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Supplemental information

Dorsal clock neurons in *Drosophila* sculpt locomotor outputs but are dispensable for circadian activity rhythms

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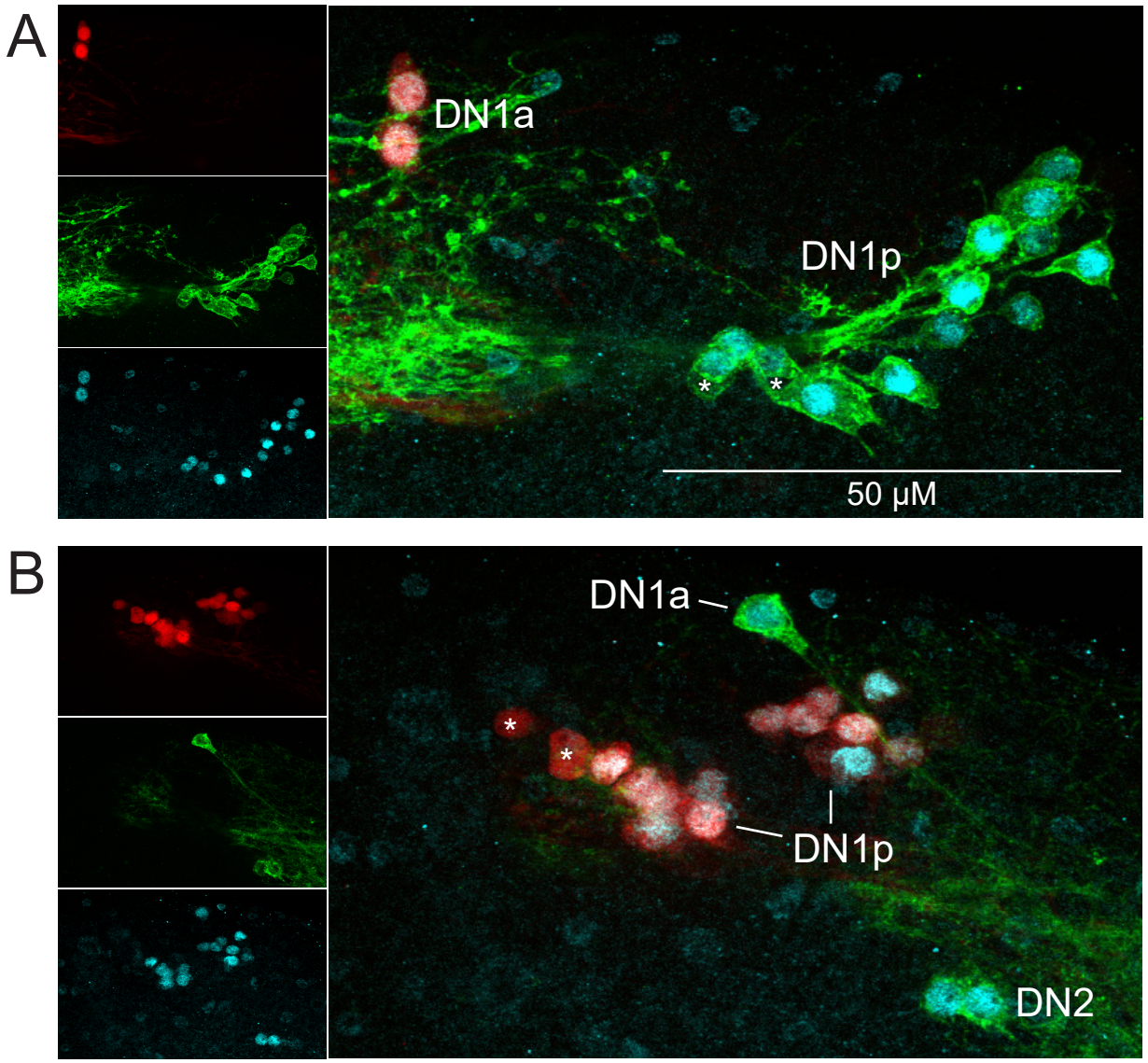

