

Supplemental Materials for

The dynamics of P granule liquid droplets are regulated by the *C. elegans* germline RNA helicase GLH-1 *via* its ATP hydrolysis cycle

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List of Supplementary Items:

Figure S1. Germline helicases promote the formation of P granules

Figure S2. P granules form enlarged and less dynamic aggregates in the *glh-1*
DQAD mutant

Figure S3. Analyses of the GLH-1 and PRG-1 complexes in *glh* mutants

Figure S4. The N terminal FGG repeats in GLH-1 promote its nuclear anchoring

Table S1. *C. elegans* strains used in this study*

Table S2. Oligonucleotide sequences used in this study*

Supplemental file 1. *glh-1* and *glh-4* alleles created in this study

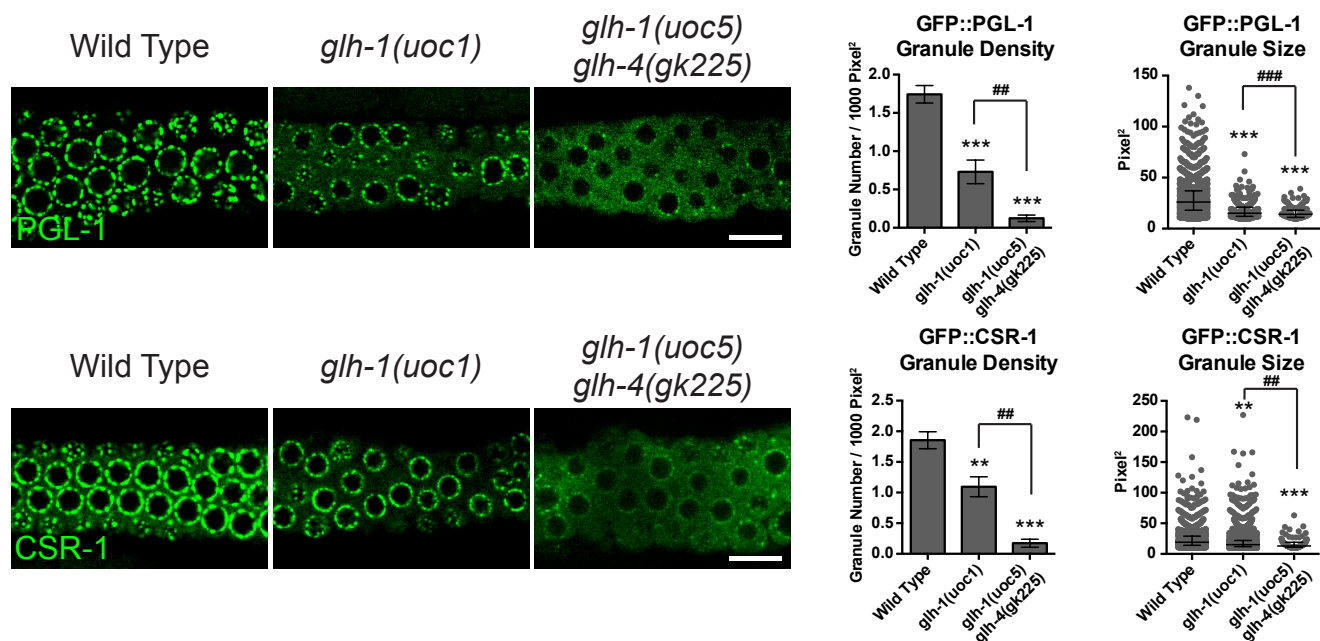
Supplemental file 2. MS results of GLH-1 complex*

