Figure EV1. The molecular lesions and transcripts expression analysis of pelo alleles.

A. Upper diagram: a schematic drawing for the molecular lesions of different pelo alleles and the primer sets used for quantitative RT–PCR analysis. pelo\(^1\) is an allele with P-element inserted in the 3\(^{rd}\) intron. pelo\(^{PB60}\) and pelo\(^{PA13}\) were generated by using P-element excision from pelo\(^{KG06646}\). pelo\(^{PB60}\) has a 107-bp deletion in the second exon, resulting in early stop codon. pelo\(^{PA13}\) has 558-bp insertion at 5\(^{\prime}\) UTR. Lower plot: quantitative RT–PCR analysis of relative pelo expression in ovaries of the indicated genotypes using two different sets of primers. Values are means ± SEM, n = 3.

HeT-A mRNA is further up-regulated in peloPA13 ovaries under a high-temperature condition.

A Quantitative RT–PCR analysis to detect the relative amount of transposon mRNAs from ovaries of peloPA13+/− and peloPA13−/− under a normal (25°C) condition or a high-temperature (29°C) condition. Values are means ± SEM, n = 3.

B Morphology of ovaries from indicated genotypes under 25°C (upper panel) or 29°C (lower panel) conditions. Scale bar represents 500 μm.
RNA profiles in wild-type and pelo mutant ovaries.

A. Reads analysis of total small RNAs from pelo^{1/+/P860} and pelo^{1/+} ovaries.

B. Small RNA size profiles correspond to genome-matching reads after excluding rRNA, tRNA, and snoRNA.

C. Quantitative RT–PCR analysis of the expression of a set of housekeeping genes in pelo mutant ovaries. Values are means ± SEM, n = 3. *P < 0.05, t-test.
Figure EV4. Pelo-mediated TE silencing in Ago3 or Aub mutant ovaries.

A–C Quantitative RT–PCR to detect the relative amount of transposon mRNAs from ovaries of the indicated genotypes. All relative fold changes were compared to the heterozygous controls. Pelo depletion further enhances TE up-regulation in Ago3 mutant ovaries (A), Aub RNAi ovaries (B) and Ago3 and Dcr2 double-mutant ovaries (C). Values are means ± SEM, n = 4. *P < 0.05, **P < 0.001, ***P < 0.0001, t-test.