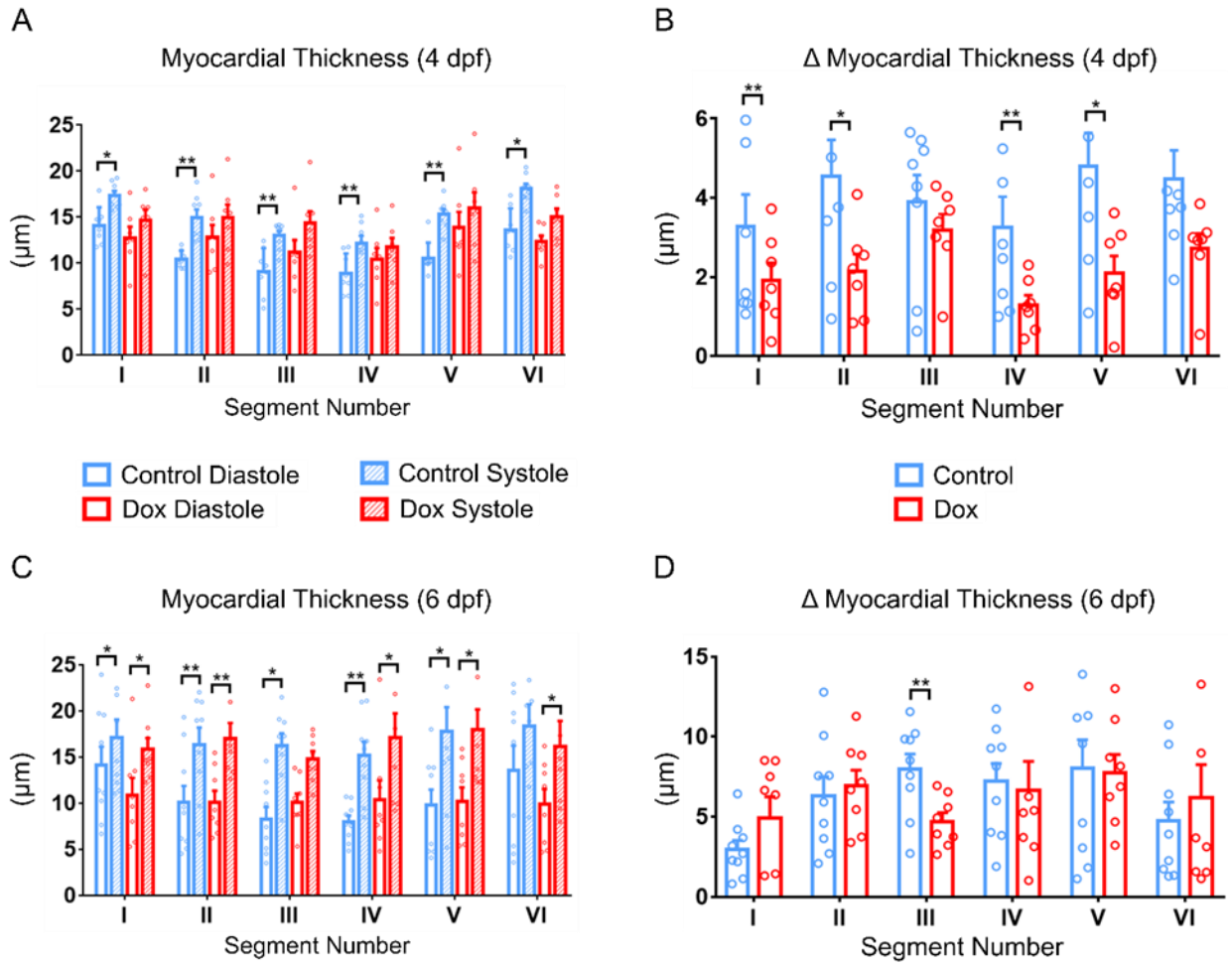
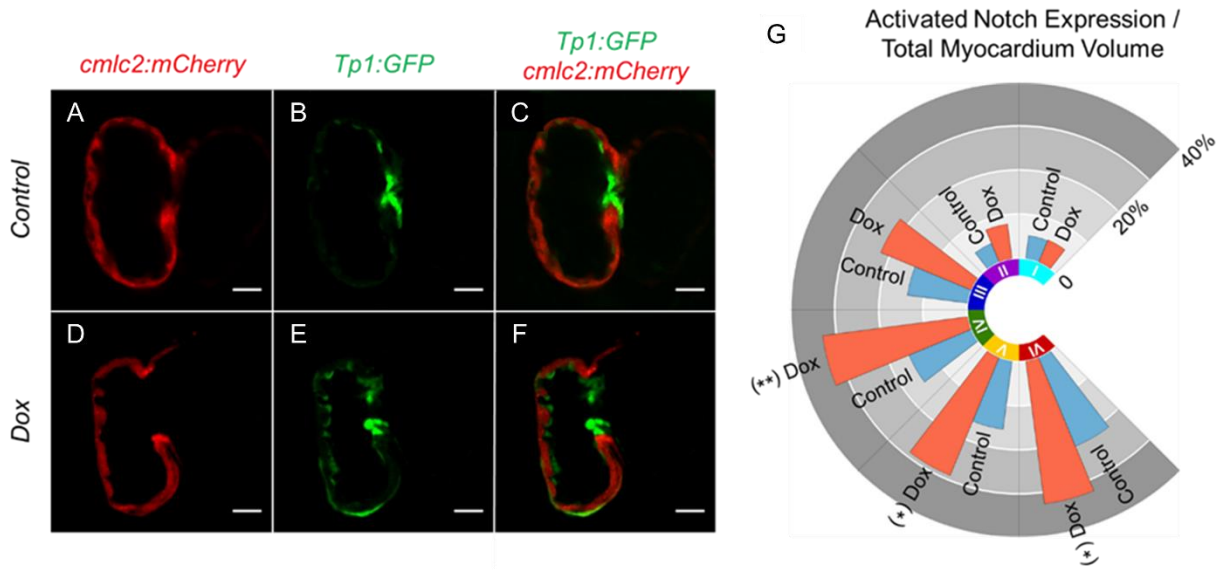
SUPPLEMENTAL INFORMATION

