Supporting Information for

Cellular and Molecular Basis of Odorant Reception in the Biting Midge *Forcipomyia taiwana*

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Other supporting materials for this manuscript include the following:

Tables S1 to S5 as a single excel file with multiple tabs

Supporting Information Text

Figures S1 to S8

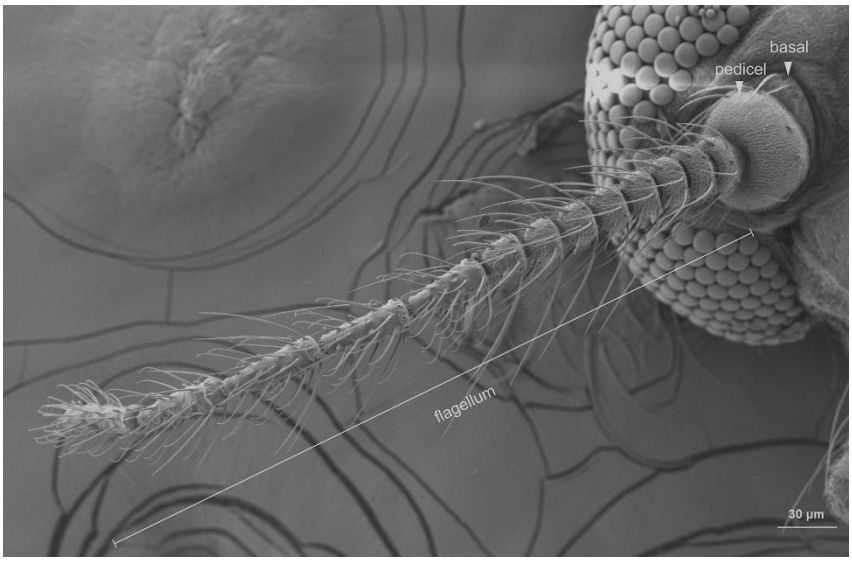

Fig. S1. Scanning electron micrographs of the antenna of *F. taiwana*. Arrows mark the basal segment and pedicel of the antenna. The flagellum consists of 13 segments. Scale bars = 30 µm.

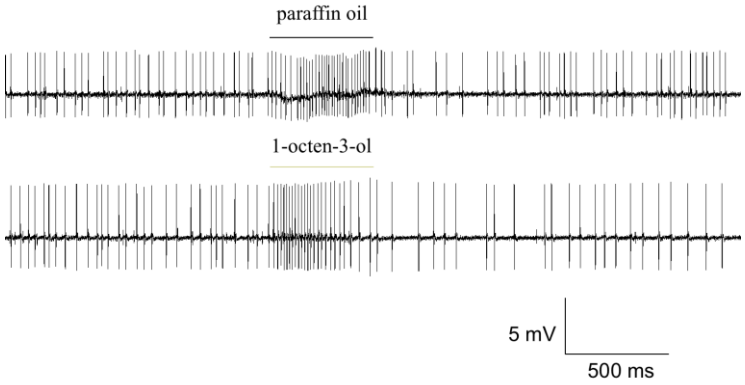

Fig. S2. Representative SSR traces indicate that the spike responses of the B neuron within in spherical sensilla of female midges to 1-octen-3-ol are comparable to the responses evoked by paraffin oil.

