LETTER

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CALHM1 ion channel mediates purinergic neurotransmission of sweet, bitter and umami tastes

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Recognition of sweet, bitter and umami tastes requires the non-vesicular release from taste bud cells of ATP, which acts as a neurotransmitter to activate afferent neural gustatory pathways[1]. However, how ATP is released to fulfil this function is not fully understood. Here we show that calcium homeostasis modulator 1 (CALHM1), a voltage-gated ion channel[2,3], is indispensable for taste-stimuli-evoked ATP release from sweet-, bitter- and umami-sensing taste bud cells. *Calhm1* knockout mice have severely impaired perceptions of sweet, bitter and umami compounds, whereas their recognition of sour and salty tastes remains mostly normal. *Calhm1* deficiency affects taste perception without interfering with taste cell development or integrity. CALHM1 is expressed specifically in sweet/bitter/umami-sensing type II taste bud cells. Its heterologous expression induces a novel ATP permeability that releases ATP from cells in response to manipulations that activate the CALHM1 ion channel. Knockout of *Calhm1* strongly reduces voltage-gated currents in type II cells and taste-evoked ATP release from taste buds without affecting the excitability of taste cells by taste stimuli. Thus, CALHM1 is a voltage-gated ATP-release channel required for sweet, bitter and umami taste perception.

Tastes are sensed by dedicated receptor cells in taste buds, which are composed of three anatomically distinct types of cells: types I, II and III. Only type III cells, which sense sour tastes, have neuron-like features, including expression of neurotransmitter biosynthesis enzymes and synaptic vesicles at classical synapses with sensory nerve fibres[1]. Type II cells, which sense sweet, bitter and umami tastes, share a common signal-transduction pathway. However, they lack classical synaptic structures, yet transmit taste information to gustatory neurons by releasing ATP as a neurotransmitter[4,5]. Our work, described below, implicates CALHM1 as a critical component responsible for this ATP release.

Calhm1 (ref. 6) encodes the pore-forming subunit of a voltage-gated, non-selective, plasma membrane ion channel involved in neuronal excitability[2] and, potentially, the pathogenesis of Alzheimer's disease[6–8]. *Calhm1* expression was identified in primate taste buds[9], suggesting that CALHM1 may have physiological functions outside the brain. We confirmed that *Calhm1* was expressed in mouse taste buds but not in surrounding epithelium (Fig. 1a, b, e–g). To examine CALHM1 function, we generated a constitutive *Calhm1*$^{-/-}$ mouse and verified loss of *Calhm1* expression in taste buds (Fig. 1a, c). *Calhm1*$^{-/-}$ mice were viable and fertile, with no overt morphological abnormalities in their taste buds nor any altered expression of taste-related marker genes (Fig. 1a and Supplementary Fig. 1). Loss of *Calhm1* signal in taste buds of *Skn-1a* (also known as *Pou2f3*) knockout mice, from which type II cells are developmentally absent[10] (Fig. 1d and Supplementary Fig. 2), and the co-expression of *Calhm1* and *Trpm5* (Fig. 1h), demonstrated that *Calhm1* expression is confined to type II cells. Expression profiling by reverse-transcription PCR of pools of isolated type II and type III cells and individual taste cells also supported the confined expression of *Calhm1* to type II cells (Supplementary Fig. 3). *Calhm1* expression was observed not only in *Tas1r3*-expressing sweet and umami taste cells, but also in other type II cells, indicating that *Calhm1* is expressed in sweet, bitter and umami taste cells (Fig. 1i).

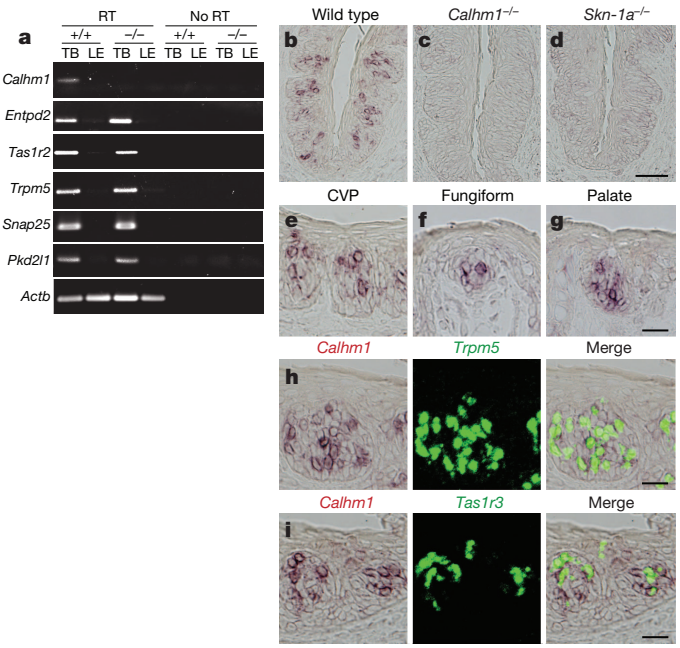

Figure 1 | CALHM1 is selectively expressed in type II taste bud cells. **a**, Reverse-transcription PCR of messenger RNA of *Calhm1*, *Actb* (β-actin) and taste cell marker genes from laser-microdissected circumvallate papillae (CVP) taste buds (TB) and lingual epithelium (LE) in wild-type (+/+) and *Calhm1* knockout (−/−) mouse tongues. RT, reverse transcriptase. **b**–**d**, *In situ* hybridization of *Calhm1* in CVP taste buds of wild-type (**b**), *Calhm1*$^{-/-}$ (**c**) and *Skn-1a*$^{-/-}$ (**d**) mice. Scale bar, 50 μm. **e**–**g**, *Calhm1* is expressed in subsets of CVP (**e**), fungiform (**f**) and palate (**g**) taste bud cells. **h**, Double-label *in situ* hybridization directly illustrates cellular co-expression of *Calhm1* and *Trpm5* in CVP taste buds. Most cells expressing *Trpm5* also express *Calhm1*, with *Calhm1* expression absent from *Trpm5*-negative cells. **i**, CVP taste buds illustrating that *Tas1r3* is expressed in a subset of *Calhm1*-positive cells. Scale bars for **e**–**i**, 20 μm.

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We used two behavioural tests and nerve recordings to evaluate the effect of the loss of CALHM1 expression on taste perception. Two-bottle preference tests revealed that *Calhm1*$^{-/-}$ mice had a nearly complete loss both of preference for sweet and umami compounds and of avoidance of bitter compounds (Fig. 2a, Supplementary Fig. 4a, c, d and Supplementary Tables 1 and 2). In contrast, there were little or no differences between wild-type and *Calhm1*$^{-/-}$ mice in their responses to salty and sour compounds (Fig. 2a, Supplementary Fig. 4a and Supplementary Tables 1 and 2). Brief-access taste tests reproduced these phenotypes (Fig. 2b, Supplementary Fig. 4b and Supplementary Table 3), demonstrating that the taste phenotype in *Calhm1*$^{-/-}$ mice is a sensory defect. Recordings of the whole chorda tympani nerve revealed strongly reduced responses to sweet, bitter and umami compounds in *Calhm1*$^{-/-}$ mice, whereas responses to NaCl and the acid compounds were not different from wild type (Fig. 2c and Supplementary Fig. 5), indicating that the sensory defect in *Calhm1*$^{-/-}$ mice was due to the knockout of CALHM1 function in the peripheral taste cell system. Together, these results indicate that CALHM1 has a crucial role in taste-sensing type II cells[1,11] (see Supplementary Information for further discussion).

Type II cells signal to the nervous system by non-vesicular ATP release to nearby afferent gustatory nerves[5,12–14]. Although connexin hemichannels and pannexin 1 have been proposed, the molecular identity of the ATP-release mechanism remains uncertain[1,12,13,15]. The CALHM1 pore diameter is ~14 Å (ref. 3), similar to that estimated for connexins. We therefore examined the possibility that CALHM1

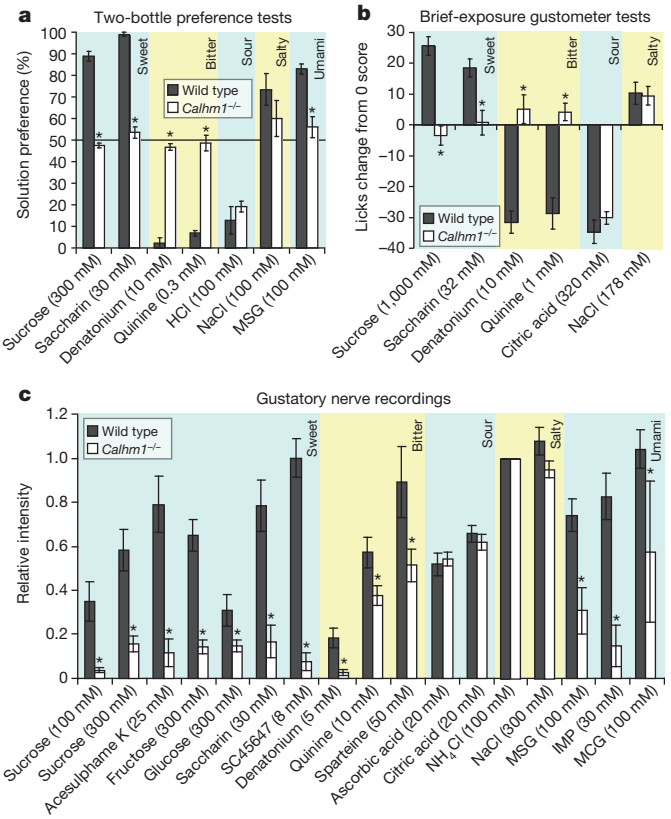

Figure 2 | CALHM1 is essential for sweet, bitter and umami taste perception. **a**, **b**, Mean preference percentage (taste compound versus water) from 48-h, two-bottle preference tests (**a**) and brief-access lick scores (**b**) for indicated compounds in *Calhm1*$^{-/-}$ mice and wild-type littermates. Error bars, s.e. (8–12 mice per group, 4–6 months old); *$P < 0.01$ (*post hoc* Bonferroni's test (**a**) and Student's *t*-test (**b**)). **c**, Summary of responses from whole-chorda-tympani nerve recordings stimulated with indicated compounds and normalized to response to NH_4Cl from wild-type ($n = 7$) and *Calhm1*$^{-/-}$ ($n = 8$) mice. MSG, monosodium glutamate; IMP, inosine 5′-monophosphate; MCG, monocalcium di-L-glutamate. Error bars, s.e.; *$P < 0.05$ (Student's *t*-test).

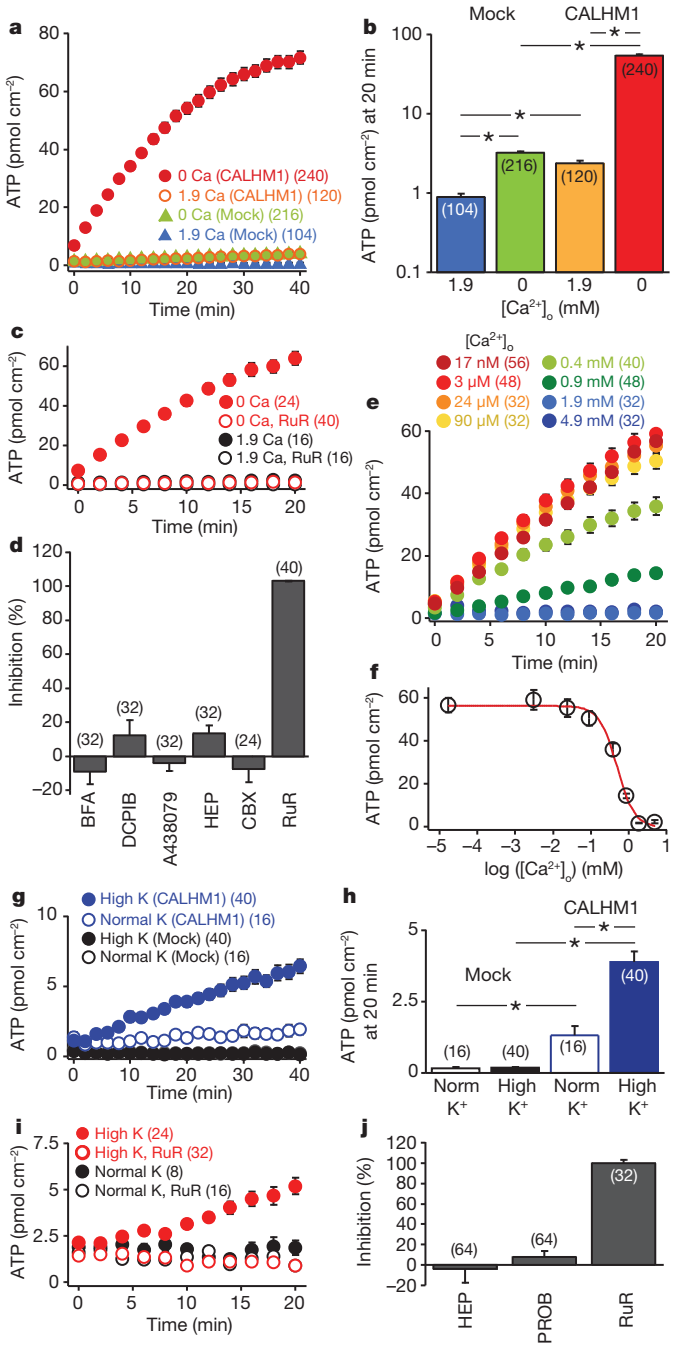

Figure 3 | CALHM1 mediates ATP release. **a**, Time courses of extracellular ATP levels due to release from mock- and hCALHM1-transfected HeLa cells exposed to normal (1.9 mM) or essentially zero (17 nM) $[Ca^{2+}]_o$. **b**, Summary of extracellular ATP levels at 20 min in **a**. **c**, Low-$[Ca^{2+}]_o$-induced ATP release in CALHM1-expressing cells is abolished by ruthenium red (RuR). **d**, Effects on low-$[Ca^{2+}]_o$-stimulated ATP release from hCALHM1 cells of 0.5 µg ml^{-1} brefeldin A (BFA), 10 µM DCPIB, 3 µM A438079, 1 mM 1-heptanol (HEP), 30 µM carbenoxolone (CBX) and 20 µM RuR. **e**, Time courses of ATP release from hCALHM1 cells induced by various $[Ca^{2+}]_o$. **f**, ATP levels at 20 min in **e** plotted against $[Ca^{2+}]_o$ and fitted to a Hill equation. **g**, Depolarization by high $[K^+]_o$ (117.5 mM K^+) induces ATP release specifically from hCALHM1 cells. **h**, ATP levels at 20 min in **g**. **i**, Depolarization-induced ATP release from CALHM1-expressing cells is abolished by RuR. **j**, Pharmacological sensitivities of depolarization-induced ATP release from hCALHM1 cells: 1 mM HEP, 1 mM probenecid (PROB), 20 µM RuR. Number of wells shown in parentheses throughout. Error bars, s.e. (where visible); *$P < 0.01$ (Student's *t*-test).

