

LETTERS

The cells and logic for mammalian sour taste detection

Angela L. Huang¹, Xiaoke Chen¹, Mark A. Hoon², Jayaram Chandrashekar¹, Wei Guo¹, Dimitri Tränkner¹, Nicholas J. P. Ryba² & Charles S. Zuker¹

Mammals taste many compounds yet use a sensory palette consisting of only five basic taste modalities: sweet, bitter, sour, salty and umami (the taste of monosodium glutamate)^{1,2}. Although this repertoire may seem modest, it provides animals with critical information about the nature and quality of food. Sour taste detection functions as an important sensory input to warn against the ingestion of acidic (for example, spoiled or unripe) food sources^{1–3}. We have used a combination of bioinformatics, genetic and functional studies to identify PKD2L1, a polycystic-kidney-disease-like ion channel⁴, as a candidate mammalian sour taste sensor. In the tongue, PKD2L1 is expressed in a subset of taste receptor cells distinct from those responsible for sweet, bitter and umami taste. To examine the role of PKD2L1-expressing taste cells *in vivo*, we engineered mice with targeted genetic ablations of selected populations of taste receptor cells. Animals lacking PKD2L1-expressing cells are completely devoid of taste responses to sour stimuli. Notably, responses to all other tastants remained unaffected, proving that the segregation of taste qualities even extends to ionic stimuli. Our results now establish independent cellular substrates for four of the five basic taste modalities, and support a comprehensive labelled-line mode of taste coding at

the periphery^{5–10}. Notably, PKD2L1 is also expressed in specific neurons surrounding the central canal of the spinal cord. Here we demonstrate that these PKD2L1-expressing neurons send projections to the central canal, and selectively trigger action potentials in response to decreases in extracellular pH. We propose that these cells correspond to the long-sought components of the cerebrospinal fluid chemosensory system¹¹. Taken together, our results suggest a common basis for acid sensing in disparate physiological settings.

A broad range of cell types, receptors and mechanisms have been proposed to mediate salt and acid sensing in taste receptor cells (TRCs)^{1–3}. These include the activation of epithelial sodium channels (ENaCs), acid-sensing ion channels (ASICs), potassium (K²P) channels, H⁺-gated calcium channels, as well as the involvement of Na⁺-H⁺ exchangers, TRPV pain receptors, and even acid inactivation of K⁺ channels^{1–3,12,13}. Notably, most of these proteins are broadly expressed in TRCs and other tissues. In contrast, we previously isolated and characterized the receptors for sweet, umami and bitter taste^{5–7,14–16}, and showed that each of these three taste modalities is mediated by highly selective receptor proteins expressed in distinct and independent populations of TRCs^{5–10}.

