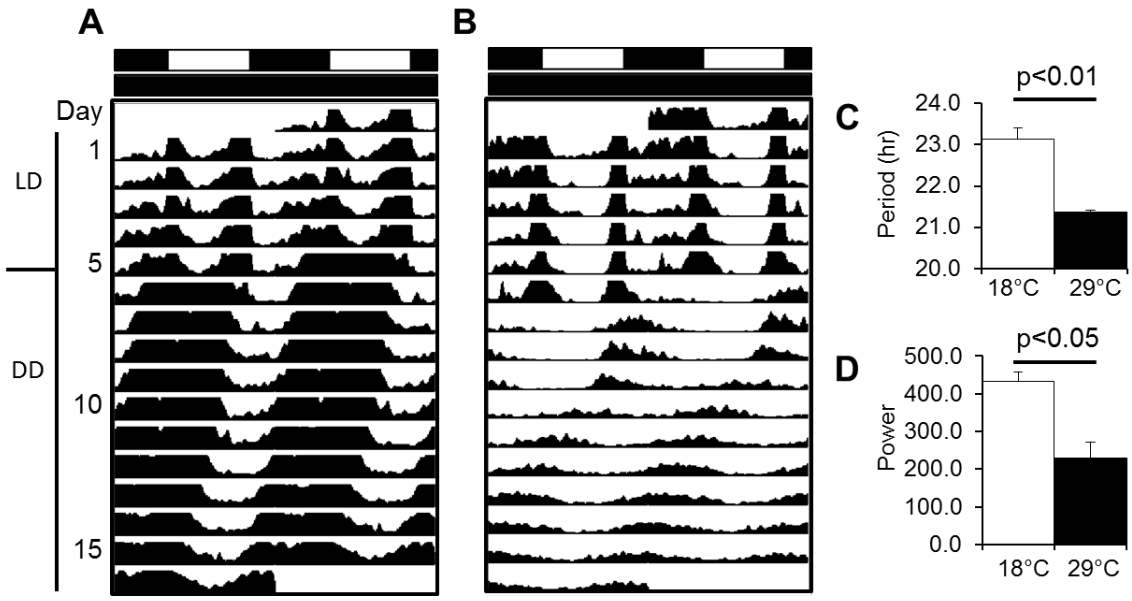


Figure S1. The locomotor activity rhythms of *Mz520Gal4/UAS-Kir2.1; tubGal80^{ts}/+* (*Mz520>Kir, tubGal80^{ts}*) flies at 18°C (A) and 29°C (B) in light-dark (LD) cycles and constant darkness (DD), related Figure 3.

(A and B) Average activity profiles of *Mz520>Kir, tubGal80^{ts}* flies at 18°C (A) (N=30) and 29°C (B) (N=13). *Mz520>Kir, tubGal80^{ts}* flies were reared at 18°C in LD cycles. The locomotor activity was measured under LD cycles for the first 5 days and subsequently in DD at 18°C (A) and 29°C (B). The white and black bars above the actograms indicate light and dark conditions, respectively.

(C and D) Mean free-running period (C) and power value (D) (mean±SEM) under DD. Student's t-test was used to determine the P-value.

	Period (hr)			Power			Rhythmic %	N (total)	N (Rhythmic)
	Ave	STDV	SEM	Ave	STDV	SEM			
Mz520gal4>Kir, tubGal80^{ts} (18°C)	23.13	0.26	0.05	433.60	224.24	40.94	100.00	30	30
Mz520gal4>Kir, tubGal80^{ts} (29°C)	21.36	0.43	0.18	229.80	64.75	26.43	46.15	13	6

Table S1. Comparison of free-running period and power value between 18°C and 29°C, related to Figure 3.

