Expression of *Calca* gene-derived peptides in the murine taste system

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Abstract

The Calcitonin Related Polypeptide Alpha (*Calca*) gene is a source of four biologically active peptides with varied physiological roles. Alternative splicing of the *Calca* messenger RNA generates either prepro calcitonin gene related peptide (CGRP) or preprocalcitonin encoding transcripts. Proteolytic processing of preprocalcitonin generates procalcitonin, calcitonin and katacalcin. Calcitonin is a ligand for the G-protein coupled receptor calcitonin receptor (CALCR) while CGRP is a ligand for the CGRP receptor (CGRP1R) formed by the calcitonin receptor like receptor (CALCRL)receptor activity modifying protein 1 (RAMP1) complex. Interestingly, procalcitonin too, is a ligand for the CGRP1R where it can antagonize CGRP. CGRP expression in taste neurons has been documented and is posited to regulate taste signaling. Single cell and bulk RNASeq of taste papillae revealed that the preprocalcitonin but not the CGRP transcript is expressed in *Tas1r3*- expressing type II taste cells, while *Calcrl* (but not *Calcr*) and *Ramp1* are expressed in stem/progenitor and type I cells in the circumvallate papillae. The CGRP1R is also expressed by fibroblasts in the lingual mesenchyme. We confirmed this expression pattern using quantitative polymerase chain reaction (qPCR), RNAScope and immunohistochemistry. qPCR of geniculate and nodose-petrosal ganglia revealed that both express *Cgrp* and CGRP1R subunit mRNAs, but not procalcitonin and *Calcr*. This interesting expression patterns suggests that procalcitonin and CGRP might reciprocally regulate the CGRP1R in taste cells and lingual fibroblasts and thereby influence taste signaling, taste cell regeneration and the taste microbiome.

Keywords

Neuropeptide, Procalcitonin, CGRP, Gustation

Introduction

The *Calca* gene is a source of up to four peptide signaling molecules. Tissue specific alternative splicing of *Calca* transcript generates mRNAs coding for either calcitonin gene related peptide (preproCGRP, proteolytically processed to alpha CGRP) or preprocalcitonin (prePCT).[1,2] PrePCT is proteolytically processed to generate procalcitonin (PCT), which is further processed to generate calcitonin and katacalcin.[3] All four belong to a larger group of peptides that include beta CGRP, amylin, adrenomedullin and intermedin, which are ligands for receptors formed by the GPCRs calcitonin receptor (CALCR) or calcitonin receptor like receptor (CALCRL).[4] CALCR and CALCRL combine with one of three receptor activity modifying proteins (RAMP1, RAMP2 or

RAMP3) to form receptor complexes specific to a subset of these peptides. For example, calcitonin is a ligand for CALCR (without an associated RAMP subunit), while CGRP is a ligand for both CALCR+ RAMP1 and CALCRL+RAMP1 (this being its primary receptor, henceforth designated CGRP1R).[4,5] CGRP is an exceptionally well-studied neuropeptide; it is expressed in virtually all peripheral sensory neurons, many central neurons, and some non-neuronal cells.[6-8] It serves a wide variety of functions including nociception, neurogenic inflammation, immunity, vasodilation, wound healing, regulation of the microbiome etc.[6-8] It is a potent neuro-immune modulator and was recently shown to modulate antigen sampling by M cells in the Peyer's patch.[9] It displays antimicrobial activity against several gram-negative and gram-positive bacteria and Candida albicans, which adds a further dimension to its immunomodulatory and wound healing roles.[10,11] From a clinical standpoint, its prominent role in triggering migraine has generated significant scientific interest.[6] This has led to the development of small molecule- and monoclonal antibody- based CGRP1R antagonists to treat migraines.[12,13] Interestingly, clinical studies have suggested that one-quarter of migraine patients have altered taste perception.[14] Indeed, CGRP was shown to shape taste transduction by inducing 5-HT secretion by type III taste cells in a phospholipase C-dependent manner.[15]

Calcitonin and katacalcin are key regulators of calcium and phosphate levels in blood and bone.[3,16,17] PCT is a much less studied neuropeptide. It is not widely secreted in the steady state; it may regulate bone density by suppressing osteoclast (macrophage) migration and maturation.[18] Interestingly, sepsis-associated cytokine storm is preceded by ubiquitous upregulation of PCT expression, leading to its adoption as an early sepsis marker.[19-22] PCT is a partial agonist of CGRP1R and exerts its mediator role in sepsis through this receptor. It also partially antagonizes CGRP at this receptor.[23-25] However, its biological role(s) in both the steady state and in sepsis remains enigmatic. Single cell RNASeq (scRNASeq) and bulk RNASeq of the circumvallate papillae (CVP) done in our lab showed that the prePCT encoding *Calca*-transcript is highly expressed in type II taste cells, while subunits of the CGRP1R are expressed in taste stem cells, type I taste cells and lingual mesenchymal fibroblasts. The CGRP transcript and *Calcr* were not expressed in taste tissues. This was confirmed using qPCR, RNAScope and immunohistochemistry. On the other hand, the nodose- petrosal and geniculate ganglia that innervate taste buds were shown to express the CGRP- transcript and CGRP1R subunits, but not the prePCT transcript and *Calcr*. This intriguing expression pattern suggests that taste cell derived PCT and taste nerve derived CGRP may reciprocally modulate CGRP1R in the taste papillae.

103 **Methods**

104 ***Animals.***

105 8-10 weeks old C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were used for this
106 study. Animals were housed in a specific pathogen free vivarium with a 12-h light/dark cycle and
107 open access to food and water. All animal experiments were performed in accordance with the
108 National Institutes of Health guidelines for the care and use of animals in research and reviewed
109 and approved by the Institutional Animal Care and Use Committee at University of Nebraska-
110 Lincoln (protocols: 2610 and 2366).

111

112 ***Bulk RNASeq of taste papillae.***

113 Pooled taste buds from CVP and fungiform papillae (FFP) of C57BL/6 mice (n=3 each) were
114 excised from CVP sections using laser micro dissection, and full length bulk RNASeq libraries
115 were prepared using the Ovation RNASeq system V2 (Tecan biosystems, Morgan Hill, CA) per
116 manufacturer instructions. Indexed illumina sequencing libraries were prepared and sequenced
117 in a Hiseq 2000 sequencer (Illumina Inc, San Diego, CA). Raw sequences were aligned to the
118 mouse reference genome (version GRCm38.p3) using the STAR program with default settings
119 and Gencode M24.gtf as the splice junction annotation file, and the reads mapping to genes
120 were counted using the featureCounts package.[26,27] Sashimi plots to show alignment of reads
121 were generated using the integrative genome viewer.[28]

