

A single-cell and spatial transcriptomic atlas of human tuberculous constrictive pericardium

Feng Xiong,^{a,e,***} Yangran Qi,^{b,e} Shuzhen Wang,^a Lijuan Zhang,^a Fuyi Cheng,^c Kunyue Tan,^a Jian Chen,^d Yali Lei,^a Wenjie Yang,^b Zelei Zhao,^b Liang Huang,^b Lijian Cheng,^d Yong Luo,^{d,**} and Yi Wang^{b,*}

^aDepartment of Cardiology, Affiliated Hospital of Southwest Jiaotong University, The Third People's Hospital of Chengdu, Chengdu, 610031, Sichuan, China

^bDepartment of Pathophysiology, West China College of Basic Medical Sciences & Forensic Medicine, Sichuan University, Chengdu, 610041, Sichuan, China

^cState Key Laboratory of Biotherapy and Cancer Centre/Collaborative Innovation Centre of Biotherapy, West China Hospital, Sichuan University, Chengdu, 610041, Sichuan, China

^dDepartment of Cardiovascular Surgery, Affiliated Hospital of Southwest Jiaotong University, The Third People's Hospital of Chengdu, Chengdu, 610031, Sichuan, China

Summary

Background Tuberculous pericarditis (TP) can progress to tuberculous constrictive pericarditis (TB-CP), a life-threatening fibrotic syndrome. However, the cellular architecture and intercellular circuits that link granulomatous inflammation to pericardial fibrosis remain poorly defined.

Methods We performed single-cell RNA sequencing (scRNA-seq) on 81,634 cells from surgically resected pericardium of six patients with TB-CP and three normal controls. Ligand-receptor and trajectory analyses were integrated with spatial transcriptomics, histology, immunohistochemistry, and multiplex immunofluorescence to map cell states, signalling pathways, and immune-stromal niches in situ.

Findings We generated an integrated single-cell and spatial atlas of the human pericardium in TB-CP, delineating 14 major cell types and heterogeneous transcriptional programmes associated with granuloma formation, vascular remodelling, and fibrotic activation. Spatial mapping revealed MMP9⁺ macrophage-rich granulomatous cores surrounded by infiltrating CCL19⁺ fibroblasts and T cells. In parallel, S100A4⁺ endothelial cells displayed endothelial-to-mesenchymal transition-like programmes and trajectories converging on ACTA2⁺ myofibroblasts, supported by immune cell-derived TGF- β and VEGF signalling. Ligand-receptor analysis and spatial co-localisation suggested macrophages may act as hubs that couple immune activation to endothelial reprogramming, fibroblast activation, and T-cell recruitment within fibrotic lesions.

Interpretation This human atlas defines the cellular landscape and key intercellular circuits underlying the immunopathogenesis of TB-CP. Our findings show that a spatially organised immune-endothelial-fibroblast network is associated with pericardial fibrosis and nominate VEGF/TGF- β pathways and associated cell states as potential biomarkers and therapeutic targets.

Funding National Natural Science Foundation of China (82270486 to F.X.); Science and Technology Bureau of Sichuan Province (2021YFS0051, 2024YFFK0209 to Y.W.; 2024NSFC0646 to F.X.).

Copyright © 2026 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords: Single-cell RNA sequencing; Spatial transcriptomics; Tuberculosis; Constrictive pericarditis

Introduction

Tuberculous pericarditis (TP) is a severe extrapulmonary manifestation of tuberculosis, which serves as a

major cause of constrictive pericarditis (CP).¹ It has been reported that tuberculosis (TB) accounts for approximately 4% of acute pericarditis, and 6% of CP

*Corresponding author.

**Corresponding author.

***Corresponding author.

E-mail addresses: wang83@scu.edu.cn (Y. Wang), loyong8@sina.cn (Y. Luo), xiong.feng05@163.com (F. Xiong).

^cCo first authors.



eBioMedicine
2026;126: 106233
Published Online xxx
<https://doi.org/10.1016/j.ebiom.2026.106233>

Research in context

Evidence before this study

Tuberculous constrictive pericarditis (TB-CP) is a severe complication of *Mycobacterium tuberculosis* infection that causes diastolic heart failure and often requires surgical pericardiectomy. Previous studies have described the clinical features, imaging characteristics, and surgical outcomes of TB-CP, and a few have examined pericardial fluid cytokines or bulk tissue pathology, suggesting granulomatous inflammation and fibroproliferation as key processes. However, the cellular composition, immune-stromal niches, and in situ signalling circuits that link granuloma formation to fibrotic constriction have not been systematically resolved. To our knowledge, no study has integrated single-cell RNA sequencing with spatial transcriptomics to map the human TB-CP pericardial microenvironment.

Added value of this study

In this study, we generated an integrated single-cell and spatial transcriptomic atlas of human TB-CP by profiling approximately 81,600 cells from surgically resected pericardium of six patients with TB-CP and three normal controls. We delineated 14 major cell types and 33 subclusters and uncovered a granuloma-centred circuit in which SPP1/MMP9 macrophages anchor necrotising granulomas and neighbour CCL19⁺ invasive fibroblasts and T cell aggregates. We identified a S100A4⁺ endothelial subset with EndoMT-like transcriptional programmes and

pseudotime trajectories converging on ACTA2⁺ myofibroblasts. Ligand-receptor analysis, spatial co-localisation, and phospho-readouts highlighted macrophage-derived VEGF and TGF- β /SMAD3 signalling as key links between immune activation, endothelial reprogramming, and fibroblast activation. These findings were validated by spatial transcriptomics, multiplex immunofluorescence, and protein assays in human pericardial tissue.

Implications of all the available evidence

Taken together, the available evidence suggests that TB-CP fibrosis is not a passive scar but the outcome of an organised immune-endothelial-fibroblast circuit embedded within granulomatous lesions. Our atlas provides a human reference map of TB-CP pericardium, identifies SPP1/MMP9 macrophages, S100A4⁺ EndoMT-like endothelial cells, and CCL19⁺ fibroblasts as key cellular players, and implicates VEGF and TGF- β /SMAD3 pathways as potential druggable nodes. The cell states and signatures we define nominate assay-ready biomarkers (for example SPP1 and p-SMAD3) for risk stratification and monitoring of patients with TB-CP and may inform the design of host-directed therapies aimed at modulating immune-vascular-stromal interactions to prevent or attenuate constrictive remodelling. These concepts may also be relevant to other infectious and non-infectious forms of pericardial fibrosis.

cases,² while in high-burden areas (such as sub-Saharan Africa and parts of Asia), the proportion of pericardial diseases attributable to TB infection may exceed 50%.^{3,4} CP is featured by pericardium fibrosis and calcification, which can result in life-threatening diastolic heart failure.⁴ Various pathological changes in TP were identified, including increase in immune-reprogramming, fibroblast proliferation and vascular remodelling.⁵ Mechanistically, the transcriptomic and proteomic profiling of pericardial fluid from patients with TP revealed an enrichment of fibrosis regulators (COL1A1 and CTGF), elevated proinflammatory effectors (IFN- γ , IL-1 β , IL-8 and IL-6) as well as abundant matrix metalloproteinase factors (MMPs and TIMPs).^{6,7} Early studies on pericardial effusions indicated IFN- γ -producing CD4⁺ T cells were the principal lymphoid population in HIV-negative patients with TP.⁸ Moreover, recent study demonstrated marked activation of the NLRP3 inflammasome in human pericarditis, while NLRP3 or IL-1 blockage could alleviate murine pericardial inflammation.^{5,9} These findings suggest that immune cell activation is a central mechanism in TP.

More recently, scRNA-seq studies have provided high-resolution insights into immune landscapes in the blood and tissues of patients with tuberculosis. For instance, a comprehensive single-cell atlas of peripheral

blood revealed a striking expansion of monocytes in patients with severe conditions, accompanied by profound functional exhaustion and apoptosis in lymphocyte subclusters.¹⁰ Additionally, combined scRNA-seq and cell surface antibody sequencing analysis revealed diminished cytotoxicity and heightened exhaustion in T cells.¹¹ Moreover, in lung tissue, hypermetabolic tuberculous lesions were characterised by an accumulation of myeloid and regulatory T cells (Treg), establishing a localised immunosuppressive microenvironment.¹² Despite these findings, detailed insights into the immune landscape of TP-associated pericardial pathology remain largely unknown.

ST has recently emerged as a powerful technology that preserves tissue architecture while enabling in situ gene expression profiling.¹³ It has been successfully applied to characterise cell-cell interactions and delineate localised tissue remodelling programmes in lung and skin. In pulmonary tuberculosis, ST displayed early pneumonia lesions were dominated by TREM2⁺ foamy macrophages, while organised granulomas were featured by compartmentalised T cell-macrophage niches in dictating bacterial clearance and disease persistence.¹⁴ On the other hand, spatial mapping of sarcoid granulomas in skin revealed metabolically reprogrammed macrophages, cytokine-producing

Th17.1 cells, and extracellular matrix (ECM)-remodeling fibroblasts as central components of granuloma organisation, indicating conserved immune-stromal interplays shape granulomatous pathology.¹⁵ However, to date, few detailed molecular characterisations of human pericardial samples have been reported, limiting mechanistic insight into the TP/CP.

By combining scRNA-seq with ST approaches on surgically resected pericardium from six patients with TB-CP and three matched controls, we generated spatially resolved single-cell atlas of human pericardium. This dataset, based on ~81,600 high-quality single-cell profiles and whole-tissue spatial maps, (i) identifies the cellular composition and state transitions involved in granuloma formation and pericardial fibrosis; (ii) delineates spatially organised intercellular interactions that couple immune activation with endothelial remodelling and stromal activation; and (iii) defines cell-type-specific signalling modules and ligand-receptor circuits within CP lesions. Together, these findings provide a comprehensive landscape for understanding molecular mechanisms associated with pericardial fibrosis post TB infection.

Methods

Patients and sample collection

The protocol for collection of human pericardial tissues and the standardised tissue-processing workflow was approved by the Institutional Review Board of the Third People's Hospital of Chengdu ([2023] S-183). Patient characteristics are summarised in [Table S1](#). The TB-CP cohort included 3 male and 3 female patients, and the normal control group included 1 male and 2 female patients. All participants in this study were of East Asian (Chinese) ancestry. Patients were excluded for prior pericardiectomy, competing causes of constrictive physiology (for example, untreated severe coronary artery disease), active malignancy, or other systemic conditions likely to confound tissue interpretation. Excised specimens were transported on ice and subdivided according to a prespecified protocol for scRNA-seq, ST, formalin fixation/paraffin embedding, and molecular assays. All patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Control group (NC) sample collection

Inclusion and exclusion criteria.

Inclusion. Adults aged 18–60 years, irrespective of sex, requiring surgical repair for atrial or ventricular septal defects or other congenital cardiac anomalies. Exclusion criteria included a history of tuberculosis, prior cardiac surgery, pregnancy, malignancy, autoimmune disease, severe renal dysfunction, or radiotherapy.

Surgical procedures. Pericardial tissue was obtained during autologous pericardial patch repair for large atrial or ventricular septal defects. The excised pericardium was trimmed to match the defect size and then sutured in place as a patch to ensure complete closure of the septal defect.

Tuberculous-associated constrictive pericarditis group (CP) sample collection

Inclusion and exclusion criteria.

Inclusion. Adults aged 18–60 years who were clinically suspected or confirmed to have tuberculous pericarditis and subsequently underwent pericardiectomy at the Third People's Hospital of Chengdu were prospectively enrolled. The diagnosis of tuberculosis was based on the Diagnostic Criteria for Tuberculosis (WS 288-2008), in conjunction with clinical evaluation. Patients were eligible if they met the following criteria: (i) diagnosed as tuberculosis according to WS 288-2008, based on clinical manifestations (e.g., cough, expectoration ≥ 2 weeks, or haemoptysis) and/or radiographic features consistent with pulmonary tuberculosis, with or without additional microbiological or histopathological evidence; (ii) had received standard anti-tuberculosis treatment prior to surgery, with documented clinical stabilisation or improvement; (iii) presented with imaging or haemodynamic evidence of pericardial constriction, and the surgical indication for pericardiectomy was confirmed by the treating physicians. In all cases, evidence of pericardial constriction was required, based on imaging or haemodynamic findings. Postoperative pathology, when available, was used as the final confirmatory evidence.

Exclusion criteria included prior cardiac surgery, immune disorders, unresolved malignancy, other causes of heart failure (e.g., coronary artery disease, pulmonary heart disease), previous pericardiectomy, or history of radiotherapy.

Surgical procedures. Pericardiectomy was performed through a median sternotomy in all patients. The operation followed a standardised sequence as previously described.¹⁶ The thickened pericardium over the right ventricular outflow tract and ascending aorta was initially excised and the resection was then extended toward the left ventricular apex. Complete release of the left ventricle was achieved before continuing the dissection over the right ventricular surface, right atrium, and atrioventricular groove. Special attention was given to the fibrotic constriction rings at the junctions of the superior and inferior vena cava with the right atrium, which were circumferentially relieved. The extent of resection routinely reached the pericardium medial to both phrenic nerves, superiorly to the pericardial reflections of the ascending aorta and pulmonary artery, and inferiorly to the diaphragmatic surface. Whenever feasible, additional dissection was

carried posterior to the left phrenic nerve to achieve more radical release.

Idiopathic constrictive pericarditis samples

Pericardial tissue samples from patients with idiopathic constrictive pericarditis were obtained from archived formalin-fixed paraffin-embedded (FFPE) specimens stored in the Department of Pathology, the Third People's Hospital of Chengdu. These specimens were originally collected for routine diagnostic purposes and were retrieved retrospectively for research use. All idiopathic CP samples were fully anonymised prior to analysis, and no patient-identifiable information was accessible to the investigators.

Tissue processing and cell preparation

All excised pericardial tissues were trimmed to remove cauterised edges and adherent clots, washed repeatedly in ice-cold saline, and immediately divided into: (i) scRNA-seq group (>500 mg), (ii) paraffin-embedded histology group (>0.5 × 0.5 cm²), and (iii) cryopreservation group for downstream assays. Peripheral blood (2 mL) was collected intraoperatively into serum-separating tubes and processed post-operatively. All tissue samples used for spatial transcriptomics were pathologically assessed for quality, snap-frozen in liquid nitrogen, and stored at –80 °C until further use.

