Expression of heat shock protein 70 is insufficient to extend *Drosophila melanogaster* longevity.

Chengfeng Xiao[a], Danna Hull[a,b], Shuang Qiu[a], Joanna Yeung[c], Jie Zheng[d], Taylor Barwell[a], R Meldrum Robertson[a], Laurent Seroude[a,*]

[a] *Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada.*

[b] *Medical Genetics Unit, Kingston General Hospital, Kingston, Ontario K7L 2V7, Canada*

[c] *Department of Medical Genetics, Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, University of British Columbia, Vancouver, British Columbia V5Z 4H4, Canada*

[d] *Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, United States of America*

* Corresponding author at: Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada.
E-mail address: seroudel@queensu.ca (L. Seroude)

Keywords:

UAS/GAL4

Muscle

nervous system

stress resistance

development

synaptojanin

Abstract

It has been known for over 20 years that flies with twelve additional copies of the hsp70 gene encoding the 70 kDa heat shock protein lives longer after a non-lethal heat treatment. Since the heat treatment also induces the expression of additional heat shock proteins, the biological effect can be due either to HSP70 acting alone or in combination. This study used the UAS/GAL4 system to determine whether hsp70 is sufficient to affect the longevity and the resistance to thermal, oxidative or metabolic stresses of the whole organism. We observed that HSP70 expression in the nervous system or muscles has no effect on longevity or stress resistance but ubiquitous expression reduces the life span of males. We also observed that the down-regulation of Hsp70 using RNAi did not affect longevity. Two of the seven UAS-hsp70 strains tested did show GAL4-dependent life span extensions. The molecular characterization and preliminary genetic analysis of one of these strains suggests that the synaptic phosphatidylinositol phosphatase Synaptojanin limits the longevity of the fly.

Introduction

Drosophila 70 kDa inducible heat shock protein (HSP70) is synthesized in salivary glands, Malpighian tubules, brain, wing imaginal discs and many tissues after a heat shock (Ashburner and Bonner, 1979; Lewis et al., 1975; Tissieres et al., 1974).The expression of *hsp70* coincides with a well-known phenomenon of heat shock-induced chromosomal puffing in *Drosophila* salivary glands (Ashburner, 1970; Ritossa, 1962, 1963; Ritossa, 1964; Tissieres et al., 1974). HSP70 is the most abundant protein induced by heat shock in fly and many other organisms (Ashburner and Bonner, 1979; Lewis et al., 1975; Velazquez and Lindquist, 1984). The amino acid sequences of HSP70 are highly conserved among different species (Daugaard et al., 2007; Lindquist and Craig, 1988). HSP70 has a nucleotide binding domain (NBD) and a substrate-binding domain (SBD) and exhibits ATPase, disaggregase, holdase, translocase and unfoldase activities (Bukau et al., 2006; Fernandez-Funez et al., 2016; Hartl et al., 2011; Mattoo and Goloubinoff, 2014; Saibil, 2013). The HSP70 protein binds preferentially to hydrophobic residues but no consensus has emerged and the molecular mechanisms responsible for substrate specificity remain unknown (Clerico et al., 2015).

Drosophila hsp70 genes have undergone extensive duplication during evolution (Bettencourt and Feder, 2001). There are six nearly identical *hsp70* genes at two adjacent chromosomal loci 87A and 87C (Gong and Golic, 2004; Holmgren et al., 1979; Ishhorowicz et al., 1977). *Drosophila hsp70* contains no introns, presumably allowing rapid and abundant induction since it allows transcripts to circumvent the interruption of RNA splicing during heat shock (Lindquist, 1986; Yost and Lindquist, 1986). Upon heat shock and other types of stress, HSP70 increases and concentrates in the nuclei and nucleoli, where it is believed to bind incorrectly folded preribosome proteins and ribonucleoproteins (Kloetzel and Bautz, 1983; Pelham, 1984; Schlesinger, 1990; Velazquez and Lindquist, 1984). Heat shock has the most effect on nucleoli causing a loss of RNP granules, rRNA synthesis and processing, and ribosome assembly and export. During recovery, HSP70 leaves nuclei and is widely distributed in the cytoplasm. Without stress, cytosolic HSP70 and its cognate family members bind incorrectly folded proteins for refolding or degradation (Hartl et al., 2011; Mattoo and Goloubinoff, 2014; Saibil, 2013; Schlesinger, 1990). HSP70 mainly seems

to assist folding by stabilization of denatured or unfolding proteins until they reach an appropriate structural conformation.

Many studies have examined the function of HSP70 under stress. Monkey cells transfected with the *Drosophila hsp70* gene showed a better recovery of nucleolar morphology and of ribosome export after heat shock (Pelham, 1984). Rat cells transfected with the human *hsp70* gene have a better thermal resistance (Li et al., 1991). Reciprocally, HSP70 immuno-neutralization of rat fibroblasts impairs its translocation to the nucleus and cell survival during thermal stress (Riabowol et al., 1988). Competitive inhibition of hsp70 expression elevates the thermosensitivity of hamster ovary cells (Johnston and Kucey, 1988). Genetic and transgenic manipulations established that HSP70 alters thermotolerance in the whole organism as well. The addition of 12 extra *hsp70* copies confers greater and faster tolerance to lethal temperatures while the deletion of all *hsp70* genes show the opposite (Bettencourt et al., 2008; Feder et al., 1996; Gong and Golic, 2006; Welte et al., 1993). Thermoprotection is also achieved by increasing hsp70 expression with the UAS/GAL4 system. Ubiquitous or motoneurons-specific expression protects larval locomotion during hyperthermia (Xiao et al., 2007). Mice lacking the stress-induced hsp70 genes display genomic instability, reduced body weight, sensitivity to ionizing radiations, impaired response to skeletal muscle injury and, enhanced onset, severity and progression of Huntington's disease symptoms (Hunt et al., 2004; Senf et al., 2013; Wacker et al., 2009). Mice overexpressing hsp70 are less affected and recover better from muscle damage, and display a better preservation of muscle strength and slower progression of muscular dystrophy (Gehrig et al., 2012; McArdle et al., 2003).

The biochemical properties and protective effects of HSP70 raise the possibility that HSP70 is one of the determinants of the longevity of an organism. HSP70 accumulates with age in the fly muscles in correlation with increased oxidative stress and molecular damages, loss of proteostasis and induction of apoptosis (Demontis and Perrimon, 2010; Wheeler et al., 1995; Zheng et al., 2005). The level of HSP70 is also increased in the skeletal muscles of old rats (Chung and Ng, 2006). Tissue-specific selective HSP70 accumulation suggests that it might help prolong the life span of the organism (Wheeler et al., 1999). Consistent with this hypothesis, *hsp70* alleles correlate with human

longevity and HSP70 level at early age is partially predictive of the remaining life span in flies (Singh et al., 2010; Singh et al., 2006; Yang and Tower, 2009). Furthermore a non-lethal heat treatment extends the adult fly life span and the amplitude of the extension is higher in a strain with twelve additional *hsp70* copies (Khazaeli et al., 1997; Tatar et al., 1997). Although these observations demonstrate that HSP70 influences longevity, the heat treatment also induces the expression of additional heat shock proteins and therefore the biological effect can be due either to HSP70 acting alone or in combination. The overexpression of *hsp26*, *hsp27* and *hsp68* with the UAS/GAL4 system is sufficient to increase longevity and the resistance to oxidative stress (Liao et al., 2008; Wang et al., 2004; Wang et al., 2003). Although the overexpression of hsp70 is detrimental to growth and survival during development, several pathways influencing longevity control growth and the hyperfunction theory proposes that growth causes aging (Feder et al., 1992; Gems and Partridge, 2013; Krebs and Feder, 1997).

This study uses the UAS/GAL4 system to control the expression of *hsp70* without the need for a heat treatment and therefore be able to examine whether HSP70 is sufficient to affect organismal longevity and stress resistance.

Results

Gal4 expression across age

Three GAL4 drivers were selected for this study: da-GAL4 (Wodarz et al., 1995), D42 (Parkes et al., 1998; Yeh et al., 1995) and DJ694 (Seroude et al., 2002). DJ694 is an adult muscle-specific driver. The pattern and dynamics of GAL4 expression during aging has been described (Seroude et al., 2002). Newly emerged DJ694 mostly express GAL4 in the abdominal muscles. The expression level increases rapidly, predominantly in the thoracic flight muscles, to reach a two- to three-fold higher level within ten days. It remains constant until around 30 days and then gradually declines. Expression patterns of da-GAL4 and D42 from the embryo to the emergence of the adult have been reported (Parkes et al., 1998; Wodarz et al., 1995; Yeh et al., 1995). However, the anatomical location and dynamic of GAL4 expression during aging is missing. Therefore the spatial-temporal expression of GAL4 across age was determined by driving a UAS-lacZ reporter encoding the *E. coli* β-galactosidase (Figure 1).

The temporal expression profiles show that da-GAL4 expresses GAL4 at the highest level upon emergence but it drastically decreases by at least twenty-fold during the first two weeks and remains constant for the remainder of the life span (Figure 1A). It appears that the initial decrease is slightly less pronounced in the females. The D42 driver displays a constant level of GAL4 expression across the entire life span that is almost identical between sexes. The expression across age shows that da-GAL4 drives GAL4 expression almost ubiquitously while D42 is strictly restricted to the nervous system (Figure 1B). The da-GAL4 driver is not completely ubiquitous because no expression is detected in the testis. Furthermore it is obvious that the expression is not homogenous. In the younger animals, the signal in the central nervous system (brain and ventral cord) appears weaker than in other tissues and in the older animals is barely detectable. The signal is also hardly visible in one of the vertical thoracic flight muscle of the oldest animals. Although D42 targets specifically the central nervous system, it is not homogenous. Since both drivers drive similar level of expression in older animals, D42 result in higher overexpression in the nervous system than da-GAL4.

hsp70 overexpression

Transgenic UAS-hsp70 fly strains that carry the Hsp70Ab cDNA under the control of a UAS promoter have already been obtained and described (Xiao et al., 2007). These strains express the *hsp70* coding region followed by a C-terminal EQLRSTSRTM linker and EQKLISEEDL myc epitope. The HSP70-myc fusion produced can be differentiated from the endogenous HSP70 by size and can be detected with myc and HSP70 antibodies (Figure 2 and S1). The level of *hsp70* expression can vary between strains because the UAS-hsp70 transgene does not contain chromatin insulator elements and can therefore be affected by the genomic sequences surrounding its insertion. The effects of the genome are advantageous by providing an opportunity to test different levels of expression with the UAS/GAL4 system without using the temperature shifts that are required to modulate GAL4 activity directly or indirectly with a thermosensitive GAL80 negative regulator of GAL4 (Duffy, 2002; Lee and Luo, 1999). Therefore, this study used seven UAS-hsp70 strains. The level of HSP70-myc expression was determined by Western-blot with a myc antibody (Figure 2). Three and ten days old animals were processed because of the early expression changes associated with the DJ694 and da-GAL4 drivers. Three days after emergence, a

specific HSP70-myc signal is clearly visible in the extracts from animals carrying the da-GAL4 transgene and one of the UAS-hsp70 transgene (da-GAL4::#_._) but not in extracts from control animals that lack one of the two transgenes (#_._/+ and da-GAL4/+)(Figure 2A). At ten days, as expected from the temporal profile of GAL4 expression, the HSP70-myc signal is significantly reduced compared with three days ($P < 0.0001$)(Figure 2B). Although most of the variation is largely due to age factor, the genomic location of the UAS-hsp70 transgene (genotype) has a significant ($P < 0.0001$) but modest effect on the expression level.

In agreement with the GAL4 expression profile, Hsp70-myc levels increased significantly between three and ten days with the DJ694 driver ($P < 0.0001$) but not with the D42 driver ($P = 0.5751$). With both drivers, the location of the UAS transgene has a significant effect on the expression level and the range of expression obtained is wider than with the da-GAL4 driver. Between the genotypes with the lowest and highest expression level, there is a ~40% (10 days) to ~130% (3 days) difference with da-GAL4, a ~135% (3 days) to ~165% (10 days) with DJ694 and a ~410% (3 days) to ~560% with D42 (10 days).

hsp70 overexpression does not cause developmental defects

Since the constitutive expression of HSP70 in the larval salivary glands at normal temperatures severely reduced cell size (Feder et al., 1992), a developmental analysis was first conducted to determine if the GAL4-driven expression induce developmental delay or lethality (Figure 3, Table S1). Whatever GAL4 driver is used and whatever the genomic location of the UAS-hsp70 transgene, the totality of viable individuals for any genotype (controls or experimental) were obtained 26-30 h (first instar L1 larvae), 5-6 days (pupae) and 10-12 days after egg laying. Earlier scoring underestimated the number of viable individuals while later scoring did not change the total number indicating that *hsp70* expression with these drivers does not delay development.

No lethality was observed with the D42 driver. With the DJ694 driver, the UAS-hsp70 insertions #9.1 and #10.1 yielded significantly less L1 larvae indicating some embryonic lethality. The #4.3 insertion with the DJ694 driver and the #9.1 with the da-GAL4 driver yielded significantly less pupae indicating some larval lethality. Since most of the UAS-hsp70 insertions do not show any significant differences between experimental and control

animals, the embryonic and larval lethality observed result from insertion effects, leading to the conclusion that the overexpression of hsp70 does not impair development.

hsp70 overexpression does not affect stress resistance

The survival to three kinds of stress was measured in both sexes after three and ten days of adulthood (Table S2). High temperature stress was tested by exposure at 36°C. Oxidative stress was assessed by feeding the free radical generator paraquat. Metabolic stress was also examined with a dry starvation assay. At least three and up to 10 replicates were processed per stress, sex, age, UAS-hsp70 insertion and GAL4 driver. The results from each replicate are compiled in the figure 4 (males) and the supplemental figure S2 (females). These data do not show any consistent effect between replicates across the seven UAS strains whatever the kind of stress and location of hsp70 expression. Furthermore only very few genotypes displayed consistently a significant effect. Consistent identical effects in all the replicates is only seen in two situations in each sex. In males, the #9.1 insertion with the ubiquitous driver decreased oxidative stress survival at 10 days while the #3.1 insertion with the nervous system driver increased it at 3 days. In females, at 10 days the nervous system driver increased the survival to heat with the #3.2 insertion while it decreased the survival to starvation with the #9.1 insertion. Overall these data indicate that hsp70 expression is insufficient to affect the resistance of the organism to heat, oxidative or metabolic stresses.

hsp70 is insufficient to extend longevity

The longevity of flies with both UAS-hsp70 and GAL transgenes was measured and compared to control flies lacking the GAL4 or UAS transgene (Table S3). Consistent results are only seen across all UAS-hsp70 insertions in males with the ubiquitous driver (Figure 5). All insertions resulted in a reduction of the life span in all replicates with the exception of #2.1 and #3.1 that showed this effect in 5 out of 6 and 6 out of 8 replicates respectively. No consistent effects are seen in females (Supplemental Figure S3) or with the DJ694 and D42 drivers. These results show that the overexpression of HSP70 in the muscles or nervous system is insufficient to affect longevity. However the reduction of the life span of males with the ubiquitous driver indicate that HSP70 is detrimental in an anatomical location that remains to be identified.

Life span extensions were consistently obtained across replicates with the #3.2 and #10.1 insertions. Both insertions driven by DJ694 or D42 extend the longevity of males in all replicates except for #3.2 that revealed an extension in 5 out of 6 replicates with D42. The extensions associated with #3.2 appear to be also consistent in the females but with a lower magnitude. The extensions associated with #10.1 is seen in females with DJ694 but not with D42. The increased longevity with these insertions is not correlated with the level of hsp70 expression. With D42, #3.2 and #10.1 express the highest and lowest levels respectively. With DJ694, #3.2 and #10.1 express high levels that are similar to the #9.1 insertion that does not display any change in all replicates.

Down-regulation of hsp70 does not affect longevity

The *hsp70* cDNA section was excised from the pCX1 plasmid (Xiao et al., 2007) previously used to generate the UAS-hsp70-myc transgenic strains and inserted into the SympUAST RNAi plasmid (Supplemental Figure S4) (Giordano et al., 2002). The resulting pCX9 plasmid allows symmetrical transcription of *hsp70* from two convergent UAS promoters and was used to obtain nine transgenic strains. The effectiveness to reduce *hsp70* expression was first tested with the DJ694 driver by co-expressing each UAS-hsp70-RNAi insertion with the UAS-hsp70 #3.1 insertion (Supplemental Figure S5A). Seven out of the nine RNAi insertions completely prevented the detection of the transgenic HSP70-myc whereas it remains detectable with R5.2 and unaffected with R5.12. Next the effectiveness of the R3.12 insertion to reduce endogenous hsp70 expression was examined with the three GAL4 drivers (Supplemental Figure S5B). As one would expect from the anatomical location of GAL4 expression, almost complete or strong reduction of heat-induced HSP70 is observed with daGAL4 and DJ694 respectively whereas D42 expression is too localized to affect whole animal extracts. Finally the effects of the DJ694-driven R3.12 and R5.14 insertions on endogenous HSP70 expression were compared (Figure 6A). Both insertions clearly suppress HSP70 expression but R3.12 appears to be more efficient when the level of expression is higher after heat shock.

The longevity with both UAS-hsp70-RNAi and GAL transgenes was measured and compared to control flies lacking the GAL4 or UAS transgene (Table S4). Two out of the three RNAi insertions tested with all drivers did not show any effects in both sexes (Figure

6B). These results indicate that the down-regulation of hsp70 is insufficient to influence the longevity of the organism.

Molecular analysis of the UAS-hsp70 #3.2 and #10.1 insertions

The GAL4-dependent extended lifespan observed with only two of the seven UAS-hsp70 insertions suggests that it is mediated by the altered expression of genes downstream of the UAS promoter. The genomic location of the *UAS-hsp70* transgene was determined by inverse-PCR in the #3.2 and #10.1 strains (Figure 7A). In #10.1, the transgene is inserted at the cytological position 53E2 in the fifth exon of the RC transcript of the *mute* (*muscle wasted*) gene (Bulchand et al., 2010). The transcriptional orientation of the UAS promoter is opposite to *mute* but within 10Kb downstream the *CG34191*, *PIG-V* and *CG6665* genes have the same orientation. *CG34191* encodes the UFM1 Ubiquitin-fold modifier that is involved in the development of neuromuscular junctions (Duan et al., 2016). *PIG-V* is a phosphatidylinositol glycan anchor biosynthesis class V gene that is involved in the synthesis and transfer of lipid bilayer glycosylphosphatidylinositols anchors (Rosenbaum et al., 2012). *CG6665* encodes a polypeptide whose function remains to be determined (Thurmond et al., 2019). In #3.2, the transgene is inserted at the cytological position 58B2-3 in the first exon of the CG11474 gene encoding a polypeptide with a protein methyltransferase domain (Thurmond et al., 2019). The adjacent CG2921 gene also encodes a polypeptide with a protein methyltransferase domain (Thurmond et al., 2019). Among the genes downstream of the insertion, only the *GlcT-1* and *Synj* genes use the same transcriptional orientation as the UAS promoter. *GlcT-1* encodes a ceramide glucosyltransferase linked to fat metabolism (Kohyama-Koganeya et al., 2011). *Synj* encodes the synaptic phosphoinositide phosphate phosphatase SYNAPTOJANIN implicated in the uncoating of nascent endocytic vesicles (Verstreken et al., 2003). Semi-quantitative RT-PCR was performed to determine whether the transgene affects the transcription of the genes downstream of the UAS promoter (Figure 7B). Ten days old DJ694::#3.2 flies were examined because they exhibited the strongest extension of longevity and the highest level of GAL4 expression. Compared to controls lacking either the driver or the UAS transgene, slight but insignificant increases of *CG2921* and *GlcT-1* transcripts are observed whereas *synj* is significantly increased (Figure

7C). To test if the increased expression of *synj* is involved in the lifespan extension observed with the #3.2 transgene, the longevity of males expressing a UAS-synj transgene with the DJ694 or D42 drivers was measured (Figure 7D). The average life span of both DJ694::UAS-synj and D42::UAS-synj were significantly longer than the UAS-synj/+ (all replicates: P<0.0001) and DJ694/+ or D42/+ controls.

Discussion

The UAS/GAL4 system allowed extensive and precise control of Hsp70 expression within a constant environment. The manipulation of HSP70 level during the pre-adult stages did not cause any visible morphological defect, lethality or developmental delays. Under normal conditions, *Hsp70* mRNA (Ireland et al., 1982; Welte et al., 1993) and protein (Feder et al., 1996) cannot be detected in embryos and larvae, and null mutants are morphologically normal, viable and fertile (Gong and Golic, 2004). Developmental lethality has been reported when twelve extra copies of *Hsp70* are present (Krebs and Feder, 1997) but the level of HSP70 expression achieved by the UAS/GAL4 system is not comparable since the DJ694 and D42 drivers are tissue-restricted and the amount of HSP70 produced with da-GAL4 is obviously lower than the amount produced by heat shock (Figure S1)(Klose et al., 2005). Since the analysis of null *Hsp70* mutants have demonstrated that HSP70 is required for basal and induced thermotolerance of larvae and adults (Gong and Golic, 2006; Shilova et al., 2018), the stress resistance of animals expressing RNAi transgenes was not investigated. The overexpression of *Hsp70* with any of the three GAL4 drivers did not alter the response of adult flies to thermal, oxidative or metabolic stresses. Although it has long been known that increasing *Hsp70* expression is sufficient to increase the thermotolerance of cultured cells (Li et al., 1991; Pelham, 1984; Solomon et al., 1991), it is not surprising that it is not the case *in vivo* in the whole organism (Halliwell, 2003; Place et al., 2017; Yazdani, 2016). This report adds further evidence that elevated *Hsp70* expression is sufficient to affect thermotolerance of embryos (Welte et al., 1993), larvae and pupae (Feder et al., 1996) but not adults (Feder and Krebs, 1998; Jensen et al., 2010). The effect of *Hsp70* overexpression on the resistance to oxidative and metabolic stresses has been investigated in the presence of additional *Hsp70* copies and did not have any consequences with or without heat shock (Minois, 2001). The UAS/GAL4 system yields a range of HSP70

expression levels that are intermediate between the uninduced low level and the heat-induced high level used by Minois. This study complements the observation reported by Minois and reinforces the conclusion that the manipulation of *Hsp70* expression alone is insufficient to affect the resistance of the organism to stress.

The reduction of *Hsp70* expression with RNAi transgenes did not affect longevity. Although the western-blot analysis confirmed the effectiveness of the transgenes, it remains possible that there is residual expression. The null *Hsp70* mutant does completely prevent expression and has been recently reported to exhibit a modest reduction of the life span (Shilova et al., 2018) indicating that *Hsp70* is required for longevity and that only minimal amount is needed accounting for the lack of correlation between longevity and *Hsp70* RNA levels in wild-type strains (Zhao et al., 2005). The UAS/GAL4 system allowed for testing of the effect of *Hsp70* levels above wild-type levels without any environmental modifications that affect the expression of additional genes. Ubiquitous GAL4-mediated *Hsp70* overexpression reduced male longevity while ubiquitous overexpression by heat treatment of males carrying extra *Hsp70* copies had the opposite effect (Tatar et al., 1997). This result suggests that *Hsp70* has detrimental effects on longevity that are mitigated or abolished by the simultaneous expression of one or several gene products induced by the heat treatment. After extended periods of recovery from heat shock HSP70 intracellular granules are formed to control HSP70 activity by sequestration (Feder et al., 1992), a mechanism that may depend on gene product(s) induced by heat. Alternatively, the overexpression of *Hsp70* with the da-GAL4 driver during the pupal stage may not be detrimental enough to cause lethality and instead caused the emergence of sick animals. It is unlikely that the detrimental effect is due to overexpression in the nervous system or muscles since it is not observed with the D42 or DJ694 drivers. It will be worth investigating additional GAL4 drivers with different tissue-specificity in order to identify the anatomical location responsible for the detrimental effect in males.

The overexpression of *Hsp70* in the nervous system, muscles in both sex or ubiquitously in females had neither beneficial nor detrimental effects on life span indicating that HSP70 is insufficient to extend longevity. It is important to note that identical results were obtained in muscles with the MHC-GAL4 driver and independent UAS-hsp70 strains (Demontis and Perrimon, 2010). However it cannot be excluded that higher levels of overexpression may

be required to affect the life span since heat treatment of animals with extra Hsp70 copies lead to higher overexpression levels than the GAL4 drivers used in this study. The level of expression induced by the DJ694 driver is the highest that is currently available (Barwell et al., 2017; Seroude, 2002; Seroude et al., 2002) and preliminary investigations using two instead of one copy of the UAS transgenes failed to further increase expression levels. It is also possible that the overexpression during the pre-adult stage is not detrimental enough to cause developmental lethality and reduced longevity but enough to prevent the extension of the life span although it is unlikely with the DJ694 driver that is not expressed in muscles at any stage of development (Barwell et al., 2017). If HSP70 alone is indeed insufficient to extend longevity, the extension after heat treatment of animals with extra *Hsp70* copies would suggest that HSP70 extends longevity in combination with one or several gene products induced by the heat treatment. The identification of HSP70 partners could easily be investigated by measuring the longevity of animals carrying a UAS-hsp70 transgene as well as a UAS transgene for a gene to be tested.

Two out of seven UAS strains resulted in a GAL4-dependent extension of the lifespan with the muscle and nervous system drivers. The molecular characterization of the #3.2 strain revealed that the UAS transgene is inserted in the vicinity of the *Synj* gene and the expression of this gene is higher in presence of GAL4. Furthermore the overexpression of a UAS-Synj transgene with the muscle and nervous system drivers extend longevity. It is noticeable that this transgene does not increase longevity as much as the #3.2 transgene. The UAS-Synj transgene expresses a protein fused at the N terminal with an enhanced green fluorescent protein (eGFP)(Dickman et al., 2005). Although the fusion protein is able to rescue the larval lethality of *Synj* mutants, the addition of eGFP may reduce the ability to affect longevity. Alternatively, the co-overexpression of *Hsp70* with the #3.2 transgene may contribute to the longevity phenotype. The generation of UAS-Synj strains expressing the native protein as well as testing the longevity of animals with the UAS-synj transgene combined with an UAS-hsp70 transgene insertion that did not affect the life span will allow to differentiate between these hypotheses.

Materials and Methods

Drosophila strains and cultures

Eleven UAS-hsp70 strains carrying independent insertions of pINDY5-hsp70 (pCX1) have been previously obtained (Xiao et al., 2007). Seven UAS strains with pCX1 inserted on the X, II or III chromosomes were used: UAS-hsp70 #2.1 (X), UAS-hsp70 #4.3 (X), UAS-hsp70 #9.1 (X), UAS-hsp70 #3.2/CyO (II), UAS-hsp70 #10.1/CyO (II), UAS-hsp70 #3.1 (III) and UAS-hsp70 #4.4 (III). They are referenced in short as #2.1, #4.3, #9.1, #3.2, #10.1, #3.1 and #4.4. The D42 (obtained from G. Boulianne), da-GAL4 (obtained from J. Merriam) and DJ694 Gal4 strains have been already described (Seroude et al., 2002; Wodarz et al., 1995; Yeh et al., 1995). The UAS-lacZ (#1777) strain was obtained from the Bloomington drosophila stock center. The UAS-synj (UAS-eGFP-HA-synj) strain was derived from the w*;synj^{LY}/CyO;pUAS-eGFP-HA-synj strain (Dickman et al., 2005)(4-E-10 obtained from H. Bellen). Nine UAS-hsp70-RNAi strains (R1.17[III], R1.26[II], R2.17[III], R3.12[X], R4.15[III], R5.2[III], R5.12[II], R5.12e[III] and R5.14[III]) carrying independent insertions of Sym-pUAST-hsp70 (pCX9) were obtained by P-element mediated transformation (Robertson et al., 1988). Standard cornmeal medium was used (0.01% molasses, 8.2% cornmeal, 3.4% killed yeast, 0.94% agar, 0.18% benzoic acid, 0.66% propionic acid).

GAL4 expression across age

D42, da-GAL4 and w males were crossed with UAS-lacZ females. Age-synchronized cohorts of the resulting adult progenies were obtained by emptying cultures and collecting newly emerged flies within 48 h. Males and females were maintained separately at 25°C at a density of 25-30 individuals per vial. Fresh media was provided by transferring to new vials at least twice a week. Individuals were randomly removed at the selected ages by aspiration.

The temporal profiles of GAL4 expression were obtained by quantification of ß-galactosidase activity with a CPRG assay (Seroude et al., 2002). Individual flies were manually homogenized in 100 μl extraction buffer (50 mM Na2HPO4/NaH2PO4, 1 mM MgCl2, pH 7.2, 1 cOmplete™ protease inhibitors cocktail tablet(Roche)/40ml). At least five individuals were processed per age, sex and genotype. Extracts were centrifuged for 1 min at 13 000 g. 10 μl of extract supernatant was mixed with 100 μl 1 mM chlorophenolred-ß-D-galactopyranoside (CPRG, Roche). Background levels from the negative control (progeny of

cross with w) were averaged and subtracted from the experimental samples. Enzymatic activity was calculated as the rate of change of OD_{562nm} per minute (ΔmOD562/min/fly), and standardized to a single fly equivalent.

The anatomical location of GAL4 expression was determined by 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) staining (Poirier et al., 2008; Seroude et al., 2002). At least two males and two females were processed per age and genotype. 10 microns cryosections were fixed for 20 minutes with 1% glutaraldehyde in Phosphate Buffered Saline Solution (1x PBS) (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.4). Sections were washed twice for 3-5 minutes with 1x PBS before being reacted with the X-gal solution (0.2 % X-gal, 3.1 mM K3Fe(CN)6, 3.1 mM K4Fe(CN)6, 150 mM NaCl, 1 mM MgCl2, 10 mM Na2HPO4/NaH2PO4) at room temperature. Reactions were terminated with 3 3-5 min washes with 1x PBS. The sections were then mounted in 70% glycerol in PBS. Images were obtained on a Zeiss Axioplan II imaging microscope with a Leica DC500 high-resolution camera and the OpenLab imaging software (Improvision, Lexington, MA). All images were captured with the same exposure time and light intensity.

Western blot

One male and one female were homogenized in 50 μl of extraction buffer (10 mM Tris·Cl, pH 8.0, 50 mM NaCl, and 1 % NP-40) supplemented with protease inhibitors (cOmplete Cocktail Tablet, Roche). Extracts were mixed with 50μl 2× SDS loading buffer (0.125 M Tris·Cl, pH 6.8, 4.6 % SDS, 20 % glycerol, 10 % β-mercaptoethanol, and 0.1 % bromophenol blue), incubated for 5 min at 100˚C and centrifuged for 5 min at 10,000 g. 20 μg samples were separated on a 7.5 % SDS-PAGE gel and transferred to nitrocellulose membrane. HSP70-myc was detected with the mouse 9E10 monoclonal anti-myc antibody (11667203001, Roche) at 1:5000 or the mouse 5A5 monoclonal anti-human HSP70 antibody (MA3-007, ThermoFisher Scientific) at 1:1000. α-Tubulin was used as loading control and detected with the mouse DM1A monoclonal anti-chicken α-Tubulin (Ab7291, Abcam) at 1:5000. Primary antibodies were incubated at 4˚C overnight. After washes, the nitrocellulose membrane was then incubated with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG antibody (#170-6516, Bio-Rad) at 1:5000 at 4˚C overnight. The ECL advance Western blotting detection kit (RPN2135, Amersham) was used for signal detection. Results were visualized and imaged with ChemiGenius bio-imaging system

(Syngene, USA). ImageJ (NIH, USA) was used for the measurement of optic density of specific bands. Hsp70-myc level was normalized to α-Tubulin for quantification. Two-way ANOVA with Bonferroni post-test was performed with GraphPad™ Prism 5 to compare Hsp70-myc expression levels.

Developmental analysis

Control animals were obtained by crossing w males with homozygous UAS-hsp70 females (UAS control) and homozygous GAL4 driver males with w females (GAL4 control). Experimental animals were generated by crossing homozygous UAS-hsp70 females with homozygous GAL4 driver males. For each cross, 20-30 males and 50 virgin females were mated for two days. At least 200 0-4 h eggs were collected from each cross and transferred with forceps onto petri plates filled with media (25 eggs per plate, 8 plates per genotype). The plates were then incubated at 25 ˚C. Hatching rate were assessed 26-30h after egg transfer by scoring the number of empty eggshells. Pupation and adult emergence rates were scored 5-6 days and 10-12 days after egg transfer respectively.

Stress tests

Control animals were obtained by crossing w males with homozygous UAS-hsp70 females (UAS control) and homozygous GAL4 driver males with w females (GAL4 control). Experimental animals were generated by crossing homozygous UAS-hsp70 females with homozygous GAL4 driver males. 0-2 days old males and females were collected from control and experimental crosses and aged at 25˚C in separate vials with media (20-30 flies per vial, 2 vials per replicate, vials replaced twice a week) until tested. Paraquat oxidative stress tests were performed as previously described (Lin et al., 1998). Dry starvation tests were done by transferring control and experimental animals to empty vial. Heat stress tests were performed by immersing vials in a 36˚C water bath (water level above the bottom of the plug). The number of dead flies was scored every 4-5 hours. Kaplan-Meier (Log-rank) analysis was done with GraphPad™ Prism 5 to compare survival upon stress between control and experimental genotypes.

Longevity measurements

Control animals were obtained by crossing w males with homozygous UAS females (UAS control) and homozygous GAL4 driver males with w females (GAL4 control). Experimental animals were generated by crossing homozygous UAS females with homozygous GAL4

driver males. 0-2 days old males and females were sorted and collected from control and experimental crosses under nitrogen anesthesia for less than three minutes. The longevity of each genotype is obtained from four vials of males and four vials of females (25-30 animals per vial) kept at 25°C (except for synj longevities that only used males and were kept at 22°C). Animals were transferred to vials with fresh media and the mortality recorded at least twice a week. Four to eight replicates were measured (two replicates for synj longevities). Each replicate used independent cultures and parents. Kaplan-Meier (Log-rank) analysis with GraphPad™ Prism 5 was used to determine the significance of experimental animals that displayed a mean longevity higher or lower than both kinds of control animals.

Inverse PCR

Genomic DNA was extracted with QIAamp DNA Mini Kit (Cat. No. 51304, Qiagen), digested with MspI (R0106S, New England Biolabs), ligated with T4 DNA ligase (M0202S, New England Biolabs), and PCR amplified with 300 nM DJ51 primer (5'-GAGTTAATTCAAACCCCACGG-3'), 300 nM DJ39 primer (5'-CCGGGGTACCCCTAGGCGGATCCAGACATGATAAGATAC-3'), 200 μM dNTPs and 1 unit of Taq polymerase (M0273S, New England Biolabs). The PCR product was sequenced by Cortec DNA service laboratories, Inc. (Kingston, Canada).

RT-PCR

Semi-quantitative RT-PCR was done as previously described (Zheng et al., 2005) with the following modifications. Total RNA was extracted from 10 days old male flies with the RNeasy Mini Kit (Cat. No. 74104, Qiagen). Two independent extractions were performed for each genotype. 900ng RNA was reverse-transcribed with the SuperScript II reverse transcriptase (Cat. No. 18064-022, Invitrogen) in a 20 μl reaction. PCR was conducted in a final 25 μl reaction containing 5 μl diluted reverse transcriptase reactions (1:100, 1:1,000 or 1:10,000), 300 nM forward primer and 300 nM reverse primer, 200 μM dNTPs and 1 unit of Taq polymerase (M0273S, New England Biolabs). These primers were used:

5'-AGTACACACGACGATCAGAGAACAG-3' (*CG2921* forward)

5'-CTTATGCTTCTTCAGTTTAGGGTTTTC-3' (*CG2921* reverse)

5'-TATTCTCAAGCCCCTCATGG-3' (*GlcT-1* forward)

5'-GGCCATATAACCTGGATGGA-3' (*GlcT-1* reverse)

5'-GGCCATATAACCTGGATGGA-3' (*synj* forward)

5'-CAACAACCAGGACTGGCAAT-3' (*synj* reverse)

10 μl of the PCR reactions were loaded and separated in a 2 % agarose gel. DNA bands were stained with ethidium bromide (0.5 μg/ml) for 1 hour, destained in distilled water for 6 hours, and imaged with Syngene Gene Genius Bio Imaging System (SynGene). The optic density (OD) of DNA bands was measured with ImageJ (Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018). Relative level was calculated as a ratio of OD values between DNA band and internal background (the RT-PCR reaction without RTase). Significant differences were assessed by two-way ANOVA with GraphPad™ Prism 5.

