

Hyperactivation of mTORC1 by an endogenous *raga-1* gain-of-function mutation does not reduce lifespan in *C. elegans*

Tatiana M. Moreno¹, Michelle E. Brown², Caroline Kumsta^{2§}

¹Graduate School of Biomedical Sciences, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California, United States

²Development, Aging and Regeneration Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California, United States

[§]To whom correspondence should be addressed: ckumsta@sbsdsc.discovery.org

Abstract

Inhibition of mTORC1, a conserved nutrient-sensing complex, extends lifespan across model organisms, but the effects of mTORC1 hyperactivation are less understood. RagA, a GTPase essential for mTORC1 activation, can be locked in its active GTP-bound state through gain-of-function mutations, such as Q63L in *C. elegans* RAGA-1. We found that transgenic expression of *raga-1*[Q63L] mutation (*egIs12*) decreases lifespan without hyperactivating mTORC1, suggesting mTORC1-independent effects or transgene toxicity. In contrast, we show that a CRISPR-generated Q63L mutation at the endogenous *raga-1* locus (*viz128*) hyperactivates mTORC1 without affecting lifespan, challenging the paradigm that mTORC1 hyperactivation accelerates aging. Thus, genetic context and potential compensatory mechanisms may contribute to mTORC1-mediated lifespan regulation, at least in metazoans.

