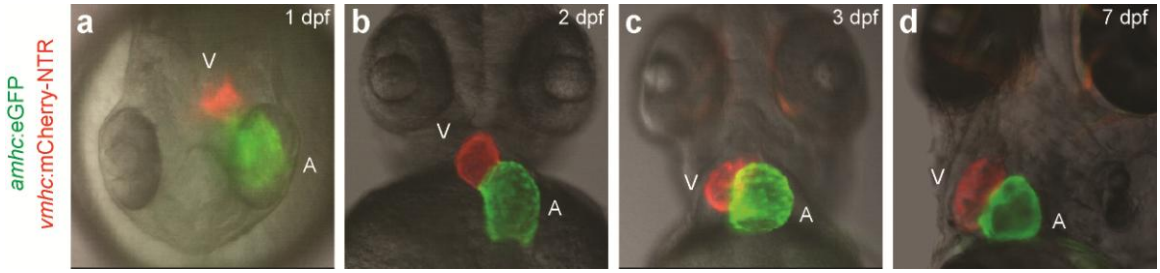
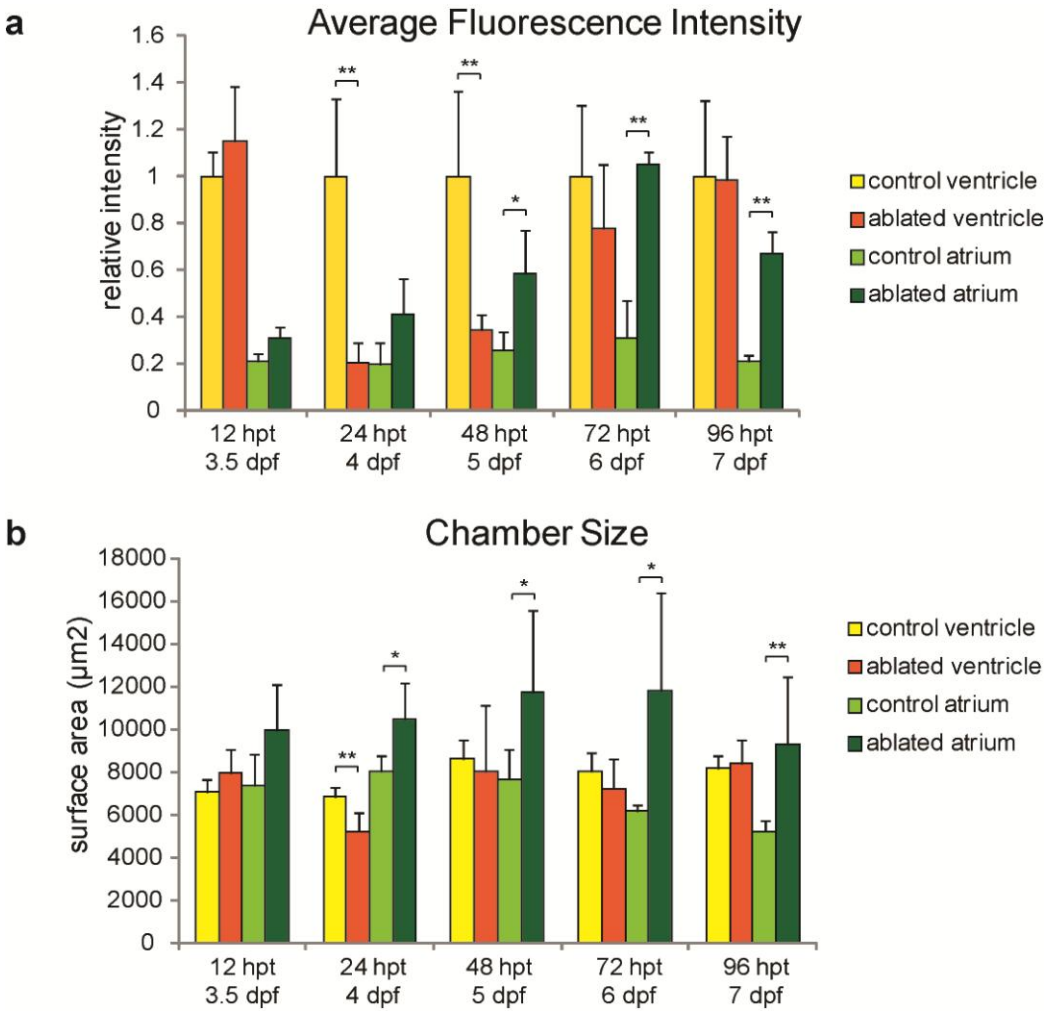
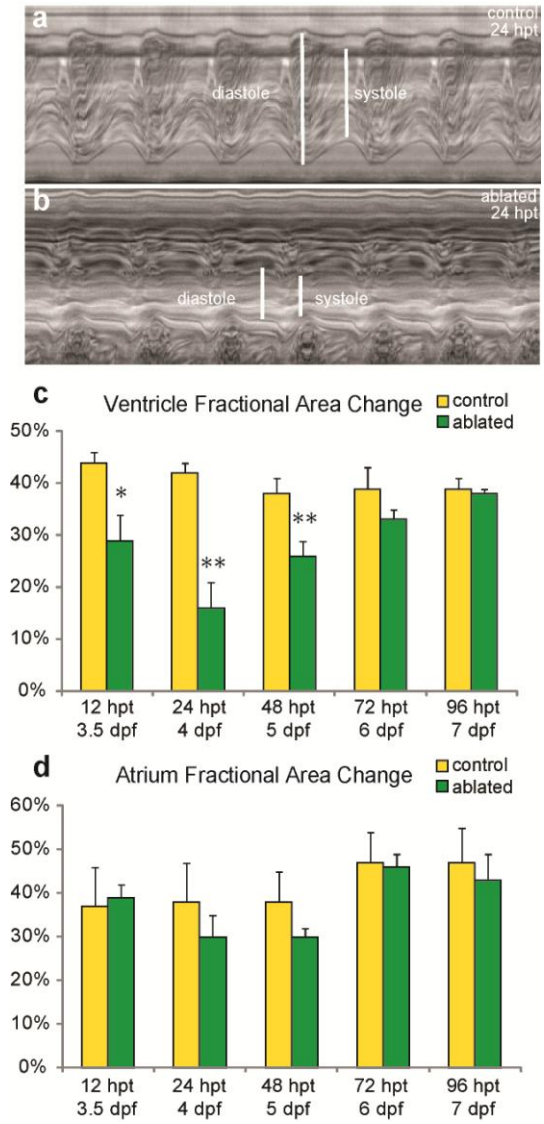


Supplementary figure 1. ***Tg(vmhc:mCherry-NTR)*** **and** ***Tg(amhc:eGFP)*** **reporter lines specifically express mCherry-NTR and eGFP in ventricular and atrial cardiomyocytes, respectively.** (a-d) Fluorescence micrographs of the same *Tg(vmhc:mCherry-NTR);Tg(amhc:eGFP)* fish at (a) 1 dpf, (b) 2 dpf, (c) 3 dpf and (d) 7 dpf showed that by 2 dpf, the *amhc* promoter can drive GFP expression specifically in atrial cardiomyocytes whereas the *vmhc* promoter can drive *mCherry-NTR* expression specifically in ventricular cardiomyocytes. (a) head on dorsal view. (b-d) ventral view, anterior to the top.

