Supplementary Figure legends

Supplementary Fig. 1 AmHsTRPA is irreversibly desensitized by heat and chemical activation
Upper panels show desensitization of AmHsTRPA activation currents during repeated heat stimulation in the whole-cell patch-clamp mode at a holding potential of –60 mV. A heat-evoked current gradually decreased by repeated application. Middle panels show desensitization of AmHsTRPA activation currents in long time period. After the first desensitization by heat, the cell was kept at 25^{o}C for 15 min, and then stimulated with heat again, which generated only a small current. A lower panel shows desensitization of AmHsTRPA activation currents during repeated camphor stimulation. A camphor (3 mM)-evoked current gradually decreased by repeated application.

Supplementary Fig. 2 Many thermosensitive TRP channel activators do not affect AmHsTRPA
Ca^{2+}-imaging experiments were performed with *AmHsTRPA*-expressing HEK293 cells. The cells were stimulated with nifedipine (100 μM), menthol (1 mM), acrolein (2 mM), nicotine (1 mM), a low pH solution (pH 5), a high pH solution (pH 10), capsaicin (0.5 mM), 4 α-phorbol 12, 13-didecanoate (10 μM), hypotonic solution (160 mOsm), guaiacol (1 mM), creosol (1 mM), and p-cresol (1 mM) . Guaiacol, creosol, and p-cresol were tested because wood tar containing these substances has been used as an insect repellent. Functional AmHsTRPA expression was confirmed by the heat-generated responses. Colored traces indicate fura-2 ratio changes in *AmHsTRPA*-expressing cells and thick black lines indicate temperature changes. The pH 10 solution raised $[Ca^{2+}]_i$ to some extent; however, equivalent $[Ca^{2+}]_i$ increases were also observed in mock-transfected HEK293 cells.

Supplementary Fig. 3 HC-030031 does not affect the AmHsTRPA activation by heat and camphor
Upper panels show temporal changes in the current in an *AmHsTRPA*-expressing cell and temperature during temperature fluctuations in the presence of 30 μM HC-030031 (purple bar) in the whole-cell patch-clamp mode at a holding potential of –60 mV. Middle panels show temporal changes in the AmHsTRPA currents activated by 3 mM camphor with (purple bar) or without 30 μM HC-030031 treatment during the inactivation period. No effects of HC compound on AmHsTRPA activation by heat and camphor are observed. Note that the same concentration of HC compound completely and reversibly inhibited human TRPA1 activation evoked by 100 μM AITC (the lower panel).

Supplementary Fig. 4 *AmHsTRPA* mRNA is not expressed in the scape and pedicel of honey bee antenna
In situ hybridization of *AmHsTRPA* antisense riboprobe to the scape and pedicel sections did not give positive signals. One representative image is shown.

Supplementary Fig. 5 Quinine and berberine do not activate AmHsTRPA
(A, B) Temporal changes in the current of *AmHsTRPA*-expressing cells and temperature for quinine (upper panels) and berberine (lower panels) treatments in a whole-cell

patch-clamp mode at a holding potential of –60 mV. Functional AmHsTRPA expression was confirmed by heat-evoked currents.

