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Reassessing the genetic lineage tracing of lingual *Lgr5*$^{+}$ and *Lgr6*$^{+}$ cells *in vivo*

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ABSTRACT

Taste buds, the neuroepithelial organs responsible for the detection of gustatory stimuli in the oral cavity, arise from stem/progenitor cells among nearby basal keratinocytes. Using genetic lineage tracing, *Lgr5* and *Lgr6* were suggested as the specific markers for the stem/progenitor cells of taste buds, but recent evidence implied that taste buds may arise even in the absence of these markers. Thus, we wanted to verify the genetic lineage tracing of lingual *Lgr5*- and *Lgr6*-expressing cells. Unexpectedly, we found that antibody staining revealed more diverse *Lgr5*-expressing cells inside and outside the taste buds of circumvallate papillae than was previously suggested. We also found that, while tamoxifen-induced genetic recombination occurred only in cells expressing the *Lgr5* reporter GFP, we did not see any increase in the number of recombined daughter cells induced by consecutive injections of tamoxifen. Similarly, we found that cells expressing *Lgr6*, another stem/progenitor cell marker candidate and an analog of *Lgr5*, also do not generate recombined clones. In contrast, *Lgr5*-expressing cells in fungiform papillae can transform into *Lgr5*-negative progeny. Together, our data indicate that lingual *Lgr5*- and *Lgr6*-expressing cells exhibit diversity in their capacity to transform into *Lgr5*- and *Lgr6*-negative cells, depending on their location. Our results complement previous findings that did not distinguish this diversity.

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Introduction

Gustatory stimuli are detected solely by taste buds, the sensory organs specialized for chemosensation in the oral cavity (Yarmolinsky et al. 2009; Liman et al. 2014; Jang et al. 2021). Despite several neuron-like features, such as cellular excitability (Seto et al. 1999), neurotransmitter release (Finger et al. 2005; Huang et al. 2005; Huang et al. 2011), and synaptic connections to the nervous system (Lee et al. 2017), taste buds are epithelial cells with fungiform (FuP), foliate (FoP), and circumvallate papillar (CVP) subtypes that arise from stem/progenitor cells in a basal layer of *Krt14*-expressing keratinocytes (Okubo et al. 2009; Golden et al. 2021). Basal keratinocytes divide symmetrically to reproduce themselves with the conservation of their cellular identities. They can also divide asymmetrically to differentiate into multiple layers of *Krt13*-expressing suprabasal keratinocytes (also known as corneocytes) in a squamous stratified epithelium or into *Krt8*-expressing taste buds.

Although how the fate of stem/progenitor cells is determined remains unclear, several signaling pathways have been suggested as the molecular determinants of taste bud differentiation and maintenance. These include the epidermal growth factor (EGF) (Liu et al. 2008), fibroblast growth factor (FGF) (Petersen et al. 2011), bone morphogenic protein (BMP) (Nguyen and Barlow 2010), Notch (Seta et al. 2005), sonic hedgehog (Shh) (Golden et al. 2021), and Wnt/β-catenin pathways (Gaillard et al. 2015, 2017). Genetic disruption of *Wnt10a*, which encodes the dominant Wnt ligand in the oral mucosa, resulted in a developmental defect in taste papillae (Xu et al. 2017). The roles Wnt/β-catenin signaling plays in adult taste bud tissue homeostasis have been investigated by taking advantage of the temporal control the Cre/lox system provides to circumvent the embryonic lethality of Wnt/β-catenin mutant mice (Gaillard et al. 2015, 2017). Removal of β-catenin led to the loss of taste buds (Gaillard et al. 2017), but the induction

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of a constitutively active form of β-catenin led to a taste cell hyperplasia comprising primarily type I cells (Gaillard et al. 2015). Consistent with this result, loss of either RNF43 or ZNRF3, which inhibit the protein stability of the direct Wnt ligand receptor Frizzled (FZD) (Hao et al. 2012; Koo et al. 2012), increased the number of CVP taste buds. This transformed pattern is quite different from that induced by the expression of a constitutively active form of β-catenin (Lu et al. 2022).

The functional significance of Wnt/β-catenin signaling led to the discovery of molecular markers for epithelial stem cells in alimentary organs. The initial discovery of *Lgr5* in intestinal stem cells not only revealed the physiology of intestinal renewal (Barker et al. 2007), which had been debated for a long time, it also opened new avenues for the development of adult stem cell-based organoids (Sato et al. 2009). This breakthrough has inspired the adoption of similar genetic approaches to uncover adult stem cells in many other organs (Jaks et al. 2008; Barker et al. 2010).

To identify taste bud stem/progenitor cells, several groups independently applied genetic lineage tracing techniques with the *Lgr5-eGFP-IRES-CreERT2* and *Lgr6-eGFP-IRES-CreERT2* strains to arrive at the conclusion that *Lgr5*- and *Lgr6*-exressing cells serve as stem/progenitor cells for intragemmal cells in taste buds (Takeda et al. 2013; Yee et al. 2013; Ren et al. 2014). Organoid forming assays also supported these tracing data in that *Lgr5*- and *Lgr6*-expressing cells were far superior to *Lgr5*- or *Lgr6*-negative cells in generating organoid colonies with more mature taste cells (Ren et al. 2014; Aihara et al. 2015).

Recently, however, conflicting reports have indicated that taste buds can arise even in the absence of *Lgr5* expression. Adenovirus-induced overexpression of R-spondins (RSPOs), a group of collateral potential Wnt/β-catenin agonists, led to the production of ectopic taste buds that did not originate from *Lgr5*-expressing cells (Lin et al. 2021). Even more directly, the regeneration of taste buds following gustatory nerve injury seems to arise from both *Lgr5*-expressing cells and surviving intragemmal taste cells (Adpaikar et al. 2023). These seemingly conflicting results caused us to question the original genetic lineage tracing experiments. Here, we decided to repeat the genetic lineage tracing of *Lgr5*- and *Lgr6*-expressing cells in adult lingual mucosa to clarify these points.

Materials and methods

This study is compliant with the ARRIVE (Animal Research Reporting of In ViVo Experiments) guidelines. See Supplemental for further details.

Mice

All animal manipulation was carried out with the approval of the Institutional Animal Care and Use Committee of Korea University (KOREA-2022-0140). Mice were raised under standard conditions (22°C; 12 hours light–dark cycle; ad libitum access to a standard chow diet and water). *Lgr5-eGFP-IRES-CreERT2* (#JAX008875) (Sato et al. 2009), *Lgr6-eGFP-IRES-CreERT2* (#JAX016934) (Snippert et al. 2010), *Rosa26-lsl-tdTomato* (#JAX007908) (Madisen et al. 2010), and *Rosa26-lsl-diphteria toxin A* (DTA) (#JAX009669) (Voehringer et al. 2008) mice were imported from the Jackson Laboratory. *Lgr5-tdTomato* and *Lgr6-tdTomato* mice were generated by crossing *Rosa26-lsl-tdTomato* to *Lgr5-eGFP-IRES-CreERT2* and *Lgr6-eGFP-IRES-CreERT2*, respectively. *Lgr5-DTA* mice were generated by crossing *Lgr5-eGFP-IRES-CreERT2* to *Rosa26-lsl-DTA*. *Lgr5-DTA-tdTomato* mice were generated via multiple crosses. Genotyping was carried out by PCR with the primer pairs listed in Supplemental Table 1.

Tamoxifen injection

Mice 8–10 weeks of age were treated with tamoxifen (T5648, Sigma, MA, USA) dissolved in corn oil (C8267, Sigma). For single tamoxifen injections, the mice were injected intraperitoneally with 0.2 mg/g tamoxifen and sacrificed 1, 4, 7, 14, 21, or 60 days after injection. For multiple tamoxifen injections, the mice were given daily intraperitoneal injections with 0.1 mg/g tamoxifen for 7 or 14 consecutive days and sacrificed the day after the final injection.

Immunohistochemistry (IHC)

Mice were euthanized and perfused with 0.1 M phosphate buffered saline (PBS) and 4% paraformaldehyde (PFA) in PBS. Whole tongue and intestine were isolated, post-fixed in 4% PFA at 4°C overnight, and then transferred to 30% sucrose in PBS at 4°C for 48 hrs. Samples were embedded in Tissue-Tek OCT (4583, Sakura Finetek, CA, USA). Anterior tongue samples were cut into coronal sections with a thickness of 40 μm, and every third free-floating section was collected for further processing. The posterior tongue was cut into coronal sections with a thickness of 12 μm and directly attached to slide glass (HMA-S9911, MATSUNAMI, Japan). Intestine samples were cut into serial sections with a thickness of 12 μm and directly attached to slide glass.

