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Figures and figure supplements

Lhx1 maintains synchrony among circadian oscillator neurons of the SCN

Megumi Hatori, et al.

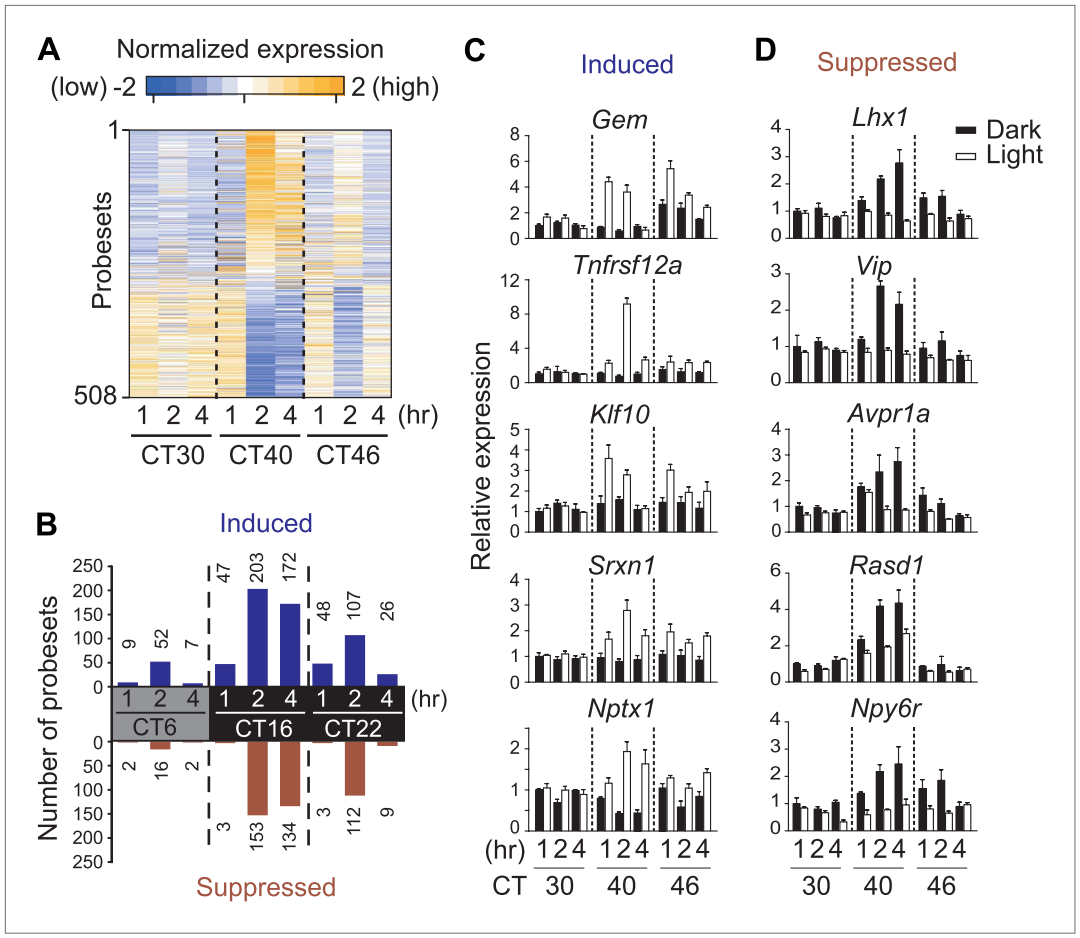

Figure 1. Light-regulated transcripts of the SCN. (**A**) Heatmap rendering of light-regulated SCN transcripts. For each time point, fold change between respective light treated and dark control was plotted. (**B**) Circadian gating of light-modulated transcripts. Cutoffs of two fold were set for up-regulation (blue) or suppression (red) after light pulse, and the number of probesets that satisfy each cutoff was plotted for each point. Quantitative RT-PCR (qRT-PCR) expression confirmation of genes detected as light-regulated by microarray. Examples of genes (**C**) induced or (**D**) repressed by light pulses at three different time points. (Mean +s.e.m., n = 4).

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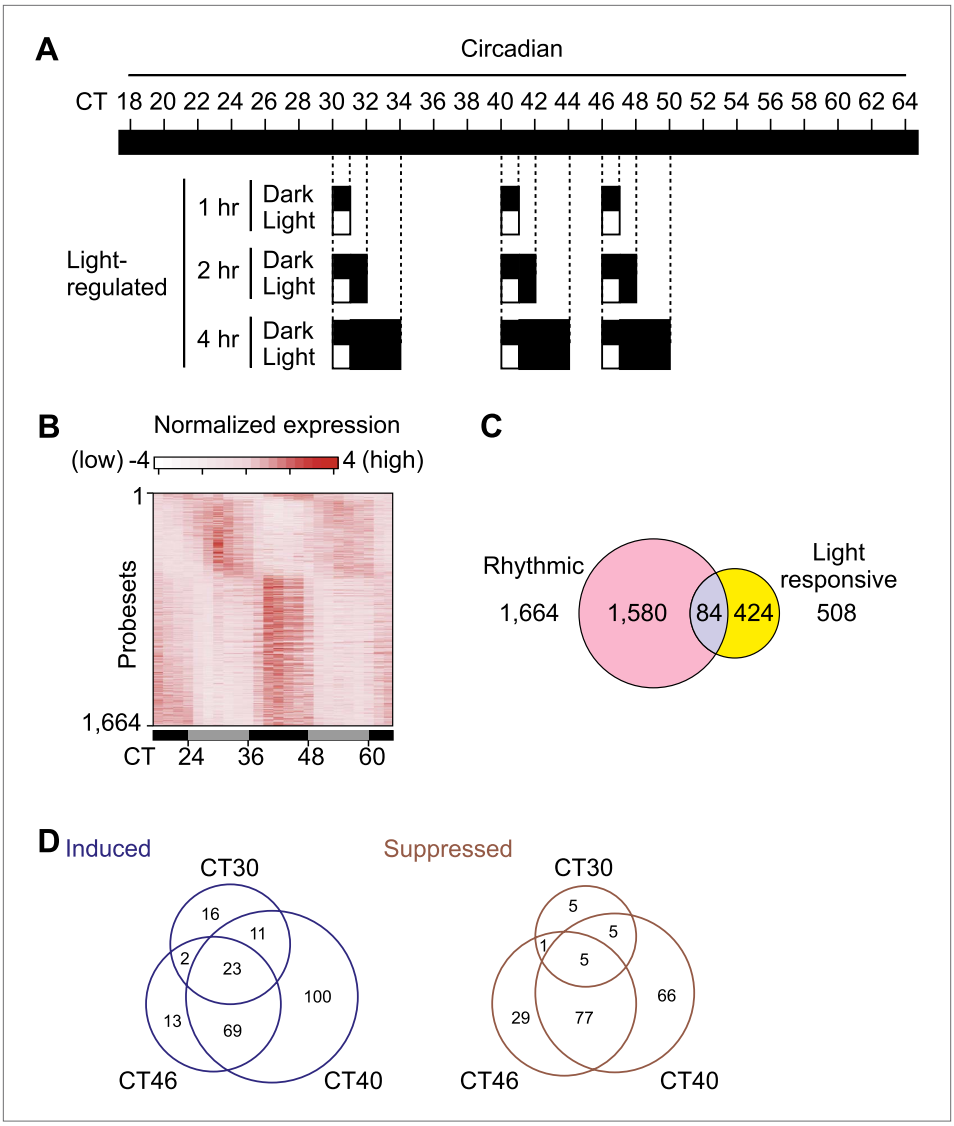