Supplemental file 3. MS results of PRG-1 complex*

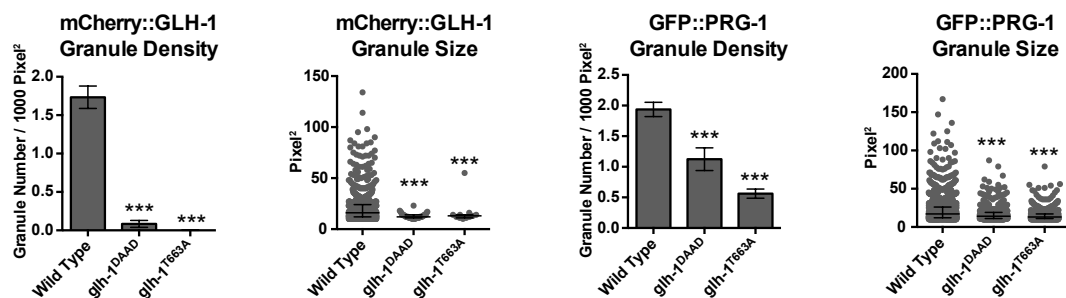
Movie S1-6* & legend

*** Files independently uploaded to figshare**

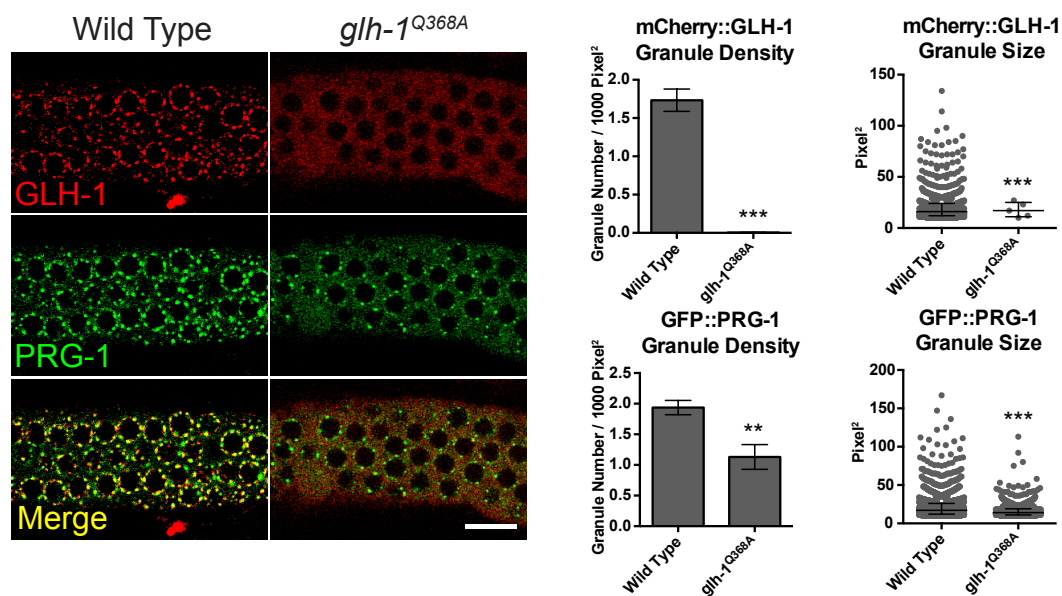
A



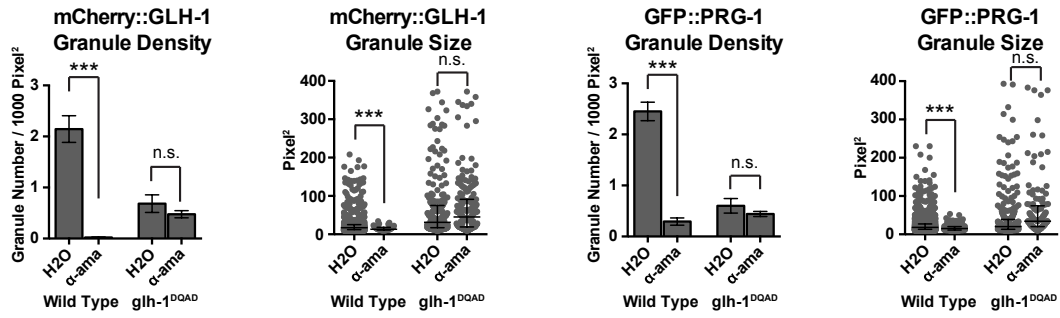
B



C



D



E

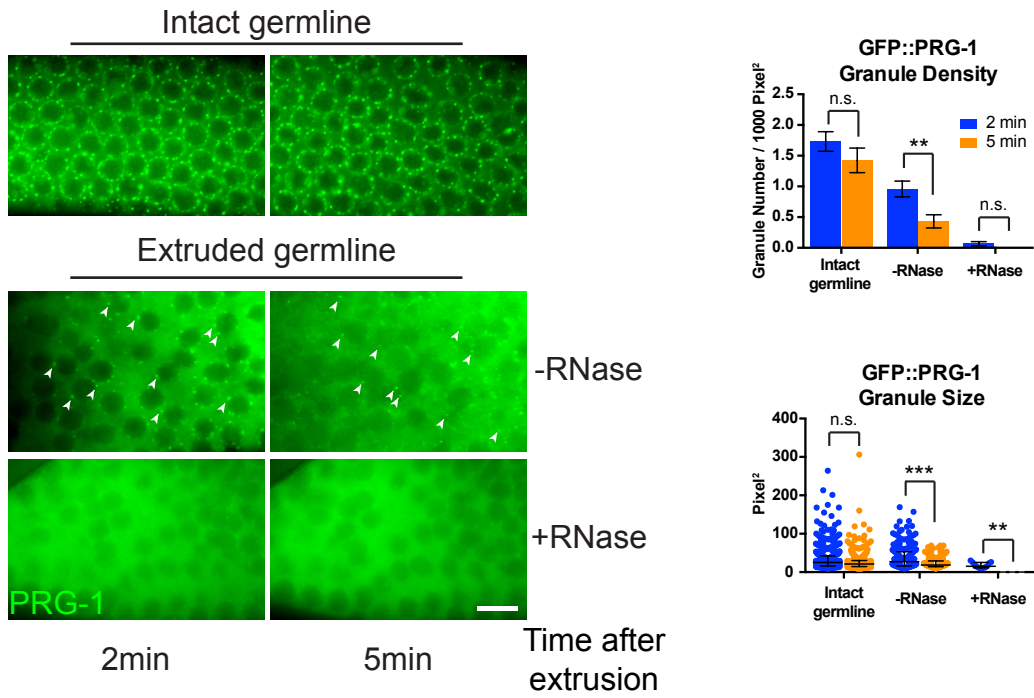


Figure S1. Germline helicases promote the formation of P granules.

(A) GLH-1 and GLH-4 act redundantly to promote the formation of P granules.

Fluorescent micrographs show the localization of the P granule component GFP::PGL-1 and GFP::CSR-1 in the indicated strains (left). Image analyses show the granule density and granule size of the GFP::PGL-1 or GFP::CSR-1 in the indicated strains. *uoc1*, *uoc5* and *gk225* are null alleles of the indicated genes. Scale bar: 10 micrometers. For the measurements of granule density, the average and the standard deviation are indicated. For the measurements of granule size, the median, 25 and 75 percentile are indicated. Note that unless specifically labeled, the significance test was measured between wild type worms and the indicated strains.

*** or ###: $p < 0.001$. ** or ##: $p < 0.01$.

(B) Image analysis shows the granule density and granule size of the mCherry::GLH-1 and GFP::PRG-1 in the indicated strains. The significance test was measured between wild type worms and the indicated strains. ***: $p < 0.001$.

(C) Fluorescent micrographs show the localization of mCherry::GLH-1 and GFP::PRG-1 in the indicated strains (left). Scale bar: 10 micrometers. The significance test was measured between wild type worms and the indicated strains. ***: $p < 0.001$. **: $p < 0.01$.

(D) Image analysis show the granule density and granule size of the mCherry::GLH-1 or GFP::PRG-1 in the indicated strains. Scale bar: 10 micrometers. The significance test was measured between wild type worms and the indicated strains. ***: $p < 0.001$. n.s.: non-significant

(E) RNase treatment promotes the *ex vivo* disassembly of PRG-1 granules.

Fluorescent micrographs (left) and image analysis (right) show GFP::PRG-1 granules at the indicated time after germlines were extruded in the RNase containing buffer or in the control buffer. Arrow heads indicate examples of remaining P granules after extrusion. Scale bar: 10 micrometer. The significance

test was measured between wild type worms and the indicated strains. ***: $p < 0.001$. n.s.: non-significant. **: $p < 0.01$.