The sections were blocked in 10% normal goat or donkey serum dissolved in 0.2% Triton X-100 in PBS

(PBS-T) for 30 min. Primary antibodies diluted in blocking solution were applied to the sections overnight. After three consecutive washes in PBS-T, the sections were incubated in the appropriate secondary antibodies diluted in PBS-T for 2 h. DAPI stain (1:5,000; D9542, Sigma) was added after three more PBS-T washes, and the slices were cover-slipped using anti-fade fluorescence mounting medium (ab104135, Abcam, Cambridge, UK). The primary and secondary antibodies that were used are listed in Supplemental Table 2. Images were acquired with an LSM 900 confocal microscope (Zeiss, Oberkochen, Germany).

Antibody generation

The coding sequence for C-terminal 70 amino acids of TRPM5 was cloned into pRSETA (Thermo Fisher Scientific, Waltham, MA, USA). His-tagged fusion protein was expressed in bacteria was purified using HisPur™ Ni-NTA Resin (Thermo Fisher Scientific), and inoculated at two guinea pigs. Unpurified antisera were verified by immunohistochemistry with CVP of TRPM5 KO mice and used as polyclonal antibodies against TRPM5.

Single-cell data analysis

We reanalyzed the single-cell RNA-seq data for the mouse CVP from NCBI GEO (accession GSE220065) using Scanpy package. After filtering the cells expressing less than 6000 genes, and more than 20 mitochondrial genes, we are acquired total 5487 cells, and used them for further analysis. By principle component analysis (PCA) with the top 50 PC values and a resolution value of 1.8, cell clusters were identified using a shared nearest neighbor (SNN) modularity optimization-based clustering algorithm. A total of 32 clusters were identified, and the clusters were visualized using Uniform Manifold Approximation and Projection (UMAP). Then, marker genes were used to distinguish intragemmal taste cell clusters from perigemmal cell clusters: *Entpd2* (type I taste cells); *Trpm5*, *Plcb2* (type II taste cells); *Pkd2l1*, *Car4* (type III taste cells); and *Shh* (type IV taste cells). We isolated 416 intragemmal cells and visualized them with an independent UMAP plot. The expression levels of *Lgr5* and *Lgr6* genes were indicated with intensity-coded colors on the entire and intragemmal UMAP plots.

Statistics

Statistical analysis was conducted using SPSS software, version 27.0 (IBM, Armonk, NY, USA). The proportion of overlapping and non-overlapping FuP staining patterns in *Lgr5-tdTomato* mice was compared using the Chi-square test.

Results

Broad expression of Lgr5 *both in intragemmal and perigemmal regions*

Previous studies indicated that *Lgr5* is expressed exclusively in basal keratinocytes (Yee et al. 2013; Ren et al. 2014). Using knock-in mice that express GFP under the control of the endogenous *Lgr5* promoter and enhancers, we carefully examined GFP expression in CVP. As native fluorescence was fainter than in the intestine, we performed the antibody staining experiments against GFP. Surprisingly, we detected GFP signals that had been invisible in the unstained sections (Figure 1(A)). In unstained sections, we observed intrinsic GFP fluorescence only in basal keratinocytes and the epithelial cells of cryptic folds, with no staining in the upper layer of the suprabasal keratinocytes or intragemmal taste cells (Figure 1(A1)). In the antibody-stained sections, however, we detected fluorescent signals in most of the epithelial cells, both perigemmal and intragemmal (Figure 1(A2)). We did not detect this expansion of fluorescence when we omitted either the primary or secondary antibody (Figure 1(A3,A4)). We also confirmed the antibody specificity by testing several anti-GFP antibodies originated from different species, and by comparing the difference of stained patterns between *Lgr5* reporter mice and the control B6 mice (Supplemental Figure 1). These data indicate that *Lgr5* is expressed more broadly in CVP than was previously known.

Intragemmal expression of Lgr5 *is not restricted to specific cell types*

Our observation of intragemmal localization of the *Lgr5* reporter GFP signal raised the question of which cell types express *Lgr5*. Therefore, we conducted a double immunostaining experiment using antibodies against GFP and representative taste cell markers. A taste bud is composed of several types of taste cell: type I taste cells (glial-like cells), type II taste cells (taste receptor cells for sweet, bitter, umami, and salty tastes), type III taste cells (presynaptic cells for sour taste), and type IV cells (precursor cells that represent an intermediate transitional state, ready to differentiate into other types of taste cells). We observed an overlap of the intragemmal GFP signal, not only with the pan-taste cell maker Krt8 (Figure 1

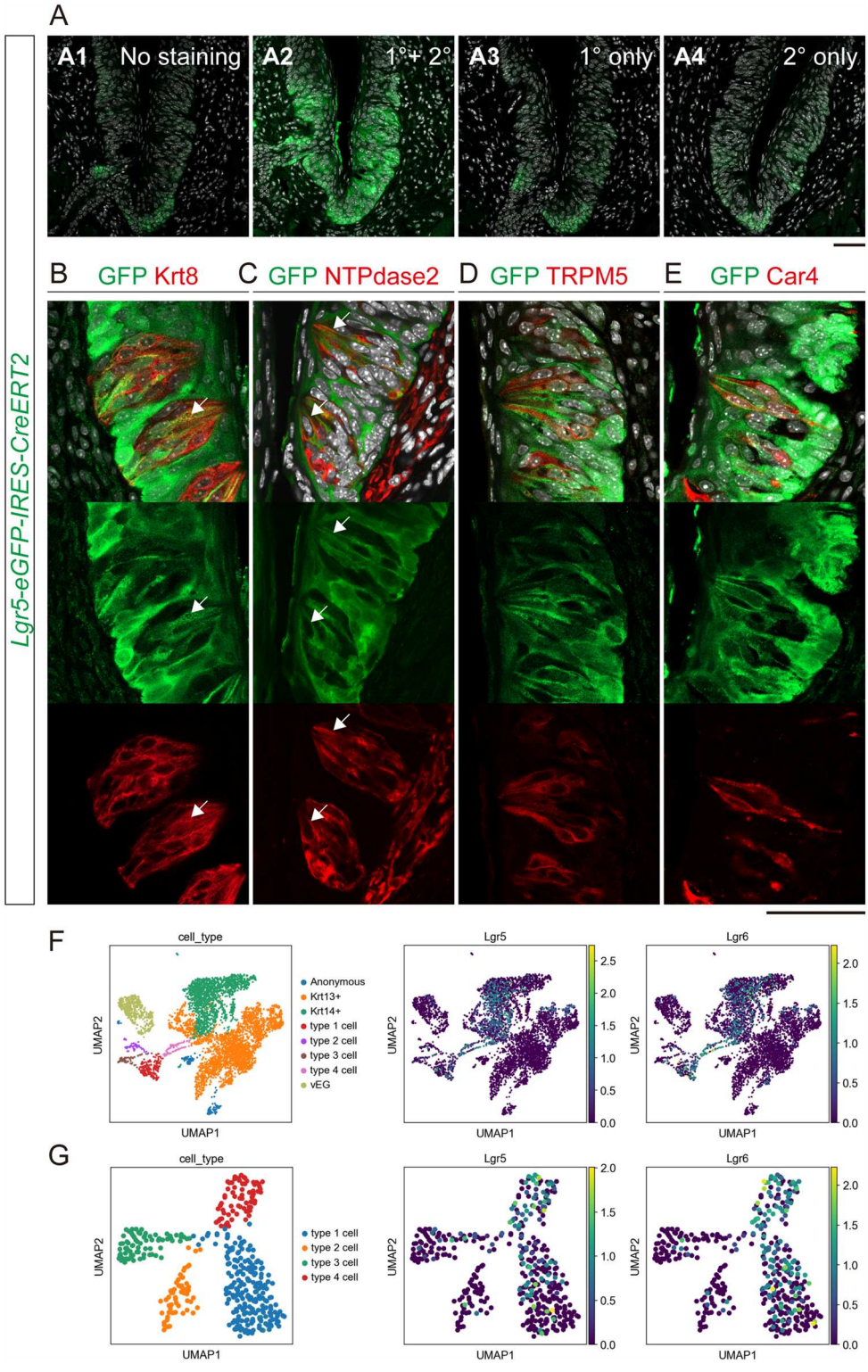

Figure 1. Intragemmal and perigemmal *Lgr5*^{low} cells in CVP. (A) Representative fluorescence images of CVP from *Lgr5-eGFP-IRES-CreERT2* mice. Unstained (A1), primary and secondary treated (A2), primary only treated (A3), and secondary only treated sections (A4). GFP (green) and DAPI (white) immunosignals. (B-E) Representative double fluorescence images of CVP from *Lgr5-eGFP-IRES-CreERT2* mice with anti-GFP and anti-Krt8 (B), anti-NTPdase2 (C), anti-TRPM5 (D), or anti-Car4 (E) signals. GFP (green), taste cell type-specific markers (red), and DAPI (white). The white arrows indicate the cells co-expressing GFP and each cell type-specific marker. Scale bars = 50 µm. (F, G) Reanalysis of single-cell RNA-seq data for mouse CVP reveals intragemmal expression of *Lgr5* and *Lgr6*. (F) UMAP plots for total cell clusters in the dataset (left). Expression levels of *Lgr5* and *Lgr6* are indicated with intensity-coded colors (right). (G) UMAP plots for intragemmal cell clusters in the dataset (left). Expression levels of *Lgr5* and *Lgr6* in intragemmal cell clusters are indicated with intensity-coded colors (right).

