

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

## Hair follicle stem cell progeny heal blisters while pausing skin development

### **This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1812492> since 2021-10-15T18:31:17Z

*Published version:*

DOI:10.15252/embr.202050882

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1 **EMBOR-2020-50882V3.**

2 **Hair follicle stem cell progeny heal blisters while pausing skin development**

3

4 Yu Fujimura<sup>1</sup>, Mika Watanabe<sup>1,2</sup>, Kota Ohno<sup>3</sup>, Yasuaki Kobayashi<sup>3</sup>, Shota Takashima<sup>1</sup>,

5 Hideki Nakamura<sup>1</sup>, Hideyuki Kosumi<sup>1</sup>, Yunan Wang<sup>1</sup>, Yosuke Mai<sup>1</sup>, Andrea Lauria<sup>2,4</sup>,

6 Valentina Proserpio<sup>2,4</sup>, Hideyuki Ujiie<sup>1</sup>, Hiroaki Iwata<sup>1</sup>, Wataru Nishie<sup>1</sup>, Masaharu

7 Nagayama<sup>3,5</sup>, Salvatore Oliviero<sup>2,4</sup>, Giacomo Donati<sup>2</sup>, Hiroshi Shimizu<sup>1†</sup>, Ken Natsuga<sup>1\*</sup>

8

9 <sup>1</sup>Department of Dermatology, Hokkaido University Graduate School of Medicine,

10 Sapporo, Japan

11 <sup>2</sup>Department of Life Sciences and Systems Biology, Molecular Biotechnology Centre,

12 University of Turin, Turin, Italy

13 <sup>3</sup>Research Institute for Electronic Science, Hokkaido University, Sapporo, Japan

14 <sup>4</sup>Italian Institute for Genomic Medicine, Candiolo (TO), Italy

15 <sup>5</sup>Japan Science and Technology Agency, CREST, Kawaguchi, Japan

16

17 <sup>†</sup>Deceased in February 2021

18

## 19 **Conflicts of Interest**

20 The authors declare that no conflicts of interest exist.

21

22

23 \*Correspondence and reprint requests to:  
24 Ken Natsuga, M.D., Ph.D.  
25 Department of Dermatology, Hokkaido University Graduate School of Medicine  
26 North 15 West 7, Sapporo 060-8638, Japan  
27 Telephone: +81-11-716-1161, ext. 5962  
28 E-mail: natsuga@med.hokudai.ac.jp  
29

30 **Abstract**

31 Injury in adult tissue generally reactivates developmental programs to foster  
32 regeneration, but it is not known whether this paradigm applies to growing tissue. Here,  
33 by employing blisters, we show that epidermal wounds heal at the expense of skin  
34 development. The regenerated epidermis suppresses the expression of tissue  
35 morphogenesis genes accompanied by delayed hair follicle (HF) growth. Lineage  
36 tracing experiments, cell proliferation dynamics, and mathematical modeling reveal that  
37 the progeny of HF junctional zone stem cells, which undergo a morphological  
38 transformation, repair the blisters while not promoting HF development. In contrast, the  
39 contribution of interfollicular stem cell progeny to blister healing is small. These findings  
40 demonstrate that HF development can be sacrificed for the sake of epidermal wound  
41 regeneration. Our study elucidates the key cellular mechanism of wound healing in skin  
42 blistering diseases.

43

44 **Keywords**

45 Wnt signaling, epidermal stem cells, epidermolysis bullosa, basement membrane zone

46



47 **Introduction**

48 Tissue responds to injury by transforming its cellular components and extracellular  
49 matrix from homeostasis into a regenerative state. Damaged tissue typically reactivates  
50 an embryonic gene program in epithelia to accelerate tissue regeneration (Fernandez  
51 Vallone *et al*, 2016; Miao *et al*, 2019; Nusse *et al*, 2018; Yui *et al*, 2018). However, it is  
52 unknown whether this phenomenon also applies to injuries in developing tissue, in  
53 which the embryonic gene expression program is switched on before damage.

54 The epidermis is a stratified epithelium of the skin and is located on the surface of  
55 the body, where it serves as a barrier against external stimuli and microorganisms  
56 (Natsuga, 2014). Cellular proliferation and differentiation in the epidermal basal layer,  
57 where epidermal stem cells (SCs) are present, are fine-tuned to maintain the integrity of  
58 the epidermis (Donati & Watt, 2015). The epidermis attaches to the dermis through  
59 proteins in the epidermal basement membrane zone (BMZ) (McMillan *et al*, 2003).  
60 Epidermal BMZ proteins function as a niche for epidermal SCs (Watt & Fujiwara, 2011),  
61 and the loss of these proteins, such as  $\alpha 6$  integrin (ITGA6),  $\beta 1$  integrin, and collagen  
62 XVII (COL17), leads to transient epidermal proliferation (Brakebusch *et al*, 2000;  
63 Niculescu *et al*, 2011; Watanabe *et al*, 2017).

64 Skin wounding causes pain and carries a significant risk of bacterial infection. The  
65 sources of skin wound healing have been extensively investigated in experimental  
66 animals (Dekoninck & Blanpain, 2019; Rognoni & Watt, 2018). Hair follicles (HFs),  
67 epidermal appendages, fibroblasts, and immune cells coordinate to heal the wound, and  
68 the contribution of each component can vary depending on the assay (Garcin *et al*,

69 2016). Wounding in adult skin induces the expression of genes regulating epidermal  
70 development, including SOX11 and SOX4 (Miao *et al.*, 2019).

71 Conventional skin wounding assays have employed full-thickness skin wounds, in  
72 which all skin components are removed, including the epidermis, epidermal  
73 appendages, dermis, and subcutaneous fat tissue. In contrast to conventional full-  
74 thickness skin wounds, epidermal detachment, as exemplified by subepidermal blisters,  
75 is distinctive because it does not affect the structures below the epidermis per se. The  
76 epidermis is detached from the dermis in several pathological conditions, such as burns  
77 (Chetty *et al.*, 1992), congenital defects in epidermal BMZ proteins (epidermolysis  
78 bullosa (EB)) (Fine *et al.*, 2014; Vahidnezhad *et al.*, 2019), autoimmunity to these  
79 proteins (pemphigoid diseases) (Schmidt & Zillikens, 2013), and severe drug reactions,  
80 such as Stevens-Johnson syndrome/toxic epidermal necrolysis (White *et al.*, 2018).  
81 Although the cells that contribute to the repair of full-thickness skin wounds have been  
82 identified (Aragona *et al.*, 2017; Dekoninck & Blanpain, 2019; Donati *et al.*, 2017;  
83 Gonzales & Fuchs, 2017; Ito *et al.*, 2005; Kang *et al.*, 2020; Page *et al.*, 2013; Park *et al.*,  
84 2017; Sada *et al.*, 2016), the cellular dynamics of subepidermal blister healing are  
85 completely unknown. In addition, full-thickness skin wounding, when applied to  
86 developmental skin, is unsuitable for distinguishing tissue regeneration and  
87 development. In contrast, blistering injury allows us to monitor both skin regeneration  
88 (reepithelization of the epidermis) and morphogenesis (HF development) within the  
89 same wound bed.

90 Here, by taking advantage of subepidermal blisters, we explore the effects of injury  
91 on developmental tissue. Unexpectedly, blistering injury is found to reduce the

92 expression of tissue morphogenesis genes in the healed epidermis and to direct HFSCs,  
93 rather than epidermal SCs, to provide progeny to heal the wound and to suspend HF  
94 development.

95

96

97 **Results**

98 **Subepidermal blister formation and its healing process**

99 The suction-blister technique was developed more than a half-century ago to selectively  
100 remove the epidermis from the dermis (Kiistala & Mustakallio, 1964, 1967), and it has  
101 been utilized to harvest epidermal pieces for transplant to repair human skin defects.  
102 We reasoned that suction blisters on neonatal mice, in which the epidermis is removed  
103 while HFs, the dermis, and subcutaneous fat tissues are maintained in the wounds,  
104 enable us to examine the direct relationship between tissue injury and skin development.  
105 Therefore, we applied constant negative pressure to the dorsal skin of C57BL/6 wild-  
106 type (WT) neonates to produce subepidermal blisters (postnatal day 1 (P1), **Figure 1A**).  
107 Histologically, skin separation occurred at the level of the dermoepidermal junction  
108 (DEJ) (**Figure 1B, Figure EV1A-C**). HFs, as shown by alkaline phosphatase (AP)-  
109 positive dermal papillae, remained on the dermal side of the blisters (**Figure 1B, Figure**  
110 **EV1D**). Dermis and subcutaneous tissues were intact after blistering (**Figure 1B**).  $\alpha 6$   
111 integrin (ITGA6), a hemidesmosome protein, was seen at the blister roof, whereas type  
112 IV collagen (COL4), a major component of the epidermal basement membrane, and  
113 laminin 332 (L332) were present at the base of the blister (**Figure 1C, Figure EV1B**). In  
114 line with the immunofluorescence data, hemidesmosomes localized on the blister roof  
115 and lamina densa (basement membrane) were observed at the blister base by electron  
116 microscopy (**Figure 1D, Figure EV1C**), as seen in human suction blisters (Kiistala &  
117 Mustakallio, 1967) and their murine counterparts (Krawczyk, 1971).

118 We then characterized the healing processes of the subepidermal blisters. One to  
119 two layers of the regenerated epidermis, marked with pan-cytokeratin, were found one

120 day after blister formation (P2, **Figure 1E**, **Figure EV1E**). The regenerated epidermis  
121 restored ITGA6 expression at the DEJ (**Figure 1E**, **Figure EV1E**). The shape of the  
122 basal keratinocytes in the intact skin was cuboidal or columnar (P2, keratin 14 (K14)-  
123 positive cells in the nonlesional area, **Figure 1F**). In contrast, the regenerated  
124 keratinocytes in the blistered skin transformed from cuboidal to a wedge/flattened shape  
125 (P2, K14-positive cells in the lesional area, **Figure 1F**) (Krawczyk, 1971). Two days  
126 after blistering (P3), the stratified epidermal layers were mostly restored but still lacked  
127 loricrin-positive granular layers, a hallmark of proper epidermal differentiation, in the  
128 lesional area (**Figure 1G**). The final step in epidermal differentiation was completed by  
129 the formation of loricrin-positive granular layers three days after blister formation (P4,  
130 **Figure 1H**). The immunofluorescence data for subepidermal healing are summarized in  
131 **Table EV1**. These results demonstrate that subepidermal blisters on neonates can  
132 serve a model for visualizing wound healing without damaging HFs and other dermal  
133 components at the developmental stage.

134

### 135 **Epidermal restoration at the expense of skin development**

136 To elucidate the effects of the blistering injury on the neonatal skin, we performed RNA-  
137 seq profiling of the wounded tissue above the dermis (the regenerated epidermis and  
138 the blister roof) one day after blistering (P2, **Figure 2A**, **EV2A**). Unexpectedly, the  
139 expression of genes involved in HF morphogenesis, such as Wnt signaling,  
140 melanogenesis, and Hedgehog signaling, was significantly downregulated (**Figure 2B**,  
141 **2C**, **EV2B-D**, **Dataset EV1**). The HF undergoes morphogenesis in utero and after birth  
142 (**Figure EV1F**) (Paus *et al*, 1999; Saxena *et al*, 2019). In murine skin, HF

143 morphogenesis is classified into nine stages: the accumulation of nuclei in the epidermis  
144 without downward growth of HFs (stage 0), HFs with the most proximal part in the  
145 dermis (stages 1-5), and HFs with the most proximal part in the subcutaneous tissue  
146 (stages 6-8) (Paus *et al.*, 1999). The downregulation of HF morphogenesis genes  
147 (**Figure 2B, 2C**) led us to hypothesize that epidermal wounding tunes down tissue  
148 development to accelerate blister healing. In agreement with this hypothesis, the  
149 number of hair canals, which are tube-like connections between the epidermal surface  
150 and the most distal part of the inner root sheath (IRS) and are present in only developed  
151 HFs (HF morphogenesis, stage 6-8) (Paus *et al.*, 1999), was reduced in the  
152 regenerated epidermis (**Figure 2D, 2E**). HF growth under the regenerated epidermis at  
153 P4 was delayed at stages 5 and 6 where the IRS is halfway up to the HF or contains the  
154 hair shaft up to the level of the hair canal. In contrast, the surrounding intact skin of the  
155 blisters or the normal skin of the littermate controls had stage 7 HFs, in which the tip of  
156 the hair shaft leaves the IRS and enters the hair canal (**Figure 2F**). The observation of  
157 smaller HFs in the skin lesion when compared to the surrounding intact skin is  
158 accompanied by diminished Wnt signalling indicated by the LacZ-positive area in Wnt  
159 reporter mice (ins-Topgal+) (P2, **Figure 2G**).

160 The expression of genes involved in cytokine-cytokine receptor interactions and  
161 chemokine, TNF, IL-17, and JAK-STAT signaling pathways was increased in the  
162 regenerated epidermis (**Figure 2B, 2C, EV2B**), and these pathways are implicated in  
163 the recruitment of immune cells. However, the number of neutrophils, lymphocytes, and  
164 macrophages was not increased in the lesional dermis (P2, **Figure EV1G**), in which 1-2  
165 layers of the regenerated epidermis covered the wound one day after blistering. There

166 was no apparent increase of these immune cells either at P4 (**Figure EV1G**), in which  
167 the whole epidermis was restored. These results suggest that the immune cells might  
168 not play a significant role in blister healing, although the involvement of immune cells or  
169 of molecules that they secrete—such as  $\gamma\delta$  T cell-derived Fgf9, which induces HF  
170 neogenesis (Gay *et al*, 2013)—cannot be fully excluded or may serve as a confounding  
171 factor affecting HF growth.

172 These data indicate that, in the context of skin morphogenesis where the immune  
173 system is not yet fully defined, subepidermal blisters heal at the expense of HF growth.

174

### 175 **Progeny of junctional zone SCs represent the main cellular contribution to blister** 176 **healing**

177 Wounded lesions require epithelial cell proliferation and migration to restore skin  
178 integrity. We then investigated the dynamics of epidermal and HF keratinocytes during  
179 blister healing. One day after blister formation (P2), BrdU+ cells were abundant in the  
180 HFs and the intact epidermis adjacent to the blisters (**Figure 3A**). Cells positive for  $\alpha 5$   
181 integrin (ITGA5), a marker of migrating keratinocytes (Aragona *et al.*, 2017), were seen  
182 in the HFs within the lesional area and in the epidermal boundary between the blister  
183 and the nonlesional area (epidermal tongue) (**Figure 3B, Figure EV1E**). As HF growth  
184 was delayed in the regenerated epidermis (**Figure 2D-G**) and proliferative cells were  
185 abundant in HFs of the lesional area (**Figure 3A**), HF keratinocytes were deduced to  
186 participate in epidermal regeneration rather than in HF development.

187 To confirm this hypothesis, we employed a short-term lineage tracing strategy with  
188 suction blistering (**Figure 3C**). K14-lineage labeled cells (K14CreER:R26R-H2B-

189 mCherry or K14CreER:R26R-confetti), mainly progeny of SCs in the interfollicular  
190 epidermis (IFE), were sparse in the regenerated epidermis (**Figure 3D, 3E, Figure**  
191 **EV3A**). In contrast, most of the cells in the regenerated epidermis were Lrig1 (leucine-  
192 rich repeat and immunoglobulin-like domain protein 1)-lineage labeled cells  
193 (Lrig1CreER:R26R-H2B-mCherry or Lrig1CreER:R26R-confetti), which are the progeny  
194 of junctional zone SCs (**Figure 3F, 3G, Figure EV3A**). In line with this, phospho-  
195 Histone H3 (PH3)-positive cells were observed in Lrig1-lineage labeled cells at P2  
196 (**Figure EV3B, 3C**). K14- and Lrig1-lineage labeled cells were increased from P1 (at the  
197 time of suction blistering) to P4 (sampling), but the expansion of Lrig1-lineage labeled  
198 cells was more evident in the regenerated epidermis (**Figure EV3D**). The expression of  
199 the Lrig1 gene was not upregulated in the regenerated epidermis at P2 in our RNA-seq.  
200 These data indicate that the HF junctional zone on the dermal side of the blister is the  
201 main pool for the keratinocytes that heal subepidermal blisters while halting HF  
202 development, although other hair follicle populations might also be involved in blister  
203 healing.

