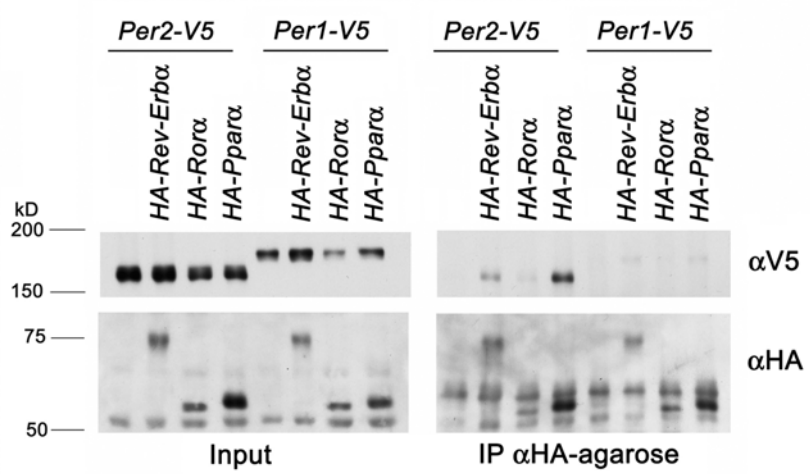
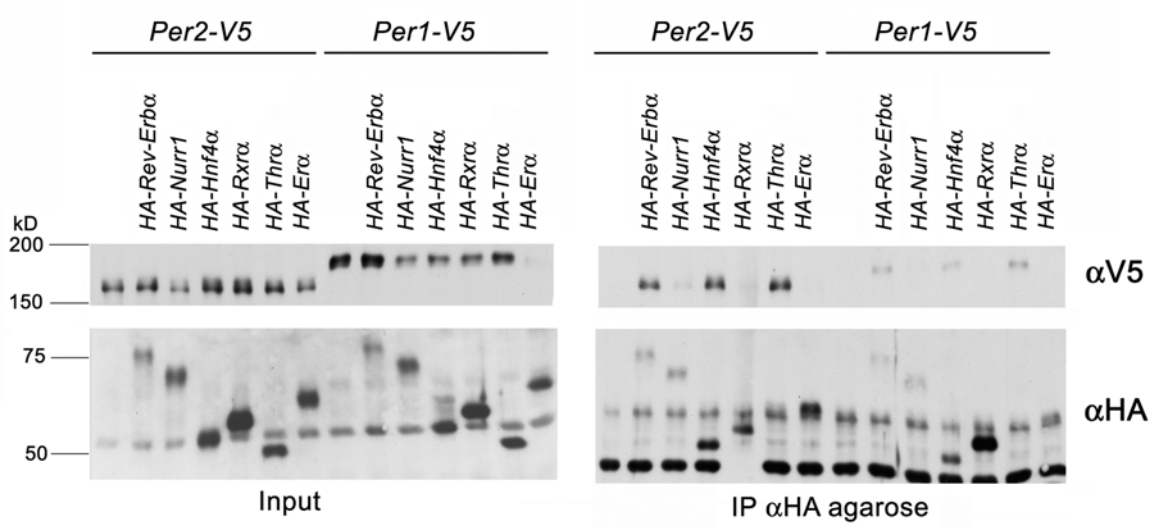
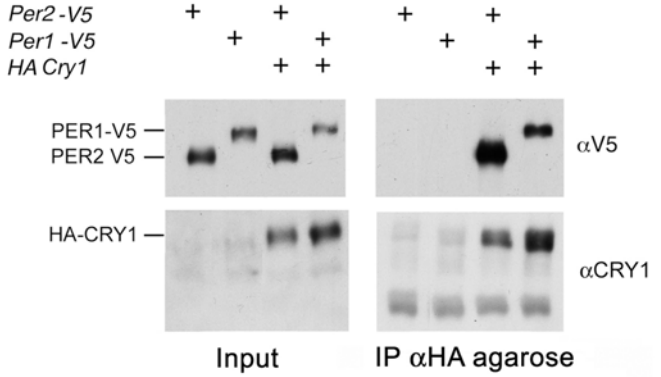
A


Per2-V5
Per1-V5
Per2-V5
Per1-V5
HA-Rev-Erbα
HA-Rorα
HA-Pparα
kD
200
150
75
50
αV5
αHA
Input
IP αHA-agarose

