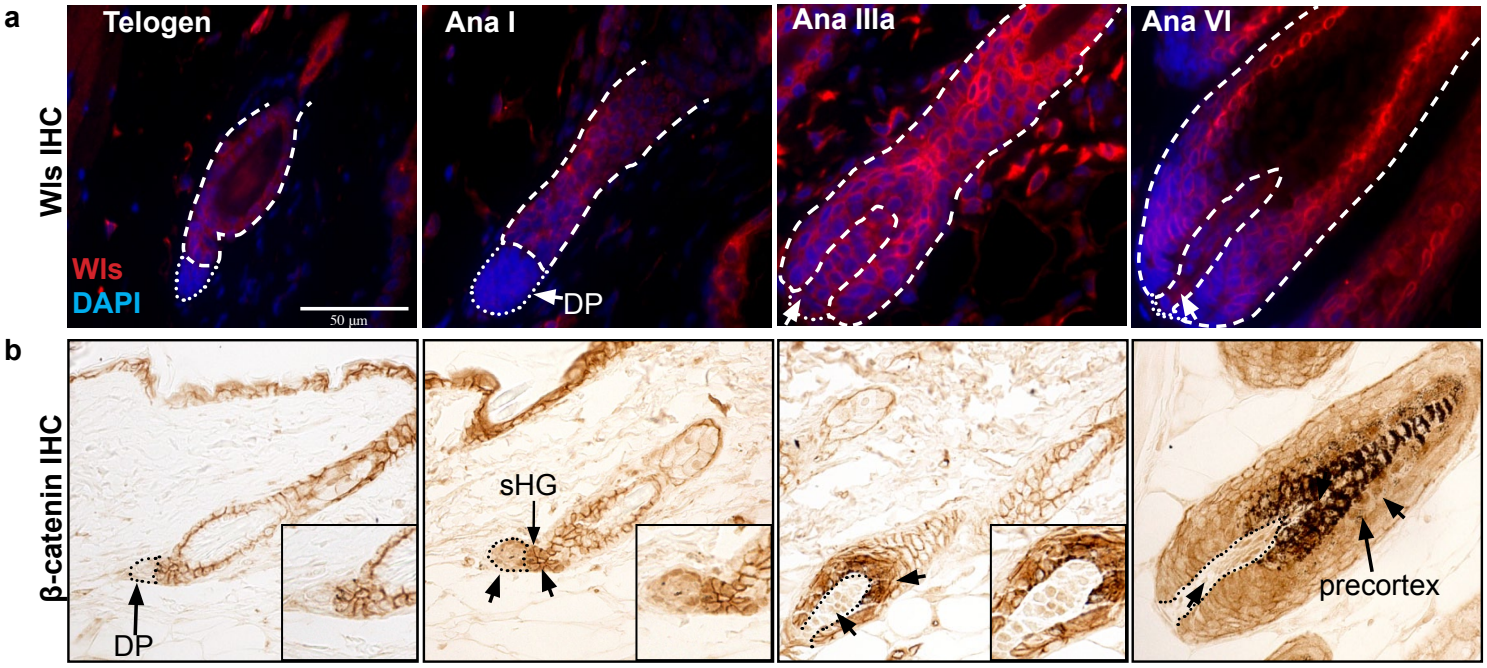


**Figure S1. Wls and nuclear β-catenin expression during spontaneous anagen correlates with depilation-induced anagen.** (a) Wls is upregulated in the follicular epithelium and DP during spontaneous anagen (bar = 50 um). Dorsal skin was analyzed at ages P21-P30 to obtain various stages of spontaneous anagen (b) Brightfield immunohistochemistry of β-catenin at various stages of spontaneous anagen. Short arrows indicate nuclear β-catenin-positive cells.

