SUPPLEMENTARY INFORMATION

Supplementary Table 1. Transcriptional profile of genes at 20 dpa show faster regeneration in *dusp6* mutant zebrafish.

Feature ID	WT 20dpa P-value	WT 20dpa EDGE test: Fold change	P-value	Dusp6 mut 20dpa EDGE test: Fold change	Uninjured RPKM	WT 20dpa RPKM	Dusp6 mut 20dpa RPKM
LPL (2 of 2)	3.08E-03	5.21	0.253	-2.61	0.41	2.11	0.14
adrb2b	0.0487	2.32	0.1	2.05	0.03	0.09	0.03
postna	5.59E-03	1.92	0.7996	1.06	7.40	14.05	7.09
postnb	6.43E-05	2.37	0.0027	1.93	4.51	10.53	7.84
cav1	6.00E-07	2.77	1.91E-06	2.66	19.65	53.67	47.00
fn1a	3.97E-04	2.30	0.654	-1.13	2.14	4.87	1.71
fn1b	2.34E-05	2.38	0.703	1.08	18.27	43.08	17.85
cybb	9.79E-03	2.25	0.77	1.10	1.34	2.97	1.33
adrb1	0.0337	1.85	0.197	1.47	4.02	7.35	5.34
smad3b	6.98E-03	1.84	0.398	1.22	6.55	11.92	7.21
mapk14a	0.0449	1.59	0.106	1.46	5.26	8.24	6.92
col11a1b	2.00E-04	6.48	0.0285	3.30	0.06	0.37	0.17
col12a1a	2.59E-06	3.08	0.261	1.34	1.05	3.19	1.27
col12a1b	2.14E-04	4.91	0.0275	2.77	0.16	0.77	0.40
col1a1a	9.19E-04	2.04	0.111	1.42	20.02	40.32	25.62
col1a1b	1.65E-03	1.86	0.187	1.31	26.78	49.30	31.62
col1a2	6.50E-03	1.76	0.164	1.34	21.28	37.05	25.78
col5a1	5.80E-04	2.05	0.126	1.38	6.17	12.49	7.72
col6a1	0.0158	1.65	0.481	1.16	16.20	26.44	16.94
col6a3_1	7.04E-03	1.71	0.268	1.25	6.17	10.41	6.94
loxl2a	3.64E-09	7.05	5.00E-03	3.00	0.43	2.99	1.16
ltbp1_1	3.28E-03	2.11	0.139	1.47	2.07	4.33	2.76
TNC	0.0907	2.43	0.714	-1.32	0.02	0.26	0.14
mmp13b	0.0606	4.32	1	-1.15	0.04	0.19	0.03

Supplementary Table 2 Primers set for Q-PCR.

Gene	Sequence 5' to 3'	Amplicon Length (bp)
β-actin -F	CGTGCTGTCTTCCCATCCA	86
β-actin -R	TCACCAACGTAGCTGTCTTTCTG	
cybb-F	AACACCCTGGTACAAAAGTAGGT	102
cybb-R	ACTCAGTTCCGCCCTCAG	
col12a1a-F	GGTGAAAGAGGAGACACTGCGT	124
col12a1a-R	AGTTGCTGGGGATCTGGTT	
dusp6-F	CTAATGCTCGATAAGTTCAAACCC	416
dusp6-R	TTGCTGAAGCCACCCTCG	
errfi1b-F	AACCTCATCCTTCCAGTA	101
erffi1b-R	GCTTCTGATGTTCCTGTA	
fn1b-F	ATTCCTGCCATTGGTACTGGATC	97
fn1b-R	AGTTGCCCTCTTCGATAAGTTCAT	
postna-F	CAAGGATCAAGACGAAGAGCAAG	78
postna-R	ATCTCAGGGTCTCCATTCATCT	
RNA pol-F	CCAGATTCAGCCGCTTCAAG	149
RNA pol-R	CAAACTGGGAATGAGGGCTT	

