Dopaminergic Neurons Regulate Aging and Longevity in Flies

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Abstract

Dopaminergic neurons (DANs) modulate essential brain functions involving memory formation, reward processing, and decision making. Here, I demonstrate a novel and important function of the DANs in regulating aging and longevity. Overexpressing a putative scaffolding protein Mask in two distinct groups of DANs can significantly extend lifespan in flies. Dopamine transmission is required to elicit beneficial effects, but elevating dopamine production or supplementation of L-dopa is not sufficient to yield similar effects on longevity. The lifespan extension induced by Mask-overexpressing is accompanied by sustained adult locomotor and fecundity in the long-lived flies; and other physiological functions in the adult flies include food intake and insulin production in the brain are not consistently altered by overexpressing Mask in either group of DANs. Recent work demonstrated a novel cellular function of Mask in promoting microtubule (MT) dynamics. Overexpressing Unc-104 or moderately reducing $p150^{Glued}$ level, interventions that can induce MTs dynamics, in the same groups of DANs also extend lifespan in flies. Altogether, these data unraveled an undocumented DANs-dependent mechanism that regulates systemic aging and longevity in flies, alterations of the cellular properties in subsets of DANs can have a profound impact on aging.

Dopaminergic neurons, a critical modulatory system in the brain[1,2], are greatly affected by age, but it is unclear whether it can impact the aging process in animals. During the course of studying a putative scaffolding protein, Mask, I uncovered a novel role of dopaminergic neurons in regulating longevity and aging in fruit flies. Overexpressing Mask in dopaminergic neurons leads to a ~40% increase in lifespan in flies (median lifespan in male flies increased from ~76 days to ~104 days) (Fig. S1). This effect seems to be specific to the dopaminergic neurons, as overexpressing Mask in neither the entire body nor the nervous system (neurons or glial cells) showed significant effects on the lifespan (Fig. S2). Dopaminergic neurons in the adult fly brain cluster into eight groups[3], and three well-characterized Gal4 drivers together show non-overlapping expression in most of the dopaminergic neurons in the fly brains[4-6], which allows the test on whether all or only subsets of dopaminergic neurons are responsible for this lifespan-control function. Overexpressing Mask in these three groups of dopaminergic neurons yield distinct results on longevity. Expressing Mask in the PAM dopaminergic neurons by the 0273-Gal4 driver does not alter the lifespan (Fig. 1A); however, expressing Mask driven by either TH-C'- or TH-D'-Gal4 drivers significantly extends the lifespan in flies- the median lifespan in male flies increased from 64 days to 96 days, or from 63 days to 86 days, respectively (Fig. 1 B&C).

Although the dopaminergic system provides essential modulation on various behaviors and physiological functions, flies devoid of dopamine in their brains[7] and worms lacking the rate-limiting enzymes for dopamine synthesis live a normal lifespan[8]. Consistently, blocking neuronal activity in the TH-C' or TH-D' dopaminergic neurons by the DREADD-Di[9] does not affect the lifespan in flies (Fig. S3). Together, these results suggest that dopamine systems is not required to drive normal aging. Overexpressing Mask in specific dopaminergic neurons possibly induces a gain-of-function cellular effect, which consequently confers a beneficial outcome on aging and

longevity. Before the underlying molecular mechanism could be fully resolved, I first set out to further confirm the potentials of the dopaminergic neurons to impact flies' lifespan. Induced Activation of the dopaminergic neurons may yield similar effects. But fully activating the TH-C' or TH-D' dopaminergic neurons continuously via the thermosensitive cation channel TrpA1shortened the lifespan in the male flies and had no effect on the female flies (Fig. S4). The inducible DREADD-Ds receptor allows dose-dependent modulation of neurons[9], which permits the moderate modification of neuronal properties, instead of full activation, in the TH-C' and TH-D' dopaminergic neurons. The 1μM CNO treatment that is significantly below the full activation dose[9] induces prominent and consistent lifespan extension in flies expressing the engineered Gαs receptor in their TH-C' or TH-D' neurons (Fig. 2). Although it's not completely clear how the cellular property of these dopaminergic neurons have been altered by the chronic low activation of the DREADD-D_s receptor, this result confirms that moderate modulation on these neurons is sufficient to impact longevity in flies.

Next, I asked whether dopamine transmission is required to mediate the lifespan extension effect induced by Mask-overexpression in the dopaminergic neurons. If dopamine transmission is needed, reducing dopamine signaling in these neurons would suppress the ability of Mask-overexpression to extend lifespan. Four genes are essential for dopamine transmission: two enzymes responsible for dopamine synthesis - Tyrosine Hydroxylase (TH) and Dopa Decarboxylase (Ddc); and two transporters for dopamine - dopamine transporter (DAT) that controls dopamine reuptake, and vesicular monoamine transporter (VMAT) that mediates synaptic vesicular loading of dopamine. Genetically heterozygosity that reduces gene dosages of either of these four genes can each blunt the extend of lifespan extension in flies that overexpress Mask in the TH-C' or TH-D' neurons compared to the wild type background (Fig. S5). These

results support the notion that the dopamine system is necessary and indispensable for Mask to induce lifespan extension. To test whether upregulation of dopamine signaling is also sufficient to induce lifespan extension in flies, the flies were fed with dopamine precursor L-DOPA[10], dopamine D1 receptor agonist SKF-82958[11], dopamine D2 receptor agonist Quinpirole[12], or the D1 and D2 agonists together. It appeared that none of the treatments was able to extend the lifespan in flies. The lifespan of female flies fed with L-DOPA was even moderately shortened (Fig. S6). This adverse effects may be attributable to global elevation of dopaminergic signaling, as long term treatment with L-DOPA has been shown to cause neural toxicity in mammals[13]. To eliminate the potential "off target" effects, selectively increase of dopamine production in the TH-C' or TH-D' neurons was achieved by overexpressing Tyrosine Hydroxylase (TH), or Dopa Decarboxylase (Ddc), the two enzymes responsible for dopamine synthesis, in these two groups of neurons. However, overexpressing these two enzymes did not affect longevity either (Fig. S7). Similar to L-DOPA feeding, these treatments lead to moderate reduction in the lifespan in female flies. These results together suggest that although neurons require dopamine transmission to communicate and to induce lifespan extension, increasing dopamine production alone is not sufficient to elicit similar effects on aging and longevity induced by Mask overexpression or moderate DREADD-Ds activation in these neurons.

To fully understand how aging and longevity are impacted at the systematic level by overexpressing Mask in the TH-C' or TH-D' neurons, I next examined age-related phenotypes including feeding, energy storage/metabolic, adult locomotor and brain insulin productions in Mask-expressing and control flies. Dopaminergic neurons regulate feeding and food-foraging in flies[14-17], raising the possibility that the long-lived flies that overexpress Mask in the TH-C' or TH-D' neurons may consume less food and therefore undertake voluntary caloric restriction,

which may serve as a potential contributing factor for lifespan extension in these flies. However, the Mask-overexpressing long-lived flies consume a comparable amount of food compared to the control flies at young and middle ages (10 and 30 days old) in the Capillary Feeder assay (CAFE assay)[18] (Fig. S8). These flies also show moderately increased whole-body glucose and Triglyceride (TAG) levels at middle and late ages (30 and 50 days old) (Fig. S9). Adult locomotor reflects overall activity and health in flies and can be quantified using the rapid iterative negative geotaxis (RING) assay[19,20]. Expressing Mask in TH-C' dopaminergic neurons enhances the locomotor performance significantly during the entire lifespan, and expressing Mask in the TH-D' neurons sustain the adult locomotor at middle and late ages (Fig. S10). To determine whether there is a correlation between the prolonged lifespan and potential changes in insulin production in the brain, I next measured transcription levels of Dilp2, 3, 5, and 6 in the adult brains of Mask-overexpression and control flies. No consistent reduction in transcription levels of the Dilp genes between gender or age was detected in the Mask-overexpressing flies (Fig. S11- 14). Altogether, the results of the above analysis suggest that the Mask-overexpressing flies live healthy and active lives throughout the entire lifespan.

Delayed reproductive maturation or reduced fecundity correlates closely with lifespan extension[21]. To test whether there is a trade-off between reproduction and lifespan in Mask-overexpressing flies, I examined the fecundity of male and female flies at different ages. No delay or reduction in reproduction in either male or female flies was detected. Instead, overexpressing Mask in the TH-C' neurons lead to increased fertility in female flies beginning right after eclosion, and the TH-D' overexpressing flies showed a significantly higher level of fecundity at older ages (Fig. 3). While previous studies reveal a trade-off between lifespan and fecundity, the Mask-overexpressing flies, as well as a few other previously reported mutant

flies[22-25] show a trait of extended lifespan at no cost of reproduction. This contradiction together with the lack of a consensus theory to explain the relationship between aging and reproduction calls for an alternative theory that can provide a unifying explanation for the intricate relationship between aging and reproduction.

Since overexpressing Mask in the dopaminergic neurons very likely induces a gain-of-function effect that leads to lifespan extension, I next investigated the primary cellular effector in the Mask-overexpressing dopaminergic neurons that mediate the outcome on aging and longevity. The recent finding that Mask promotes microtubule (MT) dynamics in fly larval motor neurons and body wall muscles[26] led to the postulation that altered MT dynamics in the Mask-expressing dopaminergic neurons is the key mediator. If this is true, reagents that alter MT dynamics, in the same manner, would also induce lifespan extension when applied to the same subsets of dopaminergic neurons. Overexpressing the Kinesin heavy chain Unc-104[27] or knocking down a component of the Dynein/Dynactin complex, p150[Glued28] are two interventions that have been previously shown to impact MT dynamics by reducing MT length and stability, and enhance MT-based transportations. When applied to the TH-C' or TH-D' dopaminergic neurons, both treatments extended lifespan in flies (Fig. 4), demonstrating that increasing MT dynamics and reducing MT stability in the TH-C' or TH-D' dopaminergic neurons is sufficient to induce lifespan extension in flies.

Mask is involved in multiple cellular functions which may also contribute to its ability to induce the dopamine-dependent lifespan extension. Mask is a large protein (4001 amino acids) containing several conserved functional domains (Fig. S15), and the structures of Mask enable its versatile functions in the cells including its ability to promote microtubule (MT) dynamics[26], autophagy[29], HIPPO pathway[30,31] or vesiculation[29,32]. Mask is a KH domain-containing protein. KH

domains bind RNA or single-stranded DNA and regulate transcription, translation, mRNA stability, and alternative splicing[33-35]. Mutating the GXXG loop to GDDG in the KH minimal motif greatly reduces the ability of the KH domain to bind RNAs[36]. The GXXG loop of Mask resides in amino acids 3053-3056 as GRGG, which is fully conserved between fly and human. To assess whether the KH domain is required for normal functions of Mask, I generated a UAS-Mask transgene that carries a GRGG to GDDG mutation in its KH domain (named Mask-KH-Mutant) (depicted in Fig. S15A). The previous work demonstrated that overexpressing Mask promotes autophagic degradation[29], and an intact KH domain is required for Mask to enhance autophagic degradation because overexpression of Mask-KH-Mutant fails to induce the same effect on autophagy (Fig. S16). However, the KH-Mutant transgene can fully rescue the MT defects in larval muscle[26], and more importantly, expressing the Mask-KH-Mutant protein in the TH-C' dopaminergic neurons extended lifespan to a similar level as the wild type Mask protein (Fig. S15 B&C). The fact that KH domain is dispensable for Mask to induce lifespan extension indicates that autophagy may not be a primary cellular effector in Mask-mediated lifespan-extension. Also, overexpressing ATG-1, a potent inducer of autophagy, in all neurons in the adult flies can extend lifespan[37]; however, overexpressing Mask in all neurons in the adult flies failed to induce similar lifespan extension (Fig. S17). These data together suggest that autophagy is unlikely a primary cellular effector in Mask-mediated lifespan-extension.

The Ankyrin repeats in ANKHD1, the mammalian homolog of Mask, regulates membrane vesiculation[32], suggesting a possible role of its fly counterpart Mask to promote vesicle formation and recycling at the nerve terminals. Indeed, overexpressing Mask in the fly larval neuromuscular junction (NMJ) increases the size of Rab5-positive vesicles pool (Fig. S18) and promotes vesicular recycling (measured by FM-143 dye loading assay, see Fig. S19) at the presynaptic terminal. Expressing the wild type or constitutively active form of Rab5 can enhance

synaptic vesicle fusion efficacy and firing strength at the presynaptic terminals[38], thus, it can potentially mimic some aspects of the Mask-overexpressing effects in the dopaminergic neurons. But, expressing only the active form of Rab5 protein in the TH-C' or TH-D' dopaminergic neurons can moderately extend lifespan in the male flies (Fig. S20). This results suggests that although Mask can enhance endocytosis and vesicle recycling at the nerve terminal, promoting these events alone is only able to induce at much less extend the cellular output by dopaminergic neurons leading to lifespan extension. Although it is unclear whether the function of Mask to promote MT dynamics and to enhance endocytosis at the nerve terminals are consequentlly related, altered vesicle trafficking can possibly be one of the downstream effectors that mediate Mask-depend lifespan extension.

In this study, I report a novel role of dopaminergic neurons in regulating aging and longevity, and enhancing the MT dynamics in subsets of dopaminergic neurons profoundly prolonged lifespan in flies. Interestingly, the lifespan extension is accompanied by the sustained fecundity in the long-lived flies, a phenotype that contradicts the disposable soma theory for aging that states an inversely related energy allocation between reproduction and somatic maintenance. To reconcile the paradox, I suggest a reproduction-centered mechanism for aging that could potentially provide a consensus explanation for the relationship between aging and reproduction. This theory states that there is no competition for resources between somatic maintenance and reproduction, rather, somatic tissues adapt and cope with the animal's need for reproduction. In the "live fast and die young[39]" scenario, the fulfilled reproduction in early life eases the somatic maintenance as the need to sustain the reproduction has been lifted, and the animals live a shorter lifespan. The nutrient-sensing pathway that mediates the interplay between prolonged lifespan and delayed reproduction[40] also represent an aspect of this theory- the sense of nutrition scarcity

translates to the signals for insufficiency in resources needed to produce quality progenies as well as possibly threatens for the progenies' survival; so that the reproduction activity will be paused and the somatic system adapts accordingly to prolong their lifespan in order to allow reproduction to be resumed later. In eusocial insects, the reproductive females undergo somatic transformation and remodeling. They live much longer in order to fulfill the prolonged reproduction demand. Thus, a critical aspect of the fitness for reproduction is the capacity of the animals to respond to different environmental challenges and to adjust and ensure successful reproduction and consequentially the survival of the species. This reproduction-centered aging theory, or adaptive soma theory, posits that the organism would possess the ability to sense an internal status, or in another word, a goal for their reproduction capacity; and to fulfill this goal, the body engages mechanisms to monitor the progress of the reproduction as well as a system that can efficiently support somatic maintenance needed to sustain their reproduction demands. The dopaminergic system, an essential part in the brain that facilitates information evaluation and decision making, is the best candidate that can centrally organize the information on internal status as well as the environmental cues, and relay out to the peripheral the decision to adjust the somatic system to match the reproduction status. The identification of the dopaminergic system as a regulator for aging regulation provides an entry point to further explore the molecular and cellular mechanisms associated with this new reproduction-centered aging theory.

Method and Material

Drosophila strains and genetics

Flies were maintained at 25°C on standard food containing agar (0.75% w/v), cornmeal (5% w/v), molasses (4.15% v/v), and yeast (1.575% w/v) at 25 °C in a 12:12 light:dark cycle. The

following strains were used in this study: UAS-Mask[41], TH-C'-Gal4[6], TH-D'-Gal4[6] (from Mark Wu), 0273-Gal4 (from Thomas R. Clandinin), UAS-DREADD-D_s[9] (from Charles Nichols), Ddc-Gal4 (Bloomington Stock #7010); UAS-unc104-GFP (Bloomington Stock #24787); UAS-p150 Glued RNAi (Bloomington Stock #24761). Control flies used in the study are w^{1118} from BestGene Inc. (Chino Hills, CA, USA) in which the UAS-Mask line was generated[41] and Iso[31] (from Mark Wu) in which TH-C'-Gal4 and TH-D'-Gal4 flies were generated[6].

Fly Longevity Assay

The procedures are adapted from previously described protocol[42]. 15-20 male or female flies were separated into one vial, and a total of 4-6 vials of flies were used for each genotype for longevity measurement. The flies are maintained at 25°C on standard food or food containing drugs and are transferred into fresh vials every 3 days.

DREADD Activation

10mM Clozapine N-oxide (CNO) (Tocris 4936) stock solution was prepared in DMSO. Food containing control (DMSO only) or 1uM CNO was prepared freshly every three days before the flies were transferred to new vials during the longevity recording experiments.

Fly Fecundity Assay

Newly eclosed individual virgin male or female flies (day1) were crossed with 2-3 w^{1118} virgin female or male flies respectively and kept together for 3 days. The total progeny produced within the 3-day intervals were counted after they eclose as adult flies. Sexually exposed and active male or female flies reared together with w^{1118} female or male were aged till day 10, 30 and 50. Each female fly was then separated into a single vial and allow to lay eggs for 3 days. The total progeny produced within the 3-day intervals were counted after they eclose. The aged male flies

were individually mated with three 5-7 day old w^{1118} virgin females for 3 days, and fecundity was measured as the percentage of males that can successfully produce progenies.

Statistical analysis

Data analysis for longevity: The Kaplan-Meier estimator was used to analyze the data and the survivorship curves were generated in Origin. The log-rank test was performed with Evan's A/B tools.

Each sample will be compared with other samples in the group (more than two) using ANOVA, or with the other sample in a group of two using a t-test. The graphs were generated in Origin (Origin Lab, Northampton, MA).

