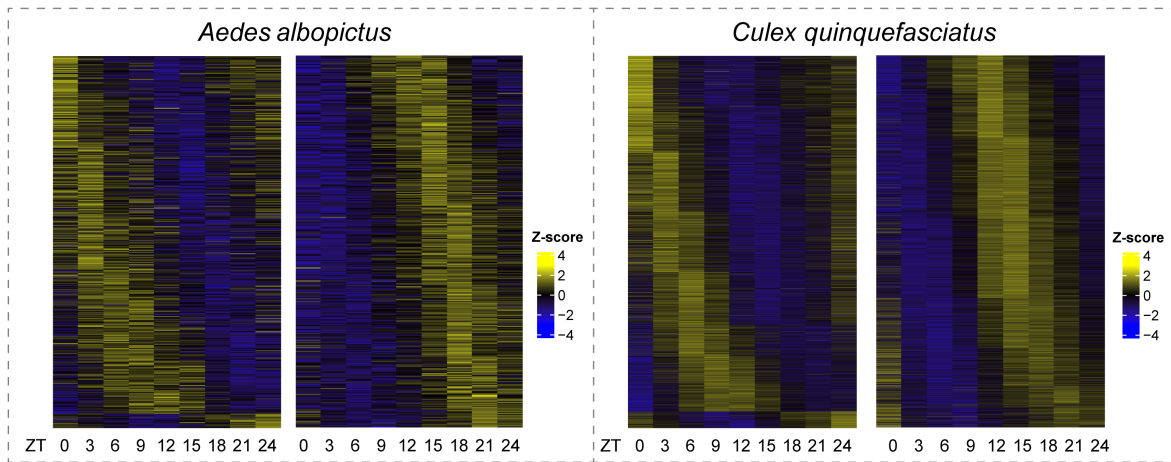


- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13
- 14
- 15
- 16
- 17
- 18
- 19

Hongkai Liu, Ling Kong, Siyu Zhao, Zhensheng Xie, Leyang Li, Chenyu Han, Junyu Fu, Jing Wu, Shuang Liu, Jiayong Zhou, Xiaolin Xiao, Chunmei Wang, Jinbao Gu, Xiaohong Zhou, Anthony A. James, Xiao-Guang Chen

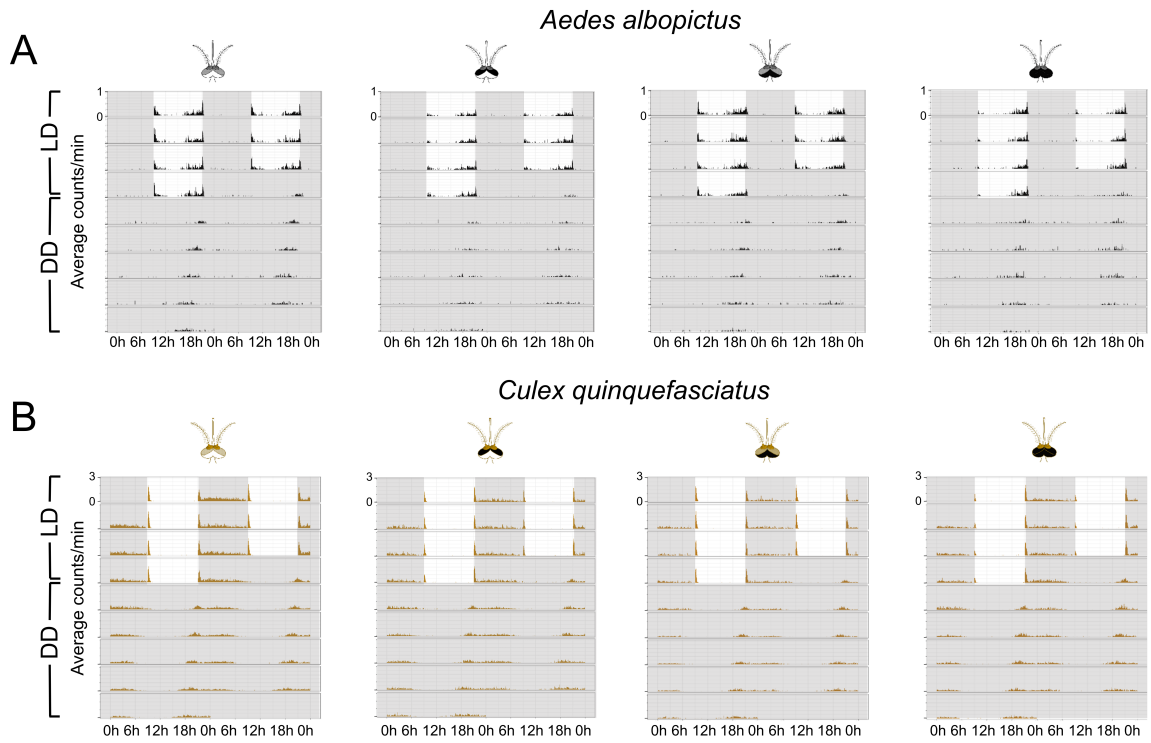
Corresponding author: Xiao-Guang Chen, xgchen@smu.edu.cn

Supplementary Figures. 1 to 12  
Supplementary Tables. 1 to 8



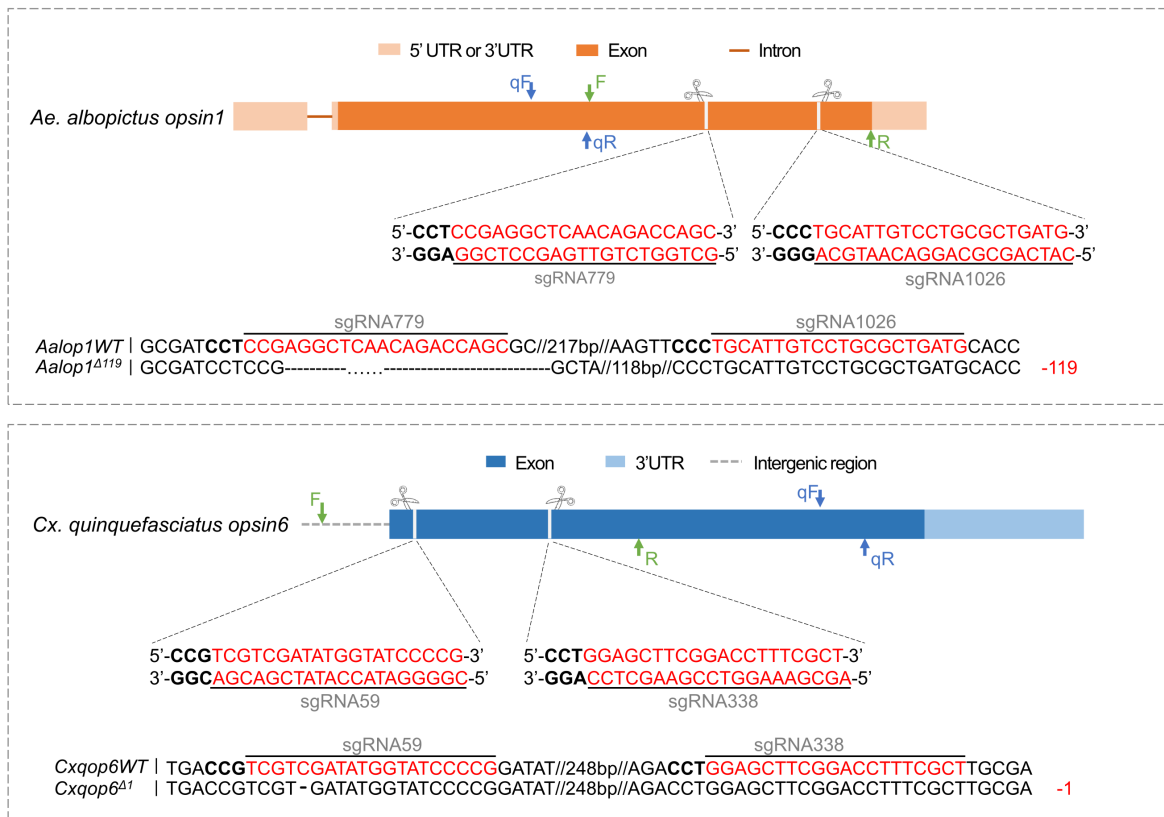
**Supplementary Figure 1. Time-dependent heatmap of RNA-seq data from *Aedes albopictus* and *Culex quinquefasciatus*.**

