Integrated Transcriptomic Analysis Revealed Lymphatic-mediated Immune Dynamics after Myocardial Infarction

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Abstract

Cardiac lymphatic vessels actively regulate immune responses after injury. Intercellular cell adhesion molecule-1 (ICAM-1), a cell surface glycoprotein, extensively expresses in endothelial cells and participates in inflammatory processes by regulating leukocyte recruitment from circulation. However, the specific role of lymphatic endothelial cells (LECs) *Icam1* in regulating the immune microenvironment associated with myocardial infarction (MI) remains elusive. In this study, we generated an LEC-specific *Icam1* conditional knockout (cko) model and performed single-cell RNA and spatial transcriptome sequencing to evaluate the cellular dynamic features at 3-, 7-, 21-, and 42- days post-MI. Our results showed that the deletion of *Icam1* in LECs led to increased inflammation and fibrosis, indicating aggravated cardiac damage. We identified alterations in cell type-specific proportions and molecular signatures in *Icam1*cko group. Notably, the activation of neutrophil and macrophage subsets with pro-inflammatory properties are key features in *Icam1*cko group. Cellular interaction analysis revealed that *Icam1* knockout might obstruct immune cell drainage via LEC-specific ICAM signaling. Additionally, accumulated pro-inflammatory cell populations further attracted immune cells via CXCL crosstalk, resulting in the further destruction of immune microenvironment in *Icam1*cko hearts. Collectively, our findings indicate that LEC *Icam1* might be a potential therapeutic target for improving post-MI outcomes.

Keywords: Myocardial infarction; Lymphatic endothelial cells; *Icam1;* Single-cell RNA sequencing; Spatial transcriptome sequencing

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INTRODUCTION

Myocardial infarction (MI) remains the leading cause of morbidity and mortality worldwide (Heusch et al., 2014). The ischemic insult results in widespread cardiomyocyte death, initiating a cascade of inflammatory responses and fibrotic remodeling (Prabhu and Frangogiannis, 2016; Weil and Neelamegham, 2019). Persistent inflammation and excessive fibrosis contribute to adverse cardiac remodeling and are key drivers in the progression to heart failure (Heusch and Gersh, 2017).

Recent studies have highlighted the critical role of the cardiac lymphatic vasculature in modulating immune responses and facilitating tissue repair after MI. Lymphatic remodeling occurs in both infarcted and peri-infarct regions in human and animal models (Houssari et al., 2020). A key function of cardiac lymphatics is to promote the resolution of inflammation through the drainage of immune cells and interstitial fluid (Vieira et al., 2018), making them an emerging therapeutic target for limiting post-MI edema and chronic inflammation.

In addition to fluid clearance, lymphatic endothelial cells (LECs) actively participate in shaping immune cell behavior. Lymphangiogenesis is initiated within 3–4 days post-MI, coinciding with the peak infiltration of innate immune cells (Klotz et al., 2015; Wang et al., 2023). Immunostaining studies have demonstrated colocalization of newly formed lymphatic vessels and myeloid cells, implicating a role for LECs in modulating local immune activity (Vieira et al., 2018). Experimental promotion of cardiac lymphangiogenesis via vascular endothelial growth factor (VEGF) delivery enhances lymphatic expansion and reduces leukocyte accumulation, ultimately improving cardiac function (Dieterich et al., 2014; Vieira

et al., 2018; Vivien et al., 2019). Collectively, these findings underscore the importance of lymphatic-immune interactions in post-infarction remodeling (Bråkenhielm et al., 2021; Henri et al., 2016).

Intercellular adhesion molecule 1 (ICAM1) is a well-characterized adhesion receptor expressed on endothelial surfaces, where it mediates leukocyte adhesion and trans-endothelial migration (Bui et al., 2020). ICAM1 is involved in diverse inflammatory processes, including leukocyte trafficking, effector activation, and tissue clearance (Lawson and Wolf, 2009). Our previous work identified *Icam1* as a direct transcriptional target of Tbx1 that mediates recruitment of immunosuppressive regulatory T cells (Tregs) in the infarcted heart (Wang et al., 2023). Notably, conditional deletion of *Icam1* led to aberrant expansion of cytotoxic $CD8^+$ T cells at 7 days post-MI, suggesting a role in immune resolution. However, the broader role of lymphatic *Icam1* in orchestrating immune cell dynamics during cardiac remodeling remains poorly defined.

In this study, we generated lymphatic-specific *Icam1* conditional knockout (*Icam1*cko) model by crossing $Icam1^{flox/flox}$ with $Prox1^{CreERT2}$ mice, and induced recombination by tamoxifen administration one week prior to MI. To comprehensively characterize the temporal and spatial immune landscape, we performed single-cell RNA sequencing (scRNA-seq) and spatial transcriptomic profiling (ST-seq) of cardiac tissue at 3-, 7-, 21-, and 42-days post-MI (dpMI). Our findings demonstrate that deletion of *Icam1* in LECs exacerbates inflammation and fibrosis, and perturbs the immune microenvironment through both direct LEC–immune cell interactions and altered inter-immune cell crosstalk. These results uncover a critical role

for lymphatic *Icam1* in shaping immune responses and tissue remodeling in the post-infarct heart, providing new insights into therapeutic strategies targeting LECs in cardiovascular disease.

MATERIALS AND METHODS

Animals

All mouse experiments were performed according to the guidelines of the Ethics Committee of BGI (permit No. BGI-IRB 20A22037), and the Institutional Animal Care and Use Committee of Shanghai Children's Medical Center affiliated to Shanghai Jiao Tong University School of Medicine. Mice used in this study were housed under specific pathogen-free conditions at the animal facility of Shanghai Children's Medical Center. Strains used were *Prox1*^{CreERT2} (Bazigou et al., 2011) and *Icam1*^{flox} (Xu et al., 2004) on the C57BL/6 background. Experiments were performed on both male and female mice between 8 and 12 weeks of age. *Prox1*^{CreERT2}; *Icam1*$^{flox/+}$ mice were bred with *Icam1*$^{flox/flox}$ mice to generate *Prox1*^{CreERT2}; *Icam1*$^{flox/flox}$ as *Icam1* conditional knockout (*Icam1*cko), and *Icam1*$^{flox/flox}$ was used as control. To activate recombination, we injected tamoxifen at 1week before MI surgery for both control and *Icam1*cko mice.

Establish myocardial infarction model

Myocardial infarction was induced via the left anterior descending (LAD) coronary artery permanent ligation as previously described (Wang et al., 2023). Briefly, animals were anesthetized with 3%-5% isoflurane. A small skin incision was made at the fourth intercostal space on the left side of the sternum to expose the heart, and the left coronary artery was ligated with nylon thread. The heart was subsequently replaced into the thoracic cavity, and the chest closed. Sham animals have only opened the chest, except for the coronary ligation.

Mice in the MI groups were euthanized by CO_2 inhalation and hearts were harvested 3, 7, 21, and 42 days after surgery, respectively.

Single-cell RNA sequencing

Heart tissues were collected from the infarct region of the left ventricle at 3, 7, 21, and 42 dpMI. Single-cell suspension was prepared as previously described (Han et al., 2022). For each experimental condition, heart tissues were first rinsed and minced into 1 mm pieces on ice, then the minced tissues were pooled and digested in tissue dissociation media (0.1% Collagenase with 2.5 U/ml Dispase II, 9:1 in volume) for 40 minutes in 37 °C water bath, pipetted every 5 minutes, to ensure an adequate number of cells and a robust representation of cardiac cell types. Single-cell suspension was passed through a 40 μm filter. Cells were stained with APC Rat anti mouse CD45 (BD Pharmingen, 559864), incubated with Anti-APC microbeads (MACS,130-090-855). The cDNA synthetization, amplification and library construction steps were performed based on a standard protocol. Libraries were sequenced on DNBSEQ-T1 sequencers.

