SUPPLEMENTARY INFORMATION

MATERIALS AND METHODS

I. Generation of mutant mice

Both mutant mouse lines were generated using B6N;B6N-*Snap29* $^{tm1a(EUCOMM)Wtsi}$ heterozygous knock-in mice (*Snap29*$^{fl/wt}$) derived from the Wellcome Trust Sanger Institute (WTSI) Mouse genetics program (MGP) and provided by the European Mutant Mouse Archive (EMMA: www.emmanet.org) (strain number EM:04440).

***Snap29*$^{-/-}$ (total *Snap29* knockout)** mice were generated by intercrossing *Snap29*$^{fl/wt}$ heterozygous mice with transgenic mice expressing Cre under the control of the adenovirus EIIa promoter (Lakso *et al.*, 1996). *Snap29*$^{fl/wt}$ mice harbor a FRT flanked lacZ/neomycin sequence followed by a loxP site in intron 1-2 and an additional loxP site inserted downstream of exon 2 (Fig. S1). After recombination Exon 2 is deleted and a frameshift introduced with early termination at the eighth codon in Exon 3 (Fig. S1).

***Snap29*$^{fl/fl}$/K14-Cre (keratinocyte-specific *Snap29* knockout)** mice were generated by mating the *Snap29*$^{fl/wt}$ mouse line with a flippase (FLP) mouse line (B6.129S4-*Gt(ROSA)*$^{26Sortm1(FLP1)Dym}$/RainJ, Jackson Laboratory). This mouse line expresses a variant of the FLP1 recombinase gene. Mating with these mice leads to an excision of the FRT flanked lacZ cassette. Resulting offspring were subsequently mated with transgenic mice expressing Cre under the control of the keratinocyte-specific keratin 14 promotor (Hafner *et al.*, 2004). Thus, exon 2 deletion and the frameshift in *Snap29* occurred exclusively in keratinocytes resulting in keratinocyte-specific *Snap29* knockout *Snap29*$^{fl/fl}$/K14-Cre mice.

II. Genotyping of mutant mice

Genotyping was performed by PCR analysis according to figure S1 using genomic DNA isolated from tail biopsies. All reactions were performed in a total volume of 20µl. Primer sequences are listed in Table S1. Primers P1, P2, and P3 were used for genotyping *Snap29*$^{-/-}$ mice. The size of the respective PCR products is indicated in figure S1. Primers P4, P5, and P2 were used for genotyping *Snap29*$^{fl/fl}$/K14-Cre mice. Primers EIIa-Cre-s and EIIa-Cre were used for genotyping EIIa-Cre mutant mice. PCR conditions were: 95°C for 3 min, followed by 39 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension step of 72°C for 10 min. Primers K14-Cre-up and K14-Cre-down were used for genotyping K14-Cre mutant mice. PCR conditions were: 95°C for 2 min, followed by 33 cycles of 95°C for 30 s, 60°C for 45 s, and 72°C for 45 s, with a final extension step of 72°C for 5 min.

III. Immunohistochemistry, immunofluorescence, Nile red, and BODIPY 493/503 staining

Immunohistochemistry: Sections were deparaffinized and subjected to antigen retrieval for 40 min in citrate buffer, pH 6, (Agilent Technologies, Inc., Santa Clara, USA). Endogenous peroxidase activity was quenched for 15 min at room temperature with 3 % H_2O_2 in PBS. The sections were incubated for 30 min in blocking buffer (TSA blocking reagent, Perkin Elmer, Waltham, USA) and incubated with the respective primary antibodies (Table S2) overnight at 4°C. Secondary antibodies: biotinylated goat anti-rabbit or anti-mouse IgG (Vector Laboratories, Burlingame, USA). Antibodies were visualized using the ABC technique (Vector Laboratories, Burlingame, USA)**.** The slides were counterstained using hematoxylin and imaged with an Axioskop 2 (Carl Zeiss, Oberkochen, Germany).

Immunofluorescence: Sections were deparaffinized, antigen unmasked, and blocked with TSA (Perkin Elmer, Waltham, USA) as described. The sections were incubated with the primary antibody (Table S2) overnight at 4°C, washed with PBS, and incubated with the Alexa-Fluor® 488 goat anti-rabbit secondary antibody (Dianova, Hamburg, Germany), diluted 1:800 in blocking buffer. Sections were mounted with Vectashield mounting medium containing DAPI (Vector Laboratories, Burlingame, USA) and were imaged with an Axioskop 100 (Carl Zeiss, Oberkochen, Germany).