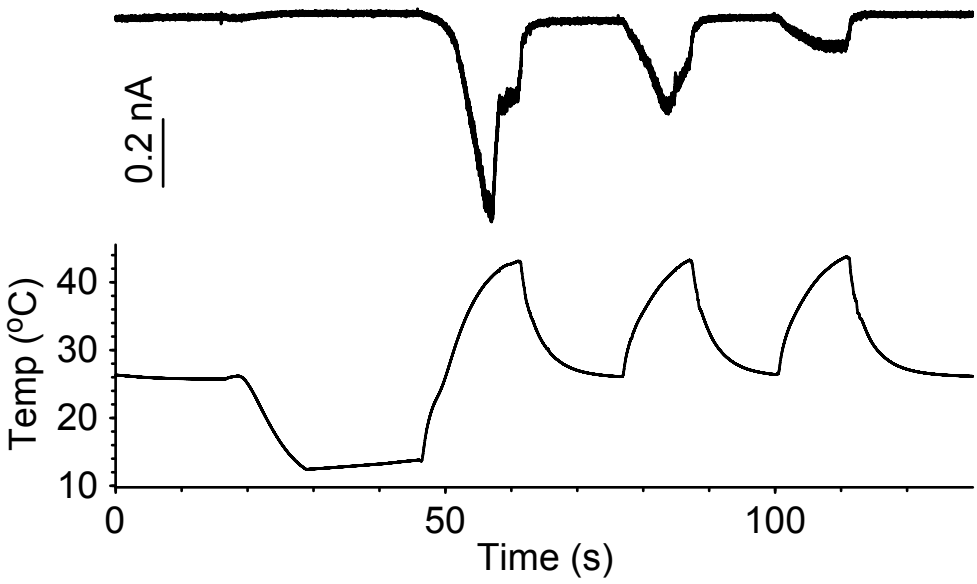
Supplementary Fig. 6 DmTRPA1 is activated by chemical compounds

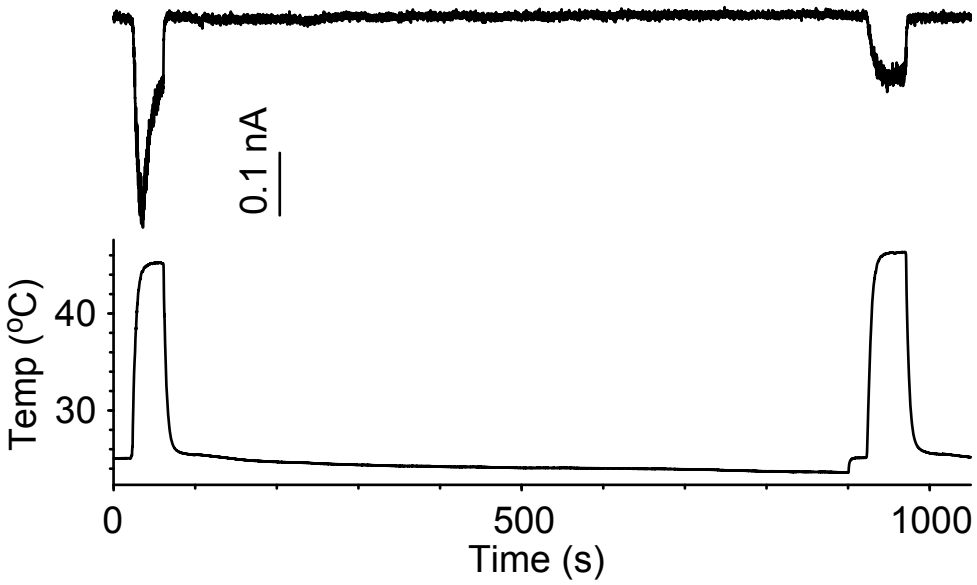
Ca^{2+}-imaging experiments were performed with *DmTRPA1*-expressing HEK293 cells. The cells were stimulated with AITC (100 μM, upper panel), CA (300 μM, middle panel), or camphor (5 mM, lower panel). Robust intracellular Ca^{2+} increases were observed in all cases tested. Functional DmTRPA1 expression was confirmed by the heat-generated responses. Colored traces indicate fura-2 ratio changes in *DmTRPA1*-expressing cells and thick black lines indicate temperature changes.

