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Supplemental information

The nutrient sensor CRTC and Sarcalumenin/thinman represent an alternate pathway in cardiac hypertrophy

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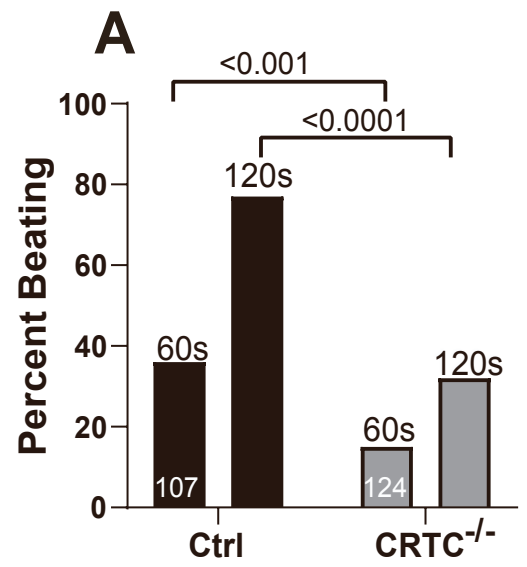
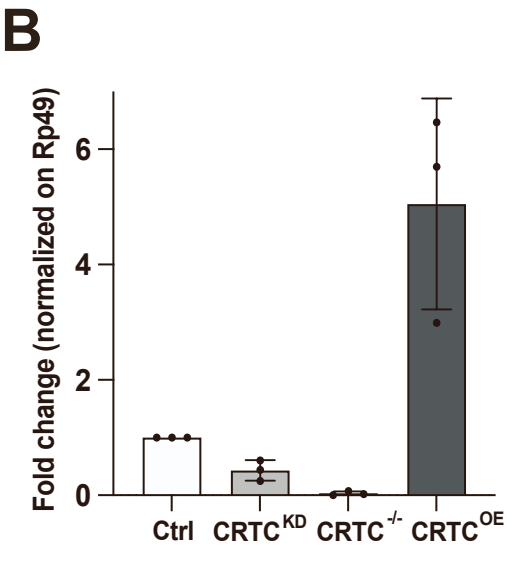
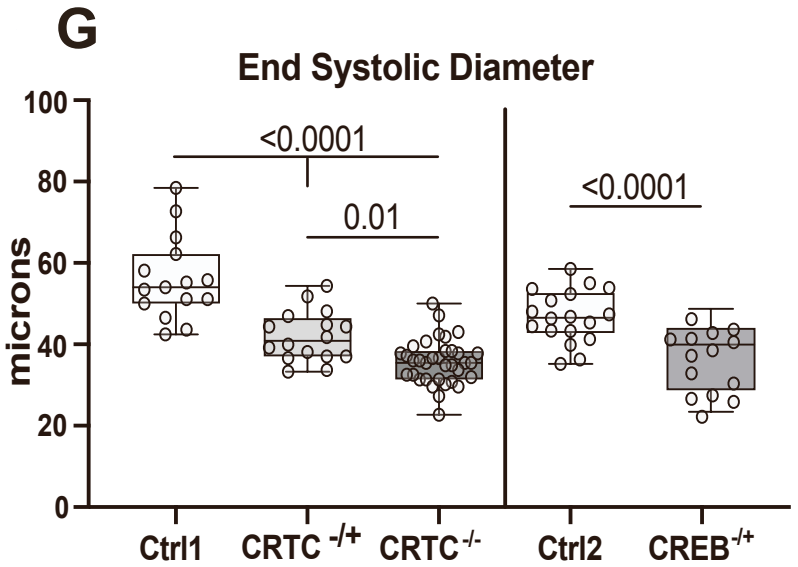
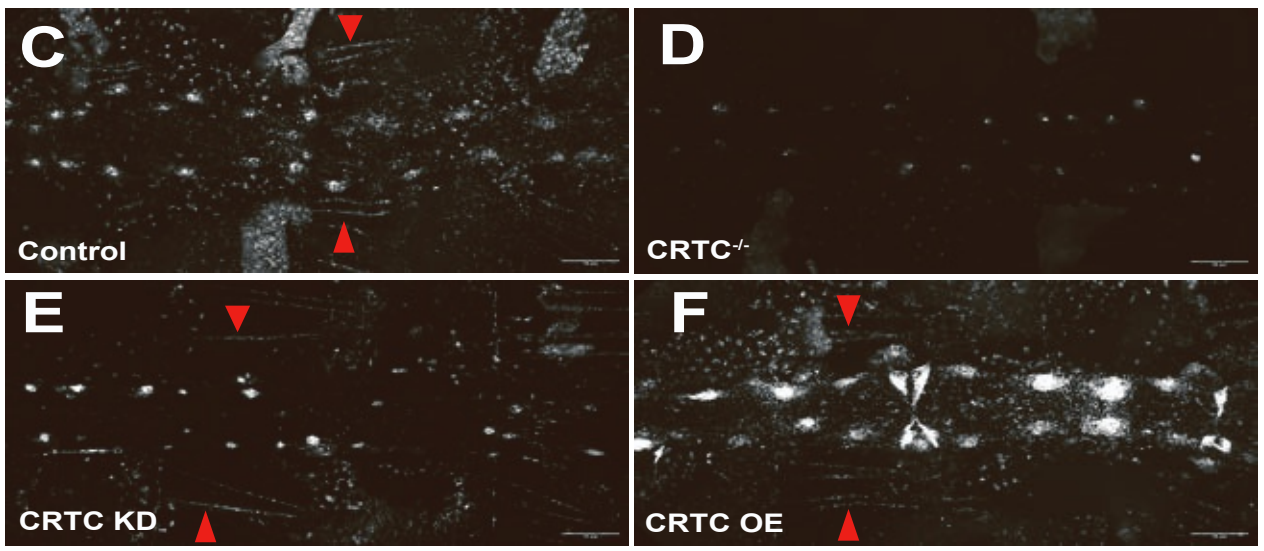
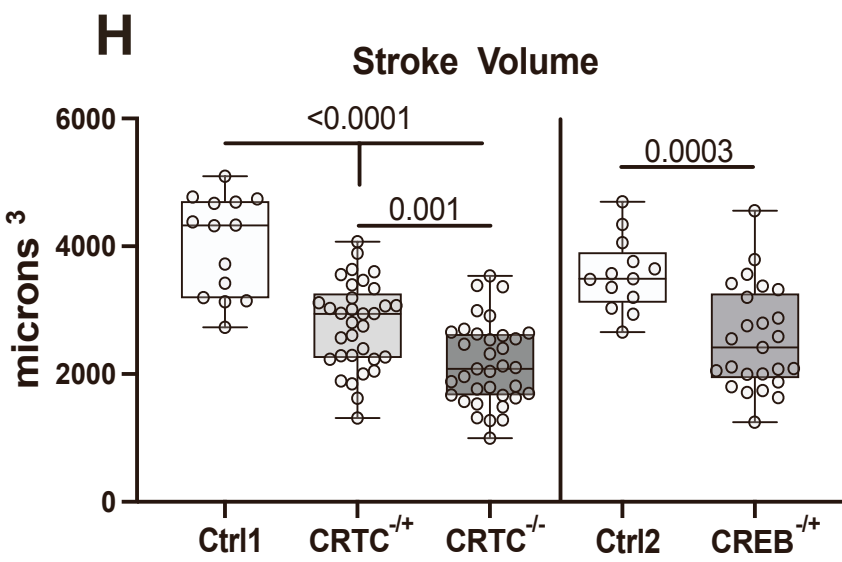
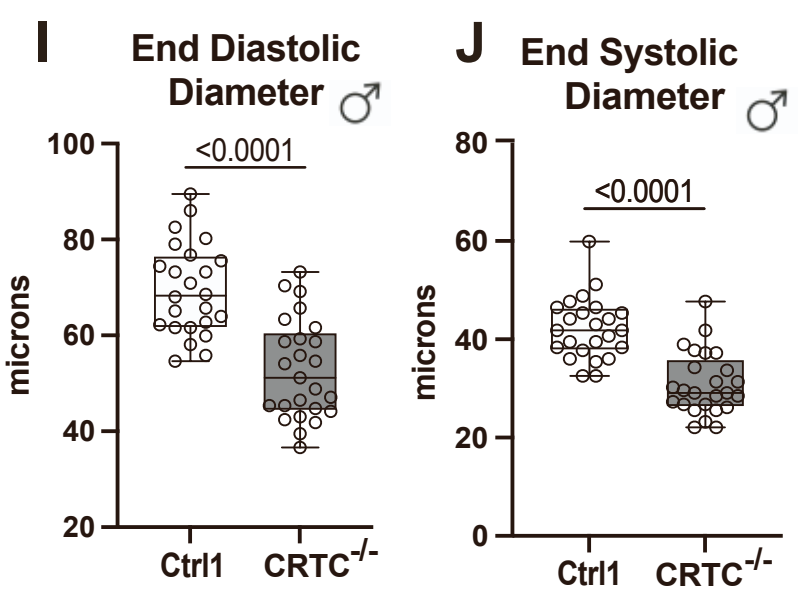

A
<0.001
<0.0001
120s
60s
120s
60s
107
124
Percent Beating
Ctrl
CRTC-/-


B
Fold change (normalized on Rp49)
Ctrl
CRTCKD
CRTC-/-
CRTCOE

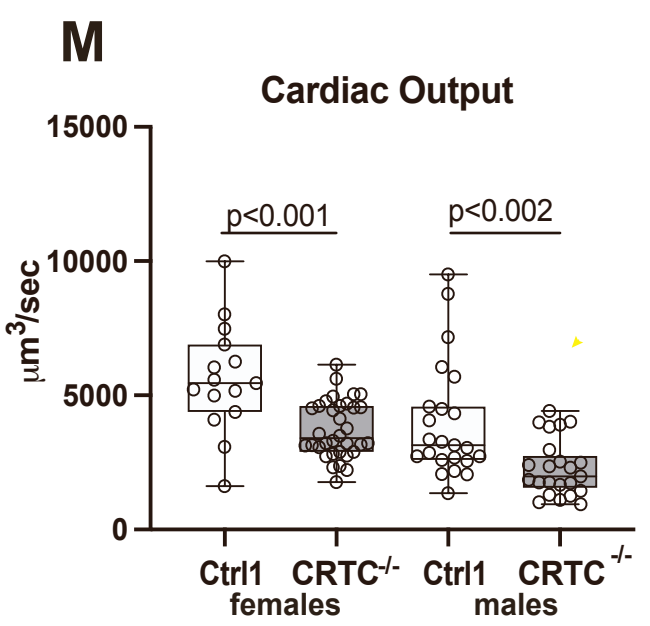

G
End Systolic Diameter
<0.0001
0.01
<0.0001
microns
Ctrl1
CRTC -/+
CRTC-/-
Ctrl2
CREB-/+

