

ZFP36 Family Members Regulate the Proinflammatory Features of Psoriatic Dermal Fibroblasts



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Dermal fibroblasts are strategically positioned underneath the basal epidermis layer to support keratinocyte proliferation and extracellular matrix production. In inflammatory conditions, these fibroblasts produce cytokines and chemokines that promote the chemoattraction of immune cells into the dermis and the hyperplasia of the epidermis, two characteristic hallmarks of psoriasis. However, how dermal fibroblasts specifically contribute to psoriasis development remains largely uncharacterized. In this study, we investigated through which cytokines and signaling pathways dermal fibroblasts contribute to the inflammatory features of psoriatic skin. We show that dermal fibroblasts from lesional psoriatic skin are important producers of inflammatory mediators, including IL-6, CXCL8, and CXCL2. This increased cytokine production was found to be regulated by ZFP36 family members ZFP36, ZFP36L1, and ZFP36L2, RNA-binding proteins with mRNA-degrading properties. In addition, the expression of ZFP36 family proteins was found to be reduced in chronic inflammatory conditions that mimic psoriatic lesional skin. Collectively, these results indicate that dermal fibroblasts are important producers of cytokines in psoriatic skin and that reduced expression of ZFP36 members in psoriasis dermal fibroblasts contributes to their inflammatory phenotype.

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INTRODUCTION

Psoriasis (Pso) is a common chronic inflammatory skin disease that affects 1–3% of the world population. The characteristic feature of established Pso is the presence of scaly and persistent skin lesions that result from abnormal activation and differentiation of keratinocytes (KCs) in the epidermis (Billi et al., 2019). Such epidermal changes are the consequence of chronic inflammatory events in the underlying dermal compartment, which involve the infiltration of mononuclear cells and the instauration of a positive inflammatory feedback loop in the skin (Afonina et al., 2021).

The participation of dermal fibroblasts in the transition from innate to adaptive immunity has recently been taken into consideration. Dermal fibroblasts are the most abundant cells in the dermis and are critically positioned underneath the basal epidermis layer to sustain KCs proliferation (Gubán et al., 2016; Miura et al., 2000). Their expanded life span and their stable topographic localization also make them more sensitive to possible insults from the microenvironment (Stunova and Vistejnova, 2018).

In stable conditions, dermal fibroblasts are primarily involved in extracellular matrix production and guarantee tissue integrity and repair. However, in activating conditions, such as in psoriatic skin, fibroblasts contribute to epidermal overgrowth by promoting KC proliferation (Gubán et al., 2016) and by producing proinflammatory proteins (Gęgotek et al., 2020; Kolář et al., 2012; Zalewska et al., 2006) in response to TNF- α and IL-17, key cytokines with elevated expression in psoriatic skin (Billi et al., 2019; Grine et al., 2015). Furthermore, fibroblasts from lesional (LS) psoriatic skin display higher oxidative damage than normal fibroblasts (Becatti et al., 2018; Dimon-Gadal et al., 2000) and were suggested to acquire augmented inflammatory responses upon rechallenge (Crowley et al., 2018).

Overall, these observations indicate a prominent role of dermal fibroblasts in the regulation of inflammatory responses, which goes far beyond the conventional classification of structural cells. To date, however, investigations addressing whether and through which mechanisms dermal fibroblasts contribute to psoriatic manifestations lag far behind studies in other skin cell types.

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Abbreviations: AD, atopic dermatitis; ARE, adenylate-uridylylate-rich element; DEG, differentially expressed gene; GEO, Gene Expression Omnibus; KC, keratinocyte; LS, lesional; NL, nonlesional; Pso, psoriasis; UTR, untranslated region

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In this study, we show that TNF- α -stimulated fibroblasts from psoriatic skin lesions display the exaggerated production of specific cytokines (such as IL-6, CXCL8, CXCL2) as compared with fibroblasts derived from paired non-LS (NL) skin. We find this increased cytokine production to be regulated by ZFP36 family members, RNA-binding proteins with mRNA-degrading properties. We further describe that ZFP36 family proteins are suppressed in chronic inflammatory conditions mimicking psoriatic lesions. Overall, our results indicate that fibroblasts are important regulators in psoriatic skin and that their enhanced inflammatory phenotype depends on altered ZFP36 family levels. These data provide insight into Pso pathogenesis and may provide therapeutic opportunities in the future.

RESULTS

Activated dermal fibroblasts share transcriptomic signatures with psoriatic LS skin

To assess how dermal fibroblasts contribute to psoriatic skin manifestations, we retrieved microarray data from whole skin of patients with NL and LS Pso (GSE13355) (Nair et al., 2009) and from unstimulated versus TNF- α -stimulated primary fibroblasts (GSE40560) (Boisson et al., 2012). Although fibroblasts can be activated by other cytokines present in psoriatic skin, such as IL-17 (Billi et al., 2019), our choice to specifically investigate the effects of TNF- α on fibroblasts relied on the high expression of TNF- α receptor (*TNFRSF1A*) in these cells, which we found approximately 20 times higher than the IL-17 receptor (IL-17RA) (data not shown). The majority (>87%) of the gene IDs were recognized by the probe sets used in the two microarray studies (Supplementary Figure S1a). From these, we found 13,442 differentially expressed genes (DEGs) between NL and LS psoriatic skin and 4,119 DEGs in unstimulated versus in TNF- α -stimulated fibroblasts. A total of 3,169 genes, corresponding to 76.9% of all DEGs in TNF- α -stimulated fibroblasts and 23.6% of all DEGs in psoriatic LS skin, were shared in both psoriatic LS skin and TNF- α -stimulated fibroblasts (Figure 1a and Supplementary Table S1).

Among the common DEGs with psoriatic LS skin, fibroblasts were found to be the main producers of proinflammatory genes, including cytokines (*CXCL8*, *CXCL1*, *CXCL2*, *CCL20*, *CCL5*), ILs (*IL6*), guanosine triphosphate cyclohydrolases (*GCH1*), cell adhesion molecules (*VCAM1* and *ICAM1*), and TNF- α -inducible regulators of cell death (*TNFAIP2*, *BIRC3*, *CYLD*), upregulated either at short (2 hours) and late (6 hours) time points after TNF- α stimulation (Figure 1b and Supplementary Figure S1b and Supplementary Table S2). The common pathways associated with these shared DEGs included typical activating signals of the immune system (Figure 1c). Together, these findings indicate that on TNF- α activation, fibroblasts induce the expression of a great variety of genes partially overlapping with typical Pso-associated signatures.

