SUPPLEMENTARY MATERIALS AND METHODS

Mice

Ovol1$^{+/-}$ mice are maintained in a CD1 strain background (Sun et al. 2021) and are intercrossed to produce homozygous mutant (*Ovol1*$^{-/-}$) progeny for the study. CD1-*Ovol1*$^{-/-}$ mice survive to adulthood but are sometimes smaller than the control littermates, so sex- and weight-matched control and mutant littermates were used for all analysis.

Imiquimod-induced psoriasis model

Mice aged 7−8 weeks received a daily topical dose of 62.5 mg 5% imiquimod cream (Perrigo, Dublin, Ireland) on shaved backs for 1–5 consecutive days or as indicated. On the basis of a previously described objective scoring system called PASI (van der Fits et al. 2009), erythema (redness of the skin) and scaling (approximated by dry, white cracks and patches on the skin surface) were blindly scored independently by one or more investigators on a score from 0 (none) to 4 (most severe). The cumulative score (erythema plus scaling) served as a measure of the severity of clinical signs (score 0−8).

Flow cytometry

To obtain a single-cell suspension, minced samples were digested with 10 ml of a solution containing 0.25% collagenase (C9091, Sigma-Aldrich, St. Louis, MO), 0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (BP310; Thermo Fisher Scientific, Waltham, MA), 0.001 M sodium pyruvate (BP356; Thermo Fisher Scientific), and 0.1 mg/ml DNase (DN25; Sigma-Aldrich) at 37 °C for 1 hour with rotation and then filtered through a 70-μm filter, spun down, and resuspended in 2% fetal bovine serum (Alpha FBS; Alphabioregen, Boston, MA). A total of 5×10^5 cells were stained by incubation for 30 minutes at room temperature with the following antibodies diluted in 2% fetal bovine serum/×1PBS: Alexa Fluor 488–conjugated anti-CD11b (101217; BioLegend, San Diego, CA), phycoerythrin-conjugated anti-F4/80 (123110; BioLegend), APC-conjugated anti-CD45 (clone 30-F11, 20-0451; Tonbo Biosciences, San Diego, CA), APC-Cy7-conjugated anti-Ly6G (clone 1A8, 25-1276; Tonbo Biosciences), and 7-aminoactinomycin D (559925; BD, Franklin Lakes, NJ).

Histology and immunostaining

Sections from paraformaldehyde-fixed, paraffin-embedded back skin were stained with H&E, and epidermal thickness was measured at over 30 positions per section, and values were averaged.

For indirect immunofluorescence, mouse back skins were freshly frozen in optimum cutting temperature compound (Tissue-Tek) and stained using the appropriate antibodies. The primary antibodies used were keratin 1, keratin 14, and loricrin (rabbit or chicken, 1:1,000; gifts of Julie Segre, National Institutes of Health, Bethesda, MD), Ki-67 (rabbit, clone #D3B5, catalog number 9129; 1:1,000; Cell Signaling Technology, Danvers, MA), and Ly6G (rat, clone 1A8; 1:200; eBioscience, Waltham, MA). The following secondary antibodies were used: FITC-conjugated goat anti-rabbit (FI-1000; 1:1,000; Vector Laboratories, Burlingame, CA), rhodamine-conjugated goat anti-chicken (103-295-155; 1:1,000; Jackson ImmunoResearch Laboratories, West Grove, PA), and Alexa Fluor 488–conjugated donkey anti-rat (A-21208; 1:1,000; Thermo Fisher Scientific). Slides were mounted in Antifade medium (Vectashield H-1000; Vector Laboratories).

RNA extraction and RT-qPCR

Back skin was collected, and epidermis was separated from the dermis after incubation in a 1:1 dilution of dispase in Epilife media (M-EPICF-500; Cascade Biologics, Portland, OR) at 37 °C for 1 hour. The epidermis was then lysed in TRIzol (15596018; Thermo Fisher Scientific), followed by chloroform extraction and RNA purification from the aqueous phase using Zymo Research's Quick-RNA MiniPrep per the manufacturer's instructions.

For RT-qPCR, 2 μg of RNA was used to generate cDNA (4368814; Applied Biosystems, Waltham, MA) as per the manufacturer's instructions. qPCR was performed using a Bio-Rad CFX96 Real-Time System and SsoAdvanced Universal SYBR Green Supermix (172-5271; Bio-Rad Laboratories, Hercules, CA). *Gapdh* was used as a loading control. Information for the gene-specific primers used is provided in Supplementary Table S1.