Single-cell RNA sequencing

10× Genomics library preparation and sequencing platform. After harvested, about 500 mg pericardium tissues were washed in ice-cold PBS (Hyclone SH30256.01) and dissociated using 20 mL digestion solution containing 2 mg/mL Collagenase II (Sigma V900892) and 10 µg/mL DNaseI (Sigma 9003-98-9). The samples were incubated at 37 °C for 1.5 h, filtered using a 70 µm cell filter (Miltenyi 130-098-462), and centrifuged at 300 g, 4 °C for 5 min to collect cell precipitates. Cell count and viability was estimated by using fluorescence Cell Analyser (Countstar® Rigel S2) with AO/PI reagent after removal erythrocytes (Solarbio R1010) and then dead cells removal was decided to be performed or not (Miltenyi 130-090-101). Finally, fresh cells were washed twice in the RPMI1640 (Gibco 11875119) and then approximately 1 × 10⁴ fresh cells were resuspended at 1 × 10⁶ cells per ml in RPMI1640 and 2% FBS (Gibco 10100147C).

Single-cell RNA-Seq libraries were prepared using Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (10× Genomics Catalogue No.1000268). Briefly, appropriate number of cells were mixed with reverse transcription reagent and then loaded to the sample well in Chromium Next GEM Chip G. Subsequently Gel Beads and Partitioning Oil were dispensed into corresponding wells separately in chips. After emulsion droplet generation reverse transcription were performed at 53 °C for 45 min and inactivated at 85 °C for 5 min. Next,

cDNA was purified from broken droplet and amplified in PCR reaction. The amplified cDNA product was then cleaned, fragmented, end repaired, A-tailed and ligated to sequencing adaptor. Finally, the indexed PCR was performed to amplify the DNA representing 3' polyA part of expressing genes which also contained Cell Bar code and Unique Molecular Index. The indexed sequencing libraries were cleanup with SPRI beads, analysed by Qubit (Thermo Fisher Scientific Q33226) and Bio-Fragment Analyser (Bioptic Qsep400). The libraries were then sequenced on illumina NovaSeq 6000 with PE150 read length.

Data alignment and quality control (Cell Ranger, Seurat). Raw FASTQ files Reads were aligned to the GRCh38 human reference genome with the cellranger (v7.2.0) count function, generating cell-by-gene expression matrices. Quality control (QC): Cells were filtered based on the following thresholds, determined by manual inspection of distribution plots: Minimum number of detected genes: 10 (to be inserted from actual QC). Maximum mitochondrial read fraction: 10%. Include cells where at least 200 features are detected. Cells failing any QC criterion were removed. Gene expression matrices from all retained cells were normalised using the NormaliseData method (Seurat v5.2.0) by default Parameter Values. Integration and clustering: Highly variable genes were identified (top 2000 genes) across datasets. Batch correction and dataset integration were performed using the CCAIntegration, Dimensionality reduction was performed using UMAP, clustering was using FindClusters function with resolution 0.6.

Doublet removal and normalisation. Doublets were predicted using DoubletFinder (v2.0.3). Normalisation was performed with the LogNormalize method. The expected doublet rate was set to 0.076, and doublets were identified with the doubletFinder_V3 function, specifying 15 statistically significant principal components while keeping all other parameters at their default values.

Dimensionality reduction, clustering, annotation. Cell type annotation: These annotations were manually refined based on canonical marker gene expression.

Differential gene expression (DGE) analysis: DEGs between groups or cell types were identified using FindMarkers function applied to scaled RNA array, with batch and QC metrics as covariates. Genes were retained if expressed in ≥10% of cells or with an average normalised log count ≥1. Adjusted p-values were computed using the Benjamini-Hochberg method.

Downstream analysis. Pathway and network analysis: Gene set enrichment analysis (GSEA) was conducted against MSigDB Hallmark (H), and C5 collections. Ligand-receptor interaction inference was performed using CellPhoneDB (v5.0.1). Pseudotime analysis was performed using Monocle 2.

Pseudotime–ssGSEA correlation analysis. To assess gene-set activity dynamics along pseudotime, we computed per-cell ssGSEA scores (GSVA, method = “ssgsea”) on the normalised Seurat matrix (log-normalised as in Section 3.2). Scores were merged with cell metadata (cell type, group, state, and Monocle2 pseudotime) to generate a working table. For visualisation, pseudotime was binned (ceiling function), median scores were aggregated per bin, and smoothed LOESS curves with 95% CI were plotted using ggplot2. Statistical associations between ssGSEA scores and pseudotime were tested by Spearman’s rank correlation (R base cor. test, method = “spearman”), with correlation coefficients and p-values annotated on plots. Multiple testing was corrected by the Benjamini-Hochberg method (FDR).

Spatial transcriptomics

Visium tissue section preparation and library construction. Spatial gene expression profiling was performed using the Visium Spatial Gene Expression Reagent Kit (10× Genomics). Pericardial tissue blocks were embedded in OCT (Tissue-Tek) and stored at –80 °C until sectioning. Sections (5–10 µm thick) were cut using a pre-cooled cryostat and mounted within the capture areas of Visium Spatial Gene Expression Slides. Prior to the main experiment, tissue optimisation was conducted using the Visium Tissue Optimisation Kit (10× Genomics) to determine the optimal permeabilization time for pericardial tissue. Cryosections from representative samples were used for fluorescence imaging-based permeabilization time testing, identifying 18 min as optimal (exact value to be confirmed from experimental log). For the main spatial transcriptomics workflow, tissue sections were fixed, subjected to H&E staining, and imaged on an Olympus IX83 microscope equipped with a Hamamatsu ORCA-Flash 4.0 sCMOS camera (10× objective). Permeabilization, reverse transcription, and second-strand synthesis were performed directly on the slide. cDNA amplification cycles were optimised for each sample using the KAPA SYBR FAST qPCR Kit (KAPA Biosystems). Spatial libraries were prepared according to the manufacturer’s protocol and sequenced on an Illumina NovaSeq 6000.

Data processing (Space Ranger). Raw data were processed with Space Ranger (v3.1.1, 10× Genomics) using GRCh38 genome annotation v2020-A.

Integration with scRNA-seq (Seurat). Normalisation of the scRNA-seq data was performed using the SCTransform method. Integration of the single-cell and spatial transcriptomic datasets was carried out with the FindTransferAnchors function, based on the SCT-normalised matrix. Cell type annotation of spatial spots was subsequently performed using the TransferData function, whereby each spot was assigned to the cell type with the highest prediction score, which was designated as the predicted cell type.

Spatial projection of granuloma and functional gene signatures. To map granuloma-associated and other functional gene signatures onto spatial transcriptomic (ST) sections, we computed per-spot single-sample gene-set enrichment scores (ssGSEA) using the GSVA package and the SCT-normalised expression data from Seurat objects. Gene sets used for projection (including the granuloma score signature and other modules) are provided in Table S2.

Flow cytometry

Fresh pericardial tissues from patients with TP were processed immediately after surgical excision. Samples were minced into ~1 mm³ fragments and digested in pre-chilled enzymatic buffer (T002, Docsense, China) containing collagenase II (1 mg/mL), DNase I (50 U/mL), and elastase (0.5 mg/mL) in RPMI-1640 medium. Digestion was performed at 37 °C with gentle orbital shaking (60 rpm) for 30–60 min, with periodic pipette trituration to facilitate dissociation. Following digestion, suspensions were filtered through a 70 µm nylon strainer (Biosharp, China), pelleted by centrifugation (300×g, 5 min, 4 °C), and resuspended in FACS buffer (PBS with 2% FBS and 0.1% sodium aside). Peripheral blood samples (2 mL) collected intraoperatively were transferred into EDTA-coated tubes, followed by red blood cell lysis using ACK buffer (A4000225501, Gibco, USA) for 3–5 min at room temperature. After washing in FACS buffer, the mononuclear cell fraction was obtained for downstream analysis. For surface staining, single-cell suspensions were incubated with Fc receptor blocking reagent (C1752S, Beyotime, China) for 10 min at 4 °C, then stained with fluorochrome-conjugated antibodies against human CD3, CD19, and CD138 (see Key Resources Table) for 30 min at 4 °C in the dark. After staining, cells were washed twice with FACS buffer, filtered through 35 µm mesh caps, and analysed on a BD FACSAria III flow cytometer equipped with FACSDiva software. Gating strategies included: (i) exclusion of debris (FSC/SSC), (ii) singlet discrimination (FSC-A vs FSC-H), (iii) live/dead discrimination using Zombie Aqua viability dye (BioLegend), and (iv) identification of immune cell subsets based on CD3, CD19, and CD138 expression. Data was analysed using FlowJo v10.8.1, and results were expressed as percentages of total live single cells.

Haematoxylin and eosin, immunohistochemistry, and multiplex immunofluorescence staining

Haematoxylin and eosin (H&E) staining. Pericardial tissues allocated for histology were fixed in 10% neutral-buffered formalin for 24 h at room temperature, dehydrated through graded ethanol, cleared in xylene, and embedded in paraffin. Sections (5 µm) were cut using a rotary microtome (Leica RM2235) and mounted on SuperFrost Plus slides (Thermo Fisher Scientific). Slides were stained with Mayer’s haematoxylin for

5 min, rinsed, differentiated in acid alcohol, blued in Scott's tap water substitute, counterstained with eosin Y for 2 min, dehydrated, cleared, and cover slipped with neutral mounting medium. Stained slides were scanned using the SLIDEVIEW VS200 system (Olympus).

Immunohistochemistry (IHC). Paraffin sections underwent deparaffinization in xylene, rehydration through graded ethanol, and antigen retrieval using 10 mM citrate buffer (pH 6.0) at 95 °C for 20 min in a microwave oven. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in methanol for 15 min, and non-specific binding was blocked with 10% normal goat serum for 30 min at room temperature. Sections were incubated overnight at 4 °C with primary antibodies against ITLN, CD68, CD5, CD3, CD4, CD8, CD19, CD20, CD79A, CD138, α -SMA, TGF- β , IFN- γ or COL1A (see Key Resources Table). After washing, HRP-conjugated secondary antibodies were applied for 1 h at room temperature. Visualisation was achieved with 3,3'-diaminobenzidine (DAB) substrate and counterstaining with haematoxylin. Stained slides were scanned using the SLIDEVIEW VS200 system (Olympus) or directly imaged with an Olympus BX53 upright microscope equipped with a DP74 digital camera.

Multiplex immunofluorescence. FFPE tissues were cut into 4- μ m sections and placed on polylysine-coated slides. Before staining, sections were deparaffinised in xylene and were then rehydrated in 100%, 90%, and 70% alcohol successively, followed by microwave-based antigen retrieval, endogenous peroxidase inactivation and nonspecific site blocking. Briefly, different primary antibodies (Key Resource Table) were sequentially applied, followed by HRP conjugated secondary antibody incubation and tyramide signal amplification. The sections were microwave heat-treated after each TSA operation. Nuclei were stained with DAPI after all the human antigens had been labelled. Following enclosure by ProLongTM Diamond Antifade Mountant (Invitrogen), slides were scanned using the Panoramic SCAN II (3DHISTECH Ltd.) and analysed with Slidedviewer software.

Fluorescence in situ hybridisation (FISH) combined with immunofluorescence (IF). FFPE pericardial sections (5 μ m) were baked at 60 °C for 1 h, deparaffinised in xylene, and rehydrated through graded ethanol to PBS. Antigen retrieval was performed in 10 mM citrate buffer (pH 6.0) at 95 °C for 15–20 min. Sections were blocked with 10% normal goat serum and incubated with primary antibodies overnight at 4 °C. After antibody incubation, slides were post-fixed (4% paraformaldehyde, 10 min), washed in PBS, and processed for RNA hybridisation. A custom-designed VEGFB probe (5'-FAM-CTGCACAGTCACGCAGCTGGGCACAGCTGTTTGGCCACGGTGCCCATGAGCTCCACAGTCAA-FAM-3') was hybridised in hybridisation buffer (50% formamide, 2 $\times$ SSC, 10% dextran sulphate, 0.1% SDS, and RNase-free competitor nucleic

acid) at 37 °C overnight (final probe concentration 0.5–1.5 ng/ μ L). Post-hybridisation washes were performed in 2 $\times$ SSC and low-salt SSC under stringent conditions. To enhance detection sensitivity, FAM signals were amplified by anti-FITC-HRP conjugate followed by TSA/Opal fluorophore deposition. Antibody signals were visualised with species-appropriate fluorescent secondary antibodies. Finally, slides were counterstained with DAPI, mounted in ProLong Antifade, and imaged on an Olympus SLIDEVIEW VS200 or BX53 microscope. Negative controls (no-probe) and positive control probes were included to confirm specificity and RNA integrity.

Western blot analysis

Fresh-frozen human pericardial tissues (normal control and constrictive pericarditis) were pulverised in liquid nitrogen using a pre-chilled mortar and pestle, and the resulting tissue powder was immediately lysed in ice-cold RIPA buffer (Thermo Fisher Scientific) supplemented with protease inhibitor PMSF and phosphatase inhibitor cocktail (Sigma-Aldrich). Lysates were incubated on ice for 30 min with intermittent vortexing and clarified by centrifugation at 12,000 rpm for 15 min at 4 °C. Protein concentrations were measured using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of total protein (30–50 μ g) were mixed with 1 $\times$ loading buffer containing 5% β -mercaptoethanol, denatured at 95 °C for 5 min, resolved by 10% SDS-PAGE, and transferred onto PVDF membranes (Millipore) using wet transfer (300 mA, 90 min). Membranes were blocked with 1 $\times$ Yeasen Fast Blocking Buffer (protein-free, Yeasen Biotechnology) for 15 min at room temperature, followed by overnight incubation at 4 °C with primary antibodies against VEGFA and TGFB1 (1:1000 dilution, see Key Resources Table). After washing, membranes were incubated with HRP-conjugated secondary antibodies (1:5000) for 1 h at room temperature. Protein bands were visualised using ECL substrate (Biosharp) and imaged with a ChemiDoc MP Imaging System (Bio-Rad). Band intensities were quantified by ImageJ software and normalised to β -actin or GAPDH. Statistical analysis of relative protein expression was performed in GraphPad Prism.

Statistical analysis

Data visualisation and statistical analysis were primarily conducted in R (v4.3.2). For single-cell and spatial transcriptomics, differential expression testing was performed with Seurat's FindMarkers function (Wilcoxon rank-sum test) and corrected for multiple comparisons by the Benjamini-Hochberg method. Cell type proportion comparisons across conditions were assessed with non-parametric tests (Mann-Whitney U or Kruskal-Wallis, as appropriate). Correlations were evaluated using cor.test in R, applying Pearson or Spearman methods according to distributional

assumptions. For validation experiments, results are presented as mean $\pm$ SEM and compared with unpaired Student's *t* tests (two groups) or one-way ANOVA with Holm-Bonferroni post hoc correction (>2 groups). All statistical tests were two-sided unless otherwise specified. Exact test details and sample sizes (*n*) are provided in Figure Legends.