Dermal fibroblasts contribute to psoriatic-associated inflammatory signatures

Some of the cytokines that displayed the highest expression in fibroblasts, such as *IL6* and *CXCL1*, and *CXCL2-8* (Figure 1b), are conventionally known to be produced by KCs (Furie et al., 2019; Patel et al., 2018). Thus, we investigated

which cell type between fibroblasts and KCs is a predominant producer of these cytokines. Microarray analysis from TNF- α -stimulated fibroblasts and KCs (GSE154558) (de Stree et al., 2020) revealed that such cytokines are predominantly expressed by fibroblasts (Figure 2a and Supplementary Table S3). Further experimental validation of these findings in isolated primary skin fibroblasts and KCs, which selectively expressed fibroblast marker *FGF7* (KGF) and basal KC marker keratin 14, respectively (Supplementary Figure S2a), confirmed that the expression of *IL6* and *CXCL8* is approximately 70–80 times higher in TNF- α -stimulated fibroblasts than in KCs (Figure 2b). While retaining a high expression in fibroblasts, other genes such as *CXCL2* and *CCL20* displayed either a similar or lower expression than in KCs (Figure 2b and Supplementary Figure S2a). Furthermore, TNF- α proved to be a main activator of fibroblasts compared with IL-17 (Supplementary Figure S2b).

Once having assessed the mRNA expression of these cytokines in fibroblasts, we next evaluated their production in dermal fibroblasts isolated from LS and NL psoriatic skin. We observed a significantly higher production of *IL6*, *CXCL8*, and *CXCL2* in TNF- α -stimulated cells derived from LS psoriatic skin than in paired NL skin (Figure 2c), whereas we did not detect baseline cytokine production in unstimulated conditions (data not shown). Overall, these results indicate that dermal fibroblasts from psoriatic LS skin sites retain inflammatory features that could perpetuate local inflammation in psoriatic skin.

Genes upregulated in psoriatic LS skin and TNF- α -stimulated fibroblasts are associated with RNA regulation by adenylate-uridylylate-rich elements

To explore the molecular mechanisms that could contribute to the inflammatory phenotype of psoriatic LS skin and in TNF- α -stimulated dermal fibroblasts, we conducted a Gene Set Enrichment Analysis for canonical pathways from the Reactome database. Both psoriatic LS skin and TNF- α -stimulated fibroblasts shared common pathways (Supplementary Table S4). As expected, pathways with the highest normalized enrichment scores included processes associated with immune activation, such as IL cascade, NF- κ B, MAPK, and Wnt/ β -catenin signaling (Figure 3a), all of which were previously described in psoriatic LS skin (Afonina et al., 2021; Gęgotek et al., 2020).

Of interest, one of the most enriched pathways concerned the processes of mRNA stability mediated by adenylate-uridylylate-rich element (ARE)-binding proteins (Figure 3a and b). We validated the enrichment of this pathway in an independent microarray study in psoriatic LS skin (Supplementary Figure S3a). This type of mRNA regulation, which relies on the presence of AREs in the 3' untranslated regions (UTRs), has been reported to tightly control cytokine mRNAs (Uchida et al., 2019). We therefore investigated whether DEGs in psoriatic LS skin would also contain AREs in the 3'UTR. We screened for a variety of AREs, including minimal pentameric consensus sequences (Carpenter et al., 2014; Uchida et al., 2019) and other ARE family motifs (Fallmann et al., 2016). We found that 71% of the DEGs in psoriatic LS skin contained at least one pentameric, heptameric, and nonameric ARE family motif in their 3'UTR (Figure 3c and d and

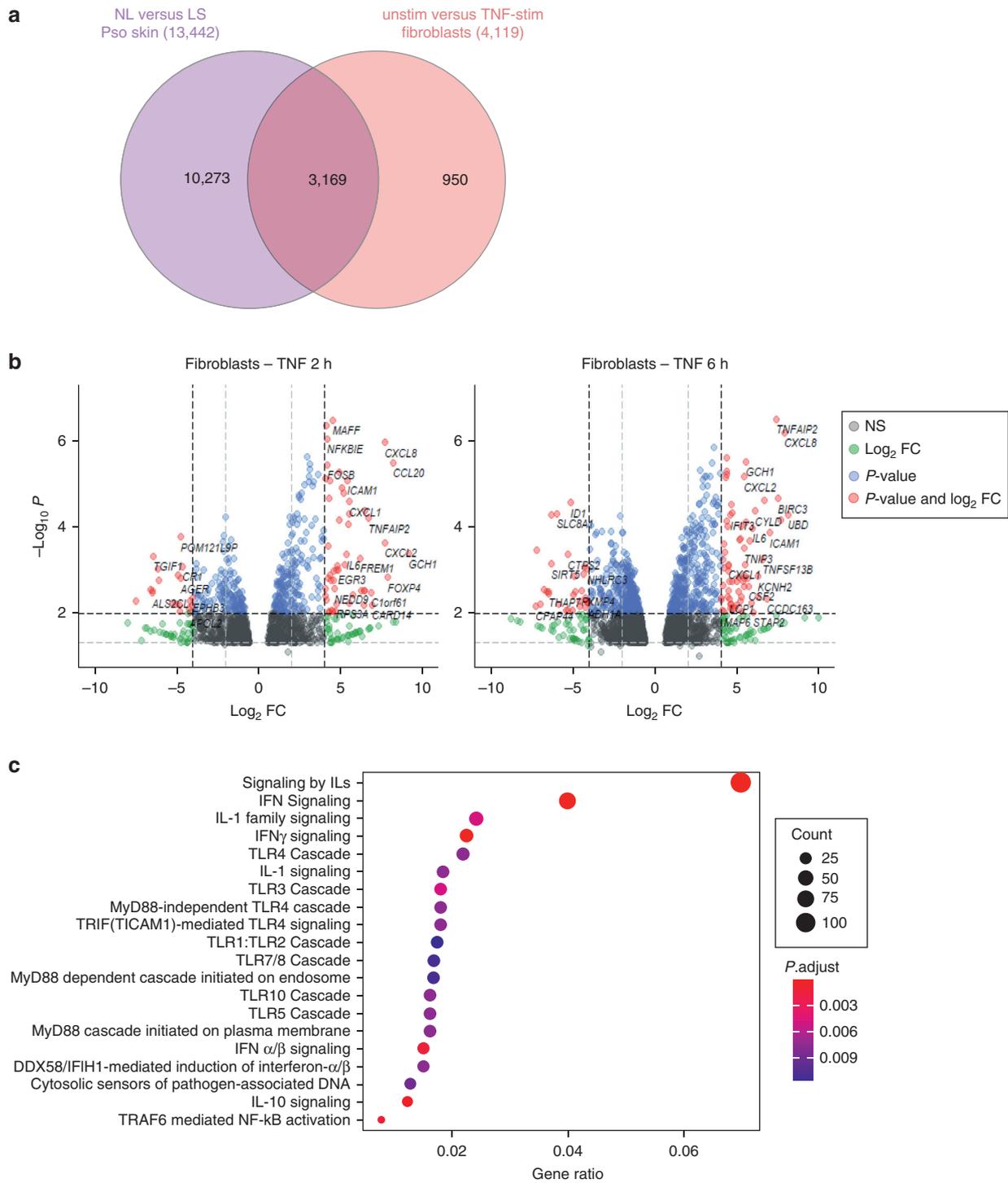


Figure 1. TNF- α -stimulated dermal fibroblasts share overlapping transcriptomic signatures with psoriatic LS skin. (a) DEGs in psoriatic NL versus those in LS skin (13,442; GSE13355) and in unstim versus those in TNF- α -stim fibroblasts (4,119; GSE40560) were compared by Venn Diagram, and 3,169 DEGs were found shared. (b) DEGs in fibroblasts stimulated with TNF- α for 2 h (left panel) or 6 h (right panel) from a are represented in Volcano plots. Significantly ($P < 0.05$) upregulated or downregulated genes ($FC > 2$) are shown with red dots. (c) DEGs from a were subjected to pathway enrichment analysis with ReactomePA package, with FDR-adjusted P -value (q -value) set at < 0.05 . A dot plot of the top 20 enriched signaling pathways is shown. DEG, differentially expressed gene; FC, fold change; FDR, false discovery rate; h, hour; LS, lesional; NL, nonlesional; NS, not significant; ReactomePA, Reactome Pathway Analysis; stim, stimulated; TLR, toll-like receptor; unstim, unstimulated.