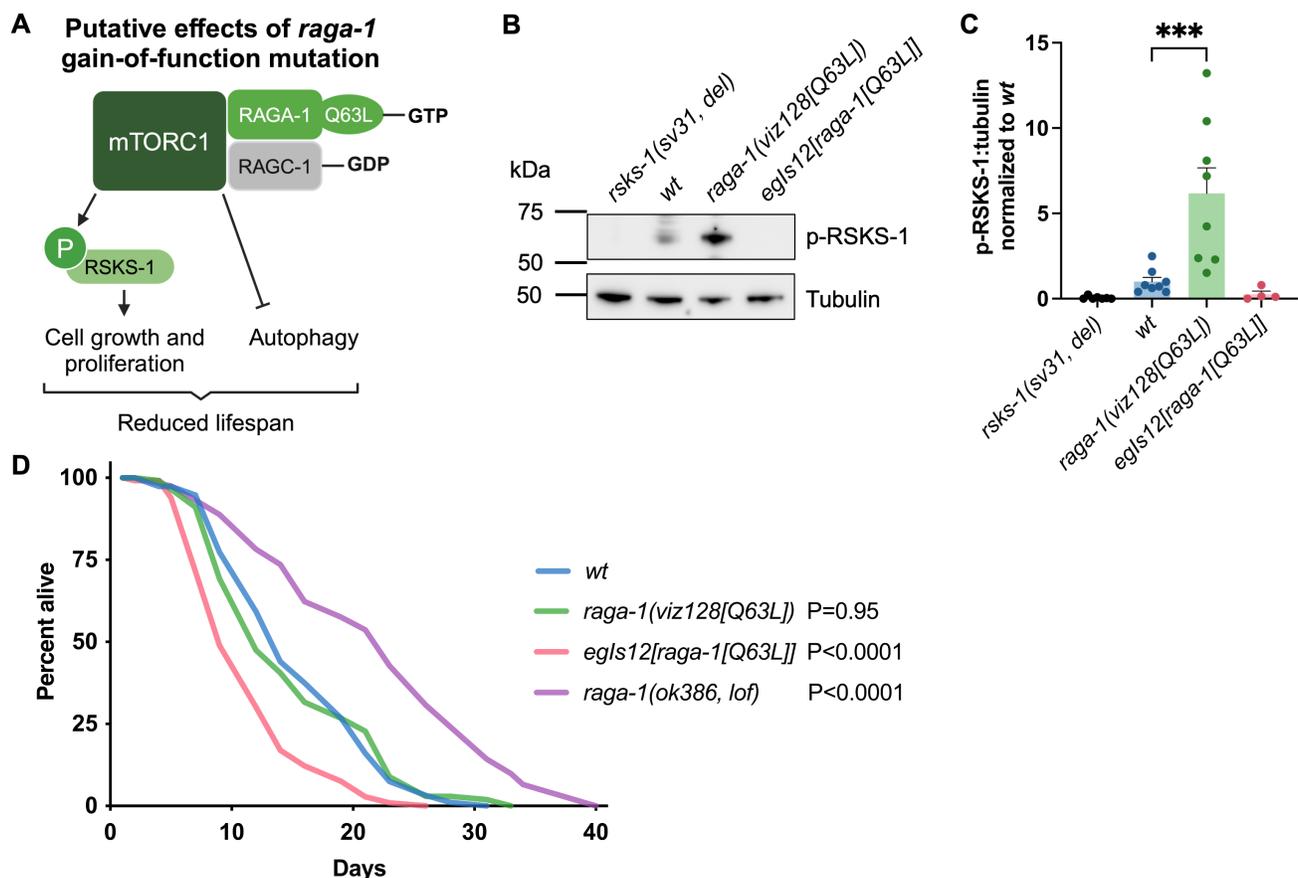


Figure 1. Endogenous *raga-1* gain-of-function mutation (*viz128*) increases phospho-RSKS-1 without affecting lifespan in *C. elegans*:

(A) Model of the putative effects of Q63L gain-of-function mutation in *C. elegans* RAGA-1 (RagA[Q66L] mutation in humans). RAGA-1[Q63L] is expected to lead to constitutive GTP binding, stabilizing its active conformation in a dimer with

GDP-bound RAGC-1, hyperactivating mTORC1, and leading to phosphorylation of downstream targets, including RSKS-1/S6K to stimulate cell growth and others to inhibit autophagy, contributing to lifespan shortening. **(B)** Representative Western blot and **(C)** quantification of phospho-RSKS-1(T404), conserved from human phospho-p70-S6 Kinase(T389), in day-1 old *rsk-1(sv31)*, wild type (*wt*), *raga-1(viz128[Q63L])*, and *egIs12[raga-1[Q63L]]* animals. *raga-1(viz128[Q63L])* and *egIs12[raga-1[Q63L]]* were outcrossed four times to wild type, see **Reagents**. *rsk-1(sv31, del)* carries a 2,673 bp deletion including T404, and is used as a negative control. $n=4-8$. $***P<0.001$ by mixed-effects analysis with Holm-Šidák's correction. No other comparisons to *wt* were significant. **(D)** Representative lifespan analysis of *wt*, *raga-1[Q63L]* and *raga-1* loss-of-function mutants. *raga-1(ok386, lof)* carries a 1,242 bp deletion spanning from 11 bp upstream of the Q63 codon through the 3' UTR. *wt* (mean lifespan (MLS): 15.5 days, $N=98/116$) compared with *raga-1(viz128[Q63L])* (MLS: 15.0 days, $N=103/112$), *egIs12[raga-1[Q63L]]* (MLS: 11.3 days, $N=108/117$), and *raga-1(ok386, lof)* (MLS: 21.9 days, $N=101/120$). Statistical comparisons by log-rank test. See **Extended Data Table 1** for additional lifespan experiments.

Description

The mechanistic target of rapamycin complex 1 (mTORC1) is a conserved protein complex that regulates cellular growth and metabolism in response to environmental and nutritional cues. Under nutrient-rich conditions, mTORC1 becomes activated and promotes anabolic processes such as cell growth and proliferation while simultaneously inhibiting catabolic pathways such as autophagy. Conversely, during nutrient scarcity or stress, mTORC1 activity is reduced, leading to the activation of autophagy to recycle cellular components and maintain energy homeostasis (Saxton and Sabatini 2017; Papadopoli et al. 2019).

Inhibition of mTORC1, either through genetic means or pharmacological compounds including rapamycin, reproducibly extends lifespan and can delay several hallmarks of aging including dysregulated nutrient sensing, disabled macroautophagy, and loss of proteostasis (Mannick and Lamming 2023; Papadopoli et al. 2019). These effects are observed across diverse model systems, including in *C. elegans* (Vellai et al. 2003; Hansen et al. 2007; Robida-Stubbs et al. 2012; Zhang et al. 2024), *Drosophila* (Kapahi et al. 2004; Bjedov et al. 2010) and mice (Harrison et al. 2009; Wu et al. 2013). In contrast, increased mTORC1 activity has been observed in several human and rodent tissues with age (Markofski et al. 2015; Joseph et al. 2019; Tramutola et al. 2015), and mTORC1 activation via gain-of-function mutations of the heterodimeric Ras-related small GTP-binding proteins (Rag GTPases), which positively regulate mTORC1 complex formation and activity, is associated with shortened lifespan in mammalian models (de la Calle Arregui et al. 2021; Ortega-Molina et al. 2024). However, the characterization of models hyperactivating mTORC1 to directly study its effects on aging and related pathways is still limited, particularly in genetically tractable systems, such as *C. elegans*. Understanding the physiological effects of mTORC1 hyperactivation is critical to uncover how mTORC1 contributes to aging and age-related pathologies.

