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Supplemental information

Condensate cooperativity underlies transgenerational gene silencing

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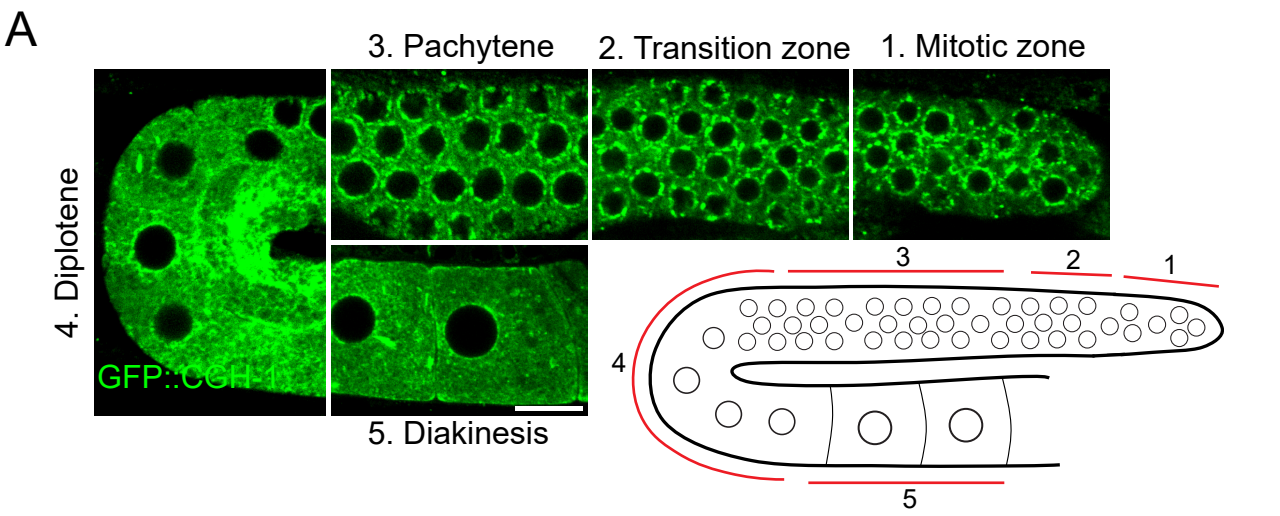
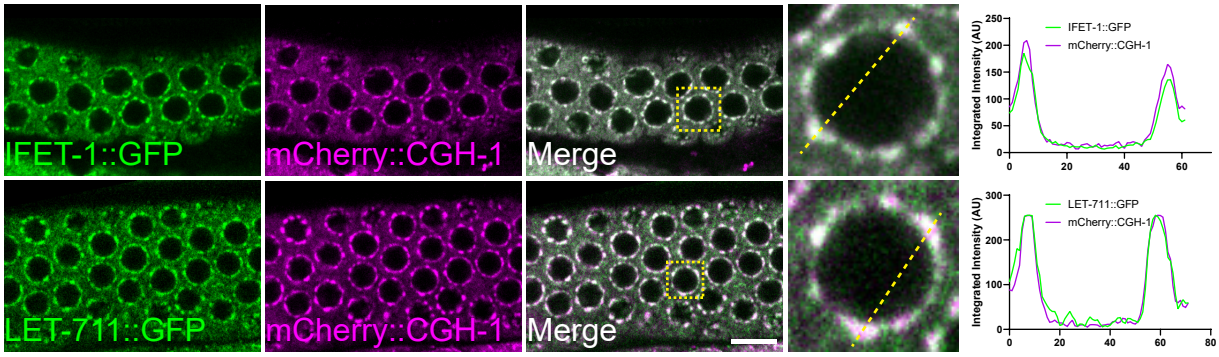
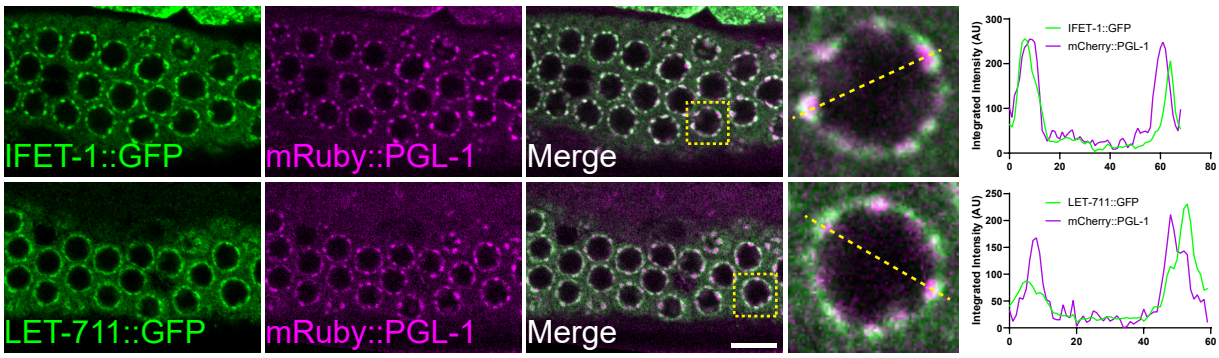
Figure S1


A
3. Pachytene
2. Transition zone
1. Mitotic zone
4. Diplotene
GFP::CGH-1
5. Diakinesis
3
2
1
4
5


B
IFET-1::GFP
mCherry::CGH-1
Merge
LET-711::GFP
mCherry::CGH-1
Merge
IFET-1::GFP
mCherry::CGH-1
Integrated Intensity (AU)
LET-711::GFP
mCherry::CGH-1
Integrated Intensity (AU)


C
IFET-1::GFP
mRuby::PGL-1
Merge
LET-711::GFP
mRuby::PGL-1
Merge
IFET-1::GFP
mCherry::PGL-1
Integrated Intensity (AU)
LET-711::GFP
mCherry::PGL-1
Integrated Intensity (AU)

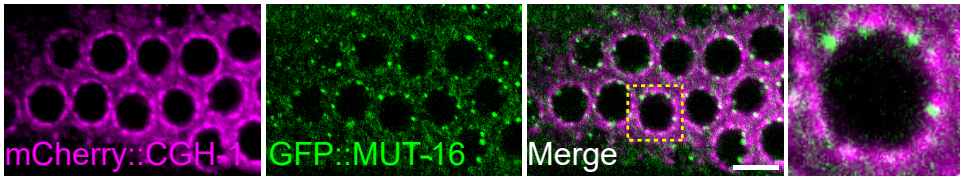

D
mCherry::CGH-1
GFP::MUT-16
Merge

Figure S1. P bodies form perinuclear condensates on the cytoplasmic side of germ granules in the germline of *C. elegans*, related to Figure 1

(a) Fluorescent micrographs show the localization of P body marker CGH-1 in the indicated regions of the *C. elegans* germline. Bars: 10 micrometers. A cartoon shows the relative positions of distinct regions in the gonad of *C. elegans* (bottom right).

