Chemosensory receptors of *Cydia pomonella* (Lepidoptera: Tortricidae)

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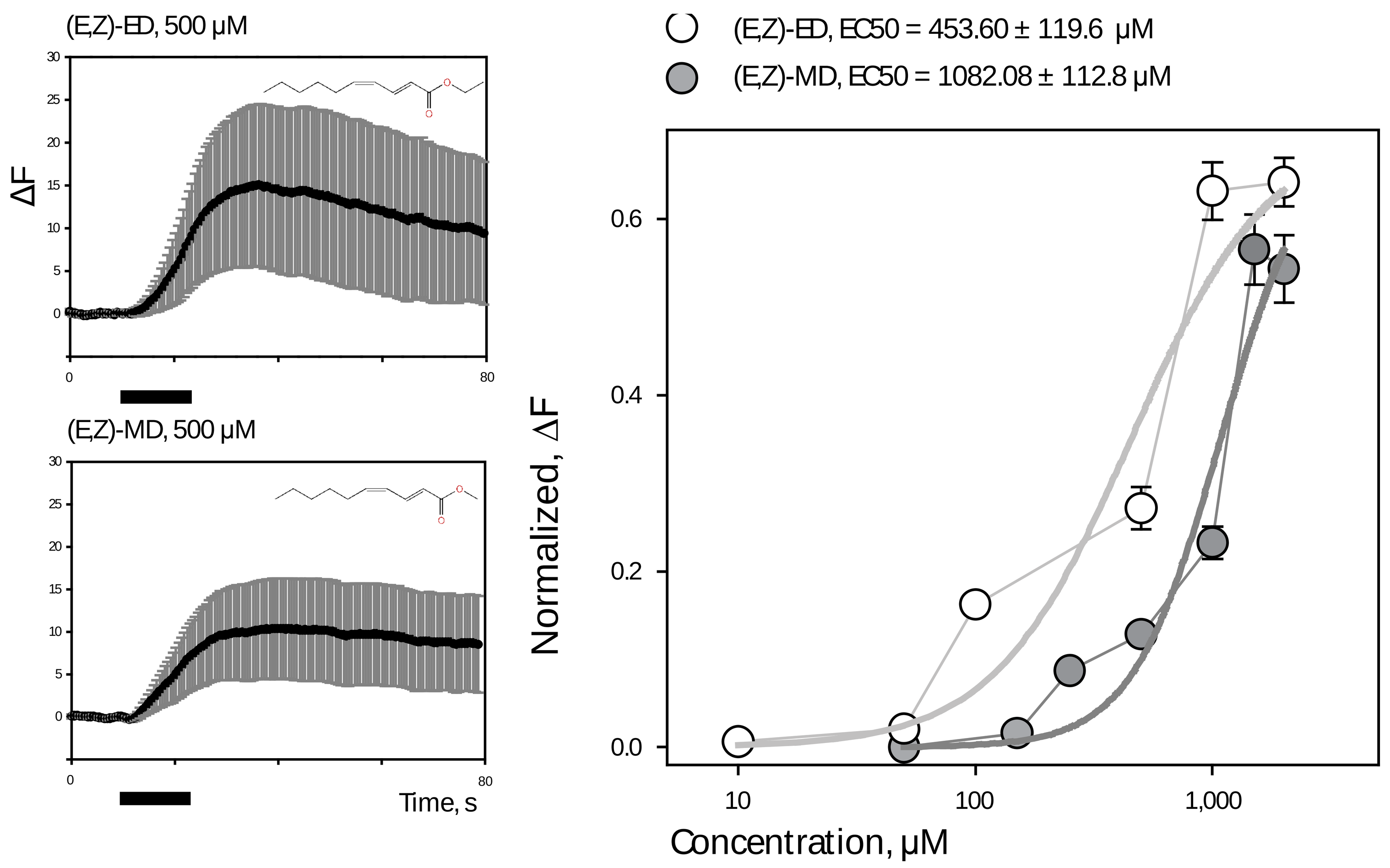
Cydia pomonella L.

