Antimicrobial peptides do not directly contribute to aging in *Drosophila*, but improve lifespan by preventing dysbiosis

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Abstract

Antimicrobial peptides (AMPs) are innate immune effectors first studied for their role in host defense against bacterial and fungal infections. Recent studies have implicated these peptides in the clearance of aberrant cells and various neurological processes including neurodegenerative syndromes. In *Drosophila*, an array of AMPs are produced downstream of the Toll and Imd NF-κB pathways in response to infection. Many studies have suggested a role for the Imd pathway and AMPs in aging in this insect, supported by the upregulation of AMPs with aging (so-called "inflammaging"). However, functional studies using RNAi or over-expression have been inconclusive on whether and how these immune effectors impact aging.

Leveraging a new set of single and compound AMP gene deletions in a controlled genetic background, we have investigated how AMPs contribute to aging. Overall, we found no major effect of individual AMPs on lifespan, with a possible exception of *Defensin*. However, *ΔAMP14* flies lacking 14 AMP genes from seven families display a reduced lifespan. Interestingly, we found an increased bacterial load in the food medium of aged *ΔAMP14* flies, suggesting that the lifespan reduction of these flies was due to a failure in controlling the microbiome. Consistent with this idea, use of germ-free conditions extends the lifespan of *ΔAMP14* flies. Overall, our results do not point to an overt role of individual AMPs in lifespan. Instead, we find that AMPs collectively impact lifespan by preventing dysbiosis over aging. This is consistent with our previous study showing that AMPs control the gut microbiome, and many works showing that dysbiosis is detrimental upon aging. In the course of our experiments, we also uncovered a strong impact of a *Drosophila nora virus* infection on lifespan, and share our experience in reconciling our data given this confounding cryptic factor.

Introduction

Antimicrobial peptides (AMPs) are innate immune effectors found in plants and animals that show microbicidal activity in vitro. They are typically cationic and amphipathic, and disrupt microbial membranes that are more negatively charged (Broekaert et al., 1997; Christensen et al., 1988; Ludtke et al., 1996; Zasloff, 1987). Recent studies in various animal models have pointed to a role for AMPs beyond microbial infection, notably in aging and aging-related diseases (Deslouches and Di, 2017; Mookherjee et al., 2020; Semple and Dorin, 2012; Smith and Gwyer Findlay, 2022). Like other animals, AMP genes are upregulated upon aging in *Drosophila,* and disparate studies have suggested a role for AMPs as causative agents of aging, neurodegeneration or mitochondrial stress (Arora and Ligoxygakis, 2020; Garschall and Flatt, 2018; Hanson and Lemaitre, 2020). Here we have leveraged a set of isogenic AMP deficient flies to analyze the contribution of AMPs to aging in *Drosophila*.

Antimicrobial peptides are well characterized in *Drosophila* for their contribution to host defence. There are currently eight families of inducible AMPs known in *D. melanogaster*: the antifungals *Drosomycin*, *Baramicin*, and *Metchnikowin*; *Cecropins* (four inducible genes) and *Defensin* that have both antibacterial and some antifungal activities; and *Drosocin*, *Attacins* (four genes) and *Diptericins* (two genes) that primarily exhibit antibacterial activity (Hanson et al., 2021; Imler and Bulet, 2005). In addition, the *Drosophila* genome encodes many other host defence peptide families such as *Daisho* (two genes) and *Bomanin* (12 genes), where overt antimicrobial activity in vitro is not yet demonstrated, but functional studies have shown they are important in vivo to resist microbial infection (Clemmons et al., 2015; Cohen et al., 2020). While most *Drosophila* defence peptide genes are strongly induced in the fat body in response to systemic infections, many show specific and complex patterns of expression in tissues such as the trachea, gut, salivary glands, or reproductive tracts (Ferrandon et al., 1998; Tzou et al., 2000).

AMPs genes are regulated at the transcriptional level by the Toll and Imd NF-kB signaling pathways upon systemic infection, or by the Imd pathway in local epithelia (Lemaitre and Hoffmann, 2007; Myllymäki et al., 2014). It is well established that Imd and Toll deficient flies show marked susceptibility to microbial infection. Until recently, the importance of immune effectors downstream of these pathways, notably AMPs, was unclear. With the advent of CRISPR gene editing, we systematically deleted seven families of AMP genes (*Defensin, Cecropin, Drosocin, Attacin, Diptericin, Drosomycin,* and *Metchnikowin*) and analyzed their contributions to host defence individually or collectively. We found that *Drosophila* AMPs are essential downstream of the Imd pathway to resist systemic infection by Gram-negative bacteria. *Drosophila* AMPs also contribute downstream of the Toll pathway to combat fungi and to a lesser extent Gram-positive bacterial infection, although *Bomanins* play a more prominent role against these micro-organisms (Carboni et al., 2021; Clemmons et al., 2015; Hanson et al., 2019a). Use of fly lines carrying combinations of AMP mutations revealed that they can function either additively or synergistically against some microbes, but in some cases AMPs exhibit striking specificity, with one peptide contributing most of the AMP-dependent

defence against a specific pathogen (Hanson et al., 2019a; Hanson et al., 2022a; Hanson et al., 2022b; Unckless et al., 2016). *Drosophila* AMPs are also important to control the fly microbiome downstream of the Imd pathway, particularly for their role in regulating Gram-negative bacteria like *Acetobacter* (Marra et al., 2021).

While *Drosophila* AMPs were initially investigated for their contribution to host defence, AMP upregulation is also observed in non-immune contexts, including anti-tumor defence (Araki et al., 2019; Krautz et al., 2020; Parvy et al., 2019), neurodegeneration (Cao et al., 2013; Kounatidis et al., 2017; Petersen et al., 2013; Shukla et al., 2019; van Alphen et al., 2022), and aging (Glittenberg and Silas, SukritGlittenberg, M. T., Silas, S., MacCallum, D. M., Gow, N. a R., & Ligoxygakis, 2011; Lai et al., 2007). This suggests that AMPs could have roles beyond their traditional function as microbicidal agents. Notably, transcriptomic studies revealed that high AMP expression, reflecting an increase in Imd pathway activity, is a hallmark of aging in flies (Landis et al., 2004; Pletcher et al., 2002; Seroude et al., 2002; Zerofsky et al., 2005). This is reminiscent of the situation observed in humans where a low-grade chronic inflammation is observed upon aging, termed "inflammaging" (Franceschi et al., 2000; Franceschi et al., 2017; Liang and Diana, 2020). The key question now is to decipher if this high and chronic activation of the immune response is simply a marker of aging, or if cytotoxic immune effectors like AMPs accelerate aging-associated syndromes.

To address this question, several reports have investigated the role of the Imd pathway and AMPs in aging in *Drosophila* with contrasting outcomes. Most of these studies targeting Imd itself, or the Imd transcription factor *Relish* report that Imd pathway mutants are short-lived, (Cai et al., 2021; Kounatidis et al., 2017; Petersen et al., 2013). However one study suggested that Imd mutation itself improves lifespan, as did fat body or whole-body AMP knockdown (Lin et al., 2018). The negative impact of Imd pathway downregulation has been associated with defects in gut homeostasis (Buchon et al., 2009), exaggerating the decline in gut resilience upon aging (Rera et al., 2012), leading to invasion of microbes into the hemolymph that drive mortality (Clark et al., 2015). Indeed the guts of aged *Relish* mutant flies display precocious loss of compartmentalization, increased permeability, and dysplasia (Liu et al., 2022). However preventing the over-activation of Imd in the gut through transgene expression can improve lifespan, suggesting a careful balance of immune signalling in the gut is needed for optimal health with aging (Cai et al., 2021; Guo et al., 2014; Iatsenko et al., 2018). Some of these symptoms could be reverted when flies were raised axenically pointing to a role of the microbiome in the precocious aging of the gut (Buchon et al., 2009; Clark et al., 2015; Iatsenko et al., 2018; Liu et al., 2022; Zhu et al., 2021). Another study reports that AMP genes are strongly induced in the head of old flies, and that silencing *Relish* in glia can extend life span (Kounatidis et al., 2017). This would suggest that Imd-mediated immune responses drive aging by directly affecting brain activity. Indeed, many studies report that suppression of the Imd pathway can prevent neurodegeneration in various neurodegenerative disease models. For instance, flies mutated in the serine/threonine kinase *ataxia telangiectasia mutated* (*ATM,* also called *tefu*) display reduced lifespan associated with increased neuronal lesions, which can be rescued by combination with *Relish* mutation (Petersen et al., 2013). *Relish* deletion also rescues the aging-dependent

134 neurodegeneration of *Cdk5-alpha* mutant flies (Shukla et al., 2019), and interesting recent
135 studies even showed that deletion of AMPs can improve fly survival after traumatic brain
136 injury (Swanson et al., 2020; van Alphen et al., 2022). An effect of AMP overexpression
137 in aging has also been proposed, albeit with conflicting results. Cao et al. (Cao et al.,
138 2013) reported AMP upregulation and neurodegeneration in the brains of *dnr1* mutant
139 flies, and showed that AMP overexpression in neurons was sufficient to induce
140 neurodegeneration. Badinloo et al. found that chronic and ubiquitous AMP
141 overexpression reduced fly lifespan alongside induction of mitochondrial stress
142 (Badinloo et al., 2018). A recent study of the AMP gene *Metchnikowin* further suggests a
143 trade-off between greater antimicrobial activity and host fitness (Perlmutter et al., 2023).
144 These results suggest AMPs could be deleterious to host fitness, which is also supported
145 by evolutionary studies showing that AMP deletions segregate in wild populations (Early
146 et al., 2017; Hanson et al., 2019b). However in direct contradiction to this idea, an earlier
147 report found that excess expression of certain AMPs could extend lifespan (Loch et al.,
148 2017).
149
150 Presently, we do not know how AMPs impact aging, and a direct link between
151 AMPs and aging remains to be demonstrated. Some of the conflicting results mentioned
152 above may arise from use of different conditions (e.g. temperature, sex, mating status,
153 nutrition) (Camus et al., 2019; Dobson et al., 2018; Landis et al., 2015; Miquel et al.,
154 1976), microbiome differences, which could help explain contradictory findings on the
155 impact of germ-free conditions on lifespan (Brummel et al., 2004; Ren et al., 2007), or
156 lack of control over genetic background, including transgene insertions (Ferreiro et al.,
157 2017; Sasaki et al., 2021), all of which can influence longevity. In the absence of
158 mutants, these studies have relied on the use of over-expression or RNAi to modulate
159 AMPs, methodologies with certain limitations (Tower et al., 2017; van der Graaf et al.,
160 2022). As a result, exactly how AMPs contribute to aging remains unclear.
161
162 Here we have leveraged a recently generated collection of AMP mutations to
163 analyze whether these immune effectors impact aging. We find that individual AMP
164 deletions do not markedly affect aging, with the possible exception of *Defensin*.
165 However, *ΔAMP14* flies lacking 14 AMP genes displayed reduced lifespan associated
166 with microbial dysbiosis. Rearing *ΔAMP14* flies in germ-free conditions significantly
167 rescues lifespan, indicating that AMPs contribute to lifespan through their impact on the
168 microbiome. In contrast, depleting the microbiome of *Relish* mutant flies did not rescue
169 lifespan, suggesting the Imd pathway can affect lifespan independent of regulating AMP
170 genes. Together, our results indicate that AMPs are likely not direct contributors to the
171 aging process, though they have a major impact on aging through AMP-microbiome
172 interactions. Thus, our study, using loss of function mutations, clarifies the role of these
173 innate immune effectors in aging.

174 Results

175

176 **Presence of cryptic infections may confound lifespan analyses in *Drosophila***

177

To address the role of AMP genes in aging, we first compared the lifespan of flies lacking eight AMPs located on Chromosome II (*ΔAMP Chr2* lacking *Def, Dro, AttA,B,C, Mtk,* and *DptA,B)* to their DrosDel isogenic wild-type (*iso* w^{1118}*)* controls. Of note, all our mutations are in the DrosDel isogenic genetic background (unless mentioned otherwise), and are negative for the endosymbiont *Wolbachia*. We show the lifespans of male flies in the main text, and female flies in the supplementary figures. In most cases, trends were similar between the two sexes. Cases where trends differed between male and females are noted in the main text. We observed a striking effect where *ΔAMP Chr2* deficient flies displayed a marked lifespan extension compared to wild-types (Fig. 1A). This first result would be consistent with studies suggesting AMPs negatively impact lifespan. However, we were surprised by the short lifespan of our *iso* w^{1118} wild-type compared to other aging studies, and to a second wild-type included in these experiments, *Oregon R* (*OR-R* in Fig. 1A). *Drosophila* can carry a number of cryptic bacterial or viral infections that affect fitness (Plus et al., 1976). Notably infection with *Drosophila nora virus* is common in lab stocks, and this virus has been shown to reduce lifespan (Habayeb et al., 2009). We thus suspected some component of the virome/microbiome of our *iso* w^{1118} flies could be affecting our *iso* w^{1118} wild-type but not *ΔAMP Chr2* flies. We therefore cleared our flies of their virome/microbiome through bleaching, allowed microbiome recolonization by microbes in the food medium, and assessed the lifespan of the cleaned *iso* w^{1118} stock (protocol in materials and methods). At the same time, we screened our *iso* w^{1118} flies for a panel of common contaminating viruses; specifically *Drosophila sigmavirus, Drosophila A virus, Drosophila C virus,* and *Drosophila nora virus*. We did detect *Drosophila nora virus* (hereafter "*nora*") in our *iso* w^{1118} flies, but not in our *ΔAMP Chr2* mutants, nor in other stocks included in those experiments. We also detected *nora virus* in four other genotypes at different times during our five years of study (*AttC*^{Mi}*, Bom*$^{\Delta 55C}$*, Group C, OR-R*, defined later). In some cases, these stocks were previously nora-negative, and so, were seemingly contaminated from standard fly tipping (Fig. 1B). Strikingly, the bleaching treatment markedly improved the lifespan of all of these stocks to rival their contemporaries, such as *ΔAMP Chr2* flies. The net lifespan reductions for *iso* w^{1118} and *OR-R* were ~39% and ~23% respectively, possibly suggesting genetic background-effects in susceptibility to *nora virus*. We conclude that our wild-type reference had artificially reduced lifespan, likely due to a cryptic *nora virus* infection. While we took care to control for genetic background effects, this stock, at the time, was not an appropriate baseline for comparison. These considerations are in line with recommendations that healthy wild-type stocks should live to median lifespans of 70-90 days (Piper and Partridge, 2018), emphasizing the importance of considering the health of our control stocks prior to making comparisons across stocks/genotypes.

After detecting this contamination in our wild-type flies and seeing the impressive deleterious effect *nora* had on lifespan in our hands, we screened various lab stocks for *nora* virus. We found that 20 out of 44 arbitrarily selected stocks (both from our lab and others) were *nora*-positive, though *nora* titres were typically found only at low levels under standard rearing conditions. We also checked RNA samples collected from aged *nora*-positive flies (aged >3 weeks), finding consistent high *nora* titres (Fig. 1C). These stocks also often showed abdominal bloating at older ages pre-empting mortality (personal observation), similar to bloating seen in flies infected with *Drosophila C virus*

(Chtarbanova et al., 2014), or after systemic infection with some strains of *Acetobacter* bacteria (Hanson et al., 2022b). We thus suspect *nora virus* contamination contributed greatly to the early mortality of our *iso* w^{1118} flies in our first lifespan experiments (Fig. 1A), with a more drastic effect than previously reported (Habayeb et al., 2009). However, we will note that we did not intentionally perform infection *nora* experiments, and so here we provide only correlation-based evidence.

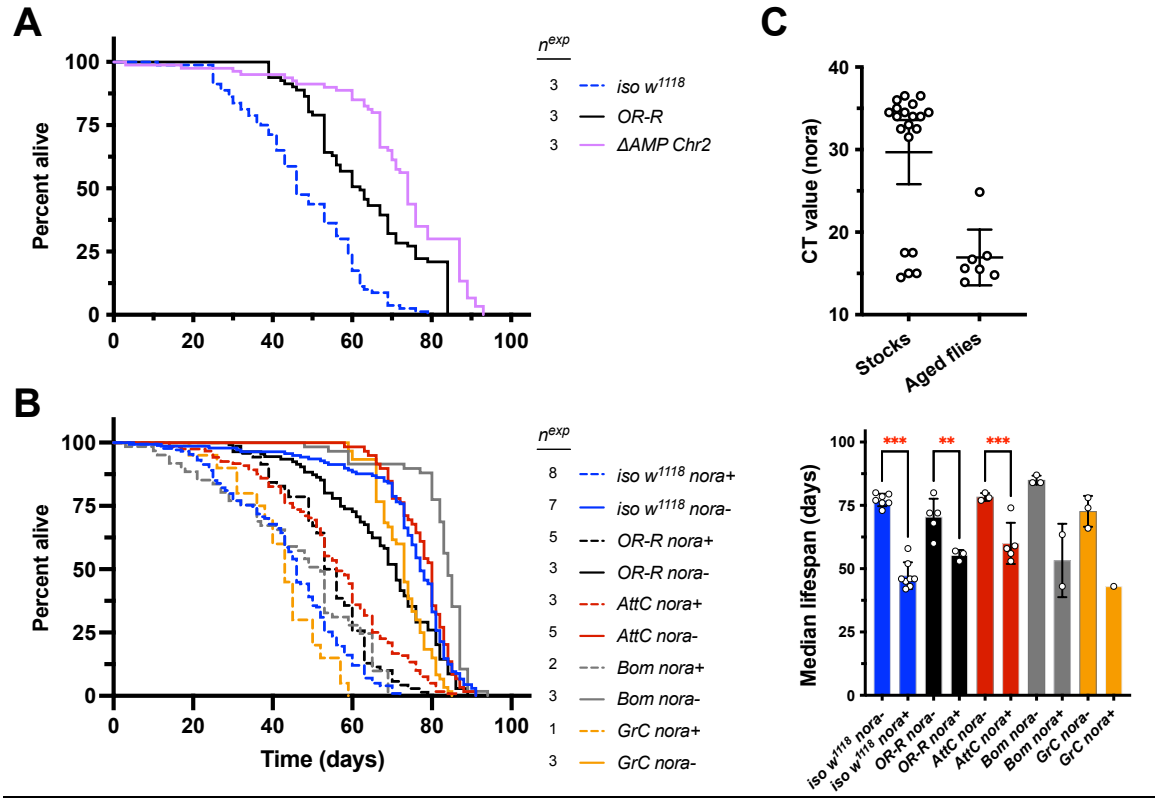

Figure 1: Drosophila nora virus significantly reduces lifespan of DrosDel iso w^{1118} flies. *Male flies are reported here and female flies in Fig. S1. A) Comparison of lifespan from early experiments using two wild-types (iso w^{1118} and OR-R) alongside compound AMP mutants lacking Def, Dro, AttA,B,C, Mtk, and DptA,B, which are deleted in ΔAMP Chr2 flies. B) Effect of nora clearance on lifespan of iso w^{1118}, OR-R, AttC, Bom, and GrC genotypes. Median lifespans are shown in the right panel (** P < .01, *** P < .001). Number of independent experiments (n^{exp}) is reported. C) Nora titres measured by CT value in 18℃ source stocks (Stocks) or nora-positive flies aged 3+ weeks kept at densities of 20 flies per vial (Aged flies). CT values represent nora titre from 5ng total fly RNA per 10µL qPCR reaction.*

Methodology for measuring lifespan and control genotypes

Following these results, we screened all our fly stocks for *nora virus* and bleached *nora*-positive stocks before use in experiments. Subsequent longevity experiments were carried out with the following conditions: we used our standard food medium (recipe in materials and methods), flipped flies three times per week, with ~20 flies per vial, using mated males or females (sexes kept separate), and performed lifespans at 25°C unless otherwise specified. As an assay for brain health, we also monitored locomotor competence during aging using the climbing pass rate assay at 5, 40, 50, and 60 days post-eclosion (dpe) unless specified otherwise.

To compare lifespan, we first used a Cox mixed model commonly used in the literature. However, we found this statistical method was overly-sensitive for our purposes, as even minor differences in lifespan were returned as highly significant ($P < .001$). In some cases, this was driven by sex*genotype interaction effects. Because we kept males and females in separate vials, we realized that any putative sex*genotype interactions present in our Cox mixed model were indistinguishable from vial effects. This is especially important as a consideration, as our AMP mutants are known to suffer dysbiosis with aging ((Marra et al., 2021) and see below), compounding the impact of vial-specific microbiome stochasticity (File S4 for further discussion). Visual inspection of survival curves suggests that, by and large, even significant Cox mixed model variation in survival largely reflects variation around the mean wild-type lifespan, which is expected when performing multiple hypothesis tests (Streiner and Norman, 2011). Thus, we paired our lifespan analyses with one-way ANOVA statistics run on median lifespans per experiment, intended to get a more stringent measure of lifespan differences by placing greater value on effects that were consistent across experiments. As such, we use one-way ANOVA *P*-values by default in the text to report significant lifespan differences.

Before we address the impact of AMP mutations on lifespan, we analyzed the lifespans of mutants affected in the Toll (*iso spz*^{rm7}) or Imd (*iso Rel*^{E20}) pathways that have been backcrossed in the DrosDel background (Ferreira et al., 2014). We also compared our DrosDel *iso w*^{1118} *white*$^{-/-}$ flies with another wild-type (*OR-R*), which has *white*$^{+/+}$ red eyes. Of note, *white* gene deletion may affect lifespan through e.g. the role of *white* in the brain (Ferreiro et al., 2017), or through *white* regulation of intestinal stem cell proliferation (Sasaki et al., 2021). We further included non-isogenic flies with previously-reported lifespan effects to show how control genotypes behave in our conditions, and set expectations for the size and consistency of lifespan effects we could reasonably observe in our hands compared to other studies. This included: *methuselah* mutants suggested to have exceptionally long lifespan (*mth*^{1}) (Lin et al., 1998), a *dnr1* mutation that reduces lifespan previously associated with neurodegeneration and aberrant AMP induction (*dnr1*$^{2-133}$) (Cao et al., 2013), and *ATM*^{8} flies with precocious neurodegeneration caused by autophagy defects, also associated with Imd pathway activation (Petersen et al., 2013); *mth*^{1}*, dnr1*$^{2-133}$, and *ATM*^{8} were not backcrossed into the DrosDel background, and have red eyes. Our experiments confirmed that *ATM*^{8} flies have significantly shorter lifespan (Fig. 2A,C) associated with poor climbing competence (Fig. 2D). We also observed that our *OR-R* wild type displays a shorter lifespan compared to our DrosDel *iso w*^{1118} wild-type. Contrary to expectation, the lifespan of *mth*^{1} flies was not longer than wild-type ($P > .10$, Fig. 2A,C). However, we did observe improved climbing competence of *mth*^{1} flies into old age (Fig. 2D), which suggests these flies do have a form of improved fitness with aging in our hands. These experiments also confirmed a reduced lifespan in *Rel*^{E20} flies as found by other studies (Kounatidis et al., 2017; Petersen et al., 2013), suggesting a significant contribution of the Imd pathway transcription factor *Relish* to aging.

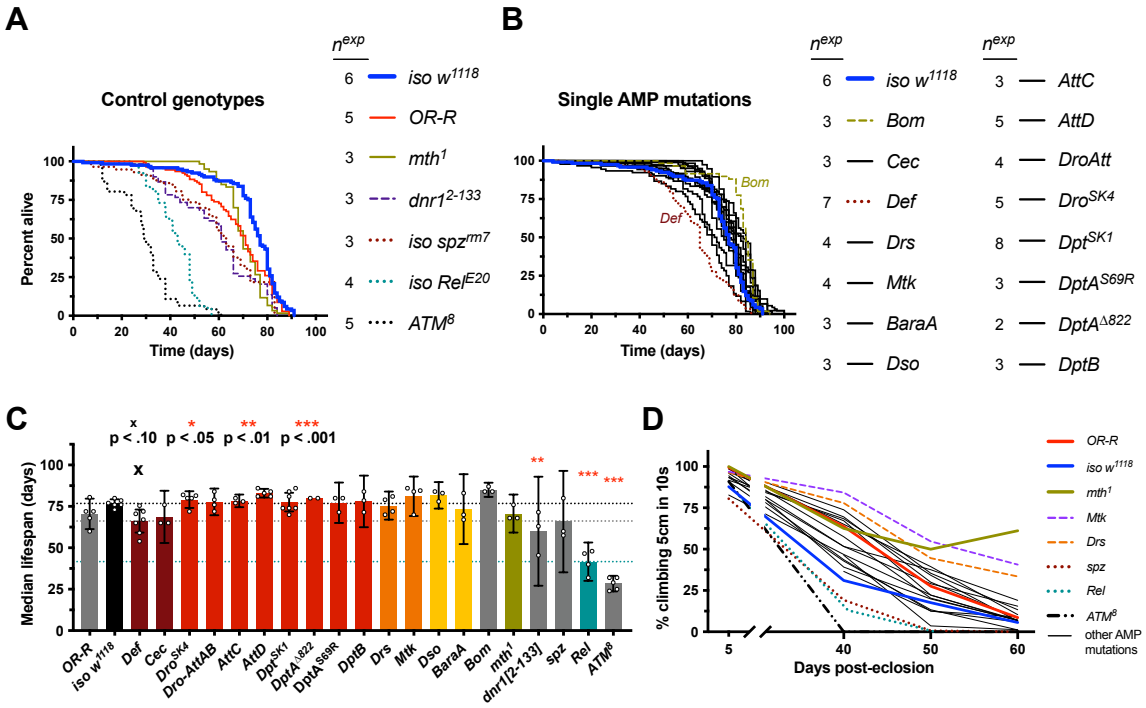

Figure 2: Figure 2: Individual AMP gene deletions do not drastically affect lifespan. *Male flies are reported here and female flies in Fig. S3. A) Cumulative lifespans of flies with various genetic backgrounds. Of note, ATM^{8} data are based on fewer individuals per experiment (see File S1). B) Cumulative lifespans of single gene AMP mutants. Most AMP mutant lifespans (black lines) cluster around the wild-type (blue line), except Def^{SK3}. Bom$^{\Delta 55C}$ is also noted as an outlier perhaps living slightly longer than iso w^{1118}, which was not seen in females (Fig. S3). C) Median lifespans where each data point represents one replicate experiment (cumulative of ~20 males). Median lifespan analysis suggests that the only AMP mutation noticeably differing from iso w^{1118} was Def^{SK3}. Of note, the impact of Def on lifespan was not corroborated using RNAi (Fig. S4). Bom$^{\Delta 55C}$ median lifespans were not different from iso w^{1118}. Horizontal dotted lines indicate median lifespans of iso w^{1118} (top), Def^{SK3} (middle), or Rel^{E20} (bottom). Statistic summaries (p-values: ^{x}, *, **, ***) reflect comparisons to iso w^{1118}. D) Climbing pass rates suggest most AMP mutants climb like wild-type flies, while methuselah mutants uniquely retain climbing competence into old age (also seen at 29°C, Fig. S2); Mtk and Drs males also show improved climbing with aging (but see supplemental text in File S4).*

We also repeated these lifespan experiments at 29°C, which represents a more stressful temperature for *Drosophila melanogaster*, causing more rapid aging (Miquel et al., 1976). When we reared flies at 29°C, as expected, we observed precocious aging both in terms of lifespan and climbing pass rate compared to 25°C for all genotypes tested: *iso w^{1118}*, *OR-R*, *dnr1$^{2-133}$*, *mth^{1}*, and *Rel^{E20}* (Fig. S2A,B). Again, *mth^{1}* flies retained climbing competence longer than other genotypes (Fig. S2C), despite no lifespan extension effect. Of note, differences in lifespan between *OR-R* and *iso w^{1118}* were lost when flies were raised at 29°C. The previous *dnr1* mutant study showing reduced lifespan used 29°C (Cao et al., 2013), and *dnr1$^{2-133}$* generally had reduced lifespan compared to *iso w^{1118}* and *OR-R* wild-types at 29°C, albeit only significantly comparing female *OR-R* and *dnr1$^{2-133}$* flies ($P < .05$), while 25°C experiments were not significant compared to *OR-R* ($P > .10$).

Collectively, we observed that, *ATM*, *dnr1*, and *Relish* deletion reduced lifespan consistent with previous studies (Cao et al., 2013; Kounatidis et al., 2017; Petersen et al., 2013; Shaposhnikov et al., 2014). Surprisingly, we did not observe lifespan extension in *methuselah* mutants. However, *mth^{1}* flies retain climbing competence far better than any other genotype assayed, indicating these flies do have a form of improved aging in our

325 hands. Thus, we could broadly replicate the trends of findings from previous studies,
326 though lifespan itself was not always repeatable in our hands.
327
328 ***Single AMP gene deletion does not drastically affect lifespan***
329
330 We next analysed the lifespan of flies lacking individual or small genomic
331 clusters of AMP genes at 25°C. This included mutants for all 'classical' AMPs from the
332 seven gene families initially identified: *Def, Cec* (removing 4 *Cecropins*), *Dro* alone, and
333 also *Dro* alongside *AttA* and *AttB,* and then *AttC, AttD, Mtk, Drs*, *DptA* and *DptB* together
334 or individually, as well as mutants for the more recently described Toll-regulated effector
335 genes *Daisho (Dso1* and *Dso2), Baramicin A (BaraA),* and *Bomanins (Bom$^{\Delta 55C}$*,
336 removing ten *Bom* genes at cytogenetic map 55C*)*.
337
338 We found no major effect on lifespan of any AMP mutation individually, as
339 survival curves tended to disperse randomly around the *iso w^{1118}* lifespan curve, and
340 median lifespans were not significantly different from *iso w^{1118}* (Fig. 2B-C). The only
341 exception to this trend was male *Def^{SK3}* flies, which had noticeably reduced lifespan
342 compared to *iso w^{1118}* (P = .059). In general, climbing competence was also distributed in
343 a wild-type range comparable to *iso w^{1118}* or *OR-R* flies, and most were not exceptionally
344 good climbers into old age like *mth^{1}* flies, though *Mtk* and *Drs* males had somewhat
345 improved climbing into old age (Fig. 2D, Fig. S5). The *Def^{SK3}* reduction in lifespan was
346 sufficiently interesting that we tested this effect using ubiquitous (*Actin5C-Gal4*) or glia-
347 specific (*Repo-Gal4*) *Def* interfering RNA (*Def-IR,* RNAi), however we saw, if anything,
348 the opposite effect by silencing *Def* by RNAi compared to the results seen with the *Def^{SK3}*
349 mutant (*Act>Def-IR* males had longer lifespans, and females shorter lifespans). However,
350 RNAi genetic controls also suggested complex genetic background effects independent of
351 the Gal4 and RNAi constructs, confounding meaningful interpretations of those results.
352 In general, we could not support a reduced lifespan effect of single AMP mutation with
353 RNAi (Fig. S4).
354
355 Collectively, our study using isogenic mutants shows that individual AMP
356 mutations do not significantly affect lifespan, with the possible exception of *Def^{SK3}*.
357 Overall, we conclude that deleting single AMP genes has no effect on lifespan beyond
358 levels of difference we also observed when comparing different wild-types.
359
360 ***ΔAMP14 flies lacking seven AMP gene families display significantly reduced life span***
361
362 *Drosophila* possess many AMPs with possibly redundant or synergistic activities.
363 Thus, AMPs could affect lifespan only when several genes are deleted simultaneously.
364 After evaluating the effect on lifespan of individual AMP genes or genomic clusters of
365 AMPs, we investigated whether combinatory loss of AMPs could impact aging. Using
366 the same approach as described in Hanson et al. (Hanson et al., 2019a), we generated four
367 groups of compound AMP mutants that remove different subsets of AMP gene families:
368 *Group A* flies were deleted for *Defensin* and the four *Cecropins*. *Group B* flies were
369 deleted for the structurally related *Drosocin, Attacin,* and *Diptericin* families. *Group C*
370 flies were deleted for the two antifungal *Drosomycin* and *Metchnikowin* peptides. And we

371 introduce a new isogenic line combining the loss of two recently-described Toll-regulated
372 antifungal peptides: *"Group D"* that are deleted for both *Baramicin A* (Hanson et al.,
373 2021) and the two *Daisho* genes (Cohen et al., 2020). We screened combined mutants
374 lacking each of these AMP groups, including all the combinations of Groups *A, B,* and *C*
375 (i.e. *AB, AC, BC,* and *ABC* (aka *ΔAMP14*)). We also screened flies lacking 10 AMP
376 genes, but which retain a wild-type *Cecropin* locus (*ΔAMP10*, as used previously
377 (Carboni et al., 2021; Hanson et al., 2019a)). In total we screened nine AMP combinatory
378 genotypes for lifespan and climbing effects (Fig. 3).
379
380 We found a significant effect of *Group A* mutations on male lifespan compared to
381 *iso* w^{1118}, which is not unexpected given this AMP group uses the same 2nd chromosome
382 as the isogenic Def^{SK3} mutation as above (Fig. 2B), except with the additional loss of
383 *Cecropins* on the third chromosome. Comparisons between the two suggest a non-
384 significant effect of *Cecropin* mutation (Def^{SK3} vs. *Group A*, P > .10). Deleting other
385 groups of AMPs typically resulted in non-significant effects on median lifespan
386 compared to their *iso* w^{1118} control (P > .10). *Group AB* flies also had reduced lifespan
387 compared to *iso* w^{1118}, but were comparable to *Group A* alone (*Group A* vs. *Group AB*, P
388 > .10). *Group AC* and *BC* flies had comparable lifespans to *iso* w^{1118} (P > .10 in both
389 cases), which is notable as *Group AC* flies have the Def^{SK3} mutation, but only after an
390 additional round of chromosome II recombination with the isogenic Mtk^{R1} chromosome
391 needed to generate the *Group AC* genotype. Deleting ten AMP genes, which included
392 *Defensin,* also had a *Group A*-like lifespan (*ΔAMP10* vs. *Group A*, P > .10). Thus, various
393 combinations of mutants had lifespans comparable to either *iso* w^{1118} or Def^{SK3} alone.
394 However, deletion of all 14 classic *Drosophila* AMP genes caused a more pronounced
395 median lifespan reduction than the effect of *Group A* alone (*ΔAMP14* vs. *Group A*, male
396 P = .059, female P = .002). There was minor variation in climbing competence amongst
397 AMP mutant groups, with *Group C* flies (*Mtk, Drs* double mutants) having slightly
398 improved climbing into old age. However, this effect was minor compared to the
399 climbing competence of mth^{1} flies into old age (Fig. 3D and see supplementary text).
400
401 While deletion of any single AMP gene had little impact on lifespan, deletion of
402 all 14 classical AMP genes causes lifespan reduction significantly different from wild-
403 type flies. Moreover, recombination of Def^{SK3} with another mutation to produce *Group*
404 *AC* flies yields lifespan comparable to *iso* w^{1118}. This result reinforces the need for careful
405 interpretation of the Def^{SK3} mutation effect of *Group A*. Despite this caveat, *ΔAMP14*
406 flies had significantly reduced lifespan compared to both wild-type flies and other AMP
407 groups. The lifespan of *ΔAMP14* flies is intermediate between *iso* w^{1118} and Rel^{E20},
408 indicating *Relish* likely impacts fly lifespan through processes other than AMP
409 regulation.
410

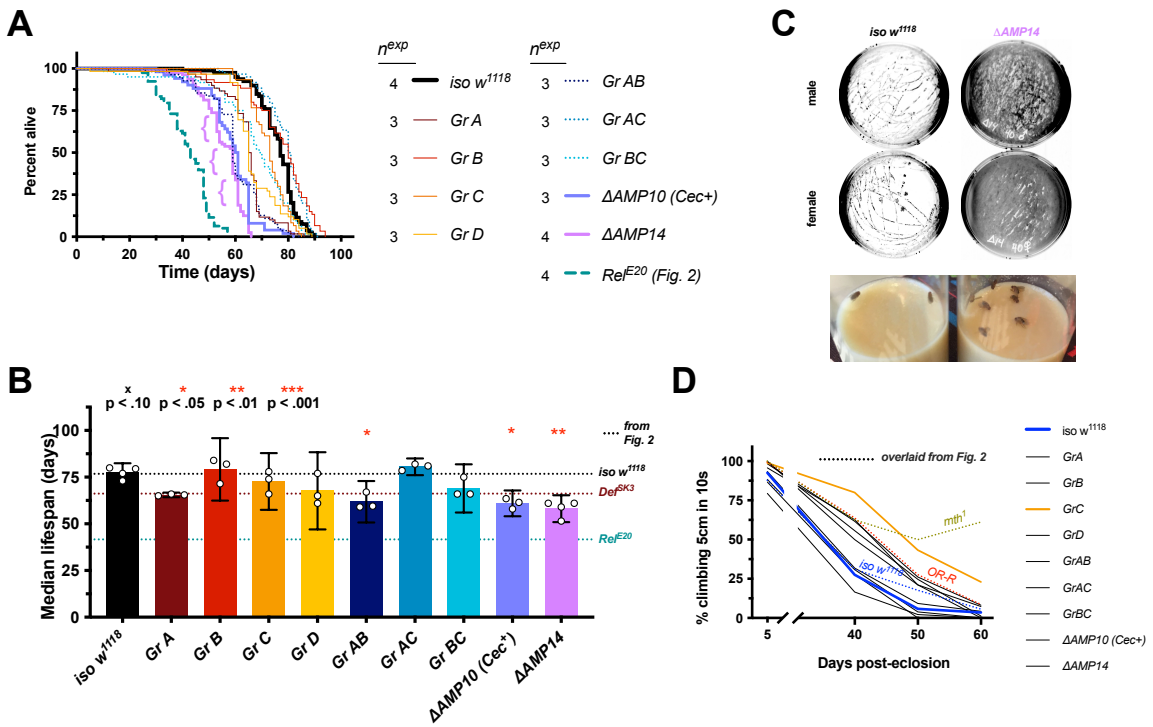

Figure 3: Figure 3: ΔAMP14 flies have significantly reduced lifespan. *Male flies are reported here and female flies in Fig. S6. A) survival curves of various compound AMP mutants. The lifespan of Rel^{E20} from Fig. 2 is overlaid for direct comparison. "{" annotations highlight major mortality events in ΔAMP14 flies. B) Median lifespans of compound AMP mutants. Dotted lines indicate average median lifespans from Figure 2 of iso w^{1118} (top), Def^{SK3} alone (middle), and Rel^{E20} (bottom) for easier comparisons across figures. Statistic summaries (p-value: ^{x}, *, **, ***) reflect comparisons to iso w^{1118} data specific to Figure 3. C) Top: representative photonegative images of agar plates seeded by the microbiome found in the vial of 40-day old flies. Thick bacterial films in ΔAMP14 vials are readily visualized by this method, which shows the significantly greater bacterial density (dark parts) compared to iso w^{1118}. Bottom: representative photo of iso w^{1118} and ΔAMP14 food vials revealing discoloured bacterial biofilm alongside a major mortality event ("{" in Fig. 3A). D) Climbing pass rates of AMP group mutants, with climbing curves from genotypes in Figure 2 overlaid for direction comparison. Group C is highlighted for having a slightly improved climbing over aging, though this improvement is still minor compared to the climbing competence of mth^{1} flies (also see females in Fig. S6 and File S4).*

ΔAMP14 flies suffer microbial dysbiosis with aging

During our studies, we noticed that the food surface of the vials containing *ΔAMP14* flies became discolored and sticky upon aging, suggesting microbial proliferation (Fig. 3C bottom). The *Drosophila* microbiome is found both in the gut and in the external environment due to constant ingestion and fecal deposition (Broderick et al., 2014; Pais et al., 2018; Storelli et al., 2018). We suspected this change in the fly food appearance in *ΔAMP14* upon aging could be linked to a change in the microbiome, as we have recently described a role for AMPs in regulating *Acetobacter* using *ΔAMP14* flies (Marra et al., 2021). Sticky and discolored food was also observed in *Rel^{E20}* flies, which do not express antimicrobial peptides, and indeed both *ΔAMP14* and *Rel^{E20}* flies suffer increased dysbiosis over aging (Marra et al., 2021). To test if microbiome load was associated with early mortality of *ΔAMP14*, we monitored bacterial abundance on the food medium. To do this, we emptied vials where flies had been present for two days, added glass beads to those vials, shook vials with beads for 10 seconds, then placed those

441 beads on agar plates and spread vial microbes by rolling the beads over the agar for 10
442 seconds. By 40 days post-eclosion (dpe), plating of vial contents using beads confirmed
443 far higher microbe loads in aged *ΔAMP14* vials compared to *iso* w^{1118} (Fig. 3C top).
444 Moreover, in the time period between 50-70dpe, most major mortality events in *ΔAMP14*
445 vials were associated with sticky and discolored bacterial food (example photo in Fig. 3C
446 bottom). These major mortality events are also seen as precipitous drops in Fig. 3A
447 (indicated with "{") survival curves in *ΔAMP14* flies, and similar trends were observed
448 for *ΔAMP10*.
449
450 We conclude that *ΔAMP14* flies thus suffered increased microbe load within
451 vials with aging, agreeing with an increase in gut microbiome abundance with aging
452 shown previously using gnotobiotic flies (Marra et al., 2021). This suggests that
453 precocious aging observed in *ΔAMP14* flies could be indirectly caused by the impact of
454 AMPs on the microbiome.
455
456 **Lifespan of *ΔAMP14* flies can be rescued significantly by rearing on antibiotic**
457 **medium**
458
459 To test whether the effect of AMP mutation on lifespan was linked to changes in
460 the microbiome, we performed the same lifespan experiments in germ-free conditions
461 using *iso* w^{1118}, *ΔAMP14,* and Rel^{E20}. For this, we first bleached embryos, and then kept
462 larvae and emerging adults on antibiotic food media for their entire lifespan. In our
463 hands, antibiotic-reared *iso* w^{1118} flies have a similar lifespan compared to conventionally
464 reared flies. Likewise, in our hands Rel^{E20} mutants had similar lifespan whether reared
465 conventionally or in antibiotic conditions. However, rearing *ΔAMP14* flies on antibiotics
466 significantly rescued their lifespan (Fig. 4A-B, *ΔAMP14* ABX vs. CR, P = .013), and a
467 similar non-significant trend was seen in females (Fig. S7A-B). Climbing competence
468 remained largely unchanged, although male Δ*AMP14* flies showed improved climbing
469 specifically at 50dpe in germ-free conditions (P = .053, Fig. 4C).
470
471 These results suggest that the short lifespan of *ΔAMP14* flies primarily relies on
472 the impact that antimicrobial peptides have on the microbiome through aging. The
473 observation that antibiotics treatment rescues *ΔAMP14* but not *Relish* mutant lifespan
474 suggests the short lifespan of Rel^{E20} flies depends on effects other than the microbiome
475 and independent of AMPs.
476

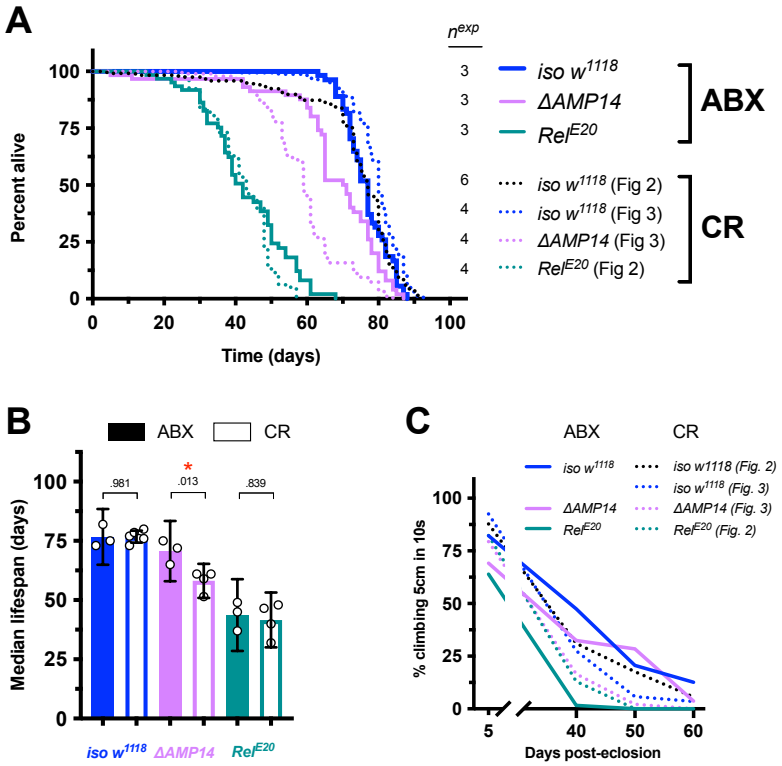

Figure 4: Figure 4: Microbiome depletion rescues the ΔAMP14 fly lifespan. *Male flies are reported here and female flies in Fig. S7. A) Survival curves, including both antibiotic-reared flies (ABX), and also conventionally-reared (CR) lifespans from previous figures as dotted lines for direct comparison. B) Median lifespans, including both ABX and CR fly lifespans for direct comparison. (conventionally-reared iso w^{1118} and Rel^{E20} lifespans shown in Fig. 2C). C) Climbing pass rates of ABX (solid lines) and CR (dotted lines) flies at 5, 40, 50, and 60 days post-eclosion.*

Discussion

A number of studies done in *Drosophila* and *C. elegans,* but also vertebrates, have implicated AMPs in processes as diverse as behavior, neurodegeneration, tumor clearance, and aging. In mammals, some AMPs function as pro-inflammatory cytokines, and as such could influence these processes by disrupting homeostasis when chronically expressed (Liang and Diana, 2020; Mookherjee et al., 2020; Smith and Gwyer Findlay, 2022). However, to date there is no evidence of a cytokine role for AMPs in *Drosophila* (Hanson and Lemaitre, 2020). AMPs can also disrupt the membranes of aberrant host cells, that may become more negatively charged due to the exposure of phospholipids such as phosphatidylserine, a well-known "eat-me" signal that promotes the phagocytosis of apoptotic cells (Fadok et al., 1992; Hakim-Mishnaevski et al., 2019; Manaka et al., 2004). Studies have now also implicated AMPs in the control of tumorous growth in vivo in *Drosophila* (Araki et al., 2019; Parvy et al., 2019), providing a proof of principle that AMP action can target self-tissue. Thus, AMPs could have a role in tissue maintenance with consequences on the aging process. Moreover, AMPs have a number of properties in common with neuropeptides, often being cationic and amphipathic (Augustin et al., 2017; Smith and Gwyer Findlay, 2022), alongside descriptions of AMP-like genes undergoing downregulation in the brain after exposure to pheromones (Gendron et al., 2014), modulating sleep (Sinner et al., 2021; Toda et al., 2019), memory (Barajas-azpeleta et al., 2018), and affecting behaviors (Ebrahim et al., 2021; Hanson et al., 2021; Kobler et al., 2020). Thus, it cannot be excluded that AMPs modulate neuronal activity, a process that could impact lifespan.

525
526 There is an increase of AMP gene expression observed upon aging, though it was
527 unclear if AMPs directly affect lifespan, or if this activation is just a secondary
528 consequence of aging. For instance, aging is accompanied by dysbiosis and gut barrier
529 dysfunction allowing opportunistic systemic infection by microbiome bacteria (Buchon et
530 al., 2009; Clark et al., 2015; Liu et al., 2022; Marra et al., 2021; Rera et al., 2012), which
531 should activate immune signalling and induce AMP expression. Using flies carrying null
532 mutations in AMP genes, we find no evidence that individual AMPs are so essential to
533 host physiology that they have a notable impact on fly lifespan. Overall, the lifespan of
534 flies mutated for individual genes or clusters of AMPs, *Bomanins*, or *Daishos*, did not
535 differ from the wild-type. The climbing activity of these mutants were also similar to
536 wild-type flies. The only possible exception to this was the apparent lower lifespan of *Def*
537 mutant males. However conflicting results from RNAi experiments and use of *Group AC*
538 flies with the Def^{SK3} mutation after additional rounds of recombination suggest the
539 somewhat shortened lifespan of *Def* mutant males was not linked to *Def* mutation itself,
540 but due to the presence of one or several cryptic mutations that were not removed during
541 the isogenization process due to their proximity to the *Def* locus. Alternately, complex
542 interactions amongst AMPs could provide a protective effect against *Defensin* mutation,
543 as AMP interactions can synergize to prevent damage to host membranes (Drab and
544 Sugihara, 2020). Overall, our study suggests that individual AMPs do not affect lifespan
545 beyond levels of difference we also saw when comparing wild-types and/or classic aging
546 mutant flies (e.g. mth^{1}), a result that contradicts other studies using RNAi or
547 overexpression methods. However, our results do support a role for AMPs in regulating
548 the microbiome over aging. Indeed, we recently showed that *Acetobacter* microbiome
549 bacteria grow out of control in the microbiome of Δ*AMP14* flies (Marra et al., 2021), and
550 later confirmed that *Diptericin B* has a highly specific and important role in suppressing
551 *Acetobacter* growth after systemic infection, which causes bloating similar to what we
552 saw in *nora virus*-infected flies (Hanson et al., 2022b). This phenotype of *Acetobacter*
553 systemic infection could help explain why flies bloat upon infection by enteric pathogens
554 (like *nora virus* or *Drosophila C virus*), or upon aging, given the eventual invasion of gut
555 microbes into the hemolymph (Clark et al., 2015; Rera et al., 2012).
556
557 We cannot exclude that additional AMP mutant combinations could reveal a
558 stronger impact of AMPs on lifespan. Indeed, a number of immune-induced peptides that
559 could be AMPs await description (Hanson and Lemaitre, 2020; Schlamp et al., 2021),
560 and additional deletion of those peptides could explain the lifespan difference between
561 *ΔAMP14* and *Relish* mutants. Use of other rearing conditions could also reveal a role for
562 AMPs not found here. As part of this study, we also monitored AMP expression upon
563 aging in four wild-type backgrounds (a lab standard w^{1118}, *Exelexis, OR-R,* and *Canton-S*)
564 by separating out dissected heads and bodies (thorax + abdomen). In our hands, we
565 observed increased expression of AMP genes in fly bodies aged 40 days compared to
566 young flies, though the extent differed by genotype (Table S1 and File S2). However, we
567 saw no marked AMP increase in the head with aging, including in a separate experiment
568 following flies over multiple time points (File S2). Indeed, when AMPs were upregulated
569 in the head upon aging, this was often coupled with a stronger upregulation in the body,
570 suggesting that this increase of AMP expression in the head could derive from head-

571 specific fat body responding like the fat body in the thorax and abdomen. We also tested
572 if glia-specific *Relish* knockdown (via *Repo-Gal4* and *UAS-Rel-IR*) could rescue lifespan,
573 as many studies suggest a role of *Relish* in neurons/glia to rescue neurodegenerative
574 syndromes (Cao et al., 2013; Kounatidis et al., 2017; Petersen et al., 2013; Shukla et al.,
575 2019), but if anything we saw the opposite effect: female *Repo>Rel-IR* flies had shorter
576 lifespan than genetic controls, and we found that *Repo-Gal4* alone had improved
577 climbing into old age, suggesting a genetic background effect on aging unrelated to RNAi
578 using this driver (Fig. S8). While *Repo>Rel-IR* can rescue various models of
579 neurodegeneration, taken together our results suggest AMPs are not especially
580 upregulated in the head upon aging (except when also upregulated in the body), and that
581 glia-expressed *Relish* does not have a major deleterious role in lifespan using our
582 standard rearing conditions. Differences between research groups (local microbiome
583 composition, including cryptic viral infections, food recipe, etc…) could account for
584 these conflicting results. As we found by including *methuselah* mutant flies,
585 improvements to healthy aging could also be consistent across research groups, but actual
586 lifespan extension may be lab-specific. These factors, and the importance of assaying
587 multiple healthy aging metrics, should be considered when comparing our AMP mutant
588 results to the larger field.
589
590 Importantly, our study suggests that AMPs collectively affect fly lifespan through
591 their impact on the microbiome. Several studies have investigated the role of the gut
592 microbiome on lifespan using germ-free conditions with mixed results (Brummel et al.,
593 2004; Cai et al., 2021; Iatsenko et al., 2018; Ren et al., 2007; Shukla et al., 2021). It could
594 be that discrepancies between studies reporting an impact of the microbiome on aging
595 results from a complex interaction between nutrition and gut bacteria. In poor or
596 unbalanced diets, the microbiome could have a positive impact on lifespan by extracting
597 more nutrients from the food (Camus et al., 2019; Chaston et al., 2016; Consuegra et al.,
598 2020; Erkosar et al., 2017; Newell and Douglas, 2014; Storelli et al., 2018; Téfit et al.,
599 2018). In contrast, on a lab standard nutrient-rich diet, as we have used in this study, the
600 microbiome could have less impact. Consistent with this, we did not see major
601 differences between germ-free and conventionally raised wild-type flies in this study.
602 Previous studies have already revealed the role of Imd signalling in controlling
603 microbiome load and diversity, preventing dysbiosis (Guo et al., 2014; Li et al., 2016;
604 Liu et al., 2022; Yamashita et al., 2021). The specific microbiome of a given research
605 group could also change the impact of germ-free conditions. For instance, different
606 *Acetobacter* strains have different virulence to the fly during systemic infection (Hanson
607 et al., 2022b), which accompanies aging and intestinal barrier dysfunction (Clark et al.,
608 2015; Rera et al., 2012). Indeed, use of *ΔAMP14* flies revealed a key role of AMPs to
609 control *Acetobacter* levels in the microbiome, and accordingly, *ΔAMP14* flies have
610 increased *Acetobacter* loads upon aging (Marra et al., 2021).
611
612 Our present study shows that the action of AMPs preserves lifespan, and that this
613 effect is largely due to their impact on the microbiome. Thus, the impact of AMPs on
614 lifespan is consistent with their well-established microbicidal activity. Interestingly,
615 raising *Relish* mutant flies in axenic conditions did not lead to lifespan extension,
616 indicating that *Relish,* and likely the Imd pathway, have a much more profound impact on

host physiology independent of AMP regulation. In line with this, *Relish* and the Imd pathway have been implicated in neural systems (Kounatidis et al., 2017; Masuzzo et al., 2019; Shukla et al., 2019), cell competition (Meyer et al., 2014; Nandy et al., 2018), metabolism (Molaei et al., 2019; Musselman et al., 2018), and enterocyte delamination (Liu et al., 2022; Zhai et al., 2018), processes that likely impact lifespan. It is interesting to note that the food media of *ΔAMP14* and Rel^{E20} flies was enriched in bacteria, agreeing with elevated bacterial loads in these flies with aging shown previously (Marra et al., 2021). External microbes colonize the *Drosophila* gut, and gut microbes are released into the external environment as part of excreta (Broderick et al., 2014; Newell and Douglas, 2014; Storelli et al., 2018; Winans et al., 2017). Thus, it was expected that an increased gut microbiome load in the absence of AMPs would result in high bacterial load in the fly food medium. Although we reared flies in a vial in artificial lab conditions here, it is tempting to speculate that AMPs expressed in the gut could not only shape the gut microbiome, but also environmental bacteria. The increased load in the food medium could therefore rely either on AMP-mediated control of the gut microbiome, or on external AMPs secreted into the food medium. This would suggest that AMP mutation can exacerbate microbiome effects from both within the fly and in the external vial environment.

During our study, we experienced a number of challenges to lifespan data interpretation. Notably, our reference wild-type and several other fly strains were infected with *Drosophila nora virus*, which had a greater deleterious effect on lifespan of *iso* w^{1118} compared to wild-type flies used in a previous study (Habayeb et al., 2009). Thus, we initially interpreted our early results as if AMP deletion extended lifespan to a great extent compared to their isogenic wild-type controls (Fig. 1A). However, our ultimate findings, including standard genotypes and different conditions, instead highlight that loss of AMPs does not extend lifespan; if anything, they show the opposite. We also note that cryptic and chronic infections common in fly stocks, such as *nora virus*, represent a serious threat to aging studies. In our study, we realized this cryptic viral infection confounded our results when comparing our isogenic wild-type lifespan to the expected absolute lifespan of *Drosophila* according to previous recommendations (Piper and Partridge, 2018). We publish this experience, which confused years of data collection, as a cautionary note for the field of aging and immunity. Our hope is that our experience can help others avoid similar confounding factors.

In conclusion, our study reveals a key role of AMPs in the aging process: but mainly indirectly, through their effect on the microbiome. We cannot exclude that certain contexts could reveal an intrinsic effect of AMPs on host tissues during aging, such as conditions found in individuals that have aging-associated diseases like cancer or precocious neurodegeneration, uncommon in standard wild-types. However, here we do not find evidence of AMPs directly impacting aging in a striking way. We are still far from understanding the complex relationship between the immune system, senescence, and aging, which requires further investigation.

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Materials and Methods

Drosophila rearing conditions

Drosophila stocks used in this study, including genotype descriptions, are listed in File S3. Food media used the following recipe (per 600mL): 3.72g agar, 35.28g cornmeal, 35.28g yeast extract, 36mL grape juice, 2.9mL propionic acid, and 15.9mL Moldex. Antibiotic media also contained final concentrations of 50ug/mL Ampicillin, 50ug/mL Kanamycin, 10ug/mL Tetracycline, and 10ug/mL Erythromycin. Flies were flipped three times per week (Monday, Wednesday, Friday), and vials were left on their side to ameliorate the effect of the food medium stickiness on mortality with aging, by allowing flies falling to the ground to drop onto plastic rather than the food surface. This precaution was taken as vial conditions differ markedly in specific immune-deficient genotypes (Marra et al., 2021).

To clear flies of *nora virus*, embryos were collected from grape juice agar plates, rinsed with distilled water, and left to soak in 3% bleach for 3 minutes. Embryos were then rinsed twice in distilled water for one minute each. This protocol was also used to clear the microbiome of antibiotic-reared flies, whereafter we placed embryos directly on antibiotic medium for germ-free experiments.

Lifespan experiments

Lifespan experiments were conducted from 2017 to 2022. Flies were allowed to emerge and mate randomly for ~3 days prior to separating males and females. Then, groups of 20 males or 20 females (mated) were flipped three times per week (Monday, Wednesday, Friday) to measure fly lifespan in 90x15mm polystyrene vials.

We used a Cox proportional hazard (CoxPH) mixed effects model to initially analyze lifespan effects, with experimental replicate and biological sex as interaction terms in R version 3.6.3. In both cases, experimental replicate and sex were significant contributors to the model ($P < .001$). Our impression from the initial data analysis was that the CoxPH model was overly sensitive to minor variation around the geometric mean lifespan of *iso* w^{1118} control flies, exacerbated by the large sample sizes used and the many comparisons performed in our study inflating the likelihood of Type I (false positive) statistical errors. Even if minor differences in lifespan were genuine, their ultimate importance was

707 questionable when compared to other genetic backgrounds (e.g. *OR-R, mth*^{1}), particularly
708 given variable mutation types and transgene insertions used for different AMP mutations
709 (i.e. point mutation, genomic deficiency, *white*$^{+}$, 3xP3-EGFP, or 3xP3-dsRed). We thus
710 preferred to focus on each experiment as if that population of flies represented a single
711 sampling. We therefore treated our data as if we had e.g. n = 3 per genotype (3
712 experiments), rather than n = 120 (60 males and 60 females) per genotype across 3
713 experiments. For this purpose, we decided to use median lifespans as our primary
714 readout. Sex-specific median lifespans were analyzed using one-way ANOVA with
715 Holm-Sidak's multiple test correction implemented in Prism v9.3.1, or one-way ANOVA
716 with Tukey's HSD correction in R 3.6.3.
717
718 ***Climbing pass rates***
719
720 We paired our lifespan data with the gravotaxic locomotor climbing assay to provide an
721 independent metric of aging (Madabattula et al., 2015). This assay assesses the general
722 locomotor competence of the fly, which is often used as a readout of neurodegeneration,
723 but can also reflect generic aging effects (e.g. muscle weakness). Climbing pass rates
724 were filmed for flies at 5, 40, 50, and 60 days post-eclosion ±2 days, and analyzed later
725 manually. A pass was considered if a fly climbed 5cm within 10s of being tapped to the
726 bottom of the vial. Flies were transferred to a chamber made of two empty vials stacked
727 atop each other to provide ample room to climb upwards without reaching the ceiling or
728 disrupting other flies. Two sets of broken markings were made with permanent marker at
729 2.5cm and 5cm on the lower vial as reference points, though we ultimately report only
730 5cm climbing rates given similar trends between the two. At each time point, an initial
731 tapping down was performed to associate the flies with their new environment and
732 encourage climbing behaviour, as we found the first repetition of this experiment often
733 had fewer climbers than subsequent repetitions. After this initial association, three
734 technical repeats of the climbing assay were performed, and final values represent the
735 average of these three technical repeats. All climbing measurements were taken between
736 2pm-5pm to ensure a consistent measurement timeframe.
737
738 This assay can be a measure of both climbing speed and/or readiness of response: for
739 instance, older flies sometimes suffered temporary seizures after being tapped down, or
740 might climb slower, more erratically, or simply to a lesser extent than younger
741 counterparts. In most cases, climbing pass data mirrored lifespan data in terms of relative
742 trends. We thus have no suspicion that AMP mutations, individually or collectively,
743 greatly affect locomotory competence with aging. The only noteworthy exception to
744 mirrored trends between lifespan and climbing was *methuselah* mutant flies, which had
745 wild-type lifespans, but improved climbing competence into older age at both 25°C and
746 29°C.
747
748 For analysis of trends of ΔAMP14 in Fig. 4C, we fit a two-way ANOVA measuring the
749 climbing at 40, 50, and 60 days, with an interaction term for age and germ-free conditions
750 in Prism 9.4.1.
751
752 ***Microbiome monitoring***

753
754 We monitored the microbiome of compound AMP mutants and antibiotic-reared flies by
755 checking plated vial food contents on MRS + Mannitol agar, a medium amenable to both
756 Lactobacilli and *Acetobacter*. Specifically, at both ~20 and ~40 days we took vials where
757 flies had been present for two days, added 5 glass beads to the vials, shook the glass
758 beads in vials for 10s, then transferred glass beads to MRS+Mannitol agar plates and
759 shook plates for 10s, then removed the beads and left plated microbes to grow overnight
760 at 29°C. Vial microbiome loads were checked on Wednesdays. We chose Wednesdays
761 rather than a precise age (e.g. exactly 20dpe) to ensure that flies entered the plating time
762 point after experiencing a similar regimen of flipping; i.e. in our experiments, we only
763 plated microbes from vials where flies had spent the past five days with ~3 days in a vial
764 accruing microbes over the weekend (Fri-Mon), and the next two days depositing
765 microbes in the vial that was ultimately measured (Mon-Wed). This design was chosen
766 based on demonstrations that flipping regimen drastically affects microbiome load
767 (Arias-Rojas and Iatsenko, 2022; Blum et al., 2013; Pais et al., 2018). Beyond ~40 days,
768 comparisons were not equal due to onset of mortality in AMP mutants and associated
769 drops in vial fly density.
770
771 In antibiotic experiments, microbes were never detected from overnight growth using this
772 method to monitor vial microbiome loads.
773
774 ***Gene expression assays***
775
776 Gene expression was performed using primers listed in File S3 with PowerUP SYBR
777 Green Master Mix, using the PFAFFL method of qPCR quantification with Rp49 as the
778 reference gene (Pfaffl, 2001). RNA was extracted using TRIzol according to
779 manufacturer's protocol. cDNA was reverse transcribed using Takara Reverse
780 Transcriptase.
781
782 Dissections of heads from bodies was performed in ice cold PBS, and tubes containing
783 pools of 20 heads or bodies were kept at -20°C until after TRIzol was added to prevent
784 RNA degradation before sample processing.

785 ## Bibliography

786

787 **Araki, M., Kurihara, M., Kinoshita, S., Awane, R., Sato, T., Ohkawa, Y. and Inoue,**
788 **Y.** (2019). Anti-tumour effects of antimicrobial peptides, components of the
789 innate immune system, against haematopoietic tumours in Drosophila mxc
790 mutants. *Disease Models & Mechanisms* **12**,.

791 **Arias-Rojas, A. and Iatsenko, I.** (2022). The Role of Microbiota in Drosophila
792 melanogaster Aging. *Front. Aging* **3**, 909509.

793 **Arora, S. and Ligoxygakis, P.** (2020). Beyond Host Defense: Deregulation of
794 Drosophila Immunity and Age-Dependent Neurodegeneration. *Front.*
795 *Immunol.* **11**, 1574.

796 **Augustin, R., Schröder, K., Murillo Rincón, A. P., Fraune, S., Anton-Erxleben, F.,**
797 **Herbst, E.-M., Wittlieb, J., Schwentner, M., Grötzinger, J., Wassenaar, T.**
798 **M., et al.** (2017). A secreted antibacterial neuropeptide shapes the
799 microbiome of Hydra. *Nat Commun* **8**, 698.

800 **Badinloo, M., Nguyen, E., Suh, W., Alzahrani, F., Castellanos, J., Klichko, V. I., Orr,**
801 **W. C. and Radyuk, S. N.** (2018). Overexpression of antimicrobial peptides
802 contributes to aging through cytotoxic effects in Drosophila tissues. *Archives*
803 *of Insect Biochemistry and Physiology* **98**,.

804 **Barajas-azpeleta, R., Wu, J., Gill, J. and Welte, R.** (2018). Antimicrobial peptides
805 modulate long-term memory. *PLoS Genetics* 1–26.

806 **Blum, J. E., Fischer, C. N., Miles, J. and Handelsman, J.** (2013). Frequent
807 replenishment sustains the beneficial microbiome of Drosophila
808 melanogaster. *mBio* **4**, e00860-00813.

809 **Broderick, N. A., Buchon, N. and Lemaitre, B.** (2014). Microbiota-induced changes
810 in Drosophila melanogaster host gene expression and gut morphology. *mBio*
811 **5**,.

812 **Broekaert, W. F., Cammue, B. P. A., De Bolle, M. F. C., Thevissen, K., De**
813 **Samblanx, G. W., Osborn, R. W. and Nielson, K.** (1997). Antimicrobial
814 Peptides from Plants. *Critical Reviews in Plant Sciences* **16**, 297–323.

815 **Brummel, T., Ching, A., Seroude, L., Simon, A. F. and Benzer, S.** (2004).
816 Drosophila lifespan enhancement by exogenous bacteria. *Proc Natl Acad Sci U*
817 *S A* **101**, 12974–12979.

818 **Buchon, N., Broderick, N. A., Poidevin, M., Pradervand, S. and Lemaitre, B.**
819 (2009). Drosophila Intestinal Response to Bacterial Infection: Activation of
820 Host Defense and Stem Cell Proliferation. *Cell Host and Microbe* **5**, 200–211.

821 **Cai, Q., Ji, S., Li, M., Zheng, S., Zhou, X., Guo, H., Deng, S., Zhu, J., Li, D. and Xie, Z.**
822 (2021). Theaflavin-regulated Imd condensates control Drosophila intestinal
823 homeostasis and aging. *iScience* **24**, 102150.

824 **Camus, M. F., Piper, M. D. and Reuter, M.** (2019). Sex-specific transcriptomic
825 responses to changes in the nutritional environment. *eLife* **8**, e47262.

826 **Cao, Y., Chtarbanova, S., Petersen, A. J. and Ganetzky, B.** (2013). Dnr1 mutations
827 cause neurodegeneration in Drosophila by activating the innate immune
828 response in the brain. *Proceedings of the National Academy of Sciences* **110**,
829 E1752–E1760.

830 **Carboni, A., Hanson, M. A., Lindsay, S. A., Wasserman, S. A. and Lemaitre, B.**
831 (2021). Cecropins contribute to *Drosophila* host defence against fungal and
832 Gram-negative bacterial infection. *Genetics* iyab188.

833 **Chaston, J. M., Dobson, A. J., Newell, P. D. and Douglas, A. E.** (2016). Host Genetic
834 Control of the Microbiota Mediates the Drosophila Nutritional Phenotype.
835 *Appl Environ Microbiol* **82**, 671–679.

836 **Christensen, B., Fink, J., Merrifield, R. B. and Mauzerall, D.** (1988). Channel-
837 forming properties of cecropins and related model compounds incorporated
838 into planar lipid membranes. *Proc Natl Acad Sci U S A* **85**, 5072–5076.

839 **Chtarbanova, S., Lamiable, O., Lee, K.-Z., Galiana, D., Troxler, L., Meignin, C.,**
840 **Hetru, C., Hoffmann, J. A., Daeffler, L. and Imler, J.-L.** (2014). Drosophila C
841 Virus Systemic Infection Leads to Intestinal Obstruction. *J Virol* **88**, 14057–
842 14069.

843 **Clark, R. I., Salazar, A., Yamada, R., Fitz-Gibbon, S., Morselli, M., Alcaraz, J., Rana,**
844 **A., Rera, M., Pellegrini, M., Ja, W. W., et al.** (2015). Distinct Shifts in
845 Microbiota Composition during Drosophila Aging Impair Intestinal Function
846 and Drive Mortality. *Cell Reports* **12**, 1656–1667.

847 **Clemmons, A. W., Lindsay, S. A. and Wasserman, S. A.** (2015). An Effector Peptide
848 Family Required for Drosophila Toll-Mediated Immunity. *PLoS Pathogens*
849 **11**,.

850 **Cohen, L. B., Lindsay, S. A., Xu, Y., Lin, S. J. H. and Wasserman, S. A.** (2020). The
851 Daisho Peptides Mediate Drosophila Defense Against a Subset of Filamentous
852 Fungi. *Front Immunol* **11**, 9.

853 **Consuegra, J., Grenier, T., Baa-Puyoulet, P., Rahioui, I., Akherraz, H., Gervais, H.,**
854 **Parisot, N., da Silva, P., Charles, H., Calevro, F., et al.** (2020). Drosophila-
855 associated bacteria differentially shape the nutritional requirements of their
856 host during juvenile growth. *PLoS Biol* **18**, e3000681.

857 **Deslouches, B. and Di, Y. P.** (2017). Antimicrobial peptides with selective
858 antitumor mechanisms: prospect for anticancer applications. *Oncotarget*.

859 **Dobson, A. J., He, X., Blanc, E., Bolukbasi, E., Feseha, Y., Yang, M. and Piper, M. D.**
860 **W.** (2018). Tissue-specific transcriptome profiling of Drosophila reveals
861 roles for GATA transcription factors in longevity by dietary restriction. *npj*
862 *Aging Mech Dis* **4**, 5.

863 **Drab, E. and Sugihara, K.** (2020). Cooperative Function of LL-37 and HNP1
864 Protects Mammalian Cell Membranes from Lysis. *Biophys J* **119**, 2440–2450.

865 **Early, A. M., Arguello, J. R., Cardoso-Moreira, M., Gottipati, S., Grenier, J. K. and**
866 **Clark, A. G.** (2017). Survey of Global Genetic Diversity Within the Drosophila
867 Immune System. *Genetics* **205**, 353–366.

868 **Ebrahim, S. A. M., Talross, G. J. S. and Carlson, J. R.** (2021). Sight of parasitoid
869 wasps accelerates sexual behavior and upregulates a micropeptide gene in
870 Drosophila. *Nat Commun* **12**, 2453.

871 **Erkosar, B., Kolly, S., van der Meer, J. R. and Kawecki, T. J.** (2017). Adaptation to
872 Chronic Nutritional Stress Leads to Reduced Dependence on Microbiota in
873 Drosophila melanogaster. *mBio* **8**, mBio.01496-17, e01496-17.

874 **Fadok, V. A., Voelker, D. R., Campbell, P. A., Cohen, J. J., Bratton, D. L. and**
875 **Henson, P. M.** (1992). Exposure of phosphatidylserine on the surface of
876 apoptotic lymphocytes triggers specific recognition and removal by
877 macrophages. *J Immunol* **148**, 2207–2216.

878 **Ferrandon, D., Jung, A. C., Criqui, M. C., Lemaitre, B., Uttenweiler-Joseph, S.,**
879 **Michaut, L., Reichhart, J. M. and Hoffmann, J. A.** (1998). A drosomycin-GFP
880 reporter transgene reveals a local immune response in Drosophila that is not
881 dependent on the Toll pathway. *EMBO Journal* **17**, 1217–1227.

882 **Ferreira, Á. G., Naylor, H., Esteves, S. S., Pais, I. S., Martins, N. E. and Teixeira, L.**
883 (2014). The Toll-dorsal pathway is required for resistance to viral oral
884 infection in Drosophila. *PLoS Pathog.* **10**, e1004507.

885 **Ferreiro, M. J., Pérez, C., Marchesano, M., Ruiz, S., Caputi, A., Aguilera, P., Barrio,**
886 **R. and Cantera, R.** (2017). Drosophila melanogaster White Mutant w1118
887 Undergo Retinal Degeneration. *Front Neurosci* **11**, 732.

888 **Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E. and**
889 **De Benedictis, G.** (2000). Inflamm-aging. An evolutionary perspective on
890 immunosenescence. *Ann N Y Acad Sci* **908**, 244–254.

891 **Franceschi, C., Garagnani, P., Vitale, G., Capri, M. and Salvioli, S.** (2017).
892 Inflammaging and 'Garb-aging.' *Trends in Endocrinology & Metabolism* **28**,
893 199–212.

894 **Garschall, K. and Flatt, T.** (2018). The interplay between immunity and aging in
895 Drosophila. *F1000Research* **7**, 160.

896 **Gendron, C. M., Kuo, T.-H., Harvanek, Z. M., Chung, B. Y., Yew, J. Y., Dierick, H. A.**
897 **and Pletcher, S. D.** (2014). Drosophila life span and physiology are
898 modulated by sexual perception and reward. *Science* **343**, 544–548.

899 **Glittenberg, M. T. and Silas, SukritGlittenberg, M. T., Silas, S., MacCallum, D. M.,**
900 **Gow, N. a R., & Ligoxygakis, P.** (2011). Wild-type Drosophila melanogaster
901 as an alternative model system for investigating the pathogenicity of Candida
902 albicans. *Disease models & mechanisms* **4**, 504–514.

903 **Guo, L., Karpac, J., Tran, S. L. and Jasper, H.** (2014). PGRP-SC2 promotes gut
904 immune homeostasis to limit commensal dysbiosis and extend lifespan. *Cell*
905 **156**, 109–122.

906 **Habayeb, M. S., Cantera, R., Casanova, G., Ekström, J.-O., Albright, S. and**
907 **Hultmark, D.** (2009). The Drosophila Nora virus is an enteric virus,
908 transmitted via feces. *J. Invertebr. Pathol.* **101**, 29–33.

909 **Hakim-Mishnaevski, K., Flint-Brodsly, N., Shklyar, B., Levy-Adam, F. and**
910 **Kurant, E.** (2019). Glial Phagocytic Receptors Promote Neuronal Loss in
911 Adult Drosophila Brain. *Cell Rep* **29**, 1438-1448.e3.

912 **Hanson, M. A. and Lemaitre, B.** (2020). New insights on Drosophila antimicrobial
913 peptide function in host defense and beyond. *Curr. Opin. Immunol.* **62**, 22–30.

914 **Hanson, M. A., Dostálová, A., Ceroni, C., Poidevin, M., Kondo, S. and Lemaitre, B.**
915 (2019a). Synergy and remarkable specificity of antimicrobial peptides in vivo
916 using a systematic knockout approach. *eLife* **8**,.

917 **Hanson, M. A., Lemaitre, B. and Unckless, R. L.** (2019b). Dynamic Evolution of
918 Antimicrobial Peptides Underscores Trade-Offs Between Immunity and
919 Ecological Fitness. *Frontiers in Immunology* **10**, 2620.

920 **Hanson, M. A., Cohen, L. B., Marra, A., Iatsenko, I., Wasserman, S. A. and**
921 **Lemaitre, B.** (2021). The Drosophila Baramicin polypeptide gene protects
922 against fungal infection. *PLoS Pathog* **17**, e1009846.

923 **Hanson, M. A., Kondo, S. and Lemaitre, B.** (2022a). *Drosophila* immunity: the
924 *Drosocin* gene encodes two host defence peptides with pathogen-specific
925 roles. *Proc. R. Soc. B.* **289**, 20220773.

926 **Hanson, M. A., Grollmus, L. and Lemaitre, B.** (2022b). *Single innate immune*
927 *effectors control ecological microbiome bacteria across evolutionary*
928 *timescales*. Immunology.

929 **Iatsenko, I., Boquete, J. P. and Lemaitre, B.** (2018). Microbiota-Derived Lactate
930 Activates Production of Reactive Oxygen Species by the Intestinal NADPH
931 Oxidase Nox and Shortens Drosophila Lifespan. *Immunity* **49**, 929-942.e5.

932 **Imler, J.-L. and Bulet, P.** (2005). Antimicrobial peptides in Drosophila: structures,
933 activities and gene regulation. *Chemical immunology and allergy* **86**, 1–21.

934 **Kobler, J. M., Rodriguez Jimenez, F. J., Petcu, I. and Grunwald Kadow, I. C.**
935 (2020). Immune Receptor Signaling and the Mushroom Body Mediate Post-
936 ingestion Pathogen Avoidance. *Curr Biol* **30**, 4693-4709.e3.

937 **Kounatidis, I., Chtarbanova, S., Cao, Y., Hayne, M., Jayanth, D., Ganetzky, B. and**
938 **Ligoxygakis, P.** (2017). NF-κB Immunity in the Brain Determines Fly
939 Lifespan in Healthy Aging and Age-Related Neurodegeneration. *Cell Reports*
940 **19**, 836–848.

941 **Krautz, R., Khalili, D. and Theopold, U.** (2020). Tissue-autonomous immune
942 response regulates stress signaling during hypertrophy. *eLife* **9**, e64919.

943 **Lai, C.-Q., Parnell, L. D., Lyman, R. F., Ordovas, J. M. and Mackay, T. F. C.** (2007).
944 Candidate genes affecting Drosophila life span identified by integrating
945 microarray gene expression analysis and QTL mapping. *Mech Ageing Dev*
946 **128**, 237–249.

947 **Landis, G. N., Abdueva, D., Skvortsov, D., Yang, J., Rabin, B. E., Carrick, J., Tavaré,**
948 **S. and Tower, J.** (2004). Similar gene expression patterns characterize aging
949 and oxidative stress in Drosophila melanogaster. *Proc Natl Acad Sci U S A*
950 **101**, 7663–7668.

951 **Landis, G. N., Salomon, M. P., Keroles, D., Brookes, N., Sekimura, T. and Tower, J.**
952 (2015). The progesterone antagonist mifepristone/RU486 blocks the
953 negative effect on life span caused by mating in female Drosophila. *Aging* **7**,
954 53–69.

955 **Lemaitre, B. and Hoffmann, J.** (2007). The Host Defense of Drosophila
956 Melanogaster. *Annual Review of Immunology* **25**, 697–743.

957 **Li, H., Qi, Y. and Jasper, H.** (2016). Preventing Age-Related Decline of Gut
958 Compartmentalization Limits Microbiota Dysbiosis and Extends Lifespan.
959 *Cell Host and Microbe* **19**, 240–253.

960 **Liang, W. and Diana, J.** (2020). The Dual Role of Antimicrobial Peptides in
961 Autoimmunity. *Front. Immunol.* **11**, 2077.

962 **Lin, Y.-J., Seroude, L. and Benzer, S.** (1998). Extended Life-Span and Stress
963 Resistance in the *Drosophila* Mutant *methuselah*. *Science* **282**, 943–946.

964 **Lin, Y.-R., Parikh, H. and Park, Y.** (2018). Stress resistance and lifespan enhanced
965 by downregulation of antimicrobial peptide genes in the Imd pathway. *Aging*
966 *(Albany NY)* **10**, 622–631.

967 **Liu, X., Nagy, P., Bonfini, A., Houtz, P., Bing, X.-L., Yang, X. and Buchon, N.** (2022).
968 Microbes affect gut epithelial cell composition through immune-dependent
969 regulation of intestinal stem cell differentiation. *Cell Reports* **38**, 110572.

970 **Loch, G., Zinke, I., Mori, T., Carrera, P., Schroer, J., Takeyama, H. and Hoch, M.**
971 (2017). Antimicrobial peptides extend lifespan in Drosophila. *PLoS ONE* **12**,.

972 **Ludtke, S. J., He, K., Heller, W. T., Harroun, T. A., Yang, L. and Huang, H. W.**
973 (1996). Membrane Pores Induced by Magainin. *Biochemistry* **35**, 13723–
974 13728.

975 **Madabattula, S. T., Strautman, J. C., Bysice, A. M., O'Sullivan, J. A., Androschuk,**
976 **A., Rosenfelt, C., Doucet, K., Rouleau, G. and Bolduc, F.** (2015).
977 Quantitative Analysis of Climbing Defects in a Drosophila Model of
978 Neurodegenerative Disorders. *J Vis Exp* e52741.

979 **Manaka, J., Kuraishi, T., Shiratsuchi, A., Nakai, Y., Higashida, H., Henson, P. and**
980 **Nakanishi, Y.** (2004). Draper-mediated and Phosphatidylserine-independent
981 Phagocytosis of Apoptotic Cells by *Drosophila* Hemocytes/Macrophages. *J.*
982 *Biol. Chem.* **279**, 48466–48476.

983 **Marra, A., Hanson, M. A., Kondo, S., Erkosar, B. and Lemaitre, B.** (2021).
984 Drosophila *antimicrobial peptides and lysozymes regulate gut microbiota*
985 *composition and abundance*. Microbiology.

986 **Masuzzo, A., Manière, G., Viallat-Lieutaud, A., Avazeri, É., Zugasti, O., Grosjean,**
987 **Y., Kurz, C. L. and Royet, J.** (2019). Peptidoglycan-dependent NF-κB
988 activation in a small subset of brain octopaminergic neurons controls female
989 oviposition. *eLife* **8**, e50559.

990 **Meyer, S. N., Amoyel, M., Bergantiños, C., de la Cova, C., Schertel, C., Basler, K.**
991 **and Johnston, L. A.** (2014). An ancient defense system eliminates unfit cells
992 from developing tissues during cell competition. *Science* **346**, 1258236.

993 **Miquel, J., Lundgren, P. R., Bensch, K. G. and Atlan, H.** (1976). Effects of
994 temperature on the life span, vitality and fine structure of Drosophila
995 melanogaster. *Mech Ageing Dev* **5**, 347–370.

996 **Molaei, M., Vandehoef, C. and Karpac, J.** (2019). NF-κB Shapes Metabolic
997 Adaptation by Attenuating Foxo-Mediated Lipolysis in Drosophila. *Dev Cell*
998 **49**, 802-810.e6.

999 **Mookherjee, N., Anderson, M. A., Haagsman, H. P. and Davidson, D. J.** (2020).
1000 Antimicrobial host defence peptides: functions and clinical potential. *Nat Rev*
1001 *Drug Discov*.

1002 **Musselman, L. P., Fink, J. L., Grant, A. R., Gatto, J. A., Tuthill, B. F. and Baranski,**
1003 **T. J.** (2018). A Complex Relationship between Immunity and Metabolism in
1004 Drosophila Diet-Induced Insulin Resistance. *Mol Cell Biol* **38**,.

1005 **Myllymäki, H., Valanne, S. and Rämet, M.** (2014). The Drosophila imd signaling
1006 pathway. *J Immunol* **192**, 3455–3462.

1007 **Nandy, A., Lin, L., Velentzas, P. D., Wu, L. P., Baehrecke, E. H. and Silverman, N.**
1008 (2018). The NF-κB Factor Relish Regulates Atg1 Expression and Controls
1009 Autophagy. *Cell Reports* **25**, 2110-2120.e3.

1010 **Newell, P. D. and Douglas, A. E.** (2014). Interspecies Interactions Determine the
1011 Impact of the Gut Microbiota on Nutrient Allocation in Drosophila
1012 melanogaster. *Appl Environ Microbiol* **80**, 788–796.

1013 **Pais, I. S., Valente, R. S., Sporniak, M. and Teixeira, L.** (2018). Drosophila
1014 melanogaster establishes a species-specific mutualistic interaction with
1015 stable gut-colonizing bacteria. *PLoS Biol* **16**, e2005710.

1016 **Parvy, J.-P., Yu, Y., Dostalova, A., Kondo, S., Kurjan, A., Bulet, P., Lemaitre, B.,**
1017 **Vidal, M. and Cordero, J.** (2019). The antimicrobial peptide defensin
1018 cooperates with tumour necrosis factor to drive tumour cell death in
1019 Drosophila. *eLife* eLife 2019;8:e45061.

1020 **Perlmutter, J. I., Chapman, J. R., Wilkinson, M. C., Nevarez-Saenz, I. and**
1021 **Unckless, R. L.** (2023). *A single amino acid polymorphism in natural*
1022 *Metchnikowin alleles of* Drosophila *results in systemic immunity and life*
1023 *history tradeoffs*. Genetics.

1024 **Petersen, A. J., Katzenberger, R. J. and Wassarman, D. A.** (2013). The innate
1025 immune response transcription factor relish is necessary for
1026 neurodegeneration in a Drosophila model of ataxia-telangiectasia. *Genetics*
1027 **194**, 133–142.

1028 **Pfaffl, M. W.** (2001). A new mathematical model for relative quantification in real-
1029 time RT-PCR. *Nucleic Acids Res.* **29**, e45.

1030 **Piper, M. D. W. and Partridge, L.** (2018). Drosophila as a model for ageing.
1031 *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1864**, 2707–
1032 2717.

1033 **Pletcher, S. D., Macdonald, S. J., Marguerie, R., Certa, U., Stearns, S. C., Goldstein,**
1034 **D. B. and Partridge, L.** (2002). Genome-wide transcript profiles in aging and
1035 calorically restricted Drosophila melanogaster. *Curr Biol* **12**, 712–723.

1036 **Plus, N., Croizier, G., Veyrunes, J. C. and David, J.** (1976). A comparison of buoyant
1037 density and polypeptides of Drosophila P, C and A viruses. *Intervirology* **7**,
1038 346–350.

1039 **Ren, C., Webster, P., Finkel, S. E. and Tower, J.** (2007). Increased Internal and
1040 External Bacterial Load during Drosophila Aging without Life-Span Trade-
1041 Off. *Cell Metabolism* **6**, 144–152.

1042 **Rera, M., Clark, R. I. and Walker, D. W.** (2012). Intestinal barrier dysfunction links
1043 metabolic and inflammatory markers of aging to death in Drosophila. *Proc*
1044 *Natl Acad Sci U S A* **109**, 21528–21533.

1045 **Sasaki, A., Nishimura, T., Takano, T., Naito, S. and Yoo, S. K.** (2021). white
1046 regulates proliferative homeostasis of intestinal stem cells during ageing in
1047 Drosophila. *Nat Metab* **3**, 546–557.

1048 **Schlamp, F., Delbare, S. Y. N., Early, A. M., Wells, M. T., Basu, S. and Clark, A. G.**
1049 (2021). Dense time-course gene expression profiling of the Drosophila
1050 melanogaster innate immune response. *BMC Genomics* **22**, 304.

1051 **Semple, F. and Dorin, J. R.** (2012). ß-Defensins: Multifunctional Modulators of
1052 Infection, Inflammation and More? *J Innate Immun* **4**, 337–348.

1053 **Seroude, L., Brummel, T., Kapahi, P. and Benzer, S.** (2002). Spatio-temporal
1054 analysis of gene expression during aging in Drosophila melanogaster. *Aging*
1055 *cell* **1**, 47–56.

1056 **Shaposhnikov, M., Latkin, D., Plyusnina, E., Shilova, L., Danilov, A., Popov, S.,**
1057 **Zhavoronkov, A., Ovodov, Y. and Moskalev, A.** (2014). The effects of
1058 pectins on life span and stress resistance in Drosophila melanogaster.
1059 *Biogerontology* **15**, 113–127.

1060 **Shukla, A. K., Spurrier, J., Kuzina, I. and Giniger, E.** (2019). Hyperactive Innate
1061 Immunity Causes Degeneration of Dopamine Neurons upon Altering Activity
1062 of Cdk5. *Cell Rep* **26**, 131-144.e4.

1063 **Shukla, A. K., Johnson, K. and Giniger, E.** (2021). Common features of aging fail to
1064 occur in Drosophila raised without a bacterial microbiome. *iScience* **24**,
1065 102703.

1066 **Sinner, M. P., Masurat, F., Ewbank, J. J., Pujol, N. and Bringmann, H.** (2021).
1067 Innate Immunity Promotes Sleep through Epidermal Antimicrobial Peptides.
1068 *Current Biology* **31**, 564-577.e12.

1069 **Smith, K. J. and Gwyer Findlay, E.** (2022). Expression of antimicrobial host defence
1070 peptides in the central nervous system during health and disease. *Discovery*
1071 *Immunology* kyac003.

1072 **Storelli, G., Strigini, M., Grenier, T., Bozonnet, L., Schwarzer, M., Daniel, C.,**
1073 **Matos, R. and Leulier, F.** (2018). Drosophila Perpetuates Nutritional
1074 Mutualism by Promoting the Fitness of Its Intestinal Symbiont Lactobacillus
1075 plantarum. *Cell Metab* **27**, 362-377.e8.

1076 **Streiner, D. L. and Norman, G. R.** (2011). Correction for Multiple Testing. *Chest*
1077 **140**, 16–18.

1078 **Swanson, L. C., Rimkus, S. A., Ganetzky, B. and Wassarman, D. A.** (2020). Loss of
1079 the Antimicrobial Peptide Metchnikowin Protects Against Traumatic Brain
1080 Injury Outcomes in Drosophila melanogaster. *G3 (Bethesda)* **10**, 3109–3119.

1081 **Téfit, M. A., Gillet, B., Joncour, P., Hughes, S. and Leulier, F.** (2018). Stable
1082 association of a Drosophila-derived microbiota with its animal partner and
1083 the nutritional environment throughout a fly population's life cycle. *J Insect*
1084 *Physiol* **106**, 2–12.

1085 **Toda, H., Williams, J. A. and Gulledge, M.** (2019). A sleep-inducing gene, nemuri ,
1086 links sleep and immune function in Drosophila. *Science* **515**, 509–515.

1087 **Tower, J., Landis, G. N., Shen, J., Choi, R., Fan, Y., Lee, D. and Song, J.** (2017).
1088 Mifepristone/RU486 acts in Drosophila melanogaster females to counteract
1089 the life span-shortening and pro-inflammatory effects of male Sex Peptide.
1090 *Biogerontology* **18**, 413–427.

1091 **Tzou, P., Ohresser, S., Ferrandon, D., Capovilla, M., Reichhart, J. M., Lemaitre, B.,**
1092 **Hoffmann, J. A. and Imler, J. L.** (2000). Tissue-specific inducible expression
1093 of antimicrobial peptide genes in Drosophila surface epithelia. *Immunity* **13**,
1094 737–748.

1095 **Unckless, R. L., Howick, V. M. and Lazzaro, B. P.** (2016). Convergent Balancing
1096 Selection on an Antimicrobial Peptide in Drosophila. *Current Biology* **26**,
1097 257–262.

1098 **van Alphen, B., Stewart, S., Iwanaszko, M., Xu, F., Li, K., Rozenfeld, S.,**
1099 **Ramakrishnan, A., Itoh, T. Q., Sisobhan, S., Qin, Z., et al.** (2022). Glial
1100 immune-related pathways mediate effects of closed head traumatic brain
1101 injury on behavior and lethality in Drosophila. *PLoS Biol* **20**, e3001456.

1102 **van der Graaf, K., Srivastav, S., Singh, P., McNew, J. A. and Stern, M.** (2022). *The*
1103 *Drosophila* attP40 *docking site and derivatives are insertion mutations of*
1104 *MSP300*. Genetics.

1105 **Winans, N. J., Walter, A., Chouaia, B., Chaston, J. M., Douglas, A. E. and Newell, P.**
1106 **D.** (2017). A genomic investigation of ecological differentiation between free-
1107 living and *Drosophila* -associated bacteria. *Mol Ecol* **26**, 4536–4550.

1108 **Yamashita, K., Oi, A., Kosakamoto, H., Yamauchi, T., Kadoguchi, H., Kuraishi, T.,**
1109 **Miura, M. and Obata, F.** (2021). Activation of innate immunity during
1110 development induces unresolved dysbiotic inflammatory gut and shortens
1111 lifespan. *Dis Model Mech* **14**, dmm049103.

1112 **Zasloff, M.** (1987). Magainins, a class of antimicrobial peptides from Xenopus skin:
1113 isolation, characterization of two active forms, and partial cDNA sequence of
1114 a precursor. *Proc Natl Acad Sci U S A* **84**, 5449–5453.

1115 **Zerofsky, M., Harel, E., Silverman, N. and Tatar, M.** (2005). Aging of the innate
1116 immune response in \textlessi\textgreaterDrosophila
1117 melanogaster\textless/i\textgreater. *Aging Cell* **4**, 103–108.

1118 **Zhai, Z., Boquete, J. P. and Lemaitre, B.** (2018). Cell-Specific Imd-NF-κB Responses
1119 Enable Simultaneous Antibacterial Immunity and Intestinal Epithelial Cell
1120 Shedding upon Bacterial Infection. *Immunity* **48**, 897-910.e7.

1121 **Zhu, Y., Cai, Q., Zheng, X., Liu, L., Hua, Y., Du, B., Zhao, G., Yu, J., Zhuo, Z., Xie, Z., et**
1122 **al.** (2021). Aspirin Positively Contributes to *Drosophila* Intestinal
1123 Homeostasis and Delays Aging through Targeting Imd. *Aging and disease* **12**,
1124 1821.

1125

1126

Supplementary figure captions

Figure S1: Lifespan of various fly genotypes at 29°C. Male flies are shown in Figure 1. A) Comparison of lifespan from early experiments using two wild-types (*iso* w^{1118} and *OR-R*) alongside compound AMP mutants lacking *Def, Dro, AttA,B,C, Mtk,* and *DptA,B*, which are deleted in *ΔAMP Chr2* flies. B) Effect of *nora* clearance on lifespan of *iso* w^{1118}*, OR-R, AttC, Bom,* and *GrC* genotypes. Median lifespans are shown in the right panel (** P < .01, *** P < .001). Number of independent experiments (n^{exp}) is reported. C) *Nora* titres measured by CT value in 18°C source stocks (Stocks) or *nora*-positive flies aged 3+ weeks kept at densities of 20 flies per vial (Aged flies). CT values represent *nora* titre from 5ng total fly RNA per 10µL qPCR reaction.

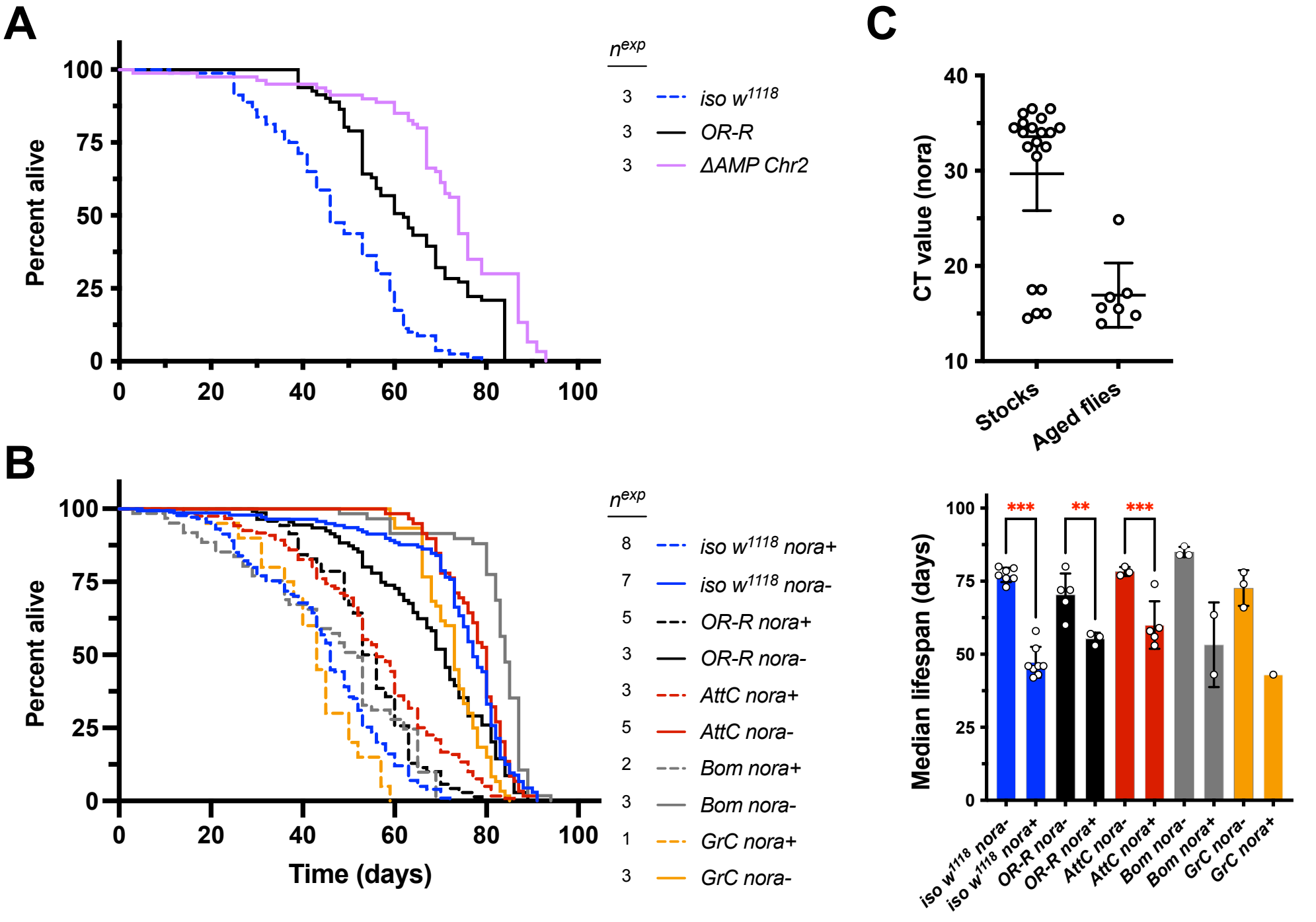
Figure S2: Lifespan of various fly genotypes at 29°C. A) *Relish* mutant (Rel^{E20}) and compound AMP mutants (*ΔAMP14*) suffer significantly reduced lifespan at 29°C. B) Median lifespan data from Figure S2A. C) Climbing pass rates of flies reared at 29°C. mth^{1} flies are the only fly line that retains climbing competence into old age.

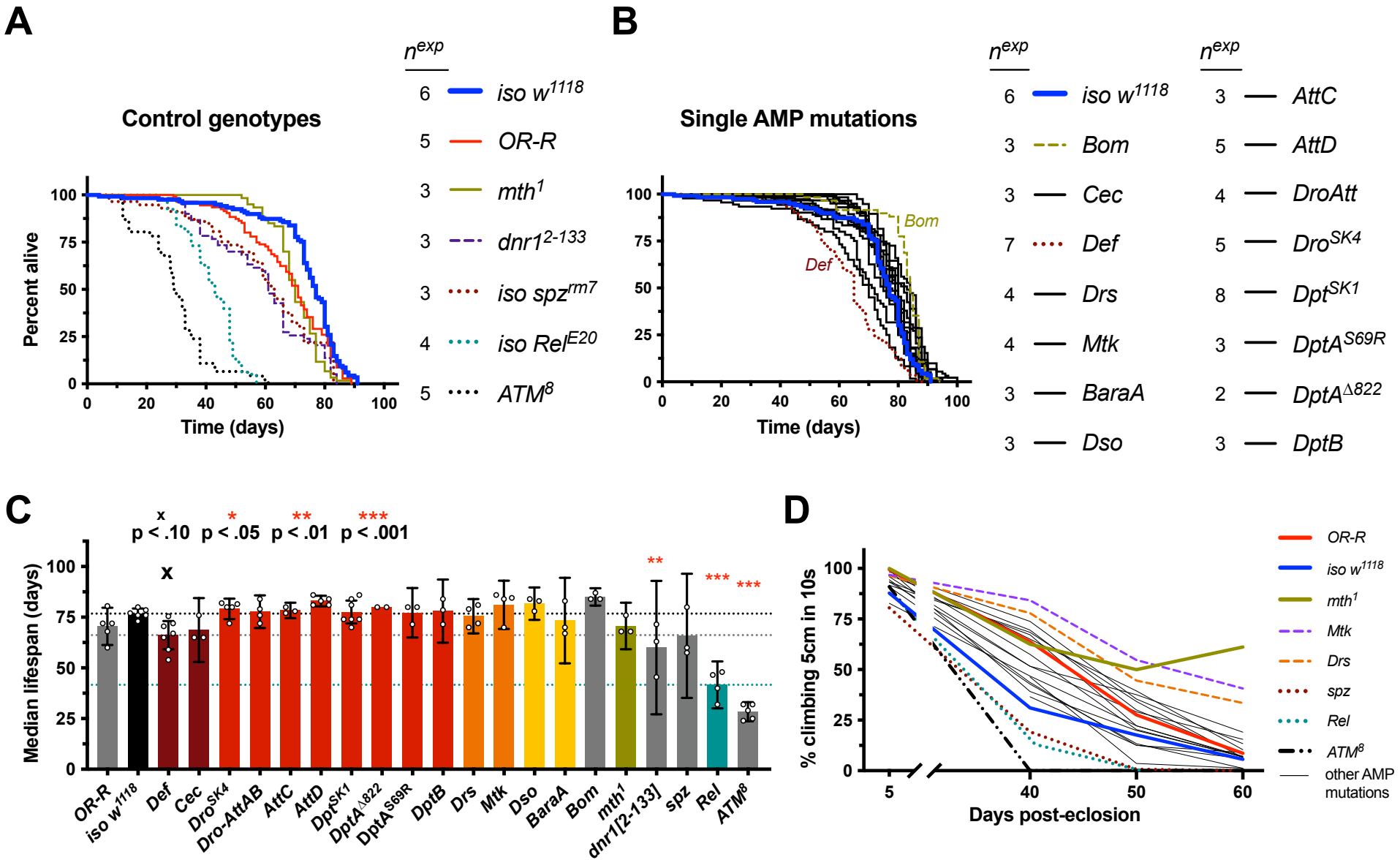
Figure S3: Individual AMP gene deletions do not drastically affect lifespan. Male flies shown in Figure 2. A) Cumulative lifespans of female flies with various genetic backgrounds. Of note, ATM^{8} data are based on fewer individuals per experiment (see File S1). B) Cumulative lifespans of single gene AMP mutants. Most AMP mutant lifespans (black lines) cluster around the wild-type (blue line), except Def^{SK3}. C) Median lifespans where each data point represents one replicate experiment (cumulative of 20 females). Median lifespan analysis suggests that the only AMP mutation statistically differing from *iso* w^{1118} was Def^{SK3}. Of note, the impact of *Def* on lifespan was not corroborated using *Defensin* RNAi (Fig. S4). Horizontal dotted lines indicate median lifespans of *iso* w^{1118} (top), Def^{SK3} (middle), or Rel^{E20} (bottom). Statistic summaries (p-values: ^{x}, *, **, ***) reflect comparisons to *iso* w^{1118}. D) Climbing pass rates suggest most AMP mutants climb like wild-type flies, while *methuselah* mutants uniquely retain climbing competence into old age (also seen at 29°C, Fig. S2).

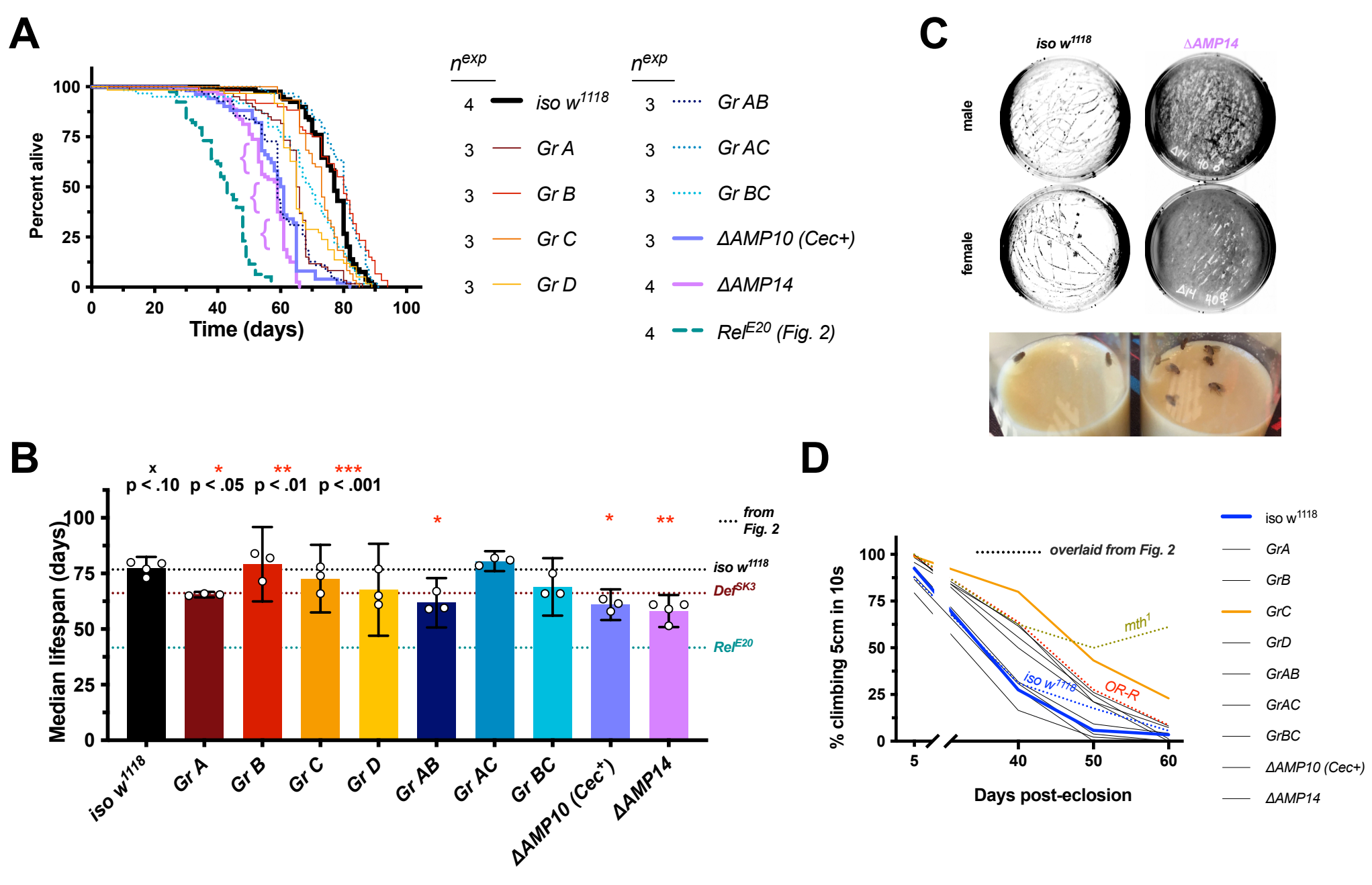
Figure S4: AMP gene silencing ubiquitously or in glia emphasizes the importance of reference genotypes for lifespan context. Males (M) and females (F) shown in separate panels. A) Comparison of multiple *Actin5C-Gal4>AMP-IR* lines shows possible major lifespan effects of Act>Def-IR, with males having extended lifespan compared to controls, while females had reduced lifespan. However these data are exactly opposite to trends from Def^{SK3} mutant data, where males had reduced lifespan (Fig. 2) and females had comparable lifespan to wild-type (Fig. S3). B) In general, lifespan differences are not especially striking in the context of additional genetic background controls and references. For instance, female *Act>Drs-IR* lifespan is not very different from *OR/Drs-IR* control, suggesting the *Drs-IR* genetic background is long-lived independent of *Drs* knockdown. Similarly, despite a seeming lifespan extension effect by *Act>DptB-IR* in males, we found no lifespan effect in Dpt^{SK1} or *DptB* mutants, both deficient for *DptB* (Fig. 2).

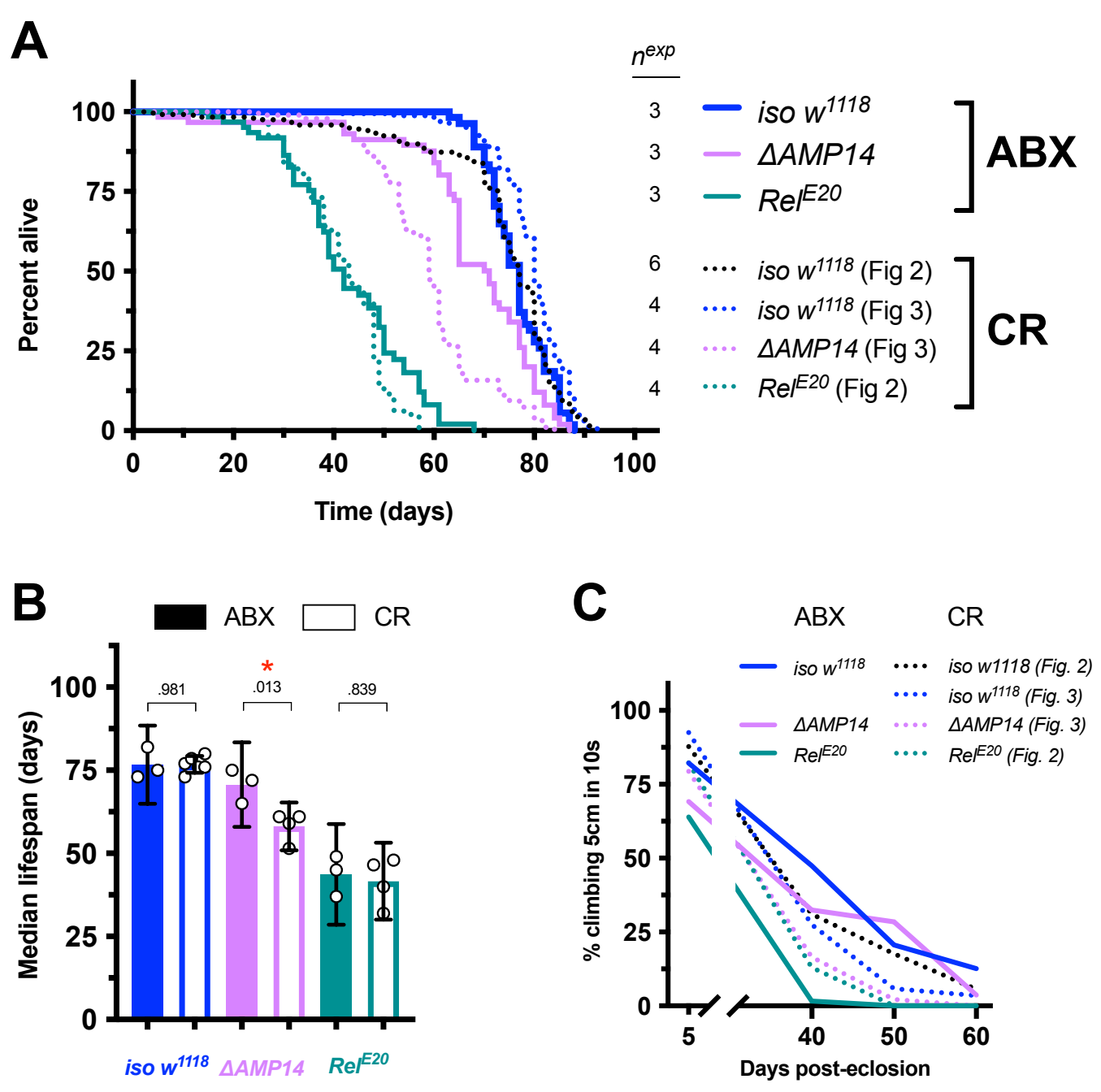
1172 **Figure S5: Climbing pass rate data overview from individual mutants.** Males (M)
1173 and females (F) shown in separate panels. A) Climbing pass rate curves over aging in
1174 males and females. AMP mutants displaying climbing rate within wild-type range are left
1175 with solid black lines (left panels). Of note, *Drs*^{R1} and *Mtk*^{R1} are the only mutations which
1176 are caused by the insertion of *white*$^{+}$ transgenes. Thus, we cannot exclude that their
1177 improved climbing effect results from the presence of *white*$^{+}$ compared to *iso w*^{1118} and
1178 other AMP mutants. In line with this idea, *OR-R white*$^{+/+}$ wild-type flies similarly trended
1179 towards better climbing competence than *iso w*^{1118} flies, although many factors likely
1180 distinguish these two genetic backgrounds.
1181
1182 **Figure S6: *ΔAMP14* flies have significantly reduced lifespan.** Male flies are shown in
1183 Figure 3. A) survival curves of various compound AMP mutants. The lifespan of *Rel*^{E20}
1184 from Fig. S3 is overlaid for direct comparison. B) Median lifespans of compound AMP
1185 mutants. Dotted lines indicate average median lifespans from Fig. S3 of *iso w*^{1118} (top),
1186 *Def*^{SK3} alone (middle), and *Rel*^{E20} (bottom) for easier comparisons across figures.
1187 Statistic summaries (p-value: ˣ, *, **, ***) reflect comparisons to *iso w*^{1118} data
1188 specific to Figure S6. C) Climbing pass rates of AMP group mutants, with climbing
1189 curves from genotypes in Fig. S3 overlaid for direction comparison. *Group C* is
1190 highlighted for having a slightly improved climbing over aging, though this improvement
1191 is still minor compared to the climbing competence of *mth*^{1} flies (but see Fig. S5 caption
1192 and File S4).
1193
1194 **Figure S7: Microbiome depletion rescues the *ΔAMP14* fly lifespan.** Female flies are
1195 reported here and male flies in Figure 4. A) Survival curves, including both antibiotic-
1196 reared flies (ABX), and also conventionally-reared (CR) lifespans from previous figures
1197 as dotted lines for direct comparison. B) Median lifespans, including both ABX and CR
1198 fly lifespans for direct comparison. (conventionally-reared *iso w*^{1118} and *Rel*^{E20} lifespans
1199 shown in Fig. 2C). C) Climbing pass rates of ABX (solid lines) and CR (dotted lines)
1200 flies at 5, 40, 50, and 60 days post-eclosion. Of note, antibiotic treatment did not rescue
1201 female Δ*AMP14* lifespan to the same extent as in males *(ΔAMP14-ABX vs. ΔAMP14-CR*,
1202 P = .354). The general trend of the antibiotic treatment remains the same as in males:
1203 Δ*AMP14* flies lived longer in ABX conditions than CR flies (consistent with visual
1204 inspection in Figure S7A). Indeed, using a more standard CoxPH mixed model, the
1205 rescue effect of antibiotics in Δ*AMP14* females is significant (*P* = .004). Dissecting the
1206 effect at the level of sex*genotype interactions in our study should still be interpreted
1207 with caution, as any sex*genotype interaction is indistinguishable from 'vial effects' due
1208 to our experimental design, particularly important as microbiome development in
1209 conventionally-reared AMP mutants in more stochastic than control flies (Marra et al.,
1210 2021). A full discussion is provided in File S4 (supplemental text).
1211
1212 **Figure S8: Silencing *Relish* in glia does not extend lifespan.** Males (M) and females
1213 (F) shown in separate panels. A) Silencing Relish in glia provides no lifespan benefit in
1214 males, and was even associated with reduced longevity in females (*Repo>Rel-IR*
1215 genotype: *UAS-Rel-IR/+; Repo-Gal4/+*). B) Median lifespans of *Repo* and *Rel-IR*
1216 combinations. P < .05 = "*". C) Climbing pass rates of flies with various combinations of
1217 *Repo* and *Rel-IR* systematic crosses showing linear progression of climbing competence

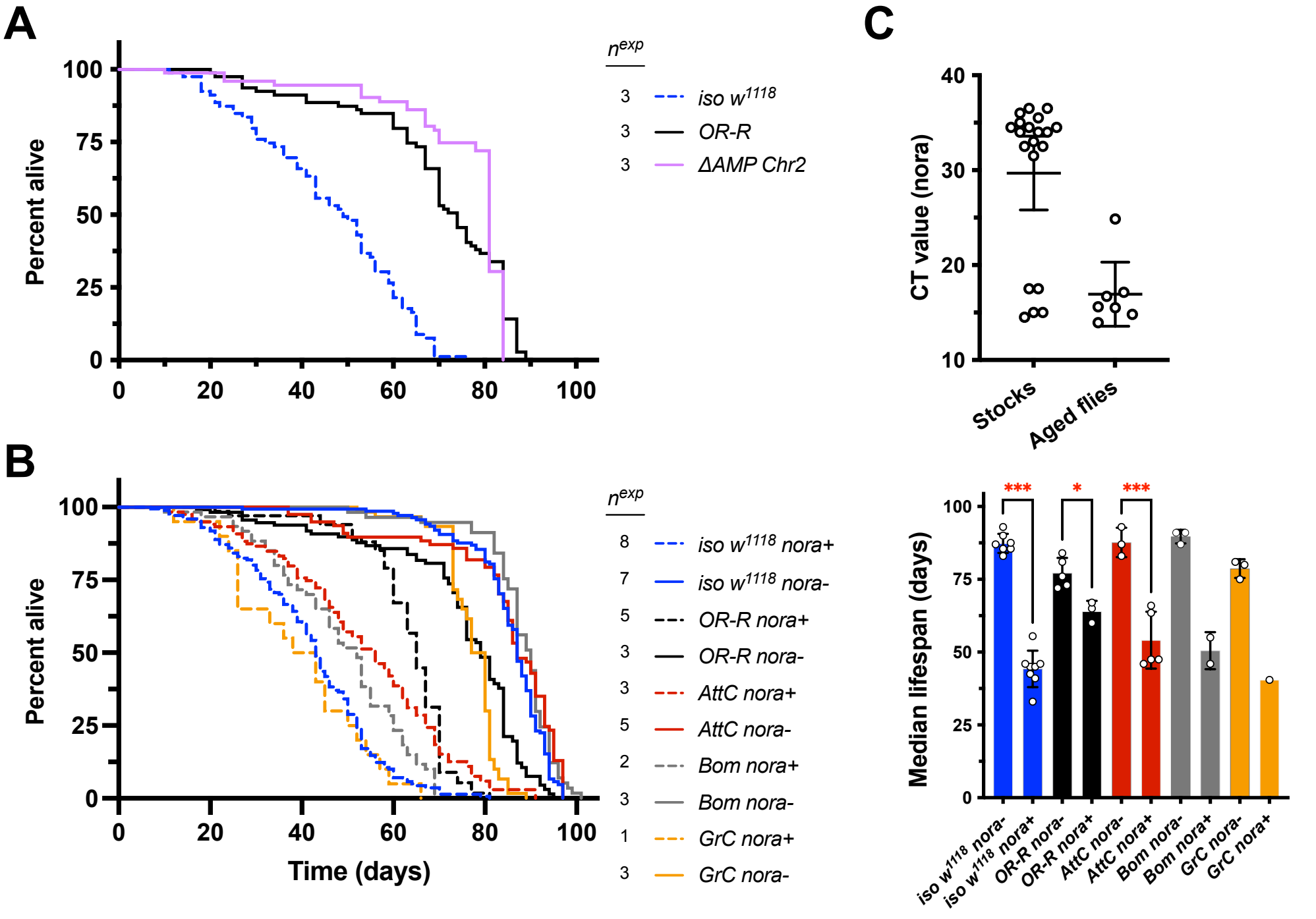
1218 loss. The *Repo-Gal4* genetic background seems to improve climbing competence into old
1219 age independent of the RNAi construct in males, and perhaps slightly in females. Of note,
1220 the lifespan and/or climbing of *OR-R* wild-type flies in these experiments was poor
1221 compared to the previous *OR-R* experiments shown in Fig. 2. We therefore included the
1222 Fig. 2 *OR-R* lifespan and climbing (and mth^1 climbing) in the present figure (A, C) for
1223 context, and for ease of direct comparisons.
1224
1225 **Table S1: No consistent signal of high AMP expression in the head upon aging.** We
1226 aged four wild-types (*Oregon-R, Exelexis,* w^{1118} *VDRC, and Canton S*) for 40 days and
1227 compared AMP expression in 40 day old flies to 5 day old flies, specifically in heads or
1228 decapitated bodies. While Imd-responsive AMPs were somewhat consistently
1229 upregulated in the body with aging, there was little consistent upregulation of AMPs in
1230 the head with aging. When present, upregulation was often minor compared to the
1231 expression seen during a systemic immune response for those AMP genes (e.g. at 40dpe
1232 *DptA* was induced anywhere from 2-50x in the head, or 5-130x in the body, while
1233 induction upon infection often reaches 500-1000x using the same normalization
1234 procedure). Underlying data and additional qPCR data from a separate experiment
1235 following AMP expression in the heads of aging flies at 8, 15, and 30 days are provided
1236 in File S2.
1237
1238 **File S1: Median lifespan and other summary statistics from all genotypes across all**
1239 **experiments, separated by genotype * experiment * sex.** Tabs within this excel file are
1240 named according to which experiment they relate to. Full survival raw data are uploaded
1241 in File S5.
1242
1243 **File S2: AMP expression data in the head. Contains data related to Table S1 for**
1244 **four wild-type flies collected at 5dpe and 40dpe.** Includes additional data on AMP
1245 expression in the heads of aged flies at 8, 15, and 30 days post-eclosion.
1246
1247 **File S3: Description of fly stocks and qPCR primers used in this study.**
1248
1249 **File S4: Supplemental text.** This supplementary discussion addresses issues and
1250 limitation of our study that we encountered when combining mutations in the DrosDel
1251 isogenic background. We discuss the use of median lifespan as our primary readout.
1252 There is also discussion of the unique, but minor, climbing effect of *Mtk* and *Drs*
1253 mutation in the light of a recent study, but also with the caveat of Mtk^{R1} and Drs^{R1} having
1254 a $white^{+}$ transgene insertion that may affect aging and neurodegeneration. Also contains
1255 discussion of the consideration of sex*genotype interactions as vial effects, and how
1256 future studies may better assess AMP interactions with sex and aging.
1257
1258 **File S5: Raw data for all experiments.**

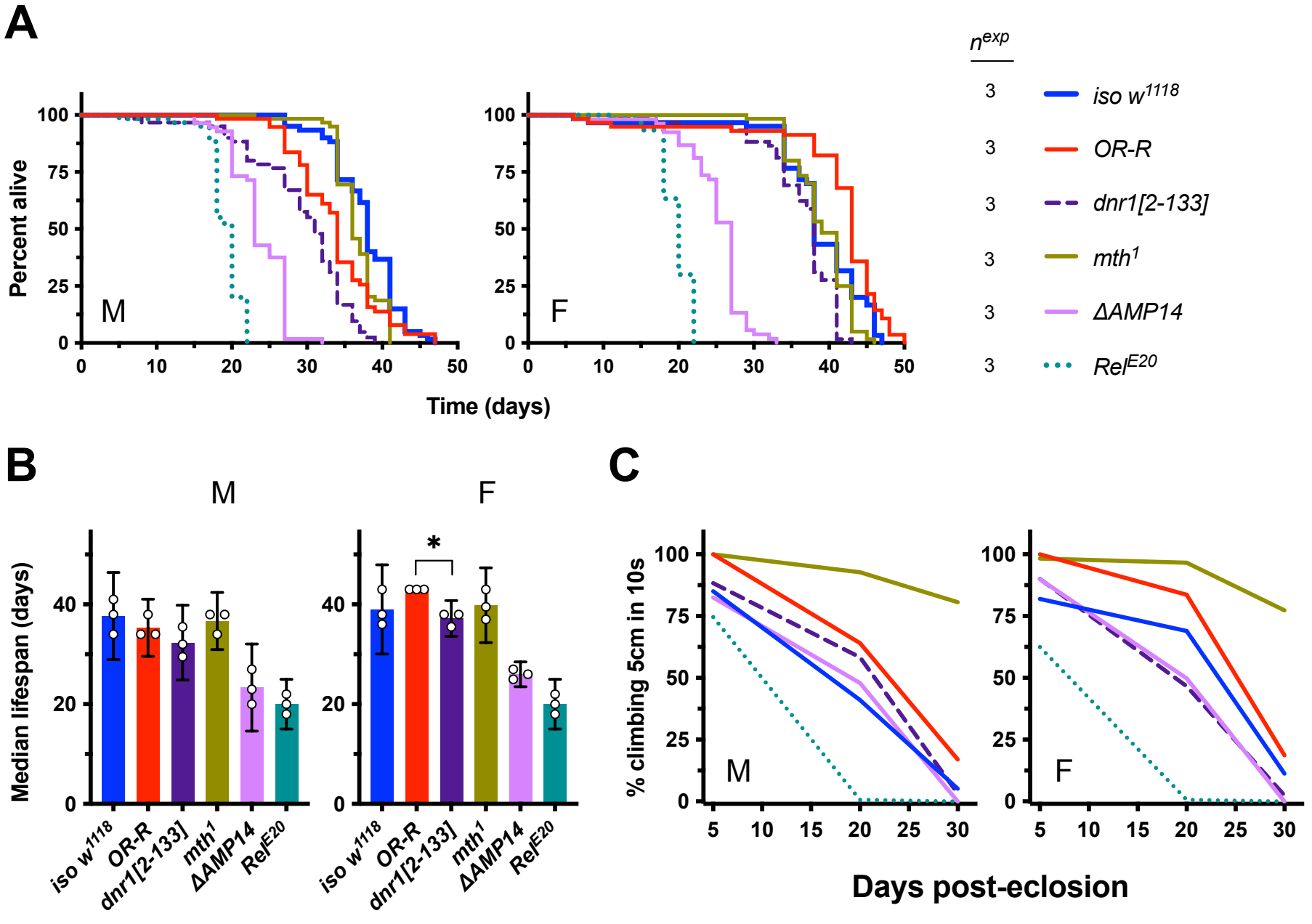

A
Percent alive
100
75
50
25
0
0
20
40
60
80
100
n^{exp}
3 iso w^{1118}
3 OR-R
3 ΔAMP Chr2
B
Percent alive
Time (days)
n^{exp}
8 iso w^{1118} nora+
7 iso w^{1118} nora-
5 OR-R nora+
3 OR-R nora-
3 AttC nora+
5 AttC nora-
2 Bom nora+
3 Bom nora-
1 GrC nora+
3 GrC nora-
C
CT value (nora)
40
30
20
10
Stocks
Aged flies
Median lifespan (days)
***
**
***
iso w^{1118} nora-
iso w^{1118} nora+
OR-R nora-
OR-R nora+
AttC nora-
AttC nora+
Bom nora-
Bom nora+
GrC nora-
GrC nora+

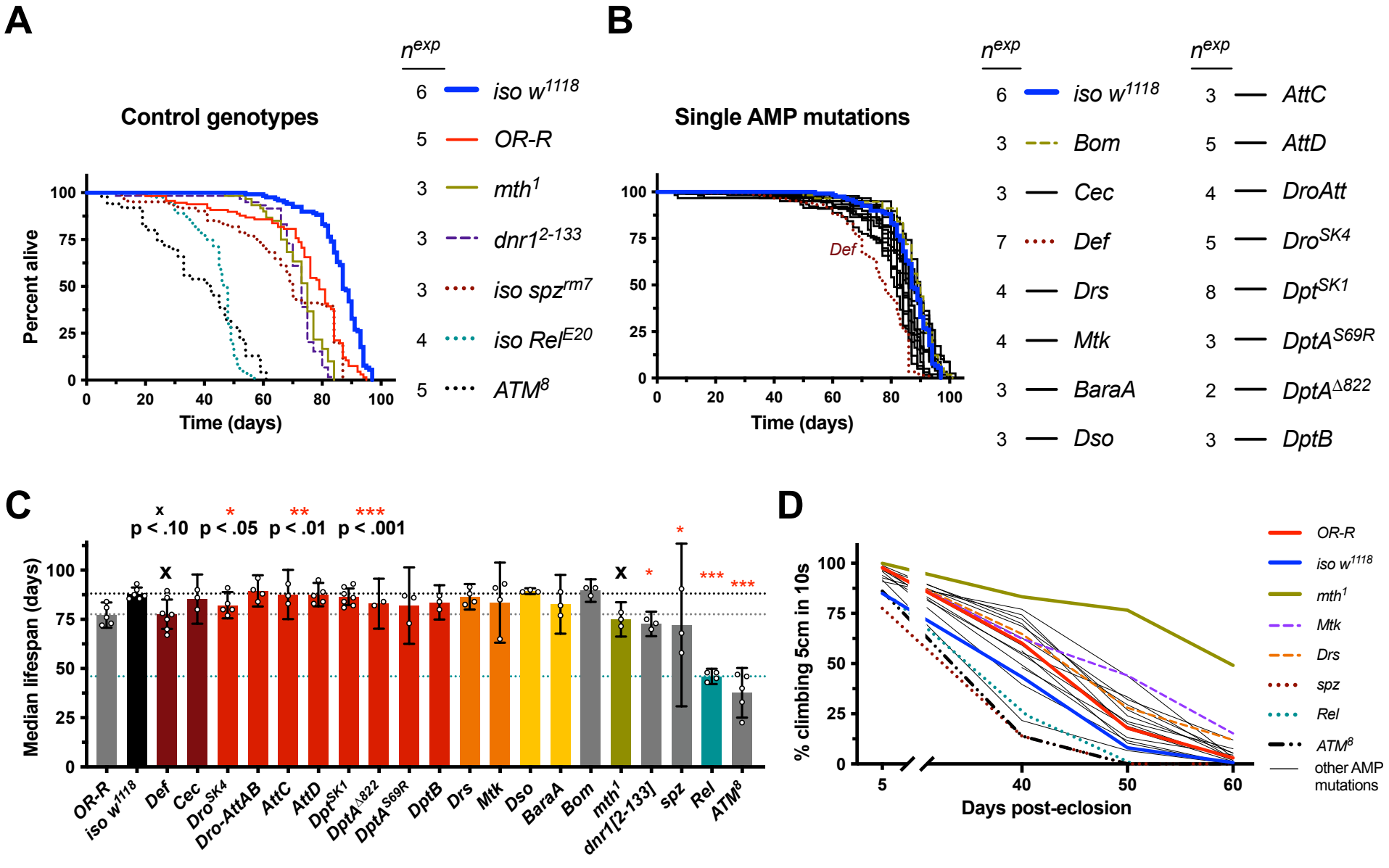

A
Control genotypes
Percent alive
Time (days)
n^exp
6 iso w^1118
5 OR-R
3 mth^1
3 dnr1^2-133
3 iso spz^rm7
4 iso Rel^E20
5 ATM^8
B
Single AMP mutations
Bom
Def
Time (days)
n^exp
6 iso w^1118
3 Bom
3 Cec
7 Def
4 Drs
4 Mtk
3 BaraA
3 Dso
n^exp
3 AttC
5 AttD
4 DroAtt
5 Dro^SK4
8 Dpt^SK1
3 DptA^S69R
2 DptA^Δ822
3 DptB
C
p < .10 p < .05 p < .01 p < .001
Median lifespan (days)
OR-R
iso w^1118
Def
Cec
Dro^SK4
Dro-AttAB
AttC
AttD
Dpt^SK1
DptA^Δ822
DptA^S69R
DptB
Drs
Mtk
Dso
BaraA
Bom
mth^1
dnr1[2-133]
spz
Rel
ATM^8
D
% climbing 5cm in 10s
Days post-eclosion
OR-R
iso w^1118
mth^1
Mtk
Drs
spz
Rel
ATM^8
other AMP mutations

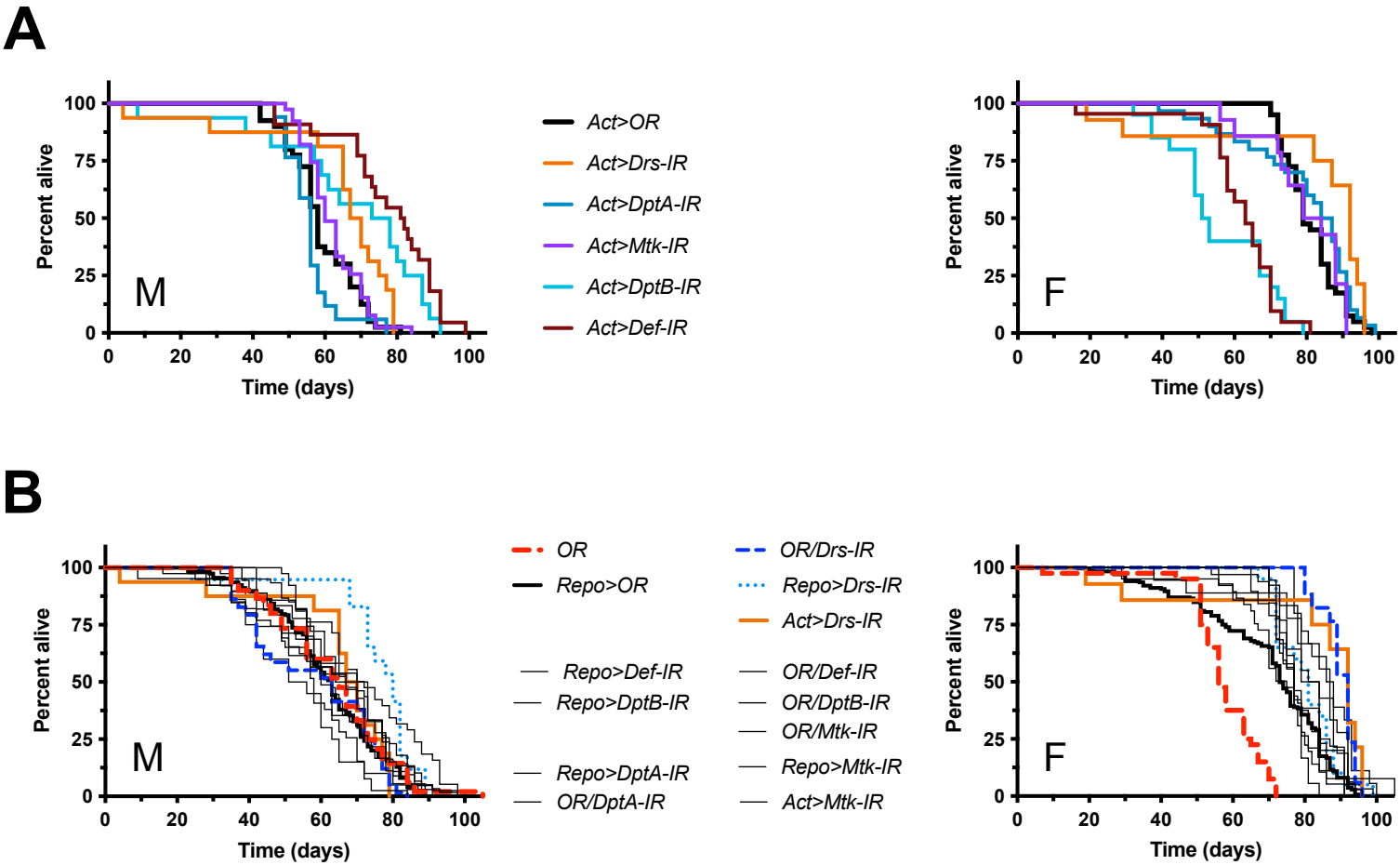

A
Percent alive
Time (days)
n^exp
4 iso w1118
3 Gr A
3 Gr B
3 Gr C
3 Gr D
3 Gr AB
3 Gr AC
3 Gr BC
3 ΔAMP10 (Cec+)
4 ΔAMP14
4 RelE20 (Fig. 2)
B
x p < .10
* p < .05
** p < .01
*** p < .001
Median lifespan (days)
.... from Fig. 2
iso w1118
DefSK3
RelE20
iso w1118
Gr A
Gr B
Gr C
Gr D
Gr AB
Gr AC
Gr BC
ΔAMP10 (Cec+)
ΔAMP14
C
iso w1118
ΔAMP14
male
female
D
% climbing 5cm in 10s
Days post-eclosion
overlaid from Fig. 2
mth1
OR-R
iso w1118
iso w1118
GrA
GrB
GrC
GrD
GrAB
GrAC
GrBC
ΔAMP10 (Cec+)
ΔAMP14

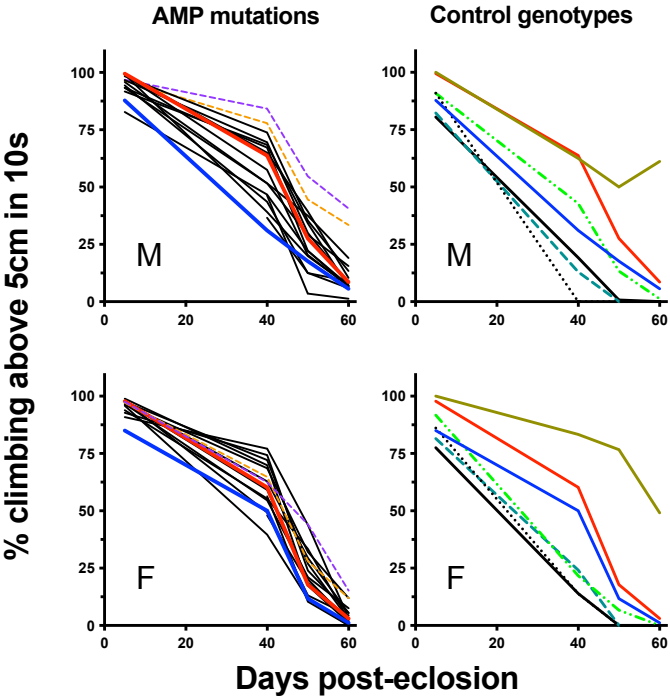

A
n^{exp}
3 iso w^{1118}
3 ΔAMP14
3 Rel^{E20}
ABX
6 iso w^{1118} (Fig 2)
4 iso w^{1118} (Fig 3)
4 ΔAMP14 (Fig 3)
4 Rel^{E20} (Fig 2)
CR
Percent alive
Time (days)
B
ABX
CR
Median lifespan (days)
.981
.013
.839
iso w^{1118}
ΔAMP14
Rel^{E20}
C
ABX
CR
iso w^{1118}
ΔAMP14
Rel^{E20}
iso w1118 (Fig. 2)
iso w^{1118} (Fig. 3)
ΔAMP14 (Fig. 3)
Rel^{E20} (Fig. 2)
% climbing 5cm in 10s
Days post-eclosion


A
Percent alive
100
75
50
25
0
0
20
40
60
80
100
n^{exp}
3 iso w^{1118}
3 OR-R
3 ΔAMP Chr2
B
Percent alive
Time (days)
n^{exp}
8 iso w^{1118} nora+
7 iso w^{1118} nora-
5 OR-R nora+
3 OR-R nora-
3 AttC nora+
5 AttC nora-
2 Bom nora+
3 Bom nora-
1 GrC nora+
3 GrC nora-
C
CT value (nora)
40
30
20
10
Stocks
Aged flies
Median lifespan (days)
***
*
***
iso w^{1118} nora-
iso w^{1118} nora+
OR-R nora-
OR-R nora+
AttC nora-
AttC nora+
Bom nora-
Bom nora+
GrC nora-
GrC nora+


A
n^{exp}
3 iso w^{1118}
3 OR-R
3 dnr1[2-133]
3 mth^1
3 ΔAMP14
3 Rel^{E20}
Percent alive
Time (days)
M
F
B
Median lifespan (days)
iso w^{1118}
OR-R
dnr1[2-133]
mth^1
ΔAMP14
Rel^{E20}
*
C
% climbing 5cm in 10s
Days post-eclosion

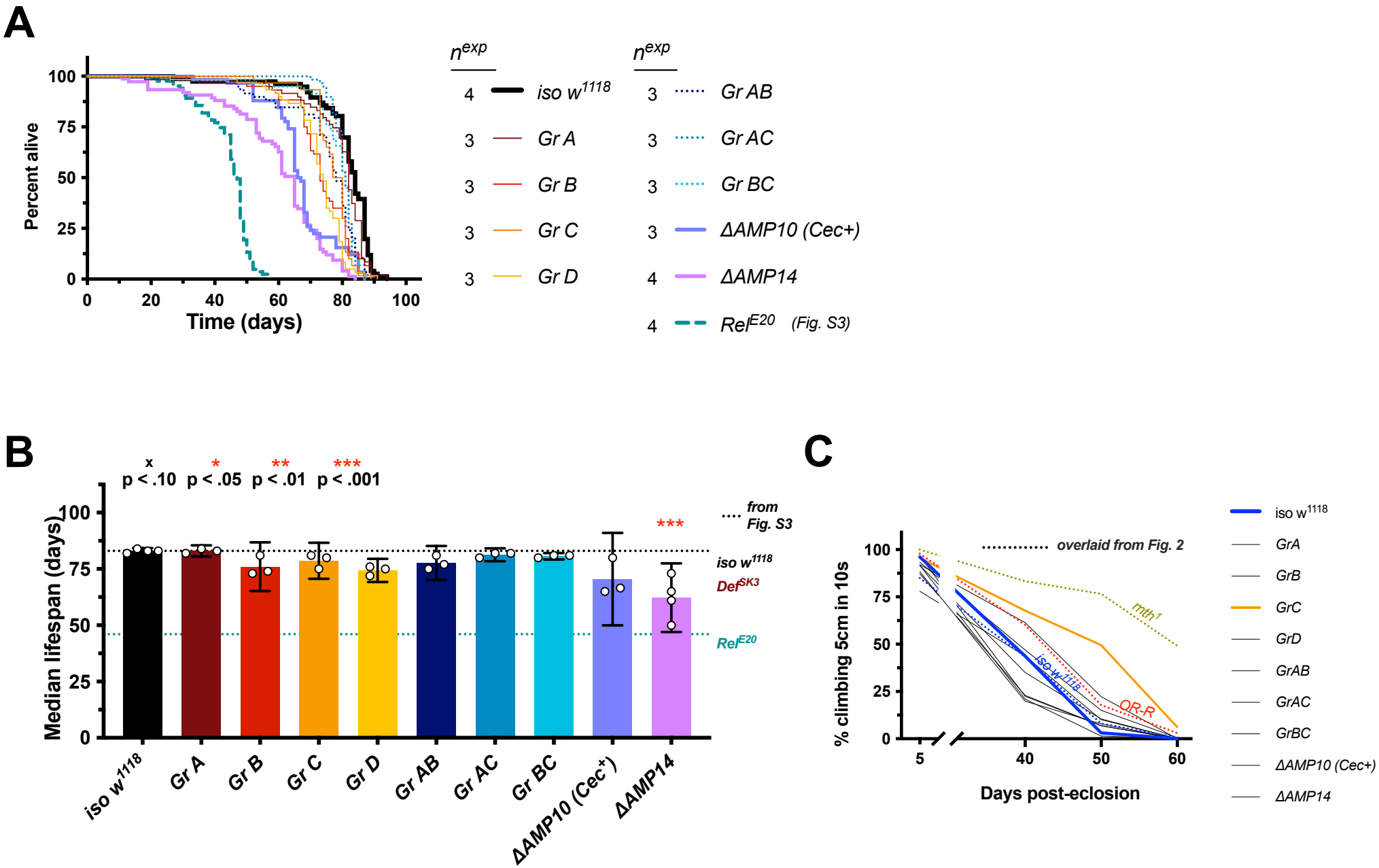

A
Control genotypes
Percent alive
Time (days)
n^exp
6 iso w^1118
5 OR-R
3 mth^1
3 dnr1^2-133
3 iso spz^rm7
4 iso Rel^E20
5 ATM^8
B
Single AMP mutations
Def
Time (days)
n^exp
6 iso w^1118
3 Bom
3 Cec
7 Def
4 Drs
4 Mtk
3 BaraA
3 Dso
n^exp
3 AttC
5 AttD
4 DroAtt
5 Dro^SK4
8 Dpt^SK1
3 DptA^S69R
2 DptA^Δ822
3 DptB
C
p < .10 p < .05 p < .01 p < .001
Median lifespan (days)
OR-R
iso w^1118
Def
Cec
Dro^SK4
Dro-AttAB
AttC
AttD
Dpt^SK1
DptA^Δ822
DptA^S69R
DptB
Drs
Mtk
Dso
BaraA
Bom
mth^1
dnr1[2-133]
spz
Rel
ATM^8
D
% climbing 5cm in 10s
Days post-eclosion
OR-R
iso w^1118
mth^1
Mtk
Drs
spz
Rel
ATM^8
other AMP mutations

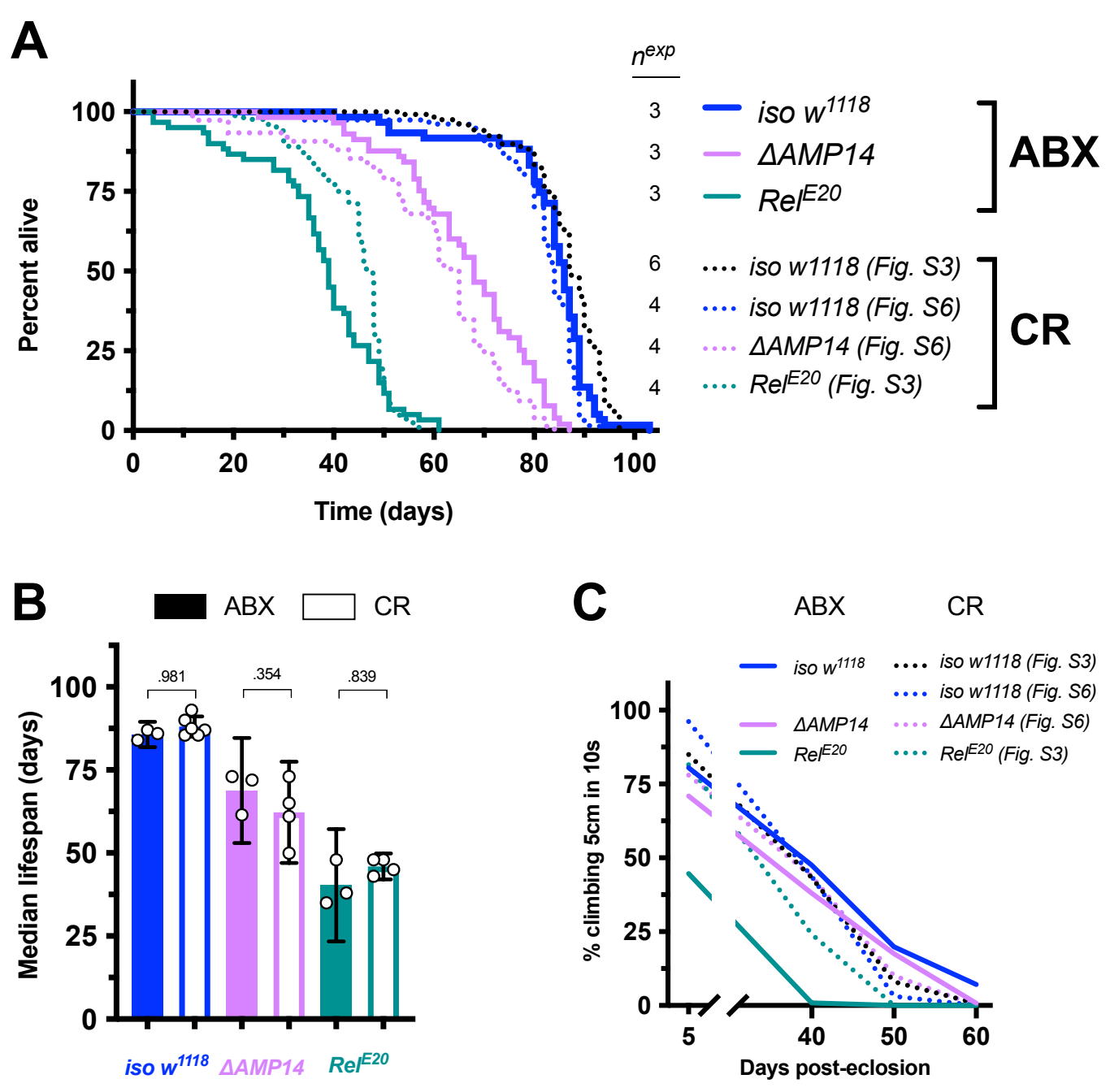

A
Percent alive
Time (days)
M
F
Act>OR
Act>Drs-IR
Act>DptA-IR
Act>Mtk-IR
Act>DptB-IR
Act>Def-IR
B
OR
Repo>OR
Repo>Def-IR
Repo>DptB-IR
Repo>DptA-IR
OR/DptA-IR
OR/Drs-IR
Repo>Drs-IR
Act>Drs-IR
OR/Def-IR
OR/DptB-IR
OR/Mtk-IR
Repo>Mtk-IR
Act>Mtk-IR

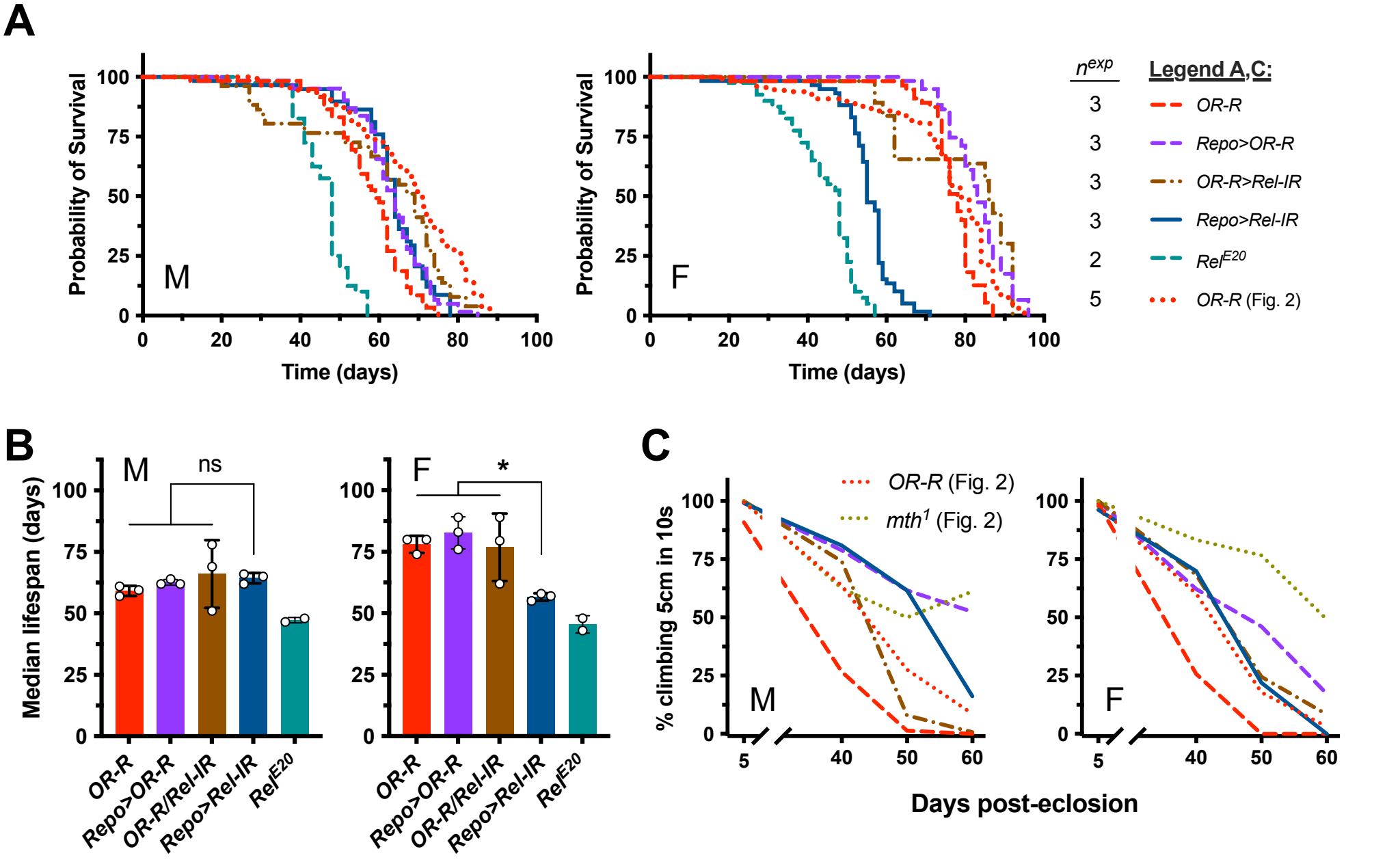


Legend (A):

- *iso w*^{1118}
- *OR-R*
- *Bom*
- *Def*
- *Cec*
- *Drs*
- *Mtk*
- *BaraA*
- *Dso*
- *AttC*
- *AttD*
- *Dro-AttAB*
- *Dro*^{SK4}
- *Dpt*^{SK1}
- *DptA*^{S69R}
- *DptA[822]*
- *DptB*
- *mth*^{1}
- *dnr1[2-133]*
- *spz*
- *Rel*
- *ATM*^{8}


A
Percent alive
Time (days)
n^exp
4 iso w1118
3 Gr A
3 Gr B
3 Gr C
3 Gr D
3 Gr AB
3 Gr AC
3 Gr BC
3 ΔAMP10 (Cec+)
4 ΔAMP14
4 RelE20 (Fig. S3)
B
x p < .10
* p < .05
** p < .01
*** p < .001
Median lifespan (days)
from Fig. S3
iso w1118
DefSK3
RelE20
iso w1118
Gr A
Gr B
Gr C
Gr D
Gr AB
Gr AC
Gr BC
ΔAMP10 (Cec+)
ΔAMP14
C
% climbing 5cm in 10s
Days post-eclosion
overlaid from Fig. 2
mth1
iso w1118
OR-R
iso w1118
GrA
GrB
GrC
GrD
GrAB
GrAC
GrBC
ΔAMP10 (Cec+)
ΔAMP14


A
n^{exp}
3 iso w^{1118}
3 ΔAMP14
3 Rel^{E20}
ABX
6 iso w1118 (Fig. S3)
4 iso w1118 (Fig. S6)
4 ΔAMP14 (Fig. S6)
4 Rel^{E20} (Fig. S3)
CR
Percent alive
Time (days)
B
ABX
CR
Median lifespan (days)
.981
.354
.839
iso w^{1118}
ΔAMP14
Rel^{E20}
C
ABX
CR
iso w^{1118}
ΔAMP14
Rel^{E20}
iso w1118 (Fig. S3)
iso w1118 (Fig. S6)
ΔAMP14 (Fig. S6)
Rel^{E20} (Fig. S3)
% climbing 5cm in 10s
Days post-eclosion


A
Probability of Survival
Time (days)
M
F
n^{exp}
Legend A,C:
3 OR-R
3 Repo>OR-R
3 OR-R>Rel-IR
3 Repo>Rel-IR
2 Rel^{E20}
5 OR-R (Fig. 2)
B
Median lifespan (days)
ns
*
OR-R
Repo>OR-R
OR-R/Rel-IR
Repo>Rel-IR
Rel^{E20}
C
% climbing 5cm in 10s
OR-R (Fig. 2)
mth^1 (Fig. 2)
Days post-eclosion

↑ increases with age ↓ decreases with age ≈ about equal after aging

Wild-type	**Body** *(sans head)*				**Head**			
	DptA	*Drs*	*AttA*	*Dro*	*DptA*	*Drs*	*AttA*	*Dro*
Oregon-R	↑	↑	↑↑↑	↑↑↑	↑	↓	↓↓↓	↓↓↓
Exelexis	↑↑	≈	↑↑	↑↑↑	↑	↑	≈	↑↑
w^{1118} VDRC	↑	↓	↑	≈	↑↑	↓	≈	↑↑
Canton S	≈	≈	↑	≈	≈	≈	≈	↓
Overall trend:	up	no change	up	mixed	up	no change	no change	mixed