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# Single-cell transcriptome analysis reveals cellular heterogeneity in the aortas of Takayasu arteritis

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## Abstract

**Objectives** Takayasu arteritis (TAK) is an inflammatory vasculitis that affects the aorta and its primary branches. The pathogenesis of TAK remains elusive, yet identifying key cell types in the aorta of TAK patients is crucial for uncovering cellular heterogeneity and discovering potential therapeutic targets.

**Methods** This study utilized single-cell transcriptome analysis on aortic specimens from three TAK patients, with control data sourced from a publicly available database (GSE155468). Additionally, bulk RNA sequencing was performed on peripheral CD4 + and CD8 + T cells from eight TAK patients and eight matched healthy volunteers. All participants were recruited at Anzhen Hospital, Capital Medical University, China, between January 2020 and December 2023.

**Results** Single-cell transcriptome analysis identified 11 predominant cell types in aortic tissues, with notable differences in proportions between TAK patients and controls. T cells, B cells, macrophages, smooth muscle cells (SMCs), and fibroblasts exhibited subtype-specific gene expression signatures, with notable changes in interactions between T cells, B cells, and monocyte-macrophages, highlighting their active involvement in the pathogenesis of TAK. Bulk RNA-Seq analysis of peripheral blood T cells from TAK patients showed an upregulation of complement system genes, underscoring the significance of the complement signaling pathway in TAK's immunopathogenesis.

**Conclusion** The findings underscore the active involvement of various immune and structural cells in the aortic tissues of TAK patients and reveal the presence of the complement signaling pathway in peripheral blood T cells. These insights are instrumental for identifying novel therapeutic targets and developing robust disease monitoring methods for TAK.

**Keywords** Takayasu arteritis, Single-cell RNA sequencing, Bulk RNA sequencing, Cellular heterogeneity, Complement pathway

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## Introduction

Takayasu arteritis (TAK) is a distinct subset of large vessel vasculitis (LVV), distinguished by its granulomatous inflammation targeting the aorta and its major branches [1]. The ensuing vascular damage has the potential to lead to significant mortality, with severe organ ischemia being a notable risk, including conditions such as stroke and myocardial infarction [2]. In rare instances, patients may experience hemorrhagic shock due to vascular rupture.

The etiology of TAK remains elusive. A variety of cytokines, including interleukin-6 (IL-6), interferon-γ (IFN-γ), and interleukin-17 (IL-17), along with immune cells such as CD4 + T cells, macrophages, CD8 + T cells, and natural killer (NK) cells, are implicated in the promotion of vascular inflammation in TAK patients [3–5]. Additionally, premature senescence of vascular smooth muscle cells is thought to contribute to the disease pathology [6]. In the aortas of TAK patients, neoangiogenesis in the adventitia and luminal occlusion due to intimal hyperplasia are commonly observed phenomena [7].

While the primary cell types involved in TAK have been identified, the heterogeneity and specific contributions of these vascular cells are not well understood. The advent of single-cell transcriptome analysis (scRNA-seq) has opened new avenues for characterizing gene expression at the individual cell level. This study leverages scRNA-seq to delineate the transcriptional profiles of various cell types within the TAK-affected aortas, including lymphocytes, macrophages, smooth muscle cells (SMCs), fibroblasts, mast cells, endothelial cells, and mesenchymal stem cells (MSCs). The data reveal a complex tapestry of cell populations and intricate gene regulatory networks, offering novel insights into the pathogenesis of TAK and paving the way for a deeper comprehension of this condition.

## Materials and methods

### Study subjects

Between January 2020 and December 2023, eleven patients diagnosed with Takayasu arteritis (TAK) were referred to Anzhen Hospital, affiliated with Capital Medical University in China. These patients fulfilled the classification criteria for TAK as established by the American College of Rheumatology (ACR) in 2022 [8]. For comparative analysis, three control subjects obtained from a public database(GSE155468) [9] and eight age- and gender-matched healthy volunteers were included as control groups for single-cell sequencing and bulk RNA sequencing, respectively. The patient information for ascending aortic samples (TAK1-3, Control1-3) designated for single-cell sequencing and peripheral blood samples (TAK4-11) intended for bulk RNA sequencing is summarized in Table 1.

### Single-cell transcriptome analysis of aortic tissue samples

Aortic tissue samples from three patients with TAK were obtained from regions exhibiting ascending aortic aneurysms or dilation of the ascending aorta. In accordance with the stringent requirements for aortic tissue in single-cell sequencing, we carefully selected tissue specimens measuring approximately $1 \times 1$ cm$^2$ (>200 mg) from each patient, ensuring the preservation of the complete trilaminar vascular wall structure. Adhering strictly to the SeekOne® Operate the MM High Flux Single Cell Transcriptome Kit User Manual V4.1, we constructed single-cell transcriptome libraries compatible with high-throughput sequencing platforms. Following successful library validation, sequencing was conducted using Illumina or Huada high-throughput sequencing systems. The qualified library's main peak fragment size was targeted to range from 350 to 750 base pairs (bp), ensuring the absence of smaller fragments. Any presence of small fragments was addressed through secondary purification until eliminated, as confirmed by Agilent4200 TapeStation analysis. The library concentration was maintained at no less than 1 ng/μL, as measured by Qubit 4.0. To ensure the precision of single-cell sequencing data analysis, we aimed for a sequencing depth of at least 50,000 reads per cell.

### Bulk RNA-Seq of peripheral blood samples

Peripheral blood samples were collected from eight TAK patients and eight matched healthy volunteers, with heparin used as an anticoagulant. CD4 + and CD8 + T cells were isolated using magnetic bead sorting. The TruSeq™ RNA Sample Preparation Kit from Illumina (San Diego, California, USA) was utilized to perform bulk RNA-seq, following the manufacturer's protocol.

### Bioinformatics analysis

Upon acquisition of the raw sequencing data, known as Sequenced Reads, we employed the SeekOne® suite of bioinformatics tools for comprehensive analysis. This process primarily encompassed several key steps: data quality control to ensure the integrity of the sequencing output, assembly of an expression matrix to map gene expression across samples, cell clustering to identify distinct cell populations, differential gene expression analysis to pinpoint genes with significant variation in expression levels, and enrichment analysis to explore the biological significance of these variations.

This research study was granted approval by the Medical Ethics Committee of Anzhen Hospital, confirming adherence to ethical standards and patient confidentiality.

**Table 1** Patient information for ascending aortic samples (TAK1-3,Conrol1-3) and peripheral blood samples (TAK4-11)

| | Age, year | Disease duration, month | Sex | Symptoms | CRP, mg/l | ESR, mm/1 h | Numano classification | Ascending aortic aneurysm/dilation | Hypertension | Surgical history | Immunosuppressive therapy |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TAK1 | 47 | 12 | M | Chest pain | 7.62 | 34 | IIa | Yes | Yes | Bentall | GC + CTX |
| TAK2 | 22 | 72 | F | Chest tightness | 5.61 | 19 | IIa | Yes | No | Ascending aortic replacement | GC + MMF |
| TAK3 | 47 | 480 | F | Fever, back pain | 1.37 | 13 | V | Yes | No | Bentall | GC + TOF |
| Control1 | 63 | NA | F | NA | NA | NA | - | No | No | Heart transplant recipient | NA |
| Control2 | 61 | NA | M | NA | NA | NA | - | No | Yes | Heart transplant recipient | NA |
| Control3 | 62 | NA | F | NA | NA | NA | - | No | Yes | Lung transplant donor | NA |
| TAK4 | 57 | 48 | F | Chest tightness, syncope | 46.5 | 73 | V | No | No | No | No |
| TAK5 | 66 | 480 | F | Dizziness, lower limb weakness | 3.8 | 25 | V | No | Yes | Resection of Abdominal Aortic Stenosis | No* |
| TAK6 | 35 | 180 | F | Claudication of the lower limbs | 0.64 | 5 | V | No | No | No | No |
| TAK7 | 48 | 24 | F | Dizziness | 19.76 | 53 | V | Yes | Yes | No | No |
| TAK8 | 24 | 24 | F | Chest tightness | 84.87 | 77 | V | No | No | No | No |
| TAK9 | 39 | 48 | F | Headache | 21.3 | 48 | V | No | Yes | No | No |
| TAK10 | 58 | 216 | F | Weakness in both upper limbs, chest tightness | 34.95 | 11 | IIa | Yes | No | No | No |
| TAK11 | 65 | 492 | F | Weakened pulse on the left side | 3 | 56 | V | No | Yes | No | No |

TAK, Takayasu arteritis; CRP, C-reactive protein; ESR, Erythrocyte Sedimentation Rate; M, Male; F, Female; GC, Glucocoticoid; CYP, Cyclophosphamide; MMF, Mycophenolate mofetil; TOF, Tofacitinib; NA, not available

* The patient had previously taken Glucocoticoid, Mycophenolate mofetil and Methotrexate, and has been off the medication for 2 years

## Results

### Cell type composition of aortic tissue via single-Cell RNA sequencing (scRNA-Seq) analysis

In a pioneering effort, we have delineated a detailed and comprehensive map of the cellular composition within aortic tissues affected by Takayasu arteritis (TAK), as illustrated in Fig. 1. Post quality control measures, a total of 28,531 cells from three TAK patients and 8,477 cells from three control subjects were subjected to further analytical scrutiny. The detailed cell numbers for each cluster are shown in eFigure 1.

