Ann Rheum Dis 84 (2025) 1151–1163

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Axial spondyloarthritis

CD4 + tissue-resident memory Th17 cells are a major source of IL-17A in Spondyloarthritis synovial tissue

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Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.04.018

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ARTICLE INFO

Article history:
Received 14 March 2025
Received in revised form 16 April 2025
Accepted 17 April 2025

ABSTRACT

Objectives: Interleukin (IL)-17A is a key driver of spondyloarthritis (SpA) joint pathology. We aimed to identify its cellular source in synovial tissue from patients with 2 forms of SpA, namely axial SpA (AxSpA) and psoriatic arthritis (PsA).
Methods: Synovial tissue from patients with SpA was profiled using single-cell RNA sequencing (scRNA-seq; AxSpA, n = 5; PsA, n = 6) or spatial RNA profiling (PsA, n = 4). CellPhoneDB was used to infer cell–cell communication. Tissue-resident memory Th17 (TRM17)-like cells were generated *in vitro* using blood memory CD4+ T cells from SpA patients. An epigenetic inhibitor library, siRNA, and clustered regularly interspaced short palindromic repeats (CRISPR) were used to identify epigenetic regulator(s) for TRM17.
Results: scRNA-seq showed that *CD4+CXCR6+* TRM17 cells are the predominant spontaneous *IL17A* producers in SpA synovium. Cell–cell communication and single-cell spatial analysis support the interaction between TRM17 and *CLEC10A+* dendritic cells, which were activated in SpA. Both sublining and lining fibroblasts in SpA synovium showed evidence of interleukin (IL)-17A activation. *In vitro*-generated CD4+ TRM17-like cells phenocopied joint tissue TRM17, producing IL-17A/F upon T cell-receptor (TCR) stimulation, which was further enhanced by cytokines. Perturbation of BRD1 inhibited the generation of TRM17-like cells.
Conclusions: CD4+ TRM17 cells are the predominant source of IL-17A in SpA synovial tissue. TCR stimulation is essential for the secretion of IL-17A by CD4+TRM17-like cells. The epigenetic regulator bromodomain-containing protein 1 (BRD1) contributes to the generation of CD4+TRM17. Depleting CD4+TRM17 cells in SpA is thus a therapeutic strategy with potential to induce long-term remission.

INTRODUCTION

The spondyloarthritides (SpA) are a group of common inflammatory arthritides that include axial SpA (AxSpA) and psoriatic arthritis (PsA). The AxSpA and PsA share pathological drivers including interleukin 17 (IL-17) and tumour necrosis factor (TNF). Drugs targeting IL-17 are effective in over 50% of SpA patients but require continued long-term administration, and therapies to target the cells producing IL-17 have not yet been developed.

IL-17A is known to promote tissue inflammation, at least in part through fibroblast activation. Among the different forms of IL-17 (A-F), IL-17A is the key driver in joint inflammation whereas a major role for IL-17F in skin inflammation has been supported by recent trials [1]. IL-17A can be produced by a variety of immune cells, including conventional T cells, innate-like lymphocytes, and innate lymphoid cells (ILCs). Whether a specific cell type dominates IL-17A production in SpA is debated [2]. Different studies have implicated all 3 subsets of innate-like lymphocytes ($\gamma\delta$ T cells, invariant natural killer T [iNKT] cells, and mucosal associated invariant T [MAIT] cells) and CD8+ tissue-resident memory T cells as the sources of IL-17A in SpA joints [3–9]. Increasing evidence, however, is highlighting a role for CD4+ tissue-resident memory Th17 (TRM17) cells as key IL-17A producers in tissues such as the skin, lung and kidney [10–12]. Furthermore, CD4+ Th17 cells expressing CXCR6, an established marker for tissue residency, have been linked to brain inflammation in a murine model of multiple sclerosis [13]. It is therefore possible that a role for CD4+ TRM17 cells in SpA may have been missed in previous studies focused on synovial fluid samples and/or utilising potent *in vitro* stimulation to determine the source of IL-17.

Notably, CD4+CXCR6+ Th17 cells in inflamed brain tissue have been found to exhibit a distinct epigenetic profile that is different from homeostatic Th17 cells with stem-like features [13]. Considering the established role of epigenetic modifiers in the regulation of T cell differentiation and function [14,15], it is plausible to predict that epigenetic regulators could be involved in the generation of CD4+ TRM17 cells.

Through profiling unstimulated synovial tissue using single-cell RNA sequencing (scRNA-seq) and spatial RNA profiling, we here show that CD4+ CXCR6+ TRM17 cells are the predominant source of IL-17A in SpA synovial tissue. We also establish a model to generate CD4+ TRM17-like cells *in vitro* and show that T-cell receptor (TCR) engagement, rather than cytokines, is essential for these cells to produce IL-17A and IL-17F. Lastly, we show that the epigenetic regulator bromodomain-containing protein 1 (BRD1) contributes to the generation of CD4+ TRM17-like cells.

METHODS

Patient recruitment

Fresh synovial tissue was obtained at arthroplasty or synovial biopsy was collected from patients attending the Oxford University Hospitals, Queen Elizabeth Hospital, and Shanghai Sixth People's Hospital with appropriate consent and ethical approvals. All patients included in this study met the criteria of the Assessment of Spondyloarthritis International Society for AxSpA

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Interleukin (IL)-17A plays a key role in the immunopathogenesis of spondyloarthritis (SpA), but its cellular source in joint tissue has not been determined previously. The induction and accumulation of CD4+ tissue-resident memory Th17 (TRM17) cells following the clearance of pathogens has been described in skin, lung, and kidney. Whether CD4+ TRM17 cells also accumulate in the joint and contribute to the pathology of SpA is not clear.

WHAT THIS STUDY ADDS

- CD4+ TRM17 cells are present in SpA synovial tissue and are the predominant source of *IL17A*.
- CD4+ TRM17 cells in SpA joints express *IL17A* without any *in vitro* exogenous stimulation.
- T-cell receptor (TCR) rather than cytokine stimulation is essential for IL-17A production by CD4+ TRM17-like cells.
- The epigenetic regulator BRD1 contributes to the generation of CD4+ TRM17-like cells.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Our findings identify CD4+ TRM17 cells as the primary source of IL-17A in SpA synovium, a previously unrecognised role for these cells. Key questions remain: How do CD4+ TRM17 cells relate to IL-17A producers in synovial fluid? What mechanisms induce and maintain them in the joint? How do they interact with other cells to promote arthritis? These questions warrant further investigation. In addition, our data suggest that targeting CD4+ TRM17 cells, the 'factory' of IL-17A in SpA synovial tissue, has the potential to induce long-term remission, encouraging future efforts to develop new therapies to deplete CD4+ TRM17 cells in SpA.

or Classification criteria for Psoriatic ARthritis (CASPAR) criteria for PsA. Patients currently or previously receiving interleukin 17 inhibitor (IL17i) therapy were excluded from the study population. The demographics of AxSpA and PsA patients recruited for this study are shown in Supplementary Table S1.

