Supplemental Material

Material and methods

Echocardiography

Success surgery and cardiac function in mice were confirmed by echocardiography (Vevo 770, Visual Sonic, Toronto, Canada) 14 days after MI. Two mice died in the MI group before performing echocardiography. After chest hair removal, these surviving mice were anesthetized with 1.5-2% isoflurane, and their heart rates were regulated to be greater than 400 beats per minute. The pictures of the ventricular long axis in M-mode echocardiography were acquired at the level of the papillary muscle tips. The ejection fraction (EF) and fractional shortening (FS) of the left ventricle were computed. Each parameter represented the mean of three measurements. Then, all mice were euthanized postoperatively at 2 weeks for further analysis.

Immunohistochemical analysis

The hearts collected from mice were fixed with formalin, embedded in paraffin wax, and sectioned into thin slices. Next, the sections were treated in xylene and gradually rehydrated through a series of alcohol washes. Antigen retrieval is performed by heating the sections in a citrate solution, to unmask the antigens and make them accessible to the antibodies. Then, the sections were incubated with the serum to block Non-specific binding sites on the tissue sections. The P3h3 antibody (Abcam, ab7817, 1:100) specific to the target protein of interest is applied to the tissue sections. The sections are then incubated at 4°C overnight to allow the antibody to bind specifically to its target antigen. The excess primary antibody is removed by

washing the sections with phosphate-buffered saline. A secondary antibody, conjugated with Horseradish Peroxidase (HRP), is applied to the sections. Similar to the previous step, the sections are washed again to remove any unbound secondary antibodies. After that, the tissue sections are incubated with the substrate, which is deposited at the site of secondary antibody binding. The stained slides were photographed using a digital microscope scanner (Grundium, OCUS) and Image J software was used to assess the positive area rate (positive area/ total area) of immunohistochemical images for P3h3.

qRT-PCR assay of the P3h3

Total RNA from the infarcted and normal left ventricle tissue samples of both groups was extracted with TRIzol Reagent (EnzyArtisan, China). According to the manufacturer's instructions, 1μg RNA (RNA samples) from total RNA were treated with gDNA Remover, as well as synthesized into cDNA using a HyperScript III RT SuperMix for qRT-PCR with gDNA Remover (EnzyArtisan, China). qRT-PCR was performed with Universal SYBR qPCR master mix (EnzyArtisan, China) and gene-specific primers on a QuantStudio 6 real-time PCR System according to the instructions. The gene-specific primers were as follow: P3H3-Forward: 5′-TCACAGGATTCTTGGACCCC-3′, P3H3-Reverse: 5′-GTCCCAGGGCTTCTTCTCTT-3′; Beta-actin-Forward: 5′-CATTGCTGACAGGATGCAGAAGG-3′, Beta-actin-Reverse: 5′-TGCTGGAAGGTGGACAGTGAGG-3′. The qRT-PCR protocol was performed in a 96-well plate using a pre-PCR step at 95 °C for 5 min, denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s and elongation at 72 °C for 20 s. qRT-PCR was adjusted to 35 cycles. The

melting temperature of P3H3 was 83°C after amplification. The primer melting curve showed a single peak. Relative mRNA expression was compared with the 2−ΔΔCt method.[1]

SnRNA-seq data analysis

Filtered feature barcode matrices were read by using the function “Read10X” and “CreateSeuratObject”, and then merge into two Seurat-compatible objects based on group classification using the function “merge”. The “Seurat” package was used to perform quality control, normalization, and downstream analysis. Quality control was performed as described previously.[2] After quality control, we performed normalization and identified highly variable features of two objects, and we integrated two objects by using the function “FindIntegrationAnchors” and “IntegratedData”. Then, we performed ScaleData for linear regression on all genes and performed RunPCA for dimensional reduction. The first 50 principal components were determined and then were used as input for Uniform manifold approximation and projection (UMAP) dimensionality reduction to visualize the spots. DEGs were detected by the function “FindAllMarkers” and were further applied to guide the manual annotation of clusters.

References

1. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25(4):402-8. doi:10.1006/meth.2001.1262

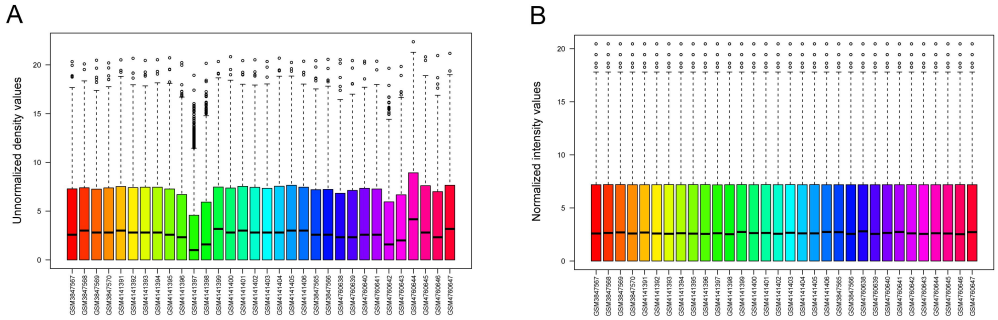
2. Yamada S, Ko T, Hatsuse S, et al. Spatiotemporal transcriptome analysis reveals critical roles for mechano-sensing genes at the border zone in remodeling after myocardial infarction. *Nature Cardiovascular Research*. 2022;1(11):1072-1083. doi:10.1038/s44161-022-00140-7

Supplementary Table 1. Top three gene interaction networks based on the MCODE plug-in

