

Targeting strategy for SHH expression

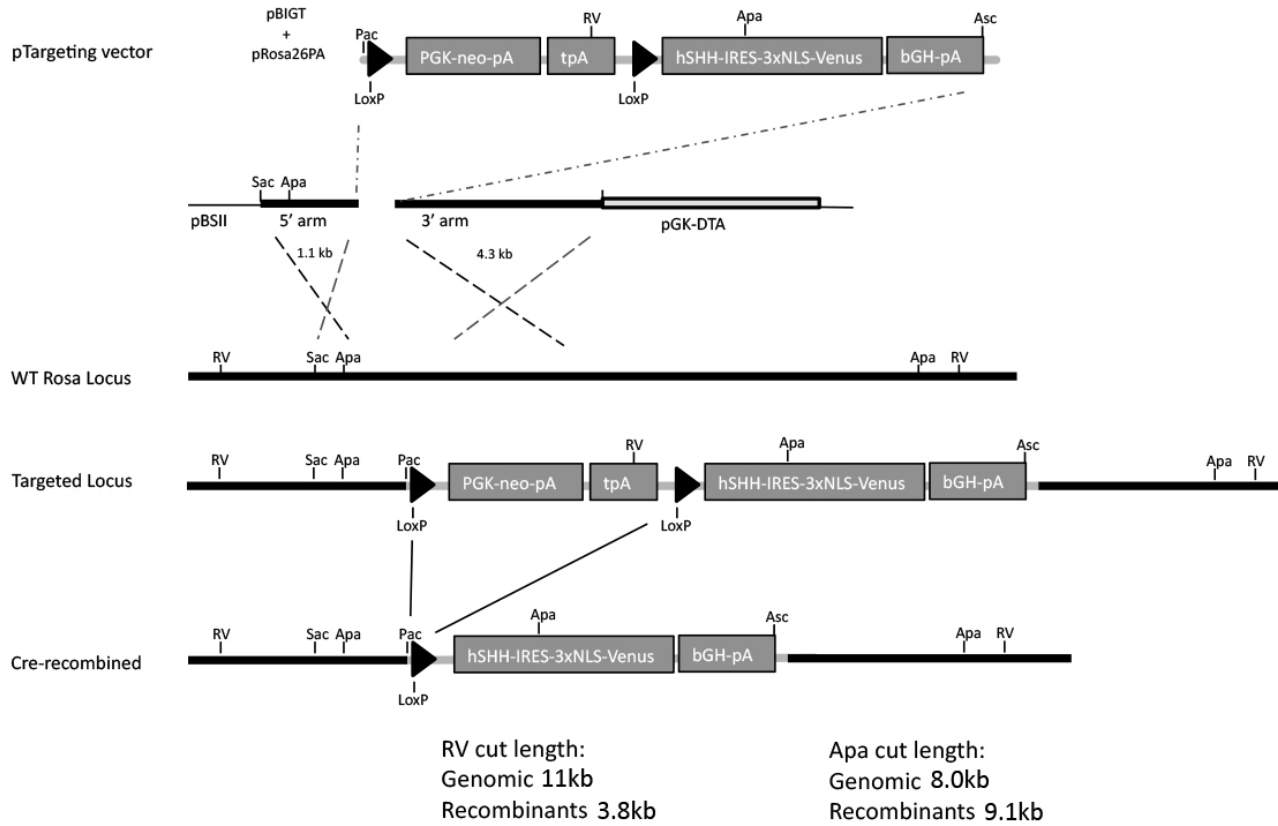


Fig. S1. Targeting strategy and map of allele. A lox-STOP-lox cassette followed by a cDNA encoding hSHH, an internal ribosomal entry site (IRES), three nuclear localization signals (NLS) and Venus yellow fluorescent protein was targeted into the Rosa26 using the pROSA26PA vector (Soriano, 1999).