Role of the funding source

The funders provided financial support for this study and had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit the manuscript for publication.

Results

Single-cell transcriptome profiling of pericardium in patients with TB-CP

We collected normal control (NC) pericardium samples from three adults who underwent atrial or ventricular septal defect repair surgery, and CP samples from six patients with tuberculous constrictive pericarditis via pericardiectomy, in which fibrotic parietal pericardium was removed¹⁷ (Fig. 1A). The TB-CP group comprised three males and three females, aged 53–60 years, whereas the control group included one male and two females aged 19–34 years. All patients with TB-CP had a documented history of tuberculosis, and several presented with pericardial effusion and/or heart failure at the time of surgery. Detailed demographic and clinical information for each individual is provided in Table S1. ScRNA-seq was then conducted, followed by ST analysis on two CP samples and multiplex immunofluorescence (IF) staining for data validation (Fig. 1A). Echocardiography (ECHO) and cardiac computed tomography (CT) examinations demonstrated remarkable pericardial thickening as well as potential epicardial effusion in patients (Fig. 1B and Figure S1A).

A total of 81,634 qualified cells were captured for scRNA-seq analysis, encompassing 14 major cell types (Figure S1B left) that were further resolved into 33 transcriptionally distinct subclusters (Fig. 1C and Figure S1B right). Endothelial cells (EC) were characterised by higher expressions of PECAM1 (CD31), CDH5, and VWF. Smooth muscle cells (SMCs) expressed classical contractile markers including ACTA2, MYH11, and MUSTN1. Fibroblasts showed robust expressions of THY1, PDGFRA, DCN, and COL1A1, whereas myofibroblasts displayed compromised expression pattern along with these cell populations.¹⁸ Mesothelial cells were detected in NC (ITLN1 and MSLN), but nearly absent in CP samples. Myeloid lineage included macrophages (CD68, C1QA, and APOE), monocytes (VCAN, S100A8, and S100A9), dendritic cells (DCs) (CD1C, FCER1A, and CLEC9A),

and neutrophils (S100A12, FCGR3B, and CXCR2). The B cell lineage encompassed multiple differentiation stages including naive B cells (CD19, CD79A, and MS4A1 (CD20), plasmablasts (MZB1, IGHG1), plasma cells (SDC1 (CD138), MZB1, IGHG1, and TNFRSF17) as well as a cycling plasma cell subcluster (plasma cell markers and MKI67). T/NK cell compartment contains natural killer (NK) cells (GNLY, NKG7, and CD160), T cells (CD3D, CD3E, and CD3G), which were further categorised into CD4⁺ and CD8⁺ subclusters (CD4, CD8A, CD8B), and a small population of doublet cells possessing ambiguous T cell markers (Fig. 1D). Comparison of cell percent ratio revealed a substantial cluster expansion of myeloid, T and B lineages in CP pericardium (Figure S1C), suggesting an aberrant immune microenvironment formed.

Haematoxylin and Eosin (H&E) staining revealed marked granulomatous architecture in CP pericardium (Fig. 1E top). Immunohistochemistry (IHC) staining demonstrated an intact ITLN1⁺ mesothelial layer, accompanied by laminated collagen deposition (COL1), sparse microvasculature (CD31), and low abundance of macrophages (CD68) in normal tissue. In contrast, CP samples showed extensive mesothelial layer loss,⁴ along with increased fibrotic matrix components, pathological angiogenesis and prominent macrophage infiltration (Fig. 1E). Immunofluorescence (IF) staining of CD68, CD3, and THY1 validated the cellular organisation within pericardial granuloma, showing condensed CD68⁺ myeloid niches were surrounded by CD3⁺ lymphocytes and THY1⁺ fibroblasts (Fig. 1F and Figure S1E). In-detailed IHC staining using various cell markers further confirmed accumulation of myeloid and lymphoid populations in CP pericardium (Figure S1D). These findings suggested significant activation among stromal and immune cells during CP progression.

The transcriptional heterogeneity of myeloid clusters and the role of granuloma-associated MMP9⁺ macrophage subcluster in CP.

Myeloid cells were categorised into four major populations: dendritic cells (DC_a and DC_b subclusters), macrophages (Mac_a, Mac_b, and Mac_c subclusters), monocytes, and neutrophils. Among them, significantly expanded cell proportions in Mac_a and Mac_b and notably decreased neutrophils were found¹⁶ (Figure S2A). IF staining confirmed increased LYVE1⁺/CD68⁺ macrophages spreading across pericardial lesions (Figure S2B). Differentially expressed genes (DEGs) heatmap delineated distinct transcriptional profiles for each subcluster (Fig. 2A). Both DC_a and DC_b expressed canonical dendritic cell markers (CLEC10A and LAMP3). All macrophage subclusters uniformly expressed CD68, C1QA, and APOE, while monocytes were characterised by CD14, FCGR3A (CD16) and VCAN. Notably, DCs shared transcriptional feature in expressing CLEC5A, CLEC6A, and the costimulatory molecules (CD80 and CD86) with Mac_b

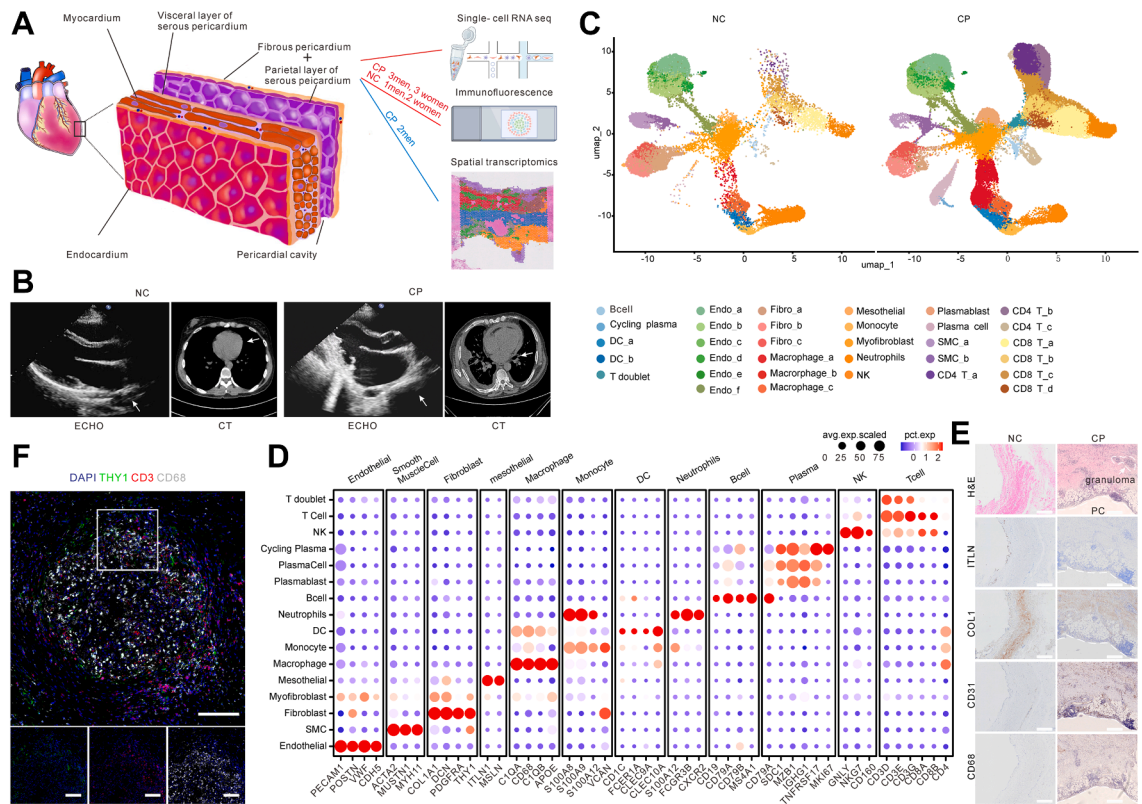


Fig. 1: Single-cell landscape of human healthy and CP pericardial tissue. A. Scheme of heart wall and pericardium (left). The normal human pericardium is a double-layered sac consisting of an outer fibrous pericardium and an inner serous pericardium. The serous pericardium is further divided into two parts: the parietal serous pericardium, which lines the inner surface of the fibrous pericardium, and the visceral serous pericardium, which closely covers the myocardium of the heart. The pericardium is typically less than 1 mm thick, and the pericardial cavity between these two serous layers normally contains less than 50 mL of clear serous fluid. Right: experimental approaches and data analysis strategies. B. Representative cardiac computed tomography (CT, left) and echocardiography (ECHO, right) images of pericardium (NC n = 3, CP n = 6). NC (negative control). CP (constrictive pericarditis), white arrows indicate normal and thickened pericardium. C. UMAP plots of 33 subclusters in NC and CP pericardium. D. Dot plot showing the expression levels of marker genes across pericardial cell clusters. The size and colour spectrum of dots indicates positive percentage and average expression. NK = natural killer cells; DC = dendritic cells; SMC = smooth muscle cell. E. H&E and IHC staining for ITLN, COL1, CD31 and CD68 on NC and CP samples (NC n = 2, CP n = 6). White dash lines indicate lost mesothelium in CP. PC = pericardial cavity. Scale bar, 500 μ m. F. IF staining for THY1 (green), CD3 (red) and CD68 (grey) in CP sample. DAPI (blue). Scale bars: 200 μ m (main) and 20 μ m (zoom-in).

and Mac_c, indicating their roles in pathogen-mediated T cell activation.¹⁹ DC_b was further distinguished by strong expression of NAPS. Mac_a only displayed canonical macrophage markers, resembling a quiescent or homeostatic subcluster. In contrast, Mac_b exhibited upregulation of granuloma-associated genes such as SPP1 and MMP9, together with fibrosis-related immunoregulation factor GPNMB.²⁰ Mac_c was characterised by high expression of tissue-resident markers (LYVE1 and MERTK)^{21,22} and M2-polarisation molecules (MRC1 and FOLR2).^{23,24} Interestingly, it also shared high expression profile of proinflammatory mediators, including IL1A, IL1B, and NLRP3, with monocytes, suggesting dual functions of this subcluster in resolved pericardial inflammation. Lastly, neutrophil-specific transcriptional programme was enriched for CXCR1,

CXCR2, S100A8, and S100A12, reflecting their canonical granulocytic identity (Fig. 2A and Figure S2C–D).

We further explored transcriptional heterogeneity among macrophage subclusters via extracted DEGs heatmap (Fig. 2B). Genes related to ribosomal biogenesis and translation (RPL11 and RPS10 *etc.*), were prominently enriched in Mac_a, suggesting a biosynthetic state. In contrast, Mac_b and Mac_c exhibited marked upregulation of oxidative phosphorylation (UQCRI10, COX8A, NUPR1 *etc.*) and antigen presentation genes (B2M, CTSS, HLA family genes) in CP group, indicating a metabolic shift toward high aerobic respiration that coordinates antigen presentation.^{25–27} Additionally, Mac_c displayed an augmented inflammatory signature across both control and CP samples, suggesting its roles in chronic inflammation. These

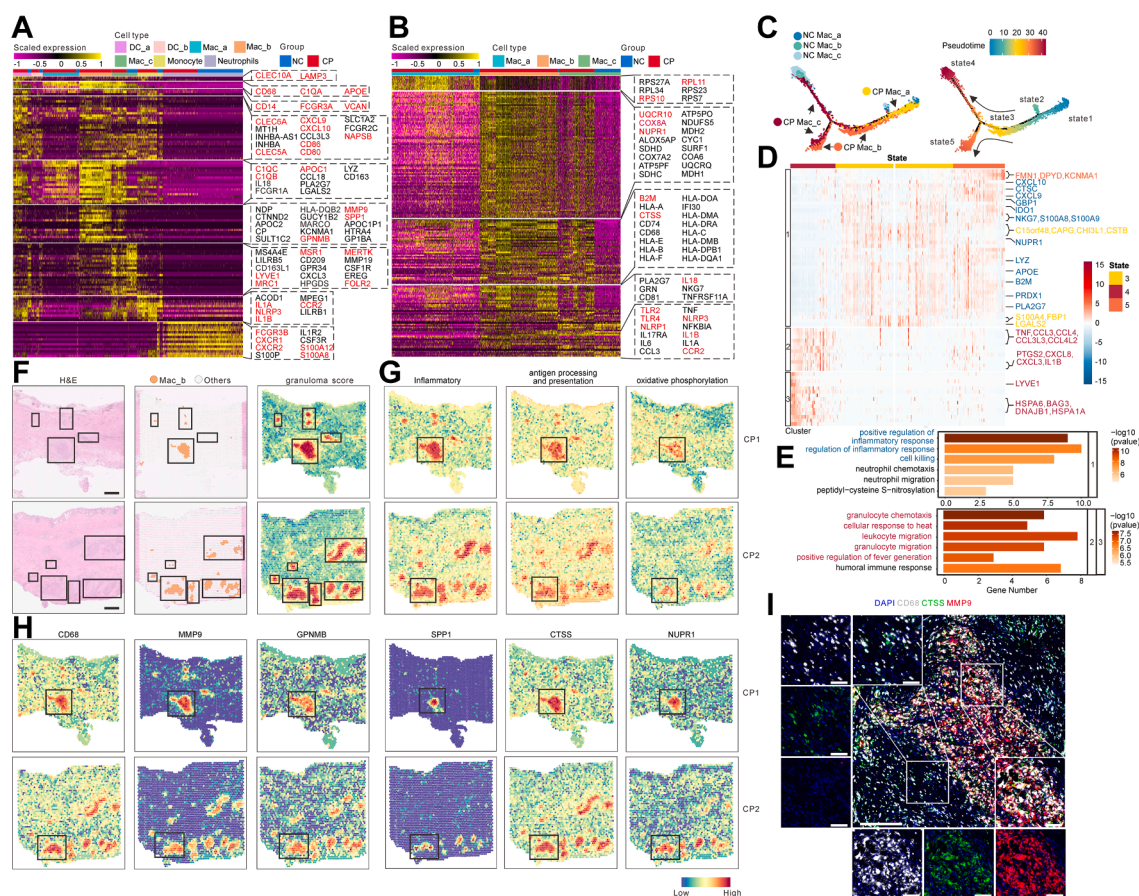


Fig. 2: Expression profiles of myeloid population and spatial organisation of granuloma-associated myeloid subset in CP pericardium.

