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Supplemental Data

SKPs Derive from Hair Follicle Precursors and Exhibit Properties of Adult Dermal Stem Cells

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Supplemental Experimental Procedures

Antibodies. Primary antibodies used were those raised against versican (1:250; a gift from R. LeBaron), PDGFRα (1:500, Santa Cruz), PDGFRβ (1:200, eBioscience), tyrosinase (1:500, Santa Cruz), mouse fibroblast antigen pan reticular (1:500, Serotec), cd34 (1:200, Serotec), keratin-15 (1:500, Covance), keratin-5 (1:500, Covance) α-smooth muscle actin (1:500, Sigma), fibronectin (1:200, Sigma), S100β (1:500, Sigma), Pax3 (1:400, Developmental Studies Hybridoma Bank), MBP (1:100, Serotec), Ki67 (1:200, BD Biosciences Pharmingen), nestin (1:500, BD Biosciences Pharmingen), P_0 (1:1000, Aves Labs), p75NTR (1:500, Promega), βIII-tubulin (1:500, Covance), e-cadherin (1:500, Santa Cruz), keratin 5 (1:500, Covance), hair keratin 15 (1:500, Covance), keratin 17 (1:1000, gift from P. Coulombe), Sox2 (1:500), collagen type I (1:400), neural cell adhesion molecule (ncam,; 1:200), vimentin (1:500), chicken green fluorescent protein (1:1000, all from Chemicon/Millipore) were used as previously described (Fernandes et al., 2004; McKenzie et al., 2006). Secondary antibodies used were Alexa488-conjugated goat anti-mouse, -rabbit, or -chicken, Alexa555 goat anti-mouse, rabbit or chicken and Alexa647 goat anti-rabbit, -mouse or -rat (1:1000; all from Invitrogen).

