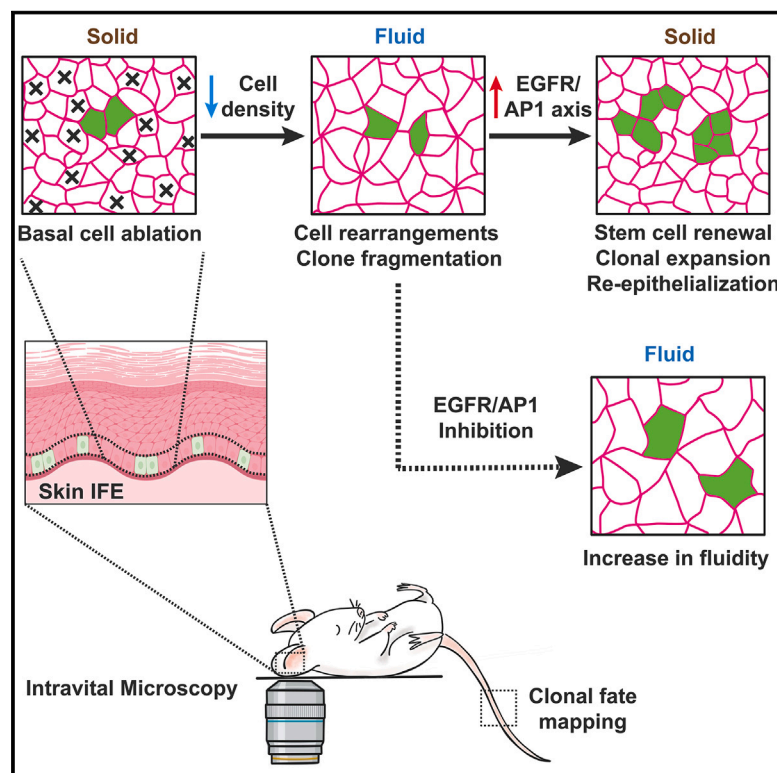


Dynamic regulation of tissue fluidity controls skin repair during wound healing

Graphical abstract



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In brief

During skin wound repair, the basal cell layer transitions from a solid-like homeostatic state to a fluid-like state that allows tissue remodeling during repair and then progressively returns to a solid-like state with re-epithelialization.

Highlights

- Clonal analysis after cell ablation uncovers dynamic of skin SCs during tissue repair
- Mathematical modeling supports a density-dependent promotion of symmetric SC division
- Wound healing involves dynamic changes in tissue fluidity
- EGFR/AP1 axis controls a regenerative state that regulates tissue fluidity and repair



Article

Dynamic regulation of tissue fluidity controls skin repair during wound healing

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SUMMARY

During wound healing, different pools of stem cells (SCs) contribute to skin repair. However, how SCs become activated and drive the tissue remodeling essential for skin repair is still poorly understood. Here, by developing a mouse model allowing lineage tracing and basal cell lineage ablation, we monitor SC fate and tissue dynamics during regeneration using confocal and intravital imaging. Analysis of basal cell rearrangements shows dynamic transitions from a solid-like homeostatic state to a fluid-like state allowing tissue remodeling during repair, as predicted by a minimal mathematical modeling of the spatiotemporal dynamics and fate behavior of basal cells. The basal cell layer progressively returns to a solid-like state with re-epithelialization. Bulk, single-cell RNA, and epigenetic profiling of SCs, together with functional experiments, uncover a common regenerative state regulated by the EGFR/AP1 axis activated during tissue fluidization that is essential for skin SC activation and tissue repair.

INTRODUCTION

The epidermis is a stratified epithelium composed of the interfollicular epidermis (IFE), which constitutes the skin barrier, the hair follicles (HFs) with their associated sebaceous glands, and the infundibulum that connects the HF to the IFE. The mature IFE comprises a single inner layer of proliferative cells, called the basal layer, expressing keratin 14 (K14), and several suprabasal layers containing terminally differentiated cells.^{1,2} In the absence of injury, the distinct epidermal compartments (IFE, HF, and infundibulum) are sustained by their own pool of compartment-restricted stem cells (SCs).^{3–5}

During adult life, the IFE undergoes a process of continual turnover to replace the damaged and dead cells that are constantly shed from the skin surface.¹ To maintain the size of tissue and density of cells constant, the frequency of cell dupli-

cation must perfectly compensate the loss rate.⁶ Quantitative clonal analysis in the epidermis has suggested that homeostasis is achieved by asymmetric renewal at the population level, mediated by SCs and committed progenitors (CPs).^{7,8} While the dynamics of SCs during adult homeostasis appear to follow a pattern of stochastic fate, with a balance between symmetric renewal and symmetric differentiation, the underlying mechanisms that coordinate fate decisions remain unresolved. One possibility is that symmetric cell differentiation, resulting in basal cell depletion, is sensed by the epidermis, inducing basal cell duplication.

As a first barrier against the external environment, the skin epidermis is also constantly subjected to injuries such as wounding, which need to be repaired rapidly to sustain the epidermal protective function and avoid systemic infection.⁹ Wounding leads to the rapid activation of a cascade of events that mediate



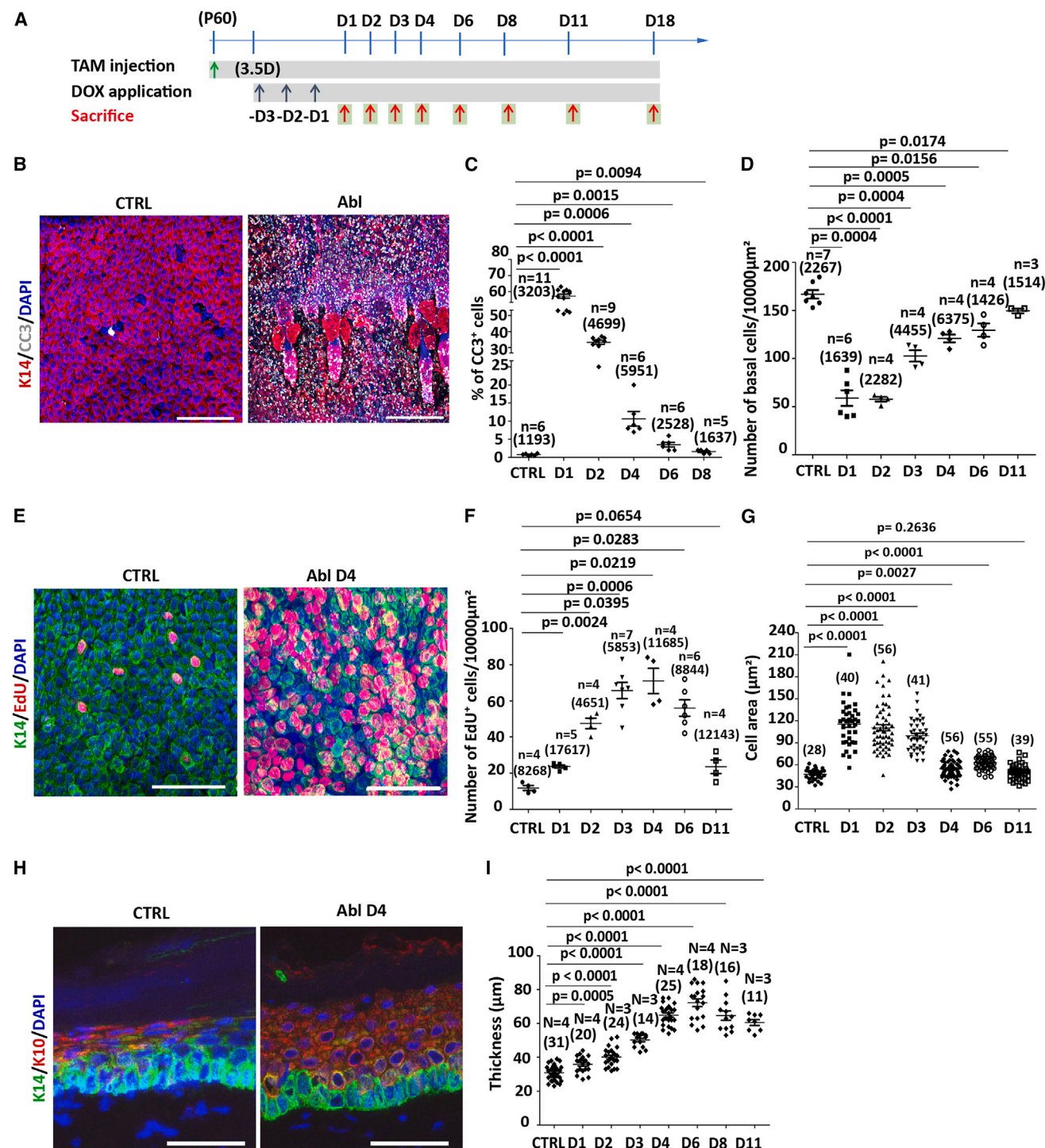


Figure 1. Kinetics of cell proliferation and epidermal regeneration following basal cell lineage ablation

(A) Experimental design.
 (B) Whole-mount immunostaining for Krt14, cleaved caspase 3 (CC3), and DAPI in control (CTRL) and ablation condition (Abl) after 3 days of Dox application. Scale bars, CTRL-50 μm , Abl-200 μm .
 (C) Percentage of CC3 positive cells after the last Dox application at different time points.
 (D) Basal cell density as number of basal cells per 10,000 μm^2 .
 (E) Whole-mount immunostaining for Krt14, EdU, and DAPI 4 h following EdU administration at day 4. Scale bars, 50 μm .
 (F) Number of EdU positive cells per 10,000 μm^2 in CTRL and Abl mice.

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the re-establishment of barrier function. In recent years, the sequences of events (coagulation/inflammatory phase, granulation phase, and a remodeling phase),¹⁰ and the types of SC involved, including HF bulge SCs, infundibulum (INF) SCs, and IFE SCs, have been increasingly well characterized.^{4,5,7,11–13} However, during the regenerative process, it remains in question how SCs become activated and adjust their fate to replenish the progenitor pool before returning to steady state. Moreover, it is unclear how these events are coordinated with potential changes in the tissue physical state during repair.

Current wound healing models cause discontinuation of the skin, which not only disrupts basal and differentiated layers but also disrupts the dermis and extracellular matrix (ECM), induces bleeding/coagulation, and leads to the recruitment of inflammatory cells and fibroblasts. Together, these responses bring many variables that can complicate and obscure our understanding of the basic molecular and physical mechanisms regulating epidermal SC activation, migration, and change in the balance of cell fate. To overcome this challenge, we developed a genetic mouse model allowing to precisely ablate basal epidermal cells, leaving intact the suprabasal differentiated cells and the basic architecture of the skin without inducing bleeding and coagulation. Basal cell lineage ablation leads to a strong depletion of IFE progenitors, leading to the activation and recruitment of neighboring and more distant SCs, which rapidly replenish basal cells. Here, using this model, coupled with continuous intravital imaging, we have identified changes in cell dynamics and the physical state of the tissue during repair. Our results indicate that the basal epidermal layer transits from a solid-like homeostatic state to a fluid-like state at low cell density during repair and then switches back to a solid-like state during re-epithelization. Concomitant with these dynamic changes in the physical state, chromatin and transcriptional remodeling that occurs in resident IFE SCs and Lrig1 infundibulum SCs induces a common regenerative state that is essential to promote SC renewal and tissue repair.

RESULTS

Kinetics of cell proliferation and epidermal regeneration following basal cell lineage ablation

To assess the clonal dynamics of epidermal cells following basal cell depletion, we developed a genetic mouse model that combines lineage tracing with genetic basal cell lineage ablation. To this end, we generated a genetically engineered mouse model that allows expression of diphtheria toxin A (DTA), which induces cell death in basal cells in a doxycycline (Dox)-dependent manner (K14rtTA/TetO-DTA). To trace the dynamics of basal cells following cell ablation, these mice were crossed with K14CREER/Rosa-confetti mice. Tamoxifen (TAM) administration labeled basal cells with one of the four fluorescent reporter proteins of the confetti system, and topical Dox administration led to the ablation of basal cells without disturbing the

dermis and suprabasal layers (Figures 1A, S1A, and S1B). However, we found there was mouse-to-mouse variability in the level of cell death upon Dox administration with some mice presenting almost no cell death. To reduce this variability, in each experiment, we treated a cohort of animals and for each mouse assessed the proportion of cell death in a small ear biopsy, studying further mice that presented at least 60% basal cell death after Dox topical application (Figures 1B, 1C, and S1C). After the last dose of Dox, there was a progressive decrease in the proportion of basal cell death during the next 4 days (Figures 1C and S1D). Basal cell death led to an initial decrease in the basal cell density, which progressively returned to its initial density 11 days after the last dose of Dox (Figures 1D and S1E). The decrease in basal cell density led to a concomitant increase in basal cell surface area (Figures 1G, S1F, and S1G).

To investigate the cellular mechanisms of tissue repair following basal cell ablation, we quantified basal cell proliferation by assessing 5-ethynyl-2'-deoxyuridine (EdU) incorporation. Basal cell ablation induced a gradual increase in cell proliferation from day 1 (D1) to day 4 (D4), peaking at day 4 and then gradually decreasing to reach homeostatic levels by day 11 (Figures 1E and 1F). The increase in cell division was accompanied by an increase in the thickness of the epidermis until day 6, pointing to an associated increase in cell differentiation (Figures 1H and 1I).

Altogether, these data show that, in this model, basal cell ablation induces severe tissue damage, leaving intact the dermis and the suprabasal differentiated epidermal cells. This damage is then repaired within 11 days through an increase in proliferation of the remaining basal cells.

Increased symmetric renewal of IFE SCs mediates epidermal regeneration following basal cell ablation

To define the cellular dynamics and fate behavior of epidermal cells following basal cell ablation, we administered TAM at clonal dose to K14rtTA/TetO-DTA/K14CREER/Rosa-confetti mice 3.5 days before the first dose of Dox and then followed the fate of marked basal cells after ablation during tissue regeneration. We collected whole-mount tail epidermis from day 1 to day 11 post Dox treatment and performed immunostaining for integrin $\beta 4$ to mark basal cells. We analyzed the clone density, size, and composition (basal and suprabasal cell number), as well as the location of clones with respect to the scale and interscale regions of the tail epidermis, as these regions present different clonal dynamics during homeostasis.^{8,14} Tissue regeneration was accompanied by a gradual increase in clone size compared with homeostatic control conditions (Figures 2A and 2B). After 11 days, the average basal clone size increased 14-fold, and the average total clone size increased 23-fold. By contrast, under homeostatic conditions, the basal and total clone size increased by only a factor 1.4- and 1.7-fold, respectively (Figures 2C and 2D). The scale and interscale compartments presented a similar increase in clone size (Figures S2A–S2D).

(G) Surface of basal cells measured by ImageJ after phalloidin staining in CTRL and Abl skin.

(H) Immunostainings for Krt14, Krt10, and DAPI on skin sections in CTRL and Abl skin at day 4. Scale bars, 50 μ m.

(I) Epidermal thickness (tissue thickness measured on 3 sections per mouse). Two-tailed Mann-Whitney test: mean per area $\pm$ SEM. For all quantification, n = number of areas, N = number of mice, and the number of cells quantified are indicated in parentheses.

See also Figure S1.

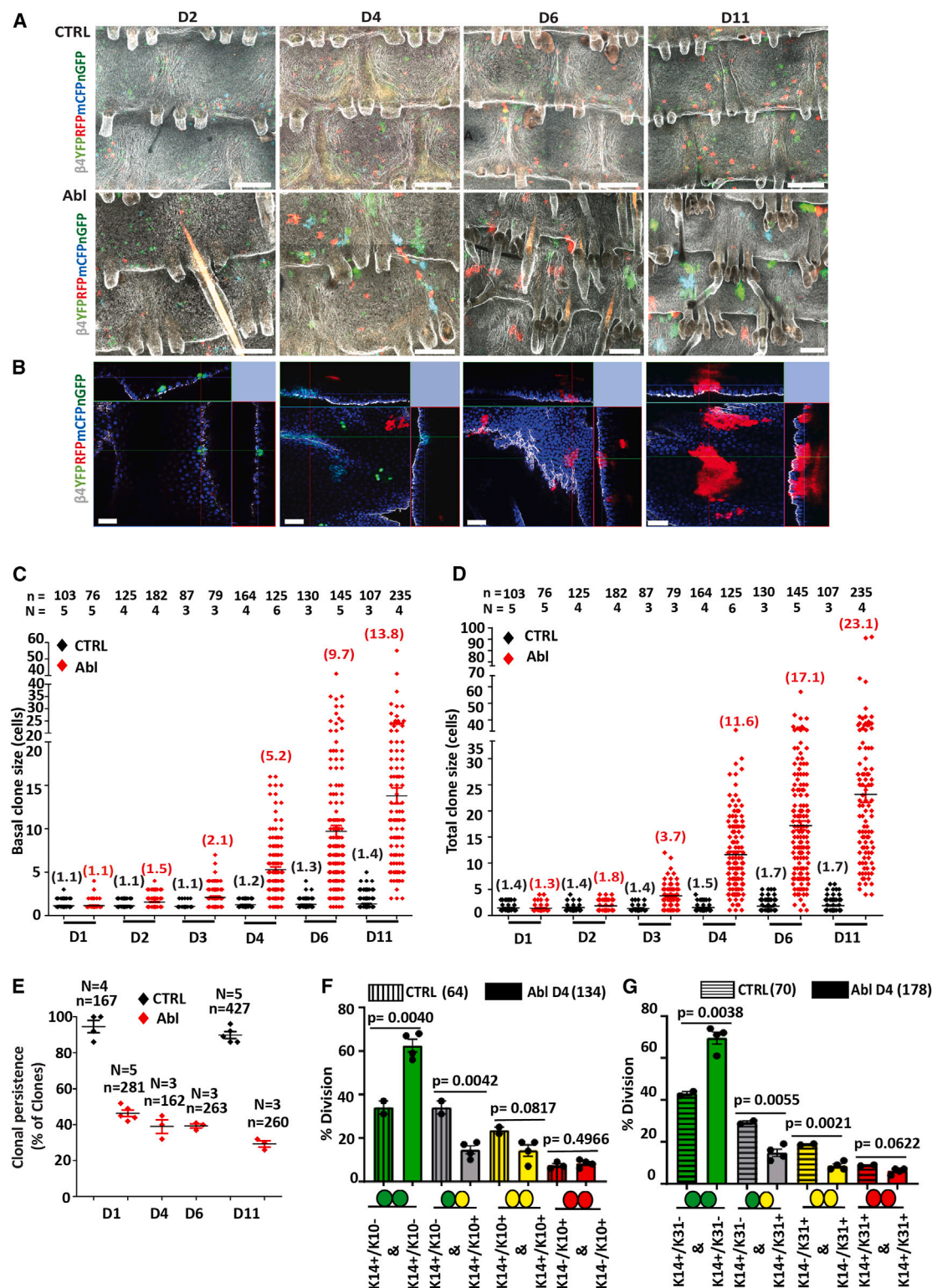


Figure 2. Increased symmetric renewal of IFE SCs mediated epidermal regeneration following basal cell ablation

(A and B) Planar (A) and orthogonal (B) views of confocal analysis of K14CREER/Rosa-Confetti/K14rtTA/Tet-O-DTA whole-mount tail epidermis stained with $\beta 4$ -integrin in CTRL and Abl mice, scale bars: 200 μ m in (A) and 50 μ m in (B).

