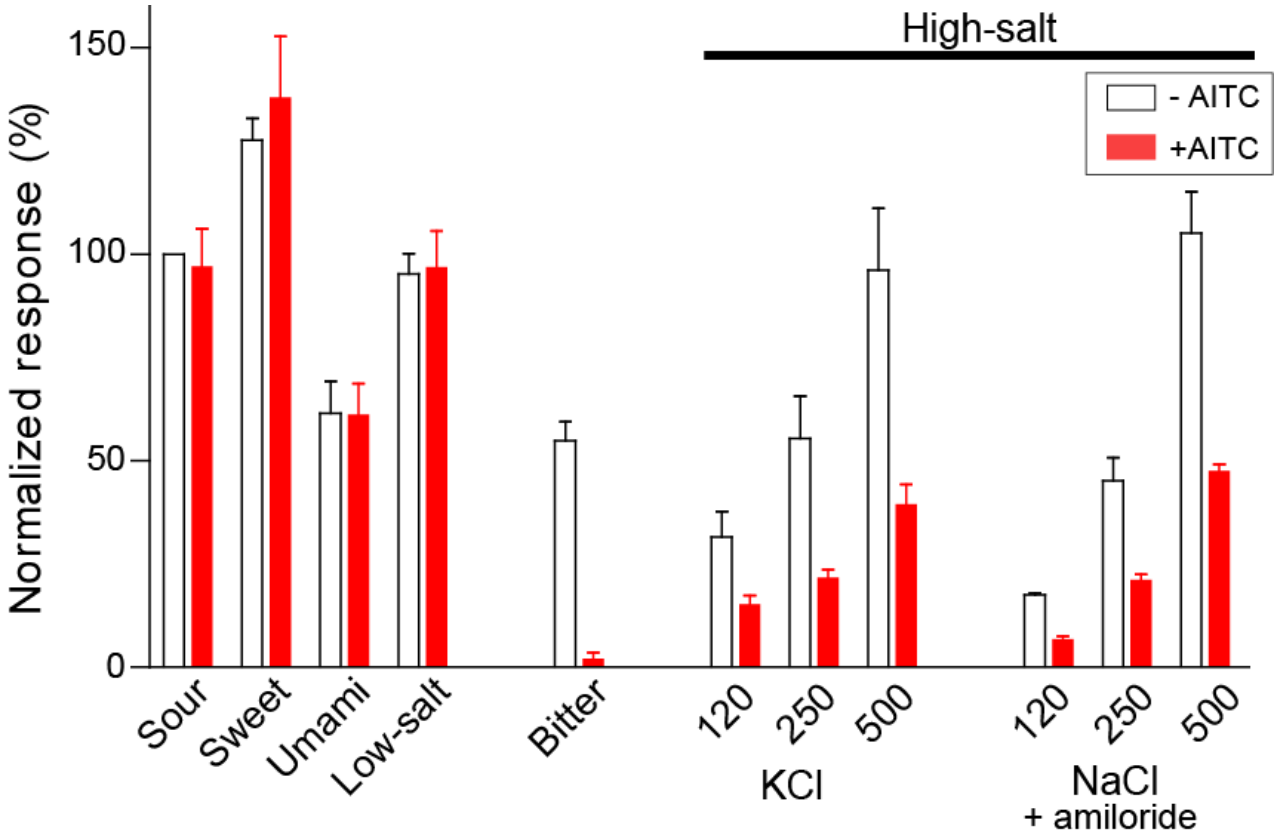
Supplementary Table 1

List of compounds screened and maximum concentration tested

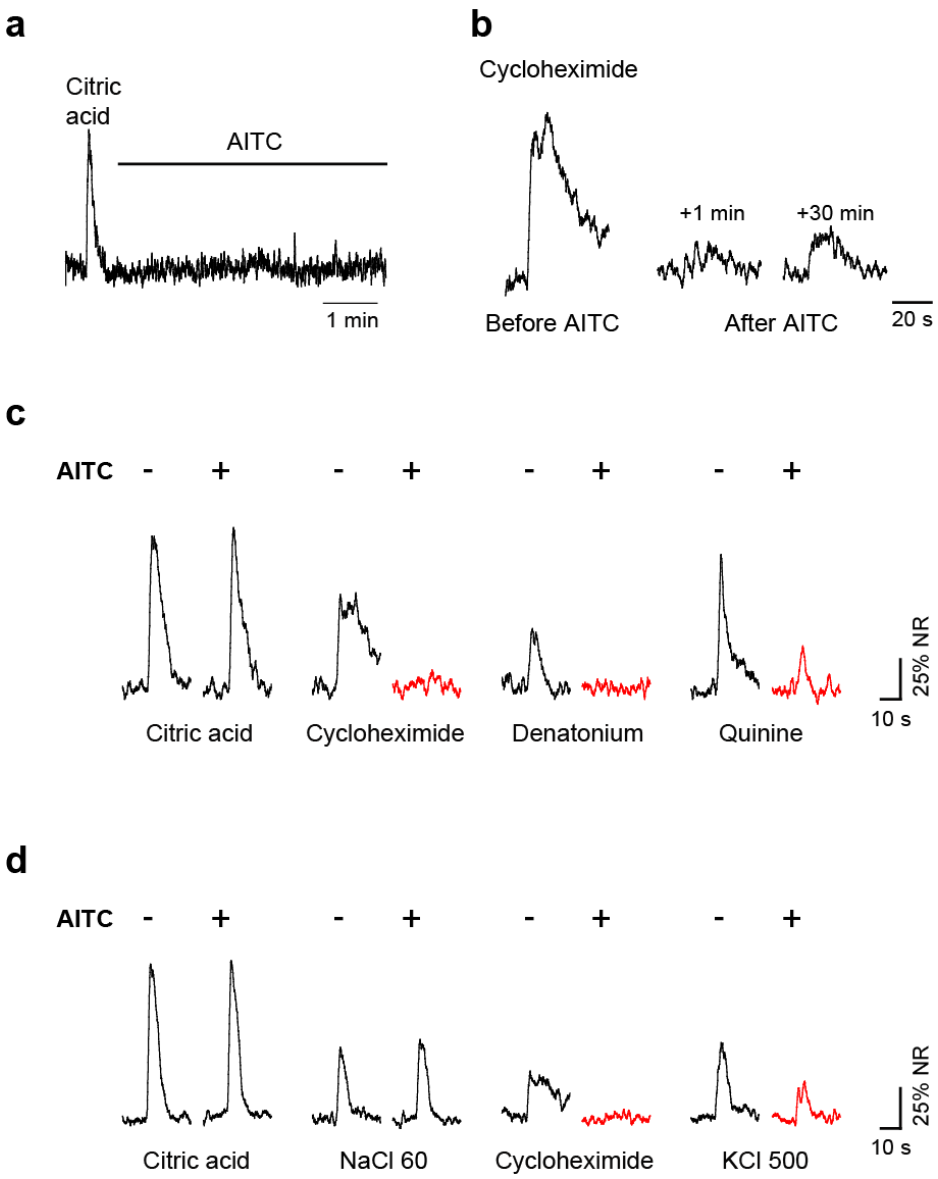
Name of Chemicals	Concentration
Mefloquine	10 mM
Probenecid	100 mM
Chloroquine	10 mM
2-APB	3 mM
alpha-glycyrrhetinic acid	2.5 mM
verapamil	1 mM
4-AP	100 mM
Citric Acid	20 mM
DIDS	30 mM
Flupirtine	100%
Carbenoxolone	30 mM
AITC	1-10 mM
capsaicin	100 uM
amiloride	30 uM
garlic extract	1%
H3030031 (TRPA1 antagonist)	200 uM
AP-18 (TRPA1 antagonist)	1 mM
LaCl3	3 mM



Supplementary Figure 1

Quantification of integrated chorda tympani nerve responses.

