Inflammatory memory sensitizes skin epithelial stem cells to tissue damage

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The skin barrier is the body’s first line of defence against environmental assaults, and is maintained by epithelial stem cells (EpSCs). Despite the vulnerability of EpSCs to inflammatory pressures, neither the primary response to inflammation nor its enduring consequences are well understood. Here we report a prolonged memory to acute inflammation that enables mouse EpSCs to hasten barrier restoration after subsequent tissue damage. This functional adaptation does not require skin-resident macrophages or T cells. Instead, EpSCs maintain chromosomal accessibility at key stress response genes that are activated by the primary stimulus. Upon a secondary challenge, genes governed by these domains are transcribed rapidly. Fuelling this memory is Aim2, which encodes an activator of the inflammasome. The absence of Aim2 or its downstream effectors, caspase-1 and interleukin-1β, erases the ability of EpSCs to recollect inflammation. Although EpSCs benefit from inflammatory tuning by heightening their responsiveness to subsequent stressors, this enhanced sensitivity probably increases their susceptibility to autoimmune and hyperproliferative disorders, including cancer.

Enhanced wound healing after inflammation

The persistence of inflammation-experienced EpSCs long after the restoration of homeostasis led us to address whether this assay may have had a lasting effect on them and/or their microenvironment. We therefore challenged inflammation-recovered skin with a secondary assault, in this case wounding. Notably, the post-inflamed mice closed their wounds approximately 2.5 times faster than naive mice. The enhanced wound-healing response by inflammation-experienced skin occurred even when the initial assault had occurred 180 days earlier (Fig. 1a and Extended Data Fig. 1b, c). Because Cre recombinase was not activated without tamoxifen (Extended Data Fig. 1d), the YFP+ EpSCs were long-lived and had survived the inflammatory assault. By contrast, Krt10-creER−activated YFP+ cells (although present initially) were shed within 6 days, indicating that once progenitors commit to terminal differentiation, they do not revert even in response to IMQ (Extended Data Fig. 1b, e).

To determine whether this heightened sensitivity might be triggered by other inflammatory stimuli, we replaced IMQ with various other treatments such as topical application of the vitamin D analogue calcipotriol (MC903; to model atopic dermatitis)7; topical application of 12-O-tetradecanoylphorbolester 13-acetate (TPA; to induce hyperplasia)8; epidermal abrasion wounding9; or infection with the fungal pathogen Candida albicans10. In all cases, wounds healed faster in inflammation-experienced skin than in naive mice. The enhanced wound-healing response by inflammation-experienced skin occurred even when the initial assault had occurred 180 days earlier (Fig. 1a and Extended Data Fig. 2a, b). These results underscored the generality of the response, indicating that once skin is sensitized to inflammation, it reacts faster when faced with a secondary assault.

After a full-thickness wound, re-epithelialization is mediated by sensitized epidermal EpSCs, which transiently express K1711.
Inflammation-experienced skin displayed enhanced epidermal thickness and accelerated re-epithelialization, but showed similar rates of proliferation (Fig. 1d, e and Extended Data Fig. 2d). Re-epithelialization by the migrating K14\(^+\) and integrin \(\alpha\delta\) \(\beta\) epithelial tongue neared completion by day 5 in wounded post-inflamed skin, and healed 2 days faster than in naive counterparts.

To exclude myofibroblast-mediated dermal contraction as a notable contributor to the augmented wound closure after inflammation, we affixed a silicone split around full-thickness wounds\(^{14}\). Inflammation-experienced mice still exhibited enhanced re-epithelialization and accelerated wound repair (Fig. 1f and Extended Data Fig. 2e). Moreover, using an assay that specifically measures keratinocyte migration (Extended Data Fig. 2f), we found that keratinocyte outgrowth from ex vivo explants after inflammation\(^{13}\) was more robust than in controls (Fig. 1g). These findings suggest that inflammation induces long-term changes in EpSCs that enhance their capacity to react swiftly to a secondary assault.

**IMQ intrinsically sensitizes EpSCs**

EpSCs receive cues from their local milieu as well as from infiltrating immune cells and circulating factors that direct wound repair. Thus, we evaluated the relative importance of these secondary effectors on the sensitization of inflammation-experienced skin to wounding. When IMQ was applied to half of the dorsal skin, the pathology remained restricted to the treatment site (local), and upon subsequent wounding, distal sites closed comparably to control skin (Fig. 2a, b and Extended Data Fig. 3a). Thus, the EpSC memory of inflammation was not transmitted through systemic (circulating) factors to naive sites.

We next tested whether the heightened responsiveness of inflammation-experienced EpSCs was secondarily dependent on the memory of resident innate and adaptive immune cells\(^{15}\). CD45\(^+\) cells including innate immune cells such as Langerhans cells, dermal dendritic cells, macrophages, and eosinophils and adaptive immune cells such as dendritic epidermal \(\gamma\delta\) T cells (\(\gamma\delta\) T cell receptor (TCR)\(^{hi/bh}\)) were restricted to the treatment site (local), and upon subsequent wounding, sensitized of inflammation-experienced skin to wounding. When IMQ-treated skin at the zenith of inflammation (day 6) and after inflammation-experienced EpSCs was secondarily dependent on inflammation-experienced skin still closed wounds approximately twice as fast as naive skin counterparts. Moreover, ROR\(\gamma\) null mice, which mount an inflammatory response to IMQ despite the absence of B and T cells, also exhibited faster wound closure after inflammation compared to naive skin (Fig. 2f–h and Extended Data Fig. 3f–i).

**EpSC memory at the chromatin level**

Memory of previous exposure to inflammatory stimuli has long been thought to be exclusive to immune cells. We posited that the heightened responsiveness of post-inflamed EpSCs to tissue damage may involve chromatin dynamics analogous to those recently described for innate immune memory\(^{17}\). To this end, we purified epidermal EpSCs from IMQ-treated skin at the zenith of inflammation (day 6) and after their return to normal homeostasis (days 30 and 180) (Extended Data Fig. 4a–c). We then used assay for transposase accessible chromatin with high-throughput sequencing (ATAC–seq)\(^{18}\). Agnostic to specific types of epigenetic modifications, this strategy enables global identification of accessible chromatin states.

Independent biological replicates showed strong correlation \((R^2 \geq 0.97)\). ATAC–seq signals were enriched at transcription start sites (TSSs) and distal regulatory element CCCTC-binding factor
Figure 2 | Resident skin macrophages and T cells are dispensable for enhanced wound closure after inflammation. a, Epidermal hyperthickening is confined to the initial site of inflammation (n = 3, ≥3 images per animal). **P = 0.0019, ***P = 0.0009. b, Wound closure at day 30 is accelerated only at sites of previous IMQ treatment (n = 12). ***P < 0.0001. c, Clodronate liposome-mediated resident macrophage depletion before wounding does not alter the wound repair advantage after inflammation (flow cytometry n = 2; wound rate n ≥ 4). *P = 0.0419 (vehicle control; veh-ctrl), *P = 0.0266 (clodronate). d, DETC, dendritic epidermal γδ T cells; RORC+ cell populations: dermal γδ TCRαβ T cells, DN, double negative (αβ and γδ TCRαβ); TC17, IL-17-producing cytotoxic T cells; TH17, IL-17-producing T helper cells. e, RORC+ T cells (white arrows) are increased at day 30 post-inflammation (n ≥ 3, 3 images per animal). **P = 0.0056. Experiments performed with Rorc-eGFP mice. Lines denote dermo-epidermal border, asterisk denotes autofluorescence, and yellow boxes denote magnified areas in the adjacent panels. f, Wounds heal faster in post-inflamed skin despite the ablation of skin RORC+ cells (flow cytometry, n = 4, ***P = 0.0008). Schematic of RORC+ T cell depletion and wound repair using Rosa-LSL–iDTR (control) and Rorc-cre;Rosa-LSL–iDTR (Rorc-DTR) mice, which activate the diphtheria toxin receptor (DTR) from the Rosa26 locus only in RORC+ cells, enabling their selective ablation (n = 5, ***P = 0.0053 (Rorc-DTR)). g, h, Rag2-null mice mount an IMQ response (n = 3, 3 images per animal) (g) and display accelerated wound healing (h) at 30 days after inflammation (n = 5, **P = 0.0136). Rate calculated from wound area. a, Extended Data Fig. 3a; c, see Source Data; g, Extended Data Fig. 3b, h, Extended Data Fig. 3i. Data are mean ± s.e.m. Scale bars are 200 μm (a), 50 μm (e), and 100 μm for D6 Ctrl, D6 IMQ, D30 Ctrl and 50 μm for D30 IMQ (g). n denotes the number of biologically independent animals. Experiments replicated at least twice, and significance was determined using a two-tailed t-test (95% confidence).