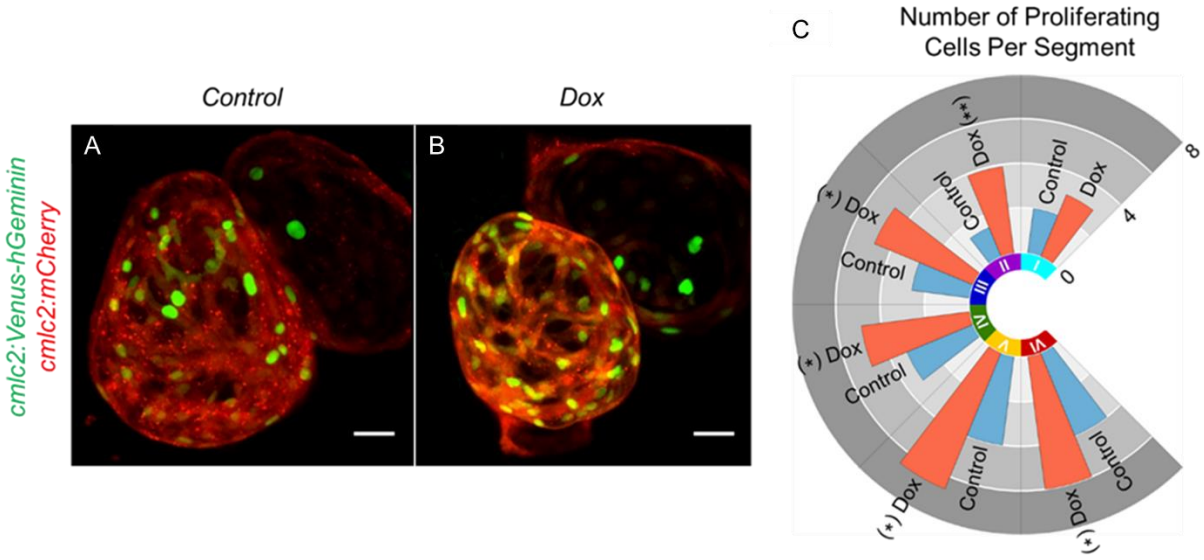
SUPPLEMENTAL FIGURES



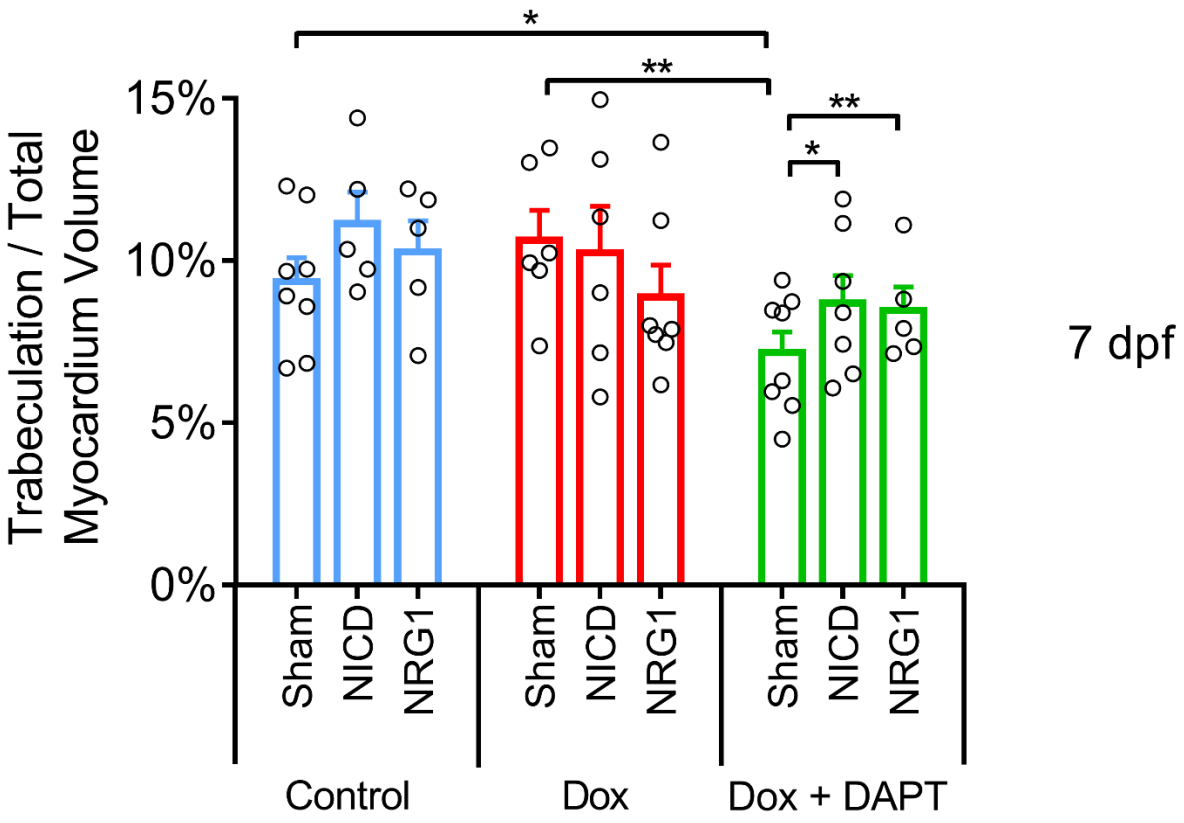
Supplemental Figure S1. Segmental myocardial thickness following doxorubicin treatment. (A, B) After 24 h of doxorubicin treatment (4 dpf), the difference in myocardial thickness between end-systole and end-diastole was significantly decreased compared to control. **(C, D)** Changes in myocardial thickness recovered in most segments 48 h after the end of chemotherapy exposure (6 dpf). Two-sided *t* tests, * $P<0.05$, ** $P<0.01$, n=6-8 per group.