OVOL1 knockdown in normal human epidermal keratinocytes and *Ovol1* deletion in primary mouse keratinocytes

Normal human epidermal keratinocytes (KCs) were cultured in Keratinocyte SFM (17005042; Gibco, Waltham, MA). *OVOL1* small interfering RNA was purchased from Thermo Fisher Scientific (small interfering RNA identification 115544) and transfected into normal human epidermal KCs on six-well plates using Lipofectamine RNAiMAX Transfection Reagent (13778030; Invitrogen, Waltham, MA) according to manufacturer's instructions. One day after transfection, the medium was replaced, and normal human epidermal KCs were treated with calcium ion (1.8 mM) for 24 hours.

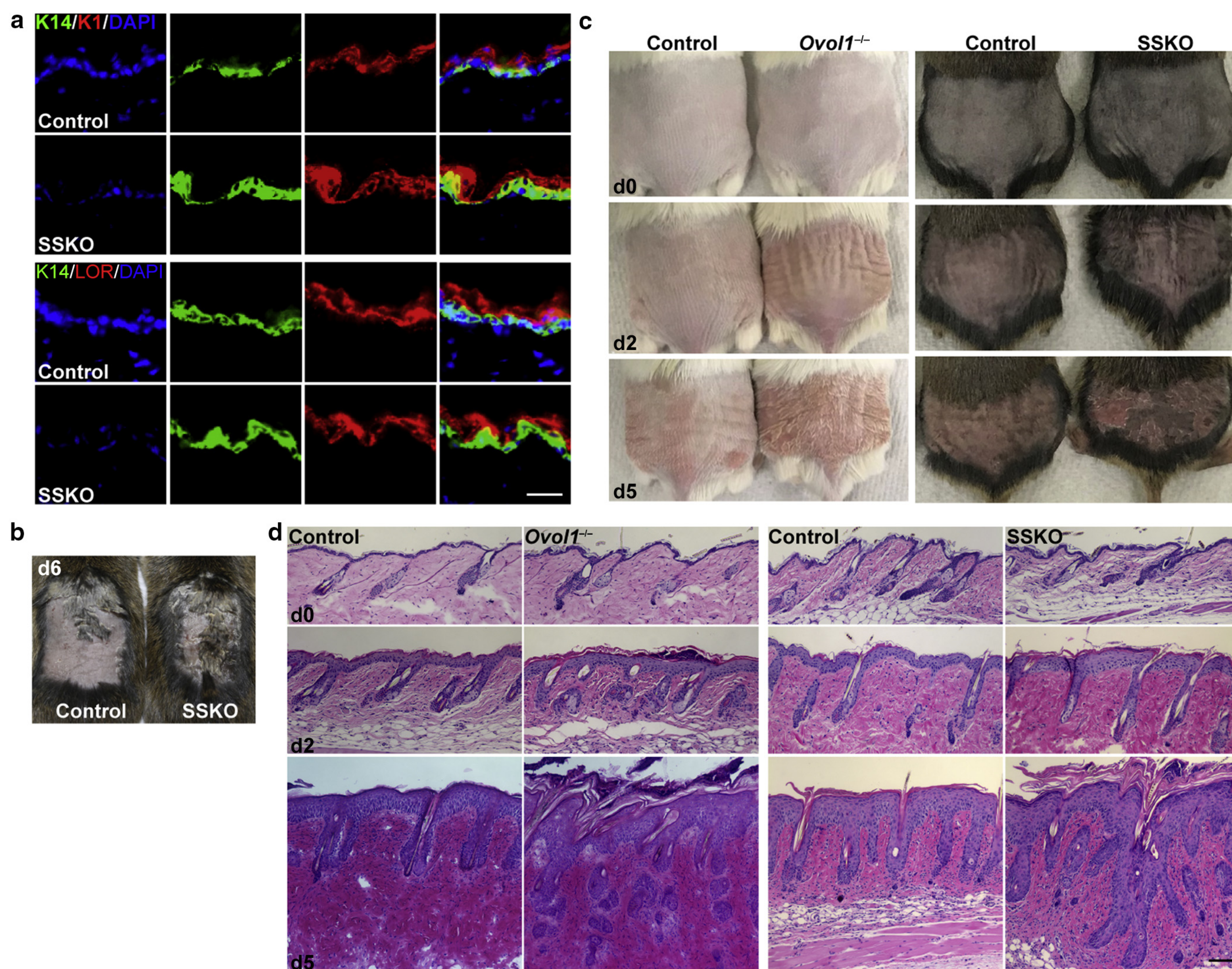
Primary mouse KCs were isolated from newborn *Ovol1*$^{f/f}$ mice as described in the study by Haensel et al. (2019) with minor modification. Briefly, the epidermis was separated from the dermis by overnight incubation with dispase. The epidermis was dissociated on a drop of TrypLE Express Enzyme (12604013; Gibco) for 30 minutes at room temperature. KCs were cultured in Epidermal Keratinocyte Medium (CnT-07, CELLnTEC, Bern, Switzerland). For acute deletion of *Ovol1*, 50,000 KCs were infected with adenoviruses expressing Cre recombinase (multiplicity of infection = 100) or control adenoviruses, centrifuged at 500*g* at room temperature for 2 hours, followed by incubation for 24 hours on 12-well plates. KCs were treated with calcium ion (1.8 mM) in a fresh medium for 24 or 48 hours.

