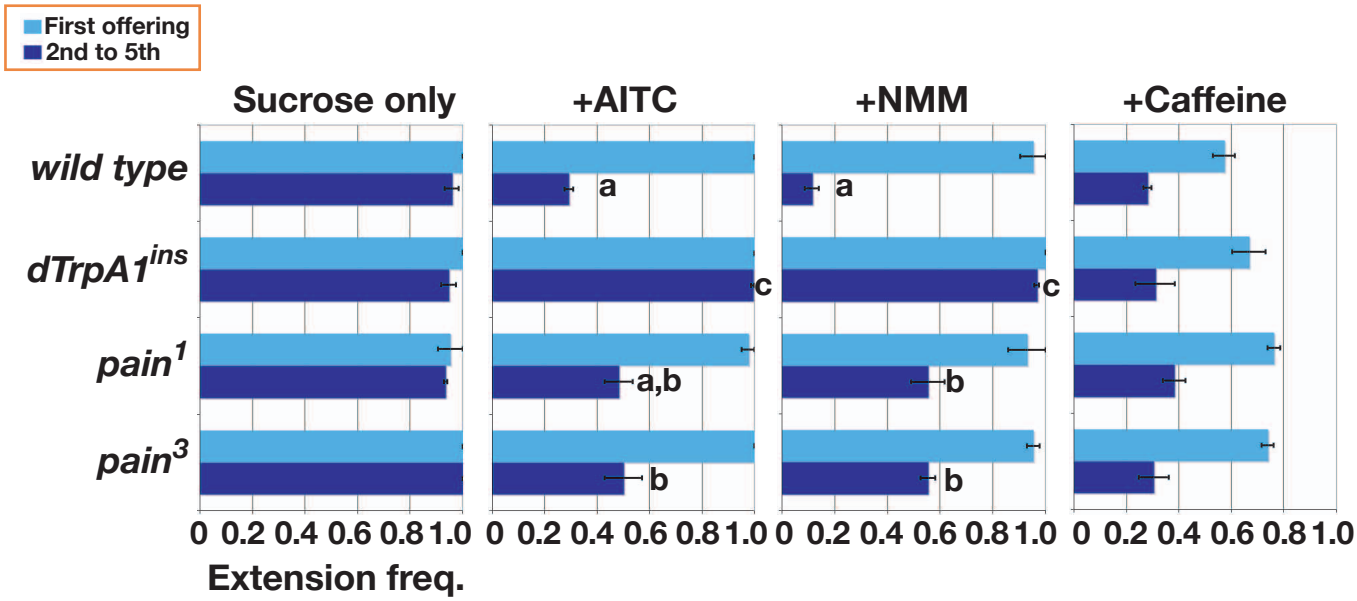
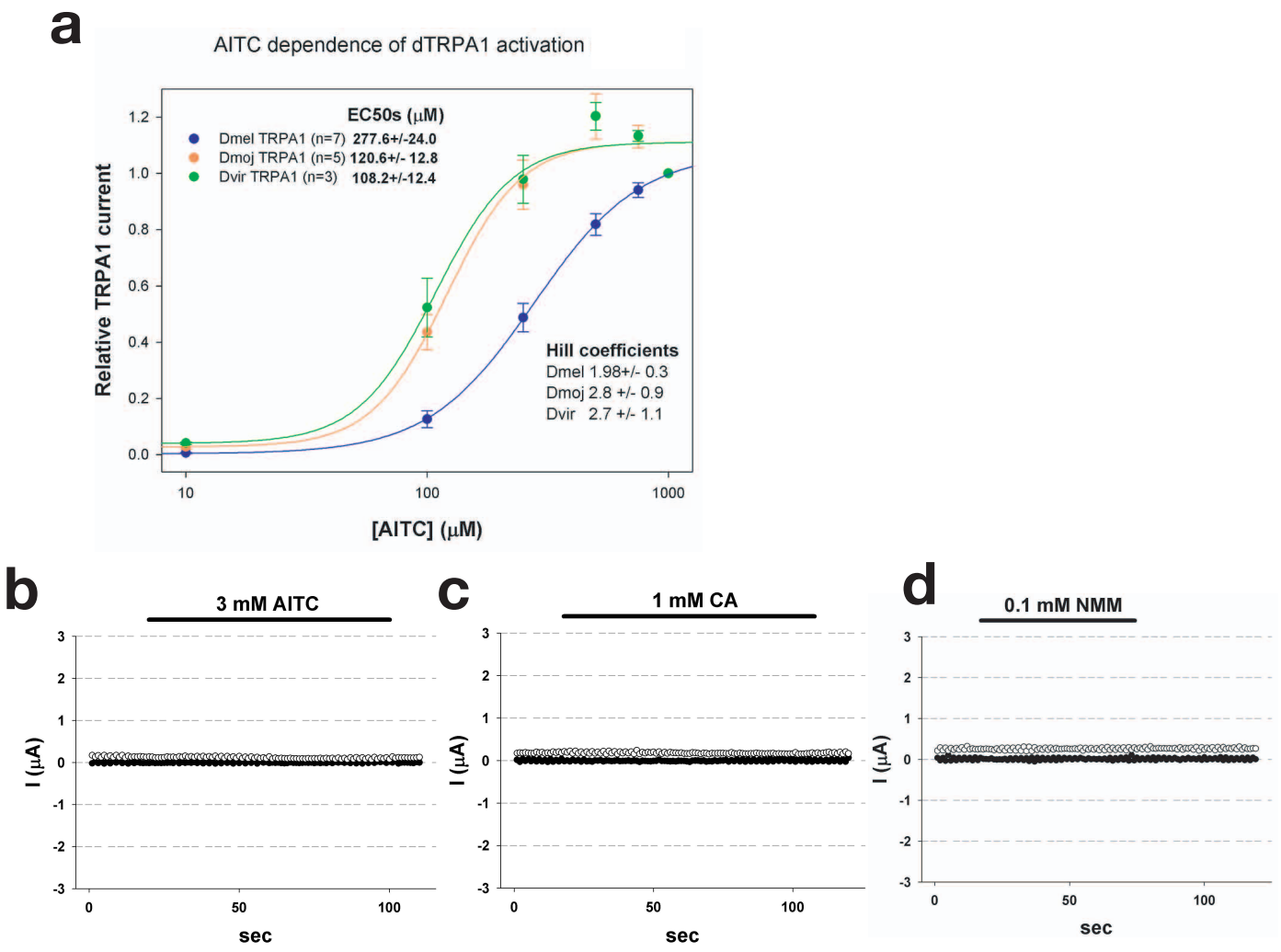
Garrity_supplementary fig. 1