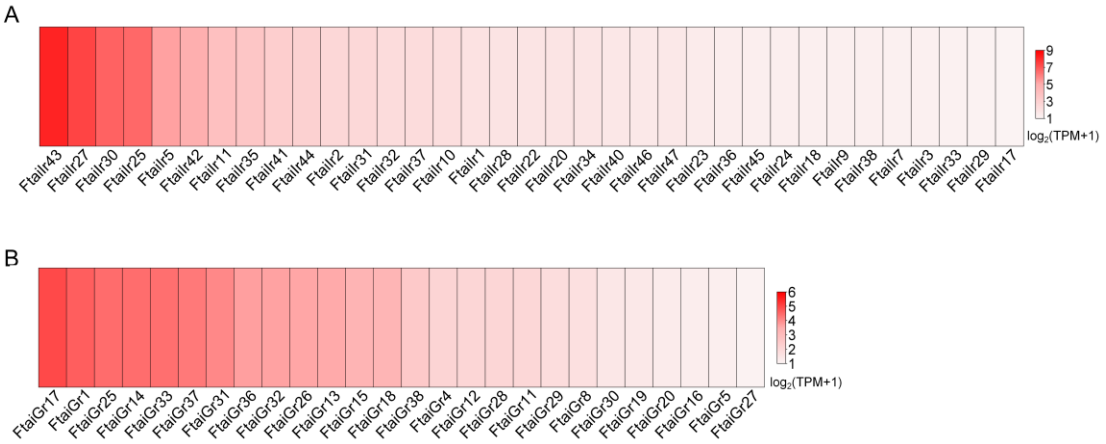

Fig. S3. Expression profiles of gustatory receptors (*GRs*) and ionotropic receptors (*IRs*) in heads of *F. taiwana*. (*A*) Heatmap of 35 *FtaiIrs* with substantial expression in head tissue (TPM>1). (*B*) Heatmap of 26 *FtaiGrs* with substantial expression in head tissue (TPM>1).

