

## **SUPPLEMENTARY INFORMATION**

**Supplementary Figures 1 – 31**

**Supplementary Table 1**

**Supplementary File 1**

### ***A C. elegans* model of *C9orf72*-associated ALS/FTD uncovers a conserved role for eIF2D in RAN translation**

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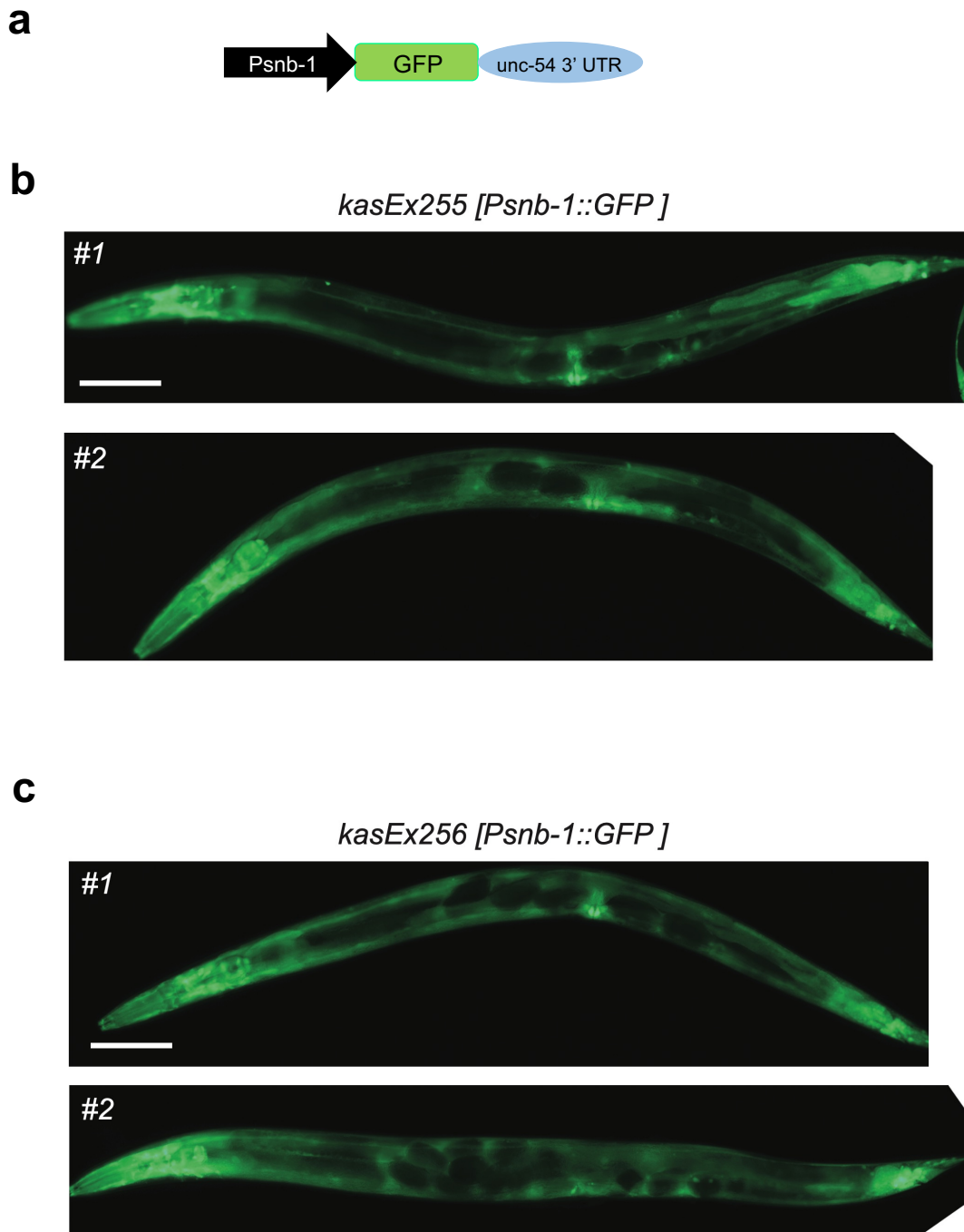
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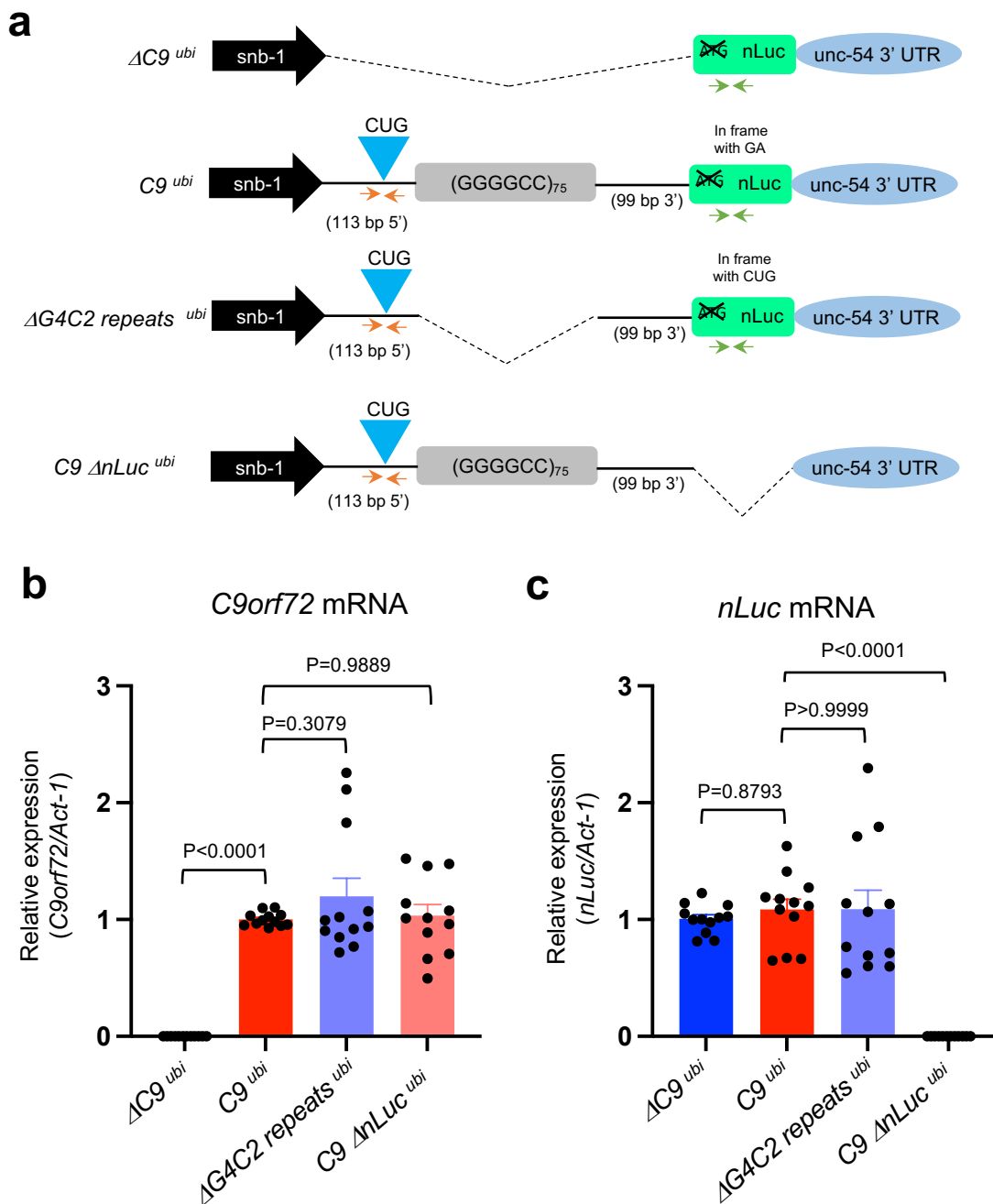
<sup>8</sup> Institute of Clinical Sciences, Imperial College London, London, UK

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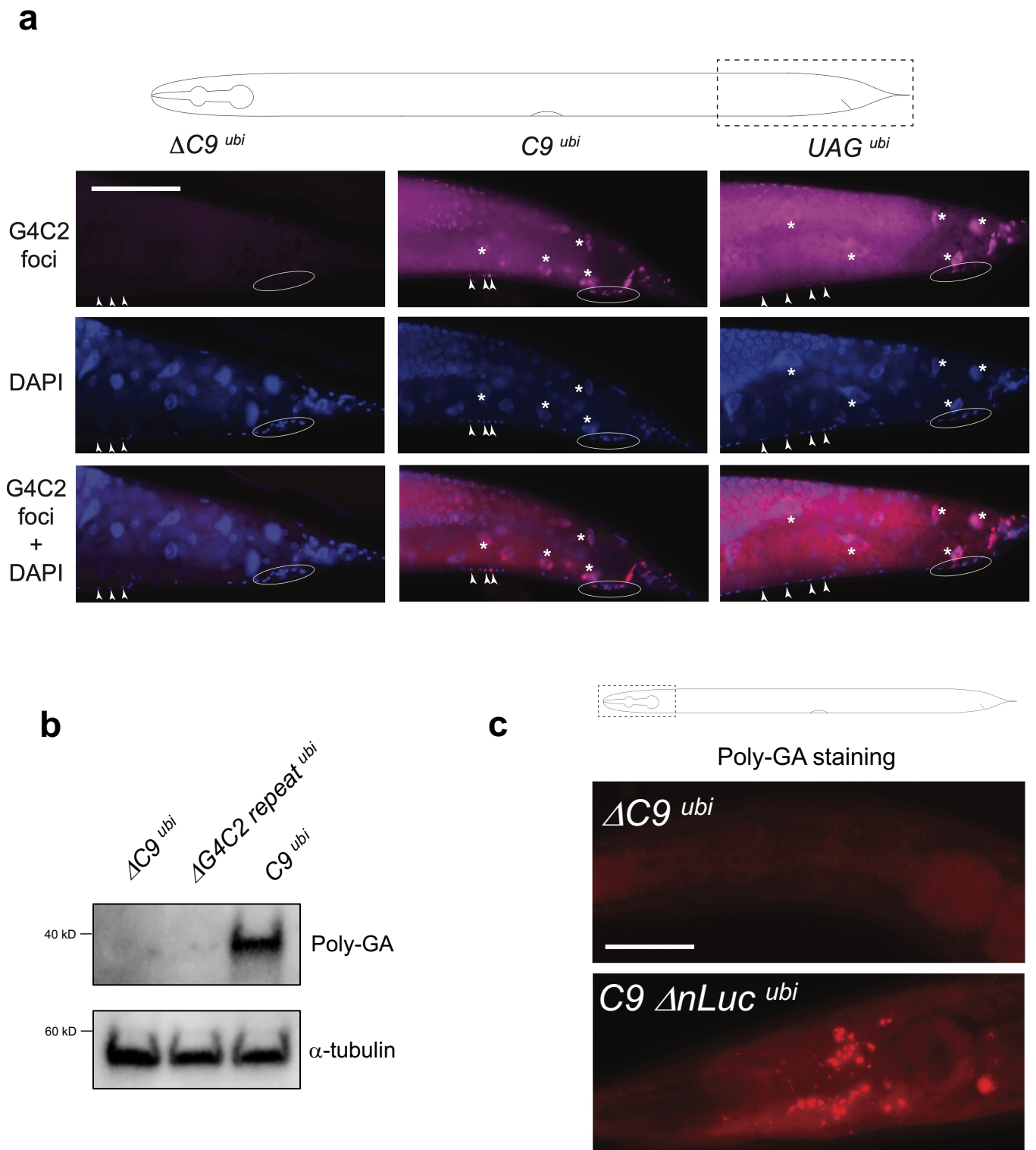
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**Supplementary Figure 1: The *snb-1* promoter drives broad GFP expression in *C. elegans*.** (a) Schematic of construct used in panels b and c. GFP: green fluorescent protein; UTR: untranslated region. (b) Representative images of two young adult (day 1) animals carrying the *kasEx255 [Psnb-1\_1094bp::GFP<sub>novo2</sub>+unc-54 3'UTR]* transgene. (c) Similar widespread GFP expression was observed with an independent transgenic line *kasEx256 [Psnb-1\_1094bp::GFP<sub>novo2</sub>+unc-54 3'UTR]*. Ten animals per line were imaged. No GFP expression is observed in the intestinal lumen and the embryos (oval shapes) inside the body of these hermaphrodite animals. Scale bar = 50 $\mu$ m.

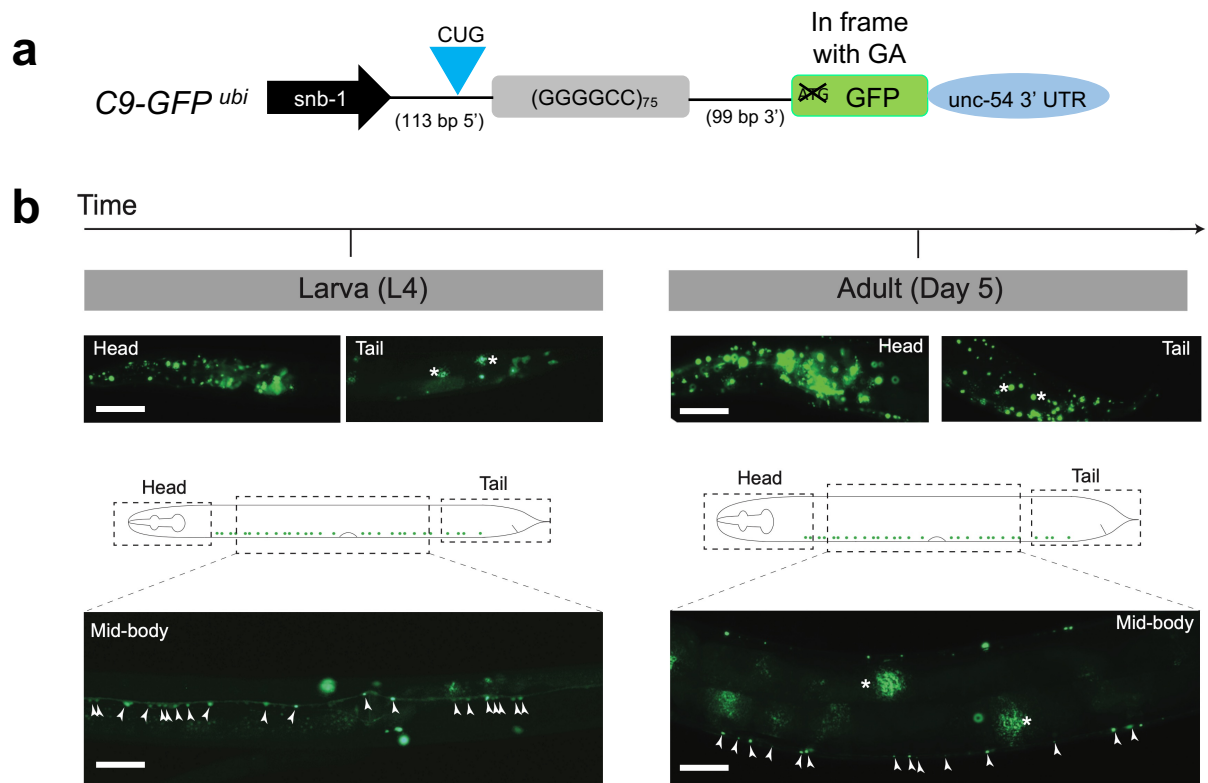


**Supplementary figure 2. Expression levels of *C9orf72* and *nLuc* mRNA in  $\Delta C9^{ubi}$ ,  $C9^{ubi}$ ,  $\Delta G4C2$  repeat  $ubi$ , and  $C9 \Delta nLuc^{ubi}$  worms.** (a) Schematic diagram showing  $\Delta C9^{ubi}$  (*KasEx154*),  $C9^{ubi}$  (*KasEx153*),  $\Delta G4C2$  repeat  $ubi$  (*KasEx240*), and  $C9 \Delta nLuc^{ubi}$  (*KasEx237*) constructs. The orange and green arrows primer location to detect intronic *C9orf72* and *nLuc* mRNAs, respectively. *nLuc*: nanoluciferase; UTR: untranslated region. (b) The *G4C2* repeat RNA (with surrounding *C9orf72* intronic RNA) and *Act-1* mRNAs were assessed by RT-PCR. (c) The *nLuc* and *Act-1* mRNAs were assessed by RT-PCR. The experiments used for (b) and (c) were repeated four times. The mean  $\pm$  s.e.m. is shown. One-way ANOVA with Dunnett's multiple comparisons test was performed.

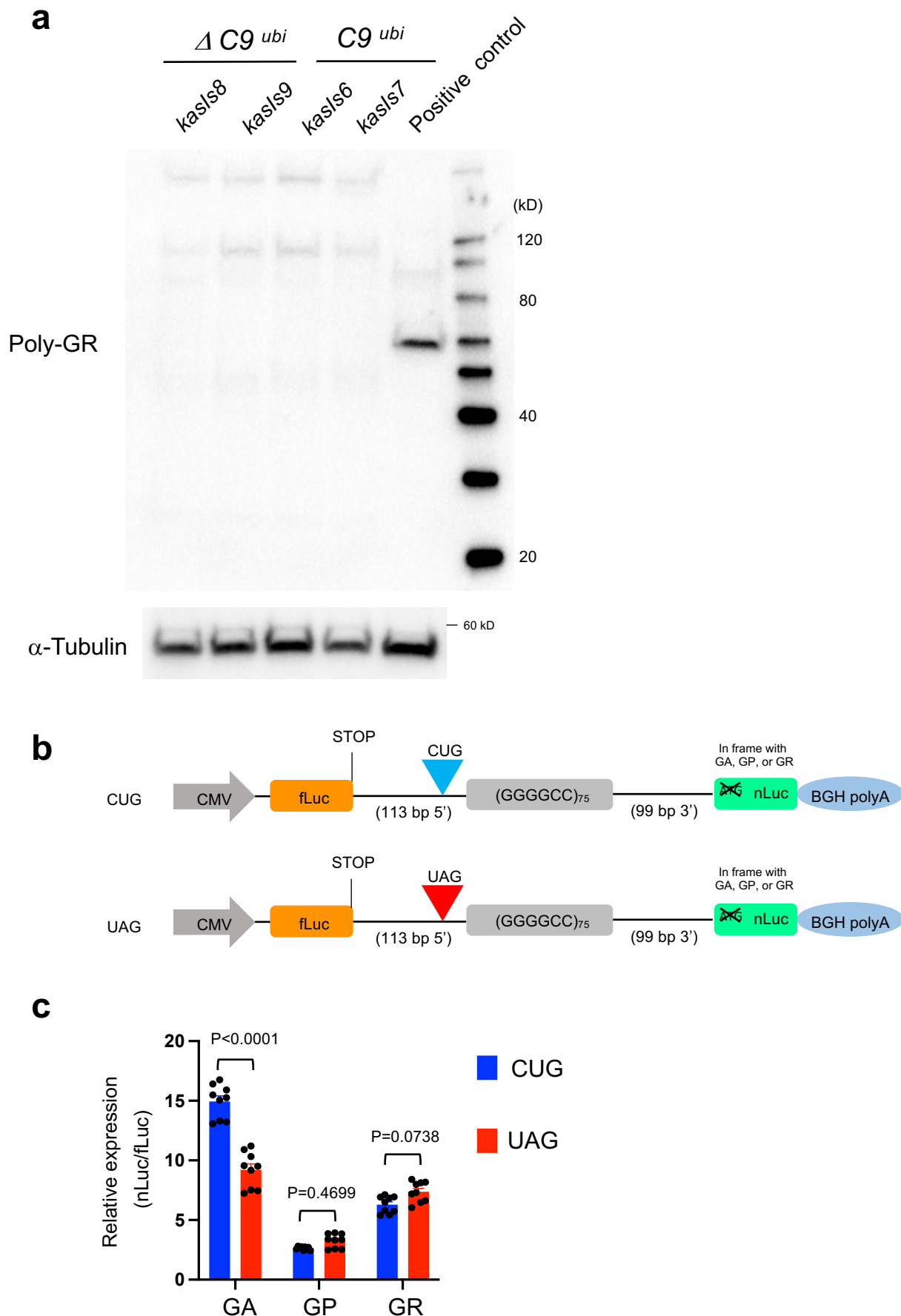


**Supplementary Figure 3: G4C2 RNA foci are detected in neuronal and non-neuronal cells of  $C9^{ubi}$  and  $UAG^{ubi}$  animals.** (a) Representative fluorescent images of the tail of adult (day 1)  $\Delta C9^{ubi}$  (*kasIs9*),  $C9^{ubi}$  (*kasIs7*) and  $UAG^{ubi}$  (*kasIs10*) animals. N = 25. G4C2 RNA foci are visualized with a Quasar 670 probe. Nuclei are demarcated with DAPI (blue). G4C2 RNA foci are detected in motor neurons of the ventral nerve cord (arrowheads) and the preanal ganglion (circled) both in  $C9^{ubi}$  and  $UAG^{ubi}$  animals. RNA foci are also detected in other cells, including intestinal cells (asterisks). (b) Lysates were processed for Western blotting from  $\Delta C9^{ubi}$ ,  $\Delta G4C2\ repeat^{ubi}$ , and  $C9^{ubi}$  worms and immunostained with poly-GA and  $\alpha$ -tubulin antibodies. Experiments were repeated 3 times. (c)  $\Delta C9^{ubi}$  (*KasEx154*) and  $C9\ \Delta nLuc^{ubi}$  (*KasEx237*) animals (day 1) were immunostained for poly-GA (red). Representative images of the head region taken at 40x magnification. N = 20. Anterior to the left. Scale bar = 25  $\mu$ m.