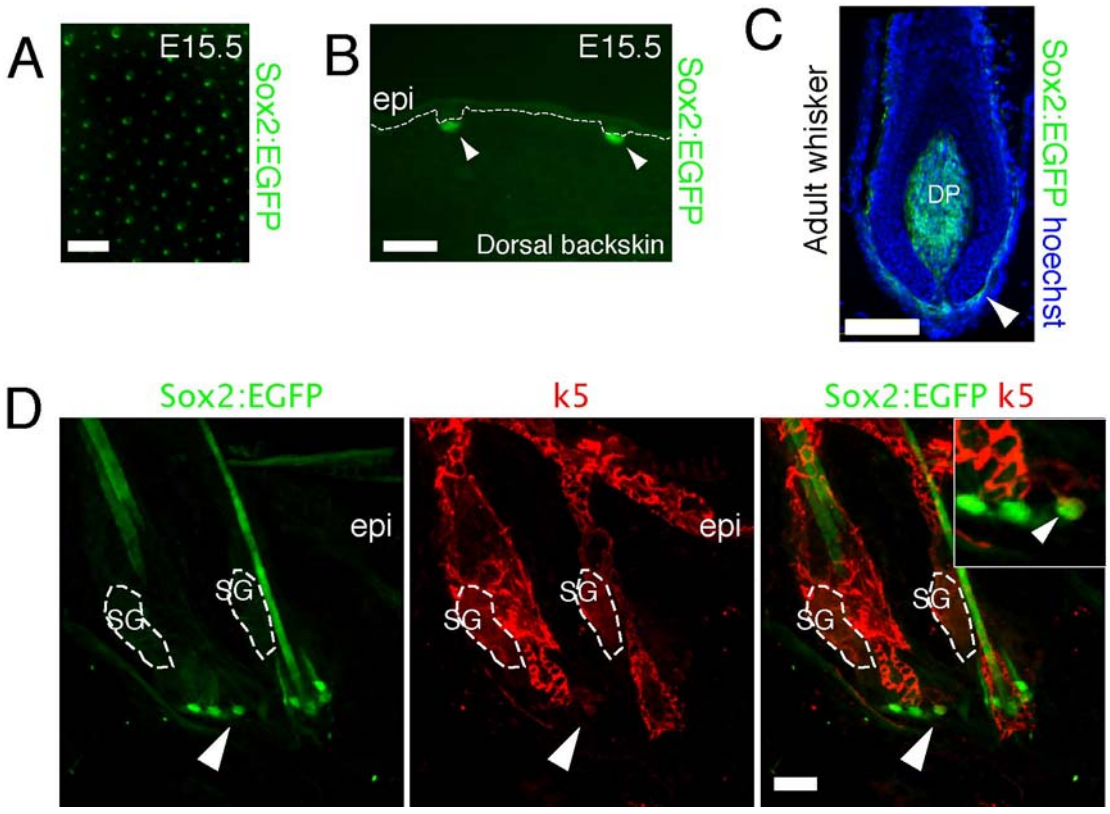


Figure S1. ***Sox2 expression in hair follicles.*** (A,B) Flat-mount (A) and transverse (B) sections of dorsal back skin from embryonic day 15.5 (E15.5) Sox2:EGFP embryos demonstrating exclusive expression of Sox2:EGFP (green) within newly-forming dermal condensates. (C) Adult whisker follicle immunostained for EGFP (green) showing EGFP+ cells in the DP and DS. (D) Confocal image showing a telogen hair follicle from adult Sox2:EGFP backskin immunostained for GFP (green) and the epidermal marker keratin 5 (red). A few Sox2:EGFP+ cells are observed decorating the regions beneath the sebaceous gland (hatched lines, SG) and some of these stain positively for K5 (red, inset). Nuclei are stained with Hoechst 33258 (blue). Scale bars = 300µm in A, 200µm in B, 100µm in C, 25µm in D.

