

Fig. S1. Targeting strategy and *Hopx* expression. (A) Targeting strategy of *Hopx*^{3FlagGFP/+} allele. Yellow represents 70 bp of intron spanning exon 2 and 3 included in targeting cassette. Red represents 3XFlag epitope and green represents GFP sequence. (B,C) *Hopx* expression (GFP) in small intestine (B) and E10.5 heart (C, merged with brightfield). (D,E) Expression of *Hopx* by in situ hybridization (D) and GFP expression (E) on adjacent slides of E11.5 embryos. (F) Telogen follicle co-stained with GFP and/or *Lrig1* from P21 *Hopx*^{3FlagGFP/+} mice. (White arrowhead indicates double-positive cell.) (G) *Hopx*^{ERCre/+}; *R26*^{mt-mG/+} mice were pulsed in telogen (P50-54) and then sacrificed 1 day later. GFP staining is specific to the basal bulge layer (white arrowheads). Scale bars: 25 μ m in B,F,G; 1 mm in C-E.

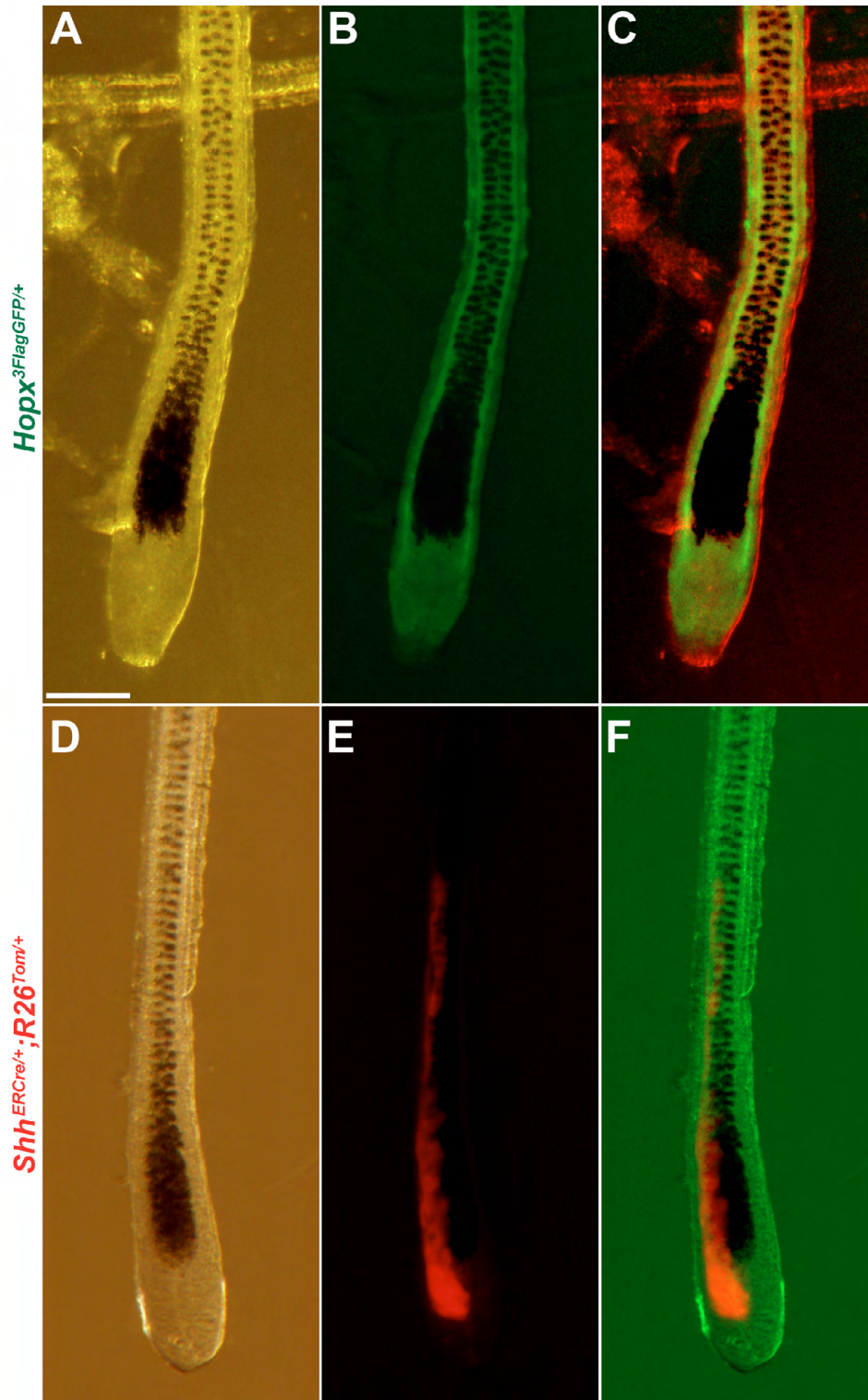


Fig. S2. Expression of *Hopx* and *Shh* at late anagen. (A-C) Whole-mount images of *Hopx*^{3FlagGFP/+} skin at P14, late anagen. Bright-field image (A) and GFP channel (B) were merged (C). (D-F) Whole-mount images of *Shh*^{ERCre/+}; *R26*^{Tom/+} skin 1 day after labeling at late anagen, P13. Bright-field image (D) and tdTomato channel (E) were merged (F). Scale bar: 50 μm.