204

205 **HF reduction from the wound bed of the blisters promotes the contribution of**

206 **IFESC progeny to blister healing**

207 The contribution of junctional zone HFSC progeny to blister healing led us to investigate  
208 how the epidermis regenerates in the absence of HFs at the blister base (dermis). Type  
209 XVII collagen (COL17) is expressed not only in the IFE but also in the bulge region of  
210 the HFs (**Figure 4A**) (Liu *et al*, 2019; Matsumura *et al*, 2016; Natsuga *et al*, 2019;  
211 Tanimura *et al*, 2011; Watanabe *et al.*, 2017). COL17 is encoded by the *COL17A1* gene,



212 and its deficiency leads to junctional EB (McGrath *et al*, 1995). The splitting of neonatal  
213 *Col17a1*<sup>-/-</sup> (Nishie *et al*, 2007) dorsal skin upon suction blistering was observed between  
214 ITGA6 and COL4/L332 (**Figure 4B, 4C**), as was the case in wild-type neonates (**Figure**  
215 **1C, 1D**). Intriguingly, suction blistering (**Figure 1A**) of *Col17a1*<sup>-/-</sup> dorsal skin detached  
216 most, but not all, of the HFs from the dermis (P1, **Figure 4D**). In agreement with this  
217 finding, dermal papilla cells (AP+) were observed on the roof side of the blisters of  
218 *Col17a1*<sup>-/-</sup> mice, whereas the blister roofs of control mice did not have these cells (P1,  
219 **Figure 4E, Figure EV1D**). Epidermal regeneration was not apparent in *Col17a1*<sup>-/-</sup> mice  
220 one day after suction blistering (P2), whereas the control mice showed regeneration of  
221 the epithelial layers (**Figure 4F, Table EV1**). *Col17a1*<sup>-/-</sup> mice had delayed expression of  
222 loricrin in the regenerated epidermis three days after blister formation (P4, **Figure 4F,**  
223 **Table EV1**). BrdU+ cells were abundant in the *Col17a1*<sup>-/-</sup> mouse epidermis surrounding  
224 blisters as was the case for controls (**Figure 3A, 4G**). Lineage tracing experiments  
225 (**Figure 3C**) revealed that IFESC progeny covered most of the regenerated area  
226 (K14CreER:R26R-H2B-mCherry:*Col17a1*<sup>-/-</sup>) at P4 (**Figure 4H, 4I**). The transgenic  
227 rescue of *Col17a1*<sup>-/-</sup> by overexpressing human COL17 (hCOL17+;*Col17a1*<sup>-/-</sup>) (Nishie *et*  
228 *al.*, 2007) ameliorated blister healing (P4, **Appendix Figure S1, Table EV1**). These  
229 data demonstrate that upon detachment of most HFs from the dermis, the IFE can  
230 compensate the lack of junctional and HF SCs and repair defects in the IFE.

231

232 **Impaired flattening of regenerated keratinocytes accompanies slower blister**  
233 **healing**

234 We further sought to identify other modulators of subepidermal blister healing. We  
235 first focused on collagen VII (COL7), encoded by *Col7a1*. COL7 forms anchoring fibrils  
236 and is located at the DEJ (**Figure 5A**) but just below the basement membrane (Shimizu  
237 *et al*, 1997; Watanabe *et al*, 2018), and its deficiency leads to dystrophic EB (Christiano  
238 *et al*, 1993; Hilal *et al*, 1993). As conventional wound healing is delayed in COL7-  
239 hypomorphic mice (Nystrom *et al*, 2013), we applied the suction-blister method to  
240 *Col7a1<sup>-/-</sup>* mice (Heinonen *et al*, 1999) (**Figure 5B-H, Appendix Figure S2**). In contrast  
241 to that in WT and *Col17a1<sup>-/-</sup>* suction blisters (**Figure 1C, 1D, 4B, 4C**), skin splitting  
242 occurred at the level below the basement membrane in *Col7a1<sup>-/-</sup>* mouse dorsal skin, as  
243 shown by the presence of L332 and COL4 on the blister roof epidermis (P1, **Figure 5B,**  
244 **5C**). The epidermal defects were not repaired in *Col7a1<sup>-/-</sup>* mice, whereas the epidermis  
245 of the control blistered skin regenerated one day after blistering (P2, **Figure 5F,**  
246 **Appendix Figure S2, Table EV1**), which is consistent with the delayed healing of full-  
247 thickness skin wounds in COL7-hypomorphic mice (Nystrom *et al.*, 2013). This finding is  
248 contrasted by the fact that COL7-depleted keratinocytes migrate faster than WT  
249 keratinocytes in vitro (Chen *et al*, 2002; Chen *et al*, 2000). We examined the HFs of  
250 *Col7a1<sup>-/-</sup>* mice to explain the slowed epidermal regeneration because HFs are the main  
251 contributor to blister healing (**Figure 3A-G**). However, HFs were present in the *Col7a1<sup>-/-</sup>*  
252 mouse wound bed (blister base) (P1, **Figure 5D, 5E**) as opposed to that of *Col17a1<sup>-/-</sup>*  
253 mouse (P1, **Figure 4D, 4E**). Moreover, the number of BrdU+ cells in HFs was  
254 comparable between *Col7a1<sup>-/-</sup>* and control mice (P2, **Figure 5G, Appendix Figure S2**),  
255 suggesting that the proliferation of HF keratinocytes does not account for the delayed  
256 blister healing of *Col7a1<sup>-/-</sup>* mice.

257 Second, we treated the blistered skin in wild-type mice with extracellular calcium  
258 (**Figure 5I-K, Appendix Figure S2**). Extracellular calcium is a potent inhibitor of  
259 proliferation and migration in cultured keratinocytes as well as an inducer of  
260 differentiation (Hennings *et al*, 1980; Magee *et al*, 1987). Consistent with previous in  
261 vitro assays, the intrablister administration of CaCl<sub>2</sub> (1.8 mM or 9.0 mM) just after  
262 suction blistering delayed epidermal regeneration in vivo (P2, **Figure 5I, Table EV1**).  
263 Premature differentiation, which might hinder wound healing, was not apparent in the  
264 CaCl<sub>2</sub>-treated blisters, as K10 labeling was seen only at the blister roof but not in the  
265 keratinocytes on the wound bed (P2, **Figure 5I**). Similar to that in *Col7a1<sup>-/-</sup>* mouse  
266 blisters, the number of BrdU+ cells in HFs was not reduced in CaCl<sub>2</sub>-treated blisters (P2,  
267 **Figure 5J, Appendix Figure 2**).

268 These two examples strongly suggest that there are factors other than HF  
269 keratinocyte proliferation that modulate blister healing. During blister healing,  
270 keratinocytes reshape into a wedge-shaped morphology (**Figure 1F**), which is mirrored  
271 by the RNA-seq data showing that the expression of genes involved in the regulation of  
272 the actin cytoskeleton is decreased in the regenerated epidermis (**Figure 2B, 2C**).  
273 Wedge-shaped/flattened keratinocytes are believed to be superior to cuboidal/columnar  
274 keratinocytes for covering epidermal defects. These data led us to wonder if the cell  
275 morphology was altered in settings of delayed blister healing. In the intact (nonblistered)  
276 skin, the morphology of *Col7a1<sup>-/-</sup>* or Ca-treated basal keratinocytes was similar to that of  
277 control cells (P2, the nonlesional area in **Figure 5F, 5H, 5I, 5K**). The regenerated  
278 keratinocytes became wedge-shaped/flattened in the control group, as shown in **Figure**  
279 **1F**. However, the keratinocytes in the regenerated epidermis of *Col7a1<sup>-/-</sup>* or Ca-treated

280 mice were not as flat as those of control mice but were still rather cuboidal (P2, the  
281 lesional area in **Figure 5F, 5H, 5I, 5K**), which correlates with the delayed blister healing  
282 in these mice.

283

### 284 **Mathematical modeling reproduces blister healing**

285 These in vivo experiments led us to speculate that HF/IFE cell proliferation during  
286 paused HF development and the morphological changes of the regenerated  
287 keratinocytes might simply account for the dynamics of subepidermal blister healing. To  
288 answer this question, we employed mathematical modeling. We adopted an agent-  
289 based model (Kobayashi *et al*, 2016; Kobayashi *et al*, 2018), where keratinocytes were  
290 modeled by spheroids and cell division was described as replication of the spheroids.  
291 Such a model allowed us to visualize the dynamics of the epidermal basal layer and to  
292 establish the epidermal defects on the basement membrane. We utilized the data on the  
293 number of BrdU+ cells among HF vs. IFE keratinocytes (approximately 7:1 per unit of  
294 epidermal length; **Figure 3A**) and on the shape of the regenerated vs. normal  
295 keratinocytes (2:1 the length of the major cell axis; **Figure 5H, 5K**). SC progeny within  
296 the epidermal defects (colored in red), simulating HF-derived cells, had a more  
297 substantial contribution to wound healing than IFE SCs (colored in yellow; **Figure 6A,**  
298 **6B, Movie EV1**), as seen in the lineage tracing experiments (**Figure 3C-G**). The  
299 absence of SCs within the epidermal defects, simulating HF reduction in the wound bed,  
300 showed delayed healing (**Figure 6C, 6D, Movie EV2**), in agreement with *Col17a1*<sup>-/-</sup>  
301 epidermal regeneration results (**Figure 4E-G**). A less flattened morphology of the  
302 regenerated keratinocytes, simulating *Col7a1*<sup>-/-</sup> and Ca-treated blister healing

303 (regenerated vs. normal keratinocytes, 1.3-1.6:1 in length; **Figure 5H, 5K**), slowed  
304 epidermal regeneration (**Figure 6E, 6F, Movie EV3**). By systematically changing the  
305 shape of regenerated vs. normal keratinocytes (from 1:1 to 2:1 in length) for different  
306 initial SC distributions within epidermal defects (**Figure EV4A**), we observed the same  
307 tendency: Less flattened morphology led to more delayed healing (**Figure EV4B, EV4C**),  
308 which suggests that the morphology affects healing dynamics irrespective of the initial  
309 SC distribution. These in silico data demonstrate that the contribution of HFSC progeny  
310 and the morphological change in the regenerated keratinocytes are sufficient to  
311 recapitulate the in vivo subepidermal blister healing.

312 We finally asked whether this HF contribution to wound healing could be applied to a  
313 human setting, in which HFs are larger but much more sparsely distributed than their  
314 murine counterparts (**Table EV2**). We examined human subepidermal blister samples  
315 and found that epidermal regeneration by HFs was observed in the samples with the re-  
316 epithelized area (**Figure EV5**). These findings from human samples are consistent with  
317 the mice data.

318 **Discussion**

319 Although recent studies have reported that injury causes damaged tissue to shift to an  
320 embryonic-like state, there is a poor understanding of how regeneration affects  
321 development at the damaged tissues. Here, we applied the blistering injury to neonatal  
322 mouse dorsal skin and showed the skewed contribution of HFSC progeny to wound  
323 healing rather than HF development.

324 Previous studies on skin wounding combined with the fate mapping of murine skin  
325 delineated the involvement of epithelial, mesenchymal, and immune cells in wound  
326 healing, depending on different settings (Dekoninck & Blanpain, 2019; Rognoni & Watt,  
327 2018). However, as full-thickness skin wounds, even when they are applied to neonatal  
328 skin, remove all skin components, it is challenging to see the effects of development on  
329 injury or vice versa. Our blistering injury has an advantage over conventional skin  
330 wounding studies in that only the epidermis is removed by constant negative pressure,  
331 with the other skin components and basement membrane being retained in the wounds,  
332 which allowed us to examine “pure” epidermal wound healing processes during skin  
333 development. Our study contrasts with wound-induced embryonic gene expression  
334 (Miao *et al.*, 2019) and follicular neogenesis upon full-thickness skin wounding in adult  
335 (Ito *et al.*, 2007; Osaka *et al.*, 2007) and neonatal mice (Rognoni *et al.*, 2016). So far, our  
336 study has not distinguished whether the waning Wnt signaling was the primary cause,  
337 or the result, of the delayed HF morphogenesis. Further studies are needed to clarify  
338 this.

339 Previous studies have suggested the possible involvement of HFs in epidermal  
340 regeneration in human suction blisters (Lane *et al.*, 1991) and extracellular matrix

341 alterations in the skin-split area (Hertle *et al*, 1992; Leivo *et al*, 2000). We show that the  
342 progeny of HF junctional zone SCs mainly repair subepidermal blisters (**Figure 3F, 3G,**  
343 **6A, 6B**), although the involvement of other HF populations cannot be excluded.  
344 However, the IFE can also serve as a reservoir of keratinocytes to repair epidermal  
345 defects when most HFs are detached from the dermis (**Figure 4H**). How the  
346 contribution of two sources of keratinocytes to blister healing is regulated is unknown,  
347 but the significant contribution of HF junctional zone progeny is reasonable because  
348 HFs are densely located in the wound bed (blister base). In contrast, the progeny of  
349 IFESCs could recover only from the blister edge, as demonstrated by mathematical  
350 modeling (**Figure 3D-3G, 6A, 6B**). The role of HFSCs is also highlighted by delayed HF  
351 growth in the regenerated epidermis, as corroborated by the downregulated Wnt  
352 signaling (**Figure 2A-2G**). These findings indicate that there is a coordinated balance  
353 between tissue development and wound healing, which has not been well recognized.  
354 In addition, the expression of IL-17 signaling pathway genes was increased, although  
355 the recruitment of immune cells was not evident one day or three days after blistering  
356 (**Figure EV1G**). Recently, IL-17 signaling has been shown to drive Lrig1-lineage cell  
357 recruitment in wound healing and tumorigenesis (Chen *et al*, 2019). Therefore, IL-17  
358 signaling might also help Lrig1-lineage cells translocate from HFs to repair epidermal  
359 defects in our study (**Figure 3F, 3G**).

360 The dynamics of cytoskeletal changes directly affect cellular morphology and  
361 migration potential (Tang & Gerlach, 2017). Previous studies have shown that cells  
362 undergo a morphological transformation into a wedge/flattened shape at the leading  
363 edge of migrating cells (Uroz *et al*, 2019) and in regenerated keratinocytes during

364 wound healing (Krawczyk, 1971; Paladini *et al*, 1996). Our study has shed further light  
365 on the significant impact of the keratinocyte morphological changes on in vivo wound  
366 healing through blistering experiments in *Col7a1*<sup>-/-</sup> and Ca-treated mice and  
367 mathematical modeling (**Figure 5F, 5H, 5I, 5K, 6A, 6E, 6F**). However, the  
368 morphological changes of keratinocytes might not directly foster blister healing but  
369 might be simply correlated with other primary causes that help regenerate the epidermis.  
370 The loss of a functional basement membrane in the *Col7a1*<sup>-/-</sup> blister bottom might slow  
371 blister healing due to the lack of substrates for cell migration. Pressure during blister  
372 induction could be a factor that changes the cellular morphology. Additional mechanistic  
373 studies are needed to verify the hypothesis we raise in our study.

374 Our in vivo blistering experiments can mimic and replace the in vitro cultured cell  
375 wound healing assay (e.g., scratch wounding), which has been used in the field of cell  
376 biology for decades because subepidermal blisters are epidermal wounds. In vivo  
377 intrablister administration of drugs, as exemplified by extracellular calcium  
378 administration (**Figure 5I**), could be an alternative to in vitro chemical treatment of  
379 scratch-wounded cultured cells to develop new therapeutic options for wound healing,  
380 especially of subepidermal blisters.

381 Our in vivo suction-blister model recapitulates the human pathological epidermal  
382 detachment seen in EB, pemphigoid diseases, burns, and severe drug reactions such  
383 as Stevens-Johnson syndrome/toxic epidermal necrolysis. Loss-of-function mutations in  
384 *COL17A1* (McGrath *et al.*, 1995) and *COL7A1* (Christiano *et al.*, 1993; Hilal *et al.*, 1993)  
385 lead to junctional and recessive dystrophic EB in humans, respectively. Therefore,  
386 *Col17a1*<sup>-/-</sup> and *Col7a1*<sup>-/-</sup> mouse blistering also serves as an EB wound model. The



387 prominent hair loss in human *COL17A1*-mutated junctional EB might be reflected by the  
388 reduction of HFs from the wound bed in *Col17a1<sup>-/-</sup>* blisters (**Figure 4D, 4E**), whereas the  
389 hair loss in recessive dystrophic EB is not as severe as that in the junctional subtype  
390 (Tosti *et al*, 2010), consistent with the maintenance of HFs in the dermis of *Col7a1<sup>-/-</sup>*  
391 mouse dorsal skin upon suction blistering (**Figure 5D, 5E**). It is plausible that recurrent  
392 blistering can exhaust the pool of HFSCs, leading to delayed blister healing and  
393 scarring, especially in recessive dystrophic EB. Taken together, the processes of  
394 subepidermal blister healing highlight HFs as a target for treating the wounds of EB and  
395 other blistering diseases.

396 Our study has some limitations, primarily due to the discrepancies between mice  
397 and humans. First, eccrine sweat glands have been reported to contribute to wound  
398 healing in human skin (Rittie *et al*, 2013). As murine back skin does not harbor sweat  
399 glands, our study was unable to estimate the contribution of the sweat glands in human  
400 settings. Second, although the skin-split level of human recessive dystrophic EB is  
401 generally just beneath lamina densa (basement membrane), as was the case for the  
402 *Col7a1<sup>-/-</sup>* blisters in our study, suction blistering on human recessive dystrophic EB  
403 induces skin detachment in the lamina lucida, that is, between hemidesmosomes and  
404 the lamina densa (Tidman & Eady, 1984). In addition, our study did not look into the  
405 contribution of mesenchymal cells to blister healing, which has been described in  
406 previous studies on epidermolysis bullosa (Chino *et al*, 2008; Fujita *et al*, 2010; Inuma  
407 *et al*, 2015; Tamai *et al*, 2011; Tolar *et al*, 2009; Webber *et al*, 2017). Further studies  
408 are warranted to elucidate the role of mesenchymal cells in blister healing.

409 In closing, our study has revealed the imbalance between development and wound  
410 regeneration in the skin blisters. Our findings of the healing processes pave the way for  
411 tailored therapeutic interventions for epidermolysis bullosa, pemphigoid diseases and  
412 other blistering diseases.