A
DN1a
DN1p
*
*
50 μM
B
DN1a
*
*
DN1p
DN2
C
LNd
LNv
D
LNd
LNv
25 μM

Figure S1. Intersectional Genetic Strategy Comprehensively Labels DN1p Cells, Related to Figure 2

(A) Representative maximum projection confocal image of the dorsal region of a DN1p Flip-In brain co-stained for mCherry (red; top left) Kir2.1^{eGFP} (green; middle left), and PER (cyan; bottom left). A merged image is shown on the right. Note that all PER+ DN1p cells are labeled with Kir2.1^{eGFP}, confirming that our intersectional approach comprehensively labels DN1p cells. There are additionally 2 GFP+ cells (marked by asterisks) which are not co-labeled by PER. In this brain, mCherry labels 2 DN1a cells, which are also PER+. (B) Representative maximum projection confocal image of the dorsal region a DN1p Flip-Out brain co-stained for mCherry (red; top left) Kir2.1^{eGFP} (green; middle left), and PER (cyan; bottom left). A merged image is shown on the right. All PER+ DN1p cells are co-labeled with mCherry, indicating efficient and comprehensive recombination in DN1p cells. There are also 2 mCherry+ cells (marked by asterisks) which are not PER+. (C-D) Representative maximum projection confocal images of the ventrolateral region of DN1p Flip-In (C) and Flip-Out (D) brains co-stained for mCherry (red; top left) Kir2.1^{eGFP} (green; middle left), and PER (cyan; bottom left). Note that the Flip-In and Flip-Out approaches label a single PER- cell (indicated by the arrow) in the vicinity of the LNd clock neurons. This cell is Kir2.1^{eGFP}+ in the Flip-In brain (C) and mCherry+ in the Flip-Out brain (D).

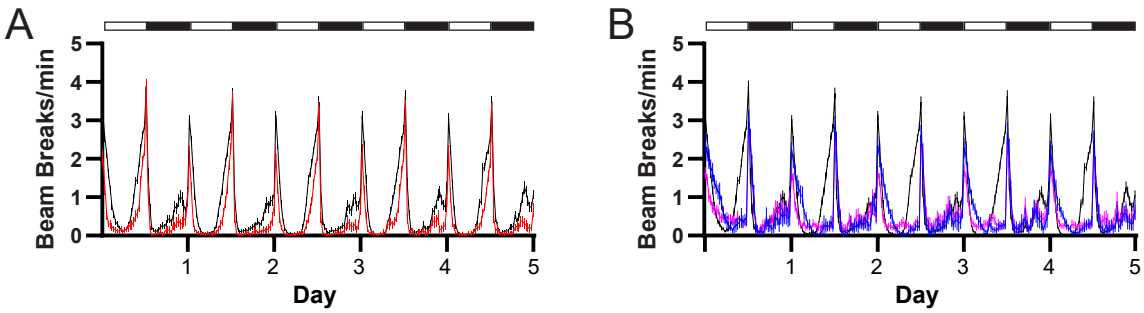
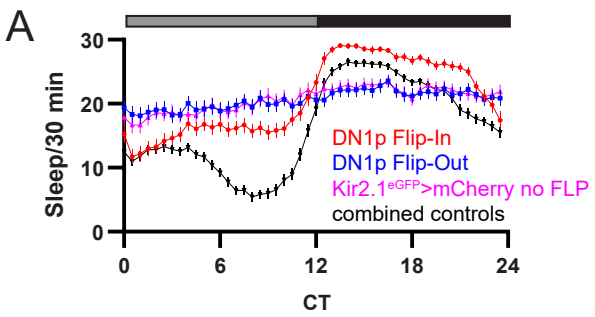
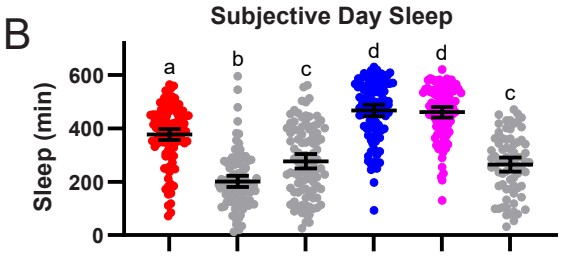
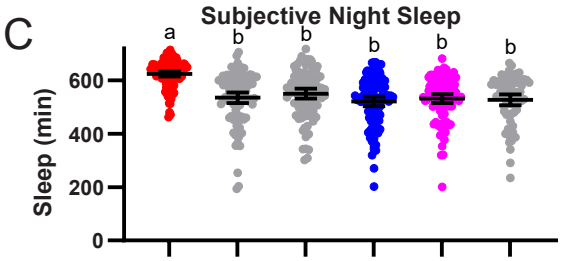


