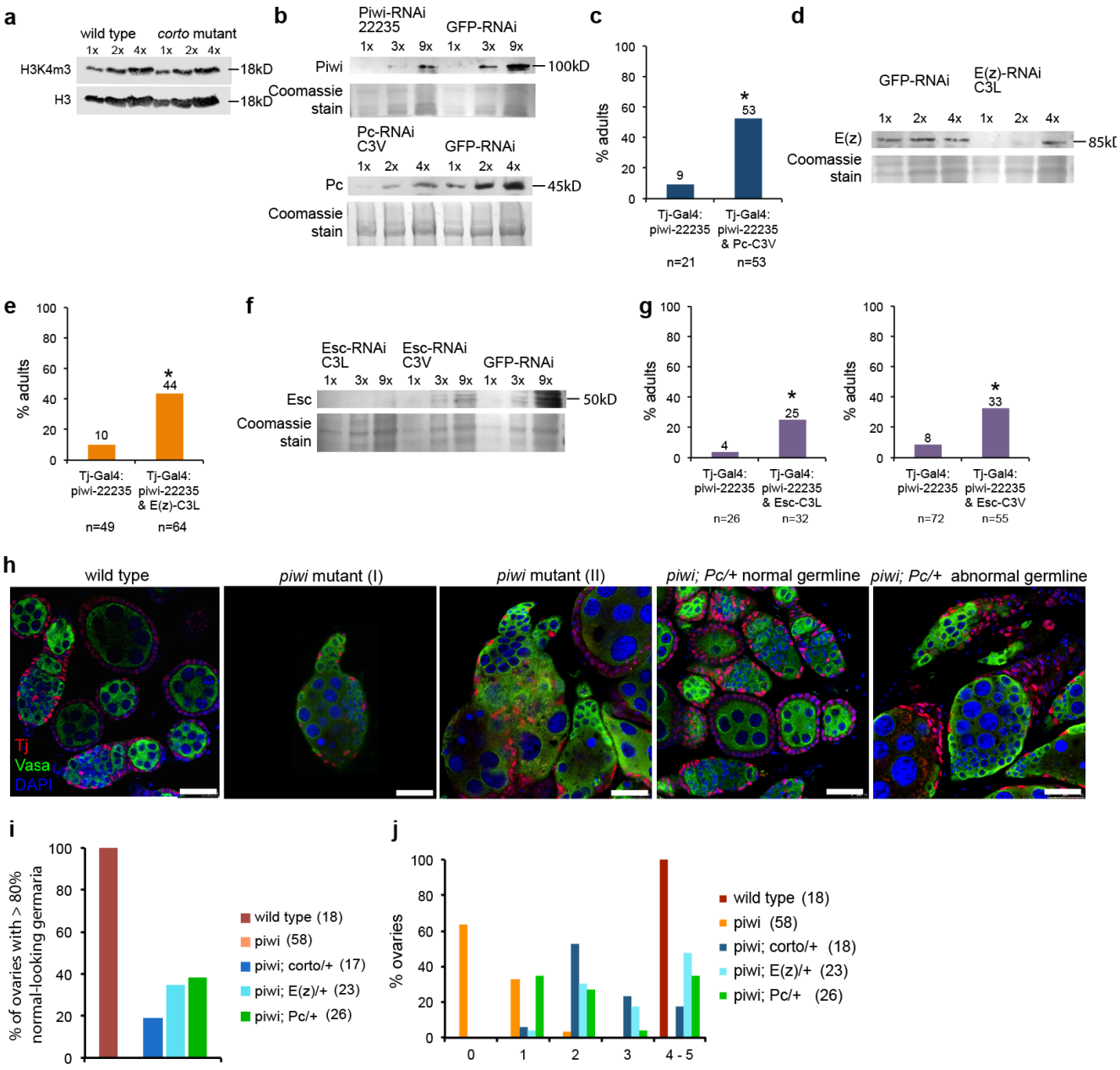
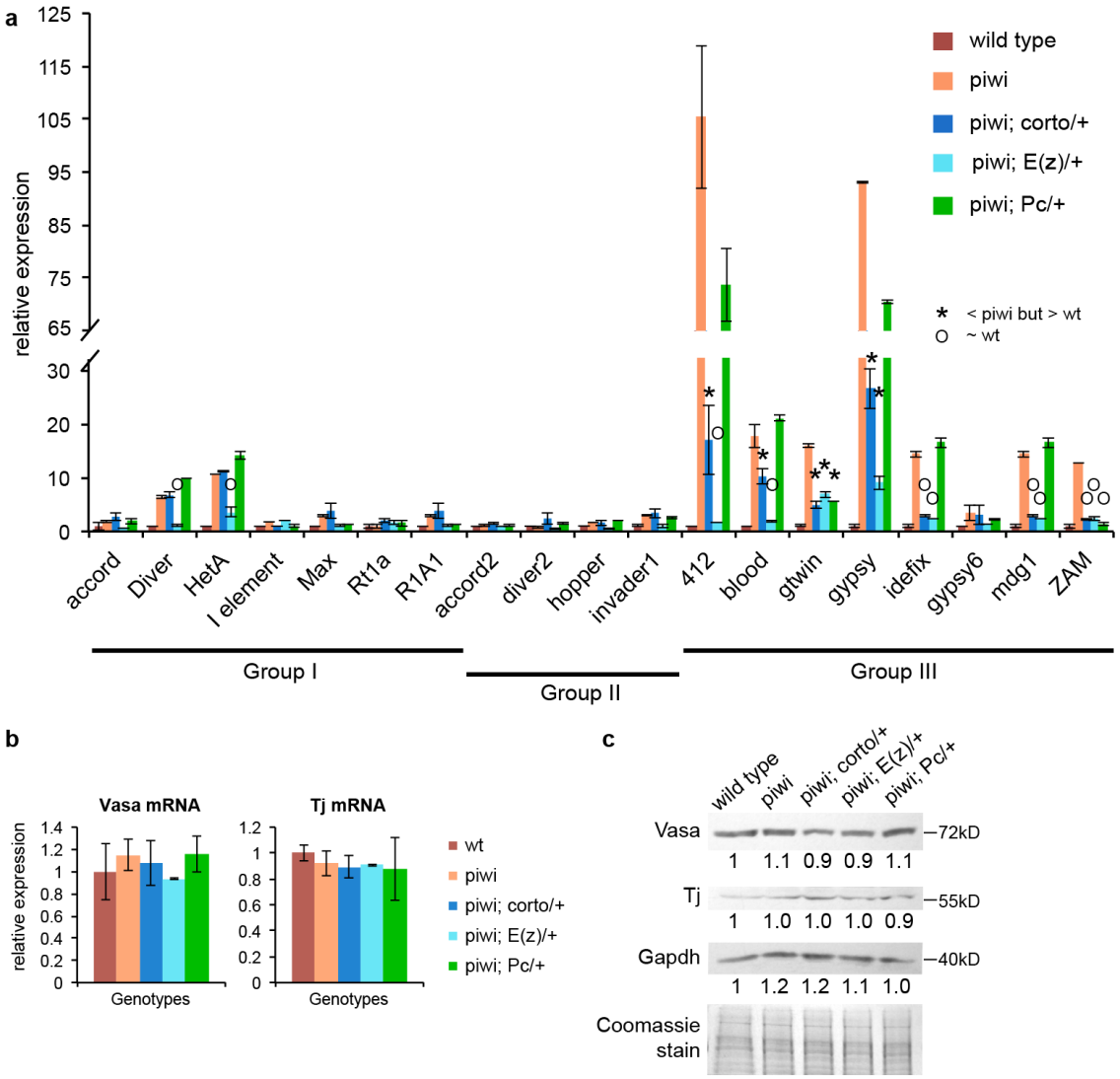


Supplementary Fig. 1. RNAi-induced reduction in *E(z)* or *Esc* or mutations in Polycomb Group genes or *corto* partially rescues oogenesis of *piwi* mutants.

