Supplementary Materials:

Materials and Methods

Animals. Male C57BL/6J at 8-12 weeks of age were housed in light-tight boxes and entrained to LD12:12 conditions. After a minimum of 7 days, animals were transferred to constant darkness (DD) conditions. Thirty-six hours after DD, liver samples (N=3-6 mice) were collected every 4 hrs. All animal experiments were in accordance with guidelines of the UT Southwestern Medical Center Animal Care and Use Committee.

Antibodies. Antibodies against PER1, PER2, CLOCK, and BMAL1 were made as described previously (7). CRY1 antibody was made as described (63). CRY2 (epitope: residues 514-592) and p300 (epitope; residues 60-242 of human p300) antibodies were generated using guinea pigs (Cocalico Biological) and serum was affinity purified using the same protein used to raise antibody. NPAS2 antibody (64) was a kind gift from Dr. Steven McKnight (UT Southwestern Medical Center). BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, CRY2, p300 and CBP antibodies were validated by western blot analysis of liver nuclei from wild-type mice. In addition, the BMAL1, PER2, and CRY2 antibodies were validated using either liver or cerebellum tissue lysates including the appropriate knockout mouse control. The PER1 antibody was validated using mouse embryonic fibroblast (MEF) extracts derived from wild-type and Per1 knockout mice. The CLOCK antibody was also validated using MEFs isolated from wildtype, Bmall knockout and Clock knockout mice. P300 antibody was validated by in vitro translated protein with a full-length p300 cDNA-pcDNA3.1 or empty pcDNA3.1. RNAPII-8WG16 (MMS-126R) antibody (50) was purchased from Covance. RNAPII-Ser5P (clone 3E8, 04-1572) antibody (51) was purchased from Millipore and RNAPII-Ser5P (ab5131) antibody

(*57*) was purchased from Abcam. H3K4me1 (ab8895), H3K4me3 (ab1012), H3K9ac (ab4441), H3K27ac (ab4729), H3K36me3 (ab9050) and H3K79me2 (ab3594) antibodies were purchased from Abcam. CBP antibody was monoclonal AC238 culture supernatant (*65*).

Chromatin Immunoprecipitation Sequencing (ChIP-seq). Livers from mice (C57BL/6J) were immediately homogenized in 4 ml per liver of 1X PBS containing 1% formaldehyde. The homogenate was kept for 8 min at room temperature, 250 µl of 2.5 M glycine was added to stop the reaction on ice. For dual crosslinking (66, 67), livers were homogenized in 4 ml per liver of 1X PBS containing 2 mM EGS, kept for 20 min at room temperature, formaldehyde was added (final conc. 1%) for 8 min at room temperature and 250 µl of 2.5 M glycine was added to stop the reaction on ice. The homogenate was resuspended in 10 ml of ice-cold 2.3 M sucrose contacting 150 mM glycine, 10 mM HEPES pH 7.6, 15 mM KCl, 2 mM EDTA, 0.15 mM spermine, 0.5 mM spermidine, 0.5 mM DTT and 0.5 mM PMSF, and layered on top of a 3 ml cushion of 1.85 M sucrose (containing the same ingredients and including 10% glycerol) and centrifuged for 1 hr at 24,000 rpm at 4°C in a Beckman SW32.1 rotor. The nuclei were resuspended in 1 ml of 10 mM Tris pH 7.5, 150 mM NaCl, 2 mM EDTA, transferred to a 1.5 ml microfuge tube, centrifuged for 3 min at 3000 rpm at 4°C, washed again and stored at -80°C until use. The nuclei from two livers were pooled before sonication. For BMAL1 and 8WG16 antibodies, the formaldehyde-crosslinked nuclei were resuspended in 0.8 ml per liver of lysis buffer (50 mM Tris pH 7.5, 10 mM EDTA, 1% SDS, 1 mM PMSF and Roche complete EDTA free protease inhibitor cocktail) and sonicated 10 times for 30 sec at 4°C using a Covaris S2 ultrasonicator. For CLOCK and NPAS2 antibodies, the dual crosslinked nuclei were resuspended in 0.8 ml per liver of Sarkosyl lysis buffer (50 mM Tris pH 7.5, 10 mM EDTA,

0.5% N-lauroylsarcosine, 1 mM PMSF and Roche complete EDTA free protease inhibitor cocktail) and sonicated 6 times for 30 sec at 4°C using a Covaris S2 ultrasonicator. The fragmented chromatin was then diluted tenfold with IP buffer (10 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM PMSF, protease inhibitor cocktail). For PER1, PER2, CRY1, CRY2, CBP and p300 antibodies, the dualcrosslinked nuclei were resuspended in 3 ml per liver of IP buffer and sonicated 48 times for 5 sec on ice using a Misonix S-4000 sonicator. For RNAPII-Ser5P antibody, the formaldehydecrosslinked nuclei were resuspended in 0.8 ml per liver of Sarkosyl lysis buffer, sonicated 6 times for 30 sec at 4°C using a Covaris S2 ultrasonicator, and diluted 10-fold with IP buffer. For H3K4me1, H3K4me3, H3K9ac, H3K27ac, H3K36me3 and H3K79me2 antibodies, the formaldehyde-crosslinked nuclei were resuspended in 0.7 ml per liver of 10mM Tris pH 7.5 and 100 mM NaCl, sonicated 5 times for 30 sec at 4°C using a Covaris S2 ultrasonicator, incubated for 40 min at 37°C with 200 kunitz units of Micrococcal Nuclease and 2 mM CaCl₂, and then stopped with 10 mM EGTA and 1% SDS. The digested chromatin was then diluted 10-fold with IP buffer. Approximately 120 μg (for transcription factors) or 80 μg (for histones) of fragmented chromatin was pre-cleared by incubating with 40 µl of protein A-agarose (Sigma) for 2 hr at 4°C on a rotating wheel. Pre-cleared chromatin was then incubated with antibody overnight at 4°C on a rotating wheel, 10 µl of Protein A/G Plus-agarose was then added and incubated for 1.5 hr at 4°C. Beads were then washed twice with IP buffer, twice with high salt wash buffer (20 mM Tris pH 7.5, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, 1 mM PMSF), twice with LiCl wash buffer (20 mM Tris pH 7.5, 250 mM LiCl, 2 mM EDTA, 0.5% Igepal CA-630, 1% sodium deoxycholate, 1 mM PMSF), and once with TE. Co-immunoprecipitated DNA fragments were eluted with 100 µl of 20 mM Tris pH 7.5, 5 mM EDTA, 0.5% SDS, then reverse crosslinked at

65°C for overnight, incubated with 10 μg of RNaseA for 30 min at 37°C, with 160μg of proteinase K for 30 min at 55°C, and then purified using a Qiaquick PCR purification Kit (Qiagen).

Sequencing library construction was performed as described (68). The immunoprecipitated DNA fragments were repaired by the End-It DNA End Repair Kit (Epicentre Biotechnology) according to the manufacturer's instructions. The end-repaired ChIP DNA fragments were purified by MinElute Reaction Cleanup Kit (Qiagen) and eluted in 20 µl in EB buffer. The resulting DNA fragments were ligated with P1 and P2 adaptors for the Applied Biosystems SOLiD system for 20 min at room temperature using the Quick Ligase Kit (NEB), followed by purification using the MinElute Reaction Cleanup Kit (Qiagen). The purified, adaptor-ligated ChIP DNA fragments were run on 5% native polyacrylamide gel electrophoresis (PAGE) for an in-gel PCR reaction. A gel slice containing 175–200 bp adaptor-ligated ChIP DNA fragments (corresponding to 115-140 bp genomic fragment sizes) was cut and shredded. PCR Platinum Supermix (100 µl, Invitrogen), 50 pmol of PCR primers with barcodes, 0.5 µl Taq DNA polymerase (NEB), and 0.15 µl p.f.u. Turbo DNA polymerase (Stratagene) were added into the shredded gel slice. The adaptor-ligated ChIP DNA fragments were amplified by 14-19 cycles of in-gel PCR. After the PCR reaction, gel pieces were filtered out with a 0.45 µm filter spin column, and the amplified ChIP-seq library was purified by the MinElute PCR purification kit (Qiagen). The library was purified by one more round of 5% PAGE. A gel slice containing 200-230 bp PCR products (110-130 bp fragment size) was cut and shredded, and the amplified library was extracted out of the gel by passive elution in elution buffer (2.5 M ammonium acetate in TE). Gel pieces were removed with a spin column, and the resulting ChIP-seq library was purified using the QIAquick PCR purification kit (Qiagen). SOLiD sequencing of ChIP-seq

libraries were performed on an Applied Biosystems SOLiD4 or 5500xl instrument with 35-bp reads according to manufacturer's instructions (Life Technologies) by the UTSW McDermott Next Generation Sequencing Core. Sequence reads were mapped to the mouse genome (NCBI m37/mm9) with Applied Biosystems BioScope v1.3. The mapping algorithm uses a seed and extend mapping scheme using the first 25 bp as the "seed" and allowing a maximum of two mismatches in the seed region for 35 bp reads. Two mapping options (ma.to.bam.output.filter=alignment score, and ma.to.bam.clear.zone = 5) were used to indicate that the mapped reads in the ma file are limited to "unique" reads for output to the bam file. These two parameters indicate that the reads are primary and unique. Uniqueness is determined by the clear zone number. If there are two primary mapping locations for a read, the read is considered unique if the mapping quality of that mapping location is at least 5 greater than the next primary mapping location. If the score is 5 or greater, all other mappings are discarded and the higher quality mapping is kept as the unique read. Otherwise all mappings are discarded. If there is only one mapping location, that location is kept and termed unique. Duplicates were removed using Picard MarkDuplicates (http://picard.sourceforge.net).

In order to adjust for differences in sequencing depth among time samples, the sequence reads were "down sampled" to the lowest number of the uniquely mapped reads with duplicates among the six time points for each ChIP factor. In determining how best to compensate for variations in sequence depth among samples during the sequencing runs, we tested four different methods and came to the conclusion that down sampling was the best method. The other three methods involved normalizing either to the "total reads," "total mapped reads with duplicates" or "total mapped reads without duplicates." In each case, we found that some type of bias could be introduced depending on how different the sequencing depth (the number of sequence reads) was

among samples. Thus, we determined empirically that it was better to randomly sample the sequence reads in order to equalize the sequencing coverage rather than to numerically divide the sample reads by one of the three sequence read metrics above.