Declarations of interest

None.

Authors contribution

CX, DH, SQ, JY and JZ performed experiments. CX, TB and LS analyzed experimental data. RMR and LS designed the study and obtained funding. LS and TB wrote the manuscript.

Acknowledgments

The authors thank the Bloomington Stock Center (National Institutes of Health P40OD018537), Hugo Bellen, Gabrielle Boulianne and John Merriam for fly strains, and Rhonda Kristensen and Frederique Seroude for technical support. This work was supported by the Institute of Aging of the Canadian Institutes of Health Research (CIHR, MOP64248, MOP79519) and the Natural Sciences and Engineering Research Council of Canada (RGPIN/250140-2010). The sponsor(s) had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Figure legends

Figure 1: Spatial-temporal expression of da-Gal4 and D42 in adult flies. A) Quantification of ß-galactosidase activity across age in males (black circles) and females (grey triangles) with a CPRG assay. Each data point is the average of five measurements ±SD. y axis: Activity (ΔmOD/min/fly); x axis: Age (days at 25˚C). B) Localization of ß-galactosidase activity across age by X-Gal staining. am: abdominal muscles; b: brain; ds: digestive system; t: testis; tm: thoracic muscles; vc: ventral chord.

Figure 2: Transgenic Hsp70 expression. A) Western-blot with monoclonal 9E10 antibody of Hsp70-myc expression driven by da-Gal4 at three and ten days. (B) Quantitative analysis. The table shows the sources of variance by a two-way ANOVA.

Figure 3: Survival during development of flies overexpressing HSP70. The mean L1 larvae (% hatching), pupae (% pupation) and adult (% eclosion) survival ±SD are plotted. Each graph compares the experimental GAL4 + UAS animals (black) to control animals missing the UAS transgene (GAL4 alone, grey) or the GAL4 driver (UAS alone, white). Statistical analysis is provided in Table S1.

Figure 4: Survival to thermal, oxidative and metabolic stresses of 3 days and 10 days old males overexpressing HSP70. Each triangle reports the outcome of the statistical comparison (Table S2) between experimental and controls for an experimental replicate. ▲: significant increase △: increase ▼: significant decrease ▽: decrease ▷: no change.

Figure 5: Longevity of males overexpressing HSP70. Typical longevity curves (x axis: days at 25˚C; y axis: percentage alive) are shown. The outcome of the statistical comparison (Table S3) between experimental and controls for each

experimental replicate is provided at the top of each graph: ▲: significant increase

△: increase ▼: significant decrease ▽: decrease ▷: no change.

Figure 6: Hsp70 RNAi. A) Western-blot analysis of seven days old male extracts. HS -: no heat-shock; HS +: heat-shock. B) Longevity summary. Each triangle reports the outcome of the statistical comparison (Table S4) between experimental and controls for an experimental replicate: ▲: significant increase △: increase ▼: significant decrease ▽: decrease ▷: no change.

Figure 7: Molecular analysis of UAS-Hsp70 insertions. A) Genomic location of the UAS-hsp70 #3.2 (58B2-3; 22,073,317-22,073,318) and #10.1 (53E2; 16,951,797-16,951,798) insertions. The insertion is depicted by the pCX1 red triangles. The exons of genes surrounding the insertion are depicted as green boxes (darker green indicates coding regions) with an arrowhead indicating the orientation of transcription. B) Semi-quantitative RT-PCR analysis of *CG2921*, *GlcT-1* and *synj* transcripts. The sizes of the ladder are indicated on the left while the sizes of the expected PCR products are displayed on the right. C) Relative transcript levels of *CG2921*, *GlcT-1* and *synj*. * P<0.05. D) The expression of UAS-synj with the DJ694 and D42 drivers extends male longevity. The P value vs the driver alone control is indicated for each experimental replicate. All replicates compared to the UAS alone control yielded P<0.0001.

References

Ashburner, M., 1970. Patterns of puffing activity in the salivary gland chromosomes of Drosophila. V. Responses to environmental treatments. Chromosoma 31, 356-376.

Ashburner, M., Bonner, J.J., 1979. Induction of Gene Activity in Drosophila by Heat Shock. Cell 17, 241-254.

Barwell, T., DeVeale, B., Poirier, L., Zheng, J., Seroude, F., Seroude, L., 2017. Regulating the UAS/GAL4 system in adult Drosophila with Tet-off GAL80 transgenes. PeerJ 5, e4167.

Bettencourt, B.R., Feder, M.E., 2001. Hsp70 duplication in the Drosophila melanogaster species group: How and when did two become five? Mol Biol Evol 18, 1272-1282.

Bettencourt, B.R., Hogan, C.C., Nimali, M., Drohan, B.W., 2008. Inducible and constitutive heat shock gene expression responds to modification of Hsp70 copy number in Drosophila melanogaster but does not compensate for loss of thermotolerance in Hsp70 null flies. BMC Biol 6, 15.

Bukau, B., Weissman, J., Horwich, A., 2006. Molecular chaperones and protein quality control. Cell 125, 443-451.

Bulchand, S., Menon, S.D., George, S.E., Chia, W., 2010. Muscle wasted: a novel component of the Drosophila histone locus body required for muscle integrity. J Cell Sci 123, 2697-2707.

Chung, L., Ng, Y.C., 2006. Age-related alterations in expression of apoptosis regulatory proteins and heat shock proteins in rat skeletal muscle. Biochim. Biophys. Acta 1762, 103-109.

Clerico, E.M., Tilitsky, J.M., Meng, W.L., Gierasch, L.M., 2015. How Hsp70 Molecular Machines Interact with Their Substrates to Mediate Diverse Physiological Functions. J Mol Biol 427, 1575-1588.

Daugaard, M., Rohde, M., Jaattela, M., 2007. The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. FEBS Lett. 581, 3702-3710.

Demontis, F., Perrimon, N., 2010. FOXO/4E-BP Signaling in Drosophila Muscles Regulates Organism-wide Proteostasis during Aging. Cell 143, 813-825.

Dickman, D.K., Horne, J.A., Meinertzhagen, I.A., Schwarz, T.L., 2005. A slowed classical pathway rather than kiss-and-run mediates endocytosis at synapses lacking synaptojanin and endophilin. Cell 123, 521-533.

Duan, R., Shi, Y., Yu, L., Zhang, G., Li, J., Lin, Y., Guo, J., Wang, J., Shen, L., Jiang, H., Wang, G., Tang, B., 2016. UBA5 Mutations Cause a New Form of Autosomal Recessive Cerebellar Ataxia. PLoS One 11, e0149039.

Duffy, J.B., 2002. GAL4 system in Drosophila: A fly geneticist's Swiss army knife. Genesis 34, 1-15.

Feder, J.H., Rossi, J.M., Solomon, J., Solomon, N., Lindquist, S., 1992. The Consequences of Expressing Hsp70 in Drosophila Cells at Normal Temperatures. Genes Dev. 6, 1402-1413.

Feder, M.E., Cartano, N.V., Milos, L., Krebs, R.A., Lindquist, S.L., 1996. Effect of engineering Hsp70 copy number on Hsp70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of Drosophila melanogaster. J Exp Biol 199, 1837-1844.

Feder, M.E., Krebs, R.A., 1998. Natural and genetic engineering of the heat-shock protein hsp70 in Drosophila melanogaster: Consequences for thermotolerance. Am Zool 38, 503-517.

Fernandez-Funez, P., Sanchez-Garcia, J., de Mena, L., Zhang, Y., Levites, Y., Khare, S., Golde, T.E., Rincon-Limas, D.E., 2016. Holdase activity of secreted Hsp70 masks amyloid-beta 42 neurotoxicity in Drosophila. Proc. Natl. Acad. Sci. U. S. A. 113, E5212-E5221.

Gehrig, S.M., van der Poel, C., Sayer, T.A., Schertzer, J.D., Henstridge, D.C., Church, J.E., Lamon, S., Russell, A.P., Davies, K.E., Febbraio, M.A., Lynch, G.S., 2012. Hsp72 preserves muscle function and slows progression of severe muscular dystrophy. Nature 484, 394-398.

Gems, D., Partridge, L., 2013. Genetics of longevity in model organisms: debates and paradigm shifts. Annu. Rev. Physiol. 75, 621-644.

Giordano, E., Rendina, R., Peluso, I., Furia, M., 2002. RNAi triggered by symetrically transcribed transgenes in *Drosophila melanogaster*. Genetics 160, 637-648.

Gong, W.J., Golic, K.G., 2004. Genomic deletions of the Drosophila melanogaster Hsp70 genes. Genetics 168, 1467-1476.

Gong, W.J., Golic, K.G., 2006. Loss of Hsp70 in Drosophila is pleiotropic, with effects on thermotolerance, recovery from heat shock and neurodegeneration. Genetics 172, 275-286.

Halliwell, B., 2003. Oxidative stress in cell culture: an under-appreciated problem? FEBS Lett. 540, 3-6.

Hartl, F.U., Bracher, A., Hayer-Hartl, M., 2011. Molecular chaperones in protein folding and proteostasis. Nature 475, 324-332.

Holmgren, R., Livak, K., Morimoto, R., Freund, R., Meselson, M., 1979. Studies of Cloned Sequences from 4 Drosophila Heat Shock Loci. Cell 18, 1359-1370.

Hunt, C.R., Dix, D.J., Sharma, G.G., Pandita, R.K., Gupta, A., Funk, M., Pandita, T.K., 2004. Genomic instability and enhanced radiosensitivity in Hsp70.1-and Hsp70.3-deficient mice. Mol Cell Biol 24, 899-911.

Ireland, R.C., Berger, E., Sirotkin, K., Yund, M.A., Osterbur, D., Fristrom, J., 1982. Ecdysterone Induces the Transcription of 4 Heat-Shock Genes in Drosophila S3 Cells and Imaginal Disks. Dev Biol 93, 498-507.

Ishhorowicz, D., Holden, J.J., Gehring, W.J., 1977. Deletions of 2 Heat-Activated Loci in Drosophila-Melanogaster and Their Effects on Heat-Induced Protein-Synthesis. Cell 12, 643-652.

Jensen, L.T., Cockerell, F.E., Kristensen, T.N., Rako, L., Loeschcke, V., McKechnie, S.W., Hoffmann, A.A., 2010. Adult heat tolerance variation in Drosophila melanogaster is not related to Hsp70 expression. J Exp Zool A Ecol Genet Physiol 313, 35-44.

Johnston, R.N., Kucey, B.L., 1988. Competitive-Inhibition of Hsp70 Gene-Expression Causes Thermosensitivity. Science 242, 1551-1554.

Khazaeli, A.A., Tatar, M., Pletcher, S.D., Curtsinger, J.W., 1997. Heat-induced longevity extension in Drosophila. I. Heat treatment, mortality, and thermotolerance. J. Gerontol. A Biol. Sci. Med. Sci. 52, B48-52.

Kloetzel, P.M., Bautz, E.K.F., 1983. Heat-Shock Proteins Are Associated with Hnrna in Drosophila-Melanogaster Tissue-Culture Cells. EMBO J. 2, 705-710.

Klose, M.K., Chu, D., Xiao, C., Seroude, L., Robertson, R.M., 2005. Heat shock-mediated thermoprotection of larval locomotion compromised by ubiquitous overexpression of Hsp70 in Drosophila melanogaster. J Neurophysiol 94, 3563-3572.

Kohyama-Koganeya, A., Nabetani, T., Miura, M., Hirabayashi, Y., 2011. Glucosylceramide synthase in the fat body controls energy metabolism in Drosophila. J Lipid Res 52, 1392-1399.

Krebs, R.A., Feder, M.E., 1997. Deleterious consequences of Hsp70 overexpression in Drosophila melanogaster larvae. Cell Stress Chaperones 2, 60-71.

Lee, T., Luo, L., 1999. Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22, 451-461.

Lewis, M., Helmsing, P.J., Ashburner, M., 1975. Parallel Changes in Puffing Activity and Patterns of Protein-Synthesis in Salivary-Glands of Drosophila. Proc. Natl. Acad. Sci. U. S. A. 72, 3604-3608.

Li, G.C., Li, L.G., Liu, Y.K., Mak, J.Y., Chen, L.L., Lee, W.M.F., 1991. Thermal Response of Rat Fibroblasts Stably Transfected with the Human 70-Kda Heat-Shock Protein-Encoding Gene. Proc. Natl. Acad. Sci. U. S. A. 88, 1681-1685.

Liao, P.C., Lin, H.Y., Yuh, C.H., Yu, L.K., Wang, H.D., 2008. The effect of neuronal expression of heat shock proteins 26 and 27 on lifespan, neurodegeneration, and apoptosis in Drosophila. Biochem. Biophys. Res. Commun. 376, 637-641.

Lin, Y.J., Seroude, L., Benzer, S., 1998. Extended life-span and stress resistance in the Drosophila mutant methuselah. Science 282, 943-946.

Lindquist, S., 1986. The heat-shock response. Annu. Rev. Biochem. 55, 1151-1191.

Lindquist, S., Craig, E.A., 1988. The Heat-Shock Proteins. Annu. Rev. Genet. 22, 631-677.

Mattoo, R.U.H., Goloubinoff, P., 2014. Molecular chaperones are nanomachines that catalytically unfold misfolded and alternatively folded proteins. Cell Mol Life Sci 71, 3311-3325.

McArdle, A., Dillmann, W.H., Mestril, R., Faulkner, J.A., Jackson, M.J., 2003. Overexpression of HSP70 in mouse skeletal muscle protects against muscle damage and age-related muscle dysfunction. FASEB J. 17, 355-+.

Minois, N., 2001. Resistance to stress as a function of age in transgenic Drosophila melanogaster overexpressing Hsp70. J Insect Physiol 47, 1007-1012.

Parkes, T.L., Elia, A.J., Dickinson, D., Hilliker, A.J., Phillips, J.P., Boulianne, G.L., 1998. Extension of Drosophila lifespan by overexpression of human SOD1 in motorneurons. Nature Genetics 19, 171-174.

Pelham, H.R.B., 1984. Hsp70 Accelerates the Recovery of Nucleolar Morphology after Heat-Shock. EMBO J. 3, 3095-3100.

Place, T.L., Domann, F.E., Case, A.J., 2017. Limitations of oxygen delivery to cells in culture: An underappreciated problem in basic and translational research. Free Radical Bio Med 113, 311-322.

Poirier, L., Shane, A., Zheng, J., Seroude, L., 2008. Characterization of the Drosophila Gene-Switch system in aging studies: a cautionary tale. Aging Cell 7, 758-770.

Riabowol, K.T., Mizzen, L.A., Welch, W.J., 1988. Heat-Shock Is Lethal to Fibroblasts Microinjected with Antibodies against Hsp70. Science 242, 433-436.

Ritossa, F., 1962. New Puffing Pattern Induced by Temperature Shock and Dnp in Drosophila. Experientia 18, 571-573.

Ritossa, F., 1963. New puffs induced by temperature shock, dnp and salicylate in salivary chromosomes of Drosophila melanogaster. Drosophila Information Service 37, 122-123.

Ritossa, F.M., 1964. Experimental Activation of Specific Loci in Polytene Chromosomes of Drosophila. Exp Cell Res 35, 601-607.

Robertson, H.M., Preston, C.R., Phillis, R.W., Johnson, S.D., Benz, W.K., Engels, W.R., 1988. A stable genomic source of P element transposase in Drosophila melanogaster. Genetics 118, 461-470.

Rosenbaum, E.E., Brehm, K.S., Vasiljevic, E., Gajeski, A., Colley, N.J., 2012. Drosophila GPI-mannosyltransferase 2 is required for GPI anchor attachment and surface expression of chaoptin. Vis Neurosci 29, 143-156.

Saibil, H., 2013. Chaperone machines for protein folding, unfolding and disaggregation. Nat Rev Mol Cell Bio 14, 630-642.

Schlesinger, M.J., 1990. Heat-Shock Proteins. J Biol Chem 265, 12111-12114.

Senf, S.M., Howard, T.M., Ahn, B., Ferreira, L.F., Judge, A.R., 2013. Loss of the Inducible Hsp70 Delays the Inflammatory Response to Skeletal Muscle Injury and Severely Impairs Muscle Regeneration. PLoS One 8.

Seroude, L., 2002. GAL4 drivers expression in the whole adult fly. Genesis 34, 34-38.

Seroude, L., Brummel, T., Kapahi, P., Benzer, S., 2002. Spatio-temporal analysis of gene expression during aging in Drosophila melanogaster. Aging Cell 1, 47-56.

Shilova, V.Y., Zatsepina, O.G., Garbuz, D.G., Funikov, S.Y., Zelentsova, E.S., Schostak, N.G., Kulikov, A.M., Evgen'ev, M.B., 2018. Heat shock protein 70 from a thermotolerant Diptera species provides higher thermoresistance to Drosophila larvae than correspondent endogenous gene. Insect Mol Biol 27, 61-72.

Singh, R., Kolvraa, S., Bross, P., Christensen, K., Bathum, L., Gregersen, N., Tan, Q., Rattan, S.I.S., 2010. Anti-Inflammatory Heat Shock Protein 70 Genes are Positively Associated with Human Survival. Curr Pharm Design 16, 796-801.

Singh, R., Kolvraa, S., Bross, P., Christensen, K., Gregersen, N., Tan, Q.H., Jensen, U.B., Eiberg, H., Rattan, S.I.S., 2006. Heat-Shock Protein 70 Genes and Human Longevity A View from Denmark. Ann. N. Y. Acad. Sci. 1067, 301-308.

Solomon, J.M., Rossi, J.M., Golic, K., Mcgarry, T., Lindquist, S., 1991. Changes in Hsp70 Alter Thermotolerance and Heat-Shock Regulation in Drosophila. New Biol 3, 1106-1120.

Tatar, M., Khazaeli, A.A., Curtsinger, J.W., 1997. Chaperoning extended life. Nature 390, 30.

Thurmond, J., Goodman, J.L., Strelets, V.B., Attrill, H., Gramates, L.S., Marygold, S.J., Matthews, B.B., Millburn, G., Antonazzo, G., Trovisco, V., Kaufman, T.C., Calvi, B.R., FlyBase, C., 2019. FlyBase 2.0: the next generation. Nucleic Acids Res 47, D759-D765.

Tissieres, A., Mitchell, H.K., Tracy, U.M., 1974. Protein-Synthesis in Salivary-Glands of Drosophila-Melanogaster - Relation to Chromosome Puffs. J Mol Biol 84, 389-398.

Velazquez, J.M., Lindquist, S., 1984. Hsp70 - Nuclear Concentration during Environmental-Stress and Cytoplasmic Storage during Recovery. Cell 36, 655-662.

Verstreken, P., Koh, T.W., Schulze, K.L., Zhai, R.G., Hiesinger, P.R., Zhou, Y., Mehta, S.Q., Cao, Y., Roos, J., Bellen, H.J., 2003. Synaptojanin is recruited by endophilin to promote synaptic vesicle uncoating. Neuron 40, 733-748.

Wacker, J.L., Huang, S.Y., Steele, A.D., Aron, R., Lotz, G.P., Nguyen, Q., Giorgini, F., Roberson, E.D., Lindquist, S., Masliah, E., Muchowski, P.J., 2009. Loss of Hsp70 Exacerbates Pathogenesis But Not Levels of Fibrillar Aggregates in a Mouse Model of Huntington's Disease. J Neurosci 29, 9104-9114.

Wang, H.D., Kazemi-Esfarjani, P., Benzer, S., 2004. Multiple-stress analysis for isolation of Drosophila longevity genes. Proc. Natl. Acad. Sci. U. S. A. 101, 12610-12615.

Wang, M.C., Bohmann, D., Jasper, H., 2003. JNK signaling confers tolerance to oxidative stress and extends lifespan in Drosophila. Dev Cell 5, 811-816.

Welte, M.A., Tetrault, J.M., Dellavalle, R.P., Lindquist, S.L., 1993. A New Method for Manipulating Transgenes - Engineering Heat Tolerance in a Complex, Multicellular Organism. Curr Biol 3, 842-853.

Wheeler, J.C., Bieschke, E.T., Tower, J., 1995. Muscle-Specific Expression of Drosophila Hsp70 in Response to Aging and Oxidative Stress. Proc. Natl. Acad. Sci. U. S. A. 92, 10408-10412.

Wheeler, J.C., King, V., Tower, J., 1999. Sequence requirements for upregulated expression of Drosophila hsp70 transgenes during aging. Neurobiol. Aging 20, 545-553.

Wodarz, A., Hinz, U., Engelbert, M., Knust, E., 1995. Expression of Crumbs Confers Apical Character on Plasma-Membrane Domains of Ectodermal Epithelia of Drosophila. Cell 82, 67-76.

Xiao, C., Mileva-Seitz, V., Seroude, L., Robertson, R.M., 2007. Targeting HSP70 to motoneurons protects locomotor activity from hyperthermia in Drosophila. Dev Neurobiol 67, 438-455.

Yang, J.S., Tower, J., 2009. Expression of hsp22 and hsp70 Transgenes Is Partially Predictive of Drosophila Survival Under Normal and Stress Conditions. J. Gerontol. A Biol. Sci. Med. Sci. 64, 828-838.

Yazdani, M., 2016. Technical aspects of oxygen level regulation in primary cell cultures: A review. Interdiscip Toxicol 9, 85-89.

Yeh, E., Gustafson, K., Boulianne, G.L., 1995. Green fluorescent protein as a vital marker and reporter of gene expression in Drosophila. Proc. Natl. Acad. Sci. U. S. A. 92, 7036-7040.

Yost, H.J., Lindquist, S., 1986. Rna Splicing Is Interrupted by Heat-Shock and Is Rescued by Heat-Shock Protein-Synthesis. Cell 45, 185-193.

Zhao, Y., Sun, H., Lu, J., Li, X., Chen, X., Tao, D., Huang, W., Huang, B., 2005. Lifespan extension and elevated hsp gene expression in Drosophila caused by histone deacetylase inhibitors. J Exp Biol 208, 697-705.

Zheng, J., Edelman, S.W., Tharmarajah, G., Walker, D.W., Pletcher, S.D., Seroude, L., 2005. Differential patterns of apoptosis in response to aging in Drosophila. Proc. Natl. Acad. Sci. U. S. A. 102, 12083-12088.

Supplemental figures and tables

Expression of heat shock protein 70 is insufficient to extend *Drosophila melanogaster* longevity.

Chengfeng Xiao, Danna Hull, Shuang Qiu, Joanna Yeung, Jie Zheng, Taylor Barwell, R Meldrum Robertson, Laurent Seroude

Figure S1. Transgenic and endogenous Hsp70 expression. Western-blot with monoclonal antibody 5A5 was performed to detect HSP70-myc (red asterisk) and endogenous HSP70 in 5-7 days old males with or without heat shock. Under both conditions, the intensity of the HSP70-myc signal is lower than the endogenous HSP70 signal. Although it remains possible that the 5A5 antibody has a lower affinity for the tagged protein, it is very likely that the level of GAL4-mediated HSP70 overexpression under non-heat shock condition is lower than the level of heat-shock induced endogenous HSP70 overexpression.

Supplemental Figure 1

Non-heat shock   Heat shock

Figure S2: Survival to thermal, oxidative and metabolic stresses of 3 days and 10 days old females overexpressing HSP70. Each triangle reports the outcome of the statistical comparison (Table S2) between experimental and controls for an experimental replicate.

Supplemental Figure 2

△: increase　▲: significant increase　　▽: decrease　▼: significant decrease　　▷: no change

Figure S3: Longevity of females overexpressing HSP70. Typical longevity curves (x axis: days at 25˚C; y axis: percentage alive) are shown. The outcome of the statistical comparison (Table S3) between experimental and controls for each experimental replicate is provided at the top of each graph: ▲: significant increase ▲: increase ▼: significant decrease ▼: decrease ▷: no change.

Supplemental Figure 3

♀

da-GAL4   DJ694   D42

#2.1

#4.3

#9.1

#3.2

#10.1

#3.1

#4.4

GAL4/+   *UAS-hsp70/+*   *GAL4/+;UAS-hsp70/+*

Figure S4. Construction of pCX9 plasmid. The *hsp70Ab* coding sequence (excised as a XhoI-BglII fragment from pCX1 (Xiao et al. 2007)) was first inserted into XhoI-BglII-opened Sym-pUASTw (Giordano et al. 2002) to obtain pCX9.1. The mini-*white* sequences from Sym-pUASTw were then removed by digestion with EcoRI and self-ligation to generate pCX9. Grey arrows show the binding sites for the oligonucleotides (DJ39, DJ51) used for inverse PCR.

Figure S5. Down-regulation of Hsp70 by RNAi. (A) Inhibition of Hsp70-myc: Western-blot with monoclonal antibody 9E10 of 5-7 days old extracts. (B) Inhibition of endogenous Hsp70: Western-blot with monoclonal antibody 5A5 of 7 days old extracts. HS: heat shock, -:without, +:with.

A

DJ694+UAS-hsp70#3.1+

no RNAi | R1.17 | R1.26 | R2.17 | R3.12 | R4.15 | R5.2 | R5.12 | R5.12e | R5.14

♂

♀

B

R3.12/+ | DJ694/+ | DJ694/R3.12 | D42/+ | D42/R3.12 | daGal4/+ | daGal4/R3.12

HS: −   +   −   +   −   +   −   +   −   +   −   +   −   +

♂

♀

Table S1. Developmental analysis of Hsp70 overexpression. Percentage hatching (L1), pupation (Pupa) and eclosion (Adult) are compared between experimental animals (Gal4+UAS) and control animals missing the driver (UAS) or UAS (Gal4) transgene (P: Kruskal-Wallis test). The first columns indicate the genotype of the mother while the second column indicate the genotype of the father used to generate the experimental and control animals. The change column indicates experimental animals that are significantly (P<0.05) lower than both controls. N: number of independent measurements, STD: Standard deviation.

Table S1

Gal4	UAS	Stage	UAS			Gal4			Gal4 + UAS			P		Change
			N	Mean	STD	N	Mean	STD	N	Mean	STD	vs UAS	vs Gal4	
da-GAL4	2.1	L1	8	98.0	2.1	8	97.0	2.8	8	93.0	4.7	0.0154	0.0568	
		Pupa	8	82.6	8.9	8	93.9	3.8	8	78.7	14.0	0.5202	0.0105	
		Adult	8	98.6	2.5	8	98.9	3.1	8	99.3	1.9	0.5372	0.7406	
da-GAL4	4.3	L1	8	94.5	3.7	8	97.0	2.8	8	96.0	4.3	0.4637	0.5899	
		Pupa	8	78.7	13.4	8	93.9	3.8	8	84.9	5.0	0.2411	0.0012	
		Adult	8	97.0	3.3	8	98.9	3.1	8	94.1	8.6	0.3900	0.1545	
da-GAL4	9.1	L1	8	97.0	3.5	8	97.0	2.8	8	98.5	2.1	0.3190	0.2462	
		Pupa	8	80.4	3.2	8	93.9	3.8	8	**76.1**	4.1	0.0359	0.0000	↓
		Adult	8	98.8	3.5	8	98.9	3.1	8	99.3	2.0	0.7035	0.7654	
da-GAL4	3.1	L1	8	94.5	5.2	8	97.0	2.8	8	95.0	5.1	0.8494	0.3504	
		Pupa	8	84.3	4.8	8	93.9	3.8	8	85.9	7.5	0.6152	0.0181	
		Adult	8	98.2	3.6	8	98.9	3.1	8	98.2	2.5	0.9984	0.5996	
da-GAL4	4.4	L1	8	98.0	3.7	8	97.0	2.8	8	96.0	4.8	0.3654	0.6186	
		Pupa	8	91.3	8.7	8	93.9	3.8	8	96.9	2.9	0.1070	0.0921	
		Adult	8	96.2	5.7	8	98.9	3.1	8	95.6	6.2	0.8593	0.1974	
da-GAL4	3.2/CyO	L1	16	96.8	3.3	8	97.0	2.8	16	95.8	3.1	0.3940	0.3476	
		Pupa	16	94.8	4.6	8	93.9	3.8	16	92.0	6.4	0.2323	0.4569	
		Adult	16	46.3	11.0	8	98.9	3.1	16	43.5	10.9	0.6573	NA	
da-GAL4	10.1/CyO	L1	16	97.0	4.0	8	97.0	2.8	16	94.3	4.4	0.0733	0.1220	
		Pupa	16	84.0	8.2	8	93.9	3.8	16	90.5	6.1	0.0154	0.1679	
		Adult	16	48.6	10.5	8	98.9	3.1	16	57.5	18.2	0.1006	NA	
DJ694	2.1	L1	8	95.0	4.7	8	80.5	9.2	8	78.0	8.0	0.0001	0.5707	
		Pupa	8	93.8	5.1	8	89.7	7.0	8	81.0	10.6	0.0086	0.0743	
		Adult	8	98.2	2.4	8	99.2	2.2	8	95.4	6.9	0.2865	0.1563	
DJ694	4.3	L1	8	95.0	5.1	8	80.5	9.2	8	80.0	12.1	0.0061	0.9271	
		Pupa	8	92.7	3.6	8	89.7	7.0	8	**70.8**	15.9	0.0020	0.0083	↓
		Adult	8	94.8	3.8	8	99.2	2.2	8	96.8	3.4	0.2968	0.1170	
DJ694	9.1	L1	8	98.0	3.0	8	80.5	9.2	8	**65.0**	10.0	0.0000	0.0060	↓
		Pupa	8	90.6	9.0	8	89.7	7.0	8	77.5	17.3	0.0776	0.0849	
		Adult	8	98.4	3.0	8	99.2	2.2	8	99.0	2.9	0.7306	0.8444	
DJ694	3.1	L1	8	94.5	6.0	8	80.5	9.2	8	78.5	8.3	0.0006	0.6540	
		Pupa	8	83.0	13.1	8	89.7	7.0	8	74.4	6.1	0.1123	0.0004	
		Adult	8	96.0	4.8	8	99.2	2.2	8	96.3	6.0	0.9216	0.2168	
DJ694	4.4	L1	8	96.0	3.0	8	80.5	9.2	8	87.5	10.1	0.0392	0.1695	
		Pupa	8	95.8	5.1	8	89.7	7.0	8	93.2	3.8	0.2667	0.2318	
		Adult	8	94.5	5.1	8	99.2	2.2	8	95.6	3.2	0.6213	0.0182	
DJ694	3.2/CyO	L1	16	94.8	5.2	8	80.5	9.2	16	78.0	7.9	0.0000	0.4943	
		Pupa	16	94.5	4.9	8	89.7	7.0	16	85.9	11.2	0.0161	0.3970	
		Adult	16	53.9	12.9	8	99.2	2.2	16	50.2	8.5	0.3201	NA	
DJ694	10.1/CyO	L1	16	96.5	3.2	8	80.5	9.2	16	**61.3**	11.5	0.0000	0.0005	↓
		Pupa	16	91.0	6.1	8	89.7	7.0	16	94.2	6.2	0.1452	0.1227	
		Adult	16	56.7	9.1	8	99.2	2.2	16	56.6	10.1	0.9958	NA	
D42	2.1	L1	8	91.5	8.9	8	96.5	2.6	8	94.0	4.8	0.4965	0.2134	
		Pupa	8	83.1	2.1	8	91.2	6.7	8	87.3	8.6	0.2012	0.3266	
		Adult	8	98.1	2.6	8	96.7	3.8	8	96.9	4.5	0.5063	0.9423	
D42	4.3	L1	8	96.5	3.3	8	96.5	2.6	8	95.5	4.5	0.6217	0.5938	
		Pupa	8	89.7	9.5	8	91.2	6.7	8	90.7	7.3	0.8189	0.8912	
		Adult	8	95.9	4.4	8	96.7	3.8	8	97.7	3.7	0.4073	0.6179	

Table S1

Gal4	UAS	Stage	UAS			Gal4			Gal4 + UAS			P		Change
			N	Mean	STD	N	Mean	STD	N	Mean	STD	vs UAS	vs Gal4	
D42	9.1	L1	8	96.0	4.3	8	96.5	2.6	8	93.0	4.1	0.1759	0.0615	
		Pupa	8	91.0	6.7	8	91.2	6.7	8	94.7	5.3	0.2444	0.2664	
		Adult	8	96.1	4.2	8	96.7	3.8	8	98.9	2.0	0.1086	0.1675	
D42	3.1	L1	8	93.5	6.4	8	96.5	2.6	8	93.5	7.4	1.0000	0.2962	
		Pupa	8	92.1	5.1	8	91.2	6.7	8	87.1	8.1	0.1540	0.2859	
		Adult	8	87.3	15.8	8	96.7	3.8	8	93.8	5.3	0.2900	0.2227	
D42	4.4	L1	8	97.5	3.0	8	96.5	2.6	8	99.0	2.8	0.3190	0.0852	
		Pupa	8	74.7	16.8	8	91.2	6.7	8	87.0	6.6	0.0745	0.2258	
		Adult	8	96.9	3.5	8	96.7	3.8	8	99.4	1.8	0.1029	0.0922	
D42	3.2/CyO	L1	16	96.3	5.4	8	96.5	2.6	16	93.0	3.7	0.0690	0.0265	
		Pupa	16	87.1	7.8	8	91.2	6.7	16	88.4	7.2	0.5426	0.3694	
		Adult	16	48.2	6.5	8	96.7	3.8	16	46.7	6.2	0.4143	NA	
D42	10.1/CyO	L1	16	97.3	3.5	8	96.5	2.6	16	98.0	2.9	0.5150	0.2310	
		Pupa	16	89.0	6.4	8	91.2	6.7	16	94.9	5.1	0.0069	0.1423	
		Adult	16	39.1	6.6	8	96.7	3.8	16	39.0	5.1	0.9748	NA	

Table S2: Stress resistance data and analysis of Hsp70 overexpression. Mean: average survival in hours, n: number of flies in the replicate, P: highest Log-rank test value, 1: replicate number, 2: 95% mortality (maximum lifespan)

