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Collagen XVII deficiency alters epidermal patterning

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Short title

COL17 and epidermal patterning

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Abstract

Vertebrates exhibit patterned epidermis, exemplified by scales/interscales in mice tails and grooves/ridges on the human skin surface (microtopography). Although the role of spatiotemporal regulation of stem cells (SCs) has been implicated in this process, the mechanism underlying the development of such epidermal patterns is poorly understood. Here, we show that collagen XVII (COL17), a niche for epidermal SCs, helps stabilize epidermal patterns. Gene knockout and rescue experiments revealed that COL17 maintains the width of the murine tail scale epidermis independently of epidermal cell polarity. Skin regeneration after wounding was associated with slender scale epidermis, which was alleviated by overexpression of human COL17. COL17-negative skin in human junctional epidermolysis bullosa showed a distinct epidermal pattern from COL17-positive skin that resulted from revertant mosaicism. These results demonstrate that COL17 contributes to defining mouse tail scale shapes and human skin microtopography. Our study sheds light on the role of the SC niche in tissue pattern formation.

Introduction

The skin is the body's outermost organ and is composed of the multilayered epithelium, the epidermis, and the underlying dermis. The epidermis serves as a physical barrier to pathogens and prevents water leakage from the body ¹. The epidermis is maintained by a fine-tuned balance between the proliferation and differentiation of epidermal stem cells (SCs), which reside in the epidermal basal layer ². Epidermal SCs need niche proteins such as integrins and collagen XVII (COL17) for their proper function ^{3,4}. Functional loss of these proteins leads to transient hyperproliferation of the developing epidermis due to disturbed SC maintenance ⁵⁻⁷.

Vertebrates have distinct skin patterns. In some, the patterns are visible through melanin distribution in the skin (e.g., zebra and tiger stripes); in others, the allocation of skin components forms patterns (e.g., human microtopography, hair follicles (HFs), and fish scales). Murine tail skin serves as a robust model for examining epidermal pattern formation ⁸. The tail epidermis consists of scale (parakeratotic) and interscale (orthokeratotic) areas, which are arranged alternately. These scale and interscale areas are distinguished by the expression of keratin 31 (K31) and keratin 10 (K10), respectively ⁹. Label-

retaining and lineage-tracing experiments have revealed that K10+ interscale epidermis is slow-cycling, whereas K31+ scale areas are fast-cycling. Two distinct stem cell populations (Dlx1+ and Slc1a3+) give rise to interscale and scale epidermis, respectively ¹⁰, although it is unclear how these cell populations are arranged into scale/interscale patterns.

The expression of epidermal SC niche proteins, including integrins and COL17, shows alternate patterns in the human epidermis, where their expression is enriched in the epidermis facing the dermal protrusion but not in the epidermal rete ridges ¹¹⁻¹³. Conversely, the scale/interscale patterns are absent in β1 integrin-null tail epidermis ¹⁴. These previous studies suggest the involvement of SC niche proteins in epidermal pattern formation. However, whether these SC niche proteins indeed regulate the epidermal patterns and the mechanisms underlying such regulation are unknown.

Here, we demonstrate that COL17, an SC niche protein ^{7,15-17}, helps in the formation of proper epidermal patterns in mice and humans. Interestingly, disturbed epidermal patterning through COL17 deletion is independent of aberrant epidermal cell polarity, but could involve wound-related skin changes.

Material and methods

Animals

C57BL/6 (wild-type, WT) mice were purchased from Clea (Tokyo, Japan). Col17a1-/-, K14-hCOL17 (h: human, a courtesy gift from Prof. Kim B Yancey), hCOL17+; Col17a1-/-, K5-Cre; aPKCλ^{ΔE5/ΔE5} (aPKCλ eKO), and *Prkcz-*/- (aPKCζ KO) were generated as previously described ¹⁸⁻²¹. aPKC $\lambda^{\Delta E5/\Delta E5}$ and *Prkcz*+/- mice were used as aPKC λ eKO and aPKC ζ KO controls, respectively, aPKCλ eKO and aPKCλ^{ΔE5/ΔE5} littermates were generated by mating aPKCλ eKO and aPKCλ^{ΔE5/ΔE5} mice. aPKCζ KO and Prkcz+/- littermates were generated by mating aPKCζ KO and Prkcz+/mice. Littermate Col17a1+/- or Col17a1+/+ mice were used as Col17a1-/or hCOL17+; Col17a1-/- control. Col17a1-/-, Col17a1+/-, and Col17a1+/+ littermates were generated by mating *Col17a1+/-* female and male mice. hCOL17+;Col17a1-/-, Col17a1+/-, and Col17a1+/+ littermates were generated by mating hCOL17+; Col17a1+/- and Col17a1+/- mice. K14hCOL17 and WT littermates were generated by mating K14-hCOL17 and WT mice. Female mice were used for the wound-healing experiments on WT mice. Otherwise, sex-matched mice were used in each experiment.

