Interactions Among Models Of Amyotrophic Lateral Sclerosis, Parkinson Disease And Ageing In *Drosophila melanogaster*

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Introduction

- Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by the loss of upper and lower motor neurons within the spinal cord, brain stem and motor cortex.
- Parkinson Disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons within the *substantia nigra* sub compacta region of the brain.
- A number of genes are known to be involved in disease progression
  - In ALS, major genes include: *SOD1*, *FUS* and *TARDBP*. Minor ALS genes include *p62*, *OPTN*, *TBK1*, and *VCP*
  - Primary PD genes include: *SCNA*, *PINK1* and *parkin*
- Both ALS and PD genes are linked to autophagy and its mitochondrial-directed sub-type mitophagy: two cellular processes suggested to play a substantial role in ALS and PD progression.
- ALS and PD may result from mitochondrial dysfunction as a result of impaired mitochondrial autophagy.
- This study focuses on four ALS-related genes: *TARDBP*, *p62*, *TBK1*, and *VCP*, all highly conserved between human and Drosophila, allowing for ALS and PD to be modeled in *Drosophila melanogaster*.

Research Goals

- This study aims to study the effects of genes directly related to ALS and PD and to study biological outcomes of the altered expression of such significant genes that associate and interact with them. By examining the effects of altered ALS gene expression in combination with altered PD gene activity this study also aims to investigate the consequences of altered ALS gene expression in the dopaminergic neurons.

Methodology

Longevity Assay

- A sample size of approximately 300 critical class male progeny were collected for each genotype and stored at 25° C for the duration of the experiment.
- Flies were scored every two days to examine if any death had occurred and transferred onto fresh media every four days.
- The data was analyzed using the Graphpad Prism 8 software (Graphpad Software Inc.) with a comparison of the survival curves analyzed by the Log-rank (Mantel-Cox) test with Bonferroni correction.

Locomotor Assay

- Critical class male progeny were collected within a 24-hour time period for a sample size of 70 males. Flies were maintained in vials with ten flies per vial, stored at 25° C, and placed on new medium once per week.
- Analysis began one week after collection and then every seven days after until flies had a minimum climbing score for two consecutive weeks, or less than 10 flies remained alive.
- The climbing ability of five cohorts was analyzed, with ten trials per cohort, resulting in a total of 500 trials per genotype per week. Flies were scored based on the height that was reached on a 30 cm glass tube after a 10 second time period.
- The data was analyzed using the Graphpad Prism 8 software (Graphpad Software Inc.) where a nonlinear regression curve was produced with a 95% confidence interval. Curves considered to be significantly different if $P < 0.05$.
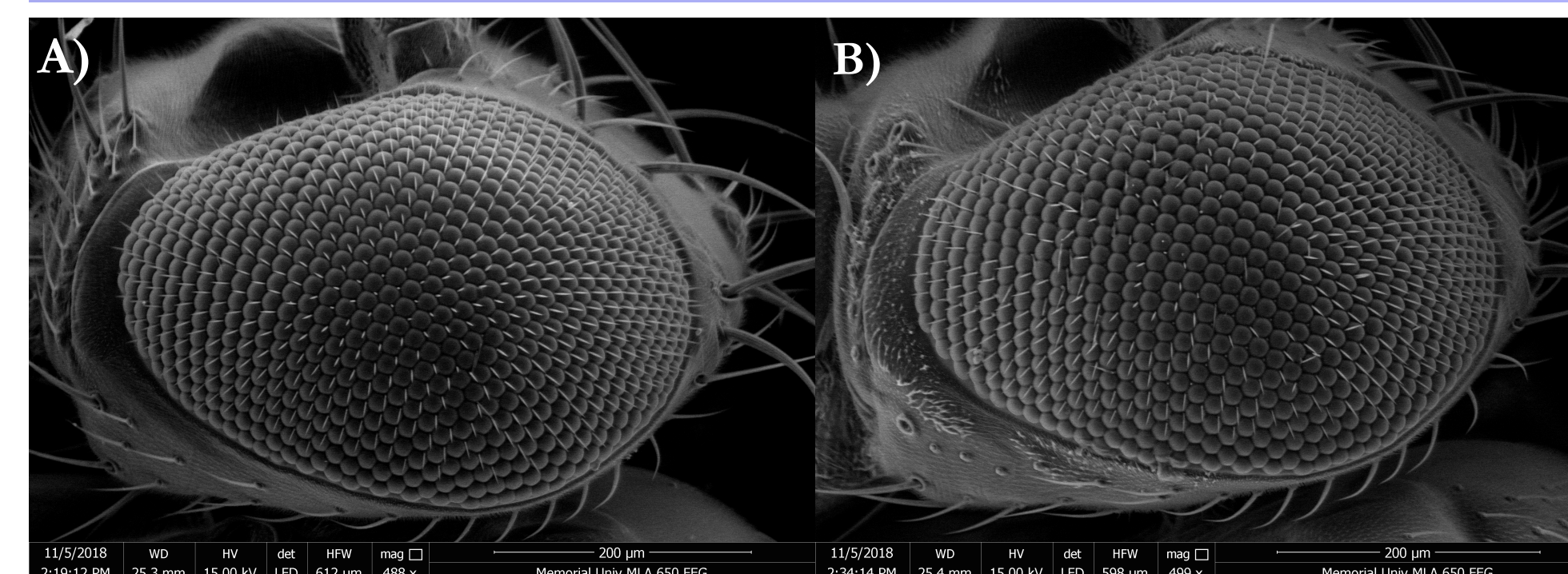
Results

