Dopaminergic Neurons Regulates 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 the fly brain in regulating aging and longevity. I show that two distinct groups of DANs, when overexpressing the putative scaffolding protein Mask, can each significantly extend lifespan. Dopamine transmission is required to elicit beneficial effects when Mask is overexpressed in these neurons, 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 in the long-lived flies is accompanied by sustained adult locomotor and fecundity. And other physiological functions in the adult such as food intake, energy storage, and insulin production in the brain are not consistently altered by overexpressing Mask in either group of DANs. Our 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. All together, these data unraveled an undocumented DANs-dependent mechanism that regulate systemic aging and longevity in flies, alterations of the cellular properties in subsets of DANs can have a profound impact on aging.

Dopaminergic neurons, the central component of a key modulatory system in the brain, are greatly affected by age. Many essential brain functions involving memory formation, motor control, reward processing, arousal, metabolism, and decision making are modulated by the dopaminergic system[1,2], but it is unclear whether it also regulates the aging process in animals. In 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. I found that 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 is neither the entire body nor in the whole nervous system (neurons or glial cells) showed significant effects on the lifespan of flies (Fig. S2). Dopaminergic neurons in the adult fly brain cluster into eight groups[3]. I next asked whether all or only subsets of dopaminergic neurons are responsible for their lifespan-control function. I express Mask in distinct groups of dopaminergic neurons in the fly brains using three well-characterized Gal4 drivers[4-6], and I found that 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 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). These results together suggest that, although dopamine systems may

not be required for a normal lifespan, overexpressing Mask in these dopaminergic neurons possibly induces a gain-of-function effect in the dopaminergic neurons which consequentially confers a beneficial outcome on aging and longevity. Before I can fully understand the underlying molecular mechanism for this phenomenon, I first set out to confirm the potentials of these dopaminergic neurons to impact lifespan in flies. Using established interventions that affect neuronal activities, I examined the effects on longevity by fully activating the TH-C' or TH-D' dopaminergic neurons in the adult flies. Next, I found that continuous activation of the TH-C' or TH-D' dopaminergic neurons using the thermosensitive cation channel TrpA1 shortens the lifespan in the male flies, while the lifespan in the female flies was not affected (Fig. S4). After, I employed the inducible activation system, DREADD-D_s[9], in the TH-C' or TH-D' dopaminergic neurons to quantitatively modulate their neuronal activity instead of fully activating these neurons. I found that the 1μM CNO treatment (only induces very modest activation of the DREADD-D_s receptors) induces prominent and consistent extension of lifespan in flies that express the engineered Gαs receptor in their TH-C' or TH-D' neurons (Fig. 2), suggesting that moderate modulation of these neurons is sufficient to impact longevity.

Next, I asked whether dopamine transmission is required for lifespan extension induced by Mask-overexpression in the dopaminergic neurons. I reasoned that if dopamine transmission is needed, reducing dopamine signaling in these neurons would suppress the ability of Mask-overexpression to extend lifespan. Indeed, genetically heterozygosity that reduces gene dosages of either 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,

can each blunt the extend of lifespan extension when Mask is overexpressed in either TH-C' or TH-D' neurons in the heterozygous backgrounds of all four genes compared to wild type background (Fig. S5). These results further support the notion that the dopamine system plays an essential role in Mask-mediated lifespan extension. I then tested whether upregulation of dopamine signaling is sufficient to induce lifespan extension in flies. It appeared that feeding flies with dopamine precursor L-DOPA[10], dopamine D1 receptor agonist SKF-82958[11], dopamine D2 receptor agonist Quinpirole[12], or D1 and D2 agonist together all fail to extend the lifespan in flies (Fig. S6). The lifespan of female flies fed with L-DOPA is even moderately shortened (Fig. S6). Since the adverse effects may be attributable to global elevation of dopaminergic signaling which has been shown to cause neural toxicity in mammals[13], I next used genetic approaches to selectively increase dopamine production in the TH-C' or TH-D' neurons via overexpressing Tyrosine Hydroxylase (TH), or Dopa Decarboxylase (Ddc), two enzymes that are responsible for dopamine synthesis, in the TH-C' or TH-D' dopaminergic neurons. I found that overexpressing these two enzymes did not affect longevity (Fig. S7). Similar to L-DOPA feeding, these treatment lead to moderate reduction in the lifespan in female flies. These results suggest that although neurons require dopamine transmission to communicate and to induce lifespan extension, increasing dopamine production alone in these dopaminergic neurons is not sufficient to elicit similar effects on aging and longevity induced by Mask overexpression or moderate DREADD-Ds activation in the same neurons.