(b) Fluorescent micrographs show the co-localization of two distinct P body markers in the pachytene region of adult germlines. Bars: 10 micrometers. The line in the merged image indicates the position of the line scan for measuring fluoresce intensity across single germline nuclei (right).

(c) Fluorescent micrographs show the localization of the indicated P body and P granule markers in the pachytene region of adult germlines. Bars: 10 micrometers. The line in the merged image indicates the position of the line scan for measuring fluoresce intensity across single germline nuclei (right).

(d) Fluorescent micrographs show the localization of the P body marker CGH-1 and Mutator foci marker MUT-16 in the pachytene region of the adult germline. Bars: 10 micrometers.

Figure S2

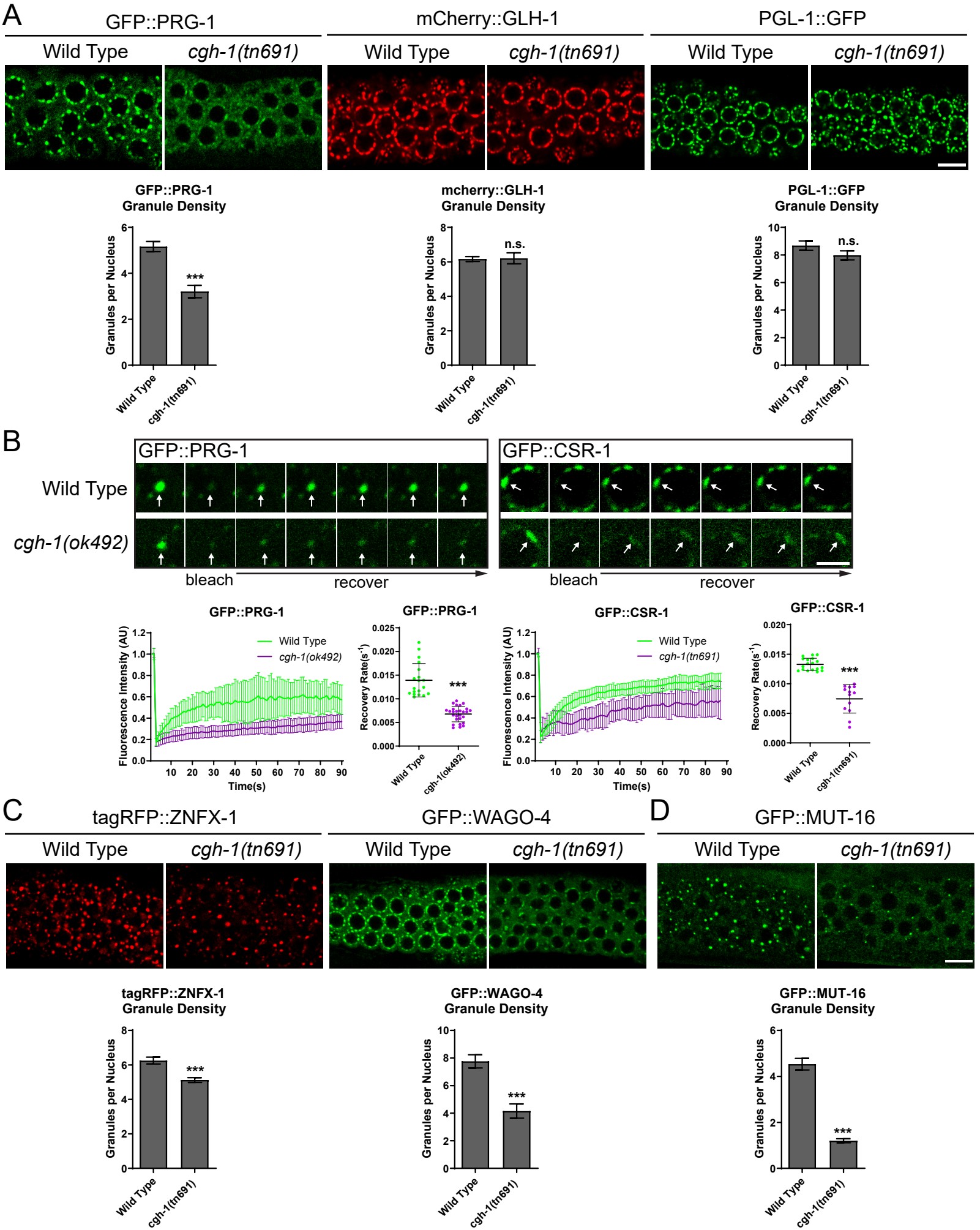

A
GFP::PRG-1
mCherry::GLH-1
PGL-1::GFP
Wild Type
cgh-1(tn691)
GFP::PRG-1 Granule Density
mcherry::GLH-1 Granule Density
PGL-1::GFP Granule Density
Granules per Nucleus
***
n.s.
B
GFP::PRG-1
GFP::CSR-1
Wild Type
cgh-1(ok492)
bleach
recover
Fluorescence Intensity (AU)
Time(s)
Recovery Rate(s-1)
cgh-1(ok492)
C
tagRFP::ZNFX-1
GFP::WAGO-4
D
GFP::MUT-16
tagRFP::ZNFX-1 Granule Density
GFP::WAGO-4 Granule Density
GFP::MUT-16 Granule Density

Figure S2. P body factor CGH-1 promotes the localization of small RNA pathway factors at germ granules, related to Figure 3