Employing established gene expression markers, the cells were categorized into 11 predominant cell types, which include: T cells, characterized by the expression of CXCR4, TRBC2, CCL5, and IL7R, comprising 13.4% of the total cell population; B cells, marked by MS4A1, CD79A, and BANK1, accounting for 3.2% of cells; Macrophages, identified by C1QC, C1QB, and C1QA, representing 7.2% of the cells; Mono-macrophages, with distinctive expression of CXCL8, CXCL3, CXCL2, and LYZ, making up 2.9% of the total; Fibroblasts 1, defined by FN1, COL1A1, and COL1A2, constituting 13.6% of cells; Fibroblasts 2, indicated by DCN and C1R, at 14.7%; Smooth muscle cells 1, with ACTA2, DSTN, and MYH11, totaling 10.4%; Smooth muscle cells 2, signified by IGFBP2 and TPM2, at 1.7%; Endothelial cells 1, distinguished by PECAM1, FABP4, VWF, and IFI27, encompassing 24% of the cell types; Endothelial cells 2, marked solely by PECAM1 and VWF, at 0.6%; Mesenchymal stem cells, identified by MT1M, IGFBP5, and C11orf96, representing 8.2%.

Each cell type exhibited a unique gene expression profile. Comparative analysis with control aortic tissues revealed significant increases or decreases in the relative proportions of these cell types within the aortas of TAK patients, highlighting the heterogeneity and complexity of the disease's cellular underpinnings.

### T cells: pivotal players in TAK

T cells are recognized for their significant role in the pathogenesis of TAK. Following the de-batching process, a stark contrast was observed in the cell counts between the TAK group, which contained a substantial 3,829 cells, and the control group, with a comparatively modest 750 cells. Through meticulous gene expression analysis, we discerned four distinct T cell subtypes within the aortic tissue, categorized as Clusters 1, 2, 3, and 4 (as depicted in Fig. 2).

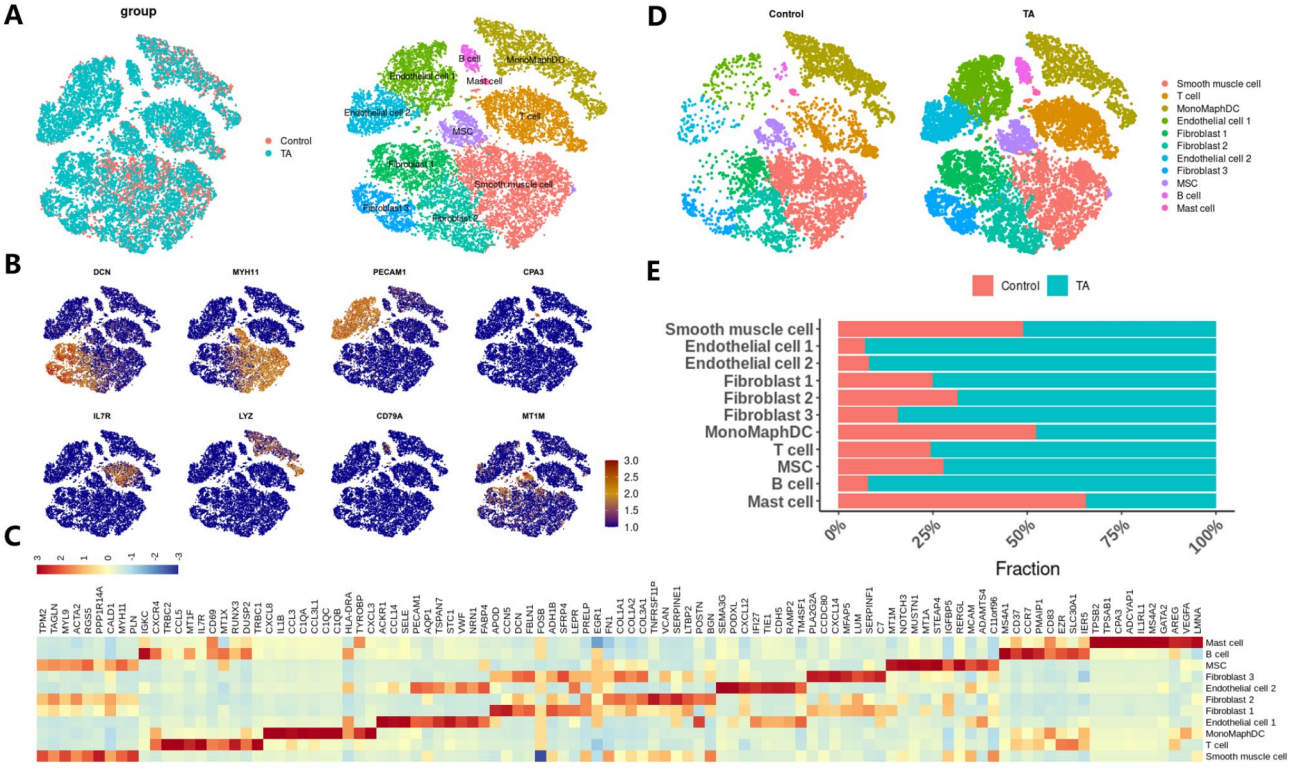


**Fig. 1** Identification of 11 major cell types in ascending aortic tissues from TAK and control groups. (**A**) t-SNE plot displaying cells color-coded by the 11 major cell types (**B**) t-SNE plot illustrating the relative expression of marker genes across selected cell types. (**C**) Mean expression levels of selected genes in major cell types (**D**) t-SNE plot comparing the distribution of 11 major cell types between control and TAK groups (**E**) Horizontal bar plot showing the proportional composition of each cell type (calculated as the ratio of control cells to the total number of cells across all specimens). MSC: mesenchymal stem cell