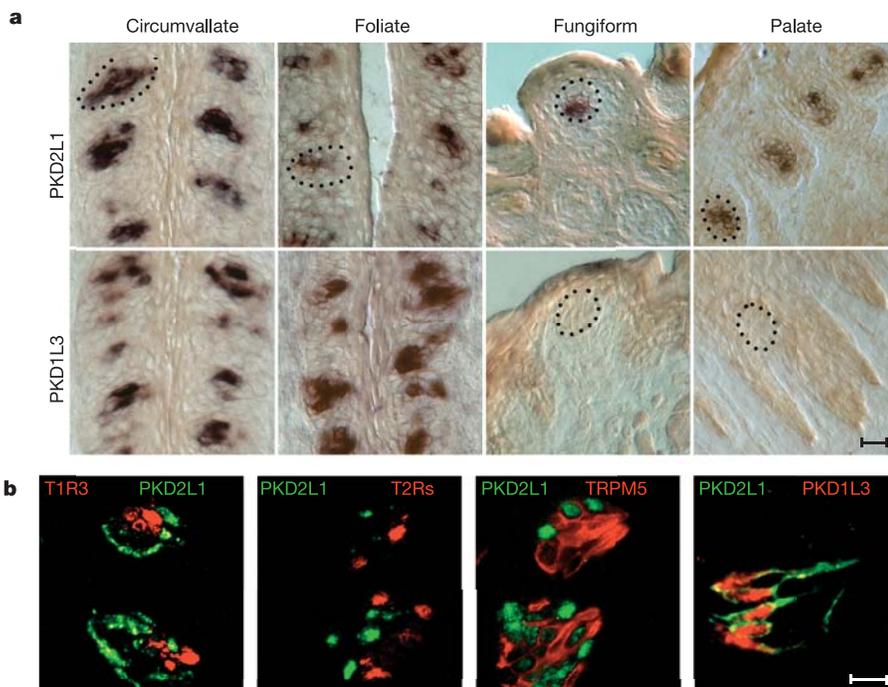


Figure 1 | PKD2L1 is expressed in a novel population of TRCs. *In situ* hybridization (PKD2L1, PKD1L3, T1Rs, T2Rs and TRPM5) and double-label fluorescent immunohistochemistry (PKD2L1) were used to examine the overlap in cellular expression of taste receptors, TRPM5, PKD2L1 and PKD1L3. **a**, *In situ* hybridization of PKD2L1 and PKD1L3 against circumvallate, foliate, fungiform and palate taste buds illustrating expression of PKD2L1 in subsets of TRCs of all taste buds, but lack of PKD1L3 in fungiform and palate TRCs. Dotted lines show the outline of sample taste buds. Scale bar, 25 μ m. **b**, The first three panels show co-labelling with a PKD2L1 antisense RNA probe (green) and T1R3 (T1R, sweet and umami cells), a mixture of 20 T2Rs (bitter cells) and TRPM5 (sweet, umami and bitter cells), respectively. The last panel shows co-labelling with anti-PKD2L1 antibodies and an antisense PKD1L3 RNA probe. Note the absence of overlap between PKD2L1-expressing cells and those expressing sweet, umami or bitter receptors. However, PKD1L3 is always co-expressed with PKD2L1 in circumvallate and foliate papillae. Scale bar, 10 μ m.

¹Howard Hughes Medical Institute and Departments of Neurobiology and Neurosciences, University of California at San Diego, La Jolla, California 92093-0649, USA. ²National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland 20892, USA.

Therefore, we reasoned that salt and sour taste should also be mediated by highly selective dedicated cells, and consequently expected the receptor proteins to be very exclusive in their expression pattern.

To identify novel taste receptors, we developed a multi-step bioinformatics and expression screening strategy. First, because sensory receptors are expected to be membrane proteins, approximately 30,000 mouse open reading frames (ORFs) were scanned for the presence of at least one putative transmembrane segment. Second, because taste receptors are predicted to be very restricted in their expression pattern, ORFs encoding candidate transmembrane proteins were cross-searched against mouse expressed sequence tag (EST) databases to eliminate those broadly expressed. Next, to identify the subset specifically enriched in taste tissue, ORFs selected as encoding transcripts infrequently represented in EST databases (~880 candidates) were used in polymerase chain reaction with reverse transcription (RT-PCR) reactions templated with messenger RNA from TRCs versus control tongue epithelium. Finally, given that our goal was to discover membrane proteins selectively expressed in subsets of TRCs (and ideally not in sweet-, bitter- or umami-sensing cells), we carried out detailed *in situ* hybridizations against taste papillae. Of 26 complementary DNAs used for *in situ* studies, five were found to robustly and selectively label subsets of TRCs. Figure 1 shows that one of these candidates,

PKD2L1, is expressed in TRCs of all taste papillae, including fungiform, circumvallate, foliate and palate taste buds.

PKD2L1 encodes a polypeptide displaying significant amino acid sequence similarity to PKD2 (ref. 4), a gene mutated in many cases of autosomal-dominant polycystic kidney disease^{17,18}. PKD2 proteins are members of the transient receptor potential (TRP) superfamily of ion channels¹⁹, and have been recently shown to function as non-selective cation channels when expressed in heterologous cells^{17,18,20}. Although the exact roles of PKD proteins remain unknown, they are believed to function as receptor-ion-channel complexes, often localized to ciliated compartments, and implicated in sensing extracellular signals (for example, in renal epithelial cells^{17,18}). We reasoned that if PKD2L1 has a specific role in taste it should be expressed in subpopulations of TRCs with unique functional characteristics. To determine which type of TRCs expresses PKD2L1, we performed double labelling experiments with sweet, umami and bitter taste receptors (T1Rs and T2Rs), as well as TRPM5, the transduction channel of sweet-, bitter- and umami-sensing cells⁸. Our results (Fig. 1) established that PKD2L1 is expressed in cells distinct from those mediating sweet, umami and bitter taste (see also ref. 21).