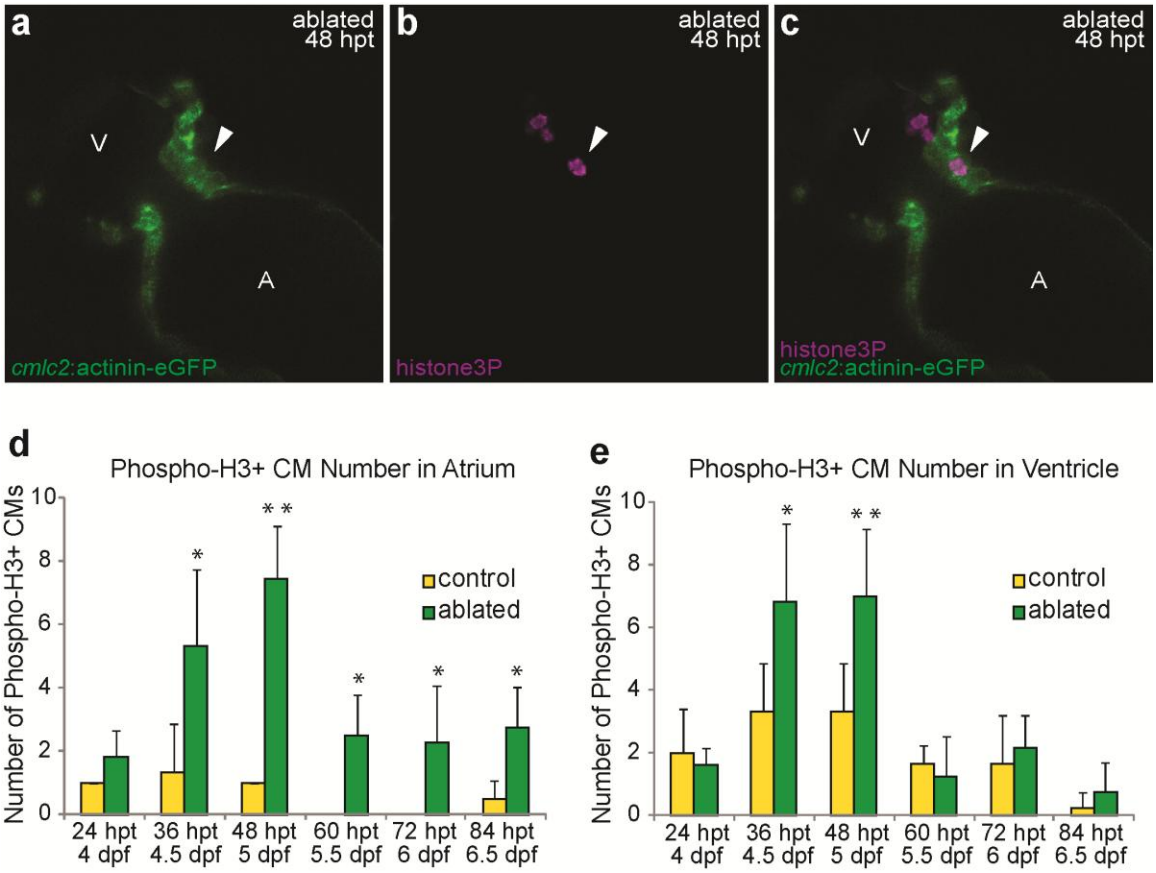


Supplementary figure 2. After ventricular cardiomyocyte ablation, ventricular fluorescence and size decreased by 24 hpt but recovered by 96 hpt, whereas atrial fluorescence and size increased throughout ventricular injury and recovery. *Tg(vmhc:mCherry-NTR;cmlc2:actinin-eGFP)* zebrafish were treated with DMSO or 5 mM MTZ at 3 dpf. (a) Quantification of average fluorescence intensity of control and ablated hearts at 12, 24, 48, 72 and 96 hpt, normalized to the intensity of control ventricle. N=5 hearts. (b) Quantification of control and ablated *Tg(vmhc:mCherry-NTR*) heart size at 12, 24, 48, 72 and 96 hpt by brightfield microscopy. N=10 hearts. Ventricular fluorescence and size were reduced significantly by 24 hpt but recovered by 96 hpt when compared to control. Atrial fluorescence and size progressively increased from 24 to 96 hpt. Mean+s.e.m. Student's *t*-test, *$p<0.05$, **$p<0.01$.

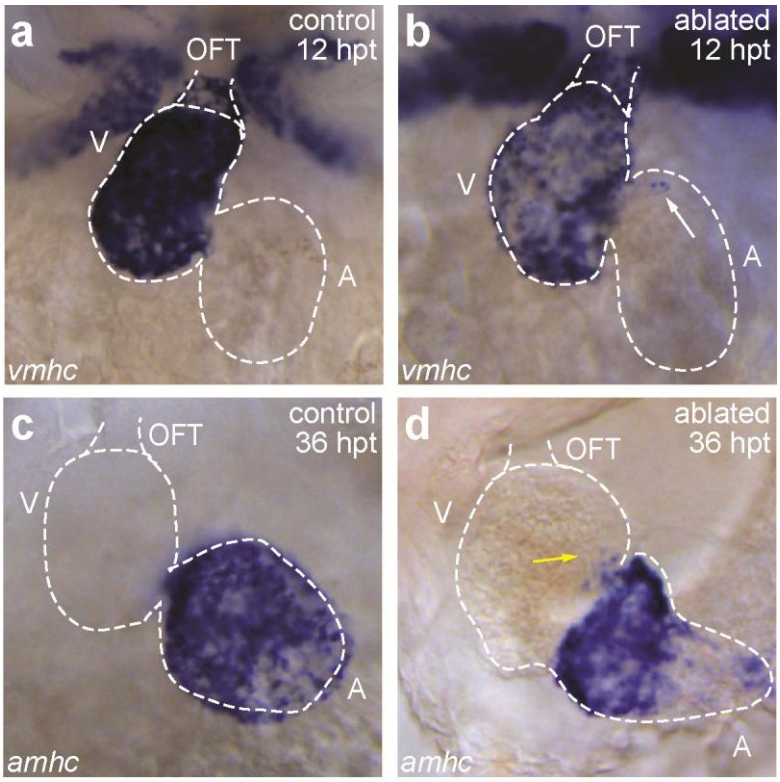


Supplementary figure 3. Cardiac function is significantly reduced after ventricular cardiomyocyte ablation but recovers by 96 hpt. (a, b) *Tg(vmhc:mCherry-NTR)* zebrafish were treated with DMSO or 5 mM MTZ at 3 dpf. M-mode pictures converted from high-speed camera recording show that ventricular function was much weaker in the (b) 24 hpt ablated hearts compared to the (a) 24 hpt control hearts at 4 dpf. Long and short vertical white bars indicate end diastolic and end systolic diameter, respectively. (c, d) Quantification of fractional area change of 12, 24, 48, 72 and 96 hpt control and ablated *Tg(vmhc:mCherry-NTR)* hearts from 3.5-7 dpf. (c) The ventricular contraction is reduced significantly between 12-48 hpt (fractional area change decreased from 42% to 16% at 24 hpt, N=5, p=0.004) but has recovered by 72-96 hpt (fractional area change of

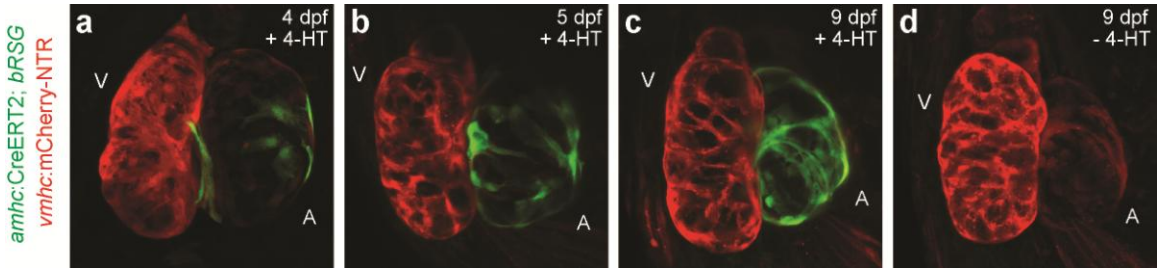
ablated and control ventricle was 38% and 39% at 96 hpt, respectively; N=5, p=0.49). (d) The atrial contraction does not significantly change throughout ventricular injury and recovery. The fractional area change was calculated as FC = (End diastolic area – End systolic area)/End diastolic area * 100%. Mean+s.e.m. Student's *t*-test, *p<0.05, **p<0.01.



