Supporting Information

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SI Materials and Methods

Animal Strains. Alleles used in this study were an inducible activated allele of *Smo*, *SmoM2* ($Gt(ROSA)26Sor^{tm1(SmoM2/EYFP)Amc}$) (1); an inducible Cre under the control of the *Keratin-15* promoter, $K15\text{-}Cre^{PR1}$ (*Tg*(*Krt1-15-cre*/*PGR*)*22Cot*) (2); an inducible Cre under the control of the *Keratin-14* promoter, $K14\text{-}Cre^{ERT}$ (*Tg*(*KRT14-cre*/*ERT*)*20Efu*) (3); the *LacZ* reporter of Cre activity (*R26R*, $Gtrosa26^{tm1Sor}$) (4); and the Hh pathway reporter *Ptch1-LacZ* ($Ptch1^{tm1Mps}$) (5).

Histology. Skin biopsies were fixed overnight in formalin for H&E staining or immunohistochemical staining. For YFP and cilia staining, biopsies were fixed for 1 h in 4% paraformaldehyde at 4 °C, then sunk in 30% sucrose overnight for OCT embedding. For whole-mount β-galactosidase (β-Gal) staining, skin and wound sites were removed, cleared of adipose tissue, fixed in 0.2% glutaraldehyde/2% paraformaldehyde for 30 min at 4 °C, and then incubated for 48 h in 1 mg/mL X-Gal (Invitrogen) diluted in 5 mM potassium ferrocyanide and 5 mM potassium ferricyanide. For on-slide β-Gal staining, skin biopsies were embedded directly into OCT. Frozen sections were fixed in 0.5% glutaraldehyde for 5 min, then stained with X-Gal, as above, before counterstaining with Nuclear Fast Red solution (Sigma-Aldrich).

Immunohistochemistry. Antibodies used for immunohistochemistry included those raised against Keratin-15 (LHK15, 1:50; Abcam), Keratin-5 (1:500; American Research Products), Sox9 (H-90, 1:25; Santa Cruz Biotechnology), NFATc1 (1:25; BD Biosciences), CD34 (1:10, BD Biosciences), P-cadherin (PCD1, 1:200; Invitrogen), GFP/YFP for detection of SmoM2 (GFP-1020, 1:500; Aves Labs), Gli2 (AB7195, 1:500; Abcam), Arl13b (1:5,000, gift of T. Caspary, Emory University, Atlanta, GA), β-catenin (Clone 6B3, 1:50; Cell Signaling Technologies; or Clone 14, 1:50; BD Biosciences), and phosphorylated H3S10 (1:200; Cell Signaling Technologies). All imaging was performed with a C1si Spectral Confocal microscope (Nikon Instruments) and NIS-Elements software (Nikon).

In Situ Hybridization. In situ staining was performed on formalin-fixed, paraffin-embedded sections using probes for *Axin2* (gift of Y. Choe, University of California San Francisco) and *Gli1* (EST W65013). Slides were dewaxed and rehydrated, permeabilized with proteinase K, postfixed with paraformaldehyde, acetylated, and probed overnight at 60 °C. Slides were subsequently probed with anti-digoxygenin antibody conjugated to alkaline phosphatase (Roche Applied Science), and detected with 5-Bromo-4-chloro-3′-indolyphosphate P-toluidine salt/Nitro-blue tetrazolium chloride (BCIP/NBT) chromogen solution (Roche Applied Science).

Quantitation. For quantitation of K14-Cre^{ERT} and K15-Cre^{PR1} activities, on-slide β-Gal staining was performed on skin biopsies 3 d after induction, and randomized fields from 10-μm sections were analyzed. The bulge and SHG was defined as the domain of cells located below the level of the sebaceous glands in telogen hair follicles. Quantitation was performed using NIS-Elements software (AR 3.2; Nikon). For quantitation of neoplastic downgrowths, clusters of cells protruding into the dermis from the IFE of unwounded skin or from the healed wound epithelium were counted in 8-μm H&E slides and normalized for the length of skin analyzed. All error bars show SE. *P* values were calculated at http://www.physics.csbsju.edu/stats/Index.html.

