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Authors

Dragan, Morgan
Sun, Peng
Chen, Zeyu
et al.

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Epidermis-Intrinsic Transcription Factor Ovol1 Coordinately Regulates Barrier Maintenance and Neutrophil Accumulation in Psoriasis-Like Inflammation

Morgan Dragan[1,2,3,8], **Peng Sun**[1,8], **Zeyu Chen**[1,4,5], **Xianghui Ma**[1], **Remy Vu**[1,2], **Yuling Shi**[5,6], **S. Armando Villalta**[3,7], **Xing Dai**[1,2,3]

[1]Department of Biological Chemistry, School of Medicine, University of California, Irvine, Irvine, California, USA

[2]NSF-Simons Center for Multiscale Cell Fate Research, University of California, Irvine, Irvine, California, USA

[3]Institute for Immunology, University of California, Irvine, Irvine, California, USA

[4]Department of Dermatology, Shanghai Tenth People's Hospital, School of Medicine, Tongji University, Shanghai, China

[5]Institute of Psoriasis, School of Medicine, Tongji University, Shanghai, China

[6]Department of Dermatology, Shanghai Skin Disease Hospital, School of Medicine, Tongji University, Shanghai, China

[7]Department of Physiology & Biophysics, School of Medicine, University of California, Irvine, Irvine, California, USA

[8]These authors contributed equally to this work.

Abstract

Skin epidermis constitutes the exterior barrier that protects the body from dehydration and environmental assaults. Barrier defects underlie common inflammatory skin diseases, but the molecular mechanisms that maintain barrier integrity and regulate epidermal–immune cell cross-talk in inflamed skin are not fully understood. In this study, we show that skin epithelia-specific deletion of *Ovol1*, which encodes a skin disease–linked transcriptional repressor, impairs the epidermal barrier and aggravates psoriasis-like skin inflammation in mice in part by enhancing neutrophil accumulation and abscess formation. Through molecular studies, we identify IL-33, a

Correspondence: Xing Dai, Department of Biological Chemistry, School of Medicine, University of California, Irvine, D250 Medical Sciences I, Irvine, California 92697-1700, USA. xdai@uci.edu.

AUTHOR CONTRIBUTIONS

Conceptualization: XD; Data Curation: MD, PS, ZC, XM; Formal Analysis: MD, PS, ZC, XM, RV; Funding Acquisition: XD; Investigation: MD, PS, ZC, XM; Validation: MD, PS, ZC, XM; Project Administration: XD; Supervision: XD, YS, SAV; Visualization: MD, PS, ZC, XM; Writing - Original Draft Preparation: XD, MD; Writing - Review and Editing: XD, MD, PS, ZC, XM, YS, SAV

CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.08.397.

cytokine with known pro-inflammatory and anti-inflammatory activities, and Cxcl1, a neutrophil-attracting chemokine, as potential weak and strong direct targets of Ovol1, respectively. Furthermore, we provide functional evidence that elevated *Il33* expression reduces disease severity in imiquimod-treated *Ovol1*-deficient mice, whereas persistent accumulation and epidermal migration of neutrophils exacerbate it. Collectively, our study uncovers the importance of an epidermally expressed transcription factor that regulates both the integrity of the epidermal barrier and the behavior of neutrophils in psoriasis-like inflammation.

INTRODUCTION

Skin epidermis is a self-renewing epithelium composed of stem/progenitor cells that terminally differentiate to produce stratum corneum, which constitutes a physical barrier at the skin's outmost surface against myriad external assaults (Gonzales and Fuchs, 2017). Common inflammatory skin diseases such as psoriasis and atopic dermatitis are associated with disruption of this epidermal barrier either as a trigger for disease or as a secondary consequence of immune cell dysregulation (Harden et al., 2015; Ring et al., 2012; Schmuth et al., 2015; Segre, 2006). Moreover, disease progression is facilitated by epidermal cell regulation of and response to immune cells (Kobayashi et al., 2019; Pasparakis et al., 2014; Zhang et al., 2019). However, the molecular mechanisms that regulate epidermally driven communication between epidermal and immune cells in coordination with barrier maintenance remain largely unknown.

Previously, we reported that germline deletion of *Ovol1*, which encodes a transcription factor known to regulate epidermal development (Dai et al., 1998; Lee et al., 2014; Nair et al., 2006; Teng et al., 2007), leads to exacerbated skin inflammation and epidermal hyperplasia in response to imiquimod (IMQ), an agent widely used to induce psoriasis-like symptoms in mice (Sun et al., 2021; van der Fits et al., 2009). Moreover, human *OVOL1* is upregulated in psoriatic lesions (Sun et al., 2021). Neutrophil accumulation in differentiated layers of the epidermis (Munro's abscesses) distinguishes a subset of human patients with psoriasis and is a key feature of psoriasis-like skin inflammation in mice (Chiang et al., 2019; Pinkus and Mehregan, 1966; Sumida et al., 2014; Uribe-Herranz et al., 2013). Epidermal keratinocytes (KCs) play an important role in neutrophil recruitment by secreting cytokine/chemokines such as IL-1α, CXCL1, CXCL8, and LTB_4 (Milora et al., 2015; Olaru and Jensen, 2010; Sumida et al., 2014; Sun et al., 2021). Although we found elevated levels of IL-1α in IMQ-treated *Ovol1* null epidermis (Sun et al., 2021), it remains unclear whether Ovol1 functions within the epidermis to directly regulate epidermal neutrophil cross-talk and whether it promotes barrier integrity.

In this work, we generated and analyzed mice with skin epithelia–specific knockout (SSKO) of *Ovol1* and examined their responses to IMQ. We found that epidermal loss of *Ovol1* dramatically aggravates IMQ-induced barrier disruption, epidermal hyperplasia, and neutrophil accumulation. In contrast, there was a reduction of lymphocytes, except for regulatory T cells (Tregs). We detected upregulated expression of *Il33*—the human counterpart of which is suppressed by OVOL1 overexpression (Furue et al., 2019; Tsuji et al., 2020)—in the skin of *Ovol1* SSKO mice but found IL-33 antibody neutralization to

further enhance the psoriasis-like skin phenotypes of these mice. We showed that Ovol1 protein binds weakly to the *Il33* promoter but strongly to the promoter of *Cxcl1*, and indeed *Cxcll* expression was elevated in *Ovol1*-deficient epidermis. Finally, we obtained evidence that *Ovol1* loss facilitates neutrophil accumulation near and migration through the epidermis and that neutrophil depletion partially mitigates the psoriasis-like phenotypes of *Ovol1*-deficient mice. Together, our findings uncover an epidermal-intrinsic transcription factor that both promotes barrier integrity and directly suppresses neutrophil accumulation in psoriasis-like inflammation.

RESULTS

Ovol1 acts in the epidermis to promote robust barrier maintenance and to suppress IMQ-induced epidermal hyperplasia

The skin of Ovol1$^{-/-}$ mice exhibit elevated *Il1a* expression in response to IMQ (Sun et al., 2021). Because barrier disruption increases *Il1a* transcription (Wood et al., 1992), we asked whether *Ovol1*$^{-/-}$ skin is barrier defective by performing transepidermal water loss (TEWL) measurements, a widely used method to assess epidermal barrier function (Alexander et al., 2018). During homeostasis (day 0), TEWL values were the same between *Ovol1*$^{-/-}$ and control littermates (Figure 1a and b). However, after IMQ treatment, which is known to induce transient barrier disruption (Barland et al., 2004), the *Ovol1*$^{-/-}$ mutants displayed significantly higher and longer-lasting TEWL increases than their control littermates (Figure 1a). Similarly, enhanced barrier disruption was observed in *Ovol1*$^{-/-}$ mice after tape stripping (Figure 1b).

