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Supplemental Information

Adult sox10^{+} Cardiomyocytes Contribute to Myocardial Regeneration in the Zebrafish

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Figure S1

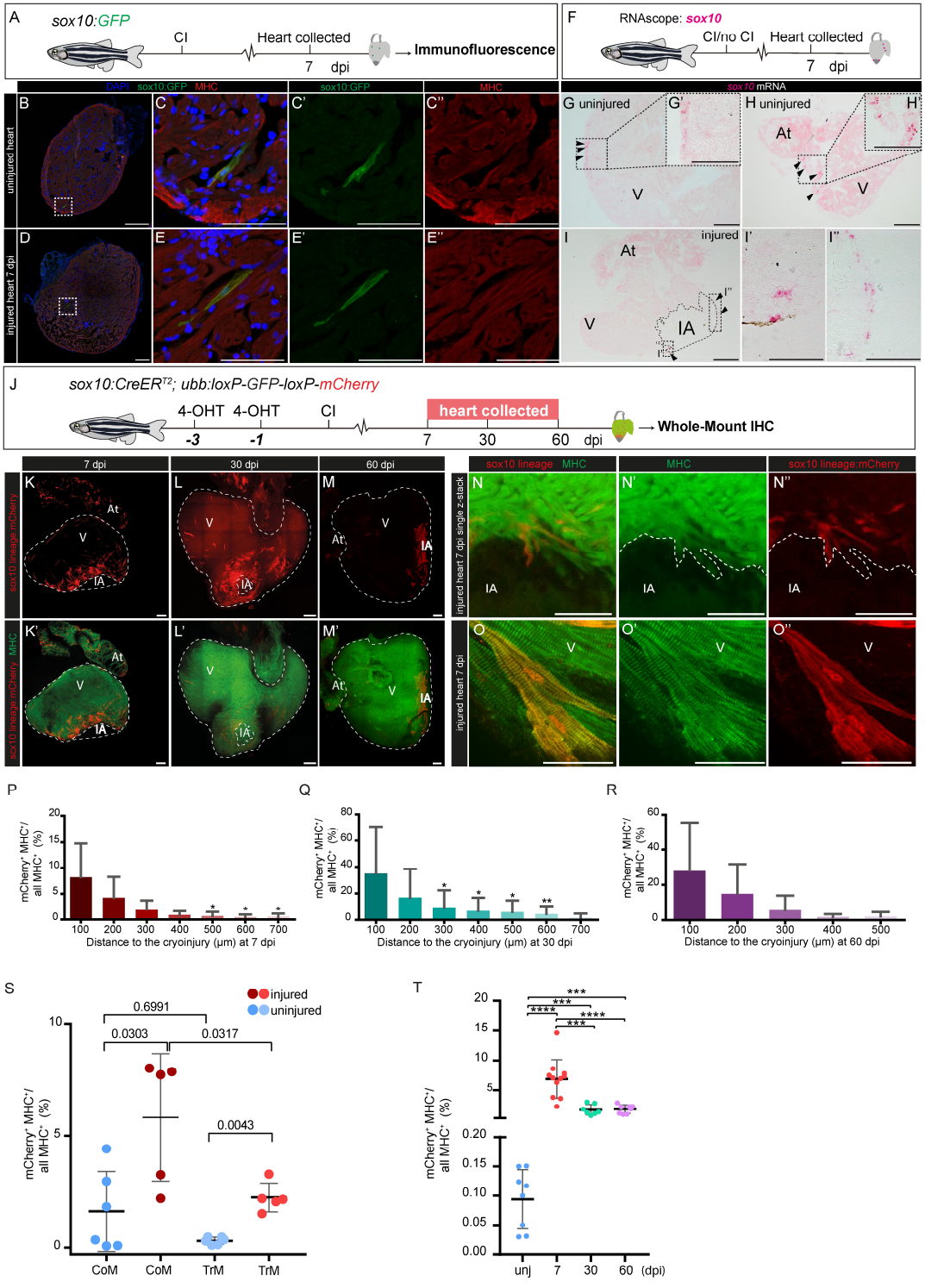

Figure S1. *sox10*-derived cells contribute to heart regeneration. Related to Figures 1 and 2.
(A-E'') Detection of a small subset of *sox10*:GFP^{+} CMs in the adult zebrafish heart.
(A) Immunostaining against GFP (green) Myosin Heavy chain (MHC, red) and nuclear counterstain with DAPI (blue) was performed on sagittal sections of *sox10:eGFP* adult zebrafish hearts.
(B-C'') Whole heart section and zoomed view of an uninjured heart.
(D-E'') Heart at 7 dpi. For both heart sections a GFP^{+} ventricular CM is shown. GFP, green fluorescent protein. All sections of the cardiac ventricles were collected on two slides. Number of GFP^{+} cells were