Figure 1—figure supplement 1. Transcriptional profiling of the mouse SCN. (**A**) Sampling schedule for the collection of SCN. C57BL/6J male mice were entrained to 12 hr light:12 hr darkness for 2–3 weeks and transferred to constant darkness. From Circadian Time (CT) 18, 30 hr after lights off, four mice at each time point were collected every 2 hr in dark over two complete days till CT64. From CT30, CT40, or CT46, one group of mice was exposed to 1 hr light, while the control group was maintained in dark, then both groups stayed in dark after 1 hr. SCNs were collected 1, 2, or 4 hr after the beginning of 1 hr light pulse. (**B**) Heatmap rendering of circadianly expressed transcripts in the mouse SCN. Each horizontal line represents one probeset from MOE430 high density array. (**C**) Venn diagram for the overlap of light-regulated and cycling transcripts in the SCN. Numbers shown are for probesets. (**D**) Venn diagram of light induced and suppressed transcripts showing that the light pulse at CT16 that causes maximal phase shift also affects the expression of a large number of SCN transcripts.

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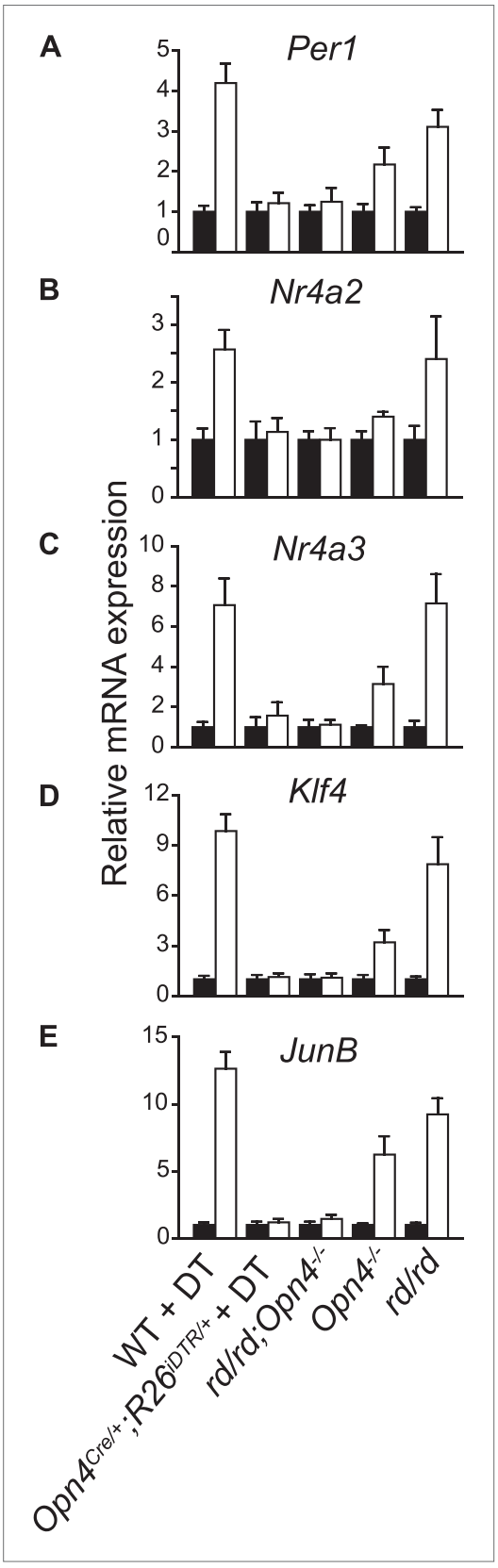

Figure 1—figure supplement 2. Light-induced changes in SCN gene expression correlate with the

Figure 1—figure supplement 2. Continued on next page

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known effect of light on phase shift in different genetic models of light signaling. qRT-PCR quantification of (**A**) *Per1*, (**B**) *Nr4a2*, (**C**) *Nr4a3*, (**D**) *Klf4*, and (**E**) *JunB* mRNA in the SCN of dark reared or 2 hr after a 1-hr light pulse delivered at CT16 are shown. (Mean + s.e.m., n = 4). The adult *rd* mice show outer retina degeneration, yet light resets their circadian clock as effectively as of the WT mice (***Foster et al., 1991***). *Opn4*$^{-/-}$ mice lack melanopsin and their circadian clock shows an attenuated light-induced phase shift (Panda et al., 2002; ***Ruby et al., 2002***). *Opn4*$^{-/-}$*;rd* mice lack rod, cone, and melanopsin photopigments and show no response to light (***Panda et al. 2003***). Opn4$^{Cre/+}$;R26$^{iDTR/+}$ mice treated with DT specifically lose melanopsin-expressing retinal ganglion cells and show no phase shifting effect of light (***Hatori et al., 2008***). All mice were dark reared for at least 7 days and their activity onset was used to calculate CT16.