(C and D) Number of basal (C) and total (basal and suprabasal) cells (D) per clone in CTRL and Abl tail epidermis.

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The clonal persistence was relatively stable over the course of the experiment, showing that the increase in clone size is not compensated by clone loss (Figure 2E), as occurs under homeostatic conditions.^{7,8,15} This suggests that basal cell ablation induces a transient switch from asymmetrical division at the population level to a more symmetric mode of division, leading to clone expansion.

To test this hypothesis, we assessed the fate of basal cells by performing a 5-bromo-2'-deoxyuridine (BrdU) pulse chase assay, which allows short-term lineage tracing, and assessed their differentiation status by immunofluorescence for undifferentiated (K14+/K10− for interscale or K14+/K31− for scale) and differentiating (K14+/K10+ or K14+/K31+) states. During repair, there was evidence of an increase in the proportion of cells that undergo renewal-biased divisions of undifferentiated basal cells (BrdU+/K14+/K10−) compared with divisions leading to more differentiated cells (BrdU+/K14+/K10+) in interscale and scale regions (Figures 2F and 2G). These data suggest that basal cell lineage ablation leads to a transient increase of self-renewing divisions, driving rapid tissue repair.

To assess the contribution and plasticity of infundibulum SCs in the regeneration of the IFE without disturbing the architecture of the skin, we performed lineage tracing of Lrig1+ SCs following basal cell ablation in K14rtTA/TetO-DTA/Lrig1CREER/Rosa-confetti mice. As previously shown,¹⁶ in the absence of basal cell ablation, Lrig1-derived cells contribute only to the infundibulum and sebaceous gland. In sharp contrast, upon basal cell ablation, Lrig1 progeny migrated along the infundibulum and contributed significantly to IFE repair (Figures S2E–S2G). Lrig1-derived clones gave rise to both basal and suprabasal cells (Figure S2H) and contributed much more to the repair of the interscale region compared with the scale region (Figure S2G). Analysis of the size and composition of clones showed that the contribution of Lrig1- and K14-derived clones to basal cell regeneration was similar, while the former made a much larger contribution to the suprabasal cell compartment (Figures S2H and S2I). The average basal and total clone size was increased by 15- and 50-fold, respectively, 11 days after basal cell ablation (Figure S2I).

To assess whether there was a threshold of basal cell depletion that induced the mobilization of Lrig1 SCs to repair the IFE, we compared the level of cell death and Lrig1 SC mobilization. We found that 60% of basal cell death was sufficient to induce a massive recruitment of cells derived from Lrig1+ cells to IFE in tail and ear skin (Figures S2J–S2M). By contrast, 40% of basal cell death did not induce the migration of Lrig1 SC-derived clones to IFE in tail or ear skin (Figures S2J–S2M), suggesting a threshold for activation and mobilization of around 50% of basal cell death. Altogether, these data demonstrate that, upon severe basal depletion, Lrig1 SCs are activated, migrate, and contribute to the repair of the IFE.

Mathematical modeling supports the density-dependent promotion of symmetric cell division and tissue fluidization

Quantitative modeling approaches have provided powerful insights into the identity and fate behavior of proliferative cells in the mouse skin IFE during homeostasis.^{7,8,15,17–20} We therefore questioned whether such models could predict the dynamics following DTA-mediated cell ablation. Previously, we and others have argued that the clonal dynamics of IFE under normal homeostatic conditions and following stretched-mediated expansion of the backskin are consistent with a two-progenitor model of cell fate.²¹ In this model, the differentiation and loss of SCs are not direct but occur through the terminal division of a CP to which it associates (Methods S1). However, here, to simplify the analysis, we focused on an effective model based on a single proliferative basal cell compartment, integrating the phase of CP cell amplification into a single pool of differentiating basal cells.¹⁵

Previously, studies of clonal dynamics in the IFE have placed emphasis on models without spatiotemporal dynamics. However, our data indicate that following cell ablation, cell density and area, as well as the tissue dynamics, change significantly during regeneration (Figures 1D, 1F, and 1G). It is likely that the response of SCs to cell death depends sensitively on local cell density and physical interactions. Indeed, the precipitous changes in basal cell kinetics during repair suggested a concomitant change in tissue mechanics.^{22,23} We therefore introduced a minimal spatial model for the basal cell layer, integrating previous models of cell fate within a 2D cell-based vertex-type (Voronoi) model.^{24,25} The Voronoi model accounts simultaneously for cell fate changes, tissue mechanics, and the spatiotemporal dynamics of cells. At homeostasis, we proposed a model of the basal layer in which SCs (S) stochastically enter cycle at a given rate while differentiated cells (D) stochastically delaminate, moving into the suprabasal cell layers where they shed (Figure 3A). A fraction of SC divisions results in asymmetric fate (S+D), while the remainder results in symmetric duplication (S+S) or terminal division (D+D). To ensure density homeostasis, we imposed a density-dependent feedback rule: when the local cell density is depleted (cell area is higher than the target value), cells become biased toward symmetric duplication until the target density is restored. Conversely, when cell density is higher than the target value, differentiation is favored over duplication, re-establishing the target density. In this model,²⁴ cells mechanically relax following perturbations in density created by division and delamination (Methods S1), thereby capturing the tendency of basal cells to relax toward similar cell areas on average. In homeostasis, the spatial model could be fit to the clone size distributions based on four key parameters: the cell division rate, the relative frequency of asymmetric SC divisions, the ratio of basal to suprabasal cells (Figures S3A–S3F), and the fraction of SCs in the basal layer.

(E) Clonal persistence (% of clones per HF area, n = number of HF areas).

(F and G) Percentage of cell fate outcome in CTRL (64 divisions from n = 3 mice) and Abl (134 divisions from n = 4 mice) at day 4 based on short-term BrdU tracing and immunostaining for Krt14 and Krt10 in the interscale (F) or Krt14 and Krt31 in the scale region (G) for identifying renewing of basal cells (K14+/K10− or K14+/K31−), committed basal cells (K14+/K10+ or K14+/K31+), and differentiated basal cells (K14−/K10+ or K14−/K31+). Two-tailed Mann-Whitney test: mean per mouse $\pm$ SEM. For all quantification, N = number of mice, and n = the number of clones/HF areas/divisions quantified are indicated in parentheses. See also Figure S2.

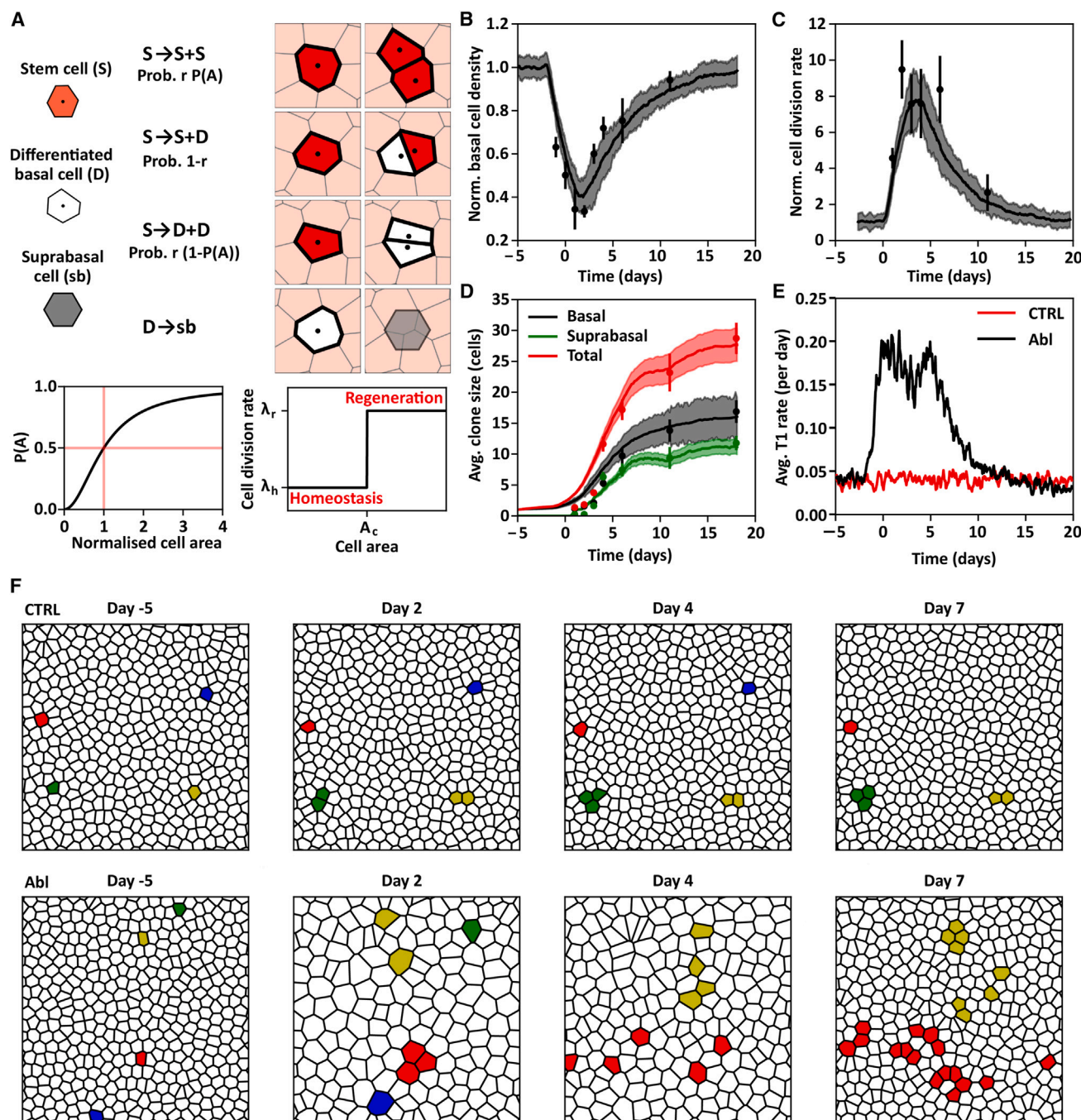


Figure 3. Voronoi model captures the dynamics of tissue regeneration

(A) Schematic of Voronoi model. SC divisions result in stochastic fate outcome with the probabilities of symmetric fates and cell division rate dependent on cell area, as depicted. (For further details of the model and its implementation, see [Methods S1](#).) A time-varying death rate is applied to the tissue according to frequency of CC3+ cells ([Figure 1C](#)).

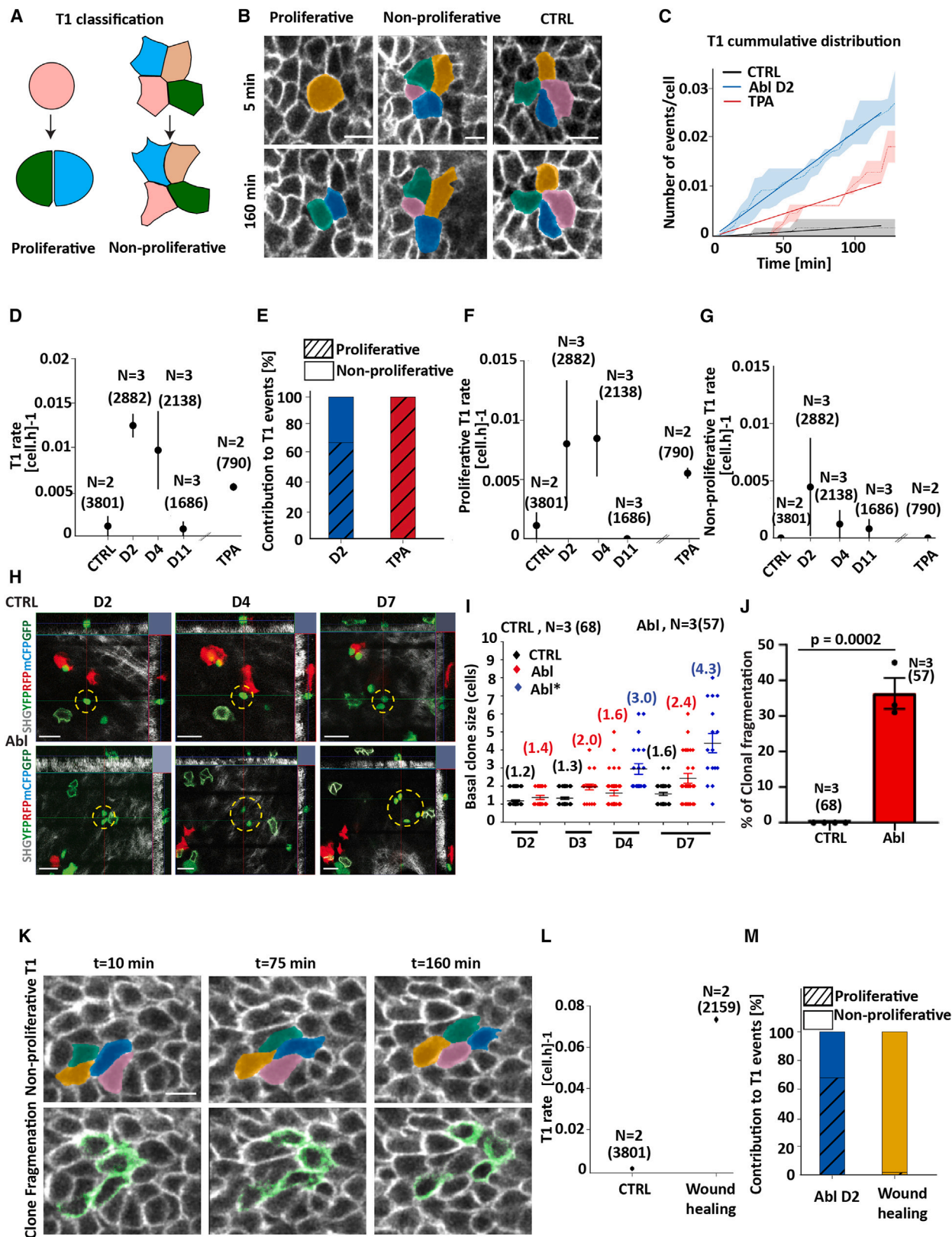
(B and C) Basal cell density (B) and division rate (C) relative to homeostatic values (cf. [Figures 1D](#) and [1F](#)).

(D) Average basal (black), suprabasal (green), and total (red) clone sizes following cell ablation. In (B)–(D), points show mean values with 95% confidence intervals, combining data from the scale and interscale regions. Lines show average results of 20 independent model simulations from best fits for a tissue of approximately 225 cells with the shading representing the 95% confidence interval (see [Methods S1](#)).

(E) Average T1 transition rate per cell per day for homeostatic (CTRL) compared with ablation (Abl) conditions.

(F) Snapshots of clone dynamics of the Voronoi model at homeostasis (CTRL) and in ablation conditions (Abl) across time. For the latter, note the degree of clone fragmentation due to the increase in T1 transitions.

See also [Figure S3](#) and [Methods S1](#) and [Video S1](#).



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By allowing SCs to undergo a density-dependent switch to a regenerative state with an enhanced division rate, the Voronoi model could be straightforwardly adapted to study regeneration under ablation conditions. The extended process of Dox-mediated loss of K14+ cells lead to a precipitous depletion in basal cell density that, in turn, promotes an enhanced proliferation rate and a bias toward symmetric SC duplication over differentiation, leading to the restoration of the steady-state basal cell density. Using the inferred fit parameters of the homeostatic system as a baseline and parameterizing the measured frequency of cell death based on Cas3 expression (Figure S3G), we questioned whether the Voronoi model could capture quantitatively the observed temporal changes in cell density and division rate, as well as the basal and total clone size distributions. To this end, we adjusted the magnitude of cell death, the critical cell area at which the division rate increases, the peak division rate during the critical period of regeneration, and the sensitivity of the SC fate bias on cell area to fit the model to the experiment (Methods S1).

We first questioned whether a model in which the basal layer comprised just a single, equipotent, proliferative population could capture the observed dynamics. We found that such a model could not faithfully recapitulate the dynamics in ablation conditions (Figures S3H–S3J; Methods S1). By contrast, when differentiated cells were allowed to have a non-zero residency time in the basal layer, the model quantitatively reproduced a wide range of data, from transient changes in cell density and division rate to the broad distribution of clone sizes and composition (Figures 3B–3D, S3L, and S3M).

