



The cellular architecture of the antimicrobial response network in human leprosy granulomas

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Granulomas are complex cellular structures composed predominantly of macrophages and lymphocytes that function to contain and kill invading pathogens. Here, we investigated the single-cell phenotypes associated with antimicrobial responses in human leprosy granulomas by applying single-cell and spatial sequencing to leprosy biopsy specimens. We focused on reversal reactions (RRs), a dynamic process whereby some patients with disseminated lepromatous leprosy (L-lep) transition toward self-limiting tuberculoid leprosy (T-lep), mounting effective antimicrobial responses. We identified a set of genes encoding proteins involved in antimicrobial responses that are differentially expressed in RR versus L-lep lesions and regulated by interferon- γ and interleukin-1 β . By integrating the spatial coordinates of the key cell types and antimicrobial gene expression in RR and T-lep lesions, we constructed a map revealing the organized architecture of granulomas depicting compositional and functional layers by which macrophages, T cells, keratinocytes and fibroblasts can each contribute to the antimicrobial response.

Granulomas are the hallmark of the chronic inflammatory response to a foreign substance that has resisted destruction by an acute inflammatory response. Granulomas have been defined as structures ‘which are formed by the immune-mediated recruitment of white blood cells, and particularly rich in macrophages’¹. In the context of infectious diseases, the function of the granuloma is to sequester and degrade microbial pathogens that have evaded the early immune response.

Leprosy offers an attractive model to investigate the mechanisms by which the human immune system combats intracellular bacteria since the disease presents as a clinical/immunological spectrum². The histological characteristic of leprosy is the granuloma, which contains macrophages that have been infected by the pathogen, *Mycobacterium leprae*, and lymphocytes. Because leprosy manifests as a spectrum of disease in the skin, the dynamics are accessible to study, in contrast to tuberculosis granulomas. At one end of the disease spectrum, T-lep typifies the host’s antimicrobial response, which controls the pathogen: there are few lesions, *M. leprae* bacilli are rare and patients eliminate the infection. At the opposite end, L-lep represents susceptibility to disseminated infection, with numerous skin lesions and abundant bacilli. This disease spectrum is dynamic since patients may undergo an RR, when patients generally upgrade, either spontaneously or during chemotherapy, from the lepromatous to the tuberculoid pole.

The structure of granulomas is distinct across the spectrum of leprosy. The granulomas in T-lep contain a core of mature macrophages with occasional multinucleated giant cells. These granulomas are organized with a mantle zone at the periphery of the granuloma containing lymphocytes and characterized by fibrosis. Granulomas in RR lesions are histologically similar to those in T-lep with the presence of intercellular edema. In L-lep, granulomas are disorganized and immature lipid-laden foamy macrophages are prominent with lymphocytes scattered throughout.

The study of leprosy lesions has provided insights regarding the host immune response to intracellular bacteria and the architecture of granulomas. Through various approaches, it has been possible to define functional subpopulations of human T cells^{3–6} and macrophages⁷, and their microanatomic distribution, as well as the patterns of cytokine secretion that influence the outcome of infections caused by pathogenic mycobacteria^{8–11}. The antimicrobial mechanisms that result in the death of the pathogen are central to understanding how granulomas contribute to host defense. Through activation via Toll-like receptors and secretion of interferon- γ (IFN- γ), the innate and adaptive immune systems trigger the vitamin D-dependent induction of the antimicrobial proteins encoded by *CAMP* and *DEFB4A*^{7,12,13}. T cells release antimicrobial proteins encoded by *GNLY* and *IL26*, which can enter infected macrophages and exert a direct antimicrobial activity^{4,5,14,15}. These human

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pathways are not present in mice, which utilize other mechanisms, such as the release of nitric oxide to kill mycobacteria. In this study, we integrated single-cell RNA sequencing (scRNA-seq) with spatial sequencing to determine the structure of the organized granuloma in leprosy including the potential contribution of specific cell types to the antimicrobial response.

Results

scRNA-seq identifies 12 cell types in leprosy lesions. To study the cellular composition and cell-state differences between RR and L-lep, we performed scRNA-seq by Seq-Well¹⁶ on skin biopsy specimens from five patients with RR and five patients with L-lep (Supplementary Table 1). We obtained 21,318 cells from 10 biopsy specimens, detecting an average of 741 genes and 3,556 transcripts per cell (Supplementary Table 1). To study the heterogeneity of these cells, we selected variable genes and performed uniform manifold approximation (UMAP) dimensionality reduction and cell clustering using the R package Seurat¹⁷. We then performed differential expression analysis to find the cluster markers and overlapped the cluster markers to canonical cell type-defining signature genes. Ultimately, we recovered 12 primary cell types across all 10 samples (Fig. 1a,b and Supplementary Table 1). These annotated cell types include: T cells, B cells, plasma cells, myeloid cells, Langerhans cells, mast cells, keratinocytes, fibroblasts, smooth muscle cells, endothelial cells, eccrine gland cells and melanocytes (Fig. 1c and Supplementary Table 2).

The major cell types, including T cells, myeloid cells, keratinocytes, endothelial cells and fibroblasts, were found in both RR and L-lep lesions (Fig. 1d). Although B cells were found in both RR and L-lep lesions, plasma cells were derived predominantly from L-lep lesions ($P < 0.05$). Given that Langerhans cells are more frequent in RR than L-lep lesions³, we isolated CD1a⁺ cells from the epidermis of three patients with RR (RR1, RR2 and RR4) by immunoselection, adding these to the epidermal and dermal cells, accounting for the high frequency of Langerhans cells from these RR lesions. *M. leprae* reads were most prevalent in the multibacillary L-lep lesions but were also detected at a lower level in one RR lesion (Fig. 1e).

Distinct cell subtypes differentiate the immune response. We defined seven T cell subclusters, two predominantly derived from RR lesions and one from L-lep lesions, which we annotated using previously defined cluster-specific genes (Fig. 2a and Extended Data Fig. 1a,b)¹⁸. T cell subcluster 0 (TC0), composed of >90% cells from RR samples, expressed the classic type 17 helper T (T_H17) cell markers *RORC*, *RORA*, *RBPJ* and *IL23R* as signature genes (Fig. 2b,c) but not the major T_H17 cytokine genes. TC1 and TC2 are designated as cytolytic T lymphocytes (CTLs) since they both contain *CD8A*, *GZMB* and *PRF1*. Because TC1 was derived mainly from RR lesions and TC2 was mainly derived from L-lep lesions, they were labeled as RR CTL and L-lep CTL, respectively (Fig. 2d). We noted several type I IFN downstream genes in L-lep CTLs including *IFI44L*, *MX1*, *IRF1* and *OAS3*. Genes upregulated in L-lep CTLs were significantly enriched in type I IFN downstream signatures¹¹ (Extended Data Fig. 1c and Supplementary Table 3). The remaining subclusters contained a mixture of cells from RR and L-lep including TC3 (central memory T (T_{CM}) cells), TC4 (naïve), TC5 (regulatory T cells) and TC6 (antimicrobial CTL (amCTL) cells) expressing *GZMB*, *PRF1* and *GNLY* containing a mixture of tri-cytotoxic T cells (Tri-CTLs)¹⁹ and $\gamma\delta$ T cells (Fig. 2c).

We previously described a functional CTL subset, amCTL cells, expressing *GZMB*, *PRF1* and *GNLY*, which exert antimicrobial activity against intracellular *M. leprae* and correlate with protective immunity to tuberculosis and leprosy^{19,20}. In TC6, the expression of *GZMB*, *PRF1* and *GNLY* was greater in cells from RR versus L-lep lesions. The module score aggregating the expression of these three

antimicrobial CTL genes was significantly greater in RR lesions (Fig. 2e). *IFNG* was most strongly expressed by T_H17 cells and RR CTLs but was also present in amCTLs and L-lep CTLs (Fig. 2c). The frequency of *IFNG*-expressing cells was similar to the frequency of *GZMB*- and *PRF1*-expressing cells in RR CTLs and amCTLs but was far greater than the frequency of *GNLY*-expressing cells in RR lesions (Fig. 2f and Extended Data Fig. 1d). These data indicate that *IFNG* is a marker for all CTLs but is not a useful marker for estimating amCTLs, the small subset of CTLs that express *GNLY* and shown to kill bacteria within infected cells.

We identified five myeloid subclusters, three predominantly derived from RR lesions and two from L-lep lesions (Fig. 3 and Extended Data Fig. 2a,b). ML0 (dendritic cells), containing cells from approximately 75% of RR lesions, consists of a mixture of dendritic cells with distinct subpopulations expressing *CD1C* and *LAMP3* (Fig. 3a–d and Extended Data Fig. 2c). ML1 (type I IFN macrophages), containing >90% cells from L-lep lesions, are macrophages that express type I IFN downstream genes including *IFI44L*, *MX2* and *IFIT3*, hence categorized as type I IFN macrophages (Fig. 3c). ML2 (TREM2 macrophages), containing >90% of cells from L-lep lesions, was characterized by high expression of *TREM2* and *APOE*, resembling the TREM2 macrophages reported in seven diseases characterized by altered lipid metabolism but not previously in human infectious disease^{21–27}. ML4, which contain approximately 85% of cells from RR lesions, are M1-like macrophages. ML3 was derived from three RR lesions and two L-lep lesions, connecting TREM2 macrophages and M1-like macrophages (Fig. 3b,d), expressing genes from both subclusters (Extended Data Fig. 2d). *TREM2* and *APOE* expression, and a TREM2 score consisting of nine conserved genes from the datasets from the seven studies of lipid metabolism diseases, were highest in TREM2 macrophages from L-lep lesions while declining in transitional macrophages and M1-like macrophages in RR lesions (Fig. 3e and Extended Data Fig. 2e). The leprosy myeloid subtypes, dendritic cells, type I IFN macrophages, TREM2 macrophages and transitional macrophages all had corresponding cell subpopulations in the scRNA-seq analysis of lung specimens from a nonhuman primate model of tuberculosis²⁸. However, we could not detect an analogous M1-like macrophage population as the one found in RR lesions in the nonhuman primate tuberculosis lung data (Extended Data Fig. 2f).

Of seven keratinocyte subclusters, two were enriched in patients with RR, KC3 (*FLG*⁺ granular layer keratinocytes) and KC4 (*KRT14/15*⁺ basal layer keratinocytes) and two were enriched in patients with L-lep (KC1 and KC5), both derived from spinous layer keratinocytes (Extended Data Fig. 3a–d). The RR-enriched KC3 and KC4 clusters had increased expression of genes that contribute to antimicrobial responses (Extended Data Fig. 3c). By contrast, no increase was observed for either inflammatory or antimicrobial gene expression in the two L-lep keratinocyte clusters (KC1 and KC5). KC0 (spinous 1 keratinocytes), KC2 (supraspinous keratinocytes) and KC6 (hair follicle keratinocytes) were derived from both RR and L-lep samples.

SFRP2⁺ fibroblasts (FB0) and *CXCL2*⁺ fibroblasts (FB2) were enriched in RR lesions (Extended Data Fig. 3e–g). The *SFRP2*⁺ fibroblast subcluster was similar to a counterpart in normal skin, sharing expression of *COL3A1*, *COL18A1* and *COMP*²⁹. *SFRP2*⁺ fibroblasts from skin have been shown to have the capacity to release extracellular matrix (ECM) proteins²⁹. *SFRP2*⁺ fibroblasts more strongly express genes that encode proteins involved in the deposition and modeling of the ECM including *VIM* (vimentin), *SPARC* (osteonectin), *FBN1* (fibrillin 1), *TNC* (tenascin C), *FBLN2* (fibulin 2) and *LOXL1* (lysyl oxidase homolog 1), as well as various collagens. By contrast, *CXCL2*⁺ fibroblasts express a number of inflammatory genes as specific marker genes including *IL6*, *CCL2*, *CXCL3*, *CXCL8* and *IL32*, which display a similar expression profile to the inflammatory fibroblasts detected in atopic dermatitis

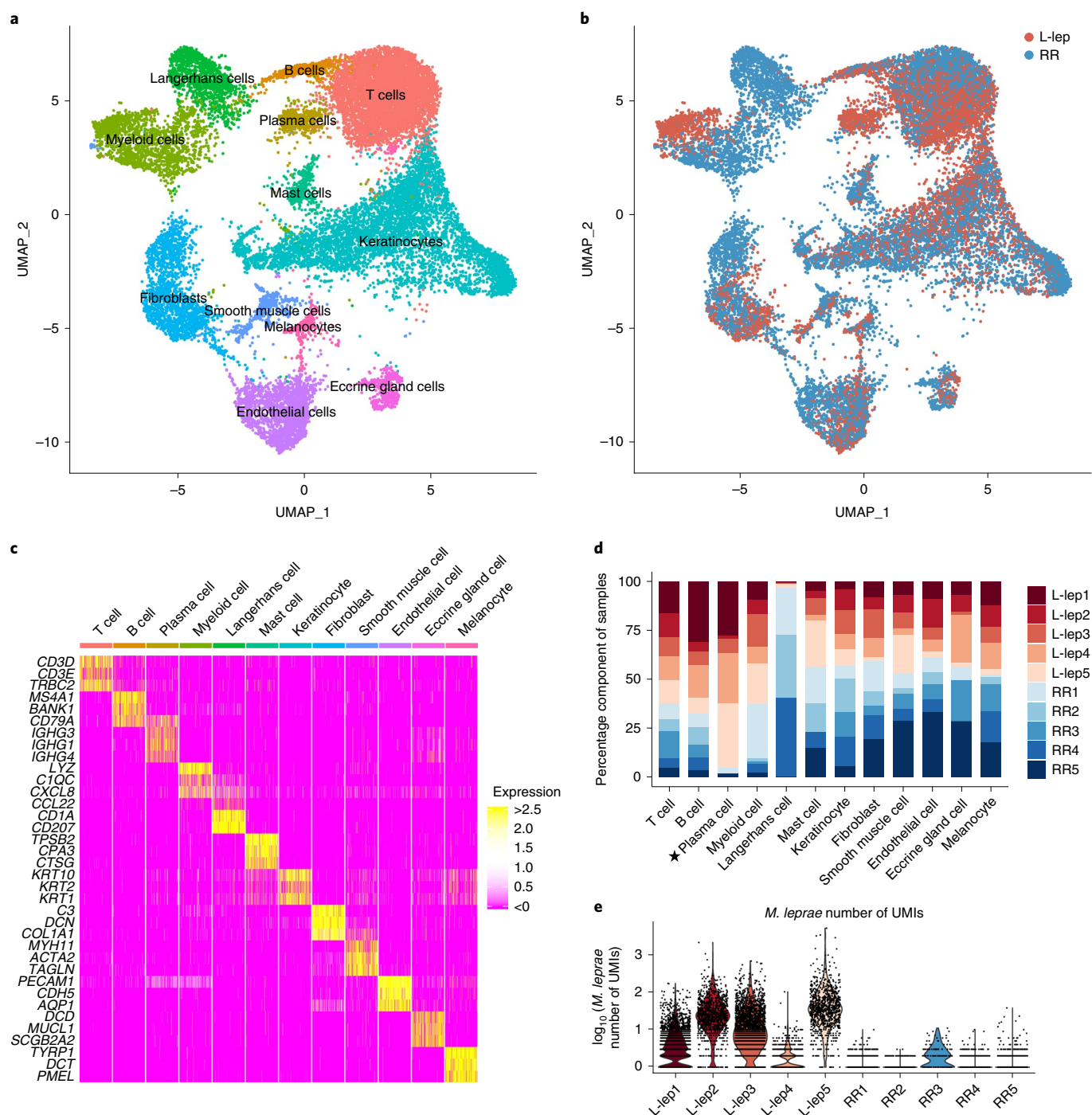


Fig. 1 | Cell types observed in leprosy lesions. a, UMAP plot for 21,318 cells colored by cell types. **b**, UMAP plot colored by clinical forms. **c**, Heatmap showing three representative marker genes for each cell type. **d**, Abundance composition across all samples for each cell type. *t*-tests were conducted between the L-lep and RR samples for each cell type (excluding Langerhans cells); only plasma cells had $P < 0.05$, indicated by a star. **e**, Violin plot showing the number of *M. leprae* transcripts detected in individual cells from each patient.

skin lesions³⁰. Two fibroblast subclusters were predominantly derived from L-lep lesions: *MGP*⁺ (FB1) and *COL11A1*⁺ fibroblasts (FB3)²⁹.

Two endothelial cell subclusters, *LYVE1*⁺ lymphatic endothelial cells (EC4) and *HEY2*⁺ endothelial cells (EC5) were mainly derived from RR lesions. *MEOX2*⁺ endothelial cells (EC3) were primarily found in L-lep lesions (Extended Data Fig. 3h–j); *MEOX2* is an inhibitor of nuclear factor kappa-light-chain-enhancer of activated B cells activation in endothelial cells³¹.

