Upregulation of Store Operated Ca^{2+} Entry pathologically impairs *Drosophila* cardiac function

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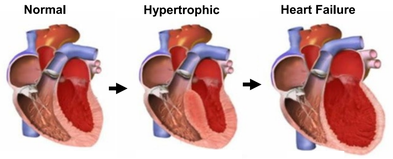
SIGNIFICANCE

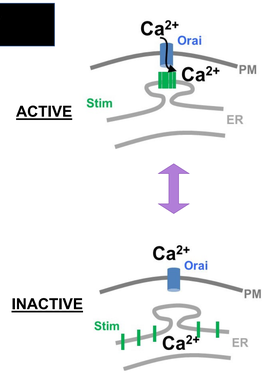
- Heart failure continues to be a leading cause of death in the western world
- Pathological cardiac hypertrophy (PCH) is a major risk factor for heart failure
  - ➢ PCH involves increases in heart muscle mass that impair cardiac function



- Upregulation of Store Operated Ca^{2+} Entry (SOCE) has been shown to induce pathological cardiac hypertrophy & heart failure

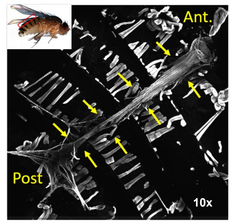
STORE OPERATED Ca^{2+} ENTRY

- SOCE is activated in response to E/SR store depletion inducing Ca^{2+} influx across the plasma membrane
  - ➢ STIM - Ca^{2+} sensor in ER lumen
  - ➢ Orai - Ca^{2+} channel on plasma membrane
- Individuals with gain-of-function mutations in *Stim* & *Orai* **do not** exhibit cardiac phenotypes
- **Goal**: to better understand how upregulated SOCE alters cardiomyocyte physiology



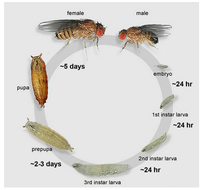
THE *DROSOPHILA* HEART

- Highly conserved developmental genetics and cardiac physiology
- Single *dStim* and *dOrai* isoforms
- Live *in vivo* imaging of the intact heart function
- *In vivo* transgene expression facilitates inducible, tissue-specific suppression



RESULTS

Drosophila Lifecycle

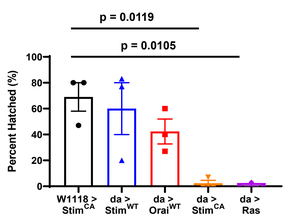


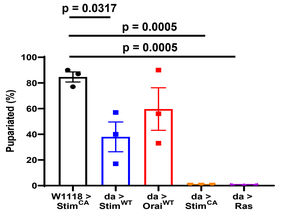
1) Ubiquitous expression of $Stim^{CA}$ induced embryonic lethality

$Stim^{CA}$ – constitutively active mutant w/ two aspartate to alanine changes in the Ca^{2+} binding EF-hand domain

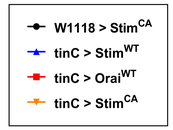
- Ubiquitous $Stim^{CA}$ & $Ras85D^{V12}$ induced embryonic lethality
  - ➢ **$Ras85D^{V12}$** (constitutively active Ras) serves a **positive control** for cardiac hypertrophy
- Ubiquitous $Stim^{WT}$ reduced pupariation by ~47%
- No effect on eclosion

n= 20-30 animals per group per biological replicate



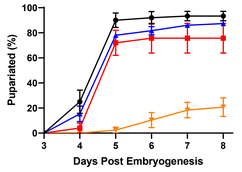


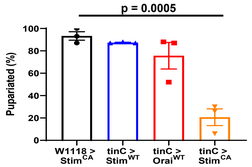
2) Heart specific expression of $Stim^{CA}$ reduced pupariation & eclosion

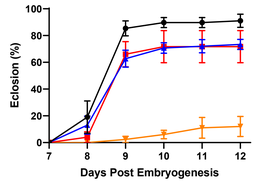


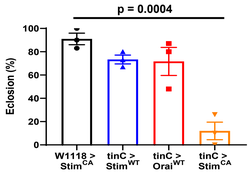
- Heart specific $Stim^{CA}$ reduces
  - ➢ pupariation by ~73%
  - ➢ eclosion by ~79%
- Heart specific expression of $Stim^{WT}$ & $Orai^{WT}$ had no significant effects on animal development