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References

1. Schultz, W. Multiple dopamine functions at different time courses. *Annu Rev Neurosci* **30**, 259-288 (2007). PubMed PMID: 17600522
2. Arias-Carrión, O., Stamelou, M., Murillo-Rodríguez, E., Menéndez-González, M. & Pöppel, E. Dopaminergic reward system: a short integrative review. *Int Arch Med* **3**, 24 (2010). PubMed PMID: 20925949
3. Mao, Z. & Davis, R. L. Eight different types of dopaminergic neurons innervate the Drosophila mushroom body neuropil: anatomical and physiological heterogeneity. *Front Neural Circuits* **3**, 5 (2009). PubMed PMID: 19597562
4. Burke, C. J., Huetteroth, W., Owald, D., Perisse, E., Krashes, M. J., Das, G., Gohl, D., Silies, M., Certel, S. & Waddell, S. Layered reward signalling through octopamine and dopamine in Drosophila. *Nature* **492**, 433-437 (2012). PubMed PMID: 23103875
5. Galili, D. S., Dylla, K. V., Ludke, A., Friedrich, A. B., Yamagata, N., Wong, J. Y., Ho, C. H., Szyszka, P. & Tanimoto, H. Converging circuits mediate temperature and shock aversive olfactory conditioning in Drosophila. *Curr Biol* **24**, 1712-1722 (2014). PubMed PMID: 25042591
6. Liu, Q., Liu, S., Kodama, L., Driscoll, M. R. & Wu, M. N. Two dopaminergic neurons signal to the dorsal fan-shaped body to promote wakefulness in Drosophila. *Curr Biol* **22**, 2114-2123 (2012). PubMed PMID: 23022067
7. Riemensperger, T., Isabel, G., Coulom, H., Neuser, K., Seugnet, L., Kume, K., Iche-Torres, M., Cassar, M., Strauss, R., Preat, T., Hirsh, J. & Birman, S. Behavioral consequences of dopamine deficiency in the Drosophila central nervous system. *Proc Natl Acad Sci U S A* **108**, 834-839 (2011). PubMed PMID: 21187381

8. Murakami, H. & Murakami, S. Serotonin receptors antagonistically modulate Caenorhabditis elegans longevity. *Aging Cell* **6**, 483-488 (2007). PubMed PMID: 17559503

9. Becnel, J., Johnson, O., Majeed, Z. R., Tran, V., Yu, B., Roth, B. L., Cooper, R. L., Kerut, E. K. & Nichols, C. D. DREADDs in Drosophila: a pharmacogenetic approach for controlling behavior, neuronal signaling, and physiology in the fly. *Cell Rep* **4**, 1049-1059 (2013). PubMed PMID: 24012754

10. Kayser, M. S., Yue, Z. & Sehgal, A. A critical period of sleep for development of courtship circuitry and behavior in Drosophila. *Science* **344**, 269-274 (2014). PubMed PMID: 24744368

11. Walters, M. R., Dutertre, M. & Smith, C. L. SKF-82958 is a subtype-selective estrogen receptor-alpha (ERalpha) agonist that induces functional interactions between ERalpha and AP-1. *J Biol Chem* **277**, 1669-1679 (2002). PubMed PMID: 11700319

12. Wiemerslage, L., Schultz, B. J., Ganguly, A. & Lee, D. Selective degeneration of dopaminergic neurons by MPP(+) and its rescue by D2 autoreceptors in Drosophila primary culture. *J Neurochem* **126**, 529-540 (2013). PubMed PMID: 23452092

13. Yamamoto, Branden J. Stansley & Bryan K. L-Dopa and Brain Serotonin System Dysfunction. *Toxics* **3**, 75-88 (2015). PubMed PMID:

14. Liu, Q., Tabuchi, M., Liu, S., Kodama, L., Horiuchi, W., Daniels, J., Chiu, L., Baldoni, D. & Wu, M. N. Branch-specific plasticity of a bifunctional dopamine circuit encodes protein hunger. *Science* **356**, 534-539 (2017). PubMed PMID: 28473588

15. Tsao, C. H., Chen, C. C., Lin, C. H., Yang, H. Y. & Lin, S. Drosophila mushroom bodies integrate hunger and satiety signals to control innate food-seeking behavior. *Elife* **7**(2018). PubMed PMID: 29547121

16. Landayan, D. & Wolf, F. W. Shared neurocircuitry underlying feeding and drugs of abuse in Drosophila. *Biomed J* **38**, 496-509 (2015). PubMed PMID: 27013449

17. Marella, S., Mann, K. & Scott, K. Dopaminergic modulation of sucrose acceptance behavior in Drosophila. *Neuron* **73**, 941-950 (2012). PubMed PMID: 22405204

18. Diegelmann, S., Jansen, A., Jois, S., Kastenholz, K., Velo Escarcena, L., Strudthoff, N. & Scholz, H. The CApillary FEeder Assay Measures Food Intake in Drosophila melanogaster. *J Vis Exp* (2017). PubMed PMID: 28362419

19. Nichols, C. D., Becnel, J. & Pandey, U. B. Methods to assay Drosophila behavior. *J Vis Exp* (2012). PubMed PMID: 22433384

20. Gargano, J. W., Martin, I., Bhandari, P. & Grotewiel, M. S. Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in Drosophila. *Exp Gerontol* **40**, 386-395 (2005). PubMed PMID: 15919590

21. Yuan, R., Meng, Q., Nautiyal, J., Flurkey, K., Tsaih, S. W., Krier, R., Parker, M. G., Harrison, D. E. & Paigen, B. Genetic coregulation of age of female sexual maturation and lifespan through circulating IGF1 among inbred mouse strains. *Proc Natl Acad Sci U S A* **109**, 8224-8229 (2012). PubMed PMID: 22566614

22. Simon, A. F., Shih, C., Mack, A. & Benzer, S. Steroid control of longevity in Drosophila melanogaster. *Science* **299**, 1407-1410 (2003). PubMed PMID: 12610309

23. Hwangbo, D. S., Gershman, B., Tu, M. P., Palmer, M. & Tatar, M. Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature* **429**, 562-566 (2004). PubMed PMID: 15175753

24. Marden, J. H., Rogina, B., Montooth, K. L. & Helfand, S. L. Conditional tradeoffs between aging and organismal performance of Indy long-lived mutant flies. *Proc Natl Acad Sci U S A* **100**, 3369-3373 (2003). PubMed PMID: 12626742

25. Grandison, R. C., Piper, M. D. & Partridge, L. Amino-acid imbalance explains extension of lifespan by dietary restriction in Drosophila. *Nature* **462**, 1061-1064 (2009). PubMed PMID: 19956092

26. Zhu, Mingwei, Martinez, Daniel, Guidry, Jessie J., Majeste, Niles, Mao, Hui, Yanofsky, Sarah, Tian, Xiaolin & Wu, Chunlai. Mask, the Drosophila Ankyrin Repeat and KH domain-containing protein, regulates microtubule dynamics. *bioRxiv*, 2020.2004.2022.056051 (2020). PubMed PMID:

27. Randall, T. S., Yip, Y. Y., Wallock-Richards, D. J., Pfisterer, K., Sanger, A., Ficek, W., Steiner, R. A., Beavil, A. J., Parsons, M. & Dodding, M. P. A small-molecule activator of kinesin-1 drives remodeling of the microtubule network. *Proc Natl Acad Sci U S A* **114**, 13738-13743 (2017). PubMed PMID: 29229862

28. Lazarus, J. E., Moughamian, A. J., Tokito, M. K. & Holzbaur, E. L. Dynactin subunit p150(Glued) is a neuron-specific anti-catastrophe factor. *PLoS Biol* **11**, e1001611 (2013). PubMed PMID: 23874158

29. Zhu, M., Zhang, S., Tian, X. & Wu, C. Mask mitigates MAPT- and FUS-induced degeneration by enhancing autophagy through lysosomal acidification. *Autophagy* **13**, 1924-1938 (2017). PubMed PMID: 28806139

30. Sansores-Garcia, L., Atkins, M., Moya, I. M., Shahmoradgoli, M., Tao, C., Mills, G. B. & Halder, G. Mask is required for the activity of the Hippo pathway effector Yki/YAP. *Curr Biol* **23**, 229-235 (2013). PubMed PMID: 23333314

31. Sidor, C. M., Brain, R. & Thompson, B. J. Mask proteins are cofactors of Yorkie/YAP in the Hippo pathway. *Curr Biol* **23**, 223-228 (2013). PubMed PMID: 23333315

32. Kitamata, M., Hanawa-Suetsugu, K., Maruyama, K. & Suetsugu, S. Membrane-Deformation Ability of ANKHD1 Is Involved in the Early Endosome Enlargement. *iScience* **17**, 101-118 (2019). PubMed PMID: 31255983

33. Brooks, A. N., Duff, M. O., May, G., Yang, L., Bolisetty, M., Landolin, J., Wan, K., Sandler, J., Booth, B. W., Celniker, S. E., Graveley, B. R. & Brenner, S. E. Regulation of alternative splicing in Drosophila by 56 RNA binding proteins. *Genome Res* **25**, 1771-1780 (2015). PubMed PMID: 26294686

34. Garcia-Mayoral, M. F., Hollingworth, D., Masino, L., Diaz-Moreno, I., Kelly, G., Gherzi, R., Chou, C. F., Chen, C. Y. & Ramos, A. The structure of the C-terminal KH domains of KSRP reveals a noncanonical motif important for mRNA degradation. *Structure* **15**, 485-498 (2007). PubMed PMID: 17437720

35. Grishin, N. V. KH domain: one motif, two folds. *Nucleic Acids Res* **29**, 638-643 (2001). PubMed PMID: 11160884

36. Hollingworth, D., Candel, A. M., Nicastro, G., Martin, S. R., Briata, P., Gherzi, R. & Ramos, A. KH domains with impaired nucleic acid binding as a tool for functional analysis. *Nucleic Acids Res* **40**, 6873-6886 (2012). PubMed PMID: 22547390

37. Ulgherait, M., Rana, A., Rera, M., Graniel, J. & Walker, D. W. AMPK modulates tissue and organismal aging in a non-cell-autonomous manner. *Cell Rep* **8**, 1767-1780 (2014). PubMed PMID: 25199830

38. Wucherpfennig, T., Wilsch-Brauninger, M. & Gonzalez-Gaitan, M. Role of Drosophila Rab5 during endosomal trafficking at the synapse and evoked neurotransmitter release. *J Cell Biol* **161**, 609-624 (2003). PubMed PMID: 12743108

39. Travers, L. M., Garcia-Gonzalez, F. & Simmons, L. W. Live fast die young life history in females: evolutionary trade-off between early life mating and lifespan in female Drosophila melanogaster. *Sci Rep* **5**, 15469 (2015). PubMed PMID: 26482533

40. Templeman, N. M. & Murphy, C. T. Regulation of reproduction and longevity by nutrient-sensing pathways. *J Cell Biol* **217**, 93-106 (2018). PubMed PMID: 29074705

41. Zhu, M., Li, X., Tian, X. & Wu, C. Mask loss-of-function rescues mitochondrial impairment and muscle degeneration of Drosophila pink1 and parkin mutants. *Hum Mol Genet* **24**, 3272-3285 (2015). PubMed PMID: 25743185

42. Linford, N. J., Bilgir, C., Ro, J. & Pletcher, S. D. Measurement of lifespan in Drosophila melanogaster. *J Vis Exp* (2013). PubMed PMID: 23328955

Figure Legend

Figure 1. Overexpressing Mask in the TH-C' or TH-D' DANs Extends Lifespan.

Survivorship curves of control flies and flies overexpressing Mask under the control of A) 0273-Gal4, B) TH-C'-Gal4, and C) TH-D'-Gal4 drivers. D) Schematic of expression patterns of 0273-, TH-C'- and TH-D'-Gal4 drivers.

Figure 2. Moderately activating the ectopically expressed DREADD system in the TH-C' or TH-D' DANs Extends Lifespan in flies.

Survivorship curves of male and female adult flies expressing the DREADD Gαs receptor and raised on food containing DMSO only (No Drug) or 1uM Clozapine N-oxide (CNO). A, B) moderately activating the DREADD-Ds receptor in the TH-C' neurons leads to extension of lifespan in flies. The median lifespan increased from ~87 days to ~98 days (~12.6% increase) in the male flies and from ~95 days to ~108 days (~12.6% increase) in the female flies. C, D) moderate activation of the DREADD-Ds receptor expressed in the TH-D' neurons leads to significant extension of lifespan in flies. The median lifespan increased from ~73 days to ~95 days (~30% increase) in the male flies and from ~67 days to ~107 days (~50% increase) in the female flies.

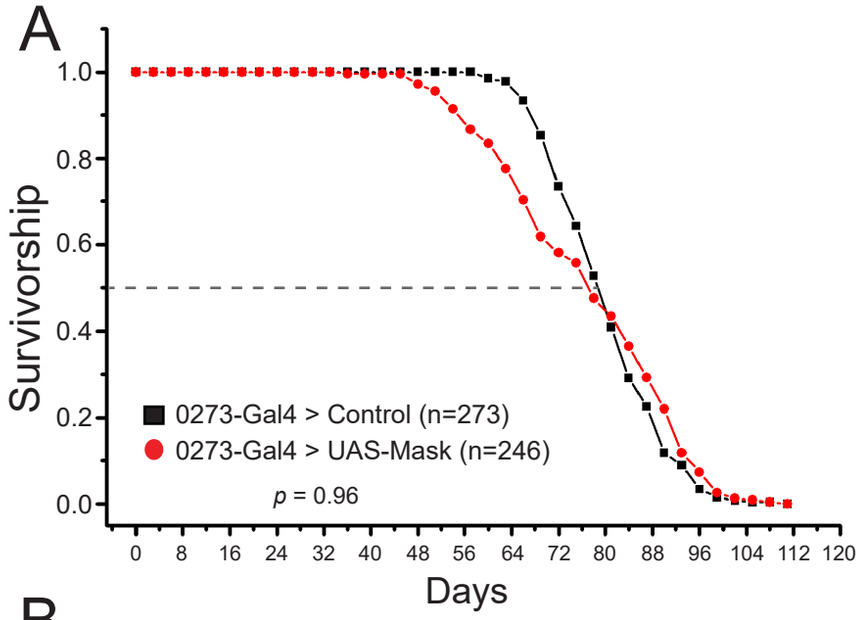
Figure 3. Mask-overexpressing Flies show sustained Fecundity at old ages. A) Numbers of progenies produced by female flies in a 3 day interval. The female flies that overexpress Mask in the TH-C' or TH-D' DANs produce significantly more progenies at 30- and 50-day old ages. B) Reproduction in male flies is measured as the percentage of male flies that are fertile. At age 50-

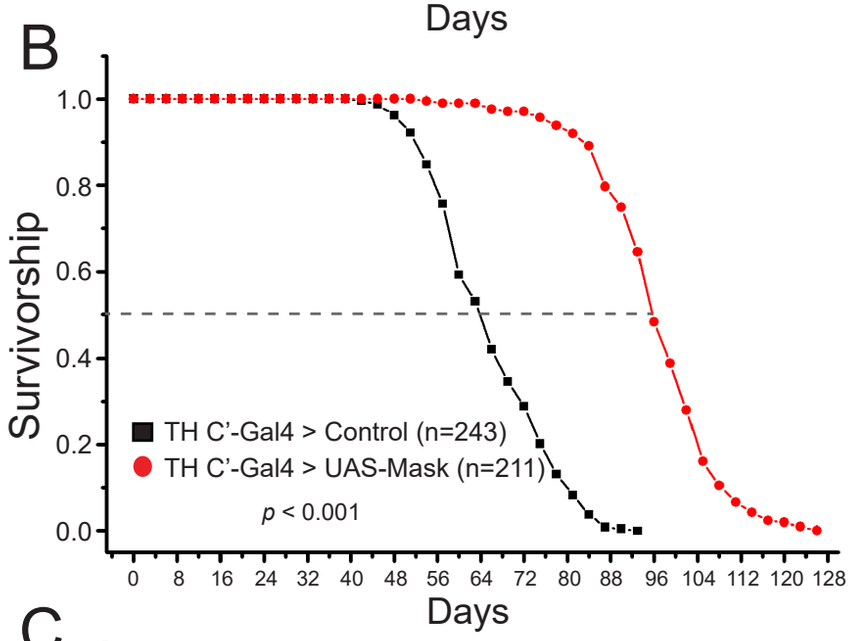
day, majority of the control male flies lose the ability to produce progeny, while over 80% of male flies that overexpress Mask in the TH-C' and 60% of those overexpress Mask in the TH-D' DANs are still reproductively active.

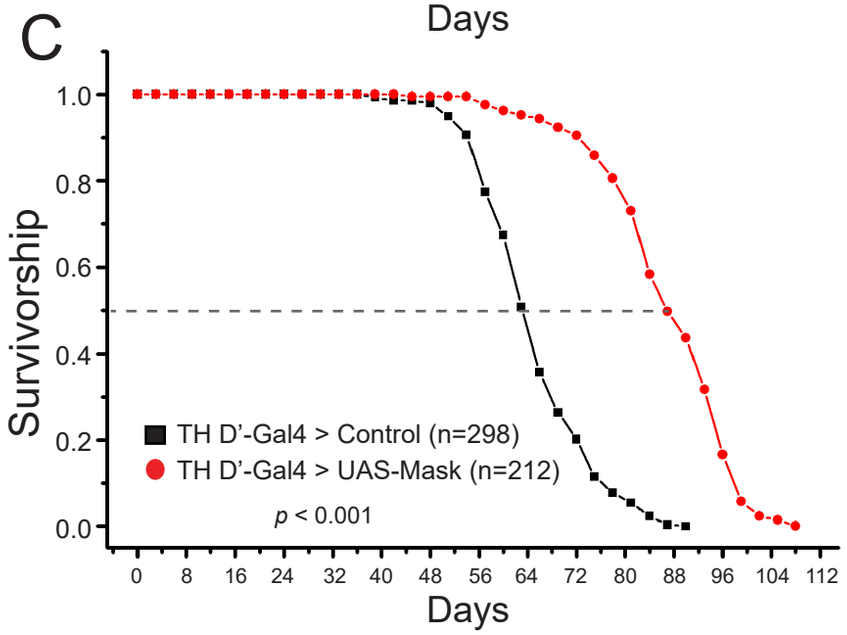
Figure 4. Overexpressing Unc-104 or Knocking down p150^{Glued} in the TH-C' or TH-D' DANs extend lifespan in flies.

Survivorship curves of female flies expressing UAS-GFP-Unc104 or a weak RNAi transgene for p150^{Glued} in the TH-C' or TH-D' DANs. A) Overexpressing the Unc-104-GFP transgene in the TH-C' or TH-D' neurons leads to extension of lifespan in flies. The median lifespan in the flies overexpressing Unc-104-GFP increased from ~67 days to ~92 days (~37% increase). In the flies overexpressing Unc-104-GFP in the TH-D' neurons, the median lifespan increased ~40% (median lifespan ~94 days). B) Moderately knocking down p150^{Glued} in the TH-C' neurons leads to extension of lifespan in flies, median lifespan increased from ~86 days to ~98 days (~13.95% increase). Same treatment in the TH-D' neurons leads to an increase of the median lifespan to ~92 days (~6.97% increase).