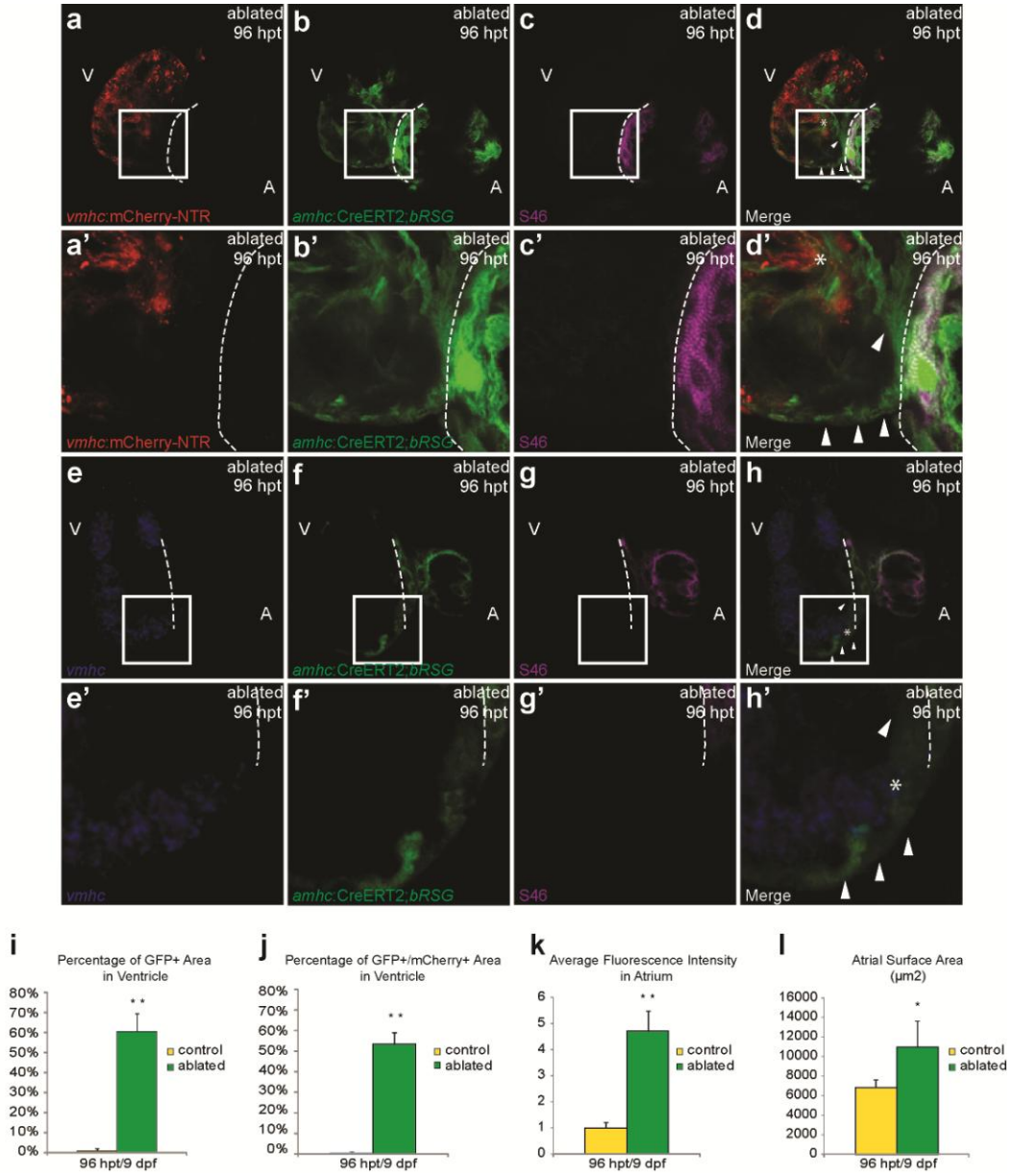
Supplementary figure 4. Cardiomyocyte proliferation increases during recovery of ventricular function. (a-c) *Tg(vmhc:mCherry-NTR;cmlc2:actinin-eGFP)* zebrafish were treated with 5 mM MTZ at 3 dpf. Representative confocal optical section of anti-phospho-histone H3 immunostaining in the 48 hpt ablated hearts at 5 dpf. The arrowhead points to proliferating cardiomyocytes as detected by anti-phospho-histone H3 immunostaining (magenta) and the cardiomyocyte marker *cmlc2:actinin-eGFP* (green). (a) green - GFP; (b) magenta - anti-phospho-histone H3; and (c) merge. (d, e) Quantification of anti-phospho-histone H3 positive cardiomyocytes per chamber in the 24, 36, 48, 60, 72 and 84 hpt control (N=5) and ablated (N=7) hearts from 4-6.5 dpf. Cardiomyocyte proliferation peaked between 36 and 48 hpt in both (d) atrium and (e) ventricle; however, (e) ventricular cardiomyocyte proliferation of ablated hearts decreased to that of controls by 60 hpt, whereas (d) atrial cardiomyocyte proliferation remained elevated up to 84 hpt when compared to controls. Mean+s.e.m. Student's *t*-test, **p*<0.05, ***p*<0.01.



Supplementary figure 5. Chamber-specific genes are ectopically expressed during recovery of zebrafish ventricular function. (a, b) *Tg(vmhc:mCherry-NTR)* zebrafish were treated with DMSO or 5 mM MTZ at 3 dpf. Whole mount *in situ* hybridization of the *vmhc* gene in the 12 hpt (a) control and (b) ablated hearts at 3.5 dpf. White arrow points to ectopic *vmhc* expression in the atrium. (c, d) Whole mount *in situ* hybridization of *amhc* gene in the 36 hpt (c) control and (d) ablated *Tg(vmhc:mCherry-NTR)* hearts at 4.5 dpf. Yellow arrow points to ectopic *amhc* expression in the ventricle. Dashed lines outline the heart. Ventral view, anterior to the top. A - atrium; OFT - outflow tract; V - ventricle.

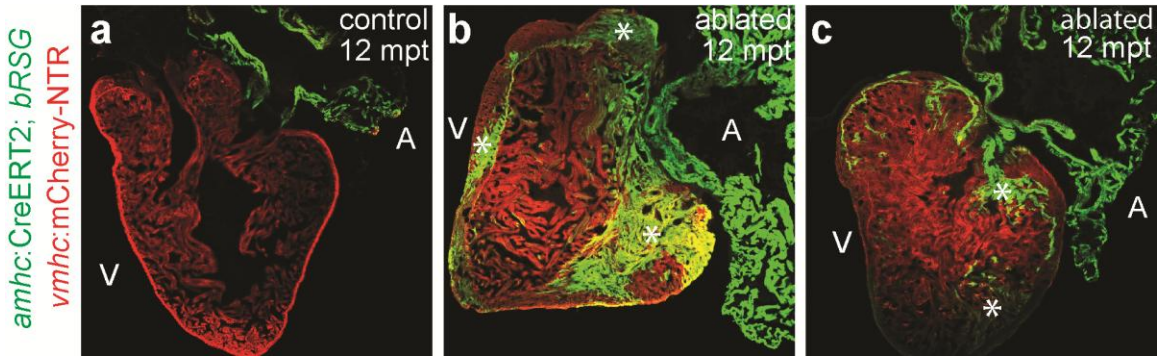


Supplementary figure 6. 4-hydroxytamoxifen treatment of the *Tg(amhc:CreERT2)* line can specifically and genetically label atrial cardiomyocytes. (a-d) *Tg(vmhc:mCherry-NTR;amhc:CreERT2;β-act2:RSG)* fish were treated with (a-c) 4-hydroxytamoxifen (4-HT) or (d) 0.1% ethanol alone from 72-78 hpf. Representative confocal maximal projections shows GFP genetically labeled atrial cardiomyocytes at (a) 4 dpf, (b) 5 dpf and (c) 9 dpf in the same 4-HT treated hearts. (d) No GFP genetically labeled atrial cardiomyocytes were detected at 9 dpf in 0.1% ethanol treatment control hearts. A - atrium; V - ventricle.



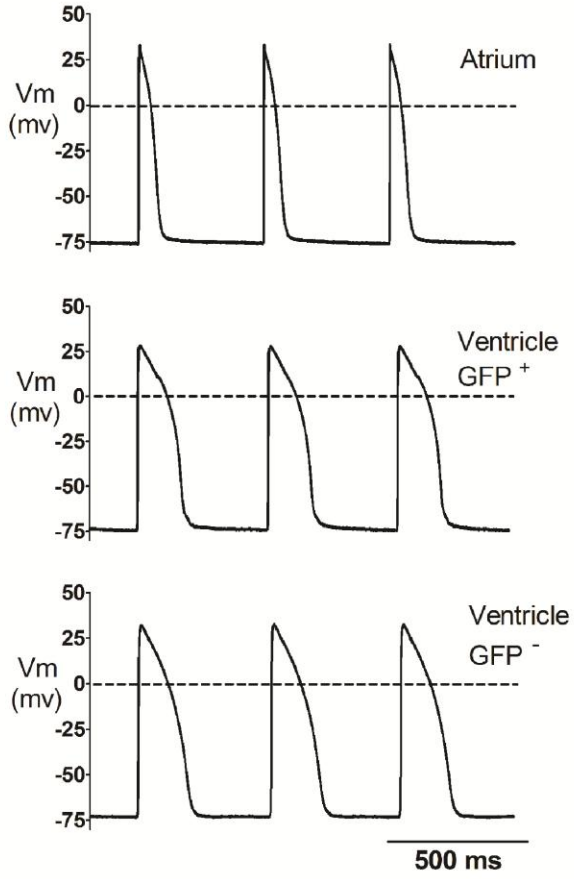
Supplementary figure 7. GFP genetically labeled atrial cardiomyocytes dedifferentiate and transdifferentiate in the ablated ventricle. *Tg(vmhc:mCherry-NTR;amhc:CreERT2;β-act2:RSG)* zebrafish were treated with 4-hydroxytamoxifen (4-HT) from 72-78 hpf to genetically label atrial cardiomyocytes with GFP. Ventricular cardiomyocytes were then treated with DMSO or 5 mM MTZ at 5 dpf. Representative confocal optical section shows that GFP genetically labeled atrial cardiomyocytes in the 96 hpt ablated ventricles co-express ventricular mCherry (red) or *vmhc* (fluorescent *in situ*, blue) but not Amhc (S46 staining, magenta) at 9 dpf (d, d', h, h', asterisk).