Inflammation–sensing chromatin elements

Our data thus far suggest that inflammation-activated transcription factors facilitate the initial opening of certain chromatin domains, which then bind epidermal transcription factors and other chromatin modifiers present in both inflammatory/stress and post-inflamed/homeostatic states. The model further predicts that domains maintained after inflammation should serve as accessible platforms for accelerated re-activation upon a secondary assault (Fig. 4a).

We tested two important facets of this model. First, we interrogated the ability of these persisting chromatin peaks to function as inflammation-sensing elements. Using our EpSC-specific in utero lentiviral delivery method22 (Methods), we engineered mice containing the Pgk-H2B-RFP and eGFP transgenes driven by one of several accessible genomic regions sustained post-inflammation. By contrast with Pgk-H2B-RFP, expressed throughout virally transduced skin epithelium, the eGFP reporters were not activated until IMQ was administered, underscoring their potential to act as sensors of tissue damage (Fig. 4b).

If the persisting open chromatin domains after inflammation are physiologically relevant to the functional EpSC memory, then they should participate in the accelerated wound-repair response that we observe in inflammation–experienced mice. To test this possibility, we wounded the skin of day 30 post-inflamed mice, and 12 h later we evaluated the transcriptional response of EpSCs within an approximately 0.5 mm² radius of the wound edge. In parallel, we transcriptionally profiled the epidermal EpSCs from control and IMQ-treated skins at days 6 and 30 after inflammation.

In line with the epidermal pathology, EpSCs from day 6 IMQ-treated skin displayed notable changes in the transcriptome relative to control counterparts. Changes correlated well with genes featuring newly acquired ATAC–seq peaks (Fig. 4c and Extended Data Fig. 5a–c). Although only a few of these genes were still transcribed at day 30 (false discovery rate (FDR) < 0.05), this picture changed rapidly...
**Figure 4** EpSC memory encodes inflammatory sensors that rapidly reactivate to enhance secondary wound responses. **a** Model of EpSC inflammatory memory. InfTFs, inflammation-induced transcription factors. **b** Persisting accessible chromatin domains induced by inflammation can drive inflammation-specific eGFP reporter activity in EpSCs in vivo (n = 2). Schematic of in vivo lentiviral transduction of skin epithelium. *Pkh-H2B-RFP* expression marks transduced EpSCs. Lines denote the dermo-epidermal border. Scale bars, 50 µm. **c** Differences between transcriptomes of day 6 inflamed or day 30 post-inflamed EpSCs, with or without wounding, relative to respective control EpSCs. Shown are MA plots (log ratio versus mean average); the numbers of significant differentially expressed transcripts (FDR < 0.05) are stated and depicted by red dots (n = 4 pooled mice per group, in duplicate). **d** Of the 140 transcripts upregulated rapidly after wounding of post-inflamed versus control skin (grey), 73 (dark grey) were encoded by genes (red) associated with chromatin-accessible domains that were unique to post-inflamed EpSCs (random permutation, P < 10^{-4}). **e** Ingenuity pathways analysis of upregulated transcripts in post-inflamed 12 hour wound-edge EpSCs relative to control cells (n = 4, P values determined by right-tailed Fisher’s exact test). **f** Increased Aim2 transcription associated with inflammation, memory and rapid wound response in post-inflamed skin, post-wounding (PW) (n ≥ 2, two-tailed t-test, day 6 and 12 h post-wounding, ***P < 0.0001; day 180, ****P = 0.0001). **g** Aim2-null mice do not show enhanced wound healing after inflammation (n ≥ 2, two-tailed t-test, ****P = 0.0002). KO, knockout. **h** Overexpression (OE) of epithelial Aim2 is sufficient to augment wound healing in naive mice (n = 2). Data are mean ± s.e.m. Rate calculated from wound area: **h** Extended Data Fig. 9c. **i** Extended Data Fig. 9f. TRE, tetracycline response element. n denotes the number of biologically independent animals. Experiments replicated at least twice.
upon wounding (Fig. 4c, d). Of the 140 genes upregulated within 12 h after injury, 73 (52%) were associated with ATAC-seq peaks that were acquired during and sustained after IMQ treatment (FDR < 0.05). Given that wound re-epithelialization did not peak until day 5 after wounding, this early activation of genes associated with inflammation-experienced chromatin accessible regions was notable.

**Downstream effectors of EpSC memory**

To understand how these ‘inflammatory memory’ chromatin elements might confer a wound repair advantage, we first performed pathway analysis of the rapid-response transcripts (adjusted P value < 0.05) of the genes that contain these chromatin elements. Within the top terms was ‘inflammasome signalling’ (Fig. 4e). This included not only AIM2, previously implicated in skin disease and cancer, but also other downstream components of the AIM2 inflammasome. AIM2 was particularly intriguing in that its transcript was sustained in EpSCs at days 30 and 180 after inflammation, and also increased after wounding (Fig. 4c, f). Consistent with these findings, repeated our experiments with and wound edge EpSCs exhibited minimal and equivalent levels of cell β triggered after infection or tissue damage.

In the intestine, AIM2 suppresses stem-cell proliferation by indirectly inhibiting phosphorylated AKT (Ser473) and equivalent numbers of proliferating EpSCs to directly inhibiting phosphorylated AKT (Ser473) (Extended Data Fig. 6a). Altogether, these data underscore a role for AIM2 as a key mediator of wound repair advantage in post-inflamed EpSCs.

A central downstream effector of AIM2 is caspase-1 (CASP1). CASP1 transcription and activity were initiated quickly within 12 h of injury in post-inflamed samples relative to naive skin (Fig. 5a). Moreover, when the activity of CASP1 was blocked by the inhibitor Ac-YVAD-cmk, rates of wound healing were unaffected in naive skin, and reduced to naive levels in inflammation-experienced skin (Fig. 5b and Extended Data Fig. 6c). This was not attributable to CASP1-dependent pyroptosis, as both control and post-inflamed wound edge EpSCs exhibited minimal and equivalent levels of cell death (Extended Data Fig. 6d).