Figure 1


A
Survivorship
Days
0273-Gal4 > Control (n=273)
0273-Gal4 > UAS-Mask (n=246)
p = 0.96


B
Survivorship
Days
TH C'-Gal4 > Control (n=243)
TH C'-Gal4 > UAS-Mask (n=211)
p < 0.001


C
Survivorship
Days
TH D'-Gal4 > Control (n=298)
TH D'-Gal4 > UAS-Mask (n=212)
p < 0.001

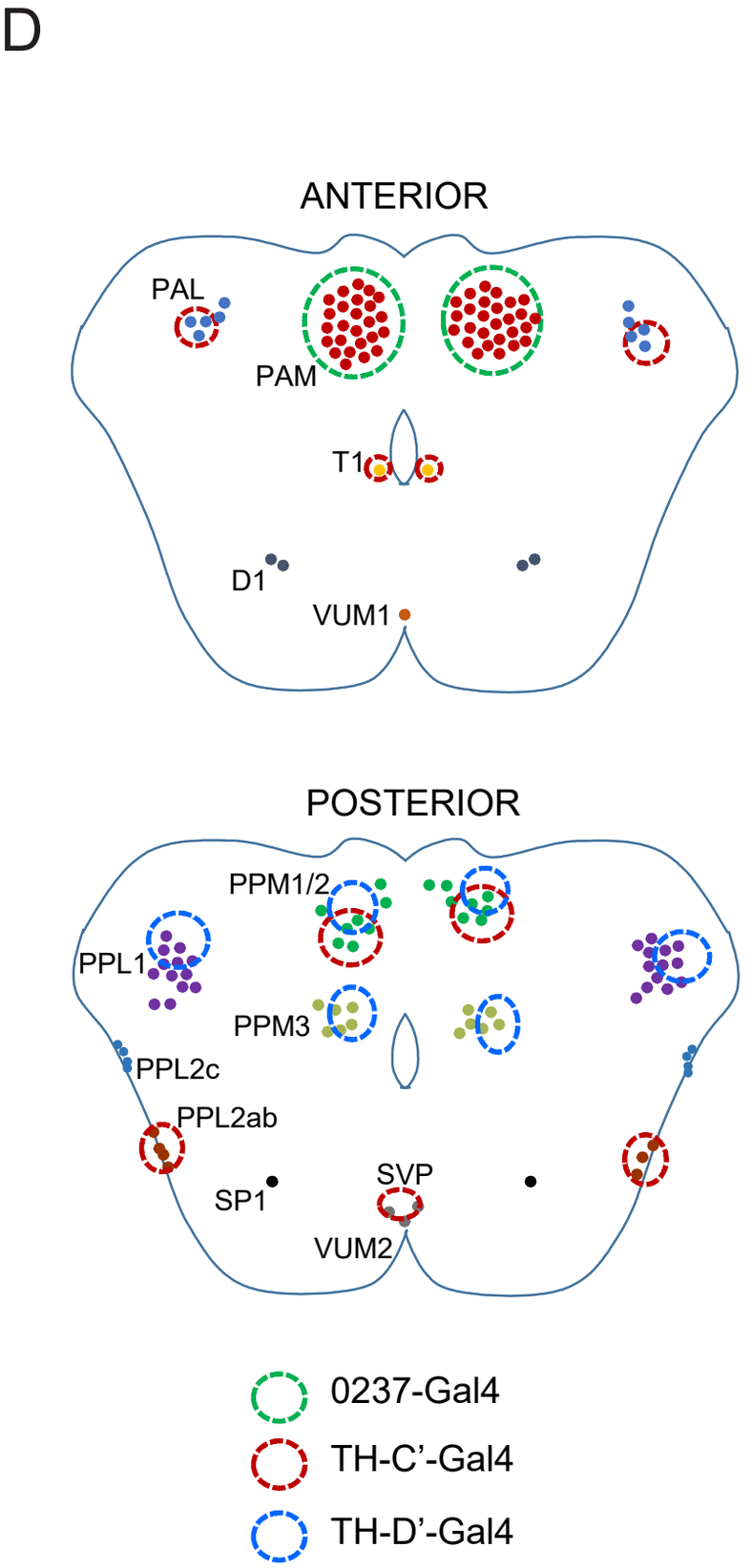

D
ANTERIOR
PAL
PAM
T1
D1
VUM1
POSTERIOR
PPM1/2
PPL1
PPM3
PPL2c
PPL2ab
SP1
SVP
VUM2
0237-Gal4
TH-C'-Gal4
TH-D'-Gal4

Figure 2

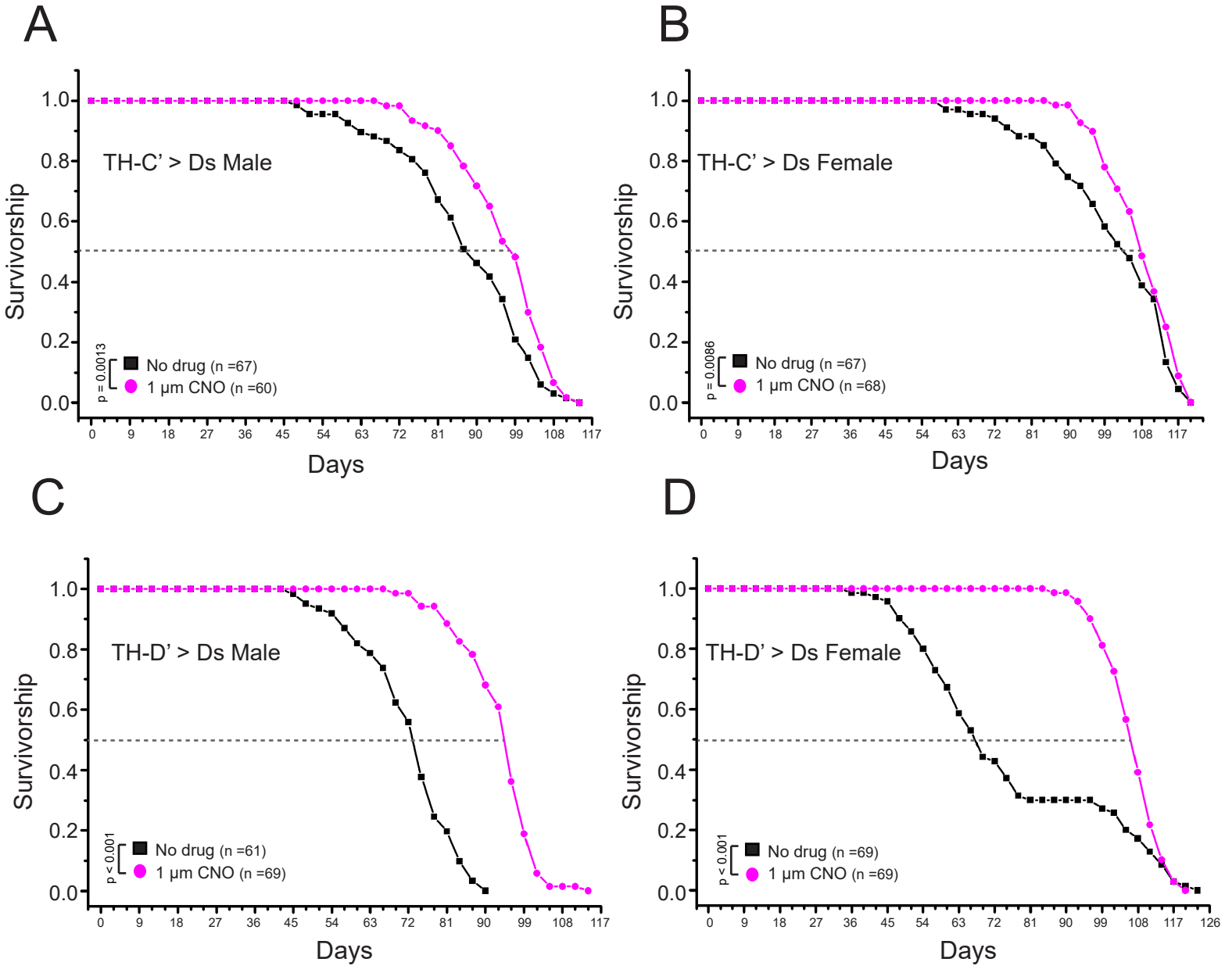

A
TH-C' > Ds Male
Survivorship
Days
p = 0.0013
No drug (n =67)
1 µm CNO (n =60)
B
TH-C' > Ds Female
Survivorship
Days
p = 0.0086
No drug (n =67)
1 µm CNO (n =68)
C
TH-D' > Ds Male
Survivorship
Days
p < 0.001
No drug (n =61)
1 µm CNO (n =69)
D
TH-D' > Ds Female
Survivorship
Days
p < 0.001
No drug (n =69)
1 µm CNO (n =69)

Figure 3

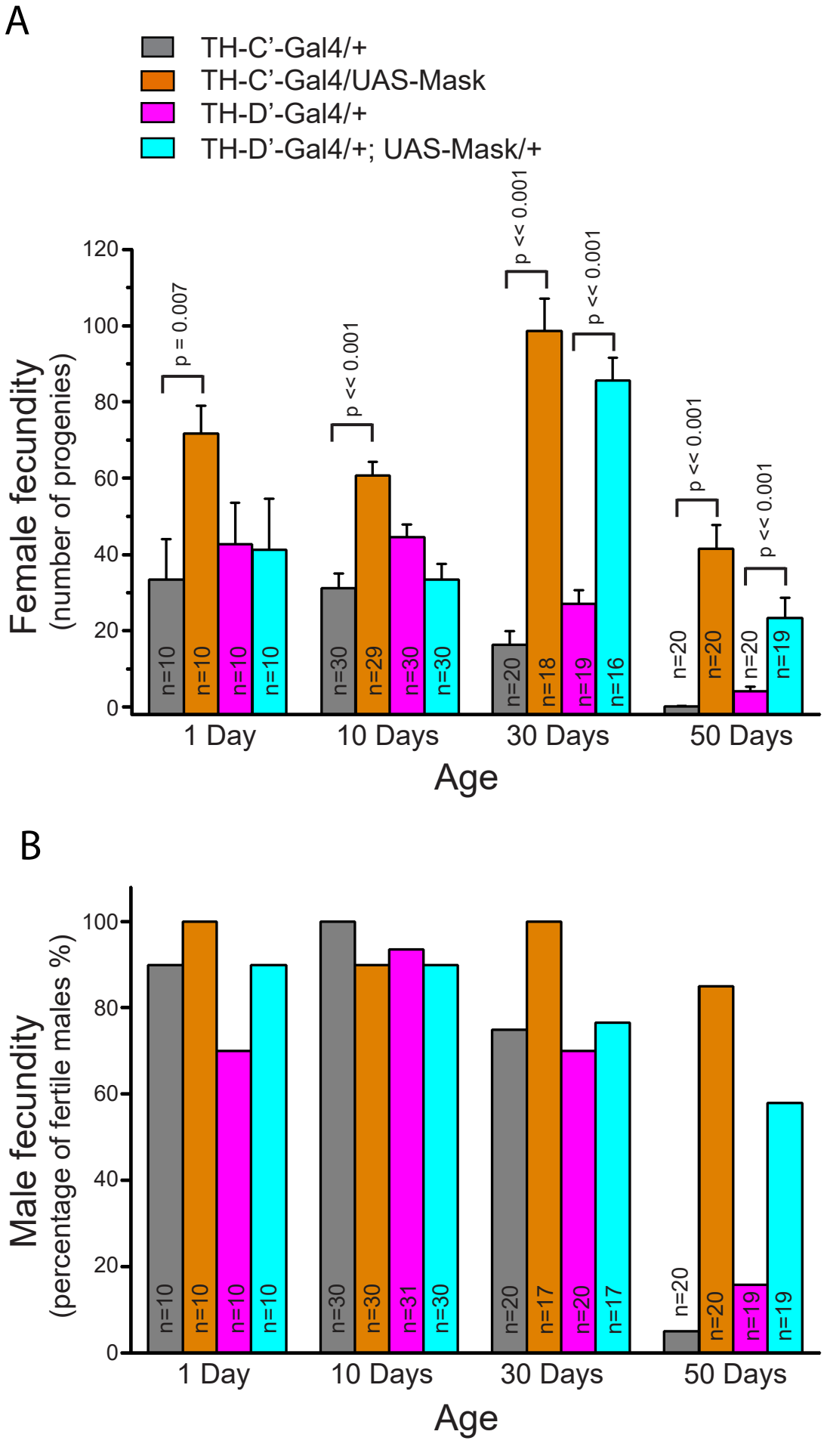
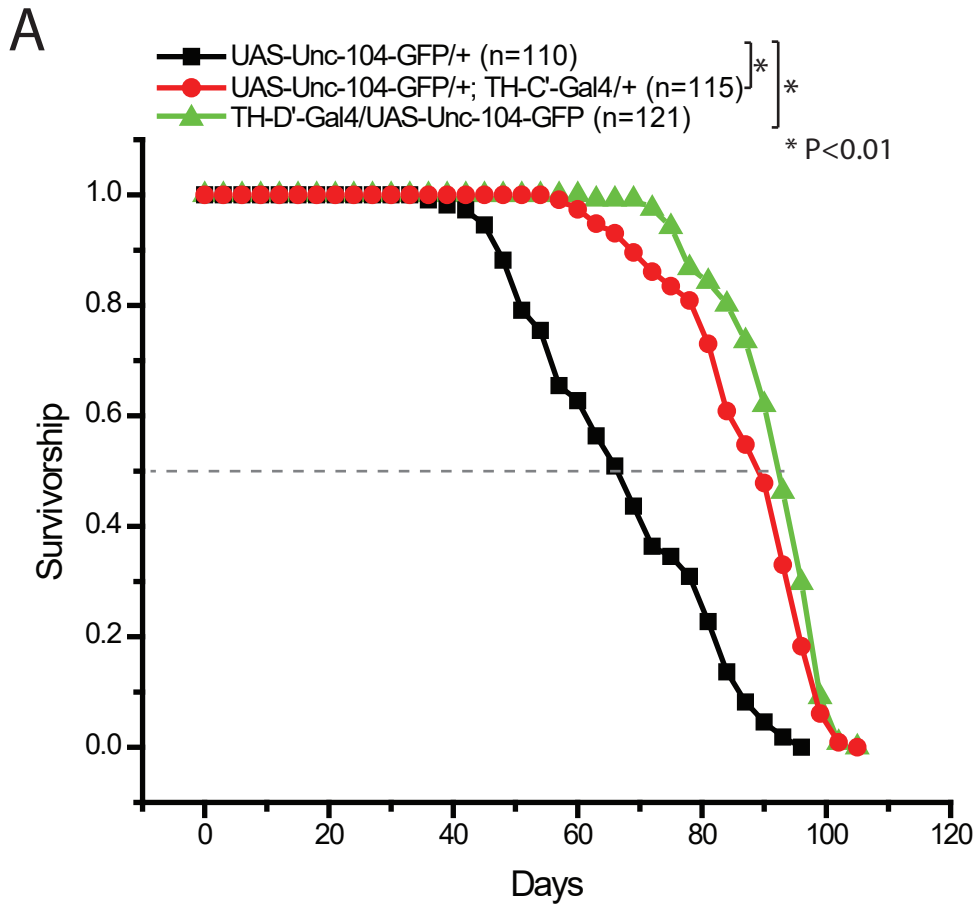
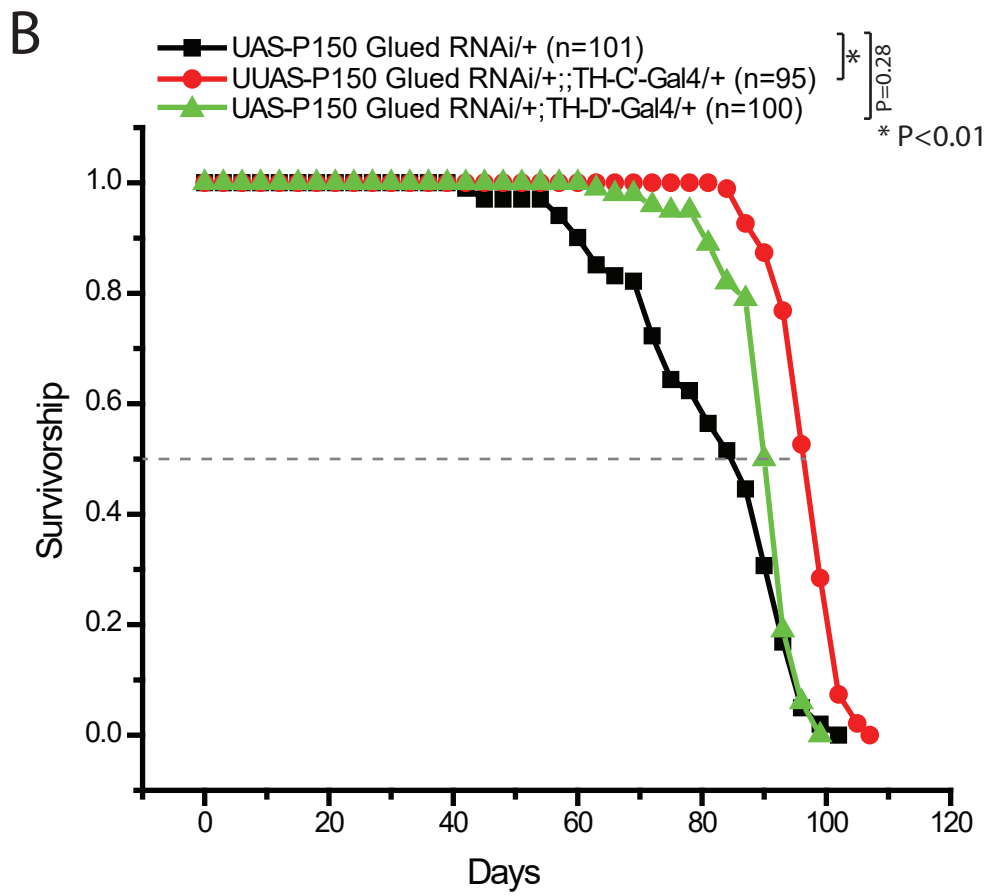

A
TH-C'-Gal4/+
TH-C'-Gal4/UAS-Mask
TH-D'-Gal4/+
TH-D'-Gal4/+; UAS-Mask/+
Female fecundity
(number of progenies)
p = 0.007
p << 0.001
p << 0.001
p << 0.001
p << 0.001
p << 0.001
n=10
n=10
n=10
n=10
n=30
n=29
n=30
n=30
n=20
n=18
n=19
n=16
n=20
n=20
n=20
n=19
1 Day
10 Days
30 Days
50 Days
Age
B
Male fecundity
(percentage of fertile males %)
n=10
n=10
n=10
n=10
n=30
n=30
n=31
n=30
n=20
n=17
n=20
n=17
n=20
n=20
n=19
n=19
1 Day
10 Days
30 Days
50 Days
Age

Figure 4


A
UAS-Unc-104-GFP/+ (n=110)
UAS-Unc-104-GFP/+; TH-C'-Gal4/+ (n=115)
TH-D'-Gal4/UAS-Unc-104-GFP (n=121)
* P<0.01
Survivorship
Days


B
UAS-P150 Glued RNAi/+ (n=101)
UUAS-P150 Glued RNAi/+;;TH-C'-Gal4/+ (n=95)
UAS-P150 Glued RNAi/+;TH-D'-Gal4/+ (n=100)
P=0.28
* P<0.01
Survivorship
Days

Supplemental Information

SI Methods and Material

Fly stocks

The following stocks were used: UAS-*mask* RNAi[1], elav-Gal4[2] (neuron specific), Repo-Gal4 (glial specific); DA-Gal4 (Ubiquitous), UAS-DREADD-Di (from Charles Nichols), UAS-MASK-KH-Mutant (this study). Stocks from the Bloomington stock center include UAS-Ple or UAS-TH (37539), UAS-Ddc (37540), UAS-tdTomato (64746), UAS-TrpA1 (26263), ple^{4} (3279), ddc^{27} (3190), $dat^{Z2-1744}$ (30867), $vmat^{SH0459}$ (29477), UAS-GFP-mCherry-ATG8 (37749), GS-elav (43642), UAS-ATG1 (51654), UAS-YFP-Rab5 (9775), UAS-YFP-Rab5-S43N (9771), UAS-YFP-Rab5-Q88L (9774).