To ask whether *Ovol1* functions within the epidermis to regulate barrier maintenance, we generated *Ovol1* SSKO (*Ovol1*$^{-/f}$; *K14-Cre*, where *Ovol1* is deleted in epithelial cells of the skin) mice in a congenic C57BL/6 (B6) background. Similar to *Ovol1*$^{-/-}$ mice, during homeostasis, TEWL values in *Ovol1* SSKO mice showed no deviation from those of their control littermates (Figure 1c). Consistently, expression of epidermal terminal differentiation markers appeared largely normal in *Ovol1* SSKO skin (Supplementary Figure S1a). On IMQ treatment, *Ovol1* SSKO mice displayed significantly more dramatic and persistent TEWL increases than their control littermates (Figure 1c). Therefore, *Ovol1* expression in the epidermis is required for maintaining a robust barrier when skin is under external physical or chemical assaults.

We also asked whether *Ovol1* suppression of IMQ-induced inflammation is intrinsic to the epidermis. *Ovol1* SSKO mice showed dramatically more severe psoriasis-like phenotypes that included erythema, scaling, and epidermal hyperplasia after 5 consecutive days of IMQ treatments than weight- and sex-matched littermate controls (Figure 1d and e). Exacerbated phenotypes of *Ovol1* SSKO mice could also be observed after only 2 consecutive days of IMQ treatments (Supplementary Figure S1 b). Interestingly, under identical IMQ treatment conditions, the psoriatic-like symptoms, including epidermal hyperplasia, of *Ovol1* SSKO mice appeared comparable with, if not more remarkable than, those of the *Ovol1*$^{-/-}$ mice, with the SSKO skin presenting finger-like projections reminiscent of the elongated rete ridges in psoriasiform hyperplasia of human patients (Murphy et al., 2007) (Figure 1e and Supplementary Figure S1c and d). Together, our findings show that *Ovol1* is required

in the epidermis to suppress IMQ-induced epidermal hyperplasia and the associated skin phenotypes.

Acute immune responses in IMQ-treated *Ovol1* SSKO skin feature increased neutrophil abundance and decreased T cell presence

Next, we performed flow cytometry to compare the immune cell compositions between IMQ-treated control and *Ovol1* SSKO mice. At 24 hours after the second IMQ treatment, we detected a ~3.7-fold increase in neutrophils ($CD45^+/Ly6G^+/CD11b^+$) in *Ovol1* SSKO skin compared with that in the control littermate skin, whereas similar numbers of total immune ($CD45^+$) cells and macrophages ($CD45^+/F4/80^+/CD11b^+/Ly6G^-$) were observed (Figure 2a and b and Supplementary Figure S2a). These data show that loss of epidermal expression of *Ovol1* results in an increased early influx of neutrophils in IMQ-treated skin.

We also examined the impact of *Ovol1* deletion on components of the adaptive immune response, which generally develops around 5–14 days after IMQ treatment (Miao et al., 2010). Specifically, we employed two consecutive IMQ applications to minimize indirect consequences from dramatic epidermal thickening and barrier disruption (Supplementary Figure S1a and b) and performed flow cytometry 6 days later. Compared with those in the control counterparts, the average numbers of both $CD45^+$ (immune) and $CD45^-$ cells were increased in *Ovol1* SSKO skin, but the differences were not statistically significant (Figure 2c and Supplementary Figure S2b and c). Importantly, the percentage of $CD3^+$ lymphocytes ($CD45^+CD3/Thy1^+$) of total immune ($CD45^+$) cells was significantly lower in *Ovol1* SSKO skin than in control skin, whereas the percentage of nonlymphocytes was significantly elevated (Figure 2d and e). A significant reduction was observed for the relative abundance of both $CD4^+$ T cells and $CD8^-CD4^-$ T cells (which likely includes γδT cells [Cai et al., 2011; Tigelaar et al., 1990]) subsets (Figure 2d and f), whereas the relative abundance of Tregs ($CD4^+Foxp3^+$) showed a trend of increase in the mutant mice (Figure 2g and Supplementary Figure S2c and d). All these differences were normalized 1 –3 months after IMQ treatment (Supplementary Figure S2e-h). Collectively, these data show that loss of epidermal *Ovol1* biases the skin's response to IMQ toward elevated neutrophil accumulation but reduced T cell abundance.

Ovol1 loss results in elevated *Il33* expression in inflamed skin, and inhibition of IL-33 signaling aggravates the IMQ-induced skin phenotypes of *Ovol1* SSKO mice

IL-33, a cytokine of the IL-1 family that is expressed in barrier tissues, has been identified as a risk factor and modulator of psoriatic inflammation (Balato et al., 2012; Duan et al., 2019; Griesenauer and Paczesny, 2017; Pichery et al., 2012). To seek the potential targets of Ovol1 in the epidermis of inflamed skin, we first considered *Il33* as a candidate because recent reports show that *OVOL1* depletion in normal human epidermal KCs induces IL33 expression (Tsuji et al., 2020). We found that both knockdown of *OVOL1* in normal human epidermal KCs and adenovirus Cre-mediated acute knockout of *Ovol1* in primary mouse KCs derived from $Ovol1^{f/f}$ mice resulted in elevated *IL33/Il33* expression (Figure 3a and Supplementary Figure S3a). Thus, similar to OVOL1 in humans, Ovol1 is capable of suppressing *Il33* expression in mouse epidermal cells.

Next, we asked whether the loss of *Ovol1* alters *Il33* expression in vivo. Interrogation of our previously published RNA-sequencing data on control and Ovol1$^{-/-}$ epidermis 6 hours after IMQ treatment (Sun et al., 2021) revealed elevated *Il33* expression in the epidermis from Ovol1$^{-/-}$ mice (×3.2, $P < 10^{-5}$). Having said this, RT-qPCR showed only a statistically nonsignificant increase for *Il33* expression in IMQ-treated *Ovol1*$^{-/-}$ epidermis, whereas the increased expression of *Il1a* was significant (Supplementary Figure S3b). We also analyzed our published single-cell RNA-sequencing data on control and *Ovol1*$^{-/-}$ mice 24 hours after IMQ treatment (Sun et al., 2021). In the inter-follicular epidermis, *Il33* was found to be predominantly expressed by basal KCs, and the number of *Il33*-expressing basal cells increased in the mutant after IMQ treatment compared with that in the control (Figure 3b and Supplementary Figure S3c-e). Moreover, control fibroblasts rarely showed detectable *Il33* expression, but fibroblasts from *Ovol1*$^{-/-}$ mice became *Il33*-positive after IMQ treatment (Figure 3c). Finally, RT-qPCR on RNAs isolated from the whole skin of control and *Ovol1* SSKO mice revealed potential increases 6 hours and 2–3 days as well as a statistically significant increase 6 days after IMQ treatment for *Il33* expression (Kakkar and Lee, 2008) (Supplementary Figure S3f). Taken together, these results suggest that epidermal loss of *Ovol1* results in mildly elevated expression of *Il33* in the epidermis but remarkably increased expression in the dermis of inflamed skin.

