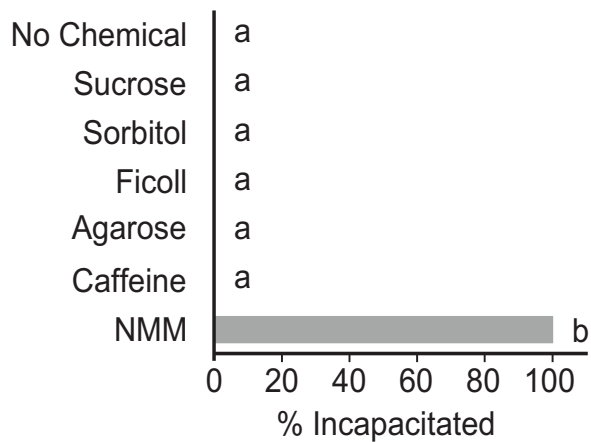
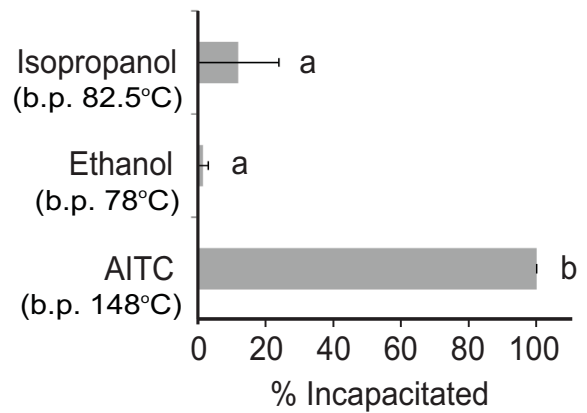


a



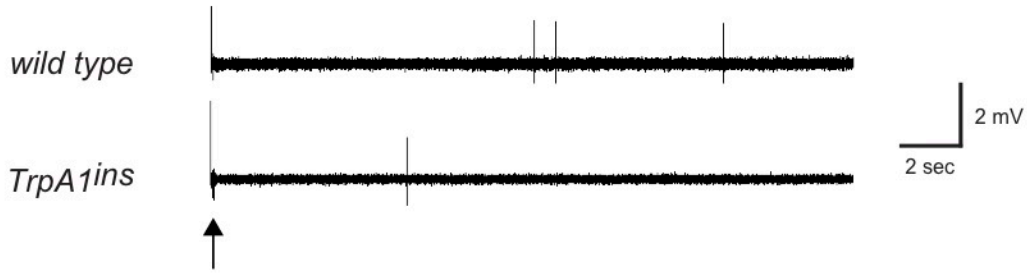
b



Supplementary Figure 1: Reactive electrophiles cause incapacitation in *Drosophila*. In all experiments, 15-30 flies were exposed to chemicals in 15 mL conical tubes. **a**, Solid chemicals were administered as ~50 mg powder for 5 min, tubes containing flies for testing were briefly vortexed to maximize exposure. "No Chemical" tubes were also vortexed as control. **b**, Undiluted Liquids were administered as ~50 μ L drop applied to KimWipes for 1 min. b.p.= boiling point. **a-b**, $**\alpha=0.01$, Tukey HSD. All data are mean \pm s.e.m. 15-30 flies/experiment, n=3 experiments/condition