Yet, despite the predictive power of the model, the quality of the fits to the basal and total clone sizes at the early time points (days 1–4) was comparatively poor. Specifically, the model significantly underestimated the number of clones during the early phases of recovery (Figure S3K) and overestimated the scale of the early-time (days 1–3) increase in the average basal and total clone size (Figure 3D). We reasoned that such behavior could arise as the result of “undetected” clone fragmentation during the early stages of regeneration. Therefore, we turned to the Voronoi model to explore whether cell ablation could promote enhanced cell rearrangements during regeneration, leading to the dispersion and

fragmentation of clones. Simulations showed that, under homeostatic conditions, clones remained relatively compact, consistent with the experimentally observed contiguity of clones under homeostatic conditions (Figure 3F; Video S1). However, following cell ablation, using the fit parameters above, the model predicted enhanced cell rearrangements (Figure 3E) alongside an elevation in division rate (Figure 3C), leading to rapid clone dispersion and fragmentation at early time points when the cell density is low (Figure 3F; Video S1). Since cell proliferation, cell death, and cell rearrangements can cause tissue fluidization,^{22,23,26,27} these model predictions suggest the presence of dynamic changes in the fluidity of the basal cell layer during repair.

Wound healing involves dynamic changes in tissue fluidity

To reveal potential changes in the physical state of the basal cell layer under ablation conditions and explore the dynamics of individual clones in living animals, we turned to the mouse ear skin, as intravital imaging in tail skin is impractical due to its greater thickness and autofluorescence (Figure S4A). Using the DTA system, we ablated around 60% of basal cells from the ear epidermis by topical Dox application and monitored the remaining basal cells using live imaging.

When there is little or no ECM between cells, as in the basal cell layer, tissue fluidity can be assessed by quantifying the degree of cellular rearrangements.^{28,29} We therefore monitored the frequency of T1 transitions (cellular rearrangements involving changes in the topology of contacts between neighboring cells; Figures 4A and 4B) in the basal cell layer both in the homeostatic state and during tissue repair following cell ablation (Figures 4A–4G). Specifically, we tracked the cumulative number of T1 transitions (Figure 4C) and obtained the T1 transition rate at each stage and condition, distinguishing T1 transitions resulting from cell division from those that occur without division (Figures 4A and 4B). The higher the T1 transition rate, the faster mechanical stress relaxation occurs in the tissue, making it more fluid-like (less viscous).^{29,30} Barely any cell rearrangements were observed in the homeostatic state (Figures 4C and 4D, CTRL; Video S2), consistent with the basal cell layer being in a solid-like

Figure 4. Tissue fluidization regulates wound healing

- (A) Schematics of T1 transition types: proliferative (caused by cell division) and non-proliferative.
 (B) Representative examples of proliferative and non-proliferative T1 events at day 2 following basal cell ablation (Abl day 2) compared with the absence of cell rearrangements in control conditions (CTRL) at $t = 5$ and 160 min. Scale bars, 10 μm .
 (C) Cumulative number of T1 events per cell in Abl day 2, TPA, and CTRL conditions. Dashed lines show experimental data with standard deviation, and straight lines are linear fits.
 (D) T1 rate (cell.h^{-1}) for CTRL, Abl day 2, day 4, day 11, and TPA conditions. (Number of T1 events: 2 for CTRL, 79 for day 2, 32 for day 4, 2 for day 11, and 20 for TPA.)
 (E) Relative contribution of proliferative and non-proliferative T1 events for Abl day 2 and TPA conditions.
 (F and G) Proliferative T1 (F) and non-proliferative T1 (G) rates (cell.h^{-1}) for CTRL, ablation, and TPA conditions.
 (H) Time-lapse imaging of the ear epidermis in CTRL and Abl conditions. Yellow circle denotes clone fragmentation. Scale bars, 50 μm .
 (I) Number of basal cells per labeled cell cluster in CTRL and Abl conditions (Abl* indicates true clone size when clusters are regrouped based on time lapse).
 (J) Percentage of clone fragmentation in CTRL (homeostasis) and Abl conditions.
 (K) Representative examples of two consecutive non-proliferative T1 events in wound-healing conditions at $t = 10, 75$, and 160 min (top row) and corresponding intercalation event (bottom row). Scale bars, 10 μm .
 (L) T1 rate (cell.h^{-1}) for the CTRL, Abl day 2, and wound-healing conditions.
 (M) Relative contribution of proliferative and non-proliferative events to the total number of recorded T1 events after 2 h for Abl day 2 and wound-healing conditions. Two-tailed Mann-Whitney test: mean per mouse $\pm$ SEM. For all quantification, N = number of mice per condition, and number of clones/cells tracked are indicated in parentheses.

See also Figure S4 and Videos S2, S3, and S4.

state. By contrast, partial basal cell ablation led to a substantial increase in T1 transitions at early stages of repair (Figures 4C and 4D; Abl day 2), with cell divisions contributing to approximately 70% of all T1 transitions (Figure 4E; Video S3), in line with model predictions (Figure 3E) and the presence of duplicative cell divisions upon ablation (Figures 2H and 2I). Enhancing cell proliferation in control conditions using 12-O-tetradecanoylphorbol-13-acetate (TPA) led to an increase in the measured T1 transition rate to the levels observed in ablation conditions at day 2, with all T1 events caused by cell divisions (Figures 4D and 4E). The T1 transition rate remained similar until day 4 (Figure 4D), with cell proliferation (maximal at this time point, Figure 1F) driving most T1 transitions (Figures 4F and 4G). Finally, the T1 transition rate was negligible at day 11 (Figures 4D–4G), with the tissue becoming solid-like as it recovered its homeostatic state. Indeed, the cell density, cell area, cell proliferation, and cell death all returned to homeostatic levels by day 11, consistent with the recovery of the homeostatic solid-like state. Taken together, these results indicate that, following DTA-mediated cell ablation, the basal cell layer transitions from a solid-like homeostatic state to a fluid-like state before returning to a solid-like state following repair.

Consistent with the increase of T1 transitions and tissue fluidity during repair, as well as with the predictions of the Voronoi model, we found that clones became fragmented during ablation conditions, albeit not in the control (Figures 4H–4J and S4B). Further, from quantifications of the size, composition, and density of labeled cell clusters (clone fragments), as well as true clones, we obtained results that were consistent with the model predictions and the tail skin data (Figures 3B–3D).

To explore whether a similar transition in the physical state of the tissue also occurs during a more classical wound-healing model, we made a 1 mm epidermal wound using laser ablation in the ear skin. Specifically, we ablated basal and suprabasal cells to create a wound without disturbing the dermis or the architecture of the skin and monitored T1 transitions in the basal cell layer during wound healing using live imaging (Figures 4K and S4C). No cell rearrangements were observed in the absence of wounding (Figure 4L), consistent with a solid-like state of the basal cell layer during homeostasis. By contrast, we observed abundant T1 transitions in the basal cell layer adjacent to the wound (Figures 4K–4M; Video S4). The measured T1 transition rate was higher in the wound-healing condition compared with the DTA-mediated ablation condition, indicating a strong fluidization of the tissue surrounding the wound site as regeneration proceeded. However, in contrast to the DTA-mediated ablation conditions, the observed T1 transitions at the leading edge during wound healing were mediated by cellular rearrangements, independent of cell divisions (non-proliferative T1 transitions; Figures 4K–4M). Analysis of labeled cells surrounding the wound revealed the presence of fragmented clones, mirroring our observations of clone fragmentation in DTA-mediated ablation conditions (Figures 4H–4J). These results indicate that tissue fluidization occurs during tissue repair in two independent models of wound healing.

Transcriptional and epigenetic landscape associated with tissue regeneration following basal cell ablation

To define the molecular mechanisms responsible for driving enhanced symmetric renewal, clonal expansion, SC mobiliza-

tion, and the dynamic regulation of tissue fluidization following basal cell ablation, we first performed bulk RNA sequencing (RNA-seq) on fluorescence-activated cell sorting (FACS)-isolated basal cells from K14- and Lrig1-derived cells at day 4, when the tissue switches from a fluid to solid-like state (Figures S5A and S5B). We FACS-isolated fluorescently labeled IFE basal cells ($CD34^{-}/\alpha6^{high}/Lrig1^{-}/YFP^{+}/GFP^{+}/RFP^{+}$ for K14CREER derived cells and the $CD34^{-}/\alpha6^{high}/Lrig1^{-}/RFP^{+}$ population for Lrig1CREER derived cells) at day 4 post lineage ablation, as proliferation is the highest at this time point. We compared RNA-seq profiling of K14 Abl vs. K14 CTRL, Lrig1 Abl vs. Lrig1 CTRL, and Lrig1 Abl vs. K14 CTRL.

Our analysis showed that 490 genes were upregulated in K14 Abl vs. K14 CTRL, whereas 990 genes were upregulated in Lrig1 Abl vs. Lrig1 CTRL, and 690 genes were upregulated in Lrig1 Abl vs. K14 CTRL with 44 genes commonly upregulated in all three conditions (Figure 5A). The latter showed enrichment for cell cycle and mitotic genes (*Aurka*, *Bub1b*, *Cdc20*) and signatures of wound healing (*Hmga2*, *Inhba*, *Mmp9*, *Flrt2*).^{4,31}

Genes upregulated in K14-derived clones under ablation conditions were found to be enriched for epidermal growth factor (EGF)-like domain secreted molecules (*Ereg*, *Mfge8*, *Areg*, *Megf6*), genes that participate in cell-cell junction regulation (*Dsc2*, *Flrt2*), mitosis (*Bub1b*, *Aurka*, *Cdc20*, *Plk1*), developmental proteins (*Bmp3*, *Gli1*, *Pdpn*, *Ephb2*), and transcription factors (TFs) (*Elk3*, *Tead2*, *Nfatc2*, *Pbx4*, *Mybl2*, *Cbx6*). In addition, keratinization genes including *Krt16*, *Krt17*, *Krt6a*, *Krt6b*, *Sprr2a1*, *Sprr2a2*, and *Sprr2a3* were upregulated following basal cell ablation (Figures 5B and S5C).

To unravel the changes in the chromatin landscape associated with tissue repair of the epidermis after basal cell ablation, we performed assay for transposase-accessible chromatin using sequencing (ATAC-seq) to identify the chromatin regions that were remodeled at day 4. We found that 8,233 peaks were upregulated and 2,707 peaks were downregulated by more than 2-fold following basal cell ablation. We then performed motif discovery analysis using Homer to define the TFs associated with remodeling of chromatin regions following basal cell ablation. The most frequent and statistically significant motifs associated with chromatin regions that become more opened in regenerative conditions corresponded to AP1 motifs, which bind to Jun and Fos TFs (Figures 5C and S5D). Alongside Activator protein 1 (AP1) motifs, highly significant TF motifs were p63 (epidermal SC renewal), AP2-gamma (epidermal development, IFE differentiation), Krüppel-like factor 4 (Klf4) (IFE differentiation), and TEA domain transcription factor 4 (TEAD4) (epidermal development and regeneration) (Figures 5D–5H). The increased activity of TFs that mediate self-renewal (p63) and differentiation (Klf4) in regenerative conditions provides a molecular explanation for the coupling of IFE basal cell renewal and IFE differentiation observed experimentally.

scRNA-seq unravels a common regenerative state in different epidermal SCs

To unravel the cellular heterogeneity and molecular features associated with distinct basal cell states during regenerative conditions, we performed single-cell RNA-seq (scRNA-seq)

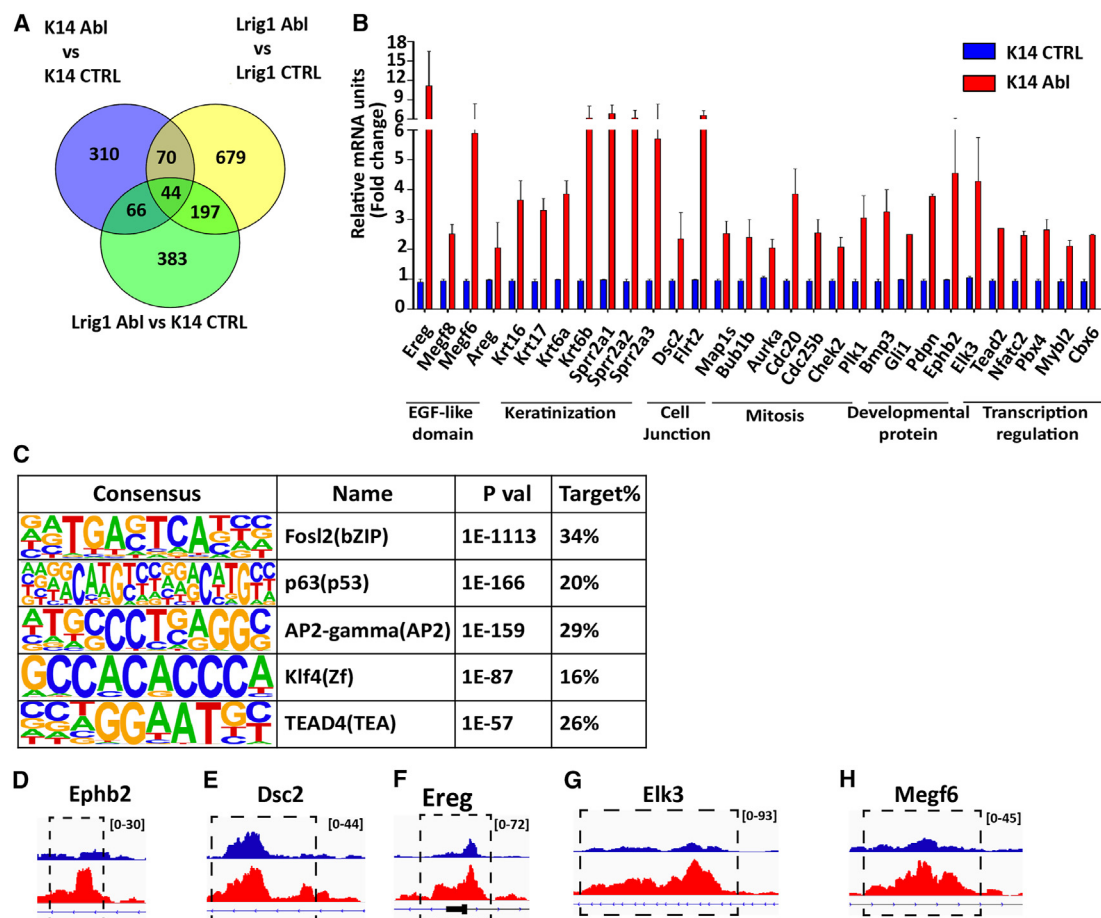


Figure 5. Transcription and chromatin profiling during clonal expansion following basal cell ablation

(A) RNA-seq of FACS-isolated basal cells from K14 CTRL, K14 Abl, Lrig1 CTRL, and Lrig1 Abl at day 4 following ablation ($N = 2$ mice per time point). Venn diagrams showing the similarities and differences between genes that were differentially upregulated by more than 2-fold.

(B) Genes upregulated in basal cells from K14 Abl conditions compared with K14 CTRL (fold change).

(C) Transcription factor motifs enriched in the ATAC-seq peaks that were upregulated by more than 2-fold in K14 Abl compared with CTRL at day 4 determined by Homer analysis ($N = 2$ mice).

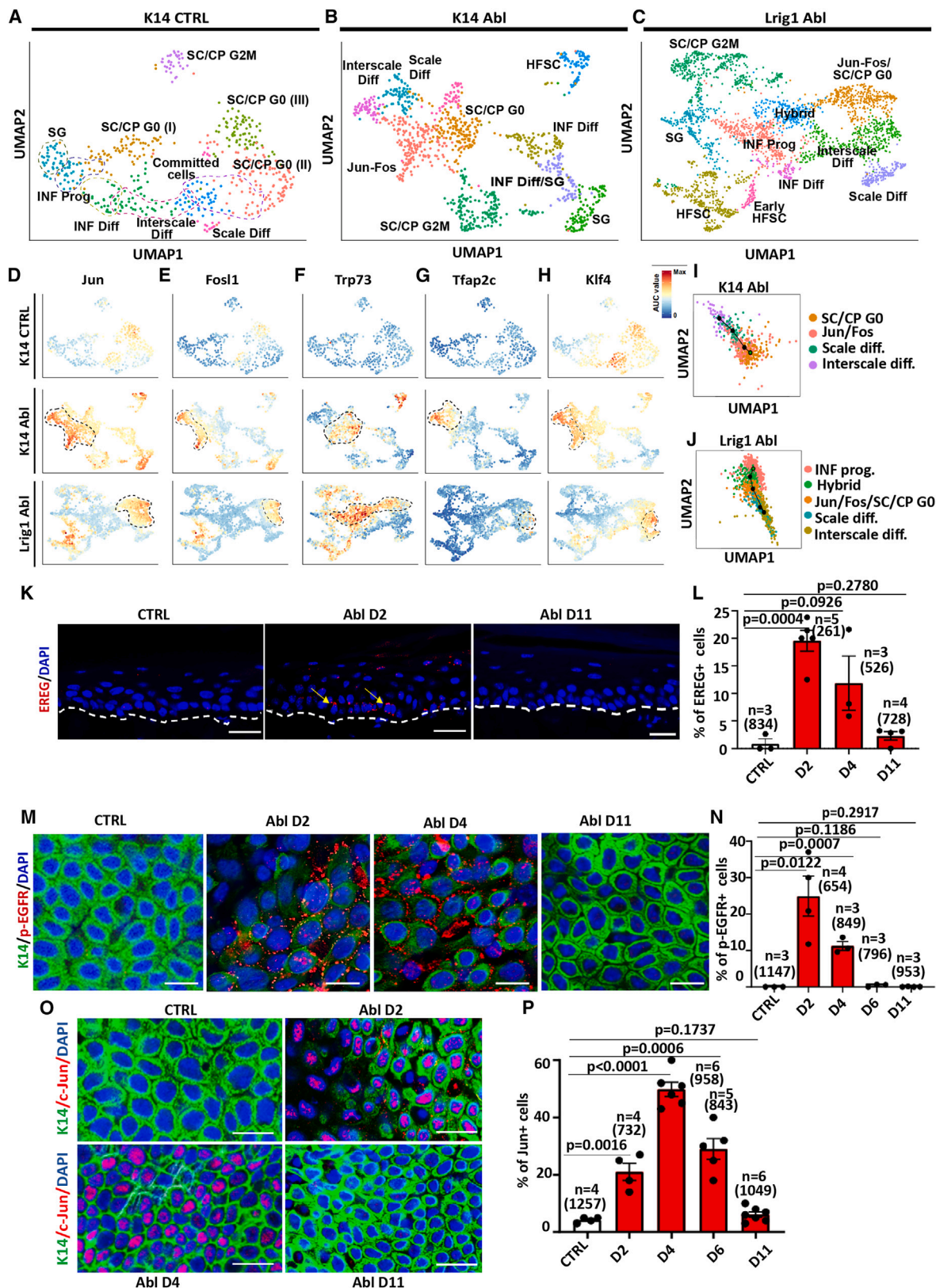
(D–H) ATAC-seq profiles showing increasing accessibility of chromatin regions remodeled during epidermal repair, peaks containing binding site of Fos (D), p63 (E), AP2-gamma (F), KLF4 (G), and TEAD4 (H).