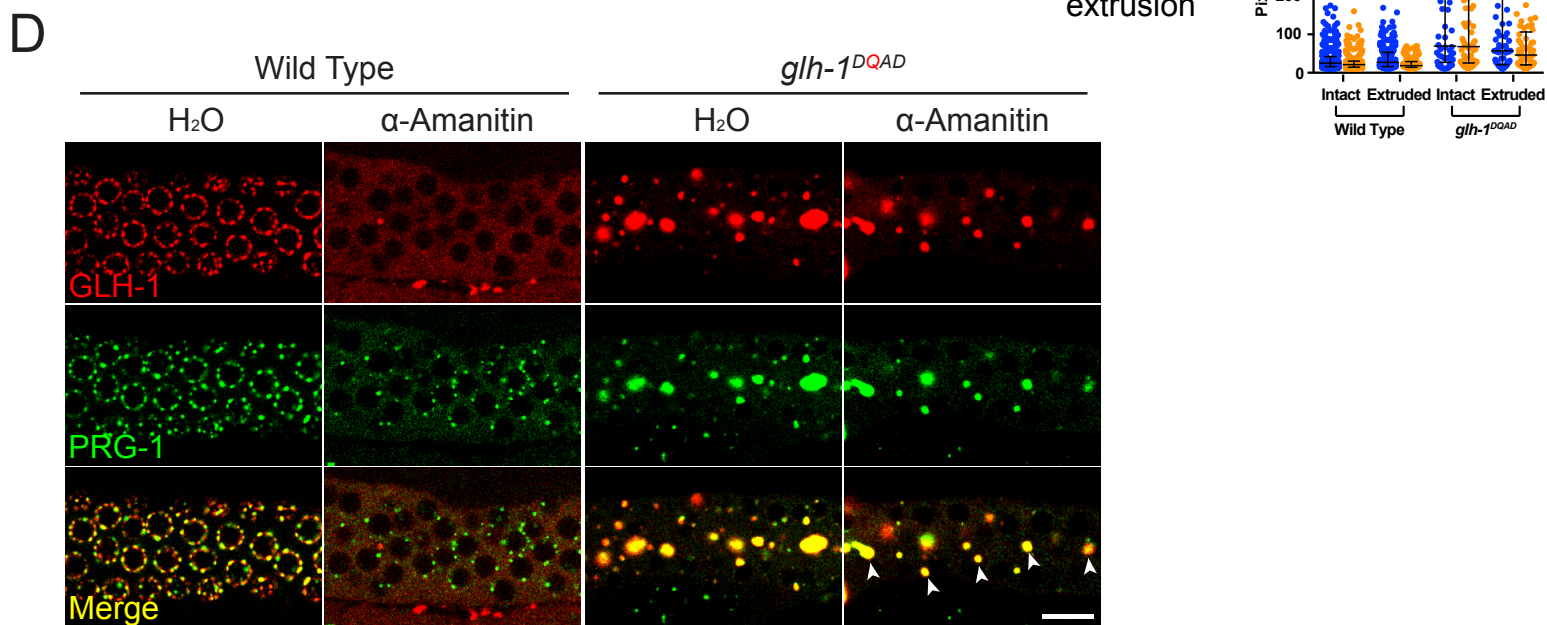
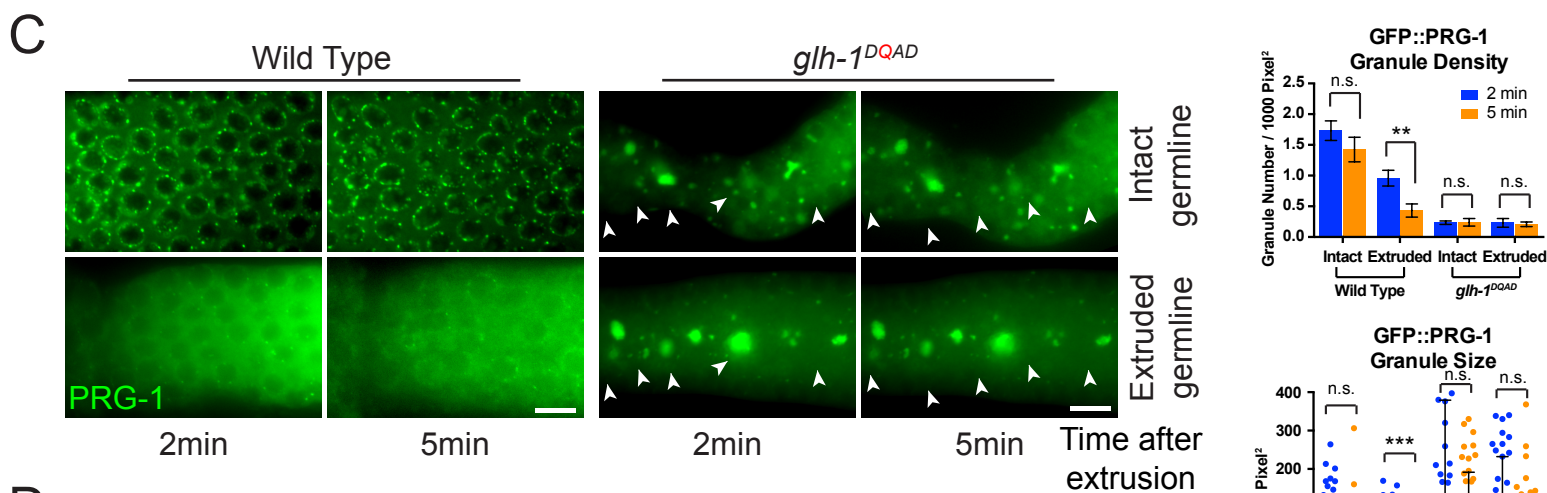
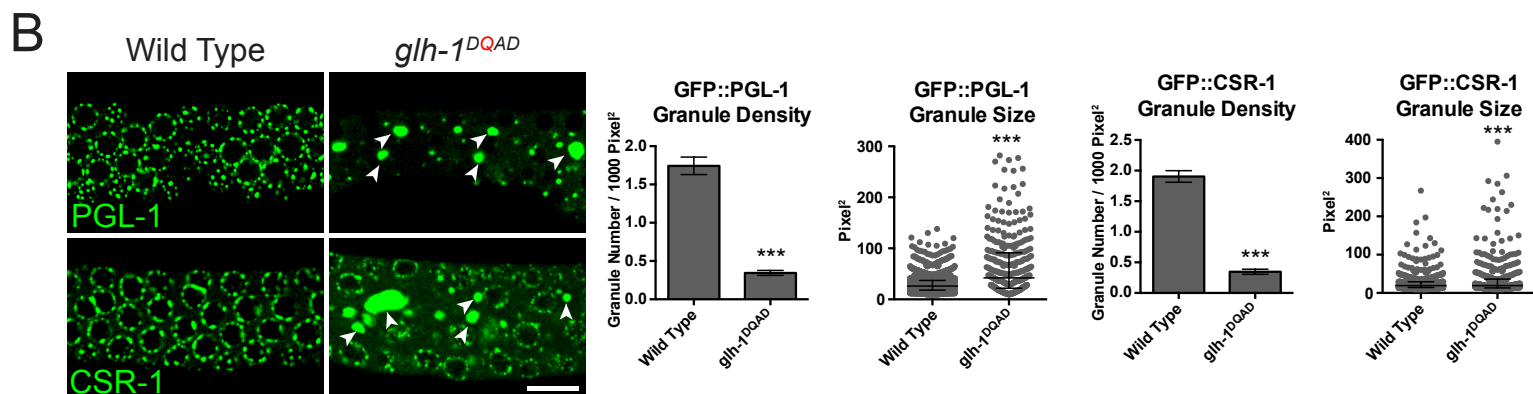
