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Single-cell and spatial profiling of peripheral blood and synovium unveils pro-arthritis CNBP⁺ myeloid cells

Linzhu Wang^{1†}, Haitao Qi^{2†}, Yuhao Zhang³, Guozhao Wei¹, Zhongxuan Pan⁴, Xingyu Li¹, Fenghua Ma⁵, Huizhen Zhang¹, Ruonan Yang¹, Xuelian Li^{1*}, Xiaoqiang E^{5*} and Yunyan Gu^{3*} 

Abstract

Background Arthritis ranks as a primary contributor to global disability, with synovitis representing a common pathological feature across all types of arthritis. Given that macrophages constitute the predominant inflammatory cells in arthritic synovium, deciphering their heterogeneity facilitates the elucidation of pathogenic mechanisms.

Methods We integrated single-cell transcriptomic profiles from human peripheral blood and synovial tissue across 173 samples encompassing six types of arthritis to characterize transcriptional remodeling, including molecular signatures, transcriptional regulation, tissue distribution, and cellular interactions. Immunofluorescence staining and spatial transcriptomic analyses were performed to substantiate the proposed mechanism.

Results We identified an expanded population of peripheral CCHC-type zinc finger nucleic acid binding protein (CNBP)⁺ monocytes exhibiting elevated scores of the “inflammatory response” pathway in arthritis patients. Synovial CNBP⁺ macrophages presented the highest peripheral CNBP⁺ monocyte signature scores. Independent single-cell and bulk transcriptomic data revealed increased expression of CNBP⁺ macrophage signatures in both human and mice arthritis samples. CNBP⁺ macrophage signature scores were positively associated with inflammatory scores, as validated by immunofluorescence staining. Abundances of CD56⁺ CD16[−] natural killer (NK) cells and CNBP⁺ macrophages were positively correlated. Multiplex immunofluorescence staining from a limited number of synovial samples, together with spatial transcriptomic analyses suggested potential communication between CD56⁺ CD16[−] NK cells and CNBP⁺ macrophages via ANXA1–FPR3 axis. Furthermore, CNBP⁺ macrophages highly expressed MHC class II molecules and interacted with interferon-high CD4⁺ T cells through CD86 signaling.

Conclusions Our findings highlight the immune crosstalk involving CNBP⁺ macrophages in arthritis and enlighten treatment development targeting NK-macrophage-T axis.

[†]Linzhu Wang and Haitao Qi contributed equally to this work.

*Correspondence:

Xuelian Li

lixuelian@hrbmu.edu.cn

Xiaoqiang E

exq_517@163.com

Yunyan Gu

guyunyan@ems.hrbmu.edu.cn

Full list of author information is available at the end of the article



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Keywords Arthritis, Synovium, Macrophage, Inflammation, Single-cell RNA sequencing, Spatial transcriptomics

Background

Arthritis, notorious for being the most prevalent joint disease worldwide [1], is expected to become a leading cause of disability in the future [2]. Among the one hundred types of arthritis, rheumatoid arthritis (RA), osteoarthritis (OA), and gouty arthritis (GA) are the most common in the clinic [1]. Almost all forms of arthritis share common characteristics, including inflammation and articular cartilage degradation [3]. Long-term synovitis and joint dysfunction can lead to progressive disability in humans, resulting in a significant economic burden [4]. Currently, curing arthritis is still challenging due to the knowledge gap of its underlying mechanism [5]. Existing treatments primarily focus on symptom relief and improving quality of life rather than addressing the root cause [1]. Therefore, uncovering the pathogenic mechanism could provide new opportunities for targeted therapies.

Under inflammatory conditions, synovial tissue undergoes cellular proliferation, which is accompanied by infiltration of inflammatory cells [1]. Macrophages are the predominant inflammatory infiltrating cells, possessing both pro-inflammatory and pro-resolving properties that enable them to maintain or terminate inflammation [6]. This has emphasized the need to explore and characterize the heterogeneity of synovial macrophages in arthritis. Single-cell RNA sequencing (scRNA-seq) has revolutionized our understanding of macrophage heterogeneity, facilitating the identification of functionally distinct macrophage subtypes in both healthy and arthritic conditions [7]. Kurowska-Stolarska et al. identified two distinct synovial macrophage subtypes in RA, namely MerTK^{neg}CD206^{neg} and MerTK^{pos}CD206^{pos} [6]. In OA, Jiang et al. proposed two mutually exclusive and reversible synovial macrophage subtypes, namely pro-inflammatory M1 and anti-inflammatory M2 [8]. Although these studies have provided valuable insights, they primarily focused on one or two types of arthritis, hindering a comprehensive and in-depth characterization of macrophage heterogeneity in arthritis. Furthermore, it remains unclear whether synovial macrophages exhibit shared states that manifest an inflammatory phenotype across different types of arthritis.

CD4⁺ T cells play crucial roles in the initiation and persistence of arthritis-associated synovitis, whereas other cells contribute to arthritis progression by presenting antigens to CD4⁺ T cells via MHC class II molecules [9]. The inflammatory cascade driven by macrophages and T cells is of importance to arthritis progression [10], whereas $\alpha\beta$ T cell deficiency promotes macrophage polarization toward a pro-inflammatory phenotype [11].

Although current studies mainly focus on the crosstalk between T cells and macrophages, guidance for arthritis treatment is still limited, highlighting the need to explore interactions between macrophages and other immune cells. Natural killer (NK) cells can secrete cytokines to drive macrophages from a homeostatic state to an inflammatory state [12]. Wicks et al. found that synovial macrophages may regulate NK cells through interleukin (IL)-18, which subsequently contributes to the persistence of arthritis [13]. However, due to the low proportion of NK cells in synovial tissue, understanding the interaction between NK cells and macrophages remains a challenging field in arthritis research.

In this study, we integrated 17 scRNA-seq datasets from 173 human samples spanning 6 types of arthritis, and analyzed both peripheral blood (PB) and synovial tissue to construct a comprehensive immune cell landscape. Ten myeloid subtypes were identified separately in PB and synovial tissue. Beyond the previously defined myeloid subtypes, we discovered an uncharacterized myeloid subtype with high CCHC-type zinc finger nucleic acid binding protein (CNBP) expression, which was enriched in arthritis. In addition, we deciphered the features of CNBP⁺ myeloid cells, including their molecular signatures, transcriptional regulation, as well as interactions with immune cells.

Methods

Human synovial sample acquisition

The protocols for handling human synovium samples in this study were reviewed and approved by the Ethics Committee of Harbin Medical University (approval number: IRB5134724) and conducted in accordance with the Declaration of Helsinki. Patients with OA met the 1986 American College of Rheumatology classification criteria for OA [14]. Two synovium samples from arthritis patients were collected at the Department of Orthopedics at the First Affiliated Hospital of Harbin Medical University (Harbin, China) between March and June 2024. Synovitis was evaluated using Krenn's synovitis score (0–9), as previously described [15]. Sample NO.10-26269263 was scored as 3, while NO.12-26477938 was scored as 1. Written informed consent was obtained from all patients for data evaluation and publication in an anonymized form.

Single-cell/nuclear RNA-sequencing data collection and processing

We collected 10x single-cell transcriptomic data of mononuclear cells from 17 public datasets comprising 173 human PB and synovial samples. We retained tumor

necrosis factor (TNF) as a pro-inflammatory factor to compare cell proportions among juvenile idiopathic arthritis (JIA), TNF-treated JIA, TNF-treated healthy, and untreated healthy samples. The E-MTAB-11791 dataset included samples from RA, psoriatic arthritis (PsA), spondyloarthritis, and undifferentiated arthritis (UA) patients. However, due to the unavailability of specific disease classifications for each sample, we categorized them as inflammatory arthritis (IA), which was consistent with the original article [16]. We analyzed samples with clearly defined arthritis types according to their original classifications. Based on this, we divided the samples into ten groups: healthy, TNF-treated healthy, GA, IA, JIA, TNF-treated JIA, OA, PsA, RA, and ankylosing spondylitis (AS) (Supplementary Table 1).

For GSE155527, GSE159117, SCP469, GSE194315, and E-MTAB-11791 datasets, we downloaded the quality-filtered expression profiles. For the SRP330356 dataset, Cell Ranger (v8.0.0) was used for sequencing read mapping against the GRCh38 human reference genome, Unique Molecular Identifier (UMI) counting, and cell identification [17]. Then cells with 500~4,000 genes, UMIs number <20,000, and mitochondrial UMI count <10% were kept; genes detected in <3 cells were filtered out. For other datasets, we applied the quality control standards used in the original publications; otherwise, we established quality control criteria based on data distribution (Supplementary Table 2).

We additionally collected four scRNA-seq datasets and one single nucleus RNA sequencing dataset of synovial tissue (Supplementary Table 3). For GSE134420 and GSE211584, raw count matrices were processed using the same filtering steps as described above (Supplementary Table 2). For E-MTAB-8322, GSE277596, and GSE243917, we obtained quality-filtered expression profiles of synovial macrophages. For GSE277596, samples were annotated with treatment response labels: responder, refractory, non-responder, and partial responder. Cells from this dataset were grouped into three categories: (1) responder, (2) non-responder (including non-responder and partial responder), and (3) refractory.

Potential doublet cells were identified using the DoubletFinder (v2.0.3) package with default parameters [18], assuming an expected doublet rate of 7.5%. The count matrix was log-normalized with default settings, and the top 3,000 variable genes were scaled and used for principal component analysis. Data integration was performed in two steps. First, Harmony (v0.1.1) package was applied to remove batch effects between samples within each dataset, with batch correction efficiency assessed using the Local Inverse Simpson's Index (Fig. S1A and S1B) [19]. Subsequently, Harmony was reapplied to correct for inter-dataset batch effects. Cell clusters were

identified using the first 20 principal components via the FindClusters function within Seurat (v5.0.1) package [20]. The annotation of resulting clusters into major cell types relied on the expression patterns of canonical marker genes, including T/NK cells (IL7R and CD3D), myeloid cells (CD14), stromal cells (PDGFRA), endothelial cells (MCAM and FLT1), and B cells (CD79A) [21–23], with an additional cluster designated “other” that lacked detectable expression of known cell-type markers. We performed unsupervised graph-based clustering on myeloid cells and then identified three common major lineages based on canonical cell markers, including monocytes (S100A8, CD14, and FCGR3A), macrophages (C1QB) and dendritic cells (DCs) (S100B and BATF3) [24–26]. Monocytes with high FCGR3A expression were classified as non-classical monocytes, while those with low FCGR3A expression were classified as classical monocytes. CD4⁺ T cells, CD8⁺ T cells, and NK cells were characterized by specific high expression of CD4, CD8A/CD8B, and NCAM1, respectively [21]. To determine the optimal clustering resolution of myeloid or T/NK cells, we applied Ratio of Global Unshifted Entropy (ROGUE), an entropy-based statistic that quantifies cluster purity. A ROGUE value of 0.9 was used as the threshold, where the number of infiltrating cells and varied genes were well constrained [27]. We calculated the number of datasets with ROGUE < 0.9 across resolutions from 0.1 to 1 (step size 0.1). For integrated datasets, we calculated the number of datasets below this threshold, whereas for individual datasets, we counted the number of samples. The resolution yielding the fewest datasets/samples with ROGUE < 0.9 was selected for downstream analysis. The final clustering resolutions were as follows: integrated PB myeloid cells (0.1), integrated synovial myeloid cells (0.2), integrated PB T/NK cells (0.1), integrated synovial T/NK cells (0.1), E-MTAB-8322 (0.6), GSE243917 (0.2), and GSE134420 (0.5) (Fig. S3). Cell subtypes were annotated based on their highly expressed marker genes.