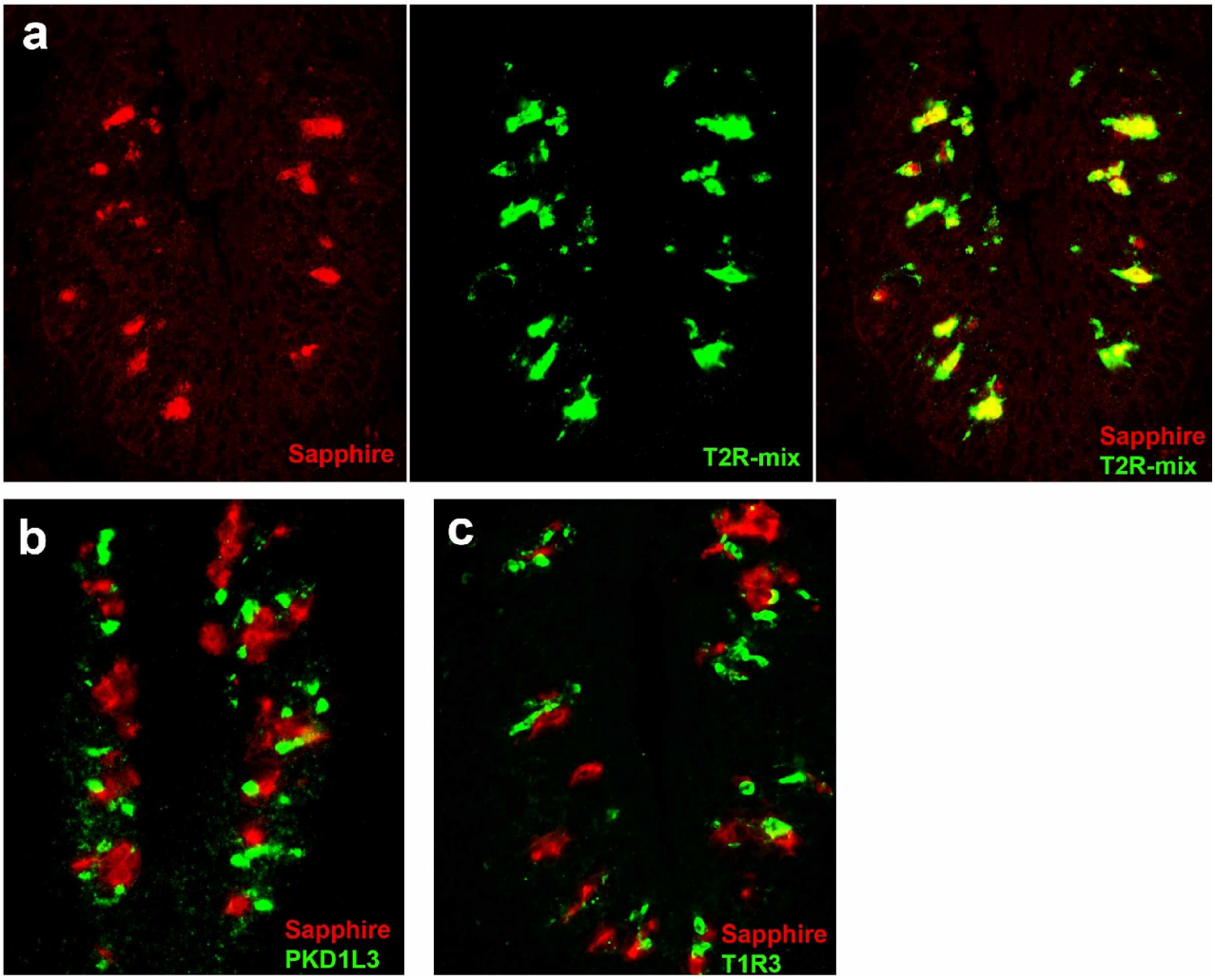
Quantification of integrated chorda tympani recordings before (open bars) and after (red bars) AITC treatment reveals significant (Student's t-test, $P<0.05$) effects of AITC on bitter and high-salt but not on other taste responses. Tastants used were: 20 mM citric acid (CA), Sour; 20 mM AcesulfameK, Sweet; 50 mM MPG + 0.5 mM IMP, Umami; 60 mM NaCl, Low-salt; 0.1 mM cycloheximide (Cyx), Bitter; and 120, 250 and 500 mM KCl or NaCl + 10 mM amiloride, High-salt; data were normalized to the response of 20 mM CA and are means ± s.e.m, n ≥ 3 mice.



Supplementary Figure 2

AITC irreversibly suppresses bitter responses.

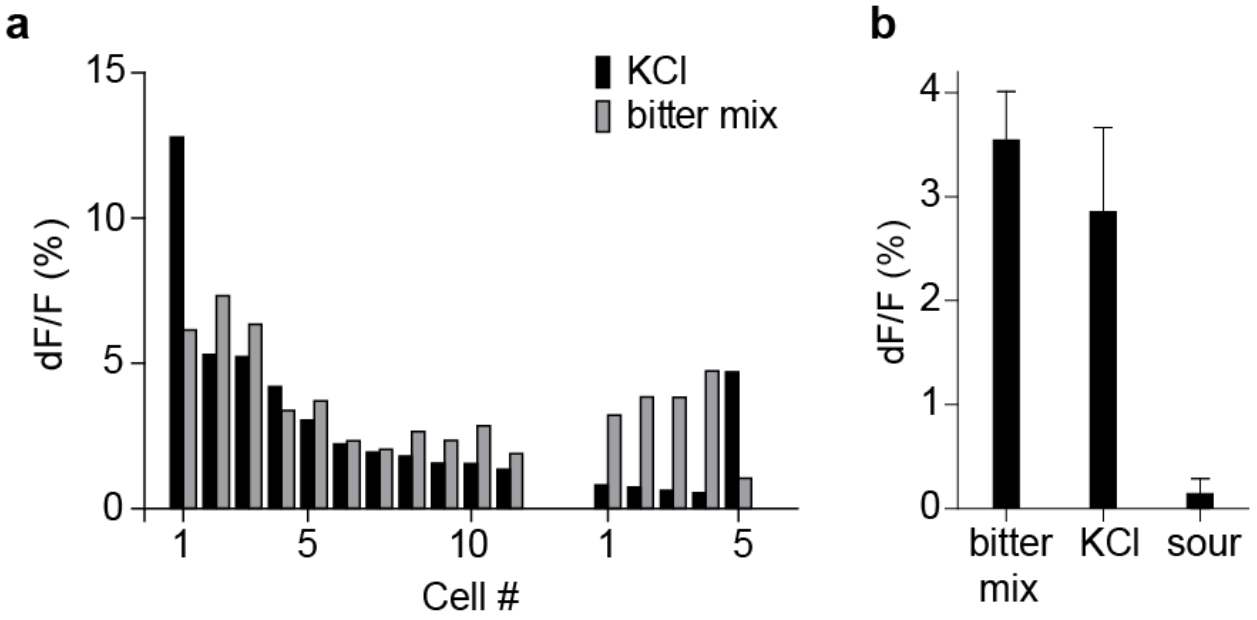
Representative chorda tympani recordings show (**a**) that 5 minutes exposure of the tongue to 3 mM AITC evokes minimal taste response but (**b**) strongly suppresses responses to high concentrations of the bitter tastant cycloheximide (1 mM) both 1 and 30 minutes after AITC treatment. (**c**) AITC treatment (indicated by + above the trace) not only suppressed responses to 0.1 mM cycloheximide but also to other bitter tastants e.g., 10 mM denatonium, and 10 mM quinine (red traces), while sour taste (20 mM citric acid) remained unimpaired. (**d**) TRPA1-KO mice exhibited robust AITC-mediated suppression of bitter and high-salt taste responses (red traces); thus TRPA1 is not required for inhibition of taste responses by AITC.



