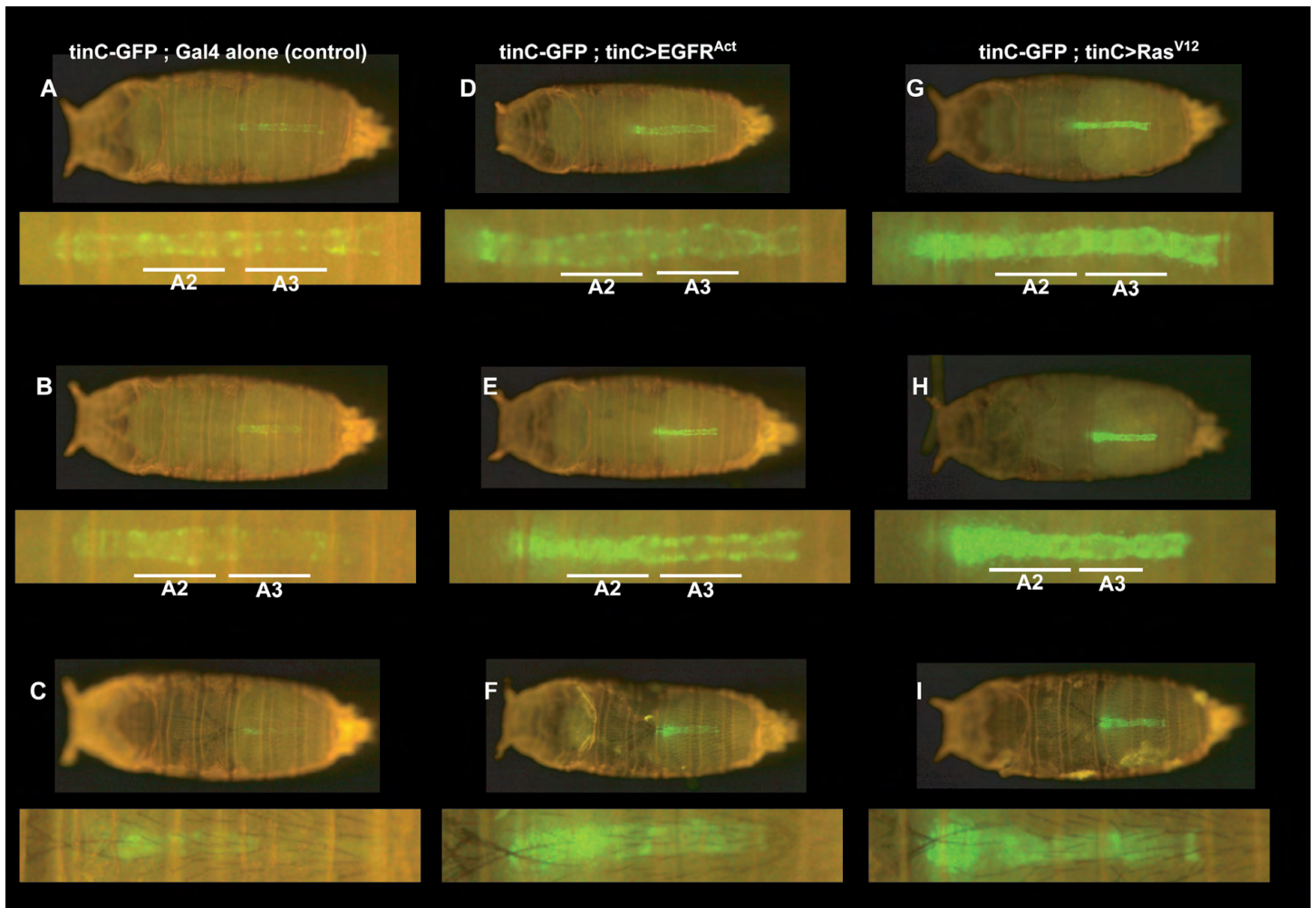
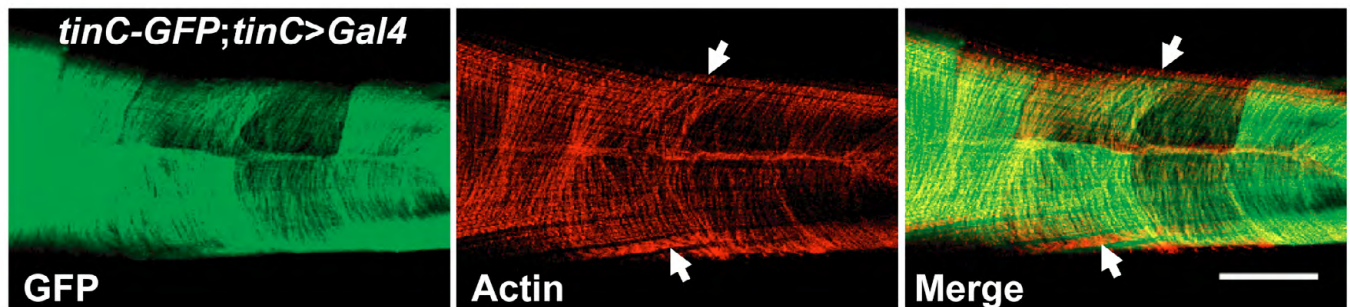


