Supplementary materials for

Transcriptional and chromatin changes accompanying *de novo* formation of transgenic piRNA clusters

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Figures S1-S5 and Table S1

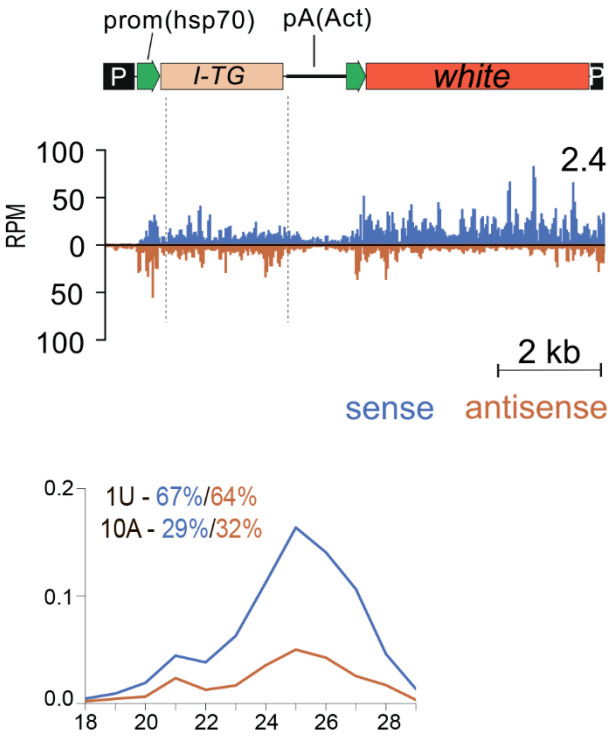

Figure S1. Transgene containing a fragment of *I*-element induces formation of strong piRNA cluster. Normalized numbers of small RNAs in a 30 bp window mapped to transgenic construct (no mismatches allowed). Schema of transgene is shown above. Length distribution of small RNAs mapping to all transgene sequences except for I-TG and *hsp70* promoters. Percentages of reads having 1U and 10A biases are indicated for each strand (only 24-29 nt reads were considered).