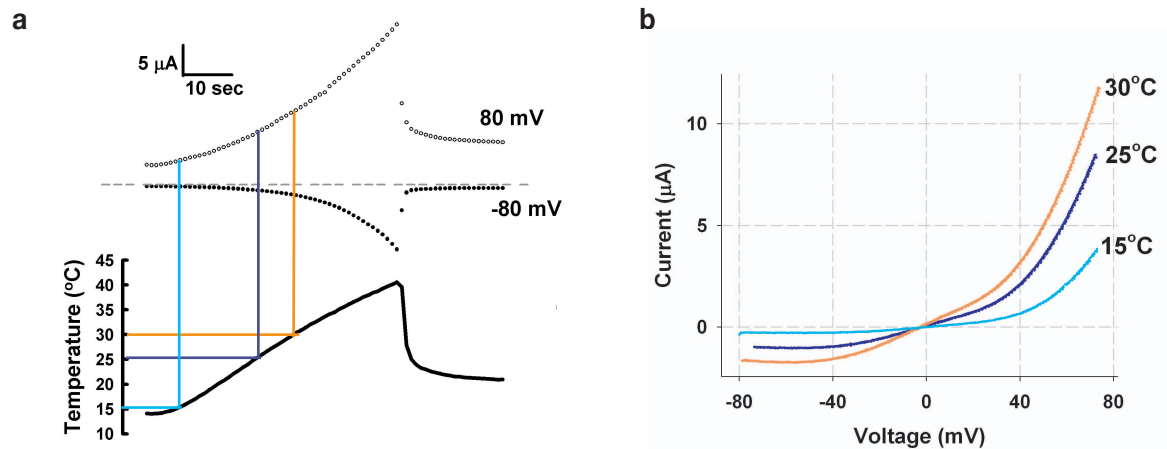
Garrity_SuppFig. 2

Electrolyte Only (30 mM Tricholine Citrate)



Supplementary Figure 2: Example responses from labellar gustatory bristles to electrolyte-only solution in wild type and *Trpa1^{ins}* mutants. Responses from berberine-sensitive i-type bristles. Arrow: artifact caused by initial contact with bristle.

Garrity_SupFig. 3

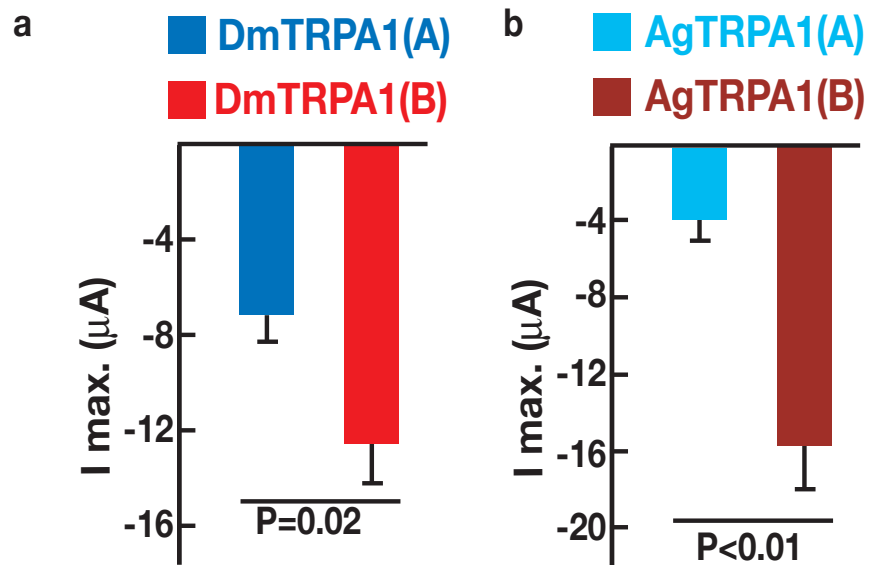


Supplementary Figure 3. TRPA1(A) currents below and above the transition temperature show similar reversal potentials and voltage dependences.

a. Temperature-dependent activity of TRPA1(A) at 80 and -80 mV in *Xenopus* oocytes. Voltage ramps between -80 and 80 mV were applied for 300 msec every second via two-electrode voltage clamp. Temperature was increased at $\sim 0.5^\circ\text{C}/\text{sec}$. *Light blue line* indicates current at 15°C , while *purple and orange lines* currents at 25 and 30°C , respectively.