To investigate the effect of excessive *Il33* expression in the psoriasis-like microenvironment of IMQ-treated *Ovol1*-deficient mice, we intraperitoneally injected *Ovol1* SSKO mice with either IgG control or an IL-33-neutralizing antibody (Byrne et al., 2011; Liu et al., 2016; Ohno et al., 2011; Peng et al., 2018; Schmitz et al., 2005) before and every day succeeding two IMQ applications (Figure 3d). Interestingly, neutralization of IL-33 signaling led to more severe skin defects characterized by exacerbated erythema and scaling, enhanced epidermal barrier disruption, and apparent contraction of the IMQ-treated skin area (Figure 3e-h and Supplementary Figure S3g-i). Consistent with enhanced epidermal hyperplasia at a histological level (Figure 3i and j), Ki-67 immunostaining trended toward elevated proliferative activity in the epidermal basal layer, including cells lining the finger-like projections (Figure 3k and l). However, the expression of keratin 1 and loricrin appeared unchanged (Supplementary Figure S3j and k), suggesting that the process of epidermal terminal differentiation is not affected by IL-33 neutralization. Despite previous reports of IL-33's effects on immune cell populations such as Tregs and neutrophils in other inflammatory models (Enoksson et al., 2013; Griesenauer and Paczesny, 2017; Lan et al., 2016; Matta et al., 2014), we did not detect any statistically significant change in nonlymphocytes and various T cell populations (including Tregs) after IL-33 neutralization in our model (Supplementary Figure S3l-p). Collectively, these data suggest that the upregulated expression of *Il33* in *Ovol1* SSKO skin functions to suppress rather than enhance the IMQ-induced skin phenotypes and epidermal hyperplasia.

Identification of an *Ovol1–Cxcl1*–neutrophil regulatory axis that enhances IMQ-induced skin inflammation in *Ovol1* SSKO mice

To seek additional candidate targets that mediate the enhanced inflammation in *Ovol1*-deficient skin, we returned to the RNA-sequencing data on IMQ-treated *Ovol1*$^{-/-}$ and control epidermis (Sun et al., 2021), this time focusing on the chemokines known to be

involved in neutrophil recruitment. We found *Cxcl1* (×2.1), *Cxcl2* (×3.0), and *Cxcl3* (×3.1) to be significantly increased in IMQ-treated *Ovol1*$^{-/-}$ epidermis ($P < 10^{-6}$). RT-qPCR analysis using independent samples confirmed the increased expression of *Cxcl1* in *Ovol1*$^{-/-}$ epidermis both 6 and 24 hours after IMQ treatment, with the upregulation being more dramatic and statistically significant at 24 hours (Figure 4a and b). A possible increase in *Cxcl3* expression was also observed (Figure 4b).

Ovol1 encodes a transcriptional repressor (Nair et al., 2007, 2006). Thus, genes that were upregulated in its absence might be direct *Ovol1* targets. Indeed, the *Cxcl1* gene but not *Cxcl2/3* contains OVOL1-binding consensus motifs (CCGTTA) (Nair et al., 2007) at two different regions (R1, −3505 to −3411; R2, +1262 − +1355). To validate Ovol1 binding, we performed chromatin immunoprecipitation coupled with real-time qPCR on epidermal cells isolated from wild-type mice 2–6 hours after IMQ treatment. Chromatin immunoprecipitation coupled with real-time qPCR revealed strong Ovol1 binding to the promoter of a known Ovol1 target, *Id1* (Renaud et al., 2015), at −1073 to −997 (R1) but not at another site (R2, +1269 − +1368) (Figure 4c). Binding to another known Ovol1 target, *c*-Myc (Nair et al., 2006), was also detected. Importantly, chromatin immunoprecipitation coupled with real-time qPCR revealed Ovol1 binding to the *Cxcl1* gene at both predicted sites (Figure 4c). Weak but statistically significant Ovol1 enrichment was also detected upstream of the *Il33* promoter at a region (−1986 to −1903) that contains an Ovol1-binding consensus motif. In contrast, no enrichment was seen for *Il1a* at three different regions (R1–R3). These results suggest that *Cxcl1* is a strong candidate as a direct *Ovol1* target in skin epidermis, whereas *Il33* and *Il1a* are likely weakly or indirectly regulated by Ovol1.

The identification of *Cxcl1* as a potential Ovol1 target led us to look more closely at neutrophil dynamics in inflamed skin with *Ovol1* ablation. At 6 hours after the first IMQ application, sizable neutrophil clusters (Ly6G positive) were already formed in *Ovol1* SSKO skin near the epidermis but were lacking in control littermates (Figure 5a). In *Ovol1*$^{-/-}$ mice, neutrophils were detected in the dermis 6 hours after IMQ treatment, whereas visible large neutrophil aggregates were observed 12–72 hours after IMQ at locations near, across, and atop the epidermis (Figure 5b). At all time points examined, only scattered neutrophils were detected around the epidermis of control counterparts (Figure 5b). These data provide evidence that loss of Ovol1 in the epidermis results in increased neutrophil accumulation, trafficking, and abscess formation near the epidermis of IMQ-treated skin.

To assess the functional contribution of neutrophils to IMQ-induced skin pathology of *Ovol1*-deficient mice, we depleted neutrophils using a neutralizing antibody against Ly6G (Daley et al., 2008) (Figure 5c). Both immunostaining and histological analysis revealed a reduced presence of neutrophils in IMQ-treated *Ovol1*$^{-/-}$ skin after Ly6G antibody injection relative to that after IgG injection (Figure 5d and Supplementary Figure S4a). Epidermal thickness and the number of Ki-67–positive basal cells were not significantly different between IgG- and Ly6G antibody–injected skin at the endpoint of these experiments (day 3 after IMQ treatment) (Supplementary Figure S4b and c). However, external symptoms such as erythema and scaling/plaque formation in *Ovol1*$^{-/-}$ mice were significantly improved by Ly6G antibody injection (Figure 5e and f and Supplementary Figure S4d). These effects

show that the accumulated neutrophils in *Ovol1*-deficient skin functionally contribute to IMQ-induced erythema and scaling/plaque formation.

DISCUSSION

Our work unravels previously unknown mechanisms by which an epidermal KC-intrinsic transcription factor modulates innate and adaptive immune responses in the skin in tandem with barrier maintenance. When the skin is chemically challenged or mechanically disrupted, Ovol1 helps to maintain a functional barrier to protect from such perturbations. It likely does so by regulating the proliferation and differentiation of epidermal cells by repressing the expression of genes such as *Ovol1*, *c-Myc* (Nair et al., 2007), *Ovol2* (Teng et al., 2007), *Flg*(Tsuji et al., 2017), and *Id1* (this work). Importantly, our work identifies a role for epidermally expressed Ovol1 to directly and indirectly regulate the expression of cytokines and chemokines such as *Il1a*, *Il33*, and *Cxcl1*, thereby modulating the ensuing immune response.

