Supplemental Methods

Mice and genotyping

All experiments were approved by the University of Wisconsin – Madison Institutional Animal Care and Use Committee (M02529). Heterozygous male *Cntnap2*$^{+/-}$ mice were purchased from the Jackson laboratories (Bar Harbor, ME) and maintained on C57BL/6J background, as previously reported(24). *Cntnap2*$^{+/-}$ mutants were genotyped using the following primers: Mutant Rev: CGCTTCCTCGTGCTTTACGGTAT, Common: CTGCCAGCCCAGAACTGG, WT Rev 1: GCCTGCTCTCAGAGACATCA. PCR amplification was performed with one cycle of 95°C for 5 min and 31 cycles of 95°C for 30 sec, 56°C for 30 s, 68°C for 30 s, followed by 68°C for 10 min. The mutant allele was obtained with a 350-bp and wild-type (WT) allele with a 197-bp PCR products.

Breeding scheme and husbandry

To minimize the stress of animal handling, all of the following were conducted by a single researcher: animal colony maintenance; breeding, prenatal stress; and behavioral tests. For breeding, a three month old *Cntnap2*$^{+/-}$ male mouse was placed together with a virgin 3 month old WT C57/Bl6J female mouse at 6 pm (one hour prior to lights off); every morning before 9 am (two hours after lights on) female mice were checked for vaginal copulation plug and separated from male. Presence of a copulation plug denoted day 1 of gestation and the pregnant female was individually housed and given a cotton nestlet. At day 12 of gestation (E12), pregnant females were randomly assigned to either a variable stress or non-stressed control group.

Litter sizes of less than 5 and more than 8 pups were removed from the experiment. Offspring were ear-tagged at postnatal day (P) 12 and left undisturbed until weaning day (P18), at which time the mice were group housed with same sex. Female offspring were left intact, and were not cycled(32). Finally, to minimize the effect of parent-to-offspring interaction per litter, a maximum of 3 pups/litter were randomly selected for the behavioral or molecular experiments (total of $N = 16$ litters for both experimental and validation cohorts). The mice used for behavioral and molecular analysis were left undisturbed until behavioral testing or sacrifice at 3 months of age.

Behavioral Tests

Early-life stressed and non-early-life stressed *Cntnap2*$^{+/-}$ and WT mice (both sexes, 12-17 weeks of age) were submitted to tests of anxiety, depression, and sociability. Male and female mice was tested on separate days from each other. All testing was performed during a period of 4 hours in dark period (beginning 2 hours after lights off). All behavioral tests included a one-week recovery interval to reduce test carryover and improve reproducibility. Each group of mice was subjected to the following same sequence of behavioral tests: open field, light/dark box, elevated plus maze, forced swim test, 3-chamber social test. For the validation of the repetitive behavior and social deficits the same group of mice were subjected to the following sequence of behavioral tests, with one-week between tests: ten-minute observational test, 3-chamber social test, and Ten-minute reciprocal social interaction test. An experimenter who was blind to the animal's group and genotype scored video recordings of each test.

Light/Dark Box Test - The light/dark box test was performed in a rectangular box divided in two compartments (light and dark). The walls of the light and dark compartments were

constructed of Plexiglass (same areas 319.3 cm^2). A removable dark Plexiglass partition was used to divide the box into light and dark sides. Each animal was placed into the light side of the box, facing away from the dark side and allowed to explore both chambers of the apparatus for 10 min. The time spent in the light side was scored for each mouse.

Open Field Test - The open field apparatus consisted of a circular arena (104 cm diameter). A marker was used to inscribe a smaller circle 25 cm from the walls. Each mouse was placed in the inner circle of the apparatus and allowed to explore for 10 min. The time spent in the center and numbers of entries to inner cycle were scored for each mouse.

Elevated Plus Maze - The elevated plus maze consisted of two open and two closed arms, elevated 52 cm above the floor, with each arm projecting 50 cm from the center (a 10 X 10 cm area). Each mouse was individually placed in the center area, facing the open arm, and allowed to explore for 7 min. The measurements scored for each mouse were as follows: time spent in closed arms, time spent in open arms, and time spent in center.