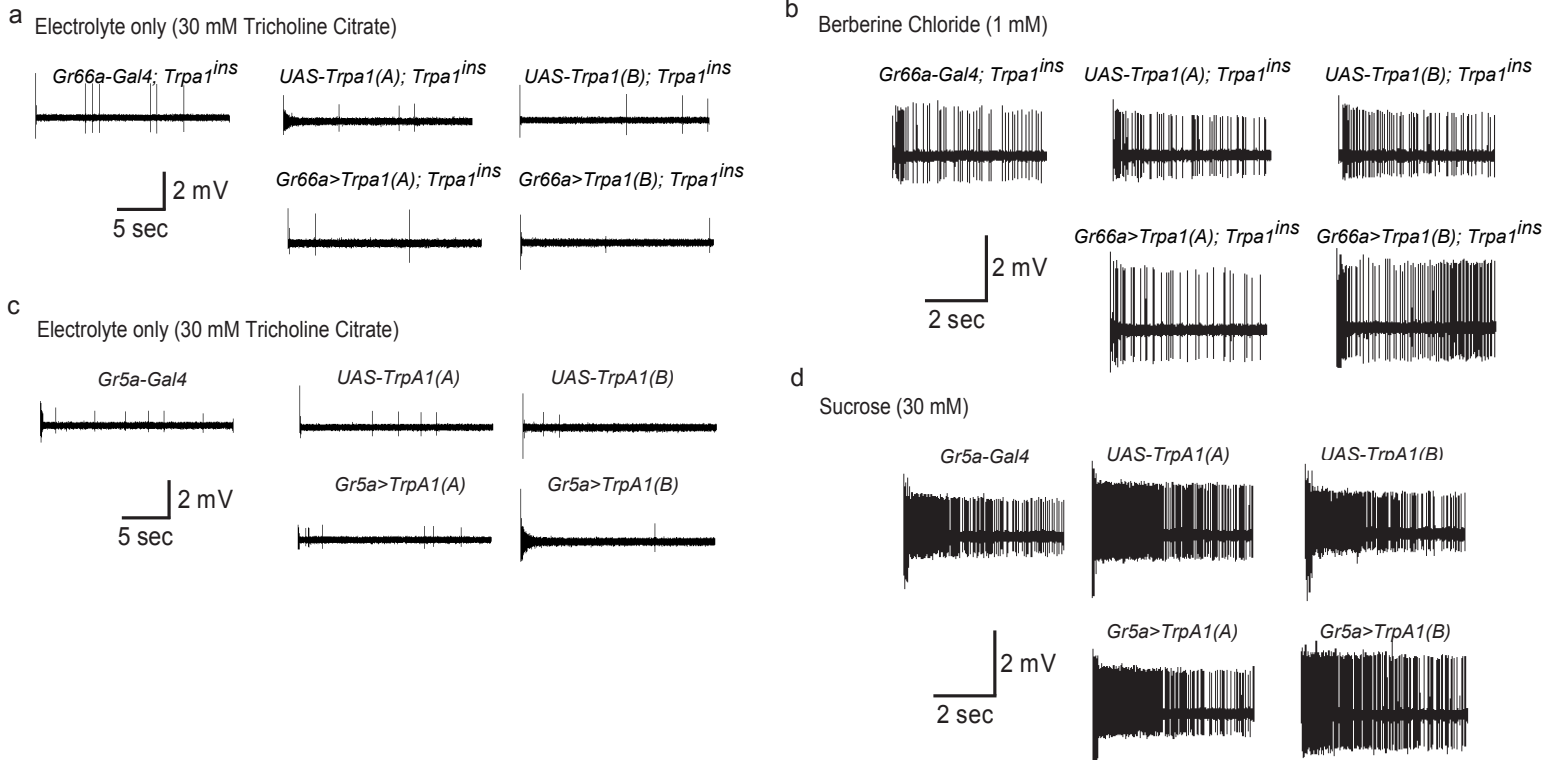
b. Current-voltage relationships of TRPA1(A) activity at 15, 25 and 30°C marked in (a).

