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Supplemental information

Alpha-1 adrenergic signaling drives cardiac regeneration via extracellular matrix remodeling transcriptional program in zebrafish macrophages

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Supplemental information and legends

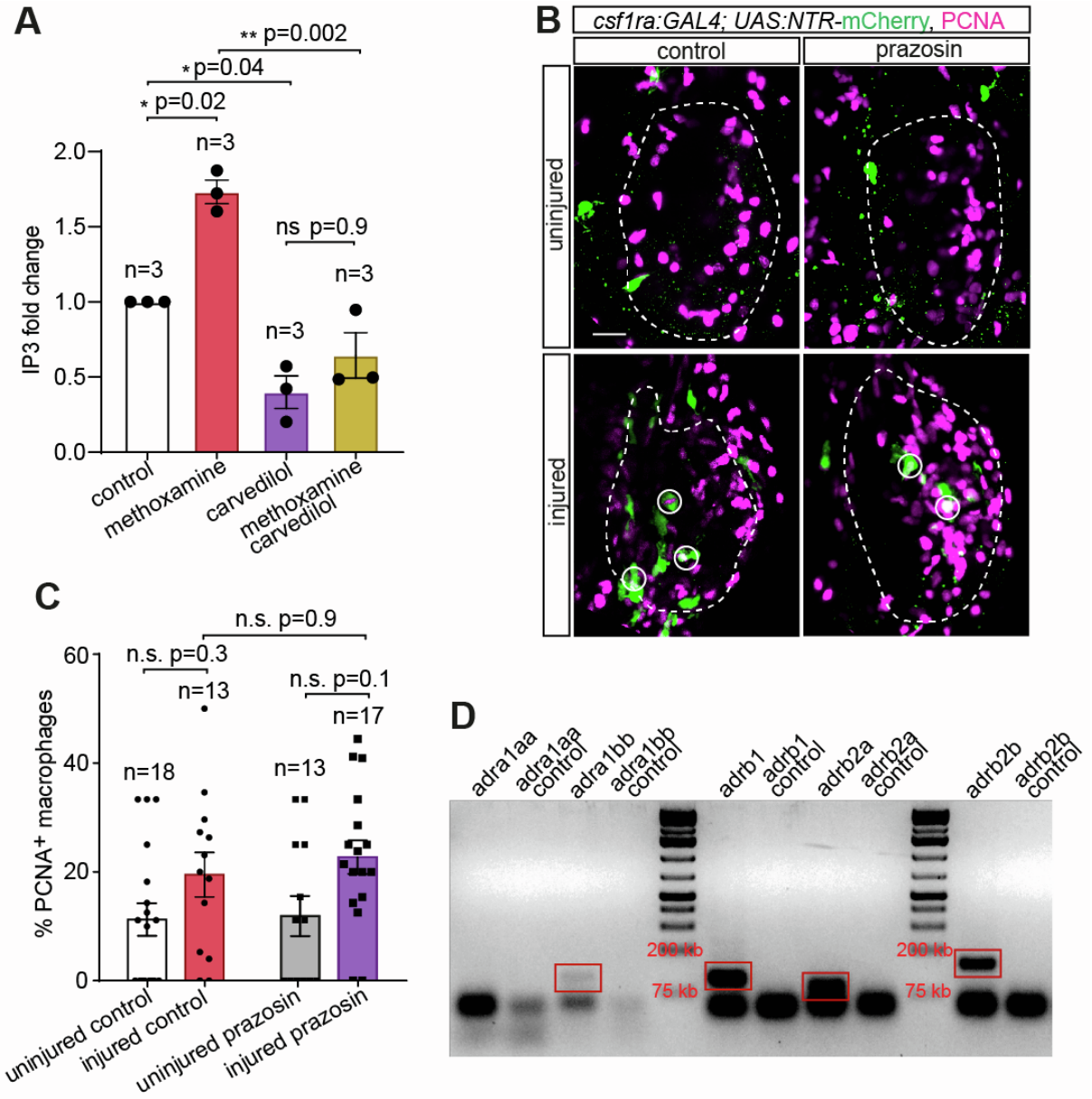

Figure S1. Assessment of efficiency of carvedilol Adra1 inhibition, prazosin effect on macrophage proliferation, and expression of adrenergic receptors in macrophages (related to figure 1). (**A**) Bar graph with data points from individual biological replicates (each was a pool of 20-30 larvae) showing IP3 amounts in 5 dpf wild-type larvae, treated with the Adra1 agonist methoxamine and/or the beta-adrenergic antagonist carvedilol, measured by ELISA and presented as fold change relative to unstimulated controls. (**B**) Proliferating macrophages in uninjured and injured *csf1ra:Gal4; UAS:NTR-mCherry* larval hearts, labeled by mCherry (green) and PCNA (magenta) immunofluorescence staining, following treatment with control solution or prazosin. Injury was induced with a two-photon laser at 7

dpf and drug treatment lasted for 1 dpi. White dashed lines outline the ventricular area. White circles mark proliferating macrophages ($mCherry^{+}/PCNA^{+}$). Scale bar: 20 μm. (**C**) Bar graph with data points from individual animals showing percentages of proliferating macrophages in the treatment groups depicted in (**B)**. (**D**) Reverse transcription polymerase chain reaction (RT-PCR) detection of mRNA encoding adrenergic receptors in macrophages isolated by fluorescence-activated cell sorting (FACS) from *csf1ra:Gal4; UAS:NTR-mCherry* larvae. Control lanes contain samples in which RT-PCR was performed without cDNA. Data in graphs are presented as mean ± S.E.M. n indicates number of biological replicates (**A**) or number of animals (**C**) measured for each group. * $p<0.05$, ** $p<0.01$, n.s. not significant, two-tailed t-test.