Background

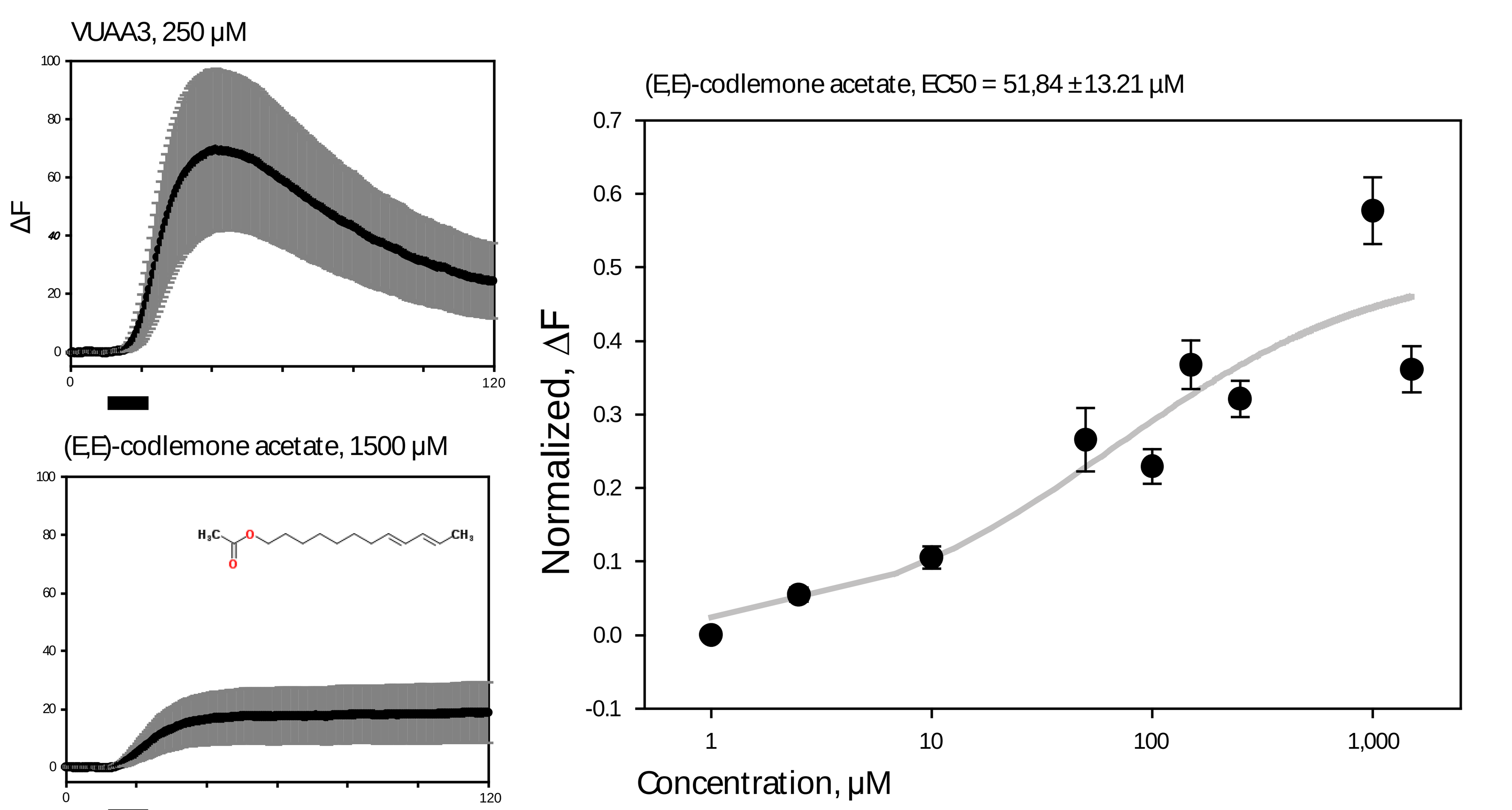
Olfaction plays a dominant role in the mate-finding and host selection behaviors of the codling moth (*Cydia pomonella*), an important pest of apple, pear and walnut orchards. Antennal transcriptome analysis (Bengtsson et al. 2012, Walker et al. 2016) revealed a number of abundantly expressed genes related to the moth olfactory system, including those encoding olfactory receptors (ORs), some of which belong to the putative pheromone receptor (PR) lineage, the co-receptor (CpomOrco) and Transient Receptor Potential (TRP) channels. Using heterologous expression, in both human embryonic kidney (HEK293T) cells and in *Drosophila* olfactory sensory neurons, coupled with calcium imaging and electrophysiological recording, respectively, we characterize the basic physiological and pharmacological properties of these receptors and demonstrate that they form functional ionotropic receptor channels. Molecular biological analysis of ORs and TRPs suggests that at least some receptors are expressed as a complex combination of splice-isoforms and their pattern may correlate with the expression of other ion channels.

Functional characterization of CpomORs in Human Embryonic Kidney (HEK293T) cells

Functional characterization of CpomOR3 heterologously expressed in HEK293T cells. Left: comparison of CpomOrco+OR3 amplitudes of the Calcium responses (mean of the maximum response ± SEM) to 500 μM **ethyl-(E,Z)-2,4-decadienoate** (pear ester, (E,Z)-ED, 15.07 ± 9.48, dF; up) and to 500 μM **methyl-(E,Z)-2,4-decadienoate** (methyl ester, (E,Z)-MD, 10.40 ± 5.91, dF; down); n = 151. Black bar: stimulus. Right: normalized dose-response of pear ester (white) and methyl ester (grey).

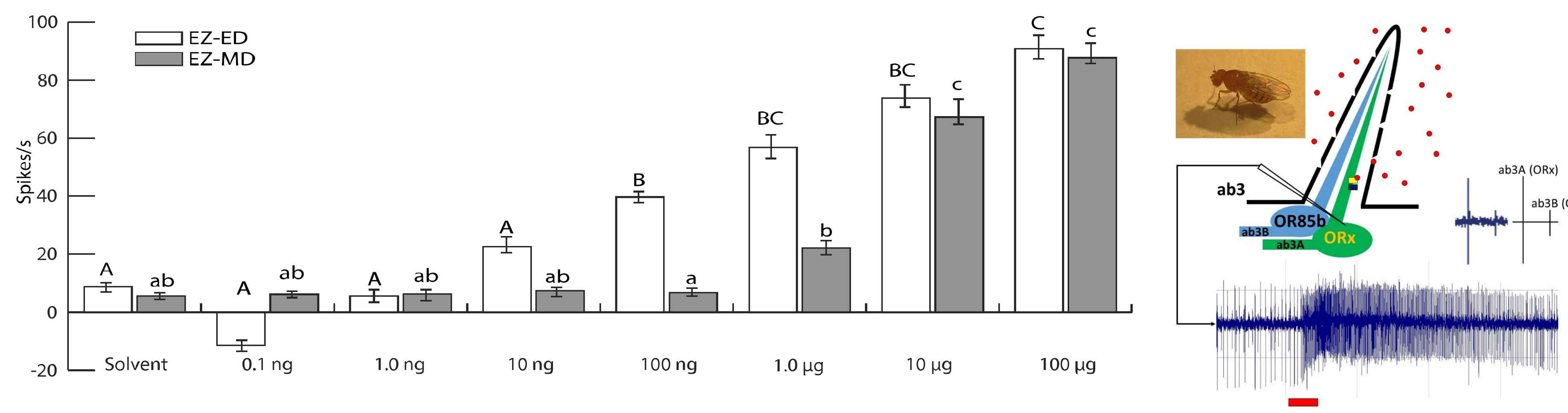


Functional characterization of CpomOR6a heterologously expressed in HEK293T cells. Left: comparison of CpomOrco+OR6a amplitudes of the Calcium responses (mean of the maximum response ± SEM) to 250 μM VUAA3 (69.71 ± 27.29, dF; up) and to 1500 μM (E,E)-8,10-dodecadien-1-yl acetate [(E,E)-codlemone acetate, 18.91 ± 10.31, dF; down]; n = 68. Black bar: stimulus. Right: normalized dose-response to (E,E)-codlemone acetate.