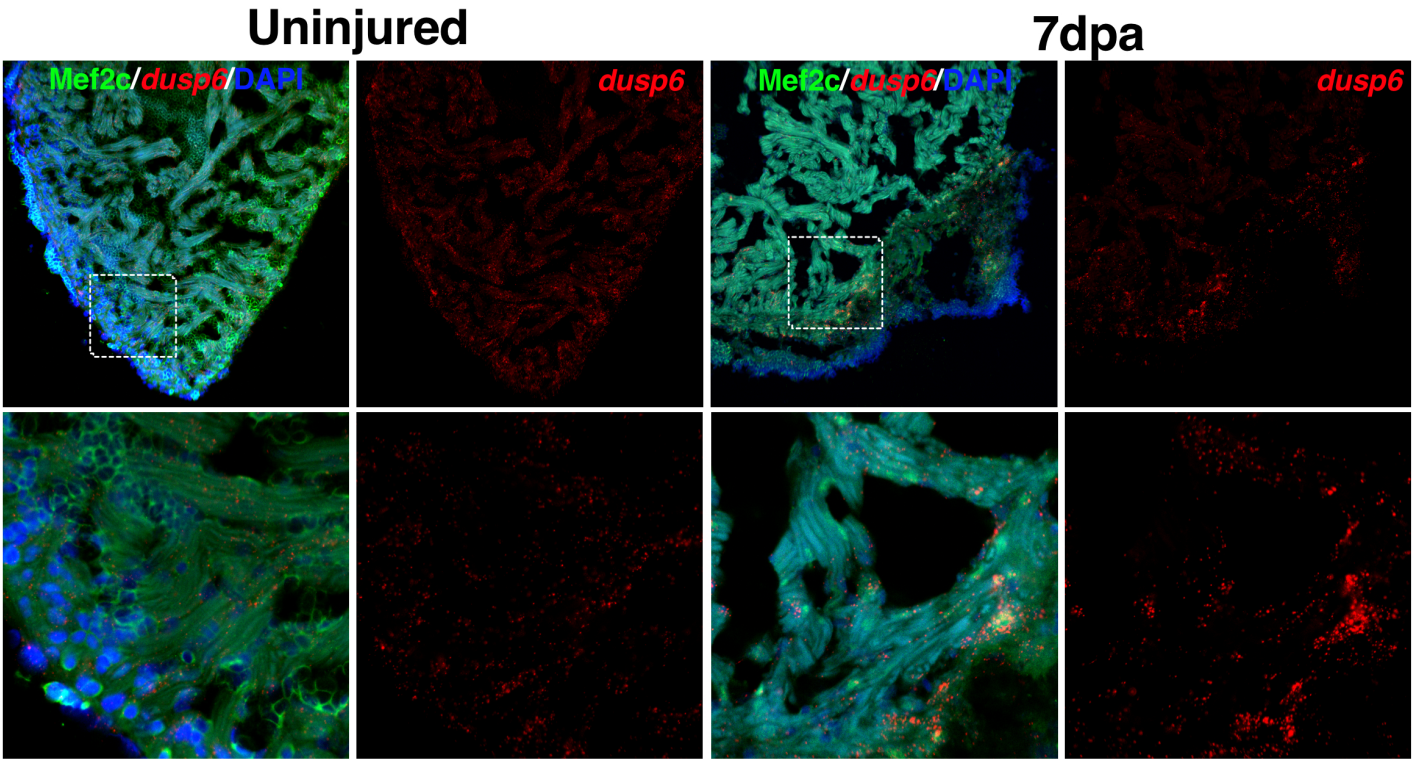

Figure S1. Dusp6 is expressed in cardiomyocytes of the adult heart.

dusp6 (red) transcripts are detected in uninjured hearts and in 7dpa hearts using RNAscope. In uninjured and 7dpa hearts, *dusp6* is detected throughout the heart and in cardiomyocytes as determined by Mef2c expression.

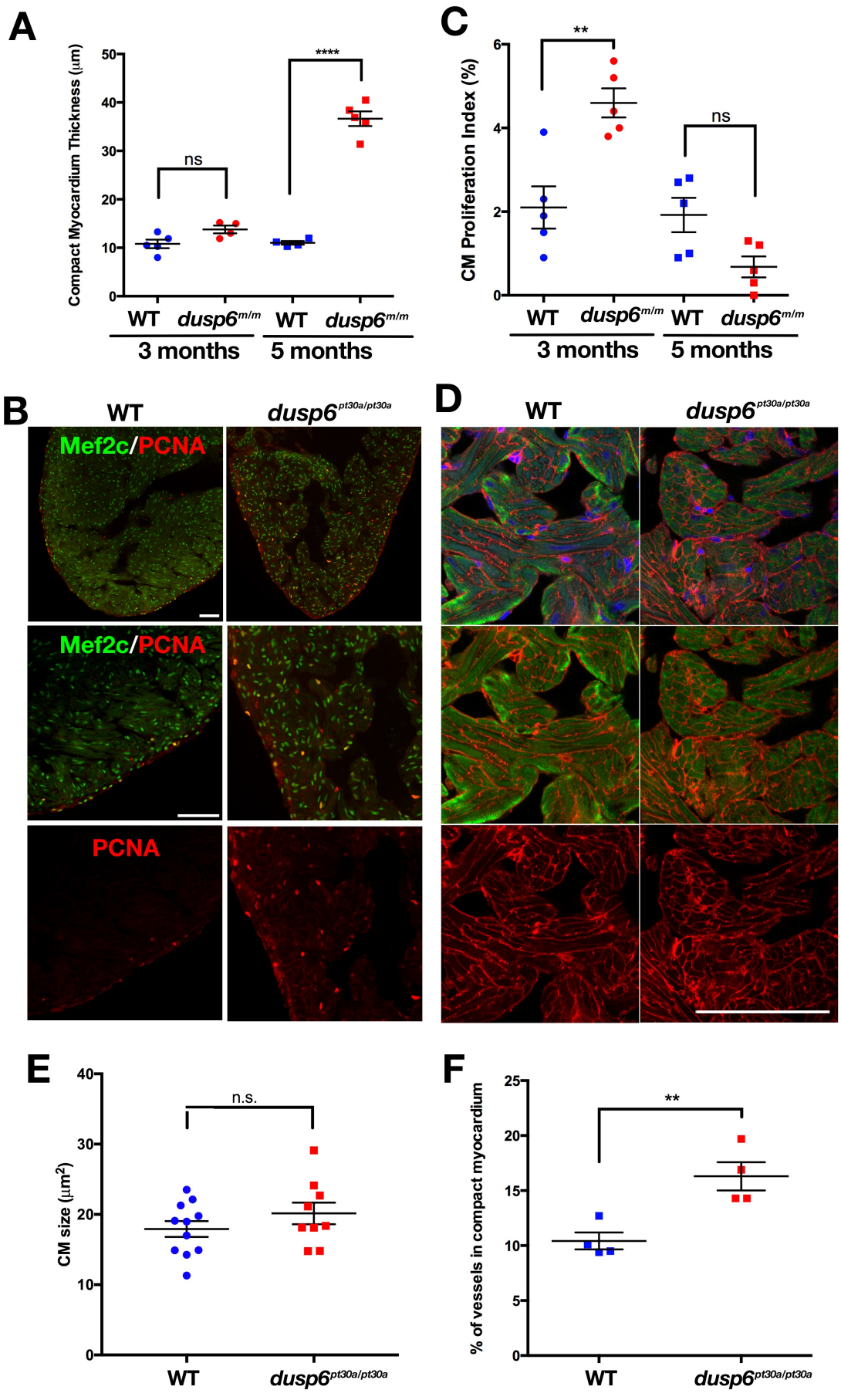

Figure S2. *dusp6* mutant heart are larger and have a thicker compact myocardium containing more vessels than WT hearts (A) Quantification of compact myocardium thickness in uninjured hearts at 3 and 5 months of age in WT and *dusp6* mutant (n≥4 for each group). At 5 months of age *dusp6* mutant fish have thicker compact myocardium than WT hearts. ****p<0.0001; ns= not significant. One-way ANOVA. **(B)** 3 months old uninjured zebrafish hearts immunostained for Mef2c (green; cardiomyocyte nuclei) and PCNA (red; proliferation marker). *dusp6* heart (n=5) have more proliferating cardiomyocyte than WT hearts (n=5). **(C)** Quantification of cardiomyocyte proliferation in WT and *dusp6* mutant hearts at 3 and 5 months of age (n=5 for each group). **p<0.01; ns= not significant. One-way ANOVA. **(D)** Uninjured hearts sections stained for MHC to visualize cardiomyocyte and wheat germ agglutinin (WGA) to stain cell membrane. **(E)** Quantification of cardiomyocyte size in uninjured hearts. *dusp6* mutant hearts (n=9) have the same size of cardiomyocyte compared to WT (n=11); Student's *t*-test. n.s.=not significant. **(F)** Quantification of vessels area per compact myocardium area in uninjured hearts at 5 months of age WT (n=4) and *dusp6* mutant (n=4). **p<0.01. Student's *t*-test. Scale bars, 100 μm.