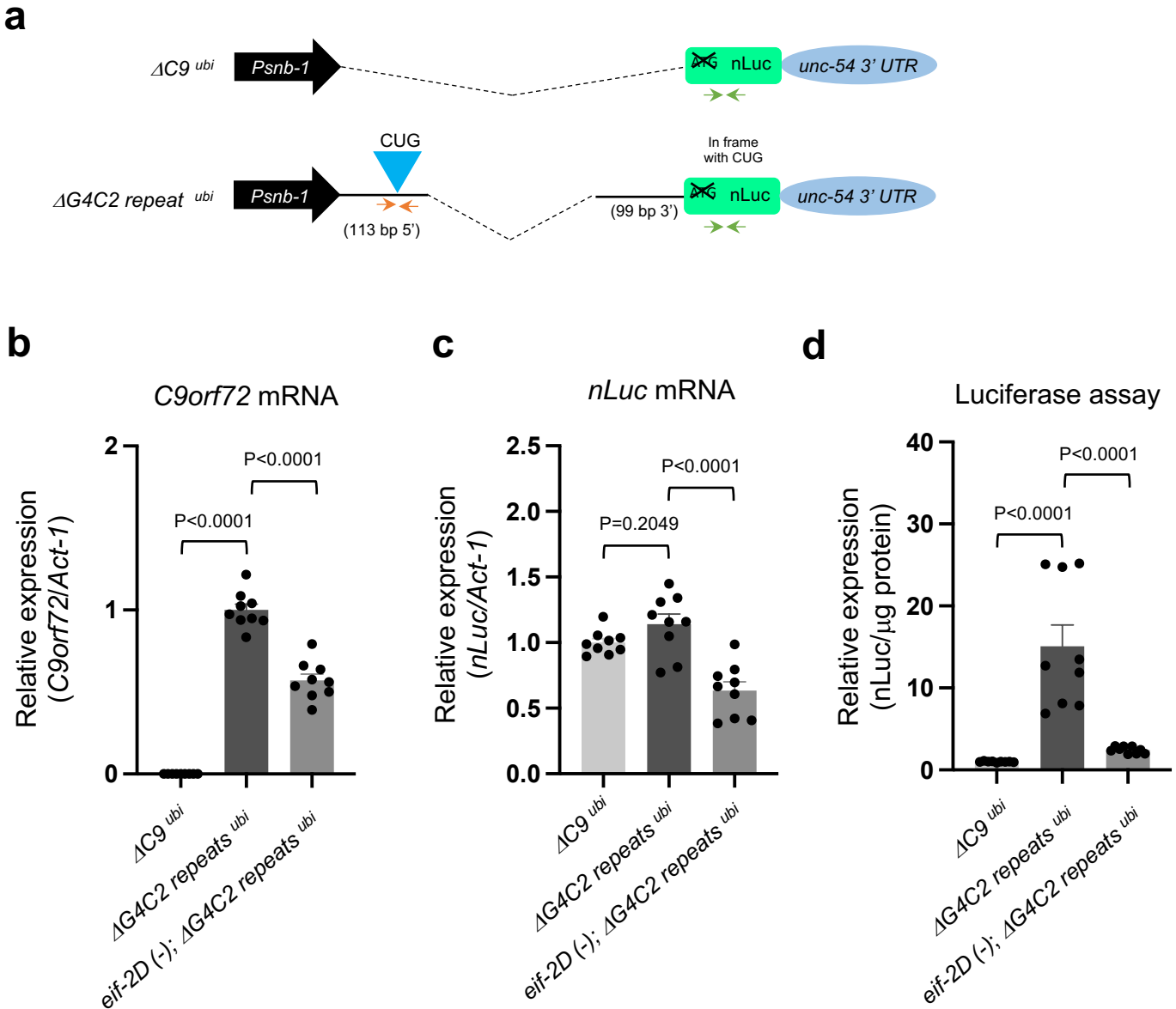




**Supplementary figure 4. Poly-GA accumulation over time and effect of UAG mutation on DPR production** (a) Schematic of construct used to generate the *C9-GFP<sup>ubi</sup>* animals. This is the same construct as *C9<sup>ubi</sup>* animals in Figure 1, but green fluorescent protein (GFP), not nLuc, is in poly-GA frame. UTR: untranslated region. (b) Representative images of head, tail and mid-body regions of *C9-GFP<sup>ubi</sup>* animals at larval (L4) and adult (day 5) stages. Aggregated GFP puncta (proxy for poly-GA expression) observed in head and tail neurons, as well as ventral nerve cord motor neurons (arrowheads). Aggregated GFP puncta were also present in non-neuronal cells, such as muscle and intestine (asterisks). GFP accumulation is more evident in adult (day 5) than L4 *C9-GFP<sup>ubi</sup>* animals. Two independent transgenic lines *kasEx247* [*Psnb-1::intron-75G4C2 repeats-intron-GFPnovo2*] and *kasEx252* [*Psnb-1::intron-75G4C2 repeats-intron-GFPnovo2*] were analyzed. N = 17 per line per time point. Scale bar = 25  $\mu$ m.



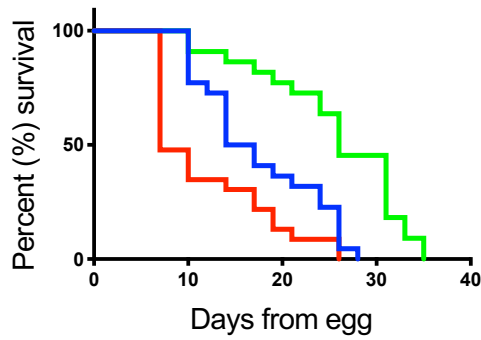
**Supplementary figure 5. Western blots to detect poly-GR in *C. elegans* and effect of CUG mutation on DPRs in HEK293 cells.** (a) The lysates from  $\Delta C9^{ubi}$  (*KasIs8*, *KasIs9*) and  $C9^{ubi}$  (*KasIs6*, *KasIs7*) worms were processed for Western blotting and immunostained with poly-GR and  $\alpha$ -tubulin antibodies. Positive control: HEK293 cells transfected with GR<sup>AUG</sup>(G4C2<sub>75</sub>-nLuc) plasmid, in which an AUG start codon was inserted before the G4C2 repeats and in the reading frame of poly-GR. (b-c) Bicistronic constructs including CUG or UAG were transfected into HEK 293 cells for 48h. (b) Schematic diagram showing bicistronic constructs. CMV: cytomegalovirus; fLuc: firefly luciferase; nLuc: nanoluciferase; BGH: bovine growth hormone. (c) The cell lysates were processed for luciferase assay. The levels of luciferase activity were assessed by dual luciferase assays. The experiments were repeated 3 times. mean  $\pm$  s.e.m. Two-way ANOVA with Šídák's multiple comparison test was performed.



**Supplementary figure 6. Analyses of nLuc mRNA and protein in *eif-2D* mutant animals carrying the  $\Delta G4C2$  repeat<sup>ubi</sup> transgene.** (a) Schematic diagram showing  $\Delta C9$ <sup>ubi</sup> (*KasEx154*) and  $\Delta G4C2$  repeat<sup>ubi</sup> (*KasEx240*) constructs. The orange and green arrows show primer location to detect *C9orf72* and nLuc mRNAs, respectively. nLuc: nanoluciferase; UTR: untranslated region. (b-c) Act-1, *C9orf72* (intronic) and nLuc mRNAs were assessed by RT-PCR. (d) A luciferase assay was performed on worm lysates. The *eif-2D* (*gk904876*) allele was used. Experiments were repeated 3 times. mean  $\pm$  s.e.m. One-way ANOVA with Dunnett's multiple comparisons test was performed.

■  $\Delta C9^{ubi}$  ■  $C9^{ubi}$  ■  $UAG^{ubi}$

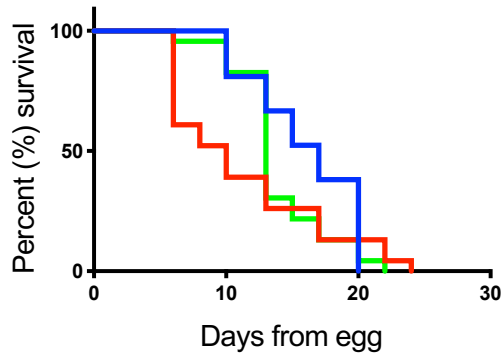
**a**



Replicate 1

|                        | $\Delta C9^{ubi}$ | $C9^{ubi}$ | $UAG^{ubi}$ |
|------------------------|-------------------|------------|-------------|
| Animals included       | 22                | 23         | 22          |
| Censored animals       | 3                 | 2          | 3           |
| Median Lifespan (Days) | 15.5              | 7          | 26          |

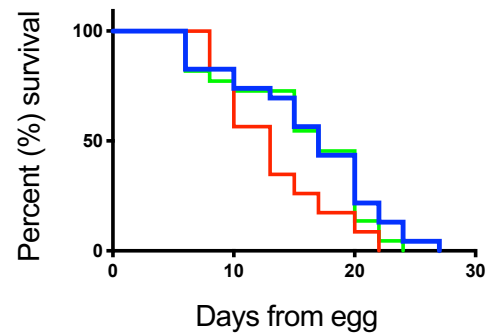
**b**



Replicate 2

|                        | $\Delta C9^{ubi}$ | $C9^{ubi}$ | $UAG^{ubi}$ |
|------------------------|-------------------|------------|-------------|
| Animals included       | 21                | 23         | 23          |
| Censored animals       | 4                 | 2          | 2           |
| Median Lifespan (Days) | 17                | 10         | 13          |

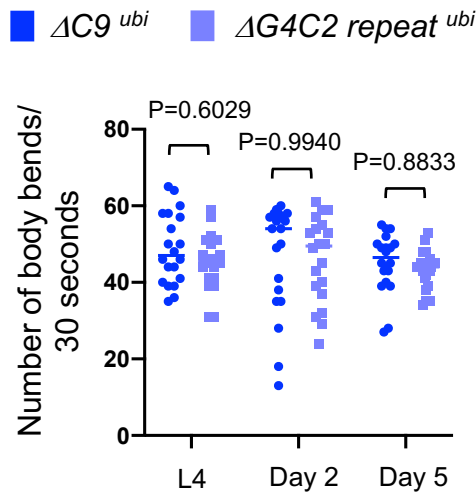
**c**



Replicate 3

|                        | $\Delta C9^{ubi}$ | $C9^{ubi}$ | $UAG^{ubi}$ |
|------------------------|-------------------|------------|-------------|
| Animals included       | 23                | 23         | 22          |
| Censored animals       | 2                 | 2          | 3           |
| Median Lifespan (Days) | 17                | 13         | 17          |

**d**



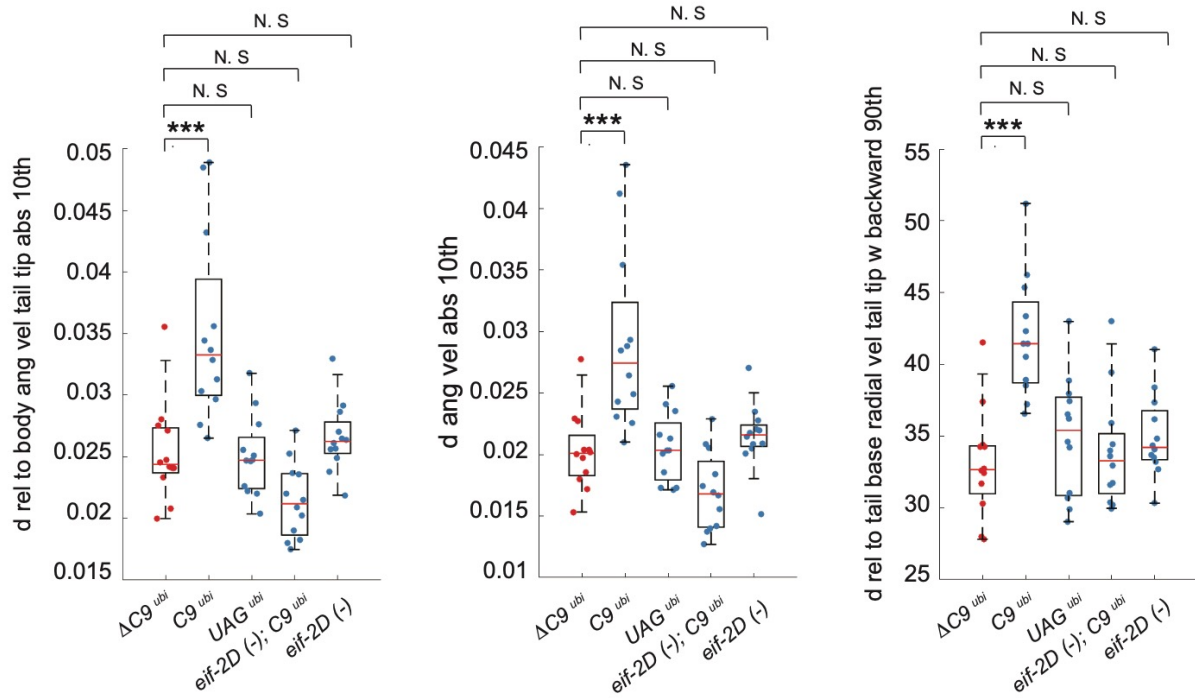
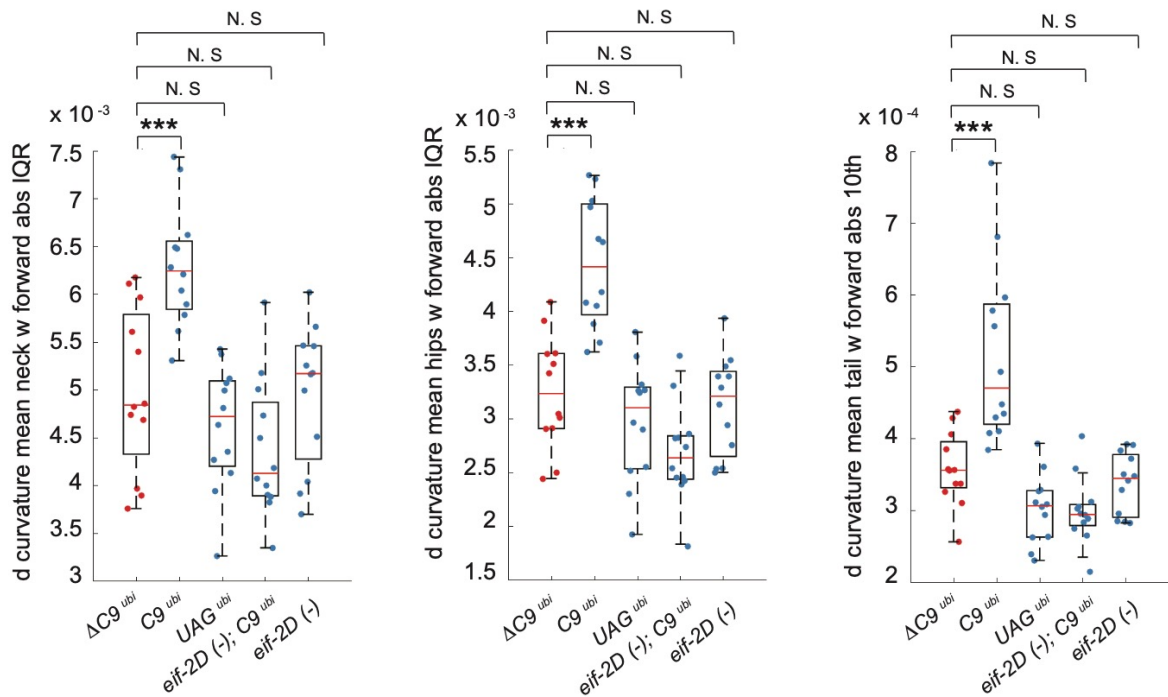
Summary of 3 replicates

|                        | $\Delta C9^{ubi}$ | $C9^{ubi}$ | $UAG^{ubi}$ |
|------------------------|-------------------|------------|-------------|
| Animals included       | 66                | 68         | 67          |
| Censored animals       | 9                 | 7          | 8           |
| Median Lifespan (Days) | 17                | 10         | 17          |

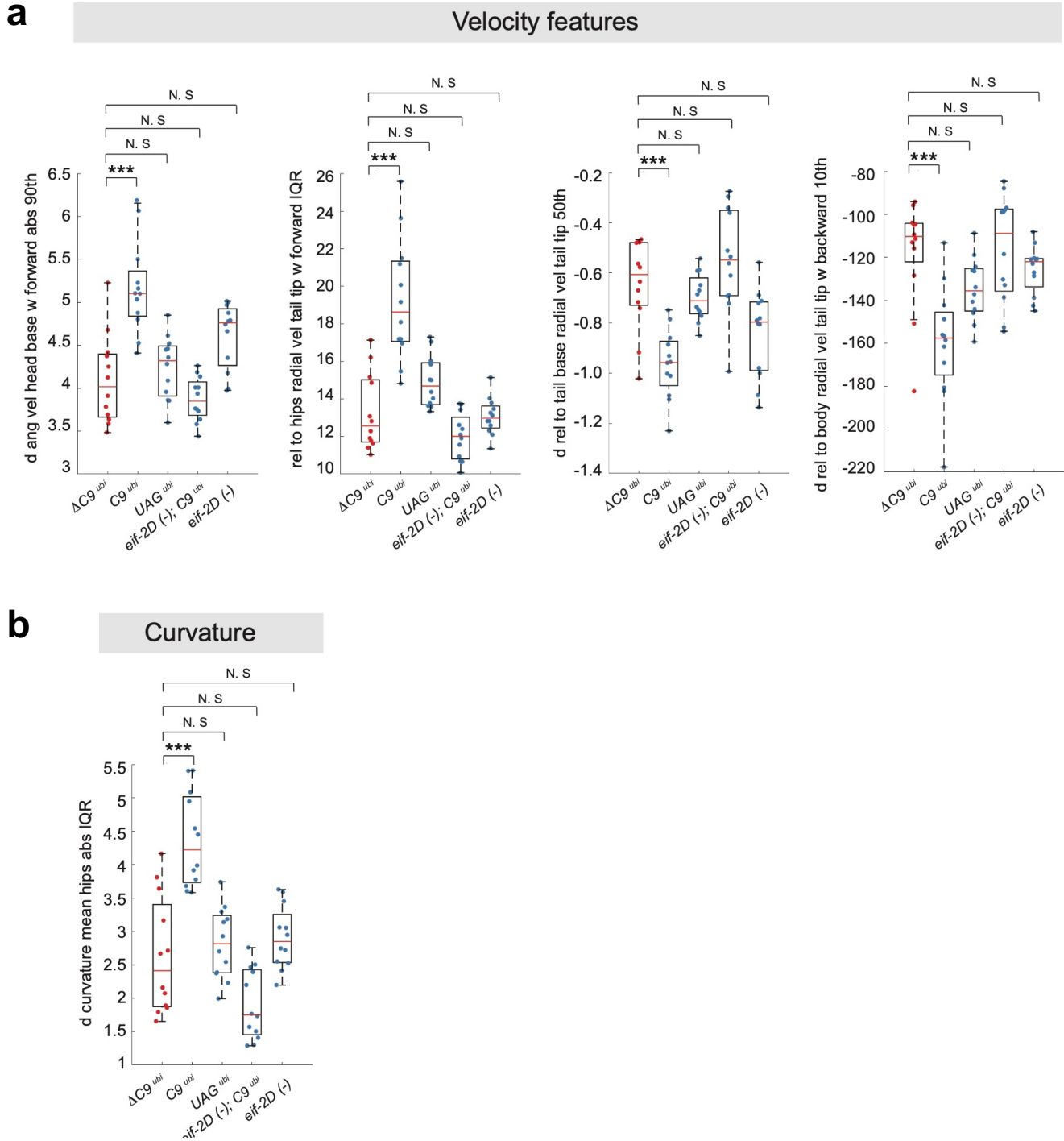
$\Delta C9^{ubi}$  vs  $C9^{ubi}$ ;  $P < 0.0001$

$C9^{ubi}$  vs  $UAG^{ubi}$ ;  $P < 0.0001$

**Supplementary figure 7. Replicates of the lifespan assay shown in Figure 2a.** (a-c) The survival graph and data tables for 3 replicates of the survival assay shown in Fig. 2a. The strains used in each replicate are as follows. Replicate 1:  $\Delta C9^{ubi}$  (*KasEx154*),  $C9^{ubi}$  (*kasEx261*),  $UAG^{ubi}$  (*KasEx264*); Replicate 2: ( $\Delta C9^{ubi}$  (*KasEx259*),  $C9^{ubi}$  (*KasEx262*),  $UAG^{ubi}$  (*KasEx155*), Replicate 3:  $\Delta C9^{ubi}$  (*KasEx260*),  $C9^{ubi}$  (*KasEx153*),  $UAG^{ubi}$  (*KasEx263*). (d) Rate of body flexion measure on  $\Delta C9^{ubi}$  and  $\Delta G4C2 repeat^{ubi}$  animals at the indicated time points. N=20. Two-way ANOVA with Šidák's multiple comparison test was performed.