Figure 1.1 Directed *TBPH* gene expression through the eye-specific transgene *GMR-Gal4*. **A:**GMR-Gal4;UAS-lacZ, **B:**GMR-Gal4;UAS-TBPH-RNAi[HMS01932]
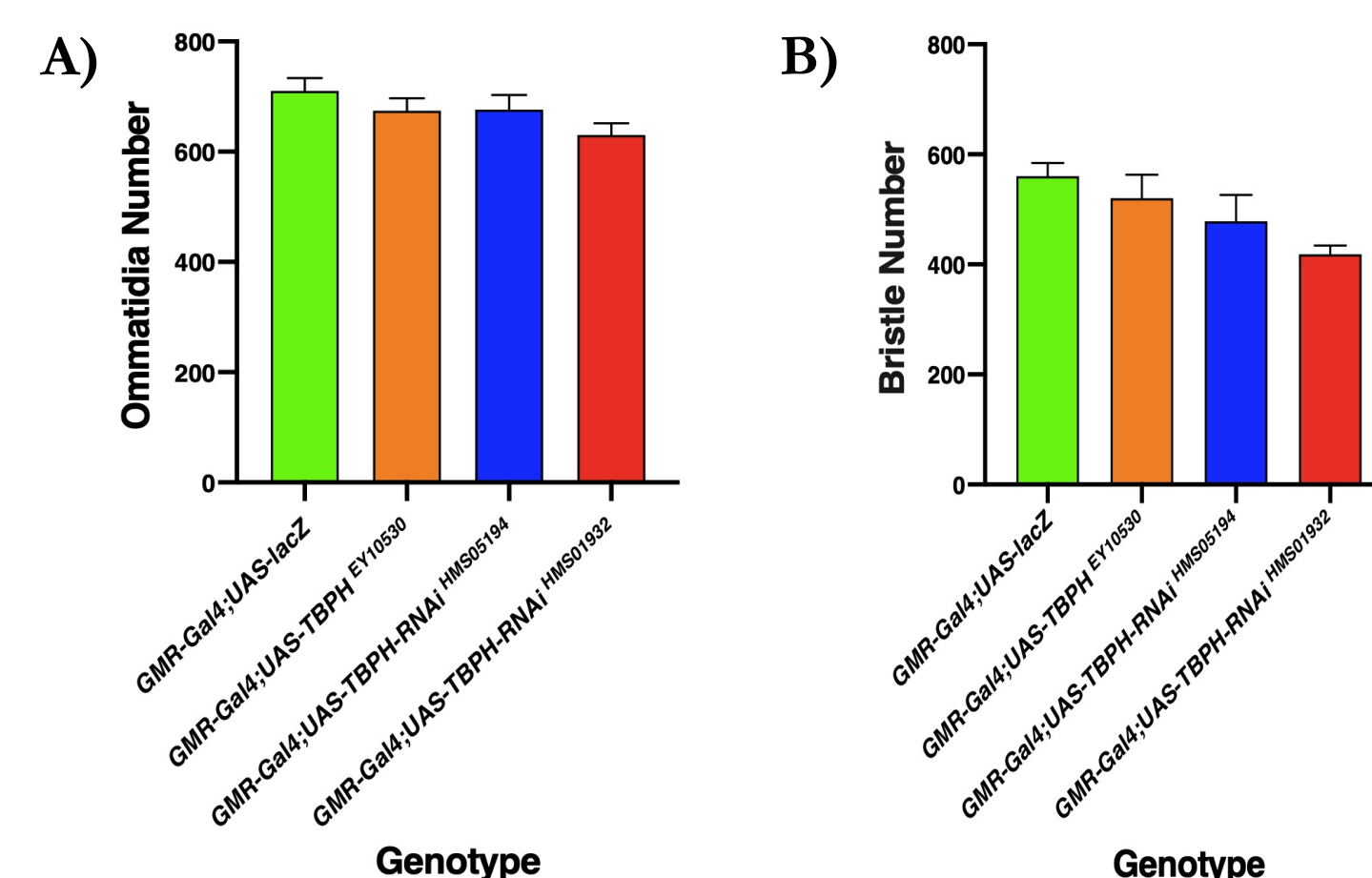

