# Keratinocyte Integrin α3β1 Promotes Efficient Healing of Wound Epidermis

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To date, studies of the role for epidermal integrin α3β1 in cutaneous wound re-epithelialization have produced conflicting results: wound studies in skin from global α3-null neonatal mice have implicated the integrin in promoting timely wound re-epithelialization, whereas studies in adult mice with constitutive, epidermal-specific α3β1 deletion have not. The objective of this study was to utilize a model of inducible α3β1 deletion in the epidermis to clarify the role of α3β1 in the healing of adult wounds. We utilized the recently developed transgenic K14$^{Cre\text{-}ERT}$::α3$^{flx/flx}$ mice (ie, inducible α3 epidermal knockout), permitting us to delete floxed *Itga3* alleles (α3$^{flx/flx}$) from epidermis just prior to wounding with topical treatment of 4-hydroxytamoxifen. This allows for the elucidation of α3β1-dependent wound healing in adult skin, free from compensatory mechanisms that may occur after embryonic deletion of epidermal α3β1 in the widely used constitutive α3β1-knockout mouse. We found that re-epithelializing wound gaps are larger in inducible α3 epidermal knockout mice than in control mice, indicating delayed healing, and that epidermal integrin α3β1 promotes healing of wounds, at least in part by enhancing keratinocyte proliferation. This work provides essential rationale for future studies to investigate integrin α3β1 as a therapeutic target to facilitate wound healing.

**Keywords:** Integrin, Keratinocyte, Proliferation, Re-epithelialization, Wound healing

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## INTRODUCTION

After cutaneous injury, epidermal repair is rapidly triggered to restore the skin barrier because wounded skin is vulnerable to pathogens. During wound re-epithelialization, keratinocytes near the wound edge actively proliferate and migrate over the open wound to aid in closure. Therefore, aberrancies or delays in the re-epithelialization process can lead to high rates of infection as well as chronic wound formation. Increased morbidity and healthcare costs associated with age-related delays in wound healing impose a substantial financial and social burden on the United States, and this burden is exacerbated by the ever-growing increase in the elderly population (Ashcroft et al, 2002; Gosain and DiPietro, 2004; Nussbaum et al, 2018). The timely development of improved wound-healing therapies is critical and requires better understanding of the re-epithelialization process.

Integrins are the major receptors for cell adhesion and migration (Hynes, 2002). Keratinocytes express several integrins that bind extracellular matrix ligands in provisional wound matrix (Litjens et al, 2006; Watt, 2002). Although epidermis-specific deletion of the β1 integrin subunit caused severe wound-healing defects (Grose et al, 2002), knockouts of individual integrins or their ligands have had surprisingly mild effects on wound re-epithelialization, and in vivo roles for most keratinocyte integrins remain unclear (Grenache et al, 2007; Litjens et al, 2006; Sakai et al, 2001; Zweers et al, 2007). However, integrin α3β1 has emerged as a regulator of keratinocyte migration and polarization in vitro (Choma et al, 2004; Frank and Carter, 2004; Hamelers et al, 2005). α3β1 is abundant in epidermis, where its main ligand is laminin-332, and it is expressed highly during wound healing, where it appears to have very similar roles in rodents and humans (Choma et al, 2004; Frank and Carter, 2004; Kreidberg, 2000; Watt, 2002).

Because homozygosity for the global α3-null variant is lethal within hours after birth owing to lung and kidney defects (DiPersio et al, 1997; Kreidberg et al, 1996), it was not possible in early studies to characterize cutaneous wound healing in α3-null mice. Therefore, the first studies to assess the effect of α3β1 deficiency on wound healing involved isolating full-thickness skins from wild-type or α3-null neonatal mice and grafting the skins onto the backs of nude mice. Wounds of grafted α3-null skin showed significantly reduced re-epithelialization compared with wounds of wild-type skin (Reynolds et al, 2008), indicating a requirement for α3β1 that is consistent with a promigratory role for α3β1 observed in keratinocytes in vitro (Choma et al, 2004; Frank and Carter, 2004; Hamelers et al, 2005). Surprisingly, later studies utilizing adult mice with constitutive, epidermal-specific α3β1 deletion exhibited no delay in wound re-epithelialization compared with those with α3β1-expressing control mice (Margadant et al, 2009; Mitchell et al, 2009).