SUPPLEMENTARY REFERENCES

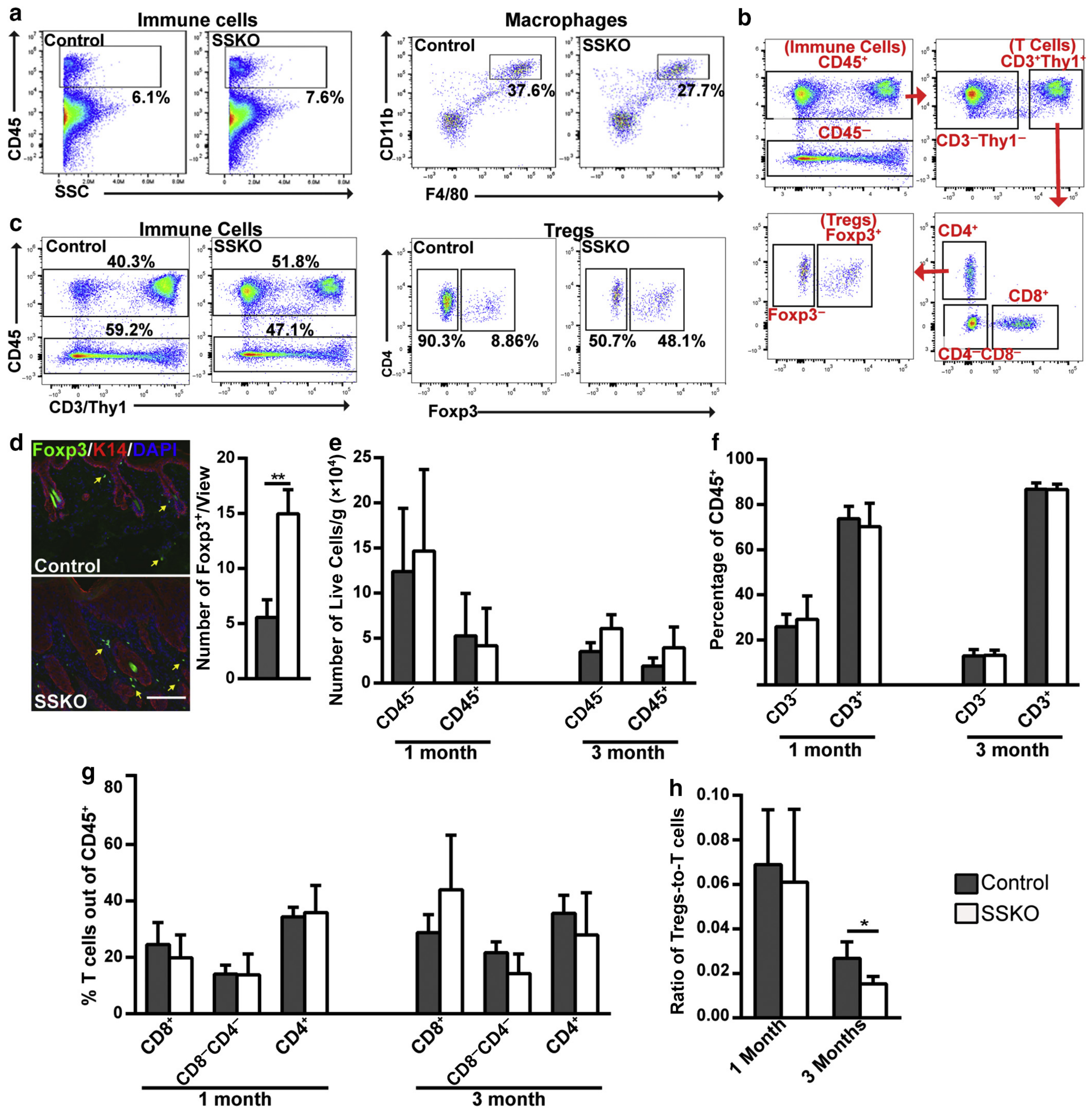
Haensel D, Sun P, MacLean AL, Ma X, Zhou Y, Stemmler MP, et al. An Ovol2-Zeb1 transcriptional circuit regulates epithelial directional migration and proliferation. EMBO reports 2019;20:e46273.