counted on one slide per heart, corresponding to one half of a heart. The number of *sox10*:GFP^{+} CMs detected on sections ranged between 0 to 14 for both conditions.
(F-I'') RNAScope for *sox10* mRNA on sections of uninjured hearts (G-H'; n=3) and hearts at 7dpi (I-I''; n=3). Arrowheads, *sox10*$^{+}$ cells.
(J-T) Analysis of the contribution of *sox10*-derived cells in the uninjured heart to the regenerated myocardium using inducible genetic fate mapping. (J) Two 4-OHT treatments were performed on alternating days to adult *sox10:CreER^{T2};ubb:loxP-GFP-loxP-mCherry* zebrafish before heart cryoinjury. Hearts were collected at 7 (n=11), 30 (n=7) and 60 (n=9) dpi.
(K-O'') Whole-mount views of an injured heart revealing *sox10*-derived mCherry^{+} cells in red and the myocardium in green (MHC^{+} expression). (K,K') Heart at 7 dpi. *sox10*-derived cells surround the injury area. (L,L') Heart at 30 dpi. *sox10*-derived CMs are present in the injured area and border zone. (M,M') Heart at 60 dpi. *sox10*-derived cells are found within the regenerated myocardium. (N-N'') Zoomed view of a 8 µm z-stack from a heart at 7 dpi. (O-O'') Magnifications showing *sox10*-derived CMs at 7 dpi.
(P-R) Graphs showing the percentage of the volume from mCherry^{+}/MHC^{+} cells relative to all MHC^{+} cells within different heart segments at 7, 30 and 60 dpi. Data are means ± SD; *p<0.05; **p<0.01; ***p<0.001 (according to one-way ANOVA followed by Tukey's honest significant difference test).
(S) Contribution of *sox10*-derived CMs to the trabecular or compact myocardium. Graph showing the percentage of mCherry^{+}/MHC^{+} versus all MHC^{+} cells in the compact (CoM) or trabecular (TrM) myocardium. Shown are percentages in injured and uninjured hearts. Dots represent measurements from individual hearts, shown are also means ± SD (p=0.0303 and 0.0043 two-tailed non-parametric t-test). For this experiment, recombination was performed at -14 and -12 days before cryoinjury.
(T) Percentage of the volume from mCherry^{+}/MHC^{+} cells relative to all MHC^{+} cells in uninjured (unj) and injured hearts. The percentage of *sox10*-derived CMs volume significantly expanded upon injury. ***p<0.001, ****p<0.0001 (according to one-way ANOVA followed by Tukey's honest significant difference test).
4-OHT, 4-hydroxytamoxifen; At, atrium; DAPI, 4',6-diamidino-2-phenylindole; dpi, days post-injury; IA, injured area; CI, cryoinjury; CMs, cardiomyocytes; MHC, myosin heavy chain; V, ventricle. Scale bars: 100 µm; C-C'', E-E'' and O-O''' 50 µm.