Nile red and BODIPY 493/503 staining: Nile red staining was performed to detect the neutral lipid distribution in the *stratum corneum*. A stock solution of Nile red (Sigma-Aldrich, Munich, Germany) (500 µg/ml in acetone) was prepared, stored at 4 °C, and protected from light. A fresh Nile red staining solution was prepared by adding 5µl of Nile red stock solution to 995µl of 70 % glycerol plus 10µl DAPI (1µg/ml) (Sigma-Aldrich, Munich, Germany). A drop of staining solution was added to frozen sections and immediately mounted with Fluoromount (Agilent Technologies, Inc., Santa Clara, USA). Nile red fluorescence was captured with an Axioskop 100 (Carl Zeiss, Oberkochen, Germany). BODIPY 493/503 staining was performed to detect lipid droplets. A BODIPY 493/503 stock solution (Thermo Scientific, Waltham, USA) (1 mg/ml) was prepared in DMSO. BODIPY 493/503 staining solution was freshly prepared by diluting BODIPY 493/503 stock solution in PBS to a concentration of 1 µg/ml. Frozen sections were fixed for 20 min with 4 % PFA, washed with PBS, stained for 20 min at room temperature, and mounted as previously described. Images were taken with a FluoView 1000 confocal microscope (Olympus) at 1024x1024 pixel resolution using a 40x 0.90 NA UPLSAPO objective, 2-fold line averaging and

a pinhole size corresponding to 1 Airy disc. BODIPY was excited using the 488 nm line of the Argon laser. Fluorescence emission was detected in the range of 500-600 nm.

IV. Autophagy assays and western blot analysis

Equal amounts of protein extracts (25 µg) from fibroblast cell cultures were separated by SDS-PAGE on 12,5 % or 15 % acrylamide gels and transferred to a polyvinylidene difluoride membrane (Roth, Karlsruhe, Germany). Primary antibodies and dilutions are listed in Table S2. Antibody binding was detected with the WesternBreeze Chemiluminescent Immunodetection System (Invitrogen, Karlsruhe, Germany) according to manufacturer's instructions. Signals were detected with a luminescent image analyzer LAS-4000 (Fujifilm, Düsseldorf, Germany). To compare the amount of protein in the different samples, the blots were reprobed with a mouse monoclonal anti-beta-actin antibody (A-5441, Sigma-Aldrich, Taufkirchen, Germany) at a dilution of 1:2500. Quantification of Ki67 positive cells was performed by counting positively stained and non-stained cells. A total of 8 sections per mouse were counted.

Table S1: Sequences of primers used for genotyping mutant mice

primer	**sequence**
P1	5’-GCAGCGTCTGCATTGGATAC-3’
P2	5’-CTTGCTATCCACCTGCCTTAG-3’
P3	5’-CACACCTCCCCCTGAACCTGAAAC-3’
P4	5’-CCACATGTAACCACCAGCCCTC-3’
P5	5’-GTTCTAGGACTCCTCGCTGCCG-3’
EIIa-Cre-s	5’-CCTGGAAAATGCTTCTGTCCG-3’
EIIa-Cre	5’-GTGAAACAGCATTGCTGTCACTT-3’
K14-Cre-up	5’-GATGAAAGCCAAGGGGAATG-3’
K14-Cre-down	5’-CATCACTCGTTGCATCGACC-3’

Table S2: Primary antibodies used for immunohistochemistry and western blot analysis

Antibody	Host	Appli-cation	Dilution	Supplier
SNAP29, p	rabbit	IHC	1:300	Synaptic Systems
Keratin 14, p	rabbit	IHC	1:1000	Covance
Keratin 10, m	mouse	IHC	1:100	Lifespan Biosciences
Keratin 6, p	rabbit	IHC	1:1500	Covance
Filaggrin, p	rabbit	IHC	1:10000	Covance
Involucrin, p	rabbit	IHC	1:4000	abcam
Kallikrein 7, p	goat	IHC	1:500	Santa Cruz Biotechnology
Corneodesmosin, p	rabbit	IHC	1:500	Emelca Bioscience
Glucosylceramide, p	rabbit	IHC	1:500	Glycobiotech™
p62	rabbit	WB	1:1000	Cell Signaling
CHOP	rabbit	IHC	1:250	Novus
Ki67	mouse	IHC	1:100	BD Bioscience
Caspase 3	rabbit	IHC	1:1000	R&D Systems
LC3B	rabbit	IHC	1:1000	Cell Signaling

m: monoclonal, p: polyclonal, IHC: immunohistochemistry, WB: western blot analysis

