

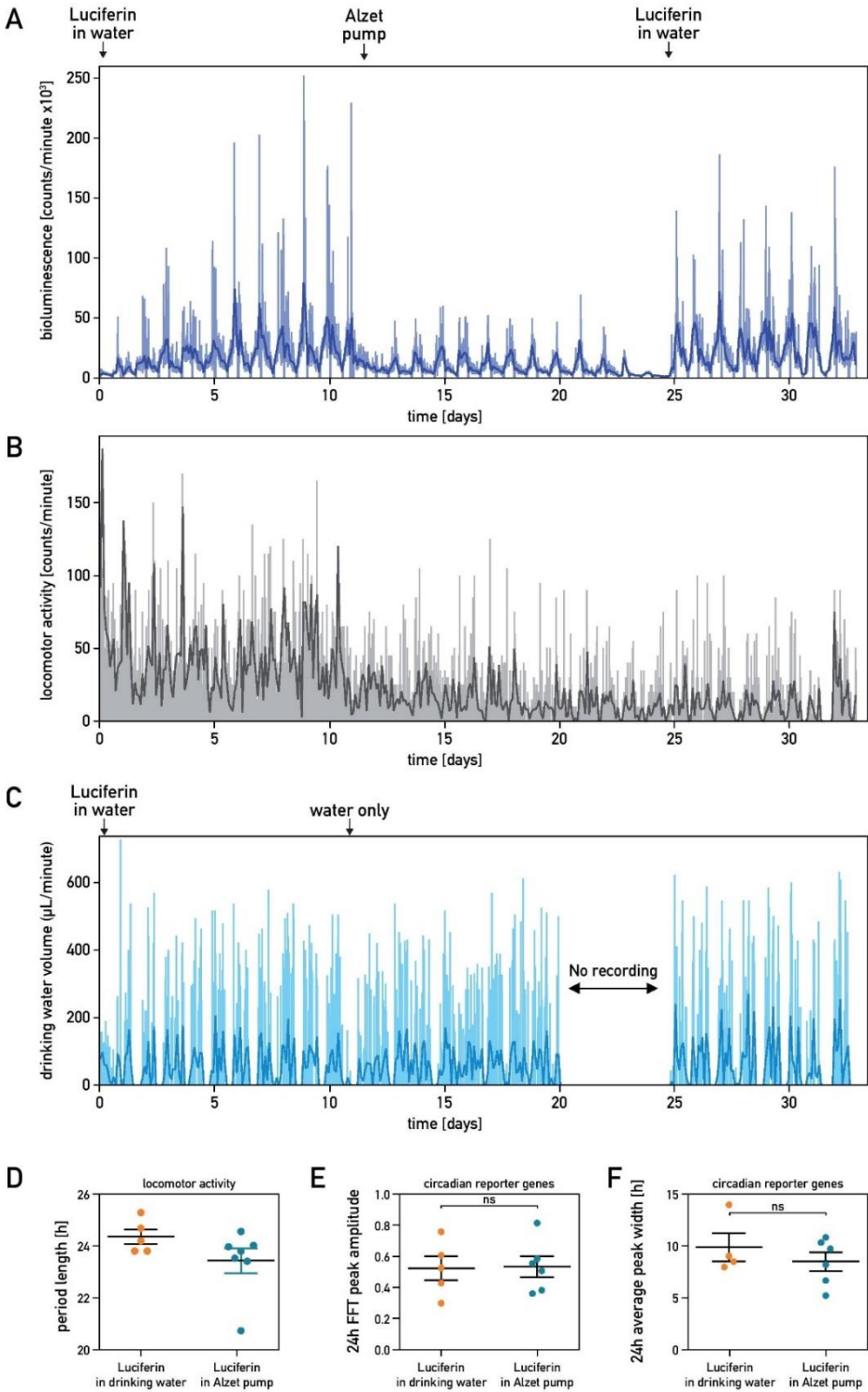
Supplemental Figures for

**Circadian hepatocyte clocks keep synchrony in the absence of a master pacemaker in the suprachiasmatic nucleus or other extrahepatic clocks**