mediates ATP release. The CALHM1 ion channel can be activated by membrane depolarization or reduction of extracellular Ca^{2+} concentration[2] ($[Ca^{2+}]_o$). We first exploited the latter mechanism to activate CALHM1 in heterologous expression systems to determine whether CALHM1 can form an ATP-release channel. Decreasing $[Ca^{2+}]_o$ from 1.9 mM to essentially zero (17 nM) activated ATP release from human CALHM1 (hCALHM1)-expressing HeLa cells, whereas little ATP efflux was induced in mock-transfected cells (Fig. 3a, b). Similar low-$[Ca^{2+}]_o$-induced ATP release was observed in hCALHM1-expressing COS-1 cells and *Xenopus* oocytes (Supplementary Fig. 6). Neither CALHM1 expression nor low-$[Ca^{2+}]_o$ exposure caused cell damage (Supplementary Fig. 7). Involvement of other possible mechanisms[16] was ruled out because ATP release was unaffected by brefeldin A (vesicular release), DCPIB (volume-sensitive Cl^- channels), A438079 (P2X7 receptors), heptanol (connexin hemichannels) or carbenoxolone (pannexins and connexins at 30 μM) (Fig. 3d). In contrast, ruthenium red (RuR), which inhibits CALHM1 ion currents[2], abolished low-$[Ca^{2+}]_o$-evoked ATP release from hCALHM1-expressing cells (Fig. 3c, d). Thus, CALHM1 expression induces a novel ATP permeability.

CALHM1 ion channel gating is regulated by $[Ca^{2+}]_o$ with an apparent half-maximum inhibitory $[Ca^{2+}]$ of $IC_{50} \approx 220$ μM and a Hill coefficient of 2.1 (ref. 2). Extracellular Ca^{2+} inhibited ATP release with $IC_{50} = 495$ μM and a Hill coefficient of 1.9 (Fig. 3e, f). CALHM1 ion currents are also activated by membrane depolarization[2]. At normal levels of $[Ca^{2+}]_o$, hCALHM1-expressing, but not mock-transfected, cells released ATP in response to high-$[K^+]_o$-induced depolarization (Fig. 3g, h). Depolarization-induced ATP release was inhibited by RuR but not by heptanol or probenecid (Fig. 3i, j), which are respectively connexin and pannexin 1 blockers[17]. Expression of mouse CALHM1 conferred ATP release with similar properties (Supplementary Fig. 8). Regulation of ATP release in hCALHM1-expressing cells is therefore similar to that of CALHM1 channel gating, indicating that the CALHM1 channel is a conduit for ATP release.

The three types of taste bud cell can be classified on the basis of whole-cell electrophysiological fingerprints[13,18]. We verified these fingerprints by recording whole-cell currents in single isolated taste bud cells from TRPM5–green fluorescent protein (GFP) mice with GFP expressed specifically in type II cells (Supplementary Fig. 9). With tetraethylammonium in the extracellular solution and Cs^+ as the major cation in the pipette solution[2] (to block K^+ currents), the electrophysiological subtypes were identified by the combination of voltage-gated Na^+ currents (I_{Na}) and non-selective, slowly activating outward currents (I_{slow}) with inward tail currents at −70 mV (I_{tail}) (Fig. 4a–c and Supplementary Fig. 9). To identify CALHM1 ion currents, we recorded whole-cell currents in isolated taste bud cells from wild-type and *Calhm1*$^{-/-}$ mice (Fig 4a–c). Loss of CALHM1 substantially reduced the amplitudes of I_{slow} and I_{tail} without affecting the amplitude of I_{Na} in type II cells (Fig. 4d–f), whereas no differences were observed in type I and type III cells (Fig. 4b, c, g). The slowly activating outward current in type II cells was inhibited by the non-specific CALHM1 blocker Gd^{3+} but not by probenecid or heptanol, ruling out contributions of pannexin 1 or connexins (Fig. 4h). These data demonstrate that CALHM1 channel conductance is present in type II cells and contributes the major component of the slowly activating outward current. Notably, the amount of depolarization-induced

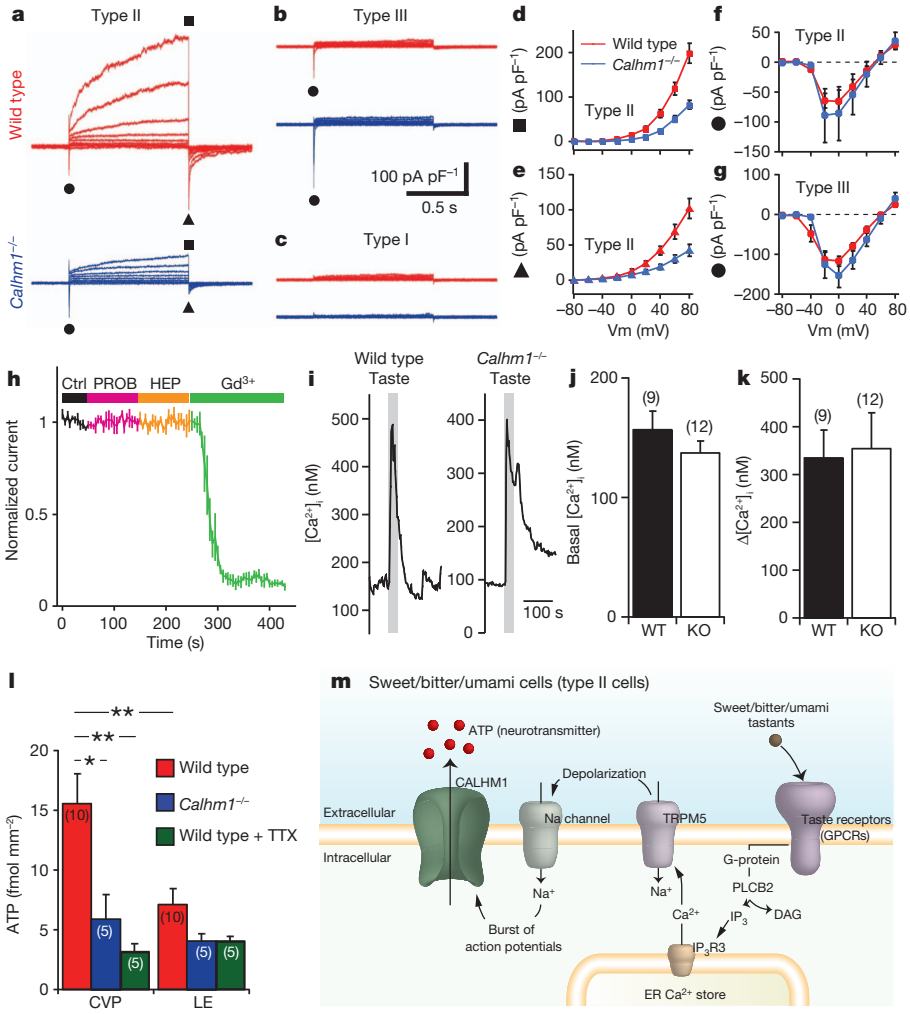

Figure 4 | CALHM1 is required for taste-evoked ATP release from taste cells.
a–c, Electrophysiological phenotypes of type I, type II and type III cells identified in wild-type (red) and *Calhm1*$^{-/-}$ (blue) taste cells. Cells held at −70 mV and pulsed from −80 to +80 mV in 20-mV increments with 1-s duration. Type I current in **c** recorded from 16 wild-type and 9 *Calhm1*$^{-/-}$ cells. **d–g**, I_{Na} (circle; **f**), I_{slow} at end of pulses (square; **d**) and I_{tail} (triangle; **e**) measured for type II cells ($n = 9$ wild type, $n = 10$ *Calhm1*$^{-/-}$), and I_{Na} measured for type III cells ($n = 9$ wild type, $n = 6$ *Calhm1*$^{-/-}$) (**g**). Vm, membrane voltage. **h**, Sensitivities of I_{slow} in GFP-positive cells from TRPM5–GFP mice to Gd^{3+} (100 μM), probenecid (1 mM), 1-heptanol (1 mM) ($n = 4$). **i**, $[Ca^{2+}]_i$ in type II cells from wild-type (left, 9 cells) and *Calhm1*$^{-/-}$ (right, 12 cells) mice. Type II cells identified by robust $[Ca^{2+}]_i$ response to a mix of sweet and bitter substances (grey bar). **j**, **k**, Basal (**j**) and taste-evoked (**k**) responses are comparable in wild-type and *Calhm1*$^{-/-}$ cells. KO, knockout; WT, wild type. **l**, Taste-evoked ATP release from gustatory CVP tissue and non-gustatory lingual epithelium. Bitter mix elicits considerable ATP release from CVP versus lingual epithelium in wild-type mice; this is abolished in *Calhm1*$^{-/-}$ mice and by 1 μM tetrodotoxin (TTX). Error bars, s.e.; $*P < 0.05$, $**P < 0.01$ (Student's *t*-test). **m**, Schematic illustration of signal-transduction cascade in type II taste receptor cells with CALHM1 as the ATP-release pathway.

ATP release from type II cells is correlated with the magnitude of I_{slow} (ref. 13).

Type II cells detect sweet, bitter and umami compounds via G-protein-coupled taste receptors[4] that stimulate a common signal-transduction cascade involving activation of PLCB2, inositol 1,4,5-trisphosphate-mediated Ca^{2+} release and Ca^{2+}-dependent activation of TRPM5 channels[11] that depolarizes the plasma membrane to generate action potentials and subsequent non-vesicular release of ATP. Importantly, no differences in basal $[Ca^{2+}]_i$ and taste-evoked $[Ca^{2+}]_i$ responses were observed between wild-type and *Calhm1*$^{-/-}$ type II cells (Fig. 4i–k). Furthermore, *Calhm1* deficiency had no effect on I_{Na} (Fig. 4f), TRPM5 expression in type II cells (Fig. 1a and Supplementary Figs 1i and 2) or the ATP content of taste buds (Supplementary Fig. 10). Strikingly, however, taste-evoked release of ATP from circumvallate papillae was abolished in *Calhm1*$^{-/-}$ mice (Fig. 4l and Supplementary Fig. 11). This strongly suggests that CALHM1 contributes to the major ATP-release mechanism in sweet/bitter/umami-sensing taste bud cells (Fig. 4l, m).

Our study sheds light on a novel cellular ATP-releasing mechanism by demonstrating that CALHM1 is a voltage-gated ion channel that mediates tetrodotoxin-sensitive ATP release in taste buds (Fig. 4l) as an essential mechanism of sweet, bitter and umami taste perception. As such, CALHM1 provides a missing link in the signal-transduction cascade in type II cells, connecting taste receptor activation and the generation of Na^+ action potentials to the activation of afferent gustatory neural pathways[1] (Fig. 4m). It has not escaped our attention that other CALHM isoforms[6] as well as pannexin 1 and connexins are also present in taste buds[9,12,13] and might also be involved in ATP release in taste, perhaps acting in parallel with or in a complex with CALHM1. Signalling by extracellular ATP is a widespread phenomenon that regulates many physiological activities[19], including neurotransmission[20,21], intercellular communication[22,23], vascular tone[24] and sensory transduction[5,25–27]. CALHM1 and its isoforms may participate in physiologically important ATP release elsewhere.

METHODS SUMMARY

All animal experiments were conducted according to protocols approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania, the Feinstein Institute for Medical Research, the Monell Chemical Senses Center and the University of Minnesota Duluth.

Full Methods and any associated references are available in the online version of the paper.

