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Supplementary Information for

A Genome-wide microRNA screen identifies the microRNA-183/96/182 cluster as a modulator of circadian rhythms

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SI Materials and Methods

Animals

All animal care and experimental procedures complied with University of Southern California guidelines for the care and use of animals and were approved by the University of Southern California Animal Care and Use Committee under protocol #20826.

The *miR-183/96/182* cluster gene trap mice (miR183C GT) were obtained as a gift from Dr. Changchun Xiao at Scripps Research. The *miR-183C*$^{GT/GT}$; *Per2*^{Luc} reporter mice were generated by crossing *miR-183C*$^{GT/+}$ mice with *Per2*^{Luc} knock-in mice. All mice were housed in a temperature- and humidity-controlled room with food and water *ad libitum*. For time-course tissue dissections, mice of each genotype at 2–3 months of age were euthanized by isoflurane at each circadian time point, and the tissues were isolated, snap-frozen, and stored at -80°C until processing for mRNA or protein analyses. For dissections of tissues during the dark phase, euthanasia of mice and dissections of eyes or retinas were performed under infrared illumination by a red lamp, and then the other tissues were dissected under the regular lights.

Wheel-Running Activity

Mice were individually housed in cages equipped with running wheels, with food and water *ad libitum*. Mice were first entrained under 12 h/12 h light/dark conditions for 2 weeks, and then were released to constant darkness for 3 weeks. A ClockLab system (Actimetrics) was used to collect wheel-running activity, and control the timing of light changes. LED lights were given as a square wave 12 h/12 h light/dark cycle with an irradiance of 5 W/m^2 during the light phase.

Total Activity

Locomotor activity was measured in polycarbonate cages (42 × 22 × 20 cm) placed into frames (25.5 × 47 cm) mounted with two levels of photocell beams at 2 and 7 cm above the bottom of the cage (San Diego Instruments, San Diego, CA, USA). These two sets of beams allowed for the recording of both horizontal (locomotion) and vertical (rearing) behaviors. The total activity was calculated as the sum of both types of behaviors. The mice were housed in the activity boxes with food and water *ad libitum*. They were first given 1 day to habituate to the testing environment. Immediately following this habituation, the mice were tested under a standard 12 h light / 12 h dark cycles for 7 days, and then constant darkness for 14 days.

miRNA screen

Per2-dLuc and *Bmal1-dLuc* reporters cells (1) were reverse transfected with miRNAs in 384-well plates. Briefly, 20 μl of transfection reagent mixture (0.045 μl RNAiMax in Opti-MEM) was added to each well which was pre-spotted with miRNA (0.4 pmol). After 5 minutes of incubation at room temperature, 20 μl of cells (2500 cells/well) were added on top of the transfection mixture, and then were incubated at 37°C in 5% CO_2 condition for 48 hours before recording. To record bioluminescence of the cells, the old medium was replaced with 60 μl HEPES-buffered explant medium supplemented with luciferin (1 mM) (GoldBio), 10% FBS and antibiotics. Plates were sealed with an optically clear film and were recorded in a plate reader (Synergy, BioTek) at a 2-hour interval for 5 days under 35°C condition. AllStars Negative Control siRNA, which does not target any gene in mammalian cells and therefore has no effects on circadian rhythms, was used as the negative control. An siRNA targeting luciferase was used as the luminescence signal control, and an siRNA targeting ubiquitous human cell survival genes was used as the cell survival control. Some miRNAs that were reported to modulate circadian rhythms (2–7) were screened in the U2OS reporter cells for positive controls prior to the

genome-wide miRNA screen. Among them, none of the miRNAs had significant and consistent effects on circadian period lengths, but only miRNA-142-3p robustly lowered the amplitude by targeting BMAL1 (2, 3). We therefore used miRNA-142-3p as a positive control for amplitude. CRY2 siRNA was used as another positive control for period length. The MultiCycle program (Actimetrics, Inc.) was used to analyze circadian bioluminescence data. Data were normalized (running average 24 hour methods) to detrend the baseline, and then the circadian parameters such as period length and amplitude were estimated using the best-fit sine wave analysis.

CRISPR-Cas9 knockout of miRNAs in human cells

The knockout of miRNA was performed by the CRISPR-Cas9 system with paired synthetic crRNAs. Synthetic crRNAs were designed by the Dharmacon online CRISPR Design Tool. The high specificity scoring crRNAs which targeted sequences either in or close to the miRNA stem-loop were selected. Cells of a clonal U2OS *Per2-dLuc* cell line were seeded in a 96-well plate at a density of 10,000 cells per well one day prior to the transfection. Two pairs of 50 nM crRNA:tracrRNA complex, and 200 ng Cas9 (PuroR) plasmid which express a puromycin resistant gene were co-transfected by 0.4 μL/well DharmaFECT Duo reagent (GE Healthcare Dharmacon, Cat #T-2010-03). After 48 hours of transfection, the medium was replaced with complete medium containing 0.5 μg/mL puromycin, and cells were incubated for 3 days. Single-cell cloning was performed by serial dilution. The genotypes of single-cell clones were first determined by PCR and 2% agarose gel electrophoresis. The positive clones were further validated by Sanger sequencing. Sequences of crRNAs and PCR primers flanking the cleavage sites are listed in SI Appendix, Table S5.

Luciferase Assays

In a 384-well plate, the wells were pre-coated with 0.02% L-Lysine solutions, and then HEK-293T cells were plated at a density of 4000 cells/well. After 24 hours, the cells were co-transfected with 10 ng 3'UTR reporter plasmid and 20 nM miRNA mimics per well. The transient transfections were performed using 0.16 µl of DharmaFECT DUO (Dharmacon) per well. Luciferase activity was measured 48 hours after transfection using the Bright-Glo™ Luciferase Assay System (Promega, Cat #E2610) on a microplate reader (Infinite M200, Tecan) according to the manufacturer's instructions.

RNA Isolation

Total RNA was extracted using the RNAzol method combined with columns. Briefly, cells or tissues were homogenized in 500 µl RNAzol RT (MRC), and mixed with 200 µl of RNase free water. The mixture was then vortexed for 15 seconds and incubated for 15 minutes at room temperature, followed by 15 minutes centrifugation at 16 000 g. The supernatant was transferred to a fresh tube with 8 µl of 4-Bromoanisol and vortexed for 15 seconds. After incubation at room temperature for 5 minutes, the solution was centrifuged for 10 minutes at 12 000 g. The supernatant contains RNA and was transferred to a fresh tube. The mixture of the RNA containing supernantant and 1 volume of isopropanol was filtered by Zymo-Spin™ ICG Column (Zymo). RNA was washed twice with Direct-zol™ RNA PreWash buffer, and once with RNA Wash Buffer on column, and then elute with 10-20 µl of RNase free water. For large size tissues such as eye and lung, the reagent volumes doubled accordingly.

