Figures and figure supplements

Coordinated hedgehog signaling induces new hair follicles in adult skin

Xiaoyan Sun *et al*

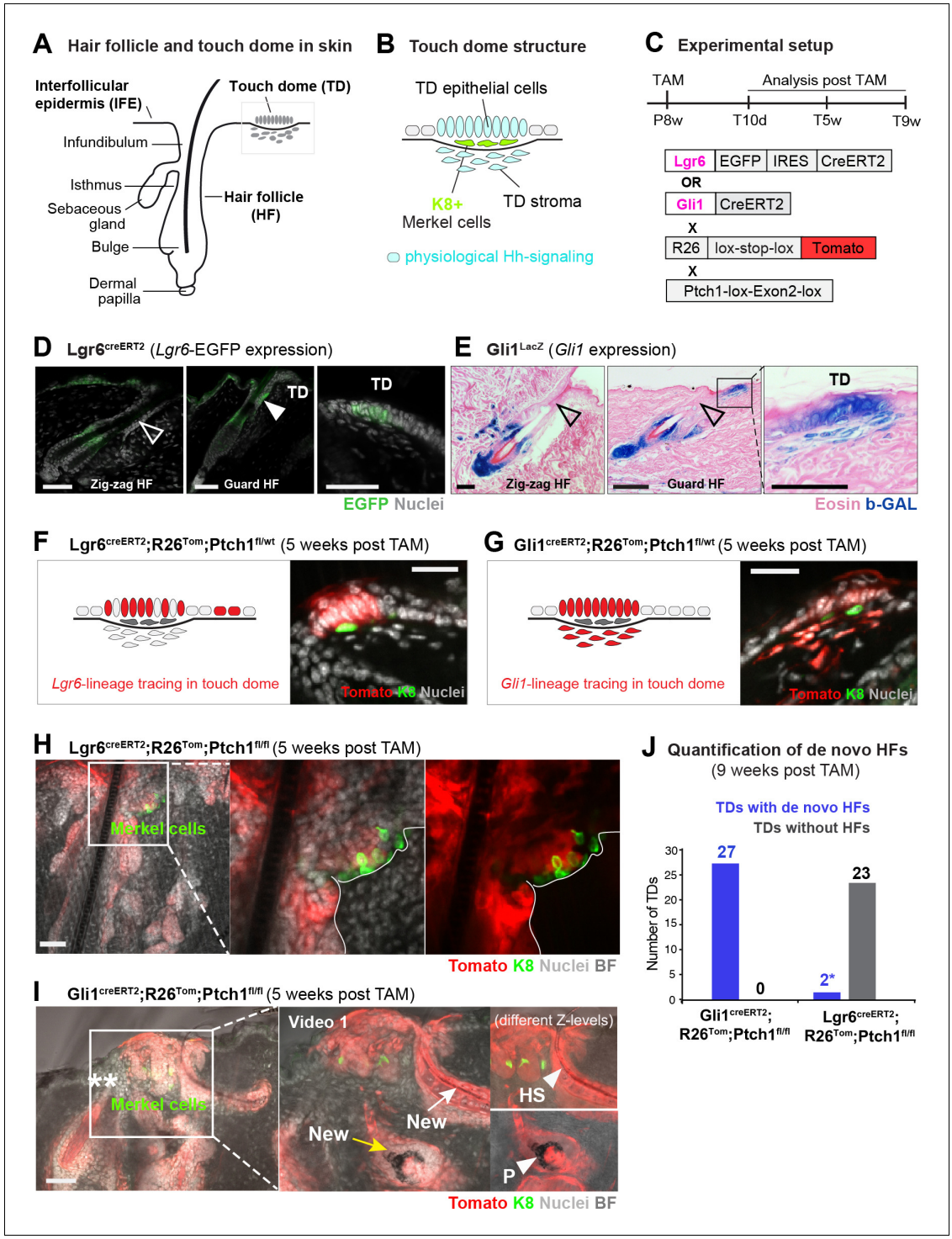

Figure 1. Formation of hair follicle (HF)-like structures in touch domes (TDs) of the Gli1 mouse model. (**A–B**) Illustrative cartoon of HF and TD structures in wild type skin. Physiological Hh signaling is present in both TD epithelium and TD stroma. The presence of K8+ Merkel cells is characteristic for TDs. (**C**) Schematic representation of the experimental timeline and the Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ and Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mouse models. (**D–E**) *Lgr6*^{EGFP} and *Gli1*^{LacZ} expression in dorsal telogen skin. Filled arrowhead: indicates *Lgr6*-expression in the HF infundibulum. Empty arrowheads: indicate lack of *Lgr6*- or *Gli1*-expression in HF infundibula (n = 3 mice per genotype). (**F–I**) Mice were treated with tamoxifen (TAM) at 8 weeks of age and dorsal skin was analyzed 5 weeks post TAM. For each genotype ≥3 mice and numerous TDs were analyzed (***Supplementary file 1***). (**F**) Illustrative cartoon and experimental Tomato-tracing of *Lgr6*-expressing cells. TDs of Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ control skin were phenotypically normal. (**G**) Illustrative cartoon and experimental Tomato-tracing of *Gli1*-expressing cells. TDs of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ control skin were phenotypically normal. (**H**)

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Tomato-traced TD of Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ skin displaying basal cell carcinoma (BCC)-like tumor growth. (**I**) Tomato-traced TD of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ skin with BCC-like tumor growth and several de novo HFs. Asterisks: mark a non-traced infundibulum in the pre-existing Guard HF adjacent to the TD. Inset: different z-levels better depicting specific de novo HF structures. A video containing all z-levels is provided (***Figure 1—video 1***). Arrows: de novo HFs with continuous tracing into the IFE. Yellow arrow: anagen bulb of a de novo HF. Arrowheads: hair shaft (HS) or pigment (P). (**J**) Quantification of de novo HFs in the TDs of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ and Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice (9 weeks post TAM; n = 3 mice for each genotype). Asterisk: the Lgr6 mouse model cannot inform on de novo HFs trough lineage tracing; based on morphology we observed in two TDs a single potentially new hair shaft, respectively. TD: touch dome. HS: hair shaft. P: pigment. TAM: tamoxifen. BF: bright field. Scale bars: 50 μm (**D–I**).

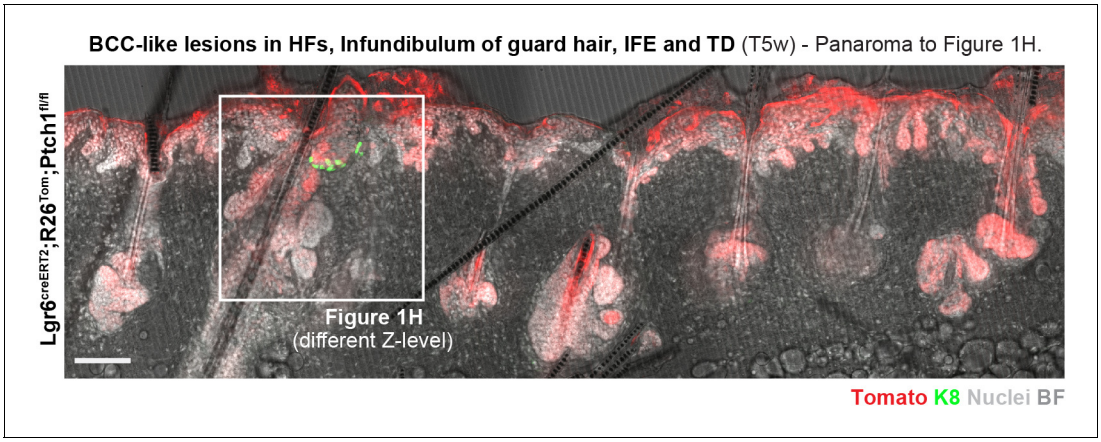

Figure 1—figure supplement 1. Panorama to ***Figure 1H***. Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice were treated with TAM at 8 weeks of age, and dorsal skin was analyzed 5 weeks post TAM (n = 3 mice). BCC-like lesions were observed in the HF isthmus, the infundibulum of guard HFs, the IFE, and in TDs. This panorama shows a different z-level than ***Figure 1H*** to better depict HF- and IFE-associated BCC-like lesions. Scale bar: 100 μm.

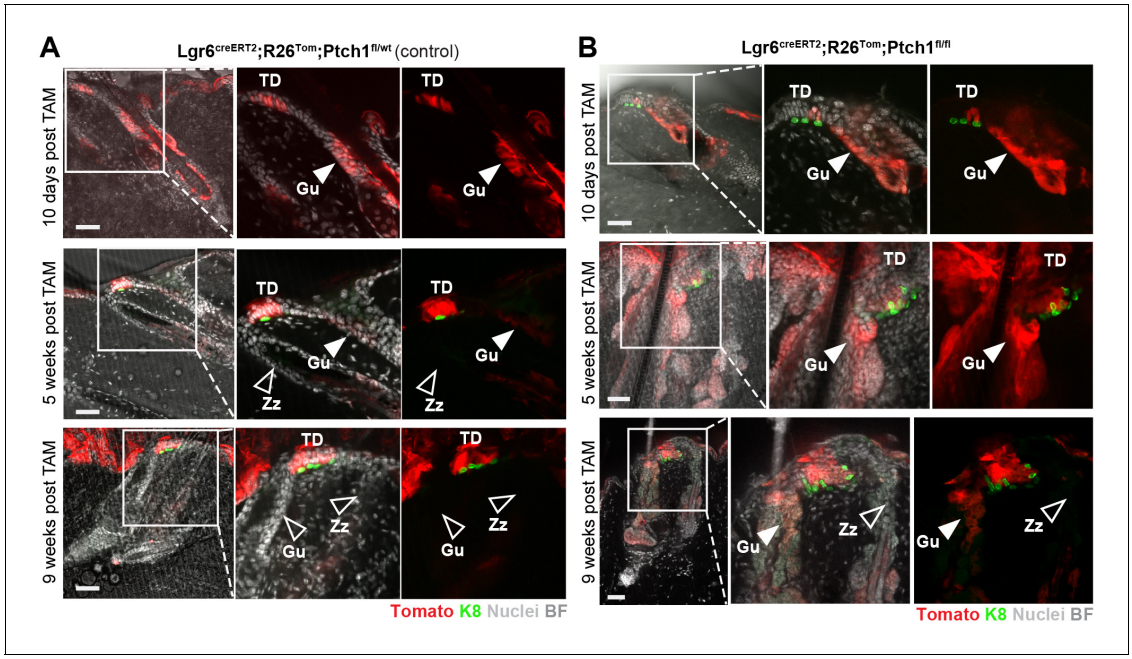

Figure 1—figure supplement 2. Phenotype and lineage-tracing pattern in Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ and Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ skin. (**A–B**) Mice were treated with TAM at 8 weeks of age and dorsal skin was analyzed post TAM as indicated in the figure panels (n = 3 mice for each genotype and time point). (**A**) Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ control mice. At all time points, the skin phenotype was normal. IFE and HFs were Tomato-traced as reported (***Füllgrabe et al., 2015***). The infundibula of Guard HFs were often partially traced (filled arrowheads) and the infundibula of TD-adjacent pre-existing Zig-zag HFs were not traced (empty arrowheads). (**B**) Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice. At 5 and 9 weeks post TAM, BCC-like lesions are present in HFs, IFE and TDs; traced and non-traced lesions are present as the Ptch1$^{fl/fl}$ alleles are 'floxed' with higher efficiency than LSL-Tomato alleles (data not shown). The infundibula of Guard HFs were partially traced (filled arrowheads) and the infundibula of TD-adjacent pre-existing Zig-zag HFs retained non-traced (empty arrowheads) even when BCC-like lesions formed. Please note that some images are used in the main figures, however with different emphasis and display ([a] extended area view of ***Figure 1F***; [b] same image as in ***Figure 1H***). TD: touch dome. Gu: Guard HF. Zz: Zig-zag HF. BF: bright field. Scale bars: 50 μm.

