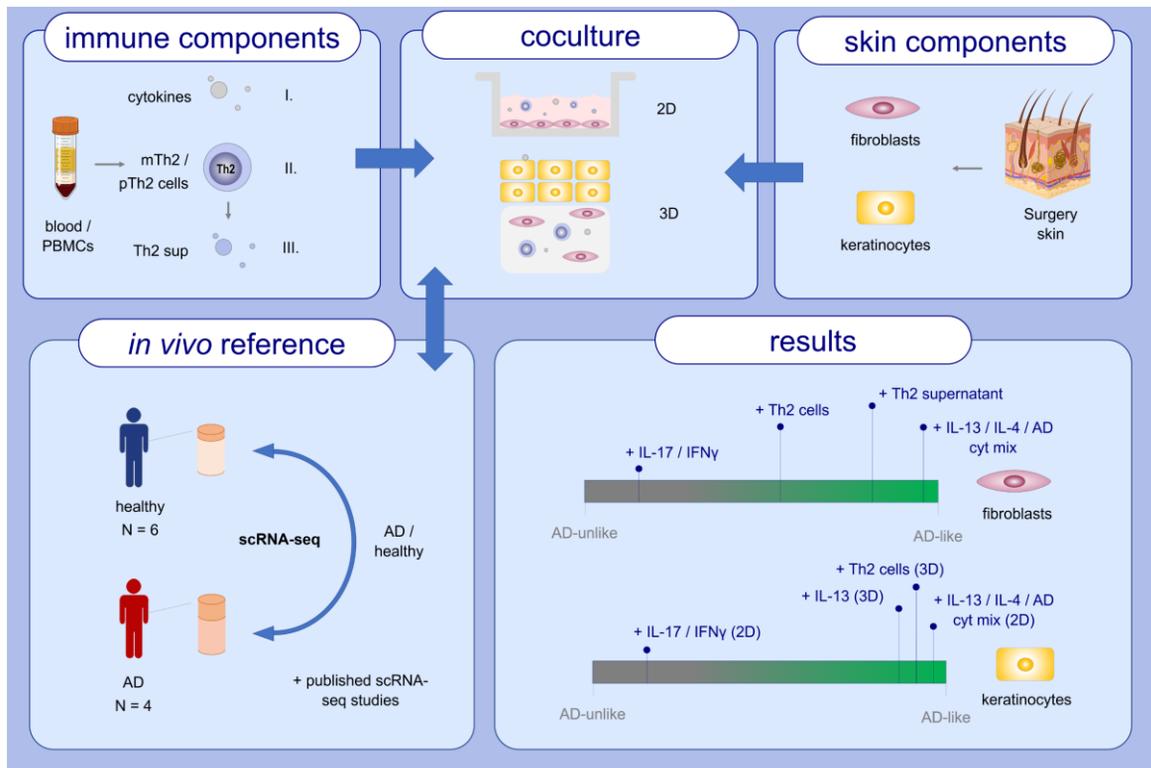


Single-cell RNA sequencing reveals 2D cytokine assay can model atopic dermatitis more accurately than immune-competent 3D setup



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 TNF α); iTh2 = induced Th2 cells; mTh2 = mature Th2 cells; PBMCs = peripheral blood mononuclear cells; scRNA-seq = single cell RNA sequencing; Th2 sup = Th2 supernatant;

