Supporting Information

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SI Materials and Methods

Mice. For tamoxifen-induced recombination, mice were given tamoxifen prepared in corn oil (20 mg/mL; Sigma) at 5 mg per 25 g of body weight by oral gavage once per day. XL139 was formulated in sterile deionized water at a concentration of 10 mg/mL and administered to mice by oral gavage every 72 h at a dose of 100 mg/kg. Dosing volume for XL139 or vehicle (sterile deionized water) was 10 μL per g of mouse weight. SAG21k was delivered by osmotic pump (Alzet) over the course of 2 or 4 wk at a dose of 2 $mg \cdot kg^{-1} \cdot d^{-1}$.

Tongue Epithelium Isolation and Whole-Mount Immunostaining. Tongues were removed from euthanized mice, rinsed in PBS, and injected with 5 units/mL dispase (BD Biosciences) made in M199-Hepes medium until fully distended. After incubation in the same solution for 20 min at room temperature, the epithelium was peeled away from the muscle tissue, rinsed in cold PBS, and fixed in 2% PFA/PBS with 0.15% Triton X-100 for 4 h at 4 °C. After washing three times with PBS, tongue epithelium were permeabilized with PBS containing 0.3% Triton X-100 for 30 min and blocked with 5% normal goat serum (NGS) in PBS containing 0.1% Triton X-100, 1.5% BSA, and 0.05% Tween 20 for at least 1 h at room temperature. Primary antibody incubations were performed in blocking solution overnight at 4 °C. Secondary antibody (Alexa Fluor 488-, 568-, or 647-conjugated, 1:500; Molecular Probes and Sigma) incubations were performed in 5% NGS/PBS for 2 h at room temperature. Washes were performed three times with 0.1% Tween 20/PBS (PBST). Nuclei staining using DAPI (D3571; 1:10,000 from 10 mg/mL stock; Invitrogen) was performed during the second wash. Tissues were rinsed twice in PBS and mounted in Fluoromount-G (Southern Biotech). Same procedures were also used for cryosections and dissected geniculate ganglia. Primary antibodies used were as follows: chicken anti–β-galactosidase (ab9361; 1:500; Abcam); rabbit anti-GFP (A-11122; 1:1,000; Invitrogen); chicken anti-GFP (GFP1020; 1:1,000; Aves Laboratories); chicken anti-K5 (SIG-3475; 1:1,000; Covance Lab); rat anti-K8 (TROMA-I; 1:250~500 of concentrated supernatant; Developmental Studies Hybridoma Bank, University of Iowa); rabbit anti-Shh (C9C5; 1:1,000 from custom-made concentrate at 1 mg/mL; Cell Signaling); biotin-conjugated anti-NeuN (MAB377B; 1:250; Millipore).

Histology. Animals were euthanized and perfused with PBS and 4% paraformaldehyde (PFA) in PBS, and then dissected tongues were postfixed in 4% PFA overnight. For cryosections, fixed tissues were placed in 30% sucrose/PBS for 12–24 h at 4 °C and embedded into Optimal Cutting Temperature medium (OCT) (Fisher Scientific). Frozen blocks were sectioned at 25-μm thickness using a cryostat (Leica). For H&E analysis, fixed tissues were placed in 70% ethanol and submitted for paraffin processing and H&E staining (HistoTec). For taste bud counting in circumvallate papilla, the entire papilla was sectioned sequentially, imaged, and counted for TRC-containing taste buds. Number of taste buds was combined from individual trench of the papilla. Taste pore was identified to avoid double counting in adjacent sections.

Whole-Mount Image Analysis. Tile-scanned images of stained lingual epithelia were projected maximally along the *z* axis, stitched, and fused using ZEN software (blue edition; Zeiss). Images were then analyzed using ImageJ (NIH). DAPI signal was used to identify fungiform papillae, and K8 signal was used to determine the presence or absence of TRCs in each fungiform papilla. Percentage of TRC-containing fungiform papillae was quantified in each animal. To obtain TRC/K8 index representing the number of TRCs in each fungiform papilla, total area of K8 signal within fungiform papillae was quantified (as seen in Fig. 5). For ectopic K8 quantification, the number of all $K8^+$ sites was divided by the number of normal K8 site within the fungiform papillae from 1 × 2 mm^2 of stained epithelia tile-scanned using 20× objective lens (as seen in Fig. 7*F*). Mean value from each experimental group was subsequently normalized to untreated group and plotted as mean ± SEM.

