Spatiotemporal dynamics of macrophage heterogeneity and a potential function of Trem2^{hi} macrophages in infarcted hearts

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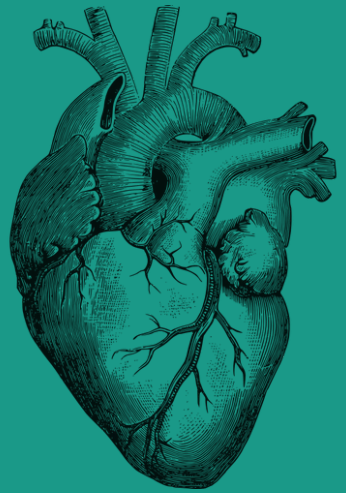
GitHub: Interpretation_of_a_spatial_omics_dataset

Introduction



Heart failure is a consequence of myocardial infarction (MI)

GOAL: identification of precise, time-dependent composition of inflammatory cells

Why?

- ***Acute Myocardial Infarction*** induces the mobilization and recruitment of a diverse innate and adaptive immune cells to the infarcted heart

  ⇨ **Critical role of macrophages** in wound repair and remodeling processes

Macrophages

According to their in vitro construction, they are divided into:

M1 macrophages	M2 macrophages
Express high levels of pro-inflammatory cytokines	Release anti-inflammatory cytokines
Promote pro-inflammatory milieu	Promote angiogenesis and wound healing

The balance between M1 and M2 during immune responses is essential for effective healing and remodeling processes.

BUT in vivo environment is more complex.

Characterization of the dynamics of macrophage heterogeneity

A more precise evaluation is needed for accurate characterization of the dynamics of macrophage heterogeneity during the acute period of MI.

⇒ Spatial and scRNA-seq analyses of cardiac immune cells in a mouse MI model to investigate the spatio-temporal dynamics of MI-associated immune cells.

Methods

Male wild-type 7-8 week-old C57BL/6

Animal models and Cell staining

- 8 mices underwent left-sided thoracotomy to induce experimental myocardial infarction (MI)
- 4 mices received intramyocardial injections of PBS, gel, or gel containing 12 µg sTrem2 at the infarct border zone.

- Tissue was collected from 1,3,5,7 days after the MI event
- Heart tissues were digested with collagenase type II solution
- Passed through a 40 µm cell strainer, washed with HBSS Buffer, and resuspended in FACS staining buffer

Single-cell RNA library construction

Droplet method using the 10× Genomic Chromium System

- ➔ Counted by hemocytometer (ThermoFisher) and 16,000 cells per sample were added to each channel
- ➔ Cells were then partitioned into **Gel beads in emulsion** (GEMs) in the Chromium instrument, where cell lysis and barcoded reverse transcription of RNA occurred
- ➔ Sequencing libraries were sequenced on an Illumina HiSeq2500 platform
- ➔ Sequencing data was processed using the Cell Ranger Single Cell software suite v3.0.1

Single-cell RNA data analysis

Quality Control and Normalization:

- Seurat R package (version 3.2.0).
- Cells with fewer than 2% or more than 98% expressed genes, and cells with over 10% mitochondrial gene reads, were excluded.
- Log-normalized and highly variable features were identified using variance stabilizing transformation (VST).

Data Integration and Clustering:

- Datasets integrated using canonical correlation analysis (CCA) with "*FindIntegrationAnchors*" and "*IntegrateData*" functions.
- PCA was performed, and clustering was done using shared nearest neighbor (SNN) modularity.Clusters were visualized using UMAP plots.

Cluster Annotation and Filtering:

- Clusters were annotated for cell types using the SingleR R package.

Single-cell RNA data analysis

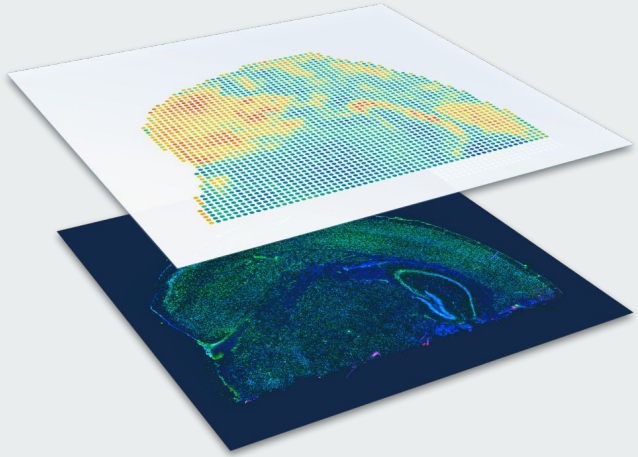
Cell Cycle and Differential Expression Analysis:

- Cell cycle analysis was conducted using the "*CellCycleScoring*".
- DEGs were identified using a logistic regression test, retaining genes with a positive average log fold-change (> 0.25) and adjusted P value < 0.05.

Pseudotime Trajectory Analysis:

- Pseudotime trajectories were generated using the Monocle3 R package.
- Ly6c2 hi monocytes were set as the root of the trajectory to infer transcriptional changes during differentiation.

Spatial transcriptome sequencing (ST-seq)