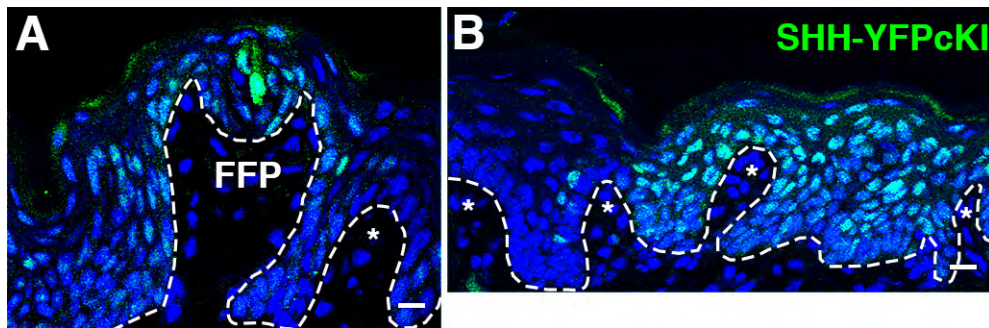


Fig. S2. Constitutive mosaic expression of SHH in K14+ lingual epithelial cells and their descendants. Tamoxifen treatment of K14CreER;SHH-YFPcKI mice results in mosaic SHH-YFPcKI expression (green) in both fungiform papillae (A), shown at 21 days post-tamoxifen, and non-taste lingual epithelium (B), shown at 28 days after tamoxifen. Scale bars=10 μm; nuclei are counterstained with Draq5 (blue); white dashed lines delimit the basement membrane; *= mesenchymal core of filiform papillae; FFP= fungiform papilla. All images are confocal Z-stacks.

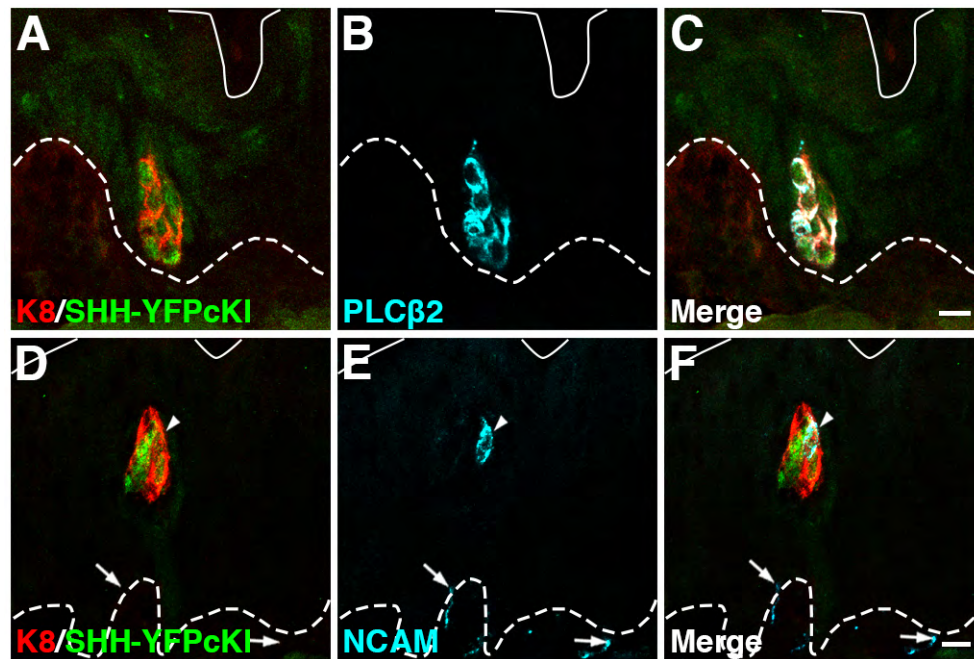


Fig. S3. Ectopic taste buds express additional markers of Type II and III taste cells. (A-C) PLC 2+ Type II taste cells (cyan) are present in an ectopic taste bud (K8+, red) which also contains SHH-YFPcKI+ cells (green). (D-E) A single Type III taste cell (NCAM+, cyan, arrowhead) forms part of an ectopic K8+ (red) and SHH-YFPcKI+ (green) taste bud. Two NCAM+ neurites (arrows) are present in the mesenchyme below and at the basement membrane, distant from the bud. Scale bars=10 μm; white dashed lines delimit the basement membrane; white solid lines delimit the epithelial surface. All images are confocal Z-stacks.

