Supplemental material

PLP-1 is essential for germ cell development and germline gene silencing in *C. elegans*

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Figure S1

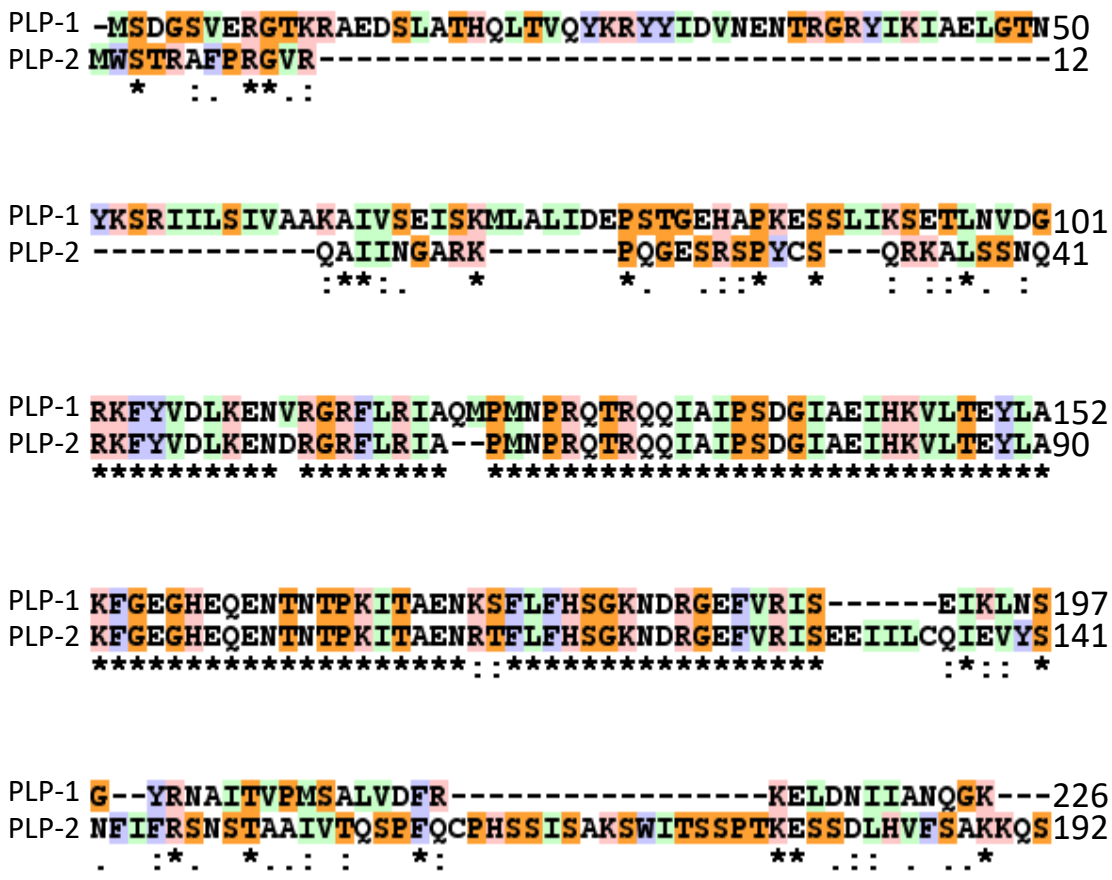


Fig. S1. Alignment of the amino acid sequences of PLP-1 and PLP-2. Amino acid sequences have been aligned using the CLUSTALW program supplied with the DNA DYNAMO software package. GenBank accession numbers: PLP-1 – NP_501241; PLP-2 – CAB01747. Identical amino acids are indicated by an asterisk (*), and the ones with very similar side chains and somewhat similar sides chains are indicated by two dots (:) and by single dot (.), respectively.