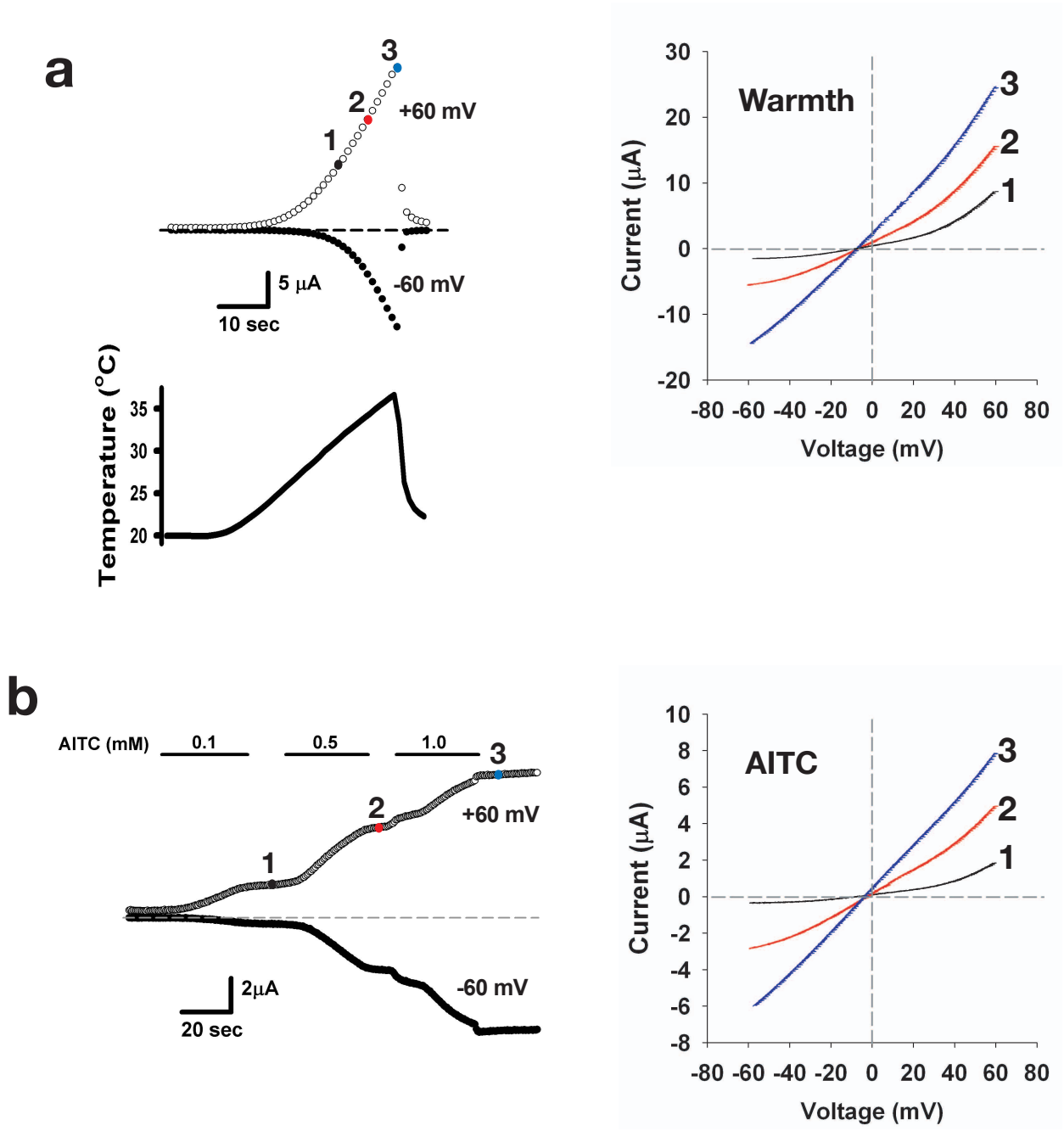
Supplementary Figure 1: ***painless*** **mutant responses to reactive electrophiles.** PER responses, ingestion permitted. Light blue, PER for first offering; dark blue, PER for second to fifth offerings combined. AITC and NMM significantly inhibited PER responses in *painless* mutant flies, although the inhibitory effect was less than in wild type. Statistically distinct groups marked by different letters (Tukey HSD, α=0.05 for +AITC, α=0.01 for + NMM). n=3 groups of 7-8 flies.

