

Fig. S1. $pdgfr\beta$ expression in the developing zebrafish heart.

(A) Representative images of $pdgfr\beta$: Citrine; fli1a:DsRed hearts used for quantification during coronary vessel development. Proximal (near) and distal (away) to atrioventricular canal (AVC) areas are highlighted with boxes at 55, 60, 69 and 76 dpf. Arrowheads indicate the $pdgfr\beta$ + mural cells. Zoomed in images are on the right. (B-B") $pdgfr\beta$ expression in early juvenile hearts (33 dpf). Double (B) and single (B', B") channel images of $pdgfr\beta$: Citrine and tcf21:DsRed. A, atrium, V, ventricle, AVC, white arrow. In bulbus arteriosus (B.A.), tcf21 signal is weak and does not overlap with the $pdgfr\beta$. n = 4. (C) Quantification of $pdgfr\beta$ + cells derived from tcf21 lineage-traced cells. In 87 dpf old $TgBAC(pdgfr\beta$:Citrine; tcf21:CreERt2; ubi:loxp- EGFP-loxp-mCherry) fish, ~76.15% of the $pdgfr\beta$ + mural cells were co-labelled with tcf21 lineage-traced cells. ~23.85% of the $pdgfr\beta$ + mural cells were not tcf21 lineage traced. Error bars, standard deviation of mean. T- test (***p \leq 0.001), n = 3.

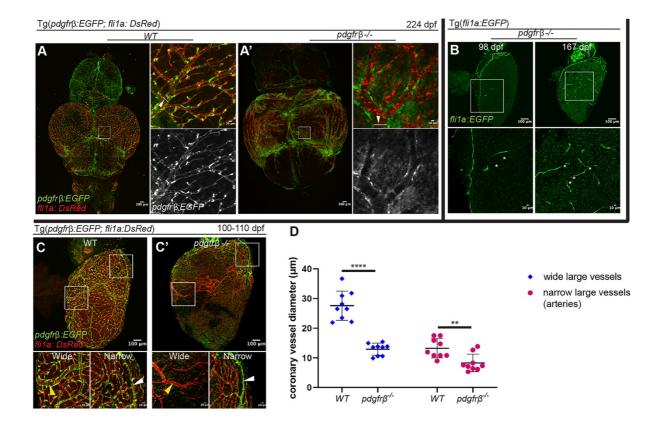
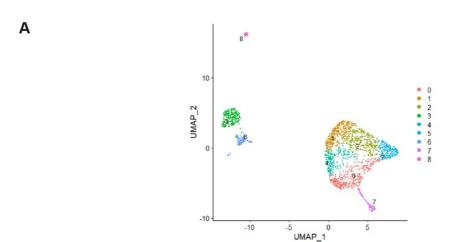
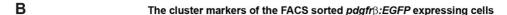
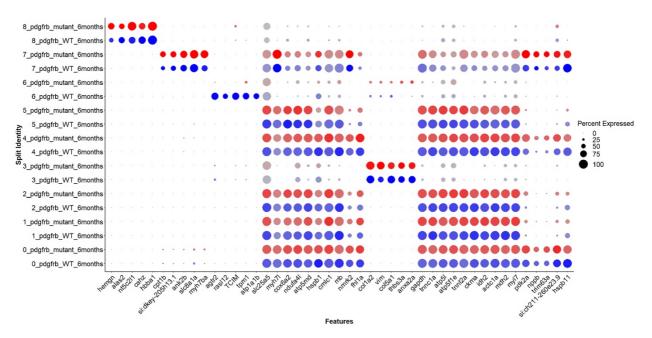


Fig. S2. Pdgfr β regulates mural cell number, association, and development of the blood vessels in the brain and coronary vasculatures.

(A) $pdqfr\beta$ mutants ($pdqfr\beta^{-/-}$) show defects in mural cell recruitment to the brain blood vessels. In adult *pdgfrβ* mutant fish (224dpf), the brain has significantly decreased mural cell [labelled with *Tg(pdgfrβ:EGFP)*] on the blood vessels [labeled with Tq(fli1a:DsRed)]. There is a decline in the vessel density, and the vessels become dilated (indicated by the white arrow and the line showing vessel diameter). (B) In pdgfrβ mutant fish, scattered isolated endothelial cells [Tg(fli1a:EGFP)+] are found to fail to form continuous coronary vessels at different developmental stages (98 dpf, 167 dpf). (C-C') In *Tg(pdgfrβ:EGFP; fli1a:DsRed)* fish, the large coronary vessels [Tg(fli1a:DsRed)+] are classified based on their diameter and appearance into the wide large vessels (yellow arrow) and the narrow large vessels (white arrow). The wide large vessels appear to have less *pdgfrβ:EGFP*+ mural cell association than narrow large vessels. In $pdqfr\beta$ mutant fish with moderate coronary vessel development, both types of large vessels are formed. While the narrow large vessels (white arrow) maintain the pdgfrβ:EGFP+ mural cell association (C), the mural cells around the wide large vessels (yellow arrow) decrease significantly (C'). (D). Quantification of the diameter of large coronary vessels. Large coronary vessels [both wide and narrow (artery) large vessels] diameter becomes smaller in $pdqfr\beta$ mutant ventricles. While the mean diameter of the wide large vessels decreases from ~27.6 mm in the $pdgfr\beta^{+/+}$ fish to ~12.9 µm in $pdgfr\beta$ mutant fish, the arteries become thin from ~13.3 μ m diameter to ~8.3 μ m (n = 3 hearts, 3 X 3 quantifications from each heart) T-test (**** $p \le 0.0001$, ** $p \le 0.01$).