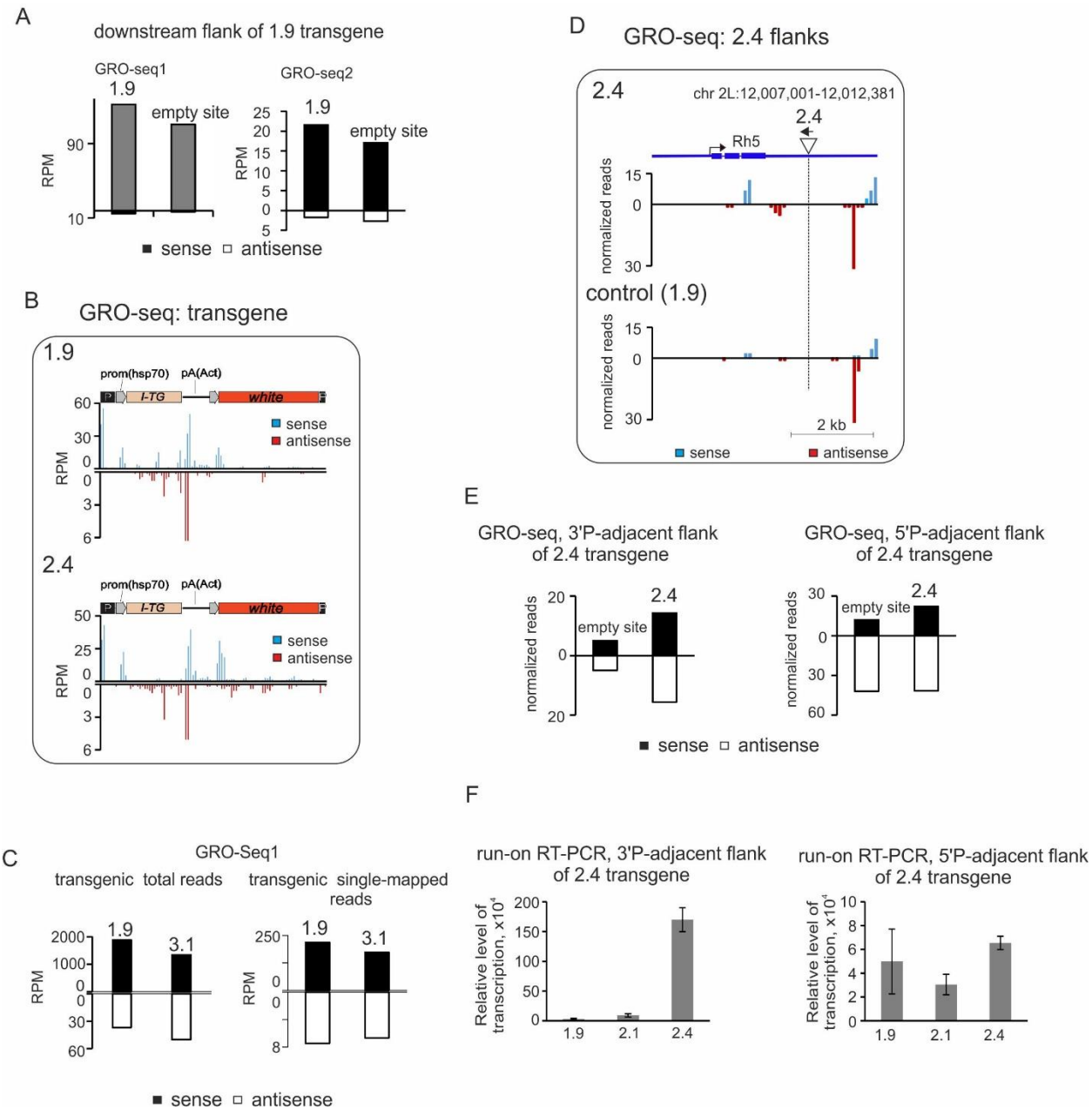

Figure S2. Transcriptional changes accompany piRNA cluster formation (related to Fig. 2). (A) GRO-seq read counts at 9 kb region downstream of 1.9 insertion site in 1.9 and 3.1 (GRO-seq1) and in 1.9 and 2.4 (GRO-seq2) strains. (B) Mapping of normalized (RPM) GRO-seq reads to the transgene calculated for 100 bp window size in strong (2.4) and in weak (1.9) strains (blue – sense; pink– antisense; no mismatches allowed). Schema of transgene is shown above. (C) Normalized numbers of transgenic small RNAs mapped to 1.9 and 3.1 transgenes. Total transgene-specific small RNAs (to the left) and single-mapped reads (to the right) are shown. (D) Normalized GRO-seq densities mapping to the 2.4 transgene insertion site (indicated by triangle

above the plots) in control (1.9) and in 2.4 strains (no mismatches allowed). Schema of genomic region is shown above; genome coordinates are given according to Drosophila R5 release. (E) Normalized GRO-seq read counts (RPM) at ~4 kb region upstream and ~2 kb region downstream of 2.4 insertion site in 1.9 ("empty" site) and 2.4 (transgene insertion) strains. (F) The level of nascent RNA from 2.4 3'P-flanking region is higher in ovaries of 2.4 strain as compared to control strains. RT-qPCR was done on ovarian run-on RNAs from indicated strains. Random hexamers were used for reverse transcription priming. Relative RNA levels normalized to *rp49* are shown. Error bars represent SEM of three technical replicates.