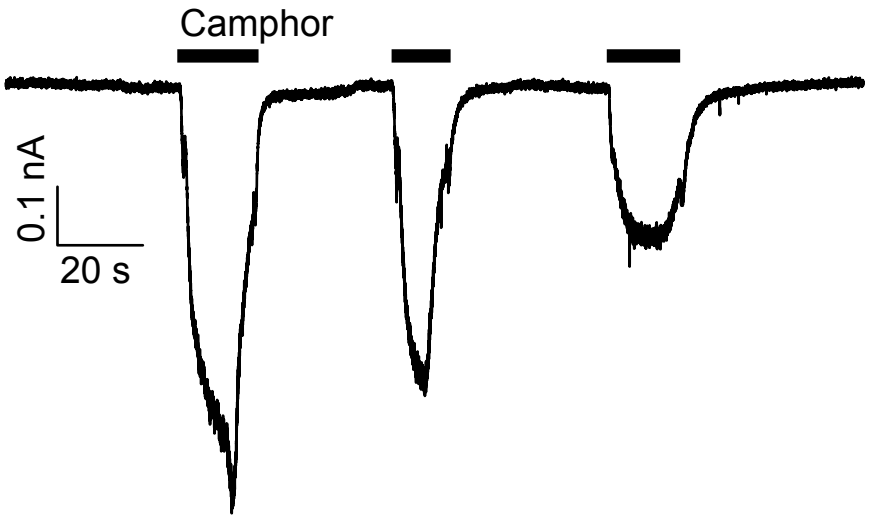
Supplementary Fig. 7 Cysteine residues covalently modified by reactive electrophiles in mammalian and *Drosophila* TRPA1 are not conserved in AmHsTRPA

Mouse (MmTRPA1), human (HsTRPA1), *Drosophila* (DmTRPA1) TRPA1 proteins, and AmHsTRPA are aligned by CLUSTALW program. The positions of cysteine residues (light blue) covalently modified by reactive electrophiles such as AITC and CA are shared between mammalian and *Drosophila* TRPA1 proteins except DmTRPA1 (472S). However, they are not conserved in AmHsTRPA (purple). Cysteine residues of AmHsTRPA (yellow) are not conserved in mammalian and *Drosophila* TRPA1 proteins.

