Supporting Information

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SI Materials and Methods

***Drosophila melanogaster* Mutant Strains.** The following mutant flies were used: $piwi^2$ and $piwi^3$ (1); $spn\text{-}E^1$ and $spn\text{-}E^1/spn\text{-}E^{hls3987}$ (2, 3); $armi^{72.1}$ and $armi^1$ (4); aub^{QC42}/aub^{HN} (5); $mael^{r20}$ (6), $mael^{EY08554}$, and $mael^{r20}/mael^{EY08554}$ transheterozygotes; zuc^{HM27}/zuc [*DTS513*] (7).

Molecular Characterization of $piwi^{Nt}$ Mutation. For RT-PCR analysis of the $piwi^{Nt}$ transcript the following primer pairs were used: *1*, forward, ACTGAGTCCAAAGCGTCGTT and *1*, reverse, GCATGATTGGACAAAACTCC, *2*, forward, CCGAGCATCGAGAATCCT and *2*, reverse, AAGTCCAAAACATTTACCCG, *3*, forward, CGACGTTGCTCACACAATC and *3*, reverse, TGTTCTGCTGTCCAATTATC, *4*, forward, TGGACAAGAACGTCACAGAA and *4*, reverse, TTCAGTTGGATTGCTATTTTG, *5*, forward, ACGACCAAGAACCGTAGC and *5*, reverse, TGTCCGTTGAGGAAGAAGC, *6*, forward, TATATTGTGGTAACCAGATCCAT and *6*, reverse, TTTAGTCATAAATGCTGAAGTG. All these primers except *1* forward and reverse revealed in mutant ovaries the presence of the *piwi* transcript with the same sequence as WT *piwi*. *1* forward and reverse primers corresponding to the *piwi* 5′ end detected no product in both RT-PCR and genome DNA PCR of $piwi^{Nt}$ mutant. 5′-RACE of $piwi^{Nt}$ transcript was performed by using the 5′-RACE System for Rapid Amplification of cDNA Ends kit (Invitrogen) according to the manufacturer's instructions. For the first PCR, *AAP*-GGCCACGCGTCGACTAGTACGGGGGGGGGG and *piwi rev*-GCAACGTCGCATTATGTCGTA primers were used. Obtained amplicon was used for nested PCR with primers *AUAP*-GGCCACGCGTCGACTAGTAC and *piwi rev*. Sequencing of PCR product was done using primers *piwi rev* and *1* reverse (as detailed earlier). 5′-RACE revealed that $piwi^{Nt}$ transcription start corresponds to the first intron of the *piwi* gene in position +1191 according to GenBank sequence NG000285. Predicted start of $piwi^{Nt}$ protein coding sequence corresponds to the position +1283 of NG000285. The result of 5′-RACE was confirmed by RT-PCR by using primers located close to $piwi^{Nt}$ transcription start site. $piwi^{Nt}$ transcript was detected by primer pair *p3* GCAGTCATTCACTTCTATTAGGA/*1* reverse, but not by primers *p4* GTTGAGTCTTTCTGGTGTTTATTG/*1* reverse located around the $piwi^{Nt}$ transcription start site. The cryptic promoter in this region was predicted using the McPromoter software (http://tools.igsp.duke.edu/generegulation/McPromoter/).

Site of P element construct insertion in the mutant $piwi^{Nt}$ chromosome was revealed by using inverse PCR. Genome DNA was restricted by using RsaI and *Cfo*I (HhaI) enzymes, ligated by T4 DNA Ligase (Roche), amplified, and sequenced. Amplification using primers *piwi in7 rev* GACATAACGGAATTATAAGGAAT and *piwi in6* for TATATTGTGGTAACCAGATCCAT revealed the insertion site in the first exon before position 105 according to GenBank sequence NG000285.

