

A spatially coordinated keratinocyte–fibroblast circuit recruits MMP9⁺ myeloid cells to drive type I interferon-driven inflammation in photosensitive autoimmunity

Received: 4 August 2025

Accepted: 16 March 2026

Published online: 24 April 2026

 Check for updates

Yuqing Wang^{1,8}, Khashayar Afshari^{2,8}, Nazgol-Sadat Haddadi^{2,8}, Carolina Salomão Lopes¹, Chee-Huat Linus Eng³, Leah M. Whiteman⁴, Nuria Martinez², Pyae P. Kyaw², Ksenia S. Anufrieva⁵, Kevin Wei⁵, Kirsten Frieda³, Misha Rosenbach⁶, Ruth Ann Vleugels⁷, Stefania Gallucci⁴, John E. Harris², Mehdi Rashighi^{2,9}✉ & Manuel Garber^{1,9}✉

Photosensitivity is central to cutaneous lupus erythematosus and dermatomyositis (DM), but the mechanisms linking UVB exposure to tissue-specific autoimmunity are poorly defined. Using single-cell RNA sequencing, spatial transcriptomics, proteomics, UVB provocation and in vitro modeling, we identify MMP9⁺CD14⁺ myeloid cells as critical mediators of photosensitivity. These cells expand significantly in lesional skin, produce interferon- β (IFN β) and colocalize with cytotoxic CD4⁺ T cells at the dermal–epidermal junction. Keratinocytes activate fibroblasts in the superficial dermis, prompting them to release chemokines (CCL2, CCL19, CCL7, CCL8) that recruit MMP9⁺CD14⁺ cells. In vitro, type I interferon-primed keratinocytes exposed to UVB release cytokines activating dendritic cells, mirroring in vivo responses. UVB irradiation of non-lesional skin of patients with DM rapidly recruits these myeloid cells. In a clinical proof-of-concept study, anti-type I interferon treatment with anifrolumab prevented UVB-induced myeloid infiltration and reduced photosensitivity. Therefore, targeting MMP9⁺CD14⁺ cells may offer therapeutic potential for managing photosensitive autoimmune skin conditions.

Ultraviolet (UV) light presents a paradox in cutaneous immunology: broadly therapeutic in psoriasis and vitiligo, yet a potent trigger of inflammation in cutaneous lupus erythematosus (CLE) and dermatomyositis (DM). Both photosensitive diseases exhibit interface dermatitis with a prominent type I interferon signature, but how type I interferon licenses UV-induced skin inflammation remains unclear¹.

To define the pathways linking UVB exposure to inflammatory injury, we integrated single-cell RNA sequencing (scRNA-seq) and proteomic profiling across photosensitive (CLE, DM) and photoresponsive (psoriasis, vitiligo) diseases, complemented by spatial transcriptomics and in vivo UV provocation. Across conditions, three features most

clearly distinguished photosensitive lesions: heightened type I interferon signaling, a marked expansion of CD14⁺ IFN β -producing myeloid cells and the presence of cytotoxic CD4⁺ T cells.

Spatial transcriptomic analyses revealed that CD14⁺ cells span a continuum of transcriptional states. In non-lesional skin, they predominantly exhibited an M2-like phenotype and were distributed away from the dermal–epidermal junction. By contrast, photosensitive lesions showed a shift toward a more inflammatory, MMP9⁺ M1-like state, with CD14⁺ cells enriched at the dermal–epidermal interface and colocalized with activated T cells. Integration of spatial profiling with in vitro perturbation experiments uncovered a keratinocyte–fibroblast–myeloid

A full list of affiliations appears at the end of the paper. ✉e-mail: mehdi.rashighi@umassmed.edu; manuel.garber@umassmed.edu

circuit that drives this transition. In a type I interferon-primed environment, UVB injury induced soluble protein release from basal keratinocytes, which in turn activated neighboring fibroblasts to produce CD14⁺ chemoattractants. This cascade promoted both recruitment and activation of CD14⁺ cells, establishing a feed-forward loop that amplifies interface dermatitis in photosensitive autoimmunity.

Results

To compare immune features of photosensitive versus photoresponsive skin, we profiled suction blister biopsies from lesional, non-lesional and healthy sites using scRNA-seq and a 250-plex soluble protein assay² (Extended Data Fig. 1a and Supplementary Tables 1 and 2). Clustering of 79,261 cells based on canonical markers³ resolved four major populations: keratinocytes (52,608), melanocytes (3,614), lymphocytes (14,256) and myeloid cells (8,783) (Fig. 1a, Extended Data Fig. 1b and Supplementary Table 3).

RNA and protein profiling reveal a robust type I interferon signature in photosensitive skin

Pseudo-bulk analysis identified 3,176 genes dysregulated in lesional and non-lesional skin compared with controls (Fig. 1b). CLE and DM exhibited a pronounced type I interferon signature at both transcript and protein levels, detectable even in non-lesional skin, consistent with a subclinical type I interferon milieu (Fig. 1c,d and Extended Data Fig. 1c). Interferon-inducible chemokines (*CXCL9-11*) were elevated across all diseases, whereas psoriasis showed an IL-17-driven inflammatory profile (Fig. 1b,c and Extended Data Fig. 1d).

Keratinocytes in non-lesional photosensitive skin are primed with type I interferon

Keratinocytes segregated into six subtypes, with activated keratinocytes (*KRT6A/6B/16/17*) enriched in psoriasis, CLE and DM, and granular keratinocytes reduced across psoriasis, CLE and vitiligo (Fig. 1e, Extended Data Fig. 1b,e and Supplementary Table 3). Strikingly, basal and suprabasal keratinocytes from both lesional and non-lesional CLE and DM skin displayed a strong type I interferon signature that was absent in psoriasis and vitiligo, revealing a pre-existing interferon-primed epithelial state unique to photosensitive diseases (Fig. 1f).

Photosensitive diseases are enriched for cytotoxic T_H1 CD4⁺ T cells

We identified distinct lymphocyte subsets, including CD4⁺ T helper cells (T_H1 cells), regulatory T cells, cytotoxic CD8⁺ T cells, natural killer cells and $\gamma\delta$ T cells (Fig. 1g and Extended Data Fig. 1f). Their composition differed significantly across disease (χ^2 test, $P < 2.2 \times 10^{-16}$; Fig. 1h and Supplementary Table 3): Tc17 cells were expanded in psoriasis, whereas cytotoxic CD8⁺ T cells were enriched in DM, CLE and vitiligo. Most strikingly, CLE and DM lesions contained abundant T_H1-skewed CD4⁺ T cells with a strong type I interferon and cytotoxicity signature, marked by high expression of *GZMB*, *GZMK* and *PRF1* (Fig. 1h,i and Extended Data Fig. 1g).

Disease-specific myeloid cell subsets distinguish inflammatory skin diseases

Re-clustering myeloid cells resolved Langerhans cells, conventional dendritic cells (cDC1, cDC2), plasmacytoid DCs (pDCs)⁴⁻⁶, a CD14⁺ monocytic cluster (*MAFB*, *CD14*, *FCGR1A* *FCGR3A*), a migratory heterogeneous cluster and a tolerogenic population (Myeloid-Tol)^{7,8} (Fig. 2a,b and Extended Data Fig. 2a).

Langerhans cells were reduced in lesional skin across all diseases (false discovery rate (FDR) < 0.2, single-cell compositional data analysis (scCODA); Fig. 2c and Extended Data Fig. 2b), probably reflecting impaired skin maintenance associated with reduced *IL34* expression in keratinocytes and melanocytes^{9,10} (Fig. 2d,e). By contrast, cDC1 cells

were expanded in vitiligo, consistent with their established role in disease pathogenesis¹¹, while cDC2 subsets were enriched in psoriasis, aligning with their function in IL-17-driven psoriasiform inflammation¹² (Fig. 2c and Extended Data Fig. 2b).

Type I interferon-producing myeloid subsets distinguish photosensitive from photoresponsive skin disease

Distinct type I interferon-producing myeloid subsets were selectively expanded in photosensitive dermatoses (Fig. 2c and Supplementary Table 3). pDCs comprised 12.5% of myeloid cells in CLE lesions (Fig. 2c and Extended Data Fig. 2b) and displayed high expression of *IRF7* and of *IFNA* family members, consistent with their established role as IFN α producers and targeting them via BDCA2/CLEC4C blockade in CLE (Fig. 2f and Extended Data Fig. 2c)^{13,14}. CD14⁺ myeloid cells (MC_{CD14}) were markedly expanded in DM (43%) and to a lesser extent in CLE (12%) (Fig. 2c,g), and emerged as the predominant source of *IFNB1* in DM, which was validated in an independent full-thickness scRNA-seq dataset¹⁵ (Methods, Fig. 2h and Extended Data Fig. 2e–g). Protein measurements mirrored transcriptomic patterns: IFN α was elevated in CLE and IFN β in DM in interstitial skin fluid (Fig. 1d and Extended Data Fig. 1d), highlighting disease-specific interferon-producing myeloid programs as defining features of photosensitivity.

Type I interferons enhance the cytotoxicity of CD4⁺ T cells

Cytotoxic CD4⁺ T cells, defined by *TBX21*, *IFNG*, *CXCR3*, *PRF1* and *GZMB* expression¹⁶, were selectively expanded in photosensitive lesions, with the highest enrichment in DM (Fig. 1h,i). To determine whether interferon signaling augments their effector function, CD4⁺ T cells from healthy donors were activated ex vivo and exposed to IFN β or IFN γ . IFN β , but not IFN γ , significantly increased *GZMB* expression (Fig. 2i) and enhanced epithelial killing in a real-time cytotoxicity assay (Fig. 2j). These findings indicate that type I interferon licenses a cytotoxic program in T_H1 CD4⁺ T cells, providing a mechanistic link between type I interferon conditioning and epithelial injury in photosensitive autoimmunity.

Spatial profiling defines cellular architecture in autoimmune skin

We mapped inflammatory cell organization in photosensitive skin using seqFISH¹⁷ with a 511-gene panel derived from scRNA-seq (Supplementary Table 4). Across 13 lesional sections from seven patients (two DM, two CLE, two psoriasis, one vitiligo), we profiled 155,440 cells (median of 594 transcripts per cell). Unsupervised clustering recovered major epidermal and dermal lineages, including populations not captured by suction blister sampling (Fig. 3a–d, Extended Data Fig. 3a–d and Supplementary Information).

Lineage-specific reclustering resolved myeloid subsets, including DC1, DC2, CD14⁺, Langerhans cells, pDCs and neutrophils (Fig. 3c,d and Extended Data Fig. 3e), and five fibroblast subtypes, including two spatially segregated proinflammatory states (Extended Data Fig. 3f). *FB_proInf_sup*, positioned in the superficial dermis, expressed *CCL2*, *CCL8* and *CXCL2*, whereas *FB_proInf_deep* enriched around eccrine glands and immune aggregates, expressed *CCL19* and *CXCL12* (Extended Data Fig. 3b,f). Cross-referencing with a recent fibroblast atlas showed that both subsets also appear in healthy skin, with *FB_proInf_sup* aligning with the ‘superficial’ fibroblast cluster and *FB_proInf_deep* with the fibroblastic reticular cell-like population, indicating that these states are not disease-specific¹⁸.

Spatial transcriptomics reveals stratified inflammatory states of CD14⁺ myeloid cells

seqFISH enabled in situ resolution of CD14⁺ myeloid heterogeneity in photosensitive skin. Although all CD14⁺ cells expressed canonical markers (*CD14*, *CD163*, *MRC1* and *MAF*), diffusion component analysis revealed a continuous transcriptional spectrum with distinct spatial

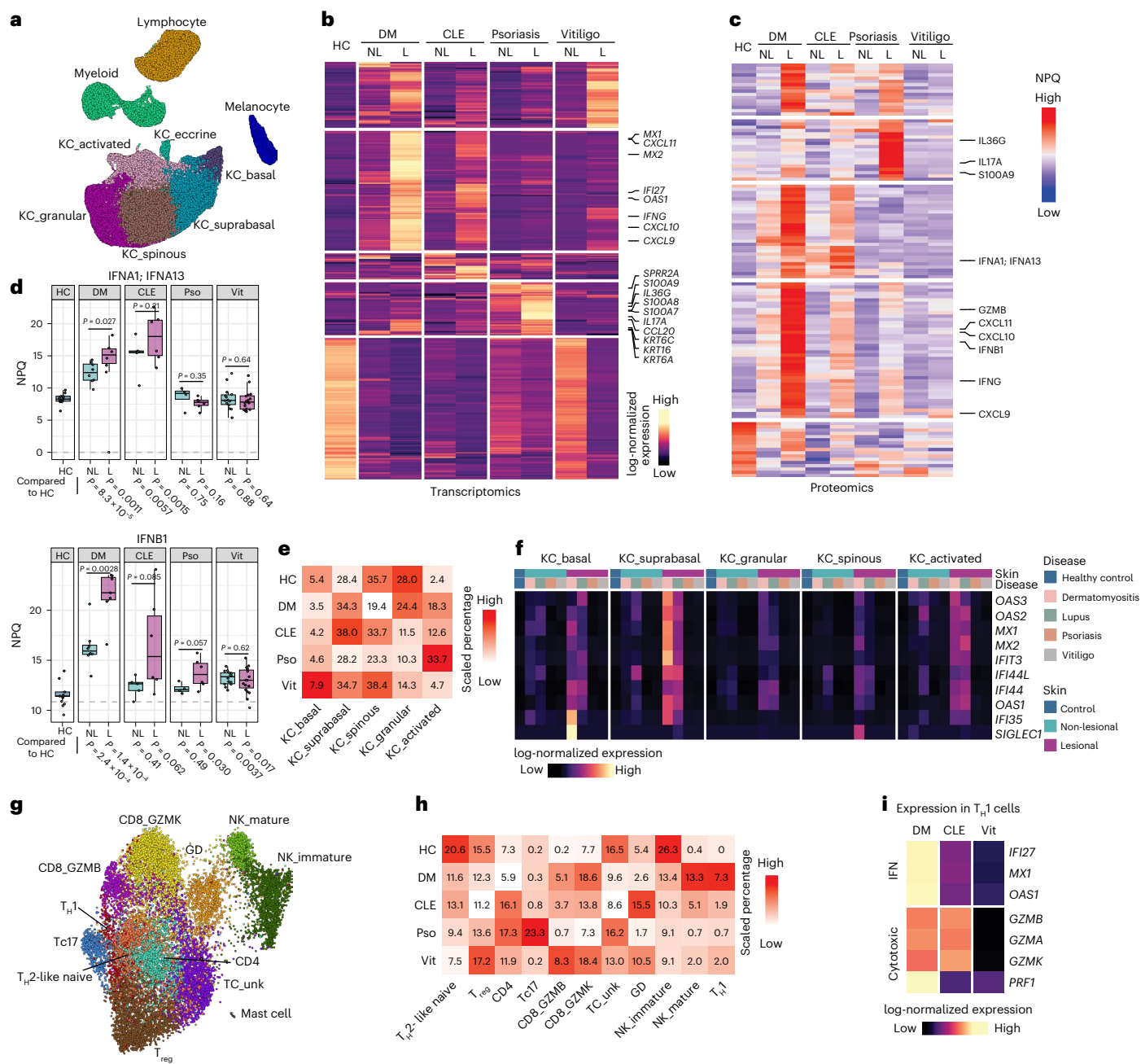


Fig. 1 | Integrated single-cell transcriptomic and proteomic profiling reveals type I interferon-associated immune and epithelial features unique to photosensitive skin diseases. **a**, UMAP embedding of all cells from blister biopsy scRNA-seq. Cells are colored by cell type. KC, keratinocytes. **b**, Heatmap of differentially expressed genes from pseudo-bulk analysis across conditions in scRNA-seq. The top 100 significant genes per disease by fold change (FDR < 0.01), identified using DESeq2, were clustered using *k*-means. log-normalized expression values are shown, scaled by row. NL, non-lesional; L, lesional. **c**, Heatmap of differentially abundant proteins in blister fluid quantified by NULISA ($P < 0.05$, $|\log_2(\text{fold change})| > 1$, *t*-test). NULISA protein quantification (NPQ) represents $\log_2$ -transformed normalized counts. Color is scaled by row. **d**, Box plots showing IFNA1, IFNA13 and IFNB1 blister fluid protein levels (NPQ) across diseases and skin types (lesional and non-lesional). Each dot represents one blister fluid sample from a single patient (one sample per patient per group). The limit of detection (LOD) is indicated by a gray dashed

line. Only samples above the LOD were included in statistical testing. Sample sizes: IFNA1, IFNA13: healthy controls (HC) ($n = 12$), DM (NL, $n = 8$; L, $n = 6$), CLE (NL, $n = 5$; L, $n = 6$), psoriasis (Pso) (NL, $n = 4$; L, $n = 6$), vitiligo (Vit) (NL, $n = 14$; L, $n = 16$). *IFNB1*: HC ($n = 9$), DM (NL, $n = 8$; L, $n = 7$), CLE (NL, $n = 5$; L, $n = 6$), Pso (NL, $n = 4$; L, $n = 6$), Vit (NL, $n = 14$; L, $n = 15$). Two-sided *t*-test was used for pairwise comparisons: NS, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Box plots show the median (center line), interquartile range (IQR) (box, 25th–75th percentiles) and whiskers extending to the most extreme values within $1.5 \times$ IQR from the box; points beyond are shown as outliers. **e**, Percent of keratinocyte subtypes (of total keratinocytes) per disease; colors scaled by cell type (column). **f**, Pseudo-bulk expression heatmap of type I interferon-induced genes across keratinocyte subtypes. **g**, UMAP embedding of reclustered lymphocytes. **h**, Percent of lymphocyte subtypes (of total lymphocytes) per disease; colors scaled by cell type (column). **i**, Pseudo-bulk expression heatmap of cytotoxic effector and type I interferon response genes. T_H1 cells were nearly absent in HC and Pso and were excluded from the heatmap.