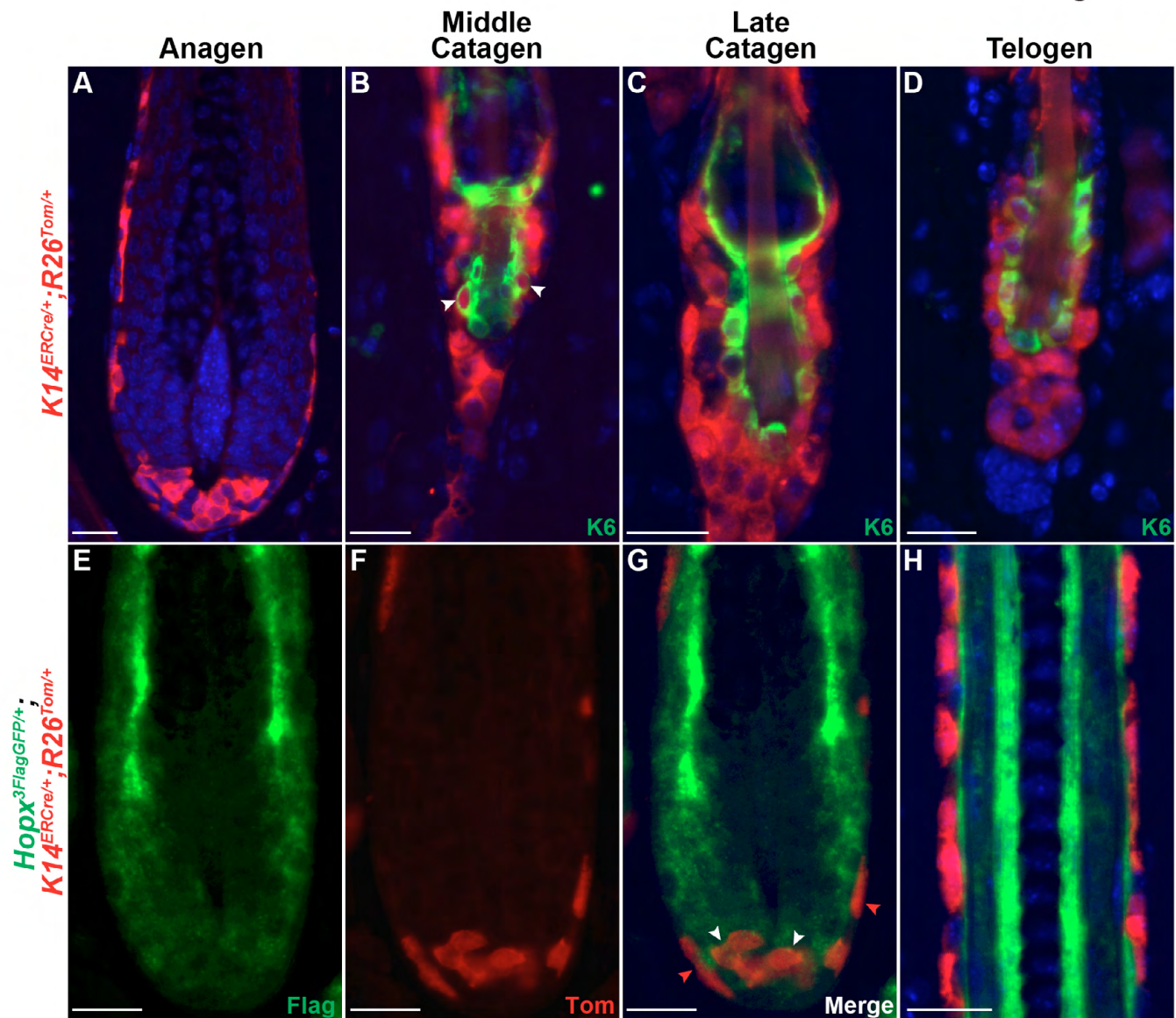


Fig. S3. Lineage tracing of *K14*⁺ cells. (A-D) *K14*^{ERCre/+}; *R26*^{Tom/+} were pulsed with tamoxifen once at P13 and sacrificed at anagen (P14, A), middle catagen (P17, B), late catagen (P19, C) and the ensuing telogen (P21, D). (A) Cells were labeled in the lower hair bulb and ORS in late anagen. (B) In middle catagen, *K14*-derived cells were found in the outermost K6⁺ layer surrounding the club hair (white arrowheads), in addition to the ORS and epithelial strand. (C,D) In late catagen and telogen, K6⁺ innermost cells were derived from *K14*-expressing precursors. (E-H) *Hopx*^{3FlagGFP/+}; *K14*^{ERCre/+}; *R26*^{Tom/+} were pulsed with tamoxifen once at P13 and sacrificed at P14. (E-G) TdTomato⁺ cells were found in the lower hair bulb (white arrowheads) as well as the ORS (red arrowheads). White arrowheads demonstrate examples of Flag⁺, tdTomato⁺ cells in the lower hair bulb. (H) There are no double-positive cells in the middle hair follicle. Scale bars: 20 μ m.