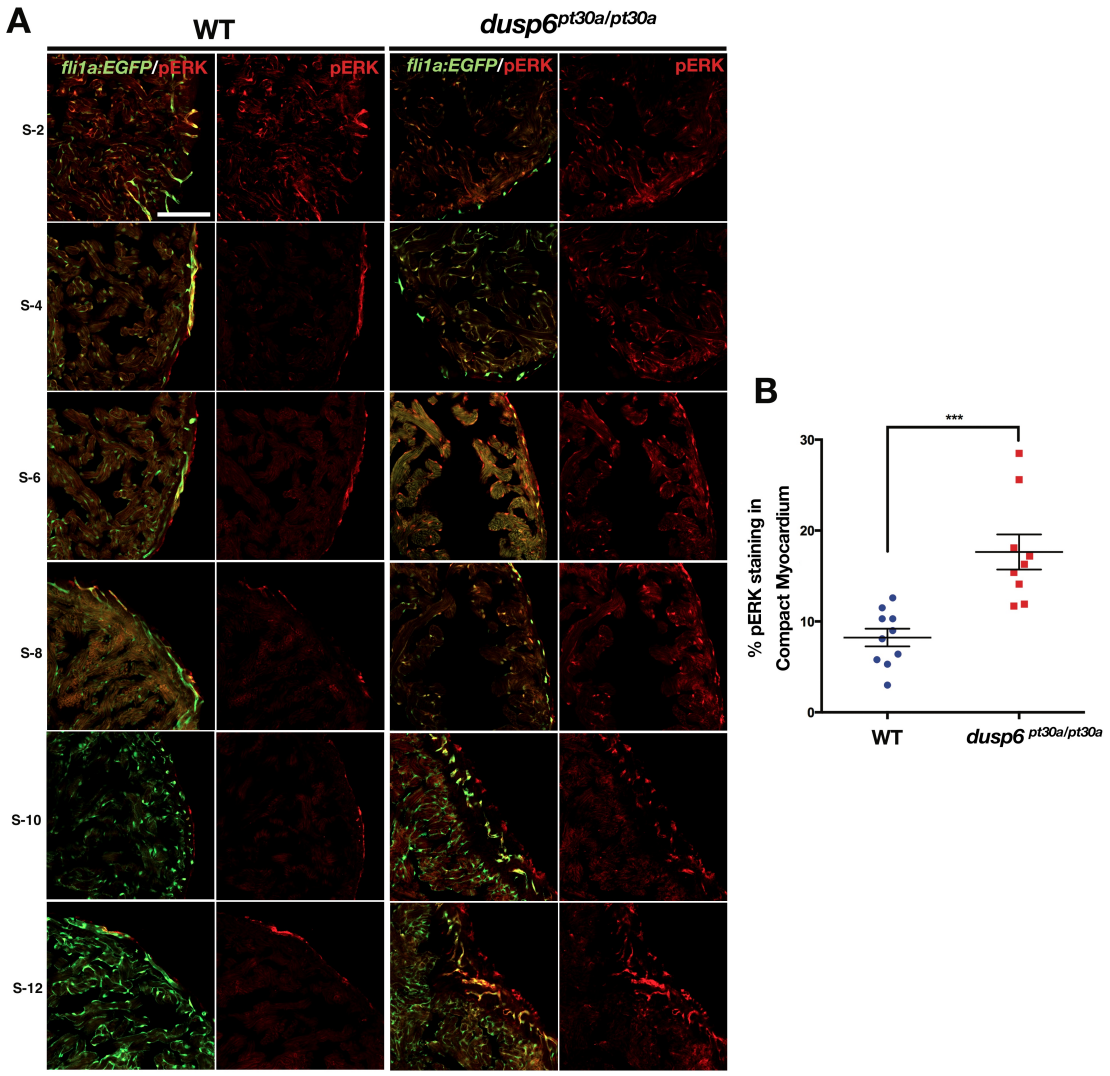

Figure S3. *dusp6* mutant hearts have increased pERK (A) Cryosections of uninjured *Tg(fli1a:EGFP)*^{y1} zebrafish hearts immunostained for pERK. *dusp6* heart (n=9) have increased pERK staining than WT hearts (n=10). Images are from one representative hearts for each group. Non-consecutive sections (S) are shown. S-2 is an initial section, in proximity of the compact myocardium. S-12 is the central section of the heart. **(B)** Quantification of pERK in WT and *dusp6* mutant hearts at 5 months of age. pERK staining was calculated as sum of pERK^{+} immunostained area divided by the compact myocardium area, and expressed as percentage. ***p<0.001. Student's *t*-test. Scale bars, 100 µm.