Each row represents a rhythmic transcript, and each column represents a ZT (Zeitgeber Time) point. Gene expression values are normalized using Z-score transformation, where green indicates relatively higher expression and red indicates relatively lower expression.. The left two panels show data for *Aedes albopictus*, and the right two panels show data for *Culex quinquefasciatus*. Time points are indicated on the x-axis (ZT 0, 3, 6, 9, 12, 15, 18, 21, 24, n = 20 at each time point).



**Supplementary Figure 2. Double-plotted actograms of paint-blocked *Ae. albopictus* and *Cx. quinquefasciatus*.**

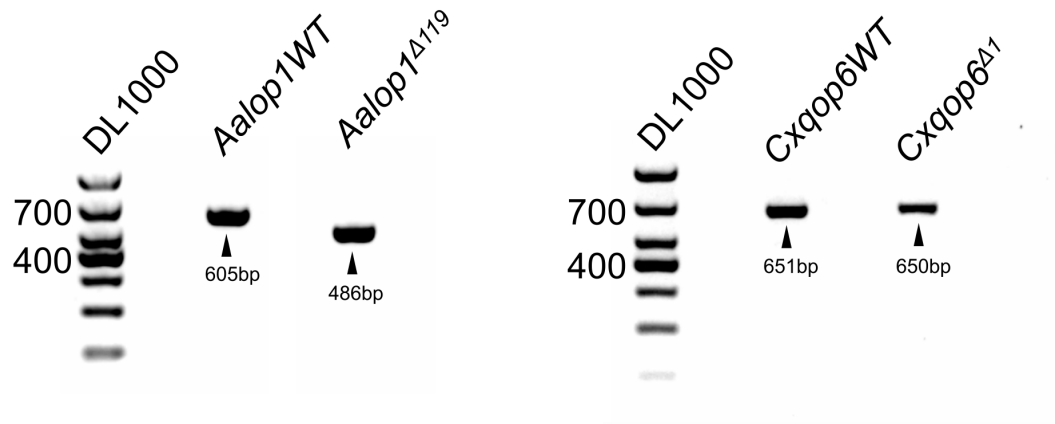
**(A)** Continuous activity profiles of paint blocked and control groups of *Ae. Albopictus*.  
**(B)** Continuous activity profiles of paint-blocked and control groups of *Cx. quinquefasciatus*. From left to right: WT group, CE-blocked group, OL-blocked group, CE+OL-blocked group. The white areas represent lights on, and the gray areas represent lights off.



**Supplementary Figure 3. Schematic diagram of sgRNA-targeting sites to induce *opsin1* gene mutation in *Ae. albopictus* and *opsin6* gene mutation in *Cx. quinquefasciatus* using CRISPR/Cas9 technology.**

Schematic diagram of sgRNA-targeting sites to induce *Aalopsin1* gene mutation in *Ae. albopictus* and *Cxqopsin6* gene mutation in *Cx. quinquefasciatus* using CRISPR/Cas9 technology. The gray scissors represent the corresponding positions of sgRNA in the exons. The red sequence is sgRNA. The dotted line indicates base deletions. The numbers at the end of the sequences indicate the number of deleted bases in the genome. Green arrows indicate the positions of mutation identification primers, and blue arrows indicate the positions of qPCR detection primers.