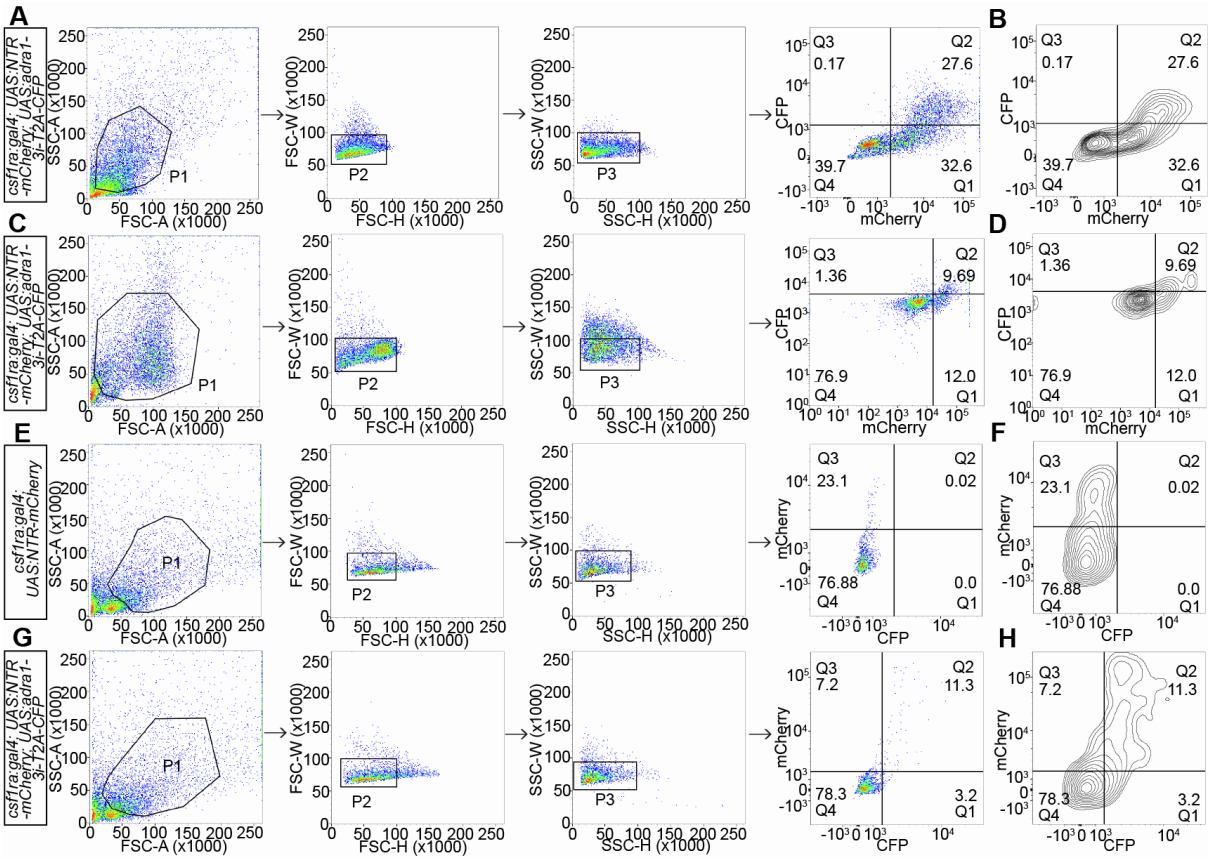

Figure S2. Mixed populations of Adra1 signaling-deficient and control macrophages in the *csf1ra:GAL4; UAS:adra1-3i-T2A-CFP*; *UAS:NTR-mCherry* line (related to figure 2). (**A-D**) Gating strategy for flow cytometry analysis of Adra1 signaling deficient cells (CFP^+) in the whole macrophage population ($mCherry^+$) from a pool of 50 larvae (**A** and **B**) and 3 adult hearts (**C** and **D**), shown as dot plots (**A** and **C**) and contour plots (**B** and **D**). In both larvae and adult hearts, *adra1-3i-T2A-CFP*$^+$ (Q2 in **B** and **D**) macrophages are circa 45% of the total (NTR-$mCherry^+$) macrophage population (Q1 + Q2 in **B** and **D**). (**E-H**) Gating strategy for flow cytometry analysis of *csf1ra:GAL4; UAS:NTR-mCherry* (**E** and **F**) and *csf1ra:GAL4; UAS:adra1-3i-T2A-CFP; UAS:NTR-mCherry* (**G** and **H**) adult hearts shown as dot plots (**E** and **G**) and contour plots (**F** and **H**). NTR-mCherry+ macrophages are circa 23% and 19% (Q2 +Q3) in *csf1ra:GAL4; UAS:NTR-mCherry* and *csf1ra:GAL4; UAS:adra1-3i-T2A-CFP; UAS:NTR-mCherry*, respectively.