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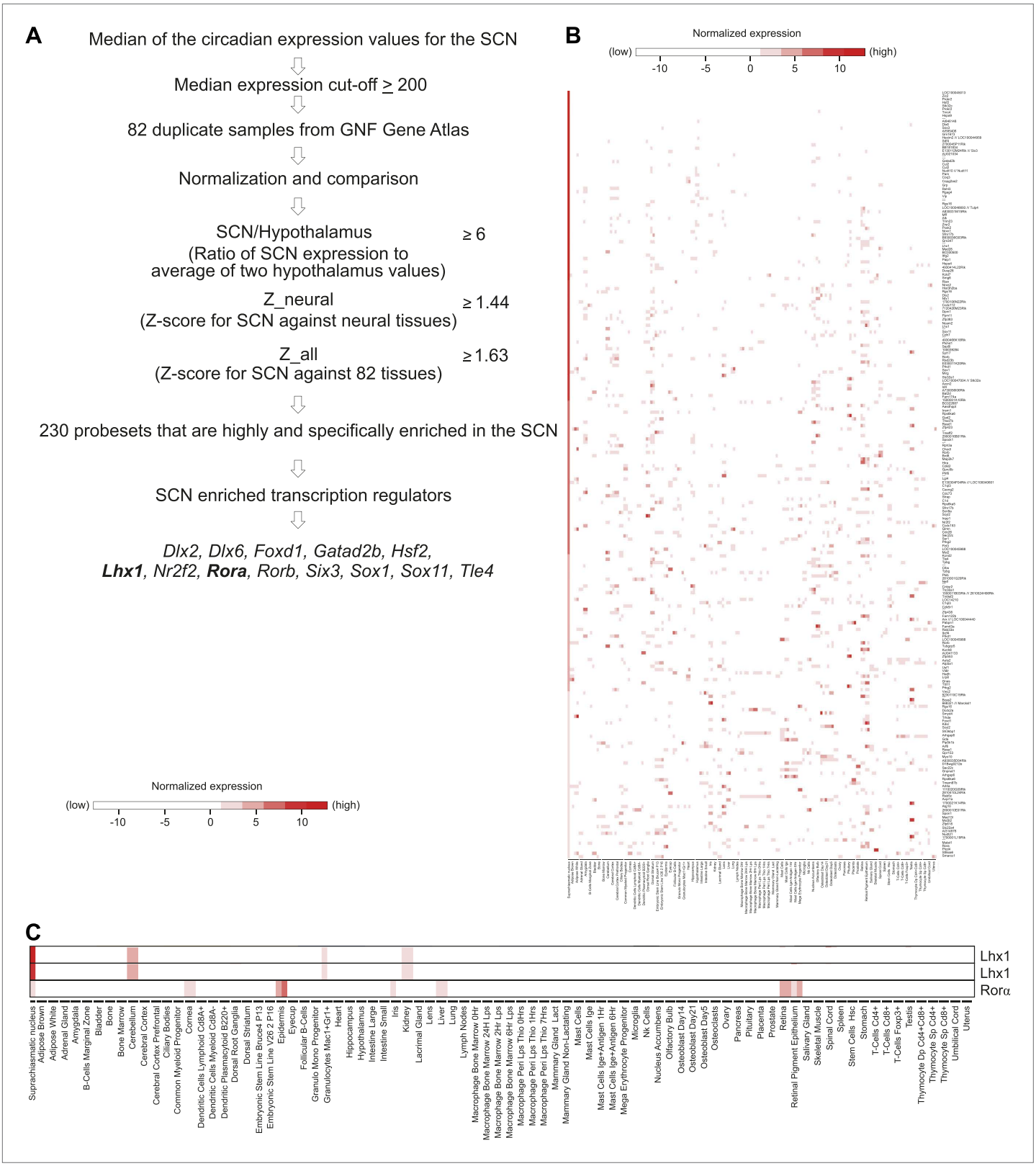

Figure 1—figure supplement 3. SCN enriched (not SCN-exclusive) transcripts. (**A**) Criteria to find SCN-enriched genes among 83 mouse tissues revealed 230 probesets among which 13 were transcription factors. (**B**) Expression patterns of SCN-enriched transcripts in 83 mouse tissues. Except SCN, duplicate data sets were used for other 82 tissues. The value used for the SCN was the (normalized) median of all the circadian values (24 in total) for the

Figure 1—figure supplement 3. Continued on next page

Figure 1—figure supplement 3. Continued

given probeset. Affymetrix probeset IDs and raw data for each gene are shown in ***Supplementary file 3***. (**C**) The SCN is the only tissue showing overlapping expression of Lhx1 and Rorα. Lhx1 (Affymetrix IDs 1421951_at and 1450428_at) and Rorα (1436325_at) in ***Figure 1—figure supplement 3C*** were extracted from ***Figure 1—figure supplement 3B***.

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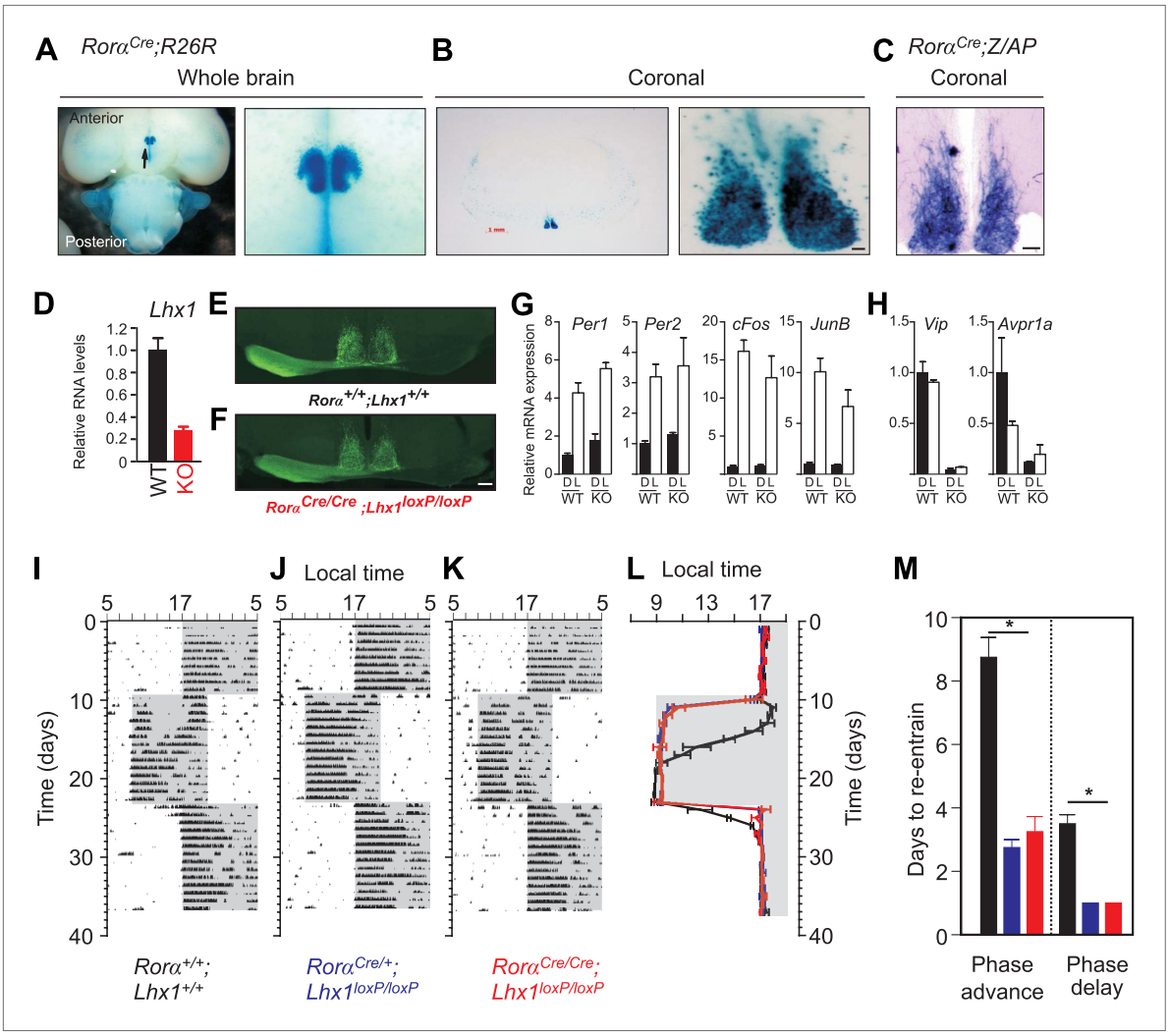