See also Figure S5.

on FACS-isolated K14CREER and Lrig1CREER derived basal cells at day 4 following cell ablation. We used 10× Genomics multiplexing technology for control ($n = 642$), K14 Abl ($n = 1,132$), and Lrig1 Abl ($n = 3,250$). We performed unsupervised clustering on individual samples using Seurat and annotated different clusters based on known marker gene expression for each epidermal subpopulation previously described by scRNA-seq.^{21,32–35} We identified similar clusters as previously reported in control conditions, whereas in regenerative conditions we uncovered different cell clusters in both K14 Abl and Lrig1 Abl that express SC/CP G0 markers together with a strong enrichment of *Jun-Fos* expression (including *Fos*, *Fosb*, and *Junb*), which we called the regenerative SC state (Figures 6A–6C and S6A–S6H).

To define the TFs and gene regulatory network responsible for epidermal repair following basal cell ablation, we performed sin-

gle-cell regulatory network inference and clustering (SCENIC) analysis that allows to determine the activity of TFs and their associated regulons expressed in the different cell states in control and regenerative conditions.³⁶ Our analysis showed that SCs, CPs, and differentiated cell states were associated with distinct TFs, with the undifferentiated states characterized by higher expression of *Trp63*, while cellular commitment was associated with a gradient of expression of *Cebpa* and *Hes1* transcriptional modules during differentiation in control conditions. Infundibulum cells were characterized by higher expression of *Sox9* and *Foxc1*. In both K14 and Lrig1 regenerative conditions, *Jun-Fos* regulons were highly enriched in SC/CP regenerative cell clusters (Figures 6D and 6E). There was a strong enrichment for *Trp73* in the IFE SC/CP G0 cluster from K14 regenerative conditions (Figure 6F). In Lrig1 regenerative conditions, *Trp73* was specifically enriched in INF progenitors, the hybrid cluster, and



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the part of Jun-Fos SC/CP G0 cluster, which suggests that *Trp73* plays an important role in the activation of Lrig1 cells (Figure 6F). *Tfap2c* TF regulons were enriched in interscale differentiation cluster in K14 regenerative conditions, while *Klf4* TF regulons were enriched in Jun-Fos SC/CP G0 and interscale differentiation clusters (Figures 6G and 6H).

To define the lineage trajectories between the different cell states during epidermal regeneration, we performed pseudo-temporal ordering of cells with Slingshot.³⁷ In K14 regenerative conditions, we found that cellular trajectories started from SC/CP G0, passed through the Jun-Fos cluster, which is associated with proliferation and differentiation, and finally to the differentiated cells of the interscale and scale (Figures 6I and S6I). In Lrig1 regenerative conditions, the origin of the lineage trajectory started with the INF progenitor, went through the hybrid state of the cells to the SC/CP G0-Jun-Fos cluster, and finally ended through IFE differentiated states (Figures 6J and S6J).

Altogether, our single-cell analysis uncovers a common regenerative cellular state that expresses markers of IFE SCs together with a strong activation of the AP1 TF axis in the progeny of IFE and infundibulum SCs. This analysis also identifies the different states and trajectories that occur in Lrig1 SCs during their activation, migration, IFE reprogramming, tissue repair, and IFE differentiation and their intermediate hybrid states.

Consistent with the transcriptional and epigenetic profiling as well as single-cell analysis, following cell ablation, we found that epiregulin (EREG), activation of epidermal growth factor receptor (EGFR), and Jun expression increased at day 2, peaked at day 4, and progressively decreased thereafter (Figures 6K–6P). Altogether, our data indicate that upregulation of EREG, phospho-EGFR, and Jun/Fos expression are maximally activated concomitant with the observed dynamic changes in tissue fluidity during tissue repair.

EGFR/MAPK/AP1 regulate tissue fluidization and re-epithelization in ablation-mediated epidermal regeneration

The molecular characterization of the cellular states that are induced during tissue regeneration and their associated transcriptional and chromatin landscape suggest that mitogen-activated protein kinase 1 (EGFR-MAPK) signaling and AP1 family TFs might be important for SC activation and the regulation of tissue fluidization during epidermal repair. To define the functional roles of the EGFR/MAPK axis during tissue repair, we assessed the impact of pharmacological inhibition of the EGFR/MAPK pathway on the clonal dynamic of IFE SC/CPs and tissue

fluidization. We treated K14rtTA/TetO-DTA/K14CREER/Rosa-confetti mice with afatinib, an erythroblastic oncogene B (ErbB)-family inhibitor that inhibits ErbB/EGFR signaling induced by the ligands for these receptors such as Ereg, Amphiregulin (Areg), that are upregulated in IFE cells following basal cell ablation. Afatinib treatment had a dramatic effect on the ability of IFE SCs to expand and mediate tissue regeneration. Afatinib administration dramatically inhibited cell proliferation (Figures 7A–7D), self-renewing divisions after basal cell ablation, as shown by the strong decrease in the basal clone size, and IFE differentiation, as shown by the decrease in the number of suprabasal cells per clone (Figures 7D–7H). The major defect in SC activation upon EGFR/ErbB inhibition prevented tissue repair following basal cell ablation, as shown by the scattering of epidermal cells in afatinib treated epidermis (Figures 7E and 7F).

To understand the effect of afatinib treatment on the physical state of the tissue, we performed lineage ablation experiments together with afatinib treatment and monitored T1 transitions in the basal cell layer by intravital imaging. T1 transition rates increased in the presence of afatinib following ablation at day 2, indicating that tissue fluidity is enhanced. These T1 transitions were not driven by cell divisions (Figures 7I–7K and S7A; Video S5).

Treatment of K14rtTA/TetO-DTA/K14CREER/Rosa-confetti mice with trametinib, an inhibitor of mitogen-activated extracellular signal-regulated kinase 1 (MEK1), and MEK2, which inhibits extracellular signal-regulated kinase (ERK) activation, also decreased basal cell proliferation and clonal expansion (Figures 7C–7H). These results show the importance of the EGFR/MEK/MAPK signaling axis in the activation of basal SCs, symmetrical self-renewing division, and differentiation during skin regeneration following basal cell ablation.

To assess the functional importance of Jun-Fos TFs in driving SC self-renewing division, clonal expansion, and epidermal repair, we treated K14rtTA/TetO-DTA/K14CREER/Rosa-confetti mice with AP1 inhibitor (T-5224),^{38,39} following basal cell ablation. The AP1 inhibitor reduced basal cell proliferation, clonal expansion, and differentiation, preventing skin regeneration following basal cell ablation (Figures 7B–7H). Together, these pharmacological studies demonstrate the key role played by the EGFR/MAPK/AP1 axis in SC activation, expansion, and repair following basal cell ablation.

To understand how AP1 inhibition affects the physical state of the basal epidermis during the early stage of tissue repair, we quantified the frequency of T1 transitions in the basal cell layer at day 2 following basal cell ablation. We found that the T1

Figure 6. scRNA-seq following basal cell ablation

(A–C) Uniform manifold approximation and projection (UMAP) dimensionality reduction plots for the K14 CTRL (A), K14 Abl (B), and Lrig1 Abl (C) at day 4 following ablation using 10X chromium. Colors represent cluster identities computed on the individual samples.

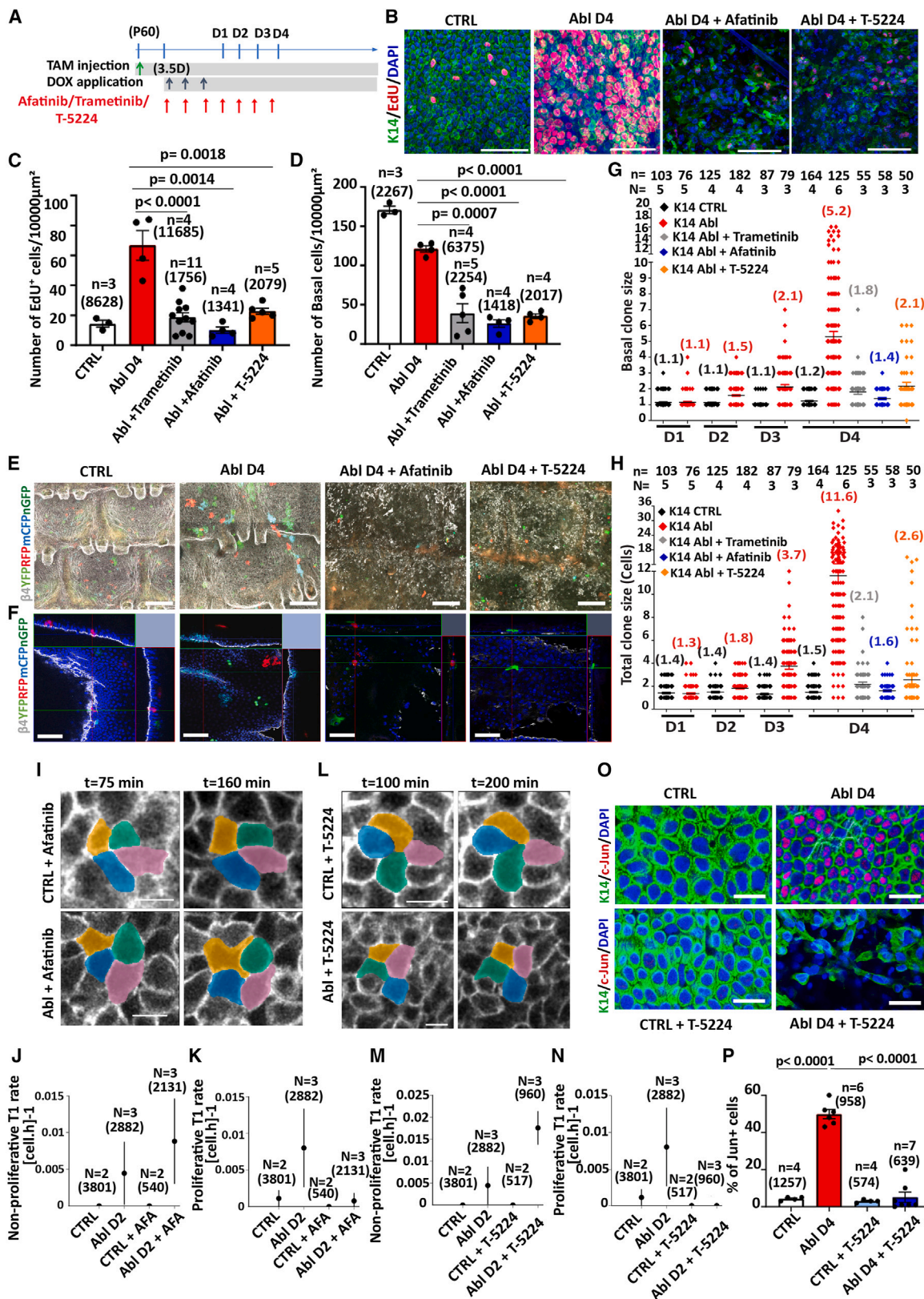
(D–H) UMAP dimensionality reduction plots colored by the degree of regulon activation for transcription factors differentially activated (area under the curve [AUC] rank-sum test false discovery rate [FDR] corrected *p* value < 0.05) in the CTRL, K14 Abl, and Lrig1 Abl of Jun (D), Fos1 (E), Trp73 (F), Tfap2c (G), and KLF4 (H). Color scaling: AUC value of target genes in the regulon being expressed as computed by SCENIC.

(I and J) Lineage trajectories (black lines) computed using Slingshot for clusters in K14 Abl (I) and Lrig1 Abl (J).

(K and L) *In situ* hybridization analysis for epiregulin (EREG) (K) and percentage of EREG+ basal cells (L) in control and following ablation.

(M and N) Immunostaining for p-EGFR and Krt14 (M) and percentage of p-EGFR+ cells (N) in control and following ablation.

(O and P) Immunostaining for c-Jun with Krt14 (O) and percentage of c-Jun+ basal cells (P) in control and following ablation (scale bars, 20 μ m). Two-tailed Mann-Whitney test: mean per areas $\pm$ SEM. For all quantification, *n* = the number of areas from at least 3 mice, number of cells quantified are indicated in parentheses. See also Figure S6.



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transition rate increased in the presence of the AP1 inhibitor following lineage ablation as compared with lineage ablation alone, indicating that interfering with AP1 leads to an increase in the tissue fluidity. As found with afatinib, the increased T1 transitions were not caused by cell divisions following AP1 inhibition (Figures 7L–7P and S7B; Video S6). These data suggest that Jun/Fos is involved in the control of tissue fluidity changes at the later stages of tissue repair.

Since tissue fluidization has been shown to be affected by levels of cell-cell adhesion and actomyosin-driven cortical contractility,²² we quantified the fluidity level of the basal cell layer following cell ablation while simultaneously perturbing non-muscle myosin II activity using ML7 (myosin light chain kinase inhibitor). Inhibiting myosin activity after cell ablation led to a decrease in the frequency of T1 transitions, consistent with the tissue becoming less fluid (Figures S7C–S7F; Video S7). This is in agreement with previous studies showing that myosin inhibition suppressed the ability of cells to overcome energy barriers and undergo cell rearrangements in non-confluent tissues, thereby rigidifying the tissue.^{28,29} The decrease in cell division rates and their contribution to T1 transition events upon myosin inhibition during repair (day 2 after ablation) indicates that cell divisions contribute to tissue fluidization, as previously proposed theoretically,⁴⁰ and in agreement with our simulations (Figure 3E). Analysis of Jun expression by immunofluorescence following myosin inhibition and cellular ablation showed a decrease in Jun expression compared with ablation conditions in the absence of myosin inhibition. This suggests that modulation of contractility following cellular ablation affects the acquisition of the common regenerative state (Figures S7G and S7H).

Finally, to reveal the changes in cell adhesion during tissue fluidization under cell ablation conditions, we analyzed the expression of E-cadherin during the regeneration process from day 1 to day 11. Our results showed that E-cadherin expression substantially decreased in the ablation condition at day 2 and gradually returned to homeostatic levels as tissue repair progressed (Figures S7I and S7J). This decrease in E-cadherin may promote tissue fluidization by facilitating cellular rearrangements in the presence of spaces between cells following ablation, as previously suggested for non-confluent tissues.^{22,29}

DISCUSSION

In this study, we have unraveled the interplay of cellular and molecular mechanisms with mechanical changes during epidermal repair following basal cell lineage ablation. Our results show that tissue repair occurs rapidly following the depletion of more than 50% of the basal cell population of the IFE. This depletion induces a transient burst of cell proliferation in the IFE and infundibulum and a switch from population asymmetric renewal to an increase in duplicative (self-renewing) divisions. Interestingly, despite the absence of depletion of the suprabasal differentiated cells, the replenishment of basal cells is accompanied by an increase in the production of differentiated cells, showing that renewing divisions are tightly coupled with epidermal differentiation, as also occurs in classical wound healing, where basal and differentiated suprabasal cells are both depleted.³¹

By developing a Voronoi model of the basal cell layer combining cell fate with physical interactions, biophysical modeling of the clonal data under normal and perturbed conditions suggests that severe basal cell depletion can induce a switch from a solid-like state, where clones remain cohesive, to a fluid-regenerative state, resulting in enhanced cell-cell rearrangements and clone fragmentation. This change in cell dynamics enables the tissue to rapidly restore a uniform basal cell density and recover homeostatic conditions after repair. Direct measurements of T1 transition rates and clone fragmentation in different conditions indicate that the tissue is in a solid-like state in homeostatic conditions and transitions to a fluid-like state during tissue repair, in agreement with the model predictions. While the solid-like homeostatic state may be important to maintain the skin barrier function, it impairs tissue repair by hindering its remodeling. Tissue fluidization during repair ensures that the basal cell layer can remodel and reset the tissue structure and architecture. Once re-established, the basal cell layer recovers the solid-like homeostatic state to restore skin barrier function.

Transitions between fluid-like and solid-like tissue states (or tissue fluidization) have been reported in many biological contexts, including embryonic development and tumor progression.²² Our results indicate that regulated tissue fluidization is also required during the initial stage of tissue repair in mice

Figure 7. EGFR/MAPK regulates symmetric renewal in ablation-mediated epidermal repair

(A) Experimental timeline.

