

## Supplementary material

### **Octopamine Signaling Regulates Phototactic Behavior via a $\beta$ -Adrenergic-like Receptor in *Diaphorina citri***

**Fei-Feng Wang,<sup>1,2</sup> Xiu-Qi Zou,<sup>2</sup> Ming Zhong,<sup>2</sup> Sheng-Feng Tu,<sup>2</sup> Min-Er Li,<sup>2</sup> Wen-Feng Zhao,<sup>2</sup> Bao-Li Qiu,<sup>1,\*</sup> Wen Sang<sup>2,\*</sup>**

<sup>1</sup> Engineering Research Center of Biotechnology for Active Substances, Ministry of Education, Chongqing Normal University, 401331 Chongqing, China

<sup>2</sup> South China Agricultural University/Engineering Research Center of Biological Control, Ministry of Education, 510640 Guangzhou, China

\* Correspondence: sangwen@scau.edu.cn (W. Sang); baoliqiu@cqnu.edu.cn (B.-L. Qiu)

**Table S1. The nucleotide sequences of primers used in this study.**

| Gene                                                                                           | Forward primer 5'–3'                                                   | Reverse primer 5'–3'                                 | Product length (bp) | Position |
|------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|------------------------------------------------------|---------------------|----------|
| <b>Primers used for open reading frame amplification</b>                                       |                                                                        |                                                      |                     |          |
| DcT $\beta$ H                                                                                  | AATGTTTCTTAGGGAA<br>CTTGGC                                             | CAGGTGGTTGTAGTCG<br>TGTTGT                           | -                   | -        |
| DcOct $\beta$ 1R                                                                               | CTCAGTAGAACCCCTC<br>CCT                                                | TCAAAACGTGGGTTCC<br>A                                | -                   | -        |
| DcOct $\beta$ 2R                                                                               | ATGACGGACTCCAGCA<br>ACCTCTTCG                                          | TCATAAGCTCGACCCG                                     | -                   | -        |
| <b>Primers used for qRT-PCR</b>                                                                |                                                                        |                                                      |                     |          |
| DcT $\beta$ H                                                                                  | GAATCGGGACAACCAT                                                       | GTCGCTACTCACTGAA<br>CTCT                             | -                   | -        |
| DcOct $\beta$ 1R                                                                               | CTACTCACCGAAGCCA<br>AC                                                 | CGCAAGACTCACGACA<br>A                                | -                   | -        |
| DcOct $\beta$ 2R                                                                               | GAGGACGCCCATCACA<br>GT                                                 | CAGGAAGAAGGGAAG<br>CCAG                              | -                   | -        |
| DcActin                                                                                        | CCCTGGACTTTGAACA<br>GGAA                                               | CTCGTGGATACCGCAA<br>GATT                             | -                   | -        |
| HEK293T-                                                                                       | GGTGAAGGTCGGTGTG                                                       | CTCGCTCCTGGAAGAT                                     | -                   | -        |
| GADPH                                                                                          | AACG                                                                   | GGTG                                                 |                     |          |
| <b>Primers used for vector construction, to heterologous expression of octopamine receptor</b> |                                                                        |                                                      |                     |          |
| DcOct $\beta$ 1R                                                                               | <u><sup>a</sup>GGATGACGACGATAA</u><br><u>GACTACAATGAGTGTT</u><br>ATGCC | <u>GATCCGAGCTCGGTAC</u><br><u>CATCAAAACGTGGGTT</u>   | =                   | =        |
| DcOct $\beta$ 2R                                                                               | <u>GACGACGATAAGACTA</u><br>CAATGACGGACTCCAG<br>CAACCT                  | <u>TCCGAGCTCGGTACCA</u><br>TCATAAGCTCGACCCG<br>AATTC | =                   | =        |
| <b>Primers used for dsRNA synthesis</b>                                                        |                                                                        |                                                      |                     |          |