A. scRNA-seq Heatmap of top DEGs in indicated cell clusters of NC (blue) and CP (red) pericardium. Representative genes are listed on right, with proposed functional markers in red. B. Heatmap showing functional DEGs across macrophage subclusters. Representative genes are listed on right, with proposed functional markers in red. C. Colour-coded macrophage subclusters (NC vs CP) (left, indicated by black arrows) and states (right, state1-6) along pseudotime trajectory (arrows denote direction of progression). D. State-resolved heatmap of clustered (Cluster 1–3) DEGs across state 3–5 as shown in C. Representative genes are labelled with the same colour code as indicated state (state 3 & 4 shared genes are labelled in blue). State 3 is duplicated (the same as state 6 in Fig. 3H and state 8 in 3 L). E. Bar plots showing Gene Ontology (GO) module enrichment of DEGs cluster as in D (top: cluster1; bottom: cluster 2 & 3). Colours of GO modules match with representative genes listed in D. F. Spatial localisation of granuloma-associated Mac_b subsets. Left: H & E staining of pericardium sections from two CP samples (black boxes indicate representative granuloma areas). Middle: spatial distribution of Mac_b. Right: spatial distribution of “granuloma score” overlay. Scale bars, 500 μ m, see also Figure 2 G–H. G. Spatial GO scores showing enrichment of “inflammatory response”, “antigen processing and presentation”, and “oxidative phosphorylation” modules in CP pericardium. H. Spatial expression maps of indicated genes in CP pericardium. I. Multiplex IF staining for CD68 (grey), CTSS (green), and MMP9 (red) in CP pericardium. DAPI (blue). Scale bars: 200 μ m (main) and 50 μ m (zoom-in).

functional characteristics were confirmed by GO enrichment and violin plots (Figure S2E–F).

Pseudotime ridge plots revealed Mac_a enrichment in early states, while Mac_b and Mac_c occupied intermediate and terminal stages (Figure S2G, top), where DEGs heatmap revealed early protein synthesis (IGFBP7 and ribosomal genes) and late immune activation (CXCL2/3, TANK, NFKB1) profiles along pseudotime curve (Figure S2G, bottom). State-specific pseudotime analysis positioned CP-derived Mac_a in early developmental (states 1–2), Mac_b in intermediate

(states 3 and 5), and Mac_c exclusively in state 4, representing a terminally differentiated population (Fig. 2C). Interestingly, NC Mac_b and Mac_c were mainly located at state 4, suggesting pre-existing functional divergence from NC Mac_a (Fig. 2C). Detailed DEGs heatmap displayed that state 3 and 5 shared functional modules of inflammatory regulation, neutrophil migration, and cell-mediated killing (Fig. 2D–E and Figure S2H, top). State 5 uniquely upregulated KCNMA1, FN1, DPYD and downregulated CAPG, CHI3L1, CSTB, while state 4 upregulated genes

linked to chemotaxis, leucocyte migration, and heat response pathways (Fig. 2D–E and Figure S2H, bottom) (Table S3).

ST analysis comprehensively delineated macrophage distribution in CP pericardial architecture, where “granuloma score” module^{28,29} co-localised with Mac_b subcluster (Fig. 2F). Dendritic cells were also found in granuloma centres, whereas monocytes and neutrophils were largely absent (Figure S2I). Consistent with GO analysis (Fig. 2E), inflammatory response module was assigned into Mac_b condensed zones, while antigen processing and presentation, and oxidative phosphorylation modules were shown spread across lesional areas (Fig. 2G). Gene-specific spatial expression patterns revealed that Mac_b marker gene MMP9 was enriched in granulomas, alongside GPNMB and SPP1. However, lysosomal antigen processing factor gene CTSS, and oxidative phosphorylation-associated gene NUPR1 were broadly expressed across CD68⁺ regions, mirroring Mac_b/c distribution (Fig. 2H). Multiplex IF staining confirmed co-localisation of CD68, MMP9, and CTSS within granuloma cores, with CD68⁺/CTSS⁺ cells at the periphery (Fig. 2I), suggesting that while MMP9⁺ Mac_b facilitates granuloma formation, it concurrently cooperates with Mac_c to regulate metabolic reprogramming and antigen presentation throughout the CP pericardium.

Heterogeneous molecular characteristics of lymphocyte subtypes

A comprehensive T lymphocytes landscape revealed three CD4⁺ T, four CD8⁺ T, and one NK subclusters. One additional doublet subcluster was also found, presumably due to disease-induced lymphocytes interaction, as higher frequency appeared in CP ($n = 535$, 1.69% of CP-T/NK) compared to NC samples ($n = 9$, 0.5% of NC-T/NK). Meanwhile, the significant expansions of CD4_a and CD4_b subclusters were detected in CP group (Fig. 3A). The top 20 DEGs heatmap revealed strong expression divergences: CD4_a was characterised by elevated expression of lymphoid chemokine genes CCL20 and IL26, which are well known for cell migration and antimicrobial immunity.^{30,31} CD4_b showed a classic Treg signature (LAYN, FOXP3, IL1R, CTLA4).³² CCNB2 and MKI67 expressions were enriched in CD4_c, indicating a proliferative state.^{33,34} Among CD8⁺ T cells, CD8_a expressed cytotoxic, chemotactic and immuno-modulation markers (TNFSF9, GZMK, CCL4).³⁵ CD8_b was featured by naive or stem-like signatures (SELL, TCF7, LEF1, and CCR7).³⁶ In contrast, CD8_c lacked distinct molecular features, with its top DEGs pattern largely overlapped with CD4_a, while CD8_d exhibited a dual programme characterised by exhaustion (SLAMF6 and TOX)³⁷ and progenitor-like markers (SELL, LEF1, TCF7). NK cells were identified by classical cytotoxic markers such as NCAM1(CD56), FCGR3A (CD16) and NKG7, as well as

antimicrobial peptide genes GNLY and CTSW (Fig. 3B and C).

Moreover, annotated immuno-functional modules from published literatures confirmed molecular signatures in T cell cluster (Table S4): CD4_a and CD4_b showed the strongest Treg profile, as well as elevated co-stimulatory molecule CD28; notably, CD4_b contributed additional co-stimulatory signalling (CD27) and Treg markers (FOXP3, LAYN, TIGIT) in the CP group. CD4_c was classified as a proliferative subcluster (PCNA, MKI67) while also sharing Treg-associated modules with CD4_a/b (Fig. 3D and Figure S3A). On the other hand, CD8_a was associated not only with terminal effector programmes, including cytotoxic and CD8⁺ Teff/Temra cells (Figure S3A), but also with exhausted and inhibitory T modules (LAG3, PDCD1, TOX). CD8_b was highlighted by canonical naive T signatures and annotated as a stem-like memory precursor. CD8_c presented a hybrid, but more comprised molecular phenotypes shown in CD4_a and CD8_a. In addition, this subcluster lacked expression of LEF1, TCF7, BATF and TOX, leaving it linked to intermediate cytotoxic programmes (CD8-EM and Cytotoxic). Strikingly, CD8_d emerged as a transcriptionally distinct subcluster with high expression of “progenitor exhaustion”-associated signatures (Fig. 3D and Figure S3A). Lastly, NK cells were demonstrated as cytotoxic and effective modules (Figure S3A). UMAP plots also displayed consistent expression patterns of featured genes, with TGFB1 broadly expressed across T lineage (Figure S3B).

Pseudotime ridges of CD4⁺ T cells showed that CD4_c was enriched in early state (Figure S3C, top), with upregulated expression of mitosis-related genes (PCNA, MKI67 *etc.*) (Figure S3C–E). In contrast, later pseudotime stages displayed upregulated lymphocyte differentiation and cytolytic genes (IL7R, RORA, GZMA, GZMK *etc.*), marking terminally differentiated CD4_a/b states (Figure S3C–E). Since most of the CD4⁺ T cells are disease-associated (Fig. 3A), seven pseudotime states reflected divergent phenotype polarisation in CP pericardium, with CD4_c enriched in state 1 and CD4_a/b spanning state 4–7 (Fig. 3E and F). State-specific analysis divided CP CD4_a/b into two major developmental fates (Fig. 3G), where DEGs heatmap (Figure S3F) showing fate1 represented Treg-like features (TIGIT, FOXP3 *etc.*) enriched in lymphocyte adhesion and T cell activation pathways (Figure S3G–H), while fate2 upregulated cytotoxic effectors (CTSH, GNLY *etc.*) and chemokines (CCL20, CCL4 *etc.*) genes, linked to cell killing and antimicrobial responses (Figure S3G–H). Consistently, cells from state 5–7 were hierarchically clustered along the fate2 trajectory, where state6 upregulated immunoregulatory genes (RTKN2, IKZF2), while state7 showed strong effector gene expression (GZMA, GZMB, LTB, TNFRSF4, TNFRSF18) (Fig. 3H).

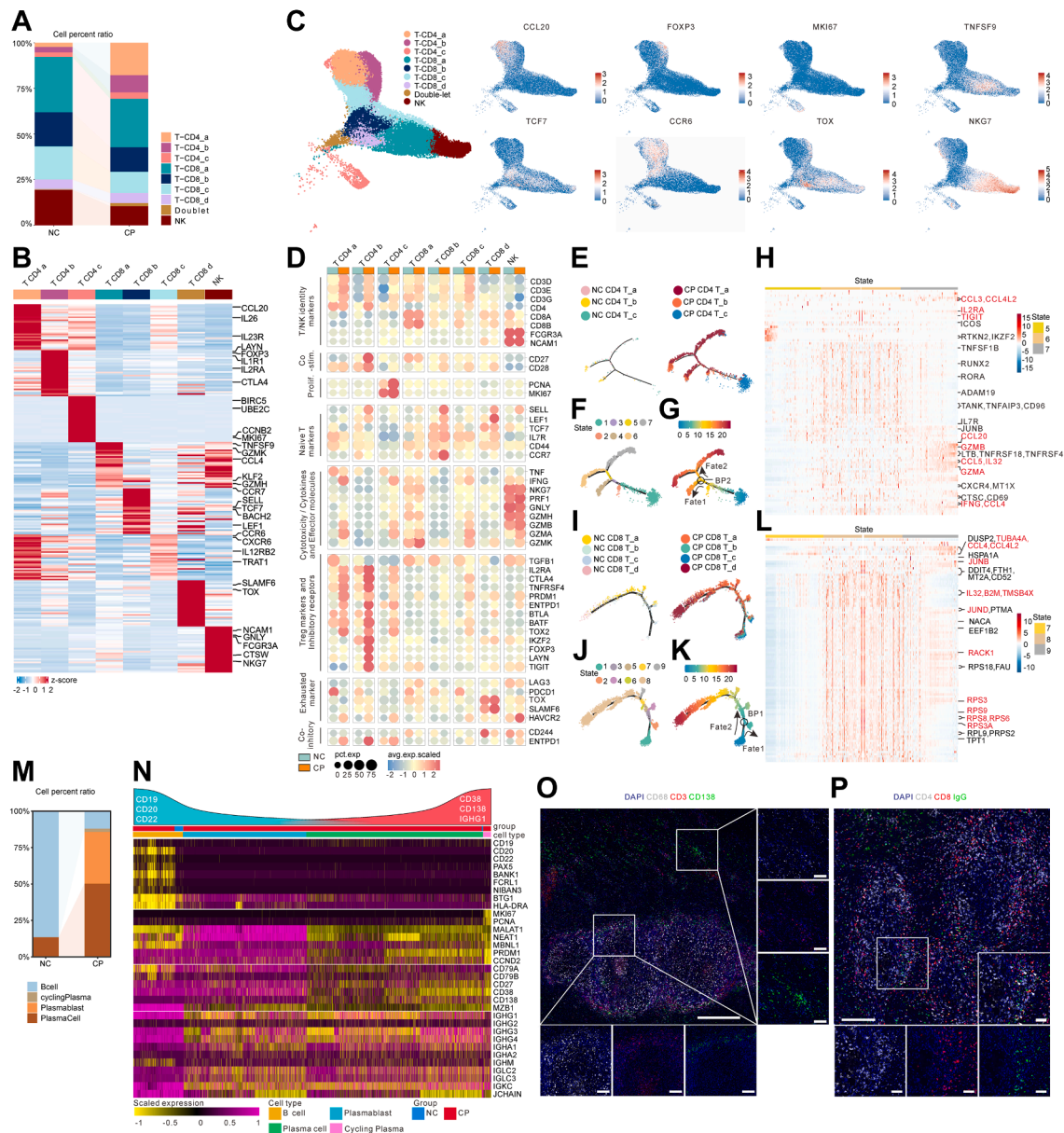


Fig. 3: Transcriptional heterogeneity in lymphocyte lineage. A. Bar graph showing relative proportions of T and NK subclusters in NC and CP pericardium. B. Heatmap illustrating the top 20 DEGs among T and NK subclusters. C. UMAP plot of T and NK subclusters (left). Feature plots of cells coloured by expression levels of indicated genes (right). Colour scale indicates the normalised expression level. D. Dot plot showing the expression of signature genes of T and NK subclusters in NC and CP groups. Genes are clustered by indicated functional modules. Colour scale and dot size indicate the effect size. Co-stim. = Co-stimulatory, Prolif. = Proliferation. E-G. Pseudotime trajectory of CD4-T cells. E. Distribution of NC and CP CD4-T cells along pseudotime trajectories. F. Pseudotime trajectory states. G. Branch-point analysis (circle indicates branch point BP2) highlighting fate-specific activation trajectory. H. State-resolved heatmap of DEGs across state 5-7 as in F. Representative genes are labelled in red. Colour scale indicates the normalised expression level. I-K. Pseudotime trajectory of CD8-T cells. I. Distribution of NC and CP CD8-T cells along pseudotime trajectories. J. Pseudotime trajectory states. K. Branch-point analysis (circle indicates branch point BP1) highlighting fate-specific activation trajectory. L. State-resolved heatmap of dynamically expressed genes across state 7-9 as in J. Representative genes are labelled in red. Colour scale indicates the normalised expression level. M. Bar graph showing relative proportions of indicated cell subclusters within B cell lineage in NC and CP pericardium. N. DEGs heatmap of marker gene expression pattern defining cell subclusters within B cell lineage in NC and CP pericardium. O. IF staining for CD68 (grey), CD3 (red), and CD138 (green) in CP pericardium. DAPI (blue). Scale bars: 500 μ m (main) and 100 μ m (zoom-in). P. IF staining for CD4 (grey), CD8 (red), and IgG (green) in CP pericardium. DAPI (blue). Scale bars: 200 μ m (main) and 50 μ m (zoom-in).