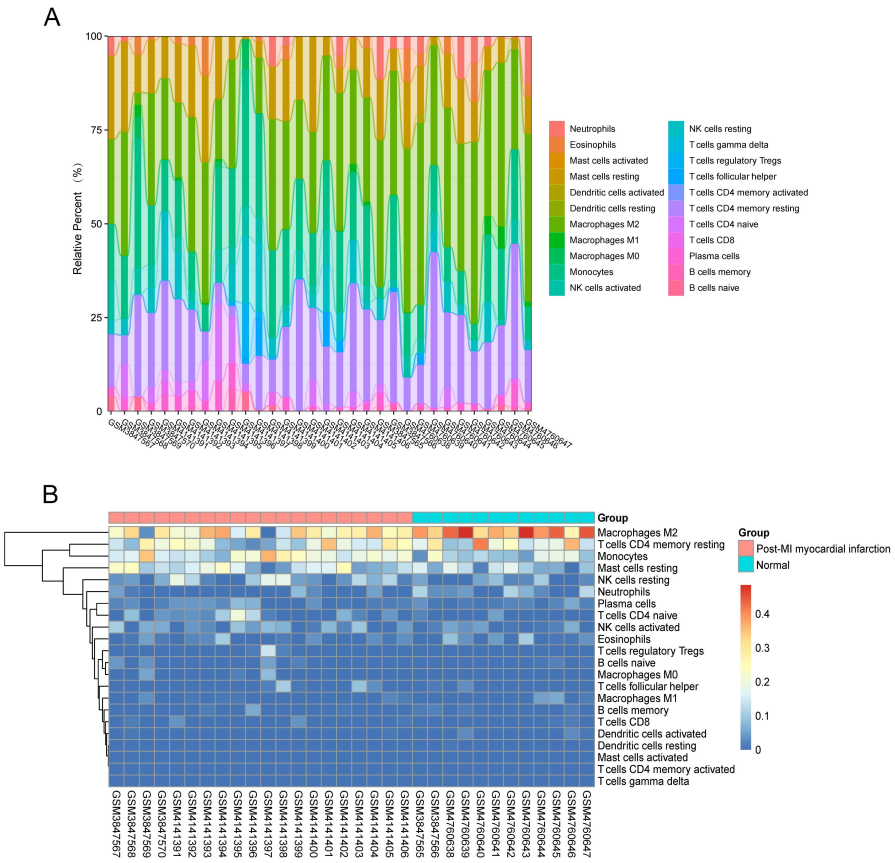
Cluster	Score	Nodes	Edges	Node IDs
1	13.385	14	174	COL9A2, COL15A1, COL16A1, COL1A2, COL6A2, COL9A1, COL5A1, COL27A1, COL22A1, COL11A2, COL1A1, P3H3, COL18A1, COL6A1
2	10.471	18	178	SETD1A, UHRF1, HIST1H3J, KMT2D, TTK, HIST1H2BH, KMT2B, SETD1B, KDM6B, CCNA2, HIST1H2BB, CENPE, HIST1H4F, HIST1H2BJ, DLGAP5, CDK1, BLM, CEP55
3	10.000	10	90	RPS8, RPL22L1, RPS16, RPS27, RPS10, TPT1, MRPL13, RPL39, RPLP1, RPS13

Supplementary Table 2. Top 10 genes generated by the MCC and DMNC algorithms

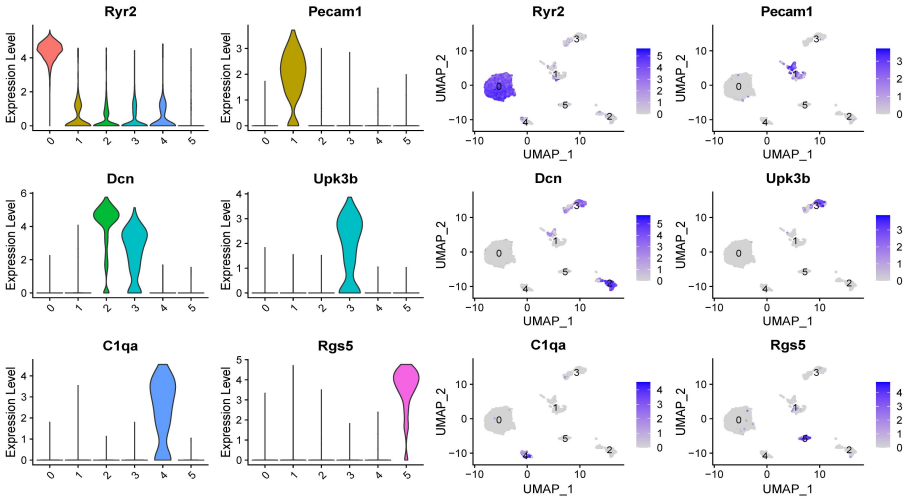
MCC	Score	DMNC	Score
COL1A1	8.07E+07	P3H3	1.89042
COL6A1	8.07E+07	COL16A1	1.84683
COL1A2	8.07E+07	COL22A1	1.78823
COL5A1	8.07E+07	COL15A1	1.66247
COL11A2	8.06E+07	COL9A2	1.57946
COL16A1	8.06E+07	COL9A1	1.57656
COL27A1	8.06E+07	COL27A1	1.56151
P3H3	8.06E+07	CENPE	1.53668
COL18A1	8.00E+07	TTK	1.53668
COL15A1	7.99E+07	CCDC124	1.53668



Supplementary Figure 1 Normalization of the GSE132143 dataset. (**A**) data before normalization. (**B**) data after normalization.



Supplementary Figure 2 Bar chart (**A**) and cluster heatmap (**B**) of the relative percentage of 22 immune cells.



Supplementary Figure 3 The Distribution and expression of marker genes in all cell clusters.