|           |                                       |                           |     |          |
|-----------|---------------------------------------|---------------------------|-----|----------|
| dsDcTβH   | <sup>b</sup> <i>TAATACGACTCACTATA</i> | <i>TAATACGACTCACTATAG</i> | 388 | 699-1086 |
|           | GGGGTCAGCCATACAA                      | GGTGGGACAGCCATCT          |     |          |
|           | CCG                                   | T                         |     |          |
| dsDcOctβ1 | <i>TAATACGACTCACTATAG</i>             | <i>TAATACGACTCACTATAG</i> | 393 | 603-995  |
| R         | GGCAAGTCCCGCCTGT                      | GGGTGCTGTCGCTGAT          |     |          |
|           | T                                     | TG                        |     |          |
| dsDcOctβ2 | <i>TAATACGACTCACTATAG</i>             | <i>TAATACGACTCACTATAG</i> | 849 | 366-1214 |
| R 1st     | GGTCATCTGTGTTGCA                      | GGTGCTTCAGGTAGGT          |     |          |
|           | TCAGTGTG                              | GTCCG                     |     |          |
| dsDcOctβ2 | <i>TAATACGACTCACTATAG</i>             | <i>TAATACGACTCACTATAG</i> | 483 | 210-692  |
| R 2nd     | GGTGCTTCTCTTGACG                      | GGCTTCGTGGTTTCGT          |     |          |
|           | GTTGG                                 | TGGC                      |     |          |
| dsGFP     | <i>TAATACGACTCACTATAG</i>             | <i>TAATACGACTCACTATAG</i> | 411 | 989-1399 |
|           | GGCTTGTACAGCTCGT                      | GGGACGACGGCAACT           |     |          |
|           | CCATGC                                | ACAAGAC                   |     |          |

<sup>a</sup>The underlined nucleotide sequence is 16 bp overlaps with the pCDNA3.1-FLAG-N vector.

<sup>b</sup>The italic nucleotide sequence is the T7 promoter sequence.

**Table S2. The accession numbers of the sequences used in this study.**

| Gene                        | Species                          | Accession Number |
|-----------------------------|----------------------------------|------------------|
| Tyramine beta-hydroxylase   | <i>Diaphorina citri</i>          | PV091843         |
|                             | <i>Trichogramma pretiosum</i>    | XP_014231771.1   |
|                             | <i>Bemisia tabaci</i>            | XP_018907091.1   |
|                             | <i>Copidosoma floridanum</i>     | XP_014216871.1   |
|                             | <i>Periplaneta americana</i>     | AFO63080.1       |
|                             | <i>Apis mellifera</i>            | DAA05780.1       |
|                             | <i>Drosophila navojoa</i>        | XP_030245696.1   |
|                             | <i>Drosophila biarmipes</i>      | XP_043949511.1   |
|                             | <i>Drosophila melanogaster</i>   | Q86B61.1         |
|                             | <i>Bombyx mori</i>               | BAK09201.1       |
|                             | <i>Tribolium castaneum</i>       | XP_974169.1      |
|                             | <i>Trichoplusia ni</i>           | XP_026739132.1   |
|                             | <i>Caenorhabditis elegans</i>    | NP_001257265.1   |
|                             | <i>Coccinella septempunctata</i> | XP_044766667.1   |
| Octopamine receptor beta-1R | <i>Diaphorina citri</i>          | PV091844         |
|                             | <i>Periplaneta americana</i>     | XP_069694870.1   |
|                             | <i>Nilaparvata lugens</i>        | ATY68966.1       |
|                             | <i>Homalodisca vitripennis</i>   | XP_046667431.1   |
|                             | <i>Helicoverpa armigera</i>      | XP_063896322.1   |
|                             | <i>Trichoplusia ni</i>           | XP_026729368.1   |
|                             | <i>Chilo suppressalis</i>        | AGV79326.1       |
|                             | <i>Dalotia coriaria</i>          | XP_065155721.1   |
|                             | <i>Cryptotermes secundus</i>     | XP_033609947.1   |
|                             | <i>Tribolium castaneum</i>       | NP_001280514.1   |
|                             | <i>Apis cerana</i>               | XP_016921836.1   |
|                             | <i>Apis mellifera</i>            | XP_397139.3      |
|                             | <i>Drosophila navojoa</i>        | XP_030242457.1   |