122

123 ***scRNASeq of taste papillae.***

124 Chromium™ Single Cell 3′ Solution (10x Genomics Inc, Pleasanton, CA, Cat. no. PN-1000268)
125 was used for scRNASeq analysis.[29]Single cell preparation from CVP was done as previously
126 described.[30,31] A protease cocktail was injected under the lingual epithelium of excised tongue
127 (n=16 mice) and incubated at 37°C for ten minutes. The epithelia were peeled, the CVP were
128 excised and minced to form single cells. Single cell capture, library preparation, sequencing,
129 and primary analyses of sequencing data were done using 10X genomics protocols, and
130 secondary analysis was done using the Seurat package in R.[32]

131

132 ***RNAScope Hiplex assay.***

133 RNAscope assay was done using the Hiplex fluorescent assay kit for mice (Advanced Cell
134 Diagnostics, Hayward, CA, Cat. no. 324443) with indicated probes (Table S1) as previously
135 described using the manufacturer's instructions.[33] Positive and negative control probes were run
136 in parallel to test probes to ensure proper hybridization and imaging conditions were attained in

137 our experiments. Confocal images were captured using a Nikon A1R-Ti2 confocal laser
138 scanning microscope using NIS-Elements A1R software image acquisition and analysis
139 software, using 40/60X objectives. Images were taken using a sequential channel series setting
140 to minimize cross-channel signal, and the channels used were: GFP 488, TxRed 550, Cy5
141 650 nm. Z-series stack with 10 images per stack was captured at a step size of 1 μm.
142 Acquisition parameters [i.e., gain, offset, photomultiplier tube (PMT) setting] were held constant
143 for experiments. Colocalization counts were made using QuPath software.[34] Cell boundaries
144 were detected automatically based on DAPI staining. The fidelity of each cell boundary was
145 confirmed by manual inspection. The number of fluorescent spots in each channel (that
146 corresponds to individual mRNA molecules) per cell were extracted and used for colocalization
147 counting. Data from more than two non-consecutive sections from two mice were pooled.
148
149 ***Immunohistochemistry***
150 Standard immunohistochemical techniques were used as previously described.[33,35] Briefly,
151 frozen sections were rehydrated with PBS. Nonspecific binding was blocked with SuperBlock
152 Blocking Buffer (Thermo scientific, Waltham, MA, Cat. no. 37580) at room temperature for 1 h.
153 Sections were incubated with primary antibodies overnight at 4 °C in a humidified chamber.
154 After three 15-min washes with PBST, slides were incubated for 1 h at room temperature with
155 one of the following fluorescent secondary antibodies in blocking buffer. All double-
156 immunofluorescent labeling was done with combinations of the secondary antibodies along with
157 DAPI (1:1,000; Invitrogen™, Thermo scientific, Waltham, MA, Cat. no. D1306) to label cell nuclei
158 for cell counting. The primary and secondary antibodies and their concentrations used in this
159 study are listed in Table S2.
160 Double labeling with two antibodies made from the rabbit (LRMP+PCT and T1R3+PCT), was
161 done as described before.[36] After blocking, the sections were incubated in succession: first
162 primary anti-rabbit antibody overnight at 4 °C, first fluorescence Fab fragment secondary for 1 h
163 at room temperature, unlabeled anti-rabbit antisera [AffiniPure Fab fragment donkey anti-rabbit
164 IgG (H+L)] for 3 h at room temperature to ensure that all binding sites in the first primary
165 antibody are occupied, second primary anti-rabbit antibody overnight at 4 °C, and then second
166 fluorescence Fab fragment secondary for 1 h at room temperature. Controls for the double
167 immunohistochemistry experiments with two rabbit primary antibodies were done without
168 donkey Fab fragment incubation following the first secondary antibody and omitting the second
169 primary antibody (Figure S6 I & II, control A) or with donkey Fab fragment incubation and
170 omitting the second primary antibody (Figure S6 I & II, Control B) to show adequate blocking of

171 rabbit IgG by donkey Fab fragment. Confocal imaging was done as described above for
172 RNAScope. Colocalization for each taste cell marker was done using images from at least 2
173 sections showing entire CVP and FOP (n=1 mouse). Only those taste cells for which the entire
174 cell body and nucleus could be visualized were counted.
175
176 **Western Blot**
177 CVP tissue from three animals was isolated and homogenized in RIPA lysis and extraction
178 buffer (Thermo scientific, Waltham, MA, Cat. no. 89900). Protein quantification was done using
179 BCA method (Thermo scientific, Waltham, MA, Cat. no. 23227). Lysate were then mixed with 4
180 X laemmli buffer (Bio-Rad Laboratories Inc, Hercules, CA, Cat. no.1610747), and 30 μg of
181 protein run on SDS-PAGE, followed by transfer onto nitrocellulose membrane. Blots were
182 blocked in Blocker (Thermo scientific, Waltham, MA, Cat no. 37520) and incubated with PCT
183 primary antibody (Table S2) at 4 °C overnight. Incubation with secondary antibody was done for
184 1 h and imaged on Odyssey F Imager (LI-COR Inc. Lincoln, NE).
185
186 ***PCR and qPCR.***
187 Total RNA was isolated from freshly dissected geniculate and nodose-petrosal ganglia, taste
188 papillae or NT epithelium using the Quick-RNA Microprep kit (Zymo Research Corp, Irvine, CA,
189 Cat. no. R1050) with on-column DNA digestion. cDNAs were prepared from total RNAs using
190 SuperScript IV VILO Master Mix kit (Thermo Fisher, Waltham, MA, Cat. no. 11756050). End
191 point PCR and qPCR were done as previously described. Exon-exon junction spanning primers
192 were designed whenever possible to avoid amplification from any contaminating genomic DNA.
193 A minimum of three biological replicates were used for all cDNA samples. The ratio of the log10
194 of the average δ-cycle threshold (Ct) value (difference between Ct values of *Bact* and each
195 gene of interest was plotted. Primers used are shown in Table S3.
196
197 **Results**
198
199 ***Bulk RNASeq and PCR results to show the expression of Calca transcripts and receptors***
200 ***in taste papillae and ganglia.***
201 Analysis of bulk RNASeq data from CVP taste buds isolated by laser microdissection showed
202 that the *Calca* transcript is strongly expressed in CVP. Alignment of the RNASeq reads to the
203 *Calca* genomic locus showed that no reads mapped to the preproCGRP specific exon 5, while
204 large number of reads aligned to prePCT specific exon 4 (Figure S1). This observation was