(B) Whole-mount immunostaining of the tail skin for K14 (green), EdU (red), and Hoechst for nuclei (blue) 4 h following EdU administration in CTRL, Abl, Abl+ afatinib, and Abl+ T-5224 conditions at day 4. Scale bars, 50 μ m.

(C and D) Number of EdU+ cells (C) and basal cells (D) per 10,000 μ m² in CTRL, Abl, Abl+ trametinib, Abl+ afatinib, and Abl+ T-5224 conditions at day 4 Abl.

(E and F) Planar (E) and orthogonal (F) views of confocal analysis of K14CREER/Rosa-Confetti/K14rtTA/Tet-O-DTA whole-mount tail epidermis stained with β 4-integrin in CTRL and Abl conditions. Scale bars: 200 μ m in (E) and 50 μ m in (F).

(G and H) Number of basal cells (G) and total cells (H) per clone in K14 tracing in CTRL, Abl, Abl+ trametinib, Abl+ afatinib, and Abl+ T-5224 conditions at day 4.

(I) Representative images at $t = 75$ min and $t = 160$ min for CTRL+ afatinib and Abl+ afatinib (day 2) conditions. Scale bars, 10 μ m.

(J and K) Non-proliferative (J) and proliferative T1 rate (cell.h)⁻¹ (K) for CTRL, Abl, CTRL+ afatinib, and Abl+ afatinib (day 2) conditions.

(L) Representative images at $t = 100$ min and $t = 200$ min for CTRL+ T-5224 and Abl+ T-5224 (day 2) conditions. Scale bars, 10 μ m.

(M and N) Non-proliferative (M) and proliferative (N) T1 rate (cell.h)⁻¹ for CTRL, Abl, CTRL+ T-5224, and Abl+ T-5224 (day 2) conditions.

(O and P) Immunostaining for c-Jun (red) with Krt14 (green) (O) and percentage of c-Jun+ cells (P) in control, day 4 post-ablation 4, control+ T-5224, and ablation+ T-5224 at day 4. Scale bars, 20 μ m. Two-tailed Mann-Whitney test: mean per area $\pm$ SEM. For all quantification, N = the number of mice, n = the number of areas, clones, and number of cells quantified are indicated in parentheses.

See also Figure S7 and Videos S5, S6, and S7.

skin. Tissue fluidization, caused by decreased tissue contractility, is believed to facilitate wound closure in epithelial monolayers.⁴¹ We find the opposite behavior in mammalian skin repair, with decreased tissue contractility rigidifying the tissue and slowing down regeneration, suggesting that a different fluidization mechanism is involved in skin regeneration.

Our results uncover a dynamic regulation of the tissue physical state, with an initial tissue fluidization followed by a transition back to a solid-like state after recovering homeostatic conditions. DTA-mediated cell ablation led to precipitous depletion in basal cell density, which increases the mechanical stress in the tissue. To mitigate the mechanical stress, cellular rearrangements drive the system toward a fluid-like state, which promotes clone fragmentation and basal cell dispersion, facilitating rapid tissue repair. Tissue fluidity activates signaling cascades through mechanical and biochemical cues for the repair of the process. Consistently, blocking actomyosin contractility reduced the rate of T1 events and the number of Jun+ cells, suggesting that tissue fluidity might initially promote the activation of a Jun/Fos regenerative state.

At the end of this phase of tissue fluidization, the secretion of EREG and other EGFR ligands leads to the activation of EGFR/AP1 pathway that is responsible for promoting proliferation, re-epithelization, and tissue regeneration. This is associated with a decrease in tissue fluidization and, eventually, a transition back to a solid-like homeostatic state. The AP1 TF plays an essential role in a wide range of cellular processes, including development, cell growth, differentiation, apoptosis, and tissue regeneration.⁴² It has been previously shown that AP1 TFs such as c-Jun and c-Fos are expressed during skin wound healing and stretch-mediated skin expansion.^{21,31,43} Recent studies have shown that AP1 regulates each step of the inflammatory memory establishment, maintenance, and recall across different cell types and wounding stimuli.^{44,45} However, the role of AP1 TFs during skin regeneration and tissue fluidization has not been investigated directly. The increase in tissue fluidity following EGFR and AP1 inhibition suggests that the EGFR/AP1 axis opposes directly or indirectly tissue fluidization following basal cell ablation, possibly providing a feedback mechanism in this process, and that re-epithelization and tissue repair are associated with a progressive restoration of solid-like state within the epidermis.

Altogether, our study identifies a dynamic regulation of tissue fluidity during wound healing that is essential for tissue repair and uncovers a common regenerative cell state across different epidermal SCs that is regulated by the EGFR/MEK/AP1 signaling axis, which controls tissue fluidization and epidermal regeneration.

Limitations of the study

While our study uncovers evidence for the dynamic regulation of the physical state of the tissue during wound healing, with perturbations of these solid and fluid-like states impairing tissue repair, some important questions remain unanswered. Future experiments will be required to define the molecular mechanisms by which physical changes in the tissue control the expression of the common AP1 regenerative state, how the acquisition of this regenerative state feeds back to control

tissue fluidization, and what are the cellular mechanisms controlling tissue fluidization in the absence of cell proliferation to promote tissue repair.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cell.2024.07.031>.

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AUTHOR CONTRIBUTIONS

R.M.S., J.H., M.V., O.C., B.D.S., and C.B. designed the experiments, performed data analysis, and wrote the manuscript. R.M.S., J.H., M.V., O.C., B.D.S., and C.B. discussed the data and their interpretation. R.M.S. performed most of the experiments. Y.S. performed RNA-seq, ATAC-seq, and scRNA-seq data analysis. M.L. provided technical support. M.A. performed initial experiments. A.H. and J.H. performed image segmentation and analysis. D.E. and A.B. helped for intravital imaging and analysis. J.H. and B.D.S. performed mathematical modeling and statistical analysis of the clonal data. M.V. and O.C. designed and performed the analysis of the T1 transitions. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Chicken Polyclonal anti-K14	Thermo Fisher	Custom batch
Rat anti-integrin b4/CD104	BD Biosciences	Cat#553745, RRID: AB_395027
Guinea Pig polyclonal anti-acidic Hair Keratin K31	Progen	Cat# GP-hHA, RRID: AB_2716780
Rat anti-BrdU unconjugated	Abcam	Cat#Ab6326, RRID: AB_305426
Purified anti-Keratin 10 Antibody	BioLegend	Cat# 905404, RRID: AB_2616955
Mouse Active Caspase-3 Antibody	R and D systems	Cat# AF835 RRID: AB_2243952
Anti-c-Jun Rabbit mAb (clone 60A8)	Cell Signaling Technology	Cat# 9165; RRID: AB_2130165
Anti-c-Fos Rabbit mAb (9F6)	Cell Signaling Technology	Cat# 2250; RRID: AB_2247211
Anti- p-EGFR (Tyr 1068) polyclonal antibody	Thermo Fisher Scientific	Cat # 44-788G RRID: AB_2533754
CD324 (E-Cadherin) Monoclonal Antibody	Thermo Fisher Scientific	Cat# 14-3249-82 RRID: AB_1210458
Anti-rat Cy5 conjugated	Jackson Immuno Research	Cat# 112-175-167, RRID: AB_2338264
Anti-rat Alexa488 conjugated	Molecular Probes	Cat#A-21208, RRID: AB_141709
Anti-rabbit Rhodamine Red-X conjugated	Jackson Immuno Research Lab	Cat# 111-295-003, RRID: AB_2338022
Anti-chicken Rhodamine Red-X conjugated	Jackson Immuno Research Lab	Cat# 703-295-155, RRID: AB_2340371
Anti-guinea pig Rhodamine Red-X conjugated	Jackson Immuno Research Lab	Cat# 706-295-148, RRID: AB_2340468
Anti-guinea pig Alexa488 conjugated	Molecular probes	Cat# A-11073, RRID: AB_2534117
Anti-CD34 Monoclonal antibody (RAM34), Biotin	Thermo Fisher Scientific	Cat# 13-0341-85, RRID: AB_466426
Anti-CD49f (integrin alpha 6) (GoH3), PE-conjugated	Thermo Fisher Scientific	Cat# 12-0495-81, RRID: AB_891478
Anti-CD49f (Integrin alpha 6) Antibody (GoH3) PE-Cy7 conjugated	Thermo Fisher Scientific	Cat# 25-0495-82, RRID: AB_10804881
Mouse LRIG1 Antibody	R and D systems	Cat# AF3688, RRID: AB_2138836
Streptavidin, APC-conjugated	BD Bioscience	Cat# 554067, RRID: AB_10050396
Mm-EREG (RNA Scope)	ACD Bio	Cat# 437981-C2
Biological samples		
Fetal Calf Serum	Merck Millipore	Cat# TMS-013-B
Chemicals, peptides, and recombinant proteins		
Phalloidin Alexa488 conjugated	Life Technologies	Cat#A12379
Tamoxifen	Sigma	Cat# T5648
Doxycycline	Sigma	Cat# D9891
Trametinib	Selleckchem	Cat# S2673
Afatinib	Selleckchem	Cat# S1011
AP1 inhibitor (T-5224)	Selleckchem	Cat# S8966
ML7 HCL	TOCRIS	Cat# 4310
ROCK1 Inhibitor (Y-27632 2HCl)	Selleckchem	Cat# S1049
TPA (12-O-Tetradecanoylphorbol-13-acetate)	Sigma	Cat# P8139
Sucrose	Merck Millipore	Cat# 107651
7-Aminoactinomycin D (7-AAD)	Sigma	Cat# A9400
EdU (5-ethynyl-2'-deoxyuridine)	Invitrogen	Cat# A10044
BrdU (5-Bromo-2'-deoxyuridine)	Sigma Aldrich	Cat# B5002

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
HBSS	GIBCO	Cat#14170-88
Trypsin	GIBCO	Cat#15090-046
DMEM	GIBCO	Cat#41965-039
Chelex	Bio-Rad	Cat#142-2842
DPBS	Capricorn scientific	Cat# PBS-1A
Critical commercial assays		
Click-it EdU Imaging Kit	Invitrogen	Cat# C10339
RNAscope® Multiplex Fluorescent Detection Kit v2	ACD Bio	Cat# 323110
Chromium Next GEM Single Cell 3' v3.1: Cell Multiplexing	10X Genomics	Cat# PN-1000268
Chromium Next GEM Chip G Single Cell Kit	10X Genomics	Cat# PN-1000120
3' Feature Barcode Library Kit	10X Genomics	Cat# PN-1000262
3' CellPlex Kit Set A	10X Genomics	Cat# PN-1000261
Dual Index Kit TT Set A	10X Genomics	Cat# PN-1000215
Dual Index Kit NN Set A	10X Genomics	Cat# PN-1000243
Deposited data		
Bulk RNA-seq	NCBI GEO	GEO: GSE270771
ATAC-seq	NCBI GEO	GEO: GSE270772
scRNA-seq	NCBI GEO	GEO: GSE270773
Experimental models: Mouse		
Mouse: K14-CreER	Jackson Laboratory	Cat# 005107
Mouse: R26R-Confetti or R26R-Brainbow2.1	Jackson Laboratory	Cat# 013731
Mouse: Rosa26-YFP	Jackson Laboratory	Cat# 006148
Mouse: Lrig1-CreERT2	Jackson Laboratory	Cat #018418
Mouse: KRT14-rtTA	Jackson Laboratory	Cat #008099
Mouse: TetO-DTA	Jackson Laboratory	Cat #008168
Mouse: Rosa26-mT/mG	Jackson Laboratory	Cat #007576
Mouse: CD1	Charles River	N/A
Software and algorithms		
Zen software (2012)	Zeiss	N/A
Fastqc	Babraham Institute	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Trimmomatic (version 0.36)	Bolger et al. ⁴⁶	N/A
STAR aligner (version 2.7.5a)	Dobin et al. ⁴⁷	N/A
Picard MarkDuplicate	Broad Institute	https://broadinstitute.github.io/picard
Htseq-count (version 0.11.1)	Anders et al. ⁴⁸	N/A
Bowtie2 (version 2.3.4.2)	Langmead et al. ⁴⁹	N/A
SAMtools (version 1.11)	Li et al. ⁵⁰	N/A
MACS2 (version 2.1.0.20151222)	Zhang et al. ⁵¹	N/A
GREAT input (version 4.0.4)	Stanford University	http://great.stanford.edu/public/html/
HOMER (version 4.11)	Duttke et al. ⁵²	http://homer.ucsd.edu/homer/
CellRanger (version 6.1.1)	Zheng et al. ⁵³ 10X Genomics	https://www.10xgenomics.com/support/software/cell-ranger/latest
R (version 4.1.0)	R Core Team ⁵⁴	https://www.r-project.org
Seurat R package (version 4.1.0)	Hao and Hao et al. ⁵⁵	N/A
pySCENIC (version 0.11.2)	Aibar et al. ³⁶	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Slingshot R package (version 2.4.0)	Street et al. ³⁷	N/A
GraphPad Prism 8	GraphPad	https://www.graphpad.com/features
Computational modelling code	Zenodo	https://doi.org/10.5281/zenodo.12629079

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Cedric Blanpain (cedric.blanpain@ulb.be).

Materials availability

This study did not generate any new unique reagents.

Data and code availability

- Single-cell RNA-seq data have been deposited at GEO and are publicly available as of the date of publication. Accession numbers are listed in the [key resources table](#).
- All original code has been deposited on Zenodo and is publicly available as of the date of publication. DOIs are listed in the [key resources table](#).
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Mouse strains

K14CREER transgenic mice were kindly provided by E. Fuchs (Rockefeller University).⁵⁶ Lrig1-CreERT2 mice were a kind gift from Kim Jensen (Copenhagen University).⁵ Rosa-Confetti mice were provided by H. Clevers (Hubrecht Institute).⁵⁷ Rosa26-mT/mG mice were provided from Isabelle Migeotte (Université Libre de Bruxelles).⁵⁸ K14rtTA transgenic mice⁵⁹ were provided by Elaine Fuchs, TetO-DTA mice were provided by J. Rajagopal⁶⁰ and Rosa YFP mice⁶¹ were obtained from Jackson Laboratory. All animals were mixed strains. No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment. Mice colonies were maintained in a certified animal facility in accordance with European guidelines. The experiments were approved by the local ethical committee (CEBEA) under protocols #604N, #605N and 795N. The study is compliant with all relevant ethical regulations regarding animal research.

METHOD DETAILS

Lineage ablation experiment

Mice were selected based on the expression Caspase3 to reduce the variability in DTA expression. Mice were first treated with topical application of Doxycycline (1mg/ml) on ears after 48 hrs. ear skin were analyzed for the % of Cas3 positive cells and selected mice based on the at least 60 % of caspase 3 positive cells in ear skin wholemount (rabbit, 1:600, R and D, AF835). Selected mice were treated with topical application Doxycycline (1mg/ml), 500ul for Tail for 3 consecutive days and 150ul for Ears for one day to ablate more than 60% of basal cells.

Clonal induction for lineage tracing

For lineage tracing in experiment and control condition, mice were induced at 2 months of age with Tamoxifen, for K14CreER mice (0.8 mg for 30g mouse), for Lrig1CreERT2 mice (7mg for 30g of mice) by intraperitoneal injection.

Mouse treatments

To inhibit MEK1/2 activity, mice were treated with Trametinib 2mg per kg body weight. To inhibit specifically EGFR (ErbB1), HER2 (ErbB2), and HER4 (ErbB4) receptors, mice were treated with Afatinib 10mg per kg body weight by daily oral gavage for 7 consecutive days. To inhibit specifically the DNA binding activity of c-Fos/c-Jun without affecting other transcription factors mice were treated with AP1 inhibitor (T-5224), 3mg per kg body weight by daily oral gavage for 7 consecutive days.

ML7 Inhibitor: ML7 powder dissolved in (0.9% NaCl) normal saline and injected Intraperitoneally at a concentration of 1 mg/Kg, while ROCK1 inhibitor dissolved in (0.9% NaCl) normal saline and injected Intraperitoneally at a concentration of 10mg/Kg.

For TPA (12-O-Tetradecanoylphorbol-13-acetate) treatments, TPA (50 μ l of 0.02 mg/ml solution in acetone) was administered daily to shaved mouse ear skin for 2 days.

Epidermal whole-mount and immunostaining

Tail and ear skin were dissected from the experimental and control mice, cut into small pieces and incubated in 20mM PBS-EDTA on a rocking plate at 37°C for 1 hr (K14 Clones-IFE) and for 1.5 hr (Lrig1 Clones-Infundibulum and IFE). Epidermis was separated from the dermis by using forceps. Washed 2 times with 1X PBS and then fixed in 4% paraformaldehyde for 1hr at room temperature. Washed 3 times with 1X PBS for 5 min each wash and then stored in PBS azide (0.2%) at 4°C. For immunofluorescence staining, we used the pieces of epidermal sheet, incubated in blocking buffer (1% BSA, 5% Horse serum, 0.8% Triton in 1X PBS) for 3 hours at room temperature on rocking plate. For primary antibody staining, samples were incubated with primary antibody anti-Integrin β 4 (rat, 1:200, BD Biosciences) for overnight at room temperature on rocking plate (100 rpm). Samples were then washed for 3 times with PBS 0.2% Tween for 20 minutes each and then incubated with secondary antibody and Hoechst (1: 2000) for nuclear staining for 2hrs at room temperature on rocking plate. Samples were washed 3 times with PBS 0.2% Tween for 20 minutes each and then mounted in DAKO mounting medium by keeping hairy side down.