Forced Swim Test -The mice were individually placed into a glass cylinder (14-cm internal diameter, 38 cm high) filled with water (28-cm deep, 25–26 °C) for 6 min. During the last 4 min of the test, the time spent floating was scored for each mouse. Floating was defined as immobility or minimal movements necessary to maintain the head above the water.

3-Chamber Social Test - The social interaction test was performed as previously described(98). Briefly, an experimental mouse was placed in the center third of a Plexiglass box (77.5 x 44.4 cm, divided into three equal chambers) for 10 minutes. Next the experimental mouse was allowed to explore all three interconnected chambers for 10 minutes. Finally, an empty wire cup was placed in one of the end chambers and an identical wire cup containing an unfamiliar mouse of the same sex was placed in the chamber at the opposite end; the

experimental mouse was allowed to explore all three interconnected chambers for 10 minutes. Time spent in each chamber and spent sniffing each cup as well as number of entries to each chamber was measured for each mouse.

Ten-minute reciprocal social interaction test: Mice were placed in a cage (previously habituated to it) with an unfamiliar mouse matched for treatment, genotype, and sex for 10 minutes. The time mice were engaged in repetitive behaviors (grooming and digging) was measured.

Ten-minute observational test: Mice were individually placed in a cage and the time engaged in repetitive behaviors (grooming and digging) was measured.

DNA and RNA extractions

DNA methylation and gene expression was obtained from early-life stressed and non-early-life stressed three-month old female *Cntnap2*$^{+/-}$ and WT mice ($N = 3$ per group). Mice were sacrificed (2 hours after lights on) and whole brains were extracted without perfusion and immediately flash frozen in 2-methylbutane and dry ice. Striatum tissue was excised by micropunch (1.53 to -0.95 mm posterior to bregma), while whole hippocampal tissue was excised by morphology. Approximately 30 milligrams of tissue was homogenized with glass beads (Sigma) and DNA and RNA were extracted using AllPrep DNA/RNA mini kit (Qiagen).

5hmC enrichment of genomic DNA, library preparation, and high-throughput sequencing

Chemical labeling-based 5hmC enrichment was described previously(15, 24). Briefly, a total of 10ug of striatum DNA was sonicated to 300 bp and incubated for 1 hour at 37ºC in the following labeling reaction: 1.5 ul of N3-UDPG (2mM); 1.5ulβ of -GT (60uM); and 3ul of 10X

β-GT buffer, in a total of 30ul. Biotin was added and the reaction was incubated at 37°C for 2 hours prior to capture on streptavidin-coupled dynabeads (Invitrogen, 65001). Enriched DNA was released from the beads during a 2 hour incubation at room temperature with 100mM DTT (Invitrogen, 15508013), which was removed using a Bio-Rad column (Bio-Rad, 732-6227). Capture efficiency was approximately 5-7% for each sample.

Library Preparation and high-throughput sequencing

5hmC-enriched libraries were generated using the NEBNext ChIP-seq Library Prep Reagent Set for Illumina sequencing, according to the manufacturer's protocol. Briefly, the 5hmC-enriched DNA fragments were purified after the adapter ligation step using AMPure XP beads (Agencourt A63880). An Agilent 2100 BioAnalyzer was used to quantify the amplified library DNA and 20-pM of diluted libraries were used for sequencing. 50-cycle single-end (striatum) or paired-end (hippocampus) sequencing was performed by Beckman Coulter Genomics or the University of Wisconsin Biotechnology Center, respectively. Paired-end sequencing for hippocampal tissue was chosen for reasons unrelated to this project. Image processing and sequence extraction were done using the standard Illumina Pipeline.

Analysis of 5hmC data: sequence alignment, fragment length estimation and peak identification

We mapped the reads to mouse NCBI37v1/mm9 reference genome using Bowtie 0.12.7(98), allowing for no more than two mismatches throughout the entire read and only keeping the uniquely mapped reads. For striatal data: the Model-based Analysis of ChIP-seq 2 (MACS2) algorithm v2.1.2 (99) was used to estimate fragment size, call peaks, and identify peak summits from aligned single-end reads using the following parameters: single-end format,

effective genome size of 1.87e9, band width of 300bp, an FDR cutoff of 0.01, auto pair model process enabled, local bias computed in a surrounding 1kb window, and a maximum of one duplicate fragment to avoid PCR bias. For hippocampal data: MACS2 also was used, employing the same parameters except in the paired-end mode, and using an FDR cutoff of 0.05, which could be relaxed (compared to striatal data) due to having paired-end sequence data, which increases the signal in the data. Summits from both striatal and hippocampal data were extracted for each peak for each sample and extended +/-500bp for downstream analysis. We defined the peaks for each group as follows using the peaks from all three subjects: peaks were merged from all subjects in a group and group peaks were identified if individual peaks overlapped between a minimum of two subjects in the group.

