Science Advances
AAAS

Supplementary Materials for

Pachytene piRNAs control discrete meiotic events during spermatogenesis and restrict gene expression in space and time

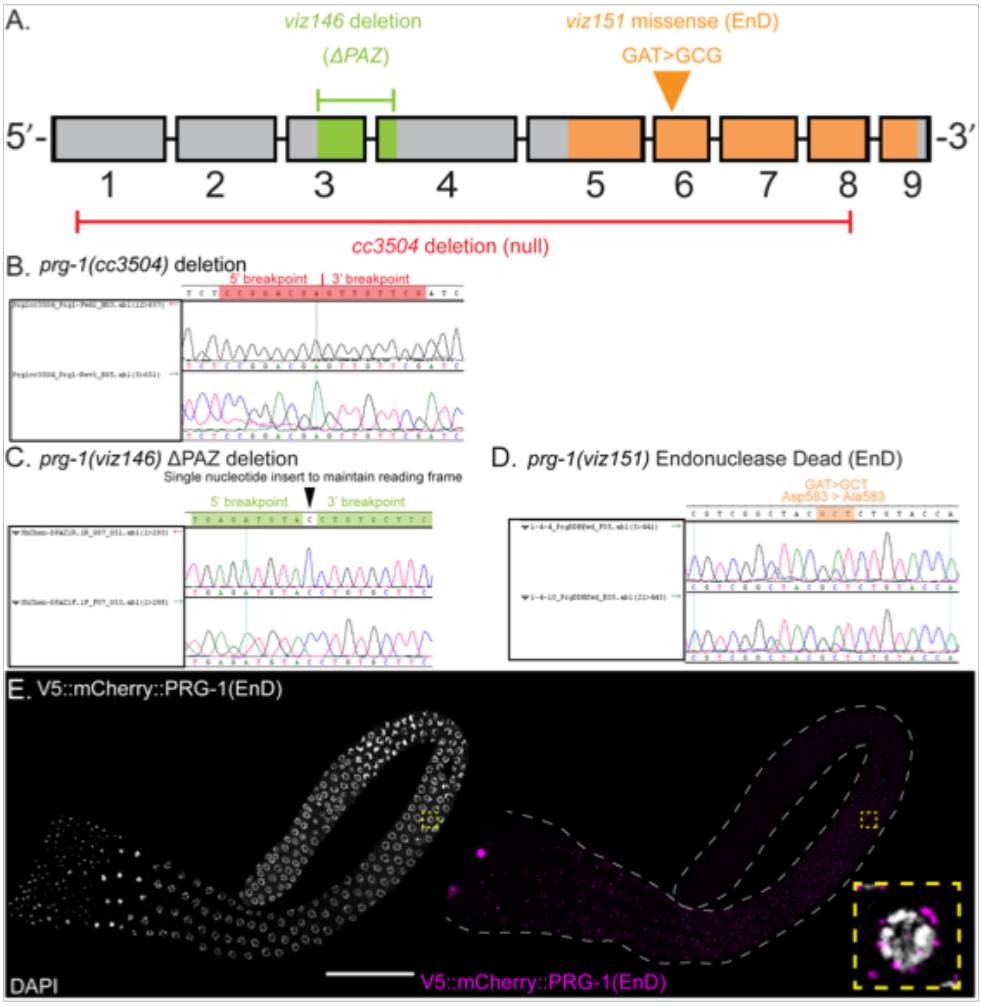
Jacob Ortega *et al.*

Corresponding author: Swathi Arur, sarur@mdanderson.org

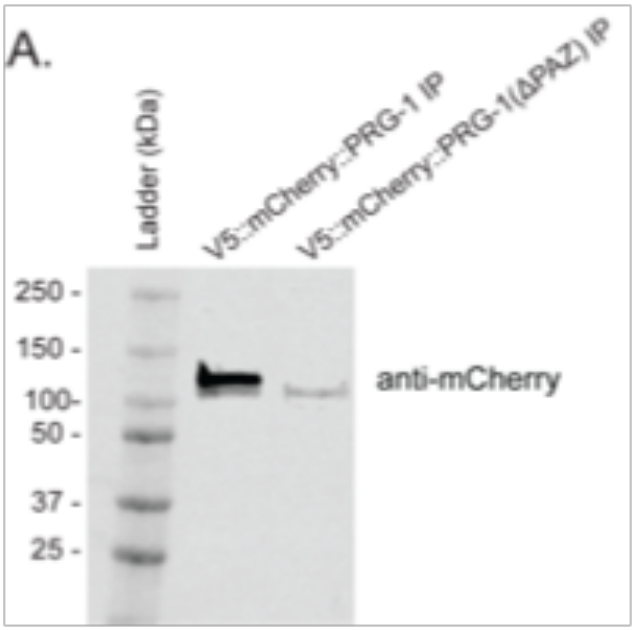
Sci. Adv. **10**, eadp0466 (2024)
DOI: 10.1126/sciadv.adp0466

The PDF file includes:

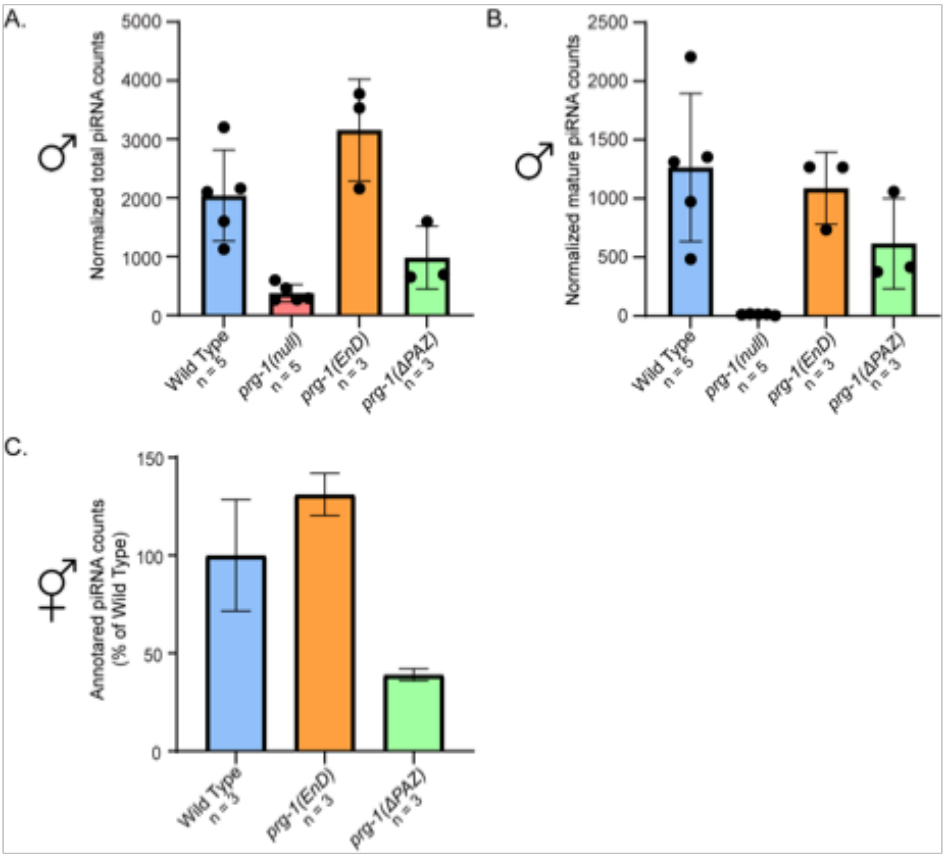
Other Supplementary Material for this manuscript includes the following:



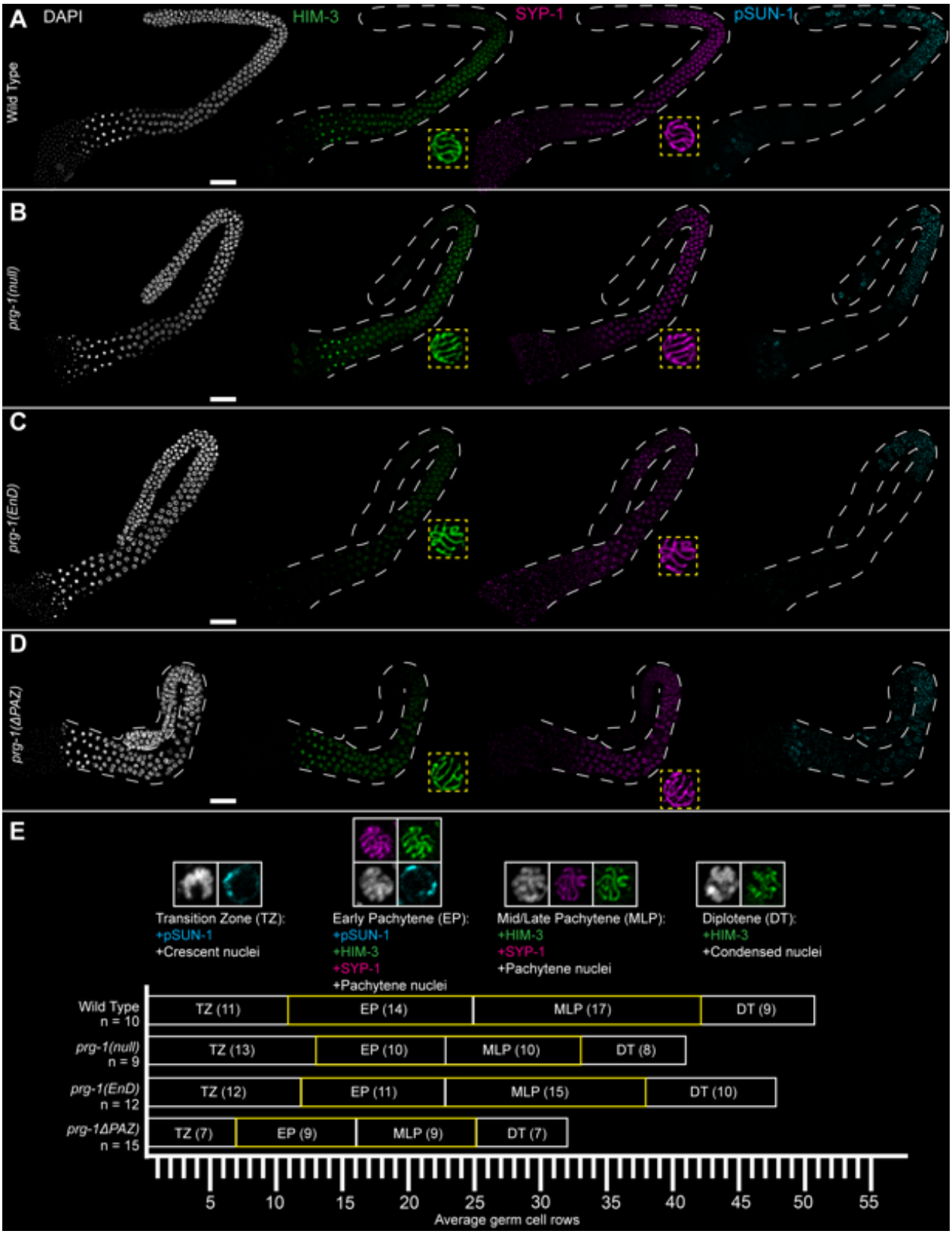
Supp. Fig. 1: Sequencing data for *prg-1* domain mutants. (**A**) Schematic of *prg-1* gene exon structure with protein-domain coding regions highlighted (PAZ-domain, green) (PIWI-domain, orange). Null and domain-specific mutations are noted (Null in red, ΔPAZ in green, EnD in orange). (**B**) Sequencing trace noting the 5′ and 3′ breakpoints for the *prg-1(cc3504)* deletion. (**C**) Sequencing trace noting the 5′ and 3′ breakpoints for the *prg-1(ΔPAZ)* PAZ-domain deletion. Black arrowhead highlights single nucleotide insertion to maintain reading frame. (**D**) Sequencing trace for endonuclease-dead allele noting single amino acid substitution at Asp583. (**E**) Representative image from DAPI-stained (white) dissected male germline expressing V::mCherry::PRG-1(EnD) (magenta). Inset (dashed line box, yellow) highlights a single pachytene germ cell nuclei. Scale bar: 50 μm.