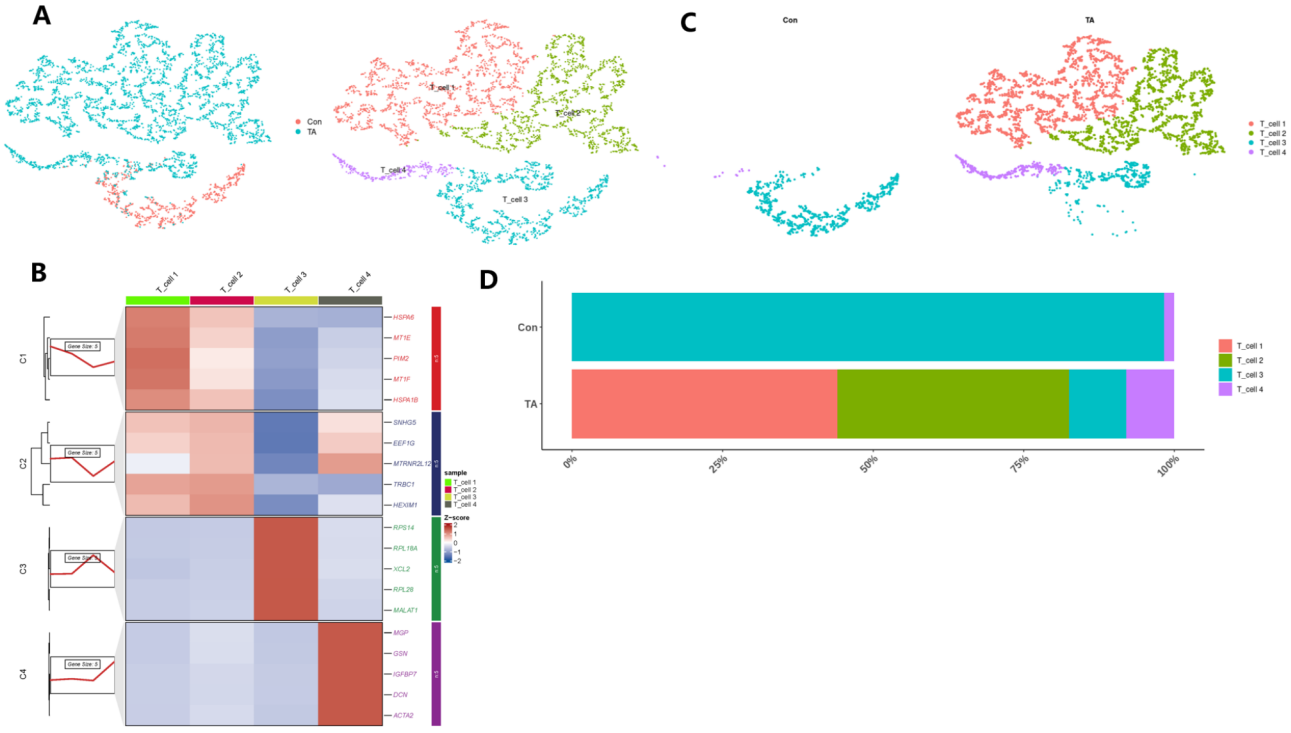


**Fig. 2** Identification of four T cell subgroups in ascending aortic tissues from TAK and control groups (**A**) t-SNE plot displaying cells color-coded by the four T cell subgroups (**B**) Mean expression levels of selected genes across the four T cell subgroups (**C**) t-SNE plots comparing the distribution of T cell subgroups between control and TAK groups (**D**) Horizontal bar plot showing the proportional composition of T cell subgroups in control and TAK groups

In the control group, the T cell distribution was predominantly skewed towards Cluster 3 (98.3%), with a minor presence of Cluster 4 T cells (1.7%). Conversely, in the TAK group, there was a notable increase in the proportion of Cluster 1 (44.1%), 2 (38.4%), and 4 (8.0%) T cells, significantly surpassing the levels observed in the control group. In contrast, the proportion of Cluster 3 T cells in the TAK group (9.5%) was markedly reduced compared to the control group.

The functional profiles of these T cell clusters were characterized by their primary activities: Cluster 1 T cells were associated with ubiquitin protein ligase binding and ATP-dependent protein folding. Cluster 2 T cells were linked to MHC class II protein complex binding, cytokine binding, and immune receptor activity. Cluster 3 T cells were characterized by CCR chemokine receptor binding, chemokine activity, and chemokine receptor binding. Cluster 4 T cells were identified by their roles as extracellular matrix structural constituents, growth factor binding, and protease binding.

Further elucidation of the molecular mechanisms was provided by KEGG pathway analysis, which revealed significant enrichment of specific pathways within the T cell clusters of TAK patients: Antigen processing and presentation pathways were prominently enriched in Cluster 1 T cells. Cytokine receptor and chemokine signaling pathways were dominant in Cluster 2 T cells. Complement signaling and vascular smooth muscle contraction pathways were notably enriched in Cluster 3 T cells, as illustrated in Fig. 3.

These findings provide a deeper understanding of the diverse roles T cells play in TAK and the intricate immunological pathways they influence.

In the bulk RNA-seq analysis, KEGG pathway enrichment revealed a striking distinction when comparing the control group to the peripheral blood CD4 + and CD8 + T cells of Takayasu arteritis (TAK) patients. Notably, the complement signaling pathway emerged as the most significantly enriched among the signaling pathways in both cell types, as depicted in Fig. 4.

In the context of CD4 + T cells from TAK patients, a pronounced upregulation was observed for several key complement system genes, including the C1q B chain (C1QB), complement C1q A chain (C1QA), complement C1q C chain (C1QC), complement factor D (CFD), complement C5a receptor 1 (C5AR1), complement C5a receptor 2 (C5AR2), ficolin 1 (FCN1), complement component 2 (C2), integrin subunit alpha M (ITGAM), clusterin (CLU), and serpin family G member 1 (SERPING1).

Similarly, within the CD8 + T cells of TAK patients, the complement signaling pathway was also found to be enriched, with significant upregulation of genes such as C5AR1, C1QA, complement component 3 (C3), C5AR2, CFD, and C1QC.

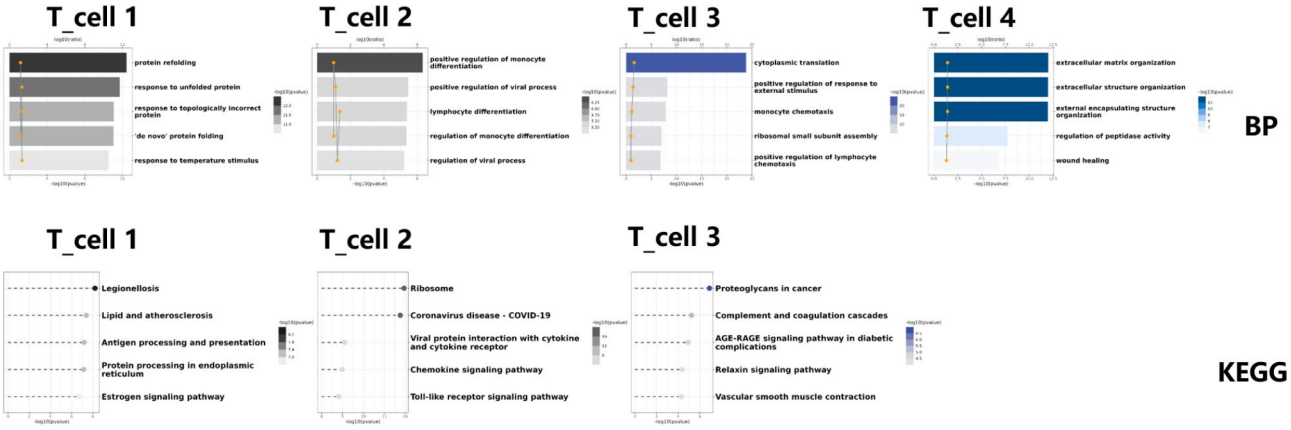


**Fig. 3** Functional enrichment analysis of four T cell subgroups in ascending aortic tissues. BP: Biological Process; KEGG: Kyoto Encyclopedia of Genes and Genomes

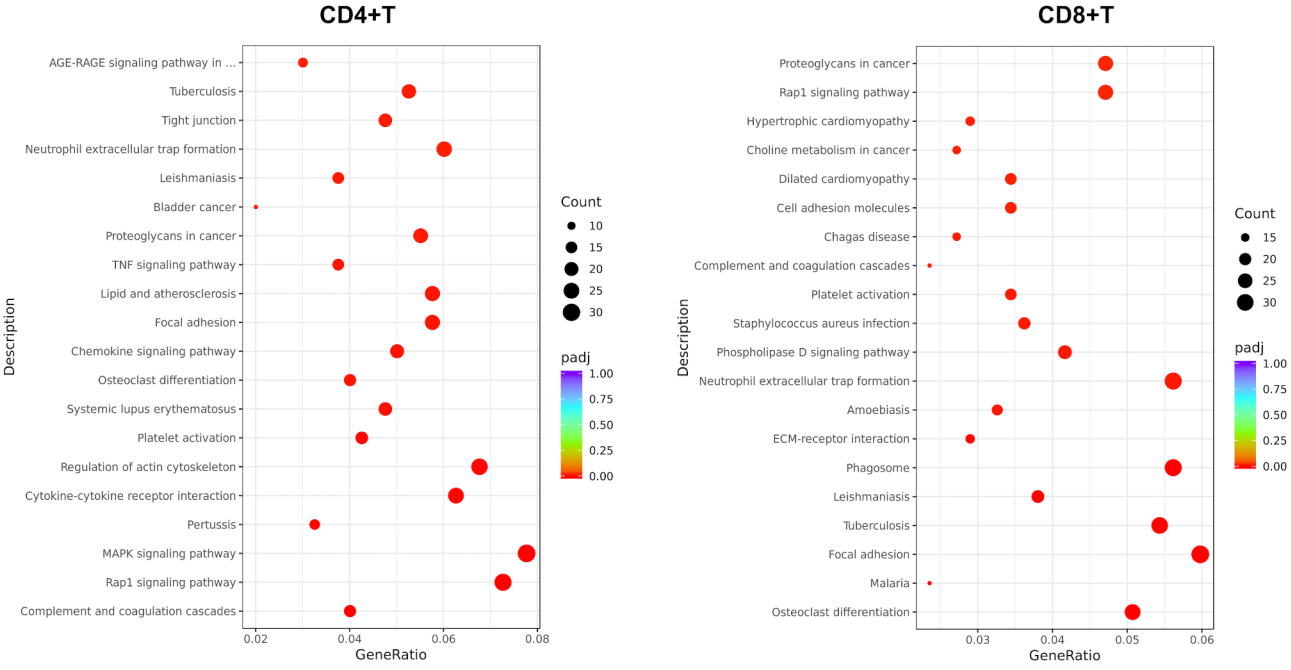


**Fig. 4** Enriched signaling pathways in TAK patients identified through bulk RNA-seq analysis of peripheral CD4 + and CD8 + T cells

These results underscore the pivotal role of the complement system in the immunopathogenesis of TAK, highlighting its potential as a therapeutic target and a valuable biomarker for disease activity and progression.

