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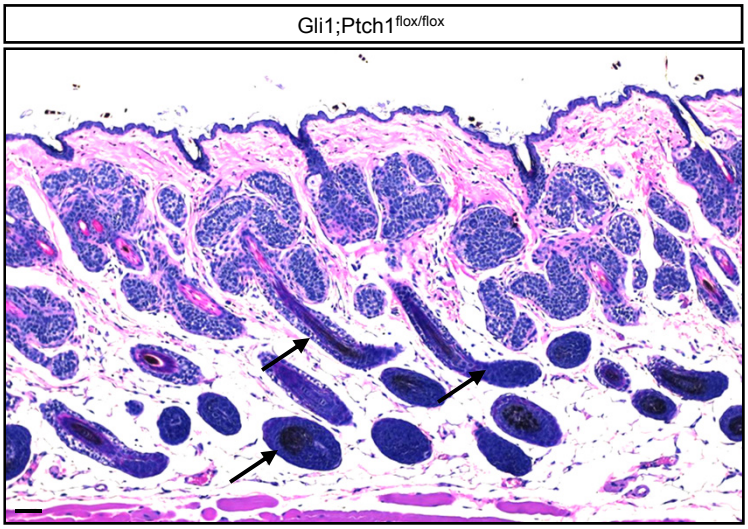

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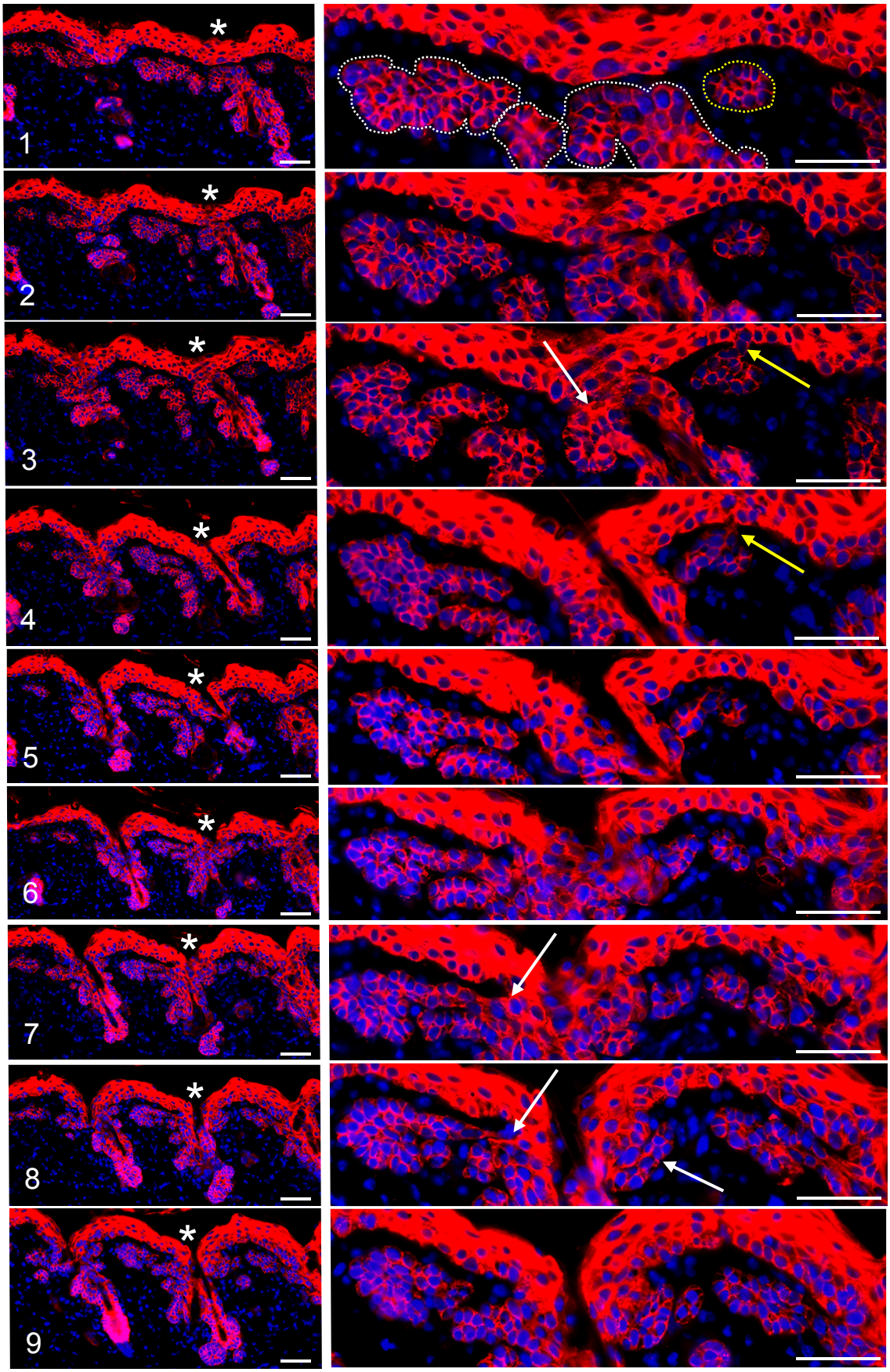

