Supporting Information

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SI Materials and Methods

Animals. *Gli1*$^{LacZ/+}$, *Gli2*$^{LacZ/+}$, *Gli1*$^{CreER/+}$, *Shh*$^{CreGFP/+}$, *Tau*$^{LacZ\text{-}mGFP}$, *Wnt1-Cre*, *Advillin*$^{Cre/+}$, *Shh*$^{flox/+}$, *Smo*$^{flox/+}$, *K5-tTA*, *TRE-cre*, *K14-CreER*, *R26*$^{SmoM2/+}$, *Hairless*, *Atoh1*$^{LacZ/+}$, *R26*$^{LacZ/+}$, and *R26*$^{YFP/+}$ mice were described previously, as shown in the table below:

Gli1$^{LacZ/+}$ (1)
Gli2$^{LacZ/+}$ (2)
K5-tTA;TRE-cre;Smo$^{flox/flox}$*;Gli1*$^{LacZ/+}$ (3–5)
Gli1$^{CreER/+}$*;R26*$^{LacZ/+}$ (6, 7)
Gli1$^{CreER/+}$*;R26*$^{YFP/+}$ (8)
Shh$^{CreGFP/+}$*;Tau*$^{LacZ\text{-}mGFP}$ (9, 10)
Hairless (*Hr*) (11)
Hr$^{hr/hr}$*;Atoh1*$^{LacZ/+}$ (12)
Wnt1-Cre;Shh$^{flox/flox}$ (12, 14)
Advillin$^{Cre/+}$*;Shh*$^{flox/flox}$ (15)
K14-CreER;R26$^{SmoM2/+}$ (16, 17)

Mice were housed and bred on an outcrossed Swiss Webster background in a pathogen-free facility at NCI, Bethesda, MD. Genotyping of mice was performed by allele-specific PCR on DNA extracted from tail tissue. Unless otherwise noted, all observations were made in at least three experimental mice and an equivalent number of littermate control mice (with the exception of denervation experiments in which the contralateral back skin served as an internal control). All experiments were performed in accordance with institutional guidelines according to Institutional Animal Care and Use Committee-approved protocols.

Tissue Processing. Skin was fixed in 4% paraformaldehyde for 15 min (for X-Gal staining) or overnight (for immunostaining). Tissue was whole-mount stained or cryoprotected overnight in 30% sucrose, embedded in O.C.T. (Fisher Scientific), and frozen. Twelve-millimeter sections were obtained. X-Gal–stained slides were counterstained with nuclear fast red. For immunohistochemistry/X-Gal combined staining, primary antibody was added to X-Gal reaction buffer and was incubated overnight at room temperature followed by standard immunohistochemistry staining procedures starting from washing of the primary antibody.

Immunofluorescent Staining. Standard and whole-mount immunostaining procedures were performed. Tissue sections on glass slides were fixed in 4% paraformaldehyde for 15 min before incubation in 10% serum in 0.1% PBT (0.1% Triton X-100 in PBS) for 1 h and then in primary antibody (in 5% serum/0.1% PBT) overnight at 4 °C. The primary antibodies used were rat anti-K8 (1:50; University of Iowa), rabbit anti–β-galactosidase (1:200; eBioscience), rabbit anti-K17 (1:200; Epitomics), chicken anti-GFP (1:1,000; Abcam), rabbit anti-S100 (1:500; Millipore), chicken anti-neurofilament (1:1,000; Abcam), and rabbit anti-K14 (1:1,000; Covance). Alexa-Fluor–conjugated secondary antibodies (1:2,000; Invitrogen) were used to detect the signals. Whole-mount immunostaining followed the online protocol as described (18). Concomitant staining of littermate control tissue and control staining in which the primary antibody was omitted were used to confirm the specificity of experimental staining. Confocal images were acquired with the Zeiss LSM 710 Confocal system (Carl Zeiss Inc.).

The Troma-1/K8 antibody developed by Philippe Brulet and Rolf Kemler was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the Eunice Kennedy Shriver National Institute of Child Health and Human Development and maintained by the University of Iowa, Department of Biology, Iowa City, IA.

