Enhanced survival of *Drosophila* Akt1 hypomorphs during amino-acid starvation requires foxo

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Title page

Title: Enhanced survival of *Drosophila Akt1* hypomorphs during amino-acid starvation requires *foxo*

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Abstract

Disordered eating includes any pattern of irregular eating that may lead to either extreme weight loss or obesity. The conserved insulin receptor signalling pathway acts to regulate energy balance and nutrient intake, and its central component Akt1 and endpoint effector foxo are pivotal for survival during nutritional stress. Recently generated *Akt1* hypomorphic mutant lines exhibit a moderate decrease in lifespan when aged upon standard media, yet show a considerable increase in survival upon amino-acid starvation media. While the loss of *foxo* function significantly reduces the survival response to amino-acid starvation, a combination of these *Akt1* hypomorphs and a null *foxo* mutation reveal a synergistic and severe reduction in lifespan upon standard media, and an epistatic relationship when undergoing amino-acid starvation. Evaluation of survivorship upon amino-acid starvation media of these double mutants indicate a phenotype similar to the original *foxo* mutant demonstrating the role of *foxo* in this *Akt1* phenotype. These results indicate that the subtle manipulation of foxo through Akt1 can enhance survival during adverse nutrient conditions to model the ability of individuals to tolerate nutrient deprivation. Ultimately, we believe that a *Drosophila* model of disordered eating could generate new avenues to develop potential therapies for related human conditions.

Key words: *Drosophila melanogaster*, starvation, Akt1, foxo

34 Introduction

35 A number of eating disorders, including the medically diagnosable forms of anorexia and
36 bulimia, or any irregular eating patterns leading to extreme weight loss or gain, can be lifethreatening
37 (Neilson, 2001; Fairburn and Harrison, 2003). The psychiatric illness with the highest mortality rate is
38 anorexia, with 10% of diagnosed individuals dying within 10 years of the onset of the disease (Sullivan
39 2002). Irregular eating and periods of malnutrition may be common, as during the lifetime of most
40 organisms there is a fluctuation in the availability of nutrition. In order to deal with situations of low
41 nutrient availability, complex signalling cascades, such as the insulin receptor signalling pathway,
42 combine both the control of nutrient intake and the control of cell growth during development to
43 appropriately manage energy stores.

44 *Drosophila melanogaster* can serve as a simple animal model system to study the molecular
45 basis of starvation during nutrient deprivation. In *Drosophila* there are seven insulin-like peptides (Ilps)
46 produced by 14 neurosecretory cells (Broeck 2001; Cao and Brown 2001; Ikeya et al., 2002). The insulin
47 receptor signalling (IRS) pathway in flies reduces the levels of trehalose in the hemolymph (Rion and
48 Kawecki 2007). Flies which lack Ilps develop into small adults with low fertility and metabolic defects
49 similar to mammalian diabetes including elevated sugar levels and initiation of the starvation response,
50 one aspect being lowered amount of triglycerides stored in the fat body (Zhang et al., 2009). The central
51 component of the insulin receptor signalling pathway, the serine/threonine kinase Akt1, is highly
52 conserved between mammals and *Drosophila melanogaster* (Scheid and Woodgett 2001; Franket et al.,
53 2003). In *Drosophila*, *Akt1* is expressed throughout the organism during embryogenesis (Staveley et al.,
54 1998; Scanga et al., 2000; Cavaliere et al., 2005). A role for *Akt1* in the control of cell growth and survival
55 is established as over-expression of *Akt1* in *Drosophila* leads to an increase in cell size, although there is
56 no effect on cell growth rates or division. Under-expression or loss of *Akt1* can result in the production
57 of smaller animals or, if severe, in lethality (Kennedy et al., 1997; Staveley et al., 1998; Verdu et al.,

1999). The role of *Akt1* in cellular survival was suggested by its inhibition of several pro-apoptotic genes (Kennedy et al., 1997; Nunez and del Peso 1998). As expected for a maternally expressed gene, *Akt1* mutant females are sterile, and *Drosophila Akt1* mutant germline clones undergo apoptosis (Staveley et al., 1998). This demonstrates that the activities of the Akt1 kinase are of great importance to the survival of the cell, and to the overall organism.