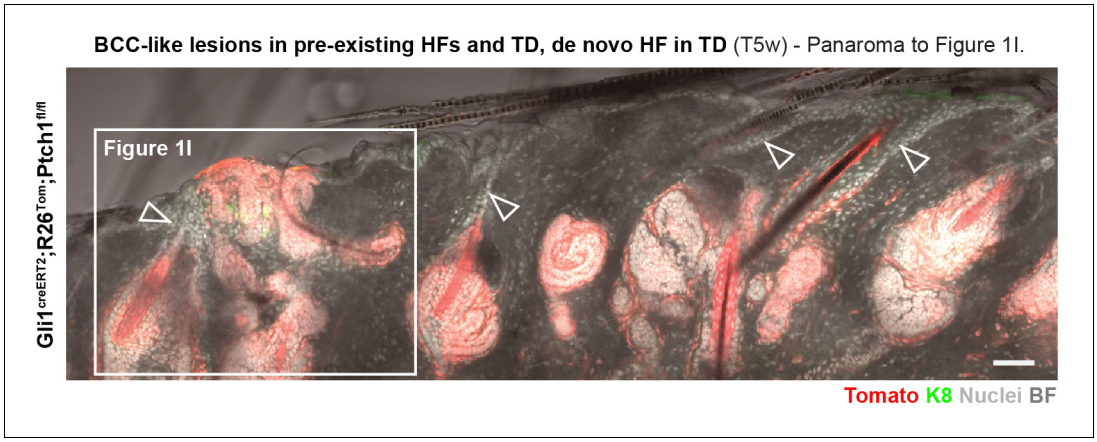

Figure 1—figure supplement 3. Panorama to ***Figure 1I***. Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice were treated with TAM at 8 weeks of age, and dorsal skin was analyzed 5 weeks post TAM (n = 3 mice). BCC-like lesions were observed in pre-existing HFs and TDs. Additionally, new HF-like structures were found in the TDs of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ skin. Note the untraced infundibulum of pre-existing HFs (arrowheads). Scale bar: 50 μm.

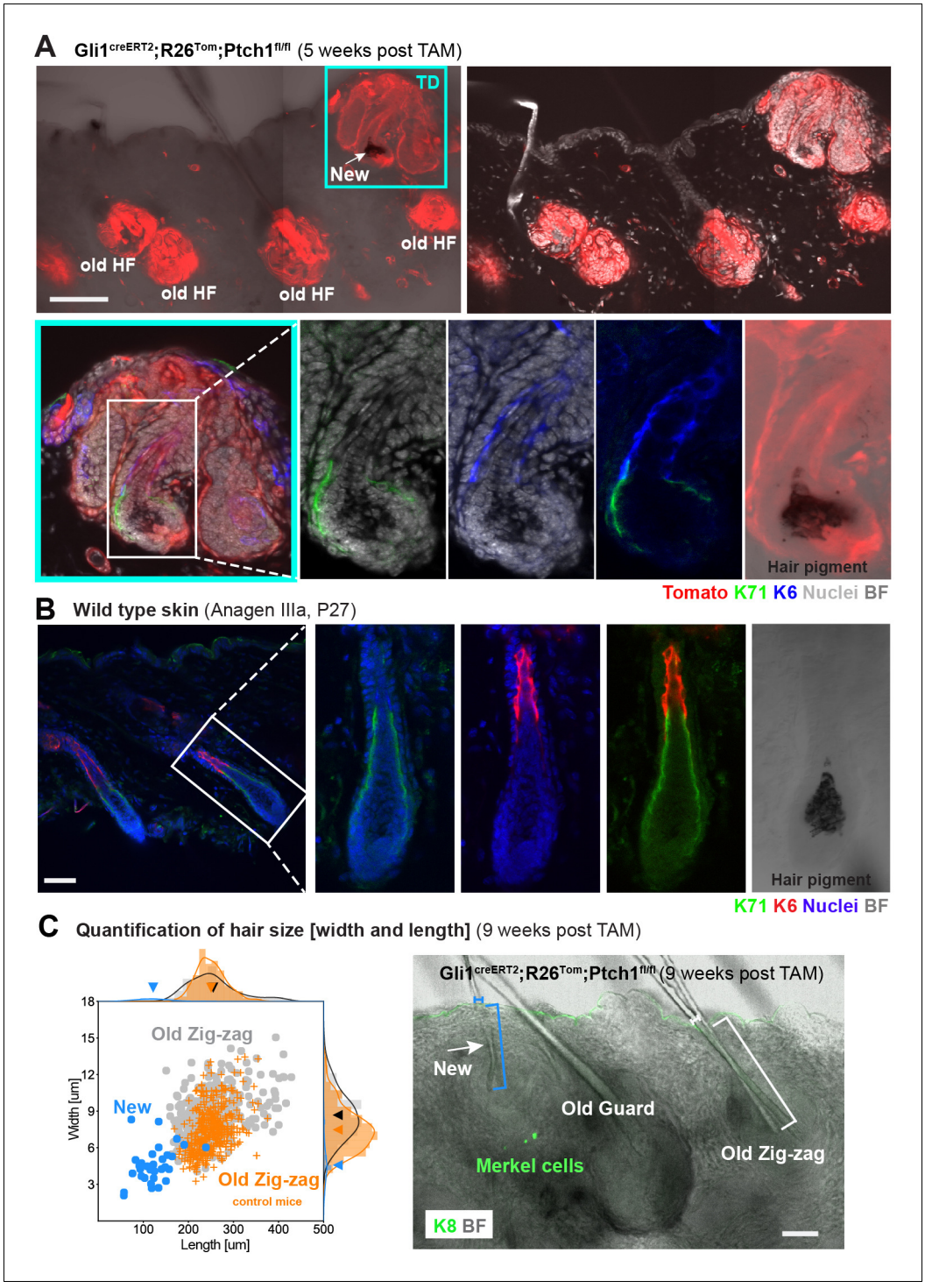

Figure 2. Characterization of de novo hair follicles (HFs) in Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ touch domes (TDs). (**A**) Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice were treated with tamoxifen (TAM) at 8 weeks and dorsal skin was analyzed 5 weeks post TAM treatment (n = 3 mice). The TD area shows a de novo anagen HF (turquois frame). Additionally, traced pre-existing (old) telogen HFs with basal cell carcinoma (BCC)-like growth are present. Inset (white frame): anagen hair bulb of de novo HF showing K71-positive Henle's layer, K6-positive companion layer, hair pigment and continuous Tomato-tracing from the hair bulb into the TD. (**B**) Immunofluorescent co-staining of K71 (Henle's layer) and K6 (companion layer) in a wild type HF of a similar hair cycle stage (Anagen IIIa, P27) (n = 2 mice). (**C**) Quantification of hair size in Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ and Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ mice that were treated with TAM at 8 weeks and dorsal skin was analyzed 9 weeks post TAM treatment. Right panel: De novo telogen

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HFs with a thin hair shaft formed in the TDs of $Gli1^{creERT2}$;$R26^{Tom}$;$Ptch1^{fl/fl}$ skin (white arrow). For the quantification, we analyzed hair shafts of de novo HFs from $Gli1^{creERT2}$;$R26^{Tom}$;$Ptch1^{fl/fl}$ mice (blue bracket), old/pre-existing Zig-zag HFs from the same mice (white bracket), and Zig-zag HFs from wild-type-phenotype control mice ($Gli1^{creERT2}$;$R26^{Tom}$;$Ptch1^{fl/wt}$) (n = 3 mice for each genotype; 34 de novo, 314 old/pre-existing, and 437 control HFs; ***Figure 2—source data 1***). Hair shaft length was measured in telogen stage hair shafts from the hair club to the HF opening (as indicated by the blue and white brackets). Left panel: Each dot represents a hair shaft and the dots are colored according to the HF type (blue for de novo HFs, gray for pre-existing Zig-zag HFs, orange for control Zig-zag HFs). De novo hair shafts were significantly smaller (p-value<1.10^{-6}) and thinner (p-value<1.10^{-6}) compared to old/pre-existing and control Zig-zag hairs (Mann-Whitney U test). Arrowheads on the x- and y-axis indicate the mean values of hair shaft length and width of de novo, old/pre-existing or control Zig-zag HFs, respectively. TD: touch dome. HF: hair follcile. TAM: tamoxifen. BF: bright field. Scale bars: 100 μm (**A**), 50 μm (**B, C**).