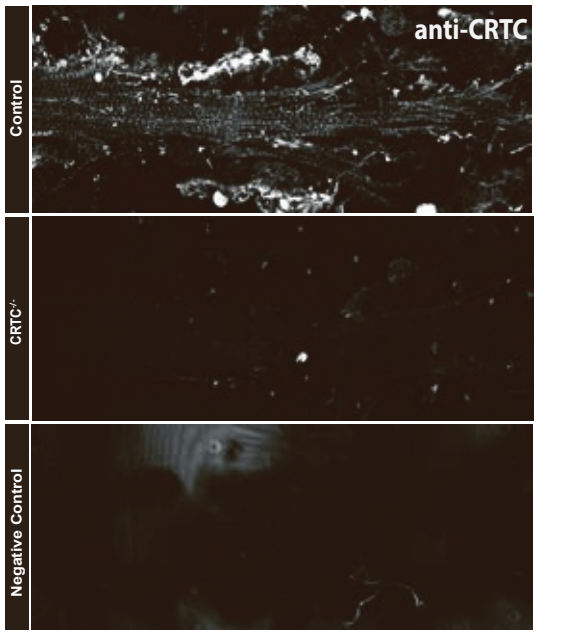

C
Control
D
CRTC-/-
E
CRTC KD
F
CRTC OE

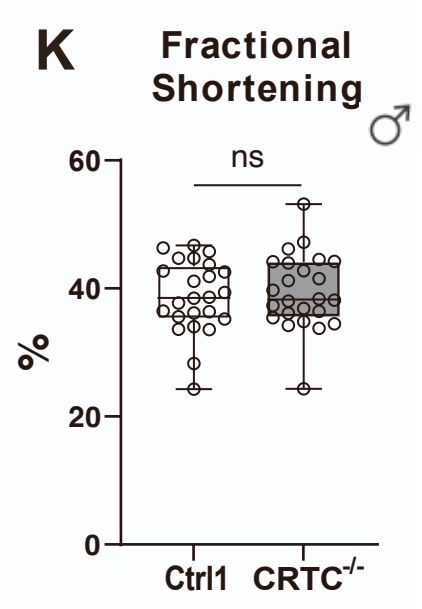

H
Stroke Volume
<0.0001
0.001
0.0003
microns 3
Ctrl1
CRTC-/+
CRTC-/-
Ctrl2
CREB-/+

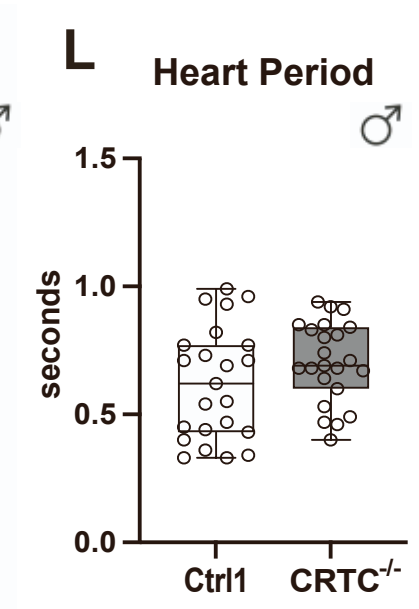

I
End Diastolic Diameter
<0.0001
microns
Ctrl1
CRTC-/-

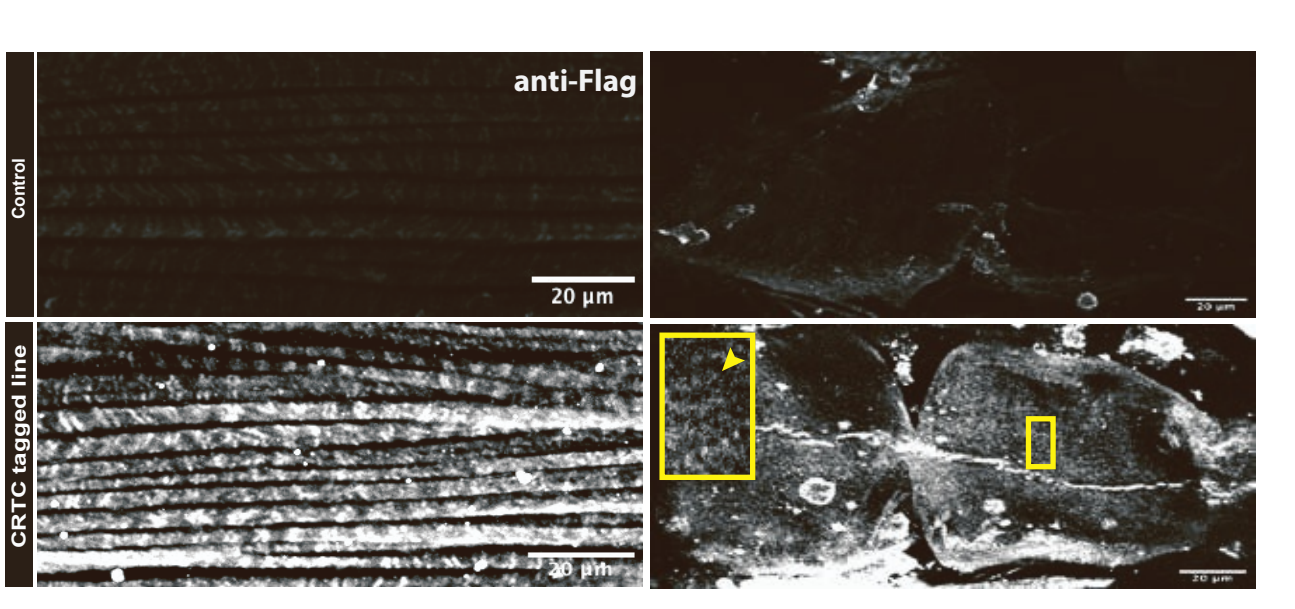
J
End Systolic Diameter
<0.0001
microns
Ctrl1
CRTC-/-


M
Cardiac Output
p<0.001
p<0.002
µm3/sec
Ctrl1
females
CRTC-/-
Ctrl1
males
CRTC -/-


N
anti-CRTC
Control
CRTC-/-
Negative Control


K
Fractional Shortening
ns
%
Ctrl1
CRTC-/-


L
Heart Period
seconds
Ctrl1
CRTC-/-


O
anti-Flag
Control
20 µm
CRTC tagged line
20 µm

Supplemental Figure 1 – Deletion of *CRTC* makes flies more susceptible to stress-induced heart failure and causes cardiac dysfunction.

(A) Heart Failure assay showing the percent of hearts still beating following a 30 sec electrical pacing stress test. Approximately 60% of hearts in wt flies (w1118, N=107) were not beating 60s after electrical pacing (6HZ for 30s); all but ~20% resumed beating by 120s post stress. Roughly 80% of hearts in *CRTC* mutants (N=124) were not beating 60s post pacing stress and more than 60% were still not beating at 120s. Significance determined by Multiple Mann-Whitney tests.

(B) RT-qPCR of isolated fly hearts validates *CRTC* mutant, KD and OE fly lines. 3 biological replicates for each condition done in triplicate.

(C) HCR for *CRTC* shows *CRTC* mRNA expression in cardiac tube, nuclei and somatic muscle (red arrowhead) in controls.

(D) *CRTC* mRNA expression is dramatically reduced in cardiac tube, nuclei, fat body, and somatic muscle in *CRTC* mutants.

(E) *CRTC* expression is significantly reduced in *CRTC* KD hearts compared to controls, but expression in somatic muscle (red arrowheads) is maintained.

(F) *CRTC mRNA* expression is significantly increased in *CRTC* OE hearts compared to controls, but expression in somatic muscle (red arrowheads) remains similar to that of controls.

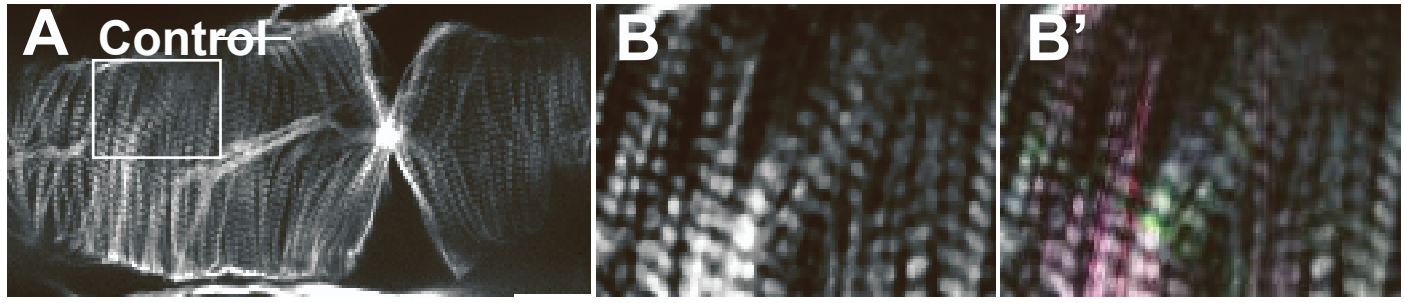
(G) Left - End systolic diameter was reduced in both *CRTC* hetero and homozygous mutants compared to controls. Right - End systolic diameter was also reduced in *CREB* heterozygous mutants compared to controls.