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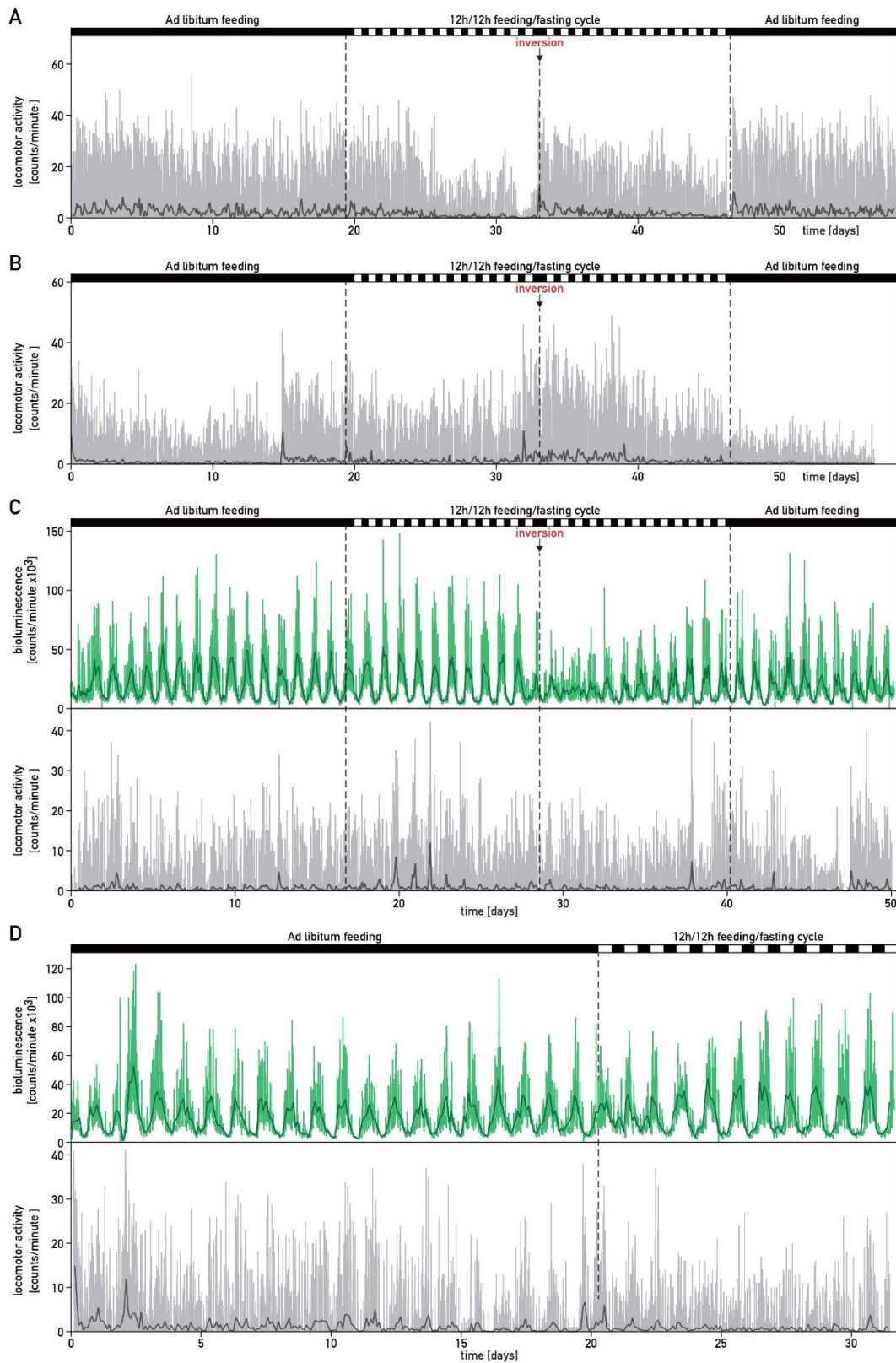
Figure S1