The institutional review board of the Hokkaido University Graduate School of Medicine approved all animal studies described below.

Cell Culture

hTERT-immortalized human primary keratinocytes (KerCT; ATCC, Manassas, VA, USA), and spontaneously transformed murine keratinocytes (PAM212) ²² were cultured in serum-free keratinocyte growth medium (KGM; Lonza). The cells were transfected with 10 μM of human *COL17A1* siRNA, murine *Col17a1* siRNA or the control (Mock) (Silencer Select siRNAs, Thermo Fisher Scientific, Waltham, Massachusetts, USA) using Lipofectamine 2000 (Thermo Fisher Scientific) and Opti-MEM (Thermo Fisher Scientific). The cells were analyzed at 48 or 72 h after the knockdown procedure.

Antibodies

The following antibodies were used: polyclonal anti-K31 (Progen, hHa1), polyclonal anti-K10 (BioLegend, Poly19054), polyclonal anti-K6 (BioLegend, Poly19057), monoclonal anti-cytoplasmic COL17 (Abcam, Cambridge, MA, USA, ab186415/EPR14758).

Quantitative RT-PCR (qRT-PCR)

RNA was isolated from the tail epidermis or cultured cells using the RNeasy

Mini kit (QIAGEN, Hilden, Germany), and cDNA was synthesized using the

SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific,

Waltham, MA, USA). qRT-PCR was carried out using the designated primers

and fast SYBR Green (Thermo Fisher Scientific) in a STEP-One Plus sequence

detection system (Applied Biosystems, Waltham, MA, USA).

The following primers were used for the analysis: Forward primer and reverse primer are: murine *Krt6a*, CACGTTAAGAAGCAGTGTGCC and GCTCTGAGCACGGGATTCT; murine *Krt6b*, AGGAGTGCAGGTTGAATGGTG and AAAAAGAGAGAGAGGAGAGACACA; murine *Krt16*,

TCCCAGCTCAGCATGAAAG and GAGCTGTGGATATTCTCGCCA; murine

Krt17, AGACAGAGAACCGCTACTGC and CGGGTGGTCACAGGTTCTTTT;

murine Col17a1, GATGGCACTGAAGTCACCGA and

TATCCATTGCTGGTGCTCCC; murine *Cyc1*,

ATCGTTCGAGCTAGGCATGG and GCCGGGAAAGTAAGGGTTGA; human KRT17, CAGAGAACCGCTACTGCGTG and

GCTCAGCATGAAAGCATCCC and GACCTCGCGGGAAGAATAGG; human

KRT6A, AGTGCAGGCTGAATGGCGAA and TGGGACCGAGAGCTAGCAGA;

human KRT6B, TTCATCGACAAGGTGCGGT and

GTCACCGGTTCTTTCTTGTACTG; human KRT16,

CAGCTCCGAGTCCAGACGAC; human COL17A1,

TCAACCAGAGGACGGAGTCA and TCGACTCCCCTTGAGCAAAC; human RNA18SN1 (18S), GGCGCCCCCTCGATGCTCTTAG and GCTCGGGCCTGCTTTGAACACTCT.

Immunofluorescence staining

Paraffin sections were deparaffinized and boiled in citrate or EDTA buffer for 20 min in a microwave oven. Frozen sections or cultured cells were fixed in 4% paraformaldehyde (PFA) for 10 min at room temperature (RT), or cold acetone or used without fixation. After washing with PBS, sections were treated with blocking buffer (0.5% fish skin gelatine, 5% goat serum, 4% BSA in PBS) for 1 h. The samples were incubated with primary antibodies at 4 °C overnight and were subsequently incubated with secondary antibodies conjugated with Alexa fluor 488, Alexa fluor 647, or FITC at RT for 1 h. Nuclei were stained with 4',6-

diamidino-2-phenylindole (DAPI). Images were obtained using confocal microscopy (FV1000, Olympus, Tokyo, Japan; LSM-710, Zeiss, Germany) or fluorescence microscopy (BZ-9000, Keyence, Osaka, Japan).

Whole mount staining

Tail skin was incubated in 5 mM EDTA/PBS on a shaker at 37 °C for 4 h to separate the epidermis from the dermis. Epidermal sheets were fixed in 4% paraformaldehyde (PFA) for 1h at RT. After blocking, epidermal sheets were incubated with primary antibodies overnight at RT, and then washed in 0.2% Tween/PBS. Samples were subsequently incubated with secondary antibodies. After washing, epidermal sheets were mounted on glass slides in Mowiol solution. The images of whole mount stained samples were obtained using FV1000 confocal laser scanning microscope (Olympus, Tokyo, Japan) or BZ9000 fluorescence microscope (Keyence, Osaka, Japan). The size and shape of the scales near the midline of the tail epidermis were analyzed using ImageJ (NIH, Bethesda, MD, USA). When the scales of the littermates were compared, the area, length, and width of the scales were normalized to the

whole tail equivalents to exclude the effects of the organismal size of each mouse.