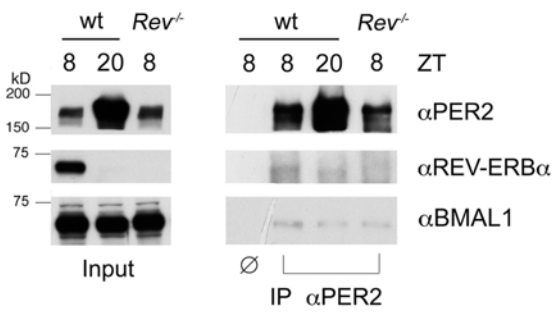
B

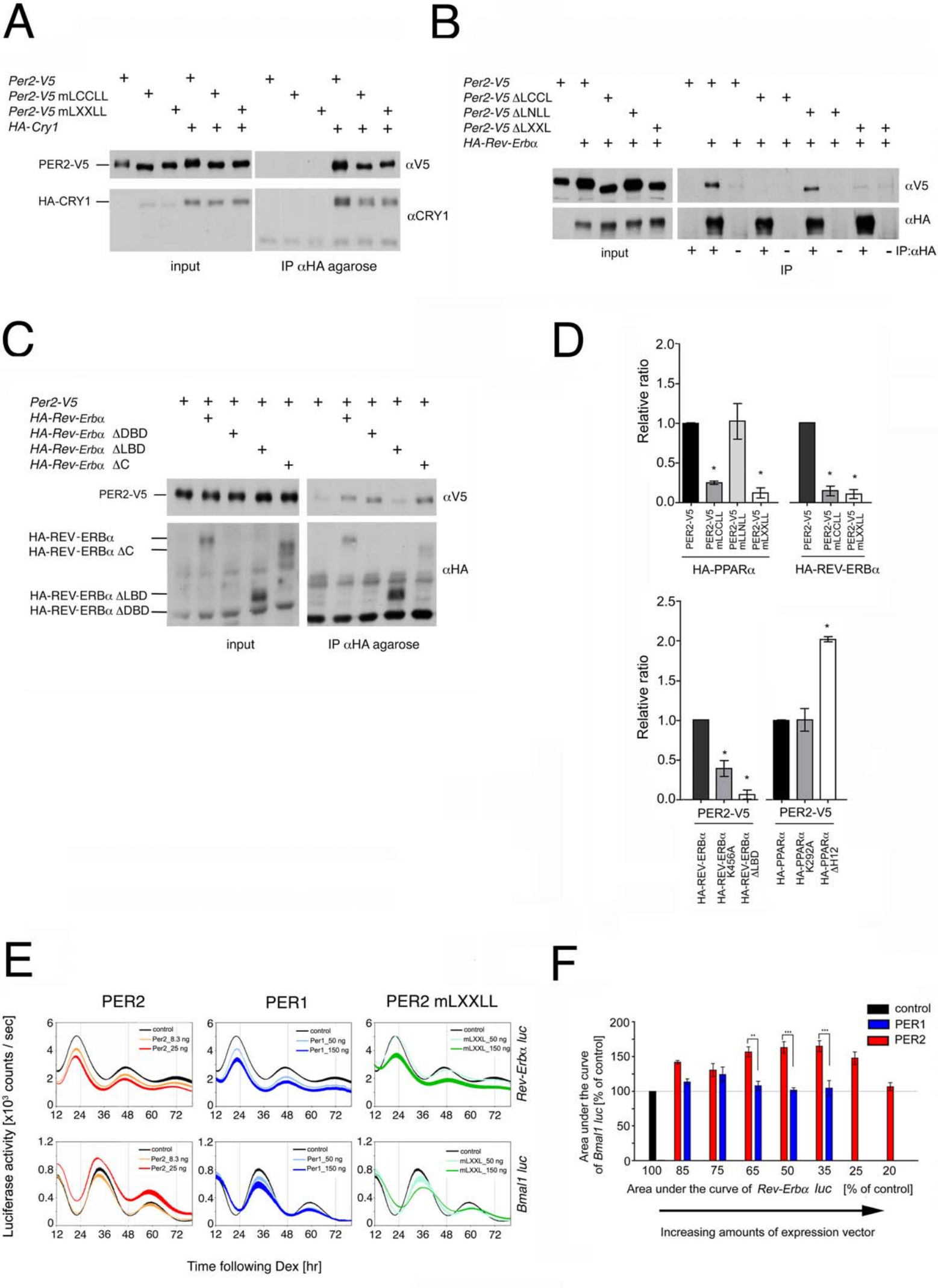

Per2-V5
Per1-V5
Per2-V5
Per1-V5
HA-Rev-Erbα
HA-Nurr1
HA-Hnf4α
HA-Rxrα
HA-Thrα
HA-Erα
kD
200
150
75
50
αV5
αHA
Input
IP αHA agarose

C

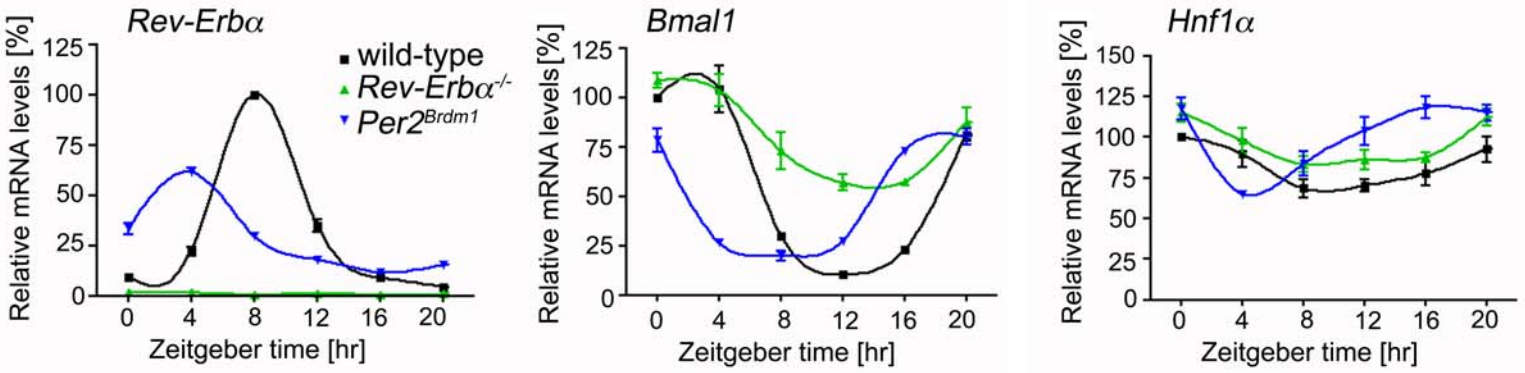

Per2 -V5
Per1 -V5
HA Cry1
PER1-V5
PER2 V5
HA-CRY1
αV5
αCRY1
Input
IP αHA agarose

D

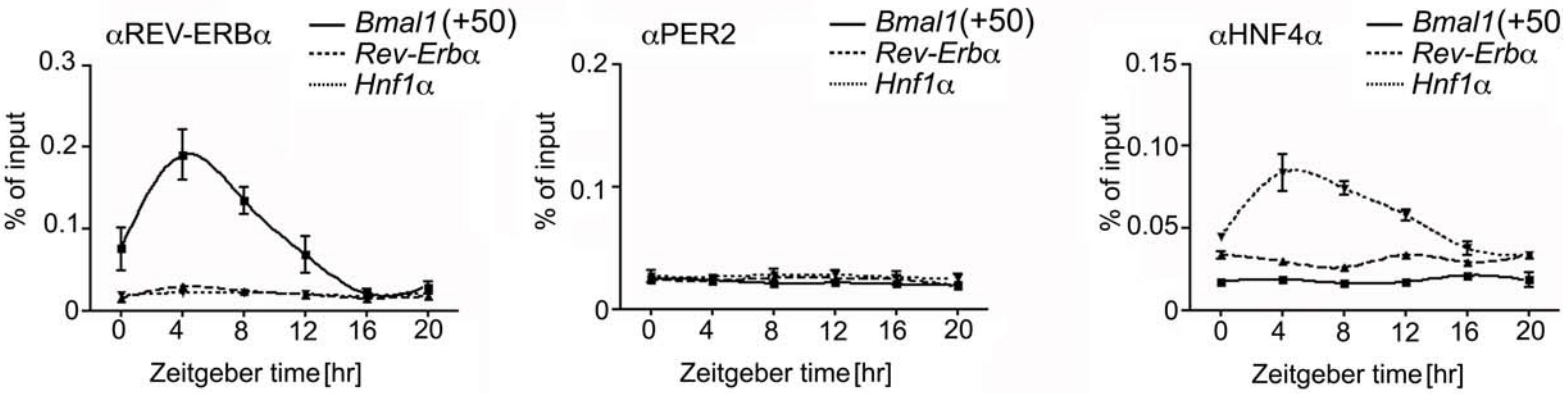

wt
Rev-/-
wt
Rev-/-
8 20 8
8 8 20 8 ZT
kD
200
150
75
75
αPER2
αREV-ERBα
αBMAL1
Input
∅
IP αPER2


A
Per2-V5
Per2-V5 mLCCLL
Per2-V5 mLXXLL
HA-Cry1
PER2-V5
HA-CRY1
αV5
αCRY1
input
IP αHA agarose
B
Per2-V5
Per2-V5 ΔLCCL
Per2-V5 ΔLNLL
Per2-V5 ΔLXXL
HA-Rev-Erbα
αV5
αHA
input
IP
IP:αHA
C
Per2-V5
HA-Rev-Erbα
HA-Rev-Erbα ΔDBD
HA-Rev-Erbα ΔLBD
HA-Rev-Erbα ΔC
PER2-V5
αV5
HA-REV-ERBα
HA-REV-ERBα ΔC
HA-REV-ERBα ΔLBD
HA-REV-ERBα ΔDBD
αHA
input
IP αHA agarose
D
Relative ratio
PER2-V5
PER2-V5 mLCCLL
PER2-V5 mLLNLL
PER2-V5 mLXXLL
HA-PPARα
HA-REV-ERBα
Relative ratio
PER2-V5
HA-REV-ERBα
HA-REV-ERBα K456A
HA-REV-ERBα ΔLBD
HA-PPARα
HA-PPARα K292A
HA-PPARα ΔH12
E
PER2
PER1
PER2 mLXXLL
control
Per2_8.3 ng
Per2_25 ng
Per1_50 ng
Per1_150 ng
mLXXL_50 ng
mLXXL_150 ng
Rev-Erbα luc
Bmal1 luc
Luciferase activity [x10³ counts / sec]
Time following Dex [hr]
F
control
PER1
PER2
Area under the curve of Bmal1 luc [% of control]
Area under the curve of Rev-Erbα luc [% of control]
Increasing amounts of expression vector

