

Expanded View Figures

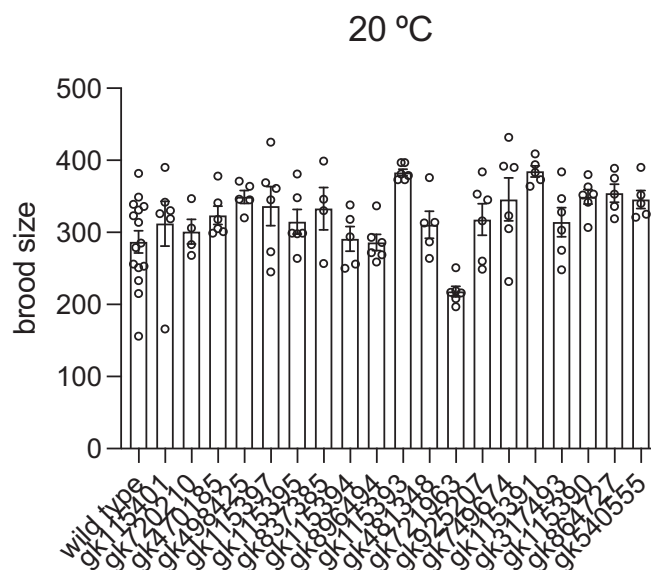


Figure EV1. *ego-1* gk mutants show normal brood size.

Brood size analysis of the indicated mutant animals that were cultured at 20 °C. The number of biological replicates (*n*) for each genotype was as follows: *gk115401*, *gk470185*, *gk115397*, *gk115395*, *gk896494*, *gk115393*, *gk721963*, *gk925207*, *gk749674*, *gk317493*, and *gk115390* (*n* = 6); *gk720210* and *gk837385* (*n* = 4); *gk498425*, *gk115394*, *gk481348*, *gk115391*, *gk864727*, and *gk540555* (*n* = 5). Error bars represent mean ± SEM. Wild-type data are shown for reference and are the same as those shown in Fig. 1D. Source data are available online for this figure.

