SUPPLEMENTARY INFORMATION

SUPPLEMENTARY MATERIALS AND METHODS

Mice and labeling experiments

$Tbx18^{GFP}$ were generated by removing the LoxP flanked LacZ cassette in the germline by crossing $Tbx18^{LacZ}$ mice with Prm-Cre mice that universally express Cre recombinase (O'Gorman et al., 1997). $R26R^{LacZ}$ (129S-Gt(ROSA) $26Sor^{tm1Sor}$/J; (Soriano, 1999) and $R26^{ACTB-mT/mG}$ (Gt(ROSA)$26Sor^{tm4(ACTB-tdTomato,-EGFP)Luo}$/J; (Muzumdar et al., 2007) reporter mice were obtained from Jackson Laboratories, Bar Harbor, ME. Genotyping PCR was performed on genomic DNA prepared from tail biopsies. MerCreMer was activated by intraperitoneal injection of tamoxifen (Sigma, T5648) that was dissolved in corn oil (Sigma, C8267) at 20 mg/ml. Pregnant females were injected at E13.5 with 9 mg per 40 g body weight at embryonic day 13.5 (E13.5). Embryos were harvested at E14.5 or later stages. All animal experiments were performed in accordance with the guidelines and approval of the Institutional Animal Care and Use Committee at Mount Sinai School of Medicine.

Tissue histology, immunofluorescence

Embryos or skins were fixed and processed for staining or directly frozen in optimal cutting temperature (OCT, Tissue-Tek) compound. For detection of β-galactosidase activity, X-Gal staining was performed on whole-mount embryos or cryosections. Briefly, embryos were fixed in freshly prepared 4% paraformaldehyde (PFA) at 4°C for 2 hours, then washed in PBS. After fixation, embryos were permeabilized in 0.2% triton X-100 in PBS for 15 min, and then stained with 1 mg/ml X-gal (Roche, Indianapolis, IN) in PBS containing 0.02% NP-40, 5 mM of K3Fe(CN)6, 5 mM of K4Fe(CN)6, and 2 mM of MgCl2 at 37°C for 16 hours. Embryos were then rinsed, postfixed, washed, and embedded in OCT. Embryos older than E16.5 were embedded directly in OCT and cryosectioned. 12μm sections were rinsed with PBS, fixed in 4% PFA for 5 minutes, washed and X-Gal stained as described above. For immunofluorescence stainings on sections, embryos and neonatal backskins were cut using a cryostat. Slides were fixed in 4% PFA at room temperature for 5 minutes then washed in PBS. For whole-mounts immunofluorescence stainings, embryonic skin was peeled off and fixed for 1 hour in PFA 4% and then washed in PBS. After blocking with 3% normal donkey serum (Jackson ImmunoResearch, West Grove, PA), 0.1% TritonX-100 in PBS, sections and skins were incubated overnight at 4°C with antibodies against Sox2 (D-17, goat 1:100), Vimentin (goat, 1:100, Santa Cruz, CA), Integrin β4, CD31 (rat, 1:100, BD Pharmingen, San Jose, CA), E-cadherin (rat, 1:100, Invitrogen, Carlsbad, CA), CD34 (rat, 1:100, eBioscience, San Diego, CA), Integrin α8 (BAF4076 goat 1:100, R&D, Minneapolis, MN), SMA (ab 5694 rabbit 1:300, Abcam Cambridge, MA), followed by Rhodamine Red-X conjugated donkey anti-goat and Cy5 conjugated donkey anti-rat secondary antibodies (1:400, Jackson ImmunoResearch) antibodies. Nuclei were counterstained with DAPI and slides were coverslipped with antifade (2.5mg/ml p-Phenylenediamine in PBS).

Microscopy, digital photography, and image processing

Gross images were obtained using a digital camera (model C-5060; Olympus) or a dissection microscope (Leica MZ16 FA) equipped with a camera (Leica DFC500) driven by Leica LAS software. Light and fluorescent microscopy images were obtained using a Leica DM5500 upright fluorescence microscope equipped with 63x HCX PL APO (1.4-0.6NA, Oil), 40x HCX PL APO (1.25-0.75NA, Oil), 20x HC PLAN APO (0.7NA), and 10x HC PL APO (0.4NA) objectives (all from Leica, Wetzlar, Germany) and were captured using digital cameras (Leica DFC360FX and DFC340FX) driven by Leica LASAF software.