Computational analysis of scRNA-seq data

The paired reads obtained were mapped to the hg38 reference genome to generate gene expression matrices using CellRanger. For PsA samples (from the Cartography Consortium), the full dataset was analysed in Panpipes [16], and the T cell compartment was partitioned out for downstream analyses along with the AxSpA data. T cells of PsA samples and full dataset of AxSpA samples were then merged and analysed using the Seurat R package (v4.0.5). Cells exhibiting low-quality transcriptional profiles were excluded based on the application of the following exclusion criteria: a number of detected genes below 500, a proportion of mitochondrial RNA exceeding 12% of the total unique molecular identifiers (UMIs), and a total UMI count below 700. These parameters for quality control were selected in accordance with current widely adopted practices in the single-cell genomics field [17–19].The raw read counts were normalised using the NormalizeData function, and variable genes were identified for each sample. To identify cell types, we mapped our cells into a synovium reference map built by Fan Zhang et al. [20] using the Symphony algorithm. For the reclustering analysis of cellular subsets, a certain population were extracted and data from different sample were subject to FastMNN algorithms for integration. We then used FindNeighbors and FindCluster functions to cluster cells based on global transcriptional profile. For differential expression analysis, the FindMarkers function was used to test the normalised data with the default Wald test method. Unless specified, default parameters were used for each function.

For cell–cell interaction inference, normalised expression matrix and cluster annotations were exported from the Seurat object as input for CellPhoneDB. CellPhoneDB v5.0.0 was used as a ligand-receptor interaction database. 'statistical analysis' method from CellPhoneDB tools was used to assess the strength and significance of interactions.

CosMx spatial analysis

Synovial biopsy tissue sections from 8 ultrasound-guided biopsies were distributed across 2 flow cells and run on the CosMx Spatial Molecular Imager. Cell segmentation and initial quality control were completed in the AtoMx Spatial Informatics Platform before exporting and using R (v4.4.0) and Seurat (v5.1.0) for further quality control, clustering, and cluster annotation. Data were processed through iterations of clustering, annotation, identification, and removal of multiplets/poorly segmented cells based on gene expression profiles, number of genes detected, and number of transcripts detected. Subclustering was performed until low quality/poorly segmented cells were minimised in the dataset.

Patient and public involvement

We have actively engaged the patients with SpA through the Oxford Patient Engagement Network for Arthritis and Musculoskeletal Conditions (OPEN ARMS, https://www.ndorms.ox.ac.uk/get-involved/open-arms-1/open-arms) and the Botnar AxSpA day. We will discuss with patients about the impact of findings from this study and codevelop a dissemination plan to maximise the potential benefits of our findings to patients.

Additional methods

Information for Th17 expansion assay for the screen of epigenetic inhibitor library, siRNA screen of the targets of OF-1, intracellular cytokine staining (ICS) and enzyme-linked immunosorbent assays (ELISA) are available in Supplementary Methods.

RESULTS

Tissue-resident memory CD4+ T cells are the main source of IL17A in SpA joint tissue

To investigate the cellular origin of IL-17A in the inflamed joints of SpA patients, we performed single-cell transcriptomic profiling on synovial tissue from 5 AxSpA and 6 PsA patients (demographics shown in Supplementary Table S1A and B). After removing low-quality cells, we obtained 85,307 cells (Supplementary Fig S1). These cells were categorised into T cells, natural killer (NK), mast and ILCs, B and plasma cells, myeloid cells, stromal cells, and endothelial cells (Fig 1A). Within this cellular milieu, we found almost all *IL17A*+ cells are localised within the *CD4*+ T cell subset (Fig 1B). Notably, the vast majority of the *IL17A*+ cells did not express *CD8A* (encoding the CD8 protein), *TRAV1-2* (encoding the TCR α-variable 1-2 protein, abundant in MAIT cells), or *TRDC* (encoding the delta constant region of the TCR, characteristic of $\gamma\delta$ T cells) (Fig 1C). As ILCs, especially ILC3 cells, are known to be capable of producing IL-17A, we investigated further into ILCs via the reclustering of the