413 **Material and Methods**

414 **Animals**

415 C57BL/6 strain mice were purchased from Clea (Tokyo, Japan). Ins-Topgal+ mice  
416 were obtained from RIKEN BRC (Tsukuba, Japan) (Moriyama *et al*, 2007).  
417 K14CreER, Lrig1CreER, and R26R-confetti mice were purchased from the  
418 Jackson Laboratory (Bar Harbor, Maine, USA). R26R-H2B-mCherry mice were  
419 provided by RIKEN (Kobe, Japan). *Col17a1<sup>-/-</sup>* and *hCOL17+;Col17a1<sup>-/-</sup>* mice were  
420 generated as previously described (Nishie *et al.*, 2007). *Col7a1<sup>-/-</sup>* mice were  
421 provided by Prof. Jouni Uitto (Heinonen *et al.*, 1999). The institutional review board  
422 of the Hokkaido University Graduate School of Medicine approved all animal  
423 studies described below.

424

425 **Suction blisters**

426 Suction blisters were produced on the neonatal murine dorsal skin (P1) using a syringe  
427 and connector tubes. The negative pressure applied to the skin (generally for minutes)  
428 was  $523.4 \pm 1.3$  mmHg (evaluated by an Ex Pocket Pressure Indicator PM-281 (AS ONE,  
429 Osaka, Japan)). The diameter of the syringe attached to the skin was 4 mm. The size of  
430 the typical blister was 3 mm in diameter.

431

432 **Histology**

433 Mouse dorsal skin specimens were fixed in formalin and embedded in paraffin after  
434 dehydration or were frozen on dry ice in an optimal cutting temperature (OCT)  
435 compound. Frozen sections were fixed with 4% paraformaldehyde (PFA) or cold

436 acetone or were stained without fixation. Antigen retrieval with pH 6.0 (citrate) or pH 9.0  
437 (EDTA) buffer was performed on deparaffinized sections. Sections were incubated with  
438 primary antibodies overnight at 4°C. After being washed in phosphate-buffered saline  
439 (PBS), the sections were incubated with secondary antibodies conjugated to FITC,  
440 Alexa 488, Alexa 647 or Alexa 680 for 1 hr at room temperature (RT). The nuclei were  
441 stained with propidium iodide (PI) or 4',6-diamidino-2-phenylindole (DAPI). The stained  
442 immunofluorescent samples were observed using a confocal laser scanning microscope  
443 (FV-1000 (Olympus, Tokyo, Japan) or LSM-710 (Zeiss, Oberkochen, Germany)).

444 For immunohistochemistry, horseradish peroxidase (HRP)-tagged secondary  
445 antibodies were used. Sections were blocked with hydrogen peroxide, labeled with  
446 antibodies, and counterstained with hematoxylin. For morphological analysis,  
447 deparaffinized sections were stained with hematoxylin and eosin (H&E) by conventional  
448 methods. Alkaline phosphatase staining was performed using a StemAb Alkaline  
449 Phosphatase Staining Kit II (Stemgent, San Diego, California, USA). Images of  
450 immunohistochemistry, and H&E- and alkaline phosphatase-stained sections were  
451 captured with a BZ-9000 microscope (Keyence, Tokyo, Japan).

452 For whole-mount staining, mouse dorsal skin samples were fixed with 4% PFA and  
453 immunolabeled or stained with the Alkaline Phosphatase Staining Kit II. For X-gal  
454 staining of ins-Topgal+ mouse skin, a beta-galactosidase staining kit (Takara-bio, Shiga,  
455 Japan) was used according to the provider's protocol. Briefly, dorsal skin samples were  
456 fixed with 4% PFA for 1 hr at 4°C and soaked in staining solution overnight at RT.  
457 Tissues were mounted in a Mowiol solution. Images were observed with LSM-710, FV-  
458 1000 or BZ-9000 microscopes.

459 HF morphological stages were evaluated as previously described (Paus *et al.*, 1999).  
460 The length of the major axis of keratinocytes in the intact and regenerated epidermis  
461 was measured using ImageJ (NIH, Bethesda, Maryland, USA) on K14-stained sections.  
462 The quantification of the cells expressing a particular marker was performed as  
463 previously described (Natsuga *et al*, 2016).

464

### 465 **Antibodies**

466 The following antibodies were used: anti-BrdU (Abcam, Cambridge, UK; BU1/75, Dako;  
467 M0744), anti-phospho-Histone H3 (Ser10) (Merck Millipore, Billerica, Massachusetts,  
468 USA), anti-Ioricrin (Covance, Princeton, New Jersey, USA), FITC-conjugated anti-CD3e  
469 (BioLegend, San Diego, California, USA; 145-2C11), Alexa Fluor 488-conjugated anti-  
470 F4/80 (Affymetrix, Santa Clara, California, USA; BM8), FITC (fluorescein  
471 isothiocyanate)-conjugated anti-Ly-6G (Beckman Coulter, Brea, California, USA; RB6-  
472 8C5), anti-COL4 (Novus Biologicals, Centennial, Colorado; NB120-6586), anti-COL7  
473 (homemade (Iwata *et al*, 2013)), anti- COL17 (Abcam; ab186415), anti-ITGA5 (Abcam;  
474 EPR7854), anti-ITGA6 (BD Biosciences Pharmingen, San Diego, California, USA;  
475 GoH3), anti-L332 (Abcam; ab14509), anti-laminin  $\beta$ 1 (Abcam; ab44941), anti-pan-  
476 cytokeratin (PROGEN, Wieblingen, Heidelberg, Germany; PRGN-10550), anti-  
477 cytokeratin 10 (Biolegend; Poly19054), anti-cytokeratin 14 (ThermoFisher, Waltham,  
478 Massachusetts, USA; LL002).

479

### 480 **BrdU labeling**

481 For proliferation analysis, 10 µg of BrdU (BD Biosciences Pharmingen) per head was  
482 intraperitoneally administered 4 hr before sacrifice.

483

#### 484 **Transmission electron microscopy**

485 The samples were taken from C57BL/6 mouse dorsal skin (P1) just after suction  
486 blistering was performed. The samples were fixed in 5% glutaraldehyde solution,  
487 postfixed in 1% OsO<sub>4</sub>, dehydrated, and embedded in Epon 812. The embedded  
488 samples were sectioned at 1 µm thickness for light microscopy and thin-sectioned for  
489 electron microscopy (70 nm thick). The thin sections were stained with uranyl acetate  
490 and lead citrate and examined by transmission electron microscopy (H-7100; Hitachi,  
491 Tokyo, Japan).

492

#### 493 **Lineage tracing**

494 K14CreER:R26R-H2B-mCherry, Lrig1CreER:R26R-H2B-mCherry, K14CreER:R26R-  
495 confetti, and Lrig1CreER:R26R-confetti mice were intraperitoneally treated with 0.5 mg  
496 of tamoxifen (T5648; Sigma-Aldrich, St. Louis, Missouri, USA) at P0. The dorsal skin  
497 samples were harvested four days later (P4).

498

#### 499 **RNA sequencing and analysis**

500 Suction-blistered and control samples were collected at P2 at the same time of day to  
501 exclude the effects of circadian oscillations on epidermal gene expression (Janich *et al*,  
502 2013). The skin samples were treated with 0.25% trypsin EDTA overnight at 4°C. The  
503 blistered and regenerated epidermis was collected by separating it from the dermis,

504 minced with a scalpel and suspended in 10% FCS DMEM. The cell suspension was  
505 filtered through a 70 µm filter, and cell pellets were collected. Library preparation was  
506 performed using an Illumina TruSeq RNA prep kit by following the manufacturer's  
507 instructions. Briefly, following TRIzol extraction and chemical fragmentation, mRNA was  
508 purified with oligo-dT-attached magnetic beads and reverse transcribed into cDNA.  
509 Following a second strand synthesis step with DNA polymerase I and RNase H, the  
510 resulting cDNA was subjected to end repair, A-tailing, and Illumina compatible adaptor  
511 ligation. Following purification and PCR-mediated enrichment, libraries were purified  
512 with AMPure XP beads and sequenced on a NextSeq 500 Illumina sequencer.

513       After quality controls were performed, the raw reads were aligned to the NCBI m37  
514 mouse reference genome (mm9) using HiSat2 (Kim *et al*, 2015) (version 2.0.0) using  
515 options -N 1 -L 20 -i S, 1, 0.5 -D 25 -R 5 --pen-noncansplice 20 --mp 1, 0 --sp 3, 0 and  
516 providing a list of known splice sites. Expression levels were quantified using  
517 featureCounts (Liao *et al*, 2014) with RefSeq gene annotation and normalized as TPM  
518 using custom scripts. Differential expression analysis was performed using the edgeR  
519 (Robinson *et al*, 2010) software package. After lowly expressed genes (1 count per  
520 million in less than two samples) were filtered out, the treatment and control groups  
521 were compared using the exact test method (Robinson *et al.*, 2010). Genes with an  
522 absolute log<sub>2</sub>-fold change greater than 1 and false discovery rate (FDR) less than or  
523 equal to 0.05 were considered differentially expressed. Hierarchical clustering of gene  
524 expression profiles was performed on differentially expressed genes using only  
525 Euclidean distances and the complete linkage method. TPM values were normalized as  
526 Z-scores across samples, and the distances were computed. GO term, KEGG pathway

527 enrichment analysis on differentially expressed genes and GO term network  
528 visualization were performed using the clusterProfiler R/Bioconductor package (Yu *et al*,  
529 2012). To validate the enriched pathways, GSEA analysis was performed.

530

### 531 **Intrablister administration**

532 Ten microliters of 1.8 or 9.0 mM CaCl<sub>2</sub> in PBS was administered by syringe into the  
533 blisters just after the suction blistering procedure was performed.

534

### 535 **Statistics**

536 Statistical analyses were performed using GraphPad Prism (GraphPad Software, La  
537 Jolla, California, USA). P-values were determined using Student's t-test or one-way  
538 ANOVA followed by Tukey's test. P-values are indicated as \*0.01<p<0.05,  
539 \*\*0.001<p<0.01, \*\*\*0.0001<p<0.001, and \*\*\*\*p<0.0001. The values were shown as the  
540 means ± standard errors (SE), violin plots or connected with lines showing individual  
541 mice.

542

### 543 **Mathematical modeling**

544 A mathematical model proposed for epidermal cell dynamics (Kobayashi *et al.*, 2016;  
545 Kobayashi *et al.*, 2018) was adapted to simulate epidermal wound healing. (For the  
546 detailed mathematical formulation, see the Appendix Supplementary Methods and  
547 **Dataset EV2.**) In this model, epidermal basal cells were represented as spherical  
548 particles, with the cell diameter set to 10 µm. Cells designated as epidermal SCs and  
549 their progeny could undergo division on the basement membrane. Cell division was



550 described as a process of two initially completely overlapping particles gradually  
551 separating into two distinct particles. When a newly created cell was not fully  
552 surrounded by other cells, it was judged as being in a regeneration process and  
553 immediately underwent a transition to an oblate spheroid shape with the long axis  
554 increased by a factor of 2 (normal) or 1.5 (simulating *Col7a1*<sup>-/-</sup> and Ca-treated blisters)  
555 while its volume was kept constant. The same division rate was assigned to all  
556 proliferative cells, with an average division period of 57.6 [arb. unit]. Forces exerted on a  
557 cell came from adhesion and excluded-volume interactions with other cells and with the  
558 basement membrane. SCs were tightly bound and unable to detach from the basement  
559 membrane, while the progeny were weakly bound so that they could detach from the  
560 membrane via the ambient pressure: the detached cells were removed from the system.  
561 The basement membrane was assumed to be a rigid flat surface, whose shape  
562 remained unchanged over time. These interactions were calculated to obtain the time  
563 evolution of the whole system by solving equations given in a previous report  
564 (Kobayashi *et al.*, 2018). The simulation region was set to 600  $\mu\text{m}$  x 600  $\mu\text{m}$  horizontally  
565 with periodic boundary conditions. To prepare the initial conditions for the simulation of  
566 subepidermal blister healing, we first ran a simulation with SCs placed on the basement  
567 membrane until their progeny covered the whole surface. Then, we set the progeny to  
568 be nonproliferative and created epidermal defects by removing the cells that were inside  
569 a disk domain with a diameter of 480  $\mu\text{m}$ .

570

## 571 **Human samples**

572 From the H&E-stained skin of patients with congenital or autoimmune subepidermal  
573 blistering diseases (73 EB or 188 bullous pemphigoid (BP) samples, respectively), the  
574 samples that met the following histological criteria were selected: (1) subepidermal  
575 blisters or a skin split at the dermoepidermal junction; (2) re-epithelization in the area;  
576 and (3) presence of HFs on the blister base. Three BP samples fulfilled all the criteria  
577 (blisters 1, 2, and 3) and were observed with a Keyence BZ-9000 microscope. The  
578 institutional review board of the Hokkaido University Graduate School of Medicine  
579 approved all human studies described above (ID: 13-043 and 15-052). The study was  
580 conducted according to the Declaration of Helsinki Principles. Participants or their legal  
581 guardians provided written informed consent.

582

### 583 **Data availability**

584 The datasets produced in this study are available in the following databases:

585

586 - RNA-Seq data: Gene Expression Omnibus GSE154871

587 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE154871>)

588

### 589 **Author contributions**

590 Y. F. designed and performed the experiments, analyzed the data, interpreted the  
591 results, and wrote the manuscript. M. W., S. T., H. N., H. K., Y. W., Y. M, A. L., V. P., H.  
592 U., H. I., W. N., and S. O. performed the experiments and analyzed the data. K. O., Y.  
593 K., and M. N. performed the mathematical modeling and wrote the manuscript. G. D.  
594 designed and performed the experiments, analyzed the data and interpreted the results.

595 H. S. interpreted the results and supervised the study. K. N. conceived and designed  
596 the experiments, analyzed the data, interpreted the results, wrote the manuscript and  
597 supervised the study.

598

### 599 **Acknowledgments**

600 We thank Ms. Meari Yoshida and Ms. Megumi Takehara for their technical assistance.  
601 We also thank Professor Yumiko Saga, Professor Kim B Yancey, and Professor Jouni  
602 Uitto for providing the ins-Topgal+, K14-hCOL17, and *Col7a1<sup>-/-</sup>* mice, respectively. This  
603 work was funded by AMED (ID: 18059057), JSPS (KAKEN 17K16317), the Uehara  
604 Foundation, the Lydia O'Leary Memorial Pias Dermatological Foundation to KN, JST  
605 CREST (JPMJCR15D2) to MN, JSPS (KAKEN 16K10120) to HN, AIRC IG 20240 to SO,  
606 and AIRC MFAG 2018 (ID: 21640) to GD. HS, the senior author of this paper, sadly  
607 died while this manuscript was in revision. We dedicate this paper to him.

608

### 609 **Conflict of interest**

610 The authors declare that they have no conflict of interest.

611

612 **References**

- 613 Aragona M, Dekoninck S, Rulands S, Lenglez S, Mascré G, Simons BD, Blanpain C  
614 (2017) Defining stem cell dynamics and migration during wound healing in mouse skin  
615 epidermis. *Nat Commun* 8: 14684
- 616 Brakebusch C, Grose R, Quondamatteo F, Ramirez A, Jorcano JL, Pirro A, Svensson M,  
617 Herken R, Sasaki T, Timpl R *et al* (2000) Skin and hair follicle integrity is crucially  
618 dependent on beta 1 integrin expression on keratinocytes. *EMBO J* 19: 3990-4003
- 619 Chen M, Kasahara N, Keene DR, Chan L, Hoeffler WK, Finlay D, Barcova M, Cannon  
620 PM, Mazurek C, Woodley DT (2002) Restoration of type VII collagen expression and  
621 function in dystrophic epidermolysis bullosa. *Nat Genet* 32: 670-675
- 622 Chen M, O'Toole EA, Muellenhoff M, Medina E, Kasahara N, Woodley DT (2000)  
623 Development and characterization of a recombinant truncated type VII collagen  
624 "minigene". Implication for gene therapy of dystrophic epidermolysis bullosa. *J Biol*  
625 *Chem* 275: 24429-24435
- 626 Chen X, Cai G, Liu C, Zhao J, Gu C, Wu L, Hamilton TA, Zhang CJ, Ko J, Zhu L *et al*  
627 (2019) IL-17R-EGFR axis links wound healing to tumorigenesis in Lrig1(+) stem cells. *J*  
628 *Exp Med* 216: 195-214
- 629 Chetty BV, Boissy RE, Warden GD, Nordlund JJ (1992) Basement membrane and  
630 fibroblast aberration in blisters at the donor, graft, and spontaneously healed sites in  
631 patients with burns. *Arch Dermatol* 128: 181-186
- 632 Chino T, Tamai K, Yamazaki T, Otsuru S, Kikuchi Y, Nimura K, Endo M, Nagai M, Uitto  
633 J, Kitajima Y *et al* (2008) Bone marrow cell transfer into fetal circulation can ameliorate

634 genetic skin diseases by providing fibroblasts to the skin and inducing immune  
635 tolerance. *Am J Pathol* 173: 803-814

636 Christiano AM, Greenspan DS, Hoffman GG, Zhang X, Tamai Y, Lin AN, Dietz HC,  
637 Hovnanian A, Uitto J (1993) A missense mutation in type VII collagen in two affected  
638 siblings with recessive dystrophic epidermolysis bullosa. *Nat Genet* 4: 62-66

639 Dekoninck S, Blanpain C (2019) Stem cell dynamics, migration and plasticity during  
640 wound healing. *Nat Cell Biol* 21: 18-24

641 Donati G, Rognoni E, Hiratsuka T, Liakath-Ali K, Hoste E, Kar G, Kayikci M, Russell R,  
642 Kretzschmar K, Mulder KW *et al* (2017) Wounding induces dedifferentiation of  
643 epidermal Gata6(+) cells and acquisition of stem cell properties. *Nat Cell Biol* 19: 603-  
644 613