(B)), but also with more cell type-specific markers, such as the type I taste cell marker NTPdase2 (Figure 1(C)). However, we did not detect significant anti-GFP signals overlapping with the type II taste cell marker TRPM5 (Figure 1(D)), and the type III taste cell marker Car4 (Figure 1(E)).

To support the histologic findings, we reanalyzed publicly available single-cell RNA-seq data for mouse CVP (Vercauteren Drubbel and Beck 2023). *Lgr5* and *Lgr6* were expressed not only in the subset of basal keratinocyte clusters (Figure 1(F)), but also in the type I and type IV taste cell clusters (Figure 1(G)). In contrast, *Lgr5* and *Lgr6* were rarely expressed in type II and type III taste cell clusters (Figure 1(G)). These findings suggest that intragemmal *Lgr5*-expressing cells belong to either type I or type IV taste cells.

Genetic lineage tracing of Lgr5*-expressing cells in CVP*

Next, we conducted genetic lineage tracing of *Lgr5*-expressing cells in CVP from *Lgr5-tdTomato* mice. In this experiment, GFP expression reflects current *Lgr5* expression, while tdTomato expression indicates the previous cellular state regarding *Lgr5* expression during tamoxifen injection labeling (Figure 2(A,B)) (Guenthner et al. 2013). If *Lgr5*-expressing cells are self-renewing stem cells, tamoxifen injection will induce tdTomato expression in GFP-expressing cells that should remain for a long time until the removal of the labeled clones (Figure 2(A)). Alternatively, but not in a mutually exclusive way, if *Lgr5*-expressing cells are ancestors of other cell types, tamoxifen injection should induce tdTomato expression in cells that ultimately become GFP-negative after cell type transformation (Figure 2(B)).

When we traced tdTomato and GFP fluorescence signals from 1 d post-injection (dpi 1) to dpi 60 in parallel, we found that a single injection of tamoxifen could induce tdTomato in CVP (Figure 2(C–H)). The resulting tdTomato-positive region spread from a small, restricted area to fill the entire CVP by dpi 7, but the signal remained mainly in the perigemmal areas (Figure 2(C–E)). While the tdTomato signal initially emerged from a subset of GFP-expressing cells in the basal epithelial layer (Figure 2(C)), it spread to fill most of the perigemmal GFP-expressing cells eventually (Figure 2(E)). Compared to its expression in the perigemmal cells, the intragemmal tdTomato signal started to emerge later from dpi 7 and was restricted only to GFP-expressing cells, as was the case for the perigemmal area (Figure 2(E)). The perigemmal tdTomato signal disappeared or weakened gradually such that we were able to observe GFP-expressing cells unlabeled by tdTomato by dpi 21 (Figure 2(B)). In contrast, the tdTomato signal persisted in the intragemmal regions and the cryptic CVP base throughout our experimental observation period until dpi 60 (Figure 2(H)).

Regardless of the regions and periods we observed, we never detected any tdTomato-labeled cells that lacked GFP signal across many antibody-stained sections from multiple animals and independent experimental sets. This was a significant contrast to the genetic lineage tracing of intestinal *Lgr5*-expressing cells, which do produce tdTomato-positive and GFP-negative cells (Barker et al. 2007; Yan et al. 2012). Quantitative measurement of fluorescent signals revealed that the fluorescent signals of anti-GFP are not always proportionally affected by those of tdTomato, ruling out the possibility of tdTomato signals spilled over to the green fluorescent filter (Supplemental Figure 2).

To quantitatively assess how much *Lgr5*-expressing cells contribute to the formation of intragemmal taste cells, we measured the proportion of tdTomato-labeled cells relative to the total cells within taste buds at different time points (Figure 2(I)). Generally, the proportion of tdTomato-labeled cells increased over time. However, from dpi 14, this increasing trend plateaued, and even at dpi 60, only approximately half (55.8 ± 3.8%) of the total taste cells were labeled with tdTomato. Considering that the typical life span of intragemmal cells, even for long-lived ones, does not exceed 21 days (Hamamichi et al. 2006), these data indicate that not all intragemmal cells originate from *Lgr5*-expressing cells. Furthermore, since all tdTomato-labeled intragemmal cells were GFP-positive, our findings suggest that only a restricted population of intragemmal cells that express *Lgr5* originates from the *Lgr5*-expressing stem/progenitor cells.

Comparing single and multiple tamoxifen injections to label Lgr5*-expressing cells in CVP*

Nevertheless, we suspected that a single tamoxifen injection might be insufficient to label cells undergoing a transformation from basal keratinocytes to intragemmal taste cells. With a single tamoxifen injection, labeling is effectively limited to a 24-hour period (Guenthner et al. 2013). Such a brief time window may result in a lower chance of differentiation of labeled cells toward taste cells than perigemmal cells because the intragemmal taste cells are scarcer and have a longer life span than perigemmal cells (Hamamichi et al. 2006; Perea-Martinez et al. 2013). We wondered whether we could capture the differentiation of cells toward taste buds by increasing the chance of labeling