Interestingly, there is a small fraction of GFP genetically labeled atrial cardiomyocytes in the 96 hpt ablated ventricle that do not co-express either ventricular mCherry (red)/*vmhc* (blue) or Amhc (S46 staining, magenta) at 9 dpf (d, d', h, h', arrowheads). (a) red - vCherry^{+}; (b, f) green - c-aGFP^{+}; (c, g) magenta - S46/anti-Amhc; (d, h) merge; and (e) blue – *vmhc* RNA. (a'-h') Enlargement of boxed area in a-h. Dashed lines - boundary between atrium and ventricle. A - atrium; V - ventricle. (i) Quantification of percentage of GFP positive area in the 96 hpt control and ablated ventricles. $^{**}p<0.01$. (j) Quantification of percentage of GFP and mCherry double positive area in the 96 hpt control and ablated ventricles. $^{**}p<0.01$. (k) Quantification of average fluorescence intensity in the atria of 96 hpt control and ablated hearts, normalized to the intensity of control atria. $^{**}p<0.01$. (l) Quantification of atrial surface area of 96 hpt control and ablated hearts. $^{*}p<0.05$. N=5 hearts. Mean+s.e.m. Student's *t*-test.



Supplementary figure 8. 12 month post-treatment (mpt) ventricle-ablated fish survive and have normal hearts. *Tg(vmhc:mCherry-NTR;amhc:CreERT2;β-act2:RSG)* larvae were treated with 4-hydroxytamoxifen (4-HT) from 72-78 hpf to genetically label atrial cardiomyocytes with GFP. Ventricular cardiomyocytes were then treated with DMSO or 5mM MTZ at 5 dpf. 12 mpt (a) control and (b, c) ventricle-ablated hearts were morphologically similar. GFP genetically labeled atrial cardiomyocytes can be detected in the (b, c) regenerated 12 mpt ablated ventricles but not in the (a) 12 mpt control ventricles. Asterisks - transdifferentiated ventricular cardiomyocytes (c-aGFP^{+}/vCherry^{+}). A - atrium; V - ventricle.

a

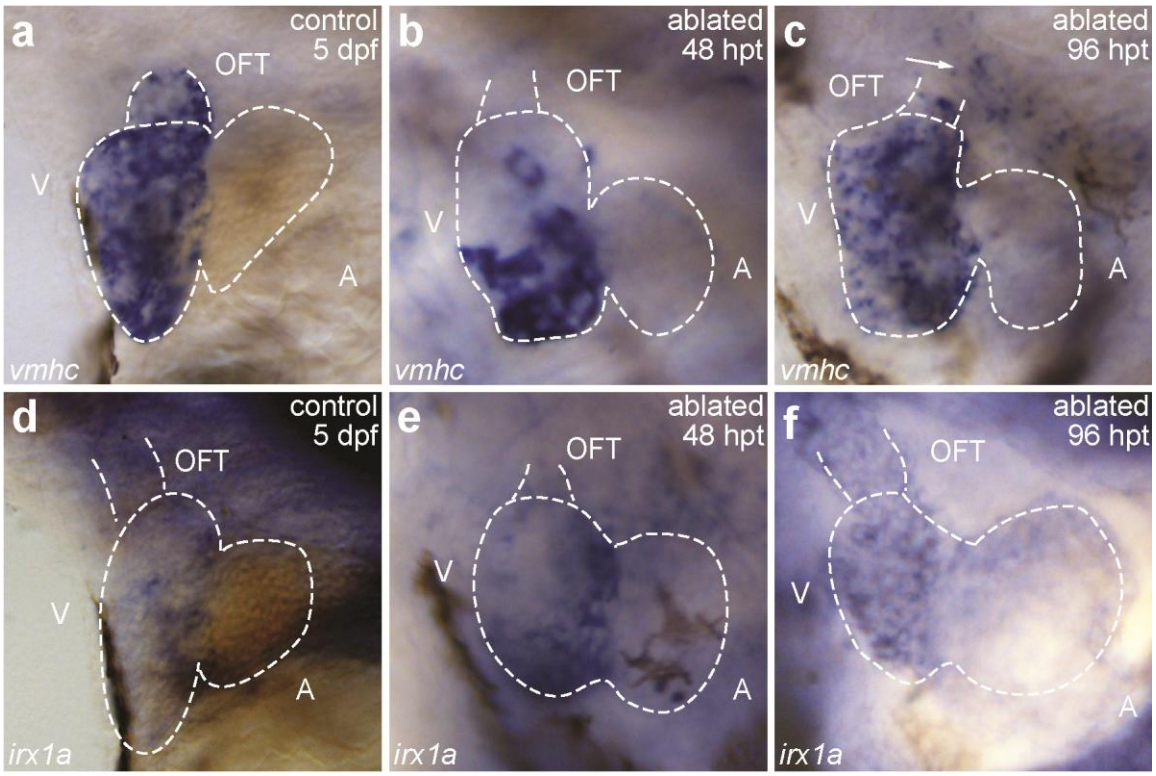


b

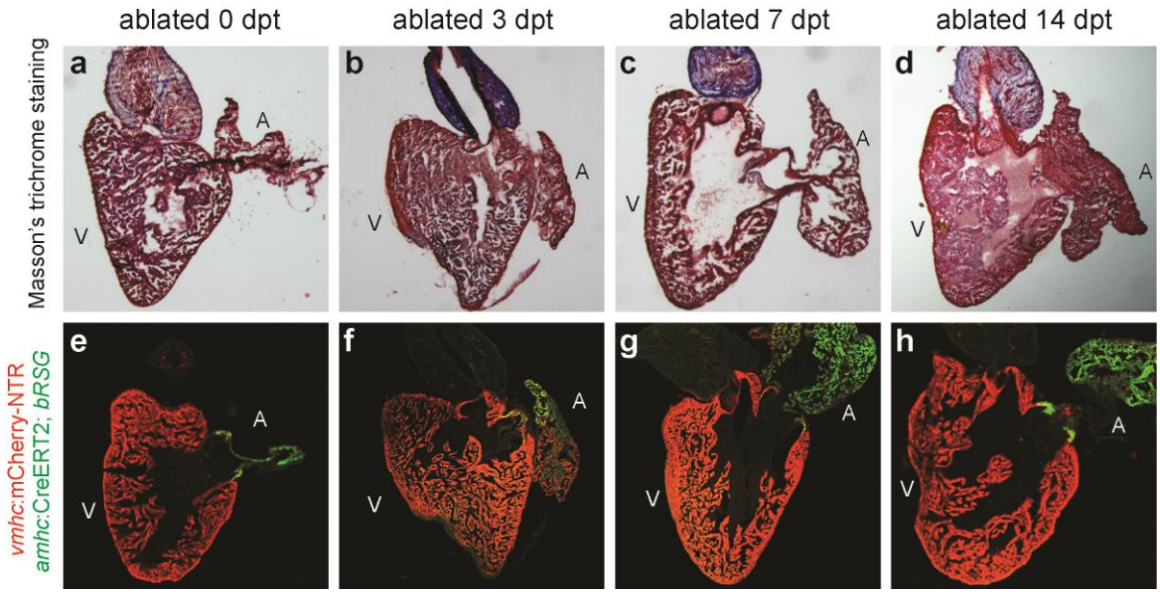
	Atrium	Ventricle GFP $^{+}$	Ventricle GFP $^{-}$
Resting membrane potential (mv)	-71.3±1.2	-70.4±1.3	-72.2±1.2
Action potential amplitude (mv)	101.2±1.7	101.7±2.5	102.3±2.4
Action potential duration at 50% of repolarization (APD_{50}) (ms)	74.1±1.3	183.5±3.6*	184.2±1.3*

Supplementary figure 9. Atrial-derived ventricular cardiomyocytes show typical ventricular electrophysiological characteristics that are different from atrial cardiomyocytes. *Tg(vmhc:mCherry-NTR;amhc:CreERT2;β-act2:RSG)* zebrafish were treated with 4-hydroxytamoxifen (4-HT) from 72-78 hpf to genetically label atrial cardiomyocytes with GFP, and ventricular cardiomyocytes were then treated with 5 mM MTZ at 5 dpf. Electrophysiologic intracellular recordings were performed on transdifferentiated ventricular cardiomyocytes (c-aGFP^{+}/vCherry^{+}), endogenous