The negatively regulated downstream target of Akt1, the transcription factor foxo (Brunet et al., 1999; Burgering and Medema 2003; Puig et al., 2003; Barthel et al., 2005; Puig and Tijan 2005), mediates the transcriptional response of the insulin receptor pathway to fluctuating nutrient conditions. As such, it controls a number of downstream targets involved in several important cellular functions. These include control of metabolism, cell cycle regulation, DNA repair, apoptosis and protection against oxidative stress (Burgering and Kops 2002; Barthel et al., 2005; Puig and Tijan 2005; Gershman et al., 2007). Through these many functions, the transcription factor foxo can affect cell growth and survival. Over-expression of *foxo*, such as its activation in the absence of insulin receptor signalling, results in phenotypes that resemble starved larvae (Kramer et al., 2003). A reduced insulin receptor signalling level is required for optimal survival during starvation, as over-expression of insulin receptor signalling components leads to the inability of *Drosophila* to survive during nutritional stress (Britton et al., 2002). Excess insulin receptor signalling results in a decrease in foxo activity, and loss of foxo function results in a decrease in survivorship upon amino-acid starvation media (Kramer et al., 2008). These findings suggest that insulin receptor signalling is involved in mediating the developmental response of larvae to starvation, and leads to the conclusion that in the absence of adequate nutrition, insulin receptor signalling is reduced, allowing foxo to enhance the transcription of downstream targets to aid in the overall survival of the organism.

The majority of studies of starvation resistance in *Drosophila* focus on complete food deprivation as opposed to reduction of any one major dietary component (Rion and Kawecki 2007).

However, the lifespan of *Drosophila* under these conditions is extremely short, thereby limiting the ability to observe any subtle differences in survival. The largest parts of a complete diet for *Drosophila* include a carbohydrate source, such as sugar, and an amino-acid/ protein source, usually yeast. Flies exposed to dietary restriction through a reduction of yeast alone, rather than sugar, are more sensitive to starvation (Min and Tatar 2006). In addition, when survivorship of flies was compared from diets of sugar alone to those with sugar and varying amounts of the protein casein, the addition of protein improved the longevity of the flies. Age-specific mortality in *Drosophila* is recognized to be related to dietary conditions (Good and Tatar 2001). Upon a full diet, including sufficient protein typically in the form of yeast, survival of *Drosophila* is considered to be optimal. However, *Drosophila* exposed to a diet lacking yeast show an increase in age-specific mortality, which decreases upon reintroduction of yeast. Taking these findings into consideration, we believe a phenotype signifying starvation resistance on amino-acid depleted media could be more enlightening than that same phenotype in other types of starvation assays. We have characterized novel *Akt1* mutants (Slade and Staveley 2015) that, upon amino-acid starvation media, show an increased starvation resistance when compared to wild type controls. When combined with a null *foxo* mutation, the novel *Akt1* hypomorphs do not show the same survivorship to suggest this phenotype is *foxo* dependent.

Materials and Methods

Generation and Source of Drosophila Strains and Culture

The initial P-element insertion line *ry⁵⁰⁶ P{PZ}Akt1⁰⁴²²⁶/TM3, ry^{RK}, Sb¹, Ser¹ (Akt1⁰⁴²²⁶)* was obtained from the Bloomington *Drosophila* Stock Center (Perrimon et al., 1996; Spradling et al., 1999). This line contains a P-element inserted within the 5' untranslated region of the *Akt1* gene on the third