To fully understand how overexpressing Mask in TH-C' or TH-D' neurons impact aging and longevity at the systematic level, 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 overexpressing Mask in the TH-C' or TH-D' neurons may consume less food and therefore undertake voluntary caloric restriction, a potential contributing factor to their prolonged lifespan. However, the Mask-overexpressing long-lived flies consume comparable amount of food compared to the control flies at young and mid age (10 and 30 day old) in the Capillary Feeder assay (CAFE assay)[18] (Fig. S8). These flies also show moderately increased levels of whole body glucose and Triglyceride (TAG) levels at mid and late ages (30 and 50 days old) (Fig. S9). I next assessed adult locomotor activity in flies using the rapid iterative negative geotaxis (RING) assay[19,20], and I found that expressing Mask in TH-C' dopaminergic neurons significantly enhances the locomotor performance during the entire lifespan (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. I did not detect any consistent reduction in transcription levels of the Dilp genes between gender or age (Fig. S11- 14). All together, the results of the aging analysis suggests that the Mask-overexpressing flies live healthy and active lives throughout the entire lifespan.

Delayed reproductive maturation or reduced fecundity correlate closely with lifespan extension[21]. To test whether there is a tradeoff between fertility and lifespan in Mask-overexpressing flies, I examined the fecundity of male and female flies at different ages. I did not detect any delay or reduction in reproduction in either male or female flies. Rather, overexpressing Mask in the TH-C' neurons 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 fertility, 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. Given the lack of consensus theory to explain the relationship between aging and reproduction, the phenomenon that increased fecundity can be a concomitant of lifespan extension 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 effects 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. We recently demonstrated that Mask promotes microtubule (MT) dynamics in fly larval motor neurons and body wall muscles[26]. This function of Mask prompted me to postulate that altered MT dynamics in the Mask-expressing dopaminergic neurons is the key effector that induces changes in neuronal behaviors leading to lifespan extensions in flies. If this is true, reagents that manipulate MT dynamics in the same manner would also induce lifespan extension when applied to the same subsets of dopaminergic neurons. I chose two interventions that have been previously shown to impact MT dynamics: overexpressing the Kinesin heavy chain Unc-104[27] or knocking down a component of the Dynein/Dynactin complex, p150^{Glued}[28], has each been shown to reduce MT length and stability and enhance MT-based transportations. When we applied these manipulations to the TH-C' or TH-D' dopaminergic neurons, both treatments extended lifespan in flies (Fig. 4). These results strongly argue 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.

I next asked whether other cellular functions of Mask also contribute to its ability to induce this dopaminergic-dependent lifespan extension. Mask is a large protein (4001 amino acids) containing several conserved functional domains (Fig. S15), and the structures of Mask enables

its versatile functions in the cells: such as 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). Our 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 fail to induce this effect (Fig. S16). However, the KH-Mutant transgene can rescue the MT defects in larval muscle[26], and more importantly, expression of Mask-KH-Mutant in the TH-C' dopaminergic neurons extended lifespan to a similar level as expression of 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 at the presynaptic terminal (Fig. S18) and promotes vesicular recycling (measured by FM-143 dye

loading assay, Fig. S19). Expressing wild type or a constitutively active form of Rab5 can both enhance synaptic vesicle fusion efficacy and firing strength at the presynaptic terminals[38]. But, when expressed in the TH-C' or TH-D' dopaminergic neurons, the active form of Rab5 protein can mildly extend lifespan in the male flies (Fig. S20). It appears 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 as Mask the cellular output by dopaminergic neurons that translates to lifespan extension. Although there is still no answer for whether the function of Mask to promote MT dynamics and to enhance endocytosis at the nerve terminals are consequentially related. It is possible that altered vesicle trafficking is 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 I show that 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 which states an inversely related energy allocation between reproduction and somatic maintenance. To reconcile such a paradox, I propose an explanation that could potentially provide a consensus theory to aging and reproduction. This theory states a reproduction-center mechanism for aging, and that there is no competition for resource between somatic maintenance and reproduction, but rather, somatic tissues adapt and cop with the animal's need for reproduction. In detail, reproduction is centered by other physiological functions, and, in a "live fast and die young" scenario, the fulfilled reproduction in early life lifts the need for somatic maintenance and the animal die early. In the nutrition-sensing scenario, when the animals sense a lack a nutrition in the environment that poses threatens for the survival of the progenies, they halt