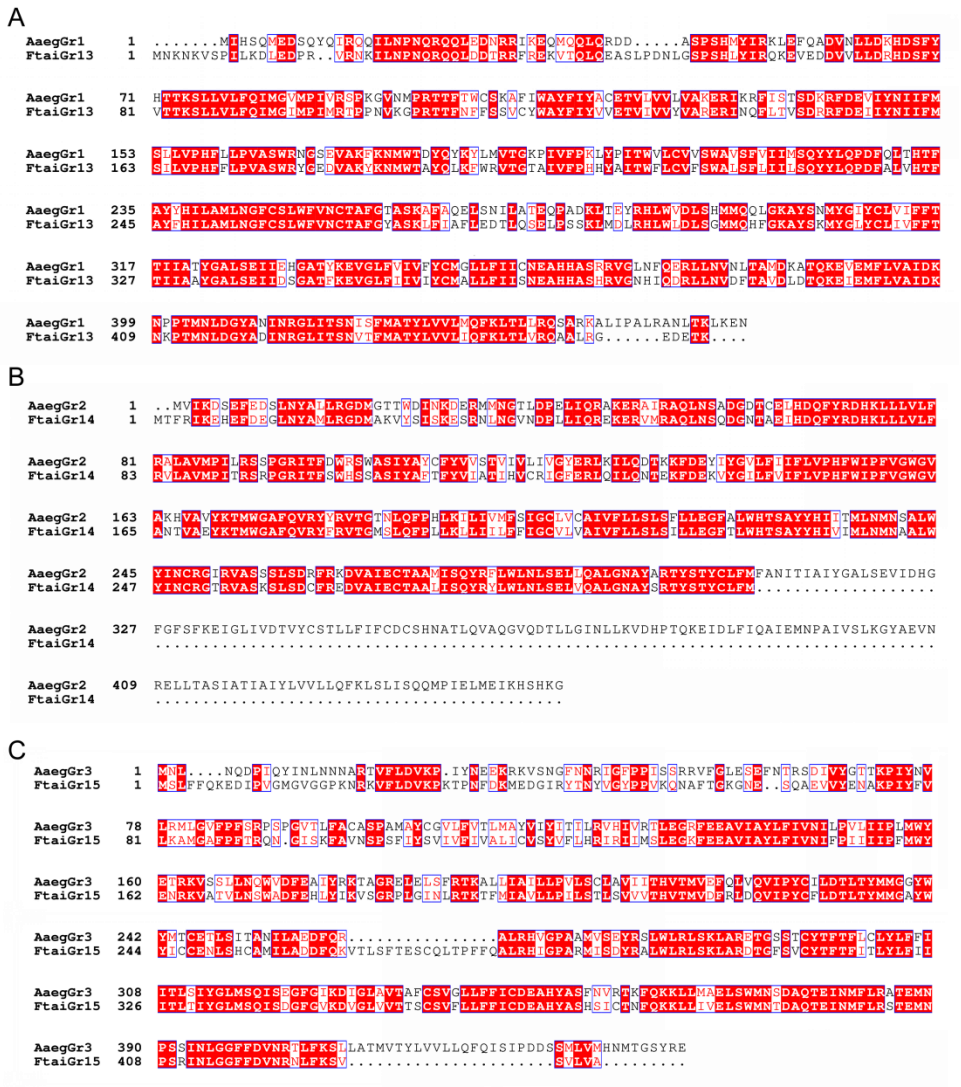

Fig. S4. Sequence alignment of carbon dioxide receptor orthologs between *Ae. aegypti* and *F. taiwana*. (*A*) Pairwise alignment of AaegGr1 and its ortholog FtaiGr13; (*B*) Pairwise alignment of AaegGr2 and its ortholog FtaiGr14; (*C*) Pairwise alignment of AaegGr3 and its ortholog FtaiGr15. Solid red shading marks fully identical amino acid residues within each pairwise alignment, while blue boxes denote residues sharing similar physicochemical properties. Among the three pairs, AaegGr2/FtaiGr14 shows the highest identity (75%), followed by AaegGr1/FtaiGr13 (69%), while AaegGr3/FtaiGr16 exhibits the lowest (60%). Conserved residues are likely involved in CO2 recognition or receptor structural stability, whereas divergent regions may account for species-specific olfactory adaptations. The alignments were performed using MUSCLE (default parameters) and visualised with ESPript.