Quantitative RT-PCR. RNA isolation, reverse transcription with oligo-dT primer, and real-time PCR was performed as described previously (8). For each mutant genotype and corresponding heterozygote, three separate RNA samples were prepared. Primers were designed to unique regions of each transposable element type to exclude the amplification of more than one transposon type in one PCR. The following primers were used: CTTCACGTTCTGCGAGCGGTCT and CGCTCGAAGGTTACCAGGTAGGTTC for *Gypsy* transcripts according to sequence (GenBank accession no. AF033821); AACAGAAACGCCAGCAACAGC and CGTTCCCATGTCCGTTGTGAT for *mdg1* transcripts according to sequence (GenBank accession no. X59545); CCTTTGGGAAGCAGGCGTAAA (*GATE s1*) and TCTTCAGACATAGGAGAGAGCGGC (*GATE as1*) for *Gate* transcripts according to sequence (GenBank accession no. AJ010298); CGCAAAGACATCTGGAGGACTACC and TGCCGACCTGCTTGGTATTG for *HeT-A* transcripts according to sequence (GenBank accession no. U06920); AATGCCCTTGTCGGACACGA (*Beagle s1*) and TGATGAAACACATTACCAGAACCTTGA (*Beagle as1*) for *HMS-Beagle* transcripts according to sequence (GenBank accession no. AF365402); TGAAATACGGCATACTGCCCCCA and GCTGATAGGGAGTCGGAGCAGATA for *I* element transcripts according to sequence (GenBank accession no. M14954); CAGAACAAGTGCCTGAGTCAATCG and GTCCAGTCGTTTAGCGTATCTCTCG for *G* element transcripts according to sequence (GenBank accession no. X06950), GCATGAGAGGTTTGGCCATATAAGC (*cop-s*) and GGCCCACAGACATCTGAGTGTACTACA (*cop-as*), corresponding to GenBank sequence XO4456 for *copia*; TGGAACTGGGTAGTTCTGAATGACA (*412 as1*) and AGTTTTTGGTAAATGCAGGGAATACAA (*412 s1*) for *412* element; AGATCCGGCAGACATTCAG (*F-element_s*) and ACTTGACCATGTTTCCCCC (*F-element_as*) for *F* element; and CACAGAAACCTAAAGACTCCAATAGCG (*Doc s1*) and GTCAGCGAAAGCACTGGCTCTT (*Doc as1*) for *Doc* element. As an internal control, we used *Adh* amplified by primers CGGCATCTAAGAAGTGATACTCCCAAAA and TGAGTGTGCATCGAATCAGCCTTATT according to the *Adh* sequence (GenBank accession no. NT033779) and *rp49* amplified by TCCGCCCAGCATACAGGC (*rp49 s2*) and CAATCCTCGTTGGCACTCACC (*rp49 as2*), corresponding to GenBank sequence Y13939 for *rp49* gene.

ChIP. ChIP assay was performed as described previously (8), by using polyclonal rabbit anti-HP1 (PRB-291C; Covance) and polyclonal rabbit antibodies (Upstate): anti–dimethyl-Histone H3 Lys4 (no. 07-030), anti–dimethyl-Histone H3 Lys9 (no. 07-441), and anti–trimethyl-Histone H3 Lys9 (no. 07-523). DNA precipitates were amplified by real-time quantitative PCR. PCR product quantities were normalized to those of the input and relations to a fragment of an intergenic spacer in the 60D region were calculated. Quantities of sample PCR products were calculated using the following expression: $E(\text{product})^{\text{sample}} * E(60D)^{\text{input}} / E(60D)^{\text{sample}} * E(\text{product})^{\text{input}}$. The following primers were used for PCR analysis in ChIP: *cop-s*/*cop-as* (as detailed earlier); *Het-s2*/*Het-as2* (as detailed earlier); *rp49 s2*/ *rp49 as2* (as detailed earlier); *Beagle s1*/*Beagle as1* (as detailed earlier); TTCCCCATCCTCGAGCCCTG (*60ds6*) and CCAGCCGAGACGAGCACCATAAT (*60das6*), corresponding to the 60D intergenic spacer; *pgd s* AGGACTCGTGGCGCGAGGTG and *pdg as* GGAATGTGTGAACGGGAAAGTGGAG for *pgd* gene; TGAGGAGGCAATGGAACTTAT (*light s*) and TGAGCATAGTTGTTCGTAGGA (*light as*) for *light* gene; *MDG1 dir* AACAGAAACGCCAGCAACAGC and *MDG1 rev* CGTTCCCATGTCCGTTGTGAT for *mdg1*; and *GATE s*/*GATE as1* (as detailed earlier) and CATCACACGTTGTTGCACCGA (*GATE s2*)/GCACTGCCAAGAAGGATAGCTCT (*GATE as2*) for *Gate*.