Garrity_SuppFig. 4



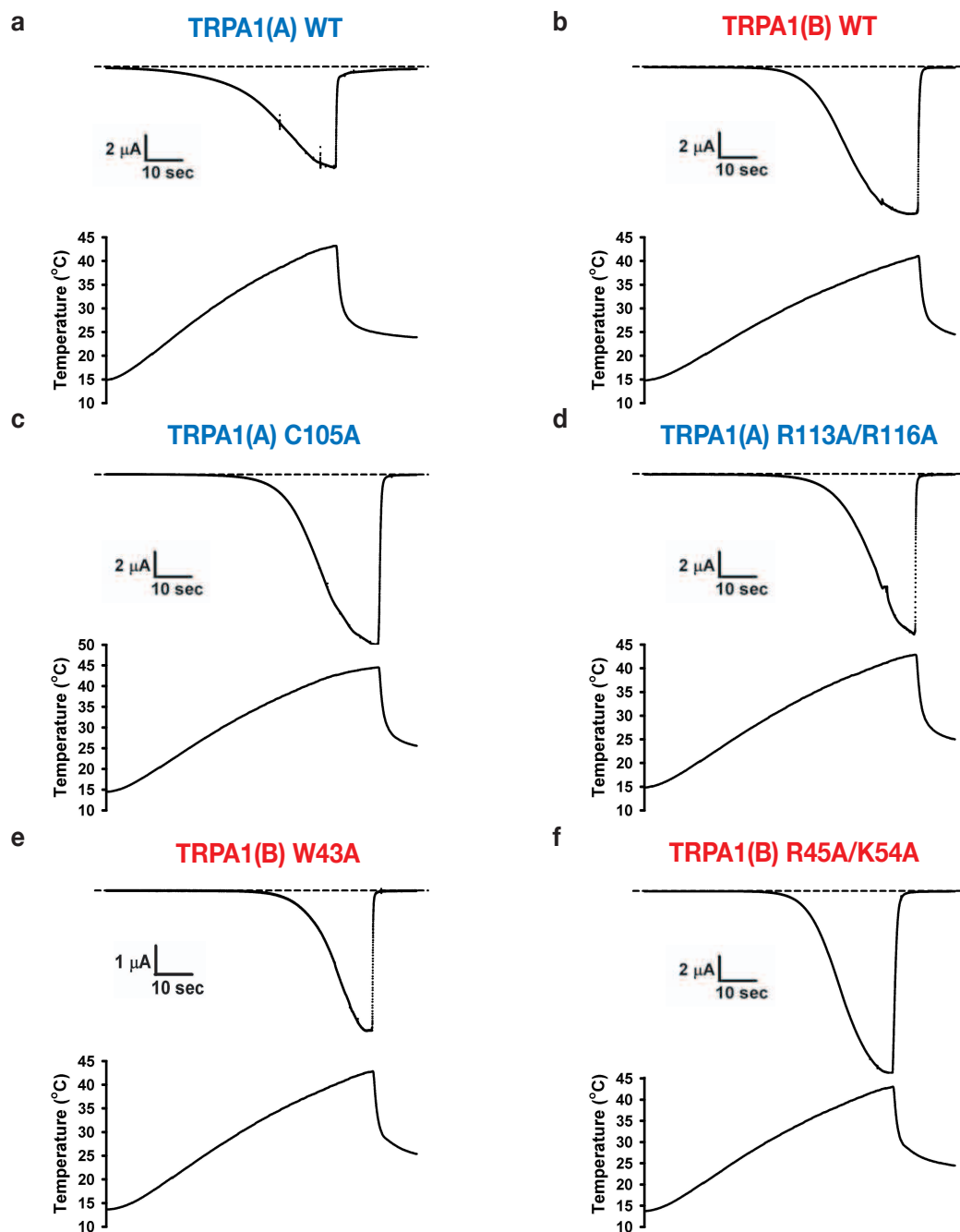
Supplementary Fig. 4. Comparison of maximum heat-responsive current amplitudes for wild type TRPA1(A) and TRPA1(B) channels from *Drosophila melanogaster* and *Anopheles gambiae*. a, b, Maximum TRPA1-dependent currents generated by temperature increase for *Drosophila* (a) and *Anopheles* (b) channels. Statistical comparisons by unpaired t-test

Garrity_SuppFig. 5



Supplementary Figure 5: Example responses from labellar gustatory bristles for TRPA1 rescue and gain-of-function. a, b, Responses of *Trpa1^{ins}* mutant, berberine-sensitive i-type bristles expressing different TRPA1 isoforms. **a, c,** Typical responses of bristles to electrolyte-only solution (30 mM tricholine citrate). **b, d,** Typical responses to positive control solutions used to confirm preparation viability. Berberine-sensitive i-type bristles confirmed with 1 mM berberine chloride (**b**) and L-type bristles confirmed with 30 mM sucrose (**d**).