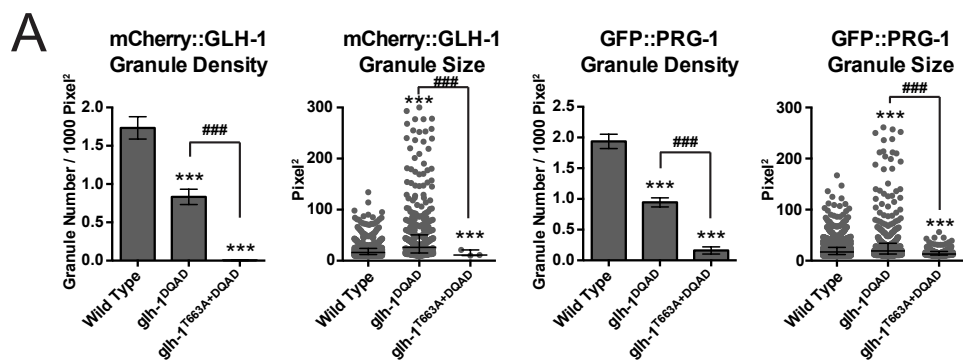
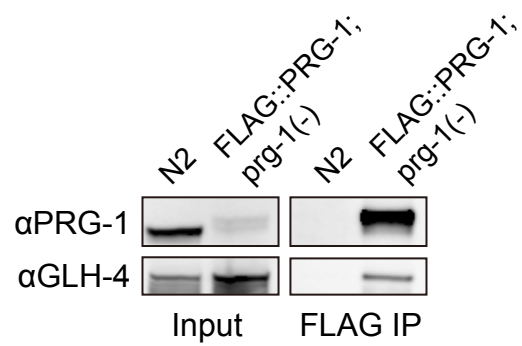


