Correction

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The authors note that Jessica Svärd and Stephan Teglund should be added to the author list between Anja Schwäger and Nick Barker. Jessica Svärd should be credited with contributing new reagents/analytical tools. Stephan Teglund should be credited with contributing new reagents/analytical tools. The corrected author and affiliation lines, and author contributions appear below. The online version has been corrected.

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Wounding enhances epidermal tumorigenesis by recruiting hair follicle keratinocytes

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Chronic wounds and acute trauma constitute well-established risk factors for development of epithelial-derived skin tumors, although the underlying mechanisms are largely unknown. Basal cell carcinomas (BCCs) are the most common skin cancers displaying a number of features reminiscent of hair follicle (HF)-derived cells and are dependent on deregulated Hedgehog (Hh)/GLI signaling. Here we show, in a mouse model conditionally expressing GLI1 and in a model with homozygous inactivation of Ptc1, mimicking the situation in human BCCs, that the wound environment accelerates the initiation frequency and growth of BCC-like lesions. Lineage tracing reveals that both oncogene activation and wounding induce emigration of keratinocytes residing in the lower bulge and the nonpermanent part of the HFs toward the interfollicular epidermis (IFE). However, only oncogene activation in combination with a wound environment enables the participation of such cells in the initiation of BCC-like lesions at the HF openings and in the IFE. We conclude that, in addition to the direct enhancement of BCC growth, the wound-promoting effect of the wound environment is due to recruitment of tumor-initiating cells originating from the neighboring HFs, establishing a link between epidermal wounds and skin cancer risk.

Lgr5 | stem cells | wound healing | carcinogenesis

In the skin, the hair follicle (HF) is a reservoir for epithelial stem and progenitor cells, which reside in different niches (1). One population marked by expression of Keratin 15 (K15) and CD34 is present in the bulge, a structure below the opening of the sebaceous gland at the attachment site of the musculus arrector pili (2). Other stem cell (SC) populations marked by expression of Lgr6 and Lrig1 are found in the junctional zone above the bulge, in close proximity to the sebaceous gland (isthmus) and the neck of the HF (infundibulum) (3, 4). In addition, a SC population marked by expression of Lgr5 is present in the bulge and secondary hair germ (SHG) in the resting telogen hair follicle but relocates to the outer root sheath (ORS) of the growing part in actively cycling anagen HFs (5). Genetic lineage tracing has revealed that bulge and Lgr5-marked SCs maintain all parts of the hair follicle below the sebaceous gland opening (5). Under normal circumstances, HFs and the interfollicular epidermis (IFE) are maintained by separate cell populations (6); however, during cutaneous wound repair, the cells originating from the HF, including the bulge SCs, leave the HF and contribute to wound reepithelialization (7, 8). Though both chronic and acute wounds have been recognized as promoters of the formation of epithelial tumors, the underlying cellular mechanisms have not been studied in detail (9, 10). By using two different genetic mouse models of human BCC, we show that the wound environment enhances the tumor initiation and progression. Lineage tracing of Lgr5+ cells revealed that keratinocytes originating from the lower bulge and the nonpermanent part of the HF contribute to wound reepithelialization and the formation of basaloid proliferations in the IFE of the wound area. Furthermore, Lgr5+ cells with an activated Hh pathway can initiate new BCC-like lesions in the wound IFE, suggesting that the enhancement of the BCC formation caused by wounding can be at least in part attributed to cellular contribution from neighboring HFs.

Results

Postnatal Activation of Hh Signaling via Overexpression of GLI1 or Deletion of Ptc1 Induces BCC-Like Lesions in Transgenic Mice. In humans, sporadic BCCs occur predominantly during the later phase of life due to the deregulation of the Hedgehog (Hh) pathway by inactivation of its negative regulator, Ptc1, and subsequent activation of the Hh pathway effectors, the GLI transcription factors (11). Hh activation in mouse skin can give rise to a spectrum of tumors with hair follicle-like differentiation ranging from trichoblastoma over hamartoma, trichofolliculoma, trichoepithelioma to tumors having characteristics of nodular and superficial BCC; however, the tumor phenotype is largely dependent on the level of Hh signaling activity (12, 13). To mimic such a situation, we activated the Hh pathway in the basal layer of the mouse epidermis after completion of the HF morphogenesis in two different genetically engineered mouse models.