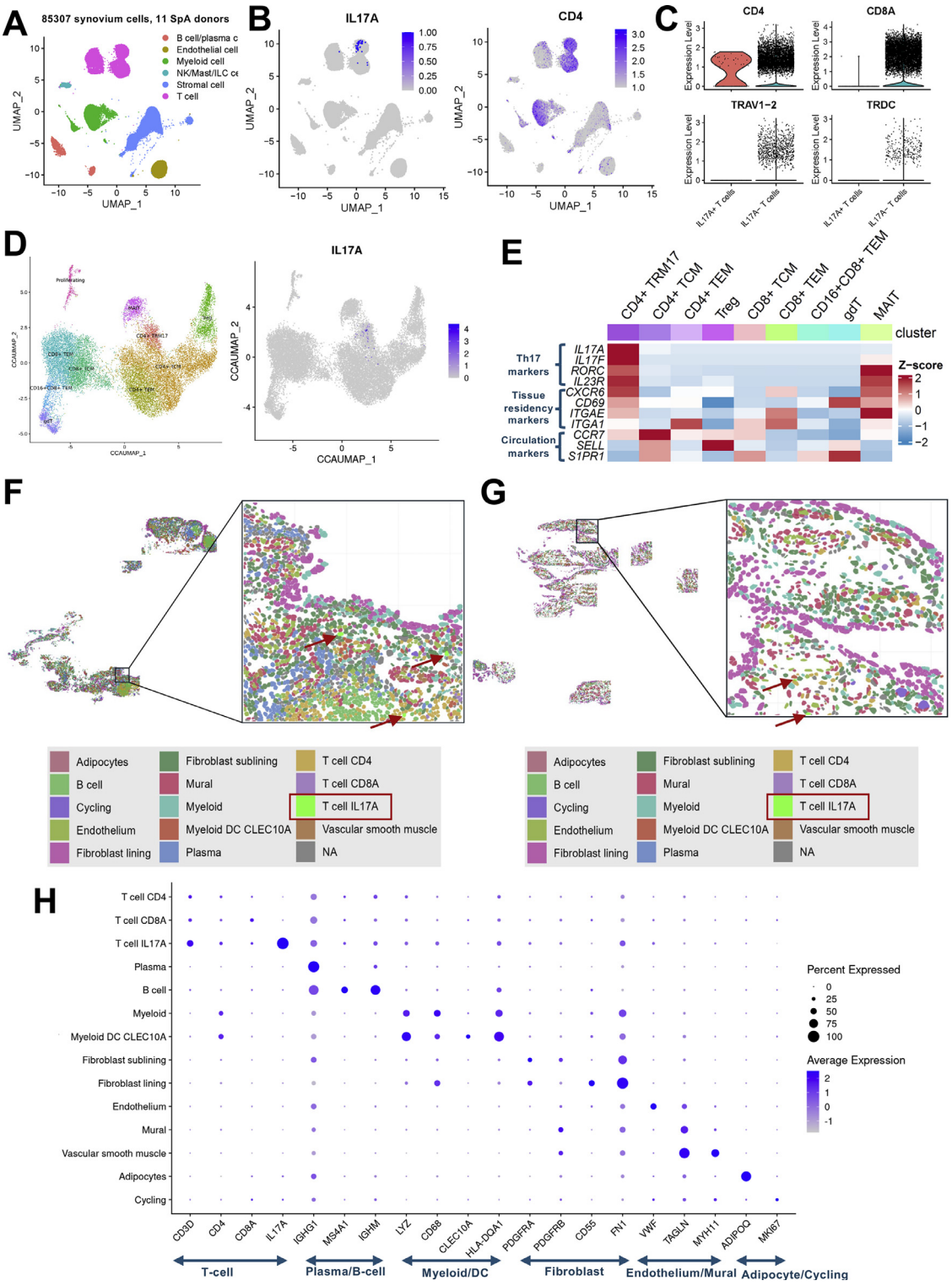

Figure 1. Tissue-resident memory CD4+ T cells are the main source of IL17A in SpA joint tissue. (A) UMAP visualisation of major cell types of cells from SpA synovial tissue. (B) UMAP visualisation of normalised expression of *IL17A* and *CD4*. (C) Violin plot of normalised expression of *CD4, CD8A, TRAV1-2, TRDC* in *IL-17A*$^{+}$ and *IL-17A*$^{-}$ T cells. (D) UMAP visualisation of subsets of T cells from SpA synovial tissues and normalised expression of *IL17A*. (E) Heatmap of normalised and scaled expression of Th17, tissue residency, and circulation signature markers in different subsets of T cells. CosMx was used for spatial profiling of synovial tissue from 4 PsA patients. (F) and (G) Representative images from 2 PsA patients. (H) Expression of cell type marker genes by IL17A+ T cells and other cell populations identified from CosMx data. The 'IL17A+ T' subset is excluded from the 'CD4+ T' and 'CD8+ T' subsets. IL17A, interleukin 17A; PsA, psoriatic arthritis; SpA, spondyloarthritis; UMAP, uniform manifold approximation and map.

cluster that contains NK, mast, and ILCs (Supplementary Fig S2A). Despite expressing high levels of *RORC* and *IL23R, IL7R* +*KIT*+ ILCs did not express detectable *IL17A* (Supplementary Fig S2B). These results identify *αβ* CD4+ T cells, not *γδ* T, MAIT, ILCs, or CD8+ T cells, as the primary source of *IL17A* in SpA synovial tissue. In line with previous findings [21], the expression of *IL17F* is not limited to T cells and extends to stroma and endothelial cells (Supplementary Fig S3). *TNF*, a key driver of SpA pathology, is predominantly expressed by myeloid cells (Supplementary Fig S4).

To pinpoint the *IL17A*-expressing T cell population, we performed subclustering analysis for all T cells from SpA synovial tissue. Ten distinct clusters were identified (Fig 1D and Supplementary Fig S5A). *IL17A*-expressing T cells are largely present in the *CD4*+ TRM17 population, exhibiting both Th17 (*IL17A, IL17F, RORC, IL23R*) and TRM (*CXCR6, CD69, ITGAE*) characteristics (Fig 1E and Supplementary Fig S5B). Separate analysis of AxSpA and PsA samples confirmed that CD4+ TRM17 cells are the primary source of *IL17A* in both disease states (Supplementary Fig S6 A-C). Analysis of the Accelerating Medicines Partnership rheumatoid arthritis (RA) dataset showed that in RA synovial tissue *IL17A* was also predominantly expressed by a *CD69*+*CXCR6*+*CD4*+ TRM17 population (Supplementary Fig S7 A-C). Notably, CXCR6+CD4+ TRM17 cells have recently been identified to drive the pathology in skin, kidney and brain inflammatory diseases [10,12,13].

To investigate the cellular niche of *IL17A*-expressing CD4+ TRM17 cells, we carried out spatial transcriptome analysis on joint synovial tissue from 4 PsA patients using CosMx Spatial Molecular Imaging (Fig 1F and G). We scanned 80 fields of view with a multiplex panel of 1000 genes (CosMx™ Human Universal Cell Characterization RNA Panel). A limitation of this approach was the absence or low expression of many CD4+ TRM cell feature genes identified in our previous scRNA-seq data. However, *IL17A* was successfully detected, enabling the definition of *IL17A*-expressing T cells which were predominantly localised in the sublining regions (24 of 27 *IL17A*+ T cells) (Figure 1F and G). We annotated cells based on canonical markers, and identified adipocyte, B, endothelial, sublining fibroblast, lining fibroblast, myeloid, *CLEC10A*+ myeloid, plasma, *IL17A*+ T, *CD4*+ T, *CD8*+ T, and mural cells (Fig 1F-H, Supplementary Fig S1C and D). Notably, in the analysis shown in Figure 1H, the '*IL17A*+ T' subset is excluded from the '*CD4*+ T' and '*CD8*+ T' subsets.

Both sublining and lining fibroblasts in SpA joint tissue exhibit an enhanced IL-17 response signature

The induction of a proinflammatory state in fibroblasts has been postulated as a critical mechanism underlying IL-17-mediated chronic inflammation. Within the synovium, sublining and lining fibroblasts are 2 distinct subsets that together constitute synovial fibroblasts. We then investigated whether sublining and lining fibroblasts from SpA synovium have been influenced by CD4+ TRM17. To this end, we integrated a publicly available dataset of fibroblasts from healthy synovium [22] with our dataset of SpA synovial fibroblasts, resulting in a combined dataset of 11,173 healthy and 27,975 SpA fibroblasts (Fig 2A). Consistent with previous studies [23,24], we identified *THY1*+*PRG4*- sublining and *THY1-PRG4*+ lining fibroblasts as 2 major subsets, with *MMP1* and *MMP3* enriched in the lining fibroblasts (Fig 2A, proinflammatory S9A and B). An IL-17 signalling score (indicative of response to IL-17) was then calculated using a set of genes induced by IL-17 in human synovial fibroblasts [25]. We found both sublining and lining fibroblast subsets in SpA exhibited an enhanced IL-17 response signature compared to their healthy counterparts (Fig 2B). Notably, established IL-17-induced effector cytokines and chemokines, such as *CXCL1, CXCL8, IL6*, and *CCL20*, were consistently upregulated in both sublining and lining fibroblast subsets within the SpA synovium. Thus, despite enrichment of CD4+ TRM17 in sublining synovium, both sublining and lining fibroblasts show evidence of IL-17-induced activation.

We then asked if the enhanced IL-17 signature in SpA synovium fibroblasts is contributed by certain subset(s) within the sublining and lining fibroblast populations. To this end, we conducted a detailed analysis of cell states within these 2 populations separately (Fig 2C and F). In the sublining fibroblasts, among 8 identified subsets, a cluster (S1) marked by the expression of *CD74* and *CHI3L2* exhibited the highest IL-17 signalling score (Fig 2D). This subset was also characterised by the upregulation of proangiogenic genes, including *SEMA3A* and *VEGFC* (Fig 2E). In the lining fibroblast compartment, the L4 cluster displayed the strongest IL-17 signalling signature (Fig 2G) and was enriched in the expression of genes associated with oxidative stress and inflammatory responses, such as *SOD2, MT2A, RCAN1*, and *TNFAIP6* (Fig 2H).