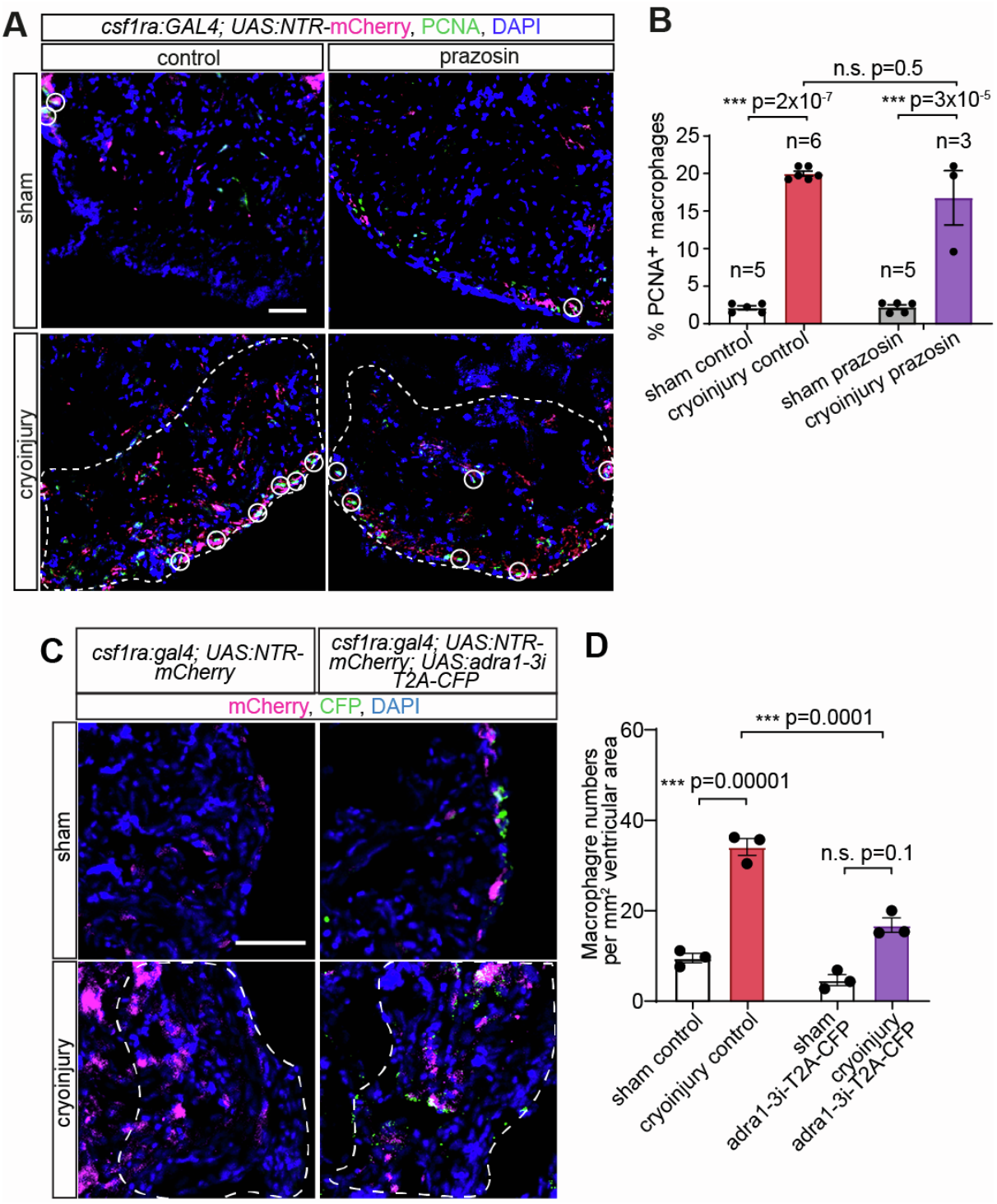

Figure S3. **Unchanged proliferation rate but lower number of macrophages found in the infarcted lesion upon macrophage-specific Adra1 signaling deficiency (related to figure 3).** (**A**) Proliferating macrophages in sham and cryoinjured *csf1ra:Gal4; UAS:NTR-mCherry* adult hearts, labeled by mCherry and PCNA immunofluorescence staining following treatment with control solution or prazosin. DAPI labels all nuclei. Lesioned areas are outlined by white dashed lines and proliferating

macrophage by white circles. Scale bar: 50 μm. (**B**) Bar graph with data points from individual animals showing percentages of proliferating macrophages. (**C**) *csf1r:GAL4; UAS:NTR-mCherry* (control) and *csf1r:GAL4; UAS:adra1-3i-T2A-CFP; UAS:NTR-mCherry* zebrafish which underwent sham operation or cryoinjury. All macrophages ($mCherry^+$) and Adra1-$3i^+$ macrophages ($mCherry^+$, CFP^+) in the heart sections were visualized by immunofluorescent staining. Scale bar: 50 μm. (**D**) Bar graph with data points from individual animals depicting numbers of macrophages ($mCherry^+$) per mm^2 measurement area. Data are presented as mean ± S.E.M. n indicates number of animals measured for each group. *** $p<0.001$, n.s. not significant, two-tailed t-test.