First, we generated K5tTA/TREGLI1 mice harboring the tetracycline-regulated transcriptional activator (TfA) under the control of the bovine cytokeratin 5 (K5) promoter and a tet-response element (TRE) controlling the expression of human GLI1 (13–16). To induce GLI1 expression, the tetracycline analog doxycycline was removed from the drinking water at postnatal day 16 (P16; Fig. 1A). The GLI1 protein could first be detected 2 wk after doxycycline removal (Fig. S1A). The K5tTA/ TREGLI1 mice developed a characteristic phenotype including standing hair, hyperkeratosis, and hyperpigmentation (Fig. 1B).

To confirm the identity of the early proliferations as precursors for BCC-like lesions, we obtained skin samples at different stages of tumor progression (Fig. 1A), which we defined by the size and character of the basaloid proliferations in the IFE. To distinguish the early proliferations from the normal IFE, we stained the sections with the following markers: K5, which labels the basal cells and basaloid proliferations; Sox9, a BCC and an ORS marker not found in the normal IFE (17, 18); P-cadherin, a secondary hair germ marker also expressed in BCC-like lesions (19); cytokeratin...
6 (K6), which marks the inner root sheath (IRS) and hyperproliferative areas during wound healing and in other dermal pathologies (20); and K67, which labels proliferative cells.

In the first stage of tumor formation, small buds consisting of 2–3 layers of K5-expressing basal cells appeared, which could be distinguished from the surrounding IFE by positive staining for Sox9 and P-cadherin and were negative for K6 (Fig. 1C and Fig. S1B), indicating a characteristic ORS-like differentiation pattern (21). When the lesions progressed to stage 2, they became clearly distinguishable from the IFE while retaining the characteristic marker expression pattern as seen in stage 1 (Fig. 1D and G). At this stage the upper cell layer of the IFE starts to express K6 due to overall epidermal hyperproliferation induced by GLI1; nevertheless, the tips of the lesions remain K6 negative. The absence of K6 expression in these areas may facilitate proliferation and migration of tumor cells, as it has been shown that K6-negative mouse keratinocytes are less rigid (22). Stage 3 lesions penetrated deeper into the dermis and began to exhibit a branched phenotype (Fig. 1E and H). More advanced lesions, which we classified as stage 4, appeared to originate both from the IFE and from the HF and consisted of keratinocyte strands invading the epidermis and occasionally Anastomosing with each other (Fig. 1F and Fig. S1C). In rare cases the lesions resembled human fibroepithelioma and nodular BCC (Fig. S1D).

Second, we generated K5Cre*PR1/Ptch1fl/fl mice, which express the Cre recombinase/progesterone receptor fusion protein under the control of the K5 promoter to allow homozygous inactivation of Ptc1 (23). In K5Cre*PR1/Ptch1fl/fl mice the recombination and subsequent activation of the Hh pathway was induced by RU486 administration (Fig. 1I and SI Materials and Methods). It has been shown previously that biallelic deletion of Ptc1 in mouse epidermis results in formation of lesions closely resembling human BCCs (24, 25). Deletion of Ptc1 in the basal compartment resulted in the formation of K5-expressing basa-

lowd proliferations in dorsal skin originating both from HFs and IFE following a largely similar timing of appearance as in K5TATREGLI1 mice, although with a different preference of cellular location during the initial stages. In stage 1, basaloid proliferations were only observed in association with HFs (Fig. 1J and M). In stage 2, early lesions in addition appeared in the IFE (Fig. 1K), and stage 3 and 4 lesions (described in ref. 25) were observed on the ears (Fig. 1L) but not in the dorsal skin at the time of sacrifice. The lesions present in the K5Cre*PR1/Ptch1fl/fl epidermis also expressed Sox9 and P-cadherin, and were negative for K6, similarly to the BCC-like lesions arising in the GLI1-overexpressing epidermis (Fig. 1M).