A key regulatory mechanism of mTORC1 involves the Rag GTPases (Sancak et al. 2008; Kim et al. 2008), which act as nutrient sensors that facilitate mTORC1 localization to lysosomes, critical for its activation. In mammals, RagA/B and RagC/D form obligate heterodimers, while *C. elegans* only has one homolog of each family *RAGA-1* and *RAGC-1*. Under nutrient-rich conditions, GTP-bound RagA/B and GDP-bound RagC/D form an active heterodimer that interacts directly with mTORC1 to activate downstream targets (Sambri et al. 2024). These include ribosomal S6 kinase (S6K), which promotes protein synthesis (Brown et al. 1995), and Unc-51-like kinase 1 (ULK1), which inhibits autophagy initiation (Kim et al. 2011). Upon low-nutrient conditions, the GTP-bound state of RagA/B is reduced, leading to mTORC1 inactivation, and dephosphorylation of mTORC1 targets (Sambri et al. 2024). Through this regulation, the Rag GTPases mediate a dynamic switch between anabolic and catabolic processes, coupling mTORC1 activity to nutrient availability.

While these regulatory principles are well-characterized in mammals, their exact roles in regulating mTORC1 in *C. elegans* remain less understood. Evidence suggests that *RAGA-1* and *RAGC-1* act as positive regulators of mTORC1 in *C. elegans*, as loss-of-function mutations phenocopy reduced mTORC1 activity in mammalian systems, leading to increased autophagy, decreased protein synthesis, and extended lifespan (Robida-Stubbs et al. 2012). However, the developmental defects caused by loss of *raga-1* or *ragc-1* are less severe than those observed with core mTORC1 components (Fukuyama et al. 2012), indicating that mTORC1 may retain some activity independent of *RAGA-1* and *RAGC-1* (Blackwell et al. 2019; Schreiber et al. 2010). Gain-of-function mutations, such as *raga-1[Q63L]*, putatively lock *RAGA-1* in its active GTP-bound state, and provide a model for mTORC1 hyperactivation (**Fig. 1A**). The transgenic *C. elegans* strain *egIs12[raga-1p::raga-1[Q63L]+ofm-1p::gfp]* overexpresses the *raga-1[Q63L]* mutation, alongside wild-type *raga-1*, and exhibits a drastically shortened lifespan (Schreiber et al. 2010; Gerisch et al. 2020; Huang et al. 2022). However, neither mTORC1 activity nor *RAGA-1* expression levels have been directly assessed in this strain, leaving it unclear whether the reported lifespan reduction is due to mTORC1 hyperactivation or alternative pathways influenced by *RAGA-1* activation.

To further investigate the role of mTORC1 hyperactivation in *C. elegans* aging, we analyzed the effects of the *raga-1*[Q63L] mutation on mTORC1 activity and lifespan. Our study included the previously characterized transgenic *egIs12*[*raga-1*[Q63L]] strain (Schreiber et al. 2010), and a newly CRISPR-generated strain, *raga-1*(*viz128*[Q63L]) (Trimmer and Arur 2021), which carries the *raga-1*[Q63L] mutation at its endogenous gene locus. The *raga-1*(*viz128*[Q63L]) strain allowed us to study the effects of **RAGA-1** hyperactivation without additional transgenic expression, providing a more direct model of mTORC1 hyperactivation. We assessed mTORC1 activity by measuring phosphorylation of mTORC1 target **RSKS-1**(T404), the *C. elegans* ortholog of mammalian S6K(T389) (**Fig. 1A**). While *rsk-1*(*sv31*) mutants, which carry a large deletion including T404, showed no p-RSKS(T404) signal, p-**RSKS-1**(T404) levels were significantly increased in the *raga-1*(*viz128*[Q63L]) mutant, but not in the *egIs12*[*raga-1*[Q63L]]-carrying strain, indicating that only the endogenous mutation is sufficient to increase mTORC1 activity (**Fig. 1B, C**). To further explore the functional consequences of altered mTORC1 activity via *raga-1* modulation, we assessed lifespan in the *raga-1*[Q63L] strains. As a control for the effects of reduced mTORC1 activity on lifespan, we included a *raga-1*(*ok386*) loss-of-function mutant, *raga-1*(*ok386, lof*) (**Fig. 1D**), which significantly extended lifespan, as previously shown (Schreiber et al. 2010). The *egIs12*[*raga-1*[Q63L]] strain exhibited a markedly reduced lifespan compared to wild type (**Fig. 1D**), consistent with previous reports linking transgenic *raga-1*[Q63L] expression to accelerated aging (Schreiber et al. 2010; Gerisch et al. 2020; Huang et al. 2022). In contrast, the *raga-1*(*viz128*[Q63L]) strain, despite increased mTORC1 activity, surprisingly displayed a normal lifespan comparable to wild type (**Fig. 1D**).