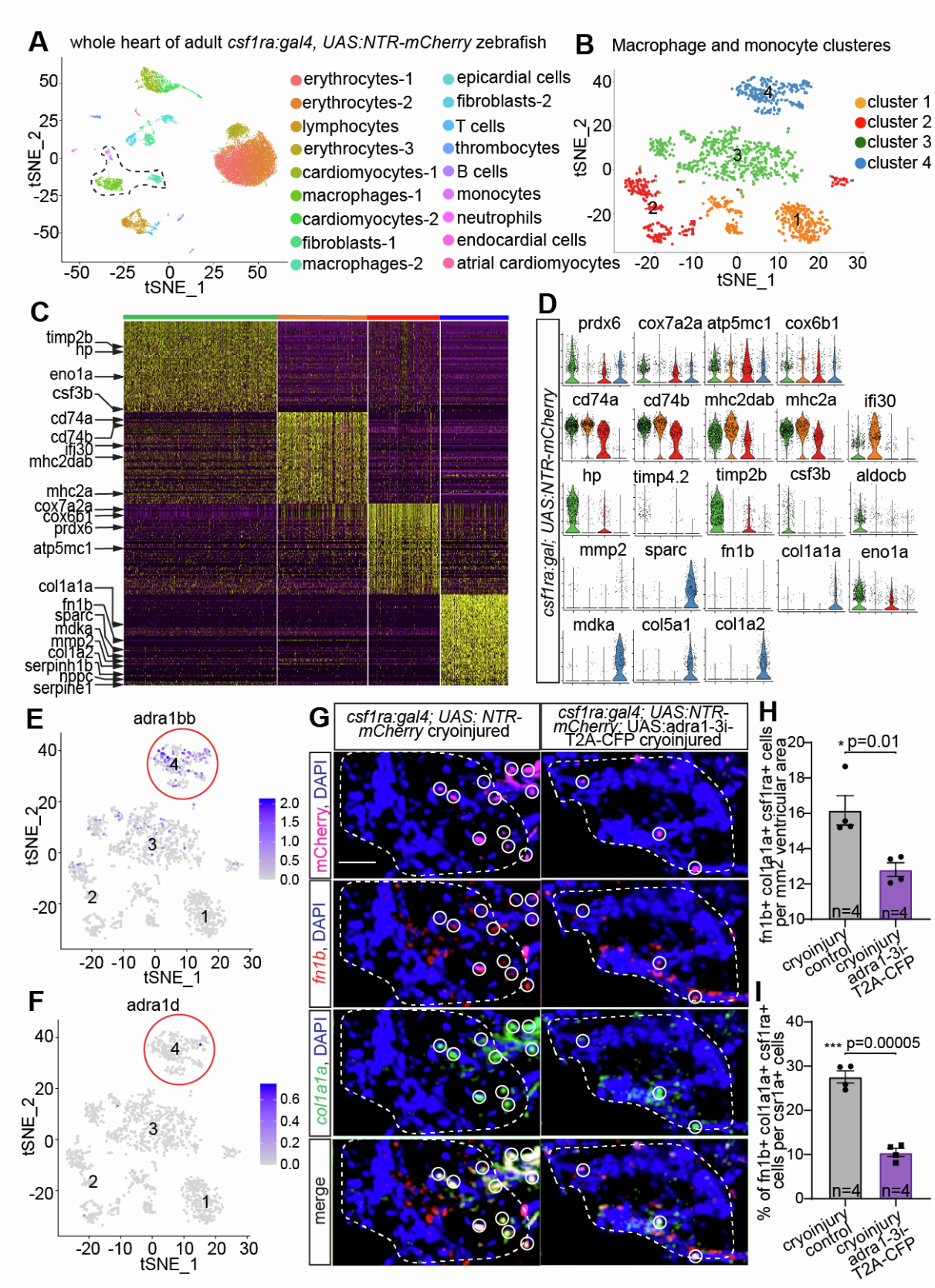

Figure S4. Macrophage subsets presented in the *csf1r:GAL4; UAS:NTR-mCherry* hearts and verification of *fn1b* and *col1a1a* expression in the 'ECM remodeling' subset (related to figure 4).