CASP1 promotes the maturation and secretion of the pro-inflammatory cytokines IL-18 and IL-1β. Indeed, post-inflamed skin displayed increases in IL18 transcription and in the levels of IL-18 and IL-1β proteins compared with naive skin (Fig. 5c). To determine whether AIM2 acts through cytokine production to accelerate wound repair, we repeated our experiments with IL18- and IL1r1-null mice. Although IL-18 was dispensable, without IL-1R1, which is required for IL-1β signalling, inflammation-experienced skin failed to enhance wound repair (Fig. 5d, e and Extended Data Fig. 6e, f). Consistent with these findings, the addition of recombinant IL-1β, but not IL-18 (50 ng ml⁻¹), accelerates the outgrowth of K14⁺ keratinocytes in day 5 ex vivo skin explants. Dotted and red lines mark outgrowth border and distance, respectively (n = 3; 3 technical replicates per mouse; P = 0.0128). Scale bar, 500 μm.

Intraperitoneal (i.p.) treatment with anti-IL-1R1 reverses the wound repair advantage conferred by overexpression of epidermal Aim2 in naive mice (n = 3; **P = 0.0055 (control IgG); ***P = 0.0007 (anti-IL-1R1)). Summary of the downstream AIM2 effectors in post-inflamed skin. ASC, apoptosis-associated speck-like protein containing a CARD. Rate constant (day⁻¹) 0.0629 ± 0.0202).

**Figure 5** Dissecting the downstream effectors of the AIM2 inflammasome in enhancing wound re-epithelialization of inflammation-experienced skin. a, Increased levels of the Casp1 transcript (**P = 0.0025) and CASP1 activity (**P = 0.0014) in day 30 post-inflamed skin, 12 h after wounding (n = 3). b, Schematic depicts the experiment. Ac-YVAD-cmk reduces CASP1 activity (left) and the wound repair rate (right) after inflammation to the levels of control wounds (CASP1 activity n = 2, wound repair n = 5; **P = 0.0018). c, Increased levels of the Il18 transcript (n = 3; **P = 0.0014), IL-18 protein (n = 4; **P = 0.0202) and IL-1β protein (n = 3; ***P = 0.0073) in post-inflamed skin after wounding, but not of the Il1b transcript (n = 2). d, Enhanced injury response of post-inflamed skin in the absence of Il1b (n = 3, *P = 0.0146 (WT); **P = 0.0019 (KO)). e, The absence of IL-1β signalling, achieved in Il1r1-null mice, abrogates the enhanced injury response of post-inflamed skin (n = 4; *P = 0.0155 (WT); **P = 0.0086 (KO)). f, Recombinant IL-1β, but not IL-18 (50 ng ml⁻¹), accelerates the outgrowth of K14⁺ keratinocytes in day 5 ex vivo skin explants. Dotted and red lines mark outgrowth border and distance, respectively (n = 3; 3 technical replicates per mouse, *P = 0.0128). Scale bar, 500 μm.

**Discussion**

Tissue adaptation to inflammation has been attributed to innate memory within macrophages and natural killer cells, and to persisting tissue-resident memory T cells. It was therefore surprising to find that inflammatory memory is also an intrinsic feature of long-lived EpSCs, enabling them to respond more rapidly to a secondary assault. Analysis of the myriad of potential contributing factors offers areas for future research. For instance, although the overall numbers of basal skin cells remained unchanged, distinct pools of EpSC that do not participate in naive skin responses might become sensitized to do so as a result of inflammation. Our current study builds on a pre-existing mechanistic model for memory T cells and trained innate immune cells, namely that EpSCs ‘remember’ a primary inflammatory stimulus by maintaining certain changes to their chromosomal
landscape that were induced at the time of assault. We show that these changes endow EpSCs with the ability to accelerate their response to subsequent stressors.

Most chromatin changes maintained in EpSCs after IMQ treatment were not accompanied by sustained transcription of their associated genes, nor did inflammation-induced transcription factors remain active. Because inflammation was necessary to render these chromatin domains accessible and yet not to maintain them once opened, the evidence indicates that EpSC transcription factors and other chromatin modifiers present in normal homeostasis can propagate these chromatin sites made accessible during inflammation.

Although the underlying mechanisms are complex, our reporter analyses indicate that sustained open-chromatin domains possess the information needed to sense tissue damage. Moreover, by remaining open long after the inflammation resolves, these sensors seem to have functional relevance in a secondary assault. Consistent with this notion, more than half of the genes induced early and selectively at the wound edge of inflammation-experienced skin were associated with these sustained open-chromatin domains.

The inflammasome pathway was featured prominently among these rapid responders. Our gain- and loss-of-function studies with AIM2, CASP1, IL-1/IL-1R1 underscore the importance of this pathway in conferring a wound repair advantage to inflammation-experienced EpSCs. Notably, intestinal epithelial cells can also sense tissue damage and they express AIM228, raising the tantalizing possibility that EpSCs in other tissues possess a similar AIM2 inflammasome-mediated memory of a primary assault.

As beneficial as it may seem to retain inflammatory memory in order to hasten barrier restoration the next time a breach occurs, heightened sensitivity to tissue damage may not always be a blessing. Genetic alterations that mobilize stem cells more rapidly are often associated not only with accelerated wound repair, but also with increased susceptibility to cancer30. Indeed, individuals with gain-of-function mutations in inflammasome components can show increased risk of epithelial cancers31,32. Our findings suggest that the inflammation-experienced memory in EpSCs may also underlie the recurrent skin inflammation displayed by patients with autoimmune disorders such as psoriasis and atopic dermatitis. Overall, the inflammation-induced rewiring of EpSCs that we report is likely to have major implications for future therapeutic approaches aimed at enhancing adaptive features and counteracting maladaptive ones.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions S.N., S.B.L. and E.F. conceptualized the study, designed the experiment, and wrote the manuscript. S.N. and S.B.L. performed all animal, flow cytometry, microscopy, and genomic experiments. N.G. and A.S. analysed the ATAC-seq and RNA-seq datasets. K.A. performed qPCR validations, cell culture experiments and cloned the overexpression construct. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations. Correspondence and requests for materials should be addressed to E.F. (fuchslb@rockefeller.edu) or S.N. (snak@rockefeller.edu).

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Animals. The following mouse strains were purchased from The Jackson Laboratory: C57BL/6J, B6.FVB-Tg(Rorc-Cre)1Litt/J (Rorc-Cre), C57BL/6-Gt(Rosa26)Sor1m1(EGFP)Avai/J, B6-Rosa26DiTR33, B6.129X1-Gt(Rosa26)Sor1m1(EGFP)Avai/J, B6.129P2-Aim2Gt(Scg5)Byg/J, B6.129S7-Il1r1tm1Imx/J, and B6.129P2-Ii18tm1Aki/J. Tg(Rorc-gFP)Ebe mice were a gift from G. Eberle. Tg(ly6-Cre)Hag mice were a gift from I. Sanchez-Garcia. Krt14-creER and Krt14-rtTA mice were previously generated in the Fuchs laboratory. Krt10-creER transgenic mice were generated by retrieving the mouse Krt10 promoter region (8,416 bp fragment upstream of the Krt10 start codon) from bacterial artificial chromatin DNA (CHORI clone RP23-336D20) and then inserting it upstream of the β-globin intron-cre-ER-polαy, analogous to our Krt14-creER transgen. All keratin promoter-driven Cre Er mice were then each crossed to Rosa26-Flox-Stop-Flox-YFP mice73. All animal studies were approved by the Institutional Animal Care and Use Committee. Mice were bred and maintained under specific-pathogen-free conditions at the AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International)-accredited Comparative Bioscience Center at the Rockefeller University and housed in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals. When possible, preliminary experiments were performed to determine requirements for sample size, taking into account resources available and ethical, reductionist animal use. Animal studies were not performed in a blinded fashion. Animals were assigned randomly to experimental groups. The number of animals shown in each figure is indicated in the legends as n = x mice per group.