Garrity_supplementary fig. 2



Supplementary Figure 2: Dose-response and dTRPA1-dependence of chemically activated currents in oocytes. a, AITC dose-reponse curves for dTRPA1 orthologs from *D. melanogaster*, *D. mojavensis* and *D. virilis*. **b, c, d,** Uninjected oocytes do not respond to reactive electrophiles. Examples of uninjected oocytes treated with 3 mM AITC (**b**), 1mM CA (**c**) and 0.1 mM NMM (**d**).

Garrity_supplementary fig. 3

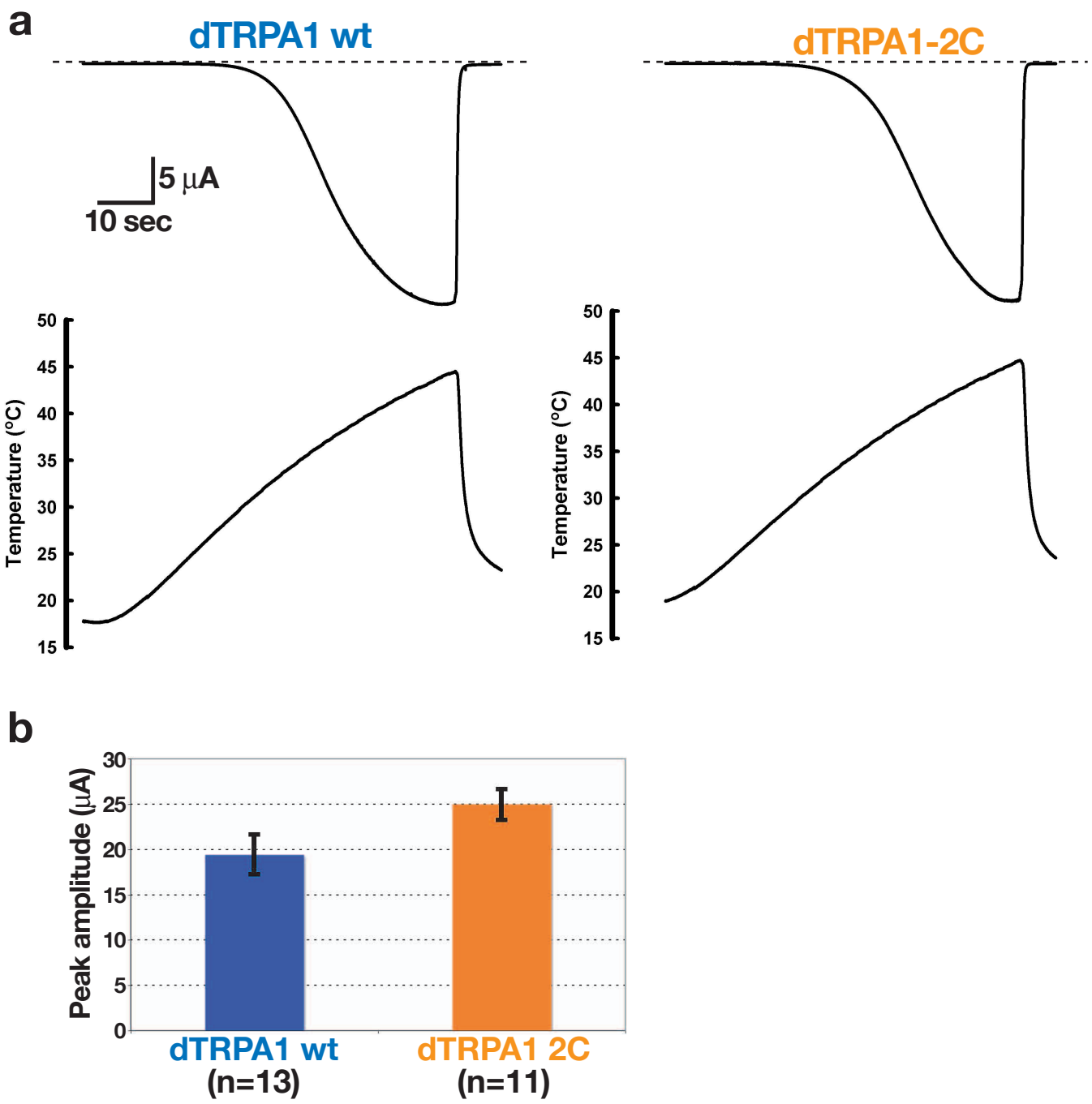


Supplementary Figure 3: Thermal and chemical activation of dTRPA1 yield currents with similar I-V properties. a, Warmth-activated dTRPA1 currents (left panel) and their I-V relationships (right panel). **b,** AITC-activated dTRPA1 currents (left panel) and