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Deletion of Siah-Interacting Protein gene in *Drosophila* causes cardiomyopathy

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Abstract

Drosophila is a useful model organism in which to study the genetics of human diseases, including recent advances in identification of the genetics of heart development and disease in the fly. To identify novel genes that cause cardiomyopathy, we performed a deficiency screen in adult *Drosophila*. Using optical coherence tomography to phenotype cardiac function in awake adult *Drosophila*, we identified *Df(1)Exel6240* as having cardiomyopathy. Using a number of strategies including customized smaller deletions, screening of mutant alleles, and transgenic rescue, we identified CG3226 as the causative gene for this deficiency. CG3226 is an uncharacterized gene in *Drosophila* possessing homology to the mammalian Siah-interacting-protein (SIP) gene. Mammalian SIP functions as an adaptor protein involved in one of the β-catenin degradation complexes. To investigate the effects of altering β-catenin/Armadillo signaling in the adult fly, we measured heart function in flies expressing either constitutively active Armadillo or transgenic constructs that block Armadillo signaling, specifically in the heart. While increasing Armadillo signaling in the heart did not have an effect on adult heart function, decreasing Armadillo signaling in the fly heart caused the significant reduction in heart chamber size. In summary, we show that deletion of CG3226, which has homology to mammalian SIP, causes cardiomyopathy in adult *Drosophila*. Alterations in Armadillo signaling during development lead to important changes in the size and function of the adult heart.

Keywords

Drosophila melanogaster; Cardiomyopathy; Siah interacting protein (SIP); Armadillo/β catenin

Introduction

Mammalian Siah-interacting protein (SIP) is an adapter protein that binds E3 ligase Siah, along with Skp1 and Ebi, to form a complex that ubiquitinates β-catenin and regulates β-catenin signaling by its degradation (Matsuzawa and Reed 2001). This complex can be regulated by activated p53, providing a link between β-catenin down-regulation and activated p53. SIP knockout mice display impaired cell cycle checkpoint response in thymocytes as well as a decrease of β-catenin degradation downstream of DNA damage induced p53 activation (Fukushima et al. 2006). SIP is developmentally up regulated in rat

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neonatal cardiomyocytes and SIP levels correlate with both an increase in cardiomyogenic differentiation and a decrease in β-catenin protein levels (Au et al. 2006). The SIP complex has been implicated in cellular processes in a variety of tissues and tumors, however the exact role of SIP in signaling pathways remains unclear (Schneider and Filipek 2011). The mammalian E3 ligase Siah (seven in absentia homolog), was originally identified as an orthologue of *Drosophila* E3 ligase Sina (seven in absentia). *Drosophila* Sina is important for its role in eye neural development, and the only known ubiquitination substrate of *Drosophila* Sina is the transcriptional regulator Tramtrack (Li et al. 1997). The *Drosophila* gene orthologous to SIP has not been characterized in the Sina complex nor in the *Drosophila* Armadillo pathway (β-catenin orthologue) (Table 1).

The mammalian SIP/Siah complex functions to regulate β-catenin levels in parallel to the Wnt pathway. Wnt signaling acts to inhibit the axin/GSK-3/APC complex, resulting in the translocation of β-catenin to the nucleus to bind Tcf and activate transcription (Stadeli et al. 2006) (Table 1). β-catenin signaling is a well characterized signaling pathway that is conserved from *Drosophila* to human, with the stability of β-catenin being the integral switch for signal transduction. In *Drosophila* the β-catenin orthologue Armadillo has been extensively characterized within Wnt signaling in the fly. Armadillo is phosophorylated and degraded by the same Wnt dependent context via proteins with close homology throughout the pathway. Whereas the axin/GSK-3/APC complex is responsible for phophorylation of β-catenin resulting in its subsequent ubiquitination and degradation, the SIP complex promotes the direct ubiquitination of β-catenin without phosphorylation (Matsuzawa and Reed 2001).

The fruit fly, Drosophila melanogaster, has many advantages to perform forward genetic screens, including short breeding times, establishment of large scale genetic tools to quickly characterize disruption of genes, and transgenic systems to allow expression of genes of interest in very specific manners. These advantages have propelled the fruit fly to become a useful tool for the study of genes involved in human disease, including in the field of cardiovascular research (Wolf and Rockman 2011).

