

1 **Supplemental Information**

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3 **Dual origins of the intracellular circadian calcium**
4 **rhythm in the suprachiasmatic nucleus**

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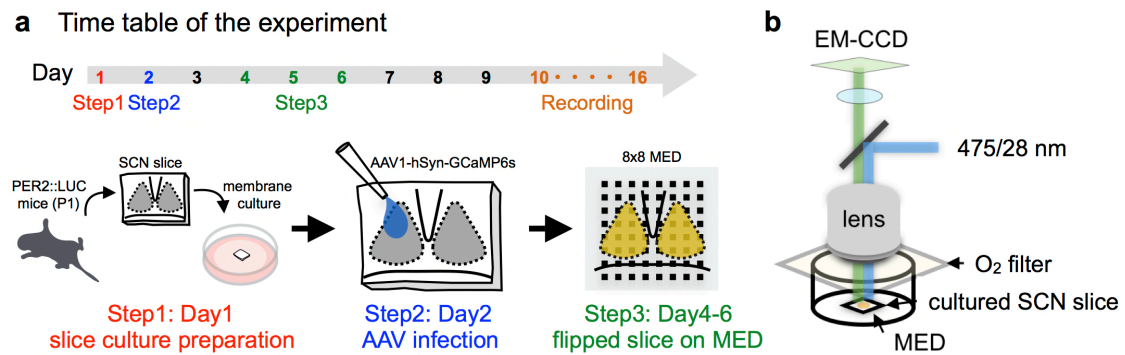
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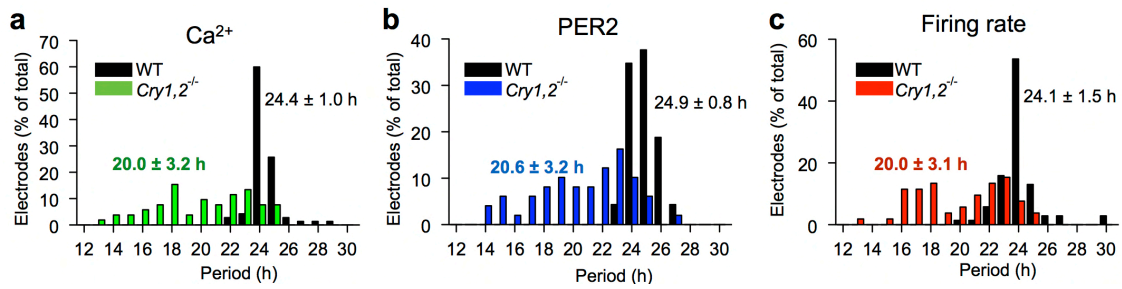
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21 **Fig. S1. Experimental procedure for simultaneous recording.**

22 (a) Step 1 (Day 1): The SCN slice was prepared from PER2::LUC knock-in
 23 newborn mice of postnatal day 1, and was explanted on a culture membrane.
 24 Step 2 (Day 2): Aliquots of the adeno-associated virus (AAV) (1 μ L) harboring
 25 GCaMP6s under the control of the human synapsin-1 promoter (hSyn) were
 26 inoculated onto the surface of the SCN cultures. Step 3 (Days 4–6): The
 27 membrane with the cultured SCN slice was cut out, flipped over, and transferred
 28 to a multielectrode array dish (MED) with 64 electrodes. Simultaneous recording
 29 of three measures was started from the 10th culture day. (b) The fluorescence of
 30 GCaMP6s and the bioluminescence of PER2 expression were measured by a
 31 high-sensitivity EM-CCD camera mounted on an upright microscope.
 32 Spontaneous firing was recorded by an MED system. The MED was sealed with
 33 an O₂-permeable filter. GCaMP6s was excited using 475/28 nm LED.



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36 **Fig. S2. Statistical comparison of the circadian period of Ca^{2+} , PER2, and**
 37 **spontaneous firing rhythms in the wild-type and $\text{Cry1,2}^{-/-}$ SCN.**

38 The fluorescence/bioluminescence signals were obtained in ROIs on each MED
 39 electrode and the distributions of the circadian period were analyzed in wild-type
 40 and $\text{Cry1,2}^{-/-}$ SCN for Ca^{2+} (a), PER2 (b), and spontaneous firing rhythms (c). All
 41 data are given as means \pm sd. Wild-type (WT): $n = 70$ in four slices, $\text{Cry1,2}^{-/-}$: n
 42 $= 52$ in four slices.