Supplementary Figure 3

Characterization of T2R32-Sapphire transgenic mice.

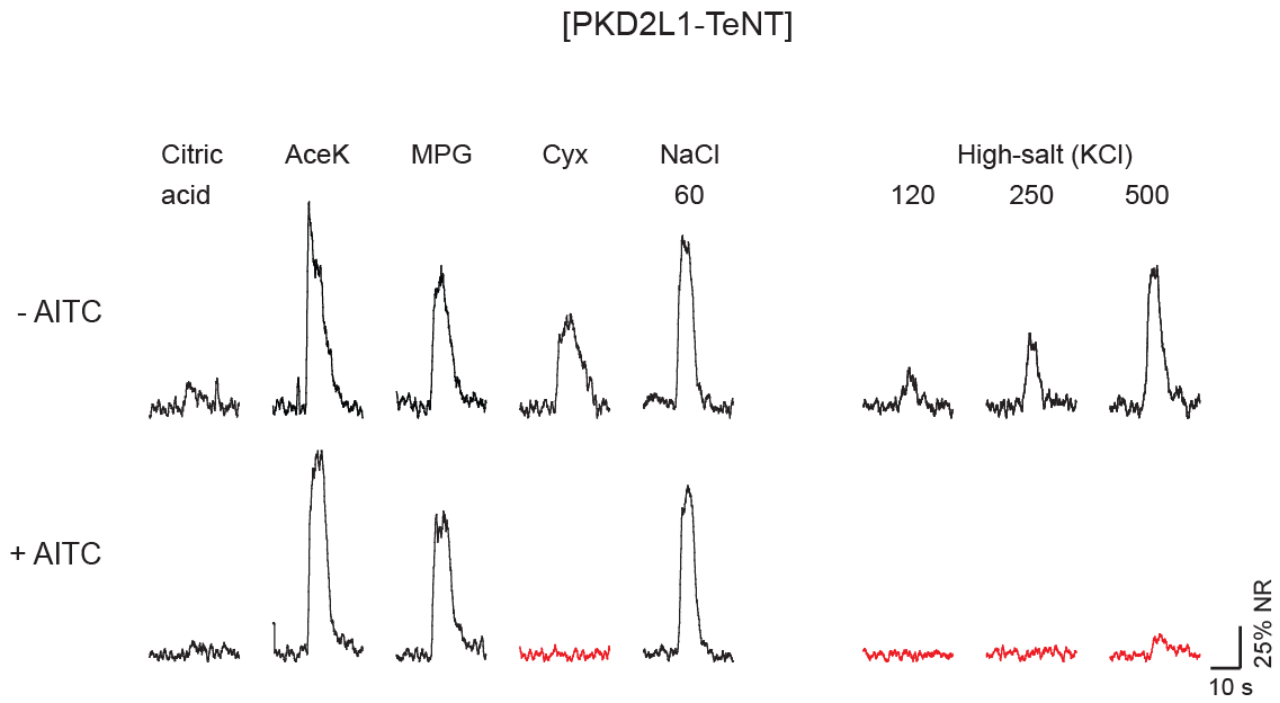
T2R32-Sapphire mice are transgenics engineered to express the blue shifted GFP-derivative, Sapphire, under the control of the T2R32 (also referred to as Tas2R139, PubMed gene #NM_181275.1) promoter. These mice, generated by Ken Mueller (UCSD Thesis, 2004) contained 10 kbp upstream of the T2R32 start codon fused to the GFP reporter. Double label in situ hybridization was used to examine the expression of the sapphire transgene in taste buds of T2R32-sapphire transgenic mice. (**a**) Sapphire (left panel, red-label) and bitter taste receptors (a mix of 20 T2Rs, middle panel, green label) are extensively co-expressed (right panel, merged image). Quantitation of labeling through the circumvallate papilla of two T2R32-sapphire mice revealed that at least 75% of positive cells were strongly detected by both probes. In contrast, (**b ,c**) Sapphire (red) was never co-expressed with (**b**) T1R3 (green), a component of sweet and umami receptors[1] or (**c**) PKD1L3 (green), a marker of sour responsive cells[2].