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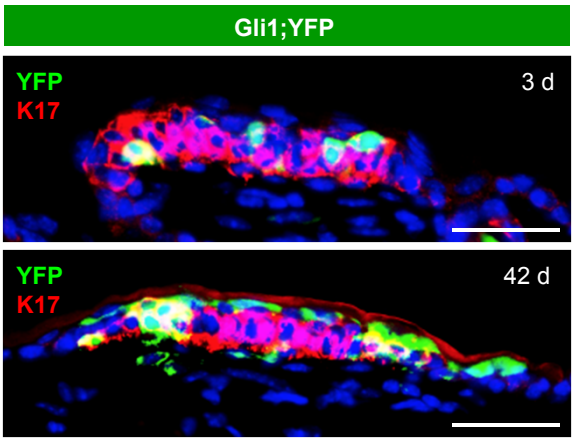

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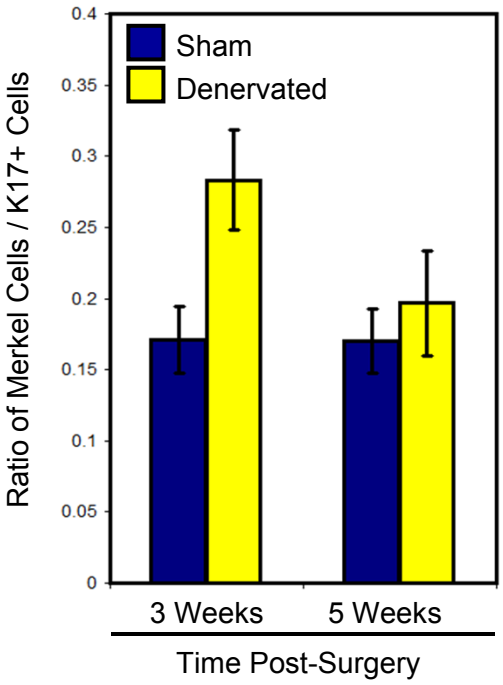

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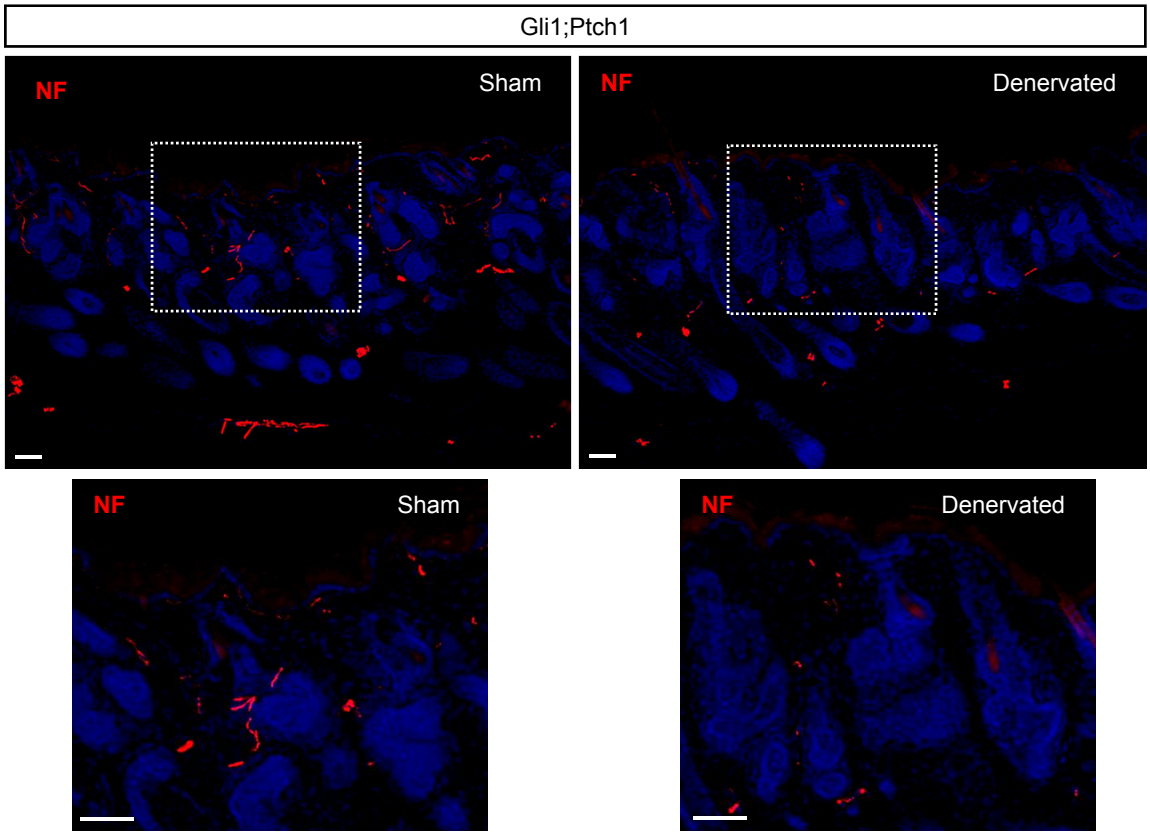

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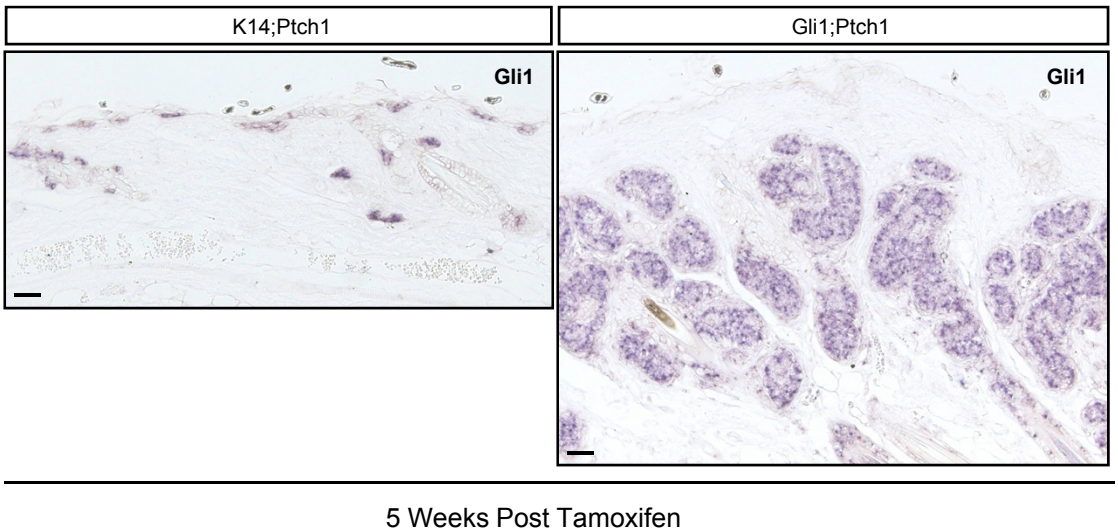

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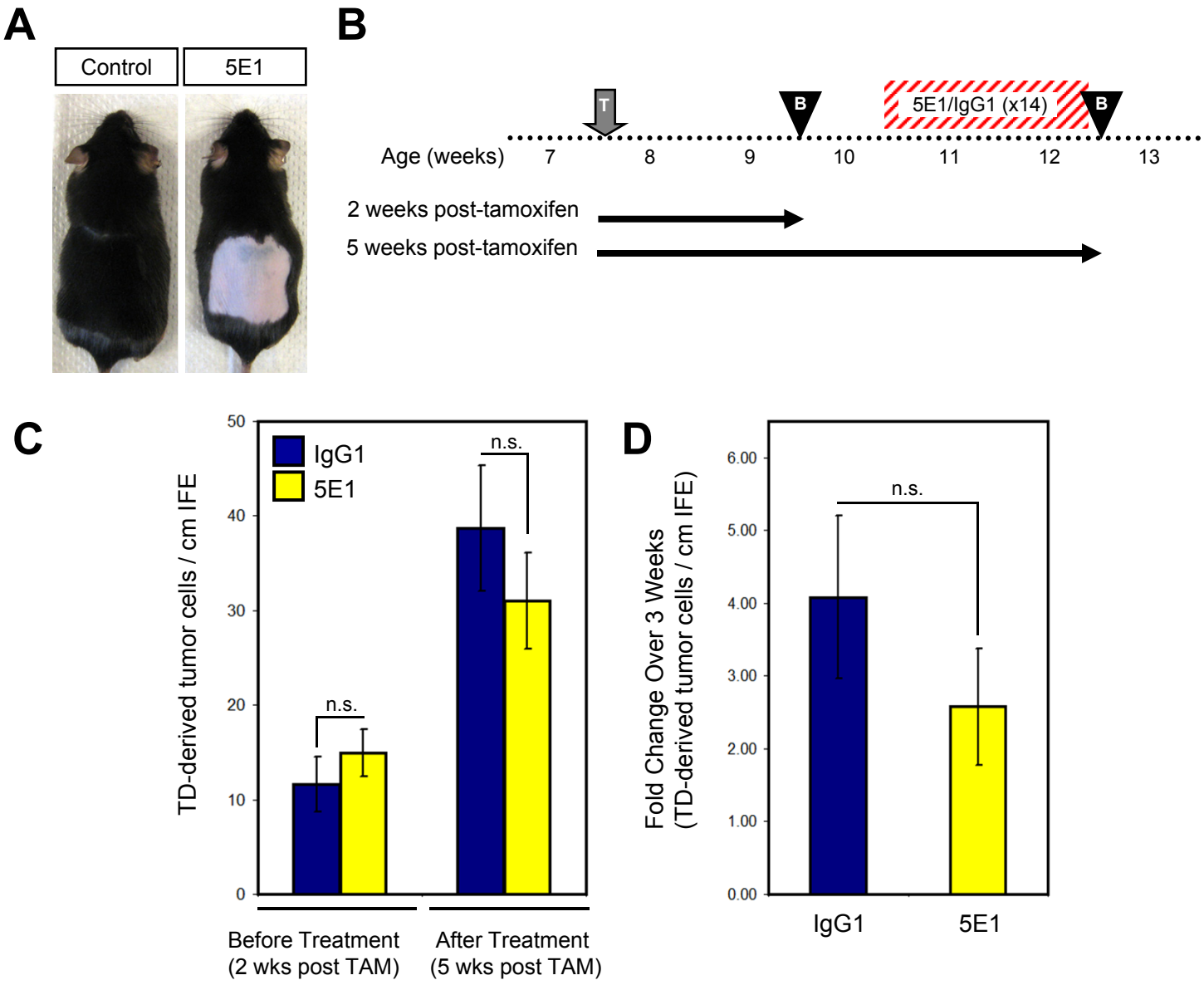

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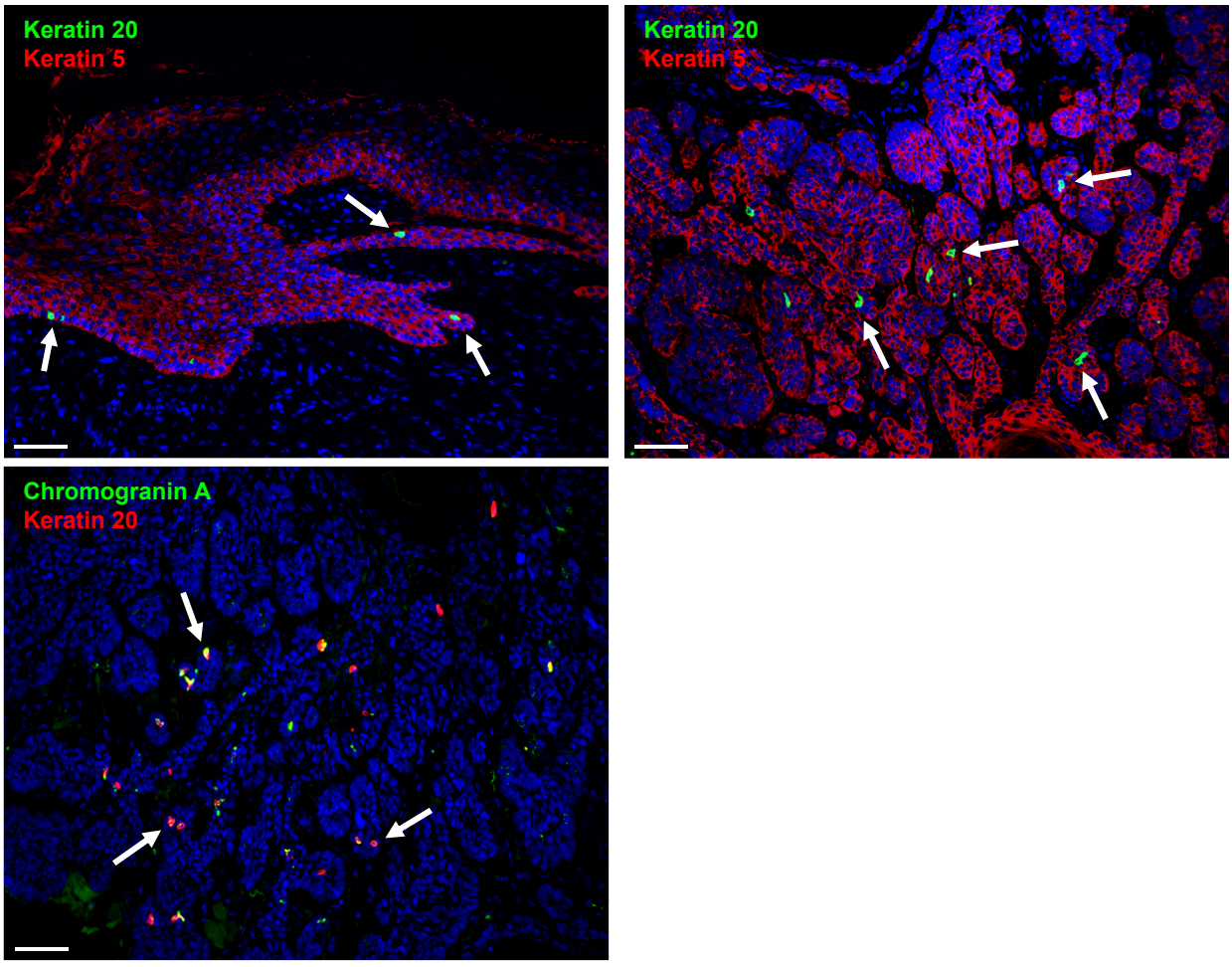

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Related to Figure 1. H&E staining showing absence of tumors in the lower anagen hair follicle (arrows) in *Gli1;Ptch1* mice. Scale bars, 50 μm.

Figure S2. Related to Figure 2. Nine serial sections of skin from a *K14;Ptch1* mouse, 12 weeks post-TAM, immunostained for K5 (red). In the left column of panels, (*) marks a similar region of the skin overlying a hair follicle that is magnified in the right column of panels. Hair follicle-associated tumors (white dotted lines) and a smaller IFE-associated ectopic bud (yellow dotted line) are indicated in the top right panel. White arrows indicate areas where tumorigenic lesions are continuous with the hair follicle. Yellow arrows indicate areas where a smaller ectopic bud is possibly continuous with the IFE (sections #3-4), but may also possibly be connected to the hair follicle (section #8). Scale bars, 50 μm.

Figure S3. Related to Figure 5. A. IHC showing stable labeling of TD epithelia (red) by Gli1-Cre^{ERT2}-induced recombination of a floxed YFP reporter allele (green), in *Gli1;YFP* mice, 3 or 42 days post-TAM. **B.** The ratio of K8+ Merkel cells to K17+ TD epithelia is increased, 3 weeks after denervation, relative to sham-operated control. Five weeks after denervation, this ratio is unchanged between denervated and sham-operated control skin. This suggests that while denervation decreases the overall number of both K17+ TD cells and K8+ Merkel cells (Figure 5B), loss of K17 expression occurs more rapidly than does loss of Merkel cells. Scale bars, 50 μm.

Figure S4. Related to Figure 6. IHC showing stable loss of nerves, as assessed by Neurofilament staining (red), in *Gli1;Ptch1* denervated skin (right panel) compared to matched sham control (left panel), approximately 4.5 weeks after denervation. Bottom panels are magnified views of the boxed areas in the top panels. Scale bars, 50 μm.

Figure S5. Related to Figure 7. *In situ* staining for *Gli1*, similar to IHC for K17, reveals that activation of downstream Hh signaling occurs in both small ectopic downgrowths in *K14;Ptch1* mice, as well as in hair follicle-associated tumors in *Gli1;Ptch1* animals, both 5 weeks after TAM induction. Scale bars, 50 μm.

Figure S6. Related to Figure 7. A. Depilated wild-type mice treated for 2 weeks with an Shh-neutralizing antibody (5E1, right) displayed a severe delay in anagen re-entry, compared to untreated mice (left), as previously reported (Wang et al., 2000). **B.** Schematic for tamoxifen-induced *Gli1;Ptch1*

mice treated with either purified 5E1 antibody or a purified IgG1 isotype control. Biopsies (B) were collected both before and after antibody treatment (2 and 5 weeks post-TAM induction (T), respectively). Daily antibody injections were given throughout the final 2 weeks of the experiment. **C.** Quantitation of TD-derived tumor cell abundance, both before and after antibody treatment. Yellow bars indicate data from animals treated with 5E1, whereas blue bars indicate data from animals treated with control antibody. n. s., not significant. **D.** Fold-change in the abundance of TD-derived tumor cells between 2-5 weeks after tamoxifen induction, for animals treated with 5E1 (yellow) or IgG1 (blue). ($p = 0.28$) ($n = 7$ mice treated with 5E1, and 5 mice treated with IgG1)

Figure S7. Related to Figure 7. IHC staining, as indicated, identifying Merkel cells (arrows) in human BCCs. Merkel cells were observed both in superficial lesions (upper left) as well as in deeper tumor nests (upper right and lower images). Merkel cells were not observed within large tumor masses (not shown). Scale bars, 50 μm.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Whole-Mounts and In Situ Analysis

For whole-mount LacZ staining, skin samples were fixed in 0.2% glutaraldehyde/2% paraformaldehyde for 30 min at 4 degrees, then incubated for 48 hrs at 37 degrees in 1 mg/ml X-Gal (Invitrogen) diluted in 5 mM potassium ferrocyanide and 5 mM potassium ferricyanide. *In situ* hybridization was performed on 5 um paraffin sections made from skin biopsies fixed overnight in formalin. Labeling was performed with DIG-labeled *Gli1* riboprobe (Hui et al., 1994) using protocols described previously (Grachtchouk et al., 2003).

Antibody injections

Hybridoma cells secreting monoclonal antibodies generated against Shh (clone 5E1) were obtained from the Developmental Studies Hybridoma Bank. Antibodies were harvested from bioreactor cell culture supernatant by the University of Michigan Hybridoma Core and subsequently purified. 200 ug of purified 5E1 antibody or purified IgG1 isotype control antibody (BioLegend), both at a concentration of 1 ug/ul, was injected intraperitoneally, once daily, for 2 weeks. Each mouse received a total of 2.8 mg of purified 5E1 or IgG1 antibody by the end of the experiment.

Keratinocyte cell culture

Keratinocytes were isolated from newborn pups and plated into 6-7 wells in a 12-well plate, as previously described (Lichti et al., 2008). Cells were grown in complete Cnt-57 keratinocyte media (CellnTec) for 3 days, then switched onto basal media (CnT-BM1.500) +/- 3 μM purmorphamine (Selleckchem) for an additional 3 days before RNA extraction.

RNA extraction and quantitative PCR

RNA was extracted from isolated epidermis and dissected dorsal root ganglia from 7-8 week old mice using the RNeasy Mini Kit (Qiagen). 250 ng of epidermal RNA and 40 ng of DRG RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Invitrogen), and quantitative RT-PCR was performed on 1:10 or 1:2 diluted cDNAs, respectively, using Power SYBR Green PCR Master Mix (Applied Biosystems). All amplification results were normalized to those of the *Hprt* loading control.

Quantitative PCR Sequences

Gene	**Sequence**
Shh	F: 5’ AAA GCT GAC CCC TTT AGC CTA-3’ R: 5’ TTC GGA GTT TCT TGT GAT CTT CC-3’
Ihh	F: 5’ CTC TTG CCT ACA AGC AGT TCA 3’ R: 5’ CCG TGT TCT CCT CGT CCT T 3’
Dhh	F: 5’ CTT GGC ACT CTT GGC ACT ATC 3’ R: 5’ GAC CCC CTT GTT ACC CTC C 3’
Gli1	F: 5’ TTG GGA TGA AGA AGC AGT TG 3’ R: 5’ GGA GAC AGC ATG GCT CAC TA 3’
Ptch2	F: 5’ GGT CCT CCG CAC CTC ATA TC 3’ R: 5’ GTC TGT CAA TTA CAG CCA CTC 3’
Hhip1	F: 5’ TCG GGT CAC ATC TTG GGA TTT GG 3’ R: 5’ GGA GCA ATC GGA GGT CAG TGG 3’
CD200	F: 5’ CTC TCC ACC TAC AGC CTG ATT 3’ R: 5’ AGA ACA TCG TAA GGA TGC AGT TG 3’
K14	F: 5’ CGC CGC CCC TGG TGT GG 3’ R: 5’ ATC TGG CGG TTG GTG GAG GTC A 3’
Tubb3	F: 5’ TAG ACC CCA GCG GCA ACT AT 3’ R: 5’ GTT CCA GGT TCC AAG TCC ACC 3’
Hprt	F: 5’ AGG ACC TCT CGA AGT GTT GGA TAC 3’ R: 5’ AAC TTG CGC TCA TCT TAG GCT TTG 3’

SUPPLEMENTAL REFERENCES

Grachtchouk, V., Grachtchouk, M., Lowe, L., Johnson, T., Wei, L., Wang, A., de Sauvage, F., and Dlugosz, A.A. (2003). The magnitude of hedgehog signaling activity defines skin tumor phenotype. EMBO J. *22*, 2741-2751.

Hui, C.C., Slusarski, D., Platt, K.A., Holmgren, R., and Joyner, A.L. (1994). Expression of three mouse homologs of the Drosophila segment polarity gene cubitus interruptus, Gli, Gli-2, and Gli-3, in ectoderm- and mesoderm-derived tissues suggests multiple roles during postimplantation development. Dev. Biol. *162*, 402-413.

Lichti, U., Anders, J., and Yuspa, S.H. (2008). Isolation and short-term culture of primary keratinocytes, hair follicle populations and dermal cells from newborn mice and keratinocytes from adult mice for in vitro analysis and for grafting to immunodeficient mice. Nat. Protoc. *3*, 799-810.

Wang, L.C., Liu, Z.Y., Gambardella, L., Delacour, A., Shapiro, R., Yang, J., Sizing, I., Rayhorn, P., Garber, E.A., Benjamin, C.D., *et al.* (2000). Conditional disruption of hedgehog signaling pathway defines its critical role in hair development and regeneration. J. Invest. Dermatol. *114*, 901-908.