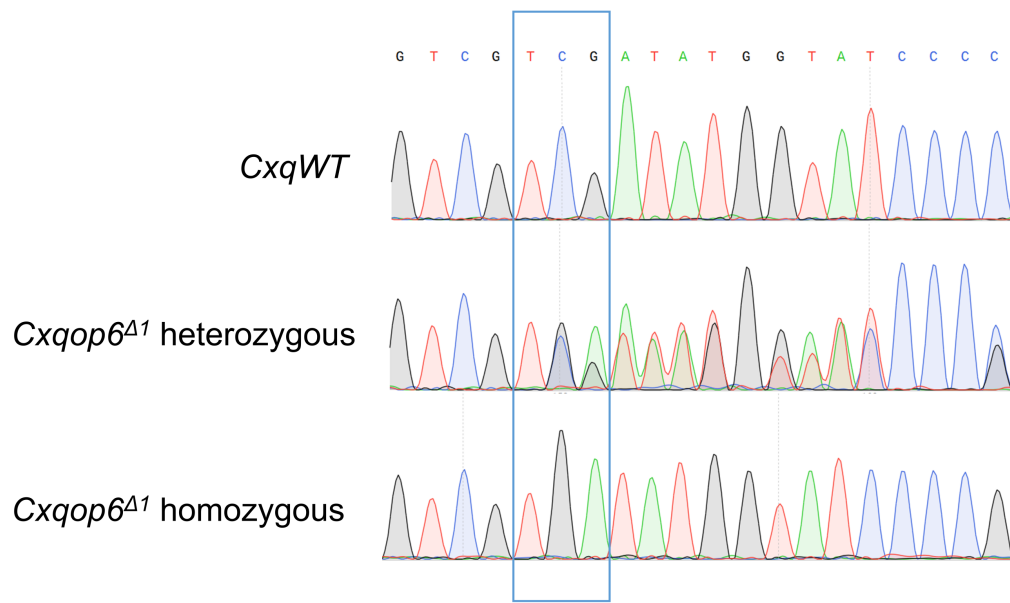
52



53

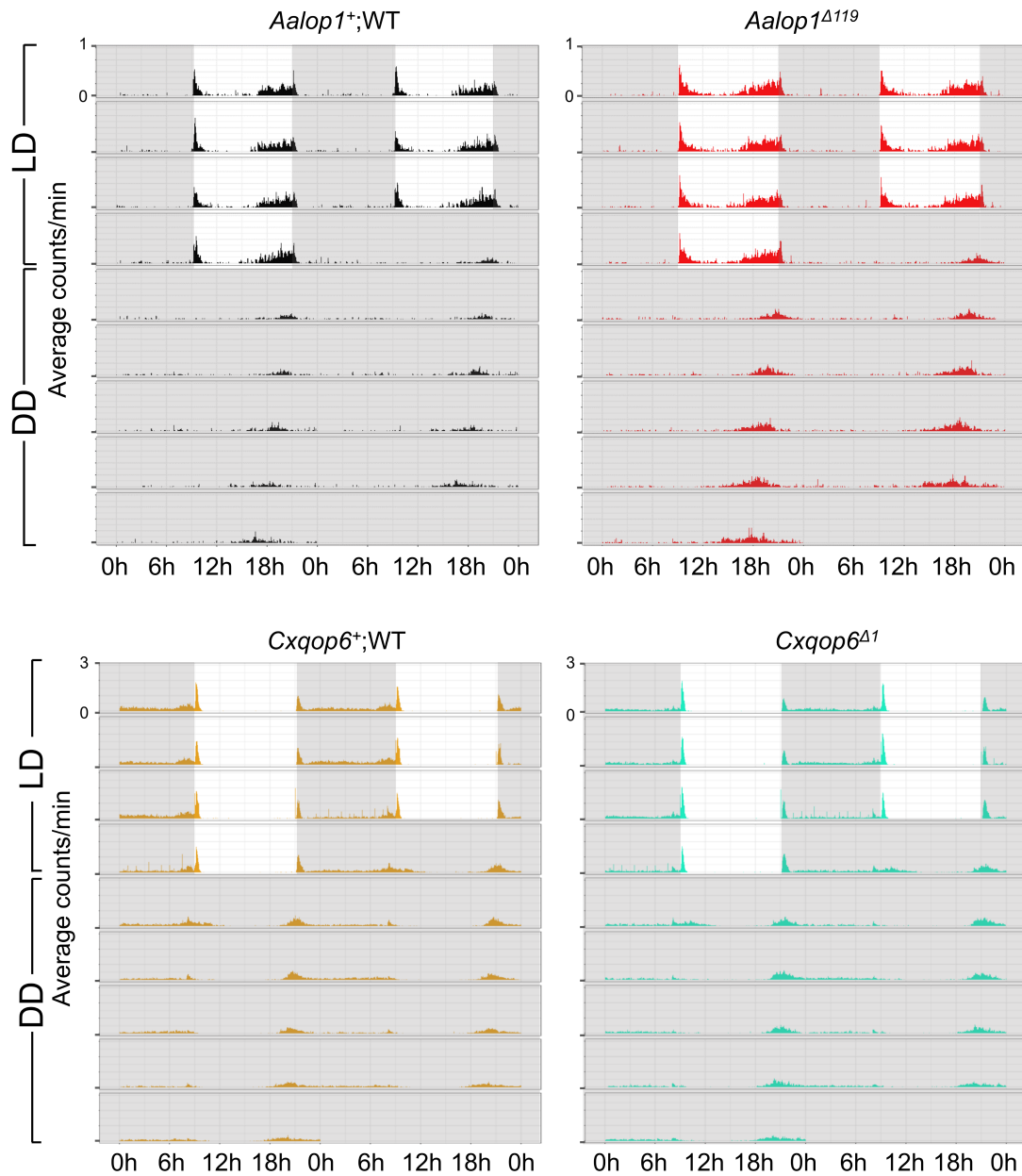
54 **Supplementary Figure 4. PCR identification of *Aalopsin1* and *Cxqopsin6* knockout**  
55 **strains.**

56 Genomic DNA extracted from *Aalop1*<sup>Δ119</sup> and *Cxqop6*<sup>Δ1</sup> and mutations were confirmed  
57 with PCR (This blot is presented in its uncropped form).  
58



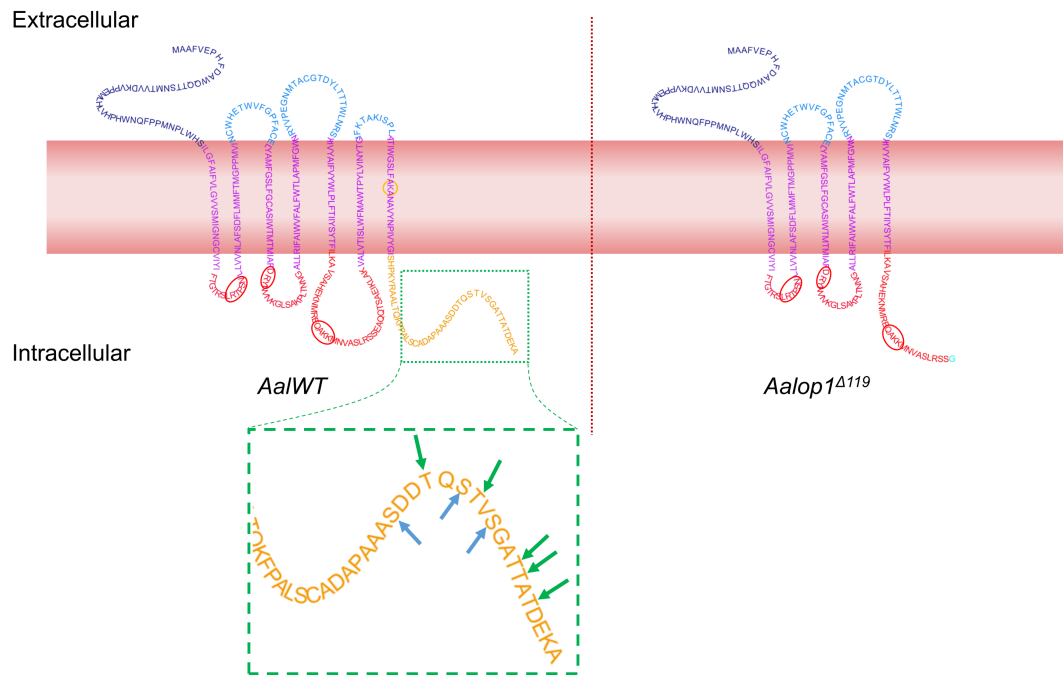
59

60 **Supplementary Figure 5. Partial DNA sequence alignments of *Cx. quinquefasciatus***  
 61 ***Cxqopsin6* wild-type (WT) and mutant knockout strain.**