Wounding Promotes the Formation of BCC-Like Lesions. To study the effect of wounding on the formation of BCC-like lesions, full-thickness excisional wounds (3 mm in diameter) were introduced to the dorsal skin of K5TATREGLI1 and K5Cre*PR1/Ptch1fl/fl mice (SI Materials and Methods). In K5TATREGLI1 mice the induction of GLI1 expression was initiated at P16. The wounds were created at the time when the hGLI1 transgene expression was first detected at the protein level (Fig. S1A), and the mice were killed when small IFE-associated lesions corresponding to stage 1 and 2 were present in the unwounded dorsal skin (Fig. 2A). In contrast to unwounded skin, the wound area displayed branching stage 3 lesions, which were associated with the HF openings and IFE (Fig. 2B). In the absence of GLI1 transgene expression, no lesions developed in the wound and neighboring areas (Fig. 2C), and wounding before Hh pathway activation had no significant effect on tumor formation (Fig. S2). We found a significant threefold increase in the size of the lesions in the wound area compared with unwounded epidermis; however, no increase in the overall number of proliferations was seen (Fig. 2D). The proliferations that emerged in the wound area exhibited the typical marker gene expression pattern of BCC-like lesions (Fig. 2E) and were distinguished from normal wound epithelium by Sox9 expression (Fig. S3).

The K5Cre*PR1/Ptch1fl/fl and K5Cre*PR1/Ptch1fl/fl mice were injected four times with RU486, and full-thickness excisional wounds were created (SI Materials and Methods). The mice were killed at late stage 1, when HF- and the first small IFE-associated basaloid lesions emerged in the unwounded epidermis of mice with homozygous deletion of the Ptc1 gene (Fig. 2F). The wound area of the K5Cre*PR1/Ptch1fl/fl epidermis contained an increased number of basaloid proliferations corresponding to stage 2, which exhibited the characteristic pattern of K5, Sox9, P-cadherin, K6, and K67 expression (Fig. 2G and J). Analysis of the lesions revealed a 2.4-fold increase in number and a 2.8-fold
increase in the average size of the lesions in the wound area compared with the unwounded epidermis. This observation suggests that, in addition to promoting tumor growth, the wound environment also increases the initiation frequency of BCC-like lesions in this model (Fig. 2I). No proliferations developed in the wound areas of the K5Cre*PR1/Ptch1fl/fl mice (Fig. 2H).

Keratinocytes from the Bulge and the Nonpermanent Part of the HF Contribute to Tumor Formation in the IFE only in the Wound Area. Because cells originating from the HF actively participate in the wound healing process, we hypothesized that HF keratinocytes might contribute to the enhancement of tumor growth caused by the wound environment. To verify this we used the previously described lineage tracing approach based on Lgr5-EGFP-IRES-creERT2/R26R mice (5, 26). Upon activation of Cre by tamoxifen, which induces recombination in the R26R locus, the Lgr5+ cells and their progeny residing in the bulge and the nonpermanent part of the HF, are marked by permanent expression of LacZ.

Lgr5+ cells were labeled at P14, full-thickness wounds were introduced in the dorsal skin at the age of 5 wk and mice were killed 2 wk later (Fig. 3A). In unwounded skin the HF below the sebaceous gland opening was fully labeled (Fig. 3B). In the HFs situated close to the wound edges, labeled cells migrated out of the HFs and repopulated the infundibular area as expected (Fig. 3C). Labeled keratinocytes also integrated into the basal layer of the newly formed wound epidermis (Fig. 3D). We did not detect activity of the Lgr5 promoter during the wound-healing process in any cellular location where it is not normally expressed (Fig. S3 C and F), supporting the HF origin of labeled keratinocytes.