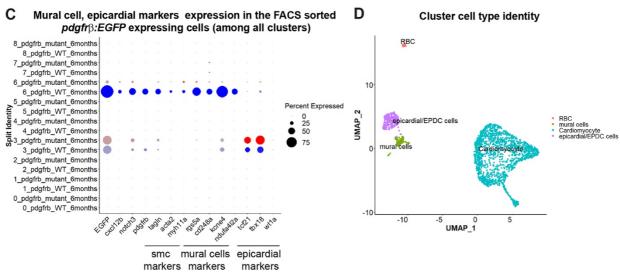
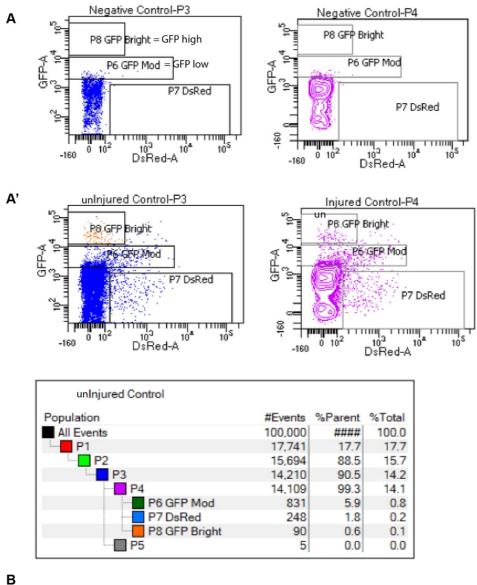


Fig. S3. Identification and characterization of the FACS sorted $pdgfr\beta$:EGFP expressing cells from wildtype and $pdgfr\beta$ mutant hearts.

(A) *pdgf\theta*:EGFP cells are FACS isolated from *Tg(pdgf\theta*:EGFP; cxcl12b:Citrine; *fli1a:DsRed)* fish. UMAP plot of all FACS isolated cells (from the wild type and *pdgf\theta*-sample integrated together), which form 9 clusters. (B) Dot plot of 5 top differentially expressed cluster marker genes of FACS sorted *pdgf\theta*:EGFP expressing cells. Blue dot, WT. Red dot, *pdgf\theta* mutants. Expression level across cells within the cluster is shown in intensity of the color while the percentage of the cells expressing the marker gene is shown by the size of the dot (0-100%), differentially expressed genes were determined with minimum percent expression cut-off = 0.15 and minimum average log fold change = 0.25. (C) Dot plot of differentially expressed cluster marker genes of mural cells, smooth muscle cells and epicardium and the *egfp* transcript. (D) Cell identity for all clusters determined by the cluster marker genes. Cluster 0, 1, 2, 4, 5, 7 cells are identified as cardiomyocytes, cluster 8 cells are red blood cells (RBCs) and cluster 3 cells are epicardial cells/epicardial derived cells. Cluster 6 cells are mural cells.

FACS sorting of GFP high and low cells from Tg(pdgfr\u00e3:GFP; fli1a:DsRed; cxcl12b:Citrine) adult (>6 months) zebrafish hearts ventricles



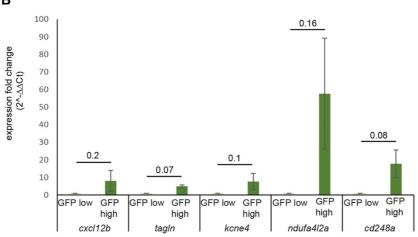
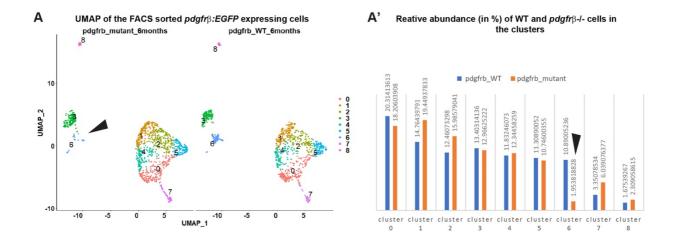
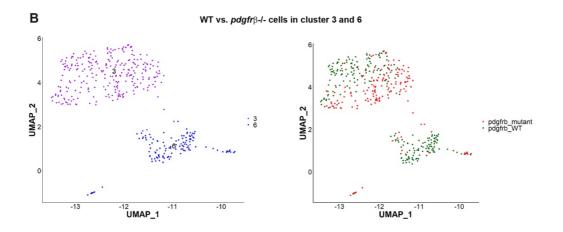


Fig. S4. Validation of the expression of smooth muscle and mural cell marker genes in high GFP expressing cells.

