

SPARC-Dependent Cardiomyopathy in *Drosophila*

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Abstract:

Background - The *Drosophila* heart is an important model for studying the genetics underpinning mammalian cardiac function. The system comprises contractile cardiomyocytes, adjacent to which are pairs of highly endocytic pericardial nephrocytes that modulate cardiac function by uncharacterised mechanisms. Identifying these mechanisms and the molecules involved is important because they may be relevant to human cardiac physiology.

Methods and Results - This work aimed to identify circulating cardiomodulatory factors of potential relevance to humans using the *Drosophila* nephrocyte-cardiomyocyte system. A Kruppel-Like Factor 15 (*dKlf15*) loss-of-function strategy was used to ablate nephrocytes and then heart function and the hemolymph proteome were analysed. Ablation of nephrocytes led to a severe cardiomyopathy characterised by a lengthening of diastolic interval. Rendering adult nephrocytes dysfunctional by disrupting their endocytic function or temporally-conditional knock-down of *dKlf15* led to a similar cardiomyopathy. Proteomics revealed that nephrocytes regulate the circulating levels of many secreted proteins, the most notable of which was the evolutionarily conserved matricellular protein SPARC (Secreted Protein Acidic and Rich in Cysteine), a protein involved in mammalian cardiac function. Finally, reducing *SPARC* gene dosage ameliorated the cardiomyopathy that developed in the absence of nephrocytes.

Conclusions - The data implicate SPARC in the non-cell autonomous control of cardiac function in *Drosophila* and suggest that modulation of *SPARC* gene expression may ameliorate cardiac dysfunction in humans.

Key words: cardiomyopathy; circulation; genetics, animal models; genetics, proteomics; genetics, transgenic models; proteomics, SPARC, *Drosophila*

Introduction

Heart disease is a major cause of mortality worldwide, so identifying mechanisms that regulate cardiac physiology is a crucial step towards its treatment and prevention. Both vertebrate and invertebrate models contribute to our understanding of cardiovascular physiology and the related disease processes affecting humans. The *Drosophila* heart comprises contractile cardiomyocytes and neighbouring pericardial nephrocytes that clear circulating colloids, macromolecules and immune peptides from the hemolymph (blood)¹⁻³. This organ system has proven to be a tractable model that permits genetic screens to identify novel pathways relevant to human cardiac performance⁴. In addition, functional studies have contributed to our understanding of how alterations in structural proteins, including adhesion proteins such as Fermitins / Kindlins⁵ and contractile proteins such as Myosin and Troponin-T, translate to cardiomyopathy⁶. Additionally, *Drosophila* has facilitated the elucidation of genetic pathways regulating cardiac ageing^{7,8} and diet-induced cardiac and kidney podocyte dysfunction⁹⁻¹¹.

There is evidence that cardiac phenotypes develop in the *Drosophila* model as a result of nephrocyte dysfunction, however the mechanisms are not well characterised¹²⁻¹⁵. It has also been reported that these cardiac phenotypes may depend on developmental changes to the nephrocyte-cardiomyocyte niche rather than a contribution by nephrocytes to cardiac homeostasis in adulthood¹⁶. Characterising the non-cell autonomous regulation of heart function in flies is important because it may provide insights into molecules that regulate human cardiac physiology.

The *Drosophila* ortholog of the mammalian transcription factor *Klf15* (also known as Kidney Kruppel-like Factor) has recently been identified as a nephrocyte-restricted gene critical for the cells' differentiation and function¹⁷. Pericardial nephrocytes in flies homozygous for a

dKlf15 loss of function allele develop normally during embryonic cardiogenesis but then fail to differentiate during larval development and undergo attrition before pupation, hence adult flies have no pericardial nephrocytes. This enables the nephrocytes' impact on the circulating proteome in adults to be analysed and for nephrocyte-dependent cardiomyocyte control mechanisms to be identified.

In this work we took advantage of the nephrocyte-cardiomyocyte system in *Drosophila* to identify circulating cardiomodulatory factors of potential relevance to humans. Proteomics was used to establish the composition of the circulating proteome in flies with and without nephrocytes. It was found that either the loss of nephrocytes or their function during development led to cardiomyopathy and, contrary to previous reports, loss of nephrocyte function in adulthood also led to cardiomyopathy. Analysis of the hemolymph proteome established that nephrocytes had a broad impact on the circulating secretome. By coupling the proteomics data with genetic experiments we showed that nephrocytes regulated circulating levels of the matricellular protein SPARC and prevent SPARC-dependent cardiomyopathy. SPARC plays multiple roles in mammals. SPARC levels increase in metabolic syndrome and ageing and it is well documented as contributing to pathological tissue fibrosis; however reduced *SPARC* expression can lead to heart rupture in pressure overload models¹⁸. The current findings suggest that SPARC's role in the mammalian heart may be evolutionarily conserved and that its modulation may ameliorate cardiac dysfunction. The work also highlights the importance of *Drosophila* for the identification and study of cardiomodulatory signals of relevance to human cardiac physiology.

Materials and Methods

Strains used in this study

The *w¹¹¹⁸* (used as wild-type strain in this study), *dKlf15^{NN}* (CG2932, FBgn0025679; previously known as *Bteb2^{f06447}* was described in¹⁷), *Dorothy-Gal4* (*dot-Gal4*, originally described in¹⁹, *UAS-mCherry* (TRiP control line), *dSparc^{MI00329}* (with a MiMIC insertion in the 5-prime region of the *dSparc* locus; described in²⁰) and *Tub-Gal80^{ts}* lines were all from the Bloomington Stock Centre (Bloomington, USA, IL). The *HandC-Gal4* (*Hand-Gal4*) line was described in²¹). The two RNAi lines for knocking-down *dKlf15* were from the Vienna Drosophila RNAi stock Centre (with a targeting hairpin inserted into the second chromosome, VDRC) and Bloomington (*Klf15^{JF02420}*, a Harvard TRiP line with a *dKlf15* targeting hairpin inserted into the third chromosome). All genetic combinations were generated by standard crosses. Generation of TARGET flies (*Hand-Gal4; Tub-Gal80^{ts}*) was achieved by standard crosses²².

Husbandry and propagation of flies

Flies were propagated routinely on a cornmeal-molasses diet at 25°C under a 12hr:12hr light-dark schedule. For TARGET experiments flies were reared at 18°C and then transferred to 29°C within 1-5 days of eclosing. Flies remained at 29°C for one to two weeks. Prior to analysis of heart function flies were transferred to 25°C for 24 hours. To reduce the effect of genetic background the *dKlf15^{NN}* mutant was backcrossed onto the *w¹¹¹⁸* for >20 generations.

rtPCR

See supplemental methods.

Imaging the adult heart

See supplemental methods file for more detailed description of the method (supplemental methods). Unless stated otherwise, 2-3 week old adult female flies were anaesthetised with

Flynap (Carolina Biological Supply Company, Burlington, NC, USA), dissected and hearts stained as described previously^{5, 23}.

Analysis of adult heart function

Two-three week old adult flies were anaesthetised with Flynap (Carolina Biological Supply Company, Burlington, NC, USA) and the beating adult heart in semi-intact preparations was visualised with an Ionoptix Myocam S high frame rate video camera (Ionoptix Ltd., Ireland) attached to a Zeiss AxioLab A1 with a water-dipping 10x objective. Approximately 15 seconds of video footage was collected using Micro-Manager open source microscopy software²⁴, with a frame rate capture of between 120 and 150 frames per second. Videos were converted to audio video interleave (AVI) files using ImageJ and analysed using semi-automated optical heart analysis software²⁵, www.sohasoftware.com; as previously described⁵. Quantified data are presented as the mean (+/-SEM) of at least 20 different flies per genotype.

Epifluorescence microscopy of adult fly tissues

See supplemental methods.

Collection of hemolymph from adult flies

One week old adult female flies were rendered immobile at 4°C for 5 minutes. To remove contaminating food and faeces flies were placed into a 1.5mL centrifuge tube and 500µL of 50% ice cold ethanol in water and the tube upturned several times. This step was repeated a further two times, first with 50% ethanol and then 50mM ammonium bicarbonate. Flies were then tipped onto an up-turned 30mm petri dish containing ice. The dorsal cuticle of the thorax of at least 100 flies was pricked with a 25G needle and then flies were collected into a centrifuge tube containing a 0.2 µm filter insert and 1 mL of 50 mM ammonium bicarbonate. Flies were centrifuged at 4°C for 10 seconds at 2000rpm. The filtrate was removed and replaced into the

upper filter cassette and centrifuged again. This step was repeated once more. The protein concentration of the filtrate was quantified by the Bradford assay using 50mM ammonium bicarbonate as a blank. Samples from flies that had not been pricked contained no protein. Samples contained 100 µg to 150 µg of total protein and were frozen at -20°C; volumes were adjusted with 50mM ammonium bicarbonate to normalise the total protein content between the samples. The mean spectral count of three independent biological replicates from the reference (*w¹¹¹⁸*) and *dKlf15^{NN}* mutant genotypes was used to infer protein abundance.

Proteomic and bioinformatics analysis of hemolymph proteome

Hemolymph samples were lyophilised and resuspended in 200µL with 50 mM ammonium bicarbonate. Protein reduction was done by adding 4 µL of Tris(2-carboxyethyl)phosphine (TCEP) to 200 µL of samples at 60°C for 30 min. Iodoacetamide was added (to 20 mM) and proteins were alkylated at 30 min at room temperature in the dark. Mass spectrometry grade trypsin (Promega) was added (1:20 ratio) for overnight digestion at 37°C on thermo-mixer set on 700rpm. After digestion, formic acid was added to the peptide solution (to 2%), followed by desalting by Microtrap (Michrom-Bruker) and then on-line analysis of peptides by high-resolution, high-accuracy LC-MS/MS, consisting of a Michrom HPLC, an Agilent Zorbax C18 peptide trap, a 15-cm Michrom Magic C18 column, a low-flow ADVANCED Michrom MS source, and a LTQ-Orbitrap XL (Thermo Fisher Scientific). A 120 min gradient of 10–30%B (0.1% formic acid, 100% acetonitrile) was used to separate the peptides. The total LC time was 140 min. The LTQ-Orbitrap XL was set to scan precursors in the Orbitrap followed by data-dependent MS/MS of the top 10 precursors. The LC-MSMS raw data were submitted to Sorcerer Enterprise v.3.5 release (Sage-N Research Inc.) with SEQUEST algorithm as the search program for peptide/protein identification. SEQUEST was set up to search the target-decoy Swiss-Prot

Drosophila melanogaster fasta protein database indexed with trypsin for enzyme with the allowance of up to 2 missed cleavages, Semi Tryptic search and precursor mass tolerance of 50 ppm. The search results were viewed, sorted, filtered, and statically analyzed by using comprehensive proteomics data analysis software, Peptide/Protein prophet v.4.6.1 (ISB). The minimum trans-proteomic pipeline (TPP) protein probability score was set to 0.8-0.90, to assure very low error (much less than FDR 2%) with reasonably good sensitivity. The differential spectral count analysis was done by QTools, an open source in-house developed tool for automated differential peptide/protein spectral count analysis and Gene Ontology²⁶. SignalP 4.1 was used to identify proteins having a signal peptide sequence in their N-terminus; default settings with a D cut-off of 0.45 were used²⁷.

Statistics

When more than two genotypes or treatments were used in an experiment one-way ANOVA was used to test the hypothesis that genotype may have affected heart function and post hoc test (Tukey's HSD) was used to establish *P* values between control and the different genotypes. An unpaired two-tailed student's t-Test was used to compare two means. GeneProf²⁸ was used to calculate the probability that hemolymph samples may be enriched with proteins predicted to have an N-terminal signal peptide versus a background dataset (the proportion of all known *Drosophila* genes predicted to encode for an N-terminal signal peptide).

Results

Genetic ablation of nephrocytes using *dKlf15* loss of function

It has recently been demonstrated that *dKlf15* is a nephrocyte-restricted transcription factor critical for the viability and differentiation of *Drosophila*'s two nephrocyte populations, the garland cells and pericardial nephrocytes¹⁷. In flies homozygous for a *dKlf15* loss of function

allele (*dKlf15^{NN}*) the nephrocyte populations undergo attrition during late embryogenesis (garland cells, compare Fig. 1B' and B'') and the L3 stage of larval development, so that adults are completely devoid of nephrocytes (compare Fig. 1A' and A'').

Loss of nephrocyte *dKlf15* expression leads to cardiomyopathy

It is increasingly clear that *Drosophila* heart function is modulated by non-cell autonomous mechanisms controlled by the neighbouring pericardial nephrocytes^{14, 15}. To confirm that nephrocytes modulate cardiac function in the *Drosophila* model, adult hearts in wild-type and *dKlf15^{NN}* mutants were monitored by videomicroscopy. Homozygous *dKlf15^{NN}* mutant females (and hemizygous *dKlf15^{NN}* mutant males) had significantly longer heart periods (the time between the initiation of successive cardiac contractions) compared to controls, primarily due to a significant lengthening of the diastolic interval (Fig. 2A and 2B; for data from males see below). The mutants also had a modest increase in the arrhythmicity index (AI; Fig. 2B), a measure of the heart's beat-to-beat variability. Additionally, end diastolic and end systolic diameters (EDD, ESD) were greater in mutants than in controls; however this was not associated with a change in fractional shortening (the ratio of EDD to ESD – the relative distance that the heart wall travels during a contraction). To establish if the heart phenotype was due to the specific loss of nephrocyte *dKlf15* expression, *dKlf15* was silenced specifically in nephrocytes using *dorothy-Gal4*. Knock down of *dKlf15* in nephrocytes led to a heart phenotype that was almost identical to that of the *dKlf15^{NN}* mutants; however the AI, although trending towards being increased, was not statistically different from that of the controls (Fig. 2B).

Nephrocytes mediate normal cardiac function in adults

There is doubt as to whether normal heart function in adult flies is dependent or not on sustained interactions between cardiomyocytes and nephrocytes. Sustained *dKlf15* expression is required

for adult nephrocyte function¹⁷, so to establish if nephrocytes were required for normal cardiac function in adult flies the TARGET system²² was used to silence *dKlf15* in the nephrocytes of adult flies (Fig. 3). Using this system it was possible to allow functional nephrocytes to develop normally and then silence *dKlf15* in adults to cause nephrocyte dysfunction. Accordingly, nephrocytes dedifferentiated (showed reduced Amnionless protein expression) and lost their ability to accumulate dextran. In association with this, the flies developed a cardiomyopathy which recapitulated that seen in the *dKlf15^{NN}* mutants as well as *dorothy-Gal4* driven *dKlf15* silencing experiments (Fig. 3B).

Reduction of nephrocyte *Amnionless* expression is associated with cardiomyopathy

Amnionless is crucial for nephrocyte function²⁹, so it was hypothesised that loss of *Amnionless* may be sufficient to cause cardiomyopathy. Silencing *Amnionless* in nephrocytes did not cause nephrocyte death but did impair nephrocyte endocytic function (Fig. 4A & B). Importantly, silencing *Amnionless* impacted cardiac function by increasing the heart period due to a lengthening of the diastolic interval (Fig. 4C), similar to the phenotype in *dKlf15* loss-of-function experiments.

Disruption of the hemolymph proteome in *dKlf15* loss of function flies

Given that disruption of nephrocyte endocytosis was associated with the development of cardiomyopathy it was hypothesised that nephrocytes may regulate levels of circulating, cardiomodulatory signals. We therefore examined the hemolymph proteome of control and *dKlf15^{NN}* mutants using a method similar to that used by others to identify over 700 larval hemolymph peptides³⁰. Signals corresponding to 495 different proteins were identified. Of these, 209 were identified in the hemolymph of both genotypes, 192 were identified only in the control strain and 94 were found only in *dKlf15^{NN}* mutants (Fig. 5A).

Proteins were allocated to five non-overlapping groups (see Table S1); (group 1) unique to *dKlf15^{NN}* mutants; (group 2) increased at least 2-fold in *dKlf15^{NN}* mutants; (group 3) present in both genotypes and within 0.8-2 fold different in mutants relative to controls; (group 4) reduced by more than 0.8 fold in the mutants relative to controls and (group 5) unique to controls.

It was predicted that ablation of the nephrocytes would lead to the accumulation of secreted proteins in circulation. To address this possibility, the SignalP 4.1 informatics tool was used to identify proteins predicted to have a signal peptide in their N-terminal region, a sequence associated with transport to the extracellular space²⁷. Of the 448 proteins identified in the circulation of the reference strain, 39% were predicted to contain an N-terminal signal peptide (Fig. 5B). In contrast, the hemolymph of the nephrocyte-free mutants was enriched for proteins predicted to contain a signal peptide (81%). In comparison to the total number of *Drosophila* genes predicted to encode signal peptides (3173 out of 17559 known genes, 18%) it was established that hemolymph proteome is enriched for proteins with signal peptides (P-value = 3.827e-30) and that in the absence of nephrocytes there is further enrichment for proteins with signal peptides, compared to wild type hemolymph (P-value = 7.408e-35). These data are consistent with nephrocytes having a broad impact on the circulating proteome and suggest that loss of nephrocyte function causes the accumulation in the circulation of a large subset of secreted proteins.

Of the proteins found only in the *dKlf15^{NN}* mutants (group 1), the most abundant signals were for the matricellular protein BM-40-SPARC (an ortholog of mammalian Secreted Protein Acidic and Rich in Cysteine (SPARC; Fig 5C & D and Fig 6A)³¹) and the cell adhesion protein DE-cadherin (encoded by shotgun, *shg*). Analysis of the SPARC peptide peak areas confirmed the absence of SPARC in the wild type hemolymph (supplemental figure S1). Proteins

significantly up-regulated in the hemolymph of *dKlf15^{NN}* mutants compared to wild type flies (group 2; Fig 6B) included three genes with unknown functions (CG18067, CG15293 and CG14961; up-regulated 27, 9 and 8.5-fold respectively; $P < 0.05$) as well as several proteins involved in immunity and clotting (Gelsolin, Immune induced peptides 10 and 23 and the Defence protein l(2)34F; $P < 0.05$). The immune modulatory serpin necrotic¹, trended towards a 3-fold accumulation in the mutants' hemolymph.