(a) *In vitro* reconstituted PRC2 complexes (represented by E(z) immunoblotting) were incubated with or without recombinant Corto, and ^{3}H-methylation signals were analyzed by radiography.
(b) Immunoblotting analysis shows that the RNAi hairpins reduce the target proteins Piwi and Pc. The genotype is: *actin:gal4; UAS:RNAi* hairpins of *GFP*, *piwi* (22235), or *Pc* (C3V).
(c) 9% of the *Tj:gal4*; UAS:*piwi*-RNAi females contain ovarioles, compared to 53% of the *Tj:gal4*, *UAS:piwi*-RNAi; UAS:Pc-RNAi females that contain ovarioles.
(d) Two-fold serial dilutions of whole-fly extract were analyzed by immunoblotting to E(z). The animals were *actin:gal4; UAS:RNAi* hairpins of *GFP* or *E(z)* (C3L).
(e) 10% of the *Tj:gal4*; *UAS:piwi-RNAi* females contain ovarioles, compared to 44% of the *Tj:gal4, UAS:piwi-RNAi; UAS:E(z)-RNAi* females that contain ovarioles.

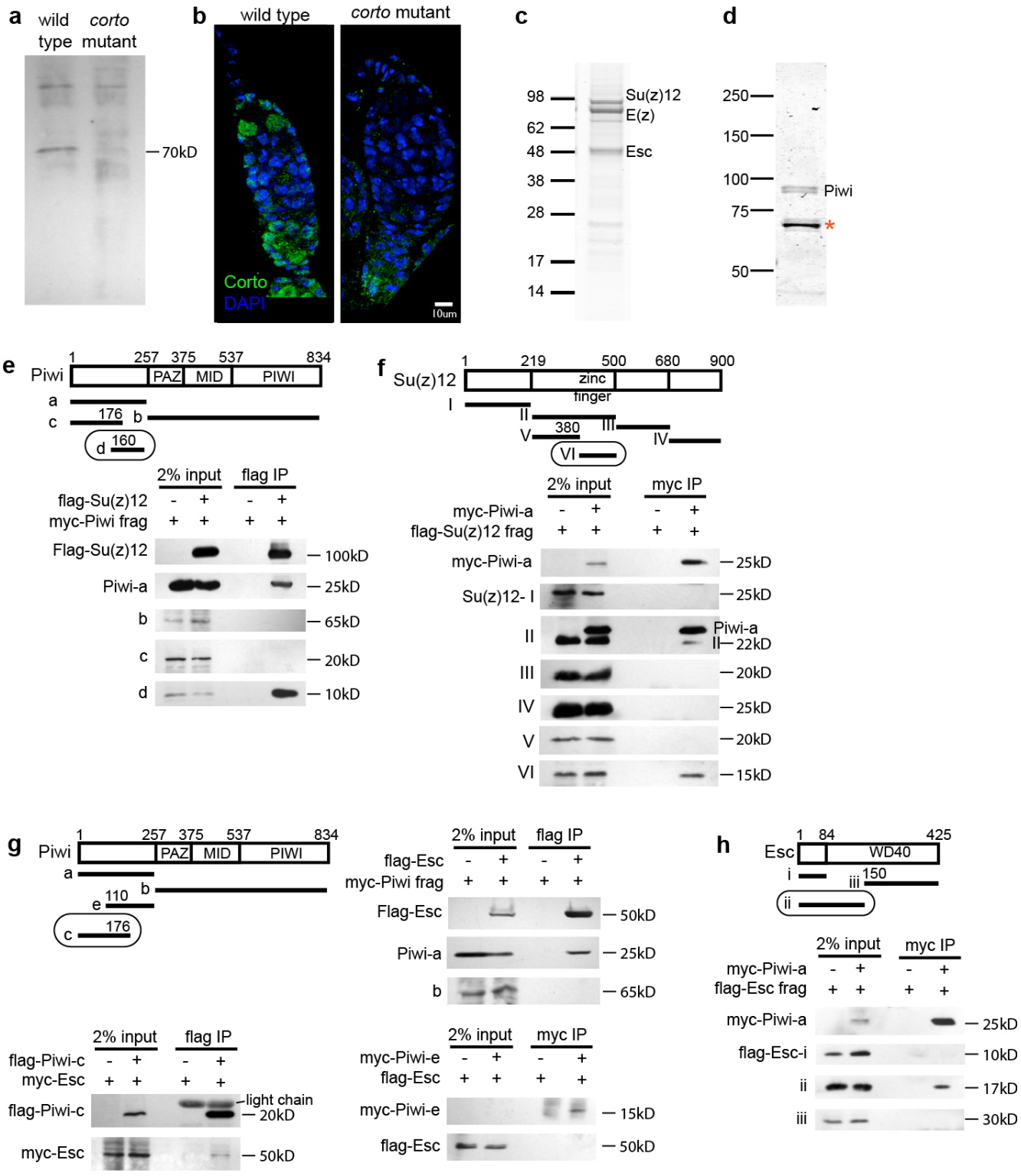
(f) Three-fold serial dilutions of whole-animal extract were analyzed by immunoblotting to Esc. The animals were *actin:gal4; UAS:RNAi* hairpins of GFP or Esc (C3L or C3V).
(g) 4 to 8% of the *Tj:gal4*; UAS:*piwi*-RNAi females contain ovarioles, compared to 25% (C3L) or 33% (C3V) of the *Tj:gal4*; UAS:*piwi*-RNAi; UAS:*Esc*-RNAi females that contain ovarioles.
(h) Representative images of wild type, *piwi*, and *piwi; Pc/+* normal and abnormal germline tissues. The *piwi* mutant ovarian tissues exhibit two typical defects: no egg chambers (I) and disorganized tissues containing well-formed egg chambers (II). Tj (red, ovarian somatic cell marker) and Vasa (green, germline cell marker) immunofluorescence microscopy of whole-mount ovaries. Bar = 25 um.
(i) The histogram shows percentages of ovaries containing >80% normal germaria. Sample sizes are in parentheses.
(j) Percentages of ovaries that contain averaged egg chamber numbers per ovariole. Sample sizes are in parentheses.
For immunoblotting analyses, lower portions of the gels were Coomassie stained to show equal loading. For the genetic analyses, the sample sizes are indicated in each experimental group. Significant differences, as indicated by asterisks, are based on the p-value cutoff of $p < 0.001$ (Chi-square test).



Supplementary Fig. 2. *Piwi*, *corto*, and Polycomb Group genes genetically interact to silence retrotransposons.

(a) RT-qPCR analysis of transposon mRNA levels in wild type, *piwi* mutants, and *piwi* with heterozygous *corto*, *E(z)*, or *Pc* mutation. The mRNA levels were normalized to *rp49* mRNA levels within each group. The heterozygous *corto* mutation suppresses transposons in Group III that are increased by the *piwi* mutations. The heterozygous *E(z)* mutation suppresses all transposons increased by the *piwi* mutations. The heterozygous *Pc* mutation suppresses gtwin and ZAM in Group III that are increased by the *piwi* mutations. The error bars represent standard deviations calculated from triplicate qPCR reactions. Asterisks (*) indicate mRNA levels that are significantly lower ($p<0.001$) than those in the *piwi* mutant but significantly larger ($p<0.001$) than those in wild type. Circles (**O**) indicate mRNA levels that are significantly lower ($p<0.001$) than those in the *piwi* mutant and similar to those in wild type. P-values were calculated using one-sided Student's *t*-test with unequal variance.