Gene Expression Analysis. Geniculate ganglia were removed and pooled from two to approximately three mice per sample. Nerve fibers were dissected away before tissues were snap-frozen. Tissue samples were homogenized and extracted for RNA using TRIzol, RNeasy Mini or Micro Kit (QIAGEN), and PureLink DNase Set (Thermo Fisher). cDNA was generated using SuperScript III (Thermo Fisher). *Shh*, *Ihh*, *Dhh*, and *Tubb3* levels were determined by qPCR by SsoAdvanced Universal SYBR Green (Bio-Rad) using primer sequences described previously (1). *Gli1* and *Hprt* levels were determined by qRT-PCR on an ABI 7900HT instrument using SuperScript III Platinum One-Step System with TaqMan Gene Expression Assays (Gli1, Mm00494654_m1; Hprt, Mm00446968_m1; Thermo Fisher). Normalized expression levels relative to untreated group were compared using one-way ANOVA nonparametric test with Dunn's multiple-comparison correction.

Proliferation Assay. Cell proliferation was measured from mice during XL139 blockade for 6 d. Five doses of EdU (25 mg/kg) were given daily by i.p. at 9 PM PST, with the first dose starting from 24 h after XL139 treatment. Tissues were processed for cryosection, and EdU assay was performed using Azide-647 triethylammonium (Thermo Fisher), (+)-sodium L-ascorbate, and copper sulfate (Sigma) as described (2), followed by immunofluorescence staining for K8 and DAPI. All EdU^+ cells located within each fungiform papilla were counted from sequential sections that contain most of the TRCs, without attempting to distinguish perigemmal progenitors vs. papillary wall progenitors. Number of fungiform papillae was quantified from at least two independent trials combining two to four mice in each group.

Cell Death Assay. TUNEL assay was performed using the Click-iT Plus TUNEL Assay for In Situ Apoptosis Detection (Thermo Fisher) according to the manufacturer's instructions. K8 staining was used to identify TRCs, and the number of $TUNEL^+$ $K8^+$ cells per fungiform papilla was quantified. We also carried out independent trials using cleaved caspase-3 (#9661S; Cell Signaling) to detect apoptotic cells and also did not observe difference in apoptosis after 1-wk Hh blockade.

Statistical Analysis. All statistical calculations were performed in Prism 7.0a (GraphPad Software).

Sample size. No statistical methods were used to predetermine the sample size. Our sample sizes are generally consistent to other studies in the field.

Randomization and blinding. Experimental samples were not randomized. The investigators were not blinded; however, mouse ear tag numbers were used when possible for tissue processing and image quantification.

Data are presented as mean ± SEM; statistical differences were calculated by the Mann–Whitney test (unpaired, two-tailed) or one-way ANOVA (Kruskal–Wallis test), and statistical significance is based on a value of $P < 0.05$.

1. Peterson SC, et al. (2015) Basal cell carcinoma preferentially arises from stem cells within hair follicle and mechanosensory niches. *Cell Stem Cell* 16:400–412.

2. Salic A, Mitchison TJ (2008) A chemical method for fast and sensitive detection of DNA synthesis in vivo. *Proc Natl Acad Sci USA* 105:2415–2420.