To identify novel genes that cause cardiomyopathy, we carried out a deletion screen using optical coherence tomography (OCT) to phenotype adult heart function (Wolf et al. 2006). Here we found disruption of the gene CG3226, orthologue of mammalian Siah-Interacting protein, to cause cardiomyopathy in the fly. Since mammalian SIP regulates β-catenin levels, we investigated how modulation of Armadillo signaling would affect heart function in the adult fly.

Materials and Methods

Drosophila stocks

The following *Drosophila* stocks were obtained from the Bloomington stock center: Df(1)Exel6240, PBac{RB}Pat1^{e02477}, l(1)G0148^{G0461}/FM7c, l(1)G0148^{G0148}/FM7c, l(1)G0148^{G0149}/FM7c, y^{1} w^{1118}; P{UAS-arm.S2}A1, P{UAS-arm.S10}C, y^{1} w^{1118}, y^{1} w^{1118}; P{UAS-pan.dTCFΔ*N}4, w**; *KrIf-1/CyO; P{UAS-Axn.GFP}3/TM3, Sb*^{1}. The following insertion stocks were obtained from the Exelixis Collection at Harvard Medical School: d02291, f00281, d06059, f08054, d09974. *Ctr1A*^{25}*/FM6* stock was obtained from the Thiele lab (Turski and Thiele 2007). tinC-Gal4 driver stock was obtained from Dr. Manfred Frasch (Yin and Frasch 1998). UAS-Armadillo siRNA stock (7767) was obtained from Vienna *Drosophila* Resource Center (Dietzl et al. 2007).

Generation of custom deletions

The following transposon insertions were used for Flp based recombination using established methods (Parks et al. 2004; Thibault et al. 2004): d02291, f00281, Pat1^{e02477},

d06059, f08054, d09974. The resulting deletions: Df(1)d02291-f00281, Df(1)e02477-d06059 and Df(1)f08054-d09974 were confirmed by PCR.

Cloning, transgenesis

Genomic constructs of CG3224 and CG3226 were cloned from w^{1118} genomic DNA via PCR with the following primers (CG3226 tctaagcgacgagcagcata, tggtgcttgtggtggtttta; CG3224 acctgcgctcactggttaat, tgtcaaatcgtgaccgacat), and confirmed by sequencing. Genomic fragments were then cloned into pTarget vector. cDNA encoding CG3224 and CG3226 was generated from RNA from w^{1118} adult flies (CG3224 cgtgccaaaacaaacgatta, caatagattggttcatcctcctc CG3226 tcaactgttattcagacaaaacaaaa, tcgttttaacttaatgccctagc). The cDNA was then cloned into pUAST, and verified by sequencing. Transgenic *Drosophila* with these constructs were generated by established methods.

Optical Coherence Tomography (OCT) measurement of adult *Drosophila* cardiac function

Adult *Drosophila* cardiac function was measured in 1 week old awake female flies, using an OCT microscopy system (Bioptigen, Inc. Durham, NC) as previously described (Kim et al. 2010; Wolf et al. 2006; Yu et al. 2010). The cardiac chamber in the first abdominal segment was first visualized using B-mode (two dimensional) OCT, then recorded as M-mode (one-dimensional) line scans that represent systolic and diastolic changes in cardiac chamber size as a function of time. OCT M-modes were analyzed using ImageJ software and a 125 micron standard. Three consecutive beats were used to calculate End-diastolic dimension (EDD) and end-systolic dimension (ESD). Fractional shortening (FS) was calculated as [(EDD)-ESD]/EDD ×100.

Histology

Adult female flies of 7 to 10 days age were collected, immersed in Telly's fixative (60% ethanol, 3.3% formalin, 4% Glacial Acetic Acid) for at least 1 week. The specimens were dehydrated in ethanol through sequential gradients. Then the samples were washed twice with xylenes before immersion in liquid paraffin. After solidification, paraffin blocks were sectioned serially at 8 μm thickness in transverse orientation. Sections were rehydrated and stained with hematoxylin and eosin. The position of the heart chamber was measured as described (Yu et al. 2010). Sections were analyzed using a Leica DM2500 microscope and images captured using a Leica DFC310FX camera. Wall thickness was calculated by measuring the cardiac chamber wall width along four positions in three serial sections to obtain the mean.

