Supporting Information

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SI Materials and Methods

Fly Stocks. FLP-mediated excision of inserts was performed using *hs-FLP Cy/noc Sco* (6876) as described previously (1). The yw^{67c23} strain was used to outcross reporter lines for pigment assays (for $^{+/-}$*1360* assays and wt controls for mutant analysis) and ChIP-qPCR. Alleles used for PEV assays are *yw; Su(var)* 205^{02}/*CyO*, *yw*; Su*(var)*205^{05}/*CyO*, w^{118};Su*(var)*$3–9^{06}$, w^{118}; aub^{QC42}/*CyO*, w^{118}; $aub^{\Delta P\text{-}3a}$/*CyO* (2), w^{118}; $piwi^{1}$/*CyO* (3), w^{118}; $piwi^{2}$/*CyO* (3).

Constructs. The *P* element landing pad construct was derived from P[T1] (1). Phage-attachment sites attP1 and attP2 were PCR-amplified (using primers attP1F, attP1R, attP2F, and attP2R) from pUASTP2 (4) and cloned into StuI and ClaI sites of P[T1], respectively. The Clontech In-Fusion PCR Cloning System was used; primers were designed according to specifications of the manufacturer (5).

Donor constructs were derived from pCiB-yin (4) by PCR amplifying *yellow* [primers y forward (F), y reverse (R)] to clone into the pCR2.1 TOPO vector to make pCR2.1-y (4). The attB1 and attB2 sites were cloned from pCiB-GFP (4), and the loxP sites from pP[wlo+GS]. The attB2 site was inserted into ApaI of pCR2.1-y to generate pCR2.1-y-attB2. A loxP site was cloned into a new pCR2.1 TOPO vector, and attB1 was inserted upstream at KpnI to make pCR2.1-attB1-loxP. PCR amplification of attB1-loxP and insertion into the SacI site of pCR2.1-y-attB2 was carried out to produce pCR2.1-attB1-loxP-y-attB2. A second loxP site from pP[wlo+GS] and a frt site from P[T1] were cloned into a new pCR2.1 TOPO vector to make pCR2.1-loxP-frt. Each deletion construct (Fig. 3*A*) was derived from the *1360{}1503* copy present in P[T1]. The full-length *Invader4* construct (Fig. 4*A*) was derived from *Invader4{}1541* (subcloned from the fourth chromosome into pCR2.1 TOPO-TA vector using primers Invader4F/R) and cloned into the XhoI site of pCR2.1-loxP-frt using primers XhoIInvader4F/R. The list of primers used to amplify deletion constructs can be found in Table S1. Each amplified deletion construct was cloned into the XhoI site of pCR2.1-loxP-frt to generate pCR2.1-loxP-frt-*1360*Δ. Primers XhoI8-24F and XhoI8-24R were used to amplify loxP-frt-*1360*Δ to clone into the XhoI site of pCR2.1-attB-loxP-y-attB to make pCR2.1-attB-loxP-y-loxP-frt-*1360*Δ-attB. Preparing the *1360*ΔTSS and *1360*ΔpiRNA constructs required consecutive inverse PCR steps; primers are listed in Table S1. Donor constructs were injected by Genetic Services.

Mobilization. Mobilization was from the X chromosome (Line 5, X:3589639). Females homozygous for the *P* element insertion were crossed to *w/Y*, *Sb Δ2–3/TM6* males. The male progeny carrying the *Sb Δ2*-3 chromosome and the landing pad construct were crossed to yw^{67c23}; *net*; *sbd*; spa^{pol} [MMR (multiply marked recessive)] females. Male progeny that carried the landing pad construct but not the *Sb Δ2-3* chromosome were backcrossed to yw^{67c23}; *net*; *sbd*; spa^{pol} females, facilitating genetic mapping as indicated by the absence of one of the recessive phenotypes, *net*, *sbd*, or spa^{pol}. Landing pad lines generated from the screen were maintained over appropriate second or third chromosome balancers (*CyO* or *TM3Sb*) or, for the fourth, a chromosome marked by a dominant mutation (ci^{D}).

Mapping Insertion Sites in Landing Pad Lines. Insertion sites in landing pad lines were mapped by inverse PCR from the 5′P end to the transposon as described previously (6). The genomic position of the landing pad *P* element in line 1198 was confirmed by amplifying and sequencing the 3′ end of the construct (primer 3′w v.2) and predicted flanking genomic region (primer 1198 F).