1. Mao J, et al. (2006) A novel somatic mouse model to survey tumorigenic potential applied to the Hedgehog pathway. *Cancer Res* 66:10171–10178.
2. Morris R-J, et al. (2004) Capturing and profiling adult hair follicle stem cells. *Nat Biotechnol* 22:411–417.
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4. Soriano P (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 21:70–71.
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Fig. S1. (*A*) Immunohistochemical staining of *K15:SmoM2* wound-induced tumors for basal layer (Keratin-5 and Keratin-14) and follicular (Keratin-17) keratins. (*B*) *K15:SmoM2* wound-induced tumor displaying nuclear palisading (dashed line). (*C*) Hyperplastic anagen follicle adjacent to the wound site of *K15:SmoM2* skin.

3 Days After Final Tamoxifen or RU486 Treatment

	IFE +	IFE Total	% IFE	# HFs	Bulge or SHG +	Bulge and SHG Total	% Bulge
K14:LacZ	624	1758	35.5	68	158	3350	4.7
K14:SmoM2:LacZ	577	1526	37.8	57	116	3100	3.7
K15:LacZ	12	1800	0.7	70	1142	3367	33.9
K15:SmoM2:LacZ	68	1857	3.7	64	1063	3197	33.2

Fig. S2. Table quantitating the activities of *K14-Cre*^{ERT} and *K15-Cre*^{PR1} in telogen skin 3 d after tamoxifen or RU486 induction, respectively. Columns depict (from left to right) the genotypes of the animals examined; the number of β-Gal+ cells counted in the IFE; the total number of IFE cells counted; the percentage of labeled cells observed in the IFE; the total number of hair follicle cross-sections examined; the number of β-Gal+ cells seen in the bulge or SHG; the total number of bulge and SHG cells counted; and the percentage of labeled cells observed in the bulge and SHG.

Fig. S3. (*Upper*) Labeled bulge and SHG cells in intact skin. (*Lower*) Whole-mount β-Gal staining of skin cross-sections reveals the migration of bulge and SHG-derived cells into the wound site 3 d after injury. Brackets indicate wound-adjacent hair follicles where labeled bulge and SHG-derived cells have migrated into the upper follicle. Asterisks indicate the labeled bulge and SHG-derived cells at the epithelial leading edge. Dotted lines delineate the edges of the wound site.

Fig. S4. (*A*) Wound-induced tumors from *K15:SmoM2* mice display increased proliferation relative to nontumorigenic re-epithelialized skin, as assessed by staining for the mitotic marker phosphorylated H3S10 (pH3S10). (*B*) Quantitation for *A*. (*C*) All neoplastic downgrowths in wound-induced tumors express SmoM2-GFP and Keratin-5.

Fig. S5. (*A Left*) In situ staining reveals that *Gli1*, a downstream target of Hh signaling, is not up-regulated either 3 or 14 d after wounding. Dotted lines delineate the epidermal–dermal junction. Asterisk indicates the leading edge of the epithelial wound margin. Arrow indicates the region of *Gli1* expression near the SHG and dermal papilla of an intact follicle adjacent to the wound site, for internal comparison. (*Right*) In situ staining reveals that *Axin2*, a downstream target of Wnt signaling, is not up-regulated either 3 or 14 d after wounding. (*B*) β-Gal staining in *Patched1-LacZ* skin showing that the Hh pathway is inactive in keratinocytes at the leading edge of the wound margin (asterisk). Arrows indicate the sites of *Ptch1* expression near the SHG and dermal papilla of adjacent follicles, for internal comparison. Arrowhead indicates a hair follicle remnant at the wound edge.