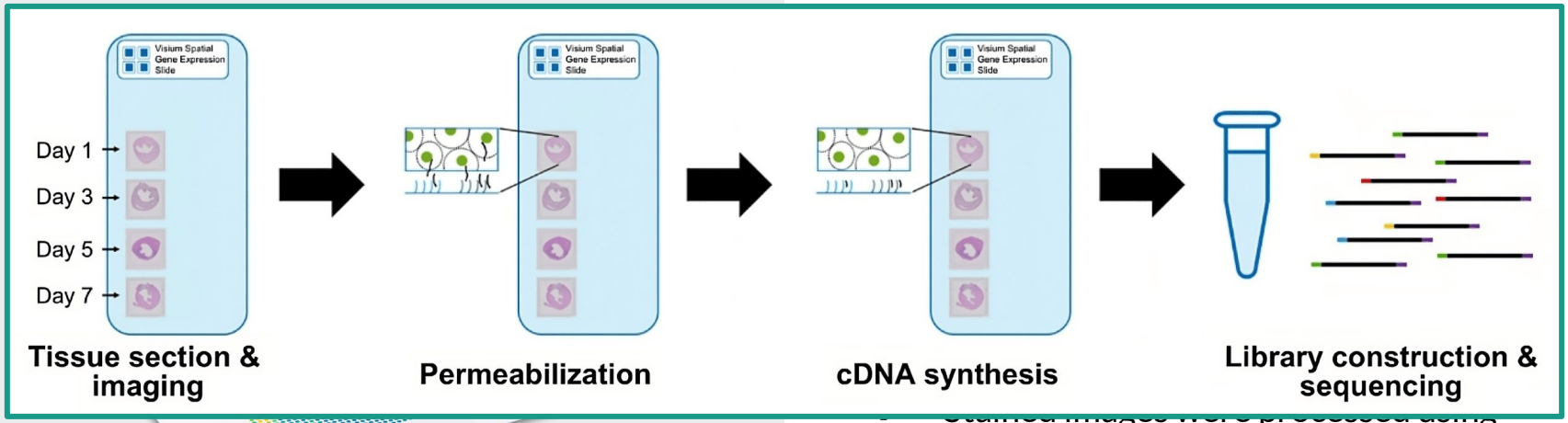


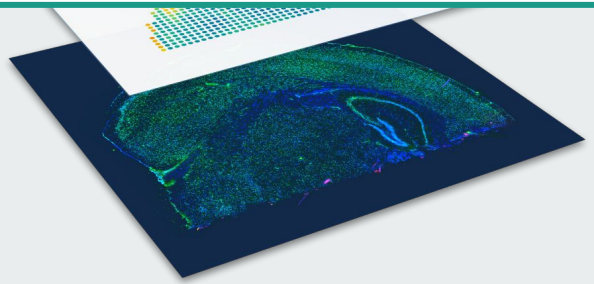
Visium Spatial Gene Expression slide

- ➔ Frozen samples from day 1, 3, 5, and 7 post-MI
- ➔ cDNA libraries were generated using Visium Spatial Gene Expression slide & Reagent Kit and sequenced on a NovaSeq 6000 system (Illumina)
- ➔ Stained images were processed using Space Ranger software

Visium Spatial Gene Expression slide

➔ Frozen samples from day 1, 3, 5, and 7 post-MI





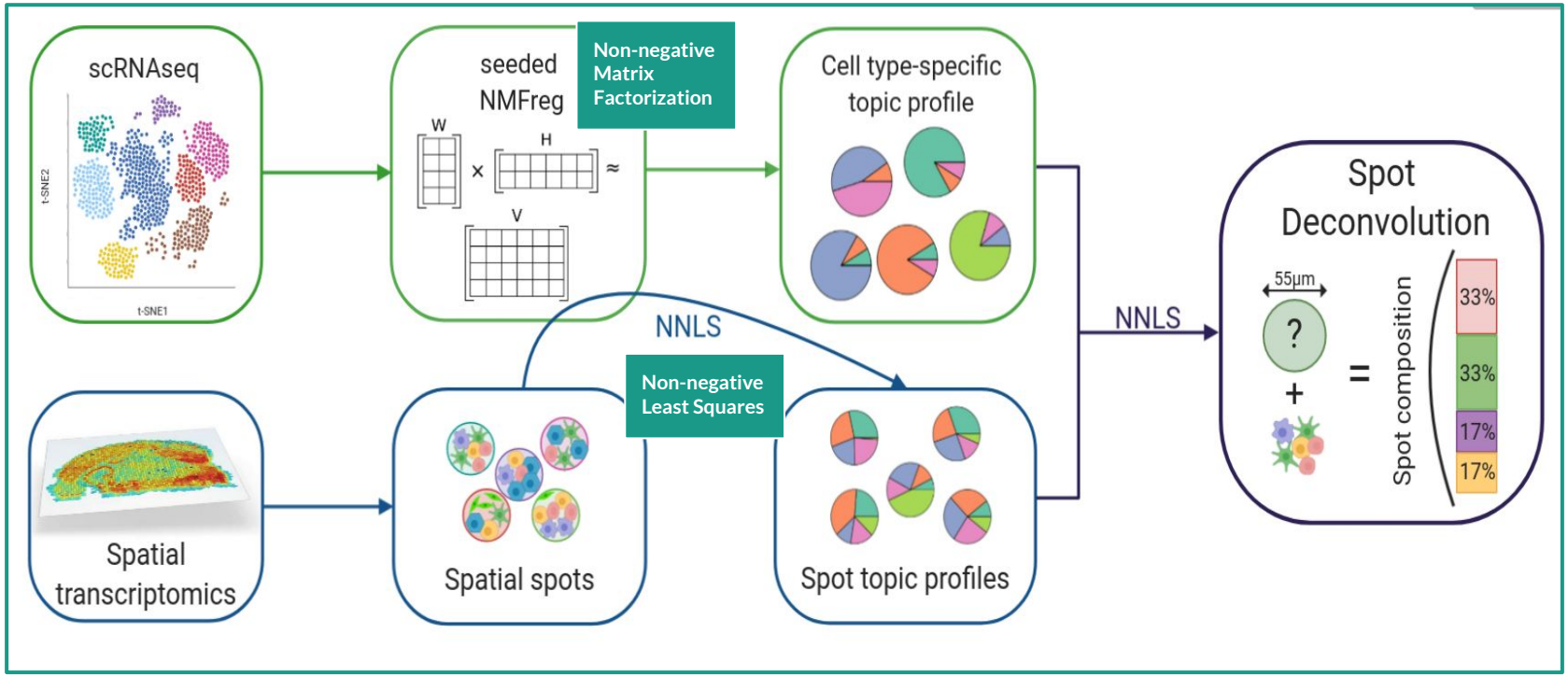
Space Ranger software, for the mapping of the sequencing data, the mm10 version 3.0.0 mus musculus reference genome was used

ST-seq data analysis

Seurat Processing

- Normalization using “***SCTransform***” function
- Dimensionality reduction using “RunPCA” function
- Graph-based clustering using:
  - “***FindNeighbors***” function with 15 PCs
  - “***FindClusters***” function with 15 PCs
- UMAP visualization
- Differentially Expressed Genes (DEGs) analysis with default parameters

SPOTlight : *Spatial Transcriptomics Deconvolution*



Two main Algorithms

NMF Algorithm:

- Unsupervised learning algorithm that decomposes a matrix VV (gene expression data) into two lower-dimensional matrices WW (basis matrix) and HH (coefficient matrix) such that V≈WHV≈WH.
- Both WW and HH contain only non-negative values.