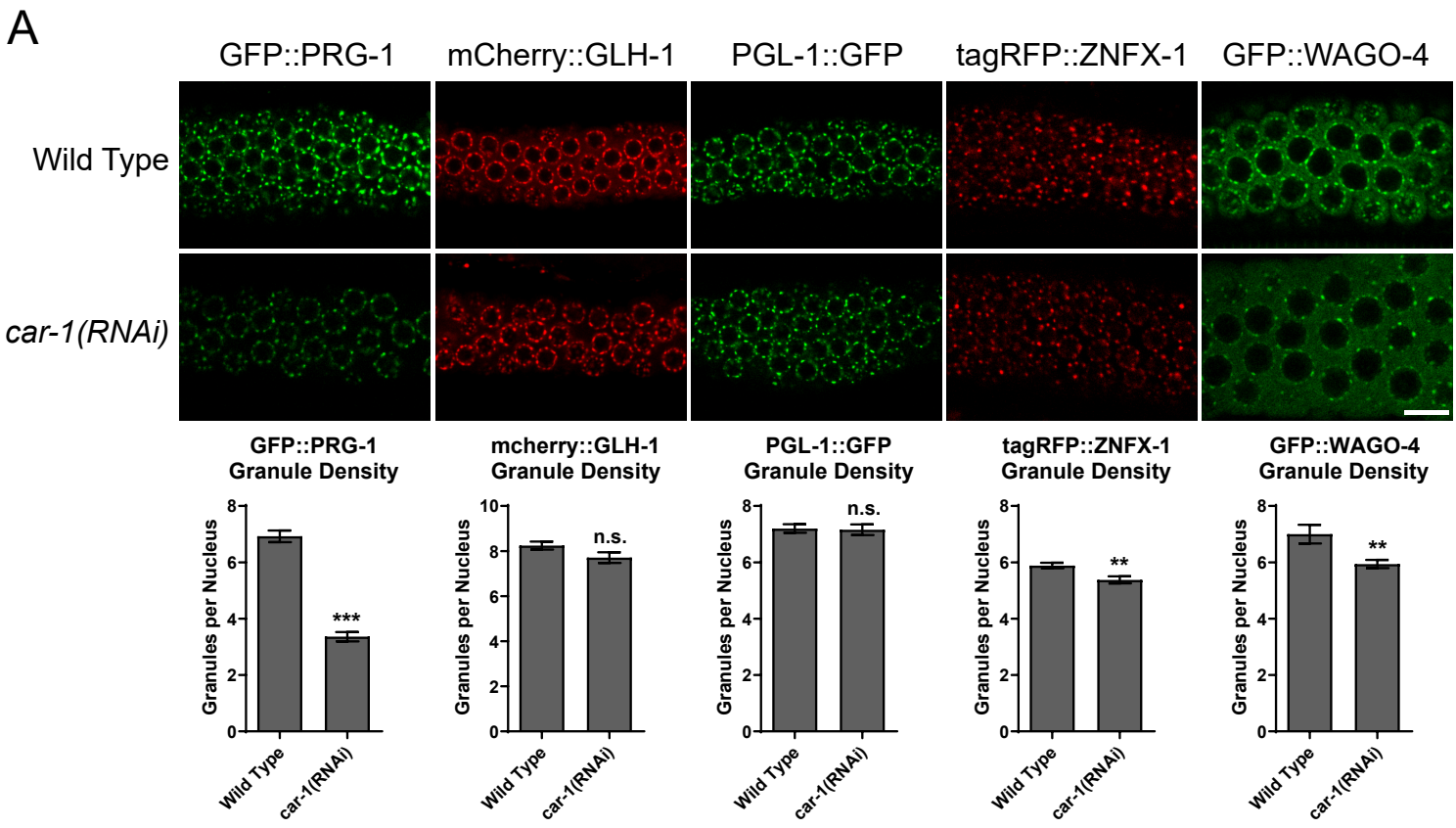
(a) Fluorescent micrographs show the localization of indicated P granule factors in wild type or in *cgh-1(tn691)* mutant animals Bars: 10 micrometers (Top). Granule density (number of granule/nuclei/section) of indicated proteins in wild type or the indicated *cgh-1* mutants (bottom). Statistical analysis was performed using a one-tailed Student's t-test. Bars indicate the mean, errors bars indicate the standard deviation. Distributions represent data collected from 8-12 independent gonad images.

(b) Fluorescence recovery after photobleaching (FRAP) analyses indicate that the dynamics of indicated P granule factors, including PRG-1 and CSR-1, are reduced in *cgh-1 (ok492)* mutant animals. The arrows indicate the P granules that are photobleached. Bars: 10 micrometers (Top). The quantification of FRAP analyses show the average fluorescence signals of GFP::PRG-1 or GFP::CSR-1 at the indicated times (seconds) after photobleaching (bottom). >10 granules at different time points were calculated for each strain. Error bars indicate the standard deviations of fluorescence intensities or recovery ratios.

(c-d) Fluorescent micrographs show the localization of indicated Z granule markers (left) Mutator foci marker MUT-16 (right) in the pachytene region of adult germlines in wild type or in *cgh-1 (tn691)* mutant animals. Bars: 10

micrometers (top). Granule number (number of granule/nuclei/section) of indicated proteins in wild type or the *car-1* RNAi-treated animals (bottom). Statistical analysis was performed using a one-tailed Student's t-test. Bars indicate the mean, errors bars indicate the standard deviation. Distributions represent data collected from 8-12 independent gonad images.