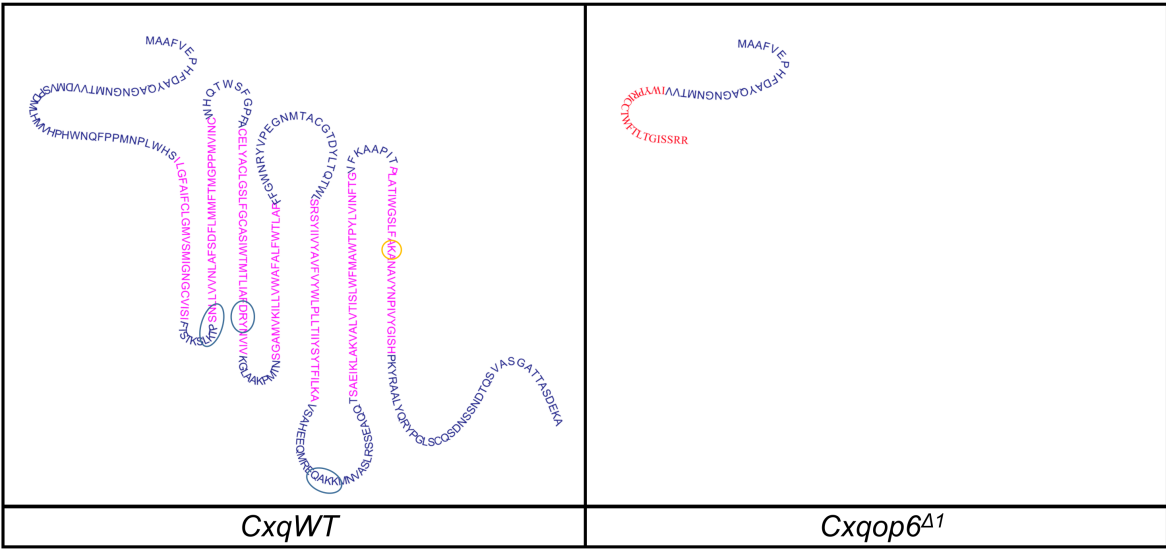
**Supplementary Figure 6. Double-plotted actograms of wild-type, *Aalopsin1* and *Cxqopsin6* knockout strains.**

The white sections represent light-on, and the gray sections represent light-off. Black represent wild-type *Ae. albopictus* (n = 59), red represent *Aalop1<sup>Δ119</sup>* strains (n = 65), brown represent wild-type *Cx. quinquefasciatus* (n = 61), and green represent *Cxqop6<sup>Δ1</sup>* strains (n = 59). LD indicates a light-dark cycle of 12 hours light and 12 hours darkness, while DD indicates continuous darkness.



**Supplementary Figure 7. Aalopsin1 protein secondary structure diagrams of wild-type and Aalopsin1 in knockout *Ae. albopictus* strains.**

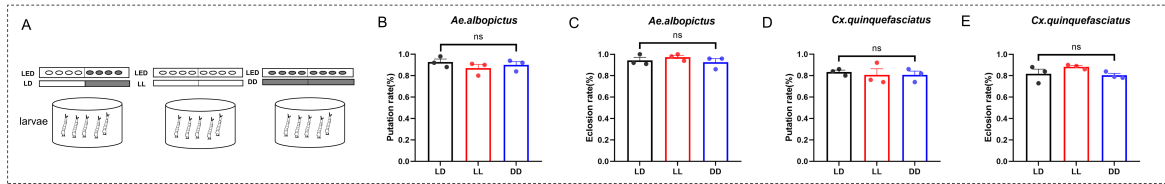
The blue sequences represent extracellular loop structures, the red sequences represent intracellular loop structures and the purple sequences represent transmembrane domains. Red circles indicate conserved binding domains within the intracellular loops, while yellow circle indicates retinal binding domains. Blue arrows indicate serine (S) residues, and green arrows indicate threonine (T) residues.



**Supplementary Figure 8. Cxqopsin6 protein secondary structure diagrams of wild-type and Cxqopsin6 knockout *Cx. quinquefasciatus* strains.**

The purple sequence indicates transmembrane domains, the red sequence represents erroneous translation due to frameshift mutations, blue circles indicate conserved binding domains in the intracellular loops, and yellow circle indicates retinal binding domains.





**Supplementary Figure 10. Effects of different lighting on development and survival.**

**(A)** Schematic diagram of larval development of *Ae. albopictus* and *Cx.*

*quinquefasciatus* under LD, LL, and DD conditions (n = 3). **(B)** Comparison of pupation

rates of *Ae. albopictus* under LD, LL, and DD conditions (n = 3). **(C)** Comparison of

eclosion rates of *Ae. albopictus* under LD, LL, and DD conditions. **(D)** Comparison of

pupation rates of *Cx. quinquefasciatus* under LD, LL, and DD conditions (n = 3).

**(E)** Comparison of eclosion rates of *Cx. quinquefasciatus* under LD, LL, and DD

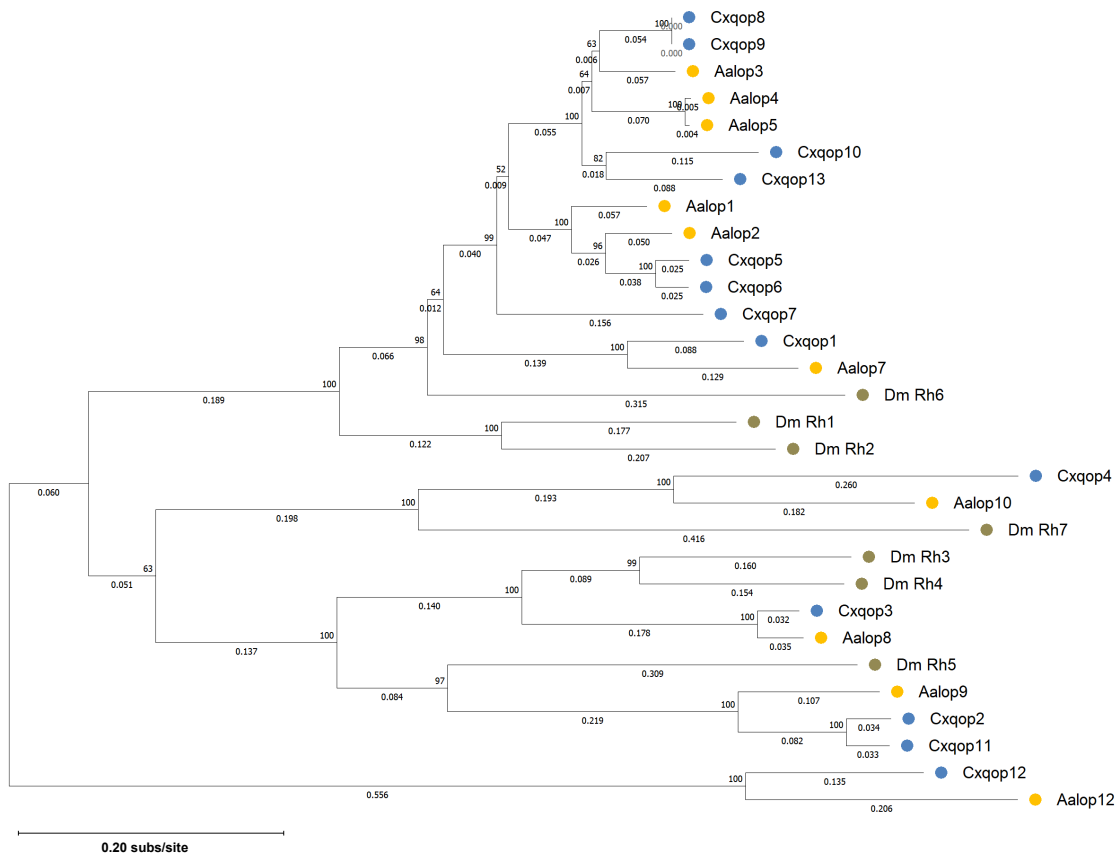
conditions (n = 3). LD (12h:12h light-dark cycle), LL (24h continuous light), and DD