QRT-PCR

Total RNA was isolated from approximately 10 female flies using RNA-Bee (Tel-Test). One microgram of RNA was treated with DNase (Invitrogen, Inc.) and used for generation of cDNA using SuperScript II reverse transcriptase (Invitrogen, Inc.). Real time PCR was performed with FastStart TaqMan Probe Master (Rox) (Roche) with the following gene assays: CG3226: primers aaacaaaatgtcattggaacagc, ggtcagcacgtcttttacgc, and Universal Probe Library #40 (Dros#47) (Roche 04687990001). RpL32: primers cggatcgatatgctaagctgt, gcgcttgttcgatccgta, and UPL #105 (Dros#10) (Roche 04692241001). CG3224: primers aaccctcgtttcttaaccaaaa, ggttgccaatggggtttta with FastStart Universal SYBR Green Master (Rox) (Roche). Reactions were amplified and analyzed in triplicate using an ABI PRISM® 7000 Sequence Detection System. Expression relative to RpL32 was calculated using $2^{-\Delta\Delta Ct}$ and levels were normalized to baseline.

Results

Deficiency *Df(1)Exel6240* causes cardiomyopathy

We initiated a screen of genomic deletions on the X chromosome from the Exelixis deficiency collection (Thibault et al. 2004) and identified *Df(1)Exel6240/FM7c* to have a phenotype of dilated cardiomyopathy (Figure 1a&b). In our screen, dilated cardiomyopathy is defined as either: an enlarged end-diastolic dimension (EDD); an enlarged end-systolic dimension (ESD); or a decreased fractional shortening (FS) compared to the control laboratory stock w^{1118} (Figure 1a&b). Since *Df(1)Exel6240/FM7c* is a heterozygous deficiency, we examined cardiac function in females crossed into a w^{1118} background to remove the effect of the X chromosome balancer. *Df(1)Exel6240*/w^{1118} females had a similar phenotype as observed in the original stock (Supplement Figure 1). The region deleted in *Df(1)Exel6240* spans approximately 125kb in the cytologic region 6B2–6C4 of the X chromosome (Figure 1c).

Identification of the causative gene within *Df(1)Exel6240*

To identify the candidate gene(s) responsible for the dilated cardiomyopathic phenotype observed in *Df(1)Exel6240*, we designed specific custom deletions using transposons containing engineered FRT-sites (Parks et al. 2004) (Figure 1c). Mutant flies with custom deletions spanning the region corresponding to the genomic deficiency of *Df(1)Exel6240* were evaluated for cardiac function by OCT. The 26kb and 51kb deletions, designated *Df(1)d02291-f00281* and *Df(1)e02477-d06059* had normal cardiac function (Figure 1a&b). However a 19kb deletion *Df(1)f08054-d09974* phenocopied the dilated cardiomyopathic phenotype as we observed in the *Df(1)Exel6240* deficiency (Figure 1a&b). These data suggest that disruption of a gene(s) within *Df(1)f08054-d09974* is responsible for the dilated cardiomyopathy observed in the larger deletion *Df(1)Exel6240*. The *Df(1)f08054-d09974* deficiency is also homozygous lethal, as it spans known lethal genes.

Since the 19kb deletion *Df(1)f08054-d09974* contains four genes, we obtained P-element insertion lines for the gene l(1)G0148, and a stock containing a deletion mutant allele of Ctr1A, $Ctr1A^{25}$ (Turski and Thiele 2007) to determine the effect of disruption of these genes on adult heart function (Figure 2). All experiments that used these mutant alleles were measured in heterozygous females, since the insertion mutations in l(1)G0148 and deletion allele of Ctr1A are homozygous lethal. *Drosophila* heterozygous for mutant $Ctr1A^{25}$ and all of the three stocks heterozygous for transposon insertions within *l(1)G0148* (G0461, G0148, G0149) had normal cardiac function (Figure 2b). These data indicate that Ctr1A and l(1)G0148 are not the causative genes for the phenotype found in the *Df(1)Exel6240* and *Df(1)f08054-d09974* deletions.

CG3226 is the candidate gene for the abnormal cardiac function of *Df(1)Exel6240*

To precisely identify the candidate gene responsible for the cardiomyopathy observed with the *Df(1)Exel6240* deficiency, we performed genetic rescue experiments of the other two genes within the smaller candidate region, CG3224 and CG3226. We designed constructs containing the genomic DNA of CG3224 and CG3226, and used them to engineer transgenic flies. Increased expression of CG3224 and CG3226 in their respective transgenic stocks was confirmed by RT-PCR (Supplement Figure 2). In contrast to the abnormal cardiac phenotype of *Df(1)Exel6240*, *Drosophila* containing the *Df(1)Exel6240* deletion and a copy of the CG3226 genomic transgene had a normal heart phenotype, indicating that additional expression of CG3226 rescued the cardiomyopathy (Figure 3). However *Drosophila* containing *Df(1)Exel6240* deficiency with one copy of the CG3224 genomic transgene continued to demonstrate abnormal cardiac function (Figure 3). Similarly, the

CG3226 genomic transgene, but not the CG3224 genomic transgene, was able to rescue heart function of the smaller 19kb deletion *Df(1)f08054-d09974* (Figure 4).

