SUPPLEMENTARY MATERIAL

Altered Circadian Activity and Sleep/Wake Rhythms in the Stable Tubule Only Polypeptide (STOP) Null Mouse Model of Schizophrenia

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Supplementary Figures and Tables:

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SUPPLEMENTARY FIGURE CAPTIONS

Figure S1. Examples of periodograms in the WT **(A)** and the two STOP KO **(B and C)** mice whose actograms are shown in Figure 1 for the 12/12 h light/dark (LD: *top*) and constant darkness (DD: *bottom*) conditions. The Qp values in the two KO mice **(B and C)** are plotted on a scale with smaller maximum than for the WT mouse **(A)** to facilitate visual detection of peak. The oblique line on the periodograms indicates significance at P < 0.05. The WT mouse in panel **A** maintained robust and relatively stable circadian rhythms of wheel-running activity under DD (see Figure 1A), as confirmed by a single, strong peak in its periodogram. The KO mouse in panel **B** maintained relatively clear, stable rhythms under DD (see Figure 1B), as confirmed by a single, smaller peak in its periodogram. The KO mouse in panel **C** showed less obvious rhythms in DD (see Figure 1C), and its periodogram showed a relatively flat spectrum with only a very weak peak in the circadian range.

Figure S2. Numbers and mean durations (seconds) of episodes during the 12 h light and 12 h dark phases in light/dark (LD) conditions and during the subjective day and night in constant darkness (DD) conditions for wake **(A and B)**, NREMS **(C and D)**, and REMS **(E and F**) in WT (white bars) and KO (black bars) mice. Data (means + SEM) were based on 2 successive 24 h periods in LD and 5 successive circadian cycles in DD and averaged for 12 h in LD or half a circadian cycle in DD in each animal. WT, n = 12; KO, n = 12. *P < 0.05 vs WT (two-tailed, unpaired t-tests).

Figure S3. Frequency histograms of the durations of sleep/wake episodes during the 12 h light and 12 h dark phases in light/dark (LD) conditions and during the subjective day and night in constant darkness (DD) conditions in WT (white bars) and KO (black bars) mice. Frequency histograms of wake **(A)**, NREMS **(B)**, and REMS **(C)** episodes are shown as a function of episode duration. All episodes of wake, NREMS, and REMS were sorted into 9 consecutive bins of logarithmically increasing duration. Data (means + SEM) were based on 2 successive 24 h periods in LD and 5 successive circadian cycles in DD and averaged for 12 h in LD or one-half of a circadian cycle in DD in each animal. WT, n = 12. KO, n = 12. *P < 0.05 vs WT (Bonferroni *post hoc* t-tests).

Figure S4. The cosinor amplitudes of daily rhythms for wake **(A)**, NREMS **(B)**, and REMS amounts **(C)**, and NREMS EEG delta power **(D)** averaged over the 2 days of LD and the 5 days of DD in WT (white bars) and KO (black bars) mice. EEG power was normalized to the 48-h average in LD. Data are shown as means + SEM. WT, n = 12 (except n = 11 in D). KO, n = 12 (except n = 11 in D). *P < 0.05 vs WT (two-tailed, unpaired t-tests).