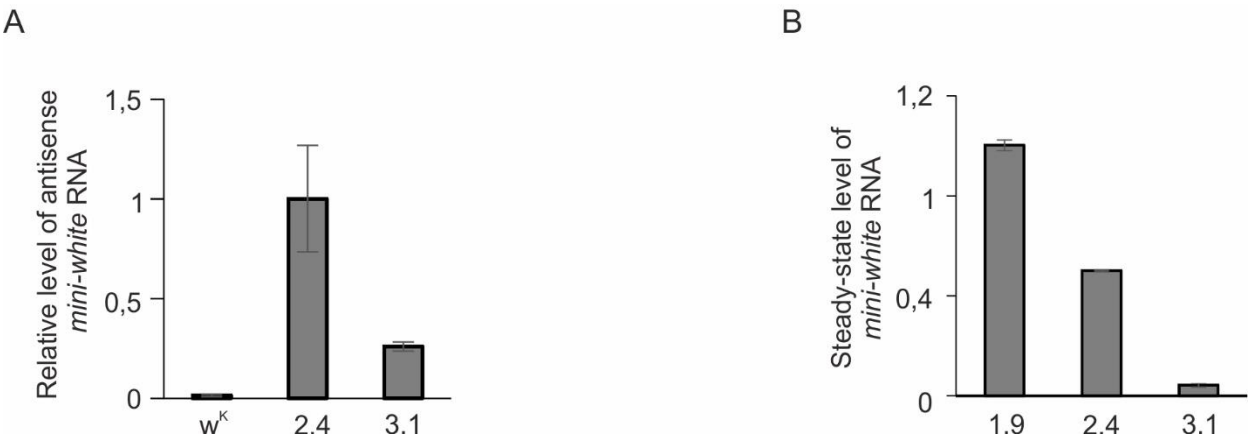

Figure S3. Expression analysis of I-TG transgenes. (A) Antisense *mini-white* RNA is not revealed in ovaries of w^K . Expression analysis of antisense *mini-white* RNA in ovaries of w^K, 3.1 and 2.4 strains using strand-specific RT-qPCR. (B) Steady-state level of transgenic *mini-white* detected by RT-qPCR using random primers and normalized to *rp49* expression level is higher in strain 1.9 than in 3.1 or 2.4.