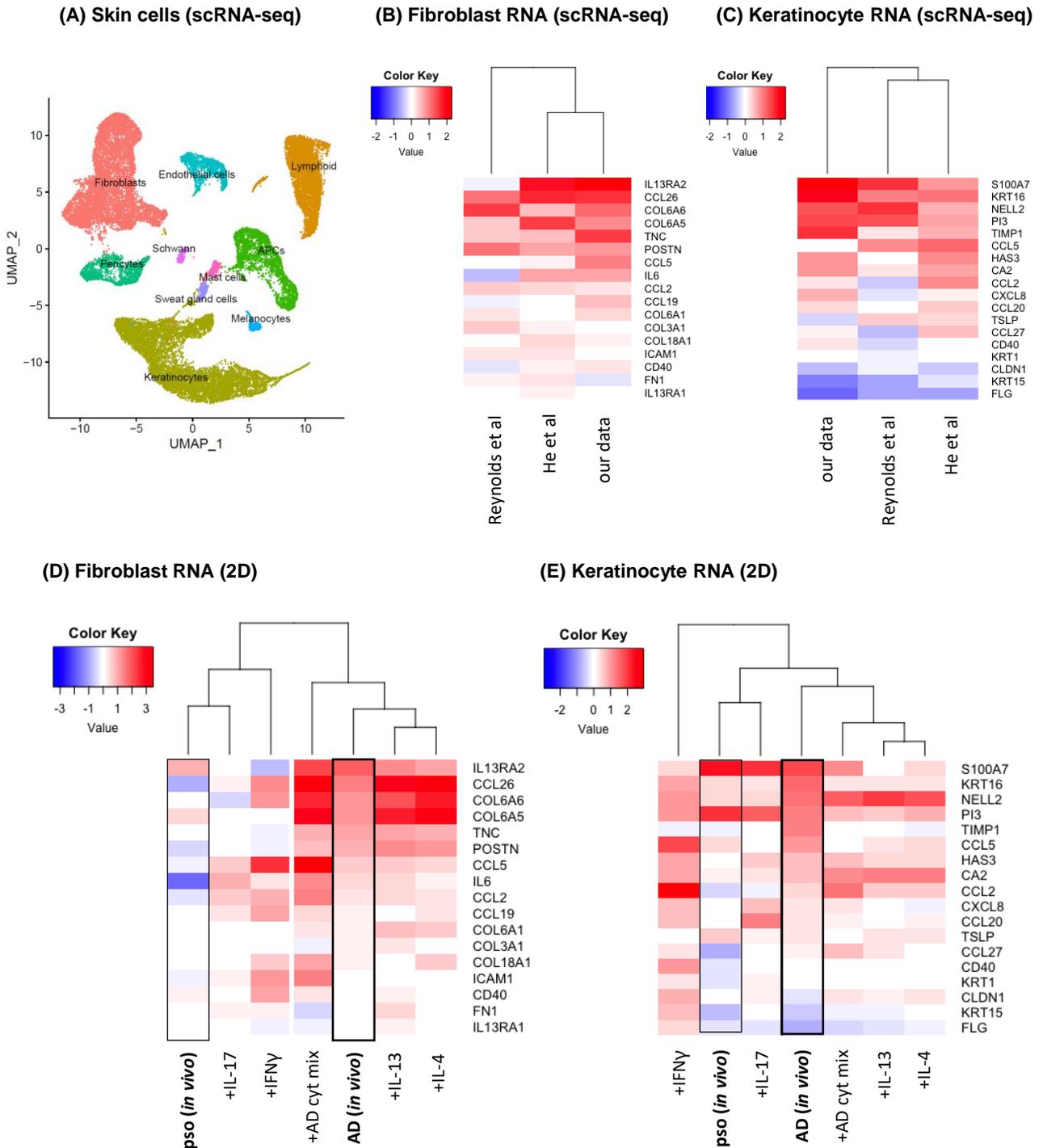


Figure 1: Relative AD marker gene expression in *in vivo* skin samples and comparison to cytokine-treated fibroblasts and keratinocytes *in vitro*.

(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 TNF α), IL-17 (100 ng/ml), IFN γ (200 U/ml), IL-4 (100 ng/ml), IL-13 (100 ng/ml).