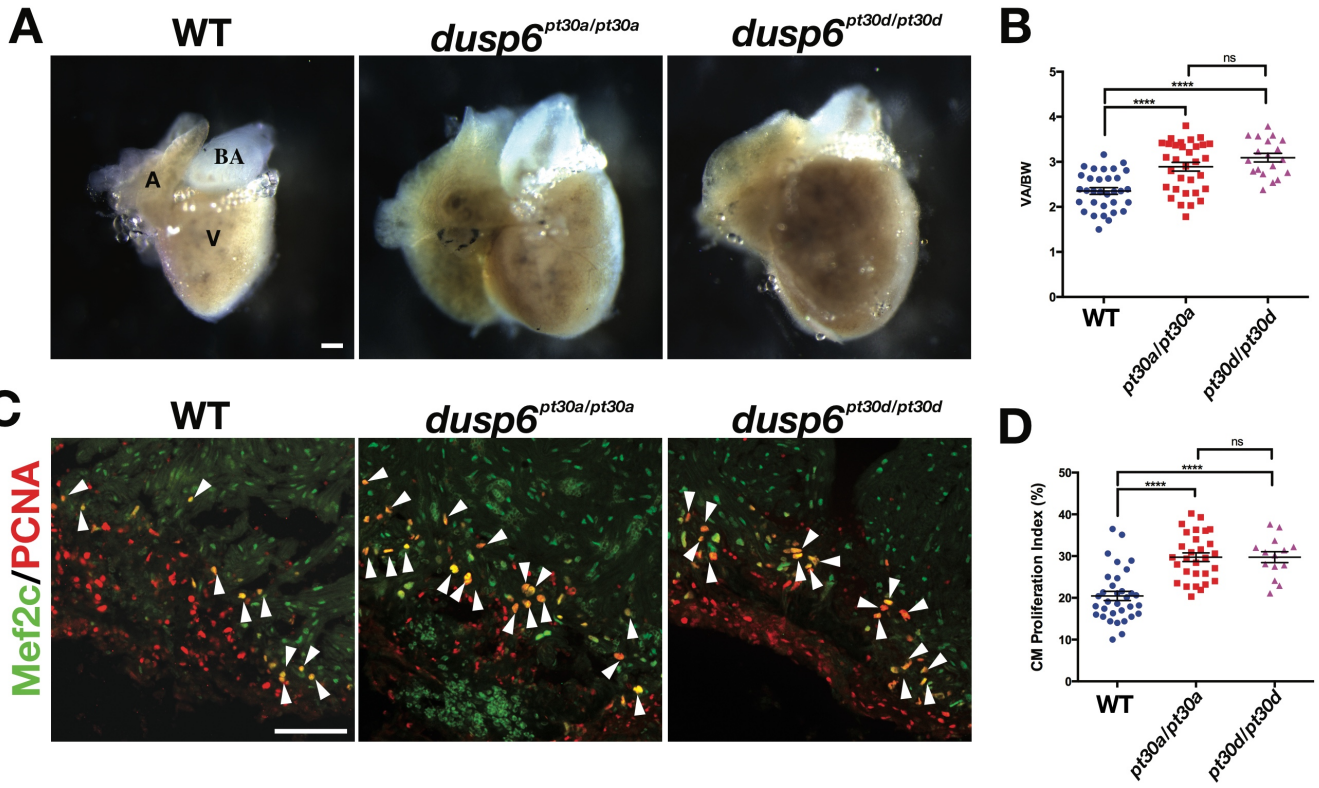

Figure S4. *dusp6*$^{pt30d/pt30d}$ hearts show the same phenotype as *dusp6*$^{pt30a/pt30a}$ (A) Whole-mount images of adult WT (n=32), *dusp6*$^{pt30a/pt30a}$ (n=32), and *dusp6*$^{pt30d/pt30d}$ (n=19) hearts at 7 dpa. *dusp6* mutant hearts show cardiomegaly. A=Atrium; V=Ventricle; BA=*Bulbus Arteriosus* **(B)** Quantification of the ratio ventricle area/body weight (VA/BW). *dusp6* mutant fish exhibit larger VA/BW than WT fish. ****p<0.0001; n.s.=not significant. One-way ANOVA. **(C)** Zebrafish hearts at 7 dpa, immunostained for Mef2c and PCNA. *dusp6*$^{pt30a/pt30a}$ (n=29) and *dusp6*$^{pt30d/pt30d}$ heart (n=14) show increased cardiomyocyte proliferation compared to WT hearts (n=33). **(D)** Quantification of cardiomyocyte proliferation index. ****p<0.0001. n.s.=not significant. One-way ANOVA. Scale bars, 100 µm.

