (with sporadic manual tumbling), the nuclear extract was cleared by centrifugation at 4ºC for 15 minutes at 20,800 *g* in a benchtop refrigerated centrifuge (Eppendorf). For immunoprecipitation, 1 part nuclear extract (300-500 μg total nuclear protein) was diluted in 4 parts Buffer III (10 mM HEPES pH 7.5, 50 mM NaF, 2 mM EDTA plus phosphatase and protease inhibitors) and incubated for a 2 h at 4 ºC while gently rocking. Simultaneously, 4 μg of the desired antibody was prebound to Protein G Dynabeads according to the manufacturer's instructions. Next, the immunoprecipitation-ready extract was incubated overnight with the antibody-bead complex at 4ºC while tumbling and subsequently washed four times with chilled 0.5X PBS, boiled in Laemmli sample buffer and subjected to SDS-PAGE-Immunoblot analysis.

Cell line generation

Jarid1a$^{+/+}$ and *Jarid1a*$^{-/-}$ fibroblasts were transfected with a destabilized luciferase reporter construct under the control of the *Per2* gene promoter as described in (*10*) (pGL4.22-Per2) (Promega) and stable transfectants selected in media supplemented with 2 μg/ml of puromycin.

siRNA transfections

U2OS cells stably expressing *Bmal1:Luc* reporter (*11*) were reverse transfected in 35-mm well format with HiPerFect transfection reagent (Qiagen). Briefly, cells were shocked with 100 nM of dexamethasone for 2 h,

trypsinized and resuspended in phenol-red free DMEM supplemented with 10% FBS, 1% Antibiotic-Antimycotic HEPES pH 7 (to 15 mM), glutamine, non-essential aminoacids, sodium pyruvate and 100 μM D-Luciferin. For each transfection 250,000 cells in 1.8 ml of media were seeded onto a 35-mm dish containing 200 μl of transfection mix (40 μl HiPerFect and 8 x 10^{-13} moles of siRNA diluted in serum-free media), sealed with vacuum grease. For qPCR analysis, transfected cells were harvested for RNA extraction 36-48 h post transfection and processed according to the RNeasy kit instructions (Qiagen).

Real-time bioluminescence

Circadian rhythms in reporter luminescence from cell population or individual cells were monitored and results analyzed as described in (*14*).

Expression and purification of JARID1a from Sf9 cells

To purify JARID1a, 500 ml of Sf9 cells in the log growth phase were infected with a baculovirus viral vector for recombinant Myc- and His-tagged JARID1a. 72 h post infection, cells were harvested by centrifugation, washed once in ice-cold PBS supplemented with 1 mM PMSF and subsequently resuspended in 20 ml of Homogenization Buffer (10 mM Tris-HCl pH 7.5, 500 mM NaCl, 10% v/v Glycerol, 0.1% Nonidet-P40, 10 mM Imidazole, 2 mM β-Mercaptoethanol, 2 mM PMSF plus cOmplete Protease Inhibitor cocktail), incubated on ice for 10 minutes with sporadic manual tumbling. Cells were then sonicated and lysis was monitored by visualization on a light microscope. Following sonication, the lysate was cleared by centrifugation and incubated for 2 h at 4ºC with 250 μl Ni-NTA (Qiagen) that had been equilibrated in Homogenization Buffer. The resin was then washed five times by tumbling for 5 minutes at 4ºC in 10 ml of Wash Buffer (10 ml Tris-HCl pH7.5, 200 mM NaCl, 10% glycerol, 0.2% Nonidet-P40, 15 mM Imidazole, 2 mM β-Mercaptoethanol, 2 mM PMSF and protease inhibitor cocktail). Bound proteins were then eluted with Elution Buffer (10 mM Tris-HCl pH7.5, 100 mM NaCl, 10% Glycerol, 0.1% Nonidet-P40, 250 mM Imidazole, 2 mM β-Mercaptoethanol , 1 mM PMSF and protease inhibitor cocktail) and collected in 300 μl fractions and thoroughly dialyzed against 10 mM HEPES pH 7.5, 75 mM NaCl, 5% Glycerol and 1 mM PMSF.

HDAC inhibition

Reactions in a final 50 μl volume were carried out as follows: First, 200 ng of HDAC1 (BPS Biosciences Cat# 50051) was pre-incubated for 25 minutes on ice with the desired amount of JARID1a (or an equivalent volume of

mock extracts) in a volume of 42 µl that contained 5 µl of 10X buffer (15 mM Phosphate Buffer pH 7.5, 15 mM HEPES pH 7.4, 5% glycerol, 40 mM KCl, 20 mM NaCl, 2 mM $MgCl_2$, 1mM NAD+ and BSA 80 ng/µl). After pre-incubation, 8 µl of acetylated histones (Millipore, Temecula, CA Cat# 17-305) suspension (10 mM HEPES pH 7.4, 5% glycerol, 75 ng/µl BSA and 0.5 µg/µl Acetylated Histones) were added to each reaction, followed by a 5 h incubation at 37ºC and then subjected to analysis via immunoblotting.

Drosophila stocks

Fly strains lid^{10424}/CyO and lid^{10424}/lid^{10424} were as described in (*15*). $y;ry^{506}$ was obtained from Dr. Allan Spradling. The *lid* transgene was generated by fusing a PCR-generated 4.5kb XhoI fragment containing the lid upstream region to a 4.8 kb Xho/NotI fragment containing the *lid* coding sequence in the P-element transformation vector pCasper4. The transgene was subsequently used to generate gLid, lid^{10424}/lid^{10424} transgenic flies by BestGene (http://www.thebestgene.com). All stocks were maintained and their circadian activities were monitored on standard cornmeal-molasses media at 22°C. $lid^{10424}/+$ and $CyO/+$ flies were generated by crossing lid^{10424}/CyO to $y;ry^{506}$.

Circadian activity assays of flies

Circadian locomotor activity measurements were made as described in (*16*). Male flies, 1–3 days old, were placed in glass tubes and entrained to a 12 h light-12 h dark cycle (LD) for 4 days and then released into constant dark conditions (DD) for at least a week. Locomotor activity was recorded with 30 minute bins using DAM2 Drosophila Activity Monitor system (Trikinetics, Waltham, MA). Only flies that had at least 14 days of recorded activity were analyzed. Data was analyzed using Clocklab software (Actimetrics, Wilmette, IL). Periodogram analysis was done by taking the DD data subjecting it to chi-square analysis as previously described (*17*) with the following modification: the locomotor activity of flies was categorized as low amplitude/weakly-rhythmic if their amplitude score was above the rhythmic threshold and was greater than 2 standard deviations below the average of the wild type flies. Daily activity profile was calculated using the last 3 days of LD where bins were normalized to the total activity for that day. An average of the normalized bins was plotted.

qPCR analyses of fly transcripts

Male files, 1–3 days old, were entrained to LD for 4 days and then released into DD for 4 days. Flies were collected at 4 h intervals starting at and frozen at -80°C. RNA extraction and qPCR measurements were carried out as described in (*16*).