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*Abbreviations: 4OHT, 4-hydroxytamoxifen; iα3eKO, inducible α3 epidermal knockout*

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There are at least 3 possible explanations for these contradictory results: (i) α3β1 expression in extraepidermal cellular compartments plays a critical role in wound re-epithelialization; (ii) α3β1 is essential in wounds of perinatal skin but dispensable in wounds from adult skin; and (iii) compensatory mechanisms occur after constitutive deletion of epidermal α3β1 during development, precluding the ability to test its requirement for re-epithelialization of adult wounds. In addition, because α3β1 is important for normal development of the cutaneous basement membrane (DiPersio et al, 1997), models in which α3β1 is constitutively deleted in the epidermis cannot distinguish whether altered re-epithelialization is due to pre-existing basement membrane defects that predispose the epidermis to defective migration. Therefore, the ability to induce deletion of epidermal α3β1 in adult animals just prior to wounding is necessary to clarify the role of α3β1 in the epidermis of adult wounds to determine whether α3β1 is a therapeutic target worthy of pursuing for advancing wound care.

To this end, in this study, we utilize the recently developed transgenic K14$^{Cre\text{-}ERT}$::α3$^{flx/flx}$ mice (Longmate et al, 2024, 2021), which permit us to delete floxed *Itga3* alleles (α3$^{flx/flx}$) from the epidermis just prior to wounding through topical treatment with active 4-hydroxytamoxifen (4OHT). Young adult mice with induced α3 knockout in the epidermis—referred to as inducible α3 epidermal knockout (iα3eKO) mice in the remaining parts of this paper—were subjected to full-thickness wounds on the dorsum, and the effect of α3β1 deletion on wound gap measurement was determined 3 days after wounding. Importantly, iα3eKO mice displayed larger wound gaps, suggesting a delay in wound re-epithelialization, than vehicle-treated control mice. A similar delay was observed in splinted wounds of iα3eKO mice, indicating that this result can indeed be attributed to re-epithelialization and not purely due to wound contraction. Consistently, iα3eKO mice have fewer proliferating keratinocytes in wound-proximal hair follicle bulges. Critically, 4OHT treatment of α3$^{+/+}$ mice lacking floxed *Itga3* alleles did not cause a change in wound gap measurement, indicating that the effect seen in iα3eKO mice was in fact due to the deletion of α3β1 rather than to 4OHT treatment per se. Overall, these studies establish an important post-developmental role for keratinocyte integrin α3β1 in timely wound re-epithelialization, indicating that α3β1 should be further explored as a potential therapeutic target to facilitate closure in hard-to-heal cutaneous wounds and that α3β1 deficiency should be investigated as a causal event in re-epithelialization defects that occur in some wound pathologies.

## RESULTS

### Induced deletion of epidermal integrin α3β1 delays wound re-epithelialization

To directly test the requirement of keratinocyte α3β1 during wound re-epithelialization, we utilized young adult mice with 4OHT-inducible deletion of epidermal α3β1 (K14$^{Cre\text{-}ERT}$::α3$^{flx/flx}$), abbreviated iα3eKO (Longmate et al, 2021). Mice were pretreated topically with 4OHT or vehicle, and then full-thickness 4-mm punch wounds were made and allowed to heal for 3 days. The wounds of 4OHT-treated iα3eKO mice appeared larger in size than vehicle-treated wounds (Figure 1a), indicating reduced wound closure. Immunofluorescence staining of day-3 wounds revealed efficient deletion of α3β1 from re-epithelialized epidermis of 4OHT-treated iα3eKO wounds (Figure 1b). Consistent with the larger wound sizes (Figure 1a), 4OHT-treated iα3eKO wounds showed significantly larger gaps in the epidermis than vehicle-treated controls (Figure 1c and d), indicating a delay in wound re-epithelialization. Importantly, no differences in either α3 expression or wound gap measurement were detected between 4OHT- and vehicle-treated K14$^{Cre\text{-}ERT}$::α3$^{+/+}$ control mice (ie, expressing α3β1 in epidermis) (Figure 2), indicating that delayed wound re-epithelialization observed in iα3eKO mice was not due to 4OHT treatment, per se.