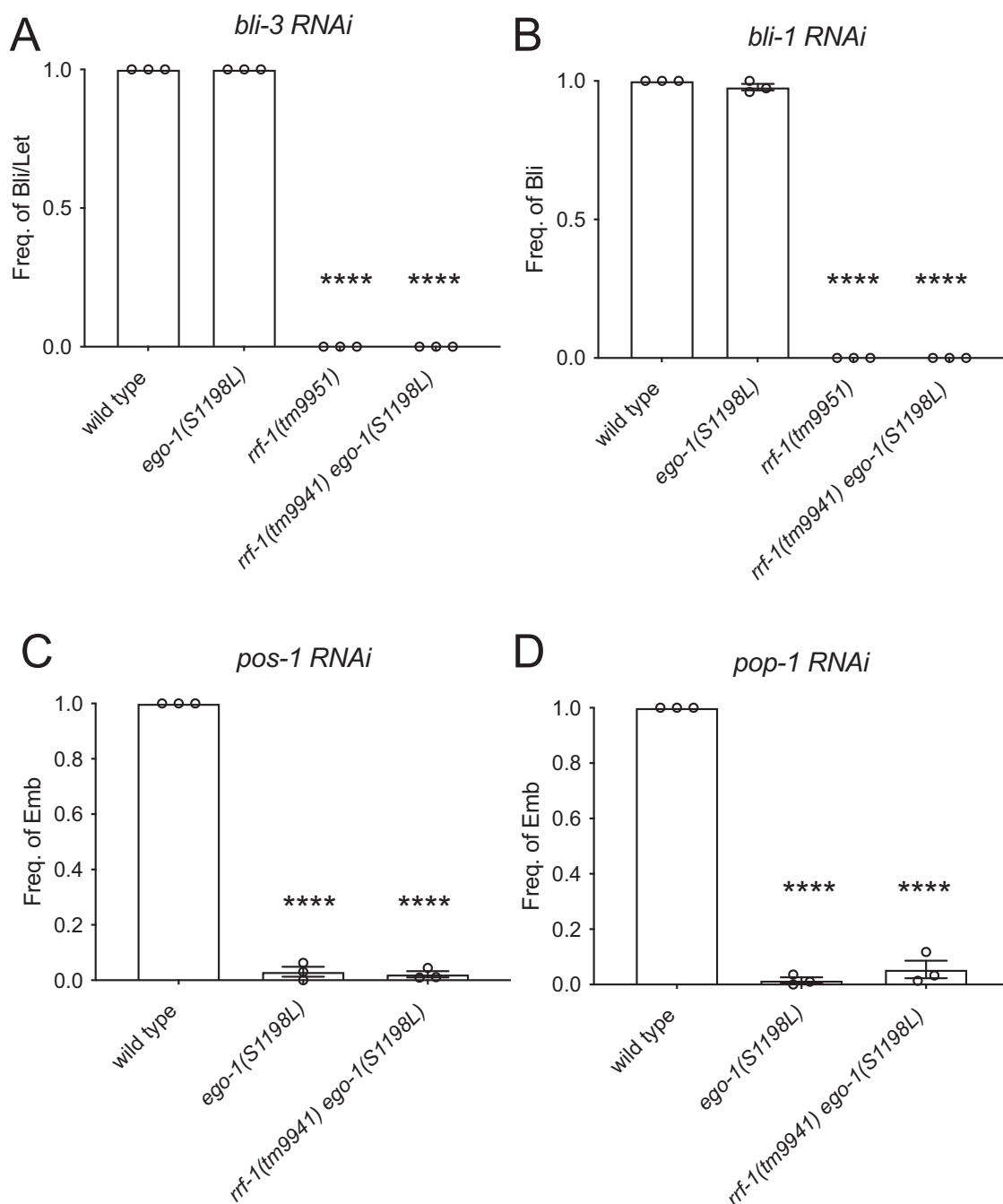


Figure EV2. *ego-1(S1198L)* is sensitive to somatic RNAi.

(A, B) Quantification of the frequency of the indicated mutant animals showing the expected phenotype under *bli-3* (A) and *bli-1* (B) feeding RNAi. Error bars represent mean $\pm$ SEM. Data represent values from three technical replicates. (C, D) Quantification of the frequency of indicated mutant animals showing the Emb phenotype under *pos-1* (C) and *pop-1* (D) feeding RNAi. Error bars represent mean $\pm$ SEM. Data represent values from three technical replicates. *P* values were determined using one-way ANOVA with Dunnett's multiple-comparisons test, compared to wildtype. **** indicates $p < 0.0001$. *P* values for specific comparisons were as follows: (A) *ego-1(S1198L)*, $p = 0.4044$; *rrf-1(tm9951)*, $p < 0.0001$; and *rrf-1(tm9941) ego-1(S1198L)*, $p < 0.0001$. (B) *ego-1(S1198L)*, $p = 0.0689$; *rrf-1(tm9951)*, $p < 0.0001$; and *rrf-1(tm9941) ego-1(S1198L)*, $p < 0.0001$. (C) *ego-1(S1198L)*, $p < 0.0001$; *rrf-1(tm9941) ego-1(S1198L)*, $p < 0.0001$. (D) *ego-1(S1198L)*, $p < 0.0001$; and *rrf-1(tm9941) ego-1(S1198L)*, $p < 0.0001$. Source data are available online for this figure.