To validate our approach further, we calculated the percentage of the reads in the sample that were localized in the peaks. This analysis shows that less than 1-2% of the signal is usually contained in the peaks. Thus the peak height or number of peaks would account for only a very small fraction of the total, and therefore would only be influenced by the normalization procedure by a few percent. Because of this fact, our procedure is equivalent to normalizing to the "background."

% reads mapped in master peak area

	CT0	CT4	CT8	CT12	CT16	CT20	КО
BMAL1	0.6475%	0.8673%	0.9845%	0.4231%	0.1512%	0.1140%	0.0365%
CLOCK	0.2968%	0.2599%	0.3647%	0.2645%	0.1668%	0.1715%	0.0262%
NPAS2	0.1375%	0.1309%	0.2316%	0.1266%	0.0862%	0.0404%	
PER1	0.1588%	0.1342%	0.0818%	0.6136%	0.3148%	0.3998%	0.0852%
PER2	0.3617%	0.1874%	0.2356%	0.6044%	1.0179%	0.8086%	0.1962%
CRY1	1.8008%	1.8779%	0.7481%	1.3286%	0.6321%	1.4935%	0.3644%
CRY2	0.4955%	0.3102%	0.6556%	0.7370%	0.9125%	0.6339%	0.2434%
CBP	0.2976%	0.4353%	0.3634%	0.1760%	0.6501%	0.4021%	
p300	0.0650%	0.0679%	0.0865%	0.0322%	0.0361%	0.0424%	
8WG16	0.2204%	0.1998%	0.1948%	0.5559%	0.4363%	0.1291%	
Ser5P	2.3970%	2.1996%	1.3907%	0.8423%	1.2076%	1.8905%	

The total reads for each sample and the down sampled reads for each factor are shown in Table S1. Results were further analyzed using HOMER (69). Genome browser views were normalized to display uniquely mapped reads per 10 million uniquely mapped reads with duplicates.

ChIP-seq peak finding. The peaks were identified from uniquely mapped reads without duplicates using MACS with following parameters: genomic size = mm (1.87 Gb), shift = 60 and input chromatin samples as control data (33). A p-value threshold of 10^{-5} (default) and a ratio

between the ChIP-Seq tag count and λlocal of 10 (fold_enrichment threshold) was used. The false peaks called by MACS in Table S11 that repeatedly emerged from low complexity sequence were removed from further analysis. The peaks were then subdivided by PeakSplitter (34) with options of –valley 0.7 and –cutoff 7. To construct a master peak list from the six time points, the peaks obtained after PeakSplitter were merged, compared for overlaps and the peak with the highest summit value was chosen if the summit coordinates were within 120 bp. Figure S3 illustrates the master peak process in which MACS peaks are called, then subdivided with PeakSplitter and then compared for overlap and summit height.

The ChIP-seq peak overlaps (peak summit +/- 120bp) from the master peak lists were determined using HOMER (69). Chow-Ruskey diagrams (70) were made with R using the Vennerable package.

Whole transcriptome sequencing (RNA-seq). RNA was isolated from livers using Trizol reagent according to the manufacturer's instructions (Life Technologies). 10 μg of total RNA pooled from three mice (individual mouse RNA samples with RIN values of 8-9) was depleted of ribosomal RNAs using RiboMinus Eukaryote Kit for RNA-seq according to the manufacturer's instructions (Life Technologies). The removal of ribosomal RNAs was confirmed on a Bioanalyser Pico Chip (Agilent). Sequencing libraries were constructed using SOLiD Total RNA-seq Kit (Life Technologies) with a modification below. A total of 1 μg of rRNA-depleted total RNA was fragmented in the 30 μl of T4 PNK buffer for 18 min at 95°C and placed on ice immediately. Fragmented RNA was phosphorylated by adding 2 μl of T4 PNK and 1 mM ATP for 60 min at 37°C and purified by RiboMinus Concentration module (Life Technologies) prior to the adaptor ligation. SOLiD sequencing of libraries was performed on an

ABI SOLiD4 instrument with 50-bp reads according to manufacturer's instructions (Life Technologies) by the UTSW McDermott Next Generation Sequencing Core. Sequence reads were mapped to the mouse genome (NCBI m37/mm9) with Applied Biosystems BioScope v1.3 to create Wig and BigWig files for visualization on the UCSC Genome Browser. In the RNAseq experiments, we did not observe obvious differences in the total amount of RNA at different time points. This is due to the great abundance of ribosomal RNAs in the samples (and this is still the case after ribosomal RNA depletion as assessed by the large number of ribosomal RNA reads in the samples). For these reasons, we used the conventional method of normalization to RPKM which does not appear to introduce bias among the time samples. For analysis with HOMER (69), sequence reads were mapped to the mouse genome (NCBI m37/mm9) with Bowtie (71) and Tophat (72) to create bam/sam/bed input files for HOMER because Bioscope derived bam/sam/bed files contained non-uniquely mapped low complexity sequence reads. To obtain reliable alignments, the reads with mapping quality less than 5 were removed by SAMtools (73). The UCSC canonical gene set (28,661 total; 21,789 with introns) was used for annotation of exons and introns. Among UCSC known canonical genes, we assumed that a gene was expressed if there were >5 reads in the gene body. To find expressed antisense transcripts, a threshold of >8 reads was used.

Quantitative PCR (qPCR). qPCR was performed with iTaq[™] SYBR® Green Supermix with ROX (BioRad) using an Applied Biosystems PRISM 7900HT Sequence Detector. Primer sequences used for RNA quantification are described (*12*, *74*). Primers used for ChIP DNA quantification were:

Name	Forward	Reverse
Dbp -2.4kb (upstream)	TGCCTCCTCTTCCACCCCAGG	CAAAGAGGCTGAGAATGGCCAGG
Dbp -0.4kb (promoter)	ACACCCGCATCCGATAGC	CCACTTCGGGCCAATGAG
Dbp +0.8kb (intron 1)	ATGCTCACACGGTGCAGACA	CTGCTCAGGCACATTCCTCAT
Dbp +2.4kb (intron 2)	TGGGACGCCTGGGTACAC	GGGAATGTGCAGCACTGGTT
Dbp +4.4kb (exon 4)	AAGAACAATGAAGCAGCCAAGAG	GGCAGCCGCACAGATAT
Per2 -4.5kb	CCACACGGTACTCAGCGGGC	GGGTCACTGCGAGCCTTGCC
Per2E2	GGTTCCGCCCCGCCAGTATGC	CCGTCACTTGGTGCGCTCGGC
Per1E1	AGCCAGCCTGCACGTGTTCC	CAGAGACAACCCCGCCCTGC
Per1E5	CAGCACCCAAGTCCACGTG	CCGGTTGGCTAAGGATCTCTT

Time Series Analysis for Circadian Cycling. RNA cycling was assessed by three programs, COSOPT (*35*) JTK cycle (*75*) and ARSER (*76*) with expression level cutoffs, >0.05 RPKM for intron, >0.5 RPKM for exon and >0.0625 RPKM for antisense. For COSOPT and JTK cycle analyses, data was detrended by linear regression. A cycling gene was considered if two out of three programs detected cycling with threshold of p<0.05. The period and phase from ARSER were used for further analysis. For ChIP-seq peak analysis, two cycles were concatenated and the cycling was analyzed with ARSER (p<0.05).

Gene Ontology analysis. WebGestalt (77, 78) was used for KEGG pathway and gene ontology analyses.

Immunoblotting and Immunoprecipitation. Immunoblotting was performed as described previously (*79*). Immunoprecipitation was performed as described previously (*12*). Briefly tissues were homogenized in EB (20 mM HEPES pH 7.5, 100 mM NaCl, 0.05% TritonX-100, 1 mM EDTA, 20 mM NaF, 1 mM Na₃OV₄, Complete Mini protease inhibitor cocktail, Roche) and centrifuged at maximum speed for 10 min at 4°C. The supernatants were transferred to fresh

tubes and incubated with 1.5 µg of antibodies for 2 hr at 4°C. Ten microliters of 50% protein-A slurry (GE Healthcare Life Sciences) was added, and the incubation continued for an additional 1.5 hr. Supernatants were discarded after centrifugation at 3000 rpm at 4°C for 1 min, and protein-A beads were washed three times with 1 ml EB. Pellets were resuspended in 20 µl of 2 X SDS sample buffer and boiled for 3 min. Protein samples were separated by 10% or 6% SDS-PAGE and then transferred to a nitrocellulose membrane (Dupont NEN).

Supplemental figure legends

Fig. S1. Rhythmic DNA binding of core clock proteins, RNA polymerase and histone modifications measured by chromatin immunoprecipitation and qPCR (ChIP-qPCR). (**A**) Circadian DNA binding of BMAL1 (a), CLOCK (b), NPAS2 (c), PER1 (d), PER2 (e), CRY1 (f) and CRY2 (g). ChIP-qPCR values are shown relative to input DNA. Rhythmic DNA binding of core clock proteins is seen at E-boxes at the *Dbp* promoter (-0.4 kb), intron 1 (+0.8 kb), intron 2 (+2.4 kb), *Per2* promoter (E2) and *Per1* promoter (E1 and E5). (**B**) Temporal profiles of CBP (a), p300 (b) and RNA polymerase II (c and d). (**C**) Temporal profiles of H3K4me1 (a) H3K4me3 (b), H3K9ac (c), H3K36me3 (d), H3K79me2 (e) and H3K27ac (f) histone modifications in *Dbp*, *Per2* and *Per1* loci.

Fig. S2. UCSC genome browser view of BMAL1 ChIP-seq for two complete circadian cycles in constant darkness at the *Per1* (**A**), *Dbp* (**B**) and *Nr1d1* (**C**) loci.

Fig. S3. Illustration of ChIP-seq peak finding and master peak list construction. ChIP-seq peaks at the *Per1* (**A**), *Dbp* (**B**) and *Nr1d1* (**C**) loci. Peaks determined by MACS (*33*) are shown in orange and peaks subdivided by Peaksplitter (*34*) are shown in green. MACS peaks with fold enrichment less than 10 were filtered out. MACS peaks were then subdivided with Peaksplitter and small peaks (peak height <7) were removed. Master peaks indicated in red were chosen from the Peaksplitter peaks by combining all peaks from all 6 time points that had summit locations within 120 bp and then selecting the highest peak for the master peak list. The numbers to the left of the bars under the peaks are the tag counts (not fold enrichment) for MACS peaks (orange) or peak height for Peaksplitter peaks (green) and master peaks (red).