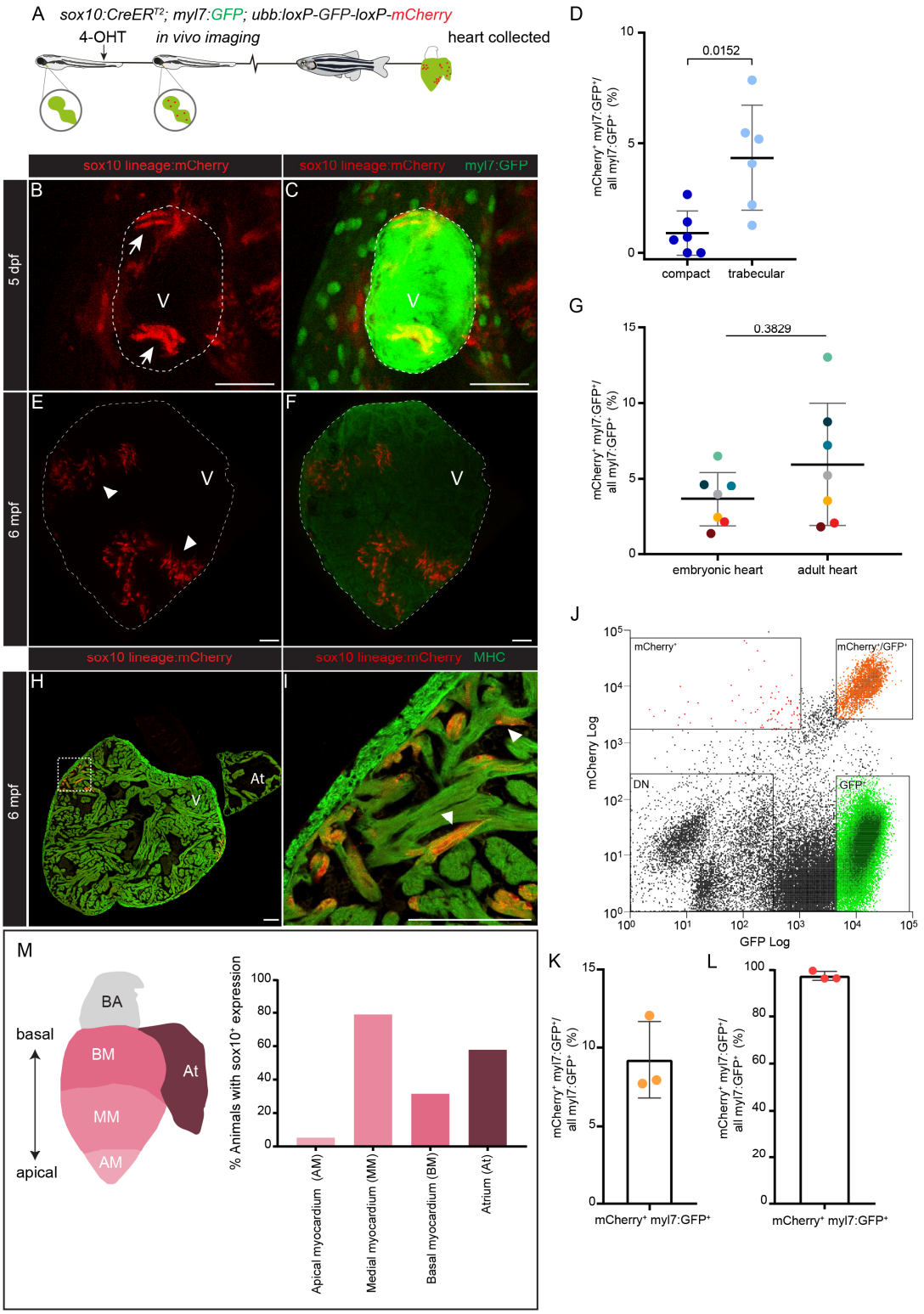

Figure S2. Embryonic *sox10*-derived cells contribute to the adult myocardium. Related to Figures 4 and 5.

(A) *sox10:CreER*^{T2}*;ubb:loxP-GFP-loxP-mCherry* embryos were treated with 4-OHT from 12 to 48 hours post-fertilization. *sox10*-derived cells are labelled with mCherry. mCherry^{+} cells were detected at 5 days post-fertilization (dpf) by confocal live imaging. Larvae were grown to adulthood and hearts collected to characterize the contribution of embryonic mCherry^{+} cells to the adult heart.

(B-C) *sox10*-derived cells within the embryonic heart. Maximal projection of a confocal z-stack through the heart at 5 dpf. Shown is a ventral view of the ventricle. Anterior is to the top. Cardiomyocytes (CMs) are shown in green, *sox10*-derived CMs in red. The cardiac ventricle is outlined by dotted lines. Arrows point to *sox10*-derived CMs clusters.

(D) Percentage of *sox10*-derived compact CMs vs all compact myocardium and *sox10*-derived trabecular CMs vs all trabecular myocardium of hearts at 5 dpf. Quantification is based on whole mount images. Shown are data from individual hearts as well as means ± SD; p= 0.0152 (two-tailed non-parametric t-test).
(E-F) Embryonic *sox10*-derived cells contribute to the adult heart (6 mpf). Maximal projection of a confocal z-stack through the adult ventricle corresponding to embryonic heart shown in (B,C).
(G) Quantification of the percentage of $mCherry^{+}/MHC^{+}$ from the total MHC^{+} volume at 5 dpf and adult ventricles. Colored dots mark individual animals (n=7). Data are means ± SD; p=0.3289 (two-tailed non-parametric t-test).
(H,I) Immunostaining of a sagittal heart section for MHC (green) and mCherry (*sox10* lineage; red). (I) Zoomed view of boxed area in (H). The cardiac ventricle is outlined by dotted lines.
(J) Representative example of a FAC sorting experiment of embryonic *sox10*-derived cells from adult hearts. Gates show *sox10*-derived CMs as orange dots (*myl7*:GFP^{+} $mCherry^{+}$), *myl7*:GFP^{+} single positive cells as green dots (GFP^{+}) and the rest of *sox10*-derived cells ($mCherry^{+}$) as red dots. 4 different hearts were pooled.
(K) Percentage of FAC-sorted *sox10*-derived CMs ($mCherry^{+}$, *my7*:GFP^{+}) *vs* the total amount of myocardium (*myl7*:GFP^{+}).
(L) Percentage of $mCherry^{+}$,*myl7*:GFP^{+} volume *vs* the total number of $mCherry^{+}$ cells in the adult heart.
(M) Localization of embryonic *sox10*-derived CMs in the adult heart (n=19). Percentage of fish, which after embryonic recombination show $mCherry^{+}$ CMs in the adult heart. Basal Myocardium (BM) category includes the atrioventricular canal myocardium.
4-OHT, 4-hydroxytamoxifen; dpf, days post-fertilization; MHC; myosin heavy chain; mpf, months post-fertilization; V, ventricle. Scale bars: 100 µm.