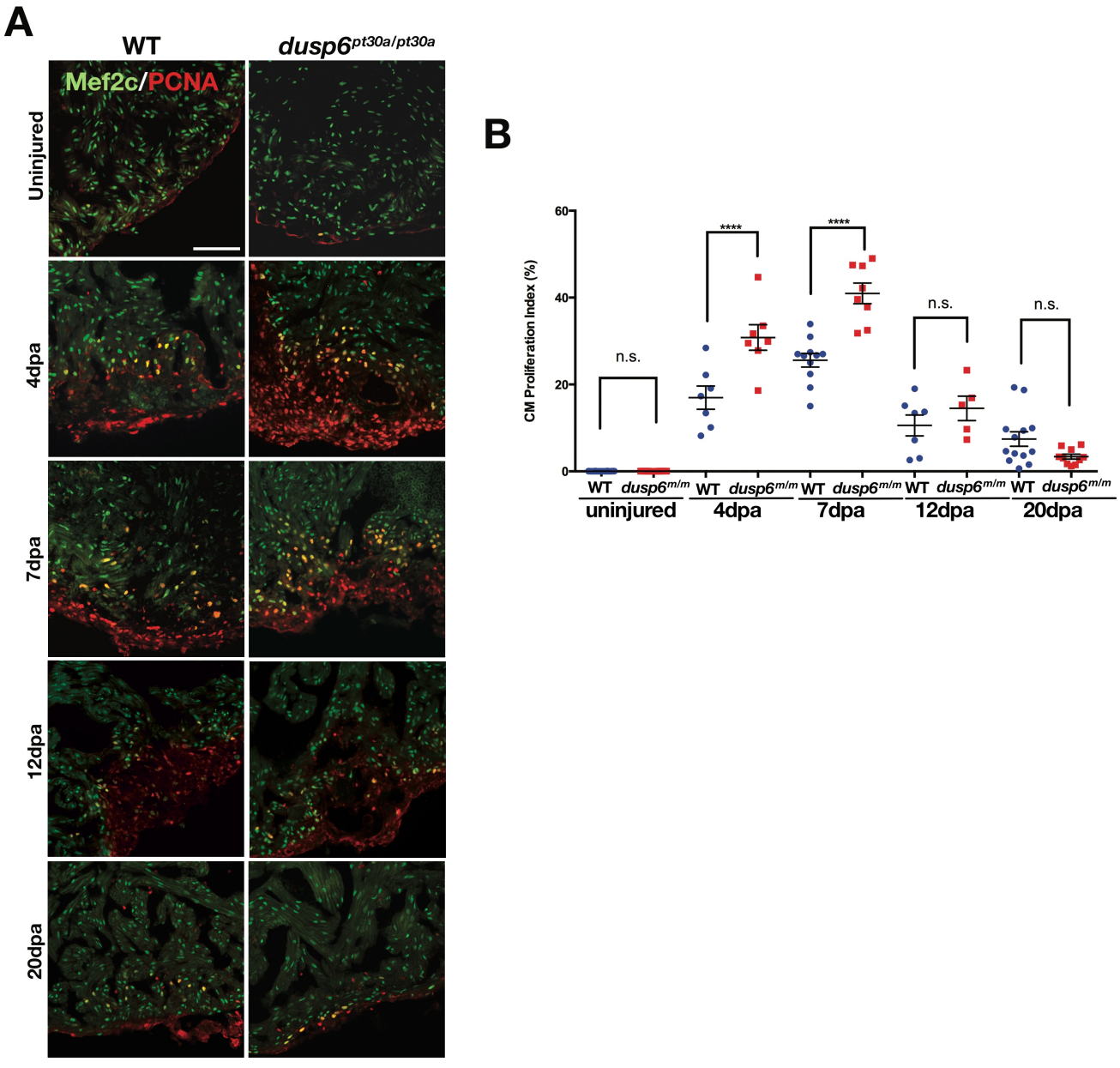

Figure S5. *dusp6* mutant hearts show increased cardiomyocyte proliferation after injury, but it is not indeterminate. (A) Hearts at 0- (WT n=24; *dusp6*$^{pt30a/pt30a}$ n=28), 4- (WT n=7; *dusp6*$^{pt30a/pt30a}$ n=7), 7- (WT n=11; *dusp6*$^{pt30a/pt30a}$ n=8) 12- (WT n=7; *dusp6*$^{pt30a/pt30a}$ n=5), and 20 dpa (WT n=13; *dusp6*$^{pt30a/pt30a}$ n=10) immunostained for Mef2c and PCNA. *dusp6* mutant hearts show increased proliferating cardiomyocytes at 4- and 7 dpa, but the proliferation index at 12- and 20 dpa is similar to WT hearts. **(B)** Graph representing the cardiomyocyte proliferation index at multiple time point between WT and *dusp6* hearts. ****p<0.0001; n.s.=not significant. One-way ANOVA. Scale bars, 100 µm.

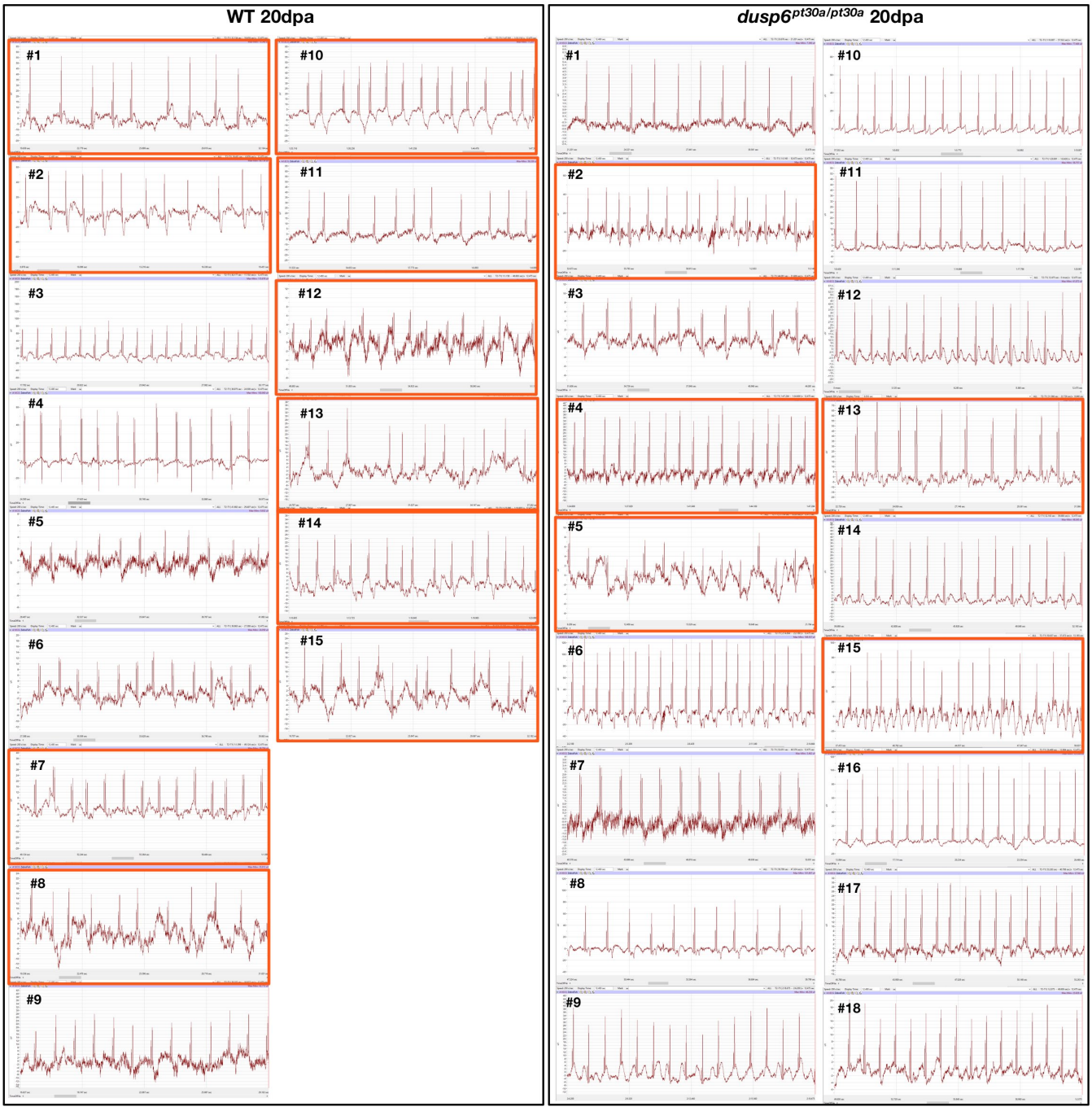

Figure S6. Electrocardiography of zebrafish hearts at 20 dpa.

Representative charts of ECG data from WT and *dusp6*$^{pt30a/pt30a}$ mutant zebrafish at 20 dpa. Data from these charts was used to calculate the R-R values. Arrhythmic events were noted with orange boxes outlining the charts.