chromosome. The control line *w; +/+; P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} Akt1⁺ (Akt1⁺)* was derived from lines obtained from Dr. Norbert Perrimon, Harvard University. The novel mutants of *Akt1*⁵², *Akt1*⁵⁷, and *Akt1*⁸⁷, as previously described (Slade and Staveley 2015), were generated by imprecise excision of the P-element via crossing to a line with a stable source of transposase, *PΔ2-3, ry⁺* (Roberston et al., 1988). These novel mutants are viable, developmentally delayed and reduced in size. The *foxo* null mutant line *w; foxo^{w124X}* was obtained from Drs. E. Hafen and M. Junger (Junger et al., 2003) of the University of Zurich. Wild-type Oregon R (*OrR*) stock was obtained from the Bloomington Drosophila Stock Center and *w¹¹¹⁸* was obtained from Dr. Howard Lipshitz from the University of Toronto. The stocks required for transgenic replacement, *w; arm-Gal4* and *w; UAS-Akt1* were previously described (Staveley et al., 1998) and complex lines were generated for each *Akt1* allele (*w; arm-Gal4; Akt1^m* and *w; UAS-Akt1; Akt1^m* where *m* is the allele). Transgenic replacement studies were carried out by generating independent lines of *w¹¹¹⁸; UAS-Akt1⁺/CyO; Akt1^m/TM6B* and *w¹¹¹⁸; arm-GAL4/CyO; Akt1^m/TM6B* where *m* represents each of the novel *Akt1* mutant alleles. Crosses between these lines generated the critical class of *w¹¹¹⁸; UAS-Akt1⁺/arm-GAL4; Akt1^m/Akt1^m* to be analyzed. Generation of flies bearing both the novel mutant *Akt1* alleles and a null *foxo* allele was performed via standard recombinant methods as previously described (Slade and Staveley 2015). Stocks and crosses were maintained on a standard medium containing cornmeal, molasses, yeast, agar and water. Routinely, stocks were kept at room temperature (22 ± 2°C) while crosses and experiments were carried out at 25°C.

Longevity Assay

Experiments were carried out on standard media containing cornmeal, molasses, yeast, agar and water at 25°C. Adult heterozygous virgin females of *w; FRT^{2A} FRT^{82B} Akt1⁺*, *w; FRT^{2A} FRT^{82B} Akt1⁰⁴²²⁶* and each of the mutants were mated to heterozygous males of the same genotype. Several single vial

matings of three to five females plus two males were made of each genotype which were transferred onto new food every two days. A cohort of adult homozygous male flies were collected upon eclosion. Only male flies were observed to avoid the effect of reproduction investment associated with female flies. Approximately between 120 and 340 critical class males were aged per genotype, at a density of $\leq$ 20 flies per vial. Adults were kept on fresh media which was replenished every four to six days. Flies were observed and scored every two days for presence of deceased adults. Flies were considered dead when they did not display movement upon agitation. Longevity data was analyzed using the GraphPad Prism 5.00 program. Survival curves were compared using the log-rank test, a statistical test that compares the actual and expected number of failures (death) between survival curves at each individual failure event. Significance was determined at 95%, at a P-value less than or equal to 0.05.

Starvation Assay

Crosses as in the longevity assay were performed and homozygous males of the desired phenotype were collected within 24 hours of eclosion. As in the longevity assay, male flies were observed to avoid the effect of reproduction investment associated with female flies. Between one hundred and nearly four hundred male flies were collected per genotype, and maintained in non-crowded conditions by a maximum number of 20 flies per vial. Adults were aged on fresh amino-acid starvation medium, consisting of 5% sucrose in phosphate buffered saline solution and 3% agar, and was replenished every four to six days. Studies of survival upon the starvation media was carried out and analyzed as described previously for the longevity assay. Flies were scored every two days for presence of deceased adults. Adults were considered dead when they did not display movement upon agitation. Results were analyzed using the GraphPad Prism 5.00 program. Survival curves were compared using the log-rank test. This is a test statistic that compares the actual and expected number of failures

(death) between survival curves at each individual failure event. Significance was determined at 95%, at a P-value less than or equal to 0.05.

Results

Novel Akt1 mutants have a reduced lifespan on standard media

Longevity assays comparing the lifespan of the mutant homozygotes to the wild-type controls indicate there is a slight decrease in lifespan (Figure 1). The control had a median lifespan of 60 days, while *Akt1*⁵² had median lifespan of 52 days, and *Akt1*⁵⁷ and *Akt1*⁸⁷ both had a median lifespan of 57 days. This corresponds to between a 13% and 16% decrease in lifespan.