Figure 1.2 Directed *TBPH* inhibition in the developing eye via *GMR-Gal4* can lead to developmental defects. **A:** Ommatidia Number, **B:** Bristle Number
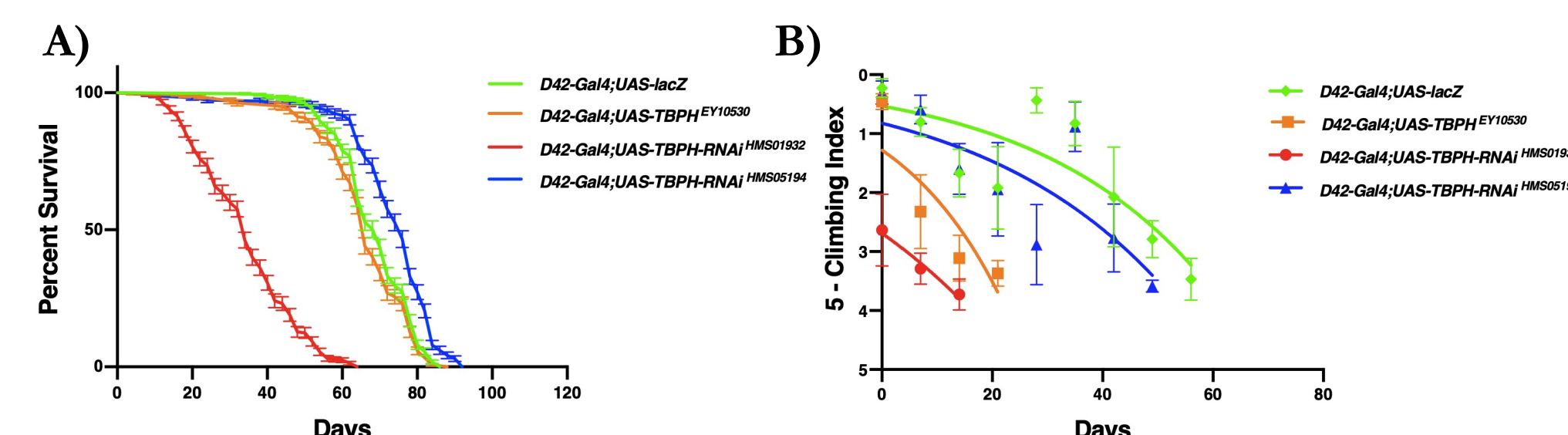