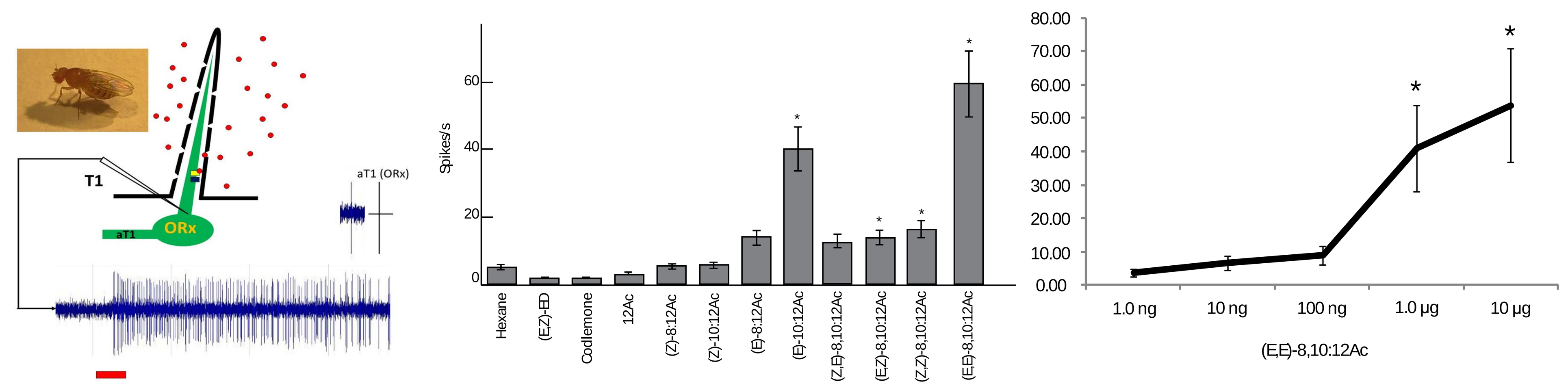


Functional characterization of CpomORs in olfactory sensory neurons of Drosophila melanogaster

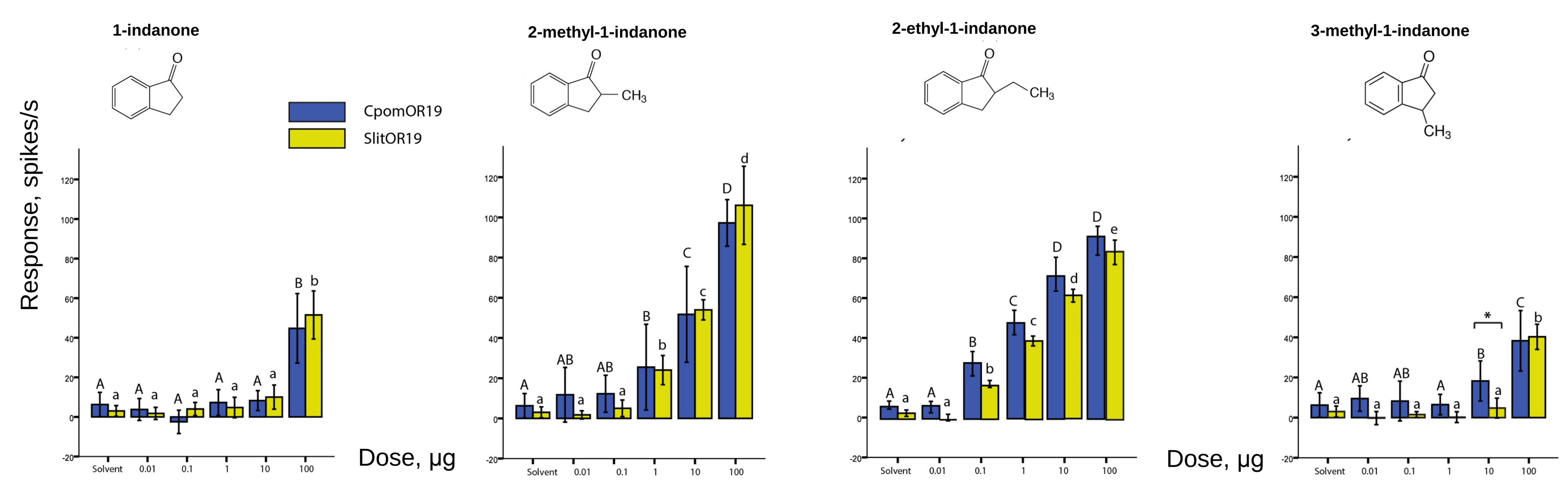
Functional characterization of CpomOR3 heterologously expressed in *Drosophila* ab3A OSNs. Single sensillum recording (SSR) of CpomOR3-expressing ab3A OSNs stimulated with different doses of (E,Z)-ED (white, n = 13) and (E,Z)-MD (grey, n = 13).
Different doses of the compound elicited significant differences (R.M. Anova: (E,Z)-ED, $F(7, 91) = 42.17$, $p < 0.001$; (E,Z)-MD, $F(7, 84) = 41.68$, $p < 0.001$).
CpomOR3 needs a minimum dose of 100 ng of (E,Z)-ED to elicit a response significantly different from the solvent (PostHoc+Bonferrotni: p = 0.026).
CpomOR3 needs a minimum dose of 10 μg of (E,Z)-MD to elicit a response significantly different from the solvent (PostHoc+Bonferrotni: p = 0.020).