Table S2

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Heat	3	daGal4	2.1	♀	1	37	72.0	84	39	61.7	84	39	63.8	93	0.0	10.7	
Heat	3	daGal4	2.1	♀	2	41	64.9	76	39	60.1	84	40	60.6	84	0.0	0.0	
Heat	3	daGal4	2.1	♀	3	42	59.0	76	38	54.1	76	39	59.7	76	1.3	0.0	**0.0048**
Heat	3	daGal4	2.1	♀	4	40	24.9	41	40	22.3	41	40	27.8	45	11.9	9.8	**0.0139**
Heat	3	daGal4	2.1	♂	1	38	61.4	84	39	60.8	84	40	57.8	76	-5.0	-9.5	0.0974
Heat	3	daGal4	2.1	♂	2	40	58.9	68	38	53.9	84	40	52.5	68	-2.6	0.0	**0.0082**
Heat	3	daGal4	2.1	♂	3	40	51.4	76	38	52.9	76	40	53.0	76	0.1	0.0	0.6036
Heat	3	daGal4	2.1	♂	4	40	23.5	34	40	23.6	45	40	25.8	41	9.7	0.0	0.1872
Heat	3	daGal4	4.3	♀	1	40	68.0	84	39	61.7	84	38	66.8	93	0.0	10.7	
Heat	3	daGal4	4.3	♀	2	40	60.7	76	39	60.1	84	39	56.3	68	-6.3	-10.5	**0.0030**
Heat	3	daGal4	4.3	♀	3	41	51.1	56	38	54.1	76	38	57.9	76	7.0	0.0	**0.0450**
Heat	3	daGal4	4.3	♀	4	39	28.1	36	40	27.9	36	40	33.6	46	19.5	27.8	**0.0005**
Heat	3	daGal4	4.3	♂	1	41	63.7	76	39	60.8	84	45	58.6	76	-3.7	0.0	**0.0175**
Heat	3	daGal4	4.3	♂	2	42	55.5	76	38	53.9	84	46	51.3	64	-4.8	-15.8	**0.0332**
Heat	3	daGal4	4.3	♂	3	41	51.9	76	38	52.9	76	40	52.1	68	0.0	-10.5	
Heat	3	daGal4	4.3	♂	4	40	25.5	36	40	29.5	41	40	29.8	41	1.1	0.0	**0.0017**
Heat	3	daGal4	9.1	♀	1	39	69.1	93	39	61.7	84	40	67.0	84	0.0	0.0	
Heat	3	daGal4	9.1	♀	2	41	61.6	76	39	60.1	84	38	65.7	76	6.7	0.0	**0.0375**
Heat	3	daGal4	9.1	♀	3	43	60.7	76	38	54.1	76	39	62.2	84	2.3	10.5	**0.0002**
Heat	3	daGal4	9.1	♂	1	39	68.7	84	39	60.8	84	38	69.7	93	1.5	10.7	**0.0079**
Heat	3	daGal4	9.1	♂	2	40	57.7	76	38	53.9	84	34	60.8	76	5.4	0.0	0.1188
Heat	3	daGal4	9.1	♂	3	40	55.4	76	38	52.9	76	40	58.3	84	5.3	10.5	**0.0352**
Heat	3	daGal4	3.2	♀	1	40	60.1	76	39	61.7	84	42	60.0	76	-0.2	0.0	0.3989
Heat	3	daGal4	3.2	♀	2	39	52.8	68	39	60.1	84	38	61.8	76	2.8	0.0	0.6183
Heat	3	daGal4	3.2	♀	3	40	50.2	56	38	54.1	76	39	54.3	76	0.3	0.0	**0.0010**
Heat	3	daGal4	3.2	♀	4	39	31.2	42	38	23.3	38	40	40.4	46	29.7	9.5	**<0.0001**
Heat	3	daGal4	3.2	♂	1	40	60.1	93	39	60.8	84	39	58.2	76	-3.2	-9.5	0.3003
Heat	3	daGal4	3.2	♂	2	45	46.5	64	38	53.9	84	40	57.3	76	6.3	0.0	0.4225
Heat	3	daGal4	3.2	♂	3	40	47.4	60	38	52.9	76	40	49.4	76	0.0	0.0	
Heat	3	daGal4	3.2	♂	4	36	21.3	30	40	19.5	30	40	27.0	34	26.6	13.3	**<0.0001**
Heat	3	daGal4	10.1	♀	1	40	61.5	84	39	61.7	84	40	61.5	76	0.0	-9.5	
Heat	3	daGal4	10.1	♀	2	40	50.7	56	39	60.1	84	38	58.4	76	0.0	0.0	
Heat	3	daGal4	10.1	♀	3	40	50.4	56	38	54.1	76	40	63.5	93	17.3	22.4	**<0.0001**
Heat	3	daGal4	10.1	♀	4	39	32.5	41	40	28.4	36	40	33.5	41	3.2	0.0	0.7430
Heat	3	daGal4	10.1	♂	1	29	61.8	84	39	60.8	84	40	60.4	84	-0.7	0.0	0.4536
Heat	3	daGal4	10.1	♂	2	40	46.4	52	38	53.9	84	39	56.4	76	4.7	0.0	0.4541
Heat	3	daGal4	10.1	♂	3	39	48.3	60	38	52.9	76	39	53.9	76	1.9	0.0	**0.0027**
Heat	3	daGal4	10.1	♂	4	40	26.2	36	40	22.0	26	40	28.8	36	9.9	0.0	**0.0218**
Heat	3	daGal4	3.1	♀	1	40	59.0	76	39	61.7	84	43	58.0	76	-1.8	0.0	0.0565
Heat	3	daGal4	3.1	♀	2	41	50.4	60	39	60.1	84	39	53.8	76	0.0	0.0	
Heat	3	daGal4	3.1	♀	3	38	46.2	56	38	54.1	76	41	52.0	64	0.0	0.0	
Heat	3	daGal4	3.1	♀	4	38	22.6	34	38	23.3	38	40	25.9	38	11.4	0.0	**0.0067**
Heat	3	daGal4	3.1	♂	1	39	61.9	76	39	60.8	84	40	61.0	76	0.0	0.0	
Heat	3	daGal4	3.1	♂	2	40	42.6	56	38	53.9	84	40	54.9	76	1.9	0.0	0.9906
Heat	3	daGal4	3.1	♂	3	18	52.2	76	38	52.9	76	39	51.6	68	-1.2	-10.5	0.3324
Heat	3	daGal4	3.1	♂	4	40	16.5	26	40	19.5	30	40	20.1	26	2.8	0.0	0.4985
Heat	3	daGal4	4.4	♀	1	41	67.1	84	39	61.7	84	38	60.3	76	-2.2	-9.5	**0.0014**
Heat	3	daGal4	4.4	♀	2	33	60.8	76	39	60.1	84	44	59.7	93	-0.7	10.7	0.6783
Heat	3	daGal4	4.4	♀	3	40	59.3	76	38	54.1	76	40	55.6	76	0.0	0.0	

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Heat	3	daGal4	4.4	♀	4	40	32.2	41	40	27.9	36	40	38.0	46	18.0	12.2	**0.0002**
Heat	3	daGal4	4.4	♂	1	43	69.9	84	39	60.8	84	42	60.5	76	-0.6	-9.5	0.3669
Heat	3	daGal4	4.4	♂	2	40	56.2	76	38	53.9	84	41	53.8	84	-0.2	0.0	0.5348
Heat	3	daGal4	4.4	♂	3	38	55.2	76	38	52.9	76	40	52.7	68	-0.5	-10.5	0.2226
Heat	3	daGal4	4.4	♂	4	40	31.3	36	40	29.5	41	40	34.9	41	11.5	0.0	**0.0003**
Heat	3	DJ694	2.1	♀	1	37	72.0	84	39	69.4	93	39	67.3	93	-3.0	0.0	**0.0434**
Heat	3	DJ694	2.1	♀	2	41	64.9	76	31	60.9	76	46	60.4	84	-0.9	10.5	0.0525
Heat	3	DJ694	2.1	♀	3	42	59.0	76	37	65.3	84	43	56.8	76	-3.6	0.0	**0.0028**
Heat	3	DJ694	2.1	♀	4	40	24.9	41	39	24.5	41	40	18.7	30	-23.7	-26.8	**<0.0001**
Heat	3	DJ694	2.1	♂	1	38	61.4	84	37	63.2	84	40	52.4	76	-14.6	-9.5	**<0.0001**
Heat	3	DJ694	2.1	♂	2	40	58.9	68	33	58.4	76	40	52.3	76	-10.5	0.0	**0.0039**
Heat	3	DJ694	2.1	♂	3	40	51.4	76	35	54.1	76	42	55.1	76	2.0	0.0	0.1223
Heat	3	DJ694	2.1	♂	4	40	23.5	34	40	21.4	34	40	21.9	41	0.0	20.6	
Heat	3	DJ694	4.3	♀	1	40	68.0	84	39	69.4	93	40	63.5	76	-6.6	-9.5	**0.0141**
Heat	3	DJ694	4.3	♀	2	40	60.7	76	31	60.9	76	36	60.6	76	-0.2	0.0	0.4922
Heat	3	DJ694	4.3	♀	3	41	51.1	56	37	65.3	84	41	52.2	76	0.0	0.0	
Heat	3	DJ694	4.3	♀	4	39	28.1	36	40	24.9	36	40	22.6	41	-9.3	13.9	**0.0106**
Heat	3	DJ694	4.3	♂	1	41	63.7	76	37	63.2	84	38	55.6	76	-12.0	0.0	**0.0026**
Heat	3	DJ694	4.3	♂	2	42	55.5	76	33	58.4	76	39	54.1	76	-2.7	0.0	0.0694
Heat	3	DJ694	4.3	♂	3	41	51.9	76	35	54.1	76	41	43.6	48	-16.0	-36.8	**<0.0001**
Heat	3	DJ694	4.3	♂	4	40	25.5	36	39	29.6	36	40	27.1	36	0.0	0.0	
Heat	3	DJ694	9.1	♀	1	39	69.1	93	39	69.4	93	41	61.1	84	-11.6	-9.7	**0.0056**
Heat	3	DJ694	9.1	♀	2	41	61.6	76	31	60.9	76	40	58.8	76	-3.5	0.0	0.2002
Heat	3	DJ694	9.1	♀	3	43	60.7	76	37	65.3	84	39	46.3	52	-23.9	-31.6	**<0.0001**
Heat	3	DJ694	9.1	♂	1	39	68.7	84	37	63.2	84	40	52.1	64	-17.5	-23.8	**<0.0001**
Heat	3	DJ694	9.1	♂	2	40	57.7	76	33	58.4	76	38	44.2	68	-23.4	-10.5	**<0.0001**
Heat	3	DJ694	9.1	♂	3	40	55.4	76	35	54.1	76	30	48.4	64	-10.5	-15.8	**0.0051**
Heat	3	DJ694	3.2	♀	1	40	60.1	76	39	69.4	93	40	54.5	60	-9.3	-21.1	**<0.0001**
Heat	3	DJ694	3.2	♀	2	39	52.8	68	31	60.9	76	40	52.5	56	-0.6	-17.6	0.9298
Heat	3	DJ694	3.2	♀	3	40	50.2	56	37	65.3	84	33	51.9	56	0.0	0.0	
Heat	3	DJ694	3.2	♀	4	39	31.2	42	39	17.7	22	40	37.4	46	20.0	9.5	**<0.0001**
Heat	3	DJ694	3.2	♂	1	40	60.1	93	37	63.2	84	39	47.8	64	-20.4	-23.8	**<0.0001**
Heat	3	DJ694	3.2	♂	2	45	46.5	64	33	58.4	76	39	40.4	48	-13.1	-25.0	**<0.0001**
Heat	3	DJ694	3.2	♂	3	40	47.4	60	35	54.1	76	39	45.4	56	-4.1	-6.7	**0.0004**
Heat	3	DJ694	3.2	♂	4	36	21.3	30	40	18.1	30	40	21.6	30	1.4	0.0	**0.0002**
Heat	3	DJ694	10.1	♀	1	40	61.5	84	39	69.4	93	40	55.6	64	-9.6	-23.8	**0.0010**
Heat	3	DJ694	10.1	♀	2	40	50.7	56	31	60.9	76	40	49.9	56	-1.6	0.0	0.2561
Heat	3	DJ694	10.1	♀	3	40	50.4	56	37	65.3	84	40	48.8	56	-3.2	0.0	0.1880
Heat	3	DJ694	10.1	♀	4	39	32.5	41	40	27.3	31	39	32.2	36	0.0	0.0	
Heat	3	DJ694	10.1	♂	1	29	61.8	84	37	63.2	84	39	48.2	60	-22.0	-28.6	**<0.0001**
Heat	3	DJ694	10.1	♂	2	40	46.4	52	33	58.4	76	40	43.3	52	-6.7	0.0	**0.0010**
Heat	3	DJ694	10.1	♂	3	39	48.3	60	35	54.1	76	30	47.1	52	-2.6	-13.3	**0.0033**
Heat	3	DJ694	10.1	♂	4	40	26.2	36	41	22.8	31	40	24.5	31	0.0	0.0	
Heat	3	DJ694	3.1	♀	1	40	59.0	76	39	69.4	93	41	51.6	56	-12.5	-26.3	**0.0003**
Heat	3	DJ694	3.1	♀	2	41	50.4	60	31	60.9	76	40	46.4	52	-8.0	-13.3	**0.0006**
Heat	3	DJ694	3.1	♀	3	38	46.2	56	37	65.3	84	40	44.3	48	-4.1	-14.3	**0.0063**
Heat	3	DJ694	3.1	♀	4	38	22.6	34	39	17.7	22	40	26.7	38	18.3	11.8	**0.0012**
Heat	3	DJ694	3.1	♂	1	39	61.9	76	37	63.2	84	41	49.1	52	-20.8	-31.6	**<0.0001**
Heat	3	DJ694	3.1	♂	2	40	42.6	56	33	58.4	76	40	41.8	44	-1.9	-21.4	0.6872

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS n	UAS Mean	UAS Max[2]	Gal4 n	Gal4 Mean	Gal4 Max[2]	Gal4 + UAS n	Gal4 + UAS Mean	Gal4 + UAS Max[2]	% Extension Mean	% Extension Max[2]	P
Heat	3	DJ694	3.1	♂	3	18	52.2	76	35	54.1	76	44	42.1	48	-19.4	-36.8	**0.0004**
Heat	3	DJ694	3.1	♂	4	40	16.5	26	40	18.1	30	40	18.3	22	0.8	-15.4	**0.0111**
Heat	3	DJ694	4.4	♀	1	41	67.1	84	39	69.4	93	38	66.7	84	-0.7	0.0	0.2066
Heat	3	DJ694	4.4	♀	2	33	60.8	76	31	60.9	76	41	64.6	93	6.1	22.4	0.1122
Heat	3	DJ694	4.4	♀	3	40	59.3	76	37	65.3	84	39	64.7	93	0.0	10.7	
Heat	3	DJ694	4.4	♀	4	40	32.2	41	40	24.9	36	40	26.3	36	0.0	0.0	
Heat	3	DJ694	4.4	♂	1	43	69.9	84	37	63.2	84	40	59.2	76	-6.3	-9.5	0.0892
Heat	3	DJ694	4.4	♂	2	40	56.2	76	33	58.4	76	43	57.5	76	0.0	0.0	
Heat	3	DJ694	4.4	♂	3	38	55.2	76	35	54.1	76	44	60.1	76	8.9	0.0	**0.0149**
Heat	3	DJ694	4.4	♂	4	40	31.3	36	39	29.6	36	40	30.8	36	0.0	0.0	
Heat	3	D42	2.1	♀	1	37	72.0	84	30	63.0	93	39	58.7	68	-6.9	-19.0	0.0854
Heat	3	D42	2.1	♀	2	41	64.9	76	36	54.3	76	43	56.0	84	0.0	10.5	
Heat	3	D42	2.1	♀	3	42	59.0	76	40	62.4	93	38	56.2	76	-4.7	0.0	**0.0024**
Heat	3	D42	2.1	♀	4	40	24.9	41	40	19.1	30	41	22.0	41	0.0	0.0	
Heat	3	D42	2.1	♂	1	38	61.4	84	37	58.8	76	39	49.1	64	-16.5	-15.8	**<0.0001**
Heat	3	D42	2.1	♂	2	40	58.9	68	32	52.0	76	39	45.8	64	-11.8	-5.9	**0.0265**
Heat	3	D42	2.1	♂	3	40	51.4	76	39	53.9	76	39	49.0	64	-4.6	-15.8	**0.0461**
Heat	3	D42	2.1	♂	4	40	23.5	34	39	21.0	41	40	20.9	41	-0.8	0.0	0.1893
Heat	3	D42	4.3	♀	1	40	68.0	84	30	63.0	93	36	66.5	84	0.0	0.0	
Heat	3	D42	4.3	♀	2	40	60.7	76	36	54.3	76	35	59.1	76	0.0	0.0	
Heat	3	D42	4.3	♀	3	41	51.1	56	40	62.4	93	39	61.9	76	0.0	0.0	
Heat	3	D42	4.3	♀	4	39	28.1	36	40	27.5	36	40	30.9	46	10.0	27.8	**0.0205**
Heat	3	D42	4.3	♂	1	41	63.7	76	37	58.8	76	39	52.3	64	-11.1	-15.8	**0.0067**
Heat	3	D42	4.3	♂	2	42	55.5	76	32	52.0	76	40	50.1	68	-3.7	-10.5	**0.0266**
Heat	3	D42	4.3	♂	3	41	51.9	76	39	53.9	76	42	49.0	56	-5.7	-26.3	**0.0082**
Heat	3	D42	4.3	♂	4	40	25.5	36	40	27.9	41	40	27.7	36	0.0	0.0	
Heat	3	D42	9.1	♀	1	39	69.1	93	30	63.0	93	39	61.3	76	-2.7	-18.3	0.4739
Heat	3	D42	9.1	♀	2	41	61.6	76	36	54.3	76	46	59.4	84	0.0	10.5	
Heat	3	D42	9.1	♀	3	43	60.7	76	40	62.4	93	39	59.9	76	-1.4	0.0	0.1615
Heat	3	D42	9.1	♂	1	39	68.7	84	37	58.8	76	41	53.4	76	-9.3	0.0	**0.0103**
Heat	3	D42	9.1	♂	2	40	57.7	76	32	52.0	76	42	45.5	56	-12.5	-26.3	**0.0004**
Heat	3	D42	9.1	♂	3	40	55.4	76	39	53.9	76	42	50.3	76	-6.8	0.0	**0.0081**
Heat	3	D42	3.2	♀	1	40	60.1	76	30	63.0	93	40	65.0	93	3.0	0.0	**0.0197**
Heat	3	D42	3.2	♀	2	39	52.8	68	36	54.3	76	37	55.9	68	2.9	0.0	**0.0180**
Heat	3	D42	3.2	♀	3	40	50.2	56	40	62.4	93	39	55.0	76	0.0	0.0	
Heat	3	D42	3.2	♀	4	39	31.2	42	39	22.6	30	40	34.5	42	10.6	0.0	**0.0208**
Heat	3	D42	3.2	♂	1	40	60.1	93	37	58.8	76	39	56.5	76	-3.9	0.0	0.1721
Heat	3	D42	3.2	♂	2	45	46.5	64	32	52.0	76	47	46.8	64	0.0	0.0	
Heat	3	D42	3.2	♂	3	40	47.4	60	39	53.9	76	40	48.0	68	0.0	0.0	
Heat	3	D42	3.2	♂	4	36	21.3	30	39	21.3	38	40	25.7	34	20.4	0.0	**0.0989**
Heat	3	D42	10.1	♀	1	40	61.5	84	30	63.0	93	41	52.0	60	-15.4	-28.6	**<0.0001**
Heat	3	D42	10.1	♀	2	40	50.7	56	36	54.3	76	41	53.3	56	0.0	0.0	
Heat	3	D42	10.1	♀	3	40	50.4	56	40	62.4	93	40	52.7	60	0.0	0.0	
Heat	3	D42	10.1	♀	4	39	32.5	41	39	25.0	36	40	31.9	36	0.0	0.0	
Heat	3	D42	10.1	♂	1	29	61.8	84	37	58.8	76	39	49.6	64	-15.6	-15.8	**<0.0001**
Heat	3	D42	10.1	♂	2	40	46.4	52	32	52.0	76	40	49.7	56	0.0	0.0	
Heat	3	D42	10.1	♂	3	39	48.3	60	39	53.9	76	39	45.0	52	-6.8	-13.3	**0.0127**
Heat	3	D42	10.1	♂	4	40	26.2	36	40	21.7	26	40	25.8	31	0.0	0.0	
Heat	3	D42	3.1	♀	1	40	59.0	76	30	63.0	93	52	57.9	76	-1.8	0.0	**0.0290**

Table S2

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS n	UAS Mean	UAS Max[2]	Gal4 n	Gal4 Mean	Gal4 Max[2]	Gal4 + UAS n	Gal4 + UAS Mean	Gal4 + UAS Max[2]	% Extension Mean	% Extension Max[2]	P
Heat	3	D42	3.1	♀	2	41	50.4	60	36	54.3	76	42	58.7	93	8.1	22.4	0.1511
Heat	3	D42	3.1	♀	3	38	46.2	56	40	62.4	93	41	52.7	76	0.0	0.0	
Heat	3	D42	3.1	♀	4	38	22.6	34	39	22.6	30	40	23.7	34	5.0	0.0	0.1976
Heat	3	D42	3.1	♂	1	39	61.9	76	37	58.8	76	40	54.7	76	-7.0	0.0	**0.0006**
Heat	3	D42	3.1	♂	2	40	42.6	56	32	52.0	76	40	52.9	76	1.7	0.0	0.7850
Heat	3	D42	3.1	♂	3	18	52.2	76	39	53.9	76	42	49.8	68	-4.6	-10.5	**0.0430**
Heat	3	D42	3.1	♂	4	40	16.5	26	39	21.3	38	40	17.5	22	0.0	-15.4	0.0041
Heat	3	D42	4.4	♀	1	41	67.1	84	30	63.0	93	38	65.1	93	0.0	0.0	
Heat	3	D42	4.4	♀	2	33	60.8	76	36	54.3	76	47	60.6	93	0.0	22.4	
Heat	3	D42	4.4	♀	3	40	59.3	76	40	62.4	93	46	55.4	76	-6.6	0.0	**0.0004**
Heat	3	D42	4.4	♀	4	40	32.2	41	40	27.5	36	40	36.5	46	13.4	12.2	**0.0011**
Heat	3	D42	4.4	♂	1	43	69.9	84	37	58.8	76	41	57.5	76	-2.3	0.0	0.6225
Heat	3	D42	4.4	♂	2	40	56.2	76	32	52.0	76	45	58.9	84	4.9	10.5	**0.0091**
Heat	3	D42	4.4	♂	3	38	55.2	76	39	53.9	76	40	53.6	76	-0.6	0.0	0.5432
Heat	3	D42	4.4	♂	4	40	31.3	36	41	28.0	41	40	35.8	46	14.4	12.2	**<0.0001**
Heat	10	daGal4	2.1	♀	1	40	20.8	30	38	18.5	30	40	21.1	30	1.4	0.0	**0.0164**
Heat	10	daGal4	2.1	♀	2	39	31.5	36	41	30.0	33	39	30.4	33	0.0	0.0	
Heat	10	daGal4	2.1	♀	3	39	33.5	40	40	30.7	36	40	32.1	40	0.0	0.0	
Heat	10	daGal4	2.1	♀	4	36	30.1	33	37	28.5	33	40	29.0	33	0.0	0.0	
Heat	10	daGal4	2.1	♂	1	39	16.4	21	37	15.9	21	40	17.9	21	9.1	0.0	**0.0027**
Heat	10	daGal4	2.1	♂	2	39	27.0	28	38	25.3	28	39	27.7	28	2.7	0.0	**0.0346**
Heat	10	daGal4	2.1	♂	3	38	27.4	33	40	25.7	28	41	27.3	33	0.0	0.0	
Heat	10	daGal4	2.1	♂	4	40	24.7	28	40	24.9	28	39	25.0	28	0.5	0.0	0.2057
Heat	10	daGal4	4.3	♀	1	26	17.5	21	39	17.8	21	40	18.7	21	4.9	0.0	0.0697
Heat	10	daGal4	4.3	♀	2	39	31.2	36	41	30.0	33	40	33.4	40	7.0	11.1	**0.0005**
Heat	10	daGal4	4.3	♀	3	41	32.2	36	40	30.7	36	42	33.0	36	2.4	0.0	**0.0008**
Heat	10	daGal4	4.3	♀	4	38	29.1	33	37	28.5	33	40	31.7	33	9.1	0.0	**0.0243**
Heat	10	daGal4	4.3	♂	1	27	14.6	17	39	16.9	21	40	15.1	17	0.0	0.0	
Heat	10	daGal4	4.3	♂	2	31	25.7	28	38	25.3	28	39	28.4	33	10.7	17.9	**0.0013**
Heat	10	daGal4	4.3	♂	3	41	26.2	28	40	25.7	28	39	27.8	33	6.4	17.9	**0.0004**
Heat	10	daGal4	4.3	♂	4	41	24.2	28	40	24.9	28	35	27.1	28	8.9	0.0	**<0.0001**
Heat	10	daGal4	9.1	♀	1	39	31.8	36	41	30.0	33	41	32.0	40	0.6	11.1	**0.0027**
Heat	10	daGal4	9.1	♀	2	40	32.8	40	40	30.7	36	41	35.0	40	6.9	0.0	**0.0051**
Heat	10	daGal4	9.1	♀	3	39	29.3	33	37	28.5	33	39	29.5	36	0.7	9.1	0.0638
Heat	10	daGal4	9.1	♂	1	40	25.8	28	38	25.3	28	39	29.0	33	12.5	17.9	**<0.0001**
Heat	10	daGal4	9.1	♂	2	41	27.6	33	40	25.7	28	38	30.5	33	10.6	0.0	**<0.0001**
Heat	10	daGal4	9.1	♂	3	43	25.1	28	40	24.9	28	41	27.5	28	9.6	0.0	**<0.0001**
Heat	10	daGal4	3.2	♀	1	32	19.0	27	40	18.0	24	40	30.6	40	61.2	48.1	**<0.0001**
Heat	10	daGal4	3.2	♀	2	39	35.3	40	41	30.0	33	40	32.0	36	0.0	0.0	
Heat	10	daGal4	3.2	♀	3	40	36.6	40	40	30.7	36	38	32.3	36	0.0	0.0	
Heat	10	daGal4	3.2	♀	4	37	32.0	40	37	28.5	33	40	29.4	33	0.0	0.0	
Heat	10	daGal4	3.2	♂	1	36	16.1	24	40	17.1	21	40	17.6	21	2.6	0.0	**0.0426**
Heat	10	daGal4	3.2	♂	2	40	33.5	40	38	25.3	28	42	29.4	33	0.0	0.0	
Heat	10	daGal4	3.2	♂	3	41	32.4	40	40	25.7	28	42	31.8	40	0.0	0.0	
Heat	10	daGal4	3.2	♂	4	43	30.3	36	40	24.9	28	46	28.1	33	0.0	0.0	
Heat	10	daGal4	10.1	♀	1	36	19.3	21	39	17.8	21	40	21.4	30	10.4	42.9	**0.0019**
Heat	10	daGal4	10.1	♀	2	41	34.1	40	41	30.0	33	40	30.4	33	0.0	0.0	
Heat	10	daGal4	10.1	♀	3	41	36.1	45	40	30.7	36	40	31.7	36	0.0	0.0	
Heat	10	daGal4	10.1	♀	4	39	33.4	40	37	28.5	33	38	28.5	33	0.0	0.0	

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Heat	10	daGal4	10.1	♂	1	40	16.6	21	39	16.9	21	38	17.9	21	6.2	0.0	**0.0296**
Heat	10	daGal4	10.1	♂	2	40	31.9	40	38	25.3	28	39	30.4	33	0.0	0.0	
Heat	10	daGal4	10.1	♂	3	42	32.5	40	40	25.7	28	39	31.9	36	0.0	0.0	
Heat	10	daGal4	10.1	♂	4	45	29.1	36	40	24.9	28	40	28.5	33	0.0	0.0	
Heat	10	daGal4	3.1	♀	1	40	15.5	21	40	18.0	24	40	21.6	32	19.7	33.3	**0.0006**
Heat	10	daGal4	3.1	♀	2	41	35.6	45	41	30.0	33	40	32.1	33	0.0	0.0	
Heat	10	daGal4	3.1	♀	3	35	36.0	40	40	30.7	36	41	31.0	36	0.0	0.0	
Heat	10	daGal4	3.1	♀	4	40	32.8	40	37	28.5	33	42	30.7	36	0.0	0.0	
Heat	10	daGal4	3.1	♂	1	39	13.3	21	40	17.1	21	40	18.8	24	9.6	14.3	**0.0068**
Heat	10	daGal4	3.1	♂	2	40	30.8	40	38	25.3	28	39	30.4	33	0.0	0.0	
Heat	10	daGal4	3.1	♂	3	40	32.6	40	40	25.7	28	40	29.7	33	0.0	0.0	
Heat	10	daGal4	3.1	♂	4	39	29.1	36	40	24.9	28	41	27.9	33	0.0	0.0	
Heat	10	daGal4	4.4	♀	1	39	17.6	21	39	17.8	21	39	18.6	21	4.6	0.0	**0.0338**
Heat	10	daGal4	4.4	♀	2	40	32.1	36	41	30.0	33	41	31.0	33	0.0	0.0	
Heat	10	daGal4	4.4	♀	3	40	34.5	40	40	30.7	36	42	29.6	36	-3.4	0.0	0.1136
Heat	10	daGal4	4.4	♀	4	38	29.8	36	37	28.5	33	41	28.1	33	-1.4	0.0	**0.0027**
Heat	10	daGal4	4.4	♂	1	39	13.9	17	39	16.9	21	40	15.8	17	0.0	0.0	
Heat	10	daGal4	4.4	♂	2	39	29.7	33	38	25.3	28	40	27.9	28	0.0	0.0	
Heat	10	daGal4	4.4	♂	3	39	29.9	33	40	25.7	28	41	29.0	33	0.0	0.0	
Heat	10	daGal4	4.4	♂	4	41	27.2	28	40	24.9	28	38	25.6	28	0.0	0.0	
Heat	10	DJ694	2.1	♀	1	40	20.8	30	38	20.4	30	40	20.1	30	-1.8	0.0	0.1930
Heat	10	DJ694	2.1	♀	2	39	31.5	36	40	33.3	36	39	30.6	36	-2.7	0.0	**0.0002**
Heat	10	DJ694	2.1	♀	3	39	33.5	40	41	35.3	40	40	32.6	40	-2.8	0.0	**0.0037**
Heat	10	DJ694	2.1	♀	4	36	30.1	33	39	31.3	33	39	31.1	33	0.0	0.0	
Heat	10	DJ694	2.1	♂	1	39	16.4	21	37	14.9	21	40	16.1	21	0.0	0.0	
Heat	10	DJ694	2.1	♂	2	39	27.0	28	40	27.9	28	41	26.6	28	-1.3	0.0	**0.0003**
Heat	10	DJ694	2.1	♂	3	38	27.4	33	40	26.3	28	40	24.6	28	-6.5	0.0	**<0.0001**
Heat	10	DJ694	2.1	♂	4	40	24.7	28	38	27.4	33	39	24.9	28	0.0	0.0	
Heat	10	DJ694	4.3	♀	1	26	17.5	21	40	18.9	21	37	17.3	21	-0.8	0.0	**0.0176**
Heat	10	DJ694	4.3	♀	2	39	31.2	36	40	33.3	36	38	31.3	36	0.0	0.0	
Heat	10	DJ694	4.3	♀	3	41	32.2	36	41	35.3	40	40	34.3	40	0.0	0.0	
Heat	10	DJ694	4.3	♀	4	38	29.1	33	39	31.3	33	41	30.7	33	0.0	0.0	
Heat	10	DJ694	4.3	♂	1	27	14.6	17	40	16.0	17	38	14.9	17	0.0	0.0	
Heat	10	DJ694	4.3	♂	2	31	25.7	28	40	27.9	28	42	27.3	28	0.0	0.0	
Heat	10	DJ694	4.3	♂	3	41	26.2	28	40	26.3	28	38	25.9	28	-1.1	0.0	0.2365
Heat	10	DJ694	4.3	♂	4	41	24.2	28	38	27.4	33	40	26.1	28	0.0	0.0	
Heat	10	DJ694	9.1	♀	1	39	31.8	36	40	33.3	36	39	33.6	36	1.0	0.0	**0.0298**
Heat	10	DJ694	9.1	♀	2	40	32.8	40	41	35.3	40	41	37.4	40	5.9	0.0	**0.0035**
Heat	10	DJ694	9.1	♀	3	39	29.3	33	39	31.3	33	40	31.8	33	1.6	0.0	0.9057
Heat	10	DJ694	9.1	♂	1	40	25.8	28	40	27.9	28	41	27.6	28	0.0	0.0	
Heat	10	DJ694	9.1	♂	2	41	27.6	33	40	26.3	28	40	27.4	28	0.0	0.0	
Heat	10	DJ694	9.1	♂	3	43	25.1	28	38	27.4	33	43	26.0	28	0.0	0.0	
Heat	10	DJ694	3.2	♀	1	32	19.0	27	40	14.9	21	40	26.9	36	41.6	33.3	**<0.0001**
Heat	10	DJ694	3.2	♀	2	39	35.3	40	40	33.3	36	40	35.2	40	0.0	0.0	
Heat	10	DJ694	3.2	♀	3	40	36.6	40	41	35.3	40	40	41.1	45	12.4	12.5	**<0.0001**
Heat	10	DJ694	3.2	♀	4	37	32.0	40	39	31.3	33	40	36.7	45	14.6	12.5	**<0.0001**
Heat	10	DJ694	3.2	♂	1	36	16.1	24	40	15.7	21	40	16.2	18	0.7	-14.3	0.5306
Heat	10	DJ694	3.2	♂	2	40	33.5	40	40	27.9	28	40	28.5	33	0.0	0.0	
Heat	10	DJ694	3.2	♂	3	41	32.4	40	40	26.3	28	40	29.9	33	0.0	0.0	

