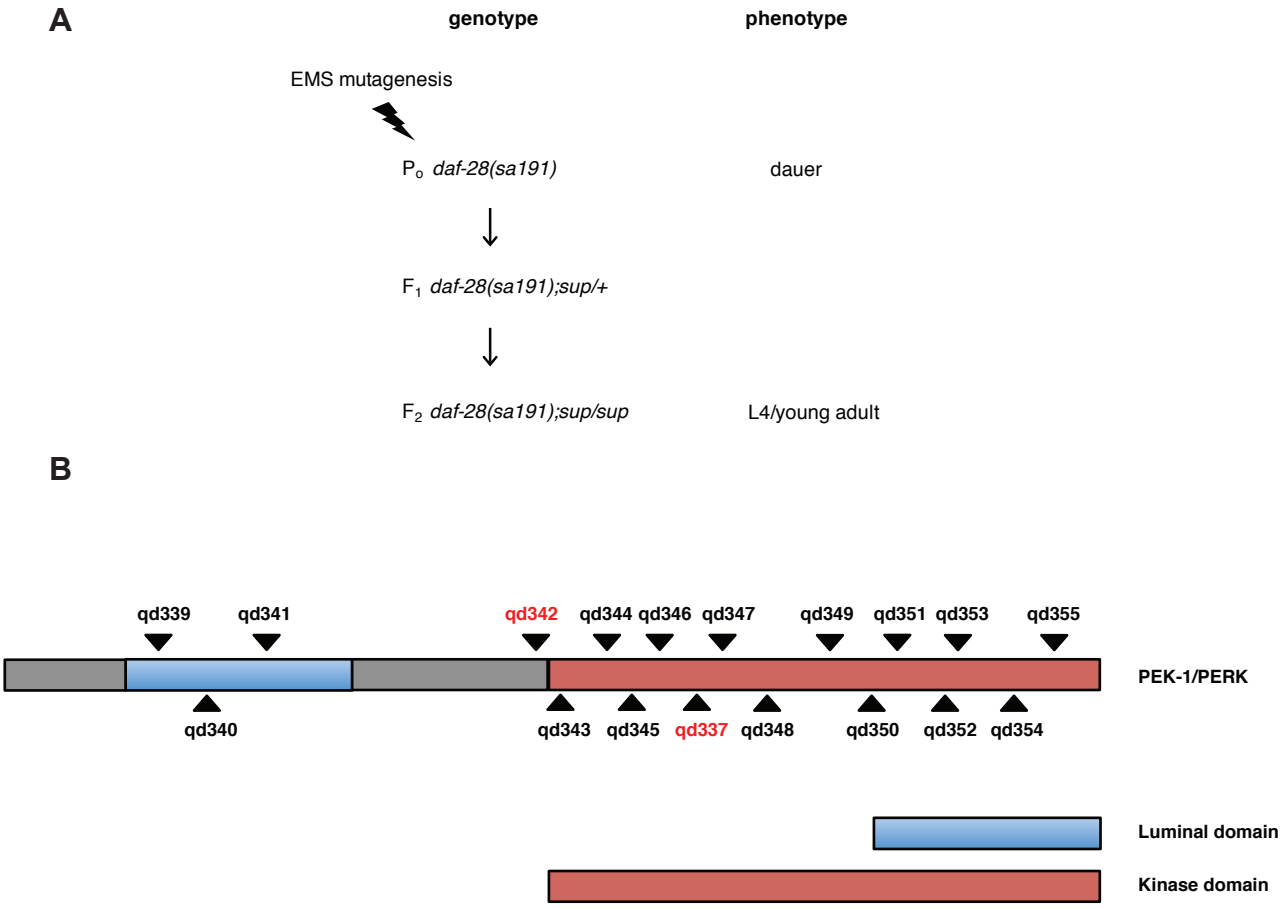
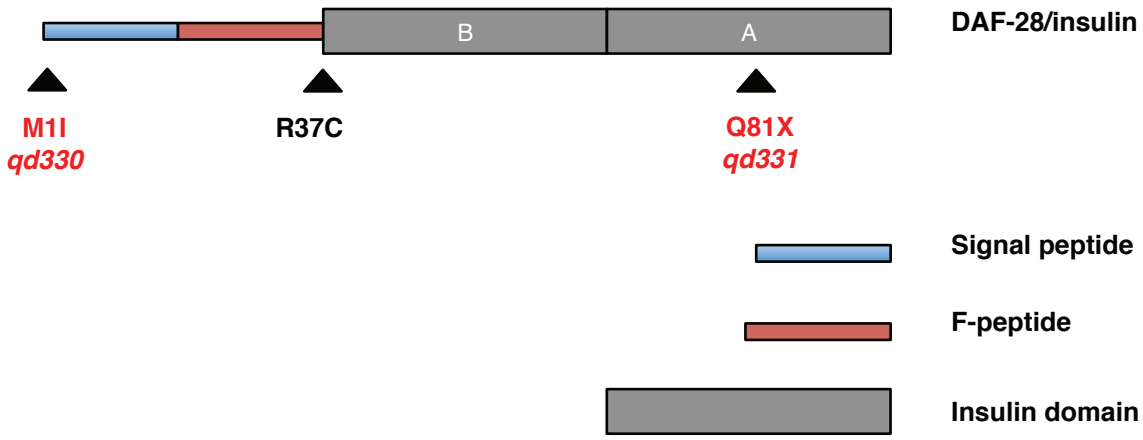