To evaluate the participation of Lgr5+ progeny in the formation of the BCC-like lesions, we generated quadruple transgenic mice by crossing Lgr5-EGFP-IRES-creERT2/R26R and K5tTA/TREGLI1 mice. To ensure that the Lgr5+ cells were labeled before the GLI1 transgene expression started, tamoxifen was injected at P14 and the doxycycline was removed at P16 (Fig. 3E). Activation of Hh signaling led to formation of BCC-like lesions as in the K5tTA/TREGLI1 mice (Fig. 3H). At 5 wk of age, when early lesions corresponding to stage 1 were detected in the IFE, Lgr5+ progeny was present below the sebaceous gland opening in the normal unwounded skin (Fig. 3F). By 6 wk of age, the Lgr5+ progeny was present above the sebaceous gland opening, extending into the infundibulum of the HF, resembling the tracing pattern of HFs close to a normal wound (Fig. 3G, compare with Fig. 3C). At 7 wk of age, when stage 2 and stage 3 basolateral proliferations had developed in the epidermis, the Lgr5+ progeny was present in the infundibulum and HF opening, and moreover found to be incorporated in the detaching differentiated keratinized layers. However, LacZ-positive traced cells were not detected in the IFE-associated BCC-like lesions, illustrating the inability of HF-derived keratinocytes, originating from the bulge and the nonpermanent part, to integrate into the IFE-associated proliferations (Fig. 3H). Note that the HF-associated lesions, which also develop in the K5tTA/TREGLI1 mice, originate from Lgr5+ cells (Fig. S4).

Next, we introduced wounds in the dorsal skin of 5-wk-old K5tTA/TREGLI1/Lgr5-EGFP-IRES-creERT2/R26R mice, treated with doxycycline and tamoxifen as described (Fig. 3I). As in normal skin, the Lgr5+ progeny was able to integrate into the basal layer of the newly formed wound epidermis (Fig. 3J). Remarkably, LacZ-labeled cells representing progeny of Lgr5+ cells also contributed to the IFE-associated Sox9- and GLI1-expressing basolateral proliferations developing in the wound epidermis (Fig. 3K and L and Fig. S4F), showing that the wound microenvironment allows recruitment of cells of follicular origin into the forming IFE-associated tumors.

**HF Keratinocytes with Activated Hh Signaling Initiate Tumor Formation in the Infundibulum and IFE upon Wounding.** The K5 promoter used in the previous experiments to direct Hh pathway activation is active throughout the basal compartment of the epidermis, including the newly formed wound epithelium. Therefore, it is possible that the cells originating from the bulge and the nonpermanent part of HF were attracted by the wound environment contributing to the forming tumors but have no autonomous tumor initiation ability. To test this possibility, we generated Lgr5-EGFP-IRES-creERT2/Ptch1fl/fl mice in which the Hh pathway was activated only in the Lgr5-expressing cells and their progeny. These mice were treated with tamoxifen so that high recombination efficiency could be achieved before the onset of the first anagen (similarly to the K5Cre*PR1/Ptch1fl/fl; SI Materials and Methods), and excisional full-thickness wounds were created at 5 wk (the same timing as for the K5tTA/TREGLI1/Lgr5-EGFP-