Figure 2. Loss of Lhx1 expression in the SCN renders faster synchronization with change in LD regimes. Enriched expression of a *Rorα*-driven marker in the SCN in *Rorα*^{Cre}*;R26R* mice. (**A**) Ventral view of a whole brain (magnified view on the right) of adult *Rorα*^{Cre}*;R26R* shows LacZ staining of the SCN. (**B**) Coronal section through the mid-SCN region (scale bar, 1 mm) and the magnified view of the SCN (scale bar, 100 μm) showing LacZ expression or (**C**) alkaline phosphatase expression in *Rorα*^{Cre}*;Z/AP* mice. (**D**) qRT-PCR estimate of Lhx1 expression in the SCN (mean +s.e.m, n = 5). (**E**) Normal SCN innervation of the retinal ganglion cells in the WT mice as revealed by monocular injection of CTB-conjugated fluorescent marker is intact in (**F**) Lhx1^{SCN-KO} mice. A 1 hr light pulse at CT16 causes (**G**) upregulation of light-induced genes (*Per1*, *Per2*, *cFos*, JunB), while (**H**) the light-suppressed transcripts (*Lhx1*, *Vip*, *Avpr1a*) in the WT SCN show reduced expression in the *Lhx1*$^{SCN-KO}$ mice. Mice were in DD for 2 days before the light pulse. Representative actograms of (**I**) *Rorα*$^{Cre/Cre}$, (**J**) *Rorα*$^{Cre/+}$*;Lhx1*$^{loxP/loxP}$, and (**K**) *Rorα*$^{Cre/Cre}$*;Lhx1*$^{loxP/loxP}$ mice subjected to 8 hr phase advance and 8 hr delay. (**I**) Average (±s.e.m., n = 5–8) activity onset and (**K**) average (+s.e.m.) number of days to re-entrain to advance or delay in light onset in three genotypes. Color codes in **L** and **M** correspond to the labels in **I**–**K**.

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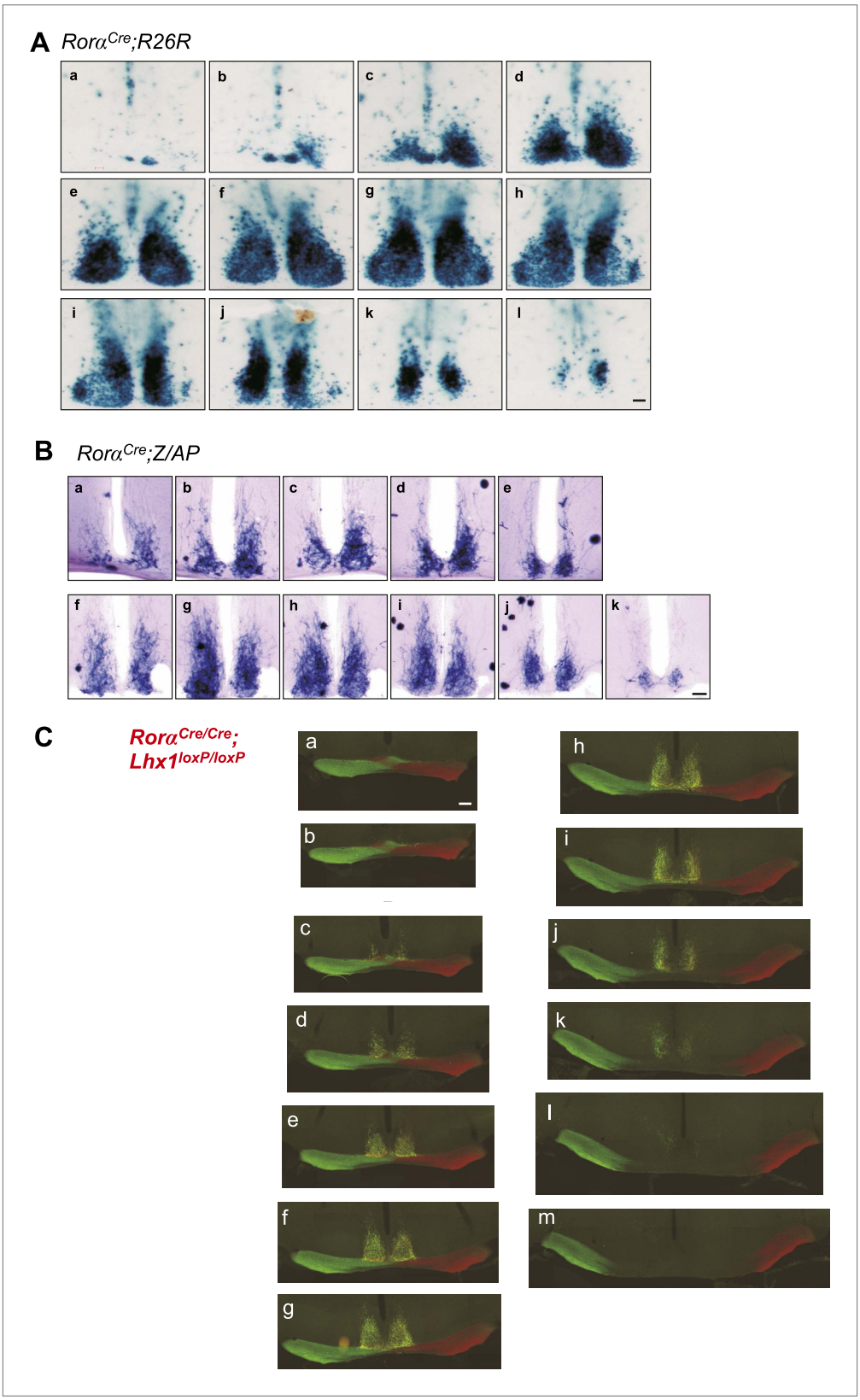