To further confirm CG3226 as the causative cardiomyopathy gene we performed heart specific rescue experiments. We cloned the cDNA of the genes CG3224 and CG3226 and produced transgenic stocks with expression of each gene under the control of a UAS promoter. *Drosophila* containing the *Df(1)Exel6240* deletion along with the heart specific tinC-Gal4 driver were crossed into transgenic stocks with UASCG3226 or UAS-CG3224. Heart specific expression of CG3226 rescued heart function in *Df(1)Exel6240*, whereas heart specific expression of CG3224 in the context of *Df(1)Exel6240* was unable to rescue heart function (Figure 5). Taken together, these results demonstrate that CG3226 is a causative gene for cardiomyopathy, and it is involved in regulating adult *Drosophila* heart function.

CG3226 and Armadillo signaling

Drosophila CG3226 is orthologous to the mammalian gene Siah-interacting-protein (SIP) (InParanoid (Ostlund et al. 2010), and Ensembl (www.ensembl.org)). Since SIP is part of a complex involved in β-catenin regulation in mammals (Matsuzawa and Reed 2001; Fukushima et al. 2006), we tested the hypothesis that Armadillo signaling (*Drosophila* β-catenin) can regulate cardiac function in the fly.

To enhance Armadillo signaling, we used two strategies to increase expression of Armadillo: 1) UAS-ArmS2 that encodes wild type Armadillo; and 2) UAS-ArmS10 that encodes a constitutively active Armadillo (Pai et al. 1997). The heart-specific overexpression of wild type Armadillo or constitutively active Armadillo did not alter cardiac function compared to controls (Figure 6a). We then tested the effects of reduced Armadillo expression using heart-specific knockdown of Armadillo using siRNA (Dietzl et al. 2007), which resulted in markedly smaller hearts with significantly reduced luminal dimensions (Figure 6b). To further clarify the effect of reducing Armadillo signaling in the *Drosophila* heart, we examined two additional transgenic stocks which result in the disruption of Armadillo signaling: $dTCF^{DN}$, a dominant negative construct of the transcriptional co-activator that is deficient for transcriptional activation with Armadillo (van de Wetering et al. 1997); and Axin-GFP that stabilizes the canonical Armadillo degradation complex resulting in enhanced Armadillo degradation and reduced Armadillo signaling (Cliffe et al. 2003). Heart-specific overexpression of these transgenes also resulted in significant reduction in heart dimensions as we observed with Armadillo knockdown (Figure 6b). End Diastolic dimension of these flies was significantly decreased compared to w^{1118} controls (Figure 6c).

We then performed histological analysis of the flies with heart specific reduced Armadillo signaling to determine what may be the cause of the small chamber size as seen by OCT. This technique allows investigation of the cardiac wall thickness and general cellular morphology, but due to artifacts caused by fixation and processing, histological analysis does not accurately reflect overall chamber size. Transverse sections of heart tissue from the heart specific knockdown of Armadillo resulted in a significant increase in heart wall thickness, which was not observed with expression of either $dTCF^{DN}$ or Axin-GFP (Figure 6d&e). We also observed accumulation of material within the lumen of the heart that occupies a large portion of the cross sectional area of the vessel chamber (Figure 6d). This was not seen in control flies, but was observed with Armadillo knockdown and Axn-GFP expression in the heart, however not in the Tcf^{DN} flies (control: 1/7flies, Arm-siRNA 5/7 flies, Axn-GFP 7/8 flies, Tcf^{DN} 0/8 flies).

Discussion

In this study we performed a deficiency screen and identified that deletion of CG3226, *Drosophila* SIP, is associated with cardiomyopathy. CG3226 is orthologous to the mammalian gene Siah-interacting protein (SIP). Since SIP is part of a complex that regulates β-catenin, we determined the effect of manipulating Armadillo signaling (*Drosophila* β-catenin) in the fly heart. We found that a heart specific decrease in Armadillo signaling resulted in decreased lumen size in the adult heart.

