## SUPPLEMENTARY TABLES AND FIGURES

### Table S1. Primers used for qPCR analysis.

| HUGO gene symbol | alias gene name | forward primer 5'-3' | reverse primer 3'-5' | *E |
|---|---|---|---|---|
| **CXCL10** | Chemokine (C-X-C motif) ligand 10 | ttcctgcaagccaattttgtc | tcttctcacccttcttttcattgt | 2.01 |
| **DEFB4** | Human beta defensin 2 | gatgcctcttccaggtgttttt | ggatgacatatggctccactctt | 1.99 |
| **LCE1A** | Late cornified envelope 1A | tgcaagagtggctgagatgc | agacaacacagttggtgtcagg | 2.18 |
| **LCE2A** | Late cornified envelope 2A | ggacctgtcccagagtgatg | gatccaggatgggctcttg | 2.10 |
| **LCE3A** | Late cornified envelope 3A | gagtcaccacagatgcc | cttgctgaccacttccc | 2.02 |
| **LCE4A** | Late cornified envelope 4A | cccccaaaatgtccctcaaagt | ggagccacagcaggaagagat | 1.88 |
| **LCE5A** | Late cornified envelope 5A | ccctctttatcctgccc | acaacacctagttctccaa | 1.87 |
| **LCE6A** | Late cornified envelope 6A | agaagcagcaatcttgga | cctttgggaactggaatg | 2.05 |
| **RPLP0** | hARP, 60S acidic ribosomal protein P0 | caccattgaaatcctgagtgatgt | tgaccagcccaaaggagaag | 2.02 |

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

**Table S2. Antibodies and their dilutions used for immunohistochemistry.**

| target protein | animal, code, manufacturer | dilution |
|---|---|---|
| **Cytokeratin-10** | Mouse-monoclonal, DE-K10, Euro Diagnostics | 1:100 |
| **Involucrin** | Mouse-monoclonal, MON-150[1] | 1:20 |
| **Ki67** | Mouse-monoclonal, MIB-1, DAKO | 1:50 |
| **Late cornified envelope protein 2** | By M. Narita (Cambridge Cancer Centre) | 1:1000 |
| **Late cornified envelope protein 3** | Mouse-monoclonal, clone-7[2] | 1:5000 |
| **Loricrin** | Rabbit-polyclonal, 145P, Convance | 1:2000 |
| **Transglutaminase-1** | Rabbit-polyclonal, H-87, Santa-Cruz | 1:100 |