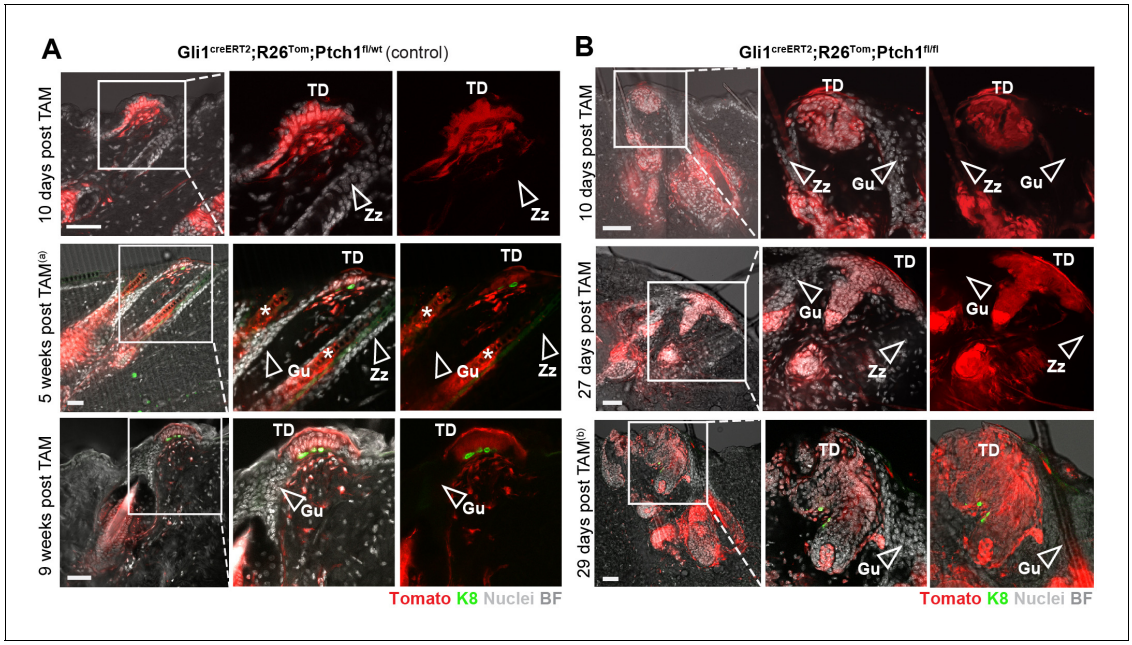

Figure 2—figure supplement 1. Lineage-tracing pattern of TD and surrounding area in Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ control and Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ skin during de novo HF formation. (**A–B**) Mice were treated with TAM at 8 weeks of age and dorsal skin was analyzed post TAM as indicated in the figure panels (n = 3 mice for each genotype and time point; except n = 1 for 29 days post TAM). The infundibula of pre-existing TD-adjacent HFs (Guard HFs as well as Zig-zag HFs) in both Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ control mice (**A**) and Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice (**B**) were never traced (empty arrowheads), even when BCC-like lesions and/or de novo HFs formed. In contrast, de novo HFs always showed continuous HF-to-TD tracing. Please note that some images are used in the main figures with different emphasis and display ([a] extended area view of ***Figure 1G***; [b] same image as in ***Figure 3D***). Asterisks: mark hair shafts that are naturally traced during anagen in these mice. TD: touch dome. Gu: Guard HF. Zz: Zig-zag HF. BF: bright field. Scale bars: 50 μm.

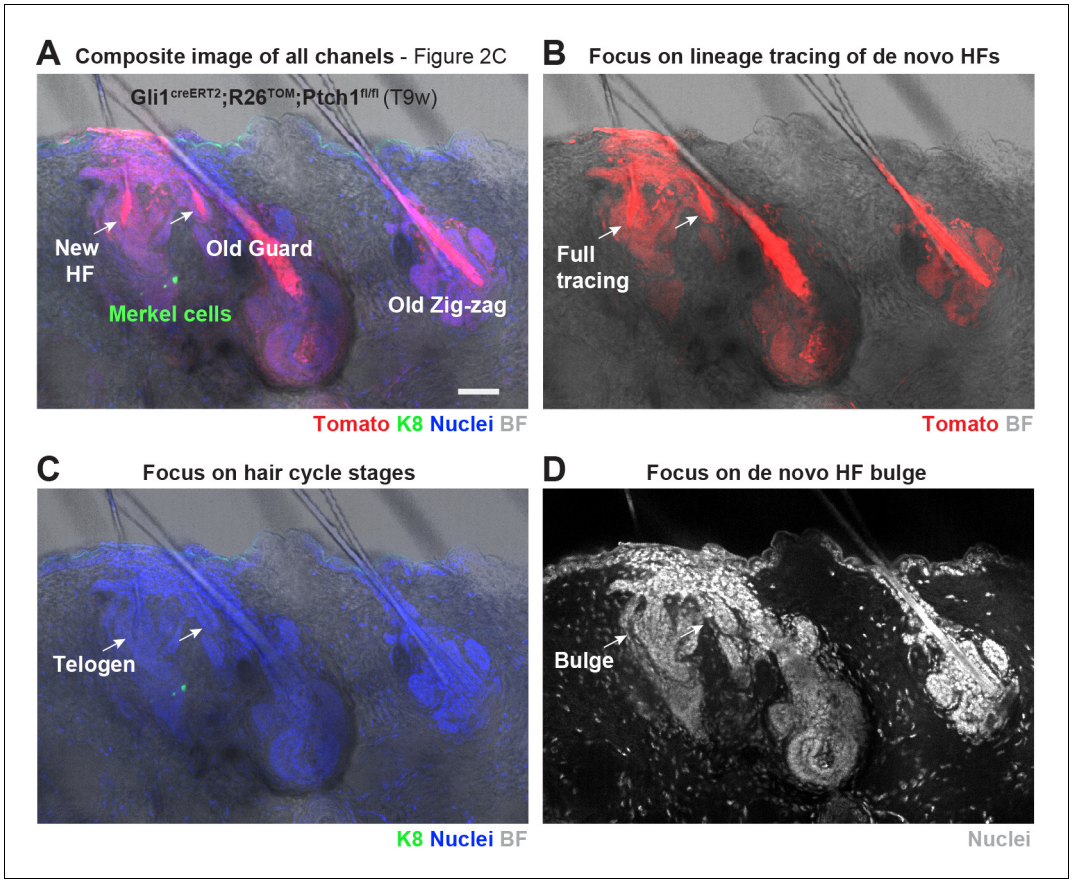

Figure 2—figure supplement 2. De novo HFs in TDs of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice 9 weeks post TAM were in telogen and displayed a HF bulge. (**A–D**) Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice were treated with TAM at 8 weeks and dorsal skin was analyzed 9 weeks post TAM treatment (n = 3 mice). Note that the BF and K8 channel of this picture are shown in ***Figure 2C***. The de novo HFs originated from the TD next to the guard hair (**A**). They are fully traced while there is a clearly non-traced gap in the infundibulum of guard HF and pre-existing Zig-zag HFs (**B**). Both de novo HFs were in telogen stage (**C**) with a noticeable telogen bulge (**D**). TAM: tamoxifen. BF: bright field. Scale bars: 50 μm.

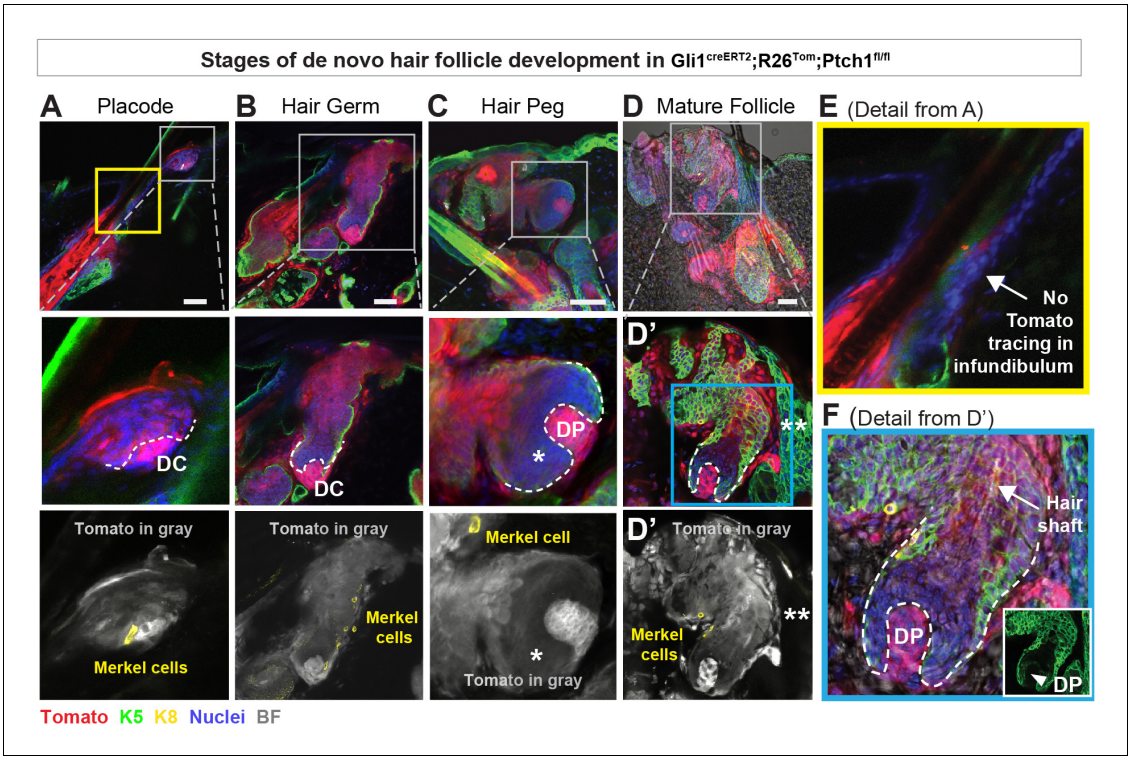

Figure 3. Developmental stages of de novo hair follicles (HFs) in Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ touch domes (TDs). (**A–F**) Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice were treated with tamoxifen (TAM) at 8 weeks and dorsal skin was analyzed 10–36 days post TAM to characterize de novo HFs originating from TDs in the following developmental stages (n = 6 mice): early placode (**A**), hair germ (**B**), hair peg (**C**), and mature follicle (**D**). These stages recapitulate embryonic HF development (***Rendl et al., 2005***). Dermal condensates (**A, B**) or dermal papillae (**C, D**) are clearly visible, and the de novo HFs – both early and mature – are continuously traced into the TD. Note: HF-matrix cells are Tomato-traced with reduced intensity (* in C), while the infundibulum of pre-existing HFs was not Tomato- traced (** in D' and arrow in E). Furthermore, mature HFs contain a hair shaft (arrow in F). Inset in F shows K5-positive staining of the HF epithelial cells and a K5-negative dermal papilla. DC: dermal condensate. DP: dermal papilla. BF: bright field. Scale bars: 50 µm (**A-D**).