Pseudotime ridges of CD8⁺ T cells revealed a gradual transition from CD8_b/c to CD8_d, through CD8_a (Figure S3I, top). Early pseudotime states were characterised by the upregulation of translation and ribosome biogenesis (*RPS7 etc.*) and lymphocyte chemotactic signalling genes (*IL12RB2, CCR6, CCL20*). In contrast, later pseudotime stages exhibited elevated expression of exhaustion and alpha-beta T cell activation (*TOX, SLAMF6, FOXP1*), along with lymphocyte proliferation genes (*RIPOR2, CCND3 etc.*) (Figure S3I–K). CD8⁺ T cells (majorly CP-derived population) were assigned into nine states along pseudotime trajectory from precursors to differentiated/functional effectors (Fig. 3I–K). At the designated branch point, CD8⁺ T cells were diverted into fate1, which enriched for humoral immune processes (*B2M, CCL20 etc.*), and fate2, which was linked to leucocyte activation/migration (*CCL4/5, LYST, and FYN*) (Figure S3L–N). Along the major differentiation path (fate2), hierarchical clustering of CD8⁺ T cells showed strong expression of genes related to effector phenotype (*CCL4/4L2, JUNB, JUND, TUBA4A, and RACK1* in state 8–9), with significant loss of ribosomal genes (*RPS7, RPS10, RPL7A, RPL20 etc.*) expression in state 8, indicating a potential exhaustion status (Fig. 3L). Dot plot analysis revealed that MHC–II-associated genes were confined to professional antigen-presenting cells and remained low in T cells in both NC and TB-CP groups, whereas MHC-I genes showed a broad upregulation across myeloid and T cells in TB-CP (Figure S4A). Feature plots of HLA-A, HLA-B and HLA-C further confirmed enhanced MHC-I expression in TB-CP, particularly within T cells (Figure S4B), consistent with an antigen-presenting milieu supportive of cytotoxic T-cell activation.

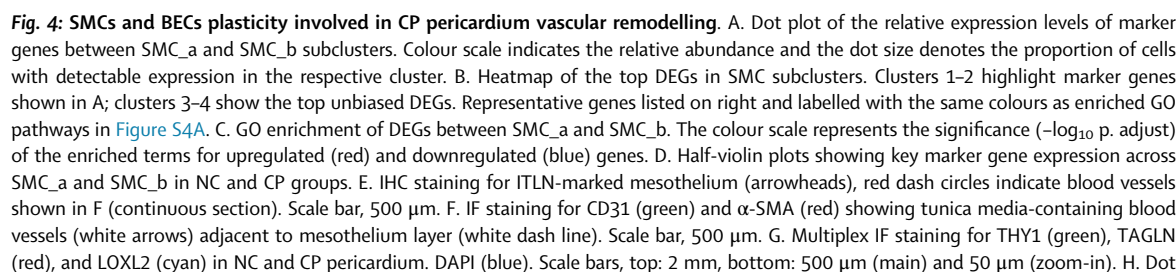
Within B cell lineage, the CP group showed reduced B cell percentage alongside an expansion of plasma-blasts and plasma cells (Fig. 3M). Flow cytometry confirmed increased B cells (CD3⁺CD19⁺) and plasma cells (CD3⁺CD138⁺) in patients' pericardium versus peripheral blood (Figure S3O). DEGs heatmap captured their lineage transition features by showing that B cells expressed CD19, CD20, CD22, PAX5 with memory-like markers (*BANK1, FCRL1, NIBAN3, BTG1*). Plasma-blasts displayed clear marker transition (CD79 A/B, CD138, and CD38), while plasma cells further upregulated post-transcriptional regulators (*MALAT1, NEAT1, MBNL1*) and differentiation drivers (*PRDM1, CCND2*) genes required for maturation.³⁸ Meanwhile both subclusters upregulated MZB1, immunoglobulin heavy/light chain genes (*IGHG1-4, IGHA1/2, IGHM, IGKC, IGLC2/3*), and JCHAIN. On top of these, cycling plasma cells were defined by high levels of MKI67 and PCNA (Fig. 3N).³⁹ CD3, CD68, and CD138 co-staining revealed distinct pericardial immune cell niches, showing CD3⁺ T cells and CD138⁺ plasma cells formed a peripheral "cuff" around granuloma-located

macrophages (CD68⁺) (Fig. 3O). Co-staining of CD4, CD8, and IgG showed CD4⁺ T cells preferentially resided in granuloma interiors, while CD8⁺ T cells co-localised with IgG-producing plasma cells in perigranuloma regions (Fig. 3P and Figure S3P). Moreover, IHC staining showed strong expression of B lineage markers (CD19, CD20, CD79A, CD138) (Figure S1D) and immunoglobulin variants (IgG1, IgG, IgM, IgG4) in pericardial tissue (Figure S3Q), implicating the potential involvement of IgG-producing cells in CP pathology.⁴⁰

SMCs and ECs involved in the vasculature remodelling in CP pericardium

VSMCs have been considered as the dominant pericardial SMCs maintaining vascular homeostasis.⁴¹ Consistent with recent scRNA-seq findings,⁴² we identified arterial SMC_a (CSPG4⁺) exhibiting contractile VSMC characteristics (*TAGLN, MYLK, MYL9, CNN1, MUSTN1*), and venous SMC_b (CHRD1⁺) showing upregulated mesenchymal programme (*THY1, FN, COL1A1/2*)⁴³ (Fig. 4A and B, cluster 1–2). Unbiased top DEGs in two subclusters (Fig. 4B, cluster 3–4) and volcano plots (Figure S5A) linked SMC_a to vascular growth pathway (*MYH11, KCNMA1, ITGA8, SORBS2*), while collagen organisation (*COL1A2, ACVRL1*), angiogenesis (*SERPINE, SASH1*), hypoxia (*CYGB, GUCY1A2, LOXL2*), and NF-κB signalling modules (*F2R, TNFAIP3*) were assigned to SMC_b (Fig. 4C). Additionally, violin (Fig. 4D) and feature plots (Figure S5B) showed loss of healthy SMC markers (*CHRD1* in SMC_b, *MYH11* and *ITGA8* in SMC_a) and gain of pathological alterations in CP (*SERPINE1* and *GUCY1A2*). IF and IHC staining confirmed neovasogenesis of tunica media-containing vessels adjacent to the mesothelial layer (Fig. 4E–F and Figure S4C). Co-localisation of *THY1* and *LOXL2* with *TAGLN*⁺ vasculature further indicated newly proliferated VSMCs undergoing hypoxia-associated fibrotic remodelling⁴⁴ (Fig. 4G).

Parallely, six endothelial subpopulations were identified, which included Endo_e marked by lymphatic endothelial cell (LEC) signatures, and Endo_a-d/f, representing blood endothelial cell (BEC) subtypes across arteriolar, venular, and transitional capillaries (Fig. 4H).⁴⁵ Violin plots validated these profiles by showing elevated *SEMA3G* in Endo_d, *IRF1* in Endo_b, *S100A4* and reduced *CDH5* in Endo_f, *ACKR1* across Endo_a-d/f, and *PROX1* in Endo_e (Figure S6A). Specifically, IF staining of CD31 and *ACKR1* showed marked capillary and venulae proliferation in CP pericardium (Fig. 4I), whereas CD31/*PROX1* co-staining revealed no evident lymphatic expansion (Figure S6B). DEGs heatmap revealed gene expression gradients and phenotype transitions across EC lineages (Fig. 4J). In both healthy and diseased states, Endo_a-f clusters retained *PECAM1* (CD31) and their respective BEC/



LEC features (cluster 1–2). However, Endo_f upregulated fibrotic/mesenchymal markers (COL1A1-3, VIM, DCN, ACTA2, TAGLN) while downregulating endothelial genes (ERG, CDH5, KDR, BMPR2) (cluster 3–4), consistent with an endothelial-to-mesenchymal transition (EndoMT) phenotype.⁴⁶ GO analysis showed BEC clusters shared expression patterns in angiogenesis, stimulus response, and leucocyte activation/migration pathways, while Endo_f uniquely enriched in lymphocyte- and humoral-related pathways (Figure S6C). Non-integrated UMAP confirmed these shared signatures across BECs but also highlighted Endo_f-specific enrichment of immune (LYZ, CXCR4, IGKC) and EndoMT (ACTA2, COL1A1, DCN) profiles, which were further supported by violin plots (Figure S6D–E). IF co-staining revealed abundant CD31⁺S100A4⁺ ECs (Endo_f) localised in α -SMA expressing venules in CP pericardium, confirming the occurrence of EndoMT in these endothelial cells (Fig. 4K).

Trajectory analysis of BEC subclusters showed Endo_a/c/d in early pseudotime, Endo_b in intermediate-late stages, and Endo_f at the terminal position (Fig. 4L and Figure S5F). Bifurcation analysis revealed fate1 (Endo_b-specific) genes were enriched in vascular development pathway, while fate2 (Endo_f) displayed upregulated collagen organisation and humoral response genes (Fig. 4M and Figure S5G). Spearman correlation-fitted dynamic expression curves confirmed inductions in ECM and immunoglobulin gene sets, as well as drop of EC marker genes in Endo_f, whereas abundant angiogenesis-related gene expression was restricted to other BEC subclusters (Figure S6H). Taken together, our data show that pericardial endothelium undergoes marked angiogenesis and immune activation during disease progression, with Endo_f exhibiting additional venular-specific EndoMT changes.

Transcriptional heterogeneity and state-transition within myofibroblasts and fibroblasts

In addition to the three fibroblast subclusters (Fibro_a/b/c), a distinct myofibroblast subcluster was identified and found to be markedly expanded in CP, suggesting its involvement in pericardial fibrotic remodelling (Fig. 5A). DEGs heatmap showed fibroblasts broadly expressed *bona fide* mesenchymal markers THY1 and

PDGFRA, while myofibroblast hallmark (COL1A1/2, ACTA2), ECM-related (POSTN, SERPINE1, TWIST1) and remodelling collagen (COL1A1, COL8A1) genes were specifically upregulated in CP, indicating a fibroblast-to-myofibroblast transition (Fig. 5B, cluster 1).¹⁸ TGF- β pathway genes (BMPR1B, TGFB3, SMAD3) were expressed across all fibroblast subclusters, indicating a shared signalling axis (Fig. 5B, cluster 2 and C). Specifically, Fibro_a and myofibroblasts expressed ribosomal genes (RPS12 *etc.*) (Fig. 5B, cluster 3). Fibro_c highly expressed HAS1/2, MMP9, and CCL19, which are enriched in cell-adhesion pathways, suggesting an invasive fibroblast identity (Fig. 5B, cluster 4 and C).⁴⁷ Myofibroblasts maintained an ACTA2⁺ contractile phenotype with reduced matrix gene expression, while uniquely expressing lymphocyte chemotactic factors (CCL3L3, CCL3, CCL4/4L2, CCL14) linked to immune regulation. It also expressed monocyte/macrophage maker (LYZ, APOC1, C1QA/B, S100A9), MHC II molecule (HLA-DRA, HLA-DPB1), and immunoglobulin (IGKC, IGLC2/3, JCHAIN) genes in CP (Fig. 5B, cluster 5 and C).⁴⁸ Feature as well as violin plots further confirmed these transcriptional patterns (Fig. 5D–E and Figure S6A).

ST analysis revealed that fibroblasts were predominantly localised near granulomas, as indicated by cluster scoring (Fibroblast) and THY1 expression patterns (Fig. 5F). In contrast, the Fibro_c marker CCL19 was accumulated within granuloma cores, whereas myofibroblasts (ACTA2⁺) were excluded to regions with high granuloma scores (Fig. 5F). IF staining revealed a concentric THY1⁺/ α -SMA⁺ layer encasing granulomas, with THY1⁺/CCL9⁺ fibroblasts localised within CD68⁺ regions (Fig. 5G and H), confirming infiltration of Fibro_c in granulomas.

Consistent with previously reported hybrid lineage features,⁴⁹ CP myofibroblasts co-expressed ECM (THY1, COL1A1, COL1A2) and endothelial (VWF, CD31, ENG, and LYZ in CP Endo_f) markers, while showing loss of mesenchymal stemness (LEPR, NTSE, CD34) and smooth muscle signatures (ACTG2, TAGLN, MYH11) (Figure S7B). To trace their origins and transitions in CP, we performed pairwise pseudotime analysis between myofibroblasts and other stromal subclusters, respectively. Firstly, normal Fibro_a/b/c subclusters were distributed along early pre-branch, while CP

plot showing relative expression levels of transitional marker genes across endothelial subclusters. Art = artery, Cap = capillary, Lym = lymphatic vessel. Colour scale indicates the relative abundance and the dot size denotes the proportion of cells with detectable expression. I. IF staining for CD31 (green) and ACKR1 (violet) in NC and CP pericardium. Arrows indicate co-expression of CD31/ACKR1. Scale bars: 200 μ m (main) and 5 μ m (zoom-in). J. Heatmap of representative DEGs among endothelial clusters in NC and CP groups. Cluster1: vasculature signature genes; 2: LECs signature genes; 3: Endothelial signature genes; 4: EndoMT signature genes. K. IF staining for CD31 (green), S100A4 (pink), and α -SMA (red) identifying CD31⁺/S100A4⁺ ECs (Endo_f) in CP, co-expressing α -SMA. Scale bars: 200 μ m (main) and 20 μ m (zoom-in). L. Pseudotime branch-point analysis (circle indicates branch point BP3) highlighting fate-specific activation trajectory of BECs (left), and distribution of BECs subcluster along inferred trajectories (right). M. Heatmap showing the scaled expression of fate-specific DEGs along three branches as in L (pre-branch, fate 1 and 2). Fate-specific representative genes are clustered and shown on right. Colour scale represents the z-score of expression level.