205 confirmed using end point PCR using primers for the *Cgrp* and prePCT transcripts (Figure S2A),
206 which showed amplification for the prePCT transcript in CVP and foliate papillae (FOP), but not
207 the FFP. *Cgrp* transcript is expressed at very low levels or were undetectable in all three taste
208 papillae. We could readily detect transcripts for *Calcrl* and *Ramp1* in all three taste papillae and
209 for *Ramp2* in CVP, but not for *Calcr* and *Ramp3*. The transcript for *Calcb* that encodes beta
210 CGRP is expressed in all three taste papillae. None of the transcripts were expressed in non-
211 taste lingual epithelium (NT, Figure S2A). Next, we checked the expression of these transcripts
212 in the geniculate and nodose- petrosal ganglia that innervate the FFP and CVP and FOP
213 respectively. The prePCT transcript and *Calcr* are not expressed in either of them, while the
214 *Cgrp* transcript, *Calcrl, Calcb*, *Ramp1*, and *Ramp2* were detected in both (Figure S2B).
215 Next, we quantified the expression of these transcripts in all four tissues using qPCR.
216 Consistent with the end point PCR results, robust expression of prPCT is detected in both
217 papillae and weak expression of *Cgrp* is detected in the CVP but not FOP (Figure 1A).
218 Conversely, *Cgrp*, but not prPCT transcript is strongly expressed in both taste ganglia (Figure
219 1B). Robust expression of *Calcrl, Calcb and Ramp1* were detected in both the taste papillae and
220 ganglia, while *Calcr and Ramp3* are not expressed in any sample. Weak *Ramp2* expression is
221 detected in the two ganglia, while it is not expressed in the taste papillae (Figure 1).
222
223 ***scRNASeq of CVP identifies Calca and CGRP1R subunit expressing taste and***
224 ***mesenchymal cells.*** To identify taste cells that express *Calca* and CGRP1R subunits, we
225 turned to scRNAseq data from CVP. We found that *Calca* is expressed only in mature and
226 immature sweet taste cells. *Calcrl* is expressed in subtypes of type I cells and taste stem cells.
227 *Ramp1* expression was low overall, while the regulatory subunit of the CGRP1R, *Crcp* is
228 expressed widely across all cell types (Figure S3).
229
230 ***Western blot and histological analyses confirm the expression of Calca and Calcrl in***
231 ***taste cells.*** Using western blot analysis using an antibody specific to PCT, we detected its
232 expression in CVP (Figure S4). Next, we turned to histological analysis to confirm the cell type
233 specific patterns of *Calca* and *Calcrl*. RNAscope Hiplex analysis of CVP confirmed the
234 scRNASeq results: *Calca* is strongly coexpressed with the sweet taste receptor subunit *Tas1r3*,
235 with 148/164 (90%) of *Tas1r3* expressing cells coexpressing *Calca,* and 148/149 (99%) *Calca*
236 expressing cells coexpressing *Tas1r3* (Figure 2A- A″). *Calca* expressing cells also coexpressed
237 the pan type II taste cell marker *Trpm5,* with 131/437 (30%) of *Trpm5* expressing cell
238 coexpressing *Calca*, while 131/149 (88%) of *Calca* expressing cells coexpress *Trpm5* (Figure 2

239 B- B″). Most type II cells that do not express *Calca* appear to be bitter taste receptor cells, as
240 less than one tenth of *Gnat3* (primarily bitter taste cell marker in CVP) expressing cells
241 expressed *Calca* (Figure 2C- C″). *Calca* is not expressed in type III taste cells marked by *Ddc*.
242 (Figure 2D- D″). *Calcrl* staining is observed in basal (presumably stem/progenitor) cells in the
243 CVP taste buds (Figure 2E, E′). Significant staining for *Calcrl* is also observed in mesenchymal
244 cells close to basal cells of taste buds that stained strongly for the fibroblast marker *Sparc*
245 (Figure 2E, E′). Comparable results were obtained using double labeled immunohistochemistry.
246 Using a PCT specific antibody, we saw strong co expression of PCT with the sweet taste
247 receptor subunit T1R3 (100% in both directions) in CVP (Figure 3 A-E) and FOP (Figure 3 F-J).
248 In addition, PCT is strongly coexpressed with the pan-type II marker LRMP, with all PCT
249 expressing cells expressing LRMP and 33/45 (73%) of LRMP expressing cells coexpressing
250 PCT in the CVP (Figure 3 K-O). In case of LRMP, colocalization is observed primarily with cells
251 that stained strongly with LRMP antibody. The weaker LRMP positive cells primarily expressed
252 GNAT3, which, as alluded to before, is primarily expressed in bitter taste receptor cells in the
253 CVP (Figure S5).

254

255 **Discussion**

256 The *Calca* derived peptides CGRP, calcitonin and procalcitonin play key roles in health and
257 disease.[3,6-8,19,21,37,38] Using a slew of techniques probing the expression of the corresponding
258 mRNAs and proteins, we show that the prePCT encoding transcript is strongly expressed in
259 CVP and FOP, almost exclusively in the *Tas1r3* expressing type II (primarily sweet and umami
260 receptor expressing) taste cells. Curiously, *Calca* expression (both prePCT and *Cgrp* mRNAs) is
261 not observed in FFP (Figure S2). The related *Calcb* gene is expressed in all three taste papillae.
262 All three taste papillae express the CGRP1R subunits *Calcrl* and *Ramp1* (Figure S2, Figure 1).
263 Interestingly, the taste ganglia that innervate the papillae expresses the *Cgrp* but not the
264 prePCT transcript and expresses both the CGRP1R subunits (Figure S2, Figure 1). A small
265 amount of *Cgrp* transcript is detected in the CVP using endpoint PCR and qPCR, although it is
266 likely derived from the nerve endings that innervate the taste papillae rather than taste cells
267 themselves (Figure S2, Figure 1). This is supported by the absence of *Cgrp* transcript in the
268 bulk RNASeq data from CVP, derived from laser microdissected taste buds devoid of
269 contamination from nerve bundles in the CVP core that would be present in the CVP samples
270 used for PCR experiments (Figure S1). CGRP expression in trigeminal neurons is well studied,
271 and it is very likely that trigeminal neurons that innervate the taste papillae also express CGRP,
272 although this has not been experimentally demonstrated to our knowledge.[7] Thus, it is possible