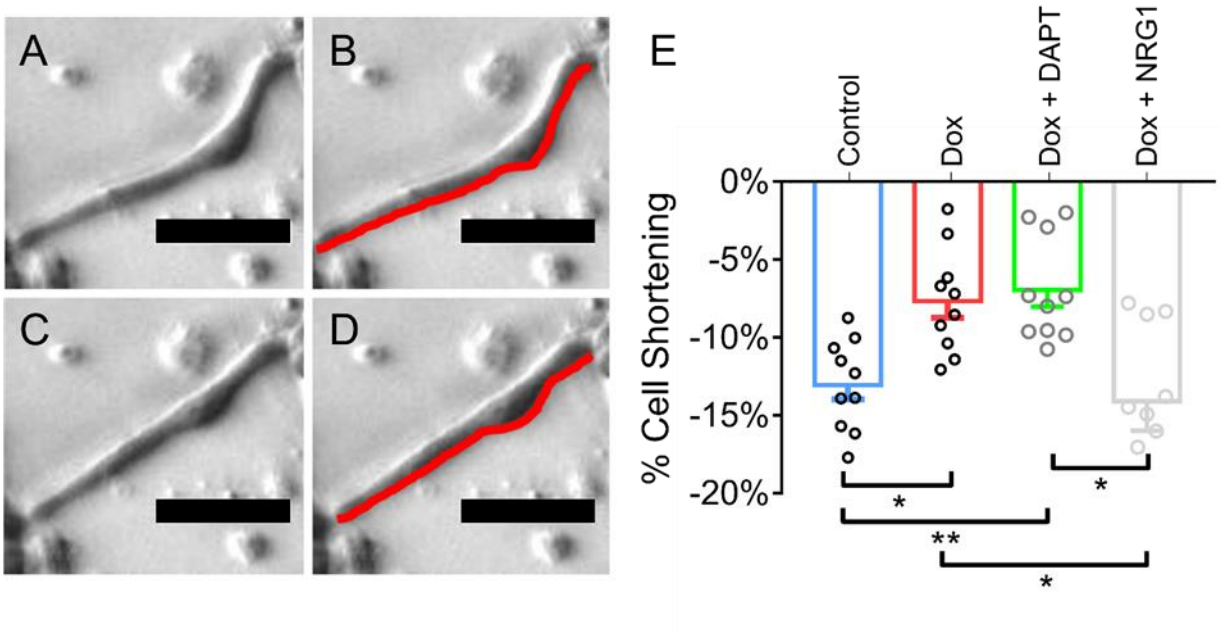
Supplemental Figure S2. Segmental comparison of Notch activation normalized to myocardial volume. (A-F) The *Tg(cmlc2:mCherry;Tp1:gfp)* line was imaged with confocal microscopy at 4 dpf. Notch activation was demonstrated by the green *Tp1-gfp* signal and the myocardium was delineated by the red *cmlc2-mCherry* signal. **(G)** Quantitative analysis of *Tp1-gfp* signal volume normalized to the segmental myocardium volume. Following 24-hour doxorubicin treatment, a robust increase in TP1 signal indicated enhanced Notch activity predominantly in segments IV – VI. Signals emanating from the atrioventricular valve were excluded from analyses. Two-sided *t* tests, * $P<0.05$, ** $P<0.01$, n=6-8 per group. Scale bar: 25 μm.



