

## **SUPPLEMENTARY TABLE LEGENDS**

**Supplementary Table S1. Primary antibodies used for immunostaining.**

## **SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure S1. Processes and tools of mouse dermal papilla (mDP) isolation from whisker hair.** (A) Surgical tools and equipment. (B) mDP wash dish 1 and dissection dish 2. (C) Dissection lines on euthanized and decontaminated mouse jaw. (D) Flipped skin exposing dermis and hair follicle ends. (E) Blue line depicts cutting area of a hair follicle from the dermis. (F) Green box outlines the end bulb of a hair follicle. (G) End bulbs of anagen hair follicles were used for DP isolation as outlined by the green box. Unpigmented telogen hair follicles were discarded, as cell emigration was not observed from these structures. (H) mDP condensates were released from the end bulb connective tissues, made free of fibrous tissues, transferred, and gently pushed down with a needle to the bottom of the dish containing attachment media. The needle was then lifted up right away.

**Supplementary Figure S2. Characterization of devitalized split-thickness human dermis.** (A) H&E staining of cryosections of surgically discarded human abdominal skin and devitalized split-thickness dermis. (B) Immunostaining for cryosections of devitalized dermal matrix with a primary antibody against human collagen VII, and then detected with an Alexa-488-conjugated donkey anti-rabbit secondary antibody. (C-D) Live/dead cell staining. This pieces of devitalized dermal matrix was incubated with (C) Calcein-AM [live, green]/Ethidium homodimer (EthD-1) [dead, orange] staining kit

(L3224, ThermoFisher Scientific) and (D) Hoechst 33258 (10 µg/ml) [live and dead, blue] and propidium iodide (PI, 10 µg/ml) [dead, orange] for 10-15 minutes, washed with PBS, and imaged under florescent microscope. Fresh mouse ear skin was used for positive control of live cell staining with Calcein-AM [green]. **(E-F)** Immunofluorescent staining with primary antibodies against Ki-67 or cytokeratin 5 (K5) followed by detection with a Dylight 555-conjugated donkey anti-rabbit secondary antibody [orange], Nuclei [blue, Hoechst 33258].

**Supplementary Figure S3. Cell/matrix composites displayed preliminary stratification after 1 week in culture.** **(A)** H&E staining of frozen sections of the cell/matrix composite after 1 week in culture. **(B-F)** Immunostaining. Cryosections of the 1-week old cell/matrix composite were incubated with primary antibodies against Collagen VII (Col VII), K15, Vimentin, K14, or K10, and then detected with Dylight 488 [green] or Dylight 555 [orange] dye-conjugated secondary antibodies. Nuclei [Hoechst 32558, blue].

**Supplementary Figure S4. 4-weeks old skin grafts contained alkaline phosphatase and Sox2-positive cells, and expressed differentiation markers.** **(A)** Clinical image of a 4-week old skin graft regenerated with hKC and mDP cells. **(B)** Alkaline phosphatases staining with frozen sections of devitalized split-thickness human dermis and 4-week old skin grafts. **(C-F)** Immunostaining. Cryosections of 4-week old skin grafts generated with hKC either alone to together with mDP cells were incubated with primary antibodies against SOX2, K10, involucrin or filaggrin, and detected with

Dylight 555 [orange] or 488 [green] dye-conjugated secondary antibodies. Nuclei [Hoechst 32558, blue].