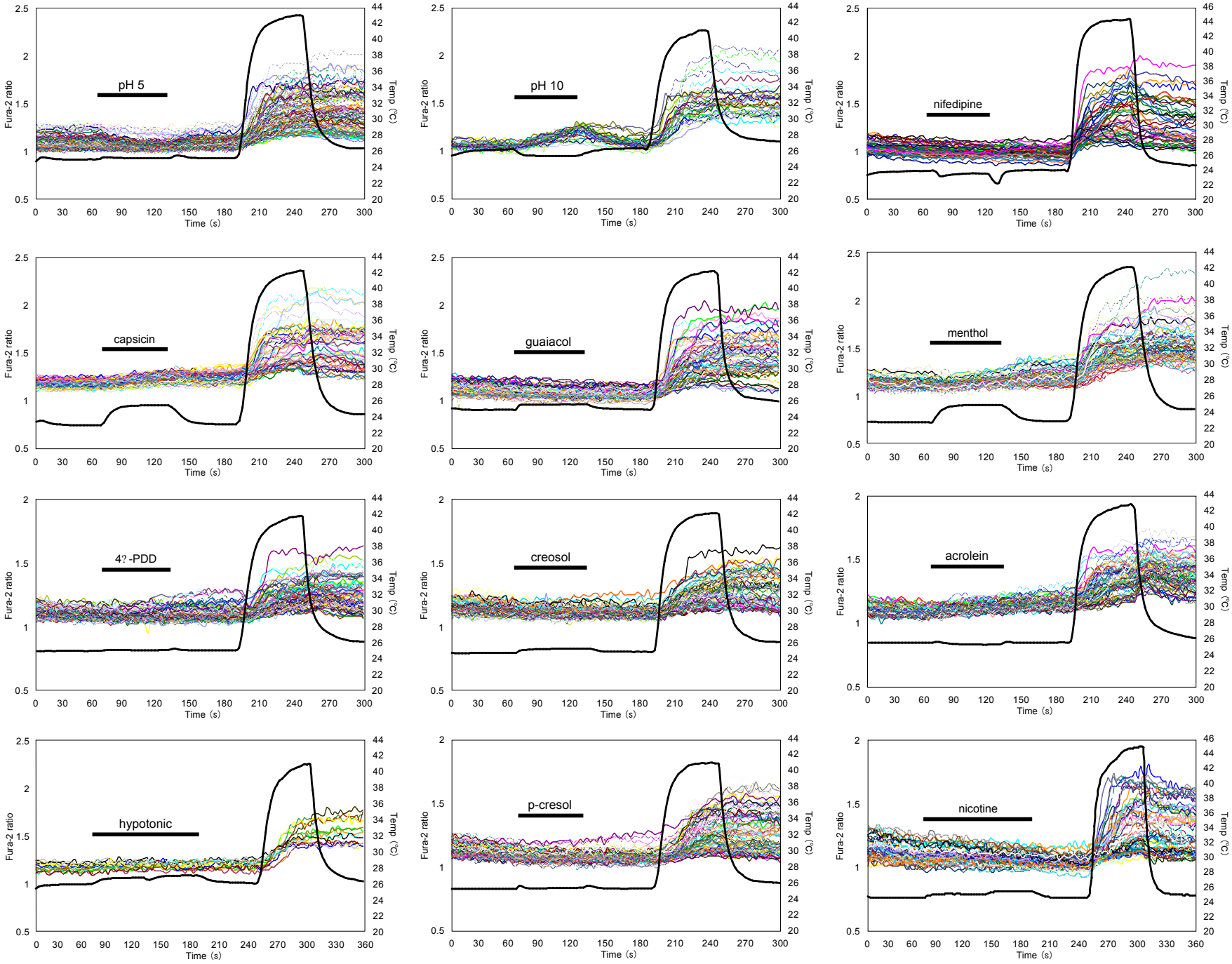
Supplementary Figure 1





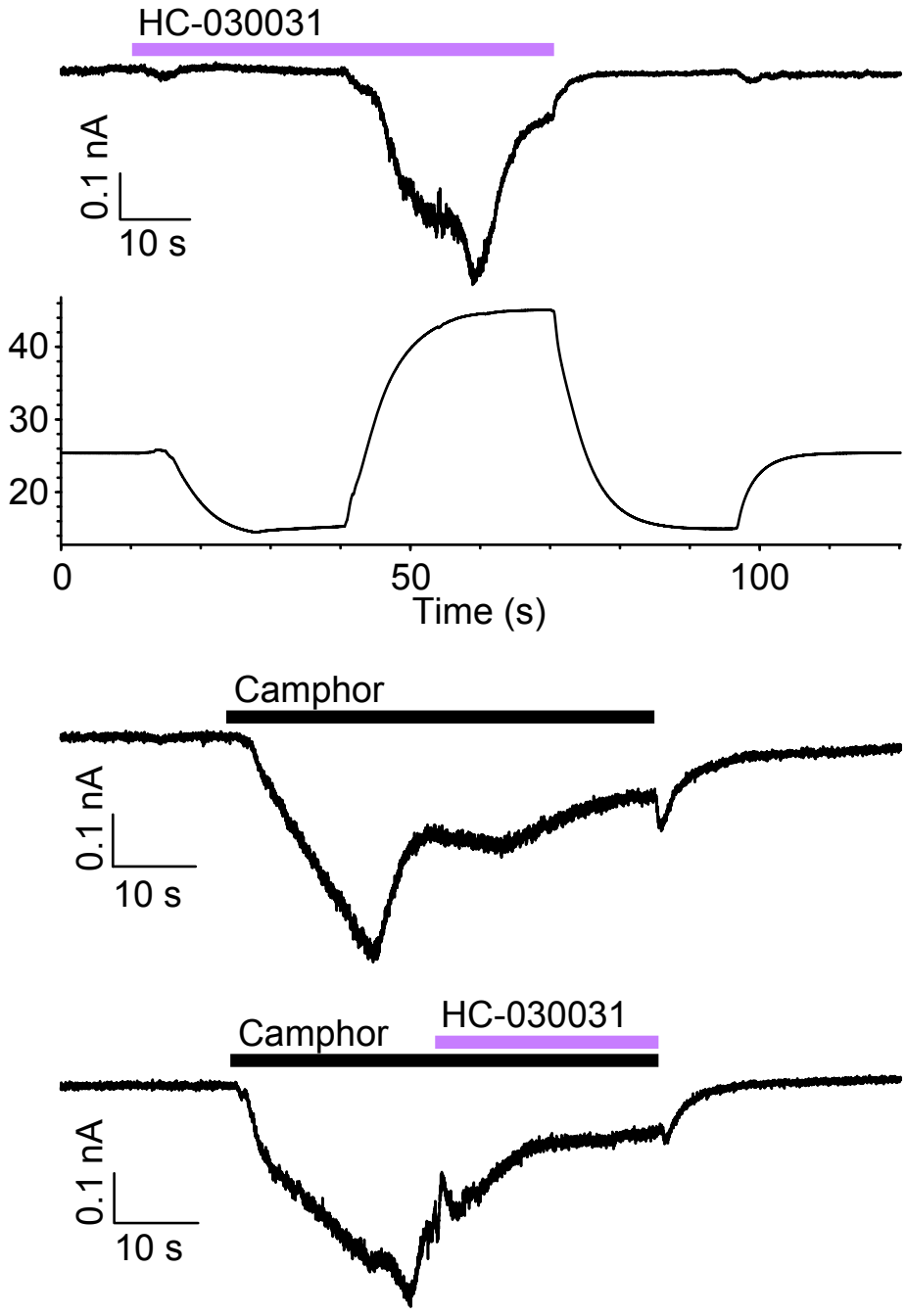