Our findings reveal distinct molecular changes and lifespan phenotypes in strains carrying the *raga-1*[Q63L] mutation, highlighting that **RAGA-1** and mTORC1 activity measured via **RSKS-1** phosphorylation, and aging in *C. elegans* are not fully interdependent, and further investigation is needed to elucidate the coupled and independent functions of each pathway component.

The *raga-1*(*viz128*[Q63L]) strain exhibited increased p-**RSKS-1**(T404) levels, indicating elevated mTORC1 activity, yet showed no significant difference in lifespan compared to wild type. This finding is inconsistent with the prevailing paradigm that mTORC1 hyperactivation universally accelerates aging, and suggests that mTORC1 hyperactivation, at least to this degree, may not be sufficient to impact lifespan in metazoans. It remains unclear whether other mTORC1 targets are similarly phosphorylated in this mutant, warranting further investigation to map the broader effects of this *raga-1* gain-of function mutation on mTORC1 activity. Further characterization of mTORC1-associated phenotypes, such as autophagy, nucleolar size, and lipid storage, could help identify which mTORC1-dependent pathways are selectively activated, inhibited or unaffected in the context of endogenous *raga-1*[Q63L] mutation. These phenotypes could help explain why lifespan remains unchanged in the *raga-1*(*viz128*[Q63L]) mutants, despite increased **RSKS-1** phosphorylation.

In contrast, the *egIs12*[*raga-1*[Q63L]] strain exhibited decreased levels of p-**RSKS-1**(T404) compared to wild type (0.26-fold, corresponding to a 73.7% reduction), although this difference did not reach statistical significance (P=0.67, mixed-effects model). This strain also displayed a markedly shortened lifespan. The combination of these two observations suggests again that mTORC1 activity, **RSKS-1** phosphorylation, and longevity are not strictly coupled. Several non-exclusive explanations could account for this. First, the shortened lifespan could result from *raga-1*[Q63L] overexpression toxicity effects, independent of mTORC1 signaling. This explanation is consistent with the shortened lifespan but does not readily account for the reduced p-**RSKS-1** levels. To test whether the reduced lifespan in *egIs12*[*raga-1*[Q63L]] is independent of **RSKS-1**, lifespan of long-lived *rsk-1* mutants (Hansen et al. 2007) expressing *egIs12*[*raga-1*[Q63L]] could be assessed; if *egIs12*[*raga-1*[Q63L]] shortens *rsk-1* mutant lifespan, it would support the hypothesis that overexpression toxicity, rather than mTORC1 signaling via **RSKS-1**, drives the phenotype. Second, the residual wild-type *raga-1* expression could mitigate the hyperactivating effects of the mutant allele, thereby limiting mTORC1 activation and **RSKS-1** phosphorylation; however, this would more likely normalize p-**RSKS-1** levels and lifespan rather than reducing both. Assessing lifespan in *raga-1*(*viz128*[Q63L])/+ heterozygotes, which, like *egIs12*, would express both wild-type and the *raga-1* gain-of-function mutation, could help distinguish between dosage-dependent effects and transgene-specific toxicity. If such heterozygotes exhibit normal lifespan, this would argue against a simple buffering model and instead support a contribution of transgene overexpression to the *egIs12* phenotype. Third, a dominant-negative effect could explain the reduced p-**RSKS-1** levels. A dominant-negative effect occurs when a mutant protein interferes with the normal function of the wild-type protein, often by forming non-functional complexes that block downstream activity. If overexpressed mutant **RAGA-1**[Q63L] bound and sequestered endogenous **RAGC-1** into non-productive heterodimers, this could suppress mTORC1 activation. While this would be consistent with the reduction in **RSKS-1** phosphorylation, an extended lifespan rather than a shortened lifespan would be expected, and a further inhibition of mTORC1 (e.g., via *let-363* RNAi) should fail to extend lifespan, which could be tested in future experiments. Finally, it is also possible that the reduced lifespan observed in *egIs12*[*raga-1*[Q63L]] is driven by tissue-specific effects of mTORC1 hyperactivation that do not translate into a detectable global increase in **RSKS-1** phosphorylation by Western blot. A recent study demonstrated that neuron-specific expression of *raga-1* in a long-lived *raga-1* loss-of-function