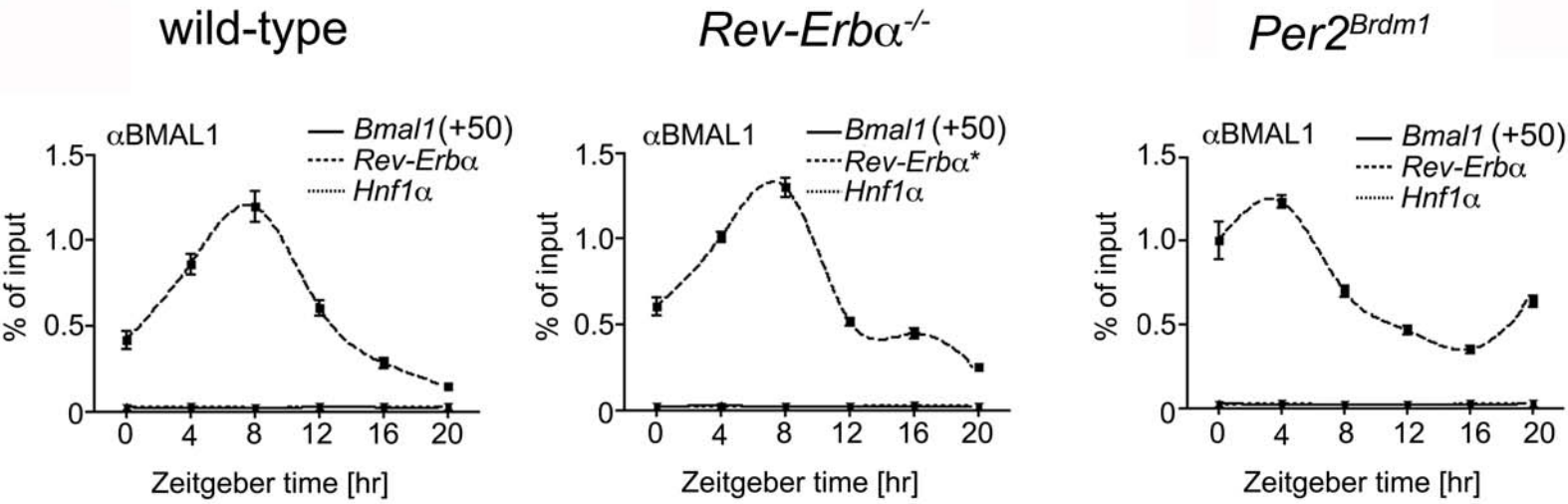
A

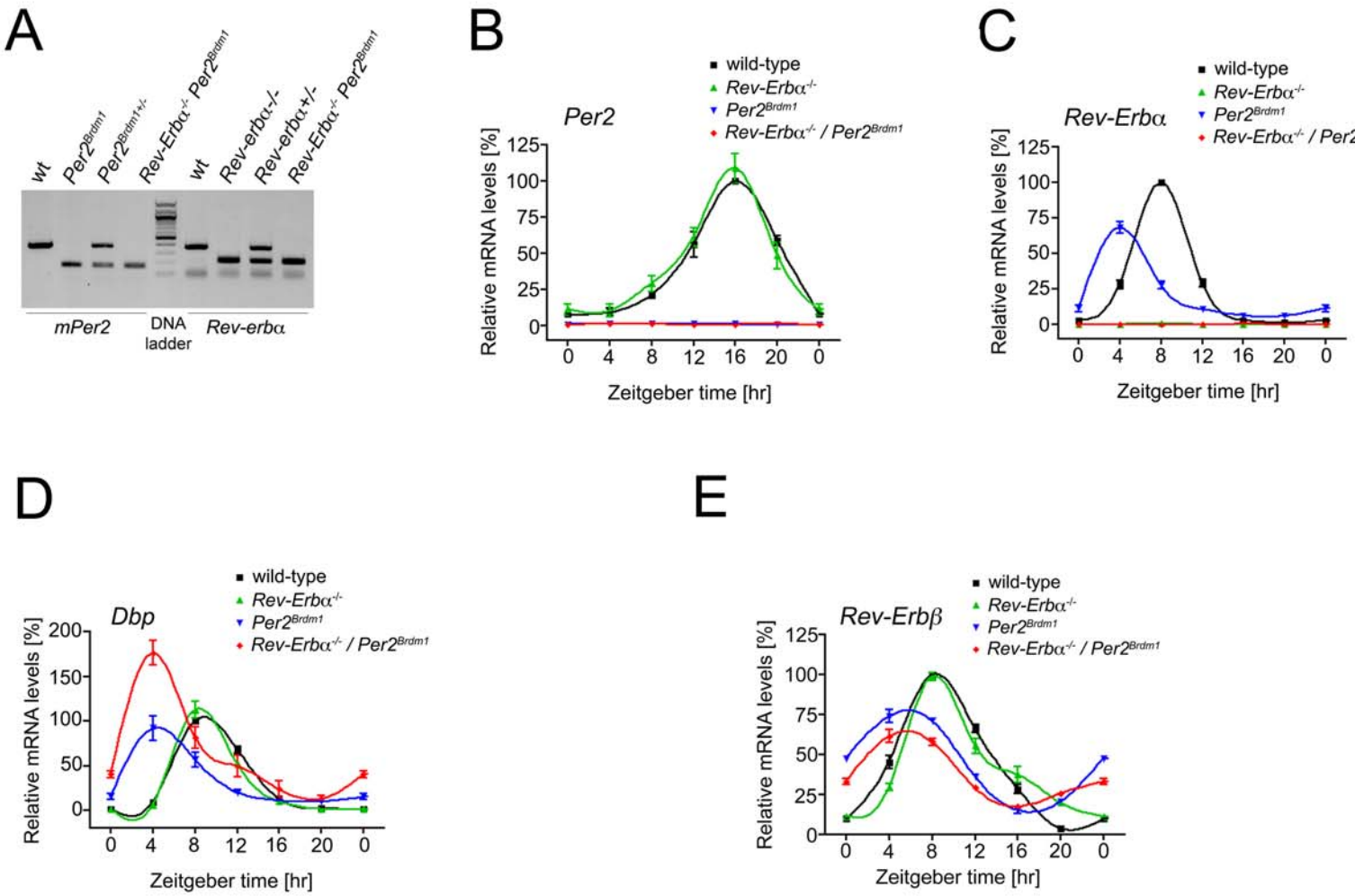

Rev-Erbα
wild-type
Rev-Erbα-/-
Per2Brdm1
Relative mRNA levels [%]
Zeitgeber time [hr]
Bmal1
Hnf1α

