Supporting information

**Figure S1. SRF expression in patients with Lichen Planus or lichenification as a result of chronic eczematous dermatitis**

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**Figure S2. K5Cre-mediated recombination at the R26R locus**

(A) Staining for β-galactosidase activity in a Rosa26R embryo (E14.5) expressing Cre recombinase under the control of the K5 promoter revealed activity in the epidermis. Staining of a complete embryo is shown in (A). (B) shows a longitudinal section of a K5CreRosa26R embryo (snout region) at E14.5. LacZ-staining is seen in the epidermis (epi, shown in (C) at higher magnification) and vibrissae (vb).

(B, C) Sections were counterstained with eosin; bar represents 50 µm in (C).
Figure S3. Embryonic lethality, edema formation and blistering in Srf flex1/flex1 mice expressing the K5Cre transgene

(A) Table showing the genotypes of Srf flex1/flex1 x Srf wt/flex1 K5Cre offspring at different stages of embryonic development and 4 weeks after birth (P28).

(B) Skin blistering (arrows) and hemorrhage of a Srf flex1/flex1 K5Cre embryo at E14.5.

(C, D) Hematoxylin/eosin staining of cross sections of control and Srf flex1/flex1 K5Cre embryos frequently revealed edema (C, E14.5, bar represents 200 µm) and occasionally subepidermal blisters (D, arrows, E15.5, bar represents 50 µm) in Srf flex1/flex1 K5Cre embryos. epi: epidermis, d: dermis, ed: edema, sp: spinal cord.

Figure S4. The inflammatory infiltrate of Srf mutant skin

(A) An increased number of leukocytes was detected by ultrastructural analysis of skin lesions in Srf mutant mice. One asterisk indicates leukocytes, two asterisks indicate an endothelial cell (right panel). Arrows indicate the basement membrane. Bar represents 10 µm.

(B) Immunofluorescence analysis of skin sections for CD3 positive cells (lymphocytes), Meca-32 positive cells (endothelial cells) and MHCII positive cells in lesional skin of Srf mutant mice. The latter are predominantly macrophages as determined by staining for a macrophage-specific lectin. An increased number of mast cells in lesional Srf mutant skin was detected by
staining of skin sections with toluidine blue. Bar represents 50 µm. d: dermis; epi: epidermis; hf: hair follicle.

**Figure S5. siRNA-mediated knock-down of SRF in primary keratinocytes does not affect apoptosis or expression of different keratins**

Western blot analysis showing expression of SRF, K5, K14, K10, cleaved caspase-3 and β-actin in primary human keratinocytes treated with scrambled or SRF siRNAs. siRNA against an unrelated protein (caspase-5) was used as an additional control. Lysates from keratinocytes that had been harvested 24h after irradiation with 50mJ/cm² UVB were used as a positive control for cleaved caspase-3 (indicated by an asterisk).

**Table S1. List of primary and secondary antibodies**

<table>
<thead>
<tr>
<th>Primary antibodies</th>
<th>Host</th>
<th>Catalog number</th>
<th>Source</th>
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<tbody>
<tr>
<td>anti-CD3</td>
<td>rabbit</td>
<td>A0452</td>
<td>DAKO</td>
</tr>
<tr>
<td>anti-cleaved caspase-3</td>
<td>rabbit</td>
<td>#9661</td>
<td>Cell Signaling</td>
</tr>
<tr>
<td>anti-BrdU</td>
<td>mouse</td>
<td>1202693</td>
<td>Roche</td>
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<tr>
<td>anti-E-cadherin</td>
<td>rat</td>
<td>ALX-804-202</td>
<td>Alexis Biochemicals</td>
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<td>mouse</td>
<td>610181</td>
<td>BD Biosciences</td>
</tr>
<tr>
<td>anti-γ-catenin</td>
<td>rabbit</td>
<td>Sc-7900</td>
<td>Santa Cruz</td>
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<tr>
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<td>mouse</td>
<td>A5441</td>
<td>Sigma</td>
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<tr>
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<td>mouse</td>
<td>#5G4</td>
<td>HyTest</td>
</tr>
<tr>
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<td>rat</td>
<td>555734</td>
<td>BD Pharmingen</td>
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<td>anti-integrin β1</td>
<td>rat</td>
<td>MAB1997</td>
<td>Chemicon</td>
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<tr>
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<td>rabbit</td>
<td>PRB-169P</td>
<td>Covance</td>
</tr>
<tr>
<td>anti-keratin 5 (AF-138)</td>
<td>rabbit</td>
<td>PRB-160P</td>
<td>Covance</td>
</tr>
<tr>
<td>anti-keratin 10</td>
<td>mouse</td>
<td>M7002</td>
<td>DAKO</td>
</tr>
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</table>
antikeratin 14 rabbit PRB-155P Covance
antikeratin 14 mouse MCA890 Serotec
antikeratin 15 guinea pig GP-CK15 Progen
antikeratin 17 rabbit - P. Coulombe, (McGowan and Coulombe, 1998)
antikeratins 8/18 guinea pig 03-GP11 American Research Products
antiloricrin rabbit PRB-145P Covance
antimacrophage rat ab 15635 Abcam
specific lectin
anti-Meca-32 rat 553849 BD Pharmingen
antimHC class II rat T-2106 BMA Biomedicals
antip63 mouse sc-8431 Santa Cruz
antisRF rat - clone 2C5, this study
anti-phospho-STAT3 rabbit #9131 Cell Signaling

