Activation of mTORC1 and Suppression of Autophagy in Muscle Tissue Shortens Lifespan in Mice and Flies

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# Abstract

# Introduction

# Methods and Materials

## Animal Housing and Procedures

Floxed *Tsc1* alleles (Tsc1*tm1Djk*/J1 from the Jackson Laboratory) were crossed with *Ckmm-Cre* driver mice (FVB-Tg(*Ckmm-cre*)5Khn/J2 from the Jackson Laboratory) to generate animals that were heterozygous for the floxed allele, and either hemizygous for the *Cre* allele or wild-type at this locus. These mice were used to generate progeny that were wild-type at both the Tsc1 and Cre loci, homozygous at the *Tsc1* loci (floxed only), hemizygous at the Cre loci (transgene only) or *Tsc1*fl/fl, *Ckmm-Cre*Tg/+. Animals were housed in a 12h light/dark cycle animal facility with ad libitum access to food (Harlan Teklad) and water according to procedures approved by the University of Michigan University Committee on Use and Care of Animals. Animals were allowed to die naturally, or were euthanized at the advice of veterinary staff.

## Drosophila Breeding and Maintenance

Fly stocks (see Table 1) were purchased from the Bloomington Stock cCnter and maintained and were raised at 25C on standard corn meal food. For crosses, virgin females were collected from the GAL4 driver strains. Ten virgin females were used per cross. Males with the appropriate genotype were chosen from each of the lines and crossed to male UAS-TRiP-shRNA lines as well as a UAS-TRiP control which contains the genomic insertion site but no shRNA3
. Flies were maintained in a humidified (50–60%)
incubator at 25C. Ten days after each cross the F1 progeny began to eclose and adults were sorted according to phenotype and gender. Flies bearing the balancer markers were discarded from the analysis. Sorted flies were put into new vials, with males and females separated and with 5–10 flies in each vial. Flies were transferred to fresh food twice weekly with deaths noted from each cross. The person handling the flies was blinded to the genotype of the flies.

## Western Blotting

Muscle samples (20-50 ug) were lysed in 20 uL/mg of RIPA buffer using a Qialyser (5 minutes at 30 Hz). After centrifugation (15 minutes at 14 000 RPM), lysates were boiled in a final concentration of 1X SDS-PAGE sample buffer (BioRad). Proteins were separated on pre-cast gradient gels from BioRad and transferred to nitrocellulose membranes. After blocking in 2% bovine serum albumin (Fisher), proteins were probed with anti-LC3, anti-pS6K, total S6K, anti-pS6 and anti-S6 antibodies. Secondary antibodies were alexa 680/700 conjugated anti-mouse and anti-rabbit secondary antibodies and blots were visualized via a LiCOR Odyssey system. Quantification was performed using ImageStudio Lite (LiCOR).

## Statistics

All statistical tests were performed using the R software package4. For pairwise tests, normality was tested via Shapiro-Wilk tests, and equal variance was tested using Levene’s test. Based on these results, either Wilcoxon tests, Student’s *t* or Welch’s *t*-tests were performed. For survival analyses and cox proportional hazard tests, the survival package was used (version 2.38-3)5,6. All raw data and reproducible code is available at http://bridgeslab.github.io/DrosophilaMuscleFunction

# Results

## Deletion of *Tsc1* in muscle tissue causes shortened lifespan

# Discussion

# Acknowledgements

# References

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