(**A**) t-distributed stochastic neighbor embedding (t-SNE) representation of whole heart single-cell clustering from *csf1r:GAL4; UAS:NTR-mCherry* zebrafish at 7 dpi. Dashed line outlines major macrophage/monocyte populations. A pool of 3 hearts was analyzed. (**B**) t-SNE dimensionality reduction showing four clusters of macrophages. (**C**) Heatmap of the 70 most differentially expressed genes in each macrophage cluster highlighted comparable expression profiles as the populations detected in *csf1r:GAL4; UAS:adra1-3i-T2A-CFP; UAS:NTR-mCherry* hearts. (**D**) Violin plots of cluster-defining genes. (**E** and **F**) Feature plots depicting expression distribution of adra1bb (**E**) and adra1d (**F**) in cardiac macrophages at 7 dpi. Cluster 4 designated as 'ECM remodeling' subset is marked with a red circle. Color bars indicate normalized expression values. (**G**) Immunofluorescence staining of mCherry (to detect *csf1ra*$^{+}$ cells) and *col1a1a*, and *fn1b* hybridization chain reaction (HCR)-fluorescent in situ hybridization (FISH) in 7 dpi *csf1r:GAL4; UAS:NTR-mCherry* (control) and *csf1r:GAL4; UAS:adra1-3i-T2A-CFP; UAS:NTR-mCherry* heart sections. White circles mark *col1a1a*$^{+}$/*fn1b*$^{+}$ macrophages. Scale bar: 20 μm. (**H** and **I**) Bar graphs with individual data points displaying density (**H**) and percentages (**I**) of 'ECM remodeling' macrophages (*fn1b*$^{+}$, *col1a1*$^{+}$, *csf1ra*$^{+}$) observed at the lesion site. Bar graphs display mean ± S.E.M. n denote number of animals measured for each group. * $p<0.05$, *** $p<0.001$, two-tailed t-test.