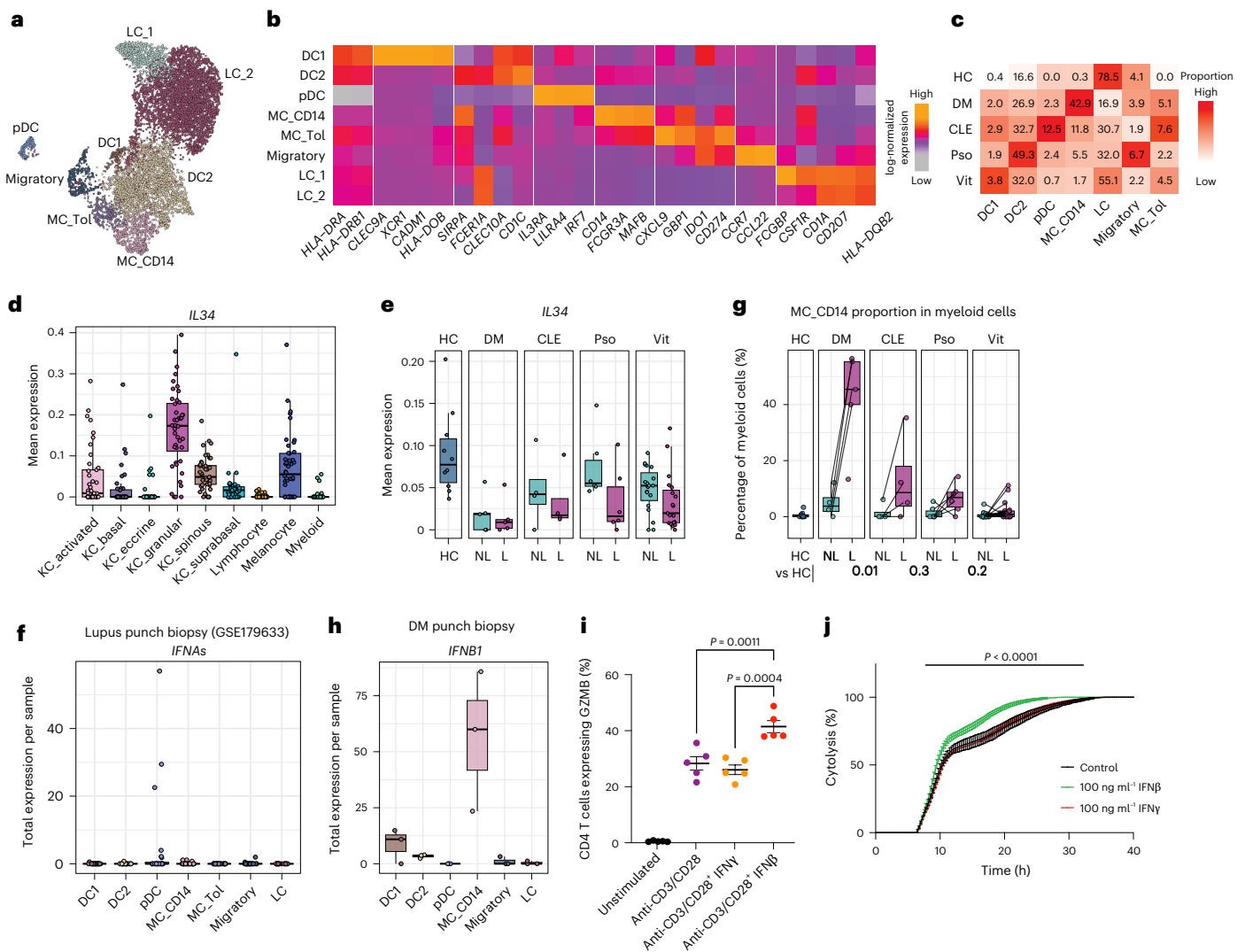


Fig. 2 | Disease-specific myeloid subsets and type I interferon-associated cytotoxicity in photosensitive skin disease. a, UMAP embedding of reclustered myeloid cells. MC, myeloid cells; LC, Langerhans cells. **b**, Pseudo-bulk expression heatmap of marker genes for myeloid subsets in scRNA-seq of blister biopsies. **c**, Percentage of myeloid subtypes within all myeloid cells per disease; colors scaled by cell type (column). **d, e**, *IL34* pseudo-bulk expression aggregated per cell type for each individual (total individuals, $n = 44$) (d) or aggregated at the patient level within each skin condition (e). Lesional and non-lesional samples are paired within the same patients. Individual numbers per condition: HC ($n = 10$), DM ($n = 5$ pairs), CLE ($n = 4$ pairs), Pso ($n = 6$ pairs), Vit ($n = 19$ pairs). **f**, Percentages of CD14⁺ myeloid cells within total myeloid cells per blister biopsy sample across skin conditions. Statistical significance was assessed using scCODA, with KC_suprabasal as the reference. Only samples containing myeloid cells were included. Sample sizes (paired NL-L within patients): HC ($n = 10$),

DM (NL, $n = 4$; L, $n = 5$), CLE (NL, $n = 4$; L, $n = 4$), Pso (NL, $n = 6$; L, $n = 6$), Vit (NL, $n = 18$; L, $n = 19$). **g, h**, log-normalized transcript counts for *IFN* (g) and *IFNB1* (h) in myeloid cell subsets per punch biopsy sample from a public skin lupus dataset²⁴ ($n = 29$ samples in g: 13 epidermis and 16 dermis), and three unique DM samples from a previous publication¹⁵ (total patients in h, $n = 3$). **i**, Proportion of GZMB expressing CD4⁺ T cells in response to activation and IFN β or IFN γ treatment ($n = 5$ donors); comparison by one-way ANOVA followed by Tukey's test. **j**, Real-time cytotoxicity of solitomab-treated BT-20 cells co-cultured with CD4⁺ T cells pre-treated with IFN β , IFN γ or control, measured by Lionheart microscope. Data are shown as means; error bars, s.d. Comparison by one-way ANOVA followed by Tukey's test (biological replicates, $n = 3$ donors, $P < 0.001$). Box plot definition (all panels): center line, median; box, 25th–75th percentiles; whiskers, most extreme values within 1.5× IQR; points beyond whiskers, outliers.

and functional poles. One end featured cells with high *LYVE1* and *CD34* but low *MMP9*, *CD68* and *IFI44L*. The opposite end comprised cells with upregulated *MMP9*, *CD68* and *IFI44L*, resembling the proinflammatory state captured in suction blister samples (Fig. 3e,f).

Spatially, *MMP9*^{low} CD14⁺ cells localized to the deeper dermis, whereas *MMP9*^{high} CD14⁺ cells accumulated near T cell aggregates at the dermal–epidermal junction¹⁹ (Fig. 3g,h and Extended Data Fig. 4a). This gradient supports in situ transition toward an inflammatory state marked by induction of *MMP9* and chemokines, such as *CCL13* and *CXCL3* (Extended Data Fig. 4b).

Mechanistically, *LYVE1* binds hyaluronic acid, a major dermal matrix component²⁰, probably retaining *LYVE1*⁺ *MMP9*⁺ cells in deeper

compartments. Conversely, *MMP9*-mediated matrix degradation may enhance motility, explaining why *MMP9*⁺ *LYVE1*⁺ CD14⁺ cells were preferentially captured by blister biopsy.

Colocalization analysis reveals spatial proximity of inflammatory CD14⁺ myeloid cells and cytotoxic CD4⁺ T cells in photosensitive skin lesions

To define spatial relationships between CD14⁺ myeloid cells and other populations, including T_H1 cytotoxic CD4⁺ T cells in DM (Extended Data Fig. 4c,d), we computed a distance-weighted colocalization score that incorporates both density and proximity (Methods and Extended Data Fig. 4e). This direction-dependent metric recapitulated

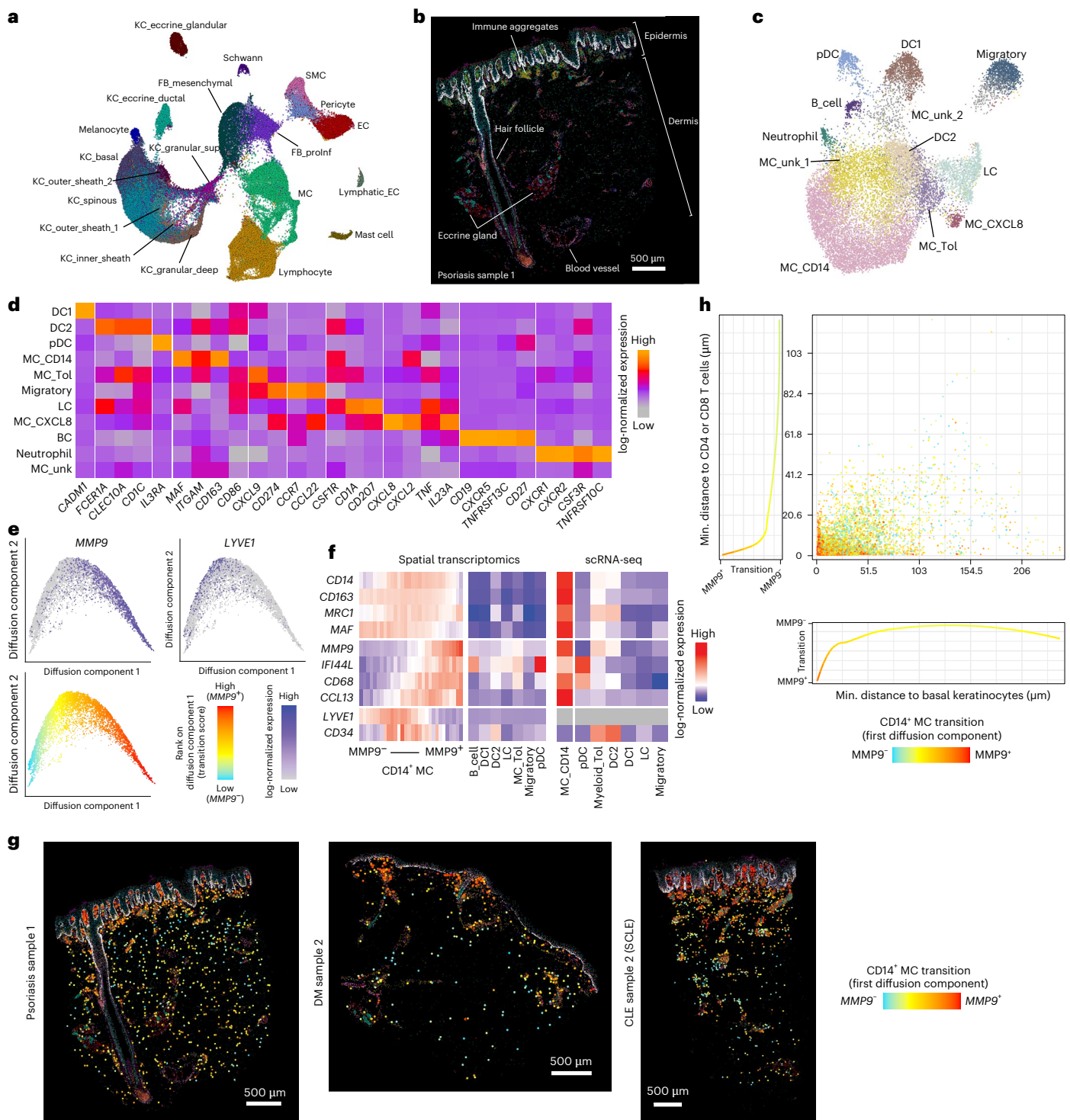


Fig. 3 | Spatial transcriptomics reveals spatial stratification and transcriptional heterogeneity of CD14⁺ myeloid cells in autoimmune skin lesions. **a**, UMAP embedding of 155,440 cells profiled by seqFISH across 13 skin sections derived from seven punch biopsies (seven patients). **b**, Spatial map showing skin features captured in a representative tissue section. Each dot represents a cell colored by cell type; basal keratinocytes are colored in white, delineating the dermal–epidermal junction. The remaining 12 sections are shown in the Supplementary Information. **c**, UMAP embedding of reclustered seqFISH myeloid lineage. **d**, Pseudo-bulk expression heatmap of marker genes for myeloid subclusters in seqFISH. **e**, Diffusion map of CD14⁺ myeloid cells identifies a transcriptional continuum from *MMP9*⁻ to *MMP9*⁺ states. Color intensity indicates log-normalized single-cell expression (upper two panels) or the first diffusion component score (bottom panel). **f**, Heatmap of selected

marker genes in myeloid cell subtypes in punch biopsy seqFISH (left) and blister fluid scRNA-seq (right). Left, CD14⁺ cells were ordered by their first diffusion component and aggregated into 50 equal-sized bins for the mean expression level of each bin. Colors were scaled separately for each dataset. **g**, Spatial embeddings of seqFISH data showing CD14⁺ myeloid cells colored by their first diffusion component in lesional tissue. Data are representative of $n = 13$ tissue sections derived from seven punch biopsies (seven patients) with similar results. **h**, Scatter plot showing minimum distance (in μ m) from a CD14⁺ cell to a basal keratinocyte (x axis) or a CD8 or CD4 T cell (y axis), colored by their projection onto the first diffusion component; marginal plots show the smoothed values of the first diffusion component of CD14⁺ cells projected onto the x axis (bottom) or the y axis (left).

known skin architecture, including epidermal layering and adnexal structures (Fig. 4a).

To examine how the spatial neighborhood of CD14⁺ myeloid cells evolves along their MMP9⁺ to MMP9⁺ trajectory, we divided them into ten equal bins along the first diffusion component and quantified colocalization with each reference population. MMP9⁺CD14⁺ cells localized predominantly near mesenchymal fibroblasts, whereas MMP9⁺ cells showed increasing colocalization with fibroblastic reticular cell-like fibroblasts (FB_proInf_deep), superficial fibroblasts (FB_proInf_sup) and immune populations, except for Langerhans cells (Fig. 4b and Extended Data Fig. 4f).

Spatial analysis further revealed transcriptional shifts associated with proximity to CD4⁺ T cells. As CD14⁺ cells approached CD4⁺ T cell aggregates, they upregulated *MMP9* but downregulated *LYVE1* (Fig. 4c,d and Extended Data Fig. 4g). Increased *MMP9* implies enhanced extracellular matrix remodeling capacity, which may facilitate immune cell migration and organization of lymphocytic aggregates at the dermal–epidermal junction^{21,22}. On the other hand, reduced *LYVE1* reflects loss of tissue-resident features and acquisition of a more migratory, inflammatory phenotype²³, consistent with activation in type I interferon-rich microenvironments.

CD14⁺ myeloid cells polarize from M2-like to M1-like states as they migrate toward the epidermis

To validate the CD14⁺ continuum past the limits of our spatial panel, we reanalyzed a published full-thickness CLE scRNA-seq dataset (GSE179633)²⁴. As expected, pDCs were enriched in discoid lupus erythematosus (DLE) and systemic lupus erythematosus (SLE) skin, whereas CD14⁺ cells selectively expanded within epidermal components, consistent with our spatial findings (Supplementary Table 3 and Fig. 5a). Targeted reclustering of CD14⁺ cells reproduced a continuous trajectory, with *MMP9* and *LYVE1* defining opposing poles along the first diffusion component (Fig. 5b and Extended Data Fig. 5a).

Differential expression across this continuum identified 3,234 genes ($P_{\text{adj}} < 0.05$; $|\text{avg. log}_2(\text{fold change})| > 1$). MMP9⁺ cells expressed an M1-like program (*IL6*, *IL1B*, *TNF*), whereas MMP9⁺ cells were enriched for M2 markers (Fig. 5c). Notably, *IFNG* and *TNF*, key M1-inducers, were expressed by immune cells, and MMP9⁺CD14⁺ cells colocalized with high *TNF* and *IFNG* at the dermal–epidermal junction and within immune aggregates (Fig. 5d,e), suggesting that local inflammatory cues drive polarization.

To identify mechanisms guiding spatial positioning, we profiled chemokine receptor expression. CD14⁺ cells expressed *CCR1*, *CCR2*, *CCR5*, *CCR7* and *CXCR4*. *CCR1* and *CXCR4* peaked at intermediate states, whereas *CCR5* and *CCR7* increased towards the MMP9⁺ pole (Fig. 5f, left).

Mapping ligand expression in the seqFISH dataset showed that fibroblasts, endothelial cells, lymphocytes and myeloid cells within ~40 μm of CD14⁺ cells produced distinct chemokines aligned with the CD14⁺ trajectory (Fig. 5f, right). *CXCL12*, the ligand for *CXCR4*, was highest in fibroblasts and endothelial cells adjacent to the intermediate state, whereas fibroblasts near the MMP9⁺ pole expressed *CCL2*, *CCL8*, *CCL13* and *CCL19*. Notably, *CCL19* was also expressed by tolerogenic/migratory myeloid cells only when in proximity to MMP9⁺CD14⁺ cells. These ligand patterns paralleled receptor dynamics: *CCR5* (for *CCL8*) and *CCR7* (for *CCL19*) were upregulated at late stages, whereas *CXCR4* was enriched earlier, suggesting an initial *CXCL12*–*CXCR4* axis followed by *CCR5*/*CCR7*-mediated reinforcement as cells approach the epidermis. Together, these findings position lesional fibroblasts as key organizers of chemokine gradients that direct CD14⁺ cells toward an inflammatory M1-like state.

Given that our seqFISH panel lacked several *CCR1*/*CCR5* ligands, we analyzed them in GSE179633, in which endothelial cells were the major sources of *CCL14*, *CCL15* and *CCL16*, implicating *CCR1*-dependent recruitment of circulating monocytes (Extended Data Fig. 5b). As this dataset reflects steady-state tissue, we cannot distinguish whether

MMP9⁺CD14⁺ cells arise from incoming monocytes or migration of dermal-resident cells; both may contribute.

T cells emerged as dominant producers of *CCL5*, a *CCR5* ligand, supporting *CCR5*-mediated colocalization of MMP9⁺CD14⁺ cells and T cells in CLE and DM. Finally, CD14⁺ cells expressed *CCL2/3/4/7/8/13*, suggesting potential self-recruitment through *CCR1/2/5* (Extended Data Fig. 5b).

Type I interferon primes UVB-induced recruitment of MMP9⁺CD14⁺ myeloid cells in photosensitive skin and is abrogated by IFNAR blockade

Given that non-lesional CLE and DM skin also exhibited heightened type I interferon signatures, we reasoned that type I interferon licenses clinically normal skin for exaggerated UVB responses. In vivo photoprovocation of healthy individuals and patients with DM demonstrated a striking difference: although CD14⁺ cells accounted for <10% of CD45⁺ immune cells in blister biopsies of both healthy skin and non-lesional DM skin at baseline, UVB irradiation induced a robust influx of CD14⁺ cells within 24 h, selectively in non-lesional DM skin (~20% of CD45⁺; $P < 0.001$; Fig. 6a and Extended Data Fig. 6a,b). As blister biopsies are enriched for MMP9⁺CD14⁺ cells, the increased frequency predominantly reflects the accumulation of inflammatory M1-like cells.

To directly test the role of type I interferon, we examined a patient with CLE before and after three monthly infusions of anifrolumab (anti-IFNAR1). IFNAR blockade resolved clinical disease activity (Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) 6→0; Fig. 6b and Extended Data Fig. 6c). Histologically, UVB induced dense myeloid–lymphoid aggregates in interface, perivascular and periadnexal regions, but this response was markedly blunted after anifrolumab (Extended Data Fig. 6d).

Transcriptional profiling confirmed that acute UVB irradiation drove CD14⁺ cells in non-lesional CLE toward an MMP9⁺ inflammatory state, an effect substantially reduced following IFNAR blockade (Fig. 6c,d). Spatially, UVB-induced MMP9⁺CD14⁺ cells localized to the interface and papillary dermis, whereas anifrolumab profoundly diminished their accumulation (Fig. 6e and Extended Data Fig. 6e).

UVB also induced fibroblast changes characteristic of lesional photosensitive skin. In non-lesional CLE, UVB provocation expanded proinflammatory fibroblasts and upregulated *CCL2* and *CCL8*, both attenuated after IFNAR blockade (Fig. 6f and Extended Data Fig. 6f). Consistent with their migratory phenotype, infiltrating MMP9⁺CD14⁺ cells expressed high *CCR1* and modestly increased *CCR5* and *CCR2*, the receptors for *CCL2* and *CCL8* ($P < 0.01$; Fig. 6g).

Together, these findings demonstrate that type I interferon signaling licenses skin for UVB-induced recruitment and polarization of inflammatory MMP9⁺CD14⁺ myeloid cells, a central initiating step in interface dermatitis.

Type I interferon-primed UVB injury in keratinocytes activates monocyte-derived DCs and recapitulates MMP9⁺CD14⁺ myeloid programs in photosensitive skin

Building on our observation that UVB triggers epidermal recruitment of inflammatory CD14⁺ myeloid cells in a type I interferon-dependent manner, we next investigated the role of keratinocytes, epithelial sentinels that absorb the majority of UVB radiation and initiate immune responses in photosensitive skin.

