| Active compounds to open | Inactive compounds to open | Active compounds to open | Inactive compounds to open |
|--------------------------|-------------------------------|--------------------------|-------------------------------|
| TmTRPA1a | TmTRPA1a | TmTRPA1b/c | TmTRPA1b/c |
| Eugenol | Cinnamaldehyde | Eugenol | Camphor |
| Carvacrol (0.5 mM) | 1,8-Cineole | Cinnamaldehyde | o-Methoxyphenol |
| 2-Undecanone | AITC | 1,8-Cineole | 2-Methoxy-4-methylphenol |
| Geranylacetone (0.5mM) | Creosote (0.1%) | AITC | Linoleic acid |
| Terpinen-4-ol | Methyl jasmonate | Carvacrol (0.5mM) | N,N-Diethyl-2-phenylacetamide |
| Nerol (1mM) | 2-Dodecanone | Creosote (0.1%) | Octanoic acid |
| | α-Terpineol | Methyl jasmonate | Decanoic acid |
| | Diallyl disulfide | 2-Undecanone | 1-Octanal |
| | 3,7-Dimethyl-6-octenal | Nerolidol | Borneol |
| | 2-Ethyl-1,3-hexanediol | Thujone | Coumarin |
| | Geraniol | 2-Dodecanone | Methyl salicylate |
| | Carveol | Geranylacetone (0.5 mM) | Verbenone |
| | Lauric acid | Myrtenal | |
| | 1-Octanol | Terpinen-4-ol | |
| | Nerolidol | β-Citronellol | |
| | Thujone | α-Terpineol | |
| | Myrtenal | Nerol (0.5 mM) | |
| | β-Citronellol | Diallyl disulfide | |
| | β-cyclocitral | 3,7-Dimethyl-6-octenal | |
| | Menthol (3 mM) | 2-Ethyl-1,3-hexanediol | |
| | Thymol | β-cyclocitral | |
| | Camphor | Menthol (3 mM) | |
| | o-Methoxyphenol | Geraniol | |
| | 2-Methoxy-4-methylphenol | Carveol | |
| | Linoleic acid | Thymol | |
| | N,N-Diethyl-2-phenylacetamide | Lauric acid | |
| | Octanoic acid | 1-Octanol | |
| | Decanoic acid | | |
| | 1-Octanal | | |
| | Borneol | | |
| | Coumarin | | |
| | Methyl salicylate | | |
| | Verbenone | | |

Table S1 List of active and inactive compounds to open TmTRPA1 channels

All compounds were tested at 1mM concentration except carvacrol, creosote, geranylacetone, nerol, and menthol. The concentrations used are indicated with parentheses.

Supplementary figure legends

Supplementary figures 1a and 1b

Activation of TmTRPA1b by 16 plant-derived compounds analyzed by calcium imaging. Red bars show the period when each compound was added, and then washed off. Arrows indicate the time points when we added ionomycin. Chemical structure of each compound is also shown except creosote which is a mixture of different compounds. The concentration of each compound was 1 mM except for creosote (0.1%), and menthol (3mM).

Supplementary figures 2a-2d

Activation of TmTRPA1c by 27 plant-derived compounds analyzed by calcium imaging. Red bars show the period when each compound was added, and then washed off. Arrows indicate the time points when we added ionomycin. The concentration of each compound was 1 mM except for creosote (0.1%), geranylacetone (0.5mM), carvacrol (0.5 mM), nerol (0.5mM), and menthol (3mM).

Supplementary figure 3

Activation of TmTRPA1a by eugenol and terpinen-4-ol analyzed by calcium imaging. Red bars show the period when each compound was added, and then washed off. Arrows indicate the time points when we added ionomycin. The concentration of each compound was 1 mM.

Supplementary figure 4

(A) Proteins expressed in HEK293 cells transfected with empty vector (Mock), TmTRPA1b wild type-, and the five deletion mutants (Δ 84-92, Δ 105-134, Δ 77-134, Δ 49-134, and Δ 21-134)-expressing constructs were analyzed by western blot. The size (kD) of protein molecular weight marker (MW) is at the left. (B) Localizations of plasma membrane-bound FITC-WGA and either TmTRPA1b wild type, or the five deletion mutant (Δ 84-92, Δ 105-134, Δ 77-134, Δ 49-134, or Δ 21-134) tagged with V5-epitope in the transfected HEK293 cells by immunofluorescence. The merged images are also shown.





Supplementary figure 2a











Supplementary figure 3



Supplementary figure 4



| | FITC-WGA | V5-epitope | Merge |
|-----------|---|--|--|
| Wild type | | \bigcirc | Ø |
| ∆ 105-134 | C | Ø | Ø |
| ∆ 77-134 | | \bigcirc | Ó |
| ∆49-134 | C | 0 | |
| ∆ 21-134 | | 0 | \bigcirc |
| ∆ 84-92 | | 0 | 0 |
| | Wild type △ 105-134 △ 77-134 △ 49-134 △ 21-134 △ 84-92 | FITC-WGAWild typeΔ105-134Δ77-134Δ49-134Δ21-134Δ84-92 | FITC-WGAV5-epitopeWild typeΔ105-134Δ77-134Δ49-134Δ21-134Δ84-92 |