Experimental Dermatology

WILEY

Experimental Dermatology

RESEARCH ARTICLE OPEN ACCESS

Dermal Fibroblasts, Not Keratinocytes, Dominate IL-17A/TNF-Driven Inflammation

Lejla Svraka[1,2] | Hakim Ben Abdallah[1,2] | Trine Bertelsen[1,2] | Christian Vestergaard[1,2] | Claus Johansen[1,2]

[1]Department of Dermatology, Aarhus University Hospital, Aarhus, Denmark | [2]Aarhus University, Aarhus, Denmark

Correspondence: Lejla Svraka (lejlasvraka@clin.au.dk)

Received: 5 November 2025 | **Revised:** 5 February 2026 | **Accepted:** 13 February 2026

Keywords: bulk RNA sequencing | hidradenitis suppurativa | in vitro | psoriasis | TNFR2 signalling

ABSTRACT

Chronic inflammatory skin diseases such as psoriasis and hidradenitis suppurativa are driven by cytokines, including IL-17A and TNF. Although biologics targeting these cytokines have transformed therapy, the transcriptional contributions of individual skin-resident cell types remain unclear, and dermal fibroblasts have been largely overlooked compared with keratinocytes. To address this, we compared the transcriptional responses of primary human dermal fibroblasts and keratinocytes following in vitro stimulation with IL-17A, TNF, or both, using bulk RNA sequencing and Western blotting. Dermal fibroblasts mounted a stronger and broader proinflammatory response than keratinocytes. This was particularly evident in response to TNF and combined TNF/IL-17A stimulation, with enrichment for immune signalling and chemotaxis pathways and robust induction of chemokine genes, including *CCL20*, *CXCL8*, and *IL6*. Keratinocytes primarily upregulated genes associated with epithelial differentiation, barrier function, and protein regulation, including *IL36G*, *S100A7A*, and *DEFB4A*. The heightened fibroblast responsiveness correlated with increased TNF sensitivity and substantially higher TNFR2 (*TNFRSF1B*) expression and signalling compared with keratinocytes, suggesting a fibroblast-specific mechanism amplifying inflammatory responses. These findings challenge the keratinocyte-centric view of skin inflammation and identify dermal fibroblasts as active contributors and potential therapeutic targets in TNF- and Th17-driven skin diseases.

1 | Introduction

Chronic inflammatory skin diseases like psoriasis and hidradenitis suppurativa (HS) are driven by dysregulated immune responses involving proinflammatory cytokines [1]. Among these, interleukin-17A (IL-17A) and tumour necrosis factor α (TNF) are key mediators of disease development [1, 2]. Although IL-17A can trigger inflammation independently, its co-activity with TNF often leads to a synergistic effect that amplifies inflammatory mediator expression, enhancing immune cell recruitment and sustaining chronic tissue inflammation [3, 4]. IL-17A is mainly secreted by the Th17 subset of $CD4^+$ T cells. This subset of T cells differentiates from naïve T cells under the influence of IL-6 and transforming growth factor-beta (TGF-β), with IL-23 further promoting their pathogenic activity [3]. In contrast, TNF is produced by a wider range of immune and stromal cells [5]. Mechanistically, TNF primarily signals through the activation of the NF-κB pathway. While IL-17A can also activate NF-κB, its downstream signalling often involves CCAAT/enhancer-binding proteins (C/EBPs), which also control the expression of proinflammatory genes [3]. The clinical significance of IL-17A and TNF is highlighted by the success of therapies targeting these cytokines in treating both psoriasis and HS [6, 7]. However, the different responses of distinct skin-resident cell types to IL-17A and TNF remain poorly understood.

The skin is composed of multiple immunoresponsive cell types, with keratinocytes and dermal fibroblasts representing two key populations [8]. Keratinocytes, the predominant

cells of the epidermis, serve not only as a physical barrier but also as active participants in immune surveillance [1, 9]. They have long been the central focus of studies on inflammatory skin diseases, recognised for their responsiveness to cytokine signalling [3, 10]. In chronic skin conditions, keratinocyte dysfunction is frequently linked to altered proliferation and differentiation, contributing to disrupted epidermal architecture [1]. IL-17A is known to drive these pathological changes by promoting keratinocyte proliferation and the expression of inflammatory genes [11]. However, IL-17A's full inflammatory potential is most evident when acting in synergy with TNF [11]. Together, these cytokines induce robust expression of proinflammatory mediators such as CXCL8, CCL20, IL-36 family cytokines, and antimicrobial peptides [1].

In addition to keratinocytes, dermal fibroblasts are a major skin cell population responsible for maintaining the extracellular matrix and tissue homeostasis [12]. Early studies, although limited in scope, have shown that stimulation of dermal fibroblasts with IL-17A and TNF leads to the upregulation of inflammatory mediators, including IL-6, CXCL8, and CXCL1 [13, 14]. These cytokines exhibit synergistic effects in dermal fibroblasts similar to those observed in keratinocytes [13–16]. Synovial fibroblasts are well-established cells of tissue remodelling and inflammation in rheumatologic diseases, where their activation contributes to chronic joint damage and the recruitment of immune cells [17]. Recent evidence increasingly recognises fibroblasts as active contributors to chronic skin inflammation as well, with distinct subpopulations displaying proinflammatory phenotypes [12, 18]. Yet, comprehensive transcriptional profiling of dermal fibroblast responses to IL-17A and TNF remains scarce. No study to date has directly compared the responses of keratinocytes and dermal fibroblasts to these cytokines, representing a significant gap in our understanding of cell-type-specific inflammatory pathways in the skin.

We hypothesize that keratinocytes and dermal fibroblasts exhibit distinct transcriptional responses to IL-17A, TNF, and their combined stimulation. Despite their abundance, dermal fibroblasts have been relatively understudied in the context of skin inflammation and may mount a different inflammatory response than keratinocytes. To address this, we compare cytokine-induced gene expression in both cell types in vitro through bulk RNA sequencing (RNA-seq), aiming to deepen our understanding of the distinct inflammatory mechanisms in these two cell types.

2 | Methods

2.1 | Cell Cultures

Skin samples obtained from healthy adults undergoing plastic surgery were used to isolate both primary human dermal fibroblasts and keratinocytes ($n = 4$). The dermal fibroblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, ThermoFisher Scientific, Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS), and antibiotics (10 μg/mL penicillin/streptomycin and 5 μg/mL gentamicin) (Gibco). The keratinocytes were cultured in Keratinocyte serum-free medium (SFM) (Gibco) supplemented with growth factors (Gibco) and 5 μg/mL gentamicin (Gibco) as previously described [19]. Prior to stimulation, the medium was changed to fibroblast basal medium (DMEM +2% FBS) and keratinocyte basal medium (Keratinocyte SFM without growth factors). The cells were incubated at 37°C and 5% CO_2 until they reached 70%–80% confluency. The cells were stimulated with a vehicle (PBS with 0.15% bovine serum albumin) as a control, 100 ng/mL IL-17A (R&D Systems, Bio-Techne, Minneapolis, Minnesota, USA), and/or 10 ng/mL TNF (R&D Systems). Cells were harvested after 24 h.