645 Donati G, Watt FM (2015) Stem cell heterogeneity and plasticity in epithelia. *Cell Stem*  
646 *Cell* 16: 465-476

647 Fernandez Vallone V, Leprovots M, Strollo S, Vasile G, Lefort A, Libert F, Vassart G,  
648 Garcia MI (2016) Trop2 marks transient gastric fetal epithelium and adult regenerating  
649 cells after epithelial damage. *Development* 143: 1452-1463

650 Fine JD, Bruckner-Tuderman L, Eady RA, Bauer EA, Bauer JW, Has C, Heagerty A,  
651 Hintner H, Hovnanian A, Jonkman MF *et al* (2014) Inherited epidermolysis bullosa:  
652 updated recommendations on diagnosis and classification. *J Am Acad Dermatol* 70:  
653 1103-1126

654 Fujita Y, Abe R, Inokuma D, Sasaki M, Hoshina D, Natsuga K, Nishie W, McMillan JR,  
655 Nakamura H, Shimizu T *et al* (2010) Bone marrow transplantation restores epidermal

656 basement membrane protein expression and rescues epidermolysis bullosa model mice.  
657 *Proc Natl Acad Sci U S A* 107: 14345-14350

658 Garcin CL, Ansell DM, Headon DJ, Paus R, Hardman MJ (2016) Hair Follicle Bulge  
659 Stem Cells Appear Dispensable for the Acute Phase of Wound Re-epithelialization.  
660 *Stem Cells* 34: 1377-1385

661 Gay D, Kwon O, Zhang Z, Spata M, Plikus MV, Holler PD, Ito M, Yang Z, Treffeisen E,  
662 Kim CD *et al* (2013) Fgf9 from dermal gammadelta T cells induces hair follicle  
663 neogenesis after wounding. *Nat Med* 19: 916-923

664 Gonzales KAU, Fuchs E (2017) Skin and Its Regenerative Powers: An Alliance between  
665 Stem Cells and Their Niche. *Dev Cell* 43: 387-401

666 Heinonen S, Mannikko M, Klement JF, Whitaker-Menezes D, Murphy GF, Uitto J (1999)  
667 Targeted inactivation of the type VII collagen gene (Col7a1) in mice results in severe  
668 blistering phenotype: a model for recessive dystrophic epidermolysis bullosa. *J Cell Sci*  
669 112 ( Pt 21): 3641-3648

670 Hennings H, Michael D, Cheng C, Steinert P, Holbrook K, Yuspa SH (1980) Calcium  
671 regulation of growth and differentiation of mouse epidermal cells in culture. *Cell* 19: 245-  
672 254

673 Hertle MD, Kubler MD, Leigh IM, Watt FM (1992) Aberrant integrin expression during  
674 epidermal wound healing and in psoriatic epidermis. *J Clin Invest* 89: 1892-1901

675 Hilal L, Rochat A, Duquesnoy P, Blanchet-Bardon C, Wechsler J, Martin N, Christiano  
676 AM, Barrandon Y, Uitto J, Goossens M *et al* (1993) A homozygous insertion-deletion in  
677 the type VII collagen gene (COL7A1) in Hallopeau-Siemens dystrophic epidermolysis  
678 bullosa. *Nat Genet* 5: 287-293

679 linuma S, Aikawa E, Tamai K, Fujita R, Kikuchi Y, Chino T, Kikuta J, McGrath JA, Uitto  
680 J, Ishii M *et al* (2015) Transplanted bone marrow-derived circulating PDGFRalpha+ cells  
681 restore type VII collagen in recessive dystrophic epidermolysis bullosa mouse skin graft.  
682 *J Immunol* 194: 1996-2003

683 Ito M, Liu Y, Yang Z, Nguyen J, Liang F, Morris RJ, Cotsarelis G (2005) Stem cells in  
684 the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis.  
685 *Nat Med* 11: 1351-1354

686 Ito M, Yang Z, Andl T, Cui C, Kim N, Millar SE, Cotsarelis G (2007) Wnt-dependent de  
687 novo hair follicle regeneration in adult mouse skin after wounding. *Nature* 447: 316-320

688 Iwata H, Bieber K, Tiburzy B, Chrobok N, Kalies K, Shimizu A, Leineweber S, Ishiko A,  
689 Vorobyev A, Zillikens D *et al* (2013) B cells, dendritic cells, and macrophages are  
690 required to induce an autoreactive CD4 helper T cell response in experimental  
691 epidermolysis bullosa acquisita. *J Immunol* 191: 2978-2988

692 Janich P, Toufighi K, Solanas G, Luis NM, Minkwitz S, Serrano L, Lehner B, Benitah SA  
693 (2013) Human epidermal stem cell function is regulated by circadian oscillations. *Cell*  
694 *Stem Cell* 13: 745-753

695 Kang S, Long K, Wang S, Sada A, Tumber T (2020) Histone H3 K4/9/27 Trimethylation  
696 Levels Affect Wound Healing and Stem Cell Dynamics in Adult Skin. *Stem Cell Reports*  
697 14: 34-48

698 Kiistala U, Mustakallio KK (1964) In-Vivo Separation of Epidermis by Production of  
699 Suction Blisters. *Lancet* 2: 1444-1445

700 Kiistala U, Mustakallio KK (1967) Dermo-epidermal separation with suction. Electron  
701 microscopic and histochemical study of initial events of blistering on human skin. *J*  
702 *Invest Dermatol* 48: 466-477

703 Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory  
704 requirements. *Nat Methods* 12: 357-360

705 Kobayashi Y, Sawabu Y, Kitahata H, Denda M, Nagayama M (2016) Mathematical  
706 model for calcium-assisted epidermal homeostasis. *J Theor Biol* 397: 52-60

707 Kobayashi Y, Yasugahira Y, Kitahata H, Watanabe M, Natsuga K, Nagayama M (2018)  
708 Interplay between epidermal stem cell dynamics and dermal deformation. *npj*  
709 *Computational Materials* 4: 45

710 Krawczyk WS (1971) A pattern of epidermal cell migration during wound healing. *J Cell*  
711 *Biol* 49: 247-263

712 Lane EB, Wilson CA, Hughes BR, Leigh IM (1991) Stem cells in hair follicles.  
713 Cytoskeletal studies. *Ann N Y Acad Sci* 642: 197-213

714 Leivo T, Kiistala U, Vesterinen M, Owaribe K, Burgeson RE, Virtanen I, Oikarinen A  
715 (2000) Re-epithelialization rate and protein expression in the suction-induced wound  
716 model: comparison between intact blisters, open wounds and calcipotriol-pretreated  
717 open wounds. *Br J Dermatol* 142: 991-1002

718 Liao Y, Smyth GK, Shi W (2014) featureCounts: an efficient general purpose program  
719 for assigning sequence reads to genomic features. *Bioinformatics* 30: 923-930

720 Liu N, Matsumura H, Kato T, Ichinose S, Takada A, Namiki T, Asakawa K, Morinaga H,  
721 Mohri Y, De Arcangelis A *et al* (2019) Stem cell competition orchestrates skin  
722 homeostasis and ageing. *Nature*



723 Magee AI, Lytton NA, Watt FM (1987) Calcium-induced changes in cytoskeleton and  
724 motility of cultured human keratinocytes. *Exp Cell Res* 172: 43-53

725 Matsumura H, Mohri Y, Binh NT, Morinaga H, Fukuda M, Ito M, Kurata S, Hoeijmakers  
726 J, Nishimura EK (2016) Hair follicle aging is driven by transepidermal elimination of  
727 stem cells via COL17A1 proteolysis. *Science* 351: aad4395

728 McGrath JA, Gatalica B, Christiano AM, Li K, Owaribe K, McMillan JR, Eady RA, Uitto J  
729 (1995) Mutations in the 180-kD bullous pemphigoid antigen (BPAG2), a  
730 hemidesmosomal transmembrane collagen (COL17A1), in generalized atrophic benign  
731 epidermolysis bullosa. *Nat Genet* 11: 83-86

732 McMillan JR, Akiyama M, Shimizu H (2003) Epidermal basement membrane zone  
733 components: ultrastructural distribution and molecular interactions. *J Dermatol Sci* 31:  
734 169-177

735 Miao Q, Hill MC, Chen F, Mo Q, Ku AT, Ramos C, Sock E, Lefebvre V, Nguyen H  
736 (2019) SOX11 and SOX4 drive the reactivation of an embryonic gene program during  
737 murine wound repair. *Nat Commun* 10: 4042

738 Moriyama A, Kii I, Sunabori T, Kurihara S, Takayama I, Shimazaki M, Tanabe H,  
739 Oginuma M, Fukayama M, Matsuzaki Y *et al* (2007) GFP transgenic mice reveal active  
740 canonical Wnt signal in neonatal brain and in adult liver and spleen. *Genesis* 45: 90-100

741 Natsuga K (2014) Epidermal barriers. *Cold Spring Harb Perspect Med* 4: a018218

742 Natsuga K, Cipolat S, Watt FM (2016) Increased Bacterial Load and Expression of  
743 Antimicrobial Peptides in Skin of Barrier-Deficient Mice with Reduced Cancer  
744 Susceptibility. *J Invest Dermatol* 136: 99-106

745 Natsuga K, Watanabe M, Nishie W, Shimizu H (2019) Life before and beyond blistering:  
746 The role of collagen XVII in epidermal physiology. *Exp Dermatol* 28: 1135-1141

747 Niculescu C, Ganguli-Indra G, Pfister V, Dupe V, Messaddeq N, De Arcangelis A,  
748 Georges-Labouesse E (2011) Conditional ablation of integrin alpha-6 in mouse  
749 epidermis leads to skin fragility and inflammation. *Eur J Cell Biol* 90: 270-277

750 Nishie W, Sawamura D, Goto M, Ito K, Shibaki A, McMillan JR, Sakai K, Nakamura H,  
751 Olsz E, Yancey KB *et al* (2007) Humanization of autoantigen. *Nat Med* 13: 378-383

752 Nusse YM, Savage AK, Marangoni P, Rosendahl-Huber AKM, Landman TA, de  
753 Sauvage FJ, Locksley RM, Klein OD (2018) Parasitic helminths induce fetal-like  
754 reversion in the intestinal stem cell niche. *Nature* 559: 109-113

755 Nystrom A, Velati D, Mittapalli VR, Fritsch A, Kern JS, Bruckner-Tuderman L (2013)  
756 Collagen VII plays a dual role in wound healing. *J Clin Invest* 123: 3498-3509

757 Osaka N, Takahashi T, Murakami S, Matsuzawa A, Noguchi T, Fujiwara T, Aburatani H,  
758 Moriyama K, Takeda K, Ichijo H (2007) ASK1-dependent recruitment and activation of  
759 macrophages induce hair growth in skin wounds. *J Cell Biol* 176: 903-909

760 Page ME, Lombard P, Ng F, Gottgens B, Jensen KB (2013) The epidermis comprises  
761 autonomous compartments maintained by distinct stem cell populations. *Cell Stem Cell*  
762 13: 471-482

763 Paladini RD, Takahashi K, Bravo NS, Coulombe PA (1996) Onset of re-epithelialization  
764 after skin injury correlates with a reorganization of keratin filaments in wound edge  
765 keratinocytes: defining a potential role for keratin 16. *J Cell Biol* 132: 381-397

766 Park S, Gonzalez DG, Guirao B, Boucher JD, Cockburn K, Marsh ED, Mesa KR, Brown  
767 S, Rompolas P, Haberman AM *et al* (2017) Tissue-scale coordination of cellular  
768 behaviour promotes epidermal wound repair in live mice. *Nat Cell Biol* 19: 155-163  
769 Paus R, Muller-Rover S, Van Der Veen C, Maurer M, Eichmuller S, Ling G, Hofmann U,  
770 Foitzik K, Mecklenburg L, Handjiski B (1999) A comprehensive guide for the recognition  
771 and classification of distinct stages of hair follicle morphogenesis. *J Invest Dermatol*  
772 113: 523-532  
773 Rittie L, Sachs DL, Orringer JS, Voorhees JJ, Fisher GJ (2013) Eccrine sweat glands  
774 are major contributors to reepithelialization of human wounds. *Am J Pathol* 182: 163-  
775 171  
776 Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for  
777 differential expression analysis of digital gene expression data. *Bioinformatics* 26: 139-  
778 140  
779 Rognoni E, Gomez C, Pisco AO, Rawlins EL, Simons BD, Watt FM, Driskell RR (2016)  
780 Inhibition of beta-catenin signalling in dermal fibroblasts enhances hair follicle  
781 regeneration during wound healing. *Development* 143: 2522-2535  
782 Rognoni E, Watt FM (2018) Skin Cell Heterogeneity in Development, Wound Healing,  
783 and Cancer. *Trends Cell Biol* 28: 709-722  
784 Sada A, Jacob F, Leung E, Wang S, White BS, Shalloway D, Tumber T (2016) Defining  
785 the cellular lineage hierarchy in the interfollicular epidermis of adult skin. *Nat Cell Biol*  
786 18: 619-631  
787 Saxena N, Mok KW, Rendl M (2019) An updated classification of hair follicle  
788 morphogenesis. *Exp Dermatol* 28: 332-344

789 Schmidt E, Zillikens D (2013) Pemphigoid diseases. *Lancet* 381: 320-332

790 Shimizu H, Ishiko A, Masunaga T, Kurihara Y, Sato M, Bruckner-Tuderman L,  
791 Nishikawa T (1997) Most anchoring fibrils in human skin originate and terminate in the  
792 lamina densa. *Lab Invest* 76: 753-763

793 Tamai K, Yamazaki T, Chino T, Ishii M, Otsuru S, Kikuchi Y, Inuma S, Saga K, Nimura  
794 K, Shimbo T *et al* (2011) PDGFRalpha-positive cells in bone marrow are mobilized by  
795 high mobility group box 1 (HMGB1) to regenerate injured epithelia. *Proc Natl Acad Sci*  
796 *U S A* 108: 6609-6614

797 Tang DD, Gerlach BD (2017) The roles and regulation of the actin cytoskeleton,  
798 intermediate filaments and microtubules in smooth muscle cell migration. *Respir Res*  
799 18: 54

800 Tanimura S, Tadokoro Y, Inomata K, Binh NT, Nishie W, Yamazaki S, Nakauchi H,  
801 Tanaka Y, McMillan JR, Sawamura D *et al* (2011) Hair follicle stem cells provide a  
802 functional niche for melanocyte stem cells. *Cell Stem Cell* 8: 177-187

803 Tidman MJ, Eady RA (1984) Evidence for a functional defect of the lamina lucida in  
804 recessive dystrophic epidermolysis bullosa demonstrated by suction blisters. *Br J*  
805 *Dermatol* 111: 379-387

806 Tolar J, Ishida-Yamamoto A, Riddle M, McElmurry RT, Osborn M, Xia L, Lund T,  
807 Slattery C, Uitto J, Christiano AM *et al* (2009) Amelioration of epidermolysis bullosa by  
808 transfer of wild-type bone marrow cells. *Blood* 113: 1167-1174

809 Tosti A, Duque-Estrada B, Murrell DF (2010) Alopecia in epidermolysis bullosa.  
810 *Dermatol Clin* 28: 165-169

811 Uroz M, Garcia-Puig A, Tekeli I, Elosegui-Artola A, Abenza JF, Marin-Llaurado A, Pujals  
812 S, Conte V, Albertazzi L, Roca-Cusachs P *et al* (2019) Traction forces at the cytokinetic  
813 ring regulate cell division and polyploidy in the migrating zebrafish epicardium. *Nat*  
814 *Mater* 18: 1015-1023

815 Vahidnezhad H, Youssefian L, Saeidian AH, Uitto J (2019) Phenotypic Spectrum of  
816 Epidermolysis Bullosa: The Paradigm of Syndromic versus Non-Syndromic Skin  
817 Fragility Disorders. *J Invest Dermatol* 139: 522-527

818 Watanabe M, Natsuga K, Nishie W, Kobayashi Y, Donati G, Suzuki S, Fujimura Y,  
819 Tsukiyama T, Ujiie H, Shinkuma S *et al* (2017) Type XVII collagen coordinates  
820 proliferation in the interfollicular epidermis. *Elife* 6

821 Watanabe M, Natsuga K, Shinkuma S, Shimizu H (2018) Epidermal aspects of type VII  
822 collagen: Implications for dystrophic epidermolysis bullosa and epidermolysis bullosa  
823 acquisita. *J Dermatol* 45: 515-521

824 Watt FM, Fujiwara H (2011) Cell-extracellular matrix interactions in normal and  
825 diseased skin. *Cold Spring Harb Perspect Biol* 3

826 Webber BR, O'Connor KT, McElmurry RT, Durgin EN, Eide CR, Lees CJ, Riddle MJ,  
827 Mathews WE, Frank NY, Kluth MA *et al* (2017) Rapid generation of Col7a1(-/-) mouse  
828 model of recessive dystrophic epidermolysis bullosa and partial rescue via  
829 immunosuppressive dermal mesenchymal stem cells. *Lab Invest* 97: 1218-1224

830 White KD, Abe R, Ardern-Jones M, Beachkofsky T, Bouchard C, Carleton B, Chodosh J,  
831 Cibotti R, Davis R, Denny JC *et al* (2018) SJS/TEN 2017: Building Multidisciplinary  
832 Networks to Drive Science and Translation. *J Allergy Clin Immunol Pract* 6: 38-69

833 Yu G, Wang LG, Han Y, He QY (2012) clusterProfiler: an R package for comparing  
834 biological themes among gene clusters. *OMICS* 16: 284-287

835 Yui S, Azzolin L, Maimets M, Pedersen MT, Fordham RP, Hansen SL, Larsen HL, Guiu  
836 J, Alves MRP, Rundsten CF *et al* (2018) YAP/TAZ-Dependent Reprogramming of  
837 Colonic Epithelium Links ECM Remodeling to Tissue Regeneration. *Cell Stem Cell* 22:  
838 35-49 e37

839

840 **Figure Legends**

841 **Figure 1. Healing of subepidermal blisters in neonatal mice.**

842 **A** Schematic diagram of suction blistering and sample collection. A blister produced on  
843 C57BL/6 wild-type (WT) mouse dorsal skin at P1. BM: basement membrane.

