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Reduced Expression of *Alpha-1,2-mannosidase I* Extends Lifespan in *Drosophila melanogaster* and *Caenorhabditis elegans*

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Summary

Exposure to sub-lethal levels of stress, or hormesis, is a means to induce longevity. By screening for mutations that enhance resistance to multiple stresses, we identified multiple alleles of *alpha-1,2-mannosidase I* (*mas1*) which, in addition to promoting stress resistance, also extend longevity. Longevity enhancement is also observed when *mas1* expression is reduced via RNA interference in both *Drosophila melanogaster* and *Caenorhabditis elegans*. The screen also identified *Edem1* (*Edm1*), a gene downstream of *mas1*, as a modulator of lifespan. Since double mutants for both *mas1* and *Edm1* showed no additional longevity enhancement, it appears that both mutations function within a common pathway to extend lifespan. Molecular analysis of these mutants reveals that the expression of *BiP*, a putative biomarker of dietary restriction (DR), is down-regulated in response to reductions in *mas1* expression. These findings suggest that mutations in *mas1* may extend longevity by modulating dietary restriction.

Keywords

alpha-1,2-mannosidase I; *Edem1*; longevity; dietary restriction; *BiP*; *Drosophila*; *C. elegans*

Introduction

Aging is a complicated process influenced by numerous genetic and environmental factors (Bishop & Guarente 2007). Several mechanisms have been proposed to regulate aging, including the accumulation of damage resulting from reactive oxygen species (ROS), the loss of genomic integrity, as well as the modulation of genetic pathways that control

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reproductive output, the ability to withstand environmental stress, and nutrient utilization (Zhang & Herman 2002; Lombard *et al.* 2005; Partridge *et al.* 2005; Sinclair 2005; Lim *et al.* 2006). During the natural aging process, the increased expression of stress-responsive genes is often observed and in many cases, long-lived individuals display increased resistance to environmental stressors (Vermeulen & Loeschcke 2007; Gems & Partridge 2008; Rattan 2008). In nematodes exposure to sub-lethal levels of stress results in enhanced longevity, a phenomenon referred to as hormesis or a hormetic state (Cypser & Johnson 2002), which also leads to lifespan extension (Gems & Partridge 2008).

In natural populations a common stressor is reduced nutrient availability. Inhibition of nutrient sensing pathways (Alcedo & Kenyon 2004; Libert & Pletcher 2007), as well as nutrient deficiency (due to dietary restriction (DR)), has been shown to extend longevity (Masoro 2005; Mattson 2008). It appears that *Sir2*-mediated life extension is modulated in part, via an endoplasmic reticulum (ER) stress response since changes in *Sir2* activity result in the altered expression of ER stress responsive genes such as *BiP/Grp78* and *abu* (*a*ctivated in *b*locked *u*nfolded protein response), and it has been shown that *abu-1* and *abu-11* can also modulate longevity (Urano *et al.* 2002; Viswanathan *et al.* 2005). *BiP/Grp78* is also down-regulated in the liver of the DR-treated mouse (Heydari *et al.* 1995; Tillman *et al.* 1996; Dhahbi *et al.* 1997) as well as in resveratrol-treated N2 worms, suggesting that it could be a DR biomarker.

Mas1 is expressed in the ER, the Golgi apparatus, and the lysosome. It is a member of the class I glycosidases and is involved in N-linked glycosylation (Herscovics 2001). During the calnexin/calreticulin cycle, Mas1 removes mannose from permanently unfolded proteins, then the de-mannosed proteins are recognized by ER degradation-enhancing alpha-1,2-mannosidase-like protein (Edem), and degraded by ER-associated degradation (ERAD) (Hosokawa *et al.* 2001; Ellgaard & Helenius 2003; Olivari & Molinari 2007). Several lines of evidence indicate that Mas1 is important during the aging process. First, altered N-linked glycosylation affects the maturation rate of proteins that influence longevity, such as insulin and insulin-like growth factor-I receptors (Duronio *et al.* 1988). Furthermore, the expression of *mas1* is decreased in aging and oxidatively-stressed *Drosophila* (Zou *et al.* 2000) as well as in the livers of aging mice and humans (Cingle *et al.* 1996; Zhu *et al.* 2006).

To identify genes involved in lifespan extension, we performed a genetic screen for resistance to multiple stressors which led to the isolation of mutants of *mas1* and *Edem1*. Individuals with targeted mutations in these genes exhibit a longer lifespan. We also show that the mutant phenotype associated with *mas1* mutants can be recapitulated via RNA interference not only in *Drosophila* but also in *C. elegans.* Molecular reduction in *mas1* expression correlates with a reduction in *BiP* expression, suggesting that dietary regimen may be altered. In addition, these mutants show a decreased response to the beneficial effects of DR on lifespan extension. These results provide a novel link between ER-associated degradation and DR-related longevity.

Results

Mutations in *alpha-1,2-mannosidase I* (*mas1*) Extend Lifespan in *Drosophila melanogaster*

Long-lived organisms frequently display enhanced resistance to environmental stressors (Martin *et al.* 1996; Wang *et al.* 2004; Bubliy & Loeschcke 2005). To identify genes involved in lifespan extension, we screened a collection of transposon-mediated mutants (Rorth 1996) for enhanced resistance to paraquat and starvation, two common experimental stressors. Under these conditions, one of the mutant lines, *EP1130*, displayed an increase of about 60% in the mean survival time in both males and females relative to the control strain w^{1118} (Fig. 1A). The long-lived phenotype remained after out-crossing the *EP1130* line to w^{1118} ten times confirming that the longevity was due to the insertion of the transposable element and not due to a background effect. The outcrossed mutant displayed a mean lifespan enhancement of 38% for males and 22% for females, and 15% maximum lifespan extension for both (Fig. 1B, 1C; Supplemental Table 1, 2).

Since the insertion site of the transposon responsible for the mutation was known, the activity of genes in the vicinity of the insertion was monitored via RT-PCR by comparing gene expression in *EP1130* and w^{1118}. Only one transcript from the region was found to be differentially expressed in both young (5-day) and old (30-day) flies (Fig. 1D, row 1). This transcript, named *p1130*, was significantly up-regulated. Blast analysis of the RT-PCR fragment revealed no significant homology to any annotated genes. To obtain the full transcript, 5′ and 3′ RACE was performed resulting in the isolation of a 1.6-kb transcript, *p1130* (Supplemental Fig. 1).