PhiC31 Cassette Exchange. To screen for putative recombinants, we crossed adults to *yw* and screened F1 males for *y*+. PCR was used to confirm that cassette exchange had occurred in the desired orientation, by screening for the loss of attP sites and gain of attL and attR (Fig. 1*A*). We crossed each recombinant to *yw* P{*y*[+*mDint2*]=*Crey*}1b for *Cre* recombinase–mediated excision of the *yellow* marker before analysis by pigment assay; this was necessary, because enhancers present in the *yellow* gene interfered with our reporter readout.

Eye Pigment Analysis. Quantitative eye pigment analysis was performed on 3- to 5-d-old adults. All mutant lines analyzed were heterozygous for the reporter and for the mutant allele. Flies were homogenized in 250 μL of 0.01 M HCl in ethanol, incubated for 10 min at 50 °C, and the extract clarified by centrifugation. A final volume of 150 μL was used to measure OD at 480 nm (adapted from ref. 7).

Assessment of RNA Products by RT-PCR. RNA was isolated from 0- to 10-h embryos (Fig. 4*B*) or 3- to 5-d adult flies (Fig. 3*C*) using TRIzol (Invitrogen) according to the protocol of the manufacture. For quantitative analysis of *hsp70-w* (primers *white* exon6 F/R) from $^{+/-}$*1360* and *1360*ΔpiRNA (Fig. 3*C*), flies were non–heat-shocked or heat-shocked at 37 °C for 55 min and allowed to recover for 2 h before RNA extraction. RNA was DNase I–treated (Promega; RQ1 RNase-Free DNase) and reverse-transcribed (Invitrogen; SuperScript II) using random hexamer primers (Fig. 4*B*) or oligo dT (Fig. 3*C*). qPCR of the 3′P end of the *P* element (Fig. 4*B*) was performed using primers 1198 F and 3′P A412 R.

1. Haynes KA, Caudy AA, Collins L, Elgin SC (2006) Element 1360 and RNAi components contribute to HP1-dependent silencing of a pericentric reporter. *Curr Biol* 16:2222–2227.
2. Pal-Bhadra M, et al. (2004) Heterochromatic silencing and HP1 localization in Drosophila are dependent on the RNAi machinery. *Science* 303:669–672.
3. Cox DN, et al. (1998) A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal. *Genes Dev* 12:3715–3727.
4. Bateman JR, Lee AM, Wu CT (2006) Site-specific transformation of Drosophila via phiC31 integrase-mediated cassette exchange. *Genetics* 173:769–777.
5. Zhu B, Cai G, Hall EO, Freeman GJ (2007) In-fusion assembly: seamless engineering of multidomain fusion proteins, modular vectors, and mutations. *Biotechniques* 43: 354–359.
6. Sun F-L, et al. (2004) cis-Acting determinants of heterochromatin formation on Drosophila melanogaster chromosome four. *Mol Cell Biol* 24:8210–8220.
7. Khesin RB, Leibovitch BA (1978) Influence of deficiency of the histone gene-containing 38B-40 region on X-chromosome template activity and the white gene position effect variegation in Drosophila melanogaster. *Mol Gen Genet* 162:323–328.

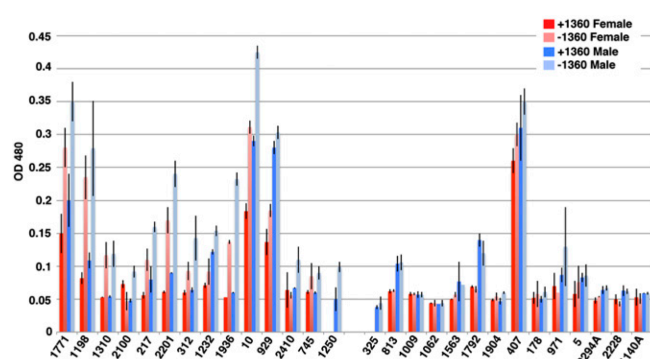

Fig. S1. Pigment assay (OD_{480}; a measure of expression of the *hsp70-white* gene) for females and males carrying the reporter element (Fig. 1) at different insertion sites, with (+) or without (–) the *1360* element. The left half of the graph is a compilation of all *1360*-sensitive landing pad lines, whereas the right half displays data from those that showed no change in pigment levels $^{+/-}$*1360*. Error bars derived from two biological replicate experiments (four technical replicates per experiment), ±SD.