Interestingly, the epidermal hyperplasia in IMQ-treated *Ovol1* SSKO mice appeared more severe than that in IMQ-treated $Ovol1^{-/-}$ mice (Sun et al., 2021). Moreover, neutrophil accumulation kinetics appears more rapid in *Ovol1* SSKO skin than in $Ovol1^{-/-}$ skin. It is possible that this is due to differential IMQ responses of B6 versus of CD1 backgrounds (Swindell et al., 2017, 2011). Alternatively, non-epidermal cells in $Ovol1^{-/-}$ mice might play a modulatory role in the IMQ responses. In human psoriatic skin, neutrophils infiltrate into the dermis from blood vessels at the early inflammatory phase and migrate into the epidermis at the chronic phase of psoriasis (Albanesi et al., 2010). Neutrophil dynamics in inflamed tissues beyond neutrophil–vasculature interactions is an emerging area of research, and swarm-like migration patterns, termed neutrophil swarming, have been described in various tissue and disease contexts (Kienle and Lämmermann, 2016). The slower neutrophil kinetics in the $Ovol1^{-/-}$ model enabled our discovery that initial dermal infiltration of neutrophils does not depend on *Ovol1*, whereas *Ovol1* loss primarily affects the accumulation of neutrophils near or at the epidermis and the migration through the epidermis to form abscesses with KCs. Our data suggest that *Ovol1* expression in the epidermis constitutes a protective mechanism that prevents epidermal-proximal neutrophil accumulation and abscess formation in inflamed skin. Combined with our previous study (Sun et al., 2021), we propose that Ovol1 does so by direct transcriptional repression of *Cxcl1* expression in KCs and by maintaining a robust barrier, which in turn suppresses KC expression of *Il1a* (Rider et al., 2011; Sawant et al., 2015; Sun et al., 2021). Although a possibility for this type of regulation was suggested (Hwang et al., 2011), to our knowledge, this study presents a previously unreported example of an epidermally expressed transcription factor modulating neutrophil behaviors in skin inflammation through direct target gene regulation.

Previous studies predominantly suggest a pro-inflammatory role of IL-33 in psoriasis (Balato et al., 2014; Duan et al., 2019; Miller et al., 2010; Sehat et al., 2018; Theoharides et al., 2010). However, it was recently reported that the introduction of recombinant IL-33 suppresses psoriatic inflammation and epidermal hyperplasia (Chen et al., 2020). Our results are consistent with this notion and suggest a protective role for IL-33 in the *Ovol1*-deficient

mouse model against IMQ responses. It is possible that this protective function only manifests when the amount of IL-33 is excessive. IL-33 is an alarmin that can function both as a traditional cytokine (through receptor signaling) and as a nuclear transcriptional regulator (Haraldsen et al., 2009). Our antibody neutralization data implicate the signaling function of IL-33 in suppressing IMQ responses when *Ovol1* and barrier are deficient. In other tissues, IL-33 can recruit a particularly suppressive form of Tregs through the ST2 receptor (Halvorsen et al., 2019; Pastille et al., 2019; Siede et al., 2016). However, despite a trending increase of Tregs in *Ovol1* SSKO mice, after neutralizing IL-33, the total number of Tregs (including the $ST2^+$ subset) did not drastically vary. In this context, the IL-33/ST2 signaling axis may alter cytokine production by Tregs or other immune cells rather than recruiting or inducing Treg proliferation (Hemmers et al., 2021). In T helper type 2 allergic response, IL-33 can induce immune cells to release secreted proteins that induce the proliferation of KCs (Ryu et al., 2015), potentially explaining the epidermal hyperplasia that we observed. Alternatively, KCs in *Ovol1*-deficient mice may respond directly to excessive IL-33 signals and hyperproliferate because ST2L and pathways such as extracellular signal–regulated kinase and c-Jun N-terminal kinase are activated by IL-33 (Du et al., 2016). Although future work outside the scope of this study is needed to characterize the downstream cellular and molecular mediators of IL-33 function, our findings identify OVOL1 as an upstream regulator that directly suppresses *Il33* expression in mice.

Collectively, this study adds Ovol1 to a growing list of skin barrier-protective transcription factors (Cangkrama et al., 2013; Gordon et al., 2014; Hwang et al., 2011; Koegel et al., 2009; Li et al., 2017; Lin et al., 2013; Segre et al., 1999) but highlights its unique role in the adult epidermis to promote barrier robustness under external challenges in coordination with fine-tuning epidermal–immune cell cross-talk. In an *Ovol1*-deficient skin microenvironment, both anti-inflammatory (*Il33*) and proinflammatory (*Cxcl1, Il1a*, neutrophils) components are upregulated and functionally contribute to skin pathology (Figure 6). These data underscore *Ovol1* function as part of a self-limiting, counter-balancing KC-intrinsic mechanism that maintains inflammation competence but at the same time keeps inflammation in check to restore tissue homeostasis. Dissecting the intricate and complex control of psoriasis-like inflammation by epidermal KCs sheds light on our understanding of psoriasis pathogenesis in human patients.

MATERIALS AND METHODS

Mice

B6N-Ovol1$^{tm1a(KOMP)Wtsi/J}$ mice, where a cassette composed of an FRT site—a *LacZ* sequence—and loxP sites are inserted into the *Ovol1* locus, were purchased from Knockout Mouse Project Re-pository of the University of California, Davis (Davis, CA). These mice were crossed with B6.Cg-Tg(ACTFLPe)9205Dym/J (Rodriguez et al., 2000) mice to generate a floxed (i.e., f) *Ovol1* allele. *Ovol1*$^{+/-}$; *K14-Cre* males were crossed with *Ovol1*$^{f/f}$ females to generate *Ovol1* SSKO (*Ovol1*$^{f/-}$; *K14-Cre*) mice, which were then analyzed along with their sex- and weight-matched control litter-mates. Genotyping primers are provided in Supplementary Table Sl. All animal studies have been approved and abide

by the regulatory guidelines of the Institutional Animal Care and Use Committee of the University of California, Irvine (Irvine, CA).

IMQ-induced psoriasis model

Mice aged 7–8 weeks received a daily topical dose of 62.5 mg 5% IMQ cream (Perrigo, Dublin, Ireland) on shaved backs for 1–5 consecutive days or as indicated. Additional details are described in the Supplementary Materials and Methods.

Tape stripping and TEWL measurement

Tape stripping was performed as described in a study by Bruhs et al. (2018). In brief, hairs were removed from the mouse back by shaving. Three days later, shaved back skin was stripped with adhesive cellophane tape 20 times. For each stripping, a fresh piece of tape was lightly pressed onto the back and gently pulled off.

TEWL was measured on shaved mouse back skin using the Delfin VapoMeter (SWL4400, Delfin, Kuopio, Finland) under basal conditions (untapped or untreated), after tape stripping, or after two-time IMQ applications. TEWL values are output as g/m^2h.