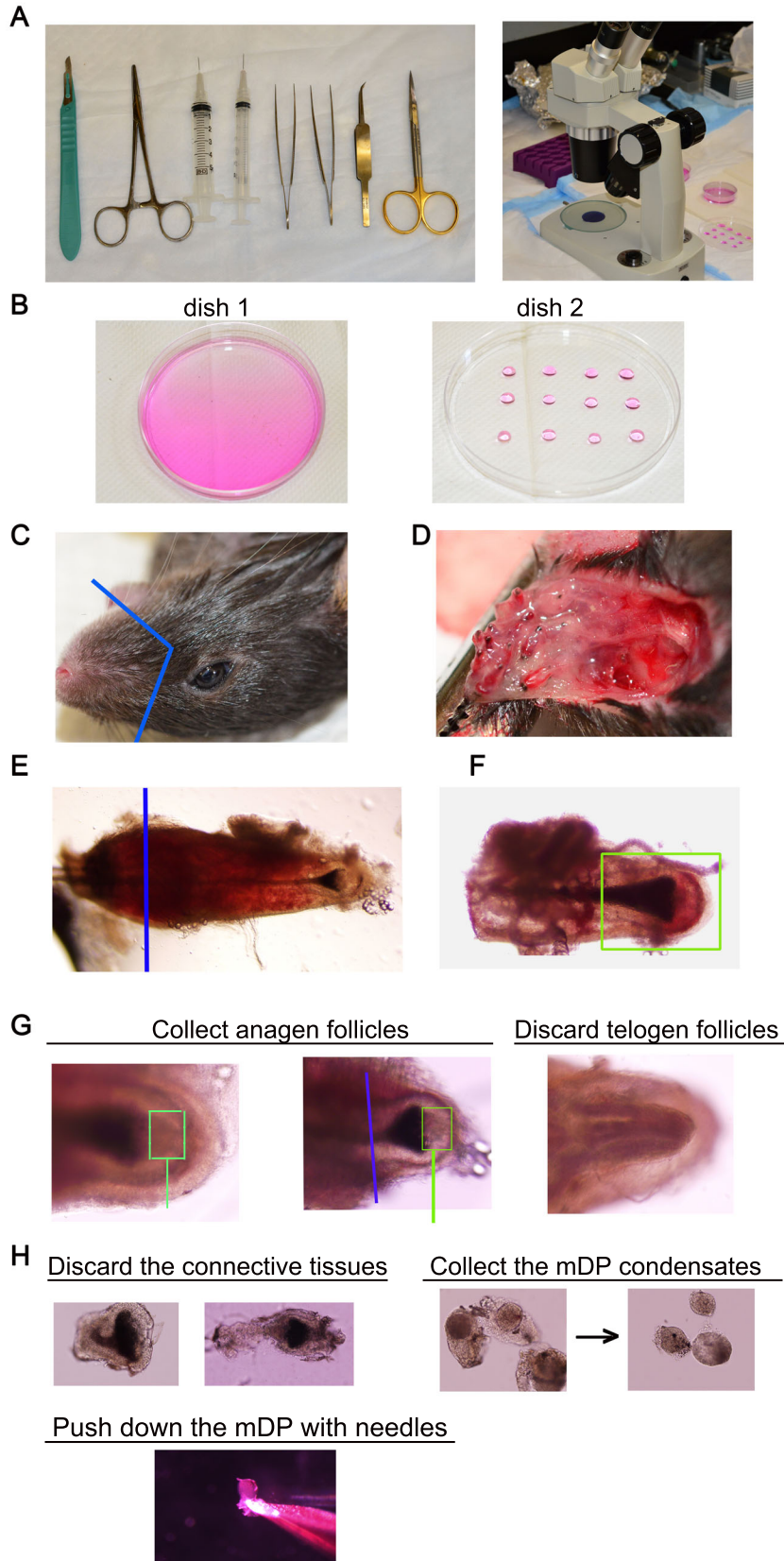
**Supplementary Figure S5. Regenerated skin grafts contained myeloid cells. (A-B)**

Immunostaining of 6-week old skin grafts regenerated with hKC and mDP cells. Frozen tissue sections were incubated with primary antibodies against the macrophage marker F4/80 or the myeloid cell marker CD45, and then detected with a Dylight 555-conjugated secondary antibody [orange]. Nuclei [Hoechst 32558, blue].