mutant is sufficient to abolish its longevity phenotype (Zhang et al. 2019), highlighting the potential for distinct outcomes depending on the tissues in which mTORC1 signaling is activated.

Our study has certain limitations, such as using tubulin for Western blot normalization rather than total levels of [RSKS-1/S6K](#) due to the lack of a robust [RSKS-1/S6K](#) antibody validated in *C. elegans*. Although [RSKS-1/S6K](#) phosphorylation may not fully reflect all functional outputs of mTORC1 activity, it remains the most comparable readout to mammalian studies available. Additionally, the [raga-1\(viz128\[Q63L\]\)](#) strain showed some variability in p-[RSKS-1](#) levels, which may reflect genetic drift, as this variability became more pronounced when the strain was cultured over several weeks. To ensure data reliability, we recommend using recently-thawed populations and multiple biological replicates when using this strain or assessing [RSKS-1](#) phosphorylation in any experimental context.

Overall, these findings challenge the current paradigm that mTORC1 hyperactivation universally reduces lifespan. We demonstrate that mTORC1 hyperactivation, as implied by increased p-[RSKS-1/S6K](#) levels, in *C. elegans* [raga-1\(viz128\[Q63L\]\)](#) mutants, is not sufficient to accelerate aging. These results underscore the complexity of mTORC1 signaling in aging, highlighting how the degree and mode of hyperactivation influence its biological outcomes and necessitates further research into its context-specific effects.

Methods

[C. elegans](#) cultivation

C. elegans were maintained at 20°C on NGM plates, fed with *Escherichia coli* OP50, and cultured following standard protocols (Brenner 1974). [AUM1693 \(raga-1\(viz128\)\)](#) and [BZ1290 \(eglIs12\[raga-1\[Q63L\]; ofm-1p::gfp\]\)](#) were outcrossed 4 times to [N2](#) wild type to generate [KUM91](#) and [KUM142](#), respectively. [VC222 \(raga-1\(ok386\)\)](#) and [VB633 \(rsk-1\(sv31\)\)](#) were not outcrossed. [raga-1](#) mutant genotypes were confirmed by PCR and Sanger sequencing. The *C. elegans* strains used in this study are listed under **Reagents**.