Functional characterization of CpomOR6a heterologously expressed in *Drosophila* at1 OSNs. Left: mean ± SEM response of CpomOR6a-expressing OSNs stimulated with 10 μg doses of different compounds. **Expression in *Drosophila* at1 OSN demonstrated activation of CpomOR6a also to (E)-10-dodecadien-1-yl acetate and (partially) to (E,Z)- and (Z,Z)-isomers of (E,E)-codlemone acetate.** Asterisks indicate significant differences between the solvent and the specific compound (Mann-Whitney U Test, p < 0.05, n = 9). Right: spiking activity of OSNs in response to different doses of (E,E)-codlemone acetate. Asterisks denote significant differences between the solvent and the dose indicated (One-way ANOVA with repeated measures, LSD post-doc test, p < 0.05, n = 10).



Functional characterization of CpomOR19 heterologously expressed in *Drosophila* ab3A OSNs. SSR of ab3A OSNs expressing CpomOR19 and its analogue from the noctuid moth *Spodoptera littoralis* (SlitOR19). When compared, ab3A expressing CpomOR19 and SlitOR19 showed increased response to indanes, when substituted with alkyl groups at position two and three of the five-membered ring (Gonzalez et al. 2015). On the contrary, indanes provided with different substitutions did not activate any of the two receptors, which suggests a conserved function for CpomOR19 and SlitOR19 orthologues, despite the phylogenetical and ecological difference between these two moths. Asterisk denotes significant differences between species for the dose indicated at P < 0.01 (Two-way ANOVA with repeated measures, LSD post-hoc test, n = 8).

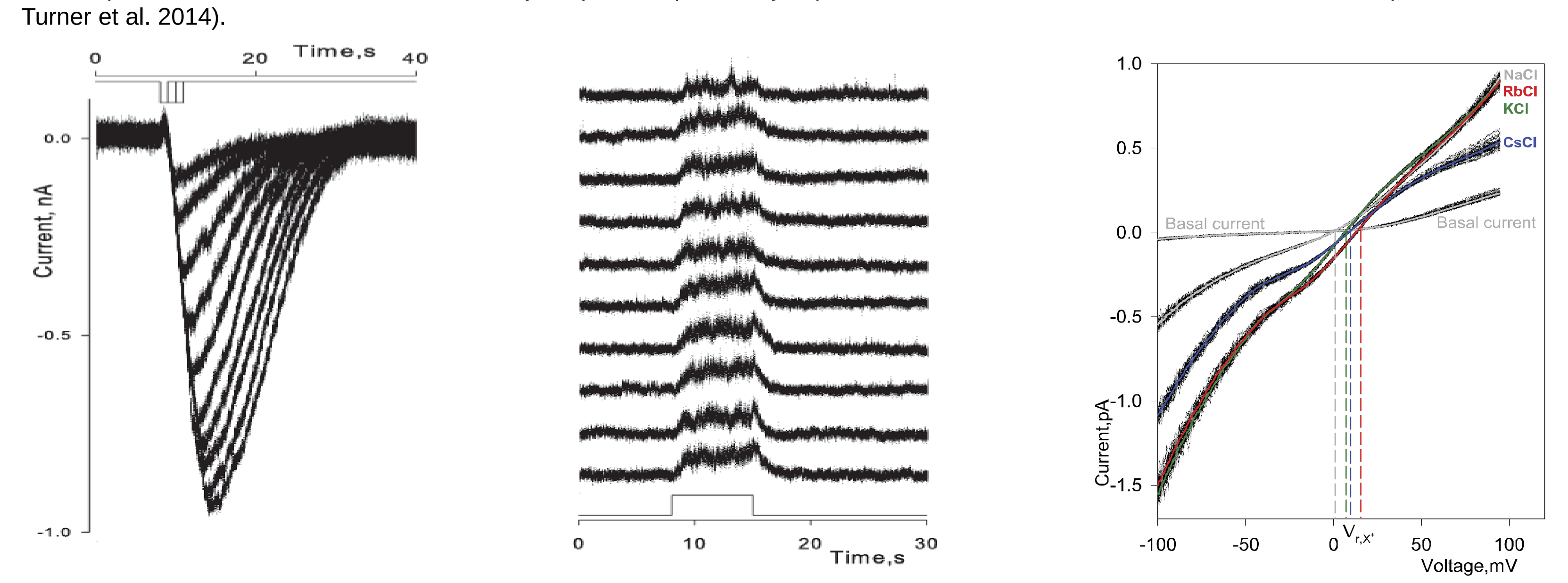


Basic electrophysiological properties of the CpomOrco+OR complexes

Left: whole-cell patch clamp recordings; currents gradually increased in a stimulus intensity dependent manner. Constant amplitudes and stable kinetic parameters indicate **the ionotropic nature of the receptor** under the current experimental conditions.
Middle: VUAA3 (200 μM) applied repeatedly to the extracellular surface of membrane patch reversibly increased the membrane current noise likely associated with the activity of ion channels.
Right: monovalent cation permeability resulted in (PX^+/PNa^+):