Figure 1.3 Altered expression of *TBPH* in the motor neurons directed by *D42-Gal4* can decrease or increase median lifespan and reduce climbing ability. **A:** Longevity Assay, **B:** Locomotor Assay
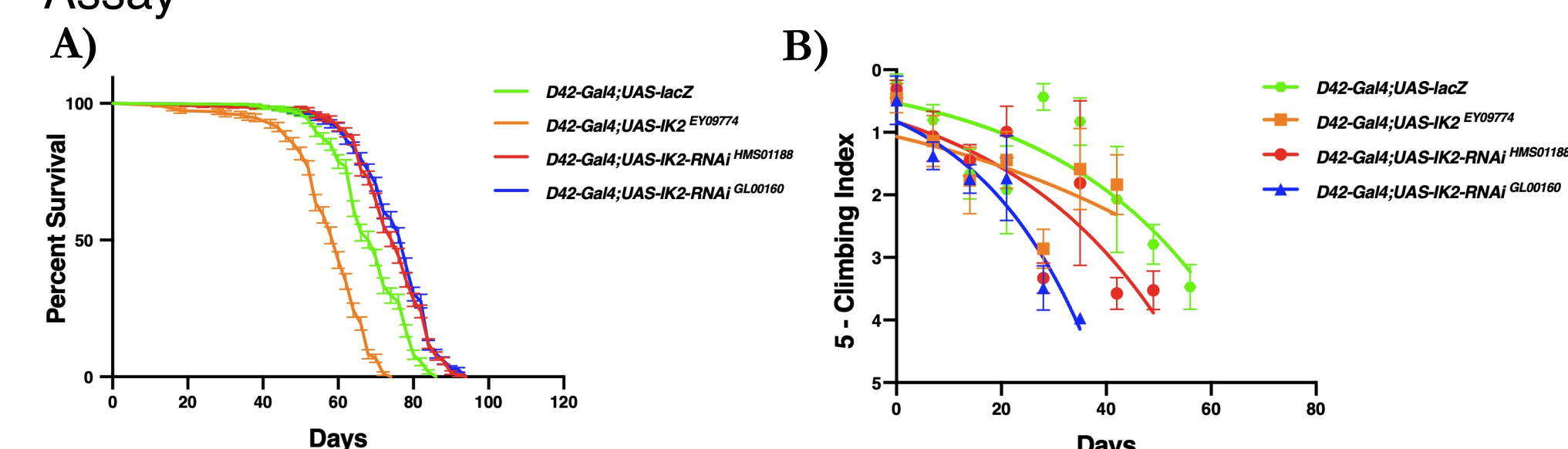

Figure 2.1 Altered expression of *IK2* in the motor neurons directed by *D42-Gal4* can decrease or increase median lifespan and reduce climbing ability. **A:** Longevity Assay, **B:** Locomotor Assay
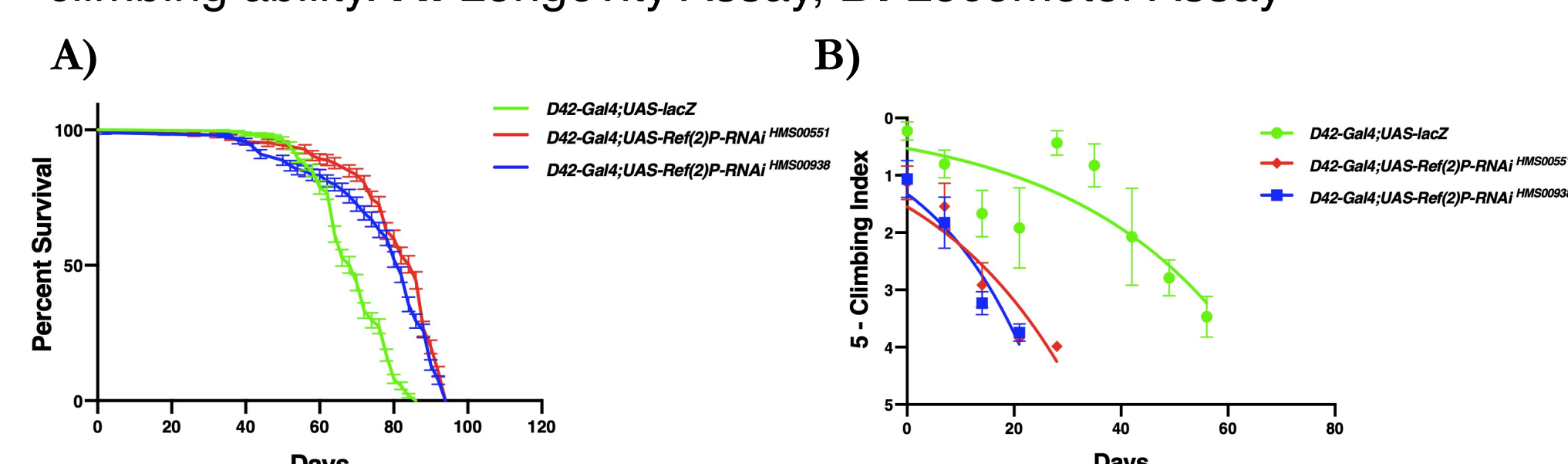