### Splinted wounds show α3β1-dependent re-epithelialization

Mice and other loose-skinned mammals have a subcutaneous muscle layer called the panniculus carnosus, which contracts after injury to aid in wound closure (Davidson, 1998; Galiano et al, 2004). Humans lack this muscle layer and rely heavily on re-epithelialization for wound closure. Therefore, we utilized a well-characterized excisional wound splinting model that resists contraction, allowing murine wounds to close by re-epithelialization (Wang et al, 2013). This mode of wound closure is more akin to human wound healing, and it allowed us to determine whether the α3β1-dependent difference that we observed in closure of unsplinted wounds can be attributed to re-epithelialization rather than to contraction.

Briefly, full-thickness 4-mm punch wounds were generated on the backs of mice that were pretreated with either 4OHT or vehicle, as earlier. Silicone splinting rings were then sutured into place surrounding the wounds, and metal washers were fixed atop (Figure 3a). After allowing wounds to heal for 3 days, the washers and splinting rings were removed, and the wounds were collected for analysis. Immunohistochemistry confirmed efficient deletion of α3β1 in 4OHT-treated skin (Figure 3b). Consistent with what we had observed in unsplinted wounds (Figure 1), splinted wounds from 4OHT-treated iα3eKO mice showed significantly larger gaps in the epidermis than those from vehicle-treated controls (Figure 3c and d), indicating a delay in wound re-epithelialization. As expected, wound gap measurements were larger in splinted wounds than in unsplinted wounds (Figure 3d vs Figure 1d), consistent with inhibited wound contraction. Overall, these results suggest that epidermal α3β1 aids in wound closure by promoting re-epithelialization.

### Proliferation is reduced in wound-proximal keratinocytes after deletion of integrin α3β1

The process of wound re-epithelialization is achieved by the coordinated migration of wound-proximal keratinocytes across the wound bed. Critically, keratinocyte proliferation at the wound margins, particularly in the stem compartments within the wound-proximal hair follicle bulges, increases the number of keratinocytes available to migrate, promoting re-epithelialization (Pastar et al, 2014). Because the same α3β1-dependent effect on wound gap was observed in both unsplinted and splinted wounds, we returned to the unsplinted wounds for the remainder of our study. Consistent with larger wound gaps in iα3eKO mice, a significant