2.2 | RNA Isolation and Bulk RNA Sequencing

RNA was isolated using the SV 96 Total RNA Isolation System (Promega, Madison, WI, USA) following the manufacturer's protocol. RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA).

Paired-end RNA sequencing was carried out by Eurofins Genomics Europe Sequencing GmbH's protocols in Konstanz, Germany. Employing the NEBNext Ultra II Directional RNA Library Prep Kit, sequencing libraries were constructed and processed on the Illumina NovaSeq 6000 platform using the 2 × 150 Sequence mode, to secure at least 20 million read pairs. Quality assessment of the raw sequence data was accomplished utilising FastQC (version 0.12.0). The R package Subread (version 2.10.3) was used for the alignment of reads to the human reference genome (GRCh38.p14 primary assembly) [20]. Unique and unambiguous alignments were retained for subsequent analyses. Genes present in > 5 samples with normalised counts ≥ 5 were included for further analysis. Correction of library size together with variance stabilisation was effectuated through the application of variance stabilising transformation (vst) using the DESeq2 R package (version 1.36.0) [21]. The vst normalised data were utilised in subsequent analyses, including principal component analysis (PCA) and heat map visualisation using pheatmap (version 1.0.12). DESeq2 was also employed for conducting differential gene expression analyses, with independent filtering enabled [21]. Pairwise comparisons were conducted using the Wald test. Significance thresholds for identifying differentially expressed genes (DEGs) were set at False Discovery Rate (FDR)-adjusted p-values < 0.05 and $|\log2(\text{fold change})| \geq 1$. Gene set enrichment analysis (GSEA) was performed using fsgea (version 1.28.0) and the genes from the differential expression analysis ranked by $\log_2(\text{fold change}) * -\log_{10}(p\text{-value})$. Reactome and Gene Ontology biological processes (GO:BP) gene sets were obtained from the Molecular Signatures Database (MSigDB, version 7.5.1) through the MSigBR R package.

2.3 | Protein Isolation and Western Blotting

Equal amounts of protein were separated by SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were incubated with primary antibodies against TNFR2 (catalogue #72337) and β-actin (catalogue #8457) (Cell Signalling

Technology, Danvers, MA, USA). Detection was carried out using either an HRP-conjugated anti-rabbit IgG (Cell Signalling Technology) or an HRP-conjugated anti-mouse IgG (Dako, Glostrup, Denmark), as appropriate. Signal development was performed using an enhanced chemiluminescence (ECL) system (Amersham Biosciences, Piscataway, NJ, USA) following the manufacturer's protocol.

2.4 | Statistical Analysis

Statistical differences were assessed using paired *t*-tests. For non-normally distributed data, the Wilcoxon signed-rank or Mann–Whitney tests were applied. A p-value < 0.05 was considered statistically significant. All analyses were conducted with GraphPad Prism version 10.6.0 or R version 4.3.2.

3 | Results

3.1 | Dermal Fibroblasts and Keratinocytes Show Distinct Transcriptomic Responses to IL-17A and TNF Stimulation

To investigate the inflammatory responses of human dermal fibroblasts and keratinocytes, we cultured both cell types in vitro and stimulated them with proinflammatory cytokines: IL-17A, TNF, or a combination of both for 24 h. Bulk RNA-seq was then performed to identify DEGs (*FDR-adjusted p-value < 0.05 and log2 fold change > 1*) relative to vehicle-treated controls. Principal component analysis (PCA) showed that transcriptional profiles were separated by cell type, indicating distinct responses to cytokine stimulation in keratinocytes and dermal fibroblasts (Figure 1a). Dermal fibroblasts showed pronounced clustering by treatment,

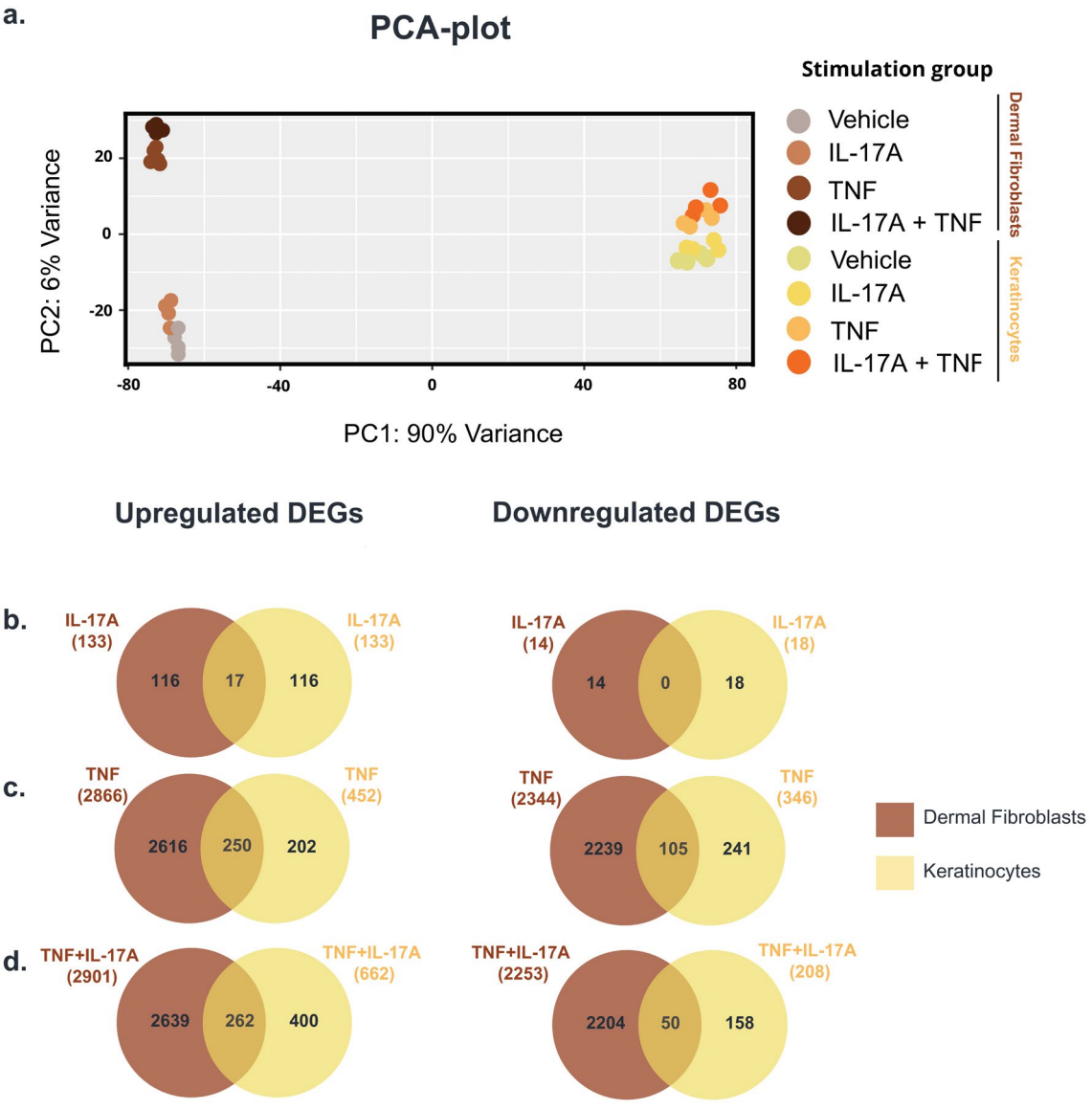

FIGURE 1 | Transcriptomic profiling of primary human dermal fibroblasts (Fb) and keratinocytes (Kc) following cytokine stimulation. Data are derived from bulk RNA-seq analysis of in vitro-stimulated dermal fibroblasts and keratinocytes. (a) Principal component analysis (PCA) of gene expression profiles across stimulation conditions. (b) Venn diagrams showing overlap of DEGs in IL-17A-stimulated dermal fibroblasts and keratinocytes. (c) Venn diagrams for TNF stimulation. (d) Venn diagrams for combined IL-17A + TNF stimulation.