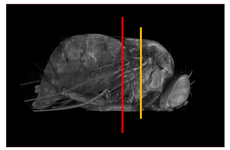
n= 30 animals per group per biological replicate







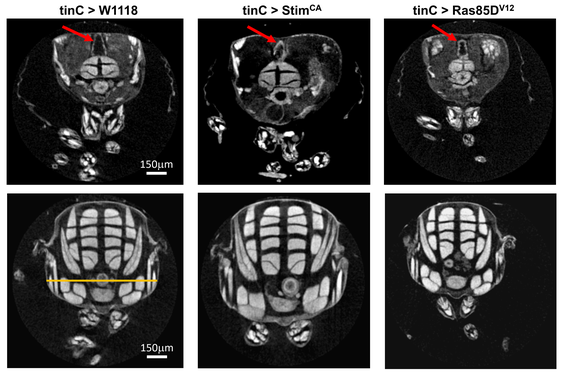




Red Line = cross section of conical chamber. Yellow line = cross section of thorax

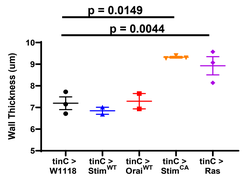
3) Heart specific expression of $Stim^{CA}$ caused cardiac hypertrophy

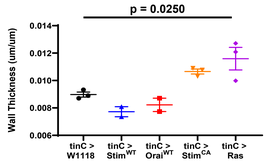
- MicroCT – micro-computed tomography
  - ➢ imaging technique using X-rays to create 3D reconstructions



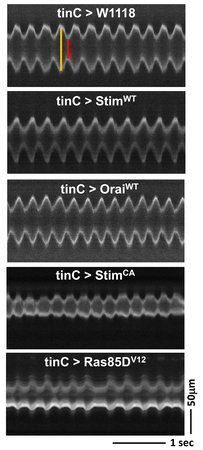
- tinC > $Ras85D^{V12}$ increased wall thickness
  - ➢ body size significantly reduced
- tinC > $Stim^{CA}$ increased wall thickness in raw measurements

Top Panel: cross section of conical chamber of tinC-GAL4 driven 7-day females; red arrow indicates heart. Lower Panel: cross section of thorax of tinC-GAL4 driven 7-day females; yellow line indicates body size measurements used to normalize heart wall thickness. Graphs indicate wall thickness raw & normalized measurements with each point representing 5 measurements from single animal

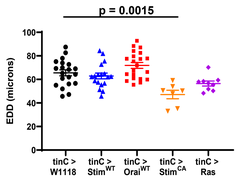


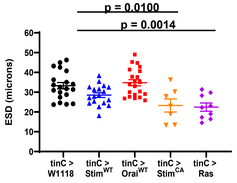


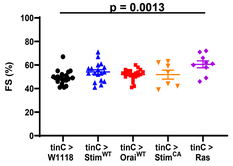
4) Heart specific expression of $Stim^{CA}$ caused hypertrophic cardiomyopathy



- Intravital Fluorescent Imaging
  - ➢ cardiomyocytes expressing RFP crossed with transgenic lines allows for fully intact, *in vivo*, imaging of cardiac function
- tinC > $Ras85D^{V12}$ reduced End Systolic Dimensions (ESD) & increased Fractional shortening (FS)
- tinC > $Stim^{CA}$ decreased End Systolic Dimensions (ESD) & End Diastolic Dimensions (EDD) with no effect on Fractional Shortening (FS)
- No change in Heart Rate (HR)







M-modes obtained from intravital fluorescent imaging of tinC-GAL4 driven Control, $Stim^{WT}$, $Orai^{WT}$, $Stim^{CA}$, or Ras as indicated. Yellow lines show EDD, Red lines indicate ESD. All measurements made on ~7-day old females

CONCLUSIONS & FUTURE DIRECTIONS

Funding:

CHRP

Conclusions:

- Overexpression of *Stim* or *Orai* alone does not induce cardiac hypertrophy
- Upregulated SOCE activity pathologically impairs cardiac function
- Upregulated SOCE induces cardiac hypertrophy & hypertrophic cardiomyopathy

Future Directions

- Identify the mechanism by which SOCE Ca^{2+} alters cardiac function
- Determine how Ca^{2+} store refilling by SOCE regulates cardiomyocyte function
- Determine which genetic interactors mediate SOCE signaling in cardiac physiology