For cell annotation, we identified differentially upregulated genes in each subtype (FindMarkers: $p.adjust < 0.05$ and $\log_2$ fold change > 0.1) and named them based on the biological pathways. For instance, PB CXCR2⁺ monocytes were enriched in the “chemotaxis” pathway, whereas BANK1⁺ monocytes showed enrichment for B cell-related pathways. Synovial C5 myeloid cells exhibited high scores for signature genes of PB C3 myeloid cells, suggesting similarity. The overlap of upregulated genes revealed CNBP, prompting their designation as CNBP⁺ myeloid cells.

Bulk transcriptomic data collection and processing

To assess whether CNBP⁺ monocytes/macrophages were associated with arthritis status, we collected 11

bulk transcriptomic datasets of arthritis, including one whole blood, three PB, and seven synovial tissue datasets (Supplementary Table 4). For E-MTAB-11611 and E-MTAB-6141 datasets, all fastq files were aligned to the human reference genome build GRCh38 with HISAT (v2.2.1). FeatureCounts (v2.0.6) was then used to generate integer-based read counts, and the count values were normalized to Transcripts Per Million values [28]. For the GSE93272 dataset, raw expression profiles by Affymetrix platform chip were acquired, and affy (v1.74.0) package was applied to normalize expression value based on the Robust Multichip Average method. For GSE117769, GSE89408, and GSE225731 datasets, the downloaded count expression profiles were normalized to Transcripts Per Million expression profiles. For other datasets, annotation files supplied from the matching detection platforms were used to convert probes to gene level. The glm function of stats (v4.3.0) package was used to evaluate the impact of CNBP⁺ monocyte signature expression on disease development, and genes with $p < 0.05$ were considered significantly associated.

Tissue preference evaluation of subtypes

To detect the tissue preference of each subtype, we calculated the ratio of observed to expected cell counts (Ro/e), where the expected cell numbers were obtained using the chi-square test, as previously described [29]. To assess whether CNBP⁺ monocytes were more prevalent in arthritis samples than in healthy samples, we constructed contingency tables for each type of arthritis and healthy samples based on the number of CNBP⁺ monocytes and performed chi-square test to evaluate statistical significance. Similarly, we applied the same approach to compare the proportion of NK cells across different types of arthritis relative to OA. Additionally, to determine whether CXCR2⁺ monocytes were abundant in a specific age group compared to other myeloid cells, we generated contingency tables categorizing CXCR2⁺ monocytes and other myeloid cells within each age group and assessed statistical significance using chi-square tests.

Signature identification and scoring

Differentially expressed genes were identified using FindMarkers function, retained genes with $p < 0.05$ and log2 fold change > 0.1 . CNBP⁺ monocyte/macrophage signatures (Supplementary Table 5) were identified based on the following criteria: (1) genes differentially expressed in CNBP⁺ monocytes/macrophages compared to other myeloid cells; and (2) not differentially expressed in B cells, T/NK cells, endothelial cells, stromal cells, and the cluster labeled as “other”. For the signature of CD56⁺ CD16⁺ NK cells, the same filtering steps were applied.

To evaluate the robustness of the CNBP⁺ monocyte/macrophage signatures, additional sensitivity analyses

were performed using the log2 fold change cut-offs of 0.1, 0.2 and 0.3. Additionally, a leave-one-dataset-out strategy was employed. In each iteration, one dataset was held out as the validation set, CNBP⁺ myeloid signatures were identified using the remaining datasets, and their classification performance to distinguish CNBP⁺ myeloid cells from other myeloid cells was evaluated in the held-out dataset. Receiver operating characteristic analysis demonstrated consistently high discriminative performance across datasets, with area under the curve remaining robust under different log2 fold change thresholds (0.1, 0.2, and 0.3) (Fig. S4A and S6G). In Fig. S4B, prevalence represented the proportion of peripheral CNBP⁺ monocytes within each dataset and varied across datasets. As area under the precision-recall curve (AUPRC) was susceptible to class imbalance, it was not optimal for evaluating signature robustness.

Metabolic and interferon pathway signatures were derived from MsigDB [30]. Signature genes of senescence and aging were obtained from recent studies [31–33]. We used AUCell to quantify signature/pathway scores in myeloid cells at single-cell level. For each cell, genes were ranked by normalized expression values, and gene set enrichment was quantified by calculating the area under the curve based on the top 10% of ranked genes. Statistical significance was calculated using Kruskal-Wallis test, with $p < 0.05$ considered significant. Scores of signatures/pathways in samples or spots were calculated using the single sample gene set enrichment analysis in GSVA (v1.44.5) package, with a Gaussian kernel density estimation and absolute ranking of gene expression values [34]. Since 5,000 genes were detected in the GSE243917 dataset, the number of detected genes involved in “regulation of acute inflammatory response to antigenic stimulus” was insufficient for robust analysis. We excluded this dataset for scoring this pathway. Function genes of NK cells were obtained from Davis et al. and Zhang et al. [35, 36].

Cell-cell communication, cell trajectory and transcription factor regulon analysis

We applied CellChat (v2.1.2) to infer communications between synovial myeloid cells and T/NK cells [37]. CytoTRACE2 (v1.1.0) was employed to evaluate the differentiation potential and stemness of the monocytes. Monocle3 (v1.3.5) and Slingshot (v2.7.0) were used to analyze the differentiation trajectory of synovial myeloid cells [38, 39]. Monocytes could differentiate into macrophages or DCs [40]. Given that CytoTRACE2 analysis revealed that synovial EML4⁺ monocytes have high differentiation potential, they were therefore designated as the origin of the differentiation trajectory in pseudotime analysis.

TF regulons were generated via the *dorothea_regulon_* human function from *dorothea* (v 1.16.0) package. TFs with confidence levels (A–C) were selected for further analysis. The activity of each TF regulon was performed with *dorothea* package with the default parameters [41]. The normalized enrichment score of MYC in each cell was inferred by *viper* (v1.38.0) package with the default parameters [42].

We intersected glucose/pyruvate metabolism pathway genes with differentially expressed genes of CNBP⁺ macrophages. MYC target genes were obtained from *dorothea*, and a one-sided hypergeometric test was performed to assess whether MYC targets were significantly overrepresented among these differentially expressed metabolism pathway genes.

Transcriptional similarity evaluation of subtypes

To assess the transcriptional similarity among myeloid subtypes, we first selected the top 20 differentially expressed genes of each subtype, ranked them by fold change, and extracted their expression profiles for further analysis. We then calculated the average expression value of each gene within each subtype and used Pearson correlation to measure expression similarity between subtypes. The distance between any two cell subtypes was defined as $(1 - \text{Pearson correlation coefficient})/2$ [29].

Spatial transcriptomic data collection and deconvolution analysis

We downloaded the spatial transcriptomic data for four RA samples from Raychaudhuri et al. [43] and two PsA samples from Ramming et al. [44]. *Cell2location* (v 0.1.3) was employed to spatially map cell types onto Visium slides using the integrated synovial scRNA-seq atlas as the reference [45]. Each Visium slide was analyzed independently using identical hyperparameters and prior settings. Models were trained on all spatial locations ($N_{\text{cells_per_location}} = 30$, $\text{detection_alpha} = 20$, and $\text{max_epochs} = 3000$).

The presence of a given cell type at the spot level was determined based on *Cell2location*-estimated cell abundance. The proportion of a specific cell type was calculated for each Visium slide independently, as the fraction of spots in which the estimated cell abundance exceeded the average abundance computed within that slide [46]. To identify spots containing CNBP⁺ macrophages, we first designated spots with above-average abundance across all myeloid cells as myeloid spots. These myeloid spots were then further classified as either CNBP⁺ macrophages or other myeloid cells, depending on whether the abundance of CNBP⁺ macrophages in a given spot exceeded the average *Cell2location*-measured cell abundance across all spots. We assessed spots containing CD56⁺ CD16[−] NK cells using the same approach. We

defined the co-expression of CNBP–CD68 and ANXA1–FPR3 pairs as the geometric mean expression values. For ANXA1–FPR3 pair, permutation tests were performed to assess interaction specificity. The observed difference in mean ANXA1–FPR3 co-expression level between CNBP⁺ macrophage and CD56⁺ CD16[−] NK cell co-localization spots and other myeloid and T/NK cell co-localization spots was compared against a null distribution generated by randomly permuting spot labels 1,000 times while preserving group sizes. The observed difference consistently exceeded those obtained from random shuffling (Fig. S13C).

As a negative control, the top five upregulated genes of T/NK cells were selected from the integrated synovial single-cell dataset. Single-sample gene set enrichment analysis was performed using GSVA to calculate spot-level signature scores. The scores were significantly higher in T/NK cell spots than in CNBP⁺ macrophage spots (Fig. S6H).

We implemented the Getis-Ord G_i^* (G_i -star) statistic to identify spatial clusters of continuous gene signatures across tissue slides. By comparing regions of high or low gene expression, pathway scores, and subtype proportions against a null hypothesis of random distribution, this analysis revealed statistically significant “hotspots” and “coldspots” [47]. These hotspots represent areas with a high concentration of specific subtypes or molecular signatures, indicating non-random aggregation, whereas coldspots represent areas with depleted subtypes or gene signatures. We then employed SpottedPy to quantify the proximity between defined gene signature hotspots and particular subtype hotspots/coldspots [47], such as between CNBP⁺ macrophage hotspots and MHC class II molecule hotspots. Quantitative hotspot proximity statistics, including mean distances, fold changes, and false discovery rates (FDR) adjusted using the Benjamini-Hochberg method, were summarized in Supplemental Table 6.

Functional enrichment and correlation analysis

To identify overrepresented pathways in PB myeloid subtypes, we performed Gene Ontology Biological Process enrichment analysis on differentially expressed genes of each subtype using the clusterProfiler (v4.8.1), and $\text{FDR} < 0.05$ was considered statistically significant. The top five pathways uniquely enriched in each subtype were retained, visualized, and ranked by FDR.

Inflammatory pathways were derived from MsigDB. We identified differentially expressed genes in PB CNBP⁺ monocytes with FindMarkers ($p < 0.05$, $\log_2$ fold change > 0.1) and ranked them by fold change. We then performed Gene set enrichment analysis (GSEA) using clusterProfiler to evaluate enrichment of inflammatory pathways, considering $p < 0.05$ as significant.

To assess the correlation between a given subtype signature score and clinical indicators in bulk data, we performed Spearman's rank correlation analysis. Multivariable linear regression analysis was conducted with age and gender included as covariates. *P* values were adjusted using the Benjamini-Hochberg procedure. For single-cell data, we analyzed the correlation between the proportions of CNBP⁺ macrophages and CD56⁺ CD16⁻/CD56⁻ CD16⁺ NK cells. Only samples containing all three cell types were included in the analysis, and Spearman's rank correlation was used to evaluate the correlation between their proportions.

Multiplex immunofluorescence staining

Synovial tissue sections from two individuals were fixed in 4% paraformaldehyde for 30 minutes at room temperature, followed by paraffin embedding. After permeabilization with 0.5% Triton-X100 for 60 minutes, the sections were blocked with bovine serum albumin for 30 minutes. Immunohistochemistry was performed using CNBP (14717-1-AP, Proteintech, China), CD68 (GB115723-50, Servicebio, China), CD56 (GB12041-50, Servicebio, China), CD3 (GB11014-50, Servicebio, China), ANXA1 (21990-1-AP, Proteintech, China), and FPR3 (CSB-PA008856LA01HU, CUSABIO, China) antibodies. Slides were scanned and analyzed using the PerkinElmer Vectra3 platform. CNBP⁺ macrophages were defined as cells co-expressing CNBP and CD68. CD56⁺ CD16⁻ NK cells were identified as CD3-negative cells expressing CD56 [36].

Hematoxylin and Eosin staining

The Hematoxylin and Eosin (H&E) staining Kit (G1120, Solarbio, China) was used to assess morphological changes. Paraffin sections of synovial tissues were stained with hematoxylin for 5–10 minutes, rinsed with tap water, and then immersed in eosin dye for 1–3 minutes. The sections were then dehydrated through graded alcohol and cleaned with xylene. Images were acquired using a microscope (Olympus).