Of the proteins common to both genotypes and at similar levels (group 3, Fig 6C), the largest spectral counts corresponded to the lipophorin, Retinoid-and fatty acid-binding glycoprotein (*Rfabg*; spectral count of 2260 $\pm$ 785 and 2097 $\pm$ 570 for wild type and mutant hemolymph; $P = 0.88$) and the yolk proteins / vitellogenins (VIT1, 2 and 3). Proteins with large spectral counts in the wild types that were significantly down-regulated in the mutants hemolymph (group 4; Fig 6D) included several intracellular cytoskeletal and metabolic proteins (Aldolase, enolase and subunits of glyceraldehyde phosphate dehydrogenase enzyme). Finally, proteins unique to wild type hemolymph (group 5, Fig 6E) included Peroxiredoxin 5 (*Prdx5*; spectral count of 16 $\pm$ 4 in wild type and 2 $\pm$ 1 in the mutant, $P < 0.05$), Iron regulatory protein 1B (*Irp1B*; spectral count of 22 $\pm$ 6 in wild type and 4 $\pm$ 1 in mutant, $P < 0.05$) and Vacuolar H⁺ ATPase 68 kDa subunit 2A (*Vha68-2*; spectral count of 13 $\pm$ 2 in wild type, undetected in the mutant, $P < 0.05$).

A *dSparc*^{M100329} mutation corrects the cardiomyopathy in *dKlf15^{NN}* mutants

Of the proteins unique to the *dKlf15^{NN}* mutants' hemolymph SPARC was notable because of its role in collagen deposition and several growth factor signalling pathways thought to affect cardiac function^{32, 33}. We therefore tested whether *SPARC* contributed to the observed cardiomyopathy. We obtained a recessive lethal *Drosophila SPARC* allele (*dSparc*^{M100329}) caused

by the insertion of a 7.3kb MiMIC transposon in the 5 prime untranscribed region of the *dSparc* open reading frame²⁰. Homozygous adults do not develop, however *dSparc*^{M100329} heterozygotes are viable, fertile and develop to adulthood, albeit with *dSparc* gene expression reduced by 60% (Fig. 7A). Homozygous *dKlf15*^{NN} mutant females were crossed with males carrying the *dSparc*^{M100329} allele and then the heart function of male progeny (i.e. those hemizygous for *dKlf15*^{NN} and heterozygous for the *dSparc*^{M100329} allele) was analysed. It was found that the *dKlf15*^{NN} mutant males had no nephrocytes and exhibited cardiomyopathy characterised by long diastolic intervals, similar to the phenotype of homozygous *dKlf15*^{NN} mutant females (cf Fig. 2 and 6). On the other hand, heart function parameters in *dSparc*^{M100329} heterozygotes (*w*¹¹¹⁸; *dSparc*^{M100329}) were not different from those of control *w*¹¹¹⁸ flies and specifically there was no increase in heart period/diastolic intervals as observed for hearts from hemizygous *dKlf15*^{NN} mutants (Fig. 7B). In contrast, when *Sparc* was silenced in wild-type cardiomyocytes, there was a significant impact on heart function (supplemental figure 2). Importantly, reducing *dSparc* expression rescued the abnormal heart phenotype in hemizygous *dKlf15*^{NN} mutants ((*dKlf15*^{NN}; *dSparc*^{M100329+/-}) Fig. 7B), despite these flies having no nephrocytes. The findings demonstrate that heterozygosity for *SPARC* leading to reduced gene expression has no direct impact on cardiac function in the wild type flies, whereas it ameliorates the cardiomyopathy caused by *Klf15* induced loss of nephrocytes.

Discussion

The *Drosophila* heart model represents a highly tractable genetic system with which to study mammalian cardiac physiology. Whilst at an anatomical level the links between fly nephrocytes and cardiomyocytes may not be evolutionarily conserved, the high degree of gene conservation supports the use of this model for the identification of genetic pathways underlying human heart

function. We used both proteomics and genetics to identify SPARC as an important component of cardiac function in *Drosophila*, highlighting the possibility that SPARC's role in the heart may be evolutionarily conserved from flies to humans. By using a cell-specific and temporally-conditional nephrocyte loss of function paradigm, we establish that pericardial nephrocytes sustain normal heart function in adult *Drosophila*. Importantly, it was shown that nephrocytes prevent the development of a SPARC-dependent cardiomyopathy, a finding of considerable importance because SPARC is emerging as a clinically important target for the control of tissue fibrosis in humans^{33, 34}. Collectively, these findings highlight the importance of the *Drosophila* heart model as a means of identifying and studying cardiomodulatory signals of relevance to human cardiac function and suggest that changes to SPARC in humans may contribute to cardiac dysfunction in disease and ageing.

Our data reaffirm that pericardial nephrocytes mediate non-cell autonomous mechanisms controlling *Drosophila* heart function. The findings also suggest that the changes in heart morphology and the increased arrhythmias seen in *dKlf15^{NN}* mutants were due not only to interactions between the cardiomyocytes and nephrocytes during pre-adult stages but also that adult heart rate is mediated by an on-going interaction between the cardiomyocytes and nephrocytes. Thus, it can be concluded that loss of nephrocytes or nephrocyte function both developmentally or acutely in adults, leads to cardiac dysfunction. Our data also suggest that the cardiomyopathy caused by loss of nephrocytes or nephrocyte function is linked to the nephrocytes' role in peripheral clearance.

To our knowledge, our dataset represents the first examination of the adult *Drosophila* hemolymph proteome. The most abundant protein in the hemolymph of both wild type and *dKlf15^{NN}* mutants was Retinoid-and fatty acid-binding glycoprotein (Rfabg). Rfabg is lipid

transporter found in insect hemolymph and known to be required for Hedgehog and Wingless signalling³⁵. There were also large signals for several important intracellular metabolic proteins (e.g. Aldolase, enolase and subunits of the glyceraldehyde phosphate dehydrogenase enzyme). The presence of intracellular proteins is a feature of the human plasma proteome, suggesting that intracellular proteins are a constituent of circulating fluids in animals. We also recorded an expected absence or near-absence from the adult hemolymph of the Larval Serum Proteins LSP1 α , LSP1 β , LSP1 γ and LSP2, all of which are amongst the most highly represented proteins in larval hemolymph³⁰. The LSPs are metabolised during the non-feeding third instar and pupal stages, hence our data indicate that by the 1st week of adulthood they are difficult to detect in the hemolymph.

In addition, there were proteins identified in both genotypes, which were significantly up or down-regulated in the hemolymph of *dKlf15^{NN}* mutants (Group 2). The most highly up-regulated signals were ascribed to genes with unknown functions (CG18067, CG15293 and CG14961) and proteins involved in immunity and clotting (Gelsolin, Immune induced peptides 10 and 23 and the Defence protein l(2)34F). Although not reaching statistical significance, necrotic, an immune modulatory serpin removed from circulation by nephrocytes¹, trended towards accumulating in the mutants' hemolymph of the mutants. In addition, there was a significant reduction in the mutants' hemolymph of Peroxiredoxin 5, an antioxidant that also negatively regulates the immune response³⁶. Collectively, these findings suggest that the mutant flies may have modified immune responses and this is currently under investigation.

The most abundant proteins identified only in the hemolymph of the *dKlf15^{NN}* mutants were DE-Cadherin and SPARC. DE-Cadherin mediates cell adhesion and is critical for embryonic development and is present in the medulla of the lymph gland³⁷, whereas SPARC

stabilises basal lamina by interacting with collagen IV, an interaction critical for normal development^{38, 39}. In contrast, the role of SPARC in post-embryonic and adult *Drosophila* remains unclear. Mammalian SPARC directly binds to collagen as well as growth factors (reviewed in⁴⁰), and is associated with a diverse range of pathologies including the maintenance of cardiac integrity⁴¹ and metabolic syndrome⁴². Our data indicate that a SPARC-dependent cardiomyopathy is prevented in the *Drosophila* model via a nephrocyte-mediated clearance mechanism. Peripheral clearance of macromolecules is fundamentally important to tissue homeostasis but difficult to study in mammals. Although few studies exist, it is interesting to note that disruption of peripheral clearance by liver sinusoidal cells in stabilin-1 and stabilin-2 knock-out mice led to local and systemic tissue fibrosis, albeit without an increase in circulating SPARC levels being detected⁴³. It remains to be verified whether the increased SPARC levels directly cause cardiomyopathy or whether it is due to other hemolymph factors that are increased in *dKlf15^{NN}* mutants that act via a SPARC-dependent pathway.

Although abnormal cardiomyocyte function in *dKlf15^{NN}* mutants could be rescued by reducing *SPARC* gene dosage, we could not confirm whether this was an effect of reduced *SPARC* expression in the cardiomyocytes, because *SPARC* knock-down in wild type cardiomyocytes led to a severe cardiomyopathy, characterised by reduced fractional shortening (supplemental figure 2). Hence, rescue of the cardiomyopathy by reducing *SPARC* gene dosage in the nephrocyte-free *dKlf15^{NN}* mutants may have been due either to a less severe reduction in *SPARC* expression in the cardiomyocytes and / or reduced *SPARC* expression in cells other than cardiomyocytes. Interestingly, moderate reductions in *ILK* expression in whole flies can extend lifespan and retard cardiac ageing, yet strong knock down in cardiomyocytes has a profound negative impact on cardiac function⁸. Thus, different phenotypes can develop in the heart as a

consequence of differing levels of gene silencing / gene dosage.

In summary, the *Drosophila* heart can develop a *SPARC*-dependent cardiomyopathy as a result of nephrocyte loss. These findings identify *Drosophila* as a highly tractable model system with which to study the important relationship between tissue homeostasis and peripheral clearance, especially as it relates to human cardiac physiology. The next step will therefore be to establish how *SPARC* contributes to cardiac function in *Drosophila* and explore whether these mechanisms are conserved and relevant to the human heart.

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Figure Legends:



Figure 1: Loss of pericardial nephrocytes and garland cells in *dKlf15^{NN}* mutants. (A) Adult control (*w¹¹¹⁸*; A') and *dKlf15^{NN}* mutant flies (A'') were dissected and the heart fixed and stained with phalloidin to visualise the heart's actin cytoskeleton and antibodies to the pericardial nephrocyte marker Amnionless (*CG11592*). All pericardial nephrocytes fail to differentiate in the mutants, undergoing attrition during late larval development so that by adulthood there are none. (B) Third instar larvae were dissected and the garland cells visualised after staining with Hoechst. In control flies (*w¹¹¹⁸*; B') garland cells (GCs) are binucleate and situated at the interface between the proventriculus (PV) and oesophagus (OE). In contrast, the garland cells fail to develop normally and are lost in the *dKlf15^{NN}* mutants (B''). Scale bar = 100 μ m.

Figure 2: Loss of nephrocytes leads to cardiomyopathy. (A) M-mode records of adult hearts. Regular contractions can be seen in wild type (*dKlf15^{+/+}*), *dKlf15^{+/NN}* heterozygote and *dot-Gal4* outcrossed once to the *w¹¹¹⁸* control line (*dot-Gal4 x w¹¹¹⁸*); whereas flies homozygous for the

dKlf15^{NN} allele, or in which *dKlf15* has been silenced in nephrocytes (*dot-Gal4; UAS-dKlf15^{RNAi}*), there is an abnormally long diastolic interval and periods of arrhythmia. **(B)** Adult heart function. EDD = end diastolic diameter; ESD = end systolic diameter; *ns* = not statistically different from *dKlf15^{+/+}* or *dot > w¹¹¹⁸* control; * = *P* < 0.01 compared to *dKlf15^{+/+}*; † = *P* < 0.01 compared to *dot > w¹¹¹⁸*; *n* = 41-45 flies per genotype.

Figure 3: Conditional loss of nephrocyte function in adults leads to cardiomyopathy. *dKlf15* was conditionally silenced in the adult fly heart using the temperature-sensitive TARGET system driven by *Hand-Gal4*. Gene silencing is prevented at 18°C but permitted at higher temperatures (29°C). Flies were reared at 18°C until they eclosed and then maintained at this temperature to prevent gene silencing or moved to the higher temperature to allow *dKlf15* silencing. The *Hand-TARGET* parent line crossed to *w¹¹¹⁸* line or *UAS-mCherry* were used as controls. **(A)** The endocytic function (ability to take-up fluorescently labelled dextran) was used to assess nephrocyte function. At the non-permissive temperature nephrocytes in all genotypes were able to accumulate dextran. When shifted to the permissive temperature the nephrocytes in control flies were still able to accumulate dextran but flies in which *dKlf15* had been silenced could not. **(B)** Quantification of heart function in adult flies reared at 18°C until eclosion and then transferred to 29°C for two weeks. The beating heart was imaged in semi-intact preparations using high frame rate videomicroscopy. *n* = 20 for each genotype. EDD = end diastolic interval; ESD = end systolic diameter. *ns* = not significantly different from Target or *w¹¹¹⁸*; * and † = *P* < 0.01 compared to Target, *w¹¹¹⁸* or Target; UAS mCherry controls, respectively.

Figure 4: Loss of Amnionless function in nephrocytes leads to cardiomyopathy. (A) *Amnionless* was silenced in nephrocytes using *dot-Gal4*. As a negative control, the parent driver line was outcrossed once to the w^{1118} line (*dot-Gal4* x w^{1118}) and offspring analysed. Micrographs show adult hearts stained with anti-Amnionless antibodies (green) and phalloidin (red). Amnionless protein was localised to nephrocytes in controls (A') whereas silencing led to reduction in its detection but not the loss of pericardial nephrocytes (A''); arrow indicates a pericardial nephrocyte); HT = Heart tube. (B) Semi-intact heart preparations were incubated with fluorescently-tagged 10kDa dextran (green) and wheat germ agglutinin (red) for 30 minutes, washed and imaged. Arrows indicate nephrocytes. Dextran accumulated in controls (B') but not in *Amnionless*-silenced nephrocytes (B''). WGA = wheat germ agglutinin. (C) Quantification of heart function in flies with *dAmnionless* silenced nephrocytes. Hearts were analysed by high frame rate videomicroscopy of semi-intact adult heart preparations. EDD = end diastolic interval; ESD = end systolic diameter; EDD/ESD = fractional shortening of the heart contraction; *ns* = not statistically different from control genotype (*dot-Gal4* x w^{1118}); * $P < 0.01$, †* < 0.05 ; $n = 18-20$ per genotype.

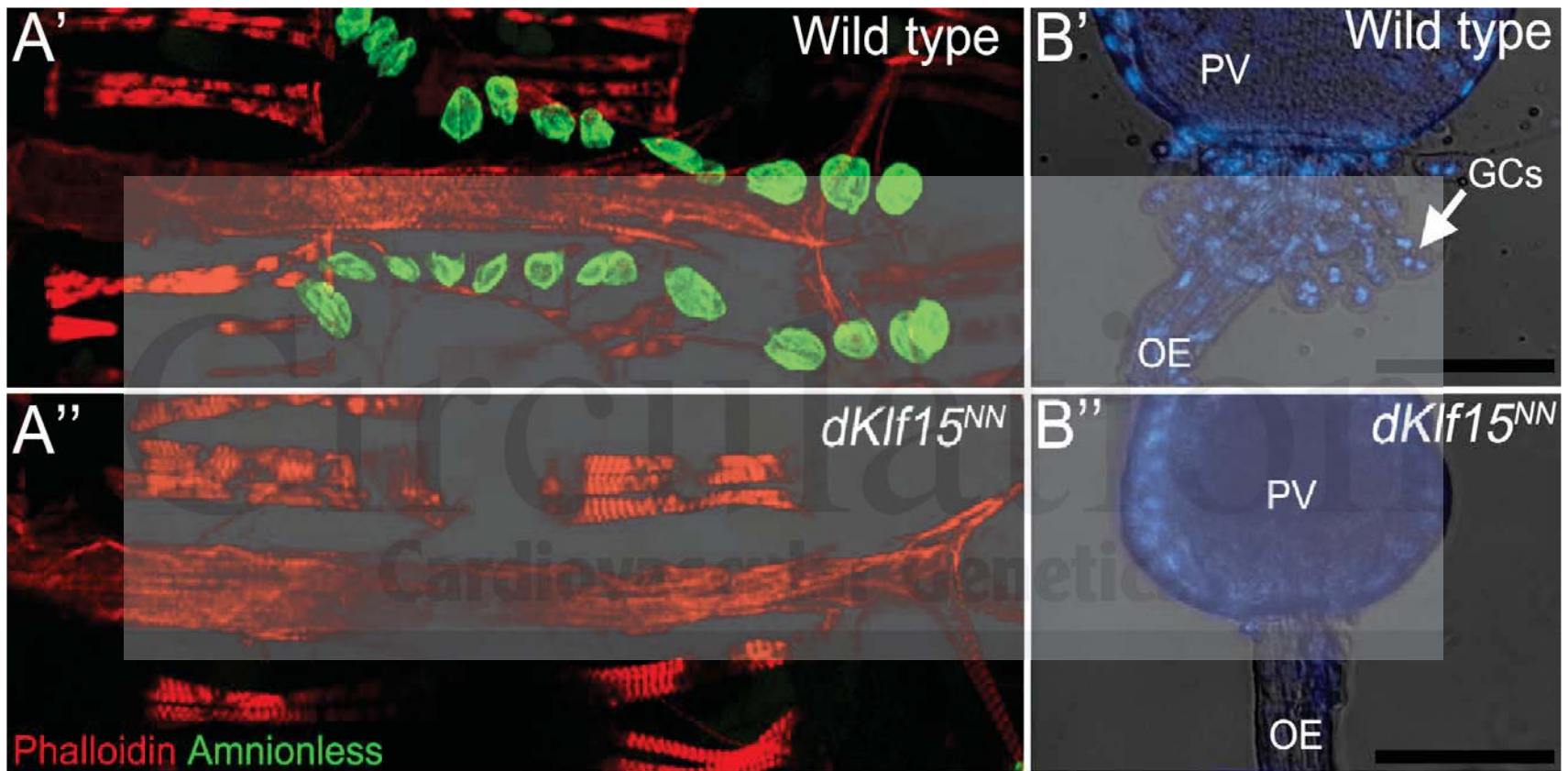
Figure 5: Proteomic analysis of adult *Drosophila* hemolymph. Hemolymph was collected from adult flies and the proteome analysed. (A) Number of different proteins identified in the hemolymph of adult control (w^{1118}) and mutant *dKlf15^{NN}* flies. (B) The number of proteins predicted to have a signal peptide by SignalP 4.1. (C) Heat map showing a truncated list of proteins identified only in the hemolymph of *dKlf15^{NN}* mutants. Proteins are rank-ordered according to spectral count; each row represents three independent samples from each genotype

(for full details see Table S1). (D) Coverage of SPARC protein sequence by detected peptides (yellow corresponds to regions detected in proteomics).