Western blots

Age-synchronized animals (N=100) were hand-picked on day 1 of adulthood into Eppendorf tubes containing 30 μ L M9 buffer (0.6% sodium phosphate dibasic, 0.3% potassium dihydrogen phosphate, 0.5% sodium chloride, 0.025% magnesium sulfate heptahydrate) using a platinum wire, and immediately washed 3-5 times with additional M9 buffer to remove all [OP50](#) bacteria. After the final wash, M9 buffer volume was adjusted to \sim 10 μ L. 2 μ L 6X Laemmli buffer (Thermo Scientific, #J61337.AC) was added, and samples were flash frozen in liquid N₂. Worms were lysed by two rounds of freeze-thaw in liquid N₂ and briefly spun down in a tabletop centrifuge after each thaw, then boiled at 95°C for 10 min. Samples were loaded onto a 4-12% Bis-Tris protein gel (NuPAGE) in MOPS running buffer (NuPAGE, #NP0001) and proteins were separated using 110 V. Proteins were transferred to a PVDF membrane (Millipore, #IPVH85R) using a Novex Mini Cell system (Invitrogen, #EI0001) at 30 V for 1.5 hours on ice. Membranes were blocked with 5% milk in Tris-buffered saline containing 0.05% Tween-20 (TBS-T) for 30 minutes, followed by immunoblotting using primary anti-phospho-p70 S6 Kinase (Thr389) (Mak et al. 2020) (1:1,000 in 1% milk in TBS-T; overnight incubation at 4°C) and secondary anti-mouse IgG HRP (1:10,000 in 1% milk in TBS-T) (see **Reagents**). Western blots were developed using SuperSignal chemiluminescence reagent (Thermo Scientific, #34577 or #34095) and imaged on a ChemiDoc imager (Bio-Rad Laboratories, Inc.). To normalize protein loading, blots were stripped for 4 min using stripping buffer (Thermo Scientific, #46430), blocked again with 5% milk in TBS-T, probed with primary anti-tubulin (1:1,000 in 1% milk in TBS-T; overnight incubation at 4°C) and secondary anti-rabbit IgG HRP (1:10,000 in 1% milk in TBS-T), and developed and imaged as described above. Protein expression was quantified using Image Lab 6.1.0 build 7 (Bio-Rad Laboratories, Inc.). Statistical analysis was performed using PRISM 10.4.0 software (GraphPad) and P-values were calculated using a mixed-effects model with Holm-Šidák's correction.

Lifespan

Lifespan was assayed at 20°C as previously described (Hansen et al. 2005). A total of 108-140 age-synchronized worms were placed on six to seven 6 cm NGM plates seeded with [OP50](#) bacteria at the L4 larval stage (lifespan day 0). Worms were transferred away from progeny to new 6 cm NGM plates seeded with [OP50](#) bacteria until post-reproductive, after which worms were scored three times per week until dead. Worms were scored as dead when no movement was observed upon gentle prodding with a platinum wire. Worms that experienced internal hatching, desiccated on the edge of the plate, escaped, or were accidentally killed were censored. Statistical analysis was performed using OASIS 2.0 at <https://sbi.postech.ac.kr/oasis2/> (Han et al. 2016), and P-values were calculated with the log-rank (Mantel-Cox) method. See **Extended Data Table 1** for a summary of all lifespan experiments.

Reagents

<i>C. elegans</i> strain	Genotype	Source
N2	<i>Wild type</i>	Kumsta Lab, originated from Hansen Lab
KUM91	<i>raga-1(viz128[Q63L]) II</i>	AUM1693 (Trimmer and Arur 2021) 4X outcrossed to N2
KUM142	<i>eglIs12[raga-1p::raga-1[Q63L]; ofm-1p::gfp]</i>	BZ1290 (Schreiber et al. 2010) 4X outcrossed to N2
VC222	<i>raga-1(ok386) II</i>	CGC
VB633	<i>rsk-1(sv31) III</i>	(Hansen et al. 2007)

Antibody	Animal and clonality	Source
Anti-Phospho-p70 S6 Kinase (Thr389)	Mouse monoclonal	Cell Signaling catalog #9206, lot #30
Anti-α-Tubulin	Rabbit polyclonal	Cell Signaling catalog #2144, lot #7
Anti-mouse IgG, HRP-conjugated	Horse	Cell Signaling catalog #7076, lot #38
Anti-rabbit IgG, HRP-conjugated	Goat	Cell Signaling catalog #7074, lot #33

Acknowledgements: We thank Drs. Swathi Arur and Adam Antebi for generously sharing *C. elegans* strains for this project. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). We thank Dr. Malene Hansen and Kumsta Lab colleagues for their feedback on this manuscript. **Figure 1A** was created using BioRender.com.

Extended Data

Description: Extended Data Table 1: Lifespan analysis of wild-type and raga-1 mutant worms.. Resource Type: Dataset. File: [Moreno TM ExtendedData.pdf](#). DOI: [10.22002/kgd2h-f8s29](#)