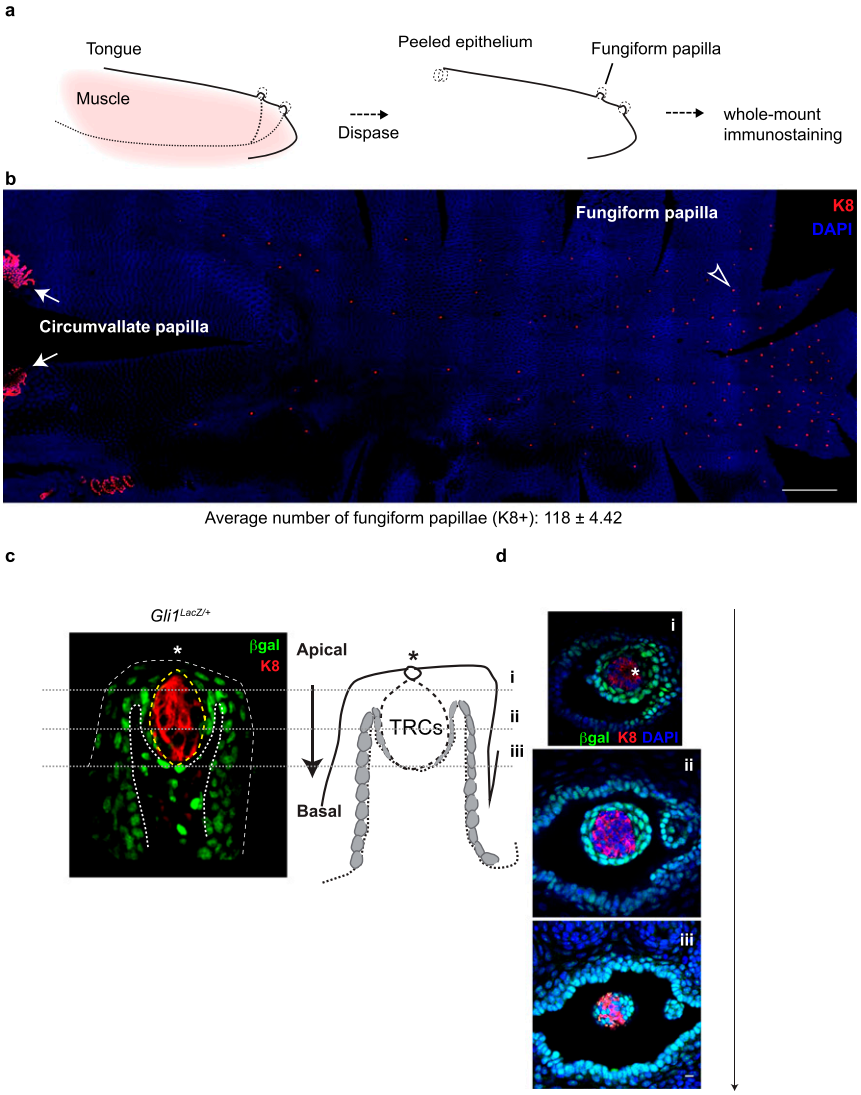

Fig. S1. Whole-mount and confocal analysis of tongue epithelium. (*A*) Separation of lingual muscle (pink) and epithelium (solid line) after dispase digestion. Peeled epithelia are subjected to whole-mount immunostaining. (*B*) An example of stained epithelium showing distribution of TRC-containing taste buds ($K8^+$, red). (*C* and *D*) Immunostaining of βgal in fungiform papillae in tissues prepared by cryosectioning (*C*) and whole-mount staining (*D*), which is shown as projected confocal images from *z*-stack images within each of the three planes (i–iii), from apical to basal direction. Note that βgal signals are absent from $K8^+$ cells, indicating lack of Hh activity within TRCs. [Scale bars: 1 mm (*B*); 10 μm (*D*).] (Magnification: *C*, 20×, as seen in Fig. S5*C*.)