Quantification of Merkel Cells in Touch Domes. The numbers of Merkel cells per touch dome were assessed by direct visualization of immunofluorescently stained K8$^+$ Merkel cells in whole-mount tissue from experimental and control animals. Counting was performed by a blinded observer. Because shaving and processing skin occasionally result in tissue damage, Merkel cells were not counted in touch domes where tissue damage was evident. In experimental animals in which touch dome maintenance was impaired, there often were guard hairs with no adjacent staining for touch dome cells. Because there was no reliable way to identify these "empty" touch dome sites, we did not include touch domes with no Merkel cells in our quantifications. Thus, the reported number of Merkel cells per touch dome in mutant animals is likely to be artificially high, because it reflects the mean number of Merkel cells in the remaining touch domes. The reported number is the number of touch domes counted. Because of the small size and low abundance of touch domes within the epidermis, Merkel cell quantification is more prone to sampling errors in stained tissue sections than in whole-mount tissue. For this reason we report quantifications of double-stained Merkel cells in sectioned tissues as estimates based on counting >50 individual Merkel cells.

Surgical Denervation of Cutaneous Nerves. Dorsal cutaneous nerves were severed by microsurgery as described (19). Briefly, a midline incision of dorsal trunk skin allowed visualization and surgical denervation of the right dorsal skin. Sham-treated left dorsal skin was used as control.

Detection of *Shh* Deletion and Relative Expression in DRG Neurons. DRG neurons were dissected from *Wnt1-Cre;Shh*$^{flox/flox}$ ($n = 3$) and control ($n = 3$) mice. Total RNA was purified using TRIzol and was reverse transcribed into cDNA following the manufacturer's manual (Invitrogen #11752). Then quantitative RT-PCR was performed with 18S rRNA as control, using SYBR Green to detect *Shh* expression with three technical replicates. Data are presented as means ± SEM. Genomic deletion of *Shh* in DRG neurons and tail biopsy total DNA were examined using ABI Taqman assay (Mm00560396_cn). The sequences of PCR primers were Shh-forward: 5′-AAGCAGGTTTCGACTGGGTC-3′; Shh-reverse: 5′-CCGGGACGTAAGTCCTTCAC-3′. Data are presented as means ± SD of three technical replicates.

Statistical Analyses. Population datasets are shown as the mean value; error bars represent SEM. For comparisons between sets, an unpaired, two-tailed t test was applied.

Animal Treatments. Tamoxifen (Sigma) was dissolved in corn oil (20 mg/mL) and injected i.p. (200 mg/kg body weight) for three daily doses to induce CreER. For *K14-CreER;R26*$^{SmoM2/+}$ mice, tamoxifen was dissolved in 100% ethanol (10 mg/mL), and ∼100 uL was applied dropwise to shaved trunk skin once daily for 3 d to induce CreER. Depilation was achieved by manual plucking of dorsal trunk skin followed by wax-strip depilation. *K5-tTA;TRE-cre;Smo*$^{flox/flox}$ mice were bred and maintained on a complete rodent diet containing 200 mg/kg dox administered ad libitum until the point of dox withdrawal, at which time mice were switched to standard rodent diet.