Figure S1

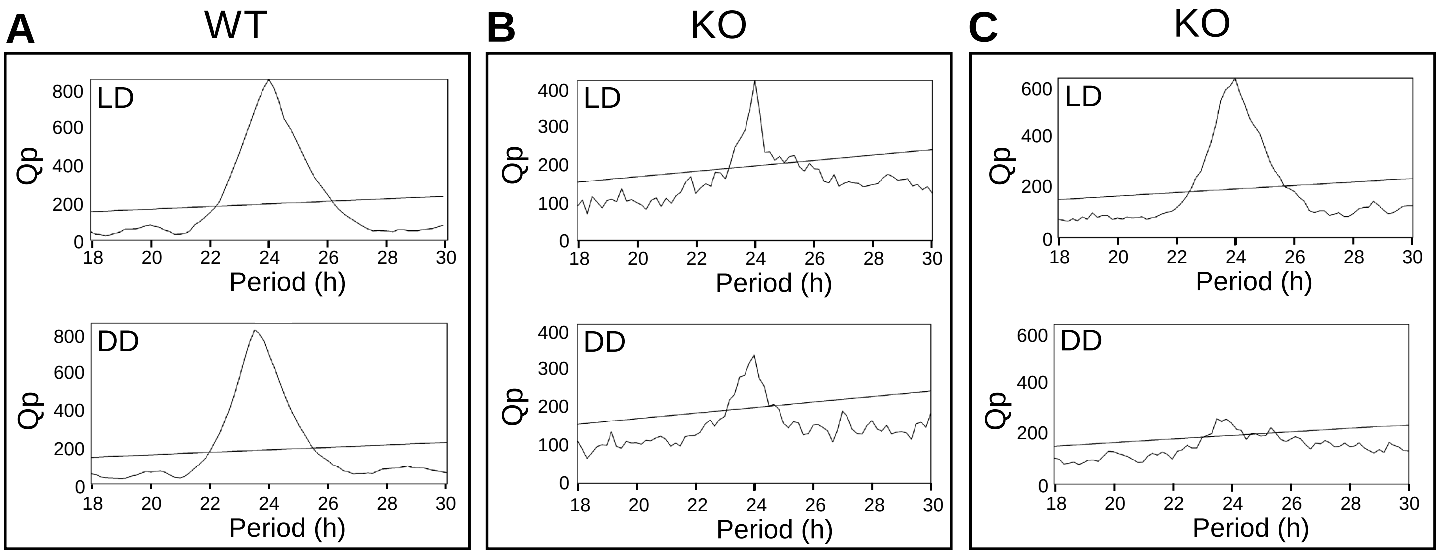


Figure S2

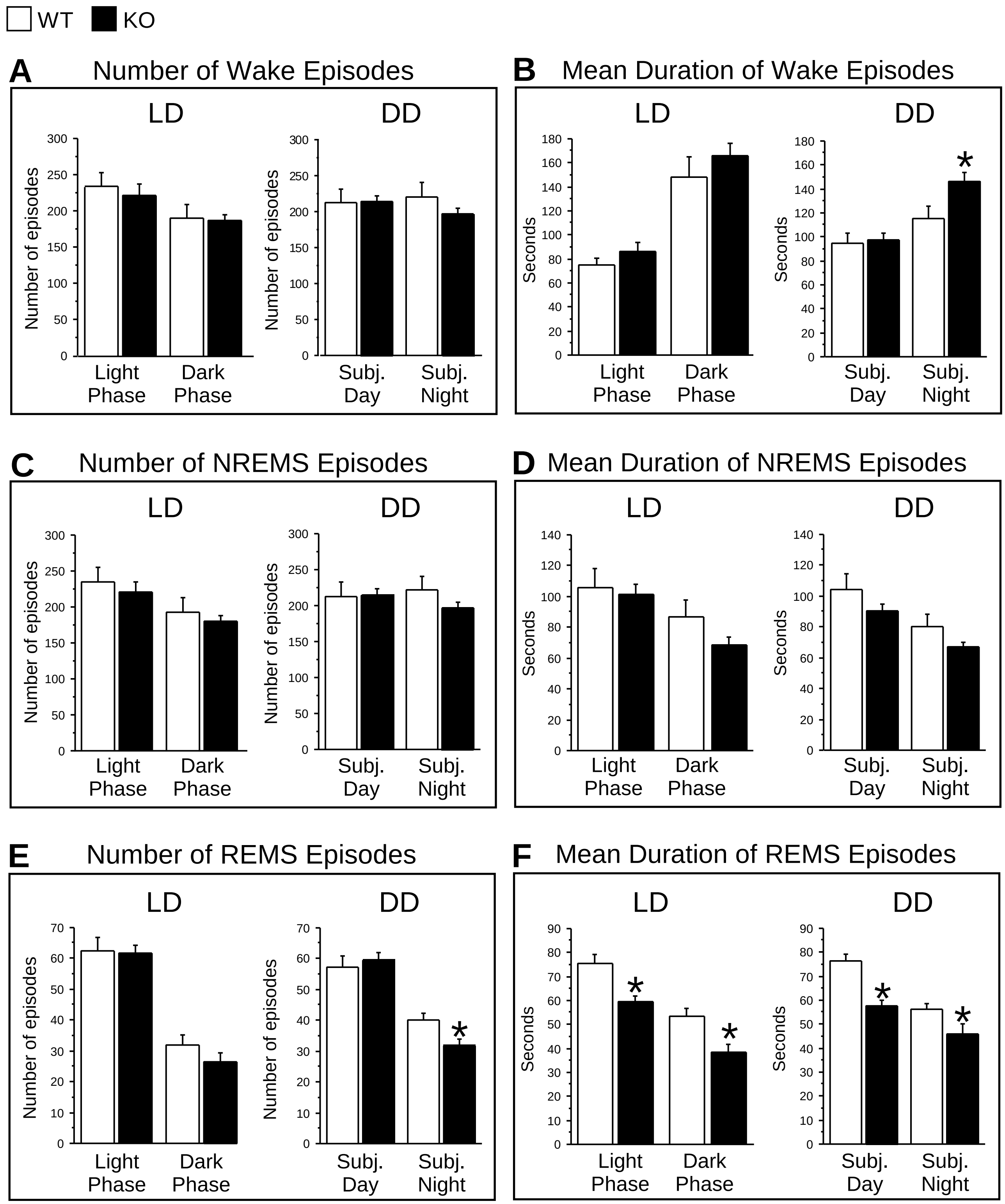
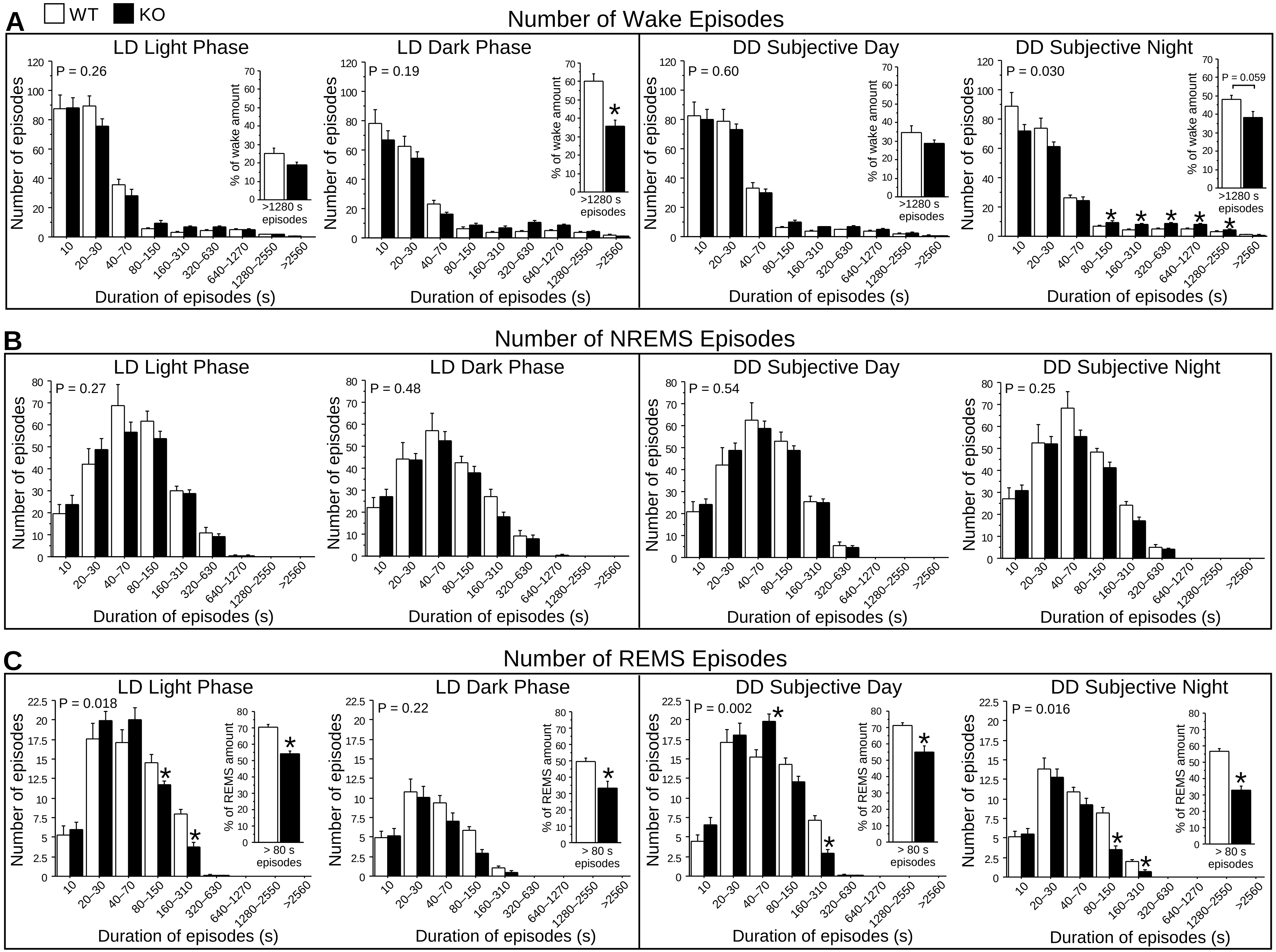

WT
KO
A
Number of Wake Episodes
LD
DD
Number of episodes
Light Phase
Dark Phase
Subj. Day
Subj. Night
B
Mean Duration of Wake Episodes
Seconds
*
C
Number of NREMS Episodes
D
Mean Duration of NREMS Episodes
E
Number of REMS Episodes
F
Mean Duration of REMS Episodes