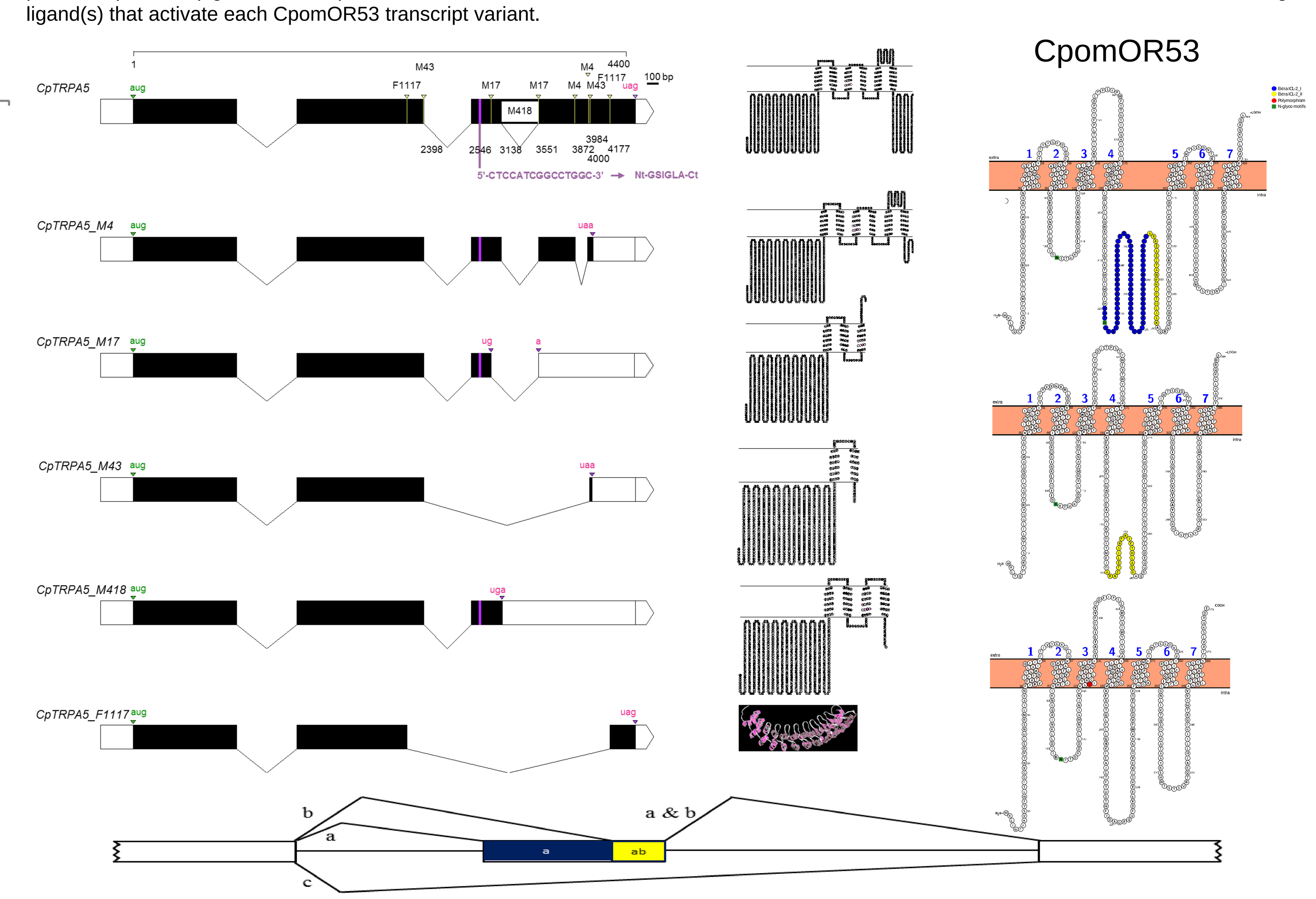
Rb^+ (2.0 ± 0.12) > K^+ (1.37 ± 0.03) > Cs^+ (1.36 ± 0.03) ~ Na^+ > Li^+ (0.93 ± 0.06) (see Cattaneo et al. 2017 for details).

The sequence is consistent with the selectivity sequences previously reported for Orco-based channels from other insects (Pask et al. 2011, Turner et al. 2014).



Alternative splicing of chemosensory receptors of Cydia pomonella

Among the TRP candidates identified in the antennal transcriptome of *Cydia pomonella,* TRPA5 is a novel insect TRPA related with Pyrexia. The mRNA of this receptor is processed to generate different splice forms (left), undertaking mRNA editing, showing combinatorial pattern in antennae and other body parts of the insect (Cattaneo et al. 2016).
Similarly, the odorant receptor CpomOR53 shows that alternative spliced products altered the length of intracellular loop-2 for two of the predicted proteins (right and below). The effects of these alterations were not determined but will be addressed in future studies determining the ligand(s) that activate each CpomOR53 transcript variant.



Conclusions

- We functionally characterized recombinant codling moth ORs by their heterologous expression in HEK293T cells and in the olfactory sensory neurons of *Drosophila melanogaster*.
- Functional expression of CpomOR3 confirmed sensitivity to pear ester (Bengtsson et al. 2014) and to the analogous methyl ester.
- Functional expression of CpomOR6a demonstrated sensitivity to (E,E)-codlemone acetate and to its geometric isomers;
- Functional expression of CpomOR19 demonstrated sensitivity to indanes, which is a feature conserved for the orthologue of *Spodoptera littoralis*, albeit differing taxonomically, in its host range and on its feeding habits;
- Whole cell and outside-out patch clamp recordings demonstrated CpomOR complexes forming functional ionotropic receptor channels.
- Different classes of chemosensory receptors of *Cydia pomonella* undergo to mRNA editing and splicing, generating variants with possible functional roles in olfactory and chemosensory mechanisms

Functional expression of CpomORs therefore represents a valuable tool that can be utilized to further investigate mechanisms of insect OR function and develop novel means to intervene and control the pest's behavior (Cattaneo 2018).