References

- Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, Foley A, Partridge L. 2010. Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metab* 11(1): 35-46. PubMed ID: [20074526](#)
- Blackwell TK, Sewell AK, Wu Z, Han M. 2019. TOR Signaling in *Caenorhabditis elegans* Development, Metabolism, and Aging. *Genetics* 213(2): 329-360. PubMed ID: [31594908](#)
- Brenner S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics* 77(1): 71-94. PubMed ID: [4366476](#)
- Brown EJ, Beal PA, Keith CT, Chen J, Shin TB, Schreiber SL. 1995. Control of p70 s6 kinase by kinase activity of FRAP in vivo. *Nature* 377(6548): 441-6. PubMed ID: [7566123](#)
- de la Calle Arregui C, Plata-Gómez AB, Deleyto-Seldas N, García F, Ortega-Molina A, Abril-Garrido J, et al., Efeyan A. 2021. Limited survival and impaired hepatic fasting metabolism in mice with constitutive Rag GTPase signaling. *Nat Commun* 12(1): 3660. PubMed ID: [34135321](#)
- Fukuyama M, Sakuma K, Park R, Kasuga H, Nagaya R, Atsumi Y, et al., Katada T. 2012. *C. elegans* AMPKs promote survival and arrest germline development during nutrient stress. *Biol Open* 1(10): 929-36. PubMed ID: [23213370](#)
- Gerisch B, Tharyan RG, Mak J, Denzel SI, Popkes-van Oepen T, Henn N, Antebi A. 2020. HLH-30/TFEB Is a Master Regulator of Reproductive Quiescence. *Dev Cell* 53(3): 316-329.e5. PubMed ID: [32302543](#)

- Han SK, Lee D, Lee H, Kim D, Son HG, Yang JS, Lee SV, Kim S. 2016. OASIS 2: online application for survival analysis 2 with features for the analysis of maximal lifespan and healthspan in aging research. *Oncotarget* 7(35): 56147-56152. PubMed ID: [27528229](#)
- Hansen M, Hsu AL, Dillin A, Kenyon C. 2005. New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a *Caenorhabditis elegans* genomic RNAi screen. *PLoS Genet* 1(1): 119-28. PubMed ID: [16103914](#)
- Hansen M, Taubert S, Crawford D, Libina N, Lee SJ, Kenyon C. 2007. Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. *Aging Cell* 6(1): 95-110. PubMed ID: [17266679](#)
- Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, et al., Miller RA. 2009. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 460(7253): 392-5. PubMed ID: [19587680](#)
- Huang W, Kew C, Fernandes SA, Lohrke A, Han L, Demetriades C, Antebi A. 2022. Decreased spliceosome fidelity and egl-8 intron retention inhibit mTORC1 signaling to promote longevity. *Nat Aging* 2(9): 796-808. PubMed ID: [37118503](#)
- Joseph GA, Wang SX, Jacobs CE, Zhou W, Kimble GC, Tse HW, et al., Glass DJ. 2019. Partial Inhibition of mTORC1 in Aged Rats Counteracts the Decline in Muscle Mass and Reverses Molecular Signaling Associated with Sarcopenia. *Mol Cell Biol* 39(19). PubMed ID: [31308131](#)
- Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S. 2004. Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. *Curr Biol* 14(10): 885-90. PubMed ID: [15186745](#)
- Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan KL. 2008. Regulation of TORC1 by Rag GTPases in nutrient response. *Nat Cell Biol* 10(8): 935-45. PubMed ID: [18604198](#)
- Kim J, Kundu M, Viollet B, Guan KL. 2011. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 13(2): 132-41. PubMed ID: [21258367](#)
- Mak KH, Zhao Q, Hu PW, Au-Yeung CL, Yang J, Duraine L, Yu Y, Abu-Remaileh M, Sabatini D, Wang JD, Wang MC. 2020. Lysosomal nucleotide metabolism regulates ER proteostasis through mTOR signaling. *bioRxiv* 2020.04.18.048561; doi: <https://doi.org/10.1101/2020.04.18.048561>
- Mannick JB, Lamming DW. 2023. Targeting the biology of aging with mTOR inhibitors. *Nat Aging* 3(6): 642-660. PubMed ID: [37142830](#)
- Markofski MM, Dickinson JM, Drummond MJ, Fry CS, Fujita S, Gundermann DM, et al., Volpi E. 2015. Effect of age on basal muscle protein synthesis and mTORC1 signaling in a large cohort of young and older men and women. *Exp Gerontol* 65: 1-7. PubMed ID: [25735236](#)
- Ortega-Molina A, Lebrero-Fernández C, Sanz A, Calvo-Rubio M, Deleyto-Seldas N, de Prado-Rivas L, et al., Efeyan A. 2024. A mild increase in nutrient signaling to mTORC1 in mice leads to parenchymal damage, myeloid inflammation and shortened lifespan. *Nat Aging* 4(8): 1102-1120. PubMed ID: [38849535](#)
- Papadopoli D, Boulay K, Kazak L, Pollak M, Mallette FA, Topisirovic I, Hulea L. 2019. mTOR as a central regulator of lifespan and aging. *F1000Res* 8:F1000 Faculty Rev-998. PubMed ID: [31316753](#)
- Robida-Stubbs S, Glover-Cutter K, Lamming DW, Mizunuma M, Narasimhan SD, Neumann-Haefelin E, Sabatini DM, Blackwell TK. 2012. TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. *Cell Metab* 15(5): 713-24. PubMed ID: [22560223](#)
- Sambri I, Ferniani M, Ballabio A. 2024. Ragopathies and the rising influence of RagGTPases on human diseases. *Nat Commun* 15(1): 5812. PubMed ID: [38987251](#)
- Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM. 2008. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 320(5882): 1496-501. PubMed ID: [18497260](#)
- Saxton RA, Sabatini DM. 2017. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 168(6): 960-976. PubMed ID: [28283069](#)
- Schreiber MA, Pierce-Shimomura JT, Chan S, Parry D, McIntire SL. 2010. Manipulation of behavioral decline in *Caenorhabditis elegans* with the Rag GTPase raga-1. *PLoS Genet* 6(5): e1000972. PubMed ID: [20523893](#)
- Tramutola A, Triplett JC, Di Domenico F, Niedowicz DM, Murphy MP, Coccia R, Perluigi M, Butterfield DA. 2015. Alteration of mTOR signaling occurs early in the progression of Alzheimer disease (AD): analysis of brain from subjects with pre-clinical AD, amnesic mild cognitive impairment and late-stage AD. *J Neurochem* 133(5): 739-49. PubMed ID: [25645581](#)

