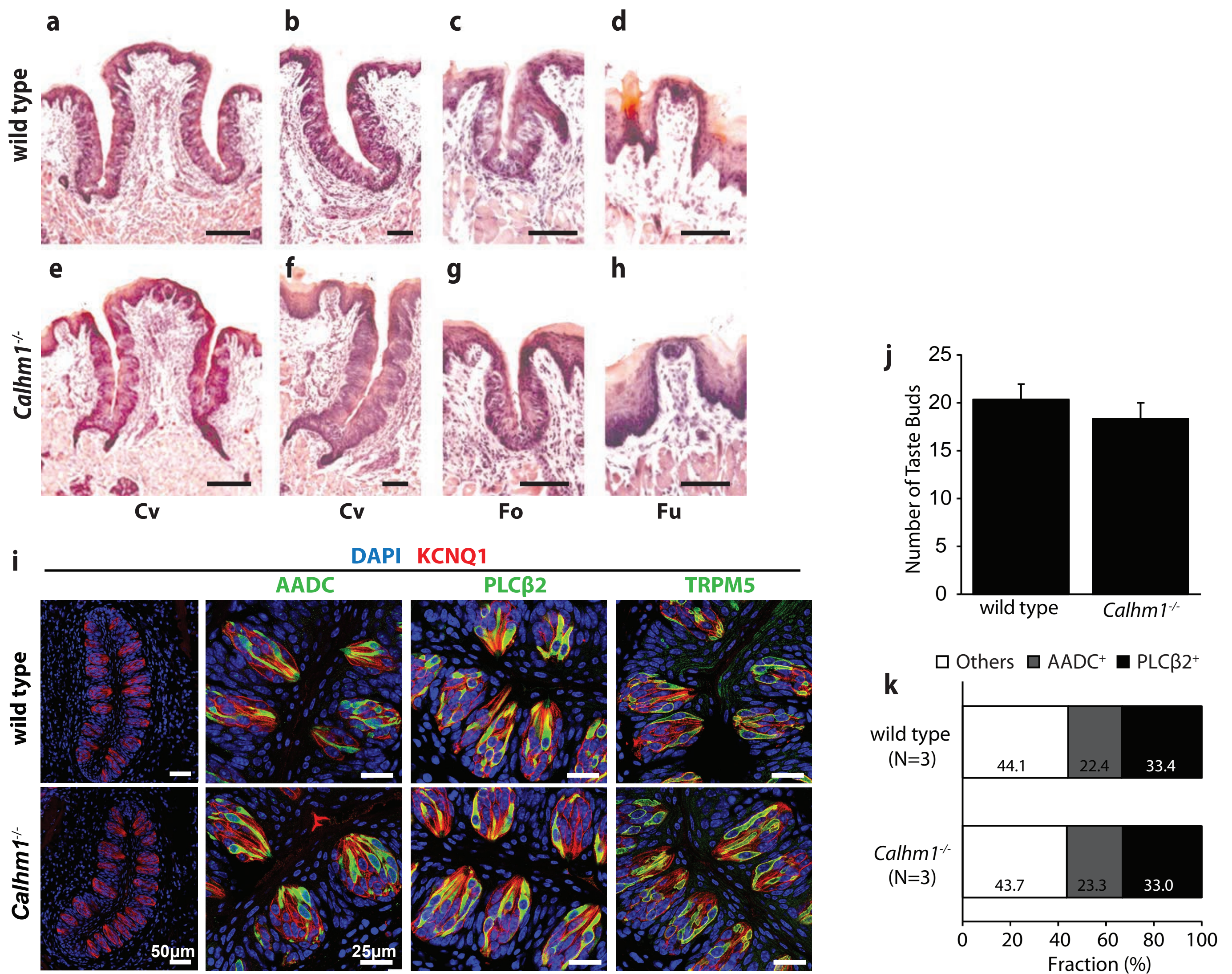

Figure S1: *Calhm1*$^{-/-}$ mice display no overt morphological abnormalities in taste buds. (a-h) Hematoxylin-eosin staining of taste buds from circumvallate [Cv; (**a**), (**b**), (**e**), and (**f**)], foliate [Fo; (**c**) and (**g**)], and fungiform [Fu; (**d**) and (**h**)] papillae of wild type (**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].