273 that the bulk of alpha CGRP in the taste papillae is nerve derived. This is also supported by
274 immunolocalization studies of CGRP in rodent, pig and human taste papillae, all of which show
275 CGRP expression restricted to nerve bundles and their termini in and around taste buds.[15,39-41]
276 PrePCT is processed to generate PCT and then to calcitonin and katacalcin. We were able to
277 detect the robust expression of prePCT transcript using end point and qPCR and could detect
278 PCT protein expression using western blot (Figures S2, S4 and 1). Although the RNAScope
279 probe for *Calca* does not distinguish between the two alternatively spliced mRNAs, we obtained
280 strong staining in TAS1R3- and LRMP- expressing taste cells with the PCT antibody (Figure 2,
281 Figure 3). However, these experiments do not allow us to determine if calcitonin and katacalcin
282 are produced by taste cells. Since both peptides are part of PCT, they cannot be readily
283 distinguished using antibody staining. Notably, their biological roles are distinct from that of PCT;
284 both are well known hormones produced by the parathyroid gland that bind to the calcitonin
285 receptor to regulate bone and serum calcium and phosphorus levels.[16,17] PCT on the other
286 hand, is an early marker of sepsis, which is only expressed at very low levels in healthy
287 individuals.[19,24] Notably, we did not detect the calcitonin receptor mRNA *Calcr,* in the taste
288 papillae or the geniculate and nodose-petrosal ganglia (Figure S2, Figure 1). Thus, the available
289 evidence indicates that calcitonin and katacalcin are either not produced by taste cells and
290 nerves or will not be biologically effective in case they are produced. PCT on the other hand,
291 can stimulate CGRP1R, and it also antagonizes CGRP at this receptor.[23-25] Considering
292 abundant CGRP1R receptor expression in taste papillae and ganglia, PCT is the only peptide
293 generated from taste cell expressed prePCT transcript capable of exerting its biological role.
294 What are the likely roles of PCT and CGRP in the taste system? The amount of peptides
295 produced by the taste cells will not be sufficient to elevate their circulating levels, and their
296 effects will be mediated by paracrine signaling within the taste papillae. We know a lot about the
297 biological roles of CGRP. One study that looked at the effects of CGRP in isolated taste buds
298 using calcium imaging and bioassay showed that CGRP may regulate taste signaling by
299 regulating 5-HT signaling by type III cells.[15] We (and the original study) did not detect CGRP1R
300 expression in type III cells. We detected *Crcp* expression in many taste cell types using
301 scRNASeq, which agrees with their findings (Figure S2). Using scRNASeq, we detected
302 CGRP1R expression in type I cells (Figure S2). Type I cells can regulate taste signaling, which
303 is an alternative explanation for CGRP's ability to regulate taste signaling.[42] We found that
304 *Calcrl* is expressed in taste stem cells using scRNASeq and RNAScope (Figure S3, Figure 2).
305 However, *Ramp1 was not detected by scRNASeq* which is likely a false negative due to the
306 limitations of scRNASeq, as we could detect it using qPCR. CGRP regulates stem cell

307 maintenance, and CGRP1R expression in basal cells in the taste buds and adjoining fibroblasts
308 raises the possibility that taste nerve derived CGRP regulates taste stem cells directly, and
309 indirectly by regulating adjoining fibroblasts (Figure 2).[43,44] CGRP can also regulate immunity at
310 the taste cells through its effects on immune cells, epithelial cells and fibroblasts, and can shape
311 the oral microbiome directly through its microbicidal effects.[8,11,37,45-47] It is also capable of
312 regulating microfold cells that mediate microbial transcytosis in the Peyer's patch.[9] We have
313 shown that type II taste cells might mediate immune surveillance similar to microfold cells, and it
314 is possible that CGRP plays a similar role in taste papillae.[33] Sepsis-associated cytokine storm
315 is preceded by ubiquitous PCT expression, leading to its adoption as an early sepsis marker.
316 However, very little is known about its role in normal physiology. In the skeletal system, it may
317 regulate bone density by suppressing osteoclast (macrophage) migration and maturation.[18] It's
318 expression in CVP and FOP but not FFP might provide some clues to its function in taste cells.
319 Unlike the FFP, the CVP and FOP have deep trenches around which the taste buds are
320 arranged. The trenches are relatively less exposed to salivary flux and may be more hospitable
321 to the oral microbiota, including those responsible for halitosis, and plausibly, pathogenic as
322 well. This raises the possibility that PCT might regulate the taste papillae microbiome in much
323 the same manner as CGRP. In addition, it has the potential to regulate taste signaling and taste
324 cell regeneration by regulating type I and stem/progenitor cell expressed CGRP1R. As stated
325 before, PCT is a partial agonist of the CGRP1R, and it can partially antagonize the effects of
326 CGRP at this receptor. Thus, it is plausible that PCT and CGRP reciprocally shape the biological
327 effects CGRP1R signaling in the taste papillae. Thus, the taste papillae might be a suitable
328 model system to determine the biological roles of PCT and its cross talk with CGRP.
329

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

SRP and AHS: performing experiments, acquiring the data, data analysis, interpretation of the results, creation of the figures, and revision of the manuscript. SKS: conceptualization and design of the study, supervision of the data acquisition, interpretation of results, writing of the manuscript.

Data availability.

The underlying bulk and scRNASeq data are being uploaded to NCBI's short read archive and will be available very soon.

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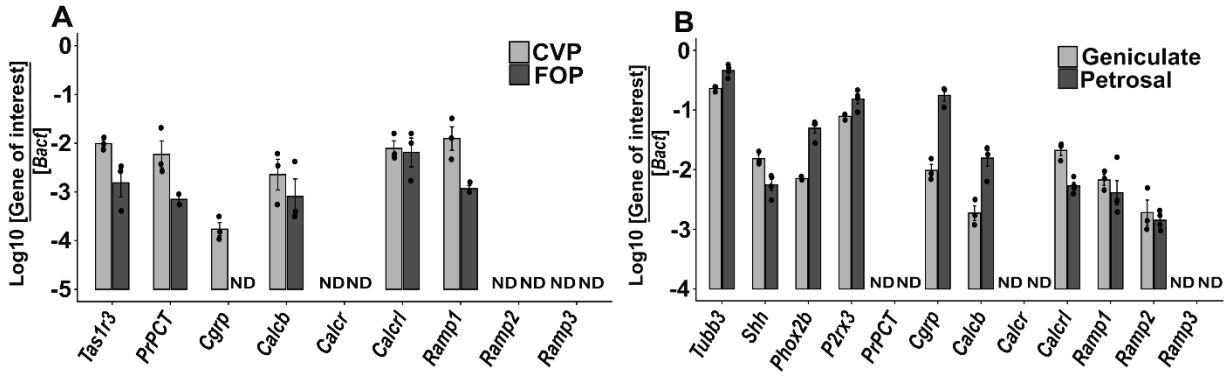

Figure 1. qPCR profiling of *Calca and Calcb* transcripts and their receptors in cDNA from taste papillae and sensory ganglia. A) Strong expression of *PrPCT, Calcb*, *Calcrl* and *Ramp1* is observed in both CVP and FOP. Weak expression of *Cgrp* is observed in CVP, while it is undetectable in FOP. *Calcrl and Ramp1* are expressed in both CVP and FOP while *Calcr, Ramp2 and Ramp3 are* not detected in either CVP or FOP. *Tas1r3* is used as a control to demonstrate the quality of taste cDNA. B) qPCR of above transcripts in geniculate and nodose-petrosal ganglia. *Cgrp* and *Calcb* are expressed in both ganglia, with stronger expression observed in the nodose-petrosal ganglion. *Calcrl, Ramp1 and Ramp2* are expressed in both ganglia and similar levels. Expression of *Ramp3*, *PrPCT* and *Calcr* is not observed in either ganglion. The taste ganglion marker genes *Tubb3, Shh, Phox2b*, and *P2rx3* are used to demonstrate the quality of ganglia cDNA. The expression of each gene is plotted as the logarithm of the ratio between its cycle threshold value and that of *Bact*. ND= not detected.

