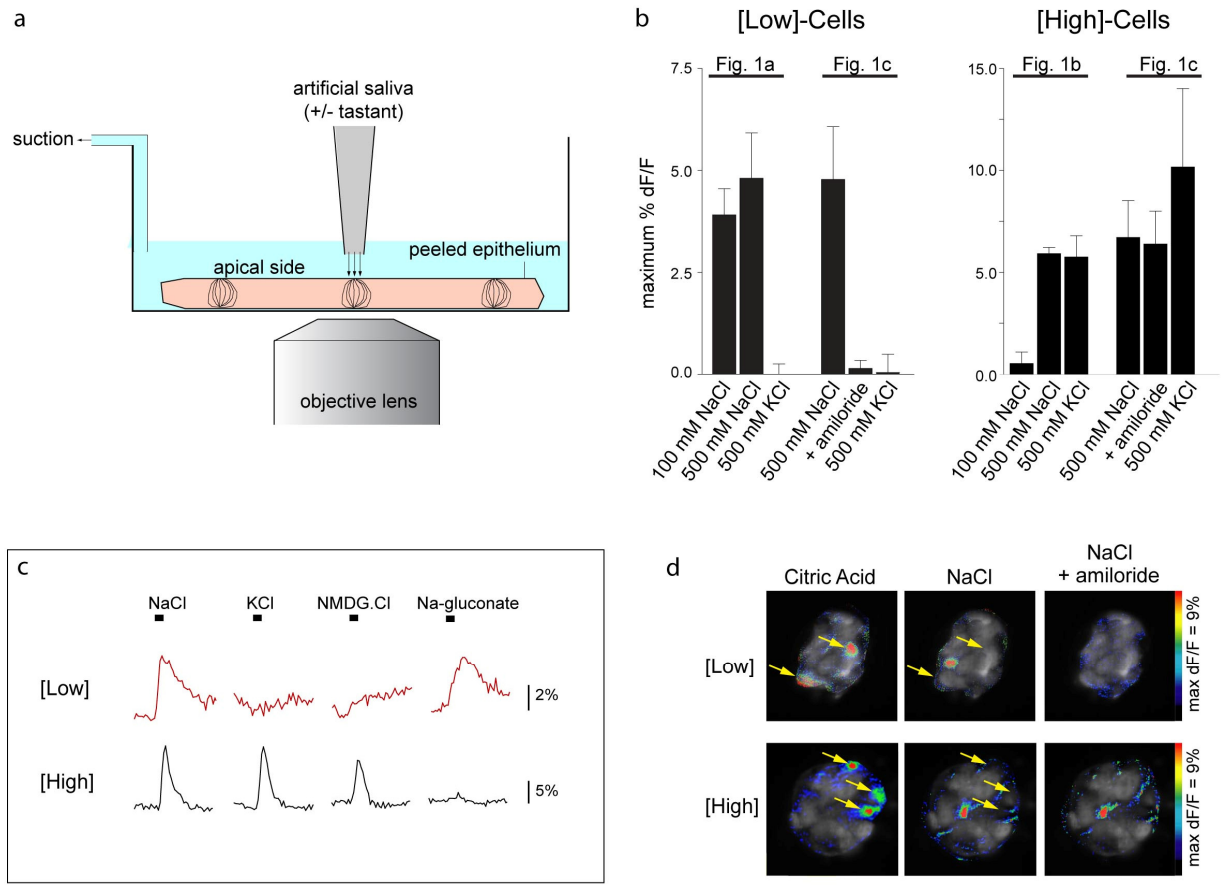