Novel Akt1 mutants outlive controls on amino-acid starvation media

Starvation assays ageing mutant homozygotes and wild-type controls resulted in between 33% and 67% increase in survivorship of the *Akt1* hypomorphic mutants (Figure 2A). The *Akt1*⁺ line, with the shortest length of survival, has a median survivorship of 18 days. In comparison, the *Akt1*⁰⁴²²⁶ line had a median survival time of 24 days, *Akt1*⁵⁷ survived for a median of 28 days, and both *Akt1*⁵² and *Akt1*⁸⁷ have a median time of survival of 30 days. When wild type *Akt1* was expressed under the control of the *armGal4* transgene in the genetic background of the *Akt1* mutant homozygotes, the extension in survival upon the starvation medium was suppressed (Figure 2B). This indicates the alteration of *Akt1* activity in the mutants is responsible for the extension in lifespan upon amino-acid starvation media.

177 *Combination of novel Akt1 hypomorphs and a null foxo mutation show a complex relationship*

178
179 To determine if *foxo* was required for the observed longevity phenotypes of the *Akt1* mutants,
180 *Drosophila* bearing mutations in both genes were generated and aged upon standard and amino-acid
181 starvation media. Lifespans of either the *Akt1* mutants or the null *foxo* mutants upon standard media is
182 decreased, with median days of survival for the *Akt1* mutants ranging between 52-57 days (Figure 1) and
183 the median lifespan of the null *foxo* mutants was 26 days (Figure 3A). The double mutant lines also
184 exhibited a decrease in lifespan when compared to the control with a median lifespan of 10 to 12 days
185 (Figure 3A). When starved of amino acids and protein, the null *foxo* mutants are sensitive with a median
186 lifespan of 12 days (Figure 3B). The novel *Akt1* mutants have an extended lifespan with median survival
187 of 24 to 30 days (Figure 2A). The double mutant resembles the *foxo* mutants with a median survival by
188 day 14 (Figure 3B). This suggests that in the absence of *foxo* signalling, the *Akt1* mutants do not outlive
189 the controls when starved of amino-acids.

191 **Discussion**

192
193 Ageing can be defined as the progressive degeneration of somatic tissues to the point that leads
194 to the eventual termination or death of the organism. Clues to the basis of the molecular control of
195 ageing was revealed in *C. elegans* when it was observed that certain mutants would undergo formation
196 of the dauer larvae stage, a type of starvation-induced developmental arrest, in the presence of free
197 access to food (Tatar 2004). In *Drosophila*, a similar diapause occurs with the manipulation of insulin
198 receptor pathway associated genes, and this diapause retards both ageing and growth. The underlying
199 mechanism may be instrumental in the increased longevities seen with mutants of some genes encoding
200 components of the insulin receptor signalling pathway. We have shown that hypomorphic *Akt1* mutant

homozygotes have a reduced lifespan when compared to controls with an 8 to 10 day difference in median lifespan. While alterations to insulin receptor signalling components have shown an increase in lifespan, the same is not true for these novel mutants. The Akt1 kinase is an important cell survival protein, and expression of *Akt1* occurs throughout embryogenesis (Staveley et al., 1998). Null mutations in *Akt1* carried by germline clone females result in production of cuticle-deprived embryos and the induction of ectopic apoptosis. Subtle reduction in *Akt1* influenced activities could certainly impede *Drosophila* development.

Interestingly, when aged upon amino-acid starvation media, these novel mutants show an extension in lifespan. A method for enhancing starvation resistance is increasing energy stores such as accumulated fats. A protein-poor diet in adult flies induces an increase in lipid reserves. *Drosophila* deficient in insulin receptor signalling maintain an increase in lipid stores which may aid in starvation resistance (Rion and Kawecki 2007). As these mutants are hypomorphs, similar to conditions of reduced insulin receptor signalling, this might contribute to the extension in lifespan.

This phenotype may also be a result of a mechanism that shares some aspects with dauer formation in *C. elegans* in response to low nutrients in which their growth is halted, and resumed again once nutrients are replenished. Newly hatched *Drosophila* larvae require nutrients in order to increase their body mass via replication of cells in mitotic tissues. In contrast, when larvae are hatched into conditions of amino acid starvation, they live in a state of developmental arrest until nutrients become available (Beadle et al., 1938; Kramer et al., 2003). When insulin receptor signalling is inhibited, developmental arrest similar to that seen during starvation in first instar larvae occurs (Britton et al., 2002). When larvae are amino acid starved, their overall growth is impeded. In previous studies, dominant-negative mutations of PI3K, and therefore inactivation of *Akt1*, has been shown to result in flies which phenocopy the effects of amino-acid starvation (Kramer et al., 2003). In addition, previous studies of *foxo*'s role during nutritional stress, by inducing ubiquitous expression of *foxo* in the first

instar larvae, caused a complete arrest of growth, yet the larvae survived for several days (Junger et al., 2003; Kramer et al., 2003). As *foxo* is a significant downstream target of Akt1, it is possible that this is responsible for the phenotypes we have observed.