Wound healing experiments

The surface of the tail skin (epidermis and papillary dermis, approximately 5 × 4 mm in size) was removed using a scalpel from 1-month-old (1MO) WT mice or K14-hCOL17 mice to produce superficial skin wounds (**Supplementary Fig. 5A**). The wounded skin was collected and analyzed when the healing process was complete (typically 4–6 weeks after wounding) and at a later time point (3 months after wounding).

Junctional epidermolysis bullosa (JEB) skin analysis

Photographs of the skin of a JEB patient ²³ who was compound heterozygous for c.1179del (p.Ala394Leufs*9) and c.4159C>T (p.Gln1387*) in *COL17A1* (NM_000494.4) were taken by TG-5 (Olympus). Three revertant mosaicism spots and three adjacent diseased skin areas from the upper arm were further analyzed as described below. Although the fingerprints have been absent in other JEB patients with *COL17A1* p.Arg1303Gln mutations ²⁴⁻²⁶, the patient in

our study maintained his fingerprints. This discrepancy is probably due to the difference of the *COL17A* mutations. The institutional review board of the Hokkaido University Graduate School of Medicine approved all human studies described above (ID: 13-043). The study was conducted according to the principles of the Declaration of Helsinki. The participant provided written informed consent.

Quantification of the skin microtopography

We selected several regions like the one surrounded by the red circle in the left panels of **Supplementary Fig. 6A and 6B** for Diseased skin and Reverted skin. We converted the image inside each circle to a grayscale image and calculated the two-dimensional autocorrelation function ²⁷ (right panels in **Supplementary Fig. 6A and 6B**). Using the two-dimensional autocorrelation function, the characteristic direction of the epidermal pattern was detected by determining the direction in which the autocorrelation in the range of a distance less than 1 mm is the largest. The one-dimensional autocorrelation function was calculated (**Supplementary Fig. 6C, 6D**) in the direction perpendicular to the characteristic direction, represented by the red lines in the right panels in **Supplementary**

Fig. 6A and 6B. The peak height (Δ) of the one-dimensional autocorrelation function in the range of distance less than 1 mm was adopted to quantify the regularity of the pattern. In the case where no peak was detected, the peak height was set to zero. The above-mentioned image analyses were performed with ImageJ NIH (Bethesda, Maryland, USA; https://imagej.nih.gov/ij/) by preparing a plug-in.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). p-values were determined using Welch's t-test, Student's t-test, or Mann-Whitney test. p-values are indicated as *0.01<p<0.05, **0.001<p<0.01, ***0.0001<p<0.001, ****p<0.0001. The values are shown as violin plots. Violin plots show median (dashed line) and quartiles (dotted line).

RESULTS

COL17 deficiency alters scale shape in the tail skin

We first characterized the scale/interscale patterning of Col17a1-/- 19 tail epidermis. We selected the time point of 1MO, when the scales became mature 8 (Supplementary Fig. 1). Immunofluorescence of the tail sections showed K10+ interscale and K31+ scale alternate patterns in both Col17a1-/- and controls (Fig. 1A; Supplementary Fig. 2A). We then examined the scale shape by whole skin imaging. We defined the length and width of a scale as the diameter of the anterior-posterior (AP) and lateral-medial (LM) axes, respectively (Fig. 1B). Since the length and width of whole tail samples varied among the mice (Supplementary Table 1), we normalized the scale length/width by dividing them by each mouse tail length/width to exclude the effects of organismal size in the analysis. Whole-mount imaging showed that the scale size was smaller and the shape was more slender (shorter on the LM axis) in the Col17a1-/- tail epidermis than in the littermate controls (Fig. 1C-1F, Supplementary Fig. 2B). The shorter scale width in Col17a1-/- explains the smaller size of Col17a1-/- scales because their length was comparable to that of the controls (Fig. 1G, 1H). Although the basal cell number of maximum diameter on the AP axis was comparable between Col17a1-/- and control scales, the cell number on the LM axis was smaller in Col17a1-/- scales than in control scales (Supplementary Fig. 2C), implying the altered alignment of the basal cells in Col17a1-/- scales. As the C57BL/6 (WT) scale shape was wider at P14 (postnatal day 14) than at 1MO (Fig. 1I), the slender scale shape in Col17a1-/- mice was not due to the delayed development of the mice. Col17a1-/- tails were generally smaller but not more slender than those of controls (Supplementary Table 1), excluding the involvement of organismal proportions in Col17a1-/- slender scales. Transgenic rescue by the expression of hCOL17 under the keratin 14 (K14) promoter in Col17a1-/- mice 19 reversed the slender scale phenotype (Fig. 2A-2C). These data indicate that COL17 helps to define the scale proportion.