Figure S2

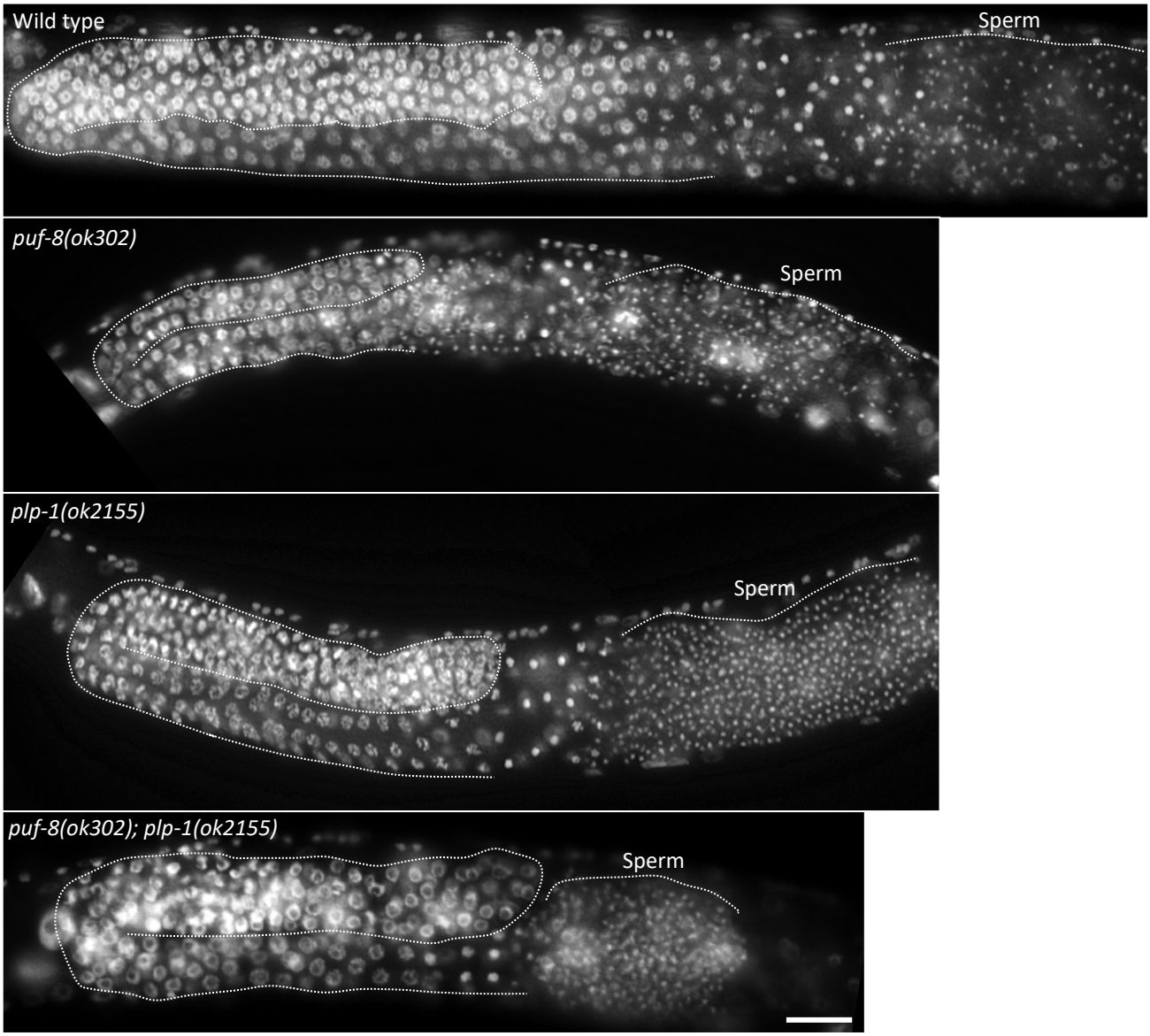


Fig. S2. PUF-8 and PLP-1 are not essential for spermatogenesis in males. Adult males of the indicated genotypes stained with DAPI are shown. Scale bar = 20μm.

Figure S3

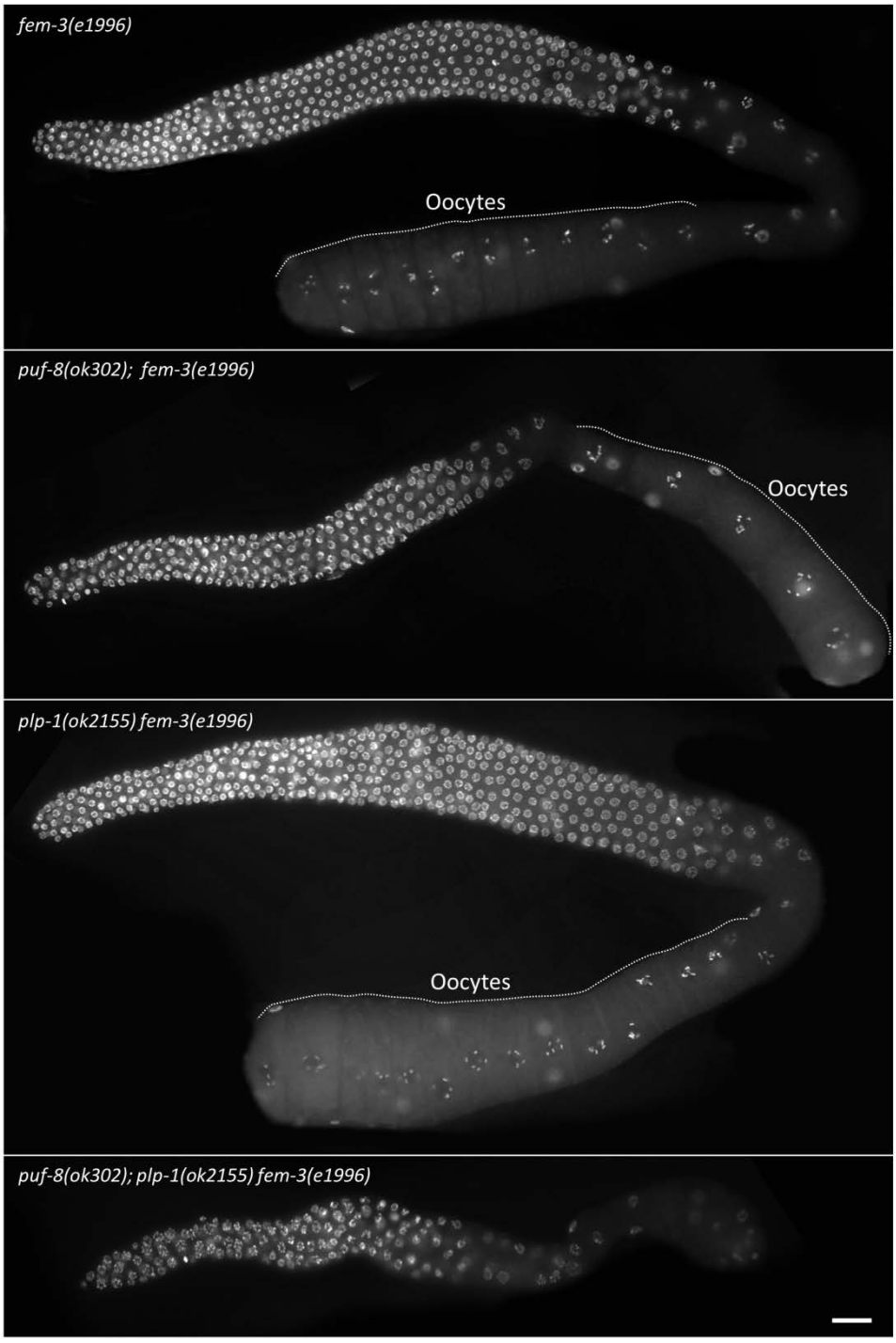


Fig. S3. Germ lines missing PUF-8 and PLP-1 fail to produce oocytes. Extruded germ lines of the indicated genotypes stained with DAPI are shown. The *puf-8; plp-1* mutant germ lines do not produce oocytes even when set in the "female mode" by the *fem-3* mutation. Scale bar = 20µm.