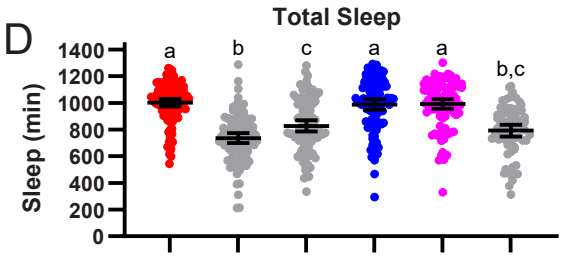
Figure S2. Kir2.1^{eGFP} Expression in DN1p Cells Reduces Overall Activity Levels Without Affecting Anticipatory Activity in Light-Entrained Conditions, Related to Figure 5

(A) Mean number of DAM beam breaks/min (± 95% confidence interval) is plotted over 5 days in LD conditions for DN1p Flip-in Flies (red) compared to control flies (black). (B) Mean DAM beam breaks/min is plotted as in (A) for DN1p Flip-Out flies (blue), flies in which all Clk856+ clock neurons are silenced with Kir2.1^{eGFP} (pink), and control flies (black). The same control data are graphed in (A) and (B) and consists of a combination of 3 control lines lacking Kir2.1^{eGFP} expression (see Methods). White and black bars indicate light and dark periods, respectively. Note that DN1p Flip-In flies have reduced activity levels compared to controls during both the day and night, but retain morning and evening anticipatory activity (ramping up activity prior to light transitions).









n	108	97	94	104	99	74
Clk856-GAL4	•	•		•	•	
InSITE911-QF	•		•	•		•
QUAS-FLP	•	•	•	•	•	•
UAS>Kir2.1eGFP-stop>mCherry				•	•	•
UAS>mCherry-stop>Kir2.1eGFP	•	•	•			
cells inhibited	DN1p	none	none	non-DN1 clock	all clock	none

Figure S3. Kir2.1^{eGFP} Expression in DN1p Cells Increases Sleep Duration in DD Conditions, Related to Figure 6

(A) Mean sleep/30 min (± 95% confidence interval) over the course of a 24-hr day is plotted for DN1p Flip-In flies (red), DN1p Flip-Out flies (blue), flies in which all Clk856-GAL4+ clock cells are silenced (pink), and control flies (black). Control plot consists of data from a combination of 3 control lines lacking Kir2.1^{eGFP} expression (see Methods). Data are averaged over 5 days in DD conditions. Grey and black bars indicate subjective day and subjective night, respectively. (B-D) Total subjective day, subjective night, or 24-hr sleep is plotted for the indicated genotypes. Lines are means ± 95% confidence intervals. Dots represent individual flies. Different letters indicate significant difference ($p < 0.05$), Tukey's multiple comparisons test following one-way ANOVA. The same letter indicates no significant different ($p > 0.05$).

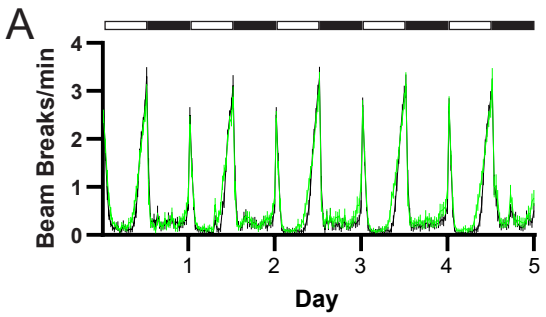


Figure S4. Kir2.1^{eGFP} Expression in DN1a Cells Prolongs Evening Anticipatory Activity and Enhances Morning Anticipatory Activity in Light-Entrained Conditions, Related to Figure 9
(A) Mean number of DAM beam breaks/min (± 95% confidence interval) is plotted over 5 days in LD conditions for experimental flies with spl-DN1a-GAL4-driven expression of Kir2.1^{eGFP} (green) and control flies with spl-DN1a-GAL4-driven mCherry (black). Note that silencing of DN1a cells causes prolonged evening anticipatory activity (preceding lights-off), starting earlier in the evening than control flies, and also increases the slope and magnitude of morning anticipatory activity (preceding lights-on).