with TNF and TNF + IL-17A inducing the most divergent expression profiles relative to controls. Keratinocytes also displayed treatment-specific shifts, although the separation was less marked than in dermal fibroblasts. Venn diagrams showed that IL-17A alone induced a moderate transcriptional response in both cell types, with 133 DEGs in both dermal fibroblasts and keratinocytes, and limited overlap between them (Figure 1b). In contrast, TNF alone induced a strong transcriptional response, particularly in dermal fibroblasts, with 2866 DEGs compared to 452 DEGs in keratinocytes (Figure 1c). The combination of TNF and IL-17A further increased the number of regulated genes in both cell types, with 2901 DEGs in fibroblasts and 662 in keratinocytes, and a moderate degree of overlap between them (Figure 1d). Across all conditions, the number of upregulated genes outnumbered downregulated genes, and the gene overlap between dermal fibroblasts and keratinocytes was generally higher among upregulated DEGs. Notably, each condition revealed a substantial number of cell-type-exclusive DEGs, underscoring distinct transcriptional programs in fibroblasts and keratinocytes in response to TNF and IL17-A.

3.2 | Dermal Fibroblasts and Keratinocytes Upregulate Distinct Gene Sets in Response to Cytokine Stimulation

To characterise the functional profiles of the transcriptional responses, GSEA enrichment analysis using GO:BP was performed in dermal fibroblasts and keratinocytes following cytokine stimulation. For each condition, the ten most significantly enriched terms with positive normalised enrichment score (NES) are presented (Figure 2a). Under IL-17A stimulation, dermal fibroblasts showed pronounced enrichment for immune- and inflammation-related processes, including *regulation of immune effector processes*, *inflammatory response*, and *defence response*, reflecting their chemotaxis- and immune signalling-dominated transcriptional profile.

In contrast, keratinocytes displayed enrichment for regulatory and metabolic pathways, including *non-coding RNA (ncRNA) catabolic process*, *ncRNA metabolic process*, *microRNA (miRNA) metabolic process*, *miRNA catabolic process*, *regulation of protein deubiquitination*, and *autophagy-related processes*. Following TNF stimulation, dermal fibroblasts again exhibited enrichment of immune and inflammatory pathways, with greater enrichment scores and statistical significance compared to IL-17A stimulation. Keratinocytes, while still enriched for protein regulatory processes such as protein deubiquitination processes, showed activation of immune-related categories. Under combined TNF + IL-17A stimulation, both dermal fibroblasts and keratinocytes adopted a convergent transcriptional profile dominated by immune and inflammatory processes.

To investigate cell-type-specific transcriptional responses to proinflammatory cytokine stimulation, we identified DEGs that were uniquely upregulated in either dermal fibroblasts or keratinocytes following stimulation with IL-17A, TNF, or both. Genes previously implicated in inflammatory or skin-relevant pathways were selected for visualisation using a z-score normalised heatmap (Figure 2b). Distinct expression patterns emerged across the two cell types. Dermal fibroblasts showed a stronger transcriptional response to TNF than to IL-17A, whereas keratinocytes responded more strongly to IL-17A. In both cell types, the combined stimulation of IL-17A and TNF resulted in the highest induction levels, consistent with previously reported synergistic effects [11, 14].

The transcriptional profile of dermal fibroblasts was characterised by the induction of genes linked to chemotaxis and immune signalling. Notably, strong unique upregulation was observed

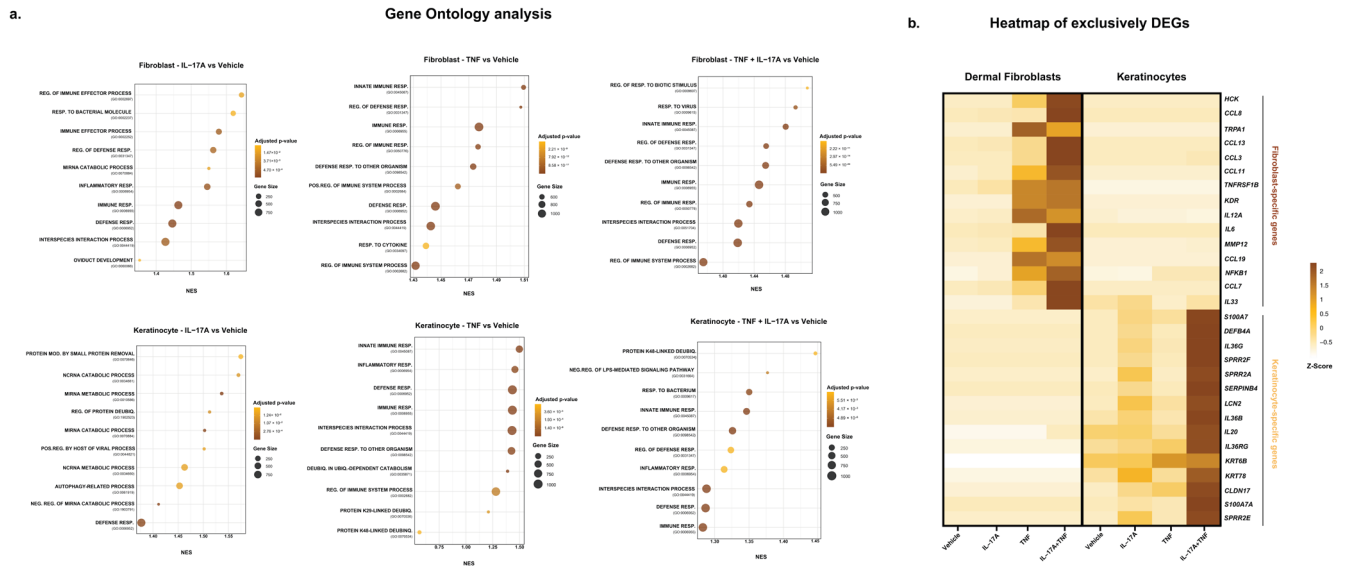

FIGURE 2 | Cell type-specific inflammatory gene signatures and enriched biological processes following cytokine stimulation. Data are derived from bulk RNA-seq analysis of in vitro-stimulated dermal fibroblasts and keratinocytes. (a) GO:BP enrichment analysis using GSEA based on the full ranked gene list from dermal fibroblasts and keratinocytes following stimulation. For each condition, the ten most significantly enriched GO:BP terms with positive NES are shown. Dot plots display the NES on the x-axis, with dot size representing gene size and colour intensity indicating adjusted *p*-value significance. Abbreviations: RESP. response, REG. regulation, POS. positive, NEG. negative, DEUBIQ. deubiquitination, UBIQ. ubiquitin, LPS lipopolysaccharide. (b) Heatmap showing *z*-score of vst normalised expression of DEGs uniquely regulated in dermal fibroblasts and keratinocytes upon stimulation. Genes selected for visualisation were based on prior implication in inflammatory or skin-relevant pathways.