Antimicrobial genes are highly expressed in RR cell types. Given that leprosy RRs are associated with a reduction in viable *M. leprae* bacilli in lesions, we sought to determine the array of antimicrobial genes that were present in defined cell populations. To do so, we first integrated a list of 1,404 ‘antimicrobial genes’ curated by GeneCards known to encode proteins that contribute to antimicrobial responses with the scRNA-seq data. We then separated each cell type (including subtype) into RR versus L-lep cells and calculated the gene expression z-scores for each cell type (Methods). We found that RR cell types have a higher

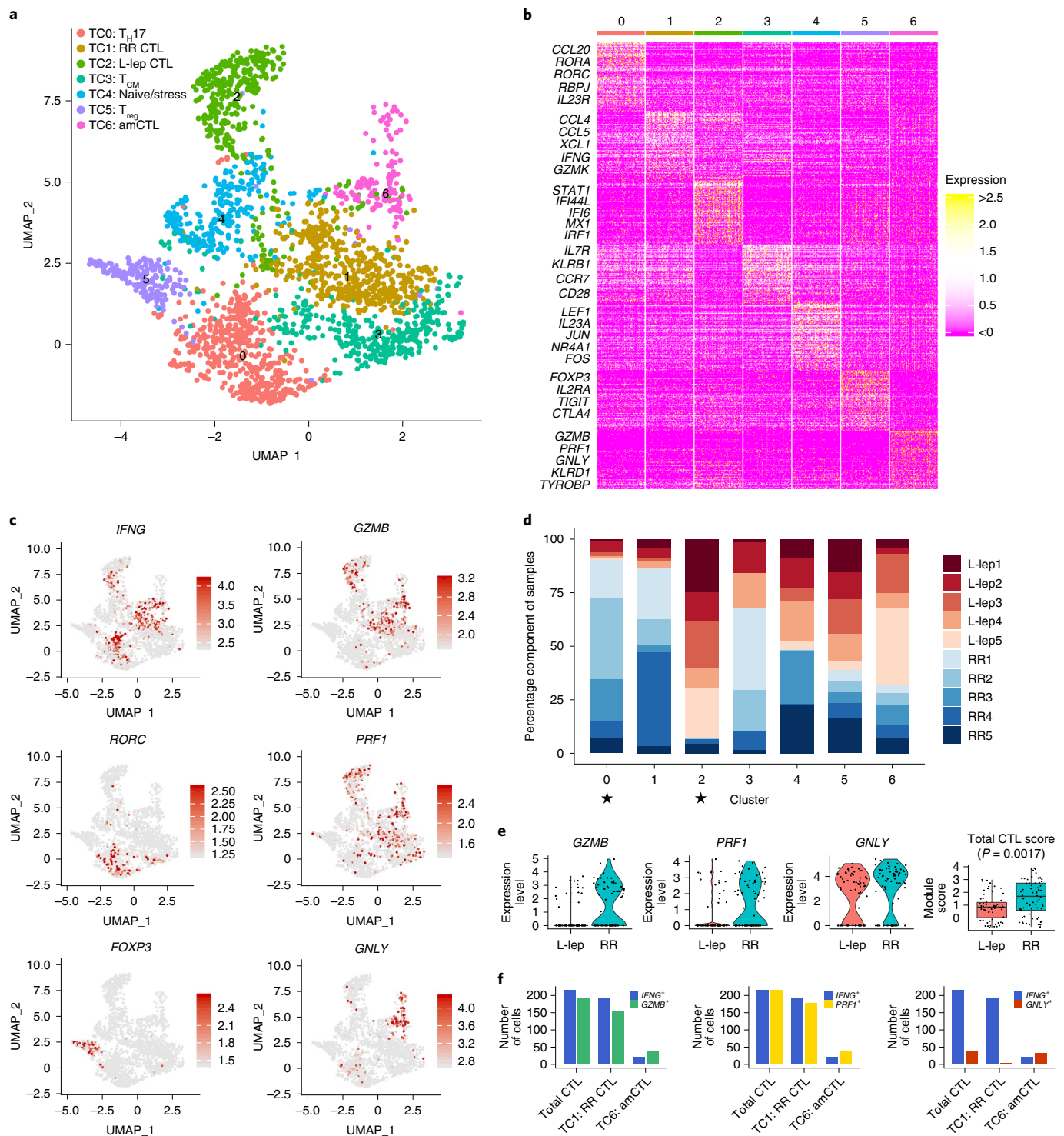


Fig. 2 | Identification of T cell subtypes. **a**, UMAP plot for 2,290 T cells colored by subtypes. **b**, Heatmap showing marker genes for each subtype. Representative genes are labeled. **c**, UMAP plots showing six marker genes. The color scale represents the normalized expression level of the gene. **d**, Abundance composition across all samples for each T cell subtype. Two-sided t-tests were conducted between the L-lep and RR samples (5 versus 5) for each subtype; TC0 and TC2, $P < 0.05$, indicated by stars. **e**, Left: violin plots showing the expression for *GZMB*, *PRF1* and *GNLY* in T cell subcluster 6 grouped by L-lep and RR. Right: boxplot showing the T-CTL score in T cell subcluster 6 grouped by L-lep and RR; the P value (0.0017) was calculated from a two-sided Wilcoxon rank-sum test (64 L-lep cells versus 70 RR cells). The bounds of the box represent the first and third quartile, and the middle bar represents the median. **f**, Number of RR cells expressing *IFNG*, *GMZB*, *PRF1* and *GNLY* in RR CTL and amCTL cells.

expression pattern for antimicrobial genes (Fig. 4a). We obtained 1,124 antimicrobial genes with a z-score ≥ 3 in at least 1 RR cell type, indicating that these cells, in aggregate, produce relatively more of the specific

transcript than other cell types. We identified the upstream regulators of the 1,124 antimicrobial genes using ingenuity pathways analysis (IPA) (Methods), with *IFNG*, *TNF* and *IL1B* having the highest

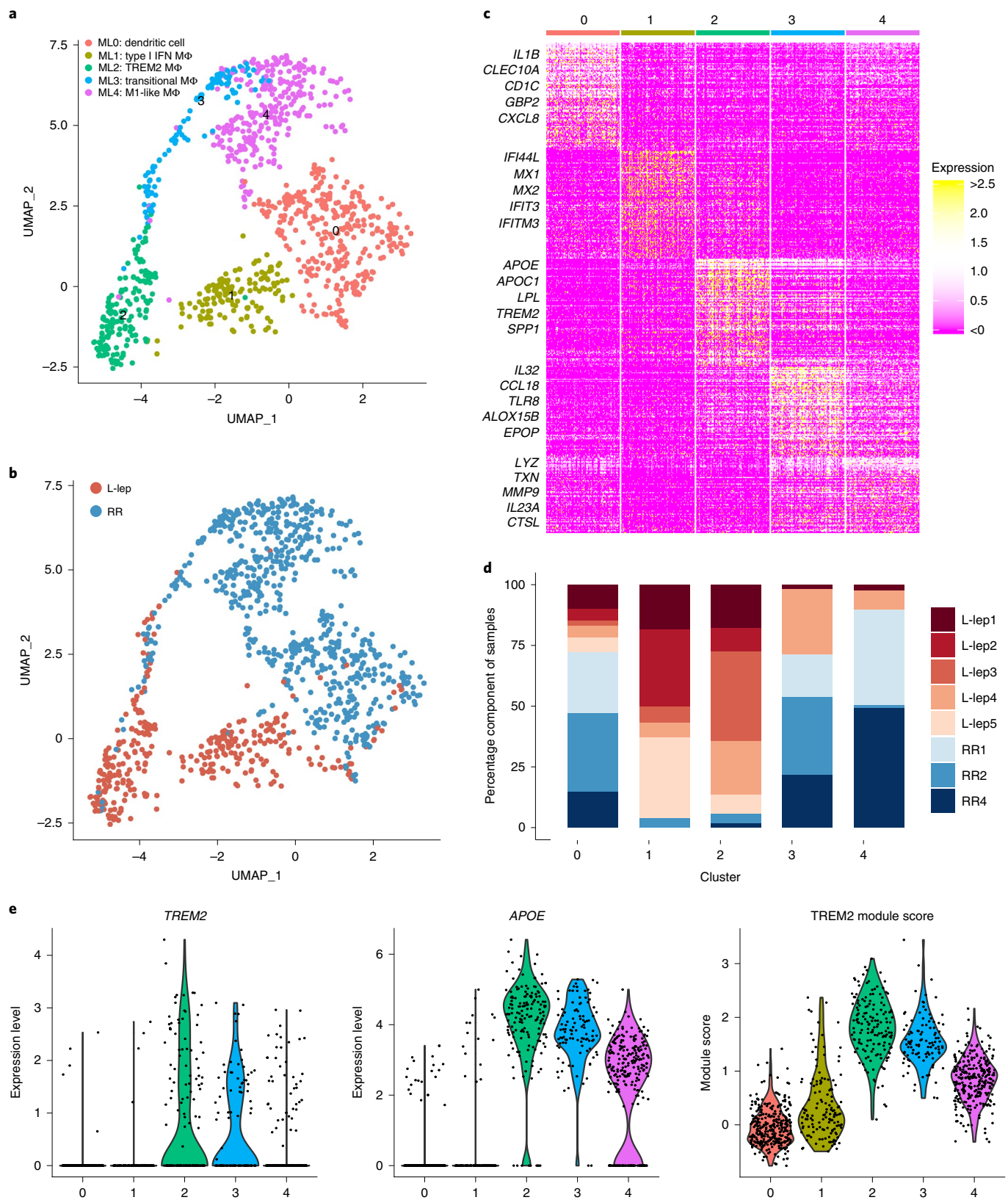


Fig. 3 | Identification of myeloid cell subtypes. a, UMAP plot for 991 myeloid cells colored by subtype. **b**, UMAP plot colored by clinical forms. **c**, Heatmap showing the marker genes for each subtype. The representative genes are labeled. **d**, Abundance composition across all samples for each myeloid cell subtype. **e**, Left: violin plots showing the expression of *TREM2* and *APOE* in myeloid subtypes. Right: violin plot showing the *TREM2* module score in myeloid subtypes.

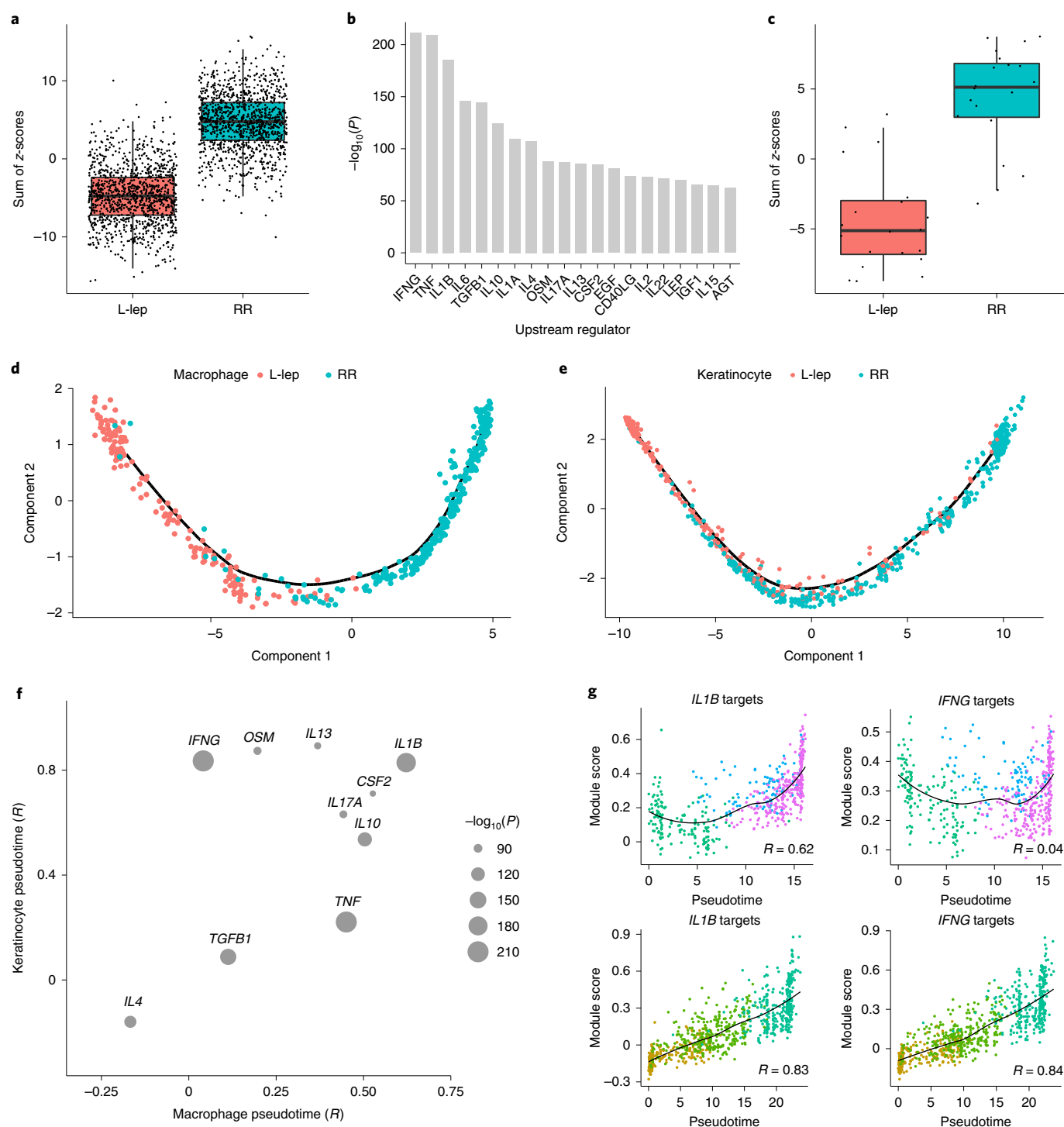


Fig. 4 | Antimicrobial gene analysis and pseudotime construction. **a**, Boxplot showing the sum of 1,124 antimicrobial gene z-scores in L-lep and RR cell types. The P value ($<2.2 \times 10^{-16}$) was calculated using a two-sided t -test ($n=1,124$). The bounds of the box represent the first and third quartile, and the middle bar represents the median. **b**, Bar graph showing the top 20 upstream regulators ranked by P value from the enrichment analysis using the 1,124 antimicrobial genes. **c**, Boxplot showing the sum of the z-scores for the top 20 upstream regulators in L-lep and RR cell types. The P value (3.5×10^{-9}) was calculated using a two-sided t -test ($n=20$). The bounds of the box represent the first and third quartile, and the middle bar represents the median. **d**, Pseudotime trajectory colored by clinical form in myeloid subclusters 2, 3 and 4. **e**, Pseudotime trajectory colored by clinical form in keratinocyte subclusters 1, 2 and 3. **f**, Dot plot showing the correlation between the module scores of the top 10 upstream regulators and macrophage/keratinocyte pseudotimes. The size of the dots represents the $-\log_{10}(P)$ from the enrichment analysis. **g**, Scatter plot showing the correlation between macrophage (top) or keratinocyte (bottom) pseudotimes and module scores calculated using *IL1B* or *IFNG* target genes from the six expression patterns. The color of the dots represents the subcluster identity of the cells.

enrichment scores (Fig. 4b). The sum of the z-scores for the top 20 upstream regulators of the antimicrobial genes was greater in the RR compared to the L-lep cell types (Fig. 4c).

Pseudotime analysis in macrophages and keratinocytes. The continuum from ML2 to ML4 observed on the UMAP suggests a continuous progression between cellular states. To better

characterize the progressive differences, we used Monocle, a pseudotime analysis program³², to create a continuous trajectory where cells were ordered from TREM2 macrophages (ML2) to transitional macrophages (ML3), terminating with M1-like macrophages (ML4) (Extended Data Fig. 4a,b). This trajectory starts from L-lep cells and ends with RR-derived cells and mirrors the clinical progression in patients (Fig. 4d). Using a similar analysis on spinous 2 keratinocytes (KC1), supraspinous keratinocytes (KC2) and granular keratinocytes (KC3), we identified a pseudotime continuum of the cells from these clusters (Extended Data Fig. 4c,d) that also progresses from L-lep- to RR-derived cells (Fig. 4e).

To identify the upstream regulators that trigger the antimicrobial response, we analyzed the variable genes from the six progressive gene programs that defined the pseudotime trajectories (Extended Data Fig. 4b,d and Supplementary Table 4). We calculated a module score for each upstream regulator using the targets found in all six patterns and calculated the correlation coefficient between the module score and the pseudotime (Methods). Of the top upstream regulators for the antimicrobial genes, only the target scores for *IL1B* were highly correlated with both macrophage and keratinocyte pseudotime ($R=0.63$ and 0.83 , respectively) (Fig. 4f,g). The target scores for *IFNG* correlated with the keratinocyte ($R=0.84$) but not the macrophage pseudotime ($R=0.04$). To this end, we selected for further study the *IFNG* and *IL1B* target genes since *IFNG* had the highest enrichment score for the antimicrobial genes and *IL1B* was not only a top upstream regulator of the antimicrobial genes but the expression of the *IL1B* target genes was highly correlated with both macrophage and keratinocyte pseudotimes.