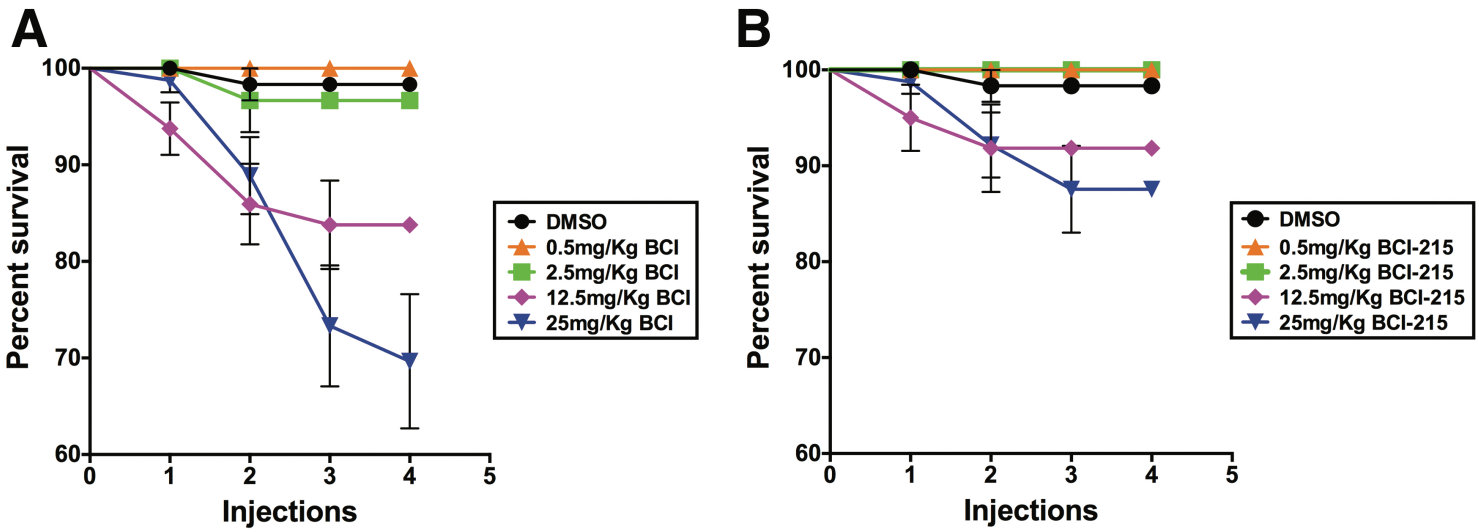

Figure S7. Maximum tolerated dose of BCI and BCI215 in adult zebrafish. (A) Survival Plot Graph showing percent survival of adult WT zebrafish after injection of BCI. Uninjured zebrafish were retro-orbitally injected daily for four consecutive days and survival rate vas measured each day. BCI was tested at the following concentrations: 0.5, 2.5, 12.5, and 25 mg/Kg ($n \geq 10$ for each condition). Vehicle DMSO, 0.5 and 2.5 mg/Kg BCI were well tolerated, while 12.5 and 25 mg/Kg showed adult lethality. **(B)** Graph showing the percent survival of adult zebrafish after injection of 0.5, 2.5, 12.5, and 25 mg/Kg BCI215 ($n \geq 10$ for each condition). DMSO, 0.5 and 2.5 mg/Kg BCI215 were well tolerated, while 12.5 and 25 mg/Kg showed minimal lethality. In all the following experiment, unless specified, 0.5 mg/Kg of BCI and 0.5 mg/Kg of BCI215 were used.

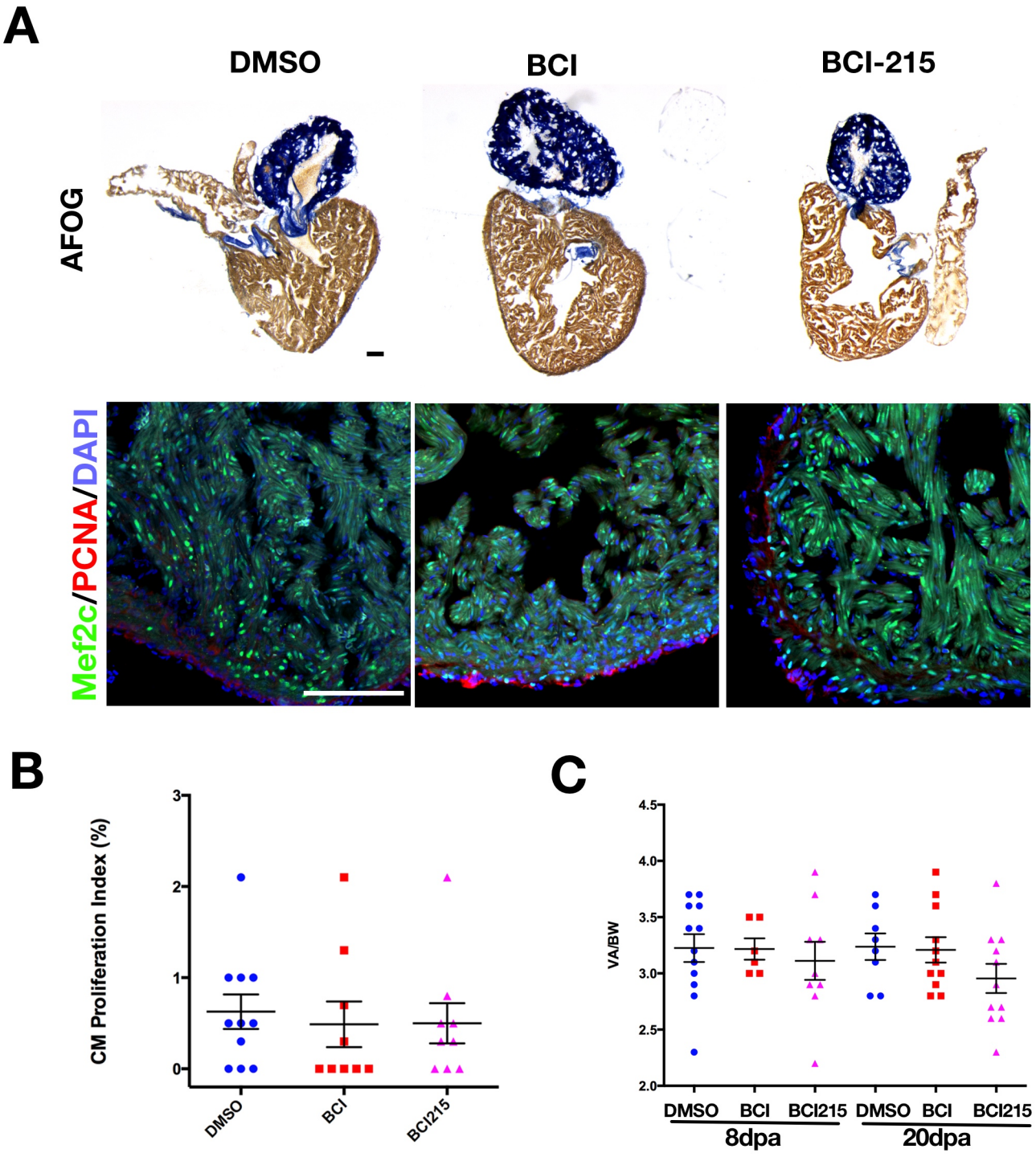

Figure S8. BCI and BCI215 do not affect cardiomyocyte proliferation or cardiac size in uninjured hearts. (A) Cryosections of uninjured hearts injected for 6 days with DMSO (n=11), BCI (n=9) or BCI215 (n=9), stained for AFOG (top) and for Mef2c and PCNA (bottom). **(B)** Quantification of cardiomyocyte proliferation in uninjured hearts injected with BCI, BCI215, or vehicle DMSO. **(C)** Graph representing the ratio of ventricle area and fish body weight (VA/BW) in samples at 8 dpa injected for 6 days with DMSO (n=12), BCI (n=6), or BCI215 (n=9), and samples at 20 dpa injected for 6 days with DMSO (n=8), BCI (n=11), or BCI215 (n=11). BCI and BCI215 compounds do not cause cardiomegaly. n.s.=not significant. One-way ANOVA. Scale bars, 100 μm.

