Ingredients of Vicks VapoRub inhibit Rhinovirus induced ATP release

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SUPPLEMENTARY DATA

Vicks VapoRub individual ingredients have limited ability to inhibit activation of TRP channels.

To determine whether individual components of Vicks VapoRub had any antagonist activity against different TRP channels, stably transfected HEK TRP cell lines where incubated with half log concentrations between 30µM and 30mM of either menthol, camphor, eucalyptus oil or turpentine oil for 300s prior to challenging with EC_{50} concentrations of known TRP channel agonist for each HEK TRP cell line or calcium signalling buffer for HEK WT cells for 120s. Cells were then activated with calcium ionophore for 60s and the responses were calculated as a percentage response to known TRP channel agonist. The concentration effect curves showed little inhibitory effect of the VVRIs against the TRP channels tested in this overexpression system (Supplementary Figure 1) and IC_{50} values could not be calculated.

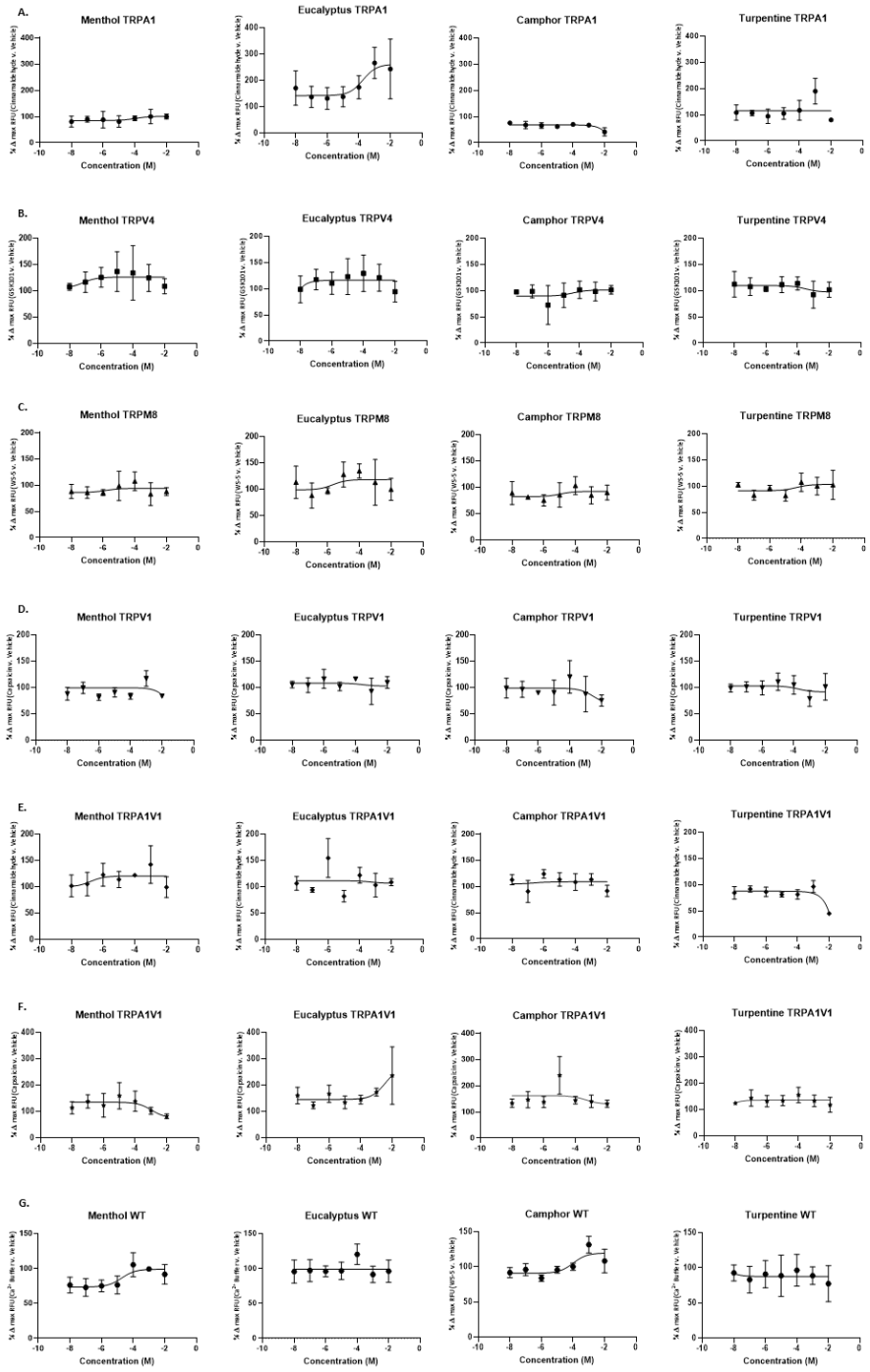

Figure S1. Antagonist dose response curves of individual VVRIs effect on transfected HEK cell lines. HEK cell lines were incubated with various concentrations of menthol, eucalyptus oil, camphor or turpentine oil for 5 minutes prior to stimulation with the specific EC_{50} of the TRP channel under test; HEK TRPA1 – 49µM cinnamaldehyde (A), HEK TRPV4 – 1.2nM GSK1016790A (B), TRPM8 – 4.8µM WS-5 (C), HEK TRPV1 – 660nM capsaicin (D), HEK TRPA1V1 – 49µM cinnamaldehyde (E) or – 660nM capsaicin (F) and HEK WT – calcium signalling buffer (G). Responses measured are presented as a percentage of maximum response to TRP channel standard agonist (EC_{50} concentration) after pre-

incubation with the equivalent vehicle. All data present as mean ± S.E.M. of three independent experiments (n=3).