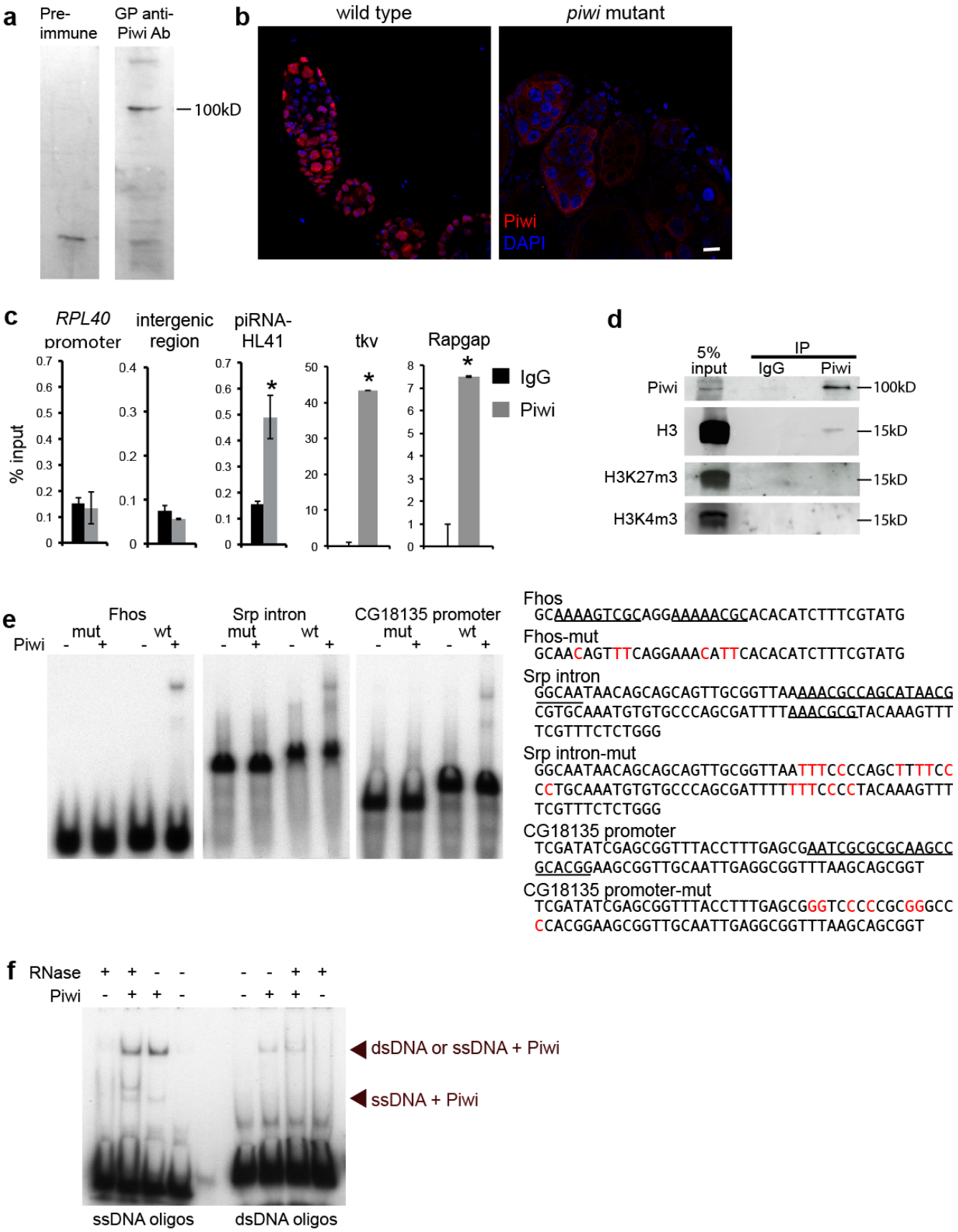
The mRNAs (by RT-qPCR in b; normalization to *rp49*) and proteins (by immunoblotting in c) of Vasa (germ cell marker) and Tj (somatic cell marker) were quantitated and shown to be at similar levels in wild type, *piwi* mutants, and *piwi* with heterozygous *corto*, *E(z)*, or *Pc* mutation. Numbers underneath individual immunoblotting bands indicate relative protein levels (normalized to wild type).



Supplementary Fig. 3. Specificity of the generated Corto antibody, gradient analyses and pull down results that identify interacting domains in Piwi, Su(z)12, and Esc.

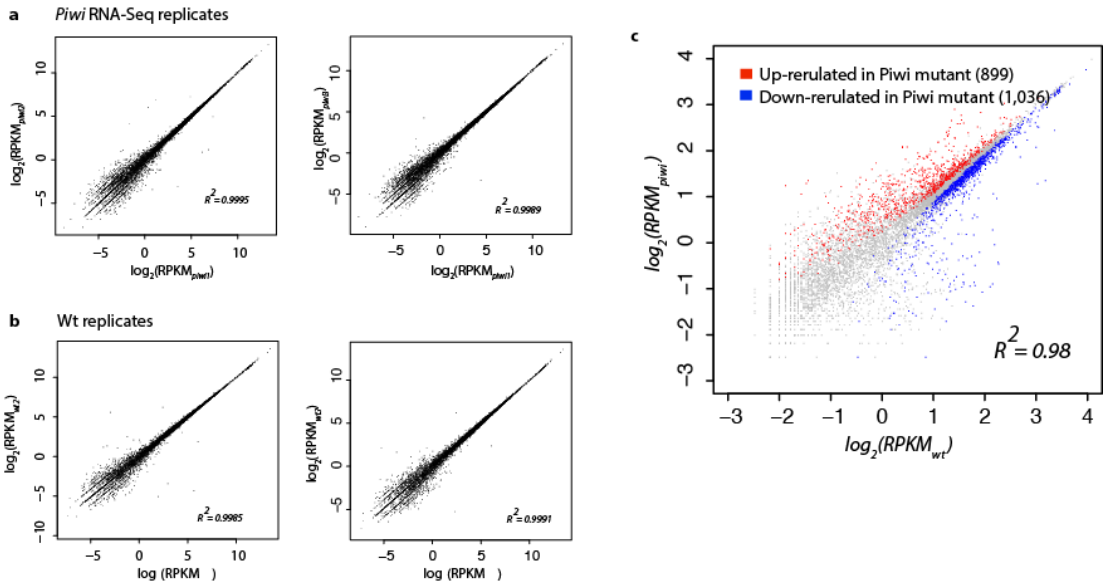
(a) Immunoblotting analysis of wild type and the *corto* mutant animal extracts.

(b) Immunofluorescence microscopy of wild type and *corto* mutant ovaries shows that Corto signals (green) are greatly reduced in the *corto* mutant.
(c) Coomassie-stained gel to visualize *in vitro* reconstituted PRC2 complex. Gel bands of Su(z)12, E(z), and Esc are indicated.
(d) Coomassie-stained gel to visualize purified recombinant Piwi-flag proteins. Asterisk (*) indicates a protein that co-purified with Piwi.
(e) Amino acid residues 160-257 (fragment d) of Piwi binds to Su(z)12. Full-length flag-Su(z)12 was used as a bait to pull myc-tagged Piwi fragments a, b, c, and d. Bound proteins were visualized by immunoblotting to myc.
(f) Residues 380-500 (fragment VI) of Su(z)12 binds to Piwi. Myc-tagged fragment a (aa 1-257) of Piwi was used as a bait to pull flag-tagged Su(z)12 fragments I, II, III, IV, V and VI. Bound proteins were visualized by immunoblotting to flag. ‘Piwi-a’ and ‘II’ labels indicate protein bands in the immunoprecipitate lane.
(g) Residues 1-176 (fragment c) of Piwi binds to Esc. Flag-tagged Esc was used as a bait to pull down Myc-tagged Piwi fragments a or b. Flag-tagged Piwi fragment c was used as a bait to pull down myc-tagged Esc. Myc-tagged Piwi fragment d was used as a bait to pull down flag-tagged Esc. Bound proteins were visualized by immunoblotting to flag or myc. ‘light chain’ label is placed next to the protein bands above flag-Piwi-C to indicate signals from the light chain of the antibody.
(h) Residues 1-150 (fragment ii) of Esc binds to Piwi. Myc-tagged fragment a (aa 1-257) of Piwi was used as a bait to pull down Flag-tagged Esc fragments i to iii. Bound proteins were visualized by immunoblotting to flag.


a
Pre-immune
GP anti-Piwi Ab
100kD
b
wild type
piwi mutant
Piwi
DAPI
c
RPL40 promoter
intergenic region
piRNA-HL41
tkv
Rapgap
% input
IgG
Piwi
d
5% input
IP
IgG
Piwi
Piwi
H3
H3K27m3
H3K4m3
100kD
15kD
15kD
15kD
e
Fhos
Srp intron
CG18135 promoter
mut
wt
Piwi
Fhos
GCAAAAGTCGCAGGAAAAACGCACACATCTTTCGTATG
Fhos-mut
GCAACAGTTTCAGGAAACATTCACACATCTTTCGTATG
Srp intron
GGCAATAACAGCAGCAGTTGCGGTTAAAAACGCCAGCATAACG
CGTGCAAATGTGTGCCCAGCGATTTTAAACGCGTACAAAGTTT
TCGTTTCTCTGGG
Srp intron-mut
GGCAATAACAGCAGCAGTTGCGGTTAATTTCCCCAGCTTTTCC
CCTGCAAATGTGTGCCCAGCGATTTTTTTCCCCTACAAAGTTT
TCGTTTCTCTGGG
CG18135 promoter
TCGATATCGAGCGGTTTACCTTTGAGCGAATCGCGCGCAAGCC
GCACGGAAGCGGTTGCAATTGAGGCGGTTTAAGCAGCGGT
CG18135 promoter-mut
TCGATATCGAGCGGTTTACCTTTGAGCGGGTCCCCCGCGGGCC
CCACGGAAGCGGTTGCAATTGAGGCGGTTTAAGCAGCGGT
f
RNase
Piwi
dsDNA or ssDNA + Piwi
ssDNA + Piwi
ssDNA oligos
dsDNA oligos