Given that type I interferon signaling was required for UVB-induced recruitment of inflammatory CD14⁺ cells in vivo, we next examined how keratinocytes shape downstream myeloid responses. We used a keratinocyte–DC co-culture system in which N/TERT2G keratinocytes were exposed to UVB (50 or 100 mJ cm⁻²) with or without IFNβ priming, and their conditioned media applied to healthy donor monocyte-derived dendritic cells (moDCs) (Extended Data Fig. 7a). moDCs were selected as surrogates for lesional MMP9⁺CD14⁺ cells

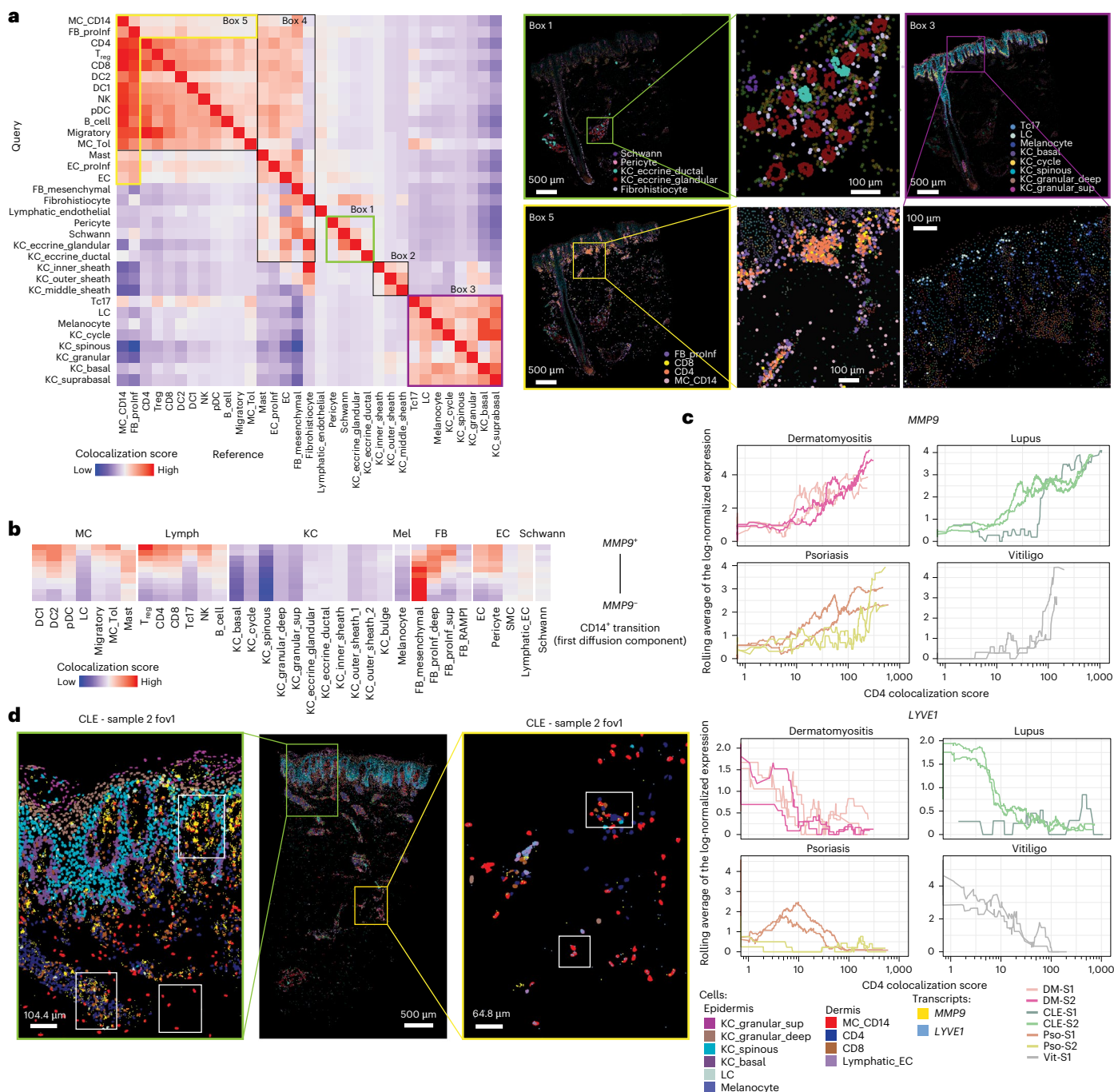


Fig. 4 | Spatial colocalization analysis reveals activation states of CD14⁺ myeloid cells associated with proximity to CD4⁺ T cells in autoimmune lesions. **a, Left, heatmap of cell-type colocalization scores across all spatial transcriptomics slides, calculated using a Gaussian-weighted distance model (Methods). Score shows the degree of query cell type (row) locating within the region of the reference cell type (column). Boxes 1–5 highlight known anatomical relationships: (1) eccrine gland: glandular and ductal keratinocytes with Schwann cells and pericytes (representative of $n = 10$ sections derived from five punch biopsies (five patients) containing eccrine glands); (2) hair follicle: concentric layers of hair follicle sheath keratinocytes; (3) epidermis: epidermal keratinocytes with melanocytes, Langerhans cells and Tc17 cells (representative of $n = 13$ sections derived from seven punch biopsies (seven patients) containing epidermis); (4) dermis: mesenchymal fibroblasts with dermal structures; (5) immune aggregates: proinflammatory fibroblasts with immune cells.**

Right, zoom-ins in representative tissue sections of the highlighted clusters (representative of $n = 13$ sections derived from seven punch biopsies (seven patients) containing immune aggregates). **b**, Colocalization score heatmap for CD14⁺ MCs (query) with other cell subtypes (reference). CD14⁺ MCs were ordered by the first diffusion component (transition) and aggregated into ten bins. **c**, Gene in CD14⁺ myeloid cell expression changes as a function of proximity (colocalization score) to CD4⁺ T cells aggregated for each slide; smoothed with rolling average. **d**, High-resolution image of CLE sample 2 showing zoom-ins of superficial and deep immune aggregates. Key cell types are displayed, with overlaid transcripts for LYVE1 (blue) and MMP9 (yellow). Lymphatic endothelial cells, typically associated with LYVE1 expression, are shown for spatial reference. Data are representative of $n = 13$ tissue sections derived from seven punch biopsies (seven patients) with similar results.

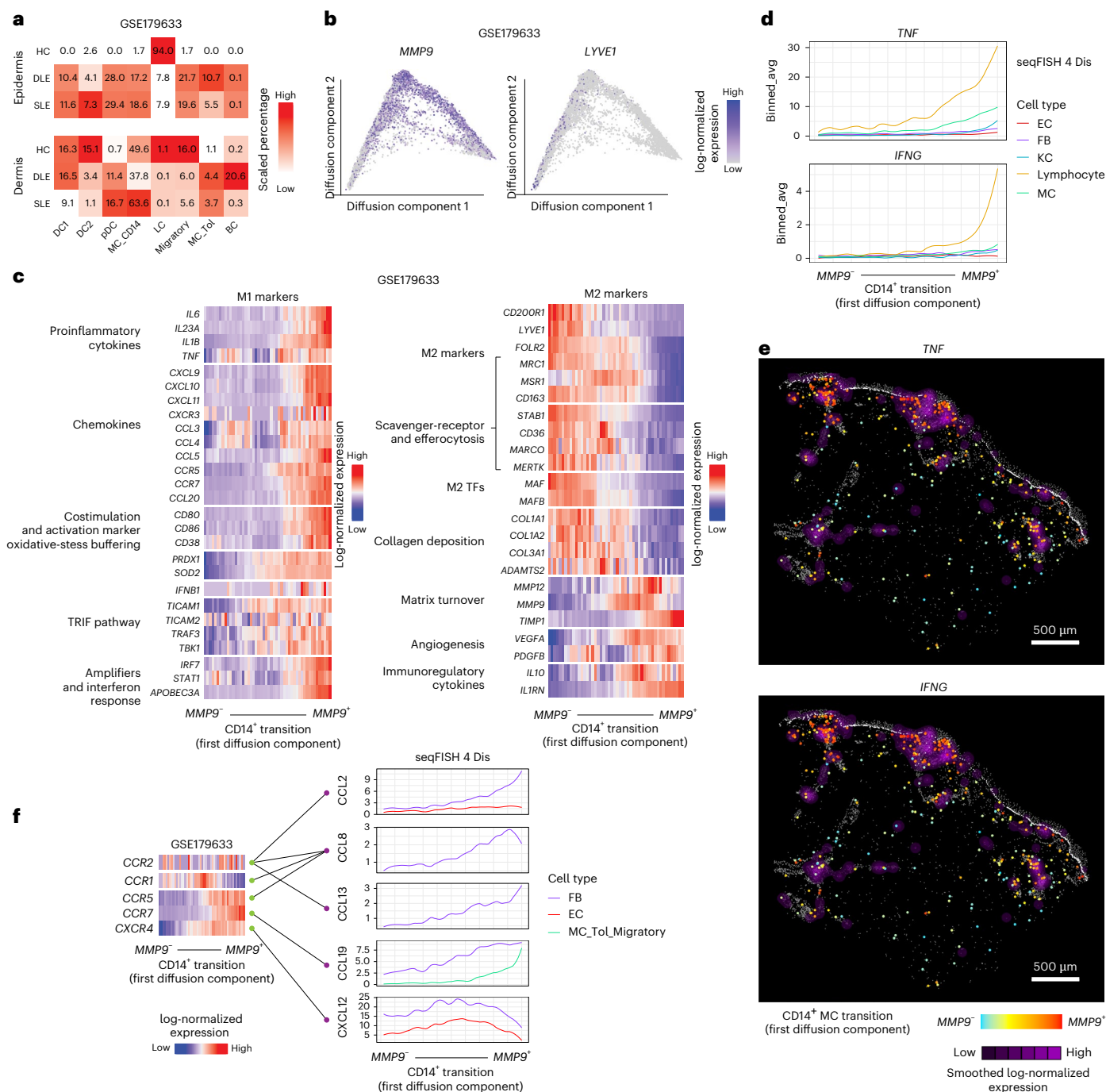


Fig. 5 | CD14⁺ cells transition from an M2-like to an M1-like state, and their skin localization is established by a multi-chemokine fibroblast gradient. **a**, Percentage of myeloid cell subtypes within all myeloid cells per condition in epidermal (top) and dermal (bottom) lupus punch skin biopsy samples from reanalyzed data in GSE179633; colors scaled by cell type (column). **b**, log-normalized expression of *MMP9* (left) and *LYVE1* (right) in CD14⁺ cells embedded in a diffusion map. **c**, Heatmap showing M1 (left) and M2 (right) markers expression in CD14⁺ cells ordered by the first diffusion component (transition) and aggregated into 50 bins (144 cells per bin). **d**, Local expression of *TNF* (top) and *IFNG* (bottom) by cells within 400 pixels (~41 μm) of CD14⁺ cells in seqFISH data. CD14⁺ cells were ordered by their first diffusion component

(transition) and averaged in bins of 193 CD14⁺ cells. **e**, Expression of *TNF* (top) and *IFNG* (bottom) in a DM lesional sample, smoothed with a Gaussian kernel; s.d., 400 pixels (~41 μm). CD14⁺ cells are overlaid on top and colored according to their projection onto the first diffusion component (transition). Data are representative of *n* = 13 tissue sections derived from seven punch biopsies (seven patients) with similar results. **f**, Left, expression of detected chemokine receptors in CD14⁺ cells from the GSE179633 dataset, shown along the first diffusion component. CD14⁺ cells were grouped into 50 bins (144 cells per bin) as in **b**. Right, seqFISH expression of corresponding ligands in different cell types within 400 pixels (~41 μm) of CD14⁺ cells, averaged per bin along the same diffusion component ordering as in **d**.

based on shared markers including *ITGAX*, *CD209* and *MAFB*^{4,6} (Fig. 2b and Extended Data Fig. 2a).

UVB increased keratinocyte membrane permeability and reduced intracellular ATP in a dose-dependent manner, with both

effects enhanced by IFNβ pretreatment (*P* < 0.0001; Fig. 7a), indicating increased epithelial stress. Supernatants from UVB-injured keratinocytes robustly upregulated HLA-DR, CD83, CD80 and CD86 on moDCs; IFNβ priming further potentiated this response, whereas

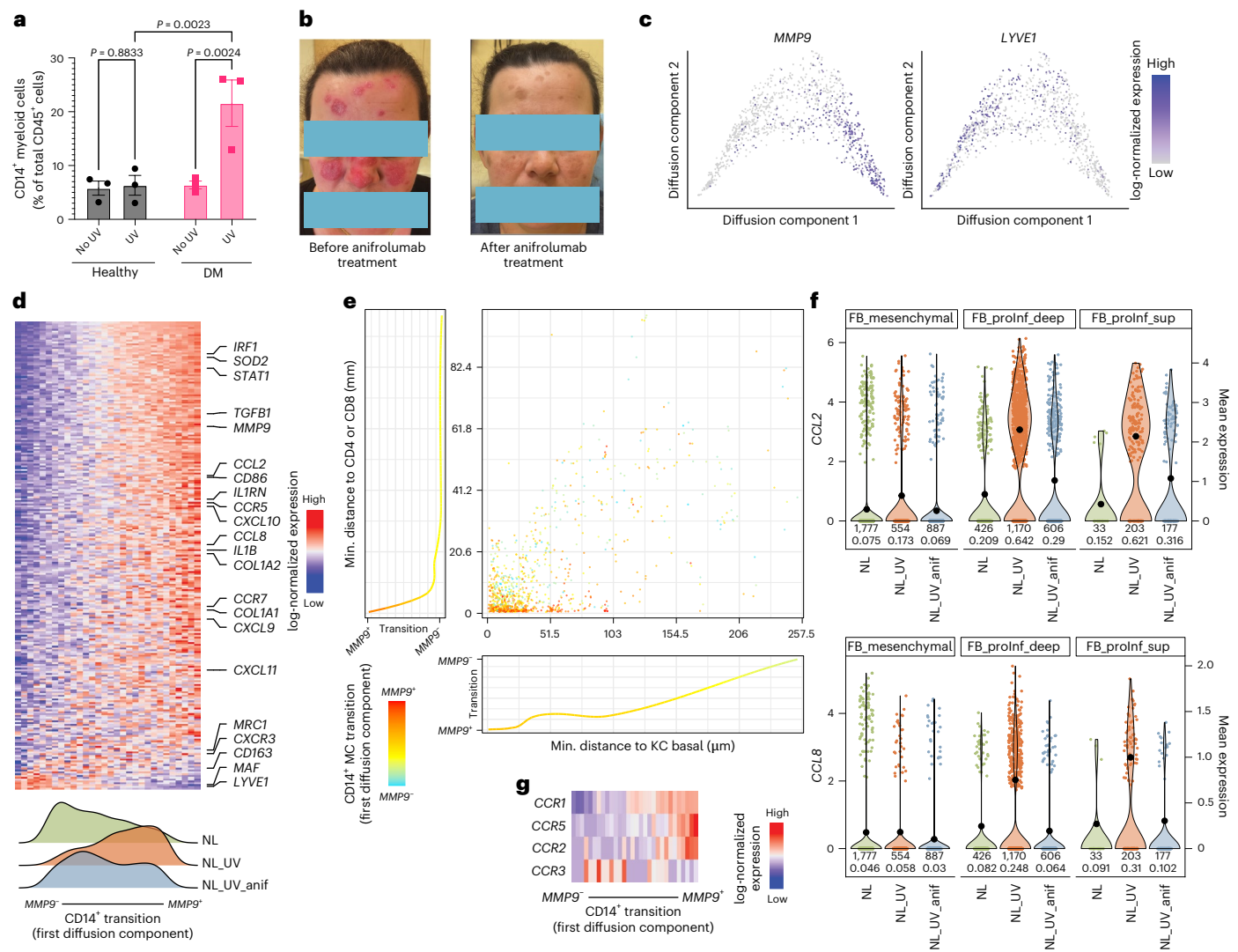


Fig. 6 | Type I interferon signaling drives UVB-induced myeloid inflammation in photosensitive autoimmune skin diseases, which is attenuated by therapeutic IFNAR blockade. **a**, Flow cytometric analysis of the percentage of CD14⁺ myeloid cells within CD45⁺ immune cells in three healthy individuals and three individuals with DM. *P* values were computed using two-way ANOVA followed by Bonferroni-corrected pairwise *t*-tests (data represented as means; error bars, s.e.m.). **b**, Clinical images before and after anifrolumab treatment. CLASI activity score from six (before) to zero (after). **c**, Diffusion map of CD14⁺ cells obtained from seqFISH data of UVB-exposed skin. **d**, Heatmap of differentially expressed genes (*P* < 0.01; tradeSeq) in CD14⁺ myeloid cells ordered by their first diffusion map component

and their expression summed in 50 bin cells isolated from untreated (NL), UVB-treated (NL_{UV}) and UVB-treated post-anifrolumab (NL_{UV}_{anf}) skin. **e**, Scatter plot showing minimum distance (in μm) from a CD14⁺ cell to a basal keratinocyte (x axis) or a CD8 or CD4 T cell (y axis) colored by their projection onto the first diffusion component; marginal plots show mean of the first diffusion component of CD14⁺ cells projected onto the x axis (bottom) or the y axis (left). **f**, Violin plots showing normalized expression of *CCL2* and *CCL8* in fibroblast subtypes. **g**, Heatmap showing expression of chemokine receptors *CCR1*, *CCR2*, *CCR3* and *CCR5* in CD14⁺ myeloid cells ordered by the first diffusion component; each tile represents aggregated expression of 33 cells.

direct IFN β stimulation of moDCs did not have a similar effect (Fig. 7b and Extended Data Fig. 7b), confirming that type I interferon acts through keratinocytes rather than myeloid cells.

Transcriptomic and proteomic profiling revealed that UVB alone induced a dose-dependent inflammatory program in moDCs, including CXCL1, CCL3 and CCL19 (Fig. 7c,d and Supplementary Table 5). Strikingly, UVB50 + IFN β elicited responses comparable to or exceeding UVB100 alone, underscoring the amplifying role of type I interferon in shaping keratinocyte output. A qualitative shift emerged: the classical monocyte chemoattractants CCL2, CCL7 and CCL8 were induced only by supernatants from IFN β -primed, UVB-irradiated keratinocytes, not by UVB alone or IFN β alone (Fig. 7d and Extended Data Fig. 7c). These chemokines are among the most enriched in MMP9⁺CD14⁺ cells in CLE and DM lesions (Extended Data Fig. 7d), underscoring their in vivo relevance.

We next asked whether keratinocyte supernatants could recapitulate the transcriptional program of in vivo MMP9⁺CD14⁺ cells. Although KC-conditioned media induced key pathogenic effectors, including *MMP9*, *PDGFB*, *CD80* and *CD86*, it failed to upregulate canonical interferon-stimulated genes (*STAT1*, *IRF7*, *TBK1*) or *CCR5* (Fig. 7e), indicating that epithelial cues alone do not fully recreate the interferon-driven state present in lesional skin.

Together, these findings show that type I interferon is required to license UVB-damaged keratinocytes to activate a central component of the MMP9⁺CD14⁺ myeloid program. The observed type I interferon signature in non-lesional CLE and DM suggests that clinically normal-appearing skin is transcriptionally poised for exaggerated UVB responses, thereby promoting rapid recruitment and activation of inflammatory myeloid cells. These data support IFNAR blockade as a strategy to interrupt early pathogenic events in photosensitive autoimmunity.

Type I interferon-licensed UVB-injured keratinocytes drive myeloid activation via soluble protein mediators

To define how type I interferon primes keratinocytes to activate moDCs, we first characterized the biochemical nature of the factors released from UVB-injured keratinocytes. Conditioned media from IFN β -primed, UVB-irradiated keratinocytes potently induced CD83 on moDCs (Fig. 7b). This effect was abolished by proteinase K but not DNase I, indicating that the active components are proteins (Fig. 7f). Activity was retained after low-speed centrifugation but lost upon 0.22 μ m filtration, suggesting a vesicle-associated or membrane-bound origin (Fig. 7g).

Proteomics identified 28 differentially expressed proteins (adjusted ANOVA $P < 0.05$) that segregated into UVB-induced and IFN β -licensed groups (Extended Data Fig. 7e). UVB alone induced TNF, IL-1 α , IL-1 β , CXCL1 and CXCL8, which were further amplified by IFN β (Fig. 7h and Extended Data Fig. 7e). A distinct group, including CCL20, CSF1 and IL-6, was induced only with IFN β priming (Fig. 7h and Extended Data Fig. 7e,f), indicating that type I interferon does more than enhancing UVB responses: it reprograms keratinocyte cytokine output.

Given the biochemical profile, we tested whether membrane-bound TNF contributed to moDC activation. TNF was strongly induced by UVB and further augmented by IFN β priming. Neutralization with adalimumab reduced moDC activation by 57% ($P < 0.0001$; Fig. 7i). However, after IFN β priming, adalimumab only partially suppressed activation (CD83⁺ 32% vs 17% without priming), implicating additional IFN β -licensed cytokines. Direct IFNAR blockade on moDCs had no effect, confirming that type I interferon acts primarily on keratinocytes to license their proinflammatory output.

Among selectively induced cytokines, IL-6 was strongly dependent on IFN β priming (Fig. 7h). Although IL-6 can exert context-dependent effects on human myeloid cells, its selective induction in type I interferon-licensed keratinocytes identifies it as a plausible contributor to the augmented moDC activation.

Keratinocyte injury cues activate fibroblasts and myeloid cells to promote T cell recruitment

Superficial fibroblasts emerged as the main producers of monocyte-recruiting chemokines (CCL2, CCL7, CCL8) in photosensitive lesions and localized adjacent to MMP9⁺CD14⁺ cells near the dermal–epidermal junction (Extended Data Figs. 3f, 5b,f and 6e,f). To define how keratinocyte UV injury shapes these fibroblast chemokine gradients,

we stimulated primary human dermal fibroblasts with conditioned media from UVB-irradiated keratinocytes with or without IFN β , or with recombinant TNF and IL-1 β , two cytokines strongly induced by UVB (Fig. 7h), and then performed RNA-seq.

Direct IFN β exposure did not alter *CCL7* or *CCL8* expression. By contrast, supernatants from IFN β -primed keratinocytes, regardless of UVB dose, robustly induced both chemokines, identifying them as key mediators of CD14⁺ recruitment in type I interferon-rich settings. *CCL7* showed modest induction by IL-1, whereas *CCL8* was minimally responsive to IL-1 or TNF, suggesting that additional keratinocyte-derived factors regulate its expression (Fig. 7j, Extended Data Fig. 7g and Supplementary Table 5). *CCL2* was consistently upregulated by all keratinocyte supernatants, with the strongest induction following UVB100, mirroring the potent effect of recombinant TNF or IL-1 on fibroblasts (Fig. 7j and Extended Data Fig. 7g).

CXCL12, a homeostatic stromal chemokine, was suppressed in fibroblasts exposed to keratinocyte-conditioned media, with maximal repression by IFN β -primed, UVB-injured keratinocyte supernatants. TNF alone also downregulated *CXCL12*, consistent with prior work showing IL-1 and TNF-mediated repression in inflamed tissues^{25–27}. *CXCL12* downregulation probably reflects a shift from a homeostatic to a proinflammatory stromal state that favors recruitment of inflammatory myeloid cells over maintenance of steady-state niches.

We next asked whether keratinocyte injury promotes T cell accumulation through moDC activation. moDCs were stimulated with keratinocyte-conditioned media for 12 h, washed and cultured in fresh media. Their secretome, reflecting moDC-intrinsic responses, was used in chemotaxis assays. moDCs activated by IFN β -primed, UVB-injured keratinocyte media induced the strongest recruitment of CD4⁺ and CD8⁺ T cells (Fig. 7k). IFN β priming lowered the UVB threshold, enabling UVB50 to elicit T cell chemotaxis comparable to UVB100 (Fig. 7k), an effect not seen with IFN β treatment alone, confirming that type I interferon amplifies UVB-induced epithelial injury to drive secondary T cell recruitment.