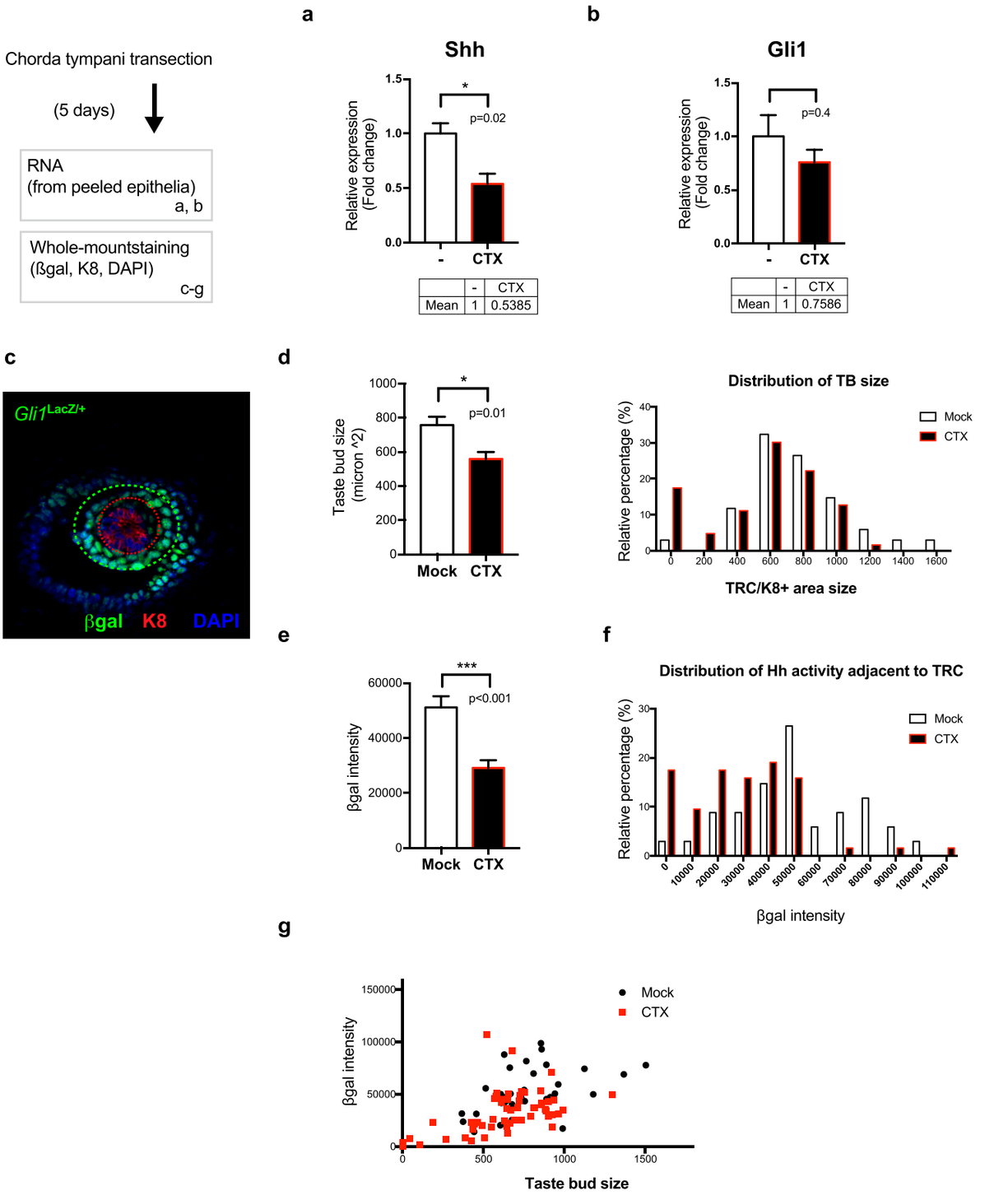

Fig. S2. Loss of Hh signaling and TRCs in the fungiform papillae following chorda tympani nerve transection (CTX). (*A* and *B*) qPCR analysis of *Shh* and *Gli1* expression from peeled epithelium 5 d after bilateral chorda tympani (CT) nerve transection. $n = 5$ mice in each group. Note that *Shh* expression in lingual epithelium of fungiform papillae is nerve dependent, consistent with previous observations in circumvallate papillae (1). (*C*–*F*) Effect of chorda tympani nerve transection on Hh activity and TRC size, 5 d after bilateral chorda tympani nerve transection. (*C* and *D*) Quantification of taste bud size marked by K8 (red, dotted red circle). (*E* and *F*) Quantification of Hh activity using integrated intensity of βgal (green) adjacent to TRC (dotted green circle). Distribution frequency of TRC area size and βgal intensity in each taste bud is divided into bins and center of bin is denoted on *x* axis. $n = 34$ and 63 fungiform papillae combined from 2–3 mice in each group. (*G*) Correlation of taste bud size and Hh activity. Note that the reduction of Hh activity upon denervation is more severe than decrease in taste bud size. (Magnification: *C*, 20×, as seen in Fig. S1*D*.)

1. Miura H, et al. (2004) A strong nerve dependence of sonic hedgehog expression in basal cells in mouse taste bud and an autonomous transcriptional control of genes in differentiated taste cells. *Chem Senses* 29:823–831.