**a****Velocity features****b****Curvature features****Supplementary figure 8. Genetic removal of *elf-2D/eIF2D* ameliorates the locomotor defects of  $C9^{ubi}$  animals.**

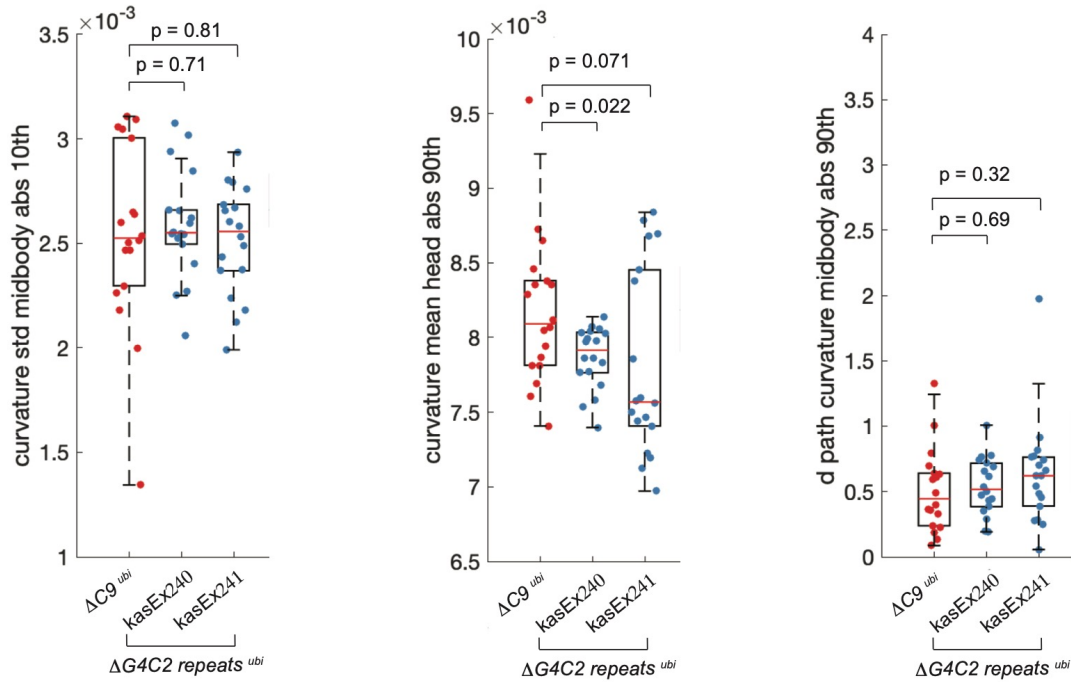
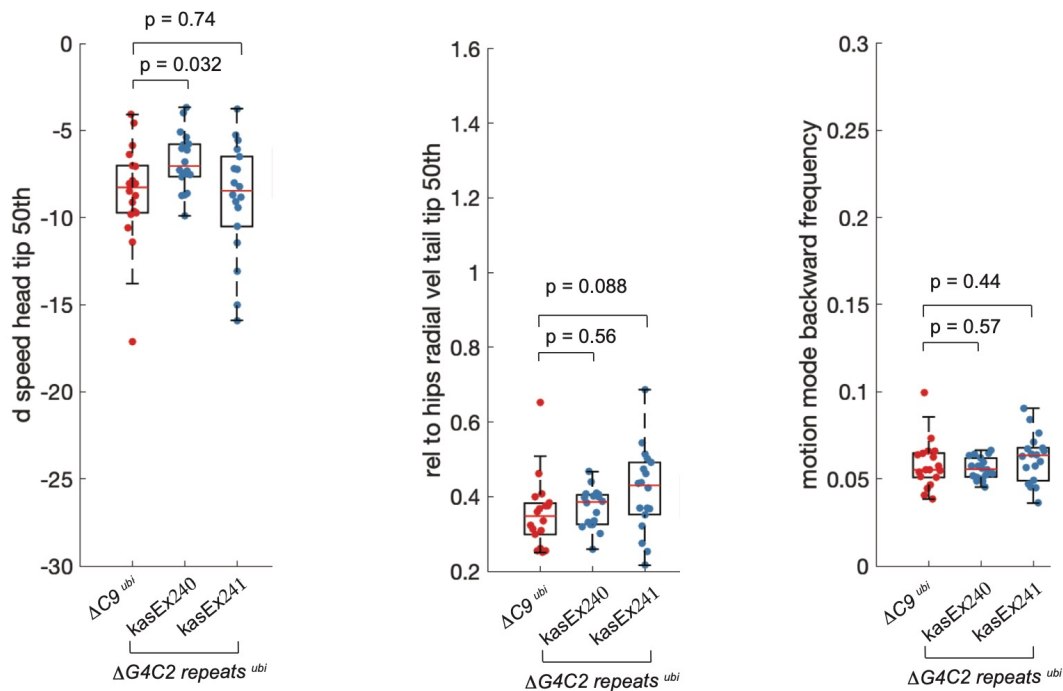
Examples of locomotion features significantly affected in  $C9^{ubi}$  animals. Genetic removal of *elf-2D* ameliorated the locomotion defects of  $C9^{ubi}$  animals [*elf-2D*(-);  $C9^{ubi}$ ]. Tracking analysis was performed at Day 2 adult animals (N = 12). Unpaired two-sided t-tests were performed. The threshold for significance was set using the Benjamini-Yekutieli procedure with a false discovery rate of 0.05. The p-value threshold for this analysis (FDR 5%) is 0.0014 (see Methods). The boxes show the median and the 25<sup>th</sup> and 75<sup>th</sup> percentiles. The whisker length is set to either 1.5 times the interquartile range or the distance to the maximum and minimum sample points, whichever is greater. (a) Three locomotion features related to animal velocity. *d rel to body ang vel tail tip abs 10th*: 10th percentile of the derivative of the absolute value of the angular velocity of the tip of the tail relative to the centroid of the mid-body points. *d ang vel abs 10th*: 10th percentile of the derivative of the absolute value of the angular velocity of the worm. *d rel to tail base radial vel tail tip w backward 90th*: 90th percentile of the derivative of the radial velocity of the tip of the tail relative to the centroid of the tail base points, while the worm is moving backwards. (b) Three locomotion features related to body curvature while the worm is moving forward. *d curvature mean neck w forward abs IQR*: interquartile range of the derivative of the absolute value of the mean curvature of the neck. *d curvature mean hips w forward abs IQR*: interquartile range of the derivative of the absolute value of the mean curvature of the hips. *d curvature mean tail w forward abs 10th*: 10th percentile of the derivative of the absolute value of the mean curvature of the tail. Lines:  $\Delta C9^{ubi}$  (*KasEx154*),  $C9^{ubi}$  (*KasEx153*),  $UAG^{ubi}$  (*KasEx155*).



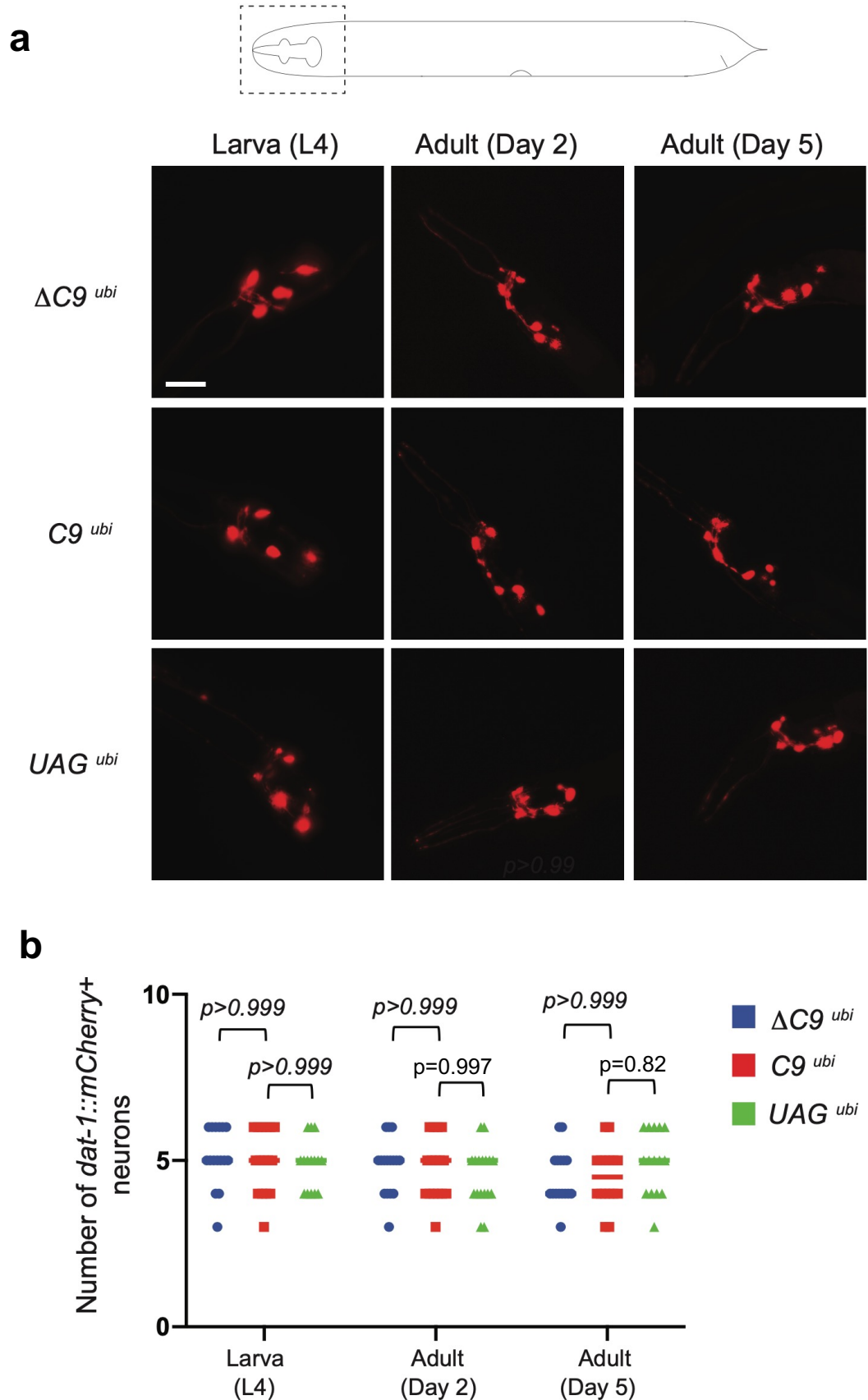
**Supplementary figure 9. Defects in velocity and body curvature are ameliorated in *C9<sup>ubi</sup>* animals upon genetic removal of *eif-2D/eIF2D*.**

Examples of seven additional locomotion features significantly affected in *C9<sup>ubi</sup>* animals. Genetic removal of *eif-2D* ameliorated the locomotion defects of *C9<sup>ubi</sup>* animals (*eif-2D*<sup>-/-</sup>; *C9<sup>ubi</sup>*). Tracking analysis was performed at Day 2 adult animals (N = 12). Unpaired two-sided t-tests were performed. The threshold for significance was set using the Benjamini-Yekutieli procedure with a false discovery rate of 0.05. The p-value threshold for this analysis (FDR 5%) is 0.0014 (see Methods). The boxes show the median and the 25<sup>th</sup> and 75<sup>th</sup> percentiles. The whisker length is set to either 1.5 times the interquartile range or the distance to the maximum and minimum sample points, whichever is greater. (a) Features related to animal velocity. *d ang vel head base w forward abs 90<sup>th</sup>*: 90th percentile of the derivative of the absolute value of the angular velocity of the base of the head, while the worm is moving forwards. *rel to hips radial vel tail tip w forward IQR*: interquartile range of the radial velocity of the tip of the head relative to the centroid of the hips points, while the worm is moving forwards. *d rel to tail base radial vel tail tip 50<sup>th</sup>*: 50th percentile of the derivative of the radial velocity of the tip of the tail relative to the centroid of the tail base points. *d rel to body radial vel tail tip w backward 10<sup>th</sup>*: 10th percentile of the derivative of radial velocity of the tip of the tail relative to the centroid of the midbody points, while the worm is moving backwards. (b) A feature related to body curvature. *d curvature mean hips abs IQR*: interquartile range of the derivative of the absolute value of the mean curvature of the hips. Lines:  $\Delta C9^{ubi}$  (*KasEx154*), *C9<sup>ubi</sup>* (*KasEx153*), *UAG<sup>ubi</sup>* (*KasEx155*).

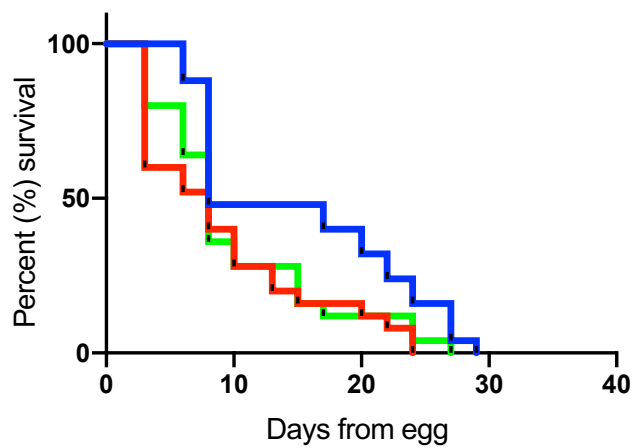
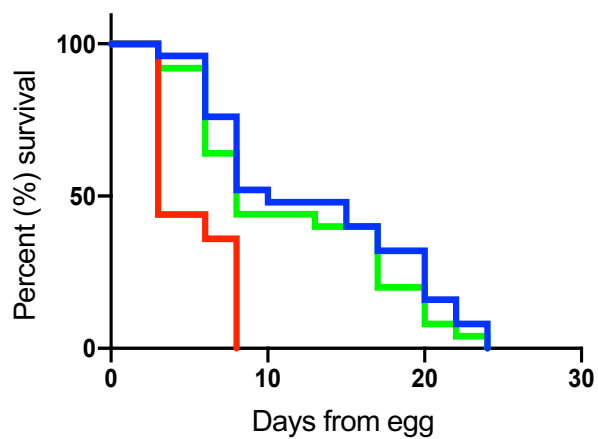
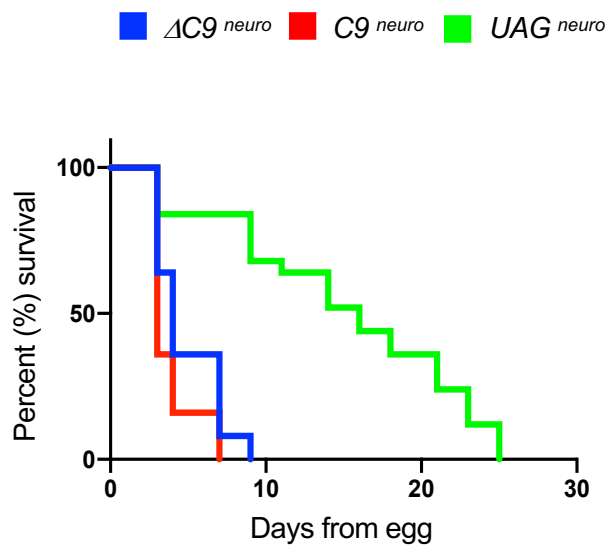


**a****Curvature features****b****Velocity features**

**Supplementary figure 10. Curvature and velocity features appear normal in  $\Delta C9^{ubi}$  and  $\Delta G4C2$  repeats  $ubi$  animals.** Examples of six locomotion features not affected in  $\Delta C9^{ubi}$  and  $\Delta G4C2$  repeats  $ubi$  animals. Tracking analysis was performed at Day 2 adult animals. Lines  $kasEx154$  ( $\Delta C9^{ubi}$ ) and  $kasEx240-1$  ( $\Delta G4C2$  repeats  $ubi$ ) were used. N = 15 animals per line. The boxes show the median and the 25<sup>th</sup> and 75<sup>th</sup> percentiles. The whisker length is set to either 1.5 times the interquartile range or the distance to the maximum and minimum sample points, whichever is greater. Unpaired two-sided t-tests were performed. The threshold for significance was set using the Benjamini-Yekutieli procedure with a false discovery rate of 0.05. The p-value threshold for this analysis (FDR 5%) is 0.0014 (see Methods). (a) Features related to body curvature. *curvature\_std\_midbody\_abs\_10th*: 10th percentile of the absolute value of the standard deviation of the curvature of the midbody. *curvature\_mean\_head\_abs\_90th*: 90th percentile of the absolute value of the mean curvature of the head. *d\_path\_curvature\_midbody\_abs\_90th*: 90th percentile of the derivative of the absolute value of the curvature of the path of the midbody. (b) Features related to velocity and frequency of movement. *d\_speed\_head\_tip\_50th*: 50th percentile of the derivative of the speed of the tip of the head. *rel\_to\_hips\_radial\_vel\_tail\_tip\_50th*: 50th percentile of the radial velocity of the tip of the head relative to the centroid of the hips points. *motion\_mode\_backward\_frequency*: frequency that a worm is moving backwards.



**Supplementary figure 11. The number of dopaminergic neurons is unaffected in  $C9^{ubi}$  animals.** (a) Representative images of the head of  $\Delta C9^{ubi}$  (*kasIs9*),  $C9^{ubi}$  (*kasIs7*) and  $UAG^{ubi}$  (*kasIs10*) animals carrying the dopaminergic marker *otIs181[dat-1::mCherry]*. (b) Quantification was performed at three different stages (L4, day 2, day 5). N= 16. Two-way ANOVA with Šidák's multiple comparison test was performed. Scale bar = 25  $\mu$ m.