(24h continuous darkness). The experiment was conducted with three biological

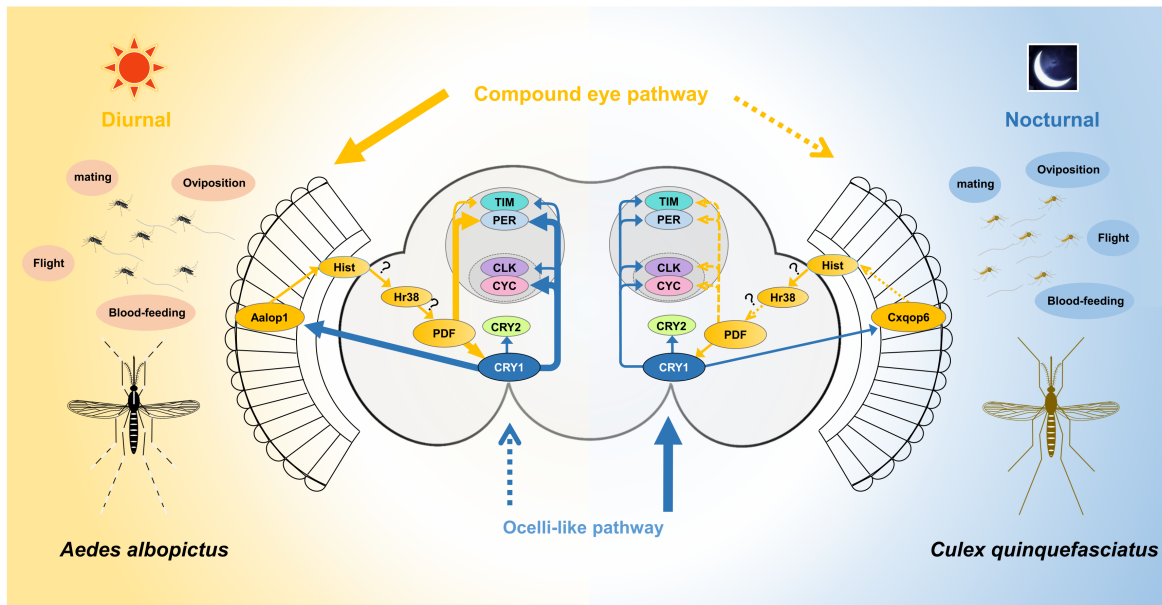
replicates. Differences in pupation and eclosion rates were determined using a Chi-square

test. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . ns, indicates no statistical difference. The

pupation and eclosion values in the figure represent the mean  $\pm$  SEM.



110  
 111 **Supplementary Figure 11. Phylogenetic tree based on protein sequences of opsins**  
 112 **from three *Dipteran* species generated with neighbor-joining tree.** Neighbor-joining  
 113 tree was constructed using MEGA11 (v11.0.11) with Poisson model+uniform rates.  
 114 Species abbreviations: Aal (*Ae. albopictus*), Cxq (*Cx. quinquefasciatus*), Dm (*D.*  
 115 *melanogaster*). The bar indicates phylogenetic distance.



**Supplementary Figure 12. Light regulates the circadian rhythms of *Aedes albopictus* and *Culex quinquefasciatus* through the compound eye and ocelli-like pathways.**

Solid lines represent primary pathways, dashed lines represent secondary pathways (The image and every element of it were created by the authors). Aalop1, Aalopsin1; Cxqop6, Cxqopsin6; PER, period; TIM, timeless; CLK: clock; CYC: cycle; CRY1, cryptochrome 1; CRY2, cryptochrome 2; Hist, Histamine; Hr38, Hormone receptor-like in 38; PDF, Pigment-Dispersing Factor.

**Supplementary Table 1. Analysis of activity rhythms of wild-type strain in *Ae. albopictus* and *Cx. quinquefasciatus* under DD conditions**

| Group         | Period*   | rhythmic/Total(%) | Qp.act/Qp.sig±SEM |
|---------------|-----------|-------------------|-------------------|
| <i>Aal</i> WT | 23.7±0.22 | 63/113(55.8)      | 1.06±0.01         |
| <i>Cxq</i> WT | 23.8±0.03 | 118/125(94.4)     | 1.15±0.01         |

\*: Period values presented are mean ± SEM.

**Supplementary Table 2. Comparison of expression profile characteristics of canonical clock genes in *Aedes albopictus* and *Culex quinquefasciatus***

| Name        | Species                     | GeneID     | Period         | Peak Phase/<br>LAG | Amplitude <sup>a</sup> | Adjusted P-value |
|-------------|-----------------------------|------------|----------------|--------------------|------------------------|------------------|
| <i>per</i>  | <i>Ae. albopictus</i>       | AALF006362 | 24             | 18                 | 3.40                   | 1.3e-13          |
|             | <i>Cx. quinquefasciatus</i> | CPIJ007193 | 24             | 19.5               | 1.23                   | 5.6e-14          |
| <i>tim</i>  | <i>Ae. albopictus</i>       | AALF000800 | 21             | 15                 | 2.29                   | 3.4e-06          |
|             | <i>Cx. quinquefasciatus</i> | CPIJ007082 | 24             | 18                 | 4.52                   | 1.3e-13          |
| <i>Clk</i>  | <i>Ae. albopictus</i>       | AALF024284 | N <sup>b</sup> | N                  | N                      | N                |
|             | <i>Cx. quinquefasciatus</i> | CPIJ002146 | 24             | 4.5                | 1.51                   | 1.8e-06          |
| <i>cyc</i>  | <i>Ae. albopictus</i>       | AALF017793 | 27             | 1.5                | 2.22                   | 3.1e-08          |
|             | <i>Cx. quinquefasciatus</i> | CPIJ014938 | 24             | 4.5                | 3.66                   | 2.1e-08          |
| <i>Cry1</i> | <i>Ae. albopictus</i>       | AALF000530 | 21             | 19.5               | 1.56                   | 0.001            |
|             | <i>Cx. quinquefasciatus</i> | CPIJ009455 | N              | N                  | N                      | N                |
| <i>Cry2</i> | <i>Ae. albopictus</i>       | AALF012140 | 24             | 19.5               | 2.07                   | 3.1e-08          |
|             | <i>Cx. quinquefasciatus</i> | CPIJ018859 | 27             | 22.5               | 2.15                   | 1.7e-11          |

a: Amplitude was calculated as maximum expression divided by minimum expression.