aPKC deregulation does not phenocopy *Col17a1-l-* scale shape
Atypical protein kinase C (aPKC) is a key regulator of epithelial polarity 28 , and the epidermis expresses two aPKC isoforms (aPKCλ and aPKCζ) 29 . The ablation of aPKCλ in the epidermis (K5-Cre; $^{aPKCλ}^{\Delta E5/\Delta E5}$, aPKCλ eKO) and $^{Col17a1-l-}$ mice share premature aging phenotypes such as gray hair and hair loss 15,19,21,28 . This phenotypic similarity has been proposed to be a

consequence of the interaction between COL17 and the aPKC complex ³⁰. We asked if the destabilized aPKC accounts for the slender scale shape of *Col17a1-/-* mice (**Fig. 3A-3F**). Whole-mount imaging showed that the scale size was smaller in aPKCλ eKO than in littermate controls, but its scale proportion (width/length ratio) was wider than that of controls (**Fig. 3A, 3C, 3D**), which is in contrast to the slender scales of *Col17a1-/-* mice (**Fig. 2**). aPKCζ knockout (aPKCζ KO, *Prkcz-/-*) mice, which have no apparent skin phenotype ²⁰, showed slightly larger scales, while the width/length ratio did not exhibit significant change (**Fig. 3B, 3E, 3F**). These results indicate that the aberrant cell polarity in *Col17a1-/-* epidermis ^{17,30} is not involved in altering the scale shape.

Expression of wound-induced keratins is pronounced in *Col17a1-/-* tail epidermis

One of the factors that may affect scale shape is the cytoskeleton of epidermal keratinocytes. Keratin 6, 16, and 17 (K6/K16/K17) are the well-known keratins expressed upon physical injury. We reasoned that these wound-induced keratins might be enriched in *Col17a1-/-* epidermis at steady state because COL17 deficiency leads to epidermolysis bullosa in humans ³¹ and shows skin

fragility in mice ¹⁹. Quantitative PCR revealed that the gene expression of these wound-induced keratins was higher in *Col17a1-/-* tail epidermis (**Fig. 4A**). In addition to gene expression, immunofluorescence analyses showed ectopic K6 expression in *Col17a1-/-* tail epidermis at 1MO. K6 expression in *Col17a1-/-* tail epidermis was also observed during the developmental stages (P1 and P14; **Fig. 4B-4D**). These data indicate that keratin profiles are skewed towards the wound-induced subsets in *Col17a1-/-* epidermis. In contrast, human or murine cultured keratinocytes knocked down for *COL17A1* or *Col17a1* did not result in the expression of wound-induced keratins at the mRNA or protein level (**Supplementary Fig. 3-4**), suggesting that this phenotype is dependent on the in vivo setting. Rather, *KRT16* and *Krt6b* expression was reduced in human and murine knockdown experiments, respectively, for unknown reasons.

Scale shape becomes slender after skin regeneration, and COL17 overexpression rescues the phenotype

The expression of wound-induced keratins in *Col17a1-/-* epidermis led us to ask whether wounding itself alters the tail scale shape upon skin regeneration (**Fig. 5A-5F**). The regenerated tail epidermis (4 to 6 weeks after wounding) exhibited

a more slender scale shape than the non-lesional areas (**Fig. 5A, 5C, 5D**), recapitulating the *Col17a1-l-* scale (**Fig. 2**). The slender scale phenotype in the regenerated epidermis was not reversed 3 months after wounding (**Supplementary Fig. 5B-5D**). To see that additive COL17 prevents the alteration of the scale shape in the regenerated epidermis, we utilized K14-hCOL17 transgenic mice, which overexpress hCOL17 under the K14 promoter. The scale shape of the regenerated K14-hCOL17 skin was not as slender as that of the regenerated WT skin (**Fig. 5B, 5E, 5F**). These results suggest that COL17 prevents wound-induced scale deformation.

COL17 influences human skin microtopography

We finally asked whether the presence or absence of COL17 also affects epidermal patterning in humans. Although scale/interscale epidermal patterns in mouse tails are not conserved, skin surface patterns consisting of grooves and ridges are visible in humans. We took advantage of the revertant mosaicism in epidermolysis bullosa (EB), in which the mutated genes are corrected spontaneously ^{32,33}. We compared COL17-negative (diseased) and COL17-positive (revertant) skin from a junctional EB (JEB) patient with *COL17A1*

mutations ²³ (**Fig. 6A-6C**, **Supplementary Fig. 6**). The diseased skin surface appeared coarse, while the revertant skin was smooth (**Fig. 6B**). We calculated the autocorrelation functions of these skin images to quantify the skin microtopography and found that the diseased skin shows a distinct pattern from the revertant skin (**Fig. 6D**). These findings demonstrate that COL17 is a deterministic factor of epidermal patterning in mice and humans.