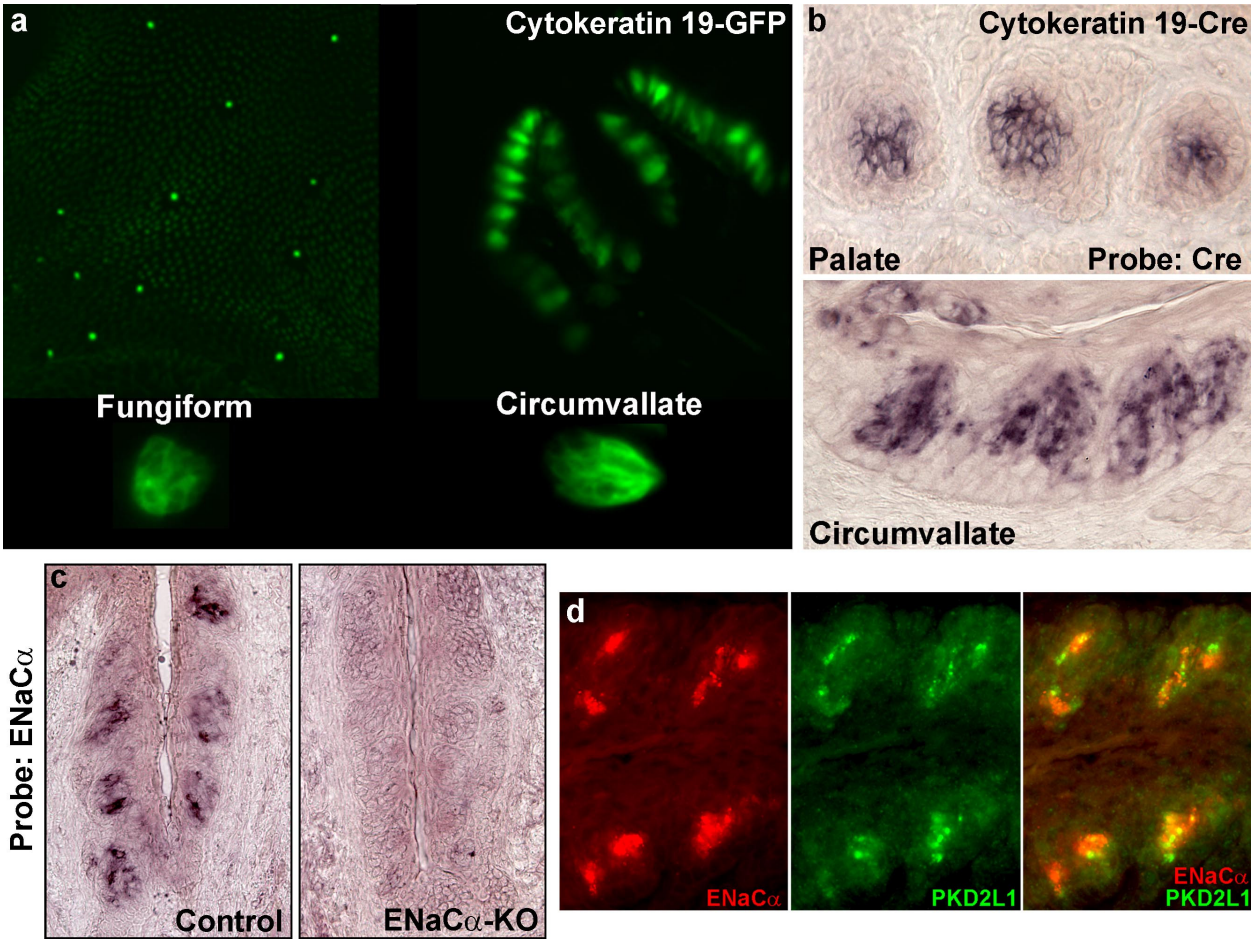
Supplementary Figure S1



