Figure E1: Expression of panel of markers during back skin development. Sections were counterstained with DAPI (blue). Scale bars: 50 mm. Photomicrographs of 10μm sections are representative of 3 biological replicates.

Figure E2: Expression of markers during back skin development. (a) Expression of additional markers during back skin development. Counterstained with DAPI (blue). (b) Co-expression of CD31, CD45, CD26, Dlk1 and Sca1. Counterstained with DAPI (blue). Scale bars 50 microns. Photomicrographs of 10micron thick sections are representative of 3 biological replicates.

Figure E3: Isolation and in vitro properties of dermal subpopulations. (a-c) Flow cytometry of E12.5, E14.5, E16.5, and P2 PDGFRαH2BeGFP mouse dermal cells labelled with antibodies to (a) CD26, (b) Dlk1 and (c) Sca1. Graphs show average % cells that co-express individual markers and GFP. Data from 3 biological replicates at each time point. One-Way A-NOVA parametric tests were used to determine P values: *<0.05; *** <0.005 (d-g) Clonal growth of fibroblast subpopulations from (d, e) E16.5 and (f, g) P2 dermis. (d, f) Representative phase contrast and LipidTox fluorescent images of clones encapulated in hydrogels and cultured in DMEM 10%BS or adipogenic differentiation medium. (e, g) Quantitation of lipid positive colonies. N=3 biological replicates. One-Way A-NOVA parametric tests of significance were used to determine P values: **<0.005 ***<0.0005. Error bars are SEM. Scale bars are 10microns.

Figure E4: Fibroblast marker expression in human skin. (a,c,e-h) neonatal human foreskin; (b,d) adult human abdomen. (a-b) PDGFRα was detected in mesenchymal (vimentin+) cells throughout the dermis. (c-d, f) Dlk1, (e, g) CD26 and (h) Pdpn were

differentially expressed in upper (CD26) or lower (Dlk1, Pdpn) neonatal dermis. Dlk1 was not expressed by adult dermal fibroblasts (d). (e, f) Enlarged areas of (c, g), respectively. Scale bars are 50 microns. N=2 biological replicates.

Figure E5: Further characterisation of P2 mouse dermal fibroblast subpopulations. (a, b) 10 micron sections of P2 skin stained for markers indicated, with DAPI counterstain (white). (c) Flow sorting strategy to isolate cells from P2 papillary dermis (CD26+Sca1-), reticular/hypo-dermis (Dlk1+/Sca1-) and hypodermis (Dlk1+Sca1+ and Dlk1-Sca1+). (d) Heat map of relative mRNA levels of the genes indicated that are represented in histograms, summarizing data in (e). (e) Characterization of flow sorted fibroblasts from P2 dermis. Relative mRNA levels of the genes indicated as normalized to GAPDH. Data are means of 3 biological replicates (± SEM). One-Way A-NOVA parametric tests of significance were used to determine P values: *<0.05 **<0.005, and ***<0.0005. (f-i) Pre-adipocyte precursors (Lin-CD34+CD29+Sca1+) express PDGFRα. Single cell suspension of P2 PDGFRαH2BeGFP mouse dermis was gated for (f) Lin-, (g) CD29+CD34+, (h) Sca1+, and (i) GFP+. Scale bar is 50 μm. All data representative of three biological replicates.

Figure E6: Skin reconstitution with different fibroblast subpopulations. (a-i) See Figure 2a for experimental set-up. (a-d) Horizontal whole mount micrographs of grafts immunostained for eGFP, Lipidtox and Itga8 with DAPI counterstain (blue). Boundary between dermis and hypodermis/adipocytes is indicated by dotted line. (e-h) Representative macroscopic views of skin grafts. (i) No. hair follicles per graft. Error bars are SEM. N = 4 grafts/fibroblast population. One-Way A-NOVA parametric tests were used to determine significance. *P<0.05. Scale bars (a-d, e-f) 100 and 400 μm.(j-

l) Representative flow cytometry plots from exclusion sorts of PDGFRaH2BeGFP dermis for grafting assays in Figure 2g-k. (j) Positive control: all dermal cells collected. (k) No reticular/hypodermis: Dlk1+ and Sca1+ cells were not collected. (l) No Papillary/Reticular dermal sorts: Dlk+Sca1-, CD26+Sca1- cells were not collected.