Flow cytometry

T cells were isolated as described in the study by Ali et al. (2017). Whole skin was digested in 2% collagenase, 0.5 mg/ml hyaluronidase, and 0.1 mg/ml DNase in RPMI with 1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1% penicillin-streptomycin, and 10% fetal calf serum at 37 °C for 45 minutes. Cells were filtered through 70- and 40-μm filters, rinsed in 5% fetal bovine serum/×1PBS, and stained for 10 minutes with Zombie NIR (423105; BioLegend, San Diego, CA). Cells were stained with cell surface markers and anti-mouse CD16/32 (101320; BioLegend) in FACS buffer (5% fetal bovine serum/×1PBS) on ice for 30 minutes. Cells were fixed with the Transcription Factor Staining Buffer Set (00-5523-00; eBioscience, Waltham, MA), stained for eFluor450 anti-Foxp3 (48-5773-82; eBioscience) for 1 hour at room temperature, and then analyzed on a FACS Aria Fusion. Surface markers include APC anti-CD45 (103112; BioLegend), PerCP/Cy5.5 anti-CD3 (100218; BioLegend), and anti-CD90.2 (140322; BioLegend), FITC anti-CD8a (100705; BioLegend), BV605 anti-CD4 (100548; BioLegend), and phycoerythrin anti-ST2 (145303; BioLegend). Additional details are described in the Supplementary Materials and Methods.

Chromatin immunoprecipitation coupled with real-time qPCR

The back skin of B6 mice was shaved and treated with IMQ for 2–6 hours. To separate epidermis from dermis, back skin was collected and digested with 2.5 U/ml dispase (07913; Stem Cell Technologies, Vancouver, Canada) for 1 hour at 37 °C, scraped, minced, resuspended in 5% fetal bovine serum/×1PBS, and filtered through 70-μm and 40-μm filters. Epidermal cells in single-cell suspension were then cross-linked in 1% formaldehyde, followed by chromatin immunoprecipitation assay using rabbit anti-OVOL1 antibody (Dai et al., 1998) and SimpleChIP Enzymatic Chromatin IP Kit (Agarose Beads) (9002; Cell Signaling Technology, Danvers, MA) according to manufacturer's instructions. DNA was then purified, and qPCR was performed using SYBRgreen reagent (Qiagen, Hilden, Germany) and gene-specific primers (Supplementary Table S1).

In vivo administration of IL-33-neutralizing and anti-Ly6G antibodies

For IL-33 neutralization experiments, same-sex/same-weight *Ovol1* SSKO littermates were intraperitoneally injected with 15 μg of goat anti-mouse IL-33 Affinity Purified Polyclonal antibody (AF3626; R&D Systems, Minneapolis, MN) or goat IgG (AB0108-C; R&D Systems) 30 minutes before the two IMQ applications and at the same time of the day for a total of six applications. For neutrophil depletion studies, same-sex/same-weight $Ovol1^{-/-}$ littermates were intraperitoneally injected with 500 μg rat anti-mouse Ly-6G antibody (clone 1A8, eBioscience) or rat IgG2a (clone eBR2a, eBioscience) once 24 hours before IMQ application. In separate control experiments, same-sex/same-weight wild-type littermates were also treated and analyzed. Skin was harvested either 6 days after the first IMQ treatment (IL-33 neutralizing) or 24 hours after the third IMQ treatment (for Ly6G) and was fixed in 4% paraformaldehyde for H&E staining, embedded in optimum cutting temperature, and frozen for immunostaining, or single cells were isolated for flow cytometry analysis.

Statistics and reproducibility

Nearly all experiments were performed on at least three biological replicates or repeated at least twice. The sample size and number of independent experiments are indicated in the relevant figure legends. Disease pathology scoring was performed blindly. No data were excluded. For analysis of the differences between groups, Student's unpaired two-tailed *t*-test was performed in Excel unless otherwise indicated. *P*-values of 0.05 or less were considered statistically significant and are indicated in the figures/legends. Those where *P*-values are not indicated are not statistically significant.

Additional information is provided in Supplementary Materials and Methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

No datasets were generated during this study. The analysis uses previously published datasets (Sun et al., 2021).

Abbreviations:

IMQ	imiquimod
KC	keratinocyte
SSKO	skin epithelia–specific knockout

TEWL	transepidermal water loss
Treg	regulatory T cell

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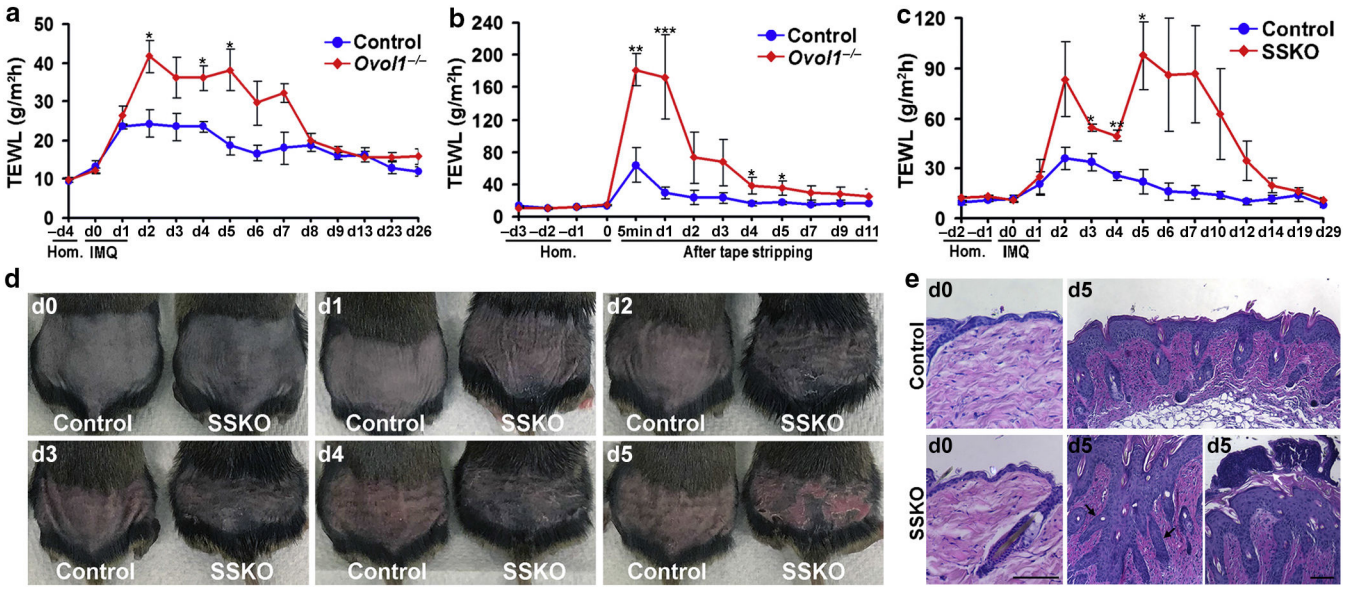

Figure 1. Loss of *Ovol1* in the epidermis aggravates IMQ-induced barrier disruption and epidermal hyperplasia.

(**a–c**) TEWL measurements of (**a, b**) Ovol1 $^{-/-}$ or (**c**) Ovol1 SSKO mice along with their control littermates after (**a, c**) IMQ treatment or (**b**) tape stripping, starting on d0, where d before perturbation represent Hom. n = 3 for control littermates in **a–c**. For mutants, (**a**) n = 5 (**b, c**) n =3. (**d**) External appearance at different d after the first IMQ application. (**e**) Skin histology on d0 and d5. Black and white arrows point to finger-like epidermal projections and neutrophil aggregates on the skin surface, respectively. Bar = 100 μm. *** $P < 0.005$; ** $P < 0.01$; * $P < 0.05$. Error bars represent mean ± SEM. d, day; Hom, homeostasis; IMQ, imiquimod; SSKO, skin epithelia–specific knockout; TEWL, transepidermal water loss.