Skin inflammation models. The dorsal skin of 8-week-old mice in the telogen (resting) phase of the hair cycle were shaved with clippers and then subjected to topical application or treatment of the skin as below.

IMQ: mice were treated with either ~1 mg cm−2 skin of 5% IMQ cream (Periget) from Farming Mac Laboratories (Pharmaceutical Specialties Inc.) for 6 consecutive days as previously described74. Epidermal abrasion wounds: mice were treated with depilatory agent Nair (Church & Dwight). A 2.5 cm2 area was then wounded shallowly with a rotary drill (Model 520 Dremel) as previously described75 to remove the epidermis and induce re-epithelialization of the wounded skin.

TPA: mice were topically treated with 40 nM TPA (Sigma-Aldrich) in acetone per 3 cm2 skin area or with acetone alone daily for 5 consecutive days10. MC903: mice were treated with 2 nM vitamin D analogue MC903 (Funck Biotechnologie) in ethanol per 3 cm2 skin area of with ethanol alone for 14 consecutive days, as previously described76.

Fungal infection: mice were infected with 106 C. albicans cells (ATCC6801) as previously described12.

Lineage tracing. At 8 weeks of age during the telogen (resting) phase of the hair cycle, mice were treated with 10 μg ml−1 of 4-hydroxytamoxifen in corn oil (Sigma-Aldrich) by intraperitoneal injection for 3 consecutive days at the following doses: 10 μg 4-hydroxytamoxifen for Krt14-creER/RosaYFP mice, and 100 μg 4-hydroxytamoxifen for Krt10-creER/RosaYFP mice. Two days after the last tamoxifen treatment, the dorsal skin of a cohort of mice was analysed for YFP expression by flow cytometry and immunofluorescence (day 0 time point).

For confirmation of YFP expression, the remaining cohort mice were treated with the aforementioned IMQ regimen and analysed at days 6, 30 and 180 after treatment. EdU pulse. For 5′-ethynyl-2′-deoxyuridine (EdU) pulse experiments, mice were injected intraperitoneally (50 μg g−1) (Sigma-Aldrich) at specified intervals (typically 1–4 h) before analysis.

Punch biopsy and splint wounds. The dorsal skin of mice was shaved at indicated time points after IMQ treatment. After visually confirming that hair follicles in the shaved area were in the telogen (resting) phase of the hair cycle, 6 mm biopsy punches (Millex) were used to make full-thickness wounds. Wounds were assessed then surgically with an engineer’s caliper daily. 3 mm wounds were splinted with 8 mm silicone splints as previously described14. Wound closure was assessed macroscopically with an engineer’s caliper daily. Wound area was calculated by applying the area of an ellipse (π × r1 × r2) to the two diameter measurements on the y axis, and x axis of the wound. One, two- and three-phase decay model of curve fitting was performed for wound healing assays. The two- and three-phase decay analyses showed minimal contributions from second (k20 = 0.00015; post-inflammation = 0.0017) phase and generated an ‘ambiguous fit’ error indicating wide confidence intervals and that the software (PRISM GraphPad) is unable to find a unique curve to fit the data. Therefore, wound healing rate constants were calculated using a one-phase decay model (PRISM GraphPad).

Diptheria toxin administration. Mice were intra-peritoneally injected with 200 ng of diptheria toxin (Sigma Aldrich) twice daily for 5 consecutive days. Mice were maintained on a 12:12 h light/dark cycle and given unrestricted access to water and food.

Supplementation in drinking water throughout the course of treatment and subsequent wounding experiment.

Caspase inhibitor administration. Mice were intra-peritoneally injected with 0.6 mM Ac-YVAD-cmk (Cayman Chemicals) 9.8 h before wounding and then daily for the first 5 days of the wound response.

Clodronate liposome administration. Mice were intra-peritoneally injected with either 200 μl clodronate or control liposomes (http://www.clodronateliposomes.org) 8 days before wounding, and then every 2 days until wounding.

In utero lentiviral transduction. Transductions were achieved by in utero injection of lentivirus into the amniotic sacs of embryonic day (E) 9.5 mice23. At E9.5, the surface ectoderm exists as a single layer of unspecialized K14+ EpSC progenitors, which become stably transduced by lentiviral within 24 h. High lentiviral titres enable highly efficient and selective transduction of the entire embryonic skin epithelium, without affecting other skin cell types. In utero injections are non-invasive and do not alter embryonic development or elicit inflammatory responses in the skin. The DNA carried by the lentivirus is stably propagated into adulthood within the skin epithelium, including the epidermis and hair follicles. In utero lentiviral deliveries were used for all ATAC–seq peak reporters and for delivery of TRE-Attn2 expression.

Doxycycline feed administration. Mice transduced with TRE-Attn2 lentivirus were placed on 2 mg kg−1 doxycycline feed (Biovet) at postnatal day 53, and maintained on a doxycycline diet throughout the course of wound repair.

Cell isolation and tissue processing. Keratinocyte isolation was adapted from a previously described protocol36. In brief, dorsal skin was shaved and digested using either 0.25% trypsin/EDTA (Gibco) or collagenase (Sigma) to obtain a single-cell suspension. Immune cells from 1 cm2 pieces of skin were isolated after digestion with liberase (Roche) based on a adapted protocol36. To isolate wound edge epithelial stem cells, 0.5 mm2 skin adjacent to wound was excised and digested in collagenase.

In vitro explant migration assay. The in vitro explant migration assay was performed as previously described39. 2 mm punch biopsies (Millex) were taken from the back skin after hair deplucking. The skin was adhered to a fibronectin-coated plate with matrigel (Corning) and cultured in 300 μM Ca2++ keratinocyte growth media made as previously described39. Explants supplemented with recombinant IL-18 and IL-1α (Peprotech) received 50 ng ml−1 of the cytokine daily. Outgrowth of K14+ cells from explants was imaged at indicated time points using the 10× objective of a Nikon Eclipse TS100 microscope equipped with an Exfo X-Cite Series 120 and a Hamamatsu ORCA-ER Digital Camera. Images were analysed with ImageJ software.

Flow cytometry and cell sorting. Female mice were used for sorting experiments at all time points and conditions to obtain maximal cell numbers. Single-cell suspensions were stained with antibodies (Extended Data Table 1) at predetermined concentrations in a 100 μl staining buffer (PBS containing 5% FBS and 1% HEPES) per 106 cells. Stained cells were re-suspended in 4′,6-diamidino-2-phenylindole (DAPI) in FACS buffer (Sigma-Aldrich) before analysis. Data were acquired on LSRII Analyzers (BD Biosciences) and then analysed with FlowJo program. FACS was conducted using Aria Cell Sorters (BD Biosciences) into either staining buffer or Trizol LS (Invitrogen).

Immunofluorescence and image analysis. Immunofluorescence staining protocols were adapted from previously described methods36. In brief, tissue was fixed in 4% paraformaldehyde in PBS for 20 min at room temperature or 4 h at 4°C. Tissue was washed three times with PBS and switched to 20% sucrose overnight and subsequently washed. Tissue was then embedded in OCT (Tissue Tek), frozen and cryosectioned (14–20 μm). Sections were permeabilized, blocked and stained with anti-C17 (DAP1) in PBS containing fluorescent conjugated antibody. Nuclei were stained using DAPI. EdU and TUNEL Click-It reaction were performed according to manufacturer’s directions (Life Technologies). For pSTAT3 immunofluorescence, sections were fixed in methanol for 20 min at −20°C before primary and secondary antibody labelling. For a complete list of antibodies, see Extended Data Table 2. Migrating epithelial tongue was determined by measuring the length of K14+ integrin α5+ cells. The percentage of wound-edge proliferating EpSCs was determined by counting EdU+ K14+ cells per total number of K17+ cells (Fig. 1e and Extended Data Fig. 2d). Data were analysed using ImageJ Software.