Primers

Chromatin Immunoprecipitation

mmPer2+500_F	AGTGGTCCTTCCCCCAGGGC
mmPer2+500_R	CGCCGACTCCCATGGTGCTG
mmPer2-500_F	GGCCCCTCTGGATCTGCTGC
mmPer2-500_R	AATGACGGTCAGCCTGGGGG
hsPer2_E_Fwd	CCATTGGCCGCGACTCCGTC
hsPer2_E_Rev	GTAGGCCCCGCCCCTCATGT
hsPUMA-346F	GTCGGTCTGTGTACGCATCG
hsPUMA-346R	ACAGTCGGACACACACACTGACT

mRNA

dmCry_F	CCACCGCTGACCTACCAAA
dmCry_R	GGTGGAAGCCCAATAATTTGC
dmLid_F	GCACATCAATGGAAGGACAA
dmLid_R	CTTGAAGCTGGCCACAATCT
dmRP11_F	CGATCTGGGCATCAAGTACGA
dmRP11_R	TTGCGCTTCCTGTGGTTCAC
dmPer_F	CAGCAGCAGCCTAATCGH
dmPer_R	GAGTCGGACACCTTG
dmTim_F	GGTCGGTCTGGTGATCCCAG
dmTim_R	CCTTTTCGTACACAGATGCC
mmPer1_F	TGAAGCAAGACCGGGAGA
mmPer1_R	CACACACGCCATCACATCAA
mmDBP_F	CCTGAGGAACAGAAGGATGA
mmDBP_R	ATCTGGTTCTCCTTGAGTCTTCTTG
mmRORg_F	GACCCACACCTCACAAATTGA
mmRORg_R	AGTAGGCCACATTACACTGCT
mmRORa_F	CTCCAGCCGAGGTGACTTTTA

mmRORa_R	AGCTTGTGAGTTCTCCTTCCT
mmRevERBa_F	TACATTGGCTCTAGTGGCTCC
mmRevERBa_R	CAGTAGGTGATGGTGGGAAGTA
mmPer2_F	GAAAGCTGTCACCACCATAGAA
mmPer2_R	AACTCGCACTTCCTTTTCAGG
mmCry2_F	TGGGCATCAACCGATGGA
mmCry2_R	CCCATTCCTTGCACAGCCTTG
mmCry1_F	CTGAAGCAAAAATCGCCACCT
mmCry1_R	CACTGGTTCCGAAAGGGACTC
mmGAPDH_F	AGACAGCCGCATCTTCTTGT
mmGAPDH_R	CTTGCCGTGGGTAGAGTCAT
mmActin_F	GGCTGTATTCCCCTCCATCG
mmActin_R	CAGTTGGTAACAATGCCATGT
mmSDF1_F	ATCAGTGACGGTAAACCA
mmSDF1_R	TTCTTCAGCCGTGCAACAATC
mmBmal1_F	CTCGACACGCAATAGATGGGA
mmBmal1_R	CTTCCTTGGTCCACGGTTT
mmJarid1a_F	CACAGACCCGCTGAGTTTTAT
mmJarid1a_R	CTTCACAGGCAAATGGAGGTT

Supplementary Figure Legends

Fig. S1. Schematic representation of JARID1 class of proteins. Genbank accession number and the number of amino acid in each protein are shown. Major domains or motifs are drawn to scale. For simplicity, the PLU-1 homology region of JARID1a is not illustrated. JmjN, jumonji N domain; ARID, AT-rich interactive domain; PHD, plant homeotic domain; JmjC, jumonji domain C; Znf, zinc finger domain.

Fig. S2. mRNA expression of *Jarid1a* in mouse liver. RT-qPCR quantified mRNA levels of *Per2*, *Bmal1* and *Jarid1a* from mouse liver sampled at 3 h interval were normalized against *Gapdh* mRNA. The minimum level over 24 h was further normalized to 1 and average (± SEM, n = 3 mice) relative mRNA expression over a full circadian day are plotted. Both *Per2* and *Bmal1* mRNA show robust circadian oscillation with more than 10 fold change between peak and trough levels. *Jarid1a* mRNA shows less than 2 fold change throughout the day.

Fig. S3. JARID1a co-immunoprecipitates CLOCK and BMAL1. HEK293T cells were transfected with Myc-tagged JARID1a in the presence or absence of Flag-tagged CLOCK and BMAL1. Lysates were then subject to immunoprecipitation (IP) with anti-Myc (9E10) monoclonal antibodies followed by immunoblot (IB) analysis.

Fig. S4. JARID1a enhances CLOCK-BMAL1 activity from both *Per1* and *Per2* promoter. (A) Co-transfection of JARID1a cDNA enhances CLOCK-BMAL1-mediated transcription from *Per1:Luc* reporter in HEK293T cells. Increasing dose of JARID1a cDNA correspondingly increases CLOCK-BMAL1 activity at *Per1* promoter. Average luciferase activity (+ SEM, n=4) from cells transfected with 25 ng *Per1:Luc* reporter, 30 ng CLOCK, 12.5 ng BMAL1 cDNA and 0, 120, 150 and 180 ng of JARID1a cDNA topped to 250 ng total DNA with empty pcDNA3.1 vector were transfected to 40,000 cells (*12*). **(B)** Full-length cDNA encoding JARID1a protein with a H483A mutation (MUT) that abolishes its demethylase activity (*7*) also co-activates CLOCK-BMAL1-mediated transcription from *Per2:Luc* reporter gene in HEK293T cells. **(C)** Both WT and MUT JARID1a co-activate CLOCK-BMAL1 activation of *Per1* transcription in *Jarid1a*$^{-/-}$ cells. As anticipated co-transfection of CRY1 cDNA represses *Per1* transcription. Expression of JARID1a MUT enhanced *Per1* transcription better than the WT JARID1a. **(D)** Effect of CLOCK, BMAL1 and JARID1a on the Ets-specific E74 binding site-Luciferase (E74-Luc) reporter construct.In **A-D**, representative results from at least three independent experiments are shown. Average luciferase activity (+ SEM, n=4) are plotted.

Fig. S5. Jmj domains of JARID1a are not necessary for enhancing CLOCK-BMAL1 function. (A) Schematic diagram showing full-length JARID1a protein or various truncations tested for co-activation of CLOCK-BMAL1

function. Jumonji N (JmjN), JmjC, ARID, plant homeo domain (PHD), zinc finger (Znf) domains and PLU-1 homology regions are shown. Numbers represent amino acid position in the full-length mouse JARID1a protein (NP_666109). **(B)** *Per2:Luc* reporter expression in HEK293T cells co-transfected with cDNAs encoding CLOCK, BMAL1 and full-length or various truncated mutants of JARID1a C-terminally tagged with Myc epitope. Average luciferase activity (+ SEM, n=3) from one of three independent experiments are shown. Mutants lacking the Jmj domains, but retaining the C-terminus PLU-1 homology region and PHD motifs enhanced CLOCK-BMAL1 activity. **(C)** Immunoblot analysis of full-length or truncated JARID1a protein shows no remarkable reduction in expression of any of the truncation mutants. **(D)** Effect of the indicated JARID1a constructs on the abundance of acetylated Lysine 9 of histone 3 at the Per2 E-box region. Results from three independent experiments are show as normalized to vector (value= 0.25 to 0.3% input) for ease of comparison (* $p \leq 0.05$) **(E)** JARID1a (WT), demethylase mutant (MUT) and $JARID1a_{600\text{-}1636}$ but not $JARID1a_{1\text{-}600}$ are able to relieve HDAC1 repression of a Per2-Luciferase reporter construct (** $p \leq 0.01$).

Fig. S6. JARID1-b and -c repress CLOCK-BMAL1 activity. (A) Co-expression of increasing levels of JARID1b or JARID1c correspondingly repressed CLOCK-BMAL1 mediated transcription from *Per2:Luc* reporter in HEK293T cells. Figure for JARID1a is re-plotted from *Fig. 1E*. **(B)** A composite plot of results shown in *fig. S6A* showing JARID1a activated while JARID1b and JARID1c repressed CLOCK-BMAL1 activity. **(C)** Overexpression of JARID1b and JARID1c attenuated H3K4me3 levels at *Per2* promoter. JARID1a enhanced H3K4me3.

Fig. S7. JARID1a expression is required for normal circadian rhythm in *Per2* or *Bmal1* transcription. **(A)** Raw luciferase counts of $Jarid1a^{+/+}$ (WT, blue) or $Jarid1a^{-/-}$ (MUT, red) mouse fibroblasts (mean $\pm$ SEM, n=3) expressing *Per2:Luc* reporter gene. **(B)** Average (*top*) and representative individual traces (*bottom*) from U2OS-*Bmal1:Luc* cells transfected with either *Jarid1a* siRNA (red) or scrambled siRNA (black).