Figure 3.1 Loss of *Ref(2)P* in the motor neurons directed by *D42-Gal4* increases median lifespan and impairs climbing ability. **A:** Longevity Assay, **B:** Locomotor Assay
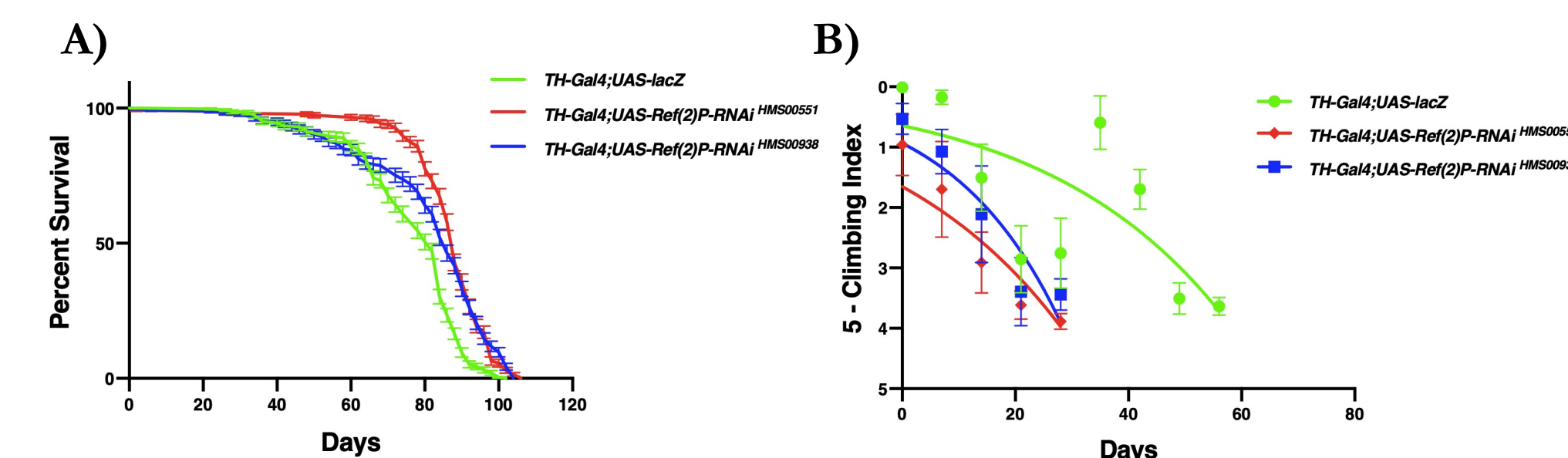