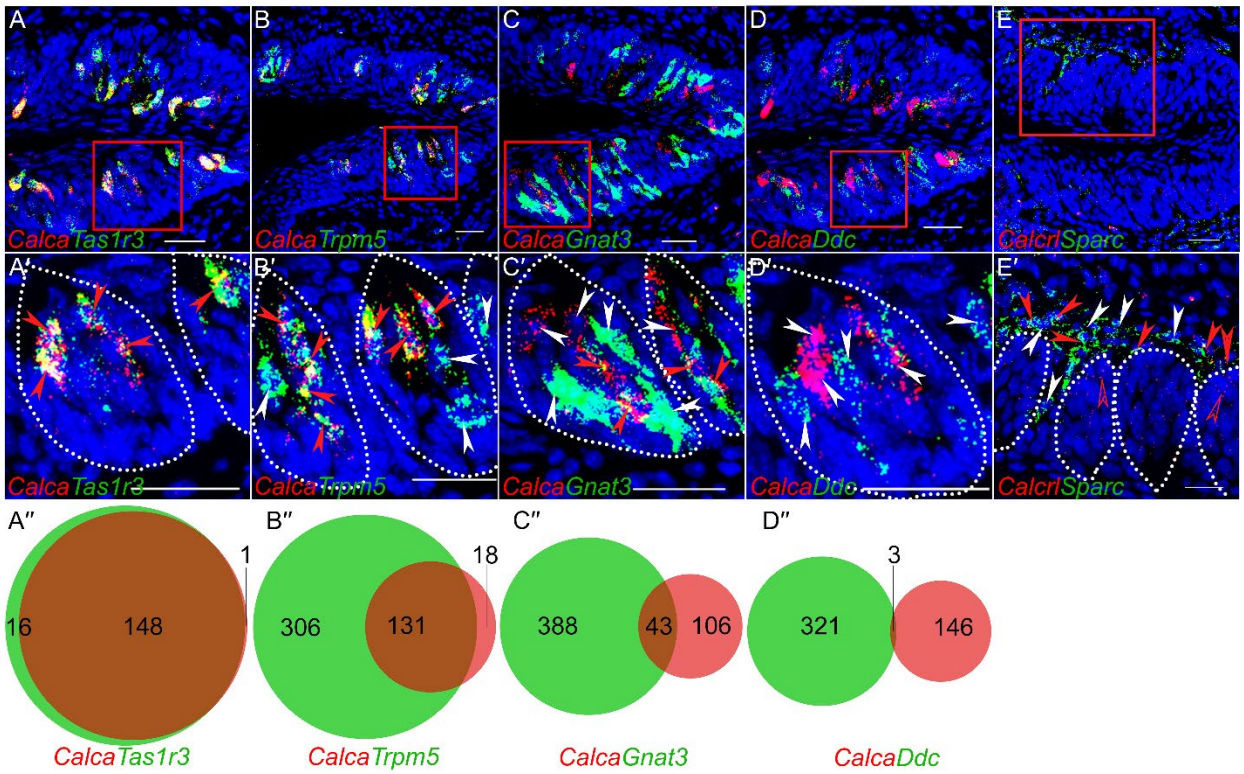

Figure 2. RNAScope analysis of *Calca* and *Calcrl* gene expression in CVP. RNAscope Hiplex fluorescence assay was used to determine the coexpression of *Calca* with the taste cell markers *Tas1r3* (A-A′), *Trpm5* (B-B′), *Gnat3* (C-C′) and *Ddc* (D-D′). The areas highlighted in red boxes in the top row is magnified in the bottom rows for each set. Taste buds are highlighted by white dotted lines. A″-D″ are venn diagrams showing the number of taste cells that co express or singly express the indicated marker genes and *Calca*. Data are from two non-consecutive sections from two mice. E & E′ shows the expression of *Calcrl* in fibroblasts adjacent to basal taste cells marked by *Sparc*. Strong coexpression of *Calca* is observed with *Tas1r3* and *Trpm5*, less strong coexpression is observed with *Gnat3* and negligible coexpression is observed with *Ddc*. *Calcrl* expression is observed in basal (presumably stem/progenitor) cells in the taste buds and adjacent fibroblasts. Filled white arrowheads highlights single positive cells, filled red arrowheads highlights double positive cells, and open red arrowheads depicts basal cells in taste buds expressing *Calcrl*. Scale bars = 30 µm.