Figure S3



WT
KO
A
Number of Wake Episodes
LD Light Phase
LD Dark Phase
DD Subjective Day
DD Subjective Night
P = 0.26
P = 0.19
P = 0.60
P = 0.030
P = 0.059
Number of episodes
% of wake amount
>1280 s episodes
Duration of episodes (s)
10
20–30
40–70
80–150
160–310
320–630
640–1270
1280–2550
>2560
B
Number of NREMS Episodes
LD Light Phase
LD Dark Phase
DD Subjective Day
DD Subjective Night
P = 0.27
P = 0.48
P = 0.54
P = 0.25
Number of episodes
Duration of episodes (s)
C
Number of REMS Episodes
LD Light Phase
LD Dark Phase
DD Subjective Day
DD Subjective Night
P = 0.018
P = 0.22
P = 0.002
P = 0.016
Number of episodes
% of REMS amount
> 80 s episodes
Duration of episodes (s)

Figure S4

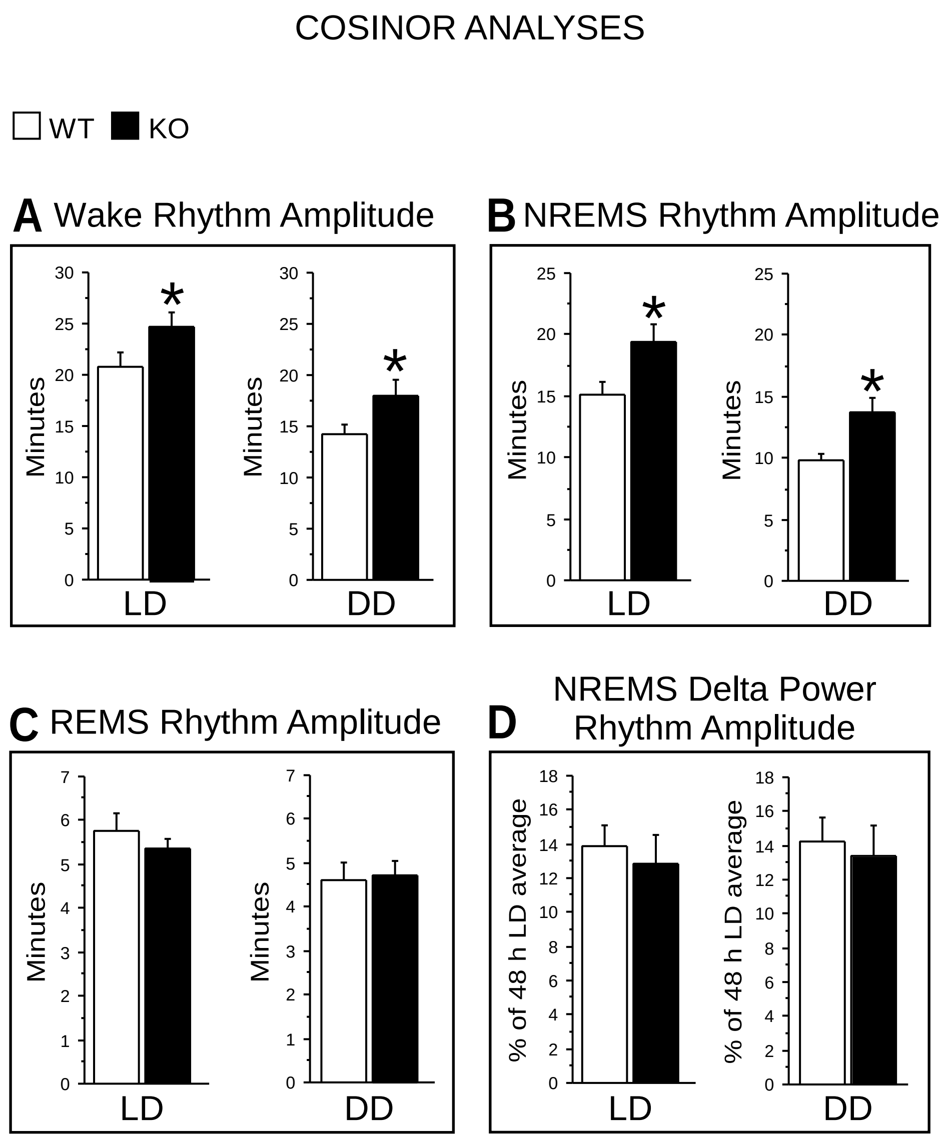

COSINOR ANALYSES
WT
KO
A Wake Rhythm Amplitude
B NREMS Rhythm Amplitude
C REMS Rhythm Amplitude
D NREMS Delta Power Rhythm Amplitude
Minutes
% of 48 h LD average
LD
DD
*

Table S1. Statistics for the data presented in Figures 2−7 and Figures S2−4

Statistical tests used for each figure with numbers of animals (n;n, KO;WT). df, degree of freedom. All t-tests are two-tailed. rANOVA, repeated ANOVA.