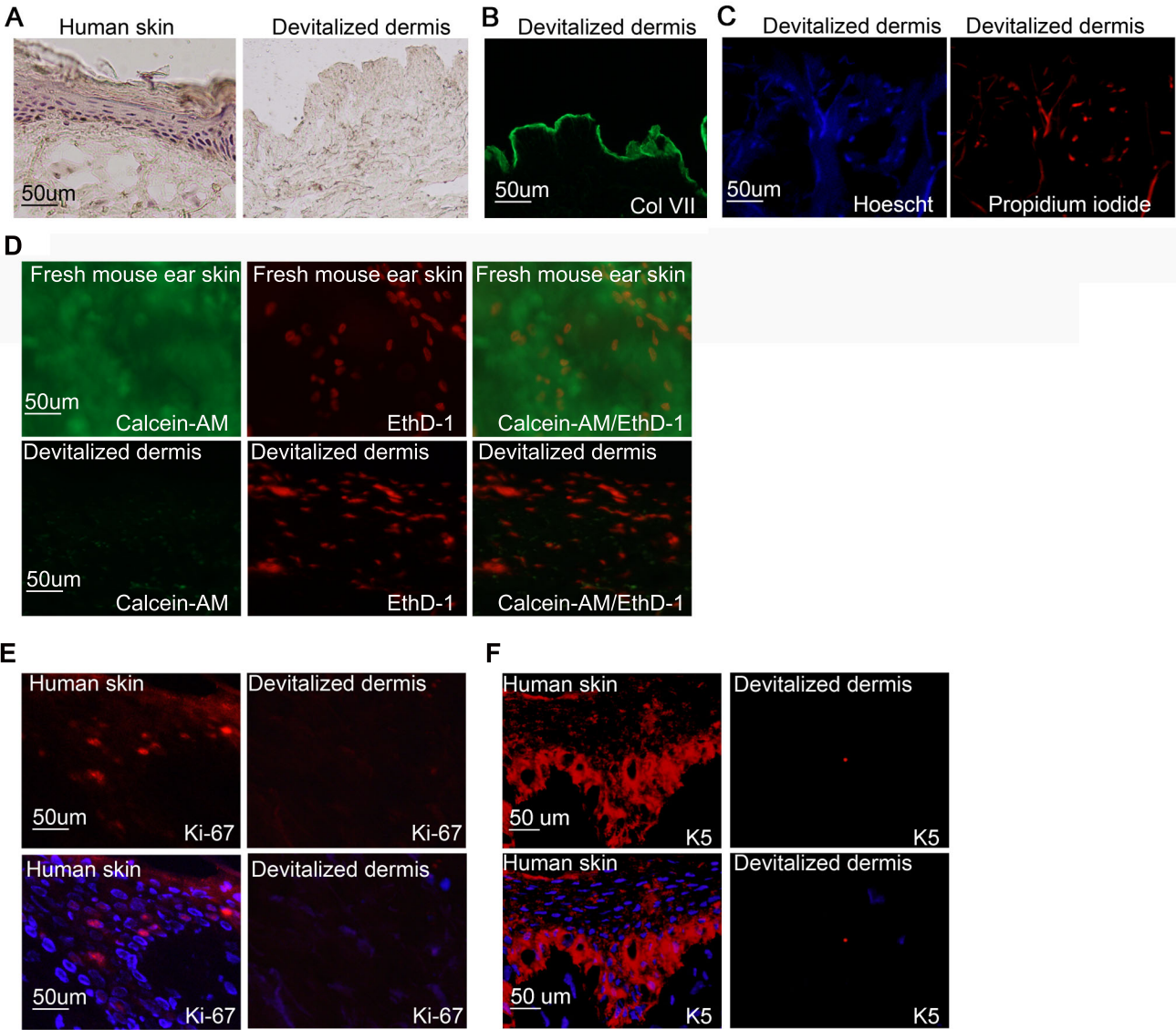
**Supplementary Table S1. Primary antibodies used for immunostaining.**