Garrity_SuppFig. 6



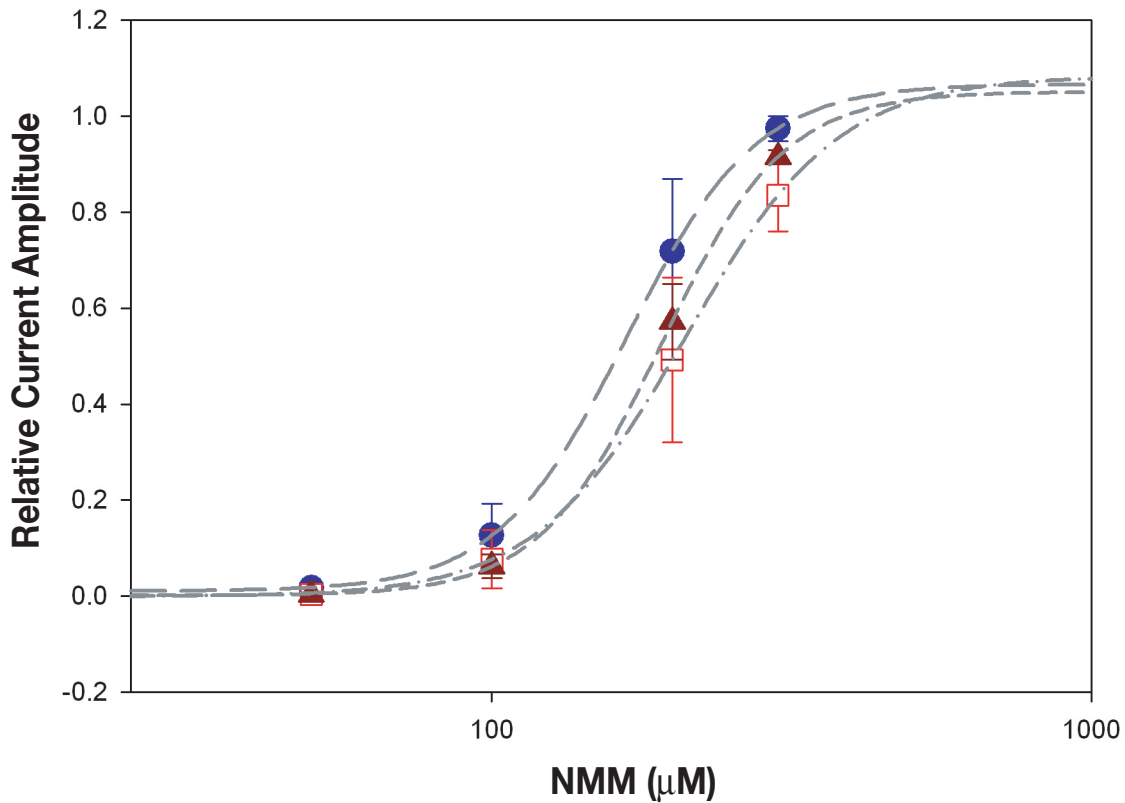
Supplementary Figure 6. Representative current recordings of wild type TRPA1 isoforms and TRPA1(A) mutants. Lower traces show temperature ramps from 15 to 45 $^{\circ}\text{C}$ ($\sim 0.5^{\circ}\text{C}/\text{sec}$) applied to frog oocytes expressing TRPA1 channels as indicated. Currents were recorded at -60 mV held by two-electrode voltage clamp. **a-d.** The corresponding arrhenius plots are presented in Figure 4.