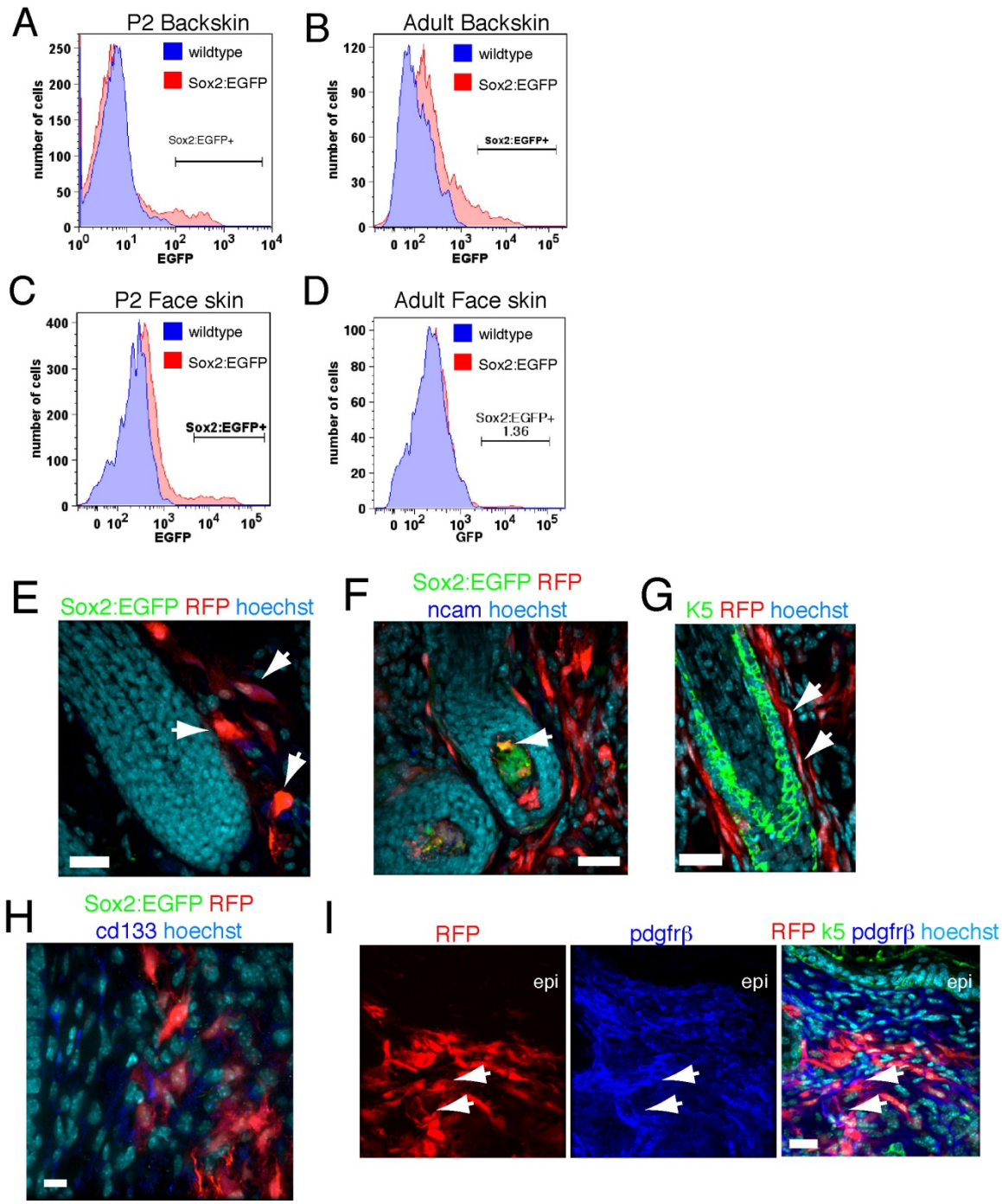