Quantitative RT-PCR

A two-tailed RT-PCR method (8) was used for miRNA quantification. Reverse transcription (RT) reactions were performed using the iScript select cDNA kit (BioRad) in a total reaction

volume of 10 µl, which contained 1x reaction buffer, 1 µl of GSP enhancer solution, mix of 0.5 µM Two-tailed RT primers, 0.5 µl reverse transcriptase and 100 ng total RNA. The reactions were incubated at 42°C for 45 minutes, and 85°C for 5 minutes in a thermocycler. Reverse transcription (RT) reactions for mRNA were performed in a total reaction volume of 10 µl, which contained 1x SuperScript IV VILO Master Mix (Invitrogen) and 200 ng RNA. The reactions were incubated at 25°C for 10 min, 50°C for 10 minutes and 85°C for 5 minutes in a thermocycler.

Methods for qPCR were the same for both miRNA and mRNA. One total volume of 10 µl reaction contained 1x Maxima SYBR Green qPCR Master Mix (Thermo Scientific), 0.5 µM forward and reverse primers, and 3 µl of diluted cDNA template. qPCR was performed in a CFX 384 Real Time Detection System (Bio-Rad) at 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, and 60°C for 60 seconds, followed by melting-curve analysis. The primers used in qPCR analysis are listed in SI Appendix, Table S6.

Western Blotting

Whole-cell extracts were prepared using lysis buffer containing 50 mM Tris (pH8), 1% TX-100, 150 mM NaCl, 12 mM Sodium Dexcycolate, 0.1% SDS, 10 mM EDTA, and protease inhibitor. After incubation on ice for 10 minutes, samples were centrifuged at 16 000 g, at 4°C for 20 minutes. Proteins were quantified by DC Protein Assay Kit (BioRad). Gel electrophoresis was run in Criterion TGX Stain-Free Gels (BioRad). Antibodies were anti-PER2 polyclonal (ProteinTech # 20359-1-AP) and anti-GAPDH (ProteinTech # 10494-1-AP).

Bioluminescence recording of tissues from *Per2*^{Luc} reporter mice

The *miR-183C*$^{GT/GT}$*; Per2*^{Luc} mice and littermate wild-type control mice were euthanized by isoflurane 2-3 hours before lights off (ZT 9–ZT10) and tissues were immediately removed into ice-cold HBSS (Gibco). Coronal brain slices containing SCN (400 μm thickness) were cut using a tissue slicer (Stoelting). The bilateral SCN tissue without optic chiasm attached was further dissected out from the brain slice with a sterile scalpel under a dissecting microscope. The SCN cultures were cultured on cell culture inserts (PICMORG50; Millipore) in sealed 35 mm culture dishes containing 1.5mL DMEM supplemented with 1 x B-27 plus (Gibco), 352.5 μg/mL sodium bicarbonate, 10 mM Hepes (Gibco), 25 U/mL penicillin, 25 μg/mL streptomycin (Gibco), and 0.1 mM D-luciferin potassium salt (GoldBio). Retinas were isolated from the eye and then gently placed on culture inserts (PICMORG50; Millipore) with ganglion cell down in Neurobasal plus medium (Gibco) containing 1 x B-27 plus (Gibco) and 2% FBS (Atlanta Biologicals), 25 U/mL penicillin, 25 μg/mL streptomycin (Gibco), and 2 mM GlutaMAX (Gibco). Bioluminescence was measured with a LumiCycle instrument (Actimetrics, Wilmette, IL). Cultures were maintained in a light-tight incubator at 37°C for one week. Data were analyzed by using the LM fit (damped sin) method in LumiCycle data analysis software (Actimetrics). The time of the peak of luminescence on the first day of constant conditions (first day in LumiCycle) was determined as the time of peak of the sine wave on that day.

Statistical Analysis

The statistical significance was determined by unpaired Student's two tailed t-test if there were only two groups of data to compare. In cases of comparing multiple conditions, ordinary one-way ANOVA and Tukey post hoc tests were applied. P values of less than 0.05 were considered significant.

References

1. A. C. Liu, *et al.*, Redundant function of REV-ERBα and β and non-essential role for Bmal1 cycling in transcriptional regulation of intracellular circadian rhythms. *PLoS Genet.* **4** (2008).
2. X. Tan, *et al.*, Clock-controlled mir-142-3p can target its activator, Bmal1. *BMC Mol. Biol.* **13**, 27 (2012).
3. V. R. Shende, N. Neuendorff, D. J. Earnest, Role of miR-142-3p in the Post-Transcriptional Regulation of the Clock Gene Bmal1 in the Mouse SCN. *PLoS One* **8**, e65300 (2013).
4. R. Nagel, L. Clijsters, R. Agami, The miRNA-192/194 cluster regulates the Period gene family and the circadian clock. *FEBS J.* **276**, 5447–5455 (2009).
5. V. R. Shende, M. M. Goldrick, S. Ramani, D. J. Earnest, Expression and rhythmic modulation of circulating micrornas targeting the clock gene Bmal1 in mice. *PLoS One* **6** (2011).
6. H. Y. M. Cheng, *et al.*, microRNA Modulation of Circadian-Clock Period and Entrainment. *Neuron* **54**, 813–829 (2007).
7. S. Kojima, D. Gatfield, C. C. Esau, C. B. Green, MicroRNA-122 Modulates the Rhythmic Expression Profile of the Circadian Deadenylase Nocturnin in Mouse Liver. *PLoS One* **5**, e11264 (2010).
8. P. Androvic, L. Valihrach, J. Elling, R. Sjoback, M. Kubista, Two-tailed RT-qPCR: A novel method for highly accurate miRNA quantification. *Nucleic Acids Res.* **45** (2017).

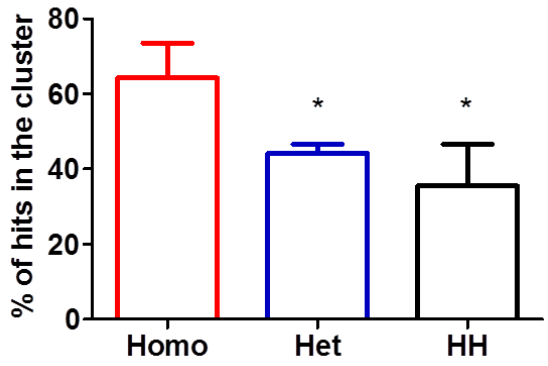

Fig. S1. Homo-seed clusters (Homo) have a significantly higher percentage of candidate miRNA enrichment than that of hetero-seed clusters (Het) and homo-hetero-seed clusters (HH). * $p < 0.05$. Data represent the mean ± SEM.