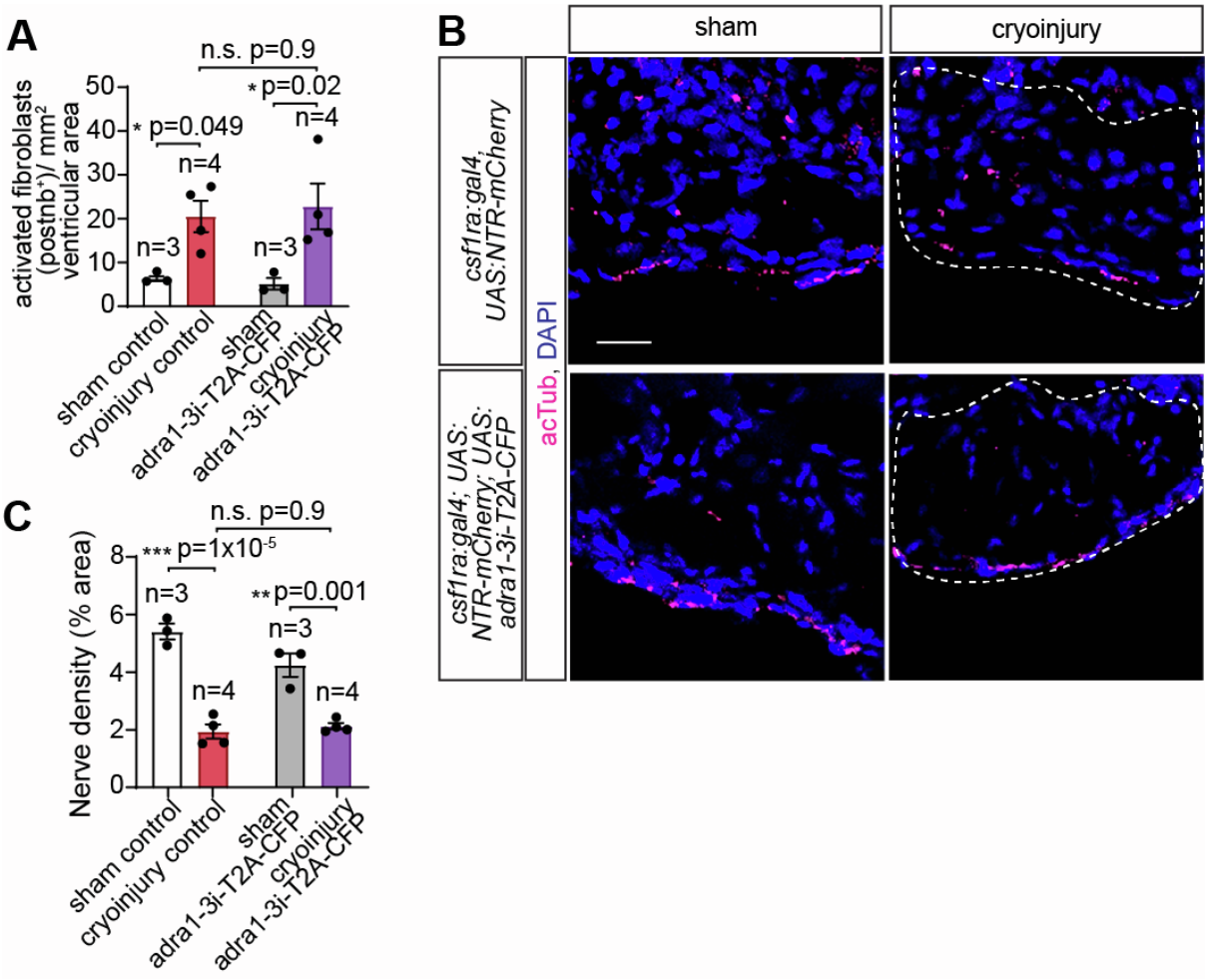

Figure S5. **Unaltered number of activated fibroblasts and re-innervation of infarcted lesion upon macrophage-specific Adra1 loss-of-function (related to figure 5).** (**A**) Bar graph with individual animal data points displaying density of activated fibroblasts (*postnb*$^{+}$ cells) per 1 mm^{2} area of the whole ventricle in sham-operated and cryoinjured control and *csf1ra:Gal4; UAS:NTR-mCherry; UAS:adra1-3i-T2A-CFP* hearts. (**B**) Nerves in sham-operated and cryoinjured control and *csf1ra:Gal4; UAS:NTR-mCherry; UAS:adra1-3i-T2A-CFP* heart sections were detected by immunostaining of acTub at 7 dpi, and quantified as percentage of acTub-labelled nerve area over total area measured (**C**). Scale bar: 20 μm. DAPI labeled all nuclei. White dashed lines demark injured areas. Bar graphs display mean ± S.E.M, and data points of individual animals. n denote number of animals measured for each group. * p<0.05, ** p<0.01, *** p<0.001, n.s. not significant, two-tailed t-test.