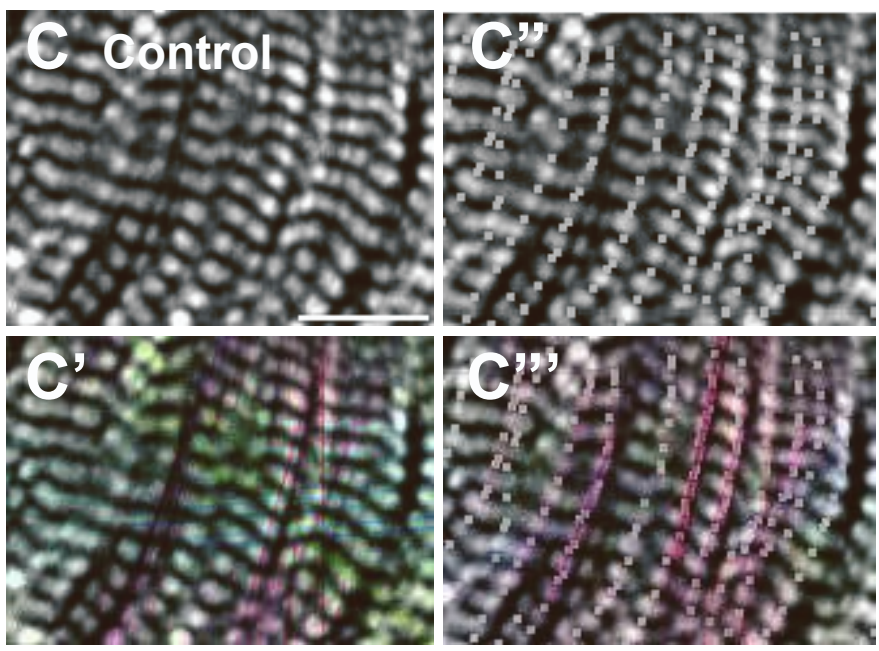
(H) Left – Stroke volume was reduced in both *CRTC* hetero and homozygous mutants compared to controls. Right – Stroke volume was also reduced in *CREB* heterozygous mutants compared to controls. Significance was determined by 1-way ANOVA for Wt1 and *CRTC* mutants with Sidak's multiple comparison post hoc test. and unpaired student t-test for Wt2 and *CREB* mutant. (All data points, Max, Min and Median with p values are shown.)

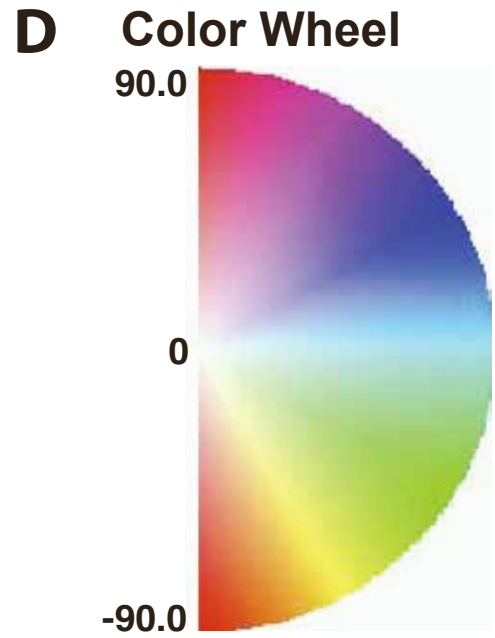
(I) End diastolic diameters, (J) end systolic diameters, and (K) fractional shortening were all reduced in hearts from 1 week old *CRTC* mutant male flies compared to Wt controls. (L) Unlike females, heart period was unaffected by *CRTC* loss in males but (M) cardiac output was still significantly reduced. Significance was determined by 1-way ANOVA for Wt1 and *CRTC* mutants with Sidak's multiple comparison post hoc test. and unpaired student t-test for Wt2 and *CREB* mutant. (All data points, Max, Min and median with p values are shown.)

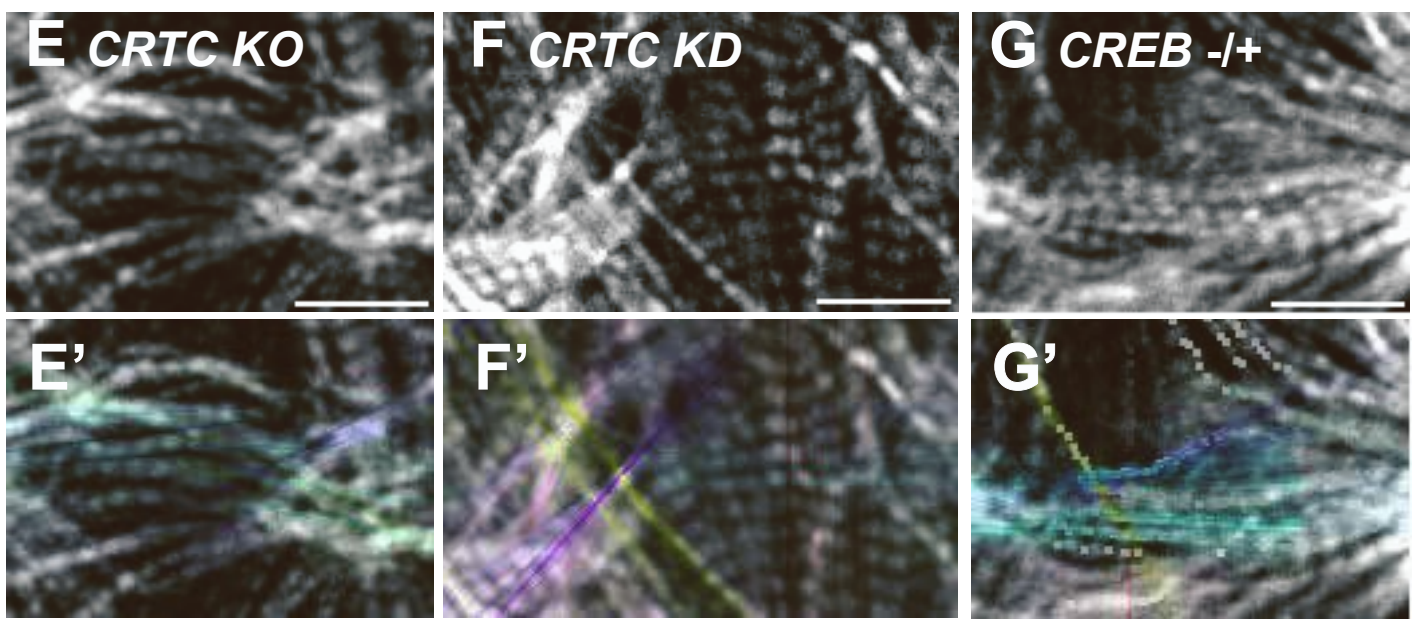
(N) Anti-CRTC staining of wt heart (top) shows a lattice like pattern over the longitudinal fibers and is present in fat bodies. A heart from a *CRTC* null mutant (middle) shows background staining. The negative control (bottom) was stained only with the secondary antibody.

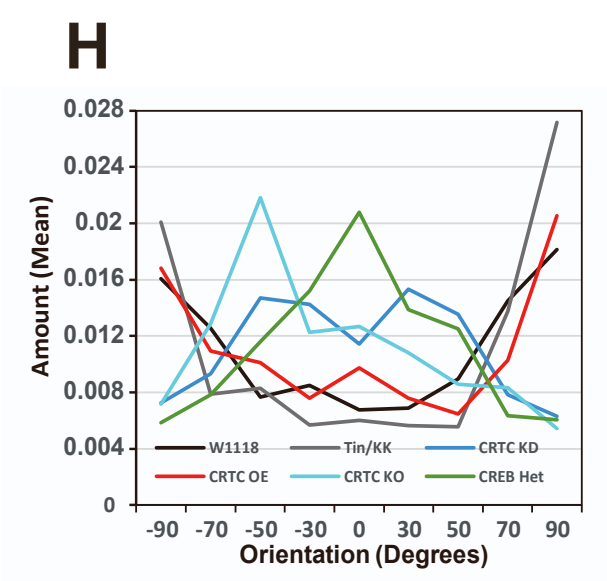
(O) Anti-Flag staining (white) of hearts from a CRTC Flag-tagged transgenic line confirmed the banded pattern for CRTC localization in non-myocardial, ventral longitudinal fibers (left). Optical section through a chamber of the fly heart tube stained with anti-Flag (right) confirmed the myocardial expression. Higher magnification of the region in the box shows a loose banding pattern (arrowheads) similar to that seen with the CRTC antibody. Scale bars are 20 µm.

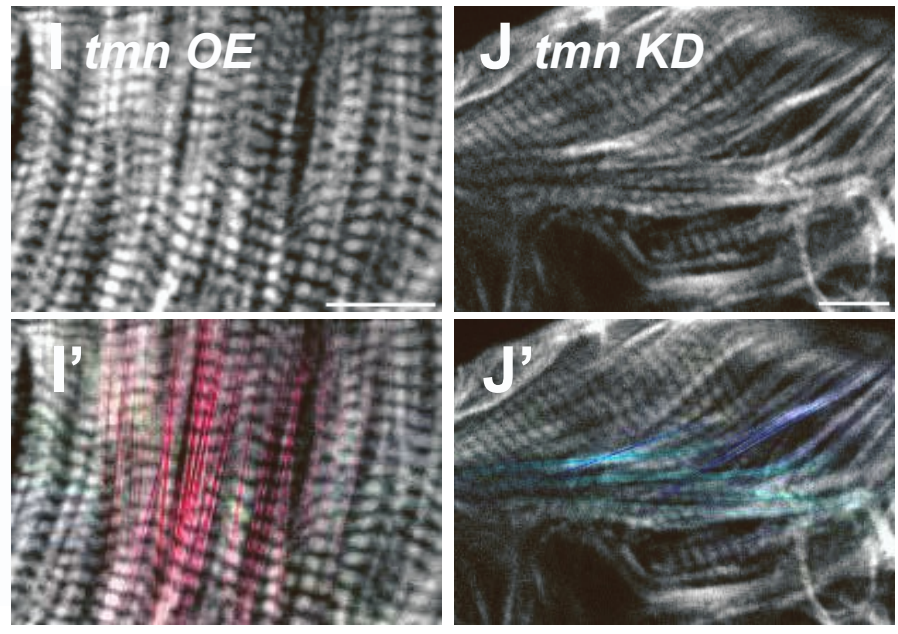

A Control
B
B'