Confocal microscopy and image processing. Images were acquired with an OLYMPUS FV1000 microscope equipped with a Hamamatsu ORCA-ER camera and an ApoTome 2 (Carl Zeiss) slide. Tiled and stitched images of sagittal sections were collected using a 20× or 40× objective, controlled by Zen software (Carl Zeiss). Maximal projection Z-stacks were presented and co-localizations were interpreted only in single Z-stacks. Z-stacks were projected.
using ImageJ software. RGB images were assembled in Adobe Illustrator CC 2015.3.

**Histology.** Skin tissue was fixed in PBS containing 10% formalin, paraffin embedded, sectioned (0.8 mm) and stained with haematoxylin and eosin by Histowiz Inc. Stained slides were scanned at 40× magnification using Aperio AT2. Slides were visualized and epidermal thickness was analysed using Aperio Image Scope software.

**Gross images.** Animals were imaged using a Leica DFC310 FX fitted with a Schott Fostec Aace fibric optical light source and a Leica microscope video lens objective. 0.63× number 1044736.

**RNA purification, quantitative PCR and RNA sequencing.** Quantitative PCR (qPCR): Individual animals were used for qPCR experiments. Total RNA was purified from either whole skin biopsies, flash frozen and then homogenized with a Bessman Tissue Pulverizer (SpectrumTM) or FACs-purified keratinocyte populations using Direct-zol RNA MiniPrep kit (Zymo Research) as per manufacturer's instructions. Equal amounts of RNA were reverse transcribed using the superscript VILO cDNA synthesis kit (Invitrogen). CDNAS for each sample were normalized to equal amounts using primers against Actb. XpressRef Universal Total RNA (Qiangen) was used as a negative control to assess FACs population purity. For a complete list of qPCR primers, see Extended Data Table 1.

**RNA sequencing (RNA-seq):** 4 animals were pooled per condition in duplicate, for each RNA-seq experiment. Total RNA was isolated from FACs-purified keratinocyte populations using Direct-zol RNA MiniPrep kit (Zymo Research) as per manufacturer's instructions. Quality of the RNA for sequencing was determined using Agilent 2100 Bioanalyser; all samples used had RNA integrin numbers (RIN) > 9. Poly-A selection and library preparation using Illumina TrueSeq mRNA sample preparation kit and sequencing on Illumina HiSeq 2500 or HiSeq 4000 machines was performed by Weil Cornell Medical College Genomic Core facility. Fifty-base pair single-end FASTQ sequences were aligned to the mouse genome (GRCm38/mm10) using STAR\(^\text{R}\), and transcripts were annotated using Gencode release 9. Differential gene expression analysis was performed using DESeq2 package\(^\text{42}\) using the gene counts output from STAR read aligner\(^\text{42}\).

Ingenuity pathways analyses were performed on differentially expressed genes with a Bessman Tissue Pulverizer (SpectrumTM) or FACS-purified keratinocyte populations using Direct-zol RNA MiniPrep kit (Zymo Research) as per manufacturer's instructions. Quality of the RNA for sequencing was determined using Agilent 2100 Bioanalyser; all samples used had RNA integrin numbers (RIN) > 9. Poly-A selection and library preparation using Illumina TrueSeq mRNA sample preparation kit and sequencing on Illumina HiSeq 2500 or HiSeq 4000 machines was performed by Weil Cornell Medical College Genomic Core facility. Fifty-base pair single-end FASTQ sequences were aligned to the mouse genome (GRCm38/mm10) using STAR\(^\text{R}\), and transcripts were annotated using Gencode release 9. Differential gene expression analysis was performed using DESeq2 package\(^\text{42}\) using the gene counts output from STAR read aligner\(^\text{42}\).

Ingenuity pathways analyses were performed on differentially expressed genes 12 h after wounding. Abbreviated pathways are presented in Fig. 4e. Complete terms are as follows: granulocyte adhesion and diapedesis; inflammasome pathway signalling; graft-versus-host disease signalling; LXR/RXR activation; role of osteoblasts, osteoclasts and chondrocytes in rheumatoid arthritis; TREM1 signalling; regulation of cytokine production in intestinal epithelial cells by IL-17A/F; hepatic cholestasis; allograft rejection signalling; and Cdc2 signalling.

**ATAC–seq.** The ATAC–seq assay was performed on 100,000 FACs-purified cells as previously described\(^\text{18,44}\). In brief, cells were lysed in ATAC lysis buffer for 5 min and then transposed with TN5 transposase (Illumina) for 30 min. Samples were barcoded and the sequencing library was prepared according to manufacturer's guidelines (Illumina) and sequenced on an Illumina HiSeq 2500.

For sequencing data analysis 50-bp paired-end FASTQs were aligned to the mouse genome (GRCm38/mm10) as previously described\(^\text{45}\). Correlation where signal intensity did not meet the threshold (FDR < 0.01) for statistical significance over background were considered closed. \(^{\text{R}}\text{x}\)-score transformations were performed to normalize data across various samples and time points. ATAC signals per base pair over the mouse genome (GRCm38/mm10), excluding the mitochondrial and Y chromosome, were scored and then averaged over 500-bp non-overlapping genomic windows. The background signal was then filtered out by excluding genomic windows that averaged less than 1 in any replicates. Data were clustered using Cluster 3.0 and visualized using GENE-E software\(^\text{46}\). Significantly different windows were assayed between samples by t-test (P < 0.05).

Sequencing data tracks were presented using University of California Santa Cruz genome browser\(^\text{47}\). Unique peaks at from day 6 and 30 IMQ-treated EpSCs, and overlapping peaking from day 6 and 30 IMQ-treated EpSCs were subjected to ontology analysis using Genomic Regions Enrichment of Annotation Tool (GREAT)\(^\text{10}\) with the whole mouse genome (GRCm38/mm10) as the background. MANTHEUS analysis was used to compare peak-associated genes with known pathways. Abbreviated terms are presented in Fig. 3d. Complete terms are as follows: apoptosis signalling pathway; PDGF signalling pathway; histamine H1 receptor-mediated signalling pathway; Notch signalling pathway; thyrotropin-releasing hormone receptor signalling pathway; oxidative stress response; oxytocin receptor-mediated signalling pathway; muscarinic acetylcholine receptor 1/3 signalling pathway; interleukin signalling pathway; Ras pathway; angiostatin II signalling through G proteins and β-arrestin; and PI3 kinase pathway. Motif analysis of these regions was performed using HOMER software\(^\text{12}\). Selected ontologies and MOTIFS are displayed. Values below the false positive range (1 × 10\(^{-10}\) to 1 × 10\(^{-21}\)) calculated based on cumulative binomial distributions by this algorithm were considered enriched.