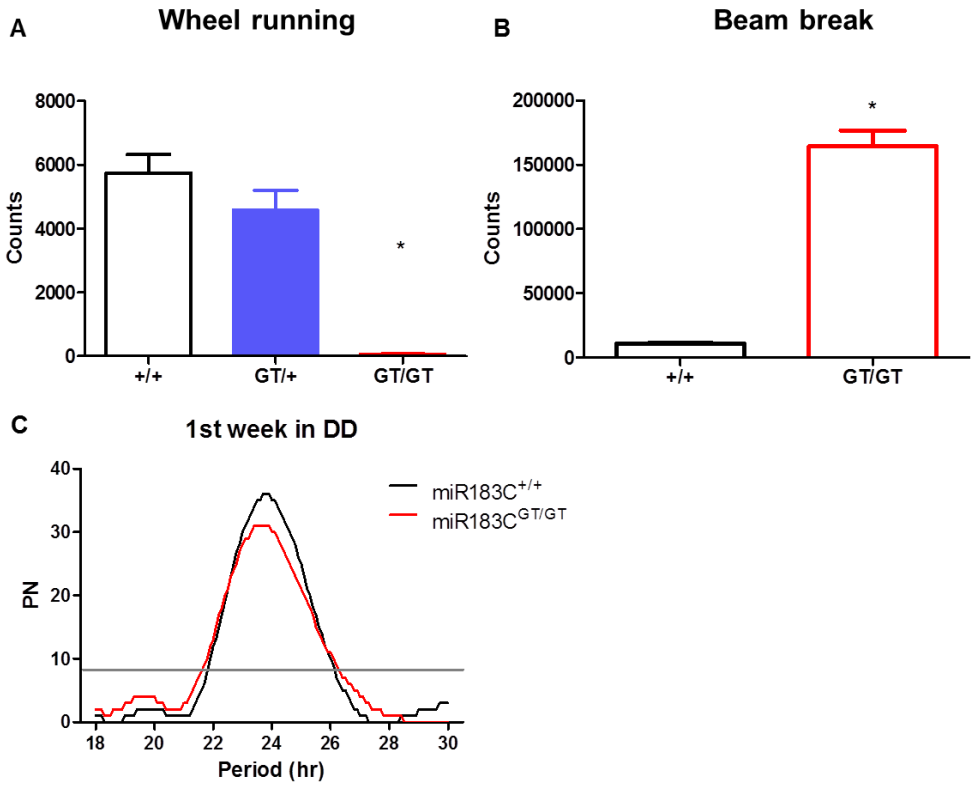

Fig. S2. (A) *miR-183C*$^{GT/GT}$ mice had significantly lower daily activity on running wheels than wild-type mice. (B) The general daily activity of *miR-183C*$^{GT/GT}$ mice was significantly higher than that of wild-type mice. * $p < 0.05$. Data represent the mean ± SEM. (C) Circadian periodicity of the beam-break total activity in the first week of DD condition was detected by Lomb-Scargle Periodogram. Both wild-type and mutant mice had similar period lengths.

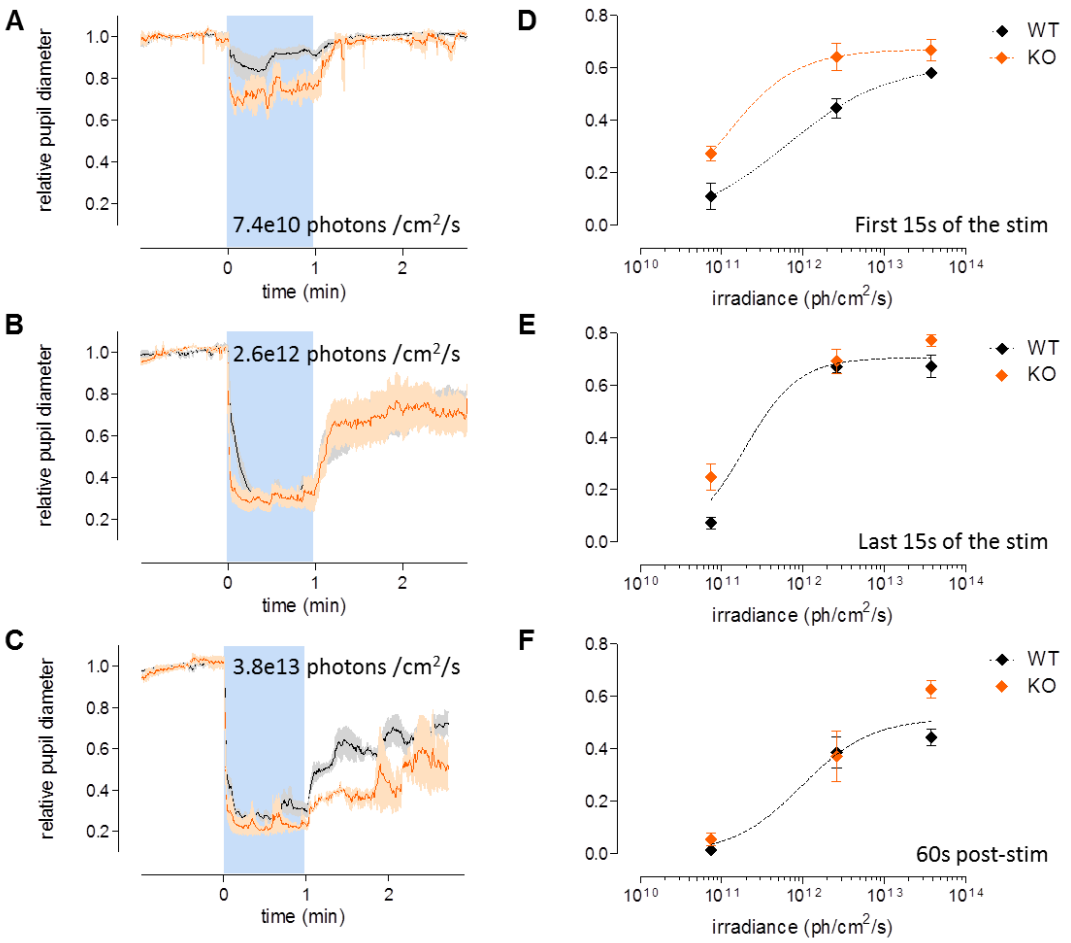

Fig. S3. Pupillary light reflex mediated by melanopsin was not diminished. (A-C) Average pupil diameter (normalized to baseline) in response to 1 min monochromatic blue light stimulation at increasing irradiance (480 nm, $7.4x10^{10}$, $2.6x10^{12}$, and $3.8x10^{13}$ photons/cm^2/s). Each 5-min recording sequence consisted of 1 min of darkness for baseline, 1 min of monochromatic 480 nm light for stimulation (blue shade), and finally 3 min of darkness for recovery. Results from the first 15 seconds during stimulation, the last 15 seconds during stimulation, and the first 60 seconds of post-stimulation were used for assessing the responses between mice of different genotypes, and were summarized in (D-F). Results from the 60s post-stimulation of $2.6x10^{12}$ photons indicated that the pupillary light reflex mediated by melanopsin were comparable between *miR-183C*$^{GT/GT}$ (WT) and *miR-183C*$^{GT/GT}$ (KO) mice. Data represent the mean ± SEM.