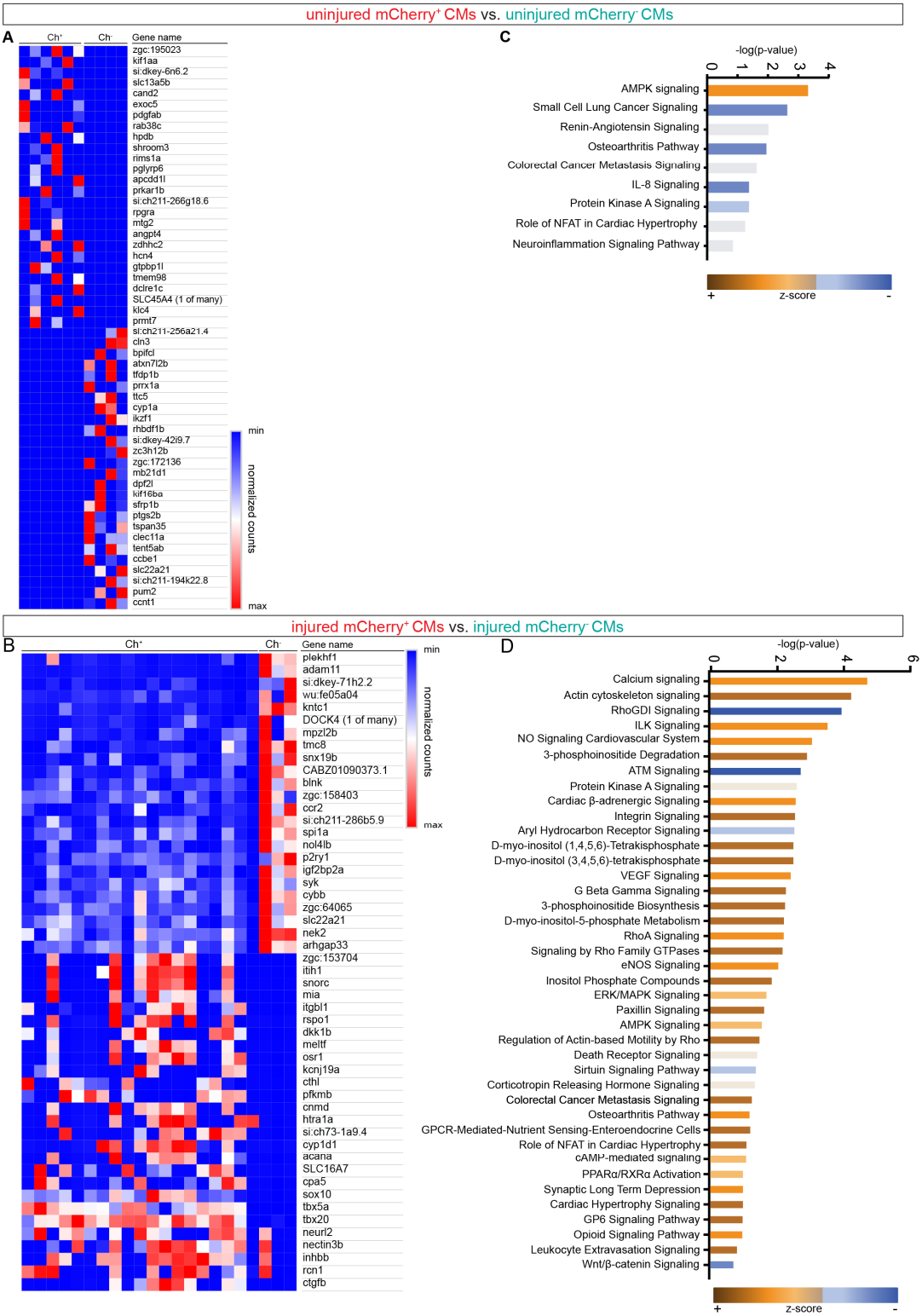

Figure S3. Heatmaps and canonical pathway analysis of *sox10*-derived CMs *vs* rest of CM in injured and uninjured hearts. Related to Figure 6.