Immunohistochemistry and Western Blot. Ovaries were dissected in PBS solution, fixed in 4% paraformaldehyde in PBS solution at

room temperature (RT) for 1 h, and treated with 50 μg/mL proteinase K solution (Boehringer) in PBS solution at RT for 5 to 12 min. Further procedures were done as described previously (9). Western blot was performed as described previously (9). The following antibodies were tested for their ability to detect the $Piwi^{Nt}$ protein in both immunostaining and Western blotting: rabbit N-terminal and C-terminal anti-PIWI polyclonal antibodies, obtained against 15 C-terminal and 15 N-terminal Piwi amino acids, respectively (10); P4D2 and P3G11 mouse monoclonal antibodies (11) and Abcam rabbit polyclonal antibodies (ab5207) obtained against the peptide corresponding to amino acids 350 to 450 in the Piwi protein sequence. P4D2 antibodies were used for $Piwi^{Nt}$ staining experiments. We also used rabbit polyclonal anti-Aub antibodies (10), rabbit anti-lamin B Receptor (Abcam), and monoclonal antibodies provided by the Developmental Studies Hybridoma Bank at the University of Iowa: mouse anti-lamin, rat anti-Vasa, and mouse anti–α-spectrin. Secondary antibodies were Alexa fluor 647-, Alexa fluor 488-, and Alexa fluor 546-conjugated anti-mouse, anti-rabbit, or anti-rat antibodies (Invitrogen). Mouse monoclonal antibodies to β-actin ab8224 (Abcam) or rabbit monoclonal antibodies to γ-tubulin (Sigma) were used as a loading control in Western blotting.

Short RNA Detection. [γ-^{32}P]dATP-labeled oligonucleotides AGGACGGAAGCACCGAGTCGCTGC, CATCCACAATTTATTTTGCCTTTG, and TTG CACATACTGTAATCAAAGGCAA complementary to frequently cloned piRNAs (10) were used to detect *TAHRE* and *HeT-A* piRNAs.

RNA in Situ Hybridization. PCR product for *HMS-Beagle* sense transcripts detection was amplified using primers TGATGAAACACATTACCAGAACCTTGA and TAATACGACTCACTATAGGGAATGCCCTTGTCGGACACGA. PCR product for *Gate* sense transcripts detection was obtained using primers CCTTTGGGAAGCAGGCGTAAA and TAATACGACTCACTATAGGGTCTTCAGACATAGGAGAGAGCGGC. In case of probes used for confocal microscopy, following hybridization, the sample was blocked during 1 h at RT in 1× PBS solution with 0.01% Tween 20 and 0.3% Triton X-100 (PBT-Tr 0.3%) with the addition of goat serum to 3% and hydrogen peroxide to 1%. Then the sample was washed four times for 15 min at RT in PBT-Tr 0.3% and incubated in the same solution with the addition of anti–DIG-POD antibodies (1:500; Roche) for 1 to 2 h at RT or overnight at 4 °C. Then, the sample was washed four times for 15 min at RT in PBT-Tr 0.3% and incubated in the same solution with the addition of 4% tyramide-FAM (0.1 mg/mL stock) for 30 min at RT, after which hydrogen peroxide was added to 0.015% and incubated for another 30 to 40 min. The sample was washed four times for 15 min at RT in PBT-Tr 0.3%, and DNA was stained with DAPI in 1× PBS solution for 10 to 20 min. The samples were transferred to SlowFade Gold reagent (Invitrogen). Confocal microscopy was done by using an LSM 510 META system (Zeiss).

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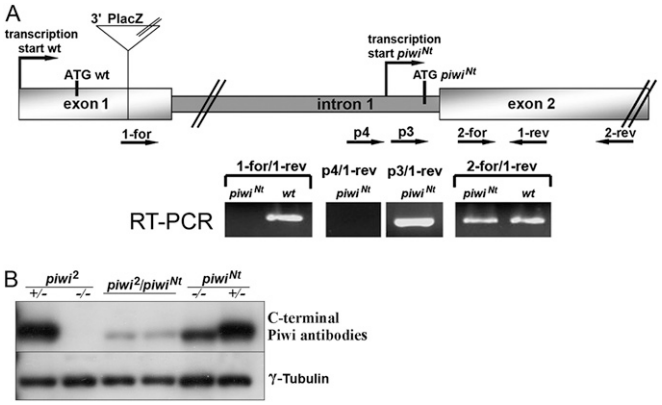

Fig. S1. The *piwi*^{Nt} mutation leads to formation of *N*-truncated Piwi protein. (*A*) As a result of *P lacZ* vector insertion, the mutant *piwi*^{Nt} transcript lacks exon 1 sequence, but has a transcription start site in intron 1, approximately 100 bp upstream of the intron 1/exon 2 boundary. Arrows indicate primers used for RT-PCR validation of the transcription start site. (*B*) Antibodies obtained against C-terminal Piwi amino acids detected shortened Piwi protein in *piwi*^{Nt} mutants and *piwi*^{Nt}/*piwi*^{2} transheterozygotes.