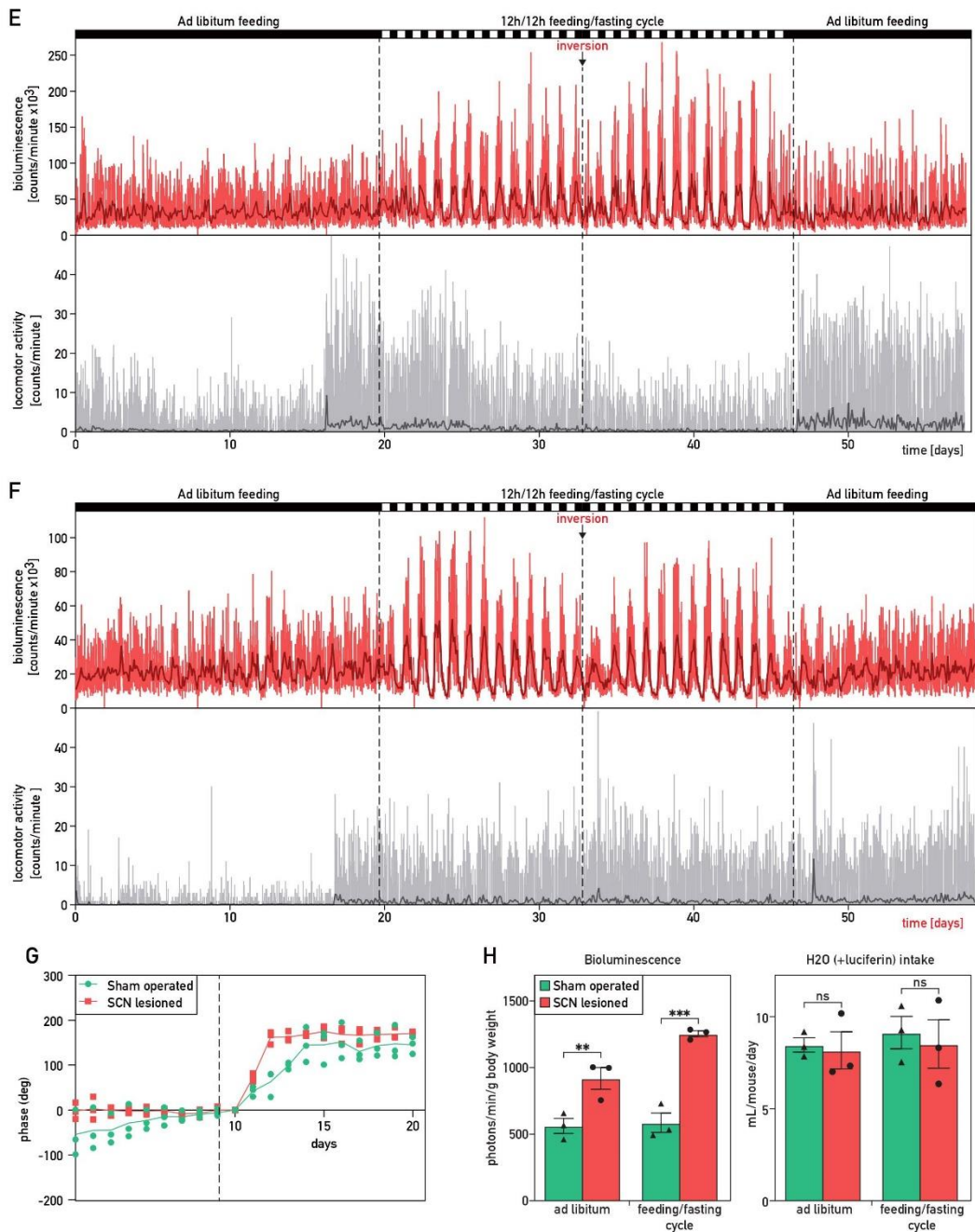


**Supplemental Figure S1. Real-time *in vivo* recording of bioluminescence with different luciferin delivery methods**

(A-C) Long-term recording of a wild-type mouse transduced with an adenoviral vector expressing the circadian Rev-erb $\alpha$ -luciferase reporter gene in the liver. The mouse was entrained by a skeleton photoperiod. Luciferin has been delivered as indicated. (A) Bioluminescence recording of Rev-erb $\alpha$ -luciferase expression, (B) Spontaneous locomotor activity and (C) drinking activity were monitored simultaneously. Colored and grey lines: raw data recorded at 1-minute intervals. Dark continuous lines: 120-minute time-binned traces. (D-F) Impact of the luciferin delivery method on the locomotor activity and circadian gene expression in animals recorded in RT-Biolumicorders in constant condition (darkness). No significant differences were detected for animals receiving luciferin via the drinking water (n = 5) or through an intraperitoneally implanted Alzet micro-osmotic pump (n = 7) with regard to the period length of locomotor activity (D), Amplitude of the FFT peak in the circadian range for bioluminescence (E) and Average oscillation width (F).