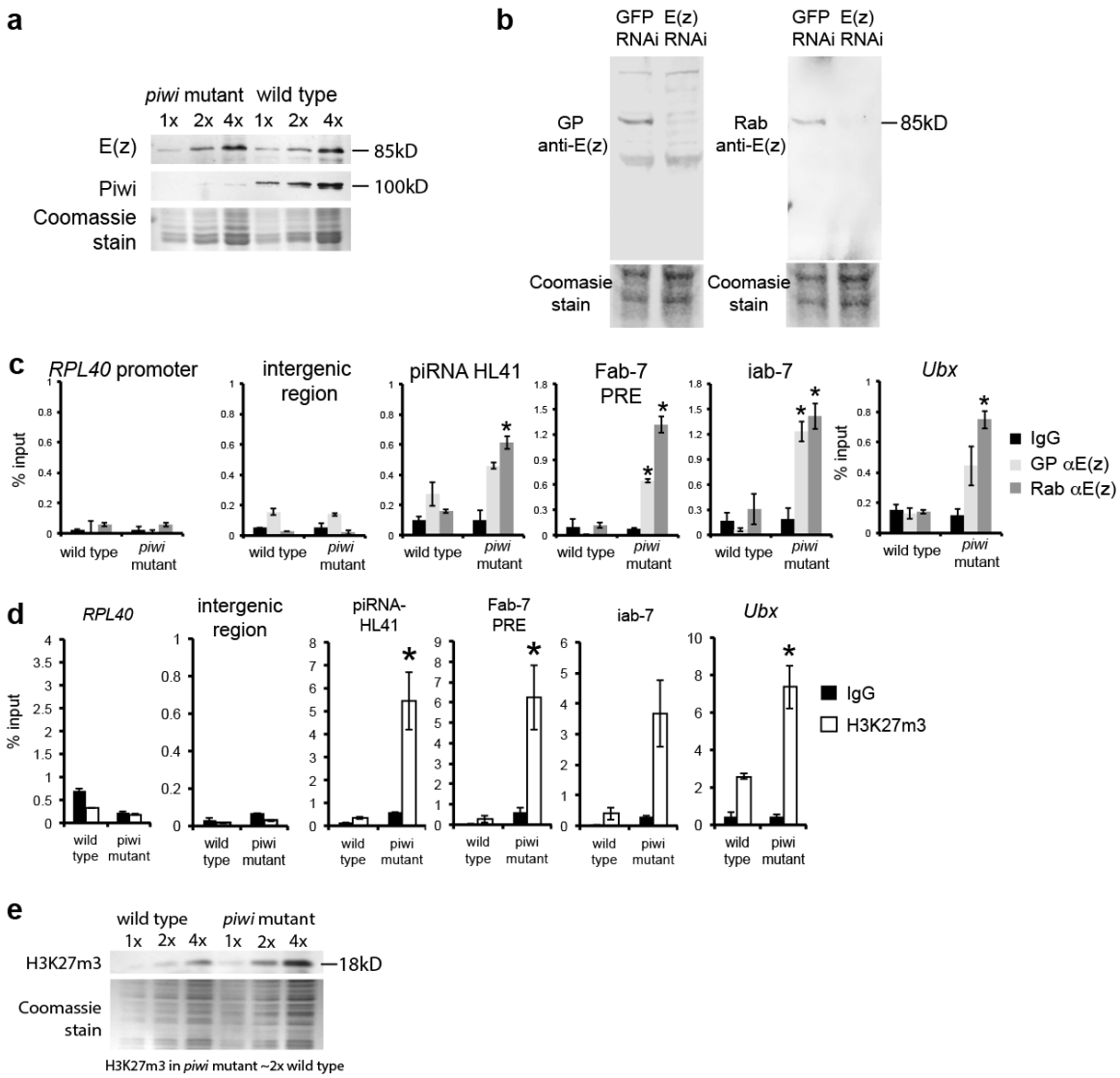
Supplementary Fig. 4. Specificity of the generated Piwi antibody, Piwi chromatin IP, and EMSA of Piwi recombinant protein on identified motif**.**

(a) Immunoblotting analysis of wild type ovarian extracts using pre-immune sera and guinea pig (GP) anti-Piwi antibodies.
(b) Piwi immunofluorescence microscopy of wild type and *piwi* mutant ovaries shows that Piwi signals are greatly reduced in the *piwi* mutant, with only a very low level of residual non-specific background visible.
(c) ChIP-qPCR analyses of Piwi levels in wild type ovarian cells at Piwi binding sites inferred from ChIP-Seq data (*tkv*, *Rapgap*), control regions (intergenic, *RPL40* promoter) or previously reported targets (*piRNA-HL41*, Huang et al. 2013). Error bars represent standard deviations calculated from three qPCR biological replicates. Asterisks indicate statistically significant differences ($p < 0.001$; Chi-square test).
(d) Piwi chromatin immunoprecipitate from ovarian cells was analyzed by immunoblotting to Piwi, histone H3, H3K27m3 and H3K4m3.
(e) EMSA analysis of recombinant Piwi protein binding to wild type and mutant motifs using *Fhos* locus, *Srp* intron, and *CG18135* promoter oligomers. For each oligomer, sequences, motif (underlined), and mutated sequence (text in red) are indicated.
(f) EMSA analysis of recombinant Piwi protein binding to single-stranded (ss) vs. double-stranded (ds) *Fhos* locus DNA, with or without RNAse A and H addition. Piwi prefers binding to ssDNA and is unaffected by RNAse.