the reproductive activities, and adjust the somatic system accordingly to survive and prolong their lifespan to reproduce later. In social insects, reproductive females undergo somatic transformation to live much longer to meet the prolonged reproduction demand. This theory roots in the notion that natural selection centers on fitness on reproduction in a way that the animals possess the ability to adapt to different environment changes and challenges to ensure successful reproduction and the survival of the species. This reproduction-centered aging theory, posit that the organism would set an internal status, or a goal for their reproduction capacity; and to fulfill this goal the body not only engage mechanisms to monitor the progress of the fulfillment of it but also possess a system that can efficiently support somatic maintenance to suit the reproduction need. The brain, with the dopaminergic systems being an essential part for information evaluation and decision making, is the best candidate to centrally organize the information on internal status as well as the environmental cues, and to relay out to the peripheral the decision of adjusting the soma 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 underpin 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[39], 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[39] 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[40]. 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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Figure Legend

Figure 1. Overexpressing Mask in 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.

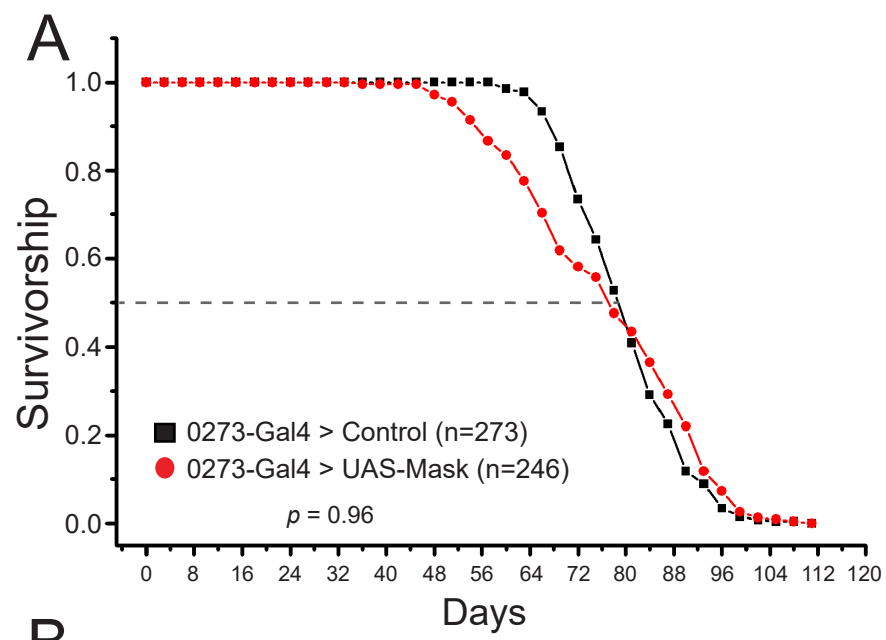
Figure 3. Mask-overexpressing Flies show sustained Fecundity at old ages. A) Numbers of progenies produced by female flies in a 3 day interval. The female flies that overexpress Mask in the TH-C' or TH-D' DA neurons produce significantly more progenies at 30- and 50-day old ages. B) Reproduction in male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