Mammalian TRCs project specialized apical microvilli to the taste pore, the site of interaction between tastants and taste receptor proteins. All known taste receptor proteins localize to and function in this TRC compartment^{1,5-7,14,16,22}. Therefore, we would expect

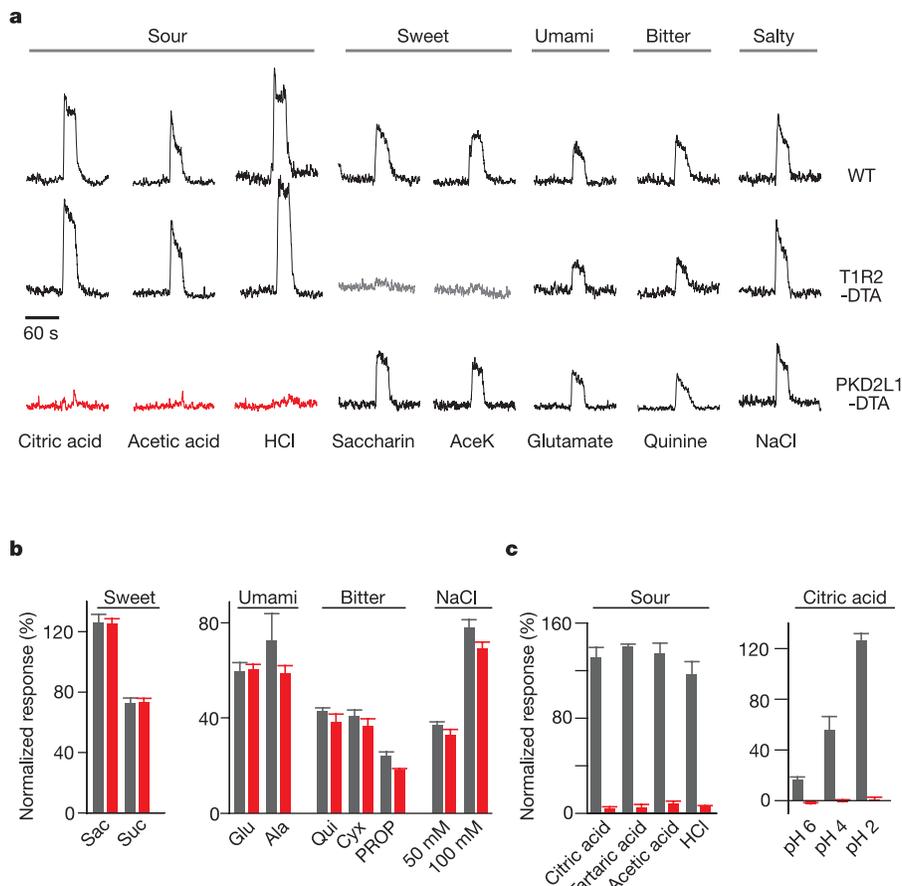


Figure 2 | PKD2L1-expressing TRCs are the mediators of sour taste.

a, Targeted expression of DTA to selective populations of TRCs produces animals with selective deficits in taste responses. Wild-type mice (WT) show robust neural responses to sour, sweet, umami (amino acid), bitter and salty tastants. However, ablation of sweet cells (T1R2-DTA) generates animals with a marked loss of sweet taste (middle panel). In contrast, ablation of PKD2L1-expressing cells eliminates responses to all acid stimuli (bottom panel). Notably, responses to all other taste qualities remain unimpaired in the DTA-expressing animals. Integrated chorda tympani responses are

shown (see Methods). **b**, Average neural responses of animals lacking PKD2L1-expressing cells to an expanded panel of tastants; note normal responses to sweet, umami, bitter and salt stimuli. Wild type, black bars; PKD2L1-DTA, red bars; values are means \pm s.e.m. ($n = 5$).

See Methods for tastant concentrations and abbreviations. **c**, Quantification of acid responses of wild-type and PKD2L1-DTA animals. The values are means \pm s.e.m. ($n = 6$). Only the differences in acid responses are significant between wild-type and PKD2L1-DTA mice ($P < 0.00001$).

bona fide candidate receptors to also be enriched in the taste pore. We generated antibodies to PKD2L1 and used them in immunofluorescence staining of tongue tissue sections. Examination of circumvallate, foliate and fungiform papillae demonstrated that PKD2L1 protein is indeed enriched in the apical surface of TRCs, with the antibodies robustly labelling the taste pore region (Supplementary Fig. 1). These results implicate PKD2L1 as part of the taste sensing machinery.

PKD2 isoforms often require PKD1 proteins for functional expression at the cell surface^{17,18,20}. The mammalian genome contains four members of the PKD1 family: PKD1, PKD1L1, PKD1L2 and PKD1L3 (refs 17, 18). We performed *in situ* hybridization studies with gene-specific probes representing each family member, and determined that PKD1L3 is specifically co-expressed with PKD2L1 in circumvallate and foliate TRCs (Fig. 1, see also ref. 21). We also generated antibodies to PKD1L3 and demonstrated selective co-expression with PKD2L1 in non-TRPM5-expressing cells of the circumvallate and foliate taste buds (Fig. 1 and Supplementary Fig. 1). Surprisingly, PKD1L3 transcript or protein is not detectable in fungiform or palate taste buds, suggesting that a different partner may be expressed in those TRCs.

