Supporting information

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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/cm^2 UVB were used as a positive control for cleaved caspase-3 (indicated by an asterisk).

Table S1. List of primary and secondary antibodies

Primary antibodies	**Host**	**Catalog number**	**Source**
anti-CD3	rabbit	A0452	DAKO
anti-cleaved caspase-3	rabbit	#9661	Cell Signaling
anti-BrdU	mouse	1202693	Roche
anti-E-cadherin	rat	ALX-804-202	Alexis Biochemicals
anti-E-cadherin	mouse	610181	BD Biosciences
anti-γ-catenin	rabbit	Sc-7900	Santa Cruz
anti-β-actin	mouse	A5441	Sigma
anti-GAPDH	mouse	#5G4	HyTest
anti-integrin α6-FITC	rat	555734	BD Pharmingen
anti-integrin β1	rat	MAB1997	Chemicon
anti-keratin 6	rabbit	PRB-169P	Covance
anti-keratin 5 (AF-138)	rabbit	PRB-160P	Covance
anti-keratin 10	mouse	M7002	DAKO

anti-keratin 14	rabbit	PRB-155P	Covance
anti-keratin 14	mouse	MCA890	Serotec
anti-keratin 15	guinea pig	GP-CK15	Progen
anti-keratin 17	rabbit	-	P. Coulombe, (McGowan and Coulombe, 1998)
anti-keratins 8/18	guinea pig	03-GP11	American Research Products
anti-loricrin	rabbit	PRB-145P	Covance
anti-macrophage specific lectin	rat	ab 15635	Abcam
anti-Meca-32	rat	553849	BD Pharmingen
anti-MHC class II	rat	T-2106	BMA Biomedicals
anti-p63	mouse	sc-8431	Santa Cruz
anti-SRF	rat	-	clone 2C5, this study
anti-phospho-STAT3	rabbit	#9131	Cell Signaling

Secondary antibodies

anti-guinea pig biotin	goat	#106-065-003	Jackson
anti-rabbit biotin	goat	#111-065-003	Jackson
anti-rabbit-Cy2	goat	#111-225-003	Jackson
anti-rabbit HRP	goat	W4011	Promega
anti-rat AP	goat	S383A	Promega
anti-rat biotin	goat	#112-065-003	Jackson
anti-rat Cy2	goat	#112-225-003	Jackson
anti-rat Cy3	donkey	#712-165-150	Jackson
anti-rat HRP	goat	NA935	GE Healthcare
anti-mouse biotin	goat	BA-9200	Vector Laboratories
anti-mouse HRP	goat	W4021	Promega
anti-mouse 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 Na_3VO_4, 10 mM $Na_4P_2O_7$, 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.

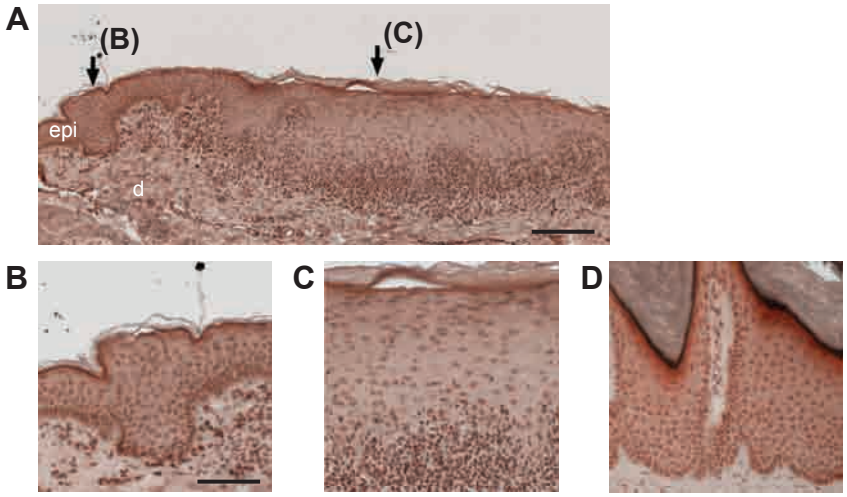
Pierrre Coulombe, Johns Hopkins University, Baltimore, MA. For the detection of proliferating cells by BrdU labeling, mice were injected intraperitoneally with BrdU (250 mg/kg BrdU in 0.9% NaCl) and sacrificed 2 hours after injection. Acidic ethanol-fixed skin sections were incubated with a monoclonal 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^{TM}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'- GCCGTCTGAACTGGAGAAG, 3'- GTGAGATGCCACAC CCACTTT; S100A9: 5'- CGCAGCATAACCACCATCAT, 3'- AAGATCAACTT TGCCATCAGC, Srf: 5'- TGTGCAGGCCATTCATGTG, 3'- ACAGACGACG TCATGATGGTG.