*Fig. 2.* Wounding enhances the formation of BCC-like lesions. (A) IFE-associated lesions in unwounded skin of K5tTA/TREGLI1 (K5GLI1) mice: small, stage 2 buds are indicated by arrowheads. (B) Lesions developing in the wound epidermis of the same mouse were more advanced, classified as stage 3 lesions, and were also found at HF openings. (C) No lesions developed in the wound epidermis of TREGLI1 mice. (D) Quantification of GLI1-induced lesions in unwounded and wounded skin showed that the number of lesions remained constant, whereas the size of the lesions was significantly increased in the wound area (**P = 0.02; n = 3; error bars indicate SD). (E) Immunostaining for marker gene expression of lesions in the wound epidermis shows the typical staining pattern for basoloidal lesions. (F) Small, stage 2 lesions in unwounded skin of K5Cre*PR1/Ptch1fl/fl mice associated with HF or IFE (arrowheads). (G) Enhanced formation of lesions at the site of wounding in K5Cre*PR1/Ptch1fl/fl epidermis (arrowheads). (H) In the wound epidermis of K5Cre*PR1/Ptch1fl/fl, no lesions formed. (I) Quantification of the lesions revealed a significant increase in the overall number and the average size of the proliferations in the wounded skin compared with unwounded skin of K5Cre*PR1/Ptch1fl/fl mice (**P = 0.01; ***P = 0.001; n = 3; error bars indicate SD). (J) Characteristic staining of lesions in the wound epidermis for basoloidal proliferation markers confirms their identity as evolving BCC-like lesions. (A–C and F–H) H&E staining. (E and J) Hematoxylin counterstain. (Scale bars: A–C and F–H, 100 μm; E and J, 50 μm.)
Fig. 3. Keratinocytes from the bulge and the nonpermanent part of the HF contribute to tumor formation in the IFE only in the wound area. (A) Experimental timeline of Lgr5+ lineage tracing in Lgr5-EGFP-ires-creERT²/R26R mice. Tamoxifen (TM) was administered at P14, wounds were created at 5 wk of age, and samples were taken at 7 wk of age. (B) LacZ staining of Lgr5-EGFP-ires-creERT²/R26R normal skin at 7 wk of age. Only the part of the HF extending up to the level of the sebaceous gland (SG) opening (dashed line) was repopulated by Lgr5+ progeny. (C and D) 2 wk after wounding, Lgr5+ progeny had advanced to the permanent and infundibular parts of the HF located close to the wound. Dashed line indicates level of 5G openings. (C). In addition, Lgr5+ progeny were integrated into the newly formed wound epidermis (D). (E) Experimental timeline of Lgr5+ tracing with subsequent tumor induction in K5Tat/TREGLI1/Lgr5-EGFP-ires-creERT²/R26R mice. (F) At 5 wk of age, when stage 1 proliferations appear in the IFE (Inset, arrowhead), the progeny of Lgr5+ cells is located below the 5G openings (dashed line). (G) As the tumors in the IFE progress to stage 2 (arrowhead), the labeled Lgr5+ progeny advances to the HF infundibulum. (H) Concomitantly with the appearance of the stage 3 lesions (arrowhead), the Lgr5+ progeny move further away from the HF and differentiate but do not integrate into the basaloid lesions in the IFE. Arrow, HF opening. (I) Experimental timeline of Lgr5+ tracing in combined tumor induction and wounding experiments. (J) Lgr5+ progeny can integrate in the wound epidermis in the presence of activated GLI1 expression. (K) The Lgr5+ progeny participate in the formation of K5-positive buds (L), which can be identified as early basaloid lesions based on their positive immunostaining for Sox9. (B–D and F Inset) LacZ and H&E staining. (F–H and L) LacZ and eosin staining. (K and L) Hematoxylin counterstain. SHG, secondary hair germ. (Scale bars: B, C, F–H, and J, 100 μm; D, K, and L, 50 μm.)