Spatial transcriptome sequencing and processing

We used the Stereo-seq platform for the spatial transcriptomics experiments, and the Stereo-seq procedures were prepared as previously described (Chen et al., 2022). Briefly, heart samples below the surgical cross-section were removed and rinsed with cold PBS. Then, the tissues were immediately snap-frozen with precooled TissueTek OCT in dry ice. The OCT blocks were stored in a -80 °C refrigerator before further operation. First, we performed quality control according to RNA integrity number (RIN) before the experiment begins. All

samples were required to have an RIN greater than 7 for the transcriptomic study. Subsequently, the OCT-embedded mouse heart was cryosectioned into 10 μm thick sections in a Leica CM1950 cryostat. We adhered the tissue section of each sample to the surface of Stereo-seq chip after cryosection, incubated at 37 °C for 5 minutes, and then were fixed in 20 °C methanol for 40 min. The sections on chips were stained with nucleic acid dye for ssDNA visualization according to the manufacturer's guidelines. Images of ssDNA were acquired with a Ti-7 Nikon Eclipse microscope. For permeabilization, the tissue sections placed on the chip were permeabilized using 0.1% pepsin (Sigma, USA) in 0.01 mol/L HCl buffer at 37 °C for 18 min and then washed with 100 mL 0.13 saline-sodium citrate (SSC) buffer supplemented with 0.05 U/mL RNAase Inhibitor. mRNAs captured by DNBs on the chip were reverse transcribed at 42 °C overnight. The chips were washed twice with 0.1 × SSC after in situ reverse transcription and incubated in tissue removal buffer at 37 °C for 30 minutes. The chips were finally washed once with 0.1 x SSC after the tissue removal buffer was removed, and then the chips were immersed in the cDNA Release Mix overnight at 55 °C to release the cDNA. The released cDNAs on the chip were further fragmented, amplified, and purified to generate a DNA nanoball (DNBs) library according to the manufacturer's protocol (Liu et al., 2019). Final DNBs were loaded into the patterned Nano arrays and sequenced on the MGI DNBSEQ-Tx sequencer (MGI, China).

ScRNA-seq data analysis

Raw sequencing data processing (alignment, filtering, and UMI counting) was performed using CellRanger toolkit (v2.1.0.) (Zheng et al., 2017). The cDNA reads were mapped with a

mouse genome reference (mm10). The public raw count matrices from each dataset included in this analysis were downloaded from the GEO database. We convert count data into Seurat objects using R package Seurat (v4.0.2) (Butler et al., 2018). For quantity filtering, dead cells and low-expression genes were identified respectively by high mitochondrial percentage (> 15%) and low gene/feature counts per cell (< 5) and filtered out. The cells that expressed less than 200 unique genes or 500 UMIs were removed. Then, Lognormalize with scale factor = 10,000 was performed on all cells to eliminate the influence of technical factors such as sequencing depth. In different batches of integrated data, "FindVariableFeatures" function in Seurat was used for feature selection, and 2000 highly variable genes were selected for downstream analysis. The integrated cells were constructed in the top 50-dimensional PCA space to construct a KNN graph performed Louvain clustering with the "FindClusters" function and visualized the data using UMAP dimensionality reduction. After confirming the existence of the batch effect, we used Harmony (v1.2.0) (Korsunsky et al., 2019) to integrate the data of different batches and re-clustered and visualized. We annotate the obtained cell clusters by combining the reported cell markers and the cluster markers identified under the default parameters of "FindAllMarkers". For the cell subpopulations, we perform the above steps under the selected main cell cluster.

Spatial transcriptome sequencing data analysis

The raw Fastq data was processed according to the method described in the Stereo-seq paper (Chen et al., 2022). CID sequences(1-25bp) were first mapped to the designed coordinate, which allows 1 base mismatch due to PCR errors. Reads containing MID sequences with low

quality (either N or more than 2 bases with quality score < 10) were filtered out. Retained reads were then aligned to the reference genome STAR. Finally, the information above were used to generate a CID-containing expression profile matrix. MID counts and CIDs of 50 × 50 DNBs were merged as one bin50. Then the new expressing profile was used to obtain spatial coordinates-genes expression matrix for subsequent analysis. We convert the raw expression matrix into Seurat objects using R package Seurat (Butler et al., 2018) (v4.0.2) function "CreateSeuratObject". The samples were filtered by gene number, MID counts (nUMI_counts), and mitochrondial percentage (mt_counts < 15%) of each cellbin (nFeature_RNA > 200, nCount_RNA > 500). The filtered spots were normalized using Lognormalize function.

Cell segmentation and cell type deconvolution

First, the StereoCell (Li et al., 2023b) Python package was used for cell segmentation and cell correction of the Stereo-seq data. Unique molecular identifiers from all DNBs in the same putative single cell (referred to as a cellbin) were aggregated, representing the transcriptomic profile for downstream analyses. Next, we implemented cell-type identification of cellbin by integrating it with our scRNA-seq raw expression matrix using Cell2location (v0.1.3) (Kleshchevnikov et al., 2022). The scRNA-seq data was trained as the reference data using a negative binomial regression model. Taking the reference data as input, we fitted a cell2location model with hyperparameter N_cells_per_location = 1, detection_alpha = 20. The training was stopped after the cell2location model converged. The cell type with the greatest cell abundance in each cellbin was identified as the cell type of that cellbin.

Differential analysis and enrichment analysis

The "FindMarkers" function in Seurat was used for differential expression analysis across distinct experimental conditions, cell types or cell subtypes of interest by comparing average transcript levels in each group cells with another group cells. For downstream analysis, those genes that satisfied the min.pct 0.1 and logfc threshold 0.5 were retained. Genes at an adjusted p value (Wilcoxon test) < 0.05 were considered as differentially expressed gene (DEGs). The DEGs were further divided into up- and down-regulated groups for Gene Ontology (GO) enrichment analysis by the enrichGO function in clusterProfiler (v4.10.0) (Yu et al., 2012) R package and pathways with q-value <0.05 in the Benjamin-Hochberg (BH) for multiple test correction were shown.

Scoring of signature gene sets

Gene signature scores were calculated using the "AddModuleScore" function in Seurat (v4.0.2) (Butler et al., 2018). "AddModuleScore" was performed using with default parameters. Functional signatures were collected from the Gene Ontology database. All signature gene sets were listed in Supplementary Table S1. Statistical analysis was performed by a two-sided Wilcoxon rank sum test followed by BH correction.

Construction of co-expression modules

We used high-dimensional weighted gene co-expression network analysis (hdWGCNA) (v0.3.2) (Morabito et al., 2023) package to perform co-expression network analysis on genes expressed in neutrophils. "TestSoftPowers" function was utilized to perform parameter sweep. Further, the weighted gene co-expression network was constructed by calculating

pairwise correlations between these genes using “ConstructNetwork” function. Gene modules were identified using hierarchical clustering and the clustered dendrogram was visualized with “PlotDendrogram” function. The module eigengenes were computed with “ModuleEigengene” function to identify the expression patterns of co-expression modules. Module hub genes within each module were identified based on their module membership and gene significance.

Inference of ligand-receptor interactions

To investigate the predict ligand-mediated regulation of gene expression in fibroblasts after MI, we applied the NicheNet (v2.2.0) (Browaeys et al., 2020) algorithm to our single-cell RNA-seq data. We identified DEGs between fibroblasts from MI and healthy samples, with the criteria mentioned above, which were used as target gene sets for subsequent NicheNet analysis. Macrophage subtypes were defined as sender cells based on tissue localization and hypothesized interactions under pathological conditions. Visualization of top-ranked ligand-target interactions was carried out using customized dot plots and heatmaps in R.

To investigate the changes in the intercellular interactions in different groups, the LEC and prominent immune cell types affected by MI were selected. The standard CellChat (v1.6.0) (Jin et al., 2021) pipeline was then applied to the selected subset data with normalized gene expression along with cell annotation metadata. The probability of ligand-receptor interactions was inferred by integrating gene expression with prior knowledge of the interactions between signaling ligands, receptors, and their cofactors.

Cell type spatial proximity analysis

To assess spatial proximity between two cell types (A and B), we conducted a neighborhood enrichment analysis based on previously described strategies (Browaeys et al., 2020). For each cell type A cell, we counted the number of cell type B cells within a 75 μm radius. To establish a null model, cell coordinates were randomly permuted while maintaining cell type identities, and cell type B cell counts within the same radius were recalculated. This randomization was repeated 1,000 times. Enrichment was defined as the log-ratio between the observed and mean permuted counts. Significance was assessed by computing a Z score based on the null distribution.