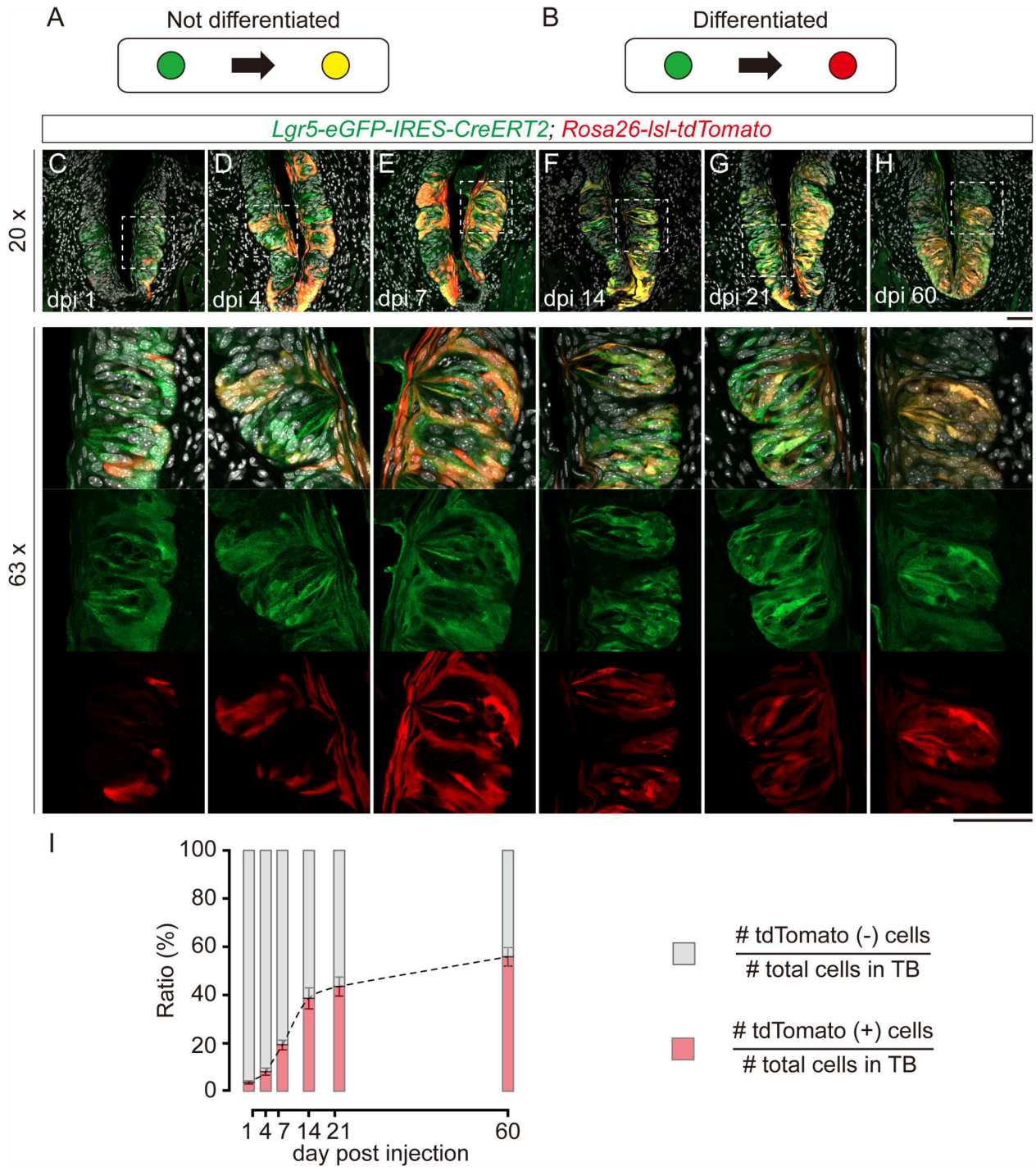

Figure 2. Time series for the tracing of *Lgr5*-expressing cells in CVP. (A, B) Theoretical schematic for the genetic lineage tracing of cells that do not differentiate (A) and those that do differentiate (B). (C-H) Double fluorescence images of anti-GFP signal and tdTomato in CVP from *Lgr5-tdTomato* mice at dpi 1 (C), dpi 4 (D), dpi 7 (E), dpi 14 (F), dpi 21 (G), and dpi 60 (H). The areas within the white, dashed boxes in the 20x images are magnified in the 63x images. These are split to show a merged image (left column), a GFP (middle column) image, and a tdTomato image (right column). Immunosignals against GFP (green), tdTomato (red), and DAPI (white). Scale bars = 50 μm. (I) Quantification of the proportion of tdTomato-expressing cells (red) and non-expressing cells (gray) within a taste bud.

via consecutive injections of tamoxifen. We expected that this would also increase the number of tdTomato-expressing cells in intragemmal regions.

We generated two independent experimental cohorts to compare tdTomato expression between single and either 7 or 14 consecutive daily tamoxifen

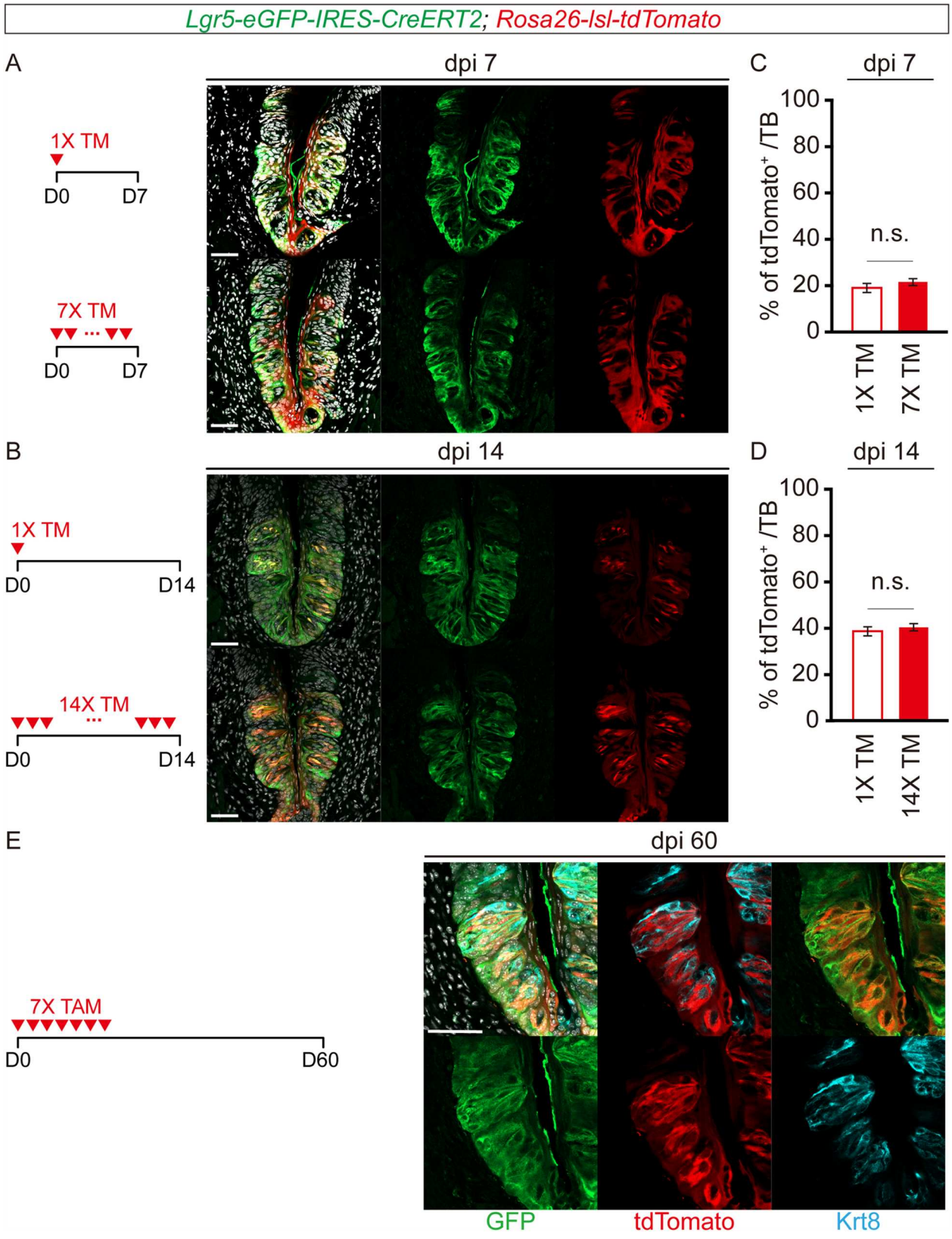

Figure 3. Comparing the labeling efficiency between single and multiple consecutive injections of tamoxifen. (A, B) Double fluorescence images showing anti-GFP signal and tdTomato in CVP from *Lgr5-tdTomato* mice at dpi 7 (A) and dpi 14 (B). Merged images (left column) with DAPI (white), anti-GFP images (green, middle column), and tdTomato images (red, right column) are shown for each condition. Tamoxifen was administrated either once (1x TM) or on the given number of consecutive days (7x TM or 14 x TM). (C, D) Comparison of the proportion of tdTomato-expressing cells within a taste bud between single and consecutive daily injections of tamoxifen at dpi 7 (C) and dpi 14 (D). Mean ± S.E.M. n.s., not significant. (E) Triple fluorescent confocal images showing anti-Krt8 (cyan), anti-GFP signal (green), and tdTomato (red) in CVP from initial 7x tamoxifen injected *Lgr5-tdTomato* mice at dpi 60. Scale bars = 50 μm.

injections (Figure 3). Compared to a single injection, both consecutive injection groups showed similar fluorescence staining patterns for GFP and tdTomato (Figure 3(A,B)). tdTomato expression was restricted to GFP-expressing cells in both the single and consecutive injection groups (Figure 3(A,B)). The number of tdTomato-expressing cells in taste buds did not differ between single and consecutive injection groups (Figure 3(C,D)). In addition, the decay of the tdTomato signal did not differ significantly in perigemmal regions. These findings suggest that increasing the tamoxifen injections did not affect our conclusions based on our single tamoxifen injection experiments.

Since *Lgr5*-expressing stem/progenitor cells give rise exclusively to a limited lineage of intragemmal cells, expressing *Lgr5* at a low level (Figure 2), we then wondered whether *Lgr5*-negative intragemmal cells genuinely do not originate from *Lgr5*-positive lineages. To assess this, we conducted immunostaining on CVP sections from *Lgr5-tdTomato* mice that were injected with tamoxifen for the initial 7 days and harvested at dpi 60, using anti-Krt8. We observed that most *Krt8*-expressing cells did not overlap with tdTomato fluorescence (Figure 3(E)). These findings suggest the presence of another pool of stem/progenitor cells contributing to the generation of a subset of intragemmal taste cells, distinct from *Lgr5*-expressing stem/progenitor cells.