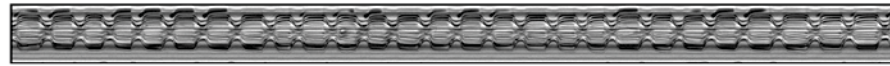
Figure 6: Mean spectral counts of hemolymph proteins. (A) Counts for two most abundant proteins found only in the *dKlf15^{NN}* mutants hemolymph. (B) Proteins showing an increase in the mutant's hemolymph. (C) The most abundant proteins in the hemolymph of wild type and *dKlf15^{NN}* mutants. (D) Proteins significantly reduced in the hemolymph of the *dKlf15^{NN}* mutants. (E) Proteins showing the largest, statistically significant, decrease in the mutant's hemolymph. *n* = 3 independent hemolymph samples from ~100 flies of each genotype; **P*<0.05, ns = no significant difference.



Figure 7: SPARC mediates cardiomyopathy in *dKlf15^{NN}* mutants. The heart function of two week old male flies of different genotypes was analysed in semi-intact fly preparations using high frame rate videomicroscopy. Quantified data for several parameters are presented. EDD = End diastolic diameter; ESD = End systolic diameter. * = different from *w¹¹¹⁸* (*P*<0.01); † = not different from *w¹¹¹⁸* (*P*>0.05); ‡ = different from *dKlf15^{NN}* (*P*<0.01); § = not different from *w¹¹¹⁸*; *dSparc^{M100329}* (*P*>0.05); ¶ = not different from *dKlf15^{NN}* (*P*>0.05); *ns* = not different from *w¹¹¹⁸* (*P*>0.05). *n* = 40-69 flies per genotype.



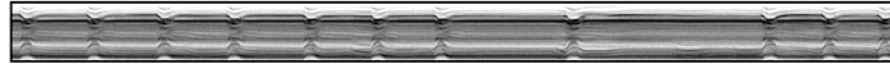
A *dKlf15*^{+/+}



dKlf15^{+/*NN*}



dKlf15^{*NN*/*NN*}



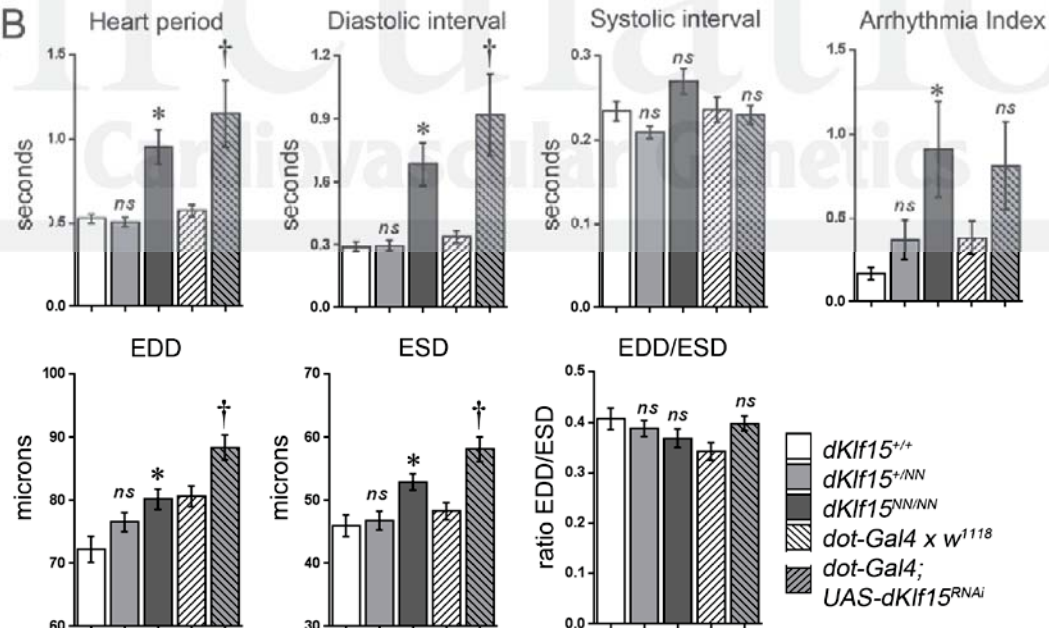
dot-Gal4 x *w*¹¹¹⁸

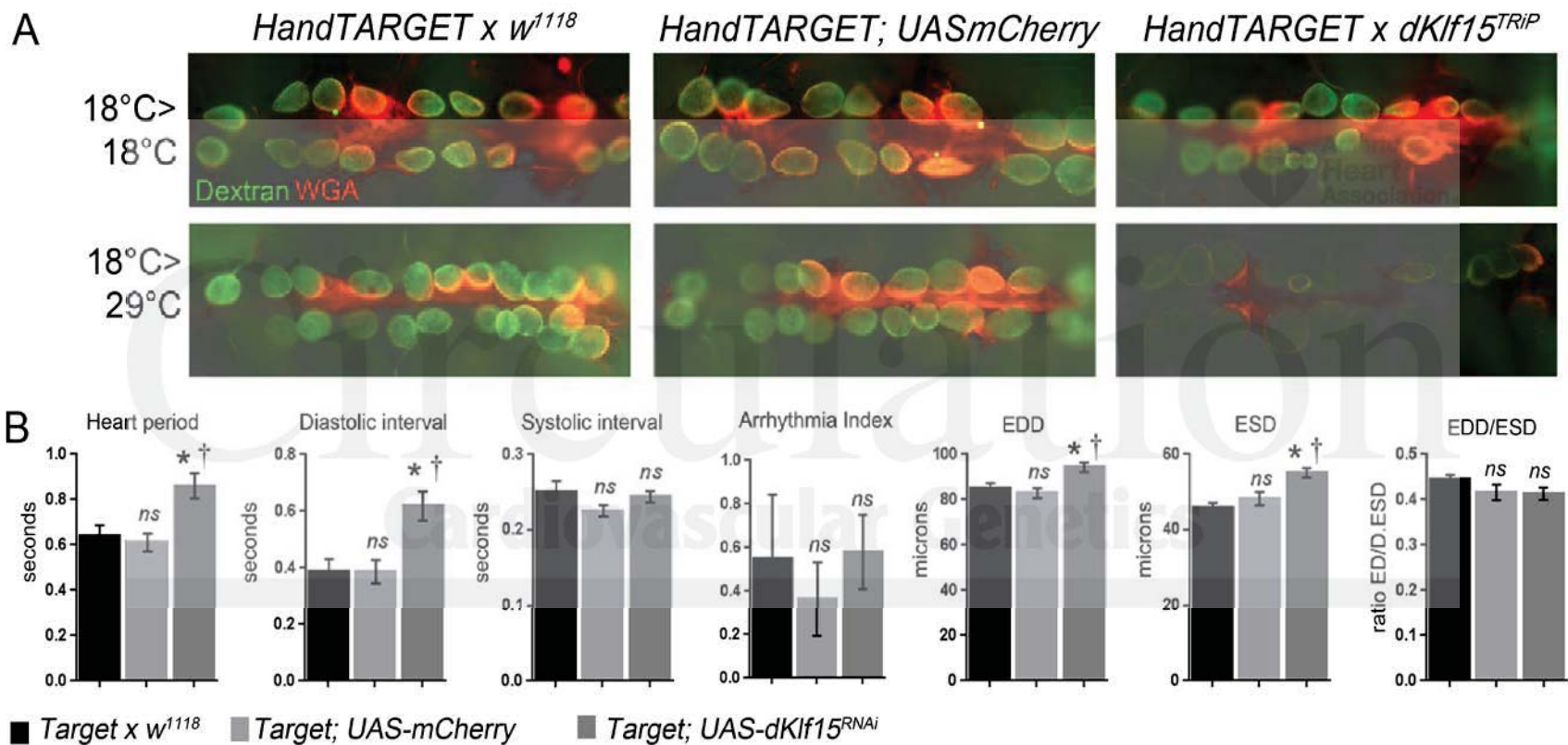


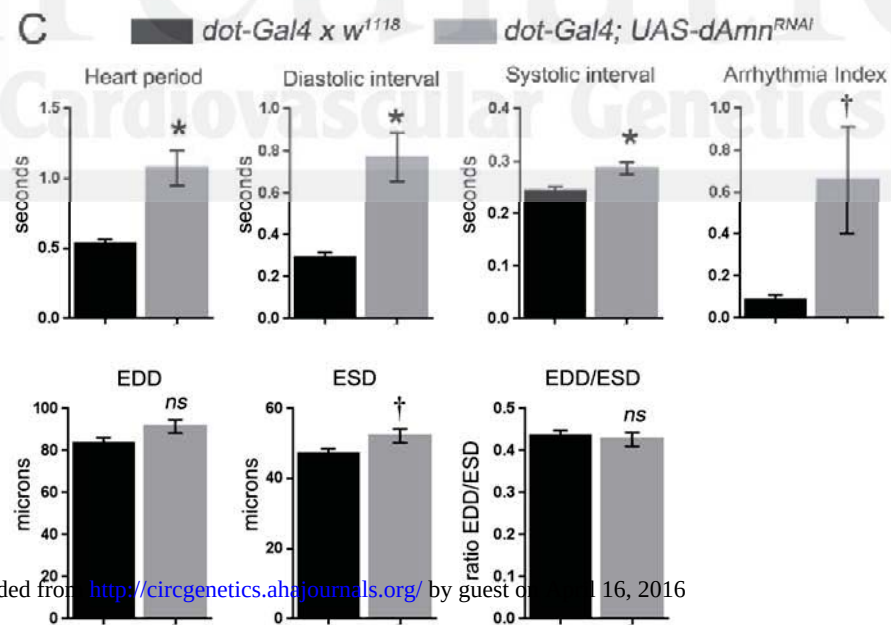
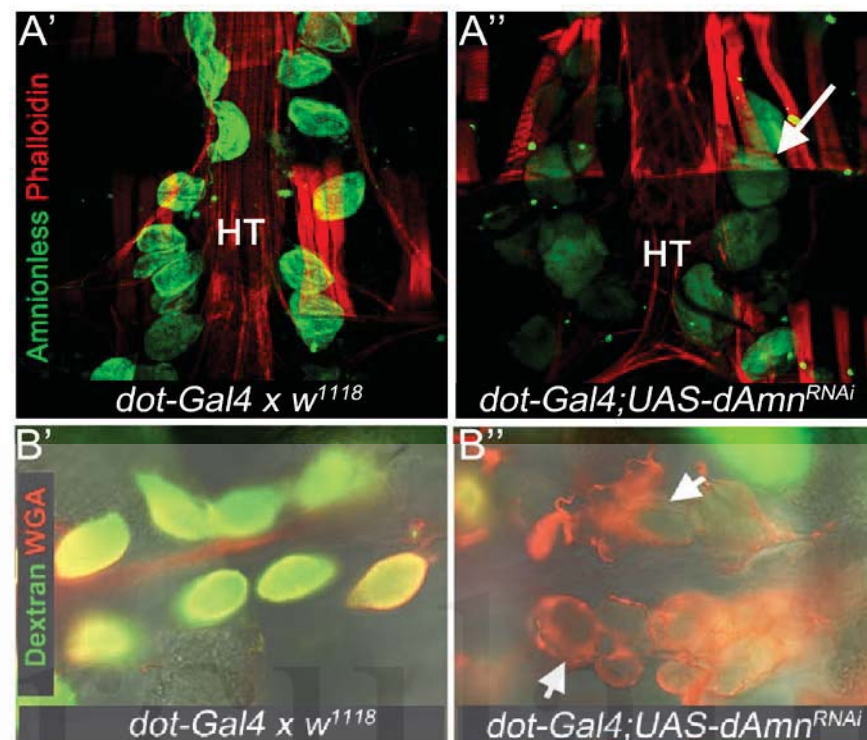
dot-Gal4; *UAS-dKlf15*^{*RNAi*}

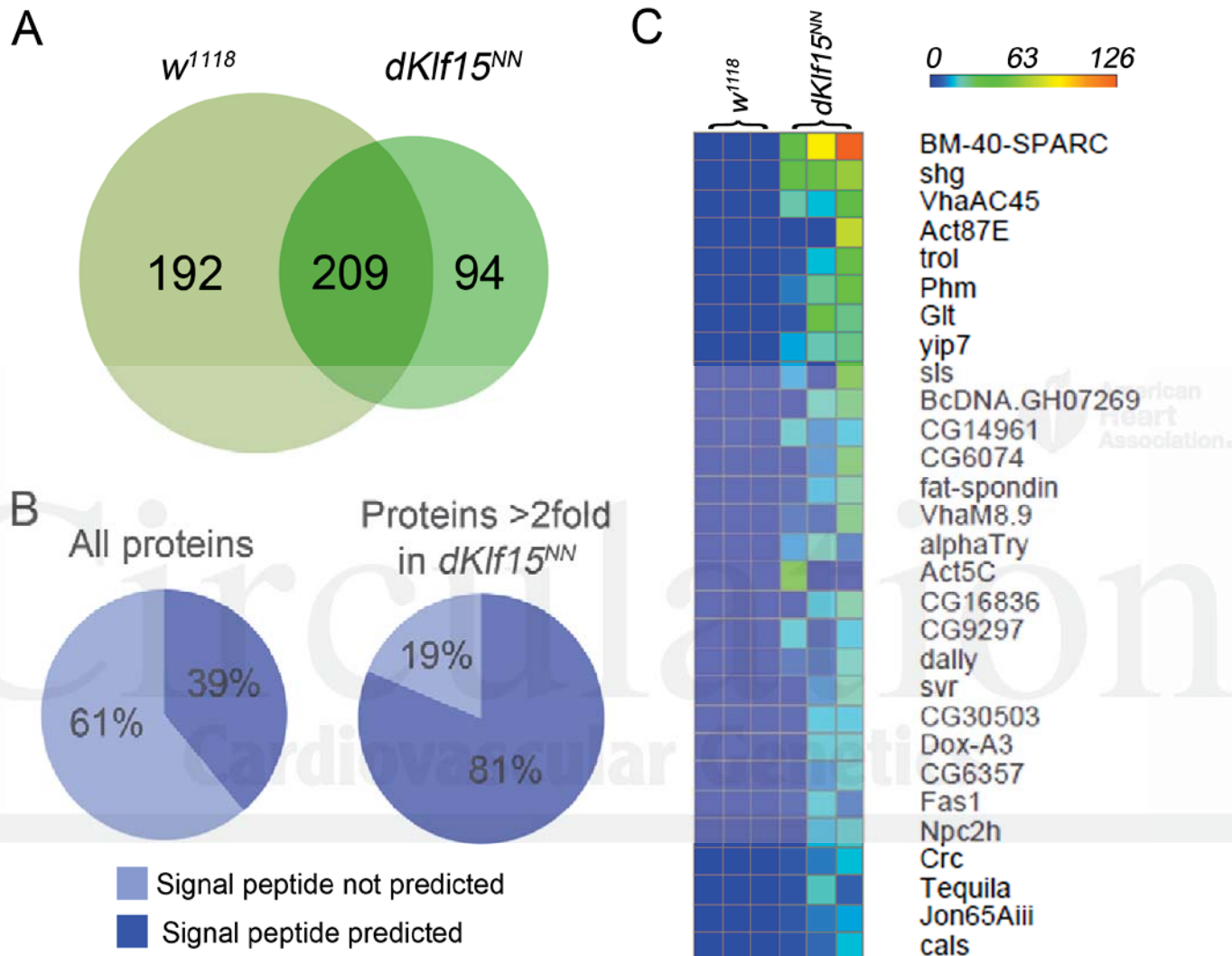


B



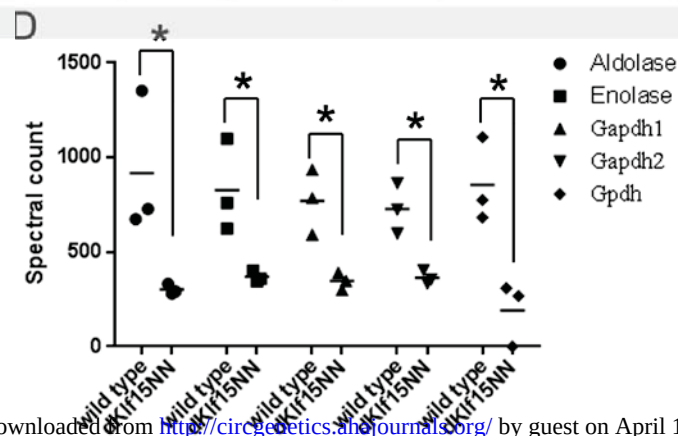
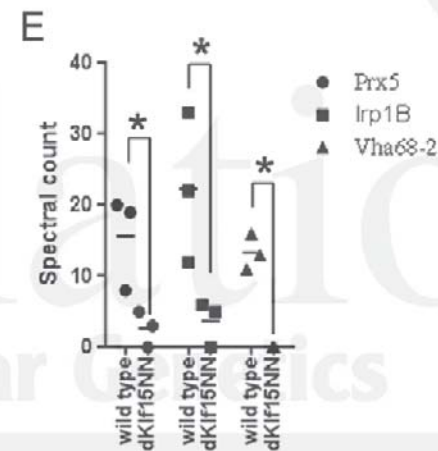
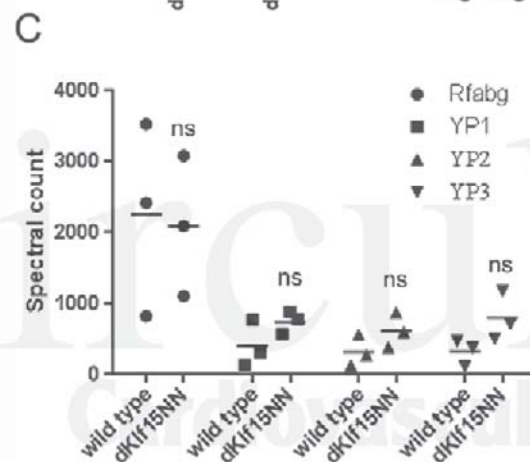
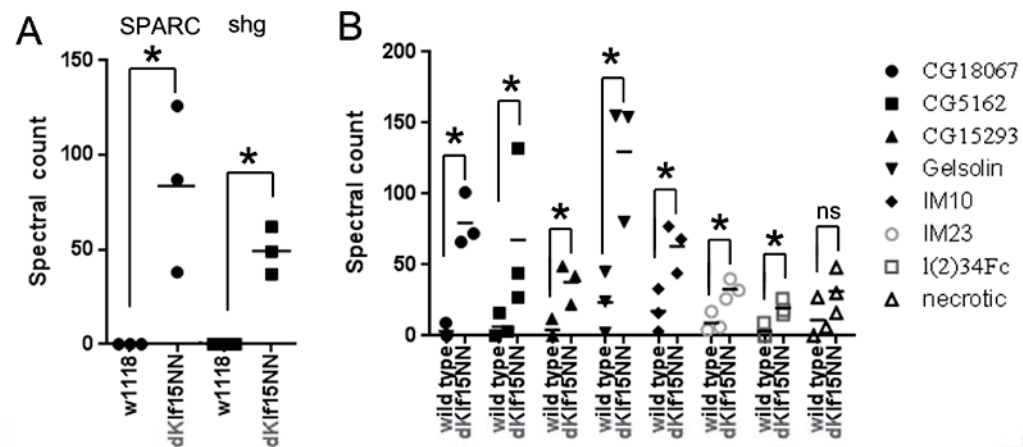