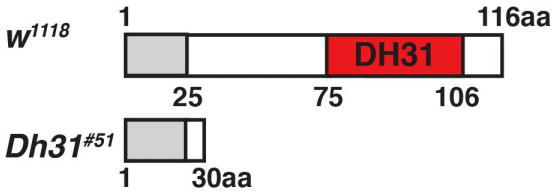
```
WT       ATGACAAACCGATGCGCTTGCTTCGCCTTGGCCTTCCTCCTCTTCTGCCT
Dh31#51  ATGACAAACCGATGCGCTTGCTTCGCCTTGGCCTTCCTCCTCTTCTGCCT

WT       CTTGGCCATCTCGAGCATCGAGGCAGCTCCGATGCCCAGGTAGTTGTACC-100
Dh31#51  CTTGGCCATCTCGAGCATCGAGGCAGCTCCGATGCCCAGGTAGTTGTACC

WT       CAATCACCTAGTTGTACCCCAACCACATGCAGACATATCCTTTCCAATTT
Dh31#51  CAATCACCTAGTTGTACCCCAACCACATGCAGACATATCCTTTCCAATTT

WT       GGCTGAACACATACAAAAGGACATAGCCTAATTAAATTCCGGCACTTAAG-200
Dh31#51  GGCTGAACACATACAAAAGGACATAGCCTAATTAAATTCCGGCACTTAAG

WT       CTGCAAAAACCGAAAAATGTGCCACACAAATATTTGAGGCAAGCAATTAT
Dh31#51  CTGCAAAAACCGAAAAATGTGCCACACAAATATTTGAGGCAAGCAATTAT

WT       CCCACTGAGTATGAATCTTGCCCATGTGGGAGATGGAAATATACTTAGGC-300
Dh31#51  CCCACTGAGTATGAATCTTGCCCATGTGGGAGATGGAAATATACTTAGGC

WT       AGCTGAAAAATAAAATATATAAAAGAAAACGCTCACATGGCGAAGGAAAA
Dh31#51  AGCTGAAAAATAAAATATATAAAAGAAAACGCTCACATGGCGAAGGAAAA

WT       TTTTCAATTAACAAAATGTAAACGTTGACAGAAAAGTGTGTCAAAAATTG-400
Dh31#51  TTTTCAATTAACAAAATGTAAACGTTGACAGAAAAGTGTGTCAAAAATTG

WT       CAGTACTTGGCCACACCACTTGAGTCGTTGATGTAGATGTAGCATATTCT
Dh31#51  CAGTACTTGGCCcatgatga

WT       AAGGACATGCCTTATGTGTGGTATGGTTTCAAACTCAATTTCCCTTCCAT-500

WT       CAAAGTAACTATTAAAAATTCATTGACCCTGGCCAACTAATTAGCAATTG

WT       CTCGAATGGAAAATGCTCCAAACATTTGGGCTTTAACATATAATTGGGTT-600

WT       TTCATGCAAATGAAGATTTTAAGCCAGTCGAATGTCAAATTCAACGGGGC

WT       CAACGTAATTAGATATCCTGTTCAGCCGCATTGTATTGTATAAATACGAA-700

WT       CAAATTCCCCATCTAATTTTCCATTTCGTTGACTTTAAAATAGCCAATCC

WT       AATGGAGGATACGGTGGCGCTGGCTATAACGAACTGGAGGAGGTGCCCGA-800

WT       CGACCTACTCATGGAACTGATGACTCGCTTTGGACGCACCATCATACGGG

WT       CTCGAAACGATCTGGAGAAGTAAGTATTGAAGACAGATATAACTGTCTAA-900

WT       TTAGAAATTGTTTAATACATATGTGCAACTAAAGTTCTCTTACTCATTTA

WT       AATGCTACCAGTAGTAAGGTTAATTGTTAGAGCCTAATCTAACCAAATCA-1000

WT       TTTGTAACTGCTAAACTTCTTTCTCTTCCAAAGTTCCAAACGAACCGTGG

WT       ACTTTGGCTTGGCCCGGGGATATTCGGGTACCCAGGAGGCGAAACATCGC-1100

WT       ATGGGTCTGGCTGCAGCCAACTTTGCCGGAGGACCCGGTCGGAGGCGACG
Dh31#51                                                  ACG

WT       ATCCGAGACCGATGTCTAAGGCGTCTGGATGAGACCGGGCTGCGAATCCT-1200
Dh31#51  ATCCGAGACCGATGTCTAAGGCGTCTGGATGAGACCGGGCTGCGAATCCT
```

Red: Exon
Black: Intron
Based on FlyBase:FBpp0079307, IDs:CG13094-PA (DH31-RA)

Figure S2. Alignment of the Dh31 genome sequence between WT and *Dh31*$^{\#51}$ mutant, related to Figure 4.

Dh31 genome sequences of WT (red: exon, intron: black) and Dh31$^{\#51}$ (blue) are shown. CG13094-PA was used for the WT genome sequence. The stop codon (WT) and putative stop codon (*Dh31#51*) are underlined.

A



B

```
WT          MTNRCACFALAFLLFCLLAISSIEAAPMPSQSNGGYGGAG-40
Dh31#51     MTNRCACFALAFLLFCLLAISSIEAAPMPR-30

WT          YNELEEVPDDLLMELMTRFGRTIIRARNDLENSKRTVDFG-80

WT          LARGYSGTQEAKHRMGLAAANFAGGPGRRRRSETDV-116
```

Figure S3. The schematic of DH31 amino acid sequences (A) and alignment of amino acid sequences (B) of w^{1118} and $Dh31^{\#51}$ flies, related to Figure 4.

The active DH31 peptide sequence (31aa) is represented in the red box (A) and shown in red (B). The active DH31 peptide sequence was removed in the $Dh31^{\#51}$ mutants. The 25-residue signal peptide is represented in the gray box (A) and is underlined (B). The peptide processing sites (N-terminal: KR, C-terminal: GRR) are shown in bold (B) as described previously [S1]. CG13094-PA was used for the WT sequences.