CLEC10A+ *dendritic cells are potential antigen-presenting cells for CD4+ TRM17 cells*

Tissue-resident memory T cells typically require antigen-presenting cells (APCs) for their generation and effector function. It has been well established that myeloid cells, especially dendritic cells (DCs), are the key APCs to support T cell function. To identify the myeloid cellular subset that serves as APC for CD4+ TRM17 cells, we extracted and reclustered the myeloid cells from SpA tissue (Fig 1A) and identified 10 transcriptionally distinct myeloid subsets: plasmacytoid dendritic cells (pDCs), *CLEC9A*+ DCs, *CLEC10A*+ DCs, *TNF*+ macrophage cluster 1 (M1), *TNIP3*+ macrophage cluster 2 (M2), *EGR2*+ macrophage cluster 3 (M3), *GIMAP*+ macrophage cluster 4 (M4), *C9*+ macrophage cluster 5 (M5), *PRODH2*+ macrophage cluster 6 (M6), and *LYVE1*+ macrophage cluster 7 (M7) (Fig 3A). We then searched for potential APCs for CD4+ TRM17 using cell–cell communication analysis for all 10 myeloid subsets and *IL17A*+ T cells (Figure 3B). The strongest interaction with *IL17A*+ cells was observed with *CLEC10A*+ DCs, which expressed high levels of ligands for CD28 (*CD80* and *CD86*), produced a chemokine (*CCL20*) known to be chemotactic for Th17 cells through CCR6, and were enriched for the key cytokine for the generation of tissue-resident memory T cells (*TGFB1*, encoding TGF-β).

Activated T cells have been shown to act on myeloid cells and induce their activation [26,27]. Thus, if *CLEC10A*+ DC cells have served as the APC for CD4+ TRM17 cells, they should have received reciprocal signals from these cells and show signs of activation. To look for evidence of *CLEC10A*+ DC activation in SpA synovium, we carried out a differential gene expression analysis that revealed 541 upregulated and 90 downregulated genes in *CLEC10A*+ DCs in SpA versus healthy synovium (Fig 3C). Notably, genes associated with an inflammatory response (*RELB, IRF4*, and *NFKB1*) were significantly increased. Pathway enrichment analysis of these upregulated genes using the Molecular Signatures database (MSigDB) revealed significant enrichment in several proinflammatory pathways, including TNFα signalling, IL-2/STAT5 signalling, IFNγ response, and IL-6/JAK/STAT3 signalling (Fig 3D). However, proinflammatory pathways were also elevated in *TNF*+ M1 and *LYVE1*+ M7 cells from SpA patients (Supplementary Fig S10). Therefore, we cannot determine whether the enrichment of proinflammatory pathways in SpA *CLEC10A*+ DCs is due to T cell interactions or the joint's inflammatory environment. Based on the hypothesis that APCs for *IL17A*+ T cells would have encountered IL-17, we analysed the expression of genes known to be induced by IL-17A in monocytes or macrophages (*MMP9, CCL4, CCL5, CSF2, IL3, IL9*) [28,29]. We observed increased expression of *MMP9* and

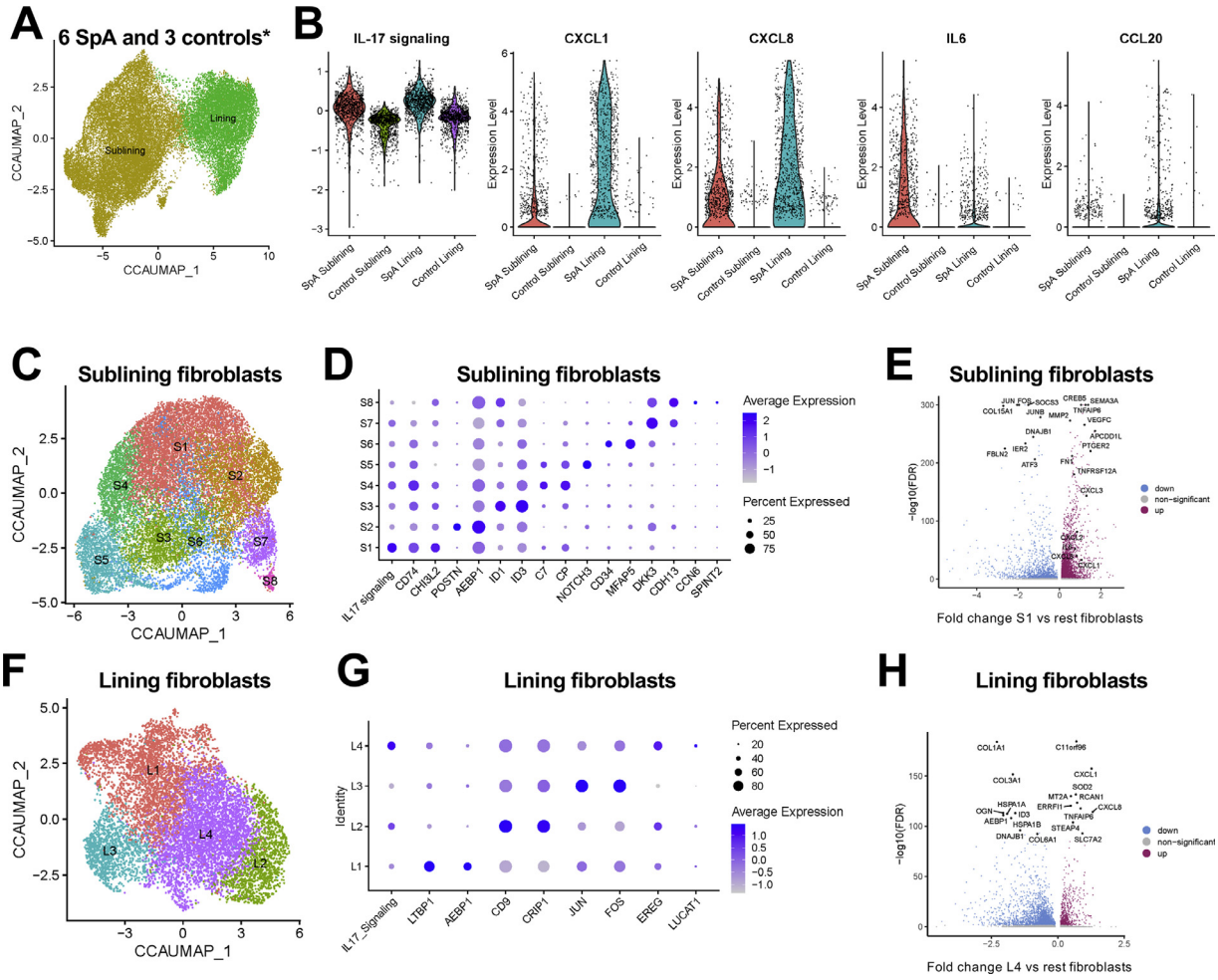

Figure 2. An enhanced IL-17 response signature is present in both sublining and lining fibroblasts from SpA synovium. (A) UMAP visualisation of fibroblast subsets of combined cells from SpA and healthy synovial tissues. (B) Violin plot of IL-17 signalling score and normalised expression of 4 selected IL-17 signalling genes in different fibroblast subsets in SpA and healthy synovium. The data for control synovium (n = 3) were sourced from GSE216651. (C) UMAP visualisation of subsets of sublining fibroblast cells from SpA synovium. (D) Relative expression of marker genes of different sublining fibroblast subsets from SpA synovium. (E) Volcano plot of differentially expressed genes in S1 sublining fibroblast cells compared to other sublining fibroblast cells in SpA synovium (FDR < 0.05). (F) UMAP visualisation of subsets of lining fibroblast cells from SpA synovium. (G) Relative expression of marker genes of different lining fibroblast subsets from SpA synovium. (H) Volcano plot of differentially expressed gene in L4 lining fibroblast cells compared to other lining fibroblast cells in SpA synovium (FDR < 0.05). FDR, false discovery rate; IL17A, interleukin 17A; PsA, psoriatic arthritis; SpA, spondyloarthritis; UMAP, uniform manifold approximation and map.