Supplementary Figure 2

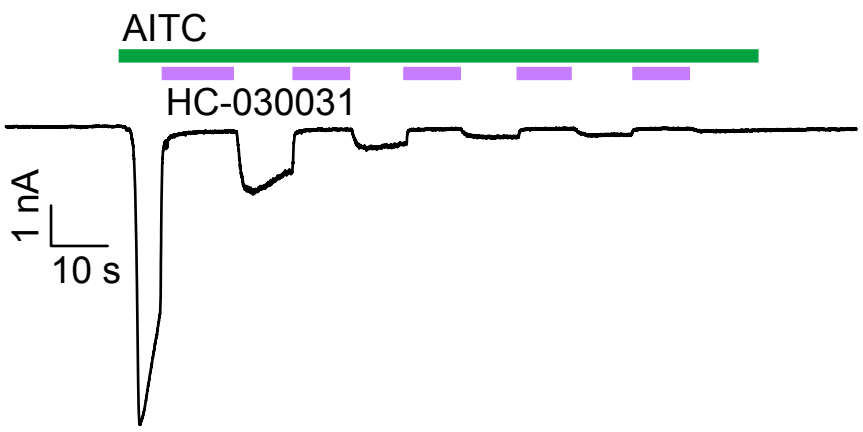


Supplementary Figure 3

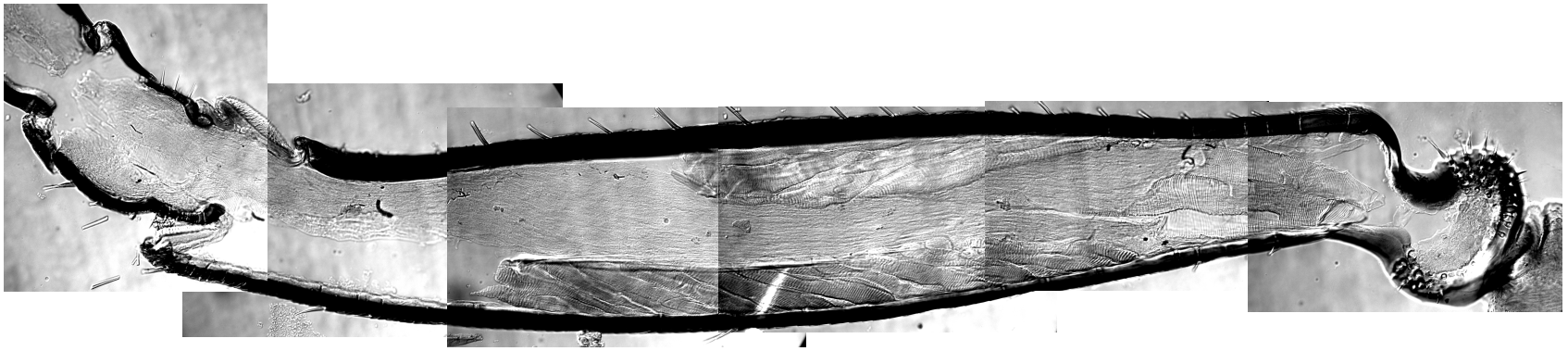