**Figure S1: Isolation of mutations that suppress the *daf-28(sa191)* constitutive dauer entry phenotype**
A. Schematic detailing the forward genetic approach used to isolate the F2 mutants that failed to enter dauer diapause constitutively under laboratory conditions.
B. Schematic is based on conserved domains. X (table) and red font (schematic) represent stop codon and nonsense mutatation respectively.




| Genotype | Phenotype |
|---|---|
| *daf-28(R37C)/daf-28(R37C)* | dauer |
| *daf-28(M1I R37C)/daf-28(M1I R37C)* | L4 |
| *daf-28(M1I R37C)/daf-28(R37C)* | dauer |
| *daf-28(-)/daf-28(R37C)* | dauer |
| *daf-28(R37C Q81X)/ daf-28(R37C Q81X)* | L4 |
| *daf-28(R37C Q81X)/daf-28(R37C)* | L4 |
| *daf-28(+)/daf-28(R37C)* | L4 |

**Figure S2: Second-site mutations in *daf-28* suppress the dauer entry phenotype**
We identified a second-site mutation in the start codon (M1I) that resulted in intragenic suppression of the constitutive dauer entry phenotype. Consistent with its loss-of-function alteration, the *daf-28(M1I R37C)* allele behaved similarly to the *daf-28* null allele, specifically in heteroallelic combination with *daf-28(R37C)*. We also isolated a second-site nonsense mutation (Q81X) that behaved similarly to the *daf-28* wild-type allele in heteroallelic combination with *daf-28(R37C)*, suggesting revertant alteration. The "dauer" phenotype refers to frequency of dauer formation higher than 90%. The "L4" phenotype refers to frequency of dauer formation lower than 10%. The phenotypes were based on at least three trials, with at least 100 animals per genotype. Data for *daf-28(-)/ daf-28(R37C)* and *daf-28(+)/daf-28(R37C)* were presented in Kulalert and Kim, 2013. Schematic above the table represents protein domains of DAF-28 and was adapted from Li et al., 2003.