CSF2 in *CLEC10A*+ dendritic cells (DCs), but a greater number of genes were enriched in *TNF*+ M1 and *TNIP3*+ M2 macrophages (Supplementary Fig S11). Lastly, we sought evidence of direct physical cell interactions between *CLEC10A*+ dendritic cells (DCs) and *IL17A*+ T cells in our spatial transcriptomic datasets. Owing to the restricted number of genes profiled in the spatial transcriptomic data, we were unable to resolve all myeloid cell subsets identified in Figure 3A. However, we were able to identify *CLEC10A*+ DCs within the spatial transcriptomic data based on their unique marker expression profile. Quantification of cell proximity revealed that *CLEC10A*+ DCs were one of the top 2 subsets within myeloid cells significantly enriched in close proximity to *IL17A*+ T cells (Figure 3E). Representative examples of *CLEC10A*+ DC and *IL17A*+ T cell interactions are shown in Figure 3F. We also observed that *IL17A*+ T cells are more frequently in proximity to sublining fibroblasts than lining fibroblasts, and to B cells and CD4+ cells than CD8+ cells. Taken together, these data suggest that *CLEC10A*+ dendritic cells are activated in SpA joints and are the potential APCs for CD4+ TRM17.

TCR engagement and cytokine stimulations codrive the production of IL-17A/F by in vitro generated CD4+ TRM17-like cells

We then asked what signals induce the production of IL-17A by CD4+ TRM17 cells. Due to the difficulties in obtaining adequate number of CD4+ TRM17 from synovial tissue for functional studies, we developeicd an *in vitro* model to induce CD4+ TRM17-like cells. It has been shown that following immunisation, effector Th17 cells can give rise to lung CD4+ TRM17 cells, which are maintained in the tissue by the survival cytokine IL-7 following the clearance of antigens [11]. Accordingly, we generated a two-phase *in vitro* model to generate CD4+ TRM17-like cells (Fig 4A). In phase I, circulating blood memory CD4+ T cells from patients with SpA were stimulated with anti-CD3 antibody, anti-CD28 antibody, and Th17 expansion cytokines (IL-1β and IL-23) to generate effector Th17 cells. These cells were then cultured with TGF-β (to model the tissue niche) and IL-7 (to provide the survival signal) in the absence of TCR stimulation in phase II. Using this model, we were able to induce IL-