for multiple chemokines (*CCL8*, *CCL13*, *CCL3*, *CCL11*, *CCL19*, and *CCL7*). In addition, dermal fibroblasts selectively expressed several genes involved in receptor-mediated immune signalling (*TNFRSF1B*, *IL12A*, *KDR*). We also observed upregulation of several other genes commonly linked to inflammation in different contexts, including *HCK*, *TRPA1*, *IL6*, *MMP12*, *NFKB1*, and *IL33*. In contrast, keratinocytes displayed a transcriptional profile centered on epithelial differentiation, keratinization, and barrier maintenance. We observed a strong unique induction of small proline-rich proteins (*SPRR2F*, *SPRR2A*, *SPRR2E*), hyperproliferation-associated keratins (*KRT6B*, *KRT78*), and barrier-defence genes (*S100A7*, *S100A7A*, *DEFB4A*, *LCN2*). Several skin-associated interleukins (*IL36G*, *IL36B*, *IL36RG*, *IL20*) were also uniquely expressed in keratinocytes (Figure 2b).

In summary, cytokine stimulation induced cell–type–specific transcriptional programs, with dermal fibroblasts enriched for immune signalling and chemotaxis, and keratinocytes for epithelial differentiation, barrier defence, and metabolic regulation of proteins.

3.3 | Dermal Fibroblasts Exhibit Higher Expression of Proinflammatory Mediators Than Keratinocytes

To assess whether dermal fibroblasts exhibit a stronger proinflammatory response than keratinocytes, we analysed DEGs that were upregulated in both cell types in response to each stimulation. We then restricted the analysis to genes annotated to inflammatory processes, including *inflammatory response* (GO:0006954), *acute inflammatory response* (GO:0002526), *innate immune response* (GO:0045087), *response to cytokine* (GO:0034097), *immune response* (GO:0006955), and *cytokine-mediated signalling pathway* (GO:0019221).

Gene expression patterns were compared between cell types using z-score normalised heatmaps. Upon TNF stimulation, both keratinocytes and dermal fibroblasts exhibited upregulation of a shared set of inflammatory mediators, including chemokines (*CCL2*, *CCL5*, *CXCL5*, *CXCL8*, *CXCL10*, *CXCL11*), cytokines (*IL32*, *TNF*), and interferon-induced effectors (*GBP4*, *GBP5*, *EBI3*) (Figure 3a). These genes were among the most highly induced compared to vehicle in both cell types, although dermal fibroblasts consistently displayed significantly higher fold changes. For instance, *CCL5* and *CXCL11* reached fold changes exceeding 13 000 and 8900, respectively, in dermal fibroblasts, compared to 14.9 and 7.9 in keratinocytes. Keratinocytes, while responsive, exhibited a lower induction, consistent with their primary role in maintaining the epithelial barrier rather than orchestrating broad inflammatory programs.

Upon co-stimulation with IL-17A and TNF, both cell types maintained induction of many core TNF-responsive mediators, but dermal fibroblasts still maintained a more inflammatory profile with higher fold inductions (Figure 3b). The response remained dominated by a strong amplification of neutrophil-attracting chemokines. In the co-stimulation group, additional chemokines emerged, including *CXCL1*, *CXCL2*, *CXCL3*, and *CXCL6*, while the recurrent mediators *CXCL8* and *CXCL5* showed markedly higher fold changes in both cell types, consistent with known IL-17A/TNF synergy. Dermal fibroblast, however, had a higher fold change in 83% of all genes in the list. The strongest fold change in dermal fibroblasts was observed for *IL1B*, *CCL20*, *CSF3*, *IL23A*, and *CXCL8*, all of which are well-established mediators of skin inflammation. However, the gene expression levels for *IL1B*, *IL1A* and *IL1RN* were greater in keratinocytes. IL-17A alone (Figure S1) induced a compact profile dominated by neutrophil-recruiting chemokines (*CXCL1*, *CXCL2*, *CXCL3*, *CXCL5*, and *CXCL6*), with higher fold changes in dermal fibroblasts compared to keratinocytes, indicating a continued fibroblast-dominant induction pattern. Together, these data demonstrate that dermal fibroblasts act as the primary amplifiers of a broader inflammatory signalling across all stimulation conditions. In contrast, keratinocytes, despite being responsive, adopt a more restrained and regulatory role.

3.4 | Enhanced TNF Responsiveness in Fibroblasts Correlates With Elevated *TNFRSF1B* Expression

To better understand the enhanced proinflammatory phenotype of dermal fibroblasts, particularly given their strong response to TNF, we performed GSEA using Reactome pathways. TNFR2 signalling emerged as one of the top 10 significantly enriched pathways following both TNF and IL-17A + TNF stimulation in dermal fibroblasts (Figure S2). This enrichment was not observed in keratinocytes, suggesting a cell-type-specific activation of TNFR2-associated signalling cascades.

We then examined the expression of TNF receptor subunits. TNF exerts its effects through two distinct receptors, TNFR1 (*TNFRSF1A*) and TNFR2 (*TNFRSF1B*), which differ in expression profiles and signalling properties [22]. RNA-seq analysis showed that both *TNFRSF1A* and *TNFRSF1B* were higher expressed in dermal fibroblasts compared to keratinocytes (Figure 4a). *TNFRSF1A* showed a modest increase (1.35-fold), whereas *TNFRSF1B* (21-fold) was markedly elevated in unstimulated dermal fibroblasts compared with keratinocytes. This finding aligns with the observation that TNFR2 signalling ranked among the top 10 enriched Reactome pathways in dermal fibroblasts. The mRNA expression levels of *TNFRSF1A* and *TNFRSF1B* under cytokine stimulation are available in Figure S3. Given the magnitude of this difference, we focused specifically on TNFR2 protein levels by western blotting, which confirmed substantially higher TNFR2 abundance in dermal fibroblasts under both basal conditions and stimulation (Figure 4b).

These findings suggest that the heightened TNF responsiveness of dermal fibroblasts may be driven by their elevated expression of TNFR1 and, more prominently, TNFR2, which could amplify downstream proinflammatory signalling.