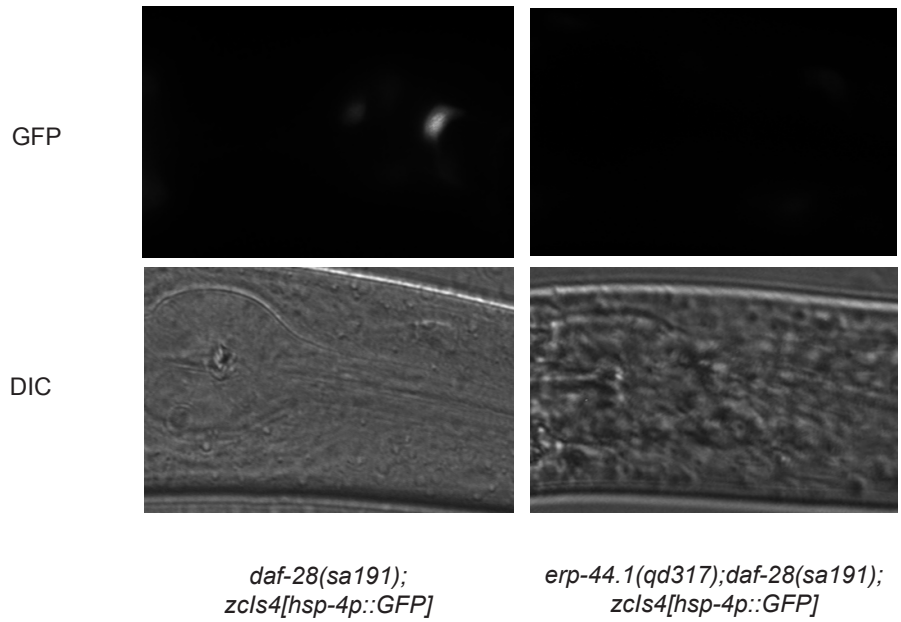



**Figure S3: Loss-of-function mutation in *erp-44.1* suppresses induction of the neuronal UPR in the *daf-28(sa191)* background**

Images of the animals with indicated genotypes, representative of multiple animals from two independent experiments. *GFP* expression is driven under the *hsp-4* promoter in the *zcIs4* transgene, as utilized in our previous study (Kulalert and Kim, 2013). The GFP induction depicted above in the *daf-28(sa191);zcIs4* background is consistent with the ASI-specific UPR activation described in Kulalert and Kim, 2013.

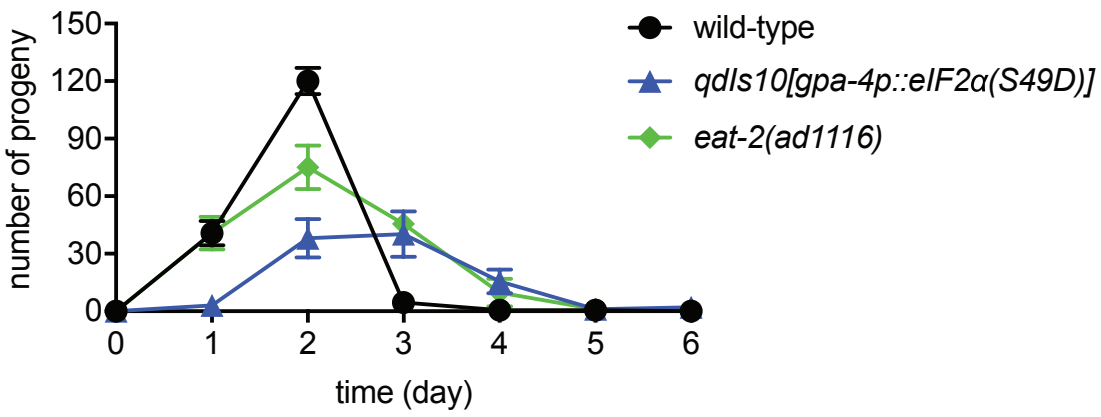



**Figure S4: Extension of the egg-laying period in the food uptake-defective *eat-2* mutant, similar to that observed in the *qdIs10* background**

The *ad1116* allele results in a splice site mutation in the *eat-2* gene, resulting in lifespan extension due to nutritional limitation. We note that while the egg-laying period was extended in the *eat-2* mutant, similar to the pattern exhibited by the animal carrying the *qdIs10* transgene, the total number of progeny was not significantly distinct from that of wild-type control. We observed reduced growth rate as well as clear appearances in the *eat-2* mutant, reminiscent of the animals carrying *qdIs10*.