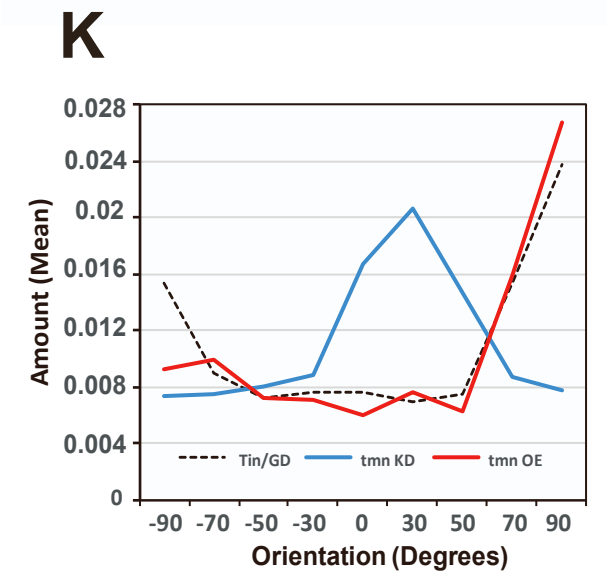

C Control
C''
C'
C'''

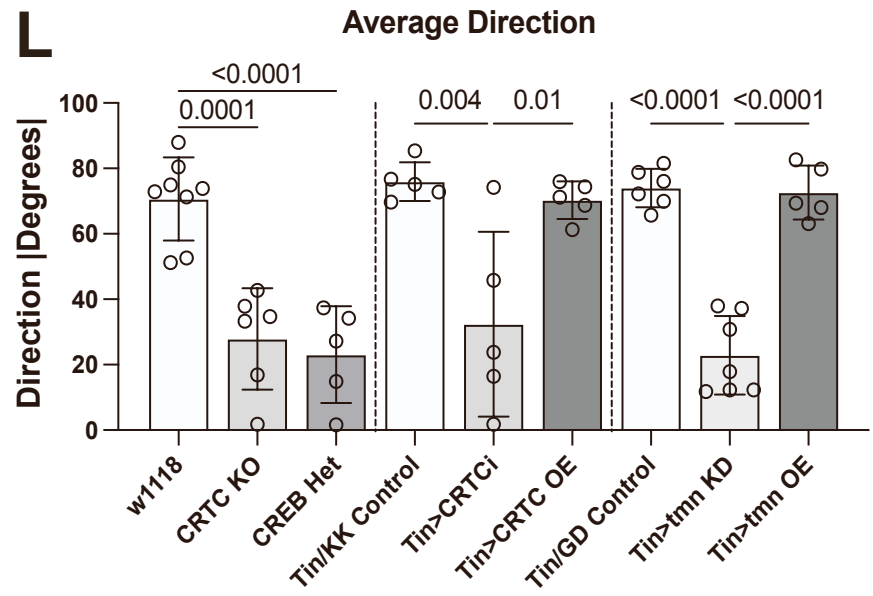

D Color Wheel
90.0
0
-90.0


E CRTC KO
F CRTC KD
G CREB -/+
E'
F'
G'


H
0.028
0.024
0.02
0.016
0.012
0.008
0.004
0
Amount (Mean)
W1118
Tin/KK
CRTC KD
CRTC OE
CRTC KO
CREB Het
-90 -70 -50 -30 0 30 50 70 90
Orientation (Degrees)


I tmn OE
J tmn KD
I'
J'


K
0.028
0.024
0.02
0.016
0.012
0.008
0.004
0
Amount (Mean)
Tin/GD
tmn KD
tmn OE
-90 -70 -50 -30 0 30 50 70 90
Orientation (Degrees)


L
Average Direction
Direction |Degrees|
100
80
60
40
20
0
<0.0001
0.0001
0.004
0.01
<0.0001
<0.0001
w1118
CRTC KO
CREB Het
Tin/KK Control
Tin>CRTCi
Tin>CRTC OE
Tin/GD Control
Tin>tmn KD
Tin>tmn OE

Supplemental Figure 2 – Quantification of Myofibrillar Disorganization.

(A) Z stack image of one heart chamber from a horizontally oriented heart (anterior – posterior). (B-B') ROIs of myofibrils from individual myocardial cells were analyzed using the Directionality Plugin in FIJI (see Methods).

(C) Example Z stack from a myocardial cell in a w^{1118} wildtype control heart stained with phalloidin for F-actin (all scale bars are 50 pixels/13 microns). (C') Orientation map from the Direction function in FIJI showing correctly identified vertically oriented myofibrils (orange and red). Additional detected elements labeled as horizontally oriented (purple, blues, and greens) are due to the regular arrangement of the unstained (dark) myosin bands. (C") The myosin bands in (C) have been filed with the same color gray as the adjacent actin bands. (C'") The Orientation map based on the image in C" shows fewer incorrectly detected horizontal myofibrils (more red, less blue and green).

(D) Color wheel showing the orientation in degrees for the differently colored elements in the orientation maps.

(E) Example of a Zstack from a myocardial cell in a *CRTC* mutant heart.

(E') Orientation map based on (E) shows correctly identified horizontal myofibrils (Blues, greens).

(F) Example of a Zstack from a myocardial cell in a *CRTC* cardiac KD heart.

(F') Orientation map based on (F) shows correctly identified horizontal myofibrils (Purples, blues, greens).

(G) Example of a Zstack from a myocardial cell in a *Creb* heterozygote heart.

(G') Orientation map based on (G), with myosin "fill in", shows correctly identified horizontal myofibrils (Yellows, blues, greens).

(H) Directionality of identified structures was analyzed in Image J and results were binned according to their orientation (from -90^{o} to $+90^{o}$, in 20^{o} bins). The mean amount is plotted for each bin. Both w^{1118} and tin-Gal4 controls as well as *CRTC* OE hearts showed peaks at -90^{o} and $+90^{o}$ (both vertical orientations), whereas hearts from *CRTC* null mutants, cardiac *CRTC* KD, and *Creb* heterozygotes had peaks around 0^{o} (more horizontal orientations).

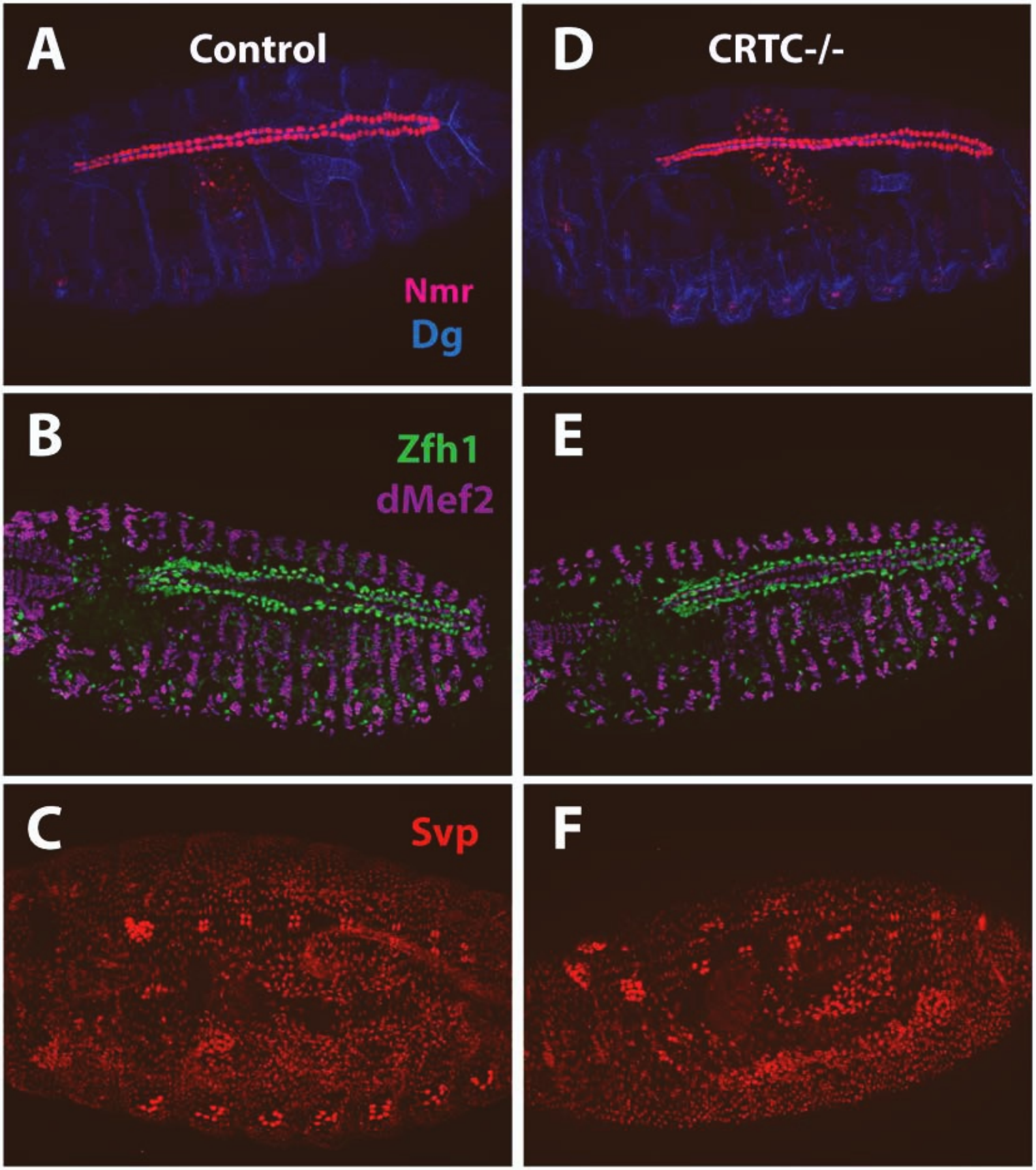
(I) Example of a Zstack from a myocardial cell in a *tmn* cardiac OE heart.

(I') Orientation map based on (I) showing correctly identified vertical myofibrils.

(J) Example of a Zstack from a myocardial cell in a *tmn* cardiac KD heart. (J') Orientation map based on (J) showing correctly identified horizontal myofibrils.

(K) Plots of directionality for tin-Gal4 outcrossed controls as well as *tmn* OE hearts showed peaks at -90^{o} and $+90^{o}$ (vertical orientations), whereas hearts from cardiac *tmn* KD hearts had a peak around 30^{o} (more horizontal orientation).