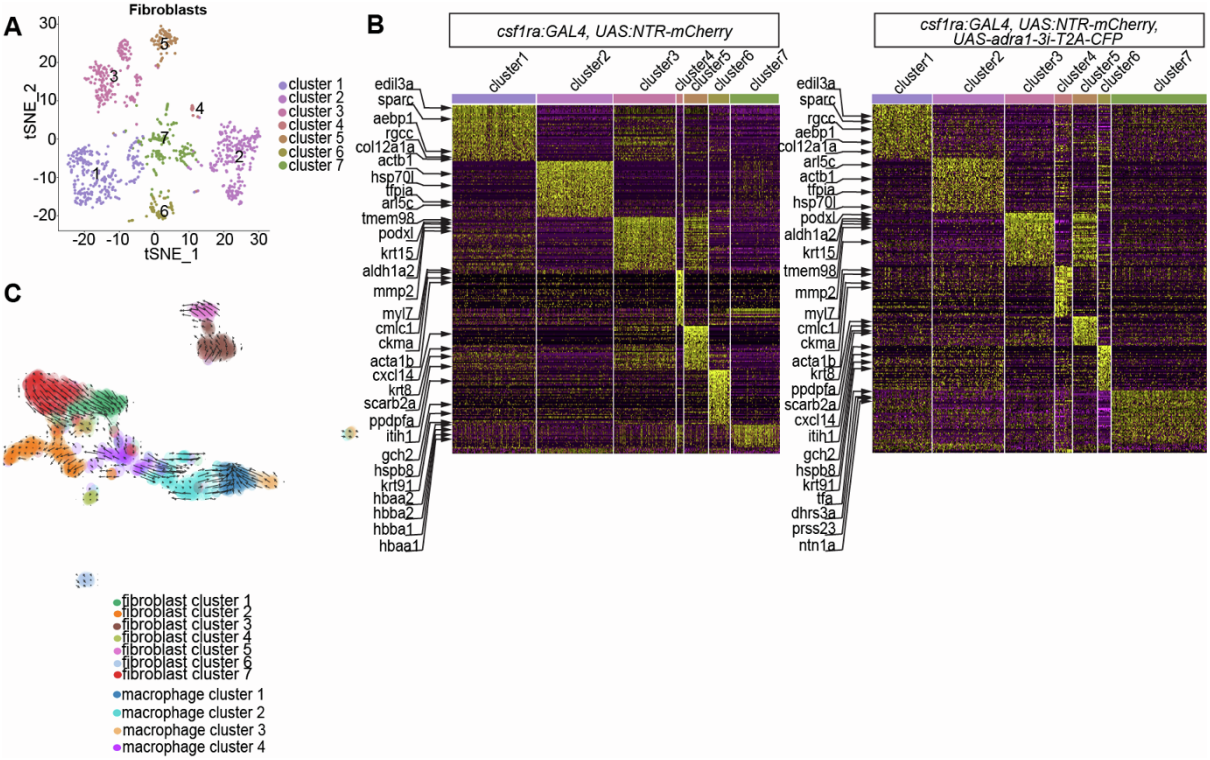

Figure S6. Fibroblast populations detected in 7 dpi hearts (related to figure 6). (**A**) t-SNE dimensionality reduction showing different fibroblast clusters in cryoinjured *csf1ra:Gal4; UAS:NTR-mCherry* hearts. (**B**) Heatmaps of the 50 most differentially expressed genes in *csf1ra:Gal4; UAS:NTR-mCherry* and *csf1ra:Gal4; UAS:NTR-mCherry; UAS:adra1-3i-T2A-CFP* hearts. (C) RNA velocity analysis suggested no cellular transition from macrophages to fibroblasts, and fibroblast cluster 7 as a precursor of cluster 1 (*col12a1a*$^{+}$ subset).

Table S1. Oligonucleotides used in this study.

Forward primer adra1 3i loop	5'-CACACGAATTCGCCGCCACCATGGTGGCCAAAATGACCACTAA-3'
Reverse primer 1 for adra1 3i loop	5'-CGTCACCGCATGTTAGAAGACTTCCTCTGCCCTCACCAGATCCTTTCTTTTCCCTGGAAAACTTG-3'
Reverse primer 2 for adra1 3i loop	5'-AGCTCTTCACCCTTGCTGACAGGGCCGGGATTCTCCTCCACGTCACCGCATGTTAGAAG-3'
Forward primer for CFP	5'-TGGAGGAGAATCCCGGCCCTGTCAGCAAGGGTGAAGAGC-3'

Reverse primer for CFP	5'-TATGATCTAGAGTCGCGGCCGCTCACTTATACAGTTCGTCCATACCC-3'
Forward primer for adra1aa	5’-TATCGTGGTGGGATGCTTCG-3’
Reverse primer for adra1aa	5’-CGTTGGGAAGATGGAACCGAT-3’
Forward primer for adra1bb	5’-TTTGCCAATTGTTTCATTCAACACC-3’
Reverse primer for adra1bb	5’-AGCAGGGGTAGATGATGGGA-3’
Forward primer for adrb1	5’-GGGTTACTGGTGGTGCCATT-3’
Reverse primer for adrb1	5’-GCGTGACGCAAAGTACATC-3’
Forward primer for adrb2a	5’-GCTTCCAGCGTCTTCAGAAC-3’
Reverse primer for adrb2a	5’-CCGAAGGGAATCACTACCAA-3’
Forward primer for adrb2b:	5’-CTCGTTCCTACCCATCCACA-3’
Reverse primer for adrb2b	5’-ATGACCAGCGGGATGTAGAA-3’