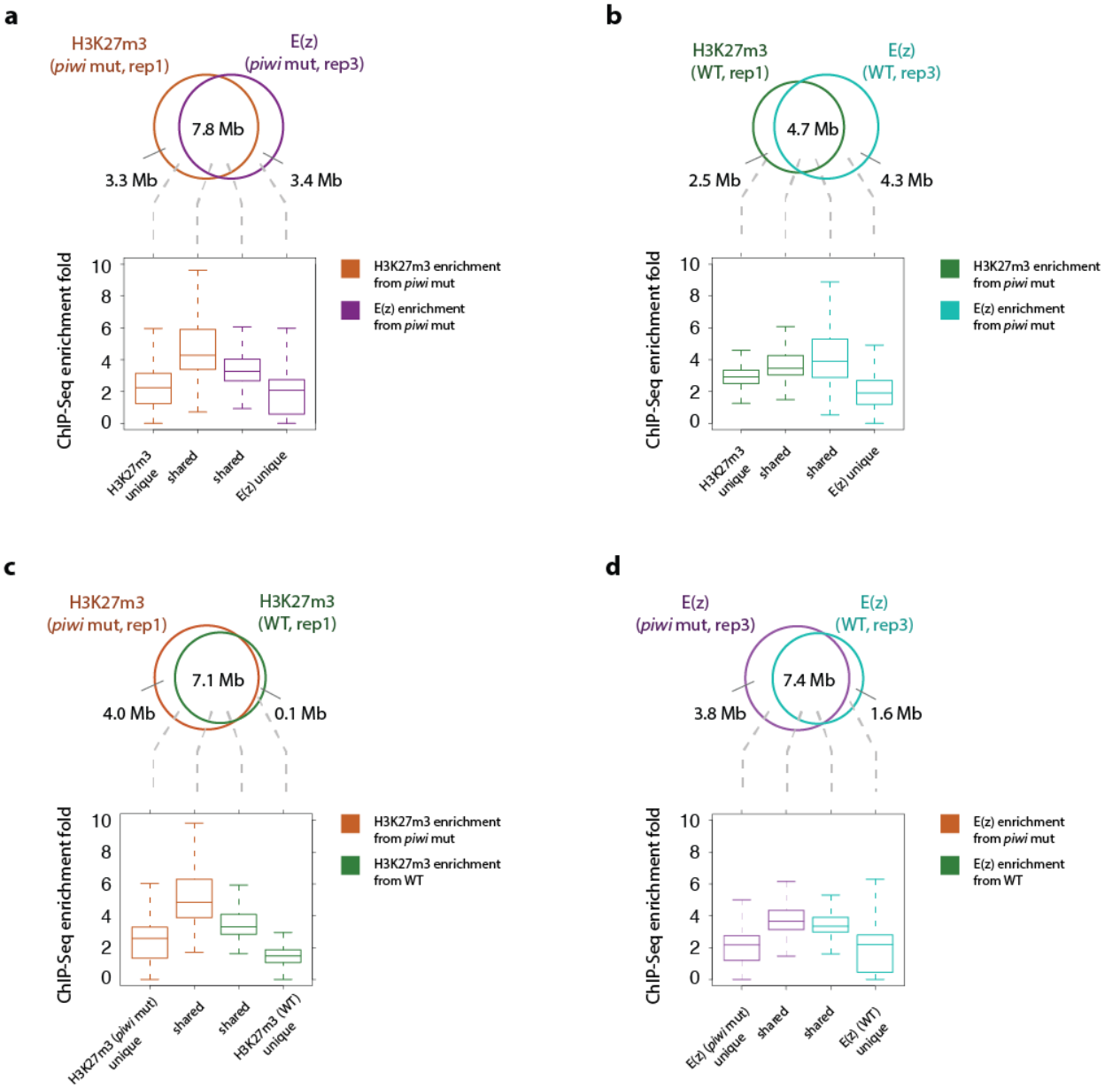


Supplementary Fig. 5. RNA-Seq analysis.

(a) Scatter plots for comparing RNA-Seq biological replicates from *piwi* mutant ovaries. High correlation was observed between the replicates.
(b) Scatter plots for comparing RNA-Seq biological replicates from wild type ovaries. Similarly high correlations were observed between biological replicates.
(c) Scatter plot for comparison of replicate-averaged RNA-Seq data between *piwi* mutant and wild type ovaries. Differentially expressed genes were determined using Student's *t*-test on replicates. Statistically significant differentially expressed genes (using p-value cutoff of 0.001) are shown in red (for up-regulated genes in *piwi* mutant) or blue (for down-regulated genes in *piwi* mutant). PANTHER analysis of enriched gene ontologies among differentially expressed genes is shown in Figure 4.



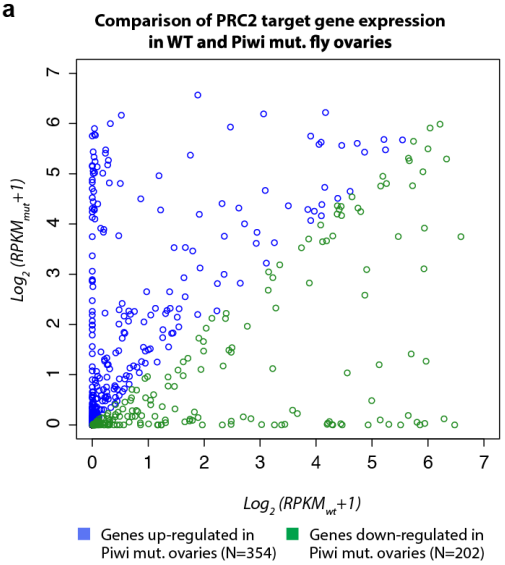
Supplementary Fig. 6. Protein levels of E(z) in wild type and *piwi* mutant ovaries, specificity of the generated E(z) antibodies, and qPCR analyses of E(z) and H3K27m3 ChIP.
(a) Two-fold serial dilutions of ovarian extracts from *piwi* mutant and wild type flies were analyzed by immunoblotting to E(z) and Piwi. Normalization of E(z) band signals to Coomassie staining signals showed that E(z) protein levels were at similar levels in wild type and *piwi* mutant.
(b) Specificity of guinea pig and rabbit anti-E(z) antibodies used for immunoblotting analysis of ovarian extracts from GFP RNAi and E(z) (C3L) RNAi flies. Bottom portions of the gel were Coomassie-stained to show equal loading.
(c) E(z) ChIP-qPCR analyses of wild type and *piwi* mutant ovarian cells using a newly generated guinea pig anti-E(z) antibody. Error bars represent standard deviations calculated from triplicate qPCR reactions. Asterisks indicate statistically significant differences ($p < 0.001$; Chi-square test).
(d) H3K27m3 ChIP-qPCR analyses of wild type and *piwi* mutant ovarian cells. Error bars represent standard deviations calculated from triplicate qPCR reactions. Asterisks indicate statistically significant differences ($p < 0.001$; Chi-square test).
(e) Two-fold serial dilutions of histone extracts from wild type and *piwi* mutant ovaries were analyzed by immunoblotting to H3K4m3 and H3.



Supplementary Fig. 7. Detailed analysis of genomic regions identified by ChIP-Seq of H3K27m3 and E(z).

(a-d) Venn diagram displayed at the top of each panel shows the number of shared bases between the two datasets. For example, in panel A we analyzed ChIP-Seq signals within regions showing enrichment of H3K27m3 histone mark and E(z) enrichment in *piwi* mutant. Despite a significant overlap between the two datasets (7.8 Mb), a considerable number of bases is called as enriched for only H3K27m3 or E(z) in piwi mutant. To further investigate unique bases, we compared intensities for both H3K27m3 and E(z) for shared regions or unique regions. The lower panel shows a distribution boxplot for ChIP-Seq enrichment signals within shared and unique regions. Shared regions have on average higher enrichment compared to unique regions. This shows that unique regions are due to threshold selection where enrichment is very low but detectable in one dataset, and also low and not detectable at the same threshold in another dataset. Similar results are shown for other overlaps between H3K27m3 and E(z) enrichment regions between WT and piwi mutants. Therefore there is a strong evidence for nearly perfect co-localization patterns of E(z) and H3K27m3 in both piwi mutant and WT.