**Peak reporters.** Genomic regions with enriched signal in post-inflamed EpSCs associated with Aim2 (peak1–chr1: 173,420,163–173,420,883; peak 2–chr1: 173,422,670–173,423,721) Arm66 (chr8:70,221,668–70,222,912), Aooah (chr13: 20,935,623–20,936,830), and Cotll (chr8: 119,810,704–119,812,768) (Fig. 4c) were PCR-amplified from keratinocytes genomic DNA and cloned into a pLKO-PGK-H2B-mRFP1-ʼPeakʼ-min-SV40-EGFP vector\(^\text{42}\). After sequence verification, individual plasmids were packaged into a lentivirus injected into utero into the amniotic sacs of E9.5 C57BL/6 embryos, as described earlier. Mice were imaged using a Leica DFC310 FX fitted with a Schott ACFP filter. Aim2 delivery was induced by qPCR 7 days after doxycycline administration to activate rtTA, which in turn binds to the TRE element. CAPS1 fluorometric assay and cytokine ELISAs. Tissue lysates from 0.5 mm\(^2\) wound edge skin 12 h after injury were prepared by freeze thawing and then dissociating tissue with a tissue lyricer (Qagen). Lysates were assayed for CAPS1 activity and cytokine levels. CAPS1/ICE fluorometric assay (R&D) and IL-1β (eBioscience) and IL-18 (MBL international) ELISAs were performed according to manufacturer's instructions. Tissue caspase activity is reported as units based on the recombinant human CAPS1 (Sigma–Aldrich) standard. All assays were normalized to total protein levels in lysate as measured by Pierce BCA Protein Assay Kit (ThermoFisher).

**Statistics.** Data are mean ± s.e.m. or mean ± 95% confidence interval. Group sizes were determined on the basis of the results of preliminary experiments. Mice were assigned at random to groups. Experiments were not performed in a blinded fashion. Statistical significance for all wound-healing studies was determined using the two-tailed unpainted Student's t-test, with a 95% confidence interval under the assumption of normality. Within each group, there was an estimate of variation, and the variance between groups was similar. Statistical significance of ATAC–seq peak and peak-gene comparisons was calculated using either a Mann–Whitney test or random permutation. Significant enrichment of transcription factor motifs was determined by cumulative binomial distributions. Significance of ingenuity pathways analysis was determined by right-tailed Fisher's exact test. Statistical analysis was calculated using Prism software (GraphPad), DESeq2, ingenuity pathways analysis or in R.

**Data availability.** All datasets generated and/or analysed during the current study are published in this published article or the accompanying Source Data or Supplementary Information files, or are available from the corresponding authors upon reasonable request. Genomic datasets generated during and/or analysed during the current study are available in the Gene Expression Omnibus (GEO) repository under accession GSE92967.

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Extended Data Figure 1 | Lineage tracing of skin stem cells and progeny during and after acute skin inflammation. a, Epifluorescence images (left) and corresponding quantification (right) of TUNEL + basal cells at day 6 of IMQ treatment (or vehicle control) and 30 days after treatment (n = 7; ****P < 0.0001). b, Schematic, immunofluorescence images and quantification of tamoxifen (TAM)-induced (corn oil control) RosaYFP reporter lineage tracing analysis with Krt14-creER (expressed by K14 + EpSCs) and Krt10-creER (expressed by K10 + terminally differentiating cells) (n = 4; all time points P > 0.05). Plots depict percentage of YFP + cells relative to pre-IMQ (day 0) baselines (corresponding flow cytometric plots shown in c and e). Arrows denote YFP + cells. c, Lineage tracing of Krt14-creER; RosaYFP at indicated times. Left, flow cytometric analysis of integrin α6 + Sca1 + CD34 – YFP + epidermal keratinocytes. Right, immunofluorescence of tamoxifen-activated EpSCs, lineage traced by YFP + to include progeny (n = 3). d, Flow cytometry of Krt14-creER; Rosa YFP cells from the skin epidermis of animals that were lineage traced starting from IMQ treatment and analysed at day 180 (n = 2). e, Analysis of skin from Krt10-creER; RosaYFP mice that were lineage traced at the beginning of IMQ or control treatment (n = 3). Left, flow cytometric analysis of side YFP + cells. Right, representative immunofluorescence images. Scale bars, 50 μm. Dotted lines demarcate the dermo-epidermal border. Arrows denote YFP + keratinocytes. Data are mean ± s.e.m. n denotes the number of biologically independent animals per group. Significance for all plots was determined using two-tailed t-test at 95% confidence interval. All experiments have been replicated at least twice.
Extended Data Figure 2 | Enhanced wound repair in post-inflamed epidermis. a, One-phase decay modelling of wound repair in day 30 inflammation-experienced or vehicle-treated control mice (see Fig. 1b). Note, relative to two- or three-phase decay models (not shown), the data (shown at right) best fit the one-phase decay model, and this was therefore used for all subsequent wound repair data. b, Temporal wound closure analysis overlaid with one-phase decay analysis at day 180 after inflammation (n = 3). c, Temporal wound closure analysis overlaid with one-phase decay analysis of skin at day 30 after treatment with a variety of different inflammation-inducing agents: MC903, TPA, epidermal abrasion (wound), or fungal infection (C. albicans) (n = 4). See Fig. 1c for rate constants. d, Immunofluorescence images of wound edge labelled with the following antibodies: anti-EdU to mark proliferating cells, anti-K17 to mark wound-sensitized keratinocytes, integrin α5 to mark the migrating wound tongue that re-epithelializes the wound bed (w.b.), and K14, which marks the epidermal progenitors, expanded at the wound site. Vertical dotted lines denote the initial wound edge; arrows mark the edge of the extended epithelial tongue (n = 3). Scale bars, 100 μm. See Fig. 1f for quantifications. e, Representative images of silicone splinted 3 mm full thickness wounds from day 30 control and post-inflamed animals (n = 4). Scale bars, 3 mm. See Fig. 1f for quantifications. f, Migration assays were performed on skin explants (see Fig. 1g), in the presence or absence of mitomycin C for 5 days under conditions that quantitatively abrogate keratinocyte cell proliferation. Note that epidermal migration rates are similar, irrespective of whether cell proliferation was impaired (n = 3, 3 technical replicates per animal; P > 0.05, two-tailed t-test). Data are mean ± s.e.m. For individual data points in b and c, see Source Data. n denotes the number of biologically independent animals per group. All experiments have been replicated at least twice.
Extended Data Figure 3 | The wound-healing advantage conferred to EpSCs is confined to the site of inflammation and occurs even when skin RORC+ cells are ablated. a, Temporal wound closure analysis overlaid with one-phase decay analysis of inflammation-experienced and vehicle control skins, comparing the wound closure rates at sites distal and local to the topical application. Wound healing was initiated at day 30 after IMQ treatment, a time at which morphological signs of epidermal homeostasis were restored (n = 3). Plot display data combined from 3 independent experiments; see rate constants in Fig. 2b. b, c, Flow cytometric analysis of total immune cells (CD45+) from 3 independent experiments; see rate constants in Fig. 2b. The proportion of γδ TCR, αβ TCR+ and double-negative (DN; innate lymphoid) cells within the RORC(GFP)+ gate are shown adjacent to the quantification of total GFP+ cells. d, Depletion of RORC+ T cells does not result in a compensatory increase in other skin T cell populations (n = 2). g, Immunofluorescence of skin sections showing effective diphtheria toxin (DT)-mediated ablation of all CD3+ RORC+ cells (yellow) in Rorc-DTR mice (n = 3), which activate DTR from the Rosa26 locus only in RORC+ cells. h, Wounds heal faster in post-inflamed skin despite ablation of skin RORC+ cells (n = 3). For corresponding rate constants in e–g, see Fig. 2f, i. Despite the absence of T and B cells, Rag2-null mice still mount a response to IMQ and display an accelerated wound-healing response after returning to homeostasis at day 30 post-inflammation (n = 3). For rate constants, see Fig. 2h. Scale bars, 100 μm (d) and 50 μm (g). Data are mean ± s.e.m. For individual data points in a, b and i, see Source Data. n denotes the number of biologically independent animals per group. Significance for all plots was determined using a two-tailed t-test at 95% confidence interval. All experiments have been replicated at least twice.