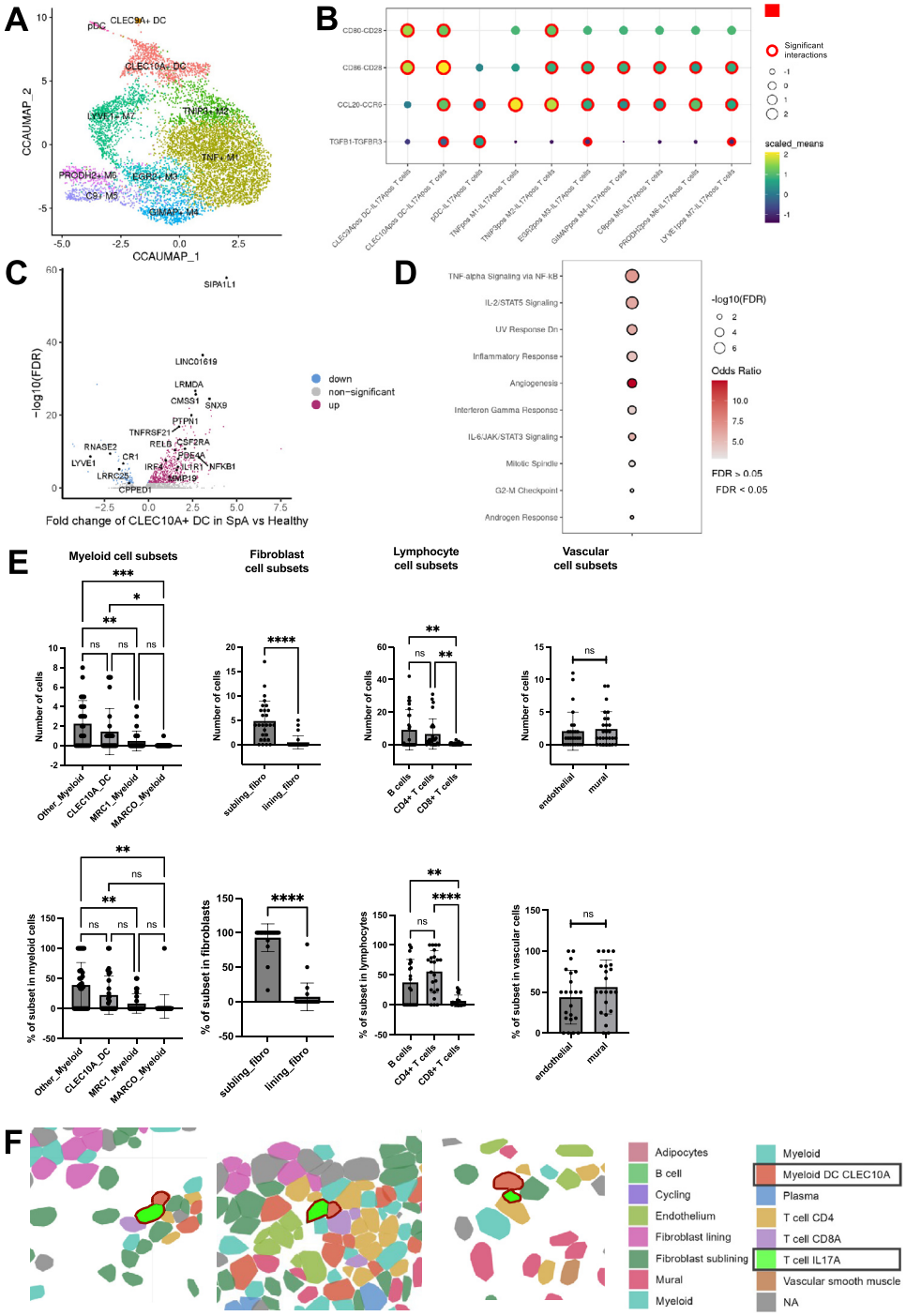

Figure 3. Cell−cell communication and single-cell spatial analysis support the interaction between CLEC10A + dendritic cells and TRM17. (A) UMAP visualisation of subsets of myeloid cells from SpA synovial tissues. (B) Cell−cell interactions between different myeloid subsets and IL17A + T cells in SpA synovium inferred by CellPhoneDB. (C) Volcano plot of differentially expressed gene in CLEC10A + DCs in SpA synovium compared to healthy synovium (FDR < 0.05). (D) Enriched pathway in CLEC10A + DCs (all upregulated genes were used for enrichment analysis using MSigDB database). (E) The number and proportion of myeloid, fibroblast, lymphocyte, and vascular cell subsets located within 50 μm of *IL17A* + T cells was quantified. (F) Three representative CosMx images of SpA synovium showing interaction between CLEC10A + DCs and IL17A + cells (highlighted in red outline). Statistical significance was assessed using one-way ANOVA (for myeloid and lymphocyte subsets) or the paired two-tailed Student's t test (for fibroblast and vascular subsets) (*$P \leq .05$, **$P \leq .01$, ***$P \leq .001$, ****$P \leq .0001$). Median ± SEM is shown in (E). ANOVA, analysis of variance; DC, dendritic cells; IL17A, interleukin 17A; MSigDB, Molecular Signatures database; PsA, psoriatic arthritis; SEM, standard error of the mean; SpA, spondyloarthritis; UMAP, uniform manifold approximation and map.

17A + CXCR6 + CD4 + TRM17-like cells which were largely absent in the blood memory compartment (Fig 4B and C). We then assessed the expression of TRM markers using our assay in comparison to a 6-day Th17 expansion assay (anti-CD3/CD28, IL-1β, IL-23). Although the CD4 + TRM17 assay slightly improved IL-17A + cell induction, it significantly increased the percentage of Th17 cells expressing CXCR6, CD49a, CD103, and CD69 (Supplementary Fig S12A, B). This was confirmed at the RNA level by quantitative polymerase chain reaction (qPCR) (Supplementary Fig S12C). Taken together, these data suggest that our assay successfully induces Th17 cells with TRM-like features *in vitro*.

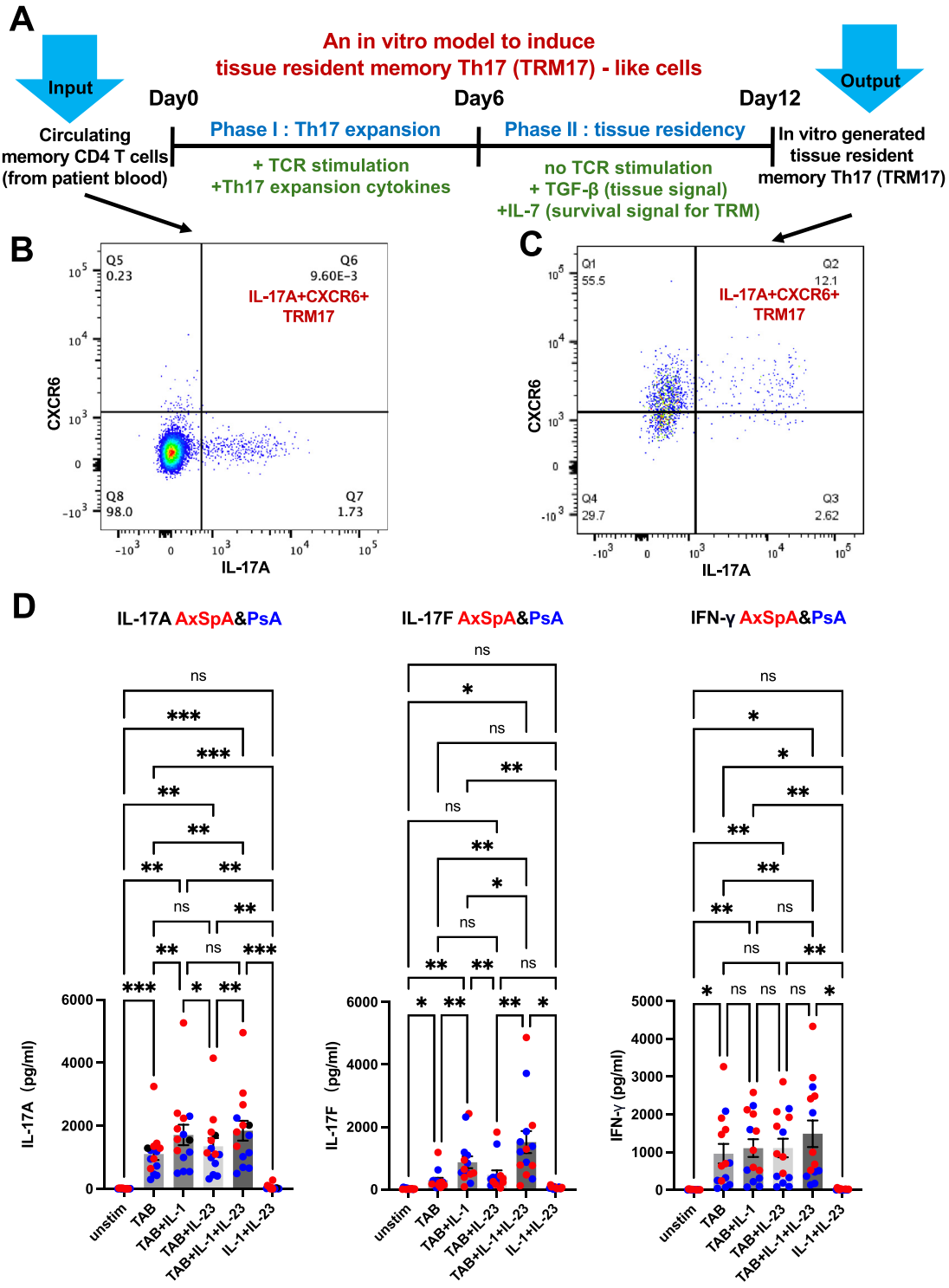

Figure 4. T-cell receptor engagement and cytokine stimulations codrive the production of IL-17A/F by in vitro generated TRM17-like cells. A, Schematic representation of the in vitro TRM17 model. Assessment of IL-17A + CXCR6 + TRM17 in circulating memory CD4 + T cells (B) and induced TMR17 cells (C). D, The secretion of IL-17A, IFN-γ, and IL-17F by TRM-like cells stimulated with vehicle control, cytokines (IL-1β and IL-23), TABs, or the combinations of cytokine and TABs. Data from 7 AxSpA (red) and 7 PsA (blue) patients are shown. Statistical significance was assessed using one-way ANOVA (*$P \leq .05$, **$P \leq .01$, ***$P \leq .001$). Median ± SEM is shown in (D). ANOVA, analysis of variance; AxSpA, axial spondyloarthritis; IFN, interferon; IL, interleukin; PsA, psoriatic arthritis; SEM, standard error of the mean; SpA, spondyloarthritis; TAB, T-cell activation beads.