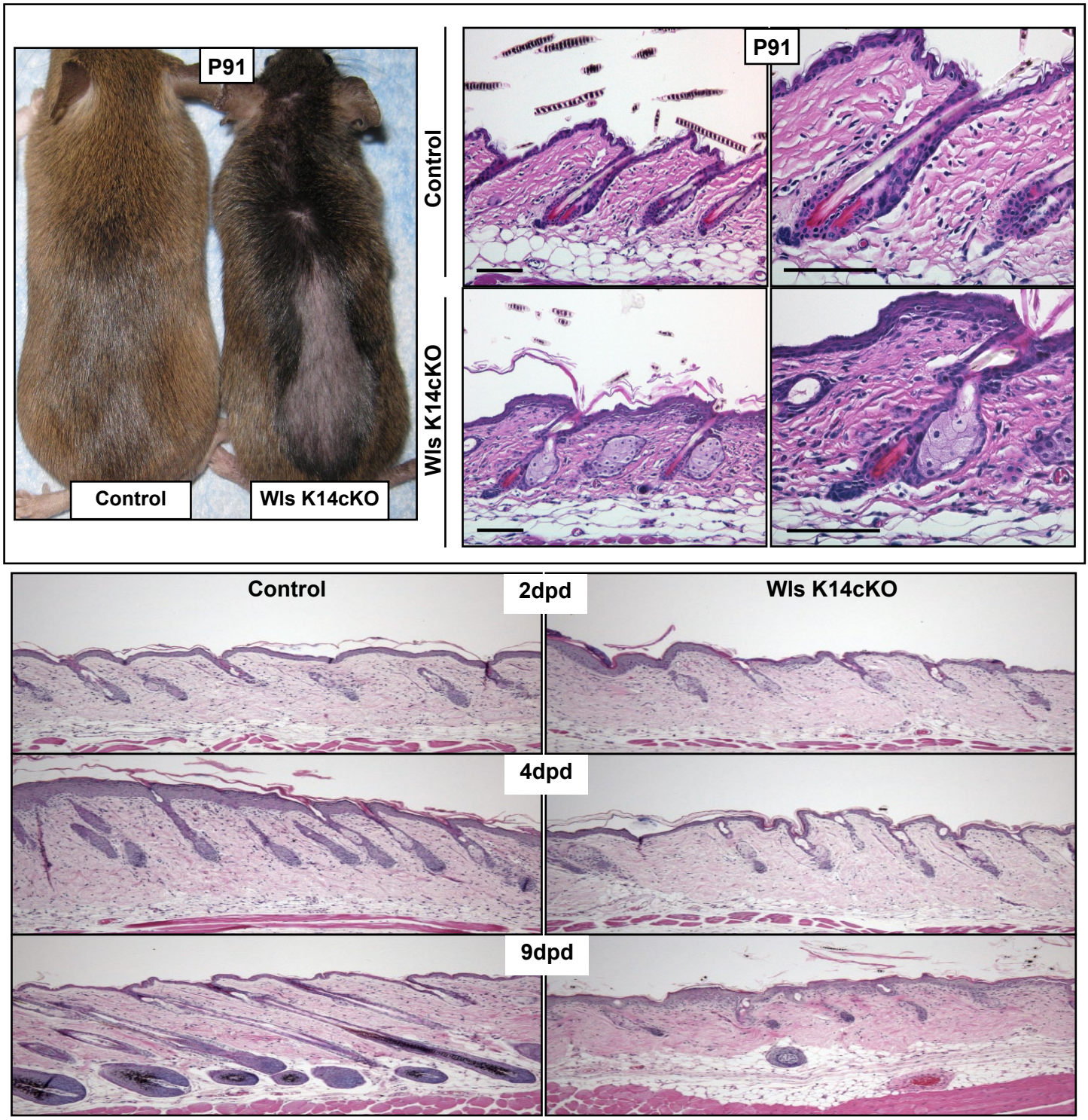


**Figure S2. Hair follicles of Wls K14cKO mice show a hair cycle arrest long-term and after plucking.** (a) Gross pictures of P91 mice induced with tam at P20 for 8 days. Posterior back skins were clipped at P20. H&E sections of P91 mice reveal that control hair follicles are in second telogen, whereas Wls K14cKO follicles remain in first telogen. The interfollicular epidermis of mutant mice remains thickened similar to younger mutant mice (bar=100μm). (b)H&E sections of control (left) and Wls K14cKO (right) depilated areas harvested either 2 days (top), 4 days (middle) or 9 days (bottom) following plucking during second telogen. Control plucked skin shows a gradual progression in anagen, whereas mutant hair follicles are arrested in telogen or early anagen phases with the exception of sporadic follicles that are able
to progress further in anagen.