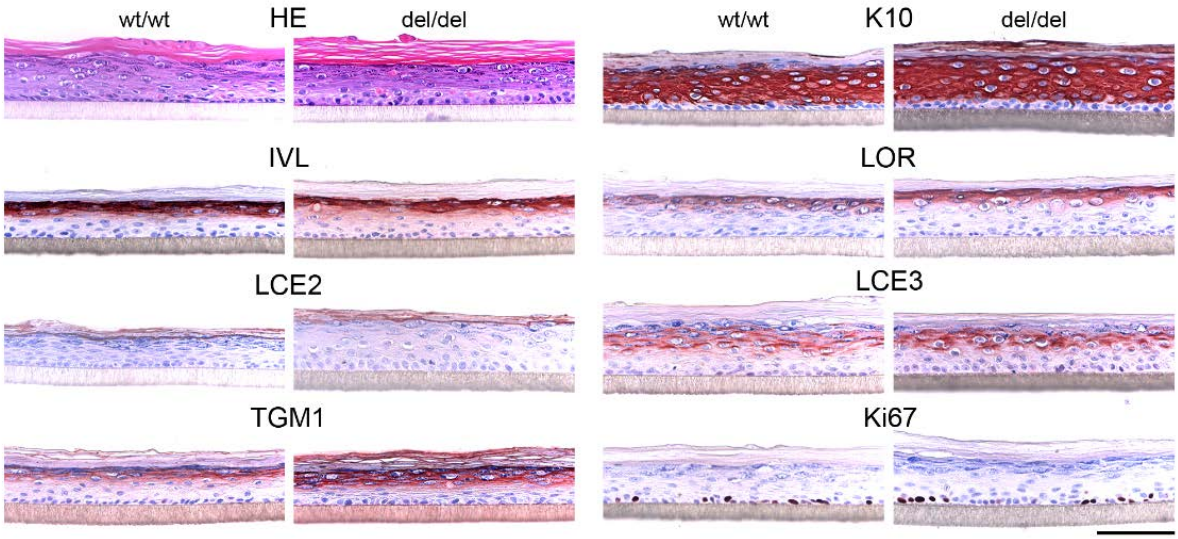


**Figure S1. General morphology, epidermal proliferation and expression of differentiation markers in *LCE3B/C wt/wt* and *del/del* 3D reconstructed epidermis.** *LCE3B/C wt/wt* and *del/del* 3D reconstructed epidermis models show equal stratification and morphology (H&E staining). Early (keratin 10, K10), late differentiation proteins (involucrin, IVL; loricrin, LOR; late cornified envelope 2, LCE2; late cornified envelope 3, LCE3, transglutaminase 1, TGM1) and the proliferation marker (Ki67) are expressed equally in both *LCE3B/C* genotype groups. Pictures are representative for N = 5 keratinocyte donors per *LCE3B/C* genotype group. Scale bar = 100 µm.