1. Bai CB, Auerbach W, Lee JS, Stephen D, Joyner AL (2002) Gli2, but not Gli1, is required for initial Shh signaling and ectopic activation of the Shh pathway. *Development* 129(20):4753–4761.
2. Bai CB, Joyner AL (2001) Gli1 can rescue the in vivo function of Gli2. *Development* 128(24):5161–5172.
3. Diamond I, Owolabi T, Marco M, Lam C, Glick A (2000) Conditional gene expression in the epidermis of transgenic mice using the tetracycline-regulated transactivators tTA and rTA linked to the keratin 5 promoter. *J Invest Dermatol* 115(5):788–794.
4. Perl AK, Wert SE, Nagy A, Lobe CG, Whitsett JA (2002) Early restriction of peripheral and proximal cell lineages during formation of the lung. *Proc Natl Acad Sci USA* 99(16):10482–10487.
5. Long F, Zhang XM, Karp S, Yang Y, McMahon AP (2001) Genetic manipulation of hedgehog signaling in the endochondral skeleton reveals a direct role in the regulation of chondrocyte proliferation. *Development* 128(24):5099–5108.
6. Ahn S, Joyner AL (2004) Dynamic changes in the response of cells to positive hedgehog signaling during mouse limb patterning. *Cell* 118(4):505–516.
7. Soriano P (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 21(1):70–71.
8. Srinivas S, et al. (2001) Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* 1:4.
9. Hippenmeyer S, et al. (2005) A developmental switch in the response of DRG neurons to ETS transcription factor signaling. *PLoS Biol* 3(5):e159.
10. Harfe BD, et al. (2004) Evidence for an expansion-based temporal Shh gradient in specifying vertebrate digit identities. *Cell* 118(4):517–528.
11. Montagna W, Chase HB, Melaragno HP (1952) The skin of hairless mice. I. The formation of cysts and the distribution of lipids. *J Invest Dermatol* 19(1):83–94.
12. Ben-Arie N, et al. (2000) Functional conservation of atonal and Math1 in the CNS and PNS. *Development* 127(5):1039–1048.
13. Danielian PS, Muccino D, Rowitch DH, Michael SK, McMahon AP (1998) Modification of gene activity in mouse embryos in utero by a tamoxifen-inducible form of Cre recombinase. *Curr Biol* 8(24):1323–1326.
14. Lewis PM, et al. (2001) Cholesterol modification of sonic hedgehog is required for long-range signaling activity and effective modulation of signaling by Ptc1. *Cell* 105(5):599–612.
15. da Silva S, et al. (2011) Proper formation of whisker barrelettes requires periphery-derived Smad4-dependent TGF-beta signaling. *Proc Natl Acad Sci USA* 108(8): 3395–3400.
16. Vasioukhin V, Degenstein L, Wise B, Fuchs E (1999) The magical touch: Genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. *Proc Natl Acad Sci USA* 96(15):8551–8556.
17. Jeong J, Mao J, Tenzen T, Kottmann AH, McMahon AP (2004) Hedgehog signaling in the neural crest cells regulates the patterning and growth of facial primordia. *Genes Dev* 18(8):937–951.
18. Li W, Mukouyama YS (2011) Whole-mount immunohistochemical analysis for embryonic limb skin vasculature: A model system to study vascular branching morphogenesis in embryo. *J Vis Exp* (51):e2620.
19. Maurer M, Peters EM, Botchkarev VA, Paus R (1998) Intact hair follicle innervation is not essential for anagen induction and development. *Arch Dermatol Res* 290(10): 574–578.