IRES-creERT²/R26R mice). In unwounded skin, conditional inactivation of PtcH1 in the Lgr5-expressing cells resulted in formation of HF-associated BCC-like lesions, whereas the skin of Lgr5-EGFP-ires-creERT²/PtcH1T2/2 mice showed a normal histological appearance (Fig. 4 A, B, and D). The morphology of the lesions ranged from prevalent simple nodular basaloid proliferations surrounded by a layer of palisading cells in the dorsal skin (Fig. 4 B and C), to less frequent, more advanced multinodular and branched BCC-like lesions in the ventral skin, confirming that Lgr5+ cells can act as cells of origin for BCC-like lesions (Fig. S5). Subsequent wounding experiments were performed on the dorsal skin, where no IFE-associated lesions were observed in unwounded skin (Fig. 4 B and C). Intriguingly, in the wound areas, multiple lesions developed, which were associated with infundibulum of the neighboring HFs and wound IFE (Fig. 4 E and F). The lesions were morphologically similar to the basaloid proliferations seen in the IFE of K5Tat/TREGLI1 and K5cre*PR1/PtcH1T2/2 mice, because they showed the characteristic expression pattern of K6, Sox9, and P-cadherin, and contained relatively high numbers of Ki67-positive cells (Fig. 4G). This shows that tumor-initiating cells can migrate from the bulge and the nonpermanent part of the HF into the permanent part of the HF and to the IFE during wound healing and contribute to tumor formation also at this location.

**Discussion**

Though chronic wounds are a well-recognized risk factor for cancer formation, little is known about the molecular and cellular mechanisms underlying this effect. Although several case reports depicting the association between BCC and skin injury have been published, to our knowledge there exist only single studies addressing this association (10, 27). The authors point out that both chronic and acute trauma should be considered as etiological factors for BCC. The possibility that an engagement of cells with high proliferative capacity or tumorigenic potential originating from neighboring areas of the tissue might be an important component of the mechanism by which a wound environment promotes tumor formation is an interesting open question addressed in this study. Using three different transgenic mouse models for human BCC, we show that a wound environment can enhance the development of BCC via two distinct mechanisms: promotion of tumor growth and enhancement of tumor initiation frequency. In the first model, the K5Tat/TREGLI1 mice, wounding does not increase the tumor initiation frequency, whereas in the K5cre*PR1/PtcH1T2/2 mice, where the number of early proliferations in unwounded areas is considerably lower, the wound increases not only the size of the tumors but also their number. Our hypothesis is that in the K5Tat/TREGLI1 epidermis, more...
cells, if not all K5+ basal cells in the IFE, which are competent to accumulate GLI1, do express GLI1. Wounding in this setting promotes tumor growth but not an increase in the number of lesions. In the case of the K5cre;Ptch1fl/fl mice, not all cells in the K5+ compartment are subjected to Ptch1 deletion due to the relative inefficiency of Cre activation, which results in a smaller number of cells having an active Hh pathway. However, in this setting, basaloid lesions emerged. Wounding recruits HF cells carrying a homozygous deletion of Ptch1 and thereby significantly increases the number of lesions in the wound area, and as shown in Lgr5-EGFP-ires-CreER<sup>T2</sup>/Ptch1<sup>fl/fl</sup> control mice, lesions associated with the infundibulum of the HFs as well as IFE-associate lesions. Immunostaining of marker genes identifies the infundibular- and IFE-associated lesions as basaloid lesions. Arrowheads show early infundibular and IFE-associated BCC-like lesions. (A–F) H&E staining. (C Right and G) Hematoxylin counterstain. (Scale bars: 100 μm.)