Figure S3


A
GFP::PRG-1
mCherry::GLH-1
PGL-1::GFP
tagRFP::ZNFX-1
GFP::WAGO-4
Wild Type
car-1(RNAi)
GFP::PRG-1 Granule Density
mcherry::GLH-1 Granule Density
PGL-1::GFP Granule Density
tagRFP::ZNFX-1 Granule Density
GFP::WAGO-4 Granule Density
Granules per Nucleus
***
n.s.
**
Wild Type
car-1(RNAi)

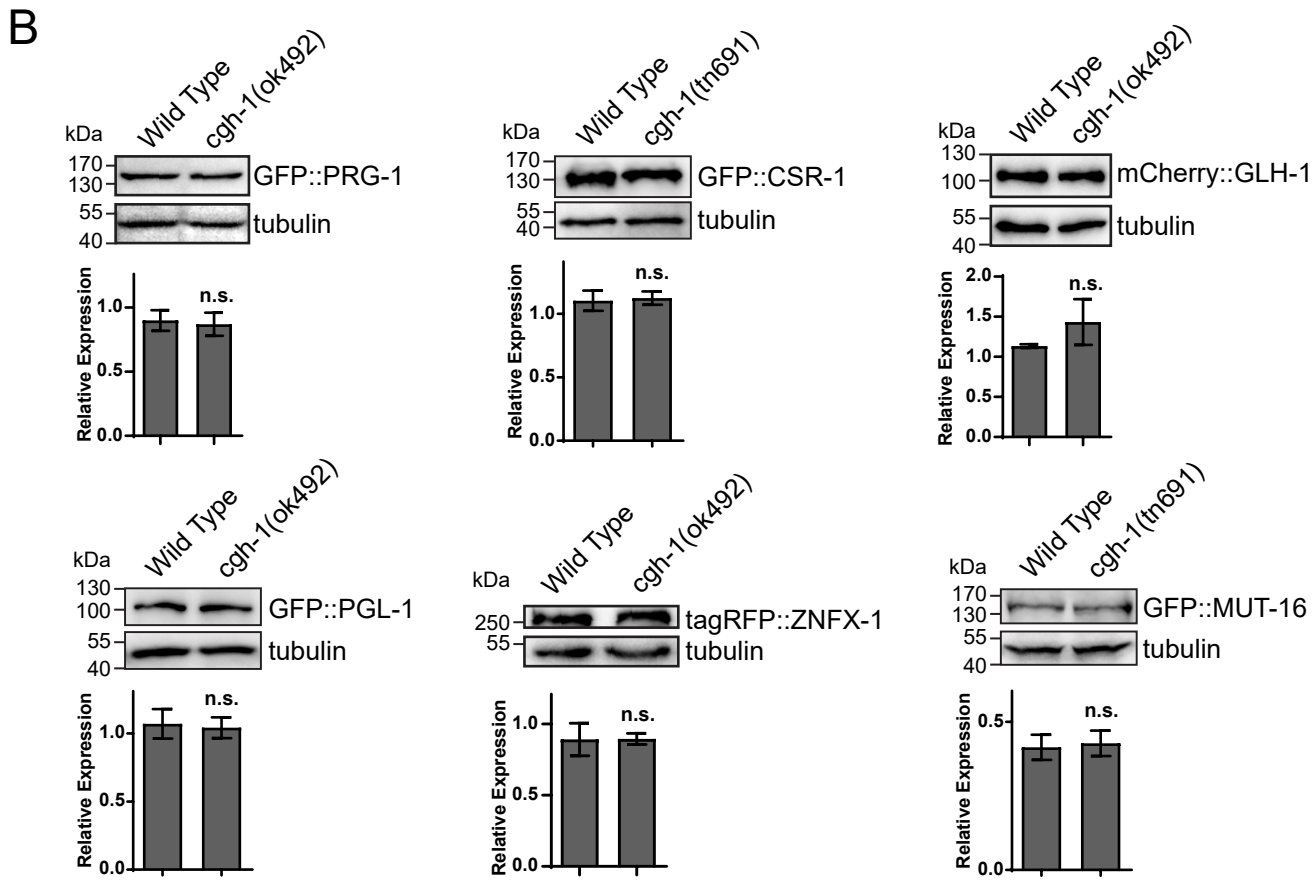

B
kDa
Wild Type
cgh-1(ok492)
cgh-1(tn691)
GFP::PRG-1
GFP::CSR-1
mCherry::GLH-1
GFP::PGL-1
tagRFP::ZNFX-1
GFP::MUT-16
tubulin
Relative Expression
n.s.

Figure S3. The localization and expression of small RNA pathway factors in P body mutants or RNAi-treated animals, related to Figure 3

(a) Fluorescent micrographs show the localization of indicated proteins in the pachytene region of adult germlines in wild type of in *car-1* RNAi-treated animals. Bars: 10 micrometers (top). Granule number (number of granule/nuclei/section) of indicated proteins in wild type or the *car-1* RNAi-treated animals (bottom). Statistical analysis was performed using a one-tailed Student's t-test. Bars indicate the mean, errors bars indicate the standard deviation. Distributions represent data collected from 8-12 independent gonad images.

(b) Western blots show the levels of indicated proteins in wild type or in the indicated *cgh-1* mutants.

