Supplemental Materials
Molecular Biology of the Cell

Lee et al.

SUPPLEMENTARY INFORMATION

MicroRNA-185 oscillation controls circadian amplitude of mouse CRYPTOCHROME1 via translational regulation

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Supplementary Figure Legends

Supplementary Figure S1. CRY1 expression is regulated by the microRNA machinery. (A) Renilla luciferase reporter fused to m*Cry1* 3′UTR was transfected with control siRNA (Con_si) or *Ago2* specific siRNA to NIH 3T3 cells by microporation. After a 24-h incubation, cells were subjected to luciferase assays. The activity of RLUC was normalized to that of FLUC, and the activity of Con-si was set to 1. (B) The knock-down of *Ago2* expression observed in panel A was confirmed by real-time PCR. *Ago2* or *Actb* mRNA levels were quantified, and *Ago2* mRNA levels normalized to those of *Actb* mRNA. The results are expressed as the mean ± SEM of 3 independent experiments.

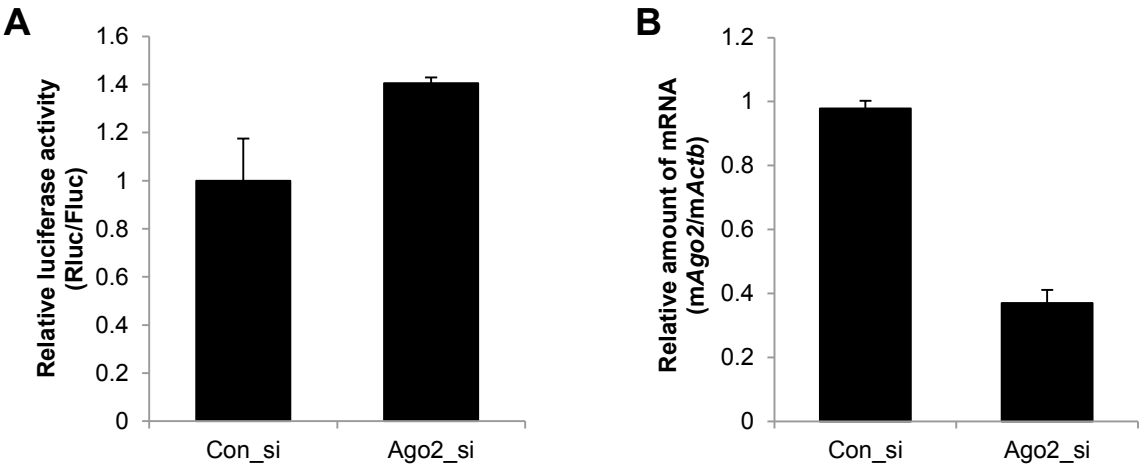
Supplementary Figure S2. miR-185 overexpression does not repress the translation of control system proteins. (A) NIH 3T3 cells were co-transfected by microporation with the RL control vector that does not contain any 3′UTR sequence and control or miR-185 expressing plasmids, and then incubated for 24 h. The luciferase activities of RLUC and FLUC were quantified, and RLUC activity normalized to that of FLUC. (B) NIH 3T3 cells were transfected with the RL control vector along with pre-miR-con or pre-miR-185. Luciferase assays were then carried out, and the ratio of RLUC over FLUC was calculated. Results shown are the mean ± SEM of 3 separate experiments.

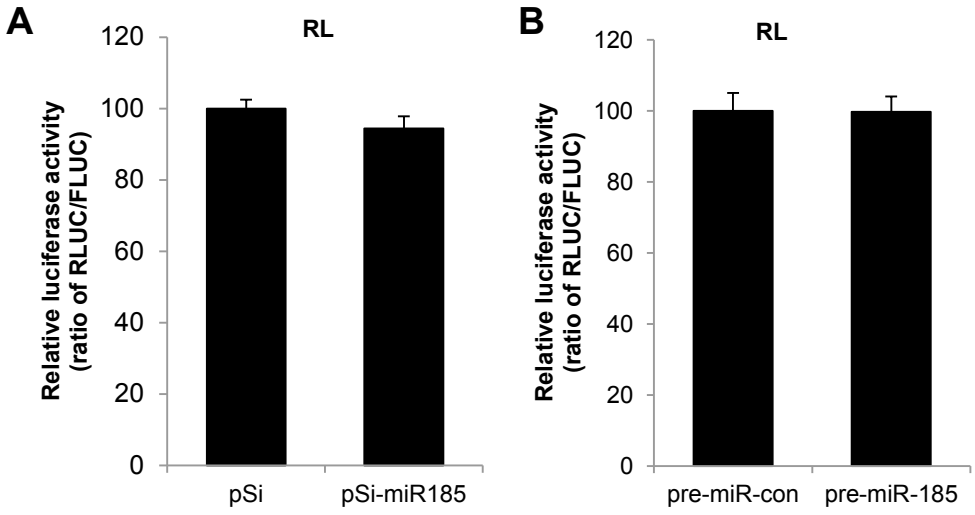
Supplementary Figure S3. Inhibition of miR-185 increase amplitude of mCRY1 expression. (A) NIH 3T3 cells were treated with water, or transfected with anti-miR-con or anti-miR-185. After 12h incubation, dexamethasone was treated and cells were harvested at every 4 h; then