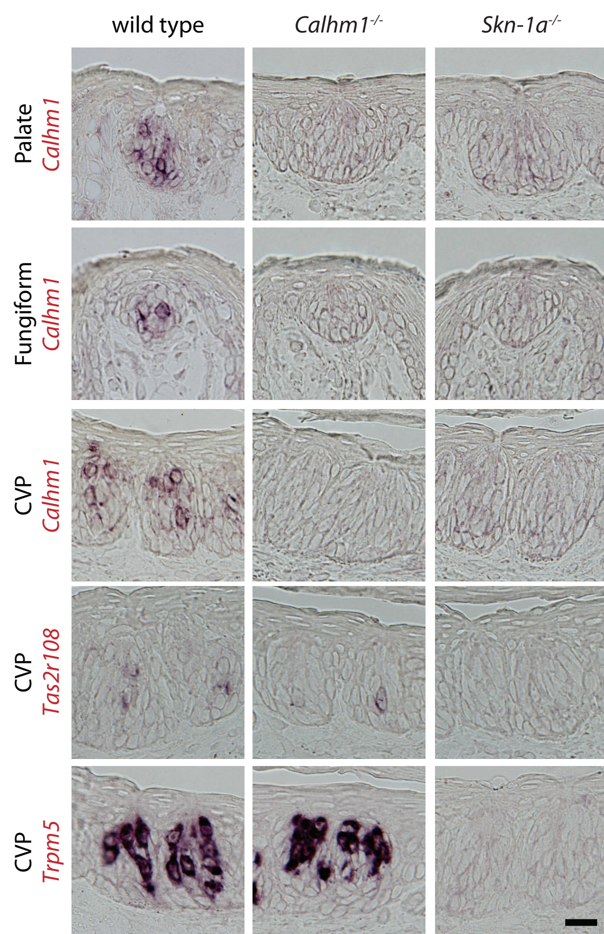

Figure S2: *in situ* hybridization analysis of type II-specific expression of *Calhm1*. *in situ* hybridization of *Calhm1* in palate (1st row), fungiform (2nd row) and palate (3rd row) TB of wild type (left column), *Calhm1*$^{-/-}$ (middle column) and *Skn-1a*$^{-/-}$ (right column) mice. Expression 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.

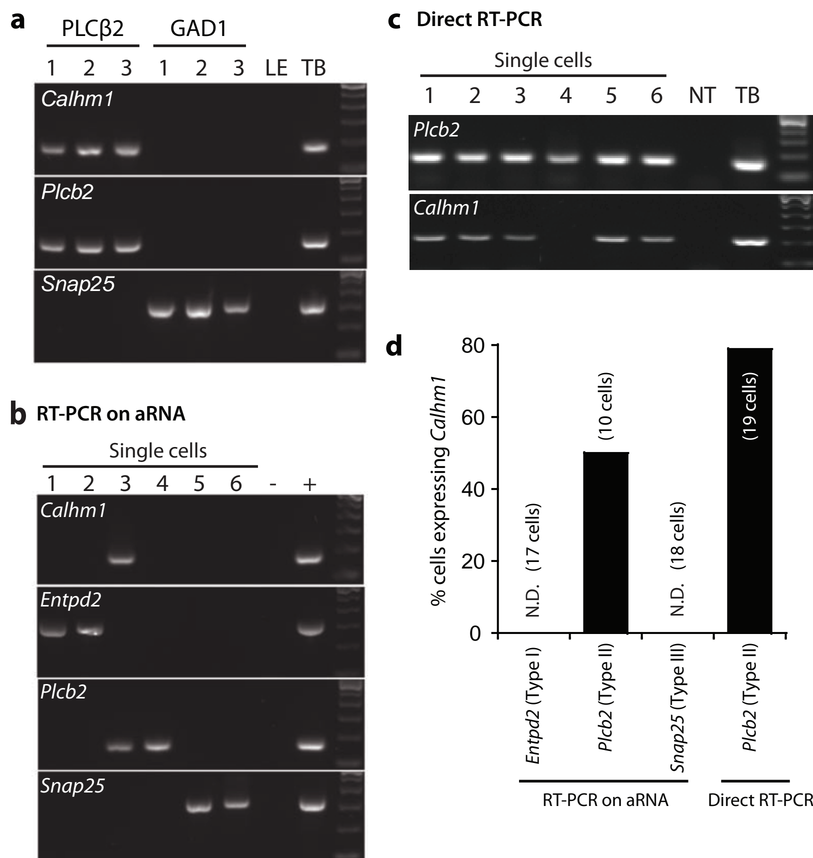

Figure S3: RT-PCR analysis of type II cell-specific expression of *Calhm1*. (**a**) mRNA from 3 pools of 10 GFP-positive taste cells isolated from CVP TB of PLCβ2-GFP (type II cells) or GAD1-GFP (type III) mice analyzed by RT-PCR. LE, LE RNA; TB, RNA from whole taste bud. (**b**) RT-PCR on linear-amplified 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.

