Supporting Information for

Original article

Renewal of embryonic and neonatal-derived cardiac-resident macrophages in response to environmental cues abrogated their potential to promote cardiomyocyte proliferation *via* Jagged-1–Notch1

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Received 2 February 2022; received in revised form 7 July 2022; accepted 18 August 2022

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Supporting Figures Fig. S1–S5

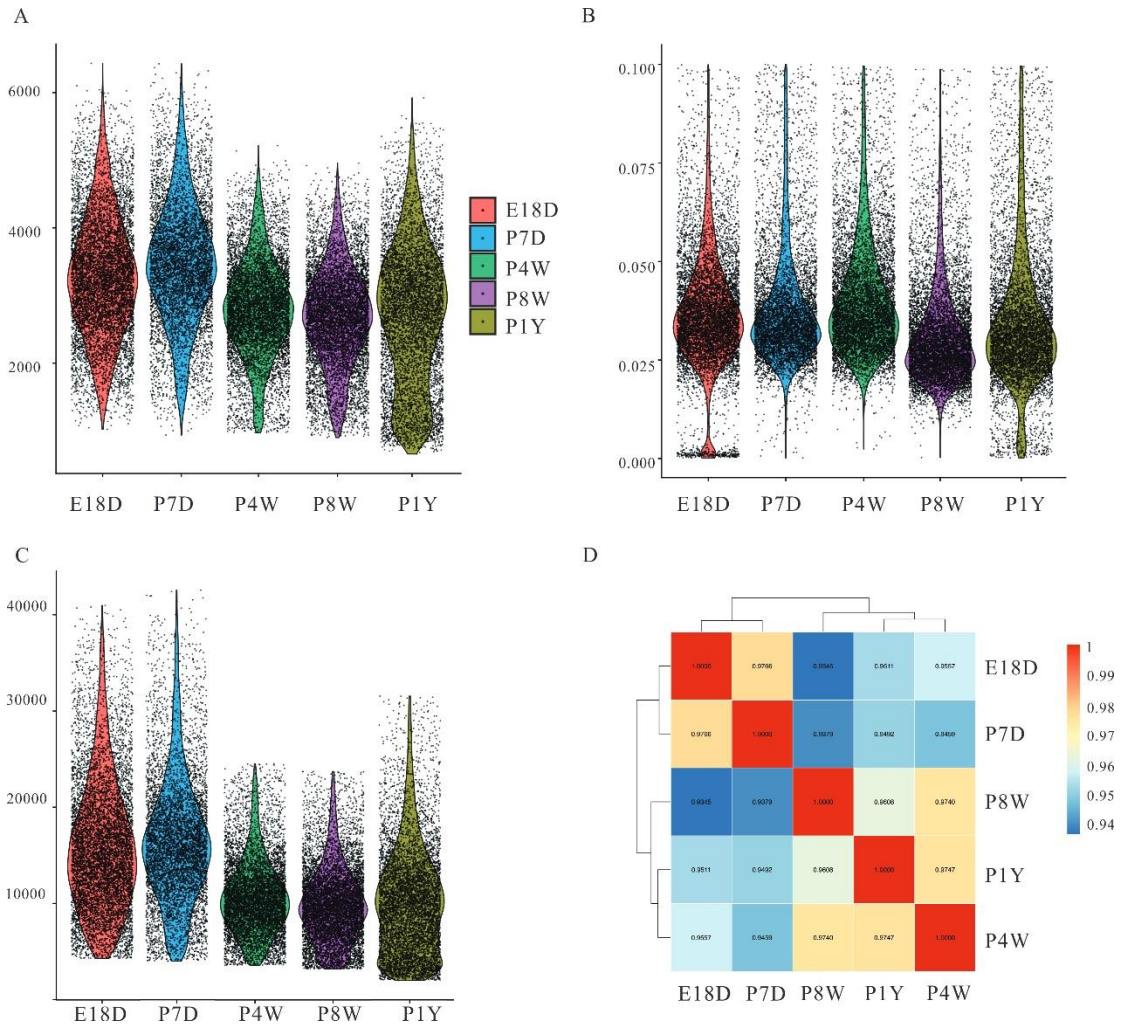

Figure S1. Quality control for scRNA-Seq data. Violin plots show several genes (A), percent mitochondrial reads (B), and unique molecular identifiers (C). 8000–10,000 cells per group. (D) Calculating mean correlation coefficient of CRMs grouped by samples. The heatmap shows mean correlation coefficient between gene expression profiles of macrophages from the different samples. Total of 800 cells were included. E18D, P7D, P4W and P1Y mean embryonic Day 18, post-natal 7 days, 4 and 8 weeks, and 1-year-old, respectively.