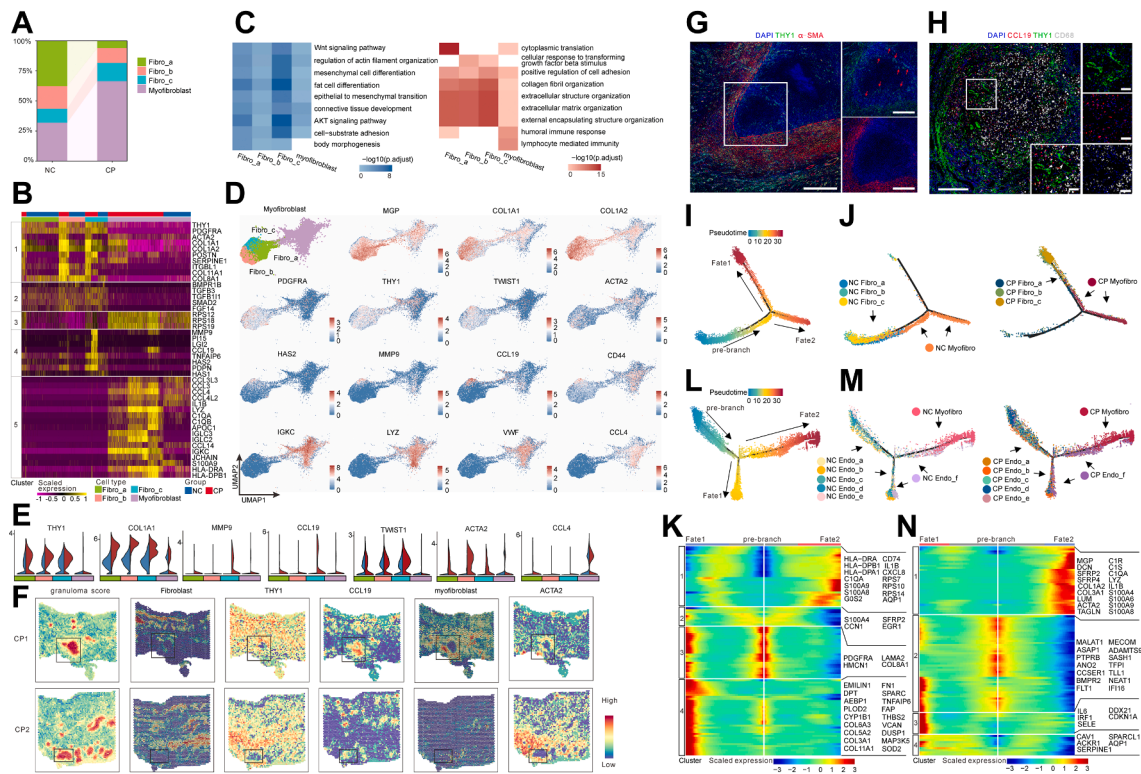


Fig. 5: Transcriptional heterogeneity and potential functional transition among fibroblasts and myofibroblasts. A. Bar graph showing relative proportions of fibroblast and myofibroblast subclusters in NC and CP pericardium. B. Heatmap of clustered marker gene expression for cell clusters shown in A. C. GO enrichment of DEGs across fibroblast and myofibroblast subclusters. The colour scale represents the significance ($-\log_{10} p$, adjust) of the enriched terms for upregulated (red) and downregulated (blue) genes. D. UMAP plot of fibroblasts and myofibroblast. Feature plots of cells coloured by expression levels of indicated genes. Colour scale indicates the normalised expression level. E. Half-violin plots of key marker gene expression of fibroblasts and myofibroblasts. Colour codes match B. F. Spatial expression maps of indicated genes in CP pericardium (black boxes indicate representative granuloma areas). Scale bars, 500 μm . G. IF staining for THY1 (green), α -SMA (red) in CP pericardium. Red dash line indicates margin of granuloma areas; red arrows indicate invasive fibroblasts. Scale bars: 1000 μm and 500 μm (zoom-in). H. IF staining for THY1 (green), CCL19 (red), CD68 (grey) in CP pericardium. White arrows indicate co-localisation of THY1 and CCL19. Scale bars: 200 μm (main) and 50 μm (zoom-in). I. Pseudotime branch-point analysis showing fate-specific activation trajectory among fibroblasts and myofibroblasts (arrows denote direction of progression). J. Distribution of indicated cell subclusters (black arrows) along inferred trajectories (NC, left and CP, right). K. Heatmap showing the scaled expression of fate-specific DEGs along three branches as in I. Fate-specific representative genes are clustered and shown on right. Colour scale represents the z-score of expression level. L. Pseudotime branch-point analysis highlighting fate-specific activation trajectory among BECs and myofibroblasts (arrows denote direction of progression). M. Distribution of indicated cell subclusters (black arrows) along inferred trajectories (NC, left and CP, right). N. Heatmap showing the scaled expression of fate-specific DEGs along three branches as in L. Fate-specific representative genes are clustered and shown on right. Colour scale represents the z-score of expression level.

counterparts shifted toward fate1. In contrast, NC and CP myofibroblasts were predominantly mapped to the fate2 trajectory, partially overlapping with fibroblast populations, indicative of a natural fibroblast-to-myofibroblast transition (Fig. 5I–J and Figure S6F top). Branch-point analysis showed fate1 upregulated ECM genes (COL1A1/3/5A2, FAP, SPARC *etc.*) with regained fibroblast markers PDGFRA, LAMA2, and COL8A1 (Fig. 5K cluster 3–4). By contrast, fate2 was enriched for immune-regulatory genes, including antigen presentation (HLA-DRA/DPA1/DPB1), complement (C1QA), DAMPs (S100A8/9), ribosomal genes,

and pro-inflammatory mediators (CD74, IL1B, CXCL8) (Fig. 5K cluster 1). These findings suggest myofibroblasts not only produce ECM but also acquire immune-modulatory properties.

Meanwhile, ECs were also shown to transit from pre-branch to two fates along pseudotime trajectory (Fig. 5L). In normal tissue, Endo_a-e remained in fate1, while some Endo_f extended toward fate2 with myofibroblasts, indicating phenotype overlap. In CP group, Endo_f shifted significantly to fate2, where myofibroblasts accumulated (Fig. 5M and Figure S6F middle). Branch-point analysis showed fate1 preserved angiogenesis programmes

(Fig. 5N, cluster 2–4), whereas fate2 upregulated ECM (MGP, DCN, SFRP2/4, COL1A2/3A1), SMC (ACTA2, TAGLN), and immune genes (S100A4/6/8/9, C1QA), suggesting Endo_f represents a EndoMT population sharing myofibroblast features (Fig. 5N, cluster 1). Lastly, regarding myofibroblast-SMCs pair, both NC and CP SMCs mainly aligned with fate1, while myofibroblasts localised to fate2 (Figure S7C–D and F bottom), with only a few SMC_b shifted toward fate2. The distinct segregation of SMC-related genes (Figure S7E, cluster 3) preserved in fate1 and myofibroblast-related programmes (Figure S7E, cluster 1–2) enriched in fate2 suggested an independent trajectory and minimal contribution of SMCs to the myofibroblast pool.

Intercellular interactions showing CP fibrotic microenvironment driven by immune cells

Ligand-receptor analysis of scRNA-seq data showed globally increased intercellular interactions in diseased tissue, especially between myeloid and stromal compartments (Figure S8A). Macrophages displayed strong interactions with fibroblasts and endothelial subclusters, while reciprocal signalling was also observed between macrophages and T cells (Fig. 6A). Specially, ECs (Endo_a–f) were major recipients of macrophage (Mac_b/c) signals via VEGF, TGF- β , and TNFSF10 (TRAIL) (Fig. 6B and Figure S7H). Western blot analysis showed increased VEGFA and TGF- β 1 in CP tissues (Figure S8B–E). IF and RNA FISH confirmed macrophage-origins of VEGFs (Fig. 6C and D), which were associated with endothelial activation (CD31/p-ERK1/2)⁵⁰ and TGF- β -induced EndoMT⁴⁶ (CD31/ α -SMA/p-SMAD3) (Fig. 6E and F). On the other hand, fibroblasts exhibited active TGF- β signalling, coinciding with ligand expression from Mac_b/c and CD4 T_a/c subclusters via TGFBR1-3 (Fig. 6G and Figure S7H), where co-localised TGF- β 1 and CD3 (Fig. 6H), as well as dramatically elevated p-Smad3 in THY1⁺/ α -SMA⁺ fibroblasts (Fig. 6I) were examined. Moreover, fibroblasts were also found to modulate immune cell recruitment, with the Fibro_c shown to attract CCR7⁺ T cell via CCL19 (Figure S8F–H).

To determine whether the immune response and fibrotic programmes observed in TB-CP are TB-specific rather than generic features of CP, we performed comparative IHC analyses using pericardial tissues from patients with idiopathic CP, which show comparable fibrotic marker α -SMA (Figure S9). In TB-CP samples, extensive immune cell infiltration (CD3/CD68) and IFN- γ -rich granulomatous structures was readily detected throughout the pericardium. These organised inflammatory foci were largely absent in idiopathic CP tissues (Figure S9). TGF- β staining was also markedly increased in TB-CP pericardium, particularly in regions adjacent to immune cell aggregates, whereas idiopathic CP samples exhibited weak or undetectable levels (Figure S9).

As for macrophage-T cell interactions, Mac_b and Mac_c subclusters were shown to express multiple chemokines (CXCL9, CXCL10, CXCL11), which corresponded to CXCR3 receptors on CD4⁺ and CD8⁺ T cells.⁵¹ Meanwhile, Mac_b was uniquely engaged with CD4⁺ T cells via CXCL16-CXCR6⁵² and further activated through glycoprotein interactions such as ICAM1-ITGAL (CD11a) and ICAM1-SPN (CD43).⁵³ In addition, Mac_c-mediated networks were enriched for costimulatory pathways such as CD86-CD28 and CD86-CTLA4, while additional co-stimulatory signals (CD80-CD28, CD80-CTLA4, CD58-CD2, and CD47-SIRPG) were shared by Mac_b/c or restricted to Mac_b (Fig. 6J and Figure S7H).

Subsequent spatial transcriptomic mapping revealed that T cells were tightly organised around macrophage-rich granulomas, with CD4 T_a and CD8 T_a positioned in closest proximity (Fig. 6K). Coincidentally, high CXCL9 and CXCL16 expressions were seen within granulomas, while CXCR3 and CXCR6 preferentially localised in peri-granulomatous regions (Fig. 6L). IF staining confirmed CD68⁺ macrophages co-expression CXCL9 with in the granuloma core, positioned near CD3⁺/CXCR3⁺ T cells (Fig. 6N). Because full T-cell activation by antigen presenting cells (APC) requires CD86-CD28 co-stimulation,⁵⁴ we next examined the spatial distribution of the APC marker CTSS, CD86, and its receptors CD28 and CTLA4. CD86 expression largely overlapped with CTSS, consistent with the localisation of Mac_b/c, while CD28 and CTLA4, though weaker in expression, were mainly enriched around CD86⁺ regions (Fig. 6M). IF staining further demonstrated the close spatial proximity of CTSS⁺/CD68⁺ macrophages and CD3⁺/CD28⁺ T cells (Fig. 6O).

Together, our findings delineate a complex and spatially organised immune-stromal architecture in TB-associated constrictive pericardium. Macrophage populations are prominently enriched within granulomatous regions and are spatially co-localised with activated fibroblast/myofibroblast subsets, remodelled endothelial cells, and infiltrating T cells. Integrated single-cell and spatial transcriptomic analyses highlight coordinated patterns of chemokine expression, antigen-presentation machinery, and profibrotic and angiogenic signalling pathways within these niches. These findings potentially provide a comprehensive framework for understanding the cellular organisation underlying TB-CP pathogenesis.

Discussion

Constrictive pericarditis represents a rare but life-threatening end stage of TP. Although adjunctive anti-tuberculosis therapy may lower the risk of progression, it seldom reverses established constriction; thus, pericardiectomy remains the definitive and primary treatment option.⁵⁵ The molecular mechanisms

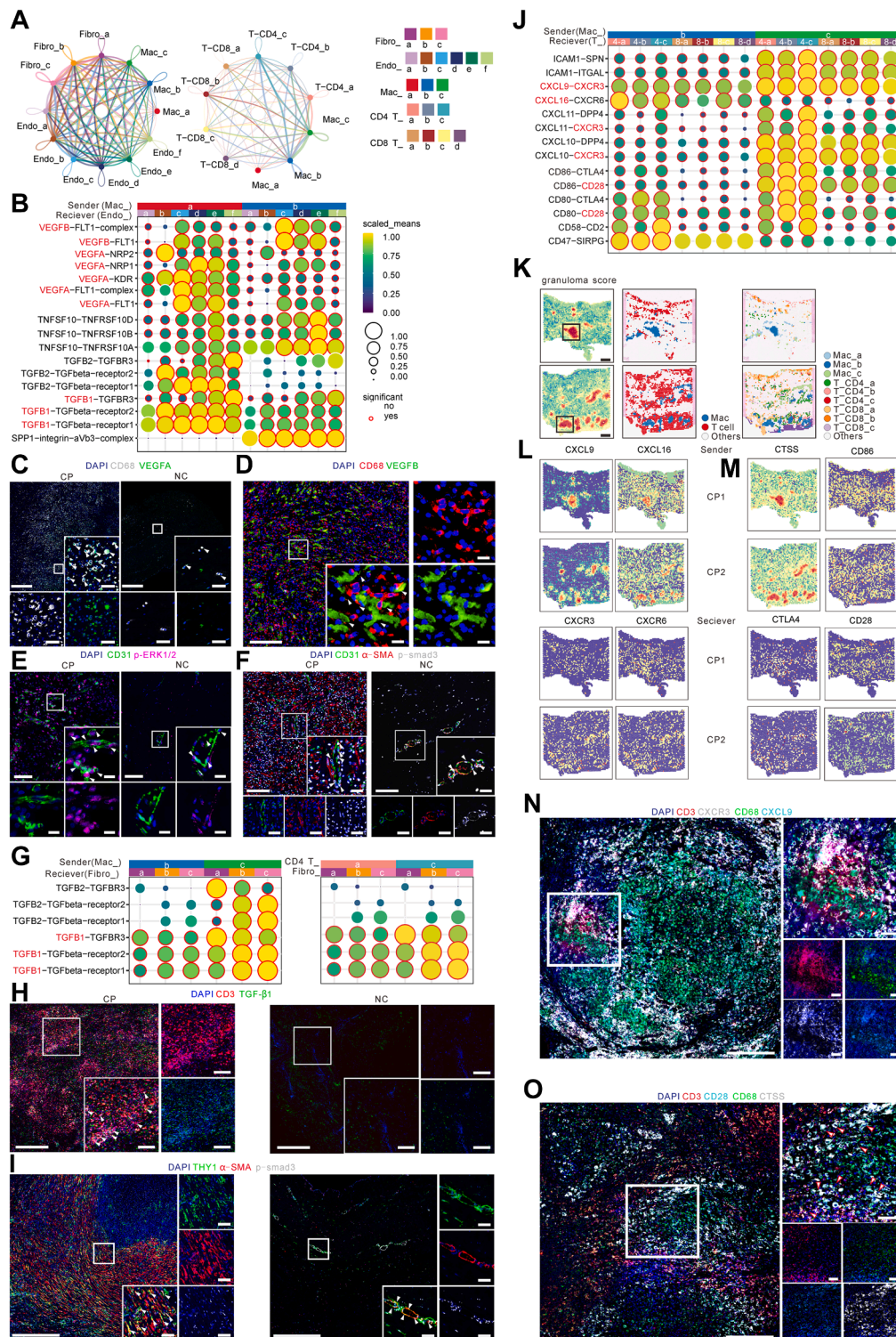


Fig. 6: Cellular crosstalk in CP pericardium. A. Circo's plots showing potential intercellular interactions. Left: interactions among stromal and macrophage compartments. Right: interactions between T cell and macrophage subclusters. Node size denotes total number of interactions for each cell type; edge width represents the number of significant ligand-receptor pairs between indicated cell subtypes. B. Dot plot showing predicted and ligand-receptor communications between senders (macrophage, Mac_) and receivers (endothelial cell, Endo_). Dot size and colour represent scaled average gene expression, with red circles indicating significant interactions (yes, $p < 0.05$) as calculated using

underlying pericardial fibrosis remain poorly defined, particularly at single-cell and spatial resolution. Here, we presented a multi-omics atlas combining scRNA-sequencing, spatial transcriptomics, and multiplexed IF staining on human CP samples. This dataset uncovers complex immune-stromal niches, underscores disease-associated plasticity in ECs and SMCs to drive angiogenic reprogramming, and identifies immune-responsive fibroblasts and myofibroblasts from divergent origins. Collectively, these findings support the hypothesis that crosstalk between immune and stromal compartments establishes a self-sustaining circuit that transforms focal infection into progressive and spatially localised fibrotic lesions.^{15,56}

