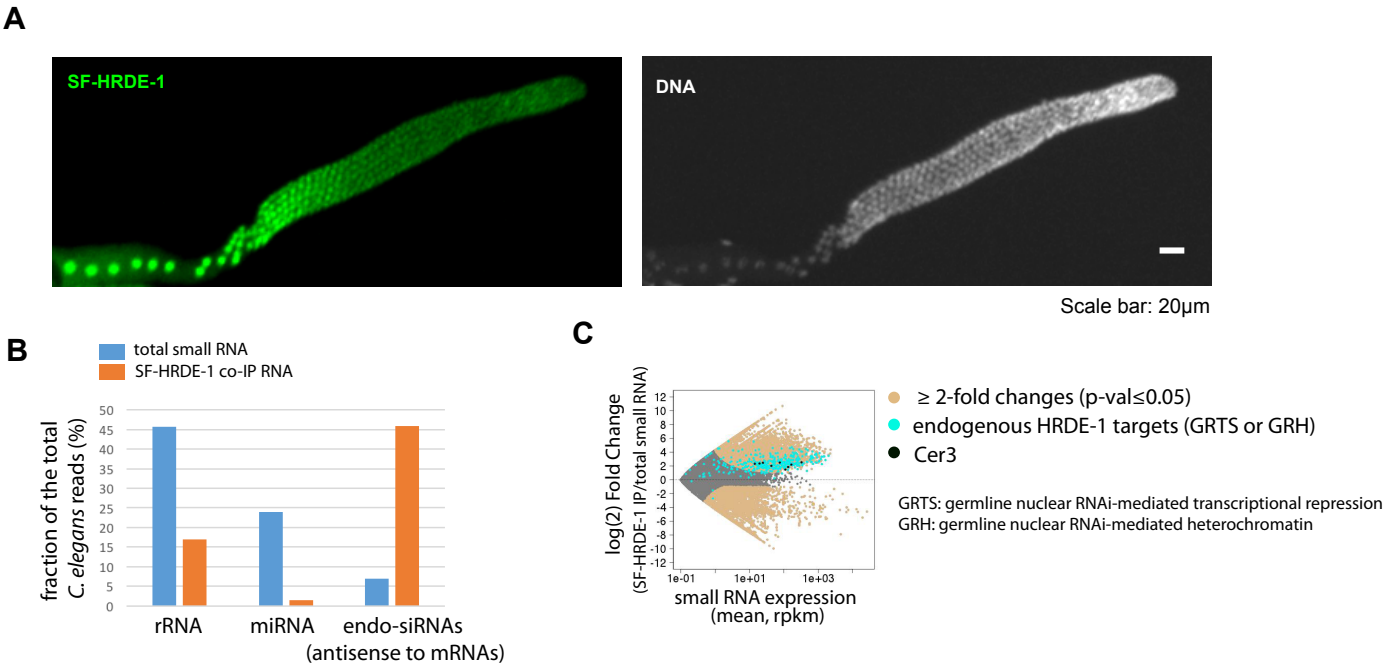


Figure S1. Enrichment of HRDE-1-bound endo-siRNAs by immunoprecipitation.

(A) A representative image of anti-FLAG immunofluorescence microscopy of a dissected gonad showing that SF-HRDE-1 is predominantly expressed in germline nuclei. HRDE-1 is tagged with Strep II-FLAG peptide (SF-tag) at the N-terminus. DNA: DAPI stain. Scale bar: 20 μm. **(B)** SF-HRDE-1 immunoprecipitation (IP) enriched endo-siRNAs (antisense to mRNAs), but not miRNAs or rRNAs. Protein extract of SF-HRDE-1-expressing adults was used for the IP. **(C)** SF-HRDE-1 IP enriched endo-siRNAs of the endogenous HRDE-1 targets. The log2 ratio of SF-HRDE-1 co-IP to total small RNA levels (y-axis) and the mean values of co-IP and total small RNA levels (x-axis) are plotted for all 1-kb regions of the genome.