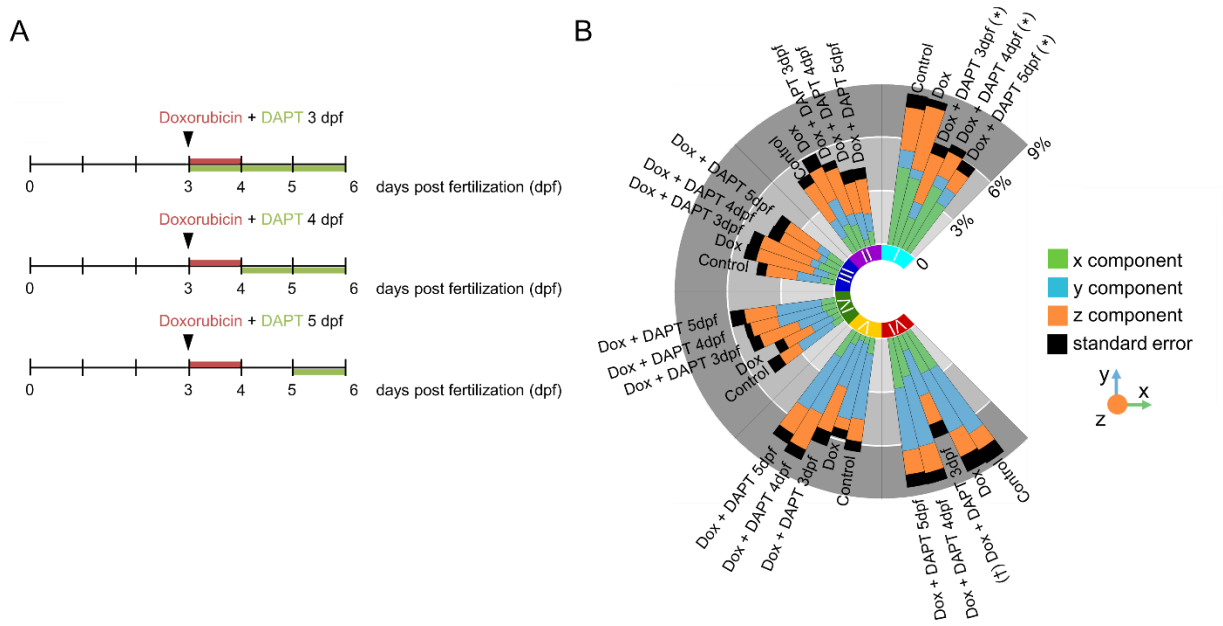
Supplemental Figure S3. Segmental comparison of cardiomyocyte proliferation following chemo-induced injury. Representative confocal microscopy images at 5 dpf, localizing proliferating cardiomyocytes (*cmlc2:Venus-hGeminin,* green) in the 3-D cardiac contour (*cmlc2:mCherry,* red) under control **(A)** and doxorubicin **(B)** conditions. **(C)** Quantitative analysis of segmental cardiomyocyte proliferation demonstrated a significant increase in segments II – VI following doxorubicin treatment. Two-sided *t* tests, * $P<0.05$, ** $P<0.01$, n=6-8 per group. Scale bar: 25 μm.