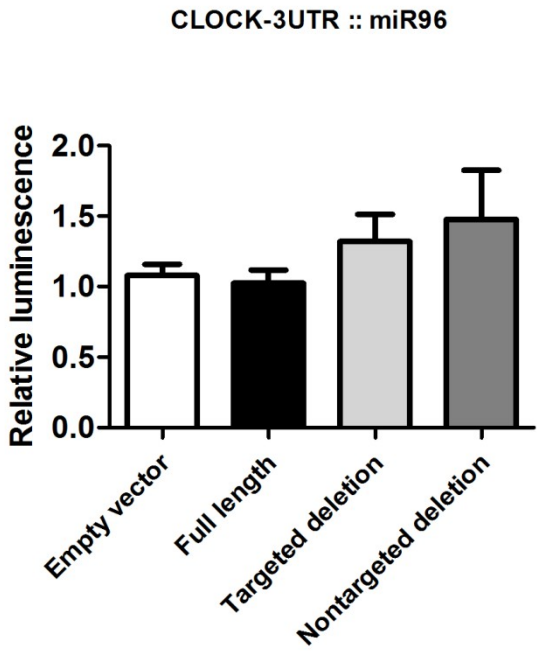

Fig. S4. HEK-293T cells were transfected with a luciferase reporter plasmid containing either no 3'UTR, or the CLOCK-3'UTR with wild-type, mutated target site of miR-96 (273_278 del: TGCCAA), or mutated non-target site of miR-96 (1054_1076 del: CACAAAACATCTTAGGCACTTTT). No statistical significance was identified in ANOVA and post hoc tests. Data represent the mean ± SEM.

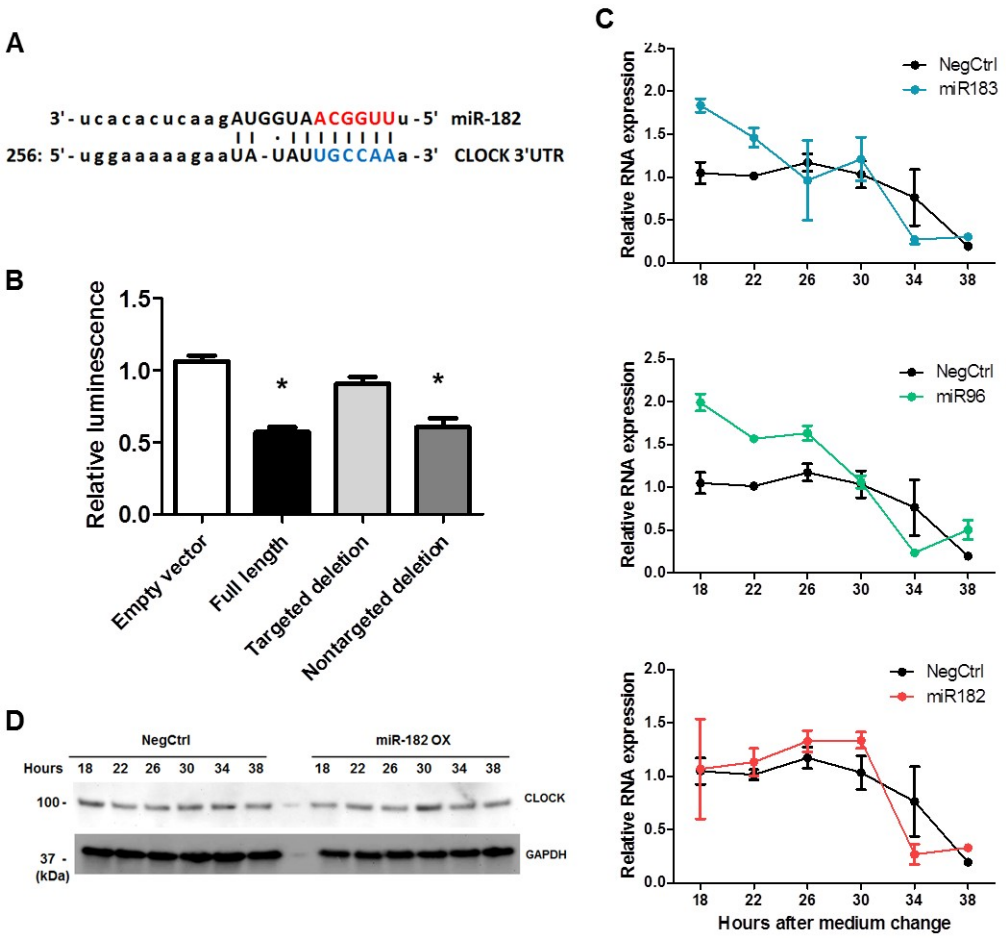

Fig. S5. (A) Sequence alignment between miR-182 and its putative binding site (blue letters) in CLOCK-3'UTR. Red letters indicate canonical seed nucleotides. (B) HEK-293T cells were transfected with a luciferase reporter plasmid containing either no 3'UTR, or the CLOCK-3'UTR with wild-type, mutated target site (273_278 del: TGCCAA), or mutated non-target site of miR-182 (1054_1076 del: CACAAAACATCTTAGGCACTTTT). * $p < 0.05$. (C) *CLOCK* mRNA expression in U2OS cells transfected with miRNA mimics of each member of the miR-183/96/182 cluster. RNA was sampled every 4 hours 18 hours after medium change. (D) U2OS cells were transfected with either control or miR-182 mimics. CLOCK protein levels were analyzed by western blot 18 hours after medium change. Data represent the mean ± SEM.