Spatial co-expression analysis using bivariate Moran's I

To quantify the spatial co-localization between fibroblasts/macrophages and neutrophils and two biological processes (ECM remodeling and inflammatory response), we computed spatial scores for each spot based on curated gene sets using the scanpy.tl.score_genes() function. We then employed the bivariate Moran's I statistic to evaluate local spatial associations between cell-type abundance and gene signature scores across tissue sections. This analysis was performed using the esda.moran.Moran_Local_BV() function from the esda Python package (PySAL library), which calculates the degree of spatial correlation between two variables at each location, while accounting for spatial neighborhood structure defined via a K-nearest neighbors graph (k=6). High-high clusters in the resulting Moran's I maps indicate spatial regions where both cell abundance and functional score are jointly elevated, suggesting local co-enrichment of cell type and biological activity. Statistical significance

was determined using permutation tests (n = 999), and clusters with adjusted p-values < 0.05 were retained for interpretation.

Statistical analysis

Statistical analyses were performed using the R software (version 3.6.1). Wilcoxon rank-sum test and Student's t-test were used in this study. *P < 0.05, **P < 0.01, and ***P < 0.001 were considered statistically significant. Data are presented as mean ± standard error of the mean (SEM).

RESULTS

Cellular landscape of WT and *Icam1*cko mouse MI hearts

To gain insights into the cellular and molecular changes of *Icam1* loss in acute and chronic stages of MI, we collected ventricular tissue and obtained nonmyocytes from wild-type (control) and *Icam1*cko mice at 3, 7, 21 and 42 dpMI, and performed scRNA-seq and stereo-seq (Figure 1A). For scRNA-seq, after rigorous quality control, we obtained 47,751 and 43,793 cells from control (ctrl) and *Icam1*cko samples, respectively. The average number of unique molecular identifiers (UMI) and gene counts per preparation were 4,664 and 1,477, respectively (Supplementary Figure S1A; Supplementary Table S2).

After unsupervised clustering analysis, was performed to dissect cardiac cellular compartments (Supplementary Figures S1B-D). 12 major cell types were identified based on the expression of canonical marker genes (Figure 1B; Supplementary Figure S1E) and the differential expression genes (DEGs) (Supplementary Table S3), including endothelial cells (EC) (*Fabp4* and *Pecam1*), lymphatic endothelial cells (LEC) (*Ccl21a* and *Mmrn1*), fibroblasts (FB) (*Col3a1* and *Dcn*), cardiomyocytes (CM) (*Tnnt2 and Actc1*), mural cells (*Myh9* and *Notch3*), proliferating (*Stmn1*, *Hmgb2* and *Top2a*), macrophages (Mac) (*Lyz2* and *C1qa*), neutrophils (Neu) (*S100a9* and *S100a8*), dendritic cells (DC) (*Cd209a* and *Napsa*), T cells (*Cd8a* and *Cd3d*), B cells (*Cd79a* and *Bank1*), neuronal cells (NC) (*Apod* and *Plp1*), and erythroid cells (Ery) (*Hbb-ba*). ECs were the numerically dominant nonmyocyte type, and the immune cell populations were highly dynamic (Figure 1C). We leveraged the *Prox1*^{CreERT2} driver, which is largely restricted in LECs (Supplementary Figure S1F). *Icam1* expression

was significantly reduced in LECs, while remaining unchanged in other cardiac cell types, confirming that the deletion was largely restricted to the LECs population (Supplementary Figure S1G). Neutrophils were the first to infiltrate the heart at 3 dpMI, followed by dramatic expansion in the macrophage and monocyte population, which was most abundant immune subset. By 42 dpMI, a substantial increase in fibroblast abundance was observed (Figure 1C). To evaluate the overall similarity between control and *Icam1*cko samples post-MI, we computed Pearson correlation coefficients based on the integrated scRNA-seq profiles. Samples from the same time points exhibited strong correlations across genotypes, indicating broadly preserved transcriptomic landscapes (Figure 1D). To identify global transcriptomic alterations induced by the *Icam1* deficiency across different stage, we carried out differential expression gene analysis by aggregating cells into a pseudo-bulk representation between control and *Icam1*cko samples. The top differential expressed genes for each time point were shown in Supplementary Figure S1H and Supplementary Table S4. To gain insight into the biological processes associated with these transcriptional changes, Gene Ontology (GO) enrichment analysis was performed. At 3dpMI, upregulated genes in *Icam1*cko hearts were significantly enriched in immune-related processes, such as leukocyte migration and cytokine-mediated signaling. By 42 dpMI, genes involved in extracellular matrix organization were upregulated, indicating enhanced fibrotic remodeling, while angiogenesis-related pathways were notably downregulated (Figure 1E).

Pathway analysis revealed early activation of VEGF and kinase/signal transducer and activator (JAK-STAT) signaling following MI, implicating their involvement in the initiation of angiogenic and inflammatory responses (Figure 1F). Notably, the expression of *Cd93*, a

key marker of angiogenesis, was significantly higher in the control group than in the *Icam1*cko group at early time points (Figures 1F, G), suggesting impaired vascular remodeling upon *Icam1* deletion. In parallel, increased activity of extracellular matrix (ECM) remodeling and apoptosis pathways was observed, consistent with cardiac injury and cell death post-MI, as previously described (Epelman et al., 2015). At the late stage, fibrosis-associated genes, including *Col3a1*, *Col1a1*, and *Bgn*—were markedly upregulated in *Icam1*cko hearts, indicative of exacerbated fibrotic remodeling and compromised cardiac function. (Figures 1F, G).

Effects of *Icam1* loss on the composition and transcriptional landscape at single cell resolution

Cell type–specific differential gene expression analysis revealed substantial transcriptional alterations between control and *Icam1*cko groups, particularly in fibroblasts, mural cells, and immune cell subsets (Supplementary Figure S2; Supplementary Table S5). Gene set scoring further demonstrated significant enrichment of inflammatory response signatures in neutrophils and macrophages, while fibroblasts exhibited elevated ECM-related activity (Figure 2A), underscoring the critical roles of these cell types in mediating immune activation and tissue repair following MI. Furthermore, spatial mapping revealed that neutrophils and macrophages were predominantly localized to regions with high inflammatory response scores, while fibroblasts were enriched in areas with elevated ECM scores (Figure 2B). Spatial co-localization between the two signals was further evaluated using bivariate Moran's

I analysis, that reinforced the functional specialization of these cell types in post-infarction pathology.

In *Icam1*cko hearts, we observed aberrant cellular dynamics, including excessive neutrophil infiltration at 3 dpMI and increased T cell accumulation at 7 dpMI, suggesting an exacerbated and prolonged inflammatory phase. At 42 dpMI, fibroblasts exhibited significant expansion, consistent with enhanced fibrosis and impaired cardiac repair at the late stage (Figure 2C).

To further delineate the molecular alterations driven by *Icam1* deficiency, we performed a time-resolved transcriptomic analysis across four post-MI time points (Figure 2D; Supplementary Table S6). Notably, neutrophils, macrophages, and fibroblasts exhibited distinct temporal transcriptional programs (Figure 2E). For instance, neutrophils in *Icam1*cko hearts upregulated genes involved in chemotaxis, activation, and intrinsic apoptotic signaling pathways during the early phase, whereas fibroblasts showed enrichment in gene sets related to cell proliferation and extracellular matrix organization at later stages (Figure 2F). These findings highlight the celltype–specific and time-dependent effects of *Icam1* deletion on post-MI remodeling.

The deletion of LEC-specific *Icam1* enriched activated neutrophil subtypes to aggravate inflammation

Neutrophils are the first innate immune responders recruited to sites of myocardial injury, playing a critical role in the early inflammatory phase following MI (Vafadarnejad et al., 2020). At 3 dpMI, we observed a marked increase in neutrophil abundance *in Icam1*cko hearts, accompanied by significant transcriptomic alterations suggestive of a shift in cellular

state (Figure 3A; Supplementary Figure S2). Neutrophils were predominantly present at this early stage, with minimal representation at later time points, a trend corroborated by spatial transcriptomics data (Figure 3B).