Resistance of Lgr5*-expressing cells in CVP to diphtheria toxin*

To determine whether *Lgr5*-expressing cells are dispensable for taste bud formation, we decided to perform a genetic ablation of *Lgr5*-expressing cells in CVP. By crossing *Lgr5-eGFP-IRES-CreERT2* mice with *Rosa26-lsl-DTA* mice, we generated *Lgr5-DTA* mice and confirmed that the genetic ablation was successful in the small intestine (Supplemental Figure 3(A)). The expression of DTA via tamoxifen in Lgr5-DTA mice not only led to a loss of GFP-positive stem cells in the intestinal cryptic base, but also induced robust expression of Tert, the marker for another stem cell pool (Suh et al. 2017). Remarkably, however, GFP-positive cells were still present in CVP of the same mice until dpi 7 (Supplemental Figure 3B). Even mice subjected to 7 consecutive daily tamoxifen injections showed similar levels of GFP in CVP (Supplemental Figure 3B). To rule out the possibility of rapid emergence of novel *Lgr5*-expressing cells from nearby cells after the removal of preexisting *Lgr5*-expressing cells, we generated triple transgenic mice carrying *Lgr5-eGFP-IRES-CreERT2*, *Rosa26-lsl-DTA*, and *Rosa26-lsl-tdTomato* (Supplemental Figure 3C). Tamoxifen injections into these mice induced tdTomato signal like in *Lgr5-tdTomato* mice (Figure 2(C–E), Supplemental Figure 3C), indicating that the expression of DTA was ineffective at killing *Lgr5*-expressing cells in CVP.

Lgr5*-expressing cells in FuP are type IV precursor cells*

Although previous studies indicated that *Lgr5* expression is limited only to embryonic stages and early life (Takeda et al. 2013), our antibody staining revealed low-level *Lgr5* expression in adult FuP (Figure 4). We found that only one-third of the entire FuP population exhibits GFP-expressing cells. The distribution of GFP-expressing FuP was random and not dependent on the anatomic location of FuP. Indeed, tamoxifen injection yielded tdTomato expression in *Lgr5-tdTomato* mice, indicating successful genetic recombination mediated by Cre recombinase. We observed two distinct lineage tracing patterns. Similar to CVP, we found an overlapping pattern in which tdTomato expression was restricted to GFP-expressing cells (Figure 4(A)). Still, we also observed a non-overlapping pattern in which tdTomato was expressed in GFP-negative cells (Figure 4(B)). In non-overlapping pattern, most GFP-expressing cells lacked tdTomato expression, implying that they were generated de novo rather than being derived from preexisting cells labeled by tamoxifen injection (Figure 4(B)). We never observed any FuP which had only tdTomato-expressing cells without GFP-expressing cells. Overall, the frequency at which we observed these two patterns was similar, but it showed a nonsignificant shift over time (Figure 4(C), Supplemental Table 3, Chi-square $= 5.999$, $P = 0.1992$). At dpi 1 and dpi 4, we observed the non-overlapping pattern more often than the overlapping pattern, but at dpi 14 and dpi 21, this trend was reversed. This reduction in the non-overlapping pattern reflects a gradual depletion of tdTomato-expressing cells lacking GFP expression. Since the mean half-life of type I cells is about eight days, which is shorter than that of other intragemmal cell types (Hamamichi et al. 2006; Perea-Martinez et al. 2013), we suspect that most of the tdTomato-expressing cells that disappeared are type I cells. These data suggest some *Lgr5*-expressing cells in FuP arise from *Lgr5*-negative cells and have the capacity to transform into other cell types.

Genetic lineage tracing of Lgr6*-expressing cells in CVP, FuP, and filiform papillae*

With *Lgr5*, *Lgr6* is another candidate molecular marker of stem/progenitor cells in the taste buds of the posterior and anterior tongue (Ren et al. 2014). To confirm this,

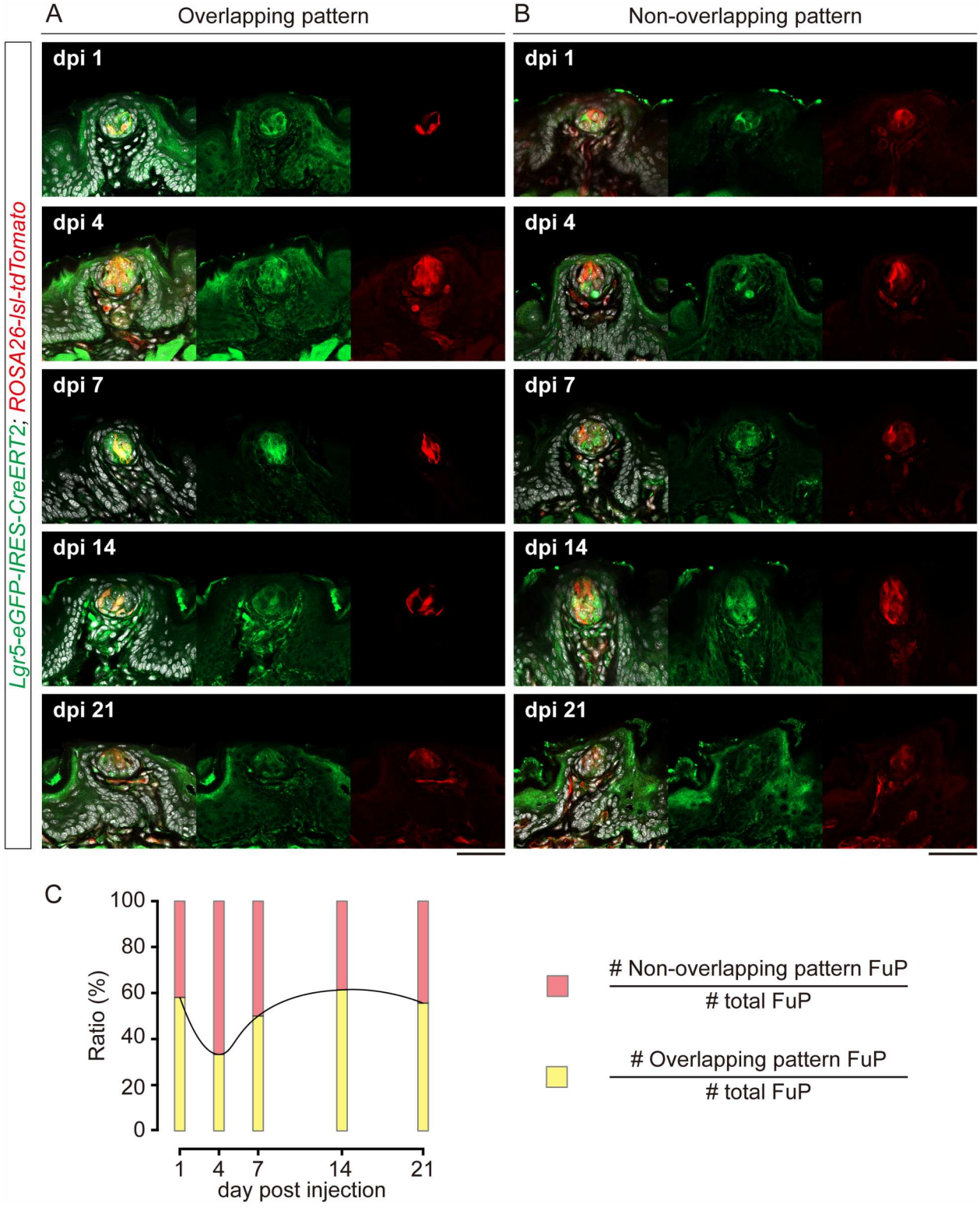

Figure 4. Two distinct lineage tracing patterns for *Lgr5*-expressing cells in FuP. (A, B) Double fluorescence images of the overlapping pattern (A) and the non-overlapping pattern (B) for anti-GFP (green) and tdTomato (red) signals in FuP from *Lgr5-tdTomato* mice at dpi 1, dpi 4, dpi 7, dpi 14, and dpi 21. Merged images (left column) with DAPI (white), anti-GFP images (middle column), and tdTomato images (right column) are shown. Scale bars = 50 μm. (C) Quantification of the proportion of FuP showing overlapping pattern (yellow bar) and non-overlapping pattern (red bar) over time.