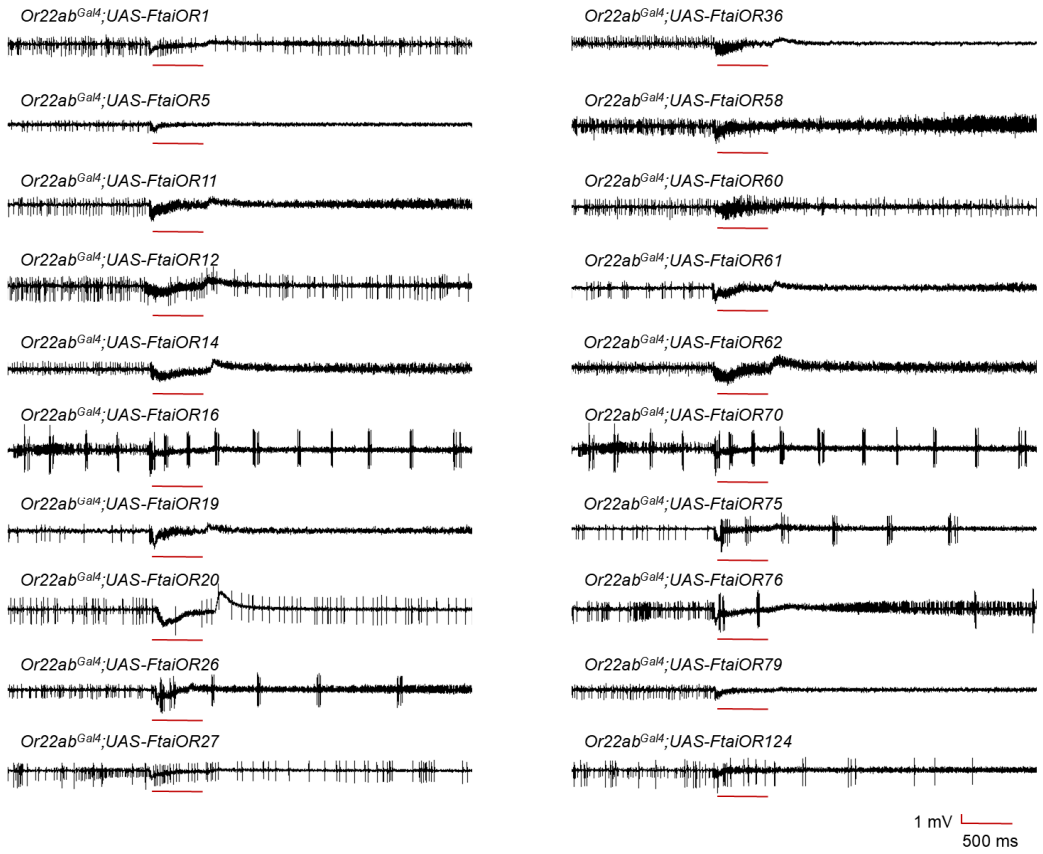

Fig. S5. Single-sensillum responses of 20 FtaiORs to 2-heptanone. Twenty FtaiORs were heterologously expressed in the empty ab3A neuron of *D. melanogaster*. The ab3 sensillum was identified using 1% 2-heptanone as the diagnostic stimulus. No A neuron spikes were recorded from ab3A neurons expressing FtaiOR5, FtaiOR11, FtaiOR14, FtaiOR19, FtaiOR36, FtaiOR58, FtaiOR61, FtaiOR62 and FtaiOR79. Although FtaiOR16, FtaiOR26, FtaiOR70, FtaiOR75 and FtaiOR76 elicited A neuron spikes, their response signals were abnormal bursts. Only FtaiOR1, FtaiOR12, FtaiOR20, FtaiOR27, FtaiOR60 and FtaiOR124 exhibited stable, consistant spikes in ab3A neurons.