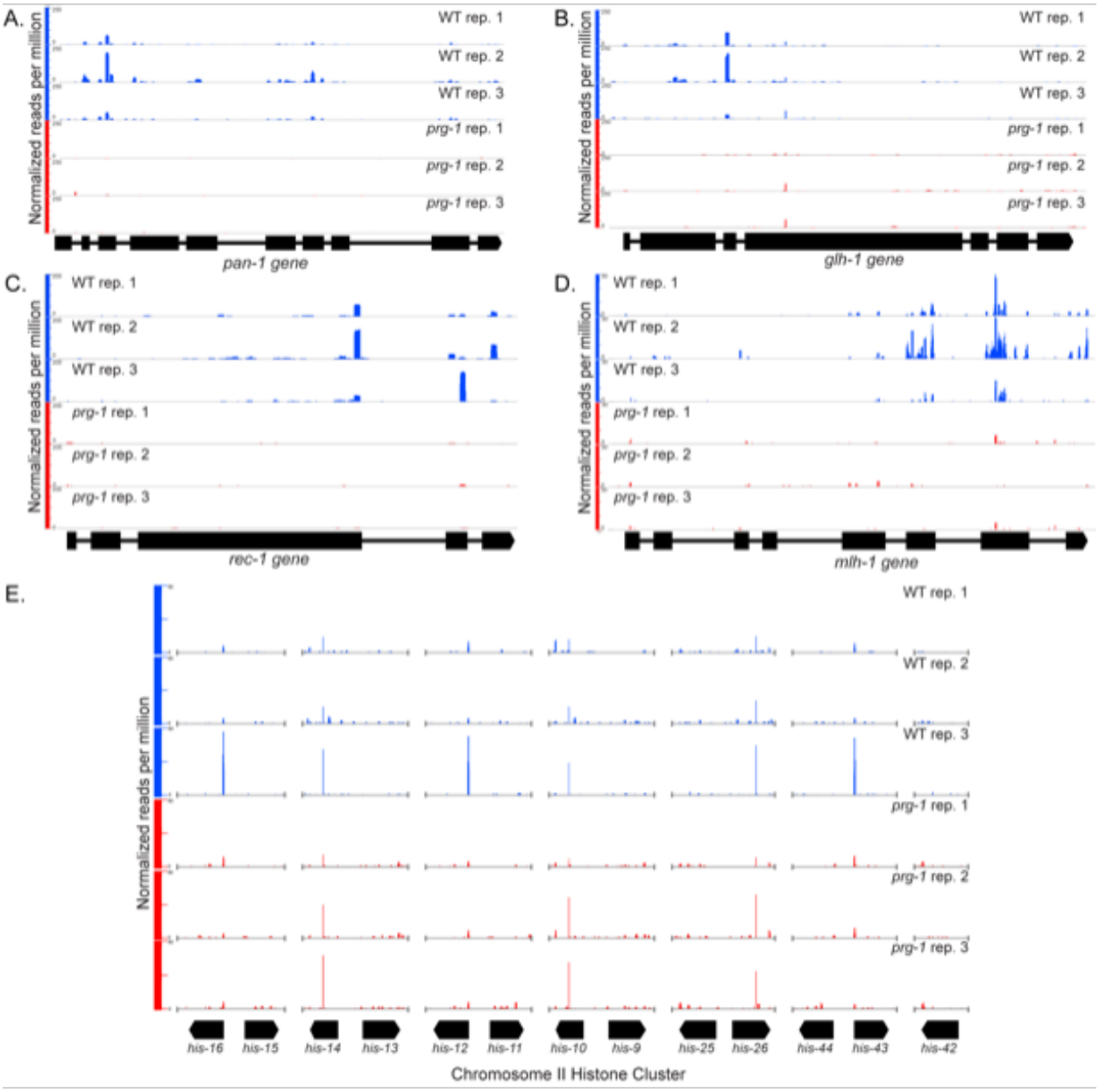
Supp. Fig. 2: Western blot for IP of V5::mCherry PRG-1 strains for RNA IP. (**A**) Western blot for IP of V5::mCherry-tagged PRG-1 strains after IP using V5-Trap beads and 1 mg of worm lysate. Because the total amount of PRG-1 protein in ΔPAZ deletion mutant is 60% reduced relative to Wild Type, to sequence small interacting RNAs from equal amount of WT and ΔPAZ PRG-1, we performed IP from seven fold as much worm lysate as WT and used 5mg of total protein for each, to perform the IP sequencing.