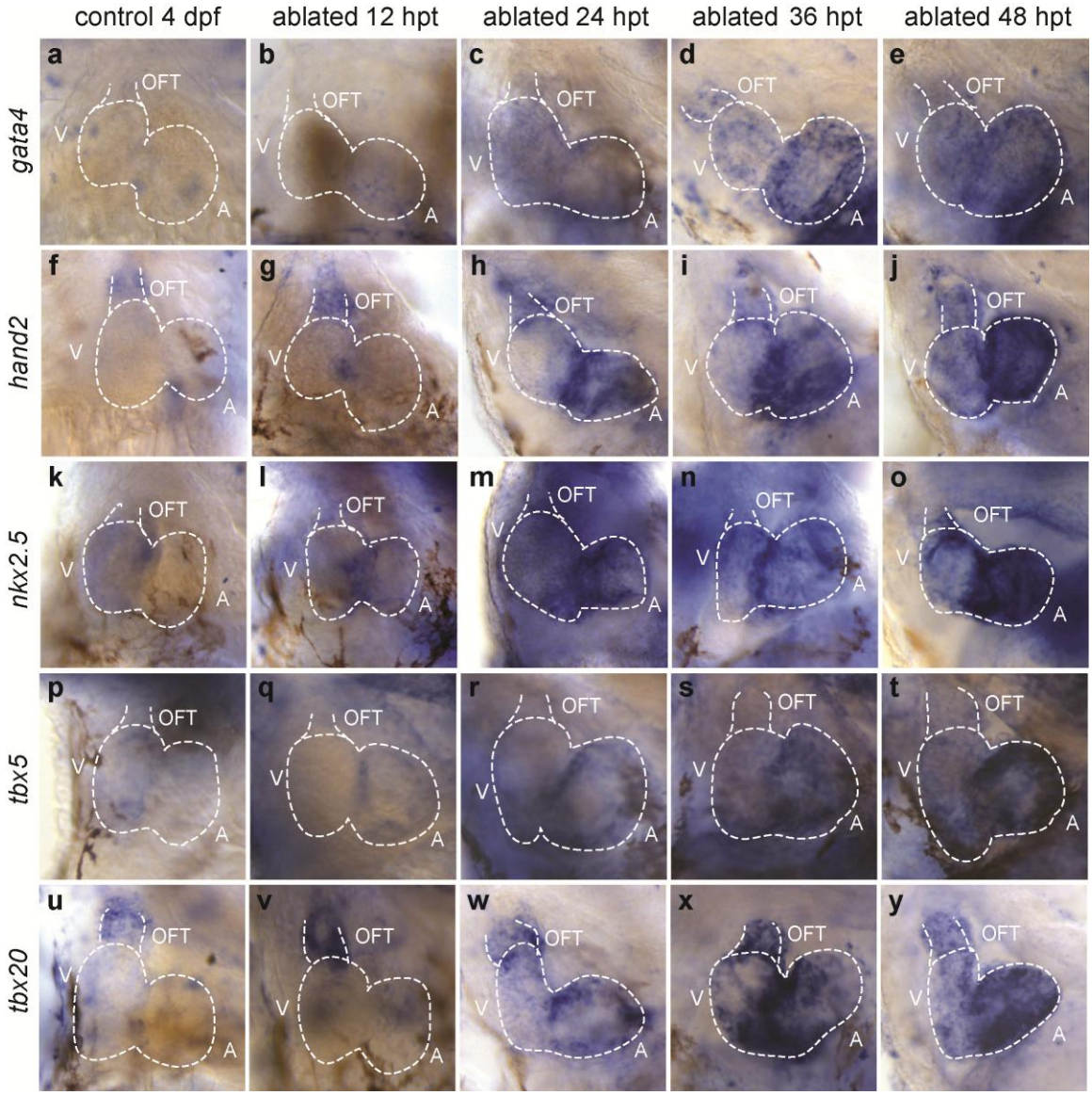
ventricular cardiomyocytes (c-aGFP$^-$/vCherry$^+$) and atrial cardiomyocytes (c-aGFP$^+$/vCherry$^-$) of the injured/regenerated hearts. (a) Representative traces show that the action potential of c-aGFP$^+$/vCherry$^+$ transdifferentiated ventricular cardiomyocytes (Ventricle GFP$^+$) is similar to that of endogenous c-aGFP$^-$/vCherry$^+$ ventricular cardiomyocytes (Ventricle GFP$^-$) but is different from that of c-aGFP$^+$/vCherry$^-$ atrial cardiomyocytes (Atrium). (b) Quantification of action potential parameters among the three groups of cardiomyocytes. The action potential duration at 50% of repolarization (APD_{50}) of c-aGFP$^+$/vCherry$^+$ (Ventricle GFP$^+$) and c-aGFP$^-$/vCherry$^+$ ventricular cardiomyocytes (Ventricle GFP$^-$) is significantly prolonged compared to that of c-aGFP$^+$/vCherry$^-$ atrial cardiomyocytes (Atrium). N=5 hearts. Mean±s.e.m. Student's *t*-test, *p<0.05.



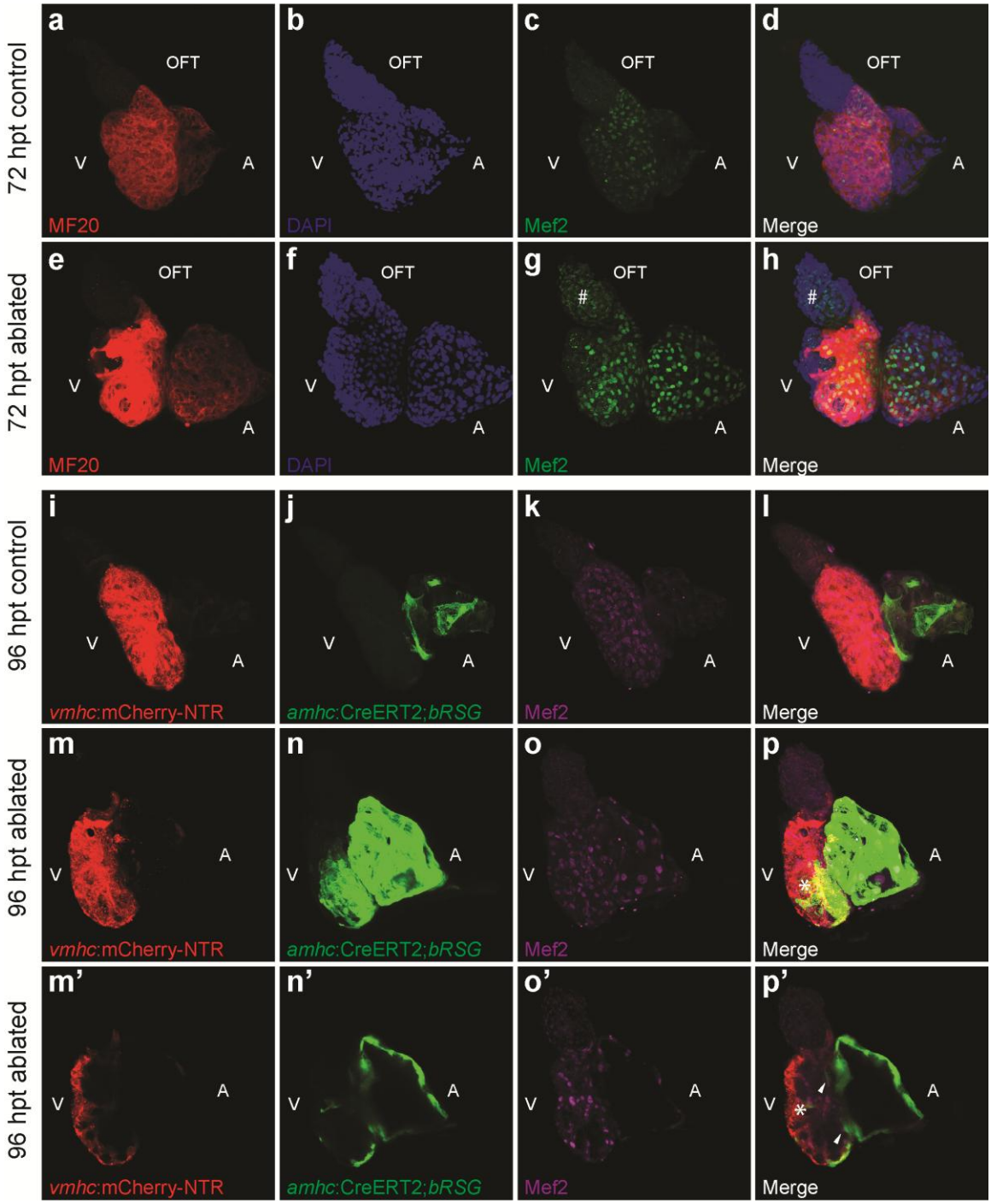
Supplementary figure 10. The ventricular specific genes, *irx1a* and *vmhc*, were expressed throughout the regenerated ventricular myocardium after injury, including regions where aGFP$^+$/vCherry$^+$ transdifferentiated ventricular cardiomyocytes typically reside. *Tg(vmhc:mCherry-NTR)* zebrafish were treated with DMSO or 5 mM MTZ at 3 dpf. Whole mount *in situ* hybridization shows that (a) *vmhc* and (d) *irx1a* were specifically expressed in the ventricles of 5 dpf control hearts. Similarly, (b, c) *vmhc* and (e, f) *irx1a* were expressed throughout the regenerated ventricular myocardium at 48 and 96 hpt. This expression included regions of the ventricle where c-aGFP$^+$/vCherry$^+$ transdifferentiated ventricular cardiomyocytes are normally present during ventricular recovery. Arrow points to ectopic *vmhc* expression outside of the heart at 96 hpt. Dashed lines outline the heart. Ventral view, anterior to the top. A - atrium; OFT - outflow tract; V - ventricle.