Fig. #	Panel	Test used	n	t/F value & df	P value
2	**A** (LD)	unpaired t-tests	14;13	$t_{25} = 1.69$	P = 0.12
2	**A** (DD)	unpaired t-tests	13;12	$t_{23} = 2.75$	**P = 0.011**
2	**B**	unpaired t-tests	13;12	$t_{23} = 1.36$	P = 0.19
2	**C** (LD)	unpaired t-tests	14;13	$t_{25} =14.18$	**P < 0.0001**
2	**C** (DD)	unpaired t-tests	13;12	$t_{23} = 12.12$	**P < 0.0001**
2	**D** (LD)	unpaired t-tests	14;13	$t_{25} = 8.89$	**P < 0.0001**
2	**D** (DD)	unpaired t-tests	13;12	$t_{23} = 17.26$	**P < 0.0001**
2	**E**	unpaired t-tests	14;13	$t_{25} = 2.90$	**P = 0.077**
2	**F**	unpaired t-tests	11;8	$t_{17} < 1$	P = 0.91
3	**A** (LD)	unpaired t-tests	14;13	$t_{25} = 10.33$	**P < 0.0001**
3	**A** (DD)	unpaired t-tests	13;12	$t_{23} = 9.37$	**P < 0.0001**
3	**B** (LD)	unpaired t-tests	14;13	$t_{25} = 4.65$	**P < 0.0001**
3	**B** (DD)	unpaired t-tests	13;12	$t_{23} = 4.72$	**P < 0.0001**
3	**C** (LD) *left* *right*	unpaired t-tests 2-way rANOVA	14;13	$t_{25} = 3.26$ Genotype x Bin: $F_{5,125} = 7.20$	**P = 0.0032** **P < 0.0001**
3	**C** (DD) *left* *right*	unpaired t-tests 2-way rANOVA	13;12	$t_{23} = 4.02$ Genotype x Bin: $F_{5,115} = 7.11$	**P = 0.0022** **P < 0.0001**
3	**D** (LD)	unpaired t-tests	14;13	$t_{25} = 8.47$	**P < 0.0001**
3	**D** (DD)	unpaired t-tests	13;12	$t_{23} = 6.78$	**P < 0.0001**
4	**A** (LD)	2-way rANOVA	12;12	Genotype: $F_{1,22} = 14.27$ Time: $F_{23,506} = 36.61$ Genotype x Time: $F_{23,506} = 1.30$	**P = 0.001** **P < 0.0001** P = 0.16
4	**A** (DD)	2-way rANOVA	12;12	Genotype: $F_{1,22} = 29.69$ Time: $F_{59,1298} = 15.48$ Genotype x Time: $F_{59,1298} = 1.43$	**P < 0.0001** **P < 0.0001** **P = 0.020**
4	**B** (LD)	2-way rANOVA	12;12	Genotype: $F_{1,22} = 6.05$ Time: $F_{23,506} = 28.78$ Genotype x Time: $F_{23,506} = 1.17$	**P = 0.022** **P < 0.0001** P = 0.26
4	**B** (DD)	2-way rANOVA	12;12	Genotype: $F_{1,22} = 13.63$ Time: $F_{59,1298} = 12.04$ Genotype x Time: $F_{59,1298} = 1.68$	**P = 0.001** **P < 0.0001** **P = 0.001**
4	**C** (LD)	2-way rANOVA	12;12	Genotype: $F_{1,22} = 25.81$ Time: $F_{23,460} = 42.66$ Genotype x Time: $F_{23,460} = 1.89$	**P < 0.0001** **P < 0.0001** **P = 0.008**
4	**C** (DD)	2-way rANOVA	12;12	Genotype: $F_{1,22} = 41.58$	**P < 0.0001**