Figure S4

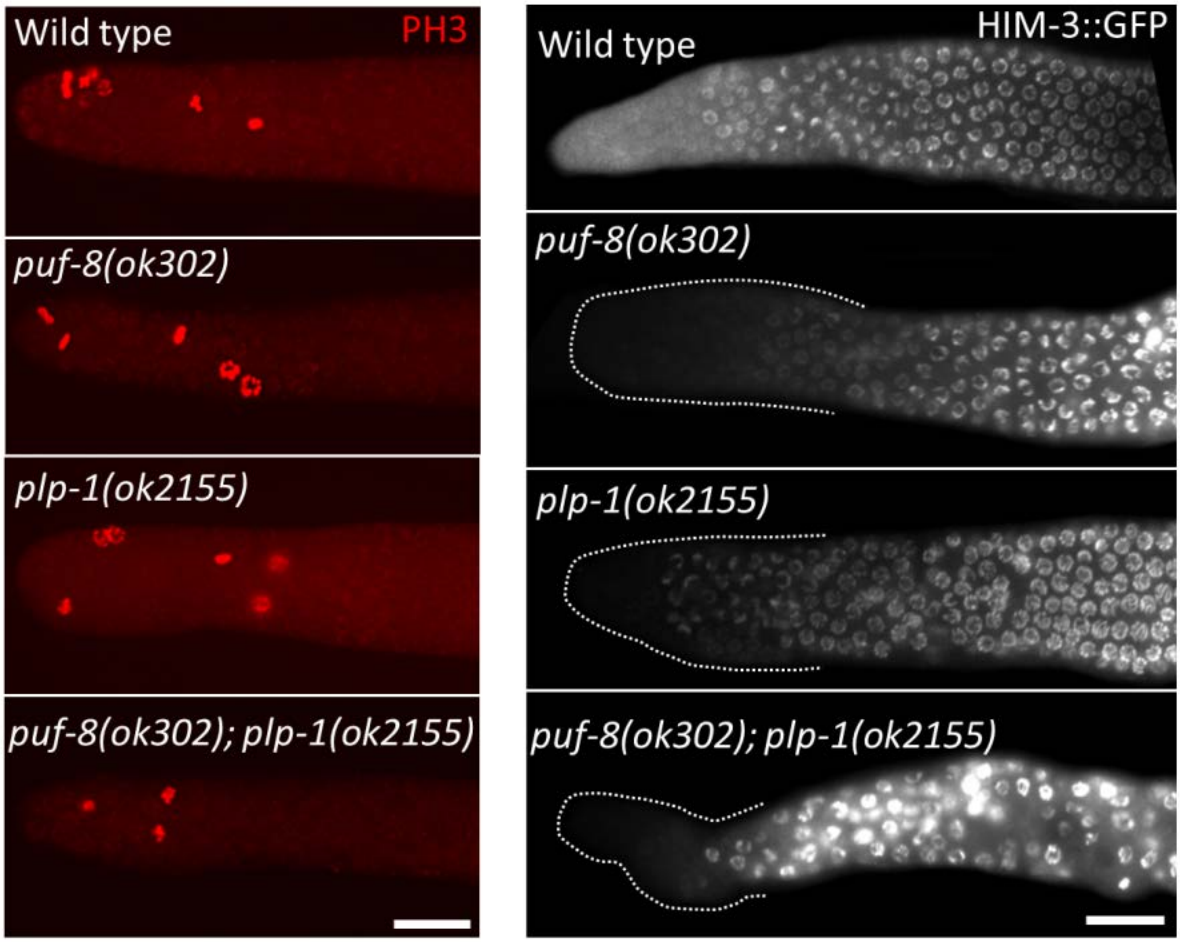


Fig. S4. Mitotic proliferation and meiotic entry are not affected in *puf-8; plp-1* germ lines. **Left** – Extruded germ lines of the indicated genotypes immunostained with anti-phosphohistone H3 (PH3) antibodies are shown. Metaphase nuclei brightly stained with anti-PH3 antibodies are visible in all genotypes. Average numbers of PH3-positive nuclei per germ line were: wild type – 4.82 (n=71); *puf-8* – 3.02 (n=46); *plp-1* – 4.33 (n=67) and *puf-8; plp-1* – 1.77 (30). Right – Germ lines extruded from live worms carrying the *him-3::gfp* transgene are shown. Expression of the meiotic marker HIM-3::GFP is seen in all genotypes. Scale bar = 20µm.