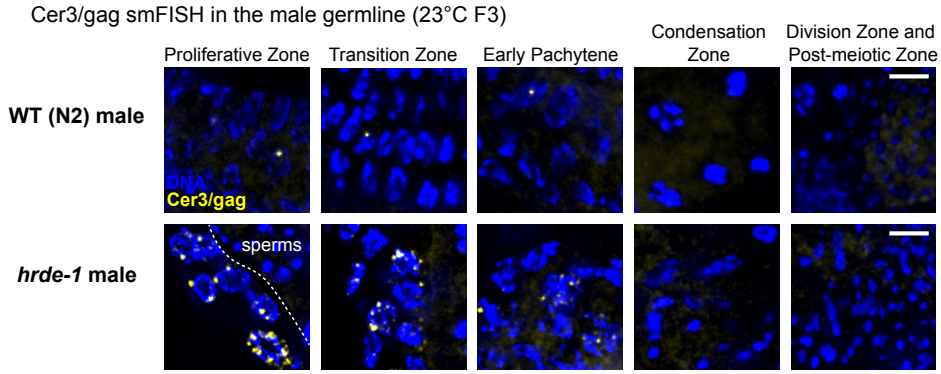


Figure S2. Cer3/gag smFISH signals at different stages of adult male germline (23°C F3).

Scale bars: 5 μm.

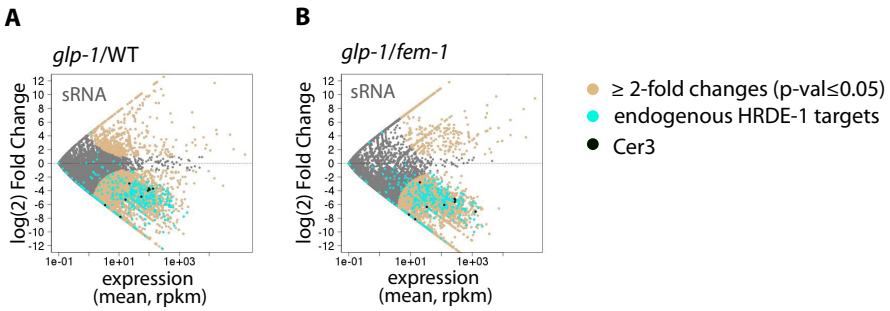


Figure S3. Endo-siRNAs of the endogenous HRDE-1 targets are enriched in the germline. **(A)** Scatter plot for wild type and *glp-1(*e2141) adults (25°C for both) with the log2 ratio between the two samples plotted in the y-axis and the mean small RNA expression of the two samples plotted in the x-axis. *glp-1(e2141)* is a temperature-sensitive germline deficient mutant. **(B)** Scatter plot for *glp-1(e2141)* and *fem-1(hc17)* adults (25°C for both) with the log2 ratio between the two samples plotted in the y-axis and the mean small RNA expression of the two samples plotted in the x-axis . *fem-1(hc17)* mutant animals are sterile at 25ºC due to the temperature-sensitive defect in producing the male germline; the female germline is normal in the mutant adults. For both (A) and (B), we used sRNA-seq data of Gent, et al. (PMC2838994).