Since the 1.6-kb transcript does not appear to encode either a functional protein or a mature microRNA, the sequence was scanned for potential antisense homology to other genes. A 480-bp sequence (labeled in red in the Supplemental Fig. 1) at the 3′ end of *p1130* is complementary to the 3′ UTR of *CG32684* (*mas1*), suggesting that the expression of this transcript could down-regulate the expression of *mas1*. Indeed, in *EP1130* decreased expression of *mas1* is observed relative to w^{1118} (Fig. 1E, row 1 and 2), while the expression of *CG12643* (another gene in the region used as an internal control), showed no changes between w^{1118} and *EP1130* (Fig. 1E, row 3). PCR results were confirmed by Northern blot, which also revealed reduced *CG32684* expression in *EP1130* compared to w^{1118} (data not shown). Thus *mas1* expression is altered in *EP1130*.

To determine whether the reduced expression of *mas1* extends lifespan, we measured the lifespan of additional strains with insertions in this gene. Two lines with insertions in *mas1*, *EP982* and *EP1628*, showed a similar level of life extension, 36% and 39% respectively (Fig. 1B-C, Supplemental Table 1 and 2). To confirm that the longevity changes were due to the down-regulation of *mas1*, the level of expression of this gene was examined in *EP982*, *EP1628*, and *EP1130* in both young and old flies by real time PCR. It was found that the expression of *mas1* is reduced in young and old flies in all three mutants (Fig. 1F). These results are consistent with the hypothesis that the down-regulation of *mas1* extends lifespan. All the mutants displayed better resistance to individual stress, oxidative stress and starvation (Supplemental Table 3). *EP1307*, another insertion in *mas1*, isolated from an independent screen, was also found to have an increase in longevity, although the life

extension was less striking in this line (Supplemental Fig. 2). The observation that three independent insertions into this gene extend lifespan strongly suggests that *mas1* is important for modulating longevity.

RNA-interference-mediated Reduction in *mas1* Expression Increases Lifespan in *Drosophila* and *C. elegans*

To determine whether *mas1* (*CG32684)* down-regulation is sufficient for the life extension, we generated an RNAi line of transgenic flies, *UAS-CG32684*^{RNAi}, to knock-down *mas1* expression. We observed the effect of downregulation of *mas1*, by crossing *UAS-CG32684*^{RNAi} transgenic flies with the ubiquitous driver line, *da-Gal4*. The mean lifespan of the progeny was 39% greater than that of control flies (Fig. 2A, Supplemental Table 4), further supporting the hypothesis that reduction of the activity of *mas1* extends lifespan.

To examine whether reduction of *mas1* can extend lifespan in an organism other than *Drosophila,* we tested the effect of down-regulating the expression of *D2030.1*, the gene most homologous to *mas1* in *C. elegans*. We measured the lifespan of the wild-type, N2 strain, fed either *E. coli* producing double-stranded RNAi against *D2030.1*, or *E. coli* containing only expression vector (*L4440*) as a control. As in the fly, the knockdown of *D2030.1* extended lifespan, although the result was less striking in the worm with 9% extension in mean lifespan ($P<0.0001$; Figure 2B, Supplemental Table 4). However, the effect is specific to this homologue since the knockdown of the other paralogues of *mas1* in the nematode by RNAi did not significantly extend lifespan (Supplemental Table 4). These results suggest that a conserved role of lifespan enhancement exists for this gene in both flies and worms.

Down-regulation of *Edem1* Extends Lifespan

To determine whether the mechanism of life extension of the *mas1* mutants is modulated via its known function in the ERAD pathway, we tested whether other mutants obtained in the screen affected components of this pathway. One mutant identified (*EP1588*) contained a transposable element inserted into *CG3810* (*Edem1*), a gene downstream of *mas1* in the ERAD pathway (Ellgaard & Helenius 2003; Olivari & Molinari 2007). The expression level of *Edem1* was significantly lower in the mutant line relative to the control (Fig. 3A) and the mean lifespan of both male and female mutant flies was increased by more than 30% (Figs. 3B and 3C, Supplemental Tables 1 and 2). Since both genes are known to act in a common pathway, the hypothesis that they mediate their effects via this pathway can be tested by looking for genetic interactions. Typically, when two genes function in different pathways double mutants show synergistic effects, while genes that function in the same pathway produce no additional enhancement.

Since the longevity phenotypes of *EP1130, EP1628, EP982,* and *EP1588* are dominant (Supplemental Table 5), the effects of combining the various mutations can be readily observed by crossing the mutant strains. If the mutations act through a common pathway, the phenotype of a fly mutant for two different genes is expected to be similar to that generated by crossing two independent mutations in one of the genes. To test this prediction, mutant flies affecting *mas1* were crossed to *Edem1* mutants. The transheterozygotes for

mas1 and *Edem1* showed similar lifespan profiles to those resulting from crossing for different alleles of *mas1* (Fig. 3D, Supplemental Table 5). These results support the hypothesis that the two genes act to extend longevity through a common genetic pathway.

Mutations in *mas1* and *Edem1* Exhibit Reduced *BiP* Expression

Mas1 is involved in the process of N-linked glycosylation (Herscovics 2001). Abolishing N-glycosylation causes unfolded protein accumulation and leads to an ER stress response (Elbein 1991; Lawson *et al.* 1998). In *C. elegans* the modulation of two ER stress response genes, *abu-1* and *abu-11*, affects lifespan (Urano *et al.* 2002; Viswanathan *et al.* 2005). To examine whether ER stress plays a role in lifespan extension, we measured the mRNA levels of several ER stress response genes, including *relish*, *perk*, *BiP/Grp78*, *xbp1*, and *crebA* (Greene *et al.* 2005) in both *EP1130* and w^{1118} and found that only *BiP* expression levels were altered (Fig. 4A). *BiP* expression was also reduced in *EP1628*, *EP982*, and *EP1588* relative to w^{1118} (Fig 4A–C). To demonstrate that *BiP* down-regulation occurs as a result of the change in *mas1* expression, the effect of knockdown by RNAi was measured. In both *Drosophila* and *C. elegans*, the treatment resulted in a reduction in *BiP* expression (Fig. 4D–E).