4 | Discussion

Keratinocytes have traditionally been regarded as the central drivers of inflammatory skin diseases, whereas dermal fibroblasts have received less attention. Our data demonstrate that dermal fibroblasts in vitro mount a robust proinflammatory response to TNF and IL-17A, alone and in combination,

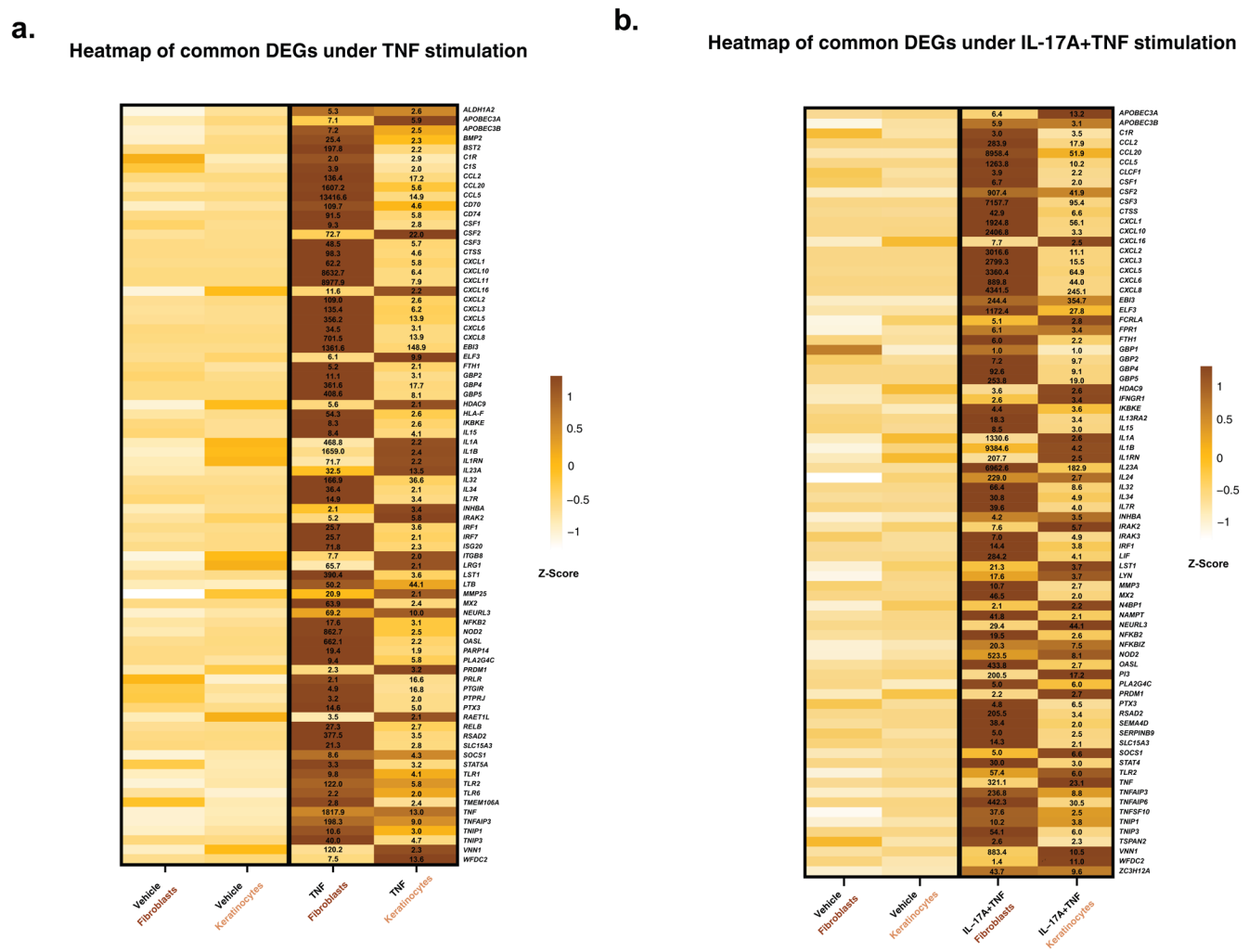

FIGURE 3 | Enhanced inflammatory gene expression in primary human dermal fibroblasts compared with keratinocytes following cytokine stimulation. Data are derived from bulk RNA-seq analysis of in vitro-stimulated dermal fibroblasts and keratinocytes. (a) Heatmap showing *z*-score vst normalised expression of DEGs annotated to inflammatory GO:BP terms and commonly upregulated in both cell types following TNF stimulation. (b) Heatmap showing the same gene set following IL-17A + TNF stimulation. Numbers within heatmap cells indicate fold change in gene expression relative to the vehicle control of the corresponding cell type.

frequently surpassing keratinocytes in the transcriptional activation of inflammatory genes. This enhanced response may reflect greater TNF sensitivity due to increased receptor expression, particularly TNFR2. These findings highlight dermal fibroblasts as important contributors to cutaneous inflammation, complementing the epithelial barrier–oriented responses of keratinocytes.

PCA and Venn analyses revealed that dermal fibroblasts and keratinocytes activate distinct transcriptional programs upon cytokine stimulation. Stimulations induced cell-type-specific transcriptional shifts, with dermal fibroblasts displaying pronounced separation in response to TNF and TNF + IL-17A, whereas keratinocytes exhibited more restrained changes. GO:BP enrichment analysis underscored the fibroblasts' engagement in broad inflammatory and chemotactic pathways, while keratinocytes were enriched for terms related to mRNA regulation and deubiquitination. The latter aligns with keratinocytes' capacity to modulate cytokine production and differentiation through miRNAs and ncRNAs [23, 24], and with the known activation of the ubiquitin–proteasome pathway in psoriasis, which regulates NF-κB and MAPK signalling [25, 26].

Building on these patterns, we identified cell-type-specific DEGs uniquely upregulated in either fibroblasts or keratinocytes. In agreement with previous studies, keratinocytes upregulated genes associated with epithelial differentiation, barrier function, and metabolic regulation following IL-17A and TNF stimulation [3, 11, 27]. Notably, members of the IL-1 family were predominantly expressed in keratinocytes, with *IL36G* showing uniquely strong keratinocyte-specific expression. In contrast, dermal fibroblasts upregulated genes primarily associated with immune signalling and chemotaxis. This included strong induction of chemokines such as *CCL3*, *CCL7*, *CCL8*, *CCL11*, *CCL13*, and *CCL19*, mediators that attract neutrophils, monocytes, eosinophils, and T cells [28–30]. Notably, *CCL13* and *CCL19* define the SFRP2^{+} fibroblast subset described in psoriasis and hidradenitis suppurativa, two Th17-driven diseases, supporting the notion that fibroblasts adopt immune-like, inflammatory phenotypes [31, 32]. Rather than serving solely structural roles,