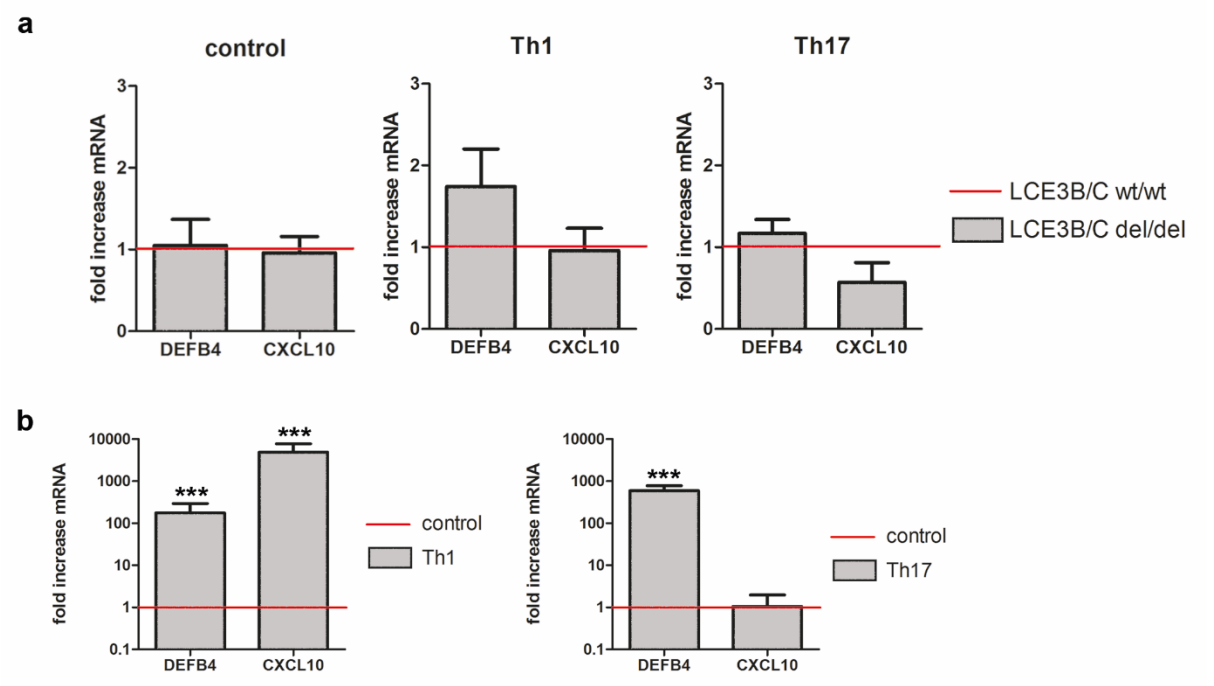


**Figure S2. Expression levels of *DEFB4* and *CXCL10* in *LCE3B/C wt/wt* and *del/del* reconstructed epidermis. (a)** Difference in mRNA expression levels of *DEFB4* and *CXCL10* comparing *LCE3B/C wt/wt* and *del/del* reconstructed epidermis under unstimulated, Th1 cytokine and Th17 cytokine stimulated conditions. N = 6 *LCE3B/C* wt/wt keratinocyte donors and N= 6 *LCE3B/C del/del* keratinocyte donors. Red line indicates *LCE3B/C* wt/wt expression level which is set to 1. **(b)** *DEFB4* and *CXCL10* are differentially induced upon Th1 (left figure) or Th17 stimulation (right figure). N = 12 keratinocyte donors (combined expression levels of N = 6 *LCE3B/C* wt/wt and N = 6 *LCE3B/C* del/del). Red line indicates expression level of unstimulated keratinocytes which is set to 1. Error bars represent SEM. * indicate statistical significant difference to control (LCE3B/C wt/wt genotype or unstimulated keratinocyte culture). ***P<0.0005. Repeated measures ANOVA, followed by Bonferroni post hoc testing was performed $P < 0.05$ was considered statistically significant.

**Table S3. Specifications of all bacterial species used in antimicrobial LCE assays.**

| panel Fig.3 | species | μM LCE for ≥ 1 log CFU reduction | | | Gram type | ATCC# | phylum | specifications |
|---|---|---|---|---|---|---|---|---|
| | | 3A | 3B | 3C | | | | |
| a | ***Acinetobacter baumannii*** | 10 | >10 | 10 | $Gram^{neg}$ | ATCC 345059 | Proteobacteria | Multidrug resistant opportunistic nosocomial pathogen |
| b | ***Escherichia coli*** | 1 | 10 | 3 | $Gram^{neg}$ | ATCC 25922 | Proteobacteria | Human gut flora |
| c | ***Pseudomonas aeruginosa*** | 10 | 3 | 1 | $Gram^{neg}$ | ATCC 27853 | Proteobacteria | Multidrug resistant pathogen; present in soil, water and skin flora |
| d | ***Proteus vulgaris*** | >10 | >10 | >10 | $Gram^{neg}$ | ATCC 3315 | Proteobacteria | Inhabitant of human skin flora |
| e | ***Corynebacterium aurimucosum*** | 0.3 | 1 | 1 | $Gram^{pos}$ | Clinical isolate | Actinobacteria | Multidrug resistant nosocomial pathogen, in skin or nose |
| f | ***Staphylococcus aureus*** | >10 | >10 | >10 | $Gram^{pos}$ | ATCC 29213 | Firmicutes | Inhabitant of human skin flora |
| g | ***Staphylococcus capitis*** | 1 | 3 | 3 | $Gram^{pos}$ | Clinical isolate | Firmicutes | Inhabitant of human skin flora |
| h | ***Staphylococcus epidermidis*** | 3 | 10 | 10 | $Gram^{pos}$ | ATCC 12228 | Firmicutes | Inhabitant of human skin flora |
| i | ***Streptococcus pyogenes*** | 10 | >10 | >10 | $Gram^{pos}$ | ATCC 12344 | Firmicutes | Opportunistic pathogen in human skin flora |
| j | ***Propionibacterium acnes*** | 3 | >10 | 10 | $Gram^{pos}$ | ATCC 6919 | Actinobacteria | Inhabitant of human skin flora |