<b>Primary Antibodies</b>	<b>Vendor</b>	<b>Catalog or clone no.</b>	<b>Dilution</b>
Involucrin	Abcam, Cambridge, MA	Ab28057	1:500
Vimentin	Cell Signaling Biotechnology, Danvers, MA	3932S	1:500
Collagen VII	EMD Millipore, Billerica, MA	234192	1:100
Filaggrin	Abcam, Cambridge, MA	Ab24584	1:500
K1	Santa Cruz Biotechnology, Santa Cruz, CA	E12	1:500
K5	BioLegend, San Diego, CA	PRB-160P	1:500
K10	ThermoFisher Scientific, Waltham, MA	DE-K10	1:500
K14	ThermoFisher Scientific, Waltham, MA	RB-9020	1:500
K17	Abcam, Cambridge, MA	Ab53707	1:500
K25	Santa Cruz Biotechnology, Santa Cruz, CA	sc-398320 (B2)	1:500
K75	Santa Cruz Biotechnology, Santa Cruz, CA	sc-166074 (H6)	1:500
Ki-67	ThermoFisher Scientific, Waltham, MA	SP6	1:100
$\alpha$ -smooth muscle actin	EMD Millipore, Billerica, MA	PA5-22251	1:500
Sox2	Abcam, Cambridge, MA	Ab75627	1:100
CD45	BioLegend, San Diego, CA	30-F11	1:100
F4/80	BioLegend, San Diego, CA	BM8	1:100

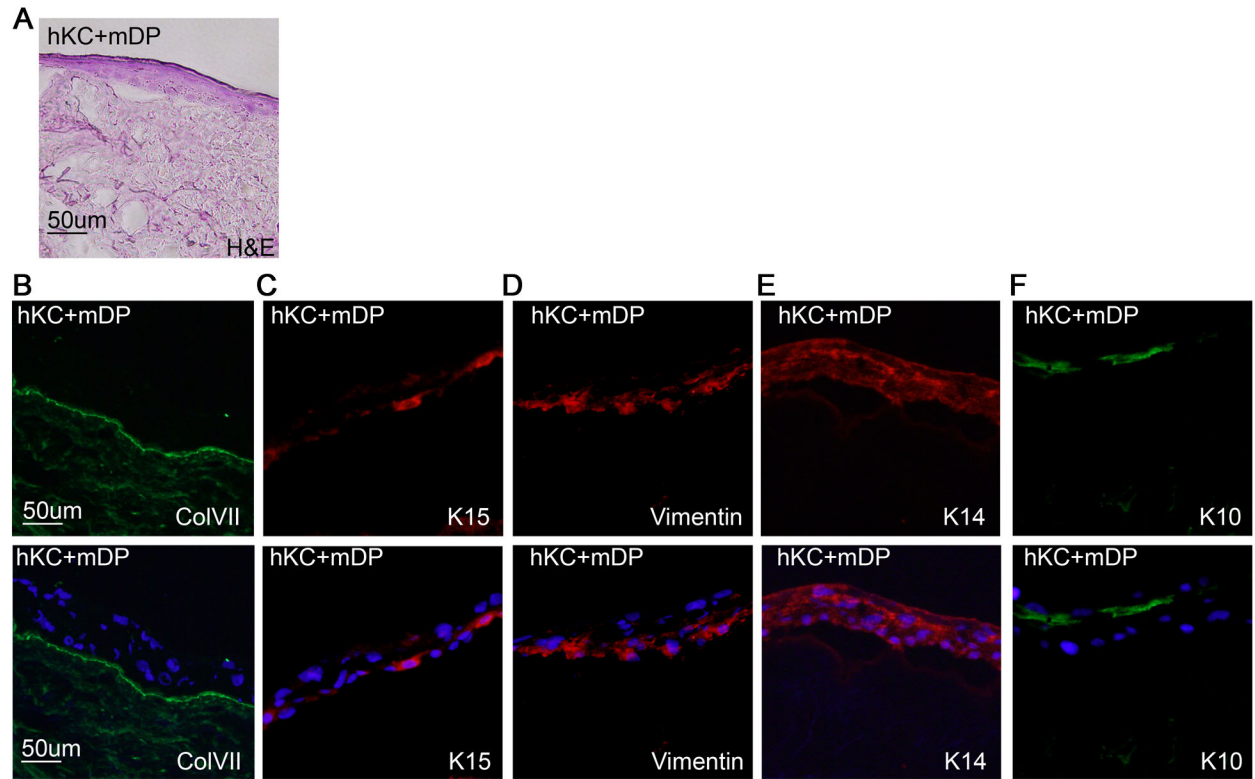
# Supplementary Figure S1. Mouse dermal papilla isolation from whisker hair.