Discussion

COL17 is a hemidesmosomal protein that anchors basal keratinocytes to the dermis. COL17 stabilizes hemidesmosomes by binding to various basement membrane zone proteins including BP230 ^{34,35}, α6 integrin ³⁶, β4 integrin ^{35,37-39}, plectin ^{35,40}, laminin-332 ^{25,41}, and type IV collagen ^{42,43}. As a consequence, COL17 deficiency results in epidermolysis bullosa ^{19,31}. Recently, COL17 has also been highlighted as an SC niche protein of HFs and epidermis, and its deficiency destabilizes epithelial SC maintenance ^{7,15-17}. Our study provides new insights into COL17 biology. *Col17a1-I-* mice have slender tail scales and are characterized by the expression of wound-induced keratins in the epidermis. In line with this, the regenerated epidermis after wounding shows slender tail scales. Human COL17 overexpression reverses the alteration of scale shapes upon wounding (**Fig. 7**).

Epidermal cell polarity regulates symmetrical and asymmetrical cell division of basal keratinocytes ⁴⁴⁻⁴⁶ and is regulated by the aPKC complex ⁴⁷⁻⁴⁹. Increased asymmetrical cell division in aPKCλ eKO epidermis ²⁸ and possible stem cell depletion explain the smaller scales in aPKCλ eKO mice (**Fig. 3**). Although COL17 interacts with the aPKC complex ³⁰ and helps maintain

epidermal cell polarity ^{17,30}, *Col17a1-/-* did not exhibit proportionally small scales as seen in aPKCλ eKO mice. This phenotypical difference indicates that the slender scale phenotype in *Col17a1-/-* mice is independent of aberrant cell polarity.

A limitation of our study is that it does not explain which contributes more to the altered epidermal patterns in *Col17a1-/-* mice: epidermal SC instability or weakened epidermal-dermal adhesion. As the HF abnormality of *Col17a1-/-* mice becomes apparent at 3 months old ¹⁵, it is not very likely that HFs are involved in the phenotypes of epidermal patterns at 1 month old in our study. Furthermore, the hard palate in the oral mucosa, where HFs are absent, shows an epithelial pattern of fast- and slow-cycling stem cells ⁵⁰, recapitulating the epidermal pattern of the tail skin. Thus, HFs might not be essential for epithelial pattern formation.

Among various skin patterns, fingerprints, also called dermatoglyphics, are the most well-characterized in humans. Fingerprints show an alternate pattern of epidermal ridges and grooves. Loss of fingerprints has been described in EB patients, including Kindler syndrome ^{51,52} and junctional EB with *COL17A1* p.Arg1303Gln mutation ²⁴⁻²⁶. Our study has also demonstrated that

the presence of COL17 alters human skin microtopography (**Fig. 6**). These facts corroborate the role of COL17 in epidermal patterning and highlight COL17 as a therapeutic target for wound-induced skin deformations.

Human skin microtopography is not identical to mouse tail scale patterns because humans lost their tails after evolutionarily branching off from other primates. It has not been determined whether human skin ridges or grooves correspond to the fast- or slow-cycling areas that characterize mouse tails. However, the data that COL17 absence disturbs proper skin patterning in both humans and mice point to the involvement of COL17 in regulating skin surface texture across species.

Wound repair in mouse back skin requires wound contraction ⁵³; however, the contribution of wound contraction has been regarded as minimal in the tail skin ⁵⁴. Recent studies suggest that two-thirds of tail wound healing is due to epithelial regeneration, while wound contraction explains the remainder ⁵⁵. We believe that wound contraction does not play a major role in the slender scale phenotype in the regenerated tail skin (**Fig. 5**) for the following reasons: 1) the scale shape in the regenerated skin would become proportionally small rather than slender if the wound contraction affected this phenotype, and 2) the

slender scale phenotype in the regenerated skin is rescued by K14-driven human COL17 overexpression, which might not influence wound contraction because the transgene expression is confined to the epidermis. The slender scales in *Col17a1-/-* mice most likely represent wound-related skin changes that involve the expression of wound-induced keratins in *Col17a1-/-* epidermis (**Fig.**4). However, the mechanisms by which wound-related skin changes affect epidermal patterning need further investigation.

In conclusion, our study highlights the unrecognized role of COL17 in epidermal patterning. We propose that COL17 modulation can be utilized to prevent epidermal deformation upon wounding.

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Ethics Approval and Consent to Participate

The institutional review board of the Hokkaido University Graduate School of Medicine approved all human studies described above (ID: 13-043). The study was conducted according to the principles of the Declaration of Helsinki. The participant provided written informed consent.

Author contributions

Y. W. performed the experiments, analyzed the data, interpreted the results, and wrote the manuscript. K.H. analyzed the data, interpreted the results, and wrote the manuscript. H.K., M.W., Y.F., S.T., SI.O., T.H., and N.W. performed the experiments and analyzed the data. M.N. and S.H. interpreted the results and supervised the study. K.N. designed the experiments, analyzed the data, interpreted the results, wrote the manuscript and supervised the study.

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Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that no conflicts of interest exist.

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Figure legends

Fig. 1. Slender tail scales of Col17a1-/- mice.