a

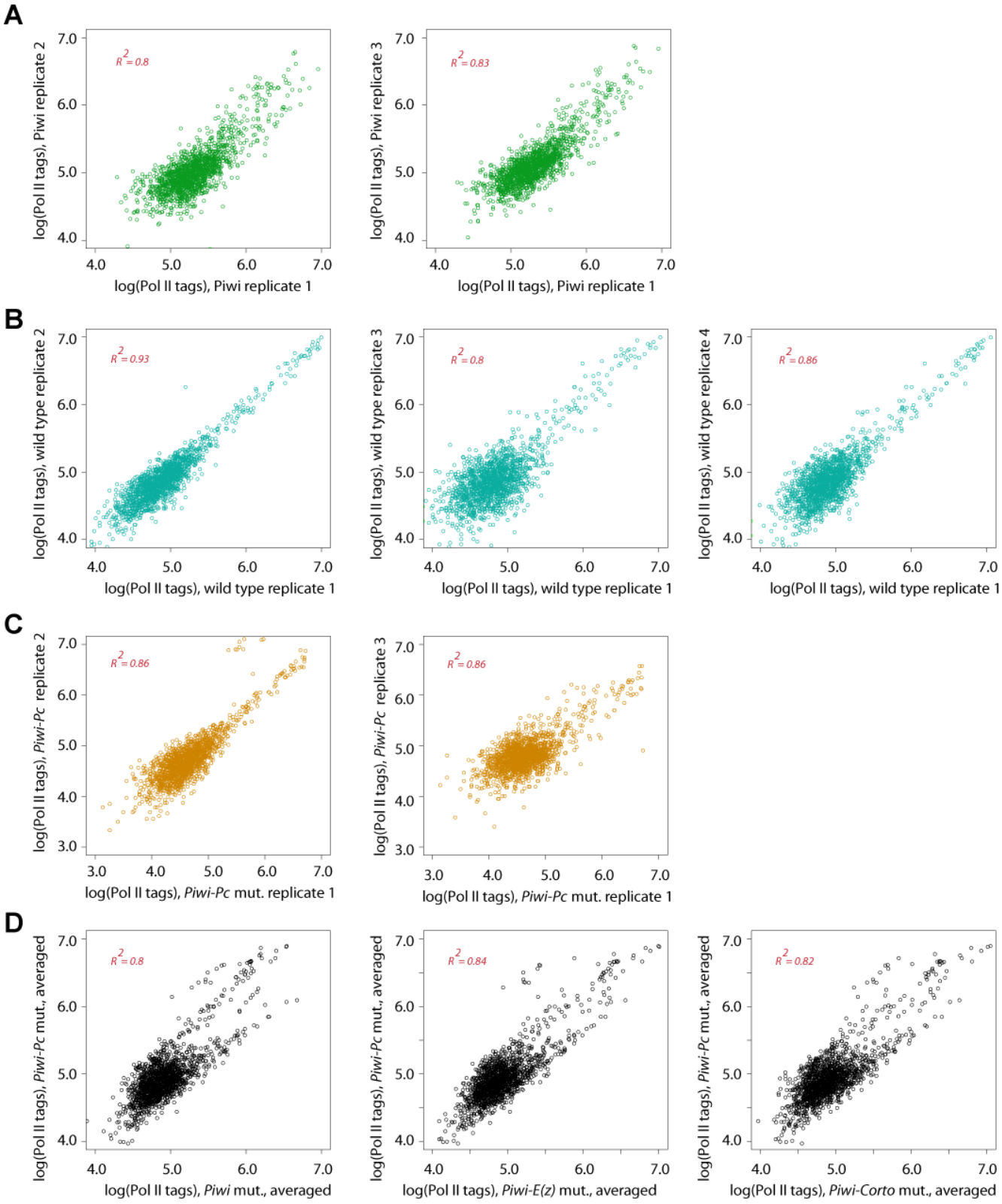


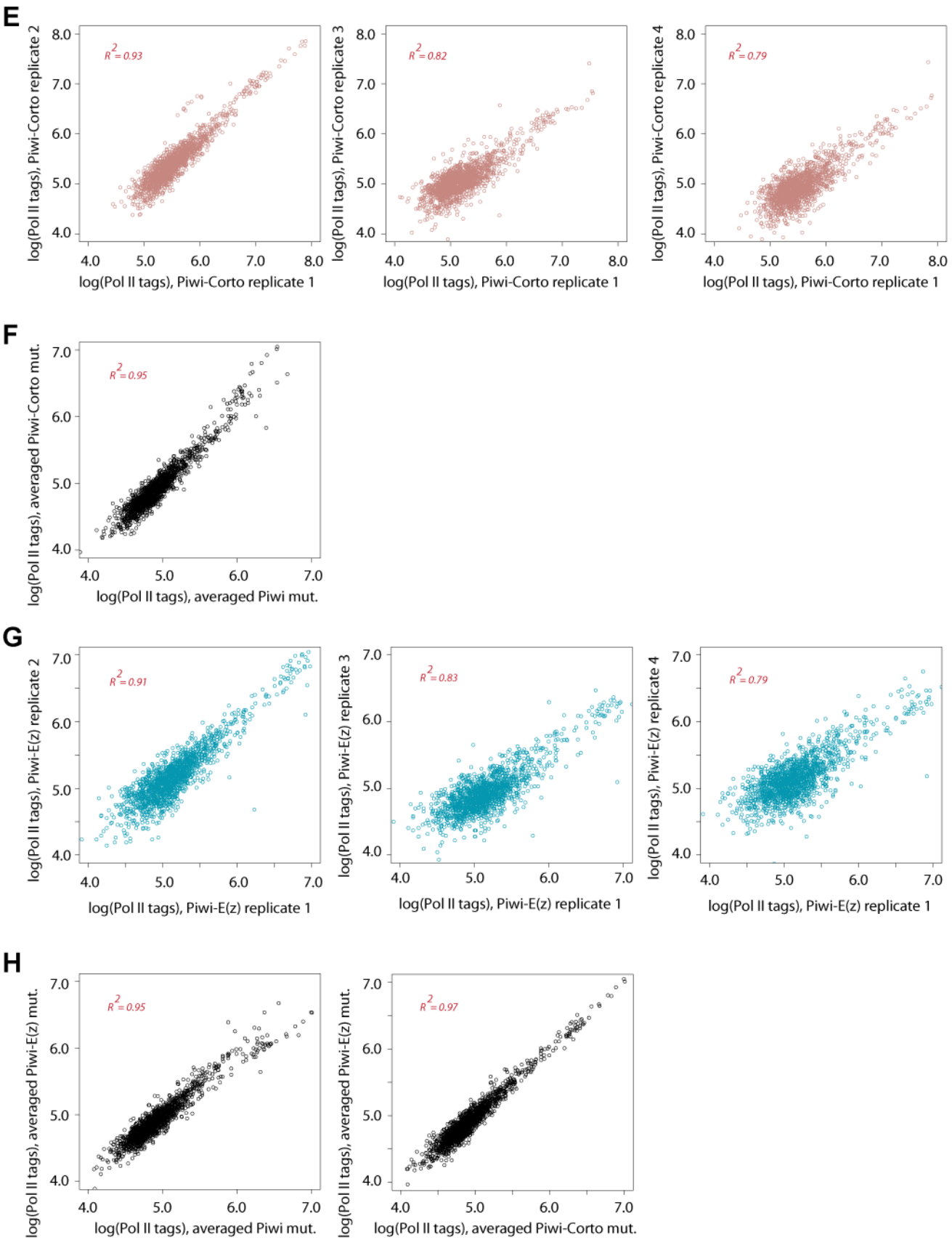
b

Germline-relevant function	Gene name	Citation with PMID #
Down-regulated in *piwi* mutant ovarian cells (27)		
germline stem cell niche homeostasis	tai	König et al., 20111; 21423150
germline stem cell division	pum	Deng & Lin, 2001; 11131529
germ cell development	abd-A	Moore et al., 1998; 9435287
	Abd-B	Moore et al., 1998; 9435287
	EcR	König et al., 20111; 21423150
	opa	Moore et al., 1998; 9435287
	srp	Moore et al., 1998; 9435287
	tin	Moore et al., 1998; 9435287
meiosis division	dia	Giansanti et al., 2004; 15004238
	mamo	Mukai et al., 2007; 17600690
oogenesis	E(Pc)	Yan et al., 2014; 24576427
	retn	Schüpbach & Wieschaus, 1991; 1783295
	stumps	Perrimon et al., 1996; 8978055
	ct	Jackson & Berg, 1999; 10471713
oocyte microtubule cytoskeleton polarization	cnc	1Guichet et al., 2001; 1518508
	heph	Besse et al., 2009; 19131435
follicle cell migration	ab	Jang et al., 2009; 19350016
	Crk	Geisbrecht et al., 2008; 18163987
	Gfat1	Geisbrecht et al., 2008; 18163987
	jing	Liu & Montell, 2001; 11152631
	tai	Bai et al., 2000; 11163181
ovary development	bab1	Couderc et al., 2002; 11973274
	cad	Chen et al., 2005; 15893978
	eya	Weyers et al., 2011; 21377458
	salm	Dong et al., 2003; 12925729
chorion-containing eggshell formation	Cp7Fb	Parks et al., 1986; 3091430
	Cp7Fc	Parks et al., 1986; 3091430
	mid	Fregoso et al., 2013; 23972992
Up-regulated in *piwi* mutant ovarian cells (12)		
germline stem cell division	dpp	Xie & Spradling, 1998; 9695953
	esg	Streit et al., 2002; 11902678
	hh	Deng & Lin, 2001; 11131529
germ cell development	Scr	Moore et al., 1998; 9435287
	ptc	Forbes et al., 1996; 8898240
	shd	Domanitskaya et al., 2014; 24373956
	vn	McDonald et al., 2006; 16712835
	sog	Carneiro et al., 2006; 16781701
female germline ring canal stabilization	Msp-300	Yu et al., 2006; 16337624
ovary development	rib	Weyers et al., 2011; 21377458
	robo	Weyers et al., 2011; 21377458
	lbe	Jagla et al., 1997; 9006070

Supplementary Fig. 8. RNA-Seq analysis of PRC2-bound genes in wild-type and the *piwi* mutant ovarian cells.