To dissect the function of PKD2L1-expressing cells in the tongue, we engineered mice where these cells were genetically ablated by targeted expression of attenuated diphtheria toxin²³ (DTA). To validate this approach as a means of uncovering TRC function, we first generated mice in which T1R2-regulatory sequences were used to target DTA expression²⁴ (see Supplementary Fig. 2). T1R2 is an essential subunit of the sweet receptor heterodimer (composed of T1R2 plus T1R3), and the selective ablation of these cells should generate animals with a specific loss of sweet taste^{6,9,10,16}. To investigate the taste responses of the genetically modified mice, we recorded tastant-induced action potentials from nerves innervating taste cells of the tongue; this physiological assay monitors the activity of the taste system at the periphery, and provides an accurate and reliable measure of TRC function. Indeed, animals expressing DTA in T1R2 cells have an extraordinary loss of sweet, but importantly retain umami, bitter, sour and salty tastes (Fig. 2). These results further substantiate the exquisite segregation of taste modalities at the

periphery, and demonstrate the utility of using DTA-mediated ablation of TRCs as a strategy for dissecting taste system function. Next, we engineered animals in which the *Pkd2L1* gene was used to target Cre recombinase into PKD2L1-expressing cells (see Supplementary Figs 2 and 3). These mice were crossed to the conditional DTA lines²⁴, and double-positive progeny were scrutinized both for the specificity and efficiency of killing, as well as the integrity of taste buds. We checked for the expression of T1Rs, T2Rs and TRPM5 in control and DTA-expressing animals, and found no significant differences in the number or distribution of T1R- or T2R-positive cells between wild-type and ablated taste tissue^{5-8,14,25} (Supplementary Fig. 2). In contrast, the DTA-targeted mice had a profound and practically complete loss of PKD2L1-expressing TRCs in the tongue (Supplementary Fig. 2). Notably, genetic ablation of the PKD2L1-expressing cells produces animals with a devastating loss of sour taste (Fig. 2). Responses to all acid tastants, including citric acid, HCl, tartaric acid and acetic acid are completely abolished, with no significant activity over a range of five orders magnitude of proton concentrations. However, responses to sweet, umami, bitter or salty tastants remain indistinguishable from wild-type control animals. These results firmly establish PKD2L1-expressing cells as the sour taste sensors, and further substantiate a model of coding at the periphery in which individual taste modalities operate independently of each other.

Acid sensing is important not only in the taste system, but also for monitoring the functional state of body fluids, including the internal milieu of the brain. This is particularly well studied in the central and peripheral control of respiration, where pH sensing is the principal mechanism for monitoring CO₂ levels in the blood and cerebrospinal fluid (CSF)^{11,26,27}. Thus, we wondered whether PKD2L1 might be expressed in additional cell types, and if so, whether such cells may also be involved in pH sensing in other physiological systems.

We carried out *in situ* hybridization and antibody staining experiments with PKD2L1 on a wide range of other tissues and identified a singular additional domain of expression: a discrete population of neurons surrounding the central canal of the spinal cord, through its entire length, from its origin in the brain stem to its end around the cauda equina (Fig. 3). Notably, these neurons send processes into the