Fig. S4. (**A**) Genomic annotation of the binding sites for BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, CRY2, CBP, p300, RNAPII-8WG16, and RNAPII-Ser5P. The peaks were annotated to promoter-TSS (-1kb to +100bp from TSS), 5'UTR, exon, intron, 3'UTR, TTS (from TTS to +1kb) as indicated. (**B**) Overlap of BMAL1 peaks from Rey et al. (*28*) with this paper. **C**) Overlap of REV-ERBα/β peaks from Cho et al. (*30*) with circadian transcription factors and CBP from this paper.

Fig. S5. Sequence motifs of the circadian transcriptional regulators, co-activators and RNAPII binding sites in Fig. 1, 3 and 4. De novo sequence motif analysis was carried out with +/- 60 bp DNA sequence from the master peak binding sites by HOMER (69).

Fig. S6. (**A**) Binding coverage profiles for BMAL1 (blue), CLOCK (green), CRY1 (red), CRY2 (purple), PER1 (orange) and PER2 (brown) from -260 to +260 bp surrounding the 1444 common binding sites. (**B** and **C**) Chow-Ruskey diagrams showing circadian time dependent overlap of activator (**B**) and repressor (**C**) binding sites. MACS derived-peaks from each time point were used to determine if there is more than 1bp overlap in the peak areas. The master peak list for each factor was used to filter out false positive peaks from the MACS peak calls at each time point.

Fig. S7. Sequence motifs of the 6-way common circadian transcriptional regulators sites or the indicated unique/combination binding sites in Fig. 1D. De novo sequence motif analysis was carried out with +/- 60 bp DNA sequence from the master peak binding sites by HOMER (69).

Fig. S8. Comparison of temporal RNA expression profiles using qPCR, intron RNA-seq and exon RNA-seq analysis. The intron RNA (red) and exon RNA (green) expression levels are indicated on the left and right Y-axis, respectively. The highest RNA abundance measured by qPCR (blue) is normalized to highest abundance from exon reads.

Fig. S9. UCSC genome browser views of CRY1 and RNAPII-Ser5P common binding sites at the *Nr1d1* (**A**), *Nfil3* (**B**), *Adck3* (**C**) and *Ppp1r3c* (**D**) loci. Tracks for BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, CRY2, RNAPII-8WG16, RNAPII-Ser5P, CBP and p300 ChIP-seq as well as RNA-seq data are shown as indicated. In addition, tracks for REV-ERBα (NR1D1) and REV-ERBβ (NR1D2) ChIP-seq at ZT8 are shown in red for comparison (*30*). The intron RNA cycling genes (~300 genes) that peak between CT20 and CT4 are similar to those seen in fig. S7B-D and account for majority of the RNAPII-Ser5P signal that peaks at CT0.

Fig. S10. (**A**) Heat map view of 24 hr (left) and 12 hr (right) cycling DNA binding sites for CBP. Histograms of the phase of the rhythms in each class are shown below the heat map (mean circular phase is shown in red). (**B**) Heat map view of 24 hr (left) and 12 hr (right) cycling DNA binding sites for CRY1. Histograms of the phase of the rhythms in each class are shown below the heat map (mean circular phase is shown in red). (**C**) Overlap of RNAPII-Ser5P with CRY1 peaks.

Fig. S11. Temporal variation in RNAPII occupancy and histone modifications. Gene model plots for RNAPII-8WG16, RNAPII-Ser5P, H3K4me1, H3K4me3, H3K9ac, H3K27ac, H3K36me3

and H3K79me2 occupancy. The average signal from ChIP-seq from 12,680 expressed genes (top), 8945 unexpressed genes (middle) and 1371 intron cycling genes (bottom) are shown as indicated. The intron-less genes were filtered out from the analysis. Signal from all genes were normalized to a standard 40 kb gene body length for display in addition to the signal from 10 kb upstream of the TSS to 10 kb downstream of the transcription termination site (TTS) are shown in the gene model. Colors indicate the circadian time (CT) of the sample.

Fig. S12. Overlap of chromatin marks with RNAPII as a function of the circadian cycle and circadian rhythms in histone modifications at promoter and enhancer sites. (**A**) Chow-Ruskey diagrams showing the overlap of RNAPII binding and histone modifications at six circadian times. The gene body +/- 1 kb was used to determine if the gene was positive for RNAPII-8WG16 occupancy, and/or H3K4me4, H3K9ac or H3K27ac modifications. (**B** and **C**) ChIP-seq binding profiles at promoter and intergenic sites. Average signal from -2 kb to +2 kb of BMAL1 (**B**) or CBP binding sites (**C**) in promoter or intergenic sites are plotted as indicated.

Fig. S13. UCSC genome browser views of BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, CRY2, RNAPII-8WG16, RNAPII-Ser5P, CBP, p300, H3K4me1, H3K4me3, H3K9ac, H3K27ac, H3K36me3 and H3K79me2 ChIP-seq as well as RNA-seq data are shown as indicated at: (A) Nr1d1 (Rev-erbα); (B) Nr1d2 (Rev-erbβ); (C) Dbp; (D) Nfil3 (E4BP4); (E) Per1; (F) Per2; (G) Per3; (H) Cry1; (I) Cry2; (J) Gm129; (K) Hlf; (L) Tef; (M) Bhlhe40 (Dec1); (N) Bhlhe41 (Dec2).

Supplemental Tables.

Table S1. Sequence statistics for ChIP-seq and RNA-seq experiments showing the number of runs, the reads per sample and the results of normalization by down sampling. The run listed at the top of each category is the experiment illustrated in this paper. Replicate experiments are shown below.

Table S2. Master peak lists for ChIP-seq experiments for BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, CRY2, CBP, p300, RNAPII-8WG16, and RNAPII-Ser5P ranked by the average tag count across all 6 times points.

Table S3. Gene ontology analysis for BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, CRY2, CBP, p300 and RNAPII-8WG16 ChIP-seq experiments.

Table S4. KEGG pathway analysis for BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, CRY2, CBP, p300 and RNAPII-8WG16 ChIP-seq experiments.

Table S5. List of the genes with cycling antisense RNA transcripts.

Table S6. List of cycling intron RNA genes.

Table S7. List of cycling exon RNA genes.

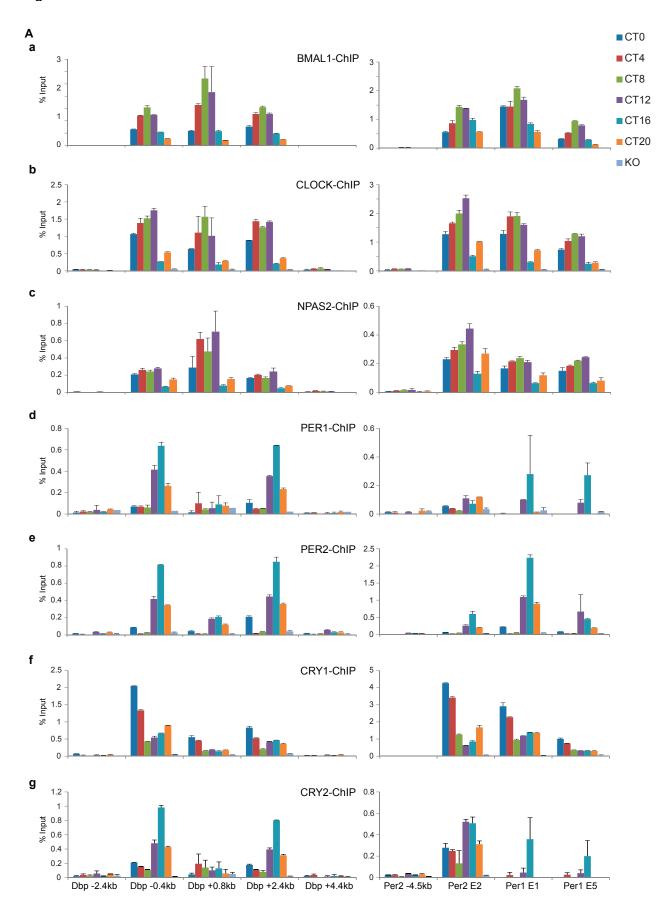
Table S8. Gene ontology analysis of the intron or exon cycling genes.

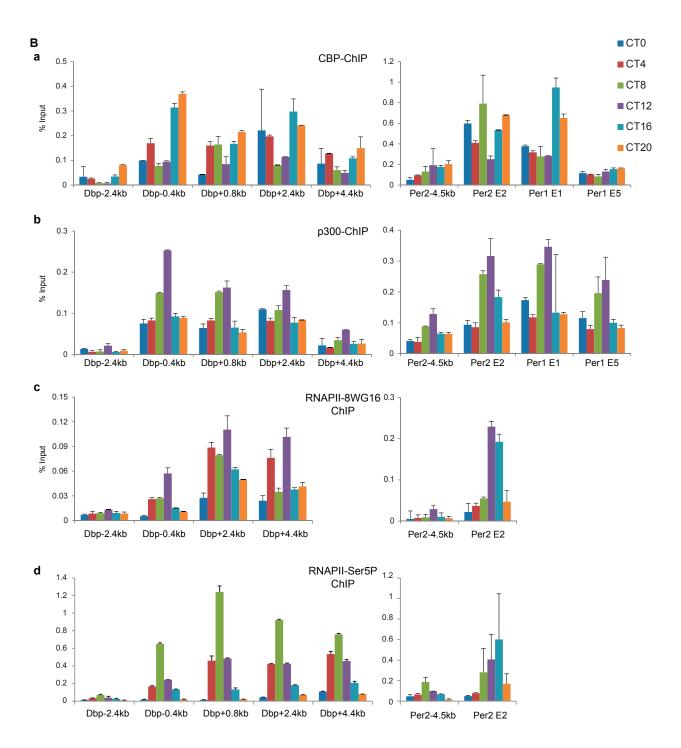
Table S9. KEGG pathway analysis of the intron or exon cycling genes.

Table S10. The majority of genes bound by circadian transcriptional regulators (CTR), coactivators and RNAPII are expressed.

Table S11. List of false positive peaks removed from the ChIP-seq peaks.