Supplementary material Fig. S1. The effect of activated EGFR on eclosion and the embryonic dorsal vessel. (A) Eclosion frequency (i.e. the emergence of an adult fly from the pupal case) is decreased due to cardiac-specific expression of activated EGFR (EGFR^{Act}) compared to control flies harboring the tinC-Gal4 driver alone. Frequencies were determined from three different groups per genotype with 89-290 pupae per group. * $P < 0.05$ for indicated genotypes versus tinC-Gal4 alone. (B) Representative confocal micrographs of the dorsal vessels from stage 16 fly embryos expressing EGFR^{Act} compared to tinC-Gal4 alone (Control) stained with anti-pericardin antibody (red) and the DNA dye TO-PRO-3 (blue).



Supplementary material Fig. S2. Cardiac morphology in *tinC-GFP*; *tinC-Gal4* alone, *tinC-GFP*; *tinC>EGFR^{Act}*, and *tinC-GFP*; *tinC>Ras85D^{V12}* during pupal stages. Panels are representative of hearts from transgenic *Drosophila* of the genotype *tinC-GFP*; *tinC-Gal4* alone (A–C), *tinC-GFP*; *tinC>EGFR^{Act}* (D–F), and *tinC-GFP*; *tinC>Ras85D^{V12}* (G–I). (A,D,G) represent ~P6, (B,E,H) represent ~P8, and (C,F,I) represent ~P13 pupae based on staging described by Bainbridge and Bownes (Bainbridge and Bownes, 1981). The approximate sections corresponding to the 2nd and 3rd abdominal segments are denoted A2 and A3, respectively.

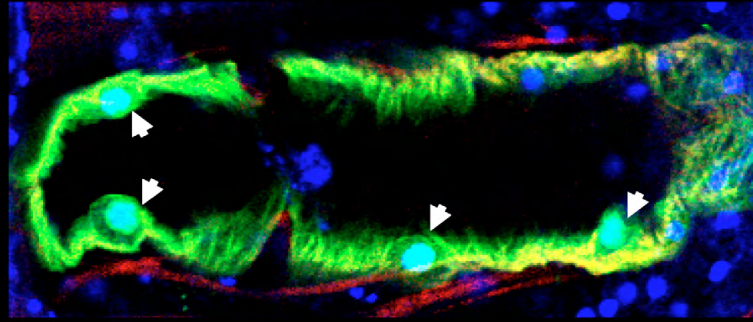


Supplementary material Fig. S3. Confocal microscopy with Z-stack reconstruction of *tinC-GFP*; *tinC-Gal4* adult fly heart in the A1/A2 abdominal segments. GFP imaging (green) and phalloidin staining of actin (red) show the circumferentially oriented fibers of the adult heart. Ventral longitudinal muscle fibers (also known as the dorsal diaphragm) that do not express *tinC-Gal4* are also seen by staining with Texas-Red phalloidin (red) denoted by white arrowheads. A 50 micron standard is shown.

Supplemental Figure 4

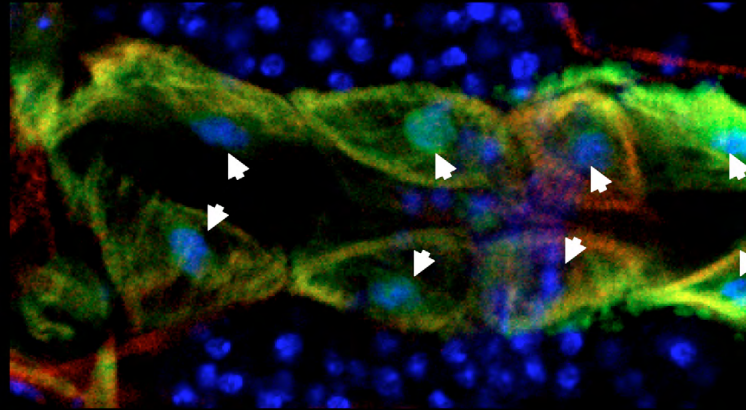
A

tinC-GFP; tinC-Gal4 (Control)