b: N indicates non-rhythmic gene expression.

| Primer name        | Sequences (5'-3')       | Annealing temperature and cycles | Primer use                                               |
|--------------------|-------------------------|----------------------------------|----------------------------------------------------------|
| <i>Aalrps7</i> -qF | ATGAACTCGGACCTGAAG      | 55°C, 40                         | Reference gene for <i>Aal</i> control with qPCR          |
| <i>Aalrps7</i> -qR | TTCTTGCTGTTGAACTCG      | 55°C, 40                         |                                                          |
| <i>Cxqrps7</i> -qF | GTCGTCGTGACCCGAACAAG    | 55°C, 40                         | Reference gene for <i>Cxq</i> control with qPCR          |
| <i>Cxqrps7</i> -qR | TTGATCAGCTGCGAACCCTC    | 55°C, 40                         |                                                          |
| <i>Aalop1</i> -qF  | GCTGTGCCTCAATCTGGACT    | 55°C, 40                         | Expression profile analysis for <i>Aalop1</i> with qPCR  |
| <i>Aalop1</i> -qR  | GAAAAGCGCGAACACCCAAA    | 55°C, 40                         |                                                          |
| <i>Aalop2</i> -qF  | TCTACGCTTGCCCTTGGATCG   | 55°C, 40                         | Expression profile analysis for <i>Aalop2</i> with qPCR  |
| <i>Aalop2</i> -qR  | GGCACCGTTGTTGGTCAAAG    | 55°C, 40                         |                                                          |
| <i>Aalop3</i> -qF  | GATCGGAAATGGCATGGTGG    | 55°C, 40                         | Expression profile analysis for <i>Aalop3</i> with qPCR  |
| <i>Aalop3</i> -qR  | TCGGTGGTCCCATCGTAAAC    | 55°C, 40                         |                                                          |
| <i>Aalop4</i> -qF  | CAGAACACCAGCACCGAAATG   | 55°C, 40                         | Expression profile analysis for <i>Aalop4</i> with qPCR  |
| <i>Aalop4</i> -qR  | CCAGAGGGCTGATTGGTGAG    | 55°C, 40                         |                                                          |
| <i>Aalop5</i> -qF  | TTCCGGTAAGCCTCTGACTA    | 55°C, 40                         | Expression profile analysis for <i>Aalop5</i> with qPCR  |
| <i>Aalop5</i> -qR  | ACAGGCGCTCATGTTTCCT     | 55°C, 40                         |                                                          |
| <i>Aalop7</i> -qF  | AATCGGTATGTGCCGGAAGG    | 55°C, 40                         | Expression profile analysis for <i>Aalop7</i> with qPCR  |
| <i>Aalop7</i> -qR  | ATGGTGAACAGCGGAACGTA    | 55°C, 40                         |                                                          |
| <i>Aalop8</i> -qF  | CAACACTTCCGTTGAGGTGC    | 55°C, 40                         | Expression profile analysis for <i>Aalop8</i> with qPCR  |
| <i>Aalop8</i> -qR  | CCAAACGCTCCAATCAAGGC    | 55°C, 40                         |                                                          |
| <i>Aalop9</i> -qF  | GGCCGCCTGAATAGAGTACAA   | 55°C, 40                         | Expression profile analysis for <i>Aalop9</i> with qPCR  |
| <i>Aalop9</i> -qR  | GTCAAAGGAGCACGTGGTAAG   | 55°C, 40                         |                                                          |
| <i>Aalop10</i> -qF | AAATCCCTCCGTACTCCAGC    | 55°C, 40                         | Expression profile analysis for <i>Aalop10</i> with qPCR |
| <i>Aalop10</i> -qR | GGACCCTGGTGGTAGCTGTT    | 55°C, 40                         |                                                          |
| <i>Aalop12</i> -qF | CTGGACCCCCATGAACATCA    | 55°C, 40                         | Expression profile analysis for <i>Aalop12</i> with qPCR |
| <i>Aalop12</i> -qR | CGGCCAAATATCCAATGGCG    | 55°C, 40                         |                                                          |
| <i>Cxqop1</i> -qF  | TTCGTGGTGAACCTTGCCAT    | 55°C, 40                         | Expression profile analysis for <i>Cxqop1</i> with qPCR  |
| <i>Cxqop1</i> -qR  | TCGCACATAAGCACTCCAG     | 55°C, 40                         |                                                          |
| <i>Cxqop2</i> -qF  | GGAATCGGAGGAGCGATCAG    | 55°C, 40                         | Expression profile analysis for <i>Cxqop2</i> with qPCR  |
| <i>Cxqop2</i> -qR  | GAACAGCGATGCTTTGGTCC    | 55°C, 40                         |                                                          |
| <i>Cxqop3</i> -qF  | CGTTGACAAACCGCATGTTT    | 55°C, 40                         | Expression profile analysis for <i>Cxqop3</i> with qPCR  |
| <i>Cxqop3</i> -qR  | GACGTGGCTTACGATTGGC     | 55°C, 40                         |                                                          |
| <i>Cxqop4</i> -qF  | CAGTACCACCTTGGACCAG     | 55°C, 40                         | Expression profile analysis for <i>Cxqop4</i> with qPCR  |
| <i>Cxqop4</i> -qR  | CGACCGAGATCATCGTGAGA    | 55°C, 40                         |                                                          |
| <i>Cxqop5</i> -qF  | CGGCAACATGTCCGTCGTG     | 55°C, 40                         | Expression profile analysis for <i>Cxqop5</i> with qPCR  |
| <i>Cxqop5</i> -qR  | TCCCAAACAGAAGATGGCGA    | 55°C, 40                         |                                                          |
| <i>Cxqop6</i> -qF  | CCTGGTCATCAACTTCACCGGAG | 55°C, 40                         | Expression profile analysis for <i>Cxqop6</i> with qPCR  |
| <i>Cxqop6</i> -qR  | ATGGCTGATTCCGTACACGAT   | 55°C, 40                         |                                                          |
| <i>Cxqop7</i> -qF  | CTGCGTACTCCGTCCAACAT    | 55°C, 40                         | Expression profile analysis for <i>Cxqop7</i> with qPCR  |
| <i>Cxqop7</i> -qR  | GGCACATCAACGGTCCAAAC    | 55°C, 40                         |                                                          |
| <i>Cxqop8</i> -qF  | GGTGAACCTGACCGTAGTGG    | 55°C, 40                         | Expression profile analysis for <i>Cxqop8</i> with qPCR  |
| <i>Cxqop8</i> -qR  | AACCAGCGAGATGAAGCACA    | 55°C, 40                         |                                                          |
| <i>Cxqop9</i> -qF  | CGGATTCGCGATCTTTGTCC    | 55°C, 40                         | Expression profile analysis for <i>Cxqop9</i> with qPCR  |
| <i>Cxqop9</i> -qR  | GACCACTAGCAGGTTGGACG    | 55°C, 40                         |                                                          |
| <i>Cxqop10</i> -qF | CCCCTCAAACCTGCTAGTGG    | 55°C, 40                         | Expression profile analysis                              |