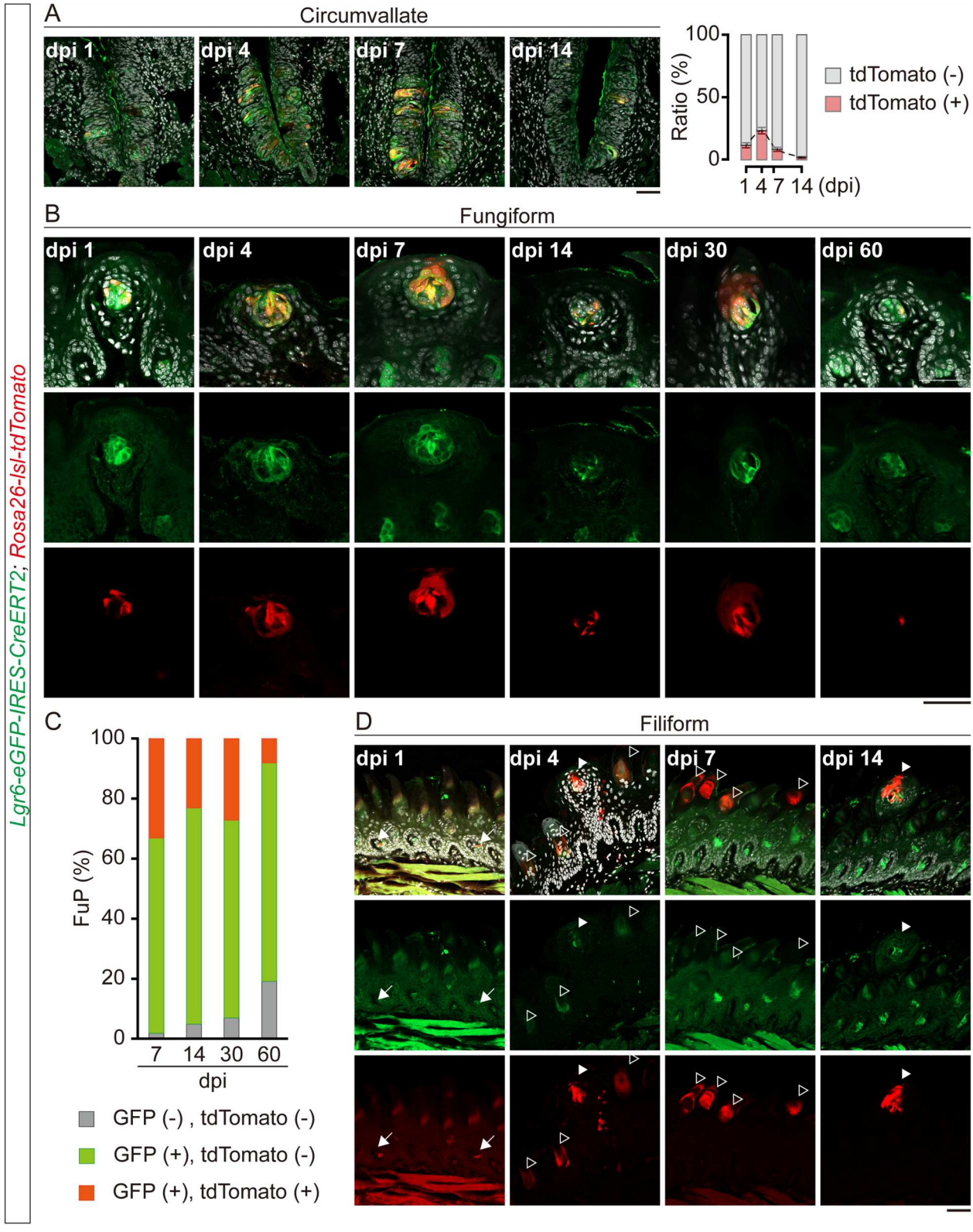

Figure 5. Time series for the tracing of *Lgr6*-expressing cells in CVP, FuP, and filiform papillae. (A) Double fluorescence images of anti-GFP signal (green), tdTomato (red), and DAPI (white) in CVP of *Lgr6-tdTomato* mice at dpi 1, dpi 4, dpi 7, and dpi 14 (left), and the quantification of the proportion of tdTomato-expressing cells (red) and non-expressing cells (gray) within a taste bud (right) (B) Double fluorescence images of anti-GFP signal (green), tdTomato (red), and DAPI (white) in CVP FuP of *Lgr6-tdTomato* mice at dpi 1, dpi 4, dpi 7, dpi 14, dpi 30 and dpi 60. (C) Quantification of GFP-expressing and tdTomato-expressing FuP. (D). Double fluorescence images of anti-GFP signal (green), tdTomato (red), and DAPI (white) in filiform papillae of *Lgr6-tdTomato* mice at dpi 1, dpi 4, dpi 7, and dpi 14. The white arrows, empty, and filled arrowheads indicate interpapillary pits (IPPs), filiform papillae, and FuP that express tdTomato, respectively. Scale bars = 50 μm.

we conducted genetic lineage tracing of *Lgr6*-expressing cells in *Lgr6-tdTomato* mice. In CVP, we found a single tamoxifen injection induced tdTomato expression in GFP-positive cells in intragemmal areas until dpi 7, but most of this tdTomato disappeared by dpi 14 (Figure 5 (A)). This was different from the genetic tracing of *Lgr5*-expressing cells where tdTomato persisted throughout the full observation period (Figure 2). In FuP, however, tdTomato fluorescence in GFP-positive cells lasted until dpi 60 (Figure 5(B)). We never detected any tdTomato-labeled cells lacking GFP signal in any antibody-stained sections from multiple animals and independent experimental sets. Finally, at dpi 60, the proportion of tdTomato-retained FuP dramatically decreased (Figure 5(C)). This means that tamoxifen-labelled clones are gradually lost.

In addition to taste-related papillae, we also observed imprints of *Lgr6*-expressing cells in filiform papillae. We observed a rapid transition of *Lgr6*-expressing cells in the middle layer of the interpapillary pits (IPPs) toward the mucosal stratum corneum. At dpi 1, tdTomato was restricted to GFP-expressing cells in IPPs, but at dpi 7, the tdTomato signal dissociated and drifted apically away from the GFP-positive cells in the basal epithelium. Finally, by dpi 14, it was entirely detached from the epithelium (Figure 5(D)). In contrast, FuP retained tdTomato labeling (Figure 5(B,D)).

Collectively, these data indicate that genetic lineage tracing using *Lgr6-eGFP-IRES-CreERT2* is not sufficient to insist that *Lgr6*-expressing cells in FuP are stem/progenitor cells for intragemmal taste cells. Additionally, *Lgr6*-expressing cells in CVP and filiform papillae represent intermediate transitionary states of maturation.

Discussion

In this study, we reevaluated whether *Lgr5*- or *Lgr6*-expressing cells serve as stem/progenitor cells for taste buds. Our data only partially complement those of previously published reports. First, cells that express low levels of *Lgr5* or *Lgr6* ($Lgr5^{low}$ and $Lgr6^{low}$ cells, respectively) are only detectable when the native GFP fluorescence signal is amplified via immunostaining. Second, $Lgr5^{low}$ and $Lgr6^{low}$ cells are located broadly in intragemmal areas and in suprabasal layers, whereas $Lgr5^{high}$ cells are limited to the basal layers and cryptic folds as previously reported. Third, in CVP, traced intragemmal cells arising from *Lgr5*- or *Lgr6*-expressing cells upon tamoxifen injection remained positive for GFP, indicating their inability to differentiate into *Lgr5*- or *Lgr6*-negative cells. Last, giving multiple consecutive injections of tamoxifen to trigger the labeling of *Lgr5*-expressing cells did not lead to more tdTomato-labeled cells than a single injection. Therefore, we have concluded that the results of genetic lineage tracing *Lgr5*- and *Lgr6*-expressing cells are insufficient to conclude that they have stem/progenitor properties in CVP. Only *Lgr5*-expressing cells in FuP were an exception. These cells originate from non-self stem/progenitor cells, and are capable of producing non-self progeny, reminiscent of type IV precursor cells (Golden et al. 2021).

Our observation of intragemmal *Lgr5*-expressing cells in CVP is supported by recently reported evidence. Shechtman et al. showed the immunofluorescent images of the *Lgr5* reporter GFP-expression in taste buds of CVP as well as RNA-fluorescence in situ hybridization of *Lgr5* transcript, although the authors did not mention (Shechtman et al. 2023). Moreover, when we reanalyzed the single-cell RNA sequencing data from Drubbel et al. (Vercauteren Drubbel and Beck 2023), we noted that *Lgr5* as well as *Lgr6* are not only expressed in the subset of basal keratinocytes, but also in intragemmal cell population, enriched especially in the subset of type I and type IV cell clusters (Figure 1(F,G)). Independent single-cell ATAC sequencing from Lin et al. also supported this expression pattern (Lin et al. 2023). In both single-cell datasets, the expression levels of *Lgr5* are lower in intragemmal cells than in stem/progenitor cells, which are consistent with our histologic results detecting *Lgr5*-driven GFP signals only by immunostaining.