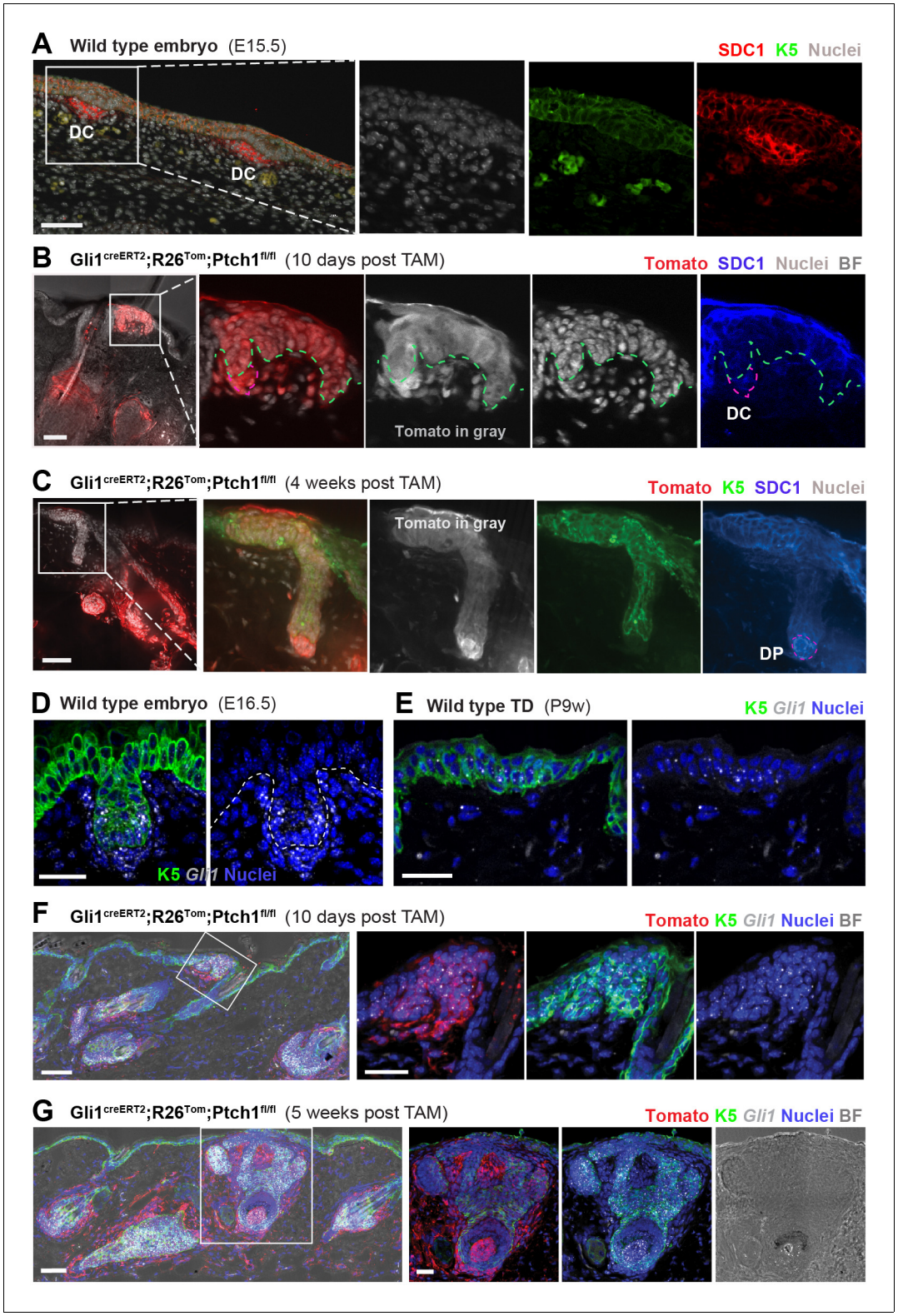

Figure 4. SDC1 protein and *Gli1* mRNA expression in developing hair follicles (HFs). (**A**) In wild type embryonic skin (E15.5), Syndecan-1 (SDC1) staining highlights dermal condensates (DCs) (n = 2 mice). (**B–C**) Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice were treated with tamoxifen (TAM) at 8 weeks and dorsal skin was analyzed for SDC1 expression in the newly formed HF buds (n = 3 mice). (**B**) Early placode stage (image from a TD 10 days post TAM) displaying faint SDC1 staining in dermal condensate cells. Comparable to epithelium of wild type embryonic HF

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buds, early de novo placodes also express some SDC1. (**C**) Positive SDC1 staining of dermal papilla (image from a TD 4 weeks post TAM). (**D–E**) *Gli1* RNA-FISH. Both the epithelial placode and dermal condensate have active Hh/Gli signaling in wild type embryonic skin (E16.5) (**D**) and at lower levels in wild type TDs of adult skin (**E**). (**F–G**) $Gli1^{creERT2}$;$R26^{Tom}$;$Ptch1^{fl/fl}$ mice were treated with TAM at 8 weeks and dorsal skin was analyzed for *Gli1* mRNA expression in the placode stage (10 days post TAM) as well as in an intermediate developmental stage (5 weeks post TAM). Active canonical Hh/Gli signaling was present in all analyzed de novo HF stages in epithelial and dermal papilla cells. Note that Tomato-tracing was visualized using an RFP-antibody. Green and white dashed lines: epithelial-stromal border. Purple dashed line: outlines the dermal condensate. DC: dermal condensate. DP: dermal papilla. TD: touch dome. BF: bright field. For RNA-FISH stainings, n = 2 mice (**D, E and G**) and n = 1 mouse (**F**). Scale bars: 25 μm (**D–G**), 50 μm (**A-C, F-G** panoramas).

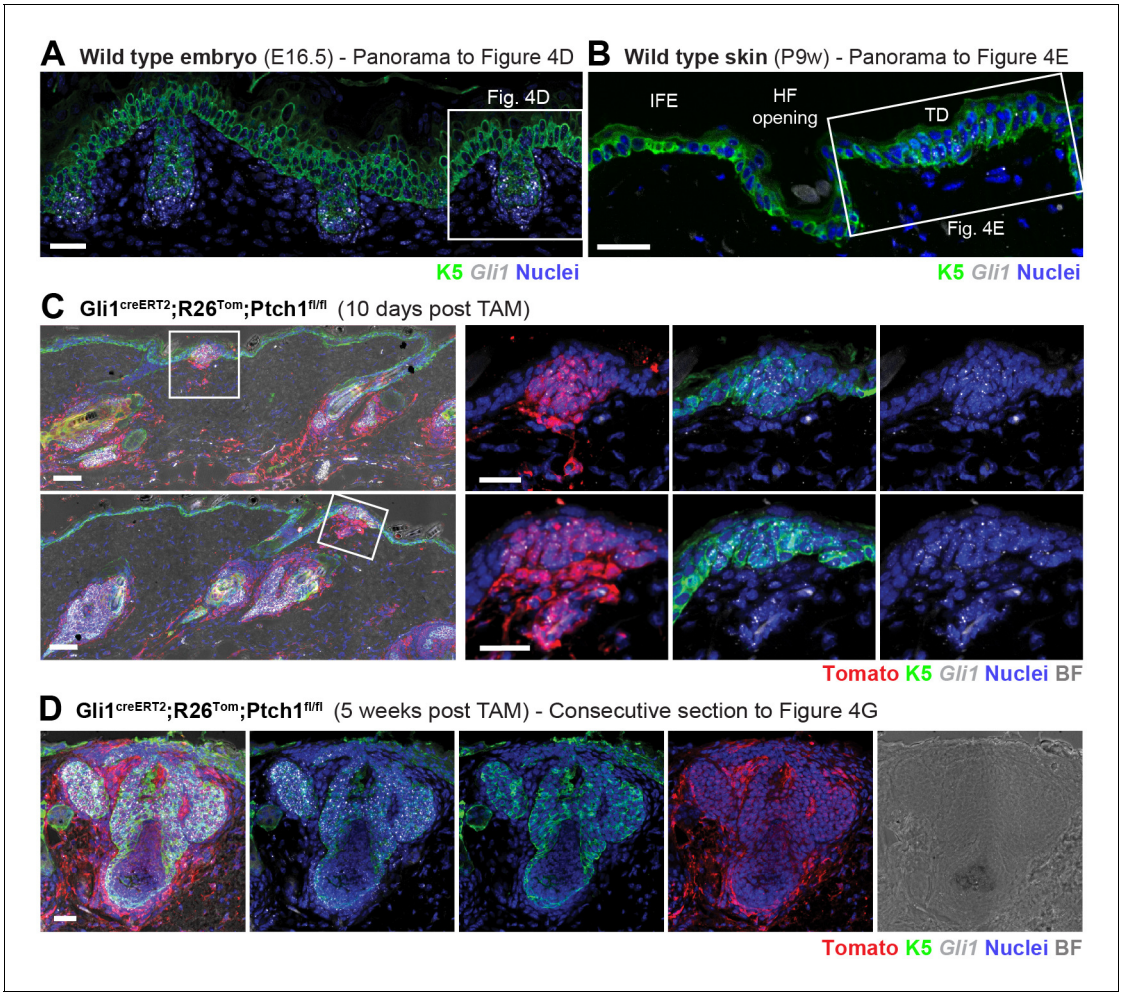

Figure 4—figure supplement 1. *Gli1* mRNA staining reveals cells with active canonical Hh/Gli signaling during de novo HF development in the TDs of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice. (**A**) Panorama to ***Figure 4D***. In wild type embryonic skin (E16.5), the epithelial placodes as well as dermal condensates show clear *Gli1* mRNA expression, while the IFE is mostly devoid of *Gli1* mRNA. (**B**) Panorama to ***Figure 4E***. In wild type skin from postnatal 9-week-old mice, *Gli1* mRNA is present in the epithelial and stromal components of the TD (white frame) but absent in the rest of the IFE. (**C**) Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice were treated with TAM at 8 weeks and dorsal skin was analyzed for *Gli1* mRNA expression 10 days post TAM with a focus on TDs. Left panels: Panoramas showing TDs in their tissue context. Right panels: Early placodes emerging from TDs clearly display active, canonical Hh signaling in the epithelium and stroma. (**D**) Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice were treated with TAM at 8 weeks and dorsal skin was analyzed for *Gli1* mRNA expression 5 weeks post TAM. Shown is a consecutive section of the HF in ***Figure 4G***. Note that Tomato-tracing was visualized using an RFP-antibody (**C-D**). For stainings n = 2 mice (**A, B, D**); n = 1 mouse (**C**). TAM: tamoxifen. IFE: interfollicular epidermis. BF: bright field. Scale bars: 50 µm (**C** panaroma), 25 µm (**A-B, C** insets and **D**).