For BrdU staining, epidermal sheets are incubated in 1M HCL at 37°C for 45 min and then washed with 1X PBS, 3 times for 10 min each. Overnight incubation with primary antibody anti-BrdU (rat, 1:200, Abcam) in blocking buffer at room temperature on rocking plate. The next day, samples were washed for 3 times with PBS 0.2% Tween for 20 minutes each and then incubated with secondary antibody and Hoechst (1:500) for 2hrs at room temperature on rocking plate. Samples were washed 3 times with PBS 0.2% Tween for 20 minutes each and then mounted in DAKO mounting medium by keeping hairy side down.

For EdU staining, performed according to the manufacturer's instructions (ThermoFisher). For co expression with EdU, first performed the K14 or other primary antibody staining then followed the EdU protocol.

Microscope image acquisition and analysis

All confocal images for whole mount epidermis and sections were acquired at room temperature with a LSM780 confocal system fitted on an AxioExaminer Z1 upright microscope equipped with C-Apochromat 40x/1.1 or Plan Apochromat 25x/0.8 water immersion objectives (Zeiss, Jena, Germany). Optical sections 512 x 512 pixels were collected sequentially for each fluorochrome. Confetti colors were acquired using tracks (mCFP: Ex: 458 nm, Em: 464-518nm/ RFP: Ex: 543nm; Em: 570-625nm/ EYFP-nGFP: Ex: 488; Em: 508-571nm). The data sets generated were merged and displayed with the ZEN2012 software (Zeiss).

Immunofluorescence intensity measurements

To quantify the intensity of the immunostaining for E-cadherin, ImageJ was used to measure the integrated density, a well-established method of measuring fluorescence intensity that accounts for differences in area of the signal in the basal layer of the skin (labelled by Krt14).

Live imaging/intravital microscopy

Mice anesthesia was done by using 4% isoflurane + 2% O₂ in chamber. For imaging, mice were under continuous low dose of anesthesia 2% isoflurane + 0.3% O₂ throughout the course of imaging sessions at 29°C temperature to avoid hypothermic condition. Mice were shaved for back skin and ear skin by using clippers and depilatory cream to image clearly the hair follicle and interfollicular epidermis.⁶²

It is important to image the same area every time to follow the same clone for multiple days we made a tattoo on the ears and back skin. Ear skin was tattooed using a small needle with carbon ink. Images were acquired on a LSM 880 confocal (Zeiss) fitted on a AxioObserver Z NLO inverted microscope (Zeiss). Multiphoton excitation from a tunable InSight X3 laser (Spectra Physics) was set at 920nm to image second harmonic generation (SHG), GFP and dtTomato using emission filters (Chroma) KP 475nm, BP 510-540nm and BP 580-640nm, respectively, in front of GaAsP non-descanned detectors (NDD) 5Zeiss). Serial optical sections were acquired through a 40x dry lens (Zeiss) with a 0.6-1 μ m step to image the progress of clonal expansion and fragmentation. Images were analyzed by using ImageJ/Fiji software.

For the timelapse imaging we acquired the data on the D2/ D4/ D11 after the Dox application continuously for 4hrs for analyzing the mechanical state of the tissue.

Wound healing assay

For wound healing study, we made wound of 1mm in size on the ear epidermis by laser ablation. To analyze the mechanical state of the tissue in wound healing condition we acquired epidermal wound area, leading edge (0-300um from wound site) continuously for 4hrs at D2 after wounding by using intravital microscopy.

Histology and immunostaining on sections

For histology and sectioning for antibody staining skin epidermis was embedded in OCT and paraffin blocks and stored at -80°C and at room temperature respectively. Sections of 6 μ m were cut using a CM3050S Leica cryostat (Leica Microsystems). Sections are fixed in 4% paraformaldehyde for 10 minutes, and washed 3 times in PBS for 5 min and incubated in blocking buffer (1% BSA,

5% Horse serum, 0.2% Triton in PBS) for 1h at room temperature. Primary antibodies were incubated overnight at 4°C. Sections were rinsed 3 times in PBS and incubated with secondary antibodies and Hoechst in blocking buffer for 1h at room temperature. Sections were again washed three times with PBS. The following primary antibodies were used: anti-K14 (chicken, 1:20000, custom batch, Thermo Fischer); anti-K1 (rabbit, 1:4000, Covance/IMTEC, PRB-165P); anti-K10 (rabbit, 1:4000, Covance/IMTEC, PRB-159P); anti-p63 (rabbit, 1:100 Abcam, ab124762); anti-KLF4 (rabbit, 1:100 Abcam, ab129473); anti c-Jun (rabbit, 1:100 Cell Signaling, #9165); anti-c-Fos (rabbit, 1:100 Cell Signaling, #2250); anti p-EGFR (rabbit, 1:100 ThermoFisher Scientific, 44-788G), anti p-Myosin light chain II (Rabbit, 1:100 Cell Signaling, 3674S). The following secondary antibodies were used diluted to 1:400: anti-mouse, anti-rat, anti-rabbit, anti-chicken conjugated to Alexa Fluor 488 (Molecular Probes), to rhodamine Red-X (Jackson ImmunoResearch) or to Cy5 (Jackson ImmunoResearch). Nuclei were stained in Hoechst solution (1:2000) and slides were mounted in DAKO mounting medium supplemented with 2.5% Dabco (Sigma). All images of section immunostaining were acquired using the Axio Imager M1 Microscope and LSM780 confocal upright microscope.

RNA-FISH

Tail skin was dissected from the ablation and control mice, embedded in OCT (Sakura) and cut into 5–8- μ m frozen sections using a CM3050S Leica cryostat (Leica Microsystems). Sections were fixed for 30 min in 4% PFA at 4 °C and the in-situ protocol was performed according to the manufacturer's instructions (Advanced Cell Diagnostics). The following mouse probes were used: Mm-EREG (437981-C2).

Proliferation and cell-density experiments

For proliferation experiments, mice were injected with a single intraperitoneal injection of BrdU (50 mg/kg in PBS) at different time points after ablation of basal cells and sacrificed 4 hours after. For the quantification at least an area of 1.5 mm per animal was analyzed with Zen2012 (Black Edition) software (Zeiss) to determine the percentage of BrdU positive cells. For basal and total cell density, number of basal cells were counted based on the β 4-integrin expression and orthogonal projection.

FACS analysis and cell sorting

Tail skin samples were incubated in 0.25% Trypsin (Gibco, Thermo Fisher Scientific) in DMEM-Dulbecco's modified Eagle's medium (Gibco, Thermo Fisher Scientific) and 2mM EDTA (Thermo Fisher Scientific) overnight at 4°C. In Lrig1 antibody staining, tail skin samples were incubated in Thermolysin (Sigma) (0.25mg/ml in PBS) for 1hr at 37°C on a rocking plate (100 rpm). Epidermis is removed from the dermis by using forceps. Epidermis was then incubated on a rocking plate (100 rpm) at room temperature for 5 min. Cells from the epidermis were mechanically separated by flushing 10 times under the epidermis. Tissues were then cut in small pieces with a scalpel and trypsin was neutralized by adding DMEM medium (GIBCO) supplemented with 2% Chelex Fetal Calf Serum (cFCS). Samples were filtrated on 70 and 40 μ m filter (Falcon). Single cells suspension was incubated in 2% FCS/PBS with primary antibodies for 30 min on ice, protected from the light, with shaking every 10 min. Primary antibodies were washed with 2% FCS/PBS and cells incubated for 30 min in APC-conjugated streptavidin (BD Biosciences), on ice, with shaking every 10 min. Living epidermal cells were gated by forward scatter, side scatter, and negative staining for Hoechst dye. Basal IFE cells were stained using PE-Cy7-conjugated anti- α 6-integrin (clone GoH3, 1:200, ebioscience), bulge cells were stained with biotinylated CD34 (clone RAM34; 1:50, BD Biosciences) and infundibulum cells were stained with Lrig1 primary antibody (Polyclonal Goat IgG AF3688, R&D systems) and A647 anti-Goat secondary antibody. Basal cells from the interfollicular epidermis were targeted using CD34 negative and α 6 integrin positive gating and Lrig1 positive cells were excluded for K14 tracing and included for Lrig1 tracing in single cell experiment. After gating on specific population, we selected cells from clones specifically to exclude Lrig1 derived cells in K14 tracing and vice versa. Fluorescence-activated cell sorting analysis was performed using FACSARIA I at high pressure (70 psi) and FACSDiva software (BD Biosciences).

Analysis of cell rearrangements (T1 transitions)

T1 transition events (originating either from cell divisions or vanishing cell junctions leading to a change in neighbours) were manually counted in a slice from confocal timelapse of optical sections of the basal cell layer. Those timelapses were obtained using live imaging of this tissue for 3 to 4 hours within the time window of the whole regeneration process. From this quantification, we obtained the cumulative time evolution of cell rearrangements for each biological replicate and condition. To obtain the rate of T1 transitions in each condition, we fitted the first two hours of the T1 cumulative time evolution with a linear function for each biological replicate and averaged the slope coefficients. The error on the rate of T1 was defined as the standard deviation between those coefficients for a given condition. All analysis and plotting were performed with Python.

RNA extraction and bulk RNA sequencing

FACS isolated cells were collected into 350 μ l of lysis buffer (RLT buffer, RNeasy Microkit, Qiagen) supplemented with β -mercaptoethanol. RNA extraction was then carried out using RNeasy Micro kit (QIAGEN) according to the manufacturer's recommendations. Prior to sequencing, the quality of RNA was evaluated by Bioanalyzer 2100 (Agilent).

Bulk RNA sequencing analysis

Indexed cDNA libraries were obtained using the Ovation Solo RNA-seq Systems (NuGen) following manufacturer's recommendations. The multiplexed libraries were loaded on flow cells and sequences were produced using a NovaSeq 6000 S2 Reagent Kit (200 cycles from Novaseq 6000 System, Illumina) on a NovaSeq 6000 System (Illumina).

Before starting the alignment and downstream analysis, the quality report of each raw dataset was generated by FastQC. The adaptor sequences and low-quality regions were trimmed by Trimmomatic PE (paired-end mode). Trimmed reads were mapped against the mouse reference genome (Grcm38/mm10) using STAR software to generate read alignments for each sample. Annotations Grcm38.87 was obtained from ftp.Ensembl.org. Duplicated reads are suppressed by picard (<http://broadinstitute.github.io/picard/>) Mark Duplicates. After transcripts assembling, gene level counts were obtained using Htseq-count (v.0.11.1) and normalized to 20 million of aligned reads. Average expression for each gene for the different populations was computed based on at least 2 biological replicates and fold changes were calculated between the subpopulations. Genes having a fold change of expression greater or equal than 2 are considered as up-regulated and those having a fold change of expression lower or equal to 0.5 are considered down-regulated.

ATAC sequencing

For ATAC-seq, 50000 sorted basal cells ($\alpha 6^{\text{High}}/\text{CD}34^{-}/\text{Lrig}1^{-}/\text{YFP}^{+}/\text{GFP}^{+}/\text{RFP}^{+}$) were collected in 1 ml PBS supplemented with 3% FBS at 4 °C. Cells were centrifuged and cell pellets were resuspended in 100 μl lysis buffer (TrisHCl 10 mM, NaCl 10 mM, MgCl₂ 3 mM, Igepal 0.1%) and centrifuged at 500g for 25 min at 4 °C. Supernatant was carefully discarded and nuclei were resuspended in 50 μl reaction buffer (Tn5 transposase 2.5 μl , TD buffer 22.5 μl , from Nextera DNA sample preparation kit, Illumina, and 25 μl H₂O). The reaction was performed at 37 °C for 30 min and was stopped by adding 5 μl clean up buffer (NaCl 900 mM, EDTA 300 mM). DNA was purified using the MiniElute purification kit (QIAGEN) following the manufacturer's protocol. DNA libraries were PCR amplified (Nextera DNA Sample Preparation Kit, Illumina), and size selected from 200 to 800 bp (BluePippin, Sage Sciences), following the manufacturer's recommendations.

ATAC-seq analysis

Before starting the alignment and downstream analysis, the quality report of each raw dataset was generated by FastQC. Adaptor sequences were removed with Trimmomatic PE using options 'HEADCROP:10 CROP:70 ILLUMINACLIP: adaptor.file:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:50'. ATAC-seq paired-end reads were then aligned to mouse reference genome mm10 using Bowtie2 (v.2.3.4.2) using options '-X 2000 -q -p 6 -t -fr -very-sensitive -no-discordant -no-unal -no-mixed'.⁴⁹ Mitochondrial reads, reads from unmapped or random counting and reads with a mapping quality <20 was removed using SAM tools. Duplicate reads were removed by Picard MarkDuplicates module.

Peak calling was performed on each individual sample by MACS2 (v.2.1.0.20151222) using options '-f BAMPE -g mm -q 0.01 -nomodel -shift 0. Peaks from the different subpopulations were merged for downstream analysis.⁵¹

Reads counts of each merged peak for each individual sample were calculated by HTSeq-count using options '-f bam -r pos -m intersection-nonempty'. These counts were normalized for one million mapped reads in merged peaks and fold-change was calculated compared to control. Peaks were associated to genes with GREAT software (v4.0.4) with the following parameters: 5.0 kb in proximal upstream, 1.0 kb in proximal downstream and 100.0 kb in distal. For further analysis, only peaks annotated to at least one gene was kept.

Differential peaks are defined as peaks having at least a two-fold change compared to control and being called peak in the expanded condition. De novo motif prediction on regulated peaks was performed using findMotifsGenome.pl program in HOMER software searching for motifs of 6, 8, 10 and 12 bp in a region of 500 bp around the peak center. Custom background peaks are applied for the motif prediction using option '-bg' and background peaks are defined by subtracting the peaks which are not regulated in K14Abl compared to K14CTL. Finding the peaks which has binding site of interested motifs was performed using annotatePeaks.pl in HOMER software using parameters '-size -250,250' and motif matrices were achieved from HOMER database.

Single-cell RNA sequencing

For the Single-cell RNA sequencing experiments cells were sorted based on the markers and from clones in CTRL, K14 Abl and Lrig1 Abl at D4 in the epidermal repair. We use MULTI-seq: multiplexing using lipid-tagged indices approach to pool all the three different samples (CTRL, K14 Abl and Lrig1 Abl) together.⁶³ We sorted $\alpha 6^{\text{High}}/\text{CD}34^{-}/\text{Lrig}1^{-}/\text{YFP}^{+}/\text{GFP}^{+}/\text{RFP}^{+}$ for K14 CTRL and K14 Abl mice and for Lrig1 Abl we sort $\alpha 6^{\text{High}}/\text{CD}34^{-}/\text{Lrig}1^{+}/\text{RFP}^{+}$ cells. Total 10,000 cells were loaded onto each channel of the Chromium Single Cell 3' microfluidic chips (V2-chemistry, PN-120232, 10X Genomics) and barcoded with a 10X Chromium controller according to the manufacturer's recommendations (10X Genomics). RNA from the barcoded cells was subsequently reverse transcribed, followed by amplification, shearing 5' adaptor and sample index attachment. The libraries were prepared using the Chromium Single Cell 3' Library Kit (V3-chemistry, PN-120233, 10X Genomics) and sequenced on an Illumina Novaseq 6000 (paired-end 100bp reads).

Single-cell transcriptomic data analysis

Demultiplexing of samples from the raw datasets are done by Cell Ranger (v6.1.1) with multi module. Sequencing reads were aligned and annotated with the mm10-2020-A reference dataset as provided by 10X and Cell Ranger with default parameters.⁵³ Further downstream analyses were carried out individually for each of the three samples (K14CTRL, K14Abl, Lrig1Abl).

Quality control and downstream analysis were performed on R (version 4.1.0), using the Seurat R package (v.4.1.0).⁵⁵ For each sample, all the cells passed the following criteria: showed expression of more than 1500 and less than 6000 unique genes and had less than 10% UMI counts belonging to mitochondrial sequences. Read counts were normalized by *NormalizeData* function of Seurat, with parameter 'normalization.method = "LogNormalize" and scale.factor=10000'. A PCA for each sample was calculated using the scaled expression data of the most variable genes (identified as outliers on a mean/variability plot, implemented in the *FindVariableGenes*). UMAP calculation and graph-based clustering were done for each sample using the appropriate functions from Seurat (default parameters) with the respective PCA results as input.⁶⁴ The clusters expressing fibroblast markers (Vim, Fn1, Ctsk) and immune-related genes (Cd74, Cxcl2, Cxcl4, Fcrlg) are excluded and dimensionality has been recalculated. The final resolutions were set to 0.7 for K14CTRL and 0.7 for K14Abl and Lrig1Abl, after testing a range from 0.3 to 0.9. Given that the obtained clustering sensitivity for a given resolution is dependent on the number of cells of that subpopulation in each respective sample, we swept over the same range of resolutions for the other samples, to assure the presence/absence of described clusters in all samples. Selected resolutions best reflected the biological heterogeneity that emerged from the different cell types consisting of the skin epidermis.

On the individual datasets, to verify proliferating stages, the S-phase and G2/M-phase scores were regressed out by *Cell Cycle Scoring* function, implemented in the Seurat.

A Wilcoxon rank-sum test was used to define marker genes for each cluster. Benjamini–Hochberg FDR correction for potential cluster marker genes across all samples using the *p.adjust* method in R and only markers expressed in at least 25% of cells of the cluster, having an average log-fold change of at least 0.25 were reported.

Gene regulatory network analysis was performed by using pySCENIC with default parameters and with python 3.7.10. To correct stochastic variation, the scenic pipelines were run 10 times for each dataset and the average AUC (Area Under the Curve) scores were calculated for further analysis.