NNLS Algorithm:

- Used to solve the regression problem where the goal is to find non-negative weights that best explain the observed gene expression in ST spots as a linear combination of the scRNA-seq-derived cell-type profiles.
- This step deconvolutes the ST data, assigning proportions of different cell types to each spatial spot.

SPOTlight : *Spatial Transcriptomics Deconvolution*

Signature Scores Calculation

- Use of "*AddModuleScore*" function in Seurat
- Generation of cell type signature scores using marker genes:
  - **Cardiomyocytes**: Myh7, Myh6, Actn2, Nkx2-5, Tnni3, Tnnt2
  - **Endothelial**: Cdh5, Ly6c1, Kdr
  - **Fibroblast**: Col1a1, Pdgfra, Lamc1

Results

Major **RESULTS**

1

Leukocytes in heart tissues exhibit significant heterogeneity, both among different cell types and within specific cell types, including 16 subsets of monocytes/macrophages, 5 subsets of neutrophils, and 6 subsets of dendritic cells.

2

Leukocyte populations become remarkably dynamic after the onset of myocardial infarction (MI), with changes occurring depending on the specific cell types and subpopulations involved.

Major **RESULTS**

3

Single-cell trajectory analysis indicates a sequential differentiation process from Ly6c2 hi monocytes to Late-Mφ, challenging the outdated M1-M2 paradigm with a more nuanced perspective.

4

Notable increase in the population of Trem2 hi macrophages and express genes. Additionally, the soluble form of Trem2, whose expression rises after the peak of full-length Trem2 expression, significantly enhances remodeling and cardiac function in the infarcted heart.

Immune cell dynamics after MI

Steady state = before induction of MI

Post-MI = after induction of MI

Macrophages are the most abundant immune cell population in the heart

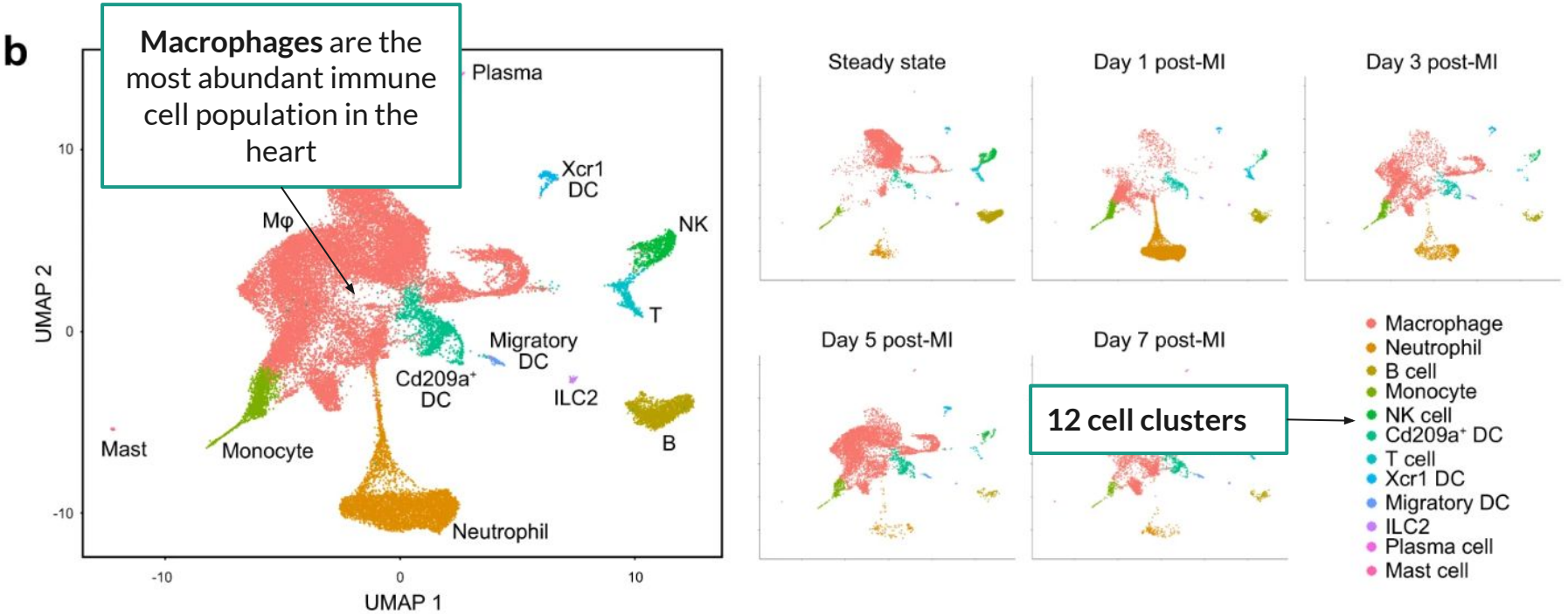


Figure 1b. Two-dimensional uniform manifold approximation and projection (UMAP) visualization of the 33,977 **cardiac CD45⁺ cells** identified 12 broad cell types after unsupervised clustering. Each point represents a single cell; cell types are color-coded.