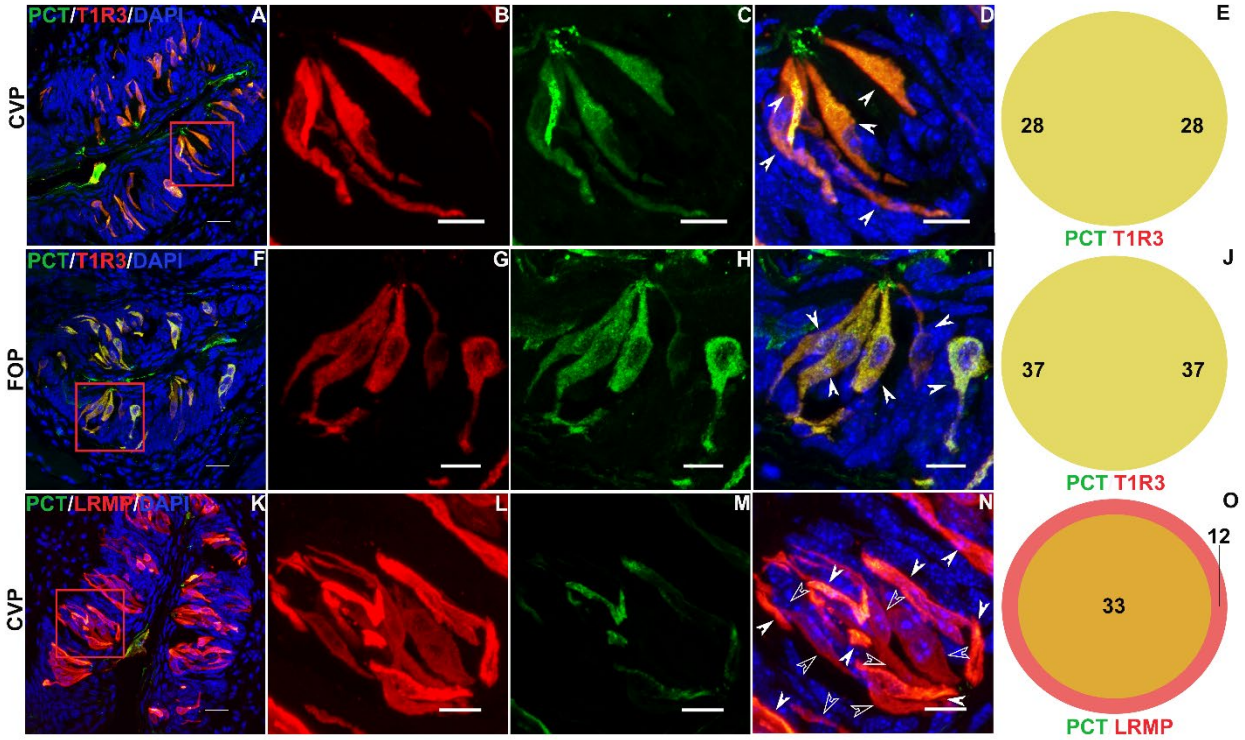

Figure 3. PCT is expressed in type II taste cells. Double labelled immunofluorescence confocal microscopy of CVP and FOP sections with antibodies against PCT (green) and type II taste cell markers T1R3 (red; A–D, F-I) or LRMP (red; K–N). Nuclei are counterstained with DAPI (blue). A, F, K are lower magnification images and dashed red boxes indicate regions shown at higher magnification in panels in the right. (D, H, L) Higher-magnification views of the boxed region showing double positive cells with solid arrows and single positive cells hollow arrows. Colocalization counts are shown in Venn diagrams (E,J,O). Scale bars = 20 µm.

Supplementary data

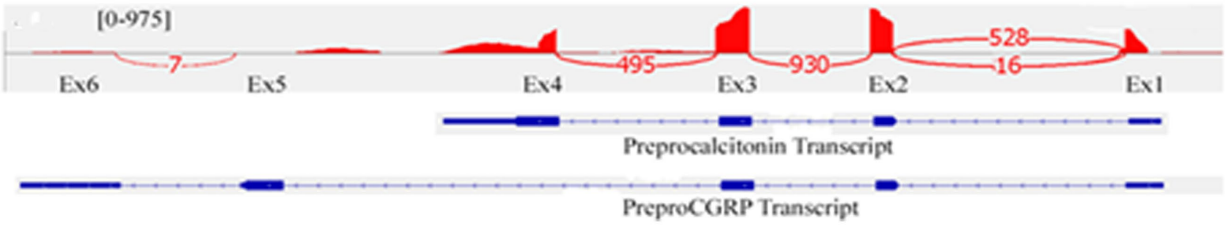

Figure S1. Sashimi plot of the *Calca* gene locus (transcribed from bottom strand in right to left direction) from bulk RNASeq data from CVP. The reads aligning to exons 1-6 (Ex1-Ex6, right to left) are shown in red, with the height of the red bars showing the strength of expression, and the number of reads aligning across splice junctions indicated in the loops between exons. No reads align to the preproCGRP specific exon 5, while large number of reads align to prePCT specific exon 4.

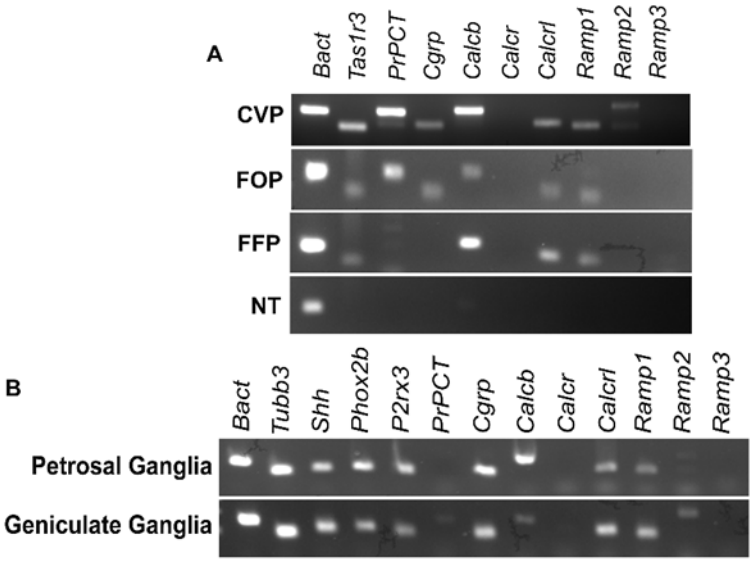

Figure S2. End point PCR showing expression of Calca- related mRNAs. **A)** Expression of indicated transcripts in taste papillae and non-taste lingual epithelium (NT). *PrPCT, Calcb*, *Calcrl* and *Ramp1* expression is observed in CVP, FFP and FOP. Weak *Cgrp expression* is observed in CVP and FOP, while it is undetectable in FOP and FFP. *Calcrl and Ramp1* are expressed in all taste papillae while *Calcr, Ramp2 and Ramp3 are* not detected in any tissue. None of the tested mRNAs are detected in NT. *Tas1r3* is used as a control to demonstrate the quality of taste cDNA, and *Bact* is used as cDNA synthesis control. **B)** qPCR of above transcripts in the geniculate and nodose-petrosal ganglia. *Cgrp*, *Calcb, Calcrl, Ramp1 and Ramp2* are expressed in both ganglia. Expression of *Ramp3*, *PrPCT* and *Calcr* is not observed in either ganglion. *Bact* is used as cDNA synthesis control, while the taste ganglion marker genes *Tubb3, Shh, Phox2b*, and *P2rx3* are used to demonstrate the quality of ganglia cDNA.

