

TER94, the *Drosophila* Homologue of the ALS-related *VCP* gene, Influences Lifespan and Leads to a Decline in Motor Function

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Research article

Keywords: *Drosophila melanogaster*, *TER94*, *VCP*, longevity, locomotor decline, parkin, alpha-synuclein, *Drosophila* Models of neurodegenerative disease

Posted Date: August 24th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-60498/v1>

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Abstract

Background: Valosin-Containing Protein (VCP) is an essential AAA+ ATPase with diverse functions within the cell. Mutations in the *VCP* gene have been detected in patients with familial amyotrophic lateral sclerosis (ALS). The aim of this study is to create a novel model of human neurodegenerative disease in *Drosophila melanogaster* by altering the expression of *TER94*, the *Drosophila* orthologue of the human *VCP* gene. *TER94* expression was altered in all neurons, the dopaminergic neurons and in the motor neurons, with longevity and locomotor function assessed over time. Altered *TER94* expression in combination with the altered expression of known Parkinson Disease (PD) genes was examined to investigate potential interactions.

Results: Inhibition of *TER94* altered median lifespan in a manner dependent upon the transgene selected for use and the tissue-specific expression directed by the *Gal4* transgene selected. Locomotor ability was significantly reduced in all cases of *TER94* inhibition tested. The inhibition of *TER94* by two *TER94-RNAi* inhibitory transgenes, in the motor neurons via *D42-Gal4* lead to increases in median lifespan, with one inhibitory transgene generating a slightly reduced lifespan. Inhibition of *TER94* in the dopaminergic neurons resulted in a severe reduction in lifespan. The co-inhibition of *TER94* and *parkin* in the neurons resulted in a major decline in lifespan by approximately 30%. While the inhibition of *TER94* and the co-expression of *alpha-synuclein* in the neurons resulted in an increase in lifespan by approximately 28%.

Conclusions: The inhibition of *TER94* in the motor neurons is an interesting model of ALS, due to the small, but reduced lifespan coupled with a strong decline in locomotor function. The inhibition of *TER94* in the dopaminergic neurons is a potential model of ALS, due to the reduction of both lifespan and locomotor function over time. The co-inhibition of *TER94* and *parkin* in the neurons provides a promising novel model of neurodegenerative disease, displaying a great reduction in lifespan and in locomotor ability over time.

Background

The neurodegenerative disease Amyotrophic Lateral Sclerosis (ALS), one of the most common adult-onset motor neuron diseases, is characterized by the progressive loss of upper and lower motor neurons from the spinal cord, brain stem and motor cortex: ALS progression eventually leads to muscle weakness and atrophy (11). To date, at least three primary ALS genes have been identified: *superoxide dismutase 1* (*SOD1*), *Fused in Sarcoma* (*FUS*) and *TAR DNA Binding Protein* (*TARDBP*) (1,10). Aside from the well-characterized disease-causing genes, there are several other, less well-established ALS-linked genes. *Valosin-containing protein* (*VCP*), also known as *Transitional endoplasmic reticulum 94* or *TER94* in *Drosophila melanogaster*, is an ALS-related gene which encodes the enzyme Valosin-Containing Protein, an essential AAA + ATPase. In the cell, *VCP* is ubiquitously expressed in the endoplasmic reticulum, mitochondria and nucleus, and associated with diverse functions in processes such as mitophagy, autophagy, and Ubiquitin Proteasome System (UPS) (3,8,10). Other functions of *VCP* include involvement with ER-associated protein degradation and DNA repair (3,10). In mitophagy, *VCP* is required for the

turnover of mitochondrial outer membrane proteins (12), and is a direct component in the PINK1/parkin-mediated process of mitophagy (6,12). In autophagy, VCP is heavily involved in the initiation phase and in the maturation of autophagosomes (5). An absence of *VCP* has been known to disturb both the aggregation of misfolded proteins, referred to as an aggresome, along with the degradation of proteins (5). Not only is the *VCP* gene associated with ALS, it has been linked to other diseases such as early onset Paget disease, Frontal Lobe Dementia (FTD) (8,9), and more recently in Parkinson Disease (PD) (9). Through whole-exome sequencing, mutations in the *VCP* gene have been linked to patients with familial ALS (4), with mutations in *VCP* accounting for approximately 1 to 2% of familial ALS cases, and the demonstration that *VCP* mutations can result in impaired autophagy (10). Dominant pathogenic mutations of *VCP* alter the amino-terminal domain, with others that lead to changes within the ATPase domains, strongly influence mitochondrial function (14). Similar to human *VCP*, the protein encoded by *Drosophila TER94* has associations with various specific proteins including the product of *Cabeza (Cas)*, the *Drosophila* orthologue of the significant ALS gene *FUS*, where it functions as a modulator of motor neuron degeneration (2). Similar to human *VCP*, *Drosophila TER94* regulates the Notch signalling pathway, which is critical in tissue development and homeostasis. Impairment of Notch signalling has been known to lead to various diseases, particularly neurodegenerative diseases (7). Furthermore, in the fly, *TER94* interacts with *Drosophila clueless (clu)* through PINK1/parkin-dependent mitophagy, where *clu* functions with *VCP* and *parkin* to degrade and promote the clearance of dysfunctional mitochondria (14). As *VCP* has prominent roles in autophagic processes, particularly in the stages of initiation, impairment in this gene, can have detrimental influence upon such pathways. Moreover, as *VCP* is a known ALS-related gene that has an active involvement with the process of PINK1-parkin-mediated mitophagy, it is beneficial to study this ALS-associated gene in light of a potential relationship to Parkinson Disease. Although studies into the role of *VCP* during neurodegeneration have been conducted, the mechanisms by which mutations in *VCP* contribute to the progression of disease must be investigated. In these experiments, *TER94* was inhibited in the motor neurons directed by the *D42-Gal4* transgene, in the dopaminergic neurons using the *tyrosine hydroxylase (TH-Gal4)* transgene, and in a wider range of neurons that include the dopaminergic through the *ddc-Gal4* transgenes (*ddc-Gal4^{HL4.3D}* and *ddc-Gal4^{HL4.36}*). It was predicted that inhibition of the *TER94* gene in *Drosophila* would decrease median lifespan and impair locomotor ability, as the loss-of-function mutations in the orthologous *VCP* gene have been observed in ALS patients. The main objective was to create a *VCP*-based ALS model in *D. melanogaster* by altering the expression of the orthologous *TER94* gene.

Results

Inhibition of *TER94* in the motor neurons influences longevity and reduces locomotor ability

The inhibition of *TER94* via the *UAS-TER94-RNAi^{GL00448}* transgene through *D42-Gal4* resulted in a slight reduction in median lifespan compared to the control *UAS-lacZ* median lifespan of 70 days, inhibition of *TER94* via *TER94-RNAi^{GL00448}* lead to a median lifespan of 68 days (Fig. 1a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into

the 8 weeks the *TER94-RNAi^{GL00448}* flies lost their ability to climb at week 4 (Fig. 1b). The inhibition of *TER94* via the *UAS-TER94-RNAi^{HMS00656}* transgene through *D42-Gal4* resulted in a significant increase in median lifespan, compared to the control *UAS-lacZ* median lifespan of 70 days, inhibition of *TER94* via *TER94-RNAi^{HMS00656}* lead to a median lifespan of 80 days (Fig. 1a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 8 weeks the *TER94-RNAi^{HMS00656}* flies lost their ability to climb at week 3 (Fig. 1b). The inhibition of *TER94* via *UAS-TER94-RNAi^{JF03402}* transgene through *D42-Gal4* resulted in a significant increase in median lifespan compared to the control *UAS-lacZ* median lifespan of 70 days, inhibition of *TER94* via *TER94-RNAi^{JF03402}* lead to a median lifespan of 78 days (Fig. 1a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 8 weeks the *TER94-RNAi^{JF03402}* flies lost their ability to climb at week 3 (Fig. 1b).

Inhibition of TER94 in the dopaminergic neurons reduces both longevity and locomotor ability

The inhibition of *TER94* via *UAS-TER94-RNAi^{GL00448}* through *TH-Gal4* resulted in a significant reduction in median lifespan compared to the control *UAS-lacZ* median lifespan of 82 days, inhibition of *TER94* via *TER94-RNAi^{GL00448}* lead to a median lifespan of 54 days (Fig. 2a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 8 weeks the *TER94-RNAi^{GL00448}* flies lost their ability to climb at week 4 (Fig. 2b). The inhibition of *TER94* via *UAS-TER94-RNAi^{HMS00656}* through *TH-Gal4* resulted in a significant reduction in median lifespan compared to the control *UAS-lacZ* median lifespan of 82 days, inhibition of *TER94* via *TER94-RNAi^{HMS00656}* lead to a median lifespan of 46 days (Fig. 2a) (Fig. 2a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 8 weeks the *TER94-RNAi^{HMS00656}* flies lost their ability to climb at week 4 (Fig. 2b).