				Time: $F_{59,1298} = 19.07$ Genotype x Time: $F_{59,1298} < 1$	**$P < 0.0001$** $P = 0.89$
4	**D** (LD)	2-way rANOVA	11;11	Genotype: $F_{1,20} < 1$ Time: $F_{23,460} = 20.01$ Genotype x Time: $F_{23,460} = 1.20$	$P = 0.86$ **$P < 0.0001$** $P = 0.24$
4	**D** (DD)	2-way rANOVA	11:11	Genotype: $F_{1,20} < 1$ Time: $F_{59,1180} = 12.44$ Genotype x Time: $F_{59,1180} < 1$	$P = 0.57$ **$P < 0.0001$** $P = 0.88$
5	**A** (LD)	unpaired t-tests	12;12	Total: $t_{22} = 3.77$ Light: $t_{22} = 2.15$ Dark: $t_{22} = 3.12$	**$P = 0.001$** **$P = 0.043$** **$P = 0.0049$**
5	**A** (DD)	unpaired t-tests	12;12	Total: $t_{22} = 2.21$ Subj. Day: $t_{22} =2.86$ Subj. Night: $t_{22} = 5.52$	**$P = 0.037$** **$P = 0.009$** **$P <0.0001$**
5	**B** (LD)	unpaired t-tests	12;12	Total: $t_{22} = 2.45$ Light: $t_{22} < 1$ Dark: $t_{22} = 2.73$	**$P = 0.023$** $P = 0.36$ **$P = 0.012$**
5	**B** (DD)	unpaired t-tests	12;12	Total:$t_{22} = 4.11$ Subj. Day: $t_{22} = 1.94$ Subj. Night: $t_{22} = 5.02$	**$P = 0.0005$** $P = 0.065$ **$P < 0.0001$**
5	**C** (LD)	unpaired t-tests	12;12	Total: $t_{22} = 5.10$ Light: $t_{22} = 4.41$ Dark: $t_{22} = 3.34$	**$P < 0.0001$** **$P = 0.0002$** **$P = 0.003$**
5	**C** (DD)	unpaired t-tests	12;12	Total: $t_{22} = 6.95$ Subj. Day: $t_{22} = 4.15$ Subj. Night: $t_{22} = 5.61$	**$P < 0.0001$** **$P = 0.0004$** **$P < 0.0001$**
6	**A**	unpaired t-tests	12;12	$t_{22} = 5.20$	**$P < 0.0001$**
6	**B** (LD)	unpaired t-tests	12;12	$t_{22} = 3.05$	**$P = 0.0058$**
6	**B** (DD)	unpaired t-tests	12;12	$t_{22} = 3.74$	**$P = 0.0011$**
6	**C** (LD)	unpaired t-tests	12;12	$t_{22} = 3.23$	**$P = 0.0039$**
6	**C** (DD)	unpaired t-tests	12;12	$t_{22} = 3.80$	**$P = 0.0010$**
6	**D** (LD)	unpaired t-tests	12;12	$t_{22} < 1$	$P = 0.70$
6	**D** (DD)	unpaired t-tests	12;12	$t_{22} < 1$	$P = 0.40$
6	**E** (LD)	unpaired t-tests	11:11	$t_{20} < 1$	$P = 0.50$
6	**E** (DD)	unpaired t-tests	11;11	$t_{20} < 1$	$P = 0.96$
7	**A** (Light)	2-way rANOVA	11;11	Genotype: $F_{1,20} < 1$ Bin: $F_{98,1960} = 201.4$ Genotype x Bin: $F_{98,1960} < 1$	$P = 0.44$ **$P < 0.0001$** $P = 0.71$
7	**A** (Dark)	2-way rANOVA	11;11	Genotype: $F_{1,20} < 1$ Bin: $F_{98,1960} = 197.0$ Genotype x Bin: $F_{98,1960} < 1$	$P = 0.40$ **$P < 0.0001$** $P = 0.73$
7	**A** (Subj. Day)	2-way rANOVA	11;11	Genotype: $F_{1,20} = 7.24$ Bin: $F_{98,1960} = 256.0$ Genotype x Bin: $F_{98,1960} = 3.17$	**$P = 0.014$** **$P < 0.0001$** **$P = 0.027$**
7	**A** (Subj. Night)	2-way rANOVA	11;11	Genotype: $F_{1,20} = 12.90$ Bin: $F_{98,1960} = 306.6$ Genotype x Bin: $F_{98,1960} = 4.03$	**$P = 0.002$** **$P < 0.0001$** **$P = 0.005$**