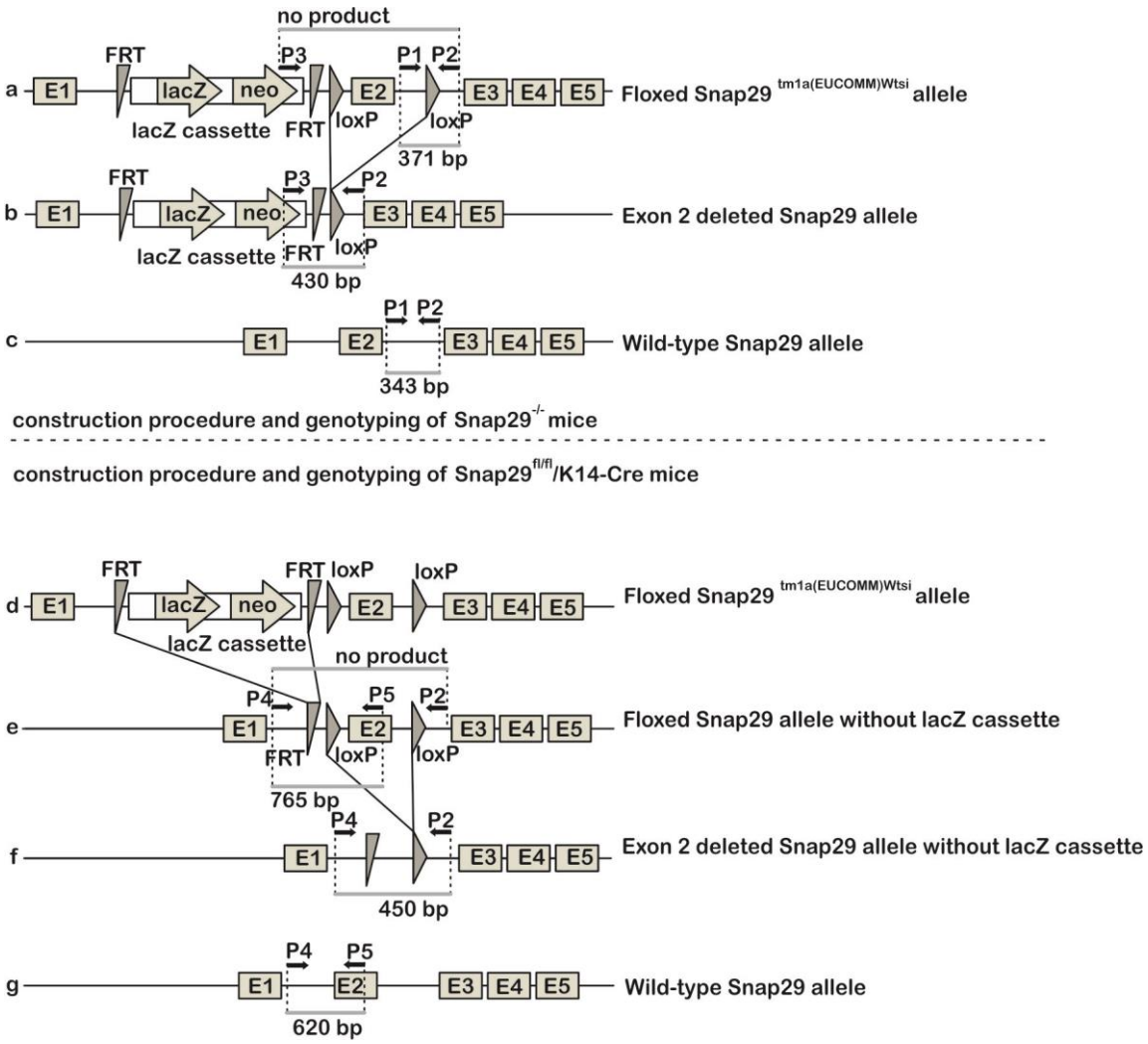

Figure S1: Allele constructs and strategies used for the generation of *Snap29*$^{-/-}$ (total *Snap29* knockout) and *Snap29*$^{fl/fl}$/K14-Cre (keratinocyte-specific *Snap29* knockout) mice. (a) Floxed *Snap*29$^{tm1a(EUCOMM)Wtsi}$ allele in *Snap29*$^{fl/wt}$ mice. (b) *Snap29* allele after deletion of exon 2. Complete loss of *Snap29* function in *Snap29*$^{-/-}$ mice after mating *Snap29*$^{fl/wt}$ mice with mice expressing Cre under the control of the adenovirus EIIa promoter. (c) Wild type allele of the murine *Snap29* gene. (d/e) Excision of the lacZ cassette via mating *Snap29*$^{fl/wt}$ mice with a flippase mouse line. (d) Floxed *Snap*29$^{tm1a(EUCOMM)Wtsi}$ allele in *Snap29*$^{fl/wt}$ mice. (e) Floxed *Snap29* allele without the lacZ cassette. (f) Functional loss of *Snap29* exclusively in keratinocytes

(*Snap29*$^{fl/fl}$/K14-Cre mice) after mating mice from (e) with transgenic mice expressing Cre under the control of the keratinocyte specific *keratin14* promoter. (g) Wild type allele of the murine *Snap29* gene. Primers for genotyping are indicated as P1, P2, P3, P4, and P5, respectively. PCR product length are indicated in base pairs (bp).