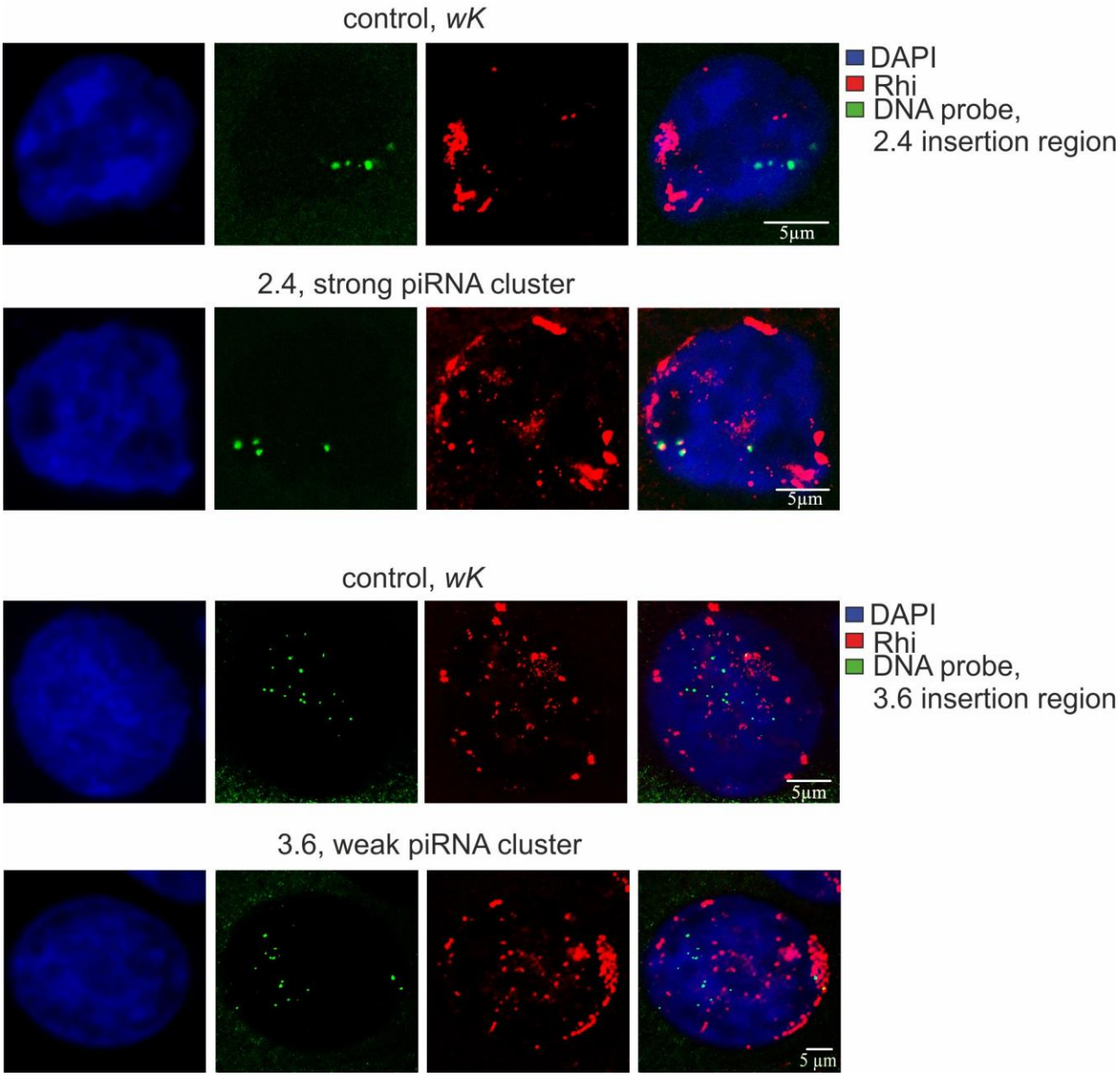

Figure S4. Strong transgenic piRNA clusters show association with Rhino foci (related to Fig. 3). Rhi immunostaining (red) in combination with DNA FISH with probes corresponding to 2.4 and 3.6 insertion regions was done on ovaries of w^K, 2.4 and 3.6 strains. Nurse cell nuclei at stage X of oogenesis are shown. DNA is stained by DAPI (blue). Bars, 5 µm.