Fig. S8. The circadian oscillators in individual $Jarid1a^{-/-}$ cells show short period rhythm. (A) Heat map rendering of *Per2:Luc* luminescence from $Jarid1a^{+/+}$ or $Jarid1a^{-/-}$ cells collected every 30 min over 3.5 days. Yellow represents peak and purple represents trough levels of *Per2:Luc* bioluminescence. **(B)** Average (+ SEM, n= 15-20 cells) period length of circadian oscillation in *Per2:Luc* expression in $Jarid1a^{+/+}$ or $Jarid1a^{-/-}$ cells are shown. **(C)** Scatter plot of period length and amplitude estimates of *Per2:Luc* oscillation in individual cells and the average ($\pm$ SEM) estimates for each genotype are shown. **(D)** Average (+ SEM) of median normalized bioluminescence from

individual cells shown in *fig. S8A* recapitulates the population levels short period phenotype of *Jarid1a*$^{-/-}$ cells shown in *Fig. 2*.

Fig. S9. The mRNA expression levels of oscillator components are altered in *Jarid1a*$^{-/-}$ cells. The mRNA levels of *Rev-erbα*, *Rorγ*, and *Bmal1* from *Jarid1a*$^{+/+}$ (black) or *Jarid1a*$^{-/-}$ (red) were quantified by RT-qPCR, normalized to *Gapdh* mRNA and plotted as levels relative to minimum value normalized to 1. Average (±, SEM, n=3) levels at 3 h intervals starting at 48 h after cell synchronization are shown.

Fig. S10. Re-introduction of wild-type or demethylase-mutant JARID1a into *Jarid1a*$^{-/-}$ cells rescues period length and improves robustness of oscillations. (A) Period estimates for *Jarid1a*$^{+/+}$ and *Jarid1a*$^{-/-}$ cells transfected with a *Per2* promoter-driven luciferase construct and either empty vector or expression plasmids for the indicated JARID1a constructs. In the presence of either wild-type (WT) or mutant (MUT) JARID1a, the period length is significantly longer than in *Jarid1a*$^{-/-}$ cells (* P<0.03, ** P<0.02). **(B)** Real-time luminescence traces for *(top)* control *Jarid1a*$^{+/+}$ and *Jarid1a*$^{-/-}$ cells, *(middle)* wild-type- or *(bottom)* demehtylase-mutant JARID1a-transfected *Jarid1a*$^{-/-}$ cells.

Fig. S11. Transgenes used for the restoration of Lid expression in *lid*^{10424} flies. (A) Schematic diagram of the genomic region encoding Lid, the P-element insertion in *lid*^{10424} mutant, and the genomic copies of Lid or Lid-JmjC*and upstream promoter region used for generating the gLid rescue constructs. Size of the P-element insertion is not to scale. **(B)** Immunoblot analysis of Lid, H3K4me3, and histone H3 in WT *y;ry, lid*^{10424}*/lid*^{10424}*;gLid, lid*^{10424}*/lid*^{10424}*;gLid-JmjC** and *lid*^{10424}*/lid*^{10424} flies. Lid protein in *lid*^{10424}*/lid*^{10424} flies is detectable and is ~10% of that in the WT *y;ry* flies. Near complete restoration of Lid expression and reduction in H3K4me3 levels are achieved in *lid*^{10424}*/lid*^{10424}*;gLid* flies whereas in demethylase mutant flies (*lid*^{10424}*/lid*^{10424}*;gLid-JmjC**) H3K4me3 levels remain elevated. Total protein from 10 dissected wing discs from 3rd instar larvae was used in each lane of the immunoblot.

Fig. S12. H3K4 methylation level and HDAC1 occupancy at *Per* promoter in *Jarid1a*$^{-/-}$ cells. (A) ChIP-qPCR estimate of H3K4me3 levels at the E-box of *Per2* promoter in *Jarid1a*$^{+/+}$ and *Jarid1a*$^{-/-}$ cells shows increased H3K4me3 level in the absence of JARID1a (** P < 0.001). **(B)** The change in H3K4me3 levels does not significantly (P > 0.05) affect HDAC1 occupancy at *Per2* promoter. Average (+ SEM, n=3) levels from a representative experiment out of three independent trials are shown. **(C)** Abundance of histone 3 Lysine 9 acetylation at a control (PUMA -346) promoter region is unaffected by JARID1a.

Fig. S13. JARID1a co-immunoprecipitates with HDAC1. Immunoblot (IB) analyses of immunoprecipitates (IP) from HEK293T cells expressing Flag-tagged HDAC1 and Myc-tagged JARID1a. JARID1a or HDAC1 protein complexes isolated with respective antibodies, or the input total cell lysate were probed for the presence of JARID1 and HDAC1.

Fig. S14. HDAC1 represses *Per2* expression. Co-transfection of increasing amount of HDAC1 cDNA reduces CLOCK-BMAL1-mediated *Per2:Luc* reporter expression in a dose-dependent fashion. Average luciferase activity (+ SEM, n=3) from HEK293T cells transfected with the indicated plasmids or empty vectors are shown.

Fig. S15. HDAC1 inhibition in WT *Jarid1a*$^{+/+}$ cells. Normalized real-time luminescence from *Jarid1a*$^{+/+}$ cells stably expressing *Per2:Luc* treated with DMSO or increasing concentration of HDAC inhibitor TSA. The reduction in amplitude and increase in period length of *Per2* expression was observed. Average luminescence (n = 4) measured every 10 min over 4.5 days are shown.

Fig. S16. His-tagged JARID1a purified from Sf9 cells. Coomasie-stained gel showing purified JARID1a, mock elutes and reference BSA.

Fig. S17. Validation of anti-BMAL1 (Santa Cruz) and anti-JARID1a (Bethyl) antibodies by immunoblot and chromatin immunoprecipitation analysis. (A) JARID1a is detected in extracts derived from WT but not *Jarid1a*$^{-/-}$ cells by immunoblot. Similarly, JARID1a is present at the non-circadian gene *SDF1* promoter in WT and *Bmal1*$^{-/-}$ but not in *Jarid1a*$^{-/-}$ cells. **(B)** BMAL1 is detected by immunoblot in extracts derived from WT but not *Bmal1*$^{-/-}$cells. Chromatin immunoprecipitations demonstrate the suitability of the anti-BMAL1 antibody for this assay. BMAL1 is present at the *Per2* promoter E-box in WT but not in *Bmal1*$^{-/-}$ cells.