### B cells in aortic tissue of TAK

In the aortic tissue of patients with TAK, the B cell population (901 cells) was notably larger than that observed in the control group (189 cells). The B cells within the aorta were meticulously classified into four distinct subtypes based on their gene expression profiles: Cluster 1 featured genes such as IRF8, NIBAN3, RIPOR2, RALGPS2, and HLA-DMB. Cluster 2 was characterized by the presence of IGHG1, IGHG3, IGLC1, IGLC2, and IGKC. Cluster 3 included genes like FKBP4, CCR7, IGFBP7, SNN, and GSN. Cluster 4 was typified by CPA3, TPSB2, YPSAB1, ADCYAP1, and ANXA1.

Significantly higher proportions of Cluster 1 (61.7%) and Cluster 3 (18.9%) B cells were found in the TAK group compared to the control group, which exhibited only 0.5% and 5.3% respectively (as illustrated in eFigure 2).

Functional enrichment analysis revealed that Cluster 1 B cells were primarily involved in MHC class II protein complex binding, a critical process in antigen presentation. In contrast, Cluster 3 B cells were predominantly associated with ATP-dependent protein folding,

indicative of their role in the endoplasmic reticulum's protein processing machinery.

Further insights were provided by KEGG pathway analysis, which identified the main signaling pathways active in Cluster 1 and 3 B cells as antigen processing and presentation, and protein processing in the endoplasmic reticulum, respectively (as detailed in eFigure 3). These findings enhance our understanding of the diverse functional roles of B cells within the aortic tissue of TAK patients and their potential contribution to the disease's pathophysiology.

### Macrophages in aorta tissue of TAK

The role of macrophages in the pathology of TAK has garnered considerable interest. Within the aortic tissue, these immune cells were categorized into five distinct subtypes based on their gene expression signatures: Cluster 1 was characterized by the expression of MALAT1, REL, PCK4, NEAT1, and CYBA. Cluster 2 was typified by RPL31, TNFSF13, FCGR3A, CD74, and HLADPB1. Cluster 3 featured IFI30, CSTB, SPP1, ANXA2, and FN1. Cluster 4 was marked by MAT2A, HMOX1, F13A1, TFRC, and CSRNP1. Cluster 5 included ACTA2, MGP, SPARCL1, IGFBP7, and TAGLN.

In patients with TAK, the proportions of macrophages within Cluster 1 (9.6%), Cluster 3 (34.5%), Cluster 4 (32.7%), and Cluster 5 (22%) were notably higher than those in the control group, which was predominantly composed of Cluster 2 macrophages (70.9%), as illustrated in Fig. 5.

Biologic process (BP) analyses revealed that Cluster 1 macrophages were primarily involved in CXCR chemokine receptor binding, chemokine receptor binding, and chemokine activity, indicating a role in chemotaxis and immune cell signaling. Cluster 3 macrophages were associated with collagen binding and protease binding functions, suggesting a role in extracellular matrix remodeling and proteolysis. The main functions of Cluster 4 and Cluster 5 macrophages were identified as mRNA 3'-untranslated region (3'-UTR) AU-rich region binding and extracellular matrix structural constituent activity, respectively. These functions point to roles in post-transcriptional regulation and tissue integrity.

KEGG pathway analysis provided further insights, showing that Cluster 1 macrophages were significantly involved in the NF-kappa B signaling pathway and NOD-like receptor signaling pathway, which are crucial for inflammatory responses and innate immune recognition. Additionally, the complement cascade signaling pathway was found to be enriched in Cluster 4 macrophages, highlighting the importance of the complement system in TAK pathogenesis, as depicted in Fig. 6.

These findings offer a more granular understanding of the multifaceted roles of macrophages in TAK and their

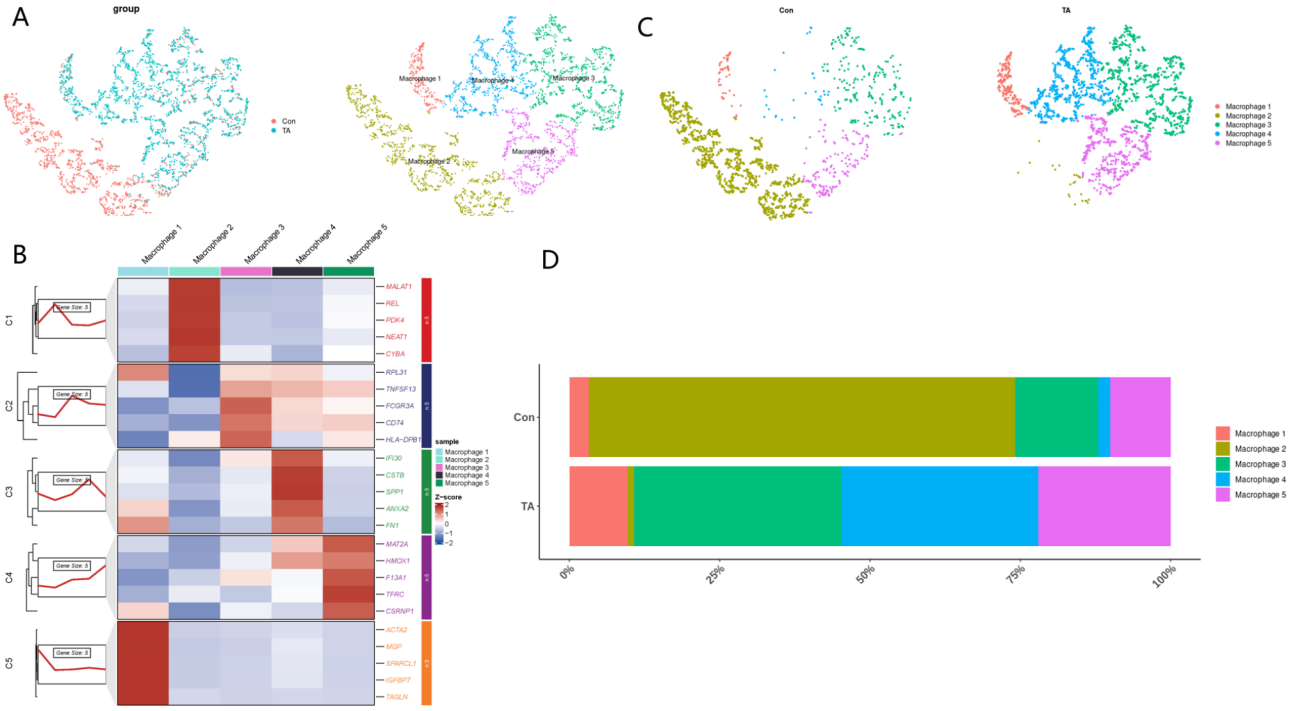


**Fig. 5** Identification of five macrophage subgroups in ascending aortic tissues from TAK and control groups (**A**) t-SNE plot displaying cells color-coded by the five macrophage subgroups (**B**) Mean expression levels of selected genes across the five macrophage subgroups (**C**) t-SNE plots comparing the distribution of macrophage subgroups between control and TAK groups (**D**) Horizontal bar plot showing the proportional composition of macrophage subgroups in control and TAK groups