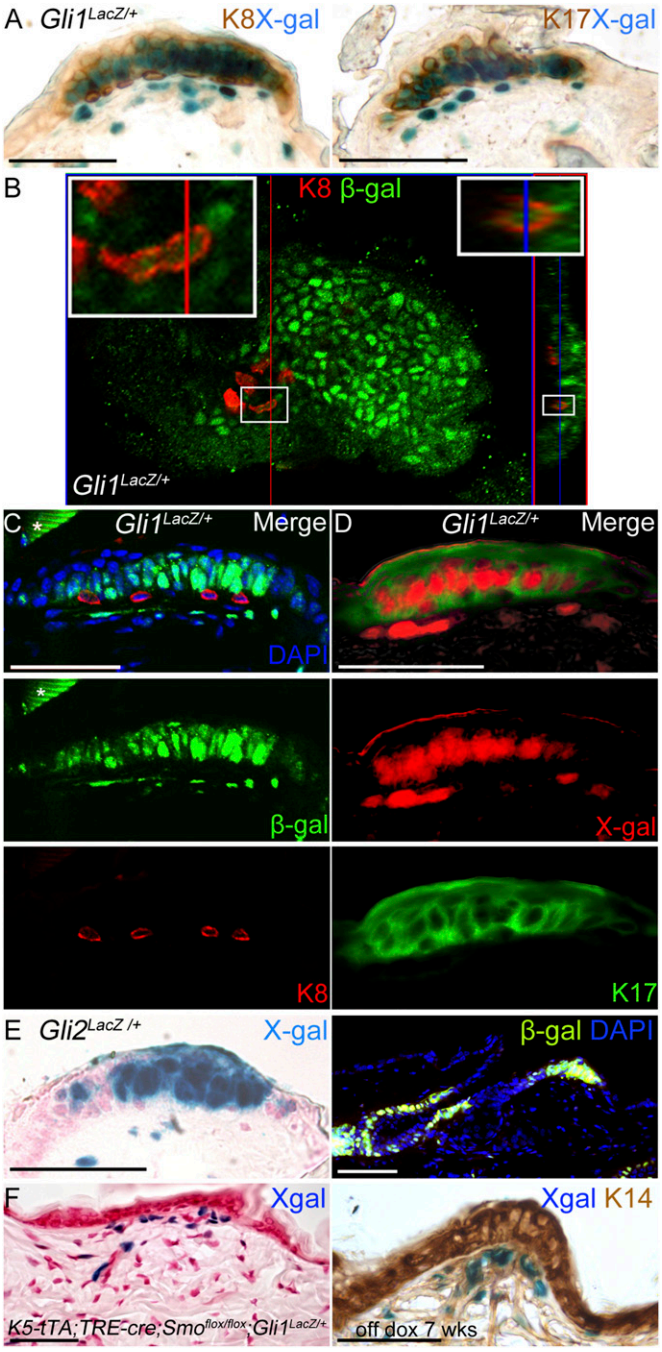

Fig. S1. The touch dome contains Hh-responding cells. (*A*) Costaining of X-Gal with K17 or K8 immunohistochemistry in the skin of an adult *Gli1*$^{LacZ/+}$ mouse. (*B*) Whole-mount *en face* confocal image of β-galactosidase (β-gal) and K8 staining in skin from the back of an adult *Gli1*$^{LacZ/+}$ mouse. (*Inset*) A rare Gli1^{+} Merkel cell. (*C*) Individual channels of Fig. 1*B* β-gal and K8 staining in skin from the back of an adult *Gli1*$^{LacZ/+}$ mouse. (*D*) Individual channels of Fig. 1C X-Gal (false-colored red) and K17 staining in the skin of an adult *Gli1*$^{LacZ/+}$ mouse. (*E*) X-Gal section staining and β-gal staining in skin from a *Gli2*$^{LacZ/+}$ mouse. (*F*) Section staining of X-Gal with nuclear fast red or with K14 immunohistochemistry in the touch dome of an adult *K5-tTA;TRE-cre;Smo*$^{flox/flox}$*;Gli1*$^{LacZ/+}$ mouse 7 wk after the withdrawal of dox demonstrating loss of Gli1 in epidermis at sites of touch domes (visualized by Gli1 reporter staining in dermis).