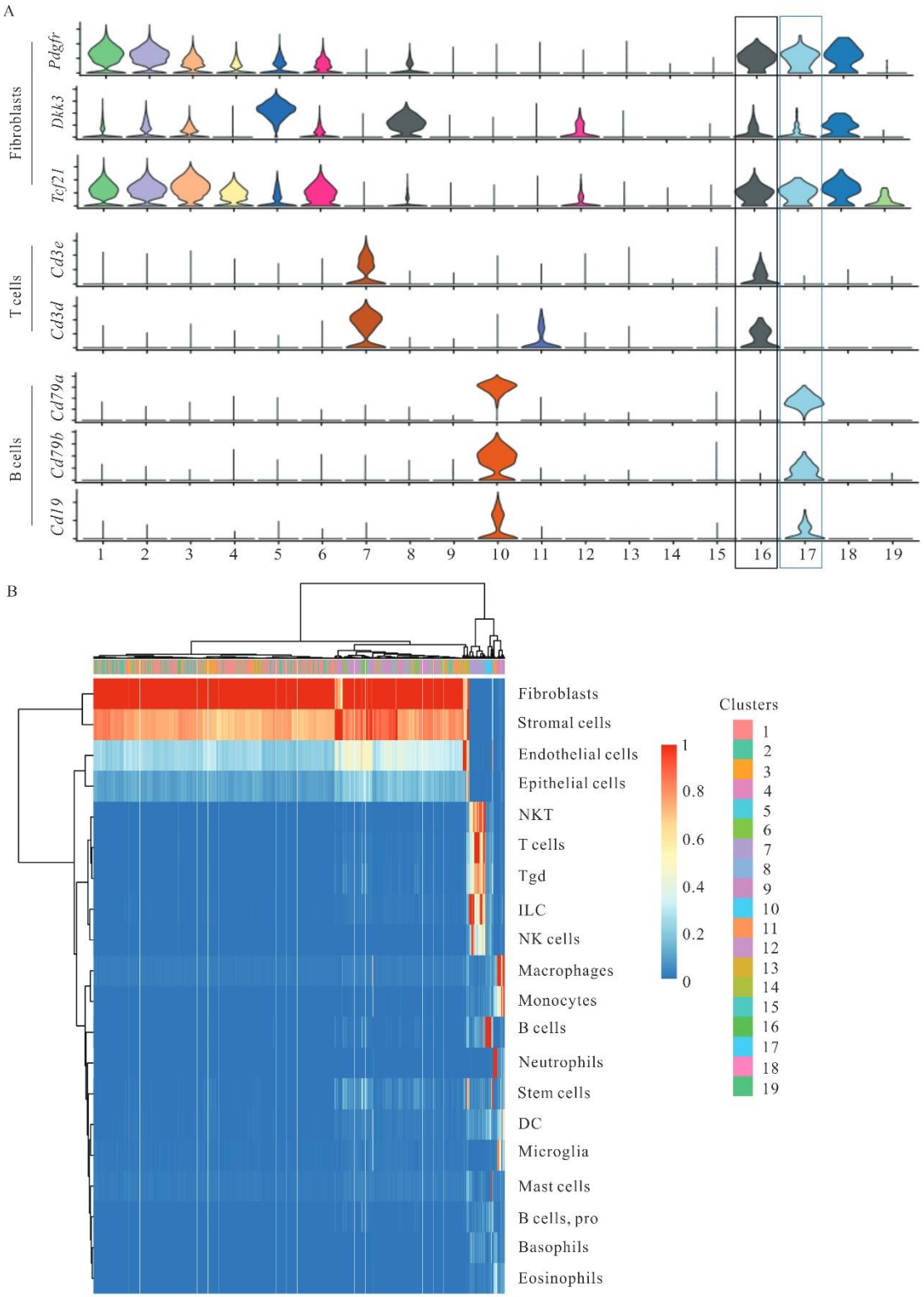

Figure S2. (A) Violin plots show the expression level of representative T cells, B cells, and fibroblast marker genes across the 19 main clusters. Y-axis is log scale normalized read count. (B) Correlation between the single-cell RNA sequencing data and reference data sets, using the SingleR package to annotate the cell type. 8000-10,000 cells per group.