Figure S4

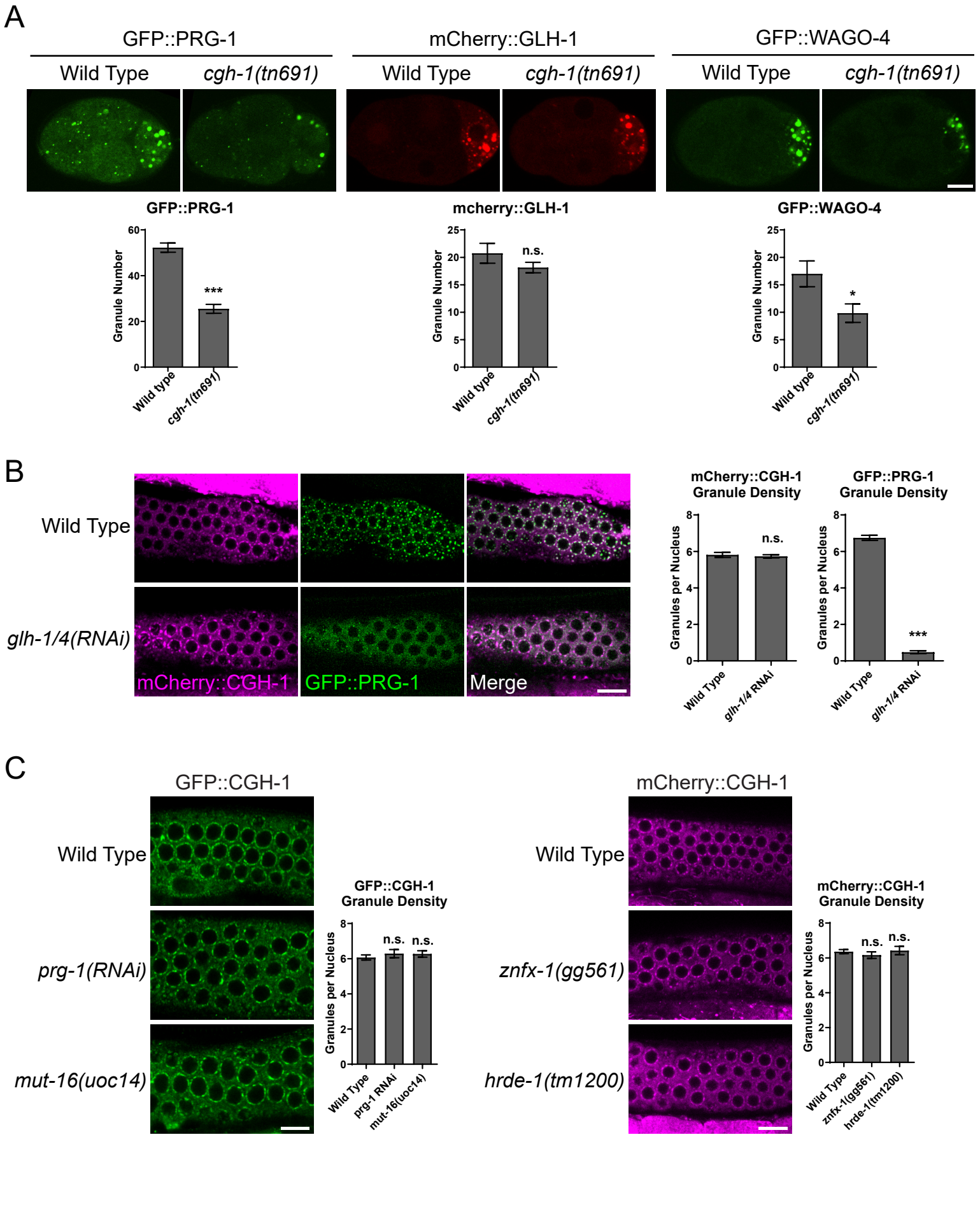

A
GFP::PRG-1
mCherry::GLH-1
GFP::WAGO-4
Wild Type
cgh-1(tn691)
Wild Type
cgh-1(tn691)
Wild Type
cgh-1(tn691)
GFP::PRG-1
Granule Number
***
Wild type
cgh-1(tn691)
mcherry::GLH-1
Granule Number
n.s.
Wild type
cgh-1(tn691)
GFP::WAGO-4
Granule Number
*
Wild type
cgh-1(tn691)
B
Wild Type
glh-1/4(RNAi)
mCherry::CGH-1
GFP::PRG-1
Merge
mCherry::CGH-1
Granule Density
Granules per Nucleus
n.s.
Wild Type
glh-1/4 RNAi
GFP::PRG-1
Granule Density
Granules per Nucleus
***
Wild Type
glh-1/4 RNAi
C
GFP::CGH-1
Wild Type
prg-1(RNAi)
mut-16(uoc14)
GFP::CGH-1
Granule Density
Granules per Nucleus
n.s.
n.s.
Wild Type
prg-1 RNAi
mut-16(uoc14)
mCherry::CGH-1
Wild Type
znfx-1(gg561)
hrde-1(tm1200)
mCherry::CGH-1
Granule Density
Granules per Nucleus
n.s.
n.s.
Wild Type
znfx-1(gg561)
hrde-1(tm1200)

Figure S4. The localization of germ granule or P body factors in the indicated animals, related to Figure 3

(a) Fluorescent micrographs show the localization of the indicated P granule factors in the 4-cell embryos wild type or *cgh-1(tn691)* mutant animals. Bars: 10 micrometers (Top). Granule number of indicated proteins in wild type embryos or the indicated *cgh-1* mutant embryos (bottom). Statistical analysis was performed using a one-tailed Student's t-test. Bars indicate the mean, errors bars indicate the standard deviation. Distributions represent data collected from 8-12 embryo images.

(b) Fluorescent micrographs show the perinuclear localization of P granule factor PRG-1, but not P body factor CGH-1, is reduced in *glh-1* and *glh-4* double RNAi treated animals. Bars: 10 micrometers (left). Statistical analysis was performed using a one-tailed Student's t-test. Bars indicate the mean, errors bars indicate the standard deviation (right). Distributions represent data collected from 8-12 independent gonad images.

(c) Fluorescent micrographs show the localization of P body marker GFP::CGH-1 in the pachytene region of adult germlines in wild type or in the indicated mutant or RNAi-treated animals. Bars: 10 micrometers. Statistical analysis was performed using a one-tailed Student's t-test. Bars indicate the mean, errors bars indicate the standard deviation. Distributions represent data collected from 8-12 independent gonad images.