Immune cell dynamics after MI

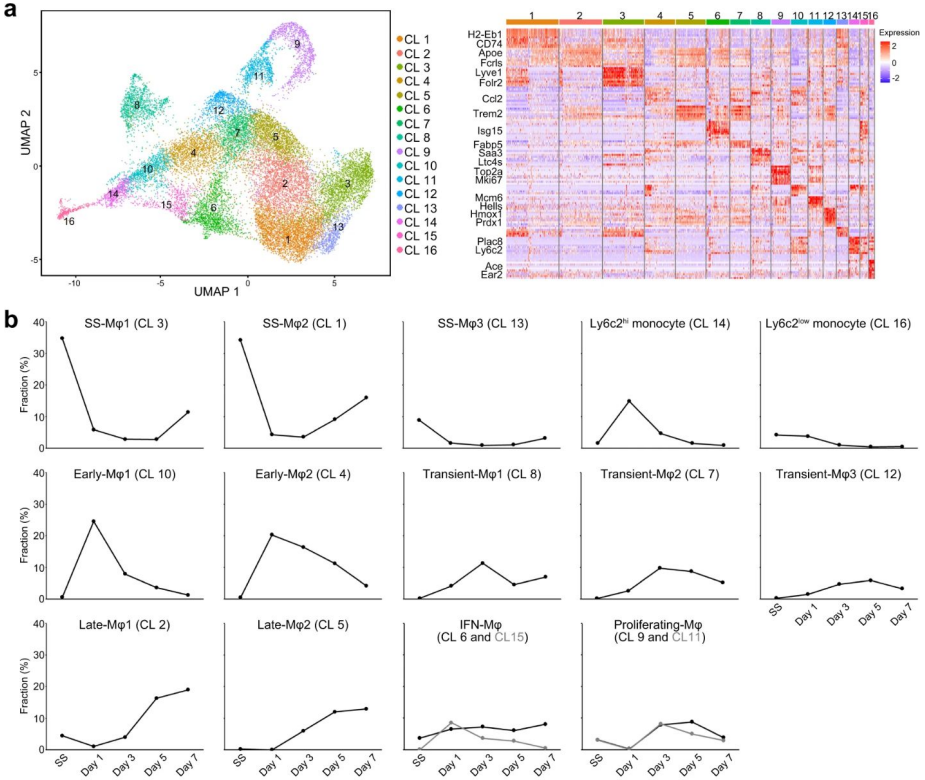
Figure 1c Left: dot plot of well-known cell-type-specific marker genes per cell type. The dot intensity (from white to red) represents the average expression value of all cells per cell type and the dot size represents the proportion of cells expressing the genes. Right: feature plot representing the expression levels of the selected cell-type-specific marker genes.

Figure 1d Bar plot representing the proportions of cells in each of the 12 broad cell types according to the time-point after MI.

The heterogeneity of monocytes and macrophages

Figure 3a The UMAP visualization of the 21,533 cardiac monocytes/macrophages identified **16 subsets** (left panel). In the right panel, the heatmap shows the top 10 most differentially expressed genes in each sub-cluster.

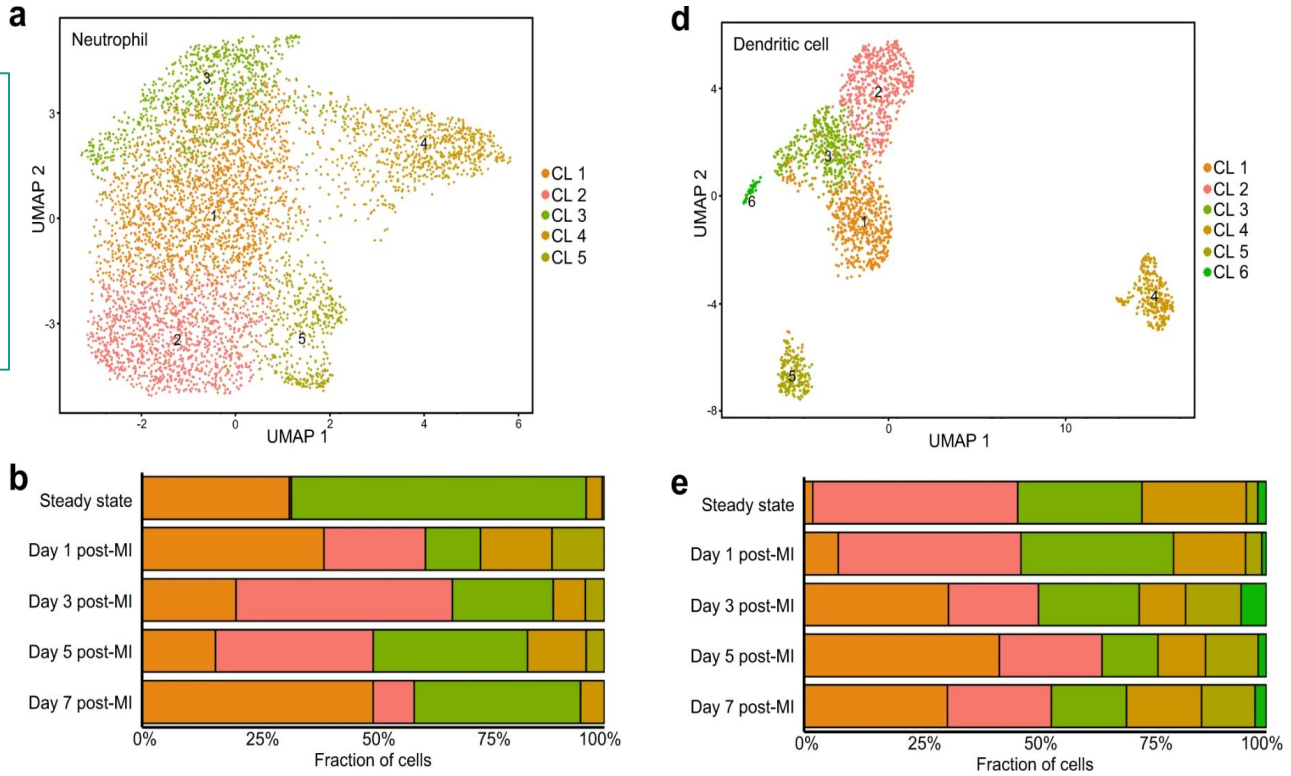
Figure 3b The proportion of each sub-cluster among total monocytes/macrophages according to the time-point after MI.