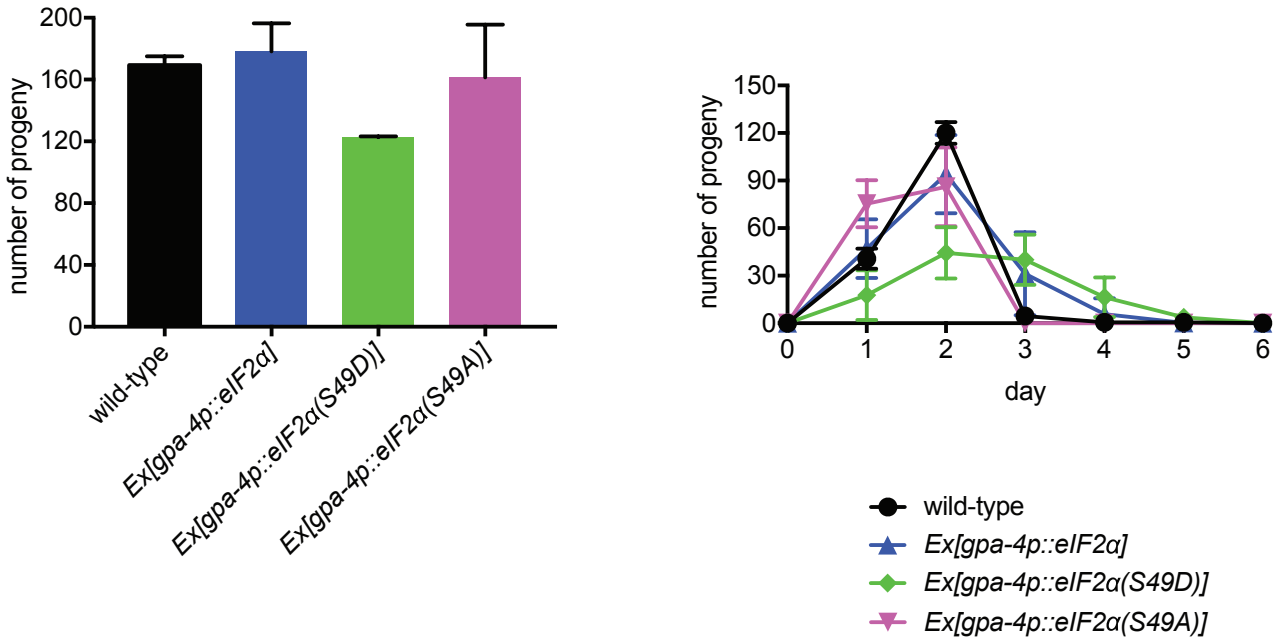



**Figure S5: The starvation-like reproductive response is specific to the phosphorylation state of neuronal eIF2α**

Animals harboring extrachromosomal arrays that express ASI-specific eIF2α of indicated phosphorylation status of Ser49 were assayed for total number of brood size and egg-laying duration. The animals carrying the extrachromosomal phosphomimetic neuronal eIF2α array appeared clear and small, and had reduced brood size, similar to the phenotypes conferred by the *qdIs10* transgene. We note that while the animals expressing the wild-type eIF2α appeared healthy, very few of them had somewhat clear and slightly smaller appearances, with normal progeny production. Importantly, the animals carrying the extrachromosomal unphosphorylatable neuronal eIF2α transgene exhibited wild-type developmental and reproductive phenotypes.

| Gene | Allele | Molecular identity |
|---|---|---|
| *daf-28* | *sa191qd330* | M1I R37C |
| | *sa191qd331* | R37C Q81X |
| *erp-44.1* | *qd329* | W4X |
| | *qd317* | splice site (G to A),<br>3´ to exon 2 |
| | *qd332* | Q213X |
| *pek-1* | *qd339* | G63E |
| | *qd340* | G147E |
| | *qd341* | P236L |
| | *qd342* | R519X |
| | *qd343* | G613D |
| | *qd344* | G616D |
| | *qd345* | D626N |
| | *qd346* | A631T |
| | *qd337* | R649X |
| | *qd347* | L654F |
| | *qd348* | E670K |
| | *qd349* | G875D |
| | *qd350* | G937R |
| | *qd351* | D956N |
| | *qd352* | G986S |
| | *qd353* | P993S |
| | *qd354* | G1037E |
| | *qd355* | splice site (G to A),<br>5´ to exon 12 |
| *eif-2a* | *qd338* | S49F |
| *eif-2Ba* | *qd333* | E28K |
| | *qd334* | T41I |
| | *qd356* | A70V |
| | *qd357* | R98C |
| | *qd358* | H132Y |
| | *qd359* | G200C |
| | *qd360* | G208D |
| | *qd361* | G215E |
| | *qd362* | S225L |
| | *qd363* | D274N |
| | *qd335* | M1* |
| *eif-2c* | *qd336* | S443L |

**Table S1: List of altered residues or splice sites caused by the suppressor mutations**
Amino acid positions and splice sites are based on the *c30h7.2a.1* isoform of *erp-44.1*. The *qd335* allele results in a new start codon just a few base pairs upstream of the original AUG, likely resulting in early translation initiation of an out-of-frame ORF that drastically truncates eIF2Bα.

| Allele of *eif-2Ba* | Molecular identity | Mode of inheritance | Residue conservation |
|---|---|---|---|
| *pk720* | insertion/deletion | dominant | N/A |
| *qd333* | E28K | dominant | variable |
| *qd334* | T41I | dominant | conserved |
| *qd356* | A70V | dominant | variable |
| *qd357* | R98C | recessive | variable |
| *qd358* | H132Y | dominant | conserved |
| *qd359* | G200C | dominant | conserved* |
| *qd360* | G208D | dominant | conserved* |
| *qd361* | G215E | dominant | conserved* |
| *qd362* | S225L | recessive | variable |
| *qd363* | D274N | dominant | conserved* |
| *qd335* | out-of-frame upstream ATG | recessive | N/A |