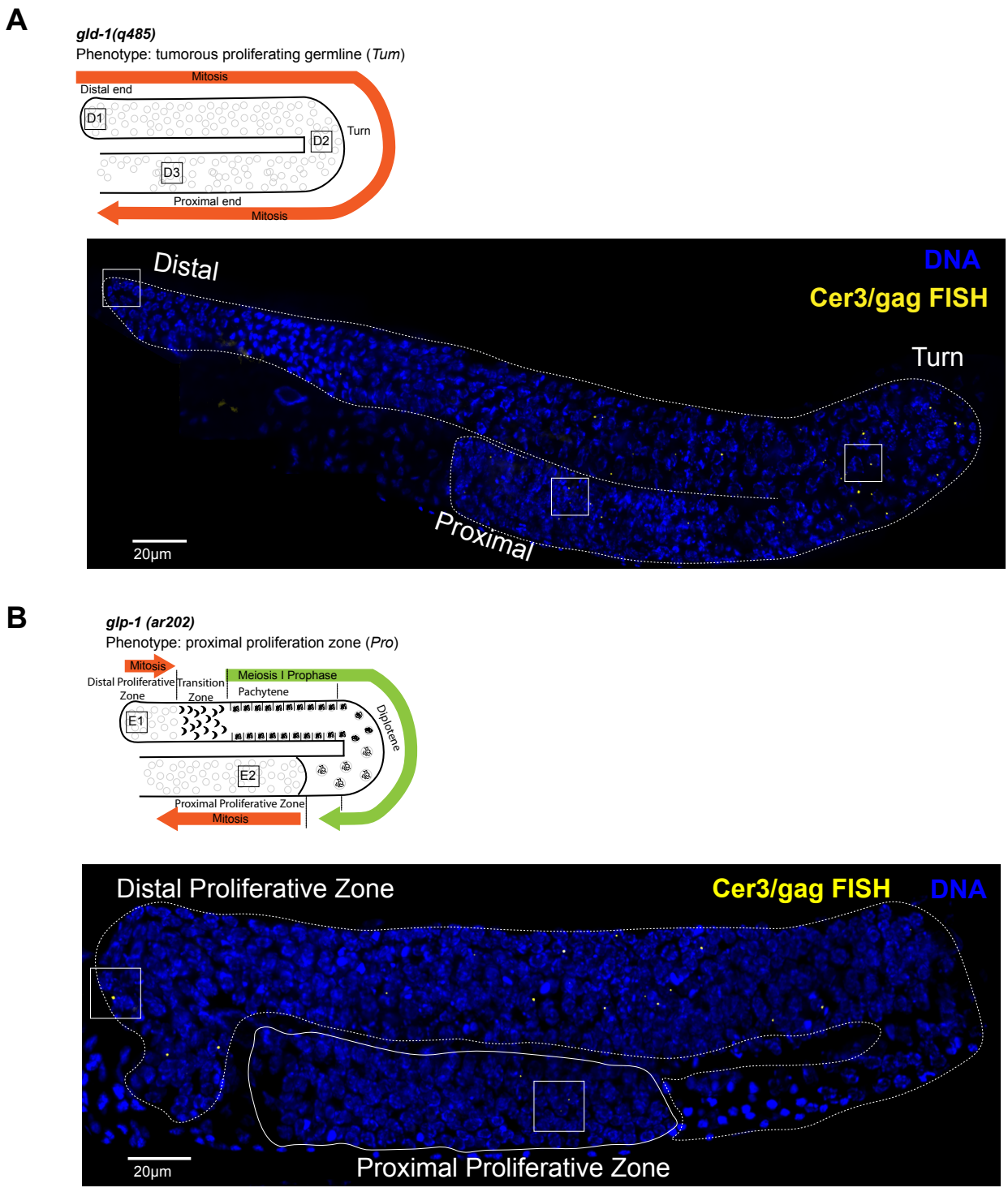


Figure S4. Cer3/gag smFISH distribution in *gld-1(q485)* and *glp-1(ar202)* gonad.

(A) A schematic diagram of *gld-1(q485)* tumorous germline phenotype and Cer3/gag smFISH in the entire *gld-1(q485)* gonad. The selected regions showed in Figure 1H are marked with white squares. **(B)** A schematic diagram of *glp-1(ar202)* gonad phenotype (proximal proliferative zone) and Cer3/gag smFISH in the entire *glp-1(ar202)* gonad (25ºC). The selected regions showed in Figure 1I are marked with white squares. Scale bars are 20µm.