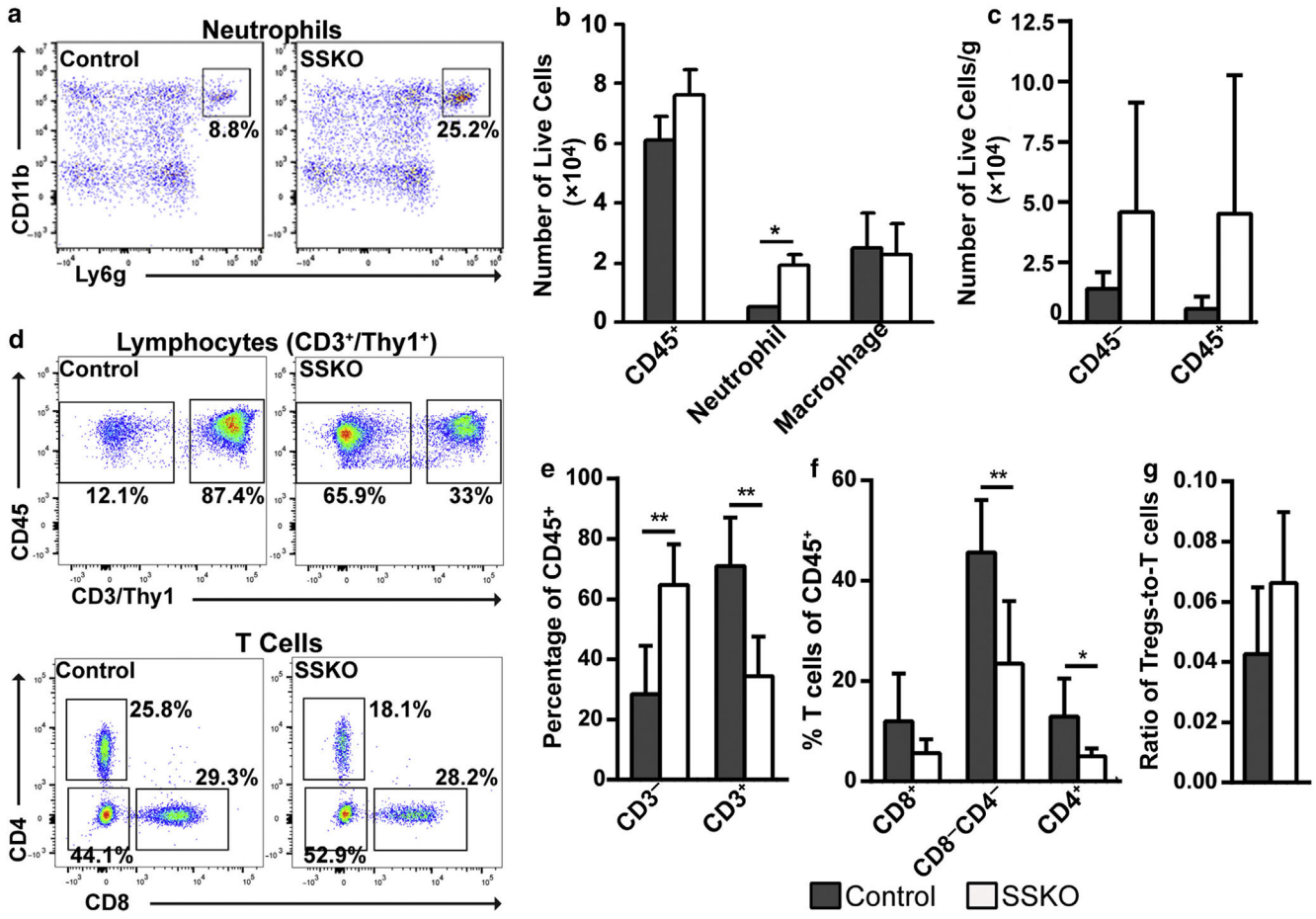

Figure 2. Immune cell profiles in IMQ-treated control and *Ovol1* SSKO skin. Shown are summaries of the flow cytometry data collected

(**a**) 24 hours and (**b–e**) 6d after the second IMQ application. Representative flow plots are shown in Supplementary Figure S2. (**a**) n = 3 pairs of *Ovol1* SSKO and control littermates and (**b–e**) n = 7 control and n = 6 mutants. ** $P < 0.01$; * $P < 0.05$. Error bars represent mean ± SD. IMQ, imiquimod; SSKO, skin epithelia–specific knockout; Treg, regulatory T cell.