Cell networks reveal an antimicrobial ecosystem in RR. To construct a gene network depicting the antimicrobial response in RR, we first determined the source of the two key upstream regulators. *IFNG* was detected ($z\text{-score} \geq 3$) in RR cells from T_H17 cells and RR CTLs and *IL1B* was detected in RR cells from Langerhans and dendritic cells (Fig. 5a).

We constructed Circos plots to depict the interactions of the *IL1B*- and *IFNG*-expressing cells with the target antimicrobial gene-expressing cells (Fig. 5b). For clarity, we limited the number of interactions to the 61 antimicrobial genes that encode proteins with direct antimicrobial activity and with a $z\text{-score} \geq 3$ in at least 1 of the major RR cell types (Extended Data Fig. 5a). In view of the variable expression of the genes encoding the receptors for IL-1 β and IFN- γ , we inferred connections between the upstream regulators and these antimicrobial genes as identified using IPA. *IL1B* and *IFNG* both regulated 22 antimicrobial gene targets and individually regulated 14 additional antimicrobial genes, for a total of 36 unique genes. For both *IL1B* and *IFNG*, most of the connections were to cell types that were predominantly derived from RR lesions; strikingly, there were no connections to a cell type that was predominantly derived from L-lep lesions. Both *CXCL2*⁺ and *SFRP2*⁺ fibroblasts expressed several chemokine genes that encode proteins with antimicrobial activity.

In an effort to validate the relevance of antimicrobial genes upregulated in RR lesions, we identified 59 genes that have been reported to contribute to host defense against leprosy and other mycobacteria (Supplementary Tables 5 and 6). Of the 59 genes, 34 encode proteins with direct antimicrobial activity³³. An additional seven genes reported as directly antimicrobial had not previously been reported to be involved in the immune response to mycobacteria. We plotted the $z\text{-scores}$ for these 66 antimicrobial genes in a heatmap arranged according to the specific cell type where the antimicrobial gene was most strongly expressed (Extended Data Fig. 5b). Strikingly, we found that each major cell type highly expressed a distinct set of antimicrobial genes. For example, *CYBB* was predominantly expressed in M1-like macrophages (ML4) and Langerhans cells, *CCL5* was predominantly expressed by RR CTLs

(TC1), *MMP2* was predominantly expressed by *SFRP2*⁺ (FB0) and *CXCL2*⁺ fibroblasts (FB2) and *KLK5* was predominantly expressed by granular keratinocytes (KC3).

Spatial sequencing identifies the coordinates of cell types. To localize the various cell types and antimicrobial responses within the granuloma architecture, we performed spatial sequencing on leprosy skin biopsy specimens (Fig. 6a). We detected 708 spatially defined spots with an average of 2,984 genes and 10,007 transcripts per 55- μm spot. We annotated the cell type composition for each spot by decomposing the spatial gene expression using the scRNA-seq gene expression of the major cell types, displayed as a scatter plot that contains a pie chart for each spot scattered across the spatial array (Extended Data Fig. 6a). Thus, we identified the distribution of the five major cell type clusters revealing distinct small aggregates of myeloid cells, T cells and fibroblasts in the dermis (Fig. 6b and Supplementary Table 7) and calculated the cell type composition scores for each cluster (Extended Data Fig. 6b). We also performed spatial sequencing on a skin biopsy specimen from a patient with T-lep (Extended Data Fig. 6c–f) since RRs represent a transition from the lepromatous to the tuberculoid pole and two additional RR samples (Extended Data Fig. 7a,b). The cell type composition in the T-lep sample was similar to the RR samples.

We also sought to localize the RR cell subtypes in the morphological context of the granuloma within the leprosy skin lesion. Strikingly, *SFRP2*⁺ fibroblasts (FB0) were located in the superficial ‘papillary’ dermis near the dermal–epidermal junction, whereas *CXCL2*⁺ fibroblasts (FB2) were located deeper in the ‘reticular’ dermis around the granulomas (Extended Data Fig. 7c). The gene signatures of the leprosy fibroblasts were distinct from the signatures that defined the spatial location of fibroblast subtypes in healthy skin³⁴. We quantified the distinct locations of the fibroblast subtypes by measuring the distance between *KRT1*⁺ and *KRT10*⁺ keratinocytes and each fibroblast subtype in the RR and T-lep spatial sequencing samples. In three of the four samples, *SFRP2*⁺ fibroblasts (FB0) were half the distance to the epidermis than *CXCL2*⁺ fibroblasts (FB2) (Extended Data Fig. 8a–d). Over the four specimens, *SFRP2*⁺ fibroblasts (FB0) were approximately half the distance to the epidermis than *CXCL2*⁺ fibroblasts (FB2) ($P < 1.4 \times 10^{-14}$, Extended Data Fig. 8e). The spatial sequencing data validate the predictions of the epidermal layers from the scRNA-seq data for granular keratinocytes (KC3), spinous keratinocytes (KC0) and basal keratinocytes (KC4). Transitional macrophages (ML3) and M1-like macrophages (ML4) localized to the large myeloid aggregates with rare dendritic cells (ML0) occasionally at the periphery. In contrast, T_H17 cells (TC0), RR CTLs (TC1) and amCTLs (TC6) were present in the mantle zone.

Spatial mapping of four representative antimicrobial genes with defined distributions among the major cell type subclusters in the scRNA-seq data (Fig. 6c) confirmed that the spatial localization of each gene mapped to the location of the corresponding cell type in the RR spatial sequencing data, including *CYBB* in the two aggregates of spots that were identified as myeloid cells, *CCL5* in T cell spots, *MMP2* in fibroblast spots and *KLK5* in regions corresponding to granular keratinocytes (KC3), particularly in the T-lep sample.

We validated the location of three antimicrobial genes expressed by keratinocytes by immunohistochemistry (Extended Data Fig. 9a–c). The DEFB1 protein was detected in the cytoplasm throughout the epidermis in all three RR specimens and in one of three L-lep specimens, which is consistent with the gene expression in both the supraspinous (KC2) and spinous (KC0) keratinocytes by scRNA-seq. The more prominent expression of the *CXCL14* protein in the basal layer in two of three RR lesions corroborated the *CXCL14* messenger RNA detection in the basal (KC4) keratinocytes. The *CXCL14* protein was absent in the L-lep epidermis. TAC1 was expressed in granular (KC3) keratinocytes, with TAC1

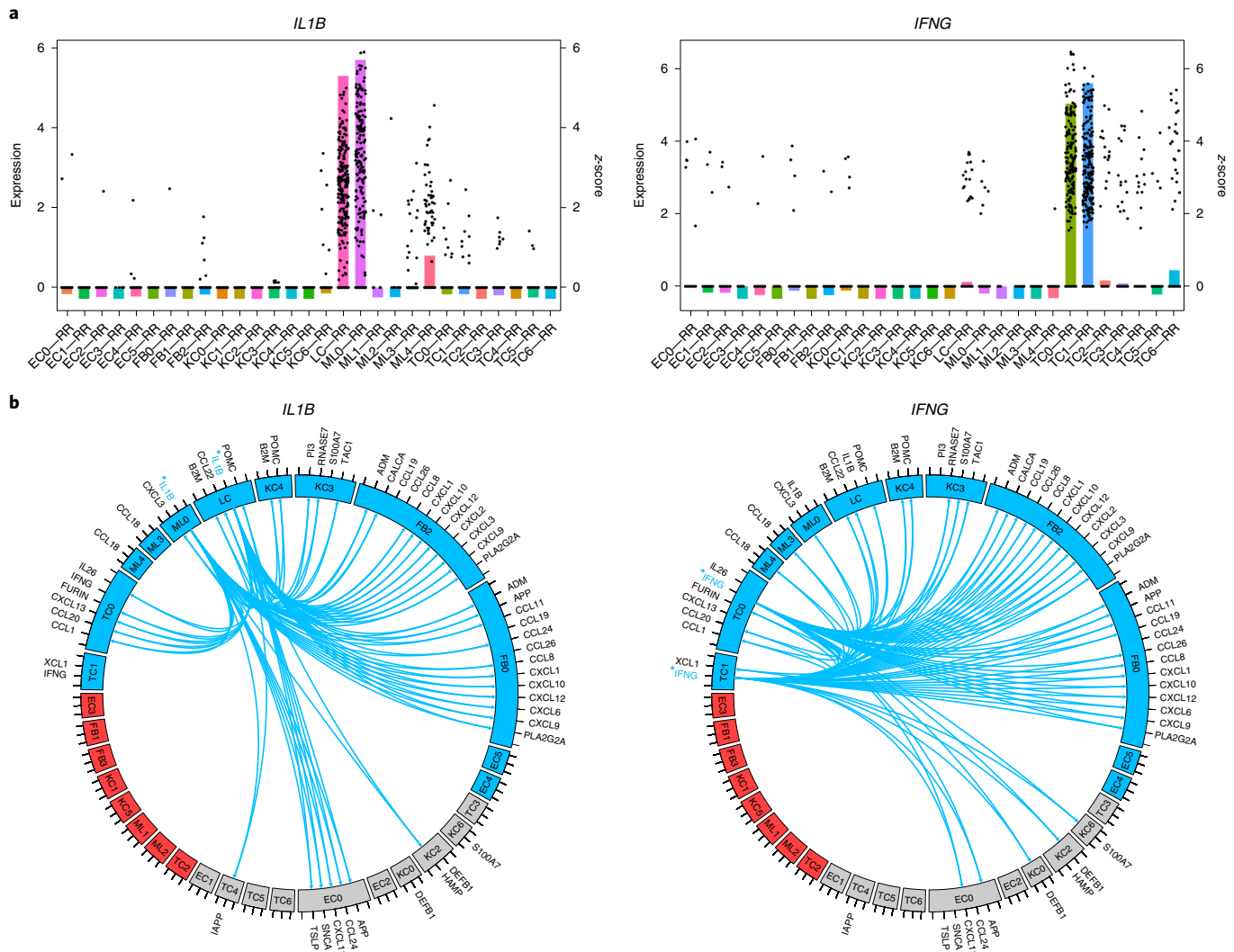


Fig. 5 | Antimicrobial network induced by *IL1B* and *IFNG*. **a**, Bar plot showing the z-scores of *IL1B* (left) or *IFNG* (right) expression levels in each cell type from RR lesions. The dots represent the *IL1B* or *IFNG* expression level in individual cells. **b**, Circos plots showing the connection between *IL1B* (left) or *IFNG* (right) and the direct antimicrobial gene targets in the cell types with a z-score > 3. The color represents the patient composition of the cell type: red, L-lep-specific; blue, RR-specific; gray, mix of L-lep and RR.

protein present, albeit subtly, in the granular layer of the RR but not the L-lep samples. We also found that keratinocyte secretion of the DEFB1, CXCL14 and TAC1 antimicrobial peptides exerted an antimicrobial activity, ranging from 50 to 100%, against *M. leprae* in infected macrophages (Extended Data Fig. 9d).

We also investigated the expression of the 66 genes encoding proteins contributing to the antimicrobial response identified in the scRNA-seq data (Extended Data Fig. 5b); 52 of these genes were detected by spatial sequencing of the RR and T-lep lesions. Of the 52 antimicrobial genes, 31 had high expression in the corresponding cell types in both the scRNA-seq and spatial sequencing data (Fig. 6d and Supplementary Table 6), with the remaining 21 genes having low or moderate expression in the corresponding spatial sequencing cell type. Thus, the spatial sequencing data validated the antimicrobial gene expression identified in the scRNA-seq data.

The spatial sequencing analysis of two L-lep skin biopsy specimens, representing the multibacillary form of the disease, revealed that the inflammatory infiltrate was composed of large aggregates of myeloid cells juxtaposed with smaller aggregates of plasma cells, along with several other cell types (Extended Data Fig. 10a,b). We validated the distribution of the TREM2 macrophages in leprosy

by interrogating the spatial sequencing data with the nine gene TREM2 macrophage consensus scores in all the spatial sequencing specimens (Extended Data Fig. 10c). The TREM2 module score was robustly expressed in both L-lep lesions compared to the T-lep/RR specimens (L-lep versus T-lep/RR, $P < 2.2 \times 10^{-16}$), although the range of the TREM2 macrophage scores in the T-lep lesions overlapped with the RR and L-lep lesions (Extended Data Fig. 10d).

We reasoned that adjacency in spatial location would facilitate cell-cell interactions. By quantifying the neighboring spots, as shown in the scatter plot (Fig. 7a), we found that in T-lep/RR samples, myeloid cells were most adjacent to T cells, keratinocytes were adjacent to fibroblasts and fibroblasts were equidistant from all cell types (Extended Data Fig. 10e). The results from the two L-lep samples indicated that the immune, myeloid, plasma and T cells were close to each other, with fibroblasts being close to the myeloid cells (Extended Data Fig. 10f).

Spatial sequencing maps the architecture of organized granulomas. The T-lep lesion contained a classic organized granuloma in the dermis of the biopsy specimen, which is composed of a core of macrophages surrounded by a mantle of aggregated lymphocytes

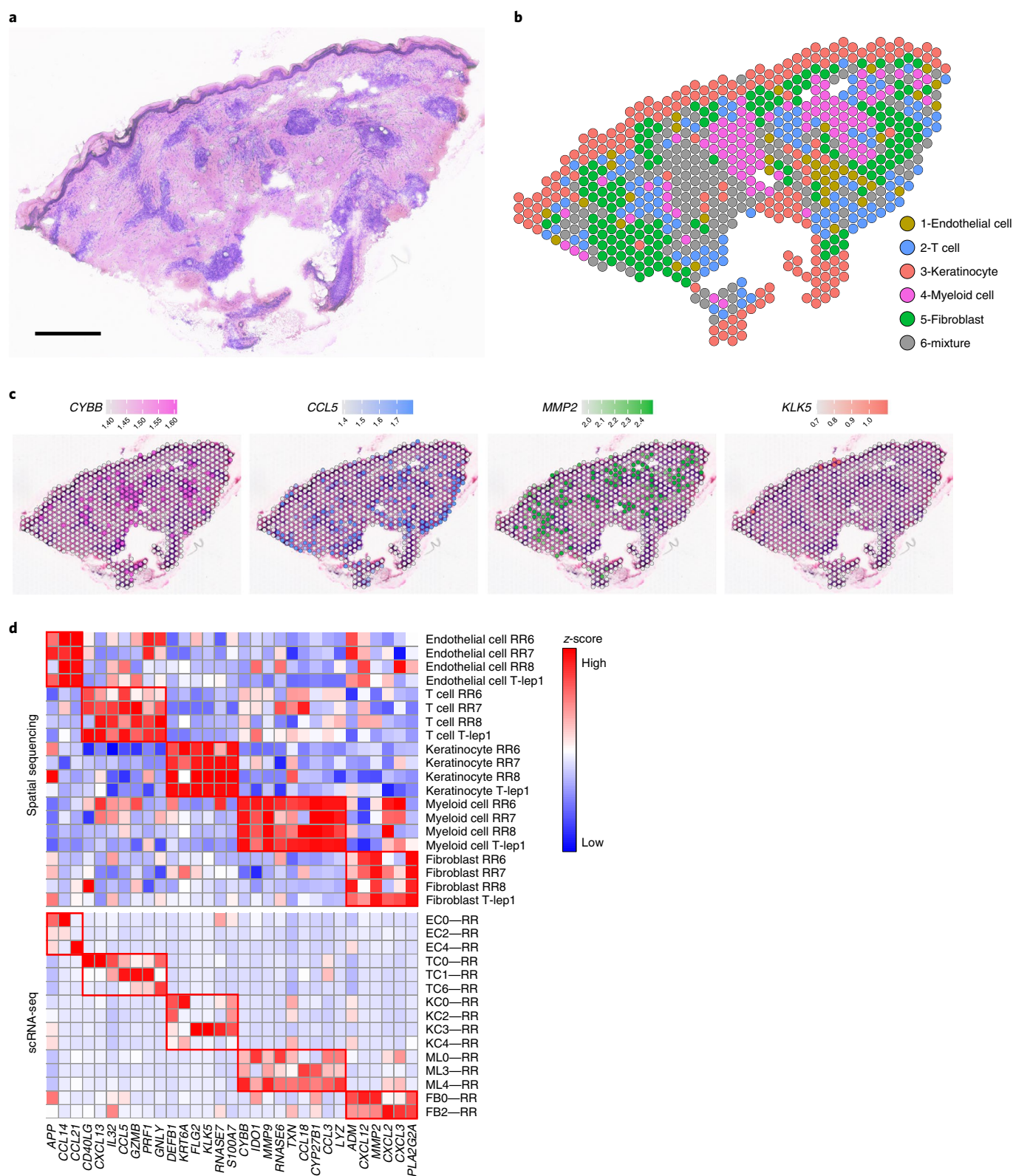


Fig. 6 | Spatial sequencing recapitulates the same set of antimicrobial genes in the corresponding cell types. a, H&E staining of the RR6 biopsy used for spatial sequencing. Scale bar, 0.5 mm. **b**, Spatial plot for 708 spots colored by clusters; the coordinates of the spot correspond to the location in the tissue. **c**, Spatial plots showing the expression level of four antimicrobial genes. The colors correspond to the cluster colors in **b**. *CYBB* was highly expressed in myeloid cells, *CCL5* in T cells, *MMP2* in fibroblasts and *KLK5* in keratinocytes. **d**, Heatmaps showing 31 antimicrobial genes expressed in the same cell types in both spatial sequencing and scRNA-seq. All the RR and T-lep spatial sequencing samples were plotted. The red boxes indicate distinct sets of antimicrobial genes highly expressed in a certain cell type.

