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Manipulation of *Sod1* expression ubiquitously, but not in the nervous system or muscle, impacts age-related parameters in *Drosophila*

Ian Martin[1,2], **Melanie A. Jones**[2], and **Mike Grotewiel**[1,2]

[1]Neuroscience Program, Virginia Commonwealth University, Richmond, VA.

[2]Department of Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA.

Abstract

Superoxide dismutase 1 (SOD1) is an important antioxidant previously shown to impact life span in *Drosophila*. We examined the consequences of manipulating *Sod1* expression throughout the body or in the nervous system or musculature on life span and age- related locomotor impairment (ARLI) in *Drosophila*. Ubiquitous overexpression of SOD1 extended life span but did not substantially forestall ARLI, whereas ubiquitous knock-down of *Sod1* shortened life span and accelerated ARLI. Interestingly, neither overexpression of *Sod1* nor expression of *Sod1* RNAi in the nervous system or muscle altered life span or ARLI. Our studies suggest that the control of reactive oxygen species by SOD1 in tissues other than the nervous system and musculature support life span and ARLI in *Drosophila*.

Keywords

oxidative stress; life span; Gal4; RNA interference

1. Introduction

Oxidative damage to macromolecules occurs largely via the action of reactive oxygen species (ROS) [1]. Cells contain a variety of antioxidants that convert ROS into less harmful molecules. Nevertheless, oxidative damage to proteins, nucleic acids and lipids occurs under both pathophysiological and normal physiological conditions [2]. Oxidative damage to macromolecules is consequently thought to contribute to numerous disease states as well as aging [3,4].

Studies in *Drosophila* show that manipulating antioxidant defenses impacts life span [5]. Conditional overexpression of superoxide dismutase 1 (SOD1, localized predominantly in cytosol) increases life span in flies [6,7]. Interestingly, overexpression of SOD1 in motor neurons is also reported to extend life span in flies [8]. Conversely, complete or partial loss of SOD1 activity in flies results in several deleterious phenotypes including sensitivity to

Corresponding Author: Mike Grotewiel, Department of Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA, 23298. ph: 804.628.4086; fax: 804.827.1124; email: E-mail: msgrotewiel@vcu.edu.

oxidative stress and early-onset mortality [8–10]. Studies in other species including *C. elegans* [11]and mice [12,13] also suggest that SOD activity influences life span. While these and other studies connect SOD1 activity to life span, it remains unclear whether SOD1-induced changes in survival are associated with alteration of other age-related features. Additionally, previous studies on SOD1 activity and life span do not address whether expression of the enzyme in selected tissues impacts the survival and function of organisms as a whole. Here, we addressed these issues by examining the consequences of augmenting and knocking-down SOD1 activity on life span and age-related locomotor impairments (ARLI) in *Drosophila*.

2. Materials and methods

2.1 Fly stocks and husbandry

Flies were grown and housed at 25°C/55% relative humidity under a 12 hour light-dark cycle. Flies were fed a sugar : yeast : cornmeal : agar medium (10% : 2% : 3.3% : 1% w/v) supplemented with 0.2% Tegosept (Sigma Chemical Co., St. Louis, MO) and active yeast. We used the Gal4/UAS system [14] to express a human *Sod1* transgene (*UAS-hSod1*) provided by Gabrielle Boulianne (Hospital for Sick Children) [8]. *Sod1* was knocked down using Gal4 to express a *Sod1* inverted repeat (*UAS-Sod1-IR*) transgene (obtained from the Vienna *Drosophila* RNAi Center (Vienna, Austria)). Gal4 lines were obtained from the following: *daughterless*-Gal4 (*da-Gal4*), John Phillips (University of Guelph); *Mef2-Gal4*, Sunita Gupta Kramer (Rutgers); *Appl-Gal4;* Lawrence Goldstein (University of California, San Diego); D42-Gal4, Gabrielle Boulianne (University of Toronto); *actin5C–Gal4*, 24B–Gal4, *repo-Gal4* and *elav-Gal4*, *Drosophila* Stock Center (Bloomington, IN); 91Y–Gal4 and 188Y–Gal4, J. Douglas Armstrong (University of Edinburgh).

2.2 SOD activity

3 groups of 25 males (1–3 days old) per genotype were collected via CO_2 anesthesia and homogenized in chilled 50 mM potassium phosphate/0.1 mM EDTA/2% Triton-X-100 buffer (pH 7.8). Samples were sonicated for 20 sec, incubated at 4°C for 45 min and then centrifuged at 16,000 x g for 15 min at 4°C. Equal amounts of total protein from the supernatants (determined with DC Protein Assay, Bio-Rad) were electrophoresed by Native PAGE (4% stacking gel (pH 6.8), 20% resolving gel (pH 8.8)). SOD activity was assessed colorimetrically using an “in-gel” assay as previously described [15]. SOD activity was quantified by densitometry using Alpha Imager software (Alpha Innotech Corp., San Leandro, CA).

2.3 Life span analysis

Adult males (1–3-days old, 150–200 flies/genotype) were collected under brief CO_2 anesthesia and placed in fresh food vials, 25 flies/vial. Every 2–4 days, flies were transferred to fresh food vials and the number of dead and surviving flies counted.

2.4 Negative geotaxis

Groups of 25 adult males (1–3 days old) were collected under brief CO_2 anesthesia and left to recover at least 18 hours at 25°C and 55% relative humidity prior to assessing negative geotaxis (bang-induced climbing) in Rapid Iterative Negative Geotaxis (RING) assays as previously described [16]. Five vials per genotype were tested to generate N=5. Following testing, flies were placed in fresh food vials for housing until the next RING test.

2.5 Statistical analyses

One- and two-way ANOVA, Tukey’s HSD multiple comparison tests, and log-rank tests were performed with JMP 5.01 (SAS Institute, Cary, NC, USA). P values greater than 0.05 were considered not significant (n.s.). Error bars represent S.E.M.

3. Results and Discussion

3.1 Ubiquitous over expression of hSod1 extends life span but minimally affects ARLI

Ubiquitous overexpression of human Sod1 (*hSod1*) via either *actin5C–Gal4* or *da-Gal4* increased SOD1 activity by approximately 3-fold (Figure 1A and 1B) without altering SOD2 activity (Figure 1A and 1C). Consistent with a previous study using a conditional expression strategy [6], we found that ubiquitous expression of *hSod1* driven by *actin5C–Gal4* or *da-Gal4* extended mean life span by 15–30% (Figure 1D and 1E). The increased life span in flies with ubiquitous *hSod1* overexpression suggested that other manifestations of aging might be delayed in these animals. To address this issue, we assessed ARLI (defined as the age-related impairment in negative geotaxis, a locomotor behavior [17]). Ubiquitous expression of *hSod1* via *actin5C–Gal4* modestly delayed ARLI (Figure 1F), whereas *hSod1* expression driven by *da-Gal4* did not (Figure 1G).

Although ubiquitous overexpression of *hSod1* extended life span, it did not substantially delay ARLI. Ubiquitous overexpression of *hSod1*, therefore, does not appear to impact all aspects of aging equally in flies. Interestingly, dietary restriction and a mutant allele of *methuselah* also extend life span in flies, but neither manipulation significantly forestalls locomotor or olfactory senescence [18,19]. The experimental dissociation between life span and behavioral senescence in flies with genetic or dietary manipulations is consistent with a model in which different aspects of aging are driven by distinct mechanisms.

3.2 Overexpression of hSod1 in the nervous system or muscle has no effect on life span

Due to their high metabolic demands with attendant high rates of ROS generation, neuronal and muscle cells are thought to experience more oxidative damage than other cells [2]. This has led to the hypothesis that these tissues may benefit the most from augmentation of antioxidant defenses. We therefore reasoned that ubiquitous *hSod1* overexpression might extend life span as a result of increased expression in the nervous system or musculature.

Consistent with the nervous system component of this hypothesis, expression of *hSod1* in motor neurons using D42-Gal4 was previously reported to extend life span in *Drosophila* [8]. To further investigate the effects of augmenting nervous system antioxidant defenses on life span, we assessed survival in flies expressing *hSod1* in motor neurons using D42-Gal4, in all neurons using *Appl*-Gal4 and *elav*-Gal4, and throughout the glia using *repo*-G*al4*. Surprisingly, we found that overexpression of *hSod1* in motor neurons did not extend life span (Figure 2A). Furthermore, neither pan-neuronal (Figure 2B and C) nor pan-glial (Figure 2D) overexpression of *hSod1* extended life span. Our results suggest that augmentation of antioxidant defenses selectively within the nervous system via overexpression of *hSod1* does not have substantive effects on life span in flies. We note, however, that there could be complex effects of genotype or environment that permit the observed life span effects of nervous system *hSod1* expression in some laboratories [8] while they preclude such effects in other experimental settings. For example, it would be interesting to determine whether the genetic background used in the previous studies [8] gives rise to elevated levels of oxidative stress as compared to the genetic background in our studies and whether this difference in oxidative stress might explain the divergent effects of *hSod1* overexpression on life span. Nevertheless, overexpression of *hSod1* in the nervous system does not appear to consistently extend life span in flies.

To determine whether increasing antioxidant defenses in muscle influences life span, we assessed survival of flies that expressed *hSod1* in this tissue via *Mef2-Gal4* or 24B–Gal4. Overexpression of *hSod1* in muscle did not alter life span (Figure 2E and 2F). Thus, overexpression of *hSod1* in the musculature is not sufficient to extend life span in *Drosophila*.

Our data suggest that the life span extension found in flies with ubiquitous *hSod1* expression is not due solely to increased SOD1 activity in the nervous system or musculature. It is possible, therefore, that augmentation of antioxidant defenses in all tissues via ubiquitous *hSod1* expression is required to extend life span in flies. An alternative possibility is that overexpression of *hSod1* in a key, unidentified tissue outside of the nervous system and musculature is required for life span extension in *Drosophila*.

3.3 Ubiquitous knock-down of Sod1 shortens life span and accelerates ARLI

Sod1 loss-of-function mutations and knock-down of *Sod1* function through RNA interference (RNAi) dramatically reduce life span in flies [20,21]. Toward further investigating the normal protective effects of *Sod1,* we knocked-down *Sod1* function via RNAi through Gal4-driven expression of a *UAS-Sod1*-inverted repeat (*Sod1-IR*) transgene. We confirmed that ubiquitous expression of *Sod1-IR* driven by *da-Gal4* substantially decreased SOD1 activity without altering SOD2 activity (Figure 3A and 3B). We also confirmed that ubiquitous expression of *Sod1-IR* substantially shortened life span (Figure 3C) and further found that it dramatically accelerated ARLI (Figure 3D).

3.4 Sod1-IR expression in the nervous system or muscle has modest effects on life span and ARLI

Overexpression of *hSod1* in the nervous system or musculature alone had no major effect on life span (Figure 2). Thus, SOD1 activity in these tissues might not be central to aging in flies. If true, then expression of *Sod1-IR* in the nervous system or muscle should not affect life span or other aspects of aging. Expression of *Sod1-IR* via the pan-neuronal Gal4 drivers 188Y and *elav* resulted in small but statistically discernable decreases in life span compared to controls harboring Gal4 or *Sod1-IR* alone (Figure 4A and 4B). *Sod1-IR* expression via two other pan-neuronal drivers, *Appl-Gal4* or 91Y–Gal4, however, did not significantly affect life span (Figure 4C and 4D). Expression of *Sod1-IR* in the muscle via 24B–Gal4 also resulted in a statistically significant, albeit small, reduction in life span (Figure 4E). In contrast, *Sod1-IR* expression in the muscle via *Mef2-Gal4* had no significant effect on life span (Figure 4F).

Expression of *Sod1-IR* throughout the nervous system using 188Y–Gal4 subtly but significantly impaired negative geotaxis across age relative to controls carrying either 188Y–Gal4 or *Sod1-IR* alone (Figure 5A). The locomotor defect in flies with 188Y–driven expression of *Sod1-IR* was restricted to thefirst 28days of their life, though, indicating that these lies have a generalized decrease in locomotor behavior as opposed to an age-dependent acceleration of ARLI. *Sod1-IR* expression in the nervous system via *elav*-Gal4 (Figure 5B), *Appl*-Gal4 (Figure 5C) or 91Y–Gal4 (Figure 5D) also did not accelerate ARLI. Furthermore, expression of *Sod1-IR* in the muscle via 24B–Gal4 (Figure 5E) had no effect on ARLI while *Mef2*-Gal4-driven *Sod1-IR* expression (Figure 5F) resulted in a subtle but statistically discernable acceleration in ARLI.

When viewed together, our studies indicate that expression of *Sod1-IR* in the nervous system or muscle has very limited effects on life span and ARLI in flies. Given that expression of the *Sod1 -IR* transgene efficiently knocks-down SOD1 activity in the whole body (Figure 3A) and that the nervous system and muscle Gal4 lines strongly express in their respective tissues (Martin et. al, submitted), the lack of effect of nervous system or muscle expression of *Sod1-IR* does not appear to be related to poor transgene function. SOD1 activity in either of these tissues alone, therefore, is not required to protect flies from premature mortality and locomotor demise with age. Since ubiquitous knock-down of *Sod1* has severe consequences on life span and ARLI, it is possible that the collective effects of *Sod1* throughout the body or in a tissue outside of the nervous system and musculature is important for normal life span and locomotor function across age in flies.

3.5 Summary

Our studies indicate that a ubiquitous increase or decrease in *Sod1* expression impacts life span and, to a limited extent, ARLI. These effects do not appear to be connected to altered expression of *Sod1* in the nervous system or musculature. Our studies also support the hypothesis that life span and ARLI are driven by distinct mechanisms in *Drosophila*.

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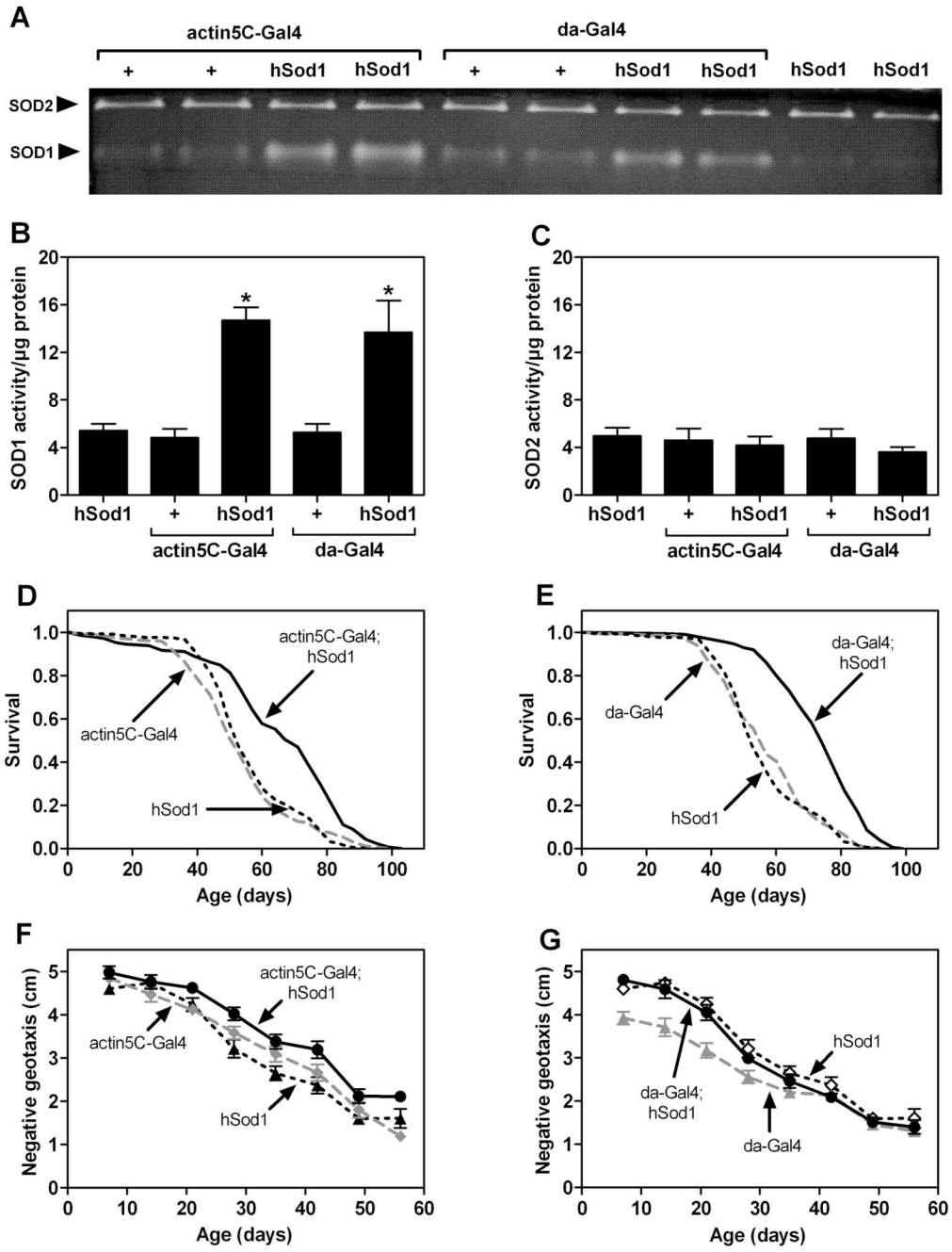

Figure 1.

Ubiquitous overexpression of *hSod1* extends life span and has minimal effects on ARLI. (A) In-gel SOD2 (upper band) and SOD1 (lower band) activity in replicate protein extracts (45µg/lane)from flies with the indicated genotypes. (B) SOD1 activity (determined by densitometry) was increased in flies expressing *hSod1* via *actin5C–Gal4* or *da-Gal4* compared to controls with single transgenes (one-way ANOVA, $p \leq 0.0045$; Tukey's HSD, * $p < 0.05$; n = 3). (C) SOD2 activity was not affected by *hSod1* overexpression (one-way ANOVA, n.s., n = 3). Survival was altered by expression of *hSod1* via *actin5C–Gal4* (D) or *da-Gal4* (E) relative to controls with single transgenes (log-rank tests, $p < 0.0001$, n = 200 flies/genotype). (F) Age and genotype affected negative geotaxis (two way ANOVA, $p < 0.0001$). Flies expressing

hSod1 via *actin5C–Gal4* performed significantly better than controls (Tukey's HSD, $p < 0.05$; n = 9–10 vials of 25 flies/genotype). (G) Although age and genotype affected negative geotaxis (two-way ANOVA, $p<0.0001$), flies with *da*-Gal4-driven expression of *hSod1* were not different from *hSod1* controls (Tukey's HSD, n.s., n = 9–10 vials of 25 flies/genotype).

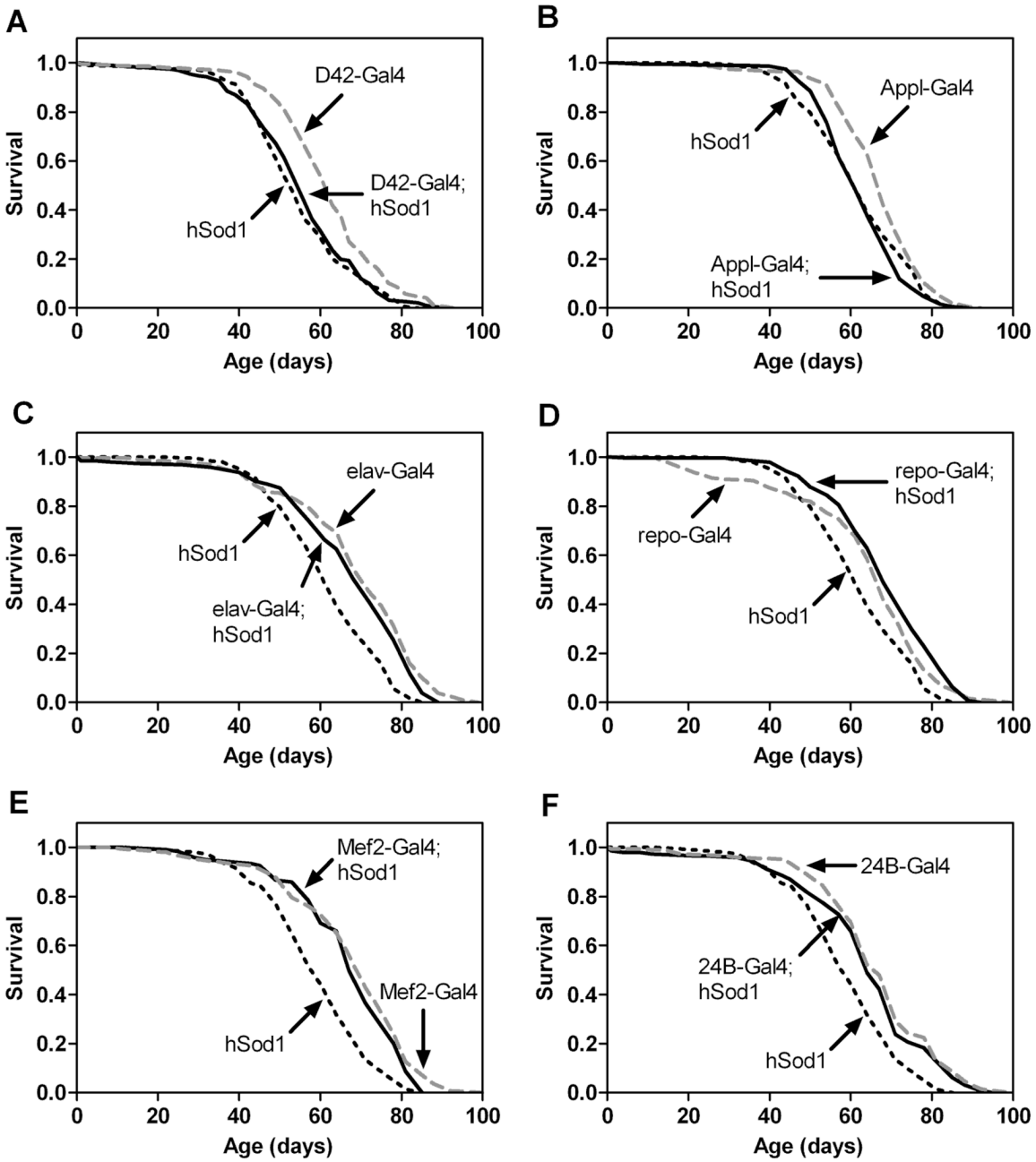

Figure 2.

Overexpression of *hSod1* in the nervous system or muscle has no effect on life span. Flies expressing *hSod1* via D42-Gal4 (A), *Appl-Gal4* (B), *elav-Gal4* (C), *repo-Gal4* (D), *Mef2*-Gal4 (E) or 24B–Gal4 (F) were not longer-lived than controls with single transgenes (log-rank tests, n.s., n = 150–200 flies/genotype).

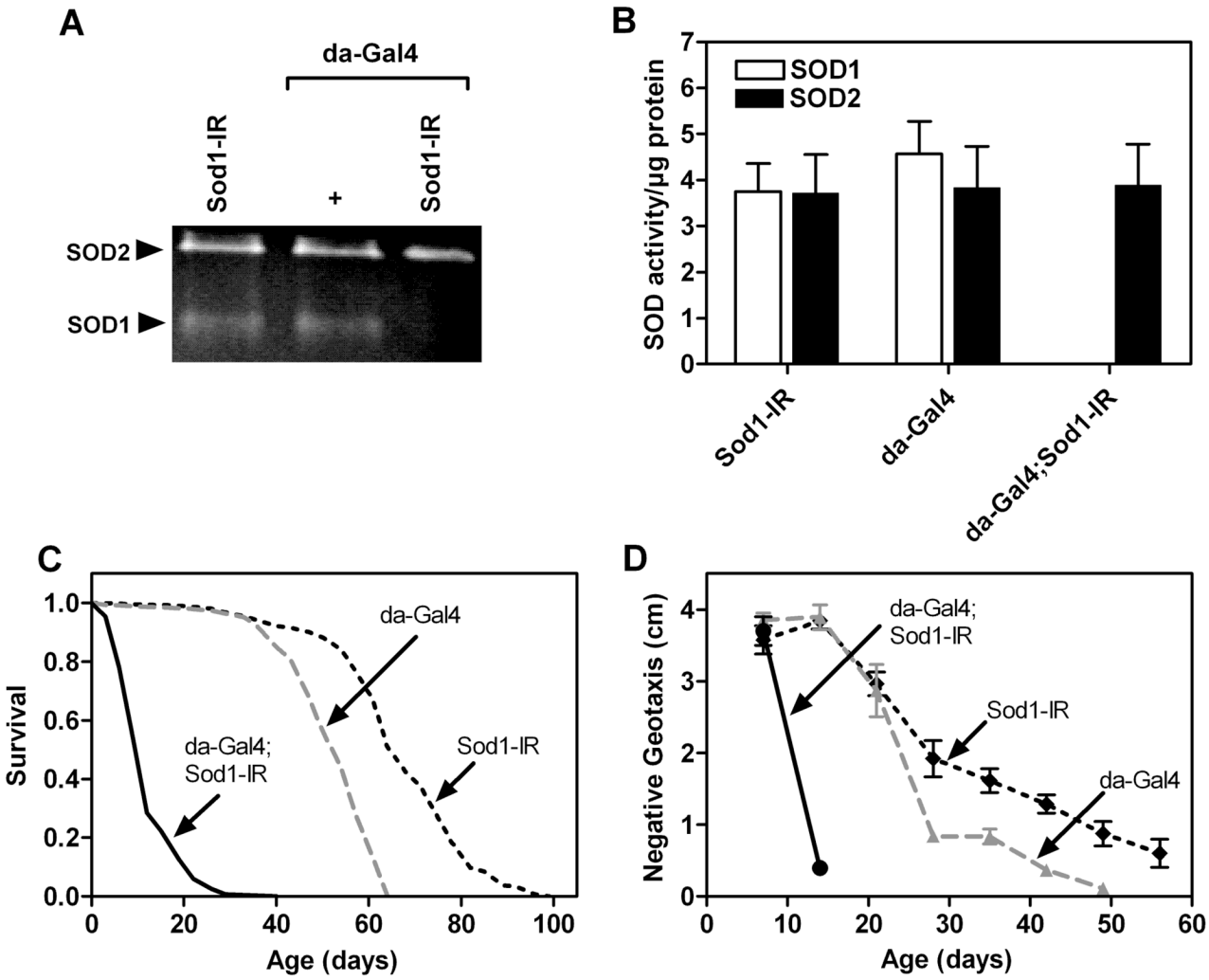

Figure 3.

Ubiquitous knock-down of SOD1 activity decreases life span and accelerates ARLI. (A) SOD1 and SOD2 activity in protein extracts (45 µg/lane) from the indicated genotypes. (B) SOD1 activity was reduced to below detectable levels in flies expressing *Sod1-IR* via *da-Gal4* (one-way ANOVA, $p < 0.0001$, $n = 3$) while SOD2 activity was unaffected (one-way ANOVA, n.s., $n = 3$). (C) Ubiquitous knock-down of *Sod1* significantly shortened life span relative to controls (log-rank tests, $p < 0.0001$, $n = 150$ flies/genotype). (D) ARLI was accelerated by ubiquitous *Sod1* knock-down relative to controls (one-way ANOVA, $p < 0.0001$; Tukey's HSD, $p < 0.05$; $n = 5$ vials of 25 flies/genotype).

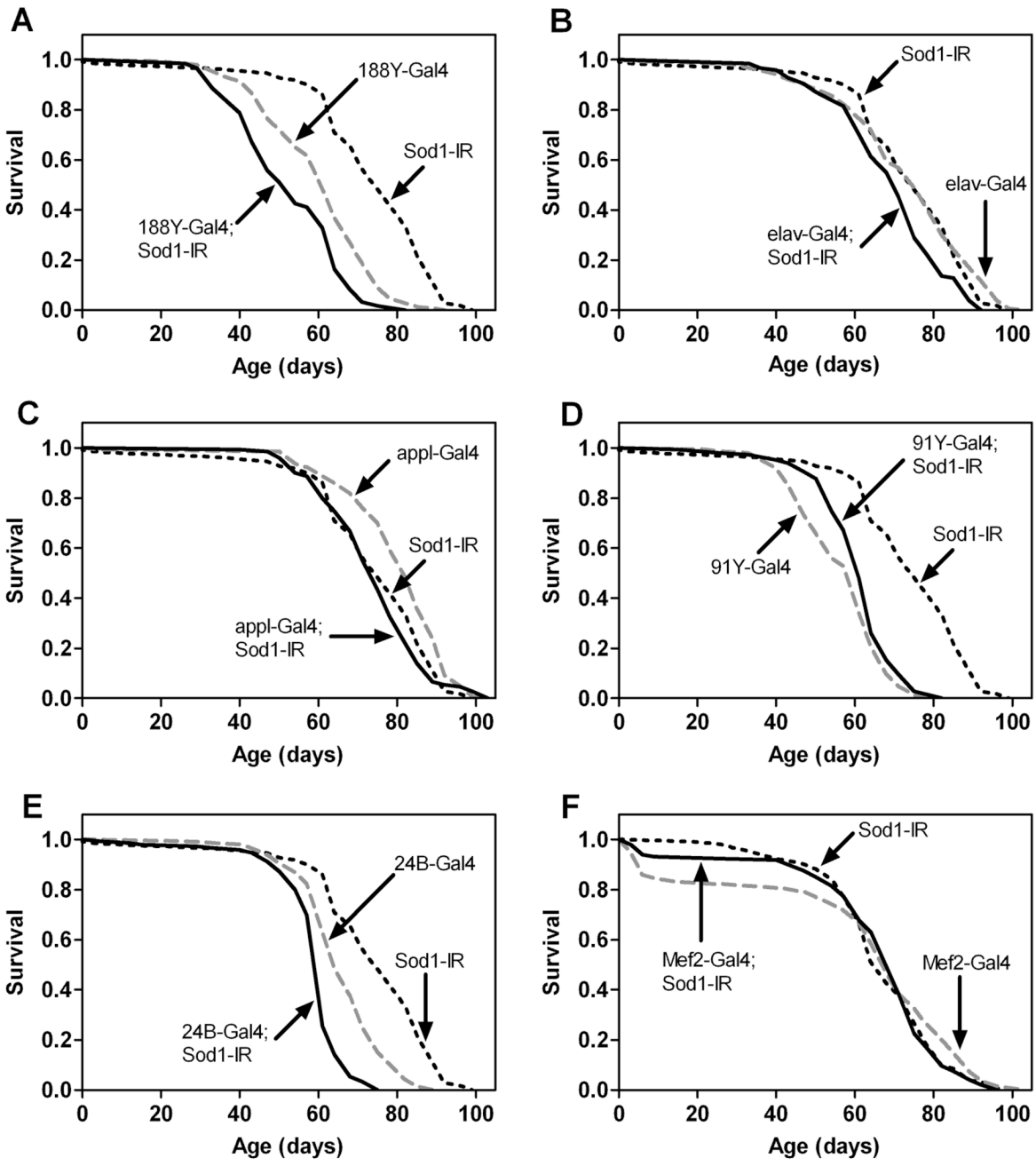

Figure 4.

Expression of *Sod1*-IR in the nervous system or muscle has limited effects on life span. Pan-neuronal expression of *Sod1-IR* via 188Y–Gal4 (A) or *elav-Gal4* (B) decreased life span relative to *UAS-Sod1-IR* and Gal4 controls (log-rank tests, $p \leq 0.0006$, n = 150 flies/genotype), whereas flies expressing *Sod1-IR* via *Appl-Gal4* (C) or 91 Y-Gal4 (D) were not shorter-lived than both control groups (log-rank tests, n.s., n = 150 flies/genotype). Expression of *Sod1-IR* in muscle via 24B–Gal4 (E) shortened life span relative to both control groups (log-rank tests, $p < 0.0001$, n = 150 flies/genotype), while expression via *Mef2-Gal4* (F) did not (log-rank tests, n.s., n = 150 flies/genotype).

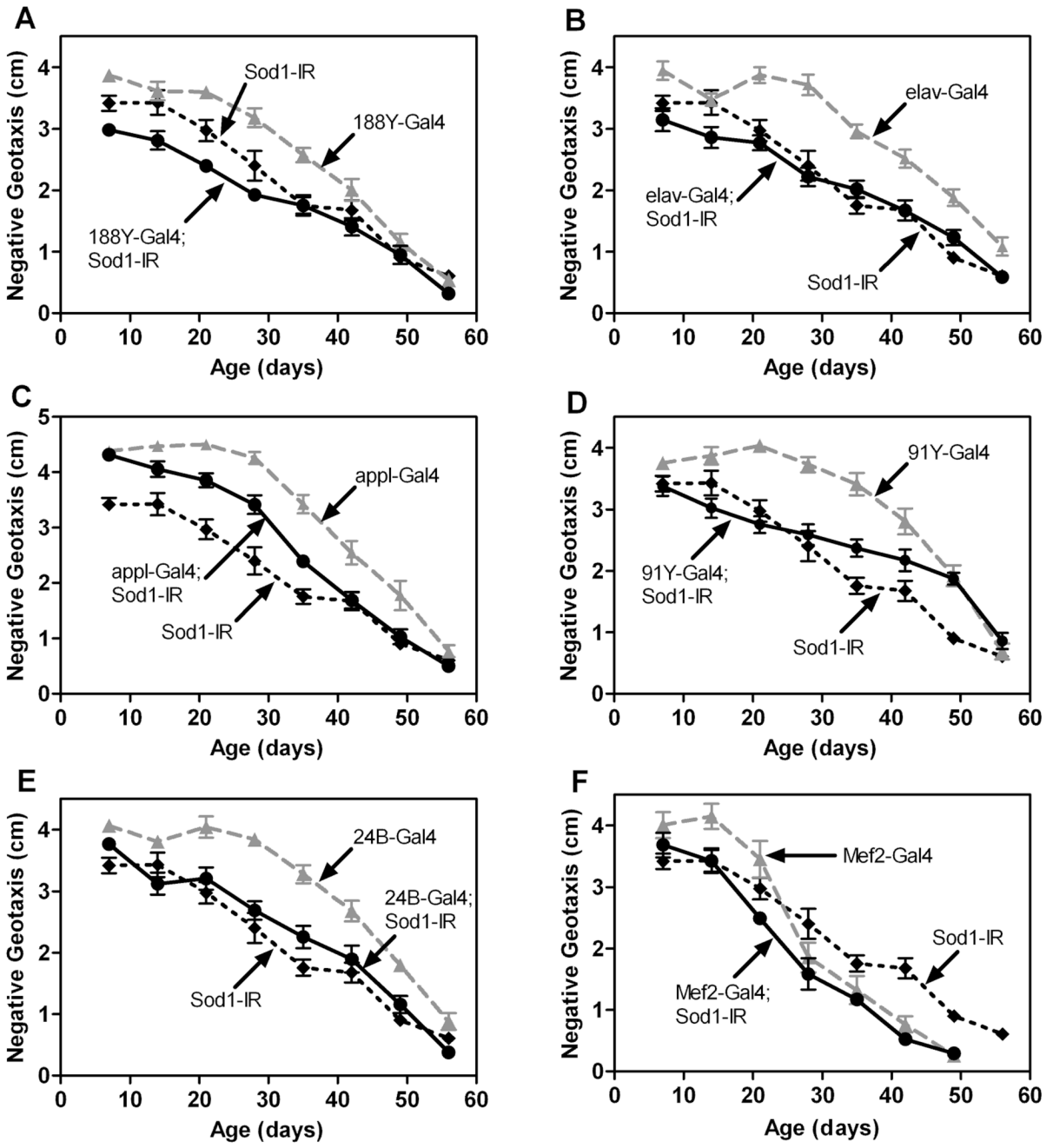

Figure 5.

Expression of *Sod1-IR* in the nervous system or muscle has minimal effects on ARLI. Overall, age and genotype affected negative geotaxis in studies with flies expressing *Sod1-IR* via 188Y–Gal4 (A), *elav-Gal4* (B), *Appl-Gal4* (C), 91Y–Gal4 (D), 24B–Gal4 (E) or *Mef2-Gal4* (F) (individual two-way ANOVAs, $p < 0.0001$, n = 5–10 vials of 25 flies/genotype). Compared to single transgene controls, negative geotaxis was reduced only in flies with *Sod1-IR* expression driven by 188Y–Gal4 (A) or *Mef2-Gal4* (F) (Tukey HSD, $p<0.05$).