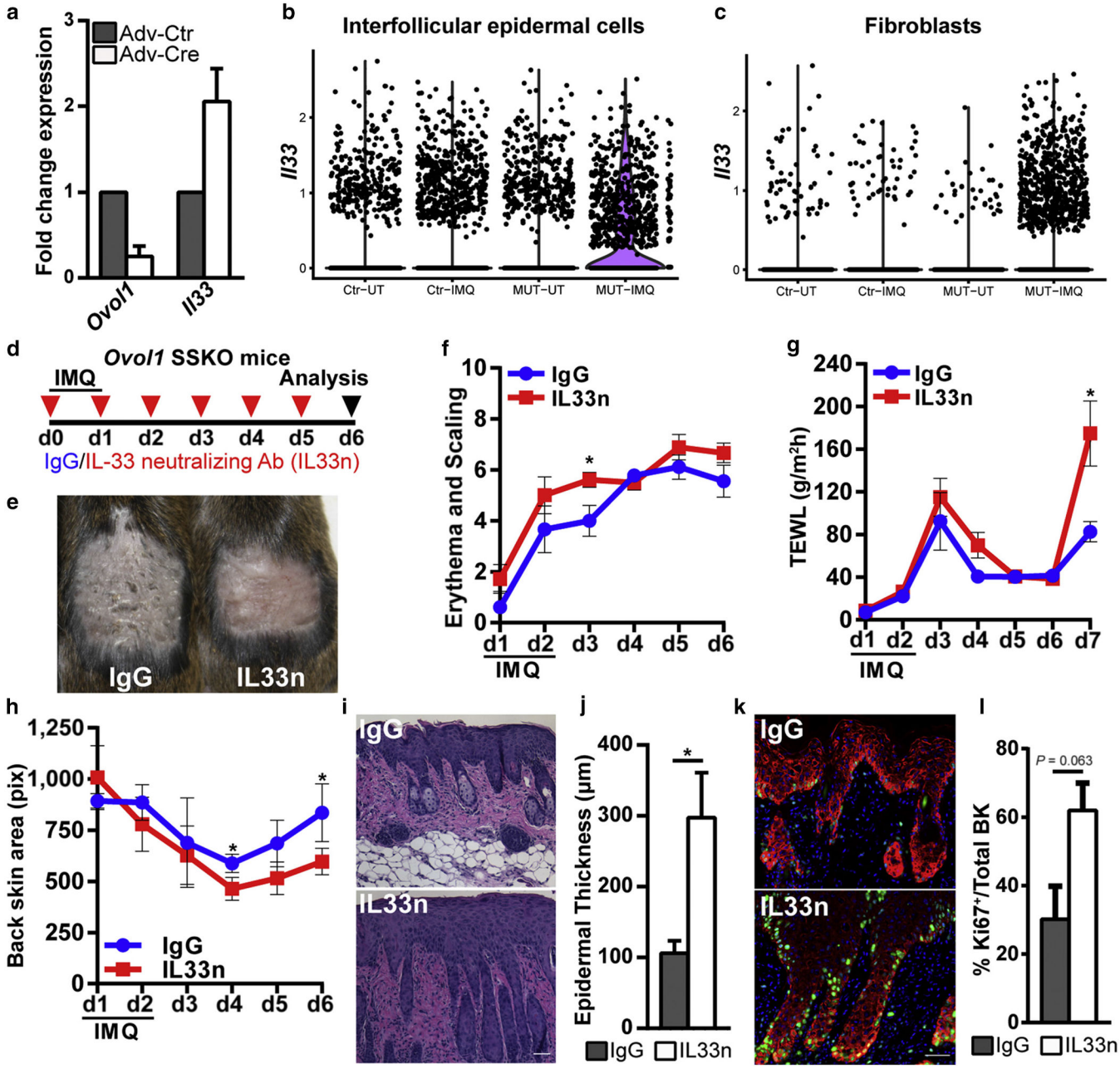

Figure 3. Expression and function of *Il33* in *Ovol1*-deficient skin.

(**a**) RT-qPCR analysis on primary mouse keratinocytes derived from *Ovol1*$^{f/f}$ mice and infected with Adv-Ctr or Adv-Cre. Results from a single experiment are shown but are representative of three independent experiments. (**b, c**) Violin plots showing the expression of *1l33* in (**b**) interfollicular epidermal cells or (**c**) fibroblasts from scRNA-seq datasets (Sun et al., 2021). (**d**) The design of experiments for IL-33–neutralizing Ab (IL33n) or IgG control on *Ovol1* SSKO mice in **e–l**. (**e**) The external appearance of the skin on d6. (**f–h**) Time courses showing (**f**) phenotype progression, (**g**) TEWL measurements, and (**h**) treated back skin area (using the images in Supplementary Figure S3e). (**i**) Skin histology and (**j**) quantification of epidermal thickness on d6. Bar = 50 μm. (**k**) Immunofluorescence

and (**l**) quantification of proliferative basal (Ki-67^{+}K14^{+}) cells on d6. n = 3 pairs of IgG- and IL33n-treated *Ovol1* SSKO mice. * $P < 0.05$. Error bars represent (**a, g, j, l**) mean ± SD and (**f, h**) SEM. Ab, antibody; Adv-Cre, Cre-expressing adenovirus; Adv-Ctr, control adenovirus; BK, basal keratinocytes; d, day; IMQ, imiquimod; K, keratin; MUT, mutant; pix, pixel; scRNA-seq, single-cell RNA sequencing; SSKO, skin epithelia–specific knockout; TEWL, transepidermal water loss; UT, untreated; Ctr, control.

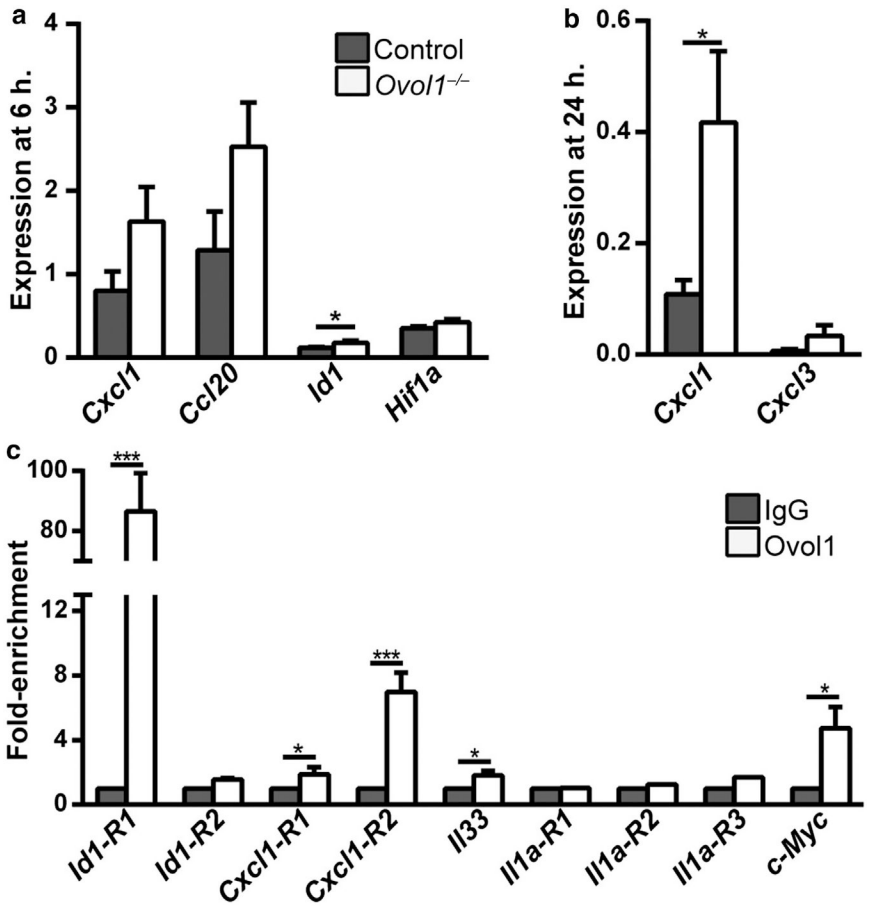

Figure 4. Molecular analysis of OVOL1 targets in the epidermis.

(**a, b**) RT-qPCR analysis at (**a**) 6 and (**b**) 24 h after IMQ treatment. n = 5 for *Ovol1*$^{-/-}$ and 4 for control littermates in **a**; note that two pairs of these mice were also used for RNA-seq analysis. n = 5 pairs in **b**. (**c**) ChIP-qPCR for the indicated genes in epidermal cells isolated from 2–6 h post-IMQ–treated adult skin. IgG control values were normalized to 1 for all. Results are summarized from one to four independent experiments. *** $P < 0.005$; * $P < 0.05$. *P*-values were calculated using a two-tailed Student's paired *t*-test. Error bars represent mean ± SEM. ChIP-qPCR, chromatin immunoprecipitation coupled with real-time qPCR; h, hour; IMQ, imiquimod; RNA-seq, RNA sequencing.