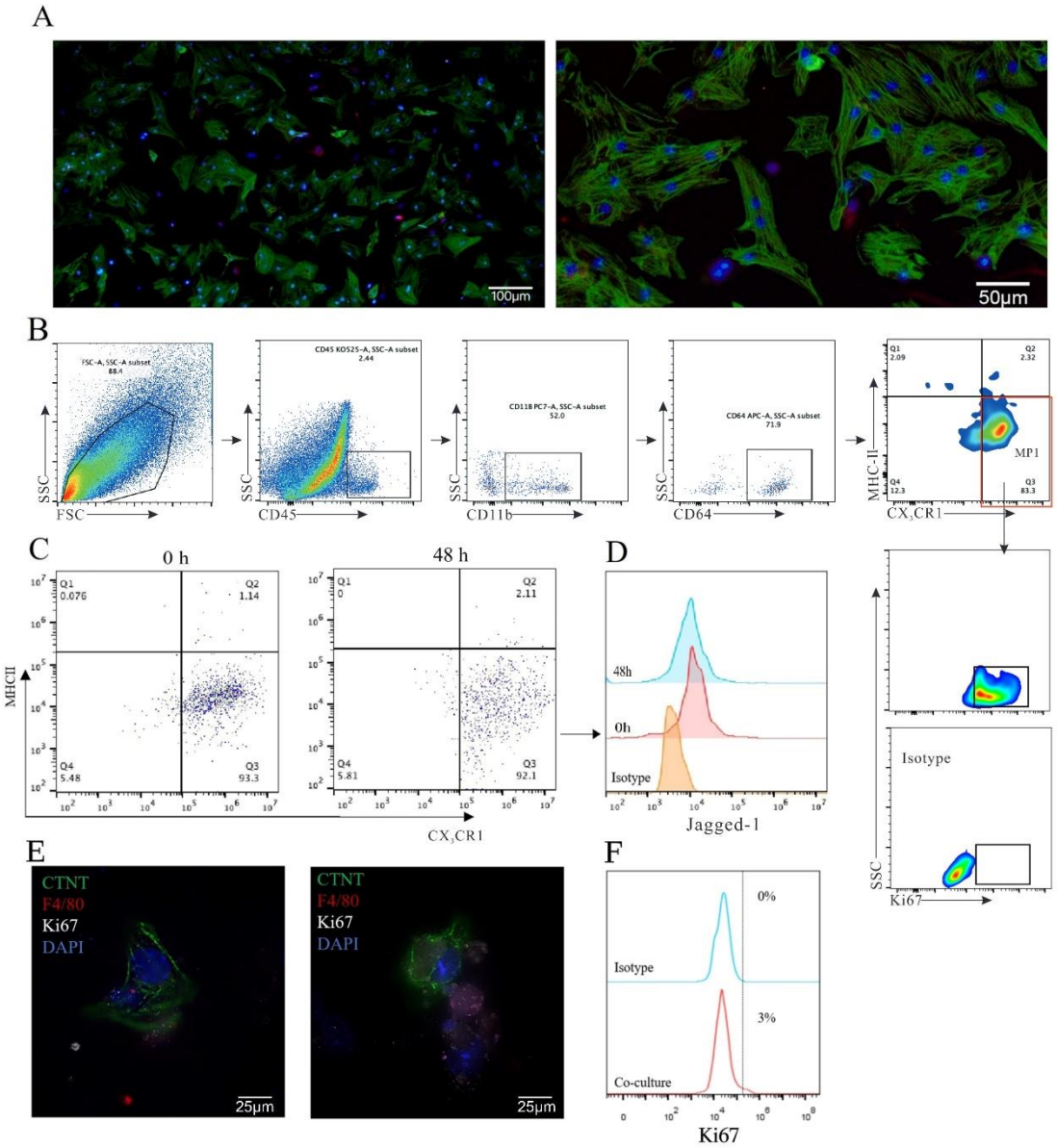

Figure S3. (A) The purity of isolated neonatal mouse cardiomyocytes was identified by immunofluorescence staining. (B) The proportions of MP1 and their expression of Ki67 in embryonic or neonatal mice. $CD45^{+}CD64^{+}CX3CR1^{+}MHCII^{-}$ were gated to reveal MP1s proportions. $Ki67^{+}$ MP1 were gated to analyze their proliferative ability. (C, D) MP1 phenotype was unchanged during in vitro culturing for 48 h by flow cytometry. (E) Immunofluorescence was analyzed by laser confocal microscopy, CRMs were labeled by F4/80, cardiomyocytes were labeled by cTNT. (F) Mean