Appendix S1.

SUPPLEMENTARY MATERIAL AND METHODS

Primary keratinocyte culture

Primary human keratinocytes were obtained from skin derived from patients undergoing abdominal skin reconstruction. Isolation and cell culture was performed as described earlier (S1).

3D human epidermal equivalent (HEE) culture

Primary human keratinocytes were seeded into a transwell system (Greiner Bio-One, 0.4um pore size) in PCT epidermal keratinocyte medium (CellNTec) at a density of 100,000 cells per insert. After 2 days medium was changed to 3D barrier/DMEM medium (60:40 ratio). At day 3 the constructs were lifted to the air-liquid interface for another 10 days, the medium was changed every other day. For details see Niehues et al. (S2). Human epidermal equivalents (HEEs) were stimulated with cytokines from day 5 till day 8 of the air-liquid interface at depicted concentrations and harvested at day 8 for further analysis. Cytokines were all purchased from Preprotech.

Immunohistochemistry

HEEs were fixed in 4% buffered formalin solution, processed and embedded in paraffin. 6 µm sections were stained with haematoxylin and eosin or processed for immunohistochemistry using an indirect immunoperoxidase technique with avidin-biotin complex enhancement (Vectastain Laboratories). For antibodies and dilutions see **STable I**. For isotype control stainings of all used antibodies, see **SFig. S1**. Images of immunohistochemical staining were taken using the Axiokop 2 MOT microscope (Zeiss) at a magnification of ×40, Axiocam MRc5 digital camera and AxioVision software rel. 4.8 (Zeiss).

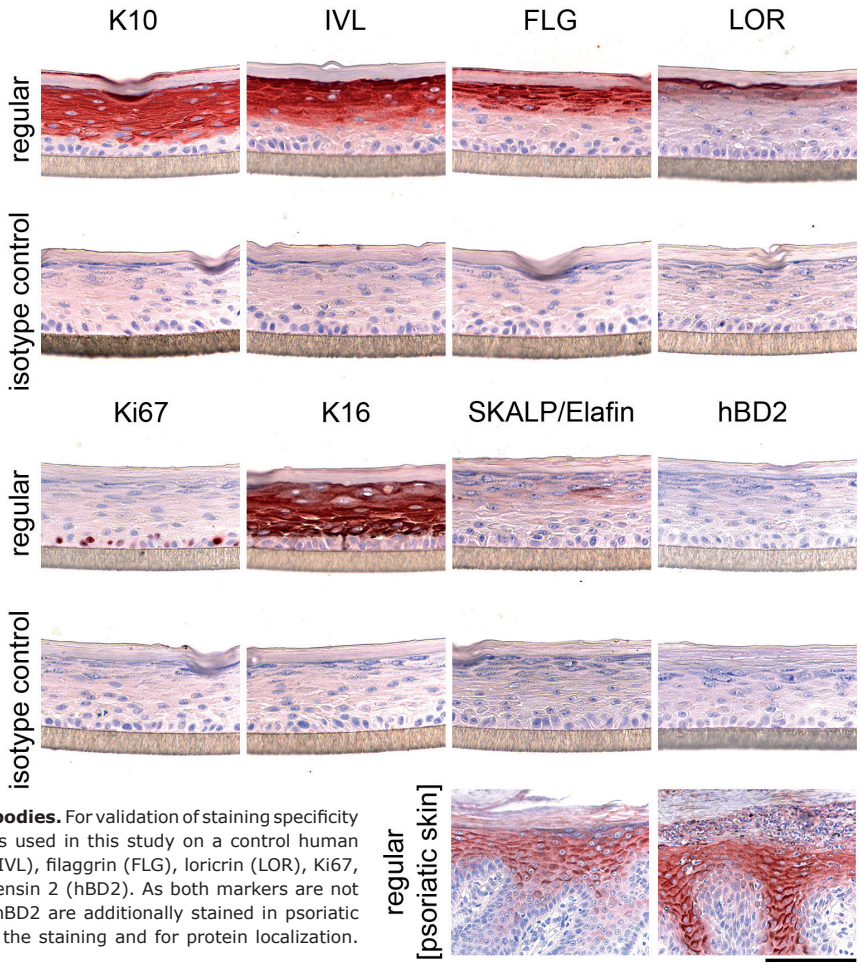
Quantitative real-time PCR

RNA was isolated using an RNA isolation kit (Favorgen) followed by DNase treatment of 1 µg of RNA. First-strand cDNA was generated with the iScript™ Reverse Transcriptase kit (Biorad) and the reverse transcriptase reaction products were used for quantitative real-time PCR amplification, which was performed with the MyiQ Single-Colour Real-Time Detection System for quantification with Sybr Green and melting curve analysis (BioRad, Hercules, California). Primers (**STable II**) were designed using PRIMER EXPRESS 1.0 software package (Applied Biosystems, Foster City, CA, USA) and the primers were designed to produce an amplicon length between 50 and 200 base pairs.