Figure S5

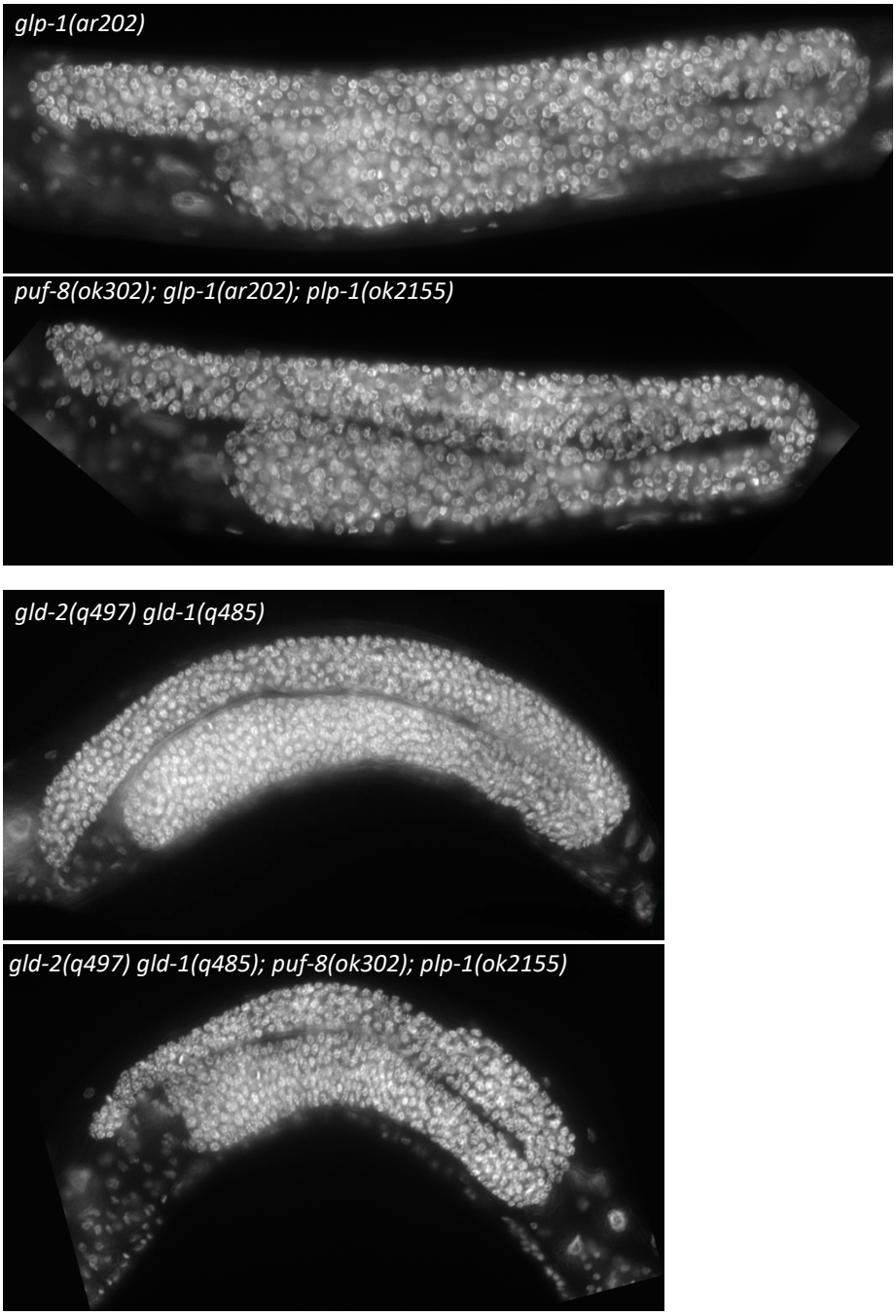
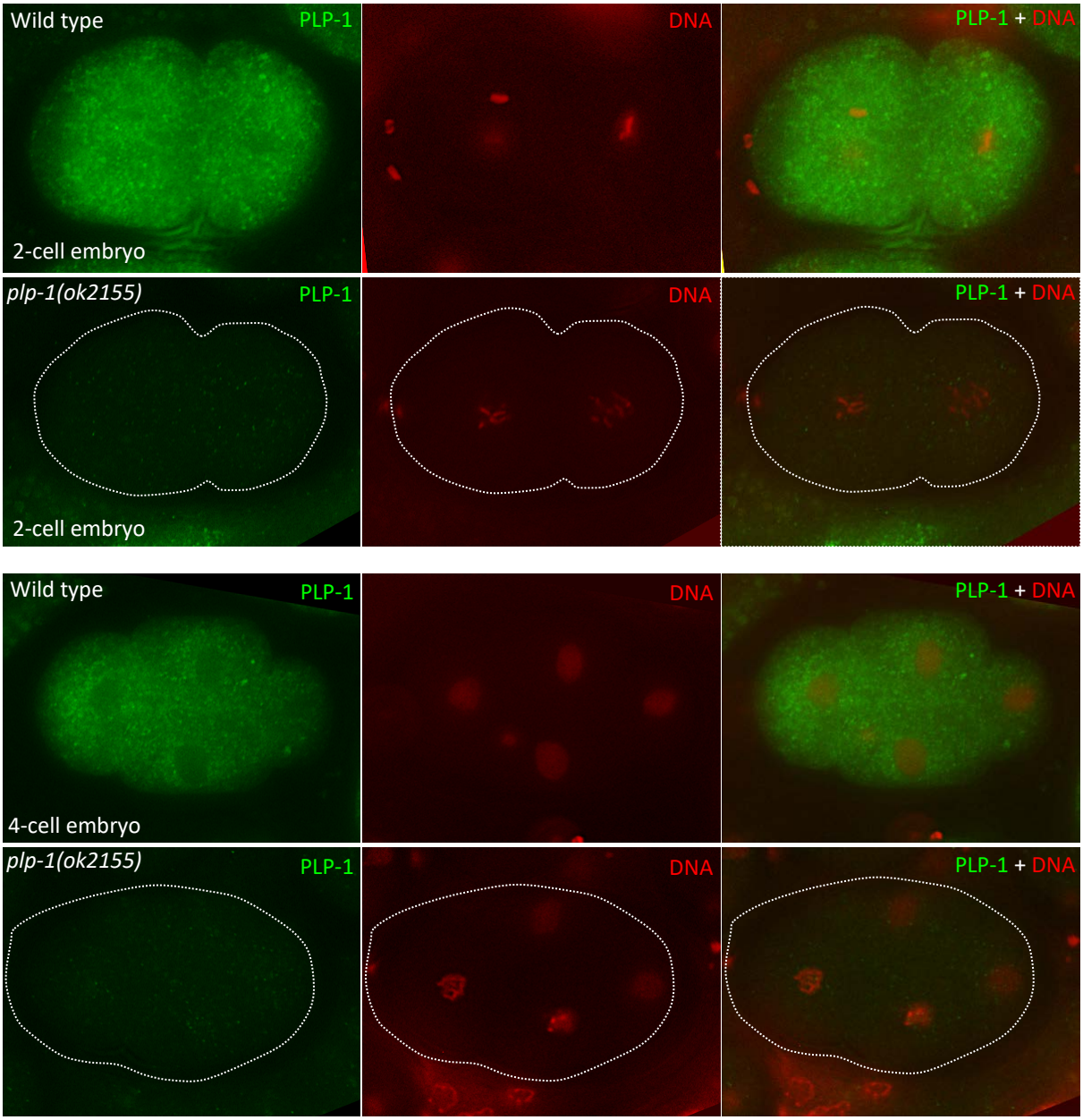


Fig. S5. PUF-8 and PLP-1 are not essential for tumor development in germ lines defective for meiotic entry. DAPI-stained animals of the indicated genotypes are shown. Only parts of animals revealing one of the gonadal arms are shown.