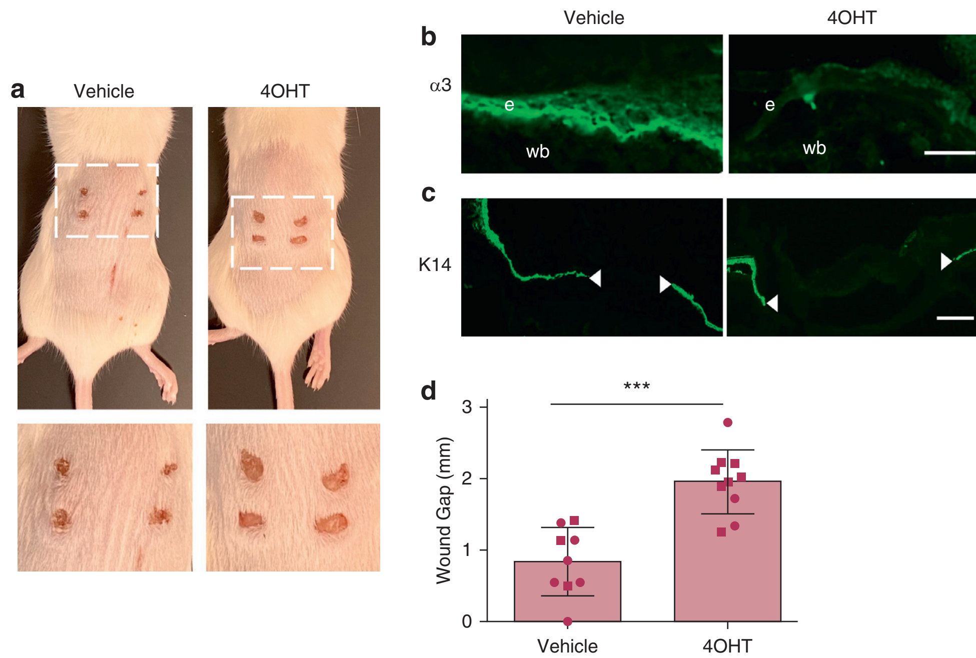


**Figure 1. Induced deletion of epidermal α3β1 results in reduced wound re-epithelialization.** K14$^{CreERT}$::α3$^{flx/flx}$ mice were pretreated 5 and 3 days prior to wounding with 4OHT or vehicle. Punch wounds (4 mm) were made on the back and allowed to heal unsplinted for 3 days. (**a**) Representative images of mouse backs 3 days after wounding; box indicates area of inset that is magnified below. (**b**–**d**) Cryosections of 3-day, partially re-epithelialized wounds were prepared. Immunofluorescence with (**b**) anti–α3 integrin subunit (bar = 100 μm) and (**c**) anti-K14 to mark the epidermis (bar = 500 μm); representative images are shown. e denotes epidermis, wb denotes wound bed, and arrowheads indicate wound edges. (**d**) Graph shows wound gap measurements between wound edges as shown in **c**. Data are shown as mean ± SEM; n ≥ 9 mice (at least 12 wounds) per treatment group. Male mice are denoted by square data points, and female mice are denoted by circle data points. Data were analyzed with Student's *t*-test. ***$P < .001$. 4OHT, 4-hydroxytamoxifen; K14, keratin 14.

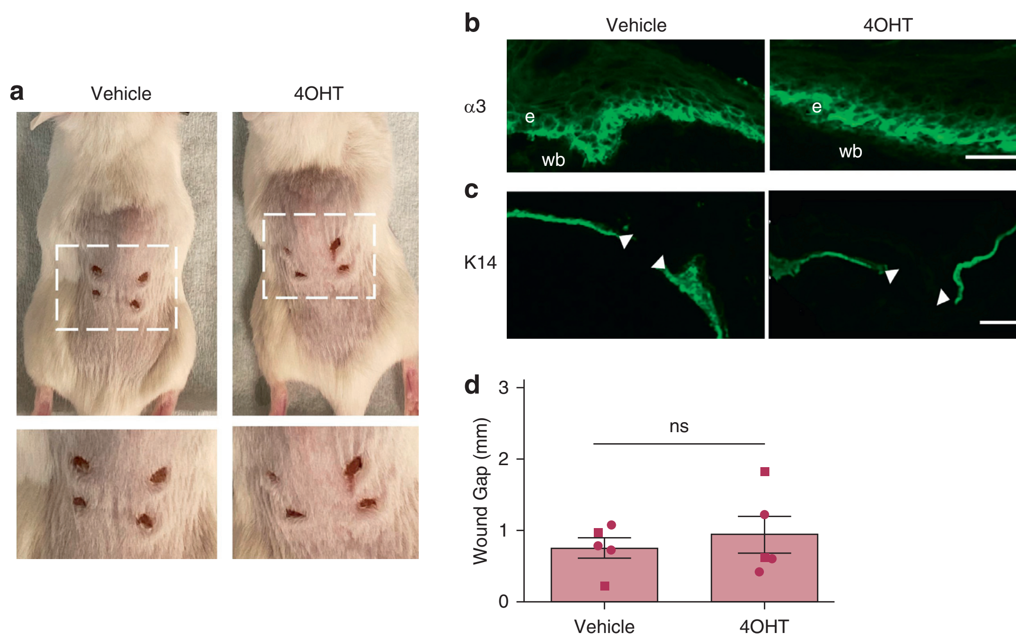


**Figure 2. Treatment with 4OHT has no effect on wound re-epithelialization in mice that express epidermal α3β1.** Control mice with wild-type *Itga3* alleles (ie, K14$^{CreERT}$::α3$^{+/+}$) were pretreated 5 and 3 days prior to wounding with 4OHT or vehicle. Punch wounds (4 mm) were made on the back and allowed to heal unsplinted for 3 days. (**a**) Representative images of mouse backs 3 days after wounding; box indicates area of inset that is magnified below. (**b**–**d**) Cryosections of 3-day, partially re-epithelialized wounds were prepared. Immunofluorescence with (**b**) anti–α3 integrin subunit (bar = 100 μm) and (**c**) anti-K14 to mark the epidermis (bar = 500 μm); representative images are shown. e denotes epidermis, wb denotes wound bed, and arrowheads indicate wound edges. (**d**) Graph shows wound gap measurements between wound edges as shown in **c**. Data are shown as mean ± SEM; n = 5 mice (5 wounds) per treatment group. Male mice are denoted by square data points, and female mice are denoted by circle data points. Data were analyzed with Student's *t*-test. 4OHT, 4-hydroxytamoxifen; K14, keratin 14; ns, not significant.