Figure 3.2 Loss of *Ref(2)P* in the dopaminergic neurons directed by *TH-Gal4* increases median lifespan and impairs climbing ability. **A:** Longevity Assay, **B:** Locomotor Assay
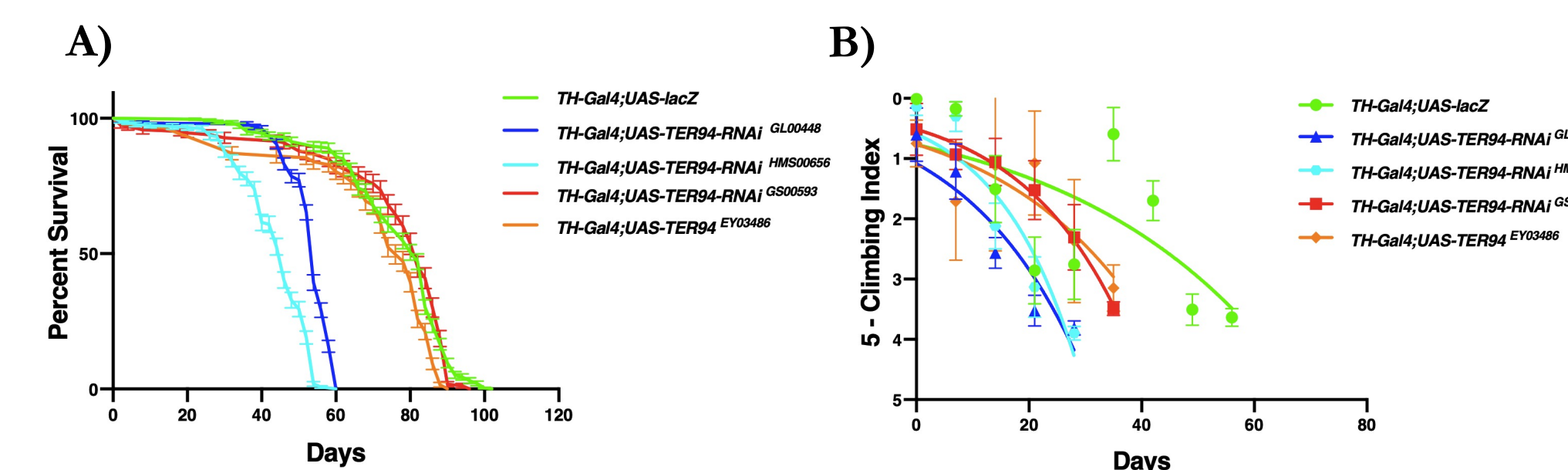

Figure 4.1 Loss of *TER94* expression in the dopaminergic neurons directed by *TH-Gal4* reduces median lifespan and climbing ability. **A:** Longevity Assay, **B:** Locomotor Assay
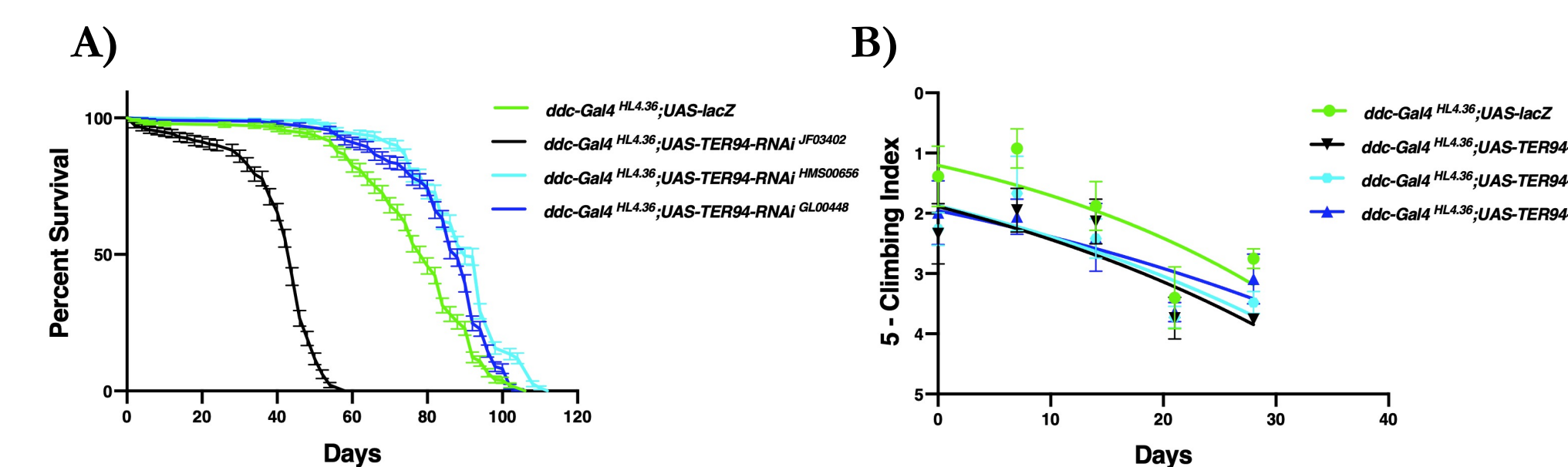