Since in the scRNAseg data, cluster 6 cells specifically express all smooth muscle and mural cell marker genes along with cxcl12b and shows significantly higher egfp expression from pdgfr\beta: EGFP reporter (than cluster 3), cells with high GFP expression (GFP^{high}) cells were collected separately from low or moderate *egfp* expressing cells (GFPlow). (A-A') FACS gate setting for isolating GFPhigh cells, GFPlow cells and fli1a:DsRed cells. (A) Wild type fish hearts without any transgenic fluorescent reporter were used for making single cell suspension as negative control and the FACS gate for GFP and DsRed were set avoiding the majority of the fluorescent negative cells. (A') The GFP gate is subdivided into GFP-Bright and GFP-moderate (GFP-Mod) regions to collect GFPhigh and GFPlow cells respectively. The figures show a representative gate setting used for the FACS sorting of different experimental replicates (n = 3). In this experiment, 0.8% and 0.1% of the total events(cells) were captured in the GFP-Mod gating and GFP-Bright gating respectively. (B) The mural cell (kcne4, ndufa4l2a, cd248a) and the smooth muscle cell markers (tagIn) and cxcl12b expression was tested in the GFPhigh cells and the GFPlow cells by isolating RNAs from them and subsequent gRT-PCR. FACS Sorting and subsequent sample preparation experiments were independently replicated three times and expression fold change (2^{Λ-ΔΔCt}) were calculated after normalizing against the housekeeping gene rpl13a expression. cxcl12b and the mural cell markers showed the trend of increased expression in the GFPhigh cells. The p values are calculated by single t-test considering expression level in GFP^{low} cells = 1.





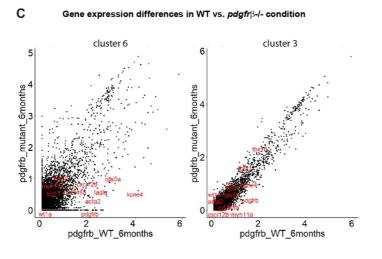
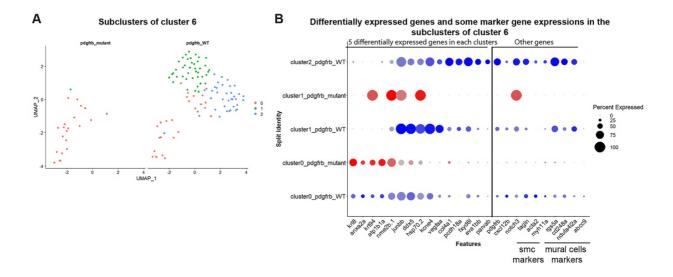
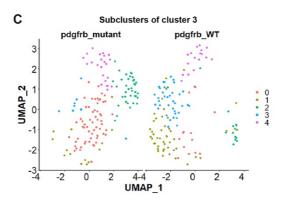


Fig. S5. Mural cells are affected in $pdgfr\beta$ mutant adult zebrafish hearts

(A-A') pdgfrβ:EGFP cells are FACS isolated from Tg(pdgfrβ:EGFP; cxcl12b:Citrine; fli1a:DsRed) fish. (A) Cluster 6 (mural cell cluster) is mostly affected in pdgfr\beta mutants (black arrowhead). In the comparative UMAP plot of all isolated EGFP+ cells (from the wild type and pdgfrβ^{-/-} sample integrated together) all other clusters except cluster 6 have comparable cell numbers. (A') Relative abundance of WT and pdgfr\(\beta \) mutant (pdgfr\beta^{-\-l}) cells in each cluster. (B) UMAP of all the FACS sorted pdgfr\beta:EGFP + cells in cluster 3 and 6 and pdgfr\beta mutant vs wildtype. pdgfr\beta expressing cluster 3 (epicardial/EPDC cells) and 6 (mural cells) shows distinct positioning of the wild type and the pdgfr\beta mutant cells in each cluster reflecting their overall gene expression differences. (C) The scatter plots of the average gene expression across the wild type and the *pdgfr* mutant conditions show more differential gene expression in the cluster 6 cells (mural cell) than the cluster 3 cells (epicardial/EPDC cells). Each black dot in the plot represents the position of the average expression of a gene across the wildtype (WT) and pdgfr\beta mutant conditions. In cluster 6, except myh11a, the smooth muscle markers (acta2, tagIn) and the mural cell marker (kcne4, rgs5a, cd248a) genes' average expression inclines towards the wildtype axis. kcne4 is an outlier towards wildtype conditions indicating its significant differential expression in the wildtype cells.