Together, these findings delineate a multicellular inflammatory cascade initiated by type I interferon-primed keratinocyte damage. Cytokines released from UVB-injured keratinocytes activate superficial fibroblasts to produce chemokines (CCL2, CCL7, CCL8) that attract CD14⁺ monocyte-derived cells into the upper dermis. Once recruited, these cells encounter keratinocyte-derived cytokines, including TNF,

Fig. 7 | IFN β -primed, UVB-irradiated keratinocytes activate inflammatory moDCs via proteinaceous, membrane-bound cytokines, leading to T lymphocyte recruitment and fibroblast-derived chemokine production.

a, Keratinocyte viability, measured by propidium iodide (PI) uptake ($n = 6$) and ATP depletion ($n = 8$). Data are presented as means and were analyzed using one-way ANOVA followed by Bonferroni-corrected pairwise comparisons; error bars, s.e.m. A P value < 0.05 was considered statistically significant. **b**, Dot plots of moDC activation marker expression (CD83, CD86) following exposure to keratinocyte-conditioned media or direct IFN β stimulation; lipopolysaccharide (LPS) was used as a positive control. Data are presented as means; error bars, s.e.m. Statistical analysis was performed using a one-way ANOVA followed by Bonferroni-corrected multiple comparisons. Each dot represents one biological replicate. Data were derived from six independent donors. **c**, Heatmap of differentially expressed genes ($P < 0.05$, $|\log_2(\text{fold change})| > 1$) in bulk RNA-seq of moDCs incubated with keratinocyte supernatants treated with UVB and IFN β ; data are counts per million (CPM) scaled by gene (row) ($n = 5$ donors per group). **d**, Heatmap of differentially abundant protein expression (NPQ) ($P < 0.05$) in moDCs after incubation with keratinocyte supernatants from the indicated conditions; colors scaled by protein (row) ($n = 4$ donors per group). **e**, Heatmap of MMP9⁺CD14⁺ signature genes found induced in moDCs after exposure to keratinocyte-conditioned media or direct IFN β . Signature genes are M1 markers and M2 markers in Fig. 5c that are high on the MMP9 end; data are counts per million scaled by gene (row). **f,g**, Percentage of moDCs expressing CD83 following stimulation with keratinocyte supernatants treated with proteinase K (PK), DNase or passed through a 0.22 μ m filter. Data are presented as means;

error bars, s.e.m. Statistical analysis was performed using a one-way ANOVA followed by Bonferroni-corrected multiple comparisons. Each dot represents one biological replicate. Data in **f** were derived from eight independent donors, and data in **g** were derived from six independent donors. **h**, Cytokine concentration in keratinocyte supernatants, measured using OLINK proteomics. Data are presented as means and were analyzed using one-way ANOVA followed by Tukey's multiple comparison test; error bars, s.e.m. Each dot represents one independent donor. Data were derived from four independent donors. **i**, Percentage of moDCs expressing CD83 following incubation with keratinocyte supernatants pre-treated with TNF neutralizing antibody (adalimumab) or IFNAR blocker (anifrolumab) under UVB and IFN β conditions. Data are presented as means; error bars, s.e.m. Statistical analysis was performed using a one-way ANOVA followed by Bonferroni-corrected multiple comparisons. Each dot represents one biological replicate. Data were derived from six independent donors. **j**, Heatmap of differentially expressed genes ($P < 0.05$, $|\log_2(\text{fold change})| > 1$) in fibroblasts treated with conditioned keratinocyte supernatants, and direct IFN β , IFN γ , IL1 α or TNF; data are counts per million scaled by gene (row). Data were derived from four independent donors. **k**, Number of CD4⁺ (left) and CD8⁺ (right) T cells by flow cytometry that migrated in Boyden chambers, recruited by supernatants of moDCs stimulated with keratinocyte supernatants (in color) or supernatants of moDCs stimulated with PBS and LPS as controls. Data are presented as means and were analyzed using one-way ANOVA followed by Tukey's multiple comparison test; error bars, s.e.m. Each symbol represents one biological replicate. Experiments were performed using cells derived from four independent donors.

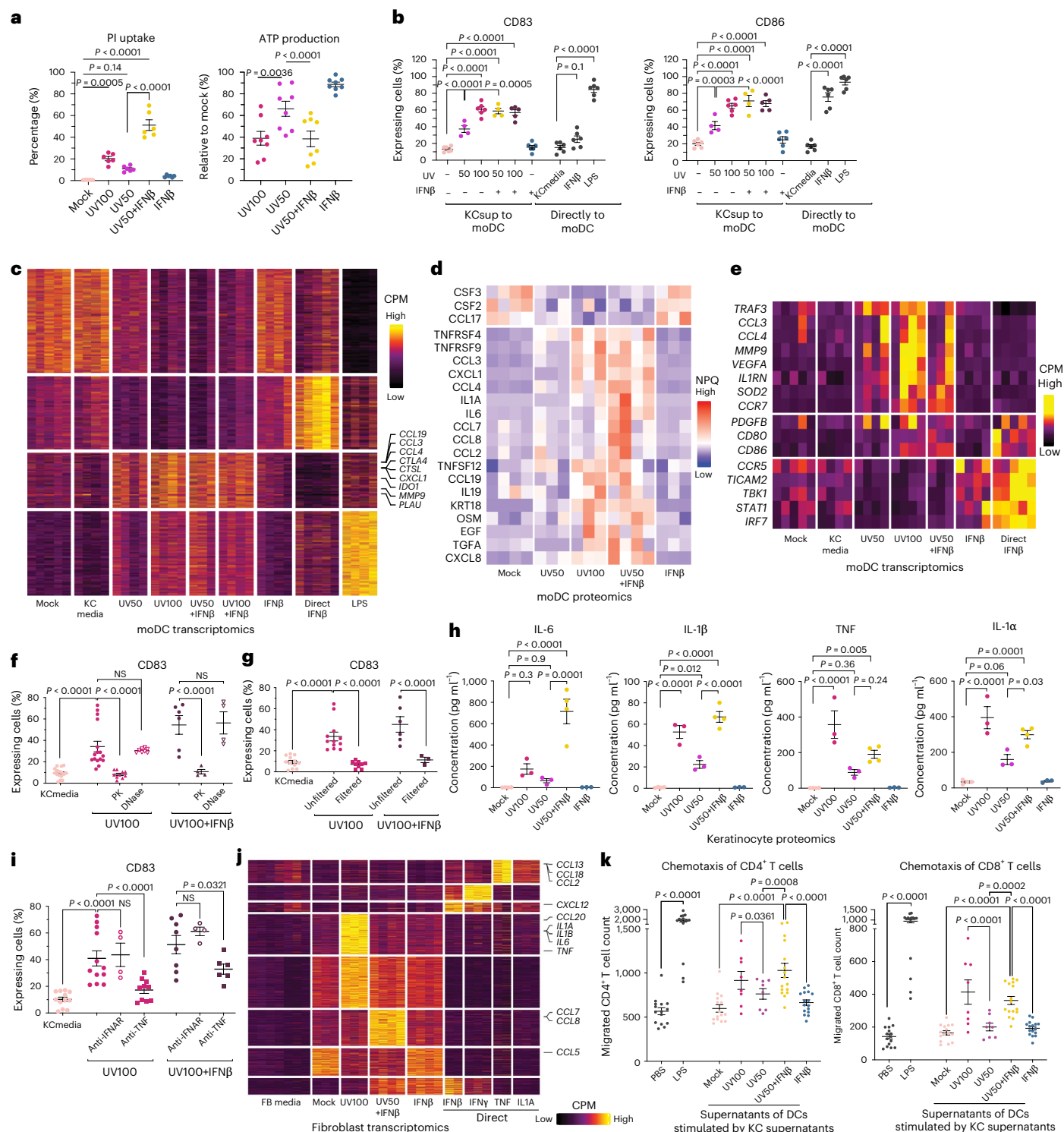
IL-1 α / β and IL-6, driving their differentiation into MMP9⁺ inflammatory effectors. Activated CD14⁺ cells, in turn, secrete chemokines that recruit cytotoxic T cells to the dermal–epidermal junction, culminating in interface dermatitis. This type I interferon-amplified feed-forward loop is effectively disrupted by IFNAR blockade, providing a mechanistic rationale for targeting type I interferon signaling to prevent UV-triggered flares in photosensitive dermatoses (Extended Data Fig. 7h).

Discussion

Photosensitivity is a defining feature of CLE and DM, yet its mechanistic basis remains unclear. Rodent models are limited by fundamental

interspecies differences in UV sensing, epidermal structure and diurnal behavior, underscoring the need for human-focused approaches^{28–32}. Comparative multiomics analysis across photosensitive (CLE, DM) and photoresponsive (psoriasis, vitiligo) diseases highlighted type I interferon activity as a unifying axis of photosensitivity and delineated a multicellular circuit linking UV injury to inflammatory recruitment.

MMP9⁺CD14⁺ myeloid cells were found as key effectors of UV-triggered inflammation in both CLE and DM. These cells accumulate at the dermal–epidermal junction, colocalize with cytotoxic CD4⁺ T cells and express *IL1B*, *IL6*, *TNF* and chemokines characteristic of an M1-like program.



Functionally, MMP9 facilitates tissue migration by degrading extracellular matrix components, including collagen IV³³, thereby contributing to basement membrane disruption, characteristic of photosensitive lesions³⁴. Although elevated MMP9 levels have been correlated with CLE disease activity³⁵, our data pinpoint MMP9⁺CD14⁺ cells as the dominant source of IFN β and establish their role as mediators of UV-induced skin inflammation. These findings provide a mechanistic explanation of prior associations of cutaneous DM severity with enrichment of monocytes and macrophages in lesional skin³⁶.

CLE and DM lesions also contained an expanded population of cytotoxic CD4⁺ T cells, co-expressing T_H1 markers (*TBX21*, *CXCR3*) and cytolytic molecules (*GZMB*, *GZMK*, *PRFI*)¹⁶. IFN β enhanced granzyme B expression in activated CD4⁺ T cells in vitro, suggesting that type I interferon not only shapes T_H1 polarization but also directly licenses cytotoxicity. This provides a mechanistic basis for T cell-mediated epithelial injury characteristic of interface dermatitis.

Consistent with reports of baseline type I interferon activation in non-lesional CLE^{37,38}, we found that keratinocytes in non-lesional DM skin similarly exhibit a tonic type I interferon signature. Yet MMP9⁺CD14⁺ cells were largely absent outside lesional skin, indicating that keratinocytes, and not myeloid cells, are the source of basal type I interferon tone. In lupus, keratinocyte-derived IFN κ has been shown to prime photosensitivity^{39,40}; our findings extend this paradigm to DM.

Spatial transcriptomics identified superficial fibroblasts as critical stromal amplifiers that guide monocyte recruitment. Keratinocytes did not express monocyte-recruiting chemokines; instead, fibroblasts beneath the dermal–epidermal junction produced high levels of CCL2 and CCL8 and colocalized with CCR2⁺, MMP9⁺CD14⁺ cells. Functional experiments demonstrated that cytokines released from IFN-licensed, UVB-injured keratinocytes—particularly TNF and IL-1 α —drive fibroblast induction of CCL2, CCL7 and CCL8. These fibroblasts thus convert epithelial injury into chemokine cues that recruit CD14⁺ myeloid cells. The recruited cells, in turn, secrete chemokines that attract T lymphocytes, which can acquire cytotoxic function in the presence of type I interferon. These findings reinforce emerging concepts that fibroblasts are not passive structural cells but context-dependent inflammatory amplifiers in CLE and DM skin^{41,42}.

Together, our data support a feed-forward inflammatory circuit in photosensitive skin. Chronic type I interferon exposure sensitizes keratinocytes; subsequent UVB damage triggers release of IL-1 α / β , IL-6 and TNF, activating fibroblasts to produce monocyte-recruiting chemokines. Incoming CD14⁺ cells acquire an MMP9⁺ inflammatory phenotype, breach the basement membrane and secrete IFN β and additional cytokines. Keratinocyte-conditioned media also induce these cells to chemoattract T lymphocytes, completing a pathogenic amplification loop. IFN-primed UVB-damaged keratinocytes also induce myeloid cells to chemoattract T cells, which can increase tissue damage and inflammation by cytotoxicity.

Our photoprovocation trial in healthy individuals and patients with DM supports our causal links, and the use of anifrolumab (anti-IFNAR) offers functional evidence that type I interferons serve as the upstream licensing signal for UV-induced exacerbation. Type I interferon blockade nearly abolished the UVB-triggered recruitment of MMP9⁺CD14⁺ myeloid cells, directly linking clinical response to interruption of this inflammatory axis. Together, these observations provide a mechanistic rationale for type I interferon-targeted therapies in photosensitive autoimmunity and identify MMP9⁺ monocyte-derived dendritic cells as a tractable, disease-relevant cellular target.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41590-026-02502-w>.

References

- Rathod, D. G., Muneer, H. & Masood, S. Phototherapy. *StatPearls* (StatPearls Publishing, 2024).
- Feng, W. et al. NULISA: a proteomic liquid biopsy platform with attomolar sensitivity and high multiplexing. *Nat. Commun.* **14**, 7238 (2023).
- Gellatly, K. J. et al. scRNA-seq of human vitiligo reveals complex networks of subclinical immune activation and a role for CCR5 in T_{reg} function. *Sci. Transl. Med.* **13**, eabd8995 (2021).
- Collin, M. & Bigley, V. Human dendritic cell subsets: an update. *Immunology* **154**, 3–20 (2018).
- Villani, A.-C. et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science* **356**, eaah4573 (2017).
- Collin, M., McGovern, N. & Haniffa, M. Human dendritic cell subsets. *Immunology* **140**, 22–30 (2013).
- Brown, C. C. et al. Transcriptional basis of mouse and human dendritic cell heterogeneity. *Cell* **179**, 846–863.e24 (2019).
- Maynard, A. et al. Therapy-induced evolution of human lung cancer revealed by single-cell RNA sequencing. *Cell* **182**, 1232–1251.e22 (2020).
- Greter, M. et al. Stroma-derived interleukin-34 controls the development and maintenance of Langerhans cells and the maintenance of microglia. *Immunity* **37**, 1050–1060 (2012).
- Wang, Y. et al. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. *Nat. Immunol.* **13**, 753–760 (2012).
- Frisoli, M. L., Richmond, J. M. & Harris, J. E. IL-12/IL-23-Independent function of BATF3-dependent dendritic cells is required for initiation of disease in a mouse model of vitiligo. *J. Invest. Dermatol.* **144**, 2574–2577.e2 (2024).
- Kim, T.-G. et al. Skin-specific CD301b⁺ dermal dendritic cells drive IL-17-mediated psoriasis-like immune response in mice. *J. Invest. Dermatol.* **138**, 844–853 (2018).
- Werth, V. P. et al. Trial of anti-BDCA2 antibody lifilimab for cutaneous lupus erythematosus. *N. Engl. J. Med.* **387**, 321–331 (2022).
- Karnell, J. L. et al. Depleting plasmacytoid dendritic cells reduces local type I interferon responses and disease activity in patients with cutaneous lupus. *Sci. Transl. Med.* **13**, eabf8442 (2021).
- Anufrieva, K. S. et al. Spatial transcriptomics identifies immune-stromal niches associated with cancer in adult dermatomyositis. Preprint at <https://doi.org/10.1101/2025.03.19.644147> (2025).
- Oh, D. Y. & Fong, L. Cytotoxic CD4⁺ T cells in cancer: expanding the immune effector toolbox. *Immunity* **54**, 2701–2711 (2021).
- Eng, C. L. et al. Transcriptome-scale super-resolved imaging in tissues by RNA seqFISH. *Nature* **568**, 235–239 (2019).
- Steele, L. et al. A single-cell and spatial genomics atlas of human skin fibroblasts reveals shared disease-related fibroblast subtypes across tissues. *Nat. Immunol.* **26**, 1807–1820 (2025).
- Wang, X.-N. et al. A three-dimensional atlas of human dermal leukocytes, lymphatics, and blood vessels. *J. Invest. Dermatol.* **134**, 965–974 (2014).
- Banerji, S. et al. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. *J. Cell Biol.* **144**, 789–801 (1999).
- Corry, D. B. et al. Overlapping and independent contributions of MMP2 and MMP9 to lung allergic inflammatory cell egression through decreased CC chemokines. *FASEB J.* **18**, 995–997 (2004).
- Dufour, A. & Overall, C. M. Missing the target: matrix metalloproteinase antitargets in inflammation and cancer. *Trends Pharmacol. Sci.* **34**, 233–242 (2013).
- Elfstrum, A. K. et al. LYVE-1-expressing macrophages modulate the hyaluronan-containing extracellular matrix in the mammary stroma and contribute to mammary tumor growth. *Cancer Res. Commun.* **4**, 1380–1397 (2024).

24. Zheng, M. et al. Single-cell sequencing shows cellular heterogeneity of cutaneous lesions in lupus erythematosus. *Nat. Commun.* **13**, 7489 (2022).
25. Fedyk, E. R. et al. Expression of stromal-derived factor-1 is decreased by IL-1 and TNF and in dermal wound healing. *J. Immunol.* **166**, 5749–5754 (2001).
26. Garcia-Cuesta, E. M. et al. The role of the CXCL12/CXCR4/ACKR3 axis in autoimmune diseases. *Front. Endocrinol.* **10**, 585 (2019).
27. Madge, L. A. & May, M. J. Classical NF- κ B activation negatively regulates noncanonical NF- κ B-dependent CXCL12 expression. *J. Biol. Chem.* **285**, 38069–38077 (2010).
28. D'Orazio, J., Jarrett, S., Amaro-Ortiz, A. & Scott, T. UV radiation and the skin. *Int. J. Mol. Sci.* **14**, 12222–12248 (2013).
29. Medetgul-Ernar, K. & Davis, M. M. Standing on the shoulders of mice. *Immunity* **55**, 1343–1353 (2022).
30. Burian, M. & Yazdi, A. S. NLRP1 is the key inflammasome in primary human keratinocytes. *J. Invest. Dermatol.* **138**, 2507–2510 (2018).
31. Fenini, G. et al. NLRP1 inflammasome activation in keratinocytes: increasing evidence of important roles in inflammatory skin diseases and immunity. *J. Invest. Dermatol.* **142**, 2313–2322 (2022).
32. Robinson, K. S. et al. ZAK α -driven ribotoxic stress response activates the human NLRP1 inflammasome. *Science* **377**, 328–335 (2022).
33. Vandooren, J., Van den Steen, P. E. & Opdenakker, G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. *Crit. Rev. Biochem. Mol. Biol.* **48**, 222–272 (2013).
34. Crowson, A. N., Magro, C. M. & Mihm, M. C. Jr Interface dermatitis. *Arch. Pathol. Lab. Med.* **132**, 652–666 (2008).
35. Ertugrul, G., Keles, D., Oktay, G. & Aktan, S. Matrix metalloproteinase-2 and -9 activity levels increase in cutaneous lupus erythematosus lesions and correlate with disease severity. *Arch. Dermatol. Res.* **310**, 173–179 (2018).
36. Patel, J., Maddukuri, S., Li, Y., Bax, C. & Werth, V. P. Highly multiplexed mass cytometry identifies the immunophenotype in the skin of dermatomyositis. *J. Invest. Dermatol.* **141**, 2151–2160 (2021).
37. Billi, A. C. et al. Nonlesional lupus skin contributes to inflammatory education of myeloid cells and primes for cutaneous inflammation. *Sci. Transl. Med.* **14**, eabn2263 (2022).
38. Der, E. et al. Tubular cell and keratinocyte single-cell transcriptomics applied to lupus nephritis reveal type I IFN and fibrosis relevant pathways. *Nat. Immunol.* **20**, 915–927 (2019).
39. Sarkar, M. K. et al. Photosensitivity and type I IFN responses in cutaneous lupus are driven by epidermal-derived interferon kappa. *Ann. Rheum. Dis.* **77**, 1653–1664 (2018).
40. Tsoi, L. C. et al. Hypersensitive IFN responses in lupus keratinocytes reveal key mechanistic determinants in cutaneous lupus. *J. Immunol.* **202**, 2121–2130 (2019).
41. Shoffner-Beck, S. K. et al. Lupus dermal fibroblasts are proinflammatory and exhibit a profibrotic phenotype in scarring skin disease. *JCI Insight* **9**, e173437 (2024).
42. Gehlhausen, J. R. et al. Cutaneous lupus features specialized stromal niches and altered retroelement expression. *J. Invest. Dermatol.* **145**, 3011–3024.e11 (2025).

