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SUPPLEMENTARY MATERIAL

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Expression profile of the zinc transporter ZnT3 in taste cells of rat circumvallate papillae and its role in zinc release, a potential mechanism for taste stimulation

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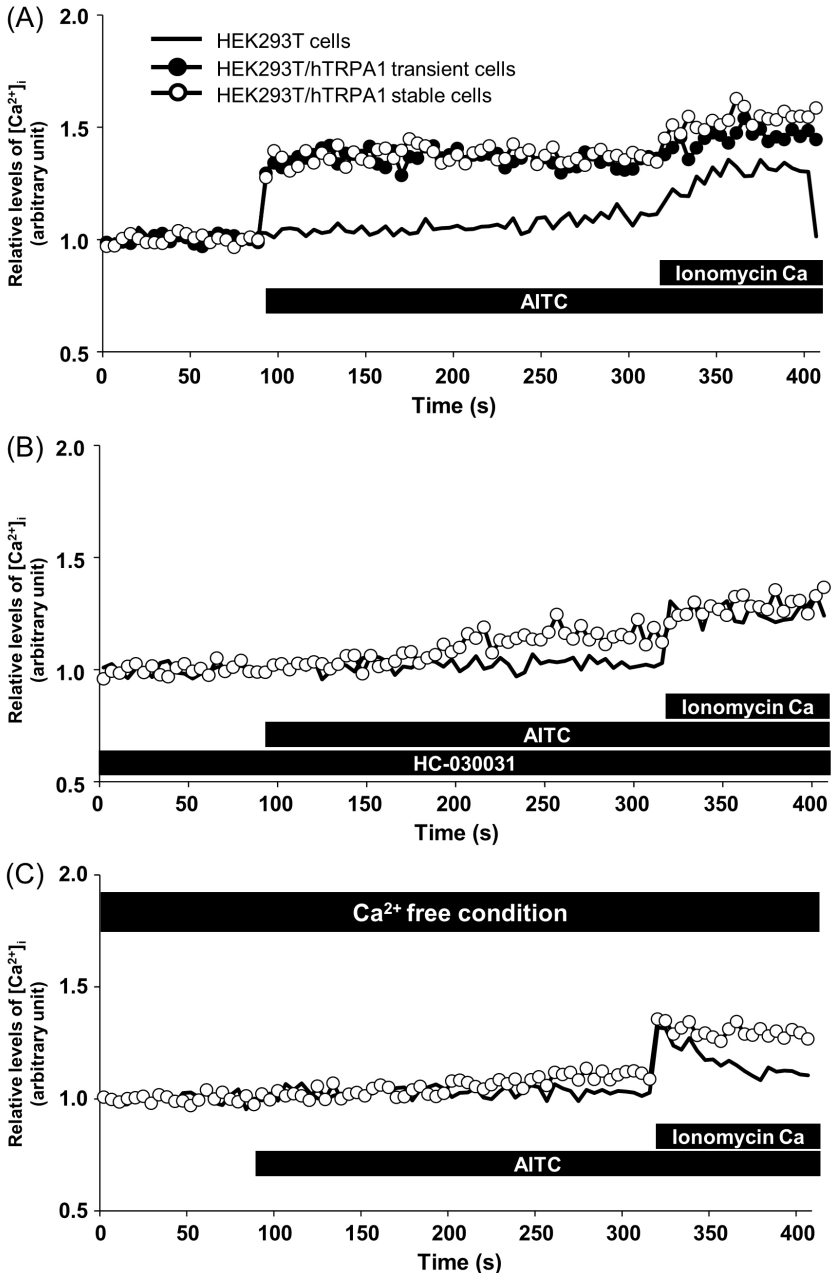
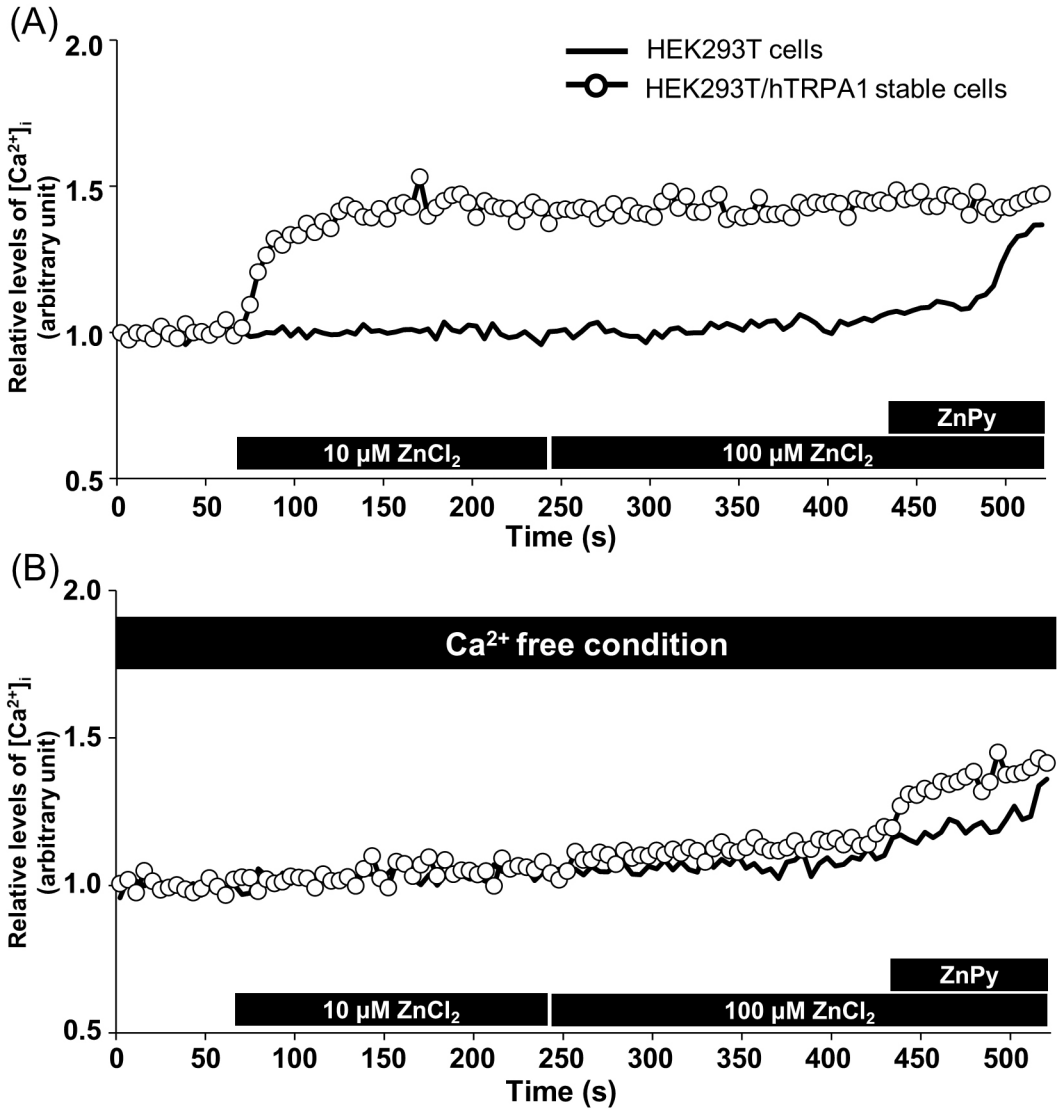
Supplementary Figure 1.
Functional expression of TRPA1 in HEK293T/hTRPA1 stable cells. A, B) To evaluate the functional expression of TRPA1, HEK293T and HEK293T/h*TRPA*1 cells were treated with 100 μM AITC, a TRPA1 agonist, and 150 μM HC-030031, a TRPA1 antagonist; to validate the $[Ca^{2+}]_i$ response, cells were treated with a calcium ionophore (4 μM ionomycin Ca). C) Cells were treated with 100 μM AITC and 4 μM ionomycin under Ca^{2+}-free conditions.