their I-V relationships (right panel). In both cases, the degree of outward rectification of the channel decreases as dTRPA1 is increasingly activated. Also note that while heat-activated currents decline rapidly upon cooling (a), chemically activated currents are more sustained (b), consistent with covalent modification of the channel by AITC .

Garrity_supplementary fig. 4

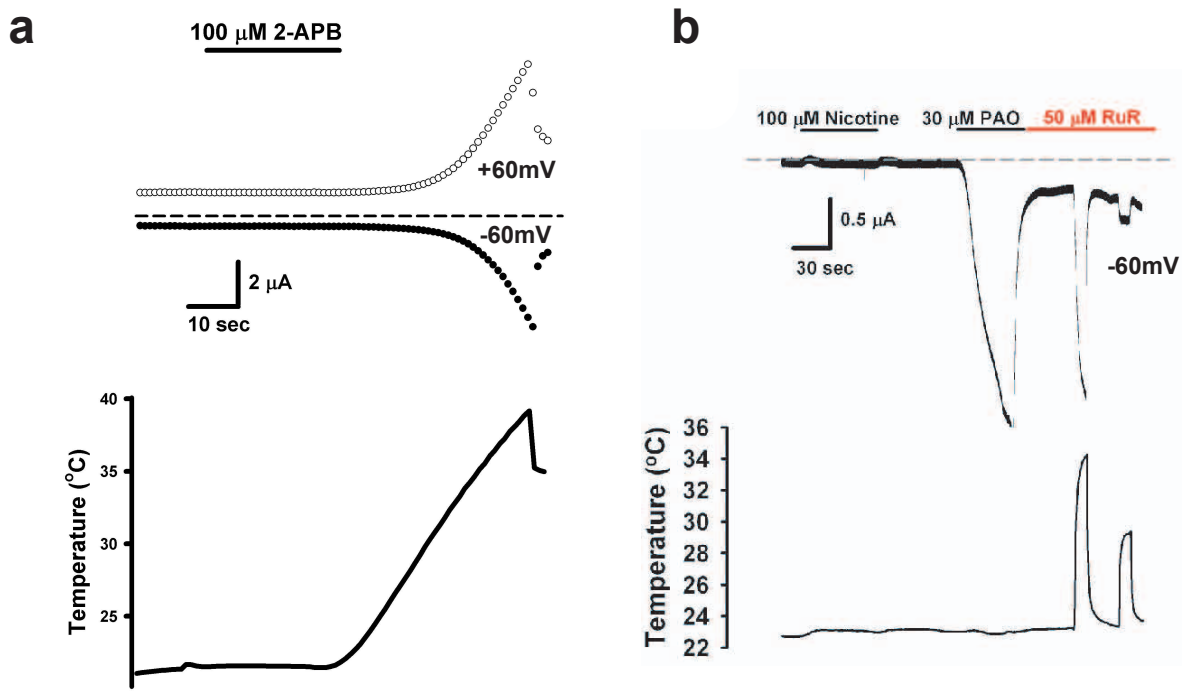
Supplementary Figure 4: Ectopic Painless expression does not confer pungent chemical sensitivity upon motor neurons. Intracellular recordings from third instar larval muscles of OK371>Painless animals before and during treatment with 500 μM cinnamaldehyde (CA). CA application does not induce excitatory junctional potentials (EJPs).