AmHsTRPA



Human TRPA1



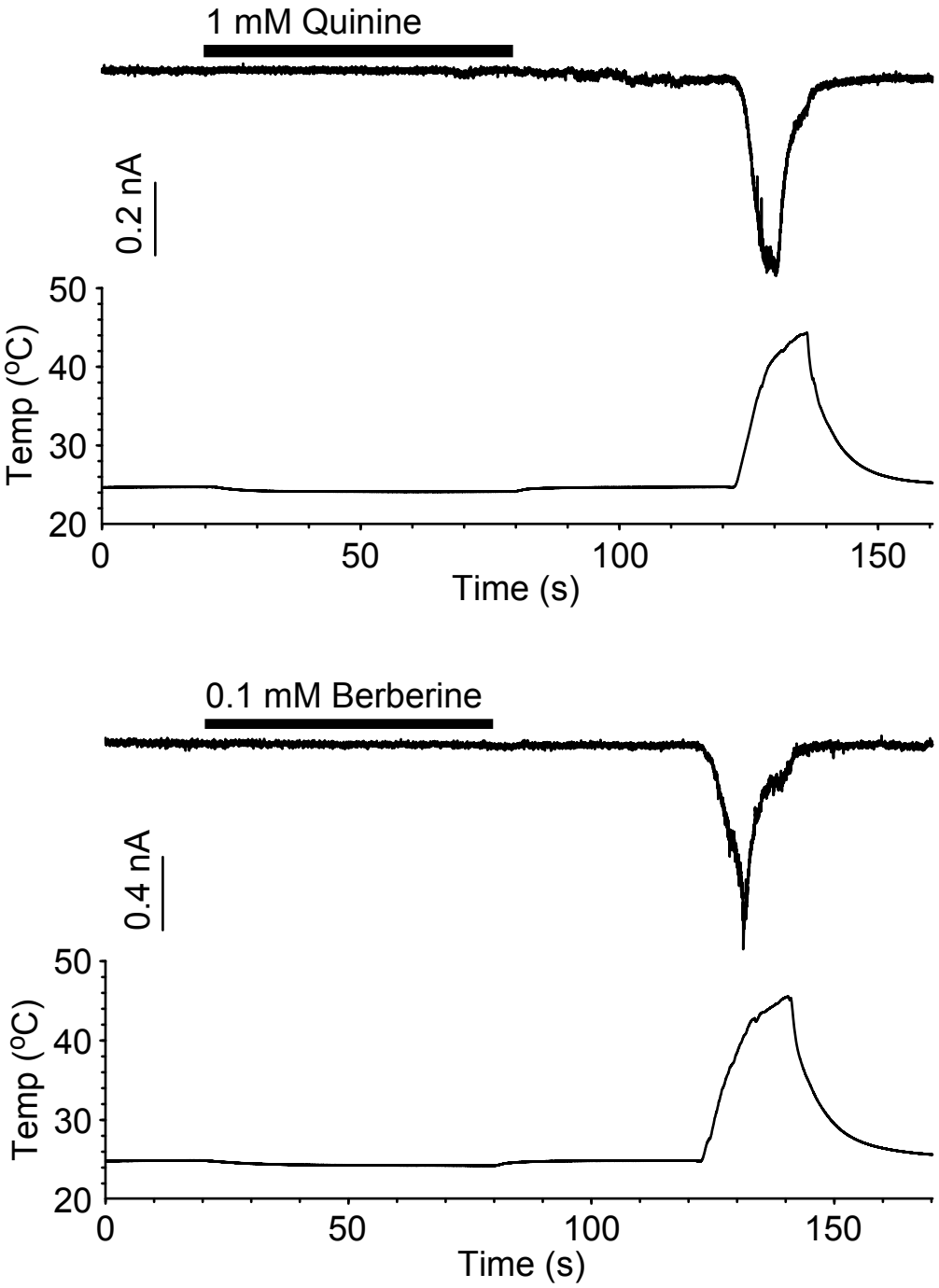
Supplementary Figure 4