Supplementary figure 11. Cardiac transdifferentiation is diminished in adult zebrafish hearts. *Tg(vmhc:mCherry-NTR;amhc:CreERT2;β-act2:RSG))* zebrafish were treated with 4-hydroxytamoxifen (4-HT) from 72-78 hpf to genetically label atrial cardiomyocytes with GFP. Ventricular cardiomyocytes were then treated with 10 mM MTZ for two days at 3-4 months of age. (a-d) Representative Masson's trichrome staining of (a) 0 dpt, (b) 3 dpt, (c) 7 dpt and (d) 14 dpt MTZ ablated hearts shows reduced myocardium and increased fibrosis at 3 dpt but ventricular regeneration/recovery by 14 dpt. (e-h) Representative confocal images reveal that relatively few c-aGFP^{+} cardiomyocytes contribute to the regenerating ventricular myocardium nearest the AV canal region.

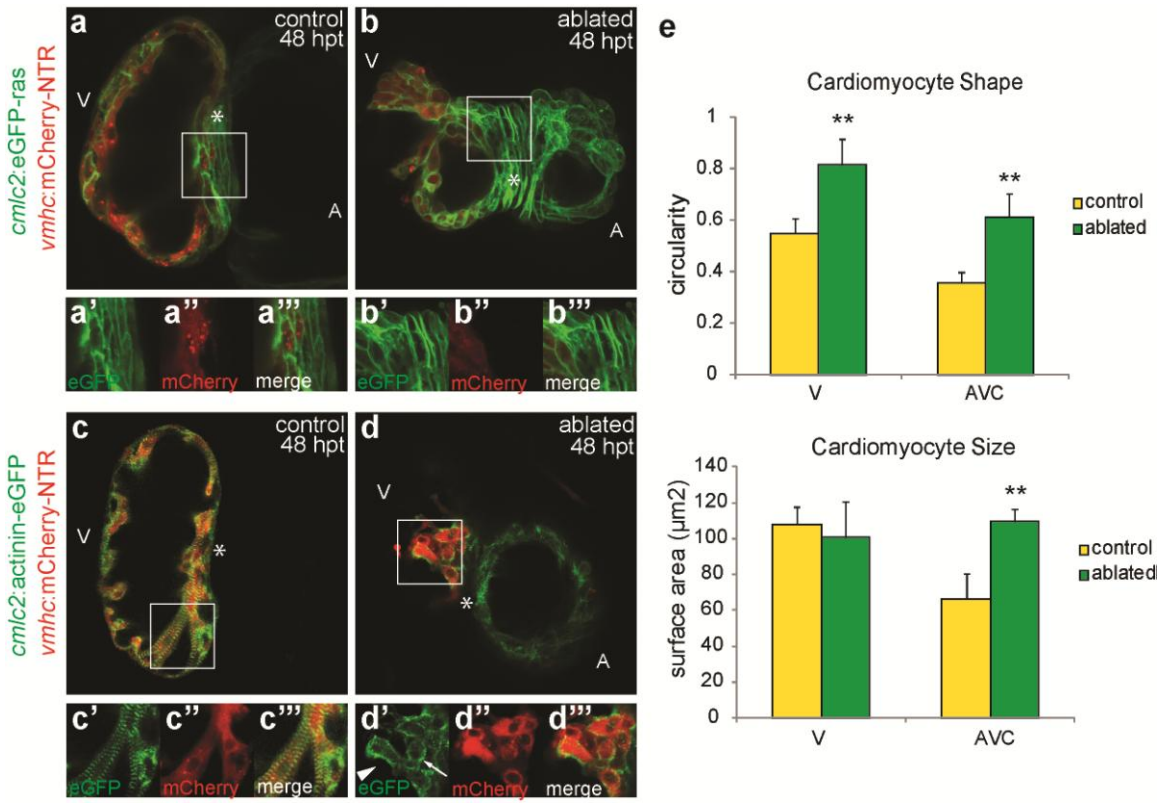


Supplementary figure 12. Early developmental cardiac transcription factors are re-activated during zebrafish ventricular regeneration. *Tg(vmhc:mCherry-NTR)* zebrafish were treated with DMSO or 5 mM MTZ at 3 dpf. Whole mount *in situ* hybridization showed that (a-e) *gata4*, (f-j) *hand2*, (k-o) *nkx2.5*, (p-t) *tbx5*, and (u-y) *tbx20* expression is progressively upregulated in the 12, 24, 36 and 48 hpt ablated hearts compared to 4 dpf control hearts. Dashed lines outline the heart. Ventral view, anterior to the top. A - atrium; OFT - outflow tract; V - ventricle.



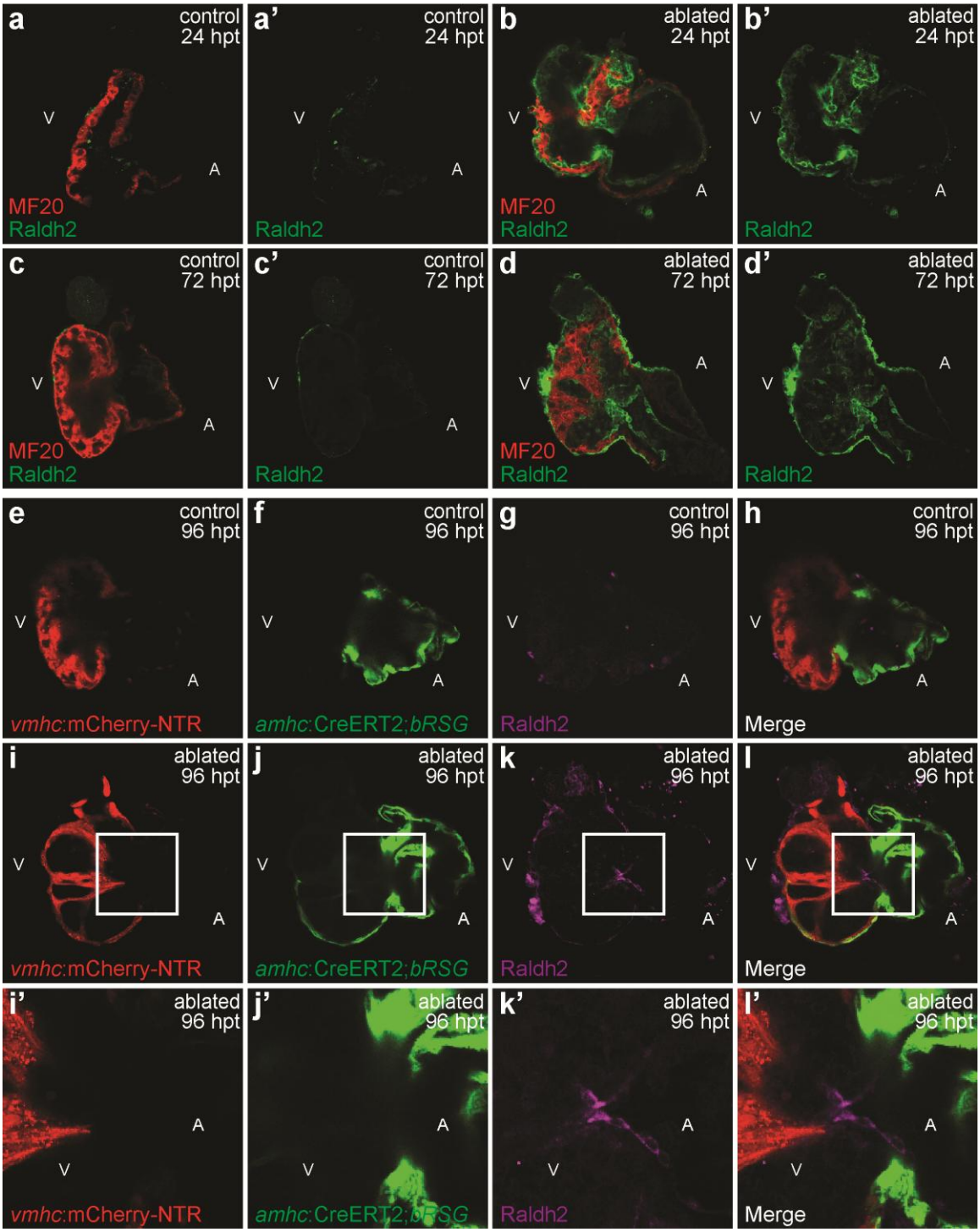
Supplementary figure 13. The expression of the Mef2 cardiac transcription factor is upregulated in the atrium, ventricle, and outflow tract during ventricular recovery. (a-h) *Tg(vmhc:mCherry-NTR)* zebrafish were treated with DMSO or 5 mM MTZ at 3 dpf. Mef2 immunofluorescence of 72 hpt (a-d) control and (e-h) ablated hearts at 6 dpf reveals