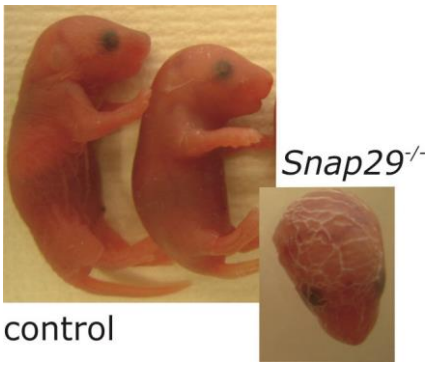

Figure S2: Macroscopic appearance of *Snap29*$^{-/-}$ newborns and control littermates. *Snap29*$^{-/-}$ newborns exhibited a slightly reduced body size and a distinct ichthyosiform phenotype. Their skin was scaly and taut, hindering the animals from extending their limbs There was no apparent difference to *Snap29*$^{fl/fl}$/K14-Cre newborns.

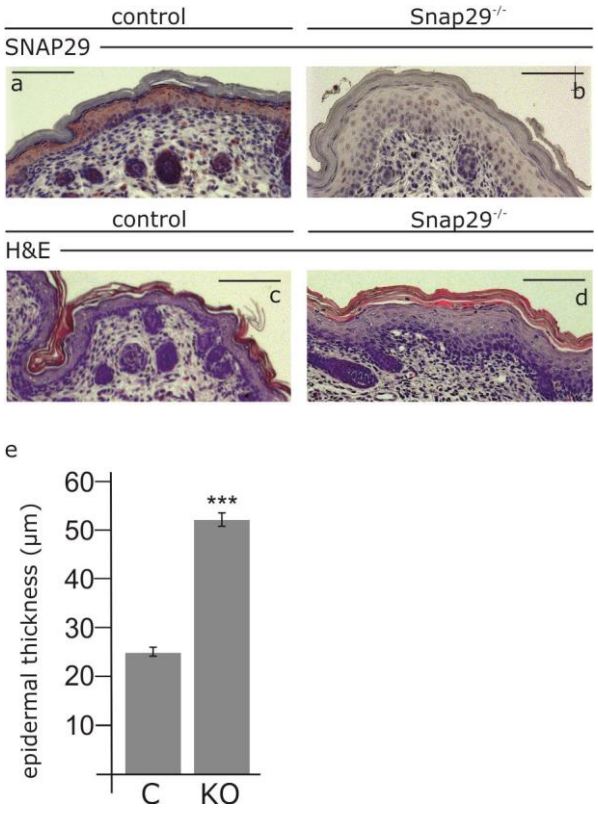

Figure S3: (a/b) Immunohistochemical staining with anti-SNAP29 antibody of dorsal skin sections from *Snap29*$^{-/-}$ mice and control littermates verified the absence of SNAP29 in mutant skin. (c/d) Hematoxylin & eosin staining of *Snap29*$^{-/-}$ mice versus control skin revealed epidermal hyperplasia (acanthosis). The *stratum corneum* of mutant mice was thickened and its structure was condensed (hyperkeratosis). Scale bars: 100 µm. (e) Quantification of acanthosis in the viable epidermis (three animals per group, three fields per animal). Data are shown as mean ± s.e.m., ***P≤ 0,001 (unpaired Student's t-test), C: control, KO: *Snap29*$^{-/-}$.