Figure S6



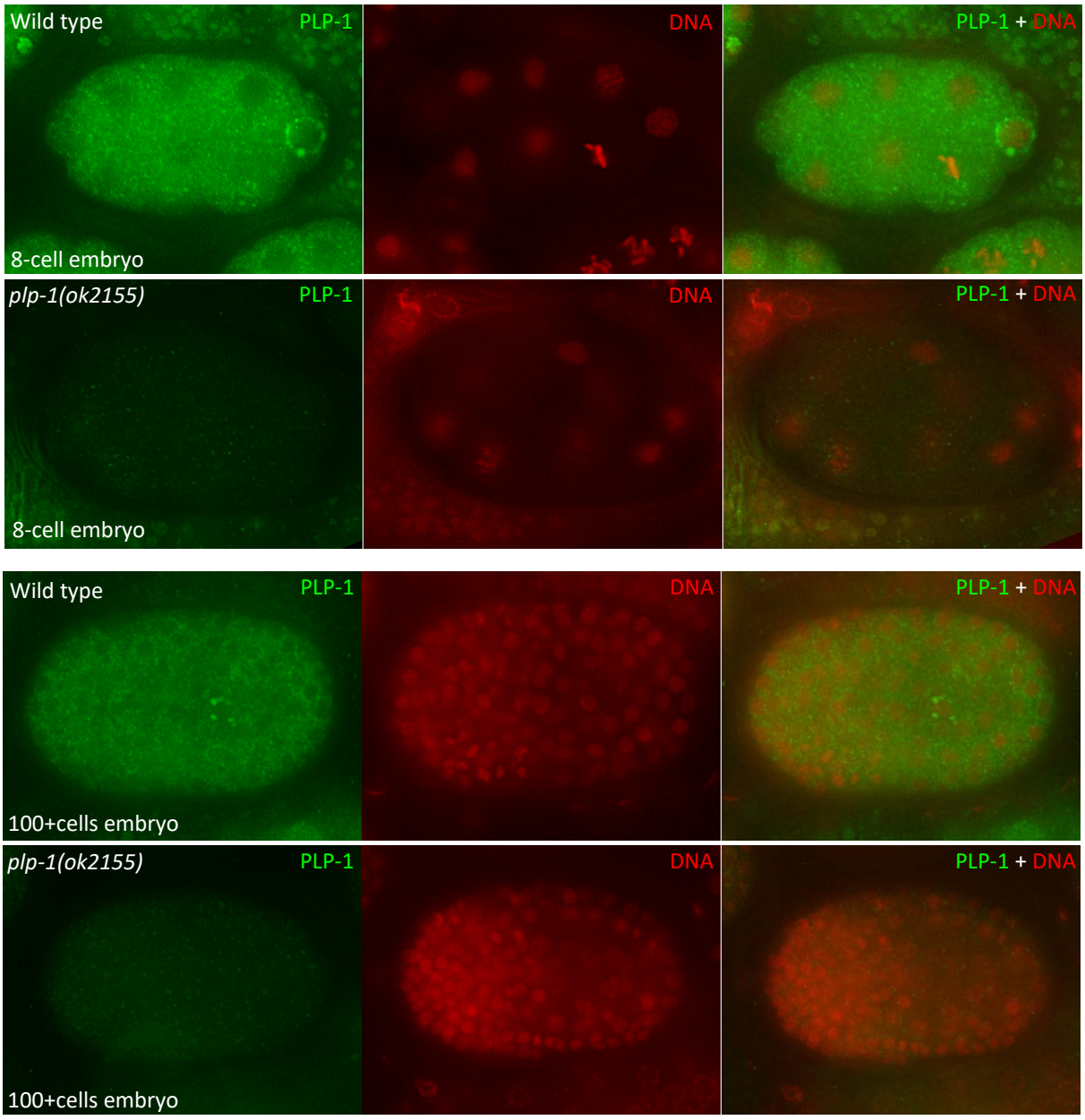


Fig. S6. PLP-1 is present in all blastomeres throughout embryo development. Embryos at different stages of development were stained with anti-PLP-1 antibodies (green) and DAPI (red). Immunofluorescence signals could be detected in the cytoplasm of all blastomeres throughout embryonic development in wild-type embryos. By contrast, no immunofluorescence was detected in *plp-1* mutant embryos, indicating that the antibody is PLP-1-specific. Perinuclear PLP-1 puncta are visible in the 8-cell (near the right end of the embryo) and 100+cells (middle right of the embryo) wild-type embryos. These puncta are not prominent in younger embryos, presumably due to the effect of formaldehyde fixation.