Supplementary Figure 4

Additional characterization of calcium responses in T2R32-Sapphire cells.

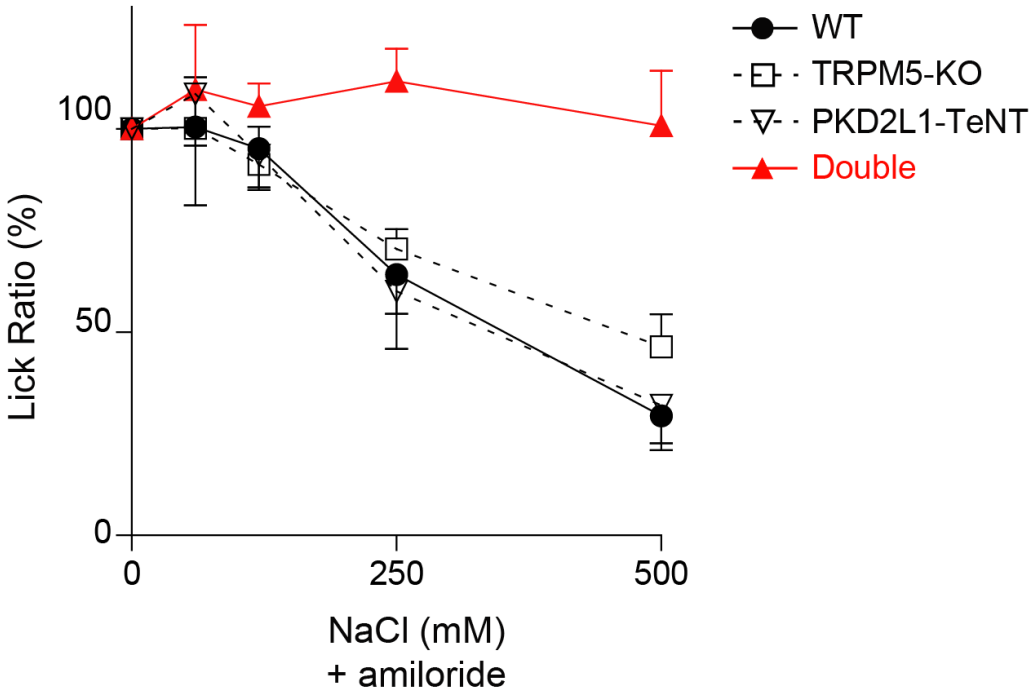
(**a**) Plot of response amplitudes (ΔF/F) for 500 mM KCl (black bars) and bitter (a mix of 10 mM denatonium, 1mM quinine and 1mM cycloheximide, grey bars) in responding T2R32-sapphire cells; 11 sapphire-labeled cells responded to both stimuli, 4 only to bitter and 1 to KCl. (**b**) ΔF/F responses of T2R32-sapphire cells shown in (**a**) to KCl, bitter and sour stimuli (mean ± s.e.m, n = 16 for bitter and KCl and 14 for sour).



Supplementary Figure 5

AITC suppresses residual high-salt responses in PKD2L1-TeNT mice.