Figure 2—figure supplement 1. Histology of the adult SCN. Serial coronal brain sections of adult. (**A**) *Rorα*^{Cre}*;R26R* or (**B**) *Rorα*^{Cre}*;Z/AP* mice showing LacZ or alkaline phosphatase staining in the SCN. Scale bar, 100 μm. (**C**) Serial coronal hypothalamic brain section of an adult Rora$^{Cre/Cre}$;Lhx1$^{fl/fl}$ mouse intra-ocularly injected with Cholera toxin B (CTB) conjugated Alexa Fluor 488 (green) or 594 (Red) showing normal innervation of the SCN.

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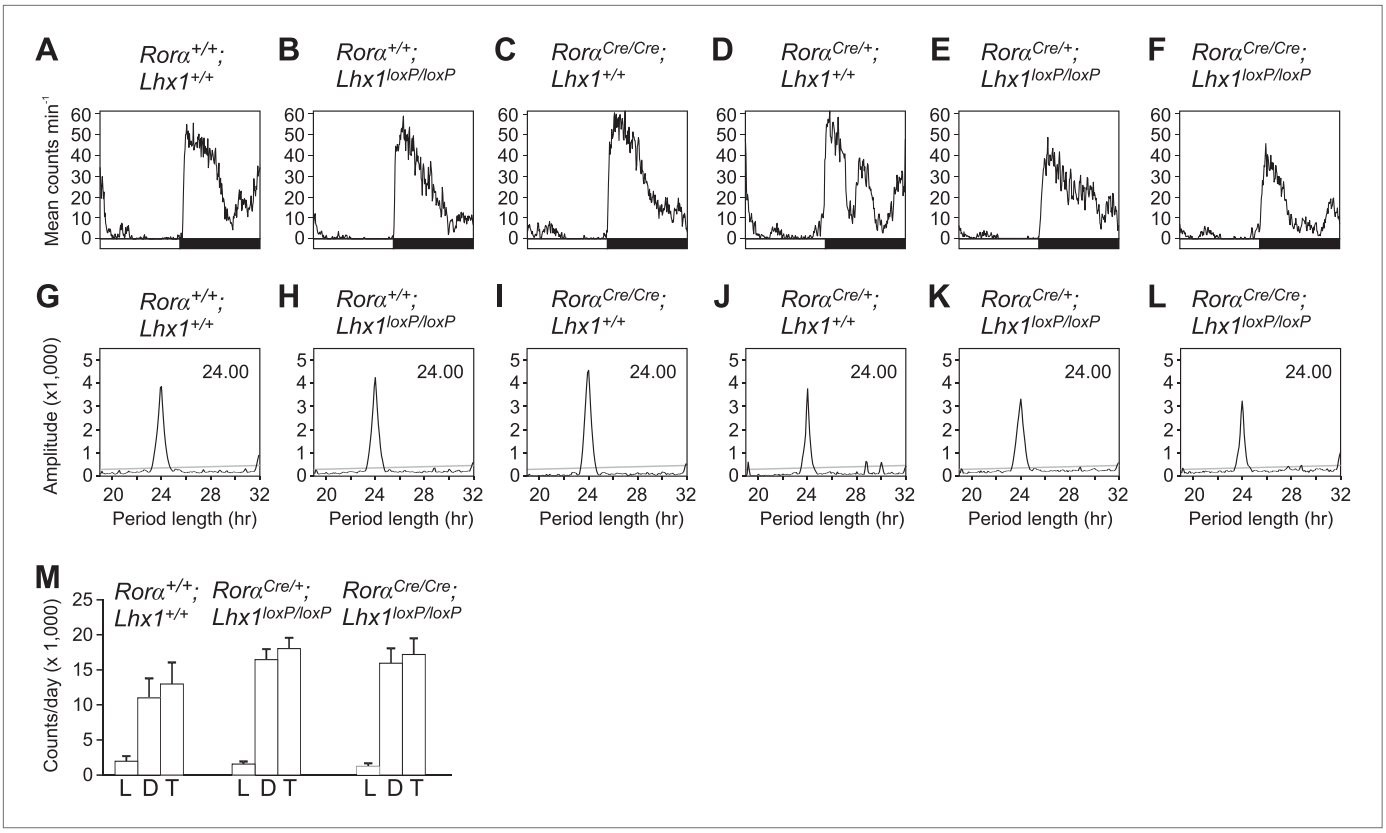

Figure 2—figure supplement 2. Activity profile under light-dark condition. (**A**–**F**) Activity profiles and (**G**–**L**) Chi-squared periodograms of representative mice of indicated genotypes during LD cycles. The period length (**H**) is shown inside panels of (**G**–**L**). Respective actograms showing wheel-running activity during LD are shown in ***Figure 3*****A**–**F**. (**M**) Quantitation of the amounts of *Rorα*^{Cre};*Lhx1*^{loxP} wheel running activity. Activity counts in LD cycles (L; light, D; dark and T; total) were plotted. Error bars indicate standard error of the mean. Activity during light, activity during dark and the total daily activity among these three genotypes were not significantly different.