Figure S1

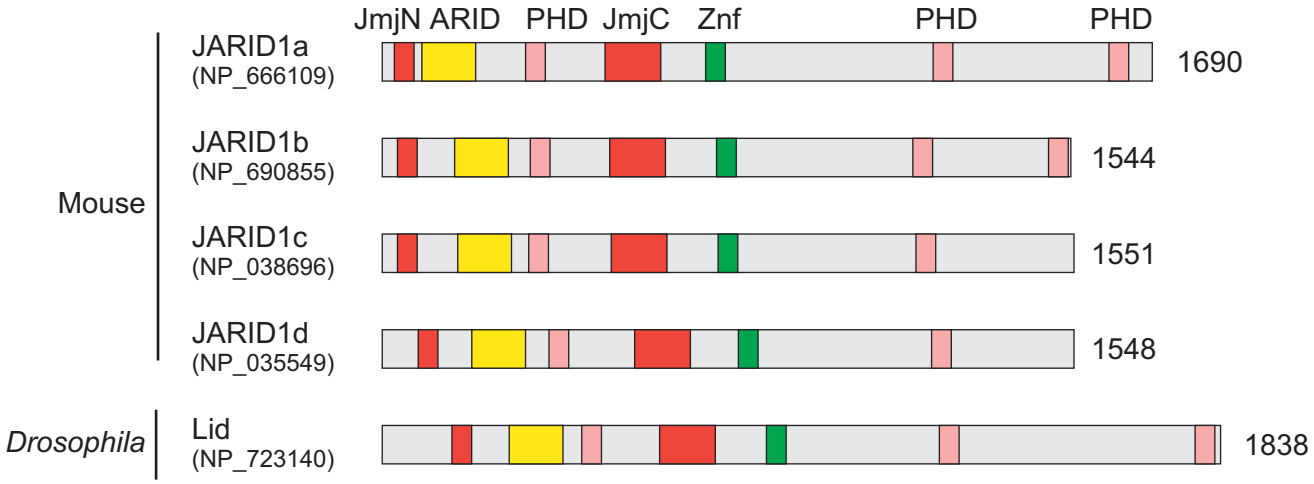
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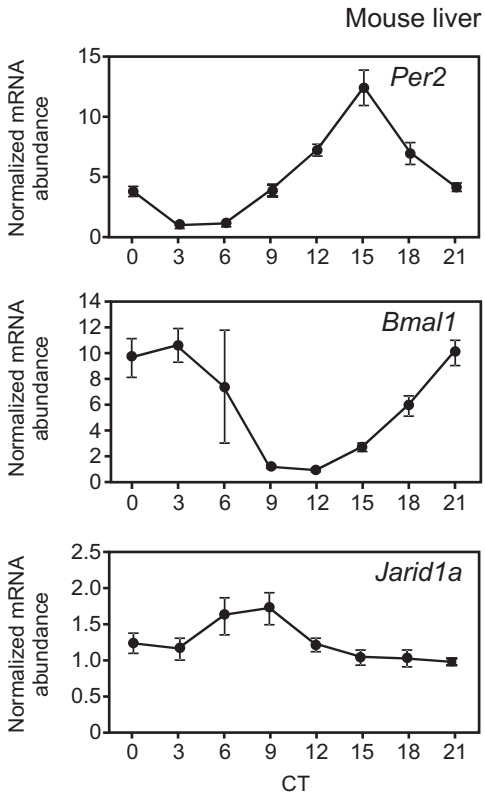
Figure S2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Bmal1
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Normalized mRNA abundance
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6
9
12
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18
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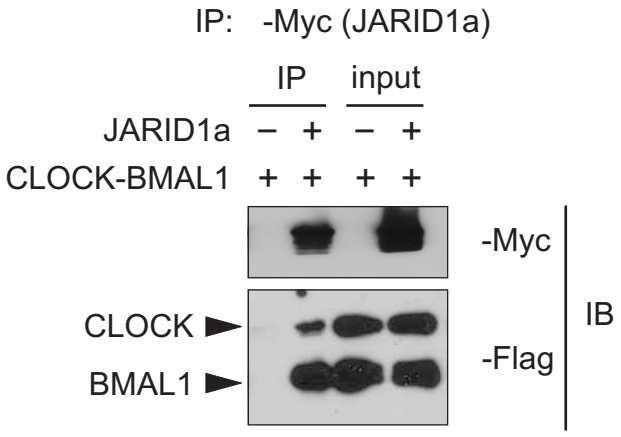
Figure S3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IB

Figure S4

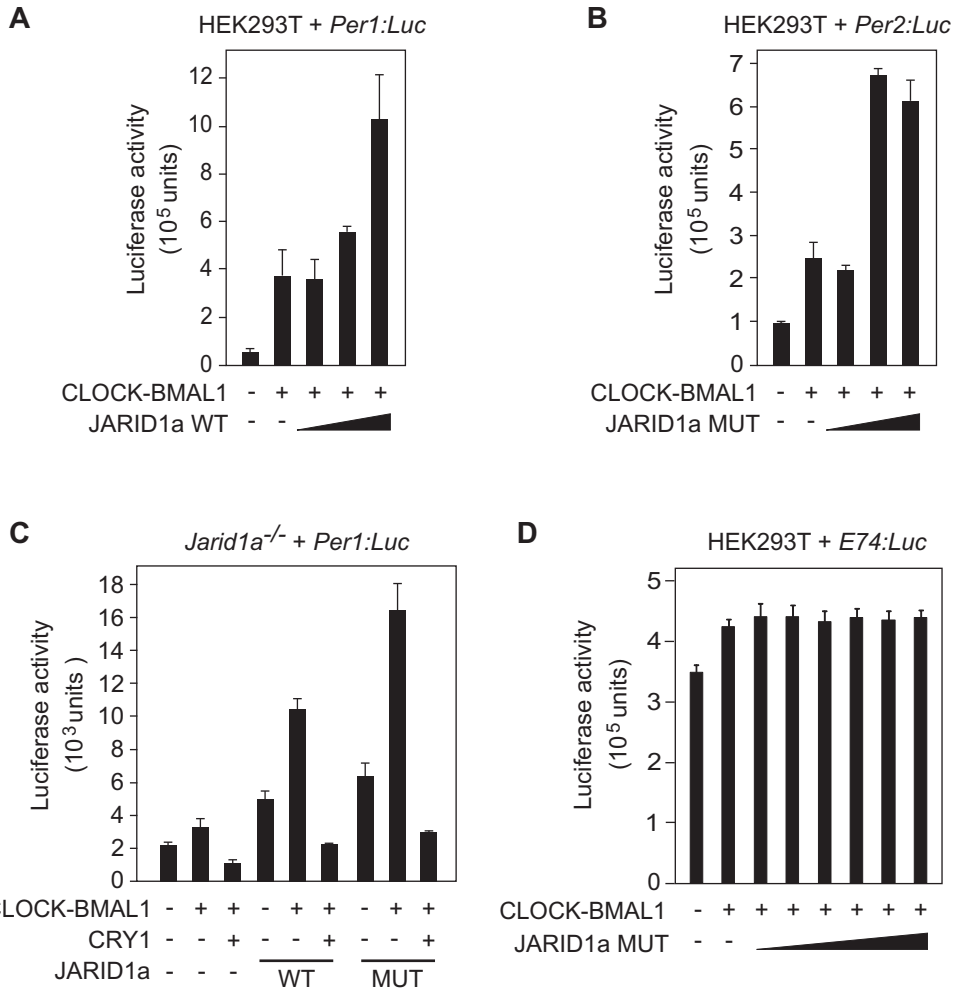

A
HEK293T + Per1:Luc
Luciferase activity (10^5 units)
CLOCK-BMAL1
JARID1a WT
B
HEK293T + Per2:Luc
Luciferase activity (10^5 units)
CLOCK-BMAL1
JARID1a MUT
C
Jarid1a-/- + Per1:Luc
Luciferase activity (10^3 units)
CLOCK-BMAL1
CRY1
JARID1a
WT
MUT
D
HEK293T + E74:Luc
Luciferase activity (10^5 units)
CLOCK-BMAL1
JARID1a MUT