|                                            |                                |                |
|--------------------------------------------|--------------------------------|----------------|
|                                            | <i>Drosophila melanogaster</i> | Q9VCZ3.1       |
| Octopamine receptor beta-2R                | <i>Diaphorina citri</i>        | PV091845       |
|                                            | <i>Periplaneta americana</i>   | XP_069695476.1 |
|                                            | <i>Nilaparvata lugens</i>      | XP_039280502.1 |
|                                            | <i>Nephotettix cincticeps</i>  | QID89710.1     |
|                                            | <i>Drosophila melanogaster</i> | Q4LBB9.2       |
|                                            | <i>Drosophila navojoa</i>      | XP_017969142.1 |
|                                            | <i>Homalodisca vitripennis</i> | KAG8316974.1   |
|                                            | <i>Helicoverpa armigera</i>    | XP_063896258.1 |
|                                            | <i>Trichoplusia ni</i>         | XP_026729763.1 |
|                                            | <i>Chilo suppressalis</i>      | AEO89318.1     |
|                                            | <i>Dalotia coriaria</i>        | XP_065155743.1 |
|                                            | <i>Tribolium castaneum</i>     | NP_001280501.1 |
|                                            | <i>Cryptotermes secundus</i>   | XP_023721065.1 |
|                                            | <i>Apis cerana</i>             | XP_016908111.1 |
|                                            | <i>Apis mellifera</i>          | XP_006558130.1 |
| FMRamide receptor                          | <i>Drosophila melanogaster</i> | NP_647758.1    |
| neither inactivation nor after potential E | <i>Drosophila melanogaster</i> | AAF55712.1     |

**Table S3. The similarity of other species shares with *Diaphorina citri* in multiple alignment.**

| Gene                        | Species                        | Accession Number | Identity (%) |
|-----------------------------|--------------------------------|------------------|--------------|
| Tyramine beta-hydroxylase   | <i>Bemisia tabaci</i>          | XP_018907091.1   | 53.18        |
|                             | <i>Tribolium castaneum</i>     | XP_974169.1      | 54.14        |
|                             | <i>Periplaneta americana</i>   | AFO63080.1       | 53.70        |
|                             | <i>Drosophila melanogaster</i> | Q86B61.1         | 44.75        |
|                             | <i>Apis mellifera</i>          | DAA05780.1       | 35.63        |
| Octopamine receptor beta-1R | <i>Nilaparvata lugens</i>      | ATY68966.1       | 58.56        |
|                             | <i>Tribolium castaneum</i>     | NP_001280514.1   | 53.88        |
|                             | <i>Periplaneta americana</i>   | XP_069694870.1   | 60.9         |

|                             |                                |                |       |
|-----------------------------|--------------------------------|----------------|-------|
|                             | <i>Drosophila melanogaster</i> | Q9VCZ3.1       | 47.51 |
|                             | <i>Apis mellifera</i>          | XP_397139.3    | 58.18 |
| Octopamine receptor beta-2R | <i>Nilaparvata lugens</i>      | XP_039280502.1 | 72.64 |
|                             | <i>Tribolium castaneum</i>     | NP_001280501.1 | 67.79 |
|                             | <i>Periplaneta americana</i>   | XP_069695476.1 | 74.83 |
|                             | <i>Drosophila melanogaster</i> | Q4LBB9.2       | 62.17 |
|                             | <i>Apis mellifera</i>          | XP_006558130.1 | 68.54 |

**Table S4. EC<sub>50</sub> and LogEC<sub>50</sub> values for stimulation of cAMP response by various agonists in HEK 293T cells expressing DcOctβ1R.**

| Agonists    | EC <sub>50</sub> (M)   | 95% CI for EC <sub>50</sub> (M)                | Log EC <sub>50</sub> (mean ± SEM) | Fitting R <sup>2</sup> |
|-------------|------------------------|------------------------------------------------|-----------------------------------|------------------------|
| Octopamine  | $2.40 \times 10^{-9}$  | $[1.25 \times 10^{-9}, 4.60 \times 10^{-9}]$   | $-8.62 \pm 0.116$                 | 0.971                  |
| Tyramine    | $5.93 \times 10^{-8}$  | $[3.62 \times 10^{-8}, 1.04 \times 10^{-7}]$   | $-7.23 \pm 0.094$                 | 0.990                  |
| Tolazoline  | $1.20 \times 10^{-7}$  | $[5.93 \times 10^{-8}, 2.77 \times 10^{-7}]$   | $-6.92 \pm 0.129$                 | 0.982                  |
| Clonidine   | $5.45 \times 10^{-7}$  | $[2.17 \times 10^{-7}, 2.01 \times 10^{-6}]$   | $-6.26 \pm 0.159$                 | 0.958                  |
| Naphazoline | $1.29 \times 10^{-10}$ | $[5.57 \times 10^{-11}, 2.95 \times 10^{-10}]$ | $-9.89 \pm 0.146$                 | 0.976                  |

