Figure S1

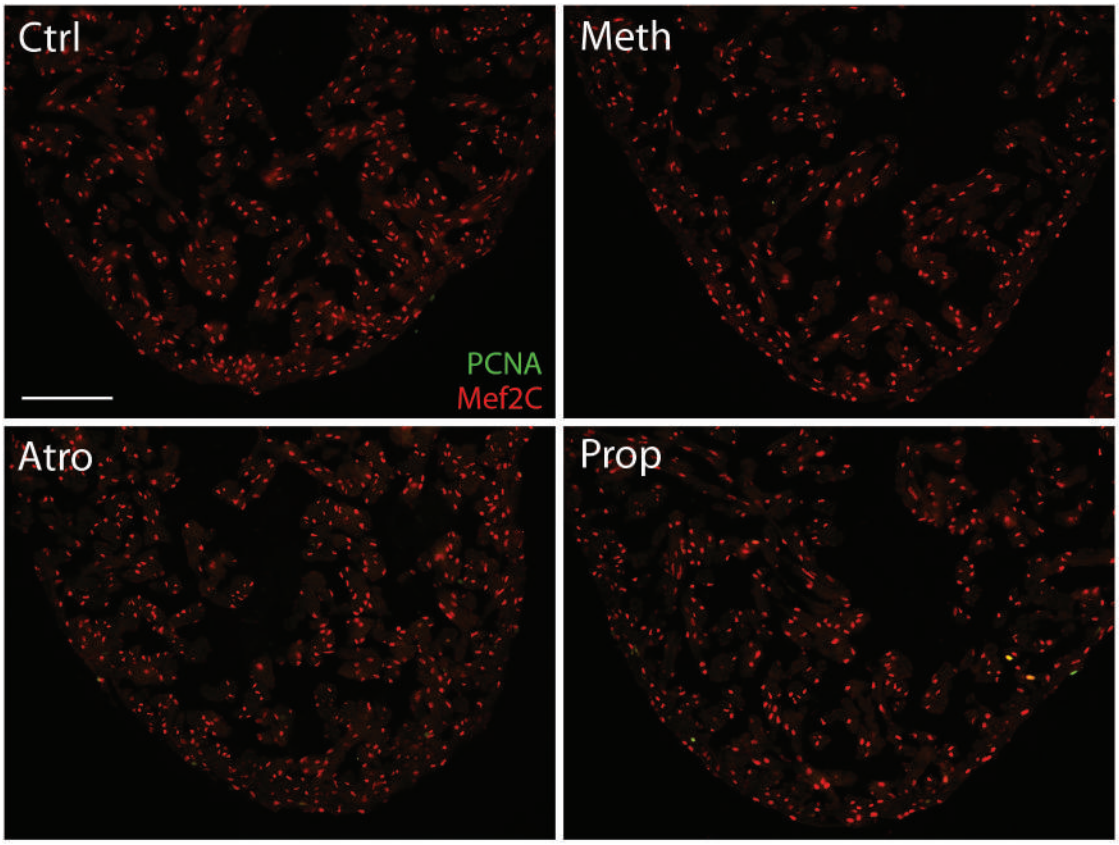
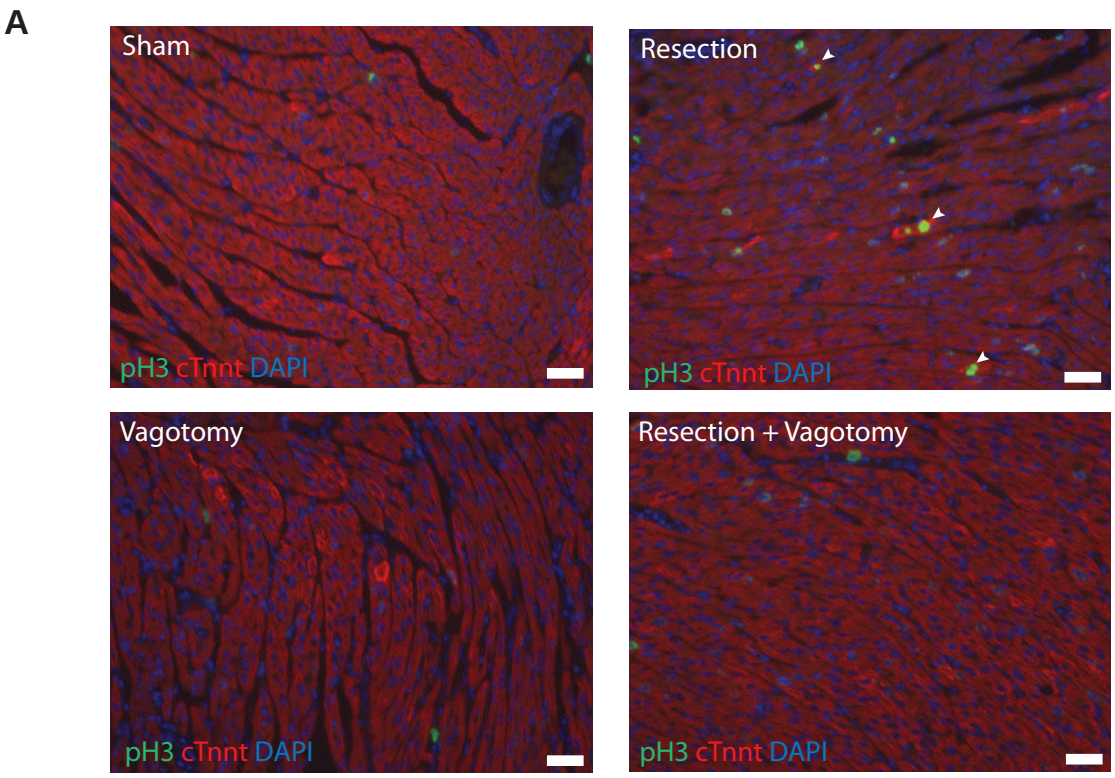