Statistical analysis

Statistical analyses were performed using R software (v 4.3.0/4.2.0), python software (v 3.9.19) and GraphPad Prism software (v9.0). Statistical significance was calculated using two-tailed Wilcoxon rank-sum test, chi-square test, Kruskal-Wallis test, and one-sided hypergeometric test as described in the Figure legends. For multiple comparisons, *P* values were adjusted using the Benjamini-Hochberg method.

Results

A single-cell atlas of peripheral blood and synovium in arthritis

We compiled publicly available scRNA-seq data from healthy individuals and patients with six arthritis types, generating a comprehensive single-cell atlas of PB and synovial tissue. After quality control, 494,021 PB cells and 430,148 synovial cells from 173 samples were retained for analysis (Fig. 1A). PB and synovial tissue data were collected simultaneously only for AS, PsA, and RA. For other conditions, only one tissue type was profiled (Fig. 1B). Cells from PsA, AS, and healthy donors were the most abundant, accounting for 28.64%, 13.65%, and 15.63% of the total, respectively (Fig. 1C). Addressing the concerns about batch effects, we observed that cells were clustered based on cell types rather than pathological status in PB (Fig. S2A). Based on unsupervised clustering of PB data, four major clusters including B cells, myeloid cells, T/NK cells, and a cluster labeled as “other” were defined (Fig. 1D, S2B, and S2C). These four clusters were consistently observed across all conditions, with T/NK cells being the dominant population. TNF-treated samples showed the lowest proportion of myeloid cells (Figs. 1E and F).

Similarly, six clusters were identified in synovial tissue based on canonical markers: B cells, endothelial cells, myeloid cells, stromal cells, T/NK cells, and others (Fig. 1G and S2D). The same cluster from different conditions was well-mixed after batch correction, indicating negligible batch effects (Fig. 1H). The cellular composition in AS patients was distinct from that in other arthritis patients, with a predominance of myeloid cells and T/NK cells, whereas stromal cells were more abundant in the other conditions (Fig. 1I). Cells assigned to the same cluster across PB and synovial tissue exhibited similar gene expression patterns. For instance, T/NK cells showed high level of IL32; myeloid cells displayed high levels of LYZ and S100A9; and B cells expressed elevated level of CD79A (Fig. 1J and S2C). PB was predominantly composed of T/NK cells and the synovium was enriched for stromal cells, whereas myeloid cell proportions were comparable between the two (Fig. 1K). Given these observations, we subsequently characterized myeloid subtypes to elucidate their roles in arthritis pathogenesis in both PB and synovial tissue.

Transcriptional diversity of peripheral blood myeloid cells

To investigate the heterogeneity of PB myeloid cells, we performed unsupervised graph-based clustering and identified ten transcriptionally distinct myeloid subtypes (Fig. 2A and S2E-H). Transcriptional similarity analysis revealed that ALDH2⁺ DCs closely resembled S100A10⁺ monocytes, while monocytes were distributed in four branches (Fig. S2G). OAZ1⁺ monocytes were comparably

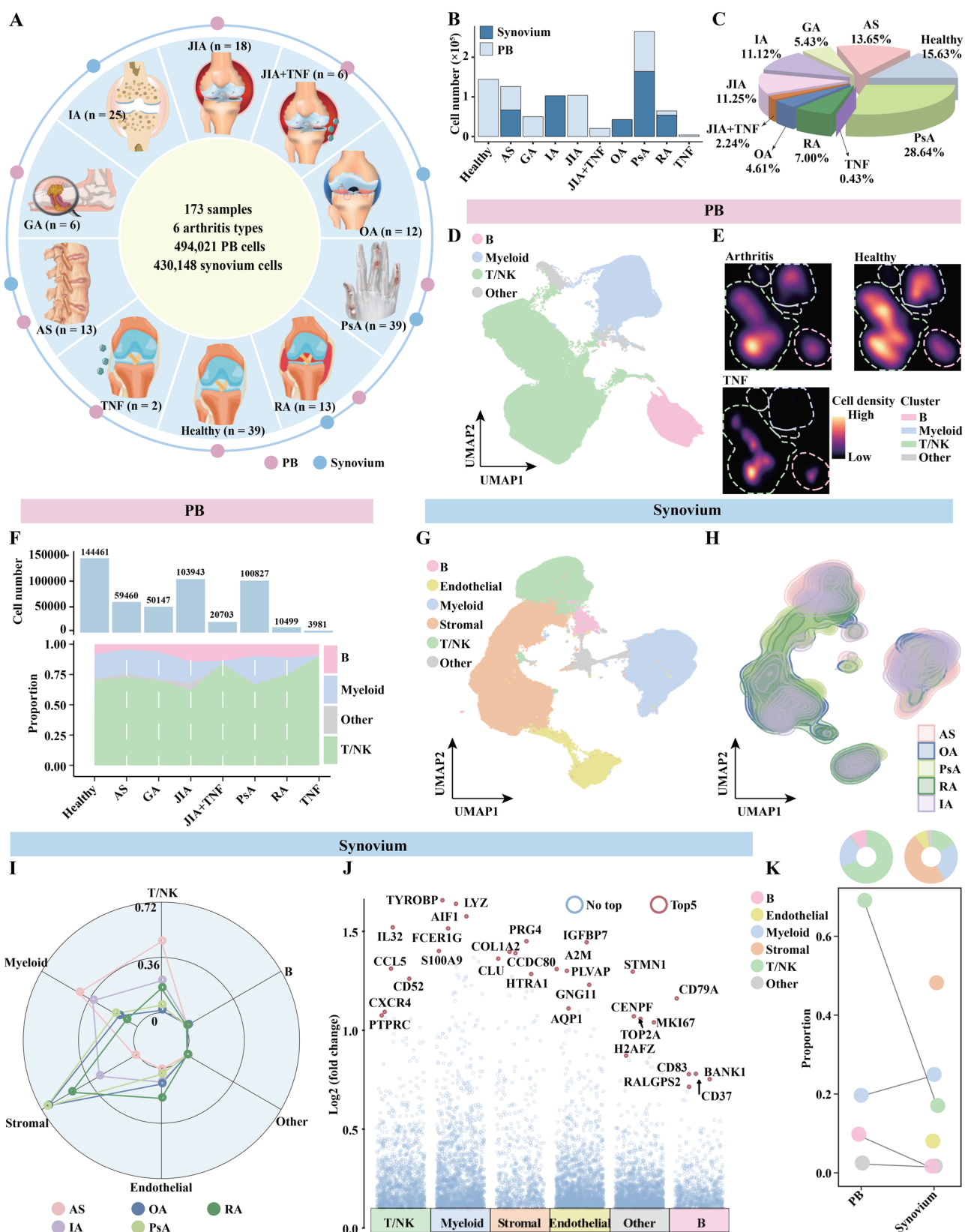


Fig. 1 (See legend on next page.)

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Fig. 1 A single-cell atlas of peripheral blood and synovium in arthritis. **(A)** Schematic of the study design. A total of 17 published datasets were analyzed, with cohort and sample information detailed in Supplementary Table 1. **(B)** Bar plots depicting the number of included cells, with the exact counts labeled above each bar (light blue: PB; dark: synovium). **(C)** Pie chart depicting proportion of cells under each condition. **(D)** Uniform manifold approximation and projection (UMAP) visualization of four major PB clusters (494,021 cells). **(E)** UMAP density plot illustrating the distribution of four major PB clusters across three main groups, with higher relative cell density represented by brighter magma colors. **(F)** Bar plots showing the proportions of the four major PB clusters (bottom) and total cell counts (top) under different conditions. **(G)** UMAP visualization of six major synovial cell clusters (430,148 cells). **(H)** UMAP density plot illustrating the distribution of synovial cells across five arthritis types. **(I)** Radar plot displaying the proportions of six synovial clusters across five types of arthritis. **(J)** Volcano plots showing differentially expressed genes for each synovial cluster (red represents the top5 differentially expressed genes, blue represents non-top5 differentially expressed genes). **(K)** Line plots showing the proportions of major clusters between PB and synovium. Circular plots at the top showing the proportions of major clusters in PB (left) and synovium(right) samples. *P* value was calculated by two-tailed Wilcoxon rank-sum test **(J)**

represented in both arthritis and healthy samples and were characterized by high expression of genes involved in “response to tumor necrosis factor” (Fig. 2B and S5A). Ro/e analysis revealed six subtypes enriched in specific arthritis types compared with healthy samples: S100A10⁺ monocytes, CNBP⁺ monocytes, RNASEK⁺ monocytes, CXCR2⁺ monocytes, S100P⁺ monocytes, and BANK1⁺ monocytes. For instance, CXCR2⁺ monocytes and S100P⁺ monocytes were scarcely detected in healthy donors, JIA patients, and AS patients, but were enriched in GA patients (Fig. 2B). In contrast, RNASEK⁺ monocytes with high expression of genes associated with “purine nucleoside triphosphate biosynthetic process” were enriched in JIA and AS but showed decreased infiltration in GA and healthy samples (Fig. 2B and S5A). CNBP⁺ monocytes, which displayed elevated expression of genes linked to “positive regulation of T cell activation”, were enriched across multiple types of arthritis samples, excluding GA samples, highlighting disease-specific heterogeneity (Figs. 2C, D, and S5A).

CXCR2⁺ monocytes and S100P⁺ monocytes, which were predominant in GA, exhibited a high score of “sphingolipid metabolism” (Fig. 2E and S5C). Studies have revealed that sphingolipids could regulate immune and senescence [48]. TNFSF14, a potential biomarker for flare gout [49], showed elevated expression in CXCR2⁺ monocytes (Fig. S5B). CXCR2⁺ monocytes were not detected in individuals aged 50–59 years (Fig. 2F). They exhibited the lowest CytoTRACE2 scores but the highest senescence and aging scores (Figs. 2G, H, and S5D), suggesting limited differentiation potential and enhanced senescence. ICAM1, a known player in senescence, was highly expressed in CXCR2⁺ monocytes (Fig. 2I). Furthermore, CSRNP1, a transcription factor governing ICAM1 expression, displayed the highest regulon activity and gene expression in CXCR2⁺ monocytes (Fig. 2J and K), indicating that the CSRNP1–ICAM1 axis potentially regulates monocyte senescence.

GSEA revealed upregulation of “inflammatory response” pathway in CNBP⁺ monocytes (Fig. 2L). RA samples showed elevated CNBP⁺ monocyte signature scores compared to healthy controls (Fig. S5E). The signatures of CNBP⁺ monocytes served as risk genes for

different types of arthritis (Fig. S5F). In addition, we identified several high-scoring regulons in CNBP⁺ monocytes that potentially regulated genes related to T cell-related pathways, including IKZF3, BCL11B, TCF7L2, TBX21, MYBL1, and PITX2 (Fig. 2M and N).