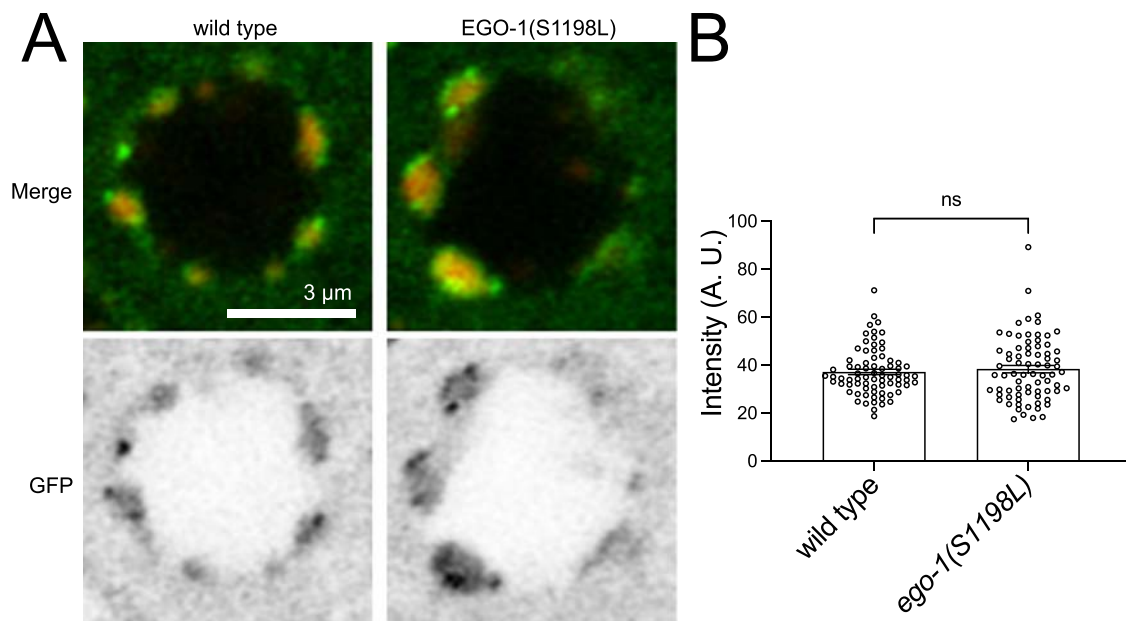


Figure EV3. GFP::EGO-1(S1198L) shows similar expression to that of GFP::EGO-1.

(A) Super-resolution image of a single pachytene nucleus of wild-type EGO-1 and EGO-1(S1198L) tagged with GFP (green) in late pachytene cells of 1-day-old adults. tagRFP::PGL-1 was used as a germ granule marker (red). (B) Bar graph shows the quantification of granular GFP::EGO-1 and GFP::EGO-1(S1198L) fluorescence signal intensity, with $n = 75$ for each genotype (25 animals, three ROIs (Nucleus and surrounded region) each). P values were determined using a Student's t -test. ns indicates not significant. Source data are available online for this figure.