(A) Heatmap representing the 50 most up- or downregulated genes when comparing mCherry^{+} with mCherry^{-} samples of uninjured hearts. (B) Heatmap representing the 50 most up- or downregulated genes when comparing mCherry^{+} with mCherry^{-} samples from injured hearts. (C) All canonical pathways activated (organge) or inhibited (blue) according to the differentially expressed gene (DEG) list when comparing mCherry^{+} with mCherry^{-} CMs from uninjured hearts (Ingenuity pathway analysis-IPA). (D) Top 40 canonical pathways (IPA) predicted to be activated (orange) or de-activated (blue) according to DEG between mCherry^{+} and mCherry^{-} CMs at 7 dpi. Ch, mCherry; CMs, cardiomyocytes; dpi, days post-injury.

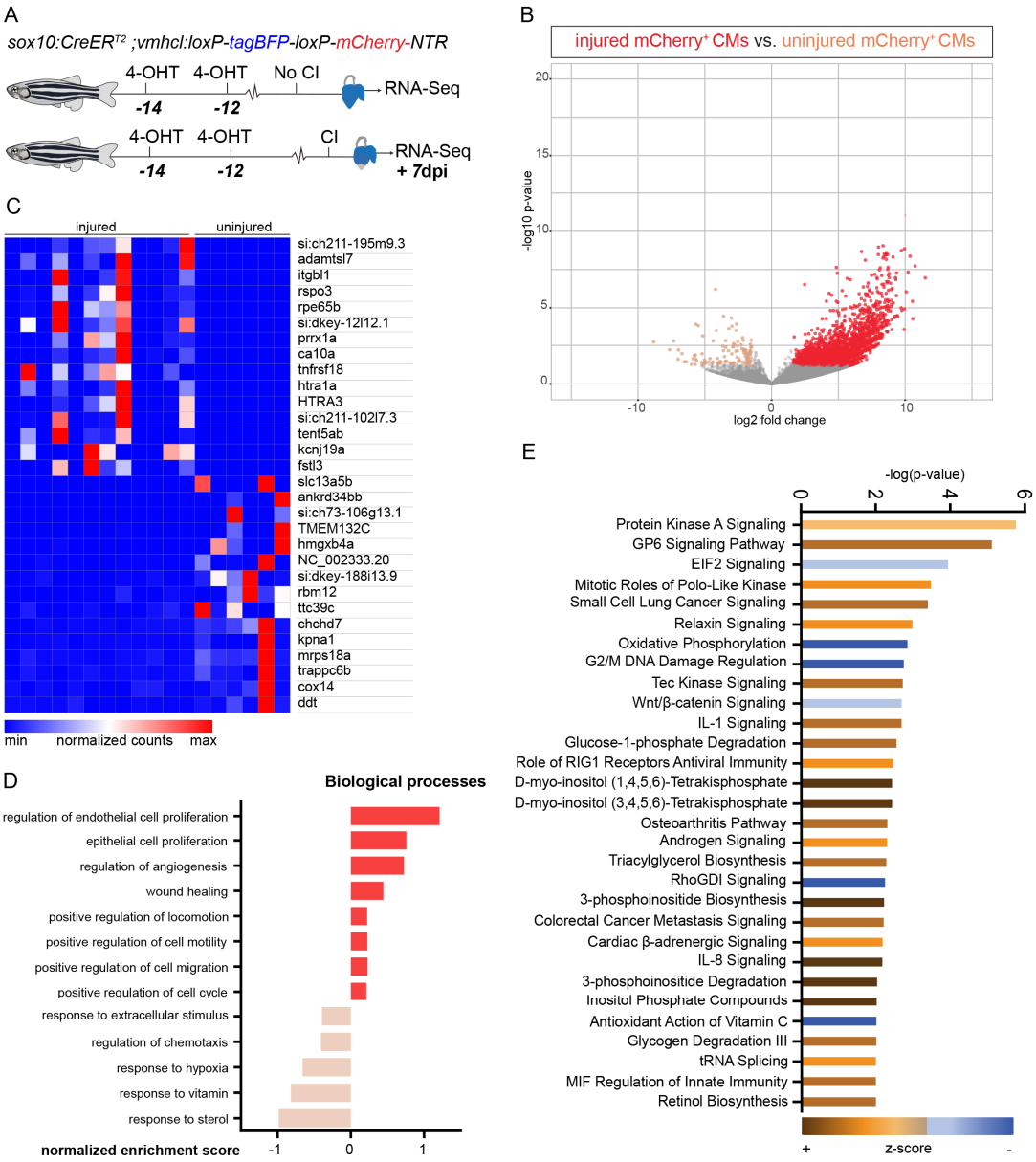

Figure S4. Changes in gene expression of mCherry$^+$ (*sox10*-derived) CMs in response to injury. Related to Figure 6.