B

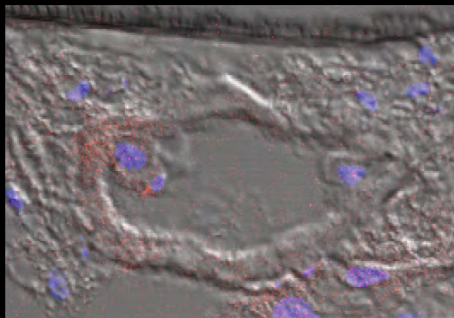
tinC-GFP; tinC-Gal4->Ras85D^{V12}



50 microns

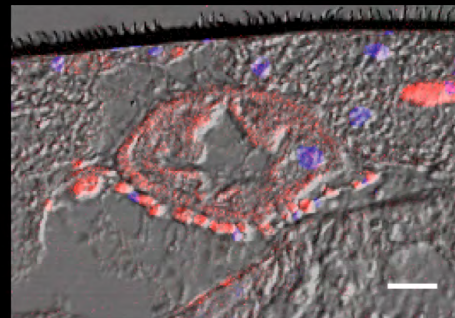
C

tinC-GFP; tinC-Gal4 (Control)

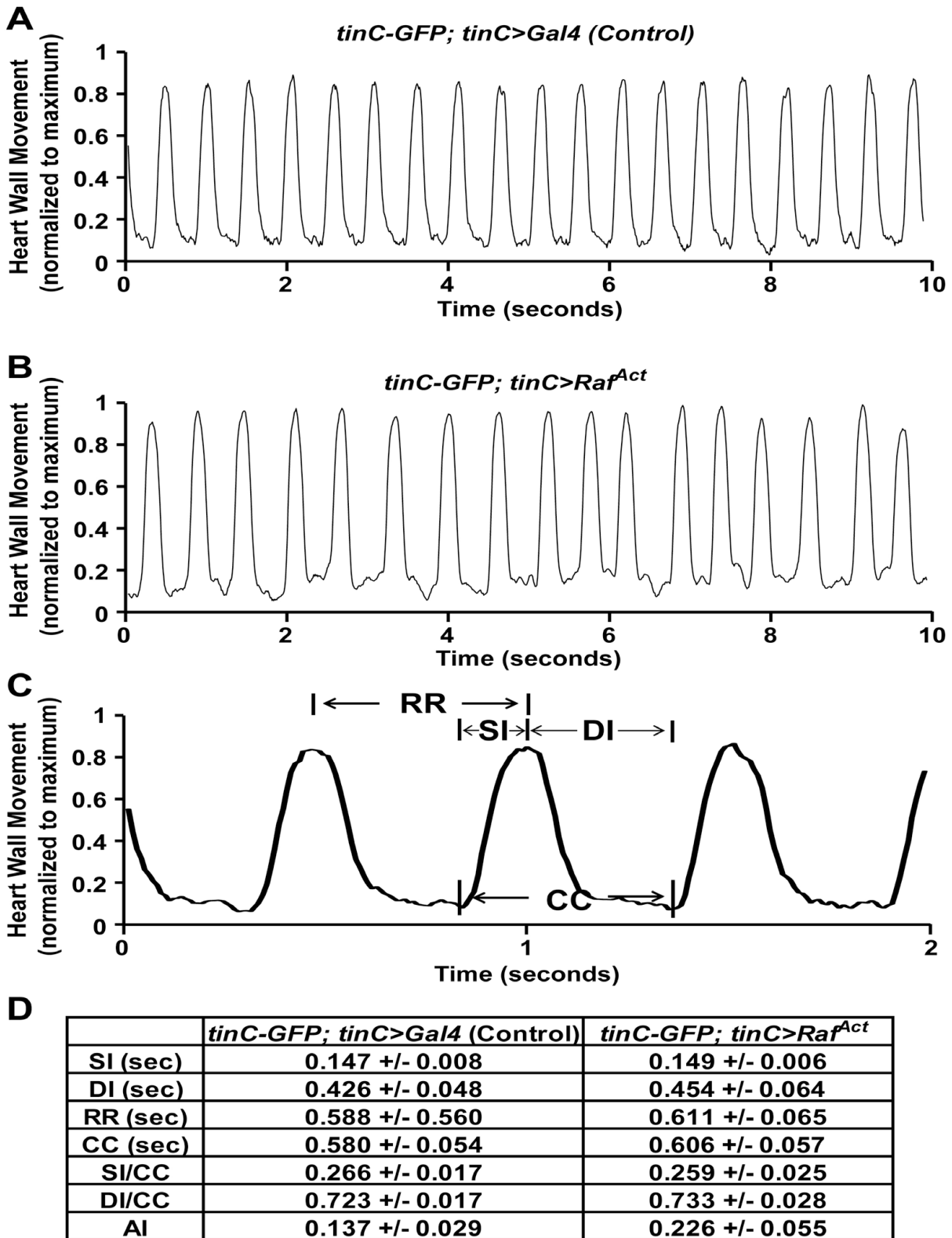


D

tinC-GFP; tinC-Gal4->Ras85D^{V12}



Supplementary material Fig. S4. Activated Ras85D causes cardiac hypertrophy in the adult fly heart. Confocal micrographs focused at the level of the cardiac nuclei in the A2 segment of hearts from *tinC-GFP; tinC-Gal4* (control) (panel A) and *tinC-GFP; tinC > Ras85D^{V12}* (panel B) stained with an antibody to GFP (Green), phalloidin-TexasRed (red), and the nuclear stain, TO-PRO-3 (blue). Arrows indicate cardiomyocyte nuclei. A 50 micron standard is shown. Brightfield and fluorescence images of histologic sections through pairs of cardiomyocytes from the A2 segment from *tinC-GFP; tinC-Gal4* (control) (panel C) and *tinC-GFP; tinC > Ras85D^{V12}* (panel D) showing laterally positioned cardiomyocyte nuclei. The sections were stained with phalloidin-TexasRed (red), and the nuclear stain, TO-PRO-3 (blue). A 10 micron standard is shown.



Supplementary material Fig. S5. Heart rate parameters in Raf-mediated cardiac hypertrophy. Representative traces of contractile rates from hearts dissected from adult *tinC-GFP; tinC-Gal4* (control) (A) and *tinC-GFP; tinC>human Raf^{Act}* (B). Each 10 second trace was recorded at 100 frames per second and contraction was normalized to maximal contraction for each trace. (C) A two second time frame is shown with the defined cardiac parameters. The systolic interval (SI), diastolic interval (DI), RR (peak-to-peak interval), and CC (cardiac cycle length) are shown. (D) Summary of heart rate parameters from *tinC-GFP; tinC-Gal4* (control) ($n=10$) and *tinC-GFP; tinC>human Raf^{Act}* ($n=9$). The SI, DI, RR, CC, fraction of cardiac cycle in systole (SI/CC), fraction of cardiac cycle in diastole (DI/CC), and the arrhythmia index (AI) are shown. AI was calculated as the standard deviation of the RR interval normalized to the median of the RR interval as previously described (Fink et al., 2009).

Cardiac Chamber Dimensions (OCT)

Heart Wall Thicknesses (Histology)