Figure S2



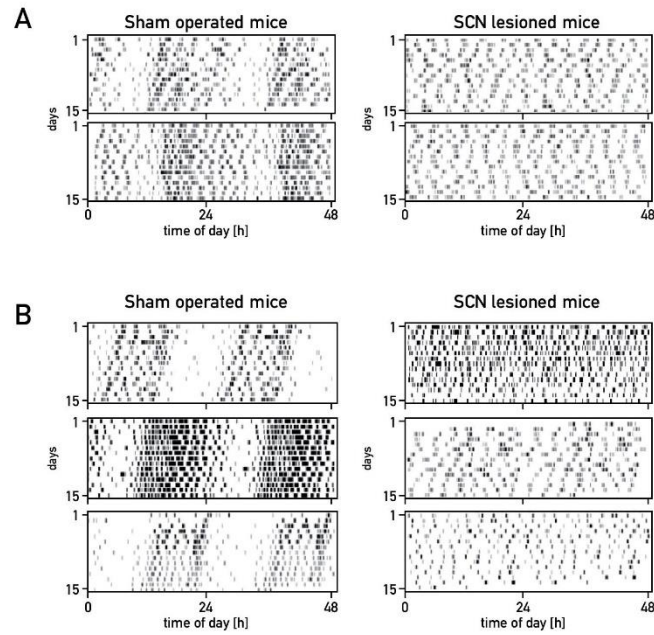


**Supplemental Figure S2. The SCN is required for the synchronization of peripheral clocks in mice kept under constant conditions**

(A-B) Recordings of spontaneous locomotor activity of (A) sham-operated and (B) SCN-lesioned *Per2::luc* mice during the bioluminescence recordings displayed in Fig.2 A-B. After sham-operation and SCN-lesion respectively, the mice were kept in constant darkness during the recordings and according to the indications on top of panel A. Gray vertical line: raw data (1-

