Single-cell RNA sequencing reveals 2D cytokine assay can model atopic dermatitis more accurately than immune-competent 3D setup

Judith Anna Seidel[1], Benjamin Al[1], Stephan Traidl[2], Nicholas Holzscheck[1], Sina Freimooser[2], Hendrik Miessner[1], Hendrik Reuter[1], Oliver Dittrich-Breiholz[2], and Thomas Werfel[2]

[1]Beiersdorf AG
[2]Medizinische Hochschule Hannover

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Abstract

Background: Modelling atopic dermatitis (AD) *in vitro* is paramount to understand the disease pathophysiology and identify novel treatments. Previous studies have shown that the Th2 cytokines IL-4 and IL-13 induce AD-like features in keratinocytes *in vitro*. However, it has not been systematically researched whether the addition of Th2 cells, their supernatants or a 3D structure are superior to model AD compared to simple 2D cell culture with cytokines. **Methods:** For the first time, we investigated what *in vitro* option most closely resembles the disease *in vivo* based on single-cell RNA sequencing data (scRNA-seq) obtained from skin biopsies in a clinical study and published datasets of healthy and AD donors. *In vitro* models were generated with primary fibroblasts and keratinocytes, subjected to cytokine treatment or Th2 cell cocultures in 2D/3D. Gene expression changes were assessed using qPCR and Multiplex Immunoassays. **Results:** Of all cytokines tested, incubation of keratinocytes and fibroblasts with IL-4 and IL-13 induced the closest *in vivo*-like AD phenotype which was observed in the scRNA-seq data. Addition of Th2 cells to fibroblasts failed to model AD due to the downregulation of ECM-associated genes such as POSTN. While keratinocytes cultured in 3D showed better stratification than in 2D, changes induced with AD triggers did not better resemble AD keratinocyte subtypes observed *in vivo*. **Conclusions**: Taken together, our comprehensive study shows that the simple model using IL-4 or IL-13 in 2D most accurately models AD in fibroblasts and keratinocytes *in vitro*, which may aid the discovery of novel treatment options.

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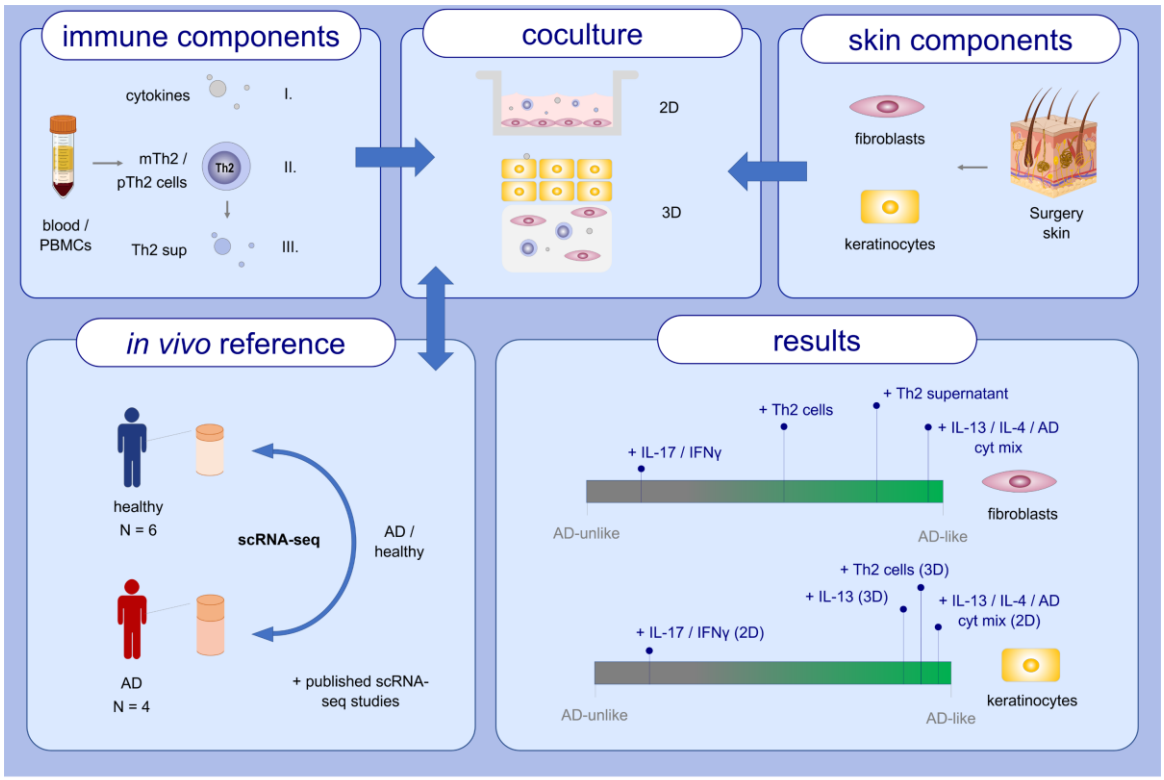