Sun P, Vu R, Dragan M, Haensel D, Gutierrez G, Nguyen Q, et al. OVOL1 regulates psoriasis-like skin inflammation and epidermal hyperplasia. J Invest Dermatol 2021;141:1542−52.

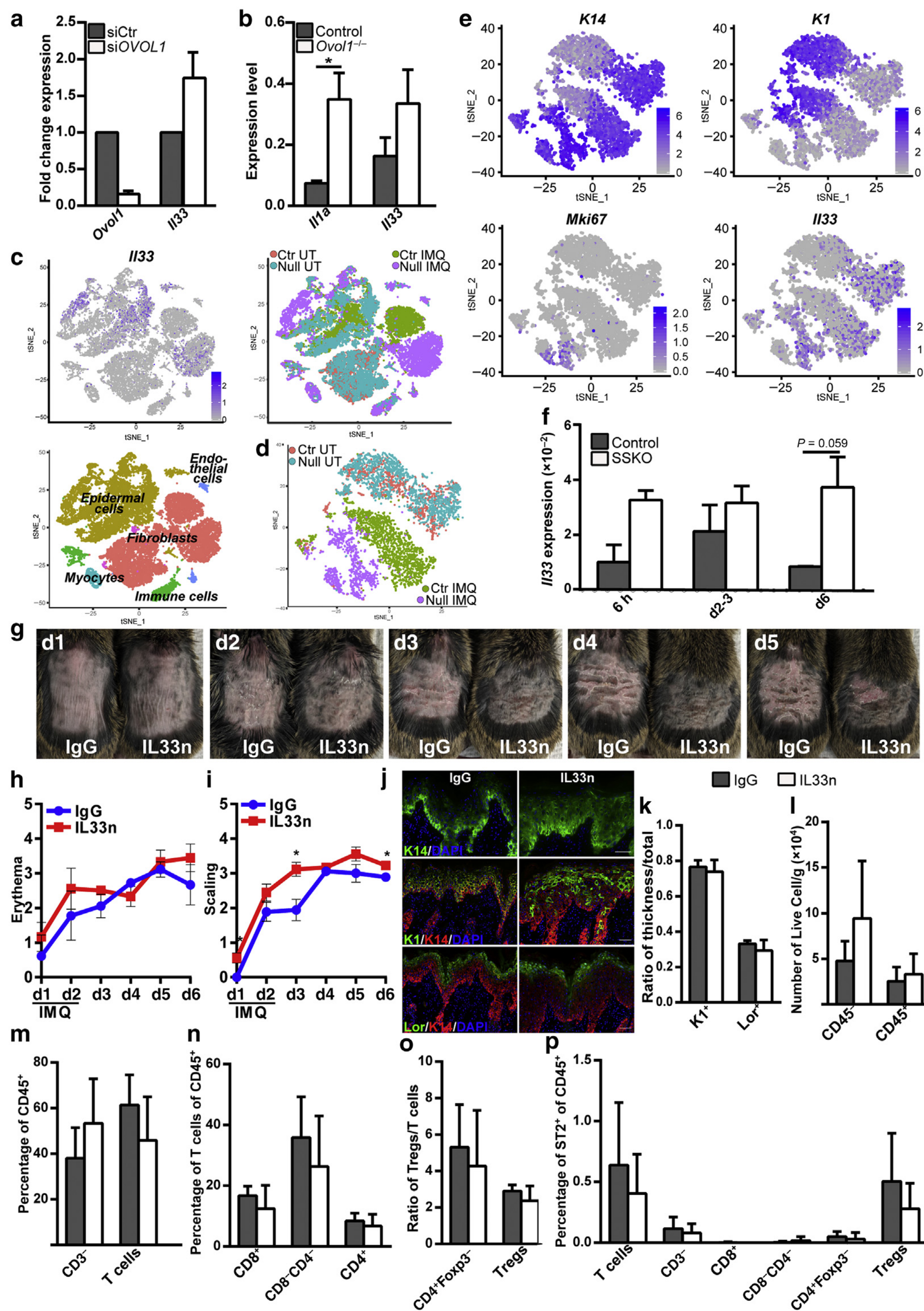
van der Fits L, Mourits S, Voerman JSA, Kant M, Boon L, Laman JD, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J Immunol 2009;182:5836−45.