While IL-23 blockade has proven highly efficacious for skin psoriasis, the effects on AxSpA and psoriatic arthritis have been less impressive [30–33]. This led us to investigate the role of IL-23 in IL-17A production by CD4 + TRM17-like cells. Figure 4D shows that TCR engagement using anti-CD3 and anti-CD28 T cell activation beads (TAB), rather than cytokine stimulation (IL-1β and IL-23), induces IL-17A production in restimulated CD4 + TRM17-like cells from SpA patients. Furthermore, the addition of IL-23 to either TAB or TAB + IL-1β conditions did not significantly enhance IL-17A production. Interestingly, in the presence of TAB and IL-1β, IL-23 significantly increased the secretion of IL-17F. This suggests that the lack of effect of IL-23 on IL-17A production is not due to the pre-existence of IL-23 in the culture media. Consistent with this, IL-23 blockade did not reduce IL-17A secretion in either the TAB alone or TAB + IL-1β conditions (Supplementary Fig S13). IL-1β blockade has been

tested in AxSpA and PsA with mixed results. We observed an IL-17-specific enhancement by IL-1β, but not IFN-γ, either in TAB or TAB + IL-23 conditions. A similar pattern was observed when AxSpA and PsA samples were analysed separately (Supplementary Fig S14), as well as in CD4+ TRM17-like cells generated from healthy controls (Supplementary Fig S15). We also performed a similar experiment using cells generated from a 6-day Th17 expansion assay (anti-CD3/CD28, IL-1β, IL-23) (Supplementary Fig S16). Like the CD4+ TRM17 assay, IL-17A secretion was predominantly driven by TAB and IL-1β. Surprisingly, IL-17F was only induced when both TAB and cytokines (IL-1β or IL-23) were present. Taken together, these data suggest that both TCR engagement and cytokine stimulation codrive the production of IL-17A/F by *in vitro* generated CD4+ TRM17-like cells.

Identification of BRD1 as a novel epigenetic regulator of Th17/CD4+ TRM17 cells

The unique epigenetic profile of CXCR6+Th17 cells reported in the murine multiple sclerosis model [13] drove us to hypothesise that epigenetic regulators could contribute to the generation of CD4+ TRM17 cells. To test this hypothesis, we screened a library of 38 epigenetic inhibitors using cells from SpA patients. Due to the low-throughput nature of the CD4+ TRM17 model, we performed the initial screen using a Th17 expansion assay and discovered 3 inhibitors (bromosporine, PFI-1 and OF-1) that suppress Th17 responses in both ICS and IL-17 enzyme-linked immunosorbent assays (ELISAs) (Figure 5A). We deprioritised the first 2 inhibitors as bromosporine is a pan-bromodomain inhibitor with multiple targets and the target of PFI-1, the bromodomain of bromodomain and extraterminal domain family, has already been shown to regulate Th17 cells [34,35]. In contrast OF-1, an inhibitor for the bromodomain and PHD finger-containing protein (BRPF) family [36], has not been implicated previously in Th17 response and thus was chosen for further investigation. To this end, we first used siRNA to screen all 3 members in the BRPF family and found BRD1 silencing inhibited IL-17A production (Fig 5B). A similar trend was observed in BRPF1-silenced cells. Silencing efficiency was greater than 50% for all 3 genes (Fig S17A), a level generally considered acceptable for primary immune cell experiments. Further transcriptome analysis showed that knockdown of BRD1, but not BRPF1, reduced both Th17 marker genes (*IL17A, IL17F, RORC*, and *IL23R*) and TRM markers (*CXCR6* and *ITGA1*) (Fig 5C). Taken together these data suggest a potential role of BRD1 in CD4+ TRM17 cell generation.

We then used our CD4+ TRM17 *in vitro* model to investigate the role of BRD1 in the generation of TRM17-like cells. As the transient nature of siRNA-mediated gene suppression is not suitable for the 12 days CD4+ TRM17 model, we deployed clustered regularly interspaced short palindromic repeats (CRISPR) technology to stably knockout BRD1 (Fig 5D) and achieved over 50% knockout efficiency (Figure S17B). The BRD1-knock out (KO) CD4+ T cells were rested for 3 days (allowing the degradation of endogenous BRD1 protein) before being used as the input for CD4+ TRM17 generation model. Figure 5E shows that, in comparison to AAVS control guide RNA, BRD1 knockout significantly inhibited the generation of CXCR6+IL-17A+ TRM17-like cells (Fig 5E). No changes in cell viability or proliferation were observed. Additionally, the restimulation of TMR17-like cells induced a lower level of IL-17A secretion in BRD1-KO cells than the adeno-associated virus integration site 1 control cells (Fig 5F). Similar results were observed for IL-17F (Supplementary Fig S17). In contrast to IL-17A and IL17-F, the percentage of IFN-γ+CXCR6+ cells and the secretion IFN-γ were not affected by BRD1 KO (Fig 5E and F).

DISCUSSION

In this study, we show that CXCR6+ tissue-resident memory CD4+ T cells are the main source of IL-17A in SpA joint tissue. Despite the predominant localisation of CD4+ TRM17 cells in the sublining regions, both sublining and lining fibroblasts in SpA joints exhibit an enhanced IL-17 response signature suggesting the diffusion of IL-17A within the tissue. We also show that TCR engagement and cytokine stimulations codrive the production of IL-17A/F by *in vitro* generated TRM17-like cells. Lastly, we identify BRD1 as a novel Th17 regulator that contributes to the generation of CD4+ TRM17-like cells *in vitro*.

Previous studies in SpA synovial fluid (SF) identified $\gamma\delta$ T cells, MAIT cells, iNKT and CD8+ tissue-resident memory T cells as key sources of IL-17 [3–9]. Our findings indicate CD4+ TRM17 cells as the primary source of IL-17 in SpA synovial tissue (ST). This discrepancy suggests that distinct inflammatory drivers are present in SF versus ST. The relationship between SF and ST inflammation within IL-17 pathology and warrants further investigation. Our SpA ST dataset makes a significant contribution to existing knowledge. In addition to demonstrating CD4+ TRM IL-17A and IL-17F production, we also highlight the activation and function of various non-T cell populations within the SpA synovium. Specifically, we observe TNF production primarily by myeloid cells (mainly macrophages). Furthermore, we detect the activation of fibroblasts and *CLEC10A*+ DCs in these AxSpA patients compared to healthy controls using infrapatellar fat pad and synovium tissues studied elsewhere.

Importantly, we found the *in vitro* generated CD4+ TRM17-like cells produced IL-17A mainly in response to TCR engagement rather than cytokine stimulation. In the presence of IL-1β, IL-23 enhanced the TCR stimulation induced production of IL-17F but not IL-17A, suggesting that cytokine stimulation has a greater influence on IL-17F production. In line with this, IL-17F has been shown to be the dominant IL-17 isoform produced by cytokine-activated innate lymphocytes (MAIT cells, $\gamma\delta$ T cells and ILC3s) [37,38], indicating a closer association of IL-17F with the innate response in lymphocytes. We also observed that IL-1β enhanced IL-17A/F production in the presence of TCR engagement, and this enhancement occurred both with and without IL-23.