Supplementary Table S5), whereas combinations of other motifs were found in lower percentages. Taken together, these data indicate that the vast majority of mRNAs induced

in psoriatic LS skin and TNF- α -stimulated dermal fibroblasts contain AREs and can therefore be potentially regulated at the level of mRNA stability.

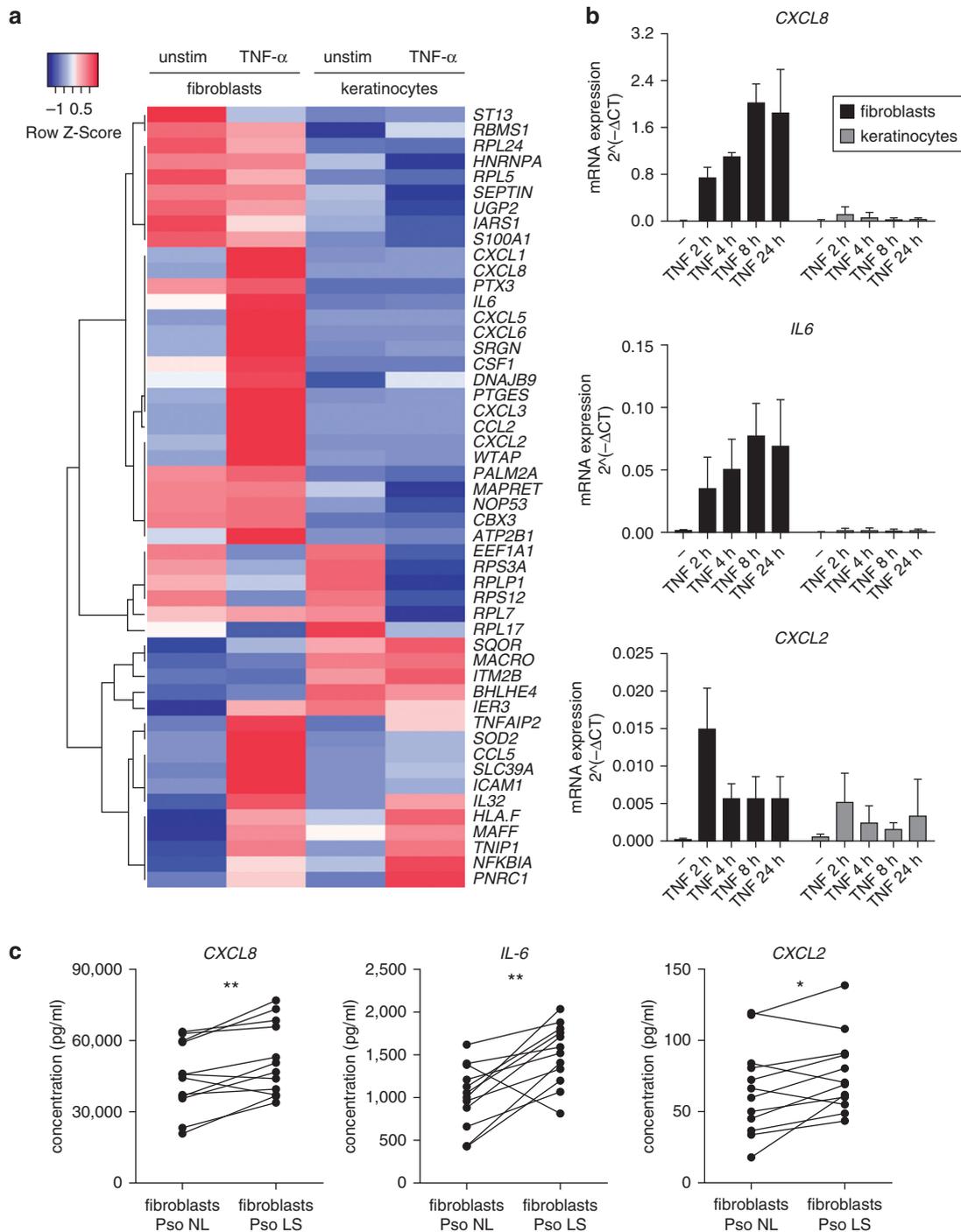


Figure 2. The production of cytokines highly expressed in dermal fibroblasts is induced in psoriatic LS fibroblasts. (a) Upregulated genes from Figure 1b ($P < 0.05$, fold change > 2) were retrieved from GSE154558. The top 50 genes with the highest expression in TNF- α -stimulated fibroblasts are shown. Average linkage clustering and Spearman distance measuring method were used for the Heatmap. (b) Primary skin fibroblasts and keratinocytes were stimulated with TNF- α at different time points, as indicated, and gene expression was assessed by qPCR ($n = 4$). (c) Dermal fibroblasts derived from either NL or LS skin of individuals with Pso were stimulated with TNF- α for 48 h, and the secretion of IL-6, CXCL8, and CXCL2 in supernatants was measured by ELISA; paired t -test. Statistical significance indicated as $*P < 0.05$; $**P < 0.01$. h, hour; LS, lesional; NL, nonlesional; Pso, psoriasis; unstim, unstimulated.