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1. Chaudhari, N. & Roper, S. D. The cell biology of taste. *J. Cell Biol.* **190,** 285–296 (2010).
2. Ma, Z. *et al.* Calcium homeostasis modulator 1 (CALHM1) is the pore-forming subunit of an ion channel that mediates extracellular Ca^{2+} regulation of neuronal excitability. *Proc. Natl Acad. Sci. USA* **109,** E1963–E1971 (2012).
3. Siebert, A. P. *et al.* Structural and functional similarities of calcium homeostasis modulator 1 (CALHM1) ion channel with connexins, pannexins and innexins. *J. Biol. Chem.* **288,** 6140–6153 (2013).
4. Chandrashekar, J., Hoon, M. A., Ryba, N. J. & Zuker, C. S. The receptors and cells for mammalian taste. *Nature* **444,** 288–294 (2006).
5. Finger, T. E. *et al.* ATP signaling is crucial for communication from taste buds to gustatory nerves. *Science* **310,** 1495–1499 (2005).
6. Dreses-Werringloer, U. *et al.* A polymorphism in CALHM1 influences Ca^{2+} homeostasis, Aβ levels, and Alzheimer's disease risk. *Cell* **133,** 1149–1161 (2008).
7. Koppel, J. *et al.* CALHM1 P86L polymorphism modulates CSF Aβ levels in cognitively healthy individuals at risk for Alzheimer's disease. *Mol. Med.* **17,** 974–979 (2011).
8. Lambert, J. C. *et al.* The CALHM1 P86L polymorphism is a genetic modifier of age at onset in Alzheimer's disease: a meta-analysis study. *J. Alzheimers Dis.* **22,** 247–255 (2010).
9. Moyer, B. D. *et al.* Expression of genes encoding multi-transmembrane proteins in specific primate taste cell populations. *PLoS ONE* **4,** e7682 (2009).
10. Matsumoto, I., Ohmoto, M., Narukawa, M., Yoshihara, Y. & Abe, K. Skn-1a (Pou2f3) specifies taste receptor cell lineage. *Nature Neurosci.* **14,** 685–687 (2011).
11. Zhang, Y. *et al.* Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell* **112,** 293–301 (2003).
12. Huang, Y. J. *et al.* The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds. *Proc. Natl Acad. Sci. USA* **104,** 6436–6441 (2007).
13. Romanov, R. A. *et al.* Afferent neurotransmission mediated by hemichannels in mammalian taste cells. *EMBO J.* **26,** 657–667 (2007).
14. Vandenbeuch, A., Zorec, R. & Kinnamon, S. C. Capacitance measurements of regulated exocytosis in mouse taste cells. *J. Neurosci.* **30,** 14695–14701 (2010).
15. Romanov, R. A. *et al.* Dispensable ATP permeability of pannexin 1 channels in a heterologous system and in mammalian taste cells. *J. Cell Sci.* **125,** 5514–5523 (2012).
16. Sabirov, R. Z. & Okada, Y. ATP release via anion channels. *Purinergic Signal.* **1,** 311–328 (2005).
17. Silverman, W., Locovei, S. & Dahl, G. Probenecid, a gout remedy, inhibits pannexin 1 channels. *Am. J. Physiol. Cell Physiol.* **295,** C761–C767 (2008).
18. Romanov, R. A. & Kolesnikov, S. S. Electrophysiologically identified subpopulations of taste bud cells. *Neurosci. Lett.* **395,** 249–254 (2006).
19. Burnstock, G. & Kennedy, C. P2X receptors in health and disease. *Adv. Pharmacol.* **61,** 333–372 (2011).
20. Edwards, F. A., Gibb, A. J. & Colquhoun, D. ATP receptor-mediated synaptic currents in the central nervous system. *Nature* **359,** 144–147 (1992).
21. Evans, R. J., Derkach, V. & Surprenant, A. ATP mediates fast synaptic transmission in mammalian neurons. *Nature* **357,** 503–505 (1992).
22. Cotrina, M. L., Lin, J. H., Lopez-Garcia, J. C., Naus, C. C. & Nedergaard, M. ATP-mediated glia signaling. *J. Neurosci.* **20,** 2835–2844 (2000).
23. Cotrina, M. L., Lin, J. H. & Nedergaard, M. Cytoskeletal assembly and ATP release regulate astrocytic calcium signaling. *J. Neurosci.* **18,** 8794–8804 (1998).
24. Burnstock, G. Dual control of local blood flow by purines. *Ann. NY Acad. Sci.* **603,** 31–44 (1990).
25. Chizh, B. A. & Illes, P. P2X receptors and nociception. *Pharmacol. Rev.* **53,** 553–568 (2001).
26. Cockayne, D. A. *et al.* Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. *Nature* **407,** 1011–1015 (2000).
27. Wynn, G., Rong, W., Xiang, Z. & Burnstock, G. Purinergic mechanisms contribute to mechanosensory transduction in the rat colorectum. *Gastroenterology* **125,** 1398–1409 (2003).

Supplementary Information is available in the online version of the paper.

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Author Contributions A.T., V.V., A.L., Z.M., M.O., I.M., H.Z., L.A., S.L., M.A., G.H., G.D. and N.C. designed and performed experiments. P.M. and V.V. generated the *Calhm1* knockout mice. J.K. and P.D. designed experiments. M.G.T., M.M.C., P.M. and J.K.F. designed experiments and helped with data interpretation. A.T., J.K.F. and P.M. wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to P.M. (pmaramba@nshs.edu) and J.K.F. (foskett@mail.med.upenn.edu).

METHODS

***Calhm1*$^{-/-}$ mice.** *Calhm1*$^{+/-}$ founder mice were generated at genOway (Lyon, France) as described previously[2]. Briefly, *Calhm1* exon 1 deletion was performed by homologous recombination in 129Sv embryonic stem cells. Positive clones were screened by PCR and confirmed by Southern blot analysis. The resultant embryonic stem cells were injected into blastocysts derived from C57BL/6J mice to obtain chimaeric mice that possessed germline transmission of the targeted *Calhm1* locus. Mice on the mixed 129Sv × C57BL/6J genetic background were used in this study. Wild-type (+/+) and *Calhm1* knockout (−/−) mice of F_1 obtained from littermate mice were used for two-bottle preference tests. All other experiments were performed with wild-type and knockout littermates. In some mice, the neomycin cassette was removed and the resulting strain was backcrossed with C57BL/6J mice. Briefly, *Calhm1*$^{+/-}$ mice were mated with mice bearing an *EIIa–cre* transgene (B6.FVB-Tg (EIIa-cre)C_{5379}Lmgd/J, The Jackson Laboratory) on the C57BL/6J background to remove the neomycin resistance cassette by *cre*–loxP-mediated excision. The resulting *Calhm1*$^{+/-}$ pups were further backcrossed to C57BL/6J (The Jackson Laboratory) for at least five generations before being made homozygous. The removal of the neomycin cassette was confirmed by PCR. Backcrossed mice were used for two-bottle preference tests. Loss of CALHM1 expression in *Calhm1*$^{-/-}$ taste buds was verified by reverse-transcription (RT)–PCR and *in situ* hybridization.

Laser capture microdissection, RNA amplification and RT–PCR. Circumvallate taste tissue from *Calhm1*$^{+/+}$ and *Calhm1*$^{-/-}$ mice was embedded in cryomolds using OCT freezing medium. Twelve micrometre-thick tissue sections were cut on a Leica CM1850 cryostat (Leica Microsystems), collected on RNase-free membrane slides (Leica) and stained with cresyl violet. Taste buds from the CVP and surrounding lingual epithelium were isolated using a Leica laser microdissection system LMD7000 (Leica). Taste buds and surrounding epithelium were pooled from a total of four mice of each genotype. Total RNA from taste bud and lingual epithelium samples was purified using a RNAqueous-Micro Kit (Ambion). Total RNA was amplified using two sequential rounds with MessageAmp II aRNA kit (Ambion), as per the manufacturer's instructions. One microgram of RNA was transcribed into complementary DNA (cDNA) using Invitrogen's SuperScript III First-Strand Synthesis System for RT–PCR with random hexamers, according to the supplied protocol. A 50-µl PCR reaction was run with the following final concentrations: 450 ng of each primer (see Supplementary Table 4 for PCR primer sequences), 2 mM $MgCl_2$, 0.3 mM dNTPs, 2.5 U Taq polymerase (Promega GoFlex DNA polymerase) and 1 µl of 10 ng µl^{-1} DNA. PCR cycling conditions used for *Calhm1*: 95 °C for 3 min; 35 cycles of 95 °C for 30 s, 65 °C for 30 s and 72 °C for 45 s; 72 °C for 7 min; 4 °C (hold).

***In situ* hybridization.** Oral epithelia containing taste buds were dissected from adult male mice deeply anaesthetized with an overdose of sodium pentobarbital (Abbott Laboratories) and embedded in the frozen OCT compound (Sakura Finetech USA). Fresh-frozen sections of 8-µm thickness were prepared using a cryostat (CM3050S, Leica Microsystems). The *in situ* hybridization procedure was described previously[28]. In brief, digoxigenin- and fluorescein-labelled antisense RNAs prepared using RNA labelling mix (Roche Diagnostics) and an RNA polymerase (Stratagene) were used for hybridization after fragmentation under alkaline conditions to a length of about 150 bases. Fresh-frozen sections were fixed with 4% PFA, treated with diethylpyrocarbonate, prehybridized with salmon sperm DNA for 2 h at 65 °C and hybridized with antisense riboprobes for 40 h at 65 °C. For single labelling of *Tas2r108* and *Trpm5*, however, prehybridization and hybridization were carried out at 58 °C. After hybridization, the sections were washed in ×0.2 SSC at 58 °C or 65 °C and blocked with 0.5% blocking reagent (Roche Diagnostics) in Tris-buffered saline. Chromogenic signals, except those from *Calhm1*, were developed for one day using alkaline phosphatase-conjugated anti-digoxigenin antibody (1:500, Roche Diagnostics) and 4-nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate as chromogenic substrates. Chromogenic *Calhm1* signals were developed for two days to clarify the cells expressing *Calhm1* messenger RNA. No *Calhm1* signal was observed in *Skn-1* or *Calhm1* knockout taste tissues after signal development for three days. Stained images were obtained with a Nikon Eclipse 80i microscope (Nikon Instruments Inc.) equipped with a DXM1200C digital camera (Nikon). Fluorescent signals were developed using biotin-conjugated anti-fluorescein antibody (1:500, Vector Laboratories), avidin–biotin complex (Vector Laboratories), tyramide signal amplification biotin system (1:50, PerkinElmer) and Alexa 488-conjugated streptavidin (4 µg ml^{-1}, Invitrogen). Fluorescent images were obtained with a Leica SP2 confocal scanning microscope (Leica). For double labelling of *Calhm1* with *Trpm5* and *Tas1r3*, fluorescent signals were first developed and obtained, then chromogenic signals were developed and obtained as described above, and finally the images were superimposed using Photoshop CS3 (Adobe). RNA probes generated were to nucleotides 1–894 of *Tas2r108* (GenBank accession number, AF227148), nucleotides 310–3,491 of *Trpm5* (accession number, AF228681), nucleotides 525–2,725 of *Tas1r3* (accession number, AF337039), and nucleotides 1–1,407 and 2,148–2,369 of *Calhm1* cDNA fragment, which contains 1,047 base pairs of the entire coding sequence and 1,322 base pairs of 3′-non-coding region.

Immunofluorescence staining. For fixed-tissue preparation, mice 12 weeks old or older were anaesthetized with an overdose of sodium pentobarbital (Abbott Laboratories) and transcardially perfused with ice-cold PBS followed by 4% paraformaldehyde in ice-cold PBS. Tongue epithelia containing CVP were dissected and postfixed with the same fixative at 4 °C overnight. Tissue samples were then cryoprotected in 30% sucrose in PBS, embedded in frozen OCT compound (Sakura Finetech) and sectioned using a cryostat (CM1900, Leica Microsystems) at 8 µm. Tissue sections were mounted on tissue-adhesive-coated glass slides (Fisher Scientific) and stored at −80 °C until analysed. For immunofluorescence staining, slides were rinsed with PBS, incubated in a preheated target retrieval solution (S1700, Dako) at 80 °C for 20 min, allowed to cool to room temperature (20–25 °C) for 20 min, still in the target retrieval solution, and washed in PBS for 3 × 10 min. After blocking by 1-h incubation in PBS containing 5% normal donkey serum (PBS-5% NDS) at room temperature, sections were incubated overnight at 4 °C with primary antibodies diluted in PBS-5% NDS: 1:3,000 for rabbit anti-TRPM5 (Alomone Labs); 1:500 for rabbit anti-PLCB2 (Santa Cruz Biotechnology); 1:500 for rabbit anti-AADC (Gene Tex); 1:500 for goat anti-KCNQ1 (Santa Cruz). The next day, the slides were washed for 3 × 10 min in PBS at room temperature and incubated for 1 h with Alexa-Fluor-conjugated antibodies diluted in PBS-5% NDS: 1:500 for Alexa Fluor 488 donkey anti-rabbit IgG; 1:500 for Alexa Fluor 568 anti-goat IgG (Invitrogen). Finally, slides were washed for 3 × 10 min in PBS at room temperature and mounted in VectaShield with 4′,6-diamidino-2-phenylindole (H-1500, Vector Laboratories). Stained sections were imaged with a confocal scanning microscope (LSM 710, Carl Zeiss).

Single-taste cell analyses. Circumvallate taste epithelium was enzymatically delaminated, taste buds were collected from peeled epithelium and dissociated single-taste cells were collected, all as detailed previously[29]. For patch clamp and fura-2 Ca^{2+} imaging experiments, isolated cells were placed on poly-L-lysine-coated coverslips and allowed to settle for 30–60 min.

Total RNAs isolated from taste buds and from adjacent non-taste epithelium were used as positive and negative controls, respectively. Individual dissociated taste cells from PLCB2–GFP (type II) and GAD1–GFP (type III) transgenic mice were selected for collection on the basis of their expression of GFP, as previously described[30]. Type I taste cells were selected from PLCB2–GFP × GAD1–GFP double-transgenic mice by the absence of GFP expression[30]. Total RNA isolated from taste cells was either subjected to T7 RNA polymerase-based linear amplification (aRNA, Message BOOSTER kit, Epicentre) or was directly converted to cDNA, both as previously described[29]. For aRNA-based analyses, 1% of the resulting cDNA was used as template for PCR. For direct RT–PCR, 15% of cDNA from each cell was used to test for diagnostic messenger RNAs and 30% was used for *Calhm1*. PCR conditions with HotStarTaq Plus (QIAGEN) were 95 °C for 5 min followed by 45 90-s cycles, each of which comprised three 30-s components, one at 94 °C, one at the annealing temperature and one at 72 °C. PCR primers, annealing temperatures and product sizes were as in Supplementary Table 4. Two different primer sets were used for *Calhm1* (Supplementary Table 4). Primers 1 and 2 were used for RT–PCR on bulk tissue RNA, as well as for aRNA from individual taste cells (Fig. 1a and Supplementary Fig. 3a, b, d). Primers 3 and 4 span an intron, and were used for the single-cell direct RT–PCR to avoid amplifying from genomic DNA (Supplementary Fig. 3c). The cell type of all single cells and pools of cells was confirmed by RT–PCR for three diagnostic cell-type markers: *Entpd2* (type I), *Plcb2* (type II) and *Snap25* (type III).