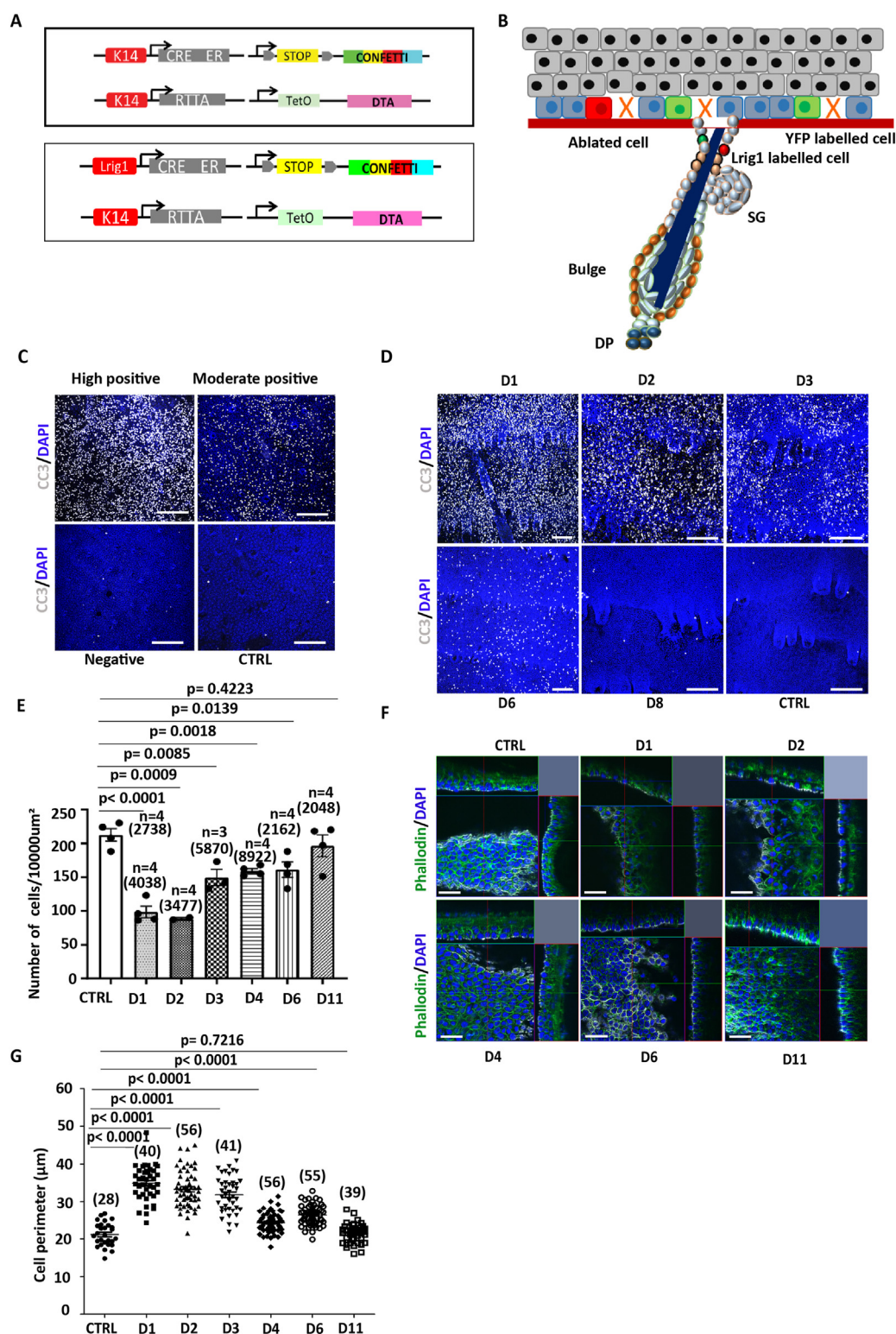
Pseudotime ordering of cells was calculated using slingshot (v.2.4.0). We checked the robustness of the resulting trajectories by performing the analysis on PCA and UMAP reductions, these different reductions and permutations did not affect the described trajectories.

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification of clone sizes were performed on live imaging as well as on whole-mount tissue acquired by confocal microscopy and counted manually using the ZEN2012 software.

Two-tailed Student's *t* test, two-tailed Mann–Whitney non-parametric tests were used for statistical analysis and performed using GraphPad Prism 8. Specific statistical analyses used are described in figure legends, and *p* values are listed in graphs.

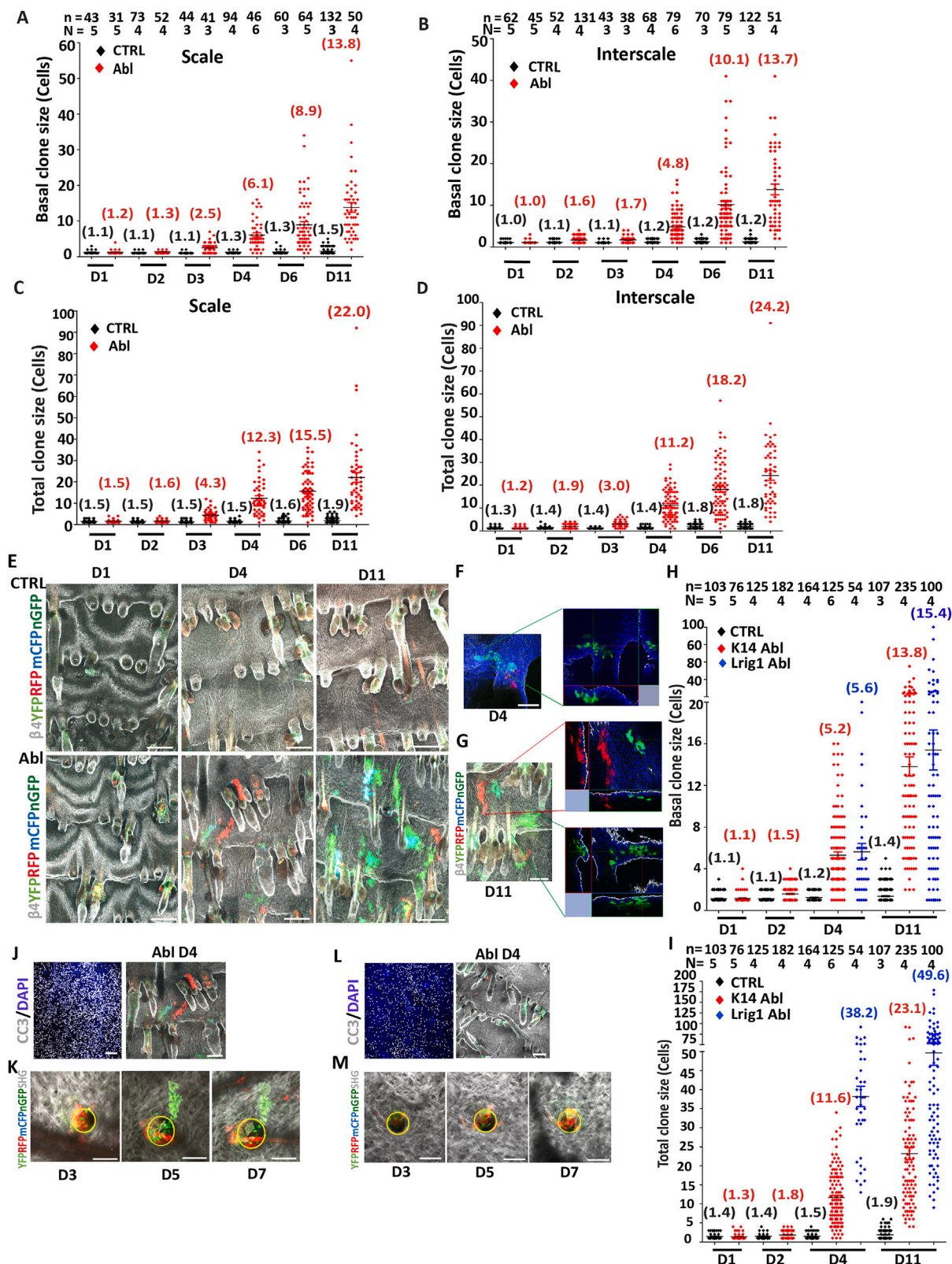
Supplemental figures



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Figure S1. Mouse model of lineage ablation in the skin epidermis, related to Figure 1

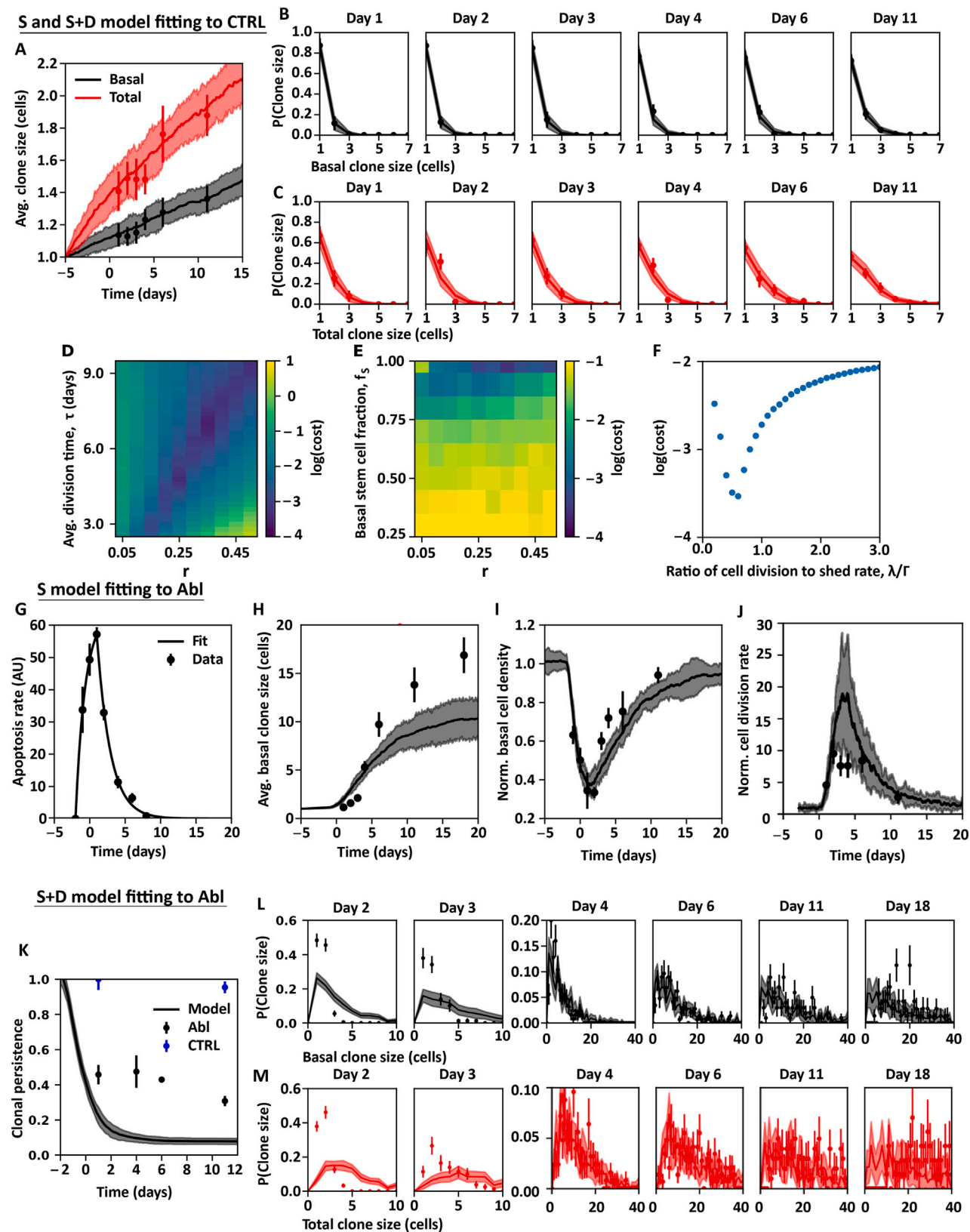
- (A) Mouse model for lineage ablation and tracing of basal and infundibulum cells.
- (B) Cartoon representing the ablation basal cells from the skin interfollicular epidermis simultaneously tracing the remaining basal cells from IFE (K14 tracing) or the infundibulum SCs (Lrig1 tracing).
- (C) Assessing CC3 expression in the ear skin after Dox application to categorize the mice based on % CC3+ cells, high positive for CC3 (% CC3+ cells is more than 60%), moderate positive (% CC3+ cells is less than 60%), and negative. Scale bars, 200 μm .
- (D) Tail whole-mount skin epidermis analysis for CC3 expression from CTRL and at days 1, 2, 3, 6, and 8 post-ablation. Scale bars, 200 μm .
- (E) Total cell density measured as number of total (basal+ suprabasal) cells per 10,000 μm^2 in CTRL and Abl mice.
- (F) Actin staining using phalloidin and DAPI for nuclei in CTRL and Abl mice.
- (G) Cell area measured with ImageJ based phalloidin staining in CTRL and Abl epidermis at different time points. Two-tailed Mann-Whitney test: mean per area $\pm$ SEM. For all quantification, n = the number of areas and the number of cells quantified are indicated in parentheses.



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Figure S2. Mobilization and increased symmetrical renewal of infundibulum SCs to repair IFE following basal cells ablation, related to Figure 2

(A and B) Number of basal cells per clone in K14 tracing in scale (A) and interscale (B) area in CTRL and Abl condition at various time points.
 (C and D) Number of total (basal and suprabasal) cells per clone in scale (C) and interscale (D) in CTRL and Abl conditions at various time points.
 (E) Representative Lrig1CREER/Rosa-Confetti/K14rtTA/Tet-O-DTA whole-mount tail epidermis stained with β 4-integrin in CTRL and Abl mice at days 1, 4, and 11, scale bars, 200 μ m.
 (F and G) Representative orthogonal views of confocal analysis of Lrig1CREER/Rosa-Confetti/K14rtTA/Tet-O-DTA clones in ablation condition with β 4-integrin staining show mobilization of Lrig1 derived cells from infundibulum to interfollicular epidermis at day 4 (F), scale bars, 50 μ m; day 11 (G), scale bars, 100 μ m.
 (H and I) Number of basal (H) and total (I) cells per clone in Lrig1 tracing Abl mice at various time points.
 (J and K) Confocal analysis (J) and intravital imaging analysis (K) show migration of clones in ablation condition. Scale bars: 100 μ m in (J) and 50 μ m in (K).
 (L and M) Confocal analysis (L) and intravital imaging analysis (M) show no migration of clones when less than 60% of basal cell were ablated. Yellow circle represents hair follicle. Scale bars: 100 μ m in (L) and 50 μ m in (M). N = the number of mice and n = the number of clones quantified are indicated in parentheses.



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Figure S3. Fit of the Voronoi model to the homeostatic and regeneration data, related to Figure 3

(A–F) Fits of one-progenitor Voronoi models to the homeostatic control data.

(A) Fit of the SC-only Voronoi model (where all basal cells are SCs) to the average basal and total clone size at homeostasis. Simulations begin at day –5 (0.5 days after TAM administration).

(B and C) Basal and total clone size distribution at homeostasis at different times post-induction for the SC-only model. In (A)–(C), points show data (mean values with 95% confidence intervals), and lines denote results of stochastic simulation with 95% confidence intervals obtained by bootstrapping (for details, see [Methods S1](#)).

(D–F) Parameter sensitivity analysis (better fits have lower cost) of the Voronoi model, with SC and differentiated cells in the basal layer, as the average division time (τ) vs. fraction of symmetric divisions (r) are varied with other parameters fixed (D), as the fraction of basal stem cells (f_S) vs. r are varied (E), and as the ratio of the division rate ($\lambda = 1/\tau$) to suprabasal cell shedding rate (Γ) is varied (F). $f_S = 1$ corresponds to the SC-only model. A discussion is provided in [Methods S1](#).

(G) Empirical fit of the apoptosis rate during DTA-mediated basal cell ablation based on the percentage of CC3+ cells ([Figure 1C](#)) used for modeling of Abl (see [Methods S1](#)). Points denote mean values with 95% confidence intervals, and line denotes empirical fit.

(H–J) SC-only Voronoi model does poorly to simultaneously capture the average basal clone size (H), basal cell density (I), and division rate (J) during regeneration (see [Methods S1](#)). The latter (I and J) are measured relative to their homeostatic values (cf. [Figures 1D](#) and [1F](#)). In (H)–(J), points show mean values with 95% confidence intervals combining data from the scale and interscale regions. Lines show the best fit of the stem-only Voronoi model using 20 independent simulations of a tissue of approximately 225 cells with the shading representing the 95% confidence intervals.

(K–M) Quantitative measures during regeneration for SC and differentiated cell Voronoi model. (K) Clonal persistence in ablation (black) and control (blue) conditions. Points show mean values with 95% confidence intervals (adapted from [Figure 2G](#)), and line shows prediction of model fit. Enhanced clone persistence in ablation conditions is consistent with substantial clone fragmentation. (L and M) Basal (L) and total (M) clone size distribution at various time points following cell ablation. Points show data (mean values with 1 SEM confidence intervals), and lines denote results obtained from stochastic simulation based on 20 independent simulations with 1 SEM confidence intervals obtained by bootstrapping (for details, see [Methods S1](#)).