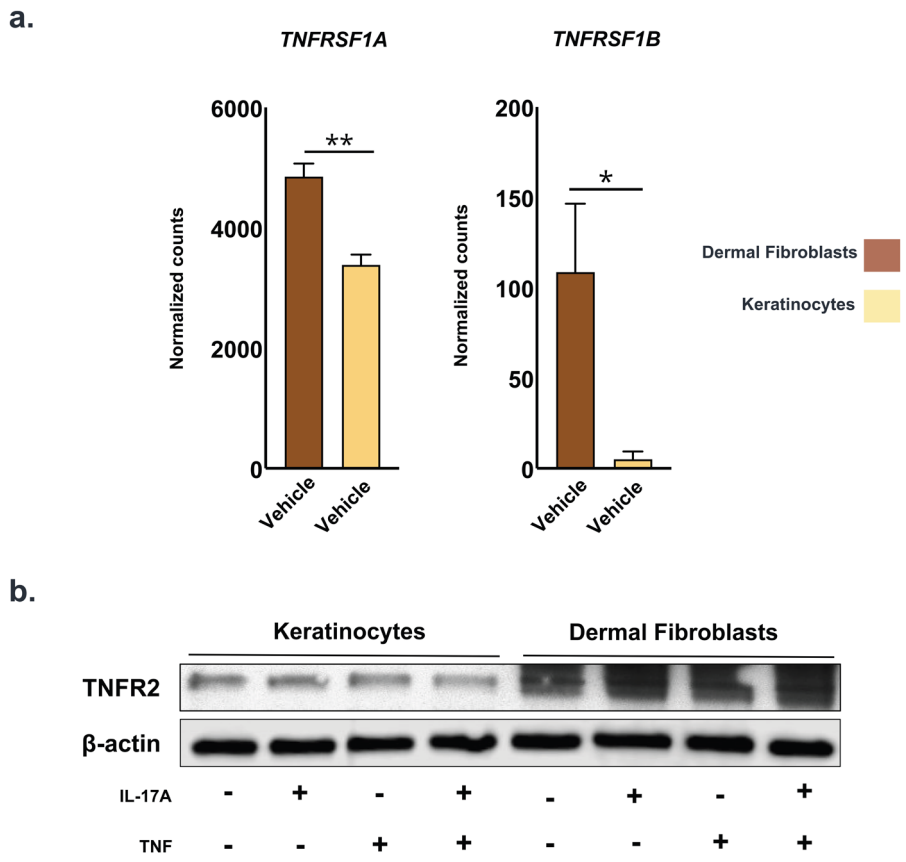

FIGURE 4 | Differential expression of receptors in primary human dermal fibroblasts and keratinocytes. (a) Bulk RNA-seq quantification of *TNFRSF1A* and *TNFRSF1B* mRNA expression in dermal fibroblasts and keratinocytes. Data are presented as bar plots of normalised counts, with values shown as the mean ± standard deviation. $^{*}p < 0.05$, $^{**}p \leq 0.01$. (b) Western blot analysis of TNFR2 protein levels in fibroblasts and keratinocytes under stimulation. β-Actin serves as a loading control.

dermal fibroblasts seem to adopt immune-like functions. This is consistent with reports of inflammatory fibroblast subsets in diseased skin that produce cytokines, chemokines, and antigen-presentation molecules [18, 31, 33]. We also observed an exclusive upregulation of *IL6* in dermal fibroblasts, which aligns with the clinical use of IL-6 receptor inhibitors such as tocilizumab in several rheumatologic diseases where synovial fibroblasts are known to play a pathogenic role [34].

When comparing genes upregulated in both cell types, restricting the focus to those annotated with inflammatory processes, dermal fibroblasts consistently expressed higher levels of key inflammatory mediators upon all stimulation groups. Interestingly, these large discrepancies in fold change between cell types were already evident under TNF stimulation alone, where high fold changes were observed in many genes. This pronounced TNF effect, which was also evident from the number of DEGs in dermal fibroblasts compared to keratinocytes, prompted us to examine TNF receptor expression of the two distinct receptors, TNFR1 (*TNFRSF1A*) and TNFR2 (*TNFRSF1B*). This analysis was particularly relevant in light of a recently published study that established TNF signalling in dermal fibroblasts as a key driver of Th17-mediated skin inflammation, contradicting the prevailing view that TNF acts predominantly through keratinocytes [35]. Interestingly, *TNFRSF1B* expression was not detected in this study, whereas our data challenge this observation, as we observed receptor expression at the protein level. Our findings were supported by previous studies that also detected TNFR2 protein levels in dermal fibroblasts [13, 36]. The pathogenic effects mediated via the classical TNFR1-NF-κB or TNFR1-MAPK pathways are well established; however, studies exploring the potential contribution of TNFR2 are scarce and yield context-dependent, often conflicting results [37]. Nonetheless, there is evidence that deletion of TNFR2 in fibroblast-like synoviocytes suppresses the expression of *CXCL9*, *CXCL10*, *CXCL11*, and *TNFSF13B* [37], all of which were highly induced in our TNF-stimulated dermal fibroblasts. It is therefore tempting to hypothesize that this receptor may contribute to the heightened responsiveness of dermal fibroblasts to TNF, particularly in light of TNFR2's markedly higher expression in dermal fibroblasts, especially under stimulatory conditions. However, the link between elevated TNFR2 expression and enhanced TNF responsiveness remains correlative. Further functional studies are required to definitively establish causality.

Several limitations should be considered when interpreting our findings. First, the proportion of dermal fibroblasts in skin tissue is lower compared to keratinocytes, which may contribute to an overall weaker inflammatory response in the skin simply due to lower fibroblast abundance. Second, while TNFR2 primarily responds to membrane-bound TNF, we used soluble TNF in our experiments. Although soluble TNF is traditionally considered a less potent activator of TNFR2, previous studies in RA synoviocytes have demonstrated that soluble TNF

can successfully drive TNFR2-dependent signalling, even at concentrations as low as 0.5 ng/mL [38, 39]. Given our use of a high concentration of recombinant TNF (10 ng/mL), we expect sufficient receptor occupancy to facilitate functional TNFR2 engagement. Nonetheless, we acknowledge that the signalling dynamics of soluble TNF may differ from the membrane-bound form encountered in vivo. Finally, our study relies on bulk RNA sequencing and in vitro systems. Further work using functional and spatial approaches will be essential to delineate TNFR2-mediated pathways and the fibroblasts' interactions with immune and epithelial cells.

In summary, this study provides a direct comparative analysis of transcriptional responses in dermal fibroblasts and keratinocytes following TNF and IL-17A stimulation. Our findings challenge the keratinocyte-centric paradigm of Th17-mediated skin inflammation by identifying dermal fibroblasts as potent amplifiers of TNF- and IL-17A–driven inflammatory signalling. Beyond their structural roles, fibroblasts emerge as dynamic immune-responsive cells and potential therapeutic targets in chronic inflammatory skin diseases.

Author Contributions

Conceptualization: H.B.A., C.J., T.B., C.V. Data curation: L.S., H.B.A. Formal analysis: L.S., H.B.A. Funding acquisition: C.J. Investigation: L.S. Methodology: L.S., H.B.A., C.J. Project; administration: C.J. Resources: C.J. Supervision: H.B.A., C.J., T.B., C.V. Validation: L.S., H.B.A., C.J. Visualisation: L.S. Writing – original draft preparation: L.S. Writing – review and editing: L.S., H.B.A., C.J., T.B., C.V.