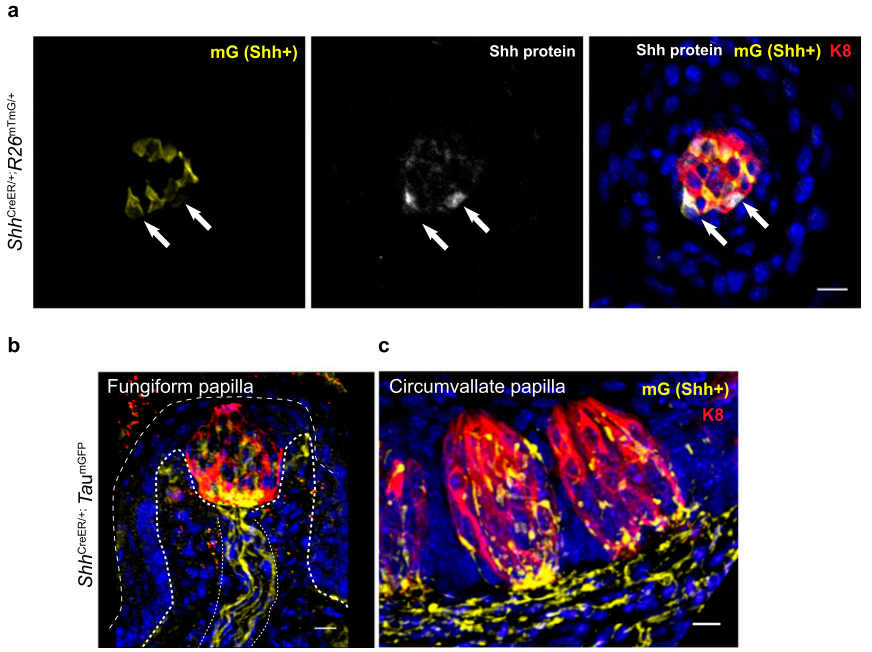

Fig. S3. Epithelial and neuronal sources of Shh. (*A*) Confocal image of a fungiform papilla immunostained with antibodies against GFP (yellow), Shh (gray), and CK8 (red) from $Shh^{CreER/+}$;$R26^{mTmG}$ mice, 7 d after tamoxifen induction. (*B* and *C*) Expression of Shh in gustatory neurons that innervate taste buds in two types of papillae was examined by marking *Shh* expression cells in a neuronal lineage reporter strain, $Shh^{CreER/+}$;Tau^{mGFP}. Immunofluorescence labeling of GFP (yellow) indicates fungiform (*B*) and circumvallate papilla (*C*) innervated by Shh-producing neurons. (Scale bars, 10 μm.)

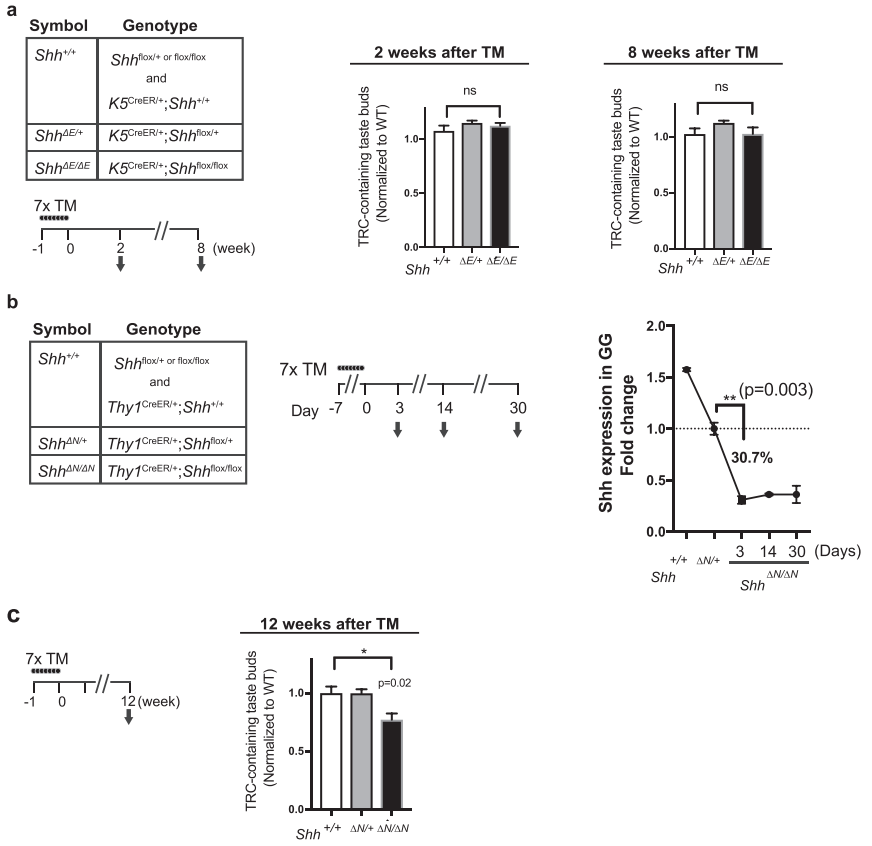

Fig. S4. Effect of conditional Shh ablation in the epithelium vs. sensory neuron. (*A*) Genotype of mice used and experimental scheme to introduce epithelial Shh ablation. Number of TRC-containing taste buds 2 and 8 wk after last dose of tamoxifen treatment in conditional epithelial *Shh* knockout $Shh^{\Delta E/\Delta E}$ mice. Sample size: $Shh^{+/+}$, $n = 7$; $Shh^{\Delta E/+}$, $n = 6$; $Shh^{\Delta E/\Delta E}$, $n = 5$ (2 wk); $Shh^{+/+}$, $n = 5$; $Shh^{\Delta E/+}$, $n = 4$; $Shh^{\Delta E/\Delta E}$, $n = 4$ (8 wk). ns, not significant. (*B*) qPCR analysis of *Shh* from dissected geniculate ganglia, taken from $Shh^{+/+}$, $Shh^{\Delta N/+}$, and $Shh^{\Delta N/\Delta N}$ at 3, 14, and 30 d after last dose of tamoxifen treatment. (*C*) Number of TRC-containing taste buds 12 wk after last dose of tamoxifen treatment in conditional neuronal *Shh* knockout $Shh^{\Delta N/\Delta N}$ mice (–22.8 ± 5.4% from $Shh^{+/+}$; $P = 0.02$). Sample size: $Shh^{+/+}$, $n = 4$; $Shh^{\Delta N/+}$, $n = 4$; $Shh^{\Delta N/\Delta N}$, $n = 5$. Note that conditional ablation of *Shh* from neurons but not epithelium causes TRC loss.