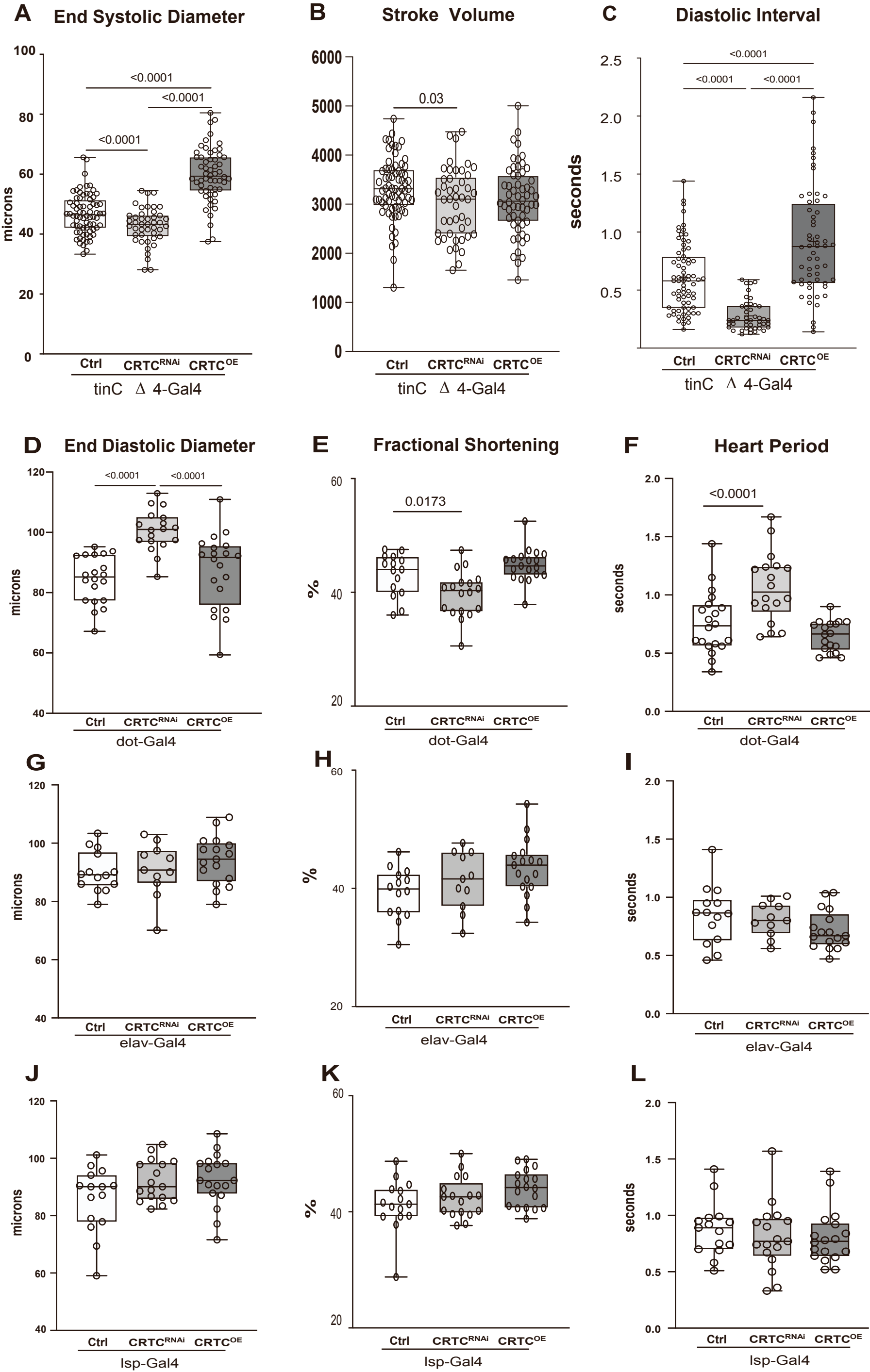
(L) "Direction" reports the orientation (in degrees) for the center of the highest peak in each sample based on a gaussian fit. The absolute values of "Direction" for each heart were averaged and plotted; significance was determined by one way ANOVA and Tukey's multiple comparisons post hoc test, p values are shown


A
Control
Nmr
Dg
D
CRTC-/-
B
Zfh1
dMef2
E
C
Svp
F

Supplemental Figure 3 - Systemic *CRTC* KO did not affect cardiac development.

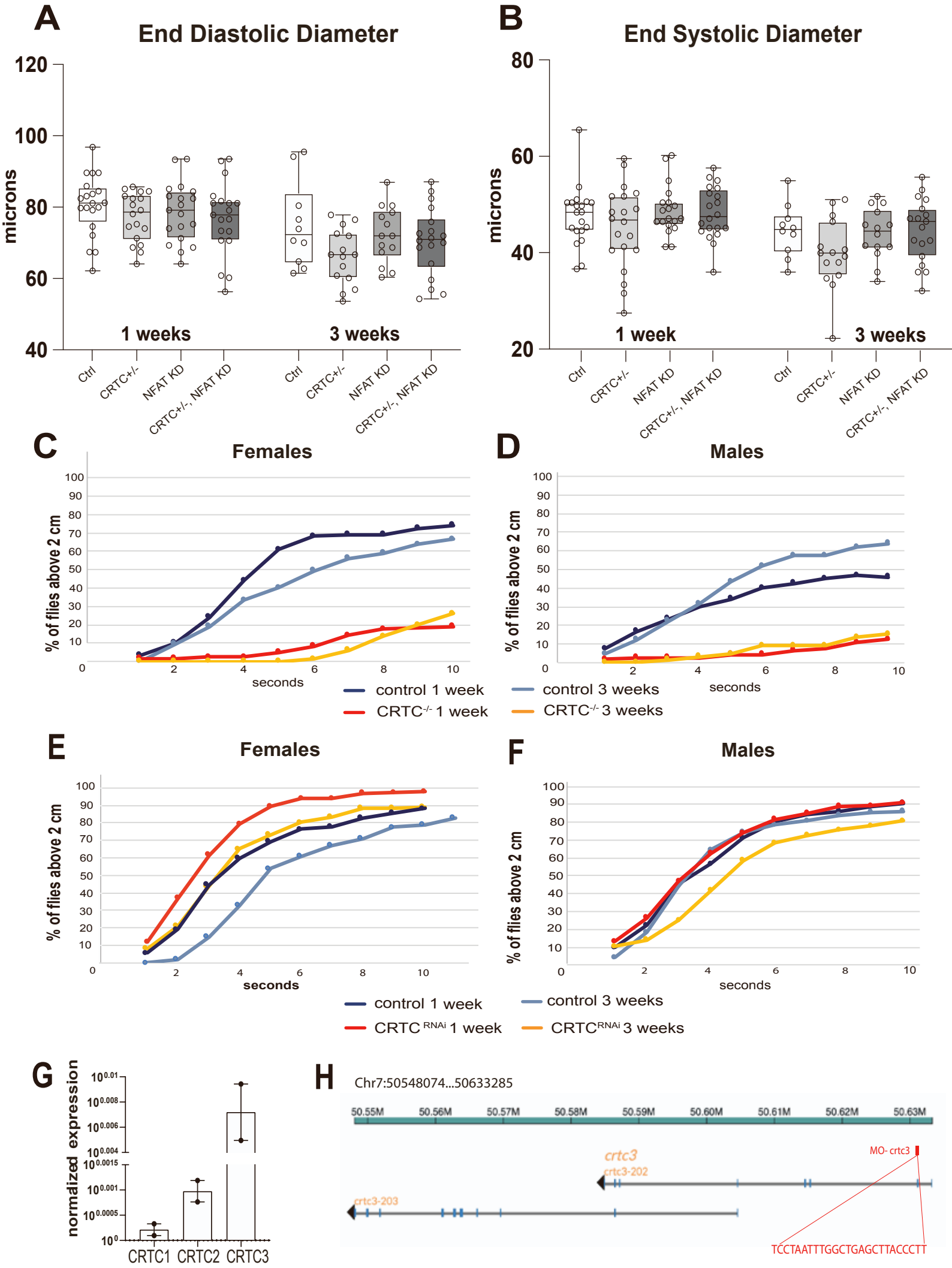
(A-C) Stage 17 wt and (D-F) *CRTC* mutant embryos were examined for cardiac development.

(A, D) Wildtype and *CRTC* mutant embryos were stained with antibodies against Neuromancer (Nmr) which labeled cardioblast nuclei (red), and dystroglycan (Dg) which labels the basal domain of epithelial cells (blue). (B,E) Anti-Zfh1 (green) labeled the nuclei of pericardial cells and anti-dMEF2 (purple) stained all muscle nuclei. (C,F) Anti-Svp labels a subset of cardiac cells that will form the ostia (inflow tract).


A
End Systolic Diameter
microns
100
80
60
40
20
0
<0.0001
<0.0001
<0.0001
Ctrl
CRTC^RNAi
CRTC^OE
tinC Δ 4-Gal4
B
Stroke Volume
6000
5000
4000
3000
2000
1000
0
0.03
Ctrl
CRTC^RNAi
CRTC^OE
tinC Δ 4-Gal4
C
Diastolic Interval
seconds
2.5
2.0
1.5
1.0
0.5
<0.0001
<0.0001
<0.0001
Ctrl
CRTC^RNAi
CRTC^OE
tinC Δ 4-Gal4
D
End Diastolic Diameter
microns
120
100
80
60
40
<0.0001
<0.0001
Ctrl
CRTC^RNAi
CRTC^OE
dot-Gal4
E
Fractional Shortening
%
60
40
20
0.0173
Ctrl
CRTC^RNAi
CRTC^OE
dot-Gal4
F
Heart Period
seconds
2.0
1.5
1.0
0.5
0.0
<0.0001
Ctrl
CRTC^RNAi
CRTC^OE
dot-Gal4
G
microns
120
100
80
60
40
Ctrl
CRTC^RNAi
CRTC^OE
elav-Gal4
H
%
60
40
20
Ctrl
CRTC^RNAi
CRTC^OE
elav-Gal4
I
seconds
2.0
1.5
1.0
0.5
0.0
Ctrl
CRTC^RNAi
CRTC^OE
elav-Gal4
J
microns
120
100
80
60
40
Ctrl
CRTC^RNAi
CRTC^OE
lsp-Gal4
K
%
60
40
20
Ctrl
CRTC^RNAi
CRTC^OE
lsp-Gal4
L
seconds
2.0
1.5
1.0
0.5
0.0
Ctrl
CRTC^RNAi
CRTC^OE
lsp-Gal4

Supplemental Figure 4 – *CRTC* cardiac phenotype was not recapitulated by *CRTC* KD in nephrocytes, neurons, or fat body.