4/25/2025 - Open Access

Trimmer K, Arur S. 2021. CRISPR-edit point mutant allele detection (CEPAD)-PCR method for rapid screening of CRISPR edited point mutations. *MicroPubl Biol* 2021. PubMed ID: [33688627](#)

Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Müller F. 2003. Genetics: influence of TOR kinase on lifespan in *C. elegans*. *Nature* 426(6967): 620. PubMed ID: [14668850](#)

Wu JJ, Liu J, Chen EB, Wang JJ, Cao L, Narayan N, et al., Finkel T. 2013. Increased mammalian lifespan and a segmental and tissue-specific slowing of aging after genetic reduction of mTOR expression. *Cell Rep* 4(5): 913-20. PubMed ID: [23994476](#)

Zhang A, Meecham-Garcia G, Nguyen Hong C, Xie P, Kern CC, Zhang B, Chapman H, Gems D. 2024. Characterization of Effects of mTOR Inhibitors on Aging in *Caenorhabditis elegans*. *J Gerontol A Biol Sci Med Sci* 79(11). PubMed ID: [39150882](#)

Zhang Y, Lanjuin A, Chowdhury SR, Mistry M, Silva-García CG, Weir HJ, et al., Mair WB. 2019. Neuronal TORC1 modulates longevity via AMPK and cell nonautonomous regulation of mitochondrial dynamics in *C. elegans*. *Elife* 8:e49158. PubMed ID: [31411562](#)

Funding: CK is supported by NIH grant R01AG083373.

Author Contributions: Tatiana M. Moreno: investigation, writing - original draft, writing - review editing, data curation, formal analysis, methodology. Michelle E. Brown: investigation, data curation, formal analysis. Caroline Kumsta: conceptualization, funding acquisition, project administration, writing - review editing.

Reviewed By: HUANHU ZHU, Anonymous, Louis R. Lapierre

Nomenclature Validated By: Anonymous, Ranjana Kishore

WormBase Paper ID: WBPaper00068045

History: Received January 24, 2025 **Revision Received** April 7, 2025 **Accepted** April 25, 2025 **Published Online** April 25, 2025 **Indexed** May 9, 2025

Copyright: © 2025 by the authors. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Moreno TM, Brown ME, Kumsta C. 2025. Hyperactivation of mTORC1 by an endogenous *raga-1* gain-of-function mutation does not reduce lifespan in *C. elegans*. *microPublication Biology*. [10.17912/micropub.biology.001520](https://doi.org/10.17912/micropub.biology.001520)