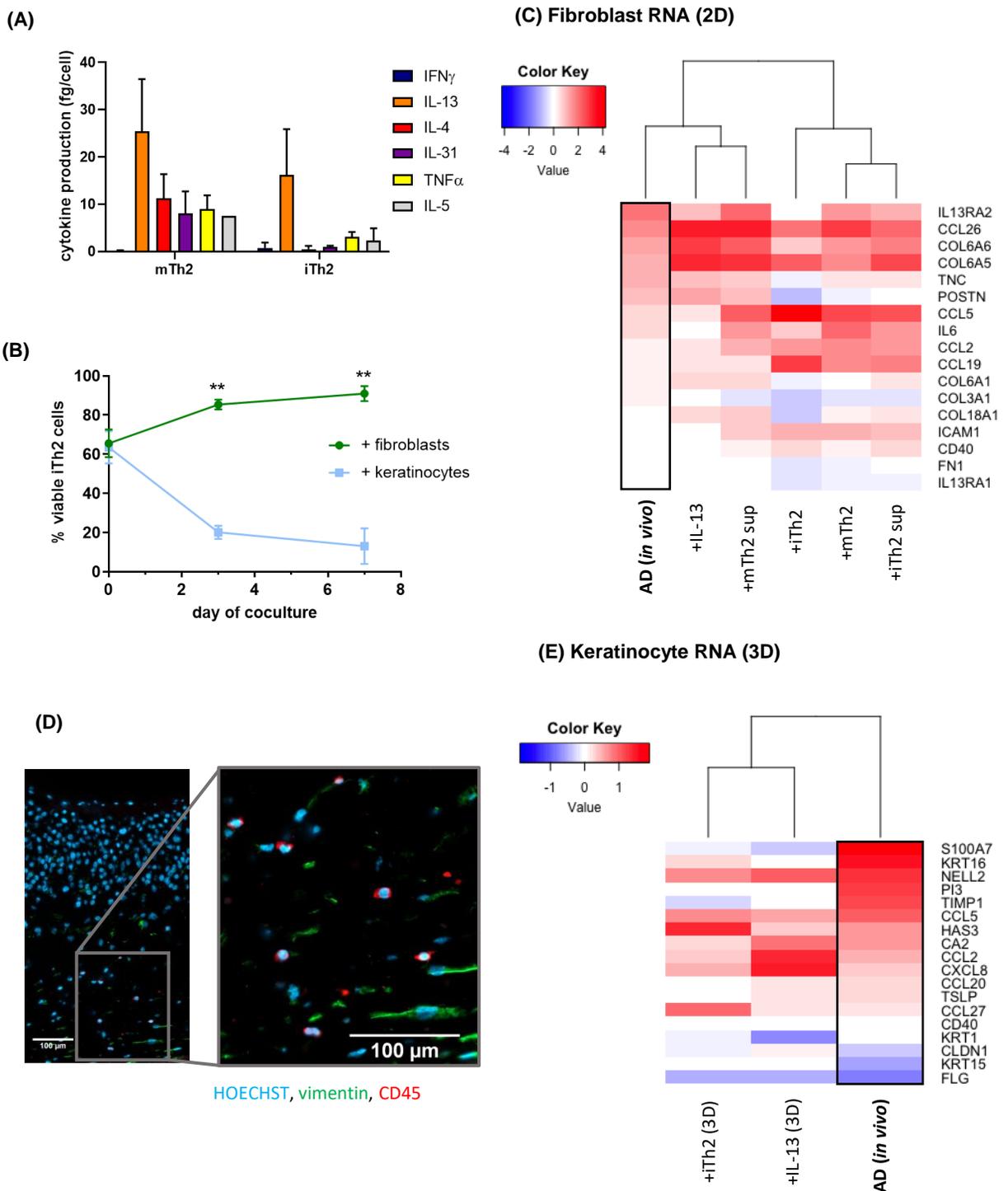


Figure 2: Effect of Th2 cell coculture on fibroblasts and keratinocytes *in vitro* compared to *in vivo*.