Inhibition of TER94 in the *ddc-Gal4^{HL4.3D}*-expressing neurons has a minimal impact longevity, and reduces locomotor ability

The inhibition of *TER94* via the *UAS-TER94-RNAi^{GL00448}* transgene though *ddc-Gal4^{HL4.3D}* resulted in a slight change in median lifespan compared to the control *UAS-lacZ* median lifespan of 70 days, inhibition of *TER94* via *TER94-RNAi^{GL00448}* lead to a median lifespan of 68 days (Fig. 3a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 8 weeks the *TER94-RNAi^{GL00448}* flies lost their ability to climb at week 3 (Fig. 3b). The inhibition of *TER94* via the *UAS-TER94-RNAi^{HMS00656}* transgene though *ddc-Gal4^{HL4.3D}* resulted in a slight increase in median lifespan compared to the control *UAS-lacZ* median lifespan of 70 days, inhibition of *TER94* via *TER94-RNAi^{HMS00656}* lead to a median lifespan of 74 days (Fig. 3a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 8 weeks the *TER94-RNAi^{HMS00656}* flies lost their ability to climb at week 4 (Fig. 3b).

Inhibition of TER94 and parkin in the *ddc-Gal4^{HL4.3D}*-expressing neurons reduces longevity and locomotor ability

The co-inhibition of *TER94* via *UAS-TER94-RNAi^{GL00448}* transgene and *parkin* through *ddc-Gal4^{HL4.3D}*-expressing neurons resulted in a large reduction in median lifespan (by ~ 30%). Compared to the control *UAS-lacZ* median lifespan of 84 days, inhibition of *TER94* via *TER94-RNAi^{GL00448}* lead to a median lifespan of 58 days (Fig. 4a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 5 weeks the *TER94-RNAi^{GL00448}* flies lost their ability to climb at week 3 (Fig. 4b).

Inhibition of TER94 in the *ddc-Gal4^{HL4.36}*-expressing neurons increases longevity, and reduces locomotor ability

The inhibition of *TER94* via the *UAS-TER94-RNAi^{GL00448}* though *ddc-Gal4^{HL4.36}* resulted in a significant increase in median lifespan compared to the control *UAS-lacZ* median lifespan of 79 days, inhibition of *TER94* via *TER94-RNAi^{GL00448}* lead to a median lifespan of 88 days (Fig. 5a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 5 weeks the *TER94-RNAi^{GL00448}* flies lost their ability to climb also at week 4, however their climbing ability was drastically poorer (Fig. 5b). The inhibition of *TER94* via *UAS-TER94-RNAi^{HMS00656}* though *ddc-Gal4^{HL4.36}* resulted in a significant increase in median lifespan compared to the control *UAS-lacZ* median lifespan of 79 days, inhibition of *TER94* via *TER94-RNAi^{HMS00656}* lead to a median lifespan of 90 days (Fig. 5a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 5 weeks the *TER94-RNAi^{HMS00656}* flies lost their ability to climb also at week 4, however their climbing ability was drastically poorer (Fig. 5b). The inhibition of *TER94* via the *UAS-TER94-RNAi^{JF03402}* transgene though *ddc-Gal4^{HL4.36}* resulted in a significant reduction in median lifespan compared to the control *UAS-lacZ* median lifespan of 79 days, inhibition of *TER94* via *TER94-RNAi^{JF03402}* lead to a median lifespan of 44 days (Fig. 5a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 5 weeks the *TER94-RNAi^{JF03402}* flies lost their ability to climb also at week 4, however their climbing ability was drastically poorer (Fig. 5b).

Inhibition of TER94 and the co-expression of alpha-synuclein in the *ddc-Gal4^{HL4.36}*-expressing neurons increases longevity, and reduces locomotor ability

The inhibition of *TER94* via the *UAS-TER94-RNAi^{GL00448}* transgene and the co-expression of *alpha-synuclein* through *ddc-Gal4^{HL4.36}*-expressing neurons resulted in a significant reduction in median lifespan compared to the control *UAS-lacZ* median lifespan of 86 days, inhibition of *TER94* via *TER94-RNAi^{GL00448}* lead to a median lifespan of 80 days (Fig. 6a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 4 weeks the *TER94-RNAi^{GL00448}* flies lost their ability to climb at week 3 (Fig. 6b). The inhibition of *TER94* via the *UAS-*

TER94-RNAi^{JF03402} transgene and the co-expression of *alpha-synuclein* through *ddc-Gal4*^{HL4.36}-expressing neurons resulted in a significant reduction in median lifespan compared to the control *UAS-lacZ* median lifespan of 86 days, inhibition of *TER94* via *TER94-RNAi*^{JF03402} lead to a median lifespan of 48 days (Fig. 6a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 4 weeks the *TER94-RNAi*^{JF03402} flies lost their ability to climb at week 2 (Fig. 6b). The inhibition of *TER94* via the *UAS-TER94-RNAi*^{HMS00656} transgene and the co-expression of *alpha-synuclein* through *ddc-Gal4*^{HL4.36}-expressing neurons resulted in a large increase in median lifespan (by ~ 28%). Compared to the control *UAS-lacZ* median lifespan of 86 days, inhibition of *TER94* via *TER94-RNAi*^{HMS00656} lead to a median lifespan of 110 days (Fig. 6a). Locomotor ability was reduced over time compared to the control *UAS-lacZ* which maintained strong climbing ability well into the 4 weeks the *TER94-RNAi*^{HMS00656} flies lost their ability to climb at week 3 (Fig. 4b).

Discussion

In patients, certain mutations in the *VCP* gene are known to give rise to cases of familial ALS (4,10), and here we demonstrate that the inhibition of the *TER94* gene results in significant changes in median lifespan, and reduction in locomotor function over time. The inhibition of *TER94* through the expression of *UAS-TER94-RNAi*^{GL00448} in the motor neurons gave a slight reduction in median lifespan, while a great decline in locomotor ability. This reduction in longevity, although small, and reduction in motor function corresponds with the characteristic loss of motor neurons associated with ALS, to suggest that the inhibition of *TER94* in the motor neurons to produce a model of neurodegenerative disease. On the other hand, the inhibition of *TER94* through the expression of *UAS-TER94-RNAi*^{HMS00656} in the motor neurons gave a slight increase in median lifespan, while reducing locomotor function over time. This slight increase in longevity, accompanied by a strong reduction in motor function may be interpreted as a trade-off, where the slight increase in longevity is a type of compensation for a severe decline in motor skills. The significant reduction in locomotor ability and small increase in longevity when *TER94* expression is inhibited corresponds with the characteristic loss of motor neurons associated with ALS, making the inhibition of *TER94* in the motor neurons to be an imperfect model of neurodegenerative disease. Interestingly, we found that the inhibition of *TER94* through the expression of *UAS-TER94-RNAi*^{JF03402} provided critical class male progeny only when expressed in the motor neurons via the *D42-Gal4* transgene and when expressed in the *ddc-Gal4*^{HL4.36}-expressing neurons via the *ddc-Gal4*^{HL4.36} transgene. When expressed through *D42-Gal4*, this *TER94* inhibitory transgene produced a significant increase in median lifespan, while the climbing ability over time was reduced in a significant way. When expressed through *ddc-Gal4*^{HL4.36}, this *TER94* inhibitory transgene severely reduced median lifespan, while reducing climbing ability. As this particular *TER94-RNAi* transgene was not viable when expressed under the control of other *Gal4* transgenes, and therefore, in other subsets of tissues, this may suggest that *TER94* has a significant role in governing cell survival and viability in some tissues.