Annotation of sequence reads, peaks, and DhMRs

Genomic features and their associated gene symbols were extracted from GENCODE M1 and R Bioconductor package org.Mm.eg.db(101), and the repetitive elements were downloaded from UCSC genome browser (https://genome.ucsc.edu/)(102). We used Binomial tests to determine the significance in read density and DhMR distribution differences over the Chromosomes, genomic features and repetitive elements. When conducting the binomial test for DhMRs, the background proportions were calculated from all the peaks in the two groups of animals in each comparison. We used ngsplot to draw the profile plots of the genotype/condition 5hmC enrichments and other peaks(103).

Simultaneous targeted methylation sequencing for molecular validation

Molecular validation of DhMR data was performed as previously described(101). PCR-amplicons were sequenced on an Illumina miSeq following standard protocols. Illumina adapter sequences were removed from paired-end FastQ files using Trim Galore! v0.4.4. Alignment of trimmed reads was performed using Bismark v0.19.0, coupled with Bowtie 2 v2.2.0(98, 102), using a seed mismatch parameter of one base pair, a maximum insertion length of 1000bp, with all other parameters set to default. Mapping efficiency ranged from ~78-85%. Read coverage and methylation calling was extracted using bismark_methylation_extractor with the following parameters: paired-end files used, no overlapping reads reported, with methylation output reported as a sorted bedGraph file(101). Coverage files were imported to R environment and *methylKit* was used to determine differentially hydroxymethylated loci (DhMLs)(103). A *P*-value threshold of 0.05 was used to determine significance.

RNA sequencing

Approximately 100 ng of total RNA was used for sequence library construction following instructions of NuGen mRNA sample prep kit (cat# 0348). In brief, total RNA was copied into first strand cDNA using reverse transcriptase and random primers. This was followed by second strand cDNA synthesis using DNA Polymerase I and RNaseH. These cDNA fragments went through an end repair process, the addition of a single 'A' base, and ligation of adapters. These products were gel purified and enriched with PCR to create the final cDNA libraries. The library constructs were run on the bioanalyzer to verify the size and concentration before sequencing on the Illumina HiSeq2500 machine where 100-cycle single-end sequencing was performed by the University of Wisconsin Biotechnology Center. In total, three libraries (the same mice and combinations that were used to generate the 5hmC data) were sequenced for each experimental

condition.

Analysis of RNA-sequence data

Read alignment and calculation of transcript expression levels: we used the mm9 assembly as our reference genome and the GENCODE M1 (NCBIM37) as our gene annotation library (the Y Chromosome was deleted for the alignment of female samples). We ran RSEM to calculate the expression at both gene-level and isoform-level (101). Bowtie was set to report all valid hits with up to 2 mismatches allowed, and to suppress all the alignments if a read has more than 200 hits. Differentially expressed (DE) genes/transcripts detection: EBSeq was used to detect the DE genes and transcripts in each of the pairwise comparisons of ELS-WT vs control WT, and ELS-HET vs control HET. We tested on both the gene level and the isoform level with a maximum number of differentially expressed groups of isoforms to be three for each gene. Genes or transcripts with more than 75% values < 10 (*i.e.,* 4 out of 6 samples in each contrast) are filtered to ensure better model fitting, and the normalization factors are the median count for each gene/transcript. We applied a FDR control to the testing and picked out genes/transcripts with FDR < 0.1.