Garrity_supplementary fig. 5



Supplementary Figure 5: Warming robustly activates dTRPA1-2C. **a**, Representative warmth-evoked currents in oocytes expressing wild type (dTRPA1 wt) and mutant (dTRPA1-2C) TRPA1 channels. **b**, Peak amplitude of warmth-evoked currents. Differences in peak amplitude did not reach statistical significance.

Garrity_supplementary fig. 6



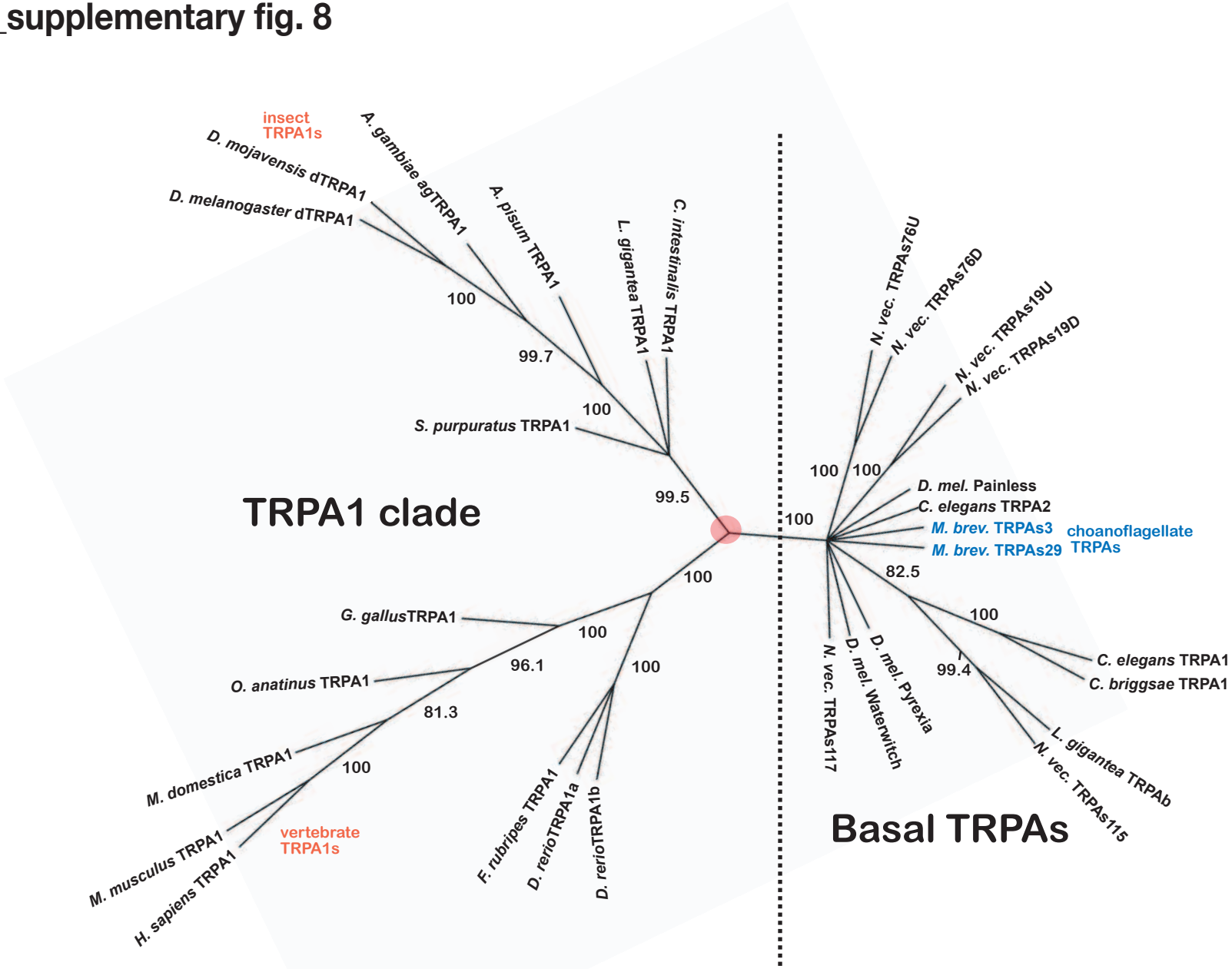
Supplementary Figure 6: dTRPA1 did not detectably respond to 2-APB or nicotine. dTRPA1 expressing oocytes exhibited did not respond to treatment with 100 μM 2-APB (panel a) or nicotine (panel b). Subsequent activation of dTRPA1 by heat and/or the cysteine-modifying reagent phenylarsine oxide (PAO, 30 μM) was used to confirm that the oocytes expressed functional dTRPA1 channels.