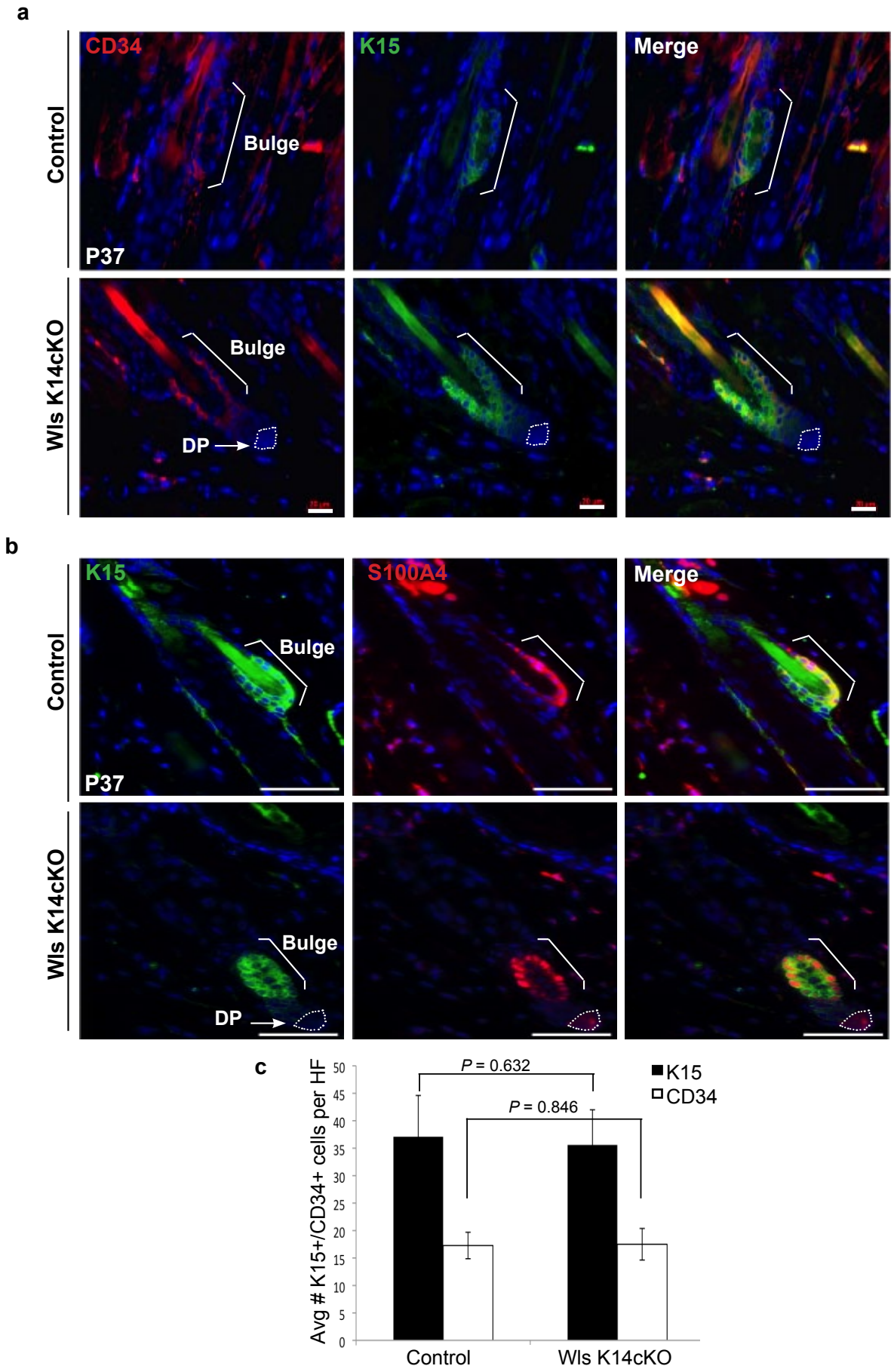


**Figure S3. HFSC markers are maintained in Wls-deficient hair follicles.** (a) Double immunofluorescent detection of HFSC markers, CD34 and K15 and (b) S100A4 and K15 are expressed similarly in both control and Wls K14cKO hair follicles at P37. S100A4 is expressed by both bulge cells and DP cells in telogen follicles. Bulge regions of control anagen follicles and Wls K14cKO telogen follicles are indicated. 40x (a) Bar=20 um, (b) Bar=50 um. (c) Average number of K15+ (black) and CD34+ cells counted per hair follicle (HF) section from 11 controls, 10 Wls K14cKO HFs (P91).