Figure S5

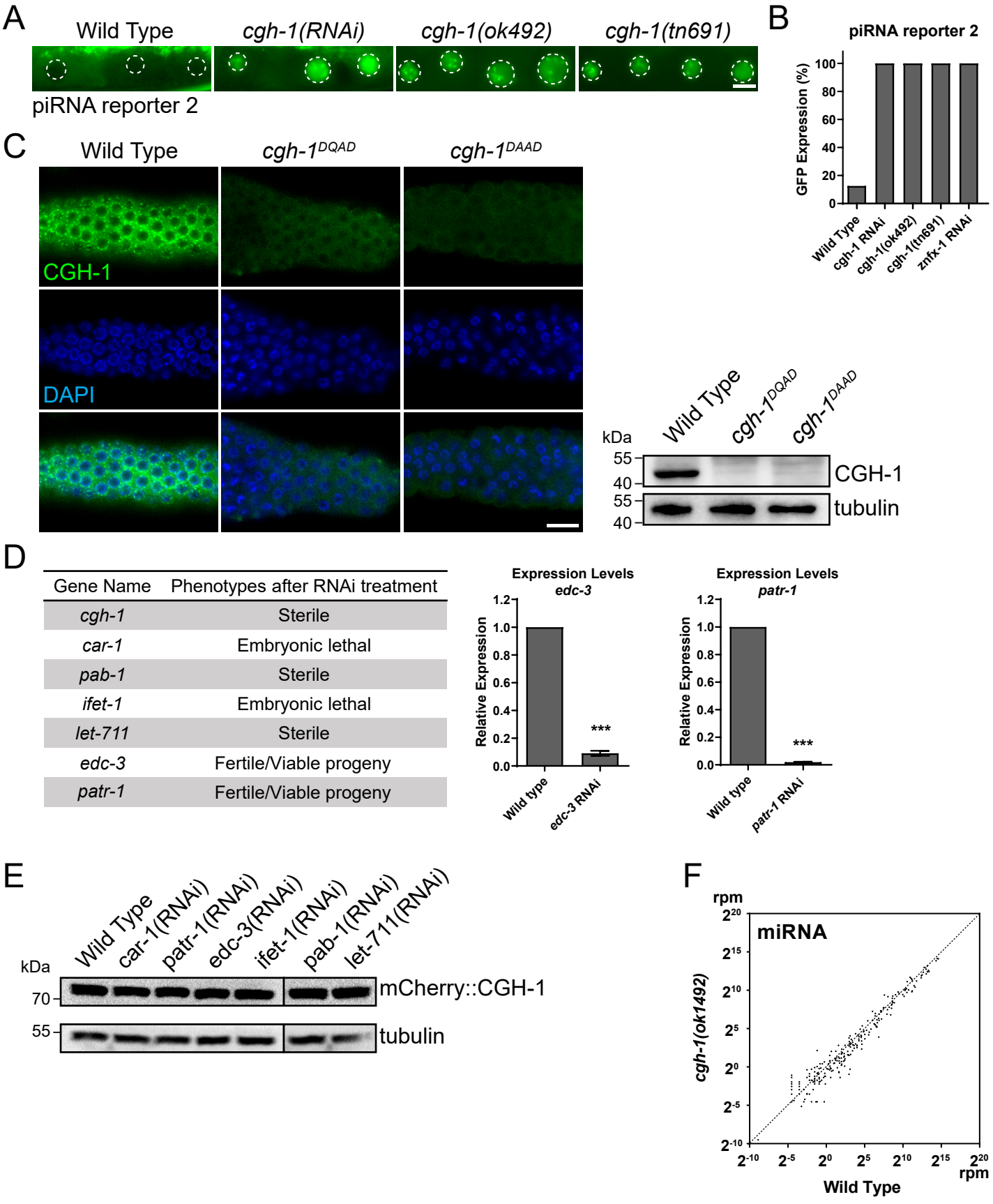

A
Wild Type
cgh-1(RNAi)
cgh-1(ok492)
cgh-1(tn691)
piRNA reporter 2
B
piRNA reporter 2
GFP Expression (%)
100
80
60
40
20
0
Wild Type
cgh-1 RNAi
cgh-1(ok492)
cgh-1(tn691)
znfx-1 RNAi
C
Wild Type
cgh-1DQAD
cgh-1DAAD
CGH-1
DAPI
kDa
55
40
55
40
Wild Type
cgh-1DQAD
cgh-1DAAD
CGH-1
tubulin
D
Gene Name
Phenotypes after RNAi treatment
cgh-1
Sterile
car-1
Embryonic lethal
pab-1
Sterile
ifet-1
Embryonic lethal
let-711
Sterile
edc-3
Fertile/Viable progeny
patr-1
Fertile/Viable progeny
Expression Levels
edc-3
Relative Expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
Wild type
edc-3 RNAi
Expression Levels
patr-1
Relative Expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
Wild type
patr-1 RNAi
E
Wild Type
car-1(RNAi)
patr-1(RNAi)
edc-3(RNAi)
ifet-1(RNAi)
pab-1(RNAi)
let-711(RNAi)
kDa
70
55
mCherry::CGH-1
tubulin
F
rpm
miRNA
cgh-1(ok1492)
2^20
2^15
2^10
2^5
2^0
2^-5
2^-10
2^-10
2^-5
2^0
2^5
2^10
2^15
2^20
rpm
Wild Type

Figure S5. Characterization of the role of CGH-1 in piRNA-dependent gene silencing and the RNAi effects of knocking down P body factors, related to Figure 4

(a) GFP expression in the piRNA reporter #2 in the indicated strains. In this reporter, the expression of Histone H2B::GFP (nuclear) is silenced by a synthetic GFP-targeting piRNA in wild type animals. Dotted circles indicate the location of maturing oocyte nuclei. Bars: 10 micrometers.

(b) Percentage of screened animals showing GFP expression in the piRNA reporter #2 in the indicated strains. We define the expression of GFP (on/off) by observing whether the indicated mutant or RNAi-treated animals exhibit visible nuclear GFP signals in their germline.

(c) Fluorescent micrographs (left) and Western blots (right) showing the expression levels of CGH-1 wild type, CGH-1 DQAD, and CGH-1 DAAD. Bars: 10 micrometers.

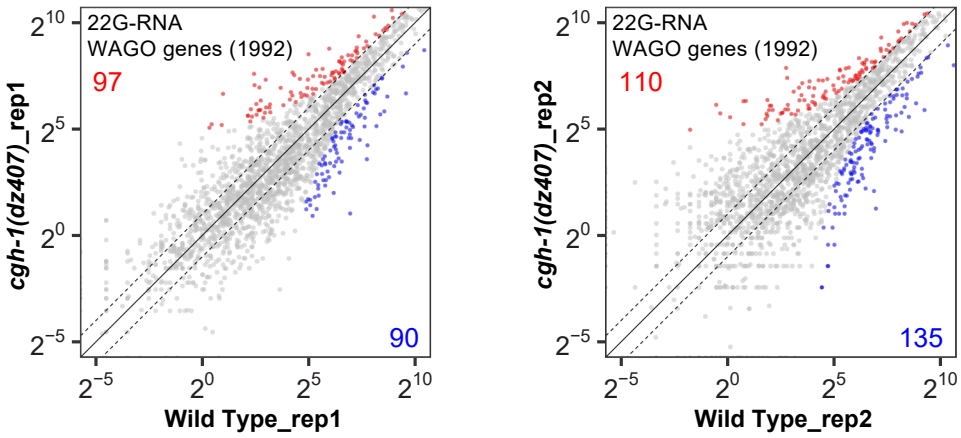
(d) The phenotypes (left) and qRT-PCR measurements of mRNA levels (right) of indicated RNAi knockdown of indicated P body factors. Statistical analysis was performed using a one-tailed Student's t-test. Bars indicate the mean, errors bars indicate the standard deviation. The qRT-PCR measurements present knockdown effects of three biological replates.