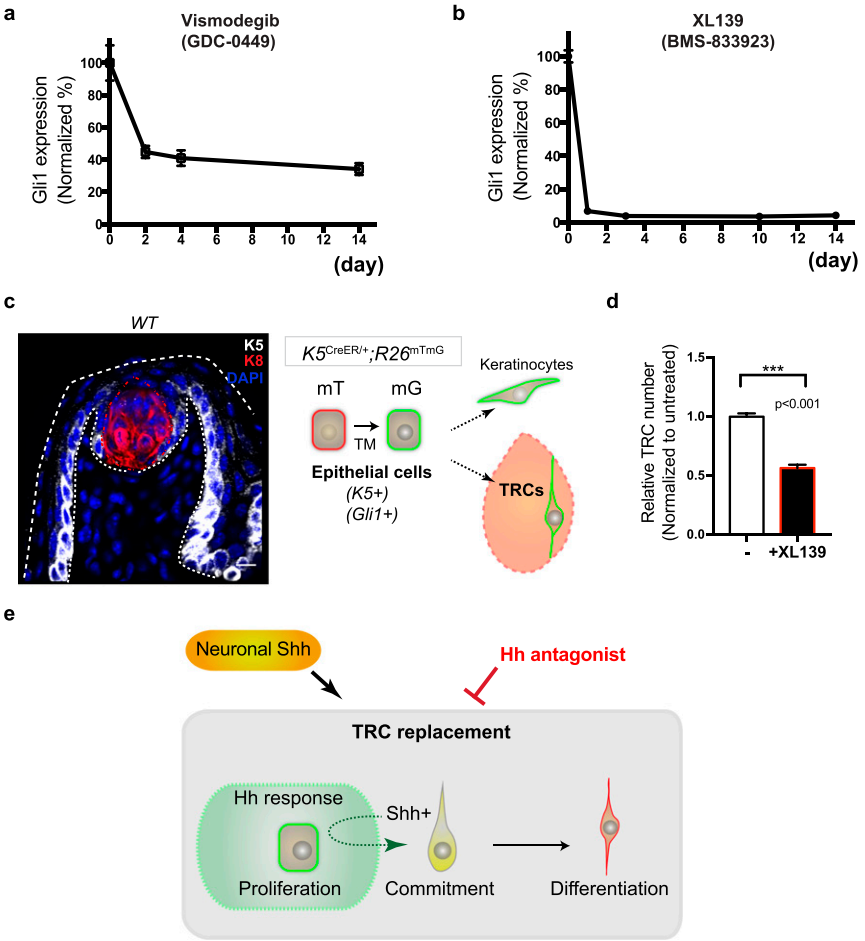

Fig. S5. Hh blockade impedes TRC replacement. (*A* and *B*) Comparison of two Smo antagonists for Hh blockade over the course of 2 wk by qPCR analysis of *Gli1* measured from peeled epithelium at indicated time points. (*A*) Vismodegib (GDC-0449) was delivered by oral gavage once per day at 100 mg/kg. (*B*) Stronger Hh blockade achieved by XL139 (BMS-833923), delivered by oral gavage every 3 d. (*C*) Confocal image of a fungiform papilla section showing that K5-expressing epithelial cells (white, for K5 native protein) occupy distinct domains from K8-expressing TRCs (red). Illustration depicts lineage tracing of K5-expressing epithelial cells and evaluation of their differentiation outcomes described in Fig. 4*D*. mG, mGFP; mT, membrane tdTomato; TM, tamoxifen. (Scale bars, 10 μm.) (*D*) Quantification of TRC number per fungiform papilla after 1 wk of XL139 treatment, scored by counting total number of DAPI$^+$ nuclei within the K8$^+$ area. Number of fungiform papillae analyzed, $n = 56$ and 81 from 3~4 mice in each group. (*E*) Summary of TRC renewal model (adapted from ref. 1). TRC replacement process begins with proliferation of epithelial cells (active in Hh response, denoted with green circle), which become Shh$^+$ committed precursor cells that can give rise to all three types of TRCs (2). Here, we examined how two sources of Hh signal (one neuronal and one epithelial) participate in TRC maintenance, with an emphasis on the role of neuronal Shh in TRC regeneration.