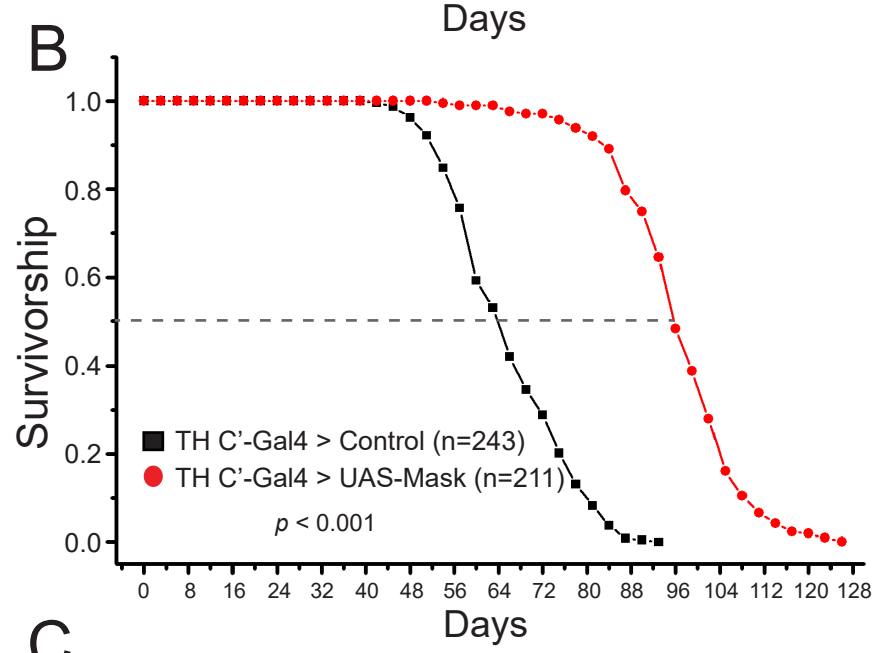
At age 50-day, majority of the control male flies lose the ability to produce progeny, while over 80% of male flies that overexpress Mask in the TH-C' and 60% of those overexpress Mask in the TH-D' DANs are still reproductively active.

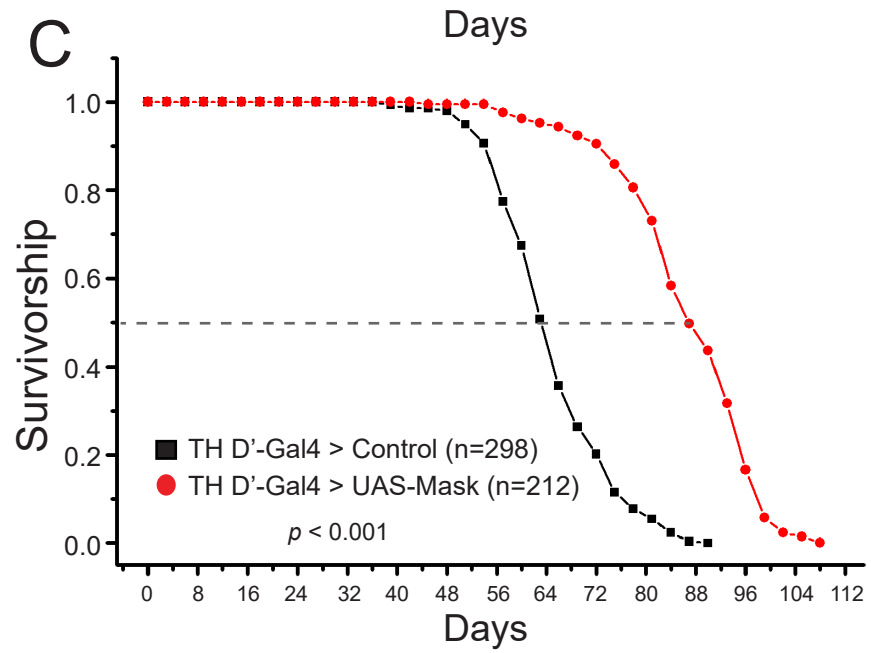
Figure 4. Overexpressing Unc-104 or Knocking down p150^{Glued} in the TH-C' or TH-D' DANs extend lifespan in flies.

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 in control flies) 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 moderate 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