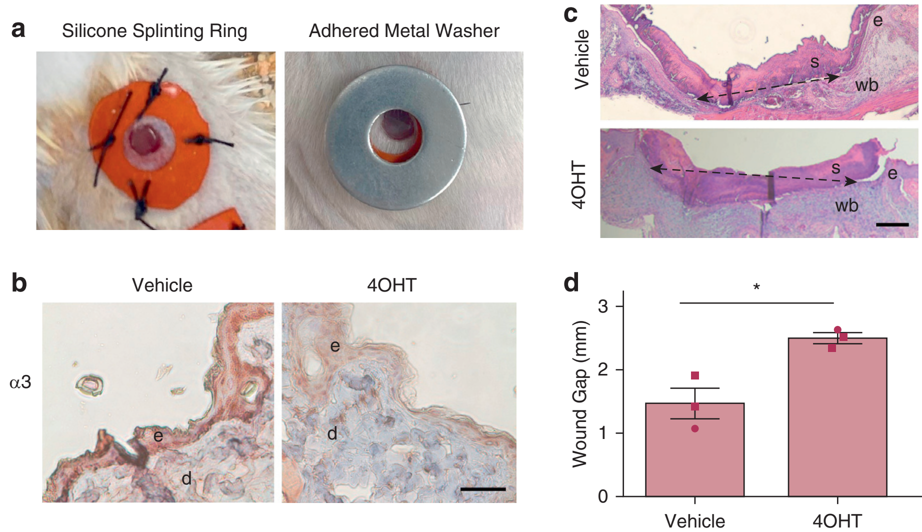


**Figure 3. Induced deletion of epidermal α3β1 results in reduced re-epithelialization of splinted wounds that cannot contract.** $K14^{CreERT}$::$α3^{flx/flx}$ mice were pretreated 5 and 3 days prior to wounding with 4OHT or vehicle as in Figure 1. (**a**) Punch wounds (4 mm) were made on the back and then splinted to prevent wound contraction by suturing on a silicone splinting ring (left) and then adhering a metal washer on top (right); representative images are shown. Wounds were allowed 3 days to heal, and then paraffin wound sections were prepared. (**b**) Immunohistochemistry for anti−α3 integrin subunit confirms deletion of α3β1 in 4OHT-treated skin; representative images are shown. e denotes the epidermis, and d denotes the dermis. Bar = 100 μm. (**c**) H&E staining shows the regenerating wound epidermis; arrowheads indicate wound edges, and dotted line indicates the wound gap. s denotes eschar, e denotes the epidermis, and wb denotes wound bed. Bar = 500 μm. (**d**) Graph shows wound gap measurements between wound edges as shown in **c**. Data are presented as mean ± SEM; n = 3 mice (3 wounds) per treatment group. Male mice are denoted by square data points, and female mice are denoted by circle data points. Data are analyzed with Student's *t*-test. $^*P < .05$. 4OHT, 4-hydroxytamoxifen.

reduction in wound-proximal keratinocyte proliferation was observed in unsplinted wounds from 4OHT-treated iα3eKO mice compared with those from vehicle-treated mice, as determined by reduced presence of the proliferation marker Ki-67 (Figure 4). This difference is attributed to deletion of keratinocyte α3β1 rather than to 4OHT treatment per se because no difference in wound-proximal keratinocyte proliferation was observed between α3β1-expressing $K14^{Cre-ERT}$::$α3^{+/+}$ mice that were treated with 4OHT and those that were treated with vehicle only (Figure 5).