FACS sorting and analysis

Backskins of E14.5 $Tbx18^{H2BGFP/LacZ}$ embryos were microdissected, then floated on dispase (Invitrogen) with 0.2% collagenase (Sigma-Aldrich, St. Louis, MO) for 40 minutes at 37C. Tissues were mechanically dispersed to obtain single cell suspensions that were passed through 40μm cell strainers (Falcon) and centrifuged at 300g. Resuspended cells were then labeled with antibodies against CD34, CD31, CD45 and E-Cadherin (Invitrogen) followed by detection with donkey anti-rat APC-conjugated secondary antibodies (Jackson ImmunoResearch). Cell purifications were performed on a FACSAria system equipped with FACS DiVa software (BD Biosciences).

SUPPLEMENTARY REFERENCES

Muzumdar, M. D., Tasic, B., Miyamichi, K., Li, L. and Luo, L. (2007). A global double-fluorescent Cre reporter mouse. *Genesis* 45, 593-605.

O'Gorman, S., Dagenais, N. A., Qian, M. and Marchuk, Y. (1997). Protamine-Cre recombinase transgenes efficiently recombine target sequences in the male germ line of mice, but not in embryonic stem cells. *Proc Natl Acad Sci U S A* 94, 14602-7.

Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 21, 70-1.