Garrity_SuppFig. 7

	Imax (heat)	Imax (NMM)	Imax (heat)/Imax (NMM)
TRPA1(A) WT	-7.3 +/- 1.1	-20.3 +/- 2.1	0.36
TRPA1(A) C105A	-7.9 +/- 2.1	-14.1 +/- 2.4	0.56
TRPA1(A) R113A/R116A	-7.8 +/- 1.4	-13.1 +/- 1.9	0.59
TRPA1(B) WT	-12.4 +/- 1.7	-24.2 +/- 0.6	0.51
TRPA1(B) W43A	-3.7 +/- 0.4	nd	---
TRPA1(B) R45A/K54A	-8.7 +/- 1.5	nd	---

Supplementary Fig. 7. Comparison of maximum current responses for wild-type and mutant TRPA1 channels. Maximum TRPA1-dependent currents generated by temperature increase and by 300 μ M NMM application in oocytes. For comparison to wild type channels, the heat responses of mutant channels that exhibited increased thermal sensitivity by Q10 (TRPA1(A)C105A and TRPA1(A) R113A/R116A) were normalized by dividing maximum heat responses by maximum NMM responses.

Garrity_SuppFig. 8

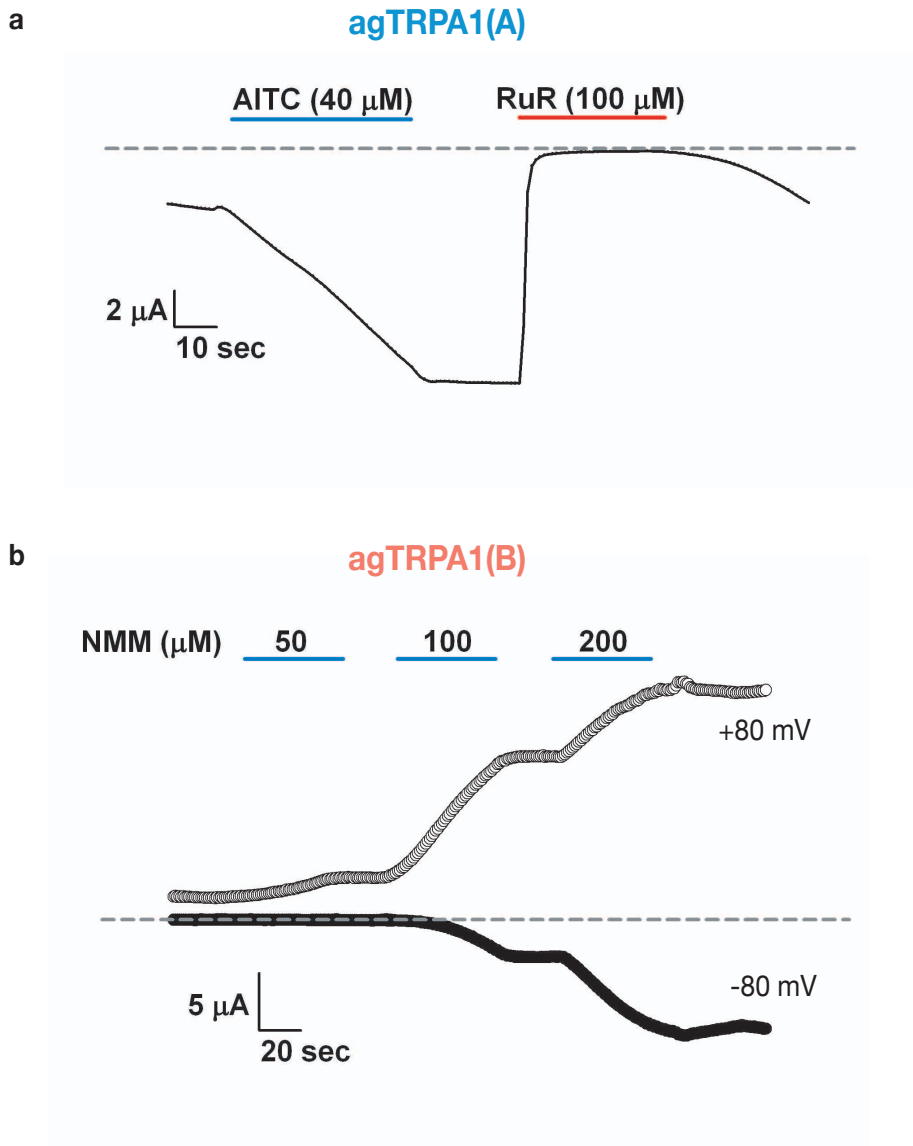


	EC50 (μM)	Hill Coefficient
● TRPA1(A) WT	168 \pm 21	4.0 \pm 1.6
□ TRPA1(A) C105A	211 \pm 73	3.4 \pm 2.6
▲ TRPA1(A) R113A/R116A	192 \pm 13	4.3 \pm 1.3

Supplementary Figure 8. NMM sensitivities of TRPA1(A) mutants are similar to that of wild type TRPA1(A).