1. Chomczynski, P., and Sacchi, N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162**:156-159.



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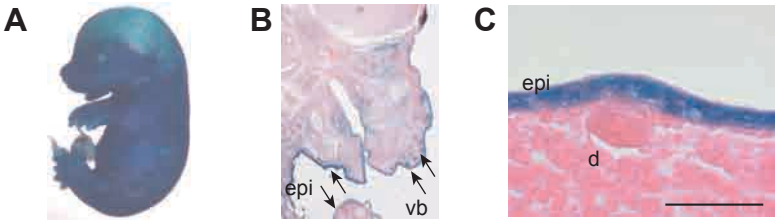

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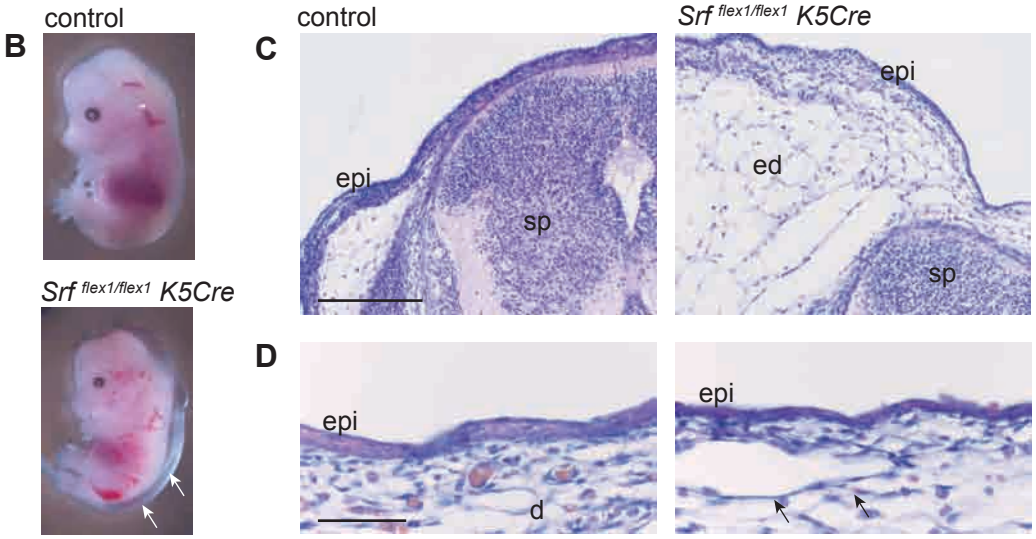
A

	total	*Srf* $^{wt/flex1}$	*Srf* $^{wt/flex1}$ *K5Cre*	*Srf* $^{flex1/flex1}$	*Srf* $^{flex1/flex1}$ *K5Cre*
E14.5	38	11	9	8	7
E15.5	30	8	12	5	5
E16.5	12	3	6	2	1
E17.5	5	1	4	0	0
P28	51	15	22	14	0