Figure S7

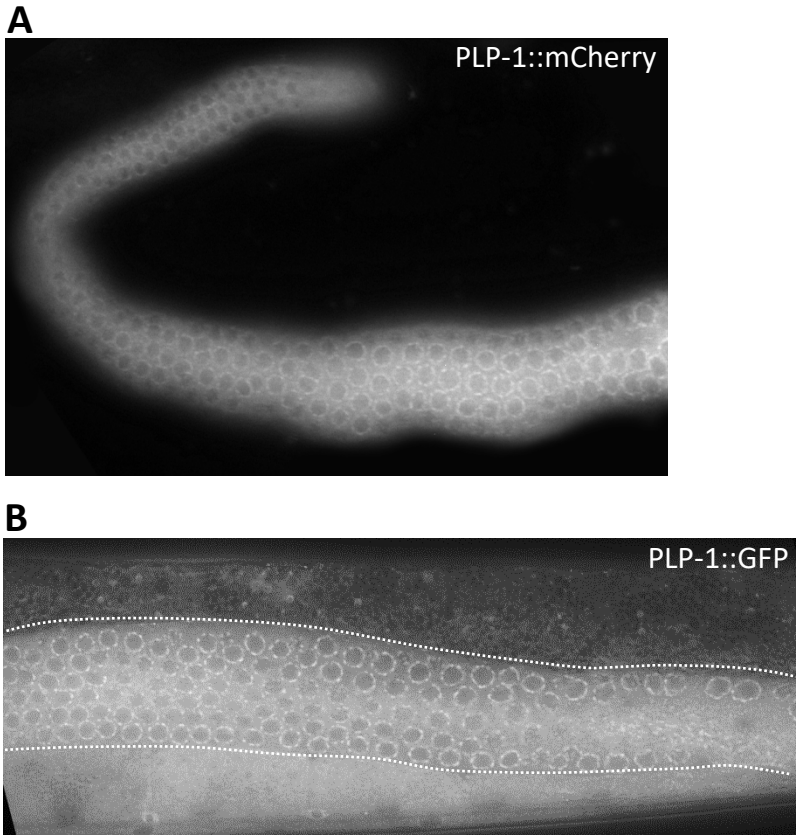


Fig. S7. Distribution patterns of PLP-1::mCherry and PLP-1::GFP. (A) Extruded germ line showing the distribution pattern of PLP-1::mCherry. (B) A section of intact hermaphrodite showing the distribution pattern of PLP-1::GFP. In this line, the endogenous *plp-1* locus has been tagged at the C-terminus with the GFP reporter using the CRISPR/Cas9 method. Note: In both cases, the perinuclear puncta of PLP-1 are prominently visible.

Figure S8

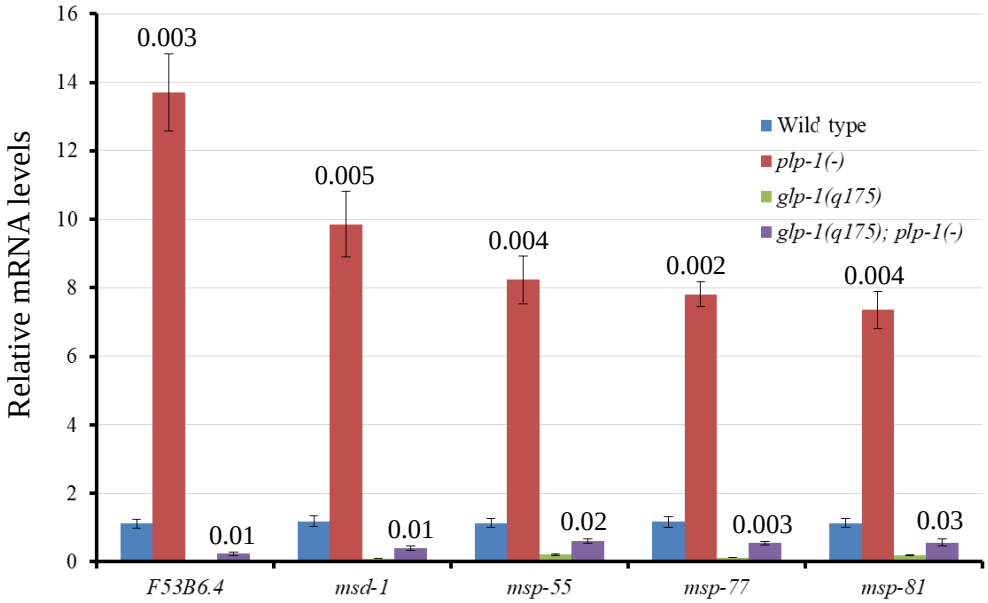


Fig. S8. PLP-1 negatively regulates the expression of sperm-related genes in the germ line. Transcript levels, quantitated by quantitative RT-PCR, of five sperm-related genes in adult hermaphrodites of the indicated genotypes are shown. All values are relative to the wild-type level which is taken as 1. These five genes were randomly selected from the list of genes identified by transcriptome sequencing as upregulated in *plp-1* mutants. Very low levels in *glp-1(q175)*, which lacks germ cells, show that these genes are mainly expressed in the germ line. Increase in transcript levels in the germ line is largely responsible for the upregulation seen in *plp-1* single mutants (compare red and purple bars). Error bars represent standard deviation. Numbers above the bars are *p*-values calculated using Student's t-test.