To further examine the effect of LEC-specific *Icam1* deletion on neutrophil populations, we integrated our data with publicly available scRNA-seq profiles of cardiac CD45$^+$ immune cells spanning multiple post-MI time points (Jin et al., 2022; Jung et al., 2022). Six transcriptionally distinct neutrophil subpopulations were identified (Figure 3C; Supplementary Figure S3A), with the subclusters being predominantly enriched at the early phase (Supplementary Figure S3B), suggesting a transient activation pattern following MI. Among these, Neu1, marked by *Cxcl3* expression—dominated early after MI and is implicated in neutrophil mobilization (Eash et al., 2010), suggesting an early pro-inflammatory response. Neu2 and Neu4 expressed interferon-stimulated genes (*Ifitm1*, *Ifitm2*, *Ifit1*, *Cxcl10*), indicating a more immune-modulatory profile. Neu5 showed increased mitochondrial gene expression. Neu6, enriched in *Cd177* and *Retnlg*, exhibited transcriptional signatures associated with neutrophil activation and degranulation (Figure 3D). Spatial transcriptomic analysis further confirmed elevated *Cd177* expression in *Icam1*cko at 3 dpMI (Figure 3E). Given that CD177$^+$ neutrophils were related to the neutrophil extracellular traps (NETs) formation—known contributors to tissue injury in inflammatory heart diseases (Li et al., 2023a; Rivadeneyra et al., 2018). Consistently, the abundance of Neu1, Neu4, and Neu6 subpopulations was moderately increased in *Icam1*cko samples (Figure 3F; Supplementary Figure S3C), indicating an exacerbated and dysregulated neutrophil response.

To elucidate neutrophil functional programs following MI, we applied hdWGCNA to construct a neutrophil-specific co-expression network (Morabito et al., 2023). This analysis identified 7 co-expression gene modules with tightly correlated expression patterns (Figure 3G; Supplementary Figures S3D, E). Module (M) 3, M4, M5 and M7 showed significantly differentiation between control and *Icam1*cko samples (Figure 3H). Among them, M3, which contained inflammation and integrin-related genes (for example, *Cxcl3*, *Itgam*, *Cd177*, *Thbs1,* and *Cd14*), was significantly upregulated in *Icam1*cko samples (Figures 3G, H). GO analysis of M3 genes revealed enrichment in neutrophil migration, myeloid leukocyte activation, cell adhesion, and cytokine production pathways (Figure 3I). M4 was redundant with inflammation-related pathways (e.g., cytokine production and toll-like receptor signaling pathway) and M7 was enriched in ribosome-related pathways (Figure 3I).

Module expression score result showed that M3 highly expressed in Neu1, Neu4, and Neu6 (Figure 3J), indicating its gene program was active in these neutrophil subtypes. Meanwhile, the neutrophil activation scores increased drastically in *Icam1*cko group, especially in Neu1, Neu4 and Neu6 (Figure 3K). Neutrophils have long been considered harmful in tissue damage due to their hallmark functions, including the secretion of proinflammatory cytokines, the formation of NETs, and the generation of reactive oxygen species (ROS) (Yang et al., 2025). In line with this, we observed elevated NETs signature scores in Neu6 of *Icam1*cko mice (Figure 3L). These results indicated the imbalance within the neutrophil subpopulations in *Icam1*cko group may aggravate pro-inflammatory properties and cardiac injury at the early stage of MI.

Inflammatory macrophages drive the regulation of post-MI cardiac fibrosis

Macrophages represented the most abundant and functionally diverse immune cell population in the post-MI heart (Jin et al., 2022; Rizzo et al., 2023). Based on DEG signatures, we identified seven macrophage subpopulations, encompassing all major macrophage types present in integrated reference datasets (Figure 4A; Supplementary Figures S4A, B). These subpopulations exhibited dynamic temporal shifts after MI (Supplementary Figure S4C). By 21 and 42 dpMI, macrophage compositions in both control and *Icam1*cko hearts largely resembled those of sham-operated controls (Figure 4B), suggesting a gradual return to immune homeostasis.

We annotated monocytes (Mono) by high *Ly6c2* and *Plac8* expression, which peaked at 1 dpMI. Mac1 was enriched in lipid metabolism genes (*Gpnmb*, *Fabp5*), while Mac2 displayed elevated MHC II-related genes. Mac3 expressed tissue-resident macrophage markers (*Lyve1*, *Cd163*). In contrast, Mac4 and Mac5 were characterized by chemokine and interferon-stimulated gene signatures, respectively, and both expressed high levels of *Ccr2* and *Ly6c2*, indicative of monocyte-derived inflammatory phenotypes (Supplementary Figure S4D) (Bajpai et al., 2019; Epelman et al., 2014). These subtypes were dominant in early post-MI stages and declined over time (Supplementary Figure S4C). To further define their functional roles, we assessed inflammatory gene signatures across macrophage subtypes. Inflammatory scores were highest in Mac4-6 and monocytes, classifying them as "inflammatory macrophages/monocytes," in contrast to the relatively quiescent Mac1-3 populations (Supplementary Figure S4E). Notably, the proportion of inflammatory

macrophages was moderately elevated in *Icam1*cko hearts (Supplementary Figure S4F), suggesting a polarization shift toward a pro-inflammatory state.

We observed that Mac4 exhibited markedly higher expression of pro-inflammatory cytokines, including *Ccl2*, *Ccl3*, and *Cxcl3*, compared to other macrophage subsets (Figure 4C). This subset was significantly expanded in the *Icam1*cko group (Figures 4D, E), and showed the largest number of DEGs (Figure 4F). Notably, *Il6*, *Csf3*, and *Il1a*—key drivers of inflammatory cascades—were robustly upregulated in Mac4 following *Icam1* deletion. GO enrichment analysis of Mac4-specific DEGs revealed strong associations with interleukin-1 signaling, cytokine activity, and T cell activation (Supplementary Figure S4G). Collectively, these findings identify Mac4 as a dominant pro-inflammatory population whose expansion and transcriptional reprogramming under *Icam1* deficiency likely contribute to persistent inflammation and adverse remodeling after MI (Figure 4G).

Recent studies in small animals have shown that macrophages can promote cardiac fibrosis and diastolic dysfunction (Hulsmans et al., 2018). To investigate fibroblast alterations after MI, we integrated two reference datasets from healthy or sham-operated hearts (Farbehi et al., 2019; Skelly et al., 2018) with our MI fibroblast dataset. Compared to fibroblasts from healthy hearts, those from MI hearts exhibited transcriptional upregulation of genes associated with apoptosis, inflammation, and extracellular matrix organization, including *Cxcl2*, *Ccl2*, *Fn1* and *Col1a2* (Supplementary Figures S4H, I). Spatial transcriptomic deconvolution revealed close physical proximity between macrophages and fibroblasts within infarcted regions, which were determined based on additional H&E staining of adjacent

tissue sections, showing clear signs of immune infiltration and fibrotic change, indicating potential cellular interactions (Figures 4H, I). To further explore macrophage–fibroblast crosstalk, we employed NicheNet (Browaeys et al., 2020) to infer ligand–receptor interactions enriched in MI hearts relative to healthy controls. Candidate fibroblast target genes were selected from upregulated DEGs post-MI, with an emphasis on those responsive to macrophage-derived ligands. Prior interaction potential analysis identified *Il1a* and *Il1b* as top-ranking macrophage-derived ligands with strong predicted regulatory effects on fibroblast transcriptional programs (Figure 4J). Both *Il1a* and *Il1b* were primarily expressed in macrophages, and their expression was markedly elevated in *Icam1*cko samples (Figure 4K). Notably, IL-1β signaling has been shown to mediate interactions between $CCR2^{+}$ macrophages and fibroblasts, promoting the emergence of $FAP/POSTN^{+}$ profibrotic fibroblasts (Amrute et al., 2024). In conclusion, these results suggest that inflammatory macrophage subtypes, especially Mac4, may drive the regulation of cardiac fibrosis via *Il1a* and *Il1b* after MI.