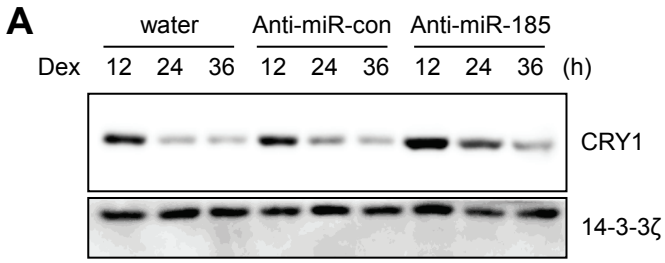
immunoblotting was performed in a single SDS-PAGE gel to check the amplitude. (B) With same extracts of panel A, immunoblotting was performed with indicated antibodies.

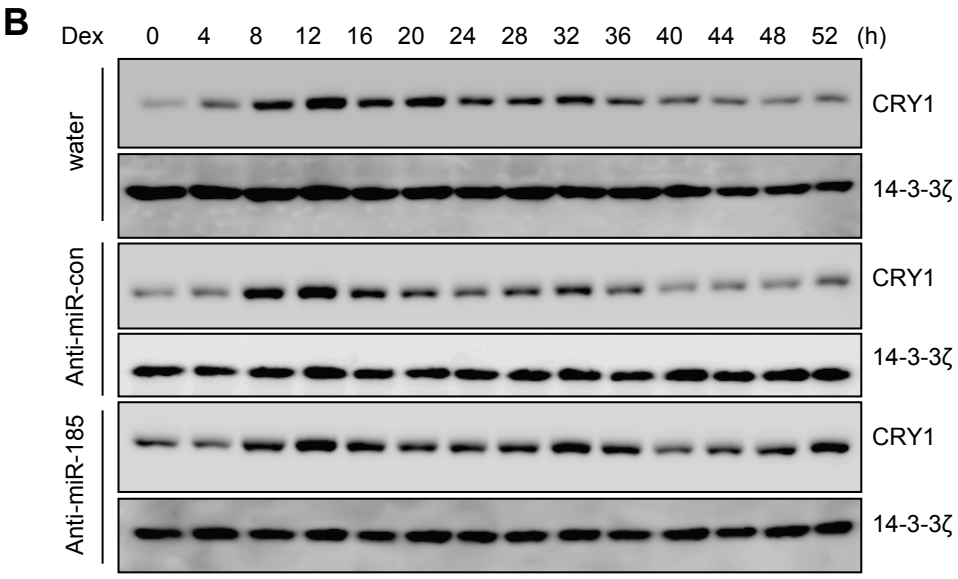
Supplementary Figure S4. miR-185 expression profile. (A) To determine the expression profile of miR-185, the synchronization of NIH 3T3 cells were confirmed by measuring m*Cry1* levels. NIH 3T3 cells were treated with 100 nM dexamethasone for 2 h before being harvested at the indicated times. Total RNA (1 μg) from each time point was subjected to real-time PCR with specific oligonucleotides for m*Cry1* and m*Tbp*, as a normalizing control. (B) miR-185 and small nucleolar RNA-202 (sno-202) levels were quantified by real-time PCR in the same extracts shown in panel A, and miR-185 levels normalized to sno-202 levels. The normalized activity of the 0 h time point was set to 1. All results shown are representative of the mean ± SEM of 3 independent experiments.