Since reduction in *BiP/Grp78* expression is observed in long-lived mice under DR (Heydari *et al.* 1995; Tillman *et al.* 1996; Dhahbi *et al.* 1997), we hypothesized that the effect of down-regulating *mas1* might be involved in modulation of the DR pathway in *Drosophila. Drosophila* raised under various nutrient regimens display dramatic changes in longevity and mutations that affect DR show an altered response to nutrient alteration. Therefore, mutations that affect DR-related pathways will display a non-proportional change in longevity on restricted (DR) media relative to control flies (Tatar 2007). To test whether *mas1* may be involved in the modulation of the DR pathway in flies, the lifespan of the mutant and control flies was measured under two dietary conditions previously shown to lie in the linear range of lifespan increase for wild-type flies: abundant (15%-sugar/yeast (15%SY)) and restricted (5%-sugar/yeast (5%SY)) food (Wood *et al.* 2004; Libert *et al.* 2007). As expected the control flies, w^{1118}, lived substantially longer under DR conditions (36% increase, Fig. 5A, Supplemental Table 6). Interestingly, the mutants were longer-lived than the controls under both conditions, and the mutants' longevity enhancement relative to controls was dramatically increased on the abundant diet. As shown in Figure 5, these increases range from a 1.6-fold increase for *EP1628* (26% extension on restricted versus 42% extension on abundant) to a 3.1-fold increase for *EP982* (17% extension on restricted versus 54% on abundant) (Fig. 5A–D, Supplemental Table 6), consistent with *mas1* and *Edem1* interacting with the dietary restriction pathway. These results are also summarized on supplemental figure 3 where the non-proportionality of the reaction norm is illustrated by plotting mean lifespan of mutant and control flies on the two media. To determine whether the mutations abrogated DR plasticity or shifted the response to DR, the longevity of the various lines was compared under both sets of conditions (Supplemental Fig. 3; Supplemental table 6). For three of the four lines, a smaller but statistically significant enhancement was observed under DR conditions, while one of the lines, *EP982*, showed no significant difference. Thus it would appear that these mutations while interacting with the DR pathway do maintain some degree of responsiveness to DR.

To further examine the relationship of *mas1* mutation and DR, the lifespan of the *mas1*-RNAi-knockdowned and control flies were measured under the abundant and restricted food conditions. Similar to the P-element mutants, the *mas1*-RNAi-knockdowned flies by RNAi also show the best life-extension on abundant media (roughly a 2-fold enhancement 19% under restricted versus 37% on abundant, Supplemental Fig. 4). Finally, to ensure that the restricted food represents DR conditions, we measured the fecundity of the mutant and control flies on both the restricted and abundant food. A similar pattern of reduced fecundity was observed for each fly strain on the restricted food compared to the abundant food (Supplemental Fig. 5), demonstrating that the restricted food environment results in DR conditions for both the mutant and control flies.

DR related life-extension can be achieved via a number of mechanisms, including reduced food consumption, reduced metabolic output, and reduced fertility (Wood *et al.* 2004; Sinclair 2005). Each of these parameters was examined and the results are shown in Table 1. No difference in food consumption was observed between *EP1130* and w^{1118} in both young and old flies. As younger flies usually eat more than older flies, to ensure that the lack of change is not due to a limiting amount of radioisotope in the food, we used similar aged females as a control since they eat more than their male peers. Female flies show a much higher dose of radiation, indicating the radioisotope in the food is in excess. Likewise, no change in metabolic rate, as measured by carbon dioxide output, was found between *EP1130* and w^{1118} (Table 1). Interestingly, a slight increase in fecundity was observed in the mutants relative to the wild-type (Table 1), a finding that has also been reported for Resveratrol (Wood et al., 2004). Since Resveratrol also improves the activity of high calorie diet-fed mice on a treadmill or rotarod test (Baur *et al.* 2006), we measured the climbing activity of *EP1130* mutants and found that the mutants display enhanced climbing activity relative to w^{1118} (Table 1). These results show that like Resveratrol treatment, reduced *mas1* activity provides DR-like benefits without some of the associated, undesirable tradeoffs.

Discussion

A number of previous studies have suggested that modulation of glycosylation via alpha-mannosidase can influence longevity. The expression of *mas1* decreases with age in *Drosophila* (Zou *et al.* 2000) while in mammals, the activity of mannosidases is lower in the livers of both aging mice and humans (Cingle *et al.* 1996; Zhu *et al.* 2006). We have also detected lower expression levels of *mas1A*, the mouse *CG32684* orthologue, in the livers of aged (28-month) mice relative to young (3-month) mice (data not shown). Direct studies aimed at examining the role of these genes have revealed that *alpha-mannosidase-II* over-expression results in extended lifespan in *Drosophila* (Landis *et al.* 2001). However, none of these studies provide a mechanism for the action of *alpha-mannosidase* nor do they address the robustness of the effects.

We provide compelling evidence that the down-regulation of a specific *alpha mannosidase*, *mas1*, can extend longevity in both *Drosophila* and *C. elegans*, suggesting that this is likely a conserved role for modulating longevity. Furthermore, we show that this effect is robust, observed in animals either heterozygous or homozygous for the mutation, as well as when down-regulation is achieved via RNA interference. These results suggest that beneficial

effects of *mas1* can be obtained over a wide range of gene activity. We have initiated studies aimed at replicating our results using pharmacological intervention. Using Mas1 inhibitors such as 1-deoxymannojirimycin and kifunensine on wild-type flies we have obtained promising results. In preliminary studies we have observed increases in lifespan of approximately 18% (data not shown); however due to high experimental variability, these results have failed to meet statistical significance. There are several possible explanations for these observations. First, the inhibitors may not be effective in the fly, or the conditions under which they were delivered were suboptimal. Alternatively, adverse side effects of the drugs may exist that mask the otherwise beneficial effects or the drugs target a wider range of molecules than we are genetically testing, since genetic or biochemical redundancy may exist for In *mas1*, since a null mutant can overcome its defect and synthesize the full range of N-linked oligosaccharides in *Drosophila melanogaster* (Roberts *et al.* 1998). Further studies using targeted RNAi may allow for the identification of specific tissues or life stages in which interventions are most effective. This work in combination with continued pharmacological studies may prove highly useful in identifying optimal intervention strategies.

Several lines of evidence suggest that the mechanism by which *mas1* modulates longevity overlaps with those that regulate dietary restriction. First, the mutants obtained show greatly enhanced longevity under the abundant diet conditions relative to control flies and much less dramatic longevity phenotypes under DR, consistent with what would be expected if the mutants functioned within that pathway. In addition, it is known that the ERAD pathway interacts with many components of the DR pathway, and we have shown that a second gene in the ERAD pathway, *Edem1,* produces phenotypes indistinguishable from those of *mas1* and fails to genetically interact with *mas1* mutants. Likewise, modulation of *Sir2* via Resveratrol administration can activate DR-induced pathways in a number of organisms (Howitz *et al.* 2003; Wood *et al.* 2004), and in the nematode resveratrol reduces the expression of worm *mas1* (*D2030.1*) (Viswanathan *et al.* 2005), which we show increases longevity when down-regulated.

