Supporting Information

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SI Materials and Methods

Morphometrics. Images were obtained using a QImaging Retiga-4000RN camera mounted on an Olympus microscope with a 20× objective. Grayscale images were taken through a blue filter to enhance contrast of the reaction product.

The intensity of the histochemical stain was measured using an elliptical region of interest (ROI) from 500–1,000 μm^2 falling within the boundary of the taste buds in both nucleoside triphosphate diphosphohydrolase-2(NTPDase2)–KO and WT mice. Measurements of mean intensity were taken from three nonadjacent taste buds within each section through the circumvallate papillae from four different individuals of each strain. Because of variability in staining intensity across histochemical preparations, the tissue was reacted with paired WT and KO tissues in each batch to permit statistical comparison between genotypes.

To test whether the size of the circumvallate papilla differed between KO and WT mice, we measured the papillary volume in the four pairs of mice from the two strains used for histochemistry. In each section, a perimeter ROI was drawn around the raised central part of the papilla starting from the base of one crypt and ending at the base of the crypt on the other side with the bottom of the ROI being a straight line from the bottom of one side of the crypt to the bottom of the crypt on the other side. The total volume of the papilla in cubic micrometers then was determined by multiplying the sum of these cross-sectional areas by the distance between sections (144 μm). To determine taste bud size, we carried out detailed morphometric measurements on two individuals of each line. For these measurements we measured the size of each taste bud profile in every section through the circumvallate papilla using the elliptical selection tool in ImageJ. We then measured the length of epithelium that contained taste buds within each section by drawing a line along the contour of the crypt extending from the first to the last taste bud on each wall of the crypt. Dividing the number of taste bud profiles by the total length of epithelium yielded the average density of taste buds per unit length of epithelium, a figure that did not differ significantly between strains.

Immunohistochemistry. Sections were washed with PBS and were incubated with 2% normal donkey serum for 1 h at room temperature. After two or three washes in 0.1 M PBS (pH 7.2–7.4), antigen retrieval was preformed as necessary using 10 mM sodium citrate (pH 9.0) at 80 °C for 25 min. Three 10-min washes in PBS preceded and followed a wash with 3% hydrogen peroxide diluted in PBS. An avidin/biotin blocking kit (Vector Labs) was used during the blocking step. A 15-min avidin-blocking step was performed before a 45-min biotin-blocking step with short rinses in PBS between the two blocking times. Specific primary antibodies for each cell type then were applied to the sections. To mark type I taste cells, guinea pig anti-glutamate aspartate transporter (GLAST) [1:1,000; AB1782 (Chemicon), generated against a synthetic peptide carboxy terminus of rat GLAST, amino acids 521–543: KPYQLIAQDNEPEKPVADSETKM; lot 25010000] was diluted in blocking solution and incubated overnight at room temperature. To mark type II and type III cells, the tissue, after washes in PBS, was incubated overnight with either rabbit anti–Gα-gustducin [1:2,500; SC-395 (Santa Cruz Biotechnology); directed against amino acids 93–112 of Gα-gustducin of rat origin; lot: K1909] or rabbit anti-synaptosomal-associated protein 25 (SNAP25) [1:5,000 (Sigma) against synthetic peptide (NELEEMQRRADQLADESLEST-K); lot: 069k4784], respectively.

For tyramide-based detection, after incubation in the primary antiserum, the tissue was washed three times for 10 min each in PBS followed by incubation in a biotinylated donkey anti-guinea pig secondary antibody (1:1,000) (lot 91844; Jackson ImmunoResearch) for 1 h at room temperature. The solutions from a Vectastain ABC kit (Vector Labs) were prepared 30 min before use and were applied to the slides for 1 h at room temperature. After washes in PBS, a tyramide reaction using a TSA kit (Invitrogen) with Alexa-Fluor 568 was performed for 15 min. Overnight washing was needed to reduce the background. Double labeling was performed after the tyramide reaction. Secondary antibody donkey anti-rabbit Alexa-Fluor 488 (1:600) (lot 1028736 Invitrogen) and a Draq5 (lot 402DR50050 Abcam;) nuclear counterstains were diluted in blocking medium and incubated for 2 h. Sections were washed in PBS and coverslipped with Fluoromount (Southern Biotech).

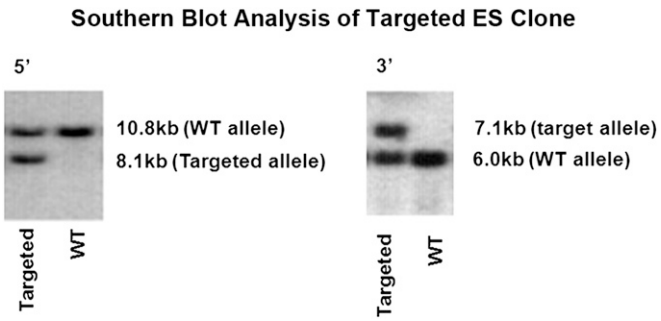

Fig. S1. The homologous recombination in the ES cells was confirmed by Southern blot analysis on both the 5′ and 3′ regions using probes generated by PCR, as described in *Materials and Methods*.

Table S1. Comparisons of circumvallate papillary volume and taste bud number between genotypes

	WT	KO
Mean papillary volume ± 95% confidence interval (um^3)	46,112,280 ± 3,910,040	38,908,640 ± 3,760,920
% difference ($P < 0.05$)		−15.6
Taste bud average minor axis (μm)	37.1	37.5
Raw taste bud profile counts		
Case 1	46	44
Case 2	42	50
Corrected taste bud counts		
Case 1	136.8	126.1
Case 2	121.6	147.8
Average no. of taste buds per papilla	129.2	137.0
Taste bud density (per 100-μm length of epithelium)	1.75	1.81

Table S2. Primers

Gene product	Accession no.	Forward primer (5′→3′)	Reverse primer (5′→3′)	Product (bp)	Anneal (°C)
NTPDase2	NM_009849	gacaaggaaaatgacacaggtatcgtgg	gttcaagacattcaaccagactc	124	61
GLAST	NM_148938	ccatcattgctgtggtgattggca	aaagtgatgggtagggtggcagaa	727	62
Gustducin*	NM_001081143	gcaaccacctccattgttct	agaagagcccacagtctttgag	286	58
TRPM5*	NM_020277	gtctggaatcacaggccaac	gttgatgtgccccaaaaact	234	58
PLCβ2*	NM_177568	gagcaaatcgccaagatgat	ccttgtctgtggtgaccttg	163	60
SNAP25	NM_011428	tgctgcagctggttgaagagagta	acttcccagcatctttgttgcacg	521	58

PLCβ2, phospholipase C β2; TRPM5, transient receptor potential melastatin 5.
*Primer sequences are taken from Tizzano et al. (1).

1. Tizzano M, et al. (2008) Expression of Galpha14 in sweet-transducing taste cells of the posterior tongue. *BMC Neurosci* 9:110.