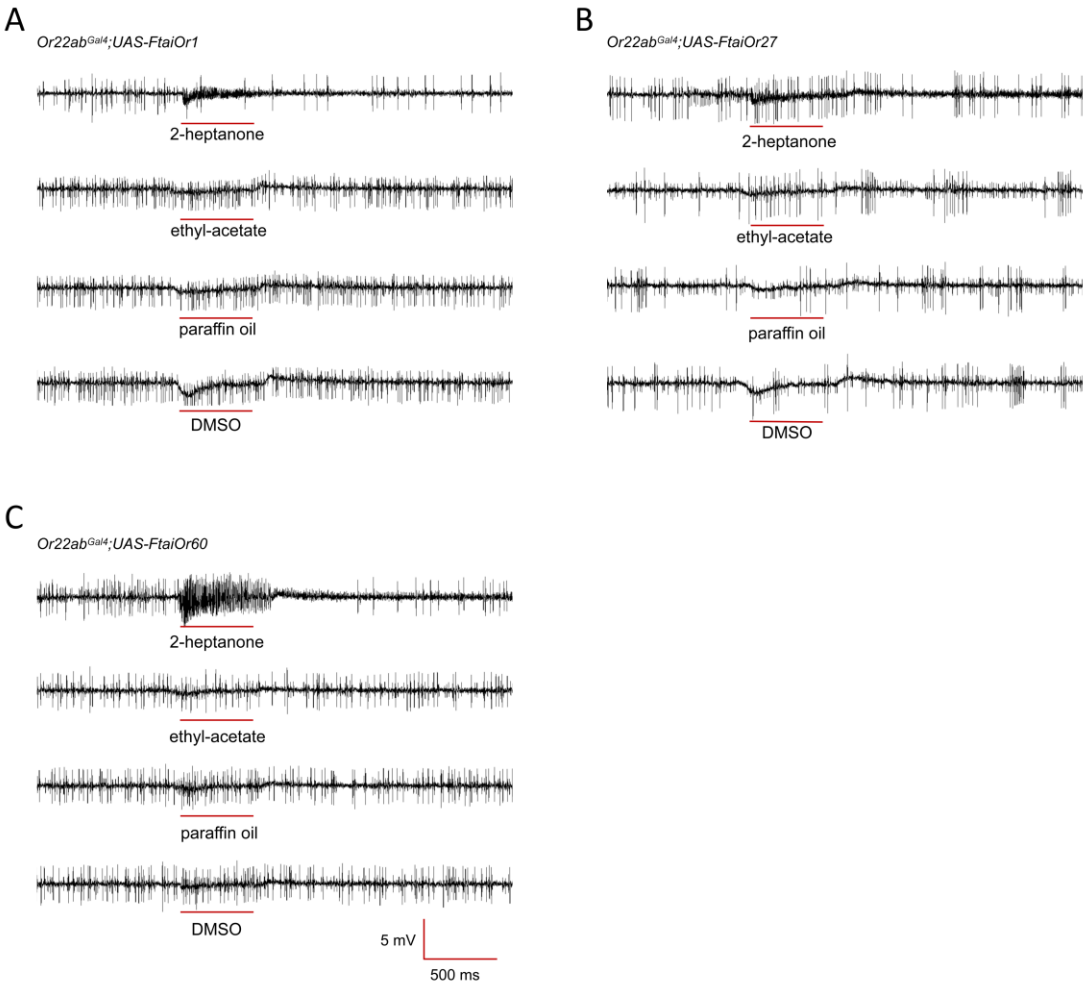

Fig. S6. Representative SSR traces of *Drosophila* ab3A empty neurons expressing FtaiOr1 (*A*), FtaiOr27 (*B*), and FtaiOr60 (*C*). 2-heptanone and ethyl acetate served as diagnostic odorants to confirm ab3 sensillum identity; paraffin oil and DMSO were used as solvent controls.