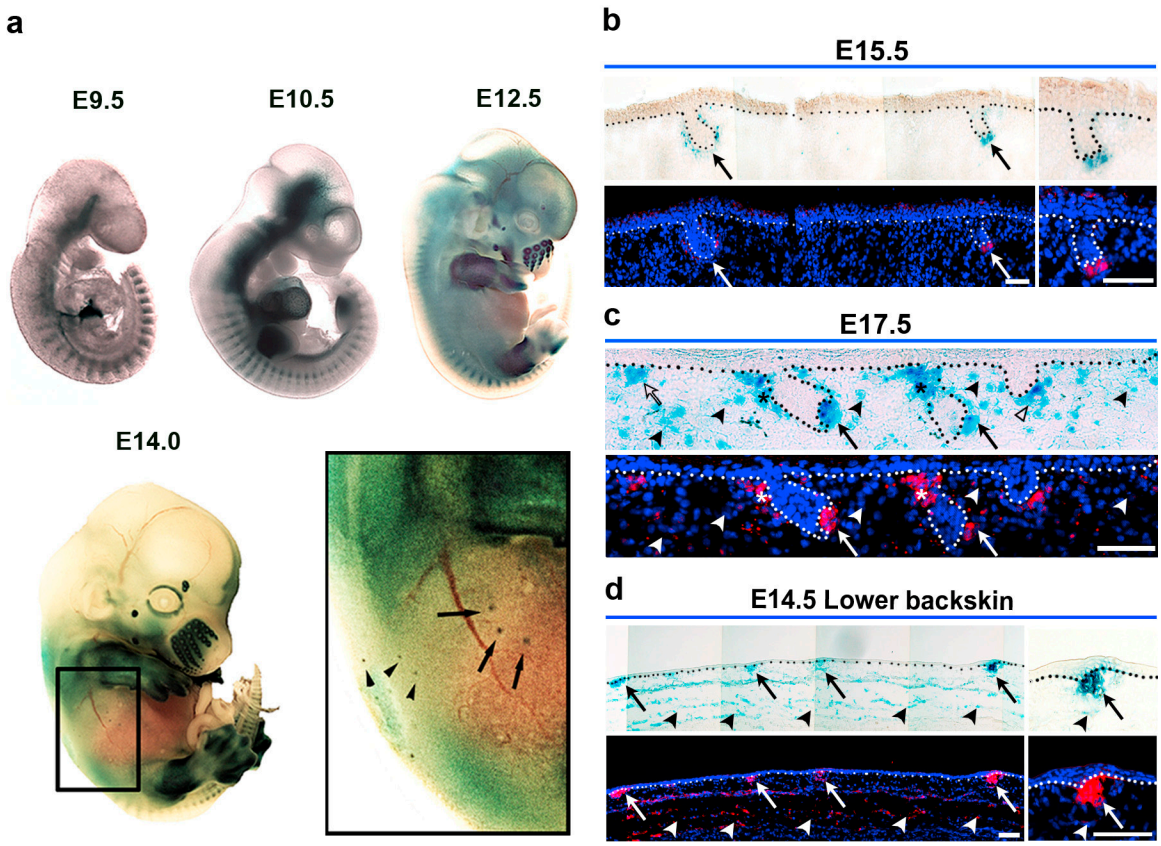
SUPPLEMENTARY TABLES

Supplementary Table 1. List of Real-Time PCR and genotyping primers

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
Gapdh	CGTAGACAAAATGGTGAAGGTCGG	AAGCAGTTGGTGGTGCAGGATG
Tbx18	ATGGCCTCCAGAATGCGTATG	TGTCCCCCATCAAGCCTGTT
Sox2	TACTGGCAAGACCGTTTTCGTG	TATTGGAATCAGGCTGCCGAG
Sox18	CTTCATGGTGTGGGCGAAGGAC	CTCCAGCCTCCGGACCTTGC
Prdm1	TGGGGCAGCAGCAGAGGGTGTG	GTCGCTGATGTCGAACCTCTCA
Bmp4	TCCACTGGCTGATCACCTCAAC	AGTCCAGCTATAGGGAAGCAGTTTG
Fgf10	ATTTCCCCCTGTATGCATCCTAAC	TTCCCACGGAGGCAGAACTC
Ptch1	ACGGGGTCCTCGCTTACAAAC	TCTCGTAGGCCGTTGAGGTAGAA
Tbx5	GCCCCCACCTAACCCATA	GCCCCGAGGTGAAATGAGC
Tbx6	GCTGAACCCCGCCTACCC	ATCCCGCTCCCTCTTACAGTTTC
Tbx15	ATCCCCTTCGGCTTCTTCTCAT	AGCCGGTTGTAGCCACTGTCA
Genotyping	**Forward primer (5' to 3')**	**Reverse primer (5' to 3')**
Tbx18-LacZ	GCCAGAGAAAGAGGAAACGGCAAA	GGATGTGCTGCAAGGCGATTAAGT
Tbx18-GFP	GCCAGAGAAAGAGGAAACGGCAAA	GAACTTCAGGGTCAGCTTGCCGTA
Tbx18-Cre	GCCAGAGAAAGAGGAAACGGCAAA	TCCCTGAACATGTCCATCAGGTTC
Tbx18-MCM	GCCAGAGAAAGAGGAAACGGCAAA	GTTCAGCATCCAACAAGGCACTGA