Acknowledgements

Acknowledgment is extended to lab technicians Annette Blak Rasmussen, Kristine Møller, and Hang Pham Jensen for their assistance in the laboratory. This study was supported by funding from Aarhus University, Danish Psoriasis Research Foundation, and Sunhedsdonationer (grant number 2024-0233). The study was conducted in Aarhus, Denmark.

Funding

This work was supported by Aarhus Universitet. Danish Psoriasis Research Foundation. Sundhedsdonationer, 2024-0233.

Ethics Statement

This study was performed in accordance with the Declaration of Helsinki. All samples were obtained from surplus skin excised during elective surgical procedures at AROS Private Hospital, Aarhus. Donors provided informed consent for the use of their tissue in research. The use of these anonymized samples was approved by the Regional Ethical Committee of Region Midtjylland, Denmark (M-20110027).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The bulk RNA-sequencing data generated in this study have been deposited in the Gene Expression Omnibus under the following accession number: *GSE315599*. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

References

1. J. M. Fletcher, B. Moran, A. Petrasca, and C. M. Smith, "IL-17 in Inflammatory Skin Diseases Psoriasis and Hidradenitis Suppurativa," *Clinical and Experimental Immunology* 201 (2020): 121–134, https://doi.org/10.1111/cei.13449.

2. T. Liu, S. Li, S. Ying, et al., "The IL-23/IL-17 Pathway in Inflammatory Skin Diseases: From Bench to Bedside," *Frontiers in Immunology* 11 (2020): 594735, https://doi.org/10.3389/fimmu.2020.594735.

3. A. Chiricozzi, E. Guttman-Yassky, M. Suárez-Fariñas, et al., "Integrative Responses to IL-17 and TNF-α in Human Keratinocytes Account for Key Inflammatory Pathogenic Circuits in Psoriasis," *Journal of Investigative Dermatology* 131 (2011): 677–687, https://doi.org/10.1038/jid.2010.340.

4. M. Noack, A. Beringer, and P. Miossec, "Additive or Synergistic Interactions Between IL-17A or IL-17F and TNF or IL-1β Depend on the Cell Type," *Frontiers in Immunology* 10 (2019): 1726, https://doi.org/10.3389/fimmu.2019.01726.

5. H. T. Idriss and J. H. Naismith, "TNF Alpha and the TNF Receptor Superfamily: Structure-Function Relationship(s)," *Microscopy Research and Technique* 50 (2000): 184–195, https://doi.org/10.1002/1097-0029(20000801)50:3<184::Aid-jemt2>3.0.Co;2-h.

6. A. M. Wride, G. F. Chen, S. L. Spaulding, E. Tkachenko, and J. M. Cohen, "Biologics for Psoriasis," *Dermatologic Clinics* 42 (2024): 339–355, https://doi.org/10.1016/j.det.2024.02.001.

7. P. Mendes-Bastos, F. Benhadou, M. Venturini, et al., "Biologic Drugs in Hidradenitis Suppurativa: What Does the GP Have to Know? A Narrative Review," *Front Med (Lausanne)* 11 (2024): 1403455, https://doi.org/10.3389/fmed.2024.1403455.

8. B. Russo, N. C. Brembilla, and C. Chizzolini, "Interplay Between Keratinocytes and Fibroblasts: A Systematic Review Providing a New Angle for Understanding Skin Fibrotic Disorders," *Frontiers in Immunology* 11 (2020): 648, https://doi.org/10.3389/fimmu.2020.00648.

9. R. K. Gupta, P. Wasnik, D. Mondal, and D. Shukla, "Critical Role of Keratinocytes in Cutaneous Immune Responses," *Exploration of Immunology* 4 (2024): 502–522, https://doi.org/10.37349/ei.2024.00155.

10. C. Albanesi, C. Scarponi, M. L. Giustizieri, and G. Girolomoni, "Keratinocytes in Inflammatory Skin Diseases," *Current Drug Targets. Inflammation and Allergy* 4 (2005): 329–334, https://doi.org/10.2174/1568010054022033.

11. M. Furue, K. Furue, G. Tsuji, and T. Nakahara, "Interleukin-17A and Keratinocytes in Psoriasis," *International Journal of Molecular Sciences* 21 (2020): 1275, https://doi.org/10.3390/ijms21041275.

12. Z. Shi, Z. Liu, Y. Wei, R. Zhang, Y. Deng, and D. Li, "The Role of Dermal Fibroblasts in Autoimmune Skin Diseases," *Frontiers in Immunology* 15 (2024): 1379490, https://doi.org/10.3389/fimmu.2024.1379490.

13. L. Yin, Y. Hu, J. Xu, J. Guo, J. Tu, and Z. Yin, "Ultraviolet B Inhibits IL-17A/TNF-α-Stimulated Activation of Human Dermal Fibroblasts by Decreasing the Expression of IL-17RA and IL-17RC on Fibroblasts," *Frontiers in Immunology* 8 (2017): 91, https://doi.org/10.3389/fimmu.2017.00091.

14. Y. Katz, O. Nadiv, and Y. Beer, "Interleukin-17 Enhances Tumor Necrosis Factor Alpha-Induced Synthesis of Interleukins 1,6, and 8 in Skin and Synovial Fibroblasts: A Possible Role as a "Fine-Tuning Cytokine" in Inflammation Processes," *Arthritis and Rheumatism* 44 (2001): 2176–2184, https://doi.org/10.1002/1529-0131(200109)44:9<2176::aid-art371>3.0.co;2-4.

15. T. Tabib, C. Morse, T. Wang, W. Chen, and R. Lafyatis, "SFRP2/DPP4 and FMO1/LSP1 Define Major Fibroblast Populations in Human Skin," *Journal of Investigative Dermatology* 138 (2018): 802–810, https://doi.org/10.1016/j.jid.2017.09.045.

16. X. Cai, M. Han, F. Lou, et al., "Tenascin C+ Papillary Fibroblasts Facilitate Neuro-Immune Interaction in a Mouse Model of Psoriasis," *Nature Communications* 14 (2023): 2004, https://doi.org/10.1038/s41467-023-37798-x.

17. M. H. Smith, V. R. Gao, P. K. Periyakoil, et al., "Drivers of Heterogeneity in Synovial Fibroblasts in Rheumatoid Arthritis," *Nature Immunology* 24 (2023): 1200–1210, https://doi.org/10.1038/s41590-023-01527-9.

18. L. Francis, D. McCluskey, C. Ganier, et al., "Single-Cell Analysis of Psoriasis Resolution Demonstrates an Inflammatory Fibroblast State Targeted by IL-23 Blockade," *Nature Communications* 15 (2024): 913, https://doi.org/10.1038/s41467-024-44994-w.

19. C. Johansen, "Generation and Culturing of Primary Human Keratinocytes From Adult Skin," *Journal of Visualized Experiments* (2017): e56863, https://doi.org/10.3791/56863.

20. Y. Liao, G. K. Smyth, and W. Shi, "The R Package Rsubread Is Easier, Faster, Cheaper and Better for Alignment and Quantification of RNA Sequencing Reads," *Nucleic Acids Research* 47 (2019): e47, https://doi.org/10.1093/nar/gkz114.