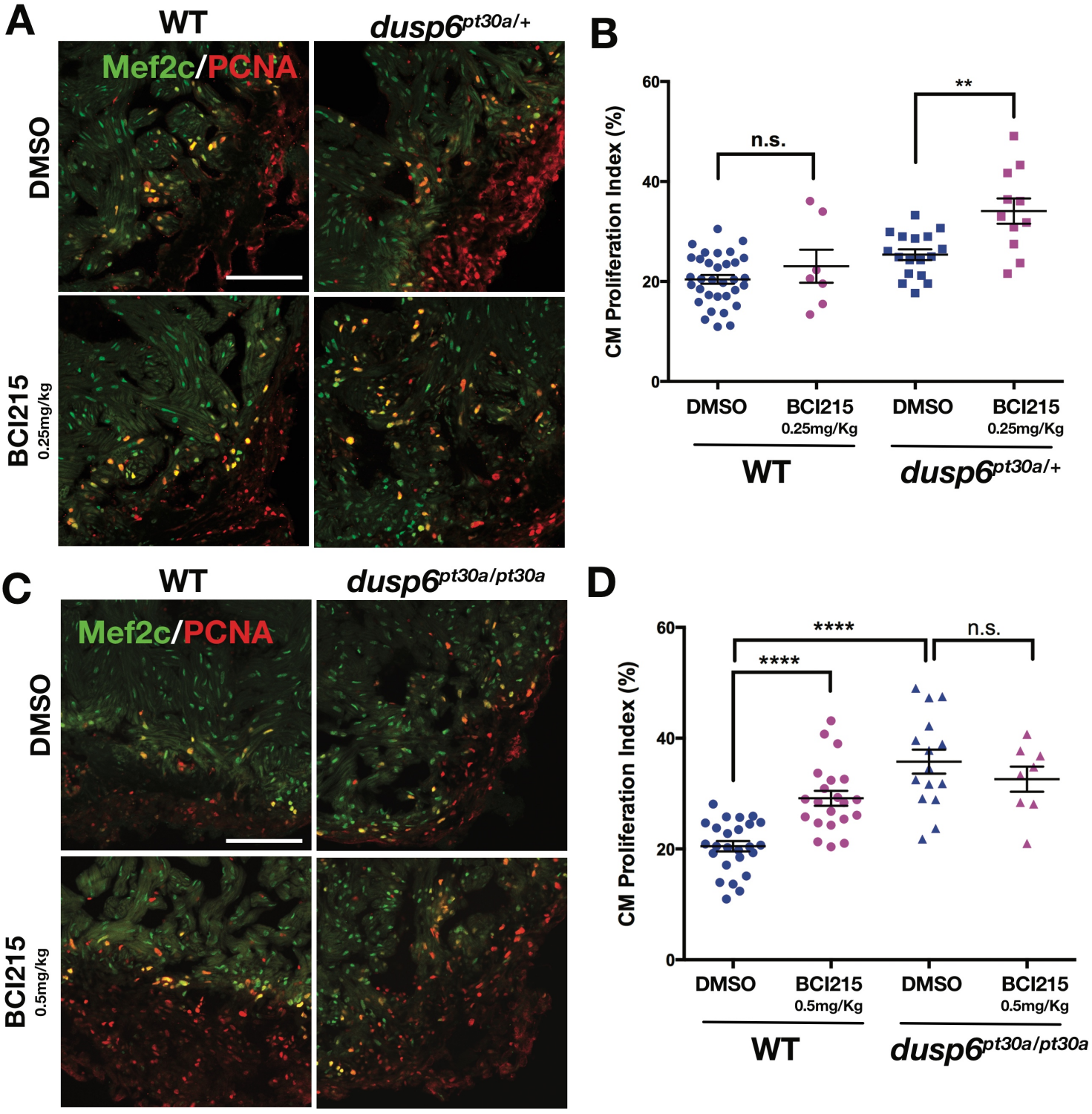

Figure S9. BCI215 is specific for Dusp6 (A) Cryosections of WT and *dusp6*$^{pt30a/+}$ hearts at 7 dpa injected for 6 days with DMSO (WT n=33; *dusp6*$^{pt30a/+}$ n=17), or 0.25 mg/Kg of BCI215 (WT n=7; *dusp6*$^{pt30a/+}$ n=11), stained for Mef2c and PCNA. **(B)** Quantification of cardiomyocyte proliferation in hearts injected with BCI215, or vehicle DMSO. Sub-optimal dose of BCI215 does not affect cardiomyocyte proliferation in WT hearts, but increases cardiomyocyte proliferation in *dusp6*$^{pt30a/+}$ hearts. **p<0.01; n.s.=not significant. One-way ANOVA. **(C)** Cryosections of WT and *dusp6*$^{pt30a/+}$ hearts at 7 dpa injected with DMSO (WT n=25; *dusp6*$^{pt30a/+}$ n=15), or 0.5 mg/Kg of BCI215 (WT n=21; *dusp6*$^{pt30a/+}$ n=8), stained for Mef2c and PCNA. **(D)** Quantification of cardiomyocyte proliferation. Optimal dose of BCI215 increases cardiomyocyte proliferation in WT hearts but it does not affect cardiomyocyte proliferation in *dusp6*$^{pt30a/+}$ hearts ****p<0.0001; n.s.=not significant. One-way ANOVA. Scale bars, 100 µm.