Figure S4

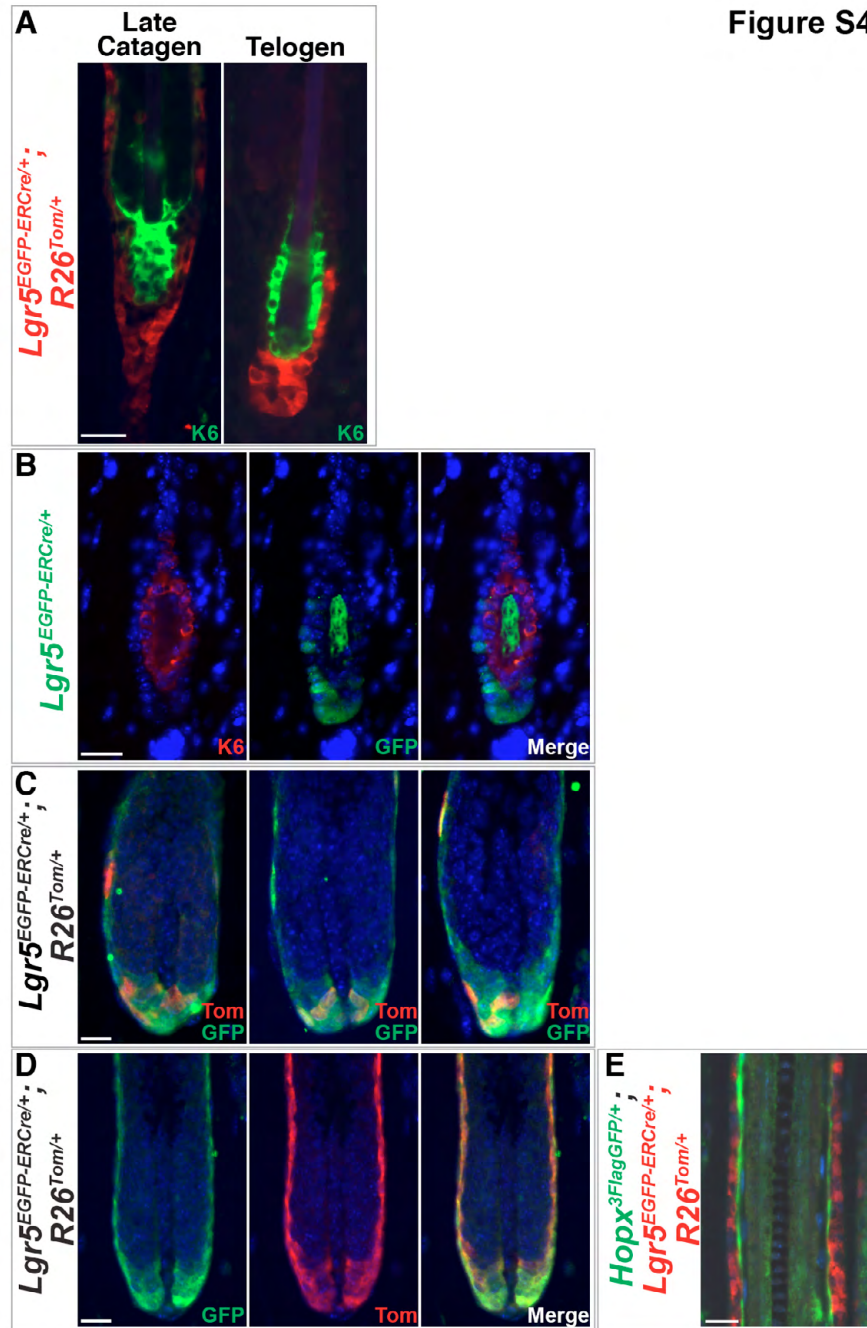


Fig. S4. Lineage tracing of *Lgr5*⁺ cells. (A) Dual staining of *Lgr5*^{EGFP-ERCre/+};*R26*^{Tom/+} skin for tdTomato and K6, 1 day (left) and 3 days (right) after labeling at late catagen, P18. (B) In telogen, P21, *Lgr5* is not expressed in K6⁺ cells. (C,D) *Lgr5*^{EGFP-ERCre/+};*R26*^{Tom/+} were pulsed with tamoxifen once at P13 and sacrificed at P14 (C) and P15 (D). TdTomato-positive cells (red) express *Lgr5* (GFP) in the ORS and the lower bulb. (E) *Hox3*^{FlagGFP/+};*Lgr5*^{EGFP-ERCre/+};*R26*^{Tom/+} were pulsed with one dose of pulse of tamoxifen at P14, sacrificed at P15. There are no double-positive cells in the middle hair follicle. Scale bars: 20 μm.