We acknowledge the inherent limitations of this study. Our sample size is relatively small, largely due to the costs of scRNA-seq and the availability of tissue. More than half of the samples came from patients with long disease duration (>10 years). While we cannot be certain that our findings fully represent early-stage SpA, we did detect CD4 TRM17 IL-17 production in samples from patients with a shorter disease duration (<5 years). A larger cohort, stratified by disease duration (and therapy), would be necessary to further confirm and expand upon our findings. In addition, we used human infrapatellar fat pad and STs as controls for the study of fibroblasts and myeloid cells. A comparison with synovium from healthy knee, hip, or spine should be conducted in the future to confirm our findings.

In summary, we demonstrate for the first time that the CD4+ TRM17 population is the main source of IL-17A in SpA ST. In addition, our data suggest that targeting CD4+ TRM17, the 'factory' of IL-17A, has the potential to induce long-term remission, encouraging future efforts to develop new therapies to deplete CD4+ TRM17 cells in SpA.

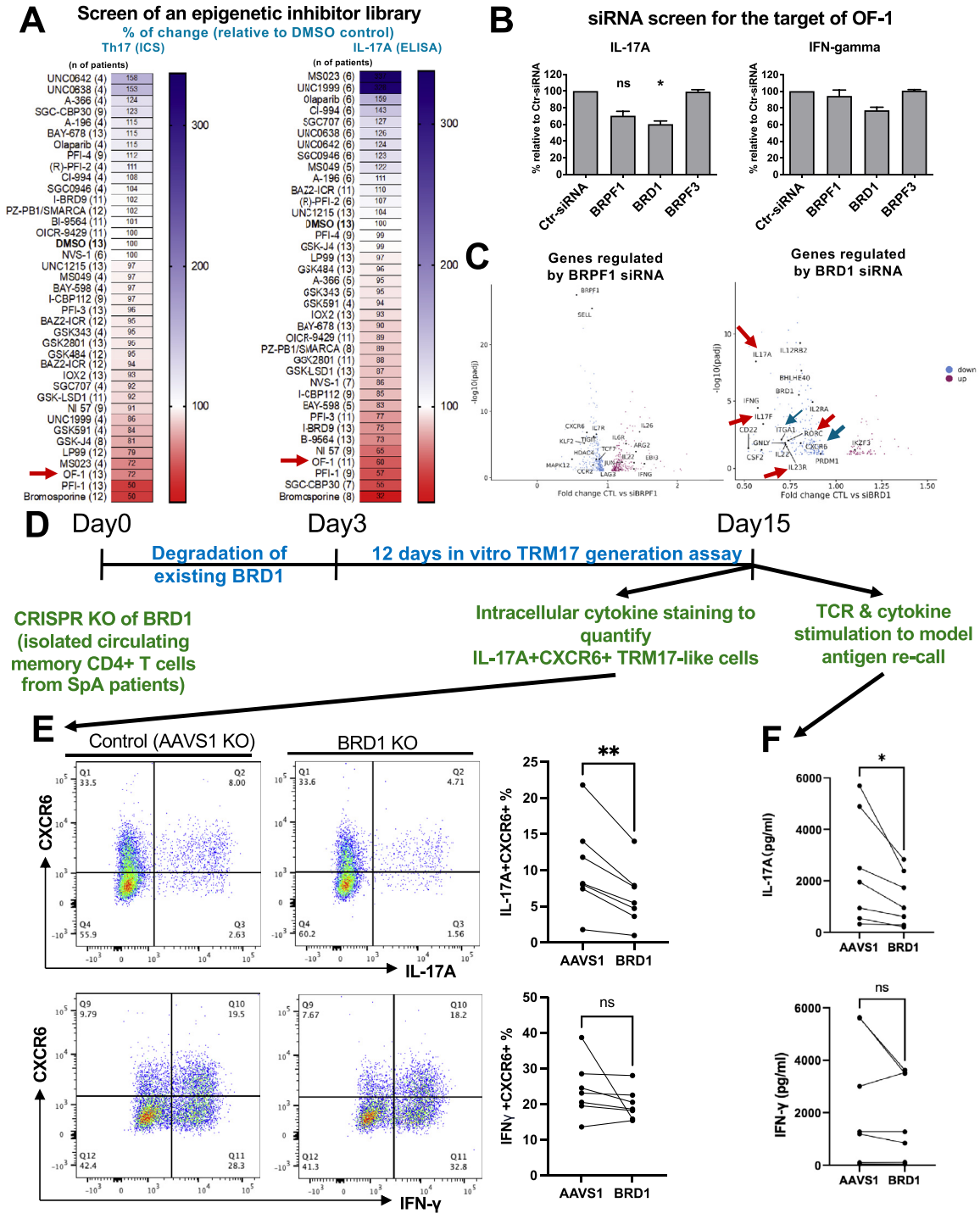

Figure 5. The epigenetic regulator BRD1 plays a role in TRM17 generation. A library of epigenetic inhibitors was screened using CD4+ T cells from SpA patients. Depending on the amount of blood obtained, a range of 4-13 donors were recruited for different inhibitors. The changes of Th17 frequency (based on IL-17A intracellular cytokine staining) and the level of IL-17A secretion (ELISA) relative to vehicle control (DMSO) are shown (A). (B) The respective influence of siRNAs for BRPF1, BRD1, and BRPF3 on IL-17A secretion by CD4+ T cells stimulated with TABs and Th17-promoting cytokines (IL-1β and IL-23) (data from 3 SpA patients). (C) The transcriptome changes induced by the siRNA silencing of BRPF1 and BRD1 (data from 6 SpA patients). D, Schematic representation of BRD1 knockout for the in vitro TRM17 model. E and F, Effect of BRD1 knockout on the generation of TRM17-like cells and their IL-17A production upon antigen recall (data from 7 SpA patients). The *P* value was assessed using the Friedman test for (B) and paired Student's t test for (E and F) (*$P \leq .05$; **$P \leq .01$). Median ± SEM is shown in (B). AxSpA, axial spondyloarthritis; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; SEM, standard error of the mean; SpA, spondyloarthritis; TAB, T-cell activation beads.

Competing interests

This work has been partially funded by J&J to the Cartography Consortium. LC has received research support from Novartis. PB has received research support from Regeneron, Benevolent AI, Novartis, and GSK. HHU has received research support or consultancy fees from J&J, UCB Pharma, Eli Lilly, BMS Celgene, and GSK. Liye Chen reports financial support was provided by J&J. If there are other authors, they declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

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Acknowledgements

We thank the patients for agreeing to participate in research and the Birmingham Tissue Analytics facility for generating the CosMx data. This work was funded by a Versus Arthritis career development award to LC 22053. FL, PB, CB, and HU were supported by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, or the Department of Health.

Funding

This work was funded by a Versus Arthritis Career Development Award to LC (22053) and by J & J to the Cartography Consortium.

Patient consent for publication

Not applicable.

Ethics approval

Venous blood was obtained under protocols approved by the Oxford Research Ethics committee (ethics reference number 06/Q1606/139). Synovial tissue were obtained under protocols approved by the Oxford Research Ethics committee (ethics reference number 06/Q1606/139), South Birmingham Research Ethics committee (ethics reference number 14/WM/1109) and Ethics Committee of Shanghai Sixth People's Hospital (2024-KY-132).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Data has been deposited at GEO (GSE290921) and will be released following the publication of the work.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.04.018.

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