Supplementary Table 1. Amino acid compositions of chimeric constructs

Supplementary Table 2. Summary of MO EC_{50} and efficacy of wild type and mutant TRPA1 channels, as determined by FLIPR

Supplementary Figure 1. Schematic representation of mTRPA1-dTRPA1 and hTRPA1-dTRPA1 chimeras.

mTRPA1, dTRPA1 and hTRPA1 are depicted as blue, red and yellow, respectively. Regions including N-term, TM1-2, TM3-4, TM5-6, TM5, Pore/TM6, and C-term are indicated. Chimera-1 to Chimera-11 did not respond to menthol or MO, suggesting that these channels are either not properly expressed, or structurally compromised.

Supplementary Figure 2. Structures of TRPA1 modulators used in this study

Supplementary Figure 3. Residues from TM5-TM6 contribute to the distinct response of mTRPA1 and hTRPA1 to menthol

A, Same as Figure 4**A**. **B**, **C**, Sensitivity of hTRPA1 (black trace), L867F (red trace), L894F/P897A/S900T/I905L (purple trace), and V942I/S943A/I946M (blue trace) to 250 μM menthol activation (**B**) and inhibition (**C**). Compared to hTRPA1, the mutants show reduced activation (but no "off-response" which is a characteristic response of mTRPA1 to 250 μM menthol activation) (**B**) and enhanced inhibition (removal of menthol led to a further increase in MO response) (**C**) by 250 μM menthol. Single mutations L894F, P897A, S900T, I905L, V942I, S943A, and I946M did not measurably affect menthol sensitivity of hTRPA1 in this calcium imaging assay (data not shown). Furthermore, none of these mutants showed significantly altered

menthol EC_{50} and IC_{50} as determined by FLIPR. These data suggest that these eight residues have only subtle effect on determining the distinct sensitivity of mTRPA1 and hTRPA1 to menthol.

Supplementary Fig 4. Swapping dTM5 for mouse TM5 domain had no dramatic effect on channel properties or surface expression levels of functional channels; hTRPA1-dTM5 revealed a general reduction in channel-mediated activity.

Voltage-gated channel behavior of mTRPA1-dTM5 (**A**-**C**) and representative examples of menthol (**D,E**) or vehicle (**F**) effects on mTPRA1-dTM5. **A,B,** mTRPA1-dTM5 reveals voltage dependent activation in cell-attached patch configuration (**A**,0 mM Ca^{2+} in pipette; 0 mM Ca^{2+} ES) and whole cell recordings (**B**, 0 mM Ca^{2+} ES/ 0 mM Ca^{2+} IS). **B** (inset): Tail current analysis reveals voltage dependence of activation of mTRPA1-dTM5 that is qualitatively similar to wild type (Macpherson, Dubin et al. 2007). **C,** Whole cell current density evoked by a voltage step to +180 mV was no different between mTRPA1 and mTRPA1-dTM5 whether or not external calcium was present. Leak subtracted currents were normalized to cell size. **D**, Representative whole cell ramp-induced currents from a mTRPA1-dTM5-transfected HEK293T cell (protocol below: -120 to +120 mV (2.8 mV/ms) from a holding potential of -50 mV). Menthol (500 μM) caused a slight non-specific decrease in conductance at all potentials (green trace). Subsequent application of 100 μM MO in the presence of menthol elicited a large outwardly rectifying current (brown trace). **E and F,** Time course of currents measured at -120 mV (red) and +120 mV (black) before, during exposure to either 500 μM menthol (**E**) or 0.05% ethanol (**F**) during the times indicated by the green or clear bars, respectively, followed by 100 μM mustard oil (brown bar) in the presence either ethanol or menthol, followed by washout. In **E**, a subsequent MO application after robust rinsing was not able to activate currents consistent with a full response to the initial MO application. Note: during the gap between 900 and 1000

sec, the cell was challenged with other voltage protocols. Comparative electrophysiological data are shown for hTRPA1 (**G, H**) and hTRPA1-dTM5 (**I, J**). **G**, hTRPA1 reveals voltage dependent activation in cell-attached patch configuration (**G**, 0 mM Ca^{2+} in pipette; 0 mM Ca^{2+} ES) and whole cell recordings (**H**, 0 mM Ca^{2+} ES/ 0 mM Ca^{2+} IS). **I**, **J**, hTRPA1-dTM5 reveals voltage dependent activation in cell attached patch configuration (**I**, 0 mM Ca^{2+} in pipette/ 0 mM Ca^{2+} ES) and whole cell recordings (**J**, 0 mM Ca^{2+} ES/ 0 mM Ca^{2+} IS). Note the voltage dependent inactivation at the most positive potentials compared to hTRPA1 wild type (**G, H,** red traces). **K**, **L**, hTRPA1-dTM5 expressed lower whole cell current density at +180 mV (**K**) and a smaller MO response compared to wild type hTRPA1 (**L**).

Supplementary Figure 5. Mosquito-TRPA1, Fugu-TRPA1 and dTRPA1-mN are insensitive to menthol.

A, Concentration-dependence curves of menthol activation of HEK293T cells transfected with hTRPA1 (squares), mTRPA1 (circles), mosquito-TRPA1 (downward triangles), Fugu-TRPA1 (leftward triangles), dTRPA1-mN (rightward triangles), or pcDNA5 (diamonds). **B**, Concentration-response relationships of menthol inhibition of 100 µM MO evoked Ca^{2+} responses in HEK293T cells transfected with the indicated constructs. MO responses of these constructs are summarized in Supplementary Table 2.

Supplementary Figure 6. AP18 does not form glutathione adducts as determined by electrospray ionization mass spectrometry (ESI-MS)

ESI-MS spectra of glutathione (GSH) alone (**A**) or reacted with either MO (**B**) or AP18 (**C**). GSH alone in water/methanol exists as monomer, MH+, m/z (336) (**A**). When reacted with MO,

glutathione adducts of predicted mass were observed (336 + 99 = 435) (**B**). GSH + AP18 displays no adduct, only the monomer of GSH and unreacted AP18 (210) were observed (**C**).