Figure S5

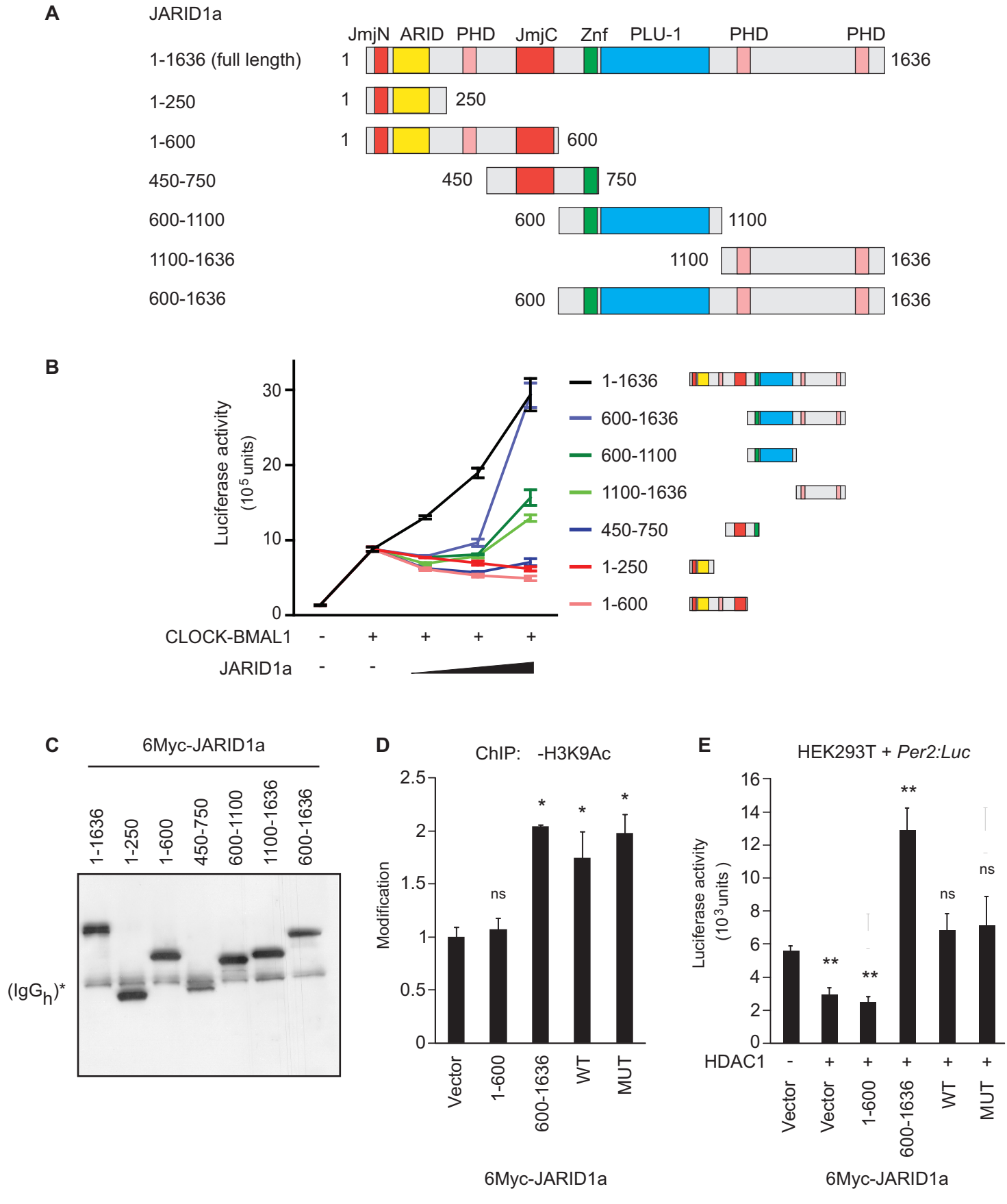

A
JARID1a
JmjN ARID PHD JmjC Znf PLU-1 PHD PHD
1-1636 (full length)
1-250
1-600
450-750
600-1100
1100-1636
600-1636
1
1636
250
600
450
750
1100
B
Luciferase activity
(10^5 units)
CLOCK-BMAL1
JARID1a
1-1636
600-1636
600-1100
1100-1636
450-750
1-250
1-600
C
6Myc-JARID1a
(IgG_h)*
D
ChIP: -H3K9Ac
Modification
Vector
1-600
600-1636
WT
MUT
6Myc-JARID1a
E
HEK293T + Per2:Luc
Luciferase activity
(10^3 units)
HDAC1
Vector
1-600
600-1636
WT
MUT
6Myc-JARID1a
ns

Figure S6

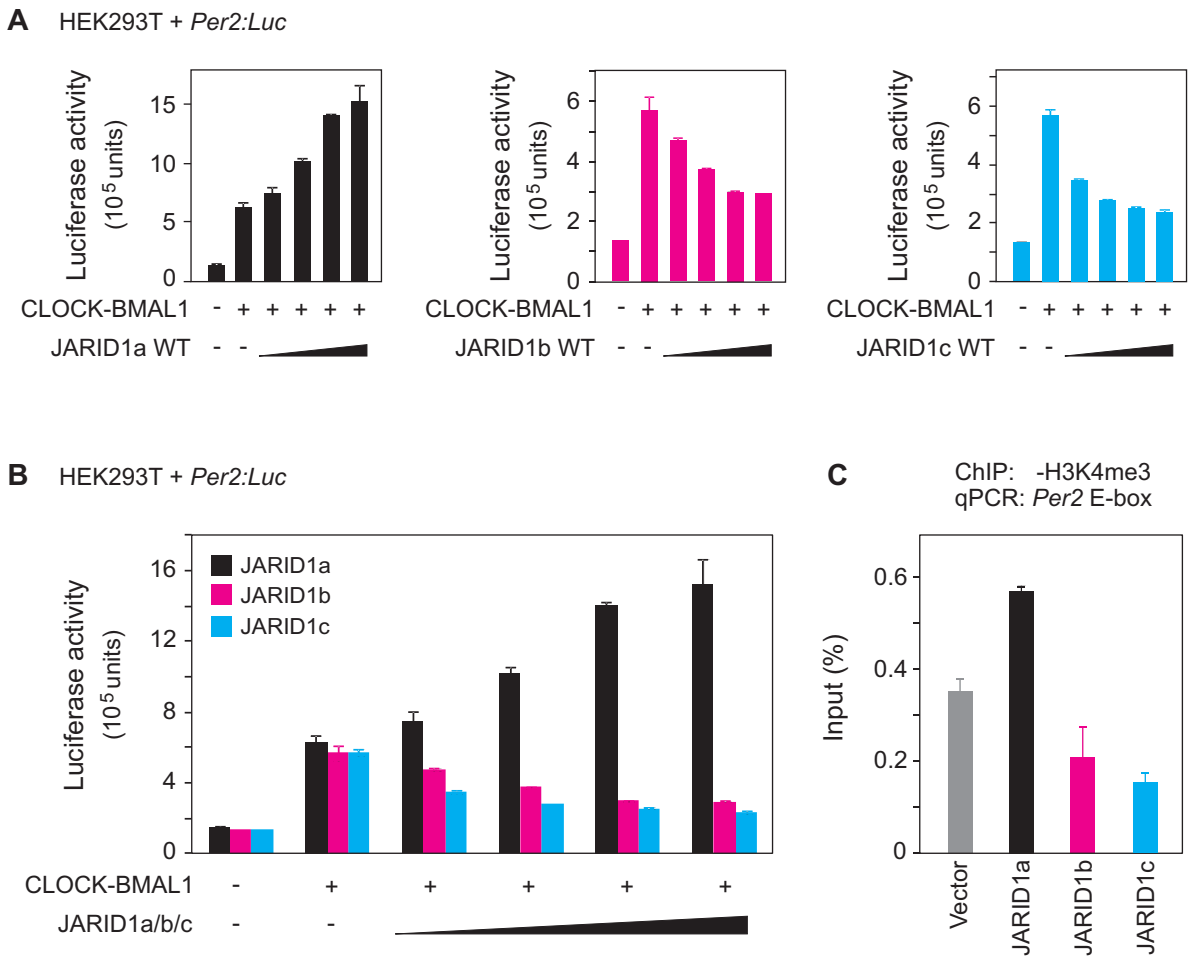

A
HEK293T + Per2:Luc
Luciferase activity
(10^5 units)
CLOCK-BMAL1
JARID1a WT
JARID1b WT
JARID1c WT
B
HEK293T + Per2:Luc
JARID1a
JARID1b
JARID1c
JARID1a/b/c
C
ChIP: -H3K4me3
qPCR: Per2 E-box
Input (%)
Vector
JARID1a
JARID1b
JARID1c