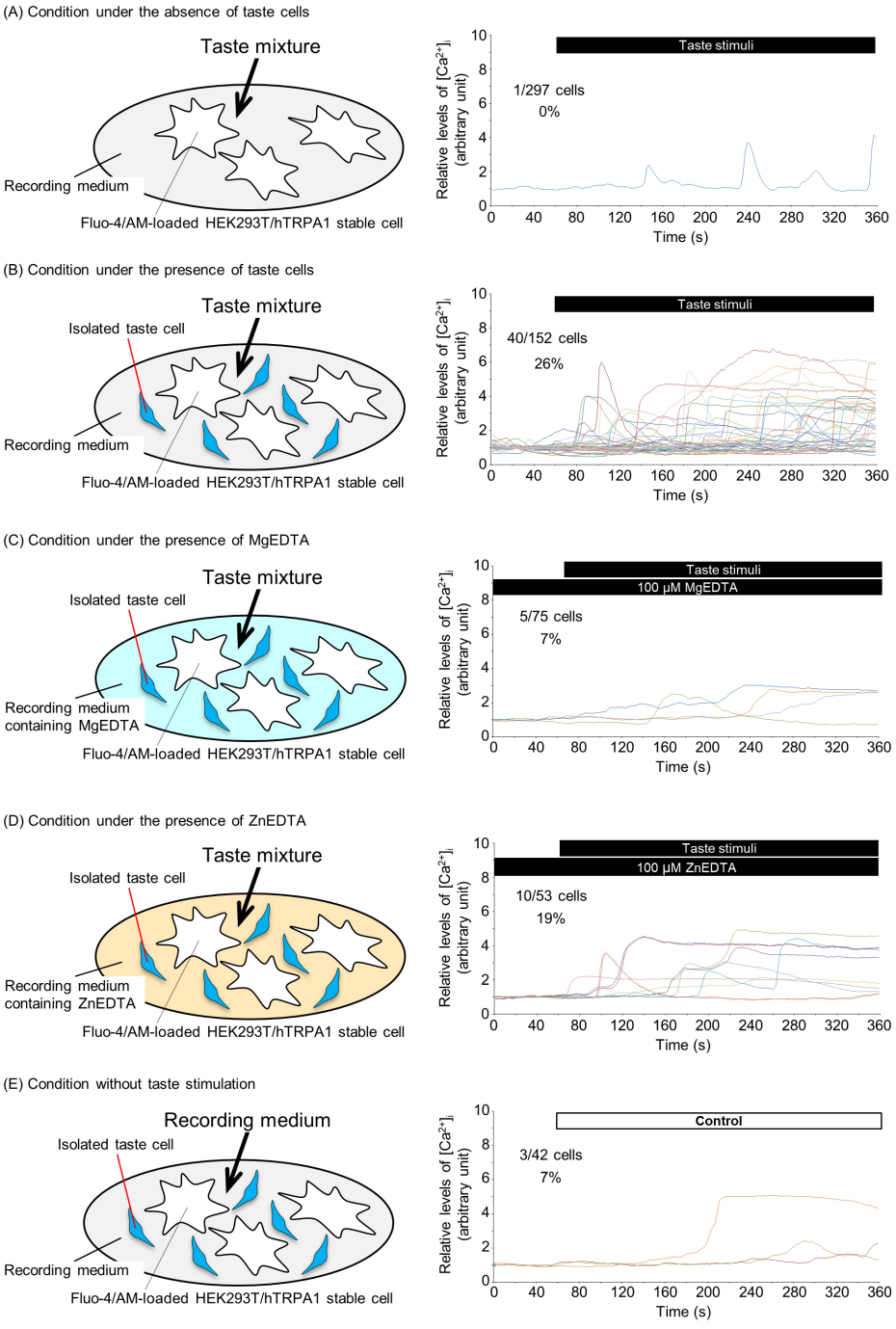
Supplementary Figure 2.