Firstly, our data demonstrated that distinct myeloid, lymphocyte, and fibroblast subclusters engaged in complex immune-stromal circuits within or adjacent to granuloma lesions. Among these, we identified two dominant macrophage subclusters (Mac_b and Mac_c) with distinct transcriptional and spatial signatures. Notably, Mac_b cells localised specifically to granuloma cores, exhibiting robust expression of matrix-remodelling and granuloma-associated gene programmes (SPP1, MMP9, GPNMB) together with high levels of MHC-II and antigen-presentation molecules. SPP1⁺ macrophages have been widely recognised as fibrosis-associated macrophages across multiple tissues.⁵⁷ Ligand-receptor analysis further revealed that Mac_b-derived SPP1 interacted with endothelial integrin receptors—most prominently ITGAV/ITGB3 (αvβ3)—implicating this subcluster in driving endothelial angiogenic reprogramming during CP. In parallel, GPNMB has been associated with fibroblast activation via ECM-trapping mechanisms and was recognised as a marker of macrophage population related to tissue injury and fibrosis.²⁰ These findings aligned with our observation of

Mac_b signature in granuloma regions that were surrounded by abundant invasive fibroblasts.⁴⁷

Compared with the other macrophage subclusters, Mac_c was distinguished by expression of tissue-resident and M2-like markers (LYVE1, MERTK, MRC1) together with an unexpectedly elevated inflammatory programme (IL1A/IL1B, NLRP3). This mixed phenotype likely reflects continuum activation driven by specific local cues. Previous studies have shown that regulatory/M2b macrophages—characterised by high MHC-II, IL-10, and scavenger receptor expression—can also produce pro-inflammatory cytokines such as IL-1β, in response to immune complexes or TLR ligands.⁵⁸ Thus, the activated transcriptional profile of Mac_c may represent a plastic state that integrates tissue repair functions with pro-inflammatory signalling, ultimately facilitating progressive pericardial fibrosis.

In addition, T cell-myeloid cell interplay is a hallmark of granuloma organisation, with chemokine signalling serving as the principal mechanism for lymphocyte recruitment and maturation. Our data revealed that granuloma-resident macrophage population expressed high levels of CXCL9 and CXCL10 to attract CD4⁺ and CD8⁺ T cells via CXCR3,^{51,52} ensuring their proximity to antigen-presenting macrophages and enabling local activation and effector responses. CD4⁺ subclusters range from regulatory/Treg-like cells (FOXP3, LAYN, CTLA4) to conventional T helper cells, while CD8⁺ subclusters span proliferative, effector, and exhausted phenotypes.^{32,34,36,37} Macrophages and dendritic cells are shown enriched within the granulomatous core, where they are well positioned and potentially present mycobacterial antigens to CD4⁺ T cells via MHC class II, consistent with classical helper T-cell activation in tuberculosis. Beyond this canonical APC-CD4⁺ T-cell interaction, several non-mutually exclusive

CellPhoneDB (the same as below). Red highlights indicate spatial or protein-level support by protein-level evidence, including IF and/or RNA-FISH and/or Western blot (same as above.). C. IF staining for VEGFA (green) and CD68 (grey) in CP pericardium. DAPI (blue). White arrows indicate co-localisation of VEGFA and CD68. Scale bars: 500 μm (main) and 20 μm (zoom-in). D. *In situ* hybridisation (RNA-FISH) staining of VEGFB (green) with IF staining of CD68 (red) in CP pericardium. White arrows indicate co-localisation of VEGFB and CD68. Scale bars: 200 μm (main) and 20 μm (zoom-in). E. IF staining for CD31 (green) and phosphorylated Erk1/2 (p-ERK1/2, magenta) in NC and CP pericardium. White arrows indicate co-localisation of p-ERK1/2 and CD31. Scale bars: 100 μm (main) and 10 μm (zoom-in). F. IF staining for α-SMA (green) and phosphorylated Smad3 (p-SMAD3, grey) in NC and CP pericardium. DAPI (blue). White arrows indicate nuclear p-SMAD3 co-localises with α-SMA and CD31. Scale bars: 100 μm (main) and 50 μm (zoom-in). G. Dot plot showing predicted ligand-receptor communications between senders (macrophage, Mac_) and receivers (fibroblast, Fibro_) (left), as well as senders (CD4-T cell, CD4_T) and receivers (fibroblast, Fibro_) (right). H. IF staining for TGF-β1 (green) and CD3 (red) in CP pericardium. DAPI (blue). White arrows indicate co-expression of CD3/TGF-β1. Scale bars: 500 μm (main) and 100 μm (zoom-in). I. IF staining for THY1 (green), α-SMA (red) and p-SMAD3 (magenta) in CP and NC pericardium. DAPI (blue). White arrows indicate co-expression of THY1/α-SMA/p-smad3. Scale bars: 500 μm (main), 50 μm (zoom-in). J. Dot plot showing predicted ligand-receptor communications between senders (macrophage, Mac_) and receivers (T cell, T_). K. Granuloma score overlay (left), spatial projection of macrophages and T cells (middle), and their detailed subsets (right) in CP pericardium. Scale bars: 500 μm. L. Spatial expression maps of chemokine-receptor pairs (CXCL9-CXCR3, CXCL16-CXCR6). Sender: macrophage; Receiver: T cell (the same as in M). M. Spatial expression maps of antigen-presentation and co-stimulatory molecules (CTSS, CD86, CTLA4, CD28). N. Multiplex IF staining validating macrophage-T cell chemokine interactions. Left: large field showing DAPI (blue), CD3 (red), CXCR3 (grey), CD68 (green), CXCL9 (cyan). Right: zoom-in view of CXCL9⁺/CD68⁺ macrophages and surrounding CD3⁺/CXCR3⁺ T cells. Arrows indicate areas of interaction. Scale bars: 200 μm (main); 50 μm (zoom-in). O. Multiplex IF staining validating antigen-presentation and co-stimulation topology. Left: main field showing DAPI (blue), CD3 (red), CD28 (cyan), CD68 (green), CTSS (grey). Right: zoom-in view of CTSS⁺/CD68⁺ macrophages and CD3⁺/CD28⁺ T cells in adjacent zones. Arrows indicate areas of interaction. Scale bars: 200 μm (main); 50 μm (zoom-in).

mechanisms may contribute to the activation of cytotoxic CD8⁺ T cells in TB-CP. Infected or antigen-loaded macrophages can directly present mycobacterial peptides via MHC class I, and dendritic cells may further engage cross-presentation pathways to prime CD8⁺ T-cell responses.⁵⁹ Notably, our single-cell analysis revealed a striking upregulation of MHC class I-related genes within both CD4⁺ and CD8⁺ T cell clusters in TB-CP compared with normal pericardium. Accumulating evidence indicates that infiltrating T cells in mycobacterial lesions undergo substantial transcriptional remodelling in response to the local inflammatory milieu. Previous single-cell profiling of tuberculous pleural effusion has shown that both CD4⁺ and CD8⁺ T cells adopt distinct activation states shaped by sustained exposure to pro-inflammatory cytokines, such as IFN- γ .⁶⁰ Consistently, our analyses of tuberculosis granulomas have demonstrated that IFN- γ -rich niches are associated with broad upregulation of antigen-processing and MHC class I-related gene programmes across multiple immune lineages, including T cells.⁶¹

At the same time, macrophage-derived IL-10 and TGF- β , together with Treg-like CD4⁺ subclusters, provide counter-regulation to restrain excessive inflammation. While effective cooperation between T cells and macrophages strengthens pathogen control, sustained or dysregulated interactions may perpetuate inflammation, tissue injury, and fibrosis. Thus, granulomas emerge as highly structured immuno-microenvironment where reciprocal T cell-myeloid interactions orchestrate both protective immunity and pathological remodelling.

Noteworthy, plasma cells and plasmablasts were found to accumulate in CP pericardium, indicating significant involvement of humoral responses. The emerging roles of B lineage in tissue damage and fibrosis have gained increasing attention.⁴⁰ Plasma cells are increasingly recognised as potential drivers of fibrosis by sustaining antibody production and engaging downstream effector pathways. In addition to these indirect effects, dysregulated antibodies may directly target tissue antigens, perpetuating injury. Mechanistically, immune complexes bound to ECM can engage fibroblast Fc γ receptors, leading to fibroblast activation, MMP release, and excessive ECM deposition; experimental depletion of plasma cells reduces fibrosis in relevant models.⁶² In the context of tuberculosis infection, plasma cells may therefore act as amplifiers of fibrotic remodelling by maintaining antibody production and fuelling immune complex accumulation within granulomatous niches. This positions plasma cells not only as pathogen-induced immune effectors but also as active participants in tissue remodelling, bridging chronic inflammation with fibrotic progression. Collectively, these insights highlight plasma cells as underappreciated contributors to fibrosis and underscore their potential as therapeutic targets.

Moreover, CP mesenchymal populations were characterised by CCL19⁺ immune-responsive fibroblasts and an expanded pool of THY1⁺/ α -SMA⁺ myofibroblasts encircling granuloma lesions. Our pseudotime analysis suggested two dominant trajectories, fibroblast-to-myofibroblast and EndoMT, with only limited evidence for SMCs contribution.^{18,46} By contrast, the Fibro_c subcluster was preferentially localised within granuloma cores, closely associated with interfacing with macrophage-rich regions. Defined by HAS1/HAS2, MMP9, CCL19, and CD44, Fibro_c combines invasive ECM remodelling with immune modulation.⁴⁷ In addition, disease-associated fibroblasts and myofibroblasts co-expressed ECM/contractile genes (ACTA2, COL1A1/2, CTGF) alongside immune-related transcripts, aligning with antigen-presenting fibroblast (apCAF) and immune-active fibroblast phenotypes.⁶³ Moreover, ligand-receptor mapping suggested coordinated fibro-immune circuits: macrophage chemokines (CXCL9/10/11) recruited CXCR3⁺ T cells; macrophage MMP/SPP1/GPNMB programmes aligned with fibroblast ECM production; VEGF and TGF- β signalling axes were enriched between macrophages to endothelial activation and EndoMT-like transitions; and fibroblast CCL19 retained CCR7⁺ lymphocytes at lesion margins.

Beyond immune activation and extensive ECM remodelling, pericarditis has long been recognised for vascular remodelling and angiogenesis,⁴ though the underlying cellular mechanisms remained unclear. In our dataset, endothelial cells were subtyped into capillary, venous, and arterial/SMC-associated populations, revealing widespread endothelial proliferation and immune activation, marked by elevated CD31 and induction of adhesion and immune genes (ICAM1, SELE, IRF1) adjacent to macrophage-rich granulomas. Notably, a venular/terminal Endo_f subcluster (S100A4⁺) exhibited a mesenchymal-related transcriptional profile (COL1A1, COL1A2, ACTA2, VIM, DCN) with reduced canonical endothelial markers (ERG, CDH5, KDR), consistent with EndoMT. IF co-staining confirmed CD31⁺/S100A4⁺ cells with α -SMA expression in venular regions. These findings are consistent with published literature, which implicates EndoMT as a driver of maladaptive vascular fibrosis and highlights it as a potential therapeutic target.⁴⁶ Since endothelial proliferation and EndoMT were closely associated with granuloma formation, we speculated that (i) vascular remodelling could serve as an early diagnostic biomarker of lesion activity, and (ii) anti-angiogenic therapies, when carefully combined with anti-TB treatment, may disrupt vascular-stromal remodelling axis driving pericardium constriction.

Lastly, since all the CP tissues were obtained from resected constrictive pericardium, the data collected may be constrained in late disease stage. Additionally, mechanistic insights in this study are majorly based on

associative inference from single-cell, spatial, and ligand-receptor analysis. Therefore, *ex vivo* perturbations and *in vivo* infection-associated CP models are required to test various scientific hypothesis. Moreover, the relatively small and heterogeneous cohort (HIV status, Mtb lineage, treatment strategies and timing *etc.*) also limits generalisability of conclusions, underscoring the need for validation in larger, earlier-stage cohorts.

To sum up, our findings suggest that granuloma-centred macrophage-lymphocytes re-programming, BECs plasticity, and multi-lineage myofibroblast expansion to establish a self-sustaining fibro-inflammatory niche that drives ECM deposition and contractile remodelling in infected pericardium. Thus, this combinatory atlas offers a hypothetic framework for identifying prioritising pathways and developing biomarkers to stratify patients at risk of progressive constriction.

Contributors

Y.W, F.X and Y.L initiated, designed and coordinated the study. Clinical coordination and patient enrolment were led by F.X, S.Z.W, L.J.Z, Y.L Lei and K.Y.T. Surgical procurement of pericardial specimens was performed by L.J.C, J.C and Y.L. Laboratory work and data acquisition were carried out by F.Y.C, Y.R.Q, L.H, W.J.Y and Z.L.Z. Bioinformatic analyses, including QC, CCA-based integration, clustering, spatial projection (ssGSEA/GSVA), trajectory inference, and ligand-receptor mapping, were performed by Y.R.Q and L.H. Immunohistochemistry, multiplex immunofluorescence, and quantitative image analyses were performed by Y.R.Q and W.J.Y. Y.W, Y.R. Q, L.H, Y.W, and F.X had full access to and verified the underlying data. F.X and Y.R. Q drafted the manuscript; all authors contributed to data interpretation, critically revised the manuscript for important intellectual content, and approved the final version.