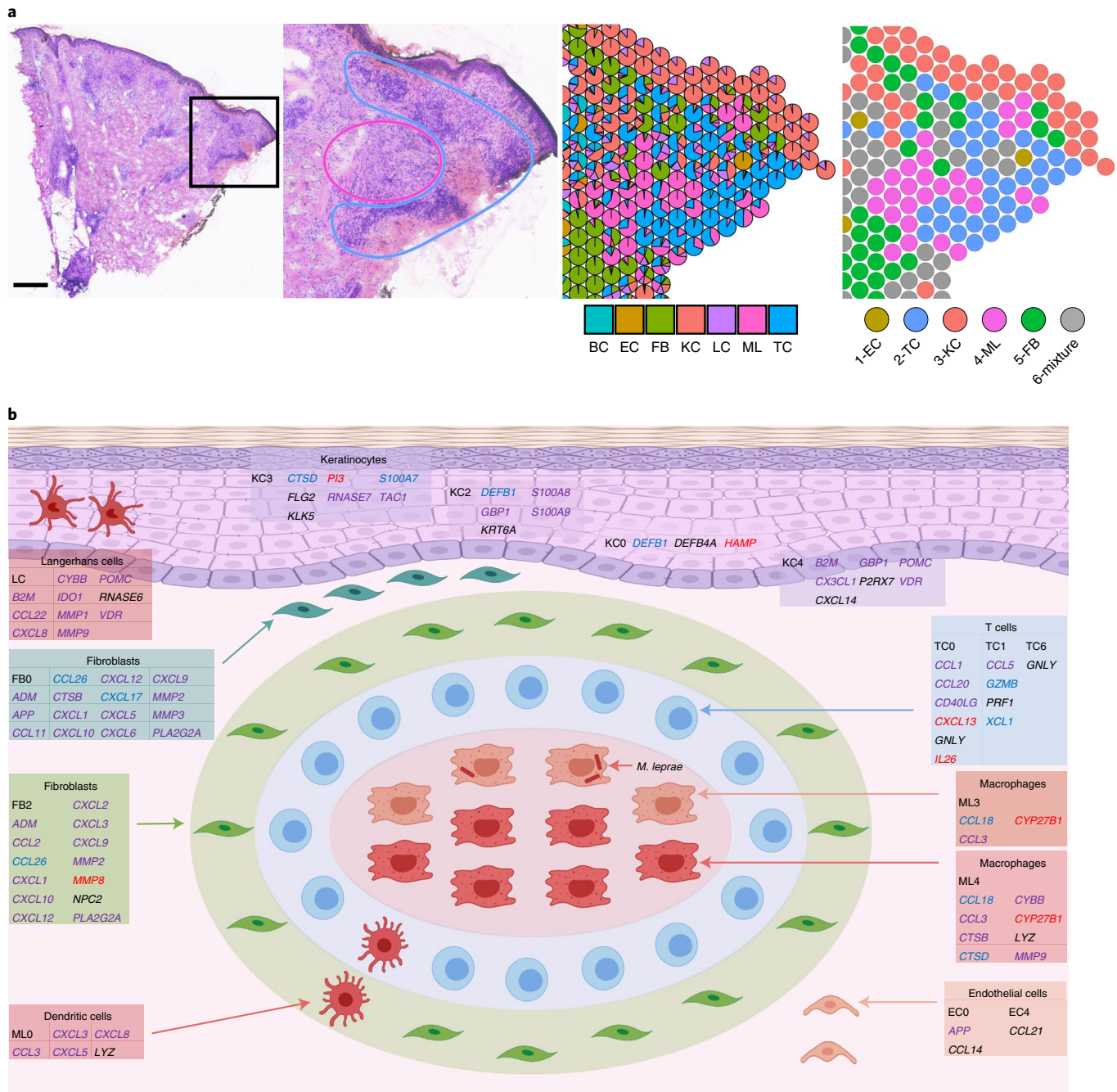


Fig. 7 | Granuloma architecture and antimicrobial ecosystem obtained by scRNA-seq and spatial sequencing. a, H&E staining of the T-lep biopsy and cell type composition highlighting myeloid cells in the center of the granuloma and T cells and fibroblasts at the periphery. Scale bar, 0.5 mm. BC, B cell; EC, endothelial cell; FB, fibroblast; KC, keratinocyte; LC, Langerhans cell; ML, myeloid cell; TC, T cell. **b**, Granuloma architecture and the antimicrobial ecosystem. The gene names in red represent targets of *IL1B*. The gene names in blue represent targets of *IFNG*. The gene names in purple represent targets of both *IL1B* and *IFNG*. The gene names in black were curated from known antimicrobial pathways. Created with Biorender.com.

(Fig. 7a). The spatial sequencing data validated the histology for this region, showing myeloid aggregates surrounded by T cells as well as regions of fibroblasts in the superficial dermis and around the myeloid aggregates. Close examination of the scatter plot representation of this region identified some heterogeneity in the composition of the spots.

Thus, by integrating scRNA-seq and spatial sequencing data, we created a model of the organized granuloma structure defined by cell subtypes and antimicrobial pathways (Fig. 7b). The model of the organized granuloma consists of a core of macrophages, both

transitional (ML3) and M1-like macrophages (ML4). The adaptive T cell response was localized predominantly in the mantle zone, contributing to the antimicrobial response against intracellular bacteria. Also surrounding the granuloma are dendritic cells (ML0) and *CXCL2*⁺ fibroblasts (FB2), which expressed genes that encode proteins with direct antimicrobial activity. Throughout the specimen were *SFRP2*⁺ fibroblasts (FB0) in the superficial dermis, various keratinocyte subtypes and endothelial cells, all expressing genes encoding antimicrobial proteins as well as chemokines known to have a direct antimicrobial response. The antimicrobial response

in the granuloma and surrounding microenvironment was largely regulated by *IL1B* and *IFNG*. Together, this suggests that the granuloma is a structured cellular ecosystem consisting of defined strata of distinct cell types that have the capacity to act to contain infection by an intracellular bacterium through the engagement of cells, both within and outside the granuloma, resulting in an integrated antimicrobial network.

Discussion

The organized granulomatous response allows the immune system to wall off and control intracellular bacteria that have initially evaded destruction. In leprosy and tuberculosis there is a spectrum, from spatially organized granulomas that can sterilize or contain the pathogen, as in T-lep and healed tuberculosis lesions, to disorganized granulomas where the pathogen flourishes and produces tissue damage, as in L-lep and miliary or progressive tuberculosis. Using scRNA-seq in organized granulomas, we identified 66 key antimicrobial genes expressed by a variety of cell types—T cells, macrophages, keratinocytes and fibroblasts—that had been previously reported to contribute to host defense in leprosy and other mycobacterial infections and/or encode proteins with direct antimicrobial activity. By integrating scRNA-seq with spatial sequencing, it was possible to delineate the cellular and molecular structure of the organized granuloma in leprosy that contributes to protective immunity. At the other end of the spectrum, we found that TREM2 macrophages, which are characterized by lipid metabolism genes, were a prominent cell type in the progressive form of leprosy with disorganized granulomas.

At the core of the organized granuloma in RR and T-lep lesions is an aggregate of mature macrophages, which we found were a mixture of M1-like (ML4) and transitional macrophages (ML3). The pseudotime trajectory analysis of the leprosy scRNA-seq data suggest that TREM2 macrophages (ML2), predominantly found in L-lep lesions, differentiate to transitional macrophages (ML3), which are found in both RR and L-lep lesions, and further mature to M1-like macrophages (ML4), which are found predominantly in RR lesions. We identified *IL1B* and *IFNG* as key upstream regulators of this pseudotime trajectory as well activation of macrophages in granulomas to express genes known to contribute to antimicrobial responses.

The organized granuloma in patients with T-lep was noted by Ridley and Jopling² in their classification of the spectrum of leprosy to exhibit a mantle zone of lymphocytes around the macrophage core. By spatial sequencing analysis, we detected dendritic (ML0) and Langerhans cells³ in the mantle zone, along with various T cell subtypes, including RR CTLs and amCTLs, both expressing *IFNG*, also a key regulator of the antimicrobial gene response. Although amCTLs were present in both RR and L-lep, there was higher expression of *GZMB*, *PRF1* and *GNLY* in RR cells; these genes encode proteins that act synergistically to kill intracellular mycobacteria^{4–6,19,20}. These data suggest that measurement of IFN- γ production by CD8⁺ T cells, which is most often used to measure T cell function in vaccine trial recipients, will simply not serve as a useful measure of amCTLs, which would need to be directly estimated by measurement of granzyme or its specific surface marker correlates¹⁹. We also found T_H17 cells in RR lesions that expressed *IL26*, which encodes an antimicrobial protein shown to have direct activity against intracellular *M. leprae*^{15,35}. Therefore, the mantle zone of the granuloma contains T cells with the capacity to deliver antimicrobial effector molecules into infected cells and secrete cytokines that trigger a downstream antimicrobial response in macrophages.

Unexpectedly, our analysis of the major cell populations indicated that fibroblasts, keratinocytes and endothelial cells, cells not typically considered to contribute to the antimicrobial response, indeed expressed antimicrobial genes in RR granulomas. There were two distinct fibroblast subpopulations in RR: *CXCL2*⁺ fibroblasts, described as inflammatory³⁰, and *SFRP2*⁺ fibroblasts, which are

involved in the deposition of ECM proteins²⁹. By spatial sequencing, we identified *SFRP2*⁺ fibroblasts in the papillary dermis of RR and T-lep lesions, just beneath and proximal to the epidermis. In contrast, *CXCL2*⁺ fibroblasts were located at the periphery of the granuloma, at a greater distance from keratinocytes. We found that both fibroblast subpopulations expressed a number of chemokines that are also directly antimicrobial. We reason that the fibroblasts surrounding the granuloma perform a dual function in host defense: establishing a physical wall of ECM proteins, including vimentin, fibrillin and collagens; and releasing antimicrobial proteins.

Several features of the subtypes that were overrepresented in L-lep lesions may contribute to disease pathogenesis in these patients. TREM2 macrophages were abundant and formed large aggregates in L-lep lesions. These macrophages have been identified in several diseases characterized by altered lipid metabolism including atherosclerosis, Alzheimer's disease, nonalcoholic steatohepatitis and obesity^{21–27}. The gene expression pattern in TREM2 macrophages suggests that these cells are programmed to transport and process lipids and contain the foamy macrophages that characterize both atherosclerosis and L-lep. One myeloid subtype, type I IFN macrophage, and one T cell subtype, L-lep CTL, both specific to L-lep lesions, are both characterized by a type I IFN gene program. Previously, we showed that IFN- β and its downstream targets were preferentially expressed in L-lep lesions and could inhibit IFN- γ -induced antimicrobial activity in macrophages¹¹. Plasma cells were numerous and formed small aggregates in L-lep lesions^{36,37}, with the local production of antibodies contributing to tissue injury in patients³⁸.

It has long been thought that the nature of the immune responses in infection, cancer and autoimmune diseases is dictated by the principal cells of the immune system, that is, lymphocytes and myeloid cells. However, a compelling aspect of our data on leprosy is that not only these immune cells but also other cell types, such as fibroblasts and keratinocytes, cells that are not thought of as traditional immune cells, have the capacity to respond to *IL1B* and *IFNG* to produce antimicrobial molecules in granulomas. Our studies support a temporal and spatial model where immunological activation of multiple cell types in and around the organized structure of the granuloma may be essential to control powerful intracellular pathogens. One could summarize our key findings simply by saying that it appears to take a village to create an effective antimicrobial granulomatous response.

Online content

Any methods, additional references, Nature Research 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/s41590-021-00956-8>.

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Methods

Institutional review board statement. Written informed consent was obtained from participants under a protocol approved by the institutional review boards of the Oswaldo Cruz Foundation, University of Southern California and University of California Los Angeles. This study was conducted according to the Declaration of Helsinki (2013).

Processing of human skin. Skin biopsy specimens were obtained from patients with leprosy at the University of Southern California and Oswaldo Cruz Foundation (Brazil). Patients were classified according to standard clinical and histological criteria² (Supplementary Table 1). Five patients with RR are designated as RR1, RR2, RR3, RR4 and RR5. The other five are designated as L-lep1, L-lep2, L-lep3, L-lep4 and L-lep5; of these, four were classified as LL and one, L-lep2, as BL.

For each sample, a 4-mm punch biopsy was obtained after local anesthesia and was placed immediately into 10 ml of Roswell Park Memorial Institute (RPMI) medium on ice. Initially, skin biopsies were incubated in 5 ml of a 0.4% dispase II solution (Roche) at 37°C for 1 h with vigorous shaking. The dermis and epidermis were then carefully separated using forceps and transferred to separate tubes for additional processing. Epidermal samples were placed in 3 ml of 0.25% trypsin and 10 U ml⁻¹ DNase for 30 min at 37°C. Trypsin was neutralized with 3 ml of FCS and the tissue was passed through a 70-µm nylon cell strainer, which was washed with 5 ml of RPMI. Epidermal cells were then pelleted at 300g for 10 min and counted. Dermal samples were minced with a scalpel and incubated in a solution of 0.4% collagenase type II and 10 U ml⁻¹ DNase for 2 h at 37°C with agitation. The cell suspension was passed through a 70-µm cell strainer and washed with 5 ml of RPMI. Cells were pelleted at 300g for 10 min, resuspended in 1 ml of RPMI and counted. Magnetic-activated cell sorting enrichment for CD1a⁺ cells was performed for the epidermis from three patients with RR.

Single-cell library preparation. Once a single-cell suspension was obtained, we utilized Seq-Well S3, a massively parallel scRNA-Seq platform for clinical samples, to capture the transcriptome of single cells. Full methods on the implementation of Seq-Well are available in Gierahn et al.¹⁶ or on the Shalek Lab website (www.shaleklab.com). Specifically, 10,000–15,000 cells were loaded onto a functionalized polydimethylsiloxane array preloaded with uniquely barcoded mRNA capture beads (catalog no. MACOSKO-2011-10; Chemgenes). After cells had settled into the wells, the array was then sealed with a hydroxylated polycarbonate membrane with pore sizes of 10 nm, facilitating buffer exchange while confining biological molecules within each well. After membrane sealing, subsequent buffer exchange permits cell lysis, mRNA transcript hybridization to beads and bead removal before proceeding with reverse transcription. The obtained bead-bound complementary DNA product then underwent exonuclease I treatment (catalog no. M0293M; New England Biolabs) to remove excess primer before proceeding with second-strand synthesis.

After exonuclease I treatment, beads were washed once with 500 µl of a Tris-EDTA-SDS (0.5% SDS) solution and twice in 500 µl of a Tris-EDTA-Tween (0.01% Tween) solution. After the second Tris-EDTA-Tween wash, the beads were solvated with 500 µl of 0.1 M of NaOH and mixed for 5 min at room temperature using an end-over-end rotator with intermittent agitation to denature the mRNA–cDNA hybrid product on the bead. After denaturing, the NaOH was removed and beads were washed once with 1 M of Tris-EDTA and then combined with a mastermix consisting of 40 µl 5× maxima reverse transcription buffer, 80 µl of 30% PEG8000 solution, 20 µl of 10 mM of deoxynucleoside triphosphate, 2 µl of 1 mM of dn-SMART oligonucleotide, 5 µl of Klenow Exo- and 53 µl of deionized ultrapure water. Second-strand synthesis was carried out by incubating the beads for 1 h at 37°C with end-over-end rotation and intermittent agitation. After incubation, beads were washed sequentially twice with 0.5 ml of Tris-EDTA buffer with 0.01% Tween 20 and once with 0.5 ml of Tris-EDTA. Immediately before PCR amplification, beads were washed once with 0.5 ml of water and resuspended in 0.5 ml of water.