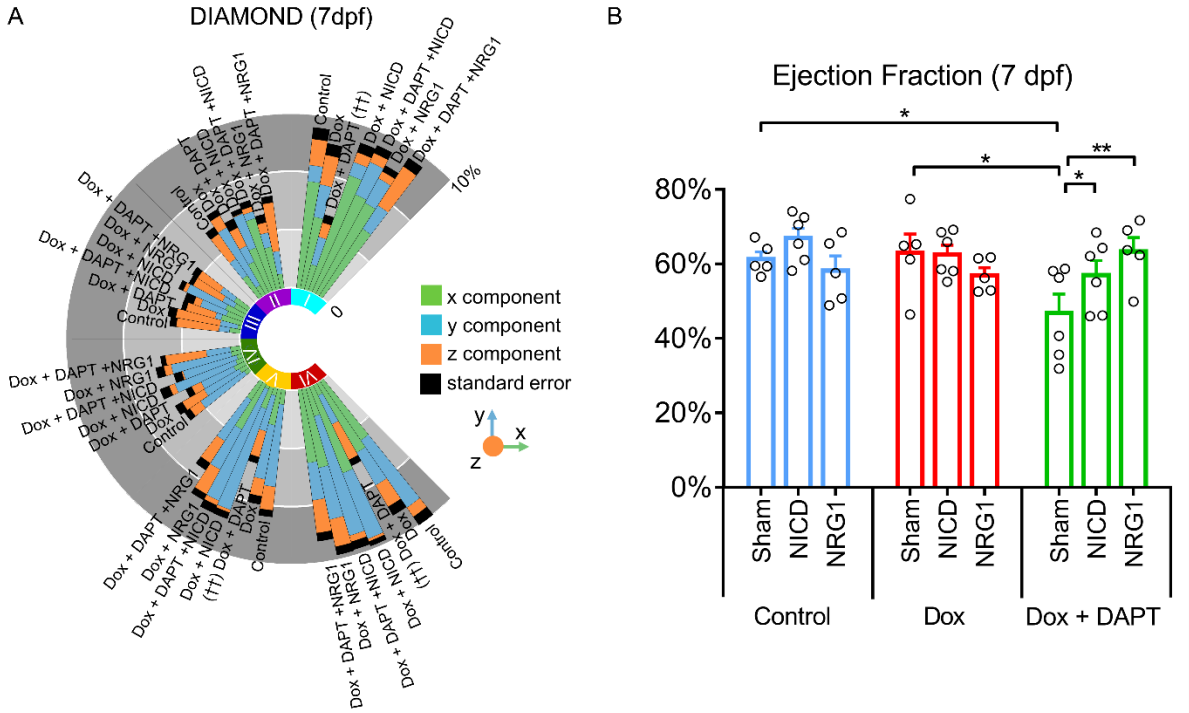
Supplemental Figure S4. The 3-D cardiac trabecular architecture at 7 dpf in response to doxorubicin induced injury and genetic manipulation. The restoration of trabecular network post chemo-induced injury remains attenuated by co-treatment with the Notch γ-secretase inhibitor DAPT at 7 dpf. Treatment with Notch downstream effectors *NICD* and *NRG1* rescues trabecular myocardium. ANOVA and Tukey's method, * $P<0.05$, ** $P<0.01$, n=6-8 per group.