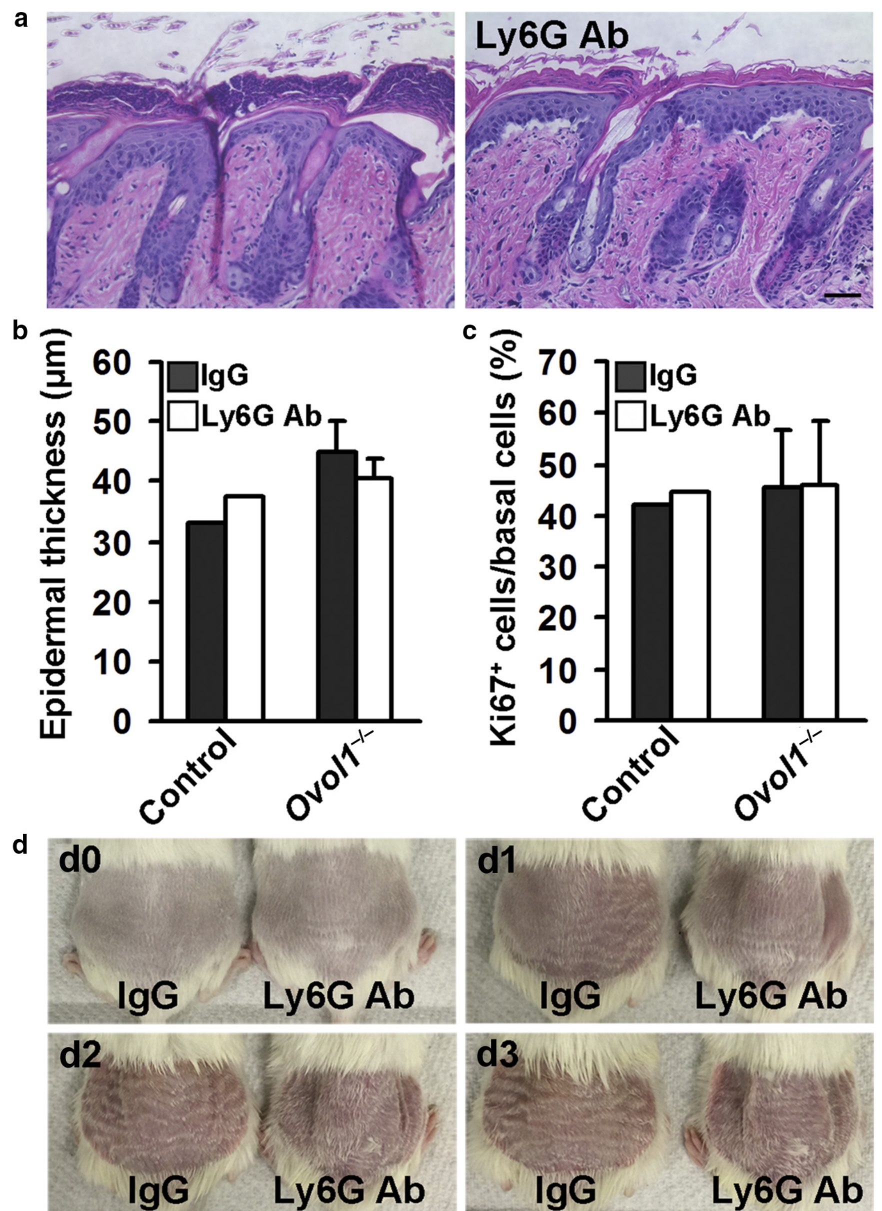
Supplementary Figure S1. *Ovol1* SSKO mice show exacerbated epidermal hyperplasia after only two IMQ applications. This figure is related to Figure 1. (**a**) Representative immunofluorescent staining of untreated *Ovol1* SSKO and littermate control back skin. Bar = 15 μm. (**b**) Representative images of *Ovol1* SSKO mice and control littermates 6d after two IMQ applications. (**c**) External appearance and (**d**) histology of the skin of *Ovol1*$^{-/-}$ (left) and *Ovol1* SSKO (right) mice and their respective control littermates at the indicated times after five IMQ treatments. Bar = 100 μm. d, day; IMQ, imiquimod; K, keratin; LOR, loricrin; SSKO, skin epithelia–specific knockout.