B

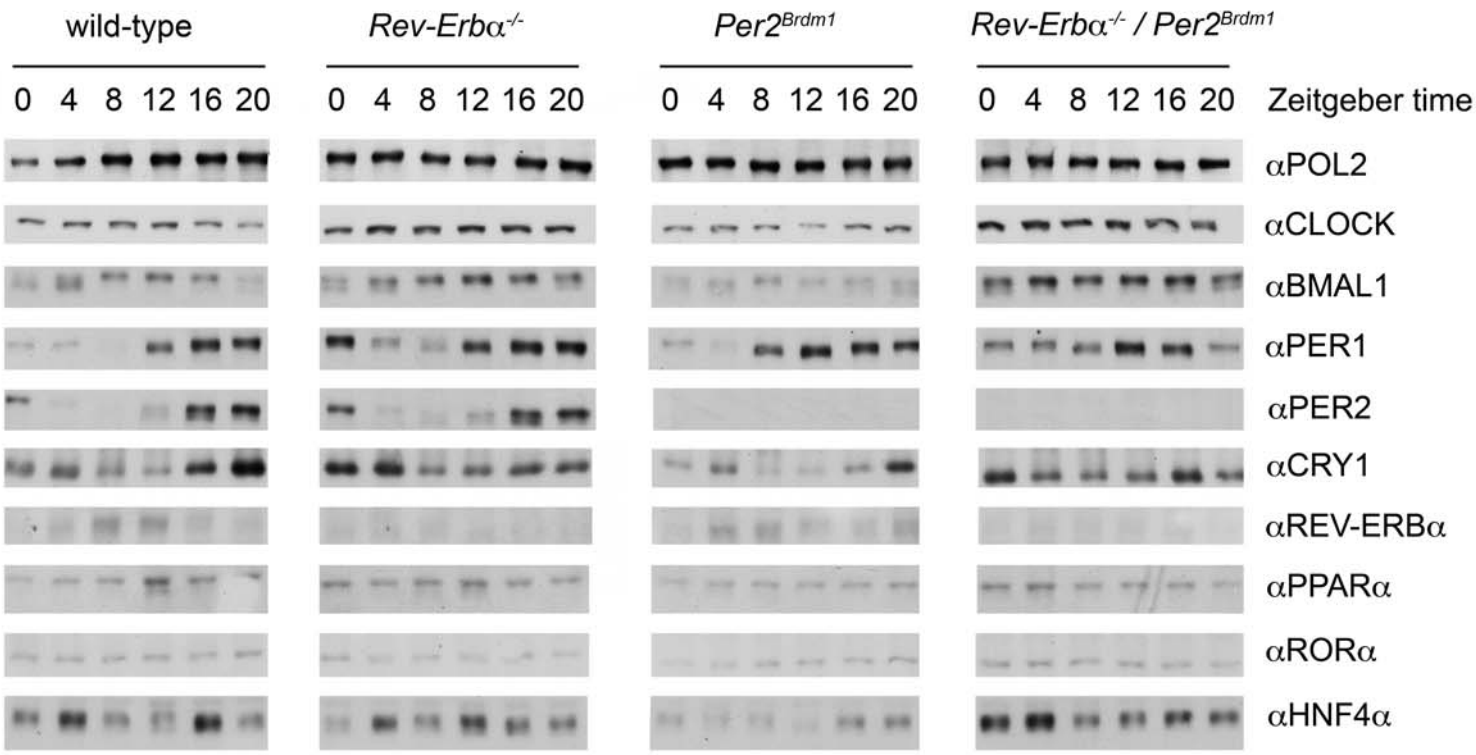

Per2Brdm1
αREV-ERBα
αPER2
αHNF4α
Bmal1(+50)
Rev-Erbα
Hnf1α
% of input
Zeitgeber time [hr]

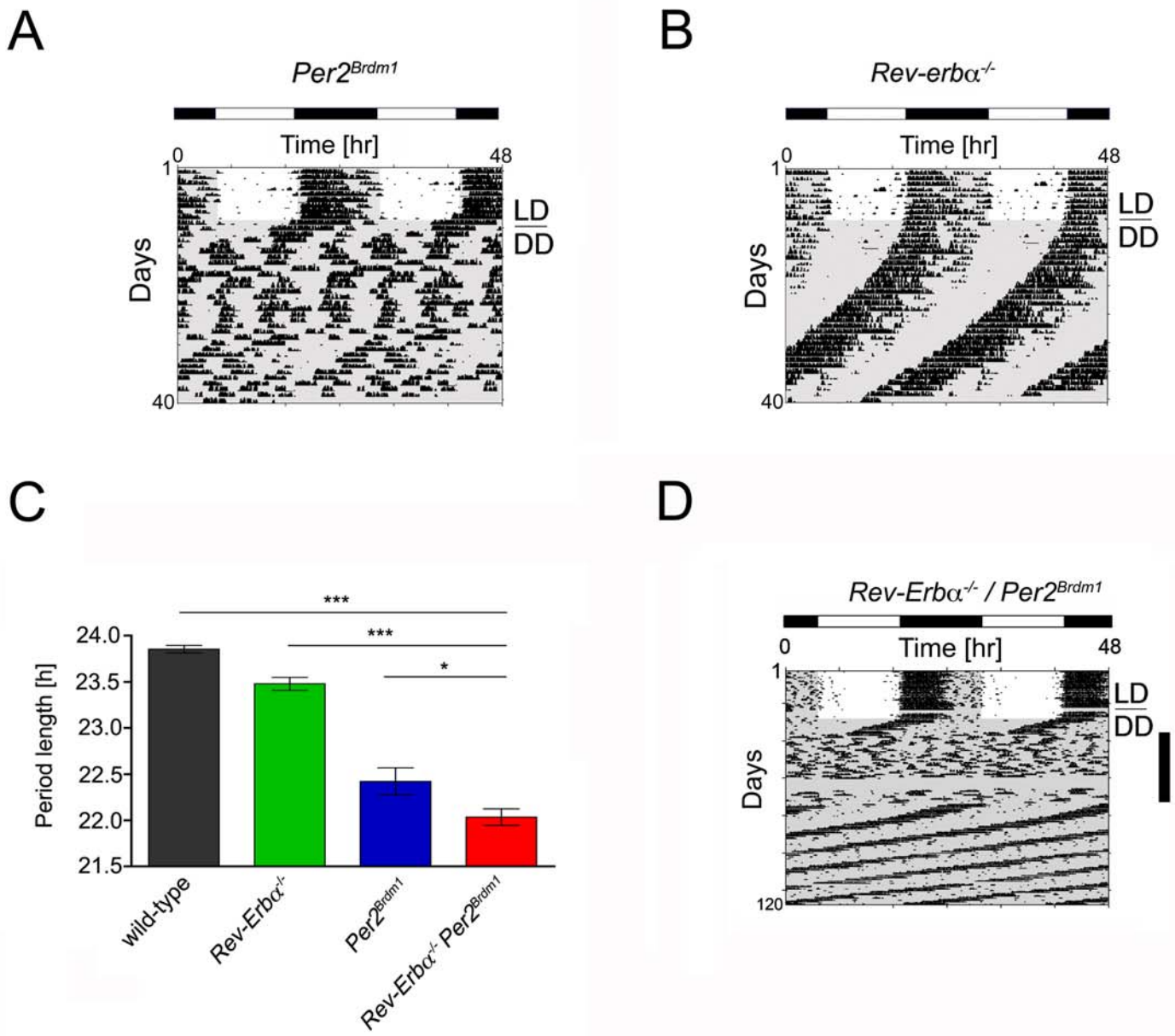
C


wild-type
Rev-Erbα-/-
Per2Brdm1
αBMAL1
Bmal1(+50)
Rev-Erbα
Rev-Erbα*
Hnf1α
% of input
Zeitgeber time [hr]

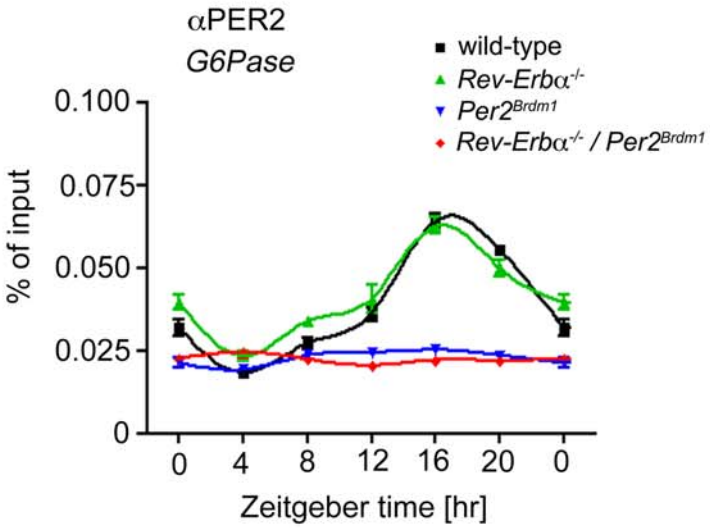

A
wt
Per2Brdm1
Per2Brdm1+/-
Rev-Erbα-/- Per2Brdm1
wt
Rev-erbα-/-
Rev-erbα+/-
Rev-Erbα-/- Per2Brdm1
mPer2
DNA ladder
Rev-erbα
B
Per2
wild-type
Rev-Erbα-/-
Per2Brdm1
Rev-Erbα-/- / Per2Brdm1
Relative mRNA levels [%]
Zeitgeber time [hr]
C
Rev-Erbα
wild-type
Rev-Erbα-/-
Per2Brdm1
Rev-Erbα-/- / Per2Brdm1
Relative mRNA levels [%]
Zeitgeber time [hr]
D
Dbp
wild-type
Rev-Erbα-/-
Per2Brdm1
Rev-Erbα-/- / Per2Brdm1
Relative mRNA levels [%]
Zeitgeber time [hr]
E
Rev-Erbβ
wild-type
Rev-Erbα-/-
Per2Brdm1
Rev-Erbα-/- / Per2Brdm1
Relative mRNA levels [%]
Zeitgeber time [hr]