**Table S5. IC<sub>50</sub> and LogIC<sub>50</sub> values for inhibition of OA-induced cAMP response by various antagonists in HEK 293T cells expressing DcOctβ1R.**

| Antagonists    | IC <sub>50</sub> (M)  | 95% CI for IC <sub>50</sub> (M)              | Log IC <sub>50</sub> (mean ± SEM) | Fitting R <sup>2</sup> |
|----------------|-----------------------|----------------------------------------------|-----------------------------------|------------------------|
| Mianserin      | $1.06 \times 10^{-5}$ | $[5.85 \times 10^{-6}, 1.89 \times 10^{-5}]$ | $-4.97 \pm 0.103$                 | 0.987                  |
| Epinastine     | $3.80 \times 10^{-8}$ | $[2.50 \times 10^{-8}, 5.76 \times 10^{-8}]$ | $-7.418 \pm 0.076$                | 0.990                  |
| Chlorpromazine | $2.56 \times 10^{-6}$ | $[6.27 \times 10^{-7}, 9.27 \times 10^{-6}]$ | $-5.59 \pm 0.229$                 | 0.949                  |
| Metoclopramide | $4.90 \times 10^{-6}$ | $[2.68 \times 10^{-6}, 8.65 \times 10^{-6}]$ | $-5.31 \pm 0.102$                 | 0.985                  |



**Figure S1. Amino acid sequence alignment of *DcTβH* (A), *DcOctβ1R* (B) and *DcOctβ2R* (C) with orthologous genes.** The numbers on the left and right indicate the position of the nucleotides and amino acids. Dashes are gaps that were introduced for alignment. The identical amino acid is colored letter showed in consensus. The quality of alignment is showed by yellow column. The shaded sequences highlight the identity level of amino acids between the orthologous sequences. Identical amino acids are highlighted in dark blue and conserved amino acids are on light blue, as determined using the 50% majority rules. The accession numbers used in the sequence alignment were listed in Table S2.

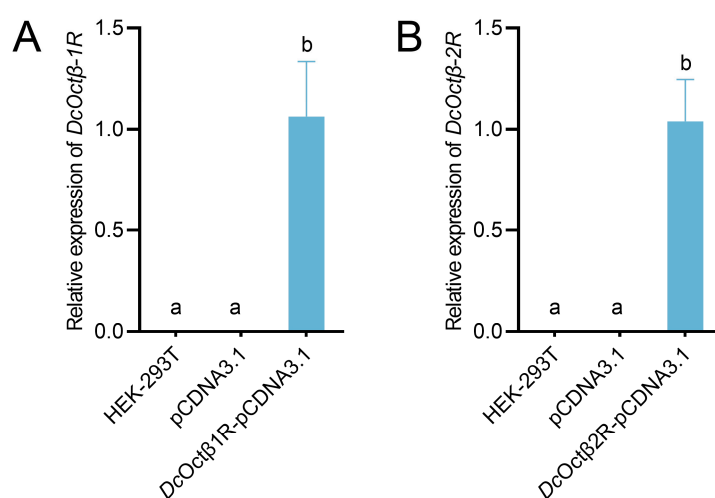


**Figure S2. The relative expression of *DcOctβ1R* (A) and *DcOctβ2R* (B) in HEK-293T cells.** Different letters above the bars indicate significant differences at  $P < 0.05$  (Tukey's post hoc test). Data are the means of three replicates  $\pm$  SEM.

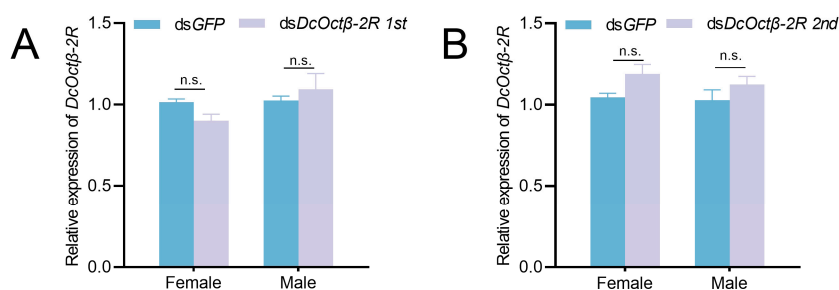


**Figure S3. Relative expression of *DcOctβ2R* after dsDcOctβ2R treatment for 24h.** “n.s.” denotes no significant difference ( $P > 0.05$ , Student's t test). Data are presented as means of three replicates  $\pm$  SEM.

SEM.