Fig. S1





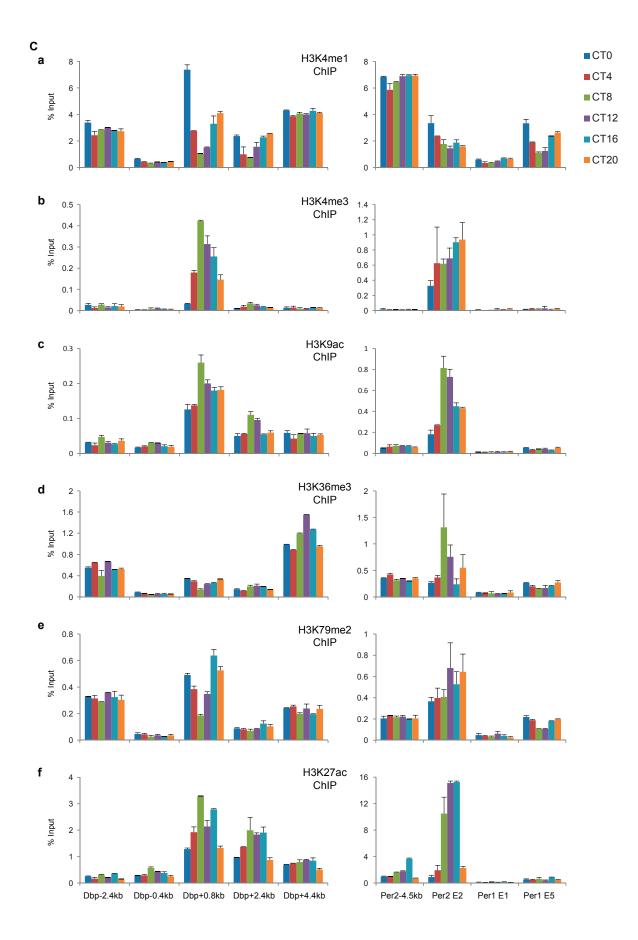


Fig. S2

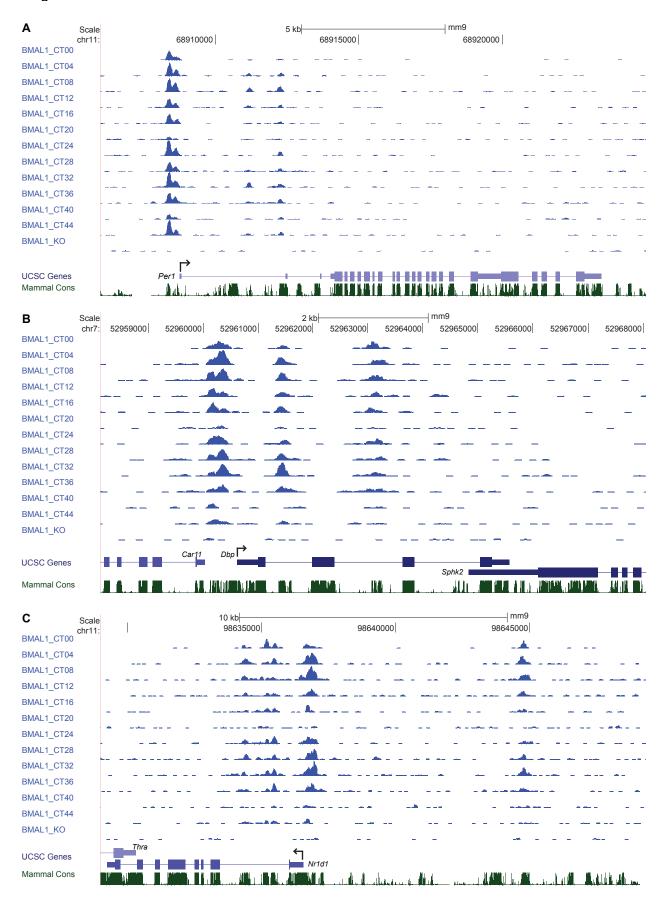


Fig. S3

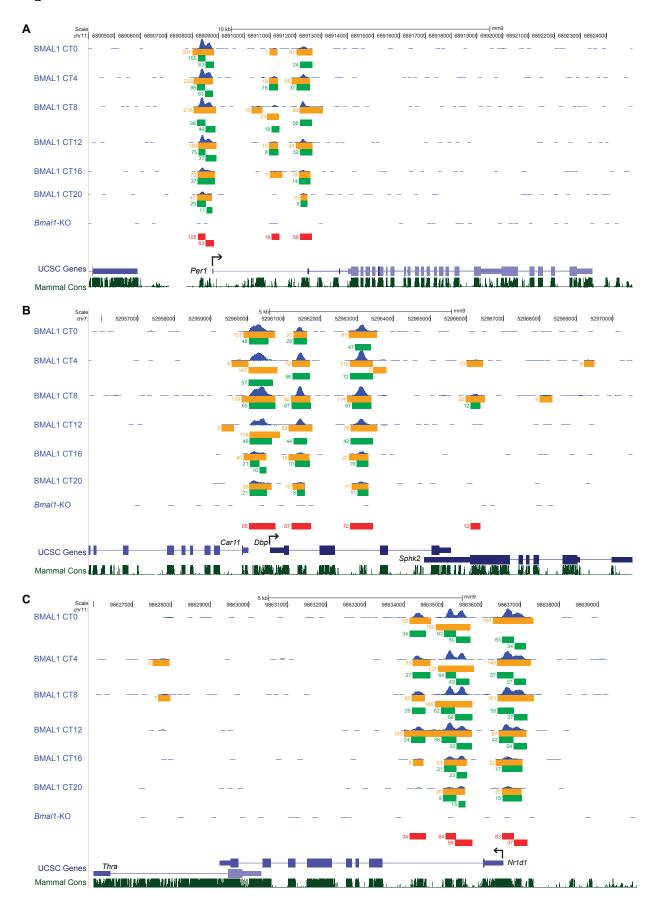
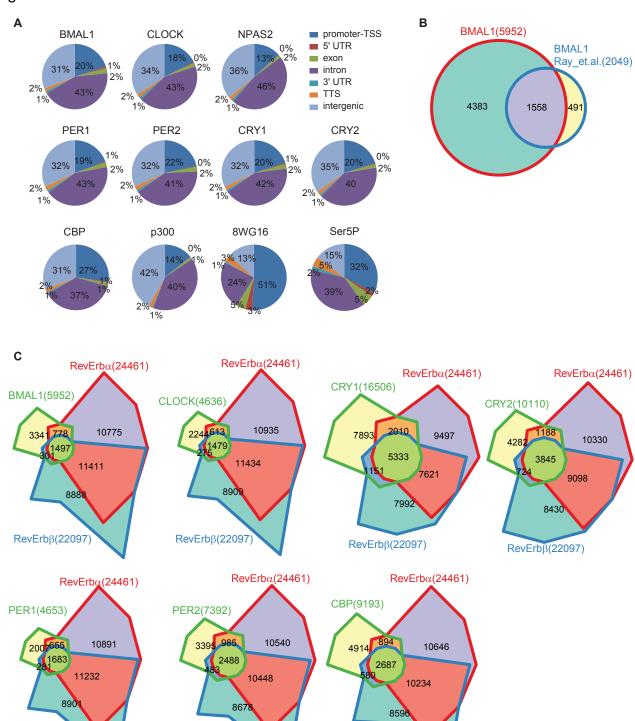


Fig. S4

RevErbβ(22097)

RevErbβ(22097)

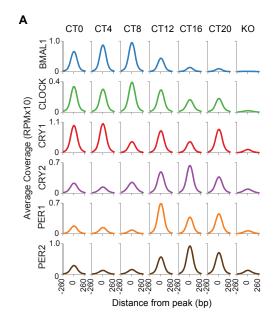


RevErbβ(22097)