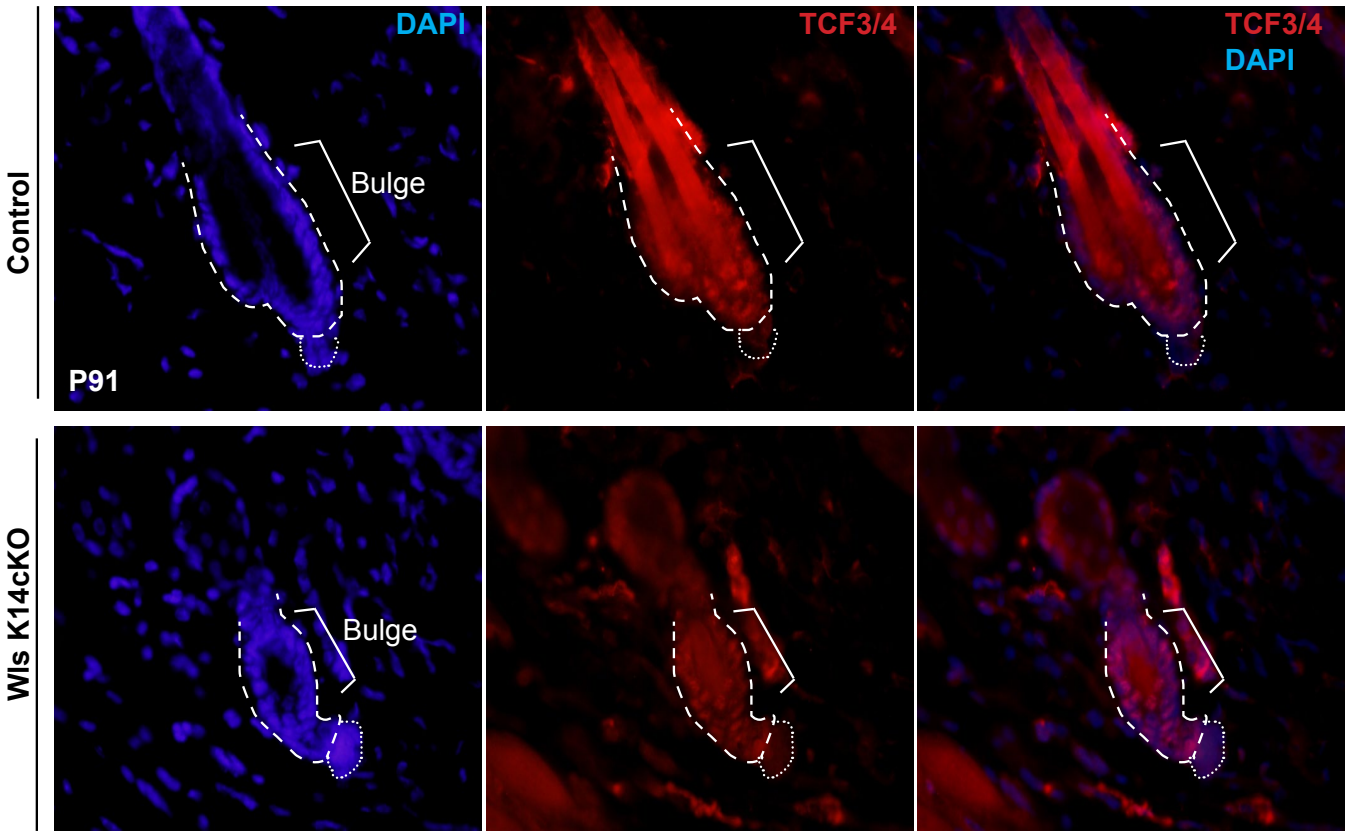


**Figure S4. HFSC marker TCF3/4 is maintained in the absence of Wls expression.** Immunohistochemical detection of TCF3/4 in the bulge and sHG regions of telogen hair follicles of control (top) and Wls K14cKO (bottom) mice at P91. Bulge region is indicated by brackets (controls and mutant photographs were taken at the same magnification).