Figure S2. P granules form enlarged and less dynamic aggregates in the *glh-1* DQAD mutant.

- (A) Image analysis shows the granule density and granule size of the mCherry::GLH-1 or GFP::PRG-1 in the indicated strains. *** or ###: $p < 0.001$. Note that unless specifically labeled, the significance test was measured between wild type worms and the indicated strains.
- (B) Fluorescent micrographs show that components of P granules, such as GFP::PGL-1, and GFP::CSR-1, are abnormally aggregated in the *glh-1*^{DQAD} mutant. Scale bar: 10 micrometers. The significance test was measured between wild type worms and the indicated strains. ***: $p < 0.001$.
- (C) PRG-1 granules in the GLH-1 DQAD mutant are more stable than wild type in solution. Fluorescent micrographs show GFP::PRG-1 granules at the indicated time after extruding germline from the body cavity. Arrows indicate the remaining P granules after extrusion in the specified strains. Scale bar: 10 micrometer. ***: $p < 0.001$. **: $p < 0.01$. n.s.: non-significant.
- (D) The effect of α -Amanitin, an RNA polymerase II inhibitor, is seen on GLH-1 and PRG-1 granules in the indicated strains. Images were taken 5 hours after the injection of α -Amanitin. Scale bar: 10 micrometers.