Figure S1, Related to Figure 2.
Mef2/PCNA staining of uninjured zebrafish in control and atropine, propranolol and methoctramine treated fish (n=4). No difference in the number of proliferating cardiomyocytes was detected.

Figure S2



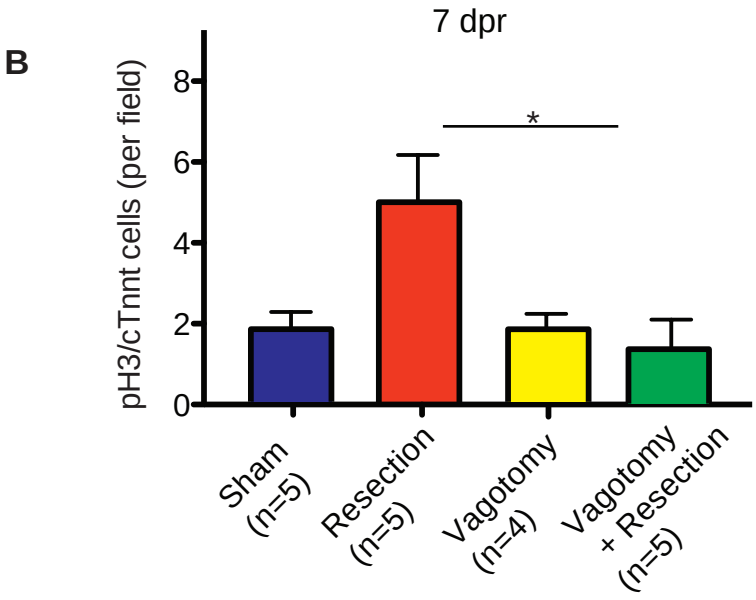


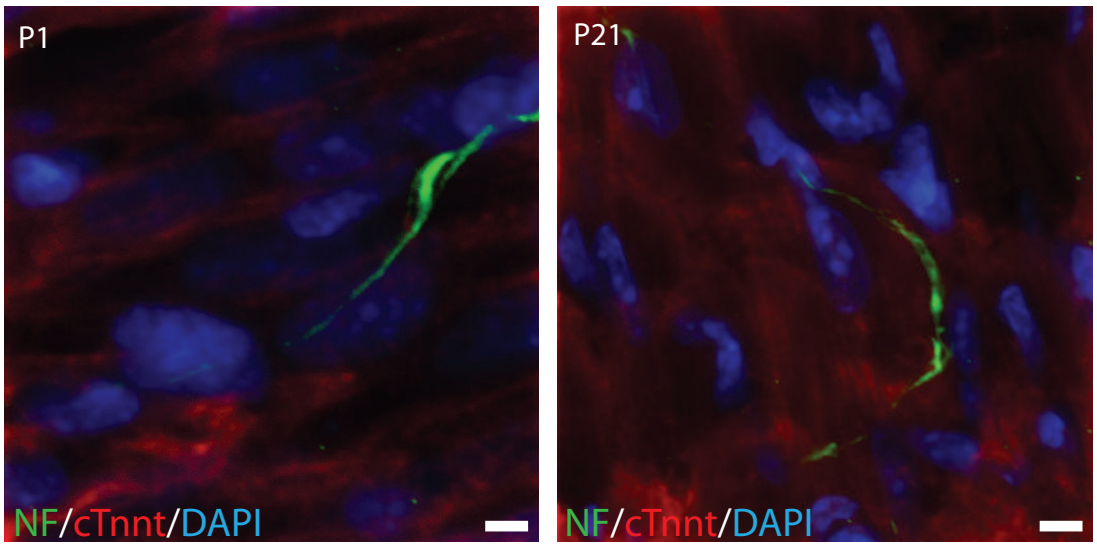
Figure S2, Related to Figure 4. Neonatal mouse vagotomy reduces cardiomyocyte proliferation following apical resection.

A) Immunofluorescence staining of pH3 and cTnnt to examine cardiomyocyte proliferation in sham, resection, vagotomy and resection paired with vagotomy hearts. Scale bar, 50 μm.