Additional characterization of salt responses of [Low] and [High] responsive salt cells.

(a) Diagram illustrating the imaging preparation (see Methods for details). Taste buds are loaded *in vivo* with calcium green-1 dextran, and allowed to recover for 24 - 36 h after electroporation; the peeled epithelium housing fungiform taste buds is then mounted with the apical side facing the stimulus and the basal side facing the imaging lens. (b) Quantitation of responses shown in Figure 1. Shown are mean ± s.e.m. dF/F responses for the [Low] and [High] salt-responding cells (n≥3). (c) TRCs activated by low concentrations of NaCl ([Low]) show robust responses to sodium salts (e.g. sodium gluconate) but not to non-sodium salts. In contrast, cells that respond to high-concentrations of NaCl ([High]) are also stimulated by KCl and NMDG.Cl, but not by sodium gluconate; black bars indicate duration of application of tastants. (d) The same set of taste buds shown in Figure 1c are shown after sour stimulation. Note that selective but different subsets of fungiform TRCs respond to salt versus sour stimuli (yellow arrows). Tastants were 100 mM citric acid and 500 mM NaCl with or without 10 mM amiloride, and 500 mM each KCl, NMDG.Cl, sodium gluconate.

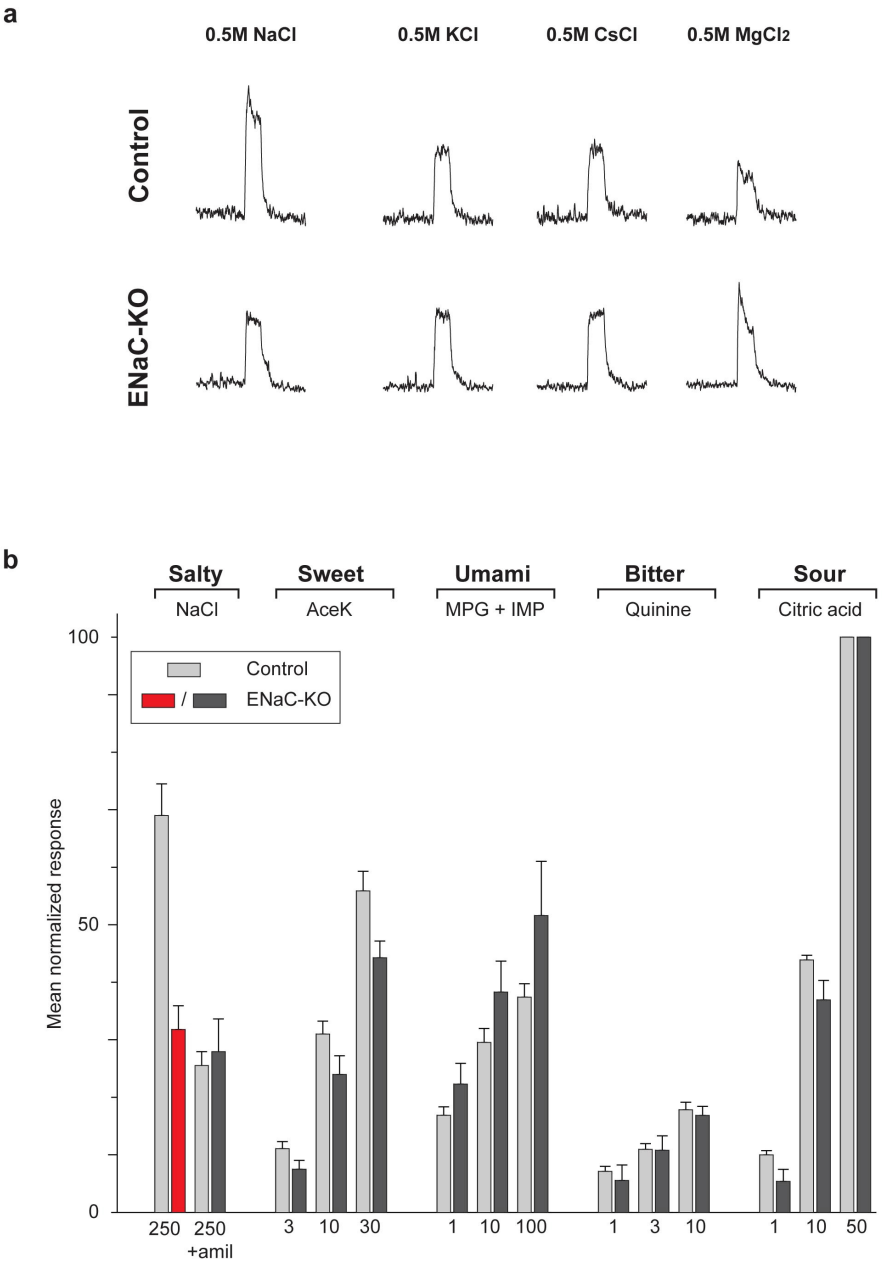
Supplementary Figure S2



Expression of ENaCα and cytokeratin 19 in TRCs of wild-type and mutant mice.

(a) Whole mount images of taste buds from tongues expressing GFP under the control of *cytokeratin 19*; see Methods for details. (b) *In situ* hybridization for *Cre-recombinase* in the *cytokeratin19-IRES-Cre* transgenic mice; note *Cre-recombinase* expression in all TRCs. No detectable expression was seen in other regions of the lingual epithelium. (c) In order to validate loss of *ENaCα* expression in the conditional knock animals, we performed *in situ* hybridizations with an *ENaCα*-specific probe. Note the loss of transcript in the knockout mice. (d) Two-colour *in situ* hybridizations demonstrating that *ENaCα* transcript is expressed in *PKD2L1/Car4*-positive cells of the circumvallate papilla in wild-type mice, just like the GFP reporter in the *ENaCα-IRES-Cre* animals. *In situ* hybridizations were performed as described previously[1, 2].