Figure 4.2 Loss of *TER94* expression directed by *ddc-Gal4* can increase or decrease median lifespan and reduce climbing ability. **A:** Longevity Assay, **B:** Locomotor Assay
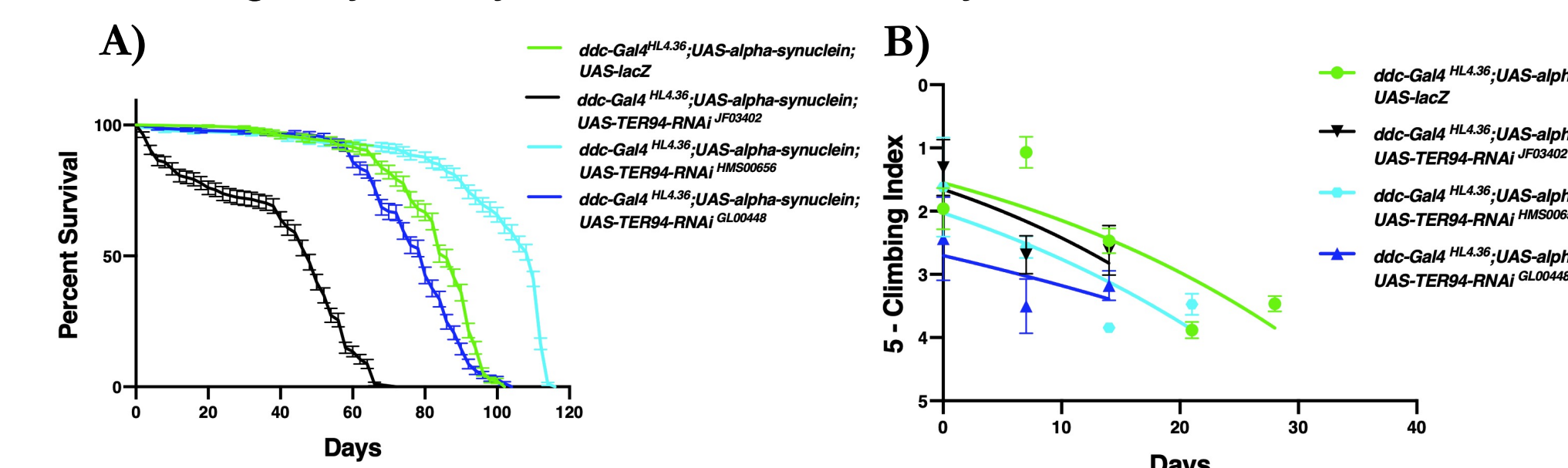