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Heat	10	DJ694	3.2	♂	4	43	30.3	36	38	27.4	33	39	27.8	33	0.0	0.0	
Heat	10	DJ694	10.1	♀	1	36	19.3	21	40	18.9	21	42	18.9	24	0.0	14.3	
Heat	10	DJ694	10.1	♀	2	41	34.1	40	40	33.3	36	40	33.8	40	0.0	0.0	
Heat	10	DJ694	10.1	♀	3	41	36.1	45	41	35.3	40	40	36.6	45	1.3	0.0	0.1489
Heat	10	DJ694	10.1	♀	4	39	33.4	40	39	31.3	33	40	32.7	36	0.0	0.0	
Heat	10	DJ694	10.1	♂	1	40	16.6	21	40	16.0	17	25	15.2	17	-4.8	0.0	0.0319
Heat	10	DJ694	10.1	♂	2	40	31.9	40	40	27.9	28	39	31.6	33	0.0	0.0	
Heat	10	DJ694	10.1	♂	3	42	32.5	40	40	26.3	28	41	30.1	36	0.0	0.0	
Heat	10	DJ694	10.1	♂	4	45	29.1	36	38	27.4	0.37	43	28.8	33	0.0	0.0	
Heat	10	DJ694	3.1	♀	1	40	15.5	21	40	14.9	21	40	19.1	24	22.7	14.3	**<0.0001**
Heat	10	DJ694	3.1	♀	2	41	35.6	45	40	33.3	36	40	32.0	33	-3.9	-8.3	**0.0025**
Heat	10	DJ694	3.1	♀	3	35	36.0	40	41	35.3	40	41	37.2	40	3.4	0.0	**0.0079**
Heat	10	DJ694	3.1	♀	4	40	32.8	40	39	31.3	33	38	31.9	36	0.0	0.0	
Heat	10	DJ694	3.1	♂	1	39	13.3	21	40	15.7	21	40	15.7	18	0.0	-14.3	
Heat	10	DJ694	3.1	♂	2	40	30.8	40	40	27.9	28	39	27.6	28	-1.1	0.0	0.1595
Heat	10	DJ694	3.1	♂	3	40	32.6	40	40	26.3	28	38	29.2	33	0.0	0.0	
Heat	10	DJ694	3.1	♂	4	39	29.1	36	38	27.4	33	39	26.7	28	-2.8	-15.2	0.0795
Heat	10	DJ694	4.4	♀	1	39	17.6	21	40	18.9	21	40	20.4	21	7.9	0.0	**0.0016**
Heat	10	DJ694	4.4	♀	2	40	32.1	36	40	33.3	36	40	30.1	33	-6.0	-8.3	**0.0009**
Heat	10	DJ694	4.4	♀	3	40	34.5	40	41	35.3	40	40	31.1	33	-9.9	-17.5	**<0.0001**
Heat	10	DJ694	4.4	♀	4	38	29.8	36	39	31.3	33	41	27.3	33	-8.3	0.0	**0.0014**
Heat	10	DJ694	4.4	♂	1	39	13.9	17	40	16.0	17	38	13.8	17	-0.6	0.0	0.9654
Heat	10	DJ694	4.4	♂	2	39	29.7	33	40	27.9	28	40	27.4	28	-1.8	0.0	**0.0493**
Heat	10	DJ694	4.4	♂	3	39	29.9	33	40	26.3	28	40	27.0	28	0.0	0.0	
Heat	10	DJ694	4.4	♂	4	41	27.2	28	38	27.4	33	41	23.8	24	-12.6	-14.3	**<0.0001**
Heat	10	D42	2.1	♀	1	40	20.8	30	38	22.7	30	40	20.3	25	-2.2	-16.7	**0.0065**
Heat	10	D42	2.1	♀	2	39	31.5	36	38	33.6	40	40	32.8	36	0.0	0.0	
Heat	10	D42	2.1	♀	3	39	33.5	40	40	35.4	40	31	32.5	36	-2.8	-10.0	**0.0001**
Heat	10	D42	2.1	♀	4	36	30.1	33	40	28.8	36	40	28.9	36	0.0	0.0	
Heat	10	D42	2.1	♂	1	39	16.4	21	36	13.7	17	40	13.5	17	-1.6	0.0	0.4803
Heat	10	D42	2.1	♂	2	39	27.0	28	42	27.8	33	50	25.1	33	-6.8	0.0	**0.0003**
Heat	10	D42	2.1	♂	3	38	27.4	33	40	27.6	33	40	28.1	33	1.8	0.0	0.2207
Heat	10	D42	2.1	♂	4	40	24.7	28	40	25.8	33	40	25.0	28	0.0	0.0	
Heat	10	D42	4.3	♀	1	26	17.5	21	39	21.3	30	40	19.3	21	0.0	0.0	
Heat	10	D42	4.3	♀	2	39	31.2	36	38	33.6	40	40	32.0	36	0.0	0.0	
Heat	10	D42	4.3	♀	3	41	32.2	36	40	35.4	40	39	34.7	40	0.0	0.0	
Heat	10	D42	4.3	♀	4	38	29.1	33	40	28.8	36	40	29.3	33	0.9	0.0	0.2837
Heat	10	D42	4.3	♂	1	27	14.6	17	39	18.5	21	40	12.6	13	-13.9	-23.5	**<0.0001**
Heat	10	D42	4.3	♂	2	31	25.7	28	42	27.8	33	40	30.2	33	8.6	0.0	**0.0004**
Heat	10	D42	4.3	♂	3	41	26.2	28	40	27.6	33	42	28.7	33	4.2	0.0	**0.0002**
Heat	10	D42	4.3	♂	4	41	24.2	28	40	25.8	33	40	25.1	28	0.0	0.0	
Heat	10	D42	9.1	♀	1	39	31.8	36	38	33.6	40	40	34.1	40	1.5	0.0	**0.0047**
Heat	10	D42	9.1	♀	2	40	32.8	40	40	35.4	40	40	34.7	40	0.0	0.0	
Heat	10	D42	9.1	♀	3	39	29.3	33	40	28.8	36	39	31.5	33	7.3	0.0	**0.0003**
Heat	10	D42	9.1	♂	1	40	25.8	28	42	27.8	33	39	27.9	33	0.5	0.0	0.6270
Heat	10	D42	9.1	♂	2	41	27.6	33	40	27.6	33	41	28.5	33	3.4	0.0	0.0760
Heat	10	D42	9.1	♂	3	43	25.1	28	40	25.8	33	38	23.8	28	-5.3	0.0	**0.0182**
Heat	10	D42	3.2	♀	1	32	19.0	27	37	18.6	27	40	30.8	36	62.1	33.3	**<0.0001**
Heat	10	D42	3.2	♀	2	39	35.3	40	38	33.6	40	40	36.3	40	3.0	0.0	**0.0011**

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Heat	10	D42	3.2	♀	3	40	36.6	40	40	35.4	40	40	37.7	40	3.2	0.0	**0.0006**
Heat	10	D42	3.2	♀	4	37	32.0	40	40	28.8	36	44	36.3	45	13.4	12.5	**<0.0001**
Heat	10	D42	3.2	♂	1	36	16.1	24	38	15.9	21	40	16.3	24	1.2	0.0	0.4681
Heat	10	D42	3.2	♂	2	40	33.5	40	42	27.8	33	41	31.9	36	0.0	0.0	
Heat	10	D42	3.2	♂	3	41	32.4	40	40	27.6	33	43	33.4	40	3.0	0.0	0.1824
Heat	10	D42	3.2	♂	4	43	30.3	36	40	25.8	33	37	31.1	36	2.5	0.0	0.4366
Heat	10	D42	10.1	♀	1	36	19.3	21	39	21.3	30	31	26.0	0.79	22.2	0.0	**<0.0001**
Heat	10	D42	10.1	♀	2	41	34.1	40	38	33.6	40	39	31.7	0.68	-5.5	12.5	**0.0149**
Heat	10	D42	10.1	♀	3	41	36.1	45	40	35.4	40	40	29.1	1.33	-17.7	0.0	**0.0022**
Heat	10	D42	10.1	♀	4	39	33.4	40	40	28.8	36	41	34.3	0.5	2.9	0.0	0.2401
Heat	10	D42	10.1	♂	1	40	16.6	21	39	18.5	21	36	19.2	0.47	3.4	14.3	**0.0002**
Heat	10	D42	10.1	♂	2	40	31.9	40	42	27.8	33	40	32.4	0.36	1.6	0.0	0.4031
Heat	10	D42	10.1	♂	3	42	32.5	40	40	27.6	33	47	33.3	0.41	2.7	0.0	0.4520
Heat	10	D42	10.1	♂	4	45	29.1	36	40	25.8	33	37	32.8	0.39	12.5	0.0	**<0.0001**
Heat	10	D42	3.1	♀	1	40	15.5	21	37	18.6	27	40	18.4	24	0.0	0.0	
Heat	10	D42	3.1	♀	2	41	35.6	45	38	33.6	40	30	32.0	33	-4.8	-17.5	**0.0371**
Heat	10	D42	3.1	♀	3	35	36.0	40	40	35.4	40	40	28.3	36	-19.9	-10.0	**<0.0001**
Heat	10	D42	3.1	♀	4	40	32.8	40	40	28.8	36	40	31.6	36	0.0	0.0	
Heat	10	D42	3.1	♂	1	39	13.3	21	38	15.9	21	40	11.6	15	-12.6	-28.6	**0.0115**
Heat	10	D42	3.1	♂	2	40	30.8	40	42	27.8	33	40	28.4	33	0.0	0.0	
Heat	10	D42	3.1	♂	3	40	32.6	40	40	27.6	33	40	28.6	33	0.0	0.0	
Heat	10	D42	3.1	♂	4	39	29.1	36	40	25.8	33	30	29.6	33	1.9	0.0	0.8362
Heat	10	D42	4.4	♀	1	39	17.6	21	39	21.3	30	40	23.3	30	9.4	0.0	**0.0109**
Heat	10	D42	4.4	♀	2	40	32.1	36	38	33.6	40	38	34.6	40	3.0	0.0	0.2075
Heat	10	D42	4.4	♀	3	40	34.5	40	40	35.4	40	40	35.1	40	0.0	0.0	0.2551
Heat	10	D42	4.4	♀	4	38	29.8	36	40	28.8	36	39	33.6	36	13.0	0.0	**<0.0001**
Heat	10	D42	4.4	♂	1	39	13.9	17	39	18.5	21	39	14.4	17	0.0	0.0	
Heat	10	D42	4.4	♂	2	39	29.7	33	42	27.8	33	40	29.4	33	0.0	0.0	
Heat	10	D42	4.4	♂	3	39	29.9	33	40	27.6	33	41	29.8	33	0.0	0.0	
Heat	10	D42	4.4	♂	4	41	27.2	28	40	25.8	33	39	31.0	33	13.7	0.0	**<0.0001**
Paraquat	3	daGal4	2.1	♀	1	40	29.4	47	40	25.0	42	40	24.6	52	-1.5	10.6	0.0802
Paraquat	3	daGal4	2.1	♀	2	40	18.9	30	40	14.4	20	40	15.3	20	0.0	0.0	
Paraquat	3	daGal4	2.1	♀	3	40	55.9	90	38	51.3	90	38	49.9	90	-2.6	0.0	0.7017
Paraquat	3	daGal4	2.1	♀	4	40	17.8	25	40	13.6	20	40	15.5	20	0.0	0.0	
Paraquat	3	daGal4	2.1	♀	5	37	56.4	84	40	43.6	90	39	42.1	90	-3.4	0.0	**0.0348**
Paraquat	3	daGal4	2.1	♀	6	42	50.9	84	39	41.6	74	39	45.3	90	0.0	7.1	
Paraquat	3	daGal4	2.1	♂	1	40	27.5	57	40	22.5	42	40	18.6	32	-17.2	-23.8	**0.0199**
Paraquat	3	daGal4	2.1	♂	2	40	17.9	25	40	14.1	20	40	14.8	25	0.0	0.0	
Paraquat	3	daGal4	2.1	♂	3	40	35.5	66	41	22.8	34	41	28.2	66	0.0	0.0	
Paraquat	3	daGal4	2.1	♂	4	40	17.8	25	40	14.1	20	40	13.8	20	-2.7	0.0	**0.0001**
Paraquat	3	daGal4	2.1	♂	5	34	40.2	84	40	26.6	50	40	27.6	50	0.0	0.0	
Paraquat	3	daGal4	2.1	♂	6	41	39.0	84	42	23.1	38	40	27.2	50	0.0	0.0	
Paraquat	3	daGal4	4.3	♀	1	40	30.8	43	40	38.8	56	40	28.5	56	-7.5	0.0	**0.0012**
Paraquat	3	daGal4	4.3	♀	2	42	49.1	84	38	51.3	90	40	40.9	74	-16.9	-11.9	**0.0135**
Paraquat	3	daGal4	4.3	♀	3	41	39.3	74	40	43.6	90	37	34.5	58	-12.0	-21.6	**0.0263**
Paraquat	3	daGal4	4.3	♀	4	40	43.2	74	39	41.6	74	41	45.1	74	4.3	0.0	0.2569
Paraquat	3	daGal4	4.3	♂	1	40	27.8	56	40	29.4	69	40	32.2	69	9.4	0.0	0.2330
Paraquat	3	daGal4	4.3	♂	2	50	42.9	84	41	22.8	34	40	30.5	74	0.0	0.0	
Paraquat	3	daGal4	4.3	♂	3	41	41.6	110	40	26.6	50	40	39.7	84	0.0	0.0	

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Paraquat	3	daGal4	4.3	♂	4	41	46.7	84	42	23.1	38	36	48.2	90	3.2	7.1	0.8014
Paraquat	3	daGal4	9.1	♀	1	39	21.2	32	36	16.9	32	39	23.0	41	8.6	28.1	**0.0002**
Paraquat	3	daGal4	9.1	♀	2	39	54.4	90	38	51.3	90	40	40.7	84	-20.7	-6.7	**0.0063**
Paraquat	3	daGal4	9.1	♀	3	40	21.5	32	37	18.5	32	40	17.8	23	-3.6	-28.1	**0.0162**
Paraquat	3	daGal4	9.1	♀	4	39	55.1	84	40	43.6	90	42	34.2	58	-21.5	-31.0	**0.0262**
Paraquat	3	daGal4	9.1	♀	5	46	49.4	90	39	41.6	74	40	41.2	84	-0.9	0.0	**0.0313**
Paraquat	3	daGal4	9.1	♂	1	40	14.7	23	39	17.6	32	40	14.3	23	-3.1	0.0	**0.0088**
Paraquat	3	daGal4	9.1	♂	2	42	31.0	66	41	22.8	34	40	31.1	58	0.5	0.0	**0.0004**
Paraquat	3	daGal4	9.1	♂	3	40	15.7	23	40	15.1	23	38	13.6	23	-9.7	0.0	**0.0249**
Paraquat	3	daGal4	9.1	♂	4	34	38.2	74	40	26.6	50	43	26.8	50	0.0	0.0	
Paraquat	3	daGal4	9.1	♂	5	39	31.2	66	42	23.1	38	39	25.7	46	0.0	0.0	
Paraquat	3	daGal4	3.2	♀	1	40	30.7	80	40	27.8	65	40	31.1	75	1.3	0.0	0.2841
Paraquat	3	daGal4	3.2	♀	2	36	69.0	118	38	51.3	90	40	50.8	110	-1.0	0.0	**0.0110**
Paraquat	3	daGal4	3.2	♀	3	38	67.4	110	40	43.6	90	42	54.8	90	0.0	0.0	
Paraquat	3	daGal4	3.2	♀	4	40	60.2	110	39	41.6	74	40	41.9	110	0.0	0.0	
Paraquat	3	daGal4	3.2	♂	1	40	33.9	80	40	25.1	51	40	42.1	94	24.0	17.5	**0.0005**
Paraquat	3	daGal4	3.2	♂	2	39	54.8	90	41	22.8	34	41	51.2	110	0.0	22.2	
Paraquat	3	daGal4	3.2	♂	3	40	62.8	110	40	26.6	50	41	41.6	110	0.0	0.0	
Paraquat	3	daGal4	3.2	♂	4	37	64.3	110	42	23.1	38	40	52.2	118	0.0	7.3	
Paraquat	3	daGal4	10.1	♀	1	40	41.6	117	40	42.5	81	39	54.3	105	27.6	0.0	**0.0252**
Paraquat	3	daGal4	10.1	♀	2	40	23.7	41	36	16.9	32	40	22.3	41	0.0	0.0	
Paraquat	3	daGal4	10.1	♀	3	28	66.1	90	38	51.3	90	42	67.0	110	1.5	22.2	**0.0101**
Paraquat	3	daGal4	10.1	♀	4	40	24.3	59	37	18.5	32	40	23.1	41	0.0	0.0	
Paraquat	3	daGal4	10.1	♀	5	39	88.3	118	40	43.6	90	41	60.0	110	0.0	0.0	
Paraquat	3	daGal4	10.1	♀	6	37	78.3	110	39	41.6	74	40	59.6	110	0.0	0.0	
Paraquat	3	daGal4	10.1	♂	1	40	63.7	117	40	34.0	69	40	69.0	129	8.4	10.3	0.2277
Paraquat	3	daGal4	10.1	♂	2	40	23.3	41	39	17.6	32	39	21.6	41	0.0	0.0	
Paraquat	3	daGal4	10.1	♂	3	39	47.5	90	41	22.8	34	43	42.5	110	0.0	22.2	
Paraquat	3	daGal4	10.1	♂	4	40	28.3	41	40	15.1	23	38	18.7	32	0.0	0.0	
Paraquat	3	daGal4	10.1	♂	5	39	73.1	130	40	26.6	50	41	42.6	110	0.0	0.0	
Paraquat	3	daGal4	10.1	♂	6	42	59.0	110	42	23.1	38	40	38.9	90	0.0	0.0	
Paraquat	3	daGal4	3.1	♀	1	40	33.4	64	40	36.4	64	40	35.0	64	0.0	0.0	
Paraquat	3	daGal4	3.1	♀	2	40	16.0	25	40	14.4	20	40	17.4	25	8.6	0.0	**0.0011**
Paraquat	3	daGal4	3.1	♀	3	40	46.1	74	38	51.3	90	40	55.4	90	8.1	0.0	**0.0016**
Paraquat	3	daGal4	3.1	♀	4	40	18.0	30	40	13.6	20	40	16.5	25	0.0	0.0	
Paraquat	3	daGal4	3.1	♀	5	36	50.6	84	40	43.6	90	41	58.1	84	14.8	0.0	**0.0242**
Paraquat	3	daGal4	3.1	♀	6	44	49.4	74	39	41.6	74	39	59.9	110	21.4	48.6	**0.0048**
Paraquat	3	daGal4	3.1	♂	1	40	23.3	39	40	26.8	55	40	38.8	82	44.8	49.1	**0.0002**
Paraquat	3	daGal4	3.1	♂	2	40	18.8	40	40	14.1	20	40	24.1	64	28.7	60.0	**0.0373**
Paraquat	3	daGal4	3.1	♂	3	45	41.4	90	41	22.8	34	41	59.2	110	42.8	22.2	**0.0009**
Paraquat	3	daGal4	3.1	♂	4	40	19.3	25	40	14.1	20	40	21.6	49	12.1	96.0	0.2077
Paraquat	3	daGal4	3.1	♂	5	39	41.6	84	40	26.6	50	41	53.8	110	29.1	31.0	**0.0115**
Paraquat	3	daGal4	3.1	♂	6	41	44.3	84	42	23.1	38	42	57.3	110	29.3	31.0	**0.0259**
Paraquat	3	daGal4	4.4	♀	1	40	45.4	69	40	38.8	56	40	45.0	81	0.0	17.4	
Paraquat	3	daGal4	4.4	♀	2	40	26.5	41	36	16.9	32	39	22.0	41	0.0	0.0	
Paraquat	3	daGal4	4.4	♀	3	36	53.7	90	38	51.3	90	41	46.9	110	-8.5	22.2	0.3531
Paraquat	3	daGal4	4.4	♀	4	40	28.2	48	37	18.5	32	43	20.0	41	0.0	0.0	
Paraquat	3	daGal4	4.4	♀	5	40	67.1	110	40	43.6	90	39	43.9	90	0.0	0.0	
Paraquat	3	daGal4	4.4	♀	6	41	57.2	90	39	41.6	74	41	40.6	84	-2.4	0.0	**0.0023**

Table S2

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS n	UAS Mean	UAS Max[2]	Gal4 n	Gal4 Mean	Gal4 Max[2]	Gal4 + UAS n	Gal4 + UAS Mean	Gal4 + UAS Max[2]	% Extension Mean	% Extension Max[2]	P
Paraquat	3	daGal4	4.4	♂	1	40	39.2	81	40	29.4	69	40	46.8	105	19.3	29.6	**0.0002**
Paraquat	3	daGal4	4.4	♂	2	39	19.1	32	39	17.6	32	39	17.7	59	0.0	84.4	
Paraquat	3	daGal4	4.4	♂	3	41	37.7	84	41	22.8	34	41	49.6	110	31.6	31.0	**0.0444**
Paraquat	3	daGal4	4.4	♂	4	40	19.3	32	40	15.1	23	41	16.7	23	0.0	0.0	
Paraquat	3	daGal4	4.4	♂	5	40	57.9	110	40	26.6	50	42	40.4	84	0.0	0.0	
Paraquat	3	daGal4	4.4	♂	6	40	43.0	110	42	23.1	38	40	32.1	84	0.0	0.0	
Paraquat	3	DJ694	2.1	♀	1	40	29.5	51	40	20.7	35	40	20.2	40	-2.5	0.0	0.7691
Paraquat	3	DJ694	2.1	♀	2	40	29.4	47	40	18.6	27	40	19.9	32	0.0	0.0	
Paraquat	3	DJ694	2.1	♀	3	40	18.9	30	40	15.6	25	40	14.1	20	-9.6	-20.0	0.0938
Paraquat	3	DJ694	2.1	♀	4	40	55.9	90	24	56.7	84	39	45.8	84	-17.9	0.0	0.1047
Paraquat	3	DJ694	2.1	♀	5	40	17.8	25	40	15.6	20	40	14.5	20	-7.2	0.0	**0.0002**
Paraquat	3	DJ694	2.1	♀	6	37	56.4	84	39	77.6	130	39	41.4	90	-26.6	0.0	**0.0100**
Paraquat	3	DJ694	2.1	♀	7	42	50.9	84	42	65.7	118	40	46.2	110	-9.2	0.0	**0.0027**
Paraquat	3	DJ694	2.1	♂	1	40	25.2	40	40	16.6	22	40	19.3	30	0.0	0.0	
Paraquat	3	DJ694	2.1	♂	2	40	27.5	57	38	19.1	22	40	17.4	22	-9.1	0.0	**0.0294**
Paraquat	3	DJ694	2.1	♂	3	40	17.9	25	40	18.0	30	40	17.6	30	-1.4	0.0	0.7459
Paraquat	3	DJ694	2.1	♂	4	40	35.5	66	45	21.5	38	50	36.6	74	3.0	12.1	0.4456
Paraquat	3	DJ694	2.1	♂	5	40	17.8	25	40	17.5	25	40	19.1	30	7.7	20.0	0.1967
Paraquat	3	DJ694	2.1	♂	6	34	40.2	84	41	20.0	34	38	24.1	42	0.0	0.0	
Paraquat	3	DJ694	2.1	♂	7	41	39.0	84	41	22.5	34	40	28.7	66	0.0	0.0	
Paraquat	3	DJ694	4.3	♀	1	40	30.8	43	40	30.9	56	40	20.9	31	-32.3	-27.9	**<0.0001**
Paraquat	3	DJ694	4.3	♀	2	42	49.1	84	24	56.7	84	35	39.9	66	-18.7	-21.4	**0.0006**
Paraquat	3	DJ694	4.3	♀	3	41	39.3	74	39	77.6	130	37	54.2	84	0.0	0.0	
Paraquat	3	DJ694	4.3	♀	4	40	43.2	74	42	65.7	118	39	40.1	66	-7.2	-10.8	0.3893
Paraquat	3	DJ694	4.3	♂	1	40	27.8	56	40	15.3	22	40	18.0	43	0.0	0.0	
Paraquat	3	DJ694	4.3	♂	2	50	42.9	84	45	21.5	38	41	25.4	38	0.0	0.0	
Paraquat	3	DJ694	4.3	♂	3	41	41.6	110	41	20.0	34	40	26.5	34	0.0	0.0	
Paraquat	3	DJ694	4.3	♂	4	41	46.7	84	41	22.5	34	39	28.8	50	0.0	0.0	
Paraquat	3	DJ694	9.1	♀	1	39	21.2	32	40	16.6	23	40	16.6	32	0.0	0.0	
Paraquat	3	DJ694	9.1	♀	2	39	54.4	90	24	56.7	84	38	43.9	66	-19.3	-21.4	**0.0006**
Paraquat	3	DJ694	9.1	♀	3	40	21.5	32	39	18.5	32	39	21.2	32	0.0	0.0	
Paraquat	3	DJ694	9.1	♀	4	39	55.1	84	39	77.6	130	40	45.3	66	-17.8	-21.4	**0.0064**
Paraquat	3	DJ694	9.1	♀	5	46	49.4	90	42	65.7	118	41	46.7	74	-5.4	-17.8	**0.0002**
Paraquat	3	DJ694	9.1	♂	1	40	14.7	23	40	13.1	16	40	12.9	16	-1.3	0.0	**0.0042**
Paraquat	3	DJ694	9.1	♂	2	42	31.0	66	45	21.5	38	41	22.5	30	0.0	-21.1	
Paraquat	3	DJ694	9.1	♂	3	40	15.7	23	40	15.1	16	39	13.4	16	-10.7	0.0	**0.0061**
Paraquat	3	DJ694	9.1	♂	4	34	38.2	74	41	20.0	34	42	20.3	42	0.0	0.0	
Paraquat	3	DJ694	9.1	♂	5	39	31.2	66	41	22.5	34	40	25.3	42	0.0	0.0	
Paraquat	3	DJ694	3.2	♀	1	40	30.7	80	40	25.7	45	40	43.0	94	40.4	17.5	**0.0049**
Paraquat	3	DJ694	3.2	♀	2	36	69.0	118	24	56.7	84	29	43.8	110	-22.7	0.0	**0.0006**
Paraquat	3	DJ694	3.2	♀	3	38	67.4	110	39	77.6	130	42	57.3	110	-15.0	0.0	**0.0135**
Paraquat	3	DJ694	3.2	♀	4	40	60.2	110	42	65.7	118	42	69.3	118	5.6	0.0	0.0540
Paraquat	3	DJ694	3.2	♂	1	40	33.9	80	40	21.6	35	40	40.7	94	20.0	17.5	0.1150
Paraquat	3	DJ694	3.2	♂	2	39	54.8	90	45	21.5	38	44	34.4	74	0.0	0.0	
Paraquat	3	DJ694	3.2	♂	3	40	62.8	110	41	20.0	34	40	35.5	84	0.0	0.0	
Paraquat	3	DJ694	3.2	♂	4	37	64.3	110	41	22.5	34	39	40.4	90	0.0	0.0	
Paraquat	3	DJ694	10.1	♀	1	40	41.6	117	40	60.1	117	40	89.8	129	49.5	10.3	**<0.0001**
Paraquat	3	DJ694	10.1	♀	2	40	23.7	41	40	16.6	23	40	23.3	41	0.0	0.0	
Paraquat	3	DJ694	10.1	♀	3	28	66.1	90	24	56.7	84	34	43.9	110	-22.5	22.2	**0.0158**

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Paraquat	3	DJ694	10.1	♀	4	40	24.3	59	39	18.5	32	39	26.0	59	6.9	0.0	**0.0003**
Paraquat	3	DJ694	10.1	♀	5	39	88.3	118	39	77.6	130	46	50.8	110	-34.5	-6.8	**0.0005**
Paraquat	3	DJ694	10.1	♀	6	37	78.3	110	42	65.7	118	30	57.5	110	-12.4	0.0	**0.0142**
Paraquat	3	DJ694	10.1	♂	1	40	63.7	117	39	33.1	81	40	79.0	141	24.0	20.5	**0.0160**
Paraquat	3	DJ694	10.1	♂	2	40	23.3	41	40	13.1	16	34	18.7	32	0.0	0.0	
Paraquat	3	DJ694	10.1	♂	3	39	47.5	90	45	21.5	38	46	35.3	74	0.0	0.0	
Paraquat	3	DJ694	10.1	♂	4	40	28.3	41	40	15.1	16	37	22.1	41	0.0	0.0	
Paraquat	3	DJ694	10.1	♂	5	39	73.1	130	41	20.0	34	26	40.2	84	0.0	0.0	
Paraquat	3	DJ694	10.1	♂	6	42	59.0	110	41	22.5	34	39	34.7	90	0.0	0.0	
Paraquat	3	DJ694	3.1	♀	1	40	31.4	45	40	20.7	35	40	22.6	35	0.0	0.0	
Paraquat	3	DJ694	3.1	♀	2	40	33.4	64	40	40.1	76	40	28.5	64	-14.7	0.0	**0.0009**
Paraquat	3	DJ694	3.1	♀	3	40	16.0	25	40	15.6	25	40	13.0	15	-16.8	-40.0	**0.0002**
Paraquat	3	DJ694	3.1	♀	4	40	46.1	74	24	56.7	84	37	53.7	90	0.0	7.1	
Paraquat	3	DJ694	3.1	♀	5	40	18.0	30	40	15.6	20	40	13.6	20	-12.8	0.0	**0.0076**
Paraquat	3	DJ694	3.1	♀	6	36	50.6	84	39	77.6	130	41	42.1	110	-16.8	0.0	0.1365
Paraquat	3	DJ694	3.1	♀	7	44	49.4	74	42	65.7	118	40	47.1	90	-4.7	0.0	**0.0008**
Paraquat	3	DJ694	3.1	♂	1	40	30.5	57	40	16.6	22	40	27.1	63	0.0	10.5	
Paraquat	3	DJ694	3.1	♂	2	40	23.3	39	40	18.9	25	40	21.5	35	0.0	0.0	
Paraquat	3	DJ694	3.1	♂	3	40	18.8	40	40	18.0	30	40	20.1	40	7.2	0.0	0.1637
Paraquat	3	DJ694	3.1	♂	4	45	41.4	90	45	21.5	38	54	42.6	84	2.7	0.0	0.7185
Paraquat	3	DJ694	3.1	♂	5	40	19.3	25	40	17.5	25	40	19.9	30	3.2	20.0	0.0502
Paraquat	3	DJ694	3.1	♂	6	39	41.6	84	41	20.0	34	39	33.5	90	0.0	7.1	
Paraquat	3	DJ694	3.1	♂	7	41	44.3	84	41	22.5	34	44	37.1	58	0.0	0.0	
Paraquat	3	DJ694	4.4	♀	1	40	35.5	58	40	45.1	67	40	55.8	97	23.8	44.8	**0.0047**
Paraquat	3	DJ694	4.4	♀	2	40	45.4	69	40	30.9	56	40	28.1	56	-9.0	0.0	0.3708
Paraquat	3	DJ694	4.4	♀	3	40	26.5	41	40	16.6	23	40	21.0	32	0.0	0.0	
Paraquat	3	DJ694	4.4	♀	4	36	53.7	90	24	56.7	84	40	52.8	110	-1.7	22.2	0.5266
Paraquat	3	DJ694	4.4	♀	5	40	28.2	48	39	18.5	32	40	23.0	32	0.0	0.0	
Paraquat	3	DJ694	4.4	♀	6	40	67.1	110	39	77.6	130	39	46.5	74	-30.7	-32.7	**<0.0001**
Paraquat	3	DJ694	4.4	♀	7	41	57.2	90	42	65.7	118	46	50.6	110	-11.5	0.0	**0.0091**
Paraquat	3	DJ694	4.4	♂	1	40	38.7	67	40	25.5	34	40	34.2	77	0.0	14.9	
Paraquat	3	DJ694	4.4	♂	2	40	39.2	81	40	15.3	22	40	20.1	31	0.0	0.0	
Paraquat	3	DJ694	4.4	♂	3	39	19.1	32	40	13.1	16	40	17.2	32	0.0	0.0	
Paraquat	3	DJ694	4.4	♂	4	41	37.7	84	45	21.5	38	42	29.3	42	0.0	0.0	
Paraquat	3	DJ694	4.4	♂	5	40	19.3	32	40	15.1	16	40	16.5	23	0.0	0.0	
Paraquat	3	DJ694	4.4	♂	6	40	57.9	110	41	20.0	34	39	24.2	34	0.0	0.0	
Paraquat	3	DJ694	4.4	♂	7	40	43.0	110	41	22.5	34	41	26.7	58	0.0	0.0	
Paraquat	3	D42	2.1	♀	1	40	29.5	51	40	23.3	35	40	24.5	45	0.0	0.0	
Paraquat	3	D42	2.1	♀	2	40	29.4	47	40	28.1	52	40	27.3	42	-3.1	-10.6	0.2322
Paraquat	3	D42	2.1	♀	3	40	18.9	30	40	16.6	20	40	15.8	20	-5.3	0.0	**0.0010**
Paraquat	3	D42	2.1	♀	4	40	55.9	90	40	52.6	110	41	24.3	42	-53.7	-53.3	**<0.0001**
Paraquat	3	D42	2.1	♀	5	40	17.8	25	40	17.5	25	40	16.9	25	-3.6	0.0	0.2549
Paraquat	3	D42	2.1	♀	6	37	56.4	84	41	61.9	110	41	24.8	46	-56.0	-45.2	**<0.0001**
Paraquat	3	D42	2.1	♀	7	42	50.9	84	41	58.4	110	40	21.7	38	-57.3	-54.8	**<0.0001**
Paraquat	3	D42	2.1	♂	1	40	25.2	40	40	22.8	30	40	23.5	40	0.0	0.0	
Paraquat	3	D42	2.1	♂	2	40	27.5	57	40	26.3	47	40	20.6	37	-21.4	-21.3	**0.0004**
Paraquat	3	D42	2.1	♂	3	40	17.9	25	40	16.3	20	40	14.6	20	-10.0	0.0	**0.0002**
Paraquat	3	D42	2.1	♂	4	40	35.5	66	40	33.0	74	38	33.4	58	0.0	-12.1	
Paraquat	3	D42	2.1	♂	5	40	17.8	25	40	15.9	25	40	14.4	20	-9.4	-20.0	**0.0003**

Table S2

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Paraquat	3	D42	2.1	♂	6	34	40.2	84	40	28.7	58	40	44.7	74	11.1	0.0	0.4630
Paraquat	3	D42	2.1	♂	7	41	39.0	84	40	38.6	84	31	45.6	84	17.0	0.0	0.1017
Paraquat	3	D42	4.3	♀	1	40	30.8	43	40	43.8	69	40	33.2	69	0.0	0.0	
Paraquat	3	D42	4.3	♀	2	42	49.1	84	40	52.6	110	40	35.3	66	-28.3	-21.4	**0.0003**
Paraquat	3	D42	4.3	♀	3	41	39.3	74	41	61.9	110	40	31.4	50	-20.0	-32.4	**0.0098**
Paraquat	3	D42	4.3	♀	4	40	43.2	74	41	58.4	110	40	27.8	58	-35.8	-21.6	**0.0001**
Paraquat	3	D42	4.3	♂	1	40	27.8	56	40	31.1	56	40	27.1	56	-2.5	0.0	0.2014
Paraquat	3	D42	4.3	♂	2	50	42.9	84	40	33.0	74	41	44.1	84	2.9	0.0	**0.0011**
Paraquat	3	D42	4.3	♂	3	41	41.6	110	40	28.7	58	39	44.1	90	6.0	0.0	0.5716
Paraquat	3	D42	4.3	♂	4	41	46.7	84	40	38.6	84	41	39.4	74	0.0	-11.9	
Paraquat	3	D42	9.1	♀	1	39	21.2	32	40	24.2	41	42	27.1	48	12.1	17.1	**0.0028**
Paraquat	3	D42	9.1	♀	2	39	54.4	90	40	52.6	110	40	28.3	58	-46.1	-35.6	**<0.0001**
Paraquat	3	D42	9.1	♀	3	40	21.5	32	40	25.8	41	41	30.0	48	16.3	17.1	**0.0495**
Paraquat	3	D42	9.1	♀	4	39	55.1	84	41	61.9	110	40	30.4	58	-44.9	-31.0	**<0.0001**
Paraquat	3	D42	9.1	♀	5	46	49.4	90	41	58.4	110	42	25.4	42	-48.5	-53.3	**<0.0001**
Paraquat	3	D42	9.1	♂	1	40	14.7	23	40	16.3	23	40	14.9	23	0.0	0.0	
Paraquat	3	D42	9.1	♂	2	42	31.0	66	40	33.0	74	43	37.6	74	14.0	0.0	**0.0066**
Paraquat	3	D42	9.1	♂	3	40	15.7	23	40	20.2	32	40	17.4	32	0.0	0.0	
Paraquat	3	D42	9.1	♂	4	34	38.2	74	40	28.7	58	40	34.5	74	0.0	0.0	
Paraquat	3	D42	9.1	♂	5	39	31.2	66	40	38.6	84	39	33.2	58	0.0	-12.1	
Paraquat	3	D42	3.2	♀	1	40	30.7	80	40	30.1	55	40	32.0	75	4.3	0.0	0.3500
Paraquat	3	D42	3.2	♀	2	36	69.0	118	40	52.6	110	40	25.0	42	-52.4	-61.8	**<0.0001**
Paraquat	3	D42	3.2	♀	3	38	67.4	110	41	61.9	110	40	20.6	30	-66.7	-72.7	**<0.0001**
Paraquat	3	D42	3.2	♀	4	40	60.2	110	41	58.4	110	40	19.7	38	-66.3	-65.5	**<0.0001**
Paraquat	3	D42	3.2	♂	1	40	33.9	80	40	25.6	45	40	36.6	88	7.9	10.0	**0.0031**
Paraquat	3	D42	3.2	♂	2	39	54.8	90	40	33.0	74	42	45.4	110	0.0	22.2	
Paraquat	3	D42	3.2	♂	3	40	62.8	110	40	28.7	58	39	36.3	74	0.0	0.0	
Paraquat	3	D42	3.2	♂	4	37	64.3	110	40	38.6	84	41	49.9	110	0.0	0.0	
Paraquat	3	D42	10.1	♀	1	40	41.6	117	40	53.0	117	40	45.2	105	0.0	-10.3	
Paraquat	3	D42	10.1	♀	2	40	23.7	41	40	24.2	41	39	31.3	59	29.7	43.9	**0.0008**
Paraquat	3	D42	10.1	♀	3	28	66.1	90	40	52.6	110	44	47.2	110	-10.1	0.0	0.1142
Paraquat	3	D42	10.1	♀	4	40	24.3	59	40	25.8	41	40	32.2	48	24.6	0.0	**0.0014**
Paraquat	3	D42	10.1	♀	5	39	88.3	118	41	61.9	110	40	28.9	66	-53.3	-40.0	**<0.0001**
Paraquat	3	D42	10.1	♀	6	37	78.3	110	41	58.4	110	40	31.1	84	-46.9	-23.6	**<0.0001**
Paraquat	3	D42	10.1	♂	1	40	63.7	117	40	54.4	105	40	58.7	117	0.0	0.0	
Paraquat	3	D42	10.1	♂	2	40	23.3	41	40	16.3	23	41	25.9	59	10.8	43.9	0.2396
Paraquat	3	D42	10.1	♂	3	39	47.5	90	40	33.0	74	46	49.8	110	4.7	22.2	**0.0005**
Paraquat	3	D42	10.1	♂	4	40	28.3	41	40	20.2	32	40	24.6	41	0.0	0.0	
Paraquat	3	D42	10.1	♂	5	39	73.1	130	40	28.7	58	41	46.9	110	0.0	0.0	
Paraquat	3	D42	10.1	♂	6	42	59.0	110	40	38.6	84	39	35.5	90	-7.9	0.0	**0.0001**
Paraquat	3	D42	3.1	♀	1	40	31.4	45	40	23.3	35	40	29.6	51	0.0	13.3	
Paraquat	3	D42	3.1	♀	2	40	33.4	64	38	37.2	76	40	30.6	64	-8.4	0.0	0.0687
Paraquat	3	D42	3.1	♀	3	40	16.0	25	40	16.6	20	40	15.6	25	-2.3	0.0	0.2561
Paraquat	3	D42	3.1	♀	4	40	46.1	74	40	52.6	110	50	40.9	74	-11.3	0.0	0.0505
Paraquat	3	D42	3.1	♀	5	40	18.0	30	40	17.5	25	40	18.9	30	4.9	0.0	0.1826
Paraquat	3	D42	3.1	♀	6	36	50.6	84	41	61.9	110	40	30.7	58	-39.3	-31.0	**<0.0001**
Paraquat	3	D42	3.1	♀	7	44	49.4	74	41	58.4	110	39	26.9	50	-45.5	-32.4	**<0.0001**
Paraquat	3	D42	3.1	♂	1	40	30.5	57	40	22.8	30	40	39.7	69	30.1	21.1	**0.0080**
Paraquat	3	D42	3.1	♂	2	40	23.3	39	38	35.0	70	40	44.6	82	27.3	17.1	**0.0195**