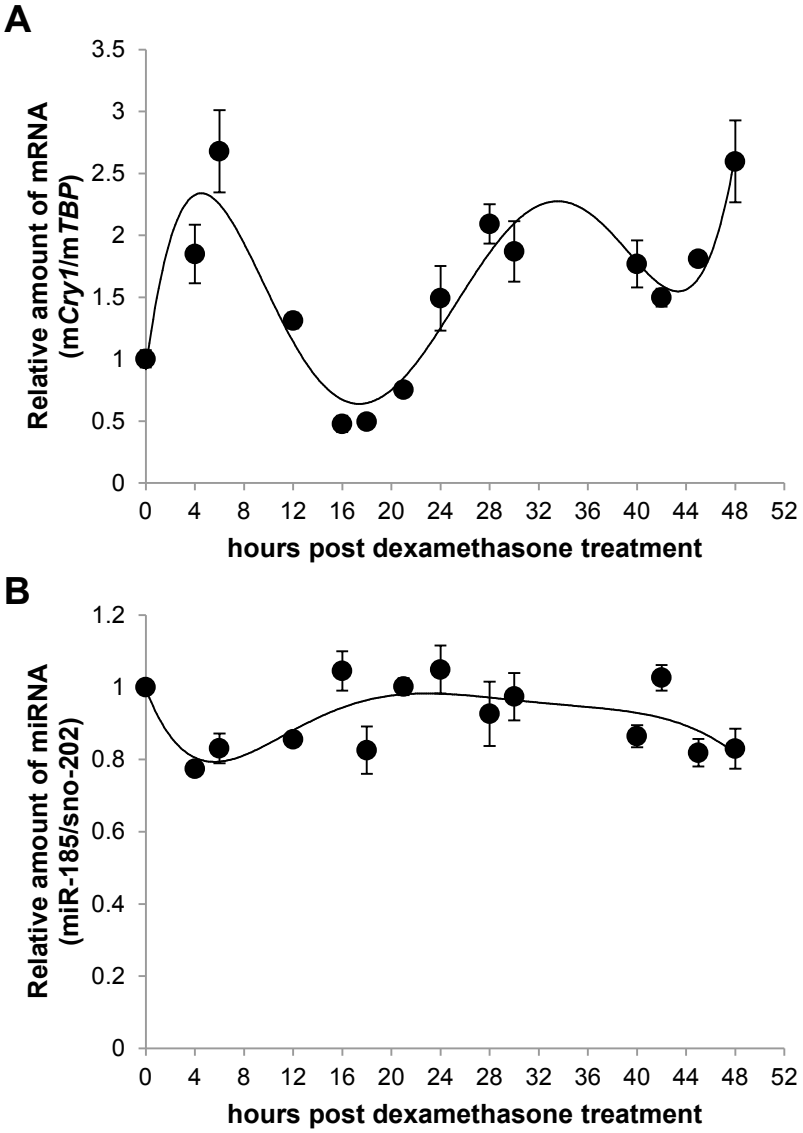
Supplementary Table S1. Sequences of real-time PCR primers


A
Relative luciferase activity (Rluc/Fluc)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Con_si
Ago2_si
B
Relative amount of mRNA (m*Ago2*/m*Actb*)
1.2
1
0.8
0.6
0.4
0.2
0
Con_si
Ago2_si


A
RL
Relative luciferase activity (ratio of RLUC/FLUC)
120
100
80
60
40
20
0
pSi
pSi-miR185
B
RL
Relative luciferase activity (ratio of RLUC/FLUC)
120
100
80
60
40
20
0
pre-miR-con
pre-miR-185


A
water
Anti-miR-con
Anti-miR-185
Dex 12 24 36 12 24 36 12 24 36 (h)
CRY1
14-3-3ζ


B
Dex 0 4 8 12 16 20 24 28 32 36 40 44 48 52 (h)
water
Anti-miR-con
Anti-miR-185
CRY1
14-3-3ζ


A
Relative amount of mRNA (m*Cry1*/m*TBP*)
3.5
3
2.5
2
1.5
1
0.5
0
0 4 8 12 16 20 24 28 32 36 40 44 48 52
hours post dexamethasone treatment
B
Relative amount of miRNA (miR-185/sno-202)
1.2
1
0.8
0.6
0.4
0.2
0
0 4 8 12 16 20 24 28 32 36 40 44 48 52
hours post dexamethasone treatment

	Sequence (5′ to 3′)
m*Cry1* (forward)	CCTTGAAAAGCCTGGGAAAT
m*Cry1* (reverse)	TCCGCTGCGTCTATATCCTC
m*Tbp* (forward)	CAGCCTTCCACCTTATGCTC
m*Tbp* (reverse)	TTGCTGCTGCTGTCTTTGTT
Rluc (forward)	GTAACGCTGCCTCCAGCTAC
Rluc (reverse)	CCAAGCGGTGAGGTACTTGT
Fluc (forward)	TTCGCTAAGAGCACCCTGAT
Fluc (reverse)	GTAATCAGAATGGCGCTGGT
m*Ago2* (forward)	AAGTCGGACAGGAGCAGAAA
m*Ago2* (reverse)	GAAACTTGCACTTCGCATCA
m*Dicer* (forward)	CTCGTCAACTCTGCAAACCA
m*Dicer* (reverse)	CAGTCAAGGCGACATAGCAA
m*Drosha* (forward)	AACAGTTCAACCCCGAAGTG
m*Drosha* (reverse)	CTCTGAGCCAGCTTCTGCTT
m*Actb* (forward)	TGTTACCAACTGGGACGACA
m*Actb* (reverse)	GGGGTGTTGAAGGTCTCAAA