CNBP⁺ macrophages are characterized by high expression of MHC class II molecules

Ten synovial myeloid subtypes with different expression patterns were identified, including one DC lineage, four macrophage lineages, and five monocyte lineages (Figs. 3A and S6A–C). Most subtypes clustered by lineage, although C1QC⁺ macrophages grouped with monocytes and CNBP⁺ macrophages grouped with DCs (Fig. 3B). CNBP⁺ macrophages exhibited the highest scores of CNBP⁺ monocyte signatures, followed by CPNE3⁺ DCs (Fig. 3C and S6D). Among macrophage subtypes, C1QC⁺ macrophages exhibited elevated expression of MERTK, MRC1, and TREM2, indicating a protective lining macrophage phenotype (Fig. S6E). Conversely, CNBP⁺ macrophages expressed high levels of CD48 and CLEC10A (Fig. S6E), markers of inflammatory macrophages [50]. The distribution of synovial myeloid subtypes varied across different arthritis types (Fig. 3D). Both CNBP⁺ macrophages and CPNE3⁺ DCs were enriched for MHC class II molecules, such as HLA-DPB1 and HLA-DPA1 (Fig. 3E). However, nearly all myeloid subtypes displayed high expression levels of MHC class I molecules (Fig. 3E), consistent with their broad expression on all nucleated cells [51]. CNBP⁺ macrophages displayed elevated scores of T cell-associated pathways, analogous to CNBP⁺ monocytes, distinguishing them from other myeloid subtypes (Fig. 3F and S6F). Multiple transcriptional regulons not only displayed high scores in CNBP⁺ macrophages but also positively correlated with T cell-related pathways (Fig. 3G), implying a complex regulatory role in T cell activation. Additionally, target genes of MYC and KLF16 were enriched in “T cell differentiation involved in immune response” pathway (Fig. 3H). We collected synovial tissues from arthritis patients to validate the presence of CNBP⁺ macrophages. X-ray imaging and H&E staining showed that the first patient had milder synovial pathology compared to the second. Double

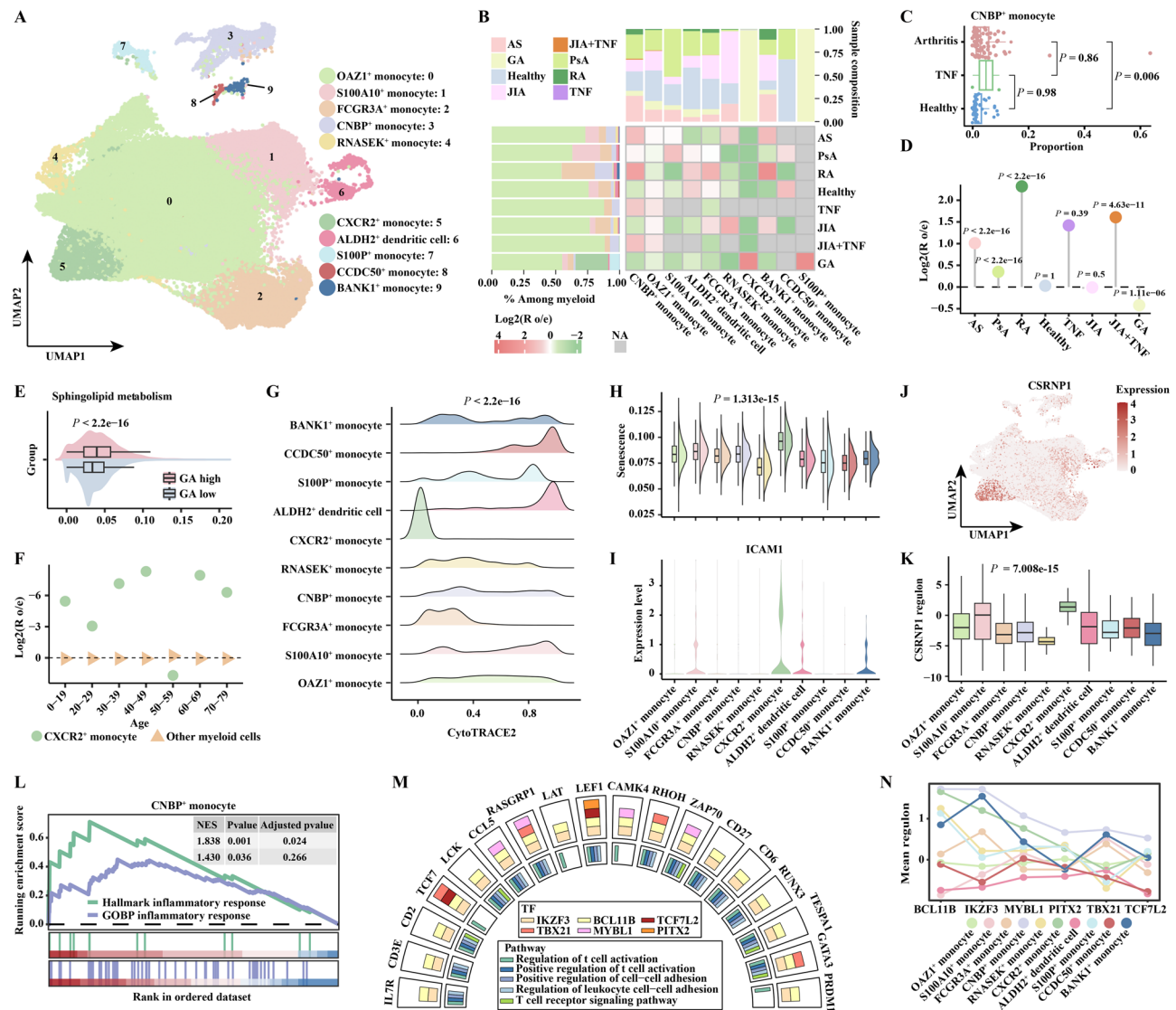


Fig. 2 Transcriptional diversity of peripheral blood myeloid cells. **(A)** UMAP visualization of PB myeloid subtypes (95,953 cells). **(B)** Heatmap displaying condition prevalence for each myeloid cell subtype, estimated by the Ro/e with the chi-square test. Bar plots showing cell composition (top) and tissue composition (left). **(C)** Box plots depicting CNBP⁺ monocyte proportions across three conditions: arthritis (AS, GA, JIA, TNF-treated JIA, PsA, and RA), TNF (TNF-treated healthy), and healthy (untreated healthy) groups. **(D)** Line plots of Ro/e scores for CNBP⁺ monocytes compared to healthy controls, with exact *P* values indicated. **(E)** Violin plots showing “sphingolipid metabolism” pathway signature scores between GA-enriched myeloid subtypes (CXCR2⁺ monocytes and S100P⁺ monocytes) and other myeloid subtypes. **(F)** Dot plots illustrating age-associated prevalence (Ro/e) for CXCR2⁺ monocytes. **(G)** Ridge plots of CytoTRACE2 scores across myeloid subtypes. **(H)** Raincloud plots of “senescence” signature scores across myeloid subtypes. **(I)** Violin plots showing ICAM1 expression levels across myeloid subtypes. **(J)** UMAP plot depicting CSRNPI expression levels. **(K)** Bar plots showing the inferred CSRNPI regulon activity across myeloid subtypes. **(L)** Gene set enrichment analysis showed “inflammatory response” pathways were upregulated in CNBP⁺ monocytes. **(M)** The circular heatmap illustrating the regulatory relationships between transcription factors and 18 target genes in the outer ring, with bar colors indicating the corresponding regulators. The inner ring shows the involvement of these genes in the top five pathways of CNBP⁺ monocytes. **(N)** Line plots illustrating average regulon activities of six transcription factors across myeloid subtypes. *P* value was calculated by two-tailed Wilcoxon rank-sum test (**C** and **E**), chi-square test (**D**), Kruskal-Wallis test (**G**, **H**, and **I**), and one-sided hypergeometric test (**L**)

immunofluorescence staining showed greater co-localization of CNBP and CD68 in the second sample (Figs. 3I and J).

To assess the spatial distribution of CNBP⁺ macrophages, deconvolution analysis of spatial transcriptomic data was performed. CNBP⁺ macrophage spots exhibited high co-expression of CNBP and CD68 (Fig.

S6I). Moreover, CNBP⁺ macrophage spots showed high expression of genes associated with “T cell differentiation involved in immune response”, “regulation of acute inflammatory response to antigenic stimulus”, and “MHC class II molecules” pathways, broadly aligning with results of scRNA-seq data (Fig. 4A and S7A). In addition, SpottedPy analysis indicated a potential spatial

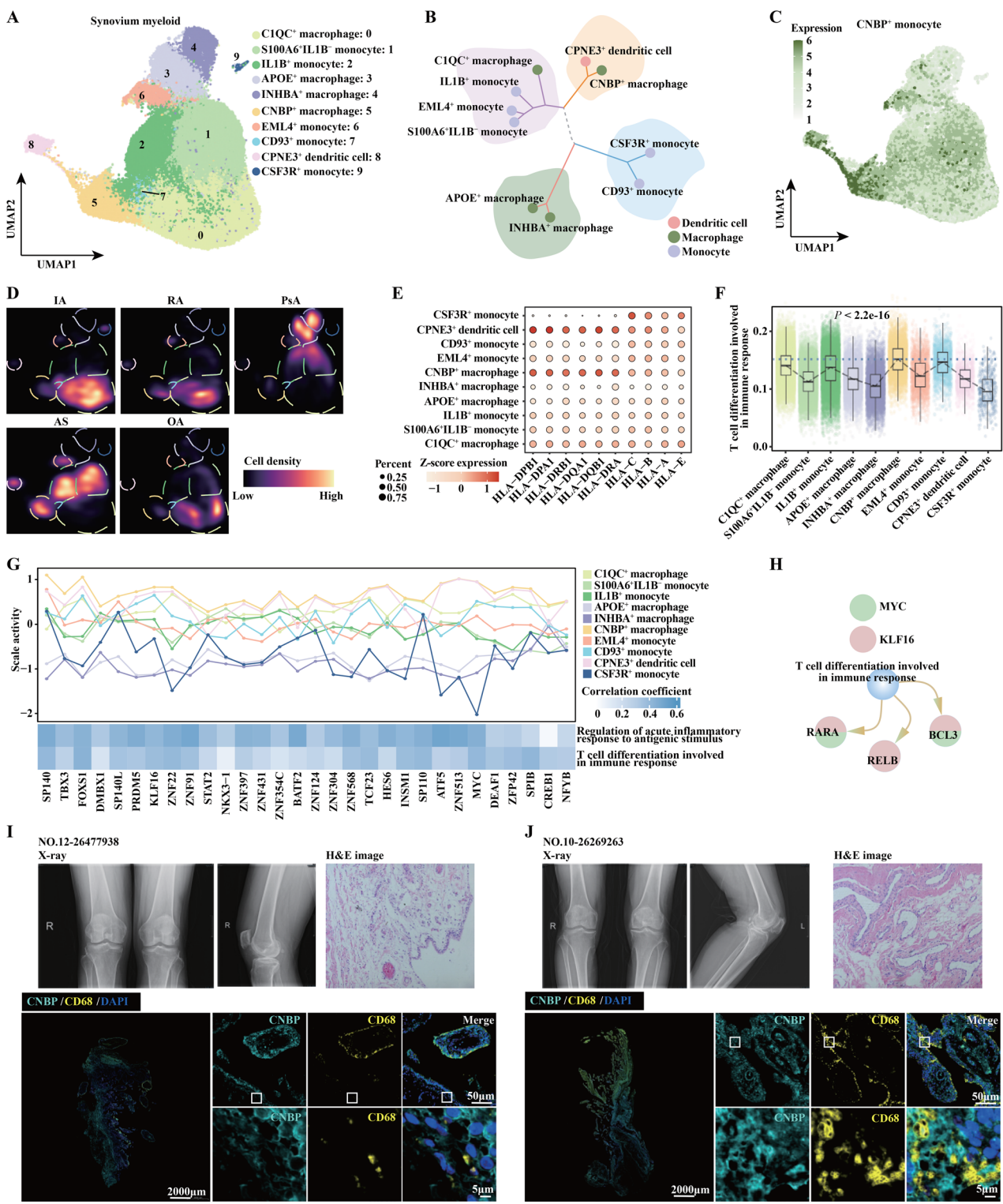


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Fig. 3 Transcriptional diversity of synovial myeloid cells. **(A)** UMAP plot depicting synovial myeloid subtypes (106,109 cells). **(B)** Transcriptomic similarities among synovial myeloid subtypes based on differentially expressed gene expressions. **(C)** UMAP plot displaying CNBP⁺ monocyte signature scores. **(D)** UMAP density plot illustrating the distribution of myeloid subtypes across five arthritis types in synovium. **(E)** Heatmap showing the MHC gene expression across myeloid subtypes. **(F)** Bar plots displaying scores of “T cell differentiation involved in immune response” across myeloid subtypes. **(G)** Line plots illustrating average transcription factor regulon activities across myeloid subtypes (top). Heatmap showing the correlation between transcription factor regulon activities and pathway scores (bottom). **(H)** Regulatory network of target genes and their associated pathway, with node colors indicating transcription factor regulators. **(I–J)** X-ray and H&E images of synovial tissue from arthritis patients NO0.12-26477938 **(I)** and NO0.10-26269263 **(J)** (top). Double immunofluorescence staining showing CNBP⁺ macrophages in patients NO.12-26477938 **(I)** and NO.10-26269263 **(J)** (bottom). *P* value was calculated by Kruskal-Wallis test **(F)** and Spearman's rank correlation analysis **(G)**

association between MHC class II molecule hotspots and CNBP⁺ macrophage hotspots (Fig. 4B). Compared with CNBP⁺ macrophage coldspots, CNBP⁺ macrophage hotspots exhibited shorter spatial distances to hotspots of “T cell differentiation involved in immune response”, “regulation of acute inflammatory response to antigenic stimulus”, and “MHC class II molecules” (Fig. 4C).