## DISCUSSION

Enhanced understanding of the cell adhesion mechanisms that underlie wound re-epithelialization is critically important to develop strategies to promote timely wound closure, thereby preventing infection or chronic wound formation. Utilizing a recently developed inducible, epidermal-specific knockout mouse model, this study indicates a critical role for keratinocyte integrin α3β1 in supporting timely epidermal wound healing. Interestingly, this role for α3β1 was not revealed in previous studies that used constitutive, epidermal-specific knockout mouse models in which α3 knockout occurs during embryonic skin development (Longmate et al, 2017; Margadant et al, 2009), suggesting the existence of a compensatory mechanism(s) such as the upregulation of different integrins or other adhesion molecules. Indeed, it has been documented in higher organisms and cells from them that chronic and irreversible deletion of genes, including the gene for integrin α3, can drive compensatory adaptation (El-Brolosy et al, 2019; Kallunki et al, 2019; Kenney et al, 2021; Ma et al, 2019). However, it is important to note that Margadant et al (2009) measured re-epithelialization at wound day 3 and assessed closure at wound day 7, whereas we compared wound gap only at wound day 3 in this study, which is a limitation that prevents direct comparison between these studies.

Importantly, previous work from the DiPersio group elucidated key roles for epidermal α3β1 in the process of wound healing using the constitutive, epidermal-specific knockout model system, including maintaining basement membrane integrity and paracrine-mediated induction of angiogenesis (Longmate et al, 2014; Mitchell et al, 2009). Taken together, these studies indicate that postdevelopmental compensation for embryonic loss of α3β1 does not extend to all α3β1-dependent wound functions.

This study indicates that α3β1 supports wound re-epithelialization, at least in part, by promoting keratinocyte proliferation in the stem compartments within wound-proximal hair follicle bulges. It is also likely that α3β1 supports the directed migration of keratinocytes across the wound bed. Indeed, in vitro studies from Choma et al (2007, 2004) have showed that α3β1 directs the stabilization of a leading-edge lamellipodium in migrating keratinocytes and that this front−back polarization requires Rac1 activation. Furthermore, in vitro and in vivo studies from Longmate et al (2018, 2014) have demonstrated that epidermal α3β1 promotes stabilization of the nascent basement membrane of regenerating wounds through regulation of matricellular protein, fibulin-2, and that α3β1 regulates the proteolytic processing of the γ2 chain of laminin-332, an essential component of the cutaneous basement membrane. Such regulation may further support the appropriate balance of keratinocyte migration and adhesion that is required for proper wound re-epithelialization.

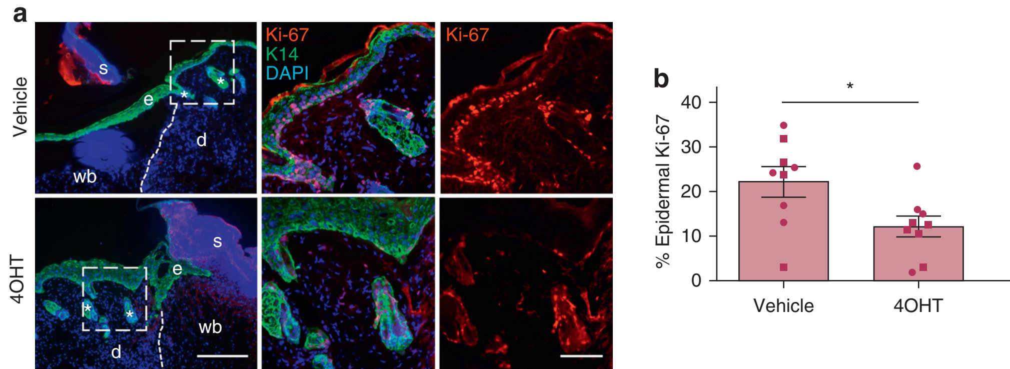


**Figure 4. Proliferation is reduced in wound-proximal keratinocytes of 4OHT-treated iα3eKO mice.** Cryosections of 3-day unsplinted wounds with wound-proximal skin from $K14^{CreERT}::\alpha3^{flx/flx}$ mice were prepared as in Figure 1. (**a**) Immunofluorescence with proliferation marker anti−Ki-67 (red), epidermal marker anti-K14 (green), and nuclear marker DAPI (blue). Representative images are shown. Left: box indicates area of inset that is magnified on right that includes wound-proximal hair follicles. s denotes eschar, e denotes the epidermis, d denotes the dermis, wb denotes the wound bed, and * denotes the hair follicle. Bar = 200 μm. Right: inset panels shown with (left) and without (right) anti-k14 and DAPI. Bar = 50 μm. (**b**) Graph shows the percentage of epidermal cells that are Ki-67 positive. Data are presented as mean ± SEM; n = 9 mice (14 wounds) per treatment group. Male mice are denoted by square data points, and female mice denoted by circle data points. Data were analyzed with Student's *t*-test. $^*P < .05$. 4OHT, 4-hydroxytamoxifen; iα3eKO, inducible α3 epidermal knockout; K14, keratin 14.