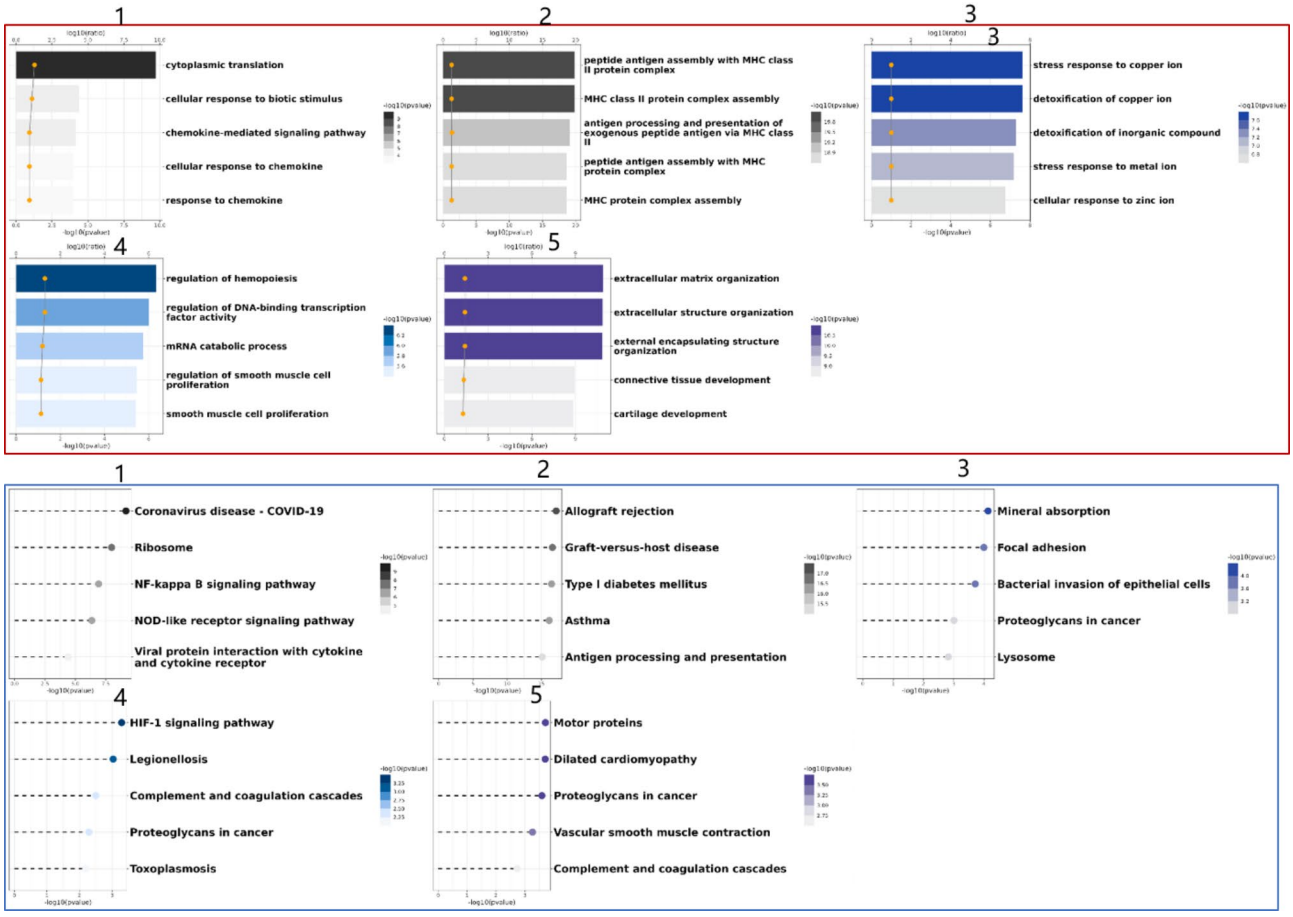


**Fig. 6** Functional enrichment analysis of five macrophage subgroups in ascending aortic tissues. BP: Biological Process; KEGG: Kyoto Encyclopedia of Genes and Genomes

potential as therapeutic targets or biomarkers for disease monitoring.

### Smooth muscle cells in aorta tissue of TAK

Vascular smooth muscle cells (SMCs) in TAK serve a dual role, being both the target of inflammatory damage and active participants in vascular inflammation through processes such as cellular senescence [6]. In our study, the aortic SMC count was 3,457 in the TAK group and 3,258 in the control group. We identified four distinct subgroups of SMCs based on their gene expression profiles: Cluster 1 was defined by the presence of CCN2. Cluster 2 was characterized by MALAT1. Cluster 3 included genes such as CD74, MT-ATP8, CCN5, DCN, and SFRP4. Cluster 4 was typified by CYR61, CTGF, WISP2, and RAMP1.

The control group predominantly featured Cluster 2 (47.2%) and Cluster 4 (39.2%), whereas the TAK group was primarily composed of Cluster 1 (61.7%) and Cluster 3 (37.9%), as detailed in eFigure 4. The primary function of Cluster 1 SMCs is to serve as extracellular matrix structural constituents, with their main signaling pathways being focal adhesion and extracellular matrix receptor interaction. Cluster 3 SMCs, on the other hand, are primarily involved in protein folding chaperoning and also function as extracellular matrix structural constituents, with their prominent signaling pathways being antigen processing and presentation.

KEGG pathway analysis revealed that the main signaling pathways for Clusters 1 and 3 SMCs were focal adhesion and antigen processing/presentation, respectively (as shown in eFigure 5). These findings underscore the complex and multifunctional nature of SMCs in the context of TAK, highlighting their active contribution to the disease's pathophysiology and their potential relevance in therapeutic strategies.

### Fibroblast in aorta tissue of TAK

Fibroblasts constitute the most abundant cell type in the TAK group, with a significantly higher cell count (8,091 cells) compared to the control group (1,520 cells). Our analysis, based on gene expression patterns, categorized these fibroblasts into five distinct subtypes: Cluster 1 was defined by the presence of CXCL14, PLAG2, HAS1, RASD1, and GFPT2. Cluster 2 was characterized by

C9orf3, AMIM37, PLA2G16, FAM213A, and SEPT10. Cluster 3 included RGS5, WFDC1, CYFIP2, and SLC14A1. Cluster 4 was typified by CRTAC1, AGT, and TNC. Cluster 5 featured SFRP4, CCN5, and ALDHA1.

In comparison to the control group, the proportion of Cluster 2 fibroblasts in the TAK group was markedly reduced. In contrast, Clusters 1, 3, 4, and 5 each represented a significant proportion of the fibroblast population (as depicted in Fig. 7). The primary functions of these clusters are as follows: Cluster 1 fibroblasts are involved in the regulation of angiogenesis and vasculature development. Cluster 3 fibroblasts play a role in cell substrate adhesion and muscle cell differentiation. Cluster 4 fibroblasts are associated with collagen fibril organization. Cluster 5 fibroblasts are linked to complement activation.

KEGG pathway analysis revealed the main signaling pathways associated with these clusters: Cluster 1 fibroblasts were primarily linked to the complement and coagulation cascades. Cluster 3 fibroblasts were involved in the focal adhesion pathway. Cluster 4 fibroblasts were associated with the extracellular matrix (ECM)-receptor interaction pathway. Cluster 5 fibroblasts were implicated in the TNF signaling pathway (as shown in Fig. 8).

### Cell-cell communication analysis

Given the pivotal immunoregulatory roles of T cells and B cells in the pathogenesis of Takayasu arteritis (TAK), we employed analytical tools such as NicheNet to systematically compare the interaction networks and ligand-receptor interaction strengths between T cells, B cells, and other immune cells in two patient groups. The study revealed significant interactions between B cells and the macrophage/monocyte-macrophage system, primarily mediated by specific signaling pathways. In healthy control tissues, T cells predominantly interact with endothelial cells through the CCL5-ACKR1 pathway and with smooth muscle cells via the PPIA-BSG; PGE2-PTGES3-PTGER3 pathway. However, in disease samples, the interactions between T cells and various cell types, including endothelial cells, smooth muscle cells, and fibroblasts, were significantly diminished. Conversely, the interaction pathways among T cells, such as HLA-CD8A, HLAA-CD8A, and HLAE-CD8A, were markedly enhanced. Similarly, the interactions between B cells and parenchymal