References

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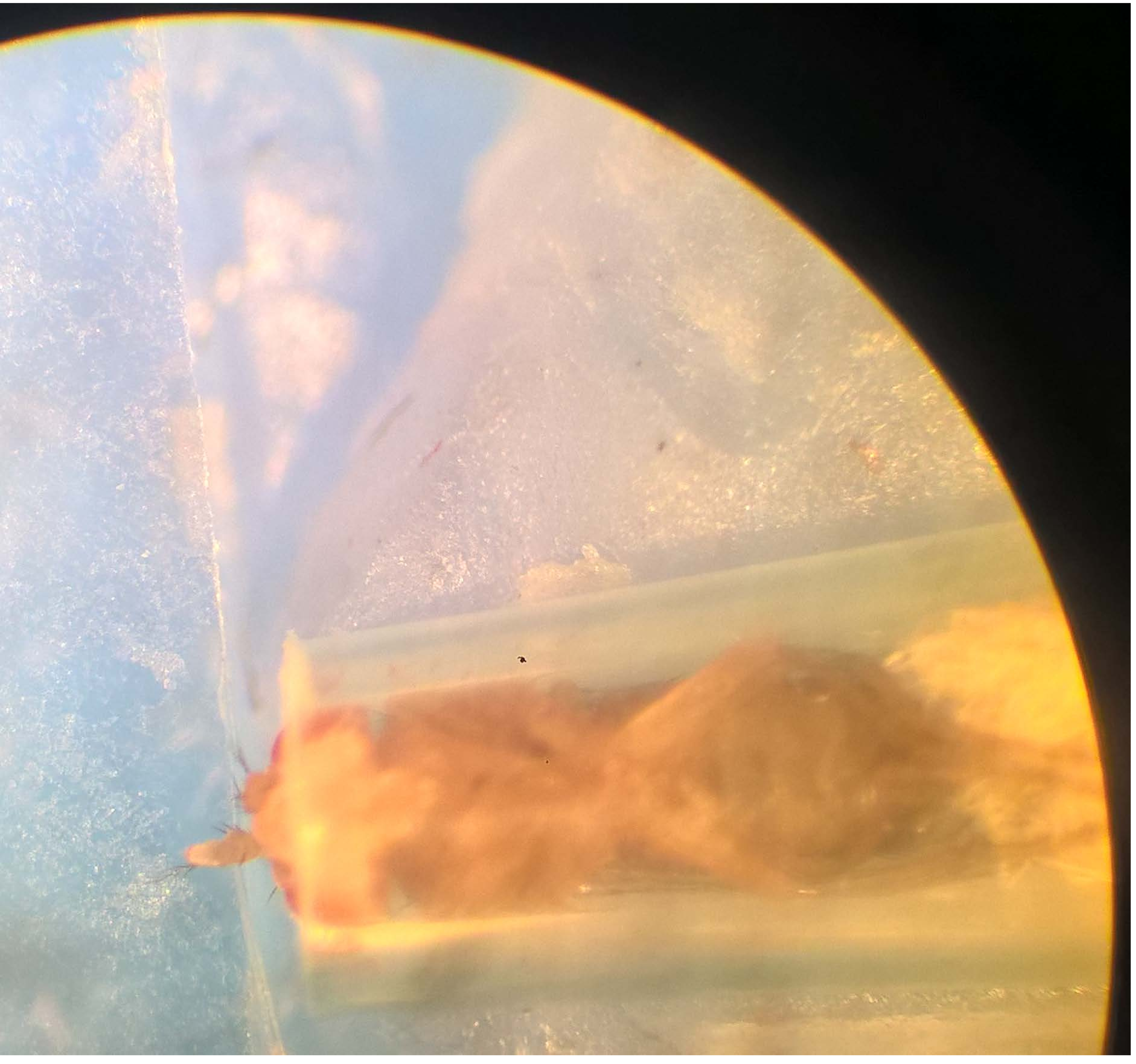
Chemosensory receptors of *Cydia pomonella* (Lepidoptera: Tortricidae) – *Supplementary information*

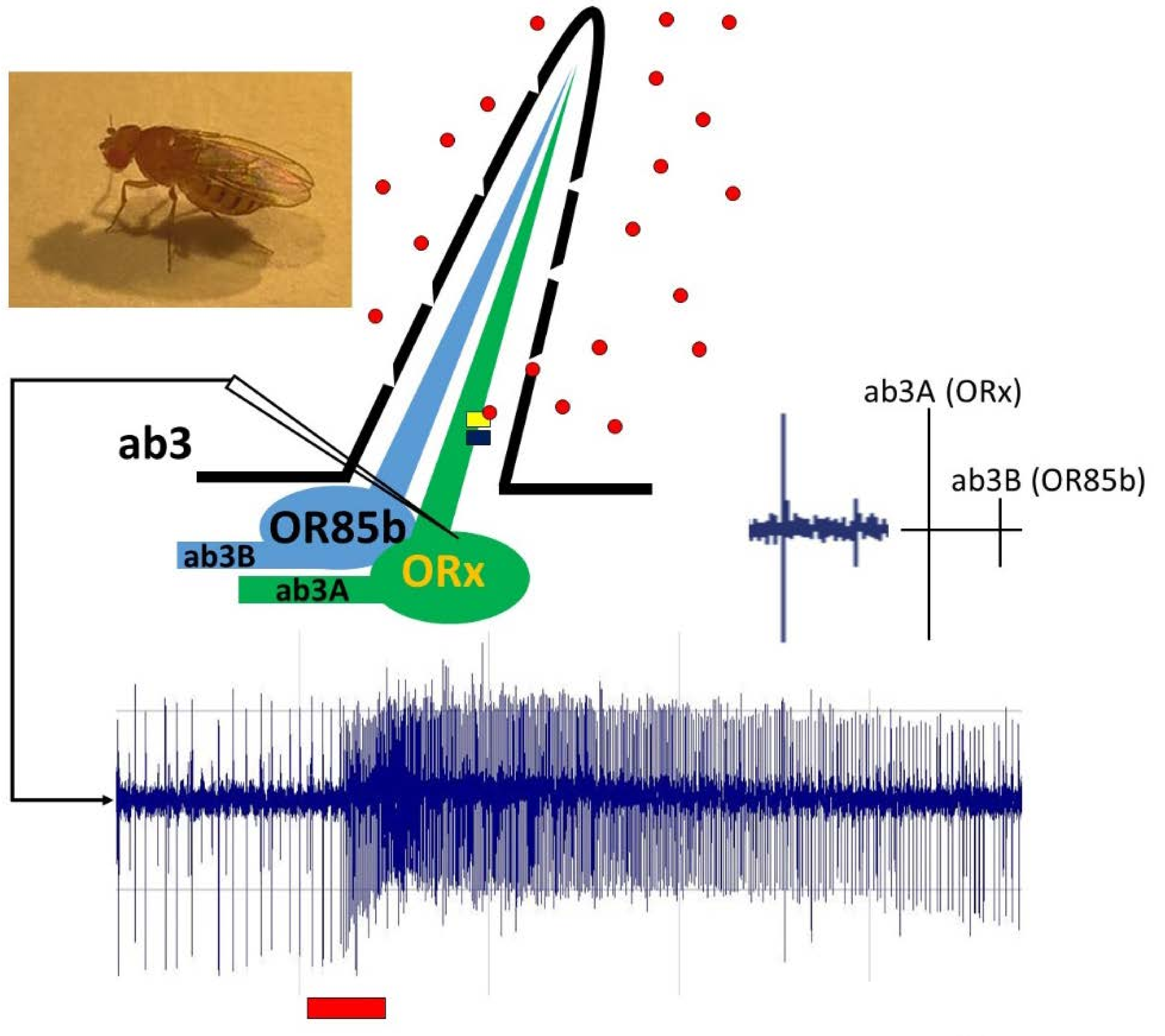
Functional expression of homomeric CpomOrco in HEK293T cells.