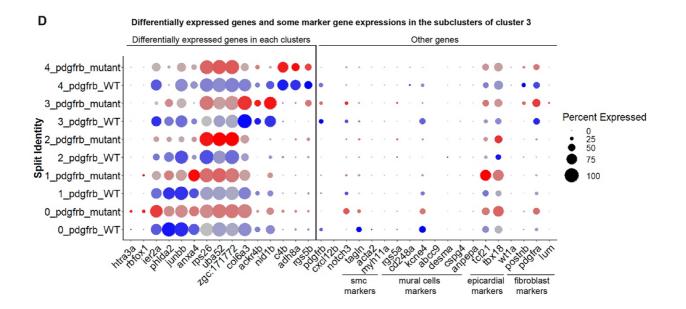
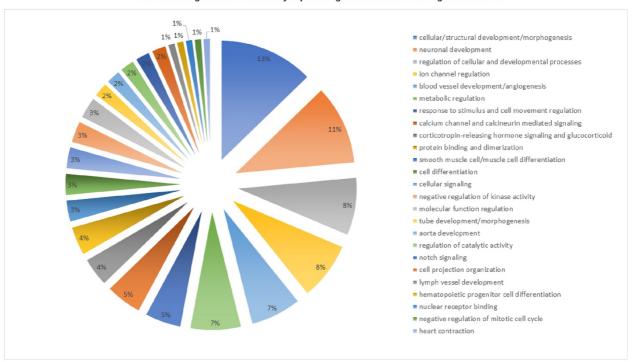


Fig. S6. Subclustering of cluster 3 and cluster 6 revealed heterogeneity in the mural cells and epicardial cells.

(A) Subclustering of the cluster 6 (mural cells) generated 3 subclusters. The comparative UMAP plot shows, there is only one cell of cluster 1 remaining in the pdgfr\beta mutant while the cluster 2 cells are completely absent. (B) Dot plot of 5 differentially expressed genes in each subcluster and mural, smooth muscle cell marker genes. Subclusters (subcluster 1, 2) having higher mural cell markers (pdgfr\(\beta\), kcne4, rgs5a, cd248a) expression are absent in pdgfr\beta mutant. WT subcluster 0 cells has higher cxcl12b expression and prominent smooth muscle marker expression which decreases in pdgfr\beta mutant. Differentially expressed genes were determined with minimum percent expression cut-off = 0.1 and minimum average log fold change = 0.25. (C) Subclustering of pdgfr\beta+ only positive cells (cluster 3 epicardial cells/EPDC) revealed 5 sub populations. The comparative UMAP plot shows, wild type hearts have more cells of subcluster 1 and 3, $pdgfr\beta$ mutant hearts have more cells of subcluster 0 and 2. Subcluster 4 cells are similarly distributed between wildtype and *pdgfr* β mutant hearts. (D) Dot plot of differentially expressed genes and mural cell, smooth muscle cell, epicardial cell and fibroblast marker genes in each subcluster. All subclusters express the epicardial markers (tcf21, tbx18). Except for subcluster 2, other subclusters also express some mural cell markers (kcne4, rgs5a, cd248a) and fibroblast (postnb, pdgfra) markers along with the epicardial markers and pdgfr\(\beta\). Differentially expressed genes were determined with minimum percent expression cut-off = 0.1 and minimum average log fold change = 0.25. Blue dot, WT. Red dot, $pdgfr\beta$ mutants. Dot size represents percentage of the expressing cells (0 -100%) and color intensity level indicates expression level of the cells expressing the marker gene.

GO-term categories for differentially expressed genes in cluster 6 cells against cluster 3



GO-term categories for differentially expressed genes in cluster 3 cells against cluster 6

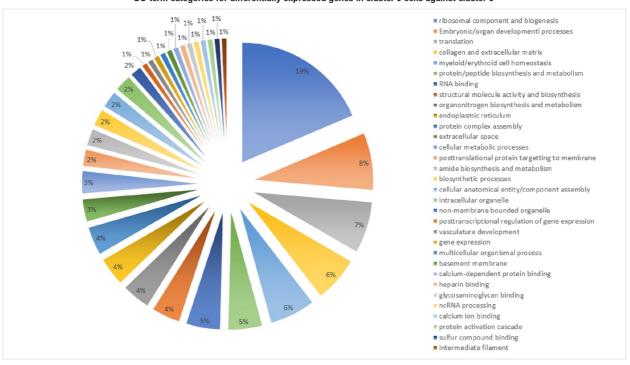


Fig. S7. GO-term analyses of differentially express genes comparing cluster 3 and cluster 6.

Gene ontology term (GO-term) analysis for the differentially expressed genes in cluster 3 and cluster 6 cells (comparing against each other). Differentially expressed genes were selected with adjusted p-value ≤ 0.1 and enriched GO-terms were selected using Benjamini Hochberg correction with p ≤ 0.05 .

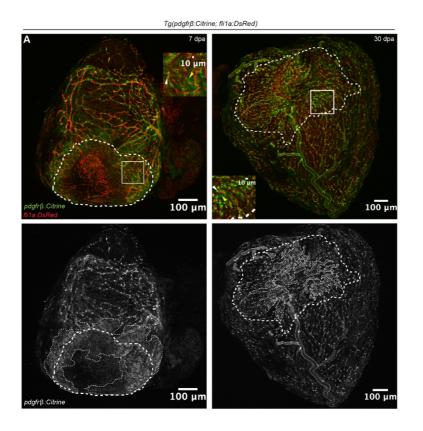


Fig. S8. Induced *pdgfrβ* expression in the injured heart decreases with regeneration completion.