$\Delta C9^{neuro}$  vs  $C9^{neuro}$ ;  $P < 0.0001$   
 $C9^{neuro}$  vs  $UAG^{neuro}$ ;  $P < 0.0001$

### Replicate 1

|                            | $\Delta C9^{neuro}$ | $C9^{neuro}$ | $UAG^{neuro}$ |
|----------------------------|---------------------|--------------|---------------|
| Number of animals included | 25                  | 25           | 25            |
| Number of censored animals | 3                   | 3            | 3             |
| Median lifespan (Days)     | 4                   | 3            | 16            |

### Replicate 2

|                            | $\Delta C9^{neuro}$ | $C9^{neuro}$ | $UAG^{neuro}$ |
|----------------------------|---------------------|--------------|---------------|
| Number of animals included | 25                  | 25           | 25            |
| Number of censored animals | 3                   | 3            | 3             |
| Median lifespan (Days)     | 10                  | 3            | 8             |

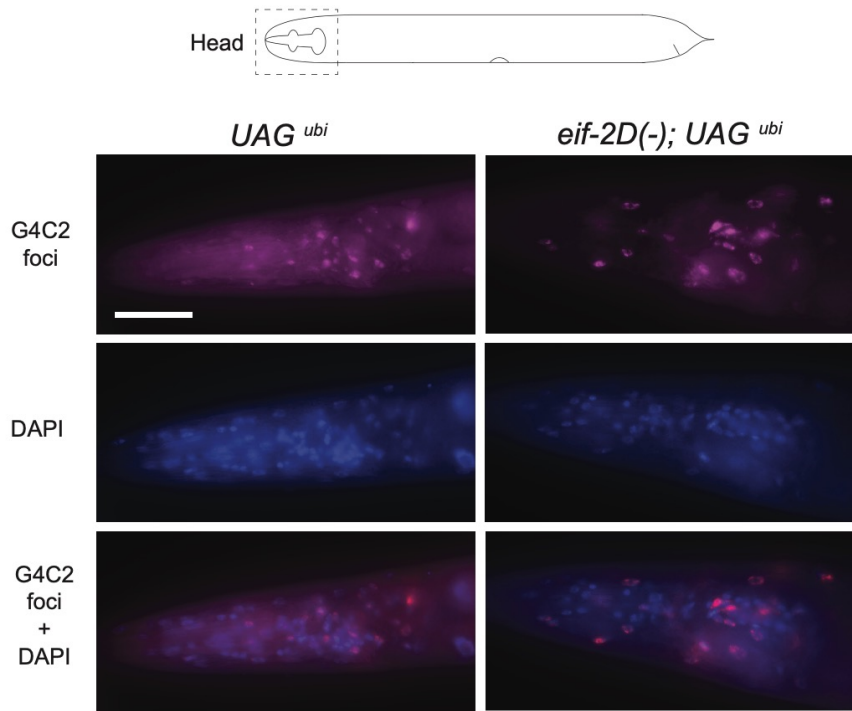
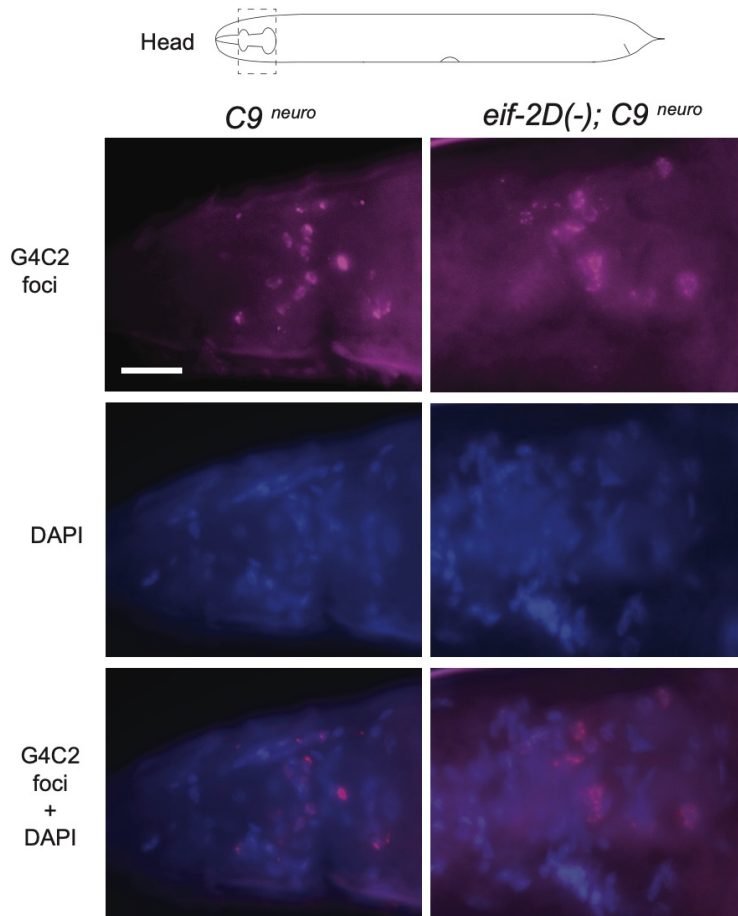
### Replicate 3

|                            | $\Delta C9^{neuro}$ | $C9^{neuro}$ | $UAG^{neuro}$ |
|----------------------------|---------------------|--------------|---------------|
| Number of animals included | 25                  | 25           | 25            |
| Number of censored animals | 3                   | 3            | 3             |
| Median lifespan (Days)     | 8                   | 8            | 8             |

### Summary of 3 replicates

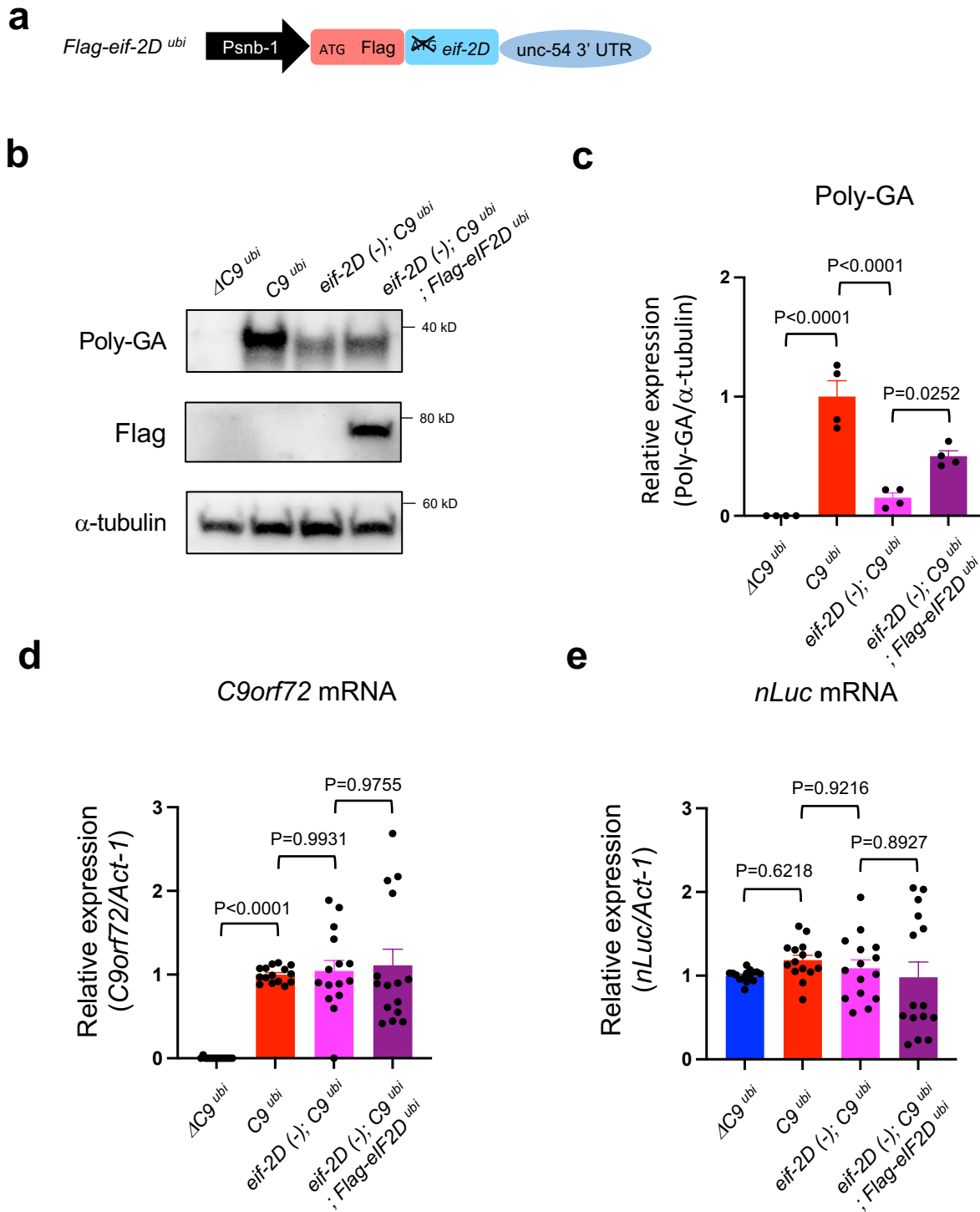
|                            | $\Delta C9^{neuro}$ | $C9^{neuro}$ | $UAG^{neuro}$ |
|----------------------------|---------------------|--------------|---------------|
| Number of animals included | 75                  | 75           | 75            |
| Number of censored animals | 9                   | 9            | 9             |
| Median lifespan (Days)     | 8                   | 3            | 9             |

**Supplementary figure 12. Replicates of the lifespan assay shown in Figure 3f.** The survival graph and data tables for 3 replicates shown in Fig. 3f. The strains used in each replicate were as follows: Replicate 1-3 ( $\Delta C9^{neuro}$ : *KasEx159*,  $C9^{neuro}$ : *KasEx157*,  $UAG^{neuro}$ : *KasEx160*). Mantel-Cox log rank test was performed.

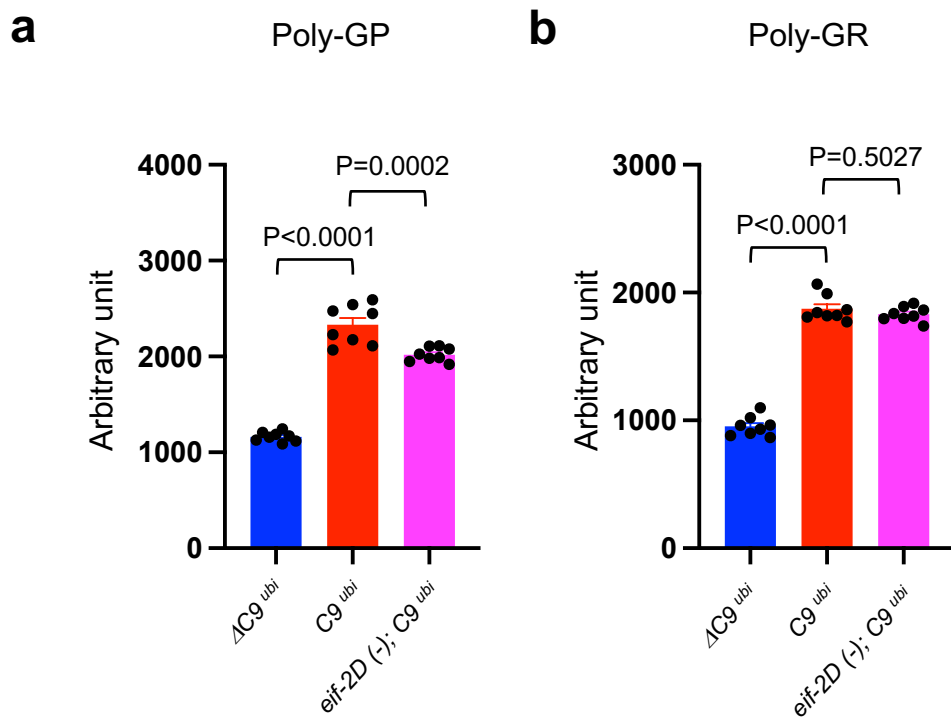
**a****b**

**Supplementary figure 13. G4C2 RNA foci are detected in cells of *eif-2D* mutant animals.**

(a) Representative fluorescent images of the head of adult (day 1) *UAG<sup>ubi</sup>* (*kasEx155*) and *eif-2D* (*gk904876*); *UAG<sup>ubi</sup>* (*kasEx155*) animals. N = 20. G4C2 RNA foci are visualized with a Quasar 670 probe. Nuclei are demarcated with DAPI (blue). Scale bar = 25μm. (b) Representative fluorescent images of the head of adult (day 1) *C9<sup>neuro</sup>* (*kasEx157*) and *eif-2D* (*gk904876*); *C9<sup>neuro</sup>* (*kasEx157*) animals. N = 20. G4C2 RNA foci are visualized with a Quasar 670 probe. Nuclei are demarcated with DAPI (blue). Scale bar = 10 μm.



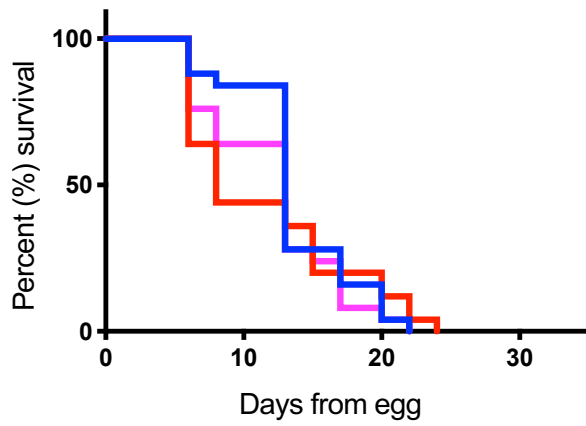
**Supplementary figure 14. Expression of *eif-2D* partially restores poly-GA expression in *eif-2D* (*gk904876*) mutant worms.** (a) Schematic diagram showing *Flag-eif-2D* construct used to generate *KasEx248* transgenic animals. (b) The worm lysates were processed for Western blotting. Representative Western blot immunostained with poly-GA and  $\alpha$ -tubulin antibodies. (c) Quantification of poly-GA on Western blots. The experiments were repeated 4 times. (d) The 75 G4C2 repeat RNA (with *C9orf72* intronic RNA) and Act-1 mRNA were assessed by RT-PCR. (e) The *nLuc* and Act-1 mRNAs were assessed by RT-PCR. The experiments were repeated 5 times. Mean  $\pm$  s.e.m. One-way ANOVA with Tukey's multiple comparisons test was performed. The *C9<sup>ubi</sup>* (*KasEx153*) line was used. nLuc: nanoluciferase; UTR: untranslated region.



**Supplementary figure 15. The level of poly-GP is reduced in *eif-2D* (*gk904876*) mutant worms.** The lysates from  $\Delta C9^{ubi}$ ,  $C9^{ubi}$  (*KasEx153*) and *eif-2D* ( $-/-$ );  $C9^{ubi}$  worms were processed for (a) poly-GP and (b) poly-GR ELISA. The experiments were repeated 4 times, mean  $\pm$  s.e.m. One-way ANOVA with Dunnett's multiple comparisons test was performed.

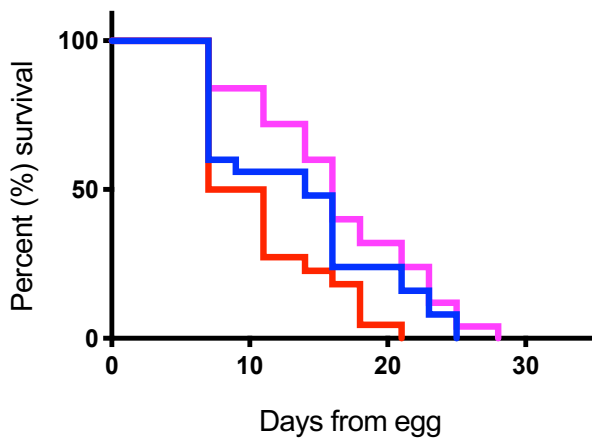


■  $\Delta C9^{ubi}$  ■  $C9^{ubi}$  ■  $eif-2D(-); C9^{ubi}$



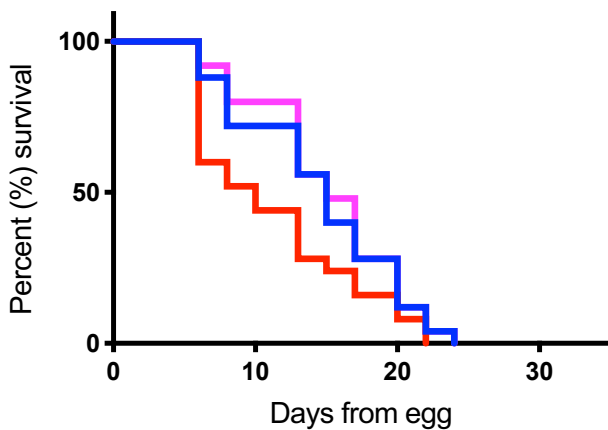
### Replicate 1

|                            | $\Delta C9^{ubi}$ | $C9^{ubi}$ | $eif-2D(-); C9^{ubi}$ |
|----------------------------|-------------------|------------|-----------------------|
| Number of animals included | 25                | 25         | 25                    |
| Number of censored animals | 2                 | 2          | 2                     |
| Median lifespan (Days)     | 13                | 8          | 13                    |



### Replicate 2

|                            | $\Delta C9^{ubi}$ | $C9^{ubi}$ | $eif-2D(-); C9^{ubi}$ |
|----------------------------|-------------------|------------|-----------------------|
| Number of animals included | 25                | 22         | 25                    |
| Number of censored animals | 2                 | 5          | 2                     |
| Median lifespan (Days)     | 13                | 8          | 13                    |



### Replicate 3

|                            | $\Delta C9^{ubi}$ | $C9^{ubi}$ | $eif-2D(-); C9^{ubi}$ |
|----------------------------|-------------------|------------|-----------------------|
| Number of animals included | 25                | 25         | 25                    |
| Number of censored animals | 2                 | 2          | 2                     |
| Median lifespan (Days)     | 15                | 10         | 15                    |

### Summary of 3 replicates

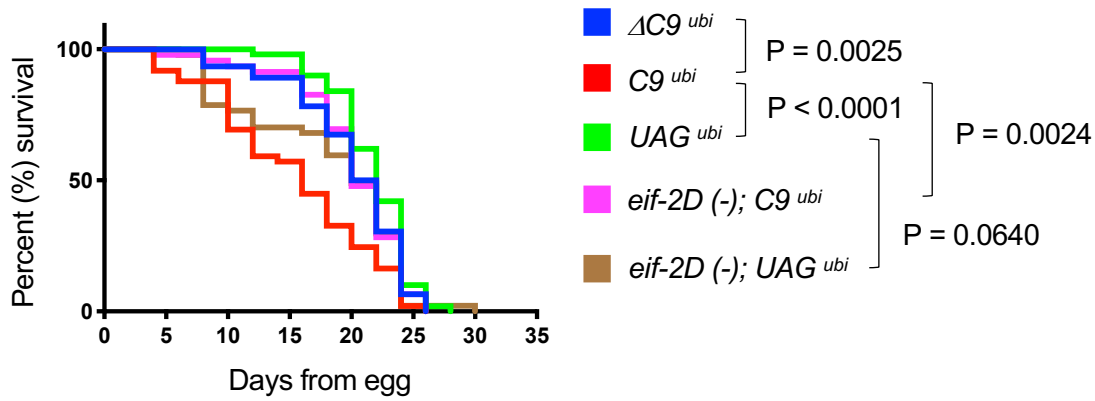
|                            | $\Delta C9^{ubi}$ | $C9^{ubi}$ | $eif-2D(-); C9^{ubi}$ |
|----------------------------|-------------------|------------|-----------------------|
| Number of animals included | 75                | 72         | 75                    |
| Number of censored animals | 6                 | 9          | 6                     |
| Median lifespan (Days)     | 13                | 8          | 14                    |

$\Delta C9^{ubi}$  vs  $C9^{ubi}$ ;  $P < 0.0079$

$C9^{ubi}$  vs  $eif-2D(-); C9^{ubi}$ ;  $P < 0.0017$

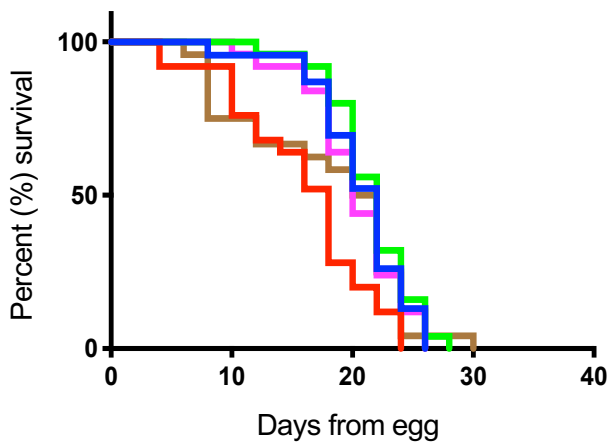
**Supplementary figure 16. Replicates of the lifespan assay shown in Figure 4e.** The survival graph and data tables for 3 replicates shown in Fig. 4e. The strains used in each replicate were as follows. Replicates 1-3:  $\Delta C9^{ubi}$  (*KasEx154*),  $C9^{ubi}$  (*kasEx262*). Mantel-Cox log rank test was performed.