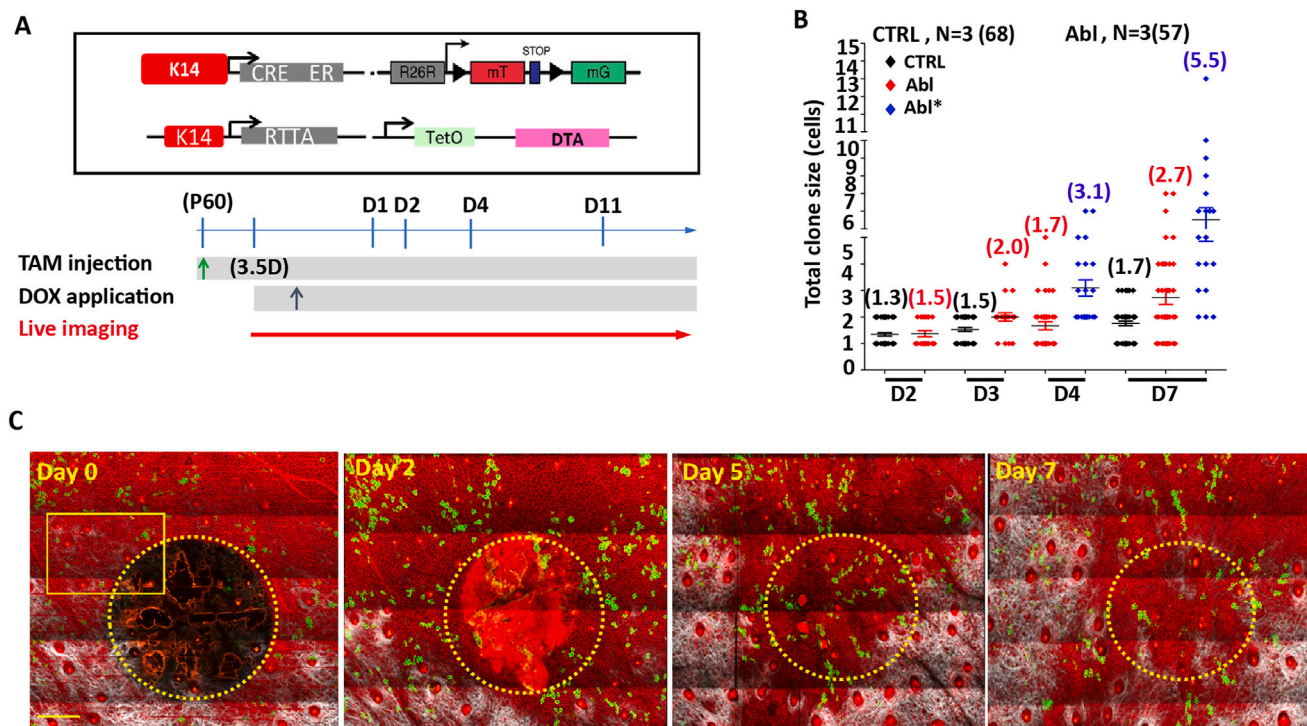


Figure S4. Tissue fluidity is associated with wound healing, related to Figure 4

(A) Experimental design. Mice followed for 4 h of time-lapse intravital imaging to analyze the physical state of the tissue.

(B) Number of total (basal and suprabasal) cells per clone in CTRL and Abl in K14 tracing at days 2, 3, 4, and 7 (Abl* indicates clone size if there is no fragmentation and considered as one clone).

(C) Live imaging map of wounded area acquired during different days during the wound-healing process from day 0 to day 7 by intravital live imaging. Scale bars, 200 μ m. N = the number of mice and n = the number of clones quantified are indicated in parentheses.

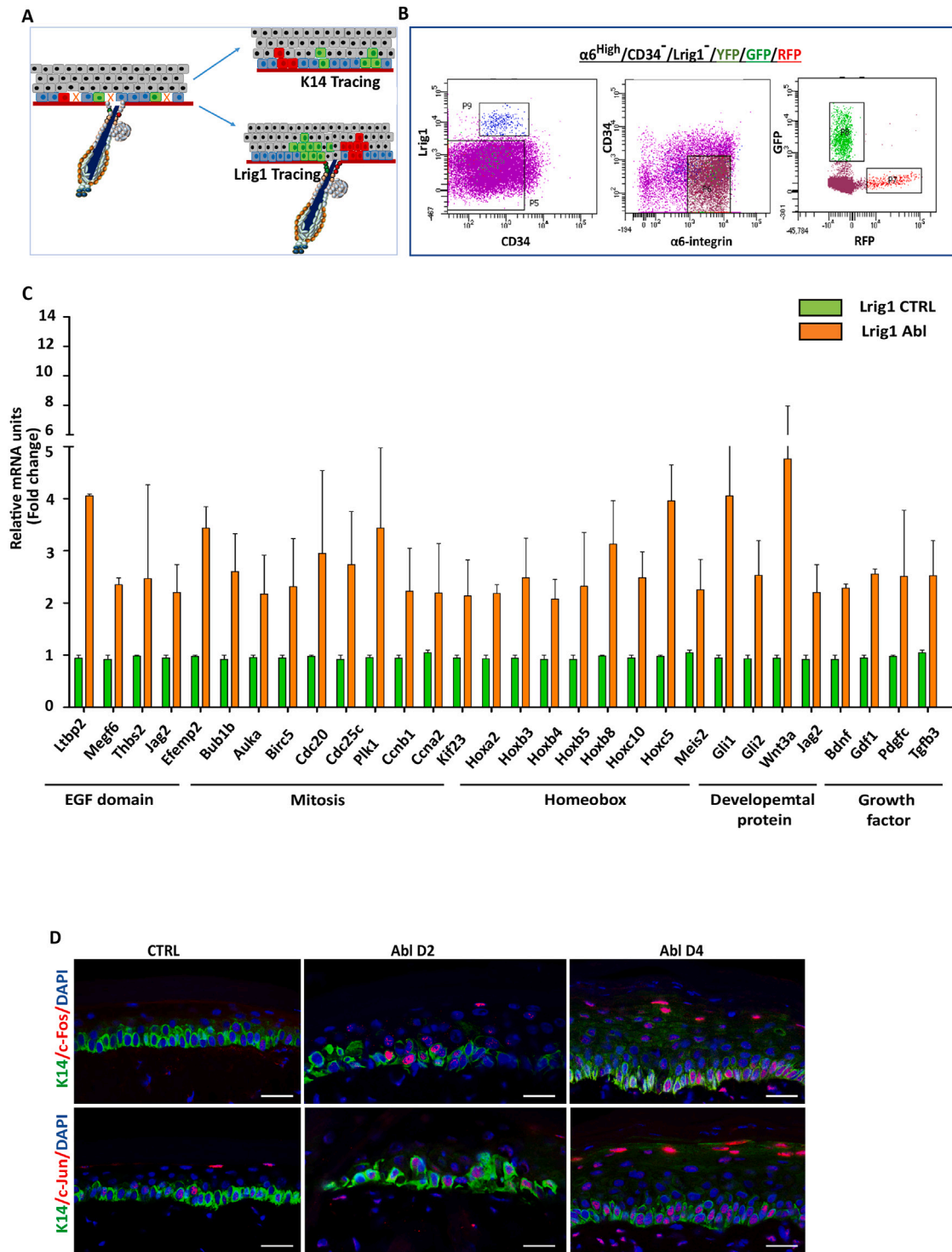


Figure S5. FACS isolation of basal cells derived from the IFE or the infundibulum for RNA-seq and ATAC-seq, related to Figure 5

(A) Cartoon represents the response of K14 and Lrig1 basal cells in the epidermal repair.

(B) Strategy to isolate basal cells from the K14 and Lrig1 clones by using flow cytometry, representative FACS plots show the sorting strategy CD34⁻/α6^{high}/Lrig1⁻/YFP⁺/GFP⁺/RFP⁺ population for K14 control and experimental conditions and CD34⁻/α6^{high}/Lrig1⁺/RFP⁺ population for Lrig1 clones at day 4.

(C) Genes upregulated in Lrig1 clones in Abl where clones migrated toward the interfollicular epidermis compared with Lrig1 CTRL at day 4 (fold change).

(D) Immunostaining for c-Fos and c-Jun with Krt14 in control and at day 2 and day 4 post-ablation (scale bars, 20 μm).

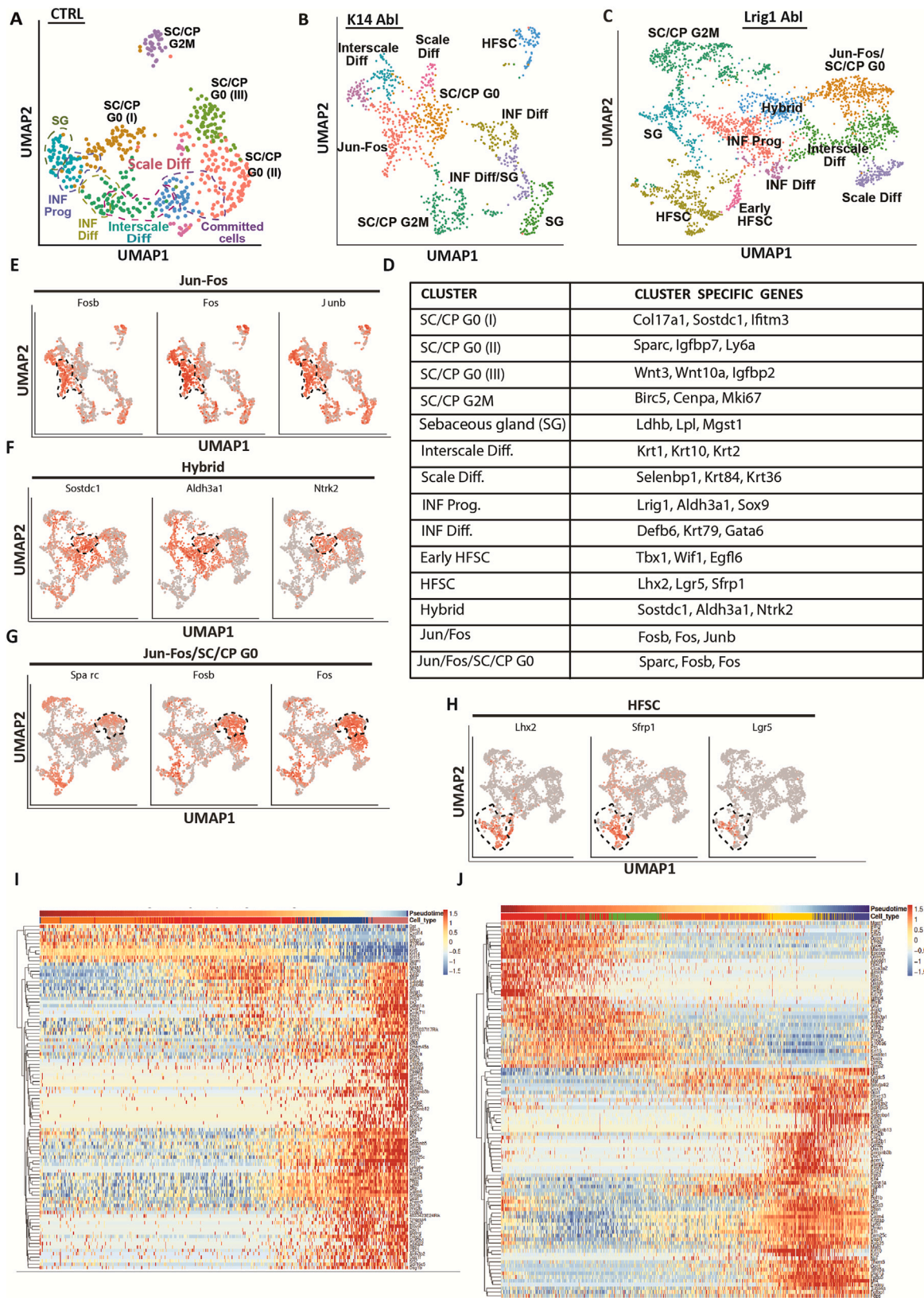


Figure S6. scRNA-seq clustering analysis on K14 CTRL, K14 Abl, and Lrig1 Abl condition, related to Figure 6

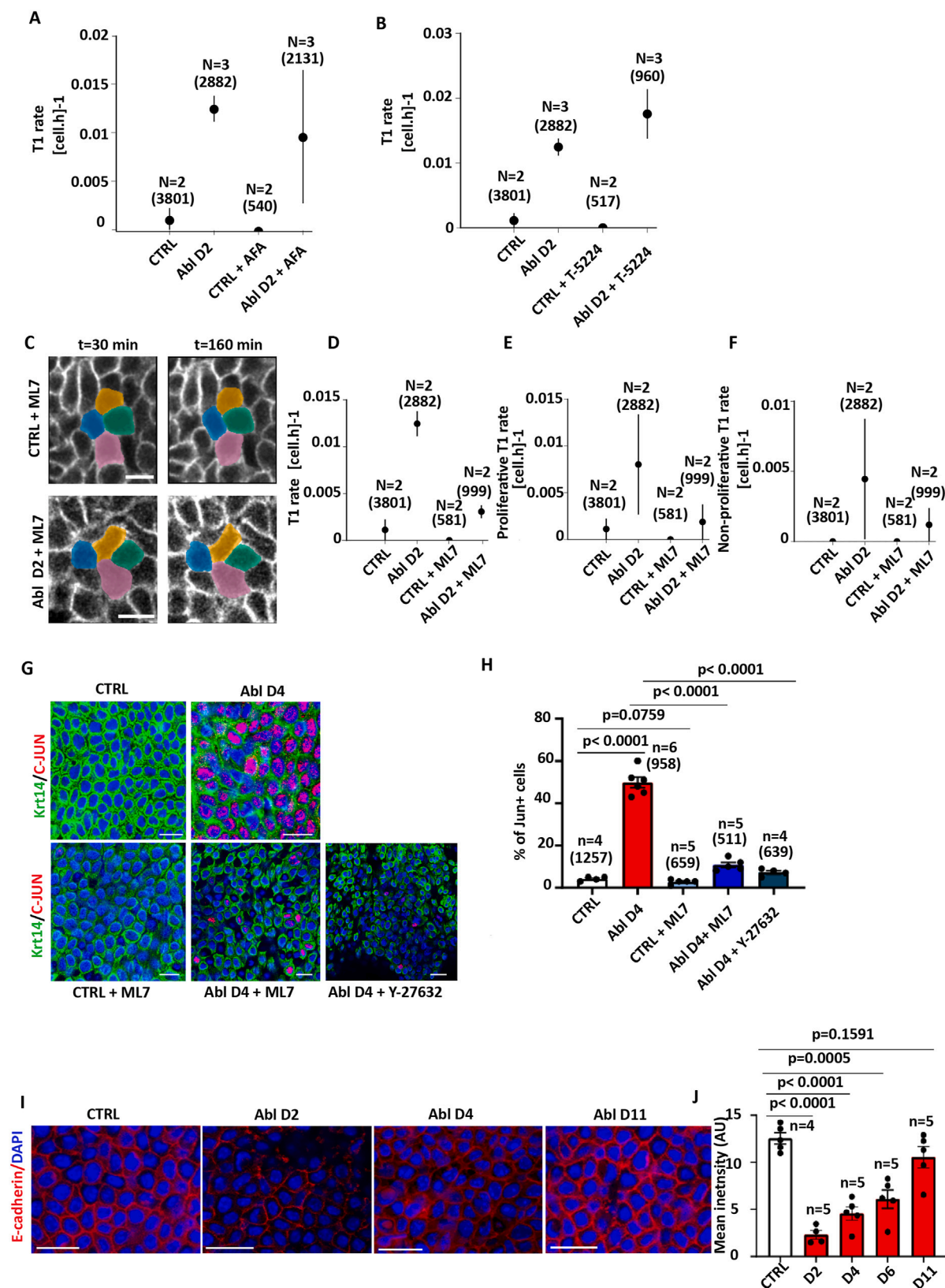
(A–C) (A) UMAP graphic representation of K14 CTRL, K14 Abl (B), and Lrig1 Abl (C) showing the graph-based clustering results annotated by cell type.

(D) Table showing the specific marker genes used to annotate different clusters.

(E–H) UMAP plot of the specific cluster colored by normalized gene expression values for genes identifying in K14 Abl condition, the Jun-Fos enriched clusters (Jun-Fos) (E), in Lrig1 Abl condition clusters hybrid (F), Jun-Fos/SC G0 (G), and hair follicle SC (HFSC) (H).

(I) Heatmap representation of the top 100 gene expression changes along the inferred pseudotime trajectory computed with Slingshot for the K14 Abl mice.

(J) Heatmap representation of the top 100 gene expression changes along the inferred pseudotime trajectory computed with Slingshot for the Lrig1 Abl mice. Columns represent cells ordered by their position along the pseudotime trajectory; rows represent genes whose expression profiles show the highest correlation (FDR-corrected $p < 0.01$) with the pseudotime variable, calculated using a generalized additive model (GAM).



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Figure S7. Modulating cell adhesion or contractility restricts tissue fluidity and regenerative state acquisition, related to Figure 7

(A) T1 rate (cell.h)⁻¹ for the conditions CTRL, Abl day 2, CTRL+ afatinib (AFA), and Abl day 2+ AFA.
 (B) T1 rate (cell.h)⁻¹ for the conditions CTRL, Abl day 2, CTRL+ T-5422, and Abl day 2+ T-5224.
 (C) Representative images of for CTRL+ ML7 and Abl day 2+ ML7 at t = 30 min and t = 160 min. Scale bars, 10 μm.
 (D–F) Total T1 (D), non-proliferative T1 (E), and proliferative T1 (F) rates (cell.h)⁻¹ for the conditions CTRL, Abl day 2, CTRL+ ML7, and Abl day 2+ ML7.
 (G and H) (G) Immunostaining for c-Jun with Krt14 (E) and percentage of c-Jun⁺ cells (H) in control, ablation condition, control+ ML7, ablation+ ML7 inhibitor, and ablation+ Y-27632 at day 4 (scale bars, 20 μm).
 (I and J) Immunostaining for E-cadherin (red) (I) and fluorescence mean intensity for E-cadherin (J) in control and at days 2, 4, and 11 post ablations. Scale bars, 20 μm. Two-tailed Mann-Whitney test: mean per area ± SEM. For all quantification, *N* = the number of mice, *n* = the number of areas, and number of cells quantified are indicated in parentheses.