844 **B-D** Blistered samples at P1. (B) Hematoxylin and eosin (H&E, top) and alkaline  
845 phosphatase (AP, bottom) staining. Hair follicles (HFs) remaining in the dermis  
846 (indicated by arrows). Scale bar: 500  $\mu\text{m}$ . (C)  $\alpha 6$  Integrin (ITGA6, indicated by  
847 arrowheads) and type IV collagen (COL4, arrows) labeling (left). Laminin 332 (L332,  
848 arrows) staining (right). Scale bar: 100  $\mu\text{m}$ . (D) Ultrastructural findings of blistered skin  
849 (left image: blister roof, right image: blister bottom) . Hemidesmosomes (white  
850 arrowheads) and lamina densa (arrows) are indicated. Scale bar: 1  $\mu\text{m}$ .

851 **E** H&E (left), pan-cytokeratin (PCK, middle), and ITGA6 staining (right) at P2. The  
852 regenerated epidermis is indicated by arrowheads. Scale bar: 200  $\mu\text{m}$ .

853 **F** Keratin 14 (K14) and keratin 10 (K10) staining of the nonlesional (intact) and lesional  
854 (blistered) skin at P2 (upper images and inlets: sections, lower images: whole-mount  
855 imaging). HFs are indicated by arrows in the whole-mount images. Scale bar: 30  $\mu\text{m}$ .

856 **G** H&E (top) and loricrin (LOR, bottom) staining at P3. Scale bar: 200  $\mu\text{m}$ .

857 **H** H&E (left), PCK (middle), and LOR staining (right) at P4. Scale bar: 200  $\mu\text{m}$ .

858 Data information: Blisters are indicated by stars. Representative images are shown from  
859 three or more replicates in each group.

860 **Figure 2. Delayed HF growth during subepidermal blister healing.**

861 **A** Heat map (Pearson's correlation) of differentially expressed genes between the  
862 blistered (regenerated) and control WT dorsal skin epidermis at P2 (n=3).

863 **B** GO analysis of differentially expressed genes in the regenerated epidermis.

864 **C** Scatter plots of differentially expressed genes in the regenerated epidermis. The red  
865 dots represent upregulated genes, and the blue dots represent downregulated genes.  
866 The gray dotted lines indicate  $|\logFC|>1$ .

867 **D** Hair canals in the regenerated (lesional) and nonlesional epidermis at P4 (indicated  
868 by asterisks). Scale bar: 300  $\mu\text{m}$ .

869 **E** Quantification of hair canals in the lesional, nonlesional, and unaffected littermate  
870 control epidermis at P4 (n=5 biological replicates). The data are shown as the mean  $\pm$   
871 SE (littermate control) or connected with lines showing individual mice. \*0.01<p<0.05,  
872 one-way ANOVA test, followed by Tukey's test.

873 **F** HF morphogenesis stages at P4 in lesional, nonlesional, and unaffected littermate  
874 control skin (n=5 biological replicates).

875 **G** Whole-mount imaging of the blistered skin of ins-Topgal+ mice at P2. Scale bar:  
876 500  $\mu\text{m}$ .

877 Data information: Representative images are shown from three or more replicates in  
878 each group.

879



880 **Figure 3. Predominant contribution of HF-derived keratinocytes to subepidermal**  
881 **blister healing.**

882 **A** (Top) BrdU labeling of blistered samples at P2. Scale bar: 100  $\mu\text{m}$ . BrdU-positive cells  
883 are indicated by arrows. Blisters are indicated by stars. (Bottom) Quantification of BrdU-  
884 positive cells in the epidermis (left) and HFs (right) (n=4 biological replicates). The data  
885 are shown as the mean  $\pm$  SE. \*0.01<p<0.05, one-way ANOVA test, followed by Tukey's  
886 test. NS, no significance.

887 **B**  $\alpha 5$  integrin (ITGA5) labeling at P2 (left image: section, right image: whole-mount).  
888 Scale bar: 100  $\mu\text{m}$ . Blister edges (epidermal tongue) and HFs are indicated by  
889 arrowheads and arrows, respectively. Blisters are indicated by stars.

890 **C** Lineage tracing strategy.

891 **D** (Top) Sections of K14CreER:H2B-mCherry mice at P4. Scale bar: 100  $\mu\text{m}$ . (Bottom)  
892 Quantification of mCherry-positive cells (n=3).. The data from individual mice are  
893 connected by lines. Student's t-test. NS, no significance.

894 **E** Whole-mount imaging of K14CreER:R26R-confetti samples at P4. Scale bar: 200  $\mu\text{m}$ .

895 **F** (Top) Sections of Lrig1CreER:H2B-mCherry mouse skin at P4. Scale bar: 100  $\mu\text{m}$ .  
896 (Bottom) Quantification of mCherry-positive cells (n=3). The data from individual mice  
897 are connected by lines. \*0.01<p<0.05, Student's t-test.

898 **G** Whole-mount imaging of Lrig1CreER:R26R-confetti mouse samples at P4. Scale bar:  
899 200  $\mu\text{m}$ .

900 Data information: Representative images are shown from three or more replicates in  
901 each group.

902

903 **Figure 4. Effects of HF reduction on subepidermal blister healing.**

904 **A** Type XVII collagen (COL17, arrowheads indicate the hair bulge) and laminin  $\beta$ 1

905 (LAMB1) labeling in WT dorsal skin sections (P1). Scale bar: 100  $\mu$ m.

906 **B, C** Blistered samples of *Col17a1<sup>-/-</sup>* mouse dorsal skin at P1. ITGA6 (indicated by

907 arrowheads) and COL4 (arrows) labeling (B). L332 staining (C, arrows). Scale bar:

908 100  $\mu$ m.

909 **D** H&E staining of blistered skin from *Col17a1<sup>-/-</sup>* mice at P1. HFs detached from the

910 dermis in *Col17a1<sup>-/-</sup>* skin are indicated by arrowheads. Scale bar: 500  $\mu$ m.

911 **E** Whole-mount AP staining of the blister roof epidermis from *Col17a1<sup>-/-</sup>* mice (right) and

912 littermate controls (left) at P1. Scale bar: 500  $\mu$ m.

913 **F** H&E (P2, left), ITGA6 (P2, middle) and LOR staining (P4, right) of *Col17a1<sup>-/-</sup>* mice

914 (top) and littermate controls (bottom) . The regenerated epidermis is indicated by

915 arrowheads. Scale bar: 200  $\mu$ m.

916 **G** (Top) BrdU labeling of *Col17a1<sup>-/-</sup>* skin at P2. Scale bar: 100  $\mu$ m. (Bottom)

917 Quantification of BrdU-positive cells in the epidermis surrounding blisters (n= 3 (control)

918 and 4 (*Col17a1<sup>-/-</sup>) biological replicates*). The data are shown as the mean  $\pm$  SE.

919 Student's t-test. NS, no significance.

920 **H, I** (H) Lineage tracing of K14CreER:R26R-mCherry:*Col17a1<sup>-/-</sup>* at P4. Scale bar:

921 100  $\mu$ m. (I) Quantification of mCherry-positive cells in the regenerated epidermis (n=3

922 biological replicates). The data from individual mice are connected by lines.

923 \*0.01<p<0.05, Student's t-test.

924 Data information: Blisters are indicated by stars. Representative images are shown from

925 three or more replicates in each group.

926 **Figure 5. Involvement of keratinocyte shape transformation in subepidermal**  
927 **blister healing.**

928 **A** Type VII collagen (COL7) labeling in WT dorsal skin sections (P1). Scale bar: 200  $\mu\text{m}$ .  
929 **B** ITGA6/COL4 (left, indicated by arrows) and L332 labeling (right, indicated by arrows)  
930 in the blistered skin of *Col7a1<sup>-/-</sup>* mice at P1. Scale bar: 100  $\mu\text{m}$ .  
931 **C** Schematic diagram of control, *Col17a1<sup>-/-</sup>*, and *Col7a1<sup>-/-</sup>* mouse skin splits. BM:  
932 basement membrane.  
933 **D** H&E staining of blistered skin from *Col7a1<sup>-/-</sup>* mice (right) and their littermate controls  
934 (left) at P1. Scale bar: 200  $\mu\text{m}$ .  
935 **E** Whole-mount AP staining of the blister roof epidermis from *Col7a1<sup>-/-</sup>* mice (right) and  
936 their littermate controls (left) at P1. Scale bar: 500  $\mu\text{m}$ .  
937 **F** K10/K14 (low magnification, left) and K14 (high magnification, middle and right)  
938 labeling of *Col7a1<sup>-/-</sup>* mouse (bottom) and littermate control (top) blistered skin at P2.  
939 Scale bar: 30  $\mu\text{m}$ .  
940 **G** Quantification of BrdU-positive cells per  $\mu\text{m}$  HF length (n=55 HFs from three control  
941 and 143 HFs from four *Col7a1<sup>-/-</sup>* mice). The data are shown as violin plots. Student's t-  
942 test. NS, no significance.  
943 **H** Length of the major axis of keratinocytes in the regenerated epidermis (n=244 (control,  
944 L), and 132 (*Col7a1<sup>-/-</sup>*, L) cells from four mice, respectively) and in the surrounding intact  
945 epidermis (basal cells; n=200 (control, NL), 299 (*Col7a1<sup>-/-</sup>*, NL), from four mice,  
946 respectively). NL: nonlesional area. L: lesional area. The data are shown as violin plots.  
947 \*\*\*\*p<0.0001, one-way ANOVA test, followed by Tukey's test. NS, no significance.

948 **I** K10/K14 (left) and K14 (high magnification, right) labeling of WT blistered skin treated  
949 with CaCl<sub>2</sub> (middle and bottom) or PBS (top) at P2. Scale bar: 30 μm.

950 **J** Quantification of BrdU-positive cells per μm HF length (n=83 (PBS), 95 (1.8 mM  
951 CaCl<sub>2</sub>), and 97 (9.0 mM CaCl<sub>2</sub>) HFs from four mice). One-way ANOVA test, followed by  
952 Tukey's test. NS, no significance.

953 **K** Length of the major axis of keratinocytes in the regenerated epidermis (n=433 (PBS,  
954 L), 451 (1.8 mM CaCl<sub>2</sub>, L), and 425 (9.0 mM CaCl<sub>2</sub>, L) cells from four mice) and in the  
955 surrounding intact epidermis (basal cells; n=311 (PBS, NL), 279 (1.8 mM CaCl<sub>2</sub>, NL),  
956 302 (9.0 mM CaCl<sub>2</sub>, NL) cells from four mice). NL: nonlesional area. L: lesional area.

957 The data are shown as violin plots. \*\*\*\*p<0.0001, one-way ANOVA test, followed by  
958 Tukey's test. NS, no significance.

959 Data information: Blisters are indicated by stars. Representative images are shown from  
960 three or more replicates in each group. The dashed and dotted lines in the violin plots  
961 show the median and quartiles, respectively.

962

963 **Figure 6. Mathematical modeling of subepidermal blister healing.**

964 **A** A particle-based model of subepidermal blister healing at the basal layer. Epidermal  
965 basal cells (colored in blue), which do not divide, are placed on the basement  
966 membrane (gray). Stem cells (SCs, green) give rise to progeny (simulating HF-derived  
967 cells; red) within epidermal defects or in the surrounding epidermis (IFE-derived cells;  
968 yellow).  $t$ : arbitrary time. See **Movie EV1**.

969 **B** Contribution of each progeny cell within the epidermal defect or of surrounding  
970 epidermis to subepidermal blister healing, measured as the ratio of the area occupied  
971 by each progeny to the area of the initial epidermal defect.

972 **C** A model of subepidermal blister healing without SCs within epidermal defects. See  
973 **Movie EV2**.

974 **D** Time course of subepidermal blister healing in control (A) and SC-depleted epidermal  
975 defects (C).

976 **E** Effects of the impaired flattening of keratinocytes upon epidermal regeneration. The  
977 diameter of basal keratinocytes (long axis of the spheroid) in the regenerated vs.  
978 surrounding epidermis was calculated as 1.5:1 (in contrast to 2:1 in Figure 6A). See  
979 **Movie EV3**.

980 **F** Time course of wound healing for control (A) and less flattened keratinocytes in the  
981 regenerated epidermis (E).

982

983 **Expanded View Figure Legends**

984 **Figure EV1. Healing processes of subepidermal blisters.**

985 **A** H&E (left) and PCK labeling (right) of blistered skin at P1 (WT). Scale bar: 100  $\mu\text{m}$ .

986 **B** ITGA6 (arrowheads in the blister roof) and COL4 (arrows in the blister bottom)

987 labeling at P1 (WT). HFs that express ITGA6 on the dermal side are indicated by

988 hashtags. Scale bar: 100  $\mu\text{m}$  (left) and 50  $\mu\text{m}$  (right).

989 **C** Electron microscopy of blistered skin at P1 (WT). Hemidesmosomes (white

990 arrowheads) and the lamina densa (arrows) are indicated. Scale bar: 10  $\mu\text{m}$  (left) and 1

991  $\mu\text{m}$  (right).

992 **D** AP staining of WT (left) and *Col17a1*<sup>-/-</sup> (right) blistered skin at P1. Scale bar: 100  $\mu\text{m}$ .

993 **E** H&E (left), ITGA6 (middle), and ITGA5 (right) staining at P2 (WT). The regenerated

994 epidermis is indicated by arrowheads. ITGA5+ cells at the tip of the HFs are indicated

995 by arrows. Blisters are indicated by stars. Scale bar: 100  $\mu\text{m}$ .

996 **F** Schematic of the suction blister experiments and HF development/cycles.

997 **G** Quantification of immune cells (CD3, F4-80, and Ly6G) in the dermis of blistered WT

998 and unaffected littermate control skin at P2 (n=4 biological replicates) and P4 (n=3

999 biological replicates). The data are shown as the mean  $\pm$  SE. \*0.01<p<0.05, Student's t-

1000 test. NS, no significance.

1001 Data information: Blisters are indicated by stars. Representative images are shown from

1002 three or more replicates in each group.

1003

1004

1005

1006 **Figure EV2. RNA-seq data on subepidermal blister healing.**

1007 **A** Volcano plot showing differentially expressed genes (DEG) between the blistered  
1008 (regenerated) and control skin epidermis at P2. Significantly ( $|\text{LogFC}| > 1$ ;  $\text{FDR} < 0.05$ ) up-  
1009 regulated and down-regulated DEG are shown in red and blue, respectively.

1010 **B** Bar plot summarizing GSEA enrichment results for selected up-regulated and down-  
1011 regulated KEGG pathways. The normalized enrichment score (NES), p-value and FDR  
1012 are shown.

1013 **C** GSEA enrichment plots of “Wnt signaling pathway”, “Hedgehog signaling pathway”  
1014 and “Melanogenesis” KEGG gene sets.

1015 **D** Network visualization of the top ten down-regulated and up-regulated ( $\text{FDR} < 0.05$ ) GO  
1016 term clusters for different GO categories (Biological process, Molecular Function and  
1017 Cellular Component). Node size reports the number of enriched genes in each GO term  
1018 (gene numbers in bottom panels). Nodes are colored as a pie chart depicting the  
1019 proportion of down-regulated (blue) and up-regulated (red) genes in each GO term.  
1020 Edge thickness depicts the number of shared genes between GO terms.

1021

1022 **Figure EV3. Lineage tracing of subepidermal blister healing.**

1023 **A** Blistered area of K14CreER:R26R-confetti (left) and Lrig1CreER:R26R-confetti (right)

1024 mouse skin samples at P4. Scale bar: 100  $\mu$ m.

1025 **B** Phospho-Histone H3 (PH3) staining of blistered skin at P2 (WT). Scale bar: 100  $\mu$ m.

1026 **C** PH3 staining\_(arrowhead) of blistered skin at P2 (Lrig1CreER:R26R-H2BmCherry).

1027 Scale bar: 100  $\mu$ m.

1028 **D** Quantification of mCherry-positive cells during lineage tracing (n=3 biological

1029 replicates). The data are shown as the mean  $\pm$  SE.

1030 Data information: Blisters are indicated by stars. Representative images are shown from

1031 three or more replicates in each group.

1032



1033 **Figure EV4. Dependence of subepidermal blister healing on flattening ratio and**  
1034 **SC arrangement.**

1035 **A** Arrangements of the SCs within epidermal defects (dispersed, 2 cells grouped, 4 cells  
1036 grouped, and 8 cells grouped) at  $t = 0$ .

1037 **B** Time course of subepidermal blister healing for different keratinocyte flattening ratios  
1038 (1.0-2.0), each with four SC arrangements.

1039 **C** Time to full recovery (100% healing rate) for different keratinocyte flattening ratios,  
1040 averaged over four SC arrangements. The data are shown as the mean  $\pm$  SD.