(A) pdgfrβ expression during heart regeneration decreases at the later stage of regeneration (30 days after amputation). Images of Tg(pdgfrβ:Citrine; fli1a:DsRed) fish hearts at 7, 30 dpa. Lower panels: pdgfrβ:citrine single channel images. White dashed line: injured area. White dotted line: pdgfrβ expressing area. White arrowhead, pdgfrβ expression in mural cells; yellow arrowhead, epicardial/non-mural cell pdgfrβ expression.

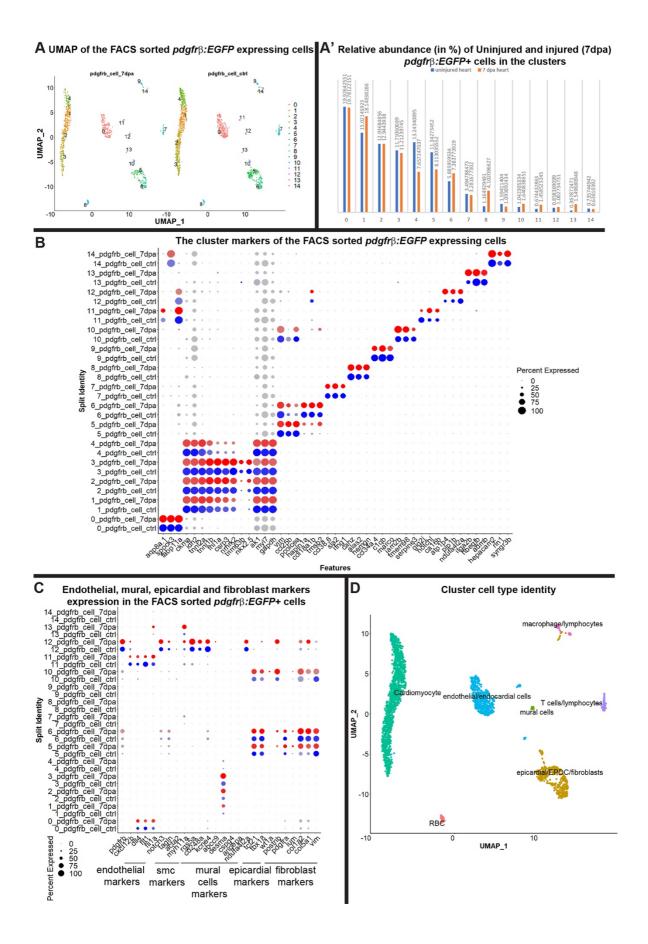
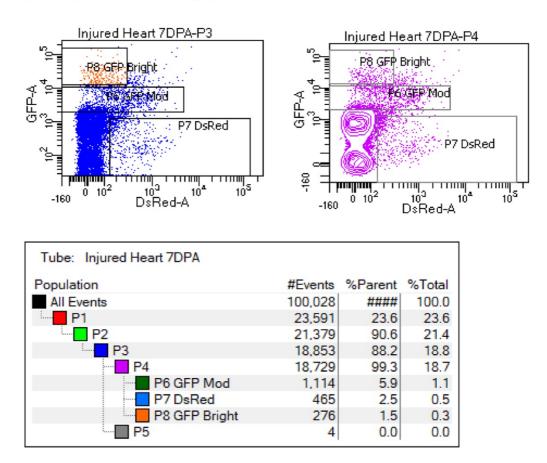


Fig. S9. Characterization of *pdgfrβ:EGFP* FACS sorted cells in uninjured and 7 dpa hearts.

(A-A') $pdgfi\beta$: EGFP cells FACS sorted from $Tg(pdgfi\beta)$: EGFP; excl12b: Citrine) fish. (A) UMAP plot of all FACS sorted cells (the uninjured and the 7 days post amputated samples integrated together), which form 15 clusters. (A') Relative abundance of uninjured and 7 dpa hearts in each cluster. (B) Dot plot of the 3 top differentially expressed cluster marker genes of FACS sorted pdgfiß: EGFP expressing cells. Blue dot, uninjured. Red dot, 7 dpa. Expression level across cells within the cluster is shown in intensity of the color while the percentage of the cells expressing the marker gene is shown by the size of the dot (0-100%). Differentially expressed genes were determined with minimum percent expression cut-off = 0.1 and minimum average log fold change = 0.25. (C) Dot plot of differentially expressed cluster marker genes of smooth muscle, mural cells, epicardial cells and fibroblasts. Pdgfiß mRNA is specifically expressed in the cluster 5, 6, 10 and 12 of which, cluster 5, 6, and 10 express the epicardial markers (tcf21, tbx18, wt1a) and cluster 12 express the smooth muscle cell markers (tagIn, acta2, myh11a) and mural cell markers (rgs5a, cd248a, kcne4, ndufa4l2a). Fibroblast marker postnb predominantly express in cluster 10 in uninjured heart but upregulated after injury in all $pdgfi\beta$ + clusters (cluster 5, 6, 10, 12). Fibroblast marker *pdgfra*, specifically express in cluster 5, 6. Other fibroblast markers (lum, col1a2, col5a1, vim) express in cluster 5, 6 and 10, where cluster 6 has most fibroblast marker expression. *lum* express rarely but induced after injury in cluster 5, 6. col1a2, col5a1 induced after injury in cluster 5, 10 and 12. (D) Cell identity for all clusters determined by the cluster marker genes. Cluster 1, 2, 3, 4 cells are identified as cardiomyocytes. cluster 5, 6, 10 cells are epicardial/EPDC/fibroblasts. Cluster 12 cells are mural cells. Cluster 0, 11, 13 are endothelial/endocardial cells. Cluster 8 cells are red blood cells (RBC). Cluster 9, 14 are macrophage/lymphocytes and cluster 7 cells are T cells/lymphocytes.