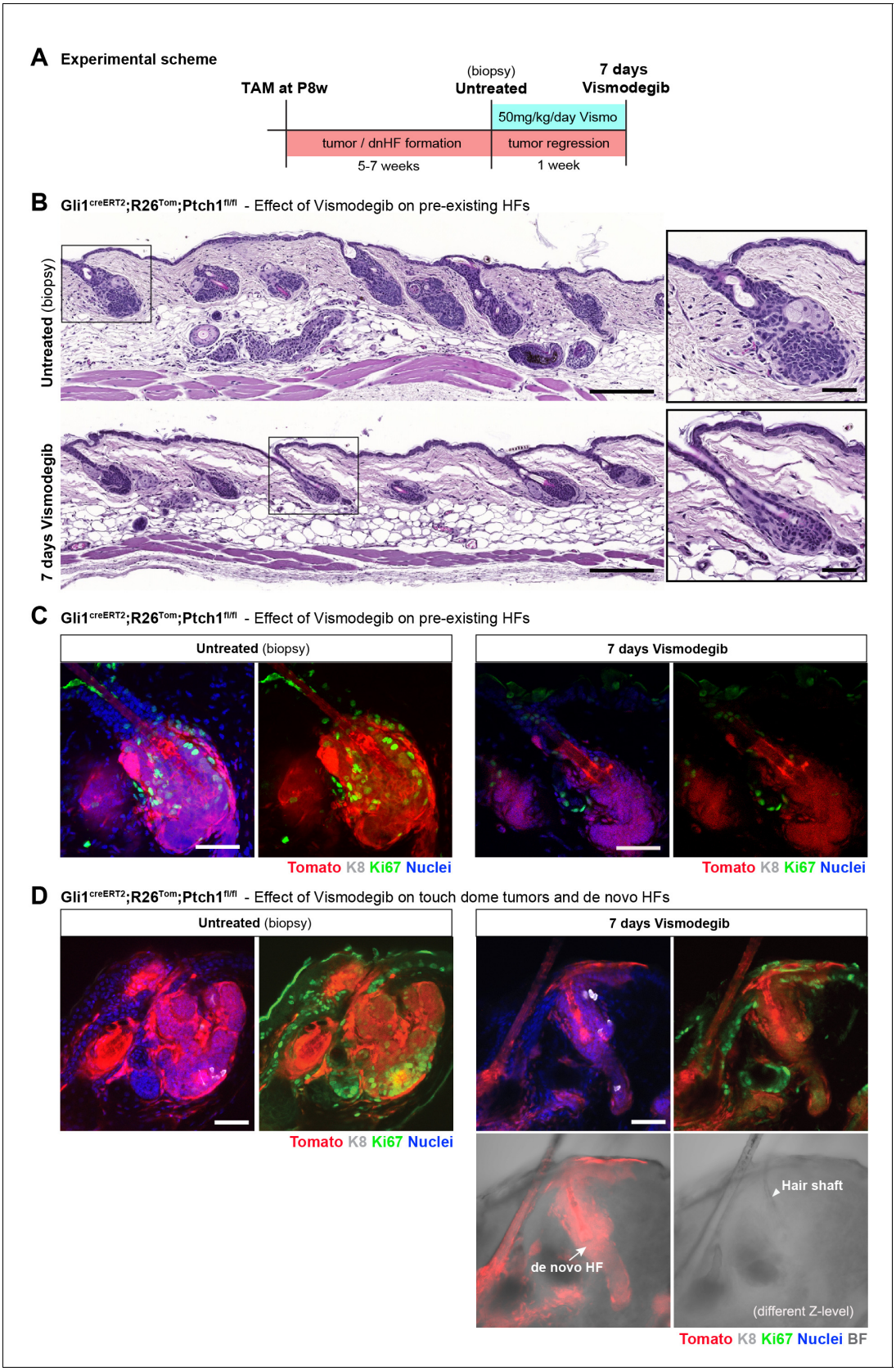

Figure 5. Established de novo hair follicles (HFs) in touch domes (TDs) persist upon short-term vismodegib treatment. (**A**) Experimental scheme of vismodegib treatment. Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice were treated with tamoxifen (TAM) at 8 weeks of age. Five to seven weeks post TAM treatment,

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when de novo HFs were clearly established in the TDs, vismodegib was given daily for a week and dorsal skin was analyzed (n = 3 mice for $Gli1^{creERT2}$;$R26^{Tom}$;$Ptch1^{fl/fl}$, n = 1 mouse for $Gli1^{creERT2}$;$Ptch1^{fl/fl}$). (**B**) Hematoxylin and eosin stainings showing that basal cell carcinoma (BCC)-like lesions were considerably reduced in response to a week of daily vismodegib treatment at a dose of 50 mg/kg body weight. (**C**) Tumor-cell proliferation in bulge area of pre-existing HFs assessed by Ki67 immunostaining. Bulge areas of untreated control biopsies showed high proliferation, which was almost entirely stalled in 7-day vismodegib samples; in the HF, only the sebaceous glands retained Ki67 expression. (**D**) BCC-like lesions were present in the TDs of dorsal biopsies taken prior to vismodegib treatment (left panel). In response to vismodegib, the tumor-growth area was considerably reduced while de novo HFs presisted (arrow). The de novo HFs are fully Tomato-traced and have a clearly visible hair shaft (arrowhead). HF: hair follicle. K8: marking TD area. Ki67: marking proliferating cells. TAM: tamoxifen. BF: bright field. Scale bars: 200 μm (**B** panaroma), 50 μm (**B** inset, **C, D**).

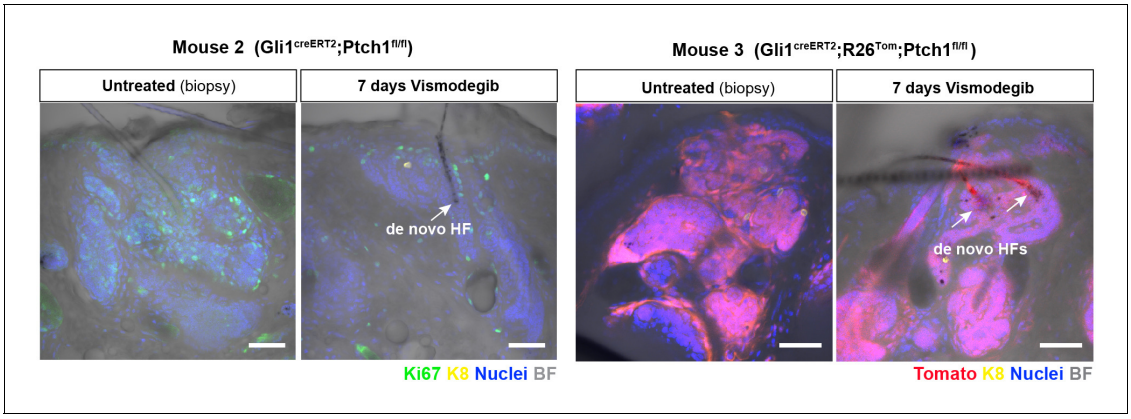

Figure 5—figure supplement 1. Effect of vismodegib on BCC-like lesions and de novo HFs in TDs. Shown are two additional examples to ***Figure 5D***. Arrowheads mark de novo HFs. Scale bars: 50 μm.

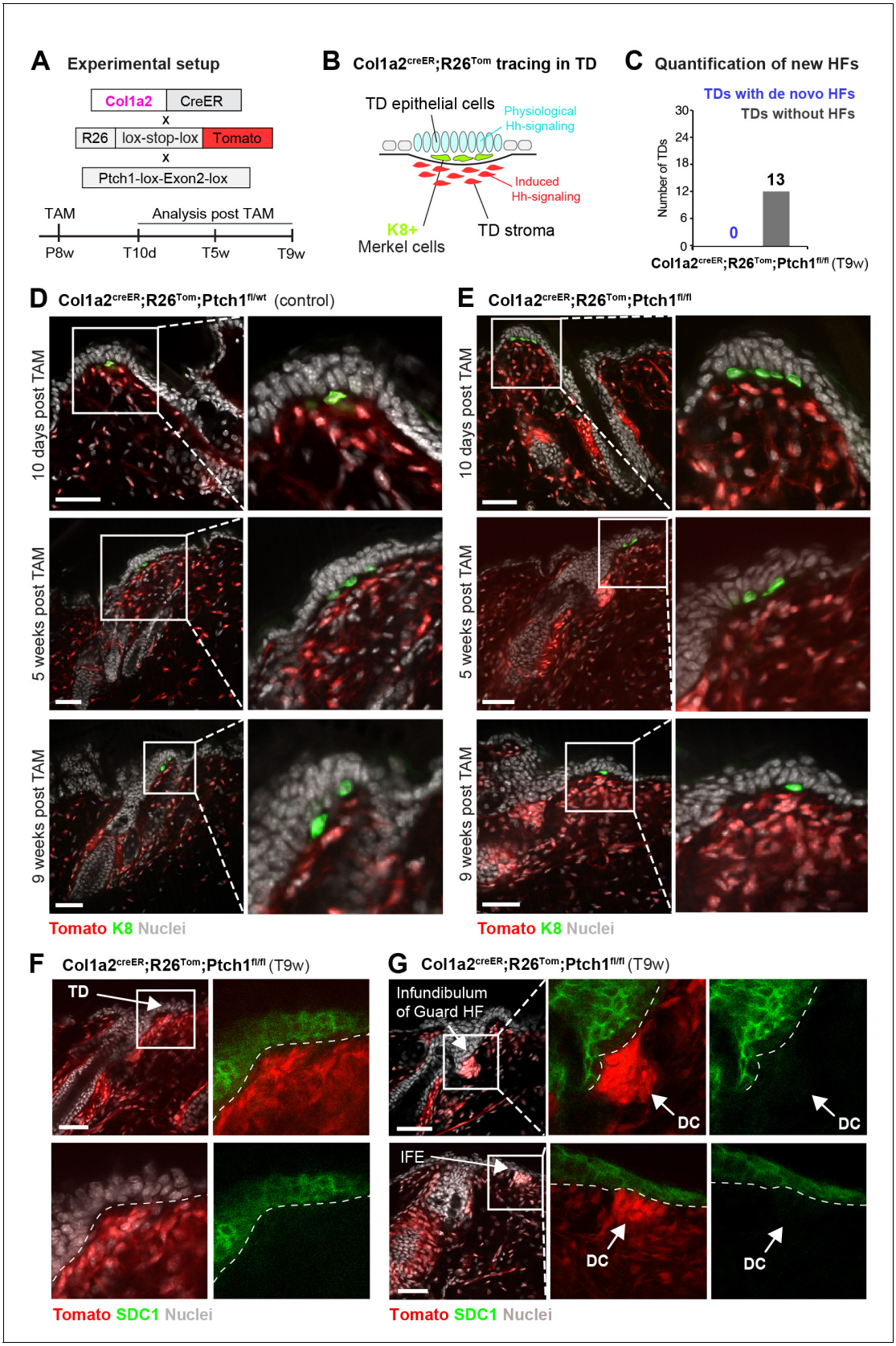

Figure 6. Stromal Hh pathway activation alone is not sufficient to induce hair follicle (HF) neogenesis in the touch domes (TDs) of Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$skin. (**A**) Schematic representation of the Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ mouse model and the experimental timeline. (**B**) Illustrative cartoon of

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Tomato-tracing of *Col1a2*-expressing cells and Hh-signaling levels in TD epithelium and TD stroma in this mouse model. (**C**) Quantification of de novo HFs in the TDs of Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ mice treated with tamoxifen (TAM) at 8 weeks. Dorsal skin was analyzed 9 weeks post TAM treatment (n = 3 mice). No de novo HFs were observed. (**D–G**) Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ and Col1a2^{creER};R26^{Tom};Ptch1$^{fl/wt}$ control mice were treated with TAM at 8 weeks and dorsal skin was analyzed 10 days, 5 weeks, and 9 weeks after TAM treatment (n = 3 mice per genotype and time point). (**D**) TDs of mice with heterozygous *Ptch1* deletion were phenotypically normal. (**E**) TDs of mice with homozygous *Ptch1* inactivation did not develop de novo HFs. Frequently, a higher cell density in stroma was observed. (**F–G**) Syndecan-1 (SDC1) staining was negative in the condensed stroma of TDs (**F**) as well as in dermal cell condensations (arrows) underneath the IFE and HF infundibula (**G**) in Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ mice. TD: touch dome. HF: hair follcile. IFE: interfollicular epidermis. DC: dermal cell condensation. Scale bars: 50 μm (**D–G**).