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Extended Data Figure 4 | Analysis of accessible chromatin in EpSCs during and after inflammation. a, Immunofluorescence analysis of basal EpSC-specific markers in inflamed and control skin. Scale bar, 100 µm. b, FACS strategy for isolation of EpSCs (integrin α6[^6]β1[^1]), with exclusion of CD45[^4], CD31[^3], CD117[^1] and CD140[^0] non-epidermal cells, as well as dead (DAPI[^5]~) doublets (side-scatter width (SSC-W[^high]) and forward-scatter width (FSC-W[^high])). c, qPCR validation of EpSC purity (left) using total mouse RNA as a control (right). d, Density plots depicting enrichment of ATAC–seq signals at TSSs ± 3 kb and around CTCF factor-binding sites. The x axis depicts respective distance ± 1 kb from each of these domains.

e, Distribution of ATAC–seq peaks within defined genomic regions. UTR, untranslated regions of predicted mRNAs. f, Genomic browser shots of peaks enriched in EpSC-specific genes Klf5[^5] and Krt14[^14] and unaffected by IMQ. Arrows denote direction of transcription. g, Top, absolute numbers of ATAC–seq peaks from day 6 inflamed, day 30/180 post-inflamed and control EpSCs. Bottom, numbers and frequencies of ATAC–seq peaks that are shared in day 6 inflamed and either day 30 or day 180 post-inflamed EpSCs. h, Transcription factor motif enrichment (cumulative binomial distributions, P < 10^{-15}) within the ATAC–seq peaks of day 6 inflamed EpSCs (n ≥ 3). For further details, see Fig. 3a–e. n denotes the number of biologically independent animals per group. All experiments have been replicated at least twice.

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Extended Data Figure 5 | Enrichment of inflammasome transcripts in wound-edge EpSCs of skin exposed to inflammation. a, b, FACS strategy and qPCR verification of wound-edge EpSC purity from skin that was treated with either IMQ or vehicle and then allowed to return to homeostasis before wounding at day 30 (n = 3 pooled mice per group). Relevant antibodies and exclusion of dead cells and doublets are described in Extended Data Fig. 4. c, Matched ATAC–seq and RNA-seq analysis reveal that 91% of differentially expressed genes in day 6 inflamed versus control EpSCs are associated with newly acquired ATAC–seq peaks. For further data, see Fig. 4c–e. d, Sustained Aim2 transcription at day 30 post-inflammation in mice depleted of RORC+ cells (n = 3). For further data, see Fig. 4f. (Two-tailed t-test with 95% confidence interval, **P = 0.0028). e, Aim2-knockout mice do not show enhanced wound healing after inflammation (n = 3). For further data, see Fig. 4g. f, Aim2 induction in EpSCs is sufficient to augment wound healing in naive mice (n = 4). For further data, see Fig. 4h. For individual data points in e and f, see Source Data. Data are mean ± s.e.m. n denotes the number of biologically independent animals per group. Experiments in a–e have been replicated at least twice, experiment in f was performed once.
Extended Data Figure 6 | Dissecting the AIM2 downstream effectors.

a. Model depicting possible effectors downstream of AIM2.

b. AKT signalling

c. Cell Death (TUNEL)

d-e. The CASP1 inhibitor Ac-YVAD-cmk reduces re-epithelialization rates of wounded, post-inflamed skin compared with the rates of naive, vehicle control skin (n = 3). See Fig. 5b for schematic and further data.

e. The absence of IL18 does not hamper the enhanced injury response of post-inflamed skin (n = 3). For further data, see Fig. 5d.

f. The absence of IL-1β signalling, achieved in Il1r1-null mice, abrogates the enhanced injury response of post-inflamed skin (n = 4). For further data, see Fig. 5c.

g. Anti-IL-1R1 treatment reverses the wound repair advantage conferred by epidermal Aim2 overexpression in naive mice (n = 4). For further data, see Fig. 5g.

For individual data points in d–g, see Source Data. Scale bars, 100 μm (b) and 50 μm (c). n = 3. Data are mean ± s.e.m. n denotes the number of biologically independent animals per group. Experiments in a–f have been replicated at least twice, experiment in g was performed once.
Extended Data Table 1  |  List of qPCR primers

| Gene Name | Forward Primer | Reverse Primer |
|-----------|----------------|----------------|
| β-actin   | ccaaccgtgaagaagatgacc | accagaggcatagggaca |
| Aim2      | caggcaatgtgcaatcgagag | cgccctcaaaagattttcact |
| Casp1     | cccactgtgataggggtgac | gcataagttactaagaatgacgactga |
| Il1β      | agttgacggacctccccaaaaag | agctggatgtccatcatcagg |
| Il18      | caaaccctcctccaatctctcct | tccctgaatgtgacgcaaga |
| Trp63     | cactctccacgcctcca | gcaccacctttgcaagaacact |
| Klf5      | gattcacaacccaaaaatttcctg | ctttgtagaatgcagctgaact |
| Lhx2      | cagccttgccgcaaaagacc | taaaggttgccgcaaatcgaact |
| Cd34      | gaaccgtgctcgtgtagag | tccaccatctccgtgtaataat |
| Sox10     | atgtgcgatgggaacccaga | gtgttggggttggtggag |
| Pdgfra    | aagacctgggcaaggaacg | gacctgtcgtcgtgagcact |
| Ptprc(CD45) | ttcagaaaatgaacagctgaaca | ccaacagtcatcctcaggatgtaa |
| Vcam1     | tggtgaataatactgtaaccc | gaccagtgaagttgaccc |
| Runx1     | ctccgtgctaccacctcact | atgcaggtgaccagagtgc |
| Lef1      | ctgaatccccccccatctctac | tgggataaagagctgaccc |
| Antibodies                                      | Source           | Identifier       |
|------------------------------------------------|------------------|------------------|
| Chicken Anti-Mouse GFP                         | Abcam            | Cat# ab13970     |
| Rabbit Anti-Mouse Keratin 14                   | Fuchs Lab        | N/A              |
| Rabbit Anti-Mouse Keratin 10                   | Fuchs Lab        | N/A              |
| Rabbit Anti-Mouse Keratin 24                   | Fuchs Lab        | N/A              |
| Rabbit Anti-Mouse Keratin 17                   | Fuchs Lab        | N/A              |
| Guinea Pig Anti-Mouse Keratin 5                | Fuchs Lab        | N/A              |
| Rat Anti-Mouse MERTK clone: DS5MMER PE         | eBioscience      | Cat# 12-5751-80  |
| Rabbit Anti-Mouse p-Akt(S473) clone: D9E       | Cell Signaling   | Cat# 4060S       |
| Rabbit Anti-Mouse RFP                          | MBL              | Cat# PM005       |
| Rat Anti-Mouse CD49e (integrin-a5) clone: 5H10-27 | BD Pharmingen    | Cat# 553319     |
| Rat Anti-Mouse CD3e clone: 17A2                | Biolegend        | Cat# 100212      |
| Armenian Hamster Anti-Mouse gdTCR clone: GL3  | Biolegend        | Cat# 1181011     |
| Armenian Hamster Anti-Mouse TCRb Clone: H57-597 PerCP/Cy5.5 | Biolegend | Cat# 109227     |
| Armenian Hamster Anti-Mouse gdTCR clone: GL3 AF647 | Biolegend       | Cat# 118133     |
| Anti-Mouse CD24 Clone: M1/69 PerCP/Cy5.5       | eBiosciences     | Cat# 45-0242-80  |
| Anti-Mouse CD11b-PacBlue Clone: M1/70          | Biolegend        | Cat# 101223      |
| Anti-Mouse CD64-PerCP-Cy5 Clone: X54-5/7.1    | Biolegend        | Cat# 139307      |
| Anti-Mouse CD11c-PECy7Clone: N418             | Biolegend        | Cat# 117317      |
| Anti-Mouse I-A/I-E (MHCII) PerCP/Cy5.5 Clone: 107625 | Biolegend | Cat# 107625     |
| Rat Anti-Mouse Siglec-F Clone: E50-2440 PE    | BD Pharmingen    | Cat# 552128      |
| Anti-Mouse Ly6c-FITC Clone: HK1.4             | Biolegend        | Cat# 128005      |
| Anti-Mouse CD34 eFlour 660 Clone: RAM34       | eBiosciences     | Cat# 50-0341-82  |
| Anti-Mouse Ly-6A/E (Sca-1) Clone D7 APC/Cy7   | Biolegend        | Cat# 108126      |
| Purified Anti-Human/Mouse CD49f Clone: GoH3   | Biolegend        | Cat# 313602      |
| Anti Human/Mouse Cd49f PE Clone: GoH3         | eBiosciences     | Cat# 12-0495-81  |
| APC/Cy7 Anti-Mouse CD45 Clone: 30-F11         | Biolegend        | Cat# 103116      |
| Biotin Anti-Mouse CD45 Clone: 30-F11           | Biolegend        | Cat# 103104      |
| Biotin Anti Mouse CD117 (c-kit) Clone: 2B8     | Biolegend        | Cat# 105804      |
| Biotin Anti-Mouse CD140a Clone: APA5          | Biolegend        | Cat# 135910      |
| Biotin Anti-Mouse CD31 Clone: 390             | Biolegend        | Cat# 102404      |
| Rabbit Anti-pStat3 (Tyr705) Clone: D3A7       | Cell Signaling   | Cat# 9145        |
| Anti-Mouse/Rat CD29 Clone: HMB1-1             | Biolegend        | Cat# 102214      |
| Purified Anti-Mouse/Rat CD29 Clone: HMB1-1    | Biolegend        | Cat# 102201      |
| FITC Streptavidin                              | Biolegend        | Cat# 405202      |
| Donkey Anti-Rabbit AF488 conjugated secondary | Jackson ImmunoResearch | Cat# 711-545-152 |
| Donkey Anti-Rabbit AF546, conjugated secondary | Jackson ImmunoResearch | Cat# 711-165-152 |
| Donkey Anti-Rabbit AF647, conjugated secondary | Jackson ImmunoResearch | Cat# 711-605-152 |
| Donkey Anti-Rat AF488, conjugated secondary   | Jackson ImmunoResearch | Cat# 712-545-150 |
| Donkey Anti-Rat AF647, conjugated secondary   | Jackson ImmunoResearch | Cat# 712-605-150 |
| DyLight 594 Goat Anti-Armenian Hamster AF594, conjugated secondary antibody Clone: Poly4055 | Biolegend | Cat# 405504 |
Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