7	**B** (Light)	2-way rANOVA	11;11	Genotype: $F_{1,20} = 2.72$ Bin: $F_{98,1960} = 303.3$ Genotype x Bin: $F_{98,1960} = 2.03$	$P = 0.11$ **$P < 0.0001$** $P = 0.13$
7	**B** (Dark)	2-way rANOVA	11;11	Genotype: $F_{1,20} < 1$ Bin: $F_{98,1960} = 184.9$ Genotype x Bin: $F_{98,1960} = 1.24$	$P = 0.34$ **$P < 0.0001$** $P = 0.30$
7	**B** (Subj. Day)	2-way rANOVA	11;11	Genotype: $F_{1,20} = 3.66$ Bin: $F_{98,1960} = 243.2$ Genotype x Bin: $F_{98,1960} = 1.09$	$P = 0.070$ **$P < 0.0001$** $P = 0.35$
7	**B** (Subj. Night)	2-way rANOVA	11;11	Genotype: $F_{1,20} = 4.99$ Bin: $F_{98,1960} = 175.4$ Genotype x Bin: $F_{98,1960} = 1.54$	**$P = 0.037$** **$P < 0.0001$** $P = 0.23$
7	**C** (Light)	2-way rANOVA	11;11	Genotype: $F_{1,20} < 1$ Bin: $F_{98,1960} = 121.8$ Genotype x Bin: $F_{98,1960} < 1$	$P = 0.34$ **$P < 0.0001$** $P = 0.46$
7	**C** (Dark)	2-way rANOVA	11;11	Genotype: $F_{1,20} < 1$ Bin: $F_{98,1960} = 107.0$ Genotype x Bin: $F_{98,1960} = 1.28$	$P = 0.55$ **$P < 0.0001$** $P = 0.29$
7	**C** (Subj. Day)	2-way rANOVA	11;11	Genotype: $F_{1,20} = 15.4$ Bin: $F_{98,1960} = 172.7$ Genotype x Bin: $F_{98,1960} = 3.61$	**$P = 0.001$** **$P < 0.0001$** **$P = 0.024$**
7	**C** (Subj. Night)	2-way rANOVA	11;11	Genotype: $F_{1,20} = 15.3$ Bin: $F_{98,1960} = 149.2$ Genotype x Bin: $F_{98,1960} = 4.50$	**$P = 0.001$** **$P < 0.0001$** **$P = 0.006$**
8	**A** (LD)	unpaired t-tests	11;11	Light: $t_{20} = 2.12$ Dark: $t_{20} = 2.37$	**$P = 0.047$** **$P = 0.028$**
8	**A** (DD)	unpaired t-tests	11;11	Subj. Day: $t_{20} = 1.66$ Subj. Night: $t_{20} = 2.18$	$P = 0.11$ **$P = 0.041$**
8	**B** (LD)	unpaired t-tests	11;11	Light: $t_{20} < 1$ Dark: $t_{20} < 1$	$P = 0.94$ $P = 0.90$
8	**B** (DD)	unpaired t-tests	11;11	Subj. Day: $t_{20} < 1$ Subj. Night: $t_{20} < 1$	$P = 0.64$ $P = 0.96$
8	**C** (LD)	unpaired t-tests	11;11	Light: $t_{20} = 1.98$ Dark: $t_{20} < 1$	$P = 0.062$ $P = 0.54$
8	**C** (DD)	unpaired t-tests	11;11	Subj. Day: $t_{20} = 2.38$ Subj. Night: $t_{20} < 1$	**$P = 0.027$** $P = 0.35$
S2	**A** (LD)	unpaired t-tests	12;12	Light: $t_{22} < 1$ Dark: $t_{22} < 1$	$P = 0.63$ $P = 0.84$
S2	**A** (DD)	unpaired t-tests	12;12	Subj. Day: $t_{22} < 1$ Subj. Night: $t_{22} = 1.19$	$P = 0.95$ $P = 0.25$
S2	**B** (LD)	unpaired t-tests	12;12	Light: $t_{22} < 1$ Dark: $t_{22} = 1.28$	$P = 0.91$ $P = 0.23$
S2	**B** (DD)	unpaired t-tests	12;12	Subj. Day: $t_{22} < 1$ Subj. Night: $t_{22} = 2.45$	$P = 0.78$ **$P = 0.023$**
S2	**C** (LD)	unpaired t-tests	12;12	Light: $t_{22} < 1$ Dark: $t_{22} < 1$	$P = 0.55$ $P = 0.57$
S2	**C** (DD)	unpaired t-tests	12;12	Subj. Day: $t_{22} < 1$ Subj. Night: $t_{22} = 1.16$	$P = 0.93$ $P = 0.26$