After second-strand synthesis, PCR amplification was performed using the KAPA HiFi HotStart ReadyMix (catalog no. KK2602; Kapa Biosystems). Specifically, a 40 µl PCR Mastermix consisting of 25 µl of KAPA 5× Mastermix, 0.4 µl of 100 µM of ISPCR oligonucleotide and 14.6 µl of nuclease-free water was combined with 2,000 beads per reaction. For each sample, the total number of PCR reactions performed varied based on the number of beads recovered after second-strand synthesis. PCR amplification was performed using the following cycling conditions: an initial denaturation at 95°C for 3 min, then 4 cycles at 98°C for 20 s, 65°C for 45 s and 72°C for 3 min, followed by 9–12 cycles at 98°C for 20 s, 67°C for 20 s and 72°C for 3 min and then a final extension of 72°C for 5 min. After PCR amplification, whole-transcriptome amplification (WTA) products were isolated through two rounds of solid phase reversible immobilization (SPRI) purification using AMPure SPRI beads (Beckman Coulter) at both 0.6× and 0.8× volumetric ratios and quantified using a Qubit fluorometer.

A total of 1 ng of WTA product at a concentration of 0.2 ng µl⁻¹ was combined with 10 µl of tagmentation DNA buffer and 5 µl of ATM buffer and incubated at 55°C for 5 min. After tagmentation, 5 µl of neutralize tagment buffer was added and incubated at room temperature for 5 min to neutralize the reaction. A total of 8 µl of nuclease-free water, 15 µl of NPM buffer, 1 µl of Custom P5 hybrid

oligonucleotides and 1 µl of N700 Index oligonucleotides were combined and PCR amplification was performed using the following cycling conditions: an initial denaturation at 95°C for 30 s, then 12 cycles at 95°C for 10 s, 55°C for 30 s and 72°C for 30 s followed by a final extension at 72°C for 5 min. PCR products were isolated through two rounds of SPRI purification (0.6× and 0.8× volumetric ratios) and quantified using a Qubit. Library size distributions were determined using an Agilent Bioanalyzer D1000 High Sensitivity Screen tape.

Sequencing and alignment. Libraries were sequenced on an Illumina NovaSeq as 50-base pair (bp) paired-end reads and were converted from BCL files to FASTQ files using bcl2fastq v2.19. We use Nextera N700 indices to identify individual samples. The alignment was performed using the Drop-seq pipeline v1.12 (ref. ³⁹). Briefly, the raw reads were aligned to the concatenated human (hg38) and *M. leprae* genome using STAR v2.5.3a⁴⁰. Each read was tagged with a 12-bp barcode and 8-bp unique molecular identifier (UMI). After alignment, reads were grouped by the barcodes and deduplicated using the UMIs. The number of UMIs was then counted for each gene in each cell to generate the digital expression matrix (DEM).

Removal of ambient RNA contamination. Ambient RNA contamination was removed using SoupX v0.3.1 (ref. ⁴¹). Specifically, we examined the distribution of UMIs for each gene and selected the genes for which the distribution most closely approximated a uniform distribution. For each sample, we calculated an array-specific ‘soup’ profile among barcodes below the UMI threshold. To calculate the estimated per-cell contamination fractions, we manually selected genes observed to be bimodally expressed across cells, which suggested that these genes are predominantly expressed in a single cell type but are observed at low levels in other cell types for which endogenous expression would not be expected. For each array, we removed individual transcripts most likely to be contamination from each single cell based on the estimated contamination fraction. Specifically, individual transcripts were sequentially removed from each single-cell transcriptome until the probability of subsequent transcripts being soup-derived was less than 0.5 to generate a background-corrected UMI matrix for each Seq-Well array.

Cell clustering and cell type annotation. After removing the ambient RNAs from each library, no further batch effects were detected in the analysis among the samples. Thus, DEMs for human genes from all ten samples were merged directly and the R (v3.6.0) package Seurat v3.2.2 (ref. ¹⁷) was used to cluster the cells in the merged matrix. Cells with less than 300 genes detected or more than 50% mitochondrial gene expression were first filtered out as low-quality cells. Genes detected in less than five cells were removed as low-abundance genes. The gene counts for each cell were divided by the total gene counts for the cell and multiplied by the scale factor 10,000. Then, natural-log transformation was applied to the counts. The FindVariableFeatures function was used to select 2,000 variable genes with default parameters. The ScaleData function was used to scale and center the counts in the dataset. Principal component analysis was performed on the variable genes and 13 principal components (based on the elbow point of variance explained by each principal component) were used for cell clustering (resolution = 0.5) and UMAP dimensional reduction. The cluster markers were found using the FindAllMarkers function and cell types were manually annotated based on the cluster markers. To generate the heatmap showing the cell type markers, the top 100 cells with the highest number of UMIs detected were plotted for each cell type. The total number of *M. leprae* UMIs were calculated for each cell and plotted for each sample.

Cell type subclustering. We performed subclustering on endothelial cells, fibroblasts, keratinocytes, myeloid cells and T cells. The same functions described above were used to obtain the subclusters. To choose the number of principal components, the rank of principal components based on the percentage of variance explained was plotted and the elbow point was chosen as the number of principal components to use in cell clustering (resolution = 0.6) and UMAP dimension reduction. Clusters that were defined exclusively by mitochondrial gene expression, indicating low quality, were removed from further analysis. To generate the heatmap with marker genes for each subcluster, the top 100 subcluster marker genes with the highest average log fold change were plotted and 5 representative genes were labeled.

Interferon signature enrichment analysis. Supervised analyses were performed to identify type I and II IFN-regulated genes as described previously^{11,42–44}. Differentially expressed genes in TC1 (RR CTLs) and TC2 (L-lep CTLs) were identified using a Wilcoxon rank-sum test with an adjusted *P* value cutoff of 0.05. A list of genes specifically induced by only IFN-α/β or IFN-γ was derived from the gene expression profile data of IFN-treated human peripheral blood mononuclear cells⁴⁵. A total of 148 IFN-α/β-specific genes and 33 IFN-γ-specific genes were identified, which were overlapped with TC1- and TC2-specific genes to determine the differential expression of IFN-regulated genes. A hypergeometric test was used to determine the enrichment level; *P* < 0.05 was considered to be significantly enriched.

Pseudotime trajectory construction on macrophage and keratinocyte subtypes. Pseudotime trajectories for macrophage and keratinocyte subclusters were

constructed using the R package Monocle v2.10.1 (ref. ³²). The raw counts for cells in the intended subclusters were extracted and normalized by the `estimateSizeFactors` and `estimateDispersions` functions with default parameters. Genes with an average expression >0.5 and detected in more than 10 cells were retained for further analysis. Variable genes were determined by the `differentialGeneTest` function with a model against the subcluster identities. The top 500 variable genes with the lowest adjusted *P* value were used to order the cells. The orders were determined by the `orderCells` function and the trajectory was constructed by the `reduceDimension` function with default parameters. Differential expression analysis was carried out using the `differentialGeneTest` function with a model against the pseudotime; genes with an adjusted *P* < 0.05 were clustered into 6 patterns and plotted in the heatmap. IPA was used to determine the upstream regulators for the genes in each expression pattern. A module score was calculated for each upstream regulator on gene targets from all six patterns. The module scores were calculated using the `Seurat` function `AddModuleScore` with default parameters, which measure the average expression levels of a set of genes, subtracted by the average expression of randomly selected control genes. Pearson correlation was then performed between the upstream regulator module scores and the pseudotimes.

Antimicrobial gene analysis. A list of 1,404 genes was curated by searching for genes with 'antimicrobial' as a keyword in GeneCards (<https://www.genecards.org/>). To study the difference in antimicrobial response between L-lep and RR, cell types were split into L-lep and RR groups. To measure the relative abundance of those encode proteins involved in antimicrobial responses ('antimicrobial genes'), the total expression of each antimicrobial gene was calculated for each L-lep and RR cell type. The sum of antimicrobial gene expression across the total number of L-lep cells was computed, as was the sum of antimicrobial gene expression across the total number of RR cells. These sums were then normalized by the total number of L-lep or RR cells, respectively. The *z*-scores of these sums were calculated across all cell types for each antimicrobial gene. A cutoff *z*-score > 3 was applied to obtain the specific antimicrobial genes for each cell type. A list of 1,124 antimicrobial genes was obtained as specific to at least one RR cell type. IPA was used to perform enrichment analysis and determine the upstream regulators of the 1,124 antimicrobial genes. To generate the Circos plots, a list of direct antimicrobial genes was obtained from the Antimicrobial Peptide Database³³ and those regulated by *IL1B* or *IFNG* were included.

Cell type composition analysis. To calculate the sample composition based on cell type, the number of cells for each cell type from each sample were counted. The counts were then divided by the total number of cells for each sample and scaled to 100% for each cell type. The same procedures were applied to calculate the sample composition for each subtype in endothelial cells, fibroblasts, keratinocytes, myeloid cells and T cells. The cell type (including the subtype) with more than 70% L-lep (or RR) composition was named L-lep (or RR) specific.

Spatial sequencing library preparation. Skin samples were frozen in optimal cutting temperature medium and stored at -80°C until sectioning. Optimization of tissue permeabilization was performed on 20-μm-thick sections using the Visium Spatial Tissue Optimization Reagents Kit (10x Genomics), which established an optimal permeabilization time of 6 min. Samples were mounted onto a Gene Expression slide (10x Genomics) and stored at -80°C until hematoxylin and eosin (H&E) staining. To prepare for staining, the slide was placed on a thermocycler adaptor set at 37°C for 5 min and then immediately fixed in ice-cold methanol for 20 min. Methanol was displaced with isopropanol and the samples were air-dried for 10 min before sequential staining with Mayer's Hematoxylin Solution (Sigma-Aldrich), Bluing Buffer (Dako) and 1:10 dilution of Eosin Y solution (Sigma-Aldrich) in 0.45 M of Tris-acetic acid buffer, pH 6.0, with thorough washing in ultrapure water between each step. Stained slides were scanned under a microscope (Keyence). Tissue permeabilization was performed to release the poly-A mRNA for capture by the poly(dT) primers that were precoated on the slide and included an Illumina TruSeq Read, spatial barcode and UMI. The Visium Spatial Gene Expression Reagent Kit (10x Genomics) was used for reverse transcription to produce spatially barcoded full-length cDNA and for second-strand synthesis followed by denaturation to allow a transfer of the cDNA from the slide into a tube for amplification and library construction. Visium Spatial Single Cell 3' Gene Expression libraries consisting of Illumina paired-end sequences flanked by P5/P7 were constructed after enzymatic fragmentation, size selection, end repair, A-tailing, adaptor ligation and PCR. Dual Index Kit TT Set A (10x Genomics) was used to add unique i7 and i5 sample indexes and generate TruSeq Read 1 to sequence the spatial barcode and UMI and TruSeq Read 2 to sequence the cDNA insert, respectively.

Spatial sequencing analysis. After sequencing, the reads were aligned to the human genome (hg38) and the expression matrix was extracted using the `spaceranger` pipeline. `Seurat` was then used to analyze the expression matrix. Specifically, the `SCTransform` function was used to scale the data and find variable genes with default parameters. Principal component analysis was applied for dimensional reduction. The `FindTransferAnchors` function was used to find a set of anchors between the spatial sequencing and scRNA-seq data, which were then transferred from the scRNA-seq to the spatial sequencing data using the

`TransferData` function. These two functions construct a weights matrix that defines the association between each query cell and each anchor. These weights sum to 1 and were used as the percentage of the cell type in the spots. The RR-specific cell type/subtypes from the scRNA-seq data were used to annotate the RR and T-lep spatial sequencing samples. The L-lep-specific cell type/subtypes from the scRNA-seq data were used to annotate the L-lep spatial sequencing samples. The predicted cell type composition for each spot was then used to cluster the spots by the *k*-means algorithm. The clusters were annotated based on the average cell type prediction score across all the spots in the cluster.

Spatial distance calculation. To measure the distance from fibroblast subtypes to the epidermis, the FB0, FB2 and epidermis spots were first defined. Epidermis spots were defined based on the expression of KRT1 and KRT10. Spots with higher expression than the 90% quantile of the sum of these two genes were considered epidermis spots. FB0 and FB2 spots were defined using the subtype prediction scores. Spots that had FB0 prediction scores higher than the 90% quantile were considered FB0 spots and spots that had FB2 prediction scores higher than the 90% quantile were considered FB2 spots. The locations of the spots were extracted from the 'tissue_positions_list.csv' file from the `spaceranger` output. The Euclidean distance from the FB0 and FB2 spots to all the epidermis spots were calculated and the shortest distance was used as the distance from the spot to the epidermis. All three RR samples and the T-lep samples were combined and a Wilcoxon rank-sum test was performed between the FB0 epidermis and FB2 epidermis distances.

Spatial nearest-neighbor measurement. For each spot, the closed six spots surrounding it were considered nearest neighbors. The number of neighboring spots for each pair of cell types were counted in each of the spatial sequencing samples to generate a nearest-neighbor matrix. Each row of the matrix was divided by the row sum. The RR average was calculated using the RR and T-lep samples; the L-lep average was calculated using the two L-lep samples.

Immunohistochemistry. Frozen tissue sections from three RR and three L-lep specimens were blocked with normal horse serum before incubation with DEFB1 (clone M11-14b-D10; Abcam), CXCL14 (clone EPR22807-28; Abcam) and TAC1 (clone NC1/34HL; Santa Cruz Biotechnology) monoclonal antibodies and respective isotype controls (Sigma-Aldrich) for 60 min, followed by incubation with biotinylated secondary antibodies (Vector Laboratories) for 30 min. Slides were counterstained with hematoxylin and mounted with VectaMount mounting medium (Vector Laboratories) and were visualized using the ABC Elite system (Vector Laboratories). Skin sections were examined using a Leica 2500 microscope (Leica Microsystems)⁴⁶.

Antimicrobial activity assay. Live *M. leprae* was provided by R. Lahiri of the National Hansen's Disease Program, Health Resources Service Administration. Peripheral blood mononuclear cells from healthy donors were isolated from peripheral blood using a Ficoll-Paque (GE Healthcare) density gradient. Monocyte-derived macrophages were generated¹⁵. To evaluate antimicrobial activity, 5 × 10⁵ monocyte-derived macrophages were pretreated with 1 μM of recombinant human CXCL14 (R&D Systems), DEFB1 (LSBio) and TAC1 (Substance P) (R&D Systems) for 30 min and infected with live *M. leprae* at a multiplicity of infection of 5 at 35°C with 4% CO₂. Rifampicin (10 μg ml⁻¹) was used as a positive control. After 4 d, TRIZol reagent (Thermo Fisher Scientific) was added to the cells and RNA and DNA were extracted according to the manufacturer's instructions. *M. leprae* viability was determined by quantitative PCR and quantified^{15,47}.

Statistics and reproducibility. Statistical differences were evaluated using two-sided *t*-tests or Wilcoxon rank-sum tests. The Benjamini-Hochberg procedure was used to correct for the false discovery rate in multiple comparisons. For scRNA-seq, statistics were conducted between the RR and L-lep samples. For spatial sequencing, statistics were conducted between the RR/T-lep and L-lep samples. scRNA-seq or spatial sequencing samples in the same disease category can be considered biological replicates. No technical replication was performed. *P* < 0.05 or *P*_{adj} < 0.05 (in multiple comparisons) was considered significant. No statistical method was used to predetermine sample size. No samples were excluded from the analyses. The experiments were not randomized.

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

Data availability

The scRNA-seq data are available at the Gene Expression Omnibus (GEO) under accession no. GSE151528. The spatial sequencing data are available at the GEO under accession no. GSE167889.

Code availability

The code used to analyze the data in this study is available at GitHub https://github.com/mafeiyang/leprosy_amg_network.

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Author contributions

F.M., T.K.H., B.B., A.K.S., B.R.B., M.P. and R.L.M. conceptualized the study. F.M., T.K.H., R.M.B.T., P.R.A., B.J.A.S., O.P., L.C.T., T.D., M.H.W., M.P. and R.L.M. devised the methodology. F.M., T.K.H., R.M.B.T., P.R.A., B.J.A.S., T.D., M.T.O. and E.N.S. carried out the investigation. F.M., T.K.H., B.B., R.M.B.T., P.R.A., B.J.A.S., T.D. and M.H.W. carried out the formal analysis. F.M., T.K.H., R.M.B.T., P.R.A., B.J.A.S., M.H.W., A.O., M.L.I.-A., E.K., B.B., A.K.S., B.R.B., J.E.G., M.P. and R.L.M. wrote the manuscript. M.P. and R.L.M. supervised the study.

Competing interests

A.K.S. reports compensation for consulting and/or scientific advisory board membership from Merck, Honeycomb Biotechnologies, Cellarity, Cogen Therapeutics, Orche Bio and Dahlia Biosciences. The other authors declare no competing interests.