(A) *sox10:CreER^{T2};vmhcl:loxP-tagBFP-loxP-mCherry-NTR* zebrafish were treated with 4-OHT. A group of zebrafish was cryoinjured and collected at 7dpi and a control group was left uninjured. mCherry$^+$ cells were sorted and RNA-seq performed.

(B) Volcano plot representing differentially expressed genes (DEG) of Cherry$^+$ cells from uninjured and injured hearts. DEG uninjured heart, orange dots. DEG injured heart, red dots. DEG defined as adj p-value ≤0.05 and a log2 fold change (LFC) ≥ 2. Grey dots, genes with an FDR ≥0.05 and LFC ≤ ± 2. A higher number of DEG in mCherry$^+$ CMs was observed at 7 dpi.

(C) Heatmap representing the 15 most up- and 15 most downregulated genes when comparing injured and uninjured mCherry$^+$ samples.

(D) Biological processes of DEG present in Cherry$^+$ CMs from uninjured (orange bars) and injured hearts (red bars). Representative biological processes were plotted and ordered according to the normalized enrichment score. Z-score represents whether a specific function is increased or decreased according to the DEG.

(E) Most significant canonical pathways activated or inhibited when comparing mCherry$^+$ CMs from injured and uninjured hearts using Ingenuity Pathway analysis (IPA).

Ch, mCherry; CMs, cardiomyocytes; dpi, days post-injury; GO; Gene Ontology.