## Summary of 2 replicates



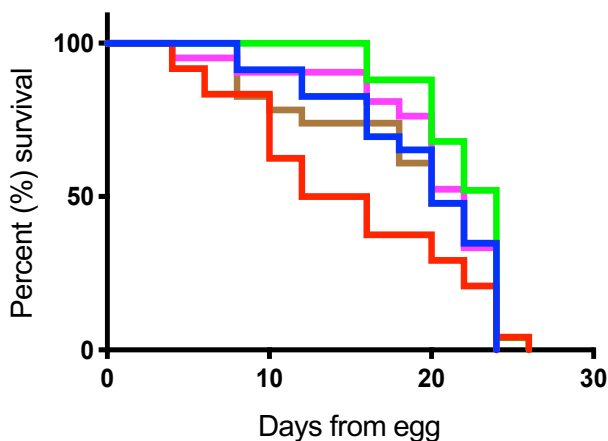
|                            | $\Delta C9^{ubi}$ | $C9^{ubi}$ | $UAG^{ubi}$ | $eif-2D (-); C9^{ubi}$ | $eif-2D (-); UAG^{ubi}$ |
|----------------------------|-------------------|------------|-------------|------------------------|-------------------------|
| Number of animals included | 46                | 49         | 50          | 46                     | 47                      |
| Number of censored animals | 4                 | 1          | 0           | 4                      | 3                       |
| Median lifespan (Days)     | 21                | 16         | 22          | 20                     | 20                      |

## Replicate 1



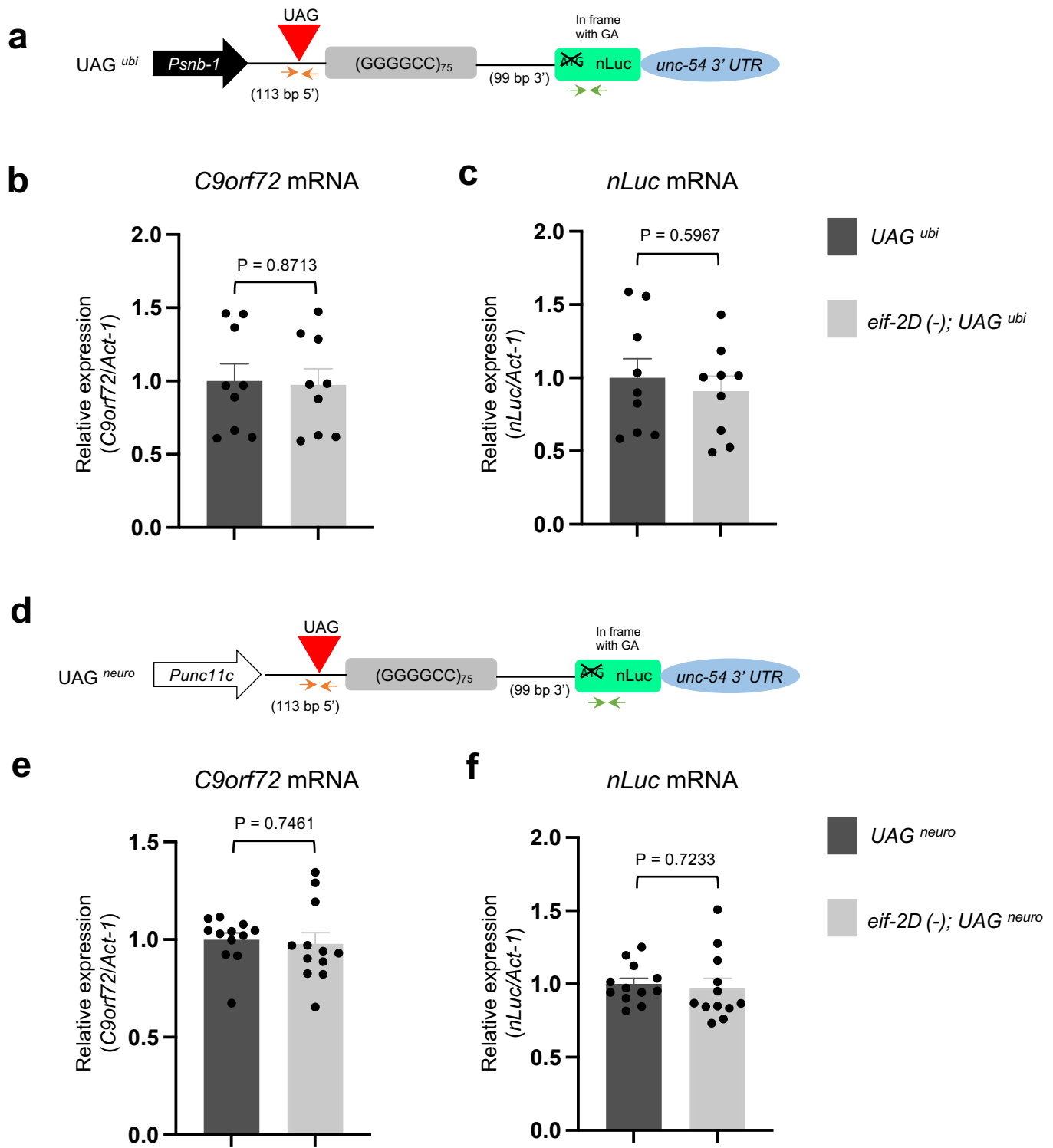
|                            | $\Delta C9^{ubi}$ | $C9^{ubi}$ | $UAG^{ubi}$ | $eif-2D (-); C9^{ubi}$ | $eif-2D (-); UAG^{ubi}$ |
|----------------------------|-------------------|------------|-------------|------------------------|-------------------------|
| Number of animals included | 23                | 25         | 25          | 25                     | 24                      |
| Number of censored animals | 2                 | 0          | 0           | 0                      | 1                       |
| Median lifespan (Days)     | 22                | 18         | 22          | 20                     | 21                      |

## Replicate 2

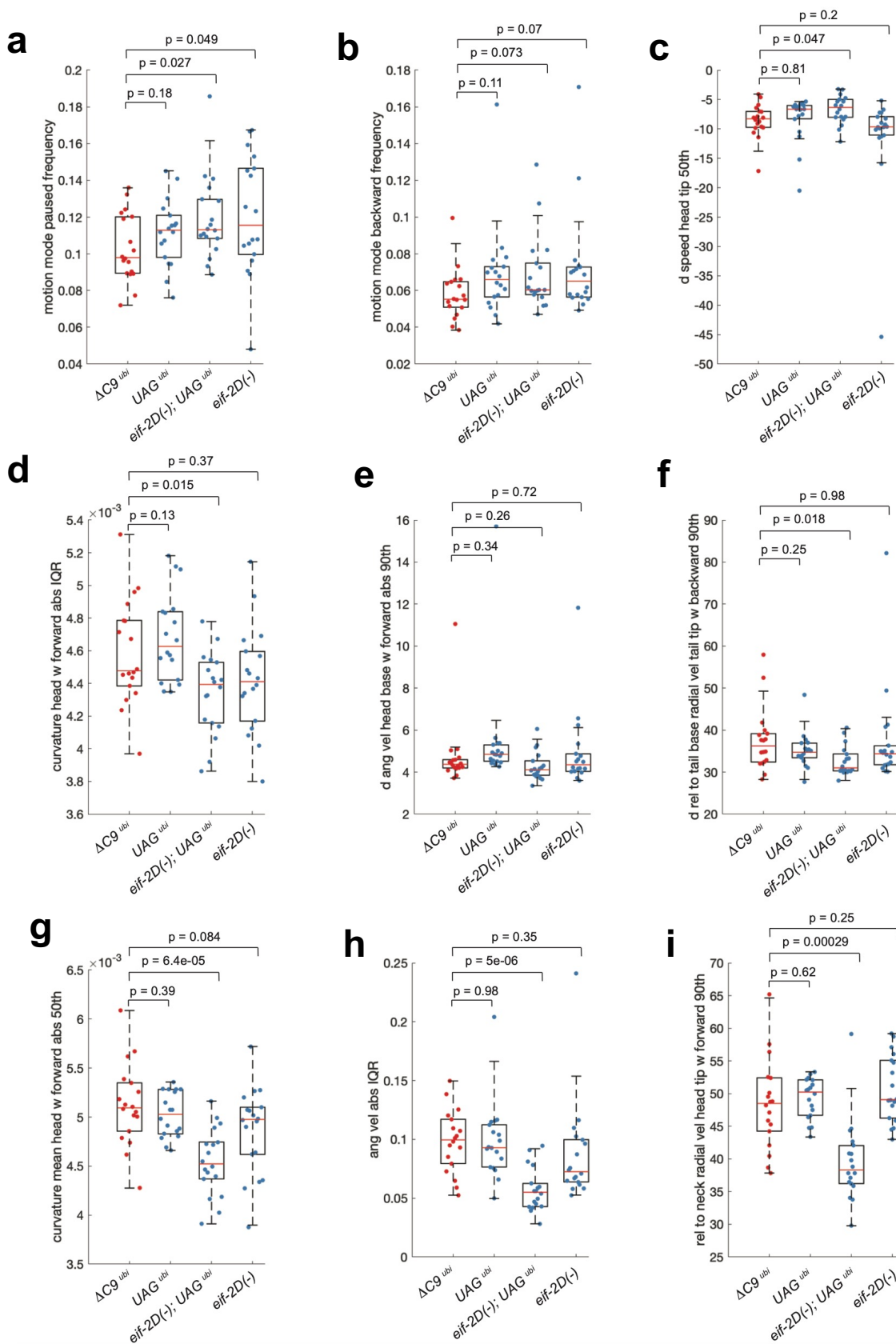


|                            | $\Delta C9^{ubi}$ | $C9^{ubi}$ | $UAG^{ubi}$ | $eif-2D (-); C9^{ubi}$ | $eif-2D (-); UAG^{ubi}$ |
|----------------------------|-------------------|------------|-------------|------------------------|-------------------------|
| Number of animals included | 23                | 24         | 25          | 21                     | 23                      |
| Number of censored animals | 2                 | 1          | 0           | 4                      | 2                       |
| Median lifespan (Days)     | 20                | 14         | 24          | 22                     | 20                      |

**Supplementary figure 17.  $C9^{ubi}$  animals display a significantly shorter lifespan in assays without FUDR.** The lifespan assays and data tables for  $\Delta C9^{ubi}$  (*KasEx154*),  $C9^{ubi}$  (*KasEx153*),  $UAG^{ubi}$  (*KasEx155*),  $eif-2D (-); C9^{ubi}$  and  $eif-2D (-); UAG^{ubi}$  animals are shown. The experiments were repeated twice. Mantel-Cox log rank test was performed. Details for the individual experiments are shown in lower panels.



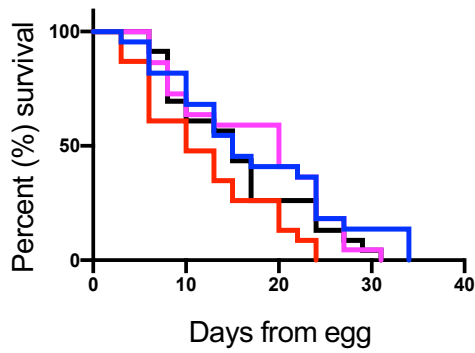
**Supplementary figure 18. Knockout of *eif-2D* does not affect the levels of *C9orf72* intronic RNA and *nLuc* mRNAs in both *UAG<sup>ubi</sup>* and *UAG<sup>neuro</sup>* animals.** (a, d) Schematic diagram showing UAG constructs. The orange and green arrows show the location of primers designed to detect *C9orf72* and *nLuc* mRNAs, respectively. *nLuc*: nanoluciferase; UTR: untranslated region. The *C9orf72* intronic RNA (b, e) and *nLuc* mRNAs (c, f) were assessed by RT-PCR and normalized to *Act-1* mRNA. The experiments in b-c were repeated 3 times, and the experiments in e-f were repeated 4 times. Mean  $\pm$  s.e.m. Two-tailed unpaired t-test was performed. Lines used: *UAG<sup>ubi</sup>* (*KasEx155*), *UAG<sup>neuro</sup>* (*KasEx161*).



**Supplementary figure 19. Locomotion features appear normal in  $\Delta C9^{ubi}$ ,  $UAG^{ubi}$ ,  $eif-2D(gk904876); UAG^{ubi}$  and  $eif-2D(gk904876)$  animals.** Examples of nine locomotion features (a-i) assessed in  $\Delta C9^{ubi}$ ,  $UAG^{ubi}$ ,  $eif-2D(-); UAG^{ubi}$ , and  $eif-2D(-)$  animals. Tracking analysis was performed at Day 2 adult animals. Transgenic lines *kasEx154* ( $\Delta C9^{ubi}$ ) and *kasEx155* ( $UAG^{ubi}$ ) were used. N = 15 animals per line. Unpaired two-sided t-tests were performed. The threshold for significance was set using the Benjamini-Yekutieli procedure with a false discovery rate of 0.05. The p-value threshold (after correcting for multiple comparisons) is 0.00096. The boxes show the median and the 25<sup>th</sup> and 75<sup>th</sup> percentiles. The whisker length is set to either 1.5 times the interquartile range or the distance to the maximum and minimum sample points, whichever is greater. The following locomotion features are shown from top left to bottom right: *motion mode paused frequency*: frequency that a worm is paused (not moving); *motion mode backward frequency*: frequency that a worm is moving backwards; *d speed head tip 50th*: 50th percentile of the derivative of the speed of the tip of the head; *curvature head w forward abs IQR*: interquartile range of the absolute value of the curvature of the head, while the worm is moving forwards; *d ang vel head base w forward abs 90th*: 90th percentile of the derivative of the absolute value of the angular velocity of the base of the head, while the worm is moving forwards; *d rel to tail base radial vel tail tip w backward 90th*: 90th percentile of the derivative of the radial velocity of the tip of the tail relative to the centroid of the tail base points, while the worm is moving backwards; *curvature mean head w forward abs 50th*: 50th percentile of the absolute value of the mean curvature of the head, while the worm is moving forwards; *ang vel abs IQR*: interquartile range of the absolute value of the angular velocity of the worm; *rel to neck radial vel head tip w forward 90th*: 90th percentile of the radial velocity of the tip of the head relative to the centroid of the neck points, while the worm is moving forwards. Lines used:  $\Delta C9^{ubi}$  (*KasEx154*),  $C9^{ubi}$  (*KasEx155*).

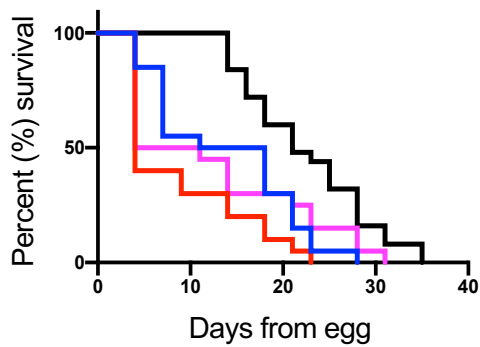
■  $\Delta C9$  *neuro*    ■ *C9 neuro*    ■ *eif-2D (-); C9 neuro*  
■ *eif-2D (-)*

### Replicate 1



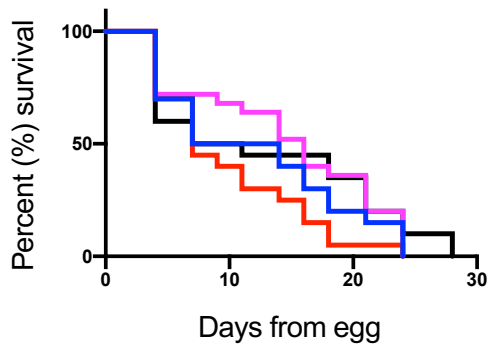
|                            | $\Delta C9$ <i>neuro</i> | <i>C9 neuro</i> | <i>eif-2D (-); C9 neuro</i> | <i>eif-2D (-)</i> |
|----------------------------|--------------------------|-----------------|-----------------------------|-------------------|
| Number of animals included | 22                       | 23              | 22                          | 23                |
| Number of censored animals | 3                        | 2               | 3                           | 2                 |
| Median lifespan (Days)     | 15                       | 10              | 20                          | 15                |

### Replicate 2



|                            | $\Delta C9$ <i>neuro</i> | <i>C9 neuro</i> | <i>eif-2D (-); C9 neuro</i> | <i>eif-2D (-)</i> |
|----------------------------|--------------------------|-----------------|-----------------------------|-------------------|
| Number of animals included | 20                       | 20              | 20                          | 25                |
| Number of censored animals | 5                        | 5               | 5                           | 0                 |
| Median lifespan (Days)     | 14.5                     | 4               | 7.5                         | 21                |

### Replicate 3



|                            | $\Delta C9$ <i>neuro</i> | <i>C9 neuro</i> | <i>eif-2D (-); C9 neuro</i> | <i>eif-2D (-)</i> |
|----------------------------|--------------------------|-----------------|-----------------------------|-------------------|
| Number of animals included | 20                       | 20              | 25                          | 20                |
| Number of censored animals | 5                        | 5               | 0                           | 5                 |
| Median lifespan (Days)     | 10.5                     | 7               | 16                          | 9                 |

### Summary of 3 replicates

|                            | $\Delta C9$ <i>neuro</i> | <i>C9 neuro</i> | <i>eif-2D (-); C9 neuro</i> | <i>eif-2D (-)</i> |
|----------------------------|--------------------------|-----------------|-----------------------------|-------------------|
| Number of animals included | 62                       | 63              | 67                          | 68                |
| Number of censored animals | 13                       | 12              | 8                           | 7                 |
| Median lifespan (Days)     | 14                       | 7               | 14                          | 17                |

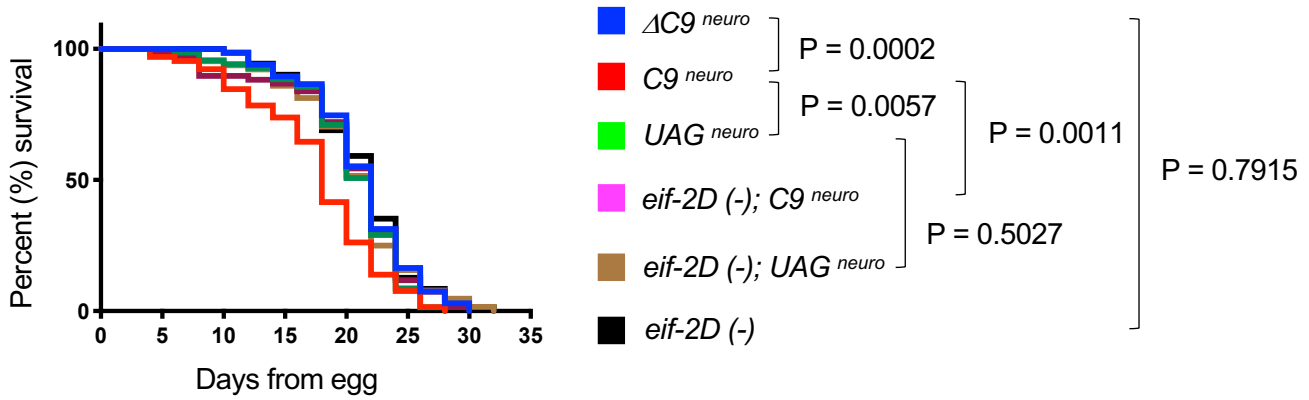
$\Delta C9$  *neuro* vs *C9 neuro*;  $P=0.0009$

*C9 neuro* vs *eif-2D (-); C9 neuro*;  $P=0.0001$

$\Delta C9$  *neuro* vs *eif-2D (-)*;  $P=0.0678$

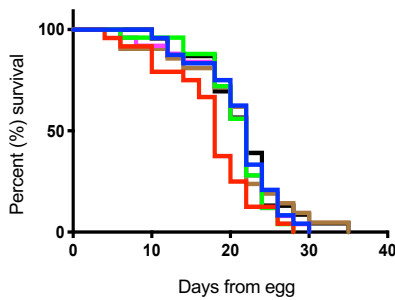
**Supplementary figure 20. Replicates of the lifespan assay shown in Figure 4j.** The survival graph and data tables for 3 replicates shown in Fig. 4j. The strains used in each replicate were as follows. Replicate 1-3:  $\Delta C9$  *neuro* (*KasEx159*), *C9 neuro* (*KasEx157*). Mantel-Cox log rank test was performed.