Altered cellular crosstalk related to LECs between control and *Icam1*cko groups

Post-MI LECs in the *Icam1*cko group exhibited reduced angiogenesis scores and elevated inflammatory scores, suggesting a shift in LECs functional state following *Icam1* deletion (Figures 5A, B). To further investigate the role of LECs in modulating the inflammatory immune microenvironment, we employed the CellChat framework (Jin et al., 2021) package to quantify intercellular communication between LECs and myeloid populations, specifically macrophages and neutrophils, under different conditions. Compared with control, *Icam1*cko

samples showed a reduction in both the number and overall strength of ligand–receptor interactions, indicating impaired LEC–immune cell communication (Figure 5C; Supplementary Figure S5A). To further investigate alterations in cell–cell communication between control and *Icam1*cko hearts, we compared signaling pathway activity originating from LECs across conditions. This analysis revealed a marked suppression of the ICAM signaling pathway, while CCL and CSF family receptor-ligand interactions increased in *Icam1*cko mice (Figure 5D; Supplementary Figure S5B). Specifically, the disrupted ligand-receptor interaction in *Icam1*cko involved *Icam1* and various members of the integrin family, such as *Icam1-(Itgam+Itgb2)*, *Icam2-(Itgam+Itgb2)* and *Icam1-Itgal* pairs (Figure 5E). Consistent with these findings, scRNA-seq data confirmed the expected downregulation of *Icam1* and *Icam2* in LECs from *Icam1*cko mice. This suggests that LEC-specific *Icam1* deletion may destabilize a broader adhesion signaling network, potentially affect other ICAM family members. Conversely, *Itgam*, *Itgb2* and *Itgal* were significantly increased in inflammatory macrophages (Mac4, Mac5, Mono and Mac6) and activated neutrophils (Neu1, Neu4 and Neu6) (Figure 5F; Supplementary Figures S5C, D), suggesting a reduction in adhesive interactions between inflammatory immune cells and LECs due to impaired ICAM–integrin signaling. As expected, the expressions of *Ccl2* and *Csf3* were upregulated in LECs from *Icam1*cko mice, indicating an increase in the proinflammatory microenvironment.

Enrichment of the LECs marker gene *Ccl21a* was observed in the subepicardial region (Figure 5G), consistent with the known distribution of LECs (Brakenhielm and Alitalo, 2019). To further investigate the spatial organization of LECs and immune cells, we calculated the distance between *Ccl21a*-positive spots and immune cells. Spatial mapping revealed that, in

control samples, inflammatory cells such as neutrophils and macrophages were spatially segregated from LEC-rich regions, whereas in *Icam1*cko hearts, these immune populations showed increased colocalization with LECs (Figure 5H). Moreover, the LEC-enriched regions in *Icam1*cko hearts exhibited elevated expression of inflammatory genes, suggesting local immune amplification (Figure 5I). These spatial transcriptomics analyses further support the notion that LEC-expressed *Icam1* may contribute to inflammatory immune cell clearance during post-MI resolution.

Furthermore, when inflammatory cell types (Neu6 and Mac4) act as senders, we identified upregulated TNF and CXCL family receptor-ligand pairs targeting multiple cell types in *Icam1*cko hearts (Figure 5J). Notably, the main contributing ligand *Tnf* and *Cxcl2* were significantly upregulated in inflammatory immune subsets within the *Icam1*cko group (Figure 5K; Supplementary Figures S5E, F). These chemokines, known to potently recruit neutrophils and monocytes, are associated with exacerbated inflammation and impaired cardiac function (Lindsey et al., 2019). This suggests that enhanced expression of these ligands contributes to the amplification of a pro-inflammatory microenvironment in the absence of *Icam1*. Altogether, these results highlight the critical role of LEC-specific *Icam1* in promoting the reflux of inflammatory cells and shaping immune microenvironment in the pathological process of MI.

DISCUSSION

Lymphatic drainage is essential to maintain tissue fluid balance under various homeostatic and pathophysiological conditions (Mikhael and Khan, 2020). However, a comprehensive atlas of the temporal-spatial role of LECs throughout cardiac remodeling stages is still lacking. Our previous study found that transcription factor *Tbx1* mediates neo-lymphangiogenesis and immune suppression by targeting *Ccl21a* and *Icam1* in the post-MI hearts (Chen et al., 2023). In the current study, we carried out scRNA-seq and stereo-seq at different time points (3, 7, 21, and 42 dpMI) to gain an integrated atlas of cardiac cellular landscape. We also generated a conditional knockout model of *Icam1* in LECs, an important gene that mediates immune cell mobility, behavior, and states during injury repair. We created a high-quality map of more than 90,000 cells and described the cellular, transcriptional, and pathway changes observed in *Icam1*cko compared with control, revealing potential mechanisms for *Icam1* regulation.

ICAM-1 is an important adhesion molecule of immunoglobulin superfamily to mediate cell-cell adhesion (Lim et al., 2022). It is upregulated in infarcted myocardium in humans (Niessen et al., 1999). Previous studies have revealed its broad distribution and regulated expression in inflammation (Marlin and Springer, 1987). For instance, miR-141 decreased the accumulation of immune cells via antithetical regulation of ICAM-1 on endothelium, thereby attenuates MI/R-induced cardiac injury and dysfunction (Liu et al., 2015). Lymphatic vessels also have an essential immunomodulatory role, and the stimulation lymphangiogenesis promotes post-MI repair (Brakenhielm and Alitalo, 2019). Notably, our previous study found

the lymphatic-specific deletion of *Icam1* significantly impaired heart function after MI. In this study, we found the cellular composition of infarcted heart was significantly altered, with increased fibroblasts at 42dpMI in *Icam1*cko. The exploratory differential expression analysis was performed to identify transcriptional changes in *Icam1*cko samples. We observed up-regulation of inflammation and fibrosis pathways in *Icam1*cko group as compared to the control group.

Neutrophils and macrophages, as important immune cell components in the inflammatory response, are considered key factors influencing the cardiac function and remodeling after MI. In animal models, neutrophils, which were activated by adherence dependent on Mac-1 (CD11b/CD18) and ICAM-1 (CD54), have been shown to contribute to tissue damage caused during myocardial infarction (Brakenhielm and Alitalo, 2019). Our data showed an increased number and activation of neutrophils in *Icam1*cko compared with control at early stage. Activated neutrophils showed up-regulated expression of pro-inflammatory module genes enriched inflammatory and cytokine pathways, such as *Trem1*, *Thbs1* and *Cxcl3*. Notably, the score of NETs was significantly elevated in Neu6 of *Icam*cko groups. Previous studies revealed the excessive NET formation may exacerbate the inflammatory response, leading to tissue damage and adverse cardiovascular events such as ventricular remodeling and arrhythmias during acute myocardial infarction (Sorvillo et al., 2019; Yuwei et al., 2024). These results suggest that activated neutrophils, especially Neu6, may play an important role in promoting inflammation for *Icam1*cko hearts.

Macrophages are the most abundant cardiac immune cells, and many studies have investigated their complex functions in cardiac immunity (Jin et al., 2022; Jung et al., 2022; Rizzo et al., 2023; Walter et al., 2018). Therefore, we further unravel macrophage dynamics in remodeled cardiac microenvironment using the intergraded data. Based on inflammation scores, we defined 7 macrophage subsets and divided them into two groups (inflammatory and noninflammatory macrophages). Inflammatory macrophages, including Mono, Mac4, Mac5, and Mac6, expressed a high level of chemokine genes and exhibited apparent expansion after MI. Among them, Mac4 exhibited higher expression of genes associated with inflammatory process in *Icam1*cko. They also were upregulated in the interleukin-1, cytokine signaling and T cell activation pathways in *Icam1*cko. Our previous study has consistently proved the accumulation of $CD8^+$ T cells in the infarcted area of *Icam1*cko hearts (Wang et al., 2023). These results indicated that *Icam1* expression in LECs plays a pivotal role in regulating the cascade of immune responses after myocardial infarction. Besides, our results revealed the critical role of Mac4 via *Il1a* and *Il1b* in leading to improved left ventricular remodeling. A previous study demonstrated that systemic IL-1α deficiency attenuated early myocardial inflammation and profibrotic gene expression in a myocardial infarction model (Lugrin et al., 2023).

