



Title	Two coupled circadian oscillations regulate Bmal1-ELuc and Per2-SLR2 expression in the mouse suprachiasmatic nucleus
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Supplementary Information

Two coupled circadian oscillations regulate *Bmal1-ELuc* and *Per2-SLR2* expression in the mouse suprachiasmatic nucleus

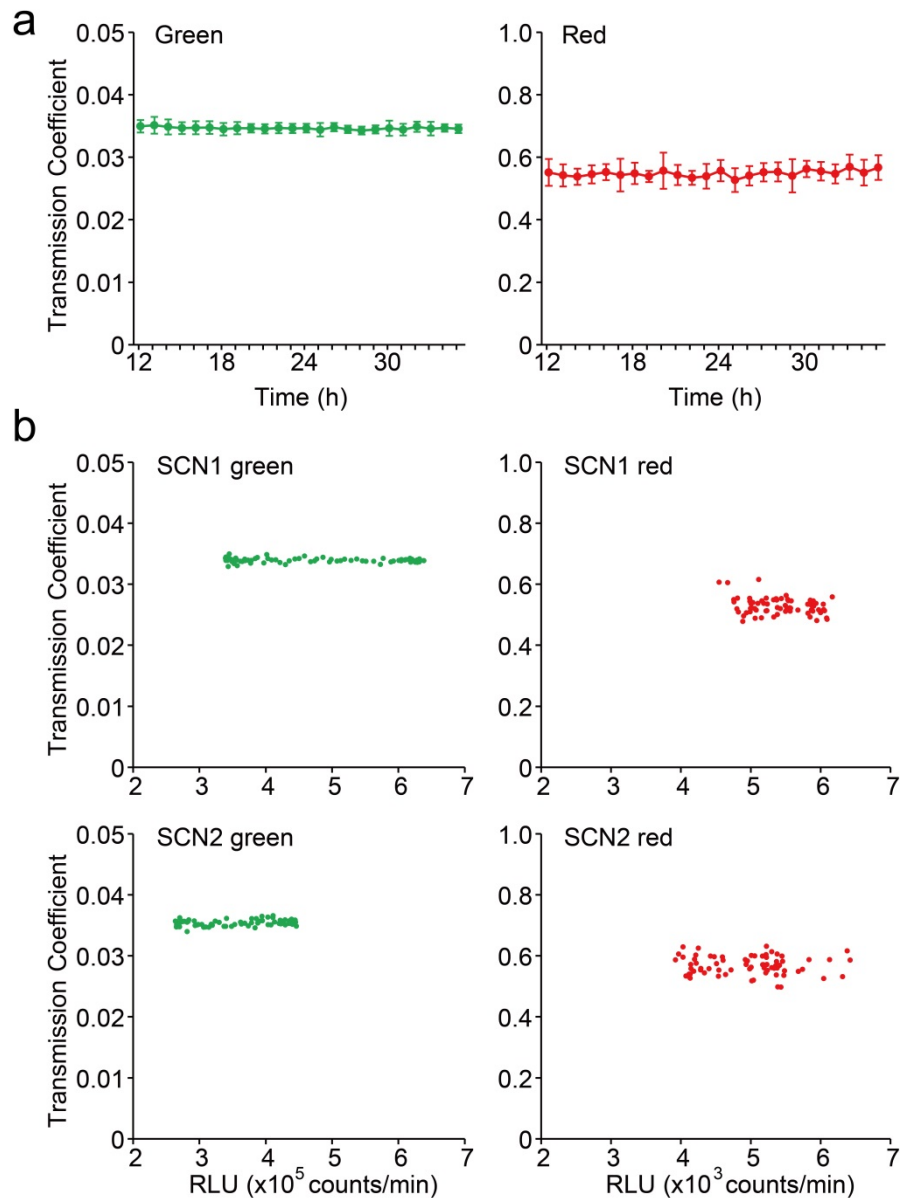
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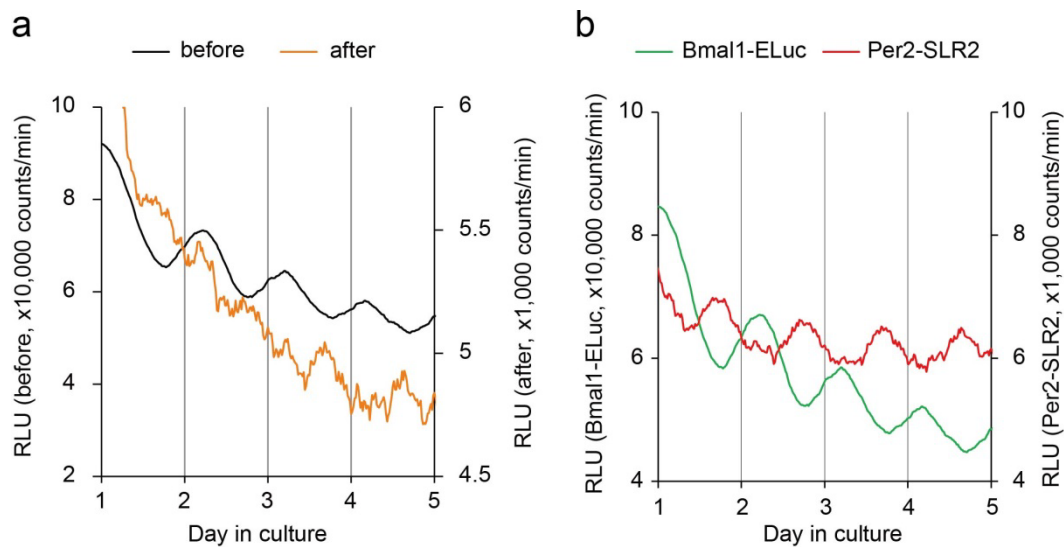
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Supplementary Figure 1 Transmission coefficient was essentially independent of the time of day and the intensity of bioluminescence