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2026, modified publication 2026

¹Department of Genomics and Computational Biology, University of Massachusetts Chan Medical School, Worcester, MA, USA. ²Department of Dermatology, University of Massachusetts Chan Medical School, Worcester, MA, USA. ³Spatial Genomics, Pasadena, CA, USA. ⁴Division of Innate Immunity, Department of Medicine, University of Massachusetts Chan Medical School, Worcester, MA, USA. ⁵Division of Rheumatology, Inflammation, and Immunity, Brigham and Women's Hospital at Harvard Medical School, Boston, MA, USA. ⁶Department of Dermatology, University of Pennsylvania, Philadelphia, PA, USA. ⁷Department of Dermatology, Brigham and Women's Hospital at Harvard Medical School, Boston, MA, USA. ⁸These authors contributed equally: Yuqing Wang, Khashayar Afshari, Nazgol-Sadat Haddadi. ⁹These authors jointly supervised this work: Mehdi Rashighi, Manuel Garber. ✉e-mail: mehdi.rashighi@umassmed.edu; manuel.garber@umassmed.edu

Methods

Study design

Study participants were recruited under IRB-approved protocols (H00021295, H-14848) at the University of Massachusetts Medical School. Written informed consent was obtained from all participants (or their legally authorized representatives, when applicable) before enrollment. Individuals with vitiligo, psoriasis, CLE or DM were diagnosed by board-certified dermatologists, and age-matched and sex-matched healthy volunteers without autoimmune or inflammatory skin disease served as controls. Skin samples included healthy skin, lesional skin and non-lesional skin (clinically normal tissue ≥ 10 cm from lesions). Samples were used for scRNA-seq, flow cytometry, proteomics and spatial transcriptomics as appropriate (Supplementary Table 1).

Suction blister biopsies and processing

Suction blisters were generated as previously described⁴³ using 10–15 mmHg negative pressure at 40 °C for 30–60 min. Blister fluid was aspirated and centrifuged at 350g for 10 min at 4 °C; the supernatant was then flash-frozen for protein analysis. The cell pellet was processed for inDrop scRNA-seq and flow cytometry.

InDrop scRNA-seq

Cells from blister fluid were processed using the inDrop protocol⁴⁴, with an extended 8 bp unique molecular identifier (UMI). Libraries were sequenced on Illumina NovaSeq or NextSeq, and raw FASTQ files were processed using the inDrop pipeline (https://github.com/garber-lab/indrop_processing). Sequences were aligned to GRCh38 with STAR⁴⁵, and digital gene expression matrices were generated using the End Sequencing Analysis Toolkit (ESAT)^{45,46}. A custom GTF file was applied to merge genes with overlapping 3' ends. Additional details are provided in Supplementary Methods.

scRNA-seq analysis

The UMI count tables were processed using Seurat (v.5)⁴⁷. Cells with 200–4,000 UMIs and < 20 hemoglobin UMIs (HBA1, HBA2, HBB, HBD) were retained. Mitochondrial and ribosomal genes (RPL*, RPS*) were excluded from the 2,000 variable genes before performing principal component analysis (PCA). The top 20–30 PCs were used for uniform manifold approximation and projection (UMAP) and clustering (FindNeighbors/FindClusters). Cells were first grouped into major lineages, followed by subtype reclustering. Doublet clusters, identified by inappropriate marker co-expression, were removed before final clustering of lymphoid and myeloid compartments.

For lymphocytes, 130 cell cycle genes were excluded from PCA. Interferon-response scores were derived from 50 high-confidence interferon-induced genes ($\log_2$ (fold change)-ranked, FDR < 0.01) and regressed out during scaling. Visualizations were constructed using ggplot2 (ref. 48).

Differential expression was performed on pseudo-bulk profiles normalized to 1 M UMIs per sample and analyzed with DESeq2 (ref. 49) (design: -skin; comparisons: healthy control versus lesional and healthy control versus non-lesional within disease and lineage). Differentially expressed genes required $P < 0.01$ and $|\log_2$ (fold change)| > 1 . Subtype-level differential expression used edgeR⁵⁰ adapted for Seurat^{3,50,51}, with significance assessed at FDR < 0.05 . Heatmaps were generated with ComplexHeatmap⁵².

Compositional differences were assessed with scCODA⁵³, which models dependencies among cell type proportions. KC_suprabasal was used as the reference because of its relative abundance stability. Proportion heatmaps show disease-level percentages scaled by cell type.

Visualization functions are packed in the R package AddOns, available on GitHub (<https://github.com/garber-lab/AddOns>).

10× scRNA-seq of three DM samples analysis

Raw count matrices were normalized using the NormalizeData function and the LogNormalize method in Seurat (v.5). Data were integrated with the CCAIntegration method on the top 30 PCs, using the top 2,000 variable genes selected with the FindVariableFeatures function, with default parameters. UMAP and clustering were performed on the integrated top 30 PCs.

GSE179633 scRNA-seq analysis

Data from a previous publication²⁴ (GSE179633) were obtained as raw UMI count matrices and analyzed using Seurat (v.5)⁴⁷ and AddOns. In total, we retained 13 epidermal samples (three healthy controls, five DLE, five SLE) and 16 dermal samples (four healthy controls, five DLE, seven SLE).

Each sample underwent standard Seurat quality control. Doublets were identified with both scDBIFinder⁵⁴ and DoubletFinder⁵⁵, and any cell flagged by either tool was removed. Cell cycle effects were regressed out, and Harmony was applied to integrate dermal and epidermal datasets. Cells were first grouped into major lineages, followed by subtype reclustering.

MC_CD14 cells were extracted and embedded separately. All dermal and epidermal samples were combined, and the top 500 variable genes (vst method) were used for PCA. The first five PCs were integrated with Harmony (k.weight = 30) and used to compute a diffusion map (destiny⁵⁶). The first diffusion component defined the LYVE1-to-MMP9 continuum; cells were ranked along this axis. Heatmaps were generated using ComplexHeatmap⁵². For trajectory heatmaps, MC_CD14 cells were divided into 50 equal bins, and mean expression per bin was calculated. Expression values were scaled per gene across all bins and reference cell types.

Proteomic analysis

The NULISA (Nucleic Acid Linked Immuno-Sandwich Assay) Inflammatory Panel (Supplementary Table 2) quantified 250 proteins in blister fluid samples. Only samples with NULISA protein quantification values above the level of detection were included in visualization and differential expression analysis.

Cytokine concentrations in cell culture media were measured using Olink's Proximity Extension Assay (Target 48 Cytokine/Immune Surveillance Bundle; Supplementary Table 6). For moDCs, secretion levels were calculated by subtracting baseline protein levels in the input media from post-stimulation concentrations.

Statistical analyses were performed using two-sided *t*-tests. Differential protein abundance was assessed using the olink_ttest function from the OlinkAnalyze package (<https://doi.org/10.32614/CRAN.package.OlinkAnalyze>) with unpaired comparisons. The BoxPlot.nulisa function in the AddOns package was used for visualization (<https://github.com/garber-lab/AddOns>).

In vivo UVB photoprovocation

UVB photoprovocation was performed under IRB approval (H00021295), using a standardized protocol⁵⁷. On day -1, six sites on the lower back were irradiated with UVB (25–150 mJ cm⁻²) using a SolRx 100-Series handheld device (285–350 nm, Philips PL-S 9W/12/2P). Then, 24 h later (day 0), the minimal erythema dose was determined for each participant.

A dose of 1.5× the minimal erythema dose was used for photoprovocation. If this dose fell within the initial testing range (≤ 150 mJ cm⁻²), samples were obtained 24 h (suction blister) or 72 h (punch biopsy) after threshold testing without additional irradiation. For participants whose 1.5× minimal erythema dose exceeded 150 mJ cm⁻², a second visit was scheduled to administer the full dose, followed by sampling at the same time points.

UVB-treated and matched untreated sites were sampled by suction blistering or punch biopsy. In a subset of

participants, photoprovocation was performed before and after three monthly 300 mg intravenous doses of anifrolumab to assess treatment-associated changes in UVB responsiveness. Cutaneous disease activity was scored using CLASI⁵⁸.

T cell isolation, activation and cytokine treatment

CD4⁺ T cells were isolated from healthy donor peripheral blood mononuclear cells (PBMCs) using the CD4⁺ T Cell Isolation Kit (Miltenyi Biotec). The cells were resuspended in T cell culture media (RPMI supplemented with HEPES, penicillin–streptomycin and FBS) and plated in 24-well Nunc cell culture plates.

For T cell activation, wells were pre-coated overnight at 4 °C with anti-CD3 (10 ng ml⁻¹; BioLegend, Ultra-LEAF Purified anti-human, Clone OKT3) and anti-CD28 (2 ng ml⁻¹; BioLegend, Ultra-LEAF Purified anti-human, Clone CD28.2) antibodies dissolved in PBS.

After plating, the cells were treated with cytokines—either IFN β (50 ng ml⁻¹; R&D Systems) or IFN γ (100 ng ml⁻¹; R&D Systems)—and cultured for 3 days. Following the incubation, cells were stained for viability and surface markers CD2 and CD4. Subsequently, the cells were fixed, permeabilized and stained intracellularly for granzyme B (GZMB, BioLegend, Clone QA16A02) before being analyzed by flow cytometry.

Cytokilling assay

The cytokilling assay was performed by measuring impedance of the cells using the xCELLigence Real Time Cell Analysis technology (RTCA, Agilent). A total of 20,000 BT-20 cells (ATCC, HTB-19) were seeded in an RTCA E-96 well plate and left to adhere for 6 h. Then, 2×10^5 freshly isolated CD4 T cells from PBMCs were added. Solitomab (Sigma-Aldrich), a bispecific antibody anti-CD3 and anti-epithelial cell adhesion molecule, was used to stimulate the cells at a concentration of 100 ng ml⁻¹. IFN β (R&D) at 10, 100 or 1,000 ng ml⁻¹ or IFN γ (R&D) at 100 ng ml⁻¹ were added to the culture. Cells were left in the RTCA analyzer until maximum cell death was reached (~40 h). The percentage of cytolysis was calculated with the measurement of the impedance, using the software provided with the RTCA technology.

Formalin-fixed, paraffin-embedded scRNA-seq and data analysis

Three lesional biopsies from three patients with DM were processed for scRNA-seq. For each sample, two formalin-fixed, paraffin-embedded curls (25 μ m each) were dissociated using the Miltenyi Biotec FFPE Tissue Dissociation Kit (CG000632 RevA, 10 $\times$ Genomics). The resulting cell suspension was divided equally into four centrifuge tubes, each containing approximately 300,000 cells. Cells in each tube were hybridized with a unique Probe Barcode, as per the instructions in the ‘Chromium Fixed RNA Profiling Reagent Kits for Multiplexed Samples’ user guide (CG000527, 10 $\times$ Genomics). Post hybridization, cells from the four tubes were washed, counted and pooled in equal proportions. Approximately 40,000 cells from the pooled suspension were loaded onto a Chromium Q chip (PN-1000422, 10 $\times$ Genomics). Sequencing libraries were prepared and sequenced on an Illumina NovaSeq platform using paired-end dual-indexing (28 cycles for read 1, ten cycles for i7, ten cycles for i5 and 90 cycles for read 2). The sequencing data were demultiplexed using bcl2fastq (Illumina). The resulting FASTQ files were processed with Cell Ranger using the multi-pipeline and the GRCh38-2020-A reference genome. Data analysis was performed using the Seurat package. Quality control and preprocessing steps included doublet removal using scDblFinder⁵⁴ and ambient RNA correction using SoupX⁵⁹. The data were then normalized, and variable features were identified. To correct for technical variation, we applied Harmony⁶⁰ integration using the first 20 principal components, followed by UMAP. Clustering was conducted using the Leiden algorithm with a resolution parameter of 1.2. Cell type annotation was performed using established marker genes. For subsequent analyses, we focused specifically on the myeloid cell population.

Keratinocyte and fibroblast isolation and culture

Pannus samples were obtained under protocol H11731 (UMass Memorial Hospital) and stored in 70% ethanol, followed by sterile saline before processing. Four to five 4 mm punch biopsies were incubated in dispase I (25 mg ml⁻¹; 04942086001, Roche) for 30 min and held overnight at 4 °C.

The following day, epidermis was separated from dermis and incubated in 0.25% trypsin-EDTA (25200056, Gibco) at 37 °C for 15 min with gentle agitation. Trypsin was neutralized with DMEM + 10% FBS to generate keratinocyte suspensions.

For fibroblast isolation, dermis was digested in collagenase (1 mg ml⁻¹; 9001-12-1, Sigma-Aldrich) for 1 h at 37 °C, neutralized with DMEM + 10% FBS and dissociated by gentle pipetting. Cells were pelleted and cultured in DMEM with 10% FBS and 1% penicillin–streptomycin, with media changes every other day and passaging at sub-confluence (~5–7 days).

Immortalized N/TERT2G keratinocytes⁶¹ were generously provided by H. Rheinwald under a Material Transfer Agreement. Unless otherwise specified, N/TERT2G cells were used in an unprimed and undifferentiated state. N/TERT2G cells were cultured in Keratinocyte Serum-Free Media supplemented with epidermal growth factor and bovine pituitary extract (17005042, Gibco) and 1% penicillin–streptomycin (15140122, Gibco). Cultures were fed every other day and passaged upon reaching sub-confluence, approximately every 5–7 days.

Fibroblast stimulation

Fibroblasts were plated at 10^5 cells per well in a 24-well plate and incubated for 2–3 days at 37 °C. Cells were then stimulated for 24 h with either keratinocyte-conditioned medium (generated from NTERT keratinocytes treated and subsequently exposed to UVB), recombinant human IFN β (50 ng ml⁻¹; R&D Systems, 8499-IF-010/CF), recombinant human TNF (50 ng ml⁻¹; BioLegend, 570102) or recombinant human IL-1 α (100 ng ml⁻¹; PeproTech, 200-01A-10UG). After 24 h, cells were lysed in RLT buffer for RNA library preparation.

Dendritic cell differentiation and stimulation

PBMCs from de-identified healthy donor leukopaks (UMass Leukocyte Core) were isolated by Ficoll-Paque PLUS centrifugation (17-1440-02, GE Healthcare), washed in PBS–EDTA and resuspended in RPMI 1640. Monocytes were enriched by CD14⁺ magnetic selection (EasySep Human CD14 Positive Selection Kit II; 17858, StemCell Technologies).

Monocytes were plated at 1×10^6 cells per ml in RPMI + 10% FBS, 1% penicillin–streptomycin, 2 mM L-glutamine, 50 ng ml⁻¹ GM-CSF (572904, BioLegend) and 50 ng ml⁻¹ IL-4 (574006, BioLegend). Medium was half-replenished with cytokine-supplemented RPMI on day 3. On day 7, moDCs were stimulated for 24 h with keratinocyte-conditioned media (from UVB-treated N/TERT2G keratinocytes), ultrapure LPS (100 ng ml⁻¹; *E. coli* O111:B4, Invitrogen), recombinant IFN β (50 ng ml⁻¹; 8499-IF-010/CF, R&D Systems) or TNF (50 ng ml⁻¹; 570102, BioLegend).

For selected studies, conditioned media were pre-treated with proteinase K (200 μ g ml⁻¹; RP107B-1, QIAGEN) at 55 °C for 1 h, then heat-inactivated DNase I (100 U ml⁻¹, 37 °C, 30 min; 79254, QIAGEN) or passed through 0.22 μ m filters (SLGVR33RS, MilliporeSigma) to assess the biochemical nature and size of active mediators. For blocking assays, moDCs were pre-incubated with adalimumab (20 μ g ml⁻¹, 1 h; HY-P9908, MedChemExpress) or anifrolumab (50 μ g ml⁻¹, 24 h; HY-P99168, MedChemExpress) before stimulation.

After incubation, cells were collected for RNA extraction or flow cytometry, and supernatants were collected for proteomic analysis. For T cell chemotaxis assays, moDCs were washed 12 h after exposure to keratinocyte supernatants and placed in fresh media; supernatants were collected 6 h later.

T cell chemotaxis with keratinocyte-conditioned DC supernatants

Fresh PBMCs from healthy donors were incubated overnight in chemotaxis medium (FBS-free RPMI + 1% ultrapure BSA, penicillin–streptomycin and L-glutamine) to enhance chemokine responsiveness. Non-adherent cells were then plated at 100,000 cells per well in the upper chamber of a 5 μ m Boyden system and allowed to migrate for 2 h toward moDC-conditioned media. These moDC supernatants were generated after exposure to keratinocyte supernatants from UVB-irradiated $\pm$ IFN β -primed N/TERT2G cells. Supernatants from untreated or LPS-stimulated moDCs served as negative and positive controls. Migrated cells were analyzed by flow cytometry (CD4, CD8, CD3, CD19, CD14) to identify responding T cell subsets.

In vitro UVB treatment

N/TERT2G keratinocytes ($0.8\text{--}1 \times 10^5$ per well) were seeded in 12-well plates 3 days before irradiation. Cells were washed once with PBS and exposed to 50 or 100 mJ cm $^{-2}$ UVB using a SolRx 100-Series handheld device (285–350 nm, Philips PL-S 9W/12/2P). PBS was then replaced with keratinocyte medium, and cells were incubated for 24 h at 37 °C with 5% CO $_2$.

Membrane permeability assays

Cells were incubated with Hoechst 33342 (1:30,000; P3566, Invitrogen) and propidium iodide (1:1500; H3570, Invitrogen). Images were acquired every 2 h for 24 h using a Lionheart FX automated microscope (BioTek) and analyzed with Gen5 software. Membrane integrity was quantified as the ratio of propidium iodide-positive to Hoechst-positive cells.

ATP production assay

Keratinocyte ATP levels were quantified using the CellTiter-Glo 2.0 Assay (G9242, Promega). Keratinocytes were plated in 12-well dishes, treated as indicated and assayed 24 h later by adding an equal volume of CellTiter-Glo reagent. After a 10 min incubation at room temperature (22 °C (range 20–25 °C)) to ensure lysis and signal stabilization, luminescence was measured on a microplate reader. Values were normalized to untreated controls and used as a readout of metabolic activity.

Flow cytometry

The cells obtained through suction blister skin sampling were treated with Fc receptor blocking solution Human TruStain FcX (422302, Biolegend) and stained with the following dye and fluorescent antibodies according to the manufacturer's instructions: Zombie NIR Fixable Viability Kit (423106, Biolegend), PerCP anti-human CD45 (368506, Biolegend; Clone 2D1, Monoclonal, Mouse IgG1, κ), Brilliant Violet 510 anti-human CD3 (344828, Biolegend; Clone SK7, Monoclonal, Mouse IgG1, κ), Spark NIR 685 anti-human CD19 (302270, Biolegend; Clone HIB19, Monoclonal, Mouse IgG1, κ), BUV737 anti-human CD56 (612766, BD Biosciences; Clone NCAM16.2, Monoclonal, Mouse BALB/c IgG2b, κ), Brilliant Violet 570 anti-human CD16 (302036, Biolegend; Clone 3G8, Monoclonal, Mouse IgG1, κ), Spark Blue 550 anti-human CD14 (367148, Biolegend; Clone 63D3, Monoclonal, Mouse IgG1, κ), PE/Fire 810 anti-human HLA-DR (307683, Biolegend; Clone L243, Monoclonal, Mouse IgG2a, κ) and eFluor 450 anti-human CD11c (48011642, Invitrogen eBioscience; Clone 3.9, Monoclonal, Mouse IgG1, κ). For flow cytometry analyses for T cell chemotaxis, Zombie Violet Fixable Viability Kit (423113, Biolegend), CD4 (344622, Biolegend; clone SK3, Monoclonal, Mouse IgG1, and R7-20041, Cytex; clone SK3, monoclonal, mouse IgG1, κ), CD8 (344742, Biolegend; clone SK1, Monoclonal, Mouse IgG1, κ), CD3 (317332, Biolegend; clone OKT3, Monoclonal, Mouse IgG1, κ), CD19 (302206, Biolegend; Clone HIB19, monoclonal, mouse IgG1, κ) and CD14 antibody (367148, Biolegend; Clone 63D3, Monoclonal, Mouse IgG1, κ) were used antibodies according to the manufacturer's instructions to stain the migrated cells. For analysis

of activated CD4 T cells, Zombie NIR viability dye, CD2 (300206, Biolegend; Clone RPA-2.10, Monoclonal, Mouse IgG1, κ), CD3 and CD4 antibodies, as mentioned above, were used for surface staining, and intracellular staining was performed using BD Cytofix/Cytoperm Fixation/Permeabilization Kit (554714, BD Biosciences) and Pacific Blue or PE/Dazzle 594 anti-human/mouse Granzyme B Recombinant Antibody (372216 and 372218, Biolegend; Clone QA16A02, Monoclonal, Mouse IgG1, κ) according to the manufacturer's instructions. Cultured DCs were detached using ice-cold PBS, and prepared and stained with Fc receptor blocking solution, live dead zombie green (423111, Biolegend), CD11b (101226, Biolegend; Clone M1/70, Monoclonal, Rat IgG2b, κ), CD11c (301608, Biolegend; Clone 3.9, Monoclonal, Mouse IgG1, κ), CD14 (367148, Biolegend; Clone 63D3, Monoclonal, Mouse IgG1, κ), HLA-DR (562804, BD; Clone L243, Monoclonal, Mouse IgG2a, κ), CD83 (BD, 612823; Clone HB15e, Monoclonal, Mouse IgG1, κ), CD86 (305442, Biolegend; Clone IT2.2, Monoclonal, Mouse IgG2b, κ) and CD80 (563315, BD Biosciences; Clone L307.4, Monoclonal, Mouse IgG1, κ). An Aurora cytometer from Cytex Biosciences was used for sample analysis, and FlowJo Software (v.10.9.0) was used for data processing. All antibodies and reagents were tested and validated per the respective producers; information is available at [biolegend.com](https://www.biolegend.com), [bdbiosciences.com](https://wwwbdbiosciences.com), [cytekbio.com](https://www.cytexbio.com) and [thermofisher.com](https://www.thermofisher.com).