1. Barlow LA (2015) Progress and renewal in gustation: New insights into taste bud development. *Development* 142:3620–3629.
2. Miura H, Scott JK, Harada S, Barlow LA (2014) Sonic hedgehog-expressing basal cells are general post-mitotic precursors of functional taste receptor cells. *Dev Dyn* 243:1286–1297.

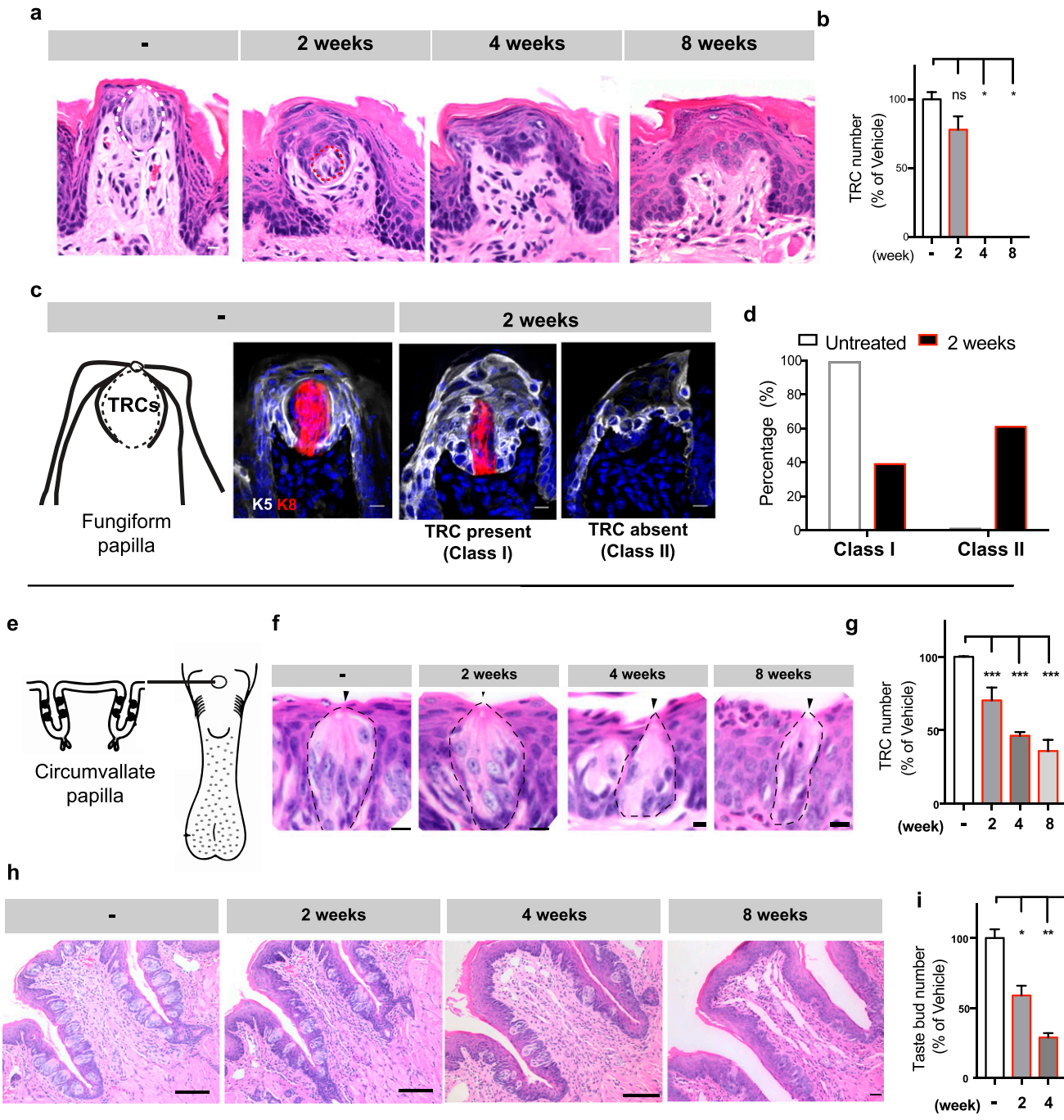

Fig. S6. Continuous TRC loss under long-term Hh blockade. (*A* and *B*) Loss of TRC in fungiform papillae (FP) from vehicle, 2-, 4-, and 8-wk XL139-treated mice. (*A*) Representative H&E staining from each group. (*B*) Quantified number of TRCs in each fungiform papilla, shown as percentage normalized to the vehicle group. Number of fungiform papillae analyzed, $n = 70$, 22, 12, and 8 from $n = 4$, 3, 3, and 3 mice. (*C*) Immunostaining of K8 (red, marks TRC) and K5 (gray, marks lingual epithelial basal cells) in fungiform papillae from vehicle and 2-wk XL139-treated mice. (*D*) Quantification of the frequency of fungiform papillae that are partially degenerated (class I) and fully degenerated (class II). (*E*) The location of circumvallate papilla on the mouse tongue; illustration adapted from Rubio-Navarro et al. (1). (*F–I*) Loss of TRCs in circumvallate papilla from vehicle-treated and 2-, 4-, and 8-wk XL139-treated mice. (*F*) Representative H&E staining of a taste bud in circumvallate papilla from each