1041

1042 **Figure EV5. Subepidermal blister healing in humans.**  
1043 H&E staining of human subepidermal blister samples with re-epithelized areas (blisters  
1044 1, 2, and 3). Blisters are indicated by stars. The regenerated epidermis from HF<sub>s</sub> is  
1045 indicated by arrows. Scale bar: 300  $\mu$ m.  
1046

Figure 1. Healing of subepidermal blisters in neonatal mice

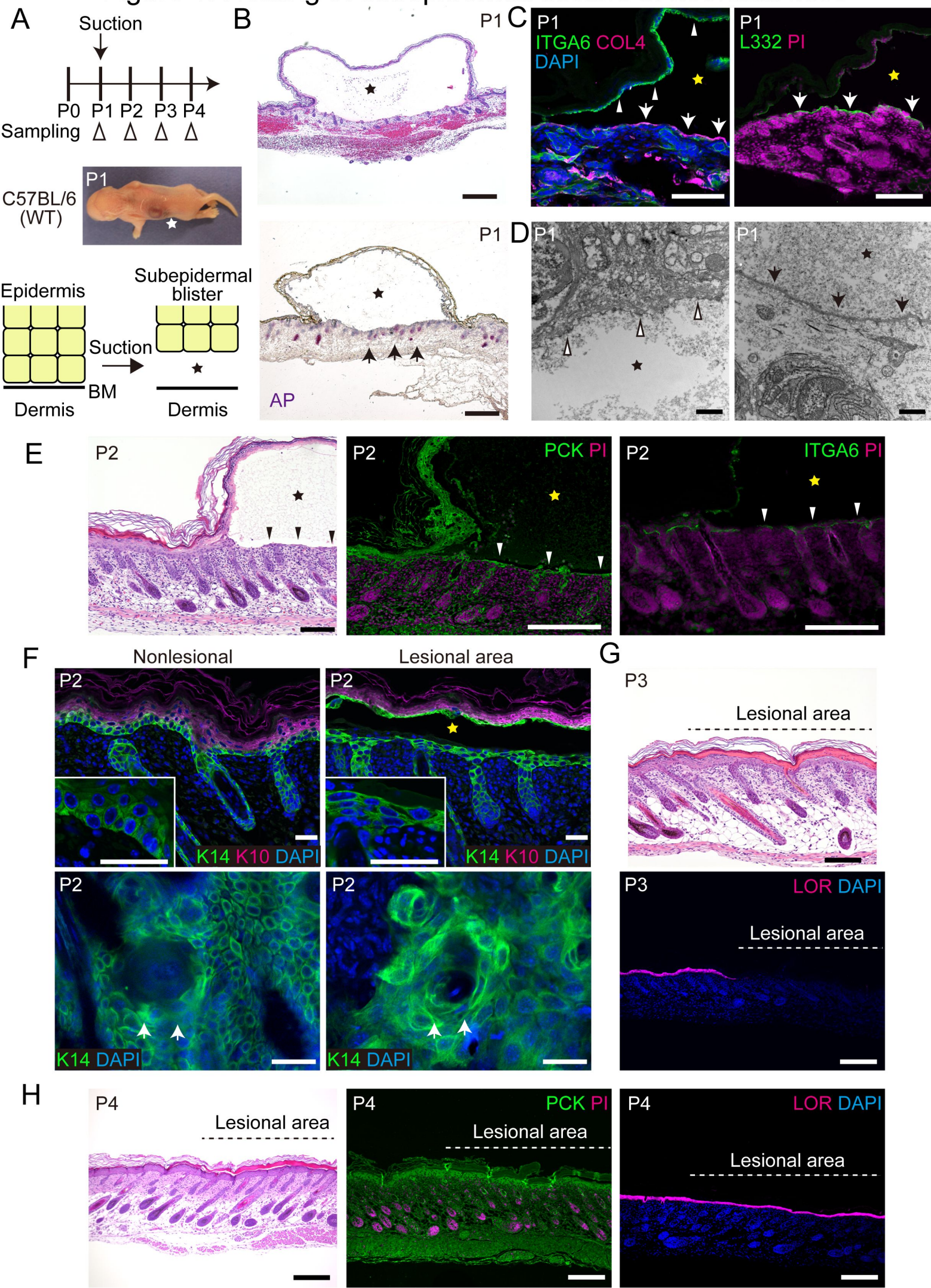




Figure 2. Delayed HF development during subepidermal blister healing

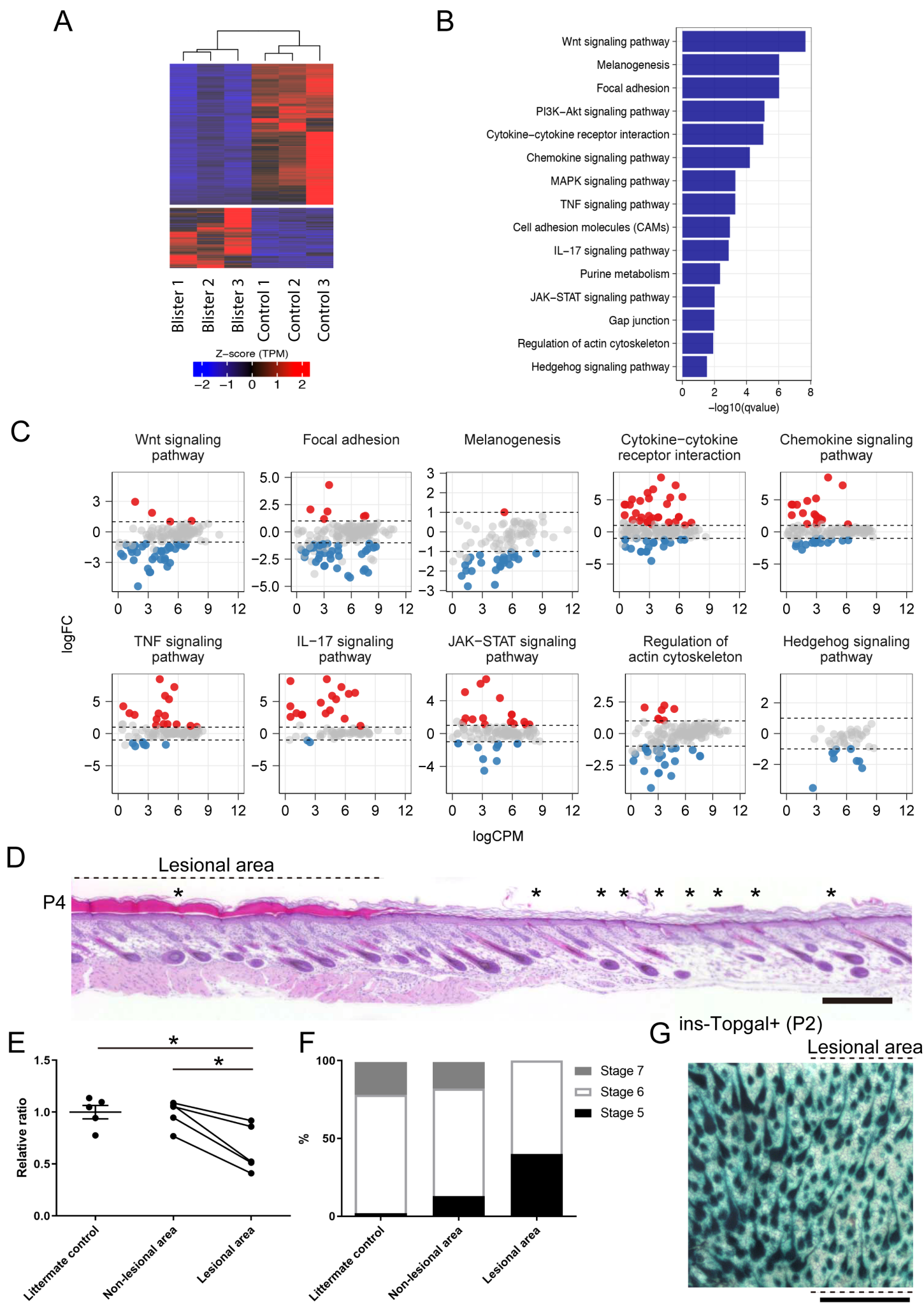


Figure 3. Predominant contribution of HF-derived keratinocytes to subepidermal blister healing

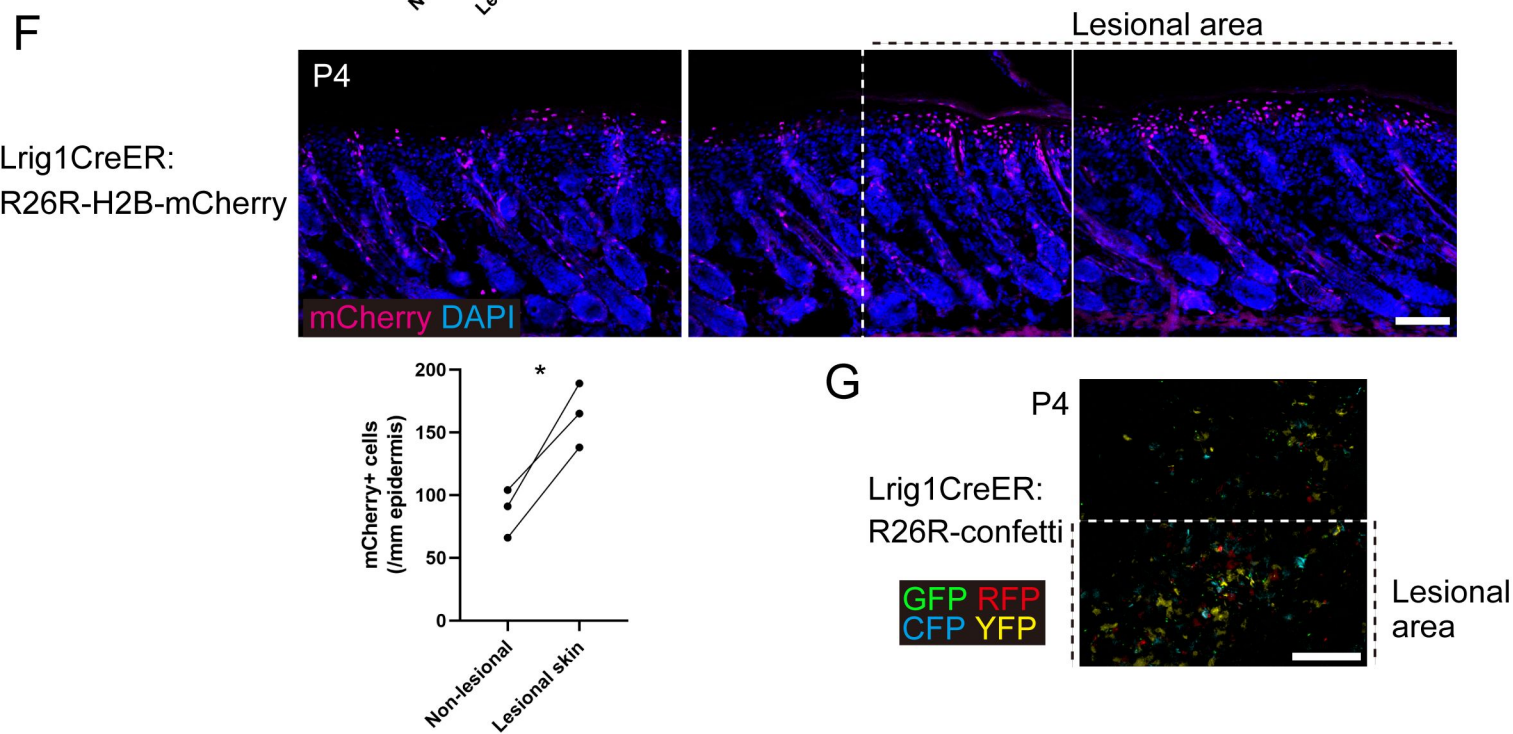
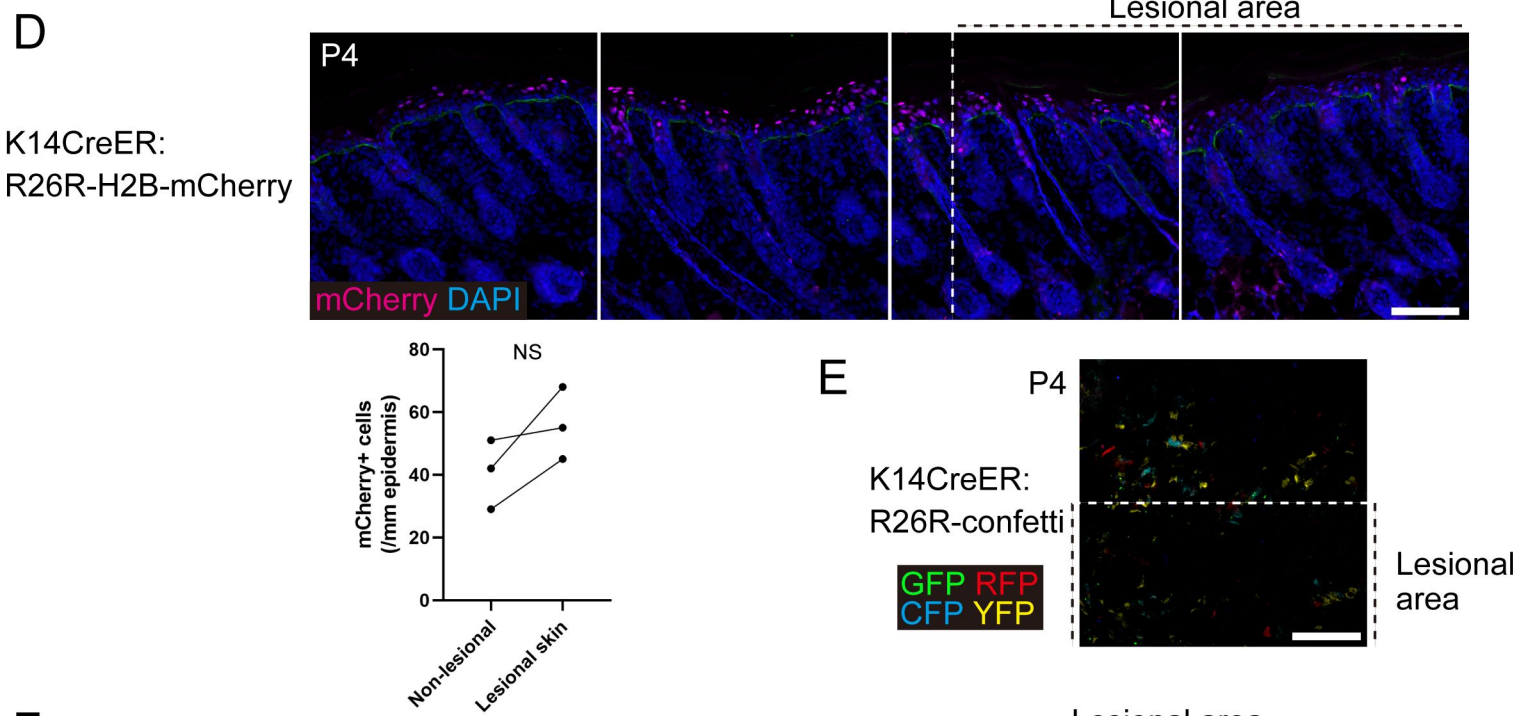
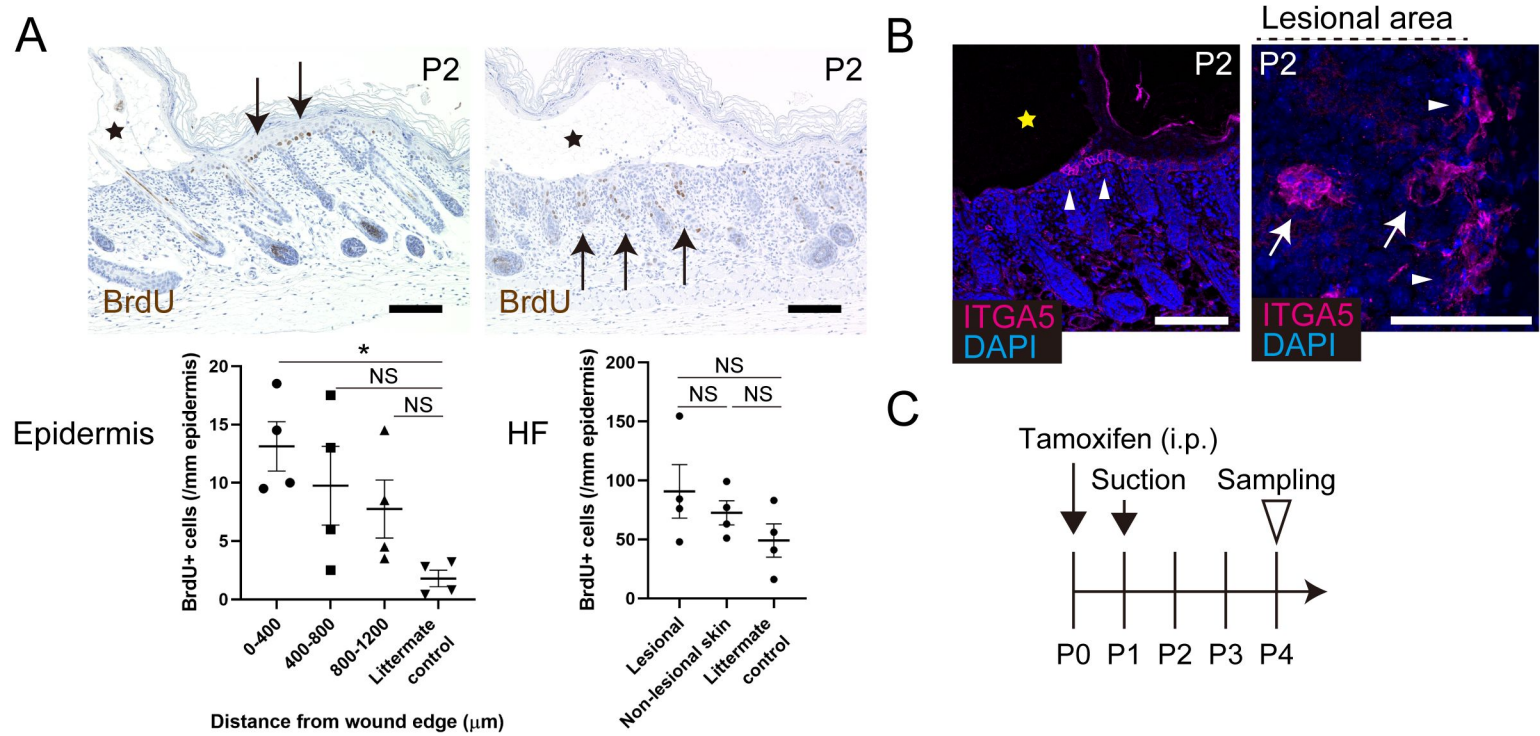