Figure S7

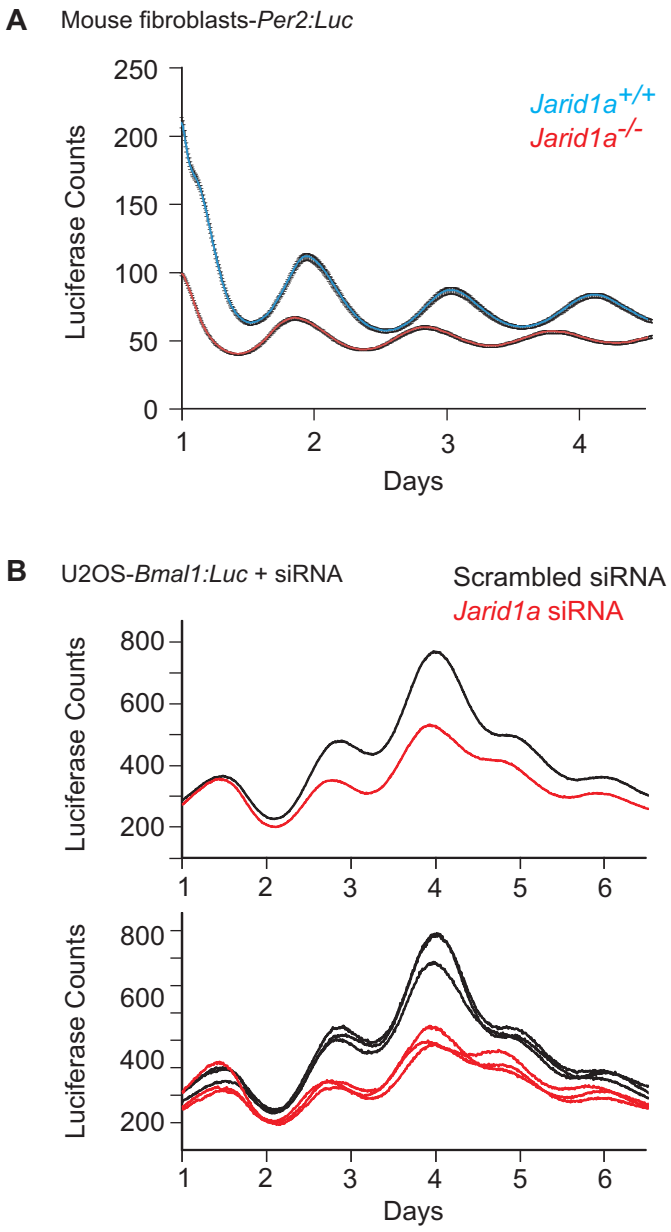

A
Mouse fibroblasts-Per2:Luc
Jarid1a+/+
Jarid1a-/-
Luciferase Counts
250
200
150
100
50
0
1
2
3
4
Days
B
U2OS-Bmal1:Luc + siRNA
Scrambled siRNA
Jarid1a siRNA
Luciferase Counts
800
600
400
200
1
2
3
4
5
6
Days

Figure S8

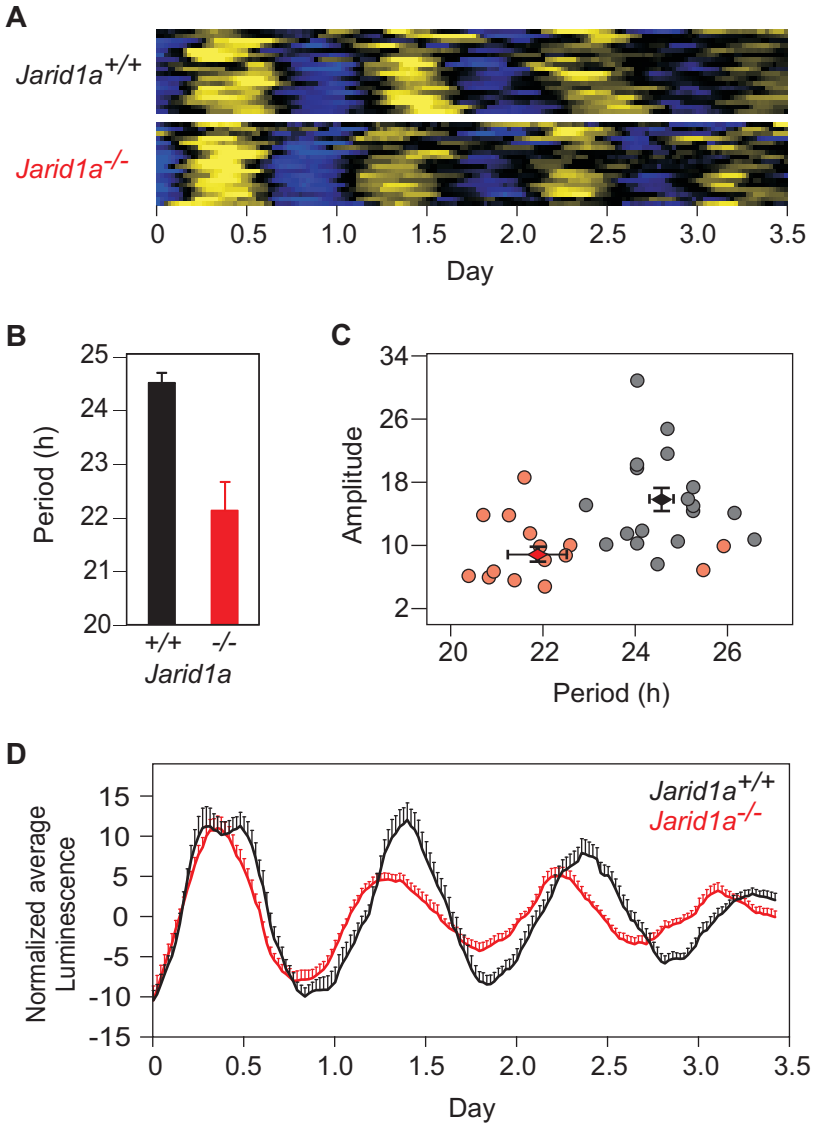

A
Jarid1a+/+
Jarid1a-/-
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Day
B
Period (h)
25
24
23
22
21
20
+/+
-/-
Jarid1a
C
Amplitude
34
26
18
10
2
20
22
24
26
Period (h)
D
Normalized average Luminescence
15
10
5
0
-5
-10
-15
Jarid1a+/+
Jarid1a-/-
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Day