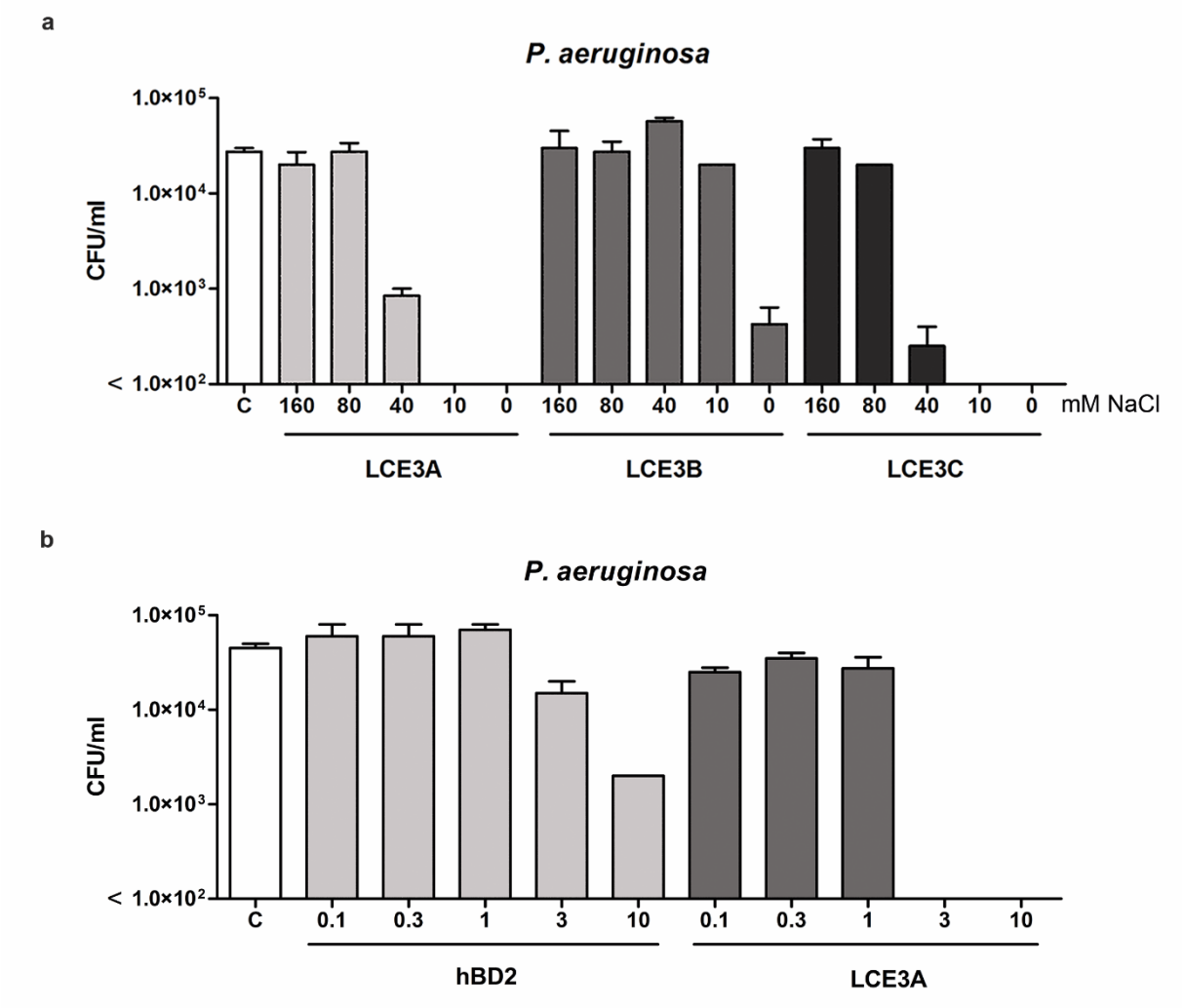


**Figure S3. *P. aeruginosa* antimicrobial assays comparing the influence of salt concentration in the assay buffer and activity of LCE proteins compared to hBD2. (a)** Dilution series of up to 160 mM NaCl added to the 10 mM sodium phosphate assay buffer with 10 µM LCE3 protein in an antimicrobial assay against *P. aeruginosa.* **b)** Antimicrobial activity of a dilution series between 10 and 0.1 µM hBD2 or LCE3A protein against *P. aeruginosa*. Note that the assay allows detection of about 2-3 logs reduction in CFU depending on the input number of bacteria in the assay. $2x10^2$ CFU/ml is the lower limit of detection, indicating zero colonies. Error bars present SEM and are based on CFU counts of several experiments. CFU = colony forming units; PB= sodium phosphate buffer.