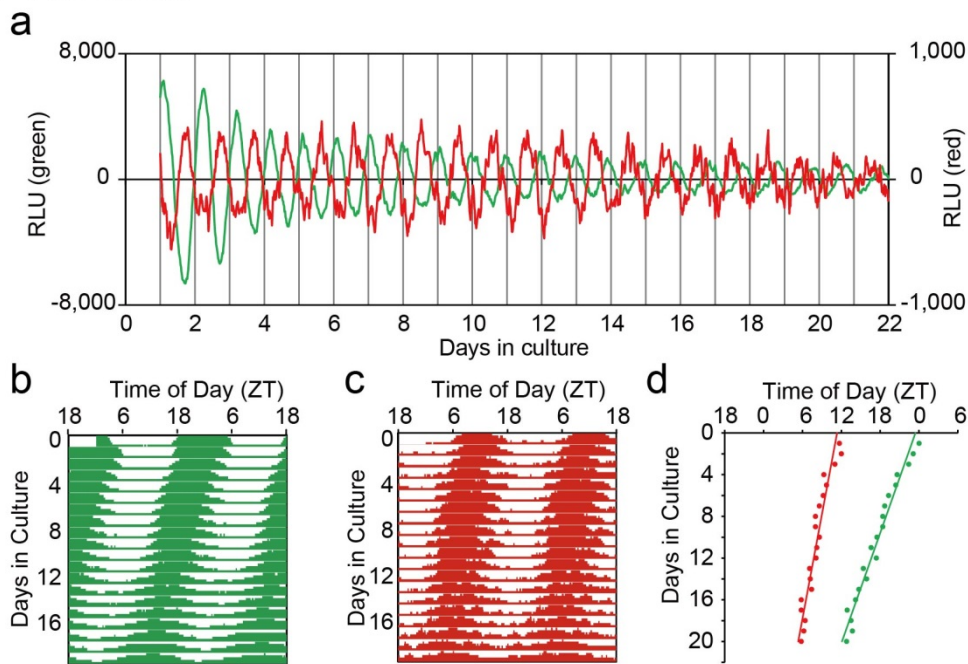
The transmission coefficient was calculated for *Bmal1-ELuc* and *Per2-SLR2* bioluminescence measured in two SCN slices of mice with a single *Bmal1-ELuc* or *Per2-SLR2* reporter. (a) Transmission coefficient in the course of culture for *Bmal1-ELuc* (left) and *Per2-SLR2* (right). Transmission coefficient was kept constant throughout the measurement. (b) The transmission coefficient at different intensities of bioluminescence. Transmission coefficient was not significantly different between the low and high bioluminescence intensity in *Bmal1-ELuc* (left) and in *Per2-SLR2* (right), indicating that the transmission coefficient was essentially independent of the intensity of bioluminescence in the range measured in the present study.