that Mef2 (green) expression is increased in the atrium, ventricle, and the outflow tract of recovering ventricle-ablated hearts. $Mef2^{+}$ cells at the outflow tract (#), which were present in ventricle-ablated but not control hearts, did not express myosin heavy chain as detected by MF20 immunostaining (red). (a, e) red - MF20/anti-MHC; (b, f) blue - DAPI; (c, g) green - Mef2; (d, h) merge. (i-p) *Tg(vmhc:mCherry-NTR;amhc:CreERT2;β-act2:RSG)* larvae were treated with 4-hydroxytamoxifen (4-HT) from 72-78 hpf to genetically label atrial cardiomyocytes with GFP. Ventricular cardiomyocytes were then treated with DMSO or 5mM MTZ at 5 dpf. Confocal maximal projection shows that c-$aGFP^{+}$ cardiomyocytes in the 96 hpt ablated ventricles co-express Mef2 in ventricular cardiomyocytes at 9 dpf (asterisks). (m'-p') Representative confocal optical section of m-p. Arrowheads point to ventricular c-$aGFP^{+}$ cardiomyocytes co-expressing Mef2 but not vCherry. Asterisks mark an area of ventricular c-$aGFP^{+}$ cardiomyocytes that co-express Mef2 and vCherry. (i, m) red - $vCherry^{+}$; (j, n) green - c-$aGFP^{+}$; (k, o) magenta - anti-Mef2; and (l, p) merge. A - atrium; OFT - outflow tract; V - ventricle.



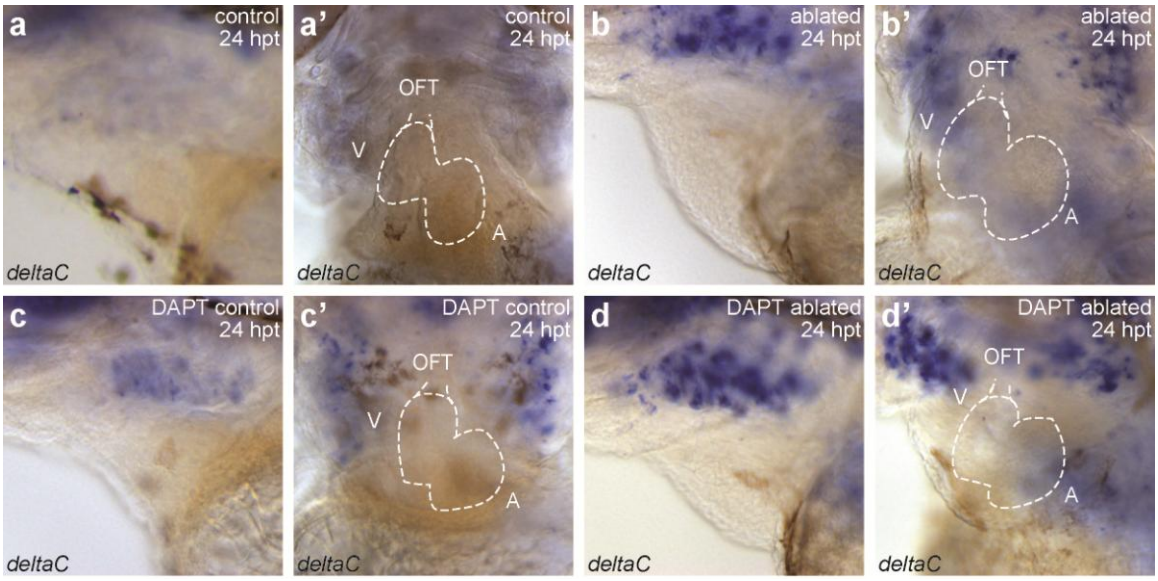
Supplementary figure 14. Dynamic cellular remodeling occurs during zebrafish cardiac regeneration and reprogramming. (a, b) Representative confocal projections of 48 hpt (a) control and (b) ablated *Tg(vmhc:mCherry-NTR;cmlc2:eGFP-ras)* hearts at 5 dpf show the cell shape change of AV canal cardiomyocytes during ventricular regeneration. (a'-a''', b'-b''') Enlargement of boxed area in a and b. (a', b') GFP channel only; (a'', b'') mCherry channel only; (a''', b''') merge. (c, d) Representative confocal projections of 48 hpt (c) control and (d) ablated *Tg(vmhc:mCherry-NTR;cmlc2:actinin-eGFP)* hearts at 5 dpf show that dynamic sarcomere assembly transpires in regenerating ventricular cardiomyocytes. (c'-c''', d'-d''') Enlargement of boxed area in c and d. (c', d') GFP channel only; (c'', d'') mCherry channel only; (c''', d''') merge. Arrow points to cardiomyocyte with sarcomeric Z-disc enriched in cell periphery. Arrowhead points to cardiomyocytes with more mature striated Z-lines. Asterisk - atrioventricular canal. A - atrium; V - ventricle. (e) Quantification of 48 hpt control and ablated *Tg(vmhc:mCherry-NTR;cmlc2:eGFP-ras)* cardiomyocyte shape and size at 5 dpf. Cardiomyocytes in regenerating ventricles and AV canals are more circular

than those in the control hearts. Cardiomyocyte surface areas are similar in control and regenerating ventricles but increased in regenerating AVC cardiomyocytes when compared to control. AVC - atrioventricular canal; V - ventricle. N=15 hearts. Mean+s.e.m. Student's *t*-test, $**p<0.01$.

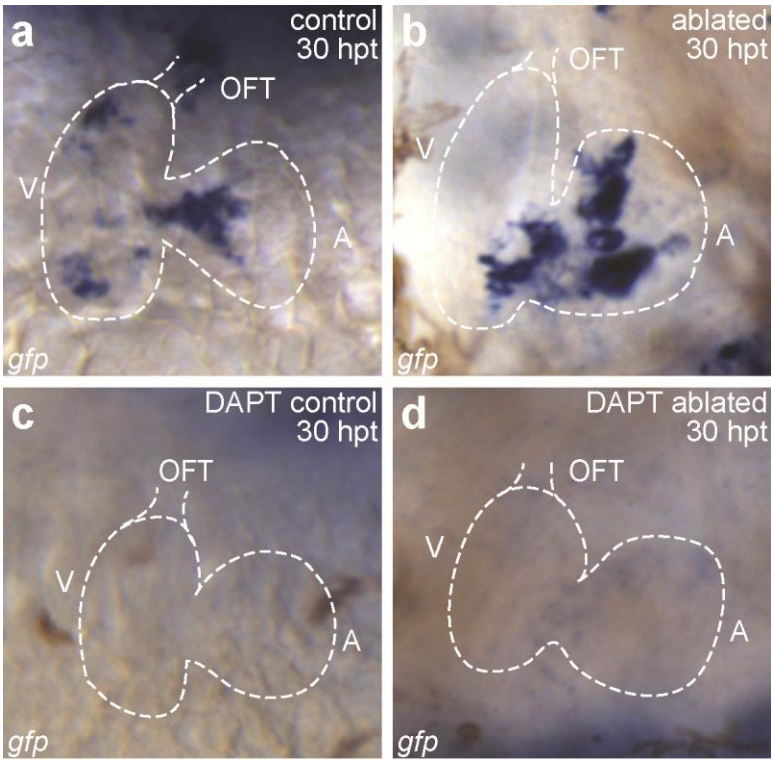


Supplementary figure 15. Raldh2 is activated throughout the endocardium and epicardium of the injured heart. (a-d) *Tg(vmhc:mCherry-NTR)* zebrafish were treated with DMSO or 5 mM MTZ at 3 dpf. Raldh2 immunofluorescence of (a, c) control and (b, d) ablated hearts at 24 and 72 hpt reveals that Raldh2 expression (green) is activated