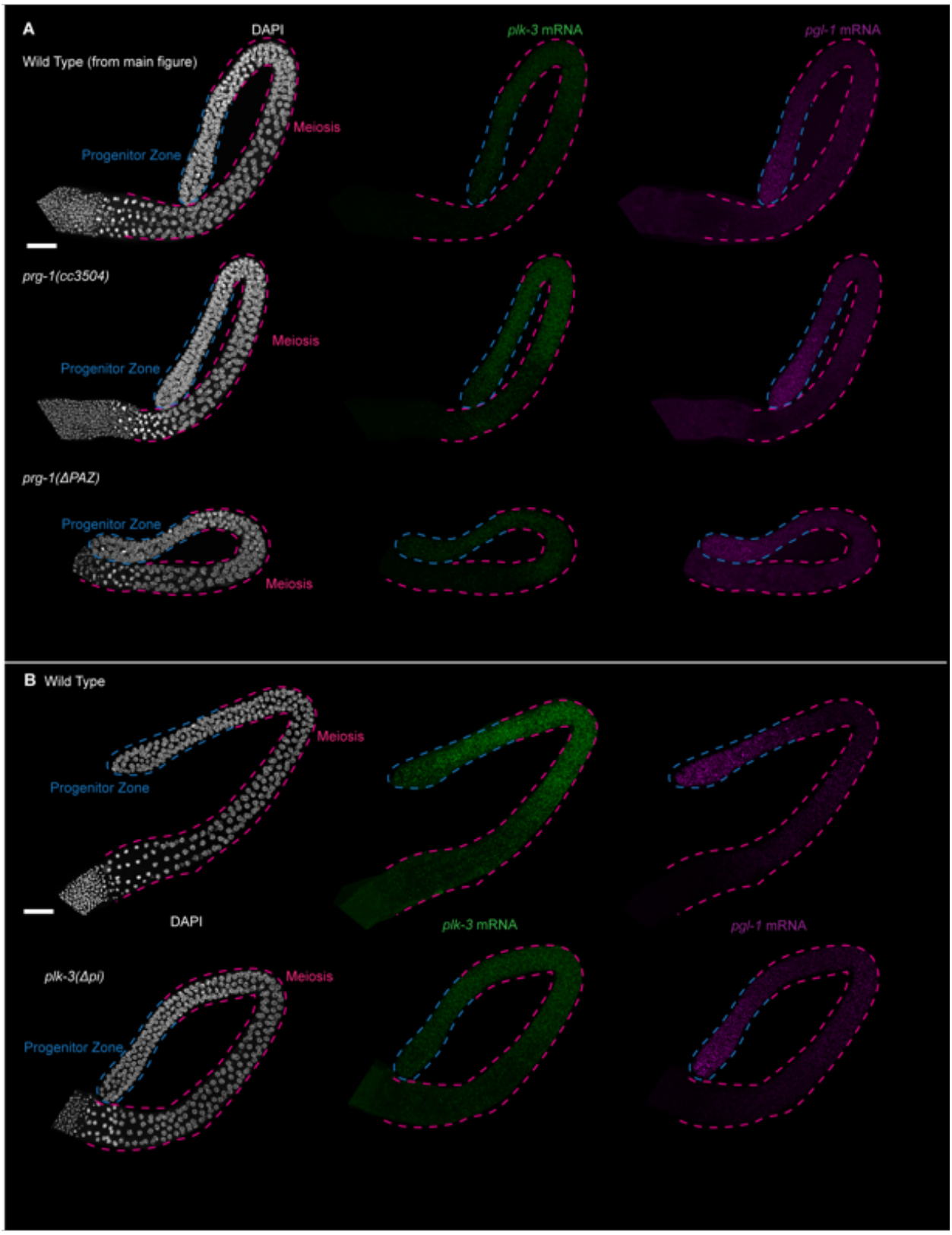
Supp. Fig. 3: Graphs for piRNA read mapping data. (**A**) Graph of total read counts mapping to annotated piRNAs after normalization to total genome mapped reads. (**B**) Graph of mature (21 nt) piRNA counts mapping to annotated piRNAs after normalization to total genome mapped reads. (**C**) Graph of total piRNA read counts mapping to annotated piRNAs after normalization, expressed as percentage relative to wild type.