Armadillo and Wnt signaling in *Drosophila* are important for development, and Wnt signaling has been studied in the context of early embryonic heart development (Bryantsev and Cripps 2009; Wu 2010). Metamorphosis of the larval heart involves remodeling of differentiated myocytes into the functional adult heart (Monier et al. 2005). In a study of trans-differentiation of the heart during metamorphosis, disruption of Wnt signaling specifically during pupation was shown to cause alterations of myofibril orientation and inflow tract formation in cardiac myocytes during the transition from larval heart to adult morphology (Zeitouni et al. 2007). We also show that disruption of Armadillo signaling in the fly produces a small heart phenotype when disrupted with transgenes driven with tinC-Gal4, indicating the importance of Armadillo signaling in determining heart shape. With histological techniques we show that knock down of Armadillo resulted in a heart chamber with increased wall thickness. Whether the increase in wall thickness is due to the reduction in chamber size or an increase in size of individual cardiomyocytes is unknown. In addition, we found the accumulation of intraluminal material in the hearts of these flies. We postulate that this is fat from the surrounding tissue, however further studies will need to be performed to determine the tissue of origin of this material. Due to the technique of OCT reflecting light, it is possible that the material inside the heart could contribute to the smaller heart chamber dimension, since this would distort the lumen size as visualized by OCT.

In mice, a variety of transgenic manipulations of β-catenin have shown its importance in both cardiogenesis and adult heart phenotypes (Grigoryan et al. 2008). While a considerable amount is known about the structure of SIP and function of mammalian SIP, it is less clear whether the *Drosophila* orthologue of SIP (CG3226) has similar function. Human SIP (calcyclin-binding protein) is highly expressed in the brain and heart, as well as in many tumors (Zhai et al. 2008). SIP has been characterized in detail with regards to binding partners and important structural domains (Bhattacharya et al. 2005; Matsuzawa and Reed 2001; Filipek et al. 2008; Schneider and Filipek 2011). Overexpression of SIP is protective against hypoxia/reoxygenation injury in rat cardiomyocytes (Au et al. 2006), and SIP regulates endometrial cell apoptosis in the mouse uterus (Yang et al. 2006). SIP was shown to change subcellular localization with an increase in calcium concentration in neurons (Filipek et al. 2002), and was shown to interact with tubulin and actin in differentiating neuroblastoma cells (Schneider et al. 2007; Schneider et al. 2010), and can bind ERK1/2 and effect Elk-1 signaling (Kilanczyk et al. 2009).

Drosophila SIP was able to replace mammalian SIP to complement an interaction with mammalian Siah and Skp in yeast-three-hybrid experiments (Matsuzawa and Reed 2001), however the *Drosophila* gene has not been further characterized. Despite the well characterized mammalian SIP complex, there has not been evidence of a genetic interaction with the *Drosophila* E3 ligase Sina in a mutagenesis screen (Carthew et al. 1994), nor a physical interaction (interfly.med.harvard.edu) with members of the Sina E3 ligase complex characterized in the fly. Additionally, while the Sina complex has been studied in the context of neuronal specification, these *Drosophila* proteins have not been implicated in control of Armadillo degradation or in adult heart function. While *Drosophila* Sina has only one known substrate, mammalian Siah proteins have a variety of ubiquitination substrates,

and have been proposed to be involved with various signaling pathways (Nakayama et al. 2009; House et al. 2009). Recently, mammalian Siah E3 ligases have been shown to be involved in neuronal migration (Famulski et al. 2010) and regulation of mitochondrial fission in cardiac ischemic injury (Kim et al. 2011). Further work will be needed to precisely define the functional role of *Drosophila* SIP (CG3226).

Drosophila is a valuable model system for investigation into human disease, including cardiomyopathy. The *Drosophila* heart is sensitive to alterations in structural and contractile proteins that can alter contractility (Wolf et al. 2006; Allikian et al. 2007; Taghli-Lamallem et al. 2008), as well as signaling in pathways such as Notch, Rhomboid 3, SMAD, and insulin and ribosomal functions can change heart function in flies (Goldstein et al. 2011; Yu et al. 2010; Kim et al. 2010; Wessells et al. 2004; Casad et al. 2011). Large scale RNAi screening and proteomics studies have also identified complexes important for *Drosophila* heart function (Neely et al. 2010; Cammarato et al. 2011). Our study adds *Drosophila* SIP, CG3226, as an intriguing new gene to investigate its role in cardiomyopathy in the fly, as well as showing the importance of Armadillo signaling in the *Drosophila* heart.