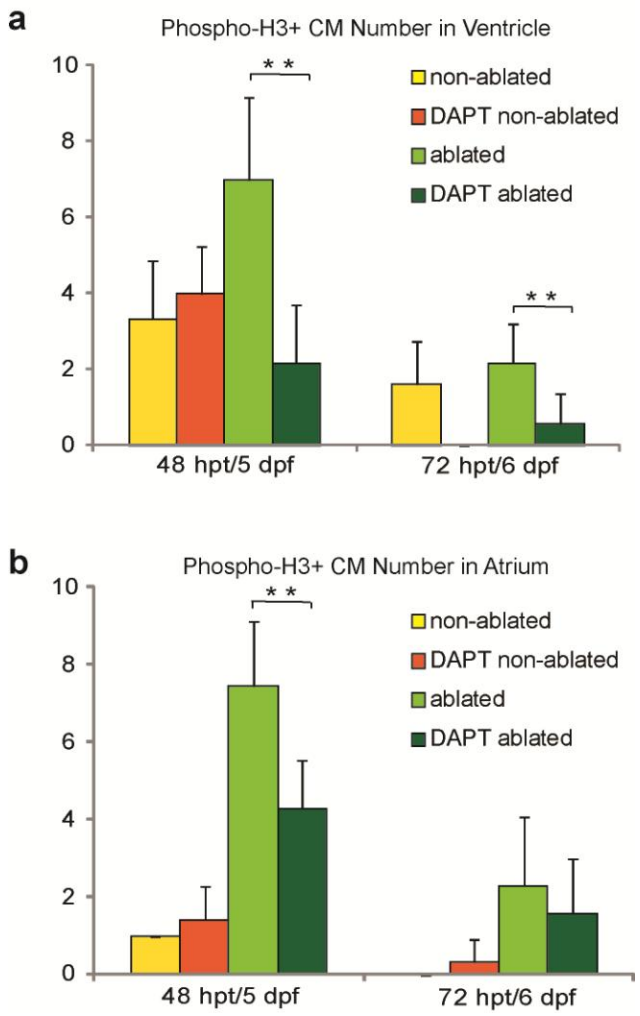
throughout the endocardium and epicardium of the ventricle-ablated heart when compared to control heart. (a-d) Merge of MF20/anti-MHC (red) and anti-Raldh2 (green) immunostaining; (a'-d') anti-Raldh2 (green) immunostaining only. (e-l) *Tg(vmhc:mCherry-NTR;amhc:CreERT2;β-act2:RSG)* larvae were treated with 4-hydroxytamoxifen (4-HT) from 72-78 hpf to genetically label atrial cardiomyocytes with GFP. Ventricular cardiomyocytes were then treated with DMSO or 5mM MTZ at 5 dpf. Raldh2 immunofluorescence of these ventricle-ablated hearts shows that no c-aGFP^{+} cells were detected in the Raldh2 expressing endocardium or epicardium of the ventricle-ablated hearts at 96 hpt. (i'-l') Boxed area of i-l. (e, i) red - vCherry^{+}; (f, j) green - c-aGFP^{+}; (g, k) magenta - anti-Raldh2; and (h, l) merge. A - atrium; V - ventricle.



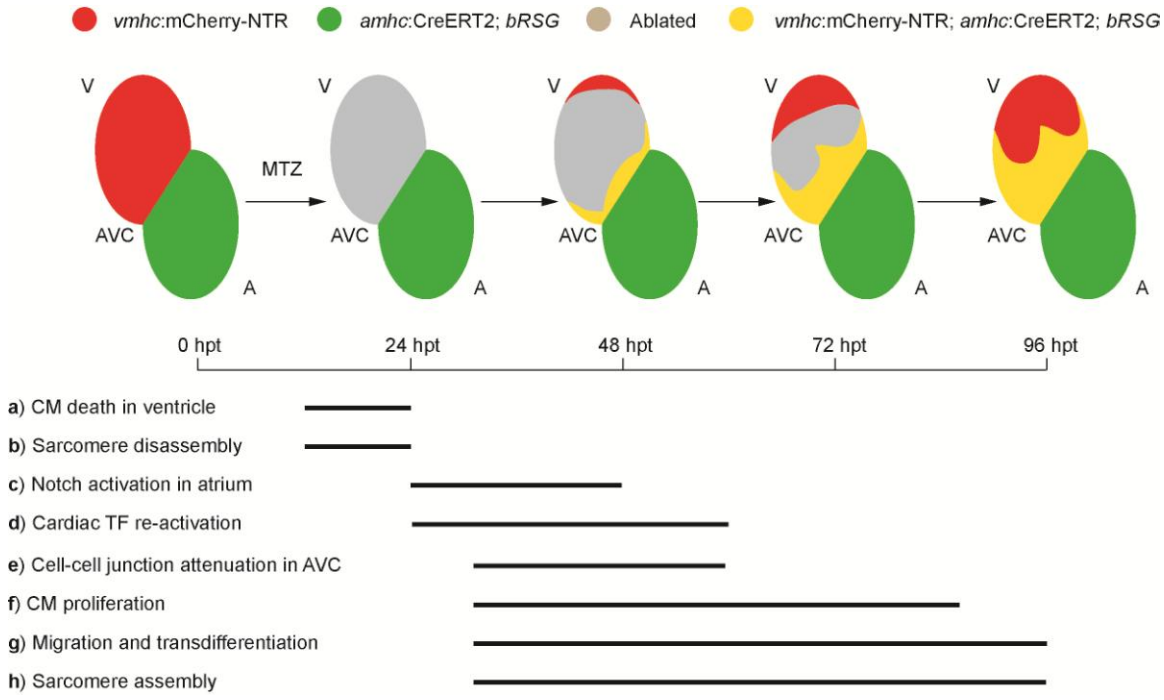
Supplementary figure 16. *deltaC* is not expressed in the heart during zebrafish ventricular regeneration. *Tg(vmhc:mCherry-NTR)* zebrafish were treated with DMSO or 5 mM MTZ at 3 dpf. Whole mount *in situ* hybridization reveals no *deltaC* expression in the 24 hpt (a) control or (b) ablated hearts at 4 dpf. DAPT treatment did not change *deltaC* expression in the 24 hpt (c) control or (d) ablated hearts at 4 dpf. (a-d) lateral view, anterior to the left. (a'-d') ventral view, anterior to the top. Dashed lines outline the heart. A - atrium; OFT - outflow tract; V - ventricle.



Supplementary figure 17. Increased Notch signaling during ventricular regeneration can be blocked by DAPT treatment. *Tg(vmhc:mCherry-NTR;tp1:eGFP)* zebrafish were treated with DMSO or 5 mM MTZ at 3 dpf . Notch signaling as detected by *gfp* whole mount *in situ* hybridization is increased in the AV canal and atrium of (b) 30 hpt ablated hearts compared to (a) 30 hpt control hearts at 4.5 dpf. DAPT treatment blocks *gfp* expression as detected by *in situ* hybridization analysis in the 30 hpt (c) control and (d) ablated hearts at 4.5 dpf. Dashed lines outline the heart. Ventral view, anterior to the top. A - atrium; OFT - outflow tract; V - ventricle.



Supplementary figure 18. Cardiomyocyte proliferation is reduced after DAPT treatment. *Tg(vmhc:mCherry-NTR;cmlc2:actinin-eGFP)* zebrafish were treated with DMSO or 5 mM MTZ at 3 dpf. Quantification of anti-phospho-histone H3 immunolabeled cardiomyocyte numbers in the (a) ventricles and (b) atria at 48 and 72 hpt with or without DAPT treatment. When compared to non-DAPT-treated ablated hearts (lime green), ventricular and atrial cardiomyocyte proliferation decreased significantly at 48 and 72 hpt in ablated hearts after DAPT treatment (green). However, there appeared to be no difference in atrial or ventricular cardiomyocyte proliferation between DAPT (orange) or non-DAPT-treated (yellow) non-ablated hearts. N=5 for non-ablated group and N=7 for ablated group. Mean+s.e.m. Student's *t*-test, $^{**}p<0.01$.



Supplementary figure 19. Cardiac transdifferentiation model of the cellular events that transpire during ventricular myocardial injury and regeneration. Illustration summarizes the cellular and molecular events that occur during ventricular injury and regeneration in 4-HT-treated MTZ-ablated *Tg(vmhc:mCherry-NTR;amhc:CreERT2;β-act2:RSG)* hearts at 0, 24, 48, 72 and 96 hpt. (a) Cardiomyocyte death occurs in the ventricle between 12-24 hpt and is accompanied by (b) sarcomere disassembly. (c) After injury, Notch signaling activates in the atrial endocardium from 24 to 48 hpt. (d) Notably, early developmental cardiac transcription factors also re-activate in the atrioventricular canal (AVC) and atrium between 24-48 hpt and then extend into the ventricle after 48 hpt. (e) During cardiac transcription factor reactivation, cell-cell junctions in the AVC cardiomyocytes dynamically attenuate from 30 to 60 hpt. (f) Cardiomyocyte proliferation begins as early as 30 hpt but peaks at 48 hpt in both ventricle and atrium; however, atrial cardiomyocyte proliferation continues for an extended period. (g) After GFP genetically labeled atrial cardiomyocytes migrate into the regenerating ventricle from 30 to 96 hpt to transdifferentiate into ventricular cardiomyocytes, (h) *de novo* sarcomere assembly in the new ventricular cardiomyocytes occurs. Red - vCherry^{+} ventricular cardiomyocytes; green – c-aGFP^{+} atrial cardiomyocytes; gray - ablated

ventricular cardiomyocytes; yellow - vCherry^{+}/c-aGFP^{+} transdifferentiated cardiomyocytes. A - atrium; AVC - atrioventricular canal; CM - cardiomyocyte; hpt - hour post treatment; MTZ - metronidazole; TF - transcription factor; V - ventricle.