(A) Images showing K31 and K10 staining of *Col17a1-/-* and control tail skin samples at 1MO (n=4). Scale bar: 500 μm. (B) Schematic of distribution of scales in mouse tail epidermis. (C) K31 whole mount staining images of *Col17a1-/-* and littermate control tail epidermis at 1MO (n=3). Scale bar: 500 μm. (D) Phase-contrast images of *Col17a1-/-* and littermate control tail epidermis at 1MO (n=4). Scale bar: 500 μm. (E-H) Quantification of the size and shape of tail scales. Scale area (E), width/length (F), length (G), and width (H) of *Col17a1-/-* and littermate control tail scales at 1MO are shown (n=298 scales from three control and 413 scales from three *Col17a1-/-* mice). (I) Width/length ratio of tail scales in P14 and 1MO WT mice (n=148 scales from three P14 WT mice and 123 scales from three 1MO WT mice). ****p<0.0001, Welch's t-test.

Fig. 2. Restoration of the scale shape in *Col17a1-/-* tail epidermis by human COL17 overexpression.

(A) Whole mount phase-contrast imaging of hCOL17+;*Col17a1-/-* and littermate control (hCOL17-;*Col17a1+/+* or hCOL17-;*Col17a1+/-*) tail epidermis at 1MO (n=3). Scale bar: 500 μm. (B, C) Quantification of the size and shape of tail scales. Scale area (B) and width/length (C) of hCOL17+; *Col17a1-/-* and littermate control tail scales at 1MO are shown (n=266 scales from three control and 359 scales from three hCOL17+; *Col17a1-/-* mice). The raw data used for **Fig. 2C** are shown in **Supplementary Table 2**. *0.01<p<0.05, Welch's t-test.

Fig. 3. Small, but not slender, tail scales of aPKCλ eKO mice.

(A) Whole mount phase-contrast imaging of aPKCλ eKO and littermate control tail epidermis (n=3). Scale bar: 500 μm. (B) Whole mount phase-contrast imaging of aPKCζ KO and littermate control tail epidermis (n=3). Scale bar: 500 μm. (C-F) Quantification of the size and shape of tail scales. Scale area and width/length of aPKCλ eKO (C, D) and aPKCζ KO (E, F) tail scales at 1MO are shown (n=226 scales from three control mice and 357 scales from three aPKCλ eKO mice, n=300 scales from three control mice and 317 scales from three aPKCζ KO mice). The raw data used for **Fig. 3F** are shown in **Supplementary Table 3**. ****p<0.0001, *0.01<p<0.05, Welch's t-test.

Fig. 4. Expression of wound-induced keratins in Col17a1-/- tail epidermis.

(A) Gene expression of *Krt6a*, *Krt6b*, *Krt16*, and *Krt17* in the tail epidermis of *Col17a1-/-* and littermate control at 1MO (n = 7). *0.01<p<0.05, Student's t-test. (B-D) K6 staining of *Col17a1-/-* and littermate control at P1 (B, n = 3), P14 (C, n = 3), and 1MO (D, n = 3). Scale bar: 100 μ m.

Fig. 5. Slender tail scales after skin regeneration and COL17 transgenic rescue.

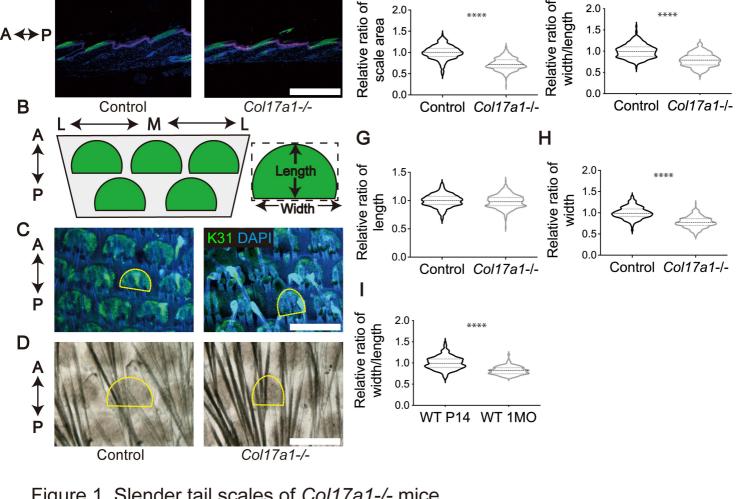
(A-B) Whole mount phase-contrast imaging of WT (A) and K14-hCOL17 (B) tail epidermis 4-6 weeks after wounding (n=4 (WT) and 6 (K14-hCOL17), respectively). Scale bar: 500 μm. (C-F) Quantification of the size and shape of tail scales. Scale area and width/length of WT (C, D) and K14-hCOL17 (E, F) tail scales after skin regeneration are shown (n=542 scales from unwounded areas and 467 scales from regenerated areas from four WT mice, n=424 scales from unwounded areas and 440 scales from regenerated areas from six K14-hCOL17 mice). ****p<0.0001, Welch's t-test.

Fig. 6. Distinct epidermal patterns of COL17-positive and negative human skin.