From immunostaining and genetic lineage tracing, our data raise the necessity to challenge the current opinions on the molecular identity of taste bud stem/progenitor cells. The proposal that *Lgr5*-expressing basal keratinocytes serve as stem/progenitor cells for taste buds was based on the findings that these cells were able to transform into intragemmal *Lgr5*-negative cells. However, it is worth noting that previous lineage tracing studies without antibody staining might have missed detecting the endogenous expression of *Lgr5* in taste buds, as well as the presence of intragemmal $Lgr5^{low}$ cells. This could potentially lead to an overestimation of the transformation capabilities of *Lgr5*-expressing basal keratinocytes.

Still, there is a potential that *Lgr5*-expressing basal keratinocytes could become intragemmal *Lgr5*-expressing cells only limitedly. Despite the half-life of taste cells being no longer than 60 days, our observation of tdTomato label retention at 60 dpi implies the need for alternative models for the role of *Lgr5*-expressing basal keratinocytes. *Lgr5*-expressing basal keratinocytes may continuously give rise to mature intragemmal taste cells that consistently express *Lgr5*. Alternatively, quiescent *Lgr5*-expressing basal keratinocytes may

migrate directly into the taste buds. The latter hypothesis does not conflict with previous reports on the half-life of taste cells, as these observations were based on BrdU or EdU labeling, which assumes cell division.

Our data led us to ask whether *Lgr5*-expressing cells in CVP are dispensable for taste bud maintenance. We attempted to genetically ablate *Lgr5*-expressing cells via targeted DTA expression. But despite finding success in other organs, this genetic manipulation unexpectedly failed in lingual tissues (Supplemental Figure 3A). This is inconsistent with previous reports that used DTA or a similar DT receptor system to successfully kill specific taste cells and alter physiologic responsiveness (Huang et al. 2006; Zocchi et al. 2017). It does not seem that this discrepancy originated in an ineffective delivery of tamoxifen because *Lgr5-tdT-DTA* mice exhibited robust tdTomato expression (Supplemental Figure 3C). Instead, the *Rosa26-lsl-DTA* strain we used lacks a *CAG* promoter (Voehringer et al. 2008), meaning DTA expression depends solely on the endogenous *Rosa* enhancer/promoter. Alternatively, *Lgr5*-expressing cells in CVP may also exhibit genuine resistance to DTA, similar to astrocytes, which are not only resistant to DTA but also undergo hypertrophy upon DTA (Chun et al. 2020). Future studies should address whether *Lgr5*-expressing cells are required for taste bud maintenance using a more potent genetic ablation technique such as *taCasp3* (Gray et al. 2010; Yang et al. 2013).

In contrast to CVP, *Lgr5*-expressing cells in FuP displayed traced patterns showing capabilities for differentiation (Figure 4). In non-overlapping pattern, tdTomato-expressing, but GFP-negative cells represent differentiation of *Lgr5*-expressing cells which were labeled by tamoxifen injection, while tdTomato-negative, but GFP-expressing cells represent *de novo* generation of *Lgr5*-expressing cells after tamoxifen labeling, implying that they could not self-renewal. Thus, it is reasonable to suggest that intragemmal *Lgr5*-expressing cells in non-overlapping patterns are type IV precursor cells, not stem/progenitor cells.

Then, how could most of the intragemmal *Lgr5*-negative mature taste cells be produced in CVP? What would be their genuine stem/progenitor? Our data do not negate the roles of basal keratinocytes near the taste buds in taste bud generation. There is a possibility that not all the basal keratinocytes near the taste buds are labeled by *Lgr5-eGFP-IRES-CreERT2* mice. Alternatively, other stem cell pools rather than *Lgr5*-expressing cells might be involved in the production of taste cells. *Lgr5* seems not to be the complete molecular marker for stem/progenitor cells dedicated to differentiated into taste buds. Whatever the scenario, at least, we conclude that genetic lineage tracing data until 60 dpi is insufficient to insist that *Lgr5*-expressing basal keratinocytes are stem/progenitor cells for differentiated cells in taste buds. While our data alone cannot provide a complete answer, this study holds significance in reevaluating and challenging the existing hypotheses for taste bud homeostatic generation through unbiased experimental recapitulation. In future studies, it will be intriguing to compare the differences in the homeostatic mechanisms of taste bud generation under normal conditions to those under regenerative conditions, such as denervation and irradiation. Indeed, adopting advanced experimental techniques, such as organoid formation and single-cell analysis in-depth, will provide deeper insights. Our discovery of the heterogeneity of *Lgr5*- and *Lgr6*-expressing cells in taste tissues would be the new starting point for further investigations to unveil the secret of taste bud generation.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Author contributions

H.J.K. designed and conducted most of the experiments. D.W.S conducted some of immunohistochemistry experiments. J.S. and S.J.M generated polyclonal TRPM5 antibody J.-S. L, S.-H. C., D-H. K. and H.-S.J. analyzed the data. Y.T.J. supervised the project and wrote the paper. All authors read and approved the manuscript.

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References

Adpaikar AA, Lee JM, Lee DJ, Cho HY, Ohshima H, Moon SJ, Jung HS. 2023. Epithelial plasticity enhances regeneration of committed taste receptor cells following nerve injury. Exp Mol Med. 55:171–182.

Aihara E, Mahe MM, Schumacher MA, Matthis AL, Feng R, Ren W, Noah TK, Matsu-ura T, Moore SR, Hong CI, et al. 2015. Characterization of stem/progenitor cell cycle using murine circumvallate papilla taste bud organoid. Sci Rep. 5:17185.

Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, Sato T, Stange DE, Begthel H, van den Born M, et al. 2010. Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell. 6(1):25–36.

Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, et al. 2007. Identification of stem cells in small intestine and colon by marker gene lgr5. Nature. 449(7165):1003–1007. doi: 10.1038/nature06196.

Chun H, Im H, Kang YJ, Kim Y, Shin JH, Won W, Lim J, Ju Y, Park YM, Kim S, et al. 2020. Severe reactive astrocytes precipitate pathological hallmarks of Alzheimer's disease via H2O2− production. Nat Neurosci. 23(12):1555–1566. doi: 10.1038/s41593-020-00735-y.

Finger TE, Danilova V, Barrows J, Bartel DL, Vigers AJ, Stone L, Hellekant G, Kinnamon SC. 2005. Atp signaling is crucial for communication from taste buds to gustatory nerves. Science. 310(5753):1495–1499. doi: 10.1126/science.1118435.

Gaillard D, Bowles SG, Salcedo E, Xu M, Millar SE, Barlow LA. 2017. β-catenin is required for taste bud cell renewal and behavioral taste perception in adult mice. PLoS Genet. 13(8):e1006990. doi: 10.1371/journal.pgen.1006990.

Gaillard D, Xu M, Liu F, Millar SE, Barlow LA. 2015. β-catenin signaling biases multipotent lingual epithelial progenitors to differentiate and acquire specific taste cell fates. PLoS Genet. 11(5):e1005208. doi: 10.1371/journal.pgen.1005208.

Golden EJ, Larson ED, Shechtman LA, Trahan GD, Gaillard D, Fellin TJ, Scott JK, Jones KL, Barlow LA. 2021. Onset of taste bud cell renewal starts at birth and coincides with a shift in shh function. Elife. 10. doi: 10.7554/eLife.64013.

Gray DC, Mahrus S, Wells JA. 2010. Activation of specific apoptotic caspases with an engineered small-molecule-activated protease. Cell. 142(4):637–646. doi: 10.1016/j.cell.2010.07.014.

Guenthner CJ, Miyamichi K, Yang HH, Heller HC, Luo L. 2013. Permanent genetic access to transiently active neurons via trap: Targeted recombination in active populations. Neuron. 78(5):773–784. doi: 10.1016/j.neuron.2013.03.025.

Hamamichi R, Asano-Miyoshi M, Emori Y. 2006. Taste bud contains both short-lived and long-lived cell populations. Neuroscience. 141(4):2129–2138. doi: 10.1016/j.neuroscience.2006.05.061.

Hao HX, Xie Y, Zhang Y, Charlat O, Oster E, Avello M, Lei H, Mickanin C, Liu D, Ruffner H, et al. 2012. Znrf3 promotes wnt receptor turnover in an r-spondin-sensitive manner. Nature. 485(7397):195–200. doi: 10.1038/nature11019.

Huang AL, Chen X, Hoon MA, Chandrashekar J, Guo W, Trankner D, Ryba NJ, Zuker CS. 2006. The cells and logic for mammalian sour taste detection. Nature. 442(7105):934–938. doi: 10.1038/nature05084.