A



B

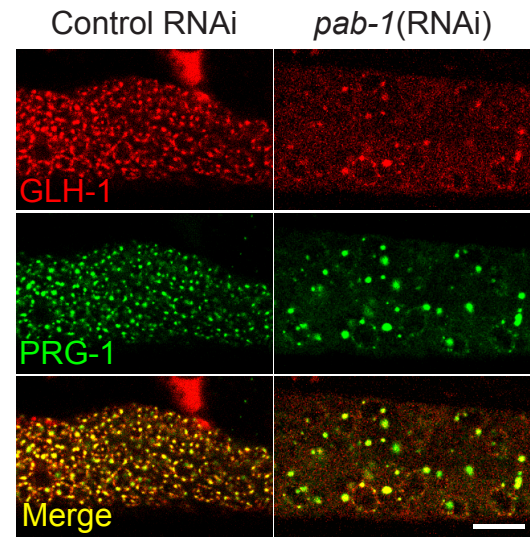
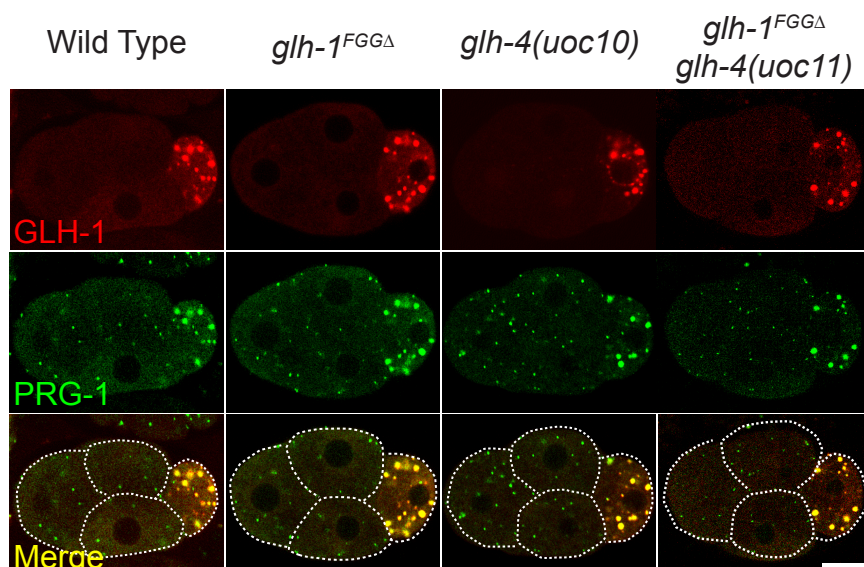


Figure S3. Analyses of the GLH-1 and PRG-1 complexes in *glh* mutants.

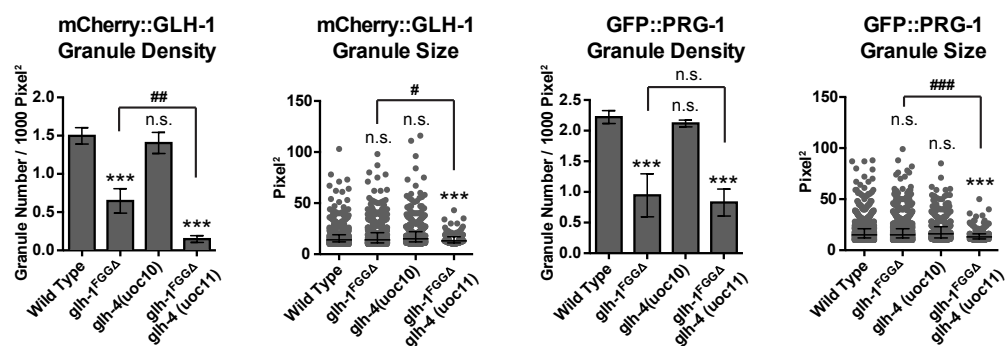
(A) Western blot analyses show the interaction between PRG-1 and GLH-4 in wild type animals.

(B) The localization of mCherry::GLH-1 and GFP::PRG-1 is seen in *pab-1* RNAi treated animals. Scale bar: 10 micrometers.