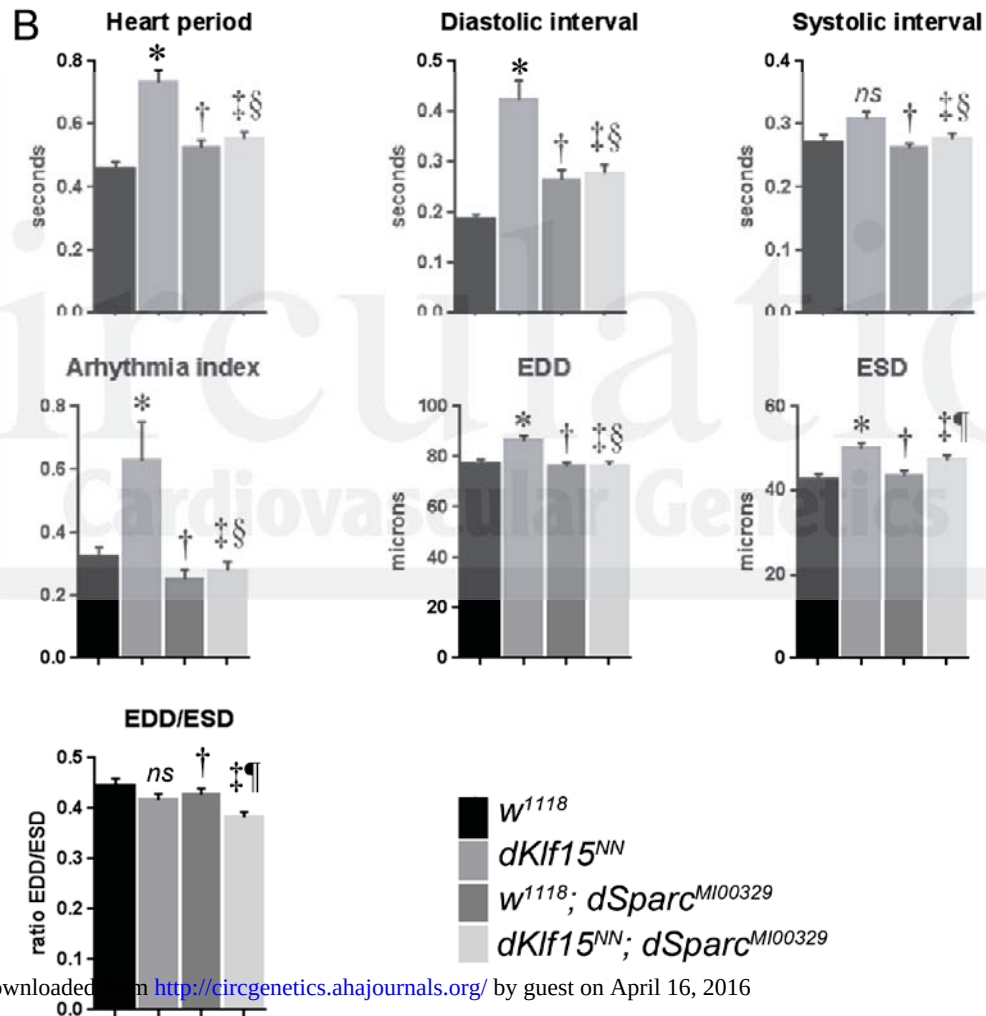
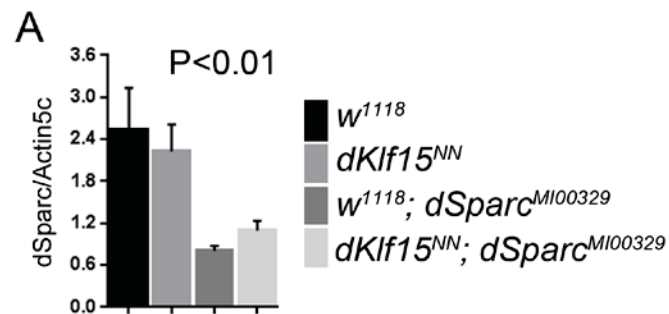




D

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L A E V Q H N L L N P V V E V D L C E T M S C G A G R I C Q M H D E K P K C V C I P E C P E E V D T R R L V C T N T N E T W P
S D C S V Y Q Q R C W C D S G E P G C T N P D N A H M H I D Y Y G A C H E P R S C E G E D L K D F P R R M R D W L F N V M
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SPARC-Dependent Cardiomyopathy in *Drosophila*

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SUPPLEMENTAL MATERIAL.

Supplemental Methods.

rtPCR

Total RNA was isolated from adult female flies using a Qiagen RNeasy Mini kit (Qiagen, Manchester, UK) according to the manufacturer's instructions. cDNA was prepared from 1 µg of total DNase-treated RNA by incubating the RNA with Oligo-dT primers at 65°C for 10 min and then placing the reaction on ice for 5 min. Second strand synthesis was performed using Roche Expand RT (Roche Products Limited, Welwyn Garden City, UK). Quantitative PCR was then performed using 10 µL reaction volumes in 384-well format in a Roche LightCycler 480. Intron-spanning primers were used to determine the relative concentration of *Drosophila SPARC* (left, CGACATCGATGAGAACGAAG; right, TCGCGCTCAATATCCTTGAT), using *Actin5C* as a reference control. Quantified data are presented at the mean (+/-SEM) of six independent samples from wild type and *SPARC*-mutant flies.

Imaging the adult heart

Adults (2-3 week old unless stated otherwise) were anaesthetised with Flynap (Carolina Biological Supply Company, Burlington, NC, USA), dissected and hearts stained as described previously^{1, 2}. For some experiments, vital dyes were used to identify functional nephrocytes or test their endocytic function (wheat germ agglutinin at 1µg / mL for 15 minutes or 50 µg / mL 10 kDa fluorescently labelled dextran for 0-30 minutes). Semi-intact preparations were then washed three times, fixed for 20 minutes with 1% formaldehyde and co-stained with antibodies (and then the relevant secondary antibodies) or Hoechst to visualise DNA and then imaged.

Epifluorescence microscopy of adult fly tissues

Semi-intact preparations were washed three times, fixed for 20 minutes with 1% formaldehyde, permeabilised with 0.1% TritonX-100 in phosphate buffered saline and co-stained with phalloidin (to visualise the actin cytoskeleton of the heart) and antibodies to the nephrocyte endocytosis protein Amnionless. To identify the

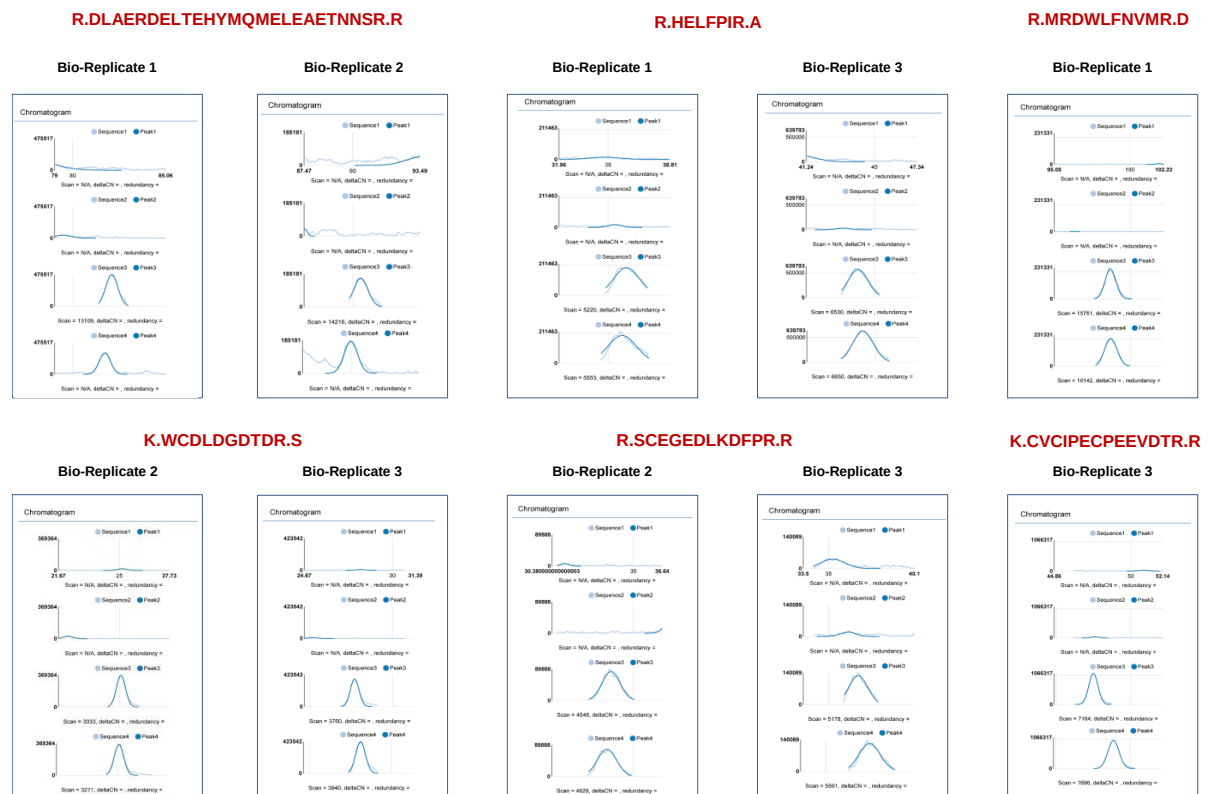
garland cells Hoechst 33342 was used to visualise DNA (garland cells having a binucleate nuclear morphology and distinct anatomical location at the interface between the oesophagus and paraventriculus). Fluorescence microscopy of flies was performed using a Zeiss LSM780 coupled to Zen image analysis software (Carl Zeiss, Welwyn Garden City, UK). Phase images were captured on a Zeiss Axiolab and images captured with an ORCA-ER CCD camera (Hamamatsu Photonics KK, Japan; Welwyn Garden City, UK) coupled to Openlab 4.1 (Improvision, Coventry, UK). Images were coloured, contrast enhanced and overlaid using Photoshop CS3. All micrographs were collected using the same microscope settings and image alterations, which were limited to contrast and brightness enhancement.

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Supplemental Figure S1.

SPARC peptide peak areas. Chromatograms for identified SPARC peptides are shown. In each panel the upper two chromatograms show peptide peaks from two technical replicates of a sample from the wild type (w^{1118}) hemolymph and the lower two chromatograms correspond to two technical replicates from a sample of the mutant ($dklf15^{NN}$) hemolymph. In each case the peptide is detected in the mutant but not the wild type hemolymph.

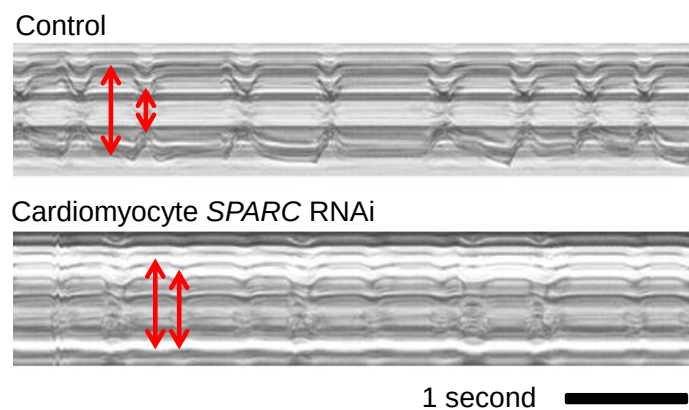


Supplemental Figure S2.

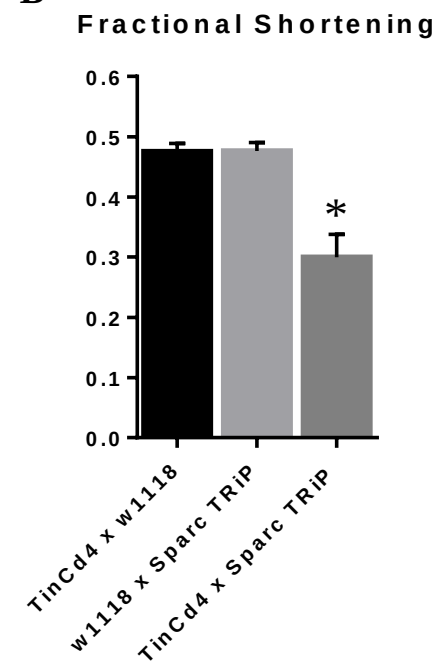
Effect of *SPARC* knock-down on heart function.

The function of the adult heart was analysed by high frame rate videomicroscopy (as described in the methods section of the main document). (A) The M-modes show the distance moved by the wall of the heart during a contraction (Fractional Shortening, red arrows). The control heart shows robust contraction between diastole to systole, whereas there is significantly less contraction when *SPARC* is silenced in the cardiomyocytes. (B) The graph shows the relative distance moved by the heart walls during diastole and systole (Fractional Shortening). The knock-down of *SPARC* in cardiomyocytes using the *TinCd4* driver (*TinCd4* x *Sparc* TRiP) had a significant impact on fractional shortening that was not seen in either of the control genotypes (*TinCd4* x *w1118* and *w1118* x *Sparc* TRiP). $n = 19$ to 29 flies per genotype. * $P < 0.001$.