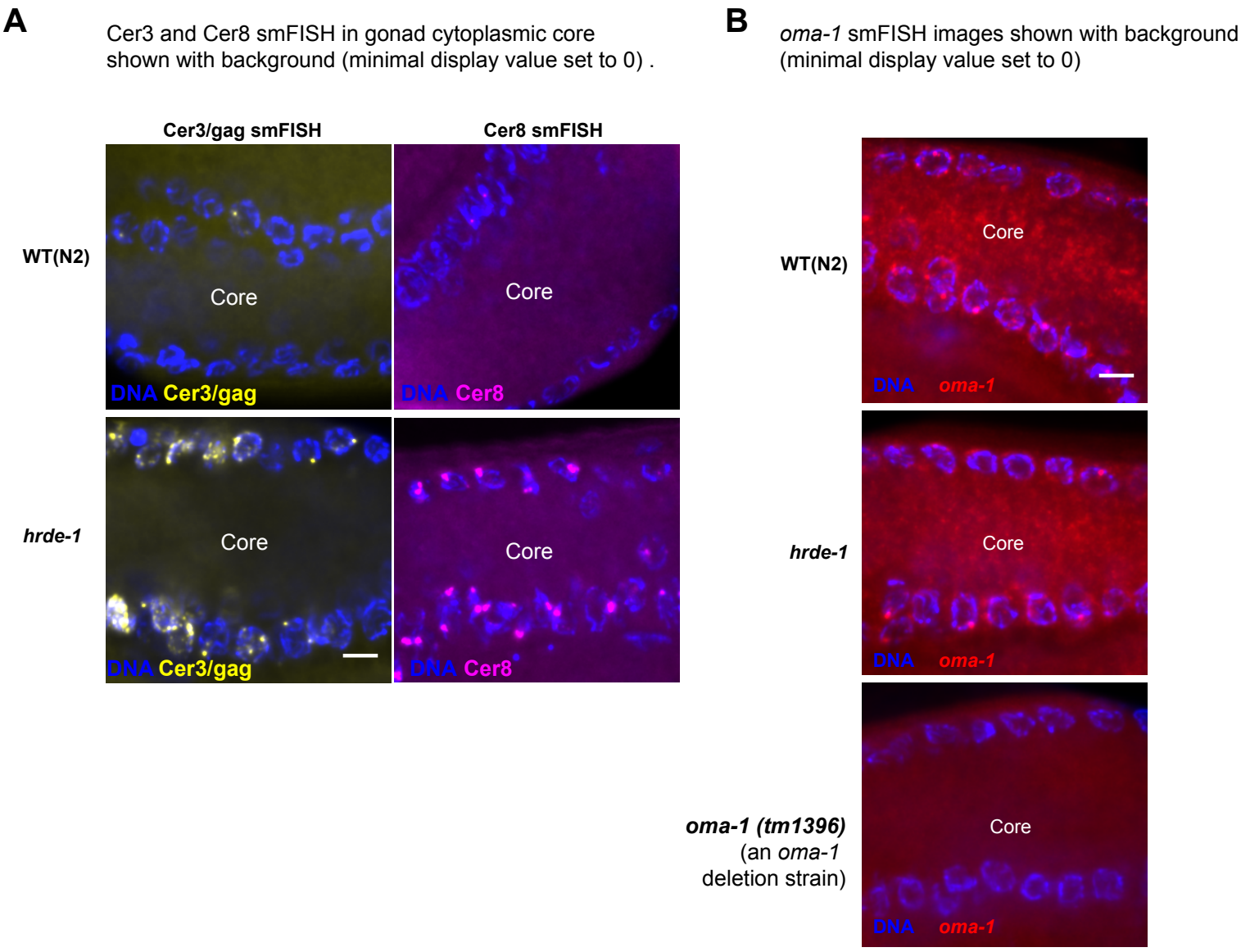
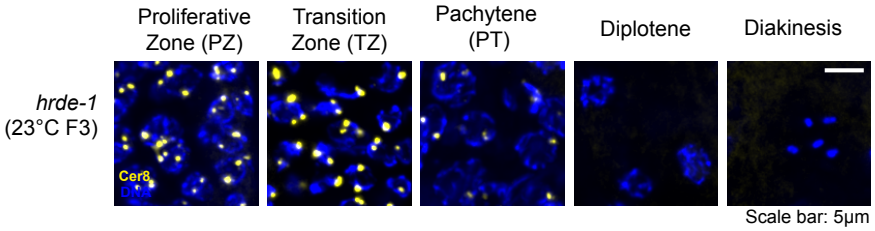


Figure S5. Cer3, Cer8, and *oma-1* smFISH images (same as the ones in Fig.4) without background reductions.

For all images, minimal display value was set to 0 in ImageJ to show background fluorescence. The image shows a representative longitudinal section through the gonad core of pachytene stage. **(A)** smFISH against Cer3/gag, Cer8 in adult WT (N2) and *hrde-1* pachytene germline (23°C generation 3). No Cer3/gag and Cer8 smFISH spot is observed in the cytoplasm core. Scale bar: 5 µm. **(B)** *oma-1* smFISH in WT (N2), *hrde-1* mutant, and *oma-1* deletion strain, *oma-1(tm1396)*. (23°C generation 3). *oma-1* smFISH signal is present in both nucleus and cytoplasm core of WT(N2) and hrde-1 mutant strain, and is absent in the *oma-1* deletion strain, *oma-1 (tm1396)*. Scale bar: 5 µm.