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Paraquat	3	D42	3.1	♂	3	40	18.8	40	40	16.3	20	40	24.5	49	30.4	22.5	**0.0013**
Paraquat	3	D42	3.1	♂	4	45	41.4	90	40	33.0	74	40	51.3	110	23.7	22.2	**0.0004**
Paraquat	3	D42	3.1	♂	5	40	19.3	25	40	15.9	25	40	24.5	40	27.1	60.0	**0.0010**
Paraquat	3	D42	3.1	♂	6	39	41.6	84	40	28.7	58	37	55.5	110	33.2	31.0	**0.0106**
Paraquat	3	D42	3.1	♂	7	41	44.3	84	40	38.6	84	44	52.6	110	18.6	31.0	**0.0059**
Paraquat	3	D42	4.4	♀	1	40	35.5	58	40	37.7	58	40	49.5	72	31.4	24.1	**0.0001**
Paraquat	3	D42	4.4	♀	2	40	45.4	69	40	43.8	69	40	46.1	69	1.4	0.0	0.6113
Paraquat	3	D42	4.4	♀	3	40	26.5	41	40	24.2	41	46	27.6	41	4.4	0.0	0.1040
Paraquat	3	D42	4.4	♀	4	36	53.7	90	40	52.6	110	42	44.8	84	-14.8	-6.7	**0.0320**
Paraquat	3	D42	4.4	♀	5	40	28.2	48	40	25.8	41	47	27.2	41	0.0	0.0	
Paraquat	3	D42	4.4	♀	6	40	67.1	110	41	61.9	110	41	46.8	74	-24.4	-32.7	**0.0005**
Paraquat	3	D42	4.4	♀	7	41	57.2	90	41	58.4	110	40	46.1	84	-19.5	-6.7	**0.0075**
Paraquat	3	D42	4.4	♂	1	40	38.7	67	40	33.3	58	40	49.2	87	27.1	29.9	**0.0038**
Paraquat	3	D42	4.4	♂	2	40	39.2	81	40	31.1	56	40	36.3	69	0.0	0.0	
Paraquat	3	D42	4.4	♂	3	39	19.1	32	40	16.3	23	42	18.5	32	0.0	0.0	
Paraquat	3	D42	4.4	♂	4	41	37.7	84	40	33.0	74	39	33.0	74	0.0	0.0	
Paraquat	3	D42	4.4	♂	5	40	19.3	32	40	20.2	32	41	19.5	32	0.0	0.0	
Paraquat	3	D42	4.4	♂	6	40	57.9	110	40	28.7	58	40	37.8	74	0.0	0.0	
Paraquat	3	D42	4.4	♂	7	40	43.0	110	40	38.6	84	38	33.4	58	-13.4	-31.0	**0.0268**
Paraquat	10	daGal4	2.1	♀	1	40	25.3	52	36	15.8	32	40	14.3	22	-9.5	-31.3	0.3221
Paraquat	10	daGal4	2.1	♀	2	40	17.9	26	40	14.7	22	42	13.1	26	-10.6	0.0	0.1758
Paraquat	10	daGal4	2.1	♀	3	36	18.6	26	40	19.1	30	41	17.4	30	-6.1	0.0	0.2041
Paraquat	10	daGal4	2.1	♀	4	41	19.3	22	45	17.3	26	44	15.8	22	-8.5	0.0	**0.0001**
Paraquat	10	daGal4	2.1	♂	1	40	24.5	47	38	20.2	42	40	21.8	47	0.0	0.0	
Paraquat	10	daGal4	2.1	♂	2	41	17.6	26	40	17.5	30	42	13.7	22	-21.5	-15.4	**0.0009**
Paraquat	10	daGal4	2.1	♂	3	40	15.4	22	45	13.3	18	42	13.4	22	0.0	0.0	
Paraquat	10	daGal4	2.1	♂	4	40	15.9	30	45	14.4	18	43	16.2	42	1.8	40.0	0.4360
Paraquat	10	daGal4	4.3	♀	1	40	20.8	31	35	15.4	22	40	17.4	31	0.0	0.0	
Paraquat	10	daGal4	4.3	♀	2	40	18.2	33	40	13.2	25	40	16.0	25	0.0	0.0	
Paraquat	10	daGal4	4.3	♀	3	40	18.5	26	40	14.7	22	40	14.1	30	-4.1	15.4	**0.0015**
Paraquat	10	daGal4	4.3	♀	4	40	15.8	25	40	13.9	25	40	16.1	25	2.2	0.0	**0.0225**
Paraquat	10	daGal4	4.3	♀	5	42	24.3	42	40	19.1	30	40	18.9	35	-1.3	0.0	**0.0010**
Paraquat	10	daGal4	4.3	♀	6	42	19.5	26	45	17.3	26	41	19.1	26	0.0	0.0	
Paraquat	10	daGal4	4.3	♂	1	40	20.5	31	40	18.2	31	40	23.6	43	15.1	38.7	**0.0010**
Paraquat	10	daGal4	4.3	♂	2	40	13.4	20	40	12.7	16	40	14.8	25	10.1	25.0	**0.0115**
Paraquat	10	daGal4	4.3	♂	3	58	18.7	26	40	17.5	30	44	13.6	22	-22.0	-15.4	**0.0015**
Paraquat	10	daGal4	4.3	♂	4	40	14.0	20	40	13.7	25	40	13.4	20	-1.6	0.0	0.5721
Paraquat	10	daGal4	4.3	♂	5	42	16.0	35	45	13.3	18	14	15.7	18	0.0	0.0	
Paraquat	10	daGal4	4.3	♂	6	40	13.6	22	45	14.4	18	48	16.6	30	16.0	36.4	**0.0136**
Paraquat	10	daGal4	9.1	♀	1	40	19.0	27	39	14.3	21	40	16.5	40	0.0	48.1	
Paraquat	10	daGal4	9.1	♀	2	41	18.7	30	40	14.7	22	41	15.6	26	0.0	0.0	
Paraquat	10	daGal4	9.1	♀	3	41	18.2	27	40	14.9	21	30	12.5	21	-15.7	0.0	**0.0072**
Paraquat	10	daGal4	9.1	♀	4	42	22.8	35	40	19.1	30	41	18.6	38	-2.7	8.6	**0.0050**
Paraquat	10	daGal4	9.1	♀	5	42	21.0	30	45	17.3	26	41	16.0	18	-7.7	-30.8	0.1465
Paraquat	10	daGal4	9.1	♂	1	40	15.6	21	41	11.7	15	40	10.5	15	-10.3	0.0	**0.0094**
Paraquat	10	daGal4	9.1	♂	2	38	16.3	30	40	17.5	30	42	13.7	22	-16.1	-26.7	**0.0017**
Paraquat	10	daGal4	9.1	♂	3	40	17.7	27	39	13.4	21	40	10.9	15	-18.7	-28.6	**0.0017**
Paraquat	10	daGal4	9.1	♂	4	40	14.1	22	45	13.3	18	40	12.5	14	-6.1	-22.2	**0.0059**
Paraquat	10	daGal4	9.1	♂	5	43	13.1	22	45	14.4	18	44	11.5	18	-12.4	0.0	**0.0096**

Table S2

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Paraquat	10	daGal4	3.2	♀	1	38	19.1	40	40	15.3	26	40	15.1	26	-1.0	0.0	**0.0198**
Paraquat	10	daGal4	3.2	♀	2	40	19.2	33	40	13.2	25	40	16.6	25	0.0	0.0	
Paraquat	10	daGal4	3.2	♀	3	35	17.1	30	40	14.7	22	40	20.5	35	19.7	16.7	**0.0387**
Paraquat	10	daGal4	3.2	♀	4	40	17.3	25	40	13.9	25	40	17.8	33	3.0	32.0	**0.0011**
Paraquat	10	daGal4	3.2	♀	5	40	22.2	42	40	19.1	30	43	24.5	35	10.4	0.0	**0.0002**
Paraquat	10	daGal4	3.2	♀	6	39	19.9	35	45	17.3	26	33	23.1	42	16.2	20.0	**0.0020**
Paraquat	10	daGal4	3.2	♂	1	38	24.3	45	40	19.9	45	40	44.7	75	83.4	66.7	**<0.0001**
Paraquat	10	daGal4	3.2	♂	2	40	18.3	25	40	12.7	16	40	17.5	48	0.0	92.0	
Paraquat	10	daGal4	3.2	♂	3	38	25.0	42	40	17.5	30	37	25.1	61	0.4	45.2	**0.0014**
Paraquat	10	daGal4	3.2	♂	4	40	18.4	33	40	13.7	25	40	18.2	33	0.0	0.0	
Paraquat	10	daGal4	3.2	♂	5	38	24.3	61	45	13.3	18	37	33.8	70	38.9	14.8	**0.0082**
Paraquat	10	daGal4	3.2	♂	6	39	29.8	61	45	14.4	18	37	34.4	70	15.4	14.8	0.1981
Paraquat	10	daGal4	10.1	♀	1	29	17.3	22	31	17.2	17	40	18.2	28	4.8	27.3	0.2535
Paraquat	10	daGal4	10.1	♀	2	40	18.9	27	39	14.3	21	40	14.2	27	-1.3	0.0	0.7502
Paraquat	10	daGal4	10.1	♀	3	36	22.9	38	40	14.7	22	38	25.3	61	10.1	60.5	0.4944
Paraquat	10	daGal4	10.1	♀	4	40	18.8	27	40	14.9	21	36	17.2	27	0.0	0.0	
Paraquat	10	daGal4	10.1	♀	5	36	27.9	61	40	19.1	30	40	23.4	42	0.0	0.0	
Paraquat	10	daGal4	10.1	♀	6	37	24.2	38	45	17.3	26	43	23.9	61	0.0	60.5	
Paraquat	10	daGal4	10.1	♂	1	37	18.8	28	31	20.1	42	38	39.7	89	97.0	111.9	**<0.0001**
Paraquat	10	daGal4	10.1	♂	2	40	25.9	40	41	11.7	15	40	14.8	21	0.0	0.0	
Paraquat	10	daGal4	10.1	♂	3	38	25.9	61	40	17.5	30	40	27.2	70	5.1	14.8	0.8843
Paraquat	10	daGal4	10.1	♂	4	40	20.6	40	39	13.4	21	39	19.7	40	0.0	0.0	
Paraquat	10	daGal4	10.1	♂	5	37	25.4	42	45	13.3	18	41	28.5	70	12.3	66.7	0.6356
Paraquat	10	daGal4	10.1	♂	6	39	24.7	42	45	14.4	18	40	20.4	38	0.0	0.0	
Paraquat	10	daGal4	3.1	♀	1	40	12.5	22	40	12.7	22	40	14.1	30	10.8	36.4	0.2253
Paraquat	10	daGal4	3.1	♀	2	43	14.0	22	40	14.7	22	39	16.1	22	9.2	0.0	**0.0228**
Paraquat	10	daGal4	3.1	♀	3	40	17.9	30	40	19.1	30	44	17.7	30	-1.0	0.0	0.2319
Paraquat	10	daGal4	3.1	♀	4	44	18.2	35	45	17.3	26	42	17.5	38	0.0	8.6	
Paraquat	10	daGal4	3.1	♂	1	33	23.5	34	39	23.6	61	40	52.9	93	123.9	52.5	**<0.0001**
Paraquat	10	daGal4	3.1	♂	2	41	19.1	35	40	17.5	30	39	14.6	22	-16.5	-26.7	**0.0019**
Paraquat	10	daGal4	3.1	♂	3	42	16.6	30	45	13.3	18	40	17.3	30	4.5	0.0	0.5164
Paraquat	10	daGal4	3.1	♂	4	42	15.1	26	45	14.4	18	43	15.2	22	0.4	0.0	0.2269
Paraquat	10	daGal4	4.4	♀	1	40	26.2	43	35	15.4	22	40	17.2	31	0.0	0.0	
Paraquat	10	daGal4	4.4	♀	2	40	19.8	27	39	14.3	21	69	16.0	27	0.0	0.0	
Paraquat	10	daGal4	4.4	♀	3	40	17.0	26	40	14.7	22	39	13.2	26	-10.3	0.0	**0.0009**
Paraquat	10	daGal4	4.4	♀	4	40	19.8	40	40	14.9	21	40	16.5	40	0.0	0.0	
Paraquat	10	daGal4	4.4	♀	5	41	23.9	30	40	19.1	30	43	17.7	30	-7.2	0.0	0.2879
Paraquat	10	daGal4	4.4	♀	6	34	20.5	35	45	17.3	26	42	15.3	22	-11.3	-15.4	**0.0005**
Paraquat	10	daGal4	4.4	♂	1	39	29.7	43	40	18.2	31	40	30.8	56	3.5	30.2	0.6732
Paraquat	10	daGal4	4.4	♂	2	40	24.8	40	41	11.7	15	56	17.0	40	0.0	0.0	
Paraquat	10	daGal4	4.4	♂	3	41	17.9	26	40	17.5	30	41	28.7	70	60.0	133.3	**<0.0001**
Paraquat	10	daGal4	4.4	♂	4	40	18.4	27	39	13.4	21	40	14.7	21	0.0	0.0	
Paraquat	10	daGal4	4.4	♂	5	40	17.0	26	45	13.3	18	40	22.9	42	34.4	61.5	**0.0005**
Paraquat	10	daGal4	4.4	♂	6	39	15.2	22	45	14.4	18	41	15.9	35	4.1	59.1	0.7465
Paraquat	10	DJ694	2.1	♀	1	40	25.3	52	40	17.0	27	40	14.6	22	-14.0	-18.5	**0.0141**
Paraquat	10	DJ694	2.1	♀	2	40	17.9	26	39	20.9	38	43	17.8	26	-0.5	0.0	**0.0195**
Paraquat	10	DJ694	2.1	♀	3	40	12.1	16	40	13.0	16	40	12.0	16	-0.8	0.0	**0.0411**
Paraquat	10	DJ694	2.1	♀	4	36	18.6	26	34	16.9	35	32	13.3	22	-21.7	-15.4	**0.0119**
Paraquat	10	DJ694	2.1	♀	5	40	14.2	20	40	13.6	24	40	10.8	12	-20.6	-40.0	**<0.0001**

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Paraquat	10	DJ694	2.1	♀	6	41	19.3	22	38	16.1	26	40	12.8	18	-20.5	-18.2	**0.0002**
Paraquat	10	DJ694	2.1	♀	7	40	13.2	20	40	11.4	16	40	10.9	12	-4.4	-25.0	0.3596
Paraquat	10	DJ694	2.1	♂	1	40	24.5	47	40	29.6	57	40	21.5	42	-12.2	-10.6	**0.0006**
Paraquat	10	DJ694	2.1	♂	2	41	17.6	26	44	14.4	18	40	12.9	18	-10.2	0.0	**0.0131**
Paraquat	10	DJ694	2.1	♂	3	40	9.5	12	40	10.6	16	40	10.8	12	1.9	0.0	**0.0038**
Paraquat	10	DJ694	2.1	♂	4	40	15.4	22	38	12.4	18	27	10.9	14	-12.5	-22.2	0.0792
Paraquat	10	DJ694	2.1	♂	5	40	10.5	12	40	12.4	16	40	10.5	12	0.0	0.0	
Paraquat	10	DJ694	2.1	♂	6	40	15.9	30	42	13.3	18	40	13.3	18	0.0	0.0	
Paraquat	10	DJ694	2.1	♂	7	40	10.2	12	40	9.5	12	40	10.1	12	0.0	0.0	
Paraquat	10	DJ694	4.3	♀	1	40	20.8	31	40	16.8	31	40	15.9	31	-5.2	0.0	**0.0018**
Paraquat	10	DJ694	4.3	♀	2	40	18.2	33	40	12.4	16	40	13.8	16	0.0	0.0	
Paraquat	10	DJ694	4.3	♀	3	40	18.5	26	39	20.9	38	41	17.1	30	-7.4	0.0	**0.0024**
Paraquat	10	DJ694	4.3	♀	4	40	12.2	16	40	13.0	16	40	10.3	12	-16.0	-25.0	**<0.0001**
Paraquat	10	DJ694	4.3	♀	5	40	15.8	25	40	13.2	16	40	15.2	20	0.0	0.0	
Paraquat	10	DJ694	4.3	♀	6	42	24.3	42	34	16.9	35	28	13.9	26	-18.1	-25.7	**0.0368**
Paraquat	10	DJ694	4.3	♀	7	40	12.8	16	40	13.6	24	30	11.3	12	-11.5	-25.0	**0.0011**
Paraquat	10	DJ694	4.3	♀	8	42	19.5	26	38	16.1	26	40	13.8	18	-14.2	-30.8	**0.0158**
Paraquat	10	DJ694	4.3	♀	9	40	13.5	20	40	11.4	16	40	10.9	12	-4.4	-25.0	0.3375
Paraquat	10	DJ694	4.3	♂	1	40	20.5	31	40	19.5	43	41	24.9	43	21.3	0.0	**0.0103**
Paraquat	10	DJ694	4.3	♂	2	40	13.4	20	40	15.1	20	40	13.9	20	0.0	0.0	
Paraquat	10	DJ694	4.3	♂	3	58	18.7	26	44	14.4	18	42	13.9	18	-3.2	0.0	0.5680
Paraquat	10	DJ694	4.3	♂	4	40	11.4	16	40	10.6	16	50	11.0	16	0.0	0.0	
Paraquat	10	DJ694	4.3	♂	5	40	14.0	20	40	14.2	20	40	12.9	20	-8.1	0.0	0.2208
Paraquat	10	DJ694	4.3	♂	6	42	16.0	35	38	12.4	18	33	15.3	38	0.0	8.6	
Paraquat	10	DJ694	4.3	♂	7	40	11.1	12	40	12.4	16	40	10.7	12	-3.6	0.0	**0.0021**
Paraquat	10	DJ694	4.3	♂	8	40	13.6	22	42	13.3	18	36	15.8	26	16.0	18.2	**0.0066**
Paraquat	10	DJ694	4.3	♂	9	40	11.3	16	40	9.5	12	40	11.0	12	0.0	0.0	
Paraquat	10	DJ694	9.1	♀	1	40	19.0	27	39	15.9	21	40	15.4	27	-3.4	0.0	**0.0008**
Paraquat	10	DJ694	9.1	♀	2	41	18.7	30	39	20.9	38	40	14.8	22	-20.8	-26.7	**0.0015**
Paraquat	10	DJ694	9.1	♀	3	40	13.0	20	40	13.0	16	40	11.5	12	-11.5	-25.0	**0.0009**
Paraquat	10	DJ694	9.1	♀	4	41	18.2	27	40	14.4	21	38	18.1	27	0.0	0.0	
Paraquat	10	DJ694	9.1	♀	5	42	22.8	35	34	16.9	35	37	12.1	18	-28.7	-48.6	**0.0003**
Paraquat	10	DJ694	9.1	♀	6	40	15.1	20	40	13.6	24	40	10.8	12	-20.6	-40.0	**<0.0001**
Paraquat	10	DJ694	9.1	♀	7	42	21.0	30	38	16.1	26	39	11.2	14	-30.3	-46.2	**<0.0001**
Paraquat	10	DJ694	9.1	♀	8	40	11.7	16	40	11.4	16	40	10.8	12	-5.3	-25.0	0.0642
Paraquat	10	DJ694	9.1	♂	1	40	15.6	21	40	15.0	21	39	13.2	15	-12.0	-28.6	**0.0011**
Paraquat	10	DJ694	9.1	♂	2	38	16.3	30	44	14.4	18	40	14.3	26	-0.4	0.0	0.0911
Paraquat	10	DJ694	9.1	♂	3	40	11.0	16	40	10.6	16	40	9.4	12	-11.3	-25.0	**0.0011**
Paraquat	10	DJ694	9.1	♂	4	40	17.7	27	39	14.7	21	40	11.8	15	-20.2	-28.6	**<0.0001**
Paraquat	10	DJ694	9.1	♂	5	40	14.1	22	38	12.4	18	37	12.8	18	0.0	0.0	
Paraquat	10	DJ694	9.1	♂	6	40	10.8	12	40	12.4	16	40	11.0	12	0.0	0.0	
Paraquat	10	DJ694	9.1	♂	7	43	13.1	22	42	13.3	18	41	12.8	18	-1.8	0.0	0.4324
Paraquat	10	DJ694	9.1	♂	8	40	11.4	16	40	9.5	12	40	11.7	16	2.6	0.0	0.5051
Paraquat	10	DJ694	3.2	♀	1	38	19.1	40	35	19.7	35	40	19.6	40	0.0	0.0	
Paraquat	10	DJ694	3.2	♀	2	40	19.2	33	40	12.4	16	40	15.8	20	0.0	0.0	
Paraquat	10	DJ694	3.2	♀	3	35	17.1	30	39	20.9	38	43	19.7	42	0.0	10.5	
Paraquat	10	DJ694	3.2	♀	4	40	14.0	24	40	13.0	16	40	12.8	34	-1.5	41.7	0.3058
Paraquat	10	DJ694	3.2	♀	5	40	17.3	25	40	13.2	16	40	18.0	33	4.1	32.0	0.5341
Paraquat	10	DJ694	3.2	♀	6	40	22.2	42	34	16.9	35	38	20.6	61	0.0	45.2	

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Paraquat	10	DJ694	3.2	♀	7	30	14.7	24	40	13.6	24	40	12.3	20	-9.6	-16.7	**0.0287**
Paraquat	10	DJ694	3.2	♀	8	39	19.9	35	38	16.1	26	34	23.6	42	18.5	20.0	0.0589
Paraquat	10	DJ694	3.2	♀	9	40	15.6	34	40	11.4	16	40	13.0	20	0.0	0.0	
Paraquat	10	DJ694	3.2	♂	1	38	24.3	45	39	24.0	63	40	47.4	75	94.6	19.0	**<0.0001**
Paraquat	10	DJ694	3.2	♂	2	40	18.3	25	40	15.1	20	40	25.1	48	37.0	92.0	**0.0016**
Paraquat	10	DJ694	3.2	♂	3	38	25.0	42	44	14.4	18	41	29.2	61	17.0	45.2	0.4007
Paraquat	10	DJ694	3.2	♂	4	40	14.0	20	40	10.6	16	40	16.1	39	15.2	95.0	**0.0009**
Paraquat	10	DJ694	3.2	♂	5	40	18.4	33	40	14.2	20	40	24.9	58	35.1	75.8	**0.0019**
Paraquat	10	DJ694	3.2	♂	6	38	24.3	61	38	12.4	18	40	34.8	61	43.1	0.0	**<0.0001**
Paraquat	10	DJ694	3.2	♂	7	40	14.9	20	40	12.4	16	40	17.3	63	16.1	215.0	**0.0057**
Paraquat	10	DJ694	3.2	♂	8	39	29.8	61	42	13.3	18	44	27.1	70	0.0	14.8	
Paraquat	10	DJ694	3.2	♂	9	40	15.2	20	40	9.5	12	40	14.1	24	0.0	20.0	
Paraquat	10	DJ694	10.1	♀	1	29	17.3	22	24	17.0	17	39	17.4	22	0.2	0.0	0.1672
Paraquat	10	DJ694	10.1	♀	2	40	18.9	27	39	15.9	21	40	16.0	27	0.0	0.0	
Paraquat	10	DJ694	10.1	♀	3	36	22.9	38	39	20.9	38	40	16.7	35	-20.5	-7.9	**0.0011**
Paraquat	10	DJ694	10.1	♀	4	40	15.4	24	40	13.0	16	40	9.9	12	-23.8	-25.0	**<0.0001**
Paraquat	10	DJ694	10.1	♀	5	40	18.8	27	40	14.4	21	40	18.8	40	0.4	48.1	**0.0004**
Paraquat	10	DJ694	10.1	♀	6	36	27.9	61	34	16.9	35	31	16.6	30	-2.0	-14.3	0.9156
Paraquat	10	DJ694	10.1	♀	7	40	17.6	34	40	13.6	24	40	12.3	20	-9.6	-16.7	0.0900
Paraquat	10	DJ694	10.1	♀	8	37	24.2	38	38	16.1	26	40	23.0	38	0.0	0.0	
Paraquat	10	DJ694	10.1	♀	9	40	15.3	20	40	11.4	16	40	11.7	20	0.0	0.0	
Paraquat	10	DJ694	10.1	♂	1	37	18.8	28	29	24.5	48	35	42.3	73	72.6	52.1	**0.0002**
Paraquat	10	DJ694	10.1	♂	2	40	25.9	40	40	15.0	21	40	22.9	40	0.0	0.0	
Paraquat	10	DJ694	10.1	♂	3	38	25.9	61	44	14.4	18	43	28.8	70	11.1	14.8	0.3863
Paraquat	10	DJ694	10.1	♂	4	40	13.3	20	40	10.6	16	40	12.7	34	0.0	70.0	
Paraquat	10	DJ694	10.1	♂	5	40	20.6	40	39	14.7	21	39	15.9	21	0.0	0.0	
Paraquat	10	DJ694	10.1	♂	6	37	25.4	42	38	12.4	18	32	19.6	42	0.0	0.0	
Paraquat	10	DJ694	10.1	♂	7	40	17.2	46	40	12.4	16	40	14.6	39	0.0	0.0	
Paraquat	10	DJ694	10.1	♂	8	39	24.7	42	42	13.3	18	42	34.0	70	37.9	66.7	**0.0053**
Paraquat	10	DJ694	10.1	♂	9	40	14.9	24	40	9.5	12	38	24.6	63	65.5	162.5	**<0.0001**
Paraquat	10	DJ694	3.1	♀	1	40	12.5	22	40	15.9	26	40	14.4	22	0.0	0.0	
Paraquat	10	DJ694	3.1	♀	2	43	14.0	22	39	20.9	38	38	13.3	26	-5.3	0.0	0.2802
Paraquat	10	DJ694	3.1	♀	3	40	13.8	20	40	13.0	16	40	9.9	12	-23.8	-25.0	**<0.0001**
Paraquat	10	DJ694	3.1	♀	4	40	17.9	30	34	16.9	35	35	12.6	18	-25.3	-40.0	**0.0036**
Paraquat	10	DJ694	3.1	♀	5	44	18.2	35	38	16.1	26	47	16.1	26	0.0	0.0	
Paraquat	10	DJ694	3.1	♂	1	33	23.5	34	38	30.0	61	39	42.1	79	40.2	29.5	**0.0110**
Paraquat	10	DJ694	3.1	♂	2	41	19.1	35	44	14.4	18	40	18.1	35	0.0	0.0	
Paraquat	10	DJ694	3.1	♂	3	40	12.4	20	40	10.6	16	40	12.4	16	0.0	0.0	
Paraquat	10	DJ694	3.1	♂	4	42	16.6	30	38	12.4	18	35	17.7	38	6.9	26.7	**0.0026**
Paraquat	10	DJ694	3.1	♂	5	42	15.1	26	42	13.3	18	33	17.5	35	15.5	34.6	**0.0002**
Paraquat	10	DJ694	4.4	♀	1	40	22.0	45	40	22.4	40	40	22.5	35	0.6	-12.5	0.5867
Paraquat	10	DJ694	4.4	♀	2	40	26.2	43	40	16.8	31	40	17.6	31	0.0	0.0	
Paraquat	10	DJ694	4.4	♀	3	40	19.8	27	39	15.9	21	40	15.5	21	-2.7	0.0	**0.0001**
Paraquat	10	DJ694	4.4	♀	4	40	17.0	26	39	20.9	38	41	16.0	30	-6.0	0.0	**0.0002**
Paraquat	10	DJ694	4.4	♀	5	40	13.0	20	40	13.0	16	40	12.7	20	-2.3	0.0	0.5633
Paraquat	10	DJ694	4.4	♀	6	40	19.8	40	40	14.4	21	40	15.9	21	0.0	0.0	
Paraquat	10	DJ694	4.4	♀	7	41	23.9	30	34	16.9	35	43	14.7	22	-13.4	-26.7	0.0999
Paraquat	10	DJ694	4.4	♀	8	40	16.0	20	40	13.6	24	40	11.7	16	-14.0	-20.0	**0.0036**
Paraquat	10	DJ694	4.4	♀	9	34	20.5	35	38	16.1	26	42	12.7	18	-21.2	-30.8	**0.0009**

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Paraquat	10	DJ694	4.4	♀	10	40	13.0	20	40	11.4	16	40	9.7	12	-14.9	-25.0	**0.0013**
Paraquat	10	DJ694	4.4	♂	1	40	25.9	55	40	19.5	30	45	23.8	35	0.0	0.0	
Paraquat	10	DJ694	4.4	♂	2	39	29.7	43	40	19.5	43	39	31.9	69	7.3	60.5	0.5747
Paraquat	10	DJ694	4.4	♂	3	40	24.8	40	40	15.0	21	40	20.5	40	0.0	0.0	
Paraquat	10	DJ694	4.4	♂	4	41	17.9	26	44	14.4	18	40	16.8	30	0.0	15.4	
Paraquat	10	DJ694	4.4	♂	5	40	13.2	20	40	10.6	16	40	11.9	16	0.0	0.0	
Paraquat	10	DJ694	4.4	♂	6	40	18.4	27	39	14.7	21	40	20.6	40	12.4	48.1	0.1945
Paraquat	10	DJ694	4.4	♂	7	40	17.0	26	38	12.4	18	40	17.1	30	0.6	15.4	**0.0001**
Paraquat	10	DJ694	4.4	♂	8	39	14.1	24	40	12.4	16	40	11.6	16	-6.5	0.0	**0.0001**
Paraquat	10	DJ694	4.4	♂	9	39	15.2	22	42	13.3	18	37	14.8	22	0.0	0.0	
Paraquat	10	DJ694	4.4	♂	10	40	12.4	20	40	9.5	12	40	12.3	20	0.0	0.0	
Paraquat	10	D42	2.1	♀	1	40	25.3	52	40	24.3	47	40	20.1	42	-17.0	-10.6	**0.0236**
Paraquat	10	D42	2.1	♀	2	40	17.9	26	40	24.5	61	40	14.6	26	-18.4	0.0	**0.0014**
Paraquat	10	D42	2.1	♀	3	36	18.6	26	41	36.7	61	40	22.4	30	0.0	0.0	
Paraquat	10	D42	2.1	♀	4	41	19.3	22	58	28.5	61	44	19.9	42	0.0	0.0	
Paraquat	10	D42	2.1	♂	1	40	24.5	47	40	25.9	52	40	21.3	37	-13.3	-21.3	**0.0211**
Paraquat	10	D42	2.1	♂	2	41	17.6	26	41	23.5	38	38	21.8	30	0.0	0.0	
Paraquat	10	D42	2.1	♂	3	40	15.4	22	40	19.9	26	40	17.4	26	0.0	0.0	
Paraquat	10	D42	2.1	♂	4	40	15.9	30	44	20.7	38	41	16.8	30	0.0	0.0	
Paraquat	10	D42	4.3	♀	1	40	20.8	31	39	22.9	43	40	19.5	31	-6.1	0.0	**0.0494**
Paraquat	10	D42	4.3	♀	2	40	18.2	33	40	14.5	25	40	14.5	16	-0.2	-36.0	**0.0006**
Paraquat	10	D42	4.3	♀	3	40	18.5	26	40	24.5	61	42	15.5	26	-16.5	0.0	**0.0234**
Paraquat	10	D42	4.3	♀	4	40	15.8	25	40	14.7	20	40	14.7	20	0.0	0.0	
Paraquat	10	D42	4.3	♀	5	42	24.3	42	41	36.7	61	44	24.0	38	-1.2	-9.5	0.8692
Paraquat	10	D42	4.3	♀	6	42	19.5	26	58	28.5	61	39	16.5	26	-15.6	0.0	**0.0036**
Paraquat	10	D42	4.3	♂	1	40	20.5	31	40	26.8	43	40	17.2	22	-16.1	-29.0	**0.0032**
Paraquat	10	D42	4.3	♂	2	40	13.4	20	40	13.2	20	40	10.7	16	-18.6	-20.0	**0.0013**
Paraquat	10	D42	4.3	♂	3	58	18.7	26	41	23.5	38	40	20.9	35	0.0	0.0	
Paraquat	10	D42	4.3	♂	4	40	14.0	20	40	14.4	72	40	10.5	20	-25.0	0.0	**0.0002**
Paraquat	10	D42	4.3	♂	5	42	16.0	35	40	19.9	26	42	22.2	61	11.5	74.3	0.2516
Paraquat	10	D42	4.3	♂	6	40	13.6	22	44	20.7	38	42	18.6	30	0.0	0.0	
Paraquat	10	D42	9.1	♀	1	40	19.0	27	40	21.8	27	40	17.5	27	-7.7	0.0	**0.0002**
Paraquat	10	D42	9.1	♀	2	41	18.7	30	40	24.5	61	41	12.7	18	-31.9	-40.0	**<0.0001**
Paraquat	10	D42	9.1	♀	3	41	18.2	27	40	20.2	40	39	21.7	40	7.4	0.0	**0.0261**
Paraquat	10	D42	9.1	♀	4	42	22.8	35	41	36.7	61	38	21.4	35	-6.2	0.0	0.3870
Paraquat	10	D42	9.1	♀	5	42	21.0	30	58	28.5	61	45	18.8	35	-10.2	0.0	0.0604
Paraquat	10	D42	9.1	♂	1	40	15.6	21	40	19.0	40	39	14.3	21	-8.4	0.0	0.0759
Paraquat	10	D42	9.1	♂	2	38	16.3	30	41	23.5	38	39	15.0	22	-8.1	-26.7	0.2309
Paraquat	10	D42	9.1	♂	3	40	17.7	27	40	18.3	40	40	14.7	21	-17.0	-22.2	**0.0016**
Paraquat	10	D42	9.1	♂	4	40	14.1	22	40	19.9	26	39	18.0	30	0.0	15.4	
Paraquat	10	D42	9.1	♂	5	43	13.1	22	44	20.7	38	41	15.7	22	0.0	0.0	
Paraquat	10	D42	3.2	♀	1	38	19.1	40	38	20.3	40	40	18.5	40	-2.9	0.0	0.3700
Paraquat	10	D42	3.2	♀	2	40	19.2	33	40	14.5	25	40	17.1	33	0.0	0.0	
Paraquat	10	D42	3.2	♀	3	35	17.1	30	40	24.5	61	41	17.5	35	0.0	0.0	
Paraquat	10	D42	3.2	♀	4	40	17.3	25	40	14.7	20	40	18.9	33	9.6	32.0	0.1735
Paraquat	10	D42	3.2	♀	5	40	22.2	42	41	36.7	61	41	18.0	30	-18.9	-28.6	**0.0357**
Paraquat	10	D42	3.2	♀	6	39	19.9	35	58	28.5	61	36	19.3	30	-2.8	-14.3	0.8258
Paraquat	10	D42	3.2	♂	1	38	24.3	45	37	24.5	45	40	42.2	69	72.3	53.3	**<0.0001**
Paraquat	10	D42	3.2	♂	2	40	18.3	25	40	13.2	20	40	22.9	58	25.1	132.0	**0.0309**