A



B



Supplemental Table. Proteomics Data

Group 1:	Present in <i>dKl/f15^{NN}</i> mutant but undetected in <i>w¹¹¹⁸</i> control (a mean of two spectral counts or lower was regarded as undetected)																			
	Uniprot ID		Annotation		w1118			dKl/f15NN			w1118		dKl/f15NN							
			#1	#2	#3	#1	#2	#3	Mean	SEM	Mean	SEM	t-Test	B-H	Correction					
trj097365 O97365_DROME	O97365	BM-40-SPARC	0	0	0	38	87	126	0	0	83	26	0.031	0.160						
sp Q24298 CADE_DROME	Q24298	shg	0	0	0	37	49	62	0	0	49	7	0.002	0.093						
trjQ7JR49 Q7JR49_DROME	Q7JR49	VhaAC45	0	1	0	22	16	40	0	0	26	7	0.023	0.143						
sp P10981 ACT5_DROME	P10981	Act87E	0	0	0	0	0	72	0	0	24	24	0.374	0.386						
trjA8JUV8 A8JUV8_DROME	A8JUV8	trol	0	0	0	6	16	48	0	0	23	13	0.135	0.266						
sp O01404 PHM_DROME	O01404	Phm	0	0	0	11	25	32	0	0	23	6	0.020	0.143						
sp P33438 GLT_DROME	P33438	Glit	0	0	0	6	32	26	0	0	21	8	0.057	0.216						
trjQ9VRS4 Q9VRS4_DROME	Q9VRS4	yip7	0	1	0	14	21	26	0	0	20	4	0.005	0.093						
sp Q9I7U4 I TTITN_DROME	Q9I7U4	sls	0	0	0	14	0	38	0	0	17	11	0.197	0.327						
trjQ9VKG4 Q9VKG4_DROME	Q9VKG4	BcDNA.GH07269	0	0	0	3	20	28	0	0	17	7	0.080	0.266						
trjQ9VZQ7 Q9VZQ7_DROME	Q9VZQ7	CG14961	0	5	0	18	12	16	2	2	15	2	0.006	0.093						
trjQ9VB76 Q9VB76_DROME	Q9VB76	CG6074	0	0	0	3	12	30	0	0	15	8	0.129	0.266						
trjA1ZAP4 A1ZAP4_DROME	A1ZAP4	fat-spondin	0	0	0	6	15	24	0	0	15	5	0.042	0.178						
trjQ9VHG4 Q9VHG4_DROME	Q9VHG4	VhaM8.9	0	0	0	9	8	28	0	0	15	7	0.084	0.266						
sp P04814 I TRYA_DROME	P04814	alphaTry	0	0	0	13	21	10	0	0	15	3	0.012	0.140						
sp P10987 ACT1_DROME	P10987	Act5C	0	0	0	43	0	0	0	0	14	14	0.374	0.386						
trjA1ZB62 A1ZB62_DROME	A1ZB62	CG16836	0	0	0	0	16	24	0	0	13	7	0.132	0.266						
trjQ8I0D4 Q8I0D4_DROME	Q8I0D4	CG9297	0	0	0	17	7	16	0	0	13	3	0.016	0.143						
sp Q24114 DALY_DROME	Q24114	dalyl	0	0	0	9	9	20	0	0	13	4	0.025	0.145						
sp P42787 I CBPD_DROME	P42787	svr	0	0	0	3	12	22	0	0	12	5	0.084	0.266						
trjQ7JX94 Q7JX94_DROME	Q7JX94	CG30503	0	0	0	4	16	16	0	0	12	4	0.040	0.177						
sp Q9W1V6 PRPD3_DROME	Q9W1V6	Dox-A3	0	0	0	0	17	18	0	0	12	6	0.116	0.266						
trjA1Z9I0 A1Z9I0_DROME	A1Z9I0	CG6357	0	0	0	5	12	18	0	0	12	4	0.038	0.177						
sp P10674 I FAS1_DROME	P10674	FasI	1	0	0	6	17	10	0	0	11	3	0.027	0.148						
trjQ8IMH5 Q8IMH5_DROME	Q8IMH5	Npc2h	0	0	0	0	15	18	0	0	11	6	0.120	0.266						
sp P29413 CALR_DROME	P29413	Crc	0	0	0	6	11	16	0	0	11	3	0.023	0.143						
trjQ8IQB8 Q8IQB8_DROME	Q8IQB8	Tequila	0	0	0	5	19	8	0	0	10	4	0.067	0.240						
trjQ9VRS7 Q9VRS7_DROME	Q9VRS7	Jon65Aiii	0	0	0	6	11	14	0	0	10	2	0.014	0.143						
sp Q9V498 CSTN1_DROME	Q9V498	calcs	0	0	0	5	9	16	0	0	10	3	0.037	0.177						
sp Q9VN93 I CPR1_DROME	Q9VN93	CG12163	0	0	0	2	13	14	0	0	10	4	0.058	0.216						
trjQ9VMJ5 Q9VMJ5_DROME	Q9VMJ5	Gal	0	0	0	10	5	12	0	0	9	2	0.010	0.133						
sp O77150 IM02_DROME	O77150	IM2	0	0	0	0	0	26	0	0	9	9	0.374	0.386						
trjQ8MKJ5 Q8MKJ5_DROME	Q8MKJ5	CG30197	0	0	0	0	7	18	0	0	8	5	0.193	0.326						
trjQ8ING0 Q8ING0_DROME	Q8ING0	CG13126	0	4	0	6	19	0	1	1	8	6	0.301	0.386						
trjQ9V1Q5 Q9V1Q5_DROME	Q9V1Q5	CG10680	0	0	0	3	7	12	0	0	7	3	0.046	0.186						
trjQ7JYV3 Q7JYV3_DROME	Q7JYV3	CG12374	0	0	0	0	9	12	0	0	7	4	0.122	0.266						
trjQ8IRD6 Q8IRD6_DROME	Q8IRD6	DrsI4	0	0	2	0	5	16	1	1	7	5	0.246	0.369						
sp P13709 I FSH_DROME	P13709	fs(1)h	0	0	0	21	0	0	0	0	7	7	0.374	0.386						
trjB7Z0T0 B7Z0T0_DROME	B7Z0T0	MP1	0	4	0	0	7	14	1	1	7	4	0.262	0.381						
trjQ9VJU6 Q9VJU6_DROME	Q9VJU6	NimB5	0	0	0	0	3	18	0	0	7	6	0.287	0.386						
trjQ9VN71 Q9VN71_DROME	Q9VN71	CG14661	0	0	0	5	7	8	0	0	6	1	0.002	0.093						
trjQ8SYQ4 Q8SYQ4_DROME	Q8SYQ4	CG31997	0	3	0	6	1	12	1	1	6	3	0.168	0.298						
trjQ9VZ66 Q9VZ66_DROME	Q9VZ66	CG15209	0	0	0	0	11	8	0	0	6	3	0.124	0.266						
trjA1Z7M8 A1Z7M8_DROME	A1Z7M8	Ance-4	0	0	0	6	8	4	0	0	6	1	0.006	0.093						
sp Q95029 I CATL_DROME	Q95029	Cp1	0	0	0	0	7	10	0	0	6	3	0.132	0.266						
trjQ9VGK3 Q9VGK3_DROME	Q9VGK3	CG14715	0	0	0	3	5	8	0	0	6	1	0.017	0.143						
trjQ9VRT2 Q9VRT2_DROME	Q9VRT2	CG10472	0	0	0	0	4	12	0	0	5	4	0.205	0.334						
sp Q9VB11 UNC80_DROME	Q9VB11	CG18437	0	0	0	0	0	16	0	0	5	5	0.374	0.386						
sp Q9VW71 FAT2_DROME	Q9VW71	kug	0	0	0	0	0	16	0	0	5	5	0.374	0.386						
trjQ9VEP8 Q9VEP8_DROME	Q9VEP8	Irc	0	0	0	2	4	10	0	0	5	2	0.106	0.266						
trjQ86P15 Q86P15_DROME	Q86P15	lrl	0	0	0	5	11	0	0	0	5	3	0.170	0.298						
trjQ7JY07 Q7JY07_DROME	Q7JY07	CG9010	0	0	0	14	0	0	0	0	5	5	0.374	0.386						
trjQ9VAQ4 Q9VAQ4_DROME	Q9VAQ4	CG11841	0	5	0	1	5	8	2	2	5	2	0.347	0.386						
sp Q24485 Q24485_DROME	Q24485	RNaseX25	0	0	0	0	4	10	0	0	5	3	0.184	0.317						
sp Q9NB71 HIW_DROME	Q9NB71	hiw	0	0	0	0	0	14	0	0	5	5	0.374	0.386						
trjQ9VAY0 Q9VAY0_DROME	Q9VAY0	CG5527	0	3	0	3	3	8	1	1	5	2	0.123	0.266						
trjA8DY49 A8DY49_DROME	A8DY49	CG34215	0	0	0	0	3	10	0	0	4	3	0.231	0.358						
trjQ7JV39 Q7JV39_DROME	Q7JV39	CG11400	0	0	0	2	3	8	0	0	4	2	0.108	0.266						
trjQ9VQT6 Q9VQT6_DROME	Q9VQT6	CG3513	4	0	0	0	4	8	1	1	4	2	0.353	0.386						
trjQ7KTA1 Q7KTA1_DROME	Q7KTA1	NimB2	0	0	0	0	0	12	0	0	4	4	0.374	0.386						
trjQ9Y136 Q9Y136_DROME	Q9Y136	CG14526	0	4	0	2	9	0	1	1	4	3	0.452	0.457						
trjQ9W2F1 Q9W2F1_DROME	Q9W2F1	CG15674	0	0	0	6	4	2	0	0	4	1	0.021	0.143						
trjQ9VGE7 Q9VGE7_DROME	Q9VGE7	Ect3	0	0	0	6	0	6	0	0	4	2	0.117	0.266						
trjA1Z876 A1Z876_DROME	A1Z876	Ndg	0	0	0	5	7	0	0	0	4	2	0.127	0.266						
trjQ5BI82 Q5BI82_DROME	Q5BI82	CG13023	0	0	0	5	0	6	0	0	4	2	0.121	0.266						
trjQ9V1Q4 Q9V1Q4_DROME	Q9V1Q4	Hf	0	4	0	5	0	6	1	1	4	2	0.374	0.386						
trjQ8IHA8 Q8IHA8_DROME	Q8IHA8	CG8273	0	0	0	4	7	0	0	0	4	2	0.140	0.266						
trjQ9VFN7 Q9VFN7_DROME	Q9VFN7	Npc2b	0	0	0	2	4	4	0	0	3	1	0.003	0.093						
trjA1Z6H6 A1Z6H6_DROME	A1Z6H6	CG7791	0	0	0	0	0	10	0	0	3	3	0.374	0.386						
trjQ8MMD2 Q8MMD2_DROME	Q8MMD2	Eps-15	0	0	0	0	0	10	0	0	3	3	0.374	0.386						
trjQ9W1H6 Q9W1H6_DROME	Q9W1H6	CG5597	0	4	0	0	4	6	1	1	3	2	0.417	0.426						
trjQ7K088 Q7K088_DROME	Q7K088	Obp56e	0	3	0	10	0	0	1	1	3	3	0.525	0.525						
sp Q23995 Q23995_DROME	Q23995	tok	0	0	0	0	1	8	0	0	3	2	0.277	0.386						
sp P33450 FAT_DROME	P33450	ft	0	0	0	0	9	0	0	0	3	3	0.374	0.386						
trjA8JR58 A8JR58_DROME	A8JR58	CG5630	0	0	0	0	9	0	0	0	3	3	0.374	0.386						
sp Q9VEG6 PERC_DROME	Q9VEG6	Pxt	0	0	0	4	5	0	0	0	3	2	0.124	0.266						
trjQ95RA9 Q95RA9_DROME	Q95RA9	CG9796	0	0	0	3	0	6	0	0	3	2	0.152	0.283						
trjQ9W314 Q9W314_DROME	Q9W314	Ser7	0	0	0	2	7	0	0	0	3	2	0.242	0.369						
trjQ0E8P5 Q0E8P5_DROME	Q0E8P5	CG5758-RC	0	0	0	4	4	0	0	0	3	1	0.116	0.266						
trjQ9VV46 Q9VV46_DROME	Q9VV46	Cpr72Ec	0	0	0	0	4	4	0	0	3	1	0.116	0.266						
trjQ95SC0 Q95SC0_DROME	Q95SC0	CG9917-RA	0	0	0	0	8	0	0	0	3	3	0.374	0.386						
trjQ9VQ27 Q9VQ27_DROME	Q9VQ27	CG14352	0	0	0	0	8	0	0	0	3	3	0.374	0.386						
trjE1J159 E1J159_DROME	E1J159	siz	0	0	0	0	0	8	0	0	3	3	0.374	0.386						
trjQ77273 Q77273_DROME	Q77273	EG:66A1.2	0	0	0	0	0	8	0	0	3	3	0.374	0.386						
trjQ9VED8 Q9VED8_DROME	Q9VED8	DNaseII	0	0	0	0	0	8	0	0	3	3	0.374	0.386						
trjQ9VWT7 Q9VWT7_DROME	Q9VWT7	CG15043	0	0	0	2	5	0	0	0	3	2	0.170	0.298						
sp Q0KH33-1 Y1004_DROME	Q0KH33-1	mesh	0	0	0	0	1	6	0	0	2	2	0.250	0.369						
trjQ23984 Q23984_DROME	Q23984	Q9VMD9	tig	0	0	2	5	0	0	0	2	2	0.217	0.342						
trjQ95SI9 Q95SI9_DROME	Q95SI9	CG3868	0	0	0	2	5	0	0	0	2	2	0.217	0.342						
trjQ9XZ34 Q9XZ34_DROME	Q9XZ34	RifI	0	0	0	0	7	0	0	0	2	2	0.374	0.386						
trjQ8T4A8 Q8T4A8_DROME	Q8T4A8	CG7542	0	0	0	0	3	4	0	0	2	1	0.132	0.266						
sp Q70PY2 PGSB1_DROME	Q70PY2	PGRP-SB1	0	0	0	2	4	0	0	0	2	1	0.140	0.266						
trjA1Z8Q2 A1Z8Q2_DROME	A1Z8Q2	CG13185	0	0	0	6	0	0	0	0	2	2	0.374	0.386						

			#1	#2	#3			#1	#2	#3			Mean	SEM			Mean	SEM	Fold change	t-Test	B-H Correction
tr A1ZBU8 A1ZBU8_DROME	A1ZBU8	CG18067	0	9	0			66	101	72			3	3			80	11	25.6	0.003	0.159
tr Q8SXXG0 Q8SXXG0_DROME	Q8SXXG0	CG5162	3	16	0			27	44	132			6	5			68	32	10.7	0.135	0.247
tr Q9V3Y7 Q9V3Y7_DROME	Q9V3Y7	CG15293	0	12	0			22	49	42			4	4			38	8	9.5	0.019	0.172
sp P54192 PBP2_DROME	P54192	Pbprp2	2	12	8			22	71	86			7	3			59	19	8.0	0.057	0.183
tr Q7JYZ0 Q7JYZ0_DROME	Q7JYZ0	CG6426	0	7	0			8	17	26			2	2			17	5	7.7	0.058	0.183
tr Q7KMM4 Q7KMM4_DROME	Q7KMM4	BcDNA.GH04962	0	8	0			12	17	24			3	3			18	3	6.7	0.026	0.172
sp Q9V3Y3 IDFP_DROME	Q9V3Y3	l(2)34Fc	0	9	0			18	15	26			3	3			20	3	6.3	0.022	0.172
tr Q9W227 Q9W227_DROME	Q9W227	CG2852	5	17	10			39	52	106			11	4			66	20	6.1	0.057	0.183
sp Q07171-1 GELS_DROME	Q07171	Gel	2	45	24			80	155	154			24	12			130	25	5.4	0.019	0.172
tr Q8SYB5 Q8SYB5_DROME	Q8SYB5	Jon65Aiv	0	0	10			11	16	24			3	3			17	4	5.1	0.052	0.183
tr Q7K533 Q7K533_DROME	Q7K533	CG11395	3	23	0			22	60	46			9	7			43	11	5.0	0.063	0.183
tr Q9VH37 Q9VH37_DROME	Q9VH37	CG12811	1	5	0			4	7	20			2	2			10	5	4.5	0.201	0.266
tr Q0K100 Q0K100_DROME	Q0K100	SP1029	6	5	0			9	32	8			4	2			16	8	4.3	0.197	0.266
tr Q7K084 Q7K084_DROME	Q7K084	Obp44a	5	12	6			23	31	46			8	2			33	7	4.3	0.022	0.172
tr Q9W0J9 Q9W0J9_DROME	Q9W0J9	CG9119	1	8	0			5	13	20			3	2			13	4	4.0	0.131	0.247
sp P29746 BNB_DROME	P29746	bnb	9	16	22			30	64	90			16	4			61	17	3.9	0.064	0.183
tr Q9VJC8 Q9VJC8_DROME	Q9VJC8	SPE	0	11	0			5	19	18			4	4			14	5	3.9	0.149	0.255
sp Q9W1C9 PEB3_DROME	Q9W1C9	PebIII	2	13	18			22	45	62			11	5			43	12	3.8	0.065	0.183
tr Q8INW9 Q8INW9_DROME	Q8INW9	fon	5	21	8			32	27	68			12	5			42	13	3.7	0.092	0.212
sp Q8ML70-1 IM10_DROME	Q8ML70-1	IM10	3	33	16			44	77	68			17	9			63	10	3.6	0.026	0.172
sp Q9V8F5 IM23_DROME	Q9V8F5	IM23	4	17	6			26	40	32			9	4			33	4	3.5	0.014	0.172
sp Q06521 VTU3_DROME	Q06521	Vm34Ca	0	8	0			6	8	12			3	3			9	2	3.2	0.146	0.255
tr Q8MYW6 Q8MYW6_DROME	Q8MYW6	CG17109	0	11	0			5	13	16			4	4			11	3	3.2	0.186	0.266
tr Q9NF33 Q9NF33_DROME	Q9NF33	EG-103E12.2	7	36	6			33	60	50			16	10			48	8	2.9	0.069	0.183
tr O97355 O97355_DROME	O97355	Tsfl	82	372	88			328	605	642			181	96			525	99	2.9	0.067	0.183
tr Q7JWX3 Q7JWX3_DROME	Q7JWX3	nec	6	27	0			30	48	16			11	8			31	9	2.9	0.169	0.259
tr Q8SY60 Q8SY60_DROME	Q8SY60	CG9928	6	31	24			38	53	82			20	7			58	13	2.9	0.063	0.183
tr Q86B19 Q86B19_DROME	Q86B19	CG18135	2	39	0			23	47	42			14	13			37	7	2.7	0.177	0.261
tr Q9NFF5 Q9NFF5_DROME	Q9NFF5	Tep4	9	63	28			54	112	102			33	16			89	18	2.7	0.079	0.199
tr Q9W306 Q9W306_DROME	Q9W306	CG9691	0	8	0			4	9	8			3	3			7	2	2.7	0.226	0.289
tr A1ZAU4 A1ZAU4_DROME	A1ZAU4	CG4847-RD	0	11	0			3	15	10			4	4			9	3	2.6	0.304	0.330
tr Q8IN51 Q8IN51_DROME	Q8IN51	CG13205-RB	10	35	26			30	61	88			24	7			60	17	2.5	0.116	0.246
sp Q10714 ACE_DROME	Q10714	Ance	0	13	0			9	19	6			4	4			11	4	2.5	0.317	0.336
tr Q8SZN1 Q8SZN1_DROME	Q8SZN1	CG13113	1	17	0			11	16	20			6	6			16	3	2.5	0.196	0.266
tr A1Z7H7 A1Z7H7_DROME	A1Z7H7	CG8586-RA	0	16	0			10	17	12			5	5			13	2	2.5	0.240	0.289
sp P06607 VIT3_DROME	P06607	Yp3	122	479	380			510	719	1184			327	106			804	199	2.5	0.102	0.225
sp Q8MLZ7 IDGF3_DROME	Q8MLZ7	Idgf3	1	29	0			16	36	22			10	10			25	6	2.5	0.266	0.313
tr Q7K127 Q7K127_DROME	Q7K127	CG7997	4	24	0			11	35	24			9	7			23	7	2.5	0.240	0.289
sp Q9VU58 NPLP2_DROME	Q9VU58	Nplp2	65	328	148			226	405	694			180	78			442	136	2.5	0.171	0.259
tr A1ZB61 A1ZB61_DROME	A1ZB61	CG15067	14	21	22			28	56	56			19	3			47	9	2.4	0.047	0.183
tr Q9Y141 Q9Y141_DROME	Q9Y141	BcDNA.GH05741	1	13	0			4	16	16			5	4			12	4	2.4	0.291	0.330
tr Q9VA42 Q9VA42_DROME	Q9VA42	Npc2g	10	41	28			30	83	78			27	9			63	17	2.4	0.127	0.247
tr Q8IPH4 Q8IPH4_DROME	Q8IPH4	Tep2	7	39	4			28	37	52			17	11			39	7	2.3	0.161	0.259
sp Q86Z9-1 PPN_DROME	Q86Z9	Ppn	4	65	28			30	60	138			33	18			76	32	2.3	0.301	0.330
tr Q9VVR8 Q9VVR8_DROME	Q9VVR8	CG17108	16	13	14			50	7	44			14	1			33	13	2.3	0.230	0.289
tr Q9VJQ3 Q9VJQ3_DROME	Q9VJQ3	yellow-c	2	9	12			11	21	20			8	3			18	3	2.2	0.089	0.212
tr Q8IPB7 Q8IPB7_DROME	Q8IPB7	LM408	13	96	50			76	117	152			53	24			115	22	2.2	0.129	0.247
tr A1ZBU5 A1ZBU5_DROME	A1ZBU5	CG13422	1	5	0			2	11	0			2	2			4	3	2.1	0.557	0.568
tr Q9V109 Q9V109_DROME	Q9V109	CG17919	0	24	4			10	23	26			9	7			20	5	2.1	0.305	0.330
tr Q9VA19 Q9VA19_DROME	Q9VA19	Obp99c	31	84	36			82	115	116			50	17			104	11	2.1	0.055	0.183
sp Q9VLJ6 ACER_DROME	Q9VLJ6	Acer	4	73	12			26	72	84			30	22			61	18	2.0	0.332	0.345
tr Q0E9C3 Q0E9C3_DROME	Q0E9C3	Sod3	0	11	0			0	5	16			4	4			7	5	2.0	0.579	0.579
tr Q9VD48 Q9VD48_DROME	Q9VD48	CG5791	0	9	6			8	11	12			5	3			10	1	2.0	0.161	0.259