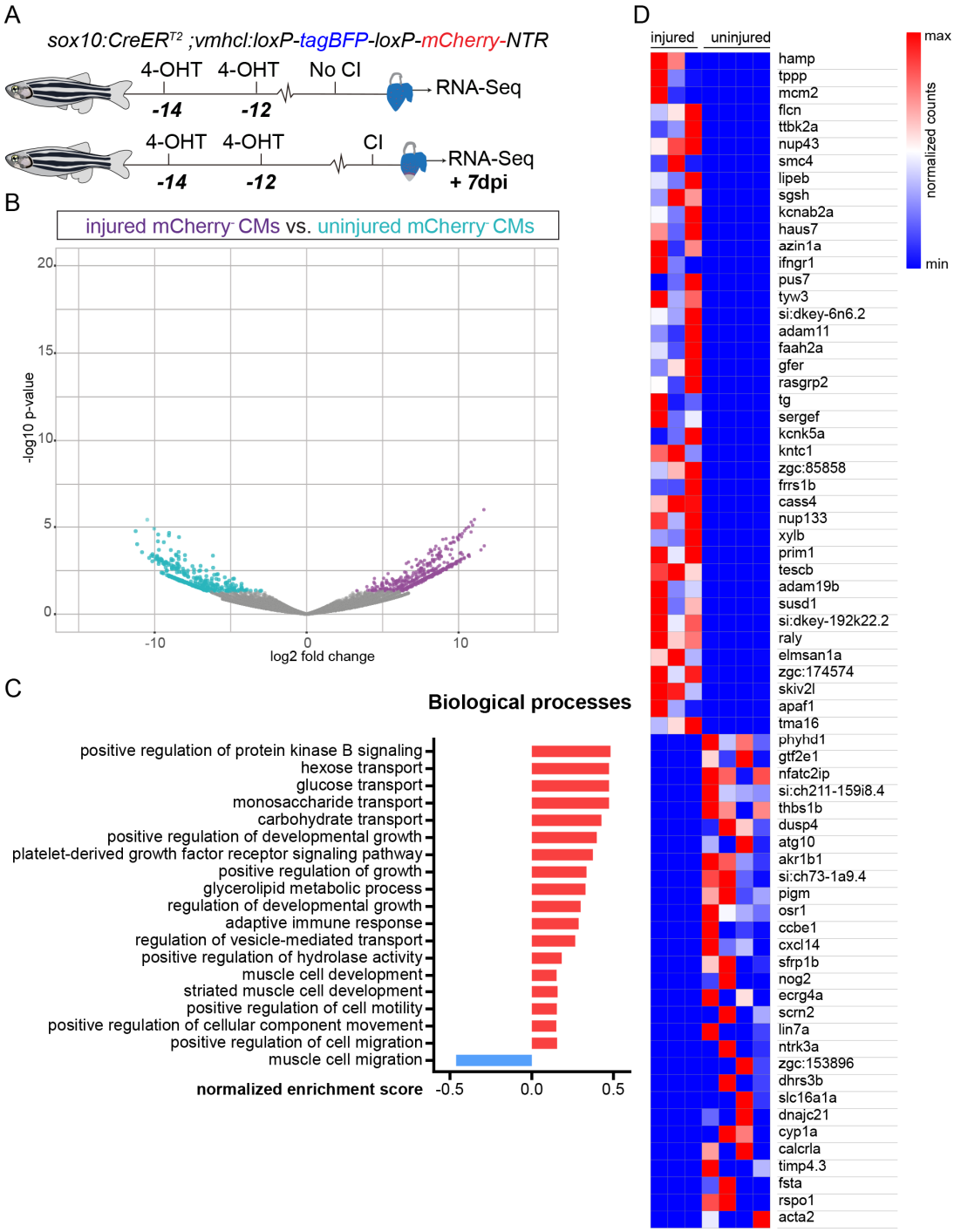

Figure S5. Changes in gene expression of mCherry$^-$ CMs (not *sox10*-derived) in response to injury. Related to Figure 6.

(A) *sox10:CreER^{T2};vmhcl:loxP-tagBFP-loxP-mCherry-NTR* zebrafish were treated with 4-OHT. A group of zebrafish was cryoinjured and collected at 7dpi and a control group was left uninjured. mCherry$^-$ cells were sorted and RNA-seq performed. (B) Volcano plot representing differentially expressed genes (DEG) in Cherry$^-$ CMs from uninjured and injured hearts. Magenta dots, DEG in uninjured Cherry$^-$ CMs; purple dots, injured Cherry$^-$ CMs. DEG: adj p-value≤0.05 and a log2 fold change (LFC) ≥ 2. Grey dots, genes with an adj p-value ≥0.05 and LFC ≤ ± 2. Similar number of DEG in the comparison of these two populations in uninjured and injured hearts. (C) GO Biological processes upregulated in injured Cherry$^-$ (red bars) and uninjured Cherry$^-$ CMs (blue bars). Representative biological processes were plotted and ordered according to the normalized enrichment score. It whether a specific function is increased or decreased according to the DEG. (D) Heatmap of the 40 most upregulated and 29 most downregulated genes when comparing injured and uninjured mCherry$^-$ samples. Ch, mCherry; CMs, cardiomyocytes; dpi, days post-injury; GO; Gene Ontology.