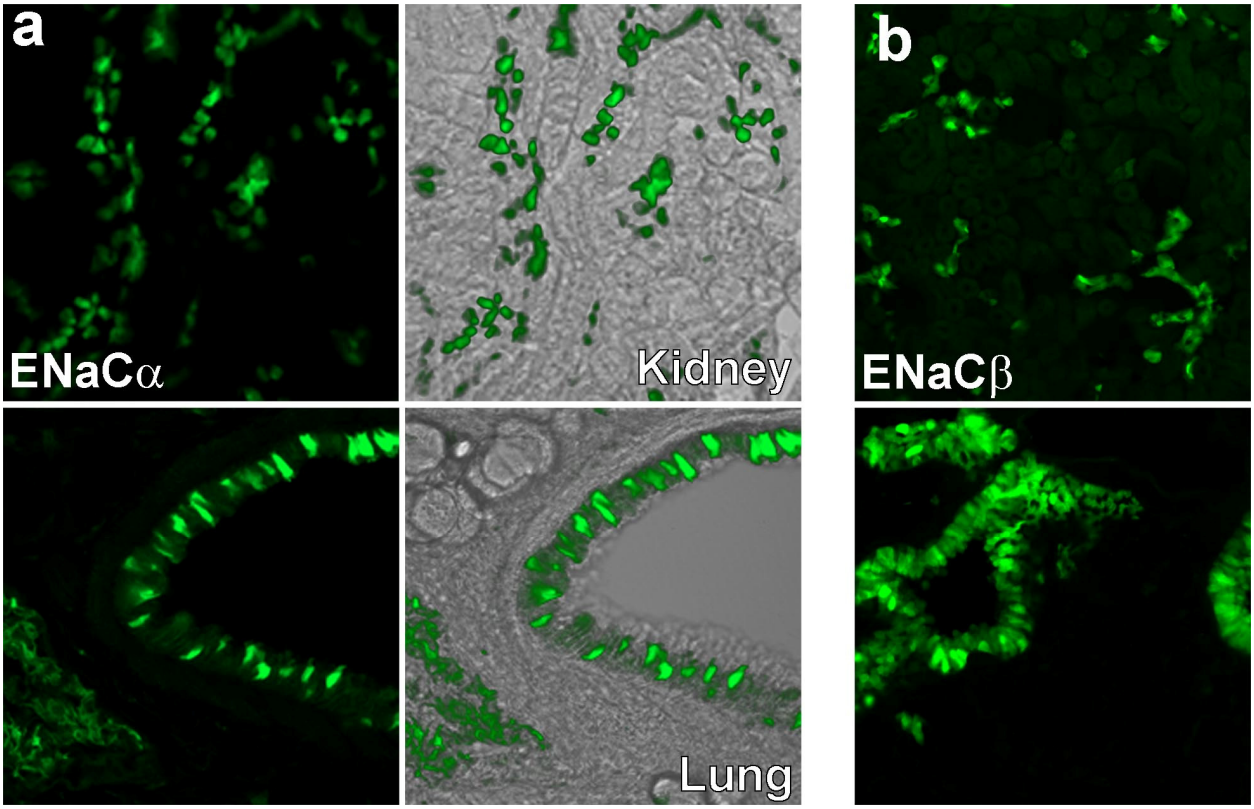
Supplementary Figure S3



ENaCα knockout mice respond normally to non-sodium cations.

(a) Shown are representative traces from control and knockout animals to 0.5 M NaCl, KCl, CsCl and $MgCl_2$. (b) Knockout animals exhibit normal taste responses to sweet, bitter, umami and sour stimuli. Shown are histograms of integrated neural recordings (mean ± s.e.m., n≥3) from