

in response to 10 nM **(a)**, 100 nM **(b)**, and 1000 nM **(c)** ZnCl₂. All responses were normalized to the open probability of 500 μM cinnamaldehyde-evoked response in the same patch. $N = 7$ for wild type, $n = 6$ for H983 mutant and $n = 7$ for C641S/C1021S mutant. **(d)** Cartoon of human TRPA1 with position of histidine and cysteine residues involved in channel activation. Darker green circles indicate cysteines specifically involved in zinc-activation.

Supplemental Figure 1. **Ruthenium red and camphor block zinc activation of TRPA1 single channel in inside out patches.** **(a)** Histogram of single channel open probability reveals blockage of zinc-activated single channel activity by 2 mM camphor. **(b)** Single channel current traces show that zinc increased single channel activity as compared with TRPA1 basal activity. Camphor blocked zinc-evoked response and induced long close state of the channel. All traces were taken at +80 mV. **(c)** Histogram illustrates the blocking effect of ruthenium red on zinc-evoked TRPA1 single channel activity in an excised inside out patch. **(d)** Single channel traces illustrate a reversible block of zinc-evoked single channel openings by 10 μM ruthenium red. All traces were taken at +80 mV. $N = 3$ for both camphor and ruthenium block.

Supplemental Figure 2. **Zinc modulates calcium activation of TRPA1 in excised inside out patches.** **(a)** TRPA1 activity in an excised inside out patch at holding potential of +80 mV in response to indicated zinc and calcium concentrations. **(b)** Histogram illustrates the effect of zinc, calcium or co-application of both on single channel open probability of TRPA1. **(c)** Bar chart illustrates the modulation of zinc and calcium on

single channel activity of TRPA1 in excised inside out patches. All values were normalized to the effect of 1 μ M calcium. ** $P < 0.01$, $n = 9$.

Supplemental Figure 3. **TRPA1-mediated zinc influx as assayed by zinc imaging of cultured mouse dorsal root ganglia neurons.** Each trace corresponds to fluorescence (Fluozin-3) in a single neuron. DRG neurons isolated from *TRPA1*^{+/+} (a) and *TRPA1*^{-/-} (b) mice were continuously perfused with assay buffer (1,26 mM Ca²⁺, 0.9 mM Mg²⁺, 5.8 mM K⁺, 138.6 mM Na⁺) that included 300 μ M ZnCl₂ for the indicated time (black bar). (c) The percentage of neurons that exhibited > 3 fold increase in fluorescence upon application of ZnCl₂ at 30 and 300 μ M. Number of zinc responsive/total neurons tested are indicated between parentheses.

Supplemental Figure 4. **Single channel conductance of wild type TRPA1 and D915A, H983A and C641S/C1021S mutants.** (a) Single-channel current amplitudes obtained from Gaussian fit to current histograms as a function of voltage. Error bars are standard errors; straight lines indicate linear fits to the data. The average values of unitary conductance were 177.5 pS (wild-type TRPA1, $n = 30$), 180.2 pS (H983A, $n = 9$), and 194.8 pS (C641S/C1021S, $n = 12$). All data were obtained from inside-out configurations clamped at various membrane potentials. (b-e) Single channel traces taken at referred voltages for wild type and indicated mutant channels with inside-out configuration.

Supplemental Figure 5. **Role of cysteine and histidine residues in TRPA1 activation by zinc.** Dose response profiles for the indicated TRPA1 mutants in response to

cinnamaldehyde (a cysteine reactive TRPA1 agonist), flufenamic acid (FFA, a non-reactive TRPA1 agonist) and $ZnCl_2$ as determined by calcium imaging (FLIPR) **(a-c)** Individual N-terminal cysteine mutants C621S, C641S, and C665S compared to wt TRPA1. **(d-f)** Triple cysteine mutant C621S/C641S/C665S compared to wt TRPA1

Supplemental Figure 6. **Schematic representation of histidine and cysteine mutants tested for zinc sensitivity.** Sequence alignment of human and mouse TRPA1 protein sequences is shown. Colors indicate mutant phenotype and bars indicate putative transmembrane segments. Statistics for agonist sensitivity of individual mutants are presented in supplemental Table 1 and 2. Mutants with a 3 fold increase in EC_{50} values and/or 60% lower in maximal responses compared with wild type TRPA1 are considered to have decreased zinc or FFA responses.

Supplemental Table 1. **EC_{50} values, Hill coefficients and normalized maximal responses for zinc, FFA and cinnamaldehyde activation of wild type and mutant TRPA1 channels depicted in Figure 5 and Supplemental Figure 5.** Maximal responses of all mutants to zinc or FFA are normalized to maximal response evoked by 100 μ M zinc or FFA in wild type TRPA1. Data of mutant TRPA1 were compared with wild type TRPA1 transfected in the same 384 well plates

Supplemental Table 2. **EC_{50} values, Hill coefficients and normalized maximal responses for zinc and FFA activation of wild type and mutant TRPA1 channels.** Maximal responses of all mutants to zinc or FFA are normalized to maximal response

evoked by 100 μ M zinc or FFA in wild type TRPA1. Data of mutant TRPA1 were compared with wild type TRPA1 transfected in the same 384 well plates.