In addition, we find that the down-regulation of *BiP/Grp78* expression, which occurs both in response to DR and to the resveratrol treatment (Heydari *et al.* 1995; Tillman *et al.* 1996; Dhahbi *et al.* 1997; Viswanathan *et al.* 2005) also occurs in response to reduced activity of *mas1*. BiP is a chaperone which maintains PERK and IRE1-alpha in an inactive state. BiP overexpression attenuates PERK and IRE1 activity and represses the unfolded protein response (UPR), whereas BiP downregulation activates the UPR (Bertolotti *et al.* 2000; Okamura *et al.* 2000). Therefore, a reduced *BiP* expression may trigger the ER stress response.

All of the mutants described in this work were shown to down-regulate the level of expression of *BiP* specifically without altering many other genes in the ER stress response pathway. These results suggest that *BiP* may be a particularly valuable biomarker for DR-related activity as mediated via the ERAD pathway. Dietary restriction protects against carcinogenesis in mammals. Recent study suggests that combination therapy with reduced *BiP/Grp78* expression may be a novel approach to eradicate residual tumors (Lee 2007; Pyrko *et al.* 2007). Our discovery that reducing *mas1* or *Edem1* reduces *BiP* expression not

only provides a new aspect of anti-aging study but also may thus offer a possible new target for cancer therapy.

Experimental procedures

Fly and worm strains

Independent EP-element insertion lines *EP1130*, *EP1628*, *EP982*, *EP1588* (Rorth 1996), were all out-crossed with the control fly w^{1118} ten times, then re-established as homozygous lines. The location of the transposon in each line was confirmed by inverse PCR. To generate *UAS-CG32684*^{RNAi}, the pWIZ vector was used to express the double-stranded RNA of a 220 base-pair sequence from *CG32684* cDNA amplified by PCR primers MA220 FOR and MA220 REV in an inverted orientation cloned into pWIZ. The construct was verified by DNA sequencing before the micro-injection to generate the RNAi transgenic flies *UAS-CG32684*^{RNAi}. A ubiquitous Gal4 driver, *da-Gal4*, was used to cross with *UAS-CG32684*^{RNAi} to express double-stranded RNA. All flies were raised and maintained in standard fly food, unless special mention elsewhere, incubated at 25°C, 65% humidity, 12-hr day/night cycle incubator.

N2 worms were fed *E. coli* HT115 containing either the vector L4440 alone or the construct expressing double-stranded RNAi against *D2030.1* (*mas1* orthologue in worm), *C52E4.5* or *T03G11.4* (*mas1* paralogues). The lifespan of RNAi-treated worms was measured as described in (Viswanathan *et al.* 2005).

Lifespan measurement of flies on regular and dietary restriction (DR) conditions and stress assays

Newly eclosed flies (4-days old) raised in standard food were collected by sex and maintained at a density of thirty per vial on the appropriate food, standard fly food (Caltech recipe by Professor Edward Lewis) for standard lifespan, and abundant food (agar 2%, yeast 15%, sucrose 15%) or the restricted food (agar 2%, yeast 5%, sucrose 5%) for DR experiments as described (Wood *et al.* 2004). The flies were maintained at 25°C/65% in a humidity controlled incubator, transferred to new food every three or four days until all were dead. At least three independent repeats were carried out for all experiments.

Young flies about 4-days old were collected at a density of 25 flies per vial for stress assays. For oxidative stress test, the flies were fed with 5mM paraquat in 5% sucrose water and counted dead fly number every four hours till all dead. For starvation, the young flies were kept in 1% agar vials and counted dead fly number every six hours till all flies were dead.

Fly character measurements

To measure food intake, 6-day- and 20-day-old control and *EP1130* flies were separated by sex with 20 flies per vial and fed with the standard fly food containing ^{32}P isotopes (7μl of 10μCi ^{32}P-dCTP mixed in 50ml fly food) for 24 hours, and then collected in a vial to measure the radioactive emission values as described in (Brummel *et al.* 2004). Carbon dioxide output, an indicator of metabolism, is measured by using groups of ten flies in 2.2-ml glass vials in the TR-2 CO2 gas respirometer (Sable Systems International) and analyzed

by DATACAN software (Walker & Benzer 2004). The measurement of fecundity was performed as described in (Wang *et al.* 2004). Ten virgin female flies were mated with ten males of the same genotype for 1 day in a vial, the mated females were collected to place in a cage to lay eggs on a grape-juice agar plate for 24 hours. A new grape-juice agar plate was replaced every 24 hours for four consecutive days. The cumulative egg number within the four days was calculated to check the fecundity. The fly climbing activity, negative geotaxis, was carried out by the countercurrent apparatus with a 15-second interval (Gargano *et al.* 2005). Approximately 50 6-day-old male flies were placed into a plastic test tube (17×100 mm, Falcon Cat#2057) fit into the countercurrent apparatus. After two-minute rest, the flies were mechanically agitated and tested for climbing activity. The performance index was calculated based on the ratio of flies climbing over the 100-mm-length tube within 15 seconds to the total number of flies.

RT-PCR and Real time PCR

Total RNA isolation and reverse transcription followed by polymerase chain reaction were described in (Wang *et al.* 2004). Complementary DNA for each sample were obtained by RT and then used in real time PCR by SYBR GREEN PCR Master Mix and quantified using an ABI PRISM 7500 sequence detector system (Applied Biosystem). The PCR was performed in triplicate reactions with 4 μl of 1/50 diluted cDNA and 2.5 μM of both primers in a final volume of 15 μl containing 7.5 μl of 2X SYBR GREEN PCR Master Mix. The average Ct value of each gene was calculated and normalized by the average Ct of the *rp49* gene as an internal standard among different samples. The information of all primers is available upon request.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

Alcedo J, Kenyon C. Regulation of C. elegans longevity by specific gustatory and olfactory neurons. Neuron. 2004; 41:45–55. [PubMed: 14715134]

Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. Nature. 2006; 444:337–342. [PubMed: 17086191]

Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol. 2000; 2:326–332. [PubMed: 10854322]

Bishop NA, Guarente L. Genetic links between diet and lifespan: shared mechanisms from yeast to humans. Nature reviews. 2007; 8:835–844.

Brummel T, Ching A, Seroude L, Simon AF, Benzer S. Drosophila lifespan enhancement by exogenous bacteria. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:12974–12979. [PubMed: 15322271]

Bubliy OA, Loeschcke V. Correlated responses to selection for stress resistance and longevity in a laboratory population of Drosophila melanogaster. Journal of evolutionary biology. 2005; 18:789–803. [PubMed: 16033550]

Cingle KA, Kalski RS, Bruner WE, O'Brien CM, Erhard P, Wyszynski RE. Age-related changes of glycosidases in human retinal pigment epithelium. Current eye research. 1996; 15:433–438. [PubMed: 8670743]

Cypser JR, Johnson TE. Multiple stressors in Caenorhabditis elegans induce stress hormesis and extended longevity. The journals of gerontology. 2002; 57:B109–114. [PubMed: 11867647]

Dhahbi JM, Mote PL, Tillman JB, Walford RL, Spindler SR. Dietary energy tissue-specifically regulates endoplasmic reticulum chaperone gene expression in the liver of mice. J Nutr. 1997; 127:1758–1764. [PubMed: 9278556]

Duronio V, Jacobs S, Romero PA, Herscovics A. Effects of inhibitors of N-linked oligosaccharide processing on the biosynthesis and function of insulin and insulin-like growth factor-I receptors. The Journal of biological chemistry. 1988; 263:5436–5445. [PubMed: 2965702]

Elbein AD. Glycosidase inhibitors: inhibitors of N-linked oligosaccharide processing. Faseb J. 1991; 5:3055–3063. [PubMed: 1743438]

Ellgaard L, Helenius A. Quality control in the endoplasmic reticulum. Nat Rev Mol Cell Biol. 2003; 4:181–191. [PubMed: 12612637]

Gargano JW, Martin I, Bhandari P, Grotewiel MS. Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in Drosophila. Experimental gerontology. 2005; 40:386–395. [PubMed: 15919590]

Gems D, Partridge L. Stress-response hormesis and aging: "that which does not kill us makes us stronger". Cell metabolism. 2008; 7:200–203. [PubMed: 18316025]

Greene JC, Whitworth AJ, Andrews LA, Parker TJ, Pallanck LJ. Genetic and genomic studies of Drosophila parkin mutants implicate oxidative stress and innate immune responses in pathogenesis. Human molecular genetics. 2005; 14:799–811. [PubMed: 15689351]

Herscovics A. Structure and function of Class I alpha 1,2-mannosidases involved in glycoprotein synthesis and endoplasmic reticulum quality control. Biochimie. 2001; 83:757–762. [PubMed: 11530208]

Heydari AR, Conrad CC, Richardson A. Expression of heat shock genes in hepatocytes is affected by age and food restriction in rats. J Nutr. 1995; 125:410–418. [PubMed: 7876915]

Hosokawa N, Wada I, Hasegawa K, Yorihuzi T, Tremblay LO, Herscovics A, Nagata K. A novel ER alpha-mannosidase-like protein accelerates ER-associated degradation. EMBO Rep. 2001; 2:415–422. [PubMed: 11375934]

Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature. 2003; 425:191–196. [PubMed: 12939617]

Landis G, Bhole D, Lu L, Tower J. High-frequency generation of conditional mutations affecting Drosophila melanogaster development and life span. Genetics. 2001; 158:1167–1176. [PubMed: 11454765]

Lawson B, Brewer JW, Hendershot LM. Geldanamycin, an hsp90/GRP94-binding drug, induces increased transcription of endoplasmic reticulum (ER) chaperones via the ER stress pathway. Journal of cellular physiology. 1998; 174:170–178. [PubMed: 9428803]

Lee AS. GRP78 induction in cancer: therapeutic and prognostic implications. Cancer Res. 2007; 67:3496–3499. [PubMed: 17440054]

Libert S, Pletcher SD. Modulation of longevity by environmental sensing. Cell. 2007; 131:1231–1234. [PubMed: 18160034]

Libert S, Zwiener J, Chu X, Vanvoorhies W, Roman G, Pletcher SD. Regulation of Drosophila life span by olfaction and food-derived odors. Science (New York, NY. 2007; 315:1133–1137.

Lim HY, Bodmer R, Perrin L. Drosophila aging 2005/06. Experimental gerontology. 2006; 41:1213–1216. [PubMed: 17126511]

Lombard DB, Chua KF, Mostoslavsky R, Franco S, Gostissa M, Alt FW. DNA repair, genome stability, and aging. Cell. 2005; 120:497–512. [PubMed: 15734682]

Martin GM, Austad SN, Johnson TE. Genetic analysis of ageing: role of oxidative damage and environmental stresses. Nat Genet. 1996; 13:25–34. [PubMed: 8673100]

Masoro EJ. Overview of caloric restriction and ageing. Mechanisms of ageing and development. 2005; 126:913–922. [PubMed: 15885745]

Mattson MP. Dietary factors, hormesis and health. Ageing research reviews. 2008; 7:43–48. [PubMed: 17913594]

Okamura K, Kimata Y, Higashio H, Tsuru A, Kohno K. Dissociation of Kar2p/BiP from an ER sensory molecule, Ire1p, triggers the unfolded protein response in yeast. Biochemical and biophysical research communications. 2000; 279:445–450. [PubMed: 11118306]

Olivari S, Molinari M. Glycoprotein folding and the role of EDEM1, EDEM2 and EDEM3 in degradation of folding-defective glycoproteins. FEBS Lett. 2007; 581:3658–3664. [PubMed: 17499246]

Partridge L, Piper MD, Mair W. Dietary restriction in Drosophila. Mechanisms of ageing and development. 2005; 126:938–950. [PubMed: 15935441]

Pyrko P, Schonthal AH, Hofman FM, Chen TC, Lee AS. The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. Cancer Res. 2007; 67:9809–9816. [PubMed: 17942911]

Rattan SI. Hormesis in aging. Ageing research reviews. 2008; 7:63–78. [PubMed: 17964227]

Roberts DB, Mulvany WJ, Dwek RA, Rudd PM. Mutant analysis reveals an alternative pathway for N-linked glycosylation in Drosophila melanogaster. European journal of biochemistry/FEBS. 1998; 253:494–498. [PubMed: 9654102]

Rorth P. A modular misexpression screen in Drosophila detecting tissue-specific phenotypes. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93:12418–12422. [PubMed: 8901596]

Sinclair DA. Toward a unified theory of caloric restriction and longevity regulation. Mechanisms of ageing and development. 2005; 126:987–1002. [PubMed: 15893363]

Tatar M. Diet restriction in Drosophila melanogaster. Design and analysis. Interdiscip Top Gerontol. 2007; 35:115–136. [PubMed: 17063036]

Tillman JB, Mote PL, Dhahbi JM, Walford RL, Spindler SR. Dietary energy restriction in mice negatively regulates hepatic glucose-regulated protein 78 (GRP78) expression at the posttranscriptional level. J Nutr. 1996; 126:416–423. [PubMed: 8632214]

Urano F, Calfon M, Yoneda T, Yun C, Kiraly M, Clark SG, Ron D. A survival pathway for Caenorhabditis elegans with a blocked unfolded protein response. J Cell Biol. 2002; 158:639–646. [PubMed: 12186849]

Vermeulen CJ, Loeschcke V. Longevity and the stress response in Drosophila. Experimental gerontology. 2007; 42:153–159. [PubMed: 17110070]

Viswanathan M, Kim SK, Berdichevsky A, Guarente L. A role for SIR-2.1 regulation of ER stress response genes in determining C. elegans life span. Developmental cell. 2005; 9:605–615. [PubMed: 16256736]

Walker DW, Benzer S. Mitochondrial “swirls” induced by oxygen stress and in the Drosophila mutant hyperswirl. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:10290–10295. [PubMed: 15229323]

Wang HD, Kazemi-Esfarjani P, Benzer S. Multiple-stress analysis for isolation of Drosophila longevity genes. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:12610–12615. [PubMed: 15308776]

Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature. 2004; 430:686–689. [PubMed: 15254550]

Zhang Y, Herman B. Ageing and apoptosis. Mechanisms of ageing and development. 2002; 123:245–260. [PubMed: 11744038]

Zhu M, Lovell KL, Patterson JS, Saunders TL, Hughes ED, Friderici KH. Beta-mannosidosis mice: a model for the human lysosomal storage disease. Human molecular genetics. 2006; 15:493–500. [PubMed: 16377659]

Zou S, Meadows S, Sharp L, Jan LY, Jan YN. Genome-wide study of aging and oxidative stress response in Drosophila melanogaster. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97:13726–13731. [PubMed: 11095759]

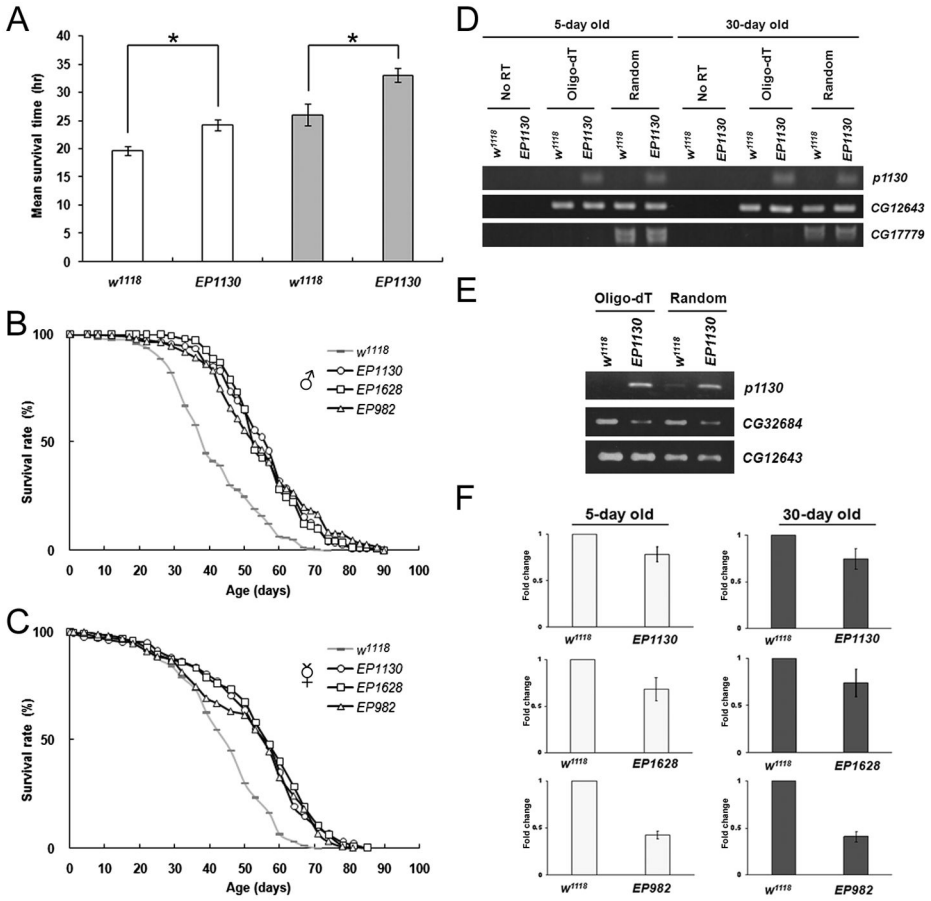

Figure 1. Mutants with reduced *mas1* (*CG32684*) expression exhibit extended lifespan

(A) Both male and female *EP1130* mutants survive longer than the control w^{1118} in the multiple-stress paradigm, 10mM paraquat and wet starvation. Open columns represent the mean survival time±SD of the male flies. Grey columns represent female. *P<0.05 by Student's *t* test. (B) Male *mas1* mutants display a similar fold of lifespan increase at 25°C. Grey bar represents w^{1118}, open circle *EP1130*, open square *EP1628*, open triangle *EP982*. (C) Virgin female mutants also show enhanced lifespan. (D) A transcript, *p1130*, is up-regulated in *EP1130* both in young (5-day old) and old (30-day old) flies as measured by RT-PCR either using oligo-dT or random primers in the RT reaction. The No RT reaction, which contains no primers in the RT reaction followed by PCR, is used to show the absence of genomic DNA in the PCR reaction. No difference in the expression of *CG12643* and *CG17779* genes near the *EP1130* insertion site is detected. These were used as internal controls for the PCR reaction comparing w^{1118} and *EP1130*. (E) An increased expression of *p1130* is correlated with the down-regulation of *CG32684* in *EP1130* by RT-PCR. The expression of *CG12643* was used as an internal control. (F) A decreased expression of *CG32684* is detected in both the young and old mutants by real-time PCR.

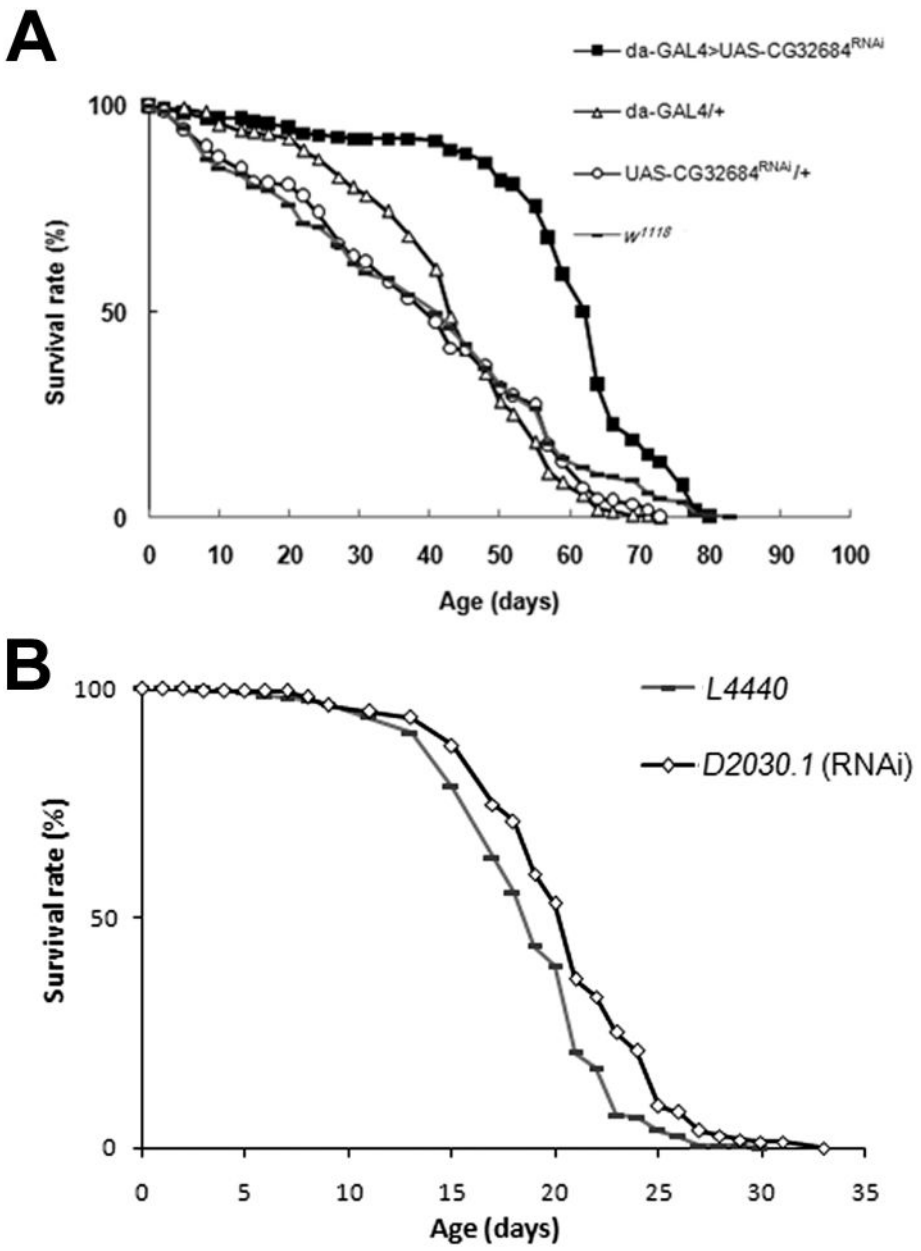

Figure 2. The RNA interference knockdown of *mas1* extends lifespan in *Drosophila melanogaster* and *C. elegans*

(A) Flies with knock-downed *CG32684* expression prolong lifespan. Solid square represents the *da*-GAL4>UAS-*CG32684*^{RNAi} knock-downed flies, open triangle *da*-GAL4/+, open circle UAS-*CG32684*^{RNAi}/+, grey bar w^{1118}. (B) N2 worms fed with *E. coli* expressing double-stranded RNA against *D2030.1* exhibit enhanced lifespan at 20°C. Grey bar represents N2 worms fed with *E. coli* containing L4440 (vector only) as a control, open diamond represents N2 worms fed with *E. coli* expressing double-stranded RNA against *D2030.1*. $P<0.01$ calculated by log rank test.

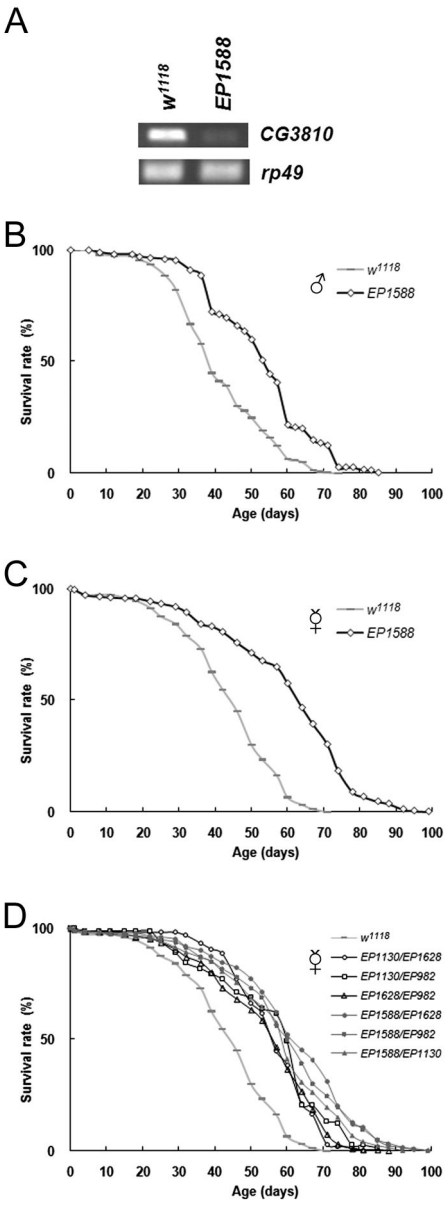

Figure 3. *Edem1* mutant (*EP1588*) also displays lifespan extension

(A) Decreased expression of *CG3810* (*Edem1*) is detected by RT-PCR in the mutant *EP1588* which is also isolated from the multiple-stress screen. *Edem1* is a downstream gene of *mas1* in the ERAD pathway. The internal control is *rp49*. (B) Male *Edem1* mutant exhibits increased lifespan. Grey bar represents w^{1118}, open diamond *EP1588*. (C) Virgin female *Edem1* mutants also exhibit extended lifespan. (D) Trans-heterozygous mutants of *Edem1* and *mas1* display similar fold levels of lifespan extension. Grey bar represents w^{1118}, open circle *EP1130/EP1628*, open square *EP1130/EP982*, open triangle *EP1628/EP982*, solid circle *EP1588/EP1628*, solid square *EP1588/EP982*, solid triangle EP1588/EP1130.

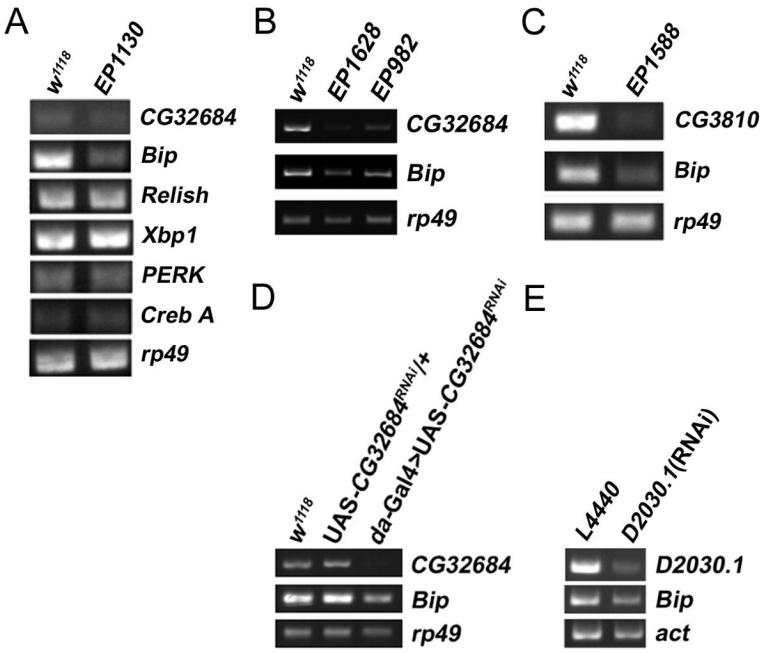

Figure 4. Reduced *BiP* expression, an indication of dietary restriction, is detected in the longevity *mas1* and *Edem1* mutant flies

(A) The expression levels of ER stress response genes, *BiP*, *Relish*, *Xbp1*, *PERK*, *Creb A*, were measured in the w^{1118} and *EP1130* by RT-PCR. Decreased *BiP* expression is found in *EP1130*. No changes appear in the expression of the other ER stress response genes. (B–C) Lowered *BiP* expression is also observed in the other longevity mutants *EP1628*, *EP982*, and *EP1588* via RT-PCR. (D–E) Reduced *BiP* expression is detected in the RNAi knockdown of *mas1* and *D2030.1* in the fly and worm by RT-PCR.

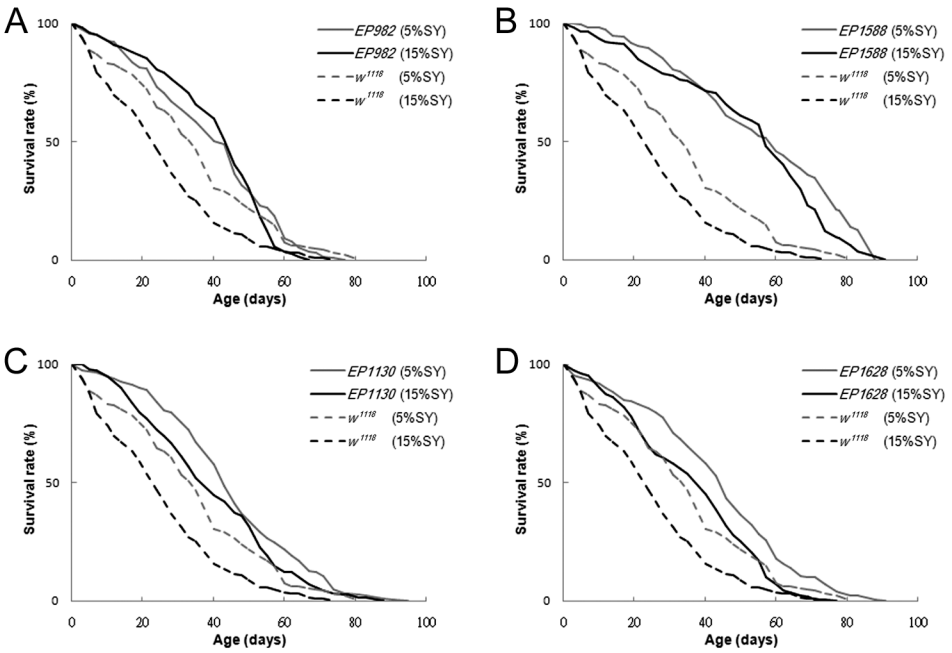

Figure 5. Mutants in *mas1* and *Edem1* extend lifespan via dietary restriction pathway

(A–D) The lifespan of the longevity mutants *EP1130*, *EP1628*, *EP982*, *EP1588* and the control w^{1118} female flies on the abundant (15%SY) and restricted (5%SY) food at 25°C. No further lifespan enhancement was observed in *EP982* and *EP1588* under the restricted food; *EP1130* and *EP1628* only display half of the fold increase than that in control w^{1118}. A dashed grey line represents the lifespan curve of w^{1118} on the restricted food, dashed black line is w^{1118} on the abundant food, solid grey line indicates mutants on the restricted food, solid black line is mutants on the abundant food.

Table 1

Food uptake, fecundity, CO_2 output, and climbing activity in *EP1130*

Strain	Food uptake (CPM)		CO_2 output (ppm/g)	Fecundity (egg number)	Climbing activity (PI)
	young	old			
w^{1118} ♂	4470±103	2734±108	286±7.1	215	25%
EP1130 ♂	4629±137	2843±63	291±8.9	243	50%
w^{1118} ♀	8022	5592			

The young flies are 6 days old and the old flies are 20 days old.

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