Removing *foxo* activity from the *Akt1* hypomorphs showed both synergistic and epistatic results. Analysis of longevity upon standard media indicates that while both Akt1 and *foxo* may overlap one another through sharing control over genes that regulate survival, reduction in activity or loss of both severely impacts lifespan. This additive effect is evidently beyond the Akt1-*foxo* pathway and most likely dependent upon one or more of the other downstream targets of either gene. Comparison of the *Akt1* hypomorphs, null *foxo* mutants and the double mutants show that the double mutant lines much more closely resemble the null *foxo* mutants. As the double mutants do not recapitulate the starvation resistance of the *Akt1* hypomorphs, this demonstrates the necessity of *foxo* in this process. Loss of *foxo* has been shown to result in an increased sensitivity to starvation caused by low levels of amino acids (Kramer et al., 2008). When nutrient levels are low, *foxo* activity is increased and upon return of nutrients, *foxo* levels return to normal. Ectopic expression of *Drosophila foxo* leads to inhibition of growth and generation of small adults. In addition, when there is an excess of *foxo* activity in larvae, feeding behaviour is altered (Kramer et al., 2003). This highlights the importance of *foxo* in times of nutritional stress, and taken together, these results show the importance of insulin receptor signalling and *foxo* in the survival of organisms during malnutrition. This may be due to a number of the genes that *foxo* transcriptionally activates. In mammalian tissue culture studies, *foxo* induces programmed cell death, or apoptosis, through the withdrawal of cytokines, up-regulation of pro-apoptotic genes, such as Bcl-2 family member Bim, cytochrome c release and caspase activation (Dijkers et al., 2002). In addition, *foxo* induces the expression of genes associated with stresses including *MnSOD* and *4EBP* (Zinke et al., 2002; Puig et al., 2003). An increase in *MnSOD* aids in the reduction of life threatening free radicals, while induction of *4EBP* decreases protein synthesis, an unnecessary process during famine. The

extended lifespan of *Akt1* mutants on amino-acid starvation media may act through *foxo* to induce the expression of pro-survival genes.

Investigation of the ability of these novel *Akt1* mutants to survive amino acid starvation may provide an excellent basis to build an understanding of how organisms counteract the stress of an inadequate diet. The ability to endure such conditions, if evolutionarily conserved, may be quite relevant to the comprehension the basic biology of individuals living with eating disorders. Typically patients do not respond well to psychological treatments while being nutritionally rehabilitated, therefore the ability to sustain them until their psychological constraints can be alleviated could be very beneficial. Developing *Drosophila* as a model to better understand the biological mechanisms underpinning resistance to starvation may provide an approach that could eventually lead to the development of therapies for these and other related human conditions.

List of abbreviations foxo: forkhead box subgroup "O"; Ilp: insulin-like peptide; IRS: insulin receptor signalling

Competing Interests

The authors declare that they have no competing interests.

Authors Contributions

JDS generated lines, performed the longevity and starvation assays, carried out the statistical analyses and drafted the initial manuscript. BES generated mutants and lines, conceived and participated in the design and supervision of the study and contributed significantly to the final draft of the manuscript.

Both authors have read and approved the final manuscript.

Animal Ethics

This study was conducted under the approval of the Animal Care Committee of Memorial University of Newfoundland as a Category of Invasiveness Level A protocol under the project title of “Genetic, biochemical and molecular analysis of cell survival and cell death in *Drosophila melanogaster*” (protocol number: 14-09-BS).