Figure 4. Effects of HF reduction on subepidermal blister healing

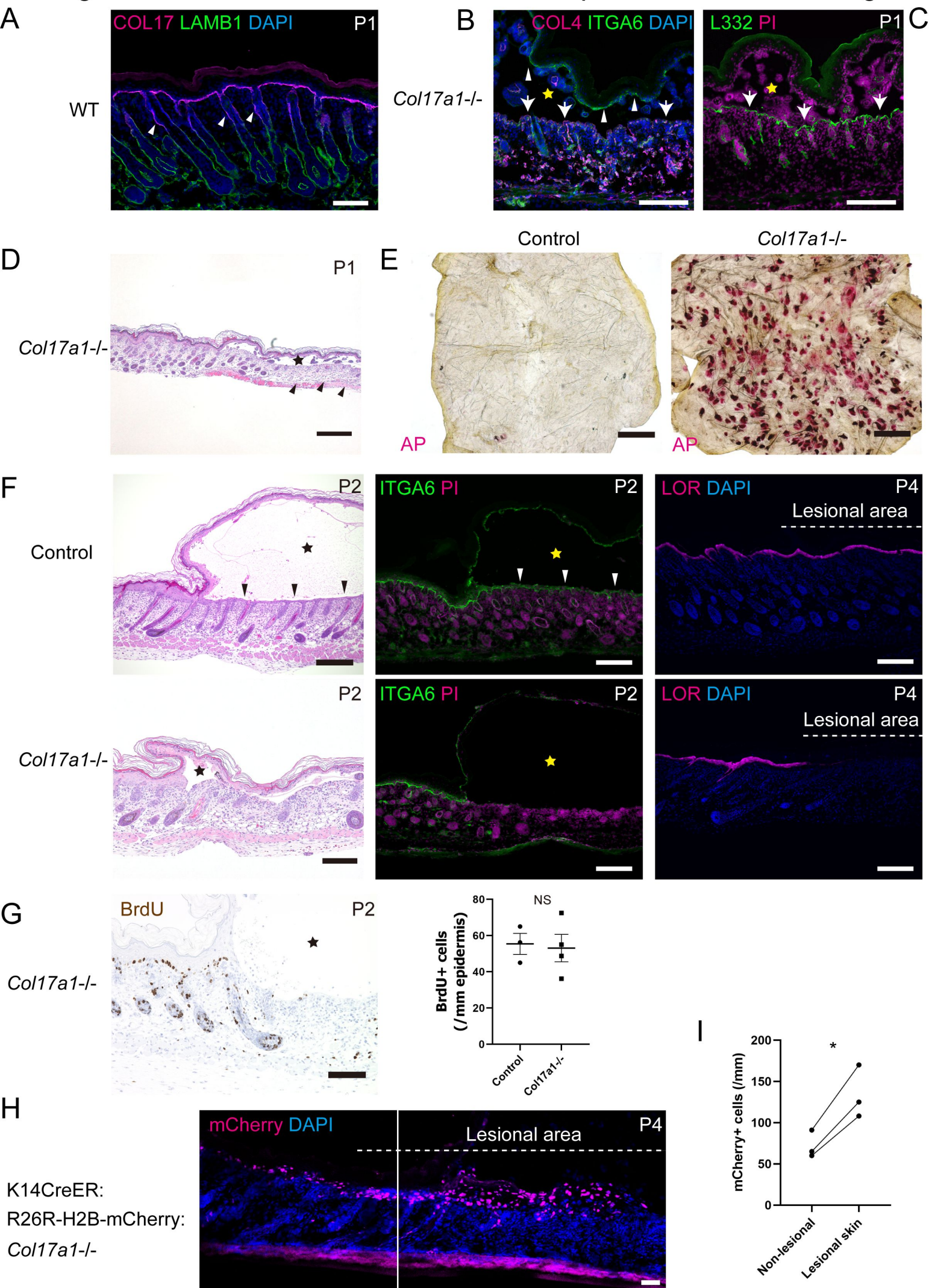




Figure 5. Involvement of keratinocyte shape transformation in subepidermal blister healing

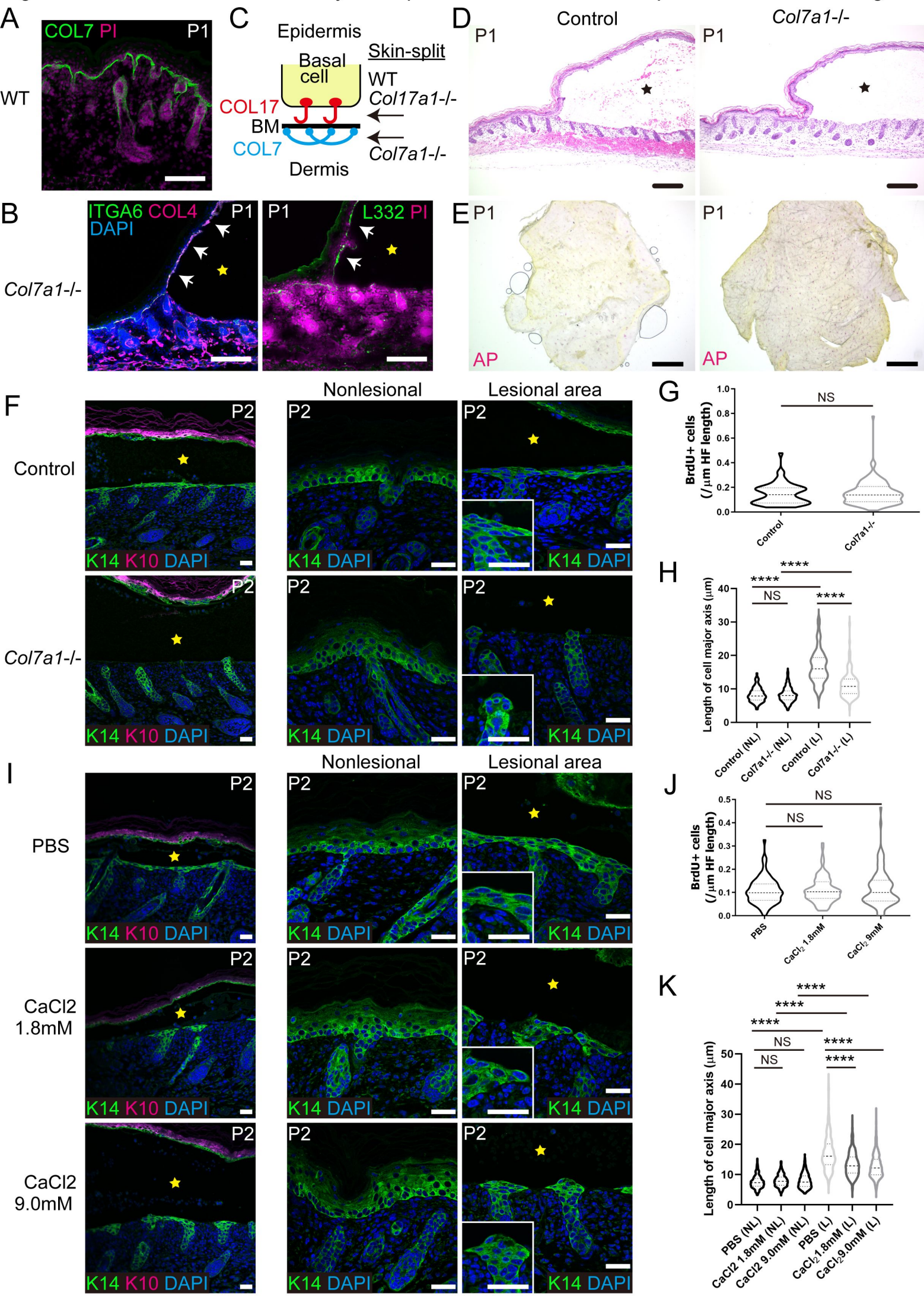
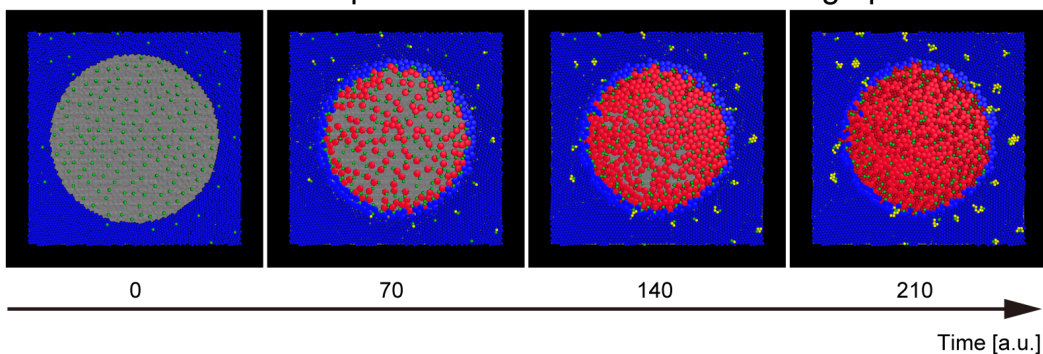




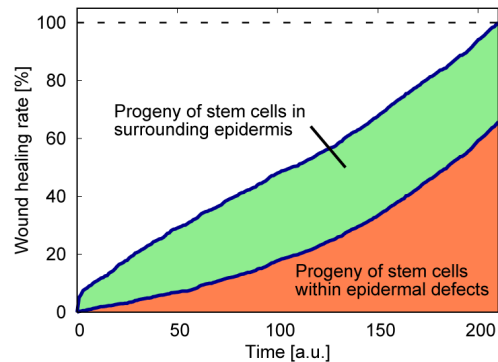
Figure 6. Mathematical modeling of subepidermal blister healing

A

Stem cells: both in epidermal defects and surrounding epidermis

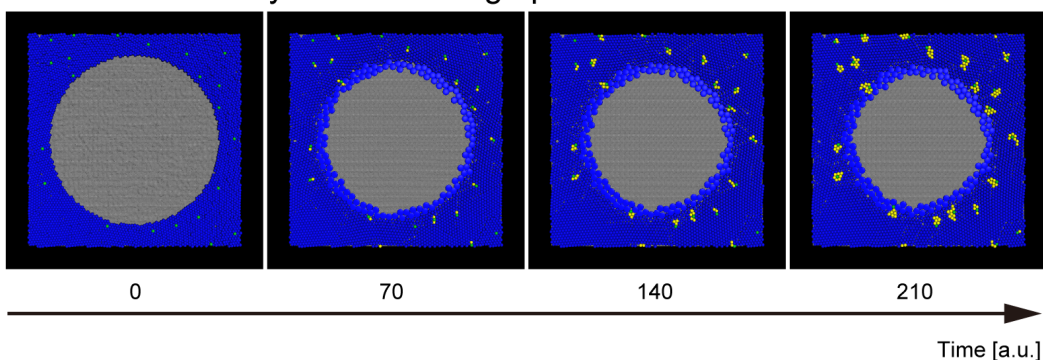


B

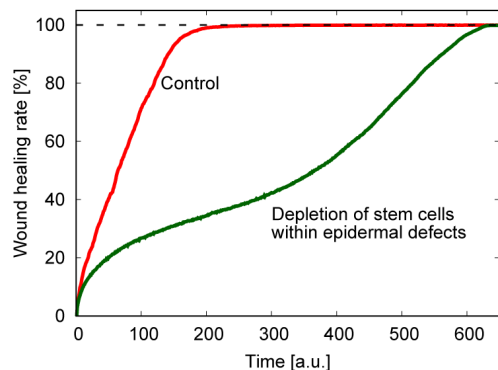


C

Stem cells: only in surrounding epidermis

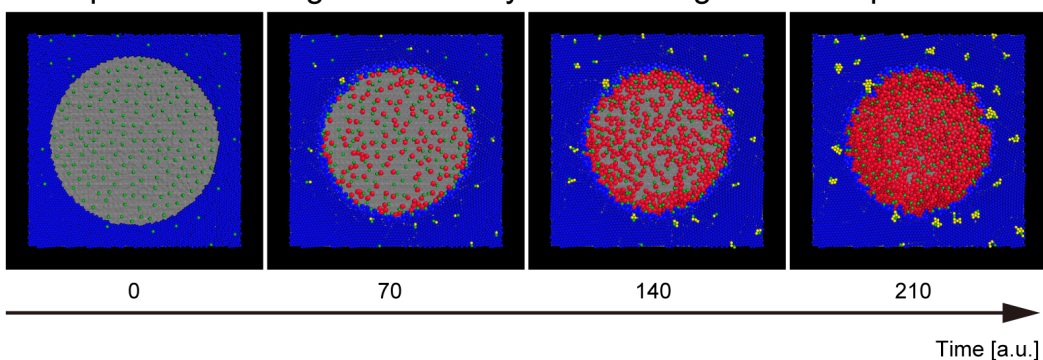


D



E

Impaired flattening of keratinocytes in the regenerated epidermis



F

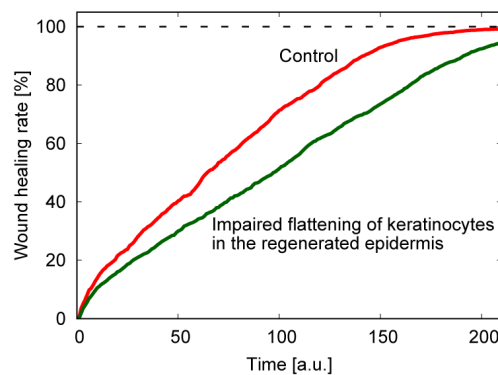
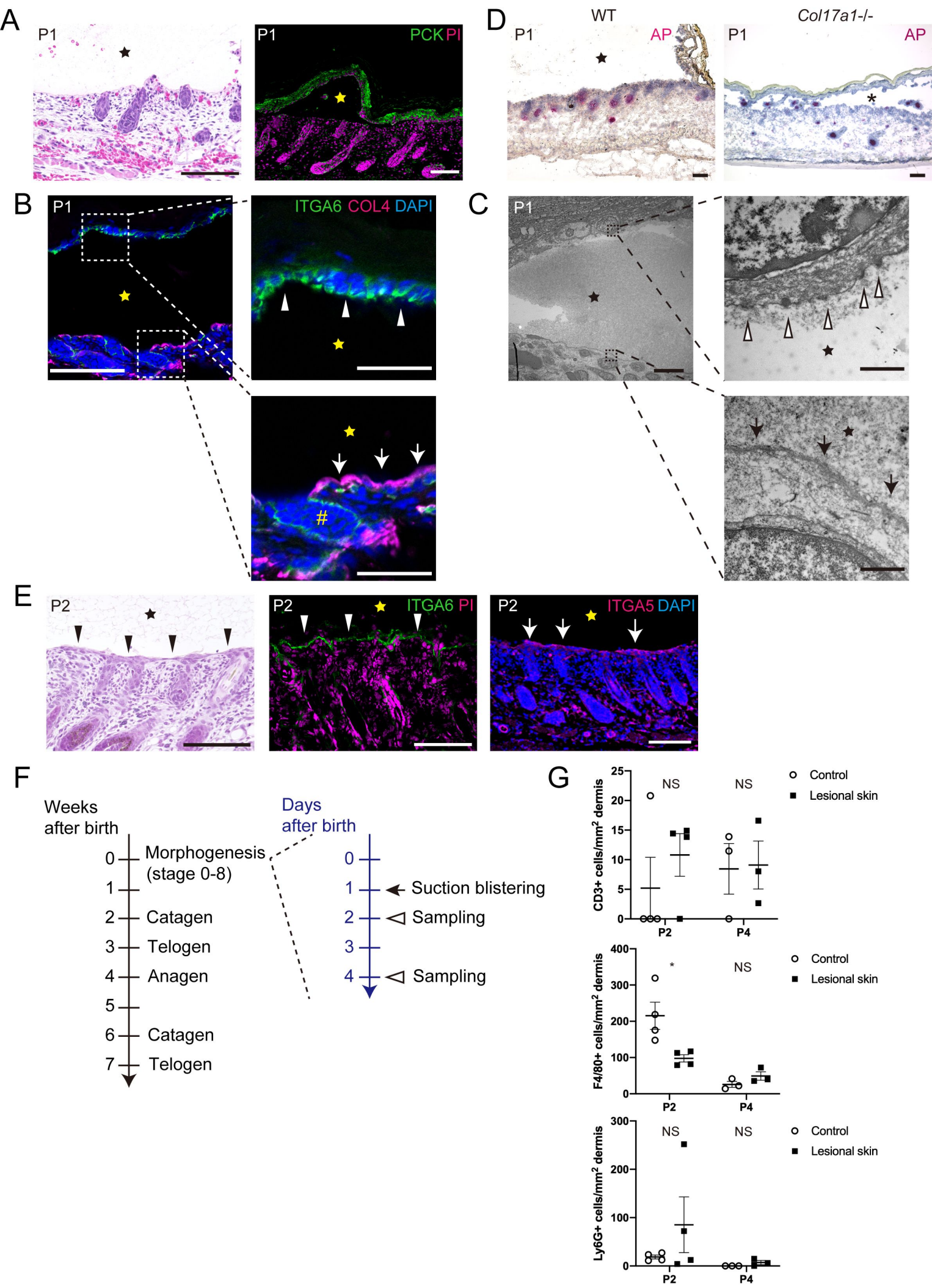