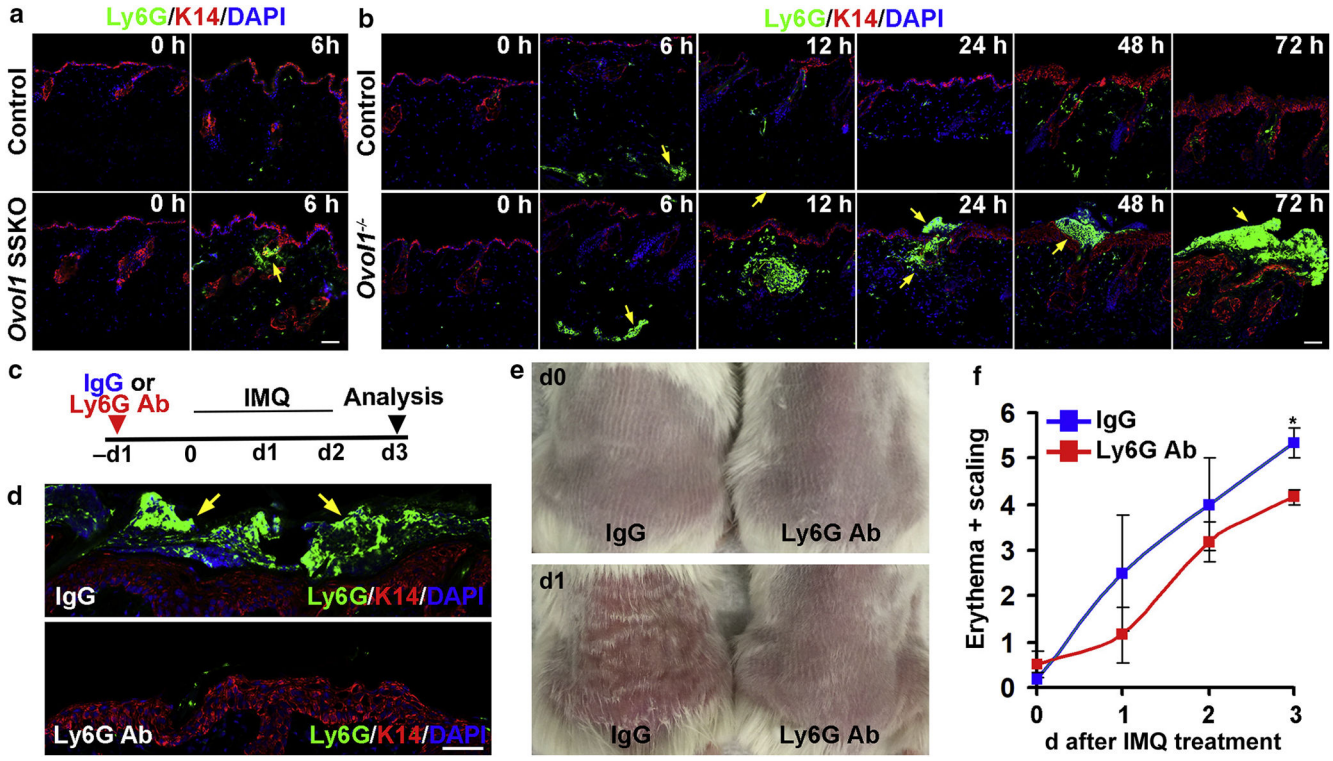

Figure 5. *Ovol1* loss alters neutrophil dynamics, and neutrophil depletion rescues psoriasis-like phenotypes in *Ovol1*$^{-/-}$ skin.

(**a, b**) Shown are representative images of immunostaining in (**a**) *Ovol1* SSKO and (**b**) Ovol1$^{-/-}$ mice. (**a, b, d**) Arrows point to neutrophils. (**c**) Design of neutrophil depletion experiments in **d–f**. IgG was used as a control. (**d**) Immunostaining on d3. (**a, b**) Bar = 50 μm and (**d**) Bar = 100 μm. (**e**) External appearance and (**f**) phenotypic score (n = 3) at the respective times. *$P < 0.05$. Error bars represent mean ± SEM. Ab, antibody; d, day; h, hour; IMQ, imiquimod; K, keratin; SSKO, skin epithelia–specific knockout.

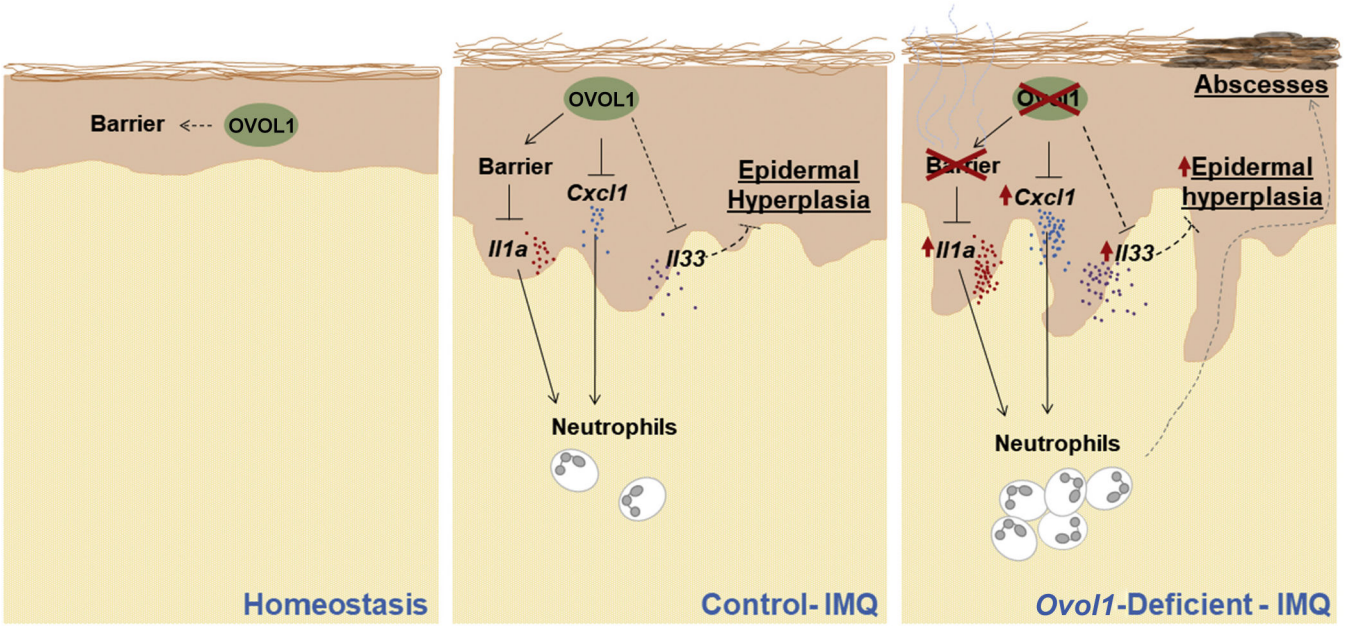

Figure 6. Working model of OVOL1 function in IMQ-induced skin inflammation.

In normal skin treated with IMQ, epidermally expressed Ovol1 not only promotes barrier maintenance, thereby suppressing alarmin (IL-1α) production, but also directly represses the gene expression of *Cxcl1* chemokine and possibly *Il33* cytokine to modulate the inflammatory and hyperproliferative responses. In *Ovol1*-deficient skin treated with IMQ, the barrier is disrupted and, *Il1a* and *Cxcl1* are upregulated, resulting in excessive and persistent neutrophil accumulation and exacerbated inflammation. In contrast, *Il33* is upregulated likely as a protective mechanism to suppress excessive epidermal hyperplasia. IMQ, imiquimod.