A
DAPI
HIM-3
SYP-1
pSUN-1
Wild Type
B
prg-1(null)
C
prg-1(EnD)
D
prg-1(ΔPAZ)
E
Transition Zone (TZ):
+pSUN-1
+Crescent nuclei
Early Pachytene (EP):
+pSUN-1
+HIM-3
+SYP-1
+Pachytene nuclei
Mid/Late Pachytene (MLP):
+HIM-3
+SYP-1
+Pachytene nuclei
Diplotene (DT):
+HIM-3
+Condensed nuclei
Wild Type
n = 10
TZ (11)
EP (14)
MLP (17)
DT (9)
prg-1(null)
n = 9
TZ (13)
EP (10)
MLP (10)
DT (8)
prg-1(EnD)
n = 12
TZ (12)
EP (11)
MLP (15)
DT (10)
prg-1ΔPAZ)
n = 15
TZ (7)
EP (9)
MLP (9)
DT (7)
5
10
15
20
25
30
35
40
45
50
55
Average germ cell rows

Supp. Fig. 4: Meiotic markers and quantification of meiotic progression defects in *prg-1* mutant germlines. (**A-D**) Representative images of dissected adult male germlines analyzed. Germlines were stained with DAPI (white), anti-HIM-3 (green), anti-SYP-1 (magenta), and pSun-1 (cyan). Insets (dashed-boxes, yellow) indicate single pachytene germ cell nuclei. (**E**) Graphical representation of the average proportion of total germ cell rows that are either transition zone stage, early pachytene stage, mid/late pachytene stage, or diplotene stage across germlines analyzed. Numbers graphed are averages across germlines analyzed. Scale bar: 50 μm. Each experiment was performed at least in triplicate and each time at least 25-30 germlines were analyzed.

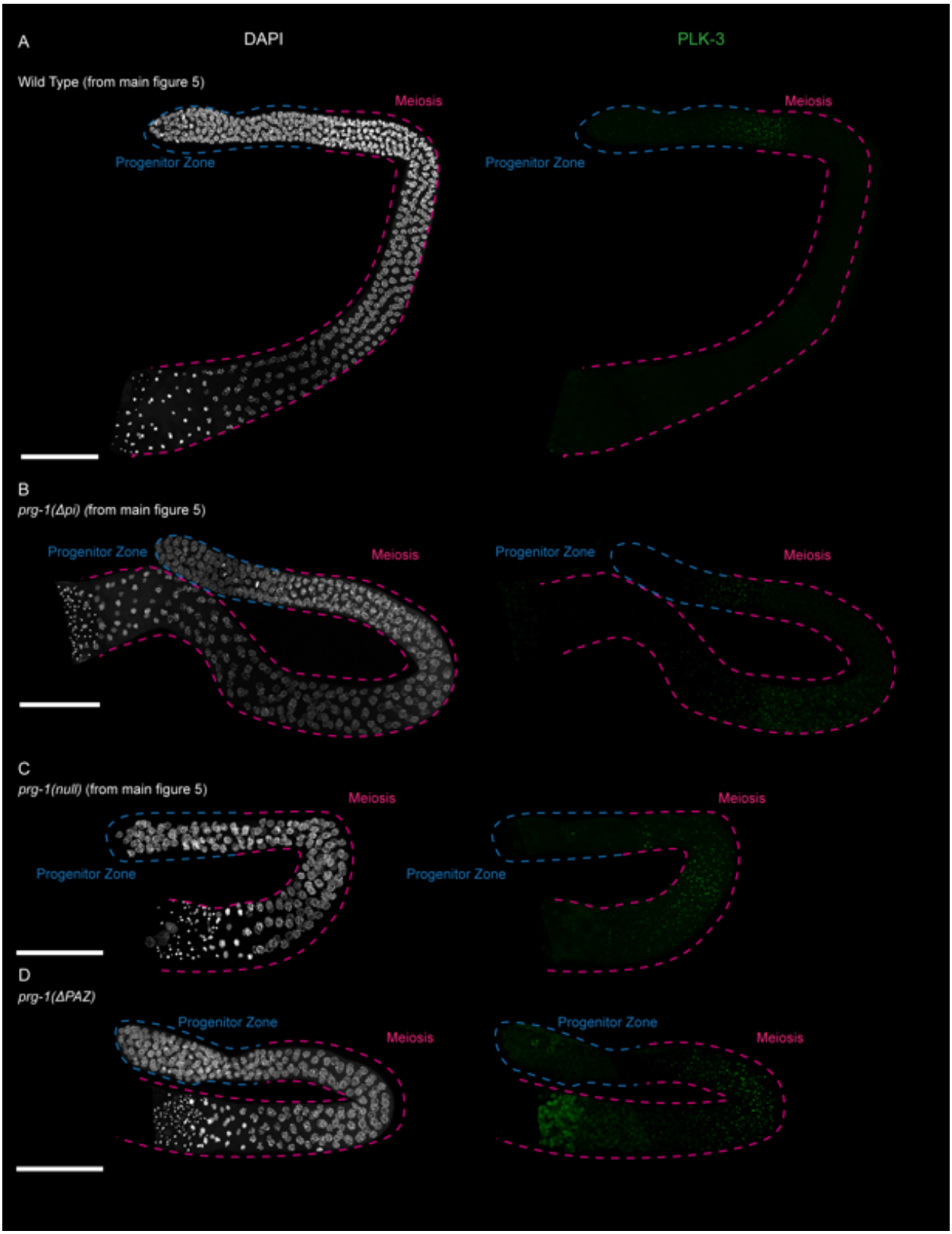


Supp. Fig. 5: Additional graphs of individual genes for 22G siRNA analysis (**A-D**) Read histograms displaying mapped 22G small RNA reads for select genes where 22G siRNAs were changed significantly reduced in *prg-1(null)*. Replicates for wild type samples are labeled in blue and replicates for *prg-1(null)* samples are labeled in red. (**E**) Read histogram of mapped 22G small RNA reads for the histone cluster on *C. elegans* chromosome II, which displayed increased 22G siRNA reads in previous studies of *prg-1* mutant hermaphrodites. None of the thirteen genes displayed significant changes in mapped reads.