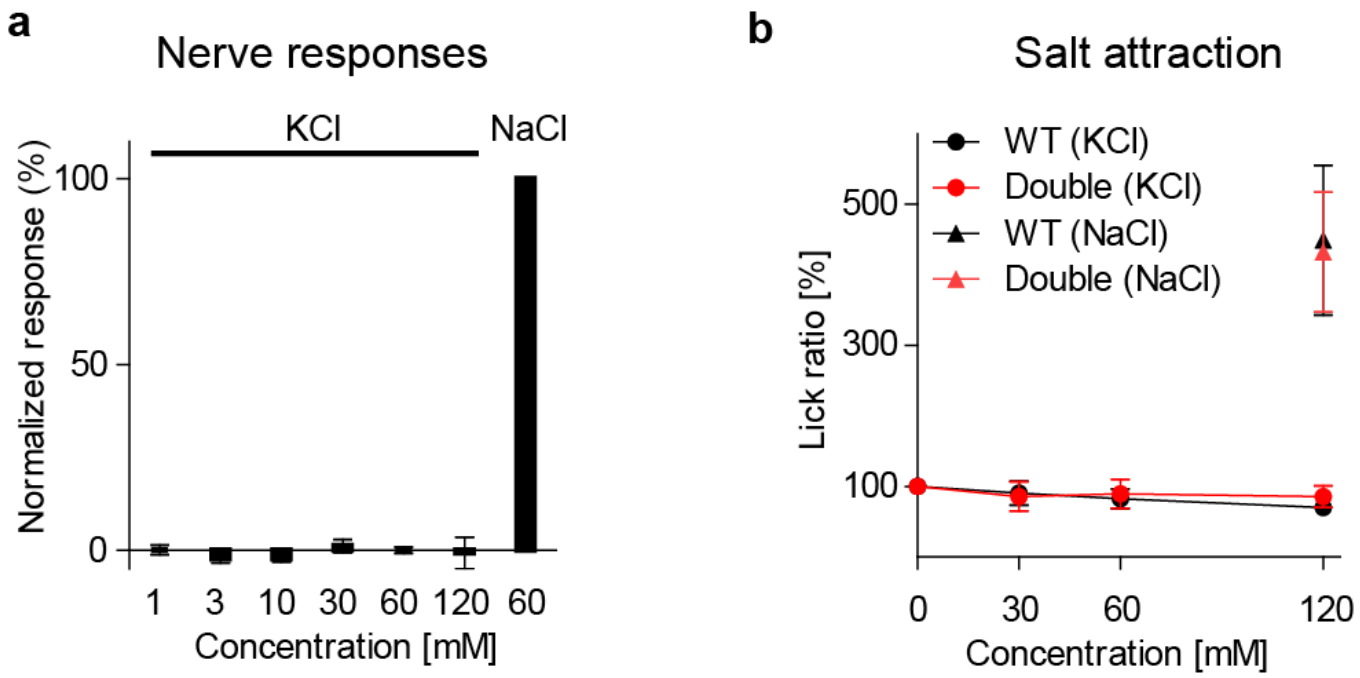
Upper panel: as expected, no chorda tympani nerve responses to sour stimuli were detected in PKD2L1-TeNT mice. These animals exhibit low responses to high-salt (KCl, see Figure 3a for additional data). Lower panel: high-salt responses of PKD2L1-TeNT mice are effectively abolished by application of AITC (red traces) indicating that high-salt responses are entirely mediated by bitter receptor cells in these animals. Tastants used were citric acid (20 mM), acesulfame K (AceK, 20mM), monopotassium glutamate + IMP (MPG, 50 mM + IMP 0.5 mM), cycloheximide (Cyx, 0.1mM) and NaCl and KCl at indicated concentrations (mM).



Supplementary Figure 6

Loss of behavioral aversion to NaCl in TRPM5-KO/PKD2L1-TeNT.

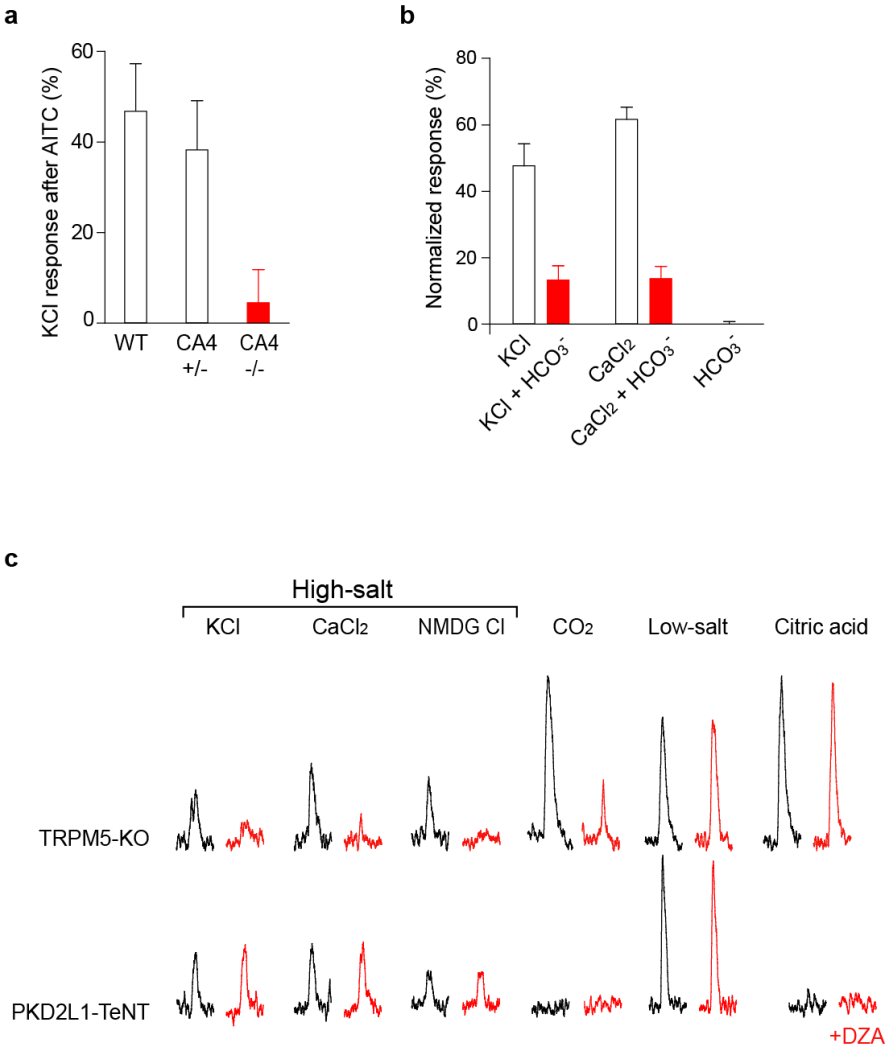
Water-deprived TRPM5-KO / PKD2L1-TeNT mice (Double) showed no aversive taste behavior in response to NaCl in the presence of 30 mM amiloride even at concentrations as high as 500 mM (red line)**.** In contrast, control (WT, black line) and single mutant mice (dotted lines) showed robust dose dependent behavioral aversion. Two-way ANOVA with post hoc tests at individual salt concentrations revealed significant differences between double mutants and other genotypes at 250 mM NaCl ($P<0.05$) and at 500 mM NaCl ($P< 0.01$). Values are means ± s.e.m. $n \geq 6$ mice for each point; data represent the percentage of licks relative to water licks.