Figure S9

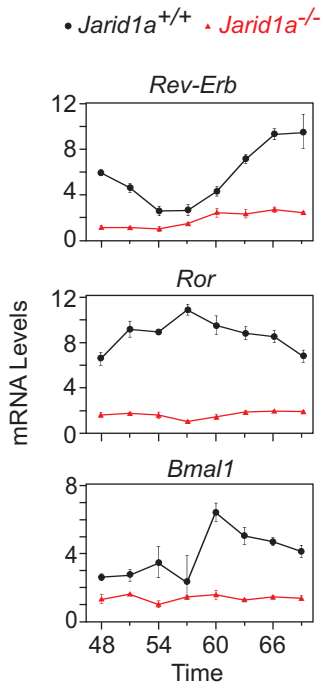

Jarid1a+/+
Jarid1a-/-
Rev-Erb
Ror
Bmal1
mRNA Levels
Time
0
4
8
12
48
54
60
66

Figure S10

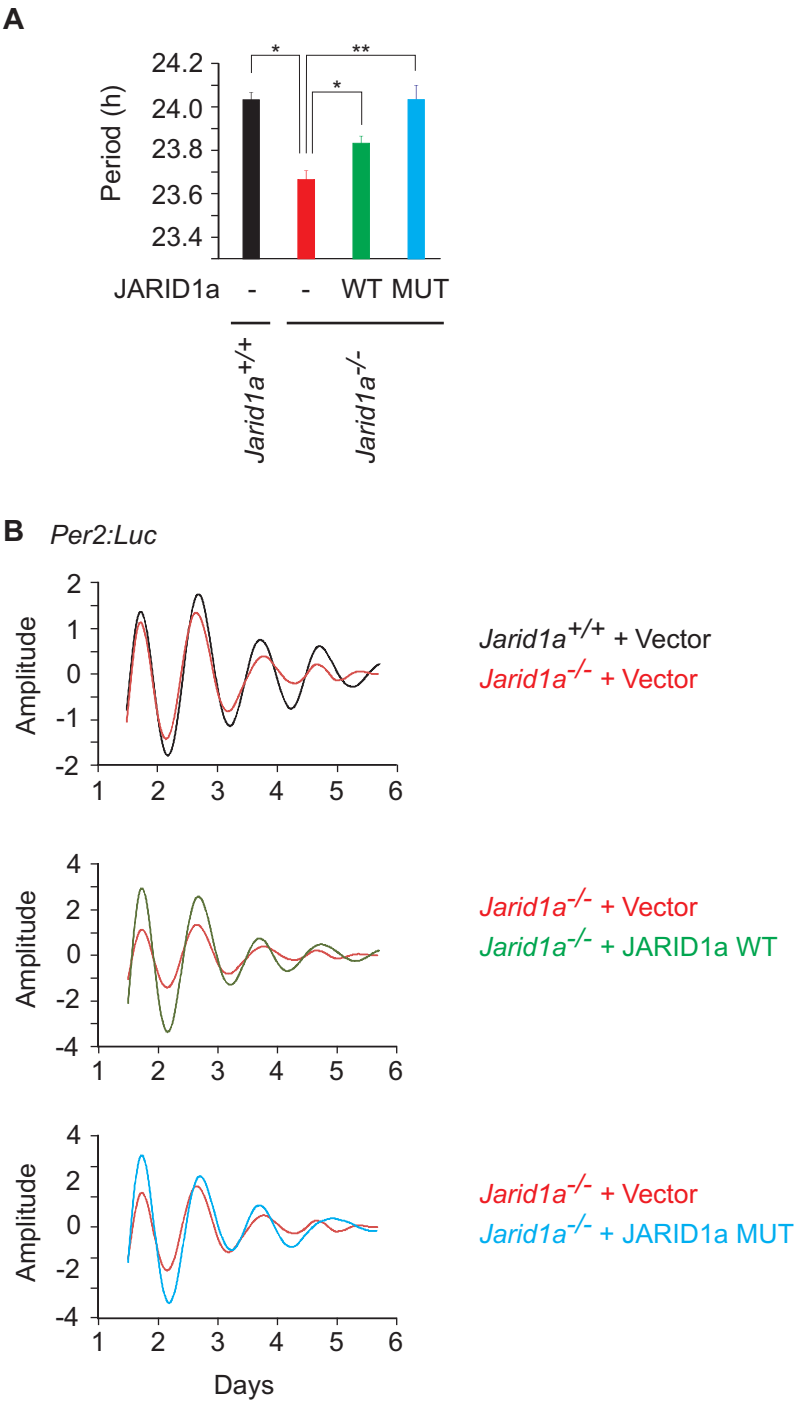
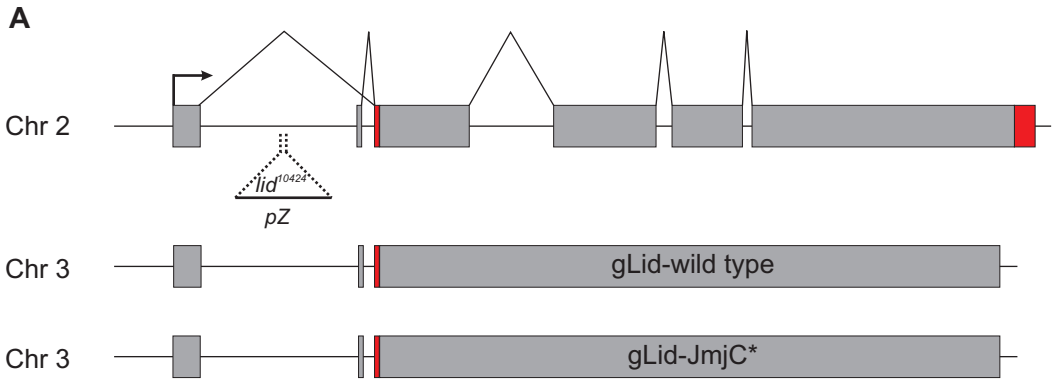

A
Period (h)
24.2
24.0
23.8
23.6
23.4
*
**
*
JARID1a - - WT MUT
Jarid1a+/+
Jarid1a-/-
B
Per2:Luc
Amplitude
Days
Jarid1a+/+ + Vector
Jarid1a-/- + Vector
Jarid1a-/- + Vector
Jarid1a-/- + JARID1a WT
Jarid1a-/- + Vector
Jarid1a-/- + JARID1a MUT

Figure S11


A
Chr 2
lid10424
pZ
Chr 3
gLid-wild type
Chr 3
gLid-JmjC*

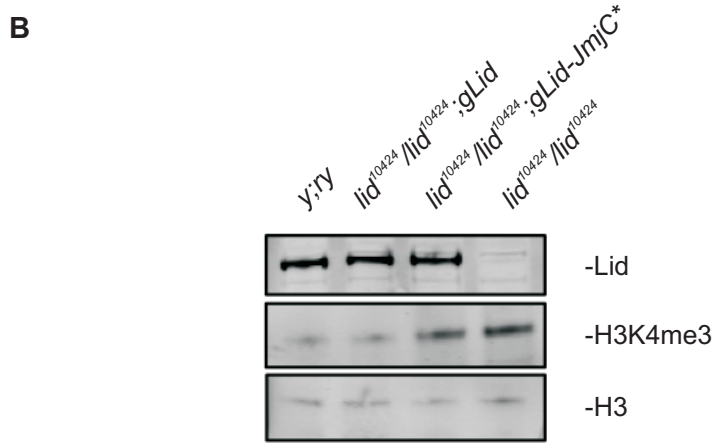

B
y;ry
lid10424/lid10424;gLid
lid10424/lid10424;gLid-JmjC*
lid10424/lid10424
-Lid
-H3K4me3
-H3

Figure S12

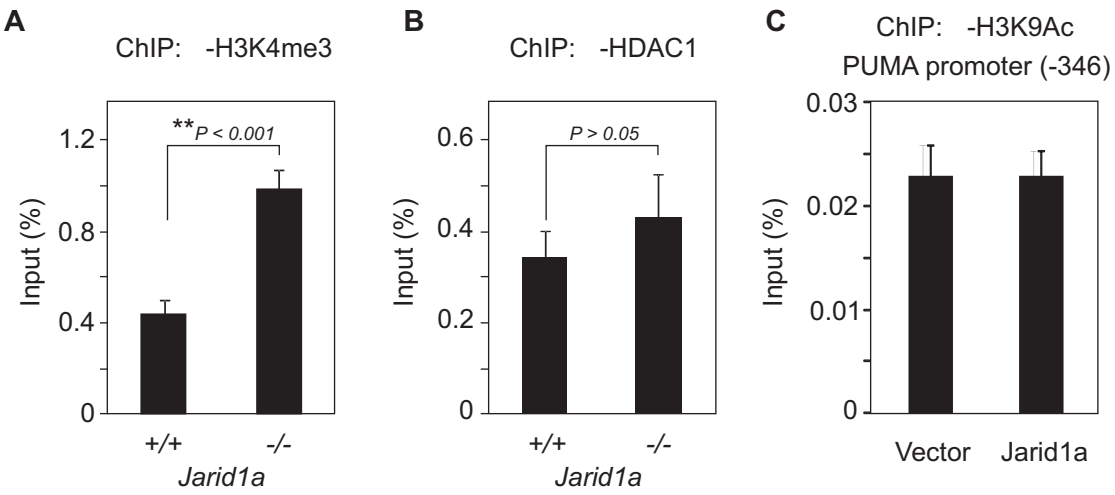

A
ChIP: -H3K4me3
**P < 0.001
Input (%)
1.2
0.8
0.4
0
+/+
-/-
Jarid1a
B
ChIP: -HDAC1
P > 0.05
Input (%)
0.6
0.4
0.2
0
+/+
-/-
Jarid1a
C
ChIP: -H3K9Ac
PUMA promoter (-346)
Input (%)
0.03
0.02
0.01
0
Vector
Jarid1a