Two-bottle preference tests. *Calhm1*$^{-/-}$ and wild-type control mice were presented for 48 h with two drinking bottles, one containing water and the other a solution supplemented with ascending concentrations of the specific taste compounds to be tested. All solutions were prepared with tap water and served at room temperature. Mice had *ad libitum* access to a standard chow diet (Laboratory Rodent Diet 5001, LabDiet, PMI Nutrition International) and drinking solutions. Two independent groups of mice were tested for the following series of taste solutions. The order of testing was as follows: sucrose, saccharin, NaCl, denatonium (group 1); MSG, quinine, HCl (group 2). Sucrose, saccharin, denatonium benzoate, quinine sulphate and MSG were from Sigma-Aldrich. HCl and NaCl were from Thermo Fisher Scientific Inc. The order of stimulus presentation was identical for wild-type and knockout mice. The mice had three days with a single bottle of drinking water between each test series. The two bottles were switched after 24 h to control for a possible effect of the bottle positions. The change in liquid levels was considered to be the mouse's liquid intake[31]. Fluid intakes were measured volumetrically to the nearest 0.1 ml. The volumes of water and taste compound solutions consumed were recorded. Solution preference was calculated as the intake of taste solution divided by total liquid intake, and this ratio was expressed as a percentage. The taste compounds were chosen as exemplars of the

sweet, bitter, umami, salty and sour taste qualities, and their concentrations were chosen to span the range between indifference and marked acceptance (for sucrose, saccharin and MSG) or avoidance (for quinine sulphate, denatonium benzoate, NaCl and HCl). Results were analysed by mixed-design analyses of variance with factors of group (wild type or *Calhm1*$^{-/-}$) and concentration. Differences between the groups in consumption of specific concentrations of taste solution were determined using Bonferroni *post hoc* tests. Differences in response of each group to individual concentrations of test compounds relative to water were determined using Student's *t*-test. Results presented in Fig. 2a are representative examples taken from the more comprehensive concentration functions displayed in Supplementary Fig. 4a and Supplementary Tables 1 and 2.

Brief-exposure gustometer tests. Acceptance of taste solution was assessed with brief-exposure tests using procedures similar to those used by other groups[11,32–35].

Twelve *Calhm1*$^{-/-}$ mice (5 male, 7 female) and 12 wild-type littermate controls (5 male, 7 female) were maintained in a vivarium at 23 °C on a 12 h:12 h light/dark cycle with lights off at 19:00. When not being tested, each mouse was housed alone in a plastic 'tub' cage (26.5 cm × 17 cm × 12 cm) with a stainless-steel grid lid and wood shavings scattered on the floor. The mouse ate pelleted AIN-76A diet and drank deionized water according to the regimen described below. Throughout the experiment, each mouse was weighed daily, immediately before it was placed into a gustometer.

Brief-exposure taste acceptance was assessed using three MS160-Mouse gustometers manufactured by DiLog Instruments. Each gustometer consists of a 14.5 cm × 30 cm × 15 cm test chamber with a motorized shutter that controls access to a taste solution. Bottles of taste solution are mounted on a rack that is precisely positioned by a stepper motor so that any one of eight different taste solutions can be presented to the mouse. The drinking spout of each bottle is part of a high-frequency alternating current contact circuit so that each lick the mouse makes is detected and recorded. Details of construction and other technical information is available elsewhere[34,36]. Taste compounds were reagent grade and were purchased from Sigma-Aldrich and dissolved in room-temperature deionized water. Deionized water was used as the water (0 concentration) and for 'washout' trials (see below). To avoid any undue influence of subtle differences among the three gustometers we used, we ensured that each mouse was always tested with the same gustometer and that equal numbers of wild-type and *Calhm1*$^{-/-}$ mice were tested with each gustometer.

To train the mice to sample taste solutions, they were first deprived of water for 22.5 h and then placed in a gustometer with its shutter open. During this first training session, each mouse had continuous access to water for 25 min from the time it first licked the drinking spout. It was then returned to its home cage and given water for 1 h. During the following two days, this procedure was repeated, except the shutter allowing access to water was closed 5 s after each time the mouse began to lick, and was re-opened after a 7.5-s interval. Once again, after 25 min, the mouse was returned to its home cage and given water for 1 h. By the second test using these procedures, all except one wild-type and one *Calhm1*$^{-/-}$ mouse had learned to obtain water during the 5-s access periods. We continued to test the two non-responders and they began to drink during the third or fifth test session (see below).

The mice received two test sessions with each of the 11 compounds presented in Supplementary Fig. 4b. These were presented in two cycles, with exemplars of the four basic tastes in the first cycle (sucrose, quinine hydrochloride, HCl and NaCl) and the other compounds in the second cycle. Only one taste compound was used during a session (see below) and there was only one session per day. The deprivation regimen used to investigate the response to sucrose, saccharin and Polycose differed from that used to investigate the response to the other taste compounds because these are hedonically positive and the other three compounds are hedonically negative[34]. Prior to a session with a hedonically positive compound, each mouse received free access to food and water for 24 h. It then received 1 g of food and 2 ml of water, and the session began 24 h later. After these sessions, the mouse had a recovery day with free access to food and water for 24 h. Its water was then removed for 22.5 h to prepare it for the next session. After sessions with hedonically negative compounds, each mouse received water for 1 h in its home cage and it was then deprived of water in preparation for the next session. All sessions lasted 25 min. When hedonically positive compounds were being investigated, the session began with a single test of the highest concentration available to kindle the mouse's interest in the drinking spout. After this, repeated series of five concentrations (including water) were presented, in a quasi-random order (a concentration could appear only once in a series of five tests). For each exposure, the shutter was open for 5 s, during which licks of the drinking spout were counted. This was followed by 7.5 s with the shutter closed, during which a new taste solution was positioned, ready for the next presentation. For sessions with hedonically negative compounds, repeated series of five or six concentrations (including water) were presented in randomized order. Additional 1-s washout trials with water were interposed between test trials. Thus, a mouse received access to a taste solution for 5 s followed by 7.5 s with the shutter closed, then access to water for 1 s followed by 7.5 s with the shutter closed, followed by the next taste solution for 5 s, and so on. We think the brief washout trials with water have the effect of cleansing the mouse's palate and thus prevent it from quitting because it expects only bad-tasting solutions.

Separate analyses were conducted for each taste compound. The mean number of licks by each mouse in response to each concentration was obtained by averaging the results of identical exposures. These values for individual mice were then used in mixed-design analyses of variance with factors of group (wild type or *Calhm1*$^{-/-}$) and concentration. Mice that did not respond during any presentation of a particular concentration of a taste compound were not included in statistical analyses of that compound. *Post hoc* least-significant difference tests were used to assess between-group differences in consumption of specific concentrations of taste solution and differences in the response of each group to individual concentrations of each taste compound (STATISTICA 10, Stat Soft Inc.). All analyses were conducted using a criterion for significance of $P < 0.05$. Results presented in Fig. 2b are representative examples taken from the more comprehensive concentration functions displayed in Supplementary Fig. 4b and Supplementary Table 3.

Whole-chorda-tympani nerve recordings. The mice were anaesthetized with an intramuscular injection of a mixture of 1.75 mg ml^{-1} ketamine and 1.75 mg ml^{-1} xylazine in saline (5 µl g^{-1} body weight). Anaesthesia was maintained with 0.4–0.6% isoflurane. Body temperature, surgical table temperature, blood oxygen, anaesthesia level and heart rate were continuously monitored. The chorda tympani nerve was dissected free from its junction with the lingual nerve to the tympanic bulla, where the central part of the chorda tympani was cut and the peripheral part then mobilized in the rostral direction so that afferent nerve impulses could be recorded. The nerve impulses were recorded between a silver wire electrode and an indifferent electrode touching the walls of the wound, fed into a custom-made amplifier and monitored over a loudspeaker and an oscilloscope. The nerve impulses were processed by a smoothed absolute value circuit integrator[37] and rectified to a d.c. potential whose amplitude was related to the nerve impulse frequency, here called the summated signal. This signal and a code related to the kind of taste compound used were fed into an oscilloscope and computer. Both the individual nerve impulses and the summated signal were recorded (Gould ES 1000). The stimuli were delivered to the tongue with an open flow system controlled by the computer under conditions of constant flow and temperature[37] (33 °C). Each stimulation lasted for 5 s with 50-s rinsing time between stimulations. Care was exercised to make sure that the flow was directed over the fungiform papillae. In each animal, we strived to run the same sequence of stimuli at least three times while we recorded the nerve impulses from the whole chorda tympani nerve. The integrated response during stimulation was calculated by subtracting the area of nerve activity preceding stimulation from that during stimulation. Thus, the data reflect the level of activity during stimulation time. The responses to all compounds were expressed relative to the response to 0.1 M NH_4Cl in each mouse. The average in each animal and group was calculated, the variance determined and the significance between the chorda tympani responses of *Calhm1*$^{-/-}$ and wild-type mice determined. $P < 0.05$ was considered significant.

Cell culture. HeLa cells (American Type Culture Collection) were grown in plastic flasks at 37 °C in a humidified incubator in culture medium containing 90% (v/v) DMEM, 10% fetal bovine serum and ×1 Antibiotic-Antimycotic (Invitrogen) with 5% CO_2-in-air.

ATP measurements using cultured cells. Extracellular ATP concentration was measured by the luciferin–luciferase reaction as previously described[38]. HeLa cells were seeded onto 96-well microplates (Corning Costar) at a density of 10,000 cells per well one day before transfection. Cells in each well were transfected with 0.2 µg of hCALHM1 cDNA, mouse CALHM1 cDNA or empty vector (pIRES2.EGFP) using 0.4 µl Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. After 20–30 h, media were removed and cells were incubated for 1 h before 75 µl of the 100-µl bath solution containing 2 mM Ca^{2+} was replaced with an equal volume of test solution to establish final $[Ca^{2+}]_o$ and drug concentrations. The plate was immediately placed in a microplate luminometer (Synergy 2, BioTEK) and 10 µl of ATP assay solution (FL-AAM and FL-AAB, Sigma-Aldrich) was dispensed into each well. ATP release was measured every 2 min. ATP concentration was calculated from a standard curve created in each plate. Separate standard curves were made in experiments involving all added drugs at the concentrations used. The bath solution contained (in mM) 150 NaCl, 5 KCl, 10 HEPES and 10 glucose, pH 7.4, adjusted with NaOH. $[Ca^{2+}]_o$ values below 90 µM were achieved by mixing $CaCl_2$ and EGTA, and free $[Ca^{2+}]_o$ was calculated using WEBMAXC software (http://maxchelator.stanford.edu). When a drug was tested, cells were pre-incubated for 1 h with the drug before being exposed to low-

$[Ca^{2+}]_o$ solution containing the same drug. The high-$[K^+]_o$ condition (117.5 mM) was established by replacing Na^+ with equimolar K^+.

ATP measurements using intact taste buds. Taste-evoked ATP release from intact taste buds was recorded with sheets of lingual epithelium as described previously[5]. The tongue epithelium was cut into pieces containing the CVP or lingual epithelium devoid of taste buds and mounted in a custom Ussing-type chamber that separated the fluid-containing serosal chamber from the apical surface. The apical surface was selectively exposed to a bitter mix (40 mM denatonium benzoate, 1 mM cycloheximide, 10 mM HEPES, pH 7.4) for 45 s. The serosal fluid (130 µl) was then collected and added to an equal volume of ATP assay solution (FL-AAM and FL-AAB, Sigma-Aldrich) to determine its ATP content by the luciferin–luciferase assay. The solution on the serosal side contained (in mM) 150 NaCl, 5 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$ 10 HEPES and 10 glucose, pH 7.4, adjusted with NaOH.

Single-cell Ca^{2+} imaging. Isolated taste cells on coverslips were secured in a perfusion chamber and mounted on the stage of an inverted microscope (Nikon Eclipse TE2000). Cells were loaded with fura-2AM (Molecular Probes; 2.5 µM) for 45 min at room temperature in the bath solution containing (in mM) 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 10 glucose, pH 7.4, adjusted with NaOH. Cells were continuously perfused with the bath solution and stimulated for 30 s by bath perfusion of taste mix (2 mM saccharin, 100 µM SC45647, 1 mM denatonium, 10 µM cycloheximide; dissolved in the bath solution at pH 7.4). Fura-2 was alternately illuminated at 340 or 380 nm, and the emitted fluorescence intensity at 510 nm was collected with a PerkinElmer Ultraview imaging system. The background fluorescence was estimated for a region without cells and subtracted during analysis. Changes in $[Ca^{2+}]_i$ are presented as changes in fluorescence ratio (F340/F380). Dye calibration was achieved by applying experimentally determined constants to the equation $[Ca^{2+}] = K_d\beta(R - R_{min})/(R_{max} - R)$. Macros used for analysis were custom macros written for IGOR PRO (WaveMetrics). Isolated taste bud cells that responded to a taste stimulus by robust $[Ca^{2+}]_i$ increase were identified as type II cells: 24% of wild-type cells (9 out of 38 cells from three animals) and 39% of *Calhm1*$^{-/-}$ cells (12 out of 31 cells from four animals).

Single-cell electrophysiology. Whole-cell recordings were made in single taste bud cells isolated from *Calhm1*$^{-/-}$ and wild-type littermates and TRPM5–GFP mice with a patch-clamp amplifier (Axopatch 200B, Axon Instruments). TRPM5–GFP mice were a gift from Dr R. F. Margolskee[39] and were used for selective recordings from type II cells on the basis of their expression of GFP. The pipette solution contained (in mM) 155 CsF, 2 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, 10 HEPES, pH 7.3, adjusted with methanesulphonic acid, ~308 mOsm. The bath solution contained (in mM) 140 Na^+, 5.4 K^+, 10 TEA^+, 20 sucrose, 1.5 Ca^{2+}, 1 Mg^{2+}, 6 Cl^- and 10 HEPES, pH 7.4, adjusted with methanesulphonic acid, ~330 mOsm. Patch pipettes were fabricated from thick-walled borosilicate glass capillaries (PG10150-4, World Precision Instruments) and had a resistance of 5–9 MΩ in the recording condition. Electrode capacitance was compensated electronically, and 70% of series resistance (10–25 MΩ) was compensated with a lag of 20 µs. Currents were low-pass-filtered at 5 kHz with eight-pole Bessel characteristics and sampled at 10 kHz. For testing pharmacological properties of I_{slow} in type II cells (Fig. 4h), whole-cell currents were recorded in GFP-positive taste cells isolated from TRPM5–GFP mice and I_{slow} was evoked every 5 s by a voltage pulse to +100 mV with 1-s duration from a holding potential of −70 mV. Current amplitudes were taken at the end of the pulse and normalized to the average of values before drug addition (initial 50 s) for each cell.