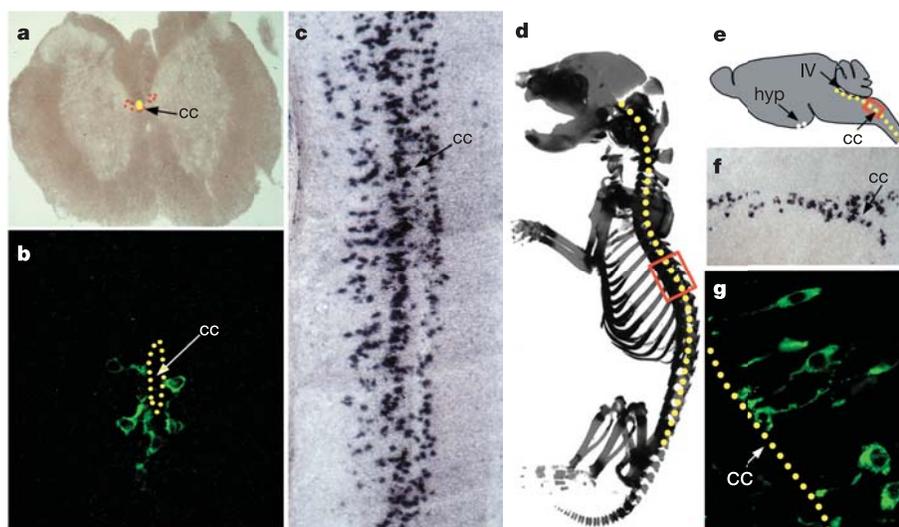


Figure 3 | PKD2L1 is expressed in neurons contacting the central canal of the spinal cord. **a**, *In situ* hybridization with PKD2L1-specific probes to a coronal section of the spinal cord; signals are pseudo-coloured in red. **b**, High-magnification staining with anti-PKD2L1 antibodies reveals a population of PKD2L1-expressing neurons surrounding the central canal of the spinal cord (cc; highlighted by yellow dots in all panels). **c**, *In situ* hybridization with PKD2L1-specific probes on a sagittal section of a P1 mouse. The PKD2L1-expressing cells are found throughout the entire length

of the spinal cord. **d**, Model mouse skeleton illustrating the approximate area (red box) of the *in situ* hybridization performed in **c**. **e**, **f**, PKD2L1 expression extends through the brain stem and into the IV ventricle (IV). There is also a very small group of positive cells in the hypothalamus (hyp; data not shown). **g**, Immunofluorescent staining with anti-PKD2L1 antibodies. PKD2L1-expressing neurons project into the central canal; note robust expression of PKD2L1 receptors at the terminals.

central canal, indicating that they may function as chemoreceptors sensing the internal state of the CSF (Fig. 3b, g; see also ref. 11). Given their anatomical distribution and cellular morphology, we reasoned that these cells might be part of the homeostatic circuitry responsible for monitoring and reporting the pH of the CSF. This hypothesis predicts that these neurons should trigger action potentials in response to acid stimulation. Therefore, we engineered mice where a green fluorescent protein (GFP) reporter was targeted to PKD2L1-expressing cells, and performed patch-clamp recordings from GFP-labelled cells in a spinal cord slice preparation²⁸. We anticipated some notable differences in the behaviour of these cells compared to TRCs—whereas the taste system is tuned to respond to acid stimulation in the range of multiple pH units (that is, pH 2–5), we expected the CSF monitor cells to respond to pH changes within a range of a few tenths of deviation from pH 7.4. Indeed, Fig. 4 shows that the PKD2L1-expressing neurons display exquisite sensitivity and selectivity to pH stimulation. Exposure to test solutions between pH 6.5 and 7.4 evoked a marked, dose-dependent and reversible increase in action potential frequency (Fig. 4 and data not shown). In contrast, the same acid stimuli have no significant impact on the response of control (unlabelled) cells, even after exposure to pH as