Figure S5

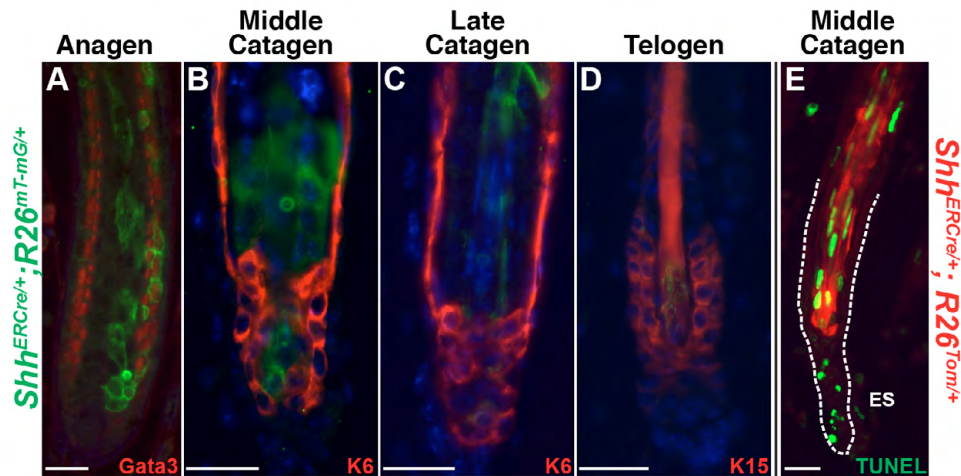


Fig. S5. Lineage tracing of *Shh*⁺ cells. (A-D) GFP staining of *Shh*^{ERCre/+};*R26*^{mT-mG/+} skin at indicated stages after a single tamoxifen treatment pulse at P14, with cell-specific markers as indicated. (D) *Shh*-derivatives are not present except in hair shafts at the following telogen, P21. (E) Dual staining of *Shh*^{ERCre/+};*R26*^{Tom/+} for tdTomato and TUNEL 3 days after labeling at P14, demonstrating that *Shh*-derivatives undergo apoptosis during middle catagen. HF is outlined by a broken white line. ES, epithelial strand. Scale bars: 20 μ m.