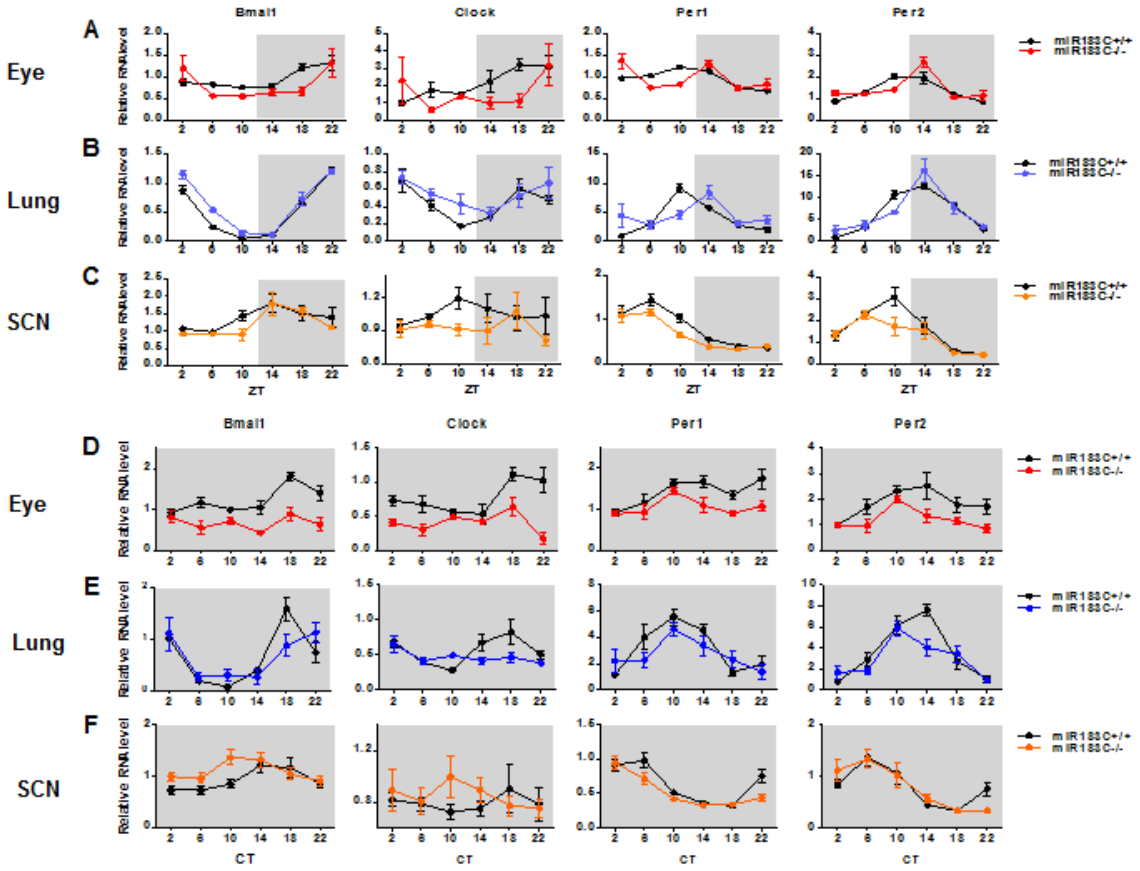

Fig. S6. Tissues of the whole eye (A and D), the lung (B and E), and the SCN (C and F) from either *miR-183C*$^{+/+}$ mice or *miR-183C*$^{GT/GT}$ mice were sampled every 4 hours across 24 hours either under LD (top panel) or DD (bottom panel) conditions. The relative mRNA of core circadian genes was measured by qPCR (n = 4~5). Data represent the mean ± SEM.

Table S1. Candidate miRNA hits affecting period length.

miRNA Names	miRNA Seq	Period Phenotype
hsa-let-7a	UGAGGUAGUAGGUUGUAUAGUU	Long
hsa-let-7c	UGAGGUAGUAGGUUGUAUGGUU	Long
hsa-let-7d	AGAGGUAGUAGGUUGCAUAGUU	Long
hsa-let-7e	UGAGGUAGGAGGUUGUAUAGUU	Long
hsa-let-7f	UGAGGUAGUAGAUUGUAUAGUU	Long
hsa-let-7g	UGAGGUAGUAGUUUGUACAGUU	Long
hsa-let-7g*	CUGUACAGGCCACUGCCUUGC	Long
hsa-let-7i*	CUGCGCAAGCUACUGCCUUGCU	Long
hsa-miR-101*	CAGUUAUCACAGUGCUGAUGCU	Long
hsa-miR-103	AGCAGCAUUGUACAGGGCUAUGA	Long
hsa-miR-103-as	UCAUAGCCCUGUACAAUGCUGCU	Long
hsa-miR-106a	AAAAGUGCUUACAGUGCAGGUAG	Long
hsa-miR-106b	UAAAGUGCUGACAGUGCAGAU	Long
hsa-miR-10a*	CAAAUUCGUAUCUAGGGGAAUA	Long
hsa-miR-1178	UUGCUCACUGUUCUUCCCUAG	Long
hsa-miR-1184	CCUGCAGCGACUUGAUGGCUUCC	Long
hsa-miR-1204	UCGUGGCCUGGUCUCCAUUAU	Long
hsa-miR-1205	UCUGCAGGGUUUGCUUUGAG	Long
hsa-miR-1224-3p	CCCCACCUCCUCUCUCCUCAG	Long
hsa-miR-1224-5p	GUGAGGACUCGGGAGGUGG	Long
hsa-miR-1227	CGUGCCACCCUUUUCCCCAG	Long
hsa-miR-1233	UGAGCCCUGUCCUCCCGCAG	Long
hsa-miR-124	UAAGGCACGCGGUGAAUGCC	Long
hsa-miR-1250	ACGGUGCUGGAUGUGGCCUUU	Long
hsa-miR-126	UCGUACCGUGAGUAAUAAUGCG	Long

hsa-miR-1276	UAAAGAGCCCUGUGGAGACA	Long
hsa-miR-1285	UCUGGGCAACAAAGUGAGACCU	Long
hsa-miR-129-5p	CUUUUUGCGGUCUGGGCUUGC	Long
hsa-miR-1296	UUAGGGCCCUGGCUCCAUCUCC	Long
hsa-miR-1304	UUUGAGGCUACAGUGAGAUGUG	Long
hsa-miR-1305	UUUUCAACUCUAAUGGGAGAGA	Long
hsa-miR-1306	ACGUUGGCUCUGGUGGUG	Long
hsa-miR-130b*	ACUCUUUCCCUGUUGCACUAC	Long
hsa-miR-132	UAACAGUCUACAGCCAUGGUCG	Long
hsa-miR-145	GUCCAGUUUUCCCAGGAAUCCCU	Long
hsa-miR-148a	UCAGUGCACUACAGAACUUUGU	Long
hsa-miR-148a*	AAAGUUCUGAGACACUCCGACU	Long
hsa-miR-148b*	AAGUUCUGUUAUACACUCAGGC	Long
hsa-miR-149	UCUGGCUCCGUGUCUUCACUCCC	Long
hsa-miR-150	UCUCCCAACCCUUGUACCAGUG	Long
hsa-miR-15a	UAGCAGCACAUAAUGGUUUGUG	Long
hsa-miR-17	CAAAGUGCUUACAGUGCAGGUAG	Long
hsa-miR-182*	UGGUUCUAGACUUGCCAACUA	Long
hsa-miR-183	UAUGGCACUGGUAGAAUUCACU	Long
hsa-miR-184	UGGACGGAGAACUGAUAAGGGU	Long
hsa-miR-185*	AGGGGCUGGCUUUCCUCUGGUC	Long
hsa-miR-186	CAAAGAAUUCUCCUUUUGGGCU	Long
hsa-miR-188-5p	CAUCCCUUGCAUGGUGGAGGG	Long
hsa-miR-18a*	ACUGCCCUAAGUGCUCCUUCUGG	Long
hsa-miR-190b	UGAUAUGUUUGAUAUUGGGUU	Long
hsa-miR-19a*	AGUUUUGCAUAGUUGCACUACA	Long
hsa-miR-19b-1*	AGUUUUGCAGGUUUGCAUCCAGC	Long
hsa-miR-19b-2*	AGUUUUGCAGGUUUGCAUUUCA	Long