A series of NMM concentrations from 50 to 300 μM was applied to *Xenopus* oocytes expressing wild type and mutant forms of TRPA1(A). The data were collected following 1-min perfusion of each NMM concentration at -80 mV, and fitted to the Hill equation. All data are means; error bars indicate \pm SEM

Garrity_SuppFig. 9

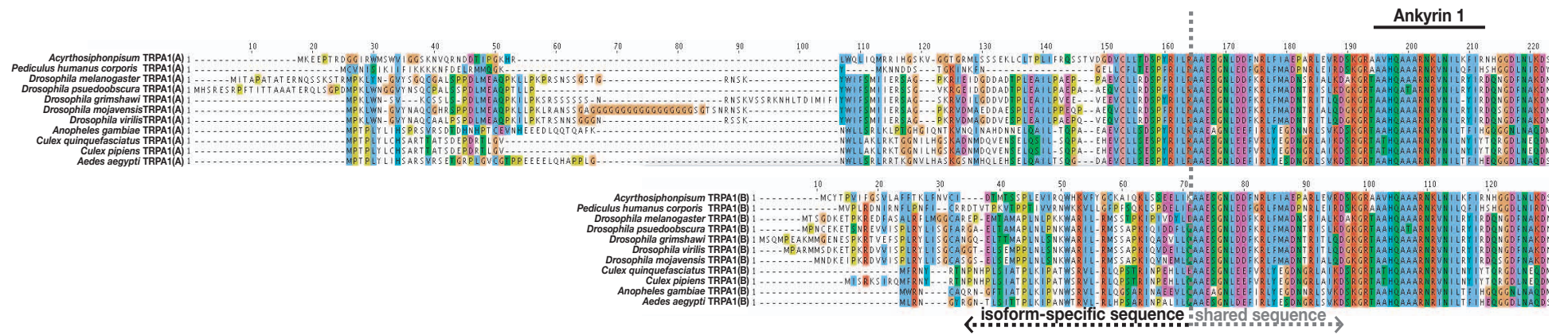


Supplementary Figure 9. Both agTRPA1(A) and agTRPA1(B) robustly respond to reactive electrophiles.

a. A frog oocyte expressing agTRPA1(A) was perfused with 40 μ M allylisothiocyanate (AITC) for 1 min, and washed for 30 sec. Subsequently, the AITC-evoked current was blocked by 100 μ M ruthenium red (RuR). The current was recorded at -60 mV held by two-electrode voltage clamp (TEVC).

b. Three concentrations of NMM from 50 to 200 μ M were sequentially exposed to a oocyte expressing agTRPA1(B) with 30 sec washing intervals as indicated. Currents were recorded while 300 msec-voltage swipes between -80 and 80 mV were applied every second via TEVC.

Garrity_SuppFig. 10



Supplementary Figure 10. Conservation of TRPA1 diversity in insect pests. Multiple sequence alignments of insect TRPA1 isoforms. Position of ankyrin repeat #1 in TRPA1 is noted.