Functional expression of CpomOrco was verified by Calcium imaging and single-cell patch clamp recording experiments (stimulus - 250 µM VUAA1, scale bar - 20 µm).

Chemosensory receptors of *Cydia pomonella* (Lepidoptera: Tortricidae) – *Supplementary information*

Functional expression of olfactory receptors in ab3A OSNs of *Drosophila melanogaster*.





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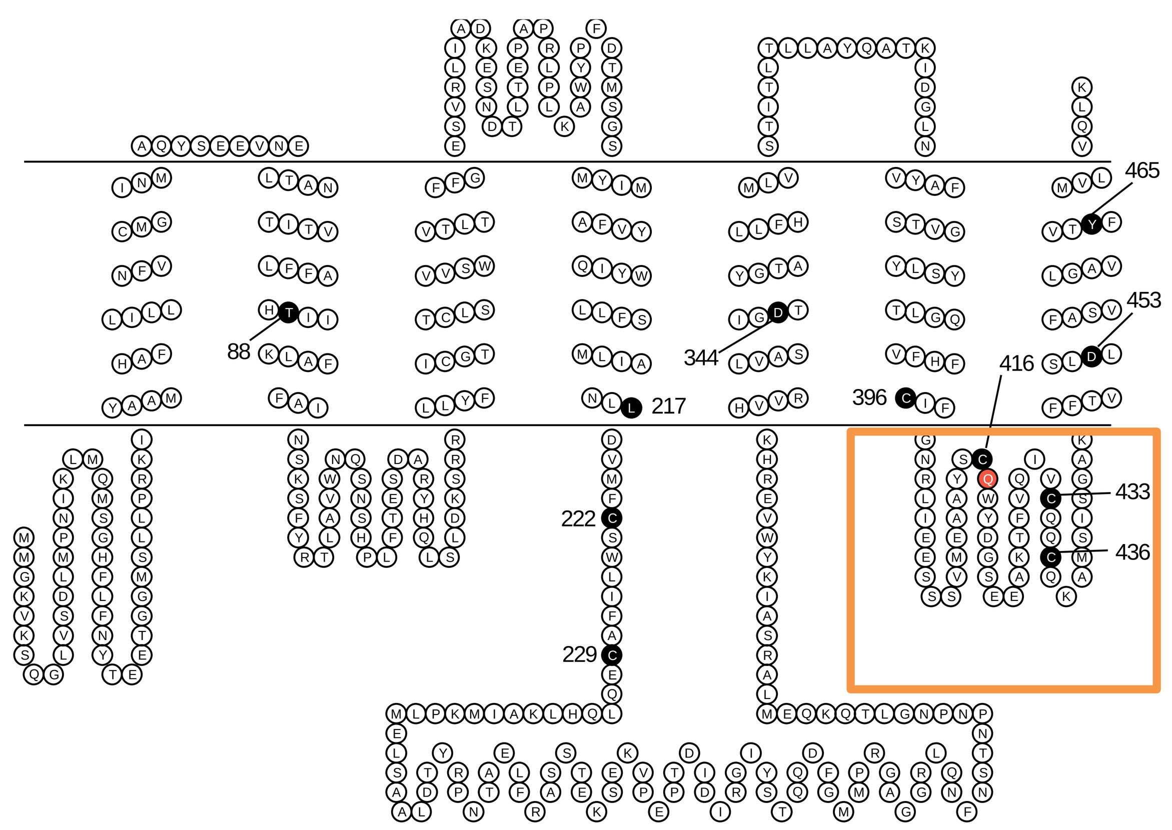
Chemosensory receptors of *Cydia pomonella* (Lepidoptera: Tortricidae) – *Supplementary information*