F

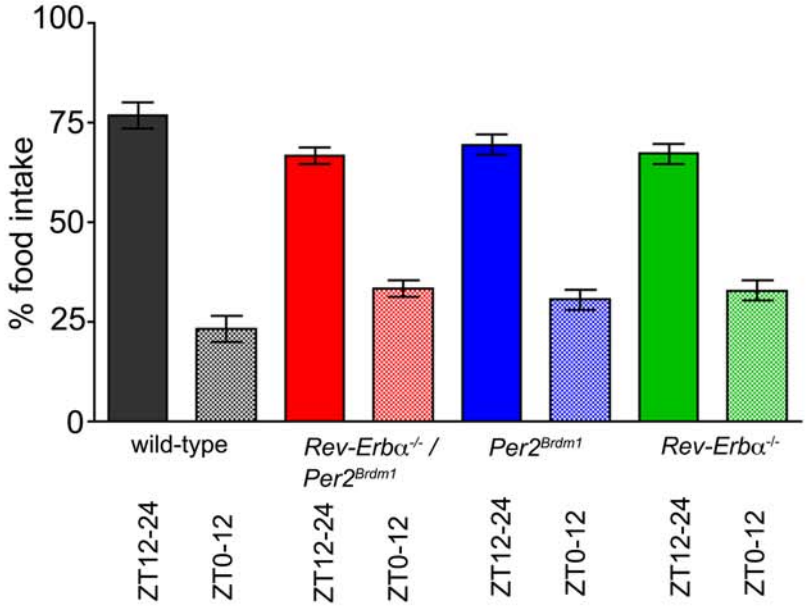

wild-type
Rev-Erbα-/-
Per2Brdm1
Rev-Erbα-/- / Per2Brdm1
0 4 8 12 16 20
Zeitgeber time
αPOL2
αCLOCK
αBMAL1
αPER1
αPER2
αCRY1
αREV-ERBα
αPPARα
αRORα
αHNF4α


A
Per2Brdm1
Time [hr]
0
48
1
40
Days
LD
DD
B
Rev-erbα-/-
Time [hr]
0
48
1
40
Days
LD
DD
C
***
***
*
24.0
23.5
23.0
22.5
22.0
21.5
Period length [h]
wild-type
Rev-Erbα-/-
Per2Brdm1
Rev-Erbα-/- Per2Brdm1
D
Rev-Erbα-/- / Per2Brdm1
0
Time [hr]
48
1
120
Days
LD
DD

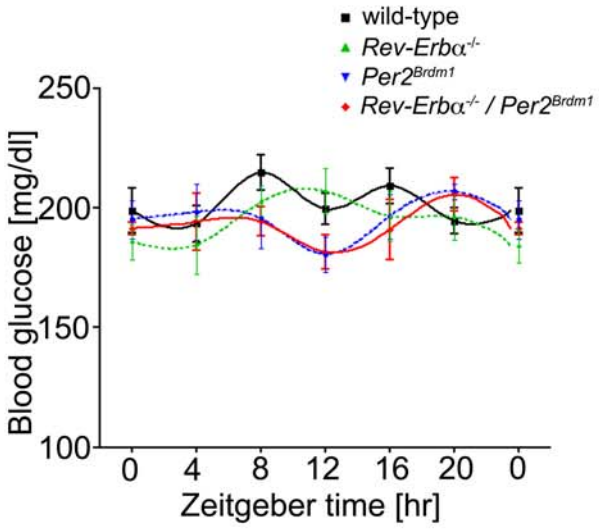
A

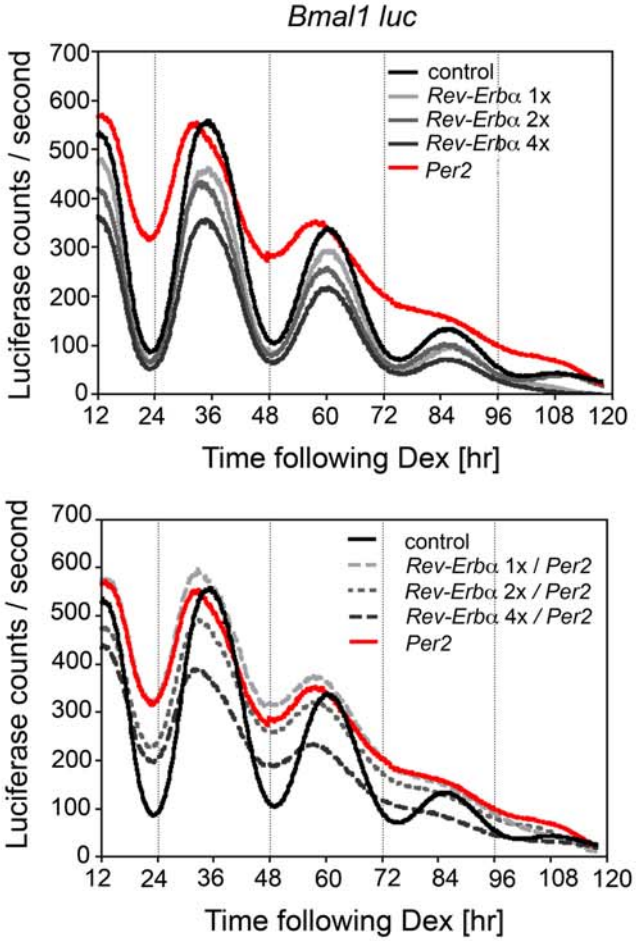

αPER2
G6Pase
wild-type
Rev-Erbα-/-
Per2Brdm1
Rev-Erbα-/- / Per2Brdm1
% of input
0.100
0.075
0.050
0.025
0
0 4 8 12 16 20 0
Zeitgeber time [hr]