Stock	N	EDD	ESD	FS	N	Dorsal	Ventral	Left	Right	Average	Wall Thickness
<i>W¹¹⁸</i>	89	87.6 ± 2.2	4.7 ± 1.3	95.5 ± 1.2	6	5.4 ± 0.3	5.8 ± 0.2	6.0 ± 0.3	5.7 ± 0.4	5.7 ± 0.2	↔
<i>tinC-GFP; tinc-Gal4/+</i>	18	95.6 ± 2.9	1.4 ± 1.4	98.6 ± 1.4	10	5.8 ± 0.8	7.1 ± 0.9	6.7 ± 0.5	7.3 ± 0.4	6.7 ± 0.6	↔
<i>tinC-GFP; tinc-Gal4>EGFR^{act} {Egfr.2.A8877.UAS}8-1</i>	10	36.4 ± 6.0*	1.2 ± 0.9	97.8 ± 1.5	6	14.0 ± 0.2*	16.4 ± 0.2*	14.9 ± 3.0*	15.5 ± 0.1*	15.2 ± 0.1*	↑
<i>tinC-GFP; tinc-Gal4>Ras85D^{V12} {UAS-Ras85D.V12}TL1</i>	10	32.3 ± 5.9*	0*	100	3	21.2 ± 1.3*	25.5 ± 3.2*	19.3 ± 2.1*	26.4 ± 0.8*	23.1 ± 1.0*	↑
<i>tinC-GFP; tinc-Gal4> Ras85D^{V12S35}</i>	11	49.2 ± 3.0*	5.2 ± 2.6	90.5 ± 4.6	11	7.1 ± 0.6	8.7 ± 0.8	10.1 ± 0.8	8.5 ± 1.1	8.6 ± 0.8	↔
<i>tinC-GFP; tinc-Gal4> Ras85D^{V12G37}</i>	16	91.5 ± 3.7	31.2 ± 6.2*	65.8 ± 6.4*	10	5.9 ± 0.4	6.1 ± 0.6	6.7 ± 0.5	6.8 ± 0.7	6.4 ± 0.5	↔
<i>tinC-GFP; tinc-Gal4> Ras85D^{V12C40}</i>	12	103.9 ± 5.6	19.7 ± 7.3	80.0 ± 7.1	8	7.0 ± 0.4	6.8 ± 0.5	7.8 ± 0.4	7.7 ± 0.7	7.3 ± 0.3	↔
<i>tinC-GFP; tinc-Gal4>Raf^{act} {UAS-cRaf1.gof}ra2</i>	18	38.8 ± 7.3*	0*	100	3	21.2 ± 3.0*	19.6 ± 2.7*	18.8 ± 0.2*	16.3 ± 1.9*	19.0 ± 0.6*	↑
<i>tinC-GFP; tinc-Gal4>Raf RNAi {TRiP.JF01483}attP2</i>	10	89.8 ± 5.3	5.5 ± 3.0	93.4 ± 3.4	4	3.5 ± 0.5*	5.2 ± 0.4	3.7 ± 0.3*	3.7 ± 0.3*	4.0 ± 0.1*	↓
<i>tinC-GFP; tinc-Gal4>Raf RNAi {TRiP.JF01185}attP2</i>	12	95.2 ± 4.7	27.3 ± 6.0*	71.5 ± 6.5*	4	3.9 ± 0.6	4.8 ± 0.4*	4.3 ± 0.9*	4.5 ± 0.5	4.4 ± 0.5*	↓
<i>tinC-GFP; tinc-Gal4>MEK RNAi {TRiP.JF01697}attP2</i>	12	102.0 ± 3.9	49.8 ± 2.5*	51.1 ± 1.7*	4	4.1 ± 0.4	5.3 ± 0.5	3.6 ± 0.3*	3.9 ± 0.6*	4.2 ± 0.4*	↓
<i>tinC-GFP; tinc-Gal4>MEK RNAi {TRiP.JF01697}attP2/tinc-Gal4>Raf^{act} {UAS-cRaf1.gof}</i>	15	82.6 ± 3.7	5.4 ± 3.1	93.4 ± 3.5	6	6.4 ± 0.8	6.7 ± 0.7	5.5 ± 0.4	5.9 ± 0.9	6.1 ± 0.5	↔
<i>tinC-GFP; tinc-Gal4>ERK^{act}</i>	11	92.1 ± 5.7	1.9 ± 1.8	98.1 ± 2.0	8	8.1 ± 0.8	8.6 ± 0.9	9.0 ± 1.0	7.9 ± 0.6	8.4 ± 0.7	↔
<i>tinC-GFP; tinc-Gal4>ERK RNAi {TRiP.HMS00173}attP2</i>	15	98.9 ± 6.2	3.1 ± 2.0	97.0 ± 2.0	4	3.6 ± 0.4*	4.2 ± 0.5*	4.8 ± 0.2*	4.7 ± 0.8*	4.3 ± 0.4*	↓
<i>tinC-GFP; tinc-Gal4>ERK RNAi {TRiP.HMS00173}attP2/tinc-Gal4>RafAct {UAS-cRaf1.gof}</i>	15	92.1 ± 4.7	5.5 ± 2.6	94.7 ± 2.4	4	3.7 ± 0.4*	5.1 ± 0.7*	4.2 ± 0.8*	4.5 ± 0.5*	4.4 ± 0.6*	↓

Supplementary material Table S1. Cardiac chamber dimensions measured by OCT and heart wall thicknesses determined by histology in adult flies. OCT values are shown as the mean±s.e.m. for EDD, ESD, and FS are shown. **P*<0.05 for indicated value versus *tinC-GFP ; tinc-Gal4* flies. Heart wall thicknesses are shown as the mean±s.e.m. for the dorsal, ventral, left and right walls. The average mean±s.e.m. of all wall measurements is also shown. **P*<0.05 for indicated heart wall thickness versus *tinC-GFP ; tinc-Gal4*. ↑ arrow indicates an increase in heart wall thickness versus *tinC-GFP ; tinc-Gal4*; ↓ arrow indicates a decrease in heart wall thickness versus *tinC-GFP ; tinc-Gal4*; and ↔ arrow indicates no significant difference in heart wall thickness versus *tinC-GFP ; tinc-Gal4*.