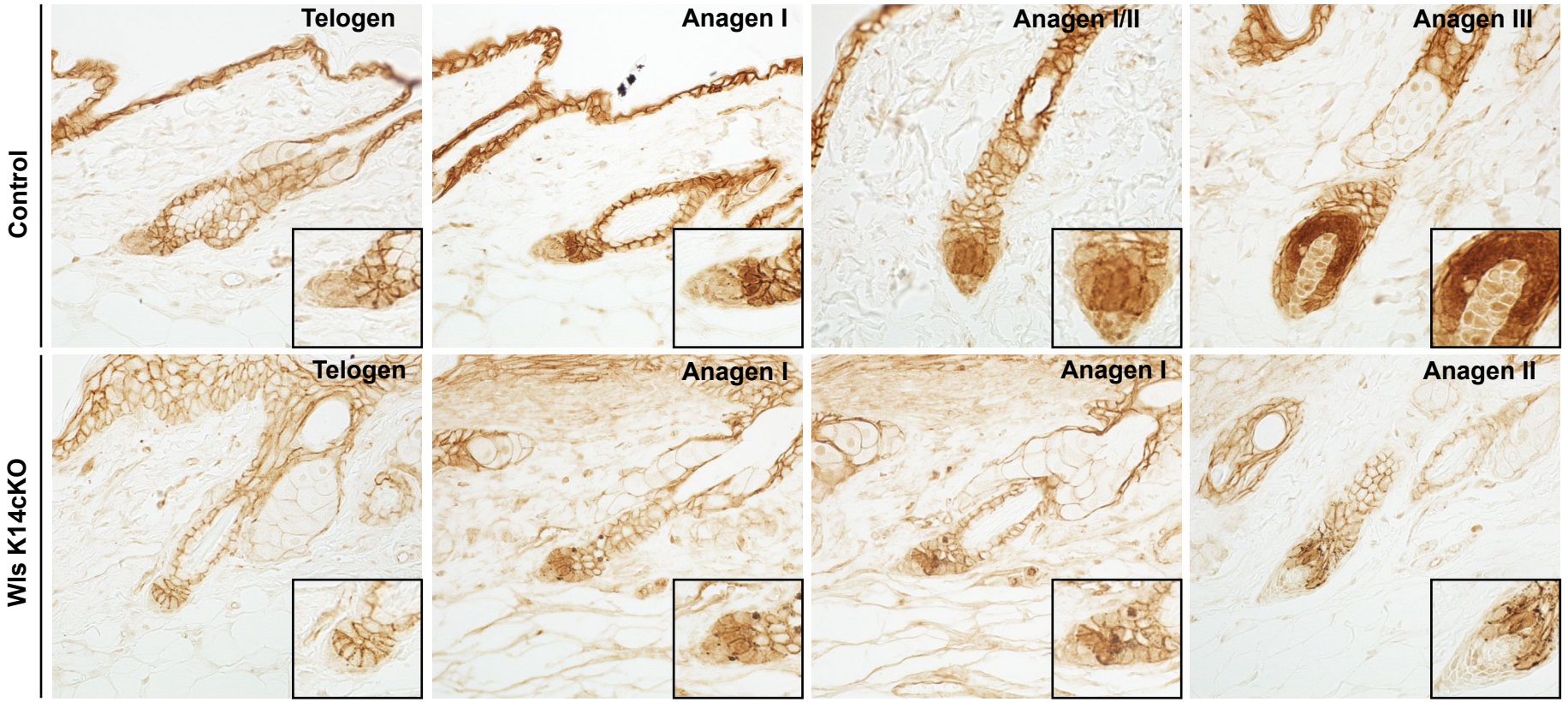


**Figure S5. Depletion of epidermal Wnt ligands leads to decreased Wnt/β-catenin signaling in the sHG and DP of arrested mutant hair follicles.** Brightfield immunohistochemical detection of nuclear β-catenin in telogen and early anagen control hair follicles (top panels). Note nuclear β-catenin can be detected in the sHG during anagen onset and then in both the sHG and DP of early anagen follicles. By anagen III, nuclear β-catenin is most prominent in cells adjacent to the DP that correspond to the precortex and appears more heterogenously within DP cells. In Wls K14cKO hair follicles (bottom panels), variable nuclear β-catenin staining was observed in which those that appeared to be in telogen by morphology showed a lack of nuclear β-catenin in the sHG and DP, similar to wildtype telogen follicles. In those that appear to have progressed to anagen I-II, some nuclear β-catenin can be seen in some sHG cells, but appears diminished and sometimes undetectable in associated DP cells when compared to stage-matched controls. All control and mutant photographs were taken at the same magnification. Insets show higher magnification of the sHG/DP regions of hair follicles.