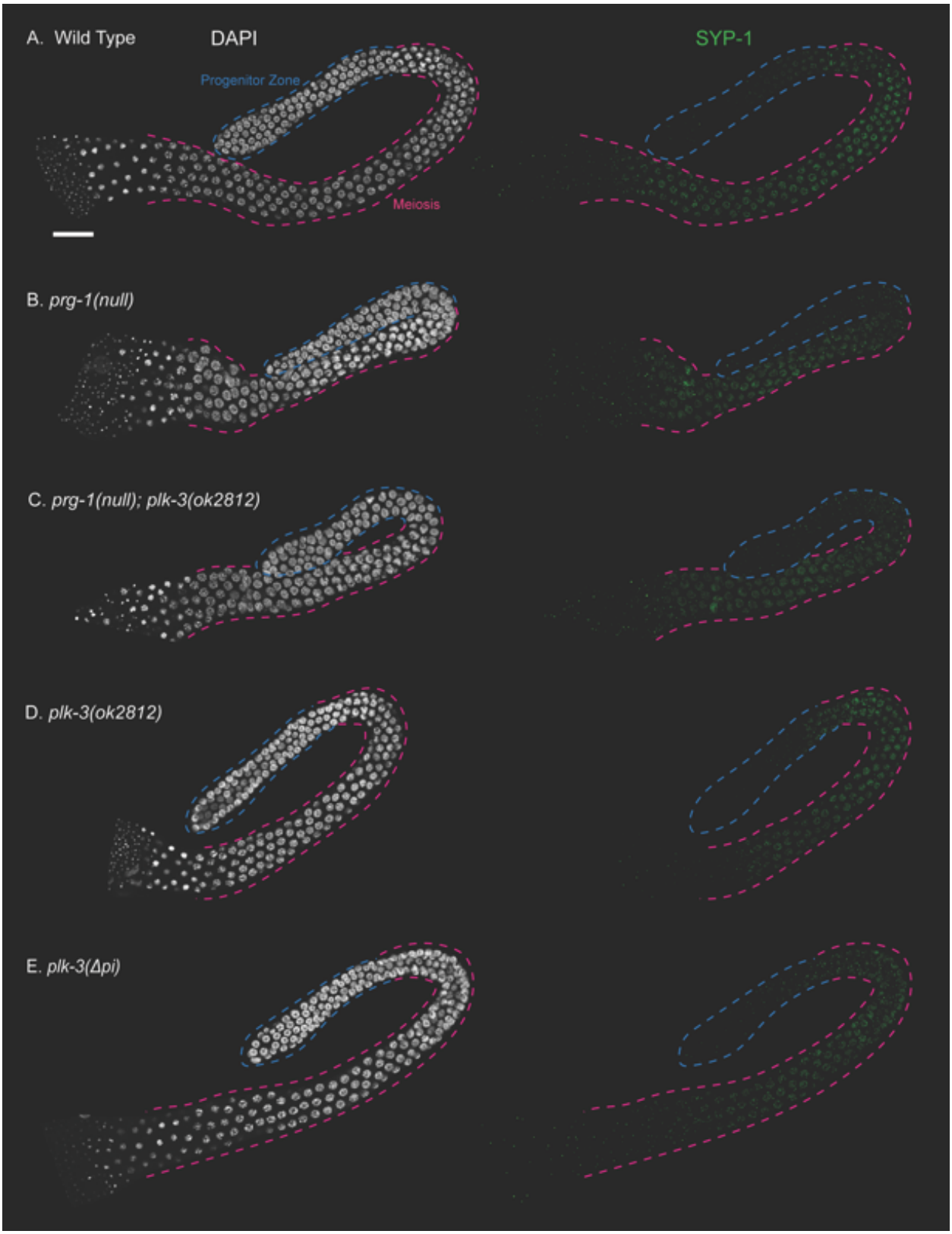


Supp. Fig. 6: HCR-FISH germlines (**A**) HCR-FISH experiment for Wild Type (from main figure) compared to parallel experiments in *prg-1(null)* and *prg-1(ΔPAZ).* The presence of *pgl-1* probe,