MYC regulates glucose metabolism in CNBP⁺ macrophages

In inflamed synovium, immune cells undergo metabolic reprogramming from oxidative phosphorylation to glycolysis, contributing to activation of inflammatory signaling and exacerbation of tissue damage [52]. We observed that CNBP⁺ macrophages exhibited high scores of “glucose metabolism” (Fig. S7B), indicative of increased energy demands. In addition, CNBP⁺ macrophages displayed the highest scores of “pyruvate metabolism” and “lactate metabolic process” pathways (Fig. 5A and B), indicating a metabolic shift toward anaerobic glycolysis. Consistently, CNBP⁺ macrophages showed increased expression of LDHA and LDHB, which encode enzymes essential for the interconversion of pyruvate and lactate (Fig. S7C and S7D). Transcriptional regulators MYC and ZNF513 were positively correlated with metabolism scores and regulated key metabolic genes, including PKM, LDHA, LDHB, and ENO1 (Fig. 5C and D). By employing CytoTRACE2, Monocle3, and Slingshot, we identified EML4⁺ monocytes as the putative initial differentiation state, which could give rise to CNBP⁺ macrophages and further transition into CPNE3⁺ DCs (Fig. S7E and S7F). Along pseudotime trajectory, dynamic changes in expression of ENO1, PKM, and LDHA were observed, with peak expression levels detected in CNBP⁺ macrophages (Fig. 5E and S7G). Moreover, SpottedPy analysis revealed a spatial tendency for hotspots of glucose metabolism and pyruvate metabolism to be more likely located near CNBP⁺ macrophage hotspots than near CNBP⁺ macrophage coldspots (Fig. S7H). Spatial transcriptomic data showed that CNBP⁺ macrophage spots were enriched for “glucose metabolism” and “pyruvate metabolism”, accompanied by upregulation of MYC, ENO1, PKM, and LDHA (Fig. 5E, S8A, and S8B). The viper analysis showed that MYC exhibited high normalized enrichment scores in CNBP⁺ macrophages among myeloid cells (Fig. S8C). MYC target genes were

significantly overrepresented among metabolism pathway genes that were differentially expressed in CNBP⁺ macrophages (Fig. S8D). These patterns indicate a potential spatial correlation that should be interpreted cautiously, as functional implications cannot be inferred without further validation.

Arthritis samples show elevated CNBP⁺ macrophage signature scores

CNBP⁺ macrophage signature scores were elevated in both RA and OA samples compared to healthy samples, with the highest levels in RA samples (Fig. 6A, B, and F). Moreover, CNBP⁺ macrophage signature score was positively correlated with several clinical indicators, including tender joint count in 28 joints (TJC28), disease activity score in 28 joints with C-reactive protein (DAS28CRP), erythrocyte sedimentation rate (ESR), and inflammation score (Fig. 6C, G, and H). Consistent associations were observed after adjustment for age and sex and FDR correction (Fig. S9A–C). In PsA patients, refractory samples showed the highest CNBP⁺ macrophage signature scores relative to those of non-responders and responders (Fig. 6D). In OA, inflammatory samples exhibited higher CNBP⁺ macrophage signature scores than in normal/reactive samples (Fig. 6E). Collectively, these findings suggest that CNBP⁺ macrophage signatures are potential indicators of disease severity across multiple forms of arthritis.

To further evaluate CNBP⁺ macrophage signatures in arthritis, we analyzed three additional scRNA-seq datasets and one single-nucleus RNA-seq dataset. Unsupervised clustering identified 13 synovial macrophage subtypes derived from healthy donors, individuals with UA, and RA patients (Fig. 6I, S10A, and S10B). Subtype 0 predominated in healthy samples, while UA samples were primarily composed of subtypes 0–2, and RA samples included subtypes 0–4 (Fig. 6J), suggesting that subtype 0 may represent a subtype shared between healthy and arthritic states. As expected, CNBP⁺ macrophage signatures were highly expressed in RA, with the highest expression in subtype 3 (Figs. 6J, K, and S10C). Furthermore, T cell-related pathways were enriched in subtype 3 (Fig. 6K and S10D). A similar analysis was performed on synovial myeloid cells from OA and RA samples (Fig. 6L, S10E, and S10F), with lower inflammation levels in OA

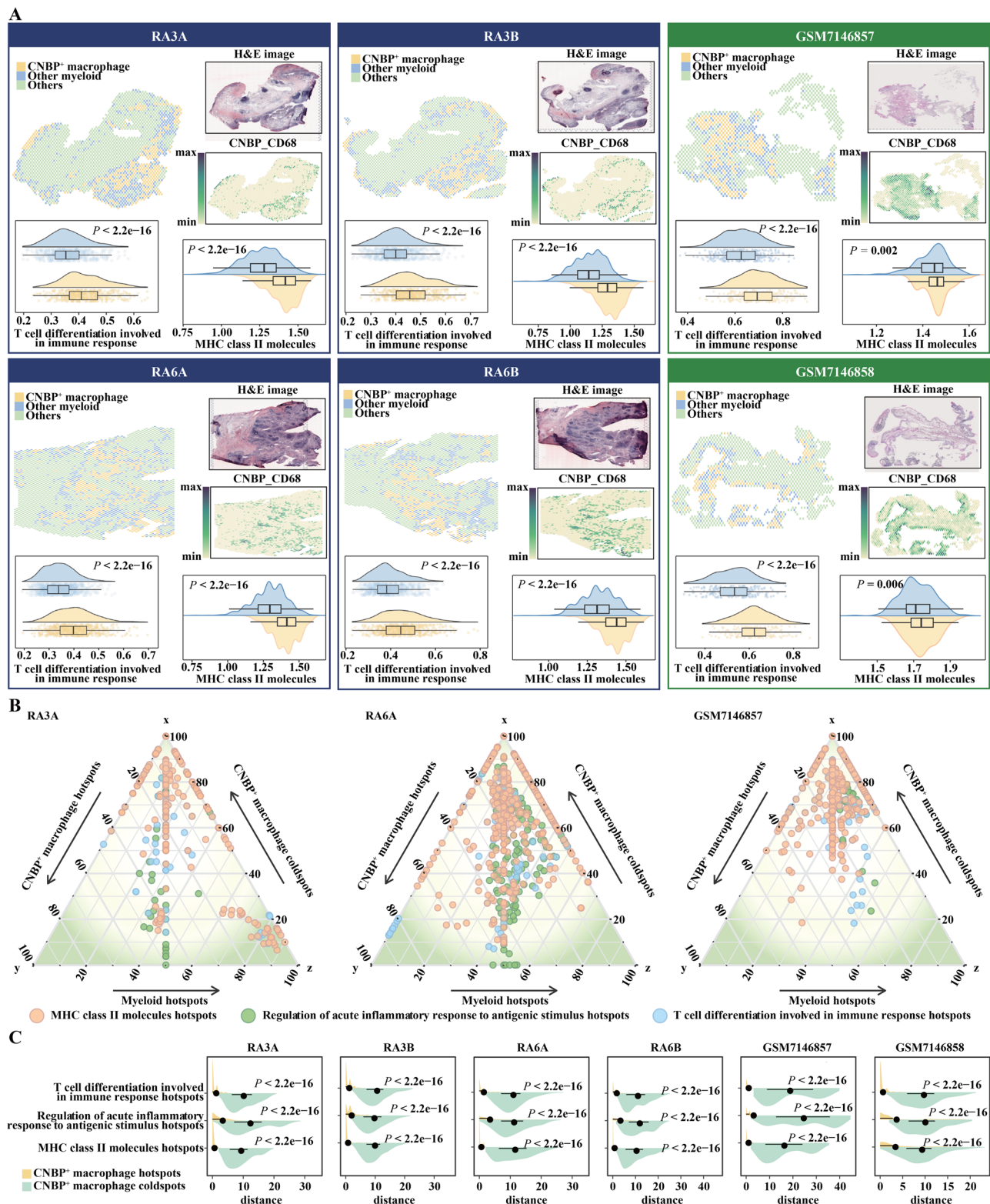


Fig. 4 Spatial profiling of arthritis synovial tissue. **(A)** Spatial mapping of cell types in ST slides utilizing the cell2location method. Inferred cell types (top left), H&E images, and CNBP-CD68 co-expression (top right) were depicted. Raincloud and violin plots displaying “T cell differentiation involved in immune response” and “MHC class II molecules” scores between CNBP⁺ macrophages and other myeloid cells (bottom). **(B)** Representative ternary plots showing distances from defined signature hotspots to specific cell type hotspots/coldspots. **(C)** Violin plots of distances from specific cell type hotspots/coldspots to defined signature hotspots. *P* value was calculated by two-tailed Wilcoxon rank-sum test (**A** and **C**)