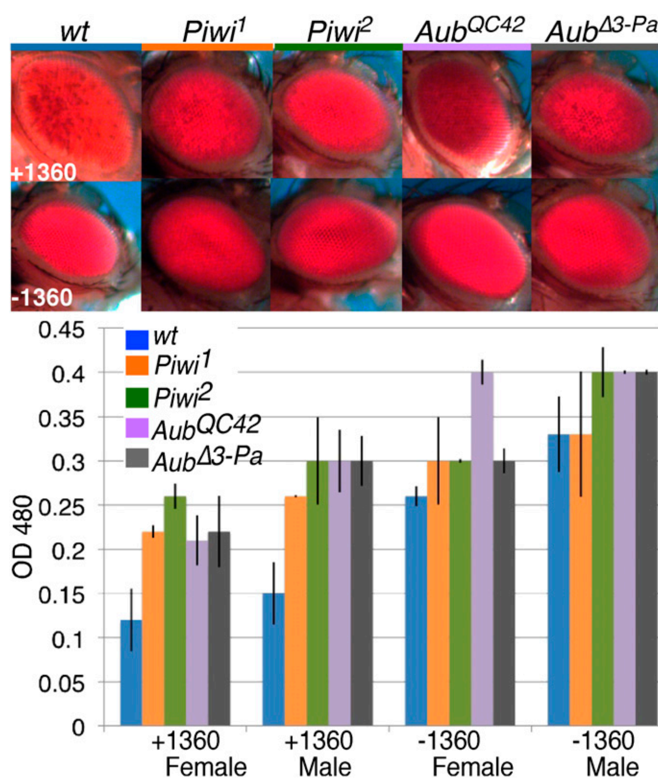

Fig. S2. Representative eye pictures for 1198 lines $^{+/-}$*1360* (*Upper*) in the presence of *piwi* and *aub* mutant alleles. Pigment assay data (OD_{480}; *Lower*) shows the mean of two biological replicate experiments, ±SEM. When *1360* is present, silencing is sensitive to mutations in *piwi* and *aub*, which code for components of the RNAi system.

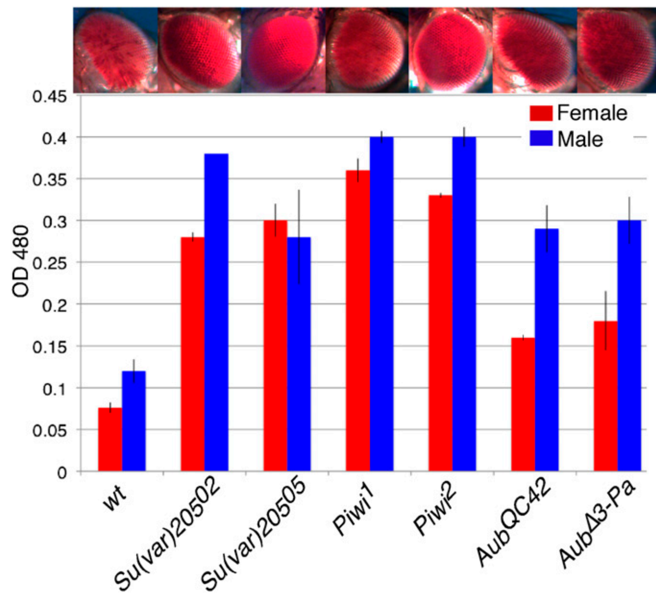

Fig. S3. Representative eye pictures and pigment assays (OD_{480}) (± SEM) for line 1198 females and males carrying the reporter construct with an *Invader4* element, demonstrating the impact of mutations in *Su(var)205*, *piwi*, or *aub*. Silencing is sensitive to mutations in both the heterochromatin system and the piRNA system.

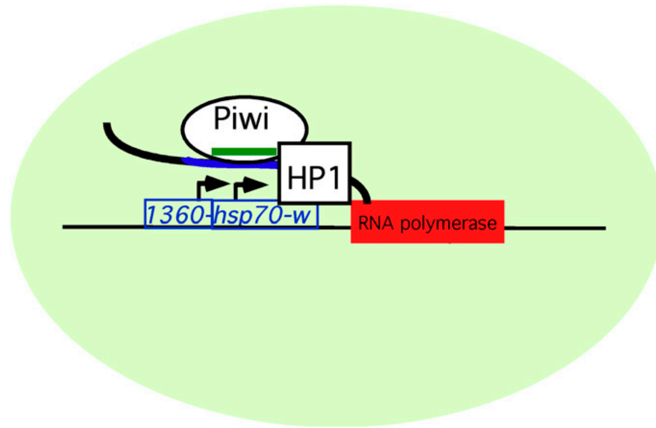

Fig. S4. Model for piRNA targeting of *1360*. piRNAs loaded onto Piwi could bind to the read-through transcript from the *nesd* promoter. Our results suggest that these RNA products help target HP1a to the *1360* element upstream of our reporter.