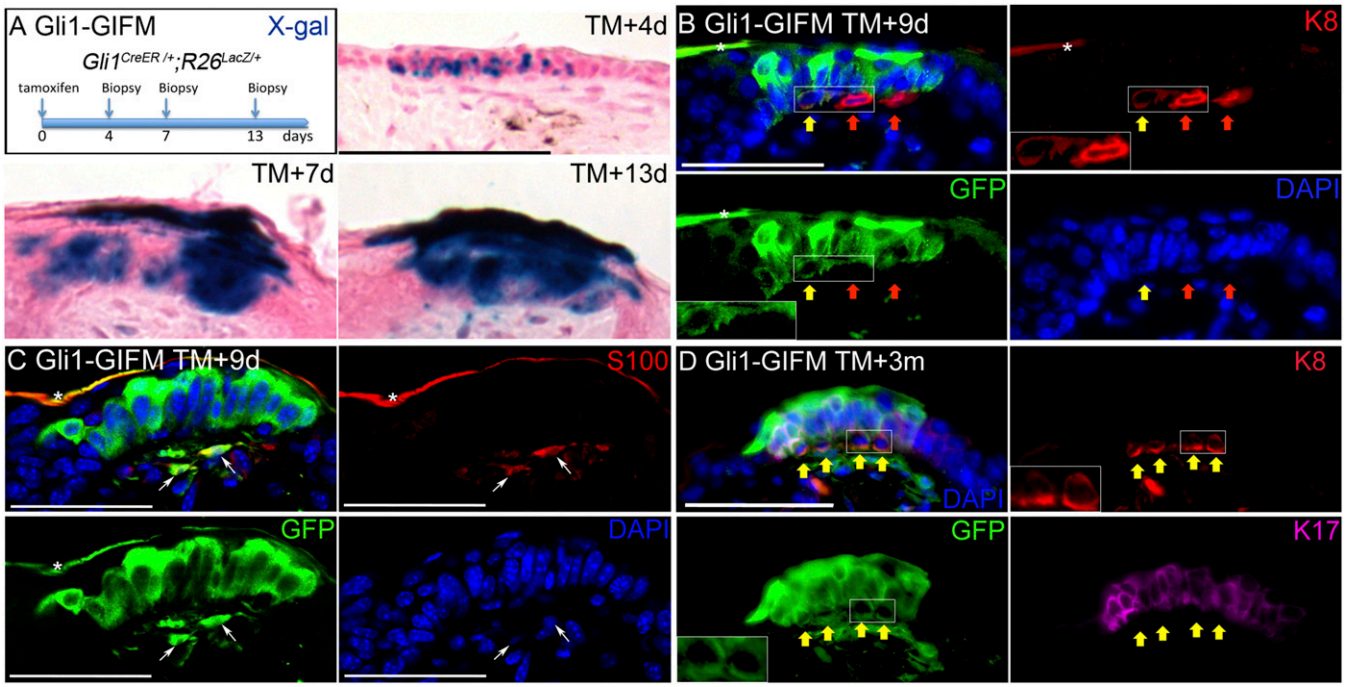

Fig. S2. Hh-responding progenitor cells maintain the touch dome and associated Schwann cells. (*A*) Experimental scheme and X-Gal staining in skin from adult Gli1-GIFM (*Gli1*$^{CreER/+}$;*R26*$^{LacZ/+}$) mice 4 d, 7 d, and 13 d after tamoxifen induction. (*B*) Individual channels of Fig. 1*D* K8 and GFP staining in the skin from a Gli1-GIFM (*Gli1*$^{CreER/+}$;*R26*$^{YFP/+}$) mouse 9 d after tamoxifen induction at P23. Red arrows indicate unlabeled Merkel cells. (*Inset*) A labeled K8^{low} Merkel cell and an unlabeled K8^{+} Merkel cell. (*C*) S100 and GFP staining in skin from a Gli1-GIFM (*Gli1*$^{CreER/+}$;*R26*$^{YFP/+}$) mouse 9 d after tamoxifen induction at P23. White arrows indicate labeled Schwann cells. Asterisks in *B* and *C* indicate nonspecific staining. (*D*) Individual channels of Fig. 1*E* K8, K17, and GFP staining in skin from an adult Gli1-GIFM (*Gli1*$^{CreER/+}$;*R26*$^{YFP/+}$) mouse 3 mo after depilation and tamoxifen induction. (*Inset*) Labeled K8^{+} Merkel cells. Yellow arrows in *B* and *D* indicate labeled Merkel cells. (Scale bars, 50 μm.)