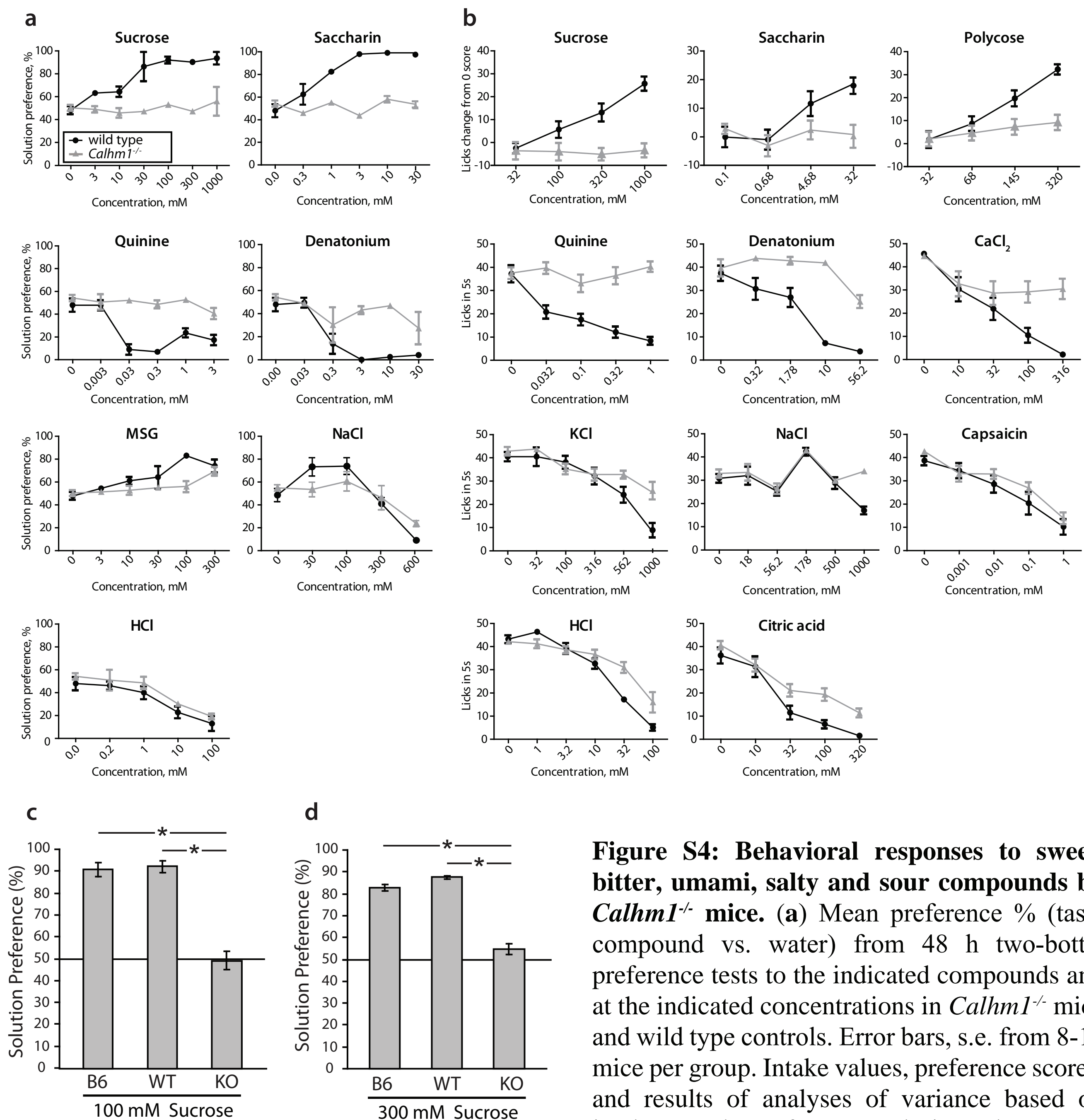

Figure S4: Behavioral responses to sweet, bitter, umami, salty and sour compounds by *Calhm1*$^{-/-}$ mice. (**a**) Mean preference % (taste compound vs. water) from 48 h two-bottle preference tests to the indicated compounds and at the indicated concentrations in *Calhm1*$^{-/-}$ mice and wild type 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).