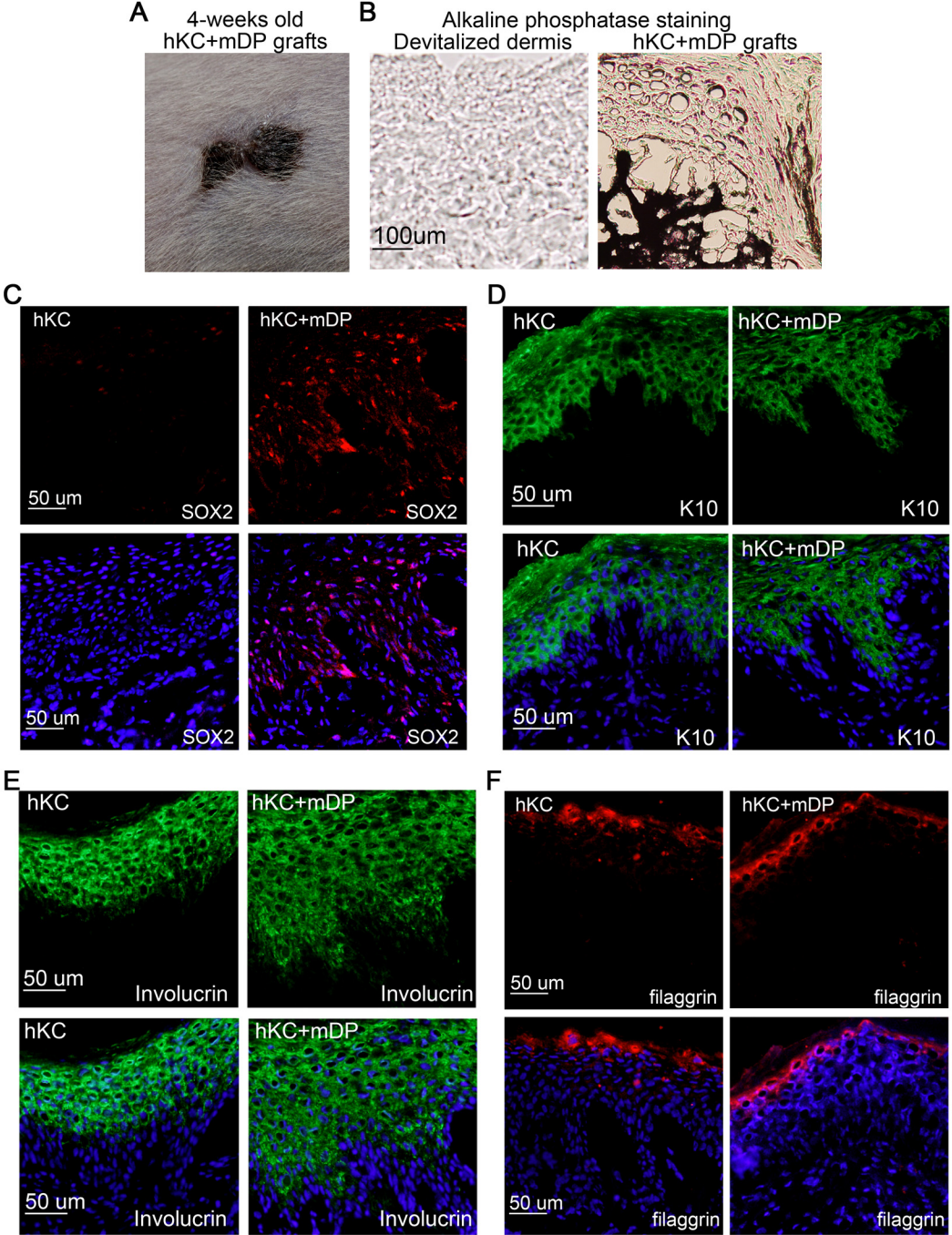
Supplementary Figure S2. Characterization of devitalized split-thickness human dermis.



Supplementary Figure S3. Cell/matrix composites displayed preliminary stratification after 1 week in culture.



Supplementary Figure S4. 4-weeks old skin grafts contained alkaline phosphatase and Sox2-positive cells, and expressed differentiation markers.





Supplementary Figure S5. Regenerated skin grafts contained myeloid cells.