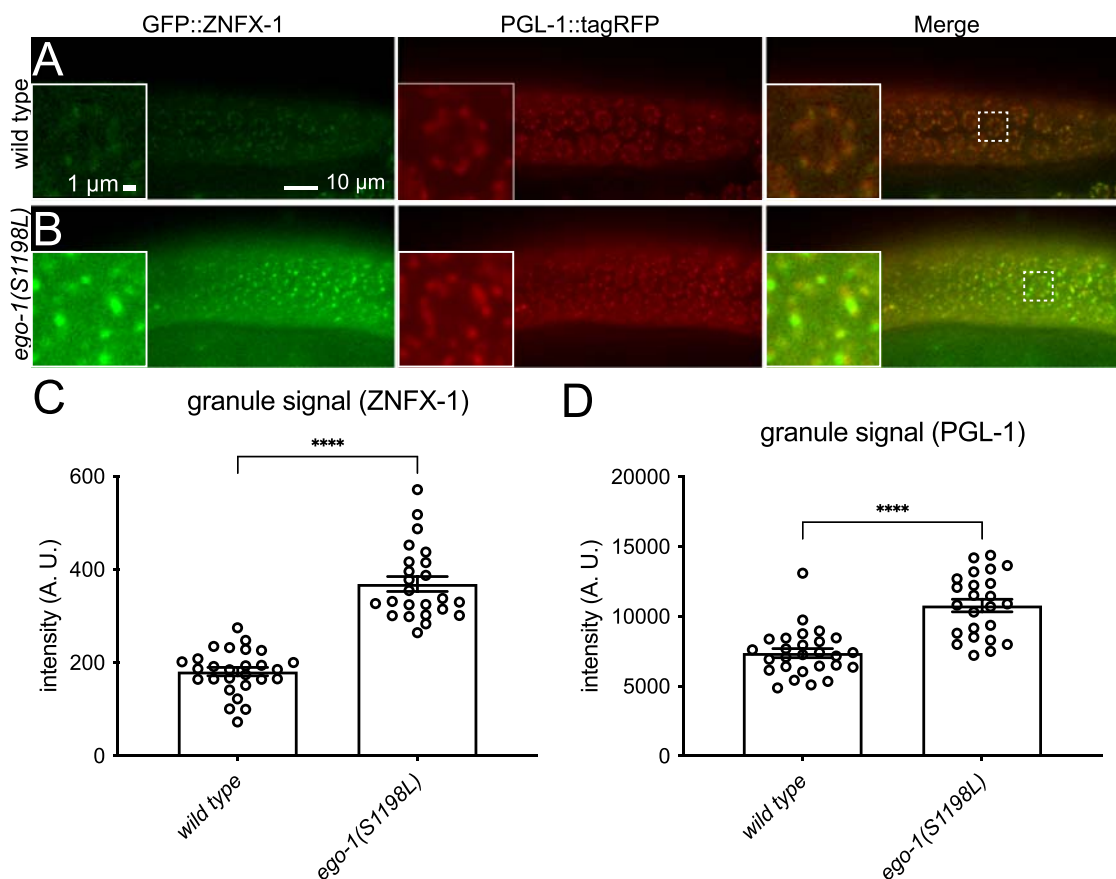


Figure EV4. Expression of GFP::ZNFX-1 and PGL-1::tagRFP in *ego-1(S1198L)*.

(A, B) Fluorescence images of germ cells showing the localization of GFP::ZNFX-1 and PGL-1::tagRFP in the wild-type (A) and *ego-1(S1198L)* (B). (C, D) Bar graphs show the quantification of granular GFP::ZNFX-1 (C) and PGL-1::tagRFP (D) fluorescence signal intensity. Error bars represent mean $\pm$ SEM. Figure EV4C shows data for wildtype ($n = 27$) and *ego-1(S1198L)* ($n = 24$) animals. Figure EV4D shows data for wildtype ($n = 24$) and *ego-1(S1198L)* ($n = 21$) animals. Statistical significance was determined using two-tailed unpaired *t*-tests. In all cases, the difference was significant (**** $p < 0.0001$). Source data are available online for this figure.

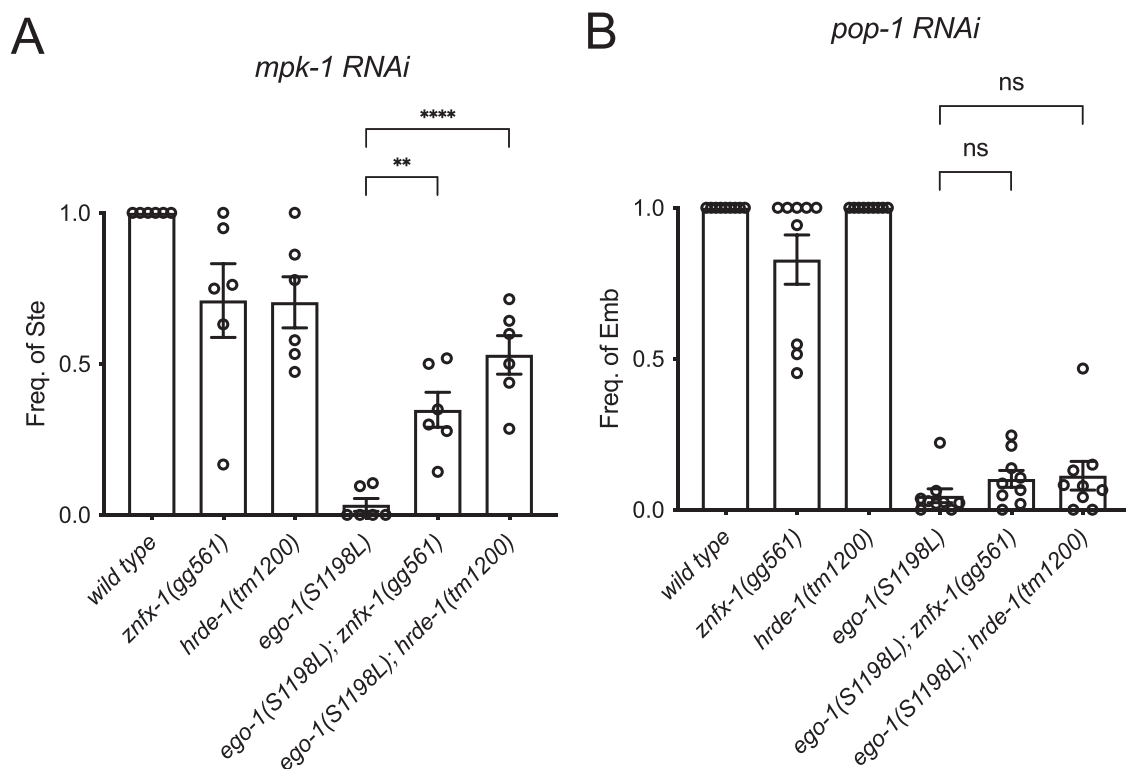


Figure EV5. Defects in germline exo-RNAi targeting *mpk-1* in *ego-1(S1198L)* are suppressed by *hrde-1* and *znfx-1* mutations.