Additional information

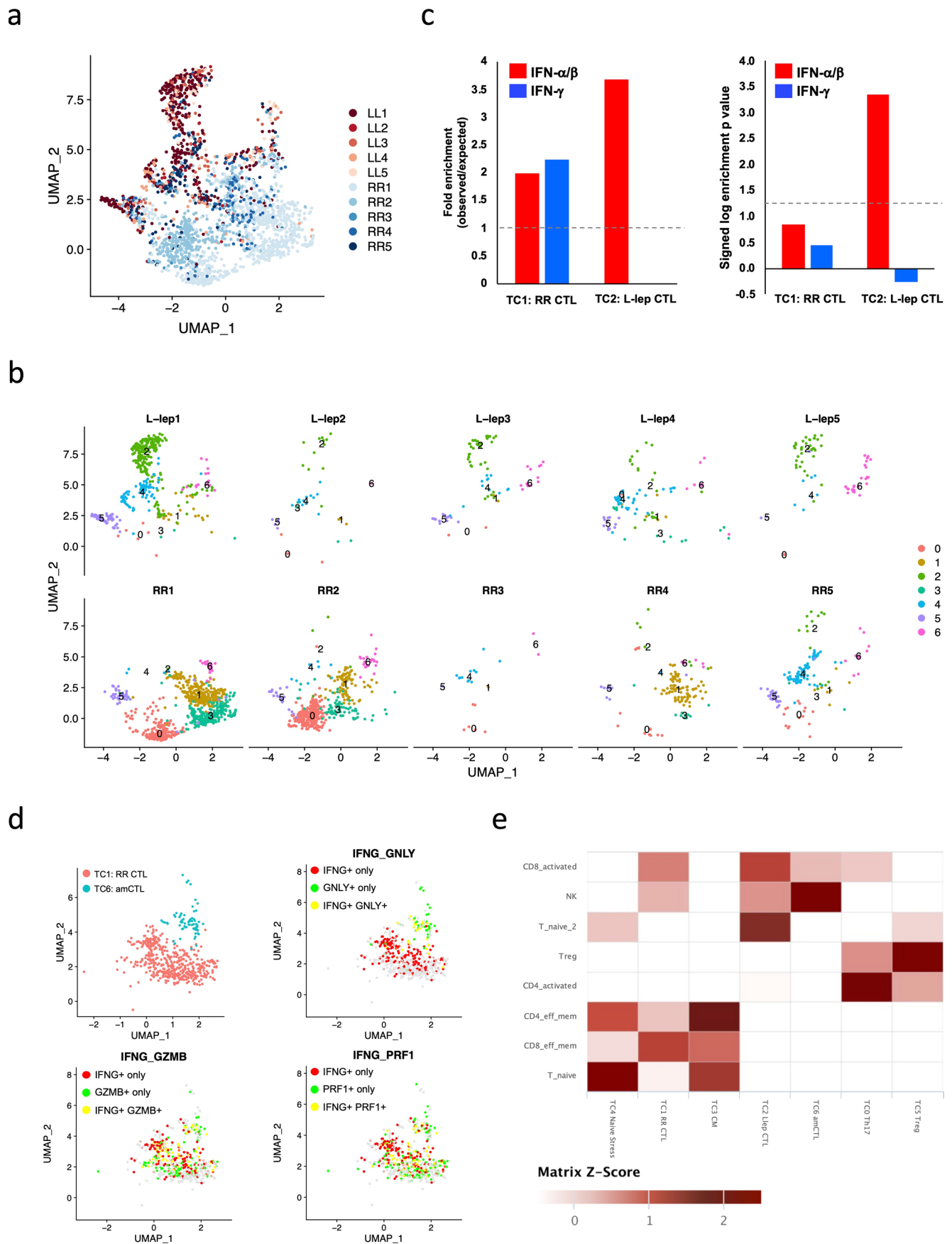
Extended data is available for this paper at <https://doi.org/10.1038/s41590-021-00956-8>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41590-021-00956-8>.

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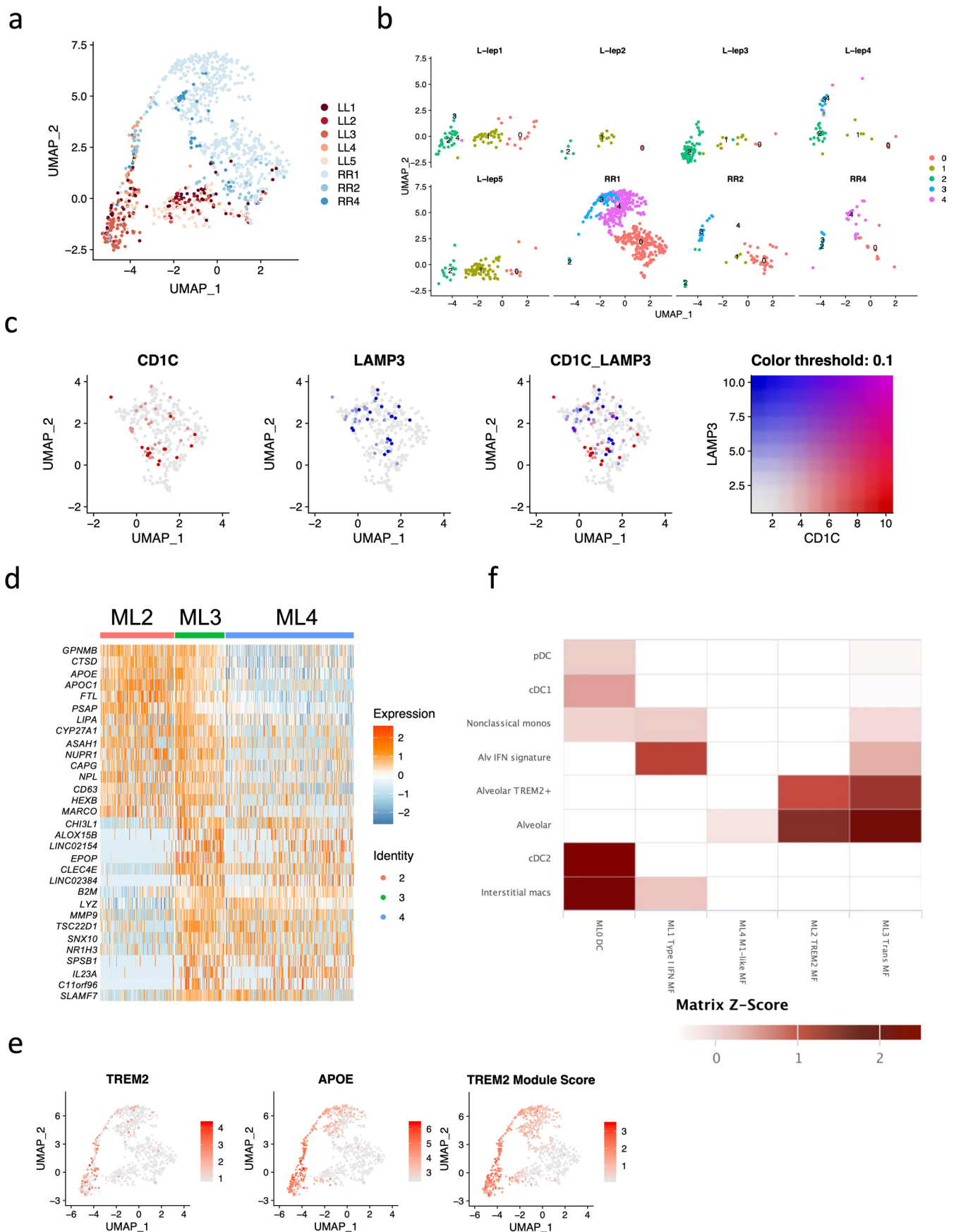
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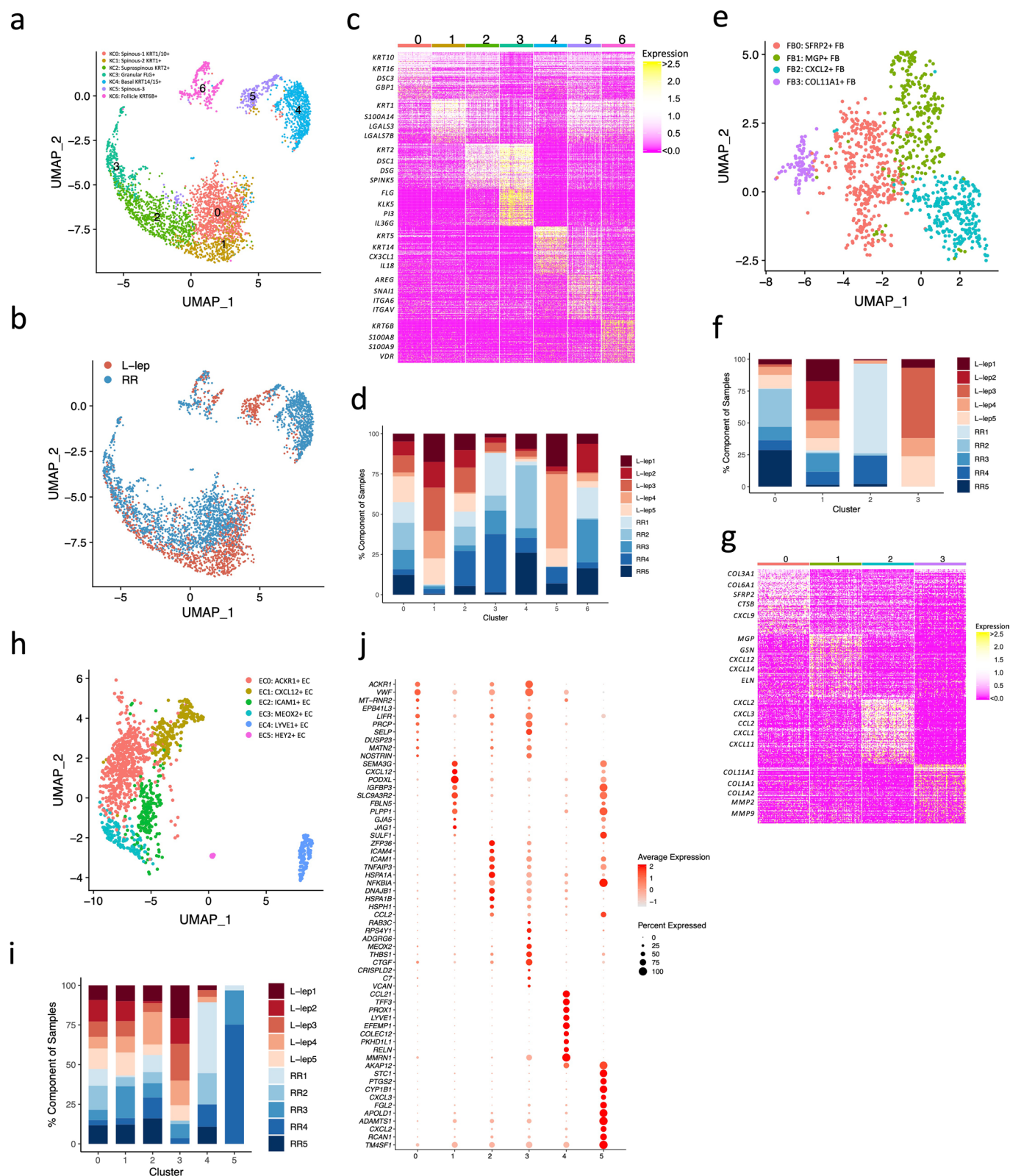
Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | IFN- α/β and IFN- γ signature on CTL subtypes and co-expression of *IFNG*, *GZMB*, *PRF1* and *GNLY*. **a**, UMAP plot for 2,290 T cells colored by patient identities. **b**, Individual UMAP for the T cells from each patient. **c**, Enrichment analysis on differentially expressed genes (adjusted p value < 0.05) between TC1 (RR CTL) and TC2 (L-lep CTL) using IFN- α/β and IFN- γ specific genes identified in human PBMC. Dotted lines indicate (left) no enrichment or (right) the hypergeometric test p value of 0.05 (log p value = 1.3). **d**, UMAP plots showing co-expression of *IFNG*, *GMZB*, *PRF1* and *GNLY* in RR CTL and amCTL. **e**, T cell subtype comparisons between scRNA-seq datasets from human leprosy skin biopsy specimens and from lung samples of a non-human primate model of tuberculosis. The color scale represents the z scores of the gene expression signature scores.