28. Ohmoto, M., Matsumoto, I., Misaka, T. & Abe, K. Taste receptor cells express voltage-dependent potassium channels in a cell age-specific manner. *Chem. Senses* **31,** 739–746 (2006).
29. Dvoryanchikov, G., Sinclair, M. S., Perea-Martinez, I., Wang, T. & Chaudhari, N. Inward rectifier channel, ROMK, is localized to the apical tips of glial-like cells in mouse taste buds. *J. Comp. Neurol.* **517,** 1–14 (2009).
30. Sinclair, M. S. *et al.* Oxytocin signaling in mouse taste buds. *PLoS ONE* **5,** e11980 (2010).
31. Tordoff, M. G. & Bachmanov, A. A. Mouse taste preference tests: why only two bottles? *Chem. Senses* **28,** 315–324 (2003).
32. Damak, S. *et al.* Trpm5 null mice respond to bitter, sweet, and umami compounds. *Chem. Senses* **31,** 253–264 (2006).
33. Eddy, M. C. *et al.* Double P2X2/P2X3 purinergic receptor knockout mice do not taste NaCl or the artificial sweetener SC45647. *Chem. Senses* **34,** 789–797 (2009).
34. Glendinning, J. I., Gresack, J. & Spector, A. C. A high-throughput screening procedure for identifying mice with aberrant taste and oromotor function. *Chem. Senses* **27,** 461–474 (2002).
35. Hallock, R. M., Tatangelo, M., Barrows, J. & Finger, T. E. Residual chemosensory capabilities in double P2X2/P2X3 purinergic receptor null mice: intraoral or postingestive detection? *Chem. Senses* **34,** 799–808 (2009).
36. Spector, A. C., Andrews-Labenski, J. & Letterio, F. C. A new gustometer for psychophysical taste testing in the rat. *Physiol. Behav.* **47,** 795–803 (1990).
37. Hellekant, G. & Roberts, T. W. in *Experimental Cell Biology of Taste and Olfaction: Current Techniques and Protocols* (eds Spielman, A. I. & Brand, J. G.) 277–290 (CRC, 1995).
38. Li, A., Leung, C. T., Peterson-Yantorno, K., Mitchell, C. H. & Civan, M. M. Pathways for ATP release by bovine ciliary epithelial cells, the initial step in purinergic regulation of aqueous humor inflow. *Am. J. Physiol. Cell Physiol.* **299,** C1308–C1317 (2010).
39. Clapp, T. R., Medler, K. F., Damak, S., Margolskee, R. F. & Kinnamon, S. C. Mouse taste cells with G protein-coupled taste receptors lack voltage-gated calcium channels and SNAP-25. *BMC Biol.* **4,** 7 (2006).

doi:10.1038/nature11906

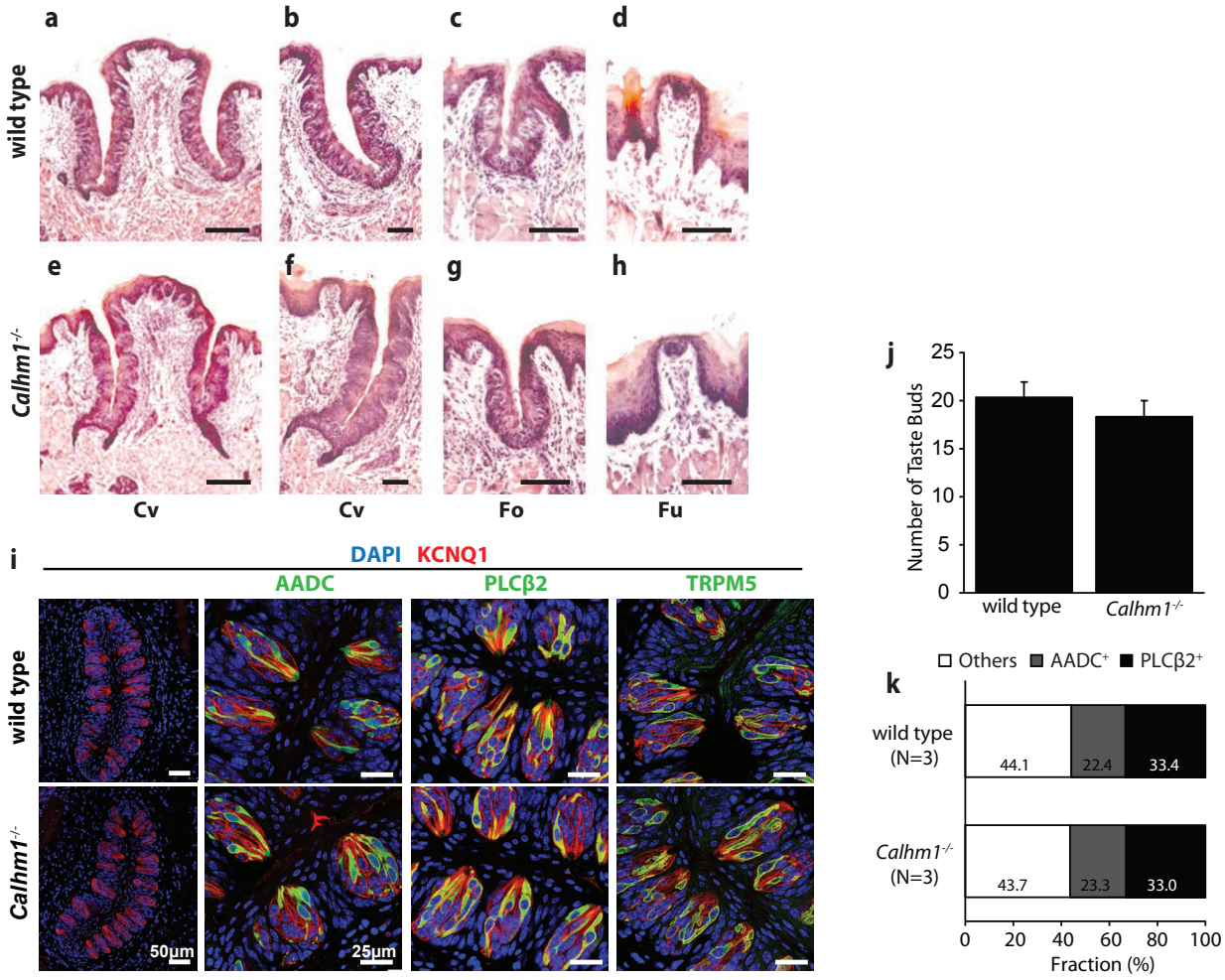


Figure S1: *Calhm1*$^{-/-}$ mice display no overt morphological abnormalities in taste buds. (a-h) Hematoxylin-eosin staining of taste buds from circumvallate [Cv; (**a**), (**b**), (**e**), and (**f**)], foliate [Fo; (**c**) and (**g**)], and fungiform [Fu; (**d**) and (**h**)] papillae of wild type (**a-d**) and *Calhm1*$^{-/-}$ (**e-h**) mice. Scale bars in (**a**) and (**e**), 100 μm; in (**b-d**) and (**f-h**), 50 μm. (**i**) Taste bud morphology was investigated by immunofluorescence of taste marker proteins: Aromatic L-amino acid decarboxylase (AADC) (also known as Ddc) for type III cells, PLCβ2 and TRPM5 for type II cells and KCNQ1 for nearly all taste bud cells[40]. Sections were counterstained with DAPI for total cell counting. (**j**) Number of taste buds in single Cv papilla was compared between wild type and *Calhm1*$^{-/-}$ mice. Taste bud number was counted as number of KCNQ1-positive structures within a maximal cross-section of a Cv papilla. Error bars, s.e. (**k**) The taste cell composition was determined in wild type (n = 3) and *Calhm1*$^{-/-}$ (n = 3) mice. The ratios of PLCβ2- and AADC-positive cells to KCNQ1-positive cells were calculated and the remaining population was counted as others, as we did previously[10].

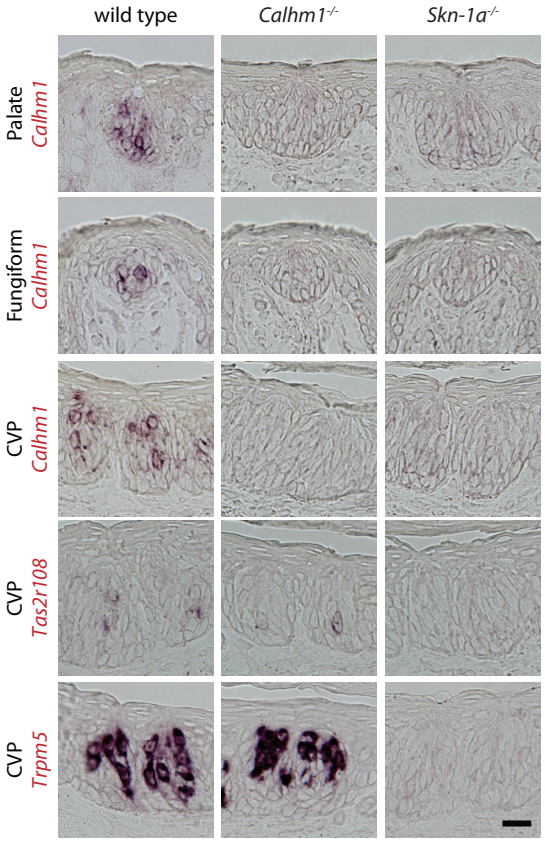


Figure S2: *in situ* hybridization analysis of type II-specific expression of *Calhm1*. *in situ* hybridization of *Calhm1* in palate (1st row), fungiform (2nd row) and palate (3rd row) TB of wild type (left column), *Calhm1*$^{-/-}$ (middle column) and *Skn-1a*$^{-/-}$ (right column) mice. Expression of genes known to be expressed only in type II cells, *Tas2r108* (4th row) and *Trpm5* (5th row), were also investigated in CVP of wild type, *Calhm1*$^{-/-}$ and *Skn-1a*$^{-/-}$ mice. Expression of all three genes detected in wild type mice is completely absent in type II cell-null *Skn-1a*$^{-/-}$ mice, while only *Calhm1* expression is lost in *Calhm1*$^{-/-}$ mice, indicating *Calhm1* is expressed only in type II cells. Note *Calhm1* knockout does not affect *Tas2r108* and *Trpm5* expression. Scale bar represents 20 μm.

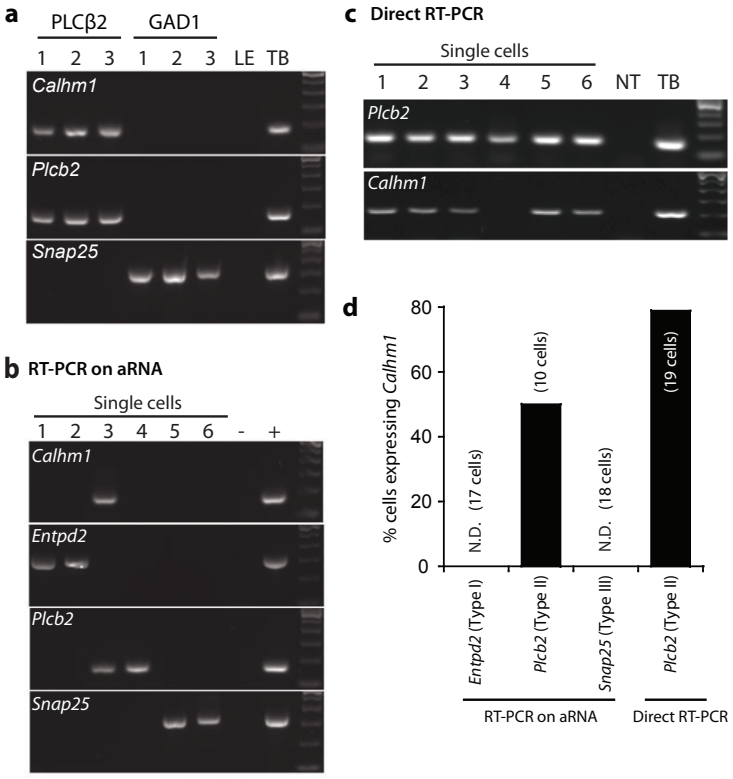
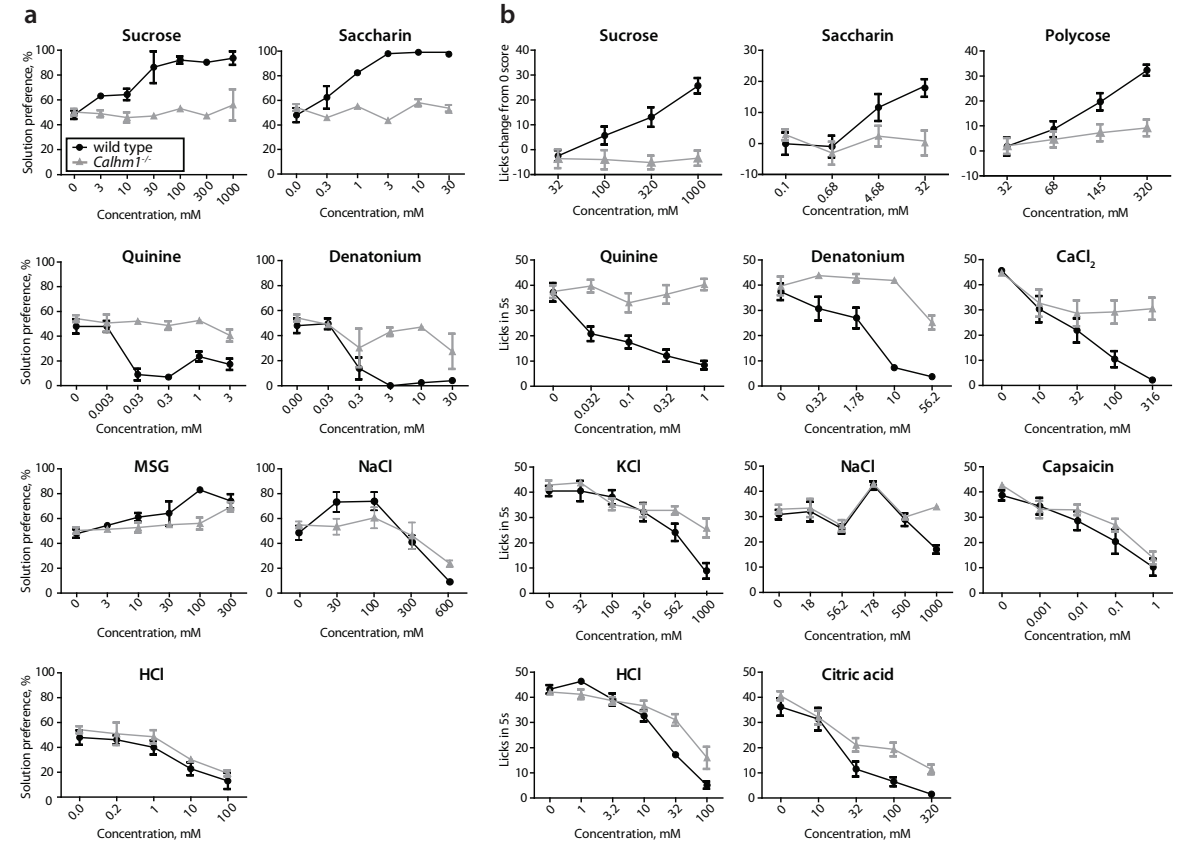