For LECs in *Icam1*cko, they showed increased inflammatory level and decreased angiogenesis ability, which were harmful in reducing infarct size (Houssari et al., 2020). These results suggest the positive role of the lymphatic-specific *Icam1* in improving heart function after myocardial injury. Indeed, lymphatics actively regulated immune responses through evacuating leukocytes. Previous studies have reported that LECs attracted immune

cells by establishing CCL21 gradients (Iwami et al., 2015) and promoted immune cell migration to lymph nodes dependent on ICAM-1/LFA-1 interactions (Teijeira et al., 2017). We found the expression of *Icam1* and *Icam2* was significantly decreased in LECs of *Icam1*cko. The lymphatic system also plays an important role in dampening inflammation by removing inflammatory cells from tissues (Podgrabinska et al., 2009). A previous study revealed the key role for ICAM-1/LFA-1 interactions in reducing the advancement of T cells and promoting transmigration across lymphatic endothelium from capillaries into lymphatic collectors (Teijeira et al., 2017). The cell-cell communication results revealed that the deletion of *Icam1* reduced the number and strength of interactions between LECs and immune cells related to adhesion molecules and integrins, including *Icam1-Itgal, Icam1-(Itgal + Itgb2), Icam2-(Itgam + Itgb2)* and *Icam2-(Itgal + Itgb2)*. The observed reduction in distance between immune cells and LECs, together with higher inflammatory response scores in the *Icam*cko group, further reflect altered cellular interactions contributing to a heightened inflammatory state. Brakenhielm *et al.* have unveiled that insufficient cardiac lymphatic drainage might be related to cardiac fibrosis and dysfunction (Brakenhielm and Alitalo, 2019). Therefore, the deletion of *Icam1* in LECs may cause the accumulation inflammatory immune cells through impaired ICAM signaling. The most significantly increased interactions in *Icam1*cko were concentrated between Mac4 and Neu6 targeting macrophage subsets and neutrophil subsets via TNF and CXCL ligand-receptor family, resulting in the further accumulation of inflammatory cells in *Icam1*cko. A recent study reported that well-developed neutrophils in fulminant viral myocarditis attract peripheral neutrophils and monocytes by chemokines, resulting in an inflammatory storm and cardiac

dysfunction (Li et al., 2023a). Therefore, these results indicated the loss of adhesion molecules prevents immune cells from homing to lymphatics, which leads to myocardial oedema, ultimately damaging heart function. These results indicated that *Icam1* expression in LECs was crucial in shaping the immune response.

In summary, lymphatic *Icam1* promotes the clearance of inflammatory immune cells mediated by cardiac lymphatics after MI. Conditional deletion of *Icam1* weakens cellular interactions across LECs, neutrophil subsets and macrophages subsets, resulting in changes of immune microenvironment. The obstruction of inflammatory immune cells drainage is one of the reasons for the dysregulation of immune cell infiltration. Therefore, *Icam1* in LECs is key in regulating the immune microenvironment after MI. This dataset provides an invaluable resource for studying the regulatory role of *Icam1* after MI and inspires more mechanistic discoveries in lymphatic-driven therapeutics.

LIMITATIONS

This study primarily relies on single-cell and spatial transcriptomic analyses to infer cellular dynamics and intercellular interactions after myocardial infarction. While these approaches provide valuable insights, some conclusions, such as altered neutrophil activation, enhanced macrophage inflammation and impaired immune drainage by ICAM–integrin signaling in *Icam1*cko hearts, are based on computational predictions and lack direct experimental validation. Functional assays will be necessary in future studies to confirm and extend these findings.

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DATA AVAILAIBILITY

The data that support the findings of this study have been deposited into CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) with accession number CNP0003981 (https://db.cngb.org/search/singlecell/CSE0000428).

COMPETING INTERESTS

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

Conceptualization, Z.Z. and M.Z.; formal analysis, X.C., Z.Z., and M.Z.; methodology, Y.W and B.H; experimental organization, S.C and S.S.; investigation, Z.C., X.L., K.L and J.Y.; resources, Z.Z., H.C., and L.W.; visualization, X.C. and M.Z.; writing original draft, X.C., Z.Z., M.Z., and J.S.; Writing-review & editing, M.Z., and J.S.; Supervision, Z.Z., M.Z., and X.F. All authors read and approved the final version of the manuscript.

REFERENCES

Amrute JM, Luo X, Penna V, Yang S, Yamawaki T, Hayat S, etc. 2024. Targeting immune–fibroblast cell communication in heart failure. *Nature*: 1-11.

Bajpai G, Bredemeyer A, Li W, Zaitsev K, Koenig AL, Lokshina I, etc. 2019. Tissue resident CCR2− and CCR2+ cardiac macrophages differentially orchestrate monocyte recruitment and fate specification following myocardial injury. *Circulation research*, **124**(2): 263-278.

Bazigou E, Lyons OT, Smith A, Venn GE, Cope C, Brown NA, etc. 2011. Genes regulating lymphangiogenesis control venous valve formation and maintenance in mice. *The Journal of clinical investigation*, **121**(8): 2984-2992.

Brakenhielm E & Alitalo K. 2019. Cardiac lymphatics in health and disease. *Nature Reviews Cardiology*, **16**(1): 56-68.

Bråkenhielm E, Chen Y, Cao Y. 2021. Lymphatics in the broken heart. *The Journal of clinical investigation*, **131**(20).

Browaeys R, Saelens W, Saeys Y. 2020. NicheNet: modeling intercellular communication by linking ligands to target genes. *Nature Methods*, **17**(2): 159-162.

Bui TM, Wiesolek HL, Sumagin R. 2020. ICAM-1: A master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. *Journal of Leucocyte Biology*, **108**(3): 787-799.

Butler A, Hoffman P, Smibert P, Papalexi E, Satija R. 2018. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nature biotechnology*, **36**(5): 411-420.

Chen A, Liao S, Cheng M, Ma K, Wu L, Lai Y, etc. 2022. Spatiotemporal transcriptomic atlas of mouse organogenesis using DNA nanoball-patterned arrays. *Cell*, **185**(10): 1777-1792. e1721.

Chen S, Francioli LC, Goodrich JK, Collins RL, Kanai M, Wang Q, etc. 2023. A genomic mutational constraint map using variation in 76,156 human genomes. *Nature*, (March 2022).

Dieterich LC, Seidel CD, Detmar M. 2014. Lymphatic vessels: new targets for the treatment of inflammatory diseases. *Angiogenesis*, **17**: 359-371.

Eash KJ, Greenbaum AM, Gopalan PK, Link DC. 2010. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *The Journal of clinical investigation*, **120**(7): 2423-2431.

Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, etc. 2014. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity*, **40**(1): 91-104.

Epelman S, Liu PP, Mann DL. 2015. Role of innate and adaptive immune mechanisms in cardiac injury and repair. *Nature Reviews Immunology*, **15**(2): 117-129.

Farbehi N, Patrick R, Dorison A, Xaymardan M, Janbandhu V, Wystub-Lis K, etc. 2019. Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury. *Elife*, **8**: e43882.

Han L, Wei X, Liu C, Volpe G, Zhuang Z, Zou X, etc. 2022. Cell transcriptomic atlas of the non-human primate Macaca fascicularis. *Nature*, **604**(7907): 723-731.

Henri O, Pouehe C, Houssari M, Galas L, Nicol L, Edwards-Lévy F, etc. 2016. Selective stimulation of cardiac lymphangiogenesis reduces myocardial edema and fibrosis leading to improved cardiac function following myocardial infarction. *Circulation*, **133**(15): 1484-1497.

Heusch G & Gersh BJ. 2017. The pathophysiology of acute myocardial infarction and strategies of protection beyond reperfusion: a continual challenge. *European heart journal*, **38**(11): 774-784.