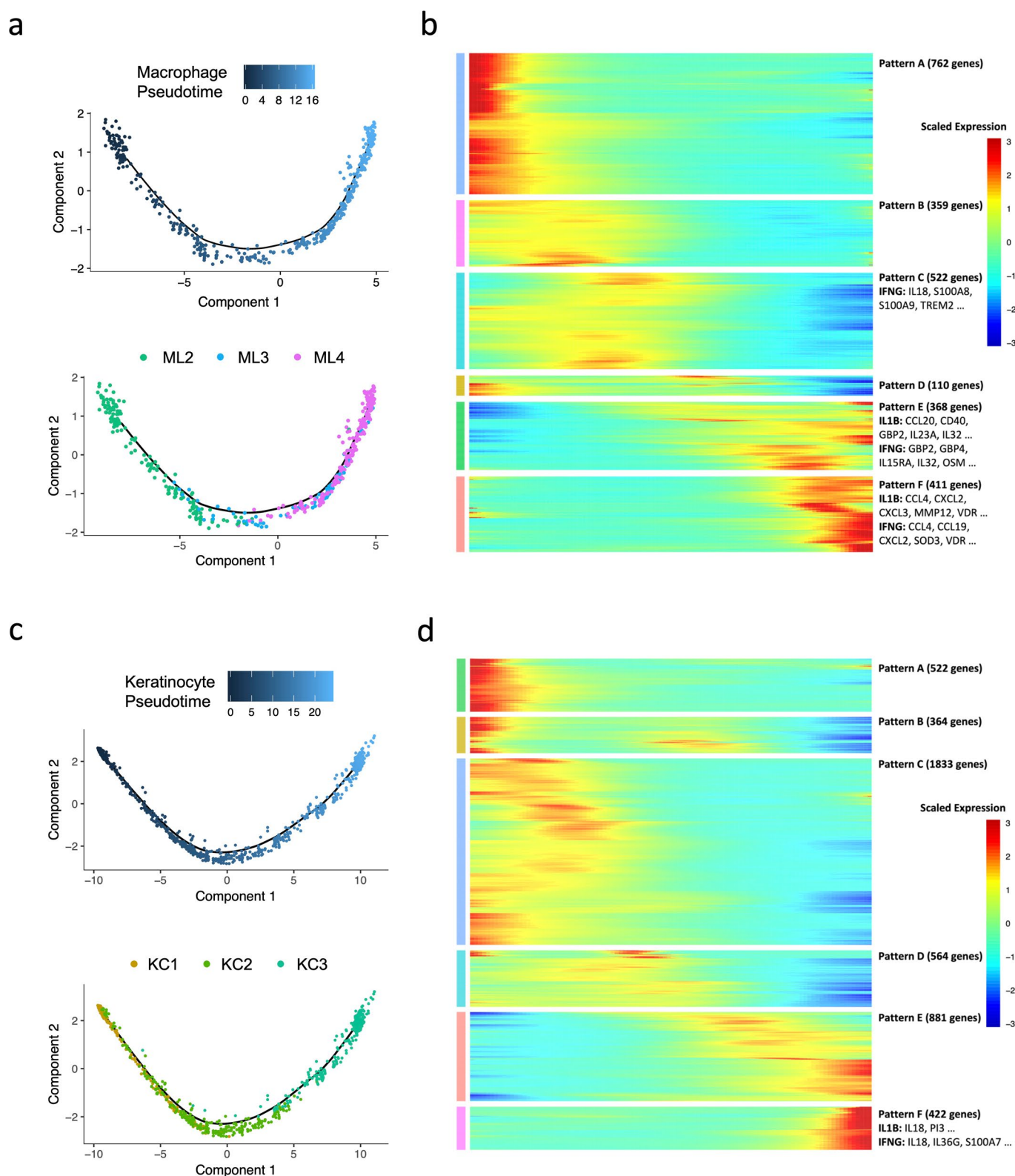


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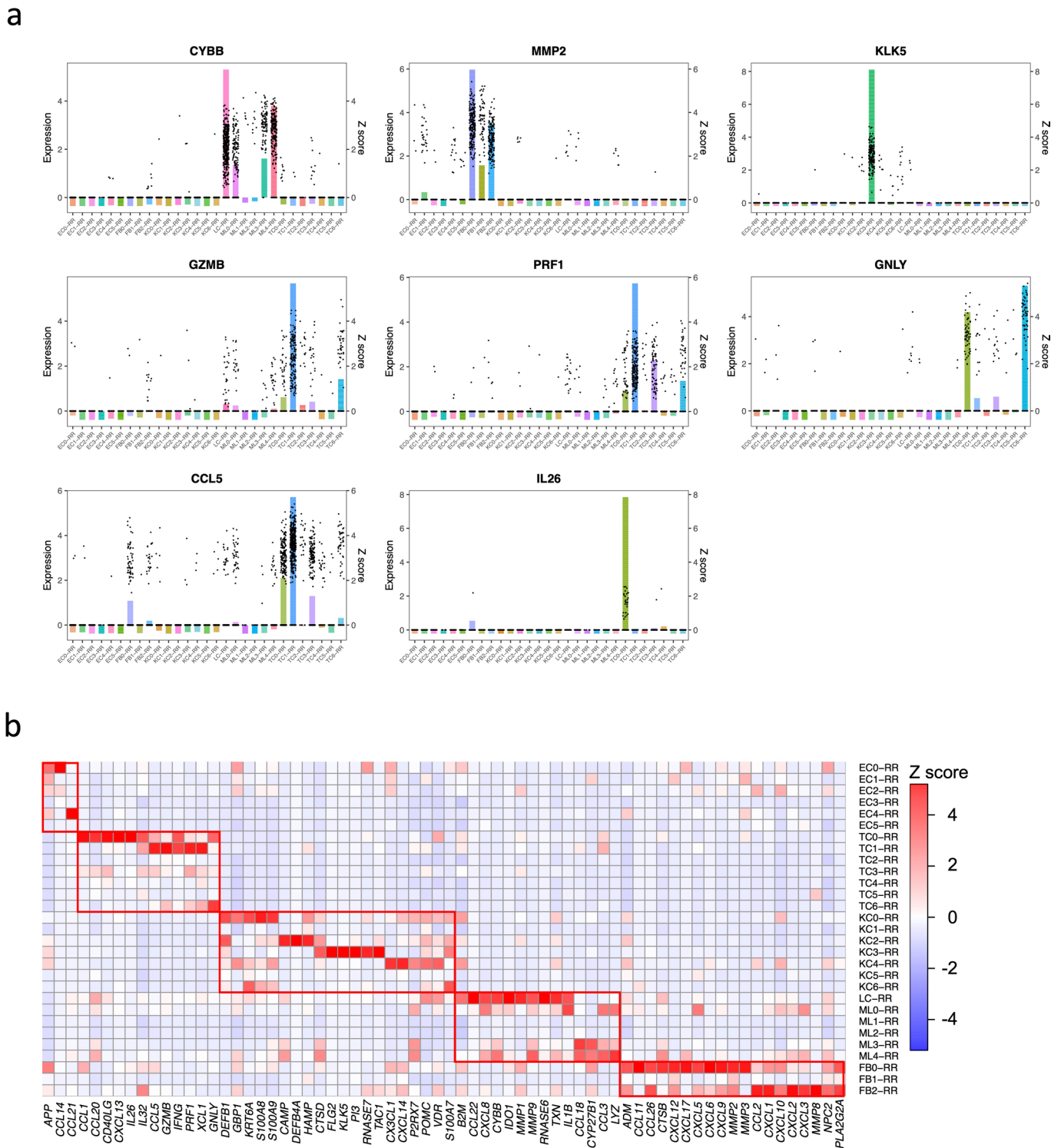
Extended Data Fig. 2 | Dendritic cell subpopulations and comparison of macrophage sub-clusters. **a**, UMAP plot for 991 myeloid cells colored by patient identities. **b**, Individual UMAP for the myeloid cells from each patient. **c**, UMAP plots showing *CD1C* and *LAMP3* expression in MLO. Only few co-expression events were observed, indicating distinct dendritic cell subpopulations. **d**, Heatmap showing top differentially expressed genes between ML2 and ML4. ML3 expressed both ML2 and ML4 specific genes. **e**, UMAP plots showing *TREM2* expression, *APOE* expression and *TREM2* module score. The color scale represents the expression level of the genes or the level of the module score. **f**, Myeloid cell subtype comparisons between scRNA-seq data sets from human leprosy skin biopsy specimens and from lung samples of a non-human primate model of tuberculosis. The color scale represents the z scores of the gene expression signature scores.



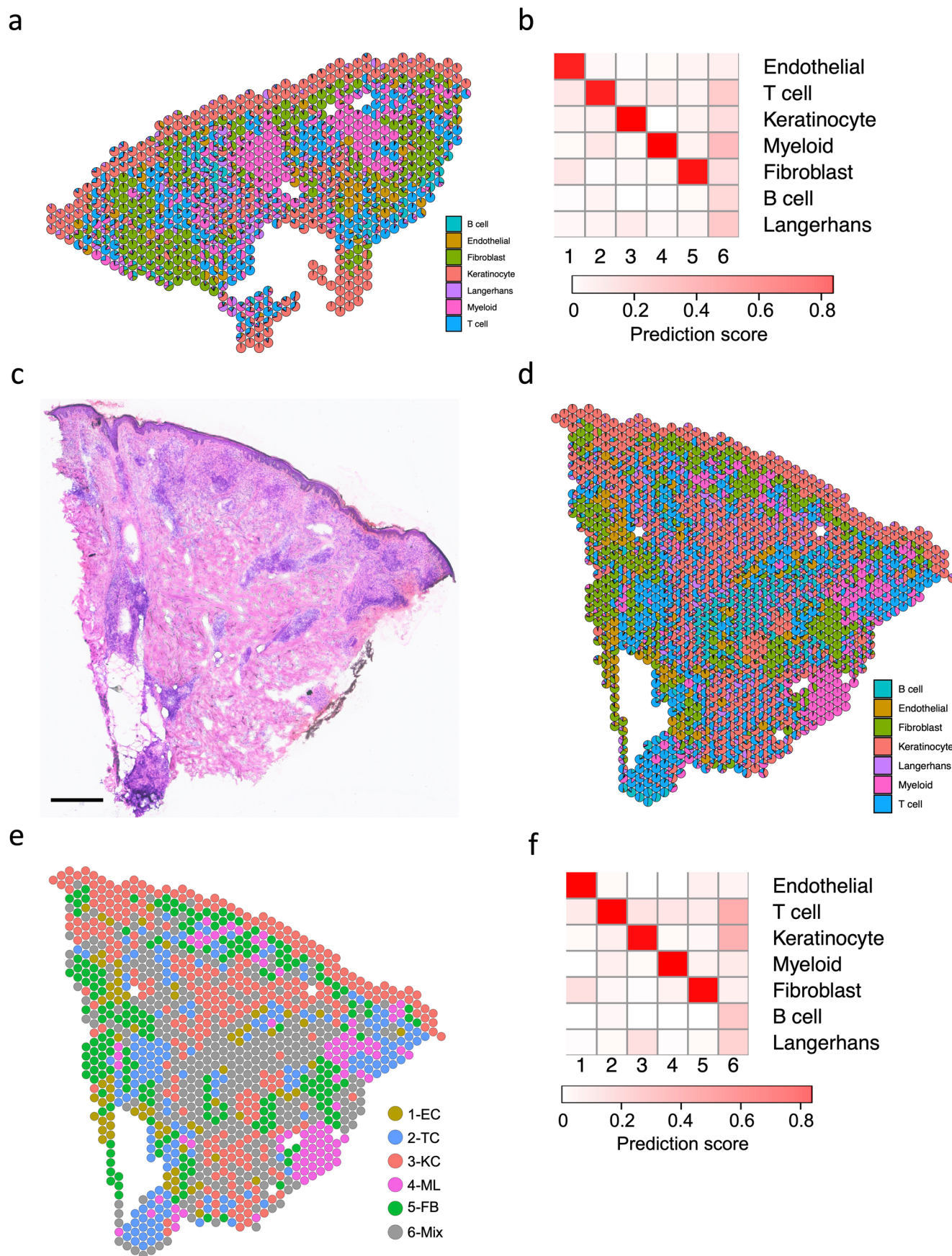
Extended Data Fig. 3 | Identification of keratinocyte subtypes. **a**, UMAP plot for 3,748 keratinocytes colored by subtypes. **b**, UMAP plot for 3,748 keratinocytes colored by clinical forms. **c**, Heatmap showing marker genes for each keratinocyte subtype. The representative genes are labelled. **d**, Abundance composition across all samples for each keratinocyte subtype. **e**, UMAP plot for 1,010 fibroblasts colored by subtypes. **f**, Abundance composition across all samples for each fibroblast subtype. **g**, Heatmap showing marker genes for each fibroblast subtype. The representative genes are labelled. **h**, UMAP plot for 1,219 endothelial cells colored by subtypes. **i**, Abundance composition across all samples for each endothelial cell subtype. **j**, Dot plot showing 10 marker genes for each endothelial subtype. The color scale represents the scaled expression of the gene. The size of the dot represents the percentage of cells expressing the gene.



Extended Data Fig. 4 | Pseudotime construction in macrophages and keratinocytes. **a**, Pseudo-temporal trajectory colored by pseudotime (top) and by sub-cluster identity (bottom) for macrophage sub-cluster 2, 3 and 4. **b**, Heatmap showing six expression patterns along the macrophage pseudotime trajectory as depicted on the x axis. Representative genes regulated by *IL1B* and *IFNG* are labelled. **c**, Pseudo-temporal trajectory colored by pseudotime (top) and by sub-cluster identity (bottom) for keratinocyte sub-cluster 1, 2 and 3. **d**, Heatmap showing six expression patterns along the keratinocyte pseudotime trajectory as depicted on the x axis. Representative genes regulated by *IL1B* and *IFNG* are labelled.

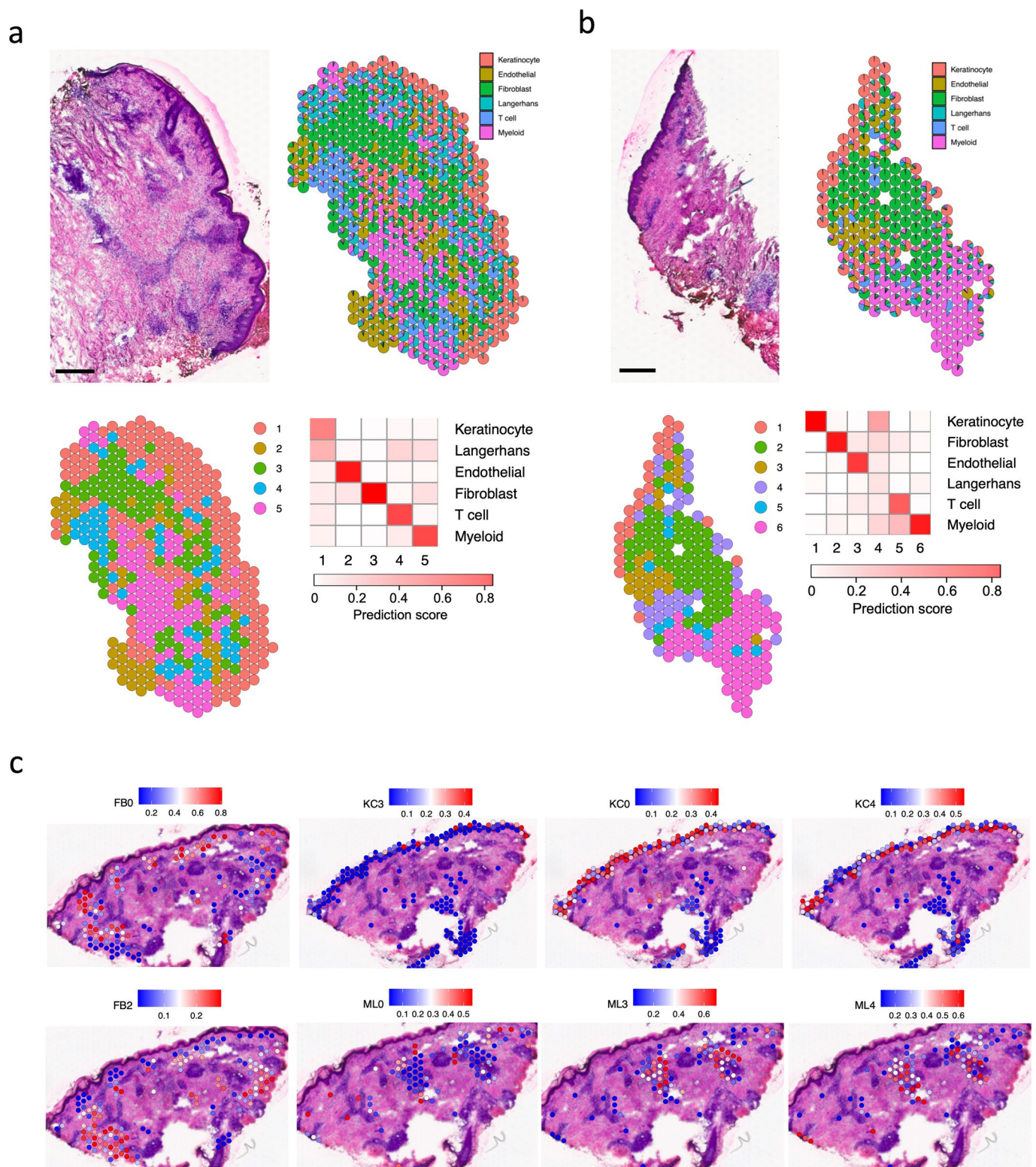


Extended Data Fig. 5 | Representative antimicrobial genes expressed by the major cell types. a, Bar plot showing the expression of the antimicrobial genes in each RR subtype. The height of the bar represents the z score of the gene in each subtype. The dots represent the gene's expression level in individual cells. **b**, Heat map showing z scores of antimicrobial genes in RR cell types. The red boxes indicate distinct sets of antimicrobial genes highly expressed in endothelial cells, fibroblasts, keratinocytes, myeloid cells and T cells.

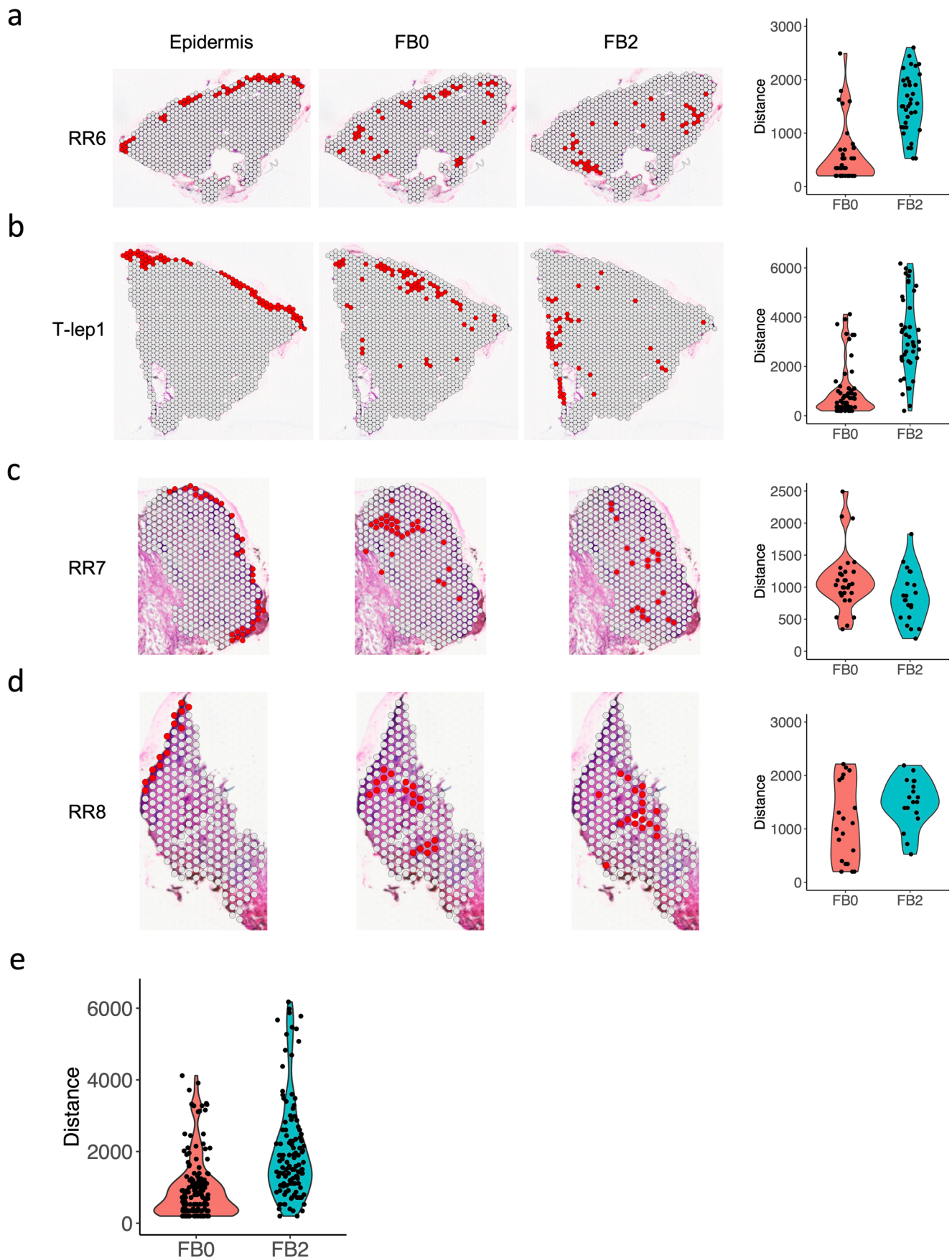


Extended Data Fig. 6 | See next page for caption.