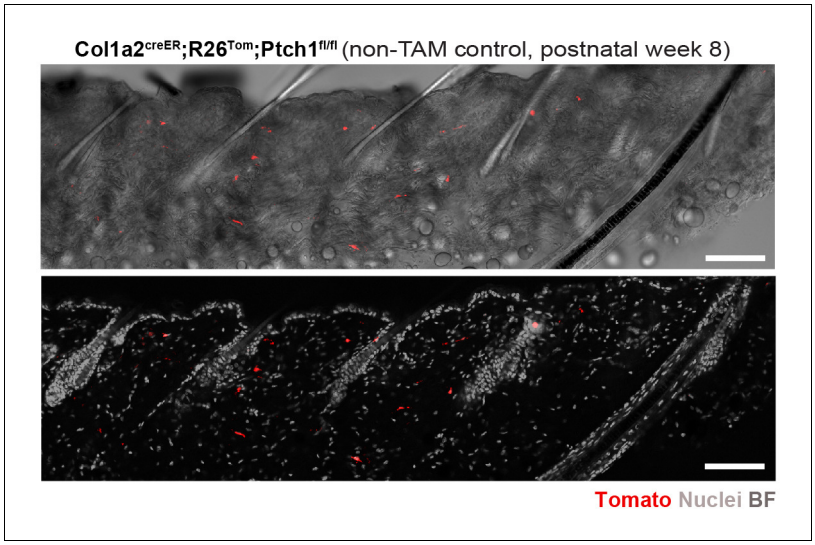

Figure 6—figure supplement 1. Leakiness in Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ skin. A small number of Tomato-traced cells was present in non-tamoxifen treated Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ mice at 8 weeks of age (i.e. start of tamoxifen treatment) (n = 2 mice). BF: bright field. Scale bars: 100 μm.

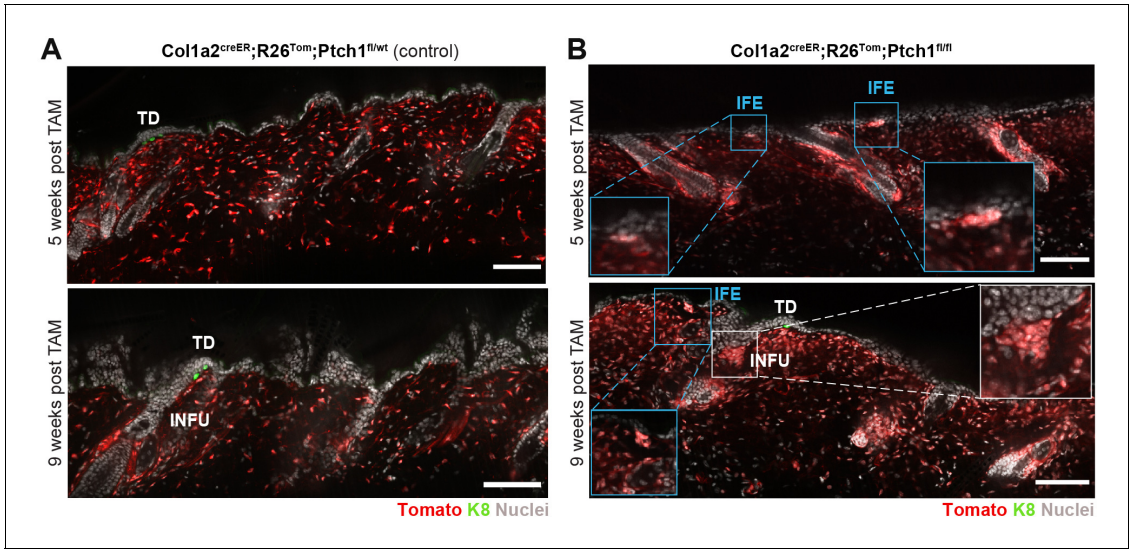

Figure 6—figure supplement 2. Stromal Hh-pathway activation in Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ skin leads to dermal cell condensations. (**A–B**) Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ mice and Col1a2^{creER};R26^{Tom};Ptch1$^{fl/wt}$ control mice were treated with TAM at 8 weeks of age and dorsal skin was analyzed 5 or 9 weeks after TAM treatment. Dermal cell condensations were not found in Col1a2^{creER};R26^{Tom};Ptch1$^{fl/wt}$ control skin (**A**) (n = 2 mice), but were frequently detected underneath the interfollicular epidermis (IFE) and HF-infundibulum (INFU) areas of Guard HFs in the skin of Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ mice (**B**) (n = 3 mice). TD: touch dome. Scale bars: 100 μm.

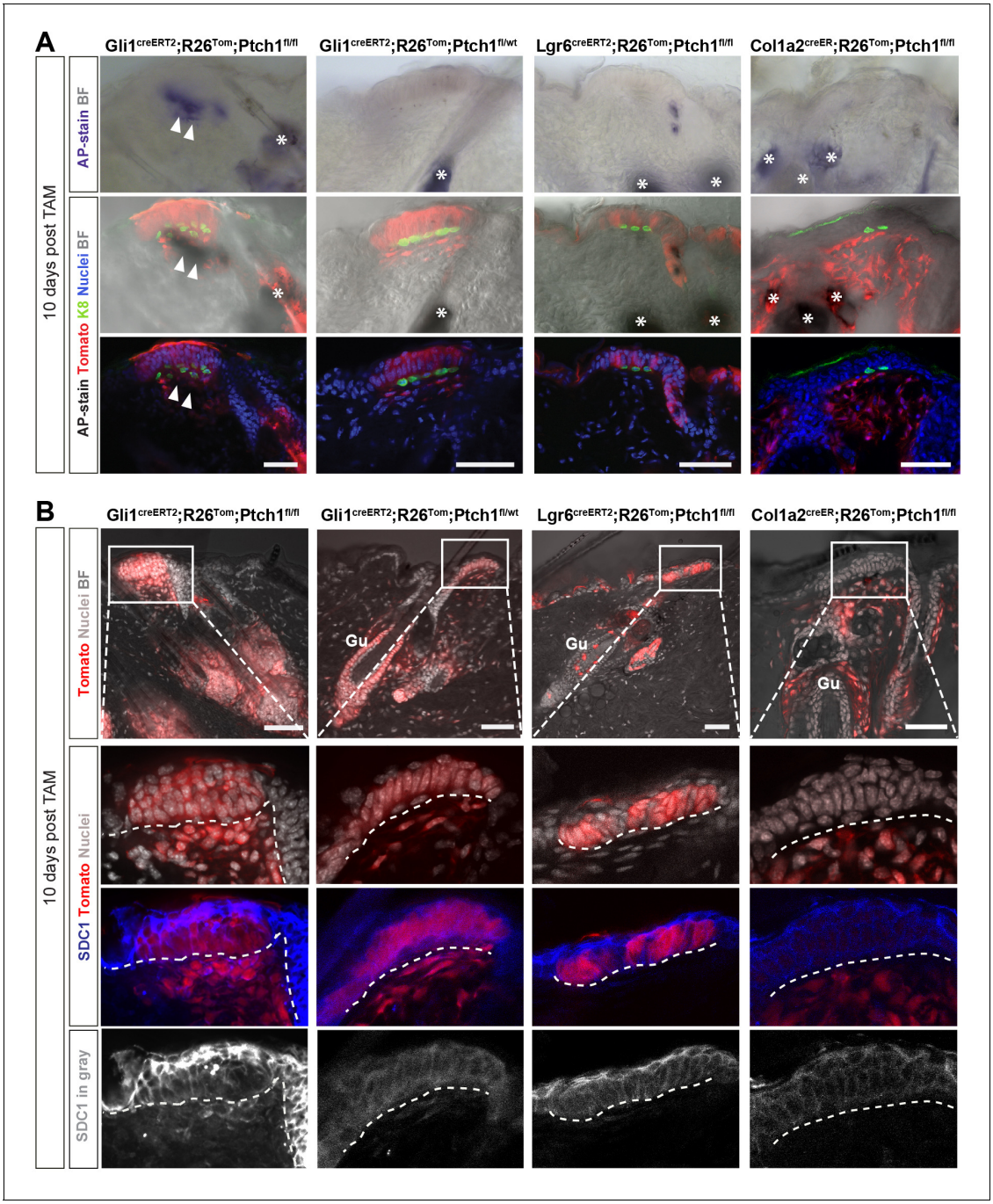

Figure 7. Expression of markers indicative for hair follicle (HF)-induction competent stroma in Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ touch domes (TDs). (**A–B**) Mice were treated with tamoxifen (TAM) at 8 weeks and dorsal skin was analyzed 10 days later using the alkaline phosphatase (AP) enzymatic assay (A; n = 2–3 mice per genotype) or Syndecan-1 (SDC1) immunofluorescence staining (B; n = 3 mice per genotype). (**A**) The TD stroma of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ skin stained AP-positive, indicating pre-dermal condensate formation. Please note that the nuclear staining (DAPI) in the areas of AP-positive signal is present but very dim (quenched). The TD stroma in skin of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$, Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ and Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ stained AP-negative. (**B**) TD stroma of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ skin was clearly positive for SDC1 staining, while the TD stroma of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ skin showed very weak to negative SDC1 staining. In the TDs of Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ and Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ skin, SDC1 staining was absent. Gu: Guard hair. TAM: tamoxifen. BF: bright field. Asterisks: sebaceous glands stain positive for AP. Arrowheads: positive AP-staining in TD stroma. Dashed line: epithelial-stromal border. Scale bars: 50 µm (**A–B**).