A



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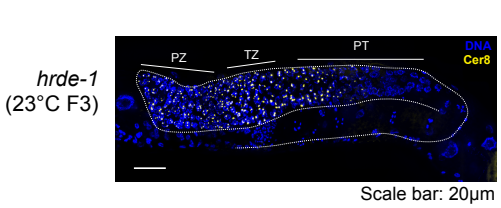

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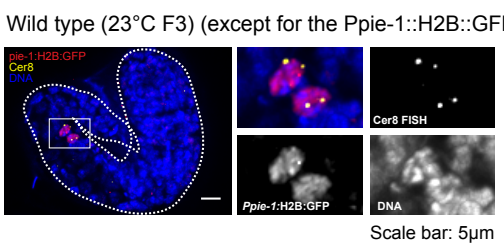

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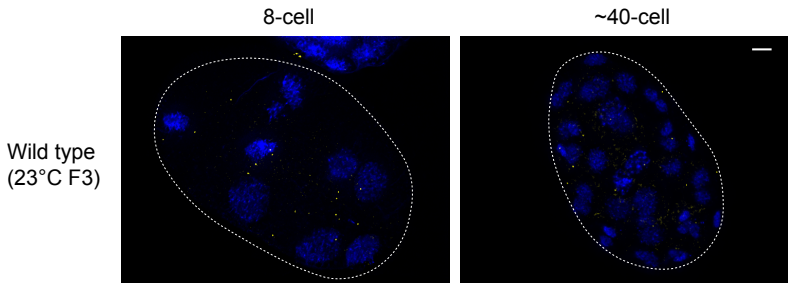

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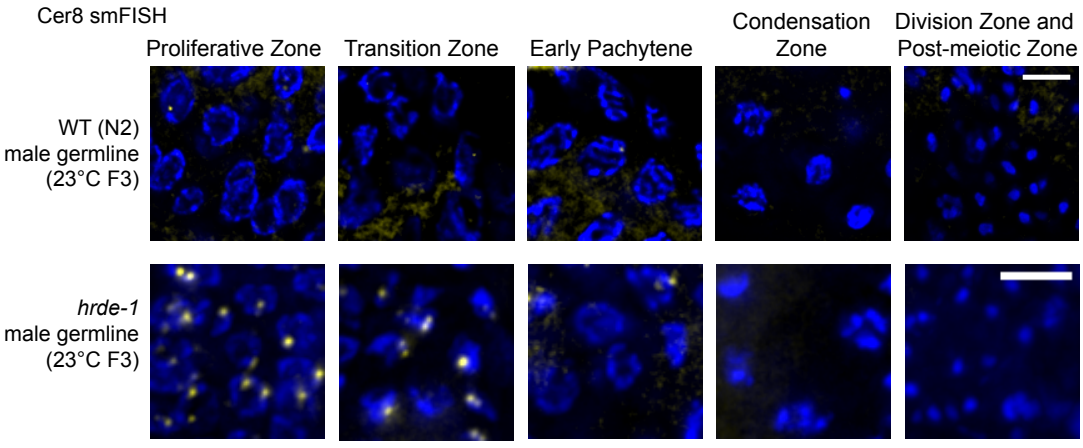
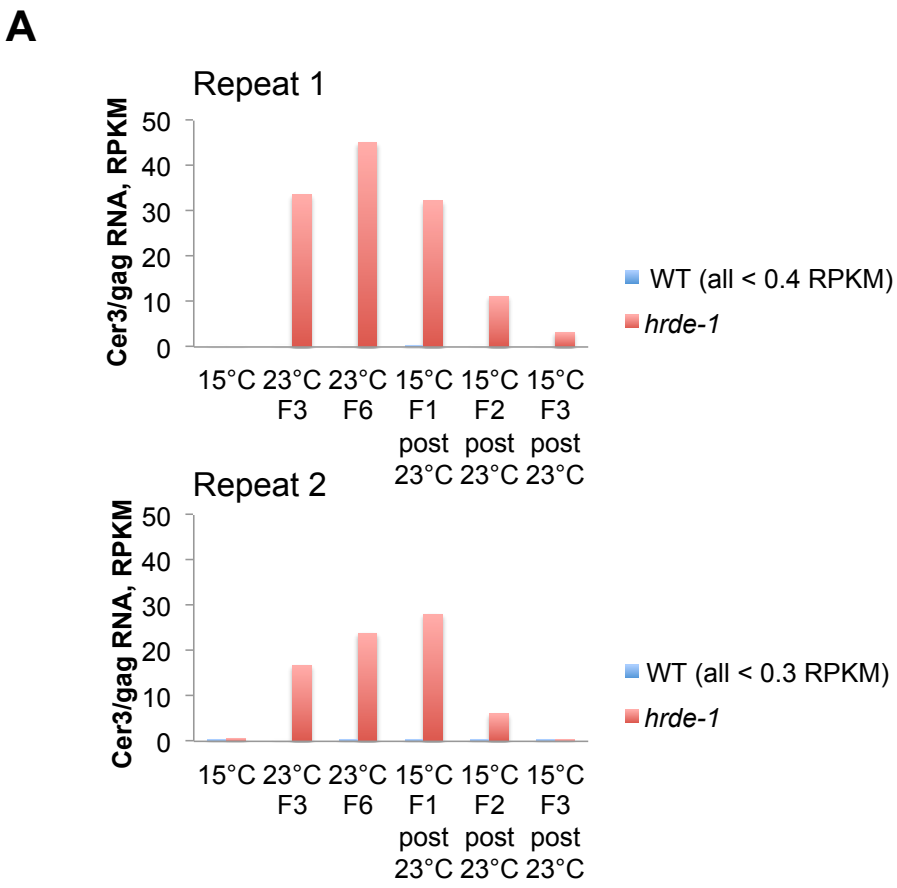