## Summary of 3 replicates



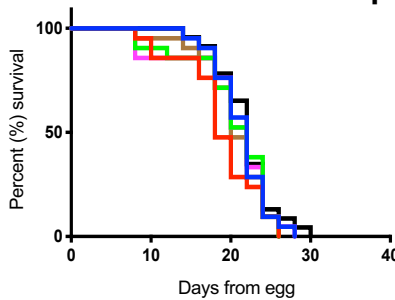
|                            | $\Delta C9^{neuro}$ | $C9^{neuro}$ | $UAG^{neuro}$ | $eif-2D^{-}; C9^{neuro}$ | $eif-2D^{-}; UAG^{neuro}$ | $eif-2D^{-}$ |
|----------------------------|---------------------|--------------|---------------|--------------------------|---------------------------|--------------|
| Number of animals included | 67                  | 65           | 69            | 68                       | 64                        | 71           |
| Number of censored animals | 8                   | 10           | 6             | 7                        | 11                        | 4            |
| Median lifespan (Days)     | 22                  | 18           | 22            | 22                       | 22                        | 22           |

### Replicate 1



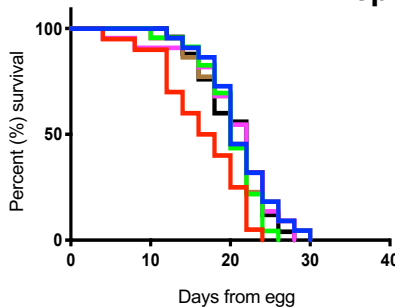
|                            | $\Delta C9^{neuro}$ | $C9^{neuro}$ | $UAG^{neuro}$ | $eif-2D^{-}; C9^{neuro}$ | $eif-2D^{-}; UAG^{neuro}$ | $eif-2D^{-}$ |
|----------------------------|---------------------|--------------|---------------|--------------------------|---------------------------|--------------|
| Number of animals included | 24                  | 24           | 25            | 25                       | 21                        | 23           |
| Number of censored animals | 1                   | 1            | 0             | 0                        | 4                         | 2            |
| Median lifespan (Days)     | 22                  | 18           | 22            | 22                       | 22                        | 22           |

### Replicate 2



|                            | $\Delta C9^{neuro}$ | $C9^{neuro}$ | $UAG^{neuro}$ | $eif-2D^{-}; C9^{neuro}$ | $eif-2D^{-}; UAG^{neuro}$ | $eif-2D^{-}$ |
|----------------------------|---------------------|--------------|---------------|--------------------------|---------------------------|--------------|
| Number of animals included | 21                  | 21           | 21            | 21                       | 21                        | 23           |
| Number of censored animals | 4                   | 4            | 4             | 4                        | 4                         | 2            |
| Median lifespan (Days)     | 22                  | 18           | 22            | 22                       | 20                        | 22           |

### Replicate 3

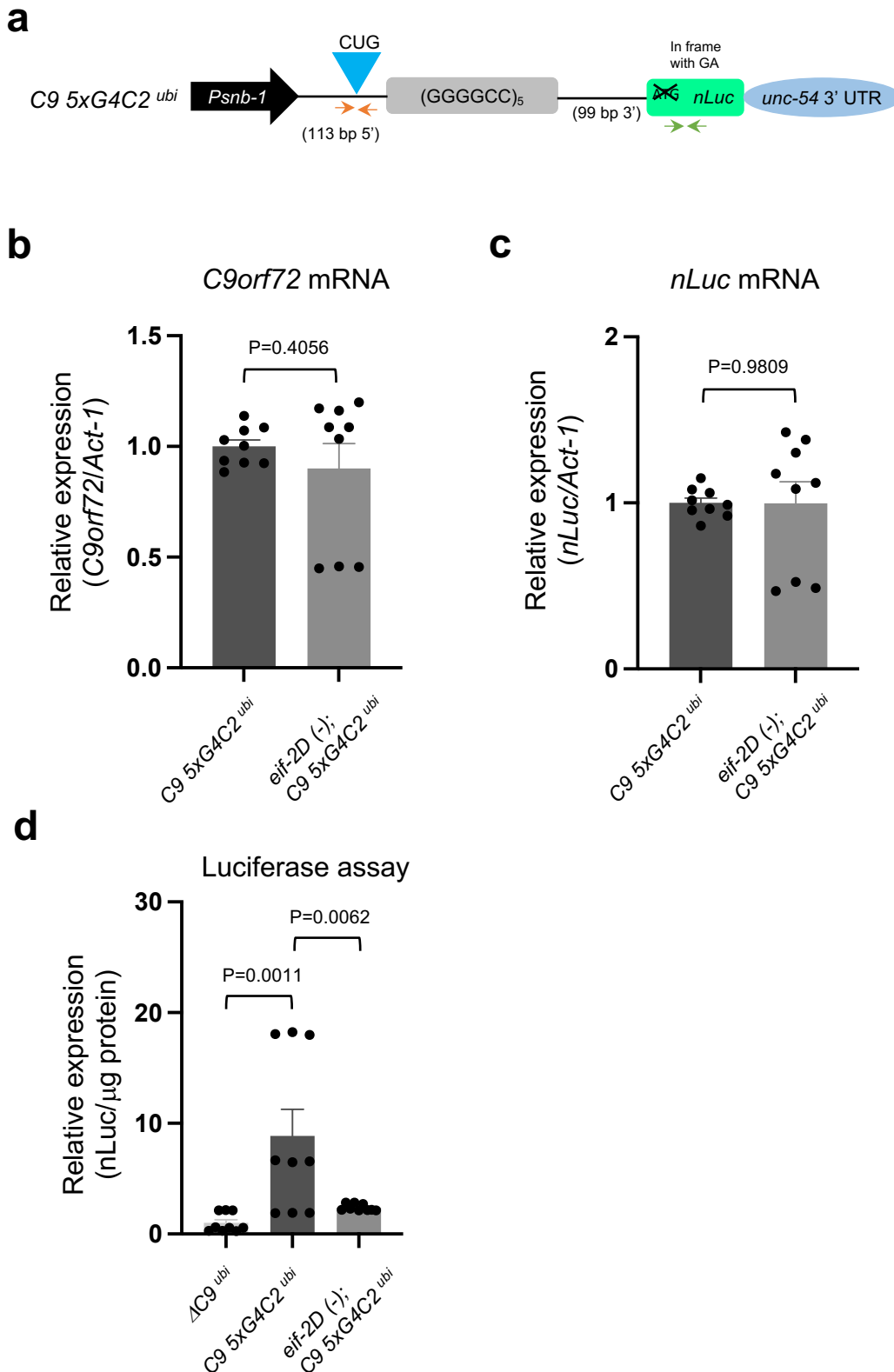


|                            | $\Delta C9^{neuro}$ | $C9^{neuro}$ | $UAG^{neuro}$ | $eif-2D^{-}; C9^{neuro}$ | $eif-2D^{-}; UAG^{neuro}$ | $eif-2D^{-}$ |
|----------------------------|---------------------|--------------|---------------|--------------------------|---------------------------|--------------|
| Number of animals included | 22                  | 20           | 23            | 22                       | 22                        | 25           |
| Number of censored animals | 4                   | 4            | 4             | 4                        | 4                         | 2            |
| Median lifespan (Days)     | 20                  | 17           | 20            | 22                       | 20                        | 22           |

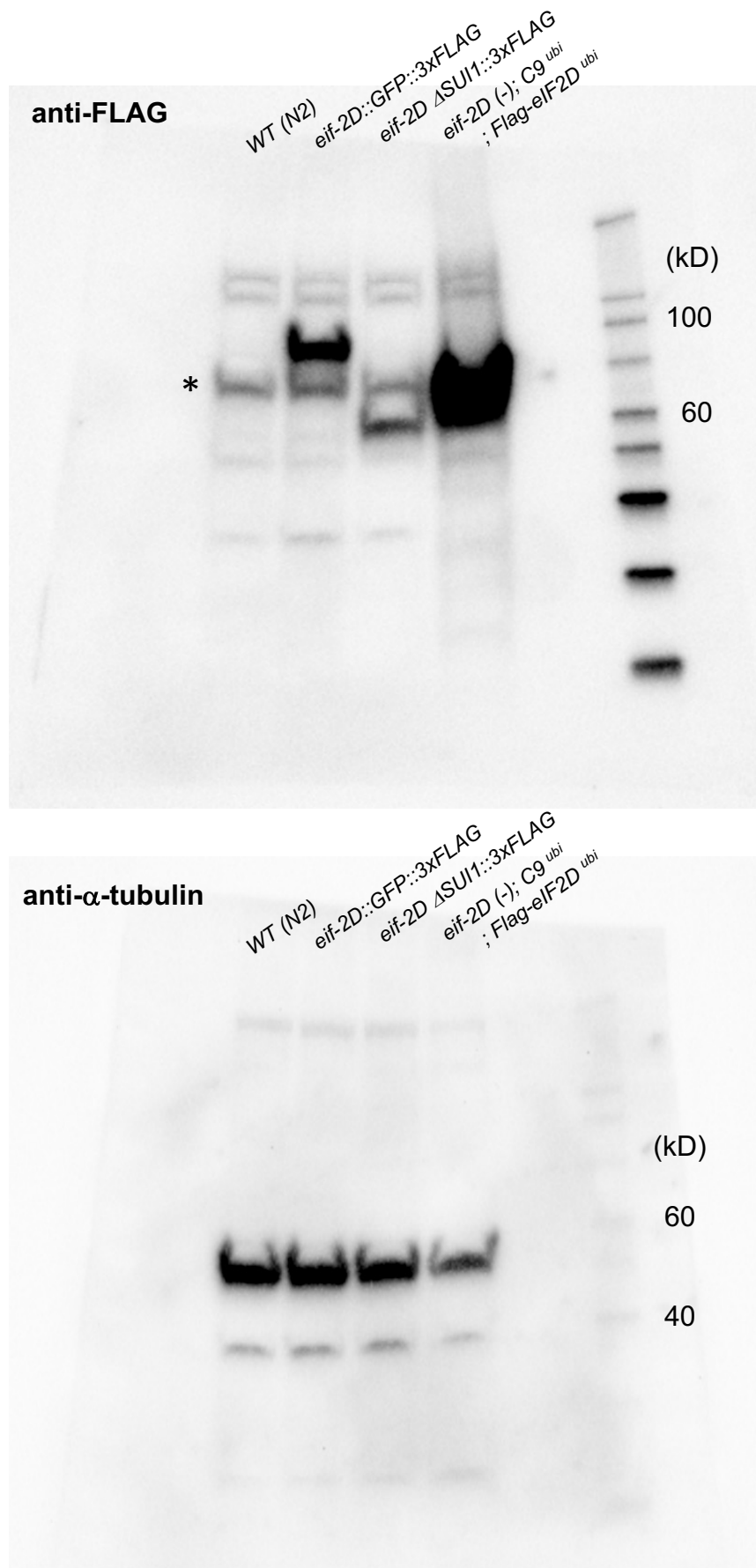
**Supplementary figure 21.  $C9^{neuro}$  animals display a significantly shorter lifespan in assays without FUDR.** The lifespan assays and data tables for  $\Delta C9^{neuro}$  (*KasEx159*),  $C9^{neuro}$  (*KasEx157*),  $UAG^{neuro}$  (*KasEx161*),  $eif-2D$  (*gk904876*);  $C9^{neuro}$ ,  $eif-2D$  (*gk904876*);  $UAG^{neuro}$  and  $eif-2D$  (*gk904876*) animals are shown. The experiments were repeated 3 times. Details for the individual experiments are shown in lower panels. Mantel-Cox log rank test was performed.



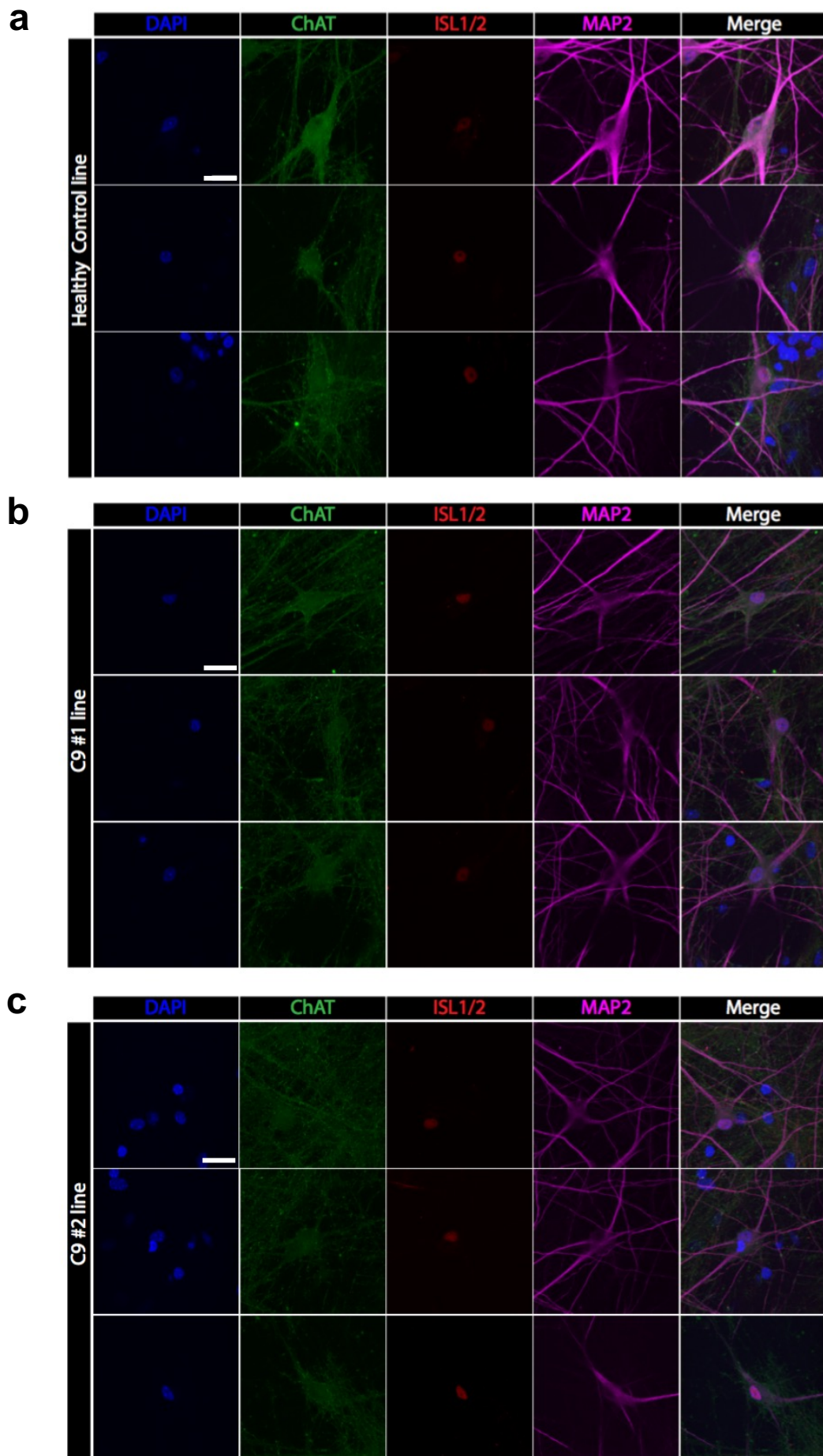




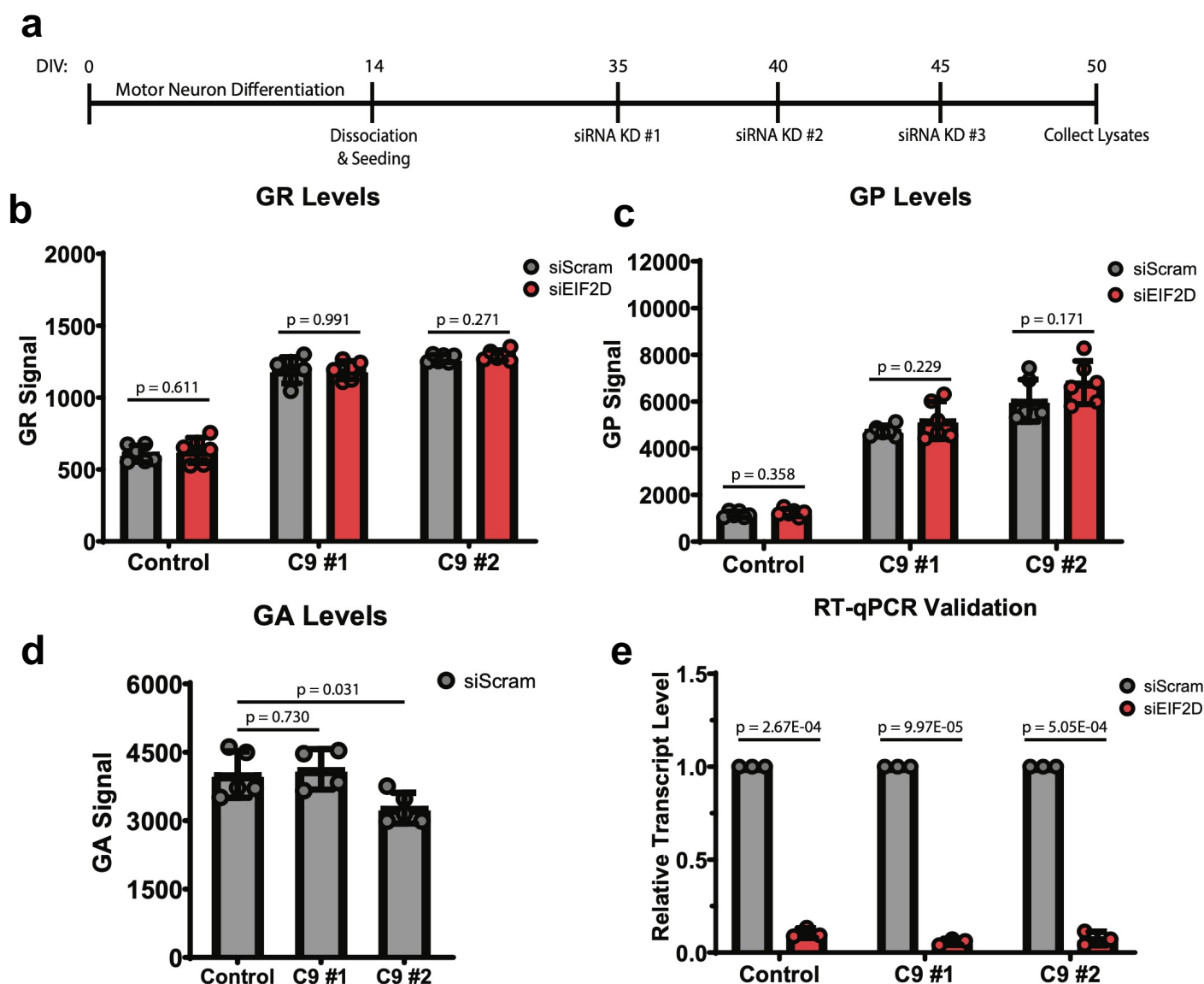
**Supplementary figure 23. Knockout of *eif-2D* does not affect the levels of *C9orf72* intronic RNA and *nLuc* mRNA in *C9 5xG4C2<sup>ubi</sup>* animals.** (a) Schematic showing the *C9 5xG4C2<sup>ubi</sup>* construct. The orange and green arrows show the location of primers designed to detect *C9orf72* and *nLuc* mRNAs, respectively. *nLuc*: nanoluciferase; UTR: untranslated region. The *C9orf72* intronic RNA (b) and *nLuc* (c) mRNAs were assessed by RT-PCR and normalized to *Act-1* mRNA. The experiments were repeated 3 times. (d) A luciferase assay was performed on worm lysates. The experiments were repeated 3 times. Mean  $\pm$  s.e.m. Two-tailed unpaired t-test was performed in (b, c) and One-way ANOVA with Dunnett's multiple comparisons test was performed in (d). The *eif-2D* (*gk904876*) allele was used. Transgenic Lines used: *DC9<sup>ubi</sup>* (*KasEx154*), *C9 5xG4C2<sup>ubi</sup>* (*KasEx244*).