ZFP36 family members regulate the expression of TNF- α -inducible cytokines in dermal fibroblasts

Previous reports identified the importance of ZFP36 (TTP), one AREBP in the regulation of psoriatic features in mice (Andrienne et al., 2017; Patial et al., 2016). ZFP36 and ZFP36-like family members ZFP36L1 and ZFP36L2 are

mRNA-destabilizing proteins that promote mRNA degradation (Fu and Blakeshear, 2017; Sanduja et al., 2011). All the three members share a highly homologous domain, which is predicted to bind the heptameric sequence UAUUUU (Ciais et al., 2013; Sedlyarov et al., 2016). By analyzing the 3'UTR regions of the DEGs in psoriatic LS

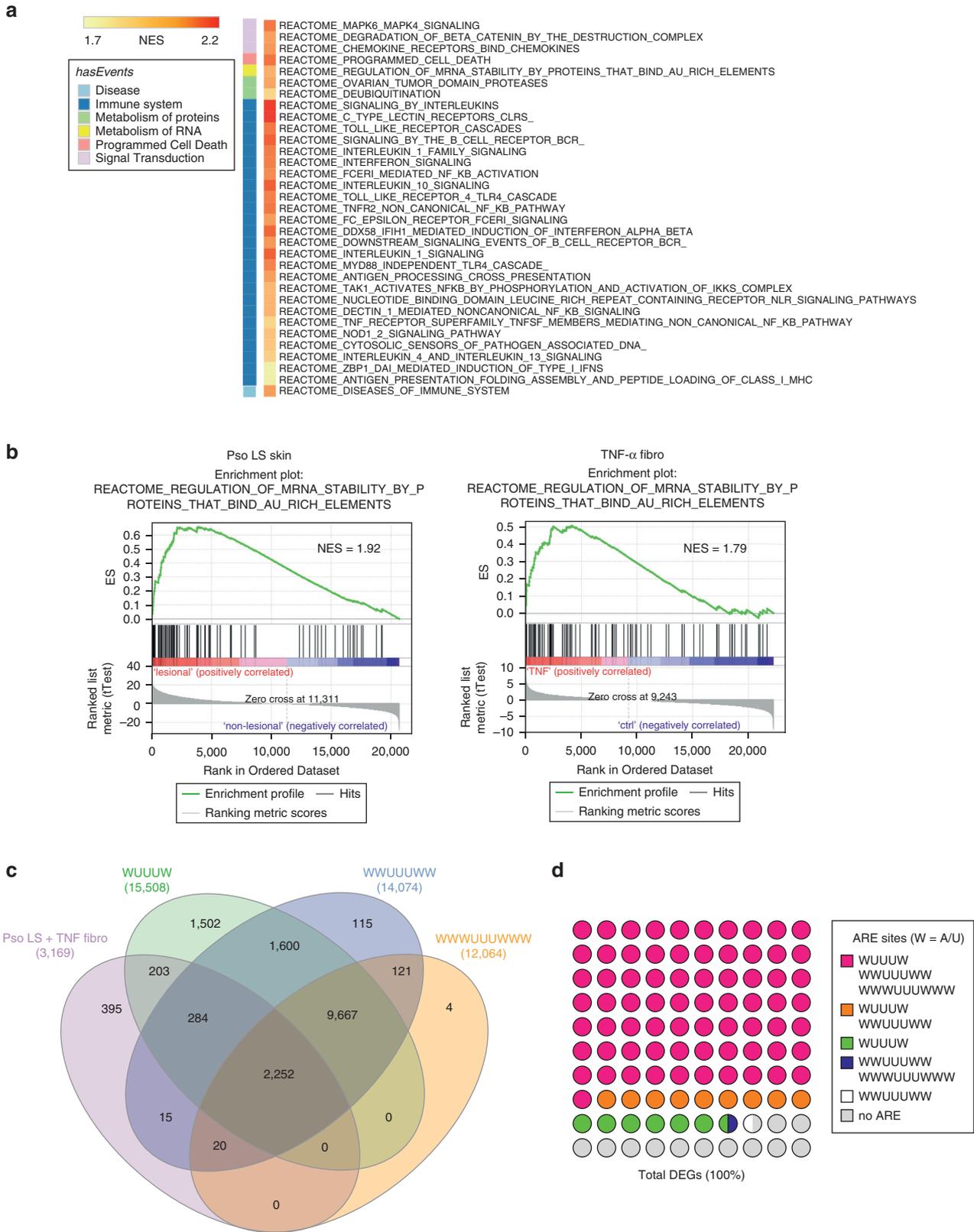


Figure 3. mRNA regulation by ARE is a common enriched pathway in psoriatic LS skin and TNF- α -stimulated fibro. (a) GSEA analyses were conducted on GSE13355 and GSE40560 using the C2.CP.Reactome gene set of the MSigDB database. Enriched pathways with FDR q-value < 0.05 were considered, and common pathways with a minimal NES of 1.7 are shown. Reactome subpathways and related superpathways (hasEvents) are indicated. (b) GSEA enrichment plots for signatures defining AU-rich-mediated mRNA stability, including NES values, are shown for GSE13355 (left panel) and GSE40560 (right panel). (c) IDs of genes containing AREs in their 3'UTR were retrieved from AREsite2 and compared with those of DEGs (3,169) from GSE13355 and GSE40560. W = A (adenine) or U (uracil) nucleotide. (d) Percentages of genes, including ARE family motifs, from total DEGs (3,169) from Figure 1a; purple = 71%; orange = 9%; green = 6.4%; blue = 0.5%; pink = 0.6%; and gray = 12.5%. ARE, adenylate-uridylylate-rich element; AU, adenylate-uridylylate; DEG, differentially expressed gene; ES, enrichment score; FDR, force discovery rate; GSEA, Gene Set Enrichment Analysis; LS, lesional; NES, normalized enrichment score; Pso, psoriasis; UTR, untranslated region.