Supplemental Figure S5. Contractile function of *ex vivo* adult zebrafish cardiomyocytes following doxorubicin treatment. (**A-B**) Relaxed cardiomyocyte with length L_1. (**C-D**) Contracted cardiomyocyte with length L_2. (**E**) The percentage cell shortening of cardiomyocytes decreased significantly following doxorubicin treatment, and co-treatment of doxorubicin with DAPT. Overexpression of *NRG1* rescued the reduction of contractility. * P<0.05, ** P<0.01, n=7-10 per group. Scale bar: 30 μm.



Supplemental Figure S6. Inhibition of Notch signaling by DAPT at 0, 24, and 48 hours post doxorubicin-induced injury leads to differential restoration of segmental cardiac function. **(A)** Doxorubicin was combined with DAPT treatment at various time-points. **(B)** Synchronous co-treatment of doxorubicin with DAPT impaired the restoration of myocardial displacement in both segment I and VI. However, Notch inhibition by DAPT at 24 and 48 hours post doxorubicin injury impaired the restoration of segmental cardiac function only in segment I. * *P*<0.05, n=6 per group.



Supplemental Figure S7. DIAMOND mechanics at 7 dpf for segmental localization and quantification of *NICD* and *NRG1* mRNA-mediated myocardial protection following doxorubicin-induced injury. The inhibition of Notch signaling by the γ-secretase-inhibitor DAPT impaired the restoration of segmental (**A**, DIAMOND) and global (**B**, ejection fraction) cardiac function, a finding that was rescued *NICD and NRG1* mRNA treatment. ANOVA and Tukey's method, †† $P<0.01$ Dox + DAPT vs. control, * $P<0.05$, ** $P<0.01$, n=6-10 per group.

SUPPLEMENTAL TABLES

	LSFM	Confocal	OCT	µCT	µMRI	µPET
Fundamental Principle	Laser with Light-Sheet	Laser with Pinhole	Optical Interference	X-ray	Directional Proton Field	Positron Annihilation
Axial Resolution / Slice Thickness	+++	++	++	++	++	+
Lateral / In-plane Resolution	+++	+++	++	++	++	+
Imaging Depth	++	+	++	+++	+++	+++
Temporal Resolution	+++	+	+++	+++	++	+
Molecular Labeling	+++	+++	++	+	+	+++
In vivo Imaging	+++	+	+++	+++	+++	+++
Phototoxicity	+	+++	++	–	–	–

Supplemental Table S1. Characteristics of imaging modalities. A semi-quantitative scale ranging from **+** to **+++** was used to compare key characteristics between imaging modalities. **Legend.** LSFM: light-sheet fluorescent microscopy. µCT: micro-computerized tomography. µMRI: micro-magnetic resonance imaging. µPET: micro-positron emission tomography. OCT: optical coherence tomography. Scale. **–**: absent. **+**: low. **++**: intermediate. **+++**: high.

