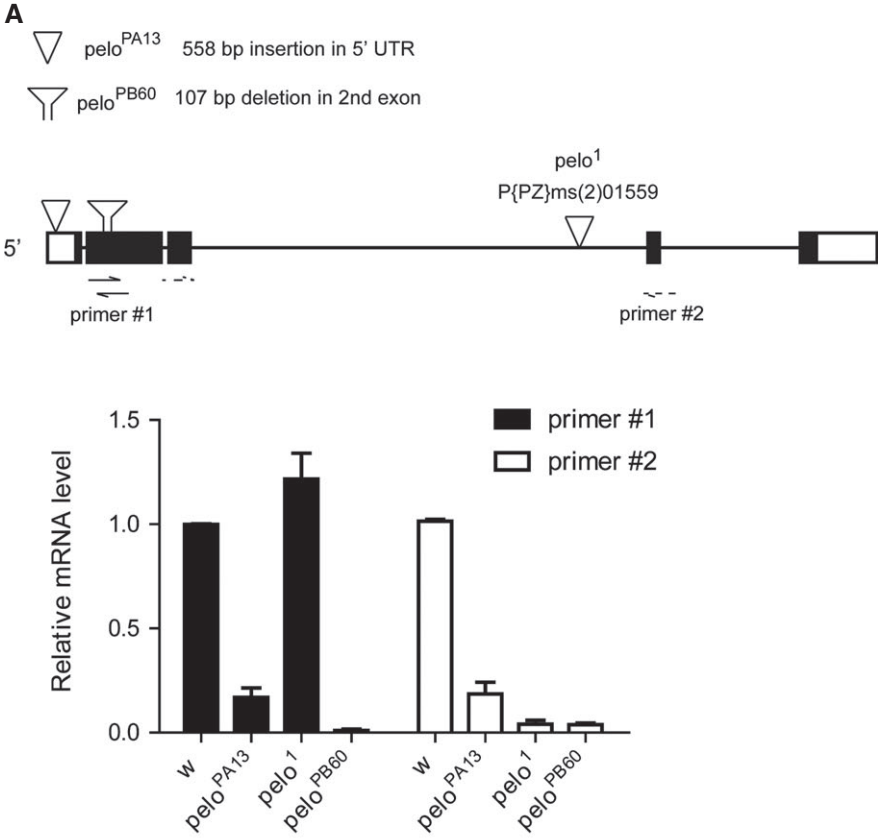
Expanded View Figures



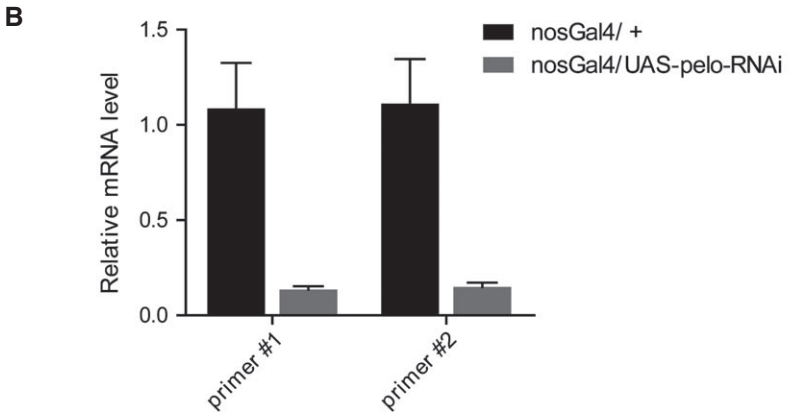


Figure EV1. The molecular lesions and transcripts expression analysis of *pelo* alleles.

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 3rd 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′ 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, $n = 3$.

B Quantitative RT–PCR analysis to measure *pelo-RNAi* efficiency. Germline knockdown of *pelo* by nos-Gal4; UAS-pelo-RNAi caused approximately 90% reduction of *pelo* expression in testes. Values are means ± SEM, $n = 4$.