Enrichment tests of genes and GO analysis

DhMRs were annotated to all genes within 10k. To test for the enrichment of known neurodevelopmental genes among the DhMR-associated genes, we used a chi-square test to compare the DhMR-associated genes to a list of orthologs of well-documented human developmental brain disorder genes ($N = 232$)(41). Bioconductor package clusterProfiler was used to test GO Biological Process (BP) term enrichment with *P*-value cutoff of 0.05 and an

enrichment fold-change > 1.5(102). For the GO enrichment analysis of DhMR associated genes, the gene universe consisted of all the genes associated with 5hmC peaks in both the early-life stressed and the control mice. For the GO analysis of DE genes, the gene universe is all genes that survived the filtering of EBSeq. To test for an enrichment of neuronal related GO BP terms among the DhMR-associated GO BP terms, we used a chi-square test and a previously published list of neuronal related GO BP terms (N = 3,046)(103).

Sequence motif analysis

For motif discovery analysis, the Hypergeometric Optimization of Motif EnRichment (HOMER) suite of tools was utilized(102). DNA sequences corresponding to DhMR coordinates were obtained from the mm9 genome and compared against background sequences, using the given size of the region. Enriched known motifs of vertebrate transcription factors (N = 428) were determined using binomial testing and an FDR cutoff of 0.05.

Western blot

Mouse hippocampal nuclear extracts were isolated from adult female mice (P90) using a nuclear and cytoplasmic extraction kit per the manufacturer's instructions (Thermo Fisher Scientific 78833). Forty micrograms of total protein from hippocampal nuclear lysates was boiled for 5-minutes to dissociate complexes before separation in a 4-20% gradient gel (Bio-Rad 4561096) for 1-hour at 150V in 1x Tris/Glycine/SDS (Bio-Rad 1610732). Following separation of proteins, gels were transferred to nylon membrane in 1x Tris/Glycine for 1-hour on ice at 100V. Following transfer, membranes were washed in 1X TBST and blocked in 5% milk/TBST for 1-hour at room temperature. Blocked membranes were incubated with primary

antibodies for 12-hours at 4°C. Primary antibodies included: 1:200 dilution anti-CLOCK (Abcam ab3517) and 1:4000 dilution anti-Actin (Abcam ab8226). Excess primary antibody was removed and membranes were washed in 1x TBST for 1-hour at room temperature. Washed membranes were incubated with secondary antibody (Abcam ab97051) in 5% milk/TBST at room temperature. Membranes were washed at room temperature 1-hour with 1x TBST. Chemilumiescence was achieved with the Pico Chemiluminescence Kit (Thermo Fisher Scientific 34579) following manufacturer's instructions. Visualization was achieved utilizing an Odyssey® Fc imaging system (Li-Cor) using a 30-second exposure.

Chromatin immunoprecipitation

Hippocampi were extracted as described above and kept at -80°C until further processing. Antibodies were bound and pre-blocked to magnetic beads using the following procedure: fresh block solution was made (225mg bovine serum albumin, 45ml ice cold 1x PBS). Dynabead protein A (50ul; Thermo Fisher Scientific 10001D) and protein G (50ul; Thermo Fisher Scientific 10003D) were added to 1ml of block solution. Magnetic beads were collected on a magnetic stand for 5min. Block solution was aspirated and this wash cycle was repeated two times. After the final wash, 5ug of anti-CLOCK antibody (Abcam ab3517) or IgG (Abcam ab171870) were added to block solution and a final volume of 250ul was added to the magnetic beads. These beads and antibody solutions were incubated with rotation at 4°C for the duration of chromatin preparation, typically ~7 hours.

Chromatin preparation and shearing was performed using a Covaris truCHIP chromatin shearing tissue kit (Covaris 520237). Quenching buffer and fixing buffer were made following manufacturer's instructions (Covaris 520237). Hippocampi (25-40mg) were removed from -80°C

and kept on dry ice. Each hippocampus was individually cut to 1mm^3 segments in a petri dish, kept frozen using liquid nitrogen, then placed in 1.5ml microcentrifuge tubes on dry ice. Tubes containing minced hippocampal tissues were placed at room temperature and 400ul of ice cold 1x PBS was added prior to centrifugation at 4^{o}C for 5-minutes at 3300rpm. During this centrifugation, 1ml of 16% formaldehyde was added to the fixing buffer, to create a final 11.1% formaldehyde fixing buffer. Following centrifugation, supernatant was aspirated and the tissue was placed on ice, resuspended in 400ul of fixing buffer (11.1% formaldehyde), and rocked at room temperature for 8-minutes. Tissue fixation was quenched using 24ul of quenching buffer and incubated at room temperature for 5-minutes while rocking. Tissue was centrifuged at 4^{o}C and 3300rpm for 5-minutes. Supernatant was removed and tissue was washed with 400ul of ice cold 1x PBS and centrifugation at 4^{o}C at 3300rpm for 5min. This 1x PBS wash was repeated twice. After the final wash, supernatant was removed and the tissues were placed in dry ice to flash freeze the tissue.