Supplementary Figure 7

Low concentrations of KCl do not elicit electrophysiological taste responses or behavioral attraction.

(**a**) Quantification of integrated chorda tympani nerve responses shows that low concentrations of KCl (below 120 mM) elicit no electrophysiological responses in TRPM5-KO / PKD2L1-TeNT double mutant mice. Data were normalized to the response of 60 mM NaCl and are means ± s.e.m, n = 3 animals. (**b**) Immediate lick assays were used to measure salt attraction to low concentrations of KCl. After salt-depletion, neither control (WT, black circles) nor double mutant mice (red circles) showed attraction to a range of KCl concentrations. As expected, robust behavioral attraction to 120 mM NaCl was observed both for control (WT, black triangle) and double mutant animals (red triangle); values are means ± s.e.m., n = 5 mice.



Supplementary Figure 8

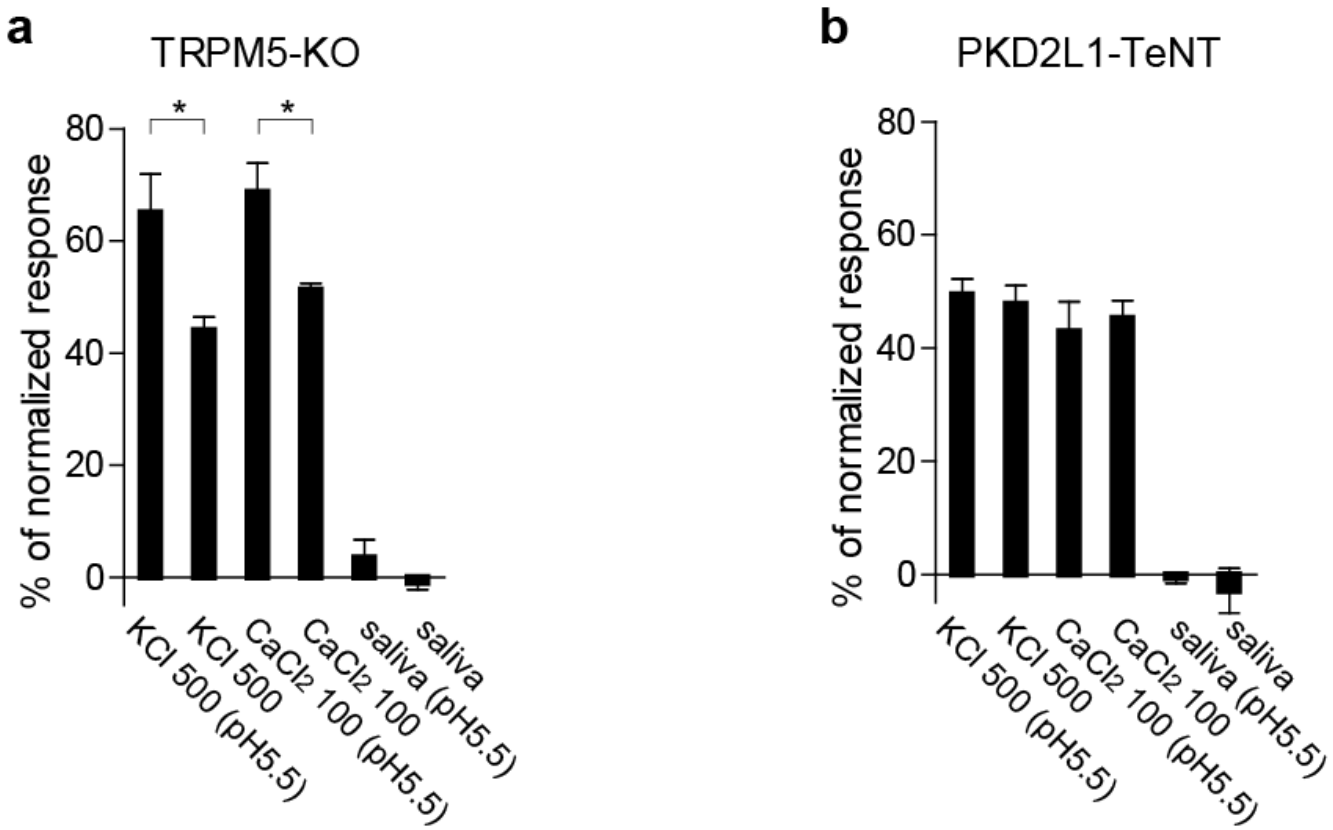
Carbonic anhydrase 4 is required for salt sensing in PKD2L1-expressing cells.