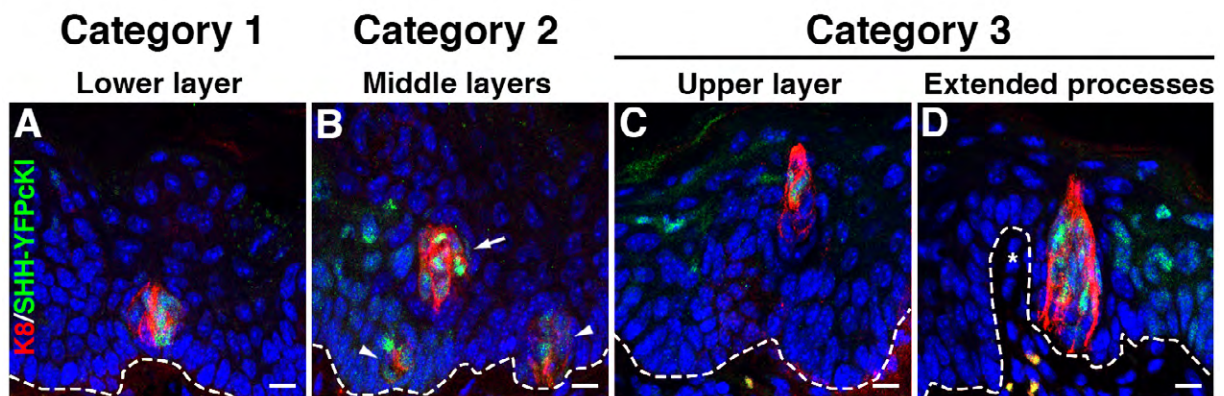


Fig. S4. Categorization of ectopic taste bud position within the lingual epithelium. (A) Category 1: ectopic buds in the lowest 2 epithelial layers. (B) Category 2: ectopic taste buds in the intermediate suprabasal lingual epithelium (arrow) (two Category 1 ectopic buds are also present, arrowheads). (C, D) Category 3: ectopic taste buds in the uppermost 2 epithelial layers (C), and those extending apical processes to the tongue surface (D). Scale bars=10 μm; nuclei are counterstained with Draq5 (blue); white dashed lines delimit the basement membrane; *= indicates mesenchymal core of filiform papilla. All images are confocal Z-stacks.

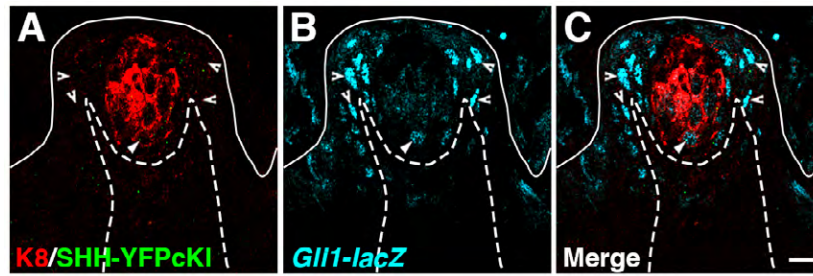


Fig. S5. Shh-responsive Gli1+ cells surround endogenous taste buds. (A-C) Double immunolabeling for K8 (red) and β -galactosidase (cyan, *Gli1-lacZ*, nicked arrowheads) reveals SHH- responding cells surrounding a K8+ endogenous taste bud; one K8+ cell in the basal compartment is dimly β -gal+ (cyan, arrowhead). Scale bar=10 μ m; white dashed lines delimit the basement membrane; white solid lines delimit the epithelial surface. A single optical section (0.76 μ m) is shown.