Graphical abstract

Highlights:

- We directly compared different *in vitro* skin cultures to scRNA-seq signatures from lesional atopic dermatitis patients.
- We found that Th2 cytokines induced a more *in vivo*-like fibroblast phenotype than direct coculture with Th2 cells.
- While three-dimensionality added stratification to keratinocytes *in vitro*, the response to AD cues was not more physiologically relevant than in 2D cultures.

Abbreviations:

AD = atopic dermatitis; AD cyt mix = atopic dermatitis cytokine mix (20 ng/ml IL-4, 25 ng/ml IL-13, 25 ng/ml IL-25, 15 ng/ml IL-31, 20 ng/ml TNFα); iTh2 = induced Th2 cells; mTh2 = mature Th2 cells; PBMCs = peripheral blood mononuclear cells; scRNA-seq = single cell RNA sequencing; Th2 sup = Th2 supernatant;

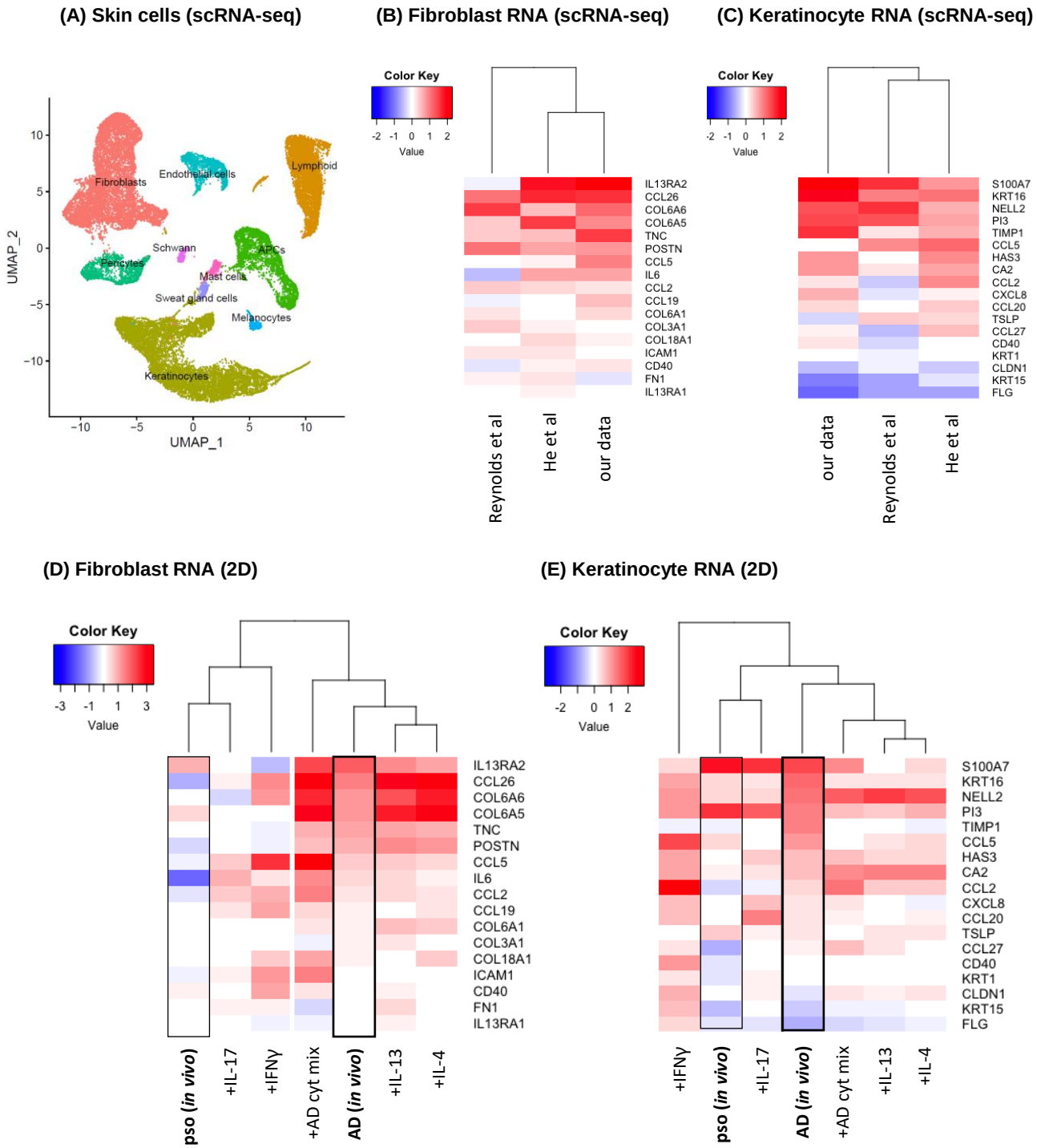

Figure 1: Relative AD marker gene expression in *in vivo* skin samples and comparison to cytokine-treated fibroblasts and keratinocytes *in vitro*.

(A) UMAP of integrated scRNA-seq data from healthy and lesional AD full thickness skin biopsies (our scRNA-seq data only).

(B), (C): Comparison of scRNA-seq data of fibroblasts (B) or keratinocytes (C) from three different studies showing the changes in AD lesional skin compared to healthy skin (log10 fold changes displayed).

(D), (E): Comparison of RNA differences in fibroblasts (D) or keratinocytes (E) induced with cytokine treatment in *in* vitro 2D culture (relative to untreated cells; using RT-qPCR) to differences in *in vivo* AD or psoriasis (pso) skin samples (compared to healthy controls; using scRNA-seq). Means of at least 3 different independent experiments for the *in vitro* data.

AD cyt mix (20 ng/ml IL-4, 25 ng/ml IL-13, 25 ng/ml IL-25, 15 ng/ml IL-31, 20 ng/ml TNFα), IL-17 (100 ng/ml), IFNγ (200 U/ml), IL-4 (100 ng/ml), IL-13 (100 ng/ml).