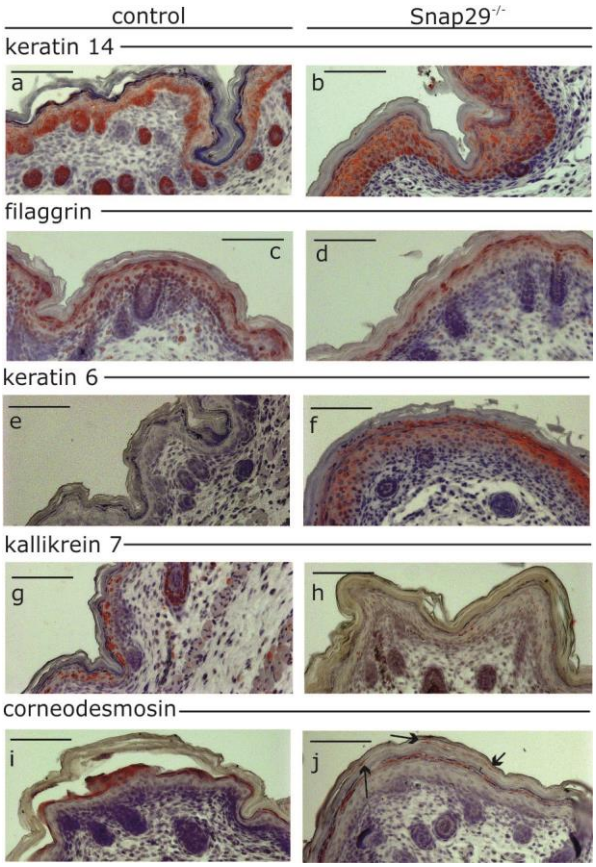

Figure S4: (a/b) In contrast to control skin, keratin 14 (K14) expression reached the suprabasal and subcorneal layers of *Snap29*$^{-/-}$ mice epidermis. (c/d) The epidermal levels of filaggrin were decreased in *Snap29*$^{-/-}$ mice epidermis. (e/f) Immunohistochemical staining with anti-K6 antibody showed pronounced expression of keratin 6 in cells of the suprabasal layers of mutant epidermis but not in controls. (g/h) Immunohistochemistry revealed strong reductions of kallikrein 7 (KLK7) and (i/j) corneodesmosin (Cdsn) levels, whereas remnants of corneodesmosin were still found in mutant *stratum corneum* (black arrows). Scale bars: 100 µm.

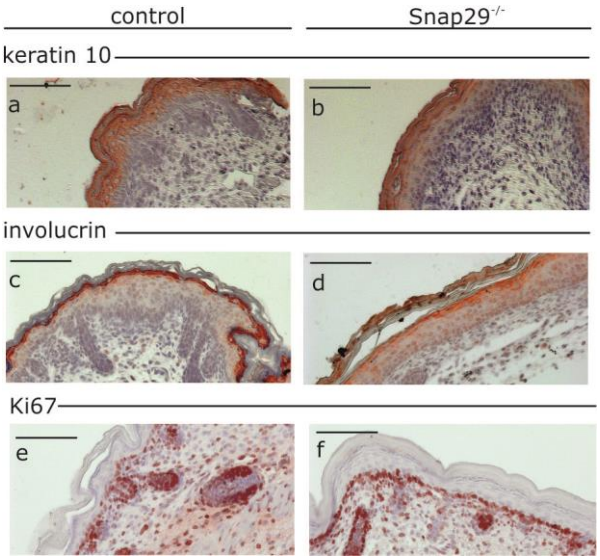

Figure S5: (a/b) Immunohistochemistry revealed that the levels and distribution of the suprabasal keratin 10 (K10) did not changed significantly in *Snap29*$^{-/-}$ mice epidermis. (c/d) Involucrin levels were decreased in the cells of the upper *stratum spinosum* and *stratum granulosum* of *Snap29*$^{-/-}$ mice. Staining with an anti-Ki67 antibody revealed 36% of positive basal cells in control epidermis as compared to 52% in *Snap29*$^{fl/fl}$/K14-Cre epidermis. Scale bars: 100 µm.