(A) The upper arm of a junctional epidermolysis bullosa patient who is compound heterozygous for c.1179del (p.Ala394Leufs*9) and c.4159C>T (p.Gln1387*) in *COL17A1* (NM_000494.4). The revertant skin areas are circled by dotted lines. (B) Representative images of the diseased and revertant skin. Scale bar: 3 mm. These images are exemplified in **Supplementary Fig. 6**. (C) COL17 labeling of the diseased and revertant skin. Scale bar: 100 μm. (D) Quantification of skin microtopography (n=75 spots (radius: 3 mm) from three skin areas, respectively). The degree of regularity on the epidermal pattern was quantified by the peak height of the autocorrelation function (**Supplementary Fig. 6**). ***0.0001<p<0.001, Mann-Whitney test.

Fig. 7. COL17-related epidermal patterning.

A graphical abstract of the study. COL17 deficiency or wounding leads to slender scale epidermis, which is reversed by overexpression of human COL17 in the epidermis.



E

Figure 1. Slender tail scales of Col17a1-/- mice.

Α

K31 (scale) DAPI K10 (interscale)

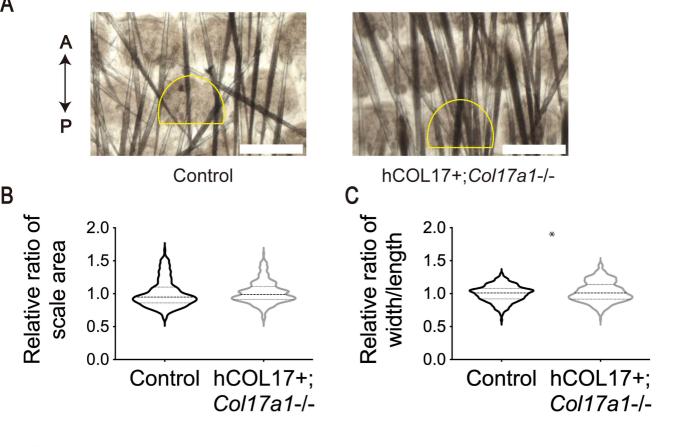


Figure 2. Restoration of the scale shape in *Col17a1-/-* tail epidermis by human COL17 overexpression

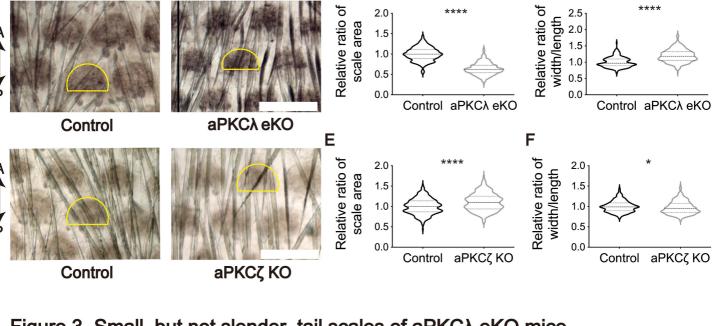


Figure 3. Small, but not slender, tail scales of aPKCλ eKO mice.

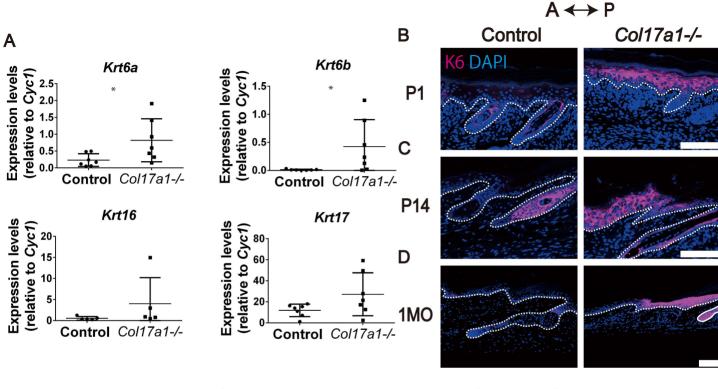


Figure 4. Expression of wound-induced keratins in *Col17a1-/-* tail epidermis.

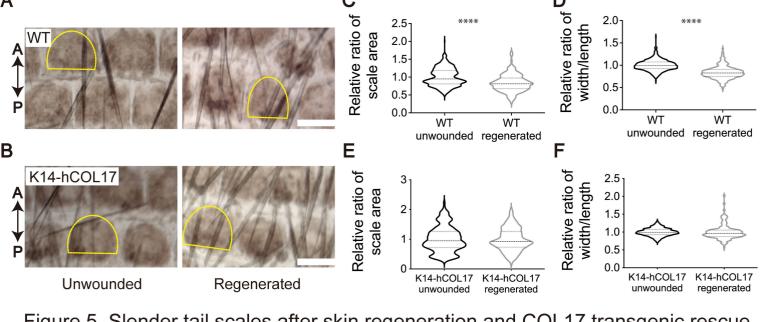


Figure 5. Slender tail scales after skin regeneration and COL17 transgenic rescue.