Overall, findings from this study indicate that therapeutically targeting integrin α3β1 to promote its function (perhaps through the administration of exogenous ligand) may promote the closure of hard-to-heal wounds by stimulating keratinocyte proliferation and wound re-epithelialization. Expedient re-epithelialization of wounds is critical to restore the barrier function of the skin and prevent infection. More broadly, this study implores the consideration of inducible gene-knockout models rather than models of constitutive gene knockout for investigating postdevelopmental roles of specific integrins (and perhaps of other target gene/proteins) because some roles may be concealed through the compensatory upregulation of alternative mechanisms.

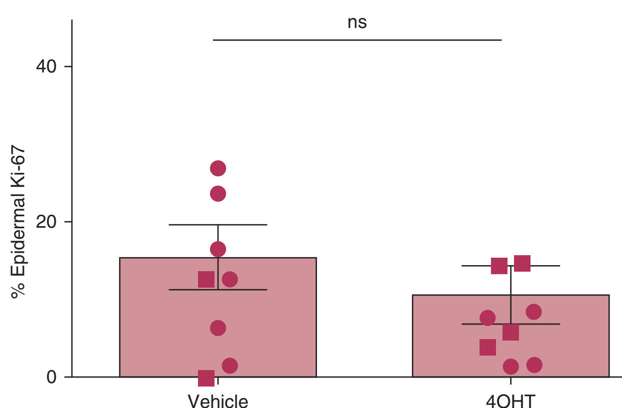


**Figure 5. Treatment with 4OHT has no effect on proliferation of wound keratinocytes in mice that express epidermal α3β1.** Cryosections of 3-day unsplinted wounds with wound-proximal skin from control $K14^{CreERT}::\alpha3^{+/+}$ mice were prepared as in Figure 2. Proliferation of wound-proximal keratinocytes was assessed by immunofluorescence with anti−Ki-67 as in Figure 4. Graph shows the percentage of epidermal cells that are Ki-67 positive. Data are mean ± SEM; n = 9 mice (9 wounds) per treatment group. Male mice are denoted by square data points, and female mice are denoted by circle data points. Data were analyzed with Student's *t*-test. 4OHT, 4-hydroxytamoxifen; ns, not significant.

## MATERIALS AND METHODS

### Murine wound healing studies

$K14^{CreERT}::\alpha3^{flx/flx}$ mice and wound-healing protocols performed in this model were previously described (Longmate et al, 2021, 2017). Briefly, 5 and 3 days prior to wounding, 1 mg of 4OHT (Caymen Chemical) in 200 μl acetone (iα3eKO) or acetone alone (vehicle control) was applied topically to shaved backs of mice aged 8−10 weeks. $K14^{Cre\text{-}ERT}::\alpha3^{+/+}$ mice lacking floxed *Itga3* alleles were used in parallel to control for off-target effects of 4OHT. On the day of wounding, mice were anaesthetized, backs were reshaved, and 4 full-thickness wounds were made on each mouse using a sterile 4-mm biopsy punch (Integra) as described (Mitchell et al, 2009). In a subset of wound experiments, we utilized a well-characterized excisional wound splinting model (Wang et al, 2013) to prevent healing by contraction. For splinted wound studies, splinting rings created in laboratory from 0.5-mm thick silicone sheets (Grace Bio-Labs) were fixed with 4 equidistant nylon sutures (Ethicon) to surround the punch wound, and then a metal washer of a similar size was adhered on top of the silicone ring with super glue (Loctite). For all studies (both splinted and unsplinted), wounds were allowed to heal for 3 days, after which, the mice were killed by carbon dioxide narcosis. Gross imaging was performed with a digital camera secured to an imaging apparatus set at a fixed and consistent distance from the specimen. Surgically excised wounds were bisected and frozen in optimal cutting temperature (Electron Microscopy Sciences) or fixed in 4% paraformaldehyde (Electron Microscopy Sciences). Male and female mice were randomized independently in roughly equal ratios. These studies were institutionally approved and conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### Histology