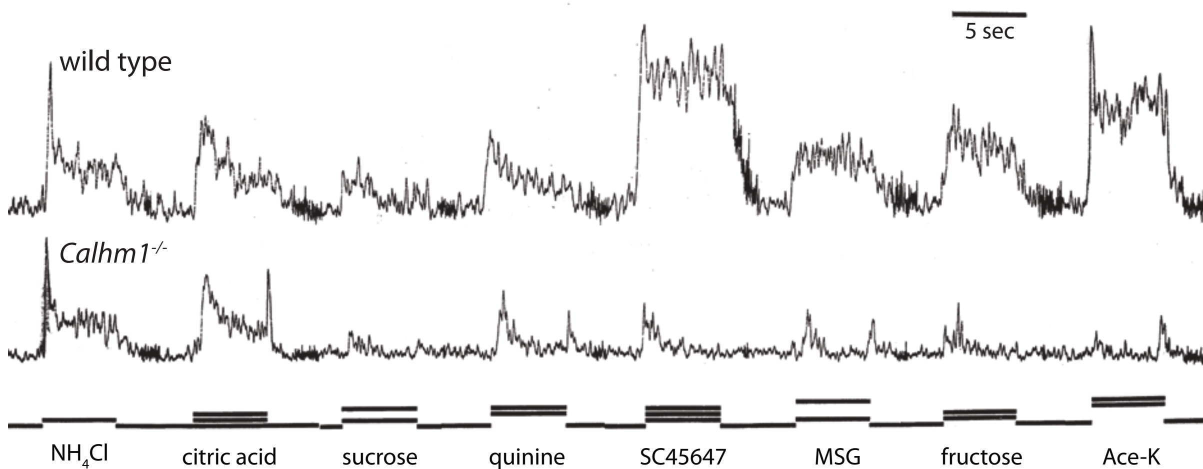

Figure S5: Gustatory nerve recordings upon lingual stimulation with sweet, bitter, umami, salty and sour compounds in *Calhm1*$^{-/-}$ mice. Whole-chorda tympani nerve recordings in *Calhm1*$^{-/-}$ and wild type mice stimulated with salty (100 mM NH_4Cl), sour (20 mM citric acid), sweet (100 mM sucrose; 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.

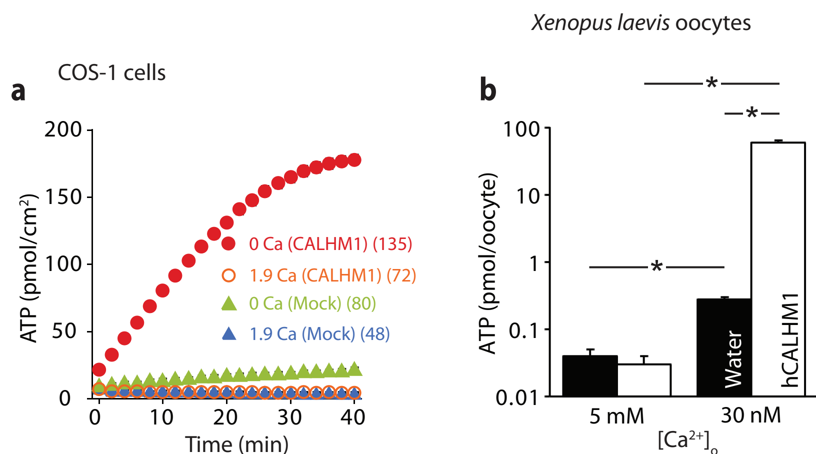

Figure S6: Low $[Ca^{2+}]_o$-evoked ATP release is strongly enhanced by CALHM1 expression in COS-1 cells and *Xenopus* oocytes. (**a**) Time courses of extracellular ATP levels due to release from mock- and hCALHM1-transfected COS-1 cells exposed to normal (1.9 mM) or zero (17 nM) $[Ca^{2+}]_o$. Cells 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).