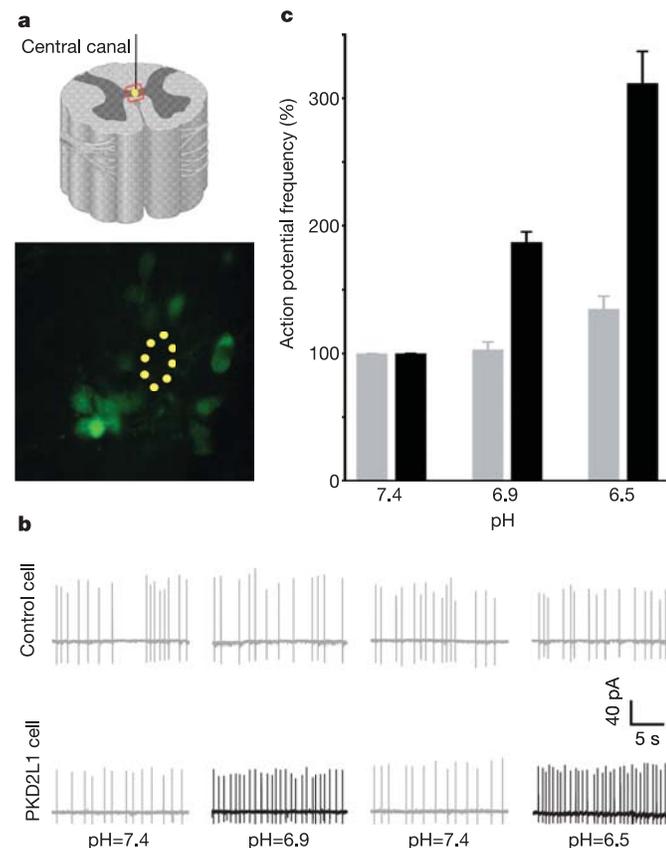


Figure 4 | PKD2L1-expressing neurons of the central canal fire action potentials in response to pH stimulation. Spinal cord neurons were patched using a loose patch configuration²⁸, tested for the presence of basal activity and recorded in the cell-attached configuration. **a**, GFP-expressing (PKD2L1-positive cells) or unlabelled (control) cells were examined for pH responses. **b**, Responses of a sample GFP-labelled or unlabelled neuron to test solutions under the indicated regime; shown are trains of spikes in a window of ~25 s. **c**, Data were analysed by examining records of ~4 min at each pH condition. Basal activity ranged between 1 to 5 Hz. Note the marked increases in pH-evoked firing frequency in GFP-labelled (black bars) neurons versus unlabelled (grey bars) cells ($P < 0.001$). A minimum of eight GFP-labelled and five unlabelled cells were characterized for each stimulus. The values are means \pm s.e.m. normalized to basal activity at pH 7.4 (taken as 100%).

low as 6.5 (lower pH values triggered irreversible damage to the slice preparation).

Most of the known CSF-contacting neurons in mammals project ciliated dendrites into the CSF, where they are proposed to sense fluid flow, pressure, pH or the composition of the CSF¹¹. Our demonstration that PKD2L1-expressing cells of the spinal cord selectively fire in response to minor changes in proton concentration strongly suggests that they function as sentinels of cerebrospinal and ventricular pH. Collectively, these results uncover an entirely unexpected role for members of the PKD family of proteins, offer a new perspective into the potential importance of PKD2 proteins in health and disease, and bring forth a surprising unity in the cellular basis of pH sensing in very different physiological systems. In the future, it will be of interest to develop an activity assay for PKD2L1 to establish the molecular mechanism of acid activation, to study the phenotype of *Pkd2l1* knockout animals, and determine whether PKD2L1 functionally associates or interacts with different partners in different cells types. In this regard, it would be worth exploring whether the differences in pH sensitivity between the tongue and spinal cord might be due to differences in PKD2L1–receptor complex composition.

The nature of the mammalian sour taste receptor and sour-sensing TRCs has been fertile ground for speculation over the years. A wide range of cell types, receptors and even receptor-independent mechanisms have been proposed to mediate acid detection in the tongue^{1–3}. The results presented here establish that sour taste, much like our previous findings for sweet, umami and bitter taste, is mediated by a unique cell type, independent of all other taste qualities. Furthermore, our demonstration that sour-less mice have normal salt responses shows that salt taste is also mediated by independent TRCs. Together, these results impose a considerable revision of the current views of taste representation at the periphery, and make a compelling case for a labelled-line mode of coding across all five taste modalities and taste receptor cell types.

METHODS

Molecular cloning of PKD2L1. We used a strategy that combined bioinformatics and differential screening to isolate genes specifically expressed in TRCs. Mouse genomic sequence information was obtained from Ensembl Mm.30 (<http://www.ensembl.org>). Approximately 30,000 predicted protein sequences were screened for the presence of at least one putative transmembrane segment, using both TMHMM server version 2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>) and *f*_TMHMM (San Diego Supercomputer centre, <http://www.sdsc.edu/pb/Group.html>). The cDNA sequence for each candidate membrane protein was then extracted from NCBI (http://www.ncbi.nlm.nih.gov/blast/blastcgihelp.shtml#nucleotide_databases) and used to screen EST databases (<http://www.ncbi.nlm.nih.gov/dbEST/index.html>). Only EST hits with *e*-values of less than or equal to e^{-100} were considered in our analysis. A total of 884 genes expressed in three tissues or less were chosen for PCR reactions with cDNA prepared from taste papillae mRNA (circumvallate and foliate) and from surrounding non-taste epithelial tissue (non-taste control). To maximise specificity of the PCR reactions, primer sets often included unique 3' untranslated region (UTR) sequences (http://irodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). A total of 98 genes showed selective enrichment in taste versus non-taste tissue, and of these five were robustly expressed in subsets of TRCs. Full-length clones were isolated from mouse taste cDNAs and/or cDNA libraries²².

In situ hybridization and immunostaining. *In situ* hybridization and immunostaining were as described previously^{8,22}. Anti-peptide antibodies to PKD2L1 (KLKMLERKGLSPGMGE) and PKD1L3 (DFQEADNYCHAQRGLAHT) were generated in rabbits and purified as described previously⁸.