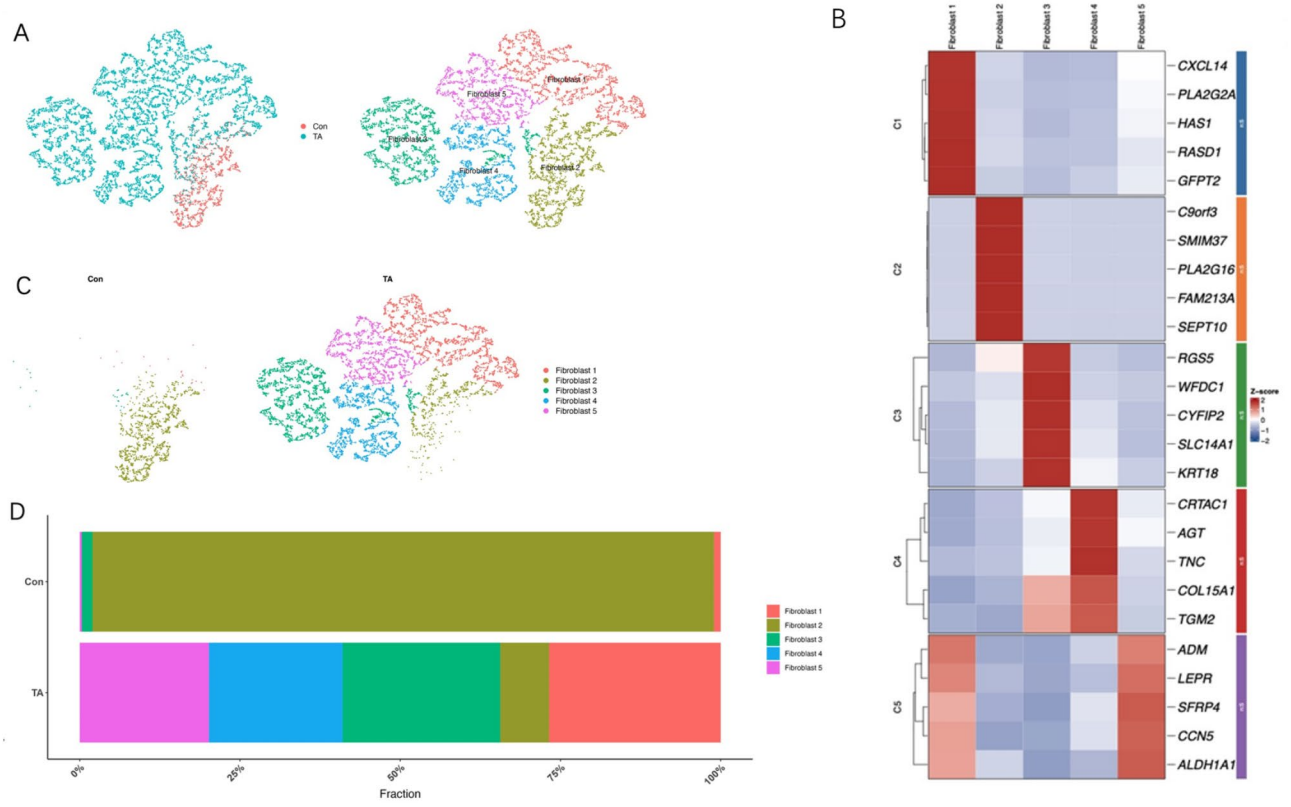


**Fig. 7** Identification of five fibroblast subgroups in ascending aortic tissues from TAK and control groups (**A**) t-SNE plot displaying cells color-coded by the five fibroblast subgroups (**B**) Mean expression levels of selected genes across the five fibroblast subgroups (**C**) t-SNE plots comparing the distribution of fibroblast subgroups between control and TAK groups (**D**) Horizontal bar plot showing the proportional composition of fibroblast subgroups in control and TAK groups

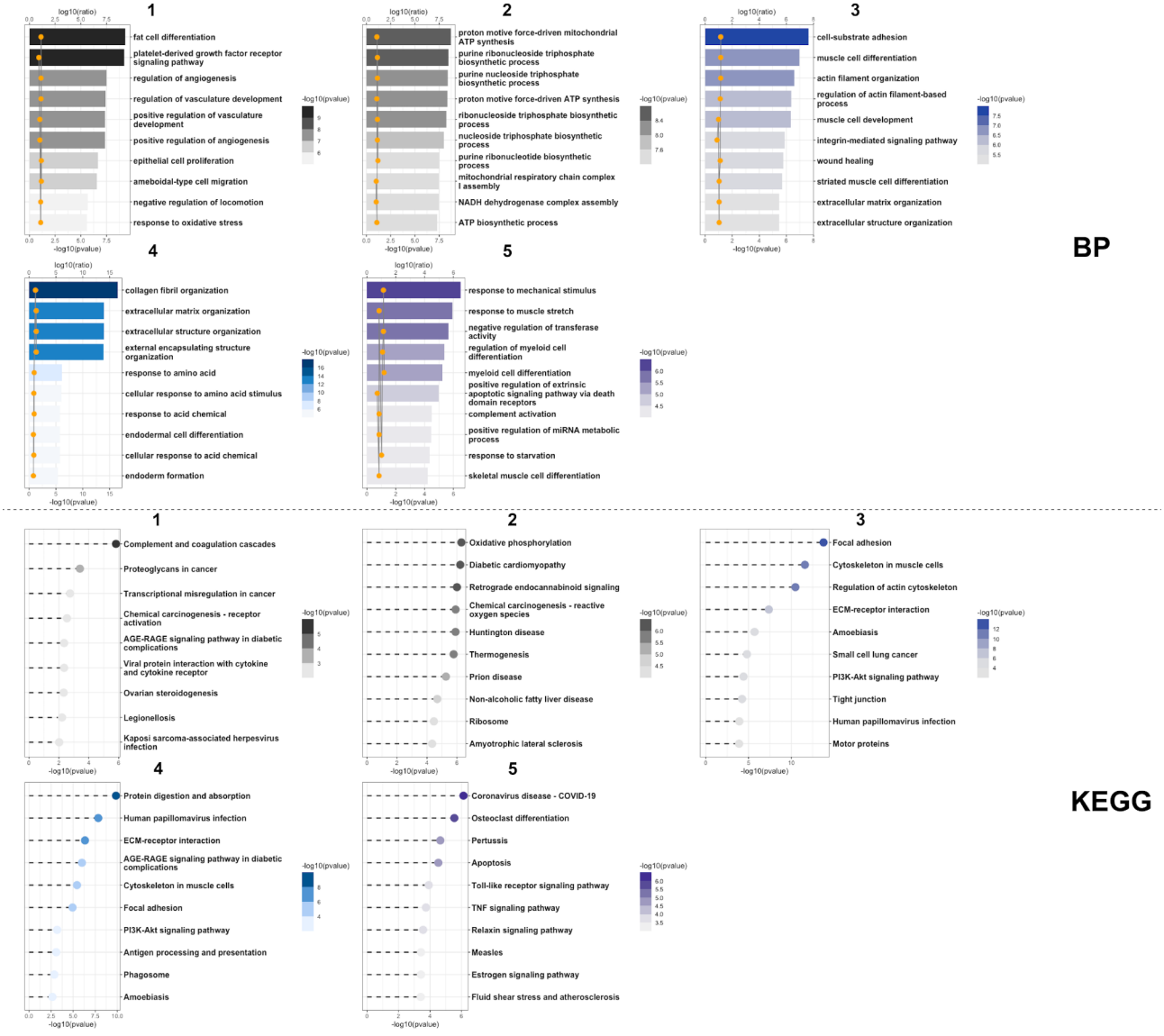


**Fig. 8** Functional enrichment analysis of five fibroblast subgroups in ascending aortic tissues BP: Biological Process; KEGG: Kyoto Encyclopedia of Genes and Genomes

cells, including endothelial cells, smooth muscle cells, and fibroblasts, were notably reduced in disease samples, while the interactions between B cells and T cells, specifically through HLAA-CD8A and HLAB-CD8A, were significantly increased. Additionally, a significant increase in receptor-ligand signaling related to inflammation, such as HLA-DRB1-CD4, HLA-DRA-CD4, and HLA-DPB1-CD4, was observed between B cells and monocyte-macrophages. These findings partially elucidate the phenotype of localized inflammation exacerbation in disease samples and its potential mechanisms, with the HLA-CD4 molecular interaction playing a crucial regulatory role (Fig. 9).

## Discussion

The acquisition and digestion of aortic tissues in TAK patients present considerable challenges, rendering related research inherently difficult. To the best of our knowledge, our study pioneers in delineating the single-cell transcriptional landscapes of ascending aortic from TAK patients. To contextualize our findings within the broader landscape of single-cell and bulk RNA sequencing studies in Takayasu arteritis (TAK), we conducted a comprehensive comparison with existing research.