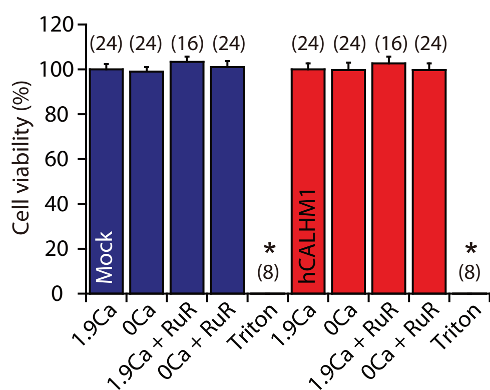

Figure S7: CALHM1 expression and function have no effect on cell viability. Exposure of mock- (blue) or hCALHM1- (red) transfected HeLa cells to normal (1.9 mM) or zero (17 nM) $[Ca^{2+}]_o$ for 60 min in the presence or absence of 20 μM ruthenium red (RuR) was without effect on cell viability. The thiazolyl 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).

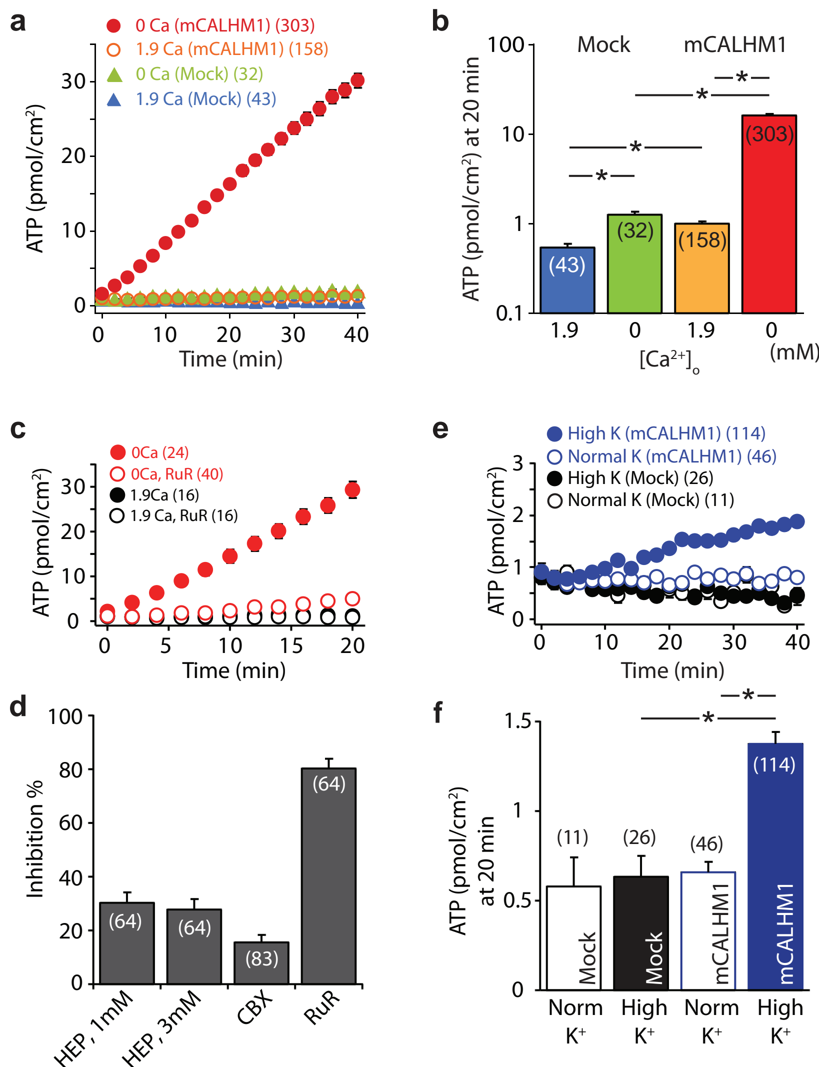

Figure S8: Mouse CALHM1 also forms an ATP release pathway with properties similar to human CALHM1. (**a**) Time courses of extracellular ATP levels due to release from mock- and mCALHM1-transfected HeLa cells exposed to normal (1.9 mM) or zero (17 nM) $[Ca^{2+}]_o$. (**b**) ATP levels at 20 min in (**a**). 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).