Bulk RNA-seq

Total RNA was extracted from frozen samples in RLT buffer using the RNeasy Mini Plus Kit (QIAGEN). To eliminate residual genomic DNA, the RNA was treated with RNase-free DNase I for 15 min at room temperature. RNA-seq libraries were constructed using an optimized CellSeq2 protocol for bulk RNA libraries⁶². Each library was prepared by pooling 24 samples, with 50 ng of input RNA per sample. Library construction primers are provided in Supplementary Data 1.

The RNA libraries were sequenced on the NovaSeq 6000 platform. Raw FASTQ files were processed using the following pipeline, <https://github.com/shuo-shan/viafoundry-celseq2-bulk-pipeline>, with a modified GRCh38 genome as the reference (details in Supplementary Information), to generate the final count matrices that were then analyzed in R (v.4.3.3). When required, batch effects owing to sequencing run and inter-donor variability were adjusted using the ComBat-seq⁶³ function from the sva package. Differential expression analysis was performed using DESeq2 (ref. 49), with a design formula in which stimulation was encoded as the condition factor. Genes were considered differentially expressed if they met all the following criteria: mean normalized expression (baseMean) ≥ 10 , P value < 0.05 and absolute log $_2$ (fold change) ≥ 1 .

Spatial genomics gene positioning system (GenePS)

A custom seqFISH panel containing 521 genes, including 21 genes to be imaged one-at-a-time, was designed with Spatial Genomics (Primary Probe Kit, v500; no. 30021131). Fresh-frozen human skin biopsy samples obtained at UMass Memorial Hospital under protocol H00021295 were cryo-sectioned 10 μ m thick and mounted on specialized coverslips (10200003, Spatial Genomics). Immediately after collection, the sections were fixed with freshly prepared 4% paraformaldehyde (28908, Thermo Scientific) in 1 $\times$ PBS for 15 min at room temperature. Following fixation, the sections were washed three times with 1 $\times$ PBS for 5 min each and dehydrated in 70% ethanol for 30 s at room temperature. After air drying, the sections were stored at -80 °C until further preparation for seqFISH experiments. Tissue processing was performed in accordance with Spatial Genomics' Sample Processing for RNA Profiling Guide (ASY-053).

Tissue sections were imaged using the Gene Positioning System (GenePS; Spatial Genomics), an automated platform for image acquisition, reagent delivery and data processing. The system was loaded with the sample, decode plate and buffers following the GenePS Instrument User Guide (INS-205) and guided by on-screen instructions. Regions of

interest within human skin sections were selected using DAPI-stained nuclei imaged with a $\times 10$ air objective. Once regions of interest were selected, the automated imaging workflow was initiated, using a $\times 60$ oil objective with standard image acquisition settings and capturing either three or six z-planes at a z-step size of 1.5 μm . The experimental protocol included iterative rounds of decode probe hybridization, imaging and signal removal, continuing until all hybridization rounds were completed.

Raw image files were aligned across hybridization rounds and processed directly on the GenePS instrument to localize RNA signals. Subsequent analysis using Spatial Genomics' Navigator software enabled transcript decoding and cell segmentation. The segmentation algorithm used machine learning to delineate nuclei based on DAPI staining, with segmentation boundaries expanded by 15 pixels ($\sim 1.5 \mu\text{m}$) past the nuclei borders. RNA transcripts were decoded with a maximum allowed FDR of 5%, and the transcripts were assigned to individual cells, generating cell-by-gene count matrices for each region of interest. The processed outputs included a cell-by-gene matrix, cell coordinates, transcript list, DAPI image and segmentation mask, providing high-resolution spatial and transcriptional data for downstream analysis.

seqFISH+ analysis

Cell-by-gene matrices, cell coordinates and transcript locations were used to create the Seurat⁴⁷ object. Cells with over 50 transcripts or over ten transcripts at the skin surface were retained. IntegrateLayers – RPCA was used for integration on the top 30 PCs, followed by UMAP and clustering (FindNeighbors/FindClusters). Cells were first grouped into major lineages, followed by subtype reclustering. Doublet clusters, identified by inappropriate marker co-expression, were removed before final clustering of lymphoid and myeloid compartments.

To order MC_CD14 cells along the MMP9 transition, the top 100 variable features were selected by FindVariableFeatures and then used for PCA. The top five PCs were used for CCAIntegration and the following DiffusionMap⁵⁶. The first diffusion component defined the transition. For heatmaps, MC_CD14 cells were divided into 30 equal bins, and mean expression per bin was calculated. Expression values were scaled per gene across all bins and reference cell types.

Spatial images were generated using software Spatial Genomics Navigator Lite.

Differential expression analysis was performed using the Model-based Analysis of Single-cell Transcriptomics (MAST) framework⁶⁴, with default parameters wrapped by Seurat. Differential analysis along the CD14⁺ myeloid cell transition axis was performed using tradeSeq⁶⁵ with cellWeights = 1.

Colocalization and visualization functions are packed in the scSpatial R package available on GitHub (<https://github.com/garber-lab/scSpatial>). The colocalization strength of each query cell within a reference cell type was calculated using the createField function (with s.d. = 400 pixels) and the getValuelnField with default parameters. The summation of colocalization strengths of all query cells was used to represent the colocalization score between two cell types. For the heatmap, colocalization scores were scaled so that the total score of all cells in the sample was zero, and the score of the query cell type itself was set to 100. Heatmap visualization and clustering were performed using the ComplexHeatmap R package^{52,66}. See the Supplementary Information and Methods for more details on colocalization analysis. Gene expression trends along colocalization scores or distances were assessed using the FeaturePlot.cont.rollmean function, with window.prop = 1.

The chemokine expression levels around a given CD14⁺ cell were summed within a radius of 400 pixels (41.2 μm). Local expression of chemokines as a function of CD14⁺ cells transition score was visualized with geom_smooth (method = "loess", span = 0.2).

The smoothed expression level of *TNF* and *IFNG* over spatial embedding was visualized with ImageFeaturePlot.contour (sigma = 400, threshold = 0.9, n_levels = 6).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data associated with this study are present in the paper or the Supplementary Information. The scRNA-seq, spatial transcriptomics, proteomics and bulk RNA-seq data are available through the Arthritis, Autoimmune and Related Diseases Knowledge (ARK) Portal (<http://arkportal.synapse.org>) under <https://doi.org/10.7303/syn71964948.1>. The ARK Portal hosts data generated by a network of research teams working collaboratively to deepen the understanding of Arthritis and Autoimmune and Related Diseases. It was established by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) and includes data from the Accelerating Medicines Partnership (AMP) RA/SLE program. The specific data used in this publication are available as a controlled-access dataset. Researchers seeking to use these data must submit a detailed intended data use statement and a completed and signed data use certificate. These access requirements ensure responsible and ethical data sharing within the research community. Instructions for access are available at <https://help.arkportal.org/help/data-use-certificate#DataUse&Acknowledgement-Acknowledgement>. A subset of the data that is available for browsing at <https://CD14photosensitivity.umassmed.edu>. Source data are provided with this paper.

Code availability

Functions used for analysis of the scRNA-seq data and seqFISH data are available on GitHub in open-source packages: <https://github.com/garber-lab/addons> and <https://github.com/garber-lab/scspatial>. An interactive data portal is available for browsing the data, with access instructions at <https://github.com/garber-lab/SkinOmicsExplorer>. All data, code and materials will be made available upon request.

References

- Strassner, J. P., Rashighi, M., Ahmed Refat, M., Richmond, J. M. & Harris, J. E. Suction blistering the lesional skin of vitiligo patients reveals useful biomarkers of disease activity. *J. Am. Acad. Dermatol.* **76**, 847–855.e5 (2017).
- Zilionis, R. et al. Single-cell barcoding and sequencing using droplet microfluidics. *Nat. Protoc.* **12**, 44–73 (2017).
- Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 (2013).
- Derr, A. et al. End Sequence Analysis Toolkit (ESAT) expands the extractable information from single-cell RNA-seq data. *Genome Res.* **26**, 1397–1410 (2016).
- Hao, Y. et al. Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nat. Biotechnol.* **42**, 293–304 (2024).
- Wickham, H. *ggplot2: Elegant Graphics for Data Analysis* (Springer, 2016).
- Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
- Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140 (2010).
- Frisoli, M. L. et al. Single-cell RNA sequencing reveals molecular signatures that distinguish allergic from irritant contact dermatitis. *J. Invest. Dermatol.* **145**, 1117–1126.e14 (2025).
- Gu, Z., Eils, R. & Schlesner, M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**, 2847–2849 (2016).
- Büttner, M., Ostner, J., Müller, C. L., Theis, F. J. & Schubert, B. scCODA is a Bayesian model for compositional single-cell data analysis. *Nat. Commun.* **12**, 6876 (2021).

54. Germain, P. L., Lun, A., Garcia Meixide, C., Macnair, W. & Robinson, M. D. Doublet identification in single-cell sequencing data using scDblFinder. *F1000Res*. **10**, 979 (2021).
55. McGinnis, C. S., Murrow, L. M. & Gartner, Z. J. DoubletFinder: doublet detection in single-cell RNA sequencing data using artificial nearest neighbors. *Cell Syst*. **8**, 329–337.e4 (2019).
56. Angerer, P. et al. Destiny: diffusion maps for large-scale single-cell data in R. *Bioinformatics* **32**, 1241–1243 (2016).
57. Kuhn, A. et al. Photoprovocation in cutaneous lupus erythematosus: a multicenter study evaluating a standardized protocol. *J. Invest. Dermatol.* **131**, 1622–1630 (2011).
58. Albrecht, J. et al. The CLASI (Cutaneous Lupus Erythematosus Disease Area and Severity Index): an outcome instrument for cutaneous lupus erythematosus. *J. Invest. Dermatol.* **125**, 889–894 (2005).
59. Young, M. D. & Behjati, S. SoupX removes ambient RNA contamination from droplet-based single-cell RNA sequencing data. *Gigascience* **9**, gaa151 (2020).
60. Korsunsky, I. et al. Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat. Methods* **16**, 1289–1296 (2019).
61. Dickson, M. A. et al. Human keratinocytes that express hTERT and also bypass a p16^{INK4a}-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. *Mol. Cell. Biol.* **20**, 1436–1447 (2000).
62. Zhang, H. et al. Worm Perturb-Seq: massively parallel whole-animal RNAi and RNA-seq. *Nat. Commun.* **16**, 4785 (2025).
63. Zhang, Y., Parmigiani, G. & Johnson, W. E. ComBat-seq: batch effect adjustment for RNA-seq count data. *NAR Genom. Bioinform.* **2**, lqaa078 (2020).
64. Finak, G. et al. MAST: a flexible statistical framework for assessing transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data. *Genome Biol.* **16**, 278 (2015).
65. Van den Berge, K. et al. Trajectory-based differential expression analysis for single-cell sequencing data. *Nat. Commun.* **11**, 1201 (2020).
66. Gu, Z. Complex heatmap visualization. *Imeta* **1**, e43 (2022).

Acknowledgements

We thank the patients of J.E.H., M. Rashighi and R.A.V. for their donation of time and tissues. We also acknowledge the University of Massachusetts Medical School Flow Cytometry Core and the University of Massachusetts Medical School Department of Dermatology's clinical research team. We would like to thank all members of K. Fitzgerald's laboratory and M. Orzalli's laboratory for their continuous advice and guidance. We also thank J. Wang for comments on early manuscript drafts. This work was funded by the National Institutes of Health (NIH) NIH-NIAMS P50AR080593 (to J.E.H. and M.G.), NIH-NIAID 5U01AI176310 (to J.E.H., M.G. and M. Rashighi), NIH-NIAID 5U01AI168640 (to M. Rashighi), NIH-NIAID R01AI181221

(to M.G.), King Trust, Bank of America Private Bank, Co-Trustees Fellowship (to K.A.) and Dermatology Foundation and Skin of Color Society (to N.-S.H.). Additional support was received from a philanthropic gift for the Edward Fischer Graft-Versus-Host Fund, with support from the Siegel Family. K.W. is supported by NIH-NIAMS K08AR077037, a Burroughs Wellcome Fund Career Award for Medical Scientists and a Doris Duke Charitable Foundation Clinical Scientist Development Award. M. Rosenbach is supported by Priovant.

Author contributions

Y.W., K.A., N.-S.H., S.G., J.E.H., M. Rashighi and M.G. designed the experiments, developed the methodology, analyzed and interpreted data and wrote the paper. Y.W., K.A., N.-S.H., C.S.L., C.-H.L.E., N.M., L.M.W., K.S.A., K.W., K.F., P.P.K. and S.G. performed experiments and acquired data. C.-H.L.E., K.F., K.W., M. Rosenbach and R.A.V. provided technical support and advice on data analysis and interpretation. M. Rashighi and M.G. supervised the study and were responsible for coordination and strategy.

Competing interests

M.G. is a scientific founder of Via Scientific, which develops analysis pipelines used in this work. J.E.H. is a scientific founder of Villaris Therapeutics, which develops therapeutic treatments for vitiligo. J.E.H. is an inventor on patent no. 62489191 ('Diagnosis and Treatment of Vitiligo'), which covers targeting IL-15 and Trm for the treatment of vitiligo. K.W. receives research support from Merck Sharp & Dohme and 10x Genomics. M. Rosenbach receives research support as honoraria from Merck, J&J, Priovant and Novartis. M. Rashighi received consulting fees from Biogen. The other authors declare no competing interests.

Additional information

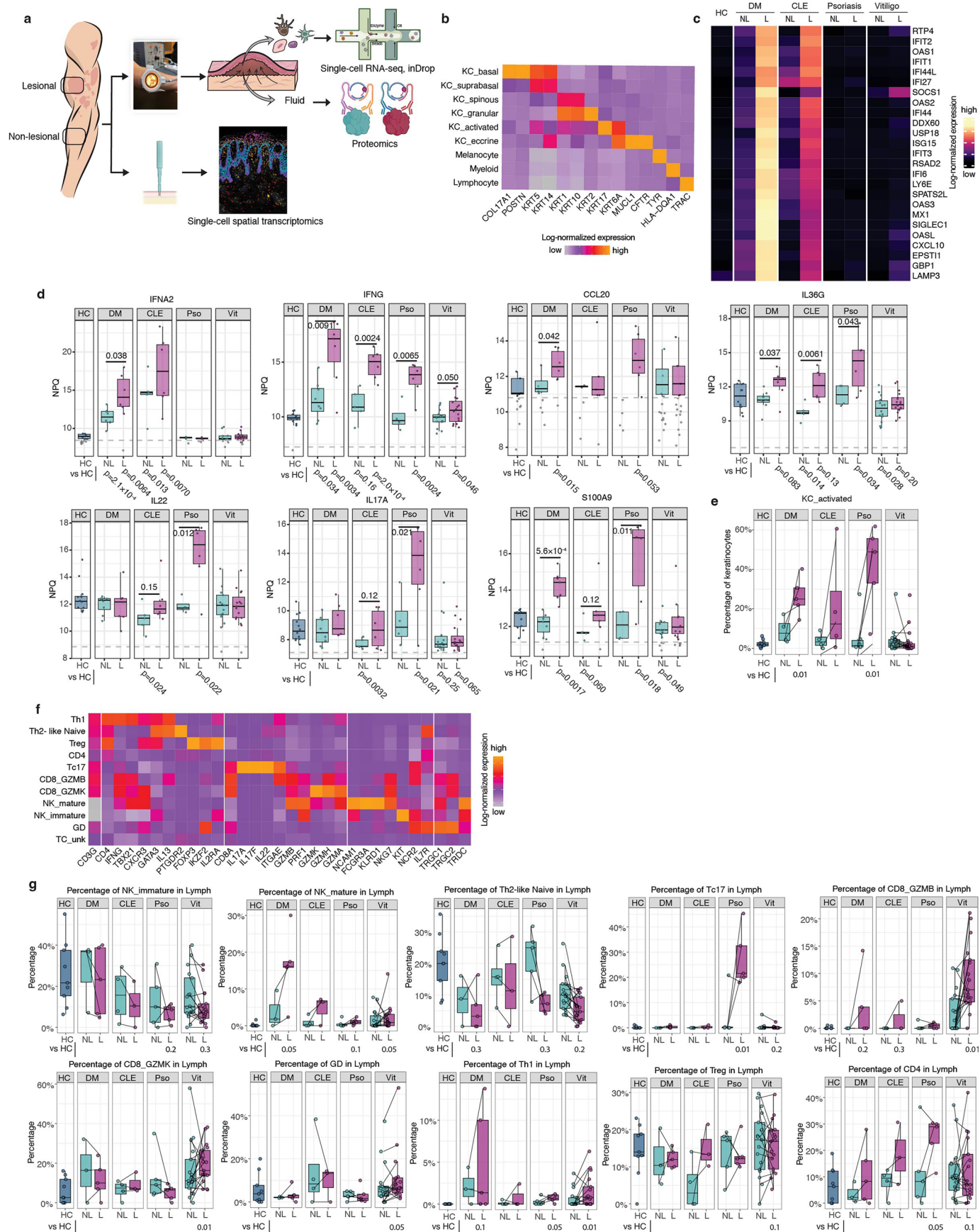
Extended data is available for this paper at <https://doi.org/10.1038/s41590-026-02502-w>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41590-026-02502-w>.

Correspondence and requests for materials should be addressed to Mehdi Rashighi or Manuel Garber.

Peer review information *Nature Immunology* thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available. Primary Handling Editor: S. Houston, in collaboration with the *Nature Immunology* team.

Reprints and permissions information is available at www.nature.com/reprints.