|                     |                           |          |                               |
|---------------------|---------------------------|----------|-------------------------------|
| <i>Cxqop10</i> -qR  | CAAACGCTCCAAACACCCAG      | 55°C, 40 | for <i>Cxqop10</i> with qPCR  |
| <i>Cxqop11</i> -qF  | ATCGGTGGAGCAATCAGCAAT     | 55°C, 40 | Expression profile analysis   |
| <i>Cxqop11</i> -qR  | ACAGCCAGGTTAGCAGCAC       | 55°C, 40 | for <i>Cxqop11</i> with qPCR  |
| <i>Cxqop12</i> -qF  | GAGGTCAATCAGTGGCAGCA      | 55°C, 40 | Expression profile analysis   |
| <i>Cxqop12</i> -qR  | CGACCGGAACGTGTTGGATT      | 55°C, 40 | for <i>Cxqop12</i> with qPCR  |
| <i>Cxqop13</i> -qF  | TGAGCCACTCACCAACAAGG      | 55°C, 40 | Expression profile analysis   |
| <i>Cxqop13</i> -qR  | GTCGGTGAGGTAGTCGGTTC      | 55°C, 40 | for <i>Cxqop13</i> with qPCR  |
| <i>Aalper</i> -qF   | CTGGACCGCTCTGGGAAAAT      | 55°C, 40 | Expression profile analysis   |
| <i>Aalper</i> -qR   | CATATCGGCCGCTTCCTTCT      | 55°C, 40 | for <i>Aalper</i> with qPCR   |
| <i>Aaltim</i> -qF   | TAAACTCGGCTTCCACCTGC      | 55°C, 40 | Expression profile analysis   |
| <i>Aaltim</i> -qR   | GGCCCAGTTTGAGAGCAATG      | 55°C, 40 | for <i>Aaltim</i> with qPCR   |
| <i>AalClk</i> -qF   | ACCTGAAAACGATACGGGGG      | 55°C, 40 | Expression profile analysis   |
| <i>AalClk</i> -qR   | AGCCCACCACAAACAGGAAA      | 55°C, 40 | for <i>AalClk</i> with qPCR   |
| <i>Aalcyc</i> -qF   | GTCCCAATCCATCACCTGCA      | 55°C, 40 | Expression profile analysis   |
| <i>Aalcyc</i> -qR   | TGTTTGCGCTTGTTCTTCGG      | 55°C, 40 | for <i>Aalcyc</i> with qPCR   |
| <i>AalCry1</i> -qF  | CCCGGAGGGCAGCACATAA       | 55°C, 40 | Expression profile analysis   |
| <i>AalCry1</i> -qR  | TCGGATTGGCCTCCATTTCGG     | 55°C, 40 | for <i>AalCry1</i> with qPCR  |
| <i>AalCry2</i> -qF  | CTGCTGGATGCCGATTGGTC      | 55°C, 40 | Expression profile analysis   |
| <i>AalCry2</i> -qR  | GCAAGTACCGGCGAATGTAG      | 55°C, 40 | for <i>AalCry2</i> with qPCR  |
| <i>Cxqper</i> -qF   | CTGTTTACGACGCCAGTATTACGC  | 55°C, 40 | Expression profile analysis   |
| <i>Cxqper</i> -qR   | CAACGGCAGCACCACTTCGAG     | 55°C, 40 | for <i>Cxqper</i> with qPCR   |
| <i>Cxqtim</i> -qF   | AGATACTACGCTGAGAACATTCCG  | 55°C, 40 | Expression profile analysis   |
| <i>Cxqtim</i> -qR   | AATCTTAGAATCCTTGGCATGAACC | 55°C, 40 | for <i>Cxqtim</i> with qPCR   |
| <i>CxqClk</i> -qF   | GACCAGTTCAACCTGCTCGTCA    | 55°C, 40 | Expression profile analysis   |
| <i>CxqClk</i> -qR   | CTTGAGAAATGCAATCGTCGACT   | 55°C, 40 | for <i>CxqClk</i> with qPCR   |
| <i>Cxqcyc</i> -qF   | ACTGATGTTTCCTCAGGGGGT     | 55°C, 40 | Expression profile analysis   |
| <i>Cxqcyc</i> -qR   | TTCCCACTTTCGGCTTCCTC      | 55°C, 40 | for <i>Cxqcyc</i> with qPCR   |
| <i>CxqCry1</i> -qF  | CCCGGCGCTCTATGTTACGAA     | 55°C, 40 | Expression profile analysis   |
| <i>CxqCry1</i> -qR  | CGTTCATCTTGTTGGCGTTGCACT  | 55°C, 40 | for <i>CxqCry1</i> with qPCR  |
| <i>CxqCry2</i> -qF  | ATACGCTGCAAGATCAACTCAACA  | 55°C, 40 | Expression profile analysis   |
| <i>CxqCry2</i> -qR  | ACTCGGTGTTTCCTCATGCCAT    | 55°C, 40 | for <i>CxqCry2</i> with qPCR  |
| <i>AalHACls</i> -qF | GGTCGAGTGGCTCAAGAACA      | 55°C, 40 | Expression profile analysis   |
| <i>AalHACls</i> -qR | AGCCACATGTAGTGGTTGGG      | 55°C, 40 | for <i>AalHACls</i> with qPCR |
| <i>AalHr38</i> -qF  | TCTCGCCAGCCTCGACTACA      | 55°C, 40 | Expression profile analysis   |
| <i>AalHr38</i> -qR  | ACGTCCACCGAGGTGGTTAG      | 55°C, 40 | for <i>AalHr38</i> with qPCR  |
| <i>Aalpdf</i> -qF   | CGGTTTGCTTGTGCCTCAGT      | 55°C, 40 | Expression profile analysis   |
| <i>Aalpdf</i> -qR   | CCACTCCGCTAACTGTCGTA      | 55°C, 40 | for <i>Aalpdf</i> with qPCR   |
| <i>CxqHACls</i> -qF | TCGGTCACGTTCCAAACGAT      | 55°C, 40 | Expression profile analysis   |
| <i>CxqHACls</i> -qR | TGGGTGTCGTGGGGATAGAT      | 55°C, 40 | for <i>CxqHACls</i> with qPCR |
| <i>CxqHr38</i> -qF  | CGTTGCTGAAGGGTGGTTAC      | 55°C, 40 | Expression profile analysis   |
| <i>CxqHr38</i> -qR  | AAGCTCCTCGGTTGACGAAG      | 55°C, 40 | for <i>CxqHr38</i> with qPCR  |
| <i>Cxqpdf</i> -qF   | TGCTTCGTGCTGTTCTGTCT      | 55°C, 40 | Expression profile analysis   |
| <i>Cxqpdf</i> -qR   | TTGGTTCGCTAGCCATTCCG      | 55°C, 40 | for <i>Cxqpdf</i> with qPCR   |
| <i>Aalop1</i> -F    | CGCGCTTTTCTGGACTTTGG      | 55°C, 35 | Genotype identification for   |
| <i>Aalop1</i> -R    | CTGCTTATGCCTTCTCGTCG      | 55°C, 35 | <i>Aalop1</i> with PCR        |
| <i>Cxqop6</i> -F    | GCCGCTAGACGCTGTGAGAT      | 55°C, 35 | Genotype identification for   |
| <i>Cxqop6</i> -R    | TCCAGAAGAGTGCGAAAGCC      | 55°C, 35 | <i>Cxqop6</i> with PCR        |