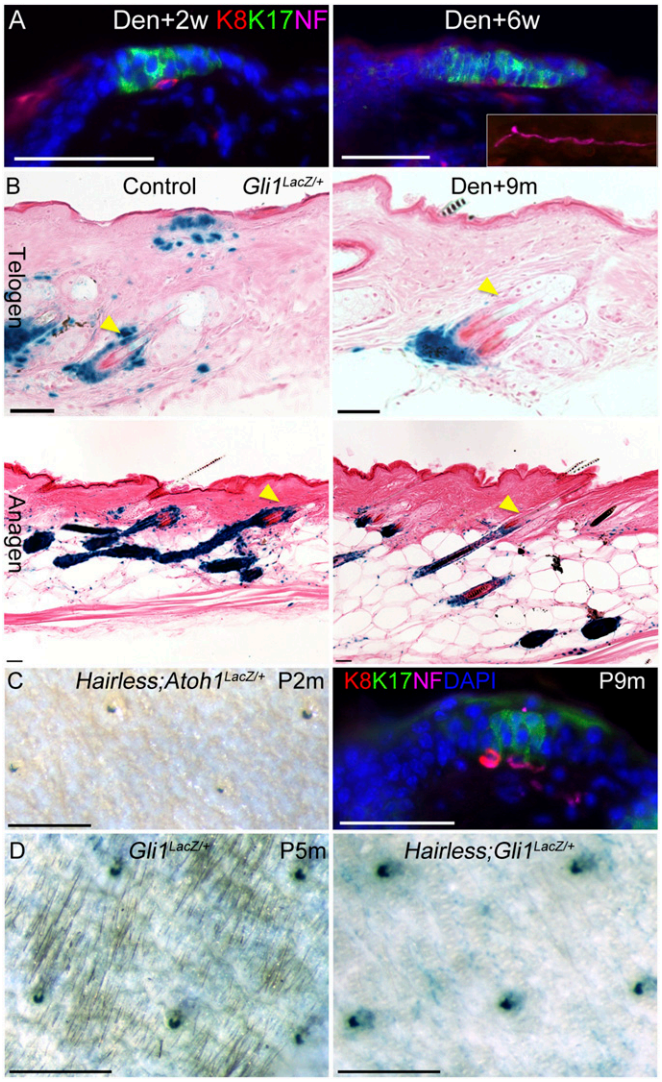

Fig. S3. The touch dome lineage is preserved in short-term denervated mouse skin and is maintained independent of cycling hair follicles. (*A*) K8, K17, and neurofilament (NF) staining in the skin of an adult *Gli1*$^{LacZ/+}$ mouse 2 wk (*Left*) and 6 wk (*Right*) after denervation (Den). (*Inset*) Stained nerve from non-denervated dermis. (*B*) X-Gal staining of telogen and anagen hair follicles in *Gli1*$^{LacZ/+}$ mouse 9 mo after denervation. The arrowheads indicate the upper bulge region. (*C*) X-Gal whole-mount staining in the skin from a 2-mo-old *Hairless;Atoh1*$^{LacZ/+}$ mouse and K8, K17, and NF staining in the skin from a 9-mo-old *Hairless* mouse. (*D*) X-Gal whole-mount staining in skin from 5-mo-old *Gli1*$^{LacZ/+}$ and *Hairless;Gli1*$^{LacZ/+}$ mice. (Scale bars, 50 μm for section staining; 0.5 mm for whole-mount X-Gal staining.)