(A) Quantification of the frequency of indicated mutant animals showing the Ste phenotype under *mpk-1* feeding RNAi. Error bars represent mean $\pm$ SEM. Data represent values from six technical replicates. *P* values were determined using one-way ANOVA with Dunnett's multiple-comparisons test. **** and ** indicate *p* value < 0.001 and < 0.01 (0.0074). (B) Quantification of the frequency of indicated mutant animals showing the Emb phenotype under *pop-1* feeding RNAi. Error bars represent mean $\pm$ SEM. Data represent values from nine technical replicates. *P* values were determined using one-way ANOVA with Dunnett's multiple-comparisons test compared to wildtype. **** indicates *p* value < 0.001 . *p* < 0.0001 for V1128E, R539Q, and S1198L. For C823Y, *p* = 0.7426. Source data are available online for this figure.

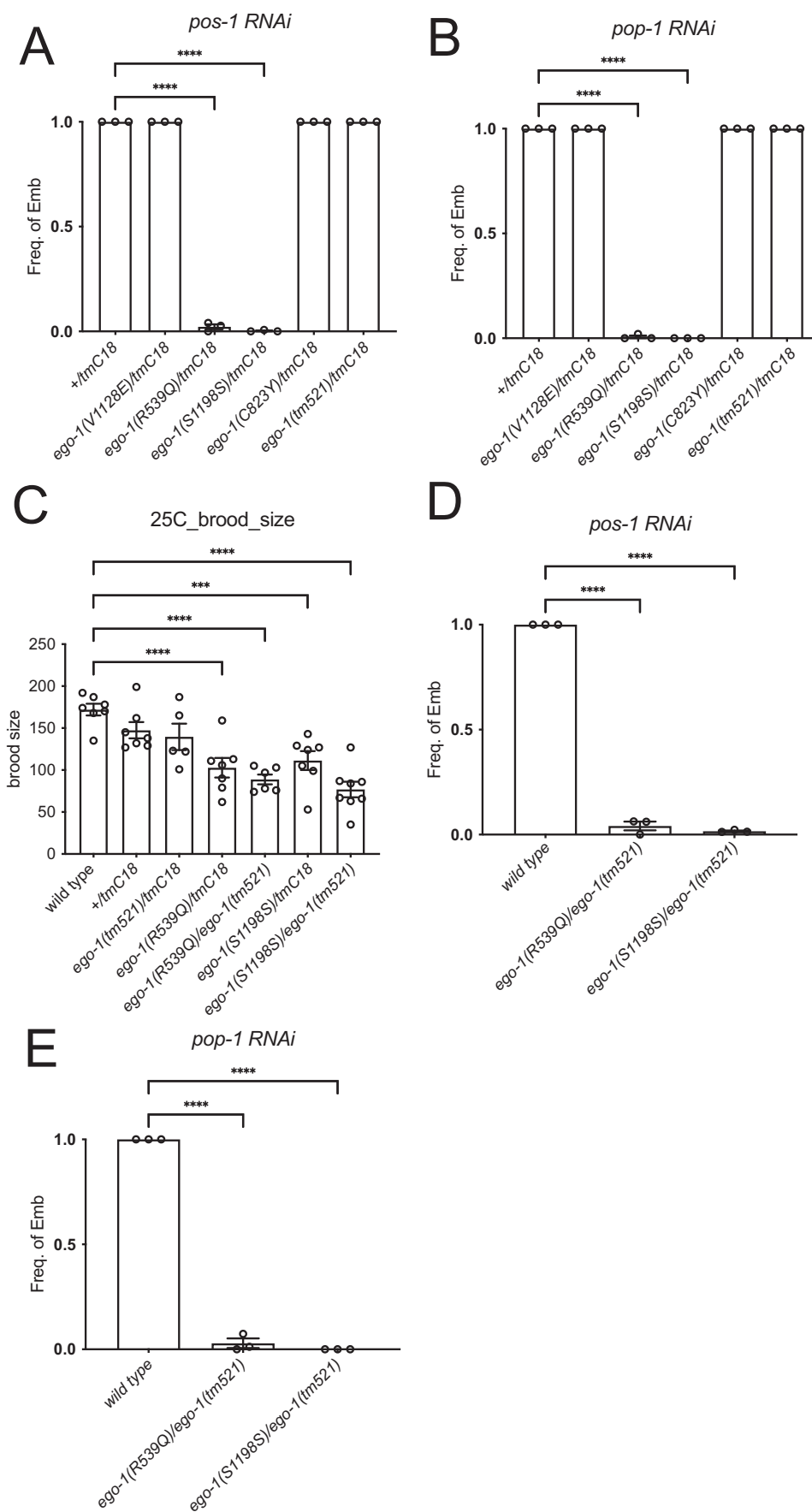
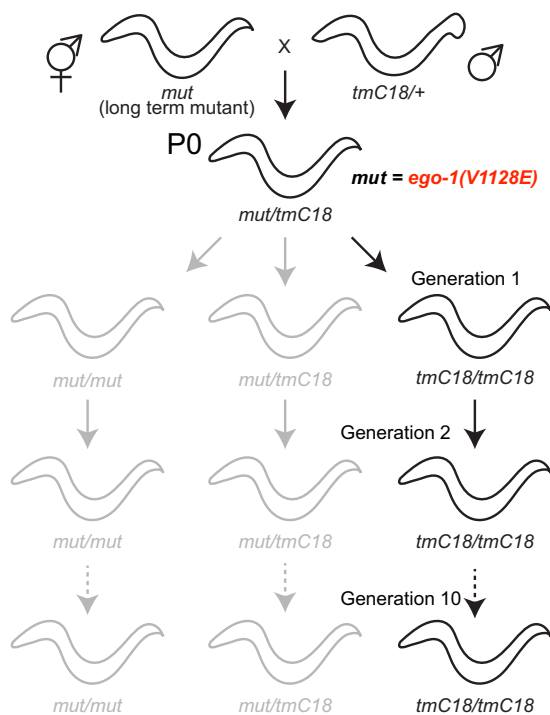
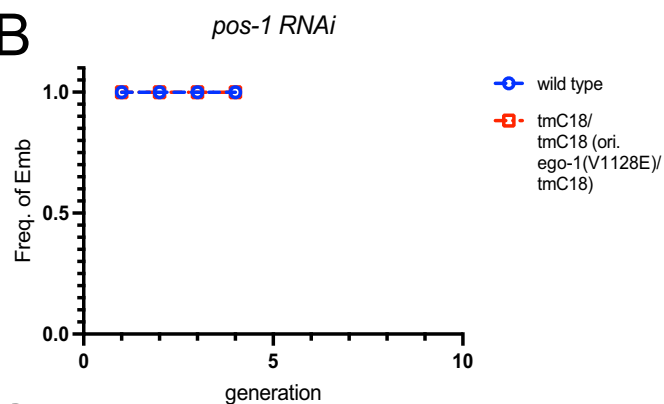
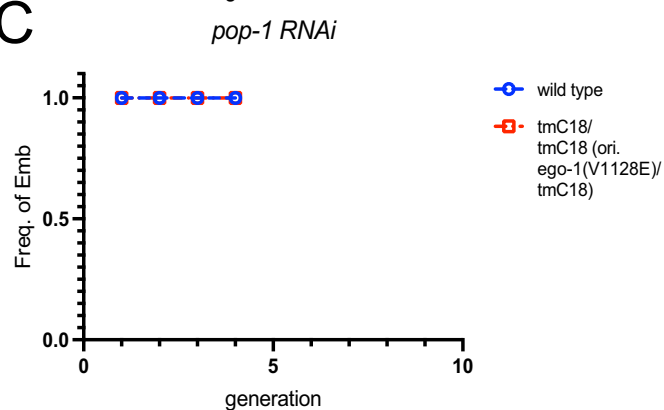
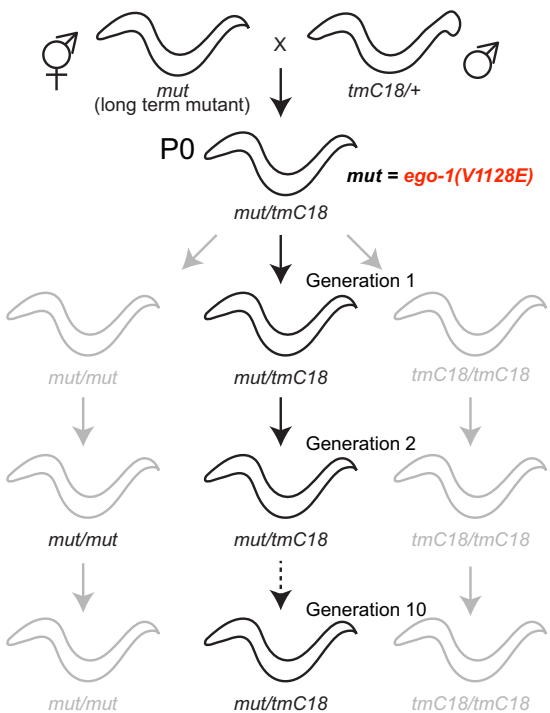
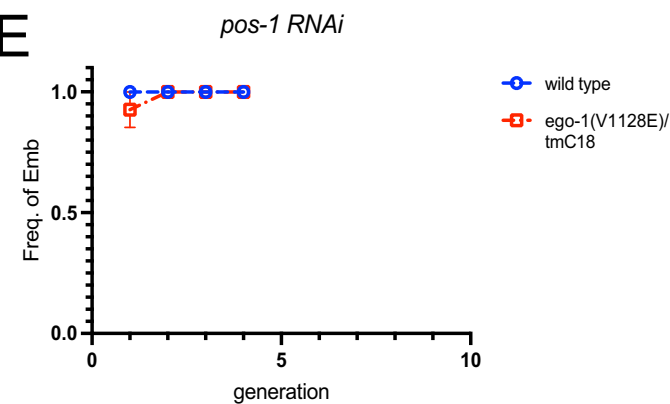
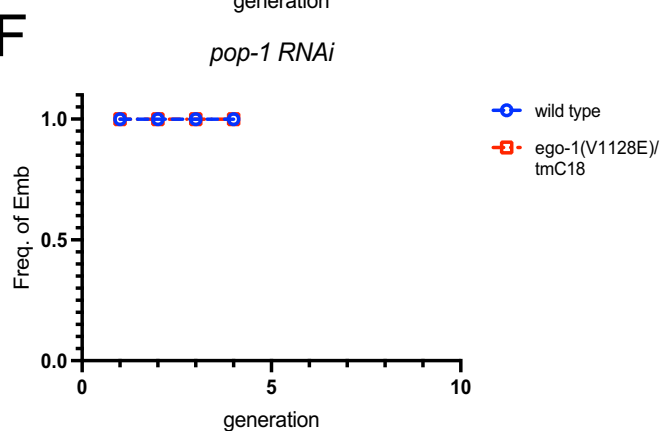