STable I. Antibodies used for immunohistochemistry

Antibody clone	Manufacturer	Host	Dilution
Filaggrin; 15C10	Novocastra, Newcastle upon Tyne, UK	Mouse	1:200
Loricrin; 145P-100	Biolegend, San Diego, USA	Rabbit	1:4000
Involucrin; Mon150	Generated by our own group (S5)	Mouse	1:10
Ki-67; MIB-1	DAKO, Heverlee, Belgium	Mouse	1:50
pAb to beta 2 defensin	Abcam, Cambridge, UK	Goat	1:200
SKALP/Elafin; 92-1	Generated by our own group (S6)	Rabbit	1:500
Keratin-10; DE-K10	LifeSpan Biosciences, Seattle, USA	Mouse	1:100
Keratin-16; LL025	Novocastra, Newcastle upon Tyne, UK	Mouse	1:50
Biotinylated horse anti-mouse IgG	Vectastain, Vector Laboratories, Burlingame, UK	Horse	1:200
Biotinylated goat anti-rabbit IgG	Vectastain, Vector Laboratories, Burlingame, UK	Goat	1:200
Biotinylated rabbit anti-goat IgG	Vectastain, Vector Laboratories, Burlingame, UK	Rabbit	1:200
HRP linked swine anti-rabbit	DAKO, Heverlee, Belgium	Swine	1:50

Primes were produced by Biolegio (Nijmegen, The Netherlands). Primer validation, PCR amplification conditions, and calculation of relative mRNA expression was performed as previously described (S3). The following qPCR program settings were used: 2 min at 50°C and 10 min at 90°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C, with data collection during the last 30 s. mRNA expression levels were normalized using the household gene RPLP0 and relative quantities were calculated according to the method of Livak & Schmittgen (S4).



SFig. 1. Isotype control stainings for all used antibodies. For validation of staining specificity we present isotype control stainings of all antibodies used in this study on a control human epidermal equivalents: keratin 10 (K10), involucrin (IVL), filaggrin (FLG), loricrin (LOR), Ki67, Keratin 16 (K16), SKALP/elafin and human beta defensin 2 (hBD2). As both markers are not always expressed in normal skin/HEEs, SKALP and hBD2 are additionally stained in psoriatic skin as positive control tissue is performed to show the staining and for protein localization. Scale bar=100 µm.

STable II. Primers used for quantitative (qPCR)

HUGO	Alias gene name	Forward primer 5'-3'	Reverse primer 3'-5'	E*
CCL2	Chemokine (C-C motif) ligand 2	gaagaatcaccagcagcaagtg	gatctccttggccacaatgg	2.1
CCL5	Chemokine (C-C motif) ligand 5	tctgcgctcctgcatctg	gggcaatgtaggcaaagca	1.9
CXCL8	C-X-C motif chemokine 10, IL-8	cttggcagccttcctgattt	ttctttagcactccttggcaaaa	2.1
CXCL10	C-X-C motif chemokine 10	ttcctgcaagccaattttgtc	tcttctcacccttctttttcattgt	2.0
DEFB4	Human beta defensin 2	gatgcctcttccaggtgttttt	ggatgacatatggctccactctt	2.0
FLG	Filaggrin	acttcactgagtttcttctgatggtatt	tccagacttgagggtctttttctg	1.9
HRNR	Hornerin	tacaaggcgtcatcactgtcatc	atctggatcgtttggattcttcag	2.1
IL9R	Interleukin 9 receptor	tggaggatgatgtggtagag	agtcagcaggagaaagatgg	2.1
IL33	Interleukin 33	ggaggcctgttactttaggagaga	aggcagcgagtaccagatgtc	1.9
IVL	Involucrin	acttatttcgggtccgctaggt	gagacatgtagagggacagagtcaag	1.9
K10	Cytokeratin-10	tggttcaatgaaaagagcaagga	gggattgtttcaaggccagtt	1.9
LCE3A	Late cornified envelope 3A	gagtcaccacagatgcc	cttgctgaccacttccc	2.0
LOR	Loricrin	aggttaagacatgaaggatttgcaa	ggcaccgatgggcttagag	2.1
PI3	Skalp/elafin	catgagggccagcagctt	tttaacaggaactcccgtgaca	2.0
RPLP0	hARP, 60S acidic ribosomal protein P0	caccattgaaatcctgagtgatgt	tgaccagcccaaaggagaag	2.0

*E is the efficiency as the fold increase in fluorescence per PCR cycle.

Statistics

Data are depicted as mean ± SD of at least 3 biological replicates. Statistical analysis of qPCR data was performed on ΔCt values using commercially available software (IBM, SPSS Statistics 22). One-way analysis of variance, followed by Bonferroni *post hoc* testing (in case of more than 2 groups), and 2-sided paired t-tests were performed (comparison of 2 groups). $p < 0.05$ was considered statistically significant.

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