**Table S2: Molecular alterations and modes of inheritance associated with different alleles of *eif-2Ba* that suppress the *daf-28(sa191)* constitutive dauer entry phenotype** We determined the phenotypic mode of inheritance by crossing the isolated suppressor strain (*daf-28(sa191);sup/sup*) to the starting strain (*daf-28(sa191);+/+*). If the F1 progeny (*daf-28(sa191);sup/+*) does not form dauers constitutively (i.e. one copy of *sup* is able to suppress the dauer entry phenotype), the allele is considered to behave dominantly. If the F1 progeny forms dauers constitutively, the allele confers a recessive suppression phenotype.

Residue conservation was based on yeast, worm, fly rat, mouse and human eIF2Bα amino acid sequences, using BLOSOM as previously analyzed in Williams et al., 2001 and other eIF2B studies. The asterisk denotes residue conservation with respect to both eIF2Bα orthologs and other regulatory subunits, eIF2B, eIF2Bβ and eIF2Bδ.

We also note that the isolated *eif-2Ba(qd334)* allele affecting the highly conserved residue, threonine 41, behaved similarly to the well-characterized T41A alteration in the *Saccharomyces cerevisiae* eIF2Bα ortholog GCN3, which contributes to resistance to eIF2α phosphorylation in response to nutrient deprivation in yeast (Hannig and Hinnebusch, 1988 and Pavitt et al., 1997). Moreover, the T41A alteration in human eIF2Bα enhanced susceptibility to viral infection due to the inability to inhibit translation in response to viral RNA-induced eIF2α phosphorylation (Elsby et al., 2011). The observed insensitivities to phosphorylated eIF2α in multiple species may suggest a critical role of the T41 residue, as well as of the alpha subunit, in translation initiation regulation mediated by eIF2α phosphorylation. Similar to the T41I substitution resulting from the *eif-2Ba(qd334)* allele, the majority of the recovered alleles alter the conserved residues of eIF2Bα, suggesting critical contributions of these residues to recognizing phosphorylated eIF2α to exert translational control (Table S2 and Kuhle et al., 2015). Our identification of diverse residues essential for eIF2(αP)-sensitivity may provide resources for further structure-function studies of eIF2B regulation in *C.*

*elegans*, and potentially higher metazoans, as in the case of the T41A eIF2(αP)-resistant mutant in yeast and human (Hannig and Hinnebusch, 1988, Pavitt et al., 1997, Elsby et al., 2011).

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Elsby, R., J. F. Heiber, P. Reid, S. R. Kimball, G. D. Pavitt *et al.*, 2011 The alpha subunit of eukaryotic initiation factor 2B (eIF2B) is required for eIF2-mediated translational suppression of vesicular stomatitis virus. J Virol 85**:** 9716-9725.

Hannig, E. M., and A. G. Hinnebusch, 1988 Molecular analysis of GCN3, a translational activator of GCN4: evidence for posttranslational control of GCN3 regulatory function. Mol Cell Biol 8**:** 4808-4820.

Kuhle, B., N. K. Eulig and R. Ficner, 2015 Architecture of the eIF2B regulatory subcomplex and its implications for the regulation of guanine nucleotide exchange on eIF2. Nucleic Acids Res 43**:** 9994-10014.

Pavitt, G. D., W. Yang and A. G. Hinnebusch, 1997 Homologous segments in three subunits of the guanine nucleotide exchange factor eIF2B mediate translational regulation by phosphorylation of eIF2. Mol Cell Biol 17**:** 1298-1313.