Figure EV6. RNAi-defective phenotype caused by *ego-1(R539Q)* and *ego-1(S1198L)* heterozygous animals.

(A) Quantification of the frequency of indicated mutant animals showing the Emb phenotype under *pos-1* feeding RNAi. Error bars represent mean $\pm$ SEM. Data represent values from three technical replicates. (B) Quantification of the frequency of indicated mutant animals showing the Emb phenotype under *pop-1* feeding RNAi. Error bars represent mean $\pm$ SEM. Data represent values from three technical replicates. In A and B, statistical analysis was performed using one-way ANOVA with Dunnett's multiple-comparisons test, compared to the $+/tmC18$ control. **** indicates $p < 0.0001$. (A) Significant differences were observed for *R539Q/tmC18* and *S1198L/tmC18* ($p < 0.0001$ for both). No significant differences were found for *V1128E/tmC18*, *C823Y/tmC18*, or *ego-1(tm521)/tmC18* ($p > 0.9999$ for all). (B) Significant differences were observed for *R539Q/tmC18* and *S1198L/tmC18* ($p < 0.0001$ for both). No significant differences were found for *V1128E/tmC18*, *C823Y/tmC18*, or *ego-1(tm521)/tmC18* ($p > 0.9999$ for all). (C) Brood size analysis of the indicated mutant animals that were cultured at 25 °C. Error bars represent mean $\pm$ SEM. Data represent values from wildtype ($n = 7$), $+/tmC18$ ($n = 7$), *ego-1(tm521)/tmC18* ($n = 5$), *R539Q/tmC18* ($n = 7$), *R539Q/ego-1(tm521)* ($n = 6$), and *S1198L/tmC18* ($n = 7$). Statistical analysis was performed using one-way ANOVA with Dunnett's multiple-comparisons test, compared to wild-type control. *** indicates $p < 0.001$ and **** indicates $p < 0.0001$. Significant differences were observed for all *R539Q* and *S1198L* genotypes shown. No significant differences were found for $+/tmC18$ or *ego-1(tm521)/tmC18*. (D) Quantification of the frequency of indicated mutant animals showing the Emb phenotype under *pos-1* feeding RNAi. Error bars represent mean $\pm$ SEM. Data represent values from three technical replicates. (E) Quantification of the frequency of indicated mutant animals showing the Emb phenotype under *pop-1* feeding RNAi. Error bars represent mean $\pm$ SEM. Data represent values from three technical replicates. In (D, E), statistical analysis was performed using one-way ANOVA with Dunnett's multiple-comparisons test, compared to wildtype. (D) *** indicates $p < 0.001$ and **** indicates $p < 0.0001$. Significant differences were observed for all *R539Q* and *S1198L* genotypes shown. No significant differences were found for $+/tmC18$ or *ego-1(tm521)/tmC18*. (E) **** indicates $p < 0.0001$. *P* values for specific comparisons were as follows: *R539Q/ego-1(tm521)*, $p < 0.0001$; and *S1198L/ego-1(tm521)*, $p < 0.0001$. Source data are available online for this figure.

A**B****C****D****E****F**

**Figure EV7. Transgenerational effects of *ego-1(V1128E)* on germline *exo-RNAi*.**

(A, D). Schematics of genetic crosses. P0 is the first generation in which cross-progenies are selected. (B, C) Quantification of the frequency of the indicated mutant animals showing the Emb phenotype under *pos-1* (B) and *pop-1* (C) feeding RNAi in the indicated generation. Data represent values from four biological replicates. These experiments were conducted using a similar experimental setup as in Fig. 5. Source data are available online for this figure.

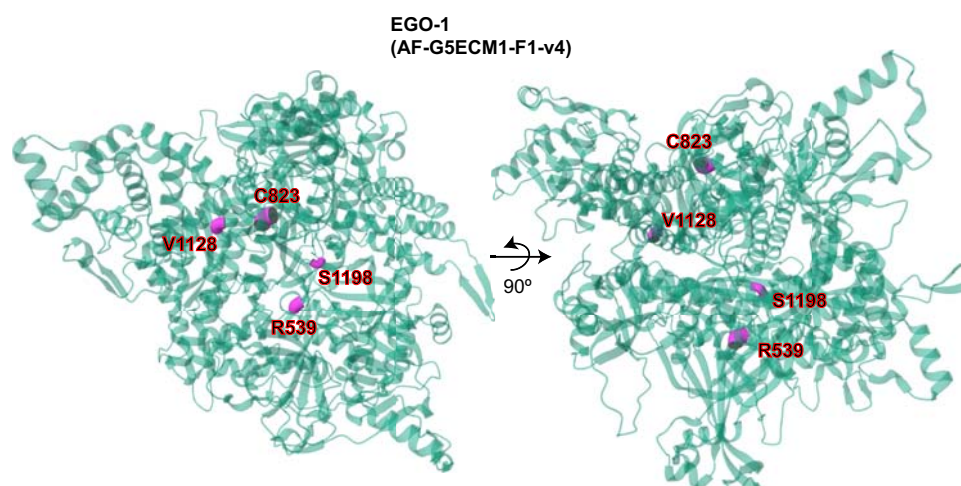


Figure EV8. Structure of wild-type EGO-1 with highlighted mutant sites (C623, V1128, R539, and S1198).

The 3D structure of the EGO-1 protein was predicted by AlphaFold. Image was obtained from Uniprot.