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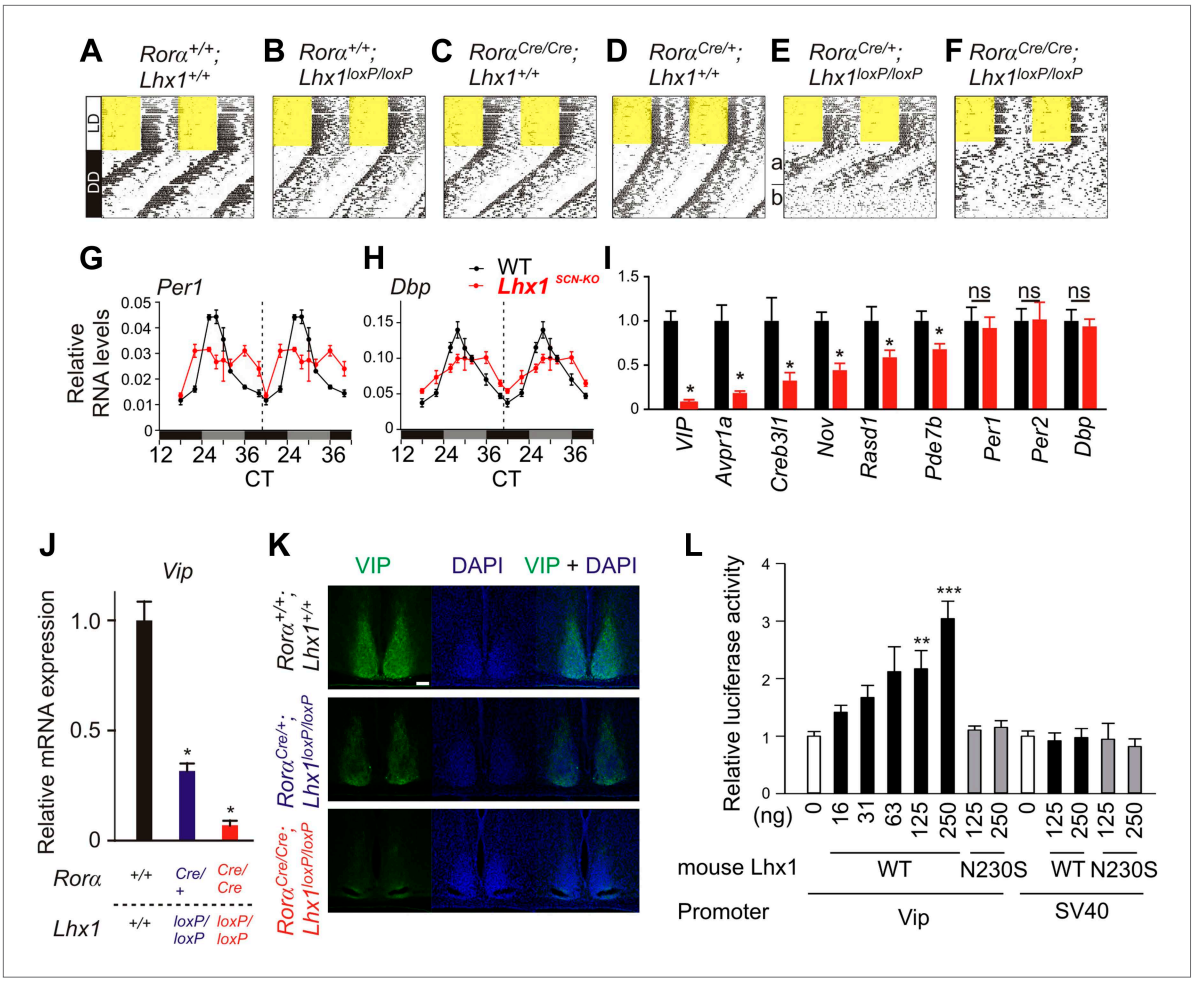

Figure 3. Lhx1 sustains normal circadian activity rhythms by regulating expression of synchronizing factors. (**A**–**F**) Representative wheel running activity pattern over several days of LD followed by DD in wild type and mice lacking *Lhx1* in the SCN. Double-plotted qRT-PCR quantification (average +s.e.m, n = 3–4 mice) of (**G**) *Per1* and (**H**) *Dbp* in the SCN of DD adapted WT and Lhx1^{SCN-KO} mice. (**I**) Average (+s.e.m. 8 time points every 3 hr over 24 hr) expression of several factors involved in intercellular communication or circadian clock in the SCN of dark adapted WT and Lhx1^{SCN-KO} mice. (**J**) Average mRNA (+s.e.m., n = 3–6 mice, *p < 0.05) expression or (**K**) immunoreactivity of VIP is reduced in the SCN of Lhx1-deficient mice. (**L**) Transcriptional activation of mouse *Vip* promoter by mouse LHX1. pGL3-promoter vector was used as a control promoter vector. Values are mean +s.e.m, ANOVA **p<0.01, ***p<0.001 vs 0 ng (white bar).