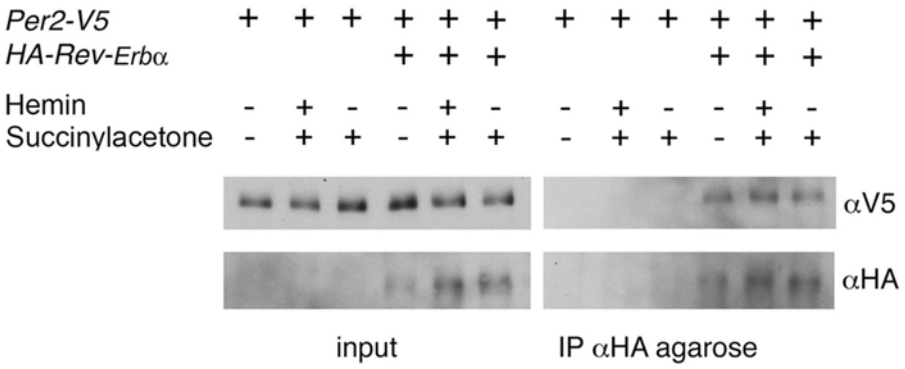
B

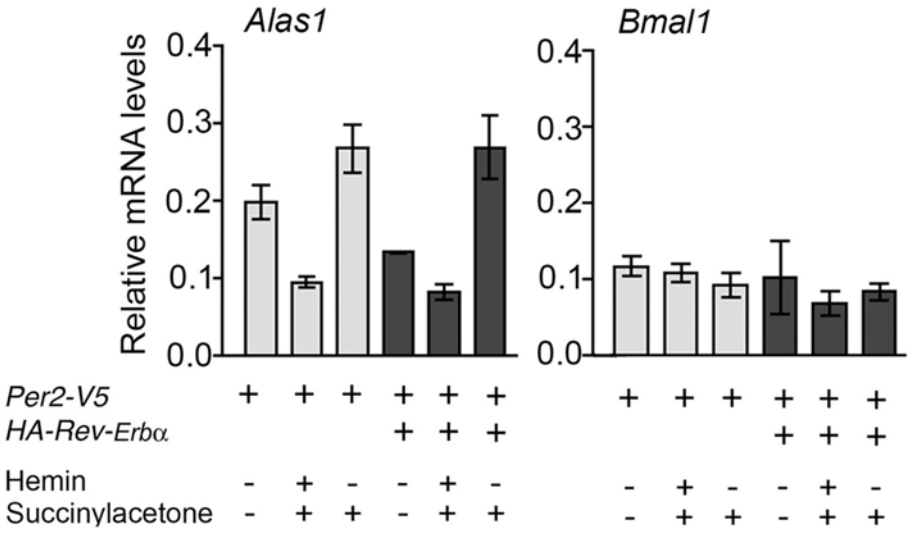

% food intake
100
75
50
25
0
wild-type
Rev-Erbα-/- / Per2Brdm1
Per2Brdm1
Rev-Erbα-/-
ZT12-24
ZT0-12
ZT12-24
ZT0-12
ZT12-24
ZT0-12
ZT12-24
ZT0-12

C


wild-type
Rev-Erbα-/-
Per2Brdm1
Rev-Erbα-/- / Per2Brdm1
Blood glucose [mg/dl]
250
200
150
100
0 4 8 12 16 20 0
Zeitgeber time [hr]


Bmal1 luc
Luciferase counts / second
Time following Dex [hr]
control
Rev-Erbα 1x
Rev-Erbα 2x
Rev-Erbα 4x
Per2
Rev-Erbα 1x / Per2
Rev-Erbα 2x / Per2
Rev-Erbα 4x / Per2


Per2-V5
HA-Rev-Erbα
Hemin
Succinylacetone
αV5
αHA
input
IP αHA agarose


Alas1
Bmal1
Relative mRNA levels
0.0
0.1
0.2
0.3
0.4
Per2-V5
HA-Rev-Erbα
Hemin
Succinylacetone

Supplemental data

The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors

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Supplemental materials and methods

Nuclear extracts from NIH 3T3 mouse fibroblasts

Nuclear extracts were prepared by swelling the cells in 100 mM Tris-HCl pH 8.8/ 10 mM DTT and subsequent hypotonic lysis (10 mM EDTA, 1 mM EGTA, 10 mM Hepes pH 6.8, 0.2% Triton-X 100, 0.5 mM DTT, protease inhibitor cocktail). After centrifugation for 5 minutes at 2,500 g and 4°C, the supernatant was stored as cytosolic fraction. The pellet was washed once with the same buffer and resuspended in 1 mM EDTA/1 mM EGTA/10 mM Hepes, pH 6.8/10% glycerol/300 mM NaCl/ 0.5 mM DTT and protease inhibitor cocktail (Roche Applied Science). Samples were incubated for 20 minutes on ice and centrifuged for 20 minutes at 16,000 g and 4°C. The supernatant was stored as the nuclear fraction at -70°C.

Co-immunoprecipitation analysis

For co-immunoprecipitation with HA-REV-ERBα, 20 µg of nuclear protein extract were incubated in the presence of 0.1% Triton-X 100 with 3 µl αHA antibody (Roche) or 15 µl αHA agarose (Sigma-Aldrich) in rotation over night at 4°C. αHA

antibody was captured with protein A agarose beads for two hours at 4°C. Beads were collected by centrifugation and washed four times using lysis buffer supplemented with 1% Triton-X 100 and 150 mM NaCl. Laemmli sample buffer was added, samples were boiled and subsequently subjected to SDS-PAGE (Laemmli 1970).

Quantification of the Area under the curve

Bioluminescence counts over several days were quantified by measuring the area under the curve (AUC) as a measure of the total reporter activity using Prism4 software (GraphPad software Inc., La Jolla, CA). To compare the effects of the overexpressed proteins on luciferase reporter expression, the AUC of the luciferase reporter vector alone was defined as 100 % and the AUC values of the co-transfections were calculated relative to 100 %.

Succinylacetone and hemin treatment

Cells were switched 24 hrs after transfection to serum free medium supplemented with or without succinylacetone (5 mM; Sigma-Aldrich) overnight. For the hemin (Sigma-Aldrich) treatment, cells were treated with either solvent or hemin (6 μM) for 6 hrs. Nuclear extracts were prepared as described above. An aliquot of cells from the treated cultures was taken for RNA extraction.

Blood glucose determination

Blood samples were collected in clotting activator coated capillaries (Microvette 100, Sarstedt) and serum was recovered after centrifugation for 10 min at 3,300 g. Glucose was measured with the glucose-hexokinase reagent (Sigma-Aldrich) and normalized to a control serum used in each experiment.

Supplemental figure legends

Supplemental figure 1. PER2 co-immunoprecipitates better than PER1 with nuclear receptors *in vitro*

(A-C) HA-tagged hormone receptors involved in the circadian oscillator mechanism (A), other nuclear receptors (B), or HA-CRY1 (C) were immunoprecipitated from NIH 3T3 nuclear extracts (right panels) in the presence of either PER2-V5 or PER1-V5. Left panels show the input of protein as used in each assay. Expression vectors used for co-transfection as well as antibodies used for Western blot analysis are indicated.

Supplemental figure 2. *In vitro* characterization of the PER2/nuclear receptor interaction

(A, B, C) Left panels show the input of the indicated proteins as used in each assay. Right panels show the co-immunoprecipitation. The immunoprecipitated proteins were analyzed by Western blot. Antibodies used for detection and immunoprecipitation are indicated. Reactions with extract and beads alone were used as negative control for unspecific background binding.

(A) Immunoprecipitation of HA-CRY1 using αHA agarose.

(B) Immunoprecipitation of HA-REV-ERBα protein using αHA antibody. Extracts were incubated with agarose A beads with or without αHA antibody.

(C) Immunoprecipitation of wild-type or truncated HA-REV-ERBα using αHA agarose.

(D) Quantification of results obtained from co-immunoprecipitation experiments using NIH 3T3 nuclear extracts. The amount of co-immunoprecipitated V5-tagged protein was normalized to the amount of protein available for co-immunoprecipitation (as seen in the input panels). This value was normalized to the amount of immunoprecipitated HA-nuclear receptor. The wild-type PER2-V5/HA-nuclear receptor ratios were set to 1 and all other values were calculated relative to it, with n = 4 and $*P < 0.001$ indicating significance (One-way ANOVA). Error bars represent standard deviation.

(E) Dose-dependent effects of PER1, PER2 and PER2 mLXXLL on *Rev-erbα luc* and *Bmal1 luc* expression. NIH 3T3 cells were transfected with the indicated reporter vector either alone or together with the specified expression vector. 48 hrs after transfection, cells were synchronized with dexamethasone and luciferase activity of each culture was monitored. Data are plotted as mean + SD (n = 2, representative experiment out of three independent experiments).