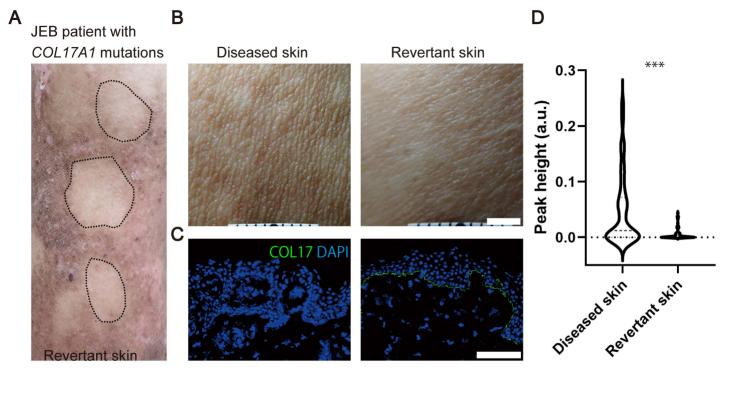


Figure 6. Distinct epidermal patterns of COL17-positive and negative human skin.

Mouse tail epidermis

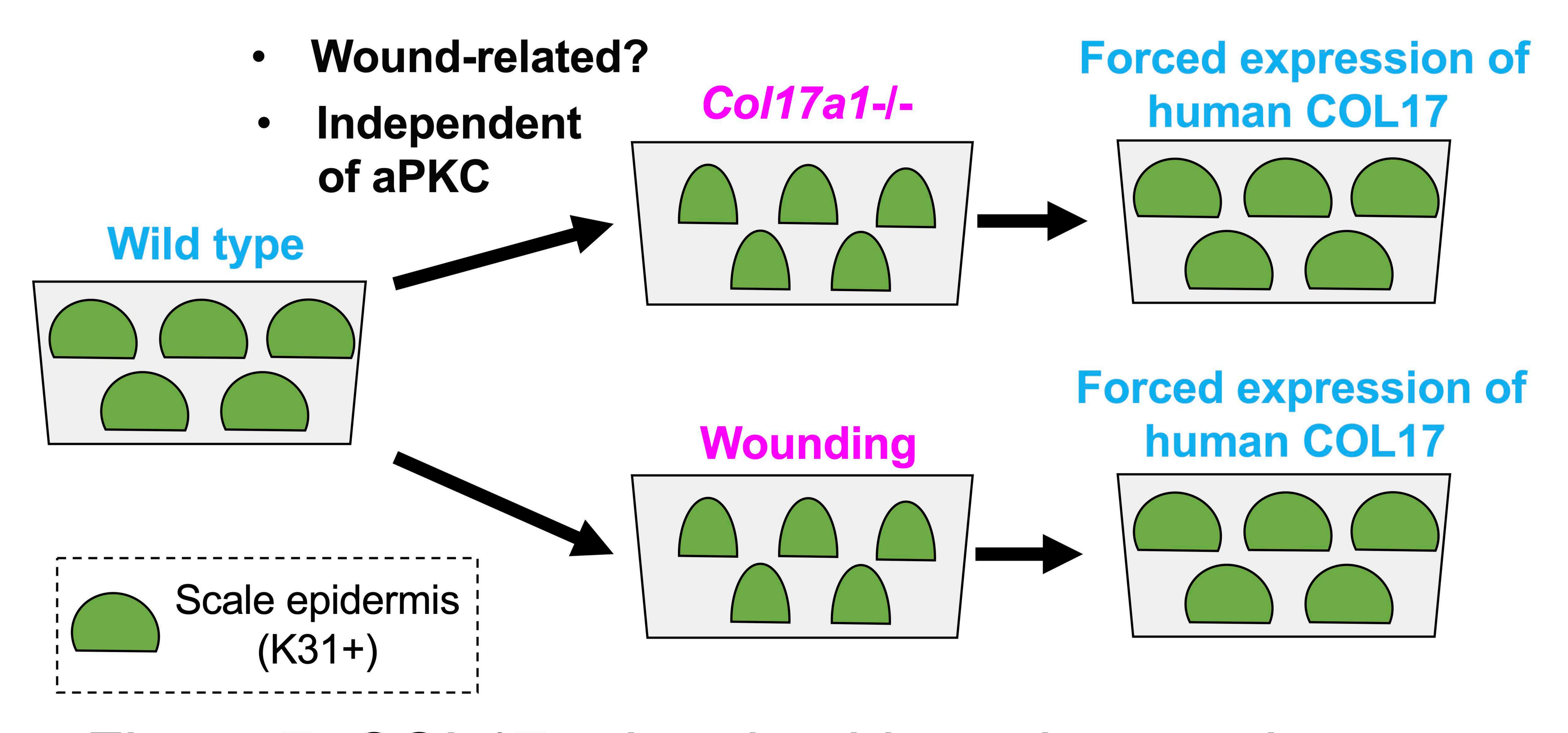


Figure 7. COL17-related epidermal patterning