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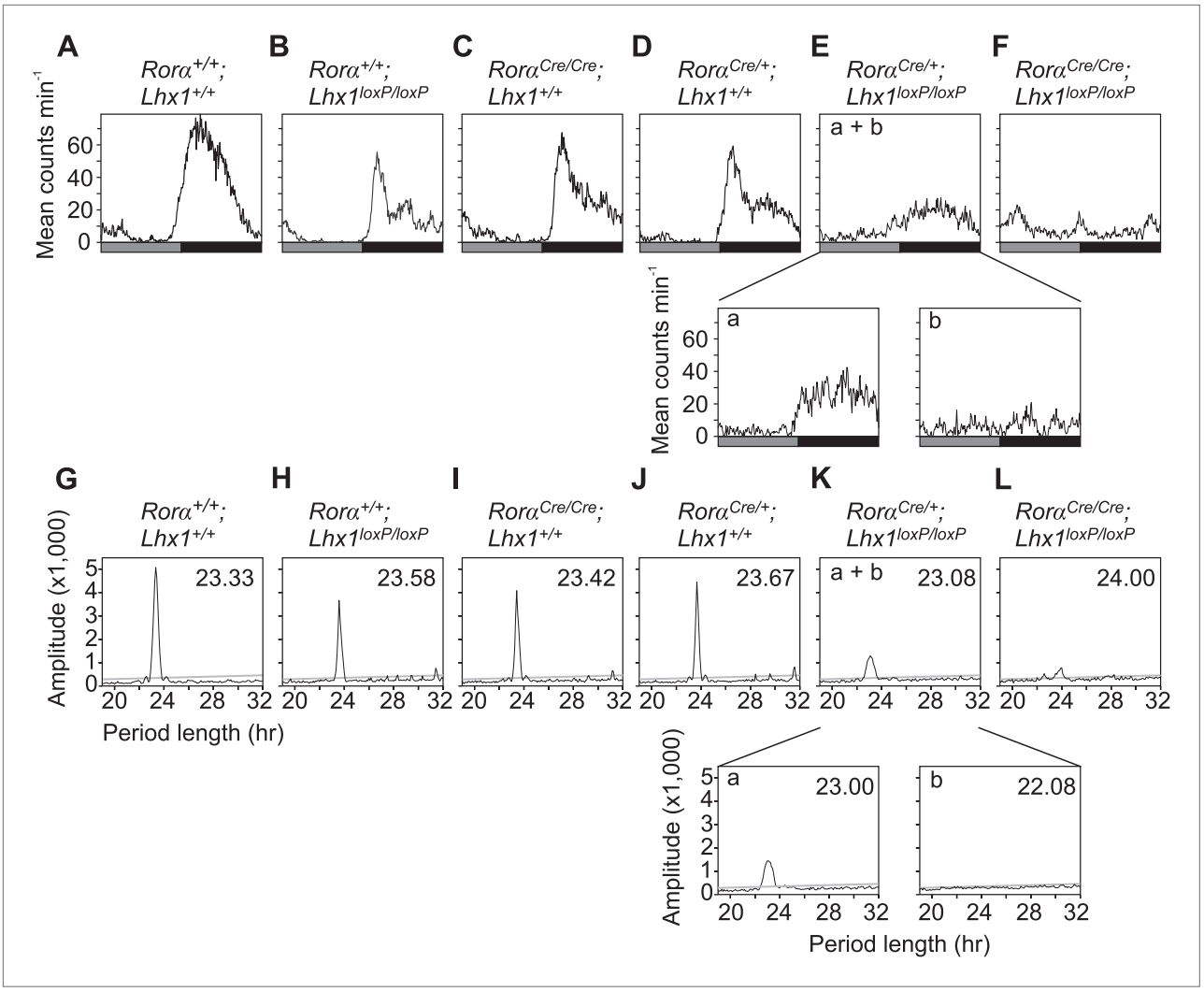

Figure 3—figure supplement 1. Activity profile under constant darkness. (**A**–**F**) Activity profiles and (**G**–**L**) Chi-squared periodograms of representative mice of indicated genotypes during DD cycles. The period length (**H**) is shown inside panels of (**G**–**L**). Respective actograms showing wheel-running activity during DD are shown in ***Figure 3A–F***. The insets in **E**a and **E**b show activity profile during the first 2 weeks in DD and last week of DD when the mice were rhythmic and arrhythmic respectively. Similarly, the insets in **K**a and **K**b show the respective chi-square periodogram. Average period lengths are shown in ***Table 1***.

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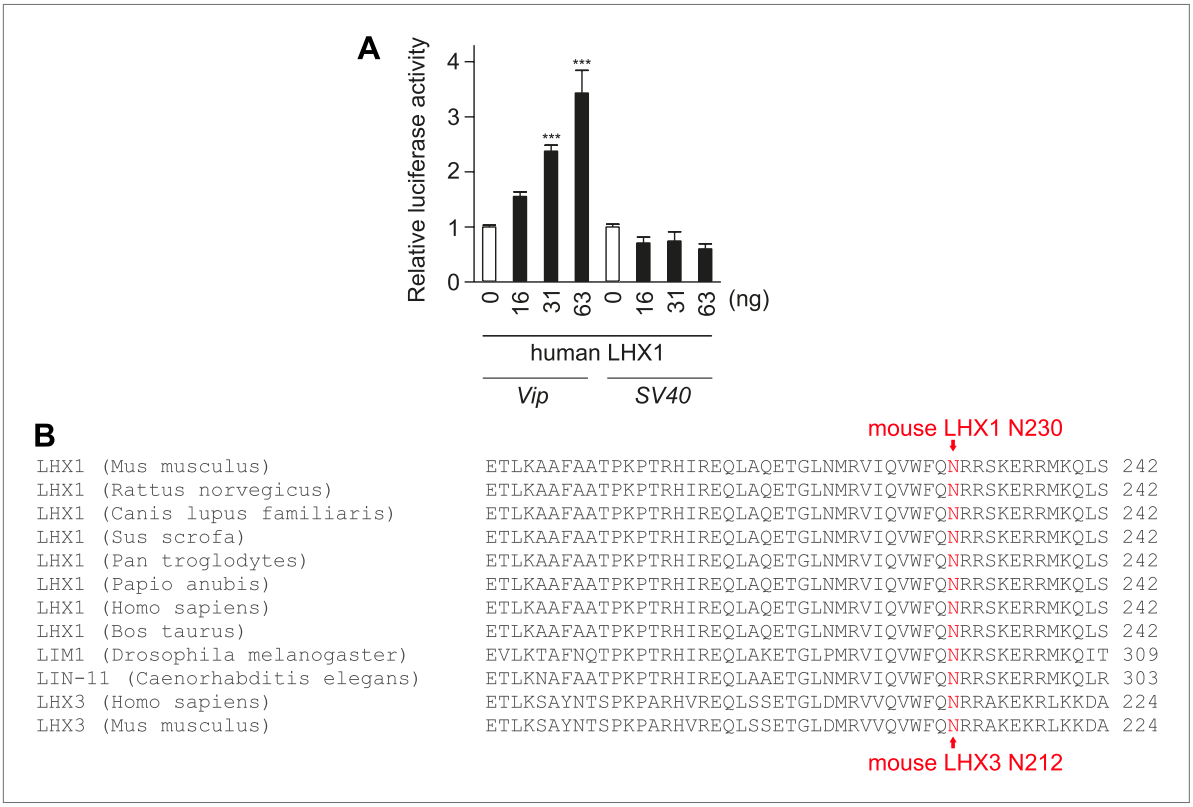

Figure 3—figure supplement 2. Lhx1 activates Vip transcription. (**A**) Human Lhx1 activates Luciferase expression from Vip:Luc but not from SV40 promoter in a dose-dependent manner. (**B**) Amino acid sequence alignment of DNA binding region of LHX1 and LHX3 showing the N230 residue in mouse Lhx1 that is critical for normal transcriptional activation function of Lhx1.