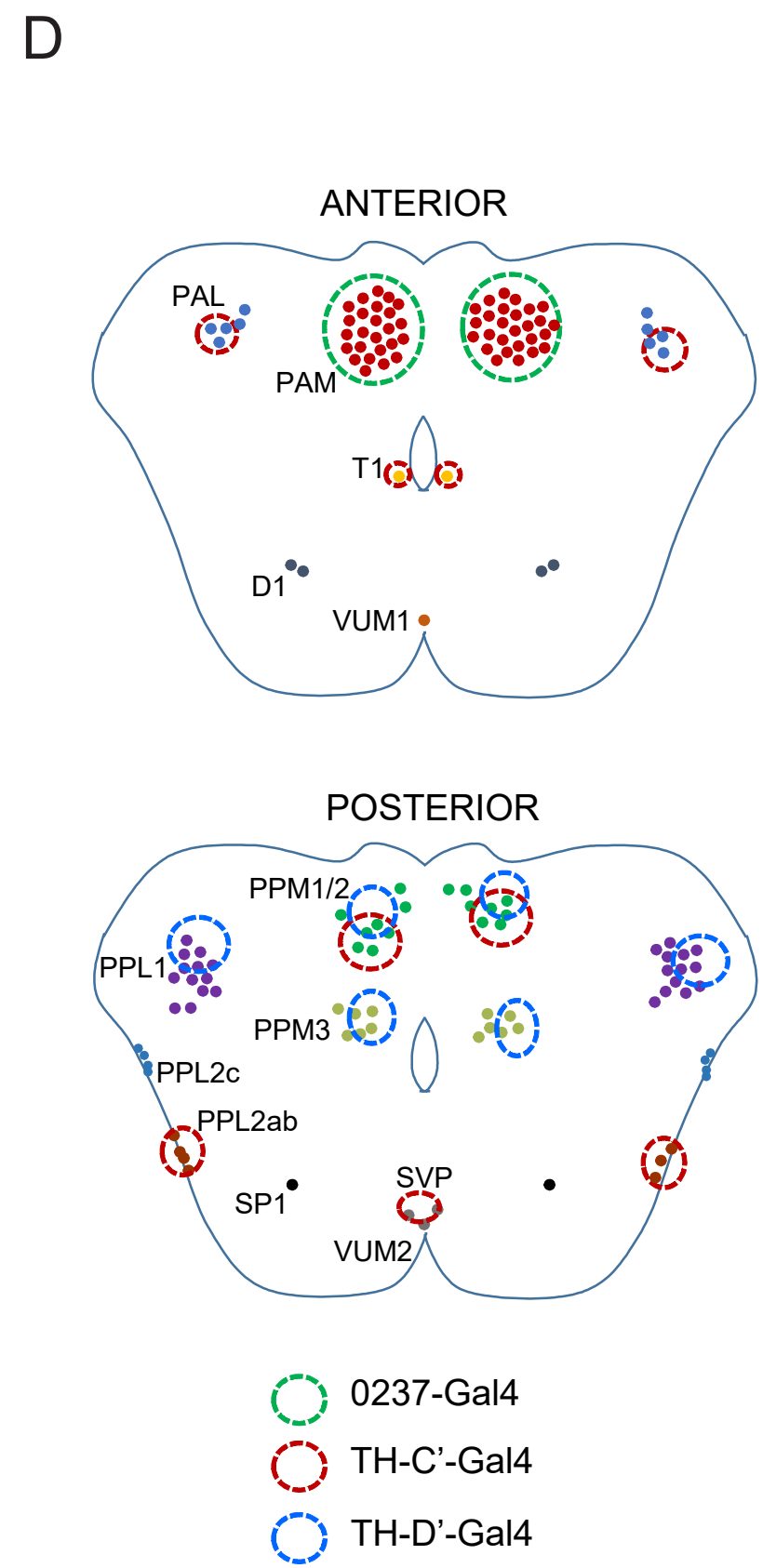

D
ANTERIOR
PAL
PAM
T1
D1
VUM1
POSTERIOR