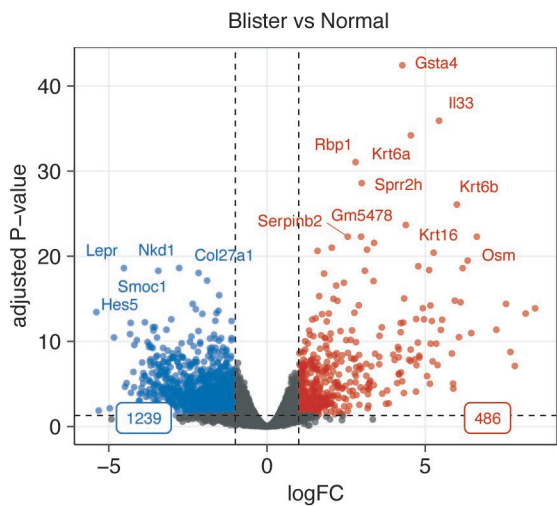


Figure EV1. Healing processes of subepidermal blisters

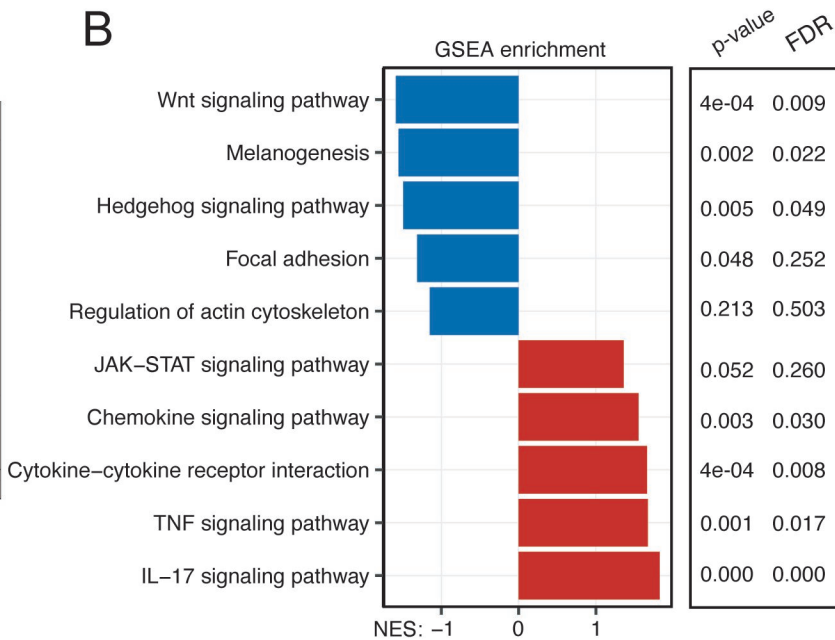


# Figure EV2. RNA-seq on blister healing

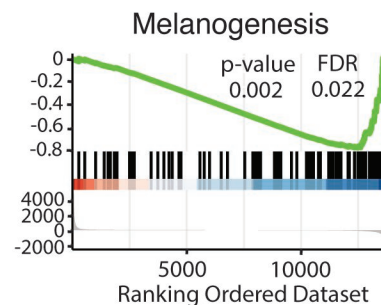
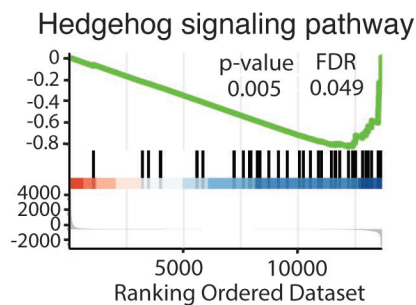
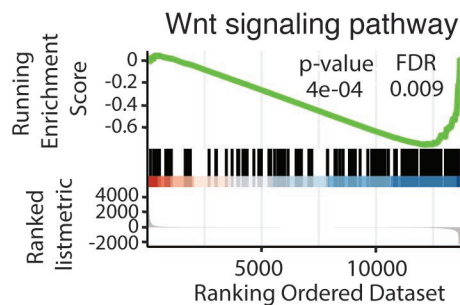
**A**



**B**



**C**



**D**

Down-regulated (blue square) Up-regulated (red square)

GO: Biological Process

GO: Molecular Function

GO: Cellular Component

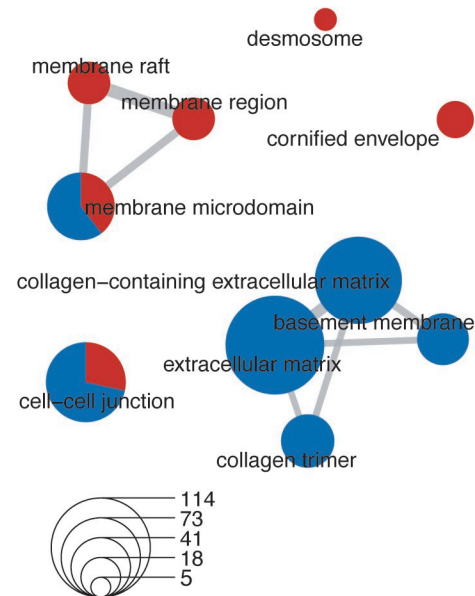
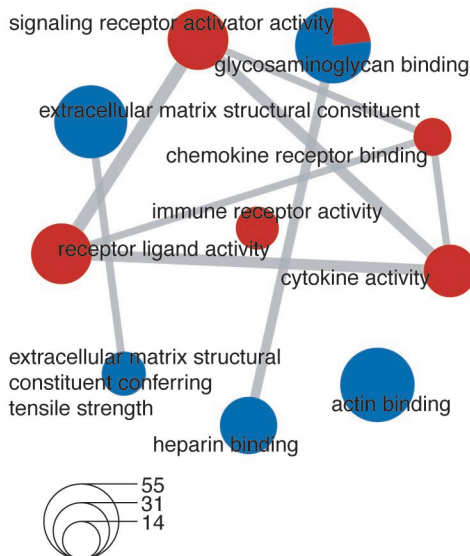
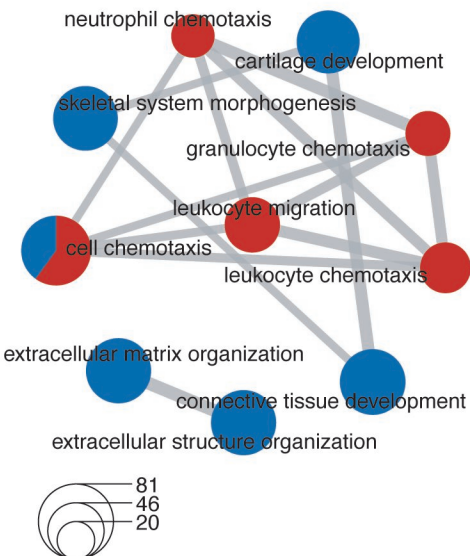




Figure EV3. Lineage tracing of subepidermal blister healing

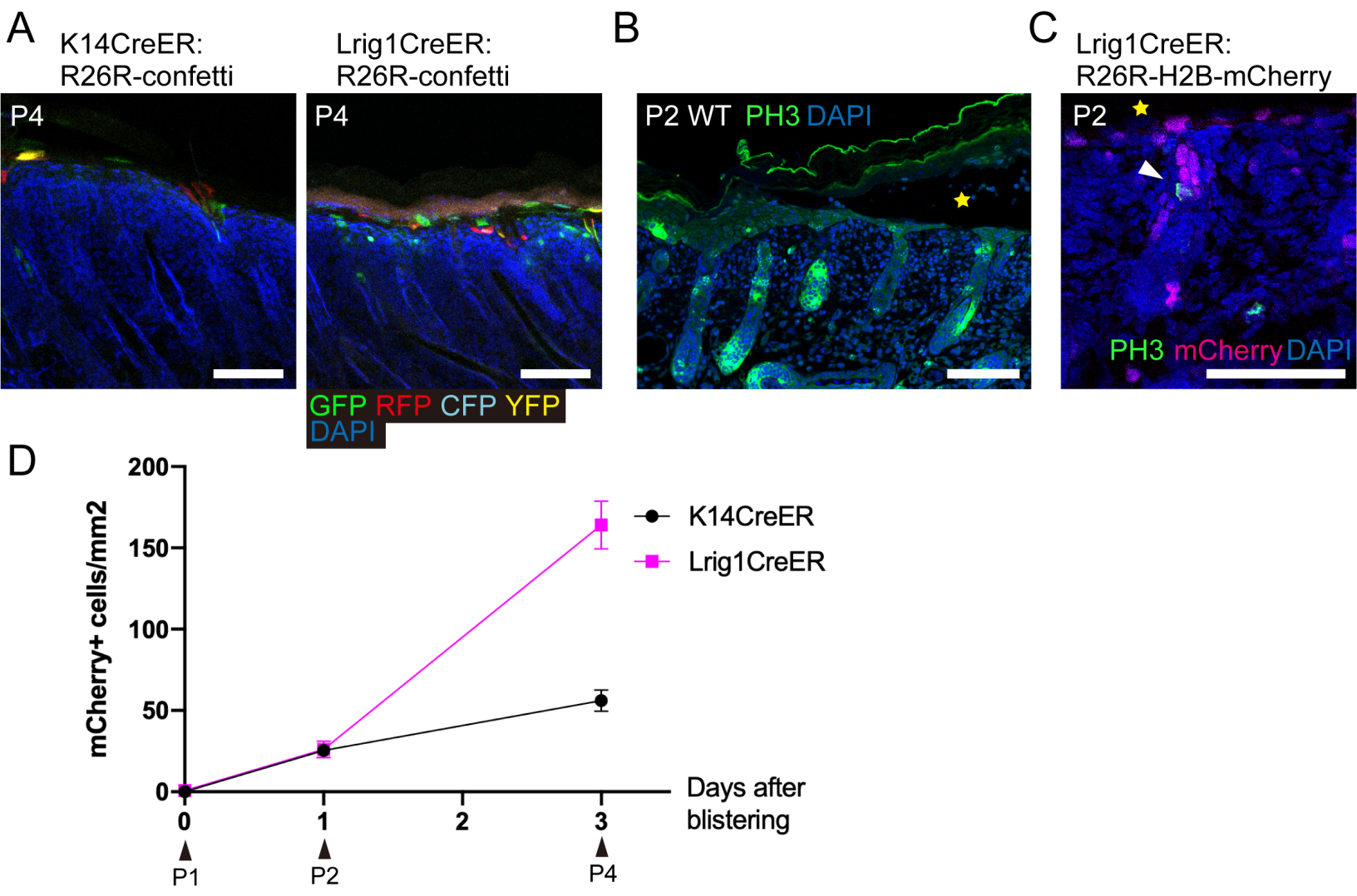


Figure EV4. Dependence of subepidermal blister healing on flattening ratio and SC arrangement

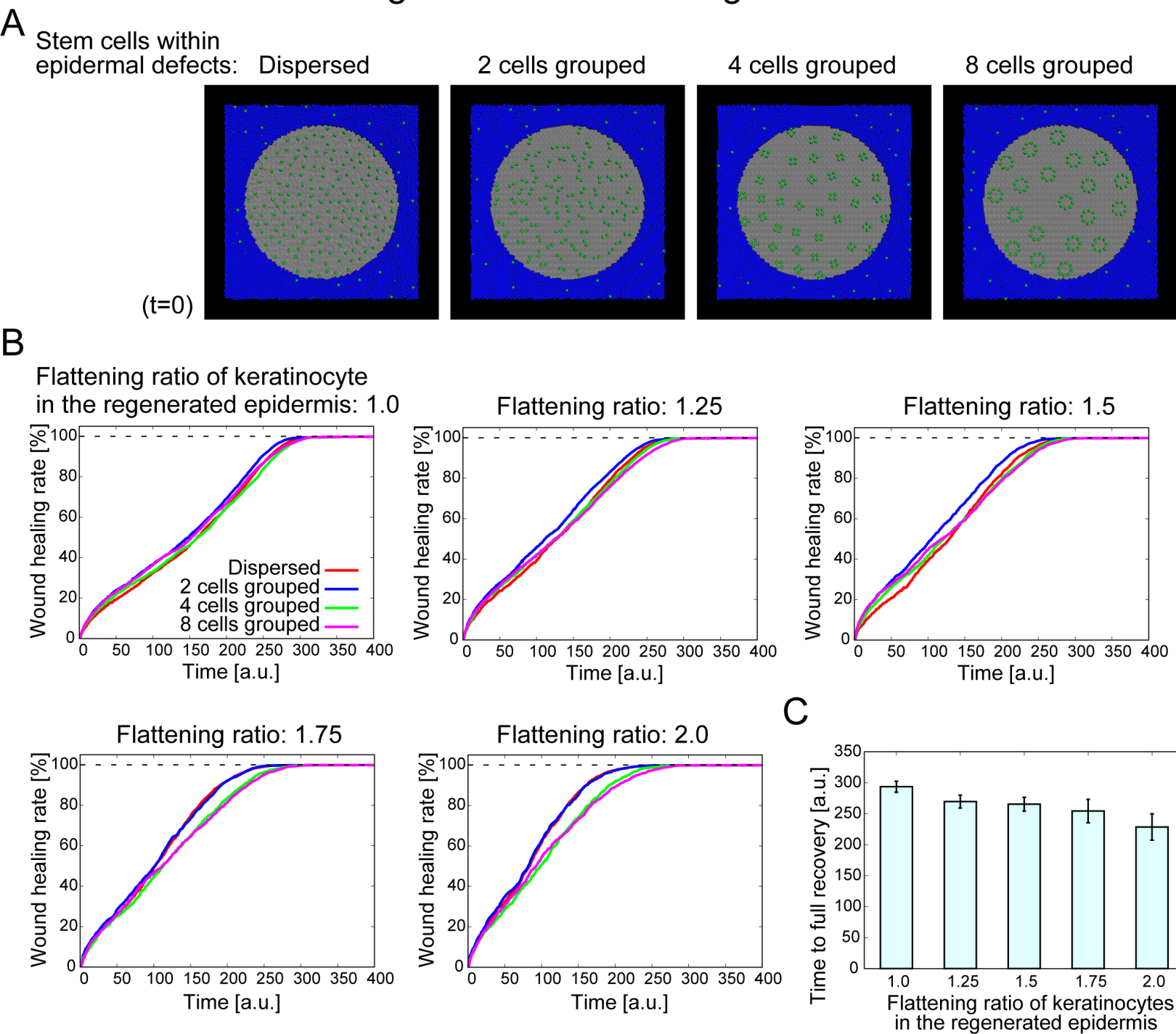
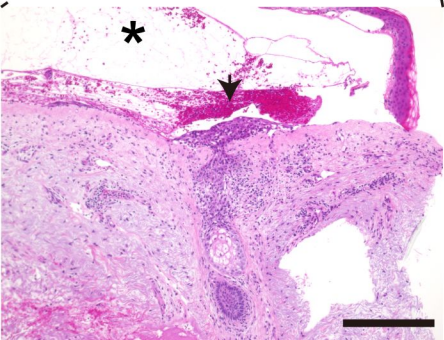
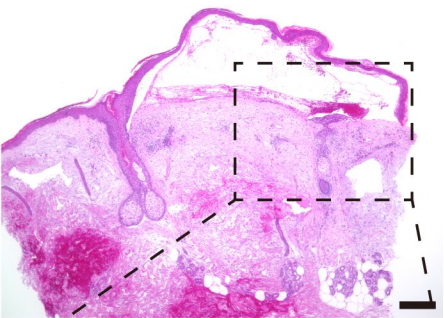
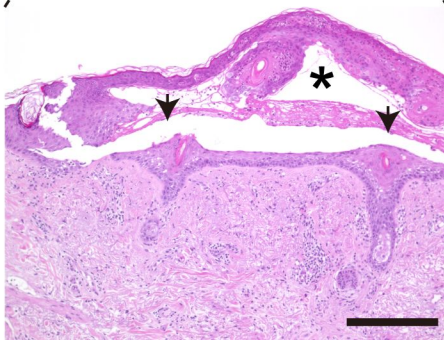
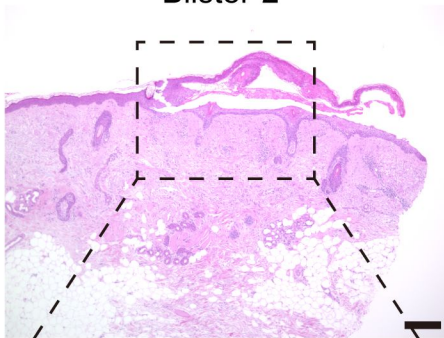


Figure EV5. Subepidermal blister healing in humans

Blister-1



Blister-2



Blister-3