Neutrophil and dendritic cell subsets

5 subsets of neutrophils

6 subsets of dendritic cells



ST-seq confirms the results

- ST-seq analysis with deconvolution algorithm indicated that the proportions of monocytes, neutrophils, and macrophages increased or decreased in a time-dependent manner, which was consistent with previous reports

- Moreover, they were concentrated in the infarcted area, indicating that these cell populations were recruited to the injured heart tissues after MI.

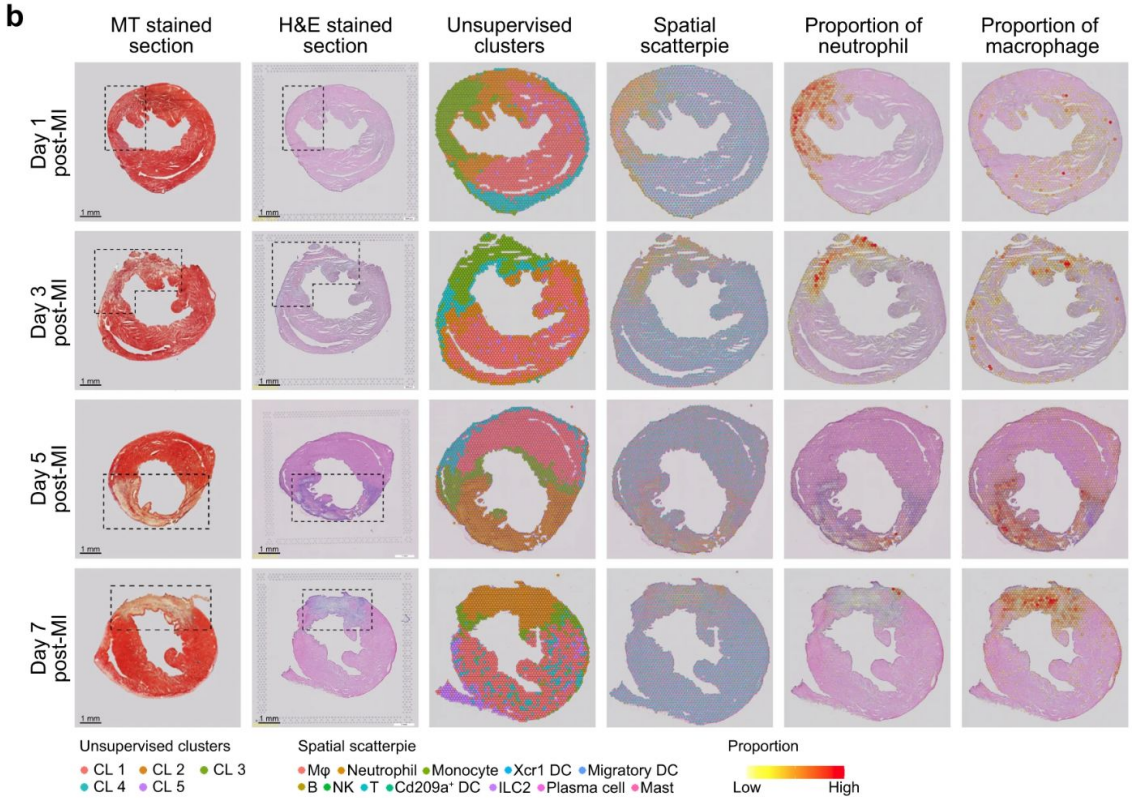
Spatiotemporal profiles of immune cells after MI

PCA and unsupervised clustering identified 5 cell clusters per sample based on the differentially expressed genes.

NMF deconvolution was applied to infer the cell-type composition of each spot.

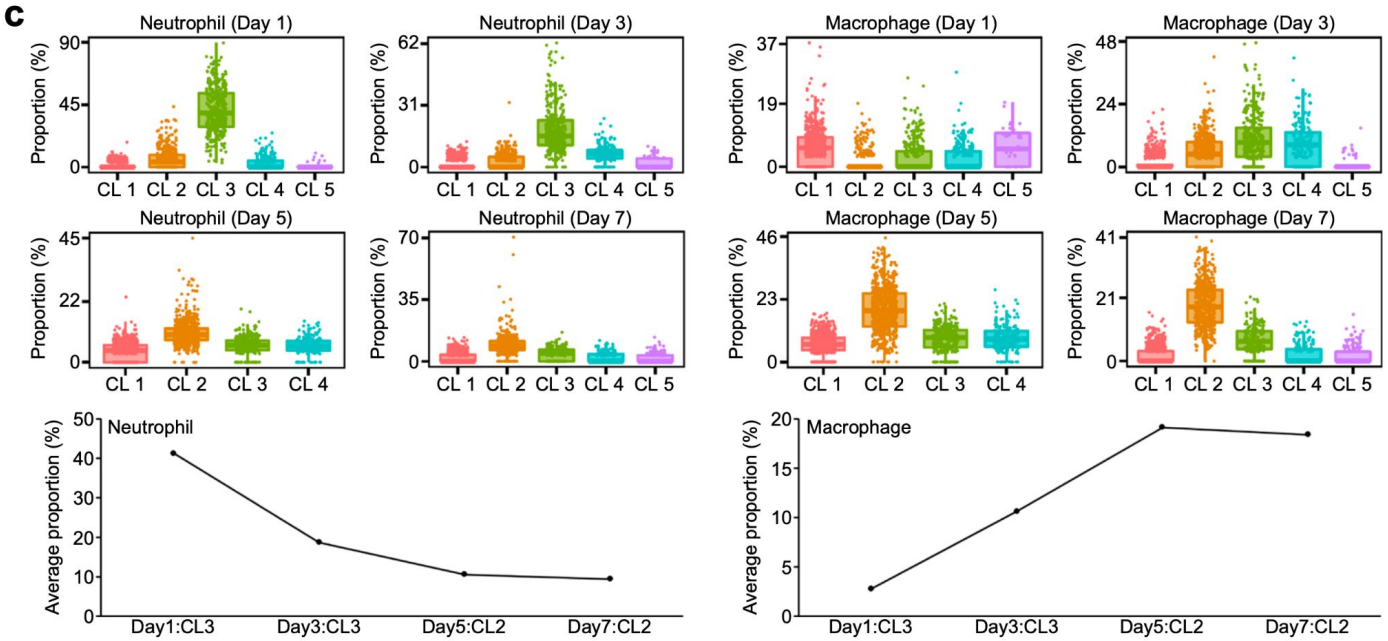
ST-seq data shows that:

- Monocytes and neutrophils infiltrated into the infarcted area at early MI
- Macrophages and fibroblasts acted oppositely



Spatiotemporal profiles of immune cells after MI

Figure 2c. The proportion of neutrophils and macrophages among total immune cells infiltrated into heart tissue according to the time-point after MI.

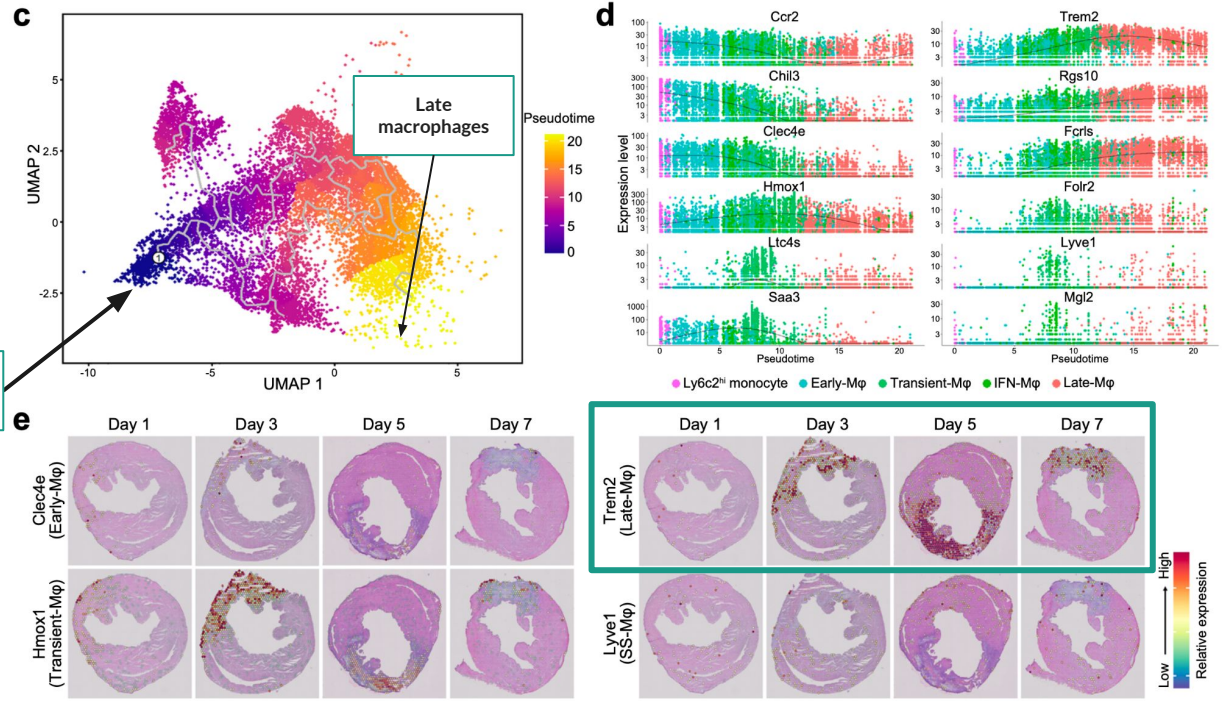


Trajectory analysis

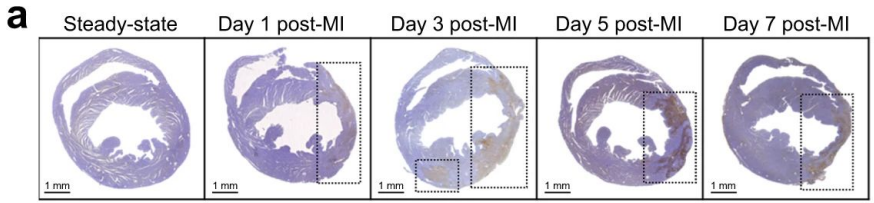
Figure 3c Pseudo-time trajectory as per the pseudo-time algorithm. Ly6c2hi monocytes were set as the root of the trajectory.

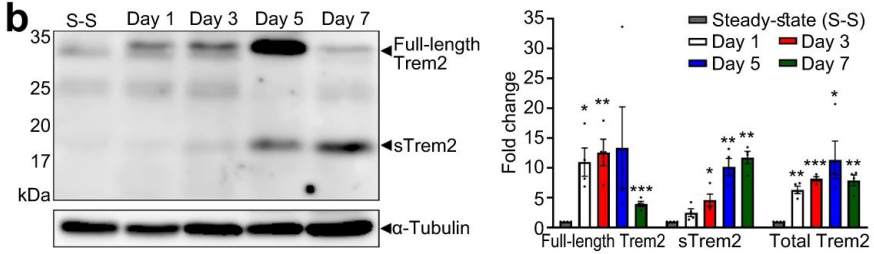
Figure 3d Spline plots showing the expression of typical markers associated with each cluster across the pseudo-time.

Figure 3e Gene expression levels of Clec4e, Hmox1, Trem2, and Lyve1 as per ST-seq.