Ingredients of Vicks VapoRub have varying agonist effects on TRP channels involved in cough

To investigate whether combinations of the VVRIs activated the TRP channels of interest, HEK TRP cell lines were challenged with either 3 VVRIs or 4 VVRIs at 10mM or 100µM concentrations and these were compared to the individual VVRIs at the same concentration. Measurements were made via calcium signalling and representative traces of 10mM responses are presented to show agonist response. Baseline measurements were taken for 20s prior to the addition of the VVRI(s), response to VVRIs were measured for 180s, subsequently calcium ionophore was added and the maximum response recorded over a further 60s (Supplementary Figure 2).

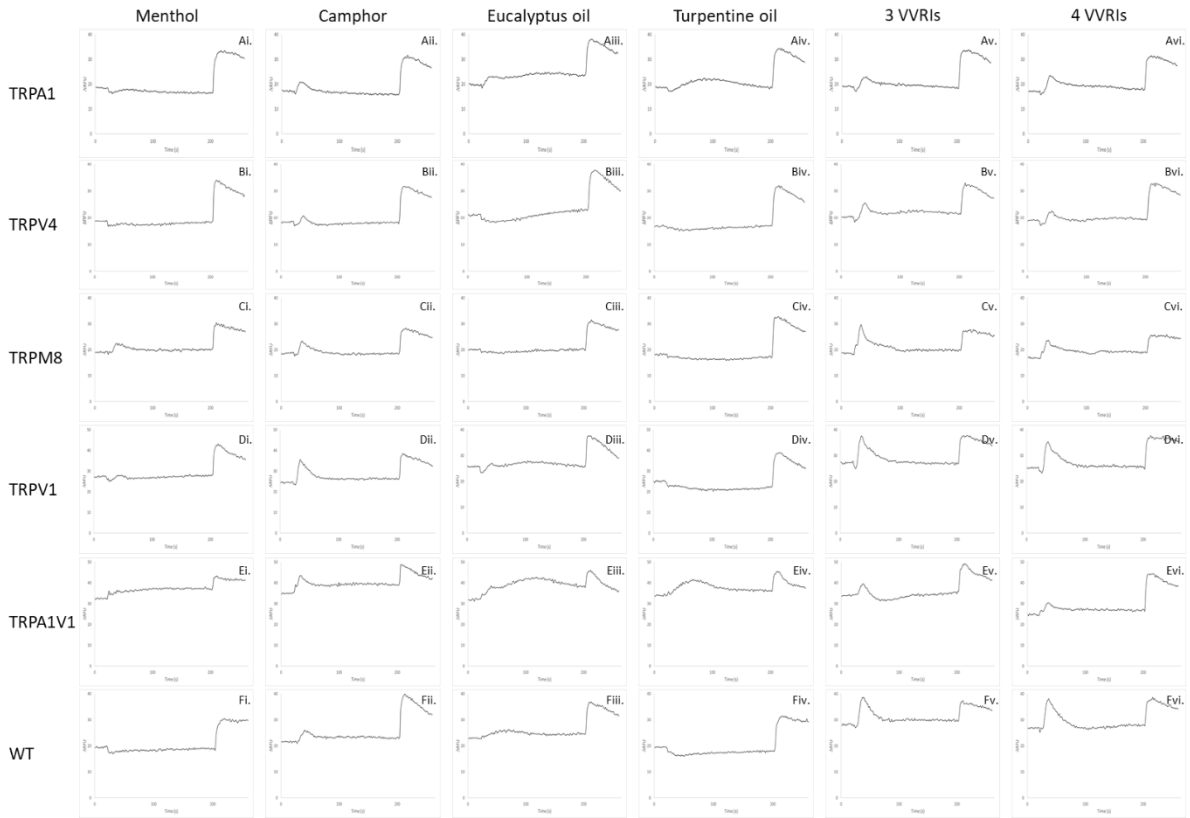

Figure S2. Agonist calcium signalling traces of individual and combined VVRIs applied to transfected HEK cell lines. HEK TRPA1 (A), HEK TRPV4 (B), HEK TRPM8 (C), HEK TRPV1 (D), HEK TRPA1V1 (E) and HEK WT (F) stimulated with 10mM concentrations of menthol (Ai – Fi), camphor (Aii – Fii), eucalyptus

oil (Aiii – Fiii), turpentine oil (Aiv – Fiv), 3 VVRIs (Av – Fv), or 4 VVRIs (Avi – Fvi). Representative traces of calcium signalling responses to VVRIs (n=6).