(A) End Systolic Diameter was decreased in cardiac-specific KD of *CRTC* using tinCΔ4-Gal4 driver and increased in cardiac-specific *CRTC* OE. (B) Stroke volume was reduced by cardiac-specific *CRTC* KD and unchanged in cardiac OE hearts. (C) Diastolic Interval was decreased in cardiac-specific CRTC KD and increased in cardiac specific CRTC OE, using the Dot-Gal4 driver. (D) End Diastolic Diameter (EDD) was increased by nephrocyte-specific KD of *CRTC.* (E) Fractional Shortening was decreased by nephrocyte-specific *CRTC* KD. (F) Heart Period was increased by nephrocyte-specific KD of *CRTC*. (G) EDD, (H) fractional shortening, and (I) heart period were unaffected by neuronal KD or OE of *CRTC*, using the elav-Gal4 driver. (J) EDD, (K) fractional shortening, and (L) heart period were unaffected by fat body-specific *CRTC* KD or OE. Plots show Max, Min and Median, significance was determined using a one-way ANOVA with Tukey's multiple comparisons post hoc test, p values shown.


A
End Diastolic Diameter
microns
120
100
80
60
40
1 weeks
3 weeks
Ctrl
CRTC+/-
NFAT KD
CRTC+/-, NFAT KD
B
End Systolic Diameter
microns
80
60
40
20
1 week
3 weeks
C
Females
% of flies above 2 cm
seconds
control 1 week
CRTC-/- 1 week
D
Males
control 3 weeks
CRTC-/- 3 weeks
E
Females
RNAi
F
Males
G
normalized expression
CRTC1
CRTC2
CRTC3
H
Chr7:50548074...50633285
crtc3
crtc3-202
crtc3-203
MO- crtc3
TCCTAATTTGGCTGAGCTTACCCTT

Supplemental Figure 5 - CRTC affects somatic muscle function but cardiac KD of *NFAT* had little effect on heart function.

(A) End Diastolic Diameters were unchanged in response to cardiac *NFAT* KD with tinCΔ4-Gal4.

(B) End Systolic Diameters in hearts from flies with cardiac *NFAT* KD were unchanged. Combined loss of function of *CRTC* and *NFAT* had no significant effects. Plots show all data points, Max, Min and Median; significance was determined using a one-way ANOVA with Tukey's multiple comparisons post hoc test.

(C-F) Climbing assay results; flies were assayed at 1 and 3 weeks.

(C) Female control flies (traces in blues; 1 week old n=40, 3 weeks old n= 35) and *CRTC systemic mutants* (traces in red/orange; 1 week old n=40, 3 weeks old n= 27) were assayed and the percent of flies above the 2 cm mark in the assay vial over a 10 sec interval is shown.

(D) Percent of male control flies (traces in blues; 1 week old n=40, 3 weeks old n= 36) and *CRTC systemic mutant flies* (traces in red/orange; 1 week old n=40, 3 weeks old n= 22) the 2 cm mark in the assay vial over a 10 sec interval is shown.

(E) Percent of female control flies (traces in blues; 1 week old n=222, 3 weeks old n= 210) and *cardiac-specific CRTC KD* flies (traces in red/orange; 1 week old n=228, 3 weeks old n= 210) above the 2 cm mark over a 10 sec interval is shown.

(F) Percent of male control flies (traces in blues; 1 week old n=201, 3 weeks old n= 186) and *cardiac-specific CRTC KD* flies (traces in red/orange; 1 week old n=234, 3 weeks old n= 219) above the 2 cm mark over a 10 sec interval is shown.

(G) *CRTC* expression in isolated adult (10-month-old) zebrafish hearts, normalized to elongation factor eF1a, showed significant expression of *CRTC 3* compared to *CRTC 1 & 2*. Data points represent 2 biological samples, 8 hearts per sample done in triplicate.

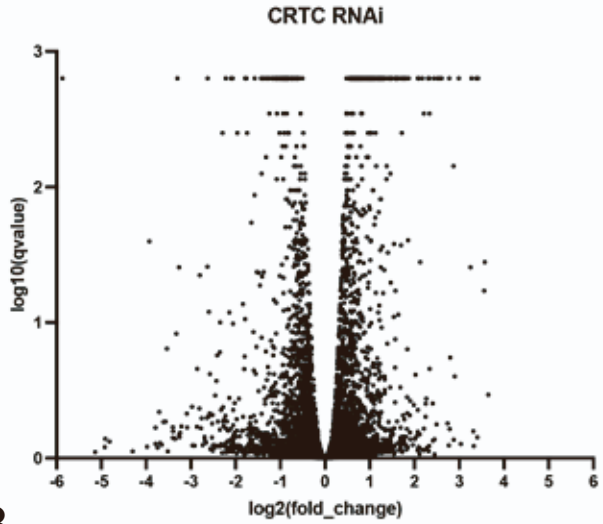
(H) Schematic representation of binding locus for the CRTC3 morpholino.

A

CRTC-RNAi

357 Upregulated

186 Downregulated



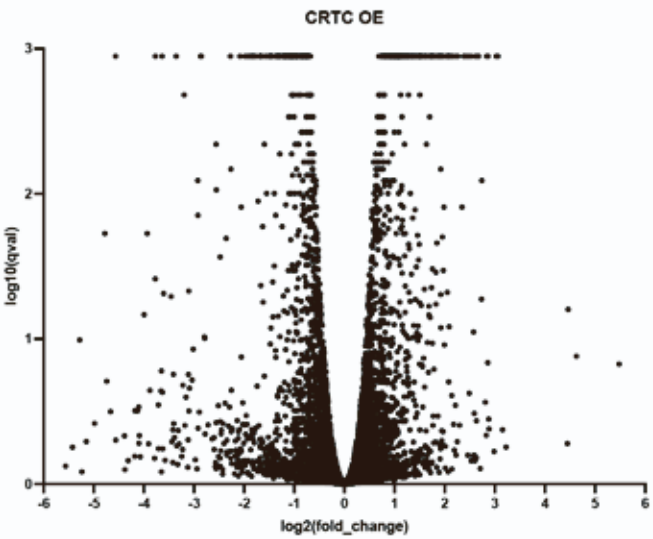
Category	Term	Count	FDR	Direction
BP_GO:0002181	Translation / Ribosome	33	1.0 x10^-23	Upregulated
BP_GO:0045087	Innate Immune Response	12	0.00864	Upregulated
KEGG dme01100	Metabolic Pathways	99	1.5 x 10^-15	Upregulated
KEGG dme00010	Glycolysis/Gluconeogenesis	21	1.6 x10^-11	Upregulated
KEGG dme01230	Amino acid Biosynthesis	21	5.1 x 10^-5	Upregulated
KEGG dme00030	Pentose phosphate pathway	10	8.7 x10^-6	Upregulated
KEGG dme00620	Pyruvate Metabolism	12	1.4 x 10 ^-4	Upregulated
KEGG dme00071	Fatty acid degradation	8	0.003	Upregulated
KEGG dme00020	Citrate (TCA) Cycle	9	0.006	Upregulated
BP_GO:0007155	Cell adhesion	7	0.00138	Downregulated
BP_GO:0030497	Fatty acid elongation	4	0.0027	Downregulated

B

CRTC-OE

426 Upregulated

390 Downregulated



Category	Term	Count	FDR	Direction
BP_GO:0030497	Fatty Acid Elongation	9	1.9 x10-4	Upregulated
BP_GO:0030239	Myofibril Assembly	6	0.023	Upregulated
KEGG dme04512	ECM-receptor interaction	7	2.0x 10-7	Upregulated
KEGG dme01212	Fatty Acid Metabolism	7	0.0012	Upregulated
BP_GO:0055114	Oxidation-Reduction	32	4.6 x 10-4	Downregulated
BP_GO:0045087	Innate Immune Response	14	0.007	Downregulated
BP_GO:0006635	Fatty acid Beta-Oxidation	8	0.015	Downregulated
KEGG dme01100	Metabolic Pathways	78	5.3 x 10^-11	Downregulated
KEGG dme00071	Fatty acid degradation	12	1.5 x 10^-5	Downregulated
KEGG dme01230	Amino acid Biosynthesis	10	0.001	Downregulated
KEGG dme00010	Glycolysis/Gluconeogenesis	9	0.001	Downregulated

C

Fly Gene	Human Gene	Diopt Score	log2 (KK FPKM)	log2 (CRTCoe FPKM)	log2 (Ratio)	q Value	Gene Product
Act42A	ACT A1, B, C1, G1	7-12	5.23	6.83	1.6	1.13E-03	Actin
Argk	Ck B, M	14	9.49	10.31	0.82	1.13E-03	Arginine/ Creatine Kinase
CanA-14F	PPPB	15	5.83	6.51	0.68	2.08E-03	Phosphatase
CRTC	CRTC1,2,3	8-11	3.47	5.18	1.72	1.13E-03	Creb Modulator
jp	JPH 1,2,3,4	7-13	5.36	6.44	1.08	1.13E-03	Ca2+ signaling
Mhc	MYH 1-8	11-14	7.13	7.96	0.83	1.13E-03	Myosin heavy Chain
Mlc2	MYL2	6	9.34	10.14	0.8	1.13E-03	Myosin light chain
Pka-C3	PRKX	13	4.47	5.95	1.48	1.13E-03	Protein Kinase
timp	TIMP 1,2,3,4	11-15	7.23	8.44	1.21	1.13E-03	Inhibitor of metalloproteases
TpnC25D	CALM	6	5.62	8.03	2.41	1.13E-03	Calmodulin Ca2+ binding protein
RyR	RYR 1,2,3	12-16	1.53	2.33	0.8	8.81E-03	Ryanodine receptor
up	TNNT 1-3	9-10	8.98	9.51	0.53	4.10E-02	Troponin T

Supplemental Figure 6 – Cardiac *CRTC* KD and OE concertedly regulate metabolism in the heart.