Expression of Trem2 in the heart after MI

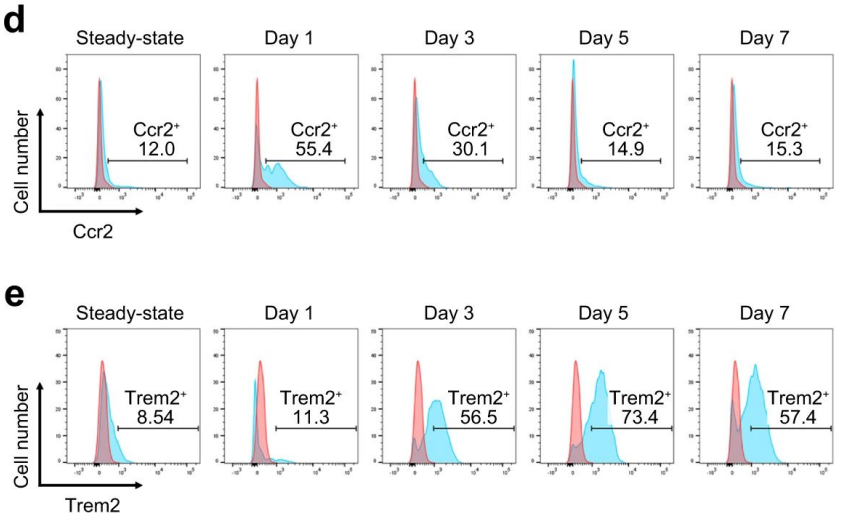




Trem2 is highly expressed by macrophages in the infarcted heart and sTrem2 is exclusively secreted from Trem2^{hi} macrophages.

Figure 5a Immunohistochemistry showed a gradual increase in the expression of Trem2 over time in the infarcted area after the induction of MI, almost absent in the steady-state.

Figure 5b Western blot targeting Trem2.
32 kDa → full- length Trem2 protein
18 kDa → soluble Trem2 (sTrem2).



Expression of Trem2 in the heart after MI

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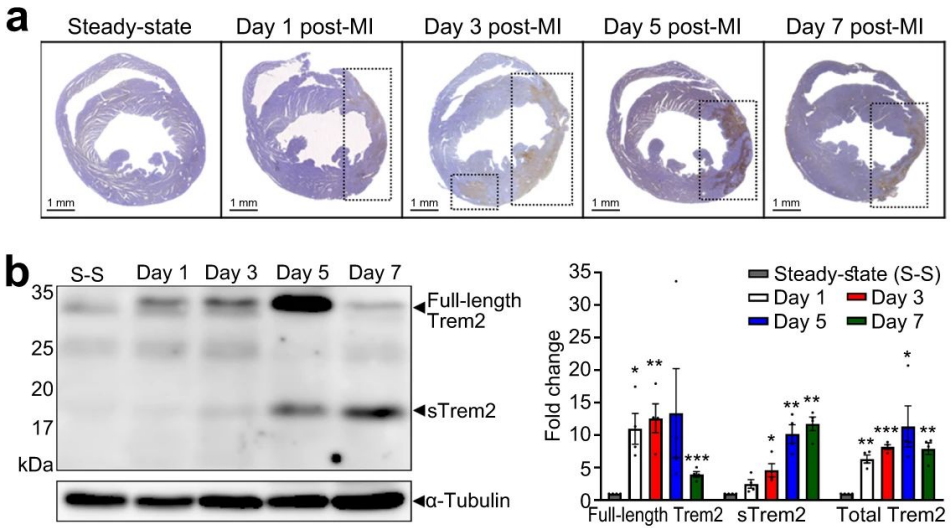
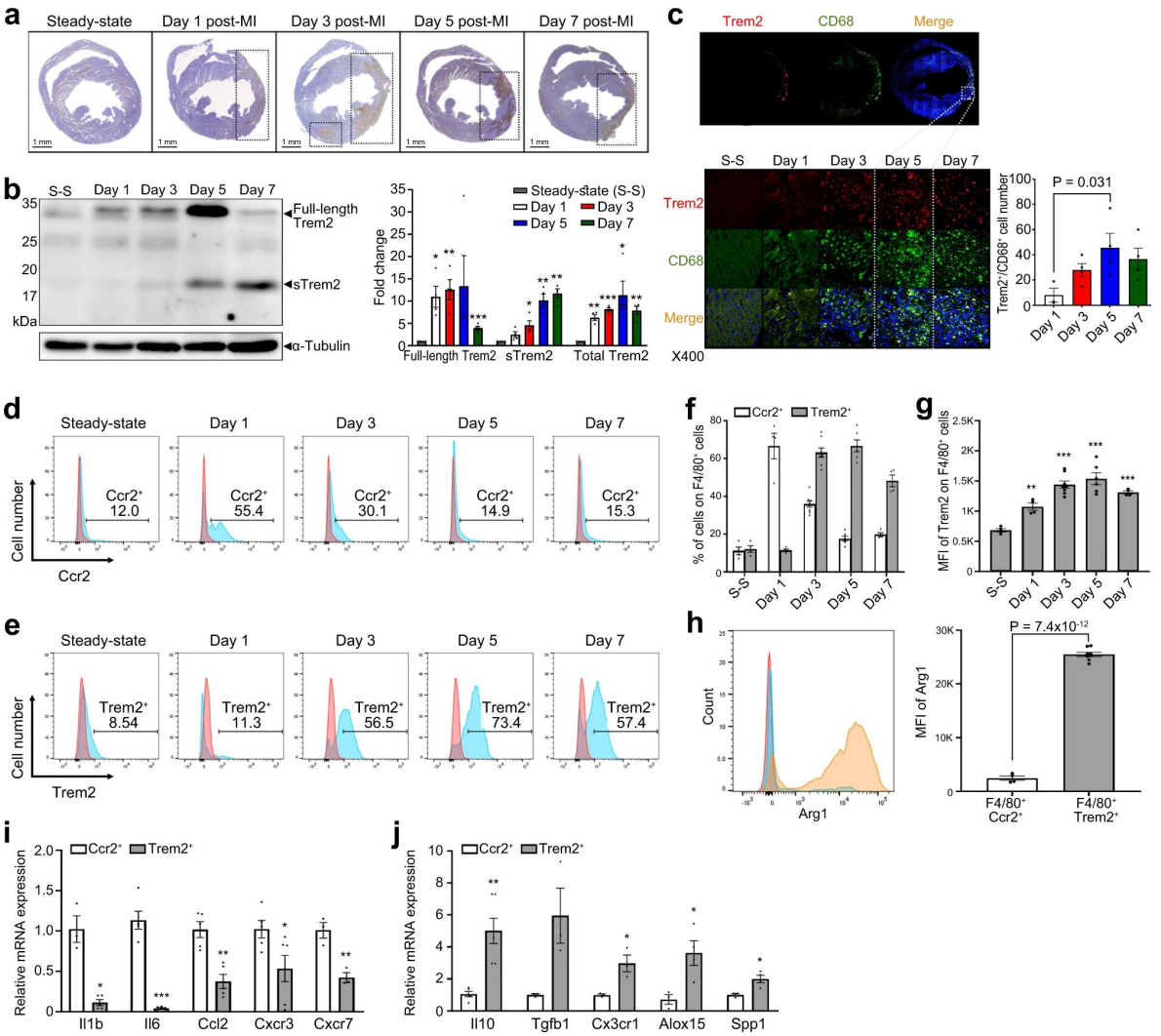