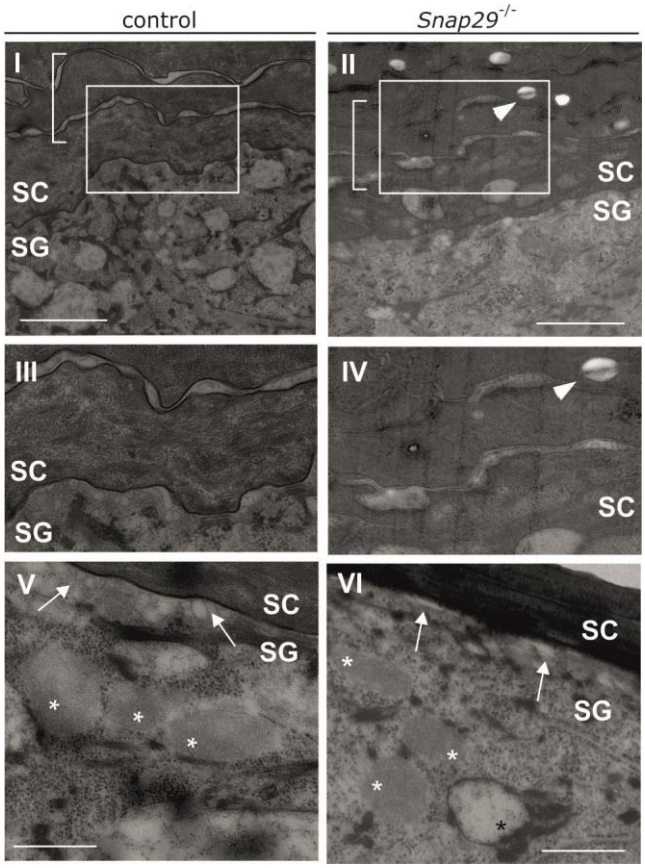

Figure S6: Ultrastructural analysis of control and *Snap29*$^{-/-}$ epidermis. (I/II) Brackets show regions of fluffy *stratum corneum* structures in control skin and corresponding condensed regions in mutant epidermis. Panels III/IV represent higher magnification views of the white rectangles in panel (I/II) showing remnants of organelles. (III/IV) White arrowhead indicate electron lucent vesicle-like structure. (V/VI) White asterisks indicate lamellar bodies without pathological findings; black asterisks indicate lamellar bodies without structured content. White arrows indicate secreted material between *stratum granulosum* and *stratum corneum*. Scale bars: 2 µm (I/II), 500 nm (V/VI). SC: *stratum corneum*, SG: *stratum granulosum*

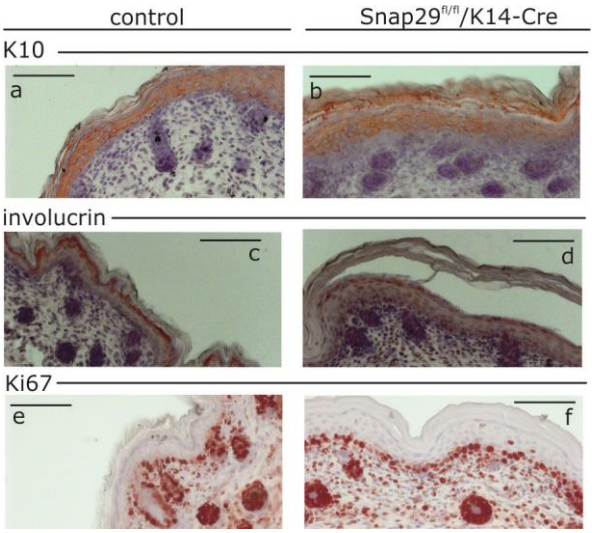

Figure S7: Immunohistochemical staining of dorsal *Snap29*$^{fl/fl}$/K14-Cre epidermis. (a/b) Immunohistochemistry revealed that the levels and distribution of the suprabasal keratin 10 (K10) did not changed significantly in *Snap29*$^{fl/fl}$/K14-Cre epidermis. (c/d) Involucrin levels were decreased in the cells of the upper *stratum spinosum* and *stratum granulosum* of *Snap29*$^{fl/fl}$/K14-Cre epidermis. (e/f) Staining with an anti-Ki67 antibody. Scale bars: 100 µm.