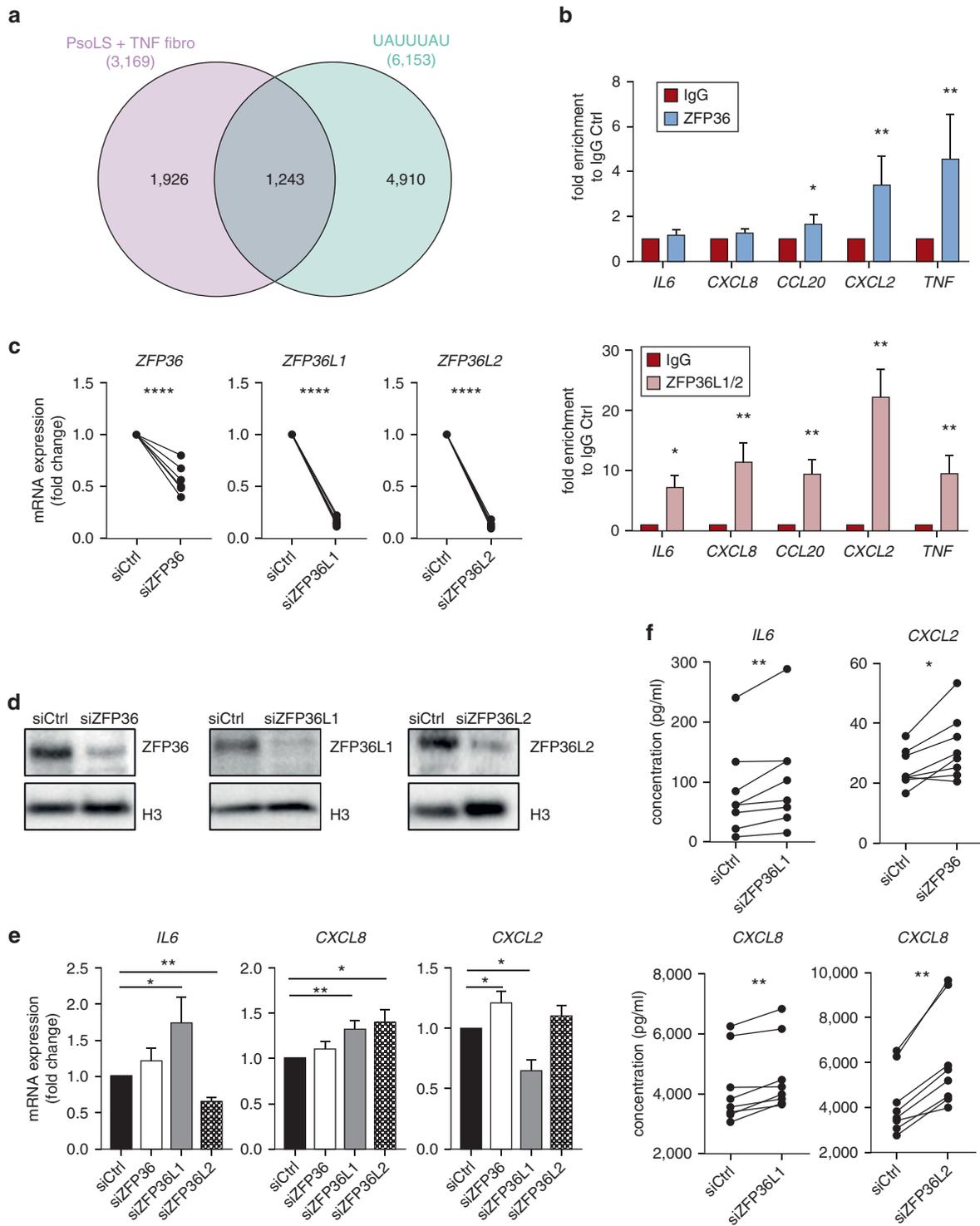
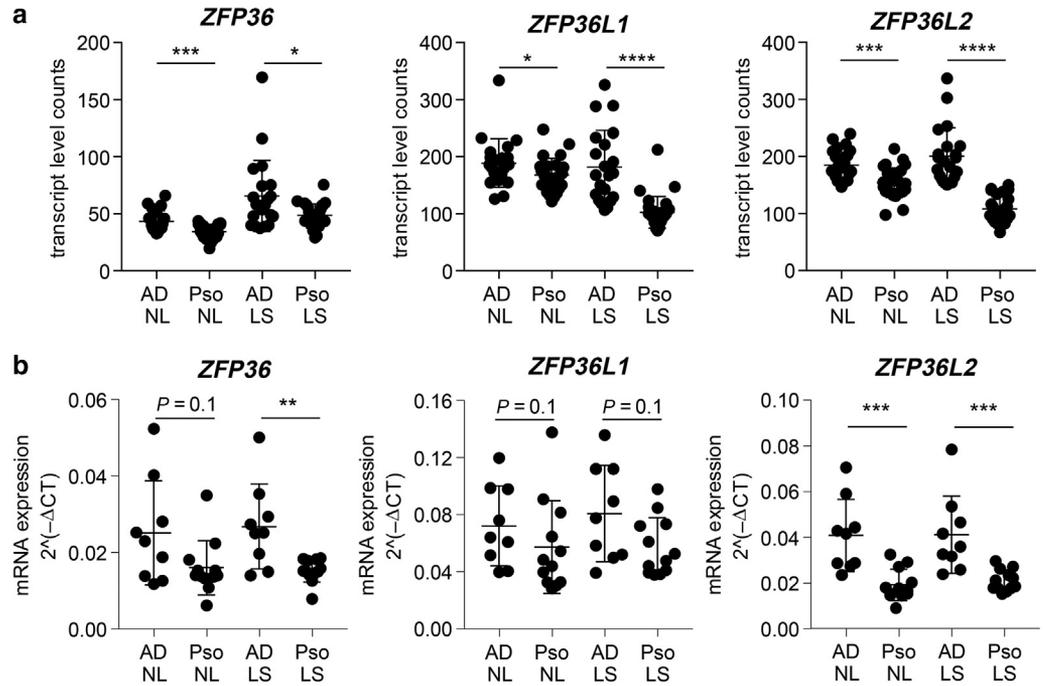


Figure 4. ZFP36 family members regulate TNF- α -inducible cytokines in dermal fibro. (a) Genes containing UAUUUAU in their 3'UTR were retrieved from AREsite2 and compared with DEGs (3,169) from Figure 1a by Venn Diagram. (b) mRNAs immunoprecipitated with proteins recognized by either ZFP36, ZFP36L1/2, or IgG antibodies were subject to qPCR analysis; Friedman test was performed followed by Benjamini–Yekutieli FDR correction ($n = 4$). (c, d) Fibro were transfected with either siCtrl or specific siRNAs targeting ZFP36 (siZFP36), ZFP36L1 (siZFP36L1), or ZFP36L2 (siZFP36L2). Knockdown efficiency was verified by qPCR; (c) paired *t*-test and (d) western blot. (e, f) Fibroblasts ($n = 8$) transfected as in c and d were stimulated with TNF- α for 8 hours. Effects of ZFP36 family knockdown on cytokine expression were assessed by (e) qPCR and (f) ELISA. In e and f, the significant differences between siCtrl and siZFP36/L1/L2 were assessed by Wilcoxon test. (d) Shows the representative pictures of three independent experiments. Statistical significance indicated as * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$. Ctrl, control; DEG, differentially expressed gene; fibro, fibroblast; LS, lesional; Pso, psoriasis; siCtrl, small interfering RNA targeting control; siRNA, small interfering RNA; siZFP36, small interfering RNA targeting ZFP36; siZFP36L1, small interfering RNA targeting ZFP36L1; siZFP36L2, small interfering RNA targeting ZFP36L2; UTR, untranslated region.

Figure 5. ZFP36 members are downregulated in psoriatic skin versus in AD skin.

(a) Transcripts levels of NL and LS skin of individuals with Pso and AD from the GSE121212 dataset were normalized using GREIN. The expression of ZFP36 members is shown. Significance was assessed by Mann–Whitney test. (b) Expression of ZFP36 members in dermal fibroblasts from NL and LS skin of individuals with Pso and AD was measured by qPCR analysis. Significance was assessed by Mann–Whitney test. Statistical significance indicated as * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. AD, atopic dermatitis; GREIN, Gene Expression Omnibus RNA-sequencing Experiments Interactive Navigator; LS, lesional; NL, nonlesional; Pso, psoriasis.



skin and TNF- α -stimulated fibroblasts, we found that 1,243 genes (39.2% of the DEGs) contained at least one UAUUUU sequence (Figure 4a). These genes included, among others, *IL6*, *CXCL8*, *CXCL2*, and *CCL20* (Supplementary Table S5).