Drug Treatment

Dopamine pathway activation: microwave melted pre-made standard fly food was blended with drugs or the same amount of water or their solvent. The final concentrations for the drugs were used in this study: 1mg/ml L-DOPA[3,4] (3mg/ml L-DOPA stock in water sucrose water, 0.1% L-ascorbic acid to prevent oxidation of L-DOPA); 30uM Quinpirole[5] (30mM Quinpirole stock was prepared in water); and 75uM SKF-82958[6] (15mM SKF-82958 stock was prepared in water).

Gene Switch system: Flies were raised on RU-486 (50ug/ml) food for the entire lifespan, control group were kept on food added equal amount of ethanol (10mg/ml RU486 stock was dissolved in ethanol).

Generation of UAS-Mask-KH-Mutant Transgene

We used the QuickChangeII XL site-mutagenesis kit (Agilent Tech) to generate KH domain mutated Mask transgenes: the pUAST-Mask plasmid was used as the template with and a specific primer that changes the 3054 Arg(CGA) and 3055 Gly(GGT) to 3054 Asp(GAC) and 3055 Asp(GAT). After a positive mutagenesis was identified, we sequenced a KpnI and XbaI DNA fragment flanking the mutated sites and ensured no undesired mutagenesis was introduced by the kit. The KpnI and XbaI DNA fragment containing the mutations was then cut and pasted to the original pUAST-Mask construct to generate Mask transgenes with KH domain mutation.

Glucose and TAG measurement

Five male or female flies were collected and snap frozen in liquid nitrogen. The flies were homogenized in 200 μL PBS + 0.05% Triton X-100, centrifuged at 1,000 × g for 1 min, and the supernatant was used to measure the protein, glucose and triacylglyceride (TAG) concentrations. 5 μL homogenate was to TAG measurement using the Infinity Trigly-cerides reagent (Thermo Scientific) The sample was added to warmed reagent, mixed briefly, incubated at room temperature for 5 minutes, and read at 520 nm. For glucose measurement, 10 μL homogenate was used to mix with 150 μL warmed Infinity Glucose reagent (Thermo Scientific), incubate for 30 min at 37 °C, and read at 340nm. For protein measurement, 2 μL homogenate was used to determine the protein concentration using DC detection kit (BioRad). All reactions were read with SpectraMAX 190 (Molecular Device).

Fly larval Neuromuscular Junction FM1-43 Dye loading

The 3rd instar wondering larvae were dissected in HL3 buffer that contains 0.43uM Ca^{2+} and then incubated in Jan's high K^+ buffer (90mM K^+, 2mM Ca^{2+}) + 4uM FM1-43 dye (final concentration) for 1 minute. The FM1-43 loading was then stopped by rinsing the larvae in Ca^{2+} free HL3 buffer rapidly twice and then slowly on a shaker for 5 minutes. The larvae were then fixed in 4% PFA (in 1X PBS) for 18 minutes, rapidly rinsed in 1X PBS twice and then incubated in HRP-Cy5 (1:1,000) for 15 minutes. The larvae then were rapidly rinsed with PBS twice and followed by a slow rinse by shaking for 5 minutes. After the rinses, the larvae were incubated in 70% glycerol (in 1X PBS) for 15 minutes and mounted for confocal imaging analysis.

Immunocytochemistry

Third instar larvae were dissected in ice-cold PBS and fixed in 4% PFA for 30 min. The fixed tissues were stained following standard procedures. Alexa-Fluor-647-conjugated goat anti-HRP at 1:1000 was used.

Confocal imaging and analysis

Single-layer or z-stack confocal images were captured on a Nikon (Tokyo, Japan) C1 confocal microscope. Images shown in the same figure were acquired using the same gain from samples that had been simultaneously fixed and stained. For quantification of YFP and FM1-43 signals at the NMJs, z-stack confocal images of microtubule in larval muscle 6/7 in segment A2 were analyzed double-blinded, IMARIS software (Bitplane, Inc) was used to quantify average signal intensity at the nerve terminals.

Fly in vivo assays

Rapid iterative negative geotaxis (RING) assay was performed as previously described[7], and the procedures of Capillary Feeder assay (CAFE) was adapted from established protocols[8].

qRT_PCR

20 adult fly heads were collected, rinse with PBS 3 times and then snap frozen in liquid nitrogen. The total RNA was extracted with Trizol reagent. To prepare cDNA, the RNA samples were first treated with DNase I (Invitrogen Cat#2020-08-31), and then used as template to synthesize the cDNA using the High Capacity cDNA Revers Transcription kit (Applied Biosystem, Cat#2020-05-31). The quantitative PCR was performed with the BioRad SYBR (Cat# 1725121) and CFX96 Real-time PCR system.

Primers used in qPCR: dilp2, dilp5, dilp5 dilp6. Primers are described previously[19];

Primers for internal control: (reference: DOI: 10.1038/ncomms10368) qRpL32_f 50 -CGCACCAAGCACTTCATCCG-30; qRpL32_r 50 -GCACGTTGTGCACCAGGAAC-30

SI Figure Legends

Figure S1. Overexpressing Mask in dopaminergic neurons extends lifespan in flies.

A) Survivorship curves of control flies (Ddc-Gal4 > UAS-tdTomato), flies expressing *mask* RNAi (Ddc-Gal4 > Mask RNAi) or wild type Mask transgenes in the DA neurons (Ddc-Gal4 >UAS- Mask). Knocking down Mask in the DA neurons does not impact the lifespan in the flies, while overexpressing Mask profoundly extend the lifespan in flies. B) Survivorship curves of control flies (Elav-Gal4 > UAS-tdTomato), flies expressing *mask* RNAi in all neurons (Elav-Gal4 > Mask RNAi), or flies overexpressing Mask in all neurons (Elav-Gal4 > UAS-Mask). Loss-of-function of Mask in all neurons leads to partial lethality during the development (data

not shown). Those that do eclose show significantly reduced lifespan. In contrast to the effect of *mask* loss-of-function, overexpressing Mask in all neurons does not affect lifespan in flies.

Figure S2. The lifespan extension induced by Mask overexpression is specifically mediated by dopaminergic neurons.

Survivorship curves of flies that overexpress Mask in A) all cells (Da-Gal4, ubiquitous driver); B) glial cells (Repo-Gal4); C) all neurons (elav-Gal4); or D) dopaminergic neurons (Ddc-Gal4). Only when Mask is overexpressed in dopaminergic neurons, the lifespan of the flies is profoundly prolonged. Overexpressing Mask ubiquitously in the entire body or in all neurons does not affect the longevity. Overexpressing Mask in glial cells only moderately extends the lifespan in flies.

Figure S3. Suppressing the Neuronal Activation of the TH-C' or TH-D' Neurons Does not impact the Lifespan in Flies.

The Survivorship curves of flies expressing the engineered DREADD-Di ($G_{\alpha i}$) in the (A, B) TH-C' or (C, D) TH-D' DANs are shown. The flies are raised on food containing DMSO only (No Drug), 1uM, 10uM or 100uM Clozapine N-oxide (CNO) for their entire adulthood. Inhibition of the activation of the TH-C' or TH-D' DANs does not affect the lifespan in flies.

Figure S4. Chronically activating the TH-C' and TH-D' dopaminergic neurons in the adult flies moderately shortens lifespan.

Survivorship curves of male A) and female B) flies expressing the thermal sensitive cation channel TrpA1 in the TH-C' or the TH-D' DANs. The activation of these neurons were achieved

by raising the flies at 29°C shortly after eclosion. Long-term activation of the TH-C' neurons leads to 33% reduction in the lifespan (median lifespan shortened from 44 days to 33 days.), and activating the TH-D' neurons only results in 10% reduction in the lifespan (median lifespan shortened from 31 days to 28 days.). No effect on lifespan was detected in females when TH-C' or TH-D' dopaminergic neurons were activated by TrpA1.

Figure S5. Reducing Dopamine Pathway Activity Blunts the Lifespan Extension effects induced by Overexpressing Mask in the TH-C' or TH-D' DANs. Decreasing dopaminergic pathway activity was achieved by genetically reducing the dosage of the four genes that are essential for dopamine transmission: two enzymes responsible for dopamine synthesis - Tyrosine Hydroxylase (TH) and Dopa Decarboxylase (Ddc); and two transporters for dopamine - dopamine transporter (DAT) that controls dopamine reuptake, and vesicular monoamine transporter (VMAT) that mediates synaptic vesicular loading of dopamine. Four loss of function alleles, ple^{4} (TH), ddc^{27}, $dat^{Z2\text{-}1744}$ or $vmat^{SH0459}$, were introduced to Mask-overexpressing paradigms to generate a heterozygous background of each gene. The survivorship curves of male flies of the following genotypes are shown: A) Overexpressing Mask in the TH-C' DA neurons in wild type or in the ddc^{27}/+ heterozygous backgrounds; B) Overexpressing Mask in the TH-C' DA neurons in wild type or in the $dat^{Z2\text{-}1744}$/+ heterozygous backgrounds; C) Overexpressing Mask in the TH-C' DA neurons in wild type or in the ple^{4}/+ heterozygous backgrounds; D) Overexpressing Mask in the TH-C' DA neurons in wild type or in the $vmat^{SH04594}$/+ heterozygous backgrounds; E) Overexpressing Mask in the TH-D' DA neurons in wild type or in the ddc^{27}/+ heterozygous background; F) Overexpressing Mask in the TH-D' DA neurons in wild type or in the $dat^{Z2\text{-}1744}$/+ heterozygous background; G) Overexpressing Mask in the TH-D' DA neurons in

wild type or in the *ple*^{4}/+ heterozygous background; H) Overexpressing Mask in the TH-D' DA neurons in wild type or in the *vmat*^{SH04594}/+ heterozygous background. With all conditions, reducing gene dosage of TH, Ddc, DAT or VMAT is able to blunt the lifespan extension induced by overexpressing Mask in DANs.

Figure S6. Ubiquitously Elevating dopamine activity is not sufficient to extend lifespan in flies. Survivorship curves of A) female and B) male w^{1118} flies raised on regular food, or on food containing 1mg/ml L-DOPA; and C) female and D) male w^{1118} flies raised on regular food, or on food containing 75uM of SKF-82958, 30uM of Quinpirole, or both drugs are shown. None of the treatments extended the lifespan of male flies. Feeding female flies with dopamine precursor L-DOPA moderately shortened the lifespan (median lifespan reduced from 88 days in the control flies to 78 days in L-DOPA fed flies, ~12% reduction). Neither dopamine receptor agonists alone nor together shows significant effects on female lifespan, although a small population of fed flies showed weaker tolerance and died earlier than untreated group. Log-Rank Test p values: C) p = 0.25 (w^{1118}+SKF-82958 vs. w^{1118}), p = 0.091 (w^{1118}+Quinpirole vs. w^{1118}), p = 0.20 (w^{1118}+SKF-82958+Quinpirole vs. w^{1118}); and D) p = 0.68 (w^{1118}+SKF-82958 vs. w^{1118}), p = 0.99 (w^{1118}+Quinpirole vs. w^{1118}), p = 0.29 (w^{1118}+SKF-82958+Quinpirole vs. w^{1118}).

Figure S7. Specifically Increasing Dopamine Production in the TH-C' Neurons fails to Extend Lifespan.

Survivorship curves of flies expressing the rate limiting enzyme for dopamine biosynthesis tyrosine hydroxylase (TH) or Dopa Decarboxylase (Ddc) in the TH-C' or TH-D' neurons are shown. The longevity of A) Male and B) female flies overexpressing TH in the TH-C' or TH-D'

DANs are recorded. This treatment does not affect the lifespan in male or female flies that overexpress TH in the TH-C' DANs. Female flies that overexpress TH in the TH-D' DA neurons show moderately reduced lifespan. C) Male flies overexpressing Ddc in the TH-C' or TH-D' DA neurons exhibit similar lifespan compared to control flies. D) Overexpressing Ddc in the TH-C' DA neurons does not affect the lifespan. However, overexpressing Ddc in the TH-D' DA neurons moderately extend the lifespan (median lifespan increased from 99 days in the control flies to 105 days in TH-D'-Gal4 > Ddc flies, ~6% increase).

Figure S8. Overexpressing Mask in the TH-C' or TH-D' Neurons Does not Change Food Intake in Flies.

The food intake in a 24 hour interval was measured using a Capillary Feeder assay (CAFE). The amount of food consumed was represented by the distance (mm) between the positions of the liquid food surface at the beginning and the end of the experiments. Food intake of flies that overexpress Mask in the TH-C' neurons is compared to the control flies at A) 10-day or B) 30-day old ages. Food intake of flies that overexpress Mask in the TH-D' neurons is compared to the control flies at C) 10-day or D) 30-day old ages. No differences in food intake was detected between the long-lived flies and the control flies. n=3 for all sample groups.

Figure S9. Overexpressing Mask in the TH-C' or TH-D' Neurons moderately increase TAG and Glucose levels at mid and late ages in Flies.

A, B) Age related whole body Glucose/Protein and C, D) TAG/Protein levels in Mask overexpressing or control flies. Mask overexpressing flies contain higher glucose and TAG levels at mid (30-day) and late (50-day) ages, with the exception that female flies overexpressing Mask in the TH-C' neurons show similar levels of TAG compared to the controls. n=4 for all sample groups.

Figure S10. Overexpressing Mask in the TH-C' and TH-D' DANs improves locomotor in aged adult flies.

Mean climb distance in Rapid Iterative Negative Geotaxis (RING) assay were measured to assess the locomotor in adult flies. Mean climb distance of a group of 15-20 A) male or B) female flies at ages of day-10, 30 or 50 are shown. Flies overexpressing Mask in the TH-C' DANs show significantly faster climbing throughout the entire lifespan compared to the control flies. Mean climb distance of C) male and D) female control flies and flies overexpressing Mask in TH-D' DA neurons were measured at ages of day-10, 30 or 50. Flies overexpressing Mask in the TH-D' DA neurons are significantly more active at mid and old ages. (n=3 for all sample groups)

Note: The RING Assay performed on 10- and 30-day old flies used 3 seconds total duration, while RING Assays on 50-day old flies used 5 seconds duration.

Figure S11. Overexpressing Mask in the TH-C' or TH-D' DANs does not lead to consistent changes in Dilp2 transcript levels in the adult brains.

The transcript levels of Dilp2 in the adult brain was measured in newly eclosed and 2-, 5-, 7-, 10-, 15-, 30- and 50-day old flies. A) Male flies overexpressing Mask in the TH-C' neurons show reduced Dilp2 levels at 7- and 15-day old ages. B) Male flies overexpressing Mask in the TH-D' neurons show reduced Dilp2 levels at 50-day old age. C) Female flies overexpressing Mask in the TH-C' neurons show reduced Dilp2 levels at 10-day old age. D) Female flies overexpressing Mask in the TH-D' neurons show no changes in Dilp2 levels in adulthood. *** $P < 0.05$

Figure S12. Overexpressing Mask in the TH-C' or TH-D' DA neurons does not lead to consistent changes in Dilp3 transcript levels in the adult brains.

The transcript levels of Dilp3 in the adult brain was measured in newly eclosed and 2-, 5-, 7-, 10-, 15-, 30- and 50-day old flies. A) Male flies overexpressing Mask in the TH-C' neurons show no changes in Dilp3 levels. B) Male flies overexpressing Mask in the TH-D' neurons show reduced Dilp3 levels at 50-day old age. C) Newly eclosed female flies overexpressing Mask in the TH-C' neurons show reduced Dilp3 levels. D) Female flies overexpressing Mask in the TH-D' neurons show reduced Dilp3 levels at 50-day old age. *** $P < 0.05$

Figure S13. Overexpressing Mask in the TH-C' or TH-D' DA neurons does not lead to consistent changes in Dilp5 transcript levels in the adult brains.

The transcript levels of Dilp5 in the adult brain was measured in newly eclosed and 2-, 5-, 7-, 10-, 15-, 30- and 50-day old flies. A) Male flies overexpressing Mask in the TH-C' neurons show reduced Dilp5 level at 15-day old age. B) Male flies overexpressing Mask in the TH-D' neurons show reduced Dilp5 levels at 15- and 50-day old ages. C) Female flies overexpressing Mask in

the TH-C' neurons show reduced Dilp5 levels. D) Female flies overexpressing Mask in the TH-D' neurons show no changes in Dilp5 levels. *** $P < 0.05$

Figure S14. Overexpressing Mask in the TH-C' or TH-D' DA neurons does not lead to consistent changes in Dilp6 transcript levels in the adult brains.

The transcript levels of Dilp6 in the adult brain was measured in newly eclosed and 2-, 5-, 7-, 10-, 15-, 30- and 50-day old flies. A) Male flies overexpressing Mask in the TH-C' neurons show no changes in Dilp6 levels. B) Male flies overexpressing Mask in the TH-D' neurons show increased Dilp6 level at 30-day old age. At 50-day old age, Dilp6 transcript level in the head is moderately reduced. C) Newly eclosed female flies overexpressing Mask in the TH-C' neurons show reduced Dilp6 level. D) Female flies overexpressing Mask in the TH-D' neurons show reduced Dilp6 level at 50-day old age. *** $P < 0.05$

Figure S15. The KH domain in the Mask Protein is not required for Mask-dependent lifespan Extension.

A) Schematic of domain structures of wild type and KH domain mutation of Mask proteins. B, C) Survivorship curves of male flies overexpressing different forms of Mask protein in the TH-C' neurons. The conserved "GRGG" motif in the KH domain was mutated to "GDDG" in UAS-Mask-KH-Mutant transgene. When overexpressed in the TH-C' DANs, the Mask-KH-Mutant transgene show similar strength to induce lifespan extension compared to the wild type Mask protein.

Figure S16. The KH domain is required for Mask Protein to promote autophagy.

(A) Confocal images of GFP-mCherry auto-fluorescence (using the same gain) from fixed larval muscles in control animals (24B-Gal4 > UAS-GFP-mCherry-ATG8), animals overexpressing wild type Mask protein (24B-Gal4 > UAS-GFP-mCherry-ATG8 + UAS-Mask) or Mask-KH-Mutant protein (24B-Gal4 > UAS-GFP-mCherry-ATG8 + UAS-Mask-KH-Mutant). (B) Quantification of mCherry intensity in the larval body wall muscles 6 is shown. Overexpressing Mask in the muscle promotes autophagy, while the KH-Mutant protein fail to induce the same effects.

Figure S17. Expressing Mask in neurons in the adult flies fail to induce lifespan extension compared to ATG1.