Data sharing statement

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive⁶⁴ in National Genomics Data Centre,⁶⁵ China National Centre for Bioinformatics/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA013508) that are publicly accessible at <https://ngdc.cncb.ac.cn/gsa-human>. Analysis code, image data used for spatial mapping, and other supporting materials that underline reported findings are available from the corresponding author upon reasonable request.

Declaration of interests

The authors have declared that no conflict of interest exists.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (82270486 to F.X.), the Science and Technology Bureau of Sichuan Province (2021YFS0051 and 2024YFFK0209 to Y.W.; 2024NSFC0646 to F.X.). We thank our clinical colleagues in the Departments of Cardiology and Cardiothoracic Surgery, as well as the staff of the Departments of Pathology and Medical Imaging, for their support in patient recruitment, sample collection, tissue processing, and image acquisition. We are deeply grateful to all patients and their families for their participation and trust. We appreciate the assistance of our institutional research administration and finance offices with project coordination and grant management. We additionally thank Dr. Tao Luo (Department of Pathogen Biology, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University) for his valuable scientific advice, critical discussion, and support during the preparation of this manuscript. We thank the patients who consented to

provide pericardial samples. We also acknowledge the SeekGene Biosciences for sequencing and imaging support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.ebiom.2026.106233>.

References

- Howlett P, Du Bruyn E, Morrison H, et al. The immunopathogenesis of tuberculous pericarditis. *Microbes Infect.* 2020;22(4–5):172–181.
- Lucero OD, Bustos MM, Ariza-Rodríguez DJ, Perez JC. Tuberculous pericarditis — a silent and challenging disease: a case report. *World J Clin Cases.* 2022;10(6):1869–1875.
- Mayosi BM, Barnes K, Ntsekhe M. Tuberculous pericarditis is multibacillary and bacterial burden drives high mortality. *eBioMedicine.* 2015;2(11):1634–1639.
- Cremer PC, Klein AL, Imazio M. Diagnosis, risk stratification, and treatment of pericarditis: a review. *JAMA.* 2024;332(13):1090–1100.
- Isiguzo G, Du Bruyn E, Howlett P, Ntsekhe M. Diagnosis and management of tuberculous pericarditis: what is new? *Curr Cardiol Rep.* 2020;22(1):2.
- Matthews K, Deffur A, Ntsekhe M, et al. A compartmentalized profibrotic immune response characterizes pericardial tuberculosis, irrespective of HIV-1 infection. *Am J Respir Crit Care Med.* 2015;192(12):1518–1521.
- Shenje J, Lai RP, Ross IL, et al. Effect of prednisolone on inflammatory markers in pericardial tuberculosis: a pilot study. *Int J Cardiol Heart Vasc.* 2018;18:104–108.
- Reuter H, Burgess LJ, Carstens ME, Doubell AF. Characterization of the immunological features of tuberculous pericardial effusions in HIV-positive and HIV-negative patients in contrast with non-tuberculous effusions. *Tuberculosis (Edinb).* 2006;86(2):125–133.
- Mauro AG, Bonaventura A, Vecchie A, et al. The role of NLRP3 inflammasome in pericarditis: potential for therapeutic approaches. *JACC Basic Transl Sci.* 2021;6(2):137–150.
- Wang Y, Sun Q, Zhang Y, et al. Systemic immune dysregulation in severe tuberculosis patients revealed by a single-cell transcriptome atlas. *J Infect.* 2023;86(5):421–438.
- Lyu M, Xu G, Zhou J, et al. Single-cell sequencing reveals functional alterations in tuberculosis. *Adv Sci (Weinh).* 2024;11(11):e2305592.
- Wang L, Ma H, Wen Z, et al. Single-cell RNA-sequencing reveals heterogeneity and intercellular crosstalk in human tuberculosis lung. *J Infect.* 2023;87:373–384.
- Eyres M, Bell JA, Davies ER, et al. Spatially resolved deconvolution of the fibrotic niche in lung fibrosis. *Cell Rep.* 2022;40:111230.
- Sawyer AJ, Patrick E, Edwards J, et al. Spatial mapping reveals granuloma diversity and histopathological superstructure in human tuberculosis. *J Exp Med.* 2023;220(6):e20221392.
- Krausgruber T, Redl A, Barreca D, et al. Single-cell and spatial transcriptomics reveal aberrant lymphoid developmental programs driving granuloma formation. *Immunity.* 2023;56(2):289–306.e7.
- Pisu D, Huang L, Narang V, et al. Single cell analysis of M. tuberculosis phenotype and macrophage lineages in the infected lung. *J Exp Med.* 2021;218(9):e20210615.
- Cho YH, Schaff HV. Surgery for pericardial disease. *Heart Fail Rev.* 2013;18(3):375–387.
- Younesi FS, Miller AE, Barker TH, Rossi FMV, Hinz B. Fibroblast and myofibroblast activation in normal tissue repair and fibrosis. *Nat Rev Mol Cell Biol.* 2024;25(8):617–638.
- Kennedy A, Waters E, Rowshanravan B, et al. Differences in CD80 and CD86 transendocytosis reveal CD86 as a key target for CTLA-4 immune regulation. *Nat Immunol.* 2022;23(9):1365–1378.
- Wang J, Zhang X, Long M, et al. Macrophage-derived GPNMB trapped by fibrotic extracellular matrix promotes pulmonary fibrosis. *Commun Biol.* 2023;6(1):136.
- DeBerge M, Yeap XY, Dehn S, et al. MerTK cleavage on resident cardiac macrophages compromises repair after myocardial ischemia reperfusion injury. *Circ Res.* 2017;121(8):930–940.
- Revelo XS, Parthiban P, Chen C, et al. Cardiac resident macrophages prevent fibrosis and stimulate angiogenesis. *Circ Res.* 2021;129(12):1086–1101.
- Murray Peter J, Allen JE, Biswas SK, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity.* 2014;41(1):14–20.

- 24 Puig-Kröger A, Sierra-Filardi E, Domínguez-Soto A, et al. Folate receptor β is expressed by tumor-associated macrophages and constitutes a marker for M2 anti-inflammatory/regulatory macrophages. *Cancer Res.* 2009;69(24):9395–9403.
- 25 Marin Franco JL, Genoula M, Corral D, et al. Host-derived lipids from tuberculous pleurisy impair macrophage microbicidal-associated metabolic activity. *Cell Rep.* 2020;33(13):108547.
- 26 Wculek SK, Heras-Murillo I, Mastrangelo A, et al. Oxidative phosphorylation selectively orchestrates tissue macrophage homeostasis. *Immunity.* 2023;56(3):516–530.e9.
- 27 Mi Z, Wang Z, Wang Y, et al. Cellular and molecular determinants of bacterial burden in leprosy granulomas revealed by single-cell multimodal omics. *eBioMedicine.* 2024;108:105342.
- 28 Tang Z, Bai Y, Fang Q, et al. Spatial transcriptomics reveals tryptophan metabolism restricting maturation of intratumoral tertiary lymphoid structures. *Cancer Cell.* 2025;43(6):1025–1044.e14.
- 29 Wu R, Guo W, Qiu X, et al. Comprehensive analysis of spatial architecture in primary liver cancer. *Sci Adv.* 2021;7(51):eabg3750.
- 30 Pant A, Jain A, Chen Y, et al. The CCR6–CCL20 axis promotes regulatory T-cell glycolysis and immunosuppression in tumors. *Cancer Immunol Res.* 2024;12(11):1542–1558.
- 31 Fries A, Saidoune F, Kuonen F, et al. Differentiation of IL-26+ TH17 intermediates into IL-17A producers via epithelial crosstalk in psoriasis. *Nat Commun.* 2023;14(1):3878.
- 32 Sumida TS, Cheru NT, Hafler DA. The regulation and differentiation of regulatory T cells and their dysfunction in autoimmune diseases. *Nat Rev Immunol.* 2024;24(7):503–517.
- 33 Li H, van der Leun AM, Yofe I, et al. Dysfunctional CD8 T cells form a proliferative, dynamically regulated compartment within human melanoma. *Cell.* 2019;176(4):775–789.e18.
- 34 Mahuron KM, Shahid O, Sao P, et al. Single-cell analyses reveal a functionally heterogeneous exhausted CD8+ T-cell subpopulation that is correlated with response to checkpoint therapy in melanoma. *Cancer Res.* 2025;85(8):1424–1440.
- 35 Guo C-L, Wang C-S, Wang X-H, Yu D, Liu Z. GZMK+CD8+ T cells: multifaceted roles beyond cytotoxicity. *Trends Immunol.* 2025;46(8):562–572.
- 36 Shan Q, Li X, Chen X, et al. Tcf 1 and Lef 1 provide constant supervision to mature CD8+ T cell identity and function by organizing genomic architecture. *Nat Commun.* 2021;12(1):5863.
- 37 Yigit B, Wang N, ten Hacken E, et al. SLAMF6 as a regulator of exhausted CD8+ T cells in cancer. *Cancer Immunol Res.* 2019;7(9):1485–1496.
- 38 Nutt SL, Hodgkin PD, Tarlinton DM, Corcoran LM. The generation of antibody-secreting plasma cells. *Nat Rev Immunol.* 2015;15(3):160–171.
- 39 Higgins BW, McHeyzer-Williams LJ, McHeyzer-Williams MG. Programming isotype-specific plasma cell function. *Trends Immunol.* 2019;40(4):345–357.
- 40 Prêle CM, Miles T, Pearce DR, et al. Plasma cell but not CD20-mediated B cell depletion protects from bleomycin-induced lung fibrosis. *Eur Respir J.* 2022;60:2101469.
- 41 Cremer PC, Kumar A, Kontzias A, et al. Complicated pericarditis. *JACC.* 2016;68(21):2311–2328.
- 42 Muhl L, Mocci G, Pietilä R, et al. A single-cell transcriptomic inventory of murine smooth muscle cells. *Dev Cell.* 2022;57(20):2426–2443.e6.
- 43 Cao G, Xuan X, Hu J, Zhang R, Jin H, Dong H. How vascular smooth muscle cell phenotype switching contributes to vascular disease. *Cell Commun Signal.* 2022;20(1):180.
- 44 Steppan J, Wang H, Nandakumar K, et al. LOXL2 inhibition ameliorates pulmonary artery remodeling in pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol.* 2024;327(4):L423–L438.
- 45 He Y, Tacconi C, Dieterich LC, et al. Novel blood vascular endothelial subtype-specific markers in human skin unearthed by single-cell transcriptomic profiling. *Cells.* 2022;11(7):1111.
- 46 Yoshimatsu Y, Watabe T. Emerging roles of inflammation-mediated endothelial–mesenchymal transition in health and disease. *Inflamm Regen.* 2022;42(1):9.
- 47 Li Y, Jiang D, Liang J, et al. Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. *J Exp Med.* 2011;208(7):1459–1471.
- 48 Mohanakrishnan V, Sivaraj KK, Jeong H-W, et al. Specialized post-arterial capillaries facilitate adult bone remodelling. *Nat Cell Biol.* 2024;26(12):2020–2034.
- 49 Zhong Y, Wei B, Wang W, et al. Single-cell RNA-sequencing identifies bone marrow-derived progenitor cells as a main source of extracellular matrix-producing cells across multiple organ-based fibrotic diseases. *Int J Biol Sci.* 2024;20(13):5027–5042.
- 50 Simons M, Gordon E, Claesson-Welsh L. Mechanisms and regulation of endothelial VEGF receptor signalling. *Nat Rev Mol Cell Biol.* 2016;17(10):611–625.
- 51 Tokunaga R, Zhang W, Naseem M, et al. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation – a target for novel cancer therapy. *Cancer Treat Rev.* 2018;63:40–47.
- 52 Di Pilato M, Kfuri-Rubens R, Pruessmann JN, et al. CXCR6 positions cytotoxic T cells to receive critical survival signals in the tumor microenvironment. *Cell.* 2021;184(17):4512–4530.e22.
- 53 Gérard A, Cope AP, Kemper C, Alon R, Köchl R. LFA-1 in T cell priming, differentiation, and effector functions. *Trends Immunol.* 2021;42(8):706–722.
- 54 Halliday N, Williams C, Kennedy A, et al. CD86 is a selective CD28 ligand supporting FoxP3+ regulatory T cell homeostasis in the presence of high levels of CTLA-4. *Front Immunol.* 2020;11:600000. Available from: <http://europepmc.org/abstract/MED/33363541>.
- 55 Wiysonge CS, Ntsekhe M, Thabane L, et al. Interventions for treating tuberculous pericarditis. *Cochrane Database Syst Rev.* 2017;(9).
- 56 Pagán AJ, Ramakrishnan L. The formation and function of granulomas. *Annu Rev Immunol.* 2018;36:639–665.
- 57 Kuppe C, Ramirez Flores RO, Li Z, et al. Spatial multi-omic map of human myocardial infarction. *Nature.* 2022;608(7924):766–777.
- 58 Wang L-X, Zhang S-X, Wu H-J, Rong X-L, Guo J. M2b macrophage polarization and its roles in diseases. *J Leukoc Biol.* 2019;106(2):345–358.
- 59 Cruz FM, Colbert JD, Merino E, Kriegsmann BA, Rock KL. The biology and underlying mechanisms of cross-presentation of exogenous antigens on MHC-I molecules. *Annu Rev Immunol.* 2017;35:149–176.
- 60 Cai Y, Wang Y, Shi C, et al. Single-cell immune profiling reveals functional diversity of T cells in tuberculous pleural effusion. *J Exp Med.* 2022;219(3):e20211777.
- 61 Gideon HP, Hughes TK, Tzouanas CN, et al. Multimodal profiling of lung granulomas in macaques reveals cellular correlates of tuberculosis control. *Immunity.* 2022;55(5):827–846.e10.
- 62 Huang Y, Fleming AJ, Wu S, Virella G, Lopes-Virella MF. Fc- γ receptor cross-linking by immune complexes induces matrix metalloproteinase-1 in U937 cells via mitogen-activated protein kinase. *Arterioscler Thromb Vasc Biol.* 2000;20(12):2533–2538.
- 63 Dart A. Presenting fibroblasts. *Nat Rev Cancer.* 2022;22(4):193.
- 64 Chen T, Chen X, Zhang S, et al. The genome sequence archive family: toward explosive data growth and diverse data types. *Genomics Proteomics Bioinformatics.* 2021;19(4):578–583.
- 65 CNGB-NGDC Members and Partners. Database resources of the National Genomics Data Center, China National Center for bioinformatics in 2022. *Nucleic Acids Res.* 2022;50(D1):D27–D38.