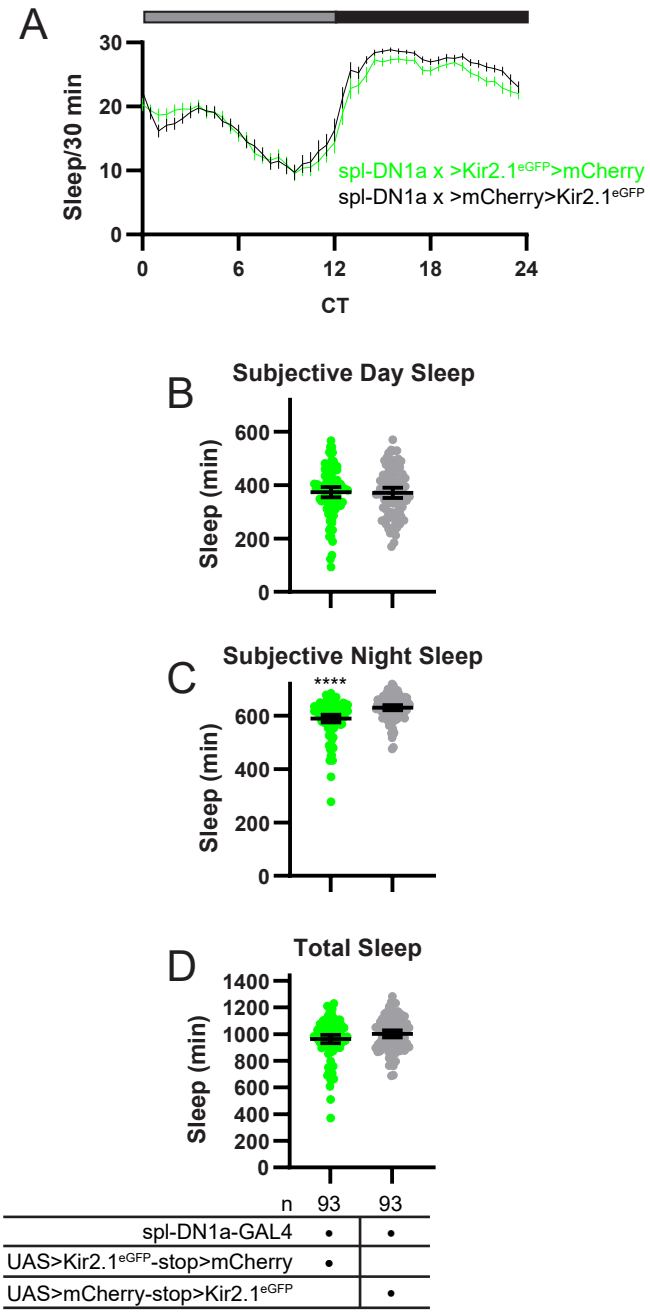


Figure S5. Kir2.1^{eGFP} Expression in DN1a Cells Decreases Sleep Duration in DD Conditions, Related to Figure 10

(A) Mean sleep/30 min (± 95% confidence interval) over the course of a 24-hr day is plotted for flies with spl-DN1a-GAL4-driven expression of Kir2.1^{eGFP} (green) and control flies with spl-DN1a-GAL4-driven mCherry (black). Data are averaged over 5 days in DD conditions. Grey and black bars indicate subjective day and night, respectively. (B-D) Total daytime, nighttime, or 24-hr sleep is plotted for the indicated genotypes. Lines are means ± 95% confidence intervals. Dots represent individual flies. ****$p <$ 0.0001, t-test.