Figure S3: RT-PCR analysis of type II cell-specific expression of *Calhm1*. (**a**) mRNA from 3 pools of 10 GFP-positive taste cells isolated from CVP TB of PLCβ2-GFP (type II cells) or GAD1-GFP (type III) mice analyzed by RT-PCR. LE, LE RNA; TB, RNA from whole taste bud. (**b**) RT-PCR on linear-amplified mRNA (aRNA) of 45 individual taste cells isolated from CVP TB (cells #1 and 2: GFP-negative cells from PLCβ2-GFP x GAD1-GFP mice; cells #3 and 4: GFP-positive cells from PLCβ2-GFP mice; cells #5 and 6: GFP-positive cells from GAD1-GFP mice). Expression of *Calhm1*, *Entpd2 (*type I cell marker*)*, *Plcb2 (*type II*)*, and *Snap25 (*type III*)* were analyzed to examine *Calhm1* expression in type I, II and III cells. *Calhm1* mRNA can be detected only in type II cells. RNA from taste buds (for the *Plcb2* RT-PCR) and from brain (for the other RT-PCRs) were used as positive controls (+). (-), no RT controls. (**c**) Direct RT-PCR on non-amplified mRNA of 19 individual type II cells isolated from CVP TB of PLCβ2-GFP mice. Expression of *Calhm1* and *Plcb2* were analyzed to examine the frequency of *Calhm1* expression in *Plcb2* positive cells. NT, no RT controls; TB, RNA from taste buds. (**d**) Aggregate data from single-taste cell profiling as in (**b and c**) on mRNA isolated from 64 individual cells of CVP TB illustrate that *Calhm1* mRNA was detected in 80% of type II cells but not in type I or III cells. The number of cells examined were indicated in parentheses. N.D., not detected.



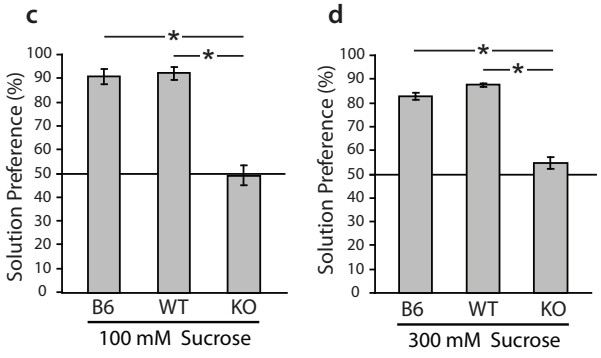


Figure S4: Behavioral responses to sweet, bitter, umami, salty and sour compounds by *Calhm1*$^{-/-}$ mice. (**a**) Mean preference % (taste compound vs. water) from 48 h two-bottle preference tests to the indicated compounds and at the indicated concentrations in *Calhm1*$^{-/-}$ mice and wild type controls. Error bars, s.e. from 8-11 mice per group. Intake values, preference scores, and results of analyses of variance based on intakes and preferences during the 48 h two-bottle preference tests are shown in Tables S1 and S2. (**b**) Brief-access lick scores for the indicated compounds and at the indicated concentrations in *Calhm1*$^{-/-}$ mice and wild type controls. Error bars, s.e. from 9-12 mice per group. Statistical analyses of the lick rates during gustometer tests are shown in Table S3. (**c and d**) Mean preference % (sucrose solution vs. water) from 48 h two-bottle preference tests as in (**a**) to 100 mM (**c**) and 300 mM (**d**) sucrose in *Calhm1*$^{-/-}$ (KO) mice backcrossed for 6 generations with C57BL/6 mice compared to the corresponding backcrossed wild type (WT) littermate controls and pure C57BL/6 (B6) mice. Error bars, s.e. from 6 mice per group. *$P < 0.0005$ (Student's *t*-test).

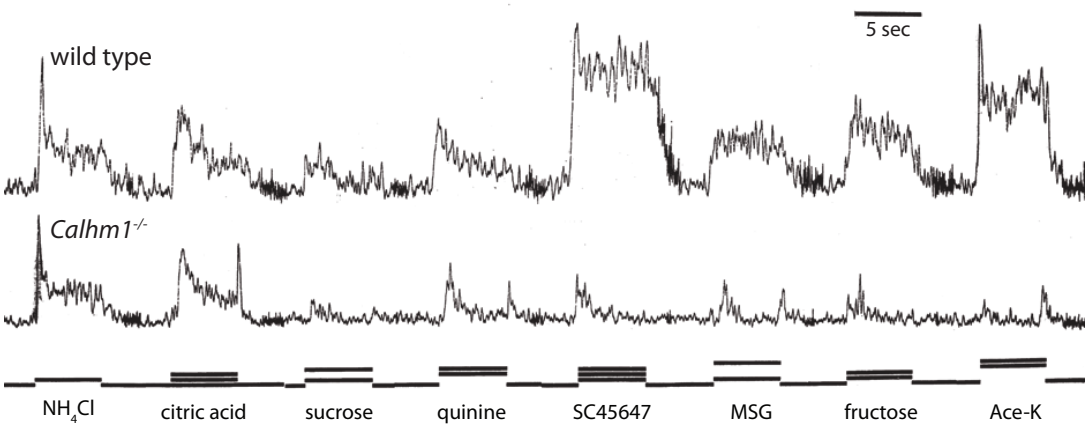


Figure S5: Gustatory nerve recordings upon lingual stimulation with sweet, bitter, umami, salty and sour compounds in *Calhm1*$^{-/-}$ mice. Whole-chorda tympani nerve recordings in *Calhm1*$^{-/-}$ and wild type mice stimulated with salty (100 mM NH_4Cl), sour (20 mM citric acid), sweet (100 mM sucrose; 8 mM SC45647; 300 mM fructose; 25 mM acesulfame-K, Ace-K), bitter (10 mM quinine), and umami (100 mM MSG) compounds. Note that the initial short-lasting peaks during stimulation with sweet and umami compounds in *Calhm1*$^{-/-}$ mice are likely to be due to mechanical stimulation artifacts and not to an actual response to the compounds.

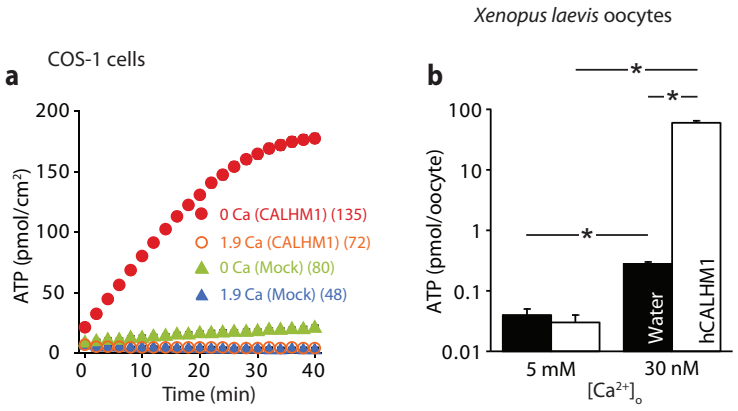


Figure S6: Low $[Ca^{2+}]_o$-evoked ATP release is strongly enhanced by CALHM1 expression in COS-1 cells and *Xenopus* oocytes. (**a**) Time courses of extracellular ATP levels due to release from mock- and hCALHM1-transfected COS-1 cells exposed to normal (1.9 mM) or zero (17 nM) $[Ca^{2+}]_o$. Cells were prepared the same way as were HeLa cells (see Methods). Numbers in parentheses = number of wells. (**b**) ATP released from water- (n = 6, total 108 oocytes) or hCALHM1 cRNA-injected (n = 9, total 162 oocytes) oocytes before and 30 min after reduction of $[Ca^{2+}]_o$ from 5 mM to 30 nM. For each sample, 18 oocytes in a small chamber (150 µl) were exposed to low $[Ca^{2+}]_o$ and bath solution was collected before and 30 min later for ATP measurements. All oocytes were injected with *Xenopus* connexin-38 antisense oligonucleotide to block endogenous hemichannel-mediated ATP release. Error bars, s.e.; $*P < 0.01$ (Student's *t*-test).

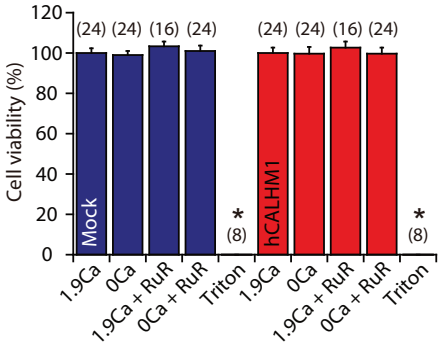


Figure S7: CALHM1 expression and function have no effect on cell viability. Exposure of mock- (blue) or hCALHM1- (red) transfected HeLa cells to normal (1.9 mM) or zero (17 nM) $[Ca^{2+}]_o$ for 60 min in the presence or absence of 20 µM ruthenium red (RuR) was without effect on cell viability. The thiazolyl blue tetrazolium bromide (MTT) assay was adapted to quantify cell viability as previously described[41]. Triton treatment used as control. Numbers in parentheses = number of wells. Error bars, s.e.; *$P < 0.01$ (Student's *t*-test).

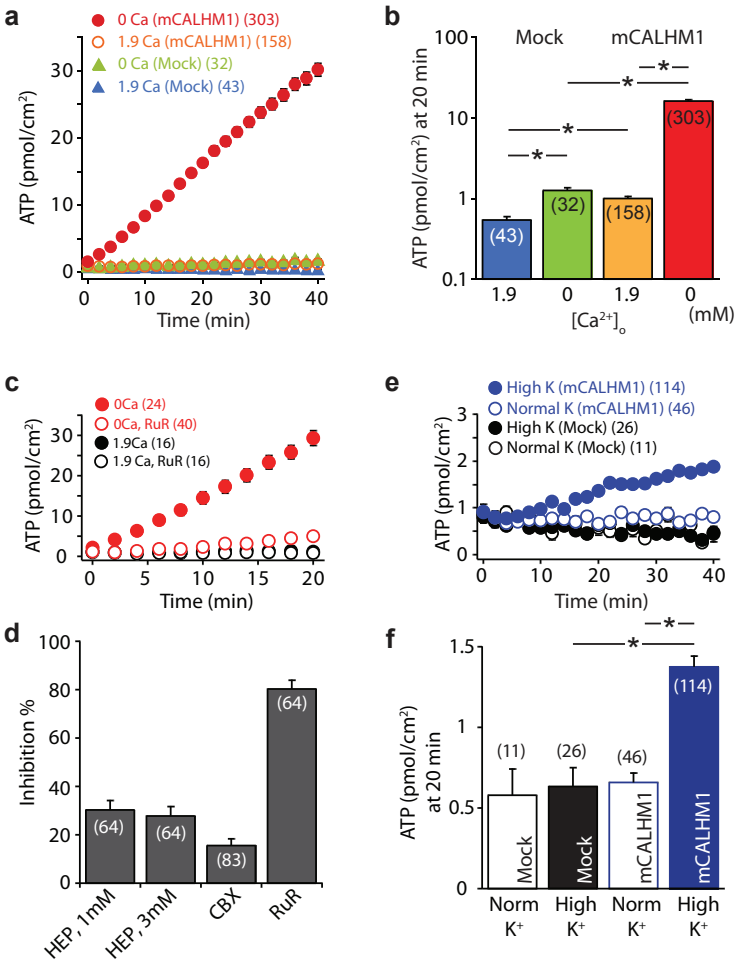


Figure S8: Mouse CALHM1 also forms an ATP release pathway with properties similar to human CALHM1. (**a**) Time courses of extracellular ATP levels due to release from mock- and mCALHM1-transfected HeLa cells exposed to normal (1.9 mM) or zero (17 nM) $[Ca^{2+}]_o$. (**b**) ATP levels at 20 min in (**a**). mCALHM1 cells respond to low $[Ca^{2+}]_o$ with robust ATP release. (**c**) mCALHM1-mediated low $[Ca^{2+}]_o$-induced ATP release is abolished by 20 µM ruthenium red (RuR). (**d**) Pharmacological sensitivities of ATP release from mCALHM1 cells. 1 and 3 mM 1-Heptanol (HEP); 30 µM carbenoxolone (CBX); 20 µM RuR. (**e**) Depolarization by high $[K^+]_o$ exposure (117.5 mM) induces ATP release specifically from mCALHM1-expressing cells. (**f**) ATP levels at 20 min in (**e**). Numbers in parentheses = number of wells. Error bars, s.e.; *$P < 0.01$ (Student's *t*-test).