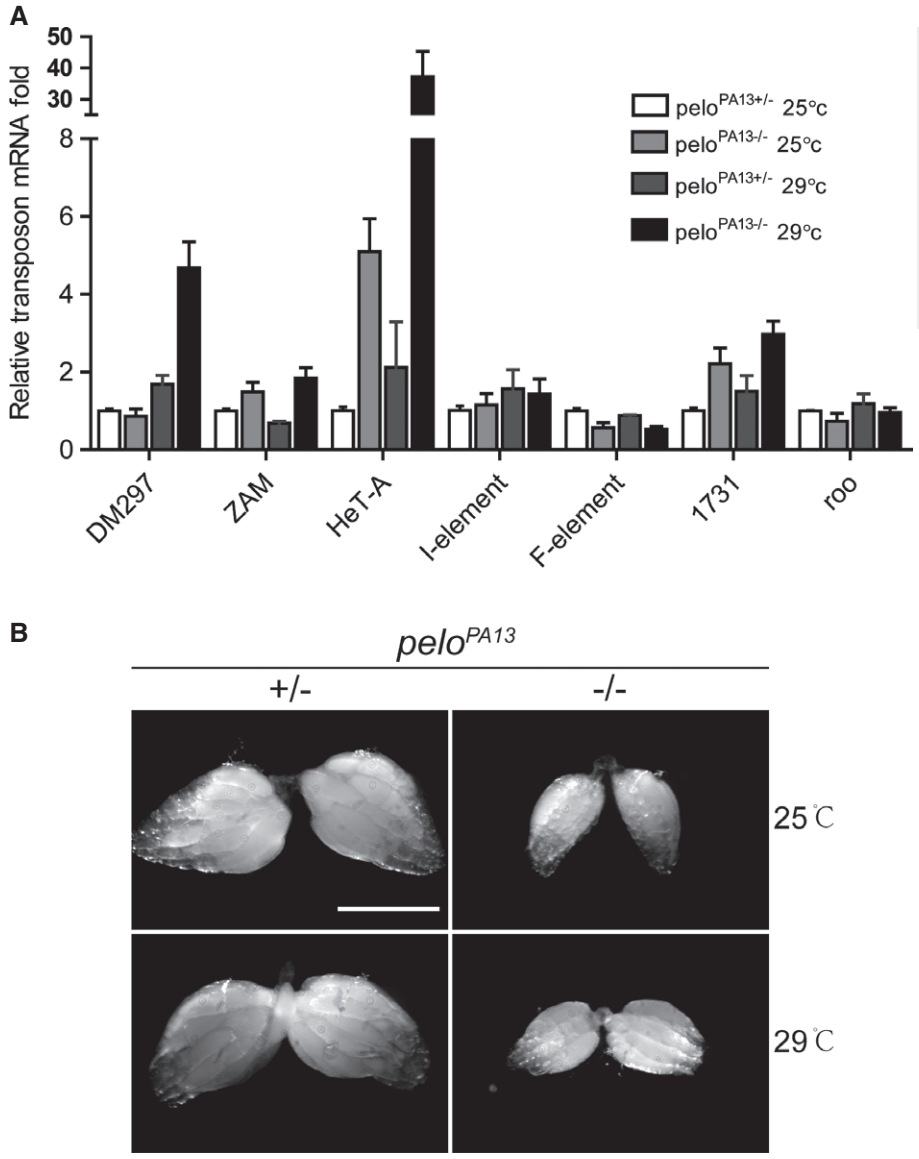


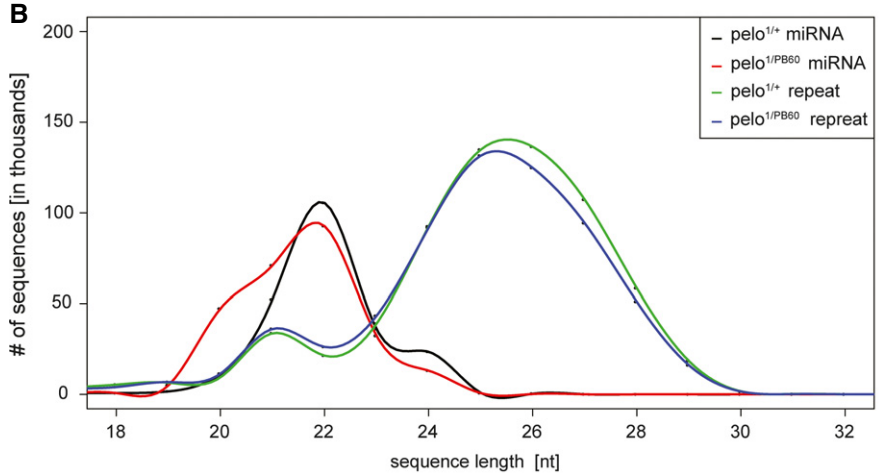
Figure EV2. *HeT-A* mRNA is further up-regulated in *pelo*^{PA13} ovaries under a high-temperature condition.

A Quantitative RT–PCR analysis to detect the relative amount of transposon mRNAs from ovaries of *pelo*$^{PA13+/-}$ and *pelo*$^{PA13-/-}$ 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.

A

	pelo $^{1/+}$	*pelo* $^{1/PB60}$
total reads	12783893	12677869
% of reads matching the genome 100%	12035410	12289972
# of reads mapping to rRNA, tRNA, snoRNA	1396993	2291858
# of analyzed reads	10638417	9998114
repeats	59.93%	59.27%
miRNA	19.69%	20.40%
genome	20.37%	20.33%