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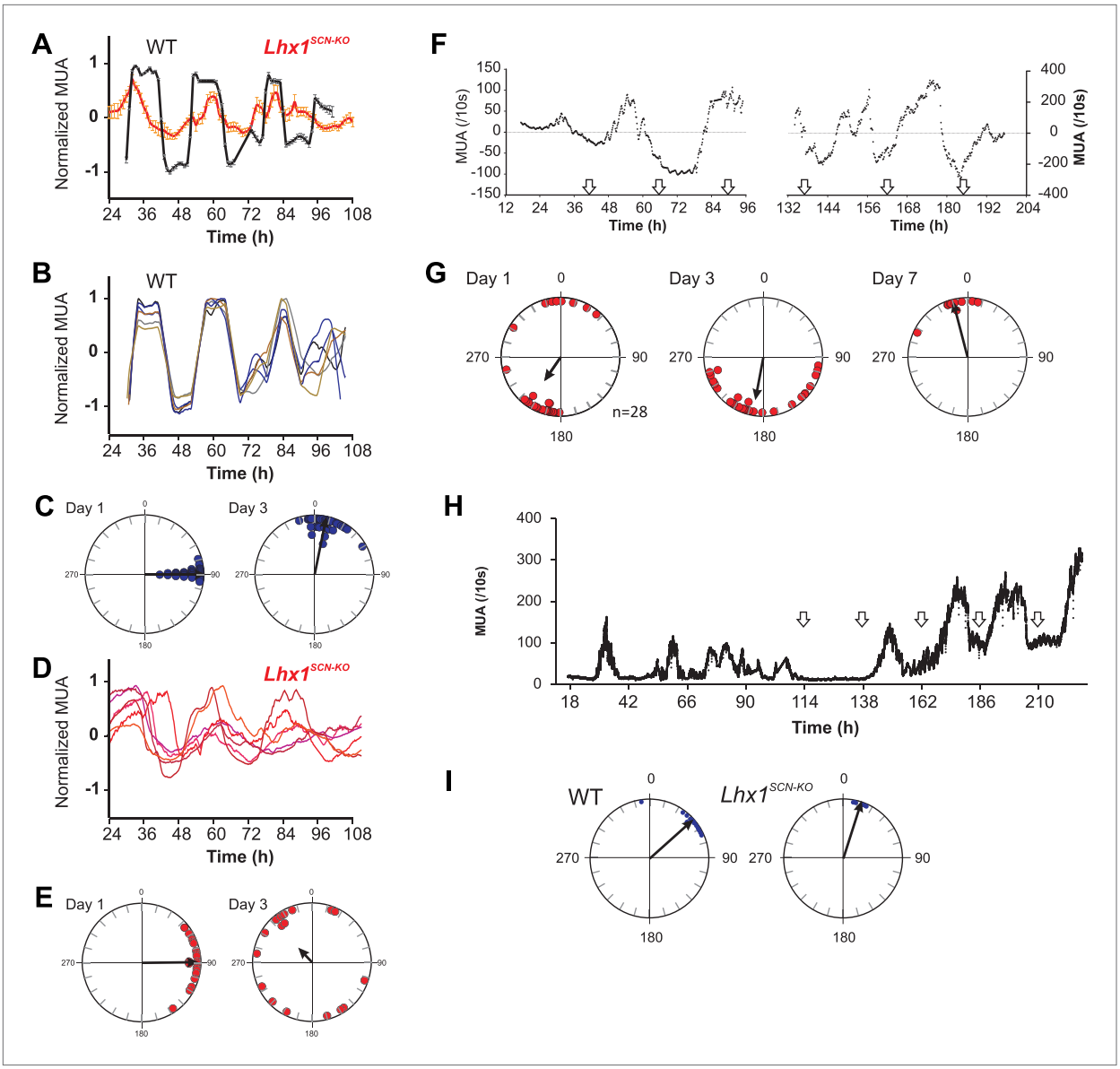

Figure 4. Lhx1 maintains synchrony among SCN neurons partly via VIP. (**A**) Average (+s.e.m.) normalized multiunit activity (MUA) recorded from representative SCN slices of LD-adapted WT (n = 40, black) and Lhx1^{SCN-KO} (n = 12, orange) mice. Data were binned every 60 min. Representative normalized MUAs and peak phase of activity from WT SCN (**B** and **C**, n = 40) and Lhx1^{SCN-KO} mouse (**D** and **E**, n = 19). For **C** and **E**, left and right panels are respectively for days 1 and 3. (**F**) Average MUA of a DD adapted Lhx1^{SCN-KO} SCN that received 1 hr perfusion of VIP daily for up to 7 days. (**G**) Peak phases of activity are gradually synchronized over 7 days. (**H**) Representative MUA from the SCN of an LD-adapted Lhx1^{SCN-KO} mouse over several days. During the first 4 days, the activity dampened, which was rescued by daily application of VIP. Down arrows in **F** and **H** indicate the time of VIP application. (**I**) Peak phases of activity in WT and Lhx1^{SCN-KO} SCN at the end of 7 days are shown.

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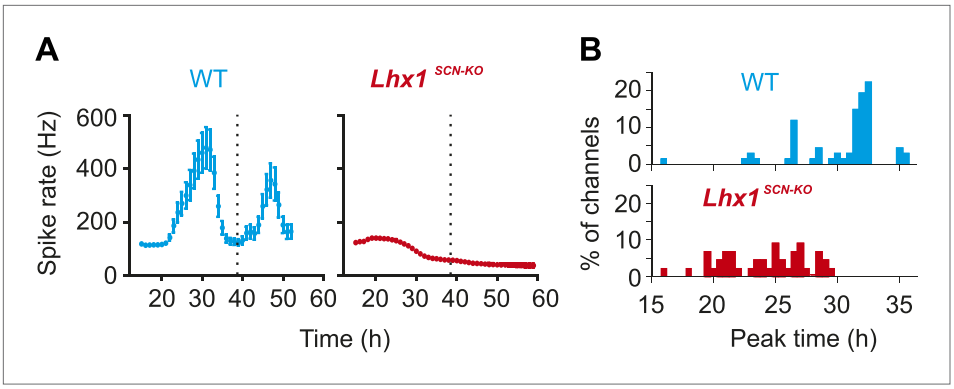

Figure 4—figure supplement 1. Normalized multi-unit activity recorded from DD adapted WT and Lhx1^{SCN-KO} (mean ± SEM). Peak time of multiunit activity from each channel shows relative synchrony in the WT mouse that is dispersed in the Lhx1^{SCN-KO} mouse.

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