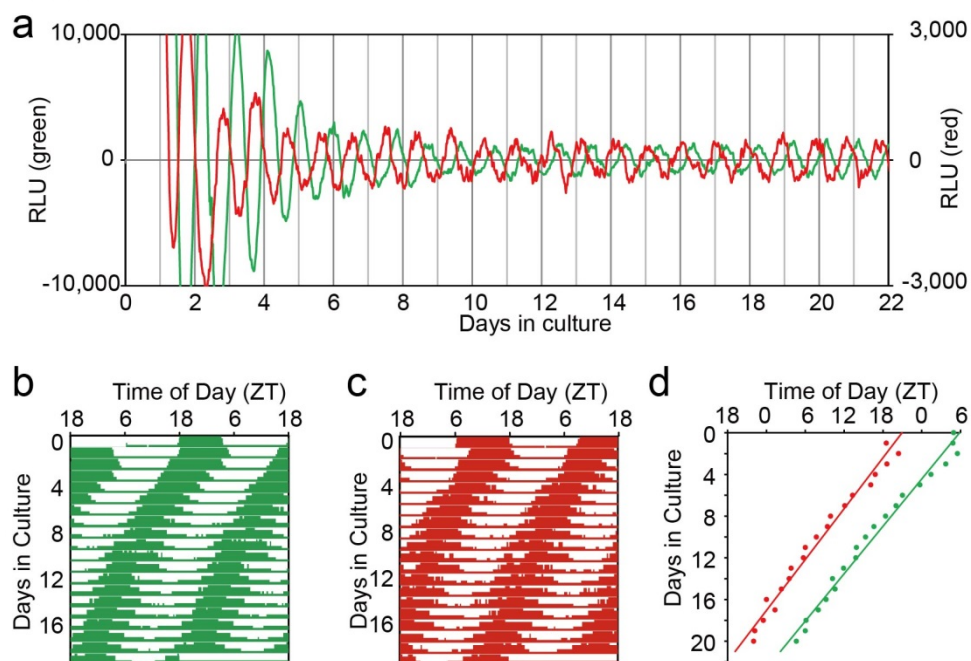
Supplementary Figure 2 Intensity of bioluminescence before and after a long-pass filter application and deduced circadian rhythms in *Bmal1-ELuc* and *Per2-SLR2* expression

(a) Bioluminescence before (black) and after (orange) a long-pass filter (620 nm) application for the first 4 culture days. The right ordinate indicates the intensity before filtering and the left after filtering. (b) Deduced circadian rhythms of *Bmal1-ELuc* (green) and *Per2-SLR2* (red). See also Materials and Methods in the text.