the chorda tympani nerve of ENaCα-KO and age-matched control mice. The only significant differences are the responses to NaCl in the absence of amiloride (red bar; $P < 0.005$); traces were normalized to the responses to 50 mM citric acid.

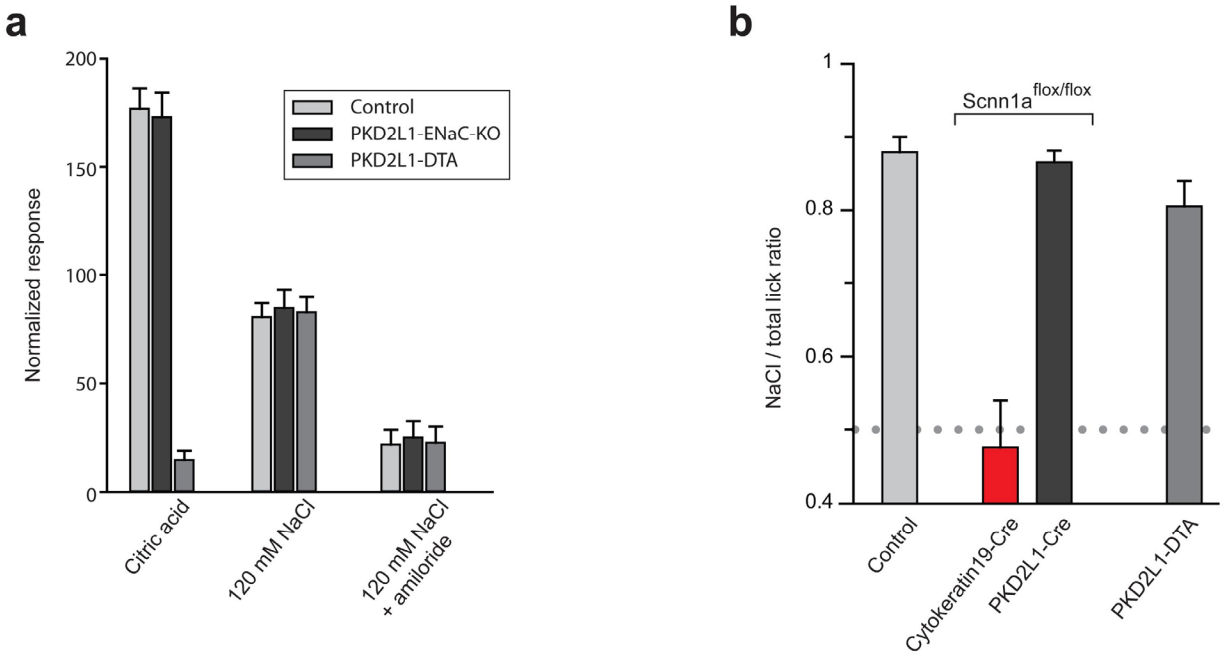
Supplementary Figure S4



ENaCα and β reporter lines mark expected populations of cells in kidney and lung.