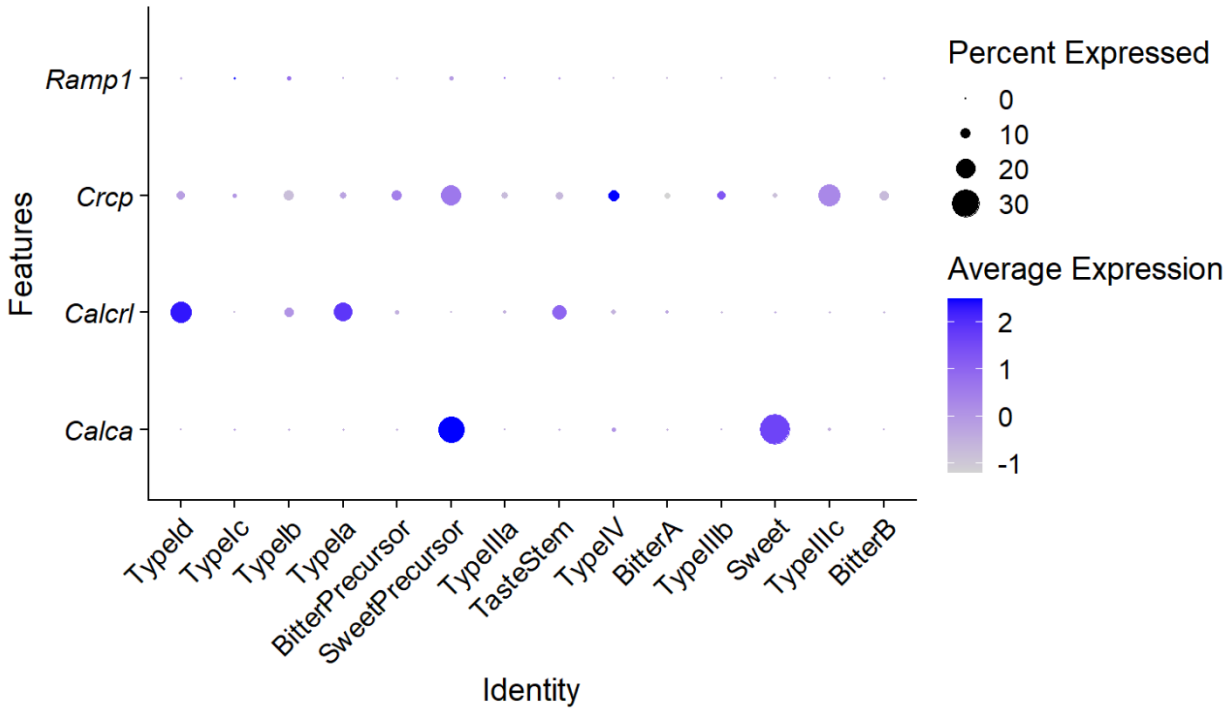

Figure S3. Dot plot from CVP. Type I a-d, type IIIa-c and type IV, sweet and bitter taste receptor cells are detected. BitterPrecursor and sweetPrecursor are clusters of Immature type II immature cells. TasteStem is a cluster of stem cells. Strong *Calca* (=prePCT) mRNA expression is seen in sweet taste receptor cells and immature type II cells, and *Calcrl* expression is observed in type I and taste stem cells, and *Crcp* expression is found in multiple cell types. *Ramp1* could not be detected in this dataset.

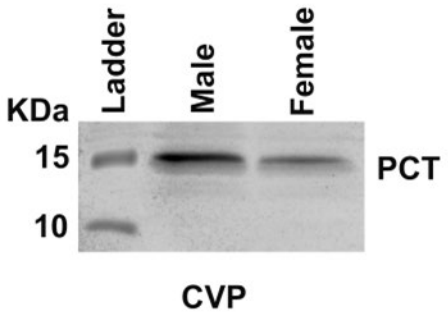

Figure S4. Western blot analysis of protein extract from CVP of male and female mice with anti-PCT antibody showing expression of PCT.

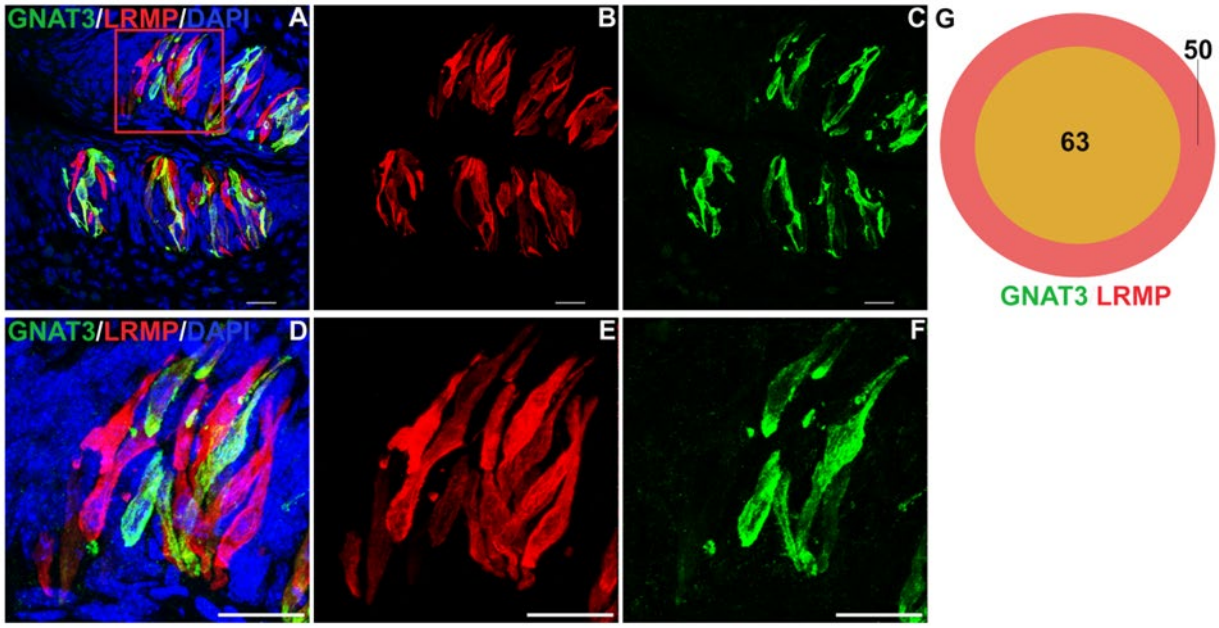

Figure S5. Double labeling for GNAT3 and LRMP. A-F) Unlike PCT staining shown in Figure 3, GNAT3 staining is restricted to weaker LRMP expressing cells with less intense staining for LRMP. **G)** Venn diagram shows quantification of coexpression data.