A: neonate



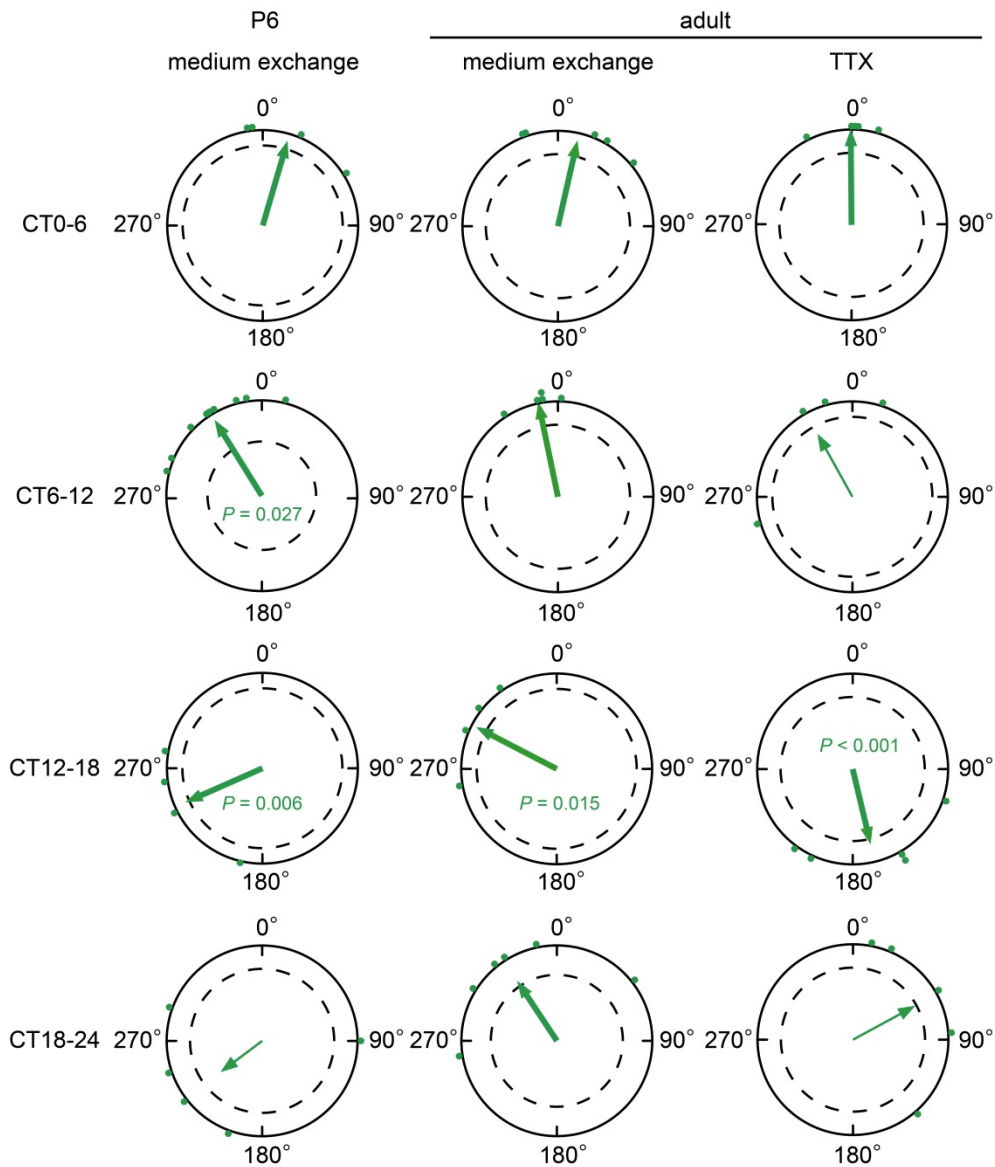
B: adult



Supplementary Figure 3 Additional examples of circadian rhythms in the neonatal and adult SCN slice of *Bmal1-Eluc:Per2-SLR2* mice