group. (*G*) Quantified number of TRCs within each taste bud. (*H*) Representative H&E staining of circumvallate papilla at lower magnification. (*I*) Quantification of taste bud loss over time. The average number of taste buds within each trench was plotted as normalized percentage of untreated group. Number of mice, $n = 4$, 2, 2, and 3. ns, not significant. (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.) [Scale bars: 10 μm (*A–F*); 100 μm (*H*).]

1. Rubio-Navarro L, Torrero C, Salas M (2011) Basic brainstem taste responsivity: Effects of perinatal influences. *eNeurobiologia* 2:1–22.

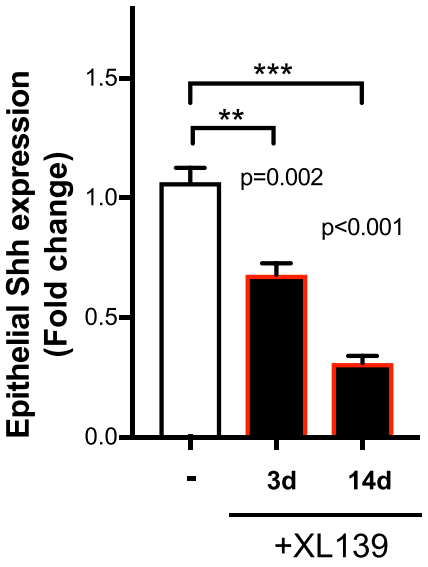

Fig. S7. Loss of *Shh* expression in lingual epithelium upon Hh blockade. qPCR analysis of *Shh* in peeled epithelium of C57B6 mice treated with vehicle or XL139 for 3 and 14 d. Number of mice: n = 8, 6, and 5.

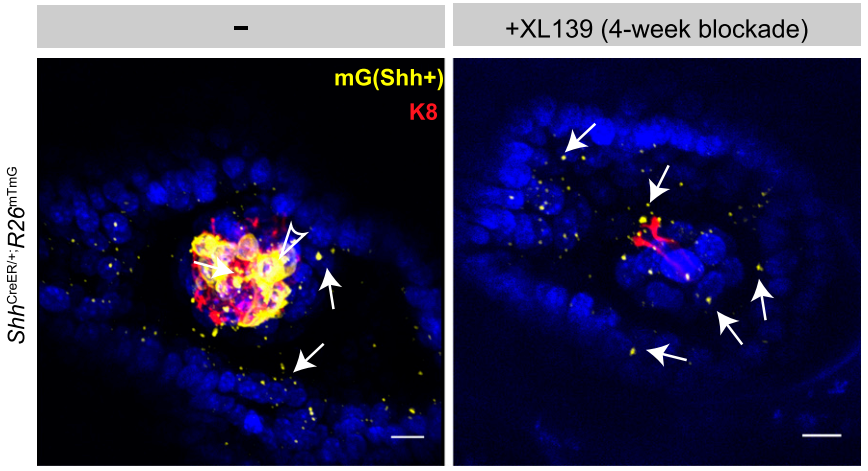

Fig. S8. Retention of neuronal Shh source under long-term Hh blockade. Immunofluorescence of GFP (yellow), K8 (red), and DAPI (blue) from peeled epithelia of $Shh^{CreER/+};R26^{mTmG}$ mice. Tamoxifen was injected after a 4-wk Hh blockade to mark Shh-expressing cells. Open arrowhead indicates marked epithelial source of Shh in untreated control group (*Left*), and arrows indicate the presence of neuronal termini in both untreated and 4-wk XL139-treated groups (*Right*). (Scale bars, 10 μm.)