In summary, we have found deletion of *Drosophila* SIP to cause cardiomyopathy in the fly. While, we show that reducing levels of Armadillo produces a small heart in adult *Drosophila*, additional studies will be needed to determine the molecular mechanism by which SIP deficiency leads to cardiomyopathy. Given the important role of SIP and armadillo signaling in Drosophila heart function, we postulate that investigation into the role of SIP in mammalian heart function could reveal new insights into human heart disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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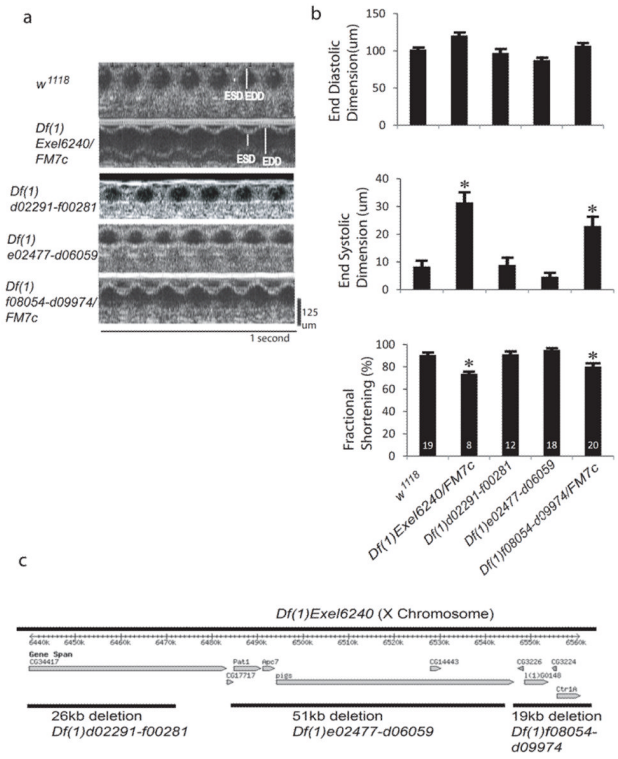

Fig. 1. Identification of a deletion on the X chromosome causing dilated cardiomyopathy

a. Representative 1 second duration M-mode recordings of *Drosophila* cardiac function reflecting the change in cardiac chamber dimension over time. Control w^{1118} and representative images of deficiency *Df(1)Exel6240*, as well as custom deletions *Df(1)d02291 -f00281, Df(1)e02477-d06059*, and *Df(1)f08054-d09974*. **b**. Summary of cardiac function for control w^{1118} and deficiencies shown in (A). Mean ± SEM for each group, n=8–20 per group show in parenthesis, *p<0.01 compared to w^{1118}, one-way ANOVA. **c**. Schematic of the *Df(1)Exel6240* deletion containing 10 genes on the X chromosome. Df(1)d02291 -f00281, Df(1)e02477-d06059, and Df(1)f08054-d09974 represents the custom recombination deletions.