(a) Scatter plot for comparison of replicate-averaged RNA-Seq data between *piwi* mutant and wild type ovaries. Differentially expressed PRC2-bound genes were determined using Student's *t*-test on replicates. Statistically significant differentially expressed genes (using p-value cutoff of 0.001) are shown in blue (for up-regulated genes in *piwi* mutant) or green (for down-regulated genes in *piwi* mutant).
(b) Table lists down- (green) or up- (blue) regulated PRC2-bound genes that exhibit functions related to germline formation, as well as the publications that reported the functions.


A
log(Pol II tags), Piwi replicate 2
log(Pol II tags), Piwi replicate 1
R2 = 0.8
log(Pol II tags), Piwi replicate 3
log(Pol II tags), Piwi replicate 1
R2 = 0.83
B
log(Pol II tags), wild type replicate 2
log(Pol II tags), wild type replicate 1
R2 = 0.93
log(Pol II tags), wild type replicate 3
log(Pol II tags), wild type replicate 1
R2 = 0.8
log(Pol II tags), wild type replicate 4
log(Pol II tags), wild type replicate 1
R2 = 0.86
C
log(Pol II tags), Piwi-Pc replicate 2
log(Pol II tags), Piwi-Pc mut. replicate 1
R2 = 0.86
log(Pol II tags), Piwi-Pc replicate 3
log(Pol II tags), Piwi-Pc mut. replicate 1
R2 = 0.86
D
log(Pol II tags), Piwi-Pc mut., averaged
log(Pol II tags), Piwi mut., averaged
R2 = 0.8
log(Pol II tags), Piwi-Pc mut., averaged
log(Pol II tags), Piwi-E(z) mut., averaged
R2 = 0.84
log(Pol II tags), Piwi-Pc mut., averaged
log(Pol II tags), Piwi-Corto mut., averaged
R2 = 0.82


E
log(Pol II tags), Piwi-Corto replicate 2
log(Pol II tags), Piwi-Corto replicate 1
R2 = 0.93
log(Pol II tags), Piwi-Corto replicate 3
log(Pol II tags), Piwi-Corto replicate 1
R2 = 0.82
log(Pol II tags), Piwi-Corto replicate 4
log(Pol II tags), Piwi-Corto replicate 1
R2 = 0.79
F
log(Pol II tags), averaged Piwi-Corto mut.
log(Pol II tags), averaged Piwi mut.
R2 = 0.95
G
log(Pol II tags), Piwi-E(z) replicate 2
log(Pol II tags), Piwi-E(z) replicate 1
R2 = 0.91
log(Pol II tags), Piwi-E(z) replicate 3
log(Pol II tags), Piwi-E(z) replicate 1
R2 = 0.83
log(Pol II tags), Piwi-E(z) replicate 4
log(Pol II tags), Piwi-E(z) replicate 1
R2 = 0.79
H
log(Pol II tags), averaged Piwi-E(z) mut.
log(Pol II tags), averaged Piwi mut.
R2 = 0.95
log(Pol II tags), averaged Piwi-E(z) mut.
log(Pol II tags), averaged Piwi-Corto mut.
R2 = 0.97

Supplementary Fig. 9. Analysis of replicate Illumina sequencing data of RNA PolII ChIP from wild type, *piwi*, , *piwi*; *Pc*/+, *piwi*; *corto*/+, and *piwi*; E(z)/+.

Comparative analyses of replicate ChIP-seq data and averaged data from replicates. R values indicate correlation between compared datasets.
(a) Three *piwi* replicate data.
(b) Four wild-type replicate data.
(c) Three *piwi*; *Pc*/+ replicate data.
(d) Averaged *piwi; Pc*/+ data compared to averaged *piwi*, *piwi*; *E(z)*/+ or *piwi; corto*/+ data.
(e) Four *piwi*; *corto*/+ replicate data.
(f) Averaged *piwi*; *corto*/+ data compared to *piwi* data.
(g) Four *piwi*; *E(z)*/+ replicate data.
(h) Averaged *piwi*; *E(z)*/+ data to averaged *piwi* or *piwi*; *corto*/+ data.

Supplementary Table 1. Fly strains used in this study.

Locus	Molecular Function	Alleles/RNAi construct	Source
Corto	Enhancer of PcG of TrxG	420	Frederique Peronnet
Corto	Enhancer of PcG of TrxG	l1	Bloomington Stock Collection # 4788
E(z)	Enzymatic subunit of PRC2	63	Richard Jones
E(z)	Enzymatic subunit of PRC2	RNAi construct	VDRC Transformant # 27646 (C3L)
Pc	Core subunit of PRC1	1	Bloomington Stock Collection # 1728
Pc	Core subunit of PRC1	3	Bloomington Stock Collection # 3399
Pc	Core subunit of PRC1	RNAi construct	VDRC Transformant # 41320 (C3V)
awd	Microtubule and GTP binding	KRS6	Bloomington Stock Collection # 9035
kni	RNA PolII transcription factor	10	Bloomington Stock Collection # 3307
Piwi	RNA-binding; germline stem cell maintenance	RNAi construct	VDRC Transformant # 22235 (C2L)
Esc	Core subunit of PRC2	RNAi construct	VDRC Transformant # 5692 (C3L)
Esc	Core subunit of PRC2	RNAi construct	VDRC Transformant # 5690 (C3V)
Tj:Gal4	Tj promoter drives Gal4		Bloomington Stock Collection # 3954

Supplementary Table 2. Antibodies used in this study.

Antibodies	Source	Application(s)
Rabbit anti-H3K27m3	Active Motif, 39155	IF 1:200; WB 1:10,000; ChIP 4ul per IP
Rabbit anti-H3K4m3	Active Motif, 39159	WB 1:10,000
Guinea pig anti-Corto	Our own lab	IP 6ul of antisera per IP; WB 1:25 of purified antibodies
Mouse anti-Piwi	Haru Siomi	4ul of purified antibodies per IP; WB 1:200 of supernatant; IF 1:3 of supernatant
Guinea pig anti-Piwi	Lin lab/Peng lab	6ul of antisera per IP
Mouse anti-Esc	Developmental Studies Hybridoma Bank	WB 1:4 of supernatant
Rabbit anti-Pc	Vincente Pirotta	WB 1:50
Rabbot anti-E(z)	Santa Cruz Biotechnology, sc-98265	WB 1:100 IF 1:5
Rabbit anti-Ezh2 also recognizes E(z),	Active Motif, 39901	IP 6ul
Rabbit anti-cMyc	Sigma-Aldrich, C3950-0.2mg	WB 1:1,1000
Rabbit anti-E(z)	Peng lab	4ul purified antisera per IP
Rabbit anti-FLAG	Sigma-Aldrich, F7425-.2MG	WB 1:1,1000
Mouse 1B1 anti-Hts	Developmental Studies Hybridoma Bank	IF 1:25 of concentrated supernatant
Rabbit anti-Vasa	Santa Cruz Biotechnology, sc-30210	IF 1:200
Guinea pig anti-Traffic jam (Tj)	Dorothea Godt	IF 1:4,000
Mouse anti-Aub	Haru Siomi	WB 1:3000
Mouse anti-RNA Polymerase II	Covance, MMS-126R	4ul per IP
Mouse anti-RNA Polymerase II	Millipore, 05-623	5ul per IP
Mouse anti-RNA Polymerase II	Active Motif, 39097	10ul per IP
Cy3-conjugated goat anti-rabbit	Jackson ImmunoResearch Laboratories	IF 1:200
Cy3-conjugated goat anti-mouse	Jackson ImmunoResearch Laboratories	IF 1:200
Alexa488-conjugated goat anti-rabbit from	Invitrogen	IF 1:400
Alexa555-conjugated goat anti-guinea pig	Invitrogen	IF 1:400
HRP-conjugated mouse anti-rabbit, light chain specific	Jackson ImmunoResearch Laboratories	WB 1:40,000
HRP-conjugated goat anti-rabbit	Jackson ImmunoResearch Laboratories	WB 1:20,000
HRP-conjugated goat anti-mouse	Jackson ImmunoResearch Laboratories	WB 1:20,000
HRP-conjugated goat anti-guinea pig	Jackson ImmunoResearch Laboratories	WB 1:20,000

Supplementary Table 3. DNA Primers used for RT-qPCR.