(e) Western blots show the levels of CGH-1 in the indicated RNAi-treated animals.

(f) A scatter plot showing the abundance of miRNAs in wild type worms compared to *cgh-1 (dz407)* mutant.

Figure S6

A


22G-RNA
WAGO genes (1992)
97
90
cgh-1(dz407)_rep1
Wild Type_rep1
22G-RNA
WAGO genes (1992)
110
135
cgh-1(dz407)_rep2
Wild Type_rep2

B

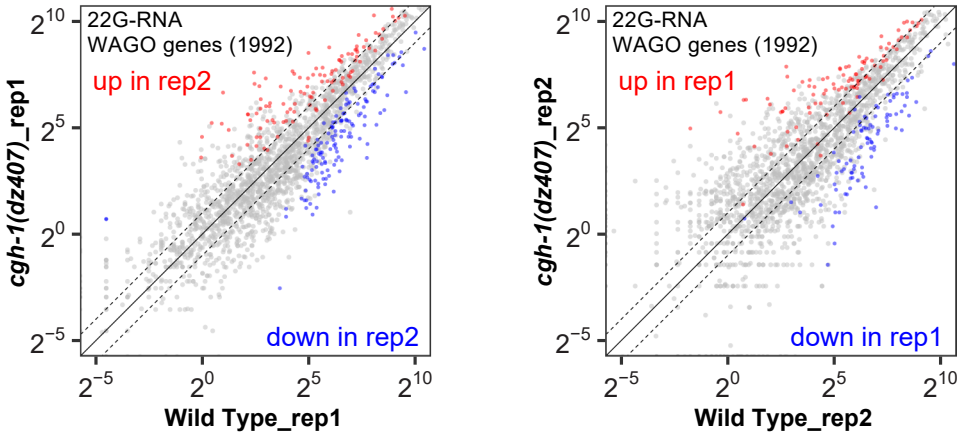

22G-RNA
WAGO genes (1992)
up in rep2
down in rep2
cgh-1(dz407)_rep1
Wild Type_rep1
22G-RNA
WAGO genes (1992)
up in rep1
down in rep1
cgh-1(dz407)_rep2
Wild Type_rep2

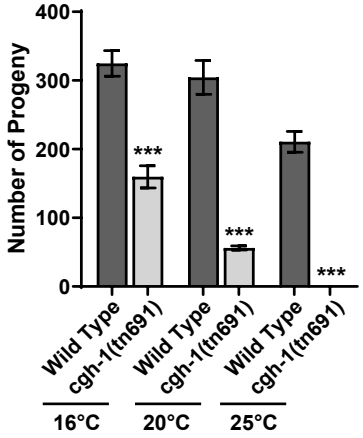
Figure S6. The comparison of changes in 22G-RNA levels in *cgh-1* mutants between two biological replicates, related to Figure 4.

(a) A scatter plot showing the abundance of 22G-RNAs mapped to WAGO target in wild-type worms compared to *cgh-1* mutant in two biological replicates. WAGO targets with significantly increased or decreased levels of 22G-RNA (adjusted P<0.05, see STAR methods for details) and at least two-fold change are highlighted in red and blue dots, respectively.

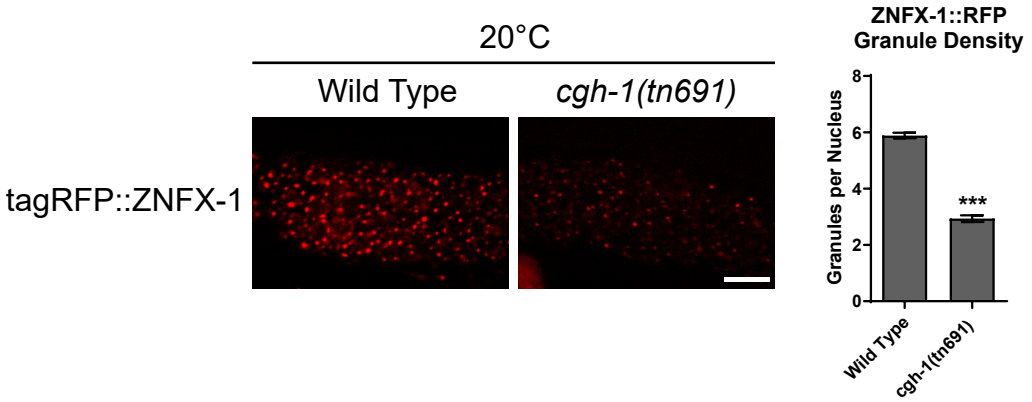
(b) A scatter plot showing the abundance of 22G-RNAs mapped to WAGO target in wild-type worms compared to *cgh-1* mutant in two biological replicates. The WAGO targets that exhibit significantly changes (adjusted P<0.05, see STAR methods for details) and at least two-fold change in 22G-RNAs in one replicate (shown in Figure S6A) are highlighted in another replicate.