ENaCα-IRES-Cre animals were crossed to *Z/EG* reporter lines[3] and double-positive progeny were examined for GFP expression. Reporter fluorescence was only detected in known sites of ENaCα expression, like the collecting duct cells and distal convoluted tubules of the kidney (a, upper panels) and in the airway epithelial cells of the lung (a, lower panels)[4, 5]. Equivalent results were obtained from four independent founders. (b) *ENaCβ-tTA/tetO-sapphire* mice (see Methods for details) show a similar pattern of expression in the kidney (upper panel) and the lung (lower panel); equivalent results were obtained for 3 independent founder lines.

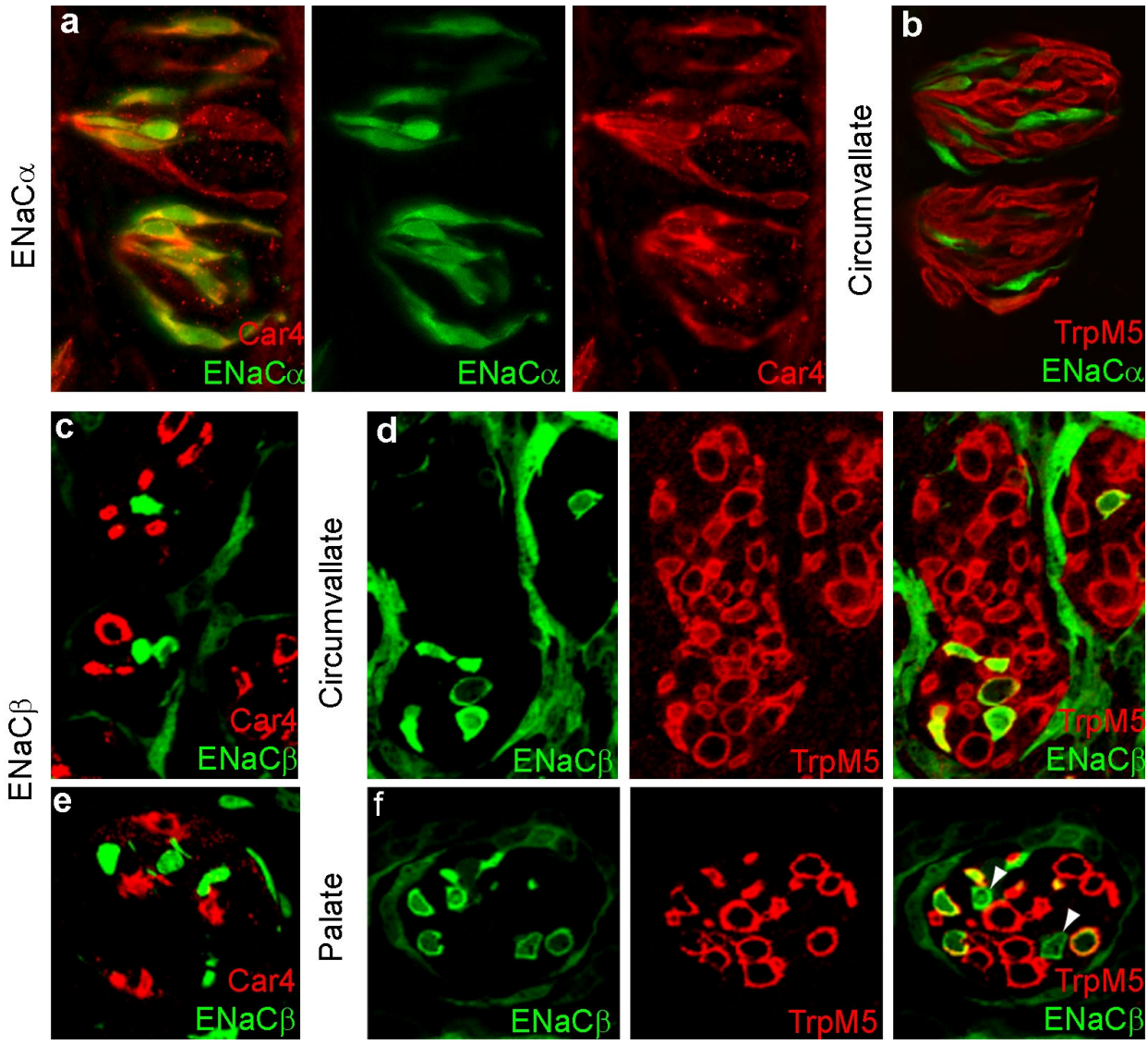
Supplementary Figure S5



ENaCα expression in sour cells plays no role in salt taste.

Animals with a conditional knockout of *ENaCα* in sour-cells (PKD2L1-ENaC-KO), or devoid of sour TRCs (PKD2L1-DTA)[6, 7] retain normal chorda tympani responses and behavioural attraction to NaCl. (a) Average neural responses to 50 mM citric acid and 120 mM NaCl in control with and without 10 μM amiloride, *PKD2L1-IRES-Cre/Scnn1a*$^{flox/flox}$ and *PKD2L1-IRES-Cre/ROSA-DTA* animals. Values are mean ± s.e.m (n=3); only the response of PKD2L1-DTA animals to citric acid was significantly different ($P<0.001$) from that of control animals. (b) Behavioural attraction of sodium-depleted mice to 120 mM NaCl in a 2-bottle immediate lick assay; shown are the fractions of licks NaCl / total licks for mice of different genotypes. As shown before (Fig. 3) animals with a global taste knockout of *ENaCα* (*Cytokeratin19-IRES-Cre/Scnn1a*$^{flox/flox}$; red bar) exhibit no preference for NaCl. In