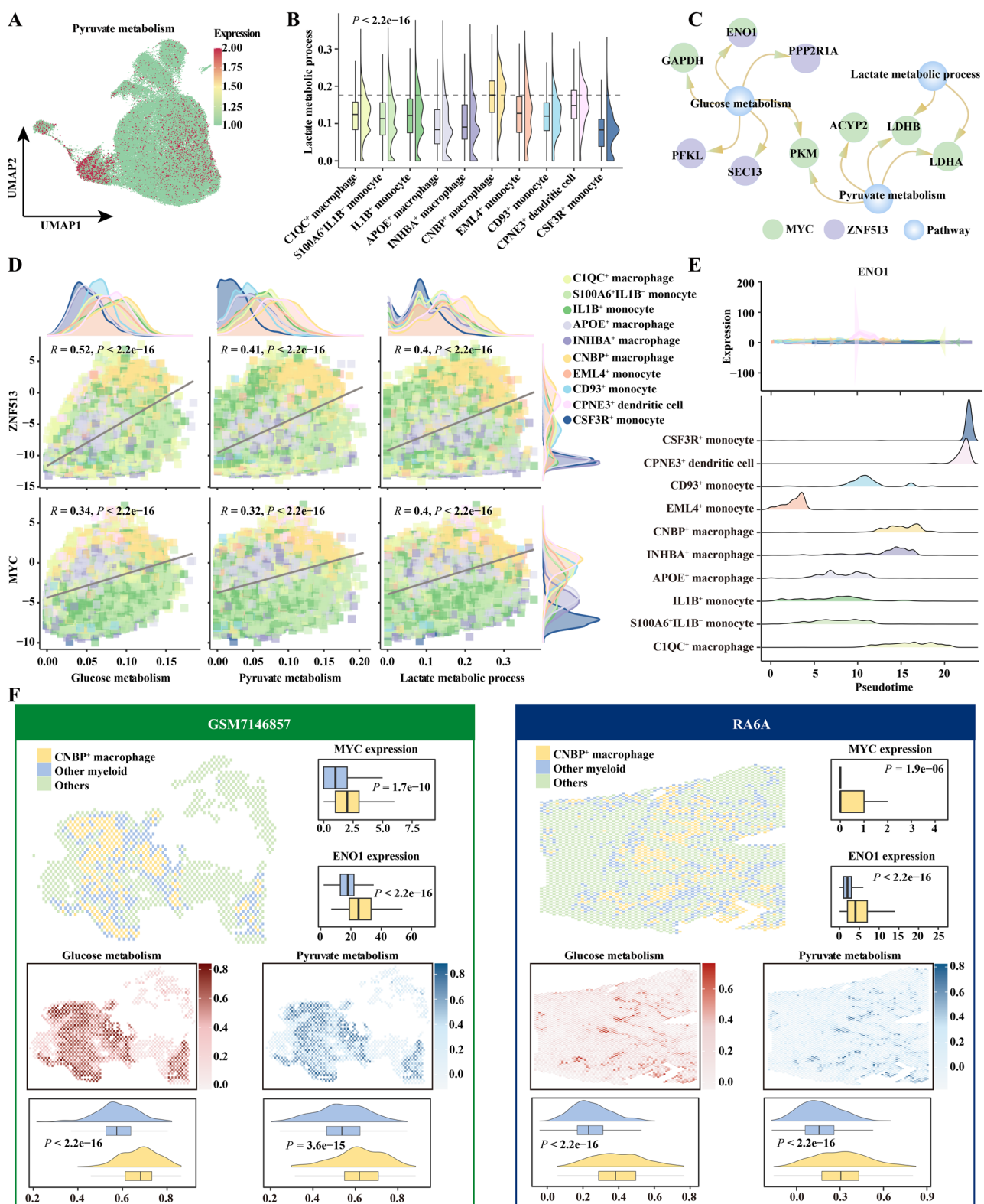


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Fig. 5 MYC regulates glucose metabolism in CNBP⁺ macrophages. **(A)** UMAP plot displaying pyruvate metabolism scores. **(B)** Raincloud plots illustrating “lactate metabolic process” scores across synovial myeloid subtypes. **(C)** Regulatory network of target genes and their involvement in pathways. Nodes of target genes were colored according to their transcription factors. **(D)** Spearman’s rank correlation analysis between metabolic pathway scores and transcription factor regulon activities. Density graphic showing the distribution of metabolic pathway scores (top) and transcription factor regulon activities (right). **(E)** Line plots show the mean expression of ENO1 along pseudotime inferred by Monocle3, stratified by myeloid subtypes. Shaded bands indicate 95% confidence intervals (top). Ridge plots displaying the distribution of inferred pseudotime across myeloid subtypes (bottom). **(F)** Inferred cell types were depicted by corresponding color (top left), with MYC and ENO1 expression levels (top right). Spatial feature plots showing the scores of metabolic pathways in ST slides, with raincloud displaying scores between CNBP⁺ macrophages and other myeloid cells (bottom). *P* value was calculated by Kruskal–Wallis test **(B)**, Spearman’s rank correlation analysis **(D)**, and two-tailed Wilcoxon rank-sum test **(F)**

samples compared to RA samples [53]. Subtype 4 was prevalent in RA patients and exhibited the highest scores of both CNBP⁺ macrophage signatures and T cell-related pathways (Fig. 6M–O).

In RA-model and sham mice, synovial CD45⁺ CD11b⁺ Ly6G[−] mononuclear phagocytes were clustered into 13 subtypes (Fig. 6P, S10G, and S10I). Subtype 5 was enriched in day 5 post K/BxN serum injection (Fig. 6Q), coinciding with the successful induction of the RA model [54]. We observed a progressive increase in CNBP⁺ macrophage signature scores over time following serum administration (Fig. S10H). Subtype 5 exhibited the highest scores of CNBP⁺ macrophage signatures and T cell-related pathways (Fig. 6R and S10J). In OA-model mice, after stringent quality control, we retained 2,903 myeloid cells (Fig. S10K, S10L and S10N), an insufficient number to identify subtypes. Nevertheless, synovial macrophages from OA-model samples displayed higher scores of CNBP⁺ macrophage signatures than sham samples (Fig. S10M).

CNBP⁺ macrophages interact with RGS10⁺ T cells through CD86 signaling

To characterize the heterogeneity of T/NK cells, we performed unsupervised clustering of PB and synovial T/NK cells, identifying seven and six subtypes, respectively (Fig. 7A–C, S11A–C, and S12A–C). Among these, PB IL7R⁺ RGS10⁺ T cells, synovial IL7R⁺ T cells, and synovial RGS10⁺ T cells showed high CD4 expression with low CD8A/CD8B expression (Fig. S11D and S12B). Furthermore, PB IL7R⁺ RGS10⁺ T cells shared marker genes with both synovial IL7R⁺ T cells and synovial RGS10⁺ T cells, suggesting potential migration of CD4⁺ T cells from PB to synovial tissue (Fig. S11C and S12C). Cellchat analysis revealed that synovial CNBP⁺ macrophages predominantly function as CD86 signaling senders due to their strong outgoing interaction strength, whereas synovial IL7R⁺ T cells and RGS10⁺ T cells served as primary receivers, exhibiting high incoming interaction strength (Fig. 7D). Spatial transcriptomic data showed co-localization of CNBP⁺ macrophages and IL7R⁺/RGS10⁺ T cells (Fig. S13A). CNBP⁺ macrophages and RGS10⁺ T cells exhibited the highest expression levels of CD86 and CD28, respectively (Fig. 7E, S11M and S11N). Compared with CNBP⁺ macrophage coldspots, CNBP⁺ macrophage

hotspots exhibited shorter spatial distances to RGS10⁺ T cell hotspots (Fig. S11O). In addition, synovial RGS10⁺ T cells displayed elevated expression of genes involved in “interferon-stimulated genes” and “interferon signaling” compared to other T cell subtypes (Fig. 7F).

Crosstalk between CNBP⁺ macrophages and CD56⁺ CD16[−] NK cells via FPR3–ANXA1 axis

AS, TNF-treated healthy, RA, TNF-treated JIA, and PsA samples exhibited low proportions of PB NK cells (Fig. S11E and S11F), whereas synovial NK cells were predominantly observed in OA samples (Figs. 7B, G, and S12D). NK cells were clustered into three subtypes based on expression of FCGR3A and NCAM1 (Fig. 7H, S11G–I, S12E, and S12F). In PB, CD56[−] CD16⁺ NK cells constituted the dominant subtype (Fig. S11H), while in synovial tissue, CD56[−] CD16⁺ NK cells and CD56⁺ CD16[−] NK cells were present at comparable levels (Fig. 7H). Both PB and synovial CD56⁺ CD16[−] NK cells exhibited elevated expression of GNLY and GZMK, contributing to cytotoxic activity (Fig. S11J and S12F). The proportion of CD56⁺ CD16[−] NK cells varied across different types of arthritis in PB and synovial tissue (Fig. 7I and S11L). CD56[−] CD16⁺ NK cells in both PB and synovial tissue expressed high levels of maturation markers, whereas CD56⁺ CD16[−] NK cells were enriched for markers of immaturity (Fig. 7J and S11K). Co-expression of activation and inhibitory molecules, including those involved in HLA-dependent and HLA-independent patterns, was observed exclusively in PB CD56[−] CD16⁺ NK cells, a feature not observed in synovial NK subtypes (Fig. 7J and S11K). PB and synovial CD56⁺ CD16[−] NK cells consistently expressed CD59, a known coreceptor (Fig. 7J and S11K).

Correlation analysis revealed CNBP⁺ macrophage abundance was more strongly associated with synovial CD56⁺ CD16[−] NK cells than with CD56[−] CD16⁺ NK cells (Fig. 7K and L). Similar to CNBP⁺ macrophages, CD56⁺ CD16[−] NK cell signature scores were elevated in both RA and OA samples compared to healthy samples, and were positively correlated with worse clinical indicators, such as swollen joint count in 28 joints (SJC28), TJC28, etc. (Fig. 7M, N, and S12G–K). CellChat analysis further identified potential ligand–receptor interactions between synovial CD56⁺ CD16[−] NK cells and CNBP⁺