Figure S7

A



B



C

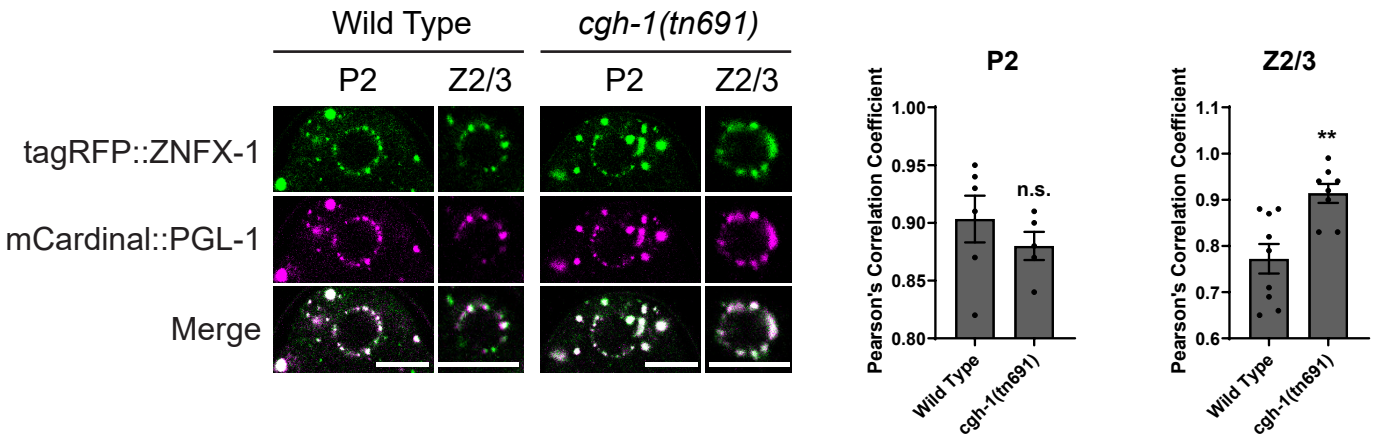


Figure S7. Characterization of temperature-sensitive *cgh-1 (tn691)* strain grown at permissive and non-permissive temperature, related to Figure 4, Figure 5, and Figure 6

(a) The brood size of wild type or *cgh-1 (tn691)* mutant animals grown at the indicated temperature.

(b) Fluorescent micrographs show the localization of indicated Z granule factor ZNFX-1 in the pachytene region of adult germlines in wild type or *cgh-1 (ok492)* mutant animals grown at 20 degree Celsius.

(c) Fluorescent micrographs show the localization of P granule marker PGL-1 and Z granule marker ZNFX-1 in the P2 cells or Z2/Z3 cells of wild type or *cgh-1 (tn691)* mutant animals (left). Pearson's correlation coefficient of PGL-1 and ZNFX-1 signals (right).

DNA Sequences of *cgh-1* wild type and the *cgh-1 (dz407)* allele

cgh-1 wild type

ATGAGTGGAGCGGAGCAACAACAGATCGTTCCTGCCAACAATGGCGACGAA
AACTGGAAGGCTGGTCTCAATCTTCCAGCAAAAGACAGAAGATTCAAAACTG
CTgtgagtttgcaaagcctatatgactttgtgaccatttcatttttcagGATGTCACTGATACGAAGGG
AGTCGAGTTTGAGGATTTCTGCCTTGGACGCGATCTTCTCATGGGGATTTTT
GAGAAAGGATGGGAAAAACCGAGTCCTATTCAAGAAGCATCCATCGGAGTC
GCTCTCACTGGTCAAGATATTCTCGCAAGAGCTAAGAACGGAACAGGAAAA
ACCGGAGCTTATTGTATCCCTGTTATCGAGAAGATCCAGCCAGCCCTCAAA
GCTATTCAAGCCATGGTCATTGTCCCAACTCGTGAATTGGCTCTCCAAACTT
CACAGATCTGCGTGGAGCTCTCCAAACACATTCAGCTCAAAGTTATGGTCAC
TACCGGAGGAACTGATCTCCGTGATGATATCATGCGTCTAAACGGAACGGT
TCACTTGGTCATCGCCACCCCAGGAAGAATTCTCGATCTTATGGAGAAAGG
AGTCGCCAAAATGGAACATTGTAAAACACTTGTTCTTGACGAGGCTGACAAA
CTTCTTAGTCAAGACTTCCAGgtaaaaaaattaacatttgaataagataacaattctcgtttacagG
GAATTCTCGACCGCCTCATCAACTTCCTCCCAAAGGAGCGTCAAGTTATGCT
TTATTCTGCCACTTTCCCTAACACCGTCACTTCGTTCATGCAGAAGCACATG
CACAAGCCGTATGAGATCAACCTCATGGAGGAGCTTACTCTTCTTGGTGTCA
CCCAATACTATGCCTTTGTTCAAGAGAAGCAAAAGGTCCACTGTTTGAATAC
GTTGTTCCGAAAACTTCAAATCAACCAGTCGATCATCTTCTGTAACTCTACTC
AACGTGTTGAGCTTCTCGCTAAGAAAATCACGGAAATCGGTTACTCGTGCTA
CTACATTCATTCCAAGATGGCTCAGAATCACAGAAACCGTGTCTTCCATGAC
TTCCGTCAAGGAAACTGCAGAAACTTGGTTTGCTCCGATCTTCTCACCCGTG
gtaagtttgactaaataatttgcgcgatgaggattgataacacatacacacactctgaagttatgtgaagagataa
ttgaagaacggatatctgagcgccatttttcttattttaaagtgtagttcatgcataattgtaatttttcagGTATCG
ATATCCAGGCTGTCAATGTTGTCATCAACTTTGACTTCCCAAGAAACGCCGA
GACTTACCTTCACAGAATCGGTCGCTCTGGACGTTTCGGTCACCTCGGAGT
TGCAATCAACCTTATCACCTACGAAGACCGTCACACCCTTCGCCGTATCGA
GCAGGAGCTTCGTACTCGCATTGAGCCCATCCCGAAAACGGTTGACCCGAA
GCTGTATGTCGCTGACCAGCAGCTCGTCGATGCCGCCGATGAGACCACTG
CTTAA

cgh-1(dz407)

attccgATGTCCGATGTCGACGCTCCAGTGGAGCGGAGCAACAACAGATCGTT
CCTGCCAACAATGGCGACGAAAACTGGAAGGCTGGTCTCAATCTTCCAGCA
AAAGACAGAAGATTCAAAACTGCTgtgagtttgcaaagcctatatgactttgtgaccatttcatttttc
agGATGTCACTGATACGAAGGGAGTCGAGTTTGAGGATTTCTGCCTTGGAC
GCGATCTTCTCATGGGGATTTTTGAGAAAGGATGGGAAAAACCGAGTCCTA
TTCAAGAAGCATCCATCGGAGTCGCTCTCACTGGTCAAGATATTCTCGCAAG
AGCTAAGAACGGAACAGGAAAAACCGGAGCTTATTGTATCCCTGTTATCGA
GAAGATCCAGCCAGCCCTCAAAGCTATTCAAGCCATGGTCATTGTCCCAAC
TCGTGAATTGGCTCTCCAAACTTCACAGATCTGCGTGGAGCTCTCCAAACAC
ATTCAGCTCAAAGTTATGGTCACTACCGGAGGAACTGATCTCCGTGATGATA
TCATGCGTCTAA