contrast, loss of *ENaCα* in sour cells (*PKD2L1-IRES-Cre/Scnn1a*$^{flox/flox}$), or even the total loss of sour TRCs (PKD2L1-DTA) has no impact on salt taste nerve responses (panel a) or behavioural preference (panel b); data are mean ± s.e.m (n≥7); responses of mice with global taste knockout of *ENaCα* were statistically significantly different from those of other strains (one way ANOVA), but the responses of the control, PKD2L1-DTA and sour-cell *ENaCα*-knockout animals were not statistically distinguishable.

Supplementary Figure S6



ENaCα and ENaCβ are expressed in completely non-overlapping populations of cells in the taste buds of the circumvallate papilla.

(a) ENaCα expression in circumvallate taste buds is confined to Car4 expressing sour-cells, and (b) is completely excluded from the TrpM5-expressing sweet, bitter and umami-cell population. The BAC transgenic lines driving Cre recombinase under the control of the *ENaCα* gene recapitulate this same pattern of expression (n=4 independent lines; see Supplementary Fig. S2). (c,d) Cross section through circumvallate papillae demonstrating that ENaCβ is not expressed in Car4 positive cells. Instead, ENaCβ is found exclusively in TRPM5-positive cells (as well as cells surrounding taste buds). Thus, the ENaCα-expressing Car4-positive cells cannot respond to salt as they lack the essential ENaCβ subunit. (e) Cross section through palate taste buds demonstrating that Car4-positive cells do not express ENaCβ. (f) As expected, there is a population of ENaC alone cells (arrows) that do not express TrpM5 or Car4 (see also Fig. 4).

References

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