Figure S6. Cer8 smFISH analysis.

(A) Cer8 smFISH at different germline developmental stages in *hrde-1* adults (23°C). Scale bar: 5 µm. **(B)** A full gonad view of Cer8 smFISH signals in *hrde-1* adult (23°C). The gonad is outlined. Scale bar: 20 µm **(C)** Simultaneous detection of pie-1 promoter driven GFP::H2B (immunofluorescence with anti-GFP) and Cer8 RNA with smFISH in a pre-hatching embryo (23°C). The strain carries a Ppie-1::GFP::H2B transgene in a wild type genetic background. Scale bar: 5 µm **(D)** Cer8 smFISH in 8-cell and ~40-cell embryos. Scale bar: 5 µm **(E)** Cer8 smFISH in WT(N2) and *hrde-1* mutant male germline (23°C). Scale bar: 5 µm



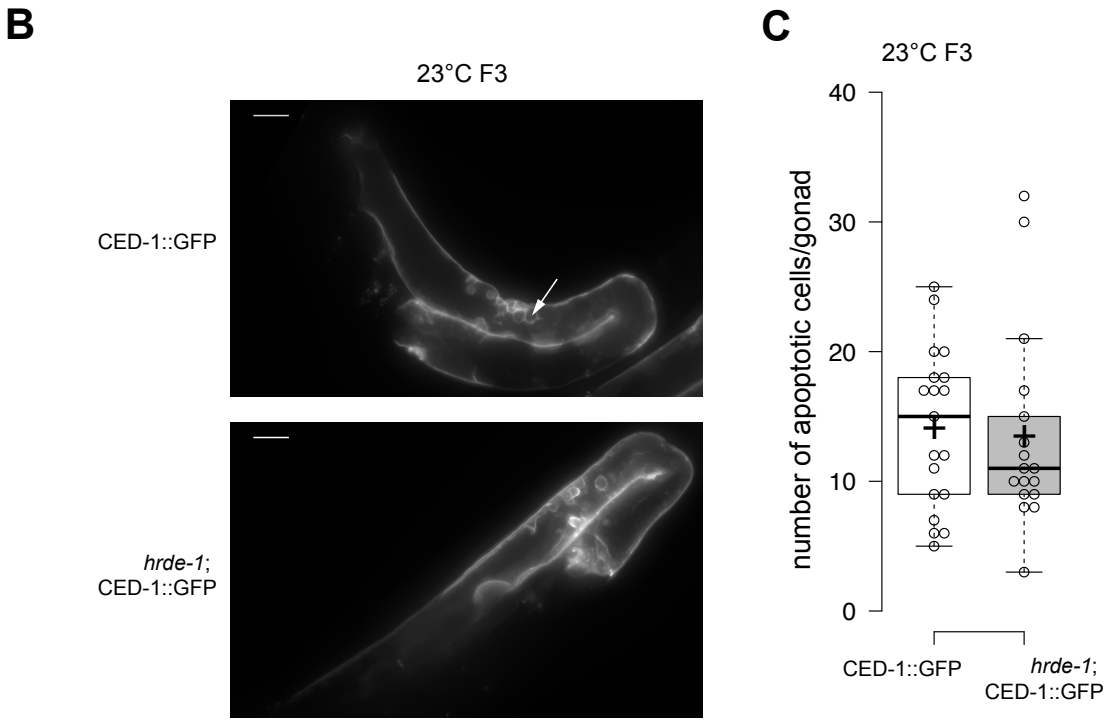


Figure S7. Effects of heat stress on Cer3/gag RNA and germline apoptosis.

(A) mRNA-seq analysis of Cer3/gag expression in WT(N2) and *hrde-1* adults during multigenerational temperature shift experiments. The two plots used mRNA-seq data from Ni et al., 2016. Two biological replicates. **(B)** CED-1::GFP expression in adult gonad sheath cells at wild-type (N2) and *hrde-1* mutant background (23°C generation 3). Arrow points to an example of engulfed apoptotic germ cell. Scale bars: 20 μm. **(C)** Number of CED-1::GFP marked apoptotic cells per gonad arm for wild-type (N2) and *hrde-1* mutant (23°C generation 3). Center lines show the medians; crosses represent sample means; box limits indicate the 25% and 75% percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. Each circle represents count from an individual gonad arm (n = 19, 17); Mann–Whitney–non-parametric tests: not significant.