Cancer development and wound healing share several common features, which has led to the view that "tumors are wounds that do not heal" (31). Indeed, the lineage-tracing experiments in K5TAT/TREGLI1 mice show that oncogenic signaling in the epidermis also induces migration of cells from the bulge and the nonpermanent part of the HF analogous to wound healing; however, these cells were unable to integrate into the HF infundibula or IFE and instead underwent terminal differentiation. This indicates that, although Hh pathway activation induces cell migration, it does not provide a receptive microenvironment allowing homing of cells to the IFE, unlike the situation in a healing wound. Evidence that the homing ability of a cell is largely dependent on the host environment comes from our experiments using Lgr5-EGFP-ires-CreER<sup>T2</sup>/Ptch1<sup>fl/fl</sup> mice where keratinocytes with an activated Hh pathway were able to migrate out of the nonpermanent part of HFs and resettle at HF infundibula and in the wound IFE, thereby initiating new BCC-like lesions. Our finding that wounding causes enhanced formation of BCC-like lesions by redirecting the fate of hair follicle progeny in the context of activated Hh signaling provides an explanation for the
association between tissue injury from excessive UV radiation exposure (32) or chronic ulceration (33) with BCC development. In these situations an increased mutational load is the result of inflammation generating reactive oxygen species or infliction of direct physical DNA damage (9, 34, 35). In line with this scenario we found that TPA treatment can stimulate migration of Lgr5+ progeny to the IFE. TPA treatment induces a strong inflammatory and hyperproliferative response in mouse skin and is typically used for epidermal tumor promotion, and TPA has been shown to be as effective as deep skin wounding in promoting papilloma formation in mice (36). Although treatment with TPA can provide the important signals responsible for the migration of the potential tumor initiating cells, the key signals for HF cell migration into the wound are as of yet unclear. Our findings suggest that molecular events during the initial phase of acute wound healing are crucial, because wounding preceding Hh pathway activation fails to promote tumor development. Moreover, only full-thickness wounds induce the migration of cells from neighboring HF’s to the place of injury. The molecular mechanism underlying both the attraction and homing of potential tumor-initiating cells or cells contributing to tumor formation remains an important topic for future studies and is likely to depend on the specific cytokine and growth factor milieu and involve alteration of epigenetic patterns (37).

Materials and Methods

Mice. Lgr5-EGFP-IRES-creER<sup>17</sup>/R26ER, K5tTA/TREGLI1, K5tTA/TREGLI1/lgr5-EGFP-IRES-creER<sup>17</sup>/R26ER, K5Cre<sup>Pri1</sup>/Ptch<sup>fl/fl</sup>, and Lgr5-EFGP-IRES-creER<sup>17</sup>/R26ER Ptch<sup>fl/fl</sup> mice were generated by interbreeding mice carrying the following alleles: Lgr5-EFGP-IRES-creER<sup>17</sup> (26), R26ER (mice were obtained from Jackson Laboratory), K5tTA (14), TREGLI1 (18), K5Cre<sup>Pri1</sup> (23), and Ptch<sup>fl/fl</sup> (Ptch<sup>fl/fl</sup> R26R CR) (a kind gift from S. Teglund, Karolinska institutes).

Hedgehog Pathway Activation. The GLI1 transgene expression in mice carrying a K5tTA and a TREGLI1 allele was induced by doxycycline removal from the drinking water (2 mg mL<sup>-1</sup> doxycycline, 5% sucrose) at P16. The deletion of the Ptch1 gene of mice carrying K5Cre<sup>Pri1</sup> or Lgr5-EFGP-IRES-creER<sup>17</sup> and Ptch<sup>fl/fl</sup> alleles was induced by i.p. injection of either 4 mg RU486 or 4 mg of tamoxifen (SI Materials and Methods).

Lineage Tracing. Mice aged 14 d (P14) were injected i.p. with 2 mg of tamoxifen (20 mg/mL in sunflower oil).

Wounding and TPA Treatment. Full-thickness wounds, 3 mm in diameter, were created on the back skin of mice. Superficial incisions, hair plucking, and TPA treatment were performed on telogen mouse skin as described in SI Materials and Methods. All animal experiments were performed according to the regulations of Sweden.

La<sub>2</sub>C<sub>2</sub> Analysis. To determine the pattern of recombination at the Rosa26-LacZ(R26ER) reporter locus, fresh dorsal (if not stated otherwise) skin tissue and by increased expression of K6. Partly overlapping images spanning Nat Cell Biol or GLI1 Mice aged 14 d (P14) were injected i.p. with 2 mg of ta-

### Immunohistochemistry

Freshly obtained skin samples were fixed in 4% formaldehyde and embedded in paraffin. Staining with antibodies recognizing K5, K6, GLI1, Ki67, Sox9, P-cadherin, CD34, or EGFP was performed as described in SI Materials and Methods.

### Quantification of Tumor Formation

Tumors were identified by Sox9 expression, and wound areas were defined by the absence of underlying pananicular cam-

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