Supplementary Figure S2. Flow plots and immune cell profiles in control and *Ovol1* SSKO skin 1−3 months after IMQ treatment. This figure is related to Figure 2. (**a**) Representative flow cytometry plots for Figure 2a. (**b**) Gating strategy for Figure 2b−e. (**c**) Representative flow cytometry plots for Figure 2c and d. (**d**) Representative immunofluorescent staining and quantification of Foxp3^{+} Tregs in the skin 6 days after two IMQ applications. Arrows point to Tregs. (**e**−**h**) Summary of flow cytometry data for the respective immune cell populations 1−3 months after two IMQ applications. n = 7 controls at 1 month and 6 controls at 3 months; n = 6 and 3 for *Ovol1* SSKO mice for 1 and 3 months, respectively. ** $P < 0.01$; * $P < 0.05$. IMQ, imiquimod; K, keratin; SSC, side scatter; SSKO, skin epithelia–specific knockout; Treg, regulatory T cell.



Supplementary Figure S3. Supplementary data on the expression and function of *Il33* in *Ovol1*-deficient skin. This figure is related to Figure 3. (**a**) RT-qPCR analysis on siCtr and siOvol1–treated NHEK cells. Results from a single experiment are shown but are representative of three independent experiments. (**b**) RT-qPCR analysis 6 h after IMQ treatment in *Ovol1*$^{-/-}$ mice. n = 5 for *Ovol1*$^{-/-}$ and 4 for control littermates. (**c**) Feature plot showing the expression of *Il33* in all cell types detected in the single-cell analysis of mouse 24 h after IMQ treatment. tSNE plots from Sun et al. (2021) depicting the samples (center) and cell (right) types are included for comparison. Ctr-UT and Ctr-IMQ represent untreated and IMQ-treated control littermate (*Ovol1*$^{+/-}$) skin, respectively. Null-UT and Null-IMQ represent untreated and IMQ-treated *Ovol1*$^{-/-}$ skin, respectively. (**d, e**) Single-cell data on interfollicular epidermal cells. Shown are (**d**) tSNE and (**e**) gene-specific feature plots. (**f**) RT-qPCR analysis on whole skin of control and *Ovol1* SSKO mice at the respective times after two IMQ applications. n = 2 pairs of *Ovol1* SSKO and controls for 6 h, n = 4 pairs of *Ovol1* SSKO and controls for 2−3 d, and n = 3 pairs of *Ovol1* SSKO and controls for 6 d. (**g**) External appearance, (**h**) erythema, or (**i**) scaling scoring at the indicated times. (**j**) Representative immunofluorescent staining of the indicated markers on d6. (**k**) The relative thickness of the K1- or LOR-positive layers relative to the total combined epidermal thickness was calculated and shown in **j**. (**l**−**p**) Flow cytometry on d6 for the indicated cell populations. n = 3 pairs of IgG-treated and IL33n-treated *Ovol1* SSKO mice. *$P < 0.05$. (**a, b, e, g, h, j, o**) Error bars represent the mean ± SD. Ctr, control; d, day; h, hour; IMQ, imiquimod; K, keratin; LOR, loricrin; NHEK, normal human epidermal keratinocyte; siCtr, control small interfering RNA; siRNA, small interfering RNA; SSKO, skin epithelia–specific knockout; Treg, regulatory T cell; tSNE, t-distributed stochastic neighbor embedding; UT, untreated.



Supplementary Figure S4. Supplementary data on neutrophil depletion experiments. This image is related to Figure 5. (**a**) Skin histology of *Ovol1*$^{-/-}$ mice treated with IgG or Ly6G antibody on d3. (**b, c**) Analysis of (**b**) epidermal thickness and (**c**) cell proliferation in *Ovol1*$^{-/-}$ mice treated with IgG or Ly6G antibody at the experimental endpoint. n = 3 for *Ovol1*$^{-/-}$ and 1 for wild-type control littermate. Error bars represent the mean ± SEM. (**d**) External appearance on different d before and after IMQ treatment. Note that these mice are distinct from those shown in Figure 5. Ab, antobody; d, day; IMQ, imiquimod.