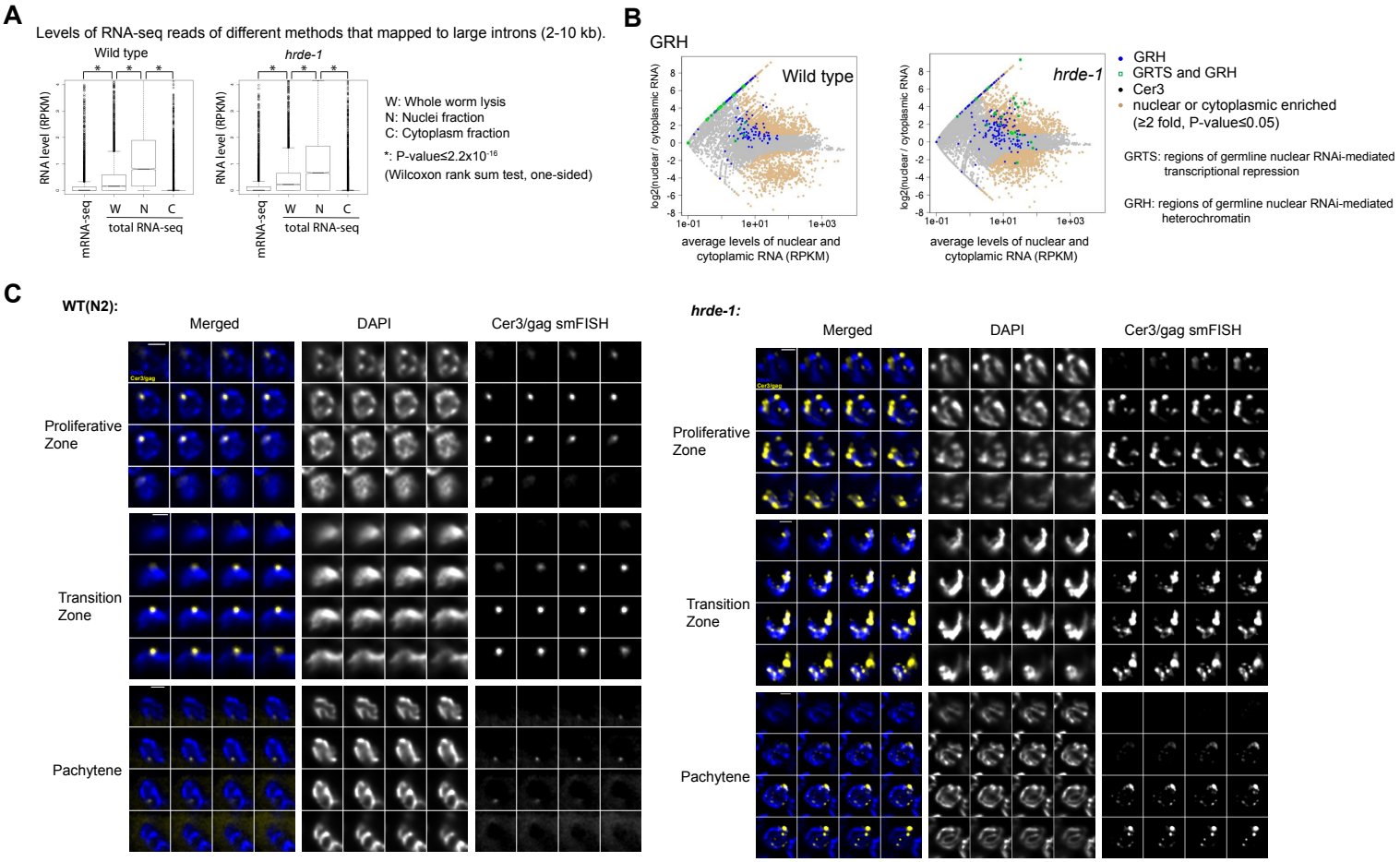


Figure S8. Nuclei and cytoplasm fractionation and RNA-seq analysis

(A) Box plot analysis for the levels of reads that are mapped to large introns (2-10 kb) from different RNA-seq methods (mRNA and total RNA; different fractions of total RNA). **(B)** RNA-seq analysis of nuclear and cytoplasmic RNA samples from WT (N2) and *hrde-1* mutant adults. The average nuclear and cytoplasmic RNA expression levels (RPKM, x-axis) and the log2 ratios of nuclear to cytoplasmic RNA level (y-axis) are plotted for all 1-kb regions in the genome. Regions with at least 2-fold differences (p-value$\leqslant$0.05) are colored in beige. **(C)** Cer3 smFISH signal shown in a montage of 16 0.2 µm optical sections of a nucleus in proliferative zone, transition zone, and pachytene of WT (N2) and *hrde-1* mutant germline. Scale bars: 2 µm.

Table S1. smFISH target sequences and probe sequences

Probe name: Cer3_GAG

Dyes: CAL Fluor® Red 610 Dye, Quasar® 670 Dye

Cer3_GAG target sequence:
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Cer3_GAG smFISH Probes:
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Probe name: Cer8

Dyes: CAL Fluor® Red 610 Dye, Quasar® 570 Dye