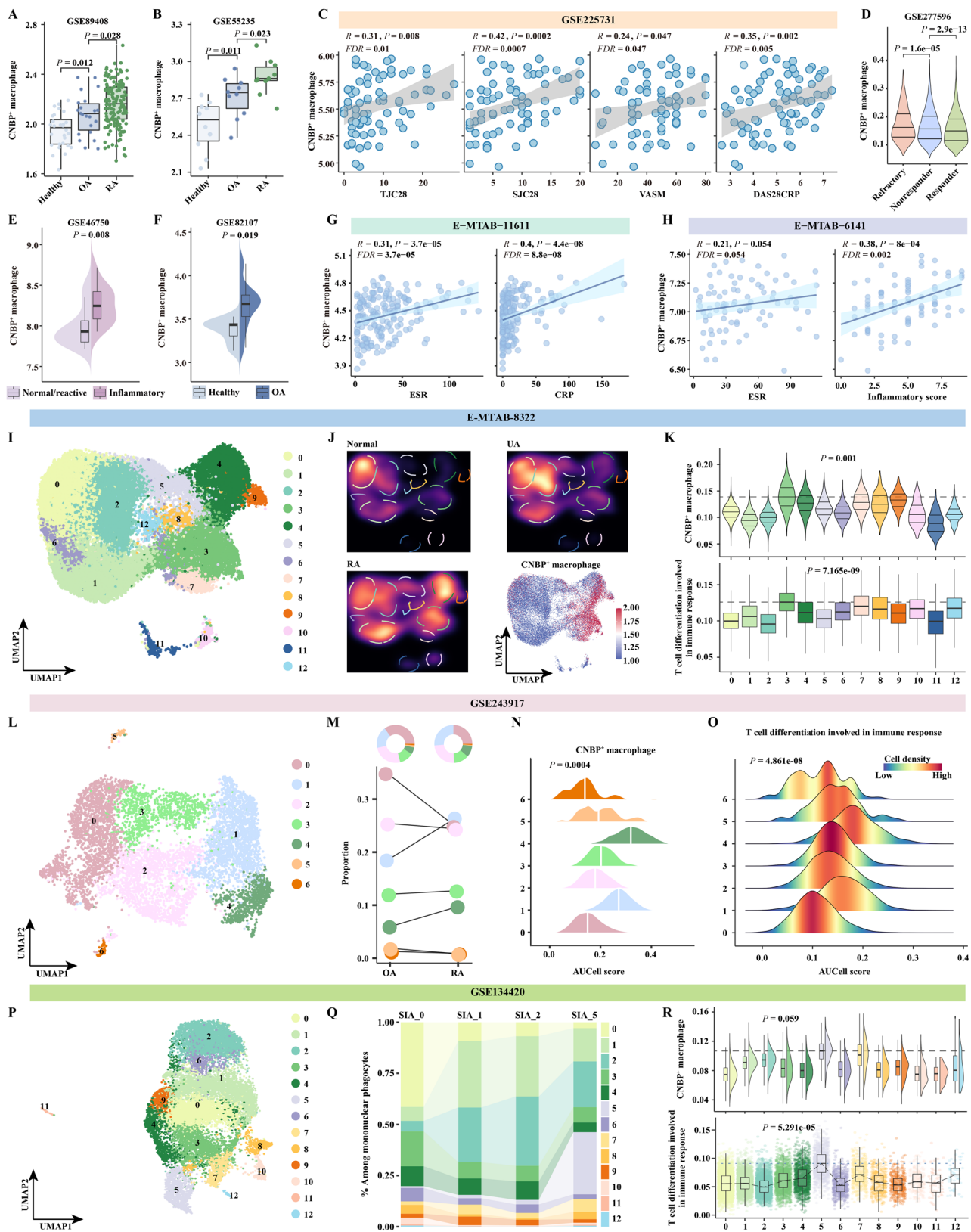


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Fig. 6 Arthritis samples show elevated CNBP⁺ macrophage signature scores. **(A–B)** Bar plots showing CNBP⁺ macrophage signature scores across healthy, OA, and RA samples in GSE89408 **(A)** and GSE55235 **(B)**. **(C)** Spearman's rank correlation analysis between CNBP⁺ macrophage signature scores and clinical indicators in GSE225731, with sample sizes of 74, 74, 67, and 74, respectively. **(D)** Violin plots depicting CNBP⁺ macrophage signature scores in GSE277596. The three horizontal lines represent the median and the upper and lower quartiles. **(E)** Violin plots showing CNBP⁺ macrophage signature scores between reactive OA and inflammatory OA samples in GSE46750. **(F)** Violin plots showing CNBP⁺ macrophage signature scores between OA and healthy samples in GSE82107. **(G–H)** Spearman's rank correlation analysis between CNBP⁺ macrophage signature scores and clinical indicators in E-MTAB-11611 **(G, n = 172)** and E-MTAB-6141 **(H, n = 83 and 74, respectively)**. **(I)** UMAP plot depicting macrophage subtypes in E-MTAB-8322 (32,139 cells). **(J)** UMAP density plot illustrating the distribution of macrophage subtypes across three conditions in E-MTAB-8322. **(K)** Violin and bar plots showing scores of CNBP⁺ macrophage signatures (top) and "T cell differentiation involved in immune response" (bottom) in E-MTAB-8322. **(L)** UMAP plot depicting myeloid subtypes in GSE243917 (7,324 cells). **(M)** Line plots showing the proportions of myeloid subtypes between OA and RA patients in GSE243917. The top circular plots depicting the relative cell proportions. **(N)** Ridge plots displaying the distribution of CNBP⁺ macrophage signature scores across myeloid subtypes in GSE243917. **(O)** Ridge plots displaying the distribution of "T cell differentiation involved in immune response" scores across myeloid subtypes in GSE243917. **(P)** UMAP plot depicting subtypes of synovial CD45⁺ CD11b⁺ Ly6G[−] mononuclear phagocytes in GSE134420 (14,637 cells). **(Q)** Bar plots showing the proportions of mononuclear phagocyte subtypes across different conditions in GSE134420. **(R)** Raincloud and bar plots showing scores of CNBP⁺ macrophage signatures and "T cell differentiation involved in immune response" across mononuclear phagocyte subtypes in GSE134420. TJC28: tender joint count in 28 joints; SJC28: swollen joint count in 28 joints; VAS: visual analogue scale for general health assessment; DAS28CRP: disease activity score in 28 joints with C-reactive protein; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; SIA_0: day 0 after K/BxN serum transfer; SIA_1: day 1 after K/BxN serum transfer; SIA_2: day 2 after K/BxN serum transfer; SIA_5: day 5 after K/BxN serum transfer. *P* value was calculated by two-tailed Wilcoxon rank-sum test **(A, B and D–F)**, Spearman's rank correlation analysis and adjusted by the Benjamini-Hochberg method **(C, G and H)**, and Kruskal-Wallis test **(K, N, O and R)**

macrophages via TNFSF14–TNFRSF14, TNFSF14–LTBR, ICAM1–SPN, ICAM1–(ITGAX+ITGB2), ICAM1–(ITGAM+ITGB2), and ANXA1–FPR3 pairs (Fig. 7O). Spatial transcriptomic data showed the co-localization of CNBP⁺ macrophages and CD56⁺ CD16[−] NK cells with elevated expression of ANXA1 and FPR3 in RA and PsA synovial tissues (Fig. 8A, S13B and S13C). A similar spatial distribution was observed by multiplex immunofluorescence staining, particularly in the sample with high synovitis (Fig. 8B).

Discussion

Our study revealed an expansion of PB CNBP⁺ myeloid cells in arthritis patients, with abundance positively correlated with inflammatory scores. We further delineated the cellular crosstalk, highlighting interactions between CD56⁺ CD16[−] NK cells and CNBP⁺ macrophages via FPR3–ANXA1 axis. CNBP⁺ macrophages also interacted with CD4⁺ T cells through CD86–CD28 signaling (Fig. S14).

Across the integrated scRNA-seq datasets, CNBP⁺ myeloid cells were expanded in most arthritis types except GA. Previous single-cell studies of myeloid cells in arthritis were limited to one or two arthritis types within a relatively small cohort [6, 22]. In contrast, our study identified ten PB myeloid subtypes across six types of arthritis and healthy donors. However, given the absence of harmonized clinical metadata across datasets, these cross-dataset patterns primarily reflect transcriptional trends rather than direct clinical associations, and potential overfitting cannot be fully excluded. PB CNBP⁺ monocytes were enriched in AS, RA, PsA, and TNF-treated JIA, but were less abundant in GA compared to healthy individuals. These observations highlight disease-specific heterogeneity. Given that inflammatory immune cells can infiltrate synovial tissue

under inflammatory conditions [1], we further assessed whether similar CNBP⁺ myeloid cells existed within the synovium. Synovial CNBP⁺ macrophages exhibited the highest PB CNBP⁺ monocyte signature scores. Future work involving lineage-tracing approaches will be necessary to determine whether PB CNBP⁺ monocytes migrate into the synovium. The presence of CNBP⁺ macrophages in arthritis was revealed by multiplex immunofluorescence staining. Multiplex immunofluorescence validation was performed on a limited number of synovial samples (*n* = 2), and therefore provides preliminary spatial evidence for the presence of CNBP⁺ macrophages. Studies reported that CNBP knockdown in the Raw 264.7 macrophages reduced the expression of NF-κB-dependent inflammatory genes [55]. In CNBP-deficient mice, IL-12β production is diminished [56]. Together with the clinical efficacy of IL-12 inhibitors in arthritis [57], these data support a pathogenic role for CNBP⁺ myeloid cells. In bulk transcriptomic datasets, CNBP⁺ macrophage signatures were upregulated in arthritis samples and positively correlated with worse clinical indicators. Validation in large-scale patients will strengthen these observations.

Consistent with the findings by Alivernini et al. [50], we identified a synovial macrophage subtype with antigen-presenting capability and inflammatory characteristics. Similar to the antigen-presenting macrophages described by Kurowska-Stolarska et al. [6], CNBP⁺ macrophages expressed high levels of CLEC10A and HLA-DPB1. In contrast, CNBP was not upregulated in HLA^{high}-CLEC10A^{high} macrophages reported in RA and UA [6], potentially reflecting differences in disease coverage. Our work encompassed six arthritis types, potentially extending the known spectrum of antigen-presenting macrophage subtypes. Growing evidence supports the central role of CD4⁺ T cells in arthritis pathogenesis [58]. Myeloid cells provide two essential signals for CD4⁺ T

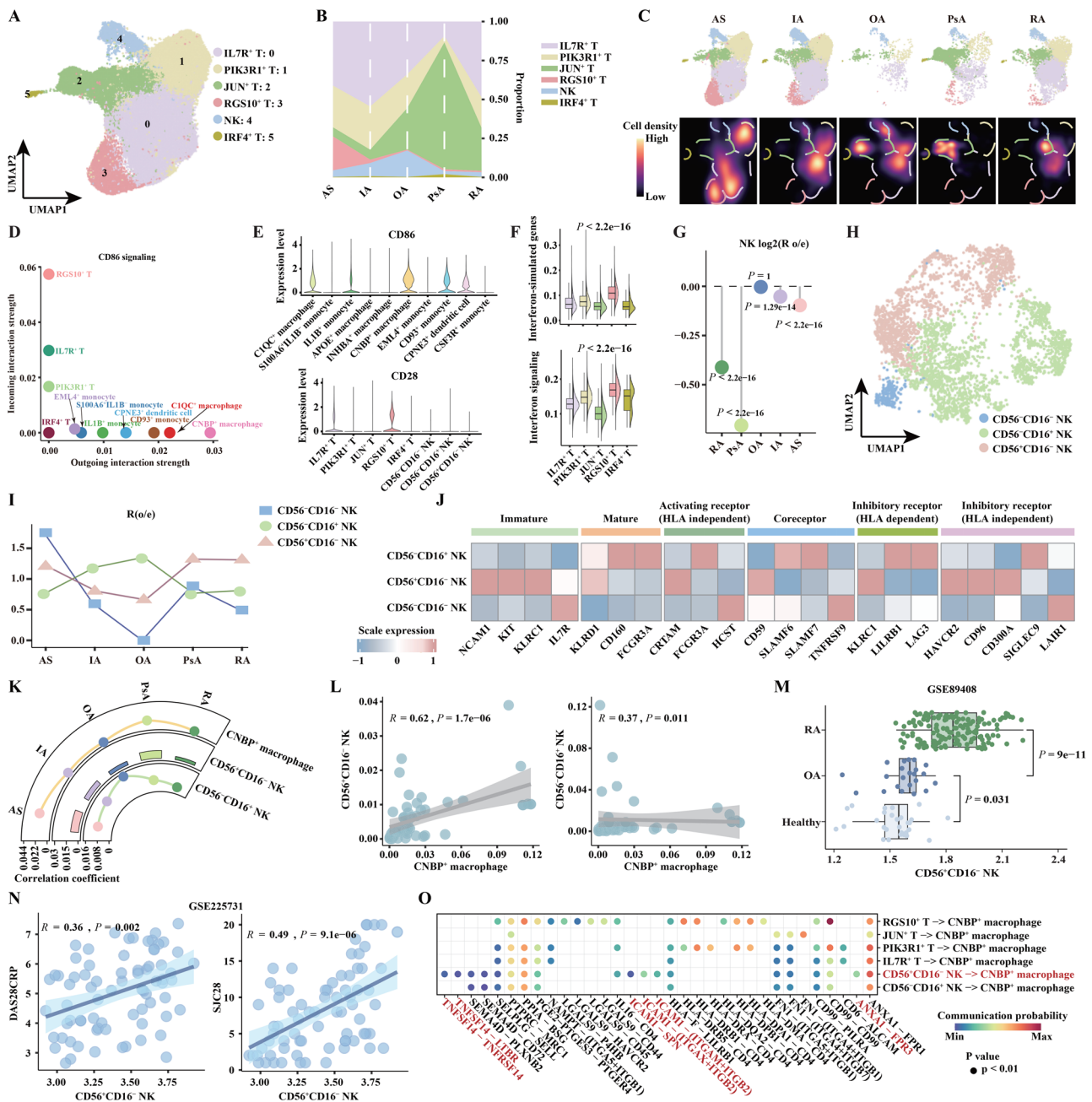


Fig. 7 Synovial immune crosstalk involving CNBP⁺ macrophages. **(A)** UMAP plot depicting T/NK subtypes in synovium (72,252 cells). **(B)** Bar plots showing the proportions of T/NK subtypes across different conditions in synovium. **(C)** UMAP view of T/NK subtypes (top) and cell density (bottom) across different conditions. High relative cell density indicates bright magma. **(D)** Dot plots of inferred cell-cell communication via CD86 signaling. Each point represents one subtype. The horizontal axis represents the intensity of the CD86 signal received by the cells, and the vertical axis represents the intensity of the CD86 signal received by the cells. **(E)** Violin plots illustrating expression of CD86 (top) and CD28 (bottom) in myeloid cells and NK/T cells, respectively. **(F)** Raincloud and bar plots showing scores of "interferon-stimulated genes" (top) and "interferon signaling" (bottom). **(G)** Line plots illustrating condition prevalence estimated by Ro/e for NK cells compared to OA samples, with exact P value indicated next to each bar. **(H)** UMAP view of NK subtypes (3,772 cells). **(I)** Line plots illustrating condition prevalence estimated by Ro/e for each NK subtype. **(J)** Heatmap showing interested gene expression across NK subtypes. **(K)** Line and bar plots illustrating proportions of interested subtypes across 5 arthritis types. **(L)** Spearman's rank correlation analysis between proportions of CNBP⁺ macrophages and two NK subtypes in synovium. **(M)** Bar plots showing CD56⁺CD16⁻ NK cell signature scores across healthy, OA, and RA samples in GSE89408. **(N)** Spearman's rank correlation analysis between CD56⁺CD16⁻ NK cell signature scores and clinical indicators in GSE225731 (n = 74). **(O)** Dot plots showing ligand-receptor pairs from T/NK subtypes to CNBP⁺ macrophages in synovium. P value was calculated by Kruskal-Wallis test (F), Spearman's rank correlation analysis (L and N), and two-tailed Wilcoxon rank-sum test (M).