Table S1. Oligonucleotides

Primer name	Sequence
Cloning	
attP1 F	aggtcgacctcgaggcctttgagatgcagtacctgacggacac
attP1 R	aacgttactcgaggccttgacctgttcggagtgattagcgt
attP2 F	tcatcaagcttatcggtcacaccacagaagtaaggttcc
attP2 R	tcctcgacggtatcgtagggaattgggaattcggcttcg
y F	aagccacctgattacccgaacact
y R	tcaagcgaccaggcgatctcaaat
attB1 F	attacgccaagcttggtaccgactcactatagggcgaattgg
attB1 R	gtggatccgagctcggtaccatcaagcttatcgataccgtcg
attB2 F	agcatgcatctagagggcccgtggatccactagttctagagc
attB2 R	ctatagggcgaattgggcccgatgtagctcggtctcgaagc
loxP F	tgtggacagagaaggaggcaaaca
loxP R	agcgacactcccagttgttcttca
XhoI8-24F	ctggcggccgctcgacaggaaacagctatgaccatga
XhoI8-24R	tagatgcatgctcgactatagggcgaattgggccctc
1360{}1503F	cttgcggccgctcgagaaaggaatacggtattaccaagacac
1360{}1503R	tagatgcatgctcgacatcggttgatgatcaataaatttc
p1360R F	cttgcggccgctcgatgtaacaataacattaaaagtgtt
p1360R R	tagatgcatgctcgacatcggttgatgatcaataaatttc
p1360L F	cttgcggccgctcgagaaaggaatacggtattaccaagacac
p1360L R	tagatgcatgctcgatcatattaagtcaaatgatttaat
p1360-IR F	cttgcggccgctcgagccatacattggtttgcccaaag
p1360-IR R	tagatgcatgctcgagccatacattggtttggcactatg
TSSΔ1	tcaaatgatttaataaatatactaaataat
TSSΔ2	attagaattaaacataaatataaatgtgtaaac
TSSΔ3	ttttcgggccgaaatcaattctgatc
TSSΔ4	tagatttcttacgctctcagcggg
TSSΔ5	attactctctttccgctcactcc
TSSΔ6	ggttaaactaaagtatttttaaag
piRNA IΔF	aacaaacttaaaaagctttaa
piRNA IΔR	catttatatttatgtttaattct
piRNA IIΔF	ccatcgatggacacgcacacttatac
piRNA IIΔR	ccatcgatggcacgattttcgggcc
Invader4 F	gtatgtgtcgaagagtcatcaggatg
Invader4 R	ggtagaagaaagcccttaaaggtatg
XhoI Invader4 F	cttgcggccgctcgagatctgcgac
XhoI Invader4 R	tagatgcatgctcgacatctgggcat
Invader4ΔLTR F	cttgcggccgctcgacggccttcct
Invader4ΔLTR R	tagatgcatgctcgatcagaagtggg
Mapping	
5′P v.2	cttcggctatcgacgggaccacctta
3′w v.2	gacgaaatgaaccactcggaacc
1198 F	ggcattgaatgagcattgtaatcgatactt
Cassette exchange	
1X1360 3′P inner F	attaacccttagcatgtccgtggg
attB4/09 R	atcaagcttatcgataccgtcgacc
attB2F	gtggatccactagttctagagc
InFusion primer bind	tagcgaattgggaattcggcttcg
1X1360 3′P R	gtatcatcagtgggaagggcgaa
AttP1F	aagatcctctagaggtaccctcgagc
ChIP-qPCR	
α-Actinin1	cagcaagcacctctgctcta
α-Actinin2	tgcaagcgttagtgagatcc
Hsp70 F	caagcgcagctgaacaagctaaac
white R	attgatggcgtaaaccgcttggag
18s1	ttcatgcttgggattgtgaa
18s2	gtacaaagggcagggacgta
RT-PCR	
RpL32 F	cgatctcgccgcagtaaac
RpL32 R	cttcatccgccaccagtcg
3′P A412 v.1 R	cccaaagctttgcgtactcgc
white exon6 Fwd	cctcagagctgccagttttt
white exon6 Rev	Ttttgagggggcaataaaca

Dataset S1. Landing pad insertion lines

Dataset S1

Landing pad lines generated from our screen, giving the eye phenotype, location in the genome (chromosome, band, and sequence position of the insertion), orientation (strand), gene and repeat densities (or number of bases annotated as genes (Flybase), or repeats (repeat masker) reported as percentages measured 10 kb on either side of the insertion site for a total of a 20-kb window), with the corresponding chromatin state of the native insertion site in BG3 and S2 cells (1), *1360*-dependent silencing is indicated as "Y" (yes), "N" (no), and "U" (unknown) (not tested). Note that sites where no chromatin state information is available are marked "NA" in those columns.

1. Kharchenko PV, et al. (2011) Comprehensive analysis of the chromatin landscape in Drosophila melanogaster. *Nature* 471:480–485.