A FACS sorting of GFP high and GFP low cells from Tg(pdgfr\u03b3:GFP; fli1a:DsRed; cxcl12b:Citrine) adult (>6 months) zebrafish hearts ventricles



B GFP bright and GFP mod cells' cDNA => (1:1) for uninjured control and injured (7dpa) heart samples => RT PCR for candidate genes

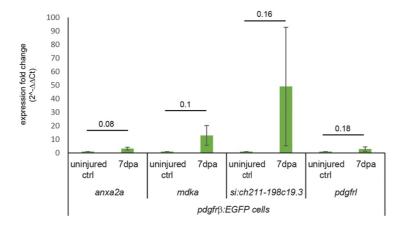


Fig. S10. Validation of expression of genes upregulated in 7 dpa heart.

(A') FACS gate setting for isolating GFPhigh cells, GFPlow cells and fli1a:DsRed cells. (A) Following a similar setting shown in Fig. S3.2 (for uninjured control) the GFP gate is subdivided into GFP-Bright and GFP-moderate (GFP-Mod) regions to collect GFP^{high} and GFP^{low} cells respectively. The figures show a representative gate setting used for the FACS sorting of different experimental replicates (n = 3). In this experiment, 1.1% and 0.3% of the total events(cells) were captured in the GFP-Mod gating and GFP-Bright gating respectively. (B) Some of the candidate genes (anxa2a, mdka, si:ch211-198c19.3, pdgfrl) found to be upregulated in pdgfr β + cells after injury (7dpa) in the scRNAseq analysis (Fig. 5B) are tested by qRT-PCR. After isolating GFP^{high} cells and the GFP^{low} cells from uninjured and injured (7dpa) hearts, cDNAs were made from their mRNAs. GFPhigh and the GFPlow cells' cDNAs are mixed in 1:1 ratio and gRT-PCR was performed. This experiment was replicated two more times. The expression fold change (2^{Λ-ΔΔCt}) were calculated after normalizing the expression against the housekeeping gene rpl13a expression. The candidate genes showed the trend of increased expression in the isolated GFP+ cells from the 7dpa injured hearts. The p values are calculated by single t-test considering expression level in the uninjured hearts' GFP+ cells = 1.]

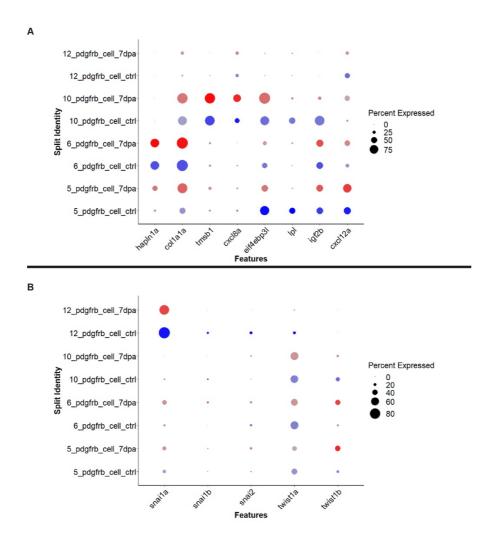
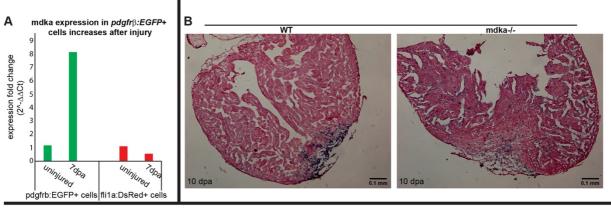


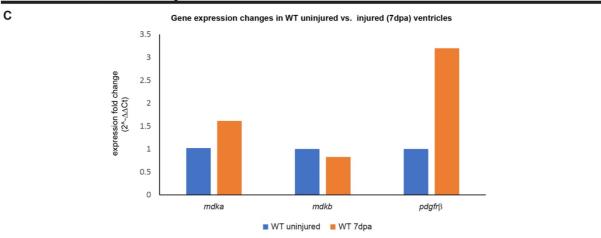
Fig. S11. Differentially expressed genes between 7 dpa and uninjured hearts in mural cell and epicardial clusters. (A) Dot plot of differentially expressed genes of FACS sorted *pdgfrβ:EGFP* expressing cells in a mural cell and epicardial derived cell (EPDC) cluster. (B) Dot plot of differentially expressed EMT genes. Blue dot, uninjured. Red dot, 7 dpa. Expression level across cells within the cluster is shown in intensity of the color while the percentage of the cells expressing the marker gene is shown by the size of the dot (0-100%).