B) Quantification of the number of pH3 positive cardiomyocytes following injury, showing the effect of vagotomy on reducing cardiomyocyte proliferation when paired with apical resection. Data presented as mean ± SEM, where $p<0.05$ was considered statistically significant.

Figure S3

A



B

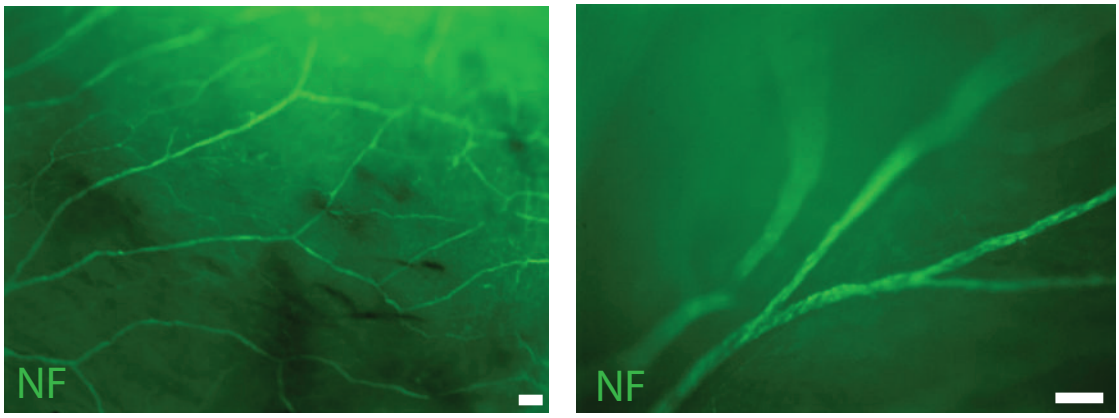


Figure S3, Related to Figure 4.
A) Confocal images of P1 and P21 heart cryosections immunostained with the neuronal marker Neurofilament and cTnnt. Scale bar, 10 µm.
B) Whole mount staining of P21 hearts with NF showing the persistence of cardiac nerves beyond the regenerative window. Scale bar, 100 µm.