Supplemental Experimental Procedures

Fly lines

All flies were raised in 12 h light/dark cycles at 25 °C; Zeitgeber Time (ZT) 0 refers to lights-on, ZT12 refers to lights-off. All fly lines used in this study were received from Bloomington *Drosophila* Stock Center except for the following lines: *Clk4.5F-Gal4, cry^{02} and cry^{b}* (from Dr. Patrick Emery), *Pdf^{01}, $pdfr^{5304}$ and $pdfr^{5304}$; UAS-pdfr (*from Dr. Paul Taghert*), Pdf-LexA* and *R6-Gal4* (from Dr. Orie Shafer), *hdc^{JK910}* (from Dr. Hugo Bellen) *Mz520-Gal4* (from Dr. Charlotte Helfrich-Förster) and *UAS-pdfr-RNAi* (from Vienna *Drosophila* RNAi Center).

Generation of DH31 mutant flies

The P-element of *y^{1};P{SUPor-P}$Dh31^{KG09001}$* flies (BL#16474) was hopped to create *$Dh31^{\#51}$* mutants. The truncated genome region of *$Dh31^{\#51}$* mutants was determined by sequence analysis. The coding sequence (31-116aa) was removed in the *$Dh31^{\#51}$* mutants (Supplemental Figures S2 and S3) and therefore, *$Dh31^{\#51}$* mutant are a severe loss-of-function mutant.

Immunohistochemistry

Immunostaining was performed as described previously [S2, S3] except 10% normal goat serum in PBST (PBS plus 0.5% Triton X-100) was used for blocking and antibody incubations. Antibodies were used at the following dilutions: mouse anti-Glass (1:200; Developmental Studies Hybridoma Bank), rat anti-TIM (1:200; from Dr. Michael Rosbash), rabbit anti-GFP (1:200; Invitrogen Cat# A6455), goat anti-rabbit FITC (1:200; Jackson ImmunoResearch), donkey anti-rabbit Cy5 (1:200; Jackson ImmunoResearch), goat anti-rat Cy5 (1:200, Jackson ImmunoResearch) and donkey anti-mouse Cy5 (1:200, Jackson ImmunoResearch). Mounted brains were scanned using a Zeiss LSM5 Pascal confocal microscope. Images are digitally projected Z-stacks.

Temperature preference assays

Temperature preference assays were performed as described previously [S2, S4, S5]. Each behavioral assay was performed for 30 minutes in an environmental room maintained at 25˚C/65-70% RH. All flies of both sexes were raised under LD (light 12 h/dark 12 h) conditions at 25˚C except for the flies used in the conditional experiments (Figure 3). The *R6-Gal4/UAS-Kir; tub-Gal80^{ts}/+* and *Mz520-Gal4/UAS-Kir; tub-Gal80^{ts}/+* flies were raised at 18˚C and were either kept at 18˚C (permissive temperature) or transferred to 29˚C (restrictive temperature) at the adult stage for two days until right before the experiments. The preferred temperature was determined by examining the distribution of flies exhibiting temperature preference behavior.

Data analysis

The method used to calculate the mean preferred temperature has been described previously [S4, S5]. After the 30-minute behavioral assay, the number of flies that were completely on the apparatus was counted. Flies that were partially or completely on the walls of the apparatus cover were not counted or included in the data analysis. The percentage of flies within each one-degree temperature interval on the apparatus was calculated by dividing the number of flies within each one-degree interval by the total number of flies on the apparatus. The location of each one-degree interval was determined by measuring the temperature at 6 different points on both the top and the bottom of the apparatus. Data points were plotted as a percentage of flies within a one-degree temperature interval. The weighted mean preferred temperature was calculated by summing the products of the percentage of flies within a one-degree temperature interval and the corresponding temperature (e.g., % of flies X 18.5ºC + % of flies X 19.5ºC + ... % of flies X 32.5ºC). We conducted the temperature preference behavioral assay at least five times in each time zone (ZT1-3, 4-6, 7-9, 10-12, 13-15, 16-18, 19-21 and 22-24). Each data curve represents a

minimum of 40 experiments. The weighted mean preferred temperature was averaged and we calculated s.e.m. in each time zone.

Locomotor activity

Flies were reared in 18°C and light-dark (LD) conditions in the agar/sucrose medium. 1- to 5-day-old flies were used to monitor the locomotor activity. Drosophila Activity Monitoring (DAM) system (http://www.trikinetics.com/) was placed in a incubator (MIR-154, Sanyo Scientific, Japan). Lights in the incubator (a 15-W cool white fluorescent lamp (FL15D, TOSHIBA, Japan)) were connected to an electric timer. Light intensity is around 800lux. The method of locomoter activity assays has been descried previously.

The free-running period was calculated by the chi-square periodogram [S6] with Actogram J software [S7]. The power value was calculated according to the previous report [S8].

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