Transgenic animals. Transgenic lines were produced by pronuclear injection of zygotes from FVB/N or CB6 (BALB/c \times C57BL/6 hybrids) mice. The PKD2L1-IRES-Cre construct was generated in RP23-297K23 and the T1R2-IRES-Cre in RP23-348G10 (<http://bacpac.chori.org/>). Recombination was carried out exactly as described previously²⁹. Z/EG reporter lines³⁰ were obtained from Jackson Laboratories, and Rosa26-flox-lacZ-flox-DTA animals²⁴ were a gift of D. Riethmacher.

Nerve recordings. Lingual stimulation and recording procedures were performed as previously described^{7,9}. All data analyses used the integrated response over a 25-s period immediately after the application of the stimulus. Tastants

used for nerve recordings were: 10 mM, 60 mM acesulfame K (AceK); 10 mM, 60 mM sodium saccharin (saccharin); 300 mM sucrose; 30 mM monopotassium glutamate plus 1 mM inosine monophosphate (Glu); 30 mM L-alanine plus 1 mM inosine monophosphate (Ala); 10 mM quinine hydrochloride (Qui); 100 μ M cycloheximide (Cyx); 10 mM 6-*n*-propyl-2-thiouracil (PROP); 50 mM, 100 mM sodium chloride (NaCl); 10 mM, 50 mM citric acid; 10 mM, 50 mM tartaric acid; 50 mM, 500 mM acetic acid; pH 2 hydrochloric acid (HCl); 10 mM citric acid pH 2, 4 and 6. The mean response to 60 mM AceK was used to normalize responses to each experimental series in the wild-type and PKD2L1-DTA animals (Fig. 2b, c).

Spinal cord slice recordings. Electrophysiological experiments were performed on P1–P4 mice as previously described²⁸. All incubations included 10 μ M CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and 50 μ M AP5 (D(-)-2-amino-5-phosphonvaleric acid). Spinal cord slices 250–300- μ m thick were generated using a Vibratome 3000 Plus at 0–4 °C in a modified Ringers' solution (0.5 mM CaCl₂, 3.7 mM MgSO₄). After at least a 1-h recovery period, slices were transferred to a recording chamber and perfused with oxygenated Ringers' solution (pH 7.4) at room temperature. Cell-attached patch-clamp recordings from GFP-labelled and unlabelled cells were performed using an EPC-10/2 amplifier and Patchmaster software (HEKA Elektronik). Slices were stimulated with a solution containing 140 mM NaCl, 3 mM KCl, 1.3 mM MgSO₄, 2.5 mM CaCl₂, 10 mM glucose, 10 mM HEPES at various pH values (7.4, 6.9, 6.5). Eight out of eight GFP-labelled cells showed pH-dependent increases in action potential frequency.

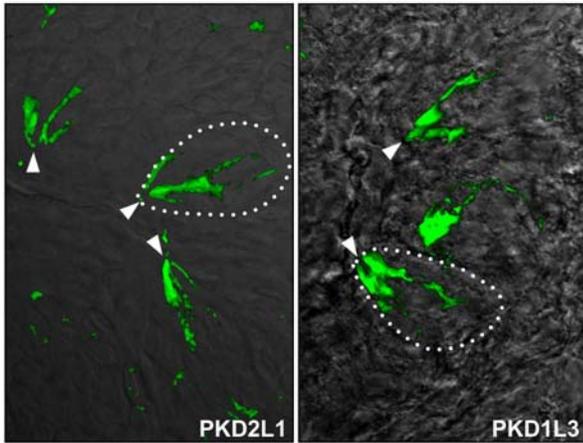
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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