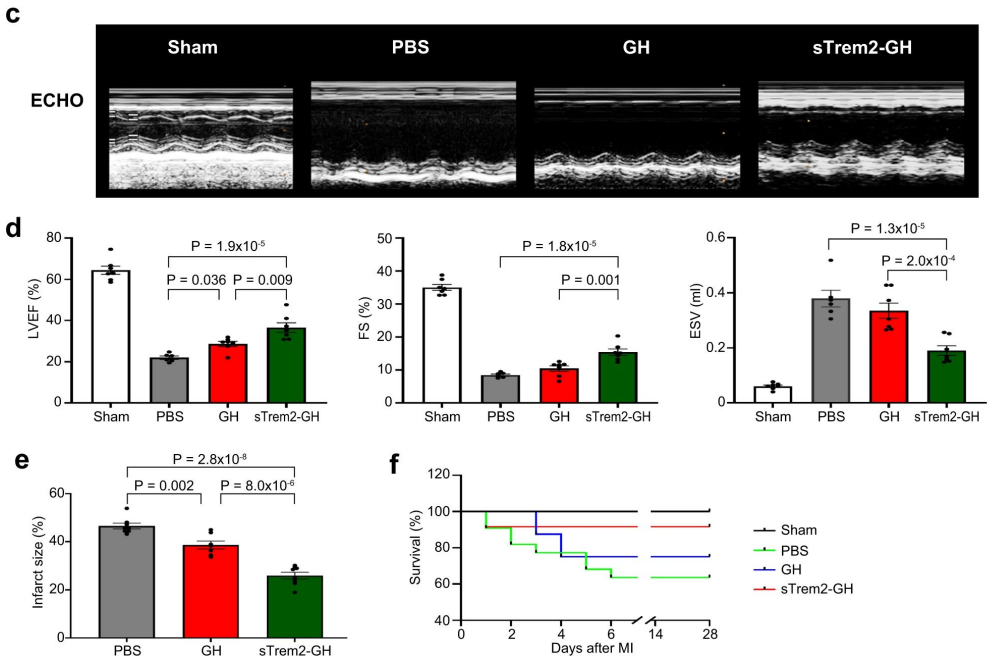

a
Steady-state
Day 1 post-MI
Day 3 post-MI
Day 5 post-MI
Day 7 post-MI
1 mm
b
S-S
Day 1
Day 3
Day 5
Day 7
35
25
20
17
kDa
Full-length Trem2
sTrem2
α-Tubulin
Steady-state (S-S)
Day 1
Day 3
Day 5
Day 7
Fold change
Full-length Trem2
sTrem2
Total Trem2
c
Trem2
CD68
Merge
S-S
Day 1
Day 3
Day 5
Day 7
X400
P = 0.031
Trem2+/CD68+ cell number
d
Steady-state
Day 1
Day 3
Day 5
Day 7
Cell number
Ccr2
Ccr2+ 12.0
Ccr2+ 55.4
Ccr2+ 30.1
Ccr2+ 14.9
Ccr2+ 15.3
e
Steady-state
Day 1
Day 3
Day 5
Day 7
Cell number
Trem2
Trem2+ 8.54
Trem2+ 11.3
Trem2+ 56.5
Trem2+ 73.4
Trem2+ 57.4
f
Ccr2+
Trem2+
% of cells on F4/80+ cells
S-S
Day 1
Day 3
Day 5
Day 7
g
MFI of Trem2 on F4/80+ cells
S-S
Day 1
Day 3
Day 5
Day 7
h
Count
Arg1
P = 7.4x10-12
MFI of Arg1
F4/80+ Ccr2+
F4/80+ Trem2+
i
Ccr2+
Trem2+
Relative mRNA expression
Il1b
Il6
Ccl2
Cxcr3
Cxcr7
j
Ccr2+
Trem2+
Relative mRNA expression
Il10
Tgfb1
Cx3cr1
Alox15
Spp1

Effect of the injection of soluble Trem2 (sTrem2) on the heart of MI mouse.

Mice treated with sTrem2 show:

- Less dilated and well-remodel left ventricle
- Smaller infarct size
- Increased survival rate

sTrem2 promotes the functional and structural improvements of infarcted hearts in vivo



CONCLUSIONS

- ➔ Leukocytes in heart tissues exhibit significant heterogeneity
- ➔ Leukocyte populations become remarkably dynamic after the onset of myocardial infarction (MI)
- ➔ Sequential differentiation process from Ly6c2 hi monocytes to Late-Mφ
- ➔ Trem2 expression significantly enhances remodeling and cardiac function in the infarcted heart.

Thank you for the attention

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