PPM1/2
PPL1
PPM3
PPL2c
PPL2ab
SP1
SVP
VUM2
0237-Gal4
TH-C'-Gal4
TH-D'-Gal4

Figure 2

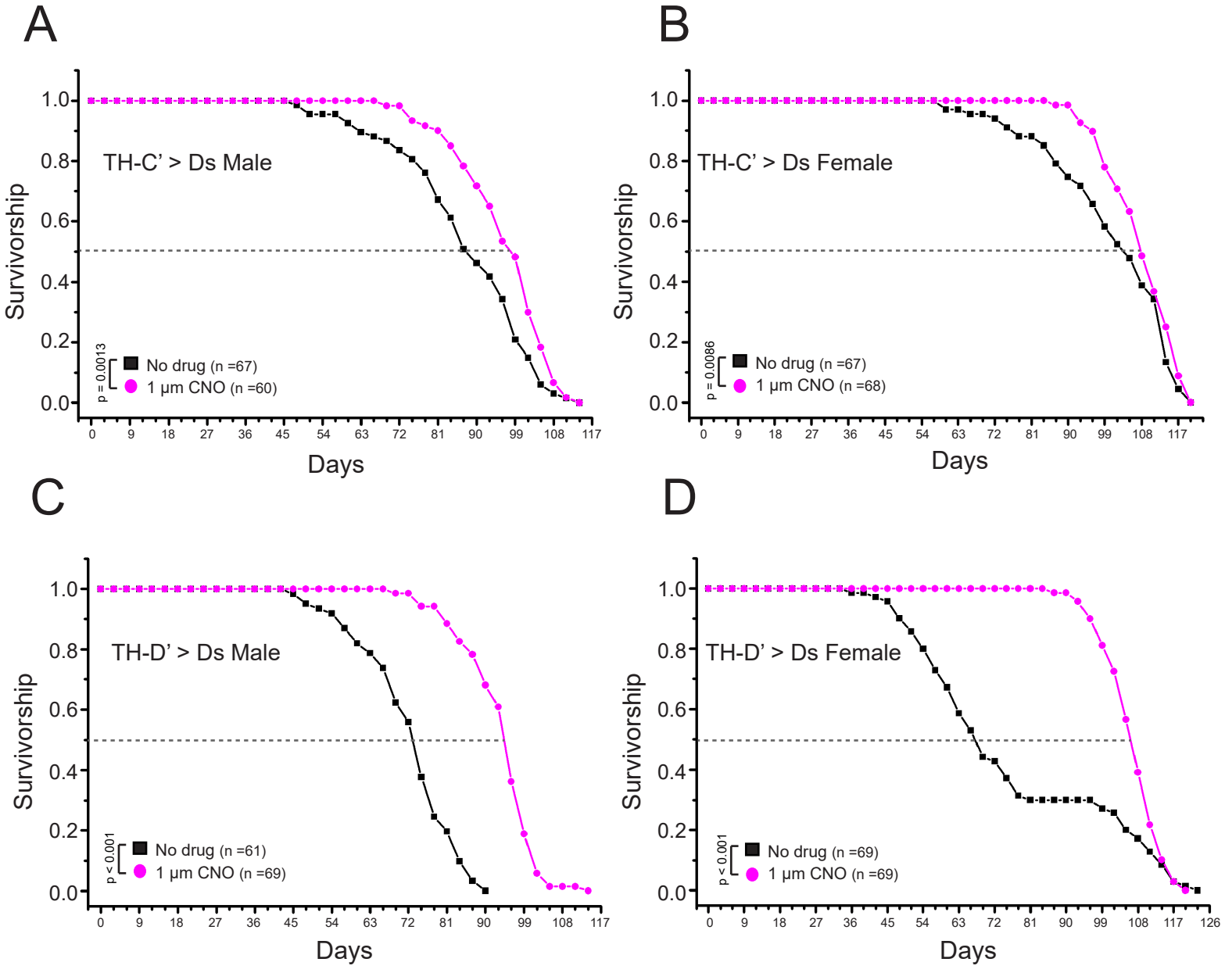
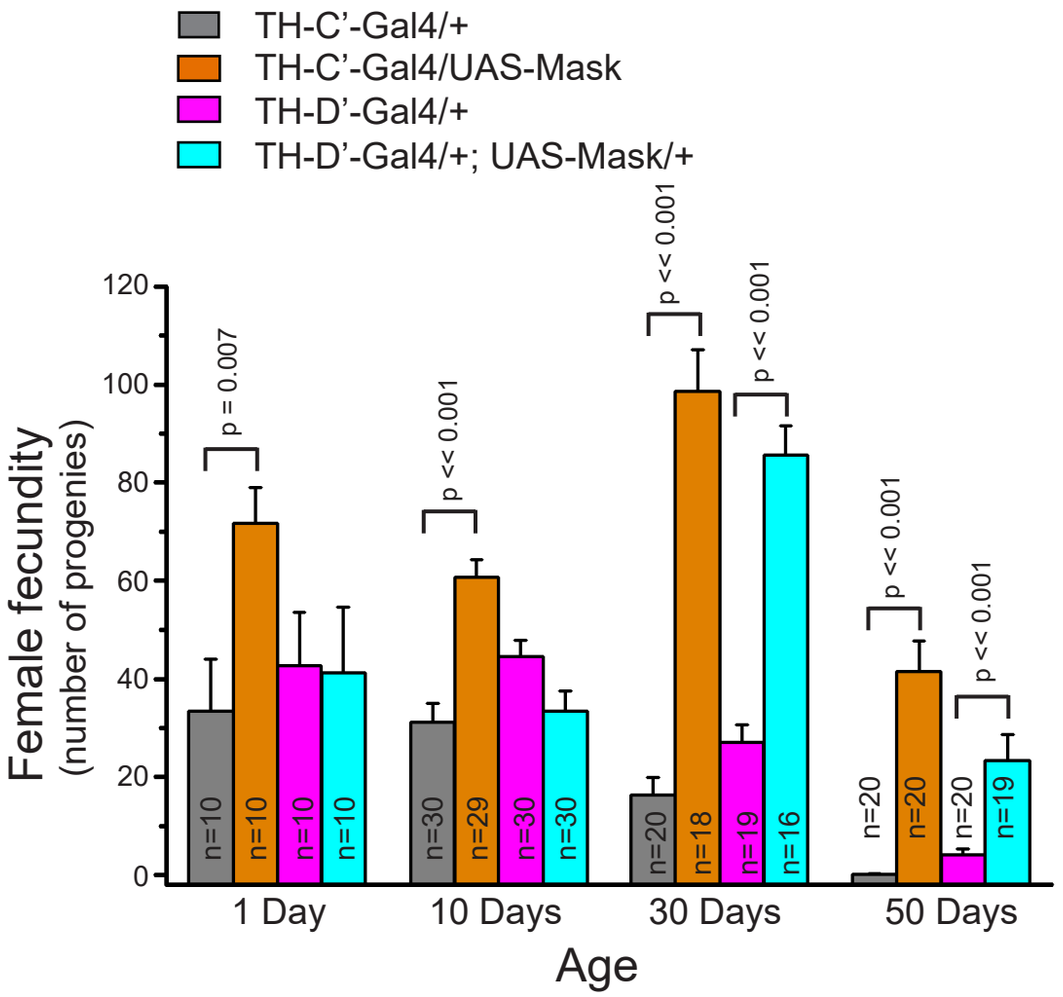