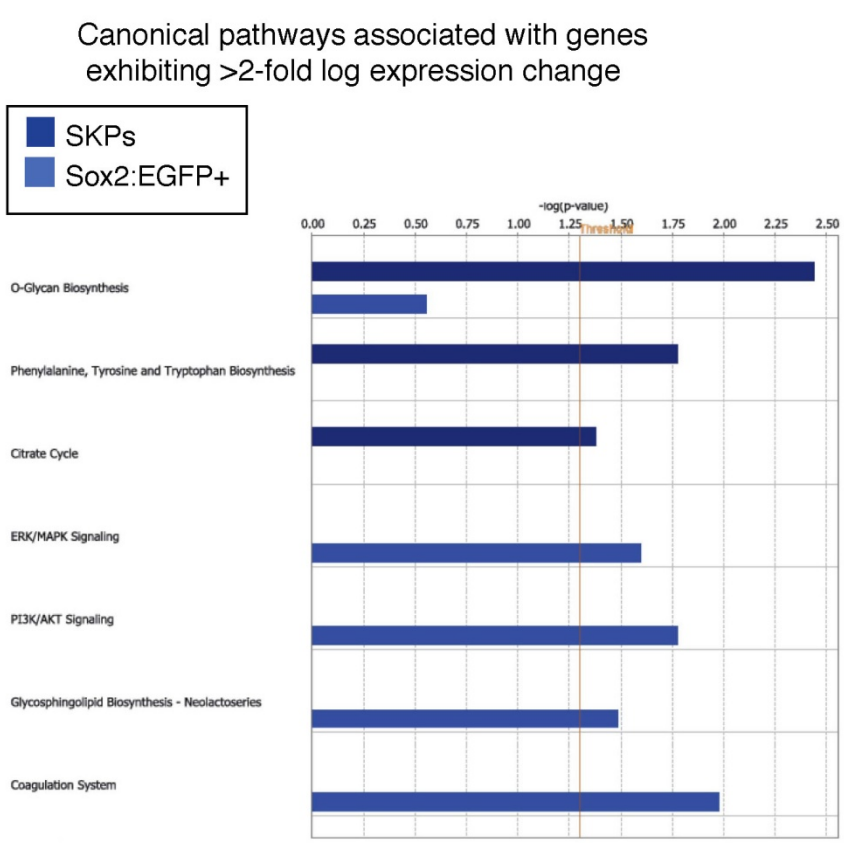