A, D) Wild type flies, flies B, E) expressing ATG1 (GS-Elav > UAS-ATG1) or C, F) Mask (GS-Elav > UAS-Mask) in their neurons are raised on regular food or food containing 50ug/ml RU488 in order to assess the effects of adulthood neuronal expression of Mask or ATG1 on longevity. GS-Elav induces pan-neuronal expression in presence of RU488. 50ug/ml RU488 induces a moderate adverse effect on lifespan in wild type flies. Expressing ATG1 in neurons leads to moderate lifespan extension (in reference to wild type flies). The lifespan of Mask overexpressing flies, to the contrary, show no difference compared to the non-expressing flies.

Figure S18. Overexpressing Mask Promotes formation of YFP-Rab5-Positive Membrane Structures at the Presynaptic Terminal at fly larval neuromuscular junctions (NMJs).

A) Representative confocal images of synapses in control (elav-Gal4 > *mask* control RNAi), *mask* knockdown (elav-Gal4 > *mask* RNAi) and Mask overexpressing (elav-Gal4 > UAS-Mask) larvae are highlighted with HRP (red). YFP auto-fluorescence (using the same gain) from fixed sample marks the YFP-Rab5-positive compartments in the nerve terminals. B) Quantification of the intensity of the YFP signals in the nerve terminal of muscle 6/7 are shown. Knocking down Mask significantly reduces, while overexpressing Mask, increases the size and intensity of YFP-YFP-Rab5-positetive compartments.

Figure S19. Mask Promotes FM1-43 Dye Loading at Fly Larval Neuromuscular Junction (NMJ).

A) Representative synapses and boutons in control (elav-Gal4/+) and Mask overexpressing (elav-Gal4 > UAS-Mask) larvae are highlighted with HRP (blue). FM1-43 dye loaded in the vesicle at the nerve terminals is shown in red. B) Quantification of FM1-43 dye intensity in the NMJs on muscle 6/7.

The membrane nonpermeable dye FM1-43 can be taken up into the synaptic vesicles through endocytosis, allowing the quantification of vesicle exocytosis-endocytosis events taking place at the nerve terminals. After stimulation, significantly higher level of FM1-43 loading was detected in presynaptic boutons in Mask overexpressing synapses compared to the controls. No significant FM1-43 loading at the NMJs was detected in a 30 minute interval at rest state in control or Mask overexpressing synapses (data not shown), indicating that overexpressing Mask does not affect endocytosis per se, but rather, the activity-dependent vesicle fusion events.

Figure S20. Enhancing endocytosis and vesicle trafficking in the TH-C' neurons moderately Extend Lifespan.

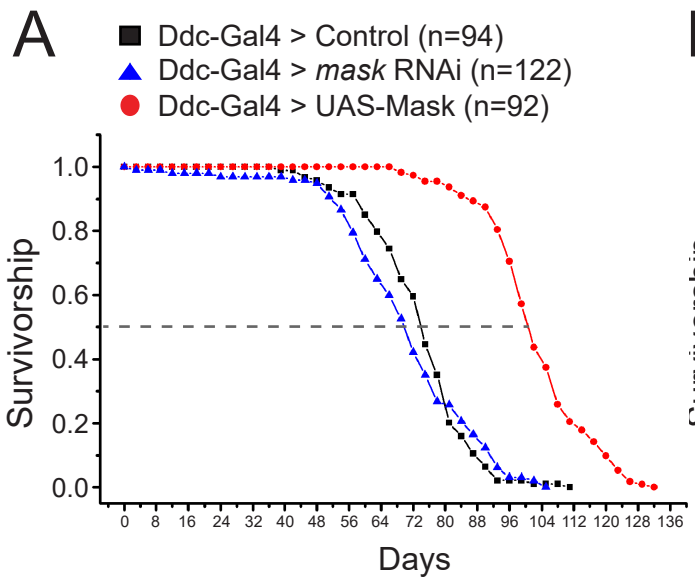
Survivorship curves of flies expressing the wild type Rab5 protein (UAS-YFP-Rab5), the dominant negative form of Rab5 (UAS-YFP-Rab5-S43N), or the constitutively active form of Rab5 protein (UAS-YFP-Rab5-Q88L) are shown. (A&B)Overexpressing the wild type Rab5 protein, or (C&D) the dominant negative form of Rab5 in the TH-C' neurons does not affect lifespan in flies. E) Overexpressing the constitutively active form of Rab5 protein in the TH-C' neurons moderately extends lifespan in male flies. The median lifespan increased from 68 days in the control flies to 74 days in TH-C'-Gal4 > UAS-YFP-Rab5-Q88L flies, ~8.8% increase. F) Overexpressing the constitutively active form of Rab5 protein in the TH-C' neurons does not affect the lifespan in female flies.

References

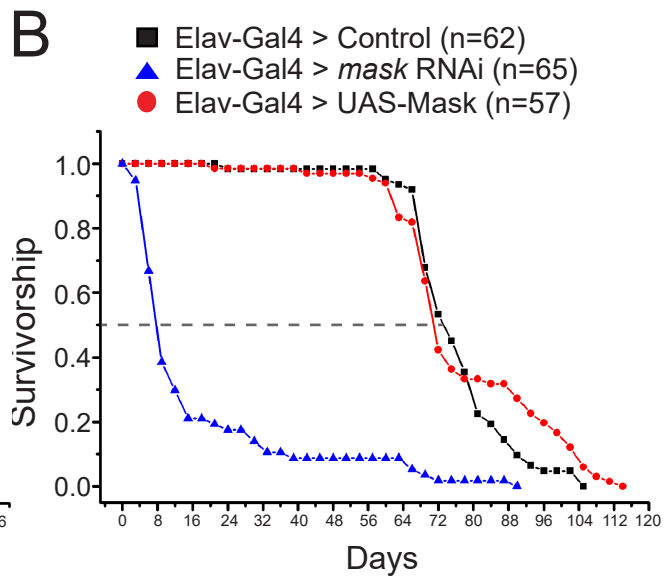
1. Zhu, M., Li, X., Tian, X. & Wu, C. Mask loss-of-function rescues mitochondrial impairment and muscle degeneration of Drosophila pink1 and parkin mutants. *Hum Mol Genet* **24**, 3272-3285 (2015). PubMed PMID: 25743185
2. Budnik, V., Koh, Y. H., Guan, B., Hartmann, B., Hough, C., Woods, D. & Gorczyca, M. Regulation of synapse structure and function by the Drosophila tumor suppressor gene dlg. *Neuron* **17**, 627-640 (1996). PubMed PMID: 8893021
3. Kayser, M. S., Yue, Z. & Sehgal, A. A critical period of sleep for development of courtship circuitry and behavior in Drosophila. *Science* **344**, 269-274 (2014). PubMed PMID: 24744368
4. Zhang, M. Z., Yao, B., Wang, S., Fan, X., Wu, G., Yang, H., Yin, H., Yang, S. & Harris, R. C. Intrarenal dopamine deficiency leads to hypertension and decreased longevity in mice. *J Clin Invest* **121**, 2845-2854 (2011). PubMed PMID: 21701066
5. Wiemerslage, L., Schultz, B. J., Ganguly, A. & Lee, D. Selective degeneration of dopaminergic neurons by MPP(+) and its rescue by D2 autoreceptors in Drosophila primary culture. *J Neurochem* **126**, 529-540 (2013). PubMed PMID: 23452092
6. Walters, M. R., Dutertre, M. & Smith, C. L. SKF-82958 is a subtype-selective estrogen receptor-alpha (ERalpha) agonist that induces functional interactions between ERalpha and AP-1. *J Biol Chem* **277**, 1669-1679 (2002). PubMed PMID: 11700319
7. Nichols, C. D., Becnel, J. & Pandey, U. B. Methods to assay Drosophila behavior. *J Vis Exp* (2012). PubMed PMID: 22433384
8. Diegelmann, S., Jansen, A., Jois, S., Kastenholz, K., Velo Escarcena, L., Strudthoff, N. & Scholz, H. The CApillary FEeder Assay Measures Food Intake in Drosophila melanogaster. *J Vis Exp* (2017). PubMed PMID: 28362419

9. Waterson, M. J., Chung, B. Y., Harvanek, Z. M., Ostojic, I., Alcedo, J. & Pletcher, S. D. Water sensor ppk28 modulates Drosophila lifespan and physiology through AKH signaling. *Proc Natl Acad Sci U S A* **111**, 8137-8142 (2014). PubMed PMID: 24821805

Supplemental Figure 1



p < 0.001 Control vs UAS-Mask
p = 0.40 Control vs RNAi



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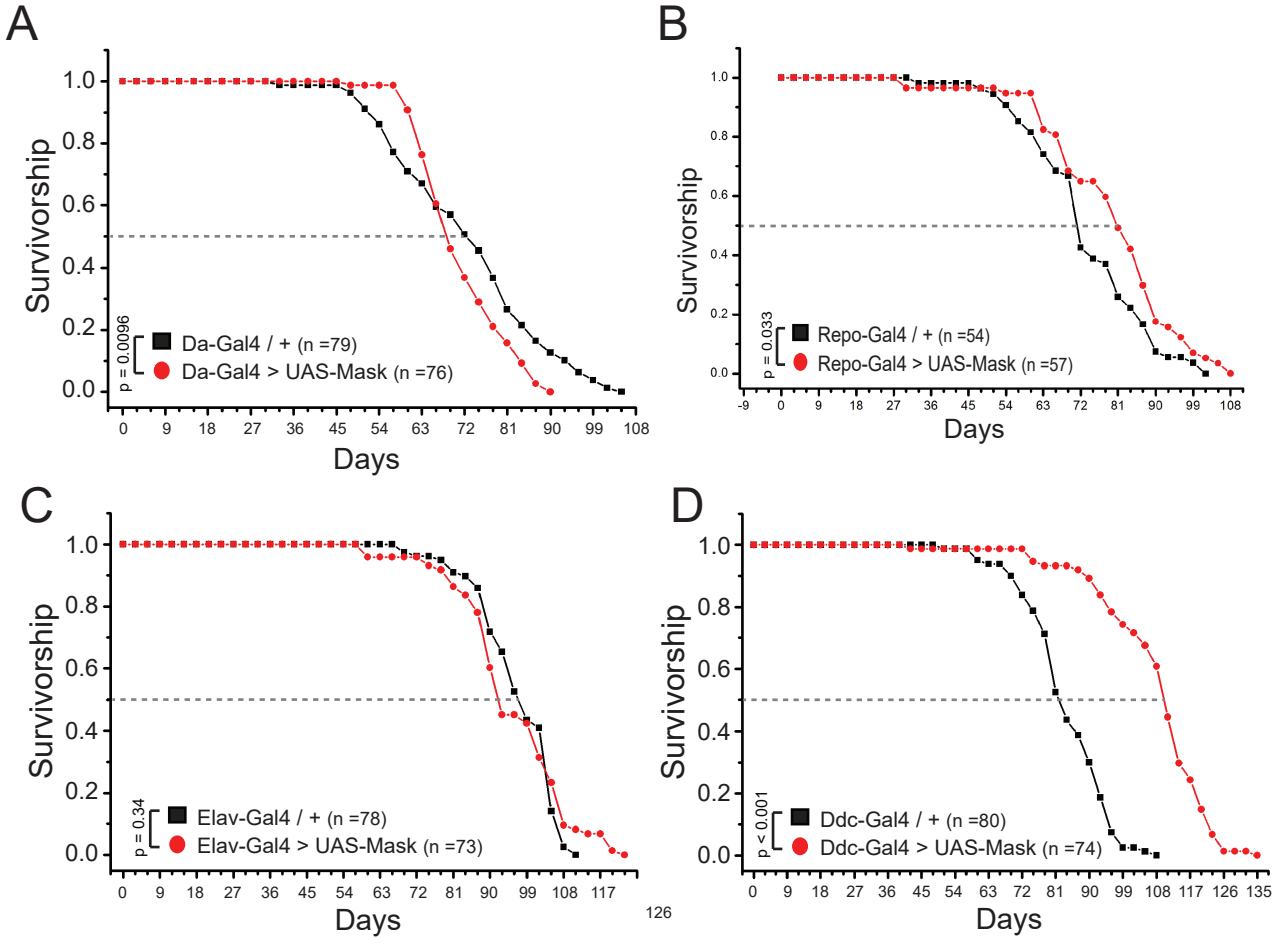

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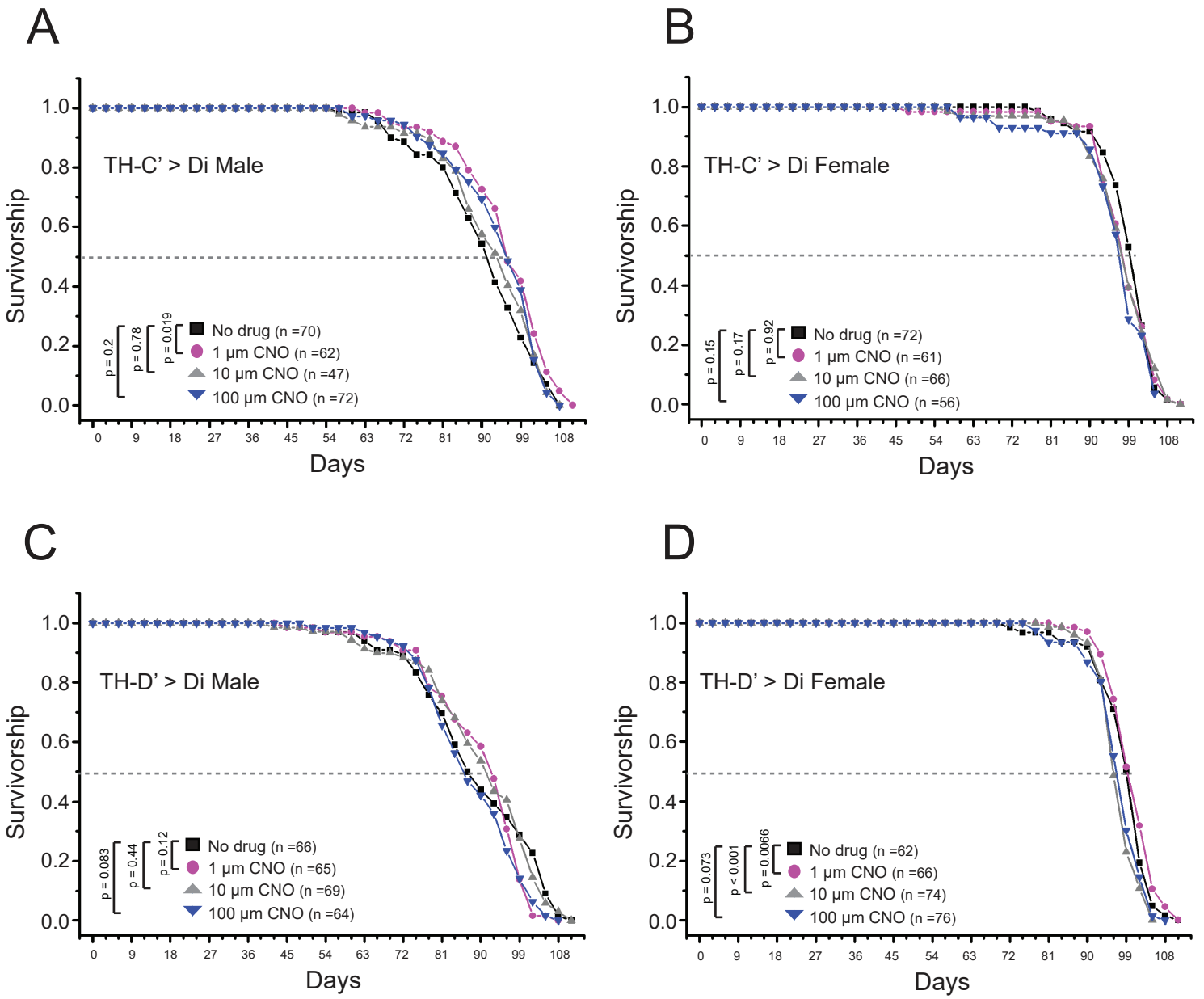

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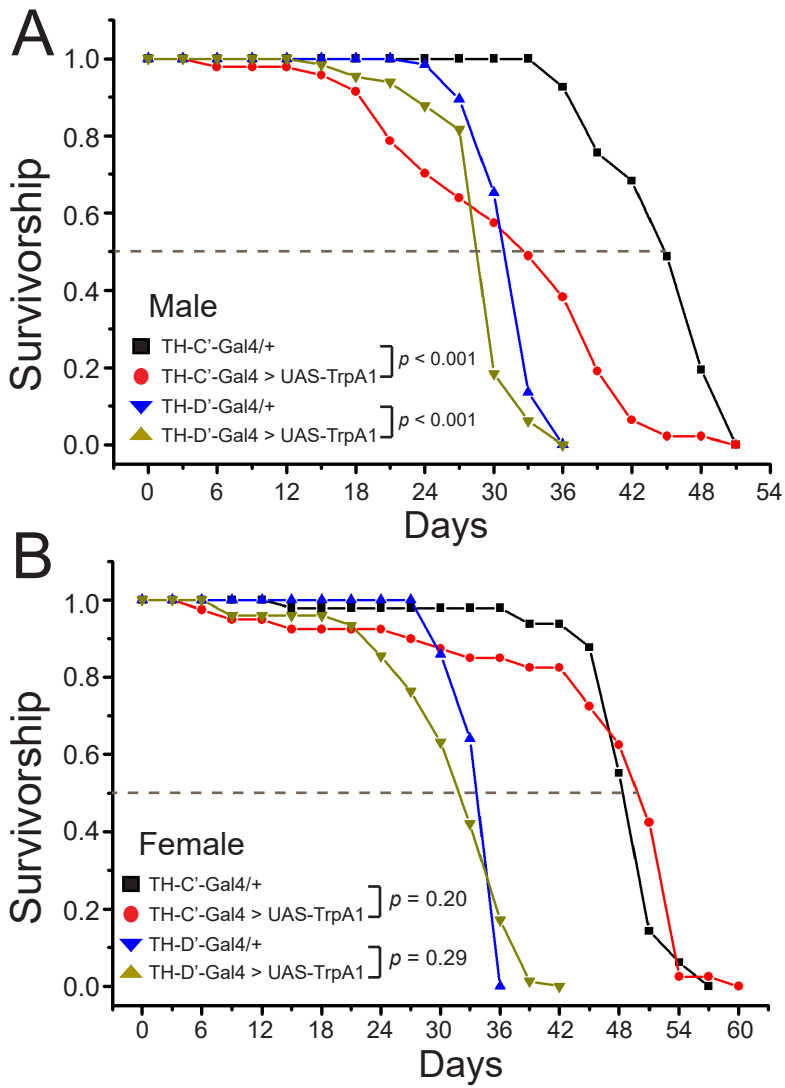