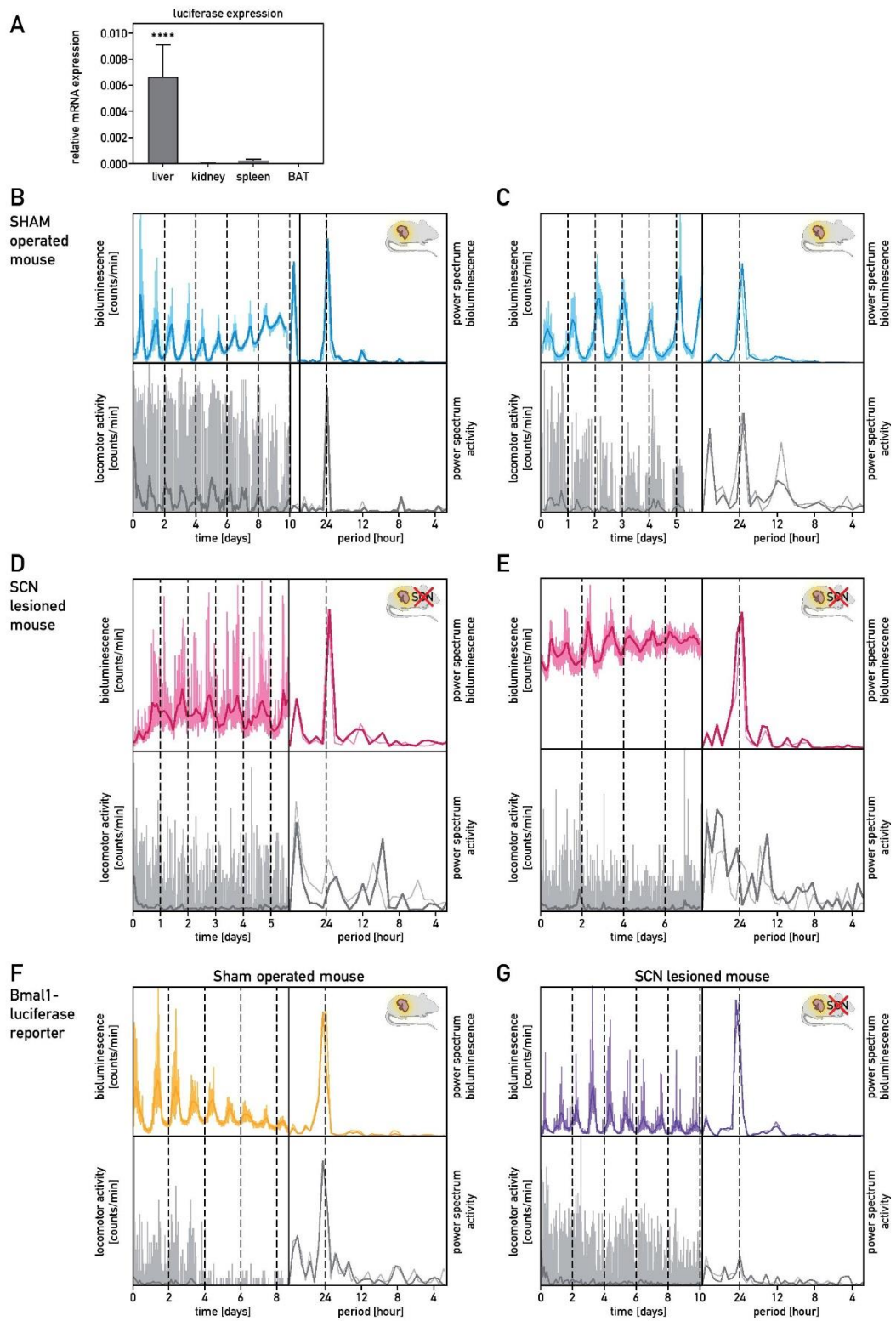
minute intervals). Black continuous lines: 120-minute time-binned traces (C-F) Additional long-time recordings of sham-operated (C-D) and SCN lesioned (E-F) *Per2::luc* mice. The mice were kept in constant darkness throughout the recordings and fed according to the indications on top of the panels. Spontaneous locomotor activity profiles monitored simultaneously are shown *below* the bioluminescence tracings as described for panels A and B. (G) Phase shifting of *Per2::luciferase* expression in sham-operated and SCN-lesioned mice entrained by feeding-fasting cycles. Diagram of the phase angle changes recorded in Figure 2 A-B and Supplemental Fig. S2 C-F as a function of time after inversion (vertical dotted line) of the feeding regimen (the peaks have been aligned in sham-operated and SCN-lesioned mice at the time of feeding inversion: day 10). Two-way ANOVA on the data after the food inversion: interaction \*\*  $p = 0.0065$ , effect of time \*\*\*\*  $p < 0.0001$ , effect of SCN surgery \*  $p = 0.0403$ . (H) Magnitude of bioluminescence in sham-operated and SCN-lesioned *PER2::luc* mice. The photons/min emitted by these mice are corrected for the weight ( $34.11 \pm 0.76$  g and  $35.39 \pm 1.38$  g for sham-operated mice fed *ad libitum* or exposed to feeding/fasting cycles, respectively, and  $31.22 \pm 0.3.6$  g and  $32.23 \pm 4.46$  g for SCN lesioned mice fed *ad libitum* or exposed to feeding-fasting cycles, respectively). Irrespective of the feeding regimen the bioluminescence generated by SCN-lesioned mice was significantly higher than that produced by sham-operated mice (\*\* $p$ -value = 0.0078; \*\*\* $p$ -value = 0.0001; two-way ANOVA). Since the water (+ luciferin) intake was highly similar for sham-operated and SCN-lesioned *PER2::luc* mice, it is unlikely that the higher bioluminescence generated by the latter is simply a consequence of higher circulating luciferin levels in these animals.