hsa-miR-204	UUCCCUUUGUCAUCCUAUGCCU	Long
hsa-miR-210	CUGUGCGUGUGACAGCGGCUGA	Long
hsa-miR-211	UUCCCUUUGUCAUCCUUCGCCU	Long
hsa-miR-216a	UAAUCUCAGCUGGCAACUGUGA	Long
hsa-miR-23a	AUCACAUUGCCAGGGAUUUCC	Long
hsa-miR-23a*	GGGGUUCCUGGGGAUGGGAUUU	Long
hsa-miR-23b*	UGGGUUCCUGGCAUGCUGAUUU	Long
hsa-miR-29b-2*	CUGGUUUCACAUGGUGGCUUAG	Long
hsa-miR-302d*	ACUUUAACAUGGAGGCACUUGC	Long
hsa-miR-323-5p	AGGUGGUCCGUGGCGCGUUCGC	Long
hsa-miR-325	CCUAGUAGGUGUCCAGUAAGUGU	Long
hsa-miR-330-3p	GCAAAGCACACGGCCUGCAGAGA	Long
hsa-miR-330-5p	UCUCUGGGCCUGUGUCUUAGGC	Long
hsa-miR-338-5p	AACAAUAUCCUGGUGCUGAGUG	Long
hsa-miR-33a	GUGCAUUGUAGUUGCAUUGCA	Long
hsa-miR-33b	GUGCAUUGCUGUUGCAUUGC	Long
hsa-miR-33b*	CAGUGCCUCGGCAGUGCAGCCC	Long
hsa-miR-34a*	CAAUCAGCAAGUAUACUGCCCU	Long
hsa-miR-374a	UUAUAAUACAACCUGAUAAGUG	Long
hsa-miR-374b	AUAUAAUACAACCUGCUAAGUG	Long
hsa-miR-377*	AGAGGUUGCCCUUGGUGAAUUC	Long
hsa-miR-378*	CUCCUGACUCCAGGUCCUGUGU	Long
hsa-miR-380*	UGGUUGACCAUAGAACAUGCGC	Long
hsa-miR-409-5p	AGGUUACCCGAGCAACUUUGCAU	Long
hsa-miR-411	UAGUAGACCGUAUAGCGUACG	Long
hsa-miR-421	AUCAACAGACAUUAAUUGGGCGC	Long
hsa-miR-431	UGUCUUGCAGGCCGUCAUGCA	Long
hsa-miR-491-3p	CUUAUGCAAGAUUCCCUUCUAC	Long

hsa-miR-493*	UUGUACAUGGUAGGCUUUCAUU	Long
hsa-miR-502-3p	AAUGCACCUGGGCAAGGAUUCA	Long
hsa-miR-511	GUGUCUUUUGCUCUGCAGUCA	Long
hsa-miR-513a-3p	UAAAUUUCACCUUUCUGAGAAGG	Long
hsa-miR-518c*	UCUCUGGAGGGAAGCACUUUCUG	Long
hsa-miR-518d-3p	CAAAGCGCUUCCCUUUGGAGC	Long
hsa-miR-518f	GAAAGCGCUUCUCUUUAGAGG	Long
hsa-miR-544	AUUCUGCAUUUUUAGCAAGUUC	Long
hsa-miR-571	UGAGUUGGCCAUCUGAGUGAG	Long
hsa-miR-572	GUCCGCUCGGCGGUGGCCCA	Long
hsa-miR-592	UUGUGUCAAUAUGCGAUGAUGU	Long
hsa-miR-605	UAAAUCCCAUGGUGCCUUCUCCU	Long
hsa-miR-610	UGAGCUAAAUGUGUGCUGGGA	Long
hsa-miR-620	AUGGAGAUAGAUAUAGAAAU	Long
hsa-miR-629*	GUUCUCCCAACGUAAGCCCAGC	Long
hsa-miR-633	CUAAUAGUAUCUACCACAAUAAA	Long
hsa-miR-641	AAAGACAUAGGAUAGAGUCACCUC	Long
hsa-miR-647	GUGGCUGCACUCACUUCCUUC	Long
hsa-miR-664	UAUUCAUUUAUCCCCAGCCUACA	Long
hsa-miR-671-3p	UCCGGUUCUCAGGGCUCCACC	Long
hsa-miR-7	UGGAAGACUAGUGAUUUUGUUGU	Long
hsa-miR-7-2*	CAACAAAUCCCAGUCUACCUAA	Long
hsa-miR-744	UGCGGGGCUAGGGCUAACAGCA	Long
hsa-miR-744*	CUGUUGCCACUAACCUCAACCU	Long
hsa-miR-770-5p	UCCAGUACCACGUGUCAGGGCCA	Long
hsa-miR-802	CAGUAACAAAGAUUCAUCCUUGU	Long

hsa-miR-887	GUGAACGGGCGCCAUCCCGAGG	Long
hsa-miR-891b	UGCAACUUACCUGAGUCAUUGA	Long
hsa-miR-92a-1*	AGGUUGGGAUCGGUUGCAAUGCU	Long
hsa-miR-934	UGUCUACUACUGGAGACACUGG	Long
hsa-miR-96	UUUGGCACUAGCACAUUUUUGCU	Long
hsa-miR-96*	AAUCAUGUGCAGUGCCAAUAUG	Long
hsa-miR-181c	AACAUUCAACCUGUCGGUGAGU	Short
hsa-miR-193a-3p	AACUGGCCUACAAAGUCCCAGU	Short
hsa-miR-29a	UAGCACCAUCUGAAAUCGGUUA	Short
hsa-miR-29c	UAGCACCAUUUGAAAUCGGUUA	Short
hsa-miR-365	UAAUGCCCCUAAAAAUCCUUAU	Short
hsa-miR-433	AUCAUGAUGGGCUCCUCGGUGU	Short
hsa-miR-933	UGUGCGCAGGGAGACCUCUCCC	Short