412-F	CACCGGTTTGGTCGAAAG	412-R	GGACATGCCTGGTATTTTGG
Accord-F	ACAATCCACCAACAGCAACA	Accord-R	AAAAGCCAAAATGTCGGTTG
Accord2-F	TTGCTTTCGGACTTCGTCTT	Accord2-R	TTCCACAACGAAAACAACCA
Blood-F	TGCCACAGTACCTGATTTCG	Blood-R	GATTCGCCTTTTACGTTTGC
Diver-F	GGCACCACATAGACACATCG	Diver-R	GTGGTTTGCATAGCCAGGAT
Diver2-F	CTTCAGCCAGCAAGGAAAAC	Diver2-R	CTGGCAGTCGGGTGTAATTT
gtwin-F	TTCGCACAAGCGATGATAAG	gtwin-R	GATTGTTGTACGGCGACCTT
gypsy-F	GTTCATACCCTTGGTAGTAGC	gypsy-R	CAACTTACGCATATGTGAGT
gypsy6-F	GACAAGGGCATAACCGATACTGTGGA	gypsy6-R	AATGATTCTGTTCCGGACTTCCGTCT
HeT-A-F	CGCGCGGAACCCATCTTCAGA	HeT-A-R	CGCCGCAGTCGTTTGGTGAGT
Hopper-F	GGCTGGCTTCAACAAAAGAA	Hopper-R	GGACTCCCGAAAACGTCATA
I-element-F	GACCAAATAAAAATAATACGACTTC	I-element-R	AACTAATTGCTGGCTTGTTATG
Invader1-F	GTACCGTTTTTGAGCCCGTA	Invader1-R	AACTACGTTGCCCATTCTGG
Max-F	TCTAGCCAGTCGAGGCGTAT	Max-R	TGGAAGAGTGTCGCTTTGTG
mdg1-F	AACAGAAACGCCAGCAACAGC	mdg1-R	CGTTCCCATGTCCGTTGTGAT
R1A1-F	AATTCCCGAGCTGTGCTAGA	R1A1-R	GTCTCAAGGCACCTTTCAGC
rp49-F	CCGCTTCAAGGGACAGTATCTG	rp49-R	ATCTCGCCGCAGTAAACGC
Rt1a-F	CCACACAGACTGAGGCAGAA	Rt1a-R	ACGCATAACTTTCCGGTTTG
ZAM-F	ACTTGACCTGGATACACTCACAAC	ZAM-R	GAGTATTACGGCGACTAGGGATAC

Supplementary Table 4. Primers used to make constructs.

FseI-piwi-for	aaaaaGGCCGGCC ATGgctgatgatcagggacg	KpnI-piwi-rev	aaaaa GGTACC ttatagataataaaacttcttttcgag
FseI-corto-for	aaaaaGGCCGGCC ATGACGATGGCCGCCTGTTA	KpnI-corto-rev	aaaaa GGTACC TCACACGTTGTAGCAGGA
FseI-E(z)-for	aaaaaGGCCGGCC ATGAATAGCACTAAAGTGCCG	KpnI-E(z)-rev	aaaaa GGTACC tcaaacaatttccatttcacgc
FseI-Su(z)12-for	aaaaaGGCCGGCC ATGGCCCCAGCGAAAAAACG	KpnI-Suz12-rev	aaaaa GGTACC ttagttgcccacacagttg
FseI-Esc-for	aaaaaGGCCGGCC ATGAGCAGTGATAAAGTGAAAAACG	KpnI-Esc-rev	aaaaa GGTACC tcagatggaagttgtttgtc
KpnI-Piwi-nt771rev	aaaaa GGTACC tttgtgagttatttcggtgcc	FseI-Suz12-nt1978For	aaaaaGGCCGGCC cagcggcagcgcgagg
FseI-Suz12-nt1438For	aaaaaGGCCGGCCacctgcggccccgtgc	KpnI-Suz12-nt2040Rev	aaaaaGGTACC ttcctgcttcttgtgcatgc
FseI-Suz12-nt640For	aaaaaGGCCGGCCcgcccaatgggtgagcag	KpnI-Suz12-nt1500Rev	aaaaaGGTACC cgtccaggcaggtcttc
KpnI-Suz12-nt642rev	aaaaaGGTACCctctccagacgatgcgc	FseI-Piwi-nt328For	aaaaaGGCCGGCC ccggaatggcggatcg
FseI-Piwi-nt769For	aaaaaGGCCGGCC aaagttatgcgcaccgagac	FseI-Esc-nt868For	aaaaaGGCCGGCC gactgtgtgcagtggttc
KpnI-Esc-nt729Rev	aaaaa GGTACCccacagcttcagcgagtg	FseI-Esc-nt727For	aaaaaGGCCGGCC tggtgtctgaatacgccc
KpnI-Suz12-nt658Rev	aaaaaGGTACC cattgggcgcatcgtctg	KpnI-Suz12-nt690Rev	aaaaaGGTACC cttgatgcgaaatagcagct
KpnI-Suz12-nt1170Rev	aaaaaGGTACC ctgaatgtgctccggtgt	FseI-Suz12-nt1138For	aaaaaGGCCGGCC acggtgtgcaagaccaca
FseI-Piwi-nt508For	aaaaaGGCCGGCC tcgaagctggacattgaataca	KpnI-Esc-nt252Rev	aaaaaGGTACC cagcgtgttgaaggctac
KpnI-Esc-nt450Rev	aaaaaGGTACC tccgcggtatccagca	FseI-Esc-nt448For	aaaaaGGCCGGCC ggagtcatccgggttattgat
FseI-Piwi-nt448For	aaaaaGGCCGGCC ggactgcaactgttcacc	FseI-Piwi-nt478For	aaaaaGGCCGGCC gagcaggaaatcacggtg
KpnI-Piwi-nt771rev	aaaaa GGTACC tttgtgagttatttcggtgcc	FseI-Esc-nt250For	aaaaaGGCCGGCC ctgctgggcaaggatgag
KpnI-Esc-nt600Rev	aaaaaGGTACCgatgttccacaatcttatcgcgtg	FseI-Esc-nt598For	aaaaaGGCCGGCC atccagagccatgtgtgc
KpnI-Esc-883Rev	aaaaaGGTACC accactgcacacagtcca	FseI-Esc-nt870For	aaaaaGGCCGGCCctgtgtgcagtggttcgg

Supplementary Table 5. Primers used for ChIP-qPCR.

RPL40-prom-F	cgaaaaatcgcaataacgtg	RPL40-prom-R	ttcgacagaaacagctccac
piRNA-41-F	ctggtgctgctttgctatgt	piRNA-41-R	cgaaggtacgggttgaagtt
Fab-7-F	ggcagtgggaagtcgtattt	Fab-7-R	caaagcttctggggctttac
iab-7-F	tttgggcctctagtttttcg	iab-7-R	cctacgacagtgcggtattc
hh-prom-F	tggctctttttccagacctt	hh-prom-R	atttcttgggcacacattga
Intergenic-F	gaacccaggacttttttcgag	Intergenic-R	ccactttgcaacatctttcc
Ubx-Piwiseq-F	ttcgcatagagccactcatc	Ubx-Piwiseq-R	tttttattggcccgtttttc
gypsy-F	cgccacaaggctagtgataa	gypsy-R	ttccttctttcgctgaggtt
ZAM-F	agtgcaagcagcaaacactt	ZAM-R	agttacctccggggagtctt

Supplementary Table 6. DNA oligomers used for EMSA.

Fhos	gcaaaagtcgcaggaaaaacgcacacatcttt cgtatg	mutant-*Fhos*	gcaacagtttcaggaaacattcacacatctttt cgtatg
Srp	gcaataacagcagcagttgcggttaaaaacg ccagcataacgcgtgcaaatgtgtgcccagc gattttaaacgcgtacaaagttttcgtttctctggg	mutant-*Srp*	gcaataacagcagcagttgcggttaatttcc ccagcttttcccctgcaaatgtgtgcccagcg attttttttcccctacaaagttttcgtttctctggg
CG18135 promoter	tcgatatcgagcggtttacctttgagcgaatcgc gcgcaagccgcacggaagcggttgcaattga ggcggtttaagcagcggt	mutant-*CG18135* promoter	tcgatatcgagcggtttacctttgagcgggtc ccccgcgggccccacggaagcggttgcaa ttgaggcggtttaagcagcggt