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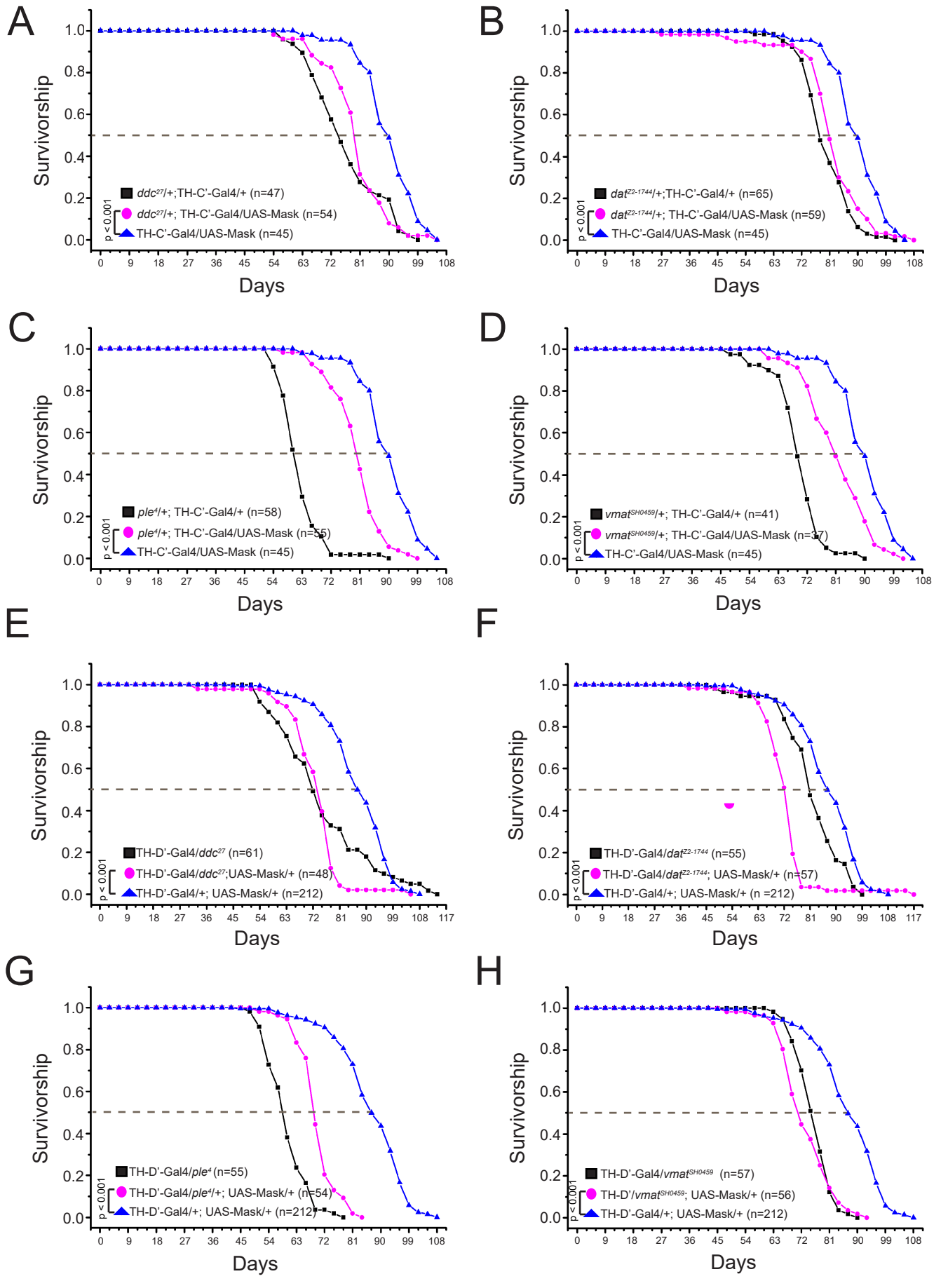

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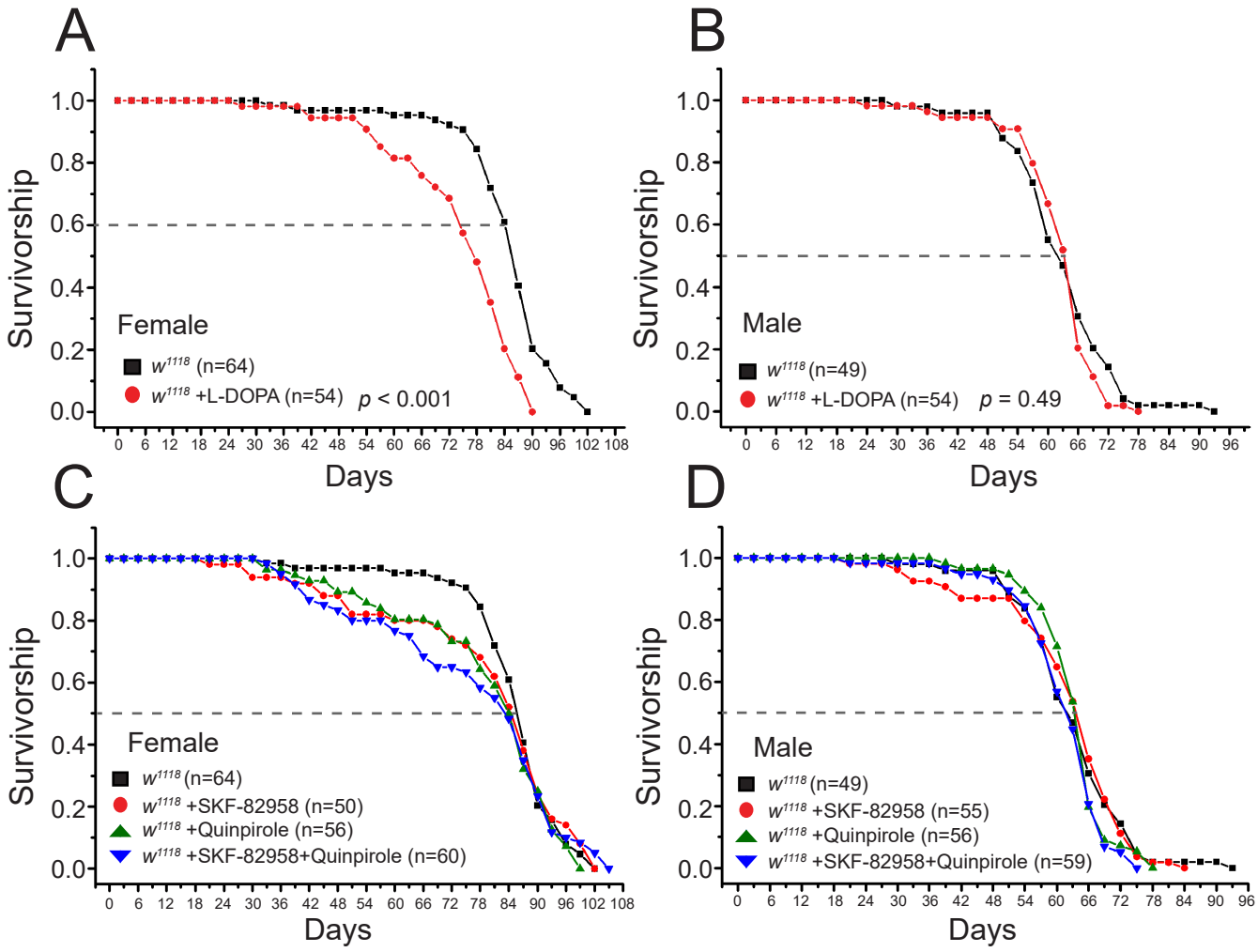

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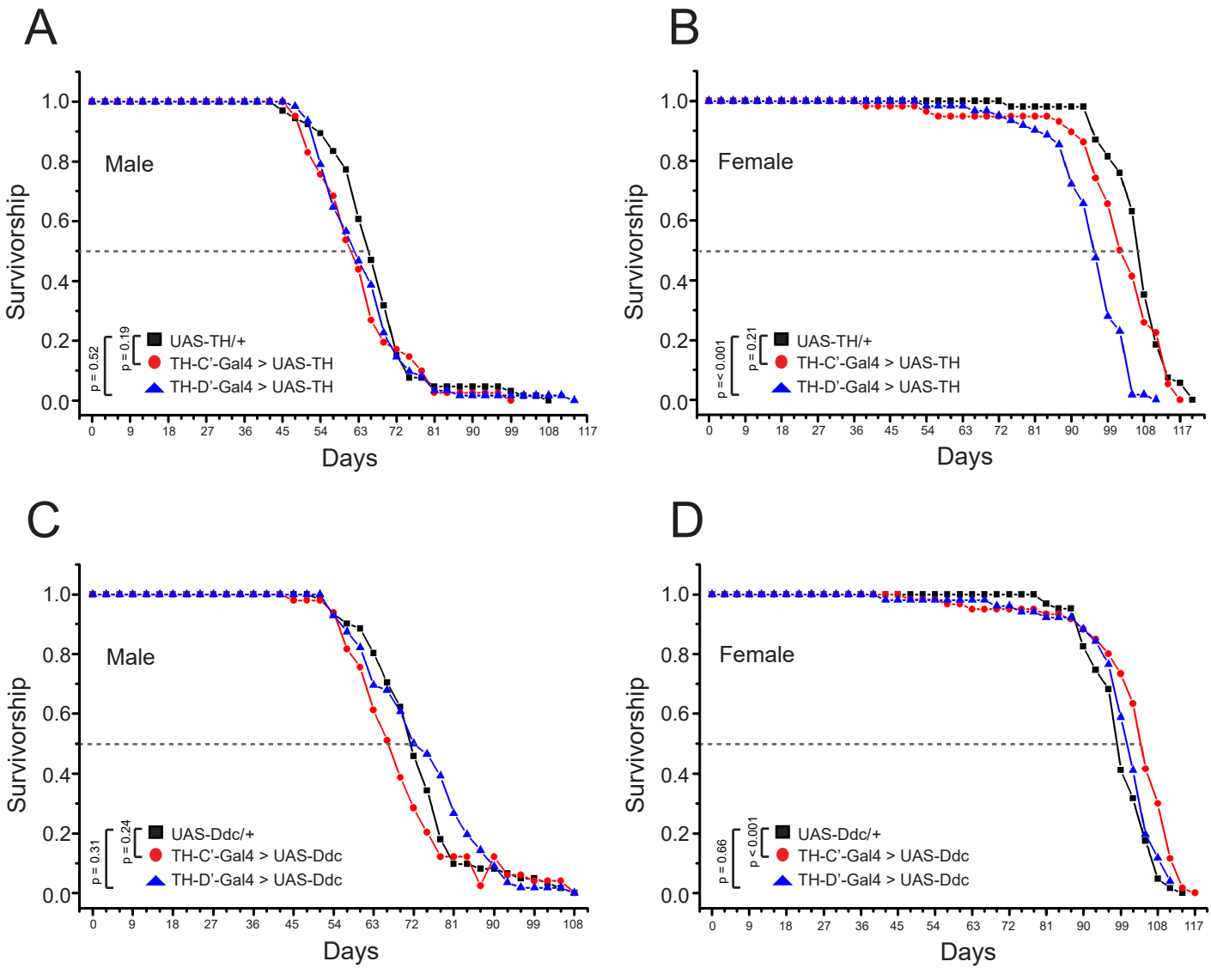

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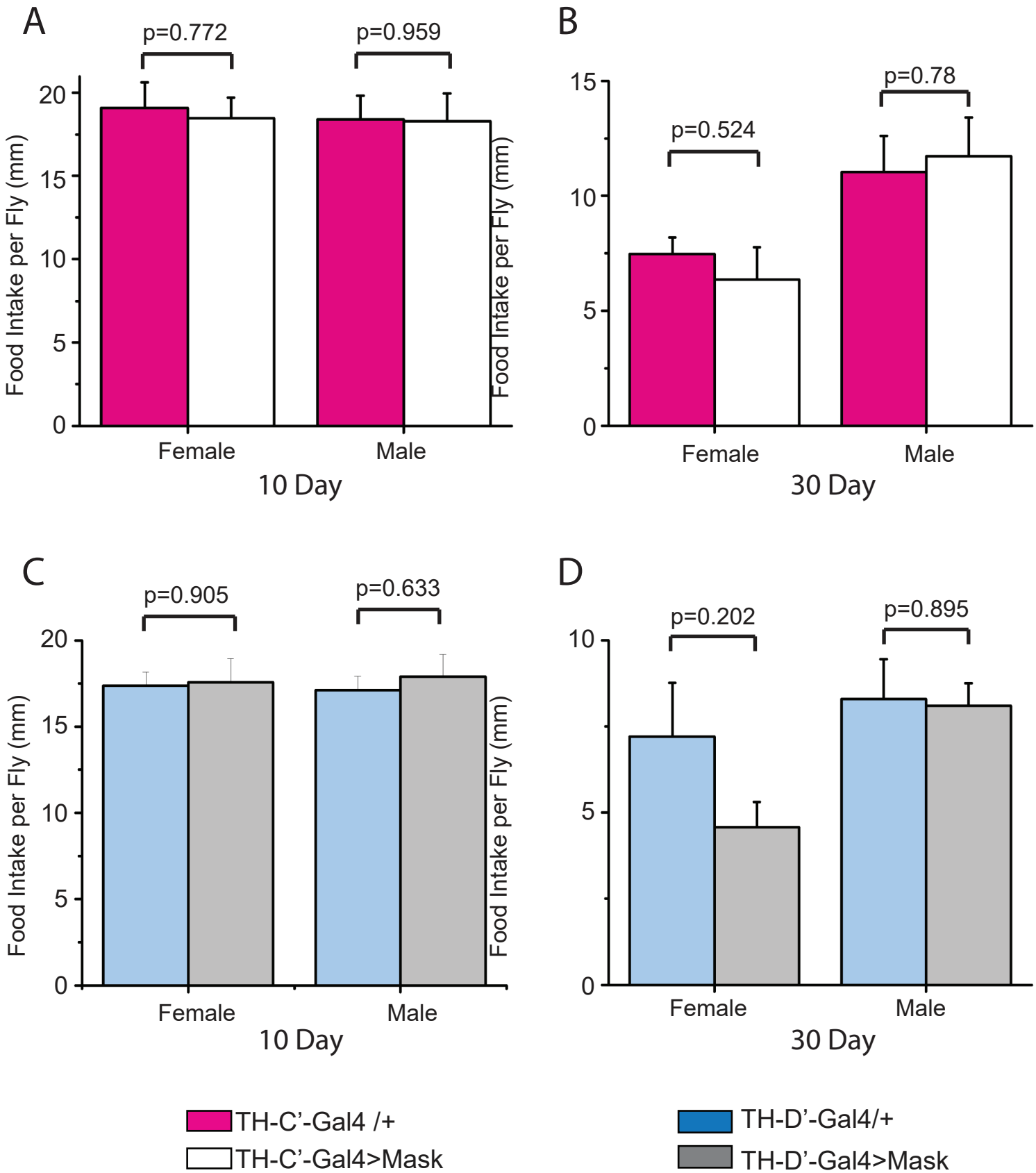

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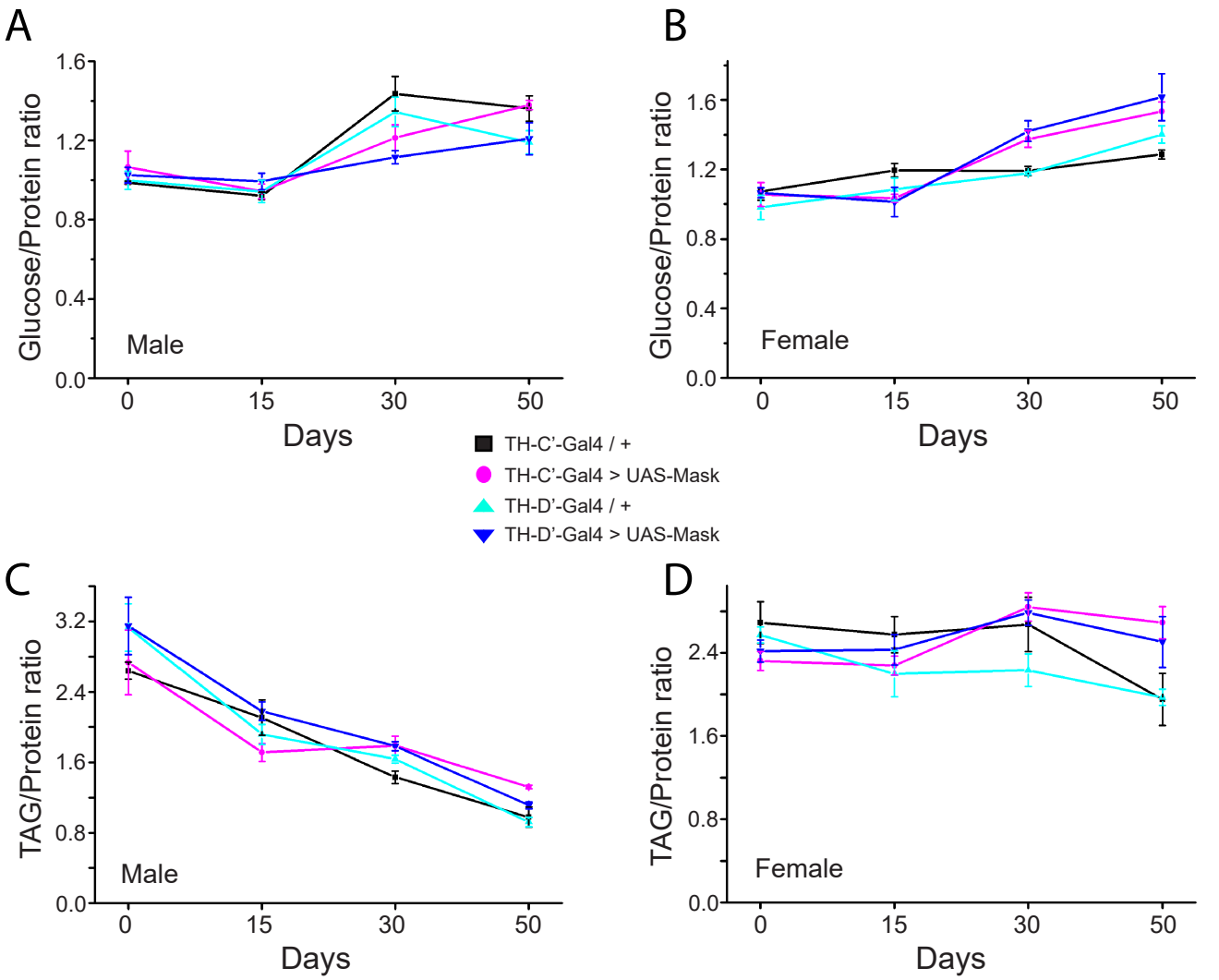