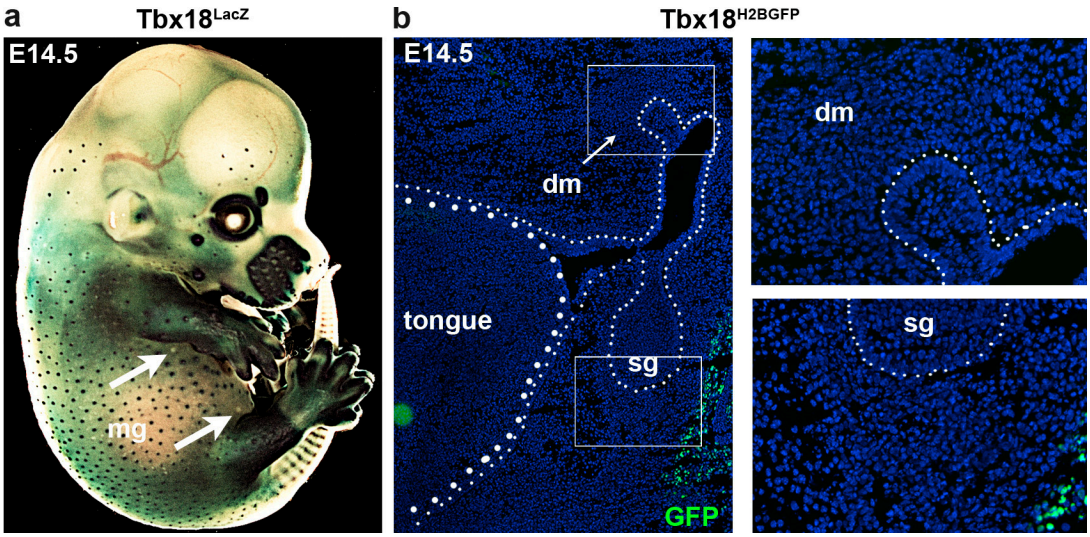
SUPPLEMENTARY FIGURES

Supplementary Figure 1



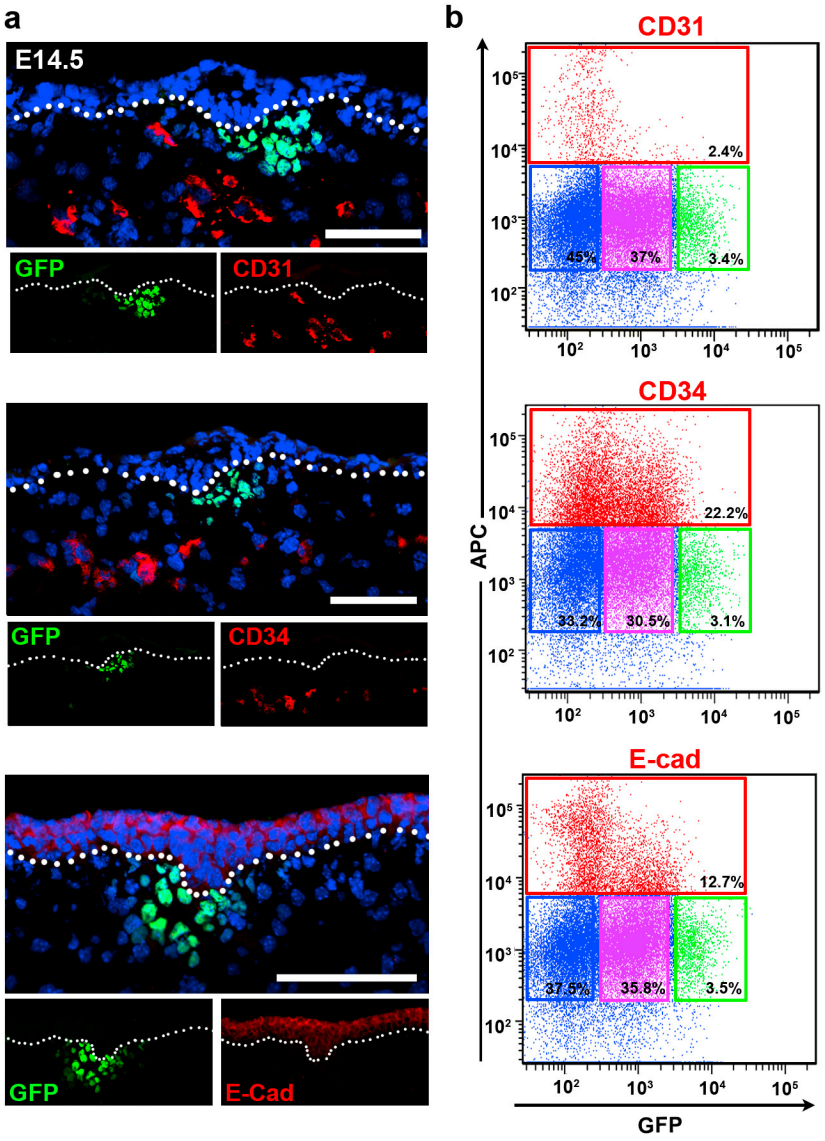
Supplementary Figure 1. Tbx18^{LacZ} expression in embryonic skin. (**a**) Whole-mount X-Gal staining of Tbx18^{LacZ} embryos between E9.5 and E14.0. High magnification inset shows few scattered positive hair follicles in embryos at E14.0 (arrows). (**b**) LacZ expression in embryonic DPs at E15.5 (arrows). **c**) At E17.5 LacZ expression was detected in dermal cells (arrowheads) and arrector pili muscles (asterisk). (**d**) Weak LacZ expression in dermal cells of the lower backskin at E14.5 (arrowheads). Bars = 50μm