Huang YA, Pereira E, Roper SD. 2011. Acid stimulation (sour taste) elicits gaba and serotonin release from mouse taste cells. PLoS One. 6(10):e25471. doi: 10.1371/journal.pone.0025471.

Huang YJ, Maruyama Y, Lu KS, Pereira E, Roper SD. 2005. Mouse taste buds release serotonin in response to taste stimuli. Chem Senses. 30(Suppl 1):i39-i40. doi: 10.1093/chemse/bjh102.

Jaks V, Barker N, Kasper M, van Es JH, Snippert HJ, Clevers H, Toftgard R. 2008. Lgr5 marks cycling, yet long-lived, hair follicle stem cells. Nat Genet. 40(11):1291–1299. doi: 10.1038/ng.239.

Jang JH, Kwon O, Moon SJ, Jeong YT. 2021. Recent advances in understanding peripheral taste decoding I: 2010 to 2020: 2010 to 2020. Endocrinol Metabo. 36(3):469–477. doi: 10.3803/EnM.2021.302.

Koo BK, Spit M, Jordens I, Low TY, Stange DE, van de Wetering M, van Es JH, Mohammed S, Heck AJ, Maurice MM, et al. 2012. Tumour suppressor rnf43 is a stem-cell e3 ligase that induces endocytosis of wnt receptors. Nature. 488(7413):665–669. doi: 10.1038/nature11308.

Lee H, Macpherson LJ, Parada CA, Zuker CS, Ryba NJP. 2017. Rewiring the taste system. Nature. 548(7667):330–333. doi: 10.1038/nature23299.

Liman ER, Zhang YV, Montell C. 2014. Peripheral coding of taste. Neuron. 81(5):984–1000. doi: 10.1016/j.neuron.2014.02.022.

Lin C, Jyotaki M, Quinlan J, Feng S, Zhou M, Jiang P, Matsumoto I, Huang L, Ninomiya Y, Margolskee RF, et al. 2023. Lipopolysaccharide increases bitter taste sensitivity via epigenetic changes in tas2r gene clusters. iScience. 26(6):106920. doi: 10.1016/j.isci.2023.106920.

Lin X, Lu C, Ohmoto M, Choma K, Margolskee RF, Matsumoto I, Jiang P. 2021. R-spondin substitutes for neuronal input for taste cell regeneration in adult mice. Proc Natl Acad Sci U S A. 118(2):e2001833118.

Liu HX, Henson BS, Zhou Y, D'Silva NJ, Mistretta CM. 2008. Fungiform papilla pattern: EGF regulates inter-papilla lingual epithelium and decreases papilla number by means of PI3K/Akt, MEK/ERK, and p38 MAPK signaling. Dev Dyn. 237(9):2378–2393. doi: 10.1002/dvdy.21657.

Lu C, Lin X, Yamashita J, Xi R, Zhou M, Zhang YV, Wang H, Margolskee RF, Koo BK, Clevers H, et al. 2022. Rnf43/znrf3 negatively regulates taste tissue homeostasis and positively regulates dorsal lingual epithelial tissue homeostasis. Stem Cell Rep. 17(2):369–383. doi: 10.1016/j.stemcr.2021.12.002.

Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, et al. 2010. A robust and high-throughput cre reporting and characterization system for the whole mouse brain. Nat Neurosci. 13(1):133–140. doi: 10.1038/nn.2467.

Nguyen HM, Barlow LA. 2010. Differential expression of a bmp4 reporter allele in anterior fungiform versus posterior circumvallate taste buds of mice. BMC Neurosci. 11(1):129. doi: 10.1186/1471-2202-11-129.

Okubo T, Clark C, Hogan BL. 2009. Cell lineage mapping of taste bud cells and keratinocytes in the mouse tongue and soft palate. Stem Cells. 27(2):442–450. doi: 10.1634/stemcells.2008-0611.

Perea-Martinez I, Nagai T, Chaudhari N. 2013. Functional cell types in taste buds have distinct longevities. PLoS One. 8 (1):e53399. doi: 10.1371/journal.pone.0053399.

Petersen CI, Jheon AH, Mostowfi P, Charles C, Ching S, Thirumangalathu S, Barlow LA, Klein OD. 2011. Fgf signaling regulates the number of posterior taste papillae by controlling progenitor field size. PLoS Genet. 7(6):e1002098. doi: 10.1371/journal.pgen.1002098.

Ren W, Lewandowski BC, Watson J, Aihara E, Iwatsuki K, Bachmanov AA, Margolskee RF, Jiang P. 2014. Single lgr5- or lgr6-expressing taste stem/progenitor cells generate taste bud cells ex vivo. Proc Natl Acad Sci USA. 111 (46):16401–16406. doi: 10.1073/pnas.1409064111.

Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, et al. 2009. Single lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 459 (7244):262–265. doi: 10.1038/nature07935.

Seta Y, Toyono T, Kataoka S, Toyoshima K. 2005. Regulation of taste bud cell differentiation by notch signaling pathway. Chem Senses. 30(Suppl 1):i48–i49. doi: 10.1093/chemse/bjh107.

Seto E, Hayashi Y, Mori T. 1999. Patch clamp recording of the responses to three bitter stimuli in mouse taste cells. Cell Mol Biol. 45(3):317–325.

Shechtman LA, Scott JK, Larson ED, Isner TJ, Johnson BJ, Gaillard D, Dempsey PJ, Barlow LA. 2023. High Sox2 expression predicts taste lineage competency of lingual progenitors *in vitro*. Development. 150(4). doi: 10.1242/dev.201375.

Snippert HJ, Haegebarth A, Kasper M, Jaks V, van Es JH, Barker N, van de Wetering M, van den Born M, Begthel H, Vries RG, et al. 2010. Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. Science. 327(5971):1385–1389. doi: 10.1126/science.1184733.

Suh HN, Kim MJ, Jung YS, Lien EM, Jun S, Park JI. 2017. Quiescence exit of Tert+ stem cells by Wnt/β-catenin is indispensable for intestinal regeneration. Cell Rep. 21 (9):2571–2584. doi: 10.1016/j.celrep.2017.10.118.

Takeda N, Jain R, Li D, Li L, Lu MM, Epstein JA. 2013. Lgr5 identifies progenitor cells capable of taste bud regeneration after injury. PLoS One. 8(6):e66314. doi: 10.1371/journal.pone.0066314.

Vercauteren Drubbel A, Beck B. 2023. Single-cell transcriptomics uncovers the differentiation of a subset of murine esophageal progenitors into taste buds in vivo. Sci Adv. 9 (10):eadd9135. doi: 10.1126/sciadv.add9135.

Voehringer D, Liang HE, Locksley RM. 2008. Homeostasis and effector function of lymphopenia-induced "memory-like" t cells in constitutively t cell-depleted mice. J Immunol. 180 (7):4742–4753. doi: 10.4049/jimmunol.180.7.4742.

Xu M, Horrell J, Snitow M, Cui J, Gochnauer H, Syrett CM, Kallish S, Seykora JT, Liu F, Gaillard D, et al. 2017. Wnt10a mutation causes ectodermal dysplasia by impairing progenitor cell proliferation and klf4-mediated differentiation. Nat Commun. 8(1):15397. doi: 10.1038/ncomms15397.

Yan KS, Chia LA, Li X, Ootani A, Su J, Lee JY, Su N, Luo Y, Heilshorn SC, Amieva MR, et al. 2012. The intestinal stem cell markers bmi1 and lgr5 identify two functionally distinct populations. Proc Natl Acad Sci USA. 109(2):466–471. doi: 10.1073/pnas.1118857109.

Yang CF, Chiang MC, Gray DC, Prabhakaran M, Alvarado M, Juntti SA, Unger EK, Wells JA, Shah NM. 2013. Sexually dimorphic neurons in the ventromedial hypothalamus govern mating in both sexes and aggression in males. Cell. 153(4):896–909. doi: 10.1016/j.cell.2013.04.017.

Yarmolinsky DA, Zuker CS, Ryba NJ. 2009. Common sense about taste: From mammals to insects. Cell. 139(2):234–244. doi: 10.1016/j.cell.2009.10.001.

Yee KK, Li Y, Redding KM, Iwatsuki K, Margolskee RF, Jiang P. 2013. Lgr5-egfp marks taste bud stem/progenitor cells in posterior tongue. Stem Cells. 31(5):992–1000. doi: 10.1002/stem.1338.

Zocchi D, Wennemuth G, Oka Y. 2017. The cellular mechanism for water detection in the mammalian taste system. Nat Neurosci. 20(7):927–933. doi: 10.1038/nn.4575.