Figure S6

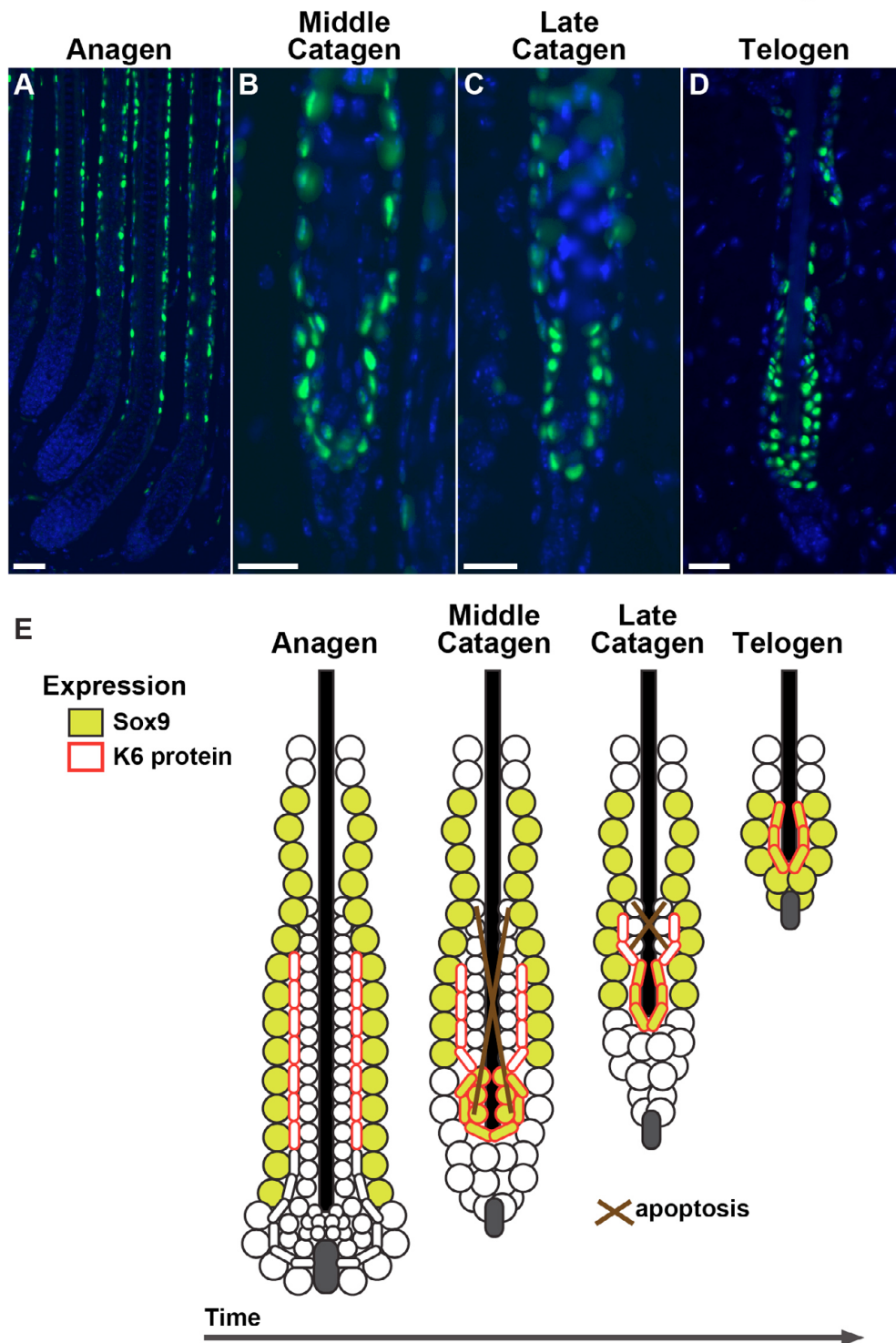


Fig. S6. Sox9⁺ cells surround the club hair. (A-D) Sox9 staining of indicated stages of hair follicle morphogenesis. (A) Sox9 is expressed in the upper and middle ORS, but not in the lower hair bulb of anagen-phase follicles at P14. (B-D) Sox9-expressing cells surround the club hair from catagen (P17-P19) to telogen (P21). (E) Model depicting Sox9 expression. In contrast to expression patterns of *Hopx* and *Lgr5*, Sox9 expression commences around the club hair with K6 protein expression during middle catagen, and remains expressed into telogen as the K6⁺ layer fully matures. Scale bars: 40 μm in A; 20 μm in B-D.