21. M. I. Love, W. Huber, and S. Anders, "Moderated Estimation of Fold Change and Dispersion for RNA-Seq Data With DESeq2," *Genome Biology* 15 (2014): 550, https://doi.org/10.1186/s13059-014-0550-8.

22. C. H. Lo, "TNF Receptors: Structure-Function Relationships and Therapeutic Targeting Strategies," *Biochimica et Biophysica Acta - Biomembranes* 1867 (2025): 184394, https://doi.org/10.1016/j.bbamem.2024.184394.

23. T. L. Timis and R. I. Orasan, "Understanding Psoriasis: Role of miRNAs," *Biomed Rep* 9 (2018): 367–374, https://doi.org/10.3892/br.2018.1146.

24. D. Ghosh, T. Ganguly, and R. Chatterjee, "Emerging Roles of Non-Coding RNAs in Psoriasis Pathogenesis," *Functional & Integrative Genomics* 23 (2023): 129, https://doi.org/10.1007/s10142-023-01057-5.

25. A. Gęgotek, P. Domingues, A. Wroński, and E. Skrzydlewska, "Changes in Proteome of Fibroblasts Isolated From Psoriatic Skin Lesions," *International Journal of Molecular Sciences* 21 (2020), https://doi.org/10.3390/ijms21155363.

26. P. J. Elliott, T. M. Zollner, and W. H. Boehncke, "Proteasome Inhibition: A New Anti-Inflammatory Strategy," *Journal of Molecular Medicine (Berlin, Germany)* 81 (2003): 235–245, https://doi.org/10.1007/s00109-003-0422-2.

27. C. Johansen, T. Bertelsen, C. Ljungberg, M. Mose, and L. Iversen, "Characterization of TNF-α– And IL-17A–Mediated Synergistic Induction of DEFB4 Gene Expression in Human Keratinocytes Through IκBζ," *Journal of Investigative Dermatology* 136 (2016): 1608–1616, https://doi.org/10.1016/j.jid.2016.04.012.

28. H. Bachelez, "Chemokine Ligand 7 (CCL7) and Innate Immune Cells in Psoriasis: Beyond Redundancy," *Experimental Dermatology* 25 (2016): 265–266, https://doi.org/10.1111/exd.12963.

29. A. M. Foster, J. Baliwag, C. S. Chen, et al., "IL-36 Promotes Myeloid Cell Infiltration, Activation, and Inflammatory Activity in Skin," *Journal of Immunology* 192 (2014): 6053–6061, https://doi.org/10.4049/jimmunol.1301481.

30. M. Sugaya, "Chemokines and Skin Diseases," *Archivum Immunologiae et Therapiae Experimentalis (Warsz)* 63 (2015): 109–115, https://doi.org/10.1007/s00005-014-0313-y.

31. F. Ma, O. Plazyo, A. C. Billi, et al., "Single Cell and Spatial Sequencing Define Processes by Which Keratinocytes and Fibroblasts Amplify Inflammatory Responses in Psoriasis," *Nature Communications* 14 (2023): 3455, https://doi.org/10.1038/s41467-023-39020-4.

32. A. Flora, R. Jepsen, E. K. Kozera, et al., "Human Dermal Fibroblast Subpopulations and Epithelial Mesenchymal Transition Signals in Hidradenitis Suppurativa Tunnels Are Normalized by Spleen Tyrosine Kinase Antagonism In Vivo," *PLoS One* 18 (2023): e0282763, https://doi.org/10.1371/journal.pone.0282763.

33. Y. Zhou, T. Cao, Z. Li, et al., "Fibroblasts in Immune-Mediated Inflammatory Diseases: The Soil of Inflammation," *Clinical Immunology* 258 (2024): 109849, https://doi.org/10.1016/j.clim.2023.109849.

34. M. Biggioggero, C. Crotti, A. Becciolini, and E. G. Favalli, "Tocilizumab in the Treatment of Rheumatoid Arthritis: An Evidence-Based Review and Patient Selection," *Drug Design, Development and Therapy* 13 (2019): 57–70, https://doi.org/10.2147/dddt.S150580.

35. K. J. Cavagnero, F. Li, C. Aguilera, et al., "Targeting Fibroblast TNF Receptor 1 Attenuates Type 17 Skin Inflammation," *Cell Reports* 44 (2025): 116111, https://doi.org/10.1016/j.celrep.2025.116111.

36. D. Wcisło-Dziadecka, J. Gola, B. Grabarek, U. Mazurek, L. Brzezińska-Wcisło, and E. J. Kucharz, "Effect of Adalimumab on the Expression of Genes Encoding TNF-α Signal Paths in Skin Fibroblasts In Vitro," *Postepy Dermatologii I Alergologii* 35 (2018): 413–422, https://doi.org/10.5114/ada.2018.77673.

37. T. Suto, A. Tosevska, K. Dalwigk, et al., "TNFR2 Is Critical for TNF-Induced Rheumatoid Arthritis Fibroblast-Like Synoviocyte Inflammation," *Rheumatology* 61 (2022): 4535–4546, https://doi.org/10.1093/rheumatology/keac124.

38. S. Zrioual, R. Ecochard, A. Tournadre, V. Lenief, M. A. Cazalis, and P. Miossec, "Genome-Wide Comparison Between IL-17A- and IL-17F-Induced Effects in Human Rheumatoid Arthritis Synoviocytes1," *Journal of Immunology* 182 (2009): 3112–3120, https://doi.org/10.4049/jimmunol.0801967.

39. R. O. Williams, F. I. L. Clanchy, Y.-S. Huang, W.-Y. Tseng, and T. W. Stone, "TNFR2 Signalling in Inflammatory Diseases," *Best Practice & Research. Clinical Rheumatology* 38 (2024): 101941, https://doi.org/10.1016/j.berh.2024.101941.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Inflammatory gene expression in dermal fibroblasts and keratinocytes following IL-17A stimulation. Data are derived from bulk RNA-seq analysis of in vitro-stimulated primary human dermal fibroblasts and keratinocytes. Heatmap showing *z*-score of vst normalised expression of DEGs annotated to inflammatory GO:BP terms and commonly upregulated in both cell types following IL-17A stimulation. Numbers within heatmap cells indicate fold change in gene expression relative to the vehicle control of the corresponding cell type. **Figure S2:** GSEA of Reactome pathways in dermal fibroblasts following cytokine stimulation. Data are derived from bulk RNA-seq analysis of in vitro-stimulated primary human dermal fibroblasts and keratinocytes. GSEA of Reactome pathways in dermal fibroblasts following cytokine stimulation. For TNF and IL-17A + TNF conditions, the ten most significantly enriched Reactome pathways are shown. Dot plots display the NES on the x-axis, with dot size representing gene size and colour intensity indicating adjusted *p*-value significance. **Figure S3:** *TNFRSF1A* and *TNFRSF1B* mRNA expression in dermal fibroblasts and keratinocytes across stimulation conditions. RNA-seq quantification of *TNFRSF1A* and *TNFRSF1B* mRNA expression in primary human dermal fibroblasts and keratinocytes upon all stimulation groups. Data are shown as bar plots of normalised counts, with values presented as mean ± standard deviation. $*p < 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$.