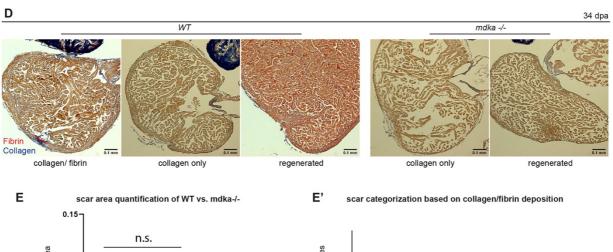
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Fig. S12. GO-term analyses of differentially express genes in epicardial and mural cell cluster

Gene ontology term (GO-term) analysis for the differentially expressed genes in cluster 5, 6, 10 and cluster 12 cells. Differentially expressed genes were selected with adjusted p-value \leq 0.1 and enriched GO-terms were selected using Benjamini Hochberg correction with p \leq 0.05.







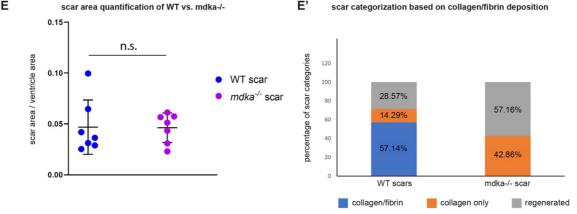


Fig. S13. Characterization of *mdka* in 7 dpa injured hearts and *mdka* mutant (A) *pdgfrβ:EGFP* cells and *fli1a:DsRed* cells were FACS sorted from 5, *Tg(pdgfrβ:EGFP; fli1a:DsRed)* fish and qRT-PCR was performed for *mdka* expression. The Y-axis shows expression fold change (2^-ΔΔCt) of *mdka*, normalized to the housekeeping gene *rpl13a*'s expression in uninjured vs. injured (7 days post-amputation of the ventricles' apical regions) hearts. (B) Representative images of *in situ* hybridization with the probe against *mdka* in injured (10 days post-amputation) wildtype fish vs. *mdka*-fish (n = 3). (C) Whole ventricles (n = 5) were collected from wild type uninjured hearts and injured (7 dpa) hearts. qRT-PCR was performed for *mdka*, *mdkb* and *pdgfrβ* expression. The Y-axis shows expression fold change normalized to the housekeeping gene *rpl13a*'s expression. (D) AFOG staining of WT and *mdka* mutant heart at 34 dpa. n=7. fibrin, red; collagen, blue. (E) Quantification of scar area in WT vs mdka mutants. n.s. not significant. (E') Quantification of scar categorization. Percentage of collagen/fibrin deposition is shown in blue, collagen only in orange, and regenerated in grey. Scale bar=0.1mm.

Table S1. Cluster marker genes of FACS sorted *pdgfr\beta*: *EGFP*+ cells in *pdgfr\beta* mutants and control integrated.

Click here to download Table S1

Table S2. Differentially expressed genes in cluster 6 vs cluster 3.

Click here to download Table S2

Table S3. Differentially expressed genes in WT cluster 6 cxcl12b+ cells vs cxcl12b- $(pdgft\beta)$ only cells.

Click here to download Table S3

Table S4. Differentially expressed genes in cluster 6 between *pdgfrβ* mutants and controls.

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Table S5. Subcluster markers of $cxcl12b+; pdgfr\beta + cells$ (cluster 6)

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Table S6. Subcluster markers of $pdgfr\beta$ + only cells (cluster 3)

Click here to download Table S6

Table S7. GO term analysis cluster 6 against cluster 3

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Table S8. GO term analysis cluster 3 against cluster 6

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Table S9. Cluster marker genes of FACS sorted *pdgfß: EGFP*+ cells in 7 dpa and uninjred hearts integrated.

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Table S10. Differentially expressed genes in all clusters combined between 7 dpa and uninjured controls.

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Table S11. GO term analysis of differentially expressed genes in cluster 5, 6, 10, 12 between 7 dpa and uninjured control hearts.

Click here to download Table S11

Table S12. GO term analysis of differentially expressed genes in cluster 5.

Click here to download Table S12

Table S13. GO term analysis of differentially expressed genes in cluster 6. Click here to download Table S13

Table S14. GO term analysis of differentially expressed genes in cluster 10.