Fig. S5							
BMAL1	P-value	% of Targets	Best Match/Details	PER2	P-value	% of Targets	Best Match/Details
<u>Ş</u> ₽₽₽ CACGTG	1e-1929	_	Mycn	ESCAPOTGAP	1e-704	36.99%	Mycn
etescavolo etieigeaae	1e-265	9.49%	bZIP_cEBP-like_subclass	ettecaraas	1e-563	21.05%	bZIP_cEBP-like_subclass
ETGACCTITE	1e-172	20.82%	Erra(NR)	IATTGACSIA	1e-325	26.98%	Foxa2
GRTCAA	1e-153	28.29%	HNF6	CAAAGGICA	1e-276	20.17%	Erra(NR)
<u>ÇATGIŞSIŞ</u>	1e-134	7.39%	INO2	AAATCGAT	1e-192	5.63%	HNF6
TGTTTGSTSA	1e-120	14.43%	fkh	ITGGCASS	1e-188	31.62%	NF1-halfsite
TGCCAS	1e-87	28.44%	NF1-halfsite(CTF)	TAAECI	1e-166	50.69%	Gsc
AGTCAC	1e-76	14.40%	ARG81	<u> </u>	1e-150	11.49%	GC-box
SCTCCCCC	1e-58	8.79%	GC-box	TGGACT	1e-61	11.80%	Hnf4a_2
GTTAATÇATT	1e-46	1.44%	HNF1A	AGAGCGTG	1e-36	4.53%	Arnt::Ahr
ACCTITGACC	1e-43	3.63%	RXR(NR/DR1)	CIGGGTCA	1e-24	4.26%	Nr2f2_2
TCAATA	1e-35	9.86%	Zfp105_2	GASTTGIAGT	1e-14	0.57%	GFY
GACTTTGGAC	1e-35	1.53%	HNF4a			% of	
IGCACGTTIT	1e-16	0.39%	Mafk_2	CRY1	P-value	Targets	Best Match/Details
CACGC&C&TG	1e-13	0.69%	Arnt::Ahr	GICACGTG	1e-1159	27.86%	USF1(HLH)
				TTASSAAJA	1e-943	19.97%	CEBPA
CLOCK	P-value	% of Targets	Best Match/Details	<u>CAAAGTICA</u>	1e-903	23.09%	Erra(NR)
	1e-1024	_	MAX	FRICA AFA	1e-606	21.48%	Foxa2(Forkhead)
ASCACGTG STIAIGFAAS	1e-1024 1e-440	14.52%	bZIP_cEBP-like_subclass	ITGGCA	1e-322	44.77%	NFIC
ATTERSTANT STORY	1e-212	21.18%	Erra(NR)	GTIAAIŞATI	1e-206	3.75%	HNF1A
ISAGTAAACA	1e-137	13.18%	Foxa2(Forkhead)	GGGCGGGG	1e-185	10.86%	GC-box
GGTCAC	1e-79	22.41%	Nuclear_Receptor_class	GATCAS	1e-153	34.90%	ECM23
CTGSCASS	1e-71	20.71%	NF1-halfsite(CTF)	GISATTGACC	1e-135	8.45%	FXR(NR/IR1)
ZATÇGATT	1e-59	5.18%	HNF6(Homeobox)	AAATOOAT	1e-130	1.85%	Hnf4a_2
<mark>₹</mark> \$£CC £ CÇÇ <u>£</u>	1e-54	11.26%	GC-box	AAATCGAT TTACGC	1e-104 1e-65	1.34% 10.06%	HNF6(Homeobox) RDR1
AATRATTAAC	1e-54	4.18%	HNF1A	TTATCI	1e-03	6.09%	GATA3(Zf)
SECATTGASS	1e-49	8.24%	FXR(NR/IR1)	AAATAIT <mark>eg</mark> t	1e-43	0.32%	Homeobox_class
CAGCTGAC	1e-19 1e-17	12.68% 0.43%	SCL fkh	GGGASAIGTS	1e-42	1.28%	GFY(?)
GTGTTTGTQC	16-17	0.43%	IKII	CGCICTCT	1e-20	1.46%	Trl
NPAS2	P-value	% of	Best Match/Details			0/ - 5	
	1e-447	Targets 46.63%	TYE7	CRY2	P-value	% of Targets	Best Match/Details
<u>ISCACGTGAS</u> TTACAIAA	1e-272		NFIL3	TATTAGA AA		21.61%	CEBPA
CAAAGGICAS	1e-115	29.39%	Erra(NR)	CAAAGUCAA	1e-569	27.96%	Erra(NR)
AGTAAAÇA	1e-72	18.93%	FKH2	SECURE SECURE	1e-421	23.43%	Mycn
ITGGCA	1e-49	44.52%	NFIC	GGTCAATA	1e-381	32.01%	HCM1
SETEAC	1e-48	39.43%	hth	ITGGC	1e-285	47.36%	NFIC
AATSATTAAC	1e-36	4.07%	HNF1A	ICANTA	1e-160	12.39%	HNF6(Homeobox)
GCAATC	1e-22	23.04%	Ddit3::Cebpa	ŞŞŞQQQQQ Ş	1e-154	14.64%	GC-box
CATGCCCTGG	1e-19	5.21%	p53	AATŞATTA	1e-49	2.30%	HAT5
ACAAACACTG	1e-14	3.01%	Forkhead_class	C&GATT&G GGACAA	1e-47	5.52%	NFY(CCAAT)
ATCGATIA	1e-13	2.37%	HNF6(Homeobox)	AGATAA	1e-39	28.25%	Nuclear_Receptor_class
DED4	P-value	% of	Best Match/Details	TTTCAC	1e-36	10.07%	GATA3(Zf)
PER1	1e-562	Targets 50.20%	Arnt	CTAGAGGGCG	1e-31 1e-22	7.94% 1.54%	STE12 btd
SECAPOTGES SETTESSEAA	1e-302 1e-373	23.68%	bZIP_cEBP-like_subclass	GGGASTTGTA	1e-22	0.45%	GFY(?)
CASACGICAS	1e-205	31.31%	Erra(NR)	TGCAATCT	1e-20	0.86%	Ddit3::Cebpa
ISTOTEONS	1e-191	18.81%	FOXA1	GAACCCGGG	1e-18	0.23%	PUT3
AATCAATA	1e-182	9.03%	HNF6	TTCCGT	1e-16	6.48%	HAL9
TGCCAA	1e-133	42.47%	NFIC		-	-	
GTEAC#	1e-118	47.26%	vis				
GEECCECCE	1e-74	11.69%	Sp1				
STSATC	1e-35	28.86%	GLN3				
GTTAATAA	1e-23	3.22%	Antp				
CGSAATGT	1e-18	2.06%	TEC1				
GAGCAAAG	1e-13	2.00%	MAC1				

		0/ 6	
CBP	P-value	% of Targets	Best Match/Details
<u>ÇAAAGTTÇA</u> \$	1e-663	27.58%	Erra(NR)
ESTISC	1e-583	20.40%	bZIP_cEBP-like_subclass
TGACGI	1e-563	39.93%	bZIP_CREB/Gbox-like_subclass
ISAGTAAACA	1e-270	15.70%	FOXA1(forkhead)
ISATTGGSI	1e-229	17.92%	NFY (CCAAT)
FGGCCCGCCC	1e-154	16.36%	GC-box
CTCTCTCTCG	1e-127	7.30%	Trl
CCGGAAGTS	1e-99	6.12%	GABPA
IGCCAASIS	1e-90	26.16%	NFIC
GTTAATIATT	1e-78	2.07%	HNF1B
GAATSGTAGT	1e-50	1.33%	GFY(?)
CGIGACGACG	1e-47	0.56%	Pax2
FGACGCG	1e-40	3.23%	MBP1
GAAGTCCG	1e-19	0.77%	SUT2
CAGTTIAAAT	1e-13	0.18%	ct
ACASTGTGTC	1e-12	0.24%	CUP9
AOATIGICIO			
-200	Divalua	% of	Dook Matab / Dataila
p300	P-value	Targets	Best Match/Details
§T@@\CTITG	1e-189	51.53%	Erra(NR)
\$TIAIG	1e-151	30.19%	bZIP_cEBP-like_subclass
TRTTIAGES A	1e-62	18.94%	fkh
TIEACC	1e-41	40.53%	Hnf4a_1
<u>IGATTGGGI</u>	1e-38	12.32%	CCAAT-box
FGGGIGGEFC	1e-31	20.93%	Klf4
GAACAGAG	1e-18	13.90%	AR-halfsite(NR)
TGECAR	1e-15	53.93%	NF1-halfsite(CTF)
AASCATIASC	1e-14	1.82%	Mybl1_1
		0/ 6	
8WG16	P-value	% of Targets	Best Match/Details
TCACTTCCG	1e-89	12.84%	GABPA
TEACTICOGE	1e-59	4.05%	YY1
CCGCCATITT	1e-34	29.06%	CHA4
<u>GGGCGGA⊊</u>	1e-32	4.66%	ELK1
AITLICCGGC	1e-30	4.43%	NRF1(NRF)
CGS&SCET	1e-29	28.98%	HAP1
TAACCG	1e-29	45.21%	
ADSESS STATESSAS	1e-29	1.03%	ovo GFX
	1e-20	11.71%	STB5
TAAACCCG	1e-21		
CCCASAATAC ACCGTTACCT	1e-20	2.34% 8.40%	ZNF143 STAF(Zf) DCE_S_III
AGCSTIAGGI TTGTCGGA	1e-16 1e-17		IRC900814 1
TTGTCGGA	1e-17 1e-17	12.39%	-
CGGGGAAAAA	1e-17 1e-15	15.40% 5.21%	EDS1 HAP4
IGATTGGC GTTAATGATT	1e-15	0.61%	HNF1A
GTTAATGATT AAAAGGCTCG	1e-13	1.90%	Nkx2-5
AA&AGGCTCG TTCCACTA	1e-13		CEBP(bZIP)
TIGFACIA	16-12	3.63%	OLDF(DZIF)

Fig. S6



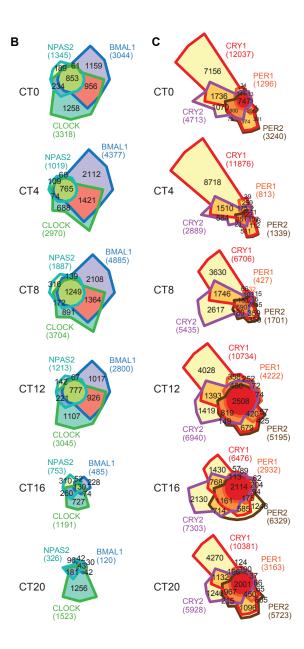
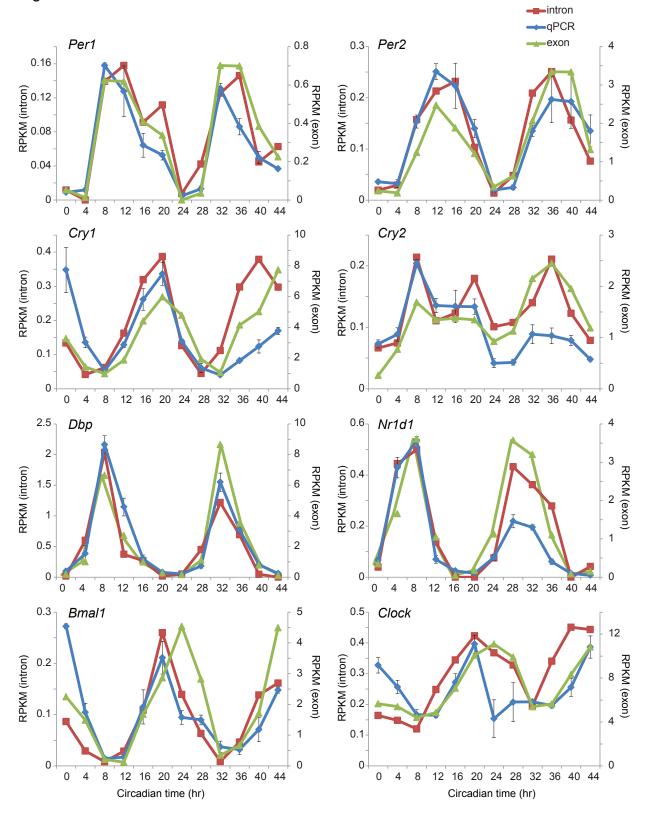
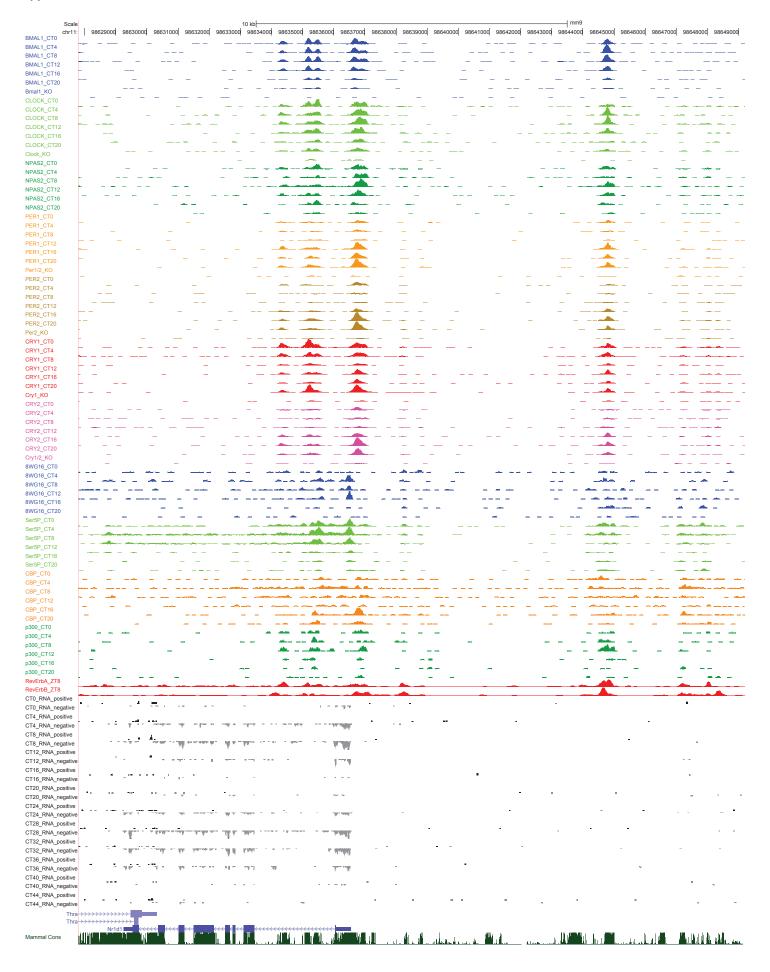
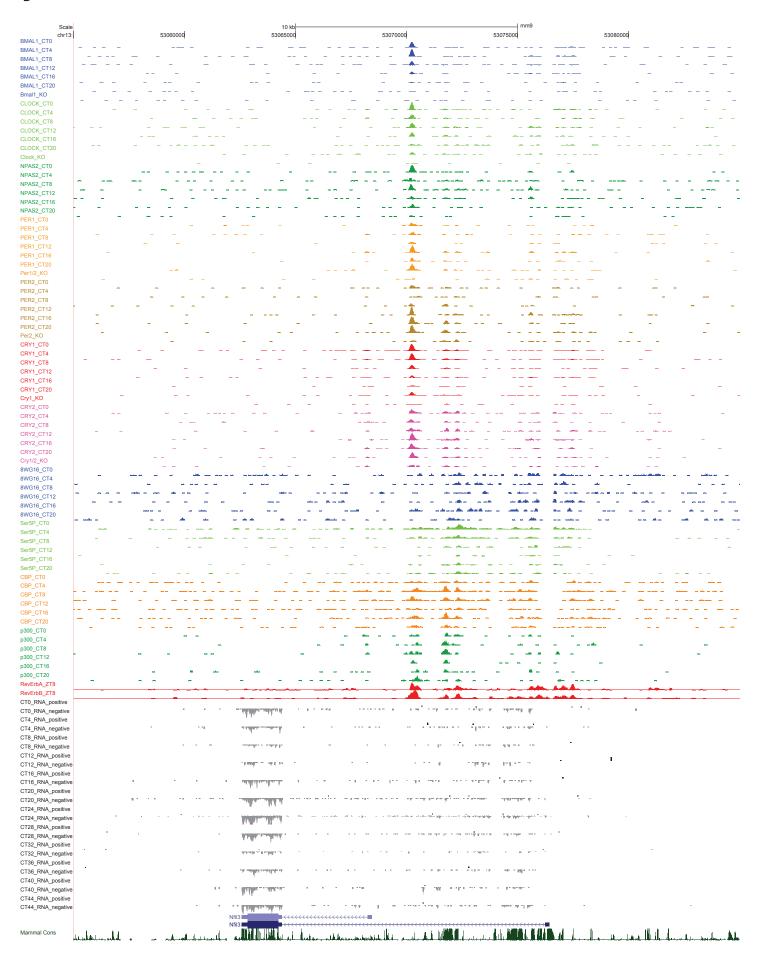