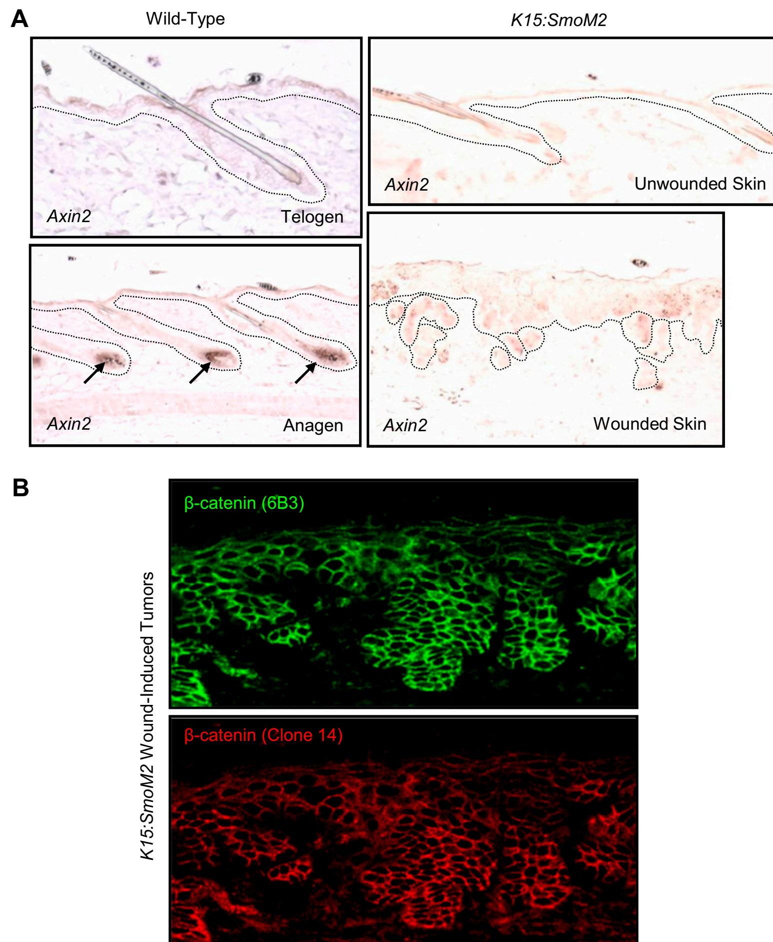

Fig. S6. (*A Left*) In situ staining for *Axin2* in wild-type telogen and anagen hair follicles. *Axin2* is expressed mostly in the matrix (arrows). (*Right*) In situ staining for *Axin2* in *K15:SmoM2* intact skin or wound-induced tumors. Dotted lines delineate the epidermal–dermal junction. (*B*) Immunohistochemical staining for β-catenin using two independent antibodies in *K15:SmoM2* wound-induced tumors.

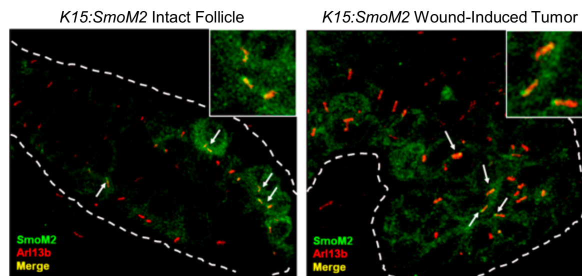

Fig. S7. Immunofluorescence staining of intact follicular bulge cells (*Left*) and wound-induced tumors (*Right*) in *K15:SmoM2* skin, showing SmoM2 localization to primary cilia, as visualized by costaining for the ciliary protein Arl13b. (*Insets*) Magnified views showing SmoM2 at the cilium.

Fig. S8. In situ staining for *Axin2* and *Gli1* in normal anagen follicles from wild-type mice (*Upper*) or in hyperplastic anagen follicles from depilated *K15:SmoM2* skin (*Lower*). Arrows indicate the *Axin2* staining in the follicular bulb and matrix. Arrowhead indicates the *Gli1* staining along the lower outer root sheath. (Image depicting *Axin2* staining in wild-type follicles from Fig. S6 is included for comparison.)

Fig. S9. Whole-mount β-Gal staining reveals that bulge or SHG-derived cells (arrows) persist at healed incisional wound sites 10 wk after wounding, irrespective of oncogene expression.

Fig. S10. (*Left*) Photograph of a healed wound site in a *K15:SmoM2* mouse (dotted oval) 10 wk after wounding. The animal was shaved before treatment with RU486 and wounding. Note that wound-adjacent follicles had re-entered anagen and regenerated hair (*Right*), whereas the rest of the shaved dorsal skin had remained in telogen. Red arrow indicates the site of biopsy at the healed wound site. Green arrow indicates the site of contralateral biopsy in intact skin.