Supplementary Figure 2



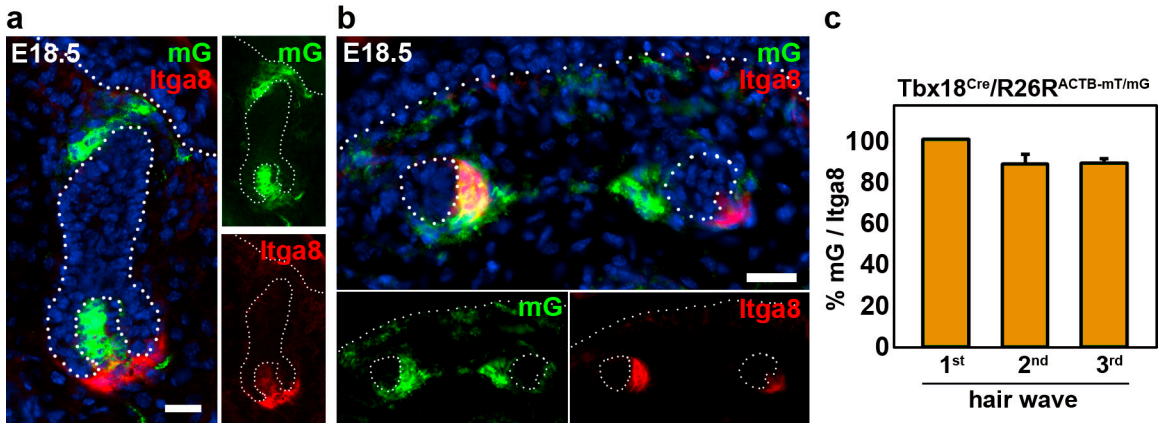
Supplementary Figure 2. Tbx18 expression is absent in mammary, salivary and dental mesenchymal condensates. (**a**) β-galactosidase staining of whole-mount $Tbx18^{LacZ}$ embryo at E14.5 showed absence of lacZ in the mesenchymal condensates of mammary gland (mg) (arrows). (**b**) Sections of $Tbx18^{H2BGFP}$ embryos at E14.5 showed absence of GFP in the dental mesenchyme (dm) and mesenchyme of the salivary gland (sg). Right: High magnification of inserts.