**Supplementary Table 4. Activity rhythms of *Ae. albopictus* and *Cx. quinquefasciatus* under DD conditions in each experimental group.**

| Mosquito                    | Group         | Period±SEM | Rhythmic/Total(%) | Qp.act/Qp.sig±SEM |
|-----------------------------|---------------|------------|-------------------|-------------------|
| <i>Ae. albopictus</i>       | WT            | 23.17±0.11 | 17/34(50.00)      | 1.14±0.02         |
|                             | CE-blocked    | 23.64±0.15 | 11/30(36.67)      | 1.10±0.02         |
|                             | OL-blocked    | 23.51±0.10 | 18/29(62.07)      | 1.12±0.01         |
|                             | CE+OL-blocked | 23.31±0.13 | 15/29(51.72)      | 1.12±0.02         |
| <i>Cx. quinquefasciatus</i> | WT            | 23.44±0.05 | 30/36(83.33)      | 1.16±0.02         |
|                             | CE-blocked    | 23.54±0.07 | 28/31(90.32)      | 1.13±0.02         |
|                             | OL-blocked    | 23.49±0.07 | 25/30(83.33)      | 1.14±0.02         |
|                             | CE+OL-blocked | 23.50±0.06 | 28/31(90.32)      | 1.14±0.02         |

138

139

**Supplementary Table 5. Mutation status in the G<sub>0</sub> generation of *Ae. albopictus* and *Cx. quinquefasciatus* after microinjection.**

| Group            | Embryos injected | Hatched (%) | G <sub>0</sub> adult survivors (%) | G <sub>0</sub> mosaic (%) | No. of pools | G <sub>1</sub> mutant pools(%) |
|------------------|------------------|-------------|------------------------------------|---------------------------|--------------|--------------------------------|
| <i>Aalopsin1</i> | 372              | 45(12.10)   | 38(84.44)                          | 9(23.68)                  | 9            | 2(22.22)                       |
| <i>Cxqopsin6</i> | 497              | 56(11.27)   | 16(28.57)                          | 8(50.00)                  | 8            | 1(12.50)                       |
| <i>AalCry1</i>   | 1175             | 374(31.83)  | 253(67.65)                         | 30(11.86)                 | 30           | 1(3.33)                        |
| <i>CxqCry1</i>   | 2007             | 426(21.23)  | 263(61.74)                         | 55(20.91)                 | 55           | 1(1.82)                        |

142

143

**Supplementary Table 6. Analysis of activity rhythms of wild-type and gene knockout strain in *Ae. albopictus* and *Cx. quinquefasciatus* under DD conditions**

| Group                           | Period ± SEM | rhythmic/Total(%) | Qp.act/Qp.sig±SEM |
|---------------------------------|--------------|-------------------|-------------------|
| <i>Aalop1</i> <sup>+</sup> ;WT  | 23.4±0.09    | 37/59(62.7)       | 1.20±0.02         |
| <i>Aalop1</i> <sup>Δ119</sup>   | 23.4±0.06    | 52/65(80.0)       | 1.19±0.02         |
| <i>Cxqop6</i> <sup>+</sup> ;WT  | 23.7±0.03    | 59/61(96.7)       | 1.26±0.02         |
| <i>Cxqop6</i> <sup>Δ1</sup>     | 23.8±0.04    | 54/59(91.5)       | 1.22±0.02         |
| <i>AalCry1</i> <sup>+</sup> ;WT | 24.5±0.95    | 21/30(70.0)       | 1.13±0.05         |
| <i>AalCry1</i> <sup>Δ13</sup>   | 25.2±0.97    | 24/31(77.4)       | 1.15±0.03         |
| <i>Cxqop6</i> <sup>+</sup> ;WT  | 24.3±0.09    | 28/31(90.3)       | 1.06±0.02         |
| <i>CxqCry</i> <sup>Δ44</sup>    | 26.7±1.59    | 12/29(41.4)       | 1.01±0.02         |

146

147 **Supplementary Table 7. Pupation rate and eclosion rate data**

| Index              | <i>Ae. albopictus</i> |           |           | <i>Cx. quinquefasciatus</i> |           |           |
|--------------------|-----------------------|-----------|-----------|-----------------------------|-----------|-----------|
|                    | LD                    | LL        | DD        | LD                          | LL        | DD        |
| Pupation Rate *(%) | 0.93±0.06             | 0.87±0.06 | 0.90±0.05 | 0.83±0.03                   | 0.80±0.10 | 0.80±0.06 |
| Eclosion Rate *(%) | 0.94±0.05             | 0.97±0.04 | 0.93±0.06 | 0.82±0.08                   | 0.88±0.02 | 0.80±0.03 |

148 \*mean ± standard error (SE)

149

150

**Supplementary Table 8. Adult mosquito life table**

| Group |   | <i>Ae. albopictus</i>        |                             | <i>Cx. quinquefasciatus</i>  |                             |
|-------|---|------------------------------|-----------------------------|------------------------------|-----------------------------|
|       |   | Average survival time (days) | Median survival time (days) | Average survival time (days) | Median survival time (days) |
| LD    | ♂ | 39.80±2.33                   | 43±4.74                     | 31.73±1.45                   | 31±0.52                     |
|       | ♀ | 49.38±2.45                   | 52±6.30                     | 37.18±1.72                   | 37±1.58                     |
| LL    | ♂ | 34.35±1.90                   | 37±1.25                     | 27.63±1.23                   | 29±0.39                     |
|       | ♀ | 41.50±2.41                   | 44±1.81                     | 29.48±1.57                   | 30±0.79                     |
| DD    | ♂ | 29.90±2.19                   | 32±1.27                     | 28.01±1.45                   | 29±1.05                     |
|       | ♀ | 30.82±1.78                   | 33±1.58                     | 36.48±1.74                   | 36±0.95                     |

151 The results are presented as mean ± standard deviation.

152