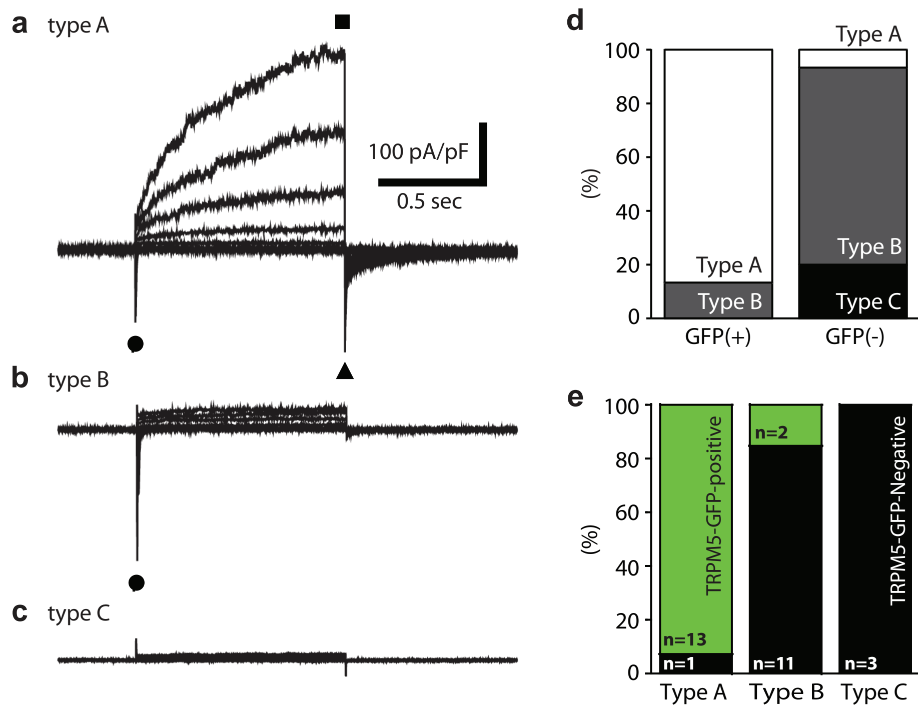

Figure S9: Type II cells are characterized by type A ion currents. Taste bud cells can be classified based on fingerprints of whole-cell voltage-gated currents as type A (corresponding to type II taste cell), B (type III taste cell), and C (type I taste cell)[13,18]. Whole cell recordings were made in 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.

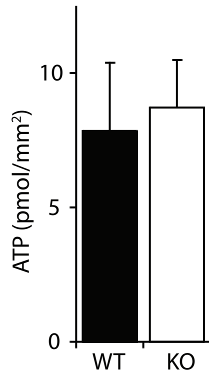

Figure S10: *Calhm1* deficiency does not affect the ATP content of taste buds. Intracellular ATP content of taste buds was estimated by treating the serosal side of CVP-containing tongue epithelial sheets from WT (n = 5) and *Calhm1*$^{-/-}$ (KO, n = 5) mice with Triton X-100/EDTA solution (0.5%/ 4 mM 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.

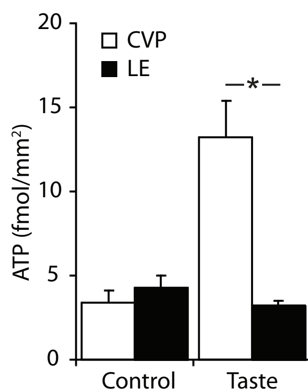

Figure S11: Taste stimulus evokes ATP release from gustatory CVP tissue but not from non-gustatory LE. Bitter mix (Taste) or only buffer (Control) was applied selectively to the apical side of tongue epithelial regions, CVP or LE, of WT mice and the amount of ATP released from the serosal side was measured. 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	Solution Intake p	Water Intake F	Water Intake p	Total Fluid Intake F	Total Fluid Intake p	Solution Preference F	Solution Preference 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 ± 3de	20 ± 3^{cd}
	32	30 ± 4^{e}	18 ± 3^{bcd}
Group x concentration		F(4,84) = 5.19, p < 0.0001	
QHCl	0	37 ± 4de	38 ± 3de
	0.032	21 ± 3^{c}	40 ± 3de
	0.1	18 ± 3^{bc}	33 ± 4^{d}
	0.32	12 ± 2^{ab}	37 ± 4de
	1	8 ± 2^{a}	41 ± 2^{e}
Group x concentration		F(4,76) = 9.62, p < 0.0001	
Denatonium	0	37 ± 3^{cd}	37 ± 4^{d}
benzoate	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 ± 2de
	316	32 ± 4^{cd}	33 ± 3de
	562	24 ± 3^{b}	33 ± 2de
	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 ± 2de
	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 ± 5de
	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.

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