Figure S9

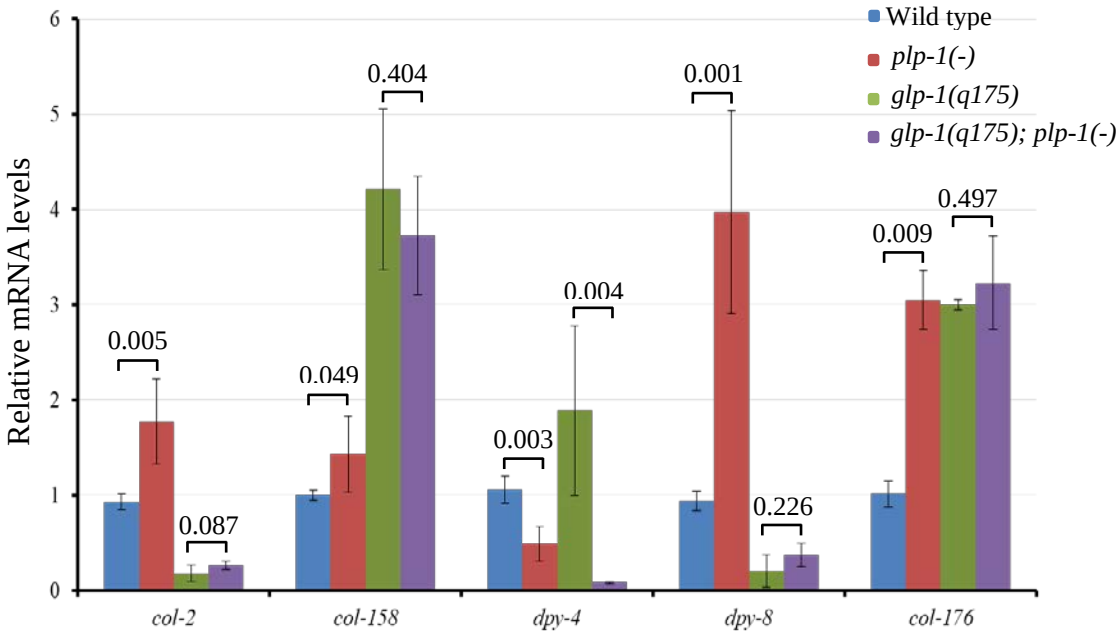


Fig. S9. Loss of PLP-1 results in the misexpression of some non-germline-enriched genes in the germ line. Transcript levels, quantitated by quantitative RT-PCR, of five non-germline-enriched genes in adult hermaphrodites of the indicated genotypes are shown. All values are relative to the wild-type level which is taken as 1. These five genes were randomly selected from the list of genes identified by transcriptome sequencing as upregulated in *plp-1* mutants. Difference between wild type and *glp-1(q175)*, which lacks germ cells, represents the level of expression in the germ line. In the absence of PLP-1, *col-2* and *dpy-8* transcript levels increase in the germ line (compare the differences between blue and red bars with green and purple bars). Intriguingly, *col-158* and *col-176* levels increase in *glp-1(q175)* animals; whether the absence of germ cells or GLP-1 led to this increase is not clear. In contrast to the transcriptome-sequencing results, qRT-PCR indicates a decrease in the transcript levels of *dpy-4* in *plp-1* mutant animals. Error bars represent standard deviation. Numbers above the bars are *p*-values calculated using Student's t-test.

Figure S10

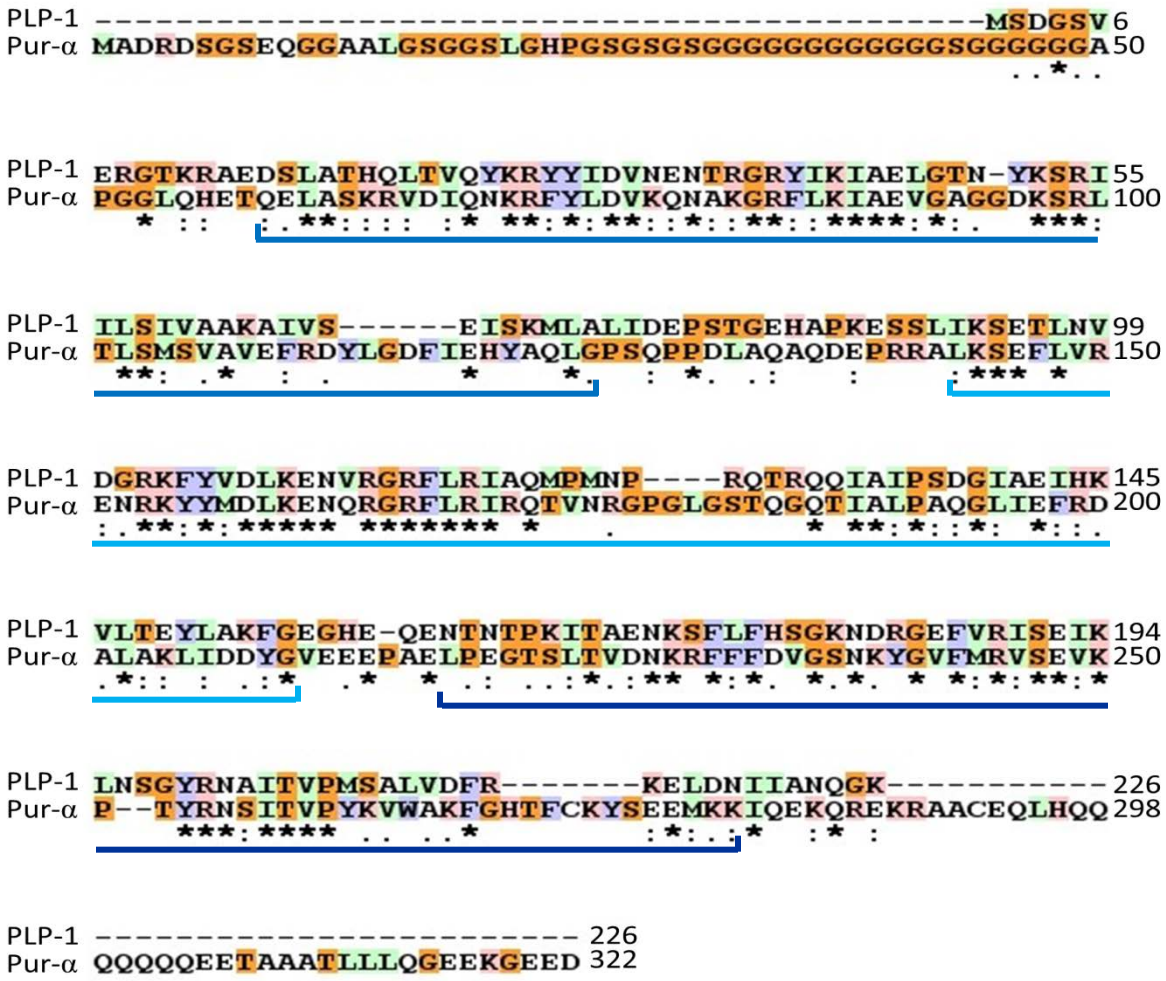


Fig. S10. Alignment of the amino acid sequences of *C. elegans* PLP-1 and human Pur-alpha proteins. The three Pur repeats are underlined. GenBank accession numbers: PLP-1 – NP_501241; human Pur-alpha – AAV38195. Identical amino acids are indicated by an asterisk (*), and the ones with very similar side chains and somewhat similar sides chains are indicated by two dots (:) and by single dot (.), respectively.