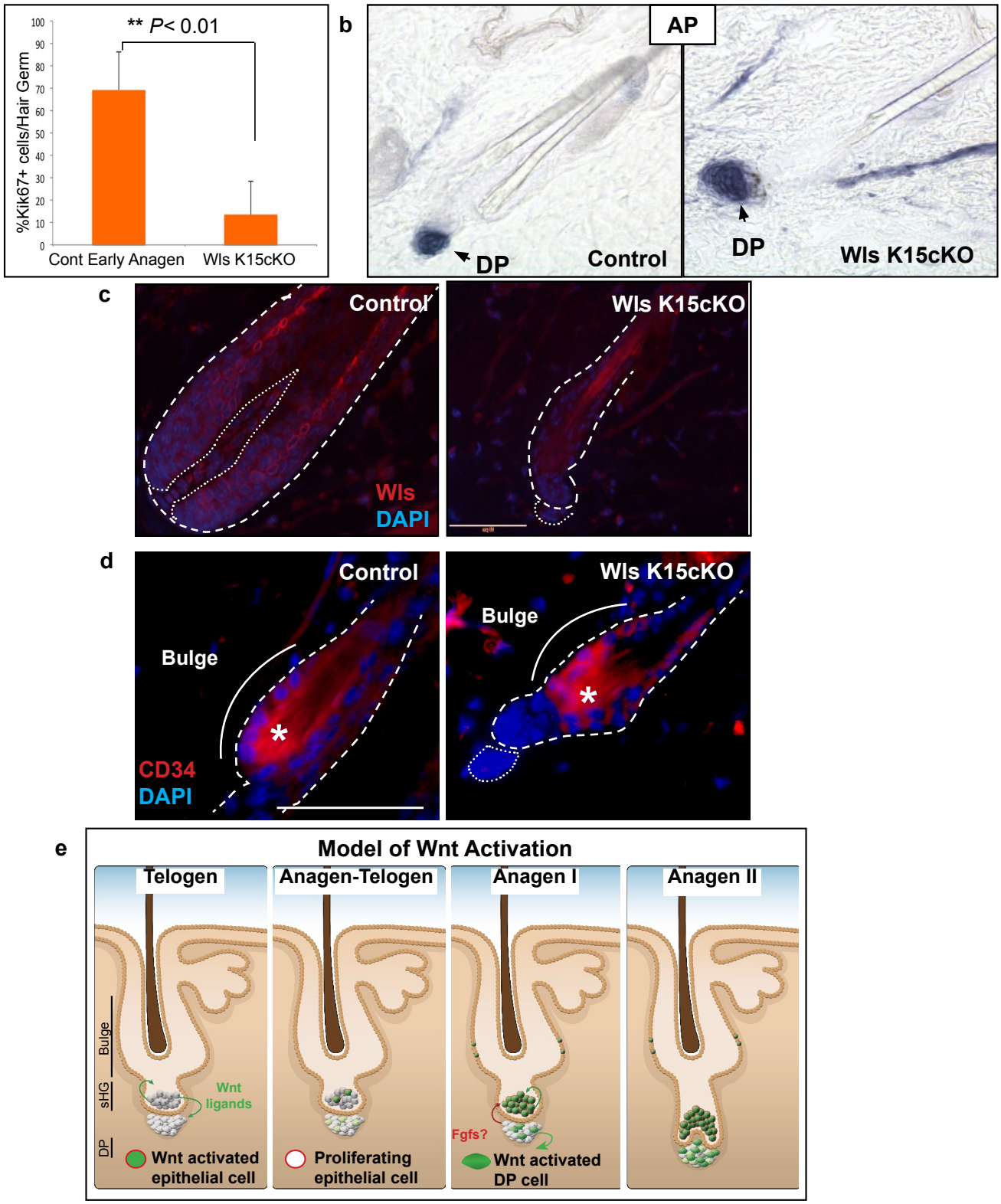


**Figure S6. Wnt ligand secretion by HFSCs is required for anagen.** (a) P37 mice from Wls K15cKO mice induced with topical RU486 for 6 days from P20-P25. Dorsal skin was harvested at P37 and sections were stained for Ki67. The proportion of Ki67+ cells per hair germ were significantly reduced compared to early anagen control hair follicles but more than P65 control telogen hair follicles (3.7% +/- SD6.41 Ki67+ in control telogen sHGs vs 13.5% +/- SD14.97 in Wls K15cKO; ** = stastically significant difference by Student t-test. (b) The DP marker, AP is maintained in Wls K15cKO hair follicles similar to control telogen follicles. (c) Wls immunohistochemistry of control and mutant hair follicles at P40. (d) CD34 staining of bulge cells in control and mutant hair follicles at P40 (* indicates autofluorescence; bar=50μm) (e) Model illustration of Wnt activation during anagen onset. Telogen hair follicles lack Wnt activated cells and are not proliferating (white cells). Wnt ligands secreted by the sHG activate sHG cells and possibly adjacent DP cells (green arrows). During anagen onset, sHG cells activate Wnt signaling (green) in response to Wnt ligands and begin to proliferate (red rim), while DP cells also begin to respond to Wnt ligands (light green). As anagen progresses, Wnt activated DP cells secrete other factors such as Fgf7/10 (red arrow) that support the continued growth and differentiation of epithelial cells. Wnt activated sHG cells continue to secrete Wnt ligands (green arrows).

## Supplementary Materials and Methods:

### Generation and induction of mouse lines

Recombination was induced by delivering 1 mg tamoxifen dissolved in corn oil (Sigma-Aldrich, St. Louis, MO) intraperitoneally daily for 7-8 days to *K14Cre-ER* transgenic mice and 1% Mifepristone (Sigma-Aldrich) in ethanol was applied topically to clipped dorsal skin of *K15Cre-PR1* mice daily for 6 days starting at P20. For each experiment, at least three to five mutants with littermate controls from one to three litters were analyzed. All animal procedures were done under the approval of Case Western Reserve University IACUC committee.