Figure S4

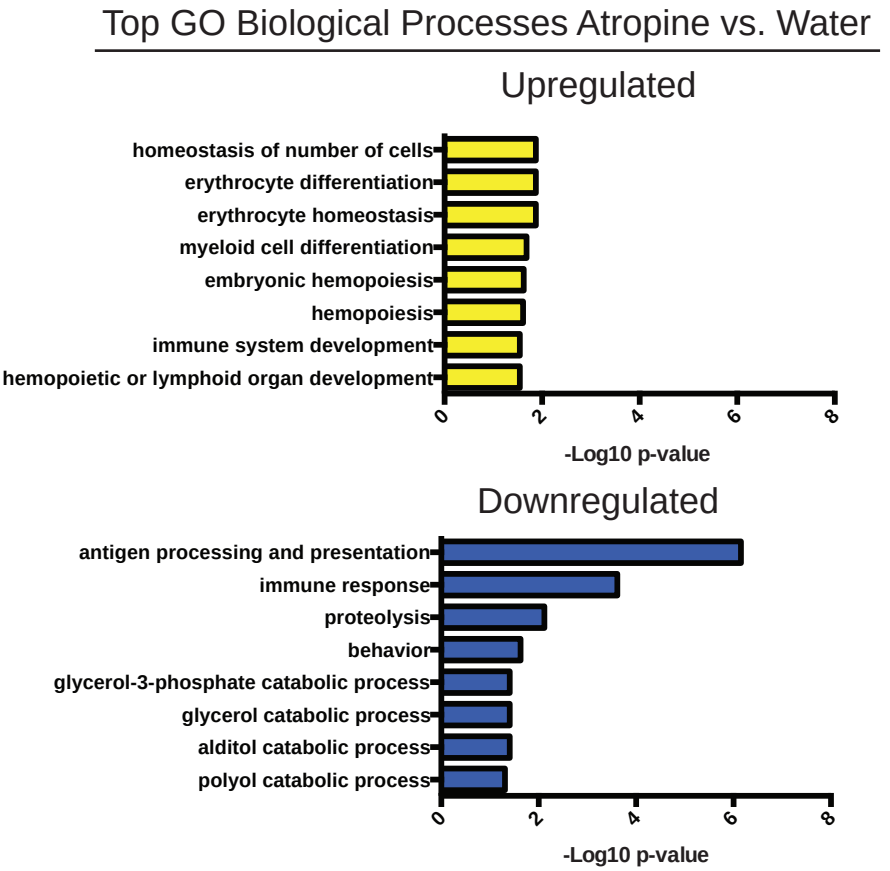


Figure S4, Related to Figure 6.
Top DAVID GO Biological Processes of genes differentially expressed by 1.5 fold in resected hearts of zebrafish treated with atropine compared to control zebrafish.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Whole Mount Staining

Whole mount nerve staining of mouse hearts was performed as follows. Heart were fixed in 4% PFA over night at room temperature. Hearts were then washed 4 X 5 minutes with PBS that contained 0.5% tween-20 (PBT). Hearts were placed in blocking media (0.5% PBT + 10% normal goat serum) for 2 hours at room temperature. Blocking solution was removed and primary antibody was added (0.5% PBT + 2% normal goat serum + anti-NF, abcam, 1:100) overnight at 4˚C. Next day, hearts were washed 3 X 5 minutes with 0.5% PBT. Secondary antibody conjugated to Alexa Fluor 488 (Invitrogen, 1:400) was incubated for 1 hour at room temperature in the dark.

Microarray Analysis

Microarray analysis was performed on RNA samples extracted from zebrafish hearts at 1-day post amputation (dpa). Following surgery, zebrafish were exposed to normal tank water or tank water supplemented with atropine. RNA samples were processed with the Affymetrix 3' IVT Express protocol (Cat # 702646). The arrays were washed and stained on Fluidics Stations 450 using the Affymetrix Hybe/Wash/Stain (Cat # 900720) and scanned on GeneChip Scanner 3000 7G. Microarray data has been uploaded to the gene expression omnibus (GEO accession GSE69775).

List of primers used for Real time PCR

Gene	Forward Primer	Reverse Primer
M2 (mouse)	TGGTTTGGCTATTACCAGTCCT	CTGAAGGTGGCGGTTGACTT
Ccnd2 (mouse)	GAGTGGGAACTGGTAGTGTTG	CGCACAGAGCGATGAAGGT
Cdk4 (mouse)	ATGGCTGCCACTCGATATGAA	TGCTCCTCCATTAGGAACTCTC
Nrg1 (mouse)	TTCCCATTCTGGCTTGTCTAGT	CCAGGGTCAAGGTGGGTAG
Ngf (mouse)	TGATCGGCGTACAGGCAGA	GCTGAAGTTTAGTCCAGTGGG
18S (mouse)	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG

Statistical Analysis

Results are expressed as mean ± SEM. An unpaired Student's *t* test was used to determine statistical significance of all samples. *P<0.05, **P<0.01 were considered statistically significant.