Fig. S7									
common bindin	ng sites	P- value	% of Targets	Best Match	CRY1		P- value	% of Targets	Best Match
CACGTO	AC	1e-419	66.07%	USF1(HLH)	<u>ÇAA</u>	AGGTÇA Ş	1e-379	28.56%	Erra(NR)
TTASA		1e-126	23.96%	CEBPA	TŢĢ	SEAATE	1e-262	20.65%	CEBPA
ETGACO		1e-80	25.21%	Erra(NR)		GTG SEE	1e-196	16.76%	Mycn
GT&AÇ	<u></u>	1e-60	63.71%	Rfx4		TACC	1e-190	22.13%	Forkhead_class
<u>AATÇAA</u>		1e-55	9.07%	HNF6(Homeobox)	G T\$		1e-103	54.75%	Rfx4_2
ITGGC4		1e-46	46.12%	NF1-halfsite(CTF)	IGC		1e-99	42.94%	NFIC
TGTTT		1e-42	40.24%	Forkhead_class		GATTIE	1e-82	4.03%	HNF6(Homeobox)
AGGGCA		1e-26	4.36%	RXR(NR/DR1)		CGCCFE	1e-59	10.63%	GC-box
TGÇAA] GG <u>&</u> AT 9		1e-17 1e-15	24.24% 2.70%	Ddit3::Cebpa TEAD1		TCCG	1e-34	3.78%	ELF1(ETS)
SICC&C		1e-15	13.02%	GC-box		CAGTGAC	1e-25	5.05%	FXR(NR/IR1)
₹CŲVĄ U	ντ	.0 .0	10.0270			GAGCG	1e-14	3.80%	Trl
		P-	% of	Best Match		AASTGTA	1e-13	0.67%	GFY(?)
BMAL1		value	Targets	Bost Water		AATATTT	1e-12	0.32%	Arid5a 1
CACGT		1e-251	46.39%	Mycn	문주문	łyżyżs		0.0270	741404
ĕ ≜TT≜(CTG⊊S		1e-19 1e-17	6.68% 20.32%	bZIP_cEBP-like_subclass Hand1::Tcfe2a	CRY2		P-	% of	Doot Match
ZAATC		1e-17	7.09%	onecut	ξ <mark>ά</mark> Τ	T <mark>ESE</mark> SAA	value 1e-150	Targets 18.08%	Best Match CEBPA
enn 🛂	NO.	P-	% of			&CTTTG	1e-137	32.39%	Erra(NR)
PER2		value	Targets	Best Match		TGAC	1e-63	30.47%	NFY(CCAAT)
SATS	Λ T T	1e-43	10.45%	HNF6(Homeobox)		CGGAGCI	1e-39	10.33%	GC-box
TTASS		1e-40	19.12%	bZIP_cEBP-like_subclass	I GT?	TACCTA	1e-34	6.34%	FOXA1(Forkhead)
						GAAGIG	1e-27	6.85%	ELK4
AAAGG		1e-29	26.27%	Nur77(NR)	TAAT	CATT	1e-26	7.46%	ATHB-5
Ţ <mark>ġĄĠ</mark> Ţ		1e-26	10.32%	FOXA1(Forkhead)		TGGCA	1e-21	10.38%	RIM101
CACGT	GCCAG	1e-19	22.70%	ref-1	CCAC	CTAGSGG	1e-17	1.17%	CTCF
TAGGG	CAAAG	1e-14	4.13%	HNF4A	IGG 1	FAGAGCG	1e-15	0.47%	SNT2
GIACT	ŢŢĢ	1e-13	6.19%	HNF4a(NR/DR1)		ACGTCA	1e-13	4.32%	CRE(bZIP)
ACTGA	SCCAG	1e-13	26.82%	Nr2f2_2	CAAA	ACACGGT	1e-13	1.31%	fkh
<u> GGGCG</u>	GGGCÇ	1e-12	9.77%	Sp1(Zf)			Б	0/ - 5	
					CRY1/CRY	Y2	P- value	% of Targets	Best Match
		P-	% of	Deed Medels	TIA	PETAAT	1e-171	22.05%	CEBPA
PER1/PER2/CI		2 value	Targets	Best Match	¿T G	EXCITIO	1e-161	41.92%	Erra(NR)
§TT <u>£</u> Ç		1e-86	27.12%	bZIP_cEBP-like_subclass	AGT	AZAAS	1e-96	24.71%	FOXA1(Forkhead)
2 AATC 2 EGCA		1e-49	29.28%	Foxa2			1e-47	6.80%	HNF6(Homeobox)
GACAC		1e-44 1e-31	59.70% 15.84%	hlh-26 Erra(NR)					,
ŞCA <u>&</u> A <u>©</u> C <u>©</u> TGCO		1e-25	24.59%	NF1-halfsite(CTF)	GT S		1e-46	50.19%	exd
GCECCE GCECCE		1e-23	13.81%	GC-box	ITG	GC <u>A</u>	1e-44	51.88%	NFIC
GCAAA		1e-21	27.50%	FKH2	ŢŢA	ATSATTA	1e-33	6.86%	Hnf1(Homeobox)
AGGGCA		1e-16	3.68%	HNF4A	A GC	TCC GCCC	1e-23	7.19%	GC-box
AGAAAT		1e-16	2.41%	SPT23		TGGGC	1e-14	15.02%	EBF1(EBF)
CCAATO		1e-13	3.42%	NFYA					
TCACAI		1e-12	8.75%	INO4		CCGGGTC	1e-13	1.58%	YBR239C
					ACA	TGGCCTG	1e-13	2.78%	SKN7

Fig. S8







	Scale					20 kb					mm9		
BMAL1_CT0	chr1:	182095000	18	82100000	182105000		182110000	182115000	182120000	182125000	182130000	182135000	182140000
BMAL1_CT4													and the second s
BMAL1_CT8 BMAL1_CT12	-									فاستفريت سيتاري	المتعاد والمسا		
BMAL1_CT16											 		
BMAL1_CT20 Bmal1_KO													
CLOCK_CT0												7.7	The second second
CLOCK_CT4 CLOCK_CT8		-			44.1	4414		وأبد أأبعا		La	4 1	10 I II	1 14 1 41
CLOCK_CT12			ىد. دەرىخارد			ر آل سماد دهاد	دادات المادية. الأناف	سو د دره درد	ماهم ما دا شده	المطالب المسايد المساي المسايد المسايد المساي			المنظ المطاعد والأساسا
CLOCK_CT16 CLOCK_CT20			بقدام	LEU I			را بيد بيد			ساهيمان بالاسم	Lilia Alam Is	العشاعلات	المراجع المسترات
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NPAS2_CT0 NPAS2_CT4			000		. +	- 444 4 -	-4	A		فتقتصت بأثث	ند بده	÷	a di manian i ua
NPAS2_CT8	-						and a second			والقهد المحادث التعداد الأنظامات الداد والا قالد	امام الساب بالمؤسطة. مراجع أن المعادلات		raki i ili kali ili ili ili ili ili ili ili ili ili
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NPAS2_CT20						<u></u>				طمعقد عدد ساهادد ساده دما			المائد المائد
PER1_CT0 PER1_CT4		•			**		×						
PER1_CT8					+					استهاد مراسبات ساخت			and the second second
PER1_CT12 PER1_CT16	-	-	-	-			A			والأمامان بالمام			La Alba Alla
PER1_CT20	ľ									ها با مديد د. د ماها مدا	an an Lakaran		ال المساهدة المساهدة المساول الما
Per1/2_KO PER2_CT0			-	-	2.2	All as		-	2 2	هاهم بيا الالتان	40 40		المراجعة المالية
PER2_CT4			-								rakan menengan dan kecamatan dan kemelah berakan dan kemelah berakan dan kemelah berakan dan berakan berakan d Berakan berakan beraka	-4 .	e a sa desta de la composición de la c La composición de la
PER2_CT8 PER2_CT12			-	-			4 - 2 - 2 - 22		-	فيستراث المارا			
PER2_CT16			<u>.</u>	<u> </u>	and and	المستعدد	and the second			Autoria de la Maria. Maraĝis de la la la Maria de la Maria de la	kaku u Mara u aku		·
PER2_CT20 Per2_KO			-	44.4	4.4	8 May 1 1	A	2 2	A sis a district	اهالا المعالم المعالم المعالم المعالم المعال	المتعادات الماسية		عد علاق مقاددات د قدمها
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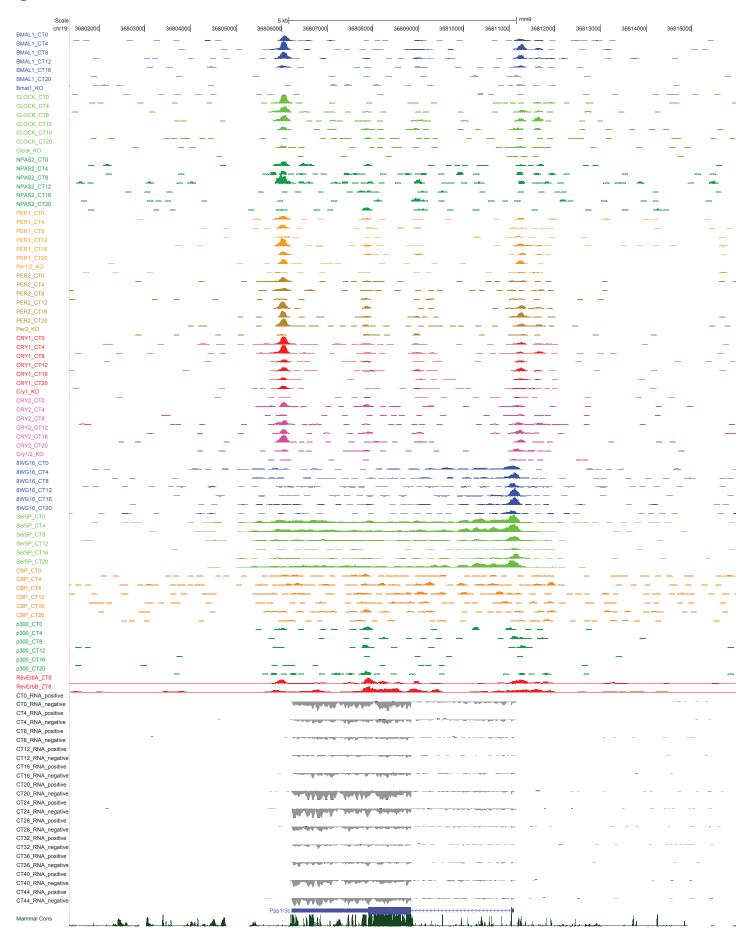
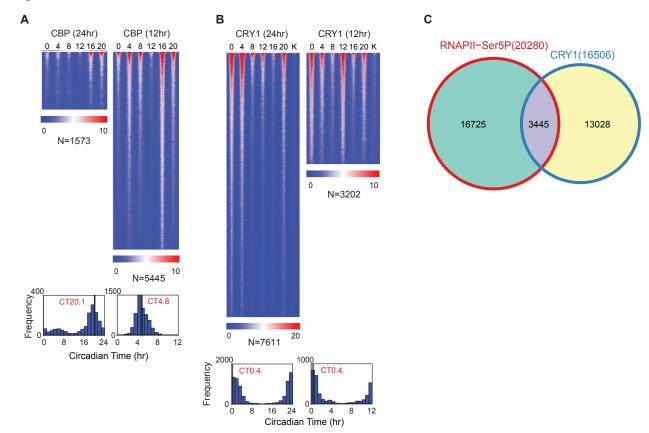


Fig. S10



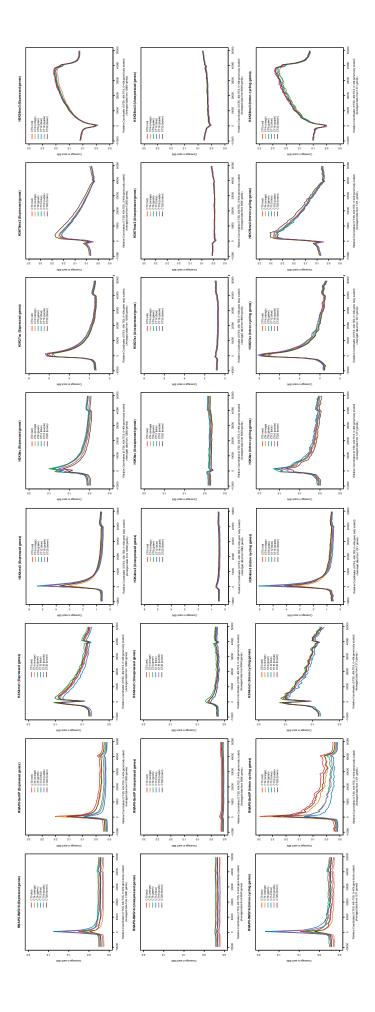
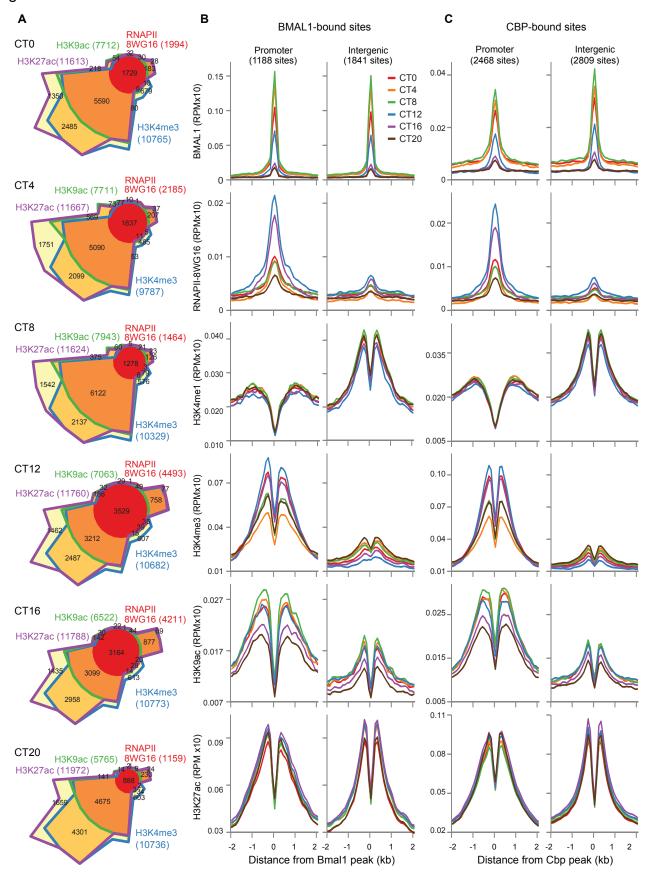
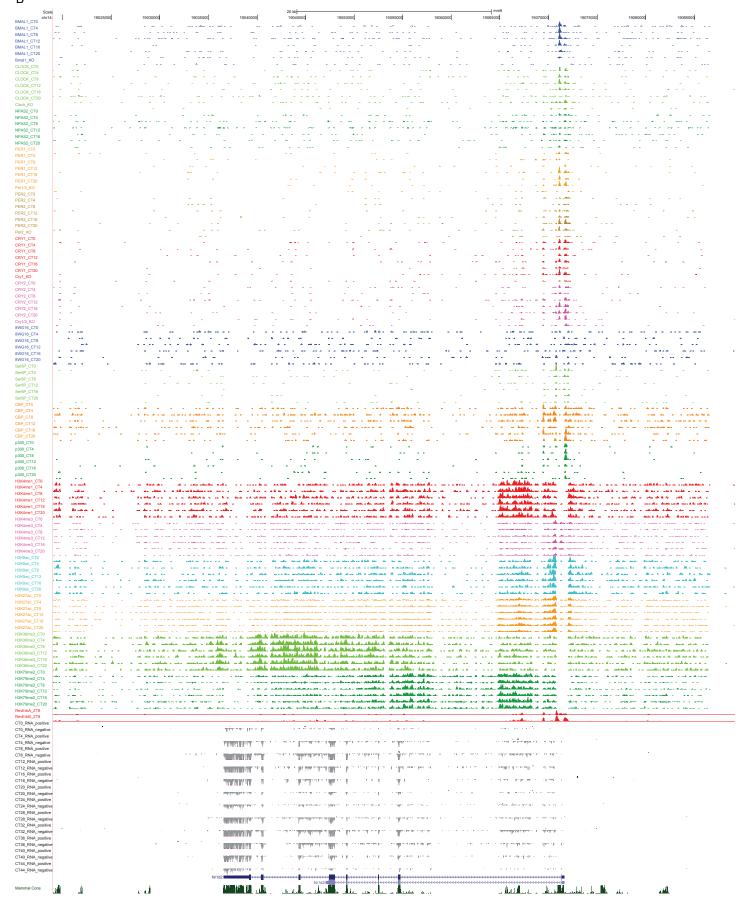
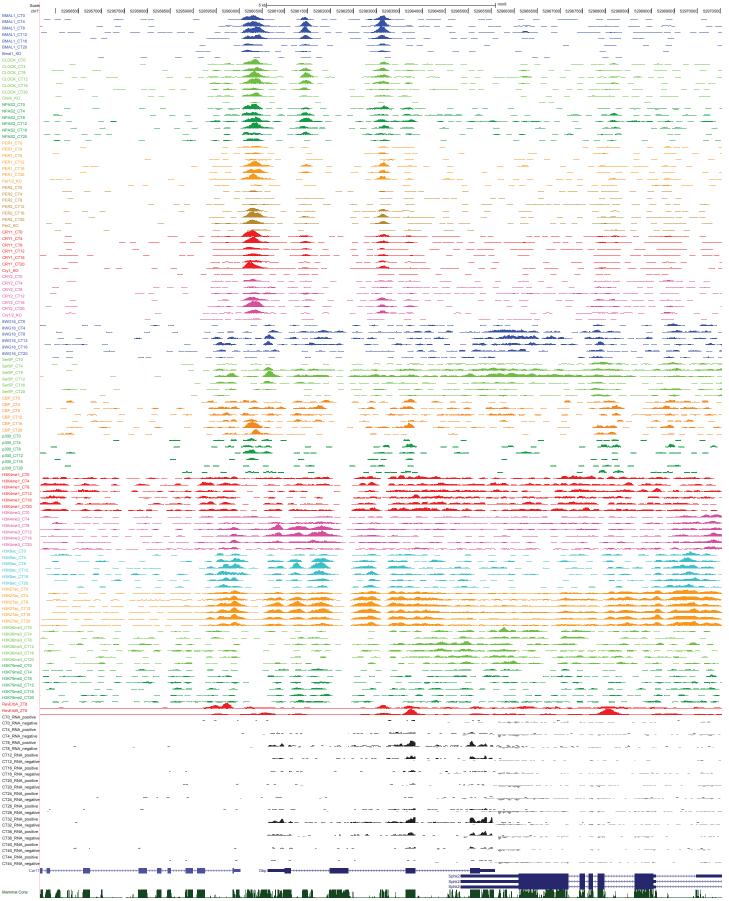


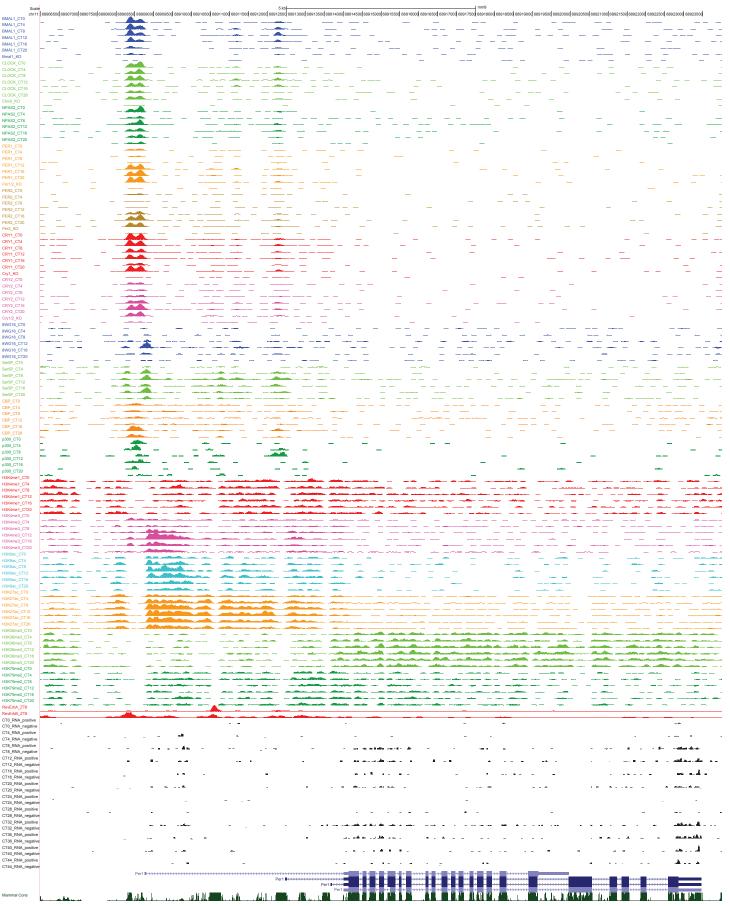
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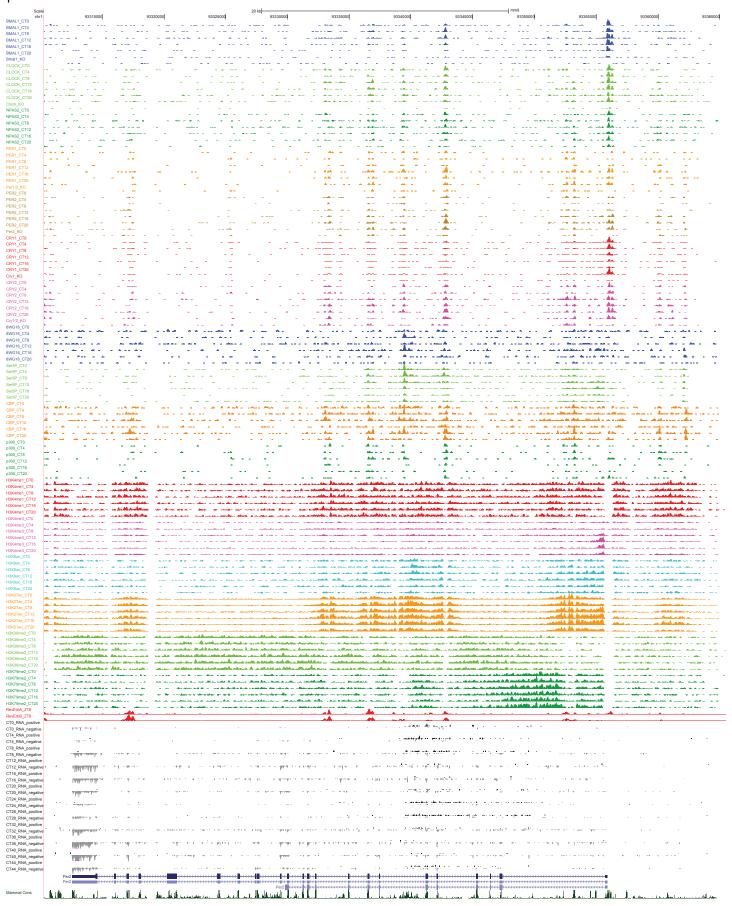


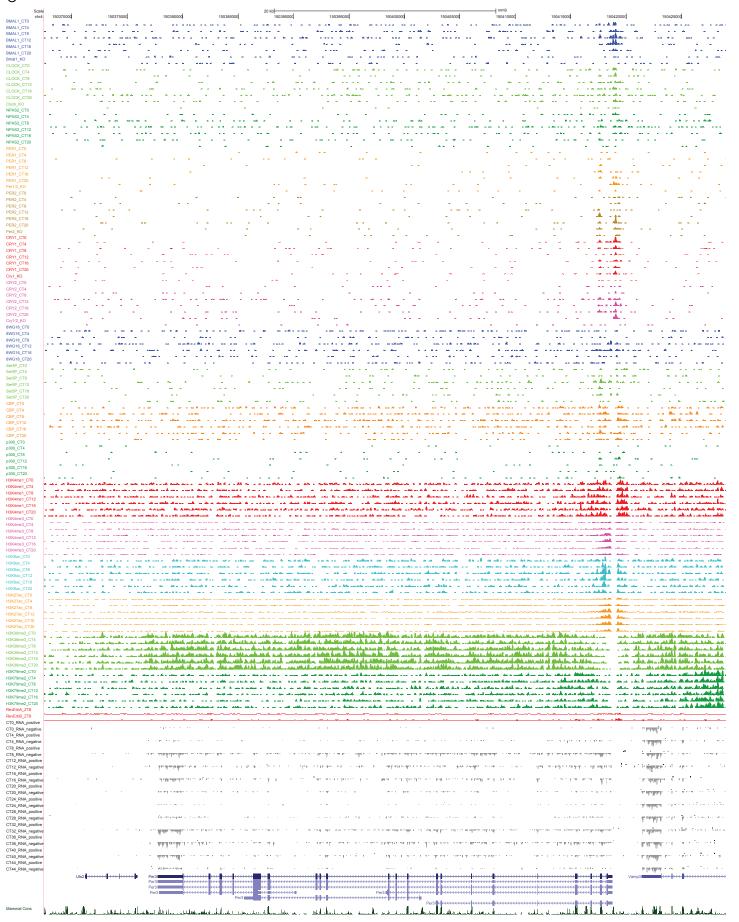




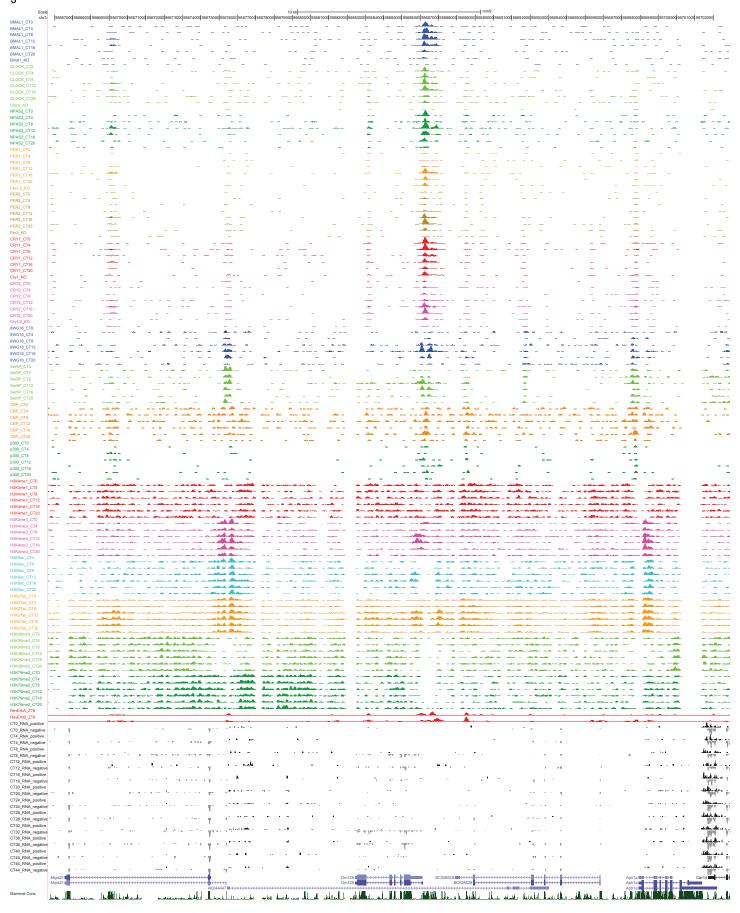
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