Figure 5.1 Loss of *TER94* expression combined with the directed expression of *alpha-synuclein* by *ddc-Gal4* can increase or decrease median lifespan and reduce climbing ability over time. **A:** Longevity Assay, **B:** Locomotor Assay
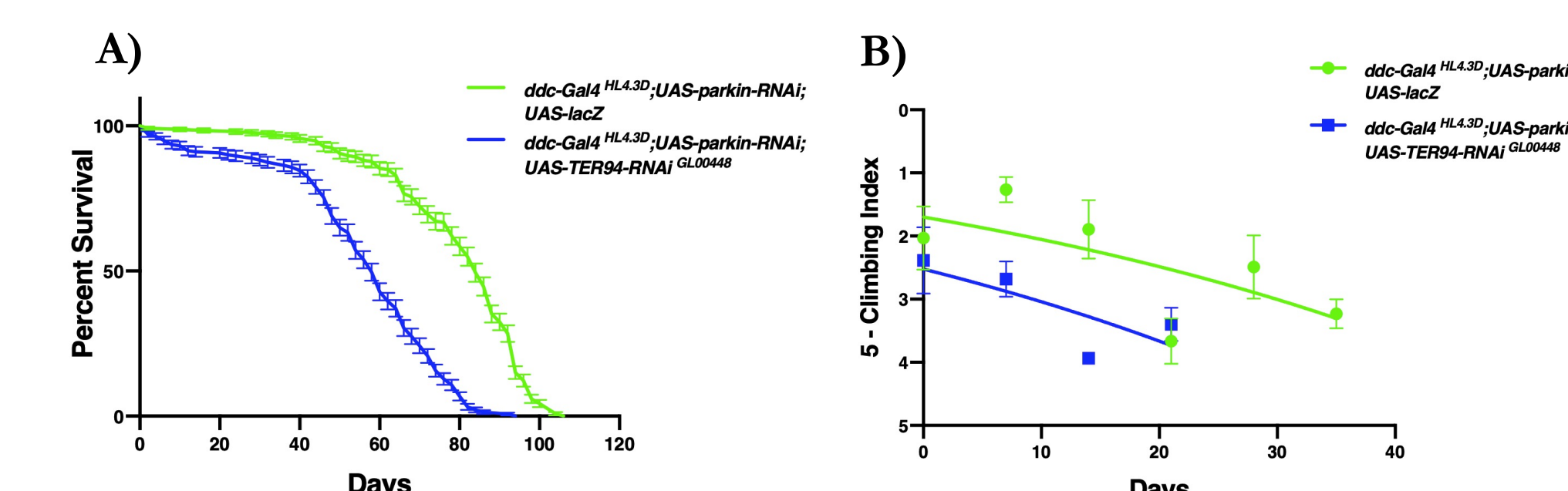

Figure 5.2 Loss of *TER94* expression combined with loss of *parkin* by *ddc-Gal4* reduces median lifespan and climbing ability. **A:** Longevity Assay, **B:** Locomotor Assay

Conclusion

- Inhibition of *TBPH* through *UAS-TBPH-RNAi[HMS01932]* in the Drosophila eye resulted in a characteristic phenotype displaying a significant reduction in ommatidia and bristle count, suggesting neural developmental defect.

- Inhibition of *TBPH* through *UAS-TBPH-RNAi[HMS01932]* in the motor neurons is a promising model of neurodegenerative disease as inhibition of *TBPH* results in a significant reduction in median lifespan and motor function, characteristic of ALS.

- Overexpression of *IK2* in the motor neurons is an imperfect model of ALS as the overexpression of *IK2* results in a significant reduction in median lifespan, however a small reduction in motor function.

- Inhibition of *Ref(2)P* in the motor neurons is a promising model of neurodegenerative disease as inhibition of *Ref(2)P* results in an increase in lifespan and a significant reduction in motor function, to suggest a trade-off between longevity and locomotor abilities.

- Inhibition of *Ref(2)P* in the dopaminergic neurons is a promising model of neurodegenerative disease as inhibition of *Ref(2)P* results in a slight increase in lifespan and a significant decline in motor function, suggesting a trade-off between longevity and locomotor abilities.

- Inhibition of *TER94* though *UAS-TER94-RNAi[HMS00656]* and *UAS-TER94-RNAi[GL00448]* in the dopaminergic neurons provides promising models of Parkinson Disease as inhibition of *TER94* results in a significant reduction in both median lifespan and motor function.

- Inhibition of *TER94* through *UAS-TER94-RNAi[HMS00656]* coupled with the expression of *alpha-synuclein* in the *ddc-Gal4*-expressing neurons results in a large increase in median lifespan by ~ 28%, while slightly reducing motor function.

- Inhibition of *TER94* and *parkin* in the *ddc-Gal4*-expressing neurons provides a very promising model of neurodegenerative disease as inhibition results in a great reduction in lifespan by ~ 30%, while having a significant decline in motor function.

References

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