(F) Dose response curves of increasing amounts of PER1 or PER2 on *Rev-Erbα* or *Bmal1* luciferase reporter genes. Areas under the curves were quantified for each reporter with or without expression vector, normalized and plotted against each other. Note that PER1 and PER2 both diminish the expression of *Rev-Erbα luc* in a dose dependent manner, whereas they act differently on *Bmal1 luc* expression. After reaching a peak of bioluminescence activity, even higher amounts of PER2 expression vector abolished this activation. ** $P < 0.01$, *** $P < 0.001$ indicating significance (One-way ANOVA). Error bars represent standard deviation (data combined from 8 independent experiments).

Supplemental figure 3. Controls for chromatin immunoprecipitation experiments

(A) Analysis of circadian mRNA accumulation in the livers of wild-type (black), *Rev-Erbα*$^{-/-}$ (green), and *Per2*^{Brdm1} (red) mutant animals. Total RNA was prepared from animals held in a LD 12:12 cycle. The relative amounts of *Bmal1, Rev-Erbα* and *Hnf1α* mRNA were measured by Taqman real-time RT-PCR. Plotted are the mean values ± SEM from four independent experiments.

(B and C) Chromatin immunoprecipitation analysis of chromatin prepared at 4 hrs intervals from wild-type, *Rev-Erbα*$^{-/-}$ and *Per2*^{Brdm1} mutant animals with the indicated antibodies. Specific Taqman probes were used to detect the indicated promoter regions. Plotted are the mean values ± SEM from three independent experiments. * represents the regulatory region preceding the *Rev-Erbα* knockout allele.

Supplemental figure 4. Analysis of mRNA and proteins levels in the liver

(A) Genotyping of wild-type, homozygous and heterozygous *Per2*^{Brdm1}, *Rev-Erbα*$^{-/-}$, *Rev-Erbα*$^{+/-}$ and *Rev-Erbα*$^{-/-}$*/Per2* mutant mice by PCR using genomic DNA obtained from tail biopsies.

(B-D) Total RNA was prepared from wild-type (black), *Rev-Erbα*$^{-/-}$ (green), *Per2*^{Brdm1} (blue) and *Rev-Erbα*$^{-/-}$*/Per2* mutant (red) animals held in LD 12:12. The relative amounts of *Per2* (B) and *Rev-Erbα* (C), *Dbp* (D) and *Rev-Erbβ* (E) mRNA were measured by Taqman real-time RT-PCR. Plotted are the mean values ± SEM from three independent experiments; ZT0 is double plotted.

(F) Nuclear extracts were analyzed by Western blot analysis using the indicated antibodies.

Supplemental figure 5. Analysis of free-running period length of wild-type, *Rev-Erbα*$^{-/-}$, *Per2*^{Brdm1} and *Rev-Erbα*$^{-/-}$*/Per2* mutant animals

(A and B, D) Representative locomotor activity records of *Per2*^{Brdm1} (A), *Rev-Erbα*$^{-/-}$ (B*)* and *Rev-Erbα*$^{-/-}$*/Per2* mutant (D) animals. The top bar indicates light and dark periods. The dark shaded areas represent darkness. Note the difference in behavior under LD 12:12 and constant conditions (DD). The side bar in panel D indicates days of arrhythmic locomotor activity of this animal.

(C) Period length of *wild-type, Rev-Erbα*$^{-/-}$*, Per2* mutant *and Rev-Erbα*$^{-/-}$*/Per2* double mutant animals as determined by χ^2-periodogramm analysis. ***P < 0.001, *$P < 0.05$ indicating levels of significance (One-way ANOVA).

Supplemental figure 6. Impact of *Per2* on feeding behavior, glucose homeostasis, and regulation of *G6Pase* expression

(A) Chromatin immunoprecipitation experiment to detect circadian PER2 binding at the *G6Pase* promoter. Co-immunoprecipitated DNA fragments were quantified by Taqman real-time PCR.

(B) Distribution of feeding activity (regular chow diet) between light and dark periods in the indicated genotypes. 100 % corresponds to the total of food consumed per day (n = 12, mean ± SEM).

(C) Resting blood glucose of wild-type (black), *Rev-Erbα*$^{-/-}$ (green), *Per2*^{Brdm1} mutant (blue) and *Rev-Erbα*$^{-/-}$*/Per2*^{Brdm1} double mutant (red) animals. Plotted values are the mean values ± SEM (n = 6); ZT0 is double plotted.

Supplemental figure 7. PER2 modulates the effect of REV-ERBα on *Bmal1 luciferase*

NIH 3T3 cells were transfected with a *Bmal1* luciferase reporter either alone or together with increasing amounts of *HA-Rev-Erbα* expression vector (grey) or a constant amount of *Per2-V5* expression vector (red). Dashed lines in the lower panel represent co-transfection of the same increasing amounts of *HA-Rev-Erbα* together with a constant amount of *Per2-V5*. Cells were synchronized by a dexamethasone shock and luciferase activity was recorded. Data are plotted as mean only (representative experiment out of three independent experiments).

Supplemental figure 8. Heme does not affect the interaction of REV-ERBα and PER2

Immunoprecipitation of HA-REV-ERBα with αHA agarose. After co-transfection, cells were pretreated with succinylacetone overnight and then treated with or without hemin for 6 hrs. The treatment is indicated for each lane. The relative amounts of *Alas1* mRNA and *Bmal1* mRNA derived from an aliquot from the same culture were measured by Taqman real-time RT-PCR and normalized to the corresponding *Gapdh* levels (lower panels). *Alas1* mRNA levels are increased or decreased in response to lower or higher than optimal intracellular hemin concentrations, respectively (for example: Raghuram et al., 2007).

Supplemental references

Hampp G, Ripperger JA, Houben T, Schmutz I, Blex C, Perreau-Lenz S, Brunk I, Spanagel R, Ahnert-Hilger G, Meijer JH et al. 2008. Regulation of monoamine oxidase A by circadian-clock components implies clock influence on mood. *Curr Biol* 18: 678-683.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.

Langmesser S, Tallone T, Bordon A, Rusconi S, and Albrecht U. 2008. Interaction of circadian clock proteins PER2 and CRY with BMAL1 and CLOCK. *BMC Mol Biol* 9: 41.

Nagoshi E, Saini C, Bauer C, Laroche T, Naef F, and Schibler U. 2004. Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. *Cell* 119: 693-705.

Table S1 Vectors			
Construct	**Based on accession number**	**Nuclear receptor nomenclature**	**Comments**
pSCT1 (Langmesser et al., 2008)			
pSCT1 Per2V5	NM_011066		
pSCT1 Per1V5	NM_011065		
pSCT1 HACry1	NM_007771	-	
pSCT1 HARev-Erbα	NM_145434	NR1D1	
pSCT1 HARorα	BC003757	NR1F1	
pSCT1 HAErα	NM_007956	NR3A1	
pSCT1 HANurr1	NM_013613	NR4A2	
pSCT1 HAPparα	NM_011144	NR1C1	
pSCT1 HAHnf4α	NM_008261	NR2A1	
pSCT1 HARxrα	NM_011305	NR2B1	
pSCT1 HAThrα	NM_178060	NR1A1	
pSEAP (Hampp et al., 2008)	-	-	
Bmal1 luciferase (Nagoshi et al., 2004)	-	-	
Rev-Erbα luciferase	-	-	To obtain *Rev-Erbα-luciferase,* a 6.6 kbp fragment of the *mRev-Erbα* gene, starting at the unique *MluI* restriction enzyme site (about 3.5 kbp upstream from the transcriptional start site) to the beginning of exon 2, was modified at the 3' site and cloned into the pGL3 basic vector (Promega, Madison, USA). This constructs expresses after appropriate splicing an in frame fusion protein containing the first 16 amino acids of mREV-ERBα and the firefly luciferase.
Bmal1 ext luciferase			5' extension of *Bmal1 luciferase* (Nagoshi et al., 2004) by 822 bp to include the PPARα-regulatory site (Canaple et al., 2006).