Tissue pulverization was performed using a Covaris CP02 cryoPREP automated dry pulverizer (Covaris 500001). Freeze dried tissue was placed in Covaris tissue bags (Covaris 520001) and pulverized twice using a setting of 5 on the cryoPREP. Following pulverization, tissue was placed in liquid nitrogen and then moved to dry ice. Lysis buffer, protease inhibitor cocktail, wash buffer, and shearing buffer were made following manufacturer's instructions (Covaris 520237). Pulverized tissue was thawed on ice and 400ul of lysis buffer was added to each tissue bag to resuspended the tissue with pipetting, and contents were transferred to a new tube on ice. Tubes were rotated at 4^{o}C for 20-minutes, then centrifuged at 4^{o}C for 5-minutes at 2000xg. The supernatant was aspirated and 400ul of wash buffer was added to the tubes before samples were incubated at 4^{o}C for 10-minutes with rotation. Samples were then centrifuged at

4°C for 5-minutes at 2000xg. This wash was repeated two times. Following the final wash, 125ul of shearing buffer was added to tubes and incubated on ice for 10-minutes. Following this incubation, 130ul of the prepared nuclei were transferred to microTUBES (Covaris 520216) and placed on ice. Shearing of chromatin was performed using a Covaris S220 focused-ultrasonicator (500217), using the following parameters: PIP 105, 2% duty factor, CPB 200, treatment time of 8-minutes, setpoint temperature of 6°C, minimum temperature of 3°C, maximum temperature of 9°C, and continuous degassing. Samples were placed on ice following sonication. ChIP dilution buffer was made following manufacturer's instructions (Covaris 520237). 160ul of ChIP dilution buffer was added to new a 1.5 microcentrifuge tube, followed by the 130ul of sheared chromatin sample. Samples were centrifuged for 10-minutes at 4°C at max speed (20,000xg). Supernatant was transferred to new a 1.5 microcentrifuge tube on ice and protein quantification was performed using a Qubit protein assay kit (Thermo Fisher Scientific Q33212) and a Qubit 4 fluorometer (Q33238).

Pre-blocked magnetic bead-bound antibodies were washed in fresh block solution as described above, for an additional three washes. Following the washes, magnetic beads were resuspended in 100ul of block solution. 600ug of protein from sheared samples were placed in corresponding tubes containing anti-CLOCK antibody or IgG control. ChIP dilution buffer was added to a final total volume of 300ul. Immunoprecipitation and IgG samples were incubated overnight at 4°C with rotation. Following overnight incubation, magnetic beads were collected using a magnetic stand for 5-minutes and the supernatant was aspirated. Bead containing tubes were removed from the magnetic stand and resuspended in 1ml of RIPA buffer (50 mM Hepes–KOH, pH 7.5; 500 mM LiCl; 1 mM EDTA; 1% NP-40 or Igepal CA-630; 0.7% Na–Deoxycholate). Magnetic beads were collected on a magnetic stand for 5-minutes and RIPA

buffer was aspirated off. This wash cycle was repeated four times. Magnetic beads were resuspended in 1ml of TBS (20 mM Tris–HCl, pH 7.6; 150 mM NaCl) and then collected on a magnetic stand for 5-minutes. TBS was aspirated and tubes were centrifuged at 1000rpm for 3-minutes at 4^{o}C. Tubes were placed on a magnetic stand and any residual TBS was aspirated off. Magnetic beads were resuspended in 200ul of elution buffer (20 mM Tris–HCl, pH 7.6; 150 mM NaCl) and samples were placed in a water bath set at 65^{o}C overnight to reverse-crosslink proteins from DNA.