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Probe name: oma-1

Dye: Quasar® 570 Dye

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tcctggcattggagagaatg
tattttcggccaattgatcg
ccacaaattgagatgcttgg

Table S2. List of NGS libraries used in this study

Sample name	Library name	Type of library
N2 adult total sRNA	SG0616b_lib11	sRNA-seq
HRDE-1 co-IP sRNA	SG0616b_lib13	sRNA-seq
N2, 25ºC, sRNA PMID:20116306	GSM503822_Gent_N2_P-independent_3	sRNA-seq
BA17 *fem-1(hc17)*, 25ºC, sRNA, PMID:20116306	GSM503834_Gent_fem-1_P-independent	sRNA-seq
CB4037 *glp-1(e2141)*, 25ºC, sRNA PMID:20116306	GSM503833_Gent_glp-1_P-independent	sRNA-seq
N2 total RNA, repeat 1	SG0117_lib34	RNA-seq
N2 nuclear RNA, repeat 1	SG0117_lib35	RNA-seq
N2 cytoplasmic RNA, repeat 1	SG0117_lib36	RNA-seq
hrde-1 total RNA, repeat 1	SG0117_lib37	RNA-seq
hrde-1 nuclear RNA, repeat 1	SG0117_lib38	RNA-seq
hrde-1 cytoplasmic RNA, repeat 1	SG0117_lib39	RNA-seq
N2 total RNA, repeat 2	SG0117_lib40	RNA-seq
N2 nuclear RNA, repeat 2	SG0117_lib41	RNA-seq
N2 cytoplasmic RNA, repeat 2	SG0117_lib42	RNA-seq
hrde-1 total RNA, repeat 2	SG0117_lib43	RNA-seq
hrde-1 nuclear RNA, repeat 2	SG0117_lib44	RNA-seq
hrde-1 cytoplasmic RNA, repeat 2	SG0117_lib45	RNA-seq