Frozen sections were prepared as described (Longmate et al, 2017). A total of 10-μm wound sections were rehydrated (0.02% Tween-20/PBS) for 10 minutes; fixed (4% paraformaldehyde/PBS) for 5−30 minutes; permeabilized (0.4% TritonX-100/PBS) for 5−30 minutes; blocked (0.5% BSA, 10% goat serum, 0.1% Tween-20) for 30−60 minutes or blocked (5% milk, 10% goat serum) for 60 minutes; and then stained with anti−α3 integrin subunit (1:750) (DiPersio et al, 1995), anti−keratin 14 (1:1000) (BioLegend), or anti−Ki-67 (1:200)

(BioLegend). Secondary antibodies (1:250) (Molecular Probes) were Alexa Fluor 488 goat anti-rabbit IgG or Alexa Fluor 594 goat anti-rat IgG. Sections were costained with DAPI to mark nuclei. Sections were mounted with ProLong Gold antifade mounting media (Molecular Probes).

Fixed tissue was embedded in paraffin, and 5-μm sections were prepared. Paraffin sections were stained with H&E or immunostained with anti−α3 integrin subunit (1:300) (DiPersio et al, 1995) followed by biotinylated anti-rabbit IgG (Vector) and hematoxylin (Vector) counterstain. Antigen retrieval was accomplished by incubation with antigen retrieval solution (Biogenex Laboratories) for 30 minutes at 100 °C.

### Imaging and quantitation

Micrograph imaging was performed on a Nikon Eclipse 80i microscope with a Photometrics Cool Snap ES camera or with a Spot camera (Diagnostic Instruments). Quantitative analyses were performed using NIS Elements AR 3.2 software or Fiji ImageJ. Length of wound gaps was determined by measuring a straight line between opposing migrating epidermal wound edges. To determine percentage epidermal Ki-67, wound-proximal keratin 14−positive epidermis was first selected as the region of interest. This included both the follicular and interfollicular epidermis. Ki-67−positive cell counts were measured in the keratin 14−positive region of interest, and total epidermal cell counts were determined by counting all DAPI-positive nuclei within the keratin 14−positive region of interest. Finally, the percentage of Ki-67−positive epidermal cells was calculated as the number of Ki-67−positive cells divided by the total number of epidermal cells, multiplied by 100.

### Statistical analysis

Data points represent either 1 wound from a mouse or 2 wounds from the same mouse that have been averaged. Data points are symbol coded to distinguish mouse sex, where square data points represent male mice and circle data points represent females. Data are presented as mean ± SEM. Normal distribution of data was confirmed with the Shapiro−Wilk normality test. Experimental groups were compared using Student's *t*-test, where $P < .05$ was considered significant. All data analyses were performed using GraphPad Prism, version 9.0, for Windows.

## DATA AVAILABILITY STATEMENT

Data underlying the results presented in this paper may be obtained from the corresponding author upon request.

## ETHICS STATEMENT

All animal experiments were approved by the Institutional Animal Care and Use Committee of Albany Medical College.

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## CONFLICT OF INTEREST

The authors state no conflict of interest.

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## AUTHOR CONTRIBUTIONS

Conceptualization: WML, CMD; Data Curation: SD, GAD, LW, MRD, WML; Formal Analysis: SD, GAD, WML; Funding Acquisition: WML, JML, CMD; Investigation: SD, GAD, LW, WML; Methodology: SD, GAD, LW, MRD, WML, JML, CMD; Project Administration: WML; Resources: WML, JL, CMD; Supervision: WML, CMD; Validation: SD, GAD, LW, WML; Visualization: WML, CMD; Writing - Original Draft Preparation: WML; Writing - Review and Editing: WML, JML, CMD

## DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) OR LARGE LANGUAGE MODELS (LLMs)

The author(s) did not use AI/LLM in any part of the research process and/or manuscript preparation.

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