Following reverse-crosslinking, samples were placed on a magnetic stand for 5min. Supernatant from immunoprecipitated and IgG samples were transferred to new 1.5ml microcentrifuge tubes, and 200ul of TE (10 mM Tris-HCl; pH 8.0; 1mM EDTA) was added, along with 10ug of RNaseA (EN0531), before the sample was incubated in a 37^{o}C water bath for 30-minutes. After the incubation, 4ul of proteinase K (Invitrogen, 25530-049) was added to each sample, mixed, and incubated at 55^{o}C for one hour. 400ul of phenol-chloroform-isoamyl alcohol was added to each tube and mixed before each sample was transferred to a 2ml phase lock gel light tube (FPR5101) and centrifuged for 5-minutes at 16,000xg. The aqueous layer was removed and transferred to a new 1.5ml microcentrifuge tube containing 16ul of 5M NaCl and 1ul of GlycoBlue (AM9516). 800ul of 100% ethanol was added and the contents were mixed and then incubated for 30-minutes at -80^{o}C to precipitate the DNA. Samples were then centrifuged at 4^{o}C for 10-minutes at 20,000xg to pellet the DNA. Pellets were washed with 500ul of 80% ethanol and centrifuged for 5-minutes at 4^{o}C at 20,000xg. Supernatant was aspirated and DNA pellets were allowed to air dry for 30-minutes at room temperature. DNA pellets were resuspended in 50ul of ultra-pure water 10 mM Tris–HCl, pH 8.0 and placed in a 55^{o}C water bath for 10-minutes prior to vortexing.

ChIP-sequencing

Next-generation sequence libraries were generated from immunoprecipitated and IgG eluted DNA using the NEBNext ChIP-seq library prep reagent set for Illumina sequencing, according to the manufacturer's instructions. Briefly, eluted DNA fragments were purified after the adapter ligation step using using AMPure XP beads (Agencourt A63880). An Agilent 2100 Bioanalyzer was used to quantify the amplified library DNA and 20pM of diluted libraries were used for sequencing. A Sage Science Pippin HT was used to size select DNA from libraries, for an average size of ~400bp per sample. 50-cycle paired-end sequencing was performed by the University of Wisconsin – Madison Biotechnology Center. Image processing and sequence extraction were done using the standard Illumina Pipeline.

ChIP-sequencing analysis

Raw paired-end sequencing files were assessed for quality using FastQC (https://www.bioinformatics.babraham.ac.uk/). Adapters were removed and sequence reads were trimmed, using a quality score cutoff of 30, in the Trim Galore! package in R (https://github.com/FelixKrueger/TrimGalore). Trimmed reads were aligned to the *mm9* genome using Bowtie 2 v2.3.4(98), local alignment. Following alignment, only uniquely mapped sequence reads were used for downstream analysis, SAMtools to filter multi-mapping reads(102) and PCR duplicates. Uniquely-mapped and filtered sequence reads were mapped to differentially hydroxymethylated regions (DhMRs) found to contain a putative CLOCK binding site (CACGTG) by motif enrichment analysis ($N = 577$). Only data from these sites were used for differential binding analyses (R package *edgeR(100)*), after controlling for background through

normalization to sequenced IgG reads. A *P*-value threshold of 0.05 was used to determine significant differential binding of CLOCK between groups.

Electrophoretic mobility shift assays

Complementary 5'-biotinylated (0.5uM) and unlabeled oligonucleotides (Integrated DNA Technologies) were annealed by heating equal concentrations of sense and anti-sense oligonucleotides to 95^{o}C and lowering the temperature by 2^{o}C in 3-minute intervals until reaching 23^{o}C. Mouse hippocampal nuclear extracts were isolated from adult female mice (P90) using a nuclear and cytoplasmic extraction kit per the manufacturer's instructions (Thermo Fisher Scientific 78833). Shift assays were performed using the LightShift® Chemilumiescent EMSA Kit (Thermo Fisher Scientific 20148). DNA binding reactions were performed in a 20ul system containing biotinylated oligonucleotides and nuclear extracts (12ug). For cold competition assays, a 1:1000 concentration of unlabeled oligonucleotides was incubated on ice for 1-hour with nuclear extracts prior to the addition on biotinylated oligonucleotides. For supershift assays, 5ug of anti-CLOCK antibody (Abcam, ab3517) or anti-BMAL1 antibody (Abcam, ab93806) were incubated on ice with nuclear extract for 1-hour prior to the addition of biotinylated oligonucleotides. In such assays, nuclear extracts without the addition of unlabeled oligonucleotides or antibody were also incubated on ice for 1-hour prior to the addition of biotinylated oligonucleotides, to ensure the absence of protein degradation during the incubation period. Following incubation on ice, biotinylated oligonucleotides (0.5uM) were added to binding reactions and incubated at room temperature for 20-minutes. Reaction products were separated by electrophoresis at 100V for 60-minutes. Following separation, protein::DNA complexes were transferred onto a positively-charged nylon membrane (Thermo Fisher