### Quantitative PCR, In situ hybridization, immunohistochemistry, proliferation assays, and statistical analysis

We followed a previously described method to prepare epidermal sheets from dorsal skin (Morris *et al.*, 2004; Rabbani *et al.*, 2011). To prepare RNA from epidermal preparations, the RNeasy Micro kit (Qiagen, Hilden, Germany) was used. cDNA from total RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription kit (ABI, Carlsbad, CA) as instructed. For quantitative PCR, TaqMan primers were used according to manufacturer's instructions (ABI, Mm00509695_m1). Relative mRNA quantities were calculated based on the $\Delta\Delta$Ct method. Mid-dorsal skins were harvested, fixed in 4% paraformaldehyde and processed for paraffin embedding or dehydrated with sucrose and embedded in OCT for cryosectioning. To show skin morphology, paraffin sections were stained with hematoxylin and eosin using standard protocols. Hair cycle stage was determined morphologically, using published guidelines (Muller-Rover *et al.*, 2001). mRNA detection by *in situ* hybridization on cryosections for *Axin2* (Addgene, Cambridge, MA) was performed as previously described (Tran *et al.*, 2010). For BrdU analysis, mice were injected intraperitoneally with BrdU (50 mg/gm body weight; Roche, Basel, Switzerland). Two or six hours later, mid-dorsal skins were harvested and

cryosections were stained for BrdU. For immunohistochemistry, sections were incubated at 4 deg overnight with primary antibodies against the following: β-catenin (BD Biosciences, San Jose, CA, 1:100), Wls (courtesy of Richard Lang, 1:1000), Filaggrin (Covance, Princeton, NJ, 1:500), Keratin10 (Covance, 1:1000), LEF-1 (Cell Signaling, Danvers, MA, 1:100), CD34 (BD Biosciences,1:50), Keratin15 (NeoMarkers, Fremont, CA, 1:100), BrdU (Roche, 1:17), Ki67 (Abcam, Cambridge, UK, 1:200), TCF3/4 (Abcam, 1:50), S100a4 (NeoMarkers, 1:100). For CD34 detection, biotinylated goat anti-rat IgG secondary antibody (Vector Labs, Burlingame, CA, 1:100) followed by TRITC-conjugated streptavidin (Vector Labs, 1:200) was used. For the remaining immunofluorescence, species-specific secondary antibodies conjugated to either Alexa488 or 594 were used (Invitrogen, Carlsbad, CA, 1:200) with DAPI nuclear counterstain (Vector Labs). For brightfield immunohistochemistry, biotinylated species-specific secondary antibodies (1:200, Jackson Labs) followed by detection using the ABC kit (Vector Labs) and DAB (Amresco, Solon, OH) were used according to manufacturer instructions. Proliferation indices were calculated by counting the percentage of Ki67+ cells within the sHG; 60 control and mutant hair follicles were analyzed at 40X magnification. Statistical significance for proliferation and WIHN follicle counts was calculated using Student's *t*-test. Statistical significance of results obtained from hair follicle stage analyses was evaluated by the Mann-Whitney test. Quantification of HFSC number was performed by counting either K15+ or CD34+ cells per hair follicle (P91) from 5 um paraffin sections. Counts were taken from hair follicles in which the club hair, sHG and DP could be all visualized at 40x. For detection of AP, cryosections were fixed in acetone for 10 min and then incubated with the 0.02% NBT/BCIP (Roche) substrate. Fluorescent images were captured with Olympus BX60 microscope and DP72 camera (Olympus, Hamburg, Germany). Brightfield images were obtained with Leica DM2000 microscope and DFC490 camera (Leica, Wetzlar, Germany). Whole-mount images were obtained with Leica MZ16F dissecting microscope. Control images were all taken at the

same magnification as mutant images for each figure. Images were processed in Adobe Photoshop and InDesign software. All schematics were created with Adobe Illustrator.

**Depilation and WIHN**

For depilation analyses, tamoxifen was administered for 8 days and then club hairs were depilated at various times as indicated to induce anagen using previously described protocols (Ito *et al.*, 2002). 1-$cm^2$ full-thickness skin wounds were made as previously described (Ito *et al.*, 2007). Tamoxifen was administered daily as described and de novo hair follicles were identified by either whole-mount AP staining of dermis preparations or whole-mount anti-K17 epidermal staining (Abcam, 1:1000) as previously described (Ito *et al.*, 2007).

**Supplemental References**

Ito M, Kizawa K, Toyoda M, Morohashi M (2002) Label-retaining cells in the bulge region are directed to cell death after plucking, followed by healing from the surviving hair germ. *J Invest Dermatol* 119:1310-1316.

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