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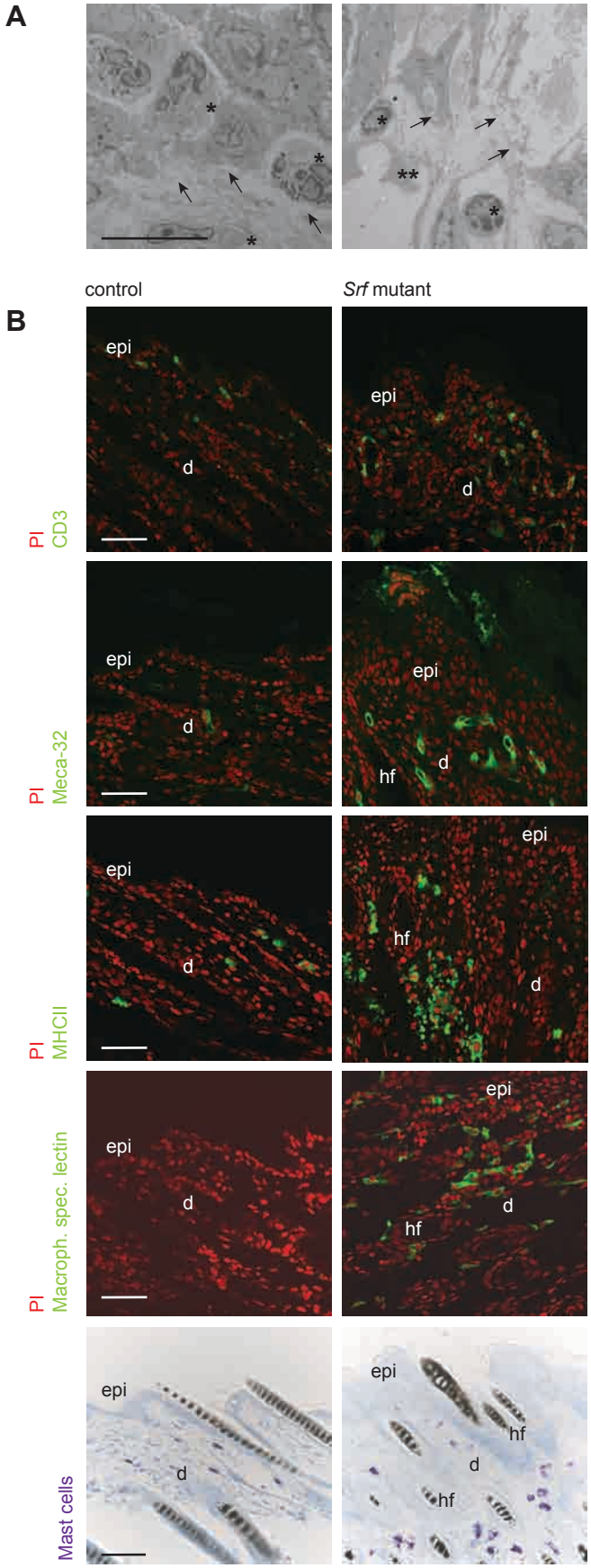

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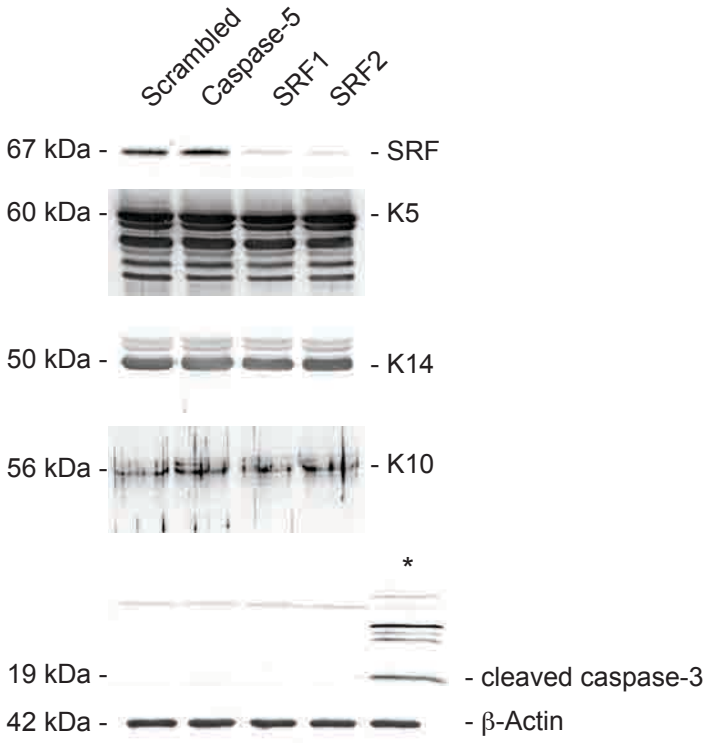

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