(A, left) Volcano plot of genes differentially regulated in hearts with cardiac-specific *CRTC* KD.

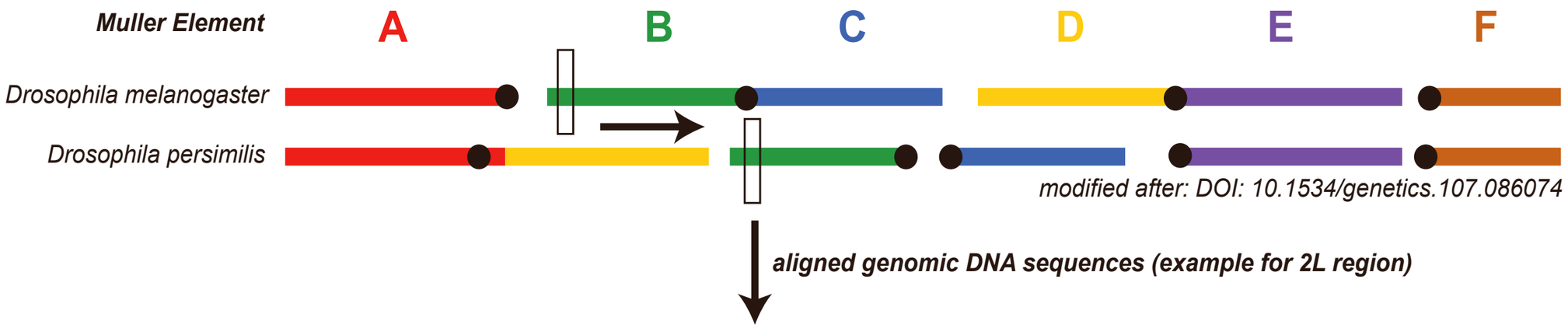
(A, right) Differentially regulated genes fall into GO categories primarily related to cell metabolism.

(B, left) Volcano plot of genes differentially regulated in hearts with cardiac-specific *CRTC* OE.

(B, right) Differentially regulated genes fall into GO categories primarily related to cell metabolism.

(C) Cardiomyopathy markers that were upregulated in response to cardiac *CRTC* OE. *CRTC* is shown as a reference and to validate the heart-specific over expression. DIOPT Score indicates similarity between fly and human gene (scores are 1-16, 16 indicates high homology).

A



B

Drosophila Genes with CREB Binding Sites											
5-HT7	CG10082	CG14777	CG32473	CG4452	CG9313	eEF1alpha1	H	mRpS9	PH4alphaEFB	Sh	tn
Abi	CG10194	CG14826	CG32679	CG45087	CG9426	eg	haf	msi	PH4alphaSG2	sha	Top3beta
abs	CG10254	CG14854	CG32982	CG45101	CG9568	eIF4E3	hdc	Msp300	Pld	shakB	Tpst
Ac13E	CG10383	CG14883	CG33116	CG4570	CG9773	Eip93F	heix	MsR1	porin	side	trpl
Acbp3	CG10467	CG14968	CG33203	CG4617	CG9911	eRF3	Herc4	MTA1-like	ppk5	side-III	Tsp66E
AdSS	CG10576	CG14985	CG33223	CG46307	CG9967	ERR	Him	Mtap	Prps	sif	TwdlC
Ae2	CG10616	CG15625	CG33543	CG46308	ChAT	Ets65A	Hipk	mtm	Rab1	SIFaR	tx
akirin	CG10778	CG15814	CG34167	CG4766	Cht7	Evi5	HLH4C	Muc4B	Rab18	siz	Uck
alphaCOP	CG10907	CG1607	CG34199	CG4813	cic	Faf2	how	Mur2B	rad	slgA	Ugt36F1
AnxB9	CG10919	CG1632	CG34210	CG4829	Cip4	fd59A	Hrb27C	MYPT-75D	Rassf	Slh	UQCR-C2
AP-1mu	CG11035	CG1640	CG34408	CG5065	ck	Fip1	Hsp22	NAT1	Rel	slif	Vha55
AP-2alpha	CG11141	CG1677	CG34461	CG5644	Clamp	fog	Ide	Nep3	Rgk2	sls	Vha68-2
Arf51F	CG1136	CG17068	CG3492	CG5758	cN-IIIB	foxo	Ih	Nop17l	rn	Smurf	vir-1
Arl1	CG11426	CG17233	CG3640	CG5860	Cog6	Fpps	IntS1	nord	Roe1	Spn	Vmat
AsnRS	CG11438	CG17271	CG3652	CG5910	Cog7	Fs	Jafrac2	Nrx-1	Rox8	sr	Vps13B
AsnS	CG11777	CG17364	CG3719	CG5916	Cp15	fs(1)Ya	Jarid2	Nsf2	RpL13	Sr-CIII	wal
AspRS-m	CG11873	CG17544	CG3829	CG6051	Cp18	fwe	jvl	nw	RpL31	sra	wbl
bai	CG12054	CG17598	CG3847	CG6055	Cpr49Ae	garz	KdelR	nyo	RpLP2	Srpk79D	Wnt10
beat-Ia	CG12307	CG17600	CG3916	CG6201	Cpr73D	GatA	l(1)G0320	Oatp30B	Saf6	SrpRbeta	Wnt6
beta'COP	CG12531	CG17672	CG42390	CG6333	Csk	GCC185	lbk	Obp56i	sand	ssh	wol
BobA	CG12948	CG17739	CG42455	CG6638	CtBP	gce	lin-28	Obp57c	sano	St3	wrapper
brm	CG13051	CG17778	CG42498	CG6678	cu	gcl	Lip1	Optix	sbb	Svil	Zasp66
brp	CG13064	CG18258	CG42637	CG7236	Cyp316a1	Gclm	Liprin-alpha	Orct	sca	svp	zfh1
bru2	CG13622	CG18659	CG42752	CG7414	Cyt-c-p	Gdi	loj	ort	Scm	Swim	
brun	CG13627	CG2918	CG42823	CG7600	Dap160	geko	mAChR-B	Osi6	Scr	Syp	
btl	CG13698	CG31055	CG43069	CG7685	daw	Gfat2	Manf	Ostgamma	sdt	Syt4	
Ca-alpha1D	CG13806	CG31086	CG43078	CG7778	Dek	Gld	Max	Pak	Sec23	tal-AA	
Ca-Ma2d	CG13887	CG31321	CG43135	CG7896	DIP-theta	Glut4EF	mbl	PAN3	Sec24AB	tank	
Cad99C	CG14075	CG31862	CG43175	CG8006	dnc	Gmap	Mbs	PAPLA1	Sec24CD	tara	
CAP	CG14182	CG31871	CG43450	CG8008	dpr1	Gp93	MED15	Pask	Sec61beta	Ten-a	
Catsup	CG14246	CG31949	CG4360	CG8012	dpr11	GstD3	MetRS	Pcyt1	Sec61gamma	Ten-m	
Cdc16	CG14247	CG32104	CG43861	CG8142	Dys	GstD4	Mnt	Pde6	Sec63	Tep4	
CDK2AP1	CG14346	CG32235	CG44270	CG8757	E(spl)m6-BFM	GstD5	mRpL49	Pfrx	SecCl	TH1	
Cdk4	CG14566	CG32281	CG44362	CG9150	E(spl)m8-HLH	GXIVsPLA2	mRpS14	Pgant7	SF2	ThrRS	
CenG1A	CG14760	CG32365	CG44433	CG9297	spl)malpha-BF	Gyc76C	mRpS24	PGRP-LA	sfl	Tlk	

C

	Differential expression	Not differentially expressed	total
with CREB-TFBS	**101**	**814**	**915**
No CREB-TFBS	**1074**	**11938**	**13912**
total	**1175**	**12752**	**13927**

D

gene	FCA heart cell counts	Human Ortholog	Function	DIOPT
CG13315	466.79	no ortholog	unknown	-
CG9297	299.60	Srl	SR Ca^{2+} binding protein	11
AdipoR	247.33	Adipor1	Glucose/lipid metabolism	16
CG32549	241.77	NT5C2	enable 5'-nucleotidase activity.	15
CG43693	240.98	SLC36A1	Amino Acid transporter	13
aralar1	229.88	ARALAR	Mitochondrial Ca^{2+} binding transport protein	18
Prat2	198.49	PRAT	Glutamine amidotransferase	17
Sodh-1	194.95	SORD	Sorbitol dehydrogenase 1	17
CG9331	142.51	GRHPR	glycerate dehydrogenase	9
trbl	113.93	TRIB	Trib family of kinase-like protein	16
CG3902	81.16	ACADSB	AA transporter	16
CG5910	79.49	no ortholog	unknown	-
Adgf-D	47.39	ADGF-C	Adenosine deaminase-related growth factor	12
Cyp6a23	31.55	CYP3A5	Cytochrome P450 6a23	9
Act42A	20.81	ACTB	Actin	12

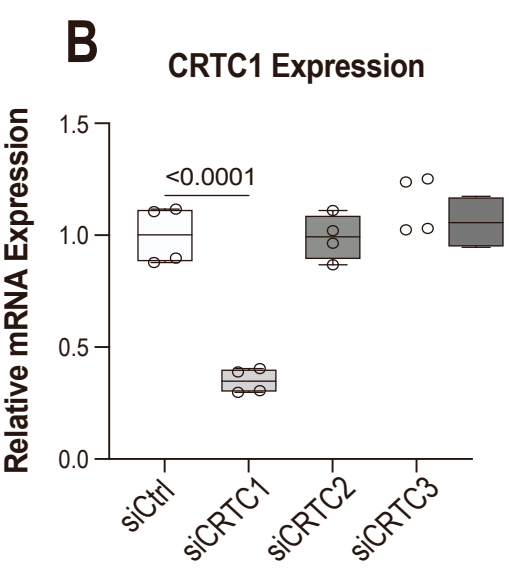
FCA (FlyCellAtlas) myocardial cell count expressing each gene from snRNAseq.
DIOPT Score (1-19) indicates the number of tools showing homolog between the genes (v.9, https://www.flyrnai.org/cgi-bin/DRSC_orthologs.pl)