To evaluate whether the mRNA of the DEGs effectively bind ZFP36 members in vitro, we immunoprecipitated ZFP36 and ZFP36L1/2 in TNF- α -stimulated fibroblasts and compared the bound mRNA from the specific pulldown with IgG control (Figure 4b). We included TNF and *CCL5* as positive and negative controls, respectively, because all ZFP36 members were shown to interact with *TNF* mRNA (Ciais et al., 2013) but not with *CCL5* mRNA, whose 3'UTR lacks AREs (Fallmann et al., 2016) (Figure 4b and Supplementary Figure S3b). All screened cytokine mRNAs bound to ZFP36L1/2, whereas *IL6* and *CXCL8* mRNAs did not bind to ZFP36. These results differ from what was previously observed in other cell types (Angiolilli et al., 2018; Sedlyarov et al., 2016), suggesting cell- and context-dependent functions of ZFP36.

We next investigated whether the knockdown of ZFP36 members would also lead to increased expression of target cytokines. We silenced all individual ZFP36 members using small interfering RNAs (Figure 4c and d) and observed that for some cytokines, the results from the silencing assay were consistent with those from the RNA immunoprecipitation assays (Figure 4e). We validated that the single knockdown of ZFP36L1 causes not only higher mRNA expression but also higher production of *IL6*, whereas the knockdown of ZFP36 and ZFP36L2 increases the expression and production of *CXCL2* and *CXCL8*, respectively (Figure 4f and Supplementary Figure S4a and b). Taken together, these data show that ZFP36 proteins regulate subsets of Pso-associated cytokine mRNAs in fibroblasts.

ZFP36 members are downregulated in psoriatic skin compared with that in atopic dermatitis skin

To gain more insights into the specific involvement of ZFP36 members in different inflammatory skin diseases, we compared psoriatic and atopic dermatitis (AD) skin transcriptomes (Tsoi et al., 2019). We observed that all ZFP36 members were downregulated in psoriatic LS skin when compared with those in AD LS skin and were downregulated in psoriatic NL skin compared with those in AD NL skin (Figure 5a). We validated these findings in dermal fibroblasts isolated from psoriatic and AD NL and LS skin (Figure 5b) and further observed that in the datasets used in this study, the expression of ZFP36L1 and ZFP36L2 was also downregulated in psoriatic NL skin compared with that in healthy skin (Supplementary Figure S5a and b). These results indicate that these proteins could contribute to the preinflammatory phase in Pso development.

Chronic exposure to TNF- α in fibroblasts leads to the suppression of ZFP36 members and the overexpression of inflammatory cytokines

Early inflammatory signatures in psoriatic skin were associated with TNF- α and IL-17 signaling and suggested to initiate psoriatic manifestations (Boyman et al., 2004; Khalil et al., 2020; Szél et al., 2019). Thus, we investigated whether chronic inflammatory exposure to such cytokines could influence ZFP36 family expression in dermal fibroblasts. We adopted a model in which cells were either stimulated daily with TNF- α , rested and restimulated with TNF- α (chronic model), or stimulated once with TNF- α (acute model) (Figure 6a). Profound changes were observed in expression for all the three ZFP36 members between the acute and chronic model, with all members being induced by acute TNF- α stimulation and unaffected or suppressed by chronic TNF- α (Figure 6b). As expected, the downregulation of ZFP36

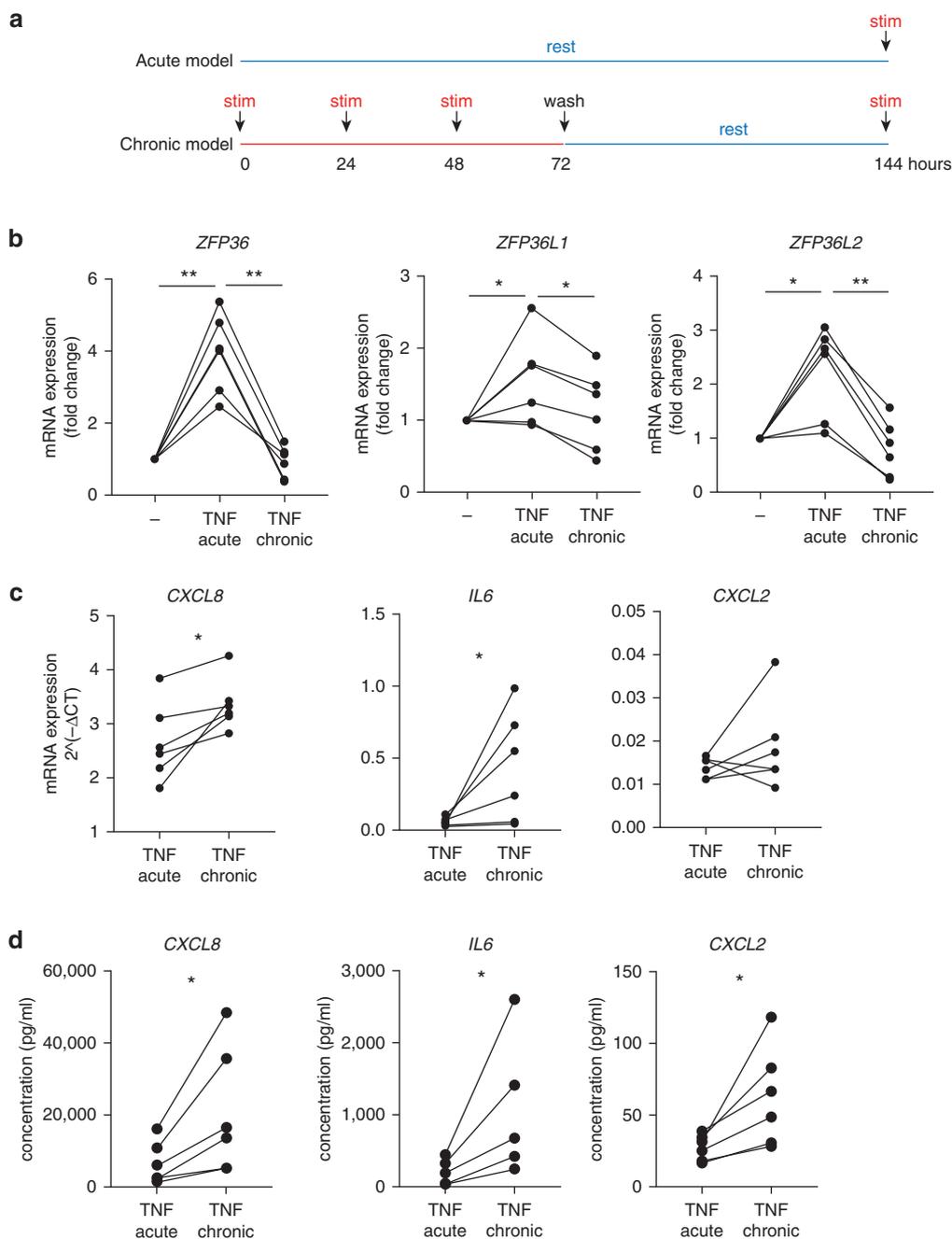


Figure 6. Chronic exposure to TNF- α in fibroblasts leads to the suppression of ZFP36 members and the overexpression of inflammatory cytokines. (a) Model for acute and chronic stimulation in dermal fibroblasts. (b, d) Dermal fibroblasts were stimulated either once (acute model) or repeatedly (chronic model) with TNF- α , and the expression of either (b) ZFP36 family members after 2 hours stimulation or (c, d) cytokines after 8 hours of stimulation was assessed by (b, c) qPCR or (d) ELISA. In b, the significance was defined by one-way ANOVA followed by Benjamini–Yekutieli FDR correction, and in c and d, the significance was defined by paired *t*-test. Statistical significance indicated as **P* < 0.05; ***P* < 0.01. stim, stimulated.

members was also concomitant with increased cytokine expression, both at mRNA and protein levels (Figure 6c and d). Ultimately, we observed that the most prominent cytokine induction and ZFP36 family suppression occurred after chronic TNF- α but not after IL-17 stimulation (Supplementary Figure S6a and b).