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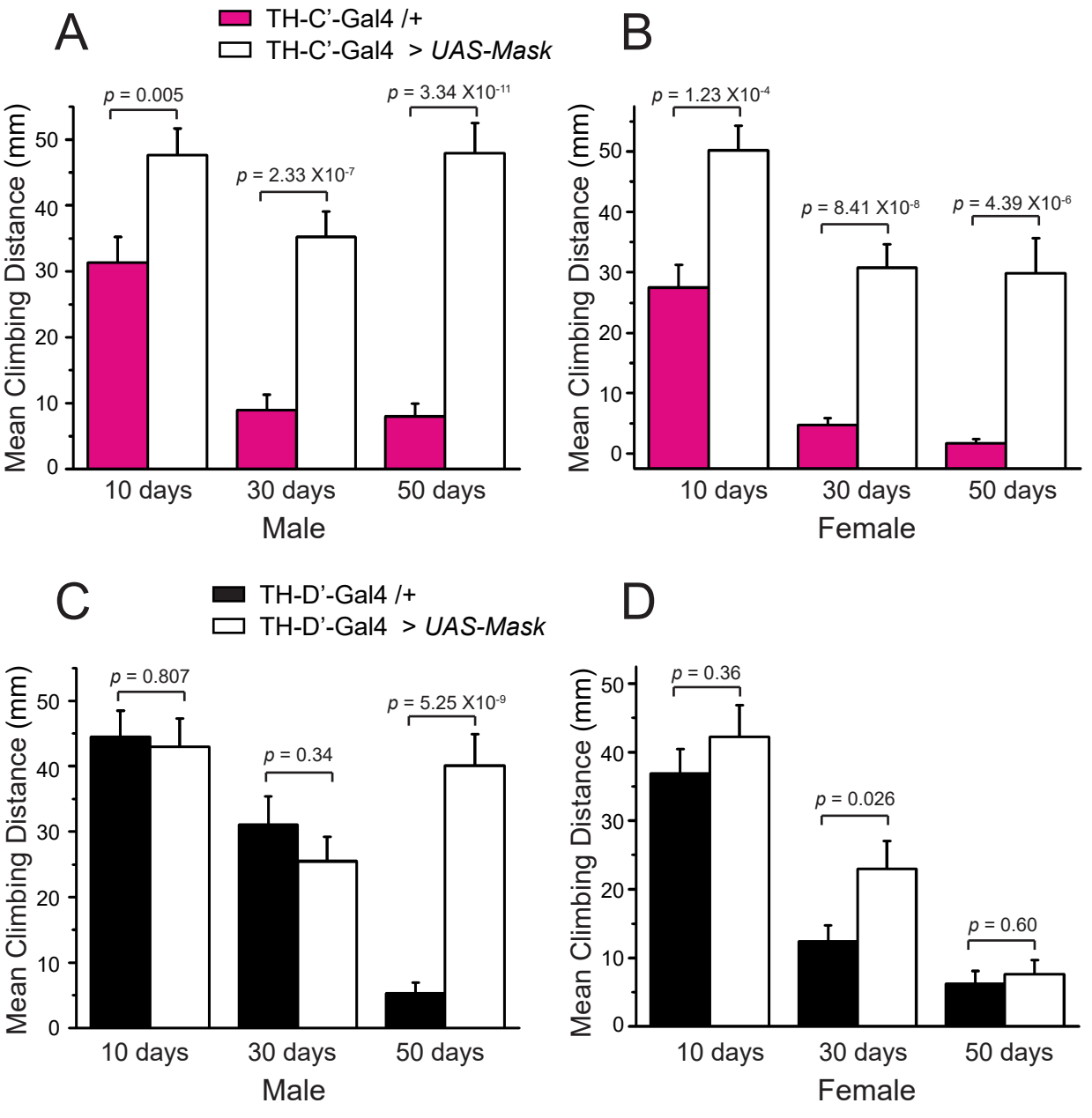

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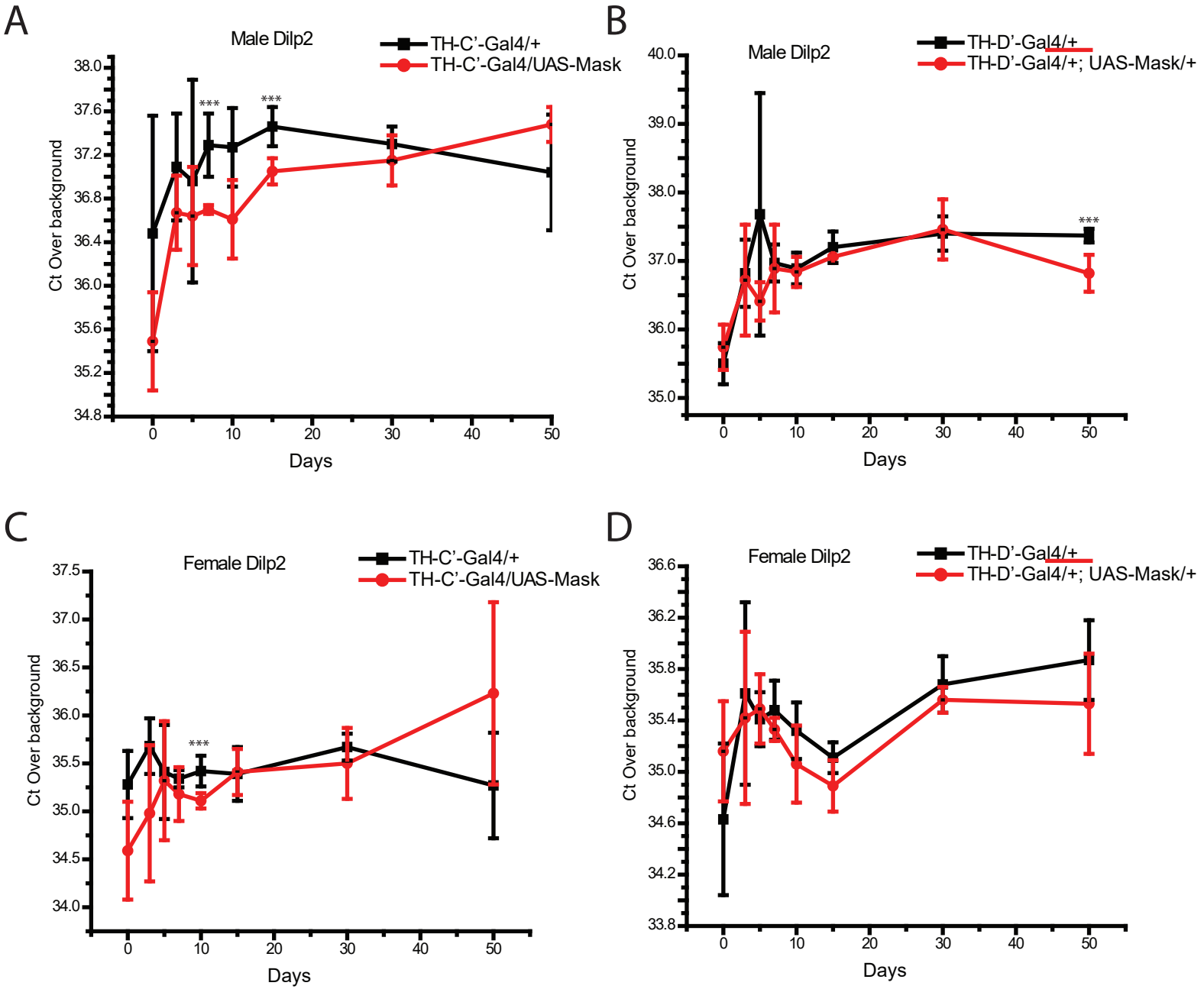

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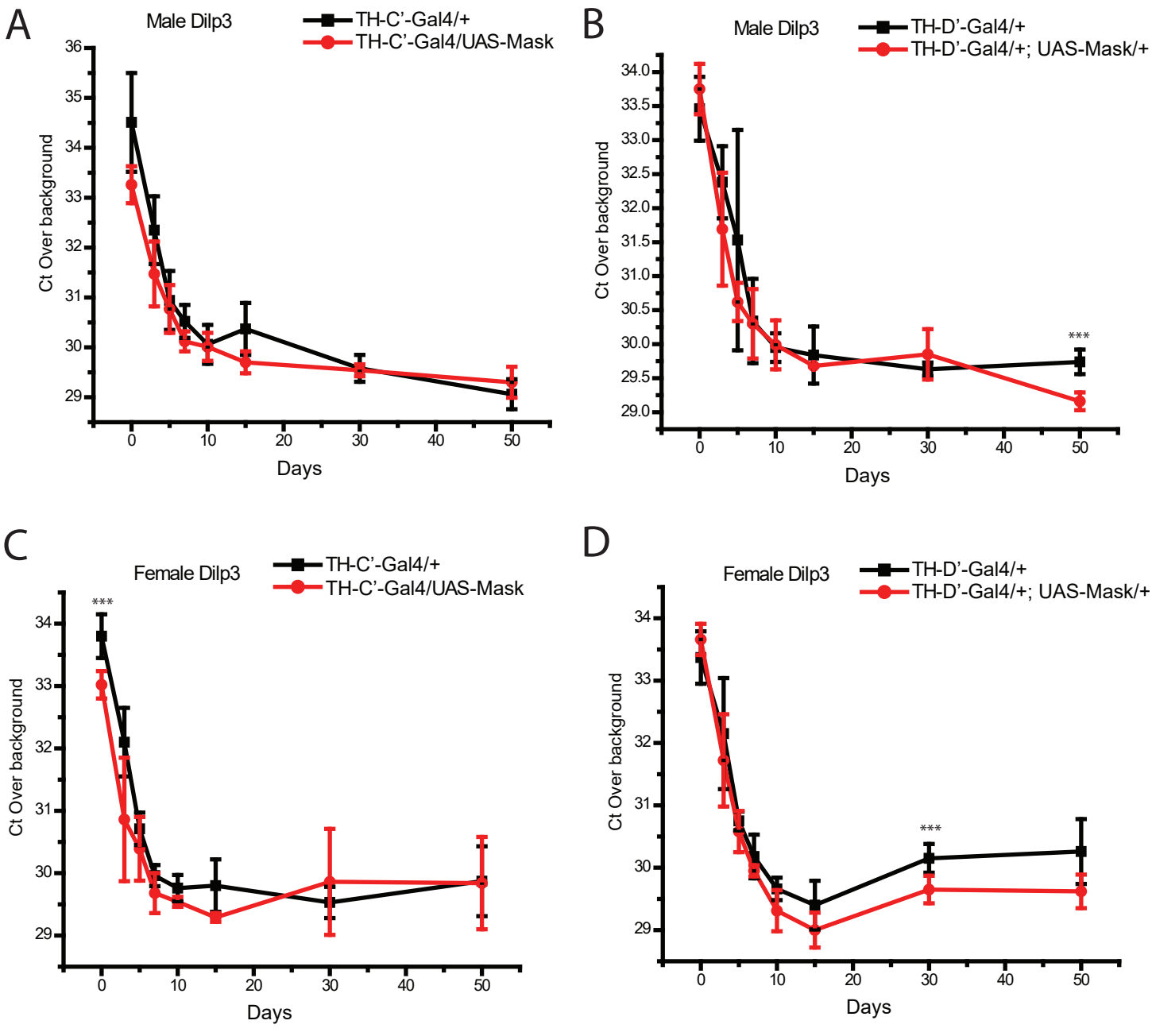

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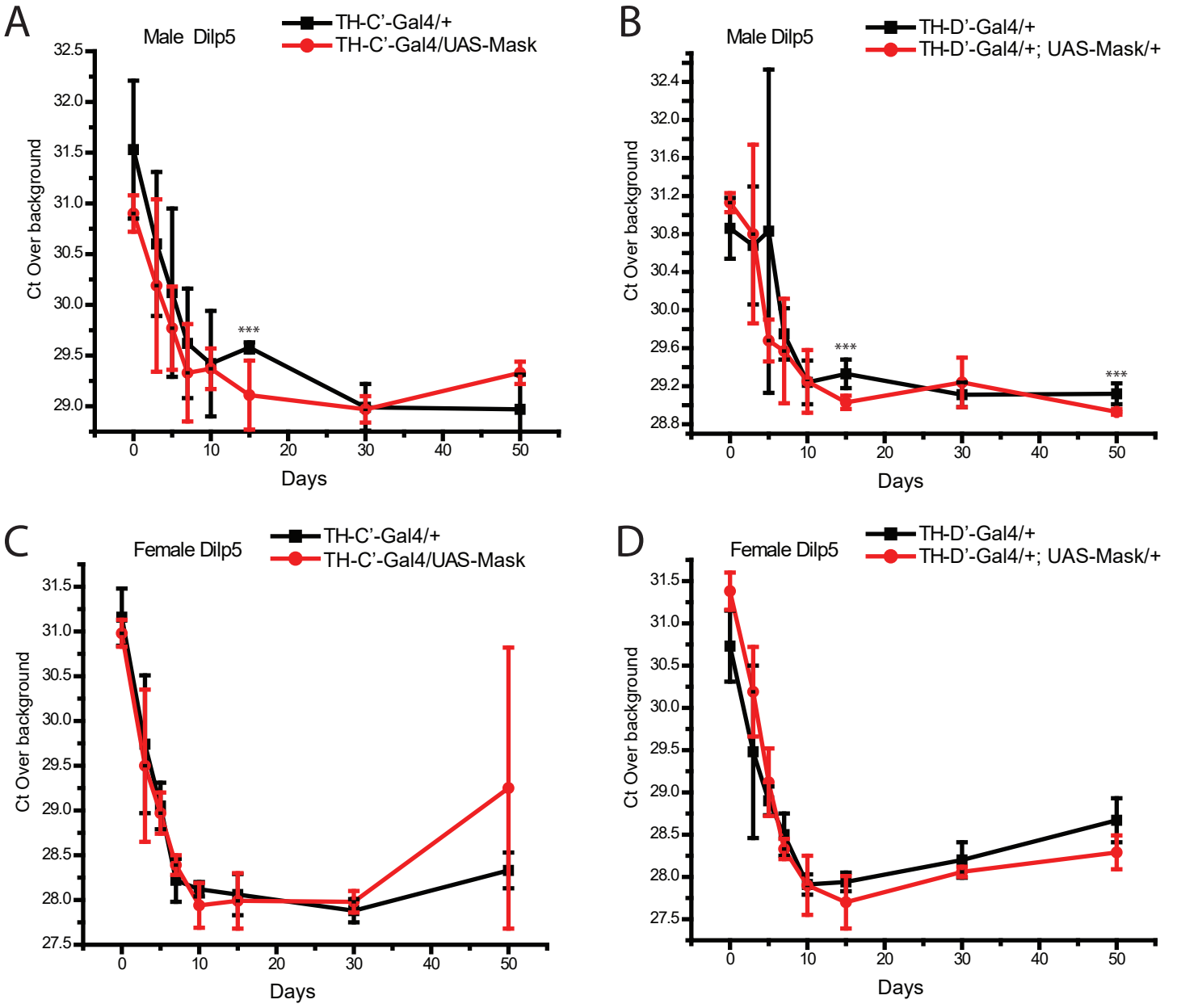

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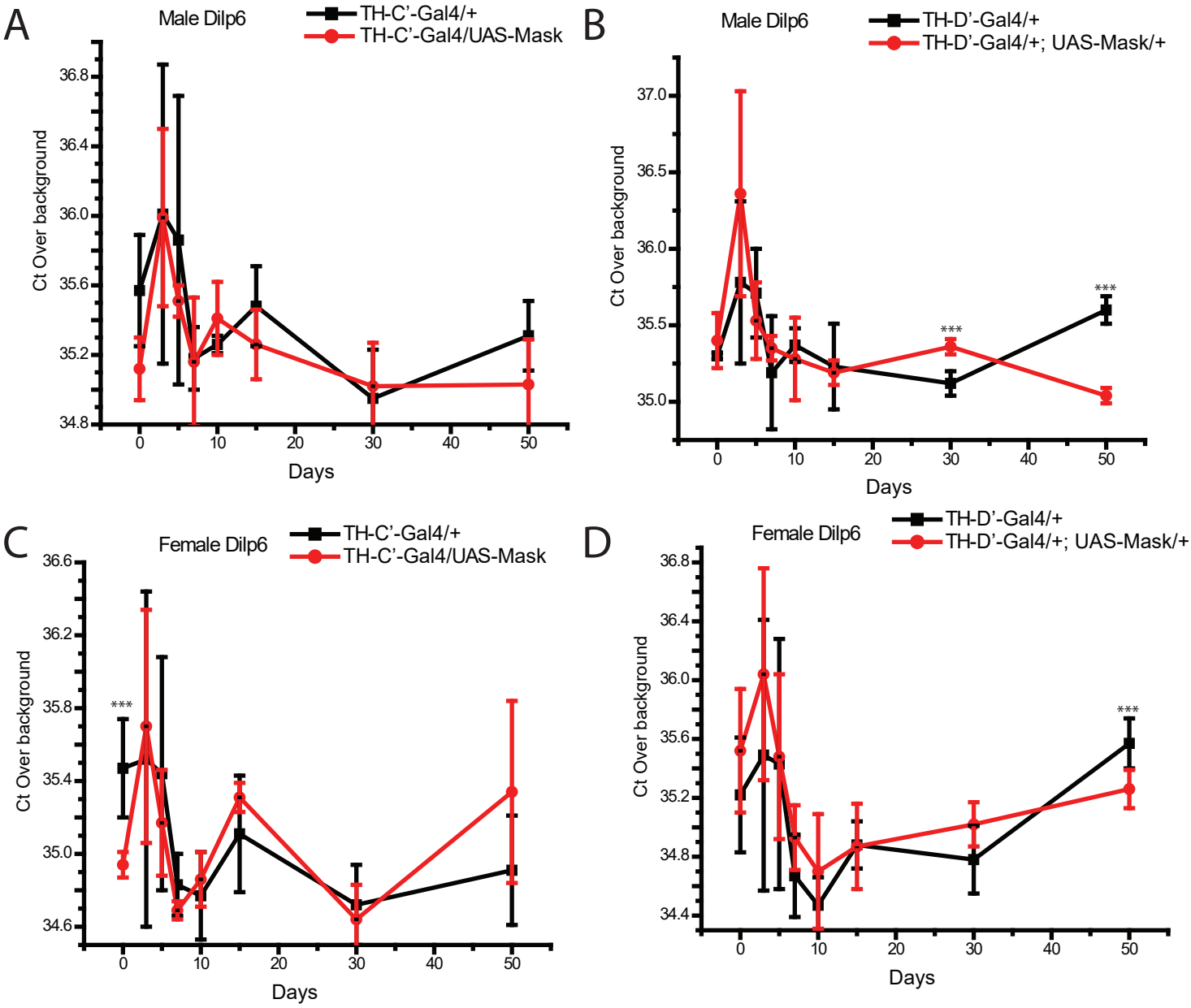