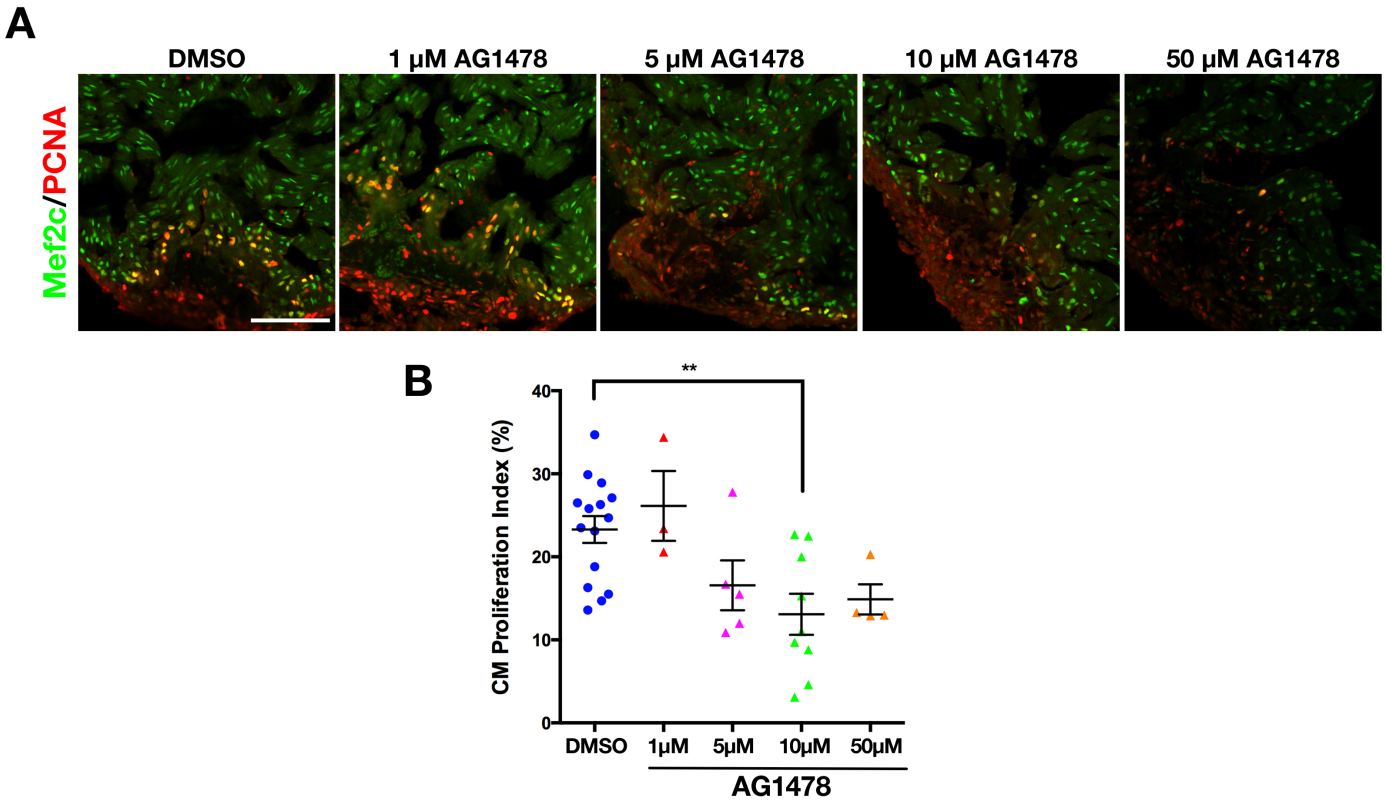

Figure S10. EGFR inhibition blocks cardiomyocyte proliferation in WT hearts (A) Dose response of AG1478 in WT hearts on cardiomyocyte proliferation. Mef2c and PCNA immunostaining in WT hearts at 7 dpa injected with DMSO (n=15), 1 µM (n=3), 5 µM (n=5), 10 µM (n=9), and 50 µM (n=4) of AG1478. **(B)** Quantification of cardiomyocyte proliferation after injection of AG1478. 5, 10 and 50 µM of AG1478 reduced cardiomyocyte proliferation in WT hearts. **p<0.01 One-way ANOVA. Scale bars, 100 µm.

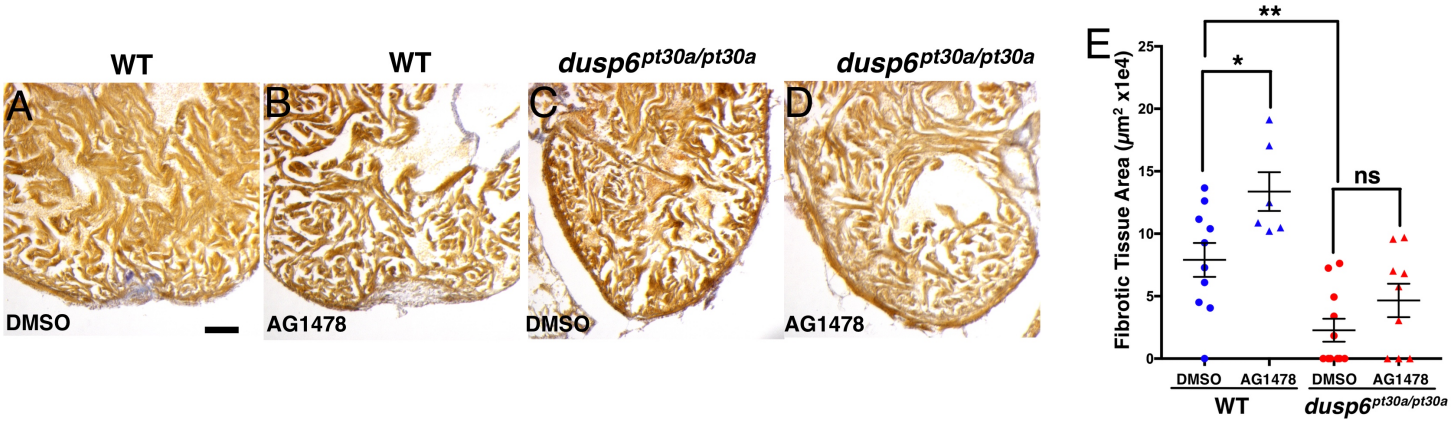

Figure S11. AG1478 blocks heart regeneration in WT hearts, but not in the *dusp6* mutant hearts. (**A-D**) Sections of hearts at 20 dpa stained with AFOG to visualize the deposition of fibrotic tissue. AG1478 treated WT hearts (**B**) (n=6) show larger area of fibrotic tissue when compared to DMSO control treatments (**A**) (n=9). However, AG1478 did not result in suppressing heart regeneration in *dusp6*$^{pt30a/pt30a}$ mutants (**D**) (n=9), compared to DMSO (**C**) (n=9). **(E)** Graph showing the measurements of fibrotic tissue areas at 20 dpa. **p<0.01, *p<0.05; n.s.=not significant. One-way ANOVA. Scale bars, 100 μm.