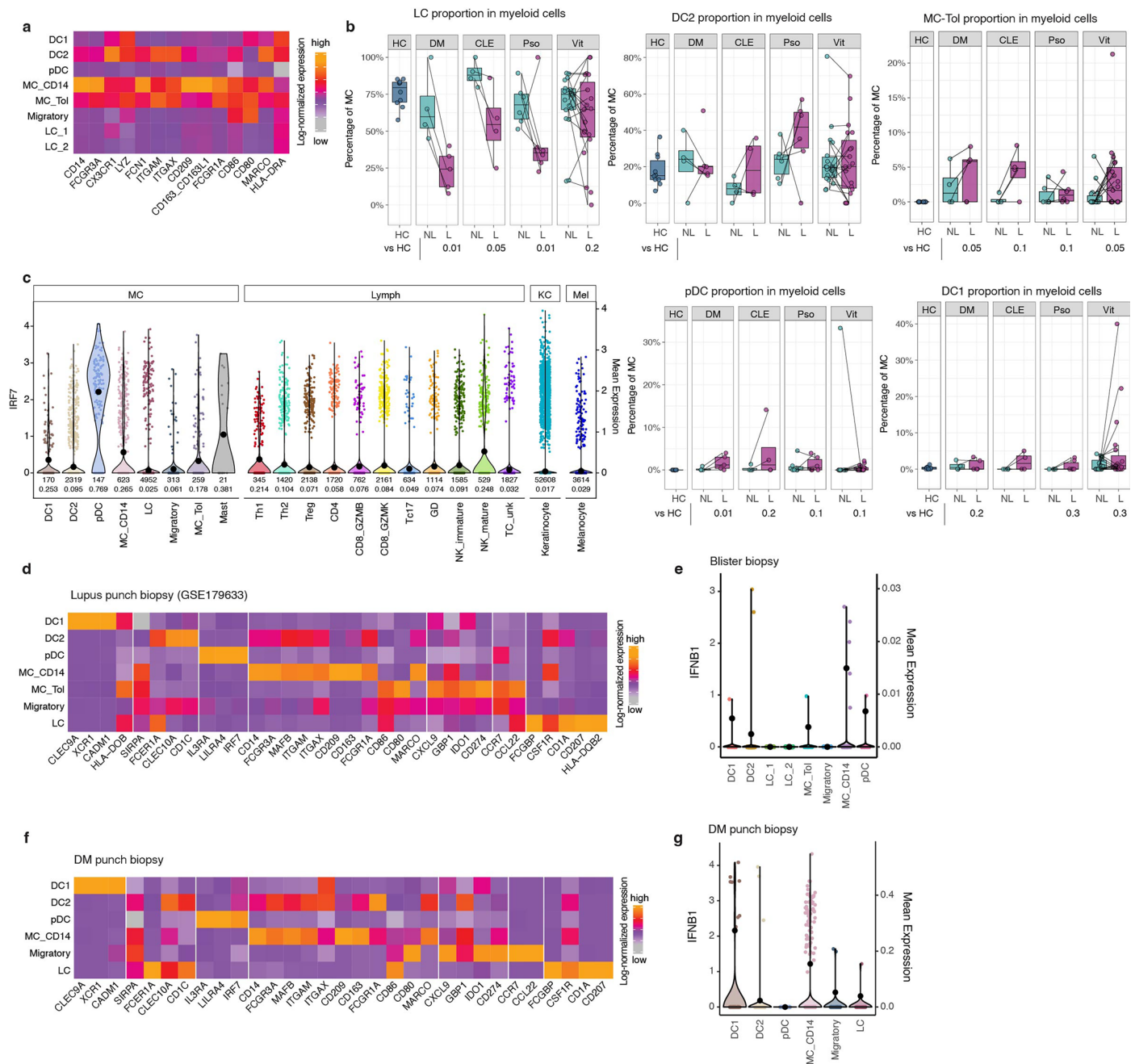


Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Keratinocyte and lymphocyte subtypes

characterization and molecular signatures. (a) Schematic overview of the study. Suction blister biopsies were collected from lesional (L) and non-lesional (NL) skin of patients with dermatomyositis (DM), cutaneous lupus erythematosus (CLE), psoriasis (Pso), vitiligo (Vit), and from healthy controls (HC). Cells in the blister biopsies were used for inDrop scRNA-seq, and the blister fluid was used for proteomics Nucleic acid Linked Immuno-Sandwich Assay (NULISA™). Punch biopsies were collected for spatial transcriptomics sequential Fluorescence In Situ Hybridization (seqFISH). Elements Created in BioRender. Afshari, K. (2026) <https://BioRender.com/bfzhnl>. (b) Pseudo bulk expression heatmap of marker genes for main cell types and keratinocyte subtypes in scRNA-seq of blister biopsies. (c) Pseudo bulk expression heatmap of type I interferon

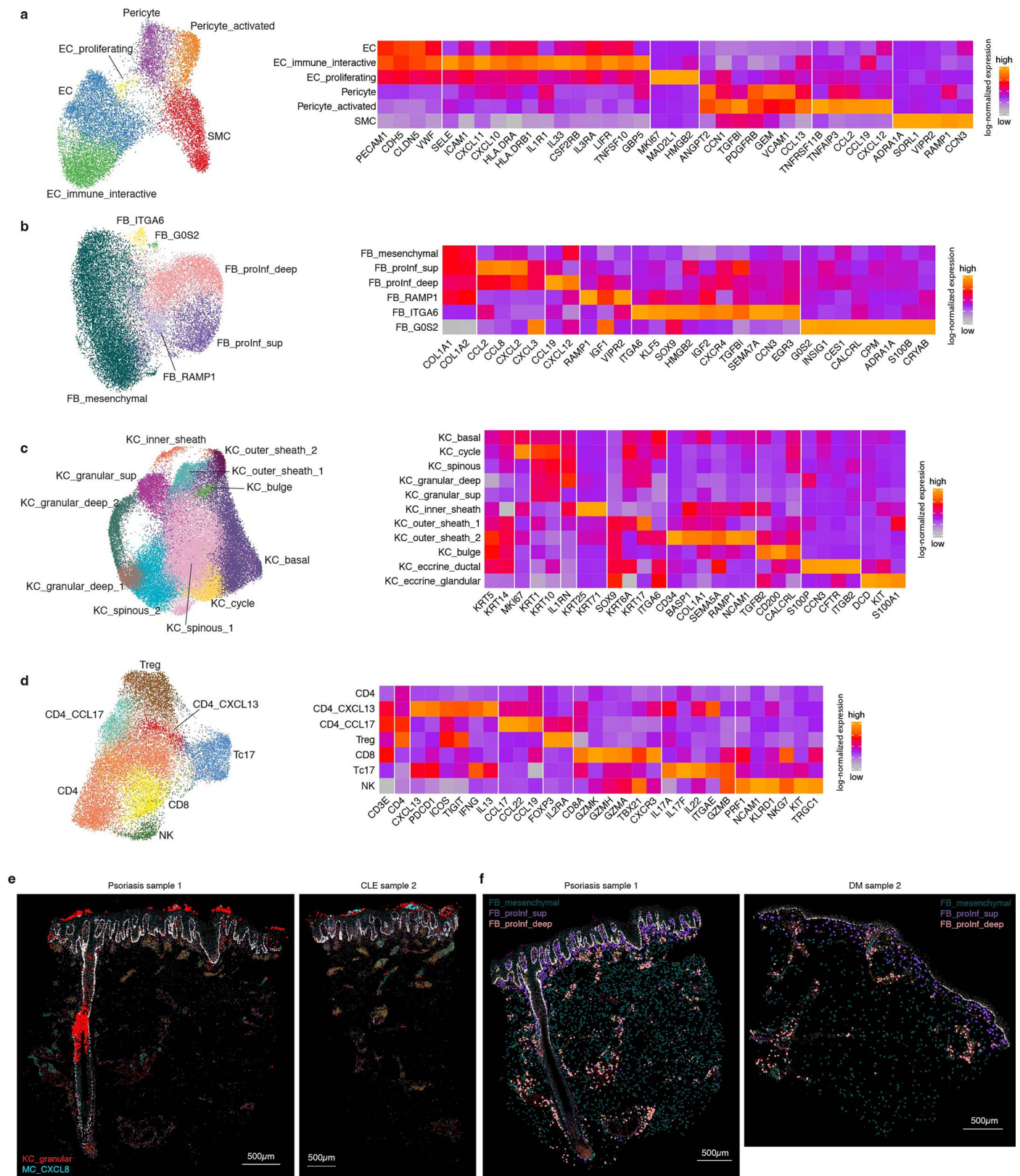
(IFN-I)–responsive genes in lesional (L) and non-lesional (NL) skin across diseases. (d) Box plots of protein levels (NPQ) in blister fluids across diseases and skins. Two-sided t-test was used for pairwise comparisons. (e) Box plots showing percentage of activated keratinocyte (KC_activated) within all keratinocytes (KC) per sample across skin conditions. Statistical significance was assessed using scCODA, with KC_suprabasal as the reference cell type. (f) Pseudo bulk expression heatmap of canonical marker genes for lymphocyte subsets. (g) Box plots showing percentage of lymphocyte subtypes per sample across conditions. Statistical significance was assessed using scCODA, with KC_suprabasal as the reference cell type. Box plot definition (all panels): center line, median; box, 25th–75th percentiles; whiskers, most extreme values within 1.5×IQR; points beyond whiskers, outliers.



Extended Data Fig. 2 | Cross-validation of myeloid subset composition and IFNB1 expression across scRNA-seq datasets in photosensitive skin diseases.

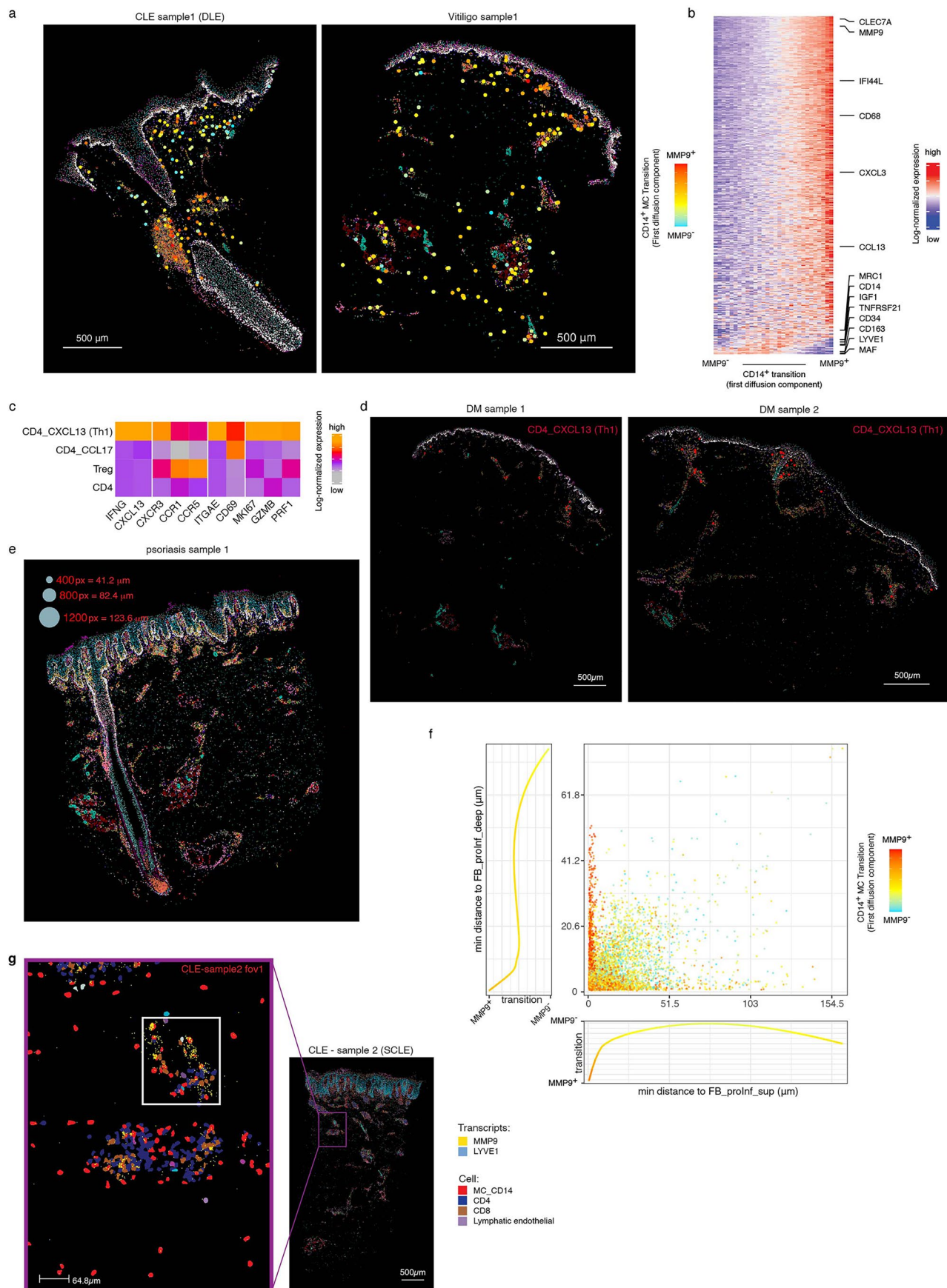
(a) Pseudo bulk expression of macrophage and dendritic cell defining marker genes in scRNA-seq of blister biopsies. (b) Box plots showing percentage of myeloid subsets within all myeloid cells (MC) per sample, across skin conditions. Each dot represents a sample. Statistical significance was assessed using scCODA, with KC suprabasal as the reference cell type. (c) Violin plot showing the expression of IRF7 in immune subsets, keratinocytes and melanocytes detected in blister biopsies. Each dot represents a single cell. (d) Pseudo bulk expression

heatmap of myeloid cell marker genes across myeloid subsets in punch biopsies obtained from lesional skin of lupus (GSE179633). (e) Violin plot showing the expression of IFNB1 from myeloid subsets detected in blister biopsies. Each dot represents a single cell. (f) Pseudo bulk expression heatmap of myeloid cell marker genes across myeloid subsets in scRNA-seq of punch biopsies obtained from lesional skin of DM. (g) Violin plot showing the expression of IFNB1 from myeloid subsets detected in DM punch biopsies. Each dot represents a single cell. Box plot definition (all panels): center line, median; box, 25th–75th percentiles; whiskers, most extreme values within 1.5×IQR; points beyond whiskers, outliers.



Extended Data Fig. 3 | Spatial transcriptomics reveals fine-grained subtypes and spatial organization of stromal, immune, and inflammatory cell states in autoimmune skin lesions. (a-d) UMAP embeddings (left) and heatmaps for pseudo bulk marker genes expression of reclustered main cell types in seqFISH **(a)** Endothelial cells, **(b)** Fibroblasts, **(c)** Keratinocytes and **(d)** Lymphocytes. **(e)** Spatial embedding of seqFISH sections highlighting MC_CXCL8 cells using KC_granular as spatial reference. **(f)** Spatial embedding of seqFISH sections

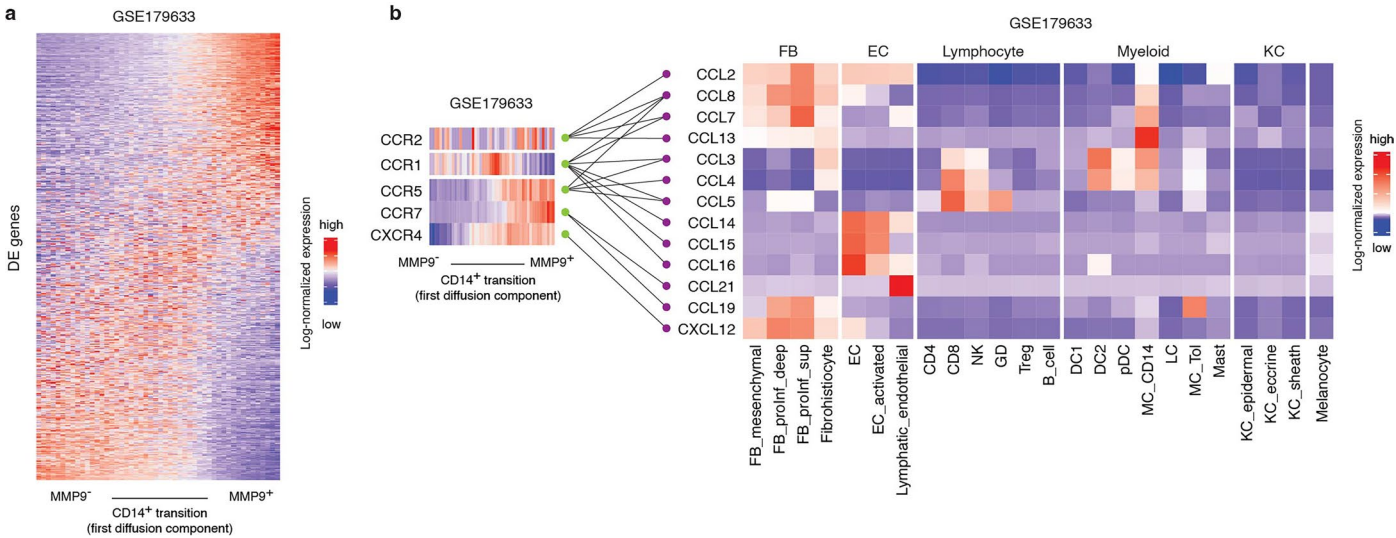
highlighting superficial proinflammatory fibroblasts (FB_proInf_sup), deep proinflammatory fibroblasts (FB_proInf_deep), and mesenchymal fibroblasts (FB_mesenchymal). Basal keratinocytes colored in white delineating the dermal-epidermal junction. Data are representative of 5 of 6 tissue sections derived from 3 punch biopsies (3 patients) exhibiting keratinocyte hyperproliferation, with similar results across those 5 sections.



Extended Data Fig. 4 | See next page for caption.

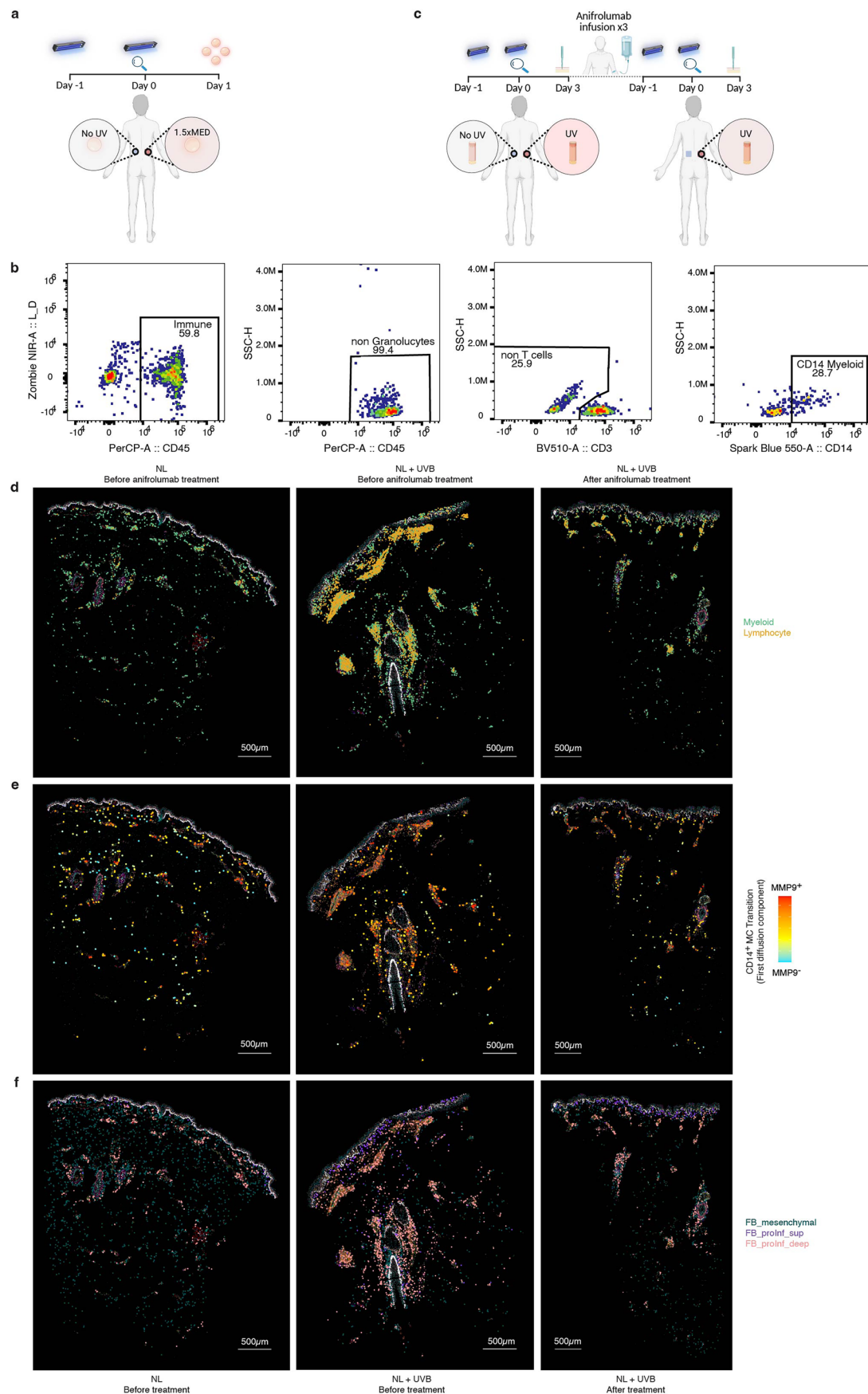
Extended Data Fig. 4 | Spatial transcriptomics reveals transcriptional and spatial continuum along the LYVE1 to MMP9 transition. **(a)** Spatial embeddings of seqFISH data showing CD14⁺ cells colored by their first diffusion component in lesional tissue sections from the DLE and vitiligo samples. Data are representative of n = 13 tissue sections derived from 7 punch biopsies (7 patients) with similar results. **(b)** Heatmap of differentially genes expressed in CD14⁺ cells ordered by the first diffusion component (transition) ($p < 0.01$, tradeSeq) and aggregated into 30 bins (193 cells per bin). **(c)** Pseudo bulk expression heatmap of cytotoxic and Th1 markers in different CD4 cell populations of the scRNA-seq data from blister biopsies. **(d)** Spatial embedding of seqFISH DM samples highlighting cytotoxic CD4_CXCL13 cell localization. Data are representative of n = 13 tissue

sections derived from 7 punch biopsies (7 patients) with similar results. **(e)** Representative spatial embedding illustrating radius used in colocalization analysis. **(f)** Scatter plot showing minimum distance in μm from a CD14⁺ cell to a superficial pro inflammatory fibroblast (x-axis) or to a deep pro inflammatory fibroblast (y-axis) colored by their projection onto the first diffusion component; marginal plots show mean of the first diffusion component of CD14⁺ cells projected onto the x-axis (bottom) or the y-axis (left). **(g)** High-resolution image of CLE sample 2 showing zoom-ins of a dermal immune aggregate. Key cell types are displayed, with overlaid transcripts for LYVE1 (blue) and MMP9 (yellow). Data are representative of n = 13 tissue sections derived from 7 punch biopsies (7 patients) with similar results.