Gao et al. employed the GEXSCOPE kit for enzymatic digestion of renal arteries, yielding a cell population predominantly composed of endothelial cells (ECs), which accounted for 57.4% of the total cells [10]. In contrast, our study utilized a combination of Collagenase

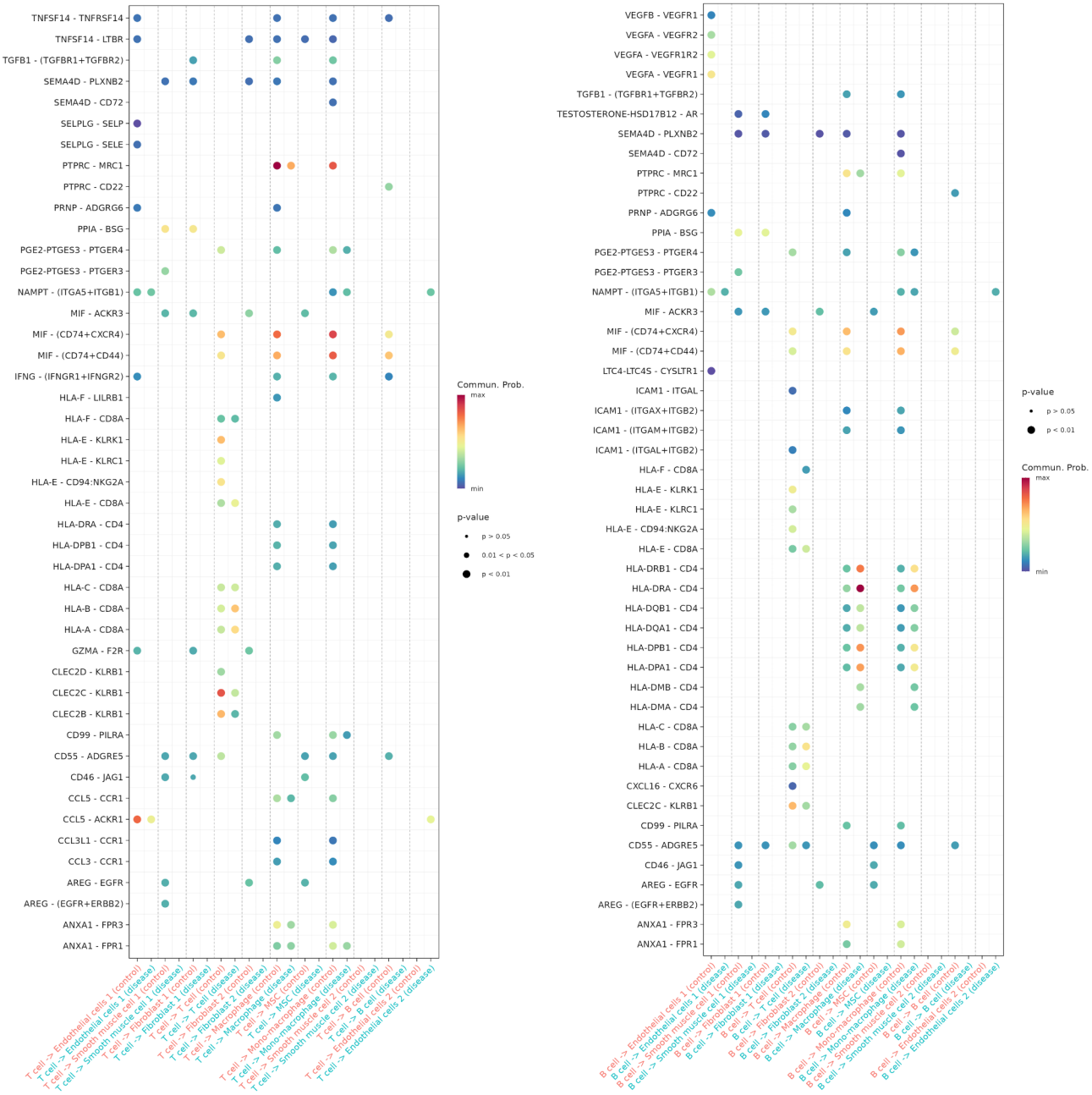


**Fig. 9** CellChat analysis of immune cell interactions in ascending aortic tissue

II (Sigma, V900892-100MG), Collagenase I (Sigma, V900891-100MG), and Collagenase IV (Sigma, C5138-500MG) for tissue digestion. Despite the differences in enzymatic protocols, endothelial cells remained the most abundant cell type in our samples, constituting 24.6% of the total cell population. Furthermore, our study broadened the cellular spectrum by identifying not only endothelial cells, smooth muscle cells, fibroblasts, mononuclear macrophages, and T cells but also uncovering the presence of B cells and mesenchymal stem cells (MSCs) within the aortic tissue. While these findings may be partially influenced by the specific enzymatic digestion protocols employed, they nevertheless highlight a more comprehensive and diverse cellular profile of the aortic tissue, offering new insights into its cellular composition and potential functional implications. In contrast to the single-cell sequencing results of renal arteries in TAK patients reported by Gao et al. [10], our study identified the presence of B-cell clusters within the aortic wall. Notably, the B-cell population was significantly larger than that observed in the control group. Specifically, we found the enrichment of pathways related to

antigen presentation and endoplasmic reticulum protein processing suggests that B cells in TAK patients may actively participate in immune responses and cellular stress responses within the aortic microenvironment, thereby driving disease progression. Arterial tertiary lymphoid organs (ATLOs) are indeed rare in normal aortas and medium-sized arteries, and their presence is typically associated with vascular lesions, as demonstrated by Kasashima et al. [11]. In the context of large vessel giant cell arteritis (LV-GCA), Graver et al. [12] have shown that the aorta exhibits substantial B-cell infiltration, particularly in the adventitia, where these cells organize into TLS containing germinal centers, proliferating B cells, and plasma cell niches. Furthermore, Srikakulapu et al. [13] have elucidated the functional role of ATLOs in upregulating B-cell genes (e.g., Cd19, Cd20, Cd79a/b, and Ighm) and recruiting diverse B-cell subsets, including IL-10(+) B-1b cells, which contribute to localized immune responses through the production of IgM and IgG. Our findings provide new insights into the functional heterogeneity of B cells in the aortic tissue of TAK patients, highlighting their potential roles in local immune activation and tissue remodeling, which may contribute to the pathogenesis of TAK. Single-cell and bulk RNA sequencing studies on TAK renal arteries samples also revealed a subset of M1-like macrophages with dual roles in tissue repair and extracellular matrix secretion, marked by IL1B expression. These macrophages exhibited a ferroptosis state characterized by PTGS2 upregulation, likely driven by IL1B-mediated NF-κB activation, which may contribute to the progression of inflammation [14]. Additionally, Matsumoto et al.'s whole-blood RNA sequencing highlighted the importance of IL-1 signaling in large vessel vasculitis, particularly in patients with poor prognosis [15].Our study also identified the NF-kappa B signaling pathway as the predominant pathway in macrophages based on KEGG analysis. In the peripheral compartment, Gao et al.'s single-cell RNA sequencing of PBMCs revealed increased $CD14^{+}$ monocytes and upregulation of inflammatory markers in surgical TAK patients [16]. These findings correlate with our identification of monocyte/macrophage activation signatures.

Our results are consistent with studies demonstrating the crucial role of vascular smooth muscle cells (VSMCs) in TAK pathogenesis. Yuqing et al. identified interleukin signaling and extracellular matrix organization as key pathways in abdominal aortic tissues, with particular emphasis on VSMC involvement in late-stage remodeling [17]. Tian et al. discovered that IL-6 induces VSMC senescence via STAT3-mediated mitochondrial dysfunction. This finding suggests that although vascular cells are primarily considered as targets of inflammatory damage, they can also serve as local initiators or amplifiers of inflammatory in vascular lesions [6]. Additionally, we identified antigen presentation-related pathways in VSMCs, suggesting their potential role in immune regulation. This finding highlights the need for further exploration into the immunomodulatory functions of VSMCs.

The methodological insights from these studies, particularly regarding tissue processing and cell type preservation, have informed our experimental approach. The consistency of findings across different vascular beds (renal, abdominal, and thoracic aorta) and between tissue and blood compartments strengthens the validity of key pathogenic mechanisms in TAK.