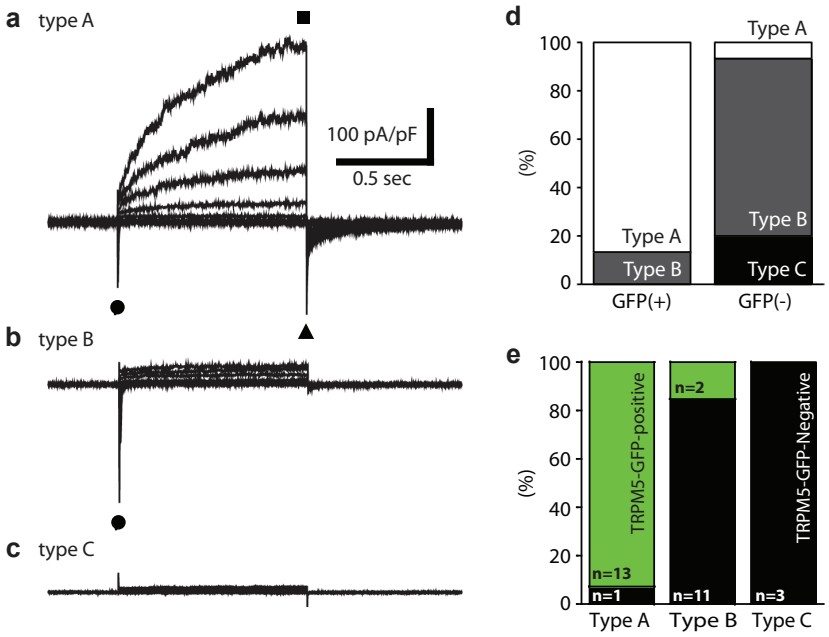


Figure S9: Type II cells are characterized by type A ion currents. Taste bud cells can be classified based on fingerprints of whole-cell voltage-gated currents as type A (corresponding to type II taste cell), B (type III taste cell), and C (type I taste cell)[13,18]. Whole cell recordings were made in taste bud cells isolated from TRPM5-GFP mice. Cells were held at -70 mV and voltage was stepped to between -80 and +80 mV at 20 mV increments with 1 sec pulse duration. (**a**) Type A current is identified by the presence of I_{Na} (●), I_{slow} (■), and I_{tail} (▲). (**b**) Type B current is identified by the presence of I_{Na} (●) and the absence of I_{slow} and I_{tail}. (**c**) Type C current has no voltage-gated currents. 87% of GFP-positive cells had type A current (**d**) and 93% of cells with type A current were GFP-positive cells (**e**), validating type A current as the characteristic current of type II cells.

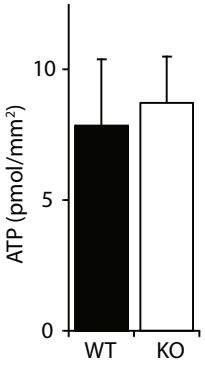


Figure S10: *Calhm1* deficiency does not affect the ATP content of taste buds. Intracellular ATP content of taste buds was estimated by treating the serosal side of CVP-containing tongue epithelial sheets from WT (n = 5) and *Calhm1*$^{-/-}$ (KO, n = 5) mice with Triton X-100/EDTA solution (0.5%/ 4 mM in water) through an orifice of 0.75 mm^2 (0.75 mm x 1 mm) for 5 min and measuring ATP released into the serosal solution by the luciferin-luciferase assay. Data presented normalized to area of orifice through which intracellular ATP was collected.

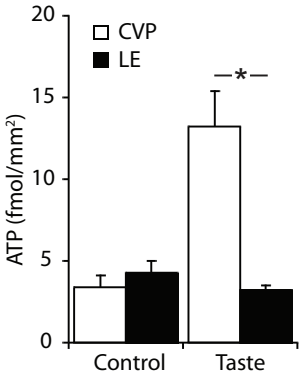


Figure S11: Taste stimulus evokes ATP release from gustatory CVP tissue but not from non-gustatory LE. Bitter mix (Taste) or only buffer (Control) was applied selectively to the apical side of tongue epithelial regions, CVP or LE, of WT mice and the amount of ATP released from the serosal side was measured. Taste stimulus elicits ATP release only from CVP region. Similar release of CVP and LE exposed to buffer suggests that basal ATP release from taste buds is below the detection limit. Thus, the difference in ATP levels between CVP and LE after taste stimulus is a measure of taste-evoked ATP release from taste buds. Error bars, s.e. from 3 experiments; *$P < 0.05$ (Student's *t*-test).

SUPPLEMENTARY TABLES

Table S1: Daily intakes and preference scores during 48-h two-bottle choice tests

Compound and concentration		Solution intake		Water intake		Total fluid intake		Solution preference	
		WT	*Calhm1* KO	WT	*Calhm1* KO	WT	*Calhm1* KO	WT	*Calhm1* KO
	0	3.4 ± 0.5	2.5 ± 0.4	3.8 ± 0.7	2.1 ± 0.4	7.2 ± 1.1	4.6 ± 0.8	47.9 ± 5.8	54.2 ± 2.8
Sucrose, mM	3	5.3 ± 0.6	3.0 ± 0.2	3.2 ± 0.3	3.1 ± 0.2	8.5 ± 0.9	6.2 ± 0.3	62.0 ± 1.5	48.9 ± 2.4
	10	7.1 ± 0.4††	4.3 ± 0.3*	4.3 ± 1.1	5.1 ± 0.6†	11.4 ± 1.5	9.5 ± 0.5†	63.5 ± 4.2	45.8 ± 3.8
	30	6.3 ± 0.5	2.6 ± 0.4**	1.6 ± 1.1	2.8 ± 0.2	8.0 ± 1.1	5.4 ± 0.7	82.3 ± 11.1	47.5 ± 1.3**
	300	19.9 ± 0.7†††	3.3 ± 0.2***	2.5 ± 0.6	3.7 ± 0.3	22.5 ± 1.3	7.1 ± 0.5***	88.9 ± 2.1††	47.5 ± 1.2***
	1000	7.9 ± 0.3††	3.5 ± 0.6***	0.4 ± 0.4	2.9 ± 0.7	8.4 ± 0.2	6.4 ± 0.2	94.6 ± 5.3††	55.2 ± 10.9***
Saccharin, mM	0.3	3.9 ± 0.0	2.1 ± 0.2*	2.6 ± 0.9	2.4 ± 0.4	6.6 ± 0.9	4.6 ± 0.6	62.3 ± 9.1	45.9 ± 2.0
	1	7.1 ± 0.4††	2.9 ± 0.3***	1.5 ± 0.1	2.3 ± 0.3	8.6 ± 0.6	5.3 ± 0.6	82.3 ± 1.1††	55.1 ± 0.8***
	3	12.8 ± 0.1†††	2.5 ± 0.2***	0.2 ± 0.1†	3.3 ± 0.6**	13.1 ± 0.1††	5.8 ± 0.9***	97.9 ± 0.8††	43.6 ± 2.2***
	10	13.4 ± 0.6†††	2.8 ± 0.4***	0.1 ± 0.1††	2.1 ± 0.4*	13.5 ± 0.5††	4.9 ± 0.8***	99.1 ± 0.8†††	58.0 ± 2.8***
	30	11.5 ± 0.4†††	3.1 ± 0.2***	0.1 ± 0.1††	2.7 ± 0.2**	11.6 ± 0.4†	5.9 ± 0.4***	99.0 ± 0.9†††	53.5 ± 2.6***
Quinine, mM	0.003	2.7 ± 0.8	2.6 ± 0.1	3.2 ± 1.1	2.6 ± 0.5	6.0 ± 1.8	5.3 ± 0.3	47.9 ± 4.4	50.8 ± 6.7
	0.03	0.6 ± 0.3†	2.8 ± 0.1*	4.6 ± 1.0	2.6 ± 0.1	5.2 ± 1.4	5.5 ± 0.2	9.0 ± 4.6††	52.0 ± 2.0***
	0.3	0.3 ± 0.0††	2.6 ± 0.3*	5.5 ± 1.1	2.7 ± 0.1	5.9 ± 1.1	5.4 ± 0.2	6.9 ± 1.2††	48.6 ± 3.5***
	1	2.1 ± 0.7	4.3 ± 0.2	6.3 ± 1.3	3.9 ± 0.1†	8.4 ± 2.0	8.2 ± 0.3†	23.6 ± 3.9	52.7 ± 0.9***
	3	1.2 ± 0.4	2.7 ± 0.4	5.9 ± 0.8	3.9 ± 0.2	7.2 ± 1.0	6.7 ± 0.5	17.2 ± 4.5†	40.6 ± 4.8**
Denatonium, mM	0.03	2.1 ± 0.1	1.7 ± 0.2	2.1 ± 0.2	1.8 ± 0.3	4.2 ± 0.1	3.6 ± 0.6	49.5 ± 4.2	48.8 ± 1.1
	0.3	0.4 ± 0.2††	1.3 ± 0.6	2.9 ± 0.5	2.7 ± 0.5	3.4 ± 0.3	4.0 ± 0.7	13.7 ± 8.7	30.2 ± 15.3
	3	0.0 ± 0.0††	1.6 ± 0.2	5.1 ± 0.2	2.2 ± 0.4**	5.1 ± 0.2	3.8 ± 0.5	0.0 ± 0.0††	43.1 ± 3.4**
	10	0.1 ± 0.1††	1.6 ± 0.1	5.1 ± 0.4	1.8 ± 0.2***	5.2 ± 0.4	3.5 ± 0.4	2.3 ± 2.3††	46.8 ± 1.6***
	30	0.2 ± 0.1††	1.7 ± 0.8	4.9 ± 0.5	3.5 ± 0.5	5.1 ± 0.6	5.2 ± 1.2	4.0 ± 2.0††	27.4 ± 14.0
MSG, mM	3	4.6 ± 1.0	4.4 ± 0.2	3.8 ± 0.7	4.2 ± 0.5	8.4 ± 1.7	8.7 ± 0.7	54.4 ± 1.5	51.4 ± 1.9
	10	4.5 ± 0.7	3.8 ± 0.9	2.8 ± 0.2	3.2 ± 0.2	7.3 ± 0.9	7.1 ± 1.1	61.0 ± 3.3	52.7 ± 3.8
	30	5.3 ± 0.9	3.7 ± 0.3	2.8 ± 0.5	3.0 ± 0.3	8.1 ± 0.5	6.7 ± 0.6	64.2 ± 8.8	55.0 ± 1.8
	100	5.7 ± 1.5	3.6 ± 0.6	1.2 ± 0.3	2.8 ± 0.4	6.9 ± 1.8	6.5 ± 0.8	83.0 ± 2.3††	56.0 ± 4.8**
	300	8.6 ± 1.5	8.6 ± 1.5	2.8 ± 0.2	3.6 ± 0.2	11.5 ± 1.4	12.2 ± 1.6†	74.0 ± 5.6	69.4 ± 4.2†
HCl, mM	0.2	3.1 ± 0.6	2.9 ± 0.6	3.5 ± 0.7	2.7 ± 0.3	6.6 ± 1.3	5.7 ± 0.6	46.1 ± 3.5	50.9 ± 8.9
	1	1.9 ± 0.3	2.4 ± 0.3	3.1 ± 0.8	2.6 ± 0.2	5.0 ± 1.1	5.1 ± 0.2	39.9 ± 5.6	48.5 ± 5.3
	10	1.4 ± 0.5	1.4 ± 0.0	4.4 ± 0.6	3.4 ± 0.1†	5.8 ± 1.1	4.9 ± 0.1	22.8 ± 5.2	30.2 ± 1.4††
	100	0.9 ± 0.4	1.1 ± 0.2	4.7 ± 1.1	4.7 ± 0.4†	5.6 ± 1.6	5.9 ± 0.7	12.9 ± 6.4†	19.1 ± 2.5†††
NaCl, mM	30	5.4 ± 0.9	3.3 ± 1.2	2.2 ± 0.8	2.6 ± 0.6	7.7 ± 1.4	5.9 ± 1.9	72.8 ± 8.0	52.8 ± 6.4
	100	6.2 ± 1.2	3.2 ± 0.1	2.2 ± 0.6	2.3 ± 0.7	8.4 ± 1.4	5.5 ± 0.6	73.4 ± 7.4	60.0 ± 8.4
	300	2.6 ± 0.5	3.3 ± 1.2	3.9 ± 0.5	3.4 ± 0.6	6.6 ± 0.8	6.8 ± 1.6	40.3 ± 5.4	45.5 ± 10.6
	600	0.4 ± 0.1††	1.3 ± 0.3	4.7 ± 0.3	4.6 ± 0.6	5.1 ± 0.2	6.0 ± 0.9	8.0 ± 2.1††	22.7 ± 2.6††

Values are means ± SE of 8 to 11 mice per group. * $p<0.02$, ** $p<0.01$, *** $p<0.001$ relative to WT group (post hoc Bonferroni's tests). † $p<0.02$ †† $p<0.01$, ††† $p<0.001$ relative to response to water (0 mM; Student's *t*-tests). Results of ANOVAs are presented in table S2.

Table S2: Results of analyses of variance based on intakes and preferences of WT and *Calhm1* KO mice during 48-h choice tests.

Measure and factor	df	Solution Intake F	p	Water Intake F	p	Total Fluid Intake F	p	Solution Preference F	p
Sucrose									
Group	1	221.6	0.0001	1.36	0.3083	29.92	0.0054	30.71	0.0052
Concentration	5	92.02	<0.0001	6.282	0.0012	38.43	<0.0001	6.623	0.0009
Group x Concentration	5	81.39	<0.0001	2.891	0.0401	25.09	<0.0001	6.703	0.0008
Saccharin									
Group	1	293.3	<0.0001	8.604	0.0427	51.02	0.002	427.8	<0.0001
Concentration	5	90.61	<0.0001	4.899	0.0043	15.63	<0.0001	18.37	<0.0001
Group x Concentration	5	76.06	<0.0001	8.237	0.0002	9.729	<0.0001	17.24	<0.0001
Quinine									
Group	1	6.418	0.0644	4.498	0.1013	0.2718	0.6297	75.11	0.001
Concentration	5	8.367	0.0002	6.904	0.0007	5.73	0.0019	13.29	<0.0001
Group x Concentration	5	8.134	0.0003	1.068	0.4074	1.054	0.4144	8.971	0.0001
Denatonium									
Group	1	3.614	0.1301	29.65	0.0055	2.136	0.2177	19.74	0.0113
Concentration	5	13.13	<0.0001	5.45	0.0025	3.699	0.0156	10.57	<0.0001
Group x Concentration	5	5.085	0.0036	3.721	0.0152	1.935	0.1333	3.915	0.0123
MSG									
Group	1	0.8063	0.4199	1.756	0.2558	0.2969	0.6148	3.855	0.1211
Concentration	5	12.73	<0.0001	3.678	0.016	8.381	0.0002	10.28	<0.0001
Group x Concentration	5	0.5422	0.7421	2.666	0.0528	0.6891	0.6374	4.212	0.0089
HCl									
Group	1	0.0076	0.9346	1.906	0.2395	0.716	0.4451	6.531	0.0629
Concentration	4	8.604	0.0007	3.803	0.0234	0.5992	0.6685	15.55	<0.0001
Group x Concentration	4	0.7965	0.5447	0.612	0.66	0.92	0.4764	0.036	0.9972
NaCl									
Group	1	1.157	0.3427	0.2057	0.6737	0.6983	0.4504	0.05589	0.8247
Concentration	4	12.41	<0.0001	12.37	<0.0001	1.429	0.2697	27.65	<0.0001
Group x Concentration	4	4.031	0.019	2.05	0.1355	2.718	0.0669	3.532	0.0301

Values are the results of mixed-design ANOVAs with factors of group (WT or KO) and taste solution concentration. Df= degrees of freedom. Means and SE are presented in Table S1.