Garrity_supplementary fig. 7

Supplementary Figure 7: TRPA channel phylogeny by Maximum Likelihood.

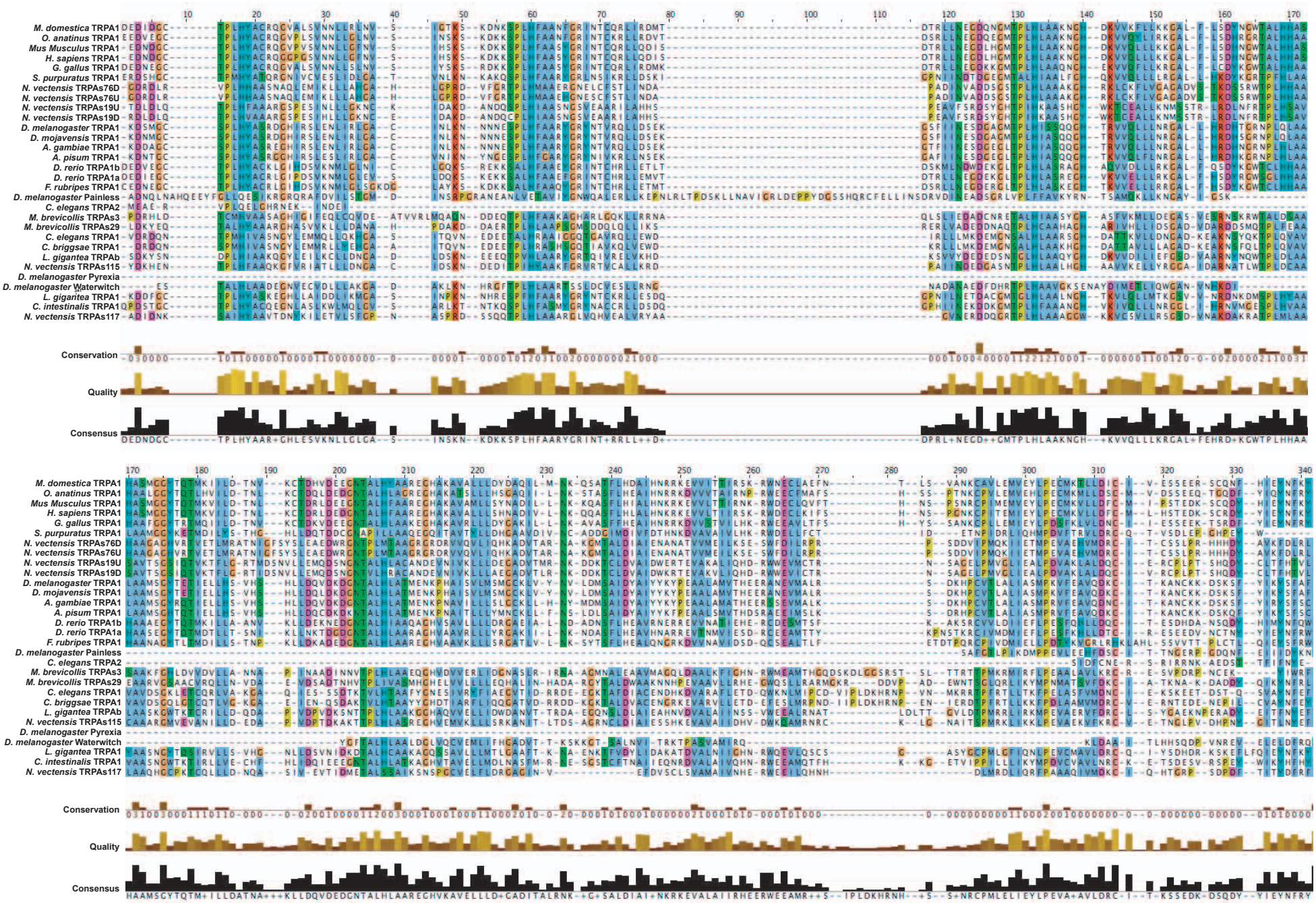
Internal branches are labeled with bootstrap percentages. Branches with bootstrap percentages below 70% unlabeled, branches below 50% collapsed.