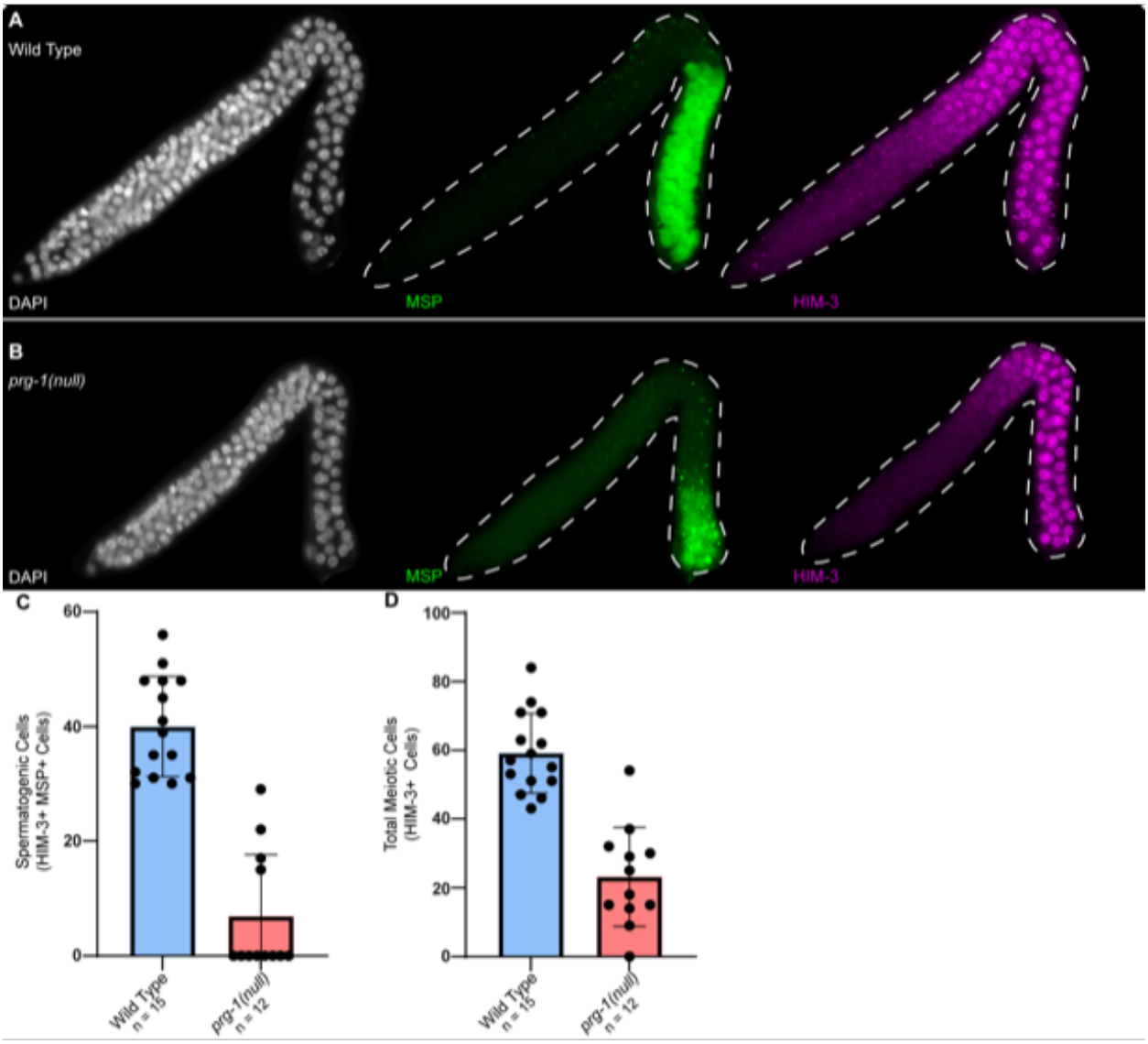
a known germline-expressed gene, was used as a positive control. (**B**) HCR-FISH experiment conducted for Wild Type and *plk-3(Δpi)* germlines using the same probes as (**A**). Scale bars are 50 μm to their respective germlines.


A
DAPI
PLK-3
Wild Type (from main figure 5)
Meiosis
Progenitor Zone
Meiosis
Progenitor Zone
B
prg-1(Δpi) (from main figure 5)
Progenitor Zone
Meiosis
Progenitor Zone
Meiosis
C
prg-1(null) (from main figure 5)
Meiosis
Progenitor Zone
Meiosis
Progenitor Zone
D
prg-1(ΔPAZ)
Progenitor Zone
Meiosis
Progenitor Zone
Meiosis

Supp. Fig. 7: PLK-3::GFP germlines (**A-D**) Representative DAPI-stained (white) germlines of PLK-3::GFP expression (green) from main figure 5 alongside data for *prg-1(ΔPAZ)*. Scale bars are 50 μm to their respective germlines.


A. Wild Type
DAPI
SYP-1
Progenitor Zone
Meiosis
B. prg-1(null)
C. prg-1(null); plk-3(ok2812)
D. plk-3(ok2812)
E. plk-3(Δpi)

Supp. Fig. 8: Representative DAPI and SYP-1 staining for germlines analyzed in main figure 6 (**A-E)** Representative DAPI (white) and SYP-1 (green) staining for each genotype of germlines analyzed for meiotic progression defects. Scale bars: 25 μm.



Supp. Fig. 9: Delay of germline development in larval hermaphrodite germlines. (**A-B**) Representative germlines from mid-L4 stage germlines hermaphrodites stained for DAPI (white), Major Sperm Protein (MSP) (green), and HIM-3 (magenta). (**C**) Quantification of spermatogenic cells (HIM-3+ MSP+) from mid-L4 germlines. (**D**) Quantification of total meiotic cells (HIM-3+) from mid-L4 germlines.

Table S1. Analysis of small RNAs mapping to annotated piRNAs from Wild Type and *prg-1* mutant males (excel file). Table of analysis comparing total piRNA counts between Wild Type and *prg-1* mutant samples in males.