Table S2. Gene family information for each candidate miRNA hit.

miRNA Names	Gene family
hsa-let-7f	let-7
hsa-let-7a	let-7
hsa-let-7d	let-7
hsa-let-7c	let-7
hsa-let-7e	let-7
hsa-let-7g*	let-7
hsa-let-7g	let-7
hsa-let-7i*	let-7
hsa-miR-10a*	mir-10
hsa-miR-101*	mir-101
hsa-miR-103-as	mir-103
hsa-miR-103	mir-103
hsa-miR-1178	mir-1178
hsa-miR-1184	mir-1184
hsa-miR-1204	mir-1204
hsa-miR-1205	mir-1205
hsa-miR-1224-5p	mir-1224
hsa-miR-1224-3p	mir-1224
hsa-miR-1227	mir-1227
hsa-miR-1233	mir-1233
hsa-miR-124	mir-124
hsa-miR-1250	mir-1250
hsa-miR-126	mir-126
hsa-miR-1276	mir-1276
hsa-miR-1285	mir-1285

hsa-miR-129-5p	mir-129
hsa-miR-1296	mir-1296
hsa-miR-130b*	mir-130
hsa-miR-1304	mir-1304
hsa-miR-1305	mir-1305
hsa-miR-1306	mir-1306
hsa-miR-132	mir-132
hsa-miR-145	mir-145
hsa-miR-148b*	mir-148
hsa-miR-148a	mir-148
hsa-miR-148a*	mir-148
hsa-miR-149	mir-149
hsa-miR-15a	mir-15
hsa-miR-150	mir-150
hsa-miR-323-5p	mir-154
hsa-miR-377*	mir-154
hsa-miR-409-5p	mir-154
hsa-miR-106a	mir-17
hsa-miR-106b	mir-17
hsa-miR-18a*	mir-17
hsa-miR-17	mir-17
hsa-miR-181c	mir-181
hsa-miR-182*	mir-182
hsa-miR-183	mir-183
hsa-miR-184	mir-184
hsa-miR-185*	mir-185
hsa-miR-186	mir-186
hsa-miR-188-5p	mir-188

hsa-miR-19b-2*	mir-19
hsa-miR-19b-1*	mir-19
hsa-miR-19a*	mir-19
hsa-miR-190b	mir-190
hsa-miR-193a-3p	mir-193
hsa-miR-204	mir-204
hsa-miR-211	mir-204
hsa-miR-210	mir-210
hsa-miR-216a	mir-216
hsa-miR-23b*	mir-23
hsa-miR-23a*	mir-23
hsa-miR-23a	mir-23
hsa-miR-92a-1*	mir-25
hsa-miR-29a	mir-29
hsa-miR-29c	mir-29
hsa-miR-29b-2*	mir-29
hsa-miR-302d*	mir-302
hsa-miR-325	mir-325
hsa-miR-33b*	mir-33
hsa-miR-33b	mir-33
hsa-miR-33a	mir-33
hsa-miR-330-3p	mir-330
hsa-miR-330-5p	mir-330
hsa-miR-338-5p	mir-338
hsa-miR-34a*	mir-34
hsa-miR-365	mir-365
hsa-miR-374a	mir-374
hsa-miR-374b	mir-374

hsa-miR-378*	mir-378
hsa-miR-380*	mir-379
hsa-miR-411	mir-379
hsa-miR-431	mir-431
hsa-miR-433	mir-433
hsa-miR-491-3p	mir-491
hsa-miR-493*	mir-493
hsa-miR-502-3p	mir-500
hsa-miR-513a-3p	mir-506
hsa-miR-511	mir-506
hsa-miR-518c*	mir-515
hsa-miR-518f	mir-515
hsa-miR-518d-3p	mir-515
hsa-miR-544	mir-544
hsa-miR-571	mir-571
hsa-miR-572	mir-572
hsa-miR-592	mir-592
hsa-miR-605	mir-605
hsa-miR-610	mir-610
hsa-miR-629*	mir-629
hsa-miR-633	mir-633
hsa-miR-641	mir-641
hsa-miR-647	mir-647
hsa-miR-664	mir-664
hsa-miR-671-3p	mir-671
hsa-miR-7-2*	mir-7
hsa-miR-7	mir-7

hsa-miR-744*	mir-744
hsa-miR-744	mir-744
hsa-miR-770-5p	mir-770
hsa-miR-802	mir-802
hsa-miR-887	mir-887
hsa-miR-891b	mir-891
hsa-miR-933	mir-933
hsa-miR-934	mir-934
hsa-miR-421	mir-95
hsa-miR-96*	mir-96
hsa-miR-96	mir-96

Table S3. Gene cluster information for each candidate miRNA hit and the categories based on seed sequence similarity. Red color denotes the miRNAs identified from the screen.

Category	miRNA cluster
Homo	let-7a-1; let-7f-1; let-7d
Homo	mir-29c; mir-29b-2
Homo	mir-132; mir-212
Homo	mir-16-1; mir-15a
Homo	mir-181c; mir-181d
Homo	mir-29a; mir-29b-1
Homo	mir-301b; mir-130b
HH	mir-379; mir-411; mir-299; mir-380; mir-1197; mir-323a; mir-758; mir-329-1; mir-329-2; mir-494; mir-1193; mir-543; mir-495; mir-376c; mir-376a-2; mir-654; mir-376b; mir-376a-1; mir-300; mir-1185-1; mir-1185-2; mir-381; mir-487b; mir-539; mir-889; mir-544a; mir-655; mir-487a; mir-382; mir-134; mir-668; mir-485; mir-323b; mir-154; mir-496; mir-377; mir-541; mir-409; mir-412; mir-369; mir-410; mir-656
HH	mir-17; mir-18a; mir-19a; mir-20a; mir-19b-1; mir-92a-1
HH	512-1; 512-2; 1323; 498; 520e; 515-1; 519e; 520f; 515-2; 519c; 1283-1; 520a; 526b; 519b; 525; 523; 518f; 520b; 518b; 526a-1; 520c; 518c; 524; 517a; 519d; 521-2; 520d; 517b; 520g; 516b-2; 526a-2; 518e; 518a-1; 518d; 516b-1; 518a-2; 517c; 520h; 521-1; 522; 519a-1; 527; 516a-1; 1283-2; 516a-2; 519a-2
HH	mir-106a; mir-18b; mir-20b; mir-19b-2; mir-92a-2; mir-363
HH	mir-106b; mir-93; mir-25
HH	mir-302b; mir-302c; mir-302a; mir-302d; mir-367
HH	mir-532; mir-188; mir-500a; mir-362; mir-501; mir-500b; mir-660; mir-502
HH	mir-891b; mir-892b; mir-892a; mir-888; mir-890; mir-892c
HH	mir-183; mir-96; mir-182
Hetero	mir-1250; mir-338; mir-657
Hetero	mir-439; mir-337; mir-665; mir-431; mir-433; mir-127; mir-432; mir-136
Hetero	mir-101; mir-3671