fluorescence intensity of Ki67 signal in CRMs from embryonic and neonatal mice co-cultured with neonatal mouse cardiomyocytes. All the data were repeated three times.

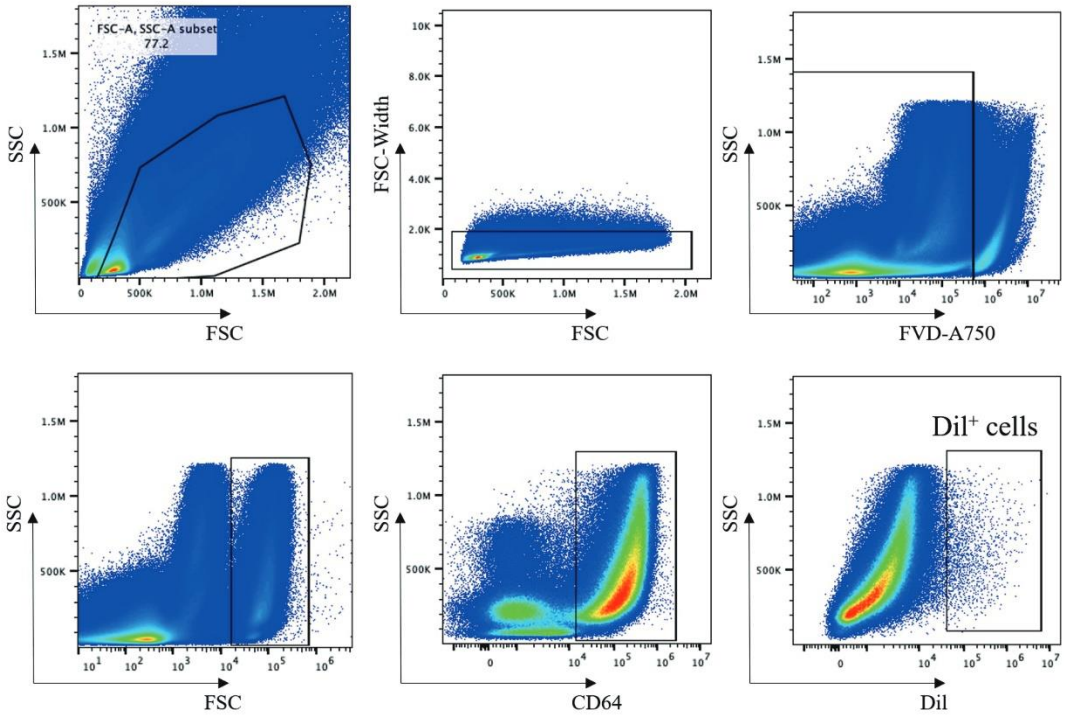

Figure S4. Dil-labeled CRMs have entered heart. Six-week-old male BALB/c mice were used to establish MI models by ligating the left coronary artery, then 2×10^4 labeled CRMs by 10 μmol/L Dil from embryonic or neonatal mice were intra-myocardial injection in the ischemic left anterior descending coronary artery territory immediately into MI mice, five mice were included. MI means myocardial infarction.