- Experimental design

1. Sample size

   Describe how sample size was determined.

   Preliminary experiments were performed when possible to determine requirements for sample size, taking into account resources available and ethical, reductionist animal use.

2. Data exclusions

   Describe any data exclusions.

   No samples or animals were excluded.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   All attempts at replication were successful.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   All animal were assigned to groups randomly.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Studies were not performed in a blinded fashion.

   Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   n/a Confirmed

   - The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)

   - A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

   - A statement indicating how many times each experiment was replicated

   - The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)

   - A description of any assumptions or corrections, such as an adjustment for multiple comparisons

   - The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted

   - A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)

   - Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
### Software

7. Software

Describe the software used to analyze the data in this study.

PRISM Graphpad software was used to perform one phase decay analysis and t-tests. R Studio and accompanying analytic packages DE-seq, Model-based Analysis of ChIP-Seq 2 (MACS2) algorithm, Genomic Regions Enrichment of Annotation Tool (GREAT), and HOMER software were used to analyze sequencing data. Image J/Fiji software was used to analyze image data.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

### Materials and Reagents

8. Materials Availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

There are no restrictions on availability of materials.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Commercial and in house (Fuch lab) generated antibodies targeted were used. Antibodies were validated by flow cytometry or immunofluorescence. For complete list of antibodies see Extended Data Table 2 and Methods Section: Immunofluorescence and FACS

### Animals and Human Research Participants

11. Description of Research Animals

Provide details on animals and/or animal-derived materials used in the study.

The following mouse strains were purchased from the Jackson Laboratories: C57BL/6, B6.FVB-Tg(Rorc-Cre)1Litt/J (Rorc-Cre), C57BL/6-Gt(Rosa), 26Sortm1(HBEGF)Awaj/J B6-Rosa26iDTR, B6.129P2-Aim2Gt(CSG445)Byg/J, B6.129S7-Il1r1tm1Imx/J, and B6.129P2-il18tm1Aki/J. Tg(Rorc-EGFP)Ebe mice were a kind gift from Dr. Gerard Eberl (Institut Pasteur). Tg(Ly6a-cre)1Isig mice were a kind gift from Dr. Isidro Sanchez-Garcia. Krt14CreER7 and Krt14rtTA mice were previously generated in the Fuchs lab. Krt10CreER7 transgenic mice were generated in the Fuchs lab for this study

12. Description of Human Research Participants

Describe the covariate-relevant population characteristics of the human research participants.

N/A
Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

- **Data presentation**
  
  For all flow cytometry data, confirm that:
  
  - 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
  - 3. All plots are contour plots with outliers or pseudocolor plots.
  - 4. A numerical value for number of cells or percentage (with statistics) is provided.

- **Methodological details**

  5. Describe the sample preparation.

  Female mice were used for sorting experiments at all time points and conditions to obtain maximal cell numbers. Single cell suspensions were stained with antibodies (Extended Data Table 1) at predetermined concentrations in a 100 μl staining buffer (PBS containing 5% FBS and 1% HEPES) per 106 cells. Stained cells were re-suspended in 4’,6-diamidino-2-phenylindole (DAPI) in FACS buffer (Sigma-Aldrich) prior to analysis. Data were acquired on LSRII Analyzers (BD Biosciences) and then analyzed with FlowJo program. Fluorescence-activated cell sorting (FACS) was conducted using Aria Cell Sorters (BD Biosciences).

  6. Identify the instrument used for data collection.

  Data were acquired on LSRII Analyzers (BD Biosciences) or Aria Cell Sorters (BD Biosciences).

  7. Describe the software used to collect and analyze the flow cytometry data.

  Data were analyzed with FlowJo program.

  8. Describe the abundance of the relevant cell populations within post-sort fractions.

  Sorted samples were >98% pure.

  9. Describe the gating strategy used.

  Epidermal stem cells were gated on FSC/ SSC- area and width, live (DAPI-), lineage negative(CD45, CD31, CD117, CD140a), integrin alpha 6+ integrin beta1+, sca1+, CD34- (Extended Data Figures 4a and 5a).

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.
Author Correction: Inflammatory memory sensitizes skin epithelial stem cells to tissue damage

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Correction to: Nature https://doi.org/10.1038/nature24271, published online 18 October 2017.

In Fig. 2g of this Article, a panel was inadvertently duplicated. The 'D30 IMQ' image was a duplicate of the 'D6 Ctrl' image. Figure 2g has been corrected online to show the correct 'D30 IMQ' image (showing skin inflammation induced by the NALP3 agonist imiquimod, IMQ). The Supplementary Information to this Amendment contains the old, incorrect Fig. 2 for transparency.

Supplementary information is available for this Amendment at https://doi.org/10.1038/s41586-018-0229-5.