In conclusion, these data indicate that reduced ZFP36 family expression in psoriatic skin could be a consequence of chronic TNF- α exposure, which further potentiates cytokine production in dermal fibroblasts.

DISCUSSION

Dermal fibroblasts constantly interact with the cells of the immune system and the epidermis. On activation, these cells

contribute to the upregulation of proinflammatory factors that recruit immune cells in the dermis and promote KC proliferation (Gubán et al., 2016). Therefore, fibroblasts are likely to play a crucial role in the development of psoriatic lesions (Geğotek et al., 2020). Despite this evidence, dermal fibroblasts are often considered the forgotten cells of the innate immune memory (Crowley et al., 2018) because they remain largely understudied.

With this work, we aimed to investigate whether and through which pathways dermal fibroblasts can impact psoriatic manifestations. Our results indicate that a large percentage (>75%) of DEGs in activated (TNF- α -stimulated) fibroblasts are also commonly dysregulated in psoriatic skin lesions. Furthermore, the expression of multiple cytokines

known for their proinflammatory functions in acute inflammatory conditions (Wang et al., 2009) and mostly annotated to KCs, such as CXCL8 and IL-6 (Patel et al., 2018), was found higher in fibroblasts than in KCs.

Conversely, IL-17 and IL-23, two major players in Pso pathogenesis (Billi et al., 2019), were not expressed in stimulated fibroblasts (data not shown). Overall, these data suggest that dermal fibroblasts can significantly contribute to the inflammatory features of psoriatic skin, albeit not directly sustaining IL-23/IL-17 production.

Of interest, we found that fibroblasts derived from psoriatic LS skin retained ex vivo proinflammatory features after different cell passages, compared with those from NL skin counterparts. We could mimic these enhanced inflammatory features by chronically stimulating NL skin fibroblasts with TNF- α . These results suggest that dermal fibroblasts can possibly acquire an innate immune memory after chronic activation. Our observations are in line with similar reports in synovial and gingival fibroblasts (Gangishetti et al., 2020; Jurdziński et al., 2020), indicating that immune memory could be a common feature in stromal cells.

By exploring the signaling pathways enriched in psoriatic LS skin and TNF- α fibroblasts, we found that one pathway, underexplored in skin fibroblasts but proven to be relevant for Pso development (Andrienne et al., 2017; Patial et al., 2016), concerned the processes of regulation of mRNA stability by AREs. This type of post-transcriptional regulation was shown to control cytokine expression in immune cells and to prevent hyperinflammation (Uchida et al., 2019). Under physiological conditions, the mRNA of proinflammatory genes is constantly degraded by AREBPs, which allows a rapid control of gene expression. About 5% of the human transcriptome contains AREs in their mRNA 3'UTRs (Bakheet et al., 2018; Ripin et al., 2019). In this study, we showed that 87.5% of DEGs common in psoriatic LS skin and TNF- α -stimulated fibroblasts contain different types of AREs, highlighting the importance of such regulatory elements in Pso manifestations.

Mammalian AREBPs that regulate inflammatory mRNAs include the ZFP36/TIS11 family members ZFP36 (TTP), ZFP36L1 (BRF1), and ZFP36L2 (BRF2) (Ciais et al., 2013). Whereas the RNA-binding domain of the ZFP36 members is highly homologous (Blackshear and Perera, 2014), suggesting that these proteins may bind to similar consensus sites and play similar roles, our data show that despite sharing in silico predicted binding sites for ZFP36 family, inflammatory mRNAs differently bind to ZFP36 and ZFP36L1/2. In addition, our silencing experiments indicate that the modulation of inflammatory mRNAs differs per ZFP36 family member. These results are in line with in vivo evidence of the different phenotypes obtained from the respective knockout models (Ciais et al., 2013), which indicates distinct functions of ZFP36 members. We further verified that the knockdown of each ZFP36 member does not lead to the upregulation of the other family members (Supplementary Figure S3c), also suggesting possibly minimal compensatory functions in fibroblasts.

KC-specific knockout of *Zfp36* was previously found to aggravate Pso manifestations in mice, and ZFP36 mRNA was found reduced in the epidermis of patients with Pso, compared with that in the epidermis of healthy individuals

(Andrienne et al., 2017). These observations, combined with our results, indicate that ZFP36 and other ZFP36 members play an important role in dermal fibroblasts and not only in KCs and that further studies are needed to define fibroblast-specific contributions of ZFP36 members in Pso manifestations. Furthermore, we did not observe differences in ZFP36 family expression between papillary and reticular fibroblasts, two major subsets of dermal fibroblasts (Ascensión et al., 2020), which suggests a conserved role of ZFP36 proteins in these cells.

Pso is notoriously characterized by an IL-17/TNF- α inflammatory signature, distinct from the IL-13/IL-4 signature in AD (Dainichi et al., 2018). However, what is not yet defined is what contributes to the specific inflammatory profile of Pso. We found that all ZFP36 members were reduced in psoriatic LS skin compared with those in AD LS skin and obtained similar results in skin fibroblasts. On chronically exposing skin fibroblasts to TNF- α , we further observed that all ZFP36 members displayed a reduced expression, compared with those exposed to the acute TNF- α model.

Although it is currently not known what may cause the differential expression of ZFP36 members in Pso and AD, GWASs of patients with Pso did not identify susceptibility variants in the ZFP36 regions. Rather, reduced ZFP36 expression in the skin was suggested to be a consequence of recurrent inflammation (Andrienne et al., 2017). As such, reduced ZFP36 family expression in NL skin may represent a first-hit molecular event, which prepares the skin to inflammatory LS manifestations on second-hit events (such as after köebnerization) (Ji and Liu, 2019). Furthermore, because ZFP36 selective depletion in the epidermis was shown to initiate not only Pso but also arthritis development (Andrienne et al., 2017), it is possible that initial ZFP36 family alterations in the skin could contribute to psoriatic arthritis, one of the most severe complications in patients with Pso (Scher et al., 2019).

The evidence that fibroblasts from different anatomic locations can retain an inflammatory memory is growing. In this study, we provide evidence that fibroblasts from LS psoriatic skin maintain proinflammatory properties ex vivo. Thus, it is possible that dermal fibroblasts contribute to the development or continuation of Pso.