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Paraquat	10	D42	3.2	♂	3	38	25.0	42	41	23.5	38	38	32.0	61	28.0	45.2	**0.0035**
Paraquat	10	D42	3.2	♂	4	40	18.4	33	40	14.4	72	40	21.8	38	18.2	0.0	**0.0002**
Paraquat	10	D42	3.2	♂	5	38	24.3	61	40	19.9	26	40	32.3	82	32.9	34.4	**0.0038**
Paraquat	10	D42	3.2	♂	6	39	29.8	61	44	20.7	38	45	33.6	70	12.6	14.8	0.1980
Paraquat	10	D42	10.1	♀	1	29	17.3	22	31	17.3	22	40	20.5	42	18.2	90.9	**0.0133**
Paraquat	10	D42	10.1	♀	2	40	18.9	27	40	21.8	27	39	19.8	27	0.0	0.0	
Paraquat	10	D42	10.1	♀	3	36	22.9	38	40	24.5	61	41	18.8	30	-18.1	-21.1	**0.0089**
Paraquat	10	D42	10.1	♀	4	40	18.8	27	40	20.2	40	40	19.1	40	0.0	0.0	
Paraquat	10	D42	10.1	♀	5	36	27.9	61	41	36.7	61	41	16.9	38	-39.4	-37.7	**<0.0001**
Paraquat	10	D42	10.1	♀	6	37	24.2	38	58	28.5	61	36	17.0	38	-29.9	0.0	**0.0003**
Paraquat	10	D42	10.1	♂	1	37	18.8	28	36	19.9	42	35	55.1	100	177.4	138.1	**<0.0001**
Paraquat	10	D42	10.1	♂	2	40	25.9	40	40	19.0	40	40	20.2	27	0.0	-32.5	
Paraquat	10	D42	10.1	♂	3	38	25.9	61	41	23.5	38	37	29.4	82	13.3	34.4	**0.0414**
Paraquat	10	D42	10.1	♂	4	40	20.6	40	40	18.3	40	40	17.5	27	-4.5	-32.5	**0.0066**
Paraquat	10	D42	10.1	♂	5	37	25.4	42	40	19.9	26	38	26.7	42	5.2	0.0	0.5106
Paraquat	10	D42	10.1	♂	6	39	24.7	42	44	20.7	38	49	26.7	61	8.4	45.2	**0.0115**
Paraquat	10	D42	3.1	♀	1	40	12.5	22	39	16.9	50	40	16.5	34	0.0	0.0	
Paraquat	10	D42	3.1	♀	2	43	14.0	22	40	24.5	61	43	13.8	22	-1.3	0.0	0.7175
Paraquat	10	D42	3.1	♀	3	40	17.9	30	41	36.7	61	28	12.9	26	-28.2	-13.3	**0.0011**
Paraquat	10	D42	3.1	♀	4	44	18.2	35	58	28.5	61	39	13.0	18	-28.8	-48.6	**<0.0001**
Paraquat	10	D42	3.1	♂	1	33	23.5	34	37	35.9	79	38	42.3	79	17.7	0.0	0.2826
Paraquat	10	D42	3.1	♂	2	41	19.1	35	41	23.5	38	40	22.1	35	0.0	0.0	
Paraquat	10	D42	3.1	♂	3	42	16.6	30	40	19.9	26	37	18.4	38	0.0	26.7	
Paraquat	10	D42	3.1	♂	4	42	15.1	26	44	20.7	38	39	18.5	35	0.0	0.0	
Paraquat	10	D42	4.4	♀	1	40	22.0	45	40	24.8	40	40	21.6	30	-1.7	-25.0	**0.0119**
Paraquat	10	D42	4.4	♀	2	40	26.2	43	39	22.9	43	40	22.0	43	-3.8	0.0	**0.0465**
Paraquat	10	D42	4.4	♀	3	40	19.8	27	40	21.8	27	40	19.0	40	-3.9	48.1	**0.0138**
Paraquat	10	D42	4.4	♀	4	40	17.0	26	40	24.5	61	44	14.3	26	-16.0	0.0	**0.0218**
Paraquat	10	D42	4.4	♀	5	40	19.8	40	40	20.2	40	40	20.3	40	0.2	0.0	0.7853
Paraquat	10	D42	4.4	♀	6	41	23.9	30	41	36.7	61	28	11.1	14	-53.3	-53.3	**<0.0001**
Paraquat	10	D42	4.4	♀	7	34	20.5	35	58	28.5	61	41	12.8	18	-37.4	-48.6	**<0.0001**
Paraquat	10	D42	4.4	♂	1	40	25.9	55	40	29.1	55	40	28.4	60	0.0	9.1	
Paraquat	10	D42	4.4	♂	2	39	29.7	43	40	26.8	43	40	22.9	31	-14.6	-27.9	**0.0001**
Paraquat	10	D42	4.4	♂	3	40	24.8	40	40	19.0	40	40	17.9	40	-5.8	0.0	**0.0007**
Paraquat	10	D42	4.4	♂	4	41	17.9	26	41	23.5	38	40	15.6	26	-13.0	0.0	**0.0311**
Paraquat	10	D42	4.4	♂	5	40	18.4	27	40	18.3	40	39	16.9	27	-7.5	0.0	0.1100
Paraquat	10	D42	4.4	♂	6	40	17.0	26	40	19.9	26	35	16.1	30	-5.4	15.4	0.0763
Paraquat	10	D42	4.4	♂	7	39	15.2	22	44	20.7	38	43	22.3	35	7.7	0.0	0.6678
Starvation	3	daGal4	2.1	♀	1	40	54.3	73	40	54.6	68	40	56.9	73	4.2	0.0	0.2560
Starvation	3	daGal4	2.1	♀	2	40	28.7	40	40	28.6	40	40	31.2	40	8.7	0.0	0.0992
Starvation	3	daGal4	2.1	♀	3	40	29.5	34	40	29.7	38	40	31.1	42	4.7	10.5	0.0574
Starvation	3	daGal4	2.1	♀	4	40	29.0	40	40	23.3	32	40	32.1	48	10.8	20.0	**0.0412**
Starvation	3	daGal4	2.1	♀	5	40	29.2	34	40	32.5	42	41	35.2	42	8.2	0.0	0.2027
Starvation	3	daGal4	2.1	♀	6	40	30.2	34	41	32.0	38	40	34.1	38	6.7	0.0	0.1151
Starvation	3	daGal4	2.1	♂	1	40	37.9	53	40	38.4	53	40	36.7	48	-3.1	-9.4	0.2710
Starvation	3	daGal4	2.1	♂	2	40	17.0	20	40	16.5	20	40	17.5	20	3.2	0.0	0.1174
Starvation	3	daGal4	2.1	♂	3	40	21.6	26	40	21.9	26	41	23.8	46	8.5	76.9	**0.0355**
Starvation	3	daGal4	2.1	♂	4	40	17.1	20	40	18.5	27	40	17.1	27	0.0	0.0	
Starvation	3	daGal4	2.1	♂	5	40	22.8	30	40	23.1	30	40	23.6	30	2.2	0.0	0.2027

Table S2

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max^2	n	Mean	Max^2	n	Mean	Max^2	Mean	Max^2	
Starvation	3	daGal4	2.1	♂	6	40	23.4	30	40	24.3	34	40	24.3	30	0.0	0.0	
Starvation	3	daGal4	4.3	♀	1	40	28.5	34	40	29.7	38	40	32.7	38	10.1	0.0	**0.0034**
Starvation	3	daGal4	4.3	♀	2	40	29.6	38	40	32.5	42	40	33.9	38	4.3	0.0	**0.0004**
Starvation	3	daGal4	4.3	♀	3	40	30.5	42	41	32.0	38	30	32.9	42	3.1	0.0	**0.0306**
Starvation	3	daGal4	4.3	♂	1	40	23.6	26	40	21.9	26	40	25.5	30	8.1	15.4	**0.0123**
Starvation	3	daGal4	4.3	♂	2	40	22.2	26	40	23.1	30	40	22.8	30	0.0	0.0	
Starvation	3	daGal4	4.3	♂	3	40	22.8	30	40	24.3	34	40	24.2	34	0.0	0.0	
Starvation	3	daGal4	9.1	♀	1	41	30.7	41	39	29.0	41	40	28.1	41	-3.3	0.0	0.3129
Starvation	3	daGal4	9.1	♀	2	40	30.2	34	40	29.7	38	40	30.3	34	0.3	0.0	0.6507
Starvation	3	daGal4	9.1	♀	3	40	30.9	32	38	30.1	41	39	28.6	41	-5.2	0.0	0.4383
Starvation	3	daGal4	9.1	♀	4	40	33.5	42	40	32.5	42	40	29.9	38	-8.0	-9.5	**0.0007**
Starvation	3	daGal4	9.1	♀	5	40	32.8	42	41	32.0	38	41	30.7	38	-4.0	0.0	**0.0115**
Starvation	3	daGal4	9.1	♂	1	40	13.8	16	39	14.4	23	39	16.3	23	13.2	0.0	**0.0049**
Starvation	3	daGal4	9.1	♂	2	40	23.7	30	40	21.9	26	40	20.2	22	-7.8	-15.4	**0.0028**
Starvation	3	daGal4	9.1	♂	3	40	15.0	23	38	14.4	23	39	15.6	23	4.1	0.0	0.2116
Starvation	3	daGal4	9.1	♂	4	40	23.7	30	40	23.1	30	40	21.2	22	-8.2	-26.7	**0.0002**
Starvation	3	daGal4	9.1	♂	5	40	25.1	30	40	24.3	34	40	21.2	26	-12.8	-13.3	**<0.0001**
Starvation	3	daGal4	3.2	♀	1	35	34.0	52	40	55.3	72	40	56.4	77	2.0	6.9	0.2976
Starvation	3	daGal4	3.2	♀	2	42	32.7	42	40	29.7	38	41	32.6	42	0.0	0.0	
Starvation	3	daGal4	3.2	♀	3	40	32.5	38	40	32.5	42	40	37.0	46	13.8	9.5	**0.0014**
Starvation	3	daGal4	3.2	♀	4	40	35.1	42	41	32.0	38	41	37.9	46	8.0	9.5	**0.0054**
Starvation	3	daGal4	3.2	♂	1	40	27.7	37	40	30.6	42	40	38.0	52	24.1	23.8	**<0.0001**
Starvation	3	daGal4	3.2	♂	2	40	23.9	30	40	21.9	26	40	23.3	30	0.0	0.0	
Starvation	3	daGal4	3.2	♂	3	40	24.0	38	40	23.1	30	40	25.0	30	4.2	0.0	**0.0186**
Starvation	3	daGal4	3.2	♂	4	40	25.0	34	40	24.3	34	20	25.2	30	0.8	-11.8	0.4527
Starvation	3	daGal4	10.1	♀	1	40	68.7	84	40	58.1	72	40	76.6	120	11.5	42.9	**0.0146**
Starvation	3	daGal4	10.1	♀	2	40	30.5	41	39	29.0	41	40	34.3	41	12.4	0.0	**0.0046**
Starvation	3	daGal4	10.1	♀	3	40	33.0	38	40	29.7	38	39	35.2	46	6.8	21.1	**0.0042**
Starvation	3	daGal4	10.1	♀	4	41	36.7	50	40	32.5	42	38	38.5	50	4.9	0.0	0.1078
Starvation	3	daGal4	10.1	♀	5	40	37.9	50	41	32.0	38	41	39.3	50	3.8	0.0	0.0693
Starvation	3	daGal4	10.1	♂	1	40	54.9	84	40	44.8	59	40	59.6	84	8.6	0.0	0.1305
Starvation	3	daGal4	10.1	♂	2	40	18.0	23	39	14.4	23	39	19.5	23	8.4	0.0	0.1063
Starvation	3	daGal4	10.1	♂	3	40	24.7	30	40	21.9	26	40	27.4	34	10.9	13.3	**0.0017**
Starvation	3	daGal4	10.1	♂	4	40	24.0	30	40	23.1	30	39	31.2	42	30.1	40.0	**<0.0001**
Starvation	3	daGal4	10.1	♂	5	40	26.0	34	40	24.3	34	41	28.4	42	9.4	23.5	**0.0002**
Starvation	3	daGal4	3.1	♀	1	40	43.8	54	40	41.1	58	40	47.4	58	8.3	0.0	**0.0014**
Starvation	3	daGal4	3.1	♀	2	40	26.7	32	40	28.6	40	40	27.1	40	0.0	0.0	
Starvation	3	daGal4	3.1	♀	3	40	27.8	34	40	29.7	38	41	32.1	38	8.2	0.0	**0.0040**
Starvation	3	daGal4	3.1	♀	4	40	23.4	32	40	23.3	32	40	23.4	40	0.0	25.0	
Starvation	3	daGal4	3.1	♀	5	40	29.8	34	40	32.5	42	40	34.5	42	6.2	0.0	0.3871
Starvation	3	daGal4	3.1	♀	6	40	31.4	34	41	32.0	38	38	34.0	46	6.4	21.1	**0.0006**
Starvation	3	daGal4	3.1	♂	1	40	28.8	38	39	29.7	38	40	28.0	34	-2.6	-10.5	**0.0351**
Starvation	3	daGal4	3.1	♂	2	40	15.9	20	40	16.5	20	40	16.2	20	0.0	0.0	
Starvation	3	daGal4	3.1	♂	3	40	21.4	22	40	21.9	26	40	23.3	30	6.4	15.4	**0.0006**
Starvation	3	daGal4	3.1	♂	4	40	15.3	20	40	18.5	27	40	16.5	20	0.0	0.0	
Starvation	3	daGal4	3.1	♂	5	40	23.0	30	40	23.1	30	40	24.2	30	4.8	0.0	0.0856
Starvation	3	daGal4	3.1	♂	6	40	22.7	26	40	24.3	34	40	22.4	26	-1.3	0.0	**0.0040**
Starvation	3	daGal4	4.4	♀	1	40	64.8	84	40	62.8	84	39	67.5	84	4.1	0.0	0.1475
Starvation	3	daGal4	4.4	♀	2	40	30.3	32	39	29.0	41	40	32.3	41	6.5	0.0	0.0642

Table S2

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Starvation	3	daGal4	4.4	♀	3	40	29.9	34	40	29.7	38	41	32.6	42	9.1	10.5	**0.0032**
Starvation	3	daGal4	4.4	♀	4	40	30.9	32	38	30.1	41	41	41.0	48	33.0	17.1	**<0.0001**
Starvation	3	daGal4	4.4	♀	5	41	34.7	42	40	32.5	42	40	34.8	42	0.3	0.0	0.1886
Starvation	3	daGal4	4.4	♀	6	40	34.7	42	41	32.0	38	40	35.7	42	2.9	0.0	**0.0012**
Starvation	3	daGal4	4.4	♂	1	40	47.5	59	40	49.7	59	37	51.9	72	4.4	22.0	**0.0443**
Starvation	3	daGal4	4.4	♂	2	40	15.4	23	39	14.4	23	44	15.2	23	0.0	0.0	
Starvation	3	daGal4	4.4	♂	3	40	21.8	26	40	21.9	26	40	22.6	30	3.2	15.4	0.0908
Starvation	3	daGal4	4.4	♂	4	40	15.5	23	38	14.4	23	41	21.0	32	35.3	39.1	**<0.0001**
Starvation	3	daGal4	4.4	♂	5	40	23.1	30	40	23.1	30	39	25.2	30	9.0	0.0	**0.0053**
Starvation	3	daGal4	4.4	♂	6	41	23.3	26	40	24.3	34	40	26.1	34	7.4	0.0	**0.0010**
Starvation	3	DJ694	2.1	♀	1	40	44.3	63	40	34.6	40	40	36.4	45	0.0	0.0	
Starvation	3	DJ694	2.1	♀	2	40	54.3	73	40	54.9	68	40	52.9	63	-2.5	-7.4	0.1174
Starvation	3	DJ694	2.1	♀	3	40	28.7	40	40	24.8	32	40	27.5	32	0.0	0.0	
Starvation	3	DJ694	2.1	♀	4	41	26.0	34	41	25.6	34	40	22.6	34	-11.9	0.0	**0.0019**
Starvation	3	DJ694	2.1	♀	5	40	29.0	40	40	22.5	32	40	27.2	40	0.0	0.0	
Starvation	3	DJ694	2.1	♀	6	40	26.4	34	42	28.0	38	42	26.4	34	0.0	0.0	
Starvation	3	DJ694	2.1	♀	7	28	27.6	34	40	29.1	34	40	27.0	34	-2.2	0.0	0.0515
Starvation	3	DJ694	2.1	♂	1	40	30.2	45	40	24.0	35	40	29.6	45	0.0	0.0	
Starvation	3	DJ694	2.1	♂	2	40	37.9	53	39	34.0	43	39	33.5	43	-1.5	0.0	**0.0004**
Starvation	3	DJ694	2.1	♂	3	40	17.0	20	40	16.7	20	40	15.9	20	-4.9	0.0	0.3501
Starvation	3	DJ694	2.1	♂	4	40	20.8	34	40	18.5	26	39	16.8	22	-9.0	-15.4	**0.0254**
Starvation	3	DJ694	2.1	♂	5	40	17.1	20	40	15.6	27	40	17.1	20	0.0	0.0	
Starvation	3	DJ694	2.1	♂	6	40	20.8	34	41	18.9	22	38	16.0	22	-15.4	0.0	**0.0001**
Starvation	3	DJ694	2.1	♂	7	39	20.1	34	40	17.9	22	37	17.6	22	-1.4	0.0	**0.0189**
Starvation	3	DJ694	4.3	♀	1	40	25.7	34	41	25.6	34	39	23.1	34	-9.8	0.0	**0.0118**
Starvation	3	DJ694	4.3	♀	2	42	25.7	34	42	28.0	38	40	22.0	26	-14.5	-23.5	**0.0001**
Starvation	3	DJ694	4.3	♀	3	40	28.1	34	40	29.1	34	40	24.7	34	-12.1	0.0	**0.0041**
Starvation	3	DJ694	4.3	♂	1	39	18.6	26	40	18.5	26	41	15.0	22	-18.7	-15.4	**<0.0001**
Starvation	3	DJ694	4.3	♂	2	40	19.2	26	41	18.9	22	40	15.2	22	-19.5	0.0	**<0.0001**
Starvation	3	DJ694	4.3	♂	3	41	18.6	26	40	17.9	22	37	16.1	22	-10.1	0.0	**0.0041**
Starvation	3	DJ694	9.1	♀	1	41	30.7	41	39	30.0	32	40	31.4	32	2.3	0.0	**0.0437**
Starvation	3	DJ694	9.1	♀	2	40	27.9	34	41	25.6	34	39	27.1	34	0.0	0.0	
Starvation	3	DJ694	9.1	♀	3	40	30.9	32	40	27.6	32	40	32.0	41	3.6	28.1	0.1301
Starvation	3	DJ694	9.1	♀	4	49	27.8	34	42	28.0	38	40	23.1	34	-16.9	0.0	**<0.0001**
Starvation	3	DJ694	9.1	♀	5	41	29.1	34	40	29.1	34	40	24.1	34	-17.2	0.0	**<0.0001**
Starvation	3	DJ694	9.1	♂	1	40	13.8	16	39	14.3	23	40	13.4	16	-2.7	0.0	0.1750
Starvation	3	DJ694	9.1	♂	2	40	18.9	26	40	18.5	26	40	14.3	22	-22.4	-15.4	**<0.0001**
Starvation	3	DJ694	9.1	♂	3	40	15.0	23	38	13.6	16	40	14.1	23	0.0	0.0	
Starvation	3	DJ694	9.1	♂	4	50	20.0	34	41	18.9	22	41	14.6	18	-22.6	-18.2	**<0.0001**
Starvation	3	DJ694	9.1	♂	5	40	19.3	26	40	17.9	22	37	14.2	18	-20.5	-18.2	**<0.0001**
Starvation	3	DJ694	3.2	♀	1	35	34.0	52	40	51.9	62	40	42.0	57	0.0	0.0	
Starvation	3	DJ694	3.2	♀	2	39	26.5	34	41	25.6	34	41	28.1	34	6.3	0.0	**0.0265**
Starvation	3	DJ694	3.2	♀	3	39	30.1	38	42	28.0	38	40	28.9	38	0.0	0.0	
Starvation	3	DJ694	3.2	♀	4	37	27.8	38	40	29.1	34	37	26.9	38	-3.5	0.0	0.0638
Starvation	3	DJ694	3.2	♂	1	40	27.7	37	40	31.1	42	40	30.0	37	0.0	0.0	
Starvation	3	DJ694	3.2	♂	2	36	21.3	34	40	18.5	26	39	18.2	34	-1.5	0.0	**0.0349**
Starvation	3	DJ694	3.2	♂	3	40	21.4	34	41	18.9	22	41	17.7	34	-6.2	0.0	**0.0153**
Starvation	3	DJ694	3.2	♂	4	40	21.2	34	40	17.9	22	41	16.9	26	-5.4	0.0	**0.0026**
Starvation	3	DJ694	10.1	♀	1	40	68.7	84	37	49.9	72	40	63.7	84	0.0	0.0	

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Starvation	3	DJ694	10.1	♀	2	40	30.5	41	39	30.0	32	40	30.7	41	0.7	0.0	0.4364
Starvation	3	DJ694	10.1	♀	3	39	27.7	34	41	25.6	34	41	30.1	38	8.6	11.8	**0.0002**
Starvation	3	DJ694	10.1	♀	4	35	29.2	34	42	28.0	38	20	27.2	34	-2.9	0.0	0.0906
Starvation	3	DJ694	10.1	♀	5	25	30.6	38	40	29.1	34	39	26.2	38	-10.1	0.0	**0.0090**
Starvation	3	DJ694	10.1	♂	1	40	54.9	84	40	36.9	47	40	48.7	72	0.0	0.0	
Starvation	3	DJ694	10.1	♂	2	40	18.0	23	39	14.3	23	40	15.0	16	0.0	-30.4	
Starvation	3	DJ694	10.1	♂	3	37	21.4	34	40	18.5	26	42	23.5	34	9.9	0.0	0.0610
Starvation	3	DJ694	10.1	♂	4	41	20.0	34	41	18.9	22	40	20.7	26	3.2	0.0	**0.0134**
Starvation	3	DJ694	10.1	♂	5	41	23.3	34	40	17.9	22	42	22.2	34	0.0	0.0	
Starvation	3	DJ694	3.1	♀	1	40	32.2	51	40	34.6	40	40	32.0	57	-0.8	11.8	0.2482
Starvation	3	DJ694	3.1	♀	2	40	43.8	54	40	43.1	50	40	40.1	46	-7.0	-8.0	**0.0004**
Starvation	3	DJ694	3.1	♀	3	40	26.7	32	40	24.8	32	40	24.6	32	-0.8	0.0	**0.0166**
Starvation	3	DJ694	3.1	♀	4	39	30.4	34	41	25.6	34	35	23.5	34	-8.2	0.0	**0.0226**
Starvation	3	DJ694	3.1	♀	5	40	23.4	32	40	22.5	32	40	22.9	27	0.0	-15.6	
Starvation	3	DJ694	3.1	♀	6	44	31.2	38	42	28.0	38	40	23.3	26	-16.8	-31.6	**<0.0001**
Starvation	3	DJ694	3.1	♀	7	39	29.6	34	40	29.1	34	38	25.8	34	-11.5	0.0	**0.0009**
Starvation	3	DJ694	3.1	♂	1	40	26.8	45	40	24.0	35	40	20.3	30	-15.2	-14.3	**0.0025**
Starvation	3	DJ694	3.1	♂	2	40	28.8	38	40	29.0	38	40	25.4	34	-11.7	-10.5	**0.0008**
Starvation	3	DJ694	3.1	♂	3	40	15.9	20	40	16.7	20	40	15.4	20	-3.5	0.0	0.1466
Starvation	3	DJ694	3.1	♂	4	40	19.3	22	40	18.5	26	37	16.8	22	-8.7	0.0	**0.0002**
Starvation	3	DJ694	3.1	♂	5	40	15.3	20	40	15.6	27	40	14.4	17	-6.0	-15.0	**0.0377**
Starvation	3	DJ694	3.1	♂	6	42	19.4	22	41	18.9	22	40	18.3	26	-2.9	18.2	0.1782
Starvation	3	DJ694	3.1	♂	7	40	19.3	26	40	17.9	22	41	17.3	22	-2.8	0.0	**0.0056**
Starvation	3	DJ694	4.4	♀	1	40	35.8	43	40	29.6	34	40	32.1	43	0.0	0.0	
Starvation	3	DJ694	4.4	♀	2	40	64.8	84	40	61.6	84	40	53.9	72	-12.5	-14.3	**0.0009**
Starvation	3	DJ694	4.4	♀	3	40	30.3	32	39	30.0	32	40	31.8	41	4.9	28.1	**0.0388**
Starvation	3	DJ694	4.4	♀	4	39	27.5	34	41	25.6	34	41	22.6	34	-11.7	0.0	**0.0019**
Starvation	3	DJ694	4.4	♀	5	40	30.9	32	40	27.6	32	39	30.4	32	0.0	0.0	
Starvation	3	DJ694	4.4	♀	6	48	30.0	34	42	28.0	38	41	24.1	34	-13.8	0.0	**0.0003**
Starvation	3	DJ694	4.4	♀	7	38	29.9	34	40	29.1	34	37	24.8	34	-14.8	0.0	**0.0002**
Starvation	3	DJ694	4.4	♂	1	40	21.3	24	40	26.4	29	40	20.3	29	-4.8	0.0	0.0786
Starvation	3	DJ694	4.4	♂	2	40	47.5	59	40	44.1	59	40	40.4	46	-8.3	-22.0	**0.0001**
Starvation	3	DJ694	4.4	♂	3	40	15.4	23	39	14.3	23	40	13.7	23	-4.1	0.0	**0.0145**
Starvation	3	DJ694	4.4	♂	4	39	18.9	26	40	18.5	26	40	15.8	22	-14.4	-15.4	**0.0010**
Starvation	3	DJ694	4.4	♂	5	40	15.5	23	38	13.6	16	40	14.2	23	0.0	0.0	
Starvation	3	DJ694	4.4	♂	6	39	17.5	22	41	18.9	22	42	16.0	26	-8.8	18.2	**0.0004**
Starvation	3	DJ694	4.4	♂	7	39	18.9	26	40	17.9	22	40	14.3	22	-19.7	0.0	**<0.0001**
Starvation	3	D42	2.1	♀	1	40	44.3	63	40	38.0	45	40	32.0	51	-15.7	0.0	**0.0002**
Starvation	3	D42	2.1	♀	2	40	54.3	73	40	53.1	63	50	55.7	63	2.7	0.0	0.2767
Starvation	3	D42	2.1	♀	3	40	28.7	40	40	24.9	32	40	33.9	40	18.2	0.0	**0.0053**
Starvation	3	D42	2.1	♀	4	40	36.3	46	40	39.0	50	42	37.1	46	0.0	0.0	
Starvation	3	D42	2.1	♀	5	40	29.0	40	40	22.5	32	40	33.8	40	16.8	0.0	**0.0008**
Starvation	3	D42	2.1	♀	6	40	37.0	42	40	39.9	50	40	38.3	46	0.0	0.0	
Starvation	3	D42	2.1	♀	7	40	36.7	42	42	39.5	50	40	40.1	50	1.5	0.0	**0.0005**
Starvation	3	D42	2.1	♂	1	40	30.2	45	40	27.6	35	40	26.5	35	-4.1	0.0	**0.0014**
Starvation	3	D42	2.1	♂	2	40	37.9	53	40	36.4	48	40	36.6	48	0.0	0.0	
Starvation	3	D42	2.1	♂	3	40	17.0	20	40	17.1	20	40	18.2	20	6.3	0.0	**0.0011**
Starvation	3	D42	2.1	♂	4	40	26.7	34	40	27.7	42	40	28.3	38	2.2	0.0	0.0840
Starvation	3	D42	2.1	♂	5	40	17.1	20	40	14.0	17	40	18.9	20	10.4	0.0	**<0.0001**

Table S2

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Starvation	3	D42	2.1	♂	6	40	28.9	38	40	27.9	38	40	27.7	34	-0.7	-10.5	0.3159
Starvation	3	D42	2.1	♂	7	40	26.7	34	40	28.3	38	40	26.4	38	-1.1	0.0	0.1402
Starvation	3	D42	4.3	♀	1	40	36.1	42	40	39.0	50	40	39.2	46	0.5	0.0	**0.0005**
Starvation	3	D42	4.3	♀	2	40	37.8	46	40	39.9	50	40	38.6	46	0.0	0.0	
Starvation	3	D42	4.3	♀	3	43	36.0	42	42	39.5	50	40	38.0	46	0.0	0.0	
Starvation	3	D42	4.3	♂	1	40	30.2	42	40	27.7	42	40	28.4	34	0.0	-19.0	
Starvation	3	D42	4.3	♂	2	41	28.0	34	40	27.9	38	40	28.2	34	0.9	0.0	0.6510
Starvation	3	D42	4.3	♂	3	40	27.1	38	40	28.3	38	40	23.8	30	-12.2	-21.1	**0.0002**
Starvation	3	D42	9.1	♀	1	41	30.7	41	40	31.8	41	43	32.2	41	1.2	0.0	0.0692
Starvation	3	D42	9.1	♀	2	40	38.0	42	40	39.0	50	40	36.0	46	-5.3	0.0	**0.0165**
Starvation	3	D42	9.1	♀	3	40	30.9	32	38	30.3	41	41	31.0	32	0.6	0.0	0.3354
Starvation	3	D42	9.1	♀	4	42	39.4	46	40	39.9	50	40	34.9	42	-11.5	-8.7	**<0.0001**
Starvation	3	D42	9.1	♀	5	40	39.2	50	42	39.5	50	40	38.3	42	-2.3	-16.0	0.1892
Starvation	3	D42	9.1	♂	1	40	13.8	16	40	12.8	16	44	14.1	23	2.8	43.8	0.0564
Starvation	3	D42	9.1	♂	2	40	29.8	42	40	27.7	42	40	24.9	34	-10.1	-19.0	**0.0109**
Starvation	3	D42	9.1	♂	3	40	15.0	23	40	13.3	16	43	13.7	16	0.0	0.0	
Starvation	3	D42	9.1	♂	4	40	27.1	38	40	27.9	38	40	24.5	34	-9.6	-10.5	**0.0047**
Starvation	3	D42	9.1	♂	5	40	28.6	42	40	28.3	38	40	23.5	30	-17.0	-21.1	**<0.0001**
Starvation	3	D42	3.2	♀	1	35	34.0	52	42	51.5	67	40	51.4	62	0.0	0.0	
Starvation	3	D42	3.2	♀	2	40	40.5	50	40	39.0	50	40	41.5	50	2.5	0.0	**0.0279**
Starvation	3	D42	3.2	♀	3	40	41.1	50	40	39.9	50	40	41.0	50	0.0	0.0	
Starvation	3	D42	3.2	♀	4	40	41.3	50	42	39.5	50	40	42.1	50	1.9	0.0	0.1144
Starvation	3	D42	3.2	♂	1	40	27.7	37	40	31.1	37	40	31.9	47	2.4	27.0	**0.0137**
Starvation	3	D42	3.2	♂	2	40	28.9	38	40	27.7	42	40	29.1	38	0.7	0.0	0.3563
Starvation	3	D42	3.2	♂	3	40	30.3	42	40	27.9	38	40	28.6	38	0.0	0.0	
Starvation	3	D42	3.2	♂	4	40	33.2	42	40	28.3	38	40	29.2	38	0.0	0.0	
Starvation	3	D42	10.1	♀	1	40	68.7	84	40	54.0	84	40	72.7	96	5.8	14.3	0.1433
Starvation	3	D42	10.1	♀	2	40	30.5	41	40	31.8	41	39	34.5	41	8.4	0.0	**0.0035**
Starvation	3	D42	10.1	♀	3	40	41.8	46	40	39.0	50	40	39.2	46	0.0	0.0	
Starvation	3	D42	10.1	♀	4	39	45.5	59	40	39.9	50	40	40.6	46	0.0	-8.0	
Starvation	3	D42	10.1	♀	5	40	42.5	50	42	39.5	50	41	41.4	50	0.0	0.0	
Starvation	3	D42	10.1	♂	1	40	54.9	84	40	46.2	59	40	53.7	72	0.0	0.0	
Starvation	3	D42	10.1	♂	2	40	18.0	23	40	12.8	16	40	17.4	23	0.0	0.0	
Starvation	3	D42	10.1	♂	3	40	31.7	42	40	27.7	42	40	33.1	42	4.4	0.0	**0.0002**
Starvation	3	D42	10.1	♂	4	41	33.6	42	40	27.9	38	42	34.7	42	3.1	0.0	0.4653
Starvation	3	D42	10.1	♂	5	40	30.5	38	40	28.3	38	40	34.5	42	13.1	10.5	**0.0005**
Starvation	3	D42	3.1	♀	1	40	32.2	51	40	38.0	45	40	36.5	57	0.0	11.8	0.0982
Starvation	3	D42	3.1	♀	2	40	43.8	54	37	41.1	50	40	44.7	54	2.1	0.0	**0.0012**
Starvation	3	D42	3.1	♀	3	40	26.7	32	40	24.9	32	40	29.2	40	9.4	25.0	**0.0003**
Starvation	3	D42	3.1	♀	4	40	36.8	42	40	39.0	50	42	37.1	46	0.0	0.0	
Starvation	3	D42	3.1	♀	5	40	23.4	32	40	22.5	32	40	29.2	32	24.6	0.0	**<0.0001**
Starvation	3	D42	3.1	♀	6	40	38.4	46	40	39.9	50	42	37.2	46	-3.0	0.0	**0.0213**
Starvation	3	D42	3.1	♀	7	40	37.7	46	42	39.5	50	40	37.5	42	-0.5	-8.7	0.0568
Starvation	3	D42	3.1	♂	1	40	26.8	45	40	27.6	35	40	25.9	35	-3.5	0.0	0.0850
Starvation	3	D42	3.1	♂	2	40	28.8	38	40	25.0	34	40	27.3	31	0.0	-8.8	
Starvation	3	D42	3.1	♂	3	40	15.9	20	40	17.1	20	40	18.7	27	8.9	35.0	**0.0001**
Starvation	3	D42	3.1	♂	4	40	24.7	34	40	27.7	42	40	26.9	34	0.0	0.0	
Starvation	3	D42	3.1	♂	5	40	15.3	20	40	14.0	17	40	18.5	27	20.8	35.0	**<0.0001**
Starvation	3	D42	3.1	♂	6	40	25.9	34	40	27.9	38	40	26.8	34	0.0	0.0	