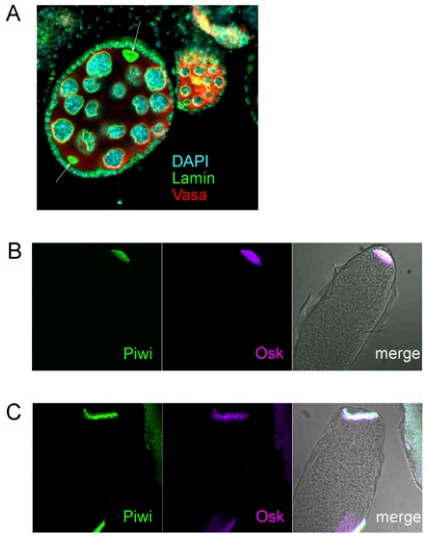

Fig. S2. Oocyte position and specification in *piwi*^{Nt} mutant. (*A*) Fused egg chambers, which can be observed in 2% of *piwi*^{Nt} ovarioles. Two oocytes are indicated by arrows. (*B* and *C*) Mature oocytes (stage 14) of *piwi*^{Nt} mutants labeled against Piwi and Oskar (Osk). Both proteins are known to be localized to oocyte posterior, where embryo pole plasm is formed. Approximately 30% of *piwi*^{Nt} oocytes (21 of 65) have correctly positioned Piwi and Osk (*B*). In most cases Osk fails to localize to the posterior pole. (*C*) An oocyte with two sites of pole plasm formation.

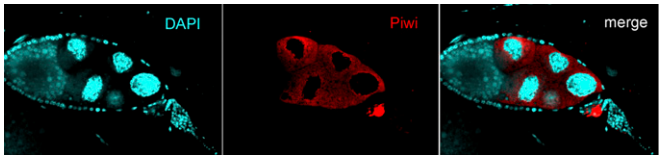

Fig. S3. Piwi cytoplasmic localization at stage 10 egg chamber of *piwi*^{Nt} mutant.

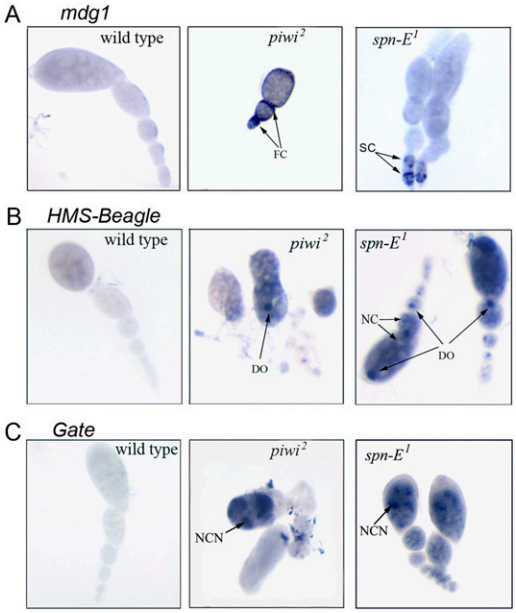

Fig. S4. Ovarioles from piRNA mutants stained by phosphatase method with riboprobes to *mdg1*, *HMS-Beagle* and *Gate* transposons. (*A*) *mdg1* transcripts are detected in somatic follicle cells (FC) in the faulty *piwi*^{2} ovaries. The *spn-E*^{1} mutation leads to a moderate increase of *mdg1* transcript abundance, predominantly in the somatic cells of the germarium (SC). (*B*) Transcripts of *HMS-Beagle* are accumulated in the nurse cells (NC) and especially in the developing oocytes (DO) of *piwi*^{2} and *spn-E*^{1} mutants. (*C*) RNA of the *Gate* retrotransposon is amassed predominantly in the nurse cell nuclei (NCN) as a result of the *piwi*^{2} and *spn-E*^{1} mutations.

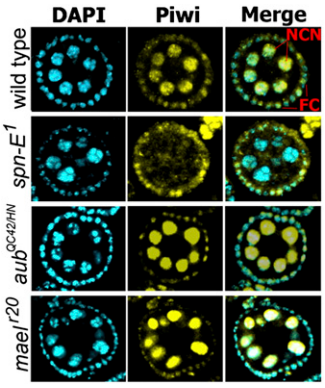

Fig. S5. *mael* and *aub* mutations do not prevent Piwi nuclear import. In concert with previous data, the *spn-E* mutation causes partial Piwi delocalization. FC, follicle cells. NCN, nurse cell nuclei.