Secondary antibodies
antiguanepig biotin goat #106-065-003 Jackson
antirabbit biotin goat #111-065-003 Jackson
antirabbit-Cy2 goat #111-225-003 Jackson
antirabbit HRP goat W4011 Promega
antirat AP goat S383A Promega
antirat biotin goat #112-065-003 Jackson
antirat Cy2 goat #112-225-003 Jackson
antirat Cy3 donkey #712-165-150 Jackson
antirat HRP goat NA935 GE Healthcare
antimouse biotin goat BA-9200 Vector Laboratories
antimouse HRP goat W4021 Promega
antimouse Cy2 goat #115-225-003 Jackson

Supplementary Methods

Preparation of protein lysates and Western blot analysis
Tissue or cells were lysed in lysis buffer containing 50 mM Tris/HCl pH 7.4, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1% NP-40, 0.5 mM AEBSF, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 10 µg/ml pepstatin, 10 mM NaF, 1 mM Na3VO4, 10 mM Na4P2O7, 20 µM phenylarsinoxide as well as phosphatase inhibitor cocktails I and II (Sigma). For analysis of keratin expression cells were lysed in Laemmli buffer. Proteins were separated by sodium dodecylsulfate polyacrylamide (SDS) gel electrophoresis under reducing conditions and transferred to nitrocellulose membrane. Blotted membranes were blocked in 3% non-fat dry milk in PBS/0.1% Tween 20 and incubated with primary antibodies (see above) overnight at 4 °C. Proteins were visualized with horseradish peroxidase-coupled anti-mouse-, anti-rabbit- or anti-rat IgG antibodies, followed by enhanced chemoluminescence detection (ECL). Alternatively, visualization was achieved with an alkaline phosphatase-coupled secondary antibody followed by incubation with the AP substrate 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT).

**Histology, immunostaining and TUNEL assay**

For routine histology, cryo- or paraffin-embedded sections (7 µm) were stained with hematoxylin and eosin. For immunohistochemical analysis, cultured cells or sections (subjected to antigen retrieval where necessary) were stained according to standard protocols and images were taken with a Zeiss Axiophot microscope or a confocal Leica TCS NT SP1 microscope. All primary and secondary antibodies used are listed in the Supplementary Table S1. The polyclonal antibody against keratin 17 was kindly provided by Dr.
Pierre Coulombe, Johns Hopkins University, Baltimore, MA. For the detection of proliferating cells by BrdU labeling, mice were injected intraperitoneally with BrdU (250 mg/kg BrdU in 0.9% NaCl) and sacrificed 2 hours after injection. Acidic ethanol-fixed skin sections were incubated with a monoclonal antibody directed against BrdU (Roche), followed by a Cy2-conjugated anti-mouse antibody. TUNEL assays were performed according to the manufacturer’s manual (Roche) using 3.5µm paraffin sections of tissue that had been fixed in 4% paraformaldehyde in PBS.

**RNA isolation and quantitative RT–PCR analysis**

Total cellular RNA from skin and primary keratinocytes was isolated as described [1], including a DNase digest. RNA (1 µg) was reverse-transcribed using the iScript™ cDNA Synthesis Kit (Bio-Rad). The reaction mix was filled up with water to 50 µl, and 0.5 µl were used for quantitative RT–PCR, using 10 nM primers and 2x Light Cycler 480 SYBR green I mix (Roche) in a 25 µl reaction volume. The reaction was followed and evaluated in the Light Cycler 480 (Roche). Amplification of the GAPDH cDNA was used for normalization. The following primers were used: Gapdh: 5’- TCG TGG ATC TGA CGT GCC GCC TG, 3’- CAC CAC CCT GTT GCT GTA GCC GTA T; IL1β: 5’- CTGAAAGCTCTCCACCTC, 3’- TGCTGATGTACCAGTT GGGG; S100A8: 5’- GCCGTCTGAAGTGGAGAAG, 3’- GTGAGATGCCACAC CCACTTT; S100A9: 5’- CGCAGCATAACCACCACCATCAT, 3’- AAGATCAACTTT TGCCATCACG, Srf: 5’- TGTGCAGGCCATTCCATGTG, 3’- ACAGACGACG TCATGATGGTG.
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