Heusch G, Libby P, Gersh B, Yellon D, Böhm M, Lopaschuk G, etc. 2014. Cardiovascular remodelling in coronary

672 artery disease and heart failure. *The Lancet*, **383**(9932): 1933-1943.
673 Houssari M, Dumesnil A, Tardif V, Kivelä R, Pizzinat N, Boukhalfa I, etc. 2020. Lymphatic and immune cell
674 cross-talk regulates cardiac recovery after experimental myocardial infarction. *Arteriosclerosis, thrombosis,*
675 *and vascular biology*, **40**(7): 1722-1737.
676 Hulsmans M, Sager HB, Roh JD, Valero-Muñoz M, Houstis NE, Iwamoto Y, etc. 2018. Cardiac macrophages
677 promote diastolic dysfunction. *Journal of Experimental Medicine*, **215**(2): 423-440.
678 Iwami D, Brinkman CC, Bromberg JS. 2015. Vascular endothelial growth factor c/vascular endothelial growth
679 factor receptor 3 signaling regulates chemokine gradients and lymphocyte migration from tissues to
680 lymphatics. *Transplantation*, **99**(4): 668-677.
681 Jin K, Gao S, Yang P, Guo R, Li D, Zhang Y, etc. 2022. Single‐cell RNA sequencing reveals the temporal diversity
682 and dynamics of cardiac immunity after myocardial infarction. *Small Methods*, **6**(3): 2100752.
683 Jin S, Guerrero-Juarez CF, Zhang L, Chang I, Ramos R, Kuan C-H, etc. 2021. Inference and analysis of cell-cell
684 communication using CellChat. *Nature communications*, **12**(1): 1088.
685 Jung S-H, Hwang B-H, Shin S, Park E-H, Park S-H, Kim CW, etc. 2022. Spatiotemporal dynamics of macrophage
686 heterogeneity and a potential function of Trem2hi macrophages in infarcted hearts. *Nature communications*,
687 **13**(1): 4580.
688 Kleshchevnikov V, Shmatko A, Dann E, Aivazidis A, King HW, Li T, etc. 2022. Cell2location maps fine-grained cell
689 types in spatial transcriptomics. *Nature biotechnology*, **40**(5): 661-671.
690 Klotz L, Norman S, Vieira JM, Masters M, Rohling M, Dube KN, etc. 2015. Cardiac lymphatics are
691 heterogeneous in origin and respond to injury. *Nature*, **522**(7554): 62-67.
692 Korsunsky I, Millard N, Fan J, Slowikowski K, Zhang F, Wei K, etc. 2019. Fast, sensitive and accurate integration
693 of single-cell data with Harmony. *Nature Methods*, **16**(12): 1289-1296.
694 Lawson C & Wolf S. 2009. ICAM-1 signaling in endothelial cells. *Pharmacological reports*, **61**(1): 22-32.
695 Li H, Zhang M, Zhao Q, Zhao W, Zhuang Y, Wang J, etc. 2023a. Self-recruited neutrophils trigger over-activated
696 innate immune response and phenotypic change of cardiomyocytes in fulminant viral myocarditis. *Cell*
697 *discovery*, **9**(1): 103.
698 Li M, Liu H, Li M, Fang S, Kang Q, Zhang J, etc. 2023b. StereoCell enables highly accurate single-cell
699 segmentation for spatial transcriptomics. *bioRxiv*: 2023.2002.2028.530414.
700 Lim E-J, Kang J-H, Kim Y-J, Kim S, Lee S-J. 2022. ICAM-1 promotes cancer progression by regulating SRC activity
701 as an adapter protein in colorectal cancer. *Cell death & disease*, **13**(4): 417.
702 Lindsey ML, Jung M, Yabluchanskiy A, Cannon PL, Iyer RP, Flynn ER, etc. 2019. Exogenous CXCL4 infusion
703 inhibits macrophage phagocytosis by limiting CD36 signalling to enhance post-myocardial infarction cardiac
704 dilation and mortality. *Cardiovascular research*, **115**(2): 395-408.
705 Liu C, Wu T, Fan F, Liu Y, Wu L, Junkin M, etc. 2019. A portable and cost-effective microfluidic system for
706 massively parallel single-cell transcriptome profiling. *BioRxiv*: 818450.
707 Liu RR, Li J, Gong JY, Kuang F, Liu JY, Zhang YS, etc. 2015. MicroRNA-141 regulates the expression level of
708 ICAM-1 on endothelium to decrease myocardial ischemia-reperfusion injury. *American Journal of*
709 *Physiology-Heart and Circulatory Physiology*, **309**(8): H1303-H1313.
710 Lugrin J, Parapanov R, Milano G, Cavin S, Debonneville A, Krueger T, etc. 2023. The systemic deletion of
711 interleukin-1α reduces myocardial inflammation and attenuates ventricular remodeling in murine myocardial
712 infarction. *Scientific reports*, **13**(1): 4006.
713 Marlin SD & Springer TA. 1987. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte
714 function-associated antigen 1 (LFA-1). *Cell*, **51**(5): 813-819.
715 Mikhael M & Khan YS. 2020. Anatomy, Abdomen and Pelvis, Lymphatic Drainage.

716 Morabito S, Reese F, Rahimzadeh N, Miyoshi E, Swarup V. 2023. hdWGCNA identifies co-expression networks
717 in high-dimensional transcriptomics data. *Cell reports methods*, **3**(6).
718 Niessen H, Lagrand W, Visser C, Meijer C, Hack C. 1999. Upregulation of ICAM-1 on cardiomyocytes in
719 jeopardized human myocardium during infarction. *Cardiovascular research*, **41**(3): 603-610.
720 Podgrabinska S, Kamalu O, Mayer L, Shimaoka M, Snoeck H, Randolph GJ, etc. 2009. Inflamed lymphatic
721 endothelium suppresses dendritic cell maturation and function via Mac-1/ICAM-1-dependent mechanism. *The*
722 *Journal of Immunology*, **183**(3): 1767-1779.
723 Prabhu SD & Frangogiannis NG. 2016. The biological basis for cardiac repair after myocardial infarction: from
724 inflammation to fibrosis. *Circulation research*, **119**(1): 91-112.
725 Rivadeneyra L, Charó N, Kviatcovsky D, De La Barrera S, Gómez RM, Schattner M. 2018. Role of neutrophils in
726 CVB3 infection and viral myocarditis. *Journal of molecular and cellular cardiology*, **125**: 149-161.
727 Rizzo G, Gropper J, Piollet M, Vafadarnejad E, Rizakou A, Bandi SR, etc. 2023. Dynamics of monocyte-derived
728 macrophage diversity in experimental myocardial infarction. *Cardiovascular research*, **119**(3): 772-785.
729 Skelly DA, Squiers GT, Mclellan MA, Bolisetty MT, Robson P, Rosenthal NA, etc. 2018. Single-cell transcriptional
730 profiling reveals cellular diversity and intercommunication in the mouse heart. *Cell reports*, **22**(3): 600-610.
731 Sorvillo N, Cherpokova D, Martinod K, Wagner DD. 2019. Extracellular DNA NET-works with dire consequences
732 for health. *Circulation research*, **125**(4): 470-488.
733 Teijeira A, Hunter MC, Russo E, Proulx ST, Frei T, Debes GF, etc. 2017. T cell migration from inflamed skin to
734 draining lymph nodes requires intralymphatic crawling supported by ICAM-1/LFA-1 interactions. *Cell reports*,
735 **18**(4): 857-865.
736 Vafadarnejad E, Rizzo G, Krampert L, Arampatzi P, Arias-Loza A-P, Nazzal Y, etc. 2020. Dynamics of cardiac
737 neutrophil diversity in murine myocardial infarction. *Circulation research*, **127**(9): e232-e249.
738 Vieira JM, Norman S, Del Campo CV, Cahill TJ, Barnette DN, Gunadasa-Rohling M, etc. 2018. The cardiac
739 lymphatic system stimulates resolution of inflammation following myocardial infarction. *The Journal of clinical*
740 *investigation*, **128**(8): 3402-3412.
741 Vivien CJ, Pichol-Thievend C, Sim CB, Smith JB, Bower NI, Hogan BM, etc. 2019. Vegfc/d-dependent regulation
742 of the lymphatic vasculature during cardiac regeneration is influenced by injury context. *Npj Regenerative*
743 *Medicine*, **4**(1): 18.
744 Walter W, Alonso-Herranz L, Trappetti V, Crespo I, Ibberson M, Cedenilla M, etc. 2018. Deciphering the
745 dynamic transcriptional and post-transcriptional networks of macrophages in the healthy heart and after
746 myocardial injury. *Cell reports*, **23**(2): 622-636.
747 Wang W, Li X, Ding X, Xiong S, Hu Z, Lu X, etc. 2023. Lymphatic endothelial transcription factor Tbx1 promotes
748 an immunosuppressive microenvironment to facilitate post-myocardial infarction repair. *Immunity*, **56**(10):
749 2342-2357. e2310.
750 Weil BR & Neelamegham S. 2019. Selectins and immune cells in acute myocardial infarction and
751 post-infarction ventricular remodeling: pathophysiology and novel treatments. *Frontiers in immunology*, **10**:
752 300.
753 Xu H, Morishima M, Wylie JN, Schwartz RJ, Bruneau BG, Lindsay EA, etc. 2004. Tbx1 has a dual role in the
754 morphogenesis of the cardiac outflow tract.
755 Yang L, Shi F, Cao F, Wang L, She J, He B, etc. 2025. Neutrophils in Tissue Injury and Repair: Molecular
756 Mechanisms and Therapeutic Targets. *MedComm*, **6**(5): e70184.
757 Yu G, Wang L-G, Han Y, He Q-Y. 2012. clusterProfiler: an R package for comparing biological themes among
758 gene clusters. *Omics: a journal of integrative biology*, **16**(5): 284-287.
759 Yuwei W, Qiongfeng W, Yimei D. 2024. Research progress of ion channels in the formation mechanism of