Figure S11

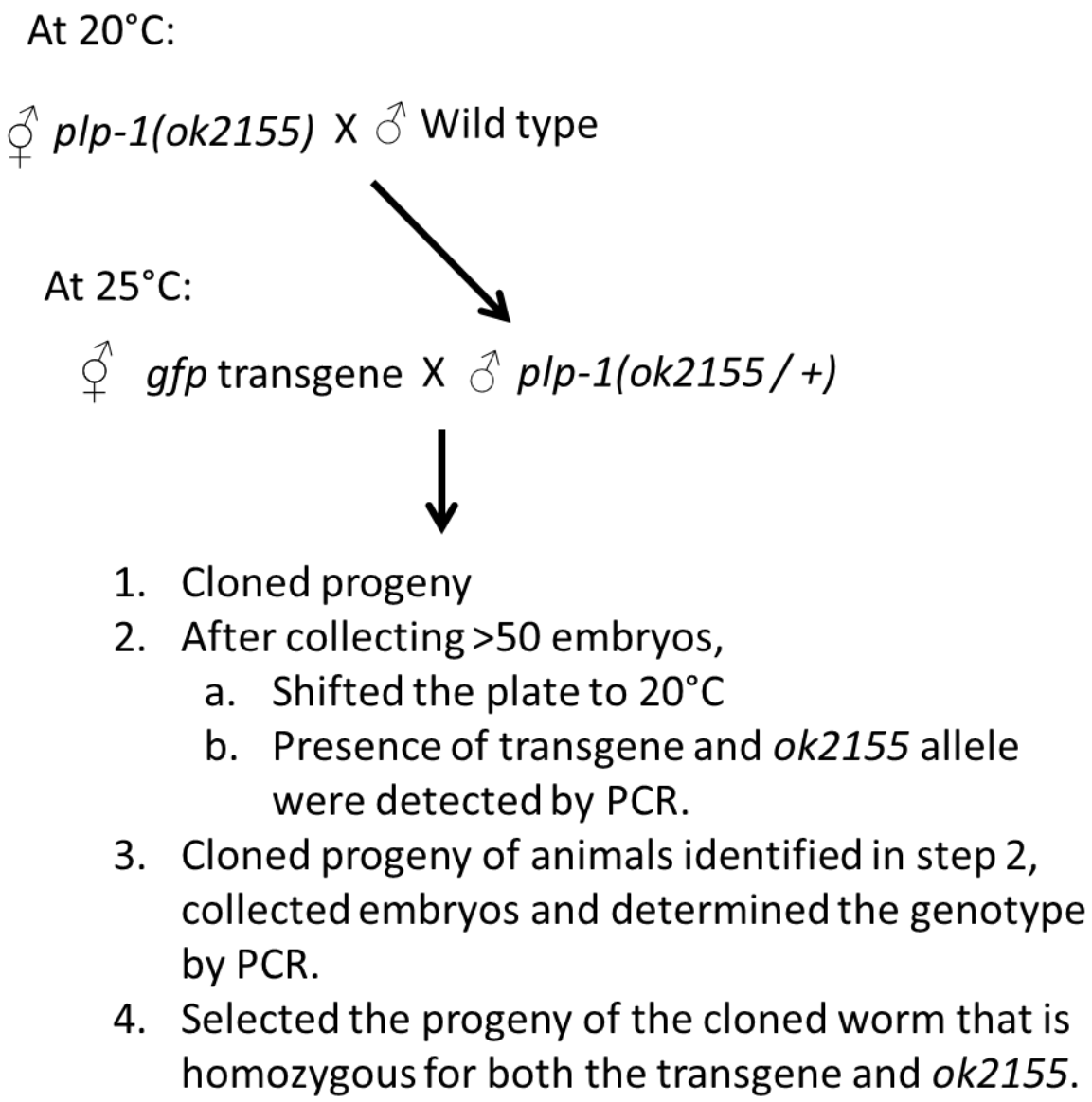


Fig. S11. Flowchart illustration of the scheme for introducing silencing-prone transgenes into the *plp-1* mutant background

Table S1. Genetic redundancy between *puf-8* and *plp-1* in the germ line

Genotype	Percent of fertile worms (n)	Percent of sterile worms (n)
Wild type	100 (500)	0 (500)
puf-8(ok302)	100 (548)	0 (548)
plp-1(ok2155)	100 (500)	0 (500)
puf-8(ok302); plp-1(ok2155)	0.56 (358)	99.44 (358)

Table S2. *plp-1(-)* hermaphrodites are sterile at 25°C

Genotype	Percent of fertile worms (n)	Percent of sterile worms (n)	Percent of worms with endomitotic oocytes (n)
Wild type 1-day-old adults	100 (114)	0 (114)	0 (114)
plp-1(ok2155) 1-day-old adults	0 (248)	100 (248)	0 (248)
plp-1(ok2155) 2-day-old adults	0 (162)	100 (162)	63 (162)
plp-1(ok2155) 3-day-old adults	0 (68)	100 (68)	Not determined

Table S3. Summary of the observations from the single-male crosses

Plate No.	Progeny present?	Genotype of the male parent	Plate No.	Progeny present?	Genotype of the male parent
1	Yes	*plp-1(-/+)*	13	No	***plp-1(-/-)***
2	Yes	*plp-1(-/+)*	14	No	***plp-1(-/-)***
3	Yes	*plp-1(-/+)*	15	No	***plp-1(-/-)***
4	Yes	*plp-1(-/+)*	16	No	*plp-1(-/+)*
5	Yes	*plp-1(-/+)*	17	No	***plp-1(-/-)***
6	Yes	*plp-1(-/+)*	18	No	*plp-1(-/+)*
7	Yes	*plp-1(-/+)*	19	No	***plp-1(-/-)***
8	Yes	*plp-1(-/+)*	20	No	***plp-1(-/-)***
9	Yes	*plp-1(-/+)*	21	No	***plp-1(-/-)***
10	Yes	*plp-1(-/+)*	22	No	***plp-1(-/-)***
11	Yes	*plp-1(-/+)*	23	No	*plp-1(-/+)*
12	Yes	*plp-1(-/+)*	24	No	*plp-1(-/+)*

Table S4. Response of *plp-1(ok2155)* mutants to RNAi

	Percent of viable embryos (n)	
Target of RNAi	**Wild type**	***plp-1(ok2155)***
mex-3	0.49 (1843)	0.31 (1287)
pos-1	1.21 (1901)	11.77 (1368)
spn-4	1.38 (1805)	15.41 (1590)
Non-RNAi control	100 (1110)	99.68 (1248)

Table S5. Small RNA high-throughput sequencing data

Click here to Download Table S5

Table S6. Protein-coding transcriptome sequencing data

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Table S7. List of genes in various gene clusters upregulated in the *plp-1* mutant

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Table S8. List of genes in various gene clusters downregulated in the *plp*-1 mutant

Click here to Download Table S8

Table S9. List of *C. elegans* strains used in this study

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Table S10. List of oligonucleotides used in this study

Click here to Download Table S10

Supplementary references

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