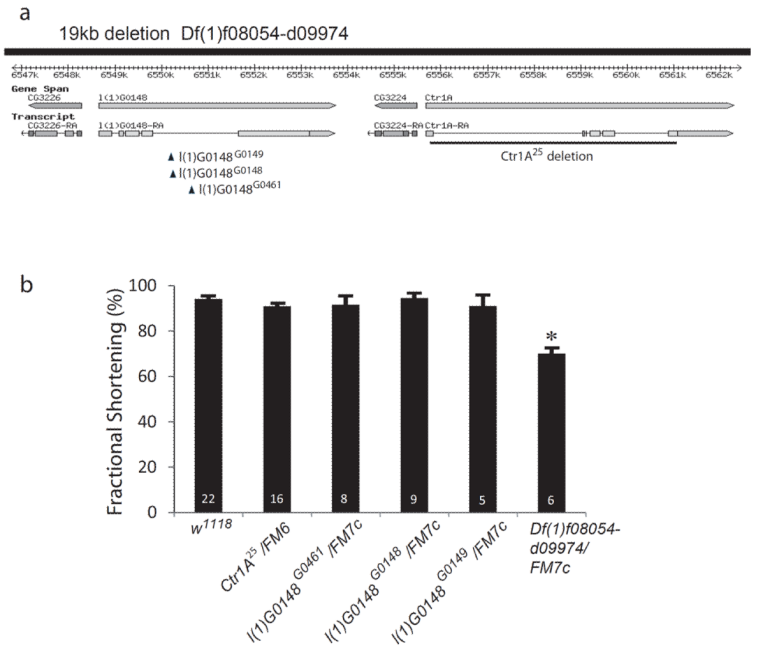

Fig. 2. Neither Ctr1A nor l(1)G0148 disruption alters cardiac function

a. Schematic of the four genes within the Df(1)f08054-d09974 deficiency. Mutants in l(1)G0148 and Ctr1A evaluated for cardiac function are delineated below each gene. **b**. Heterozygous *Ctr1A*^{25}/FM6 and flies heterozygous for alleles of l(1)G0148 do not recapitulate the dilated phenotype observed in the Df(1)f08054-d09974 deficiency. Mean ± SEM for each group, n=5–22 per group show in parenthesis, *p<0.01 compared to *w*^{1118}, one-way ANOVA.

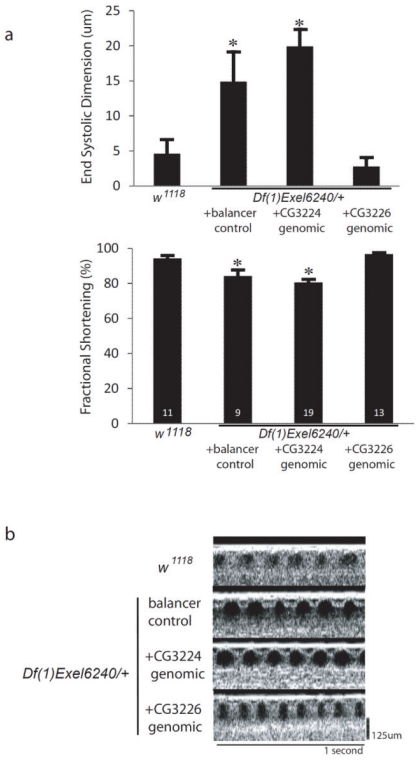

Fig. 3. Rescue of *Df(1)Exel6240* with CG3226 genomic transgene

a. Rescue of *Df(1)Exel6240* with genomic transgene for CG3226 restores ESD and fractional shortening, whereas genomic transgene for CG3224 does not.

b. Representative images of the rescue experiments described in A.

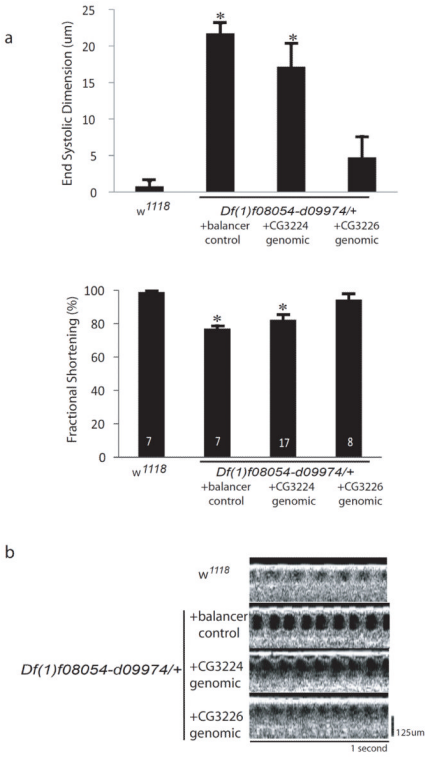

Fig. 4. Rescue of *Df(1)f08054-d09974* with CG3226 genomic transgene

a. Rescue of *Df(1)f08054-d09974* with genomic transgene for CG3226 restores ESD and fractional shortening, whereas genomic transgene for CG3224 does not.

b. Representative images of the rescue experiments described in A.