Group 3: In both w¹¹¹⁸ and mutant and within >0.8 and <2-fold difference

	Uniprot ID	Annotation	w1118			dK1f15NN			w1118			dK1f15NN			B-H		
			#1	#2	#3	#1	#2	#3	Mean	SEM	Mean	SEM	Fold change	t-Test	Correction		
sp Q9VA16 OB99B_DROME	Q9VA16	Obp99b	10	24	36	31	16	90	23	7	46	23	1.9	0.402	1.000		
sp P02844 VIT2_DROME	P02844	Yp2	128	565	274	385	601	876	322	129	621	142	1.9	0.195	1.000		
tr Q9VVKV2 Q9VVKV2_DROME	Q9VVKV2	CG5322	2	13	0	10	11	8	5	4	10	1	1.9	0.345	1.000		
sp P02843 VIT1_DROME	P02843	Yp1	143	775	316	578	784	886	411	188	749	91	1.8	0.181	1.000		
tr Q9VG08 Q9VG08_DROME	Q9VG08	yellow-f2	4	21	0	12	17	16	8	7	15	2	1.8	0.375	1.000		
tr Q9VME1 Q9VME1_DROME	Q9VME1	CG42369	2	16	0	3	9	20	6	5	11	5	1.8	0.534	1.000		
tr Q8SY86 Q8SY86_DROME	Q8SY86	CG16704	0	11	10	2	15	20	7	3	12	5	1.8	0.466	1.000		
tr Q7JQR3 Q7JQR3_DROME	Q7JQR3	CG4670	0	12	0	5	8	8	4	4	7	1	1.7	0.518	1.000		
tr Q86PE8 Q86PE8_DROME	Q86PE8	CG5390	7	25	8	15	25	30	14	6	24	4	1.7	0.247	1.000		
tr Q9VZ24 Q9VZ24_DROME	Q9VZ24	CG15201	0	3	6	2	5	8	3	2	5	2	1.7	0.457	1.000		
tr Q94881 Q94881_DROME	Q94881	Lectin-galC1	7	24	18	17	29	36	16	5	27	6	1.7	0.215	1.000		
tr A1Z6V5 A1Z6V5_DROME	A1Z6V5	Spm43Ab	4	40	26	19	51	44	23	11	38	10	1.6	0.360	1.000		
sp Q8IN44 TOTA_DROME	Q8IN44	TotA	4	77	16	36	55	62	32	23	51	8	1.6	0.484	1.000		
sp Q9V8Y2 OB56A_DROME	Q9V8Y2	Obp56a	2	29	8	14	19	28	13	8	20	4	1.5	0.478	1.000		
sp P08144 AMYA_DROME	P08144	Amy-p	0	53	20	11	43	54	24	16	36	13	1.5	0.598	1.000		
tr Q27598 Q27598_DROME	Q27598		1	46	109	70	132	160	82	19	121	27	1.5	0.309	1.000		
sp Q9V521 PRPA3_DROME	Q9V521	proPo-A3	50	185	102	82	173	232	112	39	162	44	1.4	0.445	1.000		
sp P23779 CYTL_DROME	P23779	Cys	27	103	102	70	107	154	77	25	110	24	1.4	0.401	1.000		
tr Q0E8C8 Q0E8C8_DROME	Q0E8C8	Spm77Ba	5	20	0	22	8	6	8	6	12	5	1.4	0.678	1.000		
tr Q7K3E2 Q7K3E2_DROME	Q7K3E2	CG5080	22	111	18	58	72	82	50	30	71	7	1.4	0.547	1.000		
sp P08171 EST6_DROME	P08171	Est-6	24	173	72	56	151	172	90	44	126	36	1.4	0.555	1.000		
tr Q2QBM1 Q2QBM1_DROME	Q2QBM1	Men	7	27	14	19	23	18	16	6	20	1	1.3	0.526	1.000		
sp P41572 PGD_DROME	P41572	Pgd	4	0	6	2	7	4	3	2	4	1	1.2	0.713	1.000		
sp P82705 IM04_DROME	P82705	IM4	19	45	22	27	47	32	29	8	35	6	1.2	0.553	1.000		
tr Q8IN43 TOTC_DROME	Q8IN43	TotC	0	47	16	12	33	30	21	14	25	7	1.2	0.795	1.000		
tr Q9VTC3 Q9VTC3_DROME	Q9VTC3	CG6409	17	128	100	29	105	156	82	33	97	37	1.2	0.778	1.000		
sp Q9VAJ4 OB99A_DROME	Q9VAJ4	Obp99a	0	21	0	3	8	14	7	7	8	3	1.2	0.876	1.000		
tr A5XCL5 A5XCL5_DROME	A5XCL5	UGP	0	0	86	39	36	26	29	29	34	4	1.2	0.870	1.000		
tr Q76NR6 Q76NR6_DROME	Q76NR6	regucalcin	44	56	58	49	60	76	53	4	62	8	1.2	0.378	1.000		
tr Q9VBJ6 Q9VBJ6_DROME	Q9VBJ6	CG14540	0	0	12	0	0	14	4	4	5	5	1.2	0.919	1.000		
sp Q9V3D4 IDGF2_DROME	Q9V3D4	Idgf2	3	57	22	14	36	46	27	16	32	10	1.2	0.823	1.000		
sp Q8SY61 OB56D_DROME	Q8SY61	Obp56d	18	105	36	37	81	62	53	27	60	13	1.1	0.826	1.000		
tr Q23997 CH47_DROME	Q23997	CG5210	48	243	88	93	164	164	126	59	140	24	1.1	0.836	1.000		
tr Q9VQT8 Q9VQT8_DROME	Q9VQT8	CG16712	49	156	98	68	97	170	101	31	112	30	1.1	0.813	1.000		
tr Q0E9F9 Q0E9F9_DROME	Q0E9F9	CG2915-RB	4	21	10	5	17	16	12	5	13	4	1.1	0.883	1.000		
tr Q7KTG2 Q7KTG2_DROME	Q7KTG2	Apoltp	0	25	0	5	11	12	8	8	9	2	1.1	0.939	1.000		
tr Q9VVP9 Q9VVP9_DROME	Q9VVP9	CG4306	0	7	0	3	4	0	2	2	2	1	1.1	0.947	1.000		
tr Q7JYH0 Q7JYH0_DROME	Q7JYH0	CG6503	17	96	40	15	29	116	51	23	54	32	1.0	0.953	1.000		
tr B7YZV3 B7YZV3_DROME	B7YZV3	Pdelc1	3	23	8	3	12	20	11	6	12	5	1.0	0.949	1.000		
sp P11449 VTU1_DROME	P11449	Vmt26Aa	4	19	12	11	7	18	12	4	12	3	1.0	0.966	1.000		
tr Q9VJN0 Q9VJN0_DROME	Q9VJN0	CG31821	0	15	0	1	8	6	5	5	5	2	1.0	0.994	1.000		
tr Q9VHK7 Q9VHK7_DROME	Q9VHK7	CG8369	0	17	10	0	13	14	9	5	9	5	1.0	1.000	1.000		

tr Q8MT58 Q8MT58_DROME	Q8MT58	CG17337	1	15	30		3	12	30		15	8		15	8		1.0	0.995	1.000
tr Q9VUC1 Q9VUC1_DROME	Q9VUC1	Hsc70Cb	4	7	12		6	4	12		7	2		7	2		1.0	0.942	1.000
sp P48375 FKB12_DROME	P48375	FK506-bp2	12	13	26		8	19	22		17	4		16	4		0.9	0.894	1.000
tr Q97102 Q97102_DROME	Q97102	smf3	3	3	4		2	3	4		3	0		3	0		0.9	0.775	1.000
tr Q7KRU8 Q7KRU8_DROME	Q7KRU8	Fer1HCH	28	156	214		26	108	236		133	55		123	61		0.9	0.917	1.000
sp Q9V496 APLP_DROME	Q9V496	Rfabg	829	3533	2418		1111	2095	3086		2260	785		2097	570		0.9	0.875	1.000
tr Q8INK2 Q8INK2_DROME	Q8INK2	fabp	11	11	16		11	5	18		13	2		12	4		0.9	0.797	1.000
tr Q9V3U6 Q9V3U6_DROME	Q9V3U6	26-29-p	4	3	8		2	5	6		5	2		4	1		0.9	0.830	1.000
tr Q9I7Q5 Q9I7Q5_DROME	Q9I7Q5	Cpr65Au	7	17	0		4	9	8		8	5		7	2		0.9	0.873	1.000
sp Q9W303 IDGF4_DROME	Q9W303	Idgf4	31	185	54		54	96	88		90	48		79	13		0.9	0.839	1.000
tr Q9VZR2 Q9VZR2_DROME	Q9VZR2	Drs15	0	5	20		0	0	22		8	6		7	7		0.9	0.912	1.000

Group 4: In both w¹¹¹⁸ and mutant but <0.8 lower in *dKlf15*^{NN} mutant

	Uniprot ID	Annotation	w1118			dKlf15NN			w1118			dKlf15NN			B-H		
			#1	#2	#3	#1	#2	#3	Mean	SEM	Mean	SEM	Fold change	t-Test	Correction		
sp Q05825 ATPB_DROME	Q05825	ATPSyn-beta	62	15	46	6	0	0	41	14	2	2	0.1	0.051	0.164		
sp P05661-17 MYSA_DROME	P05661-17	Mhc	264	47	158	42	0	0	156	63	14	14	0.1	0.092	0.193		
sp P18432 MLR_DROME	P18432	Mlc2	39	12	8	8	0	0	20	10	3	3	0.1	0.167	0.295		
tr Q960M4 Q960M4_DROME	Q960M4	Prx5	19	20	8	5	3	0	16	4	2	1	0.2	0.033	0.130		
tr O76935 O76935_DROME	O76935	Irf-1B	12	33	22	6	5	0	22	6	4	2	0.2	0.043	0.156		
sp P08736 EF1A1_DROME	P08736	Ef1alpha48D	26	29	152	9	25	0	69	41	11	7	0.2	0.242	0.396		
sp P13060-1 EF2_DROME	P13060	Ef2b	32	23	44	5	7	6	33	6	6	1	0.2	0.012	0.091		
tr Q9W330 Q9W330_DROME	Q9W330	Hex-A	100	92	30	33	7	0	74	22	13	10	0.2	0.066	0.180		
sp Q9W401-1 CISY_DROME	Q9W401	kdn	81	44	84	18	13	8	70	13	13	3	0.2	0.013	0.091		
sp P31409 VATB_DROME	P31409	Vha55	13	5	18	5	3	0	12	4	2	1	0.2	0.072	0.180		
tr Q24349 Q24349_DROME	Q24349	Gdi	6	9	14	6	0	0	10	2	2	2	0.2	0.073	0.180		
sp P13706-2 GPDA_DROME	P13706-2	Gpdh	683	1108	776	268	309	0	856	129	192	97	0.2	0.015	0.091		
sp Q24560 TBB1_DROME	Q24560	betaTub56D	36	36	22	10	12	0	31	5	7	4	0.2	0.015	0.091		
sp Q9VWH4-1 IDH3A_DROME	Q9VWH4	l(1)G0156	25	16	38	7	5	6	26	6	6	1	0.2	0.034	0.130		
sp Q9Y0Y2 PURA_DROME	Q9Y0Y2	CG17273	19	12	16	7	4	0	16	2	4	2	0.2	0.014	0.091		
tr Q8IQW5 Q8IQW5_DROME	Q8IQW5	HspB8	13	23	14	6	3	4	16	3	4	1	0.2	0.019	0.109		
sp P20478-1 GLNA2_DROME	P20478	Gis2	34	29	28	10	9	4	30	2	8	2	0.3	0.001	0.034		
sp P06603 TBA1_DROME	P06603	alphaTub84B	30	25	62	13	17	0	39	12	10	5	0.3	0.084	0.193		
sp P92177-1 I433E_DROME	P92177-1	14-3-3epsilon	10	27	28	0	11	6	21	6	6	3	0.3	0.075	0.180		
tr Q9VLS4 Q9VLS4_DROME	Q9VLS4	CG8498	15	9	0	6	0	0	8	4	2	2	0.3	0.287	0.428		
tr Q9VQB4 Q9VQB4_DROME	Q9VQB4	CG3609	8	16	6	0	4	4	10	3	3	1	0.3	0.089	0.193		
tr Q8IPA2 Q8IPA2_DROME	Q8IPA2	Mal-B2	6	27	2	0	9	0	12	8	3	3	0.3	0.365	0.482		
sp P42281 ACBP_DROME	P42281	Dbi	0	15	28	0	12	0	14	8	4	4	0.3	0.320	0.458		
tr B7Z001 B7Z001_DROME	B7Z001	CG3523	91	208	174	39	47	48	158	35	45	3	0.3	0.032	0.130		
tr Q9VU38 Q9VU38_DROME	Q9VU38	AdenoK	6	13	8	2	5	0	9	2	3	2	0.3	0.072	0.180		
sp P41043 GST1_DROME	P41043	GstS1	60	60	64	27	20	6	61	1	18	6	0.3	0.002	0.039		
sp P84029 CYC2_DROME	P84029	Cyt-c-p	12	67	12	2	20	4	30	18	9	6	0.3	0.325	0.459		
tr A8DZ21 A8DZ21_DROME	A8DZ21	Acon	72	31	96	18	15	26	66	19	20	3	0.3	0.074	0.180		
sp Q7KN62-1 TERA_DROME	Q7KN62	TER94	10	7	16	7	3	0	11	3	3	2	0.3	0.087	0.193		
tr Q9VKI8 Q9VKI8_DROME	Q9VKI8	CG6287	15	23	26	6	7	6	21	3	6	0	0.3	0.011	0.091		
sp P29310-2 I433Z_DROME	P29310-2	14-3-3zeta	0	107	78	0	20	36	62	32	19	10	0.3	0.270	0.414		
sp P52034-1 K6PF_DROME	P52034	Pfk	111	80	68	50	27	4	86	13	27	13	0.3	0.033	0.130		
sp P11147 HSP7D_DROME	P11147	Hsc70-4	20	49	86	17	15	18	52	19	16	1	0.3	0.138	0.258		
tr Q8IQ18 Q8IQ18_DROME	Q8IQ18	Dp	14	23	0	12	0	0	12	7	4	4	0.3	0.344	0.472		
sp P07764-1 ALF_DROME	P07764	Ald	728	1353	674	292	281	332	919	218	302	15	0.3	0.048	0.159		
sp P52029 G6P1_DROME	P52029	Pgi	119	212	122	50	48	52	151	30	50	1	0.3	0.030	0.130		
sp Q9VUY9 PGM_DROME	Q9VUY9	Pgm	27	69	38	16	21	8	45	13	15	4	0.3	0.090	0.193		
sp Q9VFC8-1 GYS_DROME	Q9VFC8	CG6904	74	64	66	24	28	20	68	3	24	2	0.4	0.000	0.000		
tr Q8IQF8 Q8IQF8_DROME	Q8IQF8	CG6084	12	24	34	8	17	0	23	6	8	5	0.4	0.141	0.259		
sp P29310-1 I433Z_DROME	P29310	14-3-3zeta	94	109	86	42	27	36	96	7	35	5	0.4	0.002	0.039		
tr Q9Y119 Q9Y119_DROME	Q9Y119	Tps1	34	51	68	22	31	4	51	10	19	8	0.4	0.063	0.180		
tr Q9VEB1 Q9VEB1_DROME	Q9VEB1	Mdh2	44	37	64	17	12	26	48	8	18	4	0.4	0.028	0.130		
sp P91938-1 TRXR1_DROME	P91938	Trxr-1	8	25	26	3	12	8	20	6	8	3	0.4	0.129	0.246		
tr Q9Y112 Q9Y112_DROME	Q9Y112	BcDNA.GH10614	4	12	26	2	11	4	14	6	5	3	0.4	0.295	0.434		
tr E1JGQ5 E1JGQ5_DROME	E1JGQ5	CG9485	0	76	0	10	9	10	25	25	10	0	0.4	0.576	0.674		
sp P83967 ACT6_DROME	P83967	Act88F	117	93	128	67	65	0	113	10	44	22	0.4	0.048	0.159		
sp P48602 VATA1_DROME	P48602	Vha68-1	10	15	0	7	3	0	8	4	3	2	0.4	0.353	0.472		
sp Q9XTL9 PYG_DROME	Q9XTL9	GlyP	423	597	520	184	221	202	513	50	202	11	0.4	0.004	0.046		
sp P29613-1 TPIS_DROME	P29613-1	Tpi	172	233	202	90	76	78	203	18	81	4	0.4	0.003	0.039		
sp O62619-1 KPYK_DROME	O62619	PyK	375	492	382	170	161	172	416	38	168	3	0.4	0.003	0.039		
tr Q8IQG9 Q8IQG9_DROME	Q8IQG9	Adk1	14	23	18	9	9	4	18	2	7	2	0.4	0.022	0.119		
tr Q9VGB6 Q9VGB6_DROME	Q9VGB6	Pglym87	20	20	38	10	8	14	26	6	11	2	0.4	0.070	0.180		
sp Q01604 PGK_DROME	Q01604	Pgk	245	244	280	125	93	102	256	12	107	9	0.4	0.001	0.034		
tr A1Z7Z4 A1Z7Z4_DROME	A1Z7Z4	CG1648	3	4	12	0	8	0	6	3	3	3	0.4	0.401	0.511		
tr Q7KSU6 Q7KSU6_DROME	Q7KSU6	CG8036	12	20	72	15	11	18	35	19	15	2	0.4	0.350	0.472		
tr Q961N7 Q961N7_DROME	Q961N7	CG5355	9	12	4	4	3	4	8	2	4	0	0.4	0.115	0.230		
sp P17336 CATA_DROME	P17336	Cat	10	45	42	8	20	14	32	11	14	3	0.4	0.197	0.338		
tr Q9VK60 Q9VK60_DROME	Q9VK60	CG6180	10	24	24	10	8	8	19	5	9	1	0.4	0.089	0.193		
sp Q917S8 PUR6_DROME	Q917S8	ade5	7	23	10	3	15	0	13	5	6	4	0.4	0.316	0.458		
sp P15007-1 ENO_DROME	P15007	Eno	760	1099	624	402	359	346	827	141	369	17	0.4	0.032	0.130		
sp P07486 G3P1_DROME	P07486	Gapdh1	785	935	592	391	300	348	771	99	346	26	0.4	0.014	0.091		
sp P02828 HSP83_DROME	P02828	Hsp83	31	43	74	21	24	26	49	13	24	2	0.5	0.116	0.230		
tr Q9VF24 Q9VF24_DROME	Q9VF24	cv-d	3	56	14	10	13	12	24	16	12	1	0.5	0.477	0.591		
tr Q9SRT1 Q9SRT1_DROME	Q9SRT1	CG11089	5	12	10	4	9	0	9	2	4	3	0.5	0.241	0.396		
sp P48610-1 KARG_DROME	P48610	Argk	465	588	532	246	243	296	528	36	261	17	0.5	0.003	0.039		
sp P07487 G3P2_DROME	P07487	Gapdh2	724	864	598	404	356	334	729	77	365	21	0.5	0.010	0.091		
tr B7Z0E0 B7Z0E0_DROME	B7Z0E0	Idh	22	27	28	13	20	6	26	2	13	4	0.5	0.044	0.156		
tr Q8MSI2 Q8MSI2_DROME	Q8MSI2	Scp1	27	235	426	14	124	208	229	115	115	56	0.5	0.425	0.534		
sp P45594 CADF_DROME	P45594	tsr	22	24	22	7	11	18	23	1	12	3	0.5	0.032	0.130		
sp P00334 ADH_DROME	P00334	Adh	303	572	472	201	251	266	449	79	239	20	0.5	0.061	0.180		
sp P02572 ACT2_DROME	P02572	Act42A	64	67	126	0	60	78	86	20	46	24	0.5	0.271	0.414		
sp P08879 NDKA_DROME	P08879	awd	0	53	126	1	15	84	60	37	33	26	0.6	0.583	0.675		
sp Q9VGS2 TCTP_DROME	Q9VGS2	Tctp	8	11	12	3	8	6	10	1	6	1	0.6	0.063	0.180		
tr Q8MQS7 Q8MQS7_DROME	Q8MQS7	Mdh1	28	48	24	18	33	4	33	7	19	8	0.6	0.265	0.414		
sp Q9V3P0 PRDX1_DROME	Q9V3P0	Jafracl	21	40	30	10	21	20	30	6	17	3	0.6	0.115	0.230		
sp Q9VAN0 SERC_DROME	Q9VAN0	CG11899	4	11	8	3	4	6	8	2	4	1	0.6	0.169	0.295		
tr Q7KT11 Q7KT11_DROME	Q7KT11	CG9331	5	15	32	10	12	8	17	8	10	1	0.6	0.402	0.511		
sp Q9VZ49 ENDOU_DROME	Q9VZ49	CG2145	1	17	0	8	3	0	6	6	4	2	0.6	0.707	0.751		
tr Q9VM18 Q9VM18_DROME	Q9VM18	CG5177	13	11	22	7	12	8	15	3	9	1	0.6	0.166	0.295		
tr Q24450 Q24450_DROME	Q24450	Pglym78	18	19	36	12	9	22	24	6	14	4	0.6	0.239	0.396		
sp Q77460 IPYR_DROME	Q77460	Nurf-38	24	44	46	20	29	20	38	7	23	3	0.6	0.127	0.246		
tr Q9U114 Q9U114_DROME	Q9U114	Spr88Ea	0	27	18	2	0	26	15	8	9	8	0.6	0.647	0.717		
tr Q8IR95 Q8IR95_DROME	Q8IR95	CG32667	0	23	8	0	9	10	10	7	6	3	0.6	0.636	0.715		
tr Q7JVX3 Q7JVX3_DROME	Q7JVX3	CG4408	7	49	24	11	11	30	27	12	17	6	0.6	0.521	0.617		
sp P46415 ADHX_DROME	P46415	Fdh	7	4	20	6	11	4	10	5	7	2	0.6	0.517	0.617		
tr Q7KT33 Q7KT33_DROME	Q7KT33	fbp	9	17	12	6	13	6	13	2	8	3	0.7	0.273	0.414		
sp Q24388 LSP2_DROME	Q24388	Lsp2	22	65	20	0	3	68	36	15	24	22	0.7	0.674	0.737		