We further provide, to our knowledge, previously unreported evidence that ZFP36 members contribute to the inflammatory features of dermal fibroblasts (graphical abstract). Transcriptomic profiles of ZFP36 family-knockout fibroblasts, fibroblast-specific depletion of ZFP36 members in animal models, as well as transcriptome-wide identification of mRNA targets will be needed to assess the broader contributions of these AREBPs in fibroblasts. Nonetheless, the development of compounds able to modulate the activity of ZFP36 members, which have already shown success in a clinical trial on patients with Pso (Colin et al., 2014), holds potential for further preclinical investigations in Pso and other inflammatory diseases.

MATERIALS AND METHODS

Patients and skin samples

Punch biopsies (4 mm) were taken from LS and NL skin locations from patients with a dermatologist-confirmed diagnosis of Pso or

AD. Informed written consent was obtained from all patients enrolled in the study. The study was approved by the medical ethics committee of the University Medical Center Utrecht, University of Utrecht, The Netherlands (METC 15-429/M and METC 12-407). All samples and clinical information were treated anonymously. Patients with Pso did not receive systemic drugs nor UV light therapy for Pso treatment and were instructed not to use topical steroids or other creams for 2 weeks in advance of their biopsy. Patients with AD were treated with topical steroids only. Once collected, skin biopsies were immediately digested for dermal fibroblasts isolation. Healthy skin biopsies were obtained as resected material after cosmetic surgery after institutional ethical approval.

RNA immunoprecipitation assay

Fibroblasts were cultured overnight in 10% fetal bovine serum DMEM, after which cells were serum starved for 16 hours. Cells were stimulated with 10 ng/ml TNF- α for 2 hours and were subsequently washed with cold PBS and lysed for 30 minutes in RNA immunoprecipitation buffer (1% NP-40, 150 mM sodium chloride, 50 mM Tris hydrochloric acid pH 8.0, 1 mM magnesium chloride, 10% glycerol, 1 mM dithiothreitol) supplemented with 50 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 0.5% aprotinin (all from Sigma-Aldrich, St. Louis, MO), and 100 U/ml RNase inhibitor Ribolock (Fermentas, Waltham, MA). Antibodies specific for ZFP36/TTP (Merck, Darmstadt, Germany), ZFP36L1/2 (Cell Signaling Technology, Danvers, MA), and control rabbit IgG (Dako Glostrup, Denmark) were added to the lysates for 6 hours at 4 °C, and A/G beads (Santa Cruz Biotechnology, Dallas, TX) were added for 2 hours at 4 °C. The samples were lysed in RLT buffer with 1:100 β -mercaptoethanol (Qiagen, Hilden, Germany) and were further processed for qPCR without normalizing the input of reverse-transcribed RNA.

Small interfering RNA transfection

Fibroblasts were transfected using DharmaFECT1 (Thermo Fisher Scientific, Waltham, MA). The day before transfection, cells were maintained in DMEM containing 10% fetal bovine serum. On transfection, DMEM was replaced by OPTI-MEM (Thermo Fisher Scientific). ZFP36-, ZFP36L1-, and ZFP36L2-specific small interfering RNA (20 nM) and control nontargeting small interfering RNA (20 nM) (all from Thermo Fisher Scientific) were mixed with DharmaFECT1 and incubated for 20 minutes at room temperature before transfection. The medium was replaced 24 hours after transfection with DMEM containing 10% fetal bovine serum, and cells were cultured for another 24 hours.

Analysis of adenylate-uridylylate-rich motifs

The lists of Ensemble gene IDs containing AREs in their 3'UTR were retrieved from AREsite2 database (Fallmann et al., 2016) and were converted to Entrez gene IDs using org.Hs.eg.db Bioconductor package in RStudio.

Gene Set Enrichment analysis

Series matrix files from Gene Expression Omnibus (GEO) dataset GSE13355 (Nair et al., 2009) and GSE40560 (Boisson et al., 2012) were analyzed with Gene Set Enrichment Analysis software (version 4.1.0). Ranking was based on *t*-test and weighted enrichment statistics. A total of 1,000 permutations on the phenotype (LS vs. NL psoriatic skin and TNF- α -stimulated vs. -unstimulated fibroblasts from GSE13355 and GSE40560, respectively) were allowed per analysis. The database used for analysis was C2.CP.Reactome (version 7.2), which includes curated gene sets belonging to

Reactome-enriched canonical pathways. Significant signatures were shortlisted on the basis of the force discovery rate-corrected *P*-value (*q*-value) and listed on the basis of normalized enrichment score. Normalized enrichment scores were plotted in heatmap using Morpheus software (Morpheus Data, Greenwood Village, CO) (software.broadinstitute.org/Morpheus). GEO dataset GSE14905 (Yao et al., 2008) used for validation purposes was subjected to the same analysis criteria by comparing LS with NL psoriatic skin samples.

GEO datasets analysis

DEGs ($P < 0.05$) in LS versus those in NL psoriatic skin and those in TNF- α -stimulated versus those in -unstimulated fibroblasts from GSE13355 and GSE40560, respectively, were analyzed in GEO2R. Illumina HumanHT-12, version 4.0 (Illumina, San Diego, CA), probe sets from GSE40560 (GPL10558 platform) and Affymetrix Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA) probe sets from GSE13355 (GPL570 HG-U133_Plus_2 platform) were converted to Entrez gene IDs using org.Hs.eg.db Bioconductor package in RStudio. Shared gene IDs (3169) were illustrated using EnhancedVolcano Bioconductor package in RStudio and analyzed by Reactome Pathway Analysis Bioconductor package in RStudio by setting the adjusted *P*-value cutoff at 0.05.

Affymetrix Human Genome U133A 2.0 Array probe sets from GSE154558 (GPL571 HG-U133A_2 platform) (de Stree et al., 2020) were converted to Entrez gene IDs using org.Hs.eg.db Bioconductor package in RStudio. The expression of gene IDs from the comparison of GSE13355 and GSE40560 studies was retrieved in TNF- α -stimulated fibroblasts and KCs from GSE154558. Expression values were plotted in heatmap using Morpheus software.

GEO RNA-sequencing Experiments Interactive Navigator () (Mahi et al., 2019) was used to retrieve normalized transcript levels from the GEO dataset GSE121212 (Tsoi et al., 2019), whereas RMAExpress software (<https://rmaexpress.bmbolstad.com/>) was used to perform Robust Multi-array Average analysis of CEL files from GSE13355. For the latter, default background correction and quantile normalization were adopted.

Additional materials and methods on skin collection, cell culture, RNA extraction and qPCR, ELISA, western blot, and statistical analysis are provided in [Supplementary Materials and Methods](#).

Data availability statement

Series matrix files are accessible from the deposited GSE13355, GSE40560, GSE154558, and GSE121212 Gene Expression Omnibus datasets. We provide the processed data used for this study as [Supplementary Materials](#).

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CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2021.06.030>.

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