Given the prominent expression of CA4 in sour responsive taste cells and the salt dependence of carbonic anhydrase activity (with high ionic strength reducing enzymatic activity[3]), we reasoned that inhibition of CA4 by high-salt might result in a localized increase of protons and concomitant activation of the sour-sensing pathway. Thus, we set out to determine if this enzyme could function as (i.e. was appropriated as) a high-salt sensor in the sour cells. If this hypothesis is true, then a knockout of CA4, or driving CA4 in the direction that raises pH (for example by adding excess HCO_3^-) or inhibition of CA4 should all reduce high-salt responses from PKD2L1 cells. Indeed, our results show that these three predictions are met:

(a) Knockout of CA4 dramatically reduces high salt taste responses mediated by the sour sensing cells. Shown is quantification of chorda tympani responses from control (WT) or heterozygous (CA4 $^{+/-}$, open bars) and homozygous CA4 $^{-/-}$ mice (red bar); AITC pretreatment was carried out to eliminate the bitter cell component of salt taste. Responses to high-salt (250 mM KCl) were greatly reduced in CA4 $^{-/-}$ mice (red bar, Student's t-test, $P < 0.05$; values are means ± s.e.m., $n \geq 3$ mice).

(b) Driving CA4 in the direction that raises pH by addition of excess CA4 substrate (HCO_3^-) also suppressed PKD2L1-cell mediated high-salt responses. TRPM5-KO mice were used to focus on responses from sour sensitive cells; quantification of chorda tympani responses to 500 mM KCl or 100 mM $CaCl_2$ before (open bars) and after addition of 30 mM $KHCO_3$ (red bars) demonstrates strong inhibition of high salt taste by bicarbonate ($P<0.05$, Student's t-test; values are means ± s.e.m., n ≥ 3 mice).

(c) Pharmacological inhibition of tongue carbonic anhydrases with the potent CA inhibitor (dorzolamide, DZA, 0.5 % w/v) also reduced high-salt induced chorda tympani responses from sour taste cells. Upper panel shows responses from TRPM5-KO mice to various salts, before (black traces), and after (red traces) treatment of the tongue with DZA. Note that high-salt (as well as CO_2) responses were affected by DZA treatment; as expected, sour (20 mM citric acid) and low salt (NaCl, 60 mM) taste responses were insensitive to DZA. The lower panel shows that taste responses from PKD2L1-TeNT mice were completely insensitive to DZA treatment, demonstrating that inhibition of carbonic anhydrase activity does not affect high-salt responses in T2R-expressing bitter cells; note that the traces shown for citric acid in come from a different animal from those used for other tastants.



Supplementary Figure 9

Effect of salivary pH on high-salt responses in TRPM5-KO and PKD2L1-TeNT mice.

If CA4 functions as a "translator" of external salt concentration into local pH changes, then lowering salivary pH should enhance the high-salt responses of sour (but not bitter) cells. (**a**) Quantitation of normalized chorda tympani responses to 500 mM KCl and 100 mM $CaCl_2$ demonstrate that reducing pH from 7.4 (normal artificial saliva) to 5.5 significantly enhances high-salt responses in TRPM5-KO mice (* indicates significance $P < 0.05$, Student's t-test). (**b**) As expected, high-salt responses were not affected by salivary pH in PKD2L1-TeNT mice, demonstrating that CA4 and PKD2L1-cell dependent high-salt responses but not those of bitter-cells are pH sensitive. Data were normalized to the response of 60 mM NaCl and are means ± s.e.m, n = 3 animals.

Supplementary References

1. Zhao, G. Q. et al. The receptors for mammalian sweet and umami taste. *Cell* **115**, 255-66 (2003).
2. Huang, A. L. et al. The cells and logic for mammalian sour taste detection. *Nature* **442**, 934-8 (2006).
3. Baird, T. T., Jr., Waheed, A., Okuyama, T., Sly, W. S. & Fierke, C. A. Catalysis and inhibition of human carbonic anhydrase IV. *Biochemistry* **36**, 2669-78 (1997).