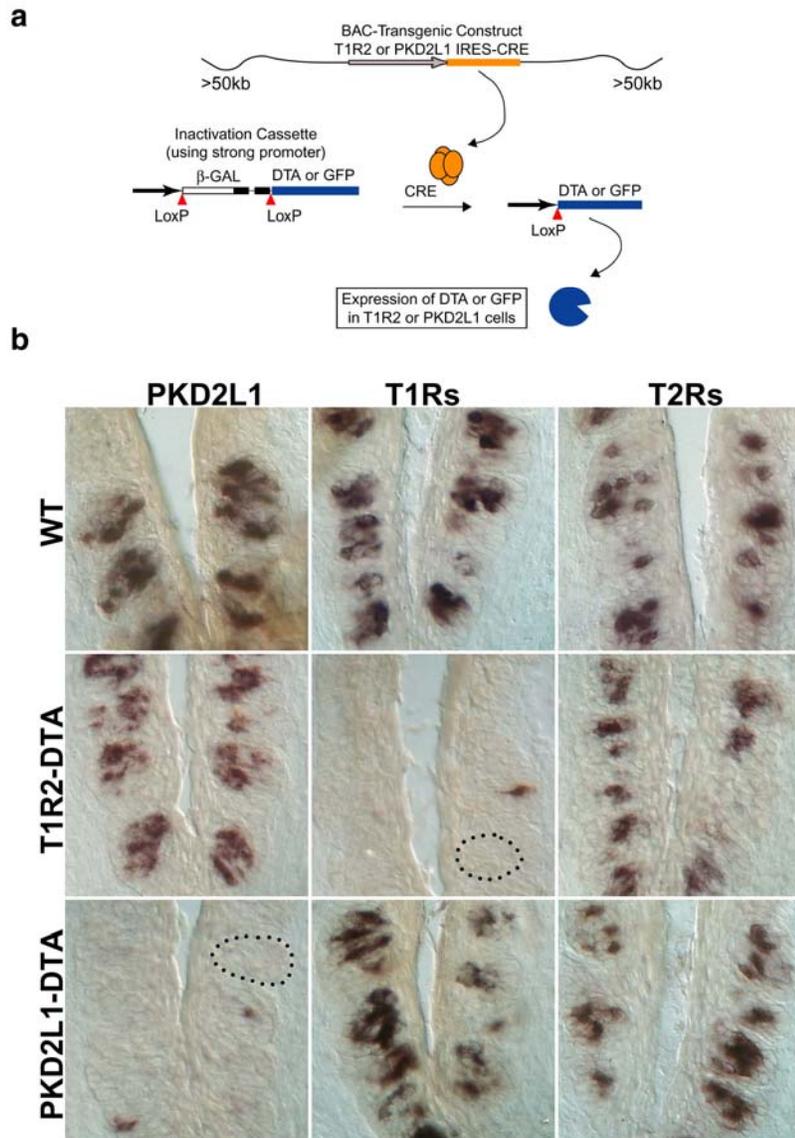
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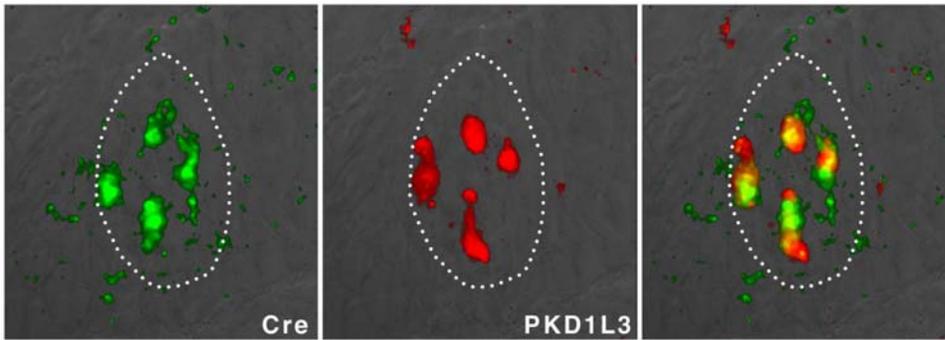
Supplementary Figure 1: PKD2L1 and PKD1L3 are enriched in the taste pore

Immunofluorescent stainings of mouse taste buds with PKD2L1 (left panel) and with PKD1L3 (right panel) antibodies. The pictures show superposition of fluorescent antibody signals on DIC images of taste tissue. Dotted lines illustrate the outline of a taste bud, and arrows point to the taste pore region.



Supplementary Figure 2: Loss of selective TRCs in DTA-expressing animals

Upper diagram (a) illustrates the strategy used to target DTA or GFP to selective populations of TRCs. BAC constructs contained the entire T1R2 or PKD2L1 genes with the IRES-Cre added downstream of the termination codon, but upstream of polyA-addition signals. In both cases, the transgenic constructs included at least 50Kb of flanking sequences upstream and downstream of the target gene (see Methods). Fidelity of Cre and reporter expression in the correct cell types was confirmed by double labeling with a variety of TRC-specific gene probes. Lower panels (b) show *in situ* hybridization experiments examining the presence of sweet (T1Rs), bitter (T2Rs) or PKD2L1-expressing cells in the two engineered lines. Targeting of DTA to T1R2- or PKD2L1-expressing cells eliminates over 95% of their respective TRC population. In situ hybridization probes were as in Figure 1.



Supplementary Figure 3: Targeting of Cre recombinase to PKD-expressing TRCs

In situ hybridization with double-labeled probes (Cre and PKD1L3) was used to examine the expression of Cre recombinase in PKD-expressing cells. Dotted lines illustrate the outline of a taste bud; note the cellular overlap in the hybridization signals. Similar results were obtained by crossing PKD2L1-Cre lines to GFP reporter lines³⁰.