Table S2. Analysis of small RNAs mapping to annotated piRNAs from Wild Type and *prg-1(ΔPAZ)* RNA IP samples (excel file). Table of analysis comparing total piRNA counts from RNA IP experiments between Wild Type and *prg-1(ΔPAZ)* samples.

Table S3. Analysis of small RNAs mapping to annotated piRNAs from Wild Type and *prg-1* mutant hermaphrodite samples (excel file). Table of analysis comparing total piRNA counts between Wild Type and *prg-1* mutant samples in hermaphrodites.

Table S4. Analysis of candidate genes with reduced 22G siRNAs (excel file). Table of analysis of genes with significantly reduced 22G siRNAs in *prg-1(null)* relative to Wild Type.

Table S5. HCR probes: Molecular HCR FISH probes used to detect expression of *plk-3* and *pgl-1* mRNA.

HCR FISH Probes: *pgl-1*	Molecular Instruments	Ordered Using Accession # ZK381.4a
HCR FISH Probes: *plk-3*	Molecular Instruments	Ordered Using Accession # NM_068795

Table S6. CRISPR-related oligonucleotides: Oligonucleotides used in the generation and detection of CRISPR-edited alleles.

Oligo type	Name	Purpose	Sequence
crRNAs			
	Dpy-10	Cas9	GCTACCATAGGCACCACGAG
	prg-1 mCherry knockin	Cas9	TGGCATCTGGAAGTGGTCGC
	ΔPAZ 1	Cas9	AAAATGAATGAGATGTACGG
	ΔPAZ 2	Cas9	CCAGTCGGGAAGCACAGTTC
	Rnase 1	Cas9	TACCACGACTCGACATTGAA
	Rnase 2	Cas9	TTCCTTTCAATGTCGAGTCG
	COSA-1 GFP knockin	Cas9	aagtgtcaATGTCAAGTTCT
	PLK-3	Cas9	CATTTATGCGAGATATCTGG
	PLK-3 ΔpiRNA	Cas9	TACGACTGAGATACTTCTTT
Primers			
	prg-1_WG_FWD	Genotyping	CGCACCGATCGATTCTGGAT
	prg-1_WG_RVS	Genotyping	TGCTGTAGTCGCTTTGAGTCA
	ΔPAZgenoFP	Genotyping	GGAACTACGATCGTGCCAGA
	ΔPAZgenoRP	Genotyping	CCCACTCTGCTTGCTTTCCT
	DDHgenoFWD	Genotyping	CCATTCCCAACCAATGCGTG
	DDHgenoRVS	Genotyping	GTAGAGGATCAAGCGGCTCG
	cosa1_wrmS11_geno_FP	Genotyping	AAACTTAGGCTCTGGTCTCGTG
	cosa1_wrmS11_geno_RP	Genotyping	GAACCTGATTCGCTGCTGA
	plk-3_cterm_geno_fp	Genotyping	CAACCAGGAGCATGTCGTCT
	GFP112R	Genotyping	CACCTTCACCCTCTCCACTG
	plk-3_cterm_geno_rp	Genotyping	TGCGAAAAGGTTGCAAGGTT
	JHS 59	Genotyping	TCTTgtgagttgaagtgtatccttt
	JHS 60	Genotyping	TTGGGCATATTCGATACGACTG
	JHS 61	Genotyping	AGTATCTGTCCCGTATCGAGTAC
ssODN			
	ΔPAZ ssODN	Repair Template	CCACAACGCGTTCAAGAGAAAATGAATGAGATGTACCTGTGCTTCCCGACTGGGCTAACGGATGAGATGCG
	RNase ssODN	Repair Template	AAAACACAATGATCGTCGGCTACGCTCTGTACCACGAtTCaACaTTaAAAGGAAAAACTGTCGGTGCTTGCGT
	ΔpiRNA ssODN	Repair Template	CGCAAACATATTGACAAACAGCTGTCCAAAGAAGTATCTGTCCCGTATCGAGTACGCACAGGCCAAAATTAAATTGCTTCGTCCTACAAAC

Table S7. Strains used in this study (excel file): List of *C. elegans* strains generated and used in this study.

Data S1. Small RNA read mapping of Wild Type and *prg-1* mutant male samples to annotated piRNAs (excel file). Table of small RNA reads from wild type and *prg-1* mutant male samples mapped to the *C. elegans* genome and annotated piRNAs for downstream analysis.

Data S2. Small RNA read mapping from Wild Type and *prg-1(ΔPAZ)* RNA IP samples to annotated piRNAs (excel file). Table of small RNA reads from wild type *prg-1(ΔPAZ)* RNA IP samples mapped to the *C. elegans* genome and annotated piRNAs for downstream analysis.

Data S3. Small RNA read mapping of Wild Type and *prg-1* mutant hermaphrodite samples to annotated piRNAs (excel file). Table of small RNA reads from Wild Type and *prg-1* mutant samples mapped to the *C. elegans* genome and annotated piRNAs for downstream analysis.

Data S4. Small RNA read mapping of Wild Type and *prg-1(null)* male samples to annotated cDNAs (excel file). Table of small RNA reads from Wild Type and *prg-1* mutant male samples mapped to the *C. elegans* genome and cDNAs to identify 22G siRNA targets in downstream analysis.