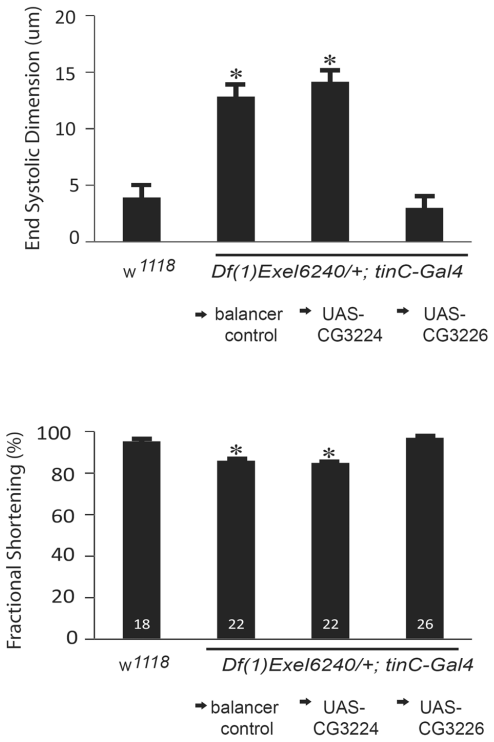

Fig. 5. Heart specific expression of CG3226 rescues heart function

tinC-Gal4 driving heart specific expression of UAS-CG3226 rescues *Df(1)Exel6240* heart function, whereas heart specific expression of UAS-CG3224 in the context of *Df(1)Exel6240* remains dilated.

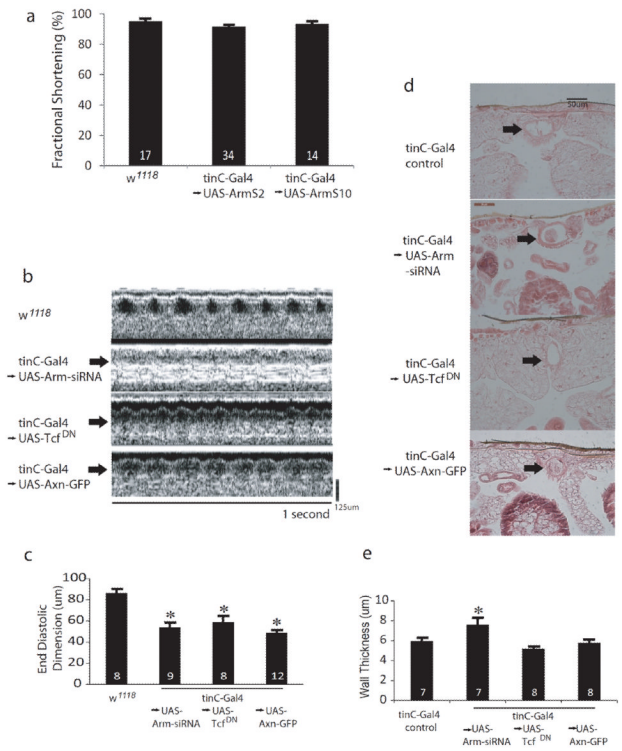

Fig. 6. Reduction of Armadillo signaling results in a small heart

a: Overexpression of wild type (S2) or constitutively active (S10) Armadillo does not phenocopy the dilated cardiac phenotype seen in *Df(1)Exel6240*. **b & c:** Reduction of Armadillo signaling in the heart by tinC-Gal4 expression of UAS-Arm siRNA, UAS-Tcf^{DN}, or UAS-AxnGFP, all result in a small heart chamber. Arrows mark the small heart chamber resulting from decreased Armadillo signaling as visualized by OCT. n=8–12 per group in parenthesis, *p<0.01 compared to w^{1118}, one-way ANOVA. **d:** Histological staining of transverse sections of *Drosophila* hearts. Arrows demarcate the hearts imaged by transverse histological section. **e:** Expression of Armadillo siRNA in the heart results in a thickened chamber wall. *p<.05 compared to control, one-way ANOVA.

Table 1

Comparison of name and functions of orthologs

Gene	Protein Function	Gene	Protein Function
Drosophila		**Mammals**	
CG3226	inferred	SIP (Siah-interacting protein)	Adapter in Siah complex
Sina (seven in absentia)	E3 ligase, neurogenic pathway	Siah (seven in absentia homolog)	E3 ligase, binds SIP
Ebi, SkpA	Skp–Cullin–F box (SCF) complex components	Ebi/Skp1	SCF complex components, bind β-catenin and SIP
Armadillo	adherens junctions, activation of wnt target genes	β-catenin	adherens junctions, activation of wnt target genes
Axn/APC/GSK3β	Canonical wnt complex for Armadillo phosphorylation and degradation	Axn/APC/GSK3β	Canonical wnt complex for β-catenin phosphorylation and degradation
Pangolin	Tcf family transcriptional regulator, binds Armadillo in the nucleus to activate transcription of wnt genes	Tcf/Lef	transcriptional regulator, binds β-catenin in the nucleus to activate transcription of wnt genes