sp P61851 SODC_DROME	P61851	Sod	35	45	76		20	31	54		52	12		35	10		0.7	0.338	0.470
sp P20432 GSTT1_DROME	P20432	GstD1	13	31	56		10	19	38		33	13		22	8		0.7	0.513	0.617
tr Q9VGA0 Q9VGA0_DROME	Q9VGA0	GstD9	12	11	0		6	8	2		8	4		5	2		0.7	0.605	0.692
sp P25007 PPIA_DROME	P25007	Cyp1	26	37	54		19	28	34		39	8		27	4		0.7	0.258	0.414
sp P0CG69 UBIQP_DROME	P0CG69	Ubi-p63E	3	4	24		2	4	16		10	7		7	4		0.7	0.741	0.763
sp Q9V429-1 THIO2_DROME	Q9V429	Trx-2	6	5	6		3	5	4		6	0		4	1		0.7	0.073	0.180
tr Q9VHA1 Q9VHA1_DROME	Q9VHA1	SpdS	3	7	0		2	3	2		3	2		2	0		0.7	0.680	0.737
sp P36951 HY1_DROME	P36951	Gip	4	17	6		3	11	6		9	4		7	2		0.7	0.639	0.715
sp P02574 ACT4_DROME	P02574	Act79B	89	77	0		55	68	0		55	28		41	21		0.7	0.702	0.751
sp Q02748 IF4A_DROME	Q02748	eIF-4a	14	17	18		5	11	22		17	1		12	5		0.8	0.482	0.591
tr Q9W370 Q9W370_DROME	Q9W370	CG15369	14	21	12		14	7	16		16	3		12	3		0.8	0.399	0.511
tr Q9U4U2 Q9U4U2_DROME	Q9U4U2	Fer2LCH	25	231	270		29	136	250		175	76		138	64		0.8	0.730	0.763
sp P05303 EF1A2_DROME	P05303	Eflalpha100E	34	27	0		10	23	16		20	10		16	4		0.8	0.736	0.763
tr Q8SXA6 Q8SXA6_DROME	Q8SXA6	CG6045	7	36	6		9	19	12		16	10		13	3		0.8	0.780	0.795
tr Q961T9 Q961T9_DROME	Q961T9	BG:DS00941.11	0	11	0		0	3	6		4	4		3	2		0.8	0.874	0.874
tr Q8SXB9 Q8SXB9_DROME	Q8SXB9	CG10433	4	28	18		5	9	28		17	7		14	7		0.8	0.813	0.821

Group 5: Present in w¹¹¹⁸ control but undetected in *dKlf15^{NN}* mutant
(a mean of two spectral counts or lower was regarded as undetected)

		Uniprot ID	Annotation	#1118			dKlf15NN			w1118			dKlf15NN						
				#1	#2	#3				Mean	SEM		Mean	SEM		t-Test	B-H	Correction	
tr E1J191 E1J191_DROME	E1J191	UGP		90	105	0		0	0	0		65	33		0	0	0.118	0.407	
sp P53501 ACT3_DROME	P53501	Act57B		0	0	106		0	0	0		35	35		0	0	0.374	0.407	
tr Q7KVP4 Q7KVP4_DROME	Q7KVP4	CG9485		48	0	44		0	0	0		31	15		0	0	0.117	0.407	
tr A1ZA66 A1ZA66_DROME	A1ZA66	Strn-Mlck		45	0	28		0	0	0		24	13		0	0	0.137	0.407	
sp P35381 ATPA_DROME	P35381	blw		37	9	16		5	0	0		21	8		2	2	0.085	0.407	
sp P19889 RLA0_DROME	P19889	RpLP0		11	3	32		0	0	0		15	9		0	0	0.154	0.407	
tr A1Z992 A1Z992_DROME	A1Z992	AGBE		6	29	10		0	0	0		15	7		0	0	0.104	0.407	
tr A8JNU6 A8JNU6_DROME	A8JNU6	Nc73EF		34	9	0		0	0	0		15	10		0	0	0.229	0.407	
sp P08570 RLA1_DROME	P08570	RpLP1		7	7	26		2	0	0		13	6		1	1	0.130	0.407	
sp Q27331 VATA2_DROME	Q27331	Vha68-2		13	11	16		0	0	0		13	2		0	0	0.001	0.075	
tr A8JRB8 A8JRB8_DROME	A8JRB8	CG5028-RC		13	9	16		4	0	0		13	2		1	1	0.008	0.224	
sp P11996 LSP1B_DROME	P11996	Lsp1beta		10	28	0		2	0	0		13	8		1	1	0.227	0.407	
sp Q96827 EF1B_DROME	Q96827	Eflbeta		6	4	26		3	0	0		12	7		1	1	0.199	0.407	
tr Q9VGQ1 Q9VGQ1_DROME	Q9VGQ1	CG5214		15	8	12		0	0	0		12	2		0	0	0.004	0.149	
tr Q8T447 Q8T447_DROME	Q8T447	CG9961		0	0	34		0	0	0		11	11		0	0	0.374	0.407	
tr E2QCF1 E2QCF1_DROME	E2QCF1	ATPCL		4	16	12		3	0	0		11	3		1	1	0.051	0.407	
tr Q9VJZ6 Q9VJZ6_DROME	Q9VJZ6	CG6523		10	15	8		6	0	0		11	2		2	2	0.031	0.407	
sp P05389 RLA2_DROME	P05389	RpLP2		4	5	22		2	0	0		11	6		1	1	0.154	0.407	
sp P55830-1 RS3A_DROME	P55830	RpS3A		4	5	20		0	0	0		10	5		0	0	0.120	0.407	
tr B7Z0V3 B7Z0V3_DROME	B7Z0V3	CG2767		5	16	8		2	0	0		10	3		1	1	0.055	0.407	
sp Q06559 RS3_DROME	Q06559	RpS3		5	0	24		0	0	0		10	7		0	0	0.252	0.407	
tr C0PUZ9 C0PUZ9_DROME	C0PUZ9	Glycogenin-RB		0	17	10		0	0	0		9	5		0	0	0.144	0.407	
tr Q95U38 Q95U38_DROME	Q95U38	skap		15	4	8		0	0	0		9	3		0	0	0.048	0.407	
sp Q9VU68-1 WDR1_DROME	Q9VU68	flr		10	7	10		2	0	0		9	1		1	1	0.004	0.149	
tr Q8MST5 Q8MST5_DROME	Q8MST5	betaTub97EF		13	13	0		0	0	0		9	4		0	0	0.116	0.407	
tr O16043 O16043_DROME	O16043	Df31		0	0	26		0	0	0		9	9		0	0	0.374	0.407	
tr Q5U0Z2 Q5U0Z2_DROME	Q5U0Z2	CG10576		0	0	26		0	0	0		9	9		0	0	0.374	0.407	
sp P53777 MLP1_DROME	P53777	Mlp60A		10	3	12		2	0	0		8	3		1	1	0.065	0.407	
tr Q9VSU6 Q9VSU6_DROME	Q9VSU6	Dhpr		7	17	0		5	0	0		8	5		2	2	0.275	0.407	
sp P38979-1 RSSA_DROME	P38979	sta		5	1	18		0	0	0		8	5		0	0	0.179	0.407	
sp P25843 PROF_DROME	P25843	chic		11	13	0		2	1	0		8	4		1	1	0.174	0.407	
sp Q8MLY8 RS8_DROME	Q8MLY8	RpS8		6	4	14		0	0	0		8	3		0	0	0.059	0.407	
tr Q9VI04 Q9VI04_DROME	Q9VI04	pyd3		9	15	0		2	0	0		8	4		1	1	0.179	0.407	
tr Q7K860 Q7K860_DROME	Q7K860	TpnC4		7	7	10		6	0	0		8	1		2	2	0.052	0.407	
tr Q8MLU2 Q8MLU2_DROME	Q8MLU2	CG11474		7	9	6		2	0	0		8	1		1	1	0.003	0.149	
sp Q9VNA5 PSB4_DROME	Q9VNA5	Prosbeta7		3	9	10		0	0	0		7	2		0	0	0.029	0.407	
sp Q9NJH0 EF1G_DROME	Q9NJH0	Eflgamma		2	4	16		3	0	0		7	4		1	1	0.227	0.407	
sp P02825 HSP71_DROME	P02825	Hsp70Ab		0	0	22		0	0	0		7	7		0	0	0.374	0.407	
sp P14318 MP20_DROME	P14318	Mp20		5	3	14		0	0	0		7	3		0	0	0.101	0.407	
tr Q7JYW9 Q7JYW9_DROME	Q7JYW9	Hex-C		4	17	0		2	1	0		7	5		1	1	0.314	0.407	
tr O46111 O46111_DROME	O46111	Uba1		13	8	0		6	0	0		7	4		2	2	0.289	0.407	
tr Q9VPZ5 Q9VPZ5_DROME	Q9VPZ5	CG5397		0	21	0		4	0	0		7	7		1	1	0.469	0.491	
sp P14130 RS14_DROME	P14130	RpS14a		4	0	16		3	0	0		7	5		1	1	0.304	0.407	
sp P55828 RS20_DROME	P55828	RpS20		0	0	20		0	0	0		7	7		0	0	0.374	0.407	
tr Q8MS44 Q8MS44_DROME	Q8MS44	CG9468		0	0	20		0	0	0		7	7		0	0	0.374	0.407	
tr Q0KIE6 Q0KIE6_DROME	Q0KIE6	CG12582		0	20	0		3	0	0		7	7		1	1	0.453	0.481	
sp O16797-1 JRL3_DROME	O16797	RpL3		5	3	12		3	0	0		7	3		1	1	0.135	0.407	
tr Q9XYZ9 Q9XYZ9_DROME	Q9XYZ9	GstE12		5	3	12		4	0	0		7	3		1	1	0.161	0.407	
sp Q94529 GS1_DROME	Q94529	Gs11		5	15	0		0	0	0		7	4		0	0	0.197	0.407	
tr Q9W1B9 Q9W1B9_DROME	Q9W1B9	RpL12		4	0	16		0	0	0		7	5		0	0	0.245	0.407	
sp P22769 PSA71_DROME	P22769	Pros28.1		3	11	6		0	0	0		7	2		0	0	0.043	0.407	
tr Q8MT28 Q8MT28_DROME	Q8MT28	CG10467		0	13	6		0	0	0		6	4		0	0	0.170	0.407	
sp P12881 PSA1_DROME	P12881	Prots35		5	8	6		0	0	0		6	1		0	0	0.001	0.075	
sp P46222 RL11_DROME	P46222	RpL11		3	4	12		0	0	0		6	3		0	0	0.091	0.407	
sp Q6AWN0 MTND_DROME	Q6AWN0	CG32068		3	4	12		2	0	0		6	3		1	1	0.116	0.407	
sp P35554 fTN_DROME	P35554	fln		7	0	12		0	0	0		6	3		0	0	0.147	0.407	
tr Q76NQ9 Q76NQ9_DROME	Q76NQ9	AMPdeam		8	4	6		2	0	0		6	1		1	1	0.022	0.407	
tr Q7K3J0 Q7K3J0_DROME	Q7K3J0	CG8258		7	3	8		0	0	0		6	2		0	0	0.023	0.407	
tr Q9VQF7 Q9VQF7_DROME	Q9VQF7	Bacc		0	4	14		0	3	0		6	4		1	1	0.296	0.407	
tr Q6WSQ9 Q6WSQ9_DROME	Q6WSQ9	Mitf		0	0	18		0	0	0		6	6		0	0	0.374	0.407	
tr Q95SI7 Q95SI7_DROME	Q95SI7	CG6028		6	8	4		1	0	0		6	1		0	0	0.008	0.224	
sp P18091-1 ACTN_DROME	P18091	Actn		18	0	0		0	0	0		6	6		0	0	0.374	0.407	
tr Q9VA37 Q9VA37_DROME	Q9VA37	dj-1beta		3	11	4		0	0	2		6	2		1	1	0.105	0.407	
sp P41042 RS4_DROME	P41042	RpS4		1	0	16		0	0	0		6	5		0	0	0.317	0.407	
tr Q8MYS9 Q8MYS9_DROME	Q8MYS9	CG7203		7	11	0		2	0	0		6	3		1	1	0.195	0.407	
sp P35122 UCHL_DROME	P35122	Uch		0	9	8		0	0	0		6	3		0	0	0.118	0.407	
sp P32100 RL7_DROME	P32100	RpL7		3	0	14		0	0	0		6	4		0	0	0.254	0.407	
sp P48159 RL23_DROME	P48159	RpL23		3	0	14		0	0	0		6	4		0	0	0.254	0.407	
tr Q9VV75 Q9VV75_DROME	Q9VV75	CG4169		9	0	8		0	0	0		6	3		0	0	0.117	0.407	
tr Q8IRQ5 Q8IRQ5_DROME	Q8IRQ5	l(1)G0255		7	9	0		0	0	0		6	3		0	0	0.121	0.407	
tr Q9W3L4 Q9W3L4_DROME	Q9W3L4	CG2233		0	11	6		0	0	0		6	3		0	0	0.146	0.407	
tr Q9VD58 Q9VD58_DROME	Q9VD58	CG6439		10	0	6		0	0	0		5	3		0	0	0.144	0.407	
tr Q7K5K3 Q7K5K3_DROME	Q7K5K3	CG1876		9	1	6		0	0	0		5	2		0	0	0.071	0.407	
sp Q94518 NACA_DROME	Q94518	Nacalalpha		2	0	14		2	0	0		5	4		1	1	0.327	0.407	
sp P09180 RL4_DROME	P09180	RpL4		8	0	8		0	0	0		5	3		0	0	0.116	0.407	
tr Q9VTZ0 Q9VTZ0_DROME	Q9VTZ0	tral		0	0	16		0	0	0		5	5		0	0	0.374	0.407	
sp P29327-1 RS6_DROME	P29327	RpS6		6	0	10		0	0	0		5	3		0	0	0.141	0.407	
tr Q9GQV2 Q9GQV2_DROME	Q9GQV2	cher		16	0	0		0	0	0		5	5		0	0	0.374	0.407	
tr Q9VN21 Q9VN21_DROME	Q9VN21	lost		1	0	14		0	0	0		5	4		0	0	0.309	0.407	
sp O18640 GBLP_DROME	O18640	Rack1		0	1	14		0	0	0		5	4		0	0	0.316	0.407	
tr Q9VW19 Q9VW19_DROME	Q9VW19	CG9372		0	13	2		0	4	0		5	4		1	1	0.435	0.466	
sp A1ZA47-1 ZASP_DROME	A1ZA47	Zasp52		6	5	4		0	0	0		5	1		0	0	0.001	0.075	