Extended Data Fig. 5 | Spatial localization of CD14⁺ myeloid cells is dependent on a complex network of chemokines. (a) Heatmap of differentially genes expressed across the CD14⁺ cell transition in the GSE179633 dataset ($p < 0.01$,

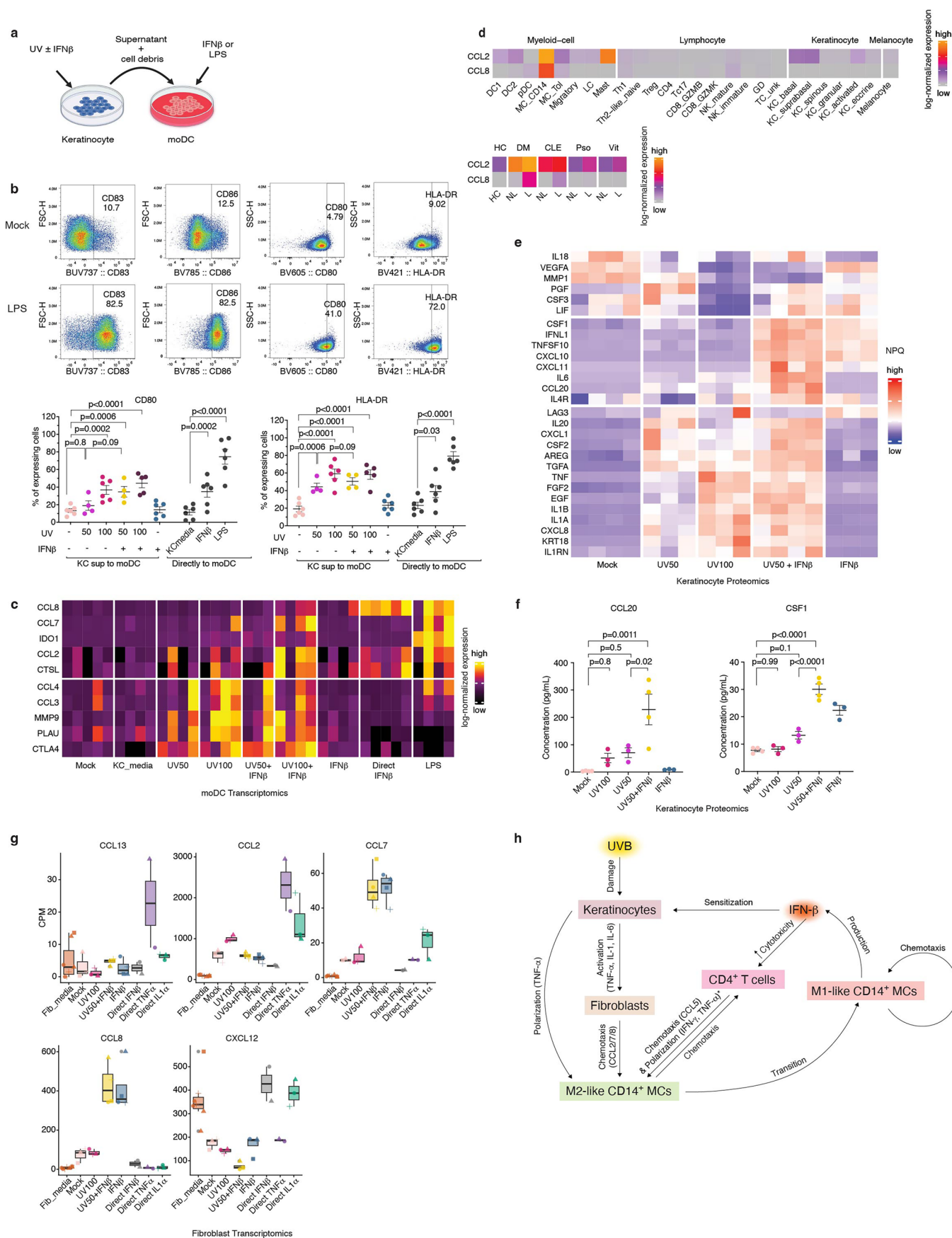
tradeSeq). Cells were ordered by the first diffusion component (transition) and aggregated into 50 bins (144 cells per bin). (b) As in 5 F, but with ligand pseudo bulk expression computed using the GSE179533 data.



Extended Data Fig. 6 | See next page for caption.

Extended Data Fig. 6 | UVB photoprovocation and interferon blockade with spatial immune cell profiling. (a) Schematic of the UVB photoprovocation protocol. Minimal erythema dose (MED) was determined for each subject, followed by exposure of non-lesional, photoprotected lower back skin to 1.5× MED UVB. Suction blister biopsies were collected 24 hours later from both irradiated and unirradiated sites. Created in BioRender. Afshari, K. (2026) <https://BioRender.com/1d40uqa>. (b) FACS gating strategy to quantify CD14⁺

cells and myeloid cells in samples from non-lesional skin of a CLE patient. (c) Schematic of anifrolumab intervention experiment. A patient with cutaneous lupus erythematosus (CLE) underwent UVB provocation before and after three monthly IV infusions of anifrolumab (anti-IFNAR1 monoclonal antibody). Created in BioRender. Afshari, K. (2026) <https://BioRender.com/7k6vkth>. (d-f) Spatial embeddings highlight relevant cell types in seqFISH data of the anifrolumab intervention experiment.



Extended Data Fig. 7 | See next page for caption.

Extended Data Fig. 7 | Type I interferon-licensed keratinocytes activate monocyte-derived dendritic cells (moDCs) and induce fibroblast-derived chemokine programs. (a) Keratinocyte–moDC co-culture system. N/TERT2G keratinocytes were treated with UVB (50 or 100 mJ/cm²) ± IFN-β, and their supernatants were applied to monocyte-derived dendritic cells (moDCs). Created in BioRender. Afshari, K. (2026) <https://BioRender.com/d27g462> (b) Left: Gating strategy for flow cytometric analysis of moDCs. Right: Quantification of CD80 and HLA-DR expression in moDCs incubated with keratinocyte supernatants or directly stimulated with interferon-β (IFN-β) or lipopolysaccharide (LPS). Data are presented as mean ± SEM and were analyzed using one-way ANOVA followed by Bonferroni-corrected multiple comparisons. Each dot represents one independent donor (n = 6 donors). (c) Heatmap of selected inflammatory genes expression in bulk RNA-seq of moDCs stimulated with keratinocyte supernatants under the indicated UVB and interferon-β (IFN-β) conditions, or directly treated with IFN-β or lipopolysaccharide (LPS); colors are normalized expression scaled by gene (row) (n = 5 donors per group). (d) Heatmap of gene expression in blister biopsy scRNA-seq data aggregated by

cell subtypes (top) or skin conditions (bottom). Colors are scaled by gene (row). (e) Heatmap of differentially abundant proteins expression (NPQ) (p < 0.05) in KC supernatants; colors scaled by protein (row) Data were derived from 4 independent donors; not all donors contributed to every condition. (f) Cytokine concentration in keratinocyte supernatants, measured using OLINK proximity extension assay. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Each dot represents one independent donor. Data were derived from 4 independent donors; not all donors contributed to every condition. (g) Bar plots of chemokine expression in fibroblasts treated with conditioned-keratinocyte supernatants, and direct IFN-β, IL-1α or TNF-α. Data were derived from 4 independent donors; not all donors contributed to every condition. (h) Schematic representation of events leading to CD14+ cell activation, chemotaxis, state switch and contribution to heightened inflammation in Type I interferon-rich environments supported by this study. * Chemotaxis by T cells including both CD4 and CD8 T cells. Box plot definition (all panels): center line, median; box, 25th–75th percentiles; whiskers, most extreme values within 1.5×IQR; points beyond whiskers, outliers.

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ A description of all covariates tested |
| ☐ | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	xCELLigence Real-Time Cell Analysis technology (Agilent), with manufacturer-provided analysis software, was used for impedance-based cytotoxicity assays. Imaging data were acquired using the Lionheart FX automated microscope (BioTek) and analyzed using Gen5 software (BioTek). Spatial transcriptomics image decoding and cell segmentation were performed using Spatial Genomics' Navigator software, with visualization performed using Navigator Lite. Proteomic data were acquired and processed using Olink NPX™ Signature software and NULISA output generated by the ARGO system. Flow cytometry data were analyzed using FlowJo v10.
Data analysis	Statistical analyses were performed using RStudio and GraphPad Prism v10. Figures were generated using Adobe Illustrator and BioRender. Functions used for analysis of the scRNA-seq data and seqFISH data are available on GitHub in open-source packages: https://github.com/garber-lab/addons; https://github.com/garber-lab/scspatial. Some package were updated during the project for compatibility of the new packages. Cell Ranger (10X Genomics) STAR (v2.5.3) ESAT (v0.1) tradeSeq (1.20.0) tidyverse (v2.0.0) ggridge (v0.5.7) Seurat (v5.3.0) dplyr (v1.1.4) cowplot (v1.2.0)

shiny (v1.11.1)
 ComplexHeatmap (v2.24.1)
 DESeq2 (v1.48.2)
 OlinkAnalyze (v4.3.2)
 ggplot2 (v3.4.0 and v4.0.0)
 scCODA (v0.1.9)
 scDbfFinder (v1.19.6)
 DoubletFinder (v2.0.4)
 destiny (v3.18.0)
 sva (v3.48.0)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data associated with this study are present in the paper or the Supplementary Materials. Data are deposited in the ARK portal (ID: syn64570520) as required by the funding agency from which it will be available. An interactive data portal is available for browsing the data, with access instructions at <https://github.com/garber-lab/SkinOmicsExplorer>. All Data, code, and materials will be made available upon request.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	Sex was recorded and reported (Table 1). Sex-based analyses were not performed due to limited sample size
Reporting on race, ethnicity, or other socially relevant groupings	Participant demographic and clinical characteristics, including age and diagnosis are summarized in Table 1.
Population characteristics	Race and ethnicity data were collected for descriptive purposes only and were not used as variables in downstream analyses.
Recruitment	Recruitment procedures and eligibility criteria are described in the Methods. Briefly Under the supervision of Institutional Review Board–approved protocol (H00021295, and H-14848) at the University of Massachusetts Medical School patients were recruited for UV photo provocation, skin biopsies and PBMC acquisition.
Ethics oversight	The study was approved by the Institutional Review Board of UMass Chan Medical School. De-identified samples were used in accordance with institutional guidelines.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For inDrop scRNA-seq, sample size adequacy was assessed by performing differential expression (DE) analyses on vitiligo patient subsets of increasing size, and identifying the minimum number of patients required to obtain stable DE results. Sample size of seqFISH experiment was low due to the limited funding. Therefore no statistical test was done comparing across diseases.
Data exclusions	In the experiment comparing multiple stimulations on fibroblasts using bulk RNA-seq, one IFN γ -stimulated replicate showed no induction of canonical IFN γ -responsive genes and clustered with unstimulated controls by PCA and hierarchical clustering. We therefore considered it a failed stimulation and excluded it from differential expression analyses. All exclusion criteria were based on biological response signatures rather than differential expression outcomes.

Replication	All attempts at replication were successful.
Randomization	Randomization was not applicable to most aspects of this study due to its observational and paired experimental design. For human tissue analyses, samples were obtained based on clinical diagnosis, and comparisons were performed either between disease groups or using paired lesional and non-lesional samples from the same individual, thereby controlling for inter-individual variability. In vivo UVB photoprovocation experiments, irradiated and untreated control sites were selected on the same individual, allowing within-subject comparisons. UVB doses were determined based on each subject's minimal erythema dose rather than random assignment. In vitro experiments were performed using predefined treatment conditions applied uniformly across biological replicates. Where applicable, samples were processed in parallel to minimize batch effects.
Blinding	Library preparation for inDrop scRNA-seq was blinded in library preparation part, using patient ID and library ID that did not reveal disease information. For computational analyses, group identities were known during analysis, but objective, predefined criteria were used for quality control and clustering, with algorithm performance assessed independently of disease labels. Patient and sample identifiers were used for evaluating sample integration, while disease labels were required and applied in downstream analyses, including differential expression and cell-type proportion analysis. For in vivo UVB photoprovocation and histological analyses, samples were evaluated using standardized protocols. Given the nature of the experimental design and the clear anatomical distinction between irradiated and non-irradiated sites, blinding was not feasible. For in vitro experiments, samples were processed and analyzed using standardized protocols, and outcomes were quantitatively assessed using flow cytometry, transcriptomic, or proteomic readouts.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

The cells obtained through suction blister skin sampling were treated with Fc receptor blocking solution Human TruStain FcX (Biolegend, #422302) and stained with the following dye and fluorescent antibodies according to the manufacturer's instructions: Zombie NIR™ Fixable Viability Kit (Biolegend, #423106), PerCP anti-human CD45 (Biolegend #368506, Clone 2D1, Monoclonal, Mouse IgG1, κ), Brilliant Violet 510™ anti-human CD3 (Biolegend, #344828, Clone SK7, Monoclonal, Mouse IgG1, κ), Spark NIR™ 685 anti-human CD19 (Biolegend, #302270, Clone HIB19, Monoclonal, Mouse IgG1, κ), BUV737 anti-Human CD56 (BD Biosciences, #612766, Clone NCAM16.2, Monoclonal, Mouse BALB/c IgG2b, κ), Brilliant Violet™ 570 anti-human CD16 (Biolegend, #302036, Clone 3G8, Monoclonal, Mouse IgG1, κ), Spark Blue™ 550 anti-human CD14 (Biolegend, #367148, Clone 63D3, Monoclonal, Mouse IgG1, κ), PE/Fire™ 810 anti-human HLA-DR (Biolegend, #307683, Clone L243, Monoclonal, Mouse IgG2a, κ), eFluor™ 450 anti-human CD11c (Invitrogen eBioscience #48011642, Clone 3.9, Monoclonal, Mouse IgG1, κ).

For flow cytometry analyses for T cell chemotaxis Zombie Violet™ Fixable Viability Kit (Biolegend, #423113), CD4 (Biolegend #344622, clone SK3, Monoclonal, Mouse IgG1, and, Cytek # R7-20041, clone SK3, monoclonal, mouse IgG1, κ), CD8 (Biolegend #344742, clone SK1, Monoclonal, Mouse IgG1, κ), CD3 (Biolegend #317332, clone OKT3, Monoclonal, Mouse IgG1, κ), CD19 (Biolegend #302206, Clone HIB19, monoclonal, mouse IgG1, κ) and CD14 antibody (Biolegend, #367148, Clone 63D3, Monoclonal, Mouse IgG1, κ) were utilized antibodies according to the manufacturer's instructions to stain the migrated cells.

For analysis of activated CD4 T cells, Zombie NIR™ viability dye, CD2 (Biolegend #300206, Clone RPA-2.10, Monoclonal, Mouse IgG1, κ), CD3 and CD4 antibodies, as mentioned above, were used for surface staining, next intracellular staining was performed using BD Cytotfix/Cytoperm™ Fixation/Permeabilization Kit (BD Biosciences #554714) and Pacific Blue™ or PE/Dazzle™ 594 anti-human/mouse Granzyme B Recombinant Antibody (Biolegend# 372216 and # 372218, Clone QA16A02, Monoclonal, Mouse IgG1, κ) according to the manufacturer's instructions.

Cultured DCs were detached using ice cold PBS, and prepared and stained with Fc receptor blocking solution, Live dead zombie green

(Biolegend #423111), CD11b (Biolegend, #101226, Clone M1/70, Monoclonal, Rat IgG2b, κ), CD11c (Biolegend, #301608, Clone 3.9, Monoclonal, Mouse IgG1, κ), CD14 (Biolegend, #367148, Clone 63D3, Monoclonal, Mouse IgG1, κ), HLA-DR (BD #562804, Clone L243, Monoclonal, Mouse IgG2a, κ), CD83 (BD #612823, Clone HB15e, Monoclonal, Mouse IgG1, κ), CD86 (Biolegend, #305442, Clone IT2.2, Monoclonal, Mouse IgG2b, κ) and CD80 (BD Biosciences #563315, Clone L307.4, Monoclonal, Mouse IgG1, κ).

An Aurora cytometer from Cytex Biosciences was used for sample analysis, and FlowJo™ Software (Version 10.9.0) for data processing. All antibodies and reagents were tested and validated per respective producers, information available at (biolegend.com, bdbiosciences.com, cytekbio.com, thermofisher.com).

Validation

All antibodies used in this study were commercially obtained from BioLegend, BD Biosciences, or eBioscience and were selected based on manufacturer-provided validation for human samples and the indicated flow cytometry applications, as documented in product datasheets. Antibodies were used according to the manufacturers' recommended staining conditions. Specificity and performance were further supported by appropriate experimental controls, including Fc receptor blocking, live/dead discrimination, and reproducible detection of expected immune cell populations based on established marker expression patterns.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Under the supervision of Institutional Review Board–approved protocol (H00021295, and H-14848) at the University of Massachusetts Medical School fresh PBMCs were obtained from 12 healthy participants (#6 male and #6 female) for in vitro experiments including T-cell activation and chemotaxis. Primary fibroblasts (under protocol #H11731) were isolated from daintified surgically discarded pannus skin as described in the methods sections. PBMCs were isolated from de-identified, healthy donor leukopaks (UMass Leukocyte Core) for dendritic cell differentiation and stimulation. Immortalized N/TERT2G keratinocytes were generously provided by H. Rheinwald under a Material Transfer Agreement.

Authentication

Phenotype and lineage of cell lines used in this study were verified by Bulk RNA sequencing (keratinocytes, fibroblast, DCs) and flowcytometry (DCs, T cells).

Mycoplasma contamination

None of the cell lines tested for mycoplasma contamination.

Commonly misidentified lines (See [ICLAC](#) register)

None

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration

Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.

Study protocol

Note where the full trial protocol can be accessed OR if not available, explain why.

Data collection

Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.

Outcomes

Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

The cells obtained through suction blister skin sampling were treated with Fc receptor blocking solution Human TruStain FcX (Biolegend, #422302) and stained with the following dye and fluorescent antibodies according to the manufacturer's instructions: Zombie NIR™ Fixable Viability Kit (Biolegend, #423106), PerCP anti-human CD45 (Biolegend, #368506), Brilliant Violet 510™ anti-human CD3 (Biolegend, #344828), Spark NIR™ 685 anti-human CD19 (Biolegend, #302270), BUV737 anti-human CD56 (BD Biosciences, #612766), Brilliant Violet™ 570 anti-human CD16 (Biolegend, #302036), Spark Blue™ 550 anti-human CD14 (Biolegend, #367148), PE/Fire™ 810 anti-human HLA-DR (Biolegend, #307683), eFluor™ 450 anti-human CD11c (eBioscience™ #48011642). For flow cytometry analyses for T cell chemotaxis Zombie Violet™ Fixable Viability Kit (Biolegend, #423113), CD4 (Biolegend # 344622), CD8 (Biolegend # 344742), CD3 (Biolegend # 317332), CD19 and CD14 antibodies as mentioned above, were utilized antibodies according to the manufacturer's instructions to stain the migrated cells. For analysis of activated CD4 T cells, Zombie NIR™ viability dye, CD2 (Biolegend #300206), CD3 and CD4 antibodies were used for surface staining, next intracellular staining was performed using BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit (BD Biosciences #554714) and Pacific Blue™ or PE/Dazzle™ 594 anti-human/mouse Granzyme B Recombinant Antibody (Biolegend# 372216 and # 372218) according to the manufacturer's instructions. Cultured DCs were detached using ice cold PBS, and prepared and stained with Fc receptor blocking solution, Live dead zombie green (Biolegend #423111), APC/Cyanine7 anti-mouse/human CD11b (Biolegend, #101226), PE/Cyanine7 anti-human CD11c (Biolegend, #301608), BV421 Anti-Human HLA-DR (BD Biosciences #562804), BUV737 Anti-Human CD83 (BD Biosciences #612823), Brilliant Violet 785 CD86 (Biolegend, #305442) and BV605 Anti-Human CD80 (BD Biosciences #563315).

Instrument

An Aurora cytometer from Cytex Biosciences was used for sample analysis.

Software

FlowJo™ Software (Version 10.9.0) for data processing.

Cell population abundance

No cell sorting was performed in this study, and therefore no post-sort fractions were generated. Cell population abundance was quantified by flow cytometry analysis of unsorted samples. Immune cell populations were identified based on sequential gating of live, singlet immune cells using established lineage and marker combinations. Population abundance was expressed as the percentage of each population relative to the relevant parent gated compartment. Data on cell population proportions are presented in the Results.

Gating strategy

For the cultured DCs, FSCA-SSCA (cell sized events) -> Singlets based on Area and Height (FSCA-FSCH) -> live cells based on viability dye staining (negative cells selected) -> moDCs i.e. CD14 and CD16 double positive cells -> CD40/CD80/CD83/CD86/HLA-DR expression.

For activated CD4 T cells: FSCA-SSCA (cell sized events) -> Singlets based on Area and Height (FSCA-FSCH) -> live cells based on viability dye staining (negative cells selected) ->CD4 + Cells -> GZMB expression.

For Blister biopsies: FSCA-SSCA (cell sized events) -> Singlets based on Area and Height (FSCA-FSCH) -> live cells based on viability dye staining (negative cells selected) -> CD45+ immune cells -> non granulocytes (Low SSC selected) -> non T cells (CD3 negative selected) -> non B cells (CD19 negative) selected -> CD14 + cells selected.

For chemotaxis: FSCA-SSCA (cell sized events) -> Singlets based on Area and Height (FSCA-FSCH) -> live cells based on viability dye staining (negative cells selected) -> subsets analyzed based on CD45/CD3 or CD19 or CD14/ CD3 positive cells subset to CD4 vs CD8.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.