Supplemental Table S2. *NICD* mRNA sequence.

TGTGGGGTGCTGCTGTCCCGCAAGCGCAGGCGGCAGCATGGCCAGCTCTGGTTCCCTGA
GGGTTTCAAAGTGTCAGAGGCCAGCAAGAAGAAGCGGAGAGAACCCCTCGGCGAGGACT
CAGTCGGCCTCAAGCCCCTGAAGAACGCCTCAGATGGTGCCCTGATGGACGACAATCAGA
ACGAGTGGGGGGACGAAGACCTGGAGACCAAGAAGTTCCGGTTTGAGGAACCAGTGGTT
CTCCCTGACCTGGATGATCAGACTGACCACCGGCAGTGGACCCAGCAGCACCTGGATGCC
GCTGACCTACGTGTGTCTGCCATGGCCCCAACGCCGCCTCAGGGGGAGGTAGATGCTGA
CTGCATGGACGTCAATGTTCGAGGACCAGATGGCTTCACTCCCCTAATGATTGCCTCCTGC
AGCGGAGGGGGCCTGGAGACAGGCAACAGTGAGGAAGAAGAAGATGCACCTGCTGTCAT
CTCCGACTTCATCTATCAGGGTGCCAGCTTGCACAACCAGACGGACCGCACAGGGGAGAC
TGCCCTGCACCTGGCTGCCCGATACTCTCGTTCAGATGCTGCCAAGCGCTTGCTGGAGGC
CAGCGCAGATGCCAACATCCAAGACAACATGGGGCGTACCCCATTACATGCCGCTGTTTC
TGCAGACGCTCAGGGTGTCTTCCAGATCCTGCTCCGGAACAGAGCCACAGATCTGGATGC
CCGAATGCATGATGGCACAACCCCTCTGATCCTGGCGGCACGCCTGGCCGTGGAAGGCA
TGCTAGAGGACCTCATCAACTCTCACGCTGATGTCAATGCTGTGGATGACCTAGGCAAGTC
AGCTCTGCACTGGGCAGCCGCTGTGAACAATGTGGACGCTGCTGTTGTGCTCCTGAAGAA
CGGAGCCAACAAAGACATGCAGAACAACAAGGAGGAGACTCCCCTGTTCCTGGCCGCCC
GTGAGGGCAGCTATGAGACTGCCAAAGTGTTGCTGGACCACTTTGCCAACCGGGACATCA
CGGATCACATGGACCGATTGCCACGGGACATTGCACAGGAGCGCATGCACCACGATATCG
TGCGGCTTTTGGATGAATACAACCTGGTGCGCAGCCCACAGCTGCATGGCACTGCCTTGG
GTGGCACACCCACTCTGTCTCCCACACTCTGCTCGCCCAACGGCTACCTGGGCAACCTCA
AGTCTGCCACACAGGGCAAGAAGGCCCGAAAGCCCAGCACCAAAGGGCTGGCTTGCAGT
AGCAAGGAAGCTAAGGACCTCAAGGCCCGGAGGAAGAAGTCCCAGGATGGCAAGGGCTG
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CTTGTCAGATGTGGCCTCACCACCCCTCCTTCCCTCCCCGTTCCAGCAGTCTCCATCCATG
CCTCTCAGCCACCTGCCAGGTATGCCTGACACCCACCTGGGCATCAGCCACTTGAATGTG
GCAGCCAAGCCCGAGATGGCAGCTCTGGCCGGAGGCAGCCGGTTGGCCTTTGAGCCACC
CCCACCACGCCTCTCCCACCTGCCTGTAGCCTCCAGTGCCAGCACAGTGCTGAGTACCAA
TGGCACAGGGGCTATGAATTTCACCGTGGGTGCACCGGCAAGCTTGAATGGCCAGTGTGA
GTGGCTTCCCCGGCTCCAGAATGGCATGGTGCCCAGCCAGTACAACCCGCTAAGGCCAG
GTGTGACTCCGGGCACACTAAGCACACAGGCAGCTGGCCTCCAGCATGGCATGATGGGC
CCGATACACAGCAGCCTCTCCACCAATACCTTGTCCCCGATTATCTACCAGGGCCTGCCCA
ACACAAGGCTGGCCACACAGCCCCACCTGGTGCAGACCCAGCAGGTGCAGCCACAGAAC
TTACAAATCCAGCCTCAGAACCTGCAGCCACCATCGCAGCCACACCTCAGTGTGAGCTCA
GCAGCCAATGGGCACCTGGGTCGGAGCTTCCTGAGCGGGGAGCCCAGCCAGGCAGACGT
ACAGCCGCTGGGCCCCAGCAGTCTGCCTGTGCACACCATTCTGCCCCAGGAAAGCCAGG
CTCTGCCGACATCACTGCCATCCTCCATGGTCCCACCCATGACCACTACCCAGTTCCTGAC
CCCTCCTTCTCAGCACAGCTACTCATCCTCACCTGTGGACAACACCCCCAGCCACCAGCT
GCAGGTGCCAGAGCACCCCTTCCTCACCCCATCCCCTGAGTCCCCTGACCAGTGGTCCAG
CTCCTCCCGGCATTCCAACATCTCTGATTGGTCCGAGGGCATCTCTAGCCCGCCCACGAG
CATGCCGTCCCAGATCACCCACATTCCAGAGGCATTTAAGTAA