Garrity_supplementary fig. 8

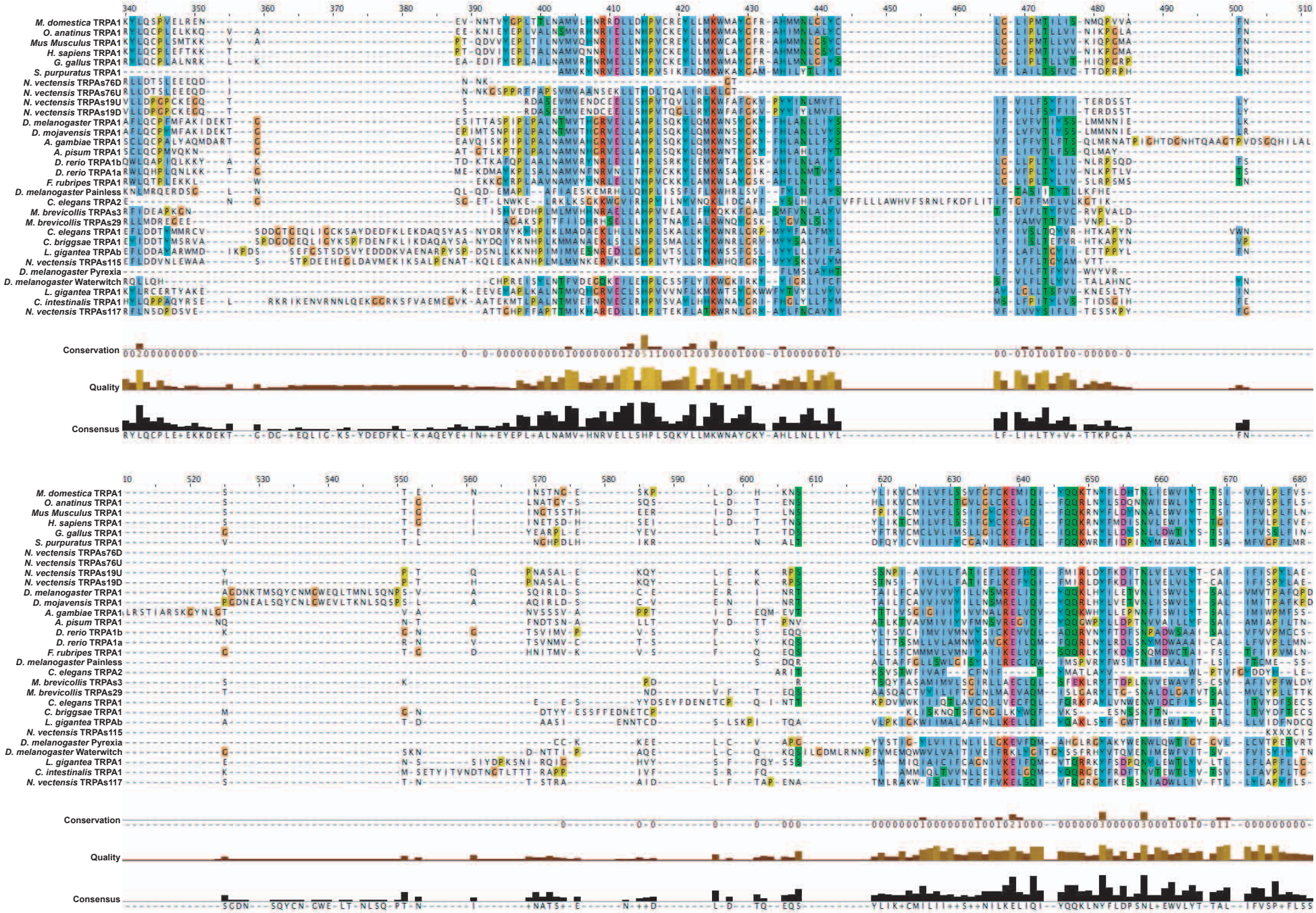


Supplementary Figure 8: TRPA channel phylogeny by Neighbor Joining. Internal branches are labeled with bootstrap percentages. Branches with bootstrap percentages below 75% collapsed.

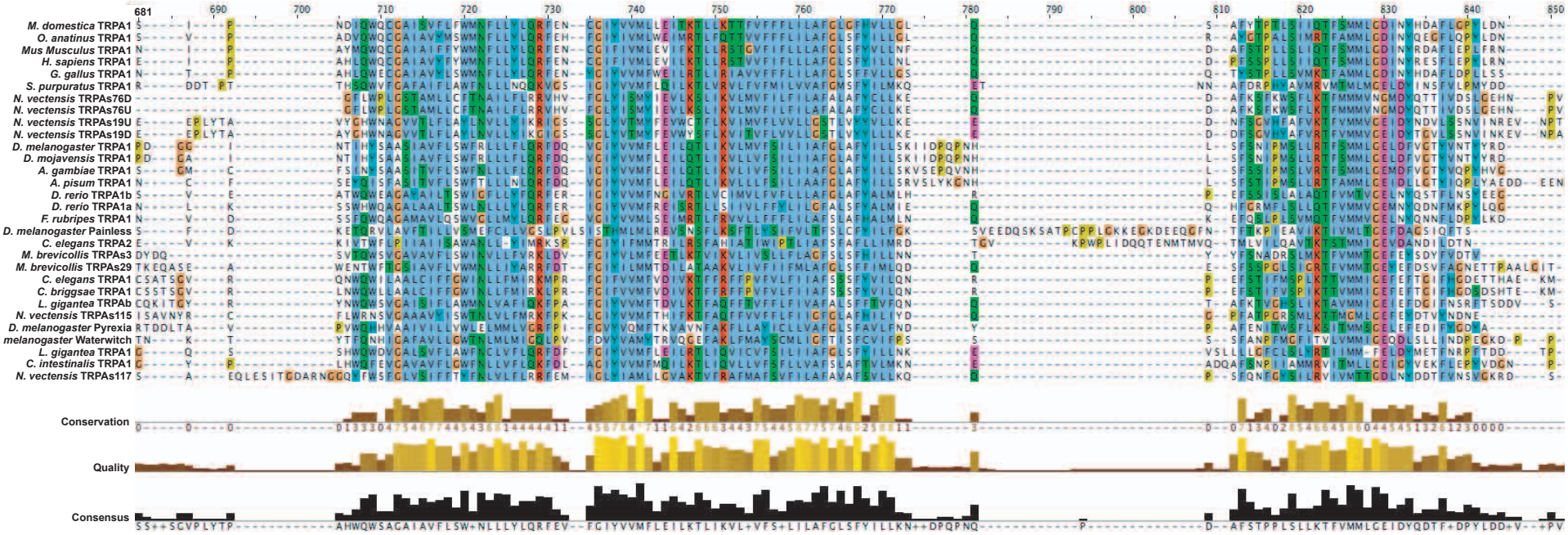
Garrity_supplementary fig. 9 (part 1 of 3)



Garrity_supplementary fig. 9 (part 2 of 3)



Garrity_supplementary fig. 9 (part 3 of 3)



Supplementary Figure 9: TRPA channel multiple sequence alignment. Multiple sequence alignments were visualized using JAL2.4[37]. Conservation is a measure of conservation of physico-chemical properties of residues calculated as described[38]. Quality is a measure of the likelihood of mutations at a given residue[37]. Consensus reflects percentage conserving modal residue.