Figure S2. (A-D) ***Prospective isolation of Sox2:EGFP+ skin cells.*** FACS profiles of neonatal (A,C) and adult (B,D) Sox2:EGFP dorsal back skin (A,B) and facial skin (C,D), sorted for EGFP. As controls, EGFP- back skin or facial skin (wt) were also sorted. Bars denote the positive and negative populations that were analyzed. **(E-I)** ***Sox2:EGFP+, but not Sox2:EGFP- cells home to the follicle DP.*** Equal numbers of prospectively-isolated Sox2:EGFP- or Sox2:EGFP+, PDGFRα+, cd34- cells were transplanted into back skin of adult NOD/SCID mice and analyzed 2 weeks later. All cells were transduced with an RFP-expressing retrovirus so that they could be followed. (E) Longitudinal section

through a hair follicle showing that while RFP+ (red, arrows), Sox2:EGFP- transplanted cells were close to hair follicles, they never integrated into the DP. (F,G) In contrast, prospectively-isolated Sox2:EGFP+, PDGFRα+, cd34- cells (red) integrated into the hair follicle DP (F, arrow) and DS (G, arrows), adjacent to keratin 5+ epidermal cells (G, green). While in the DP, these cells expressed Sox2:EGFP (F, green). (H,I) Prospectively-isolated Sox2:EGFP+, PDGFRα+, cd34- cells (red) also integrated into the interfollicular dermis, where they expressed PDGFRβ (I, blue, arrow), but not the DP marker cd133 (H, blue) or Sox2:EGFP (H, green). Nuclei were stained with Hoechst 33258 as indicated. Scale bars = 25μm in E-I.

A


Canonical pathways associated with genes exhibiting >2-fold log expression change
SKPs
Sox2:EGFP+
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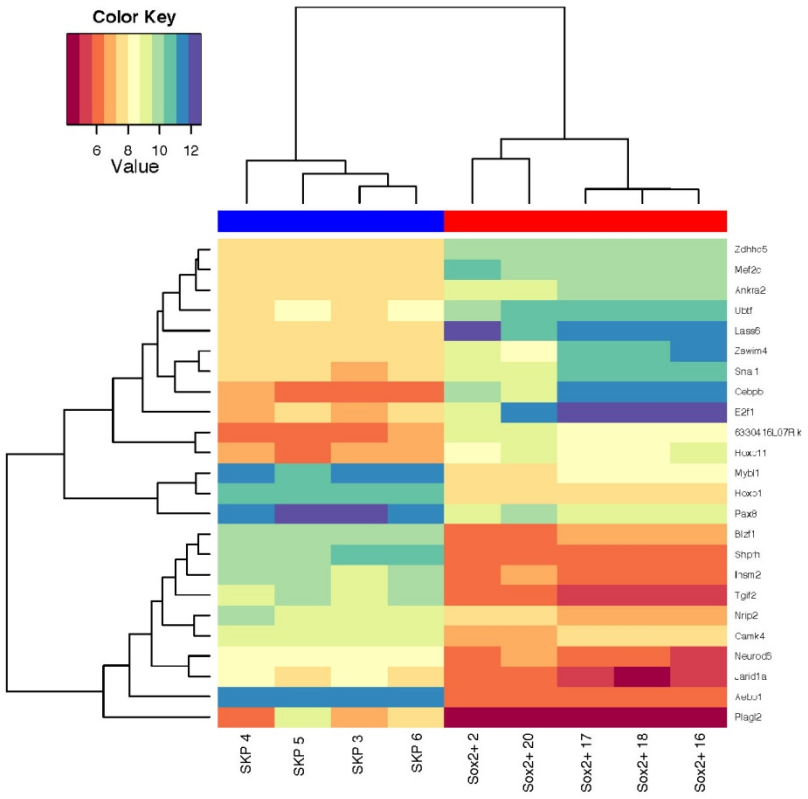
Threshold
O-Glycan Biosynthesis
Phenylalanine, Tyrosine and Tryptophan Biosynthesis
Citrate Cycle
ERK/MAPK Signaling
PI3K/AKT Signaling
Glycosphingolipid Biosynthesis - Neolactoseries
Coagulation System