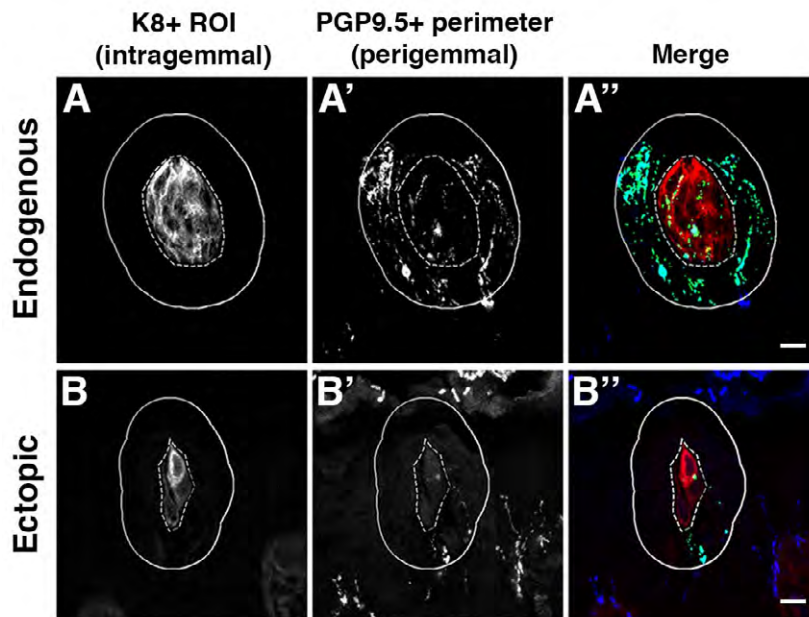


Fig. S6. Depiction of the MATLAB analysis method. See Methods for full details. (A, B) Visualizing only the K8+ red channel, the user defines a region-of-interest (ROI, dashed line) to encircle an endogenous (A) or an ectopic (B) taste bud. A ring of interest with a width of 17.6 μ m is automatically circumscribed around the taste bud ROI (outer boundary: solid line, inner boundary: dashed line). (A', B') Next, visualizing signal only in the long red channel, all PGP9.5+ neurites are categorized as intragemmal (found within the ROI, dashed line) or perigemmal (found in the ring around the taste bud ROI). (A'', B'') Merged images show taste bud ROI and perimeter boundaries around a K8+ (red) endogenous (A'') and ectopic (B'') taste bud, with intragemmal and perigemmal PGP9.5+ neurites pseudocolored green, but quantified separately via MATLAB. PGP9.5+ neurites that are neither intragemmal nor perigemmal are pseudocolored blue and are not included in the analysis. Scale bars=10 μ m.

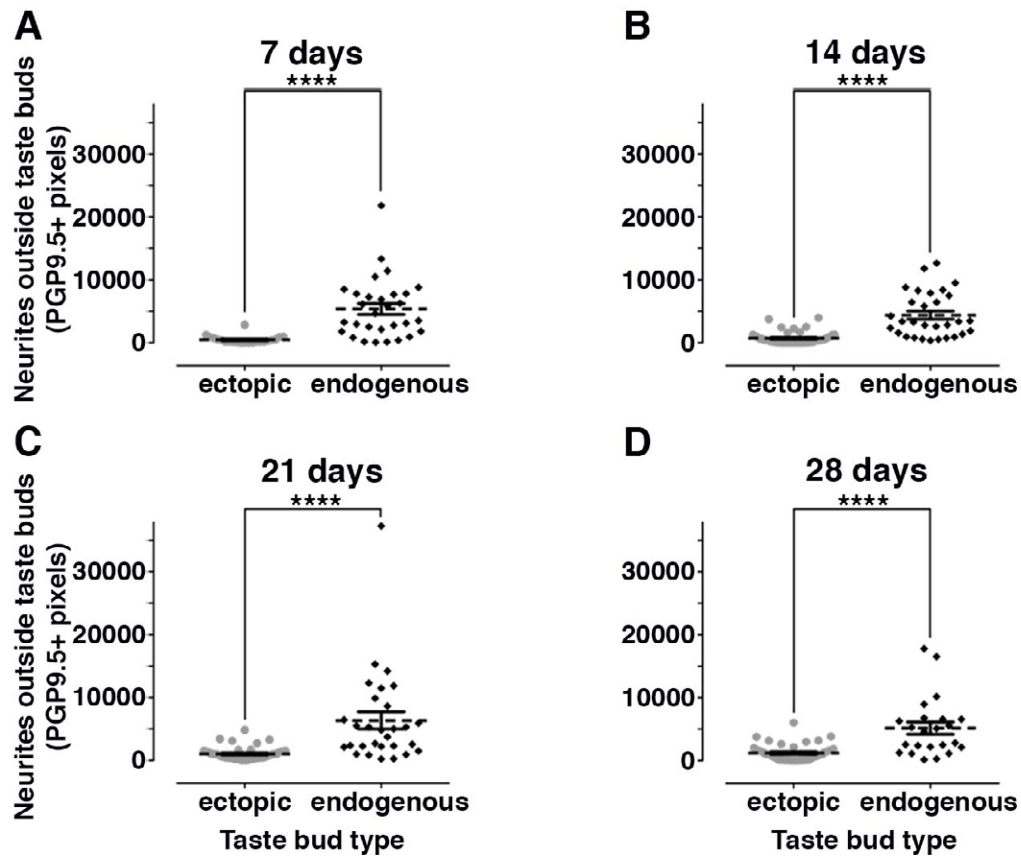


Fig. S7. The presence of ectopic taste buds is independent of perigemmal innervation. Fewer PGP9.5+ neurites are found surrounding ectopic taste buds (grey symbols) than surrounding endogenous taste buds (black symbols) (two-tailed Mann-Whitney U test: (A) 7 days $U = 69.00$, (B) 14 days $U = 146.00$, (C) 21 days $U = 179.0$, (D) 28 days $U = 163.0$, 22-45 ectopic taste buds and 23-30 endogenous taste buds randomly selected from 3 mice per time point, **** $P < 0.0001$).

Table S1. Data set used for assessing the percentages of ectopic taste buds co-expressing specific molecular markers.

[Download Table S1](#)

Table S2. Data set used for quantification of the size and innervation density of ectopic and endogenous taste buds.

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