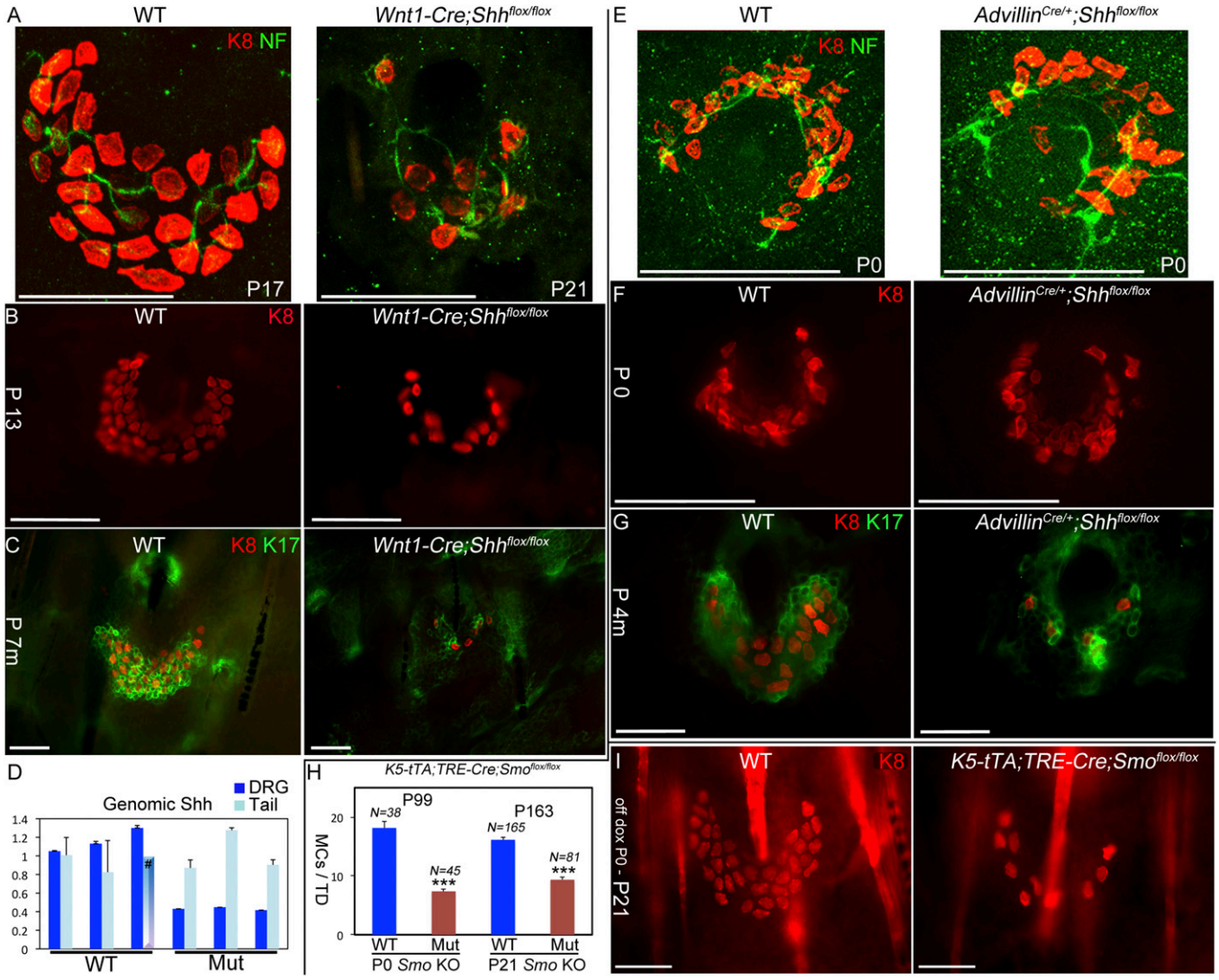

Fig. S4. Conditional knockout of *Shh* from DRG neurons or of *Smo* from the epidermis results in failure to maintain the touch dome lineage. (*A*) Confocal maximum projection of whole-mount K8 and NF staining in the skin from a P21 *Wnt1-Cre;Shh*$^{flox/flox}$ mouse. Skin from a P17 WT mouse is shown for comparison. (*B*) K8 whole-mount staining in skin from P13 WT and *Wnt1-Cre;Shh*$^{flox/flox}$ mice. (*C*) K8 and K17 staining in skin from 7-mo-old WT and *Wnt1-Cre;Shh*$^{flox/flox}$ mice. (*D*) Quantitative PCR detection of the genomic *Shh* locus in DRG neurons and tail DNA of P16 WT and mutant (Mut; *Wnt1-Cre;Shh*$^{flox/flox}$) mice. The mean copy number and SD of the *Shh* target region are normalized to that of the *GAPDH* locus in each sample. #, data missing. (*E*) Confocal maximum projection of whole-mount K8 and NF staining in skin from P0 WT and *Advillin*$^{Cre/+}$*;Shh*$^{flox/flox}$ mice. (*F*) K8 whole-mount staining in skin from P0 WT and *Advillin*$^{Cre/+}$*;Shh*$^{flox/flox}$ mice. (*G*) K8 and K17 whole-mount staining in skin from 4-mo-old WT and *Advillin*$^{Cre/+}$*;Shh*$^{flox/flox}$ mice. (*H*) Number of K8^{+} Merkel cells per touch dome (mean ± SEM) in skin from WT and mutant (Mut; *K5-tTA;TRE-cre;Smo*$^{flox/flox}$) mice after *Smo* deletion at P0 or P21, assessed at the indicated postnatal day of life. ***$P < 0.0001$. (*I*) K8 whole-mount staining in skin from P21 WT and *K5-tTA;TRE-cre;Smo*$^{flox/flox}$ mice; dox was withdrawn at P0. (Scale bars, 50 μm.)