B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Shprh
Insm2
Tgif2
Nrip2
Camk4
Neurod6
Arid1a
Aebp1
Plagl2
SKP 4
SKP 5
SKP 3
SKP 6
Sox2+ 2
Sox2+ 20
Sox2+ 17
Sox2+ 18
Sox2+ 16

Figure S3. ***Identification of pathways enriched in Sox2:EGFP+ dermal cells versus SKPs.*** (A) Identification of gene expression pathways that are selectively enriched in Sox2:EGFP neonatal murine skin cells versus neonatal murine SKPs. Genes whose expression was significantly increased in one cell population versus the other were analyzed by Ingenuity Pathway Analysis software. (B) Heat map showing the relative expression levels of transcription factors that were differentially expressed 2 to 4.3-fold in 5 independent Sox2:EGFP cell isolates (red bar on top of the heat map) and 4 independent SKP cultures (blue bar on the top of the heat map). Relative expression levels are color-coded as per the color key. The cluster analysis on the top of the heat map shows that the Sox2:EGFP+ cell samples and the SKPs samples clearly segregate with each other with regard to these genes.

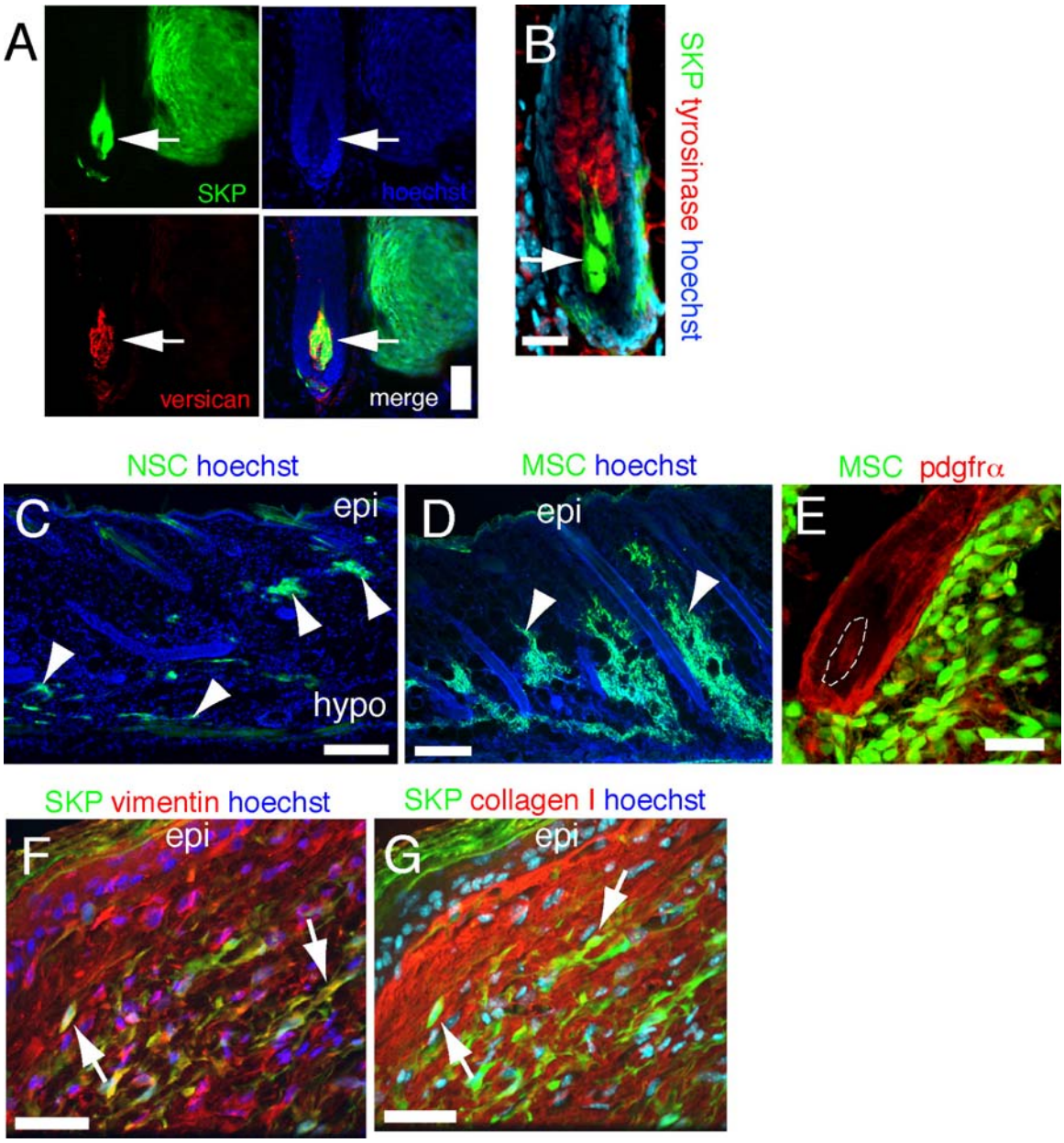

Figure S4. ***Transplantation of SKPs, NSCs and MSCs into adult mouse skin.*** (A-E) Adult rat GFP-tagged SKPs (A,B), MSCs (D,E) or neonatal murine YFP-tagged NSCs (C) were transplanted into adult NOD/SCID mouse back skin for 3 weeks. SKPs (green) integrated into hair follicle DP where they expressed versican (A, red), but not the melanocyte marker tyrosinase (B, red). In contrast, SKPs that integrated into the interfollicular dermis did not express versican (A). NSCs (green) survived only poorly in the dermis (C). MSCs (green) demonstrated better survival, and were frequently seen surrounding hair follicles (D) and expressing PDGFRα (E, red), but never integrated into the DP (E, hatched lines). (F,G) YFP+ neonatal murine SKPs were transplanted adjacent to a punch wound in NOD/SCID mouse back skin, and were analyzed three weeks later. Double-label immunocytochemistry for YFP (green) and vimentin (F, red) or collagen 1

(G, red) demonstrated that many transplanted cells found within the wound coexpressed these dermal fibroblast markers (arrows). Nuclei were stained with Hoechst 33258. Scale bars = 100µm in A, 200µm in C,D and 25µm in B,E,F,G.

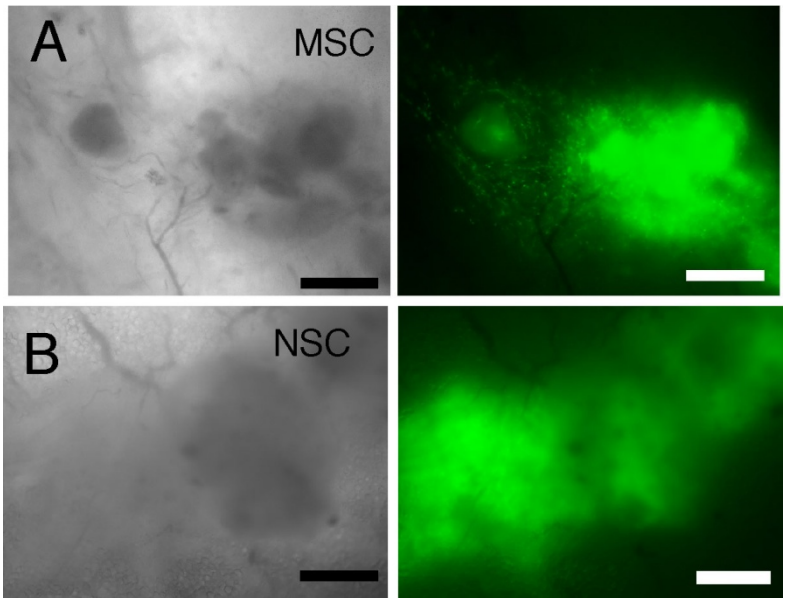

Figure S5. ***MSCs and NSCs do not induce hair follicle morphogenesis.*** Photomicrographs of patch assays at 12 days. 10^6 dissociated adult rat GFP-tagged MSCs (A) or neonatal murine YFP-expressing NSCs were mixed with epidermal aggregates from newborn C57/Bl6 mice, and visualized by phase illumination (A,B, left panels) or fluorescence illumination (A,B, right panels). Neither population induced follicle formation. Scale bars = 500µm in A,B.

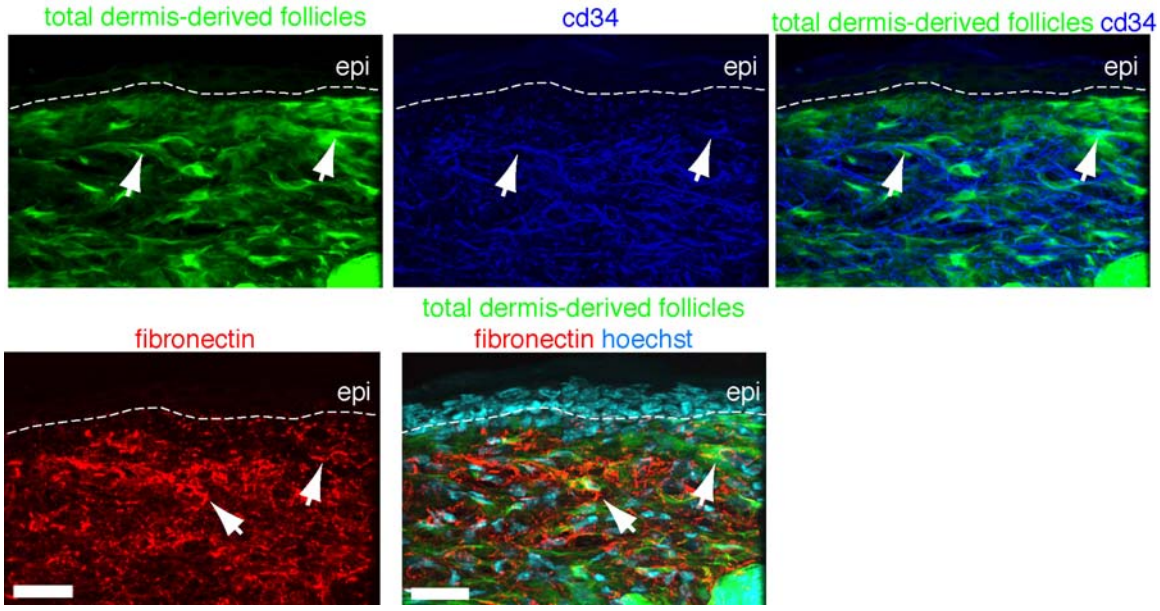

Figure S6. ***Lineage tracing of hair follicles generated from uncultured GFP+ neonatal rat dermal cells.*** Hair follicles were generated in patch assays from total uncultured GFP+ rat dermal cells, were carefully dissected and then transplanted into the back skin of NOD/SCID mice as shown in Figure 7. Four weeks later the interfollicular dermis adjacent to the transplanted follicles was analyzed by triple-labeling for GFP (green), cd34 (blue) and fibronectin (red). Arrows indicate double-labeled cells in these confocal micrographs. GFP+ cells migrated out of the hair follicles into the interfollicular dermis, where the cells expressed the phenotype of interfollicular dermal fibroblasts. Scale bar = 25 μm.