Figure S3



**Supplemental Figure S3. Spontaneous locomotor activity records** of the sham operated and SCN lesioned mice kept in free-running conditions and displayed in (A) Supplemental Fig. S2 C-F and (B) Fig. 3 A-B and Supplemental Fig. S4 A-D. After the surgical operations, mice were housed in Light/Dark (LD) and then released into Dark/Dark (D/D) for the 15 days of the locomotor activity recording in order to assess the success of the surgical operations.

Figure S4

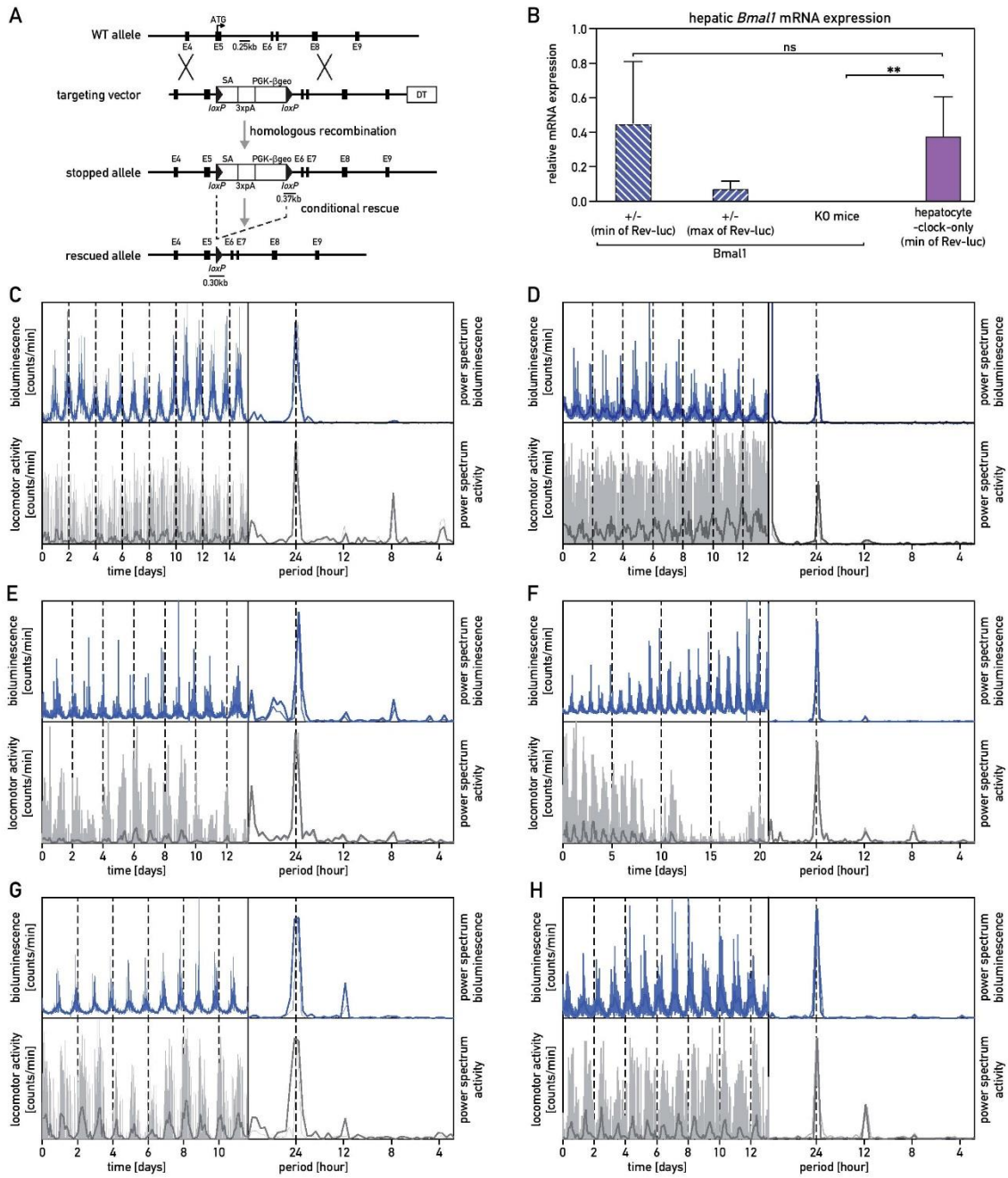


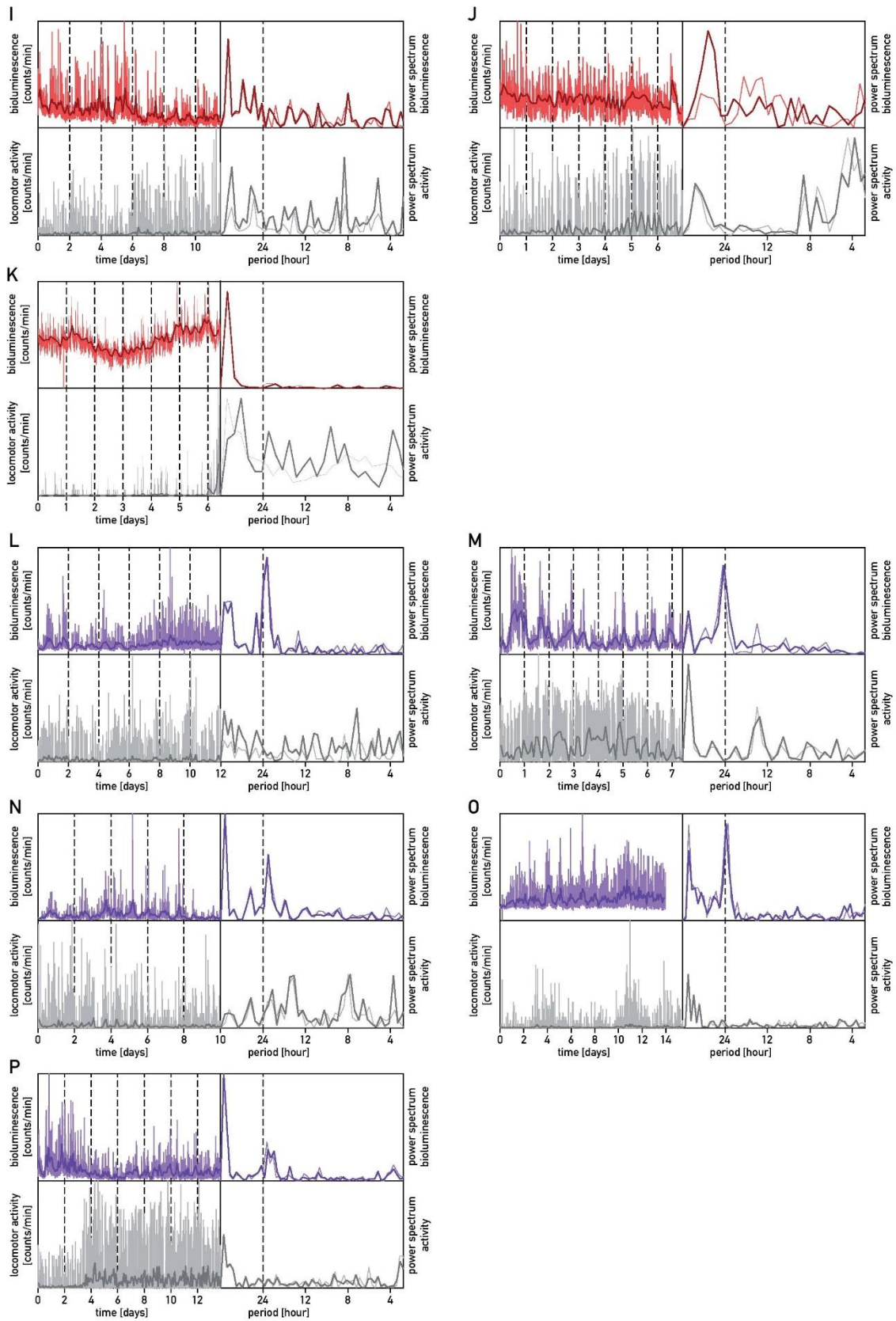


#### **Supplemental Figure S4. Hepatocyte oscillators keep phase coherence in SCN lesioned animals**

(A) *Luciferase* mRNA levels in the liver, kidney, spleen and brown adipose tissue (BAT) of mice transduced with Adv-Rev-erb $\alpha$ -luc vectors, measured by quantitative RT-PCR analysis. The measurements of the mRNA levels represent means  $\pm$  SEM for  $n \geq 12$  mice and were normalized to *Gapdh* mRNA levels. The statistical test was a Kruskal-Wallis test; \*\*\*\* $p < 0.0001$ . (B-E) Recordings of hepatic expression of Rev-erb $\alpha$ -luciferase in sham operated (B-C) and SCN-lesioned (D-E) mice. Mice were transduced with Adv-Rev-erb $\alpha$ -luc and shaved in a dorsal region on top of the liver. After 48 h, bioluminescence was monitored in a RT-Biolumicorder under constant conditions (darkness, food and water *ad libitum*). Luciferin was provided in the drinking water for the animal shown in (B) and through an Alzet micro-osmotic pump (C-E). Spontaneous locomotor activity profiles monitored simultaneously are shown below the bioluminescence tracings. The FFT analysis of the bioluminescence and locomotor activity recordings are represented at the right of the bioluminescence and activity traces. The lighter vertical lines display the photons accumulated at 1-minute intervals, and the darker lines correspond to 120-minute time-binned traces. (F-G) Recordings of circadian hepatocyte expression of Bmal1-luciferase in a sham operated (F) and a SCN-lesioned (G) mouse. Mice were transduced with the Adv-Bmal1-luc vector and shaved in a dorsal region on top of the liver. 48h after transduction, bioluminescence was monitored in a RT-Biolumicorder under constant conditions (darkness, food and water *ad libitum*). Luciferin was supplied via Alzet micro-osmotic pumps. Spontaneous locomotor activity profiles monitored simultaneously are shown *below* the bioluminescence tracings. The lighter lines display the photons accumulated at 1-minute intervals and the darker lines correspond to 120-minute time-binned traces.

Figure S5





**Supplemental Figure S5. The liver maintains partial phase coherence in mice bearing oscillators exclusively in hepatocytes**

(A) Scheme of the conditionally stopped *Bmal1* allele generation. Targeting strategy for LoxP-flanked, stopped allele and conditional rescue. The transcriptional stop-cassette was knock into the intron following the first coding exon. Boxes indicate exons; DT, diphtheria toxin. (B) *Bmal1* mRNA levels in the liver of *Bmal1*<sup>+/-</sup>, *Bmal1*<sup>-/-</sup> mice and “hepatocyte clock-only-mice”, measured by quantitative RT-PCR analysis. The animals were sacrificed at the end of the RT-Biolumicorder recordings. Livers of *Bmal1*<sup>+/-</sup> mice have been harvested at the times of maximum or the minimum Rev-erb $\alpha$ -luciferase expression as represented by the bioluminescence tracings. The mRNA levels, normalized to *Gapdh* mRNA levels, represent means  $\pm$  SEM for  $n \geq 3$  per group. The statistical test was a two-tailed Mann Whitney test; \*\*  $p < 0.01$ ; ns, non-significant. (C-P) Recordings of expression of Rev-erb $\alpha$ -luciferase in the livers of (C-H) *Bmal1*<sup>+/-</sup> mice, (I-K) *Bmal1*<sup>-/-</sup> mice, and (L-P) hepatocyte-clock-only mice transduced with the Adv-Rev-erb $\alpha$ -luc vector and shaved in a dorsal region over the liver. 48h after transduction bioluminescence was monitored in the RT-Biolumicorder in mice kept under constant conditions (darkness, food and water *ad libitum*). Luciferin was provided in the drinking water. Spontaneous locomotor activity profiles monitored simultaneously are shown below the bioluminescence tracings. The FFT analysis of the bioluminescence and locomotor activity recordings are represented at the right of the bioluminescence and activity traces. The lighter vertical lines represent photons accumulated at 1-minute intervals and the darker lines correspond to 120-minute time-binned traces.