Extended Data Fig. 6 | Cell type composition and clustering of the RR6 and T-lep1 spatial-seq sample. **a**, Scatter pie plot showing the cell type composition of the RR6 spatial-seq sample. Each spot is represented as a pie chart showing the relative proportion of the cell types. **b**, Heatmap showing the average cell type prediction score for each cluster of the RR6 spatial-seq sample. **c**, H & E staining of the T-lep1 biopsy used for spatial sequencing. Scale bar: 0.5 mm. **d**, Scatter pie plot showing the cell type composition of the T-lep1 spatial-seq sample. Each spot is represented as a pie chart showing the relative proportion of the cell types. **e**, Spatial plot for 1,154 spots colored by clusters, the coordinates of the spot correspond to the location in the T-lep1 sample tissue. **f**, Heatmap showing the average cell type prediction score for each cluster of the T-lep1 spatial-seq sample.

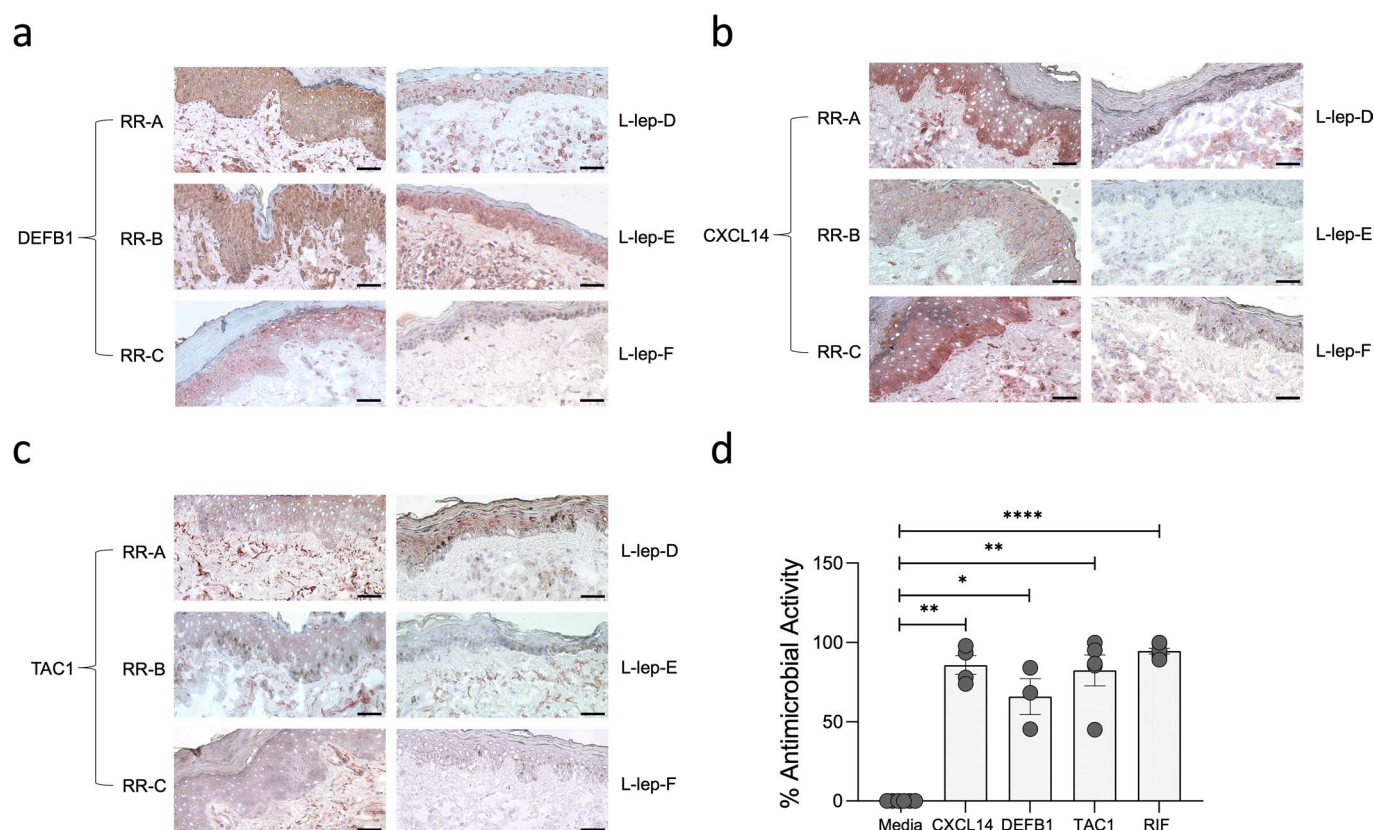


Extended Data Fig. 7 | Spatial sequencing for two additional RR samples and subtypes location for the RR6 sample. a, H & E staining of the RR7 biopsy used for spatial sequencing (top left), scale bar: 0.5 mm. Scatter pie plot showing the cell type composition of the RR7 spatial-seq sample. Each spot is represented as a pie chart showing the relative proportion of the cell types (top right). Spatial plot for 418 spots colored by clusters, the coordinates of the spot correspond to the location in the tissue (bottom left). Heatmap showing the average cell type prediction score for each cluster (bottom right). **b**, H & E staining of the RR8 biopsy used for spatial sequencing (top left), scale bar: 0.5 mm. Scatter pie plot showing the cell type composition of the RR8 spatial-seq sample. Each spot is represented as a pie chart showing the relative proportion of the cell types (top right). Spatial plot for 214 spots colored by clusters, the coordinates of the spot correspond to the location in the tissue (bottom left). Heatmap showing the average cell type prediction score for each cluster (bottom right). **c**, Subtype prediction scores for the RR6 spatial-seq sample. The spots in the corresponding cluster were used to plot the subtype scores. For example, the FB0 prediction score was plotted in the spots from cluster 5, which was annotated as fibroblasts.

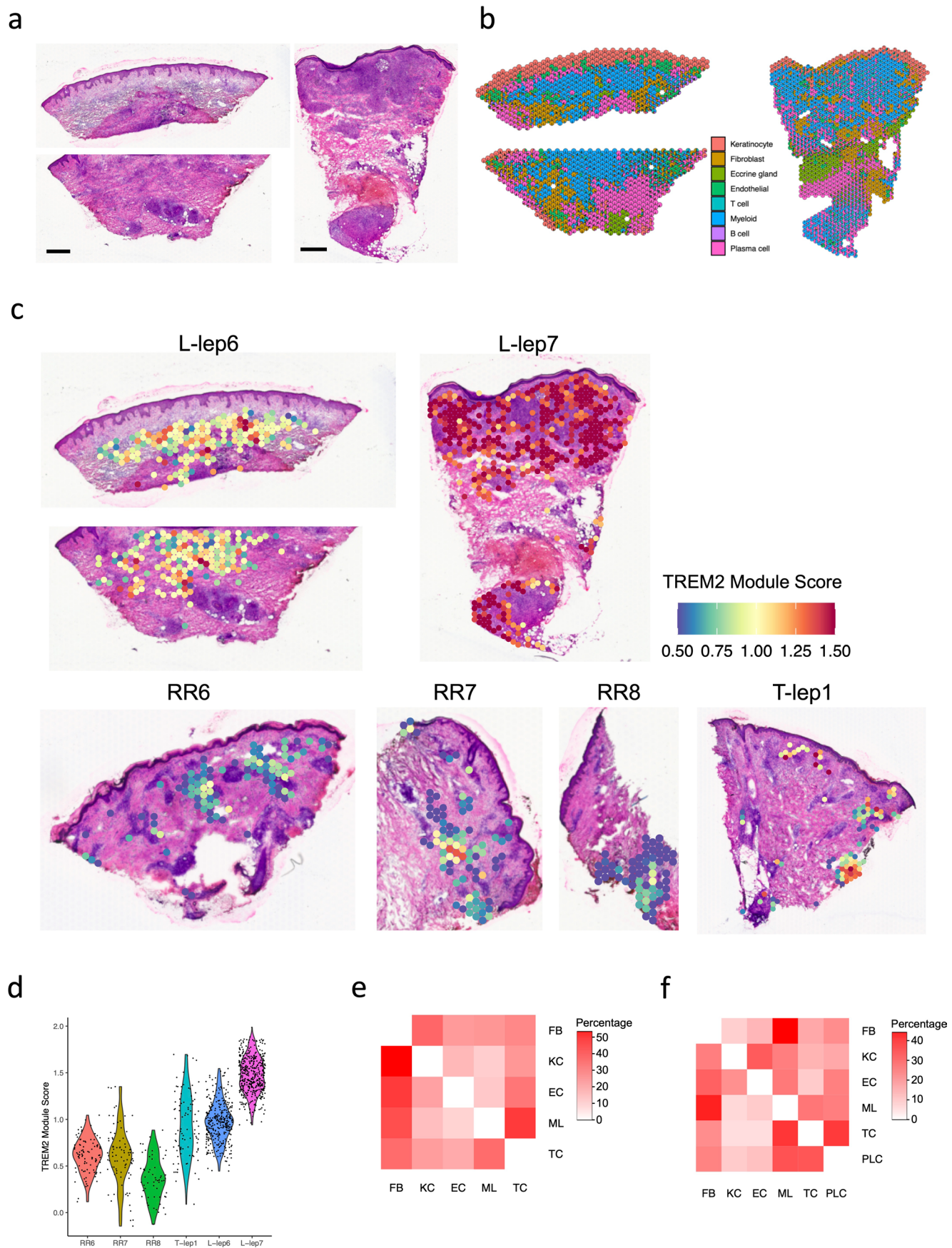


Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | Spatial distance between the FBO and FB2 sub-clusters and the epidermis in the RR and T-lep samples. **a**, Spatial plots showing the identified epidermis, FBO and FB2 spots in the RR6 sample (left). Violin plot showing the distance of each FBO and FB2 spot to the nearest epidermis spot. **b**, Spatial plots showing the identified epidermis, FBO and FB2 spots in the T-lep1 sample (left). Violin plot showing the distance of each FBO and FB2 spot to the nearest epidermis spot. **c**, Spatial plots showing the identified epidermis, FBO and FB2 spots in the RR7 sample (left). Violin plot showing the distance of each FBO and FB2 spot to the nearest epidermis spot. **d**, Spatial plots showing the identified epidermis, FBO and FB2 spots in the RR8 sample (left). Violin plot showing the distance of each FBO and FB2 spot to the nearest epidermis spot. **e**, Violin plot showing the distance of each FBO and FB2 spot to the nearest epidermis spot in all three RR and the T-lep spatial-seq samples. The p value ($1.32\text{e-}14$) was calculated from a two-sided Wilcoxon rank sum test (152 FBO spots vs 120 FB2 spots).



Extended Data Fig. 9 | Immunohistochemistry validation of DEFB1, CXCL14 and TAC1 protein expression in the epidermis of three RR and three L-lep biopsy specimens. **a**, Immunohistochemistry using monoclonal antibodies to DEFB1. The corresponding isotype controls were negative. Scale bar: 20 μ m. Original magnification: x200. **b**, Immunohistochemistry using monoclonal antibodies to CXCL14. The corresponding isotype controls were negative. Scale bar: 20 μ m. Original magnification: x200. **c**, Immunohistochemistry using monoclonal antibodies to TAC1. The corresponding isotype controls were negative. Scale bar: 20 μ m. Original magnification: x200. **d**, Antimicrobial activity was determined by pretreatment of MDMs from healthy donors with 1 μ M of DEFB1 ($n=3$), CXCL14 ($n=4$) and TAC1 ($n=5$) for 30 minutes followed by *M. leprae* infection (MOI 5:1). Rifampicin (RIF, 10 μ g/ml) was used as positive control. *M. leprae* viability was determined by qPCR after 4 days and % antimicrobial activity was calculated by assigning 100% viability to the media control. All data represent the mean $\pm$ SEM. Statistical analyses were performed using mixed effects analysis with Dunnett's multiple comparisons test in the GraphPad Prism 8 software. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.



Extended Data Fig. 10 | See next page for caption.

Extended Data Fig. 10 | TREM2 module score comparison in the RR, T-lep and L-lep spatial-seq samples. **a**, H & E staining of the two L-lep biopsies used for spatial sequencing. The first tissue slide was folded in the dermis region, thus a replicate slide from the same tissue covering the dermis region was processed in the same spatial-seq run. Scale bar: 0.5 mm. **b**, Scatter pie plot showing the cell type composition of the two L-lep spatial-seq samples. Each spot is represented as a pie chart showing the relative proportion of the cell types. **c**, Spatial plots showing the TREM2 module score in the myeloid spots from all spatial-seq samples. The same color scale was applied across the samples. **d**, Violin plot showing the TREM2 module score in the myeloid spots for individual samples. The p value ($<2.2 \times 10^{-16}$) was calculated from a two-sided Wilcoxon rank sum test (329 RR and T-lep spots vs 760 L-lep spots). **e**, Heatmap showing the number of neighbors for each pair of cell types in the RR and T-lep spatial-seq samples. The percentage of the numbers were calculated for each row, thus each row sum to 1. **f**, Heatmap showing the number of neighbors for each pair of cell types in the L-lep spatial-seq samples. The percentage of the numbers were calculated for each row, thus each row sum to 1.

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Software and code

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Data collection N/A

Data analysis The reads were aligned using STAR (v2.5.3.a). Drop-seq-pipeline (v1.12) was used to generate the expression matrix. The expression matrix was analyzed in R (v3.6.0) language with these packages: SoupX (v0.3.1), Seurat (v3.1.2), monocle (v2.10.1). The spatial data was processed by spaceranger (v1.1.0) provided by 10X Genomics. Codes are available in github page: https://github.com/mafeiyang/leprosy_amg_network

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The scRNA-seq data are available in GEO under accession number GSE151528. The spatial-seq data are available in GEO under accession number GSE167889. The reference genome (hg38) used for scRNA-seq was downloaded from Ensembl (http://ftp.ensembl.org/pub/release-104/fasta/homo_sapiens/dna/). There is no restriction in data availability.

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Life sciences study design

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Sample size	We obtained 10 samples for scRNA-seq, five RR and five L-lep. We also obtained 6 samples for spatial-seq with three RR, one T-lep and two L-lep. The sample sizes were sufficient as we obtained hundreds or thousands of cells or spots in each sample.
Data exclusions	We did not exclude any samples.
Replication	The samples in the same disease condition can be considered biological replicates. The replications were successful as the biological replicates showed similar gene expression profiles.
Randomization	Statistics were computed accounting for false discovery rates resulting from multiple testing, so no randomization was needed.
Blinding	Our goal was not to predict whether a sample was part of the disease or control group, but rather to study differences between different disease groups and therefore blinding could not be applied.

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Materials & experimental systems

Methods

n/a	Involved in the study	n/a	Involved in the study
☐	☒ Antibodies	☒	☐ ChIP-seq
☒	☐ Eukaryotic cell lines	☒	☐ Flow cytometry
☒	☐ Palaeontology and archaeology	☒	☐ MRI-based neuroimaging
☒	☐ Animals and other organisms		
☐	☒ Human research participants		
☒	☐ Clinical data		
☒	☐ Dual use research of concern		

Antibodies

Antibodies used	Anti-CXCL14 antibody-Rabbit monoclonal IgG-clone EPR22807-28, catalog number: ab264467, lot number: GR 33018268-2, Abcam. Anti-TAC1 (Substance P)-Rat monoclonal IgG-clone NC1/34HL, cat number: SC-21715, lot number: L0420, Santa Cruz. Anti-DEFB1-Mouse monoclonal IgG1-clone M11-14B-D10, catalog number: ab14425, lot number: GR 3344765-1, Abcam.
Validation	All primary antibodies were validated and titrated in human tonsil samples according to the manufacturer's instructions before tested on leprosy skin tissue. The work concentration for the monoclonal primary antibodies was: CXCL14 (5ug/ml), TAC1 (10ug/ml), and DEFB1 (10ug/ml).

Human research participants

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Population characteristics	Skin biopsy specimens were obtained from patients with leprosy at University of Southern California and Oswald Cruz Foundation (Brazil). Patients were classified according to standard clinical and histologic criteria. The population characteristics are saved in supplementary table 1.
Recruitment	Patients with leprosy are seen at the Hansen's disease clinics in Los Angeles and Rio de Janeiro. All patients are asked if they wish to participate in the ongoing research studies. Five patients with reversal reaction are designated here as RR1, RR2, RR3, RR4 and RR5. The other five are designated here as L-lep1, L-lep2, L-lep3, L-lep4 and L-lep5, of which four were classified as LL and one, L-lep2, as BL.

Ethics oversight

Informed written consent was obtained from human subjects under a protocol approved by the institutional review boards of the Oswaldo Cruz Foundation, University of Southern California and University of California Los Angeles. This study was conducted according to the Declaration of Helsinki Principles.

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