- (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

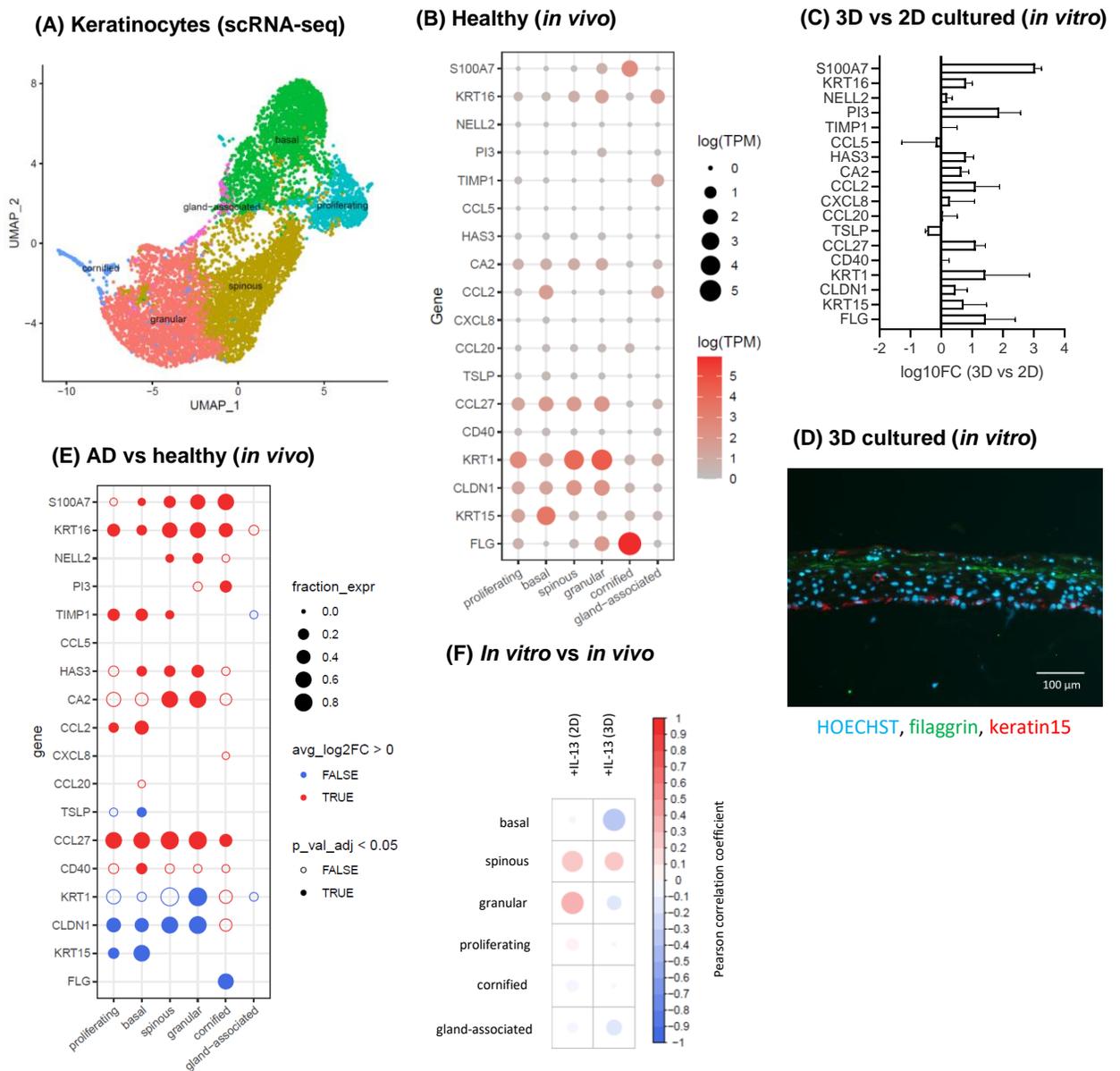


Figure 3: Relative AD marker gene expressions in subsets of healthy and lesional keratinocytes compared to keratinocytes cultured in 2D or 3D *in vitro*.

- (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.