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Figure Legends

Figure 1: Longevity assay of novel *Akt1* hypomorphs display a shortened lifespan when compared to controls. Longevity is shown as percent survival ($P < 0.05$ as determined by log rank). The dotted line represents the median survival of the flies. The median lifespan of novel hypomorphic mutants falls between 8-10 days behind the wild type control. Full genotypes are *Akt1*⁺: *w*; +/+; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁺ (N=148); *Akt1*⁵²: *w*; +/+; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁵² (N=146); *Akt1*⁵⁷: *w*; +/+; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁵⁷ (N=167); *Akt1*⁸⁷: *w*; +/+; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁸⁷ (N=133). Error bars represent the standard error of the mean.****

Figure 2: Amino-acid starvation survivorship assays of novel *Akt1* hypomorphs indicate a long life phenotype that is suppressed when wild type *Akt1* is expressed. A: Amino-acid starvation survivorship of novel hypomorphs compared to a wild type control. Full genotypes are *Akt1*⁺: *w*; +/+; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁺ (N=237); *Akt1*⁰⁴²²⁶: *w*; +/+; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁰⁴²²⁶ (N=195); *Akt1*⁵²: *w*; +/+; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁵² (N=198); *Akt1*⁵⁷: *w*; +/+; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁵⁷ (N=206); *Akt1*⁸⁷: *w*; +/+; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁸⁷ (N=204). **B:** Amino-acid starvation survivorship of hypomorphs with background expression of wild type *Akt1*⁺. Full genotypes are *Akt1*⁺: *w*; *arm-GAL4/UASAkt1*⁺; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁺ (N=256); *Akt1*⁰⁴²²⁶: *w*; *arm-GAL4/UASAkt1*⁺; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁰⁴²²⁶ (N=243); *Akt1*⁵²: *w*; *arm-GAL4/UASAkt1*⁺; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁵² (N=254); *Akt1*⁵⁷: *w*; *arm-GAL4/UASAkt1*⁺; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁵⁷ (N=225); *Akt1*⁸⁷: *w*; *arm-GAL4/UASAkt1*⁺; *P[FRT; w⁺]^{2A} P[ry⁺neoR FRT]^{82B} *Akt1*⁸⁷ (N=235). Survivorship is shown as percent survival ($P < 0.05$ as determined by log rank). The dotted line represents the median survival of the flies. Error bars represent the standard error of the mean.**********

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316 **Figure 3: Longevity and amino-acid starvation survivorship assays of *Drosophila* bearing both novel**

317 ***Akt1* and a null *foxo* mutations: a synergistic effect upon standard media and an epistatic relationship**

318 **when starved of amino-acids. A: Longevity assay of double and single mutants. B: Amino-acid**

319 **starvation survivorship assay of single and double mutants. Full genotypes are *Akt1*⁺: *w*; +/+; *P*[*FRT*;**

320 ***w*⁺]^{2A} *P*[*ry*⁺*neoR FRT*]^{82B} *Akt1*⁺ (N=149 or 237); *Akt1*⁵⁷: *w*; +/+; *P*[*FRT*; *w*⁺]^{2A} *P*[*ry*⁺*neoR FRT*]^{82B} *Akt1*⁵⁷ (N=166**

321 **or 205); *foxo*^{W124X}: *w*; +/+; *foxo*^{W124X}/*foxo*^{W124X} (N=218 or 221); *Akt1*⁵⁷, *foxo*^{W124X}: *w*; +/+; *P*[*FRT*; *w*⁺]^{2A}**

322 ***P*[*ry*⁺*neoR FRT*]^{82B} *Akt1*⁵⁷/*foxo*^{W124X} (N=120 or 97). Survivorship is shown as percent survival (*P* < 0.05 as**

323 **determined by log rank). The dotted line represents the median survival of the flies. Error bars represent**

324 **the standard error of the mean.**

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Figure 1: Longevity assay of novel *Akt1* hypomorphs display a shortened lifespan when compared to controls.

54x39mm (300 x 300 DPI)

Figure 2: Amino-acid starvation survivorship assays of novel *Akt1* hypomorphs indicate a long life phenotype that is suppressed when wild type *Akt1* is expressed.
54x57mm (300 x 300 DPI)

Figure 3: Longevity and amino-acid starvation survivorship assays of *Drosophila* bearing both novel *Akt1* and a null *foxo* mutations: a synergistic effect upon standard media and an epistatic relationship when starved of amino-acids.
50x56mm (300 x 300 DPI)