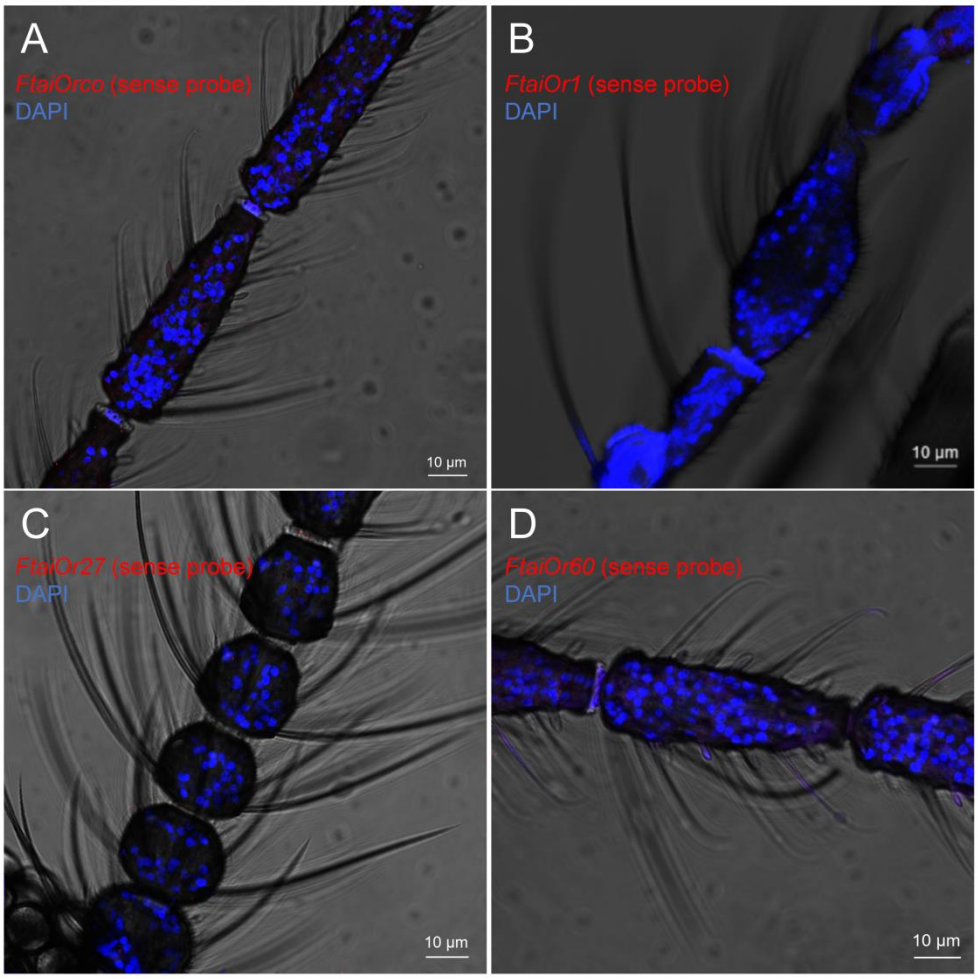

Fig. S7. Negative controls for whole-mount in situ hybridization on midge antennae and maxillary palps. Panels *A*-*D* show merged bright-field and DAPI (blue) fluorescence images of tissues hybridized with sense RNA probes (red) for *FtaiOrco*, *FtaiOr1*, *FtaiOr27*, and *FtaiOr60*, respectively. No positive hybridization signal was detected, confirming the specificity of the corresponding antisense probes. Scale bars = 10 µm.

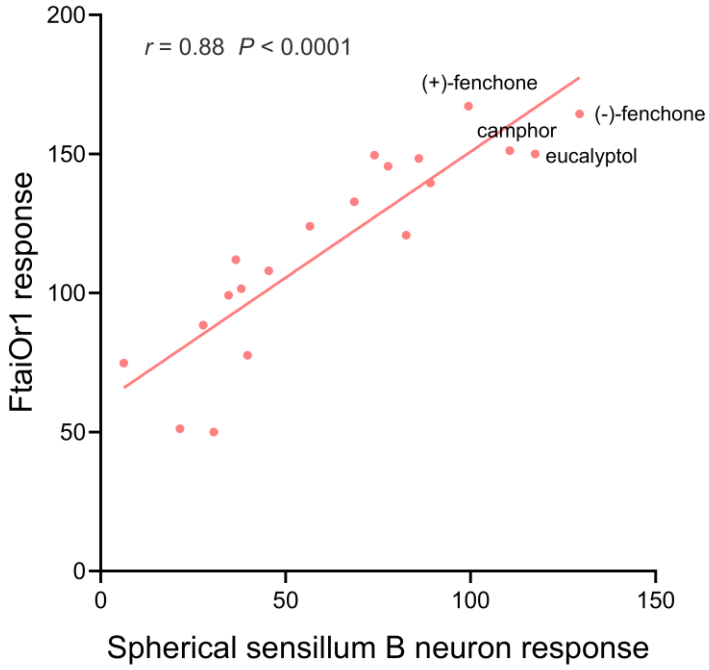

Fig. S8. Pearson correlation of odor response profiles. Y-axis:Responses of FtaiOr1 to 20 odorant compounds, the top-20 ligands that elicit the strongest responses in FtaiOr1; X-axis: Spherical sensillum B neuron responses to the same set of compounds (Spike/s). The two response spectra showed a strong significant positive correlation (r = 0.88, R^2 = 0.78, P < 0.0001).

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Tables S1 to S5

Table S1. Revised gene nomenclature for odorant receptor (Or), gustatory receptor (Gr), and ionotropic receptor (Ir) genes in *F. taiwana*.

Table S2. Expression profiles of *FtaiOr*, *FtaiIr*, and *FtaiGr* genes in the heads of *F. taiwana*.

Table S3. The 180 compounds used in electrophysiological assays of the Drosophila empty neuron system expressing FtaiOr.

Table S4. List of primers used to amplify coding sequences (CDS) of 20 *FtaiOrs*.

Table S5. Anti-sense probe sequences (5'-3') of for whole-mount fluorescence in situ hybridization (WH-FISH) targeting *FtaiOrco*, *FtaiOr1*, *FtaiOr27*, and *FtaiOr60*.