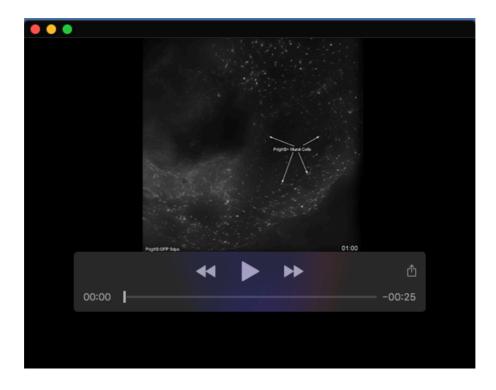
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Table S15. GO term analysis of differentially expressed genes in cluster 12.

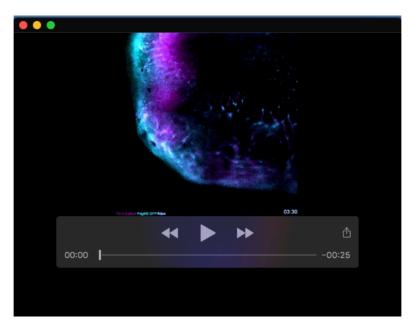
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Table S16. qRT-PCR primers

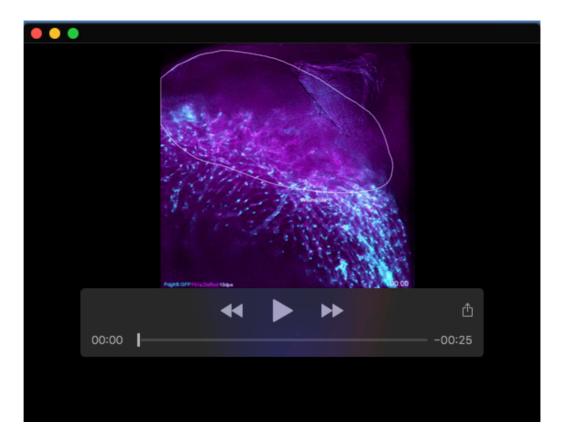
Genes	Primer sequence
acta2 forward primer	5' AGATAGTTACGTTGGTGATGAGG 3'
acta2 reverse primer	5' CTCCCTGTTGGCTTTAGGATTA 3'
kcne4 forward primer	5' GGTGAAACATCCCGGTAACA 3'
kcne4 reverse primer	5' CCGTTTGGTGCGCAAATA 3'
cxcl12b forward primer	5' TGCCCTTTCCAAGTCATT 3'
cxcl12b reverse primer	5' TGTGAGACTCCAGGACAC 3'
ndufa4l2a forward primer	5' TCTGGCCATTAACACTGACTAC 3'
ndufa4l2a reverse primer	5' ATTAGAGGAGATGCTGTTGAGAAA 3'
tagIn forward primer	5' CCAAAGAGGACGGAGCTTTC 3'
tagIn reverse primer	5' TGATGTGAGTGTGTTCAGG 3'
cd248a forward primer	5' TGCTGGTGCTGGTGATAAAG 3'
cd248a reverse primer	5' TGGACTGAAGAATGTCAAAGGG 3'
anxa2a forward primer	5' TGACCCGGATCATGGTGT 3'
anxa2a reverse primer	5' GAGATCTCCATCTGGACCTCAG 3'
si:ch211-198c19.3 forward primer	5' TGCAGACATGCAATCTGAAAG 3'
si:ch211-198c19.3 reverse primer	5' GATCCTGTGATGTGACTATTGC 3'
pdgfrl forward primer	5' CGATTGAGGCTTCTTCAAATTC 3'
pdgfrl reverse primer	5' GTTTCAACATCATCCACAATCA 3'
rpl13a forward primer	5' GGTGTGAGGGTATCAACATCTC 3'
rpl13a reverse primer	5' GTGATATGATCCACGGGAAGG 3'
mdka forward primer	5' GGGAACTAAAGGGAGCAAGAG 3'
mdka reverse primer	5' CTGAACAACACAGAGTGGAGAT 3'
mdkb forward primer	5' TCCCATGCAACTGGAAGAAG 3'
mdkb reverse primer	5' CCTGTAGTAGTGTCACATTCGG 3'
pdgfrβ forward primer	5' TATGTGTGCACCGAGAAGAAG 3'
pdgfrβ reverse primer	5' GAACCACACGTCAGGATCAG 3'



Movie 1. Movie from apex view showing behavior of two distinct *pdgfrfiEGFP* expression patterns, epicardial (diffuse) and mural (punctate), during regeneration at 5 to 6dpa. Time in minutes indicated on the bottom right.



Movie 2. Movie from apex view showing *pdgfrfiEGFP* (*green*) and *fli1a:DsRed* (*red*) expression during regeneration 6 to 8dpa. Time in minutes indicated on the bottom right.



Movie 3. Movie from lateral view showing *pdgfrfi:EGFP* (*red*) and *fli1a:DsRed* (*green*) expression during regeneration 10 to 12dpa. Time in minutes indicated on the bottom right.