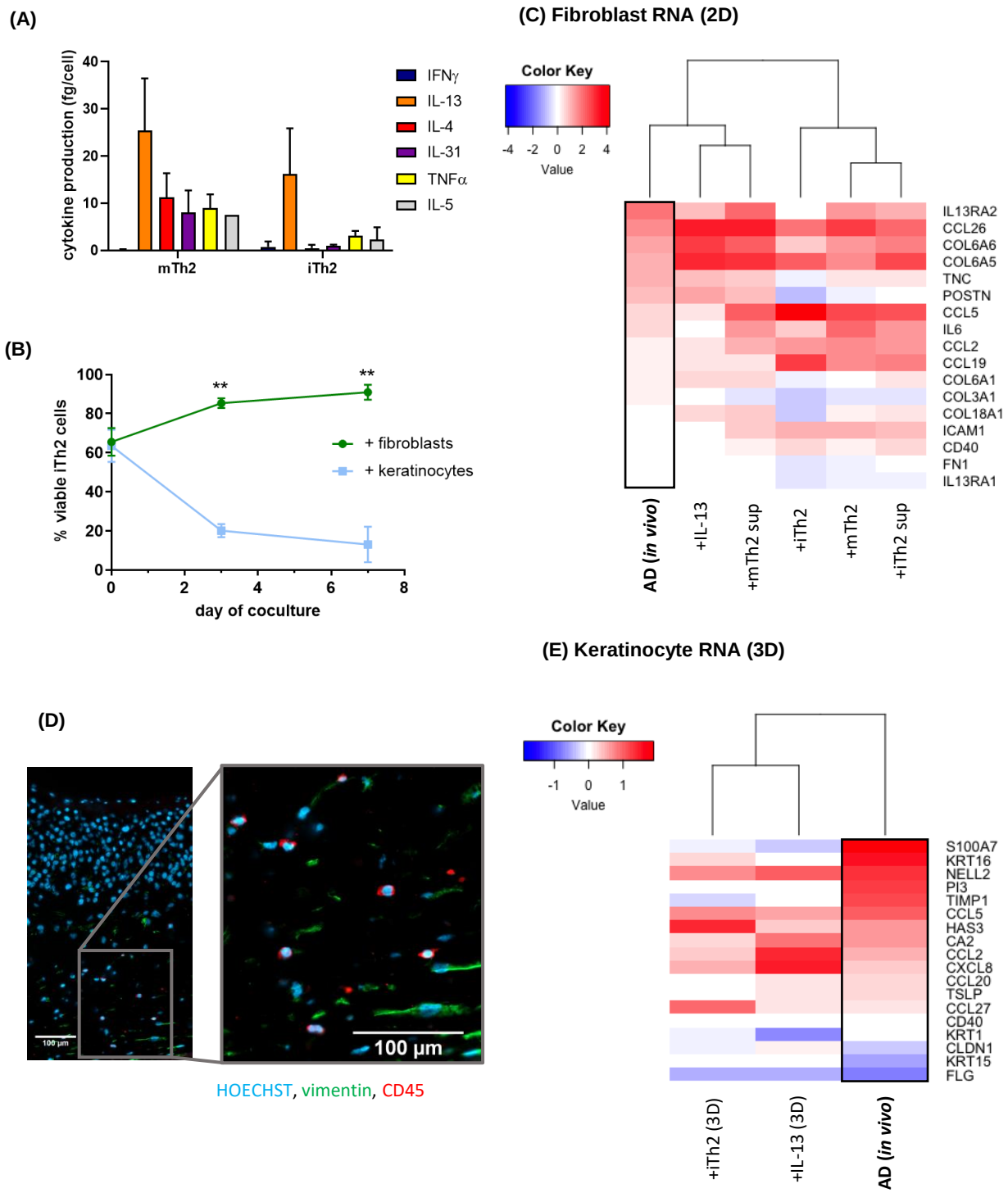

Figure 2: Effect of Th2 cell coculture on fibroblasts and keratinocytes *in vitro* compared to *in vivo*.

(A) Cytokine profiling of supernatants from iTh2 or mTh2 cells after 3d of (re-)activation using ELISA.

(B) Viability of iTh2 cells after 2D coculture with keratinocytes and fibroblasts using flow cytometry.

(C) Heatmap showing relative changes of AD RNA in fibroblast cultured in 2D after incubation with IL-13 or coculture with iTh2 or mTh2 cells or after incubation with their supernatants (sup) compared to untreated controls (log10) using RT-qPCR compared to changes in AD skin using scRNA-seq.

(D) Representative immunostaining of fibroblasts (vimentin) and T cells (CD45) in a 3D skin model containing keratinocytes, fibroblasts and iTh2 cells, which were continuously activated using ConcanavalinA.

(E) Heatmap containing relative changes of AD RNA in keratinocytes extracted from epidermis in 3D skin models after incubation with IL-13 or with iTh2 cells compared to untreated controls (log10 values) using RT-qPCR compared to changes in AD skin using scRNA-seq.

All data summarized at least three independent experiments. Mann-Whitney U test, **P<0.01