Hetero	mir-1179; mir-7-2
Hetero	mir-143; mir-145
Hetero	mir-193b; mir-365a
Hetero	mir-217; mir-216a
Hetero	mir-23b; mir-27b; mir-24-1
Hetero	mir-24-2; mir-27a; mir-23a
Hetero	mir-33b; mir-6777
Hetero	mir-3618; mir-1306
Hetero	mir-374a; mir-545
Hetero	mir-374b; mir-421
Hetero	mir-493; mir-337; mir-665
Hetero	mir-507; mir-506; mir-513a-2
Hetero	mir-647; mir-1914
Hetero	mir-6789; mir-1227
Hetero	mir-99a; let-7c; mir-125b-2
Hetero	mir-99b; let-7e; mir-125a

Table S4. Predicted targets for members of miR-183 cluster by different methods.

	miR-183	**miR-96**	**miR182**
BMAL1	-	-	-
CLOCK	Diana	miRanda, Diana	miRanda, Diana
CRY1	-	-	-
CRY2	-	-	-
PER1	-	-	-
PER2	-	miRanda, Diana	Diana

Table S5. RNA sequences for CRISPR.

miRNA	crRNA	Target Sequence	PAM	Genomic Location (Strand)	Specificity	Specificity Score	Deletion size
has-miR-183	crRNA-478166	TCCTGTTCTGTGTATGGCAC	TGG	chr7:129774978-129775000 (-)	High	30.8	56nt
	crRNA-478167	ATCTGTCTCTGCTCTGTTTA	TGG	chr7:129774911-129774933 (+)	High	36.35	
has-miR-96	crRNA-478172	GCCATCTGCTTGGCCGATTT	TGG	chr7:129774757-129774779 (-)	High	10.25	62nt
	crRNA-478174	ATCATGTGCAGTGCCAATAT	GGG	chr7:129774695-129774717 (-)	High	15.7	
has-miR-182	crRNA-454327	GGCAATGGTAGAACTCACAC	TGG	chr7:129770445-129770467 (-)	High	17.7	43nt
	crRNA-454328	GTTCTAGACTTGCCAACTAT	GGG	chr7:129770402-129770424 (-)	High	9.15	

Table S6. Primers for qPCR.

Primer	Sequence	Species	Usage
Gapdh_F	TGACCTCAACTACATGGTCTACA	Mouse	qPCR primer for mRNA
Gapdh_R	CTTCCCATTCTCGGCCTTG	Mouse	qPCR primer for mRNA
Bmal1_F	CCCGCTGAACATCACAAGTA	Mouse	qPCR primer for mRNA
Bmal1_R	TGAGCCTGCCCTGGTAATAG	Mouse	qPCR primer for mRNA
Clock_F	GGCCTTCTTATGAAGATAGAGTTTG	Mouse	qPCR primer for mRNA
Clock_R	AACTCTTCATTTGGTTCTTCAAC	Mouse	qPCR primer for mRNA
Per1_F	TGAAGCAAGACCGGGAGAG	Mouse	qPCR primer for mRNA
Per1_R	CACACACGCCATCACATCAA	Mouse	qPCR primer for mRNA
Per2_F	CTTCTGGTCTGGACTGCAC	Mouse	qPCR primer for mRNA
Per2_R	CAGGTCACTTGACGTGGAGA	Mouse	qPCR primer for mRNA
GAPDH_F	AATCCCATCACCATCTTCCA	Human	qPCR primer for mRNA
GAPDH_R	TGGACTCCACGACGTACTCA	Human	qPCR primer for mRNA
CLOCK_F	GGCACCACCCATAATAGGGTA	Human	qPCR primer for mRNA
CLOCK_R	TGTTGCCCCTTAGTCAGGAAC	Human	qPCR primer for mRNA
PER2_F	CGACGTGGCAGAATGTGTT	Human	qPCR primer for mRNA
PER2_R	GATTCAAGGGGGATCCATTT	Human	qPCR primer for mRNA
miR183-5p_RT	CAGTGCCATACGACGAATACTGCTA GAGTTGCTAGCAGAGCCCTTAA AGTGAA	Human and mouse	rtPCR primer for miRNA
miR183-5p_F	CAGTGCCATACGACGAATACTGC	Human and mouse	qPCR primer for miRNA
miR183-5p_R	GCGCTATGGCACTGGTAGA	Human and mouse	qPCR primer for miRNA
miR96-5p_RT	TAGTGCCAAAGATATGTGAGACGTA CGTTGAGTACGTCAAGTGAAGTAGC AAAA	Human and mouse	rtPCR primer for miRNA

miR96-5p_F	CGTAGTGCCAAAGATATGTGAGACG	Human and mouse	qPCR primer for miRNA
miR96-5p_R	GGCCTTTGGCACTAGCACAT	Human and mouse	qPCR primer for miRNA
miR182-5p_RT	CATTGCCAAACAACGACCAGAGCTA GAGAACCTAGCTCACCCACTAC CGGTGT	Human and mouse	rtPCR primer for miRNA
miR182-5p_F	CATTGCCAAACAACGACCAGAG	Human and mouse	qPCR primer for miRNA
miR182-5p_R	GCGTTTGGCAATGGTAGAACTC	Human and mouse	qPCR primer for miRNA
miR191-5p_RT	TCCGTTGTCAAGCTCTCGACGTACGT TGAGTACGTCACTCCACGCCAGCTG	Human and mouse	rtPCR primer for miRNA
miR191-5p_F	TCCGTTGTCAAGCTCTCGACG	Human and mouse	qPCR primer for miRNA
miR191-5p_R	GCTGCAACGGAATCCCAAAAG	Human and mouse	qPCR primer for miRNA
MIR182-5p_RT	CATTGCCAAACAACGACCAGAGCTA GAGAACCTAGCTCACCCACTACAGTG TGA	Human	rtPCR primer for miRNA
MIR182-5p_F	CATTGCCAAACAACGACCAGAG	Human	qPCR primer for miRNA
MIR182-5p_R	GCGTTTGGCAATGGTAGAACT	Human	qPCR primer for miRNA