Scientific 77016) for 60-minutes at 380A on ice. Protein::DNA complexes were detected using the Nucleic Acid Detection Module Kit (Thermo Fisher Scientific 89880) per the manufacturer's instructions, and imaged using an Odyssey® Fc imaging system (Li-Cor) and a 60-minute exposure.

ImageJ band quantification

Band intensity percentages were quantified using ImageJ software. First, images were set to grey-scale. Bands of interest were selected across all lanes and their inverted means were obtained. Background noise intensity was subtracted out by subtracting the intensity of staining above the bands of interest. Band intensities were normalized to loading controls lanes (*i.e.,* beta-actin (western blot)). For EMSA, a two-sided *t*-test (R environment) was used to determine significant alterations in binding affinity to methylated- and hydroxymethylated-oligonucleotides, using three independent EMSA replicates. For western blot, a two-sided *t*-test (R environment) was used to determine significant changes in CLOCK expression between groups/genotypes, using three independent immunoblot replicates.

Oligonucleotide sequences

<u>Unmodified Probes:</u>

Fry1 FW: 5'-Biotin-TATGTTCATCCACGTGATGC-3'

Fry1 RVL 5'-Biotin-GCATCACGTGGATGAACATA-3'

Gigyf1 FW: 5'-Biotin-GGGTACACGTGCGCCATGGC-3'

Gigyf1 RV: 5'-Biotin-GCCATGGCGCACGTGTACCC-3'

Palld FW: 5’-Biotin-GAATGTCCACACGTGTATGC-3’

Palld RV: 5’-Biotin-GCATACACGTGTGGACATTC-3’

5hmC Probes

Fry1 FW: 5’-Biotin-TATGTTCATCCA^{hme}CGTGATGC-3’

Fry1 RV: 5’-Biotin-GCATCA^{hme}CGTGGATGAACATA-3’

Gigyf1 FW: 5’-Biotin-GGGTACA^{hme}CGTGCGCCATGGC-3’

Gigyf1 RV: 5’-Biotin-GCCATGGCGCA^{hme}CGTGTACCC-3’

Palld FW: 5’-Biotin-GAATGTCCACA^{hme}CGTGTATGC-3’

Palld RV: 5’-Biotin-GCATACA^{hme}CGTGTGGACATTC-3’

5mC Probes

Fry1 FW: 5’-Biotin-TATGTTCATCCA^{me}CGTGATGC-3’

Fry1 RV: 5’-Biotin-GCATCA^{me}CGTGGATGAACATA-3’

Gigyf1 FW: 5’-Biotin-GGGTACA^{me}CGTGCGCCATGGC-3’

Gigyf1 RV: 5’-Biotin-GCCATGGCGCA^{me}CGTGTACCC-3’

Palld FW: 5’-Biotin-GAATGTCCACA^{me}CGTGTATGC-3’

Palld RV: 5’-Biotin-GCATACA^{me}CGTGTGGACATTC-3’

Unlabeled Probes:

Fry1 FW: 5’-TATGTTCATCCACGTGATGC-3’

Fry1 RVL 5’-GCATCACGTGGATGAACATA-3’

Gigyf1 FW: 5’-GGGTACACGTGCGCCATGGC-3’

Gigyf1 RV: 5’-GCCATGGCGCACGTGTACCC-3’

Palld FW: 5’-GAATGTCCACACGTGTATGC-3’

Palld RV: 5’-GCATACACGTGTGGACATTC-3