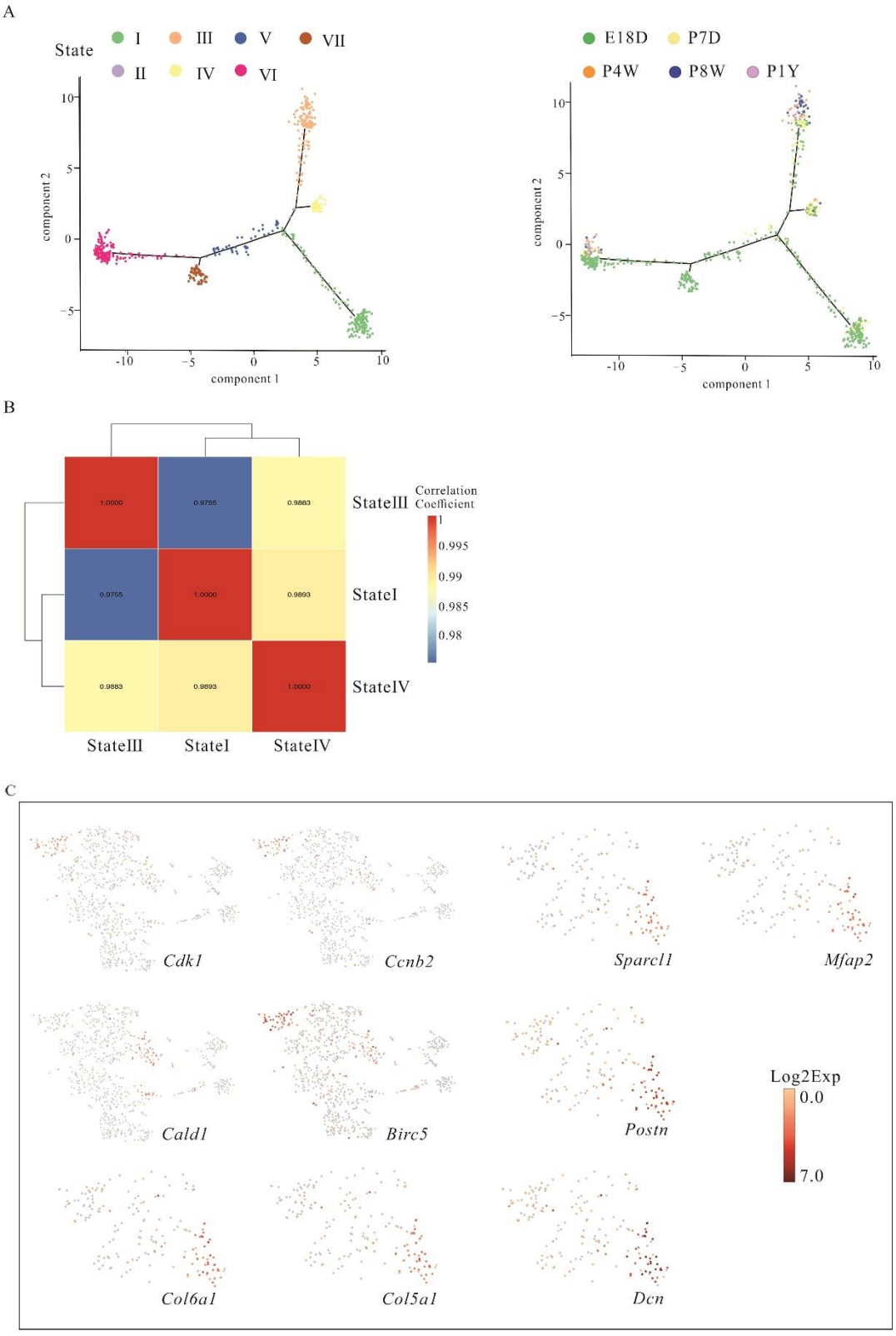

Figure S5. Trajectory analysis of CRMs. (A) Ordering macrophages along a cell conversion trajectory using Monocle package. Each color indicates a time point or a CRM state. (B) The heatmap shows mean correlation coefficient between gene expression profiles of MP1 from different states. (C) Cell distributions of pseudotime-

based “state I”, “state III” and “state IV” MP1 on t-SNE map. T-SNE sub-clustering of “state I” based on their marker genes. T-SNE plots show upregulated expression of representative cell cycle genes (red). 30–50 cells per category were included. E18D, P7D, P4W and P1Y mean embryonic Day 18, post-natal 7 days, 4 and 8 weeks, and 1-year-old, respectively.