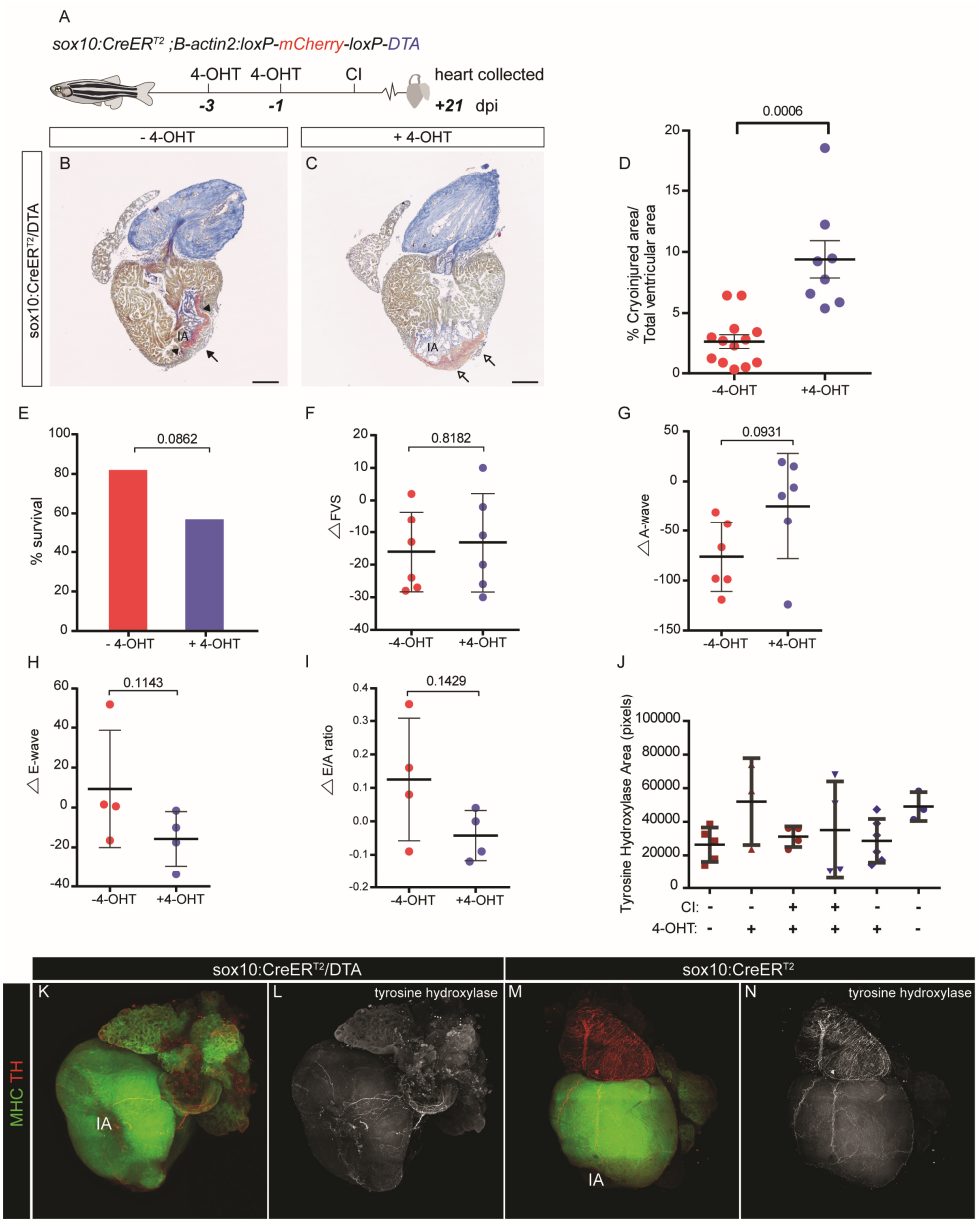

Figure S6. Ablation of adult *sox10*-derived cells does not affect cardiac function but impairs cardiac regeneration. Related to Figure 7.

(A) At day -1 and -3 prior to cryoinjury, *sox10:CreER^{T2}*;*bactin:loxP-mCherry-loxP-DTA* zebrafish (*sox10:CreER^{T2}/DTA*) were either treated with 4-OHT (n=8) or with adjuvants (control group; n=13). Cryoinjured hearts were collected at 21 days post-injury (dpi).

(B,C) AFOG histological staining on sagittal heart sections. (B) Control heart. Arroheads, collagen deposition close to the injury area. Arrows, regenerated myocardial layer. (C) 4-OHT-treated heart. Note a larger amount of collagen deposition close to the IA and absence of myocardial regeneration (empty arrows).

(D) Quantification of the IA in control hearts (n= 13) and hearts from *sox10*-derived cell depleted animals (n=8). Data are means ± SD; p= 0.0006 (two-tailed non-parametric t-test).

(E) Viability of *sox10:CreER^{T2}/DTA* animals with and without 4-OHT administration. Data are means ± SD; p= 0.0862 (Fisher's exact test). For the +4-OHT group, 33 out of 58 survived; for the - 4-OHT group, 14 out of 17 survived.

(F-I) Echocardiographic measurements. Cardiac function assessment in 4-OHT-treated animals and untreated controls. F, Relative fractional volume shortening (FVS) as a measurement of ventricular pumping efficiency. G, A-wave (late ventricular filling velocity). H, E-wave (early ventricular filling velocity). I, E/A ratio. Data are means ± SD; non-parametric t-test.

(J-N) Cardiac innervation upon *sox10*-derived cell ablation. (J) Quantification of anti-tyrosine hydroxylase (TH) signal. No difference in TH signal intensity was observed in hearts from *sox10:CreER^{T2}/DTA* (n=5) and *sox10:CreER^{T2}* (n=4) zebrafish according to one-way ANOVA. (K-N) Whole-mount immunofluorescence using anti-tyrosine hydroxylase (TH) and anti-MHC staining on 14 dpi hearts from 4-OHT-treated animals. Shown is a z-Stack comprising 35 z-planes (285 µm) for *sox10:CreER^{T2}/DTA* and a z-stack comprising 32 z-planes (260 µm) for the *sox10:CreER^{T2}* heart (control).
4-OHT, 4-Hydroxytamoxifen; At, atrium; dpi, days post-injury; FVS, fractional volume shortening; IA, injured area; MHC, Myosin Heavy Chain; TH, tyrosine hydroxylase. V, ventricle. Scale bars: 100 µm (B and C); 200 µm (K-N).