Table S2

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS n	UAS Mean	UAS Max[2]	Gal4 n	Gal4 Mean	Gal4 Max[2]	Gal4+UAS n	Gal4+UAS Mean	Gal4+UAS Max[2]	% Extension Mean	% Extension Max[2]	P
Starvation	3	D42	3.1	♂	7	40	27.0	34	40	28.3	38	40	25.6	34	-5.2	0.0	**0.0147**
Starvation	3	D42	4.4	♀	1	40	35.8	43	40	29.7	34	40	31.3	34	0.0	0.0	
Starvation	3	D42	4.4	♀	2	40	64.8	84	39	72.6	84	40	68.8	84	0.0	0.0	
Starvation	3	D42	4.4	♀	3	40	30.3	32	40	31.8	41	42	33.9	48	6.6	17.1	**0.0015**
Starvation	3	D42	4.4	♀	4	40	39.5	50	40	39.0	50	40	37.4	42	-4.1	-16.0	0.1325
Starvation	3	D42	4.4	♀	5	40	30.9	32	38	30.3	41	41	39.2	41	26.9	0.0	**<0.0001**
Starvation	3	D42	4.4	♀	6	40	41.4	46	40	39.9	50	40	36.1	42	-9.5	-8.7	**0.0015**
Starvation	3	D42	4.4	♀	7	40	39.2	50	42	39.5	50	40	34.4	42	-12.2	-16.0	**<0.0001**
Starvation	3	D42	4.4	♂	1	40	21.3	24	40	22.4	29	40	23.5	29	5.0	0.0	**0.0033**
Starvation	3	D42	4.4	♂	2	40	47.5	59	34	44.5	59	40	47.2	72	0.0	22.0	
Starvation	3	D42	4.4	♂	3	40	15.4	23	40	12.8	16	40	15.8	23	2.6	0.0	0.6505
Starvation	3	D42	4.4	♂	4	40	27.0	38	40	27.7	42	40	27.8	34	0.4	-10.5	0.4817
Starvation	3	D42	4.4	♂	5	40	15.5	23	40	13.3	16	41	22.7	32	46.2	39.1	**<0.0001**
Starvation	3	D42	4.4	♂	6	40	27.9	38	40	27.9	38	40	26.5	38	-5.0	0.0	0.3560
Starvation	3	D42	4.4	♂	7	40	27.6	34	40	28.3	38	40	25.5	38	-7.6	0.0	**0.0402**
Starvation	10	daGal4	2.1	♀	1	40	49.5	63	40	48.8	63	40	46.2	58	-5.4	-7.9	**0.0208**
Starvation	10	daGal4	2.1	♀	2	40	31.0	38	40	33.3	38	41	29.2	38	-5.7	0.0	0.0995
Starvation	10	daGal4	2.1	♀	3	40	29.3	34	41	36.3	42	43	31.2	38	0.0	0.0	
Starvation	10	daGal4	2.1	♀	4	40	30.2	34	40	35.5	42	40	31.2	38	0.0	0.0	
Starvation	10	daGal4	2.1	♂	1	39	27.9	38	36	28.3	33	40	27.5	38	-1.8	0.0	0.4315
Starvation	10	daGal4	2.1	♂	2	41	15.7	18	38	16.5	18	40	15.8	22	0.0	22.2	
Starvation	10	daGal4	2.1	♂	3	40	17.9	22	39	17.3	18	40	16.3	18	-5.7	0.0	**0.0012**
Starvation	10	daGal4	2.1	♂	4	40	16.7	18	39	17.6	22	40	16.0	22	-4.2	0.0	**0.0114**
Starvation	10	daGal4	4.3	♀	1	40	48.6	59	38	51.1	72	40	60.8	84	18.9	16.7	**0.0001**
Starvation	10	daGal4	4.3	♀	2	40	29.6	38	40	25.5	38	40	27.8	48	0.0	26.3	
Starvation	10	daGal4	4.3	♀	3	40	30.7	38	40	33.3	38	40	33.5	38	0.6	0.0	**0.0489**
Starvation	10	daGal4	4.3	♀	4	40	32.6	43	40	24.8	33	40	29.2	48	0.0	11.6	
Starvation	10	daGal4	4.3	♀	5	40	30.0	38	41	36.3	42	40	35.1	42	0.0	0.0	
Starvation	10	daGal4	4.3	♀	6	39	31.5	38	40	35.5	42	40	35.9	46	1.1	9.5	**0.0002**
Starvation	10	daGal4	4.3	♂	1	37	26.6	34	40	23.6	34	40	34.3	47	28.9	38.2	**<0.0001**
Starvation	10	daGal4	4.3	♂	2	40	11.6	16	40	10.4	16	40	13.9	33	19.6	106.3	**0.0147**
Starvation	10	daGal4	4.3	♂	3	40	17.3	22	38	16.5	18	41	17.1	18	0.0	0.0	
Starvation	10	daGal4	4.3	♂	4	40	17.2	18	39	17.3	18	39	17.7	22	2.4	22.2	0.5355
Starvation	10	daGal4	4.3	♂	5	39	16.1	18	39	17.6	22	39	17.6	22	0.0	0.0	
Starvation	10	daGal4	9.1	♀	1	39	32.7	40	39	25.6	40	39	27.3	40	0.0	0.0	
Starvation	10	daGal4	9.1	♀	2	39	31.6	38	40	33.3	38	40	30.8	38	-2.7	0.0	**0.0004**
Starvation	10	daGal4	9.1	♀	3	40	31.3	40	40	26.6	40	34	22.7	40	-14.6	0.0	**0.0176**
Starvation	10	daGal4	9.1	♀	4	40	33.3	42	41	36.3	42	40	30.2	38	-9.3	-9.5	**0.0003**
Starvation	10	daGal4	9.1	♀	5	40	34.2	42	40	35.5	42	40	29.1	34	-14.9	-19.0	**<0.0001**
Starvation	10	daGal4	9.1	♂	1	39	11.3	15	39	11.2	15	37	10.1	10	-9.3	-33.3	**0.0087**
Starvation	10	daGal4	9.1	♂	2	40	16.4	18	38	16.5	18	40	16.0	18	-2.4	0.0	0.3321
Starvation	10	daGal4	9.1	♂	3	40	11.8	15	39	10.9	15	40	10.0	10	-8.2	-33.3	**0.0053**
Starvation	10	daGal4	9.1	♂	4	40	15.5	18	39	17.3	18	40	14.7	18	-5.2	0.0	**0.0465**
Starvation	10	daGal4	9.1	♂	5	40	16.0	18	39	17.6	22	39	15.1	18	-5.4	0.0	0.1044
Starvation	10	daGal4	3.2	♀	1	35	39.2	48	40	47.3	63	40	46.5	63	0.0	0.0	
Starvation	10	daGal4	3.2	♀	2	40	28.8	33	40	25.5	38	40	30.1	43	4.4	13.2	**0.0038**
Starvation	10	daGal4	3.2	♀	3	40	32.8	38	40	33.3	38	39	33.6	38	0.9	0.0	0.1294
Starvation	10	daGal4	3.2	♀	4	40	24.7	33	40	24.8	33	40	29.4	38	18.9	15.2	**0.0005**
Starvation	10	daGal4	3.2	♀	5	40	34.2	42	41	36.3	42	40	34.0	42	-0.6	0.0	**0.0463**

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Starvation	10	daGal4	3.2	♀	6	40	33.9	42	40	35.5	42	40	36.3	46	2.3	9.5	**0.0197**
Starvation	10	daGal4	3.2	♂	1	40	20.9	29	40	24.9	33	40	28.8	38	15.8	15.2	**0.0013**
Starvation	10	daGal4	3.2	♂	2	40	11.7	16	40	10.4	16	40	11.3	20	0.0	25.0	
Starvation	10	daGal4	3.2	♂	3	40	19.6	26	38	16.5	18	40	19.1	26	0.0	0.0	
Starvation	10	daGal4	3.2	♂	4	40	13.1	20	40	10.7	16	40	14.1	25	8.0	25.0	**0.0015**
Starvation	10	daGal4	3.2	♂	5	40	18.7	22	39	17.3	18	39	19.7	26	5.6	18.2	0.0764
Starvation	10	daGal4	3.2	♂	6	40	19.1	22	39	17.6	22	40	21.4	34	12.0	54.5	**0.0322**
Starvation	10	daGal4	10.1	♀	1	36	26.9	47	34	25.1	33	40	31.5	47	16.9	0.0	**0.0005**
Starvation	10	daGal4	10.1	♀	2	40	33.6	40	39	25.6	40	40	25.6	40	0.0	0.0	
Starvation	10	daGal4	10.1	♀	3	44	35.2	42	40	33.3	38	40	34.7	42	0.0	0.0	
Starvation	10	daGal4	10.1	♀	4	39	28.2	40	40	26.6	40	32	22.6	40	-15.2	0.0	**0.0049**
Starvation	10	daGal4	10.1	♀	5	40	35.1	42	41	36.3	42	40	36.3	42	0.0	0.0	
Starvation	10	daGal4	10.1	♀	6	40	36.0	42	40	35.5	42	40	36.9	42	2.5	0.0	0.1098
Starvation	10	daGal4	10.1	♂	1	36	19.8	22	36	21.6	22	39	24.6	33	13.9	50.0	**0.0004**
Starvation	10	daGal4	10.1	♂	2	40	11.6	15	39	11.2	15	40	13.7	21	17.4	40.0	**0.0014**
Starvation	10	daGal4	10.1	♂	3	40	19.1	22	38	16.5	18	43	19.9	26	4.0	18.2	0.1526
Starvation	10	daGal4	10.1	♂	4	40	13.3	21	39	10.9	15	39	10.3	15	-5.9	0.0	0.0783
Starvation	10	daGal4	10.1	♂	5	40	18.7	26	39	17.3	18	42	20.2	30	8.0	15.4	0.0615
Starvation	10	daGal4	10.1	♂	6	37	18.4	22	39	17.6	22	40	20.3	26	10.1	18.2	**0.0170**
Starvation	10	daGal4	3.1	♀	1	37	32.7	46	33	34.7	46	40	35.9	54	3.4	17.4	0.0574
Starvation	10	daGal4	3.1	♀	2	40	30.5	38	40	33.3	38	40	31.3	38	0.0	0.0	
Starvation	10	daGal4	3.1	♀	3	40	31.4	38	41	36.3	42	40	34.4	42	0.0	0.0	
Starvation	10	daGal4	3.1	♀	4	40	34.3	46	40	35.5	42	39	33.4	42	-2.7	0.0	**0.0087**
Starvation	10	daGal4	3.1	♂	1	40	18.6	26	40	18.5	30	40	21.4	30	15.1	0.0	**0.0082**
Starvation	10	daGal4	3.1	♂	2	41	18.1	22	38	16.5	18	40	18.5	22	2.2	0.0	0.2871
Starvation	10	daGal4	3.1	♂	3	40	16.5	22	39	17.3	18	41	18.3	26	5.8	18.2	**0.0031**
Starvation	10	daGal4	3.1	♂	4	40	16.6	22	39	17.6	22	38	16.7	18	0.0	-18.2	
Starvation	10	daGal4	4.4	♀	1	40	30.5	40	39	25.6	40	36	24.9	40	-2.5	0.0	**0.0005**
Starvation	10	daGal4	4.4	♀	2	36	53.6	59	38	51.1	72	39	54.5	72	1.6	0.0	0.1343
Starvation	10	daGal4	4.4	♀	3	40	34.1	38	40	33.3	38	41	32.1	42	-3.5	10.5	0.0820
Starvation	10	daGal4	4.4	♀	4	41	32.9	40	40	26.6	40	40	25.4	40	-4.5	0.0	0.3065
Starvation	10	daGal4	4.4	♀	5	40	34.7	42	41	36.3	42	31	35.8	46	0.0	9.5	
Starvation	10	daGal4	4.4	♀	6	40	36.5	42	40	35.5	42	37	35.0	42	-1.5	0.0	0.2074
Starvation	10	daGal4	4.4	♂	1	40	11.6	15	39	11.2	15	42	10.4	15	-7.4	0.0	**0.0040**
Starvation	10	daGal4	4.4	♂	2	37	27.2	34	40	23.6	34	40	30.7	47	12.8	38.2	**0.0286**
Starvation	10	daGal4	4.4	♂	3	40	16.7	18	38	16.5	18	39	17.2	22	2.9	22.2	0.1972
Starvation	10	daGal4	4.4	♂	4	40	12.4	15	39	10.9	15	40	13.2	21	6.5	40.0	**0.0004**
Starvation	10	daGal4	4.4	♂	5	40	17.1	22	39	17.3	18	39	17.3	22	0.0	0.0	
Starvation	10	daGal4	4.4	♂	6	41	16.0	18	39	17.6	22	39	18.9	26	7.6	18.2	**0.0228**
Starvation	10	DJ694	2.1	♀	1	40	49.5	63	40	49.0	58	40	47.5	58	-3.1	0.0	**0.0256**
Starvation	10	DJ694	2.1	♀	2	40	23.9	30	40	22.4	26	40	20.5	22	-8.5	-15.4	**0.0009**
Starvation	10	DJ694	2.1	♀	3	40	23.8	30	41	24.7	30	40	22.9	26	-3.8	-13.3	**0.0008**
Starvation	10	DJ694	2.1	♀	4	40	23.5	30	40	26.0	30	49	24.2	30	0.0	0.0	
Starvation	10	DJ694	2.1	♂	1	39	27.9	38	38	26.9	33	39	27.3	33	0.0	0.0	
Starvation	10	DJ694	2.1	♂	2	40	15.3	18	40	11.8	14	40	12.1	14	0.0	0.0	
Starvation	10	DJ694	2.1	♂	3	40	14.3	18	40	15.7	18	40	13.3	18	-7.0	0.0	**0.0199**
Starvation	10	DJ694	2.1	♂	4	40	14.5	18	40	12.9	14	40	13.6	18	0.0	0.0	
Starvation	10	DJ694	4.3	♀	1	40	48.6	59	38	50.3	72	40	52.8	72	4.9	0.0	0.4071
Starvation	10	DJ694	4.3	♀	2	40	29.6	38	40	20.6	33	40	25.1	33	0.0	0.0	

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Starvation	10	DJ694	4.3	♀	3	38	24.4	30	40	22.4	26	40	20.0	22	-10.7	-15.4	**<0.0001**
Starvation	10	DJ694	4.3	♀	4	40	32.6	43	40	21.6	33	40	23.1	33	0.0	0.0	
Starvation	10	DJ694	4.3	♀	5	40	25.0	30	41	24.7	30	30	21.3	22	-13.7	-26.7	**<0.0001**
Starvation	10	DJ694	4.3	♀	6	40	26.7	34	40	26.0	30	40	22.5	26	-13.5	-13.3	**<0.0001**
Starvation	10	DJ694	4.3	♂	1	37	26.6	34	40	25.9	34	40	25.6	34	-1.1	0.0	0.4436
Starvation	10	DJ694	4.3	♂	2	40	11.6	16	40	11.0	16	40	10.0	20	-8.7	25.0	0.0795
Starvation	10	DJ694	4.3	♂	3	40	16.4	18	40	11.8	14	40	13.7	14	0.0	0.0	
Starvation	10	DJ694	4.3	♂	4	40	10.5	16	40	9.4	16	40	9.5	16	0.0	0.0	
Starvation	10	DJ694	4.3	♂	5	40	16.1	18	40	15.7	18	40	14.1	18	-10.2	0.0	**0.0003**
Starvation	10	DJ694	4.3	♂	6	40	15.0	18	40	12.9	14	40	13.4	14	0.0	0.0	
Starvation	10	DJ694	9.1	♀	1	39	32.7	40	39	26.3	40	40	24.8	40	-5.8	0.0	0.1103
Starvation	10	DJ694	9.1	♀	2	39	26.3	30	40	22.4	26	40	19.9	22	-11.2	-15.4	**<0.0001**
Starvation	10	DJ694	9.1	♀	3	40	31.3	40	40	23.0	27	41	24.0	40	0.0	0.0	
Starvation	10	DJ694	9.1	♀	4	40	27.0	34	41	24.7	30	40	20.6	22	-16.7	-26.7	**<0.0001**
Starvation	10	DJ694	9.1	♀	5	40	27.6	30	40	26.0	30	40	19.3	26	-25.8	-13.3	**<0.0001**
Starvation	10	DJ694	9.1	♂	1	39	11.3	15	38	10.0	10	39	10.0	10	0.0	0.0	
Starvation	10	DJ694	9.1	♂	2	40	16.2	18	40	11.8	14	40	10.9	14	-7.6	0.0	**0.0345**
Starvation	10	DJ694	9.1	♂	3	40	11.8	15	40	10.6	15	40	10.1	10	-4.7	-33.3	**0.0004**
Starvation	10	DJ694	9.1	♂	4	40	15.9	18	40	15.7	18	40	13.1	14	-16.6	-22.2	**<0.0001**
Starvation	10	DJ694	9.1	♂	5	40	16.7	18	40	12.9	14	40	12.1	14	-6.2	0.0	0.0805
Starvation	10	DJ694	3.2	♀	1	35	39.2	48	38	44.4	53	40	47.0	53	5.9	0.0	0.3138
Starvation	10	DJ694	3.2	♀	2	40	28.8	33	40	20.6	33	40	25.9	33	0.0	0.0	
Starvation	10	DJ694	3.2	♀	3	40	26.4	34	40	22.4	26	40	23.6	34	0.0	0.0	
Starvation	10	DJ694	3.2	♀	4	40	24.7	33	40	21.6	33	40	25.4	33	2.8	0.0	**0.0147**
Starvation	10	DJ694	3.2	♀	5	40	26.1	30	41	24.7	30	40	24.7	30	0.0	0.0	
Starvation	10	DJ694	3.2	♀	6	40	27.6	30	40	26.0	30	40	25.9	38	-0.4	26.7	0.1205
Starvation	10	DJ694	3.2	♂	1	40	20.9	29	36	19.6	29	40	24.1	38	15.3	31.0	**0.0015**
Starvation	10	DJ694	3.2	♂	2	40	11.7	16	40	11.0	16	40	11.1	25	0.0	56.3	
Starvation	10	DJ694	3.2	♂	3	40	18.5	22	40	11.8	14	40	16.5	18	0.0	0.0	
Starvation	10	DJ694	3.2	♂	4	40	13.1	20	40	9.4	16	40	10.8	20	0.0	0.0	
Starvation	10	DJ694	3.2	♂	5	40	18.1	22	40	15.7	18	40	17.0	22	0.0	0.0	
Starvation	10	DJ694	3.2	♂	6	40	19.3	22	40	12.9	14	40	16.5	22	0.0	0.0	
Starvation	10	DJ694	10.1	♀	1	36	26.9	47	32	26.3	47	40	29.3	47	8.7	0.0	0.0586
Starvation	10	DJ694	10.1	♀	2	40	33.6	40	39	26.3	40	40	23.4	27	-11.1	-32.5	**0.0032**
Starvation	10	DJ694	10.1	♀	3	40	24.5	30	40	22.4	26	40	22.3	26	-0.4	0.0	**0.0017**
Starvation	10	DJ694	10.1	♀	4	39	28.2	40	40	23.0	27	40	21.5	27	-6.6	0.0	0.0272
Starvation	10	DJ694	10.1	♀	5	40	27.6	38	41	24.7	30	39	24.6	30	-0.7	0.0	0.7832
Starvation	10	DJ694	10.1	♀	6	40	27.1	34	40	26.0	30	40	24.0	30	-7.7	0.0	**0.0002**
Starvation	10	DJ694	10.1	♂	1	36	19.8	22	32	20.4	22	38	22.7	29	11.6	31.8	**0.0015**
Starvation	10	DJ694	10.1	♂	2	40	11.6	15	38	10.0	10	40	10.0	10	0.0	0.0	
Starvation	10	DJ694	10.1	♂	3	40	17.5	22	40	11.8	14	40	15.1	18	0.0	0.0	
Starvation	10	DJ694	10.1	♂	4	40	13.3	21	40	10.6	15	40	11.8	21	0.0	0.0	
Starvation	10	DJ694	10.1	♂	5	40	18.8	22	40	15.7	18	40	17.1	18	0.0	0.0	
Starvation	10	DJ694	10.1	♂	6	40	18.5	22	40	12.9	14	40	17.5	22	0.0	0.0	
Starvation	10	DJ694	3.1	♀	1	37	32.7	46	40	41.1	54	39	34.2	50	0.0	0.0	
Starvation	10	DJ694	3.1	♀	2	40	21.7	26	40	22.4	26	40	22.8	30	1.8	15.4	0.0746
Starvation	10	DJ694	3.1	♀	3	40	23.6	26	41	24.7	30	40	22.5	30	-4.7	0.0	**0.0004**
Starvation	10	DJ694	3.1	♀	4	40	24.2	30	40	26.0	30	40	24.8	34	0.0	13.3	
Starvation	10	DJ694	3.1	♂	1	40	18.6	26	40	18.2	26	40	16.0	22	-12.2	-15.4	**0.0029**

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS n	UAS Mean	UAS Max[2]	Gal4 n	Gal4 Mean	Gal4 Max[2]	Gal4 + UAS n	Gal4 + UAS Mean	Gal4 + UAS Max[2]	% Extension Mean	% Extension Max[2]	P
Starvation	10	DJ694	3.1	♂	2	40	17.5	18	40	11.8	14	20	13.0	14	0.0	0.0	
Starvation	10	DJ694	3.1	♂	3	40	17.6	18	40	15.7	18	40	15.7	18	0.0	0.0	
Starvation	10	DJ694	3.1	♂	4	40	16.0	18	40	12.9	14	40	16.4	18	2.5	0.0	0.5390
Starvation	10	DJ694	4.4	♀	1	40	29.8	35	40	24.8	35	40	21.8	30	-12.1	-14.3	**0.0025**
Starvation	10	DJ694	4.4	♀	2	40	30.5	40	39	26.3	40	39	22.4	27	-15.0	-32.5	**0.0002**
Starvation	10	DJ694	4.4	♀	3	36	53.6	59	38	50.3	72	40	54.8	72	2.1	0.0	**0.0406**
Starvation	10	DJ694	4.4	♀	4	40	23.9	26	40	22.4	26	40	23.1	30	0.0	15.4	
Starvation	10	DJ694	4.4	♀	5	41	32.9	40	40	23.0	27	40	24.3	40	0.0	0.0	
Starvation	10	DJ694	4.4	♀	6	40	25.9	30	41	24.7	30	40	25.5	30	0.0	0.0	
Starvation	10	DJ694	4.4	♀	7	40	26.8	30	40	26.0	30	40	24.8	30	-4.6	0.0	0.0578
Starvation	10	DJ694	4.4	♂	1	40	19.5	25	40	19.8	25	40	18.6	25	-4.5	0.0	0.1191
Starvation	10	DJ694	4.4	♂	2	40	11.6	15	38	10.0	10	39	10.1	10	0.0	0.0	
Starvation	10	DJ694	4.4	♂	3	37	27.2	34	40	25.9	34	38	24.4	34	-5.8	0.0	0.0516
Starvation	10	DJ694	4.4	♂	4	40	15.6	18	40	11.8	14	40	15.5	18	0.0	0.0	
Starvation	10	DJ694	4.4	♂	5	40	12.4	15	40	10.6	15	40	10.3	15	-3.5	0.0	0.2382
Starvation	10	DJ694	4.4	♂	6	30	16.5	18	40	15.7	18	40	16.6	22	0.4	22.2	0.0956
Starvation	10	DJ694	4.4	♂	7	40	16.2	18	40	12.9	14	40	14.3	18	0.0	0.0	
Starvation	10	D42	2.1	♀	1	40	49.5	63	40	46.6	58	40	49.8	58	0.6	0.0	0.1613
Starvation	10	D42	2.1	♀	2	40	25.8	32	40	25.1	32	39	25.3	28	0.0	-12.5	
Starvation	10	D42	2.1	♀	3	40	26.1	32	40	29.4	36	40	26.8	32	0.0	0.0	
Starvation	10	D42	2.1	♀	4	40	27.5	32	40	29.8	36	40	29.4	32	0.0	0.0	
Starvation	10	D42	2.1	♂	1	39	27.9	38	36	30.5	38	40	27.8	33	-0.7	-13.2	**0.0013**
Starvation	10	D42	2.1	♂	2	37	15.2	16	40	17.4	20	39	14.7	16	-3.3	0.0	0.4107
Starvation	10	D42	2.1	♂	3	40	16.5	24	39	15.7	20	40	14.8	16	-5.9	-20.0	**0.0023**
Starvation	10	D42	2.1	♂	4	40	15.6	16	40	16.9	20	40	15.8	16	0.0	0.0	
Starvation	10	D42	4.3	♀	1	40	48.6	59	39	51.3	72	39	54.8	72	6.8	0.0	**0.0457**
Starvation	10	D42	4.3	♀	2	40	29.6	38	40	24.9	43	40	23.0	33	-7.9	-13.2	0.1441
Starvation	10	D42	4.3	♀	3	40	27.1	32	40	25.1	32	38	25.7	32	0.0	0.0	
Starvation	10	D42	4.3	♀	4	40	32.6	43	40	23.5	33	40	23.7	33	0.0	0.0	
Starvation	10	D42	4.3	♀	5	40	28.8	32	40	29.4	36	40	29.2	32	0.0	0.0	
Starvation	10	D42	4.3	♀	6	40	29.9	32	40	29.8	36	40	27.0	32	-9.4	0.0	**<0.0001**
Starvation	10	D42	4.3	♂	1	37	26.6	34	36	25.5	34	40	24.1	34	-5.5	0.0	**0.0405**
Starvation	10	D42	4.3	♂	2	40	11.6	16	40	9.9	16	40	7.9	16	-19.5	0.0	**0.0042**
Starvation	10	D42	4.3	♂	3	40	14.5	16	40	17.4	20	39	14.3	16	-1.8	0.0	0.6028
Starvation	10	D42	4.3	♂	4	40	10.5	16	40	10.6	16	40	9.6	16	-8.6	0.0	0.2443
Starvation	10	D42	4.3	♂	5	39	15.2	16	39	15.7	20	40	13.6	16	-10.4	0.0	**0.0003**
Starvation	10	D42	4.3	♂	6	40	14.8	16	40	16.9	20	40	13.7	20	-7.8	0.0	0.1176
Starvation	10	D42	9.1	♀	1	39	32.7	40	40	36.1	40	40	29.2	40	-10.8	0.0	**0.0299**
Starvation	10	D42	9.1	♀	2	40	27.6	36	40	25.1	32	40	23.2	28	-7.6	-12.5	**0.0058**
Starvation	10	D42	9.1	♀	3	40	31.3	40	40	28.9	40	40	26.2	40	-9.6	0.0	**0.0033**
Starvation	10	D42	9.1	♀	4	40	29.5	36	40	29.4	36	40	24.3	28	-17.3	-22.2	**<0.0001**
Starvation	10	D42	9.1	♀	5	40	30.4	36	40	29.8	36	38	25.3	32	-15.2	-11.1	**<0.0001**
Starvation	10	D42	9.1	♂	1	39	11.3	15	40	10.6	15	40	10.0	10	-5.9	-33.3	**0.0013**
Starvation	10	D42	9.1	♂	2	40	15.4	16	40	17.4	20	40	12.3	16	-19.9	0.0	**<0.0001**
Starvation	10	D42	9.1	♂	3	40	11.8	15	40	11.9	21	40	10.5	15	-10.8	0.0	**0.0100**
Starvation	10	D42	9.1	♂	4	40	14.8	16	39	15.7	20	38	13.5	16	-8.5	0.0	**0.0002**
Starvation	10	D42	9.1	♂	5	40	15.4	16	40	16.9	20	40	16.0	16	0.0	0.0	
Starvation	10	D42	3.2	♀	1	35	39.2	48	40	44.5	63	40	44.1	58	0.0	0.0	
Starvation	10	D42	3.2	♀	2	40	28.8	33	40	24.9	43	40	31.9	38	10.7	0.0	**0.0053**

Stress	Age	Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
						n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
Starvation	10	D42	3.2	♀	3	40	26.3	32	40	25.1	32	40	26.1	36	0.0	12.5	
Starvation	10	D42	3.2	♀	4	40	24.7	33	40	23.5	33	40	30.1	38	21.8	15.2	**0.0004**
Starvation	10	D42	3.2	♀	5	40	26.7	32	40	29.4	36	40	26.9	32	0.0	0.0	
Starvation	10	D42	3.2	♀	6	40	30.6	36	40	29.8	36	40	28.4	32	-4.7	-11.1	**0.0026**
Starvation	10	D42	3.2	♂	1	40	20.9	29	39	22.9	33	40	26.1	38	13.8	15.2	**0.0487**
Starvation	10	D42	3.2	♂	2	40	11.7	16	40	9.9	16	40	12.4	16	5.8	0.0	**0.0006**
Starvation	10	D42	3.2	♂	3	40	17.5	20	40	17.4	20	40	16.8	20	-3.5	0.0	0.0887
Starvation	10	D42	3.2	♂	4	40	13.1	20	40	10.6	16	40	11.7	16	0.0	0.0	
Starvation	10	D42	3.2	♂	5	40	18.1	20	39	15.7	20	40	17.9	20	0.0	0.0	
Starvation	10	D42	3.2	♂	6	39	19.6	24	40	16.9	20	40	20.2	24	3.1	0.0	0.2151
Starvation	10	D42	10.1	♀	1	36	26.9	47	33	26.7	47	40	25.8	33	-3.4	-29.8	0.5199
Starvation	10	D42	10.1	♀	2	40	33.6	40	40	36.1	40	40	27.4	40	-18.3	0.0	**0.0002**
Starvation	10	D42	10.1	♀	3	39	24.0	32	40	25.1	32	40	25.8	32	2.8	0.0	**0.0406**
Starvation	10	D42	10.1	♀	4	39	28.2	40	40	28.9	40	40	23.0	27	-18.6	-32.5	**<0.0001**
Starvation	10	D42	10.1	♀	5	40	28.9	36	40	29.4	36	39	25.8	32	-10.6	-11.1	**0.0004**
Starvation	10	D42	10.1	♀	6	40	30.6	40	40	29.8	36	40	29.9	40	0.0	0.0	
Starvation	10	D42	10.1	♂	1	36	19.8	22	39	21.0	22	39	24.5	33	16.5	50.0	**<0.0001**
Starvation	10	D42	10.1	♂	2	40	11.6	15	40	10.6	15	40	11.3	15	0.0	0.0	
Starvation	10	D42	10.1	♂	3	40	18.5	24	40	17.4	20	40	19.9	24	7.6	0.0	0.0559
Starvation	10	D42	10.1	♂	4	40	13.3	21	40	11.9	21	40	15.6	21	17.5	0.0	**0.0031**
Starvation	10	D42	10.1	♂	5	27	16.7	24	39	15.7	20	40	22.2	28	32.9	16.7	**<0.0001**
Starvation	10	D42	10.1	♂	6	41	20.0	28	40	16.9	20	40	24.4	32	22.0	14.3	**<0.0001**
Starvation	10	D42	3.1	♀	1	37	32.7	46	35	37.7	50	40	37.9	58	0.6	16.0	**0.0082**
Starvation	10	D42	3.1	♀	2	39	22.8	28	40	25.1	32	38	22.5	28	-1.1	0.0	**0.0008**
Starvation	10	D42	3.1	♀	3	40	26.2	32	40	29.4	36	39	24.4	28	-6.8	-12.5	**0.0323**
Starvation	10	D42	3.1	♀	4	40	25.8	32	40	29.8	36	40	28.1	32	0.0	0.0	
Starvation	10	D42	3.1	♂	1	40	18.6	26	40	18.3	26	40	21.1	30	13.7	15.4	**0.0003**
Starvation	10	D42	3.1	♂	2	40	16.5	20	40	17.4	20	40	15.4	16	-6.5	-20.0	**0.0028**
Starvation	10	D42	3.1	♂	3	40	16.9	20	39	15.7	20	40	16.3	20	0.0	0.0	
Starvation	10	D42	3.1	♂	4	40	16.0	20	40	16.9	20	40	18.2	20	7.9	0.0	0.0572
Starvation	10	D42	4.4	♀	1	40	29.8	35	40	24.3	35	40	19.9	25	-18.0	-28.6	**<0.0001**
Starvation	10	D42	4.4	♀	2	40	30.5	40	40	36.1	40	40	25.4	27	-16.8	-32.5	**0.0006**
Starvation	10	D42	4.4	♀	3	36	53.6	59	39	51.3	72	40	55.5	72	3.5	0.0	0.0973
Starvation	10	D42	4.4	♀	4	40	26.5	32	40	25.1	32	40	24.8	32	-1.3	0.0	0.0686
Starvation	10	D42	4.4	♀	5	41	32.9	40	40	28.9	40	39	23.7	40	-18.2	0.0	**<0.0001**
Starvation	10	D42	4.4	♀	6	40	28.5	36	40	29.4	36	40	27.8	32	-2.5	-11.1	**0.0206**
Starvation	10	D42	4.4	♀	7	40	31.2	40	40	29.8	36	30	28.9	32	-2.9	-11.1	**0.0065**
Starvation	10	D42	4.4	♂	1	40	19.5	25	40	19.9	25	40	19.8	25	0.0	0.0	
Starvation	10	D42	4.4	♂	2	40	11.6	15	40	10.6	15	40	10.0	10	-5.9	-33.3	**0.0217**
Starvation	10	D42	4.4	♂	3	37	27.2	34	36	25.5	34	39	27.8	34	2.3	0.0	0.0617
Starvation	10	D42	4.4	♂	4	40	14.5	16	40	17.4	20	40	15.3	20	0.0	0.0	
Starvation	10	D42	4.4	♂	5	40	12.4	15	40	11.9	21	39	10.9	15	-8.6	0.0	**0.0082**
Starvation	10	D42	4.4	♂	6	40	14.6	16	39	15.7	20	41	16.1	20	2.6	0.0	**0.0007**
Starvation	10	D42	4.4	♂	7	40	15.7	16	40	16.9	20	40	16.4	20	0.0	0.0	

Notes: 1 Experimental replicate

2 Day with 95% mortality

Table S3: Longevity data and analysis of Hsp70 overexpression. Mean: average survival in days, n: number of flies in the replicate, P: highest Log-rank test value, 1: replicate number, 2: 95% mortality (maximum lifespan)