Table S1. Vectors used in this study. Corresponding accession numbers and the nuclear receptor nomenclature are indicated.

Table S2 Cloning			
Construct	**Primer Name**	**Sequence**	**Mutation**
***pSCT1 Per2V5 (ACCAA)* mLCCLL**	Per2(ACCAA)-FW	5`-GAG CCA GGC ATG CTG CGC AGC GCT GGC AGA GAG GGT AC-3`	mutation of aa L306, L309 & L310 to alanine residues
site-directed mutagenesis	Per2(ACCAA)-RV	5`-GTA CCC TCT CTG CCA GCG CTG CGC AGC ATG CCT GGC TC-3`	
***pSCT1 Per2V5 (LEAAA)* mLLNLLL**	Per2(LEAAA)-FW	5`-CAA GTG ACC TGC TCG AGG CGG CGG CCG GCG AGG ACC TCT GCT C-3`	mutation of aa N1052E and, L1053A , L1054A, L1055A
site-directed mutagenesis	Per2(LEAAA)-RV	5`-GAG CAG AGG TCC TCG CCG GCC GCC GCC TCG AGC AGG TCA CTT G-3`	
***pSCT1 Per2V5* mLXXLL**			combination of ACCAA and LEAAA mutations
***pSCT1 PER2V5* ΔLCCL**	PER2(LCCL)-S	5`-CCT GGT CAA GGT GCA AGA GCA GCA GGG TGC TGA GAG CCA GGA GAG GGT ACA C-3`	deletion of aa L306-A312
deletion	PER2(LCCL)-AS	5`-CCG AGT GTA CCC TCT CCT GGC TCT CAG CAC CCT GCT GCT CTT GCA CCT TGA-3`	
***pSCT1 PER2V5* ΔLNLL**	PER2(LNLL)-S	5`-CAA CGA GCC CTC AGA CGG C-3`	deletion of aa T1038-T1063
deletion	PER2(LNLL)-AS	5`- TGA GCC GTC TGA GGG CTC GTT GCA TG-3`	
***pSCT1 PER2V5* ΔLXXL**			combination of ΔLCCL and ΔLNLL deletions
deletion			
***pSCT1 HARev-Erbα* ΔLBD**			deletion of aa I290-Q615
out of frame deletion			
pSCT1 HARev-Erbα (456 KA)	Rev-Erbα(KtoA)-FW	5`-GGT GGT AGA GTT TGC CGC TCA CAT CCC AGG CTT CC-3`	mutation of aa K456 to alanine
site-directed mutagenesis	Rev-Erbα (KtoA)-RV	5`-GGA AGC CTG GGA TGT GAG CGG CAA ACT CTA CCA CC-3`	
***pSCT1 HARev-Erbα* ΔDBD**	Rev-Erbα (ΔDBD)-S	5`-CCG GTA CCG AGC AGC CTG TGC CCT-3`	deletion of aa T2-N231
deletion	Rev-Erbα (ΔDBD)-AS	5`-CTA GAG GGC ACA GGC TGC TCG GTA-3`	
***pSCT1 HARev-Erbα* ΔC**	Rev-Erbα (ΔC)-FW	5`-CATGTATCCCCATGGACGCAGCGG-3`	deletion of aa K588-Q615
cloning by PCR	Rev-Erbα (ΔC)-RV	5`-GGATCCGGTGAAGCGGGAAGTCTC-3`	
pSCT1 HAPparα (K292A)	Pparα(KtoA292)-FW	5`-GAG CTC ACA GAA TTT GCC GCG GCT ATC CCA GGC TTT GC-3`	mutation of aa K292 to alanine
site-directed mutagenesis	Pparα(KtoA292)-RV	5`-GCA AAG CCT GGG ATA GCC GCG GCA AAT TCT GTG AGC TC-3`	
***pSCT1 HAPparα* ΔH12**	Pparα(ΔH12)-S	5`-GCT GCA CCC ATA ACA CGT-3`	deletion of aa L459-Y468
deletion	Pparα(ΔH12)-AS	5`-ACG TGT TAT GGG TGC AGC-3`	

Table S2. Strategies to change specific regions or amino acids of the indicated expression vectors.

Table S3 Primer list	
Primer for ChIP	**Sequence**
Bmal1 (+50) FW	5'-CAGCGAGCCACGGTGA-3'
Bmal1 (+50) RV	5'-CCCGAGACGGCTGCT-3'
Bmal1 (+50) TM	5'-FAM-CCGCAGCCATGCCGACAC-BHQ1-3'
Bmal1 (-1,600) FW	5'-GCCAATTCACATTTCAACCA-3'
Bmal1 (-1,600) RV	5'-GACACAAGGCAGCATTTCAA-3'
Bmal1 (-1,600) TM	5'-FAM-TGCAAAGGGCTGGACATGGG-BHQ1-3'
Rev-Erbα FW	5'-TCACATGGTACCTGCTCCAG-3'
Rev-Erbα RV	5'-CTTTTGCCCGAGCCTTTC-3'
Rev-Erbα TM	5'-FAM-ACAGAGGGCTCTGCGCAGGC-BHQ1-3'
Hnf1α FW	5'-GCAAGGCTGAAGTCCAAAGT-3'
Hnf1α RV	5'-ATTGGAGCTGGGGAAATTCT-3'
Hnf1α TM	5'-FAM-CCCTTCGCTAAGCGCACGGA-BHQ1-3'
G6Pase FW	5'-TGCCCTTGCAAGAGTCATGGTTGA-3'
G6Pase RV	5'-AGTGCTAGAGGCTGCCCTCC-3'
G6Pase TM	5'-FAM-AGCCAGGCCAGAGGCCCAGGGC-BHQ1-3'
Primer for genotyping	**Sequence**
Per2 FW	5`-GCTGGTCCAGCTTCATCAACC-3`
Per2 RV (wild-type)	5`-GAACACATCCTCATTCAAAGG-3`
PKGhprt RV	5`-CGCATGCTCCAGACTGCCTTG-3
Rev-Erbα FW	5`-CCAGGAAGTCTACAAGTGGCCATGGAAGA-3`
Rev-Erbα RV (wild-type)	5`-CACCTTACACAGTAGCACCATGCCATTCA-3
lacZ RV	5`-AAACCAGGCAAAGCGCCATTCGCCATTCA-3`
Primer for real-time RT PCR	**Sequence**
Hnf1α FW	5'-GCGGGCCGCTCTGTACACC-3'
Hnf1α RV	5'-CCTGCCCTGCGTGGGTGA-3'
Hnf1α TM	5'-FAM-ACGTCCGCAAGCAGCGAGAGGTGGC-BHQ1-3'
G6Pase FW	5'-ACTGTGGGCATCAATCTCCT-3'
G6Pase RV	5'-CGTTGTCCAAACAGAATCCA-3'
G6Pase TM	5'-FAM-TGGGTGGCAGTGGTCGGAGA-BHQ1-3'
Pepck FW	5'-GAAGAAAGGTGGCACCAGAG-3'
Pepck RV	5'-GGATGACCTTGGCAGAGAAG-3'
Pepck TM	5'-FAM-TGCCTCCTCAGCTGCATAACGGT-BHQ1-3'
Alas FW	5'-GGTGGAGGGATCGGTGAT-3'
Alas RV	5'-TGGCAATGTATCCTCCAACA-3'
Alas TM	5'-FAM-CGCTTGGTAAAGCGTTCGGCTG-3'

TabḷeS3. Primers used for genotyping of mutant mouse strains and Taqman real-time PCR. FAM: 6-fluorescein; BHQ1: black hole quencher1