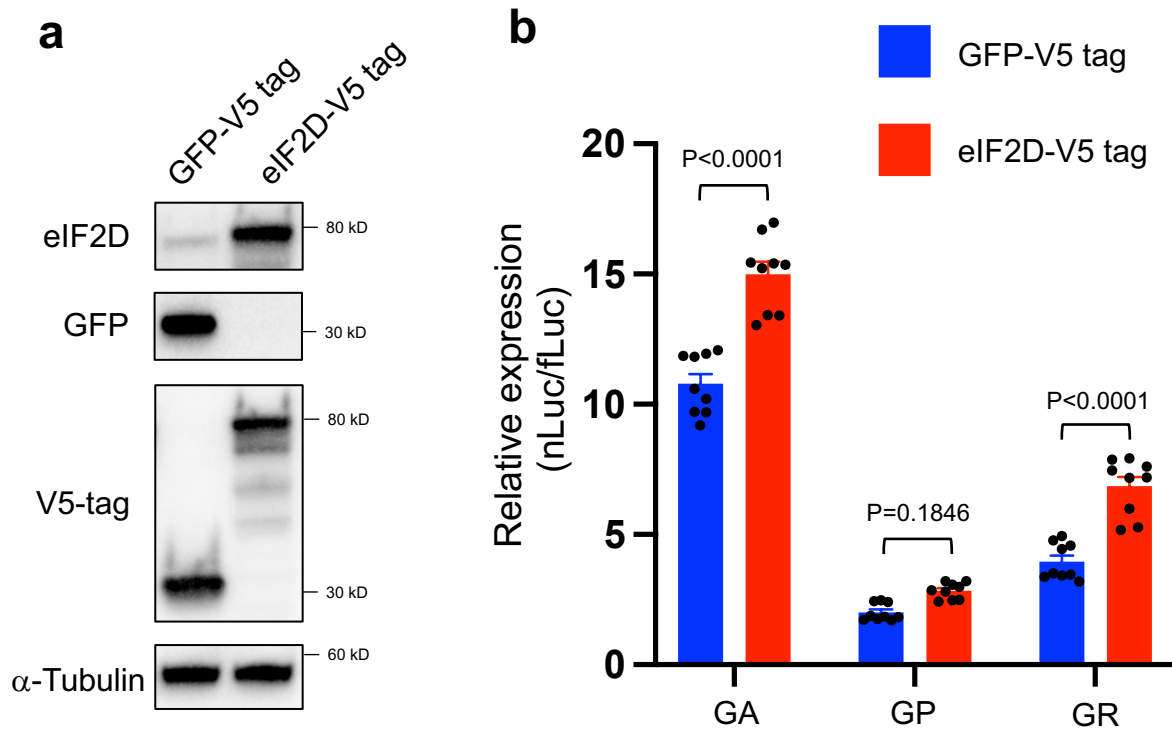
**Supplementary figure 24. Western blots to detect the FLAG epitope in worm lysates.** The lysates from WT (N2), *eif-2D::GFP::3xFLAG*, *eif-2 ΔSUI1::3xFLAG* and *eif-2D (gk904876); C9<sup>ubi</sup>; Flag-eIF2D<sup>ubi</sup> (KasEx248)* animals were processed for Western blotting and immunostained with Flag and α-tubulin antibodies. Asterisk shows non-specific band.



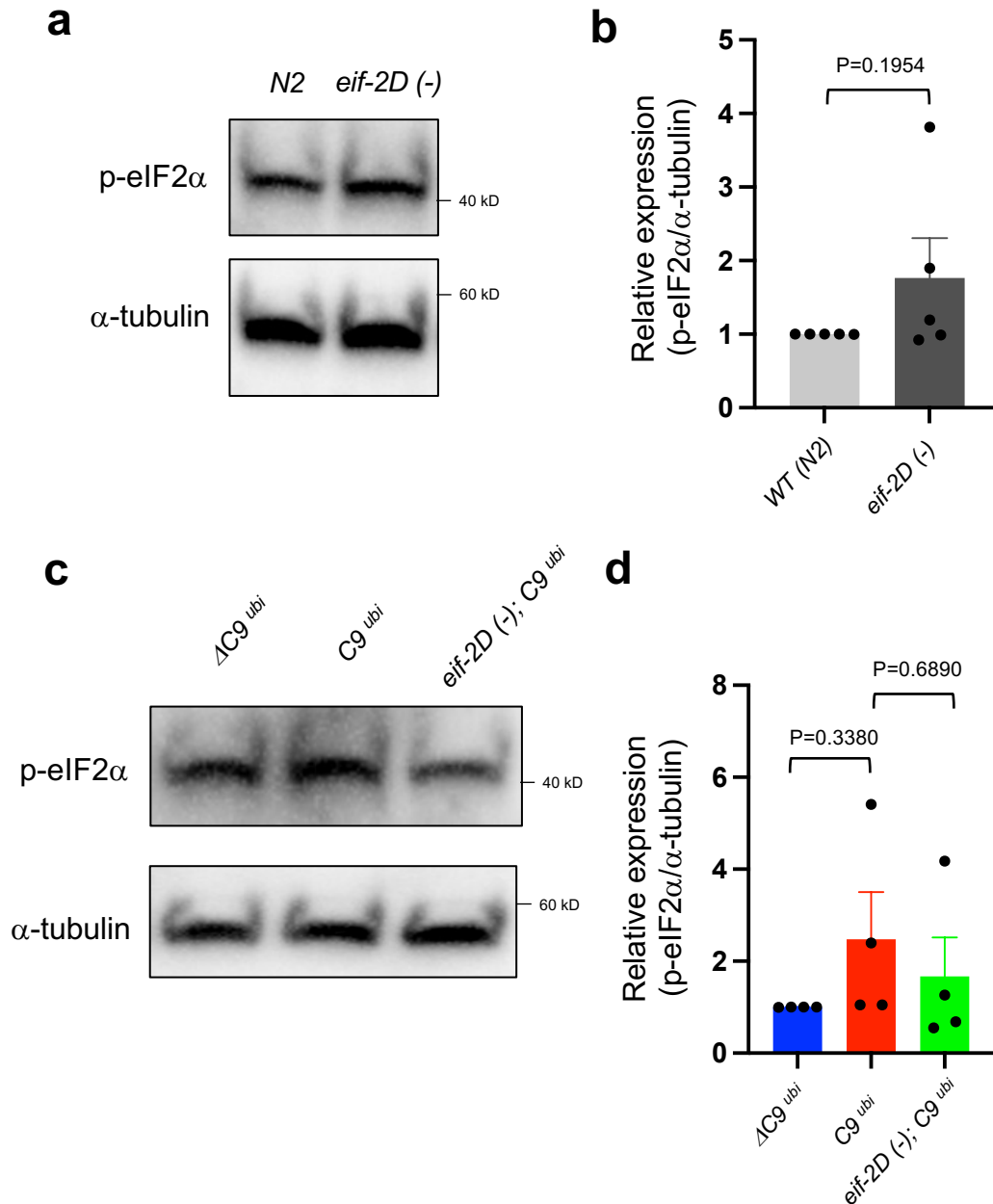
**Supplementary figure 25. Human iPSC-derived motor neurons express neuronal markers ChAT, ISL1/2 and MAP2 at 50 days in culture.** (a) Immunofluorescence staining against ChAT, ISL1/2, and MAP2. Three separate images of neurons derived from a healthy control iPSC line. Scale bar = 25  $\mu$ m. (b) Three separate images of neurons derived from an iPSC line with C9ORF72 hexanucleotide expansion (C9#1 line). (c) Three separate images of neurons derived from an iPSC line with C9ORF72 hexanucleotide expansion (C9#2 line).



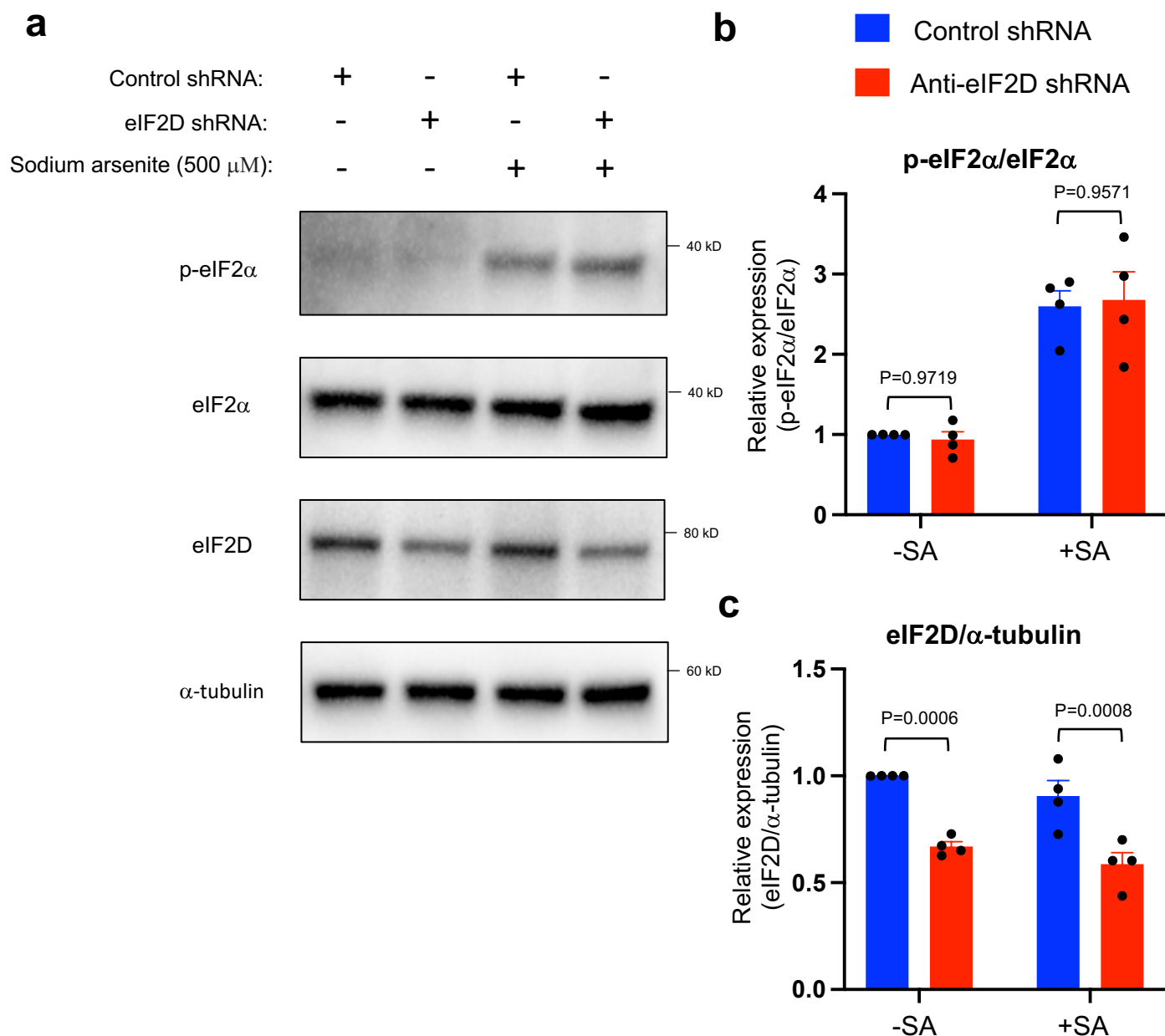
**Supplementary figure 26. Knockdown of eIF2D does not alter the levels of poly-GP and poly-GR in iPSC-derived motor neurons.** (a) Schematic of experimental design. D0-D15: small molecule driven lower motor neuron differentiation. D35, D40, D45: siRNA knockdown of EIF2D. D50: collect material for analysis. DIV = days in vitro. (b-d) The lysates from iPSC-derived motor neurons were processed for poly-GR(b), poly-GP(c), and poly-GA(d) ELISA. (e) The EIF2D, GAPDH, and Actin mRNA levels were assessed by RT-PCR. The EIF2D transcript levels were normalized to GAPDH/Actin transcript levels. The experiments were repeated 3 times. Each dot represents individual replicates within each experiment; mean  $\pm$  s.e.m. Two-tailed unpaired t-test was performed. siRNA: small interfering RNA; KD: knockdown; siScram: siScramble.



**Supplementary figure 27. Overexpression of eIF2D enhances DPR production in HEK293 cells.** Bicistronic constructs carrying 75 copies of the G4C2 repeat and flanking intronic sequences were cotransfected with constructs expressing either GFP (tagged with V5 epitope) or eIF2D (tagged with V5 epitope) and cultured for 48h. (a) Cell lysates were processed for Western blotting, and immunostained with antibodies to eIF2D, GFP, V5-tag, and α-tubulin. (b) The levels of luciferase activity were assessed by dual luciferase assays. The experiments were repeated 3 times. mean ± s.e.m. Two-way ANOVA with Šidák's multiple comparison test was performed.

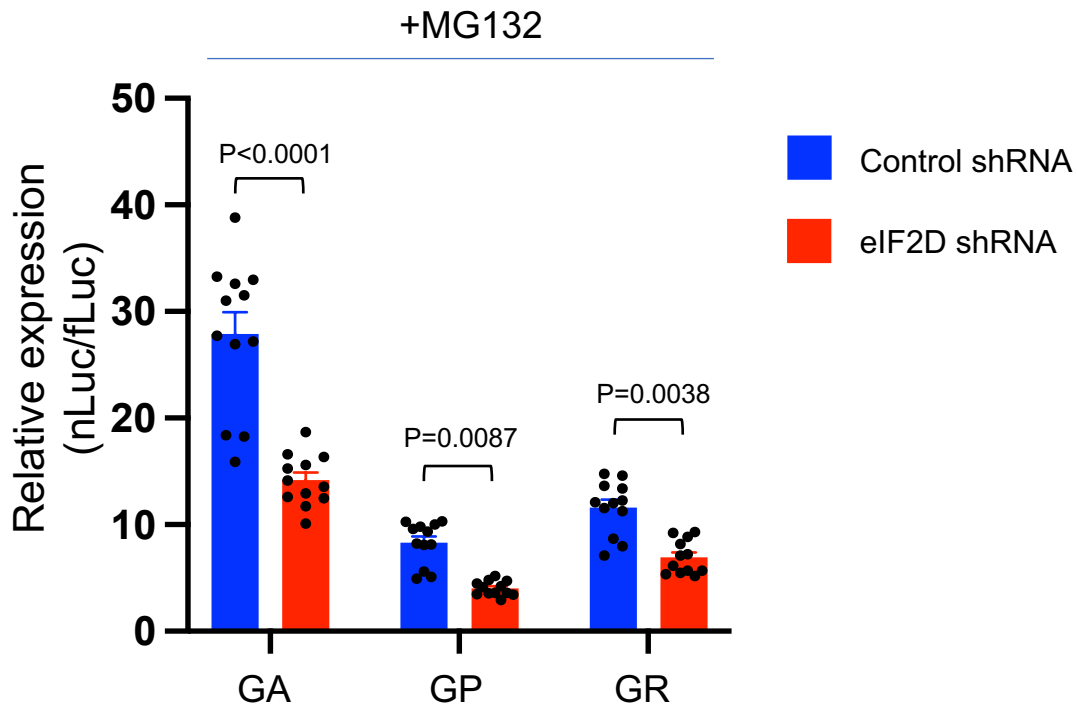


**Supplementary figure 28. Knockout of *eif-2D* does not affect the levels of p-eIF2α in *C. elegans*.** Lysates were processed for Western blotting from *N2* wild type, *eif-2D* (*gk904876*),  $\Delta C9^{ubi}$  (*KasEx154*),  $C9^{ubi}$  (*KasEx153*), and *eif-2D* (-);  $C9^{ubi}$  worms. A representative blot in (a) and (c) was immunostained with p-eIF2α and α-tubulin antibodies. Quantification is shown respectively in (b) and (d). The experiments were repeated 5 times in b and 4 times in d. Mean  $\pm$  s.e.m. Two-tailed unpaired t-test was performed in (b) and One-way ANOVA with Dunnett's multiple comparisons test was performed in (d).