Table S3

Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
				n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
daGal4	2.1	♀	1	115	55.4	78	110	66.3	95	120	66.7	81	0.6	0.0	**0.0013**
daGal4	2.1	♀	2	114	66.2	94	95	70.8	90	90	75.7	90	6.9	0.0	0.7069
daGal4	2.1	♀	3	112	58.9	81	119	71.5	88	117	62.1	74	0.0	-8.6	
daGal4	2.1	♀	4	118	60.2	87	113	70.6	87	118	65.9	80	0.0	-8.0	
daGal4	2.1	♀	5	90	63.1	76	102	69.3	87	103	65.9	80	0.0	0.0	
daGal4	2.1	♀	6	111	58.8	72	109	71.9	86	111	64.3	79	0.0	0.0	
daGal4	2.1	♂	1	117	49.4	75	113	50.6	75	116	45.6	51	-7.7	-32.0	**<0.0001**
daGal4	2.1	♂	2	113	52.3	63	107	53.9	70	118	47.1	60	-9.9	-4.8	**<0.0001**
daGal4	2.1	♂	3	113	56.0	67	118	64.2	81	117	50.5	57	-9.7	-14.9	**<0.0001**
daGal4	2.1	♂	4	112	57.0	69	117	60.5	80	116	52.0	66	-8.9	-4.3	**<0.0001**
daGal4	2.1	♂	5	102	55.5	69	100	60.5	73	117	52.9	66	-4.7	-4.3	**0.0035**
daGal4	2.1	♂	6	117	54.0	68	107	59.7	72	112	54.5	68	0.0	0.0	
daGal4	4.3	♀	1	121	53.9	66	102	71.8	94	122	66.0	85	0.0	0.0	
daGal4	4.3	♀	2	111	54.2	69	107	70.3	97	100	68.0	87	0.0	0.0	
daGal4	4.3	♀	3	112	54.8	70	119	71.5	88	118	56.7	74	0.0	0.0	
daGal4	4.3	♀	4	119	51.0	66	113	70.6	87	117	62.5	76	0.0	0.0	
daGal4	4.3	♀	5	119	48.2	62	102	69.3	87	109	54.4	66	0.0	0.0	
daGal4	4.3	♀	6	122	51.7	68	109	71.9	86	117	61.2	79	0.0	0.0	
daGal4	4.3	♂	1	120	48.0	59	114	59.1	85	118	41.9	46	-12.7	-22.0	**<0.0001**
daGal4	4.3	♂	2	120	48.2	63	115	57.7	76	115	41.5	45	-13.9	-28.6	**<0.0001**
daGal4	4.3	♂	3	118	46.8	53	118	64.2	81	119	40.3	49	-14.0	-7.5	**<0.0001**
daGal4	4.3	♂	4	113	43.0	52	117	60.5	80	112	42.0	52	-2.3	0.0	0.6680
daGal4	4.3	♂	5	118	48.1	56	100	60.5	73	107	38.7	52	-19.5	-7.1	**<0.0001**
daGal4	4.3	♂	6	120	46.2	55	107	59.7	72	119	38.0	47	-17.7	-14.5	**<0.0001**
daGal4	9.1	♀	1	119	54.6	74	119	71.5	88	115	53.9	67	-1.2	-9.5	0.9776
daGal4	9.1	♀	2	118	56.2	73	113	70.6	87	120	55.3	69	-1.6	-5.5	0.5707
daGal4	9.1	♀	3	126	52.0	66	102	69.3	87	120	52.5	66	0.0	0.0	
daGal4	9.1	♀	4	118	54.8	75	109	71.9	86	116	57.0	72	0.0	-4.0	
daGal4	9.1	♂	1	116	47.0	60	118	64.2	81	116	46.4	53	-1.2	-11.7	0.2053
daGal4	9.1	♂	2	117	48.6	59	117	60.5	80	120	44.9	56	-7.8	-5.1	**<0.0001**
daGal4	9.1	♂	3	116	44.6	56	100	60.5	73	126	41.5	52	-7.1	-7.1	**0.0019**
daGal4	9.1	♂	4	115	50.5	61	107	59.7	72	116	44.1	55	-12.6	-9.8	**<0.0001**
daGal4	3.2	♀	1	118	70.5	93	116	55.8	79	117	58.0	62	0.0	-21.5	
daGal4	3.2	♀	2	117	74.2	101	116	61.7	88	115	58.8	66	-4.7	-25.0	**<0.0001**
daGal4	3.2	♀	3	113	69.5	81	119	71.5	88	119	50.5	57	-27.2	-29.6	**<0.0001**
daGal4	3.2	♀	4	118	74.0	87	113	70.6	87	122	51.3	56	-27.4	-35.6	**<0.0001**
daGal4	3.2	♀	5	110	67.2	87	102	69.3	87	115	52.4	56	-22.0	-35.6	**<0.0001**
daGal4	3.2	♀	6	115	71.0	86	109	71.9	86	118	50.6	55	-28.7	-36.0	**<0.0001**
daGal4	3.2	♂	1	116	62.4	84	118	48.1	62	118	42.6	51	-11.4	-17.7	**<0.0001**
daGal4	3.2	♂	2	103	63.5	88	121	50.0	66	119	43.4	52	-13.2	-21.2	**<0.0001**
daGal4	3.2	♂	3	116	62.3	81	118	64.2	81	116	39.9	53	-35.9	-34.6	**<0.0001**
daGal4	3.2	♂	4	118	65.0	83	117	60.5	80	120	42.4	52	-29.9	-35.0	**<0.0001**
daGal4	3.2	♂	5	110	60.5	73	100	60.5	73	115	35.9	41	-40.7	-43.8	**<0.0001**
daGal4	3.2	♂	6	116	64.1	79	107	59.7	72	117	39.9	51	-33.2	-29.2	**<0.0001**
daGal4	10.1	♀	1	117	68.4	88	119	71.5	88	121	62.3	81	-8.9	-8.0	**<0.0001**
daGal4	10.1	♀	2	110	63.2	87	113	70.6	87	115	65.2	80	0.0	-8.0	
daGal4	10.1	♀	3	111	63.0	80	102	69.3	87	97	61.0	80	-3.2	0.0	0.3652
daGal4	10.1	♀	4	116	64.1	86	109	71.9	86	115	55.9	72	-12.8	-16.3	**<0.0001**
daGal4	10.1	♂	1	113	69.3	84	118	64.2	81	119	58.1	67	-9.6	-17.3	**<0.0001**
daGal4	10.1	♂	2	121	67.3	83	117	60.5	80	118	57.7	69	-4.5	-13.8	0.0720
daGal4	10.1	♂	3	96	64.9	80	100	60.5	73	109	57.5	66	-5.0	-9.6	**<0.0001**
daGal4	10.1	♂	4	122	63.0	82	107	59.7	72	119	58.6	68	-1.8	-5.6	**0.0248**

Table S3

Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
				n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
daGal4	3.1	♀	1	117	60.5	87	117	57.0	84	118	73.5	91	21.6	4.6	**<0.0001**
daGal4	3.1	♀	2	113	63.8	87	116	69.1	94	119	75.2	94	8.8	0.0	0.2671
daGal4	3.1	♀	3	118	83.7	106	102	71.8	94	108	73.5	91	0.0	-3.2	
daGal4	3.1	♀	4	120	78.2	105	107	70.3	97	121	69.3	84	-1.4	-13.4	0.5209
daGal4	3.1	♀	5	110	68.0	84	119	71.5	88	115	60.5	81	-11.0	-3.6	**0.0003**
daGal4	3.1	♀	6	118	58.4	80	113	70.6	87	110	61.3	80	0.0	0.0	
daGal4	3.1	♀	7	94	62.1	73	102	69.3	87	101	64.3	83	0.0	0.0	
daGal4	3.1	♀	8	102	54.9	75	109	71.9	86	118	57.3	75	0.0	0.0	
daGal4	3.1	♂	1	116	48.6	78	122	48.6	68	121	46.6	63	-4.0	-7.4	**0.0275**
daGal4	3.1	♂	2	115	45.6	57	116	56.7	80	119	47.6	60	0.0	0.0	
daGal4	3.1	♂	3	116	72.8	98	114	59.1	85	122	62.3	77	0.0	-9.4	
daGal4	3.1	♂	4	121	67.8	87	115	57.7	76	118	56.9	72	-1.4	-5.3	0.9662
daGal4	3.1	♂	5	115	66.3	81	118	64.2	81	116	53.8	67	-16.1	-17.3	**<0.0001**
daGal4	3.1	♂	6	119	61.2	80	117	60.5	80	119	54.0	66	-10.6	-17.5	**<0.0001**
daGal4	3.1	♂	7	102	59.3	80	100	60.5	73	117	51.5	62	-13.1	-15.1	**<0.0001**
daGal4	3.1	♂	8	117	59.9	79	107	59.7	72	119	51.6	65	-13.5	-9.7	**<0.0001**
daGal4	4.4	♀	1	118	63.7	81	119	71.5	88	119	56.8	74	-10.8	-8.6	**<0.0001**
daGal4	4.4	♀	2	120	55.7	80	113	70.6	87	86	62.1	76	0.0	-5.0	
daGal4	4.4	♀	3	97	62.9	80	102	69.3	87	112	54.3	73	-13.6	-8.8	**<0.0001**
daGal4	4.4	♀	4	119	52.7	79	109	71.9	86	113	59.8	79	0.0	0.0	
daGal4	4.4	♂	1	117	61.2	74	118	64.2	81	117	50.5	63	-17.4	-14.9	**<0.0001**
daGal4	4.4	♂	2	119	53.6	69	117	60.5	80	114	52.3	66	-2.3	-4.3	0.3705
daGal4	4.4	♂	3	108	56.1	66	100	60.5	73	122	51.0	56	-9.1	-15.2	**<0.0001**
daGal4	4.4	♂	4	117	54.1	68	107	59.7	72	117	53.4	65	-1.3	-4.4	**0.0029**
DJ694	2.1	♀	1	115	55.4	78	112	72.7	88	120	79.2	91	9.0	3.4	**<0.0001**
DJ694	2.1	♀	2	114	66.2	94	114	76.3	94	120	85.8	94	12.4	0.0	**<0.0001**
DJ694	2.1	♀	3	120	65.1	75	119	74.0	83	119	74.0	83	0.1	0.0	0.2063
DJ694	2.1	♀	4	118	58.0	71	117	68.3	82	120	68.0	82	0.0	0.0	
DJ694	2.1	♀	5	119	62.2	82	118	72.3	90	120	68.5	82	0.0	0.0	
DJ694	2.1	♀	6	121	57.2	70	115	69.3	81	120	67.4	77	0.0	0.0	
DJ694	2.1	♂	1	117	49.4	75	111	51.3	64	119	61.0	75	19.0	0.0	**<0.0001**
DJ694	2.1	♂	2	113	52.3	63	113	66.0	74	115	60.6	74	0.0	0.0	
DJ694	2.1	♂	3	121	53.5	68	121	58.2	68	120	54.8	72	0.0	5.9	
DJ694	2.1	♂	4	119	51.5	64	122	60.9	78	119	57.0	71	0.0	0.0	
DJ694	2.1	♂	5	119	47.9	61	119	60.0	74	118	57.2	71	0.0	0.0	
DJ694	2.1	♂	6	122	51.8	70	121	62.6	77	121	56.0	63	0.0	-10.0	
DJ694	4.3	♀	1	121	53.9	66	118	80.7	101	123	71.3	85	0.0	0.0	
DJ694	4.3	♀	2	111	54.2	69	90	75.2	97	109	72.3	84	0.0	0.0	
DJ694	4.3	♀	3	119	47.4	58	119	74.0	83	116	58.6	68	0.0	0.0	
DJ694	4.3	♀	4	114	42.4	57	117	68.3	82	120	52.8	71	0.0	0.0	
DJ694	4.3	♀	5	120	44.4	57	118	72.3	90	118	59.0	71	0.0	0.0	
DJ694	4.3	♀	6	115	41.5	59	115	69.3	81	118	55.0	77	0.0	0.0	
DJ694	4.3	♂	1	120	48.0	59	114	75.5	91	114	49.1	63	0.0	0.0	
DJ694	4.3	♂	2	120	48.2	63	105	74.6	97	116	51.7	65	0.0	0.0	
DJ694	4.3	♂	3	122	34.0	44	121	58.2	68	120	42.2	51	0.0	0.0	
DJ694	4.3	♂	4	111	36.6	50	122	60.9	78	120	39.9	50	0.0	0.0	
DJ694	4.3	♂	5	113	32.1	40	119	60.0	74	123	39.4	43	0.0	0.0	
DJ694	4.3	♂	6	125	35.5	49	121	62.6	77	120	41.8	49	0.0	0.0	
DJ694	9.1	♀	1	118	52.5	68	119	74.0	83	127	63.6	83	0.0	0.0	
DJ694	9.1	♀	2	113	47.6	67	117	68.3	82	123	59.4	78	0.0	0.0	
DJ694	9.1	♀	3	120	49.8	67	118	72.3	90	118	56.5	82	0.0	0.0	
DJ694	9.1	♀	4	111	47.5	66	115	69.3	81	115	60.9	77	0.0	0.0	

Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
				n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
DJ694	9.1	♂	1	118	46.8	58	121	58.2	68	118	53.4	65	0.0	0.0	
DJ694	9.1	♂	2	120	45.3	57	122	60.9	78	121	52.0	61	0.0	0.0	
DJ694	9.1	♂	3	119	39.1	50	119	60.0	74	122	51.0	57	0.0	0.0	
DJ694	9.1	♂	4	123	44.9	56	121	62.6	77	120	49.0	59	0.0	0.0	
DJ694	3.2	♀	1	118	70.5	93	118	64.3	84	120	82.6	97	17.2	4.3	**<0.0001**
DJ694	3.2	♀	2	117	74.2	101	118	61.8	81	119	87.6	101	18.1	0.0	**0.0001**
DJ694	3.2	♀	3	128	67.6	83	119	74.0	83	117	75.4	83	1.9	0.0	0.8979
DJ694	3.2	♀	4	124	62.9	78	117	68.3	82	118	69.1	82	1.2	0.0	0.1114
DJ694	3.2	♀	5	119	60.7	74	118	72.3	90	118	69.7	82	0.0	0.0	
DJ694	3.2	♀	6	120	60.3	81	115	69.3	81	121	68.1	85	0.0	4.9	
DJ694	3.2	♂	1	116	62.4	84	118	54.4	69	117	74.1	91	18.8	8.3	**<0.0001**
DJ694	3.2	♂	2	103	63.5	88	119	54.5	66	117	85.4	94	34.5	6.8	**<0.0001**
DJ694	3.2	♂	3	120	53.0	68	121	58.2	68	119	64.9	83	11.5	22.1	**<0.0001**
DJ694	3.2	♂	4	123	58.6	74	122	60.9	78	124	64.2	74	5.5	0.0	0.0851
DJ694	3.2	♂	5	120	53.6	61	119	60.0	74	121	61.9	82	3.1	10.8	0.0987
DJ694	3.2	♂	6	126	64.4	81	121	62.6	77	121	66.2	85	2.7	4.9	0.5922
DJ694	10.1	♀	1	119	70.0	83	119	74.0	83	119	79.6	91	7.6	9.6	**<0.0001**
DJ694	10.1	♀	2	121	65.9	82	117	68.3	82	121	72.8	82	6.5	0.0	0.1117
DJ694	10.1	♀	3	118	62.7	82	118	72.3	90	118	75.0	82	3.7	0.0	0.7199
DJ694	10.1	♀	4	120	67.8	85	115	69.3	81	123	72.8	81	5.0	0.0	**0.0290**
DJ694	10.1	♂	1	120	58.2	72	121	58.2	68	119	70.3	83	20.8	15.3	**<0.0001**
DJ694	10.1	♂	2	118	59.6	78	122	60.9	78	121	70.7	82	16.2	5.1	**<0.0001**
DJ694	10.1	♂	3	125	58.4	71	119	60.0	74	113	69.9	82	16.4	10.8	**<0.0001**
DJ694	10.1	♂	4	124	64.9	81	121	62.6	77	122	71.2	85	9.6	4.9	**<0.0001**
DJ694	3.1	♀	1	117	60.5	87	116	59.7	84	119	68.5	91	13.3	4.6	**0.0029**
DJ694	3.1	♀	2	113	63.8	87	119	72.2	91	120	71.4	94	0.0	3.3	
DJ694	3.1	♀	3	118	83.7	106	118	80.7	101	117	77.7	98	-3.7	-3.0	0.1472
DJ694	3.1	♀	4	120	78.2	105	90	75.2	97	121	76.0	93	0.0	-4.1	
DJ694	3.1	♀	5	119	61.2	72	119	74.0	83	116	67.4	83	0.0	0.0	
DJ694	3.1	♀	6	107	50.4	67	117	68.3	82	120	55.8	71	0.0	0.0	
DJ694	3.1	♀	7	112	51.7	67	118	72.3	90	121	60.8	82	0.0	0.0	
DJ694	3.1	♀	8	119	57.2	77	115	69.3	81	121	58.3	70	0.0	-9.1	
DJ694	3.1	♂	1	116	48.6	78	119	54.7	71	118	59.1	84	8.1	7.7	**0.0004**
DJ694	3.1	♂	2	115	45.6	57	116	63.3	84	119	70.5	100	11.4	19.0	**<0.0001**
DJ694	3.1	♂	3	116	72.8	98	114	75.5	91	111	70.7	85	-2.9	-6.6	**0.0170**
DJ694	3.1	♂	4	121	67.8	87	105	74.6	97	122	77.0	97	3.3	0.0	**0.0711**
DJ694	3.1	♂	5	120	62.7	72	121	58.2	68	118	61.5	75	0.0	4.2	
DJ694	3.1	♂	6	123	52.7	67	122	60.9	78	121	56.9	78	0.0	0.0	
DJ694	3.1	♂	7	119	50.6	64	119	60.0	74	119	61.0	71	1.6	0.0	0.2226
DJ694	3.1	♂	8	126	50.9	70	121	62.6	77	118	61.3	81	0.0	5.2	
DJ694	4.4	♀	1	119	51.8	75	119	74.0	83	122	65.4	83	0.0	0.0	
DJ694	4.4	♀	2	117	48.4	71	117	68.3	82	122	60.5	82	0.0	0.0	
DJ694	4.4	♀	3	118	45.5	64	118	72.3	90	118	62.0	82	0.0	0.0	
DJ694	4.4	♀	4	113	46.1	66	115	69.3	81	120	61.7	81	0.0	0.0	
DJ694	4.4	♂	1	121	44.1	61	121	58.2	68	124	53.2	65	0.0	0.0	
DJ694	4.4	♂	2	121	49.7	61	122	60.9	78	120	55.8	71	0.0	0.0	
DJ694	4.4	♂	3	116	45.1	57	119	60.0	74	123	53.0	61	0.0	0.0	
DJ694	4.4	♂	4	118	49.9	63	121	62.6	77	120	59.5	77	0.0	0.0	
D42	2.1	♀	1	115	55.4	78	103	73.0	95	120	75.7	88	3.7	0.0	0.0934
D42	2.1	♀	2	114	66.2	94	100	76.2	97	119	85.5	97	12.2	0.0	0.3860
D42	2.1	♀	3	116	71.2	92	104	59.1	92	107	76.4	92	7.4	0.0	**<0.0001**
D42	2.1	♀	4	121	73.9	90	101	61.1	94	118	84.5	104	14.4	10.6	**<0.0001**

Table S3

Gal4	UAS	Sex	Rep[1]	UAS n	UAS Mean	UAS Max[2]	Gal4 n	Gal4 Mean	Gal4 Max[2]	Gal4+UAS n	Gal4+UAS Mean	Gal4+UAS Max[2]	% Ext Mean	% Ext Max[2]	P
D42	2.1	♀	5	118	71.4	87	117	61.9	84	110	64.4	84	0.0	0.0	
D42	2.1	♀	6	117	64.4	72	114	67.1	90	116	64.2	83	-0.3	0.0	0.0644
D42	2.1	♀	7	116	62.2	76	125	63.7	83	129	67.8	83	6.5	0.0	0.3398
D42	2.1	♀	8	116	66.4	82	117	64.7	75	117	65.5	82	0.0	0.0	
D42	2.1	♂	1	117	49.4	75	103	50.3	68	112	53.9	71	7.1	0.0	0.1028
D42	2.1	♂	2	113	52.3	63	109	53.4	94	117	58.0	74	8.6	0.0	0.1147
D42	2.1	♂	3	117	56.0	71	108	54.2	78	125	60.4	78	7.7	0.0	**0.0038**
D42	2.1	♂	4	115	67.0	87	111	63.2	84	122	66.3	84	0.0	0.0	
D42	2.1	♂	5	116	61.3	77	124	61.5	70	108	57.1	70	-7.0	0.0	**<0.0001**
D42	2.1	♂	6	125	61.3	76	117	61.9	76	102	55.7	72	-9.1	-5.3	**<0.0001**
D42	2.1	♂	7	114	61.1	76	120	58.7	76	114	57.6	72	-1.9	-5.3	0.2165
D42	2.1	♂	8	126	55.7	71	120	59.8	68	112	56.1	68	0.0	0.0	
D42	4.3	♀	1	121	53.9	66	120	82.1	98	116	80.1	98	0.0	0.0	
D42	4.3	♀	2	111	54.2	69	99	78.7	97	120	77.7	97	0.0	0.0	
D42	4.3	♀	3	119	51.2	74	104	59.1	92	123	66.8	88	13.0	0.0	0.5597
D42	4.3	♀	4	112	57.1	80	101	61.1	94	93	67.7	90	10.7	0.0	**0.0002**
D42	4.3	♀	5	119	51.4	63	117	61.9	84	110	58.3	70	0.0	0.0	
D42	4.3	♀	6	120	50.1	65	114	67.1	90	119	58.3	72	0.0	0.0	
D42	4.3	♀	7	113	47.8	58	125	63.7	83	116	58.6	76	0.0	0.0	
D42	4.3	♀	8	120	49.1	64	117	64.7	75	116	57.1	75	0.0	0.0	
D42	4.3	♂	1	120	48.0	59	113	67.6	98	113	38.3	50	-20.1	-15.3	**<0.0001**
D42	4.3	♂	2	120	48.2	63	118	66.4	93	106	35.9	45	-25.4	-28.6	**<0.0001**
D42	4.3	♂	3	121	46.3	57	108	54.2	78	122	45.3	60	-2.2	0.0	0.9349
D42	4.3	♂	4	95	49.5	59	111	63.2	84	106	41.1	66	-17.0	0.0	**0.0001**
D42	4.3	♂	5	124	47.8	56	124	61.5	70	114	51.6	70	0.0	0.0	
D42	4.3	♂	6	122	41.7	55	117	61.9	76	117	42.7	55	0.0	0.0	
D42	4.3	♂	7	119	40.5	55	120	58.7	76	109	43.5	55	0.0	0.0	
D42	4.3	♂	8	119	34.4	44	120	59.8	68	110	38.9	54	0.0	0.0	
D42	9.1	♀	1	129	57.1	81	104	59.1	92	116	62.8	95	6.2	3.3	0.592
D42	9.1	♀	2	115	70.6	90	101	61.1	94	117	84.2	101	19.3	7.4	**<0.0001**
D42	9.1	♀	3	120	54.8	77	117	61.9	84	115	64.3	77	3.9	0.0	0.1598
D42	9.1	♀	4	115	49.7	62	114	67.1	90	118	58.7	72	0.0	0.0	
D42	9.1	♀	5	118	55.9	69	125	63.7	83	122	60.3	76	0.0	0.0	
D42	9.1	♀	6	118	51.5	68	117	64.7	75	119	55.7	75	0.0	0.0	
D42	9.1	♂	1	124	43.1	57	108	54.2	78	120	51.8	71	0.0	0.0	
D42	9.1	♂	2	123	53.9	73	111	63.2	84	121	69.2	90	9.5	7.1	0.1051
D42	9.1	♂	3	120	48.5	59	124	61.5	70	115	55.6	66	0.0	0.0	
D42	9.1	♂	4	109	45.4	58	117	61.9	76	111	51.3	65	0.0	0.0	
D42	9.1	♂	5	116	44.2	55	120	58.7	76	120	51.9	62	0.0	0.0	
D42	9.1	♂	6	121	47.8	61	120	59.8	68	120	48.5	61	0.0	0.0	
D42	3.2	♀	1	118	70.5	93	119	63.4	84	112	81.2	91	15.2	0.0	**0.0013**
D42	3.2	♀	2	117	74.2	101	120	66.0	88	118	83.0	94	11.9	0.0	0.7125
D42	3.2	♀	3	122	70.3	84	117	61.9	84	100	69.4	84	0.0	0.0	
D42	3.2	♀	4	116	60.1	72	114	67.1	90	119	70.4	83	5.0	0.0	0.4230
D42	3.2	♀	5	116	62.0	83	125	63.7	83	122	68.1	83	7.0	0.0	0.8400
D42	3.2	♀	6	119	62.0	75	117	64.7	75	119	62.6	75	0.0	0.0	
D42	3.2	♂	1	116	62.4	84	120	56.0	69	116	77.3	91	24.0	8.3	**<0.0001**
D42	3.2	♂	2	103	63.5	88	115	57.7	73	119	79.4	94	25.0	6.8	**<0.0001**
D42	3.2	♂	3	120	58.0	70	124	61.5	70	101	61.7	77	0.4	10.0	0.4165
D42	3.2	♂	4	120	55.5	69	117	61.9	76	125	65.2	83	5.2	9.2	**<0.0001**
D42	3.2	♂	5	125	53.7	69	120	58.7	76	114	57.0	76	0.0	0.0	
D42	3.2	♂	6	117	57.1	71	120	59.8	68	126	63.9	75	6.9	5.6	**<0.0001**

Table S3

Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
				n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
D42	10.1	♀	1	120	57.6	77	106	77.9	95	116	78.9	88	1.2	0.0	**0.0114**
D42	10.1	♀	2	109	66.6	84	106	74.0	91	110	71.6	88	0.0	0.0	
D42	10.1	♀	3	119	68.5	84	117	61.9	84	115	67.8	84	0.0	0.0	
D42	10.1	♀	4	105	66.8	86	114	67.1	90	117	70.2	90	4.6	0.0	**0.0329**
D42	10.1	♀	5	123	63.9	83	125	63.7	83	114	59.4	76	-6.8	-8.4	**0.0016**
D42	10.1	♀	6	124	59.5	75	117	64.7	75	110	65.6	89	1.3	18.7	**0.0262**
D42	10.1	♂	1	121	57.2	74	119	59.2	81	115	67.9	81	14.6	0.0	**0.0053**
D42	10.1	♂	2	124	56.8	67	116	60.4	88	123	68.7	81	13.8	0.0	0.0531
D42	10.1	♂	3	120	66.4	77	124	61.5	70	117	71.1	84	7.1	9.1	**<0.0001**
D42	10.1	♂	4	122	65.1	86	117	61.9	76	109	75.7	98	16.4	14.0	**<0.0001**
D42	10.1	♂	5	115	59.2	76	120	58.7	76	117	61.3	76	3.5	0.0	0.3541
D42	10.1	♂	6	127	62.7	89	120	59.8	68	115	68.3	82	8.9	0.0	**0.0116**
D42	3.1	♀	1	117	60.5	87	117	66.9	87	119	74.4	91	11.2	4.6	**<0.0001**
D42	3.1	♀	2	113	63.8	87	112	74.0	97	120	79.4	100	7.3	3.1	0.0642
D42	3.1	♀	3	118	83.7	106	120	82.1	98	119	89.0	106	6.4	0.0	0.6531
D42	3.1	♀	4	120	78.2	105	99	78.7	97	114	84.7	97	7.5	0.0	**0.0105**
D42	3.1	♀	5	118	67.5	80	117	61.9	84	128	67.2	87	0.0	3.6	
D42	3.1	♀	6	127	62.0	72	114	67.1	90	122	70.6	98	5.2	8.9	**0.0095**
D42	3.1	♀	7	125	61.8	76	125	63.7	83	119	71.6	83	12.5	0.0	**0.0225**
D42	3.1	♀	8	110	59.2	75	117	64.7	75	119	69.3	89	7.1	18.7	**0.0003**
D42	3.1	♂	1	116	48.6	78	120	55.2	74	118	54.2	68	0.0	-8.1	
D42	3.1	♂	2	115	45.6	57	115	60.7	73	118	66.0	91	8.7	24.7	**<0.0001**
D42	3.1	♂	3	116	72.8	98	113	67.6	98	113	66.5	77	-1.6	-21.4	0.1023
D42	3.1	♂	4	121	67.8	87	118	66.4	93	104	69.0	84	1.6	-3.4	0.8434
D42	3.1	♂	5	108	60.4	70	124	61.5	70	120	58.1	73	-3.9	4.3	**0.0055**
D42	3.1	♂	6	125	61.2	76	117	61.9	76	117	62.4	76	0.7	0.0	0.2374
D42	3.1	♂	7	117	59.7	76	120	58.7	76	120	58.6	72	-0.1	-5.3	0.3712
D42	3.1	♂	8	118	56.1	75	120	59.8	68	113	60.1	75	0.5	0.0	**0.0246**
D42	4.4	♀	1	117	59.8	88	104	59.1	92	115	75.0	99	25.4	7.6	**<0.0001**
D42	4.4	♀	2	115	67.8	97	101	61.1	94	105	80.6	104	18.9	7.2	**<0.0001**
D42	4.4	♀	3	137	61.8	77	117	61.9	84	117	60.4	87	-2.2	3.6	0.9710
D42	4.4	♀	4	119	53.9	69	114	67.1	90	117	63.1	83	0.0	0.0	
D42	4.4	♀	5	127	56.3	76	125	63.7	83	118	58.2	76	0.0	0.0	
D42	4.4	♀	6	123	56.6	75	117	64.7	75	109	62.0	82	0.0	9.3	
D42	4.4	♂	1	123	54.7	78	108	54.2	78	125	65.2	85	19.1	9.0	**<0.0001**
D42	4.4	♂	2	120	68.5	84	111	63.2	84	123	70.8	90	3.4	7.1	**0.0278**
D42	4.4	♂	3	113	55.0	70	124	61.5	70	112	54.7	70	-0.6	0.0	0.5016
D42	4.4	♂	4	109	52.3	69	117	61.9	76	119	50.8	69	-2.9	0.0	0.2690
D42	4.4	♂	5	117	52.6	69	120	58.7	76	116	56.5	76	0.0	0.0	
D42	4.4	♂	6	121	53.0	68	120	59.8	68	109	53.3	68	0.0	0.0	

Notes: 1 Experimental replicate

2 Day with 95% mortality

Table S4: Longevity data and analysis of Hsp70 RNAi. Mean: average survival in days, n: number of flies in the replicate, P: highest Log-rank test value, 1: replicate number, 2: 95% mortality (maximum lifespan)

Table S4

Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
				n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
daGal4	R1.26	♀	1	120	50.0	72	117	76.4	92	118	56.0	81	0.0	0.0	
daGal4	R1.26	♀	2	116	52.9	84	119	79.6	98	118	55.0	91	0.0	0.0	
daGal4	R1.26	♀	3	116	52.9	84	119	79.6	98	118	55.0	91	0.0	0.0	
daGal4	R1.26	♂	1	120	48.8	75	107	62.1	83	120	50.6	75	0.0	-0.5	
daGal4	R1.26	♂	2	122	48.9	67	115	58.0	67	119	51.3	67	0.0	0.0	
daGal4	R1.26	♂	3	122	48.9	67	115	58.0	67	119	51.3	67	0.0	0.0	
daGal4	R3.12	♀	1	119	55.8	79	116	55.8	79	121	67.9	91	21.5	15.2	**<0.0001**
daGal4	R3.12	♀	2	118	52.5	73	116	61.7	88	118	71.4	94	15.8	6.8	**<0.0001**
daGal4	R3.12	♂	1	120	53.2	69	118	48.1	62	120	60.5	76	13.9	10.1	**<0.0001**
daGal4	R3.12	♂	2	120	50.1	66	121	50.0	66	118	63.1	81	26.1	23.0	**<0.0001**
daGal4	R5.14	♀	1	118	50.5	72	117	76.4	92	119	57.9	92	0.0	0.0	
daGal4	R5.14	♀	2	119	53.3	87	119	79.6	98	120	55.4	98	0.0	0.0	
daGal4	R5.14	♀	3	29	59.0	73	32	75.2	88	29	57.1	77	-3.2	0.0	0.7638
daGal4	R5.14	♂	1	120	36.9	57	107	62.1	83	123	48.6	68	0.0	0.0	
daGal4	R5.14	♂	2	117	42.8	56	115	58.0	67	121	51.3	67	0.0	0.0	
daGal4	R5.14	♂	3	30	38.7	55	29	73.0	88	30	50.6	60	0.0	0.0	
DJ694	R1.26	♀	1	120	50.0	72	120	71.4	85	116	63.9	85	0.0	0.5	
DJ694	R1.26	♀	2	116	52.9	84	122	81.5	98	115	64.8	92	0.0	0.0	
DJ694	R1.26	♀	3	116	52.9	84	122	81.5	98	115	64.8	92	0.0	0.0	
DJ694	R1.26	♂	1	120	48.8	75	116	62.6	68	122	54.4	71	0.0	0.0	
DJ694	R1.26	♂	2	122	48.9	67	116	62.6	67	116	56.7	67	0.0	0.0	
DJ694	R1.26	♂	3	122	48.9	67	116	62.6	67	116	56.7	67	0.0	0.0	
DJ694	R3.12	♀	1	119	55.8	79	118	64.3	84	120	83.1	97	29.4	15.5	**<0.0001**
DJ694	R3.12	♀	2	118	52.5	73	118	61.8	81	120	76.1	97	23.0	19.8	**<0.0001**
DJ694	R3.12	♂	1	120	53.2	69	118	54.4	69	119	73.9	97	35.9	40.6	**<0.0001**
DJ694	R3.12	♂	2	120	50.1	66	119	54.5	66	119	75.4	94	38.3	42.4	<0.0001
DJ694	R5.14	♀	1	118	50.5	72	120	71.4	85	118	65.0	92	0.0	8.2	
DJ694	R5.14	♀	2	119	53.3	87	122	81.5	98	112	64.4	95	0.0	0.0	
DJ694	R5.14	♀	3	29	59.0	73	30	76.9	93	28	77.6	88	0.9	0.0	
DJ694	R5.14	♂	1	120	36.9	57	116	62.6	68	117	49.9	68	0.0	0.0	
DJ694	R5.14	♂	2	117	42.8	56	116	62.6	67	119	53.0	67	0.0	0.0	
DJ694	R5.14	♂	3	30	38.7	55	29	58.0	60	30	47.7	60	0.0	0.0	
D42	R1.17	♀	1	28	73.2	88	30	81.2	95	30	84.6	90	4.2	0.0	0.7681
D42	R1.17	♂	1	29	59.7	76	30	78.3	91	29	64.8	74	0.0	-2.6	
D42	R1.26	♀	1	120	50.0	72	119	75.9	92	115	61.1	76	0.0	0.0	
D42	R1.26	♀	2	116	52.9	84	119	84.7	98	116	72.5	98	0.0	0.0	
D42	R1.26	♀	3	116	52.9	84	119	84.7	98	116	72.5	98	0.0	0.0	
D42	R1.26	♂	1	120	48.8	75	118	59.4	68	115	52.9	68	0.0	0.0	
D42	R1.26	♂	2	122	48.9	67	119	60.2	67	117	54.5	67	0.0	0.0	
D42	R1.26	♂	3	122	48.9	67	119	60.2	67	117	54.5	67	0.0	0.0	
D42	R3.12	♀	1	119	55.8	79	119	63.4	84	118	69.9	91	10.4	8.3	**0.0003**
D42	R3.12	♀	2	118	52.5	73	120	66.0	88	119	71.0	94	7.6	6.8	**0.0145**
D42	R3.12	♀	3	27	69.4	89	30	81.2	95	30	65.9	90	-5.0	0.0	**0.0171**
D42	R3.12	♂	1	120	53.2	69	120	56.0	69	118	61.8	76	10.4	10.1	**<0.0001**
D42	R3.12	♂	2	120	50.1	66	115	57.7	73	118	65.5	88	13.6	20.5	**<0.0001**
D42	R3.12	♂	3	30	70.1	88	30	78.3	91	30	62.4	83	-10.9	-5.5	**0.031**
D42	R5.2	♀	1	30	65.0	86	30	81.2	95	27	59.9	83	-7.9	-3.5	0.2044
D42	R5.2	♂	1	30	73.9	93	30	78.3	91	29	58.7	78	-20.6	-15.2	**<0.0001**
D42	R5.12e	♀	1	30	60.9	83	30	81.2	95	25	55.8	93	-8.3	0.0	0.7819
D42	R5.12e	♂	1	31	59.0	76	30	78.3	91	30	60.1	82	0.0	0.0	

Table S4

Gal4	UAS	Sex	Rep[1]	UAS			Gal4			Gal4 + UAS			% Extension		P
				n	Mean	Max[2]	n	Mean	Max[2]	n	Mean	Max[2]	Mean	Max[2]	
D42	R5.14	♀	1	118	50.5	72	119	75.9	92	114	65.4	82	0.0	0.0	
D42	R5.14	♀	2	119	53.3	87	119	84.7	98	117	64.3	91	0.0	0.0	
D42	R5.14	♀	3	29	59.0	73	32	79.0	88	29	58.9	77	-0.1	0.0	
D42	R5.14	♂	1	120	36.9	57	118	59.4	68	119	46.8	63	0.0	0.0	
D42	R5.14	♂	2	117	42.8	56	119	60.2	67	119	53.9	63	0.0	0.0	
D42	R5.14	♂	3	30	38.7	55	29	58.6	60	29	44.6	50	0.0	-8.3	

Notes: 1 Experimental replicate

2 Day with 95% mortality

Figure 1

Figure 2

Figure 3

Figure 4

△: increase ▲: significant increase ▽: decrease ▼: significant decrease ▷: no change

Figure 5

Figure 6

Figure 7