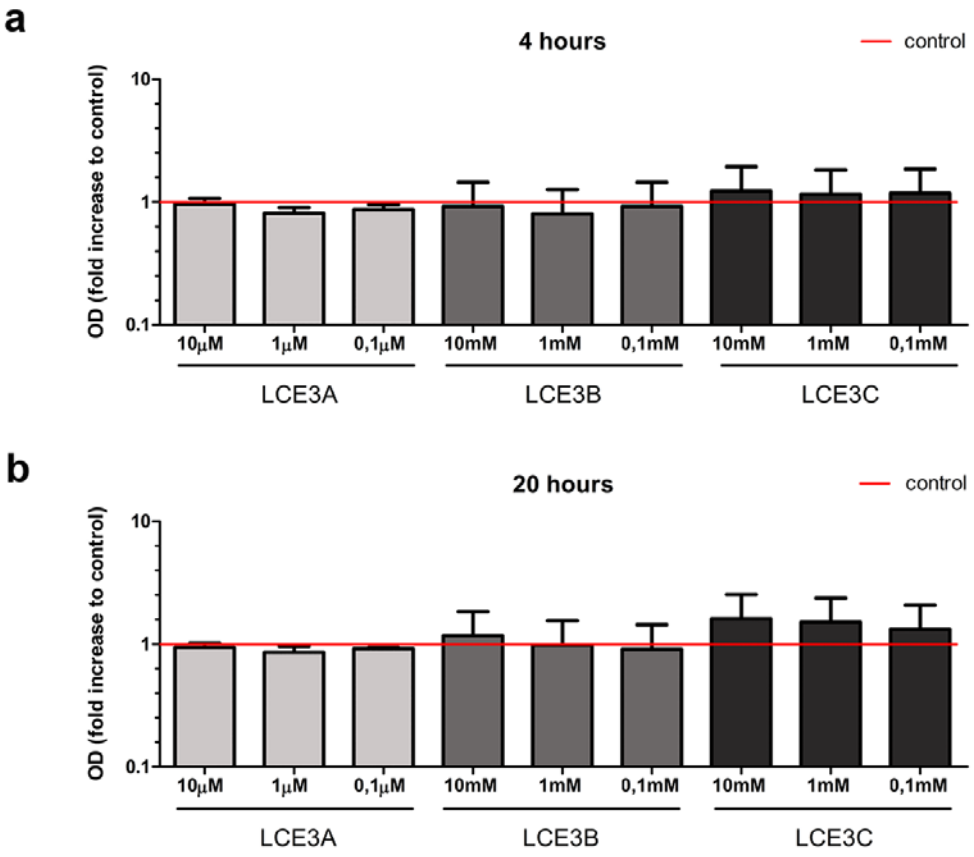


**Figure S4.** ***In vitro*** **toxicology of LCE protein on monolayer cultures of normal human epidermal keratinocytes.** Measurement of lactate dehydrogenase (LDH) release of keratinocytes exposed to the indicated doses of either reduced or oxidized LCE3A, LCE3B or LCE3C protein. The graph shows the fold change of optical density (OD) of lactate dehydrogenase amount after **(a)** 4 or **(b)** 20 hours incubation compared to control. As positive control cells were treated with Triton X-100. Fold increase of OD values of dead cells (cells treated with Triton X-100 as positive control) were >15. N = 3 keratinocyte donors. Error bars represent SEM.

## SUPPLEMENTARY REFERENCES


1. van Duijnhoven JL, Schalkwijk J, Kranenborg MH, van Vlijmen-Willems IM, Groeneveld A, van Erp PE, et al. MON-150, a versatile monoclonal antibody against involucrin: characterization and applications. Arch Dermatol Res 1992; 284:167-72.
2. Niehues H, van Vlijmen-Willems IM, Bergboer JG, Kersten FF, Narita M, Hendriks WJ, et al. Late cornified envelope (LCE) proteins: distinct expression patterns of LCE2 and LCE3 members suggest nonredundant roles in human epidermis and other epithelia. Br J Dermatol 2016; 174:795-802.