Supplementary Figure 3



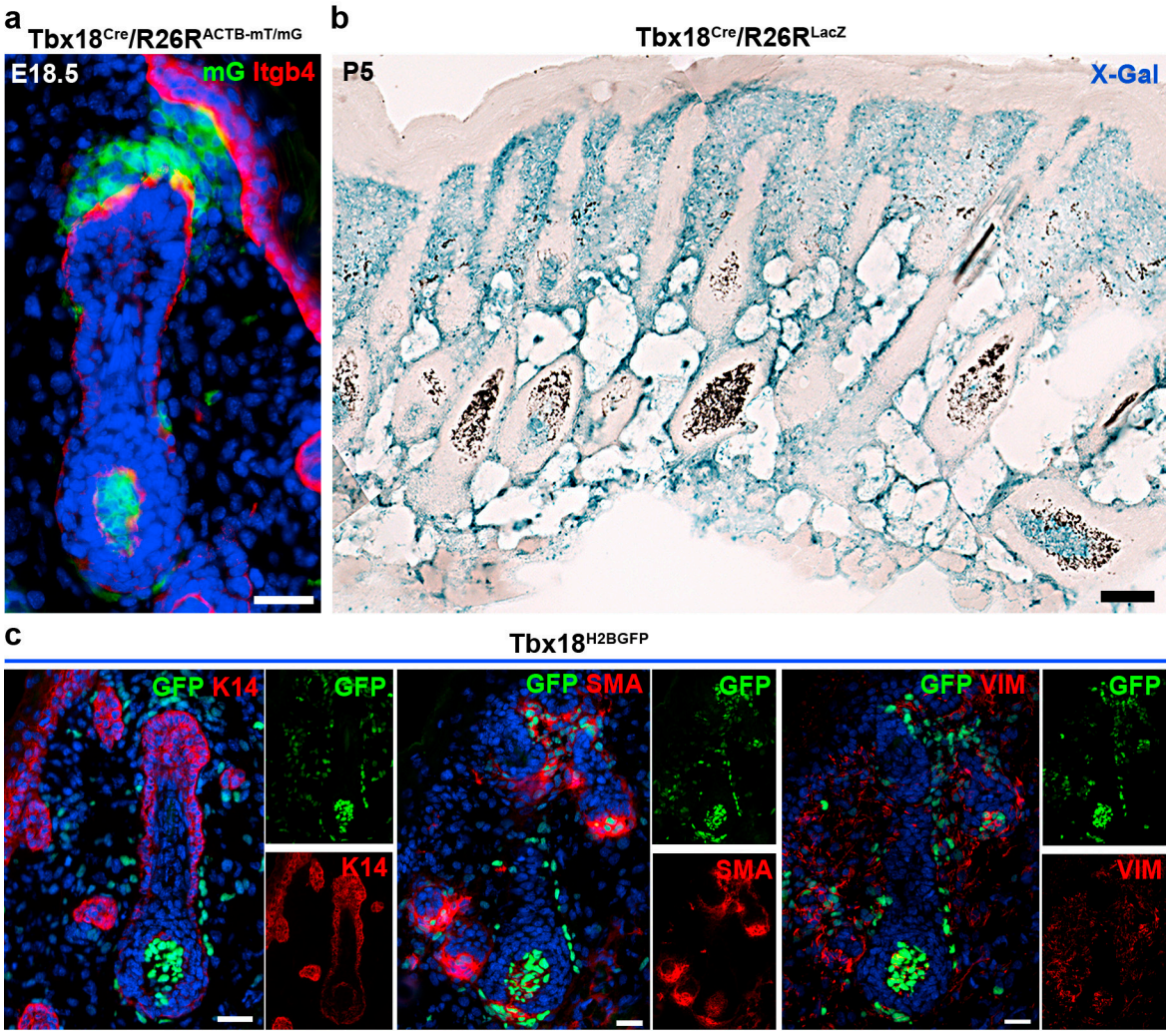
Supplementary Figure 3. FACS and immunofluorescence analysis in Tbx18^{H2BGFP} embryonic skin. (**a**) Immunofluorescence stainings on E14.5 sections of Tbx18^{H2BGFP} embryos for CD31, CD34 and E-cadherin highlighting endothelial cells and epidermal cells, respectively. Dotted line represents basement membrane border between epidermis and dermis. (**b**) FACS profile of E14.5 backskin cells from Tbx18^{H2BGFP} embryos stained for CD31, CD34 and E-cadherin in APC, respectively (red gates). GFP^{high} (green gate), GFP^{low} (pink gate) and negative (blue gate) fractions were selected as APC negative populations. Bars = 50μm.