Investigating the consequences of altered *TER94* expression under the control of the *TH-Gal4* transgene has shown that the inhibition of *TER94* through the expression of *UAS-TER94-RNAi^{GL00448}* and *UAS-TER94-RNAi^{HMS00656}* provided a severe reduction in both median lifespan and motor function over time. This significant reduction in longevity and motor ability seen when *TER94* is inhibited through *TH-Gal4* corresponds with the characteristic loss of dopaminergic neurons associated with PD, making the inhibition of *TER94* in the dopaminergic neuron a promising model of neurodegenerative disease. The alteration of *TER94* in the *ddc-Gal4^{HL4.3D}*-expressing neurons has demonstrated that the inhibition of *TER94* through the expression of *UAS-TER94-RNAi^{HMS00656}* has a minimal increase in median lifespan, while dramatically reducing locomotor function over time. This slight increase in longevity, followed with a significant reduction in motor ability may be a type of compensation for the severe decline in motor skills. The significant reduction in motor function and slight increase in longevity when *TER94* expression is inhibited in the *ddc-Gal4^{HL4.3D}*-expressing neurons may be an imperfect model of neurodegenerative disease. Furthermore, the inhibition of *TER94* in the *ddc-Gal4^{HL4.3D}*-expressing neurons through the expression of *UAS-TER94-RNAi^{GL00448}* did not significantly change median lifespan when compared to the control *UAS-lacZ*; however, it did significantly reduce locomotor function over time. The combined inhibition of *TER94* in the *ddc-Gal4^{HL4.3D}*-expressing neurons through the expression of *UAS-TER94-RNAi^{GL00448}*, and the inhibition of *parkin* resulted in a severe reduction in median lifespan, by approximately 30%, while reducing motor ability over time. The considerable reduction in longevity observed when *parkin* has been inhibited further suggests a significant functional connection between *TER94* and *parkin*. This substantial decrease in lifespan and decline in motor function appears to mimic aspects of the pathology of ALS and PD, thus reinforcing the promise of this as a promising model of neurodegenerative disease. Moreover, the alteration of *TER94* in the *ddc-Gal4^{HL4.36}*-expressing neurons demonstrates that the inhibition of *TER94* through the expression of *UAS-TER94-RNAi^{GL00448}* and *UAS-TER94-RNAi^{HMS00656}* provided a slight, but significant increase in median lifespan, while reducing locomotor function over time when compared to the control *UAS-lacZ*. The expression of these *TER94-RNAi* transgenes and the co-expression of *alpha-synuclein* in the *ddc-Gal4^{HL4.36}*-expressing neurons demonstrated that the inhibition of *TER94* through the expression of *UAS-TER94-RNAi^{GL00448}* and the co-expression of *alpha-synuclein*, resulted in a significant decrease in median lifespan, when compared to the control *UAS-lacZ*, followed with a reduction in locomotor ability. This reduction in median lifespan seen when *alpha-synuclein* was co-expressed suggests that *alpha-synuclein* interacts with the *TER94*

protein to some extent. Furthermore, the inhibition of *TER94* through the expression of *UAS-TER94-RNAi^{HMS00656}* and co-expression of *alpha-synuclein* provided a drastic increase in median lifespan, by approximately 28%, while reducing locomotor function over time. This major increase in longevity seen by the inhibition of *TER94* and expression of *alpha-synuclein* suggests a strong connection between the two gene activities and a clear where the combined effects of *TER94* inhibition and the expression of *alpha-synuclein* are greater than either alteration in isolation. From the results of this study, it is clear that the inhibition of *TER94* has variable effects on the survival and locomotion of *D. melanogaster* dependent on the tissues targeted.

Conclusions

The inhibition of *TER94* under some conditions can alter median lifespan coupled with greatly reduced motor function. As *VCP* mutations are known to exist in ALS patients, consequences of *TER94* reduction in flies supports the hypothesis that inhibition of the human *VCP* gene may contribute to alterations to a subcellular mechanism that leads to the pathology associated with ALS. Interestingly, the inhibition of *TER94* in the motor neurons can result in a slightly increased median lifespan accompanied by a reduction in motor function over time. The inhibition of *TER94* in the dopaminergic neurons lead to a severe reduction in lifespan and motor function, which suggests that the inhibition of *TER94* in the dopaminergic neurons may yield a model of PD. Although the inhibition of *TER94* through *UAS-TER94-RNAi*^{GL00448} in the *ddc-Gal4*-expressing neurons did not significantly change lifespan, however the co-inhibition of *parkin* lead to a severe reduction in lifespan and motor function. These findings suggest that the co-inhibition of *TER94* and *parkin* in the neuron may be a promising model of neurodegenerative disease. The inhibition of *TER94* through *UAS-TER94-RNAi*^{HMS00656} in the *ddc-Gal4*-expressing neurons lead to a significant increase in lifespan, while the co-expression of *alpha-synuclein* lead to an increase in lifespan, followed by a reduction in motor function. These phenotypes suggest a clear synergistic effect between the two gene activities.

Materials And Methods

Drosophila Stocks and Culture

The $P\{y[+ t7.7] v[+ t1.8] = TRiP.GL00448\}attP2$ (*UAS-TER94-RNAi*^{GL00448}), $P\{TRiP.JF03402\}attP2$ (*UAS-TER94-RNAi*^{JF03402}), $P\{TRiP.HMS00656\}attP2$ (*UAS-TER94-RNAi*^{HMS00656}) and $P\{UAS-lacZ.B\}meltBg4-1-2$ (*UAS-lacZ*) responder lines and the *Gal4* lines *D42-Gal4*, *TH-Gal4*, *ddc-Gal4*^{HL4.3D}, *ddc-Gal4*^{HL4.36}, were all obtained from the Bloomington Drosophila Stock Center at Indiana University (IN, USA). The *Gal4* lines *w;ddc-Gal4*^{HL4.3D}/*CyO*;*UAS-parkin-RNAi*/*TM3* (*ddc-Gal4*^{HL4.3D};*UAS-parkin-RNAi*) and *w;ddc-Gal4*^{HL4.36}/*Tm3 iso1*;*UAS-alpha-synuclein*/*CyO* (*ddc-Gal4*^{HL4.36};*UAS-alpha-synuclein*) were created in the Staveley Laboratory, Memorial University of Newfoundland (St. John's, Canada). *Drosophila melanogaster* was maintained on a standard media comprised of 65 g/L cornmeal, 50 ml/L fancy grade molasses, 10 g/L yeast and 5.5 g/L agar which was then treated with 2.5 ml propionic acid and 5 ml of 0.1 g/ml methylparaben. This mixture was then allowed to solidify at the bottom of vials and stored in 4 to 6° C until use. Stocks were stored at room temperature (~ 21° C), while crosses and experiments were performed at 25° C.

Drosophila Crosses

Virgin female flies from the *D42-Gal4*, *TH-Gal4*, *ddc-Gal4*^{HL4.3D}, *ddc-Gal4*^{HL4.36}, *ddc-Gal4*^{HL4.3D};*parkin-RNAi*, and *ddc-Gal4*^{HL4.36};*UAS-alpha-synuclein* lines were collected every 8 to 12 hours for several days. Once confirmed virgin flies were then bred with male flies which expressed *TER94* inhibition. Three *TER94* inhibition lines *UAS-TER94-RNAi*^{GL00448}, *UAS-TER94-RNAi*^{HMS00656}, *UAS-TER94-RNAi*^{JF03402}, and the

control line *UAS-lacZ* were used. Critical class male progeny from these crosses were assessed for longevity and locomotor ability through ageing and climbing assays.

Longevity Assays

The survival of *Drosophila* was analyzed to examine the lifespan of experimental flies in comparison to control flies. Critical class male progeny were collected daily and placed in vials with fresh medium in cohorts of twenty flies or less to avoid over-crowding. A sample size of approximately three hundred males was collected in total and stored at 25° C for the duration of the experiment. The flies were scored every two days to examine if any death had occurred. A fly was considered dead when no movement was observed. Males were transferred onto fresh media every four days to obtain a healthy environment. Graphpad Prism 8 (Graphpad Software Inc.) was used to analyze longevity data, and survival curves were analyzed and compared using the Log-rank (Mantel-Cox) test, with a *P*-value less than or equal to 0.05 with Bonferroni correction being considered statistically significant.

Locomotor Assays

Approximately seventy critical class male progeny to be collected from crosses between female *D42-Gal4*, *TH-Gal4*, *ddc-Gal4^{HL4.3D}*, *ddc-Gal4^{HL4.36}*, *ddc-Gal4^{HL4.36};UAS-alpha-synuclein* and *ddc-Gal4^{HL4.3D};parkin-RNAi* flies and male, *UAS-TER94-RNAi^{GL00448}*, *UAS-TER94-RNAi^{HMS00656}*, *UAS-TER94-RNAi^{JF03402}*, and *UAS-lacZ* flies. This assay was used to measure the ability of flies to climb up a narrow glass tube over the course of their lifespan, with fifty male flies from each genotype being evaluated once every seven days, beginning at the seventh day post-eclosion. Critical class males were maintained in vials with ten flies per vial, stored at 25° C, and placed on new medium once per week throughout the experiment. Climbing analysis followed the standard protocol outlined by our laboratory (Todd & Staveley, 2004). Graphpad Prism 8 (Graphpad Software Inc.) was used to analyze the data, and to generate climbing curves fitted using non-linear regression. 95% confidence intervals were used to test for significance with the curves considered to be significantly different if *P* < 0.05.

Abbreviations

ALS

amyotrophic lateral sclerosis

Ddc

dopa decarboxylase

PD

Parkinson disease

Pink1

PTEN-induced putative kinase 1

RNAi

ribonucleic acid interference

TER94

Transitional endoplasmic reticulum 94

TH

tyrosine hydroxylase

VCP

Valosin-Containing Protein

Declarations

Ethics approval and consent to participate

The study was approved by the Animal Care Committee of the Memorial University of Newfoundland as a Category of Invasiveness Level A protocol. Consent was not applicable for the study.

Consent for publication

Not Applicable

Availability of data and materials

All data generated or analyzed during this study are included in the article.