Supplemental Table S3. *NRG1* mRNA sequence.

ATGGAGATTTATTCCCCAGACATGTCTGAGGTCGCCGCCGAGAGGTCCTCCAGCCCCTCC
ACTCAGCTGAGTGCAGACCCATCTCTTGATGGGCTTCCGGCAGCAGAAGACATGCCAGAG
CCCCAGACTGAAGATGGGAGAACCCCTGGACTCGTGGGCCTGGCCGTGCCCTGCTGTGC
GTGCCTAGAAGCTGAGCGCCTGAGAGGTTGCCTCAACTCAGAGAAAATCTGCATTGTCCC
CATCCTGGCTTGCCTGGTCAGCCTCTGCCTCTGCATCGCCGGCCTCAAGTGGGTATTTGT
GGACAAGATCTTTGAATATGACTCTCCTACTCACCTTGACCCTGGGGGGTTAGGCCAGGAC
CCTATTATTTCTCTGGACGCAACTGCTGCCTCAGCTGTGTGGGTGTCGTCTGAGGCATACA
CTTCACCTGTCTCTAGGGCTCAATCTGAAAGTGAGGTTCAAGTTACAGTGCAAGGTGACAA
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TCTTTCTTGCCGTCCACTGCGCCATCCTTCCCTTCACCCACCCGGAACCCTGAGGTGAGAA
CGCCCAAGTCAGCAACTCAGCCACAAACAACAGAAACTAATCTCCAAACTGCTCCTAAACT
TTCTACATCTACATCCACCACTGGGACAAGCCATCTTGTAAAATGTGCGGAGAAGGAGAAA
ACTTTCTGTGTGAATGGAGGGGAGTGCTTCATGGTGAAAGACCTTTCAAACCCCTCGAGAT
ACTTGTGCAAGTGCCCAAATGAGTTTACTGGTGATCGCTGCCAAAACTACGTAATGGCCAG
CTTCTACAGTACGTCCACTCCCTTTCTGTCTCTGCCTGAATAG

SUPPLEMENTAL VIDEOS

Supplemental Video S1. 4-D cardiac cycle with DIAMOND and raw data.

Supplemental Video S2. 4-D and time-dependent 2-D intracardiac views in control fish at 4 dpf.

Supplemental Video S3. 4-D and time-dependent 2-D intracardiac views in doxorubicin treated fish at 4 dpf.

Supplemental Video S4. 4-D and time-dependent 2-D intracardiac views in control fish at 6 dpf.

Supplemental Video S5. 4-D and time-dependent 2-D intracardiac views in doxorubicin treated fish at 4 dpf.