Figure E7: Lineage tracing controls. (a) Dlk1CreER and ROSAtdTomato mice were bred and at 16.5 mothers were injected with 5mg/ml Tamoxifen in corn oil. (b-c) Embryos were collected at E17.5 and single cell suspensions of labeled embryonic dermis was analyzed for overlapping tdTomato expression and endogenous Dlk1 expression.(b) Representative flow cytometry plot. (c) Graph is representative of n=4 embryos. Error bars are SEM. (d-g) Blimp1 expression in embryonic and postnatal skin. Horizontal whole mounts were stained for endogenous Blimp1 (d,g), CD26 (e), and Itga8 (f). (h-i) Horizontal whole mount immunostains of (h) Bimp1 GFP mice and (i) Blimp1CreCAGCATeGFP mice costained for CD31. (j-k) E18.5 single cell suspensions of Blimp1GFP (j) and Blimp1CreCAGCATeGFP (k) cells labelled with CD26. (l) Experimental approach for Lrig1CreER labeling of papillary dermis. (m) Horizontal whole mount stain of Lrig1CreER/ROSAtdTomato mice labeled at E16.5 and collected at P2. (n-p) Flow cytometric analysis of labeling specificity of tdTomato compared to (n) Lrig1, (o) PDGFRa and (p) Sca1. (q) Flow cytometric analysis of overlapping expression of Lrig1GFP with CD26. Data are representative of 3 biological replicates. Scale bars are 100 μm.

Figure E8: Clonal analysis of papillary dermis. (a) Experimental strategy and flow cytometry of dermal cells isolated from mice treated with increasing concentrations of tamoxifen. (b) %PDGFRa+ cells 4 days after treatment with different concentrations of

tamoxifen (N=7 per concentration). 25mg/g tamoxifen resulted in less than 1% of fibroblasts being labelled. (c-d) Whole mount analysis of GFP labelled cells in E16.5 skin. (e-f, j) Histological analysis of the fate of papillary clones from PDGFRaCreERxCAGCATeGFP mice injected with 25mg/g tamoxifen at E12.5 andharvested at P2 (e), P10 (j), and P21 (f). (g, h, i) Quantitation of the fate of papillary fibroblast clones derived from E12.5 embryonic mesenchyme (h) cross-compartmental clone of papillary and arrector pili clones (g) and arrector pili clones (i). N=3 biological replicates (± SEM). Scale bars: 50mm. One-Way A-NOVA parametric tests were used to test for significance. ***P<0.0005.

Figure E9: Bone marrow transplantation, wound healing and beta-catenin activation. (a-k) Bone marrow reconstitution experiments. (a) Schematic representation of the experiments. Bone marrow (BM) of mice that express eGFP under the control of the CAG promoter (ACP-eGFP) or PDGFRaH2BeGFP mice was transplanted into sub-lethally irradiated wild type mice. One month later 8mm diameter full thickness circular wounds were made in the back skin with a biopsy punch. Skin was analysed 5 and 10 days after wounding. (b, c) Y- chromosome staining of reconstituted spleen from ACP-eGFP (b) and PDGFRaH2BeGFP mice (c). (d-e) Horizontal whole mounts of wild type mouse back skin reconstituted with BM from ACP-eGFP (d) or PDGFRaH2BeGFP (e) mice, stained for GFP and PDGFRa with DAPI counterstain (blue). (f-i) Horizontal whole mounts of wild type mouse back skin reconstituted with BM from ACP-eGFP (f,h) PDGFRaH2BeGFP (g,i) mice 5 (f-g) and 10 (h-i) days after wounding, stained for GFP and PDGFRa with DAPI counterstain (blue). Arrows indicate wounded regions of skin. Scale bars: (b, c) 40 μm; (d-i) 100 μm. (j-k) Flow cytometry of GFP positive dermal cells.

(j) representative plots. (k) quantitation of GFP positive cells. ACP-GFP: N=6 biological replicates. PDGFRaH2BeGFP: N=5 biological replicates. One-Way A-NOVA parametric tests were used to test for significance. ***P <0.0005. (l-p) Fibroblast responses to wounding and epidermal beta-catenin activation. (a-c) Fibroblast lineages during full thickness wound repair. PDGFRaCreER/ROSAtdTomato lineage tracing was performedas indicated (a) and wound beds were stained for tdTomato (green) and Lrig1 (red). Scale bars are 150 and 50 mm. Micrographs are representative of n=3 female biological replicates. (d-e) Histological analysis of wild type and K14bcatER transgenic mice costained with Itga8 (green) and Sca1 (red). n=3 biological replicates. Scale bars are 100 μm. Number of Tamoxifen (4OHT) treatments is indicated.

Figure E10: Fibroblast lineages during full thickness wound repair. (a-d) Dlk1CreER/ROSAtdTomato (a, b) and Blimp1/CAGCATeGFP(c, d) lineage tracing was performed as indicated (a, c) and wound beds were stained for PDGFRα, Itgα8, asma, CD45 and CD31. Scale bars are 50 mm. Micrographs are representative of n=3 female biological replicates. (e-g) Quantitative analysis of single cell suspensions of wild type and K14bcatER/PDGFRaeH2BeGFP double transgenic mice costained with (e) Lrig1-PE or (f) Itga8-APC (g) Sca1-PE. n=3 biological replicates (h) Horizontal whole mounts of Control and K14bcatER skin immunstained for Lrig1 (green) and Sca1 (red). n=6 female biological replicates. Scale bars are 100 μm. Number of Tamoxifen (4OHT) treatments is indicated.