sp P06742-1 MLC1_DROME	P06742	Mlc1	11	0	4		0	0	0		5	3		0	0		0.197	0.407
tr Q9VB64 Q9VB64_DROME	Q9VB64	CG31063	0	7	8		0	1	4		5	2		2	1		0.320	0.407
tr Q961R8 Q961R8_DROME	Q961R8	Aats-gly	5	9	0		0	0	0		5	3		0	0		0.147	0.407
tr Q9I7R0 Q9I7R0_DROME	Q9I7R0	CG18815	2	4	8		0	0	0		5	2		0	0		0.050	0.407
sp Q26365-2 ADT_DROME	Q26365-2	sesB	14	0	0		0	0	0		5	5		0	0		0.374	0.407
sp P29844 HSP7C_DROME	P29844	Hsc70-3	0	0	14		0	0	0		5	5		0	0		0.374	0.407
sp P50882 RL9_DROME	P50882	RpL9	0	0	14		0	0	0		5	5		0	0		0.374	0.407
tr Q86BQ4 Q86BQ4_DROME	Q86BQ4	CG2862	6	8	0		4	0	0		5	2		1	1		0.293	0.407
sp Q9W0P5 GALE_DROME	Q9W0P5	Gale	4	9	0		0	0	0		5	3		0	0		0.163	0.407
tr A8JUT4 A8JUT4_DROME	A8JUT4	Ntf-2	4	9	0		0	0	0		5	3		0	0		0.163	0.407
sp P49630 RL36_DROME	P49630	RpL36	1	0	12		0	0	0		4	4		0	0		0.299	0.407
tr Q7JX87 Q7JX87_DROME	Q7JX87	Prx2540-2	1	12	0		4	0	0		4	4		1	1		0.474	0.494
tr Q7KTK9 Q7KTK9_DROME	Q7KTK9	CG5261	13	0	0		0	0	0		4	4		0	0		0.374	0.407
sp Q27415-1 NLP_DROME	Q27415	Nlp	2	0	10		0	0	0		4	3		0	0		0.249	0.407
sp P48149 RS15A_DROME	P48149	RpS15Aa	2	0	10		3	0	0		4	3		1	1		0.401	0.434
sp P06754-1 TPM1_DROME	P06754-1	Tm1	1	3	8		0	0	0		4	2		0	0		0.113	0.407
sp Q9WSR8 RL5_DROME	Q9WSR8	RpL5	0	0	12		0	0	0		4	4		0	0		0.374	0.407
tr Q8MT23 Q8MT23_DROME	Q8MT23	RpL30	0	0	12		0	0	0		4	4		0	0		0.374	0.407
tr Q8INP8 Q8INP8_DROME	Q8INP8	CG11980	5	7	0		0	0	0		4	2		0	0		0.122	0.407
sp Q27580 SAHH_DROME	Q27580	Ahcy13	4	8	0		0	0	0		4	2		0	0		0.166	0.407
tr Q9VXQ5 Q9VXQ5_DROME	Q9VXQ5	Tcp-1zeta	4	0	8		0	0	0		4	2		0	0		0.166	0.407
sp Q9GU68 IF5A_DROME	Q9GU68	eIF-5A	4	0	8		5	0	0		4	2		2	2		0.457	0.483
sp P55841 RL14_DROME	P55841	RpL14	1	0	10		0	0	0		4	3		0	0		0.286	0.407
sp P20241-1 NRG_DROME	P20241	Nrg	1	7	4		0	1	0		4	2		0	0		0.130	0.407
tr Q9VSL2 Q9VSL2_DROME	Q9VSL2	GstO3	0	5	6		0	0	0		4	2		0	0		0.117	0.407
sp Q9VA91 RS7_DROME	Q9VA91	RpS7	3	0	8		0	0	0		4	2		0	0		0.192	0.407
sp Q9V9X4 MTNA_DROME	Q9V9X4	CG11334	1	1	8		0	0	0		4	2		0	0		0.176	0.407
tr Q8IRH0 Q8IRH0_DROME	Q8IRH0	Psa	7	4	0		3	0	0		4	2		1	1		0.323	0.407
tr Q9W2M4 Q9W2M4_DROME	Q9W2M4	CG10527	0	7	4		0	3	0		4	2		1	1		0.279	0.407
sp Q9VMR8 TOTM_DROME	Q9VMR8	TotM	0	11	0		0	1	0		4	4		0	0		0.434	0.466
tr Q8SXXV8 Q8SXXV8_DROME	Q8SXXV8	CG3246	0	11	0		0	5	0		4	4		2	2		0.678	0.690
sp P29843 HSP7A_DROME	P29843	Hsc70-1	5	5	0		0	0	0		4	2		0	0		0.116	0.407
sp Q94522 SUCA_DROME	Q94522	Sscalp	2	0	8		1	0	0		3	2		0	0		0.260	0.407
sp P02518 HSP27_DROME	P02518	Hsp27	0	0	10		0	0	0		3	3		0	0		0.374	0.407
sp P55935 RS9_DROME	P55935	RpS9	0	0	10		0	0	0		3	3		0	0		0.374	0.407
tr Q0E9I6 Q0E9I6_DROME	Q0E9I6	Dscam1	0	0	10		0	0	0		3	3		0	0		0.374	0.407
tr Q7JW48 Q7JW48_DROME	Q7JW48	CG10911	0	0	10		0	0	0		3	3		0	0		0.374	0.407
tr Q9VBR8 Q9VBR8_DROME	Q9VBR8	CG11902	0	0	10		0	0	0		3	3		0	0		0.374	0.407
sp P22464-1 ANXB9_DROME	P22464	AnnlX	4	1	4		0	0	0		3	1		0	0		0.029	0.407
sp P09491-1 TPM2_DROME	P09491	Tm2	4	5	0		0	0	0		3	2		0	0		0.119	0.407
tr Q9VPX6 Q9VPX6_DROME	Q9VPX6	capt	4	5	0		0	0	0		3	2		0	0		0.119	0.407
sp Q24407 ATP5J_DROME	Q24407	ATPsyn-Cf6	4	0	6		0	0	0		3	2		0	0		0.137	0.407
tr Q7KVVX1 Q7KVVX1_DROME	Q7KVVX1	l(1)G0334	7	0	2		0	0	0		3	2		0	0		0.230	0.407
tr Q9V3W0 Q9V3W0_DROME	Q9V3W0	UK114	0	1	8		0	0	0		3	2		0	0		0.277	0.407
sp Q00174 LAMA_DROME	Q00174	LanA	0	9	0		0	0	0		3	3		0	0		0.374	0.407
tr Q9VNVK6 Q9VNVK6_DROME	Q9VNVK6	CG11459	0	9	0		0	0	0		3	3		0	0		0.374	0.407
tr O96299 O96299_DROME	O96299	Sodh-2	2	7	0		0	0	0		3	2		0	0		0.204	0.407
tr Q9VM19 Q9VM19_DROME	Q9VM19	CG5171	2	7	0		1	0	0		3	2		0	0		0.243	0.407
sp P54385-1 DHE3_DROME	P54385	Gdh	4	0	4		0	0	0		3	1		0	0		0.117	0.407
sp P17704 RS17_DROME	P17704	RpS17	2	0	6		0	0	0		3	2		0	0		0.192	0.407
sp P41044 CAB32_DROME	P41044	Cbp53E	8	0	0		0	0	0		3	3		0	0		0.374	0.407
sp O61231 RL10_DROME	O61231	RpL10	0	0	8		0	0	0		3	3		0	0		0.374	0.407
sp O76454 PHS_DROME	O76454	Pcd	0	0	8		0	0	0		3	3		0	0		0.374	0.407
sp P02517 HSP26_DROME	P02517	Hsp26	0	0	8		0	0	0		3	3		0	0		0.374	0.407
sp P23128-1 DDX6_DROME	P23128	me31B	0	0	8		0	0	0		3	3		0	0		0.374	0.407
sp P49847 TAF6_DROME	P49847	Taf6	0	0	8		0	0	0		3	3		0	0		0.374	0.407
sp Q9V3S0 CP4G1_DROME	Q9V3S0	Cyp4g1	0	0	8		0	0	0		3	3		0	0		0.374	0.407
sp Q9V597 RL31_DROME	Q9V597	RpL31	0	0	8		0	0	0		3	3		0	0		0.374	0.407
tr A1Z892 A1Z892_DROME	A1Z892	Prx2540-1	0	0	8		0	0	0		3	3		0	0		0.374	0.407
tr Q24150 Q24150_DROME	Q24150	Nap1	0	0	8		0	0	0		3	3		0	0		0.374	0.407
tr Q9VTM9 Q9VTM9_DROME	Q9VTM9	CG32088	0	0	8		0	0	0		3	3		0	0		0.374	0.407
tr A1ZAA5 A1ZAA5_DROME	A1ZAA5	Got1	5	3	0		0	0	0		3	2		0	0		0.156	0.407
sp Q00637 SODM_DROME	Q00637	Sod2	4	0	4		0	0	0		3	1		0	0		0.117	0.407
tr A1Z6X6 A1Z6X6_DROME	A1Z6X6	CG1707	2	5	0		0	0	0		3	2		0	0		0.178	0.407
sp Q8T3U2 RS23_DROME	Q8T3U2	RpS23	1	0	6		0	0	0		2	2		0	0		0.238	0.407
sp O02649 CH60_DROME	O02649	Hsp60	1	0	6		0	3	0		2	2		1	1		0.468	0.491
tr Q9VJ31 Q9VJ31_DROME	Q9VJ31	CG10623	4	3	0		0	0	0		2	1		0	0		0.141	0.407
tr Q9W3B3 Q9W3B3_DROME	Q9W3B3	CG1885	3	4	0		1	0	0		2	1		0	0		0.169	0.407
sp Q9VLM8 SYAC_DROME	Q9VLM8	Aats-ala	7	0	0		0	0	0		2	2		0	0		0.374	0.407
sp P15278-1 FAS3_DROME	P15278	Fas3	0	7	0		0	0	0		2	2		0	0		0.374	0.407
tr A4V464 A4V464_DROME	A4V464	Acon	0	3	4		0	0	0		2	1		0	0		0.132	0.407
tr Q8ST39 Q8ST39_DROME	Q8ST39	CG9336	0	3	4		0	1	0		2	1		0	0		0.230	0.407
tr Q7KV27 Q7KV27_DROME	Q7KV27	CG1640	4	3	0		0	0	0		2	1		0	0		0.127	0.407
tr Q8IP97 Q8IP97_DROME	Q8IP97	Pex19	4	3	0		2	0	0		2	1		1	1		0.264	0.407
tr O76902 O76902_DROME	O76902	rush	2	0	4		0	0	0		2	1		0	0		0.147	0.407
tr Q8SYA6 Q8SYA6_DROME	Q8SYA6	CG7322	2	4	0		1	0	0		2	1		0	0		0.202	0.407
sp O18405 SURF4_DROME	O18405	Surf4	0	0	6		0	0	0		2	2		0	0		0.374	0.407
sp P29742 CLH_DROME	P29742	Chc	0	0	6		0	0	0		2	2		0	0		0.374	0.407
sp P46150-1 MOEH_DROME	P46150	Moe	0	0	6		0	0	0		2	2		0	0		0.374	0.407
sp P46223 RL7A_DROME	P46223	RpL7A	0	0	6		0	0	0		2	2		0	0		0.374	0.407
sp P48598-1 IF4E_DROME	P48598	eIF-4E	0	0	6		0	0	0		2	2		0	0		0.374	0.407
sp P62152 CALM_DROME	P62152	Cam	0	0	6		0	0	0		2	2		0	0		0.374	0.407
sp Q9VG97 GSTT3_DROME	Q9VG97	GstD3	0	0	6		0	0	0		2	2		0	0		0.374	0.407
sp Q9VWG3 RS10B_DROME	Q9VWG3	RpS10b	0	0	6		0	0	0		2	2		0	0		0.374	0.407
tr A8DZ02 A8DZ02_DROME	A8DZ02	kuz	0	0	6		0	0	0		2	2		0	0		0.374	0.407
tr Q8MR43 Q8MR43_DROME	Q8MR43	CG1622	0	0	6		0	0	0		2	2		0	0		0.374	0.407
tr Q9V9W2 Q9V9W2_DROME	Q9V9W2	RpL6	0	0	6		0	0	0		2	2		0	0		0.374	0.407
sp Q26365-1 ADT_DROME	Q26365	sesB	0	0	6		2	0	0		2	2		1	1		0.607	0.621
sp A1ZA47-4																		

tr Q4TWT4 Q4TWT4_DROME	Q4TWT4	su(r)	5	0	0		0	0	0		2	2		0	0		0.374	0.407
tr Q9VSL4 Q9VSL4_DROME	Q9VSL4	GstO2	5	0	0		0	0	0		2	2		0	0		0.374	0.407
tr Q7KUQ2 Q7KUQ2_DROME	Q7KUQ2	CG9674-RB	4	1	0		0	0	0		2	1		0	0		0.197	0.407
tr O97428 O97428_DROME	O97428	cib	2	3	0		2	4	0		2	1		2	1		0.879	0.883
sp Q02645-1 HTS_DROME	Q02645	hts	1	4	0		0	0	0		2	1		0	0		0.267	0.407
sp Q9VNT5 TRXR2_DROME	Q9VNT5	Trxr-2	4	0	0		0	0	0		1	1		0	0		0.374	0.407
tr A1Z8U4 A1Z8U4_DROME	A1Z8U4	Cct5	4	0	0		0	0	0		1	1		0	0		0.374	0.407
tr A8DYP0 A8DYP0_DROME	A8DYP0	Unc-89	4	0	0		0	0	0		1	1		0	0		0.374	0.407
tr Q24062 Q24062_DROME	Q24062	b	4	0	0		0	0	0		1	1		0	0		0.374	0.407
tr Q9VFF0 Q9VFF0_DROME	Q9VFF0	CG3731	4	0	0		0	0	0		1	1		0	0		0.374	0.407
tr Q9VH01 Q9VH01_DROME	Q9VH01	Bruce	4	0	0		4	0	0		1	1		1	1		0.940	0.940
tr Q7JV16 Q7JV16_DROME	Q7JV16	GstE13	3	1	0		0	0	0		1	1		0	0		0.171	0.407
sp Q95083 PSA5_DROME	Q95083	Prosalpha5	1	3	0		0	0	0		1	1		0	0		0.147	0.407
sp Q9XZJ4 PSA6_DROME	Q9XZJ4	Prosalpha1	1	3	0		0	0	0		1	1		0	0		0.147	0.407
tr Q967S0 Q967S0_DROME	Q967S0	Prat2	1	3	0		0	0	0		1	1		0	0		0.147	0.407
tr Q7KSB5 Q7KSB5_DROME	Q7KSB5	CG4390	1	3	0		0	3	0		1	1		1	1		0.694	0.703
sp Q6NN85-1 SSH_DROME	Q6NN85	ssh	0	4	0		0	0	0		1	1		0	0		0.374	0.407
sp Q9V813 MTAP_DROME	Q9V813	CG4802	0	4	0		0	0	0		1	1		0	0		0.374	0.407
sp Q9VXX8 RL371_DROME	Q9VXX8	RpL37a	0	0	4		0	0	0		1	1		0	0		0.374	0.407
tr A1Z8G7 A1Z8G7_DROME	A1Z8G7	Listericin	0	4	0		0	0	0		1	1		0	0		0.374	0.407
tr A8QI34 A8QI34_DROME	A8QI34	CG40625	0	4	0		0	0	0		1	1		0	0		0.374	0.407
tr B7YZX6 B7YZX6_DROME	B7YZX6	CG10600	0	4	0		0	0	0		1	1		0	0		0.374	0.407
tr B7Z076 B7Z076_DROME	B7Z076	CG6852	0	0	4		0	0	0		1	1		0	0		0.374	0.407
tr O97479 O97479_DROME	O97479	Sodh-1	0	4	0		0	0	0		1	1		0	0		0.374	0.407
tr Q8INQ3 Q8INQ3_DROME	Q8INQ3	CG11760-RB	0	4	0		0	0	0		1	1		0	0		0.374	0.407
tr Q9U9B0 Q9U9B0_DROME	Q9U9B0	gig	0	0	4		0	0	0		1	1		0	0		0.374	0.407
tr Q9VPI8 Q9VPI8_DROME	Q9VPI8	net	0	0	4		0	0	0		1	1		0	0		0.374	0.407
tr Q9VWS5 Q9VWS5_DROME	Q9VWS5	CG15040	0	0	4		0	0	0		1	1		0	0		0.374	0.407
tr Q9W137 Q9W137_DROME	Q9W137	CG4707	0	0	4		0	0	0		1	1		0	0		0.374	0.407
tr Q0E8H9 Q0E8H9_DROME	Q0E8H9	Hexo1	0	4	0		0	3	0		1	1		1	1		0.795	0.802
sp Q9NIV1 E2AK3_DROME	Q9NIV1	PEK	4	0	0		0	0	0		1	1		0	0		0.374	0.407
sp Q9V3J1-1 VATH_DROME	Q9V3J1	VhaSFD	4	0	0		0	0	0		1	1		0	0		0.374	0.407
tr Q9VIQ8 Q9VIQ8_DROME	Q9VIQ8	ColV	4	0	0		0	0	0		1	1		0	0		0.374	0.407
tr Q9VKM3 Q9VKM3_DROME	Q9VKM3	l(2)06225	4	0	0		0	0	0		1	1		0	0		0.374	0.407
tr A1Z784 A1Z784_DROME	A1Z784	ACC	2	1	0		0	0	0		1	1		0	0		0.141	0.407
tr Q0E993 Q0E993_DROME	Q0E993	Aats-val	2	1	0		0	0	0		1	1		0	0		0.141	0.407
tr Q1RL06 Q1RL06_DROME	Q1RL06	GS	1	0	2		2	0	0		1	1		1	1		0.476	0.494