A



B



C

25 °C

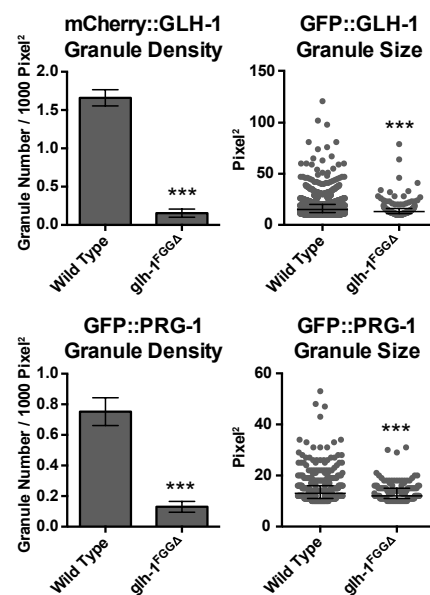
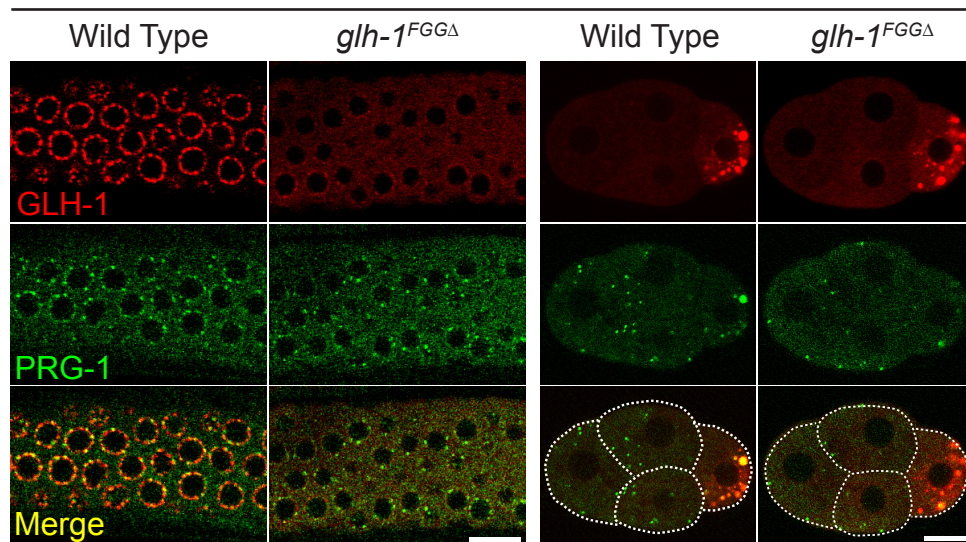
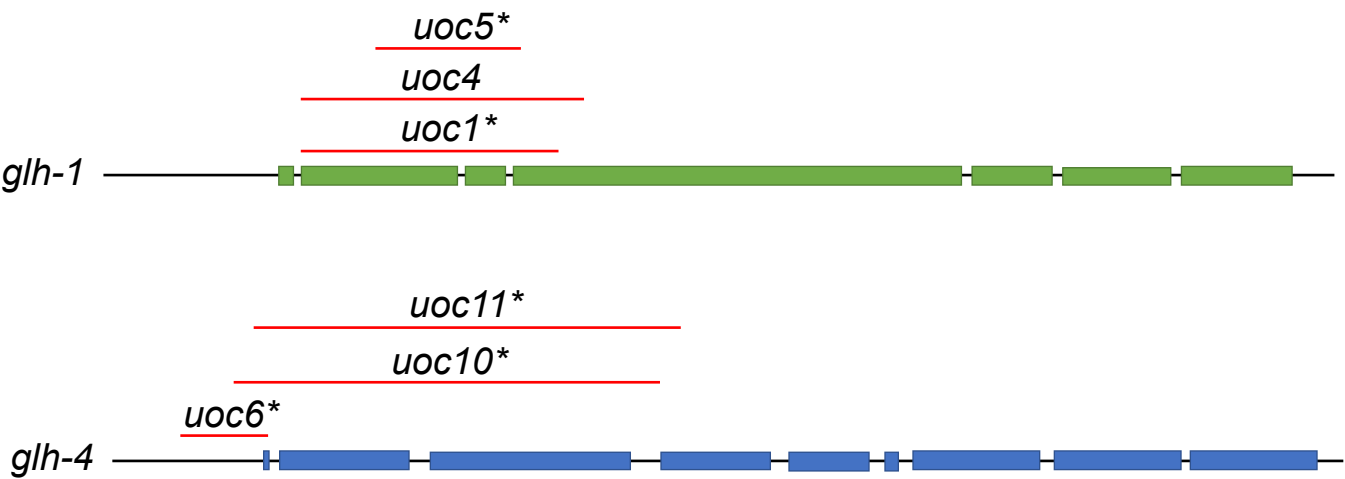


Figure S4. The N terminal FGG repeats in GLH-1 promote its nuclear anchoring.