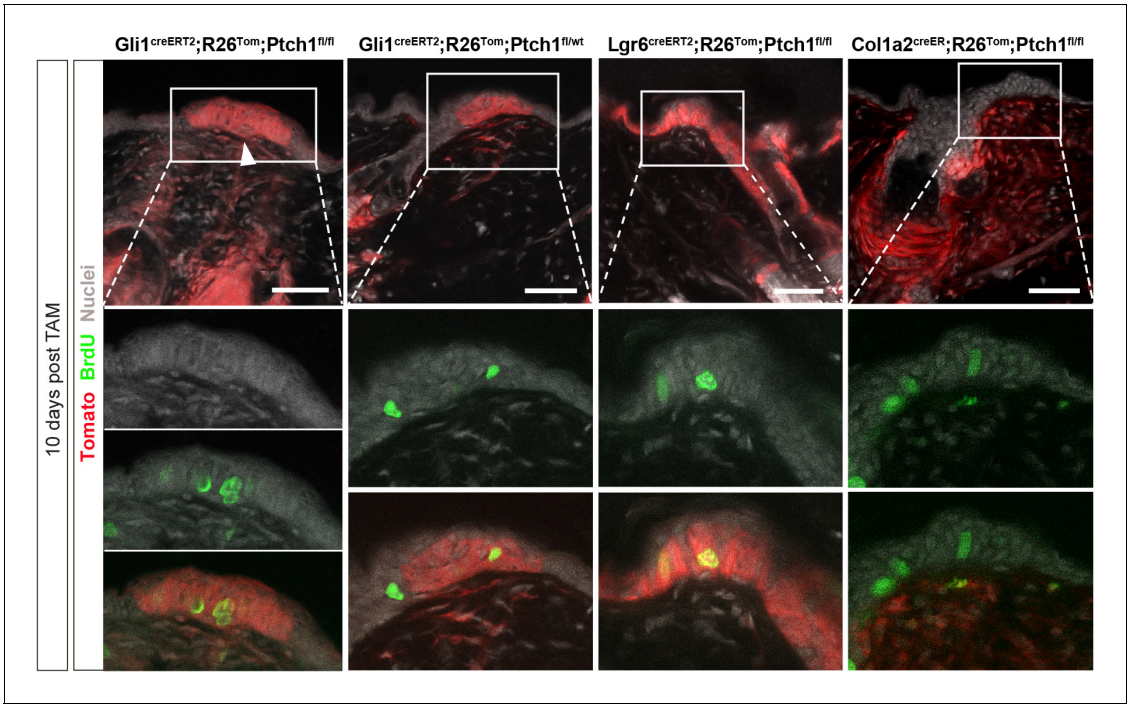

Figure 7—figure supplement 1. Capturing very early epithelial bud formation in TDs using BrdU incorporation. BrdU staining in TD areas. Mice were treated with TAM at 8 weeks of age and dorsal skin was collected 10 days after TAM treatment. Two hours prior to animal sacrifice, BrdU was given i.p. Clustered BrdU-positive cells were present in a potential developing TD-associated epithelial bud of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ skin (arrowhead). Fewer and non-clustered BrdU-positive cells were detected in the TDs of the Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ control mice, Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$, and Col1a2^{creER};R26^{Tom};Ptch1$^{fl/fl}$ mice. Note: HCl treatment for BrdU-staining results in more 'blurry' nuclei and loss of endogenous Tomato fluorescence. Tomato-tracing was visualized using an RFP antibody. n = 1 mouse per genotype, except n = 2 mice for Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$. BrdU: Bromodeoxyuridine; labels proliferating cells. Scale bars: 50 μm.

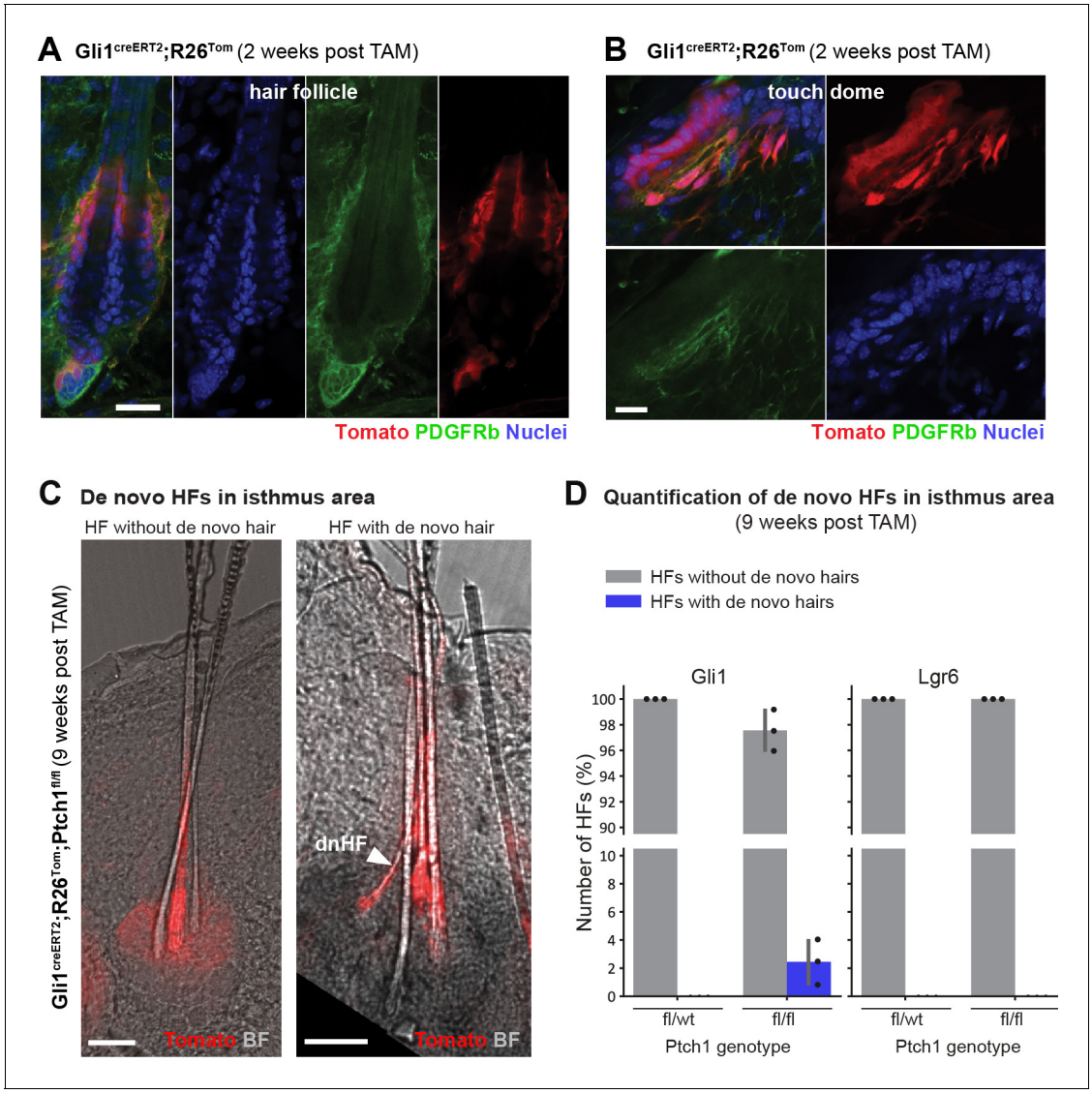

Figure 8. De novo hair follicle (HF) formation in isthmus area of pre-existing HFs. (**A–B**) PDGFRb (CD140b antibody) staining of Gli1^{creERT2};R26^{Tom} mice, treated with tamoxifen (TAM) at 8 weeks of age and traced for 2 weeks. Tomato-traced PDGFRb+ stromal cells are present in the HF isthmus area (**A**) and in the touch dome (TD) (**B**). (**C–D**) Mice were treated with TAM at 8 weeks of age and dorsal skin was analyzed 9 weeks post TAM. (**C**) Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ dorsal skin. Left panel: HFs without de novo HF contain three hair shafts (3 rounds of anagen). Right panel: HFs with de novo HF often contain four hair shafts including one thinner hair shaft with bent shape (arrowhead). (**D**) Quantification of de novo HFs in the isthmus area of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ (n = 668 HFs from n = 3 mice), Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ (n = 207 HFs from n = 3 mice), Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ (n = 335 HFs from n = 3 mice) and Lgr6^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ (n = 83 HFs from n = 3 mice) (***Figure 8—source data 1***). De novo HFs were only detected in isthmus areas of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice. p-value=0.09 (t-test comparing Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ and Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$). n = 3 mice (**A–C**). dnHF: de novo hair follicle. TAM: tamoxifen. BF: bright field. Scale bars: 20 μm (**A**), 10 μm (**B**), 50 μm (**C**).

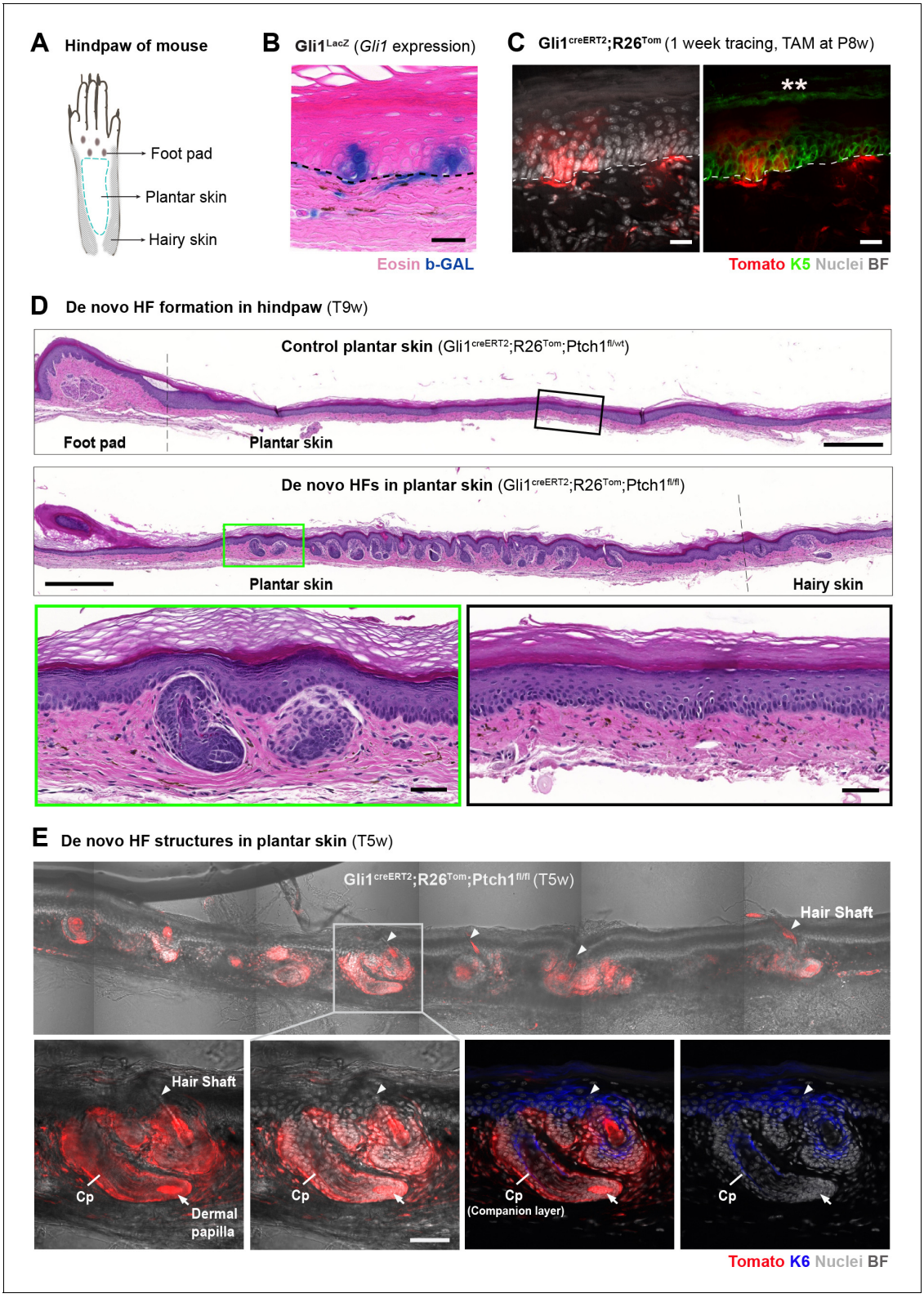

Figure 9. Formation of de novo hair follicles (HFs) in the plantar skin of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice. (**A**) Illustrative cartoon of a mouse hindpaw. (**B**) *Gli1*^{LacZ} expression in the plantar skin (n = 3 mice). (**C**) Gli1^{creERT2};R26^{Tom} mice were treated with tamoxifen (TAM) at 8 weeks of age. The hindpaws were collected 1 week post TAM and immuno-stained with K5 antibody (n = 3 mice). Asterisks mark autofluorescence on the outermost keratinized layer. (**D–E**) Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ and control Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ mice were treated with TAM at 8 weeks of age. Hindpaws were collected 5 or 9 weeks post TAM (n = 3 mice for each genotype; except n = 2 for Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ 5 weeks post TAM). (**D**) Numerous de novo HFs formed in

Figure 9 continued on next page

Figure 9 continued

the plantar skin of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice, while the same skin region in the control mice remained phenotypically normal. Green and black frames: zoom-in of plantar epidermis. Hematoxylin and eosin stained. (**E**) De novo HF structure in plantar skin of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice are fully Tomato-traced and contain a K6+ companion layer as well as hair shafts (n = 2 mice). Arrowheads: de novo hair shafts. Arrows: dermal papilla. Cp: companion layer. Dashed line: epithelial-stromal border. TAM: tamoxifen. BF: bright field. Scale bars: 20 μm (**B–C**), 500 μm (**D** panaromas), 50 μm (**D, E** insets).