In our investigation, we attempted to classify CD4 + and CD8 + T cells using canonical markers (CD4, CD8A, and CD8B); however, we encountered significant challenges in achieving clear separation between these subsets in TAK aortic tissue. So a distinct population of cells with elevated IL7R expression was characterized as T cells. The cytokine IL-7 and its receptor, IL-7R, are pivotal for both naive and memory T cells [18]. Beyond IL7R, the T cells in our study exhibited heightened expression of additional genes, including CXCR4, TRBC2, and CCL5. Notably, CXCR4 is a chemokine receptor that binds to CXCL12, a process integral to retaining hematopoietic stem and progenitor cells within the bone marrow [19]. Deviating from traditional classification schemes for immune cell subsets, our study introduces a novel categorization, dividing T cells into four distinct subsets based on the aforementioned gene expressions. This technical difficulty contrasts with findings from reference [9], which successfully identified distinct CD4 + and CD8 + T cell populations in ascending aortic tissue from ascending thoracic aortic aneurysm patients. We believe this discrepancy highlights several important aspects of TAK pathophysiology: The unique phenotypic characteristics of tissue-resident T cells in TAK, which may differ substantially from both circulating T cells and aortic T cells in other diseases; Potential marker downregulation or altered expression profiles in TAK-associated T cells due to chronic inflammation and tissue remodeling; The distinct microenvironmental influences in TAK aortic tissue that may drive T cell adaptation and differentiation.

This innovative approach provides a more comprehensive and nuanced understanding of the cellular dynamics in TAK, offering new avenues for exploring the disease's immunopathogenesis and potential therapeutic targets.

This study employs single-cell sequencing to uncover a dichotomy in the regulation of genes associated with the complement activation pathway within the aortic tissues of TAK patients. Specifically, we observed an up-regulation in macrophages and fibroblasts, juxtaposed with a down-regulation in T cells. Concurrently, bulk RNA sequencing has demonstrated an up-regulation of complement signaling in peripheral blood CD4 + and CD8 + T cells in TAK patients when compared to control subjects.

The complement system, traditionally recognized as a component of the innate immune system, is now understood to play a pivotal role in modulating the adaptive immune response. It exerts its influence by regulating antigen-presenting cells and by activating B and T lymphocytes. Intriguingly, subcellular compartments such as the endoplasmic reticulum and lysosomes in human CD4 + T cells have been found to express low levels of C3, which can be intracellularly cleaved into bio-active fragments C3a and C3b. These fragments engage in crosstalk with complement receptor CD46CYT – 1, thereby mediating a series of genes that support the assembly and activation of the mammalian target of rapamycin (mTOR) pathway—a nutrient-sensing mechanism crucial for glycolysis, cell survival, and particularly for the demanding IFN-γ and Th1 responses [20].

The complement system's critical role is further underscored by instances of dysregulation observed in human immune-mediated diseases. For example, Anti-neutrophil cytoplasmic autoantibodies (ANCA)-associated vasculitis (AAVs) is characterized by C5a generation, which triggers neutrophil activation. The C5a inhibitor avacopan is thus considered a strategic therapeutic agent to mitigate glucocorticoid exposure in AAVs [21]. In CD4 + T cells of patients with rheumatoid arthritis and systemic lupus erythematosus (SLE), increased intracellular C3 activation and CD46 signaling are observed, promoting a hyperactive Th1 phenotype through heightened mTOR activity [22].

While the precise mechanism of complement involvement in the pathogenesis of TAK remains to be fully elucidated, recent clinical studies have identified complement components as novel biomarkers for TAK. X Luo et al. discovered up-regulation of complement C4a, C3c, C7, and complement factor H related protein-1 in active TAK patients using two-dimensional gel electrophoresis. Circulating levels of complement C4b binding protein (C4bp) were found to be higher in active patients compared to those in an inactive state, with inactive patients exhibiting higher C4b levels than healthy controls. The composite area under the curve (AUC) for C4bp outperformed that of ESR or CRP as a diagnostic indicator [23]. Elevated C3 levels have been found valuable for identifying active TAK, with the sensitivity and specificity further enhanced by combining it with CRP levels [24]. A prospective observational study indicated that in TAK patients treated with tocilizumab, those with higher baseline levels of complement 3 (C3, with a cut-off value of 1.22 g/L) were more susceptible to vascular stenosis progression (VSP) [25].

Our investigation has revealed intriguing findings within the aortic tissues of patients with TAK. Specifically, we observed a reduced proportion of Cluster 3 T cells, which are typically enriched in the complement pathway, in the TAK group. In contrast, the number of Cluster 4 macrophages, also complement pathway-enriched, was found to be elevated. Macrophages, as key players in both innate and adaptive immunity, are involved in processes such as phagocytosis and antigen presentation. Notably, Revel M et al. have identified a correlation between C1q + tumor-associated macrophages (TAMs) and T cell exhaustion across a spectrum of cancers [26].

Our study also detected enrichment of complement pathways in fibroblasts within the aortic wall of TAK patients. Fibroblasts, known for their role in tissue repair and remodeling, have been reported by Monteran L to exhibit complement signaling from cancer-associated fibroblasts (CAFs) that may lead to T cell dysfunction [27]. Furthermore, Friščić J et al. have demonstrated that the metabolic reprogramming of synovial fibroblasts depends on intracellular complement C3 and C3a receptor activation, which plays a role in inflammatory tissue priming in human arthritis [28].

A notable discovery was the opposing activation of the T cell complement pathway in peripheral blood versus aortic tissue. This divergence in activation patterns may mirror the distinct microenvironments and the unique roles these cells assume in local versus systemic immune responses. As we know, the complement system is primarily regulated post-translationally through proteolytic cleavage, and transcriptional changes in complement components may not directly reflect functional activity. However, transcriptional variations in complement components provide a broader perspective on potential regulatory changes at the mRNA level, which could still offer valuable insights into the overall immune response and signaling pathways involved. Transcriptional changes may reflect underlying regulatory mechanisms or cellular states that could influence complement system dynamics.

### Limitations

This study focuses on analyzing specimens from TAK patients with aortic dilation or aneurysms, as opposed to the more common presentation of arterial stenosis or occlusion. Tissue samples are typically obtained during aneurysm surgery, whereas patients with stenosis or occlusion often undergo vascular bypass or interventional procedures like balloon dilation or stenting, which do not allow for tissue collection. Control aortic tissues were carefully selected from our institutional database. To ensure data consistency, we implemented stringent batch-effect correction measures during post-processing, minimizing potential sequencing variations across batches and enhancing the reliability of our comparative analysis.

The notable difference in final cell numbers between groups in single-cell RNA sequencing likely reflects both

biological reality (increased cellularity in inflamed TAK tissue) and technical aspects of tissue processing. Nevertheless, we believe our data remain robust, as we have maintained appropriate sequencing depth and quality metrics across all samples.

While these databases provide valuable orientation tools, the enriched pathways identified in our analysis may only reflect partial functional overlaps with Takayasu arteritis (TAK) rather than directly elucidating its pathogenesis. For instance, pathways like leishmaniasis, tuberculosis, or VSMC contraction in white blood cells, though enriched, do not necessarily imply a direct mechanistic link to TAK. These findings highlight the need for cautious interpretation and further validation through multi-faceted approaches, including experimental studies, to rigorously establish their relevance to TAK's underlying mechanisms.

Investigating temporal changes in cell populations would provide valuable insights. However, our clinical samples, obtained during surgery, were restricted to a single time point, as repeated invasive sampling from the same patient is neither practical nor ethically feasible.

The complement pathway identified through KEGG analysis of peripheral blood cells warrants further investigation. While this study highlights its potential significance, comprehensive exploration of the functional roles of key genes, protein-level interactions, and ligand-receptor dynamics within the complement system remains to be addressed in future research.

## Conclusion

The findings underscore the active involvement of various immune and structural cells in the aortic tissues of TAK patients and reveal the presence of the complement signaling pathway in peripheral blood T cells. These insights are instrumental for identifying novel therapeutic targets and developing robust disease monitoring methods for TAK.

## Supplementary Information

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Supplementary Material 1

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**Author contributions**

Na Gao, Taotao Li and Yanqiu Guo wrote the main manuscript text.He Tang and Bokang Qiao prepared figures. Yi Yang, Honglei Zhao and Longfei Wang provided aortic tissue samplesLili Pan revises the article.All authors reviewed the manuscript.

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**Data availability**

No datasets were generated or analysed during the current study.

## Declarations

**Ethical approval**

This study was approved by the Medical Ethics Committee of Anzhen Hospital. Written informed consent for publication was obtained from the participant. All authors have critically reviewed and approved the final manuscript to be published.

**Competing interests**

The authors declare no competing interests.

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