neutrophil extracellular traps in acute myocardial infarction. *J Clin Cardiol*, **40**(5): 358-365.

Zheng GX, Terry JM, Belgrader P, Ryvkin P, Bent ZW, Wilson R, etc. 2017. Massively parallel digital transcriptional profiling of single cells. *Nature communications*, **8**(1): 14049.

FIGURE LEGENDS

Figure 1. Single cell sequencing identifies cell types and disease-related transcriptomic changes. (A) Schematic diagram of experimental design and workflow. (B) UMAP clustering of 91,544 cells isolated from both control and *Icam1*cko hearts, across two genetypes and time after LAD coronary artery permanent ligation operation (n=3). (C) Proportion changes of each cell type in each sample. (D) The correlation of different groups based on gene expression. (E) GO enrichment terms of upregulated (left) and downregulated (right) expressed genes of *Icam1*cko at 3, 7, 21, and 42dpMI. (F) Heatmap displays the expression activity of selected signaling pathways (VEGF, JAK-STAT, ECM and apoptosis) and their associated genes across time points and genotypes. (G) The imputed expression of *Cd93* (angiogenesis marker) and *Col3a1* (fibrosis marker) of control and *Icam1*cko groups at 3dpMI (top) and 42dpMI (bottom), respectively. Scale bar: 500 μm.

Figure 2. Single cell sequencing identifies cell types and disease-related transcriptomic changes. (A) Violin plots showing the scores of inflammatory (left) and ECM (right) processes for major cell types. (B) The first column represents the cell abundance deconvoluted from the scRNA-seq data using cell2location. The second column represents the functional gene set scores. The third column represents the spatial correlation cluster of Bivariate Moran's I between corresponding cell abundance and gene scores. HH and LL indicate co-localized high/low expression, HL/LH indicate opposing patterns, and ns denotes non-significant correlation. Scale bar: 500 μm. (C) Relative differences in cell proportions for each cluster between *Icam1*cko and control. Red clusters have a false discovery rate (FDR) < 0.05 and mean | Log2 fold enrichment | > 1 compared to control (permutation test; n =

10,000). (D) Heatmap showing the DEG number between *Icam1*cko and control groups of major cell types at each time point. (E) UpSet charts showing the overlap of the upregulated genes in neutrophils, macrophages and fibroblasts of *Icam1*cko at each time point. (F) Network plots showing the enriched GO terms of upregulated genes in aged tissues in neutrophils, macrophages and fibroblasts of *Icam1*cko at each time point.

Figure 3. High heterogeneity and functional difference of cardiac neutrophils during MI progression. (A) The proportion of neutrophils at different time points in control and *Icam1*cko groups. (B) The spatial distribution of neutrophils. Red represents neutrophiles, and grey represents other cell types. Scale bar: 500 μm. (C) UMAP of 6,542 neutrophils identifies 6 subclusters. (D) Heatmap showing the expression of top 60 marker genes across six neutrophil subpopulations (left). Line plots show the mean Z-score trajectory of these markers (middle). To the right, the top five Gene Ontology biological processes enriched in C1-C6 (adjusted $P < 0.05$) are listed. (E) The imputed expression of *Cd177* of control (left) and *Icam1*cko (right) groups at 3dpMI. Scale bar: 500 μm. (F) The cell ratio of neutrophile subsets in control and *Icam1*cko groups at 3pMI. (G) Network visualization of 7 modules of neutrophils. (H) Boxplots showing hME of 7 modules between control and *Icam1*cko groups. ns: Not significant; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$. (I) The GO terms of the M3, M4, M5 and M7 eigengenes. (J) Boxplot showing hME scores of M3 across different neutrophil subclusters. (K-L) Comparison of scores of neutrophil activation and neutrophil extracellular trap formation process scores across neutrophil subpopulations from control and *Icam1*cko groups.

Figure 4. Characterization of monocyte/macrophage cell subsets in the infarcted heart. (A) UMAP of 46,314 macrophages identifies 7 subclusters. (B) The Pearson correlation of different groups based on the cell proportion of macrophage subsets. (C) Heatmap displaying differentially expressed cytokines involved in inflammatory macrophage subsets. (D) The proportion of macrophage subsets across different time points at each group. (E) The spatial visualization of Mac4 of control (left) and *Icam1*cko (right) groups at 3dpMI. Scale bar: 500 μm. (F) Bar plot showing the number of upregulated and downregulated genes (top), and volcano plot illustrates the upregulated and downregulated genes in different cell types between control and *Icam1*cko samples (bottom). Red dots indicate statistically upregulated genes in *Icam1*cko group (log2FC > 0.5 and adjusted $P < 0.05$). (G) Radar plot represents the change of inflammatory scores among the macrophage subsets between control and *Icam1*cko groups. (H) Heatmap showing the cell proximity of major cell types. *: $P<0.01$. (I) The first and second columns represents the spatial colocation distribution of macrophages and fibroblasts at 7dpMI. Scale bar: 500 μm. The third column represents H&E staining images on adjacent heart sections. The enlarged area corresponds to the infarct zone, as determined by showing immune infiltration and fibrotic changes. (J) Heatmap showing the regulation of ligand genes expressed in cardiac macrophages (left) and the upregulated genes after MI in fibroblasts (right). (K) The expression of *Il1a* and *Il1b* across different macrophage subclusters from control and *Icam1*cko groups.

Figure 5. Significantly altered intracellular crosstalk in the *Icam1*cko microenvironment. (A-B) Boxplots showing the angiogenesis (A) and inflammatory (B) score of LECs for each condition sample. (C) Bar plots represent the changes in both the total number of cell

interactions (left) and the interaction strength (right) between control and *Icam1*cko groups. (D) The relative information flow for each signaling pathway in the control and *Icam1*cko groups. Blue fonts and yellow fonts represent pathways significantly enriched in control and *Icam1*cko (P<0.05), respectively. Paired Wilcoxon test. (E) Overview of ligand-receptor interactions in between LECs, macrophage subpopulations and neutrophil subpopulations. (F) Violin plots shown *Icam1, Icam2, Ccl2 and Csf3* expression in LECs for each condition sample. (G) The spatial expression of *Ccl21a* in control and *Icam1*cko groups at 21dpMI. Scale bar: 500 μm. (H) Boxplot showing the distance between LECs with immune cells in control and *Icam1*cko groups at 21dpMI. (I) Inflammatory score at different distances from LECs. (J) Chord plot showing the up-regulated signaling pathways in *Icam1*cko group. (H) Heatmaps showing the expression of *Tnf* (top) and *Cxcl2* (bottom) in neutrophil subpopulations and macrophage subpopulations of different groups, respectively.