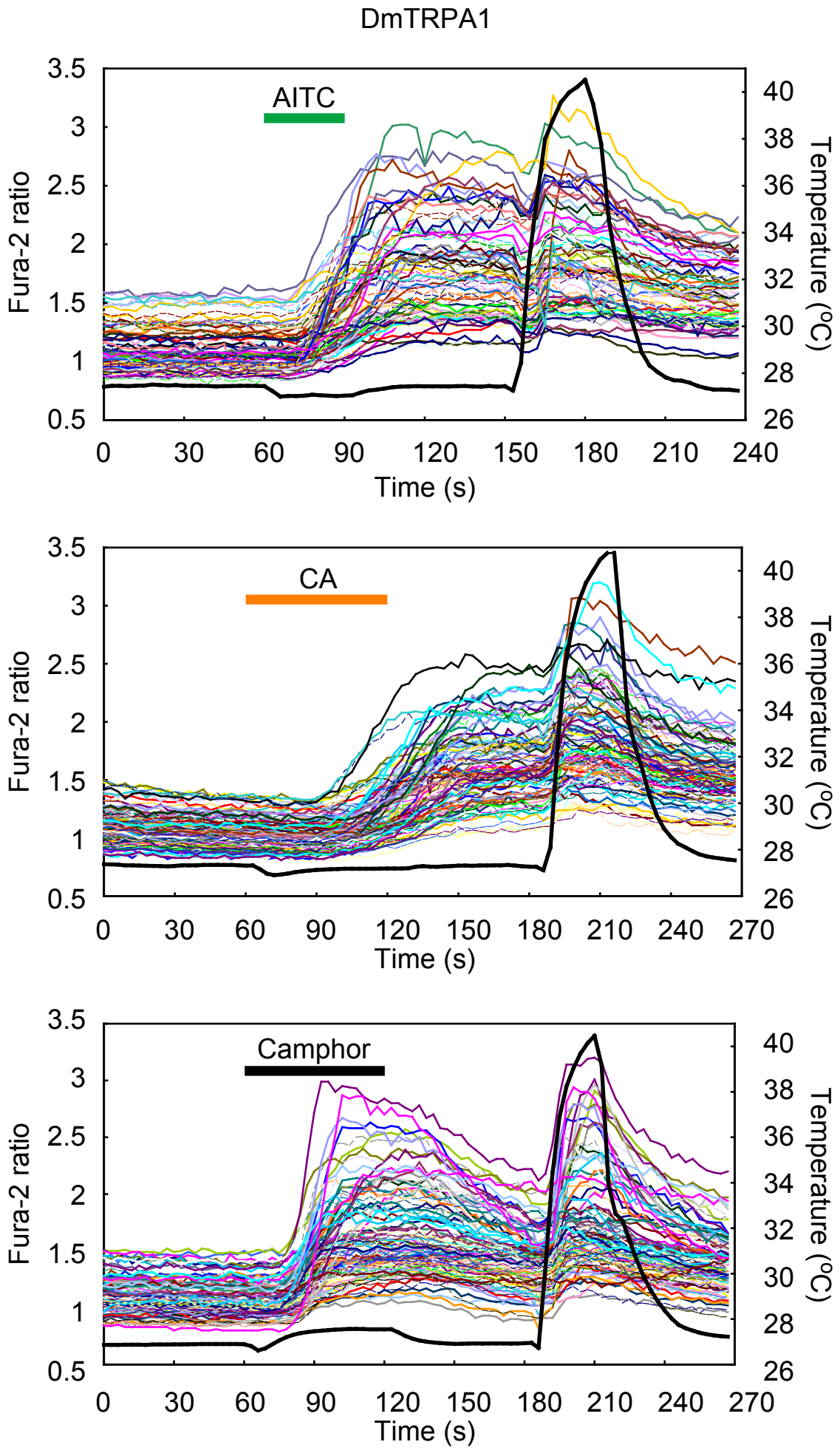
Pedicel

Scape

Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7