Effect of zinc administration on $[Ca^{2+}]_i$ in HEK293T/h*TRPA1* stable cells. A) Time courses of 10 and 100 μM $ZnCl_2$-evoked $[Ca^{2+}]_i$ HEK293T cells and HEK293T/h*TRPA1* stable cells; to evaluate whether HEK293T/hTRPA1 stable cells were zinc-sensitive, cells were treated with 10 μM $ZnCl_2$ and 100 μM $ZnCl_2$; to validate the $[Zn^{2+}]_i$ response, cells were treated with 5 μM zinc pyrithione (ZnPy) as a zinc ionophore. B) $ZnCl_2$ was applied under Ca^{2+}-free conditions to validate the $[Ca^{2+}]_i$ response.



Supplementary Figure 3.

Representative time-lapse data on zinc release from isolated taste cells by taste stimuli. Left schemes show each experimental condition (A–E). Representative time-lapse analysis of $[Ca^{2+}]_i$ in Fluo-4/AM-loaded HEK293T/h*TRPA1* stable cells without (A) or with taste cells stimulated by the taste mix solution in the absence (B) or presence of 100 μM MgEDTA (C; an extracellular zinc chelator) or 100 μM ZnEDTA (D; a negative chelator without extracellular zinc-chelating ability). Data are presented as a representative image of at least three independent experiments. As a negative control, the recording medium alone was used to stimulate Fluo-4/AM-loaded HEK293T/h*TRPA1* stable taste cells (E). The percentages of taste stimuli or medium-responding cells are shown in each panel. Fluo-4/AM-loaded HEK293T/hTRPA1 stable cells were considered responders when $[Ca^{2+}]_i$ was more than 2-fold higher than the basal $[Ca^{2+}]_i$ levels before taste stimuli.



Supplementary Figure 4.

Immunohistochemical analysis of ZnT3 and NTPDase2, PLC-β2, or AADC in longitudinal sections through the circumvallate papillae. Representative photomicrographs for double staining of ZnT3 (green) and the type I cell marker NTPDase2 (A; red), type II cell marker PLC-β2 (B; red), or type III cell marker AADC (C; red) are shown. Arrowheads show the colocalization of ZnT3 and taste cell markers in the cell bodies of taste cells. Data are presented as a typical image of three independent experiments. Scale bars: 50 μm.