Table S3: Lick rates during gustometer tests of WT and *Calhm1* KO mice

Compound	Concentration, mM	Licks made (per 5-s test) WT	KO
Sucrose	0	12 ± 3^{a}	21 ± 3^{b}
	32	9 ± 2^{ab}	17 ± 2^{bc}
	100	17 ± 2^{b}	17 ± 3^{bc}
	320	25 ± 4^{c}	16 ± 4^{abc}
	1000	37 ± 1^{d}	18 ± 3^{bc}
Group x concentration		$F(4,80) = 11.4$, $p < 0.0001$	
Saccharin	0	11 ± 2^{ab}	18 ± 3^{abcd}
	0.1	12 ± 2^{ab}	20 ± 3^{cd}
	0.68	11 ± 2^{a}	15 ± 3^{abc}
	4.68	24 ± 3^{de}	20 ± 3^{cd}
	32	30 ± 4^{e}	18 ± 3^{bcd}
Group x concentration		$F(4,84) = 5.19$, $p < 0.0001$	
QHCl	0	37 ± 4^{de}	38 ± 3^{de}
	0.032	21 ± 3^{c}	40 ± 3^{de}
	0.1	18 ± 3^{bc}	33 ± 4^{d}
	0.32	12 ± 2^{ab}	37 ± 4^{de}
	1	8 ± 2^{a}	41 ± 2^{e}
Group x concentration		$F(4,76) = 9.62$, $p < 0.0001$	
Denatonium benzoate	0	37 ± 3^{cd}	37 ± 4^{d}
	0.32	31 ± 5^{bc}	44 ± 1^{d}
	1.78	27 ± 4^{b}	43 ± 2^{d}
	10	7 ± 1^{a}	42 ± 1^{d}
	56.2	4 ± 1^{a}	26 ± 4^{b}
Group x concentration		$F(4,68) = 9.72$, $p < 0.0001$	
NaCl	0	31 ± 2^{bcd}	33 ± 2^{d}
	18	32 ± 4^{cd}	33 ± 4^{d}
	56.2	25 ± 2^{b}	26 ± 2^{bc}
	178	42 ± 2^{e}	43 ± 1^{e}
	500	29 ± 3^{bcd}	30 ± 1^{bcd}
	1000	17 ± 2^{a}	34 ± 2^{d}
Group x concentration		$F(5,110) = 3.99$, $p = 0.0023$	
KCl	0	41 ± 2^{ef}	43 ± 2^{f}
	32	41 ± 4^{ef}	44 ± 1^{f}
	100	38 ± 3^{def}	35 ± 2^{de}
	316	32 ± 4^{cd}	33 ± 3^{de}
	562	24 ± 3^{b}	33 ± 2^{de}
	1000	8 ± 3^{a}	25 ± 4^{bc}
Group x concentration		$F(5,105) = 3.85$, $p = 0.0030$	

Compound	Concentration, mM	Licks made (per 5-s test) WT	KO
HCl	0	43 ± 2^{fg}	42 ± 1^{efg}
	1	46 ± 1^{g}	41 ± 2^{efg}
	3.2	39 ± 2^{ef}	39 ± 2^{def}
	10	33 ± 2^{cd}	37 ± 2^{de}
	32	17 ± 1^{b}	31 ± 2^{c}
	100	5 ± 1^{a}	16 ± 4^{b}
Group x concentration		$F(5,100) = 6.55$, $p < 0.0001$	
Citric acid	0	36 ± 4	41 ± 2
	10	31 ± 5	32 ± 3
	32	12 ± 3	21 ± 3
	100	7 ± 2	19 ± 3
	320	2 ± 0	11 ± 2
Group x concentration		$F(4,84) = 1.86$, $p = 0.1245$ (ns)	
Capsaicin	0	39 ± 2	43 ± 1
	0.001	34 ± 3	34 ± 3
	0.01	28 ± 4	34 ± 2
	0.1	20 ± 5	25 ± 3
	1	10 ± 3	13 ± 3
Group x concentration		$F(5,80) = 0.39$, $p = 0.8125$ (ns)	
Polycose	0	9 ± 2^{a}	17 ± 3^{c}
	32	10 ± 3^{ab}	19 ± 3^{c}
	68	17 ± 2^{c}	28 ± 3^{d}
	145	28 ± 2^{d}	24 ± 3^{d}
	320	41 ± 1^{e}	26 ± 3^{d}
Group x concentration		$F(4,68) = 11.8$, $p < 0.0001$	
$CaCl_2$	0	46 ± 1^{e}	45 ± 1^{e}
	10	28 ± 5^{cd}	35 ± 5^{de}
	32	20 ± 5^{bc}	31 ± 5^{cd}
	100	13 ± 4^{b}	28 ± 5^{cd}
	316	4 ± 2^{a}	31 ± 5^{cd}
Group x concentration		$F(4,80) = 3.49$, $p = 0.0111$	

Values are means ± SEs; values are based on the analysis of 9 - 12 WT and 10 - 12 KO mice; mice that did not lick to any presentation of a taste compound concentration are not included in analyses. 0 mM = deionized water. Values with same superscript within a test did not differ significantly from each other (post hoc LSD tests). Note that the interaction term was not significant in the analyses of capsaicin and citric acid. However, for citric acid, there was significant main effect of group, $F(1.21) = 13.0$, $p = 0.0016$.

Table S4: PCR primer sequences

Protein/*Gene*	Forward primer sequence	Reverse primer sequence	GenBank Accession No.	PCR Product Size
β-actin / *Actb*	CGTTGACATCCGTAAAGACC	AGGGGCCGGACTCATCGTA	NM_007393	244
CALHM1 / *Calhm1*	Pr #1 ATGAACCATGACCTGGAACTGGGT Pr #3 CCCTGCCCTGAGATCTATGA	Pr #2 TGTGCCAGCTTGTGAGTAGCCTAT Pr #4 CTTGCGCTCAATGTCAATGT	NM_001081271	175 210
NTPDase2 / *Entpd2*	AGCTGGAGGATGCCACAGAG	GAGAGCAACCCAGGAGCTGA	NM_009849	299
PLCβ2 / *Plcb2*	GAGCAAATCGCCAAGATGAT	CCTTGTCTGTGGTGACCTTG	NM_177568	163
PKD2L1 / *Pkd2l1*	GGTGAGATTCCAACAGAGG	CACCACATATTAGTCCAAAAGA	NM_181422	202
SNAP25 / *Snap25*	GGCAATAATCAGGATGGAGTAG	AGATTTAACCACTTCCCAGCA	NM_011428	310
T1R2 / *Tas1r2*	TAGGAAAAGACAGGGGGAGTGG	GGGGGTGTAGAGAAGCGAGAAT	NM_031873	208
TRPM5 / *Trpm5*	CTCCCAGCAGCCCCAAGAAATG	TGGGTCAGGGGTCAGAAAGAAA	NM_020277	312

SUPPLEMENTARY DISCUSSION

The results of all three functional assays— chorda tympani electrophysiology (Figs. 2c and S5), two-bottle preference tests (Figs. 2a and S4a and Tables S1 and S2) and brief access tests (Figs. 2b and S4b and Table S3)—were remarkably consistent with respect to sweet, bitter and umami taste stimuli, although there were some minor discrepancies (discussed below). Regarding the data presentation of the gustatory nerve recordings shown in Fig. 2c, it is worth mentioning that the absolute values of the average responses to NH_4Cl are not significantly different between wild type (59 ± 17 (s.e.), 31 responses from 8 animals) and *Calhm1*$^{-/-}$ (76 ± 24, 31 responses from 8 animals) mice, indicating that *Calhm1* ablation has selective effects on sweet, bitter and umami tastes rather than global effects on all taste qualities, and thus validating the normalization to responses to NH_4Cl.

There was also evidence for CALHM1 mediation of responses to tastes that are not considered sweet, bitter or umami. *Calhm1*$^{-/-}$ mice given brief-access tests did not show the normal avidity for Polycose that is observed in intact animals. Polycose is a complex carbohydrate with a unique taste to rodents (see [42,43] for reviews). The transduction mechanism for Polycose taste is unknown[44] but it most likely involves the G protein-coupled receptor (GPCR) signaling cascade[45]. Thus, it is reasonable to suspect that Polycose detection also requires CALHM1. Similarly, calcium is detected primarily by T1R3[46,47] so the lack of response to $CaCl_2$ by *Calhm1*$^{-/-}$ mice is consistent with CALHM1 being a necessary component of GPCR-mediated taste transduction. A similar argument can be made to explain our observation that *Calhm1*$^{-/-}$ mice had a reduced avoidance of high concentrations of KCl. We suspect this reflects the absence of a signal involving the bitter taste of this salt, although the receptor remains to be discovered. The involvement of CALHM1 in "nontraditional" Polycose and calcium tastes implies that the deficit displayed in the knockout animals might best be considered as a loss of all GPCR-mediated taste transduction rather than simply sweet, bitter and umami taste modalities.

Calhm1$^{-/-}$ mice were unperturbed by all quinine concentrations tested, even those that reduced preferences and acceptance of control mice to virtually zero. The same was true for denatonium, although there was some suggestion of residual effects at the highest concentrations tested (30 mM in the two-bottle choice tests or 56.2 mM in the brief access tests). Perhaps surprisingly given the behavioral results, the electrophysiological response of *Calhm1*$^{-/-}$ mice to quinine was not completely eliminated. The discrepancy most likely reflects the behavior being the result of combined information from three gustatory afferent nerves (chorda tympani, glossopharyngeal and superficial petrosal) whereas the electrophysiology was confined to only one. Our electrophysiological experiment was based on recordings made from the chorda tympani nerve but the glossopharyngeal nerve is usually considered a more dominant mediator of bitter taste[48]. It also is likely that the electrophysiological response includes none-bitter components, including movement/touch artifacts and effects of the HCl salt, which could account for the residual chorda tympani response to quinine.

The response of *Calhm1*$^{-/-}$ mice to the oral irritant, capsaicin, did not differ from controls which argues that the deficits induced by *Calhm1* deletion are gustatory rather than a general debilitation. Unlike control mice, the knockout mice did not avoid the highest concentration (1000 mM) of NaCl tested in the brief access tests. The attraction to low concentrations of NaCl is believed to involve ENaC-expressing TB cells, which are distinct from type II and III cells[49], and thus is unlikely to be mediated directly by CALHM1. There are undoubtedly other sodium transduction mechanisms involved in the response to high concentrations of NaCl. Perhaps elimination of *Calhm1* modulates or interferes with one or more of these salt transduction mechanisms. *Calhm1* elimination also tended to attenuate the avoidance of sour tastes in the brief access tests (statistically significant for HCl but not citric acid). Considering that expression of *Calhm1* is confined to type II taste bud cells, it is unlikely that *Calhm1* knockout affects sour taste transduction directly because this occurs in type III cells. Perhaps the deficiency of ATP release from type II cells deteriorates not only the neurotransmission of sweet, bitter and umami tastes but also cell-to-cell communication within taste buds[12] and thus may affect transduction of other taste qualities, such as salty and sour tastes. This is an area for additional research.

REFERENCES FOR SUPPLEMENTARY INFORMATION

40 Ohmoto, M., Matsumoto, I., Yasuoka, A., Yoshihara, Y. & Abe, K. Genetic tracing of the gustatory and trigeminal neural pathways originating from T1R3-expressing taste receptor cells and solitary chemoreceptor cells. *Mol Cell Neurosci* **38**, 505-517, (2008).

41 Li, A. *et al.* Mechanisms of ATP release by human trabecular meshwork cells, the enabling step in purinergic regulation of aqueous humor outflow. *J Cell Physiol* **227**, 172-182, (2012).

42 Sclafani, A. Carbohydrate taste, appetite, and obesity: an overview. *Neurosci Biobehav Rev* **11**, 131-153, (1987).

43 Sclafani, A. The sixth taste? *Appetite* **43**, 1-3, (2004).

44 Zukerman, S., Glendinning, J. I., Margolskee, R. F. & Sclafani, A. T1R3 taste receptor is critical for sucrose but not Polycose taste. *Am J Physiol Regul Integr Comp Physiol* **296**, R866-876, (2009).

45 Sclafani, A., Zukerman, S., Glendinning, J. I. & Margolskee, R. F. Fat and carbohydrate preferences in mice: the contribution of alpha-gustducin and Trpm5 taste-signaling proteins. *Am J Physiol Regul Integr Comp Physiol* **293**, R1504-1513, (2007).

46 Tordoff, M. G., Alarcon, L. K., Valmeki, S. & Jiang, P. T1R3: A human calcium taste receptor. *Sci Rep* **2**, 496, (2012).

47 Tordoff, M. G. *et al.* Involvement of T1R3 in calcium-magnesium taste. *Physiol Genomics* **34**, 338-348, (2008).

48 St John, S. J. & Spector, A. C. Combined glossopharyngeal and chorda tympani nerve transection elevates quinine detection thresholds in rats (Rattus norvegicus). *Behav Neurosci* **110**, 1456-1468, (1996).

49 Chandrashekar, J. *et al.* The cells and peripheral representation of sodium taste in mice. *Nature* **464**, 297-301, (2010).