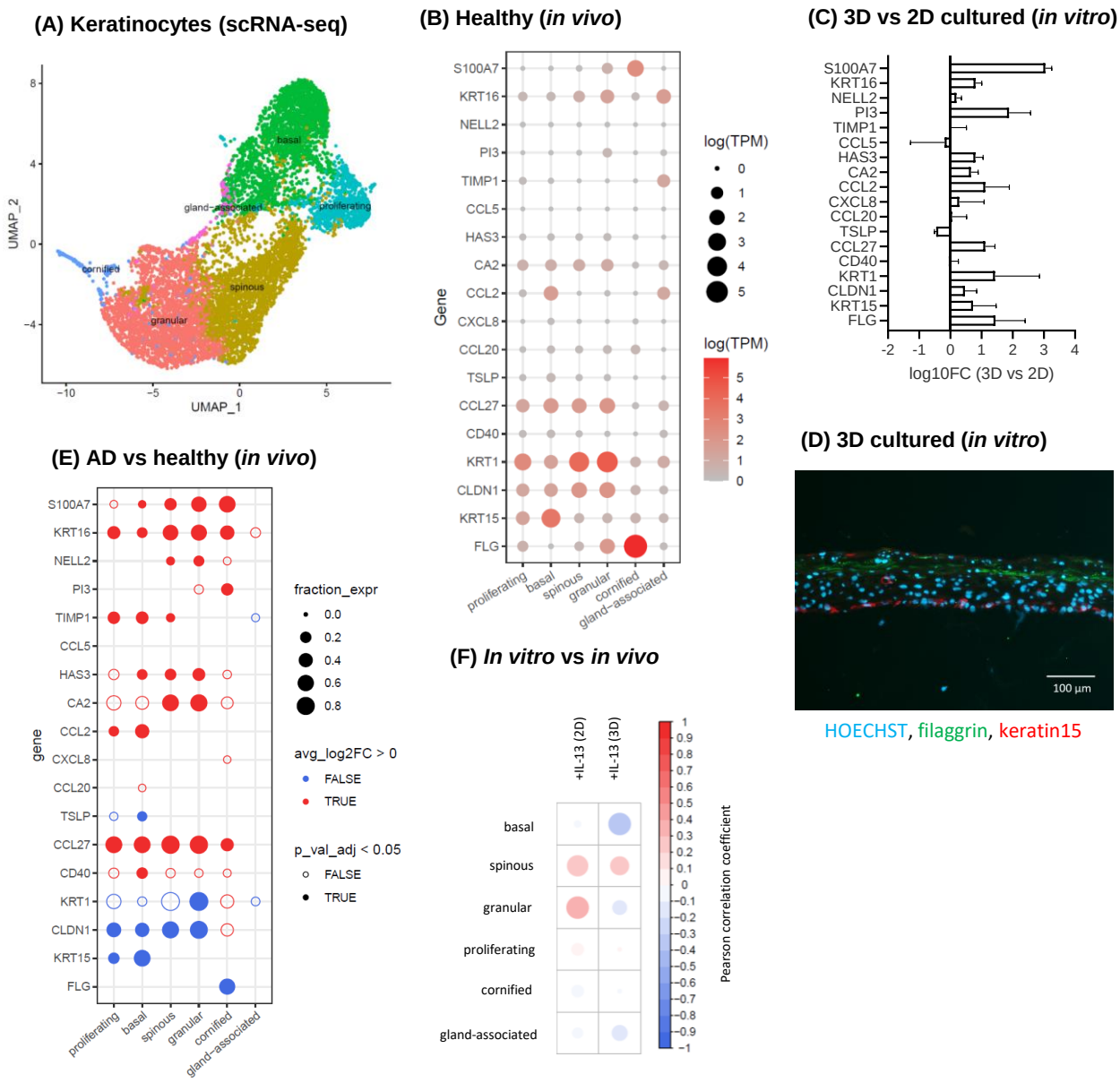

Figure 3: Relative AD marker gene expressions in subsets of healthy and lesional kerationcytes compared to keratinocytes cultured in 2D or 3D *in vitro*.

(A) UMAP plots of keratinocyte subsets from lesional and healthy full thickness skin biopsies using our scRNA-seq data.

(B) Dot plot showing the expression strength for all keratinocyte subtypes from healthy skin samples using scRNA-seq data from this study.

(C) Log10 fold changes of AD related genes in keratinocytes cultured in 3D (HSEs) compared to 2D using RT-qPCR. Means of 3 independent experiments.

(D) Representative immunostaining filaggrin (green) and keratin15 (red) in epidermis of human skin equivalents.

(E) Dot plot showing expression differences in lesional and healthy samples using scRNA-seq (this study only), with upregulation being colored in red and downregulation in blue, percentage of expressing cells being encoded by circle size and significance being depicted by filled circles.

(F) Correlogram depicting Pearson correlation coefficient of gene expression changes among different keratinocyte subsets cultured in 2D and 3D *in vitro* (RT-qPCR) compared to AD keratinocytes *in vivo* (scRNA-seq), with positive correlation colored in red and negative correlation in blue.