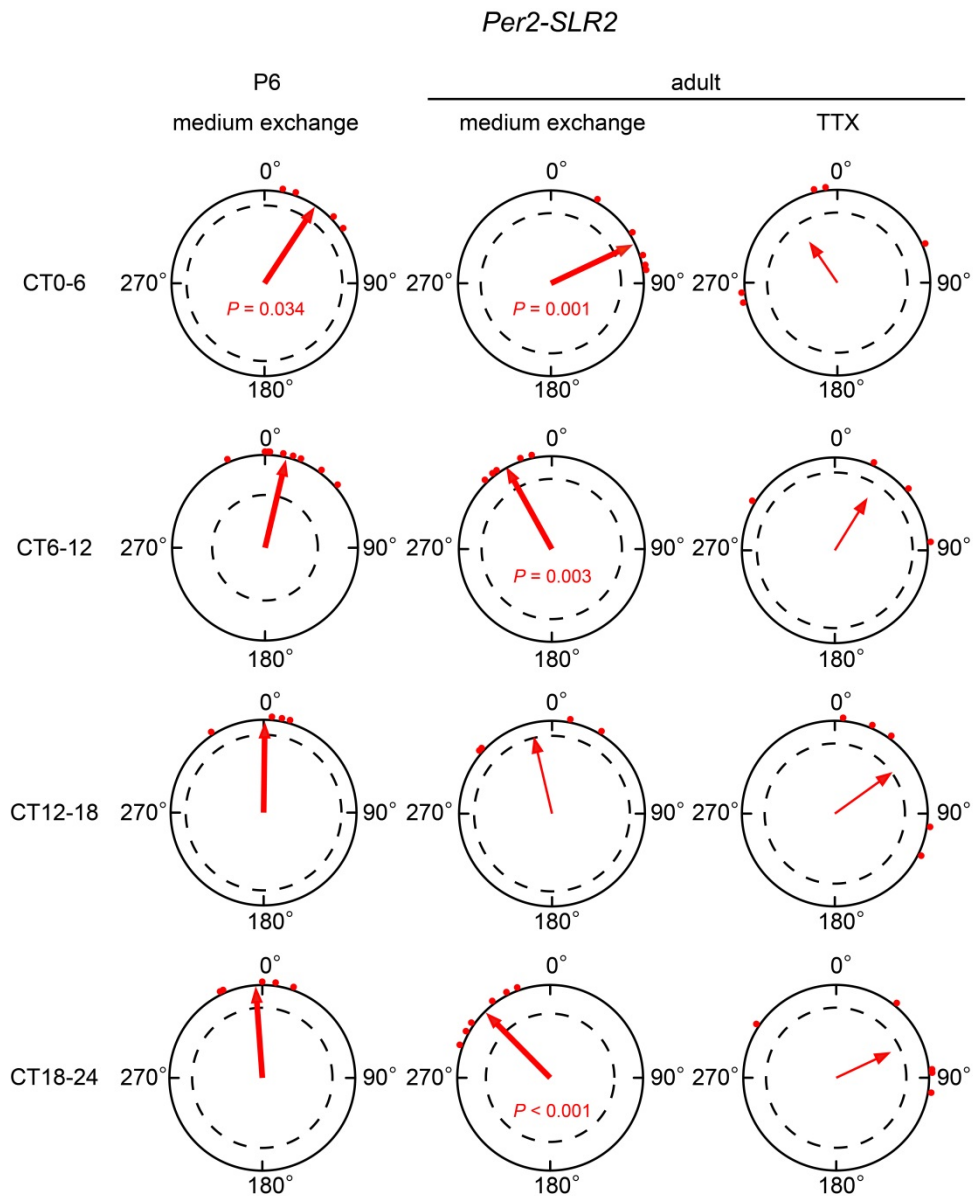
(a) Sequential records of circadian *Bmal1* (green) and *Per2* (red) rhythm. (b) (c) (d) A double plotted circadian *Bmal1* (b) and *Per2* (c) rhythm in a raster format, and successive plots of circadian peak phases (d) (*Bmal1*, green; *Per2*, red) and regressions lines fitted to them in the same SCN slice as used in (a). See also the legend of Fig 1.

Bmal1-ELuc



Supplementary Figure 4 Rayleigh plots of circadian peak phases of *Bmal1* rhythm before and after medium exchange

Phase-shifts of the circadian *Bmal1* rhythm in response to medium exchange are illustrated by Rayleigh plot in 4 different circadian phases for the neonatal SCN (left), adult SCN pretreated with distilled water (middle) and with TTX (right). Solid circles indicate the amount of phase shift with degree and broken circles a significance level ($p < 0.05$). Arrows indicate the mean phase shift. Direction and length of an arrow indicate the final phase of shift and the variability of phase-shifts, respectively. A thick arrow indicates significant concentration of final phases ($p < 0.05$, Hotelling's paired test) and p-value in the circle significant phase-shift from the initial phase (0°).



Supplementary Figure 5 Rayleigh plots of circadian peak phases of *Per2* rhythm before and after medium exchange

Phase-shifts of the circadian *Per2* rhythm in response to medium exchange are illustrated by Rayleigh plot in 4 different circadian phases for the neonatal SCN (left), adult SCN pretreated with distilled water (middle) and with TTX (right). See also the legend for Supplementary Fig 1.