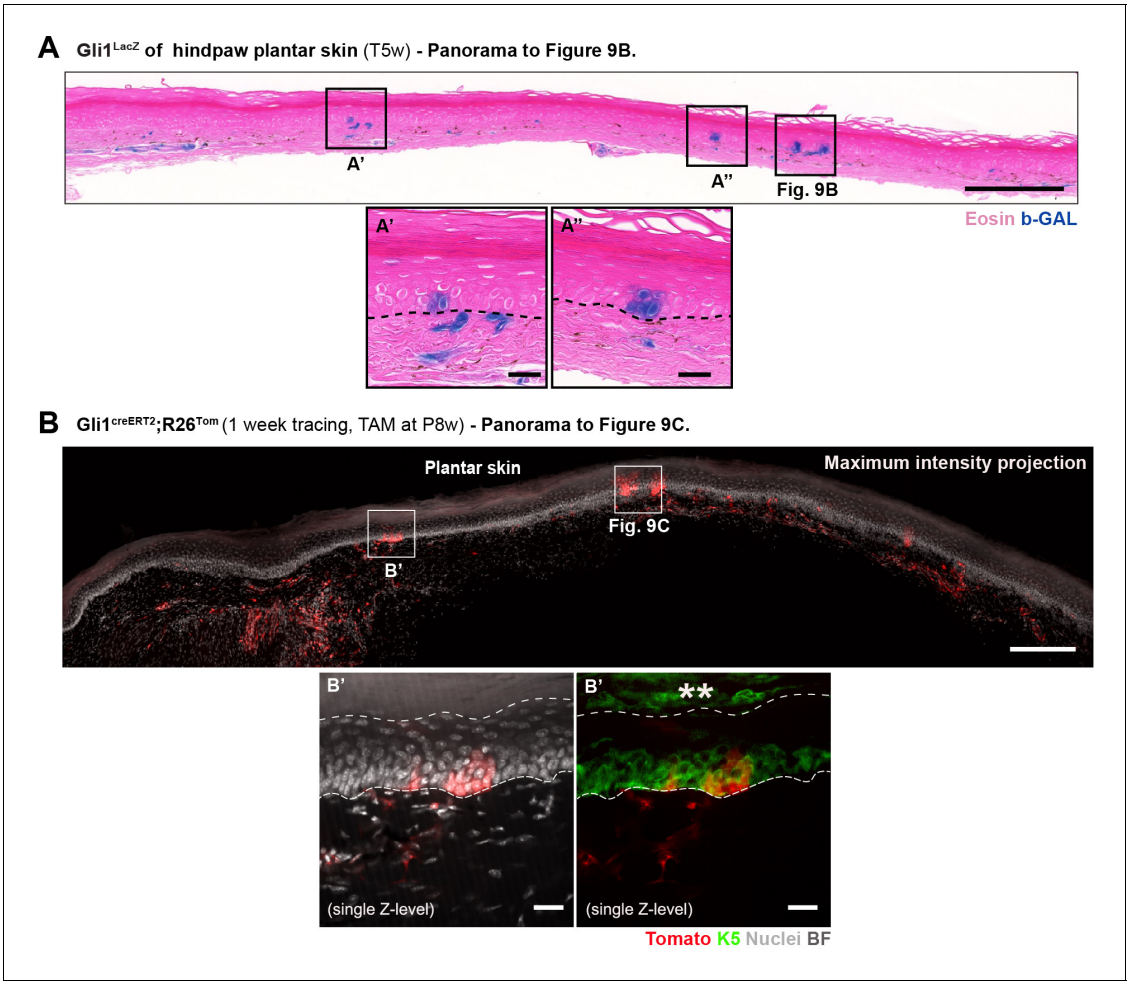

Figure 9—figure supplement 1. Physiological Hh/Gli signaling in the hindpaw plantar skin. (**A**) *Gli1*^{LacZ} expression in the plantar skin epidermis and dermis (n = 3 mice). Insets show *Gli1*-expressing cells at higher magnification. Dashed lines indicate the border between epidermis and dermis. (**B**) Gli1^{creERT2};R26^{Tom} mice were treated with TAM at 8 weeks and traced for a week (n = 3 mice). Hindpaw skin was collected and immunostained with K5 antibody. Upper panel: maximum intensity projection of Tomato-tracing pattern in plantar skin. Lower panel: details of frame B' (a single z-level is shown). Asterisks mark auto-fluorescence of the outermost keratinized layer. TAM: tamoxifen. BF: bright field. Scale bars: 200 μm (Panaroma in A and B), 20 μm (Insets A',A'',B').

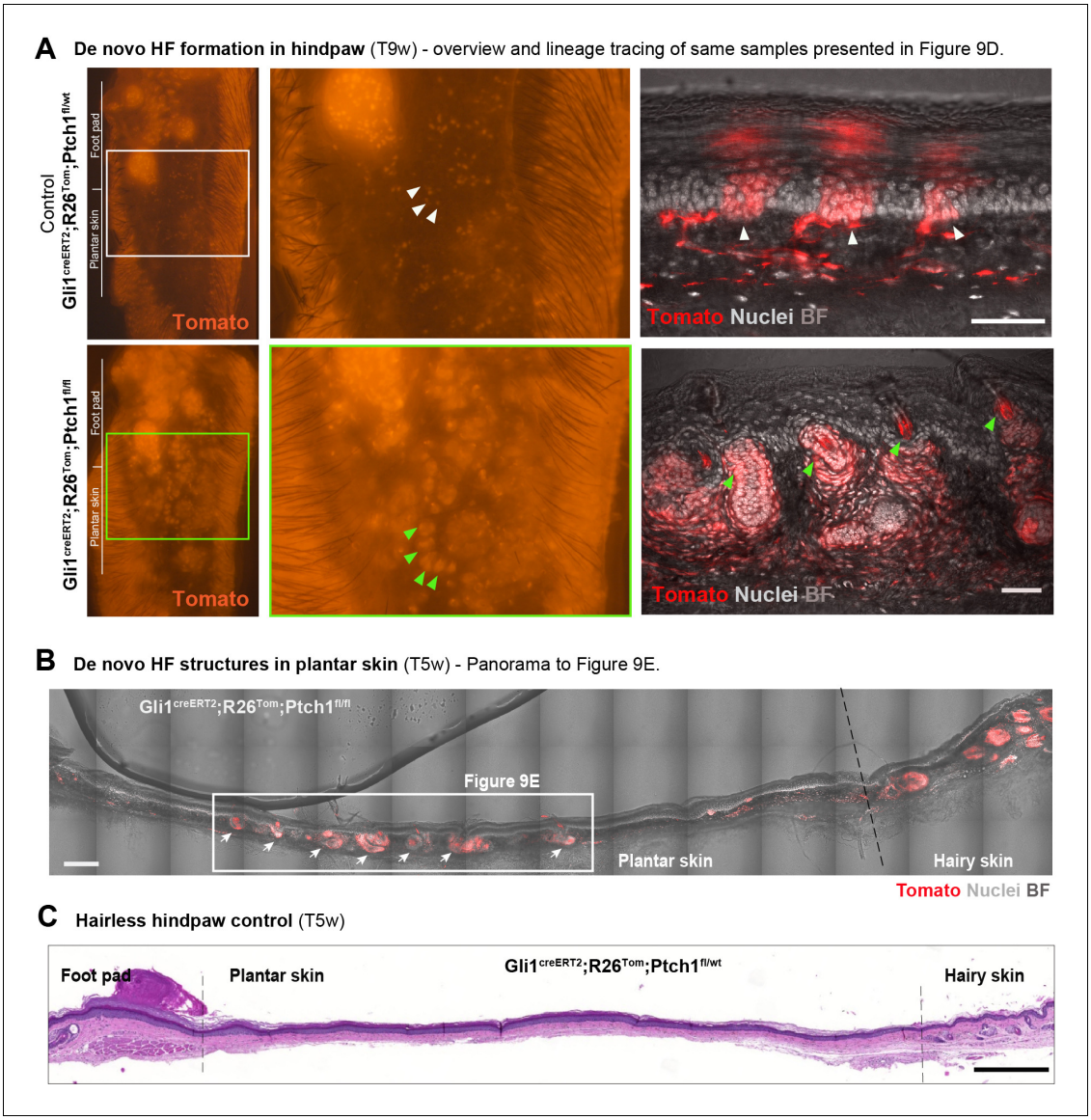

Figure 9—figure supplement 2. De novo HF formation in hindpaw of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice. (**A–C**) Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ and control Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/wt}$ mice were treated with TAM at 8 weeks of age. The hindpaws were collected 5 or 9 weeks post TAM (n = 3 mice for each genotype). (**A**) Overview images of Tomato fluorescence in paws aquired with a stereo microscope (left panels) and lineage tracing pattern (right panels) of the same samples that were presented in ***Figure 9D***. While in plantar skin of the control mice only Gli1-traced IFE cell clusters were present (white arrowheads), numerous de novo HFs with hair shafts formed in the plantar skin of Gli1^{creERT2};R26^{Tom};Ptch1$^{fl/fl}$ mice 9 weeks post TAM (green arrowheads). (**B**) Panorama to ***Figure 9E***: de novo HF structures in plantar skin. Arrows: de novo HFs. (**C**) The control plantar skin shows no signs of de novo HF formation 5 weeks after TAM treatment. TAM: tamoxifen. BF: bright field. Scale bars: 50 μm (**A**), 200 μm (**B**), 500 μm (**C**).