Williams, D. D., G. D. Pavitt and C. G. Proud, 2001 Characterization of the initiation factor eIF2B and its regulation in Drosophila melanogaster. J Biol Chem 276**:** 3733-3742.

| **Strain name** | **Genotype** |
|---|---|
| N2 | wild-type |
| JT191 | *daf-28(sa191)* |
| VS20 | *hjIs67[atgl-1p::atgl-1::GFP]* |
| ZD1094 | *daf-28(sa191);pek-1(qd351)* |
| ZD1124 | *daf-28(sa191);pek-1(qd350)* |
| ZD1125 | *daf-28(sa191qd331)* |
| ZD1126 | *eif-2Ba(qd333);daf-28(sa191)* |
| ZD1167 | *eif-2Ba(qd358);daf-28(sa191)* |
| ZD1193 | *eif-2Ba(qd356);daf-28(sa191)* |
| ZD1197 | *eif-2Ba(qd359);daf-28(sa191)* |
| ZD1198 | *eif-2Ba(qd361);daf-28(sa191)* |
| ZD1207 | *eif-2Ba(qd334);daf-28(sa191)* |
| ZD1208 | *daf-28(sa191);pek-1(qd341)* |
| ZD1213 | *daf-28(sa191);pek-1(qd342)* |
| ZD1214 | *daf-28(sa191);pek-1(qd343)* |
| ZD1215 | *daf-28(sa191);pek-1(qd348)* |
| ZD1217 | *eif-2Ba(qd333)* |
| ZD1219 | *daf-28(sa191);pek-1(qd345)* |
| ZD1220 | *daf-28(sa191);pek-1(qd344)* |
| ZD1223 | *daf-28(sa191);pek-1(qd339)* |
| ZD1236 | *eif-2Ba(qd363);daf-28(sa191)* |
| ZD1264 | *daf-28(sa191);pek-1(qd353)* |
| ZD1265 | *daf-28(sa191);pek-1(qd347)* |
| ZD1275 | *eif-2Ba(qd357);daf-28(sa191)* |
| ZD1276 | *eif-2Ba(qd362);daf-28(sa191)* |
| ZD1277 | *daf-28(sa191qd330)* |
| ZD1280 | *eif-2Ba(qd360);daf-28(sa191)* |
| ZD1281 | *daf-28(sa191);pek-1(qd346)* |
| ZD1346 | *eif-2Ba(qd335);daf-28(sa191)* |
| ZD1347 | *eif-2Ba(pk720);daf-28(sa191)* |
| ZD1355 | *daf-28(sa191);pek-1(qd340)* |
| ZD1356 | *erp-44.1(qd332);daf-28(sa191)* |
| ZD1357 | *daf-28(sa191);pek-1(qd349)* |
| ZD1362 | *daf-28(sa191);pek-1(qd354)* |
| ZD1365 | *daf-28(sa191);pek-1(qd337)* |
| ZD1373 | *daf-28(sa191);pek-1(qd352)* |
| ZD1374 | *erp-44.1(qd317);daf-28(sa191)* |
| ZD1377 | *erp-44.1(qd329);daf-28(sa191)* |

| | |
|---|---|
| ZD1392 | *daf-28(sa191);pek-1(qd355)* |
| ZD1451 | *erp-44.1(qd317);daf-28(sa191);zcIs4[hsp-4p::GFP]* |
| ZD1503 | *erp-44.1(gk411949);daf-28(sa191)* |
| ZD1565 | *eif-2a(qd338);daf-28(sa191)* |
| ZD1684 | *qdIs10[gpa-4p::eIF2.1(S49D)]* |
| ZD1752 | *daf-2(e1368);qdIs10* |
| ZD1788 | *eif-2c(qd336)* |
| ZD1789 | *hjIs67;qdIs10* |
| ZD1866 | *eif-2a(qd338)* |
| ZD1869 | *eif-2Ba(qd333);qdIs10* |
| ZD1870 | *eif-2c(qd336);qdIs10* |
| ZD1871 | *qdEx51[gpa-4p::eIF2.1(S49A)]* |
| ZD1885 | *daf-7(e1372);qdIs10* |
| ZD1886 | *eif-2Ba(qd335);qdIs10* |
| ZD1991 | *eif-2Ba(qd335);daf-28(sa191);qdEx146[str-3p::eif-2Ba]* |
| ZD1992 | *eif-2Ba(qd335);daf-28(sa191);qdEx147[str-3p::eif-2Ba]* |
| ZD1993 | *eif-2Ba(qd335);daf-28(sa191);qdEx148[str-3p::eif-2Ba]* |
| ZD2005 | *eif-2Ba(qd335)* |
| ZD738 | *mgIs40[daf-28p::GFP];qdEx47[daf-7p::csp-1b] line #4* |
| ZD851 | *eat-2(ad1116)* |
| ZD965 | *qdEx145[gpa-4p::eif-2a(S49D)]* |
| ZD1871 | *qdEx51[gpa-4p::eif-2a(S49A)]* |

**Table S3: Strains used in the study**