S2	**D** (LD)	unpaired t-tests	12;12	Light: $t_{22} < 1$ Dark: $t_{22} = 1.53$	P = 0.79 P = 0.14
S2	**D** (DD)	unpaired t-tests	12;12	Subj. Day: $t_{22} = 1.24$ Subj. Night: $t_{22} = 1.61$	P = 0.23 P = 0.12
S2	**E** (LD)	unpaired t-tests	12;12	Light: $t_{22} < 1$ Dark: $t_{22} = 1.23$	P = 0.90 P = 0.23
S2	**E** (DD)	unpaired t-tests	12;12	Subj. Day: $t_{22} < 1$ Subj. Night: $t_{22} = 2.84$	P = 0.64 **P = 0.010**
S2	**F** (LD)	unpaired t-tests	12;12	Light: $t_{22} = 3.79$ Dark: $t_{22} = 3.36$	**P = 0.0010** **P = 0.0028**
S2	**F** (DD)	unpaired t-tests	12;12	Subj. Day: $t_{22} = 4.92$ Subj. Night: $t_{22} = 2.12$	**P < 0.0001** **P = 0.045**
S3	**A** (Light) (*insert*)	2-way rANOVA unpaired t-tests	12;12	Genotype x Bin: $F_{8,176} = 1.38$ $t_{22} = 1.39$	P = 0.26 P = 0.18
S3	**A** (Dark) (*insert*)	2-way rANOVA unpaired t-tests	12;12	Genotype x Bin: $F_{8,176} = 1.77$ $t_{22} = 3.70$	P = 0.19 **P = 0.0012**
S3	**A** (Subj. Day) (*insert*)	2-way rAnova unpaired t-tests	12;12	Genotype x Bin: $F_{8,176} < 1$ $t_{22} = 1.31$	P = 0.60 P = 0.20
S3	**A** (Subj. Night) (*insert*)	2-way rANOVA unpaired t-tests	12;12	Genotype x Bin: $F_{8,176} = 2.20$ $t_{22} = 1.99$	**P = 0.030** P = 0.059
S3	**B** (Light)	2-way rANOVA	12;12	Genotype x Bin: $F_{8,176} = 1.35$	P = 0.27
S3	**B** (Dark)	2-way rANOVA	12;12	Genotype x Bin: $F_{8,176} < 1$	P = 0.48
S3	**B** (Subj. Day)	2-way rANOVA	12;12	Genotype x Bin: $F_{8,176} < 1$	P = 0.54
S3	**B** (Subj. Night)	2-way rANOVA	12;12	Genotype x Bin: $F_{8,176} = 1.43$	P = 0.25
S3	**C** (Light) (*insert*)	2-way rANOVA unpaired t-tests	12;12	Genotype x Bin: $F_{8,176} = 3.41$ $t_{22} = 5.90$	**P = 0.018** **P < 0.0001**
S3	**C** (Dark) (*insert*)	2-way rANOVA unpaired t-tests	12;12	Genotype x Bin: $F_{8,176} = 1.53$ $t_{22} = 3.46$	P = 0.22 **P = 0.022**
S3	**C** (Subj. Day) (*insert*)	2-way rANOVA unpaired t-tests	12;12	Genotype x Bin: $F_{8,176} = 5.79$ $t_{22} = 4.04$	**P = 0.002** **P = 0.0005**
S3	**C** (Subj. Night) (*insert*)	2-way rANOVA unpaired t-tests	12;12	Genotype x Bin: $F_{8,176} = 4.13$ $t_{22} = 7.55$	**P = 0.016** **P < 0.0001**
S4	**A** (LD)	unpaired t-tests	12;12	$t_{22} = 2.08$	**P = 0.049**
S4	**A** (DD)	unpaired t-tests	12;12	$t_{22} = 2.08$	**P = 0.049**
S4	**B** (LD)	unpaired t-tests	12;12	$t_{22} = 2.39$	**P = 0.026**
S4	**B** (DD)	unpaired t-tests	12;12	$t_{22} = 2.86$	**P = 0.0091**
S4	**C** (LD)	unpaired t-tests	12;12	$t_{22} < 1$	P = 0.36
S4	**C** (DD)	unpaired t-tests	12;12	$t_{22} < 1$	P = 0.87
S4	**D** (LD)	unpaired t-tests	11;11	$t_{20} < 1$	P = 0.71
S4	**D** (DD)	unpaired t-tests	11;11	$t_{20} < 1$	P = 0.65

Table S2. Experiment 1: Body weights in the WT and STOP KO groups

Genotype	Body weight (g)			
	Day 1	**Day 14**	**Day 29**	**Day 33**
WT	30.8 ± 1.4	29.9 ± 1.1	30.5 ± 1.2	30.3 ± 1.2
KO	25.9 ± 1.2	25.5 ± 0.7	26.3 ± 0.6	26.7 ± 0.7

Means ± SEM are shown. The body weight of each mouse was recorded on the first day of the experiment (Day 1 in LD), at two time points during the experiment (Days 14 and 29 in LD and DD, respectively), and on the day following the end of the experiment (Day 33). KO mice had lower average body weights (by 15%) at the start of the experiment compared to WT mice. During the course of the experiment, despite the differences in the amount of wheel-running activity, both genotypes maintained respective pre-protocol weight levels similarly. WT, n = 13; KO, n = 12.

Repeated ANOVA (Factor Genotype: $F_{1,23} = 9.14$, $P = 0.0060$; Factor Day: $F_{3,69} = 1.08$, $P = 0.36$; Genotype x Day: $F_{3,69} < 1$, $P = 0.61$)

Table S3. Experiment 2: Body weights in the WT and STOP KO groups

Genotype	Body weight (g)	
	Initial	Final
WT	39.9 ± 1.3	39.1 ± 1.0
KO	28.9 ± 0.9	28.4 ± 0.9

Means ± SEM (n = 12/group) are shown. The body weight of each mouse was recorded on the first day of the habituation period ('Initial') and again 14−18 days later on the day following the end of the experiment ('Final'). KO mice had lower average body weights than WT mice at both the beginning and end of the experiment, as in Experiment 1. There was a small but significant decrease (by ~2%) from the pre-protocol values in body weights in both genotypes. Repeated ANOVA (Factor Genotype: $F_{1,22} = 10.69$, $P = 0.0035$; Factor Day: $F_{1,22} = 5.76$, $P = 0.025$; Genotype x Day: $F_{1,22} < 1$, $P = 0.61$)