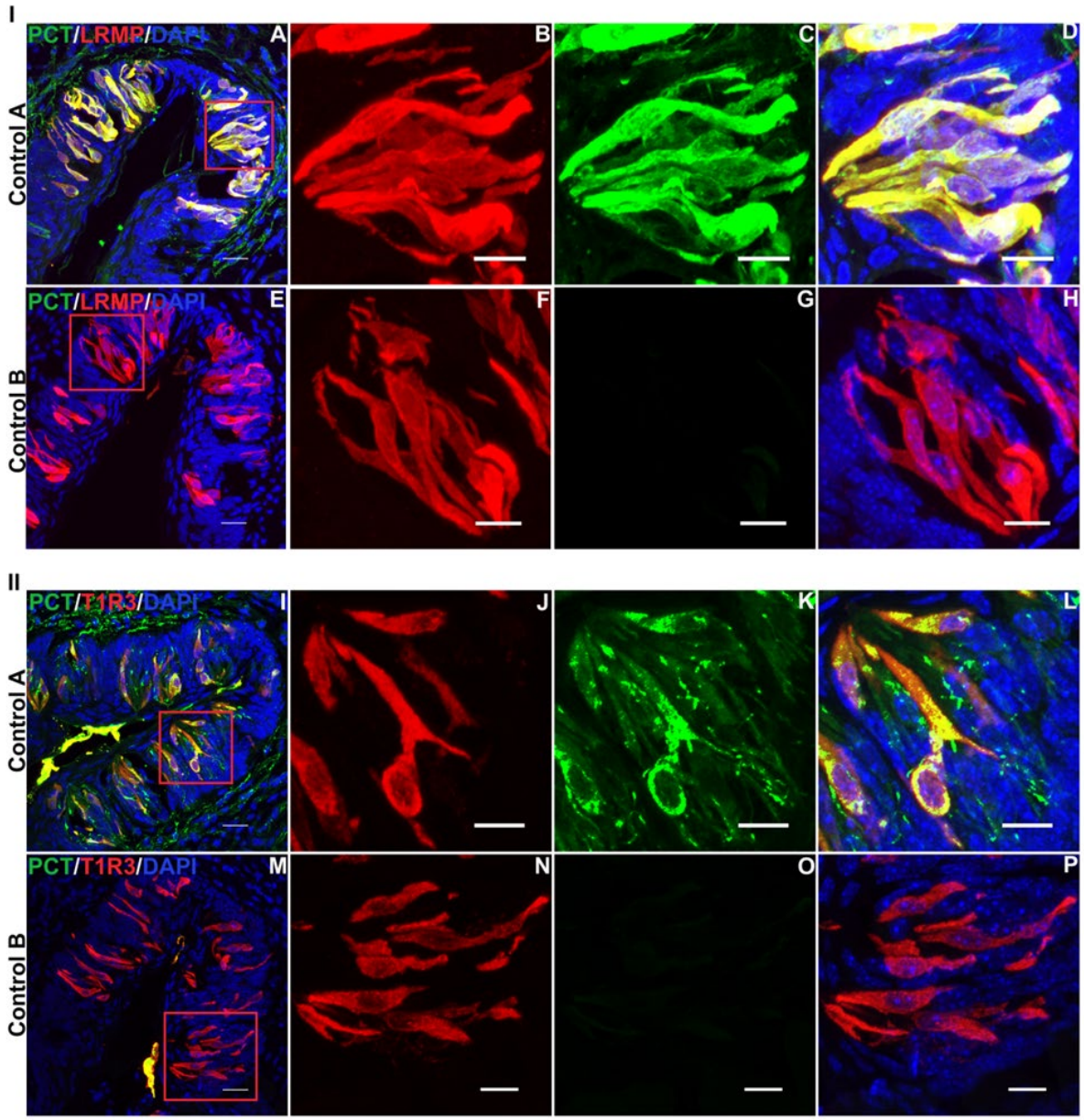

Figure S6. Controls for immunostaining with two rabbit primary antibodies. Double indirect immunofluorescence confocal microscopy of CVP shows cross-labeling when unlabelled donkey Fab fragment is omitted (control A, *I & II*), whereas adequate blocking of rabbit IgG is achieved with excess donkey Fab fragment (control B, I and II).

Probe	CAT number	LOT number
RNAscope™ HiPlex Probe- Mm-Ddc-T1	318681-T1	22363A
RNAscope™ HiPlex Probe- Mm-Tas1r3-T2	515431-T2	22174B
RNAscope™ HiPlex Probe- Mm-Sparc-T4	466781-T4	24017A
RNAscope™ HiPlex Probe- Mm-Calcrl-T5	452281-T5	24017A
RNAscope™ HiPlex Probe- Mm-Calca-T6	578771-T6	223634
RNAscope™ HiPlex Probe- Mm-Gnat3-T8	531661-T8	22171A
RNAscope™ HiPlex Probe- Mm-Trpm5-T9	453251-T9	22363A

613

614 **Table S1:** List of RNAscope HiPlex Probes used in this study.

615

Antibody	Host Species	Catalog No.	RRID	Source	Dilutions
PCT	Rabbit	LS-C296040	-	LS Bio, Newark, CA	1: 50
GNAT3	Goat	OAEB00418	AB_1088282	Aviva Systems Biology, San Diego, CA	1:500
LRMP	Rabbit	ORB166443	-	Biorbyt, Durham, NC	1:600
T1R3	Rabbit	-	-	Gift from Dr. Ichiro Matsumoto, Monell Chemical Senses center	1:500
Alexa Fluor 488 Conjugated AffiniPure Fab Fragmant Goat anti-Rabbit IgG (H+L)	Goat	111-547-003	-	Jackson Immuno Research Inc. West Grove, PA	1:500

Alexa Fluor 647 Conjugated AffiniPure Fab Fragmant Donkey anti-Rabbit IgG (H+L)	Donkey	711-607-003	-	Jackson Immuno Research Inc. West Grove, PA	1:500
Alexa Fluor 647 Conjugated AffiniPure Donkey anti-Goat IgG (H+L)	Donkey	705-606-147	-	Jackson Immuno Research Inc. West Grove, PA	1:500
Alexa Fluor 488 Conjugated Donkey anti-Goat IgG	Donkey	A11055	-	Jackson Immuno Research Inc. West Grove, PA	1:500
AffiniPure Fab Fragment Donkey anti-Rabbit IgG(H+L)	Donkey	711-007-003	-	Jackson Immuno Research Inc. West Grove, PA	1:500

616

617 **Table S2:** Primary and secondary antibodies for IHC and western blot used in this study.

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Gene	Forward Primer	Reverse Primer
Bact	GGCTGTATTCCCCTCCACG	CCAGTTGGTAACAATGCCATGT
Calcrl	CATCGTGGTGGCTGTGTTT	GTAATACAAGCTTCTGGCAATGG
Calcr	GCTGAGTGCAGAAACCCACT	TTTGCCTCATCTTGGTCACA
Ramp1	AGCCGCTTCAAGGAGAACAT	CGTGCTTGGTGCAGTAAGTG
Ramp2	TGAGGACAGCCTTGTGTCAA	CAGCACAGCAGAAAGGTTCC
Ramp3	AAGTTGGTTTTGGACGGTGA	GCATACCTGGGCACACTCA
Tubb3	GAACCTGGAACCATGGACAG	GTTGTTGCCAGCACCACTCT

Shh	AAGGATGAGGAAAACACGGG	GGTCACTCGCAGCTTCACTC
Phox2b	CACTTTTGGGGCCACGTC	CGTGGTCGGTGAAGAGTTTG
P2rx3	CCAAATATTCCTTCACTCGGC	GCTGCCATTCTCCATCTTGT
PrePCT	GGAGCAGGAGGAAGAGCAGG	GCCAGGTGCTTCAACCCCAA
Cgrp	TATGCAGATGAAAGCCAGGG	GTGGCAGTGTTGCAGGATCT
Calcb	CAGGAAGCTGGAACAGGAGG	AAGGCTTCAGAGCCCACATC
Tas1r3	CAAGTTCTTCAGCTTCTTCC	GGCGGCCACCCAGTTCCAGC

Table S3: List of primers used for PCR and qPCR.