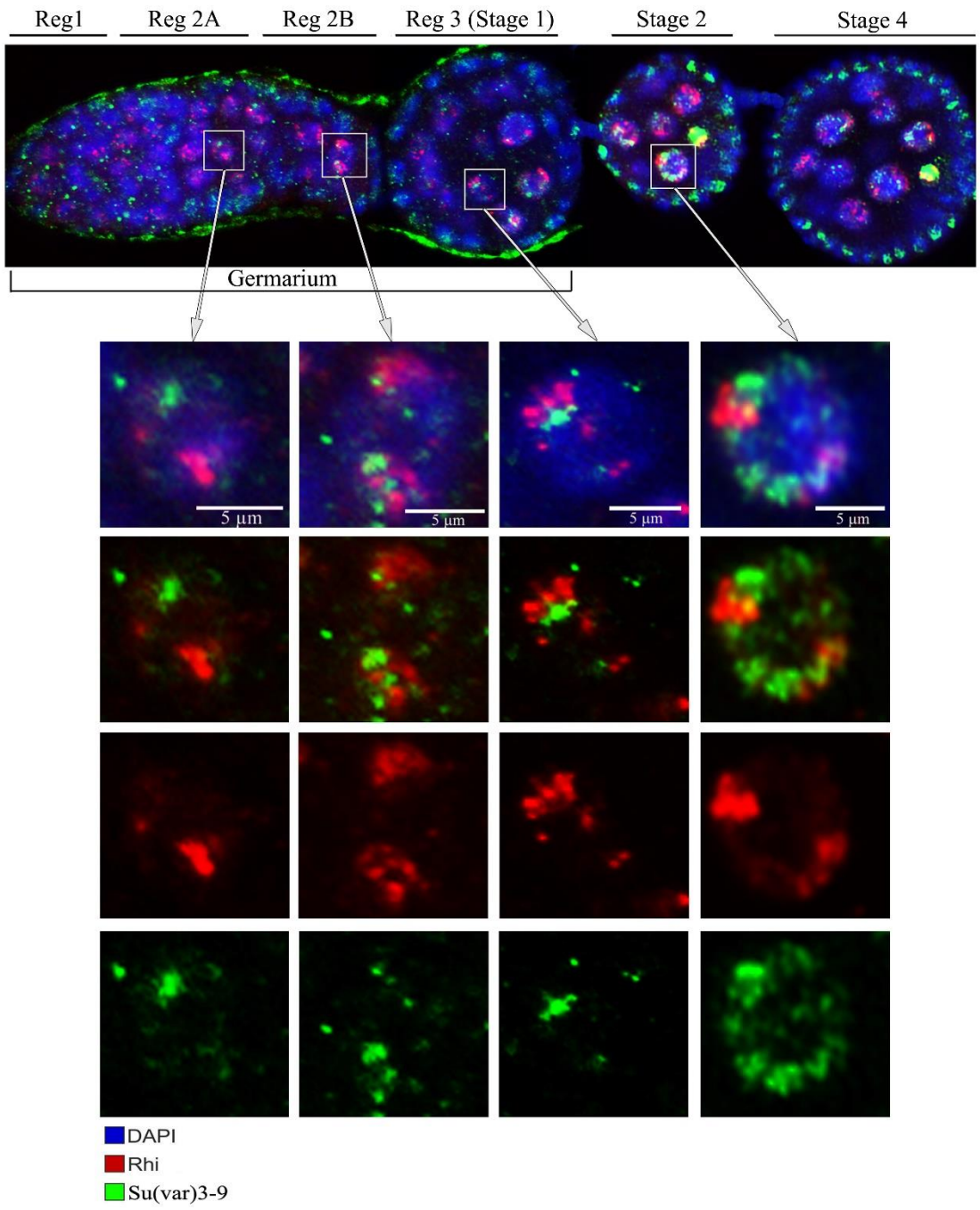

Figure S5. Rhi does not associate with histonemethyltransferase Su(var)3-9 during oogenesis. Ovaries of w^K strain were immunostained for Rhi (red) and Su(var)3-9 (green). Nuclei of germ cyst cells and nurse cells at indicated stages of oogenesis are shown. DNA is stained by DAPI (blue).

Table S1. Primers used in the study (5' to 3').

name/target	orientation	amplification region	sequence
rp49	*forward*		AAACGCGGTTCTGCATGAG
	reverse		GACGCTTCAAGGGACAGTATCTG
2.4_ins_up1	*forward*	*2.4 empty site (Fig1A); region a (Fig 1B)*	GTCTCAATGCTTCCGTGCCTG G
	reverse		CTCCTGGCCGCCGGATGTTC
2.4_claster	*forward*	*region e (Fig1B),Fig.S2F*	ACATTACCAGTTTGCCATCTCGC
	reverse		GCCATCATCATCTGCCATCG
2.1_ins_claster	*forward*	*2.1 empty site (Fig 1A)*	GACCGCCGAACTGGAGGATT
	reverse		CGAAGCGAGTGGCAAAGAAA
1.9_ins_claster	*forward*	*1.9 empty site (Fig1A)*	ATTTGAGTTTCTGTCCCACTGTGG
	reverse		TGTTGGGAGAGAGAATAGAGATAAGGC
67.2.1_ins	*forward*	*67.2.1 empty site(Fig1A)*	GAGCGATATAAAGAGAGTGGCAAAGA
	reverse		TTGTGGGTGCTCTCCAATGC
3.6 empty site	*forward*	*3.6 empty site (Fig 1A)*	TCCCCAGAGAGAAAACCCACG
	reverse		GCTCGTTCGGCTCTCGGC
5'P	*forward*	*5'P (after insertion, Fig 1A) region d (Fig 1B)*	AGAGGAAAGGTTGTGTGCGGAC
	reverse		CTGCGAATCATTAAAGTGGGTATCA
Mini-white intr	*forward*	*region c (Fig1B)*	ATTCTGGTAGCTGTGCTCGC
	reverse		GTGCATCTAGCTAGAGTCGAGC
Transgenic *mini-white*	*forward*	*transgenic mini-white (FigS3B)*	TGCAACTACTGAAATCAACCAAGA
	reverse		GCACTTTGTGTTTAATTGATGGCG
exon 6 of white gene	*forward*	*strand-specific RT -qPCR of mini-white (FigS2A)*	GCTGCCAGTTTTTATGAGGGAGG
	reverse	*mini-white (FigS2A)*	CGCCAGGCAGTTGAAGAAGTG
3'P	*forward*	*region b (Fig2B)*	TAATTCAAA CCCCACGGACA
	reverse		ATAACATAAGGTGGTCCCGTC
3.6 flank1	*s* *as*	*3.6 insertion region, probe for DNA FISH*	CCGCTAGACCACGTAACGC CGGGCACTATCTGAGCGG
3.6 flank2	*s* *as*	*3.6 insertion region, probe for DNA FISH*	CTCAGACCGGACCTCTACCAG GCTTCGTGGACAGCGCTTC
antisense mini-white	*RT primer* *PCR-s* *PCR-as*	*For detection of antisense mini-white transcripts (Fig.2F)*	CATGATCAAGACATCTAAAGGC CATGATCAAGACATCTAAAGGC GTGCATCTAGCTAGAGTCGAGC
2.4 ins up	*s* *as*	*For RT-PCR of 2.4 3' flank (Fig. S2F)*	GGTGGGTAGATCGAGTCCGGATACC GCAAGAAGAAGCCCCACTTTGCG