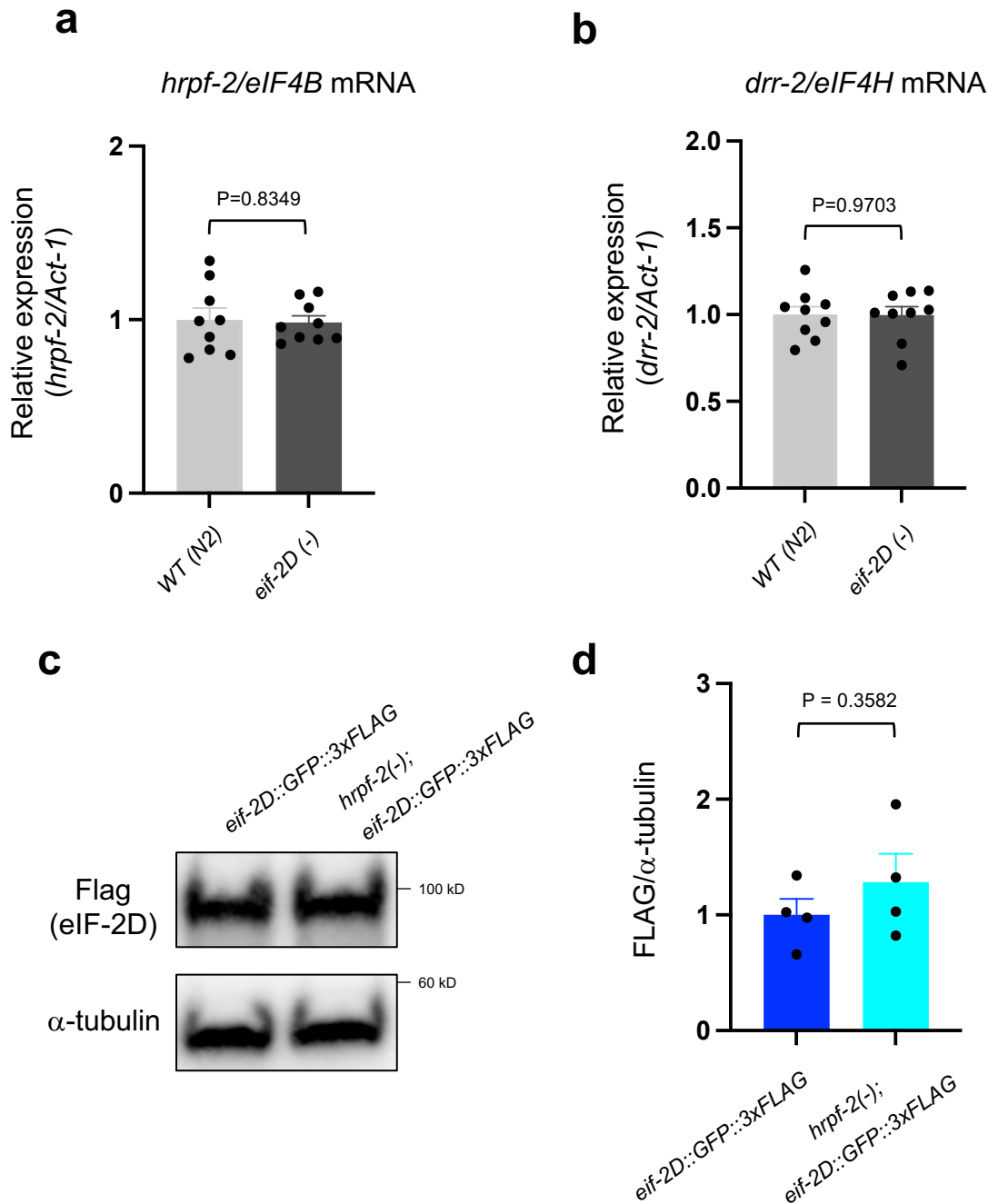


**Supplementary figure 29. Knockdown of eIF2D does not affect the levels of p-eIF2 $\alpha$  in HEK293 cells.** HEK293 cells were transfected with control shRNA or eIF2D shRNA. The cells were treated 48h later with 500  $\mu$ M sodium arsenite (SA), and after 30 min the cell lysates were processed for Western blotting. (a) A representative western blot immunostained with p-eIF2 $\alpha$ , eIF2 $\alpha$ , eIF2D, and  $\alpha$ -tubulin. Quantification is provided for p-eIF2 $\alpha$ /eIF2 $\alpha$  (b) and eIF2D/ $\alpha$ -tubulin (c). The experiments were repeated 4 times. mean  $\pm$  s.e.m. Two-way ANOVA with Šídák's multiple comparison test was performed.





**Supplementary figure 30. Treatment with a proteasome inhibitor (MG132) does not influence the effect of eIF2D knockdown on DPR production.** Bicistronic constructs carrying 75 copies of the G4C2 repeat and flanking intronic sequences were cotransfected with constructs expressing either control shRNA or eIF2D shRNA and cultured for 48h. For the last 6h, 10  $\mu$ M MG132 was added to the culture. The levels of luciferase activity were assessed by dual luciferase assays. The experiments were repeated 4 times, mean  $\pm$  s.e.m. Two-way ANOVA with Šídák's multiple comparison test was performed.



**Supplementary figure 31. The translation initiation factors *eif-2D/eIF2D*, *hrpf-2/eIF4B*, and *drr-2/eIF4H* do not cross-regulate their expression.** (a, b) Total RNA was extracted from N2 wild type and *eif-2D* (*gk904876*) animals. The *hrpf-2* (ortholog of human eIF4B) and *drr-2* (ortholog of human eIF4H) mRNAs levels were assessed by RT-PCR and normalized to *act-1*. The experiments were repeated 3 times. Two-tailed unpaired t-test was performed. (c, d) Lysates were processed for Western blotting from animals carrying the *eif-2D::GFP::3xFLAG* allele and *hrpf-2* (*gk5105*) mutants carrying the same allele. A representative blot was immunostained with Flag and  $\alpha$ -tubulin antibodies. (d) Quantification of panel c. The experiments were repeated 4 times. The data show mean  $\pm$  s.e.m. Two-tailed unpaired t-test was performed.

| <b>Supplementary Table 1. List of all primers used in this study.</b> |                |                                                                                                                                              |
|-----------------------------------------------------------------------|----------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Name</b>                                                           | <b>Purpose</b> | <b>Sequence (5'-&gt;3')</b>                                                                                                                  |
| Ce-eIF2A-F                                                            | Genotyping     | aaggaaaaaagctagctgggcagtttacggacaaaa                                                                                                         |
| Ce-eIF2A-R                                                            | Genotyping     | aaggaaaaaacttaaggaagttggtcgccaccttct                                                                                                         |
| Ce-eIF2D (gk904876)-F                                                 | Genotyping     | aaggaaaaaagctagcaccgtttacagtcaagaaaaata<br>ca                                                                                                |
| Ce-eIF2D (gk904876)-R                                                 | Genotyping     | aaggaaaaaacttaaggtgtagacattcatctgggttct                                                                                                      |
| Ce-eIF2D (gk561128)-F                                                 | Genotyping     | acataagatggcctgacggc                                                                                                                         |
| Ce-eIF2D (gk561128)-R                                                 | Genotyping     | ggcactgataattcaccaagcg                                                                                                                       |
| Ce-eif-2D-SUI>Flag-F                                                  | Genotyping     | ggctgctcaaaaggggatct                                                                                                                         |
| Ce-eif-2D-SUI>Flag-R                                                  | Genotyping     | cccatacttagaaacacacggg                                                                                                                       |
| Ce-pSnb-1-F                                                           | Cloning        | aaaccactagtcagttcgggtatctcagcaa                                                                                                              |
| Ce-pSnb-1-R                                                           | Cloning        | acagggaagcttgcgtcaagatggtcttct                                                                                                               |
| Ce-pUnc11c-F                                                          | Cloning        | aaaccactagtcgtgtctctccgtctatc                                                                                                                |
| Ce-pUnc11c-R                                                          | Cloning        | aaaccaagcttcaaataaaaggagctgtgt                                                                                                               |
| Ce-Unc54-F                                                            | Cloning        | aaaccctcgaggcgccggtcgctaccattac                                                                                                              |
| Ce-Unc54-R                                                            | Cloning        | acaggggagctcggaacagttatgtttggt                                                                                                               |
| Ce-eif2D-F                                                            | Cloning        | aaaccgcggccgcattcaaaaaaccgtttacagt                                                                                                           |
| Ce-eif2D-R                                                            | Cloning        | aaaccggatccttatttcttcttttcaccg                                                                                                               |
| Hs-eIF2D-F                                                            | Cloning        | aaaccgaattcgccatgtttgccaaggccttcg                                                                                                            |
| Hs-eIF2D-R                                                            | Cloning        | aaaccgcggccgctgcttcttctgccaggttga                                                                                                            |
| GFP-F                                                                 | Cloning        | aaaccgaattcaccatggtgagcaagggcgagga                                                                                                           |
| GFP-R                                                                 | Cloning        | cccaaagcgccgctgctgtacagctcgtccatgc                                                                                                           |
| GFP-novo2-F                                                           | Cloning        | aaaccgcggccgcaagtaaaggagaagaactttt                                                                                                           |
| GFP-novo2-R                                                           | Cloning        | aaaccggatccctatttgtatagttcatcca                                                                                                              |
| ΔG4C2-1-F                                                             | Cloning        | agcttagtactcgctgagggtgaacaagaaaagacctgat<br>aaagattaaccagaagaaaacaaggagggaacaacc<br>gcagcctgtagcaagctctggaactcag                             |
| ΔG4C2-1-R                                                             | Cloning        | gttcagagcttgctacaggctgcggtgtttccctcctgtttt<br>cttctggttaatctttatcaggctctttctgttcaccctcagcga<br>gtacta                                        |
| ΔG4C2-2-F                                                             | Cloning        | gagtcgcgcgctaggggctggtcggggcgggcccg<br>ggcgggcccgggcggggctgcggtgcggtgcctgcgc<br>ccgcggcgggcgaggcgaggcggtggcgagtggggg<br>atcaaacgc            |
| ΔG4C2-2-R                                                             | Cloning        | ggccgcgtttgatccccactcgccaccgcctgcgcctccg<br>ccgccgcggcgaggcaccgcaaccgcagccccgcc<br>ccgggcccggccccggggcccgcccgaccacgccccta<br>gcgcgcgactcctga |
| ΔnLuc-F                                                               | Cloning        | ggccgcgatcctagttaagtagcg                                                                                                                     |
| ΔnLuc-R                                                               | Cloning        | gatccgctacttaactaggatcgc                                                                                                                     |
| 3xFlag-F                                                              | Cloning        | agcttatggattataaagacgatgacgataagcgtgactac<br>aaggacgacgacgacaagcgtgattacaaggatgacga<br>tgacaagagagc                                          |
| 3xFlag-R                                                              | Cloning        | ggccgctctctgtcatcgtcatccttgaatcacgcttgcgtc                                                                                                   |

|                    |         |                                                                         |
|--------------------|---------|-------------------------------------------------------------------------|
|                    |         | gtcgtcctttagtagtcacgcttatcgatcgtctttataatccat<br>a                      |
| Hs-eIF2D-shRNA-F   | Cloning | gatccgggacgacaactggacataaagttcaagagacttt<br>atgtccagttgtcgtccttttttgaaa |
| Hs-eIF2D-shRNA-R   | Cloning | agcttttcaaaaaaggacgacaactggacataaagtctct<br>tgaactttatgtccagttgtcgtccc  |
| Mm-eIF2D-shRNA-1-F | Cloning | ccggcgccgaaagatcatcacggactactcgagtagtcc<br>gtgatgatctttcggttttg         |
| Mm-eIF2D-shRNA-1-R | Cloning | aattcaaaaaccgaaagatcatcacggactactcgagtag<br>tccgtgatgatctttcggcg        |
| Mm-eIF2D-shRNA-2-F | Cloning | ccggcgcccatttctaaagaaagggaaactcgagttccctt<br>tctttagaatgggttttg         |
| Mm-eIF2D-shRNA-2-R | Cloning | aattcaaaaaccatttctaaagaaagggaaactcgagtt<br>cccttctttagaatggg            |
| Mm-eIF2D-shRNA-3-F | Cloning | ccggcgctgcctaccaagtaacatttctcgagaaatgtta<br>cttggtaggcagggttttg         |
| Mm-eIF2D-shRNA-3-R | Cloning | aattcaaaaacctgcctaccaagtaacatttctcgagaaat<br>gttacttggtaggcaggcg        |
| Ce-qPCR-Act-1-F    | qPCR    | acgacgagtcgcccaccc                                                      |
| Ce-qPCR-Act-1-R    | qPCR    | gaaagctggtggtgacgatggtt                                                 |
| qPCR-C9-F          | qPCR    | gaaacaaccgcagcctgtag                                                    |
| qPCR-C9-R          | qPCR    | tagcgcgcgactcctga                                                       |
| qPCR-nLuc-F        | qPCR    | cagccggctacaacctggac                                                    |
| qPCR-nLuc-R        | qPCR    | agcccattttaccgctcag                                                     |
| Ce-qPCR-hrpf2-F    | qPCR    | gcagtgaaaactcaatccgca                                                   |
| Ce-qPCR-hrpf2-R    | qPCR    | ggcttcttggtggttctcca                                                    |
| Ce-qPCR-drr2-F     | qPCR    | tcagtggccttaatggagcc                                                    |
| Ce-qPCRdrr2-R      | qPCR    | gaagtttctccgcgtcctc                                                     |
| Hs-qPCR-EIF2D-F    | qPCR    | cggacaggaagcttcga                                                       |
| Hs-qPCR-EIF2D-R    | qPCR    | ccaccactcacgtacacagt                                                    |
| Hs-qPCR-ACTIN-F    | qPCR    | tttgagacctcaacaccccagcc                                                 |
| Hs-qPCR-ACTIN-R    | qPCR    | aatgtcacgcacgattcccgc                                                   |
| Hs-qPCR-GAPDH-F    | qPCR    | gtctacatggcaactgtgagga                                                  |
| Hs-qPCR-GAPDH-R    | qPCR    | gatgacatcaagaaggtggtga                                                  |

**Supplementary File 1.** Pluripotency certificate for ALS patient iPSC line C9 #1 (TALS9-11.2) obtained from Target ALS. Cat# ND50008; RRID:CVCL\_FA04. Female, 61 years old. See next page.

|                          |                                                         |
|--------------------------|---------------------------------------------------------|
| NHCDR ID - ND50008       | Source cell type - FCL                                  |
| Cell line ID - FA0000018 | Reprogramming method - Sendai viral vectors             |
| Lot numbers - R013356330 | Growth conditions - mTeSR and Matrigel (SOP# G103-0179) |
| R013535970               | Passage method - Dispase (SOP# G103-0179)               |
| Depositor - Target ALS   | Total Passage Number - 17                               |
|                          | Number of passages at RUCDR - 10                        |
|                          | Number of cells /vial - $2.0 \times 10^6$               |

| Property                                  | Test Name                 | Test Method                                                                                   | Criteria for Passing                                                                                                                             | Test Result |
|-------------------------------------------|---------------------------|-----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| Sterility                                 | Mycoplasma Contamination  | qPCR                                                                                          | Negative                                                                                                                                         | Pass        |
| Identity                                  | SNP Trace                 | 96 SNP assay                                                                                  | Matches source cells, if available                                                                                                               | Pass        |
| Pluripotency                              | Live colony image         | Visual inspection                                                                             | Majority of colonies have smooth edges, are mostly circular and consist of small, tightly compacted cells with large nuclear to cytoplasm ratios | Pass        |
|                                           | AP Stain                  | Live cell fluorescent stain for Alkaline Phosphatase                                          | Positive                                                                                                                                         | Pass        |
|                                           | Immunofluorescence        | Fixed cell staining for Oct4 and Tra-1-60                                                     | Expression of Oct4 and Tra-1-60                                                                                                                  | N/A         |
|                                           | FACS                      | Flow cytometry for the expression of Oct4 and Tra-1-60                                        | Minimum 90% Oct4/Tra-1-60 double positive                                                                                                        | 95%-97%     |
|                                           | RUCDR Pluritest           | HT12 array for gene expression                                                                | >18 Pluripotency                                                                                                                                 | N/A         |
|                                           |                           |                                                                                               | <2.1 Novelty                                                                                                                                     | N/A         |
|                                           | iPSC Scorecard            | 96 well qPCR assay on undifferentiated cells for the expression of pluripotency markers       | Positive score for pluripotency and negative score for differentiation                                                                           | N/A         |
|                                           | Differentiation Scorecard | 96 well qPCR assay on differentiated cells for the expression of markers of all 3 germ layers | Negative score for pluripotency and positive score for differentiation                                                                           | N/A         |
| Exogenous reprogramming factor expression | Sendai Persistence        | qPCR assay to detect sendai viral sequences                                                   | < 1.0 RNA molecules per cell                                                                                                                     | N/A         |
|                                           |                           |                                                                                               | $R^2$ value $\geq 0.95$                                                                                                                          | N/A         |
|                                           | Episome Persistence       | qPCR assay to detect episome sequences                                                        | < 0.5 DNA molecules per cell                                                                                                                     | N/A         |
|                                           |                           |                                                                                               | $R^2$ value $\geq 0.95$                                                                                                                          | N/A         |
| Genetic Stability                         | Karyotyping               | G-band analysis                                                                               | Expected chromosome complement (Dependant on subject)                                                                                            | N/A         |
| Viability                                 | Test Thaw                 | Live cell dye followed by confluency quantitation                                             | At least 10 pluripotent colonies at 72hr post-thaw                                                                                               | Pass        |

# Cryopreservation

---

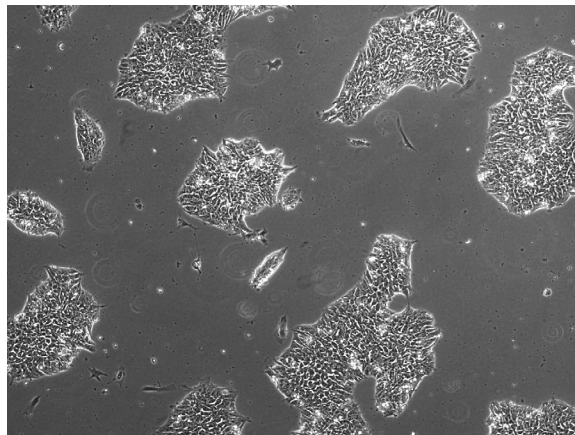
Cryopreservation date -01/04/2016

Cryopreservation method - accutase and mFreSR (SOP# G103-0179)

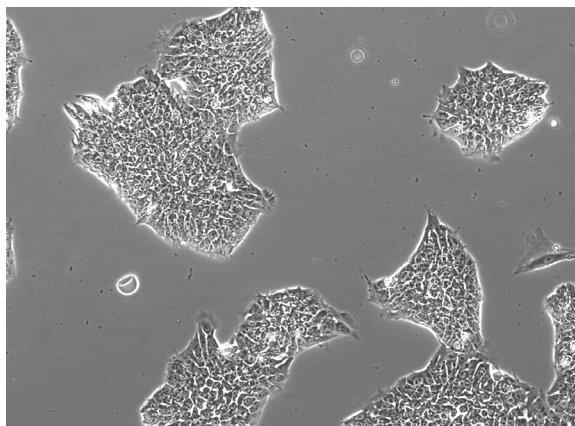
Cell density -  $2.0 \times 10^6$  /vial

|                           | R013356330                                                  | R013535970                                                  |
|---------------------------|-------------------------------------------------------------|-------------------------------------------------------------|
| Recomended thaw density   | 1 vial in 1 well(s) of a 6 well plate (9.6cm <sup>2</sup> ) | 1 vial in 1 well(s) of a 6 well plate (9.6cm <sup>2</sup> ) |
| # Colonies after 24 hours | >10                                                         | >10                                                         |
| # Colonies after 72 hours | >30                                                         | >30                                                         |

## Post thaw viability



10x magnification 3 days after (R013356330 )

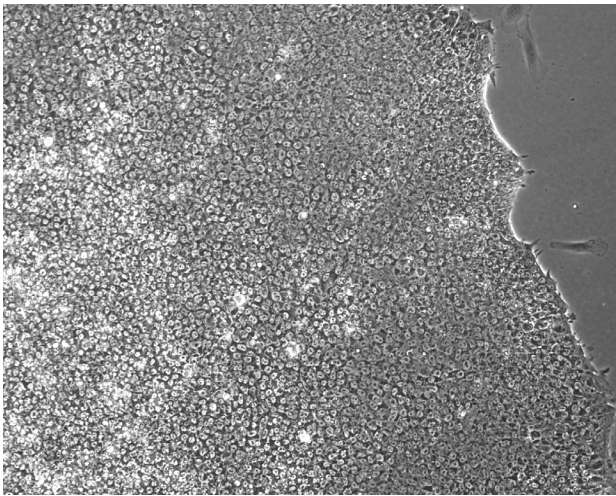


10x magnification 3 days after thaw (R013535970)

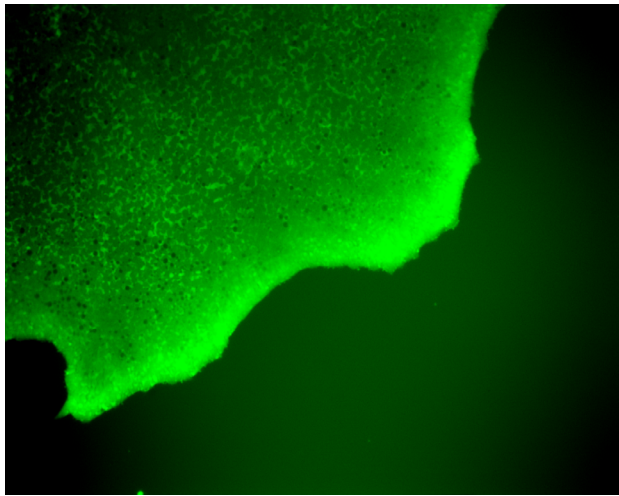


# Pluripotency

Live Colony Image (10x)



AP stain (10X)



FACS Analysis