- (A) Images show the localization of mCherry::GLH-1 and GFP::PRG-1 in the 4-cell embryos of the indicated strains grown at their optimal growth temperature, 20°C. The arrow indicates the cytoplasmic P granules in the 4-cell embryo. Scale bar: 10 micrometers.
- (B) Image analysis show the granule density and granule size of the mCherry::GLH-1 or GFP::PRG-1 of the adult germline in the indicated strains. Note that unless specifically labeled, the significance test was measured between wild type worms and the indicated strains. ***: $p < 0.001$. **: $p < 0.01$. ####: $p < 0.001$. ##: $p < 0.01$. # : $P < 0.05$. n.s.: non-significant.
- (C) An image shows the localization of mCherry::GLH-1 and GFP::PRG-1 in the adult germline (left) and in the four-cell embryo (right) of the indicated strains grown at 25°C. Scale bar: 10 micrometers. The significance test was measured between wild type worms and the indicated strains. ***: $p < 0.001$.

Supplementary File 1



* Out of frame deletion that leads to a premature stop codon

Deletion

Insertion

glh-1(uoc1)

gtgatagcgaaagtgctgctaagggtgagttttatgttgaactttccaccggtttatgttattataaaactttatcagccaaaactggatt
cggtag(713_bp_deleted)ggtaggggacggtaggtttggtagggcgaagaacgtggtccaatgaaatgttcaactgtaaaggc
gaggacatcgctctgctgaatgtccggagccac

glh-1(uoc4)

tggtaggagtgatagcgaaagtgctgctaagggtgagttttatgttgaactttccaccggtttatgttattataaaactttatcagccaaa
actgga(748_bp_deleted)gaagaacgtggtccaatgaaatgttcaactgtaaaggcgaggacatcgctctgctgaatgtccgg
agccaccccgtaggtttcaattgtggcgagc

glh-1(uoc5)

ggatctggattcggtaggaaacactggcggatctggattcggtaggagaaagactggcgggttctggattggaggtggaaatacttgt
ggatccggct(443_bp_deleted)acgcaagccgctgagggtcgactggtgattcggggcgaggagctggattggaaacaatg
gaggaaatgacggtttcggtaggggacggtaggttttgg

glh-4(uoc6)

atcacctgtgaactcccgccaaccgccagccgtgacctccaccgctacatttacaacatcatg(479_bp_deleted)CTACAT
TTACAACacaaaactagatcatttgcaaattcttatttttttagttttccgatgacggatggggcgcgaggccgaagtgaaagtcgcg
gaagatgttccgaaaag

glh-4(uoc10)

gttttgagatgcattttcaactttctcgttgaaacgacaactatcctctaaactcgtgctattttctgttcgttctgcaaaaatttgattt
cagg(1892_bp_deleted)ggatggcgagagcgaccaagaggatgccataattgcggcgaagaaggcatatttcgaaagaatg
tgataagccaaaagtcccacgttttccttgccga

glh-4(uoc11)

tgaacgacaactatcctctaaactcgtgctattttctgttcgttctgcaaaaatttgatttcaggtataatcaaacacgactctgtatc
gtatca(1850_bp_deleted)gcgtggcaattgggtagggagagcgaccaagaggatgccataattgcggcgaagaaggatcat
atttcgaaagaatgtgataagccaaaagtcccacgt

Movie S1-6 legend:

Movie S1. Time-lapse of embryos expressing GFP::PRG-1 in the wild type GLH-1 background. Note that larger PRG-1 granules are formed in the cells of the germline lineage, but some PRG-1 puncta can be observed in cells of the somatic lineage.

Movie S2. Time-lapse of embryos expressing GFP::PRG-1 in the *glh-1 (uoc1)* null background. Note that P granules do not form, but somatic puncta can still be observed.

Movie S3. Time-lapse of eggs of wild type animals undergoing the oocyte-to-embryo transition in the gonad of a hermaphrodite expressing mCherry::GLH-1 and GFP::PRG-1

Movie S4. Time-lapse of a newly fertilized embryo of wild type animals expressing mCherry::GLH-1

Movie S5. Time-lapse of eggs of the *glh-1* DQAD mutant animals undergoing the oocyte-to-embryo transition in the gonad of a hermaphrodite expressing mCherry::GLH-1 and GFP::PRG-1

Movie S6. Time-lapse of a newly fertilized embryo of the *glh-1* DAAD mutant animals expressing mCherry::GLH-1