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      -8   0         10         20         30         40         50
Tdom  QVFLFCIFGN DLIEESLSVM KAAYECPWYN GSEEAKTFIQ IVCQQCQRAL SISGAKFF-- -
Psic  QVFQFCYYGN QLIDESSSVL EAAYSCRWYD GTEEAKAFVQ IICQQCQKSM TISGAKFFTV -
Nlec  QVFLFCIFGN RLIEESSSVM DAAYSCQWYD GSEEAKTFVQ IVCQQCQKAM SVSGAK---- -
Znev  QVFLFCFFGN RLIEESSSVM EAAYSCQWYD GSEEAKTFIQ IVCQQCQKAM SISGAKFFTV -
Csec  QVFLFCFFGN RLIEESSSVM EAAYSCQWYD GSEEAKTFIQ IVCQQCQKAM SISGAKFFTV -
Phum  QVFVFCTYGN QLIEESSSVV EAAYSCQWYD GSEEAKTFVQ IVCQQCQKAL SISGAKFF-- -
Dmel  QVFHFCIFGN RLIEESSSVM EAAYSCHWYD GSEEAKTFVQ IVCQQCQKAM SISGAKFF-- -
Cpom  QVFHFCIFGN RLIEESSSVM EAAYSCQWYD GSEEAKTFVQ IVCQQCQKAM SISGAKFF-- -
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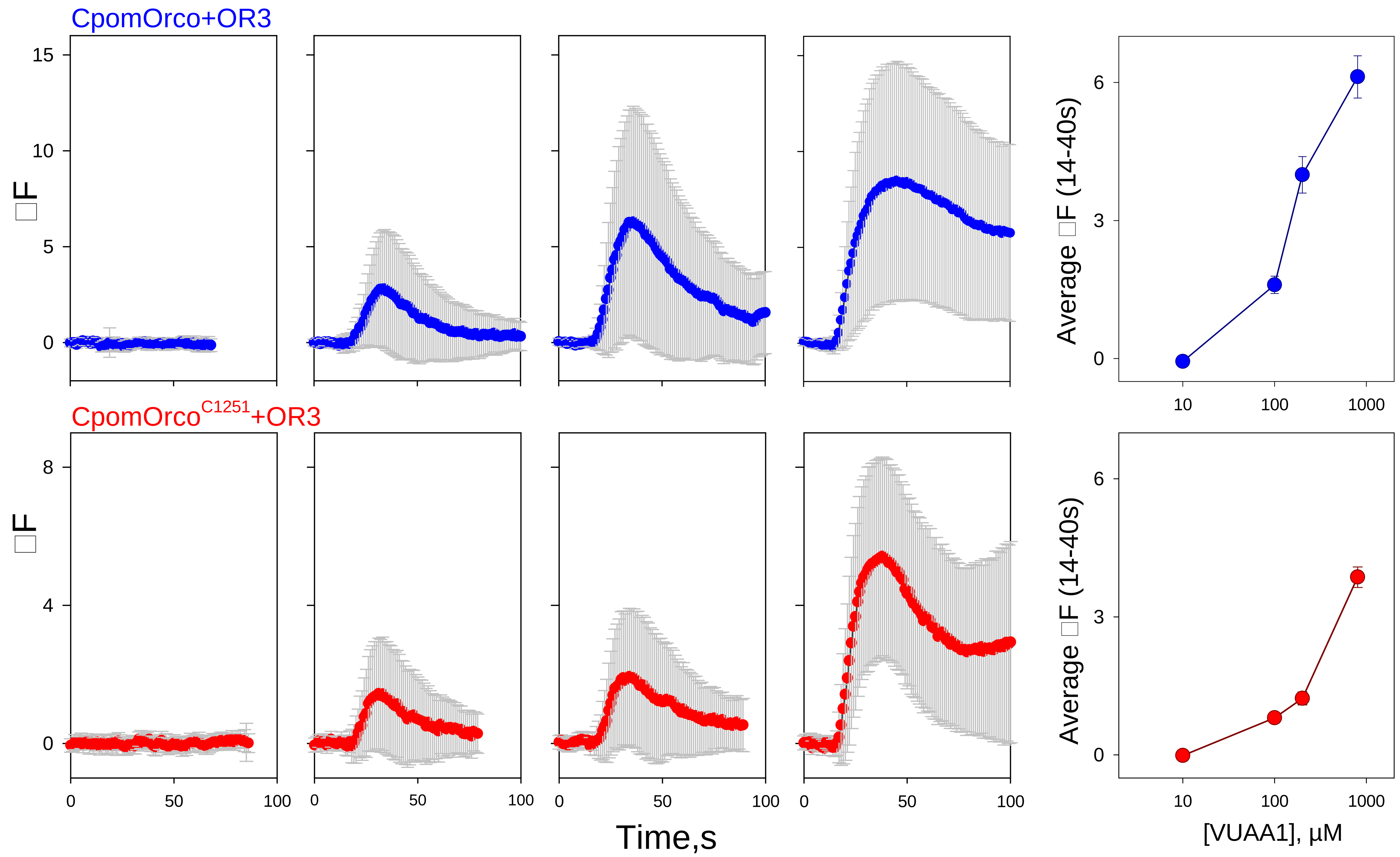
Motif A-predictions*

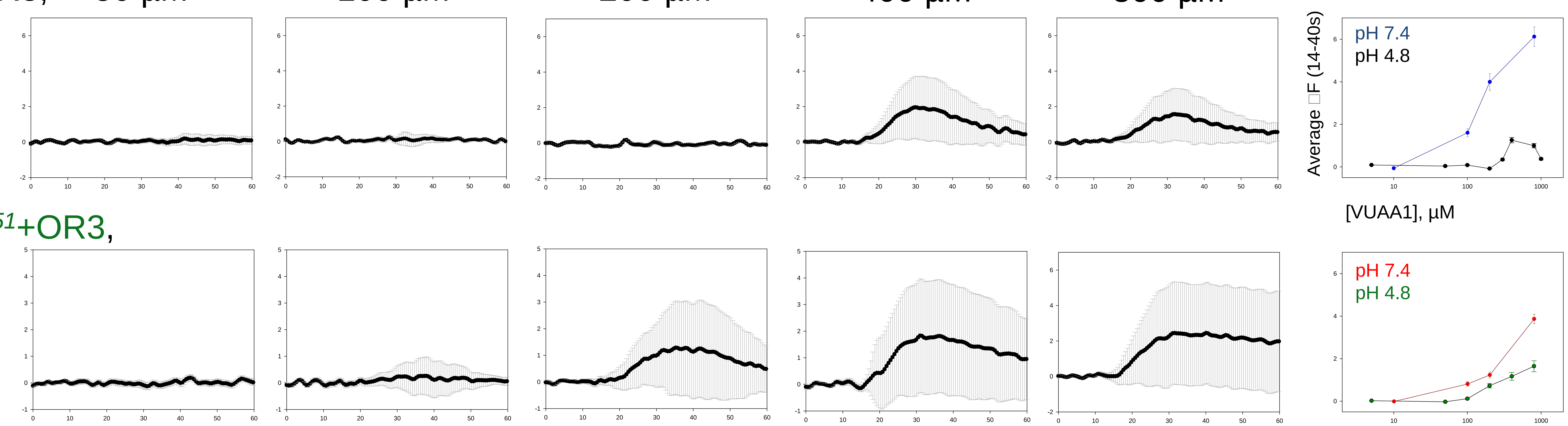
pH = 7.4



pH = 4.8

CpomOrco+OR3, N = 84

CpomOrco^{C1251}+OR3, N = 63



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