A
TH-C' > Ds Male
Survivorship
Days
p = 0.0013
No drug (n =67)
1 µm CNO (n =60)
B
TH-C' > Ds Female
Survivorship
Days
p = 0.0086
No drug (n =67)
1 µm CNO (n =68)
C
TH-D' > Ds Male
Survivorship
Days
p < 0.001
No drug (n =61)
1 µm CNO (n =69)
D
TH-D' > Ds Female
Survivorship
Days
p < 0.001
No drug (n =69)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

Figure 3


TH-C'-Gal4/+
TH-C'-Gal4/UAS-Mask
TH-D'-Gal4/+
TH-D'-Gal4/+; UAS-Mask/+
Female fecundity
(number of progenies)
120
100
80
60
40
20
0
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

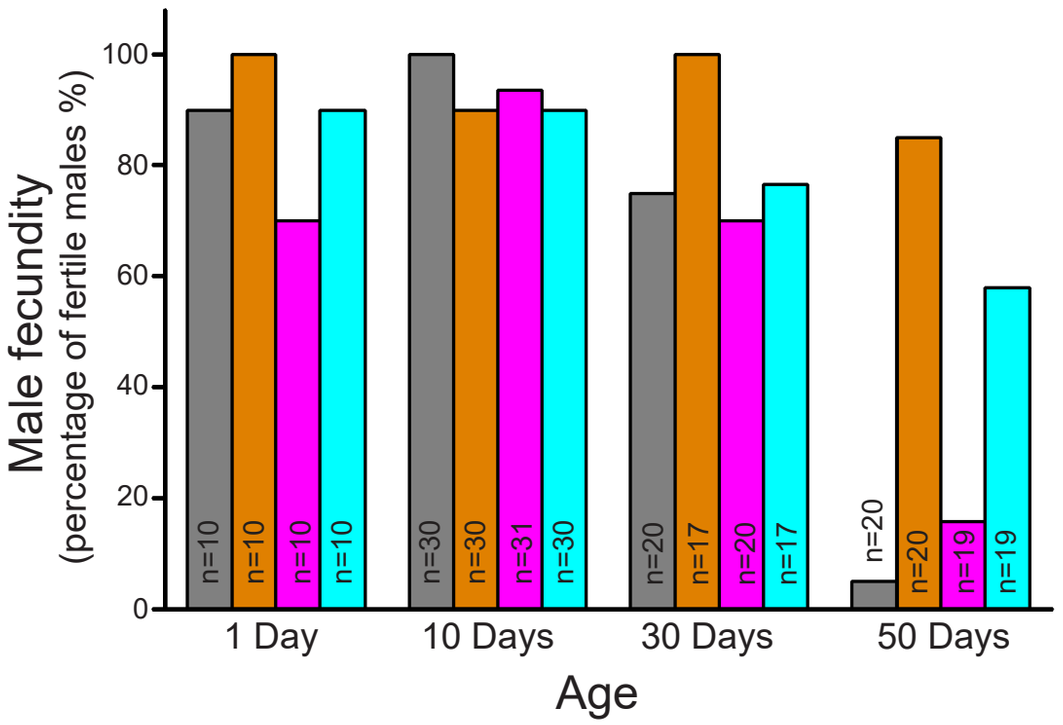
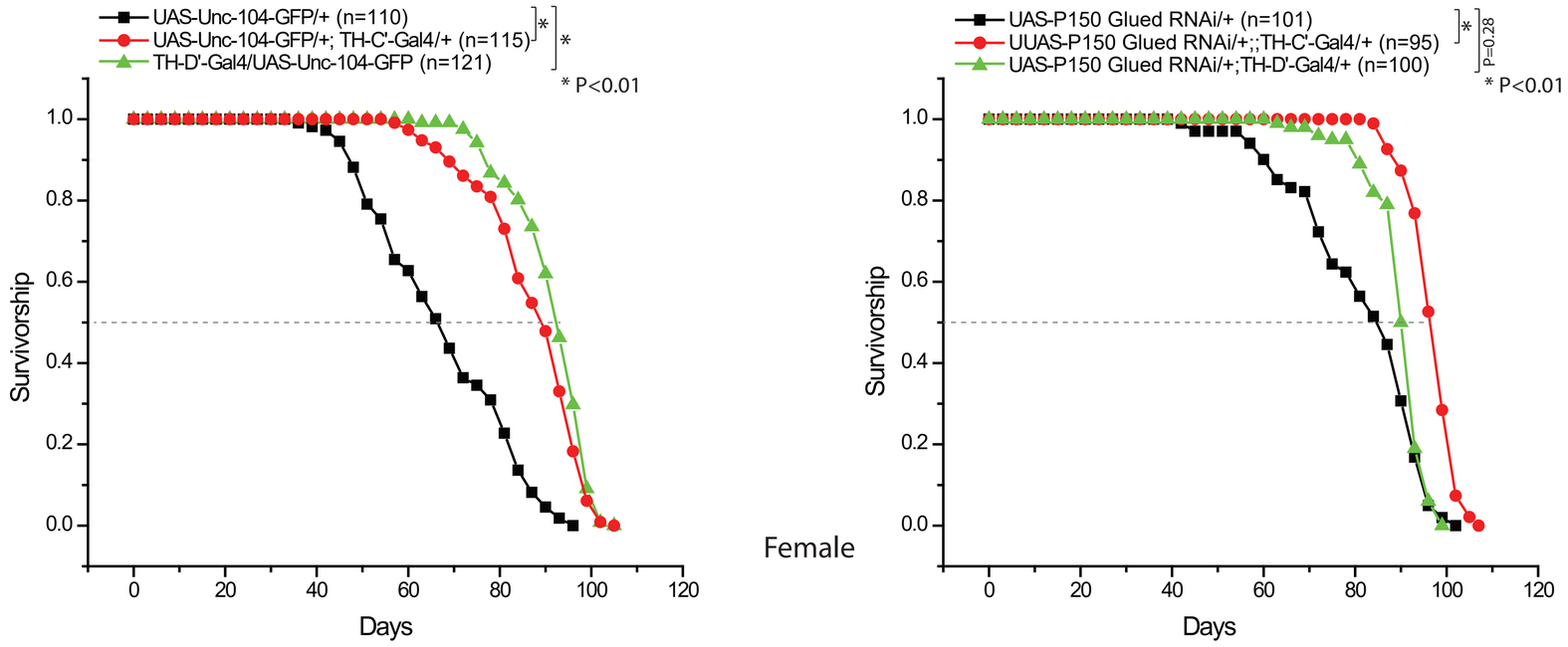

Male fecundity
(percentage of fertile males %)
100
80
60
40
20
0
n=10
n=10
n=10
n=10
n=30
n=30
n=31
n=30
n=20
n=17
n=20
n=17
n=20
n=20
n=19
n=19
1 Day
10 Days
30 Days
50 Days
Age

Figure 4


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
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
Female