Figure S13

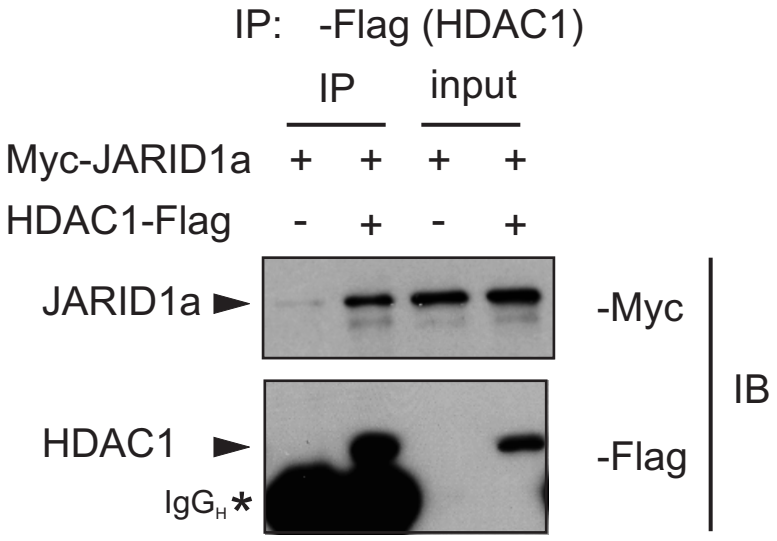

IP: -Flag (HDAC1)
IP
input
Myc-JARID1a + + + +
HDAC1-Flag - + - +
JARID1a
HDAC1
IgG$_H$ *
-Myc
-Flag
IB

Figure S14

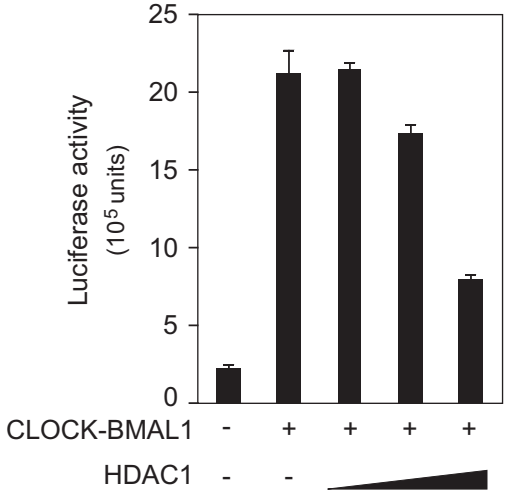

25
20
15
10
5
0
Luciferase activity
(10^5 units)
CLOCK-BMAL1 - + + + +
HDAC1 - -

Figure S15

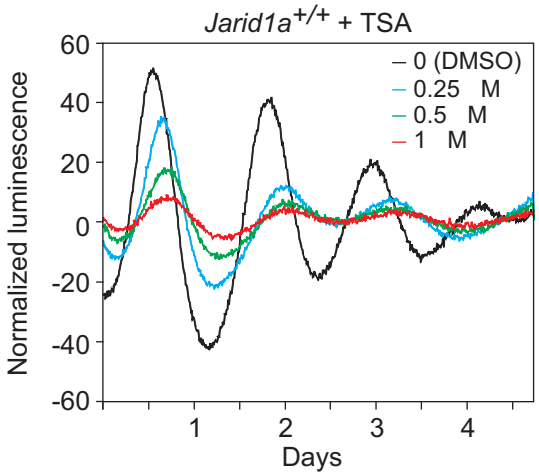

Jarid1a+/+ + TSA
0 (DMSO)
0.25 M
0.5 M
1 M
Normalized luminescence
60
40
20
0
-20
-40
-60
1
2
3
4
Days

Figure S16

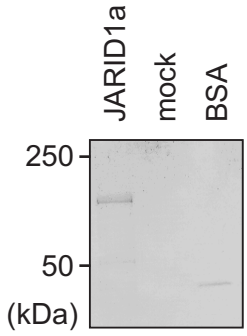
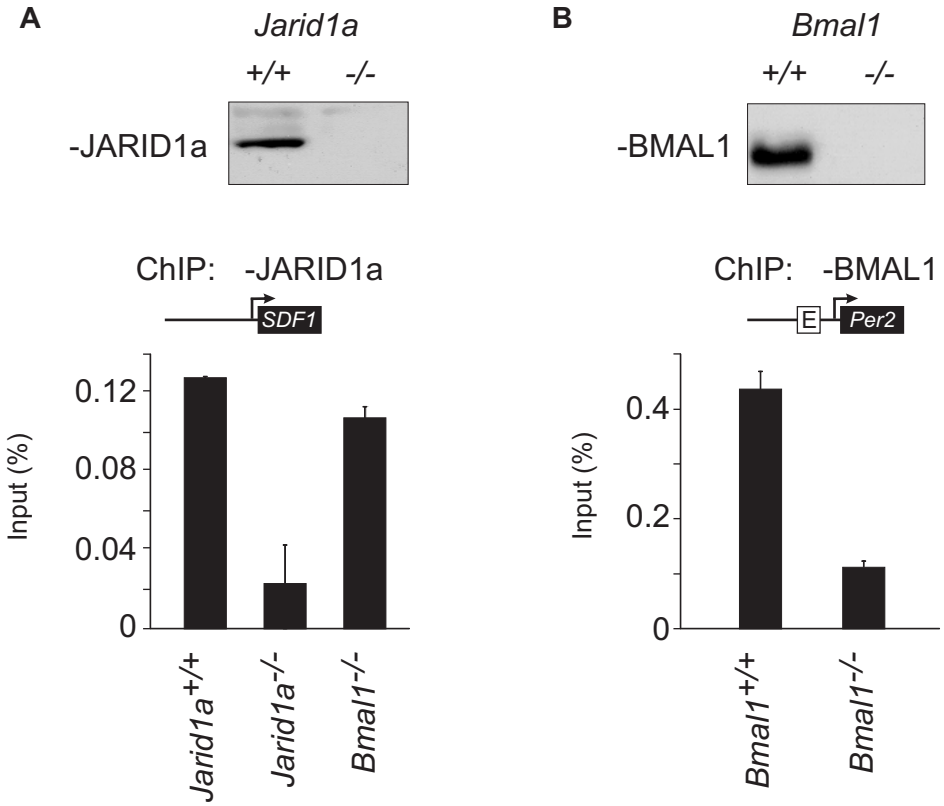


Figure S17


A
Jarid1a
+/+
-/-
-JARID1a
ChIP: -JARID1a
SDF1
Input (%)
0.12
0.08
0.04
0
Jarid1a+/+
Jarid1a-/-
Bmal1-/-
B
Bmal1
+/+
-/-
-BMAL1
ChIP: -BMAL1
E
Per2
Input (%)
0.4
0.2
0
Bmal1+/+
Bmal1-/-

References and Notes

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