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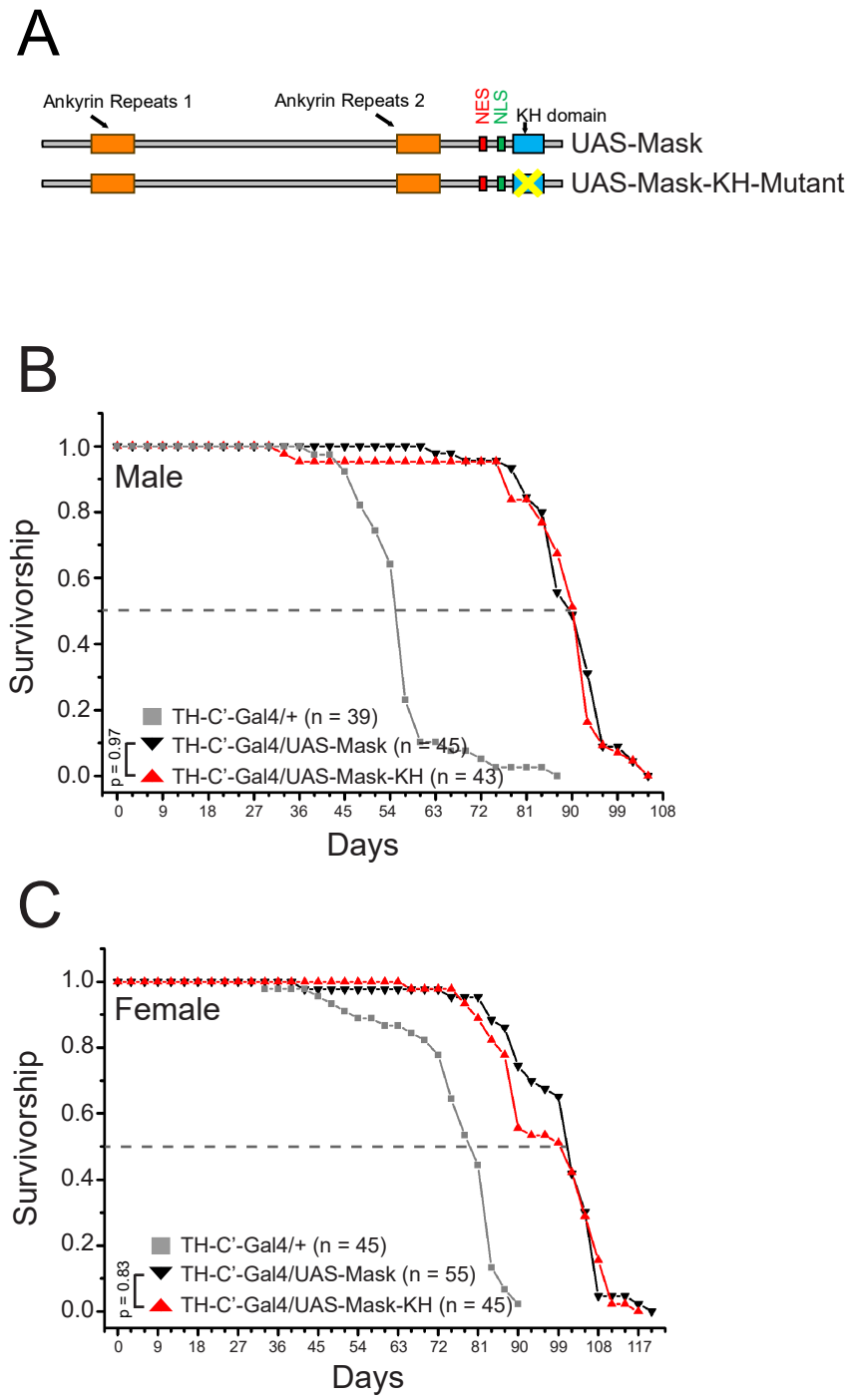

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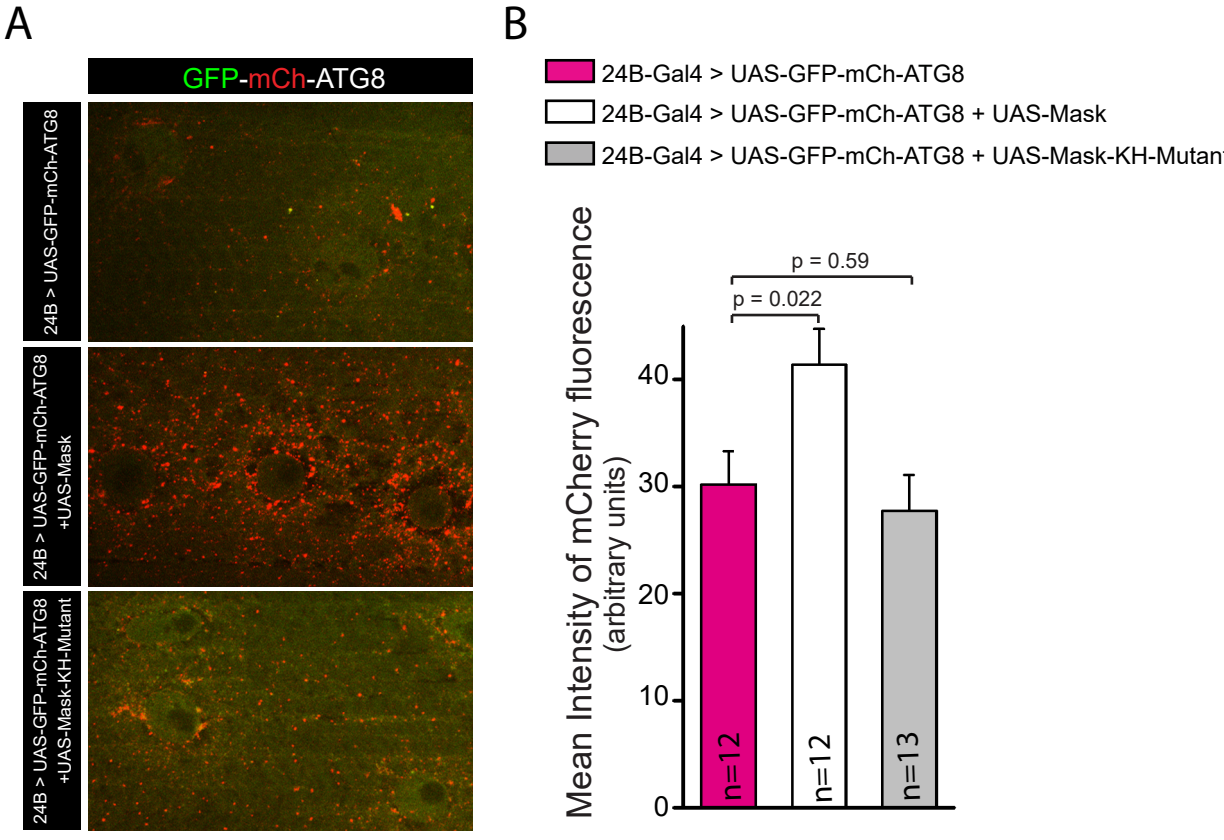

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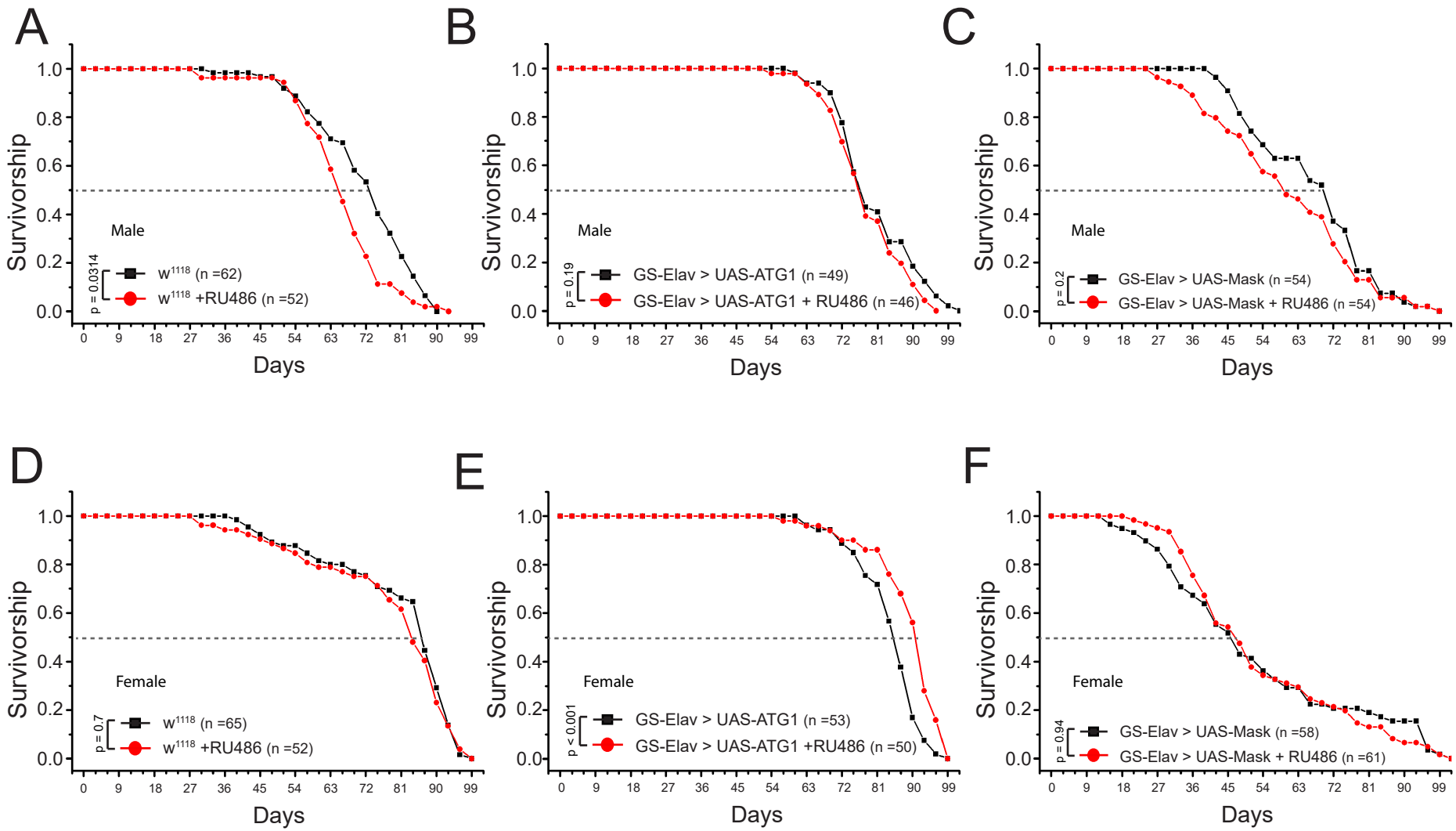

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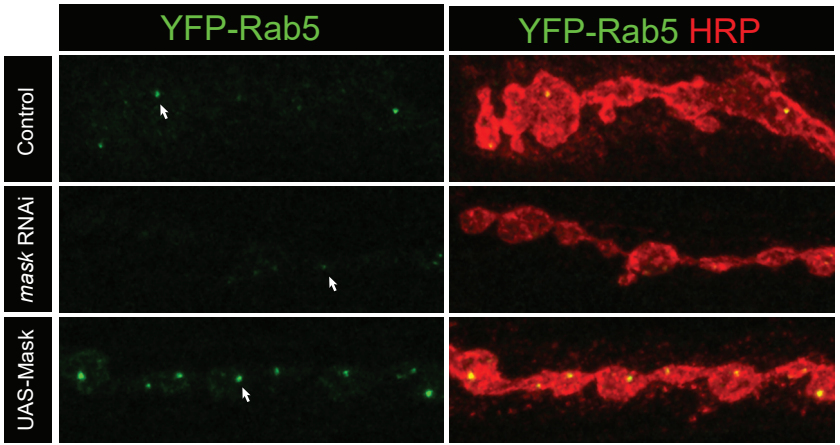
A



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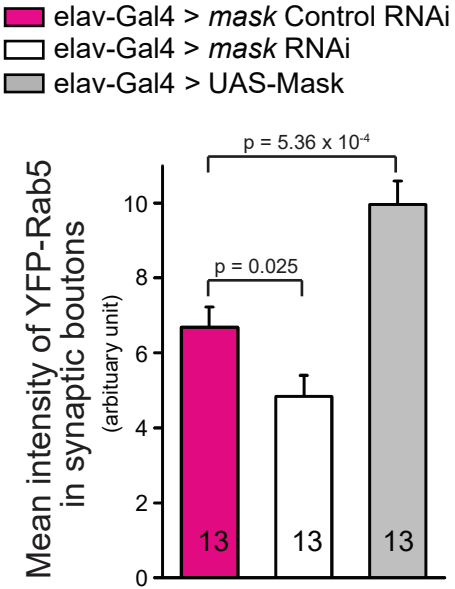

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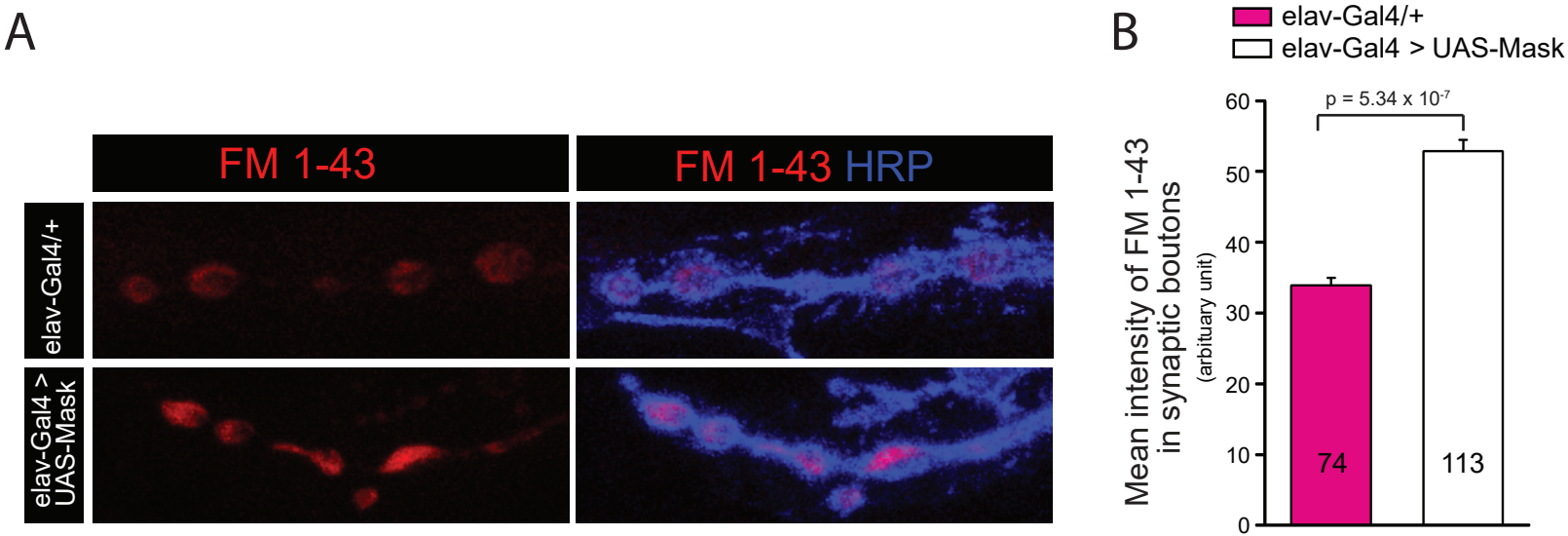
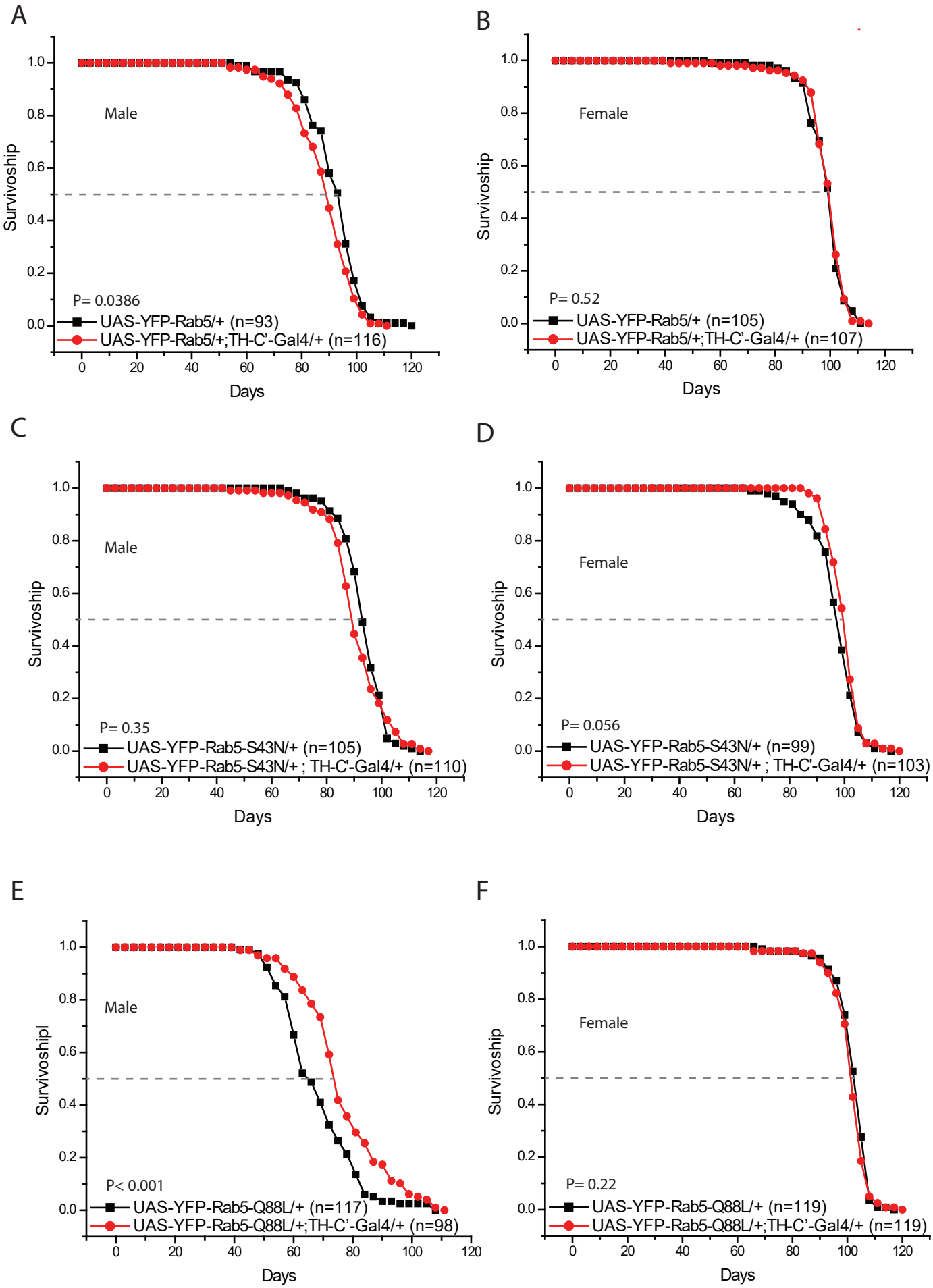
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