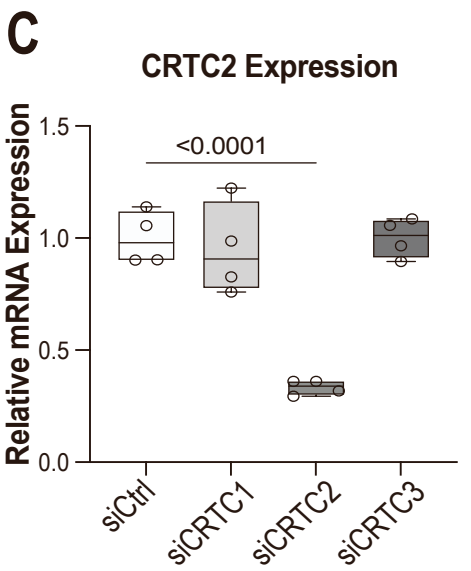
Supplemental Figure 7- Identification of conserved TFBS

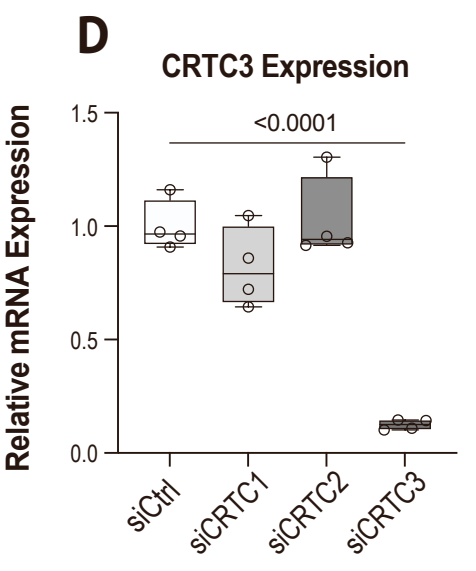
(A) Workflow for the identification of conserved TFBS.
(B) Fly genes with CREB binding sites conserved between *Drosophila melanogaster* and *Drosophila persimilis*
(C) Contingency table for genes with CREB TFBS and differential expression after *CRTC* KD -or- OE. Hypergeometric test for enrichment analysis using the values from the contingency table indicates that genes with CREB TFBS are more likely found to be overexpressed than genes without.
(D) CRTC Contra-regulated cardiac genes with conserved CREB binding sites.

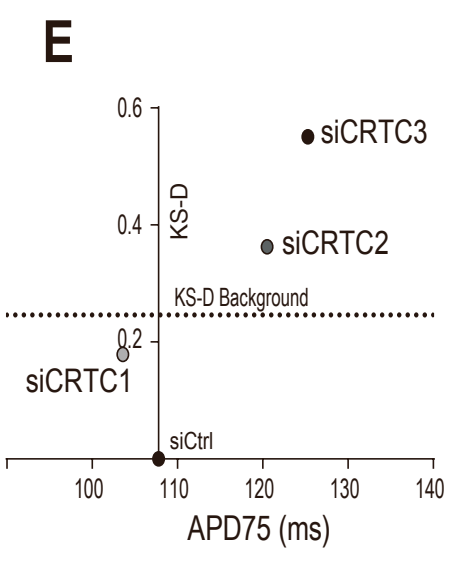
A

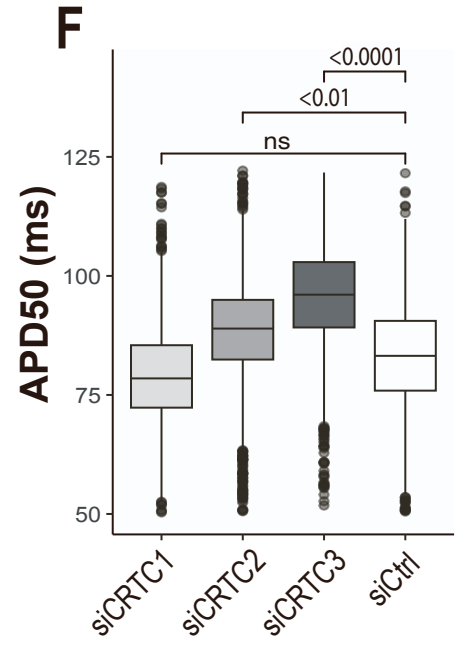
Trascript	Gene	Chrom.	Start	Stop	Strand	Lenght	VCM 1	ACM 1	VCM 2	ACM 2
NM_001098482	CRTC1	19	18683615	18782334	+	6992	0.230	0.181	0.103	0.231
NM_015321	CRTC1	19	18683615	18782334	+	6944	0.480	0.595	0.615	0.643
NM_181715	CRTC2	1	1.54E+08	1.54E+08	-	2678	1.788	1.677	1.548	1.574
NM_001042574	CRTC3	15	90529886	90645346	+	5248	1.829	1.425	1.838	1.574
NM_022769	CRTC3	15	90529886	90645346	+	5251	2.519	2.477	2.082	1.572

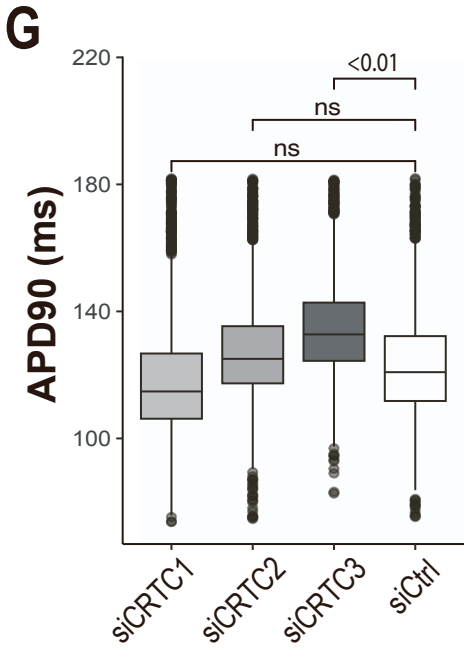


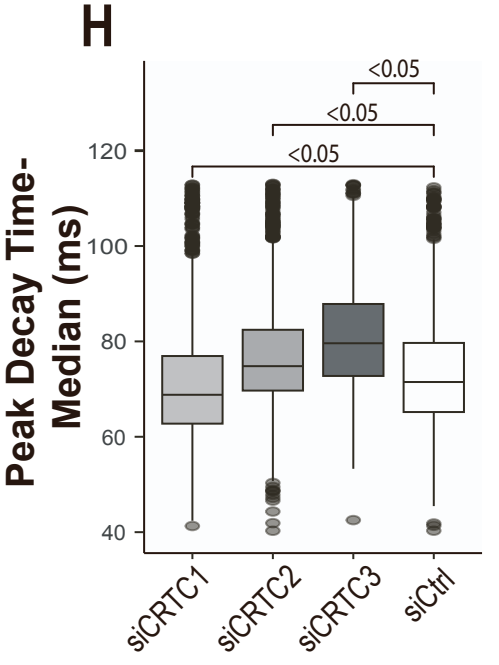


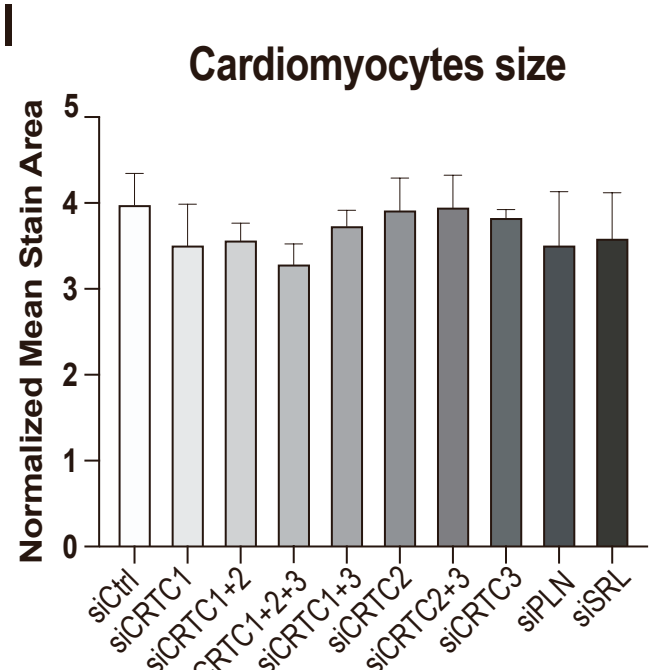












Supplemental Figure 8 – *CRTC 2 & 3* affect cardiac function in hiPSC-Cardiomyocytes (hiPSC-CM).

(A) Relative gene expression in ventricular- like (VCM1) and atrial-like cardiomyocytes (ACM1) at day 12 (early cardiac progenitors) and at day 25 (VCM-2 and ACM-2).

(B-D) Relative mRNA expression shows that siRNA against *CRTC1,2&3* efficiently KD the specific isoform in hIPSC-CMs.

(E) K-S distance plot of APD_{75} versus K-S distance shows significant increases in APD75 with KD of either *CRTC2* or *CRTC3* but not KD of *CRTC1*.

(F) Action Potential Duration (APD) was significantly increased with siRNA-mediated KD of *CRTC 3 and CRTC 2* at 50% repolarization and at (G) 90% repolarization. siRNA-mediated KD of *CRTC1* had no effect on APD. (APD50 siCtrl n=1382, siCRTC1 n=1527, siCRTC2 n=1498, siCRTC3 n=1390; APD90 siCtrl n=1333, siCRTC1 n=1466, siCRTC2 n=1421, siCRTC3 n=1299).

(H) *CRTC3* KD and *CRTC2* both caused significant increases in peak decay times (siCtrl n=1339, siCRTC1 n=1456, siCRTC2 n=1436, siCRTC3 n=1329). Significance determined by unpaired t-test, *p<0.05, **p<0.01, ****P<0.0001.

(I) Cardiomyocytes size did not change in response to *CRTC* and *Srl* KD. (siCtrl n=4, siCRTC1 n=6, siCRTC1+2 n=3, siCRTC1++3 n=5, siCRTC1+3 n=3, siCRTC2 n=2, siCRTC2+3 n=3, siCRTC3 n=3, siPLN n=4, siSRL n=4, Krustal-Wallis test)