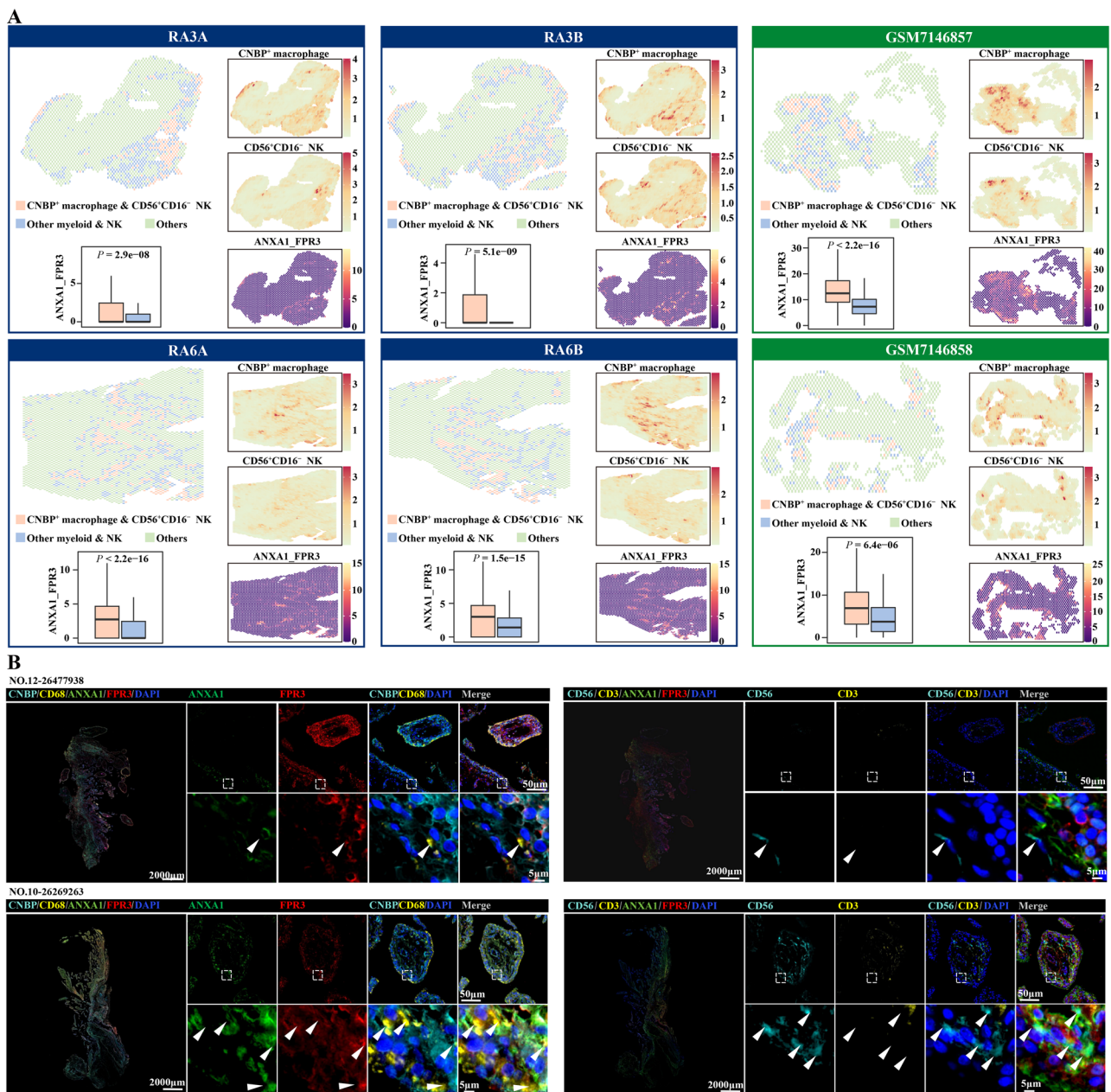


Fig. 8 Crosstalk between CNBP⁺ macrophages and CD56⁺ CD16⁻ NK cells via FPR3–ANXA1 axis. **(A)** Spatial localization of inferred CNBP⁺ macrophages and CD56⁺ CD16⁻ NK cells, with ANXA1–FPR3 co-expression. Bar plots displaying ANXA1–FPR3 co-expression level in spots simultaneously defined as CNBP⁺ macrophages and CD56⁺ CD16⁻ NK cells. **(B)** Multiplex immunofluorescence staining showing co-localization of CNBP⁺ macrophages and CD56⁺ CD16⁻ NK cells with ANXA1–FPR3 pair. *P* value was calculated by two-tailed Wilcoxon rank-sum test **(A)**

cell activation: antigen presentation through MHC class II and co-stimulatory signaling via surface ligands [59]. In our study, CNBP⁺ macrophages highly expressed MHC class II molecules and frequently communicated with CD4⁺ T cells through CD86 signaling.

CNBP⁺ macrophages showed a high score of glycolysis pathway, with MYC identified as a potential regulator. Synovium inflammatory cells often shift from oxidative phosphorylation to glycolysis under inflammatory

conditions [52]. Previous studies have shown that enhanced glycolytic activity facilitates rapid cell proliferation and activation of inflammatory cells under the inflammatory condition [52]. CNBP⁺ macrophages exhibited high scores of “glucose metabolism” and “pyruvate metabolism” in both scRNA-seq and spatial transcriptomic data, suggesting a potential shift toward glycolytic phenotype. To further explore the regulatory mechanism underlying metabolic remodeling, we found

that MYC, a known regulator of PKM and LDHA [60], was highly expressed in CNBP⁺ macrophages. CNBP⁺ macrophages highly expressed PKM, which encodes a pyruvate kinase essential for the production of ATP and pyruvate, as well as LDHA, a critical enzyme that converts pyruvate to lactate, facilitating sustained glycolysis. Although these findings suggest that MYC may be related to the glycolytic features observed in CNBP⁺ macrophages, any causal role requires further validation. MYC is known to promote the Warburg effect in cancer cells [61], but any similarity between metabolic programs in cancer cells and synovial CNBP⁺ macrophages remains speculative.

Synergistic interaction between CNBP⁺ macrophages and CD56⁺ CD16⁻ NK cells in aiding synovitis. Given that NK cells can modulate macrophage states between homeostatic and inflammatory phenotypes [62], we clustered NK cells to investigate their association with CNBP⁺ macrophages. CD56⁺ CD16⁻ NK cells were strongly correlated with CNBP⁺ macrophage abundance and were associated with worse clinical indicators. CellChat analysis indicated communication between CD56⁺ CD16⁻ NK cells and CNBP⁺ macrophages via ANXA1–FPR3 axis. Spatial transcriptomic data and multiplex immunofluorescence staining showed spatial patterns consistent with this interaction. While ANXA1 derived from NK cells is known to reprogram macrophages in cancer [36], our study uncovers a similar mechanism that may contribute to synovitis.

There were some limitations in this study. First, the absence of comprehensive clinical information in public scRNA-seq datasets hinders our ability to directly assess the relationship between CNBP⁺ macrophages and clinical indicators. Therefore, clinical associations derived from scRNA-seq analyses in this study should be considered exploratory and may be subject to overfitting. Bulk transcriptomic datasets provide indirect support, but more definitive conclusions will require large single-cell cohorts with comprehensive clinical annotations. Second, the single-cell data analyzed were derived from PB and synovial tissue, with shared cell types predominantly consisting of immune cells. While this study focused on immune cell interactions, future work should explore immune-stromal communication to provide a more integrated view of tissue pathology. Third, this study concentrated mainly on synovial tissue. Because arthritis affects multiple joint compartments, future research should integrate data from additional regions (such as subchondral bone and the infrapatellar fat pad) to build a more comprehensive understanding. Finally, multiplex immunofluorescence and spot-level spatial transcriptomic provided preliminary evidence for the interaction between CNBP⁺ macrophages and CD56⁺ CD16⁻ NK cells via FPR3–ANXA1 axis. Future studies should further

validate this interaction using both in vivo and in vitro experiments.

Conclusion

Together, this study constructed a single-cell transcriptional atlas of PB and synovial immune cells across healthy and arthritic conditions. This work highlights a coordinated immune network involving CD56⁺ CD16⁻ NK cells, CNBP⁺ macrophages, and CD4⁺ T cells, mediated through ANXA1–FPR3 axis and CD86 signaling. This panoramic immune landscape bridges the gap in shared pathogenic mechanisms across diverse arthritis types, enhancing our understanding of synovial biology.

Abbreviations

AS	Ankylosing spondylitis
AUPRC	Area under the precision-recall curve
CNBP	CCHC-type zinc finger nucleic acid binding protein
DAS28CRP	Disease activity score in 28 joints with C-reactive protein
DC	Dendritic cell
ESR	Erythrocyte sedimentation rate
FDR	False discovery rate
GA	Gouty arthritis
Gi-star	Gi*
GSEA	Gene set enrichment analysis
H&E	Hematoxylin and Eosin
IA	Inflammatory arthritis
IL	Interleukin
JIA	Juvenile idiopathic arthritis
NK	Natural killer
OA	Osteoarthritis
PB	Peripheral blood
PsA	Psoriatic arthritis
RA	Rheumatoid arthritis
Ro/e	Ratio of observed to expected cell counts
ROGUE	Ratio of Global Unshifted Entropy
scRNA-seq	Single-cell RNA sequencing
SJC28	Swollen joint count in 28 joints
TJC28	Tender joint count in 28 joints
TNF	Tumor necrosis factor
UA	Undifferentiated arthritis
UMAP	Uniform manifold approximation and projection
UMI	Unique Molecular Identifier

Supplementary information

The online version contains supplementary material available at <https://doi.org/10.1186/s12967-026-08075-4>.

Supplementary Material 1

Supplementary Material 2

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Author contribution

YG, XL and XE conceived and designed the study. LW and HQ completed data processing, analysis, and draft of the paper. YZ, ZP and FM collected data. GW, XL, HZ, and RY extracted data and visualized the results. All authors read and approved the final manuscript.

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Data availability

Data will be made available on request.

Declarations

Ethical approval

The study was approved by the Ethics Committee of Harbin Medical University (approval number: IRB5134724).

Code availability

The R and python codes used in this study are available in GitHub (<https://github.com/hmudd33-lab/arthritis>).

Competing interest

Authors declare that they have no competing interests.

Author details

¹State Key Laboratory of Frigid Zone Cardiovascular Diseases (SKLFZCD), College of Pharmacy, Harbin Medical University, Harbin, Heilongjiang 150081, China

²Department of Nutrition and Food Hygiene, School of Public Health, Key Laboratory of Precision Nutrition and Health, Ministry of Education, Harbin Medical University, Harbin, Heilongjiang 150081, China

³State Key Laboratory of Frigid Zone Cardiovascular Diseases (SKLFZCD), Department of Systems Biology, College of Bioinformatics Science and Technology, Harbin Medical University, Harbin, Heilongjiang 150081, China

⁴Department of Immunology and Microbial Sciences, King's College London, Guy's Campus, London SE1 1UL, UK

⁵Department of Orthopedics, The First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang 150081, China

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