Supplementary Figure 4



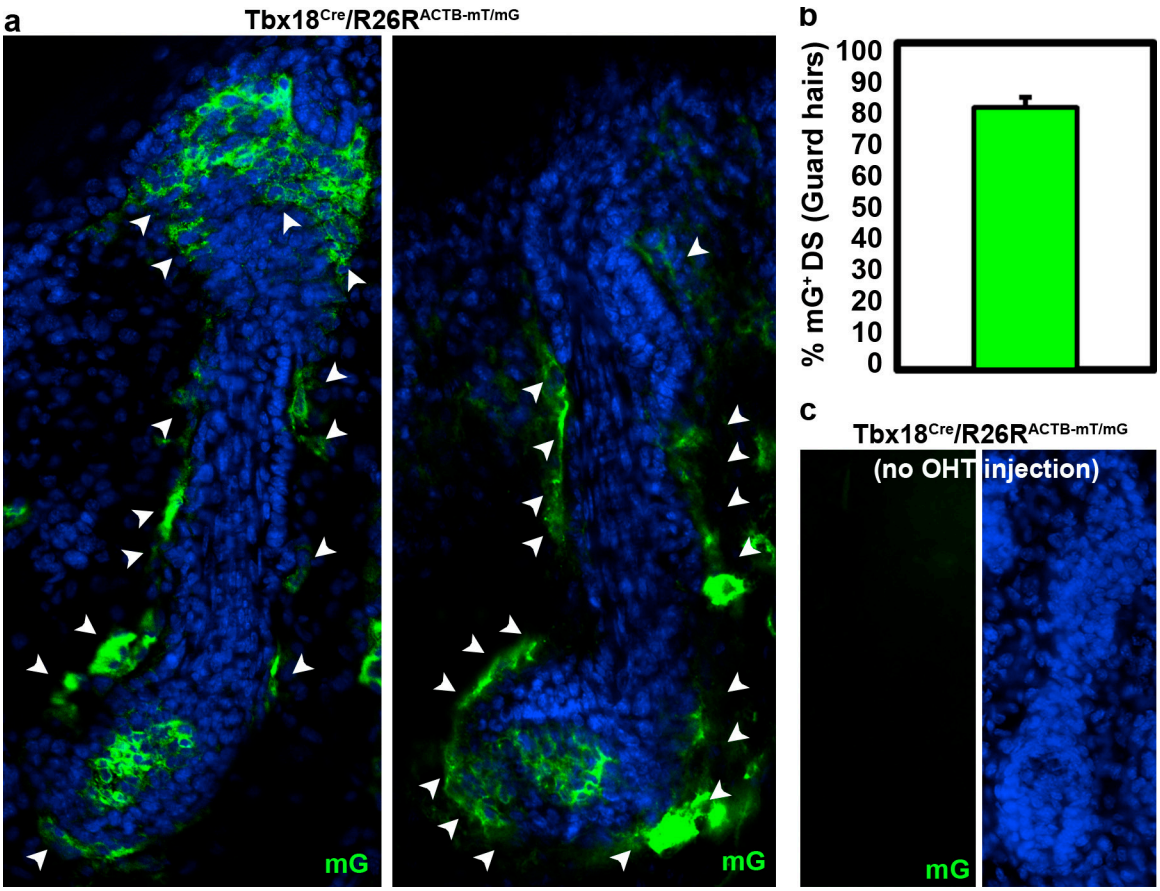
Supplementary Figure 4. Tbx18^{Cre} activity in all three hair subtypes at E18.5. a) First wave guard hair hair follicle. (**b**) Cre recombination in second wave hair types. DP cells are highlighted with Itga8 immunofluorescence and express the mG reporter. (**c**) Quantification of GFP-positive DPs in all three hair subtypes (*n*=2). Scale bars, 25μm. Data shown are mean ± SD.

Supplementary Figure 5



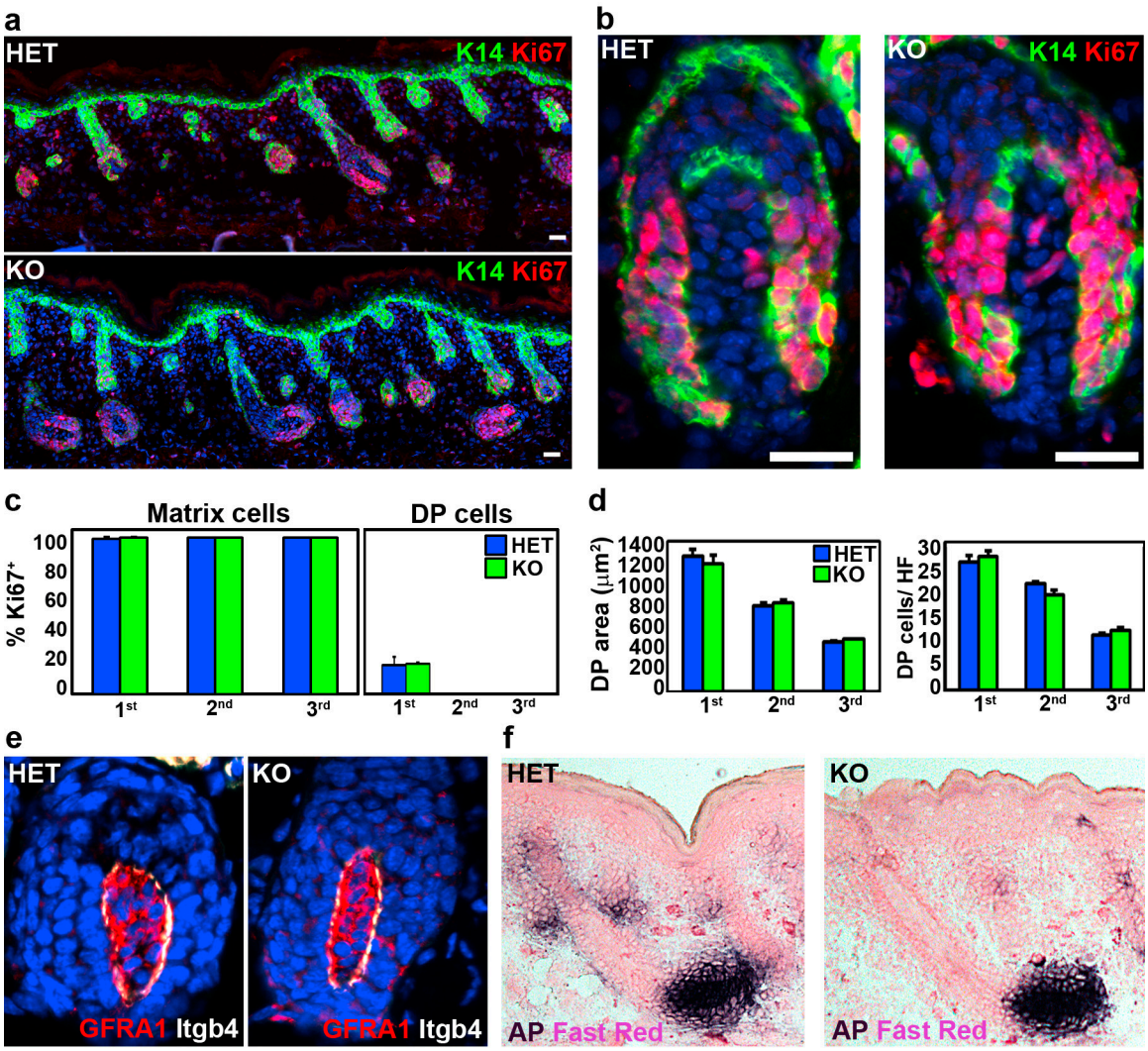
Supplementary Figure 5. $Tbx18^{Cre}$ activity is not detectable in skin epithelial cells. (**a**) mG (GFP) reporter activity in E18.5 skin of $Tbx18^{Cre}/R26^{ACTBmT/mG}$ embryos is only found in the dermis and hair follicle mesenchyme outside the epithelial domain which is demarcated by Itgb4. (**b**) X-gal staining of $Tbx18^{Cre}/R26R^{LacZ}$ skin showed Cre activity only in the dermis, DP and dermal sheath of postnatal P5 mice. (**c**) Immunostaining for K14, SMA and Vimentin in $Tbx18^{H2BGFP}$ embryos at E18.5. GFP expression is in the mesenchyme. Scale bars, 25μm.

Supplementary Figure 6



Supplementary Figure 6. Dermal sheath cells are derived from dermal condensates. (**a**) mG (GFP) reporter expression in the DP and dermal sheath cells (arrowheads) of guard hairs at E18.5 in $Tbx18^{MCM}/R26^{ACTBmT/mG}$ embryos that received a single activation tamoxifen dose at E14.5. (**b**) Quantification of follicles with labeled dermal sheath cells (n=3 embryos). (**c**) Absence of $Tbx18^{MCM}$ leakiness in skin of $Tbx18^{MCM}/R26^{ACTBmT/mG}$ embryos injected with corn oil only without tamoxifen. Bars = 25μm. Data shown are mean ± SD.

Supplementary Figure 7



Supplementary Figure 7. No difference in marker expression, proliferation and DP size and cell numbers in Tbx18 KO. (**a**) Immunofluorescence of Ki67 and keratin-14 (K14) in HET ($Tbx18^{LacZ}$) and KO ($Tbx18^{LacZ/H2BGFP}$) skin at P1. (**b**) Higher magnification of guard hairs from HET and KO. (**c**) Quantification of $Ki67^{+}$ cells in matrix and DP of follicle subtypes from all three waves showed no differences between HET and KO (n=2). (**d**) Similar DP size and cell numbers in HET and KO. (**e,f**) Immunofluorescence of GFRA1 and Alkaline phosphatase (AP) staining revealed normal staining in KO hair follicles. Data shown are mean ± SD. Bars = 25μm.