Supplemental Figure 1. Zebrafish TRPA1 amino acid sequences and domain organization. (A) Alignment of zebrafish TRPA1a amino acid sequences. TRPA1a AY677196 was previously reported as TRPA1 (Corey et al., 2004), TRPA1a ENSDART00000004407 is the ENSEMBL annotated sequence, TRPA1a Wild Type is reported in this paper and TRPA1a Mutant is the sequence of the protein encoded by the TRPA1a mutant (*trpa1a*^{hu2163}). (B) Alignment of zebrafish TRPA1b amino acid sequences. TRPA1b AY677197 was previously reported as TRPA2 (Corey et al., 2004), TRPA1b ENSDART00000105997 is the ENSEMBL annotated sequence, TRPA1b Wild Type is reported in this paper and TRPA1b Mutant is the sequence of the protein encoded by the TRPA1b mutant (*trpa1b*^{vu197}). The extra 13 amino acids in TRPA1b AY677197 result from an alternative splice junction at the beginning of exon 9. In (A) and (B), grey shading indicates sequence differences. (C) Alignment of zebrafish TRPA1a and TRPA1b amino acid sequences reported in this paper. Black shading indicates identity to TRPA1a. (D) Alignment of TRPA1 amino acid sequences from several species. Hs-*Homo sapiens*, Mm-*Mus musculus*, Dr-*Danio rerio*, Dm-*Drosophila melanogaster*, Ce-*Caenorhabditis elegans*). Black shading indicates identity to *Homo sapiens* TRPA1. In (A-D), green lines indicate ankyrin repeats, blue lines indicate transmembrane domains and red lines indicate the channel pore loop. Domain predictions were obtained using the sequence search function of the Wellcome Trust Sanger Institute Pfam 22.0 database (http://pfam.sanger.ac.uk; Finn et al., 2006) and the normal prediction function of the Phobius transmembrane topology predictor (http://phobius.sbc.su.se; Kall et al., 2004). Alignments were generated using MegAlign (DNAstar, Madison, WI).

Supplemental Figure 2. Zebrafish TRPA1 channels respond directly to several chemical irritants. HEK293T cells were transfected with TRPA1a (left column) or TRPA1b (right column) and challenged with the following agonists (red bars): acrolein (100 μM); cinnamaldehyde (cinn, 250 μM); diallyl disulfide (DADS, 500 μM); 4-Hydroxynonenal (4-HNE, 100 μM). After exposure to each agonist, cells were challenged with a saturating concentration of mustard oil (green bars, MO, 100 μM) to identify all TRPA1-transfected cells and normalize the responses.

Supplemental Figure 3. Zebrafish larval locomotor activity assay. (A) *trpa1a*$^{+/-}$; *trpa1b*$^{+/-}$ fish are mated, embryos are collected, and individual larvae are placed in each of 80 wells of a 96-well plate containing a mesh base. The plate is placed in a dark box that is illuminated by infrared lights. The larvae are monitored by an infrared camera and the locomotor activity of each larva is recorded by a computer. Sample 40 second activity traces for a single larva during periods of locomotor activity and inactivity are shown. Movement is recorded as an upward deflection of the trace. When that deflection reaches a threshold (green), it is recorded as movement by the software. The small white deflections represent background noise. Larvae are genotyped by PCR after the experiment to identify *trpa1a* and *trpa1b* mutants. (B) Top view of Videotracking chamber. The 96-well plate is placed in a chamber filled with circulating water pumped from a 28.5^{o}C waterbath. A valve is used to switch to waterbaths containing heated or cooled water, or water containing mustard oil. Modified from Prober et al. (2006).

Supplemental Figure 4. Zebrafish TRPA1 paralogs are required for normal behavioral responses to chemical irritants. (A-C) Each data point represents the average duration of locomotor activity every 5 seconds for the indicated number of larvae of each genotype. Yellow shading indicates exposure of larvae to 1% DMSO. Red shading indicates exposure of larvae to 300 μM acrolein (A), 500 μM cinnamon oil in 1% DMSO (B) or 200 μM DADS in 1% DMSO (C). Larvae were obtained by mating *trpa1a*$^{+/-}$*; trpa1b*$^{-/-}$ to *trpa1a*$^{-/-}$*; trpa1b*$^{+/-}$ adults.

Supplemental Figure 5. Zebrafish TRPA1 paralogs are required for normal behavioral responses to mustard oil but not to noxious temperatures. (A, C, E) Each data point represents the average seconds of locomotor activity per minute of a larva immersed in noxious hot water (A), noxious cold water (C) or 100 μM mustard oil (E). Bars indicate mean +/- SEM. (B, D, F) Graphs represent the distribution of average locomotor activities of larvae shown in (A, C, E). Larvae were obtained by mating *trpa1a*$^{+/-}$*; trpa1b*$^{+/-}$ adults. a/a; b/b represents *trpa1a*$^{-/-}$*; trpa1b*$^{-/-}$ larvae. b/b represents *trpa1a*$^{+/+}$*; trpa1b*$^{-/-}$ and *trpa1a*$^{+/-}$*; trpa1b*$^{-/-}$ larvae. a/a represents *trpa1a*$^{-/-}$*; trpa1b*$^{+/+}$ and *trpa1a*$^{-/-}$*; trpa1b*$^{+/-}$ larvae. Het & WT represents all other genotypes. Data from 3 independent experiments are shown for the indicated number of larvae (N). (** $p<0.01$ vs. b/b; ^ $p<0.01$ vs. a/a; b/b, by two-tailed Student's *t* test).

Supplemental Figure 6. Zebrafish TRPA1 mutants respond normally to subtle temperature changes. Each data point represents the average seconds of locomotor activity every 5 seconds for the indicated number of larvae of each genotype. Grey line

and right axis indicates water temperature. Shifting the temperature to 34.5°C (A), 32.5°C (B) or 20°C (C) moderately increased locomotor activity. None of the changes in locomotor activity are statistically significantly different among the different genotypes. Larvae were obtained by mating *trpa1a*$^{-/-}$*; trpa1b*$^{-/-}$ to *trpa1a*$^{+/-}$*; trpa1b*$^{+/-}$ adults.

Supplemental Figure 7. *mariner* mutant larvae do not respond to a mechanical stimulus. The average duration of locomotor activity (A) and the percentage of larvae that exhibit any locomotor activity (B) during each of 5 seconds before and after the application of a mechanical stimulus is shown. The stimulus was applied at time 0. (C) Each bar represents the average percentage of larvae that exhibit any locomotor activity during the second after application of the stimulus +/- SEM. The average responses to 3 stimuli spaced 30 seconds apart are shown for 24 *mariner*$^{-/-}$ and 24 wild-type larvae. (* $p<0.01$ by two-tailed Student's *t* test).

Supplemental Movies 1 and 2. Wild-type larvae (Supplemental Movie 1), but not *trpa1a*$^{-/-}$*; trpa1b*$^{-/-}$ larvae (Supplemental Movie 2), exhibit a behavioral response to immersion in 4-hydroxynonenal. In contrast to this behavioral deficit, *trpa1a*$^{-/-}$*; trpa1b*$^{-/-}$ larvae exhibit a normal response to light touch (end of Supplemental Movie 2).

References

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