Competing interest

The authors declare no competing interest

Funding

EPH was funded by an Aging Research Centre-Newfoundland and Labrador Graduate Fellowship, Department of Biology Teaching Assistantships and a school of Graduate Studies Fellowship from Memorial University of Newfoundland. The study was funded by School of Graduate studies, Memorial University and by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant. Funding organizations were not involved in the design of the study.

Author's contributions

Emily Hurley performed the experimentation, statistical analysis of survival, climbing and biometric data. Brian Staveley devised and participated in the experimental design, supervision of the study and revision to the manuscript.

Acknowledgments

Not Applicable

Author's Information

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Figures

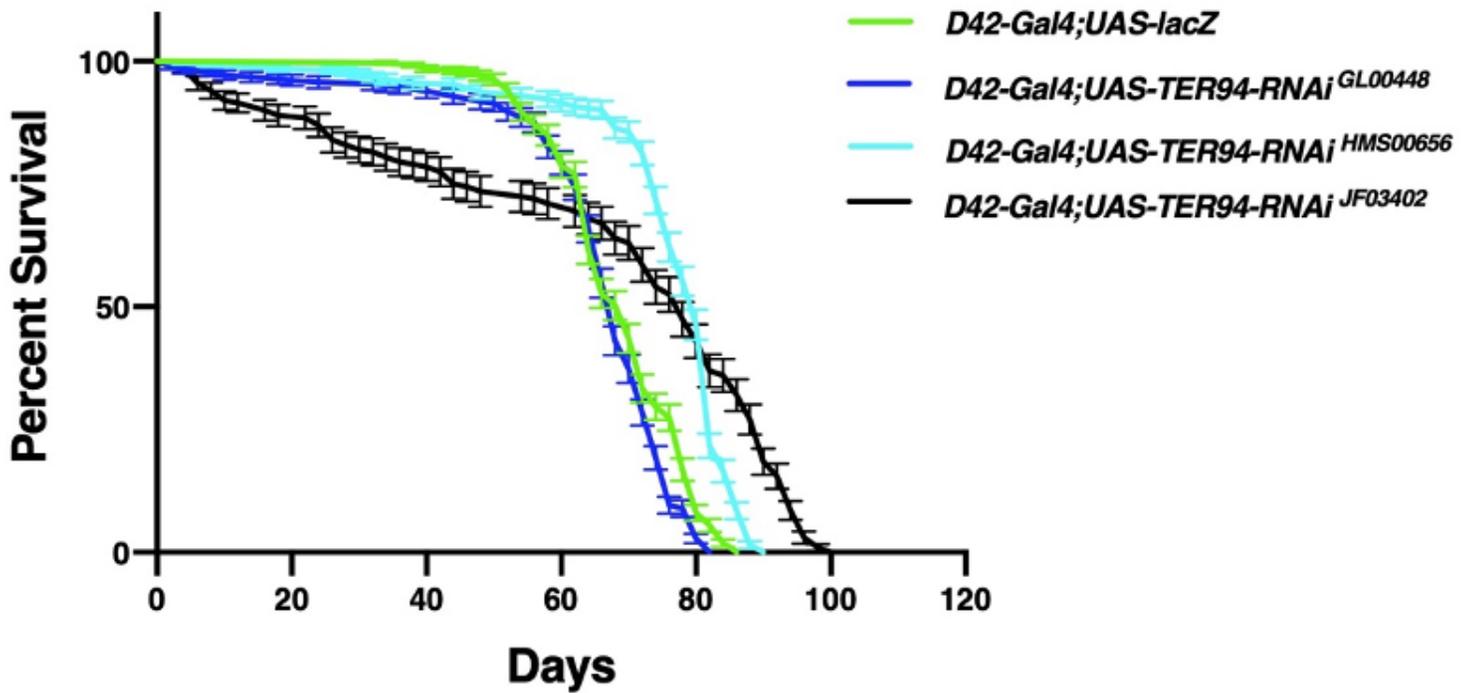


Figure 1

Altered expression of TER94 directed through the D42-Gal4 transgene affects longevity and climbing ability. A: Longevity assay of *Drosophila melanogaster* males displaying altered TER94 expression in the motor neurons. Longevity is depicted by percent survival. Significance is $P < 0.05$ using the log-rank test with Bonferroni correction. Error bars represent standard error of the mean.

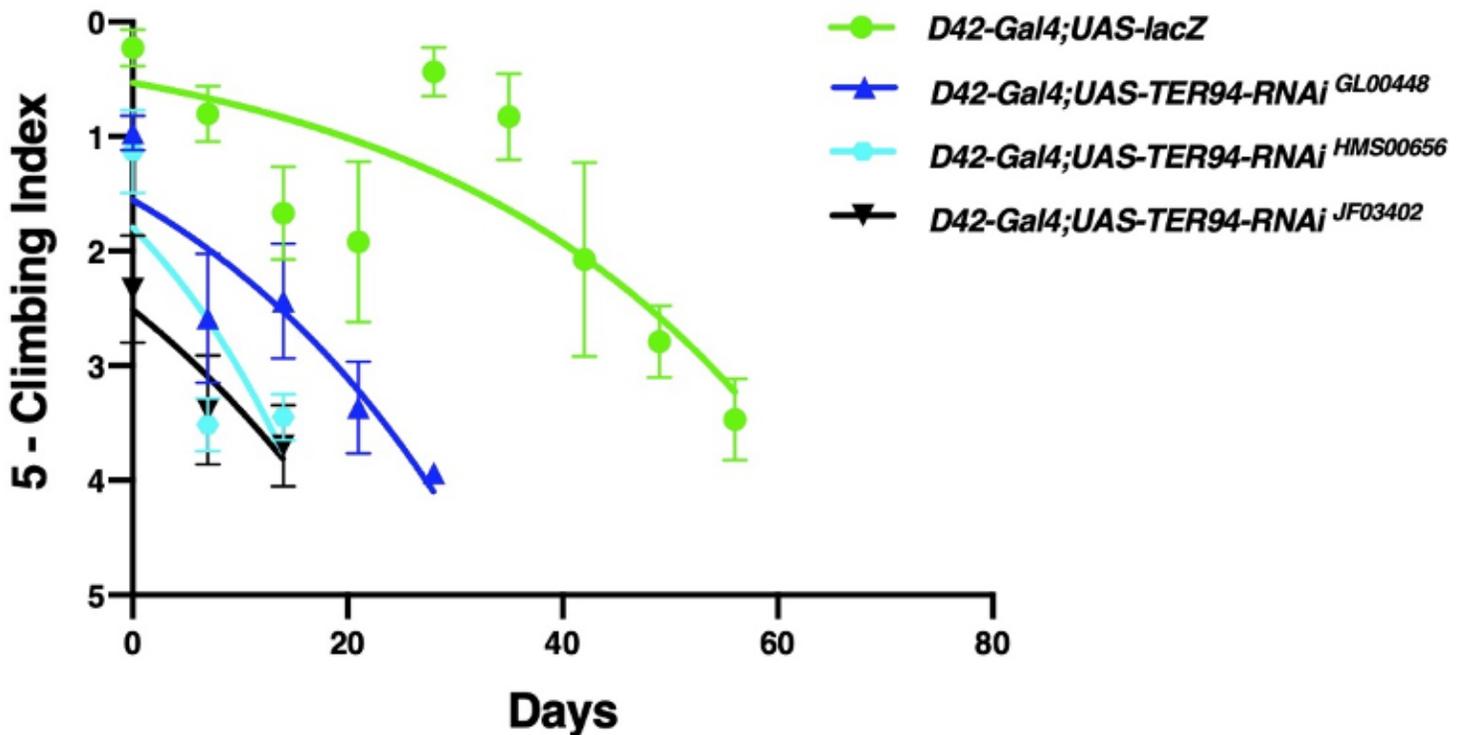


Figure 2

Altered expression of TER94 directed through the D42-Gal4 transgene affects longevity and climbing ability. B: Locomotor assay of *D. melanogaster* males displaying altered TER94 expression in the motor neurons. Climbing ability was determined by a nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean.

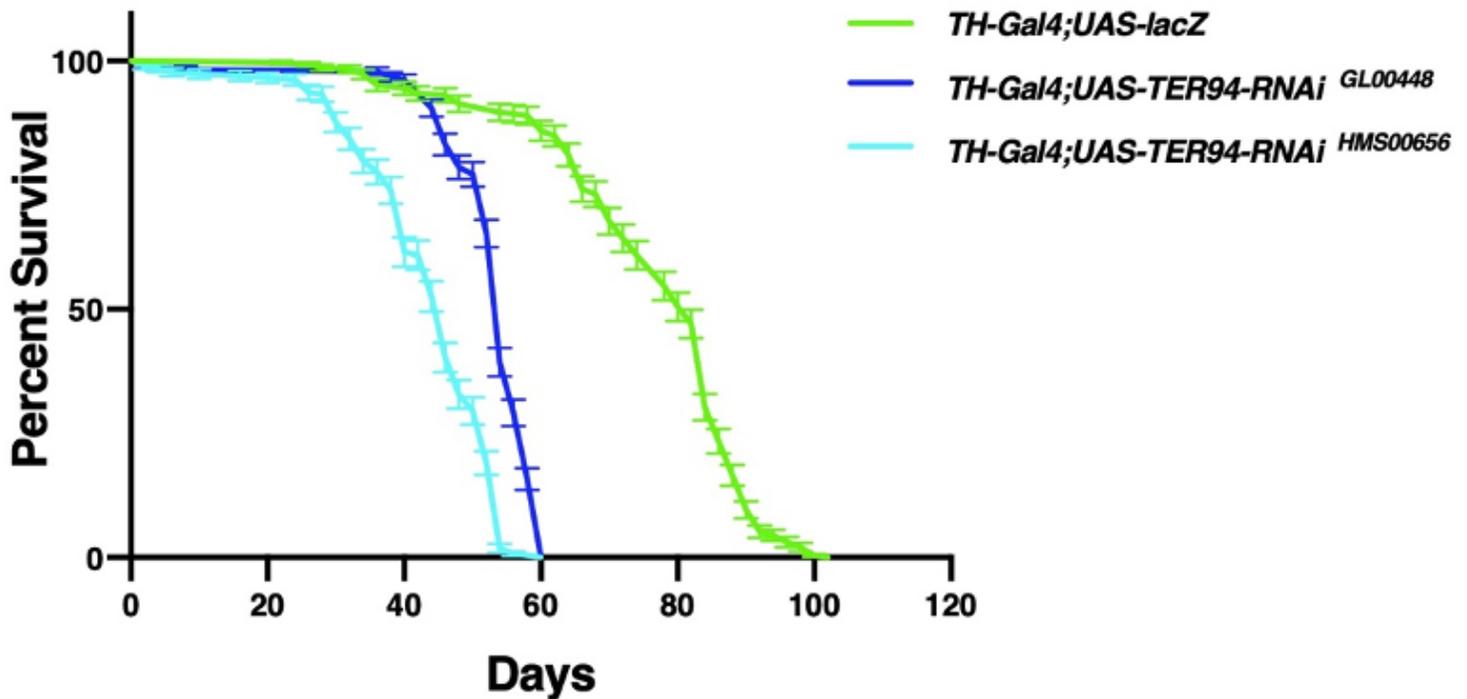


Figure 3

Altered expression of TER94 directed through the TH-Gal4 transgene affects longevity and climbing ability. A: Longevity assay of *Drosophila melanogaster* males displaying altered TER94 expression in the dopaminergic neurons. Longevity is depicted by percent survival. Significance is $P < 0.05$ using the log-rank test with Bonferroni correction. Error bars represent standard error of the mean.

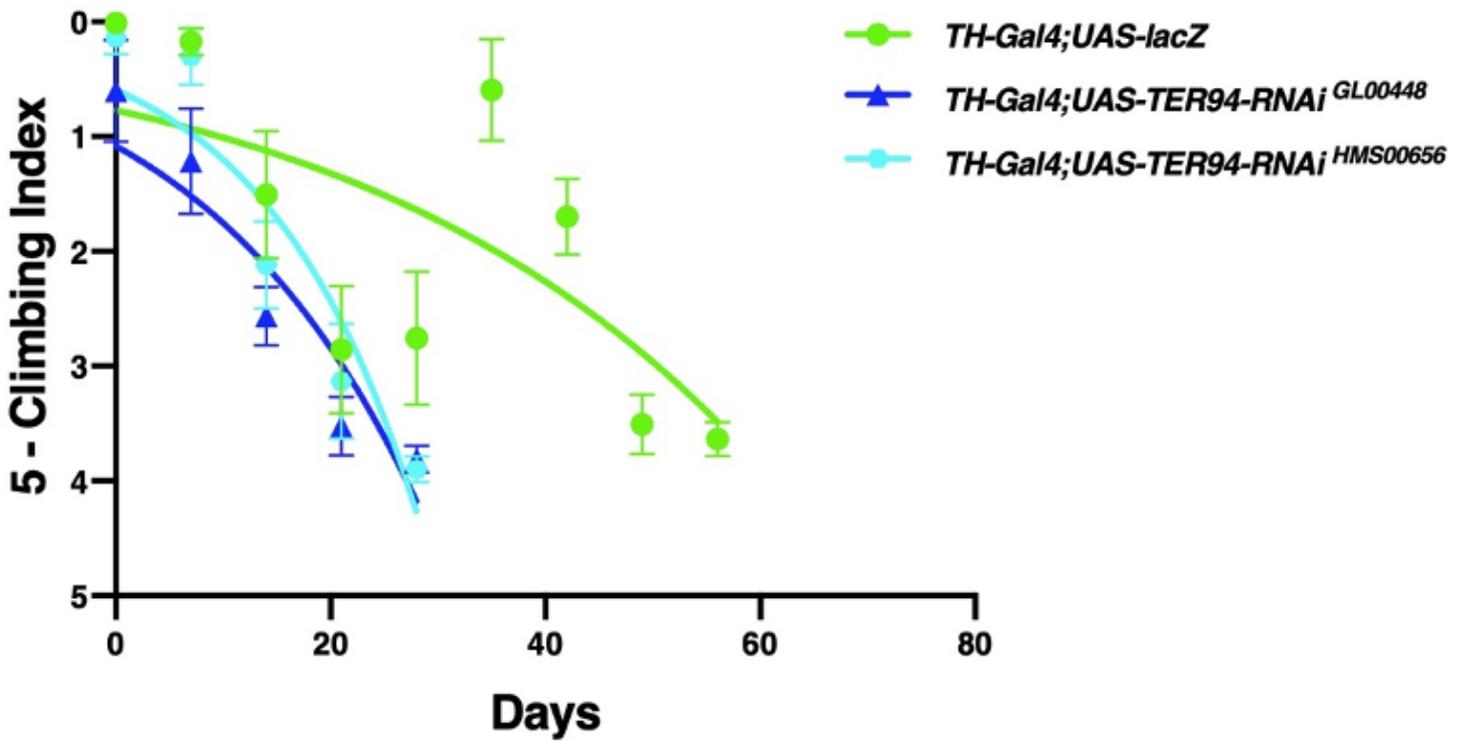


Figure 4

Altered expression of TER94 directed through the TH-Gal4 transgene affects longevity and climbing ability. B: Locomotor assay of *D. melanogaster* males displaying altered TER94 expression in the dopaminergic neurons. Climbing ability was determined by a nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean.

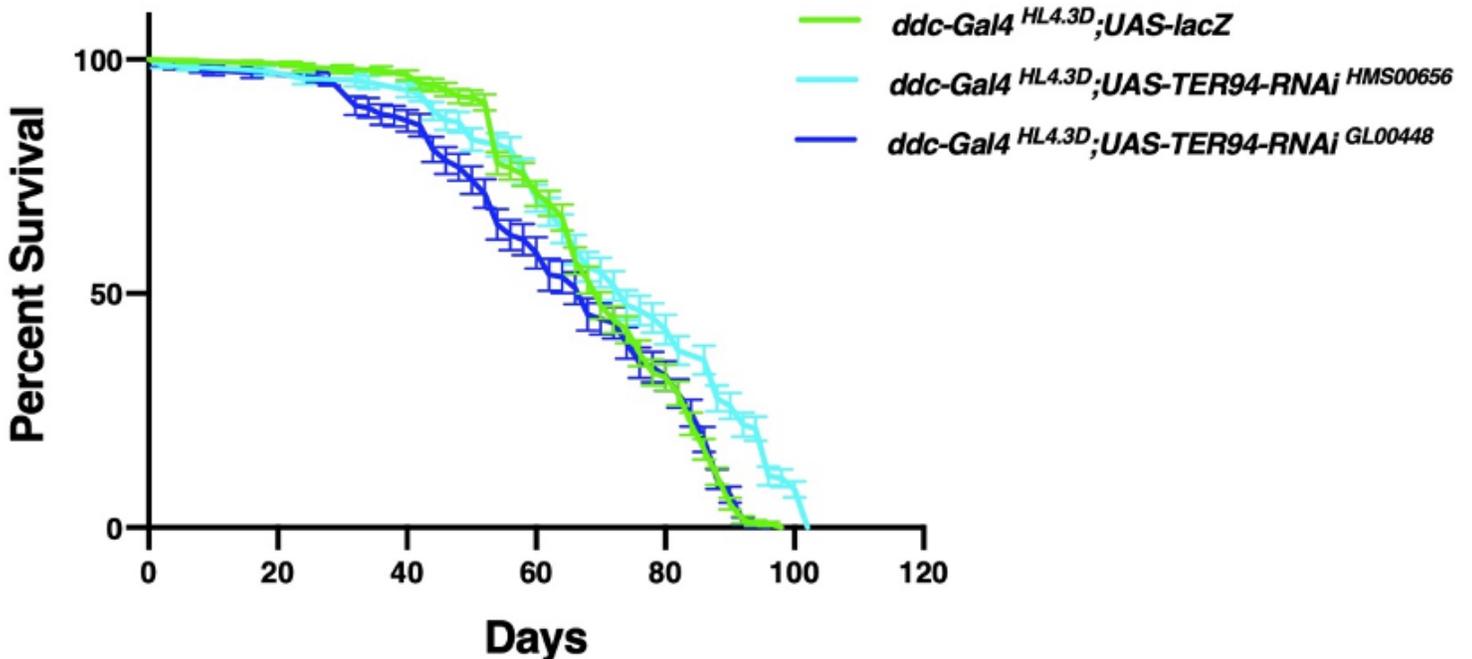


Figure 5

Altered expression of TER94 directed through the *ddc-Gal4*^{HL4.3D} transgene affects longevity and climbing ability. A: Longevity assay of *Drosophila melanogaster* males displaying altered TER94 expression in the neurons. Longevity is depicted by percent survival. Significance is $P < 0.05$ using the log-rank test with Bonferroni correction. Error bars represent standard error of the mean.

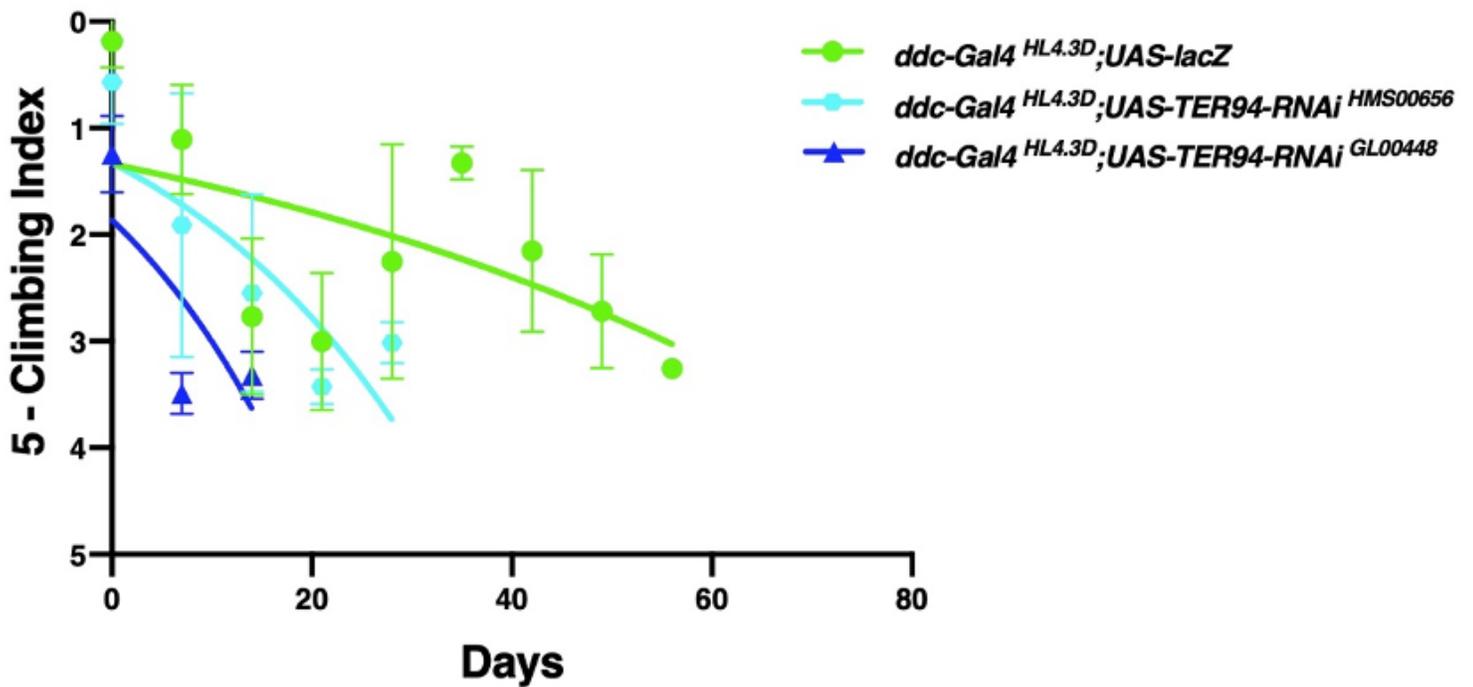


Figure 6

Altered expression of TER94 directed through the *ddc-Gal4*^{HL4.3D} transgene affects longevity and climbing ability. B: Locomotor assay of *D. melanogaster* males displaying altered TER94 expression in the neurons. Climbing ability was determined by a nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean.

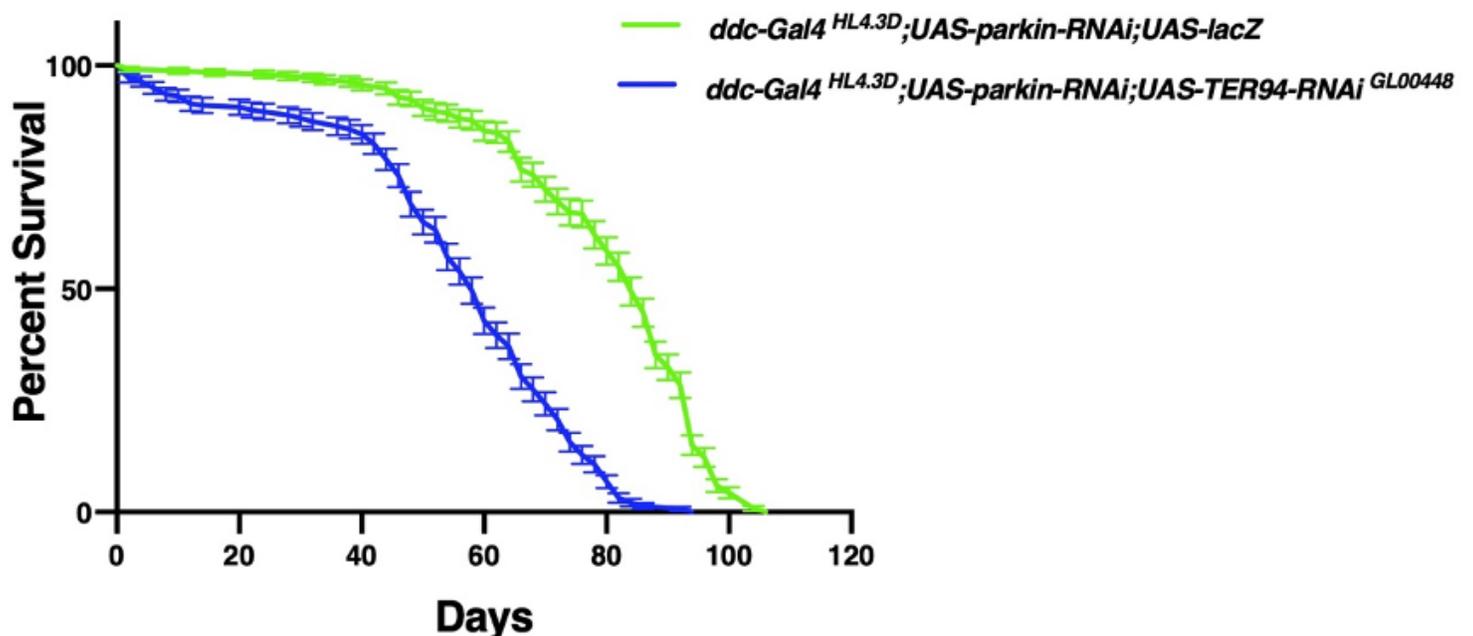


Figure 7

Altered expression of TER94 and parkin directed through the *ddc-Gal4^{HL4.3D}* transgene affects longevity and climbing ability. A: Longevity assay of *Drosophila melanogaster* males displaying altered TER94 and parkin expression in the neurons. Longevity is depicted by percent survival. Significance is $P < 0.05$ using the log-rank test with Bonferroni correction. Error bars represent standard error of the mean.

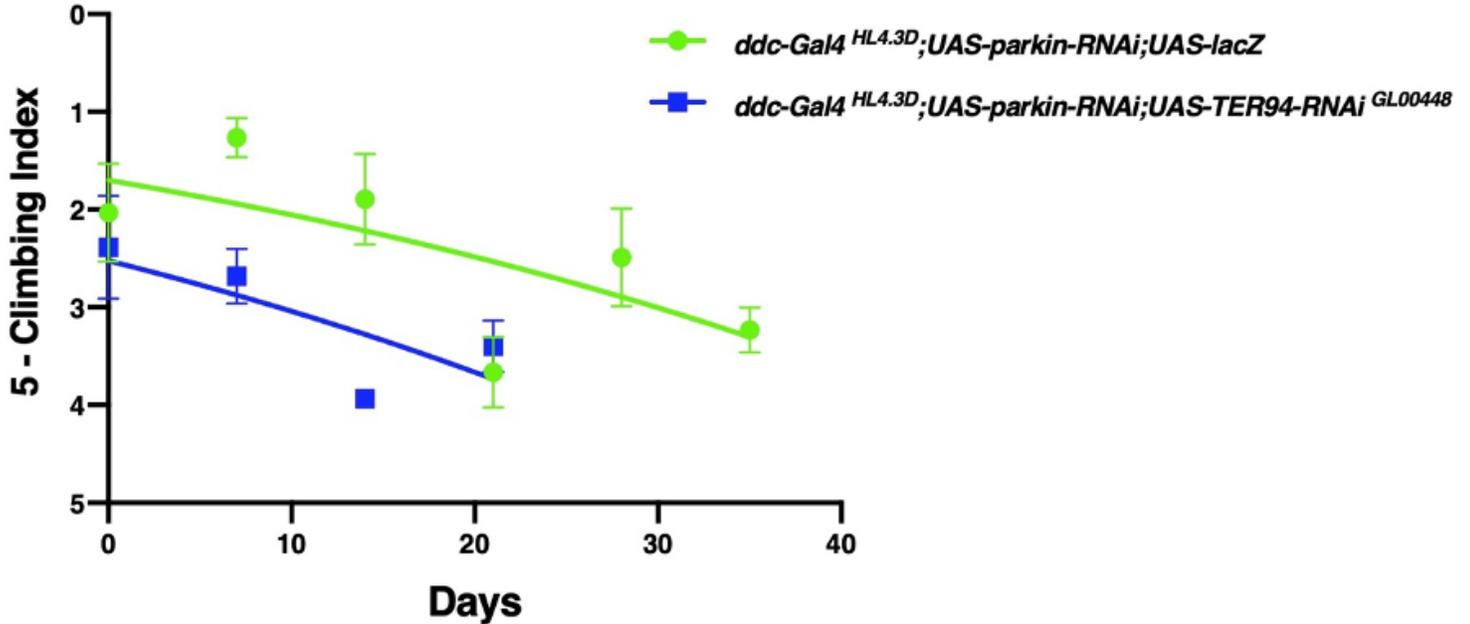


Figure 8

Altered expression of TER94 and parkin directed through the *ddc-Gal4^{HL4.3D}* transgene affects longevity and climbing ability. B: Locomotor assay of *D. melanogaster* males altered TER94 and parkin expression in the neurons. Climbing ability was determined by a nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean.

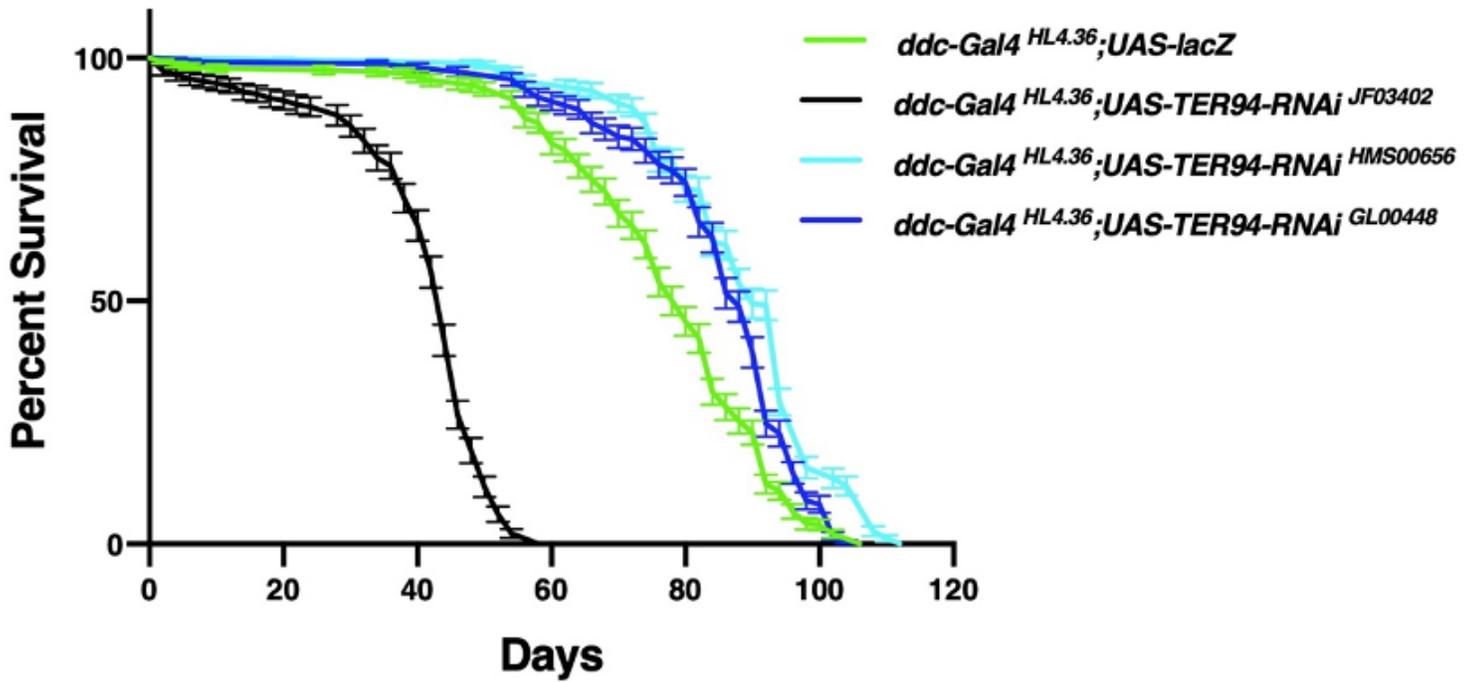


Figure 9

Altered expression of TER94 directed through the *ddc-Gal4^{HL4.36}* transgene affects longevity and climbing ability. A: Longevity assay of *Drosophila melanogaster* males displaying altered TER94 expression in the neurons. Longevity is depicted by percent survival. Significance is $P < 0.05$ using the log-rank test with Bonferroni correction. Error bars represent standard error of the mean.

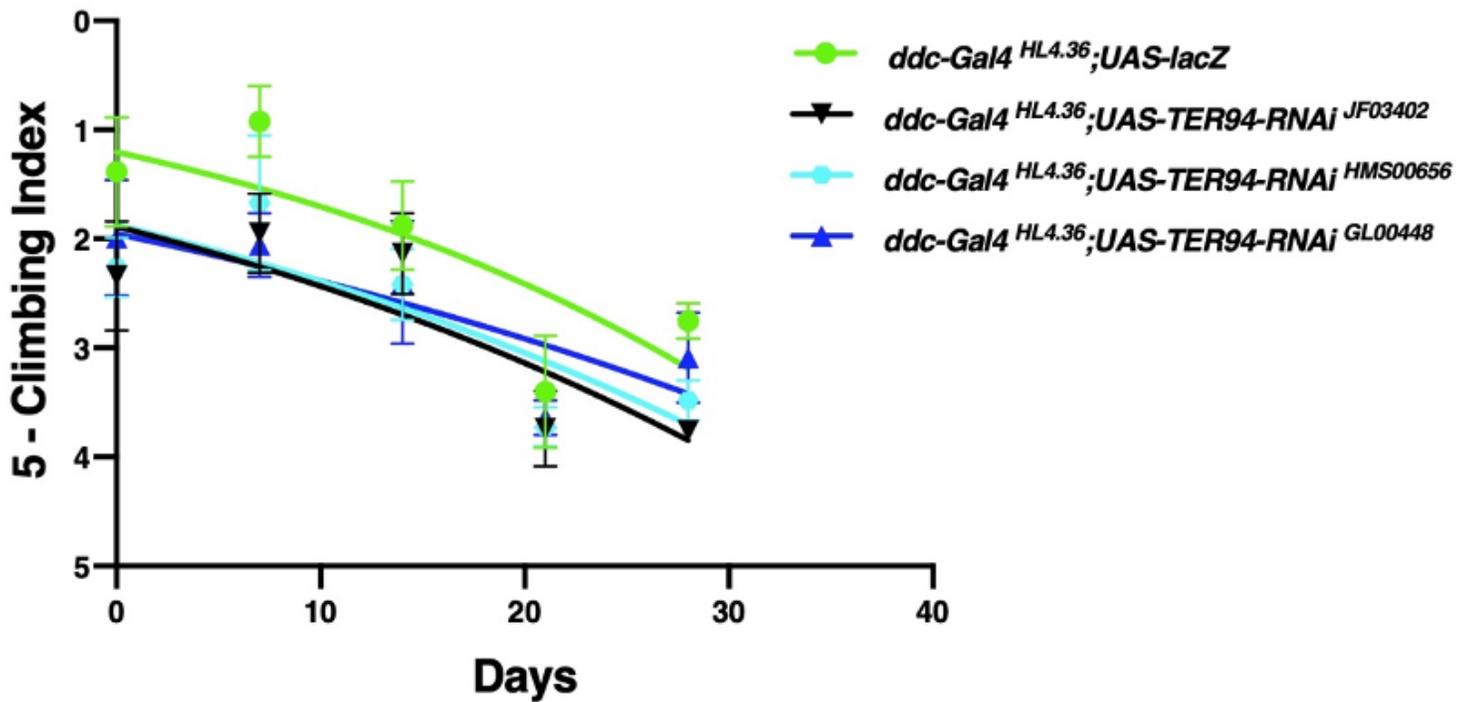


Figure 10

Altered expression of TER94 directed through the *ddc-Gal4^{HL4.36}* transgene affects longevity and climbing ability. B: Locomotor assay of *D. melanogaster* males displaying altered TER94 expression in the neurons. Climbing ability was determined by a nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean.

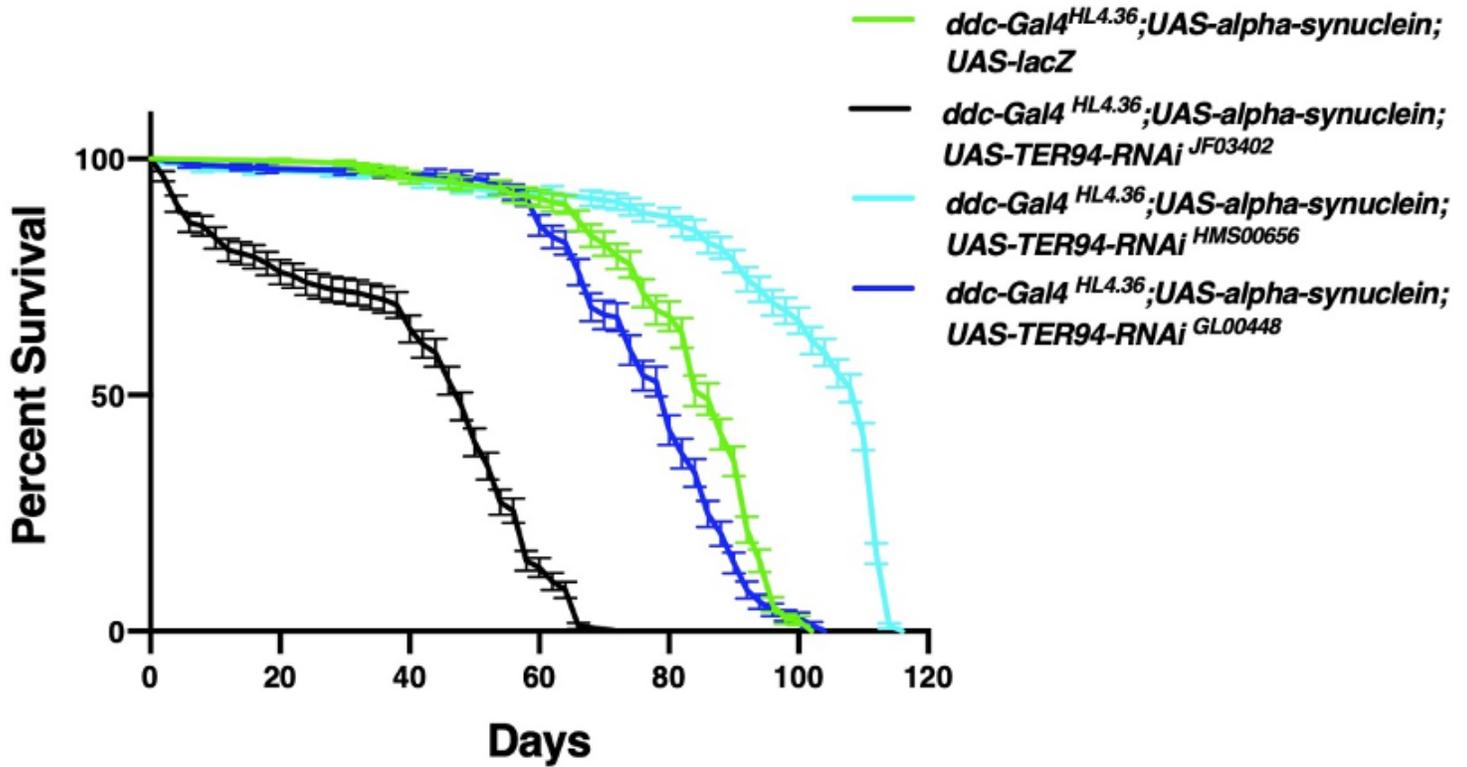


Figure 11

Altered expression of TER94 and the expression of alpha-synuclein directed through the *ddc-Gal4^{HL4.36}* transgene affects longevity and climbing ability. A: Longevity assay of *Drosophila melanogaster* males displaying the expression of alpha-synuclein and the altered TER94 expression in the neurons. Longevity is depicted by percent survival. Significance is $P < 0.05$ using the log-rank test with Bonferroni correction. Error bars represent standard error of the mean.

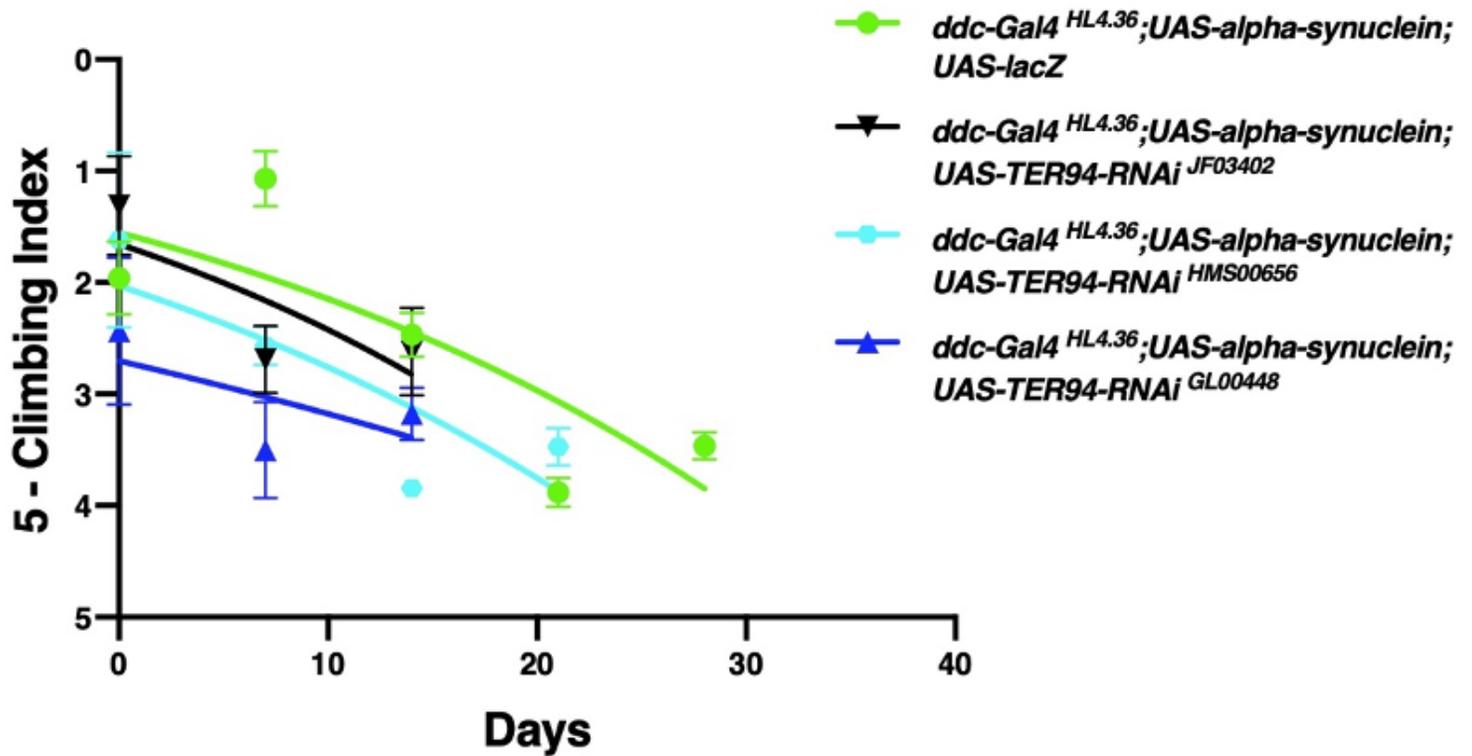


Figure 12

Altered expression of TER94 and the expression of alpha-synuclein directed through the *ddc-Gal4^{HL4.36}* transgene affects longevity and climbing ability. B: Locomotor assay of *D. melanogaster* males displaying the expression of alpha-synuclein and the altered TER94 expression in the neurons. Climbing ability was determined by a nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [HurleyStaveleyTER94Checklist.pdf](#)