



Figures and figure supplements

Embryonic attenuated Wnt/ β -catenin signaling defines niche location and long-term stem cell fate in hair follicle

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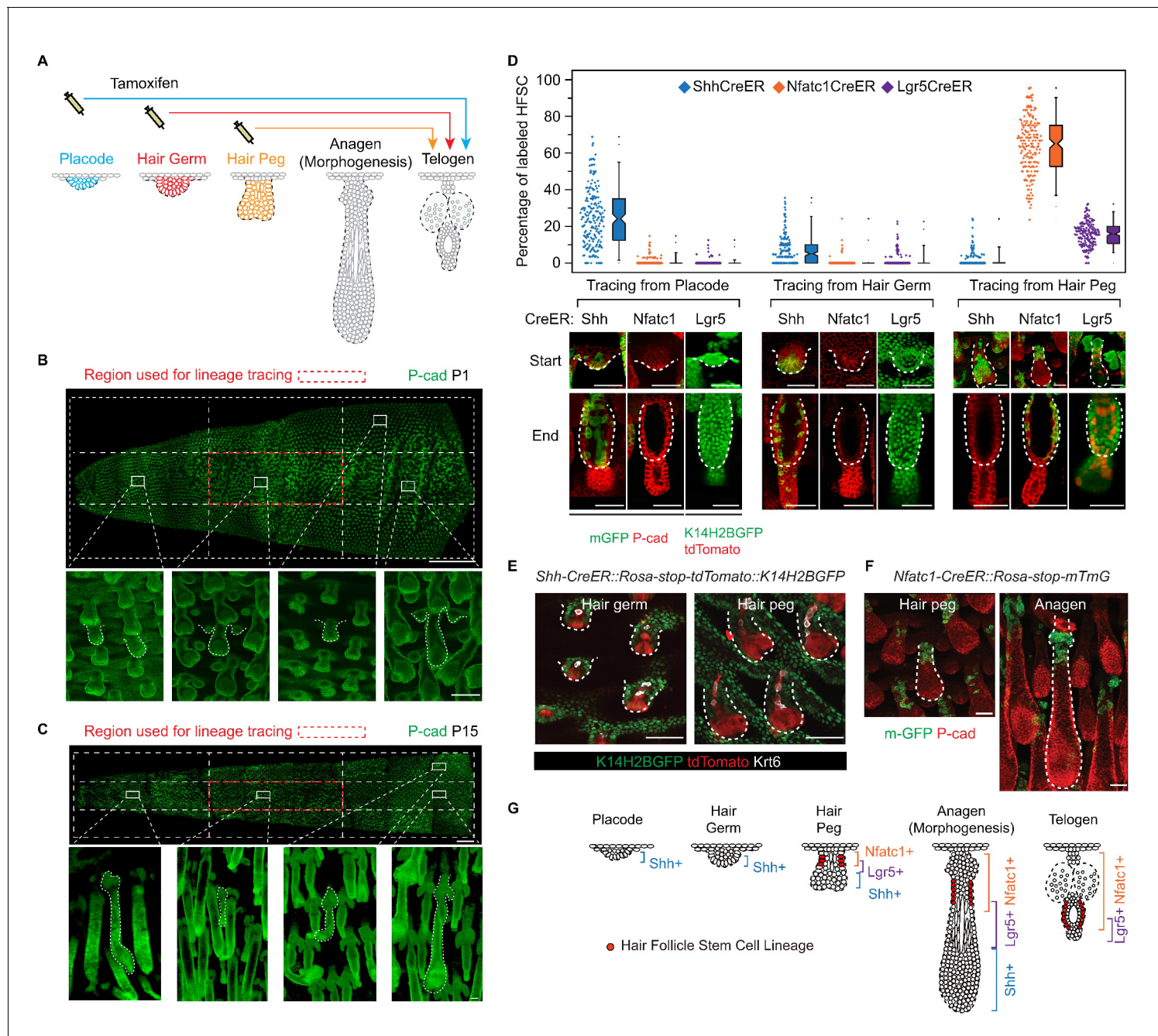


Figure 1. Embryonic cellular origin of adult hair follicle stem cells. **(A)** Diagram of hair follicle morphogenesis and the lineage-tracing experiment. All lineage-tracing experiments ended at the first telogen, but started at different stages including the placode, hair germ, and hair peg stages. **(B,C)** Representative images of tail skin hair follicle organogenesis. Red boxes indicate the regions used for quantification in the lineage-tracing experiments. The hair cycle in tail skin progresses along the anterior to posterior and in the dorsal to ventral directions. At postnatal day 1 (P1), in the chosen region, the primary central hair follicles are in the hair peg stage while the secondary outer follicles are in the placode stage. At P15, in the chosen region, primary central hair follicles are in the telogen phase. Scale bar: 1500 μ m for the whole mount image; 100 μ m for the enlarged images. **(D)** Summary of the lineage-tracing experiments with: *Shh-CreER::Rosa-stop-mTmG*, *Nfatc1-CreER::Rosa-stop-mTmG*, and *Lgr5-GFP-CreER::Rosa-stop-tdTomato::K14H2BGFP* mice. Percentages of labelled HFSCs at the telogen phase were quantified using whole mount sample images. Representative images are single confocal Z slices from the data used for quantification. N=5 mice, >200 HF. Raw data are plotted to the left of each box-and-whisker plot: the median and the 25th and the 75th percentiles are denoted by notches and the bottom and top boxes, respectively; the 5th and 95th percentiles are denoted as whiskers. Scale bar: 50 μ m. **(E)** In both the hair germ and the hair peg, the progenies of Shh+ cells in the center of HFSCs express the differentiation marker Keratin 6 (Krt6). Scale bar: 50 μ m. **(F)** Lineage-tracing experiment starting at the hair peg stage and ending at morphogenesis

Figure 1 continued on next page

Figure 1 continued

anagen (growth phase) using *Nfatc1-CreER::Rosa-stop-mTmG* mice. Note that the labeled cells stay dormant at their original position without contributing to HF down growth. Scale bar: 50 μ m. (G) Model depicting the spatial and temporal pattern of hair follicle stem cell emergence.

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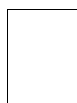


Figure 1—figure supplement 1. Difference between back and tail skin hair follicle organogenesis. (A) In mouse back skin, hair follicle initiation occurs via three separate waves that start, respectively, around E13.5, 15.5, and 18.5. At any given embryonic time point between E13.5 to E18.5, there is a mixture of hair follicles at different developmental stages in back skin, lacking any discernable pattern. After birth, the three separate waves synchronize into similar anagen and then telogen. Thus, it is impossible to pinpoint the exact early developmental stage of any telogen back skin hair follicle at the beginning of the lineage-tracing experiments when Tamoxifen was injected at an embryonic time point. (B) Tail skin hair follicles are arranged in triplets, and the growth of two secondary outer follicles is typically initiated next to a primary central follicle after it has already begun development. Within the dorsal middle one-third section of tail skin, at P1, the primary central hair follicles are at the hair peg stage and the secondary outer follicles on the side are at the placode stage. At P6, all three hair follicles in a triplet progress to anagen. At P15, the primary central hair follicles have already entered telogen or even the telogen to anagen transition stage, while the secondary outer hair follicles on the side are still in the catagen to telogen transition. At P21, the primary central hair follicles are in full anagen and the two secondary outer follicles are at the telogen to anagen transition stage. Scale bars: 50 μ m.

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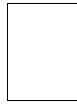


Figure 1—figure supplement 2. Diagram, quantification, and specificity of CreER lines for the lineage-tracing experiments. (A) Diagram of the lineage-tracing experiment using *Nfatc1-CreER::Rosa-stop-mTmG* mice. (B) Diagram of the lineage-tracing experiment using *Lgr5-GFP-CreER::Rosa-stop-tdTomato::K14H2BGFP* mice. Representative images used here are the same representative images shown in **Figure 1D** to indicate these diagram are drawn to explain the experimental designs for **Figure 1D**. Scale bar: 50 μ m. (C) Diagram depicting the individual time points used for the lineage-tracing experiments starting from different early developmental stages. Green color highlights the target hair follicles used in the designated lineage-tracing experiments. (D–F) Percentage of HFs with labeled HFSCs at telogen. Tracings starting from the placode stage (D), the hair germ stage (E), and the hair peg stage (F). (G) *Nfatc1-CreER::Rosa-stop-mTmG*, *Shh-CreER::Rosa-stop-mTmG*, *Lgr5-GFP-CreER::Rosa-stop-tdTomato::K14-H2BGFP* mice tail skin hair follicles with or without Tamoxifen induction. Scale bar: 200 μ m.

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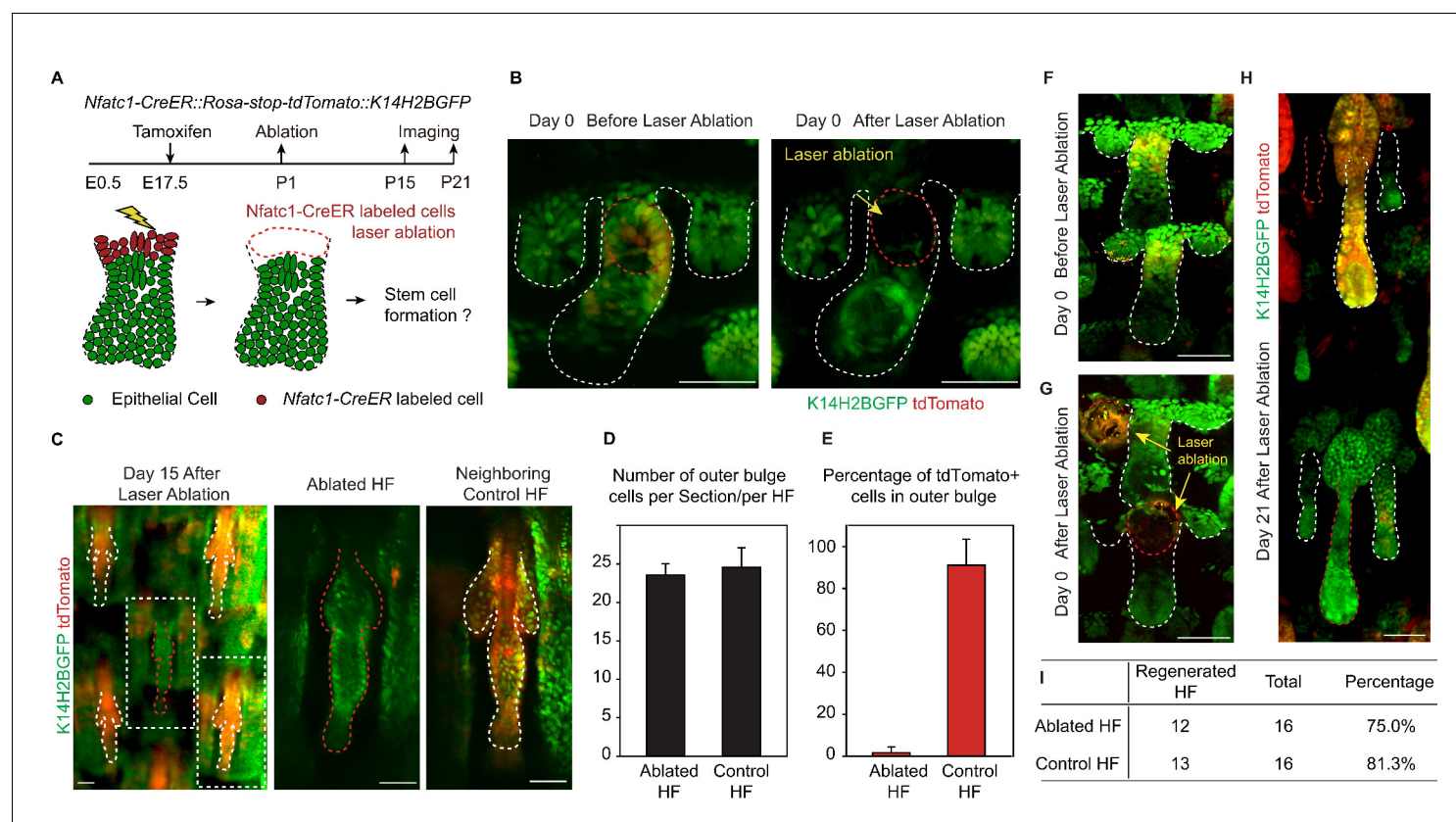


Figure 2. Niche position in the hair peg determines hair follicle stem cell fate. (A) Diagram of two-photon-mediated cell ablation experiment using *Nfatc1-CreER::Rosa-stop-tdTomato::K14H2BGFP* mice. (B) Representative images of hair follicles before and after cell ablation in a live animal. (C) Representative whole-mount images of hair follicles 15 days after cell ablation. Notice that the control hair follicle has tdTomato+ cells in the bulge while the ablated hair follicle has a normal bulge composed of tdTomato- cells. (D) Quantification of hair follicle stem cell number at telogen using whole mount samples. N=6 mice, >12 HF. (E) Quantification of percentage of tdTomato+ outer bulge cells at telogen using whole mount samples. N=6 mice, >12 HF. (F–H) Representative images of hair follicles before (F), immediately after (G), and 21 days after (H) cell ablation from the same mouse. Notice that both the control hair follicle and ablated hair follicle enter anagen. (I) Quantification of hair follicles that have started regeneration 21 days post cell ablation. N=5. Scale bars: 50 μ m

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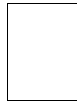


Figure 2—figure supplement 1. Ablation specificity, Tamoxifen induction efficiency, and whole-mount views of the two-photon-mediated cell ablation experiments. (A) Representative images of hair follicles before, immediately after, and 2 days post ablation in the same live mouse. Notice the ablated hair placodes disappear, while the follicles adjacent to them develop normally. Scale bar: 50 μm . (B) Whole-mount tail skin image of *Nfatc1-CreER::Rosa-stop-mTmG* mice with lineage tracing starting at the hair peg stage. The percentage of labeled HFSCs decreased along the dorsal-ventral axis starting from the dorsal anterior-posterior midline. Scale bar: 500 μm . (C) Whole-mount image of tail skin used for quantification 21 days after ablation. Ablated hair follicles are numbered and highlighted in red. Scale bar: 500 μm .

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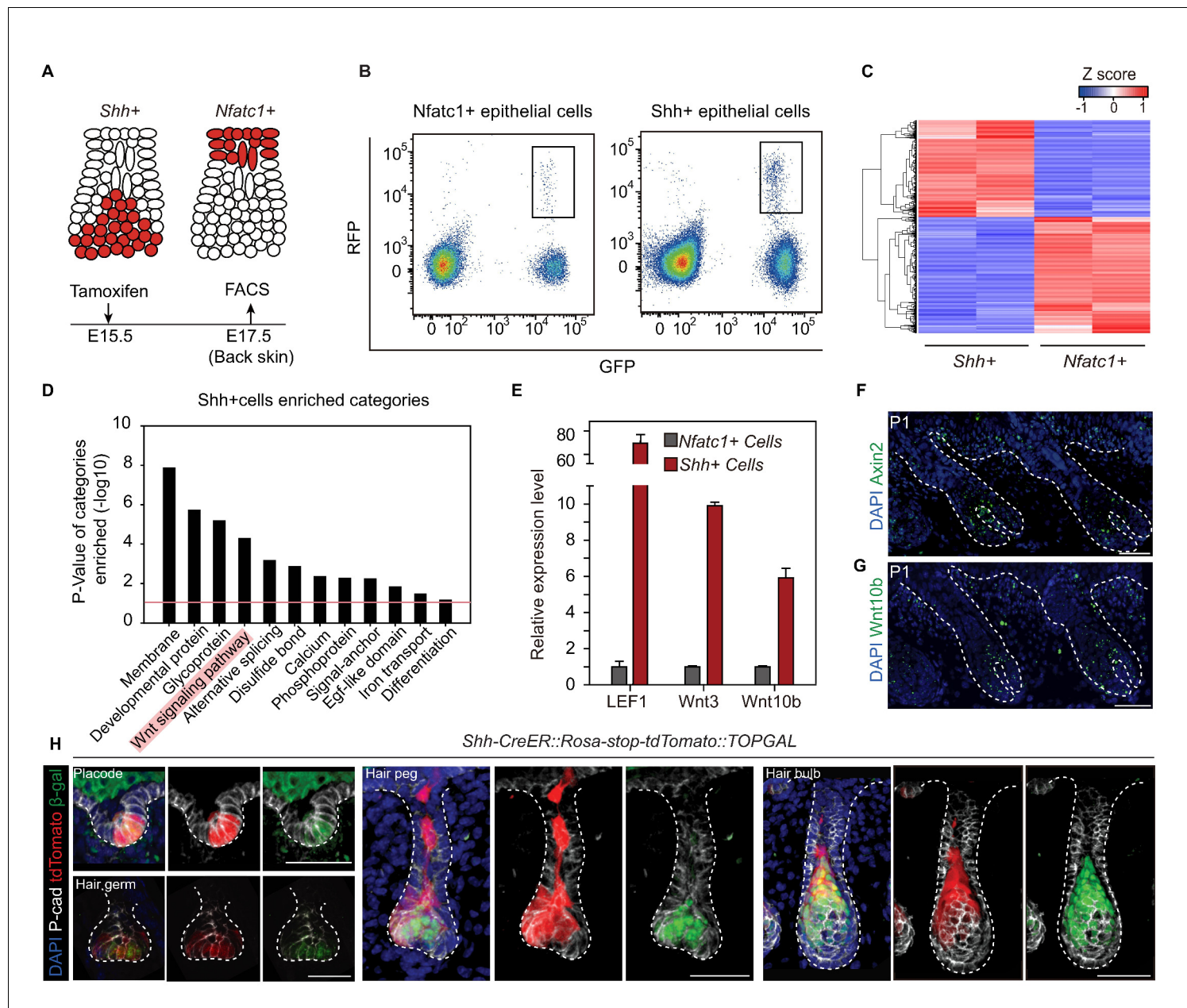


Figure 3. Unbiased RNA-seq analysis reveals factors that define the hair peg niche. (A) Diagram of the FACS experiments using *Nfatc1*- and *Shh*-*CreER::Rosa-stop tdTomato::K14H2BGFP* mice. (B) FACS isolation of distinct GFP+RFP+ populations to obtain *Shh*+ and *Nfatc1*+ epithelial cells. (C) Unsupervised hierarchical clustering and heat map display of genes that were differentially expressed between *Shh*+ cells and *Nfatc1*+ cells. N=2 (D) Gene Ontology analysis of ≥ 2 -fold up-regulated genes in *Shh*+ cells compared to *Nfatc1*+ cells. The Wnt signaling pathway is highlighted. (E) Validation of differentially expressed genes using qPCR. N=3. (F–G) In situ staining of *Axin2* (F) and *Wnt10b* (G) in developing hair follicles. (H) *Shh*+ cells are Wnt/ β -catenin signal responsive cells. *Shh* expression was represented by tdTomato in *Shh-CreER::Rosa-stop-tdTomato* mice. Wnt/ β -catenin-responsive cells were detected by β -gal staining in TOPGAL mice. Scale bars: 50 μ m.

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The following source data is available for figure 3:

Source data 1. RNA-seq results of differentially expressed genes between *Nfatc1*+ and *Shh*+ cells.

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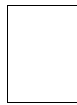


Figure 3—figure supplement 1. RNA seq results of representative genes from different populations. (A) RNA seq results of *Nfact1* and *Shh* from different populations. N=2 (B) Fold enrichment of canonical Wnt pathway genes from the RNA-seq results. N=2.

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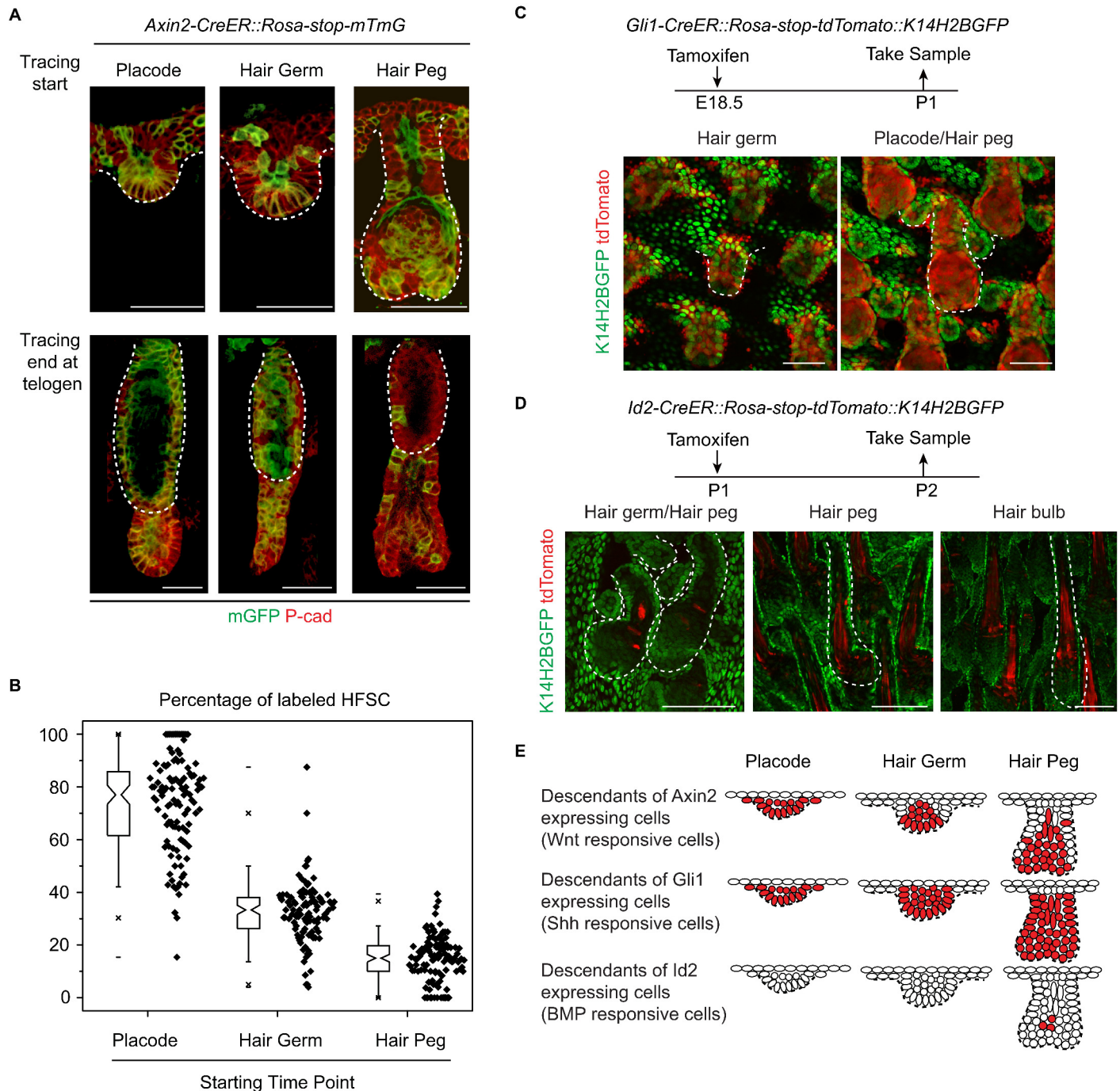


Figure 4. Attenuated Wnt/ β -catenin signaling is uniquely associated with hair follicle stem cell specification. (A–B) Lineage tracing with *Axin2CreER::Rosa-stop-mTmG* mice. N=5 mice, >120 HF. (C) Descendants of *Gli1*+ cells were represented by the expression of tdTomato in *Gli1-CreER::Rosa-stop-tdTomato::K14H2BGFP* mice. Note that *Gli1*+ descendants are located in almost all the hair follicle cells at the placode, hair germ, and hair peg stages. (D) Descendants of *Id2*+ cells were represented by the expression of tdTomato in *Id2-CreER::Rosa-stop-tdTomato::K14H2BGFP* mice. Note that *Id2*+ descendants were only located at the hair bulb stage matrix and pre-cortex area. (E) Diagram summarizing the descendants of the Wnt-, Shh-, and BMP-signal responsive cells at different early developmental stages. Scale bars: 50 μ m.

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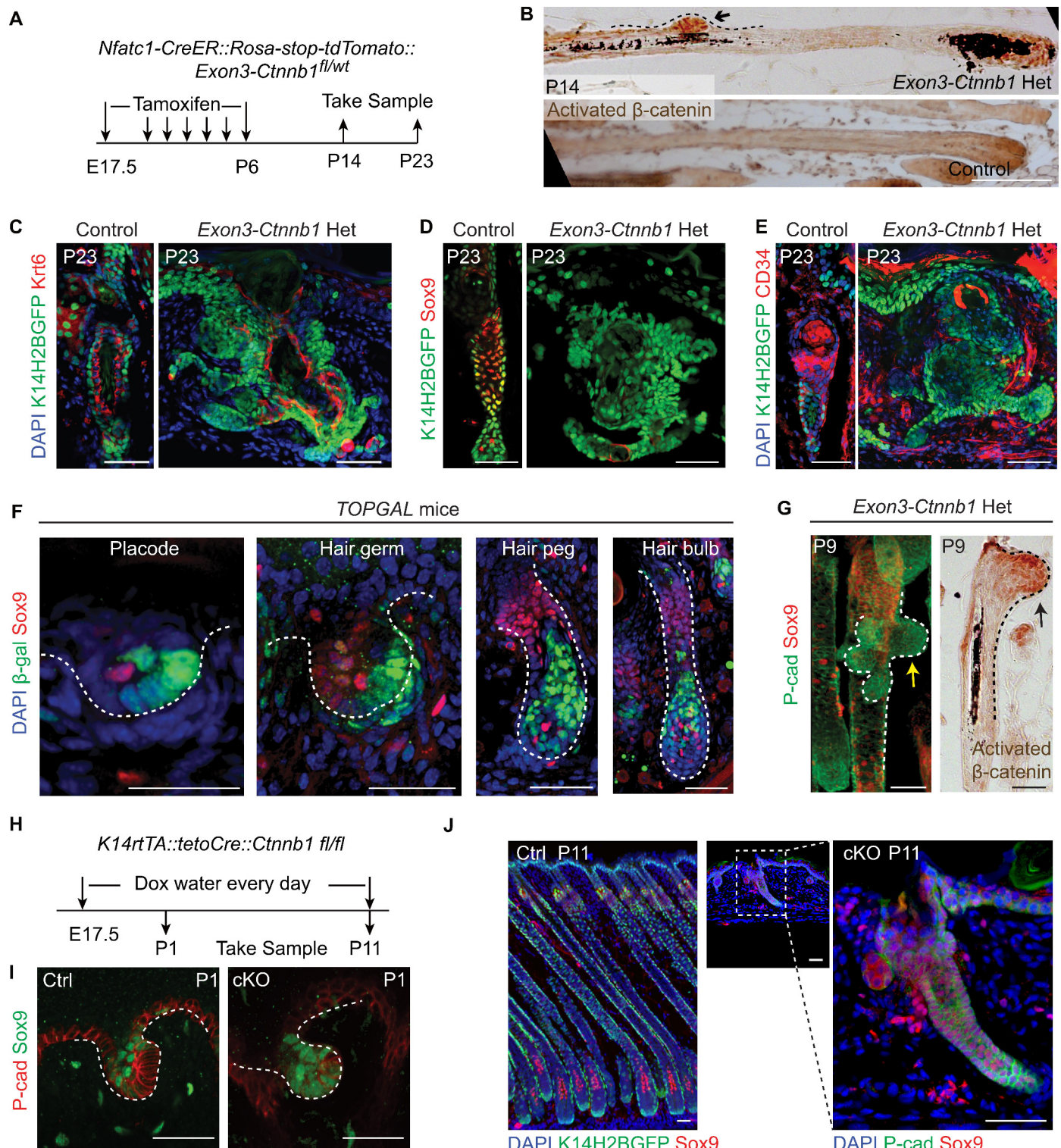


Figure 5. Elevated Wnt/ β -catenin signaling abolishes hair follicle stem cell specification and suppresses Sox9 expression in hair follicles. (A) Diagram of the experiments using *Nfatc1-CreER::Rosa-stop-tdTomato::Exon3-Ctnnb1^{fl/wt}* mice. (B) Nuclear β -catenin staining indicates successful activation of Wnt/ β -catenin signaling in upper hair follicle. (C–E) Abolished bulge niche formation and hair follicle stem cell specification in *exon3-Ctnnb1 Het* HF. Figure 5 continued on next page

Figure 5 continued

compared to WT HF. Krt6 (C) is a marker for inner layer bulge cells serving as a niche that can maintain quiescence for outer layer HFSCs. Sox9 (D) and CD34 (E) are adult hair follicle stem cell markers. (F) Wnt/ β -catenin signal responsive cells, represented by β -gal positive cells in TOPGAL mice, do not express Sox9 during normal development. (G) Activation of Wnt/ β -catenin signaling suppresses Sox9 expression in vivo. The arrows point to protrusions resulting from elevated Wnt/ β -catenin signaling in *exon3-Ctnnb1* Het HF. (H) Diagram of the experiments using *K14-rtTA::teto-Cre::Ctnnb1^{fl/fl}* mice. β -catenin is conditionally deleted in epithelial cells by feeding mice with Doxycyclin from E17.5 to P11. Samples were taken at P1 and P11. (I–J) Loss of Wnt/ β -catenin signaling leads to expanded Sox9 expression in HF at both the hair germ stage (I) and in postnatal skin (J). Scale bars: 50 μ m.

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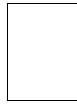


Figure 5—figure supplement 1. Effects of both gain and loss of function studies targeting Wnt/ β -catenin signaling in skin. (A) Hematoxylin and eosin (H&E) staining of exon3-*Ctnnb1* Het and control tail skin sections from P15 to P55. Control hair follicles enter into the first anagen from P15 to P25. Exon3-*Ctnnb1* Het follicles enter into anagen around P45. Afterwards they return to telogen at P55, and new bulge structures reform. Scale bar: 50 μ m. (B) Tail skin hair coats of control and exon3-*Ctnnb1* Het mice at different time points. Notice in Het mice the disappearance of the hair coat at P30 and its reappearance at P100. Scale bar: 1 mm. (C) De novo formation of a normal niche in exon3-*Ctnnb1* Het mice at P100. Note the recovered expression of the stem cell markers CD34 and Sox9; scale bar: 50 μ m. (D) Spontaneous disappearance of exon3-*Ctnnb1* Het-expressing cells shown by genotyping of isolated cells at different time points. (E) Conditional ablation of epithelium β -catenin leads to hair follicle development defects. β -catenin can be detected in the control mice but not in the epithelia of knockout mice. Scale bar: 50 μ m. (F) Hair coat differences between WT and β -catenin cKO mice at P11.

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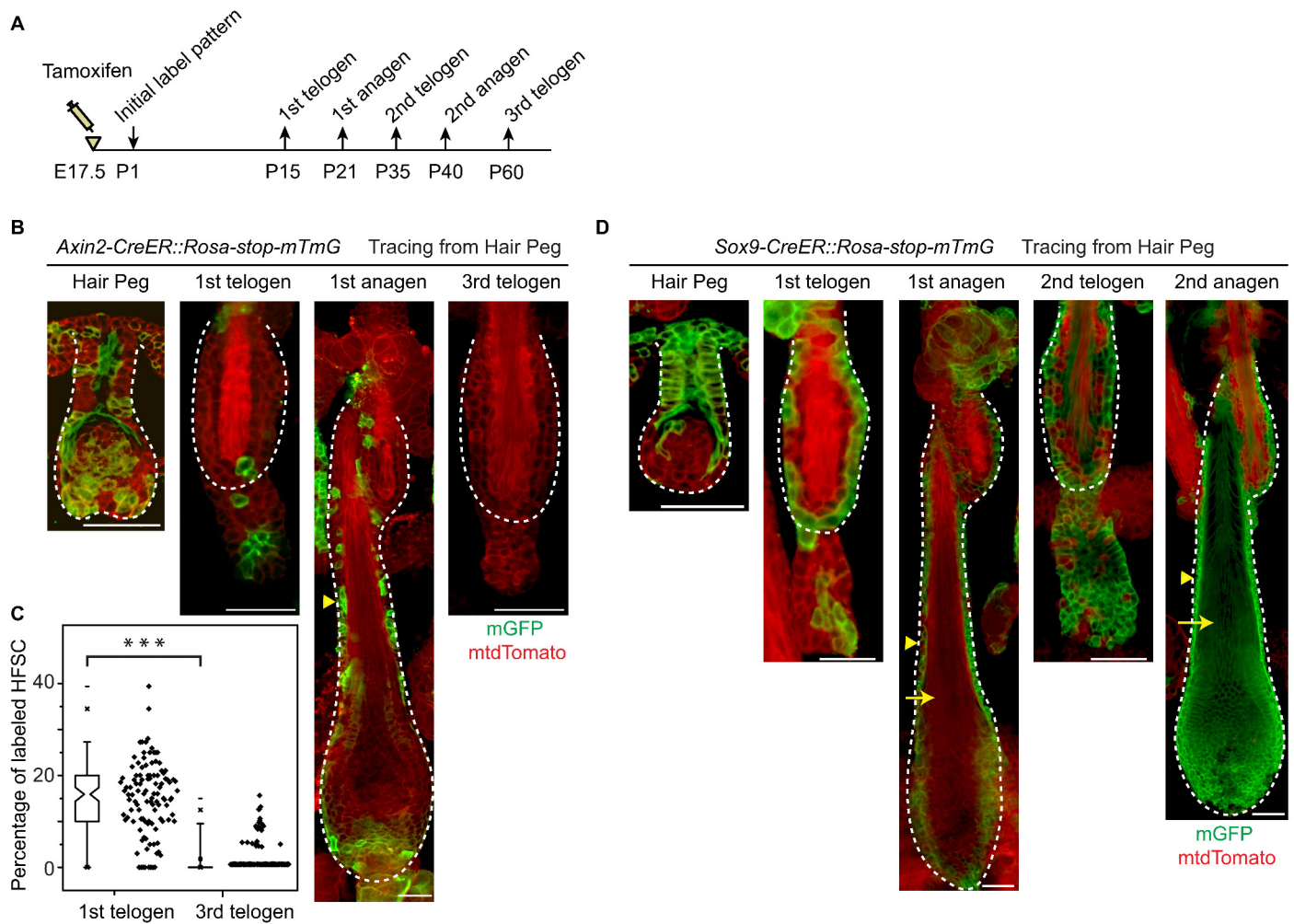


Figure 6. Embryonic Wnt/ β -catenin signaling diminishes the long-term self-renewal ability of hair follicle stem cells in adult. **(A)** Diagram depicting the time points of the lineage tracing experiment. **(B–C)** Long-term lineage tracing experiments using *Axin2-CreER::Rosa-stop-mTmG* mice. Note the disappearance of labelled HFSCs in the third telogen. Representative images shown are single confocal Z slice from the data used for quantification. N=3 mice, >110 HF. *** $p < 0.0001$. **(D)** Long-term lineage tracing experiments with *Sox9-CreER::Rosa-stop-mTmG* mice. Arrowheads indicate ORS and arrows indicate terminally differentiated layers. Note the change in downstream progeny fates of labeled cells at different hair cycles. Scale bars: 50 μ m. DOI: <http://dx.doi.org/10.7554/eLife.10567.014>

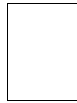


Figure 6—figure supplement 1. Long term cell fate of Lgr5-CreER labelled hair peg cells. (A) Diagram depicting the time points of the lineage tracing experiment. (B–C) Long-term lineage tracing experiments using *Lgr5-CreER::Rosa-stop-tdTomato* mice. Note the persistence of a small percentage of labelled HFSCs up to the ~6th telogen. Representative images shown are single confocal Z slice from the data used for quantification. N=3 mice, >100 HF. Scale bars: 50 μ m.

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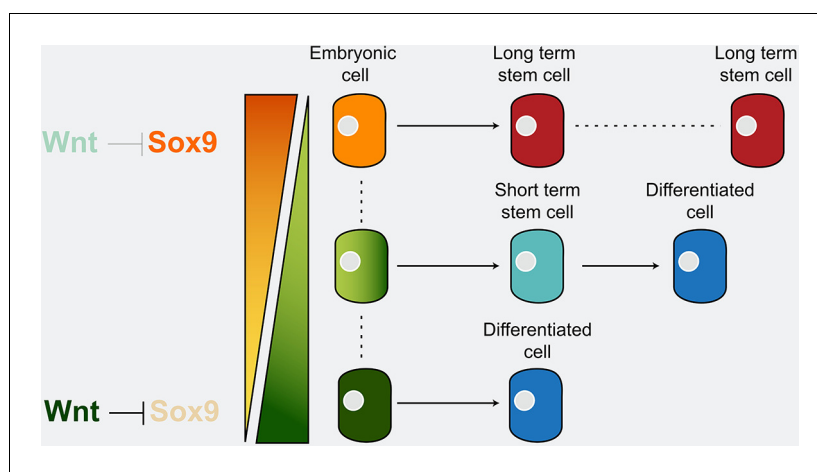


Figure 7. Model illustrating that long-term hair follicle stem cell emergence results from progenitors occupying an embryonic niche location, which is defined by the absence of Wnt/ β -catenin signaling that would otherwise block the expression of a key factor required for stem cell specification.

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