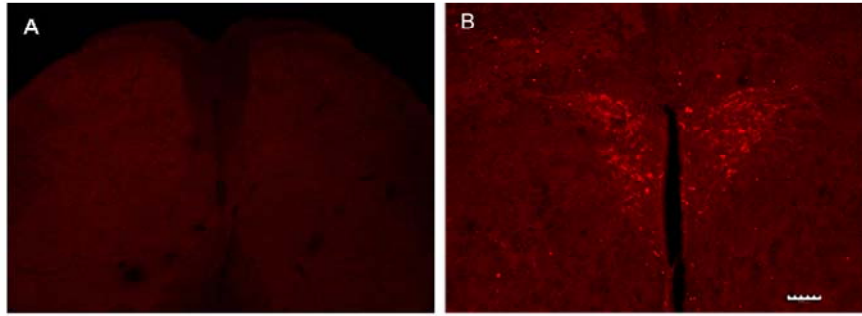


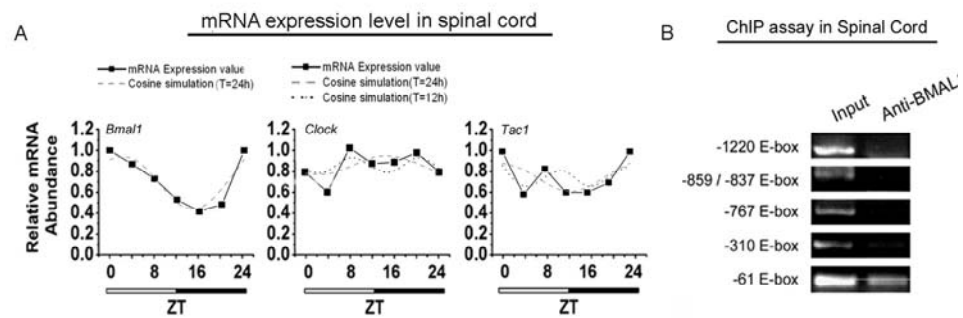
## Supplemental Digital Content 2



**Fig. S1. Negative control and positive control of rat-anti-SP-immunoreactivity.**

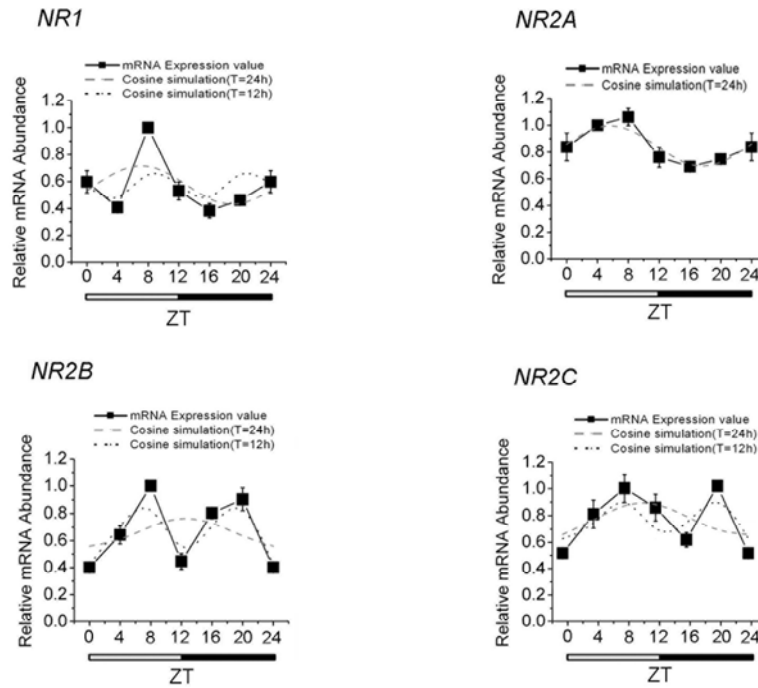
(A) The spinal cord sections of mice were incubated, without rat-anti-SP, in 0.01 M phosphate buffer saline containing 5% normal goat serum, 0.3% Triton X-100, 0.05% (w/v)  $\text{NaN}_3$  and 0.25% (w/v) carrageenan (pH 7.4) for 48 hrs at 4°C, followed by biotinylated rabbit-anti-rat IgG (1:200; Vector, Burlingame, CA) for 6 hrs at room temperature. Finally, the sections were incubated with Cy<sup>TM</sup>3-conjugated Streptavidin (1:1000; Jackson Immunoresearch, Newmarket, United Kingdom) for 4 hrs at room temperature. (B) As a positive control, SP-immunohistochemistry stain results in the paraventricular nucleus of hypothalamus in the wild type mice. Scale bar: 100  $\mu\text{m}$ .

*Abbreviations: substance P (SP).*



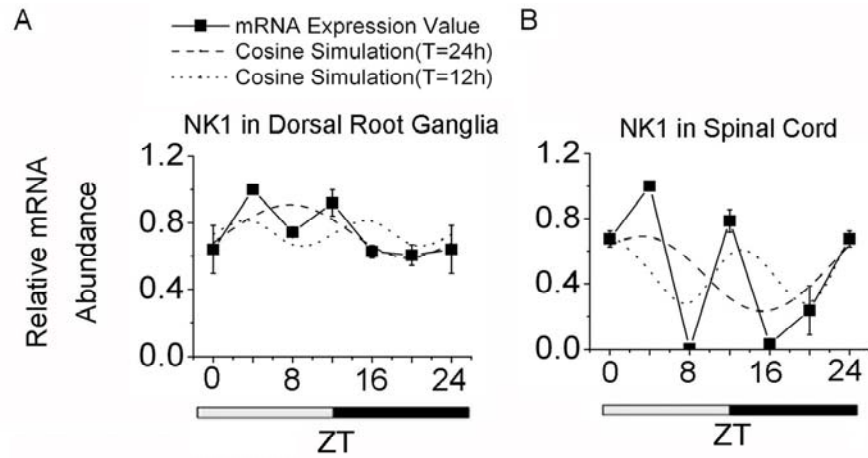
**Fig. S2. Losing the circadian oscillating trait of the positive regulators *Bmal1* and *Clock* in SC resulted in arrhythmic expression of *Tac1*.**

(A) The message RNA of *Bmal1*, *Clock* and *Tac1* were expressed in weak or non-circadian fashion in the spinal cord. Values are shown as mean  $\pm$  SE. of 6 mice per time point. Data at ZT0 are plotted twice (ZT0 and ZT24). Same methods for time-effect analysis were applied as well. See legend to Figure 1 for details. (B) Using the same procedure of Chromatin immunoprecipitation assay in DRG, the class I E-box element binding activity to BMAL1 was examined in spinal cord. *Abbreviations:* dorsal root ganglion (DRG); messenger RNA (mRNA); spinal cord (SC); zeitgeber time (ZT).



**Figure S3. Temporal mRNA expression patterns of NRs genes in spinal cord.**

The message RNA of *NRs* genes were expressed in weak (NR2A) or non-circadian fashion (NR1, NR2B, NR2C) in the spinal cord. Values are shown as mean  $\pm$  SE. of 6 mice per time point. Data at ZT0 are plotted twice (ZT0 and ZT24). Same methods for time-effect analysis were applied as well. See legend to Figure 1 for details. *Abbreviation: messenger RNA (mRNA), N-methyl-D-Aspartate receptors (NRs); zeitgeber time (ZT).*



**Figure S4. Temporal message RNA expression patterns of *NK1* receptor gene in DRG and SC.**

The mRNA of *NK1* receptor gene was expressed in non-circadian fashion in dorsal root ganglia (A) and spinal cord (B). Values are shown as mean  $\pm$  SE of 6 mice per time point. Data at ZT0 are plotted twice (ZT0 and ZT24). Same methods for time-effect analysis were applied as well. See legend to Figure 1 for details. *Abbreviations: dorsal root ganglia (DRG); messenger RNA (mRNA); Neurokinin-1 (NK1); spinal cord (SC); zeitgeber time (ZT).*