Supplementary Information

Cold-induced RNA-binding proteins regulate circadian gene expression by controlling alternative polyadenylation

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I. Extended Experimental Procedures

Retrovirus plasmids and infection

For the over-expression of Flag-tagged Cirbp/Rbm3 in the MEFs, the mouse Cirbp and Rbm3 cDNAs were amplified by PCR (The KOD Hot Start DNA Polymerase kit, TOYOBO) from mouse cDNA library and cloned into the p3xFLAG-CMV-5' vectors, then the cDNAs of the FLAG-tagged Cirbp/Rbm3 were cloned into pMSCV/Pro vectors (Clontech). For knockdown of Cirbp and Rbm3 in MEFs, retrovirus-mediated siRNA expression was used. The target sequences were determined using the Whitehead Institute siRNA designing tool(Yuan et al., 2004) and verified by BLAST searches to ensure specificity. As recommended by Clontech for the RNAi-Ready pSIREN-RetroQ system, two complementary oligos for each target sequence were designed, annealed and ligated into the BamHI/EcoRI-linearized pSIREN-RetroQ vector. The sequences of the oligonucleotides cloned into pSIREN-RetroQ were

control: 5'-GTGCGCTGCTGGTGCCAAC-3'

siCirbp-1: 5'-GACGCCATGATGGCTATGA-3'

siCirbp-2: 5'-GGCGGCAGATCAGAGTTGA-3'

siRbm3-1: 5'-GGAGGGCTCAACTTCAACA-3'

siRbm3-2: 5'-CAGTCGTCCTGGAGGATAT-3'

Retroviruses were generated following the co-transfection of recombinant pMSCV or pSiren-RetroQ plasmids with pCL10A1 helper plasmid into 293T cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's recommendations. The supernatants of the cell culture containing retroviruses were harvested 48 h later and passed through a 0.45 μm filter. 2 x 10^5 MEFs were plated into 6-well plates before retroviral infection. Virus-containing supernatants were supplemented with 20 μg/ml polybrene and 20 μg/ml HEPES, and then added to the cells for a spin infection procedure, which was performed in the 6-well plates by centrifugation at 2500 rpm at 30 ℃ for 1.5 h. The mock cells were performed with all the procedures, and were treated with the normal DMEM medium rather than the virus containing supernatants in the retroviral infection step. The MEFs were selected with 50 μg/ml puromycin.

RNA extraction and qPCR

Total RNA was isolated from cells and tissues by Trizol (Invitrogen). Total RNA quantities were measured by a Nanodrop spectrometer. RNA quality was assessed by an Agilent Bioanalyzer. 500 ng of total RNA was reverse transcribed using random hexamer and the Superscript II reverse transcriptase (Invitrogen). 5 μl of RT product (1:10 diluted) and SYBR Green I Master Mix (Roche) were used in qPCR on a LightCycler 480 (Roche). The specificity of PCR was checked by melting curve analysis. In every qPCR assay, Actb or Gapdh was used as the control for the significant bias of starting materials across samples. Primers for qPCR analyses are listed in Table S10.

Western blot and antibodies

Liver samples of mice were washed with ice-cold PBS three times to remove blood and then homogenized in lysis buffer (8M urea, 4% CHAPS, 65mM DTT, 40mM Tris with protease inhibitor mixture, 200 mg of wet tissue/ml) with a motor-driven homogenizer (Glas-Col) until no sample pieces were visible. The homogenate was sonicated for 3 min followed by centrifugation at 15,000 g for 1 h at 4 ℃, and the supernatant was separated by SDS-PAGE. The proteins were transferred to PVDF membrane using wet blotting system (BioRad). The membrane was blocked in 5% non-fat milk and incubated with primary antibody for 1 h to overnight, washed with TBST twice and incubated with secondary antibody for 1 h. The protein bands were visualized using ECL reagents (GE Healthcare) and LAS-4000 CCD camera (FUJIFILM). All bands were quantified using Multi Gauge Software (FUJIFILM). Primary antibodies used were the antibody to Cirbp (LifeSpan BioSciences), dilution 1:500; the antibody to Rbm3 was a gift from Dr. Peter W. Vanderklish, dilution 1:500; the antibodies to Actb and Gapdh were from Dr. Gang Wang, dilution 1:1000. The secondary antibody was HRP-conjugated anti-mouse IgG (Abcam), dilution 1:10000.

Tandem UTRs database construction

Mm9 KnownGene annotations were extracted from UCSC table browser (Karolchik et al., 2004). The 3' end transcripts were treated as PASs naturally. We extracted 3’ UTRs from KnownGene annotations and merge them into clusters if any overlap occurred. PolyaDB2 (Lee et al., 2007) is a comprehensive PASs database across different animals. We downloaded all PASs for mouse mm5 genome. The genomic coordinates were converted to mm9 using liftOver (Fujita et al., 2011). As a result, we have 29,558 PASs from PolyaDB2. A dataset of PASs in five tissues of

mouse generated by polyA-seq was downloaded from UCSC table browser (Derti et al., 2012). We extracted the PASs and merged them into clusters if the adjacent PASs were within the distance of 40 nucleotides. This dataset generated 126,028 PAS clusters in total. We retained 39,710 PASs by requiring that one PAS cluster should be present in at least two tissues. We then merged the PASs from the KnownGene 3' ends, the PolyADB2 and the polyA-seq dataset into the final PASs database. We assigned each PAS to the associated 3' UTR clusters if it falls within the 3'UTR or within 3,000 nucleotides downstream. In the latter case, the 3' UTR was extended to the downstream PAS. Then we defined sub-UTRs by 3' UTRs partitioned by PASs. The tandem UTR events were defined by the combination of any pair of sub-UTRs from the same 3'UTR. Finally, we obtained 42,735 tandem UTR events in 12,250 mouse genes. For the microarray analysis, we mapped the probes from mouse430 2.0 microarray onto mm9 genome by bowtie software (--sam -f -t --chunkmbs 1024 --best -n 0 -p 6 -k 20 -m 20 mm9). The mapping results were filtered so that the mapping is perfect (no mismatch) and unique on 3' UTR. We selected the sub-UTRs with at least 3 mapped probes. The probes were then grouped into probesets. Finally, we obtained 6220 tandem UTR events in 4364 genes, and generated a CDF annotation for Affymetrix Mouse Genome 430 2.0 platform.

RNA-seq

Polyadenylated RNA from the mock cells at 37 ℃ or 32 ℃ with two replicates, siCirbp-1, siCirbp-2, siRbm3-1 and siRbm3-2 MEFs at 37 ℃ were sequenced on Illumina GAII using 76 bp single-end kits according to the manufacturer's instructions. The detailed information was shown in Table S5. All obtained reads were mapped to the mm9 genome with the Tophat software (Trapnell et al., 2009). The Cufflinks (Roberts et al., 2011) was used to estimate gene and isoform FPKM (fragments per kilobase of exon per million fragments mapped)-levels and confidence intervals using the aligned reads and the RefSeq gene models. Log2 [fold change] were computed from the inferred isoform-level FPKM values (isoform that had FPKM $\geq$ 1) from siCirbp/siRbm3 and the mock cells or the mock cells from different temperatures. For the analyses of APA events, we use the CoverageBed software from BedTools (Quinlan and Hall, 2010) to estimate the read counts in each sub-UTR from our tandem UTR events database. Log2 [fold change] were calculated from the inferred reads counts in sub-UTR (sub-UTR that had read counts > 10) from siCirbp/siRbm3 and the mock cells or the mock cells from different temperature. The ext/com UTR-ratio change upon

certain condition was calculated by the Log2 [fold change] of the extended 3'UTRs subtract the Log2 [fold change] of the common 3'UTRs upon certain condition. Fisher's exact test was used to estimate the statistical significance of the changed PAS-usage switching events from different conditions. For example, to identify the significantly changed PAS-usage switching events upon Cirbp depletion, the 2 x 2 table was constructed from the read counts in the common 3'UTRs in the mock cells, the read counts in the extended 3'UTRs in the mock cells, the read counts in the common 3'UTRs in the Cirbp-depleted cells and the read counts in the extended 3'UTRs in the Cirbp-depleted cells.

Microarray Analysis

Microarray raw data were downloaded from ArrayExpress repository (E-MEXP-842) and GEO (GSE11923). The data were extracted using RMA implemented in R. Present/absent calls were generated by MAS5 in R for Mouse Genome 430 2.0 expression arrays. The annotations including gene names, gene symbols, RefSeq accessions were obtained from R Bioconductor package (mouse4302.db). The COSOPT(Straume, 2004) algorithm was used to measure the goodness-of-fit between experimental data and cosine curves with varying phases and different period lengths. P-values were then calculated by fitting it to cosine curves in order to determine the probability that the observed data matches a cosine curve by chance alone. Pearson correlation was used to estimate the correlation of the expression level between liver clock active and arrested mice. For identifying the genes that showed oscillating ext/com UTR-ratio, the expression level of the sub-UTR was obtained from the microarray data of the mouse liver (GSE11923). Then we calculated the ext/com UTR-ratio in each time point, and estimated the statistical significance of the oscillation by the COSOPT algorithm.

RIP and qPCR

The cells that are stably expressing the FLAG epitope-tagged protein of interest were harvested, washed in ice-cold PBS and collected by centrifugation (1500g, 4℃ and 10 min). RNA-IP experiments were performed with the Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore) according to the manufacturer's instructions. Single stranded cDNA was synthesized from the total RNA using random hexamer and Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's instructions. The selected transcripts that identified by PAR-CLIP were quantified by qPCR, using a set of primers (see Oligonucleotides) that amplify a 100-200

nucleotides sequence.

PAR-CLIP procedure

PAR-CLIP protocol was performed as described (Hafner et al., 2010). The cells that are stably expressing the FLAG epitope-tagged protein of interest were grown in medium supplemented with 100 µM 4SU for 16h prior to harvest.

UV 365 nm crosslinking

For UV-crosslinking, the growth medium was removed completely while the cells were still attached to the plates. The cells were irradiated on ice with 365 nm UV light (0.2 J/cm2) in a Stratalinker 2400 (Stratagene), equipped with light bulbs for the appropriate wavelength. The cells were scraped off with a rubber policeman in 2 ml PBS per plate and collected by centrifugation at 800g for 4 min.

Cell lysis and first partial RNase T1 digestion

The pellets of the cells crosslinked with UV 365 nm were resuspended in 3 cell pellet volumes of NP40 lysis buffer (50 mM Tris HCl, pH 7.5, 140 mM LiCl, 2 mM EDTA, pH 8.0, 0.5% (v/v) NP40, 0.5 mM DTT, complete EDTA-free protease inhibitor cocktail (Roche)) and incubated on ice for 10 min. The typical scale of such an experiment was 3 ml of cell pellet. The cell lysate was cleared by centrifugation at 13,000g. RNase T1 (Fermentas) was added to the cleared cell lysates to a final concentration of 1 U/µl and the reaction mixture was incubated in a water bath at 22°C for 10 min and subsequently cooled for 5 min on ice before the addition of antibody-conjugated magnetic beads.

Preparation of Dynabeads Protein G magnetic beads

10 µl of Dynabeads Protein G magnetic particles (Invitrogen) per ml cell lysate were washed twice with 1 ml of citrate-phosphate buffer (4.7 g/l citric acid, 9.2 g/l Na2HPO4, pH 5.0) and resuspended in twice the volume of citrate-phosphate buffer relative to the original volume of bead suspension. 0.25 µg of anti-Flag M2 monoclonal antibody (Sigma) per ml suspension was added and incubated at room temperature for 40 min. Beads were then washed twice with 1 ml of citrate-phosphate buffer to remove unbound antibody and resuspended again in twice the volume of citrate-phosphate buffer

relative to the original volume of bead suspension.

Immunoprecipitation, second RNase T1 digestion and dephosphorylation

10 µl antibody-conjugated Protein G magnetic beads were added per ml of partial RNase T1 treated cell lysate. Incubation was performed in 1.5 ml microfuge tubes on a rotating wheel for 1 h at 4 ℃. Magnetic beads were collected on a magnetic particle collector (Invitrogen). Manipulations of the following steps were carried out in 1.5 ml microfuge tubes. The supernatant was removed from the bead-bound material. The beads were washed 2 times with 1 ml of IP wash buffer (50 mM HEPES-KOH, pH 7.5, 300 mM KCl, 0.05% (v/v) NP40, 0.5 mM DTT, complete EDTA-free protease inhibitor cocktail (Roche)) and resuspended in one volume of IP wash buffer. RNase T1 (Fermentas) was added to obtain a final concentration of 50 U/µl, and the beads suspension was incubated at 22 ℃ for 8 min, and subsequently cooled for 5 min on ice. The beads were washed 3 times with 1 ml of high-salt wash buffer (50 mM HEPES-KOH, pH 7.5, 500 mM KCl, 0.05% (v/v) NP40, 0.5 mM DTT, complete EDTA- free protease inhibitor cocktail (Roche)) and resuspended in two bead volumes of dephosphorylation buffer (50 mM Tris-HCl, pH 7.9, 100 mM NaCl, 10 mM MgCl2, 1 mM DTT). Calf Intestinal Alkaline Phosphatase (CIP) was added to obtain a final concentration of 0.5 U/µl, and the suspension was incubated for 60 min at 37°C. Beads were washed twice with 1 ml of phosphatase wash buffer (50 mM Tris-HCl, pH 7.5, 20 mM EGTA, 0.5% (v/v) NP40) and twice with 1 ml of Polynucleotide Kinase (PNK) Buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM MgCl2, 5 mM DTT). The beads were resuspended in one original bead volume of PNK buffer.

Radiolabeling of RNA segments crosslinked to immunoprecipitated proteins

To the bead suspension described above, γ-^{32}P-ATP was added to a final concentration of 0.25 µCi/µl and T4 Polynucleotide Kinase (PNK) to 1 U/µl in one original bead volume. The suspension was incubated for 30 min at 37 ℃. Thereafter, nonradioactive ATP was added to obtain a final concentration of 100 µM and the incubation was continued for another 5 min at 37°C. The magnetic beads were then washed 5 times with 800 µl of PNK Buffer and resuspended in 20 µl of SDS-PAGE Loading Buffer (10% glycerol (v/v), 50 mM Tris-HCl, pH 6.8, 2 mM EDTA, 2% SDS (w/v), 100 mM DTT, 0.1% bromophenol blue).

SDS-PAGE and Western Blotting

Immunoprecipitation of epitope-tagged protein of interest was performed as described in the above section and radioactively labeled at the 5'end. Following radiolabeling, the beads were washed 5 times with 800 µl of PNK Buffer. The beads were resuspended in 50 µl of SDS-PAGE loading buffer and boiled 5 min at 95 ℃. The supernatant was loaded on a 4-12 % NuPAGE Bis-Tris gradient gel (Invitrogen) and exposed to a phosphorimager screen for the detection of radioactive signal. The gel was subsequently transferred to a nirocellulose membrane and protein-RNA complexes were immunodetected by incubation with Flag specific antibody, and the tagged protein was visualized using ECL Western Blot detection reagent (GE-Healthcare).

Electroelution of crosslinked RNA-protein complexes from gel slices

The radioactively-labeled RNA-protein complex migrating at the expected molecular weight of the target protein was excised from the gel and electroeluted in 800 µl SDS running buffer using D-Tube Dialyzer Midi (Novagen) according to the instructions of the manufacturer.

Proteinase K digestion

An equal volume of 2x Proteinase K Buffer (100 mM Tris-HCl, pH 7.5, 150 mM NaCl, 12.5 mM EDTA, 2% (w/v) SDS) with respect to the electroeluate was added, followed by the addition of Proteinase K (Roche) to a final concentration of 1.2 mg/ml, and incubation for 30 min at 55 ℃. The RNA was recovered by acidic phenol/chloroform extraction followed by chloroform extraction and ethanol precipitation. The pellet was dissolved in 6 µl water.

cDNA library preparation and deep sequencing

The recovered RNA was carried through a cDNA library preparation protocol, originally described for cloning of small RNAs (Dolken et al., 2008; Hafner et al., 2008). In contrast to the published cloning procedure, the 3'adapter ligation was carried out with a barcoded 3'adapter in a 20 µl reaction volume using 6 µl of the recovered RNA. PAR-CLIP libraries were sequenced on Illumina Genome Analyzer GAII platform using the 1X50BP single read protocol.

PAR-CLIP computational analysis

Generation of binding clusters and crosslinking positions

All PAR-CLIP sequencing data were analyzed with a computational analysis pipeline (Lebedeva et al., 2011). Illumina PAR-CLIP cDNA sequencing reads were aligned to the mouse genome assembly (mm9) allowing for up to one mismatch, insertion or deletion. Only uniquely mapped reads were retained. We identified the clusters of aligned reads continuously covering genomic regions based on the condition that a cluster requires 20 and more overlapping reads and at least one T-to-C transition event. The number of T-to-C mismatches served as a crosslink score, and the position with the highest frequency of T-to-C transition was referred to as the preferred crosslinking position. As the reads should originate from protein-bound transcripts, we regarded clusters aligning antisense to the annotated direction of transcription as false positives.

Motif analysis

To identify the shared binding motif in Cirbp or Rbm3 binding clusters, the Multiple Em for Motif Elicitation (MEME) software (version 4.8.1) (Bailey et al., 2009) was used (meme clusters.fa -mod anr -oc ./output_dir -nmotifs 5 -minw 6 -maxw 8 -dna -minsites 100 -maxsites 3000 -maxsize 1000000). The PATSER (v3e, http://rsat.ulb.ac.be/patser_form.cgi) was used to identify the clusters that contained the shared motif predicted by the MEME software (./patser-v3e -m ../matrix.txt -v -f clusters.txt -A a:t 1 c:g 1 -ls 0.0 > output.txt).

Conservation profiles analysis

Genome-wide conservation profiles per nucleotide PhyloP scores (Pollard et al., 2010) were obtained via UCSC table browser. The PhyloP scores in a 201nt window around the preferred crosslink site of each binding site were obtained and averaged over specific category of binding clusters. The result was a high resolution profile of average nucleotide conservation scores at a given distance from the preferred crosslink sites. The randomly selected region within the same transcript was used as a measure of background conservation.

II. Supplementary References

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III. Supplementary Figures

Supplementary Fig. S1. The RBPs and HSPs that showed highly similar circadian expression patterns in liver clock active and arrested mice. Related to Table 1. (a) Heatmap of the expression patterns of 401 genes that exhibited highly similar circadian expression patterns in liver clock active and arrested mice (COSOPT, $P < 0.01$; Pearson correlation > 0.5). (b and c) The circadian expression patterns of the RBPs and HSPs noted in the main text in liver clock active (black) and arrested (red) mice. Pearson correlation (Cor) between the expression patterns of the genes in the two mice was indicated.

Supplementary Fig. S2. The depletion of Cirbp or Rbm3 reduced the oscillating amplitude of core circadian genes in the temperature synchronized cells. Related to Fig. 2. (a) The oscillating mRNA levels of Cirbp and Rbm3 in mouse liver and cortex. Actb was used as control. (b and c) Western blot gel images (b) and quantitative densitometric (c) analyses of the circadian expression pattern of Cirbp and Rbm3 protein in mouse liver. Gapdh was used as control. (d) The oscillating mRNA levels of Dbp and Per2 in the temperature synchronized mock (black), control (gray), siCirbp-2 (green) and siRbm3-2 (pink) cells. The lowest expression value was normalized to 1. (e) The expression changes between 16 h and 28 h after the temperature synchronization of core circadian genes (Per1, Dbp, Nr1d1 and Nr1d2) in the temperature synchronized mock, control, siCirbp-2 and siRbm3-2 cells. The statistical significances were calculated between the control and Cirbp- or Rbm3-depleted MEFs. Data are represented as mean +/- SEM (n=3); Student's *t*-test * $P < 0.05$, ** $P < 0.01$.

Supplementary Fig. S3. Identification, analysis and validation of Cirbp and Rbm3 binding sites. Related to Fig. 3. (a) Phosphorimages of SDS-PAGE gels that resolved ^{32}P-labeled RNA-Flag-Cirbp or RNA-Flag-Rbm3 PAR-CLIP immunoprecipitates. Arrows indicated the excised regions. kD, kilo Daltons. Western blot was probed with an antibody to Flag. (b and c) The counts of the nucleotide mismatches in Cirbp- and Rbm3-binding clusters were shown, respectively. T-to-C transition is the signature of the efficient crosslinking. Proportion test *** $P < 0.001$. Dashed lines represent the average counts of each nucleotide mismatch. (d) Validation of Cirbp- and Rbm3-target genes by RIP and qPCR. The data were represented as the enrichment of each transcript in Flag

specific IP relative to input. The highly abundant 18s rRNA was used as a negative control. Data are represented as mean +/- SEM (n=3). (e and f) Histogram of the circadian peak times of the oscillating Cirbp- or Rbm3-target genes. Dashed lines represent the average counts of oscillating genes. Proportion test *** $P < 0.001$.

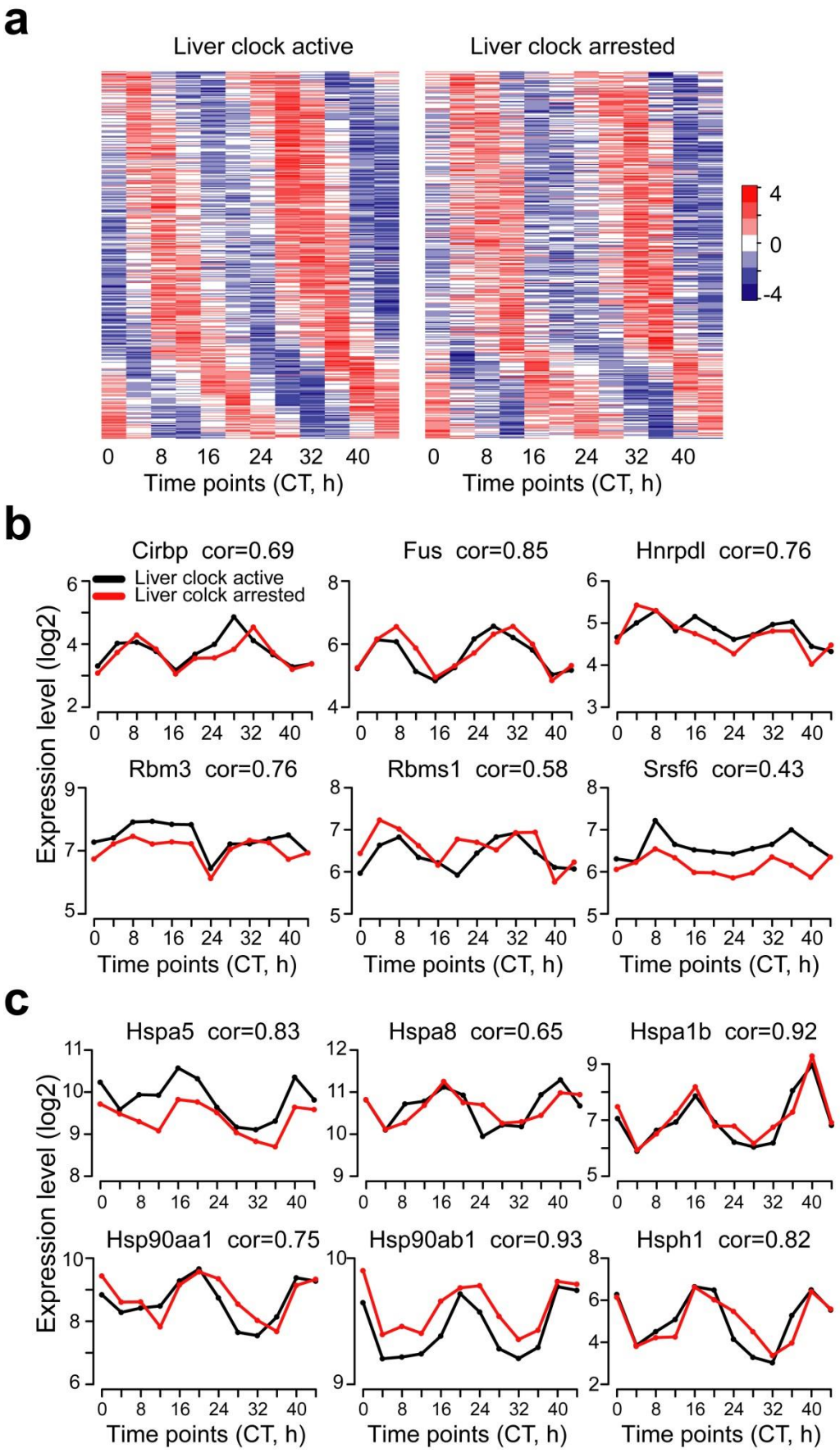
Supplementary Fig. S4. RNA-seq analyses of the functions of Cirbp and Rbm3. Related to Fig. 4. (a-d) Biological replicates in RNA-seq experiments for mock at 37 ℃, mock at 32 ℃, Cirbp depletion (siCirbp) and Rbm3 depletion (siRbm3). Pearson correlation (Cor) between the expression levels of the biological replicates was indicated. (e and f) Cumulative distribution of transcripts bound by Cirbp or Rbm3 upon Cirbp or Rbm3 depletion. The Cirbp- or Rbm3-unbound transcripts were used as controls. The P-values indicate the significance of the difference between the bound and unbound transcripts, as given by Kolmogorov-Smirnov Tests and are corrected for multiple testing.(g) QPCR analyses of the genes that showed down-regulated the ext/com UTR-ratio upon Cirbp or Rbm3 depletion. The statistical significance was calculated against the mock cells. Data are represented as mean +/- SEM (n=3); Student's t-test * $P < 0.05$, ** $P < 0.01$.

Supplementary Fig. S5. Several examples of depth-of-coverage profiles in RNA-seq. Related to Fig. 5. (a) The mRNA levels of Cirbp and Rbm3 at two different temperature 37 ℃ and 32 ℃. (b) QPCR analyses of the selected genes that showed opposite direction of PAS-usage switching upon cold shock and RBP depletion. The statistical significance was calculated against the mock cells. Data are represented as mean +/- SEM (n=3); Student's *t*-test * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (c -e) Tandem UTRs for Mapre1, Rgl1 and Sfpq are shown together with Cirbp- (blue) or Rbm3- (red) binding clusters and RNA sequencing depth-of-coverage profiles in mock at 37 ℃, mock at 32 ℃, Cirbp and Rbm3 depleted cells. The read counts scale was shown in the upper right of each panel.

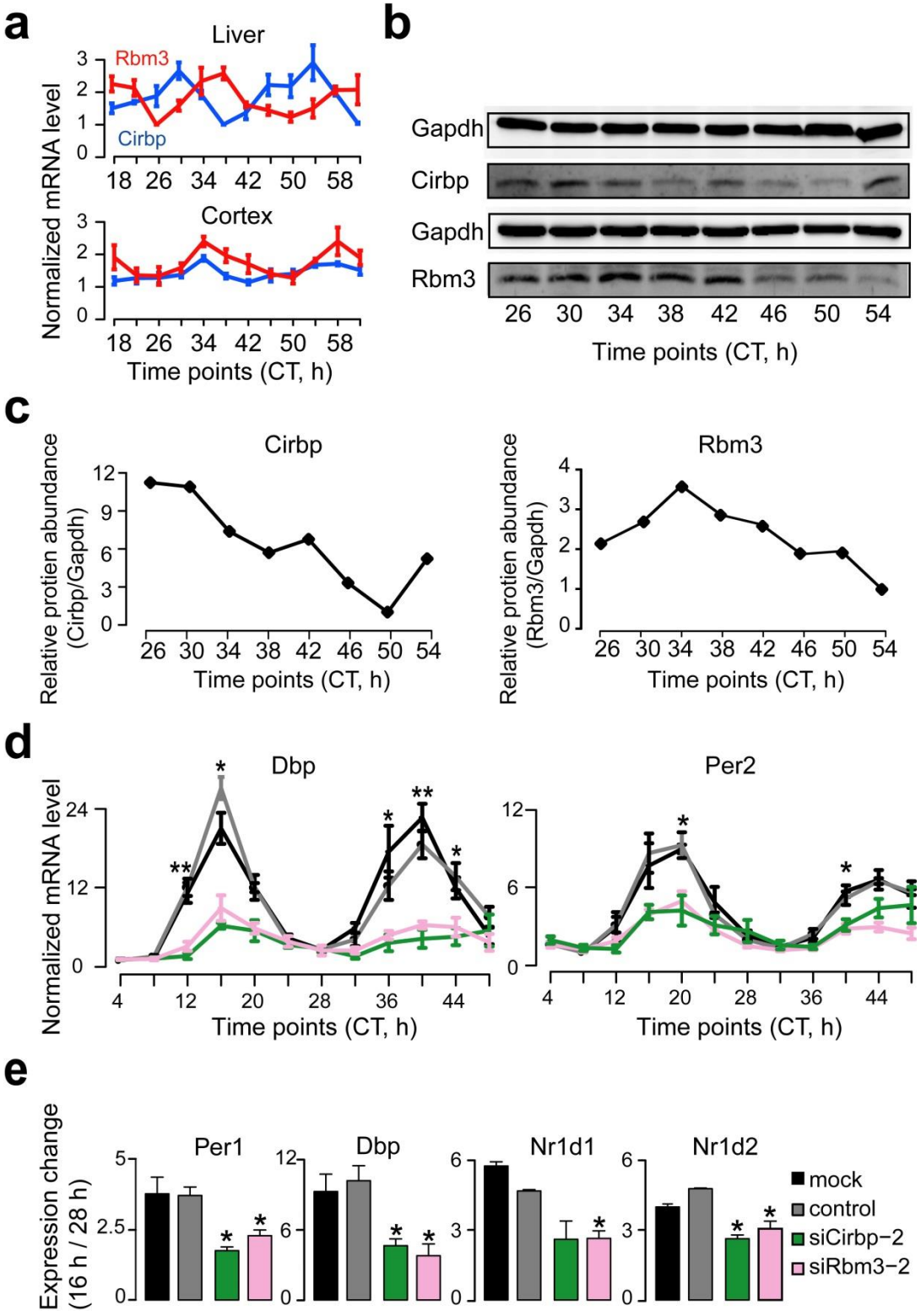
Supplementary Fig. S6. Cirbp and Rbm3 are important in the APA regulation in the circadian clock and they regulate other RBPs. Related to Fig. 5. (a) Histogram of the peak times of the genes that showed oscillating ext/com UTR-ratio in mouse liver. Dashed line represents the average counts of oscillating genes in each group. Proportion test *** $P < 0.001$. (b and c)

Heatmap of the circadian oscillating ext/com UTR-ratio of 35 genes regulated by Cirbp (b) and 23 genes regulated by Rbm3 (c) in mouse liver. (d) Connectivity diagram of Cirbp- or Rbm3-target RBP transcripts. Nodes represent Cirbp- or Rbm3-target transcripts. Each edge represents that an RBP transcript is both bound directly and regulated by Cirbp or Rbm3. Blue indicates that the transcript is regulate by Cirbp or Rbm3 in APA level. Red and green edges reflect down- and upregulation of mRNA levels upon Cirbp or Rbm3 depletion.

Supplementary Fig. S1



Supplementary Fig. S2



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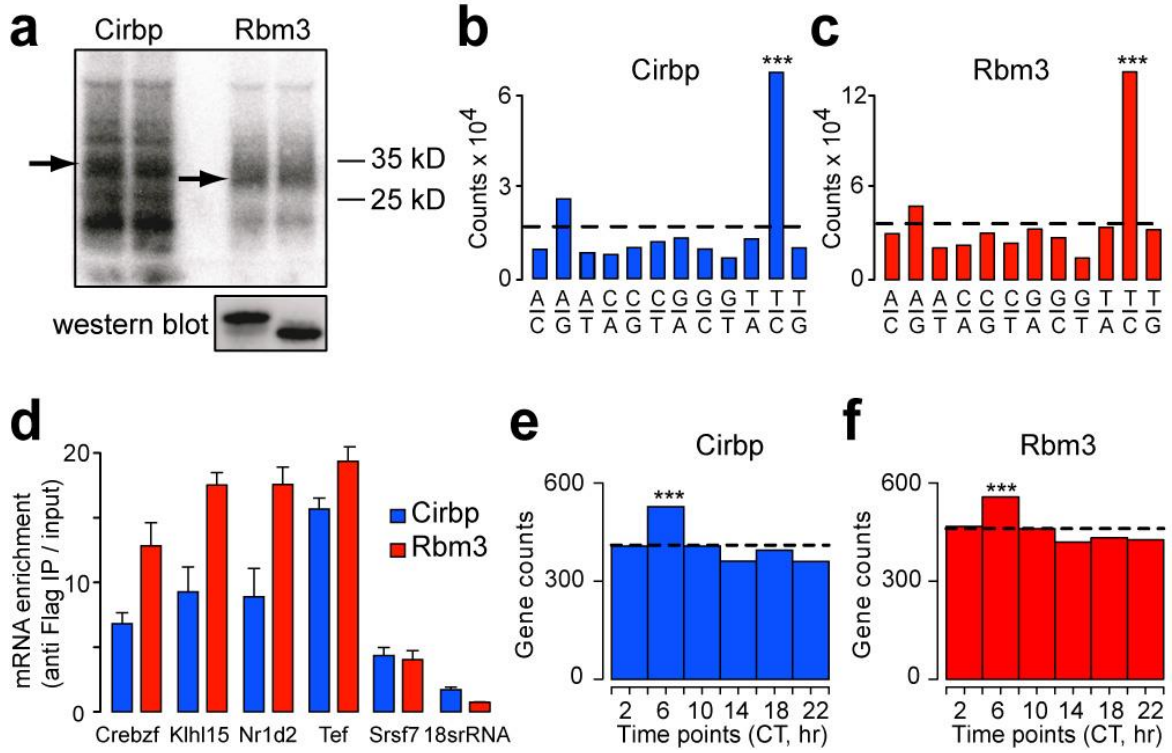
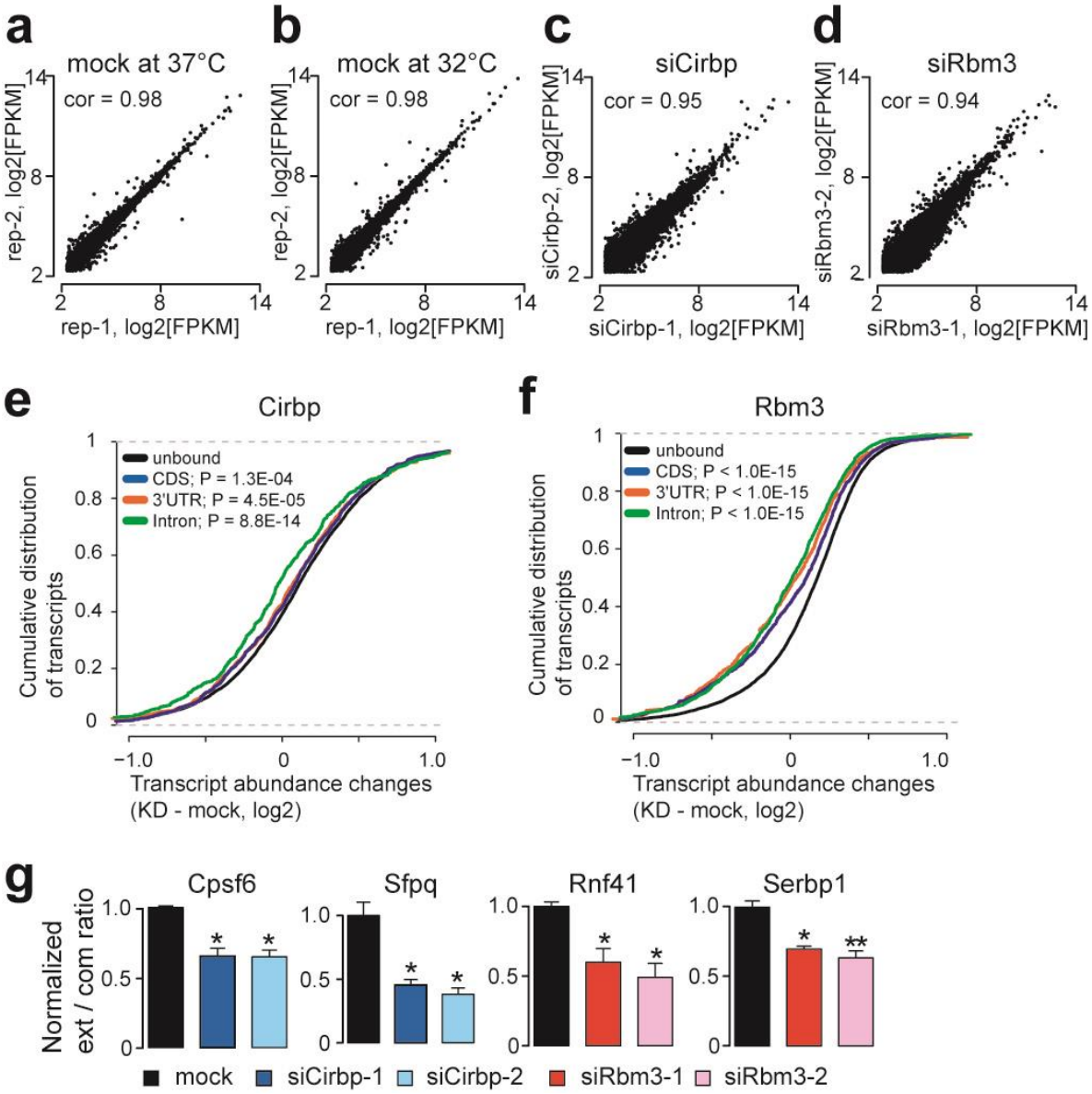
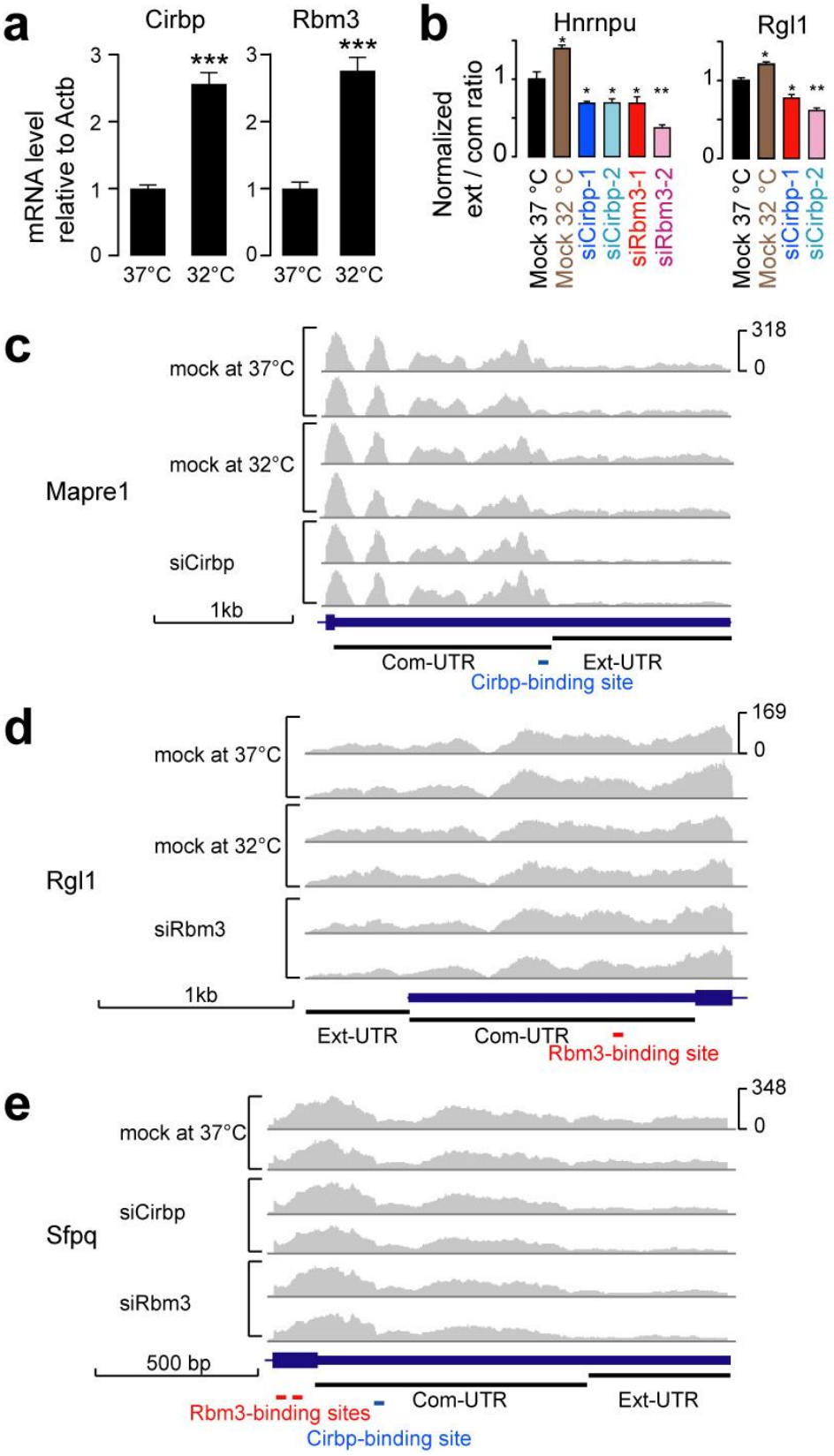


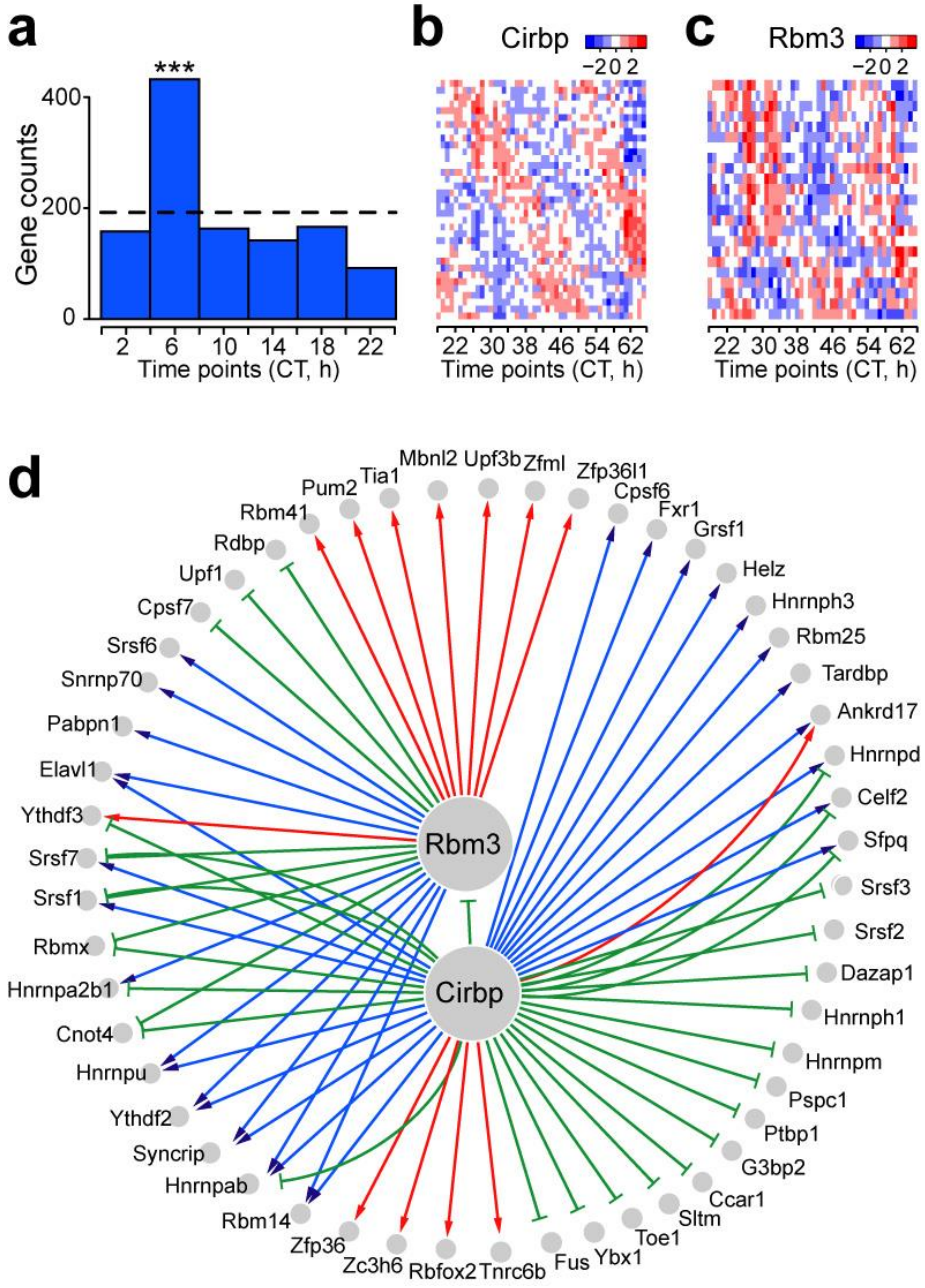
Supplementary Fig. S4



Supplementary Fig. S5



Supplementary Fig. S6



IV. Supplementary Tables

Supplementary Table S1. Reads and clusters generated by PAR-CLIP.

	Cirbp par-clip 1	Cirbp par-clip 2	Rbm3 par-clip 1	Rbm3 par-clip 2
Total reads	24725395	5313780	14175927	13341089
Kept after adapter removal	23615476	4982412	13088730	12615292
Mapped to mm9 (allow only one mismatch)	2630336	1134706	2887688	4017077
Filtered for maximum edit-distance and uniqueness	1287811	487578	1267241	1905520
Kept clusters	7240	5760	4921	10251

Supplementary Table S3. The top five enriched GO categories of Cirbp- or Rbm3-target genes

Enriched GO terms in Cirbp-target genes	Counts	P-value	FDR
GO:0045449~regulation of transcription	657	5.3E-25	9.8E-22
GO:0044265~cellular macromolecule catabolic process	221	1.2E-18	2.2E-15
GO:0016071~mRNA metabolic process	117	3.2E-12	6.0E-09
GO:0051276~chromosome organization	124	1.8E-13	3.3E-10
GO:0006397~mRNA processing	102	6.3E-11	1.2E-07

Enriched GO terms in Rbm3-target genes	Counts	P-value	FDR
GO:0045449~regulation of transcription	813	2.3E-39	4.3E-36
GO:0051252~regulation of RNA metabolic process	512	9.9E-18	1.9E-14
GO:0051276~chromosome organization	177	2.3E-16	4.2E-13
GO:0016071~mRNA metabolic process	135	1.6E-13	3.0E-10
GO:0070727~cellular macromolecule localization	120	2.0E-8	3.7E-05

FDR: false discovery rate.

Supplementary Table S4. The Cirbp- or Rbm3-target genes that oscillated in at least eight mouse tissues.

Cirbp-target genes	Circadian peak time	Tissue counts		Rbm3-target genes	Circadian peak time	Tissue counts
Nr1d2	9.6	14		Nr1d2	9.6	14
Arntl	22.37	13		Tef	11.48	11
Timp3	12.66	11		Timp3	12.66	11
Tspan4	11.77	10		Cry1	17.98	11
Clock	23.35	10		Bhlhb2	10.29	10
Fkbp5	13.6	10		Clock	23.35	10
Tsc22d3	15.68	9		Fkbp5	13.6	10
Herpud1	12.74	9		Col4a1	1.92	9
Col4a1	1.92	9		Inmt	12.51	9
Pdcd4	6.41	9		Pdcd4	6.41	9
Nedd4l	1.08	9		Pim3	8.08	9

Pim3	8.08	9		H3f3b	4.25	9
H3f3b	4.25	9		Per1	11.44	9
Per1	11.44	9		Mid1ip1	14.3	8
Hnrpdl	6.82	8		Por	12.06	8
Ccrn4l	12.32	8		Hnrpdl	6.82	8
Fbn1	2.27	8		Fbn1	2.27	8
Tfrc	19.19	8		Tfrc	19.19	8
Pbef1	14.26	8		Pbef1	14.26	8
Hsp110	18.02	8		Hsp110	18.02	8
Marcks	1.31	8		Cirbp	5.81	8
				Marcks	1.31	8

Supplementary Table S5. Detailed information about the RNA-seq libraries.

Library	**Total reads**	**Uniquely mapped to mm9**	**Transcripts with FPKM>5**
Mock_37_1	4.22E+07	2.82E+07	12533
Mock_37_2	4.14E+07	2.73E+07	12609
Mock_32_1	4.28E+07	2.81E+07	12439
Mock_32_2	4.20E+07	2.66E+07	12470
siCirbp_1	4.23E+07	2.69E+07	12650
siCirbp_2	4.31E+07	2.81E+07	12501
siRbm3_1	4.17E+07	2.71E+07	12580
siRbm3_2	4.12E+07	2.51E+07	12384

Supplementary Table S7. The enriched GO categories of the expression changed genes upon Cirbp or Rbm3 depletion.

The genes that were up-regulated upon Cirbp depletion (876)

Enriched GO terms	Genes	P-value	FDR
GO:0007049~cell cycle	139	1.0E-61	1.8E-58
GO:0006259~DNA metabolic process	97	1.1E-42	1.8E-39
GO:0051301~cell division	79	3.1E-41	5.4E-38
GO:0006260~DNA replication	52	1.4E-31	2.5E-28
GO:0006396~RNA processing	59	4.2E-14	7.3E-11

The genes that were up-regulated upon Rbm3 depletion (237)

Enriched GO terms	Genes	P-value	FDR
GO:0006260~DNA replication	13	9.8E-08	1.6E-04
GO:0006259~DNA metabolic process	18	3.3E-06	5.2E-03
GO:0007049~cell cycle	20	3.6E-05	0.056

There were no significantly enriched GO categories in the genes that were down-regulated upon Cirbp or Rbm3 depletion.

Supplementary Table S9. The genes that showed oscillating ext/com UTR-ratio in mouse liver and oscillating expression in more than six mouse tissues.

Genes	ext/com ratio oscillating peak time	ext/com ratio oscillating p-value	Gene oscillating peak time	Tissue counts
Tef	11.0	1.75E-09	11.48	11
Fkbp5	13.5	5.27E-07	13.6	10
Clock	7.3	6.61E-08	23.35	10
Col4a1	3.7	3.14E-10	1.92	9
Gsta3	19.0	2.11E-13	17.82	9
Rorc	6.7	0	18.27	9
Hnrpdl	7.0	7.77E-07	6.82	8
Litaf	11.5	7.03E-07	1.42	8
Marcks	6.0	2.94E-05	1.31	8
Nampt	3.8	2.18E-07	14.26	8
Acsl1	13.0	1.03E-05	7.25	7
Caprin1	9.3	3.09E-06	18.9	7
Eif1a	6.0	1.97E-05	20.89	7
Phf17	8.7	4.12E-07	3.24	7
Pnrc1	11.2	3.15E-07	0.22	7
St13	9.0	4.32E-08	19.28	7

Supplementary Table S10. List of oligonucleotides used in this study.

18srRNA-F	ATGGCCGTTCTTAGTTGGTG	Cpsf6_ext-F	TTGGAACCCTTTGTTTGCTC
18srRNA-R	GAACGCCACTTGTCCCTCTA	Cpsf6_ext-R	CAGCAGCAAGCAAGGCTAAT
ACTB-F	CAGCTTCTTTGCAGCTCCTT	Elavl1_com-F	ACTCATTTTGCGCCAATTTT
ACTB-R	CACGATGGAGGGGAATACAG	Elavl1_com-R	CATTTGTGGGATTTCAAGCA
Bmal1-F	CCCGCTGAACATCACAAGTA	Elavl1_ext-F	CCAGTTAGCGTGTTTCAGCA
Bmal1-R	TGAGCCTGCCCTGGTAATAG	Elavl1_ext-R	GATGGGAAGGGGTAAGGTGT
CIRBP-F	CTTTTCGTGGGAGGACTCAG	March5_com-F	TTGTTGCCATAAAAGGAGCA
CIRBP-R	CTCCCTGTCCTTTACCACCA	March5_com-R	GGCCTCAGAACAACCAGAAG
Crebzf-F	AAGGTGTCGGTGGAGTTCTG	March5_ext-F	TACGGTGTCTGTGGGAATGA
Crebzf-R	GGAGGAGAAAGGGGTAAACG	March5_ext-R	CACACTGGCAATGATGGAAG
Dbp-F	GGCTCTTGCAGCTCCTCTT	Mapre1_1-F	AGTTGCACCCCTGCTCTAAA
Dbp-R	ATTAGCACCTCCACGGTGTC	Mapre1_1-R	GCAGGTCCCAGAAACACAAT
Gapdh-F	AGGTCGGTGTGAACGGATTTG	Mapre1_2-F	AAGGGTGATGCGACTACCAC
Gapdh-R	TGTAGACCATGTAGTTGAGGTCA	Mapre1_2-R	AAACACACCCCAAGTTCTGC
Hsp90aa1-F	GTGTGCAACAGCTGAAGGAA	Rnf41_com-F	TCTCCACTCCTGATGCTGTG
Hsp90aa1-R	GATGTCACCAGTCGGTTTGA	Rnf41_com-R	TTCATGGGCATTTTCAATCA

Hsp90ab1-F	CCAGAGGACGAGGAAGAGAA	Rnf41_ext-F	ATGCACCTTCCACTGACCTC
Hsp90ab1-R	GTCCAGCCATAGGTGCTTGT	Rnf41_ext-R	ATTCCGGCTAAAGGGACACT
Klhl15-F	CTTGGCTGCGACATGATAGA	Rpl22_com-F	AGAACAACCTCCGAGACTGG
Klhl15-R	TGCTGGTGAACATTTTGGAA	Rpl22_com-R	CCTCGTCTTCCTCCTCCTCT
Nr1d1-F	CACTTACCGAGGAGGAGCTG	Rpl22_ext-F	GGATTGGACAGGAAGTTGGA
Nr1d1-R	CGGTTCTTCAGCACCAGAG	Rpl22_ext-R	CCCAGCCTCTCGTCTACTTG
Nr1d2-F	TCCAGCTCAGTGATGAGGAA	Serbp1_com-F	TTTTGAAAACGAACTTCTCTAGC
Nr1d2-R	AGGCCTCATTTGGATGGTTT	Serbp1_com-R	GCATATTTGCAAGCAGCAAT
Per1-F	GCCTCCTCCTCCTCCTACAC	Serbp1_ext-F	GTTTTGTGGGTGTGGGTTTT
Per1-R	CACCACGCTCTCTGCCTTAT	Serbp1_ext-R	TTTTGGGGGCTTTCATACAC
Per2-F	CTTCTGGTCTGGACTGCACA	Sfpq_com-F	CGCAGTGTTCAAAACAGGAG
Per2-R	CAGGTCACTTGACGTGGAGA	Sfpq_com-R	AAATTCGGTGGCACAAGGTA
Rbm3-F	CAGCCAGGGTGGCTATGAC	Sfpq_ext-F	TGGAGCAAAGTCTGTAGTCTGAA
Rbm3-R	AGCGCAGCTCCAAAAATCT	Sfpq_ext-R	CTTATGGTGGGAGGAAAGCA
RORa-F	ACACCAGCATCTGGCTTCTT	Syncrip_com-F	ACCAGAAGCTTGCAGTGGAG
RORa-R	CCAGGTGGGATTTGGATATG	Syncrip_com-R	TCCCGTATTTGATTCCATGA
RORc-F	AATGTGGCCTACTCCTGCAC	Syncrip_ext-F	AATGGGTGCAGTTTTTGAGC
RORc-R	TCCCTCTGCTTCTTGGACAT	Syncrip_ext-R	TGCATTGCCTGTTTTTCTTG
Sfrs7-F	AGGATGCAGTTCGAGGATTG	Tardbp_com-F	AATCCTGTGGCTTTGGTGAG
Sfrs7-R	GGTGGCCTATCAAAACGAGA	Tardbp_com-R	GAGGGGGAGGGAAGTACAAG
Tef-F	GTTTGCAGAGGAGGACCTGA	Tardbp_ext-F	CAGCCCTGAATGCAAAGAAT
Tef-R	GAGCGTTTAGCTGCCACATT	Tardbp_ext-R	GAAGCAAAATAAACTACTGCCAAGA
Bnip2_com-F	GCTGGGTTGTCCTAAAGCAG	Tm9sf3_com-F	CATGGCTAATCTTCCCCAAA
Bnip2_com-R	TTTCAGGGAGGAGAAGCTCA	Tm9sf3_com-R	AAACACCAAGTGCCAGGAAG
Bnip2_ext-F	CACAGTCCTTGGGGTTCTGT	Tm9sf3_ext-F	AATGCCAGGAGGAAAAAGGT
Bnip2_ext-R	GACAGAATGTGAGCGTCCAA	Tm9sf3_ext-R	ATGTCTTCCCACCTGCTGAC
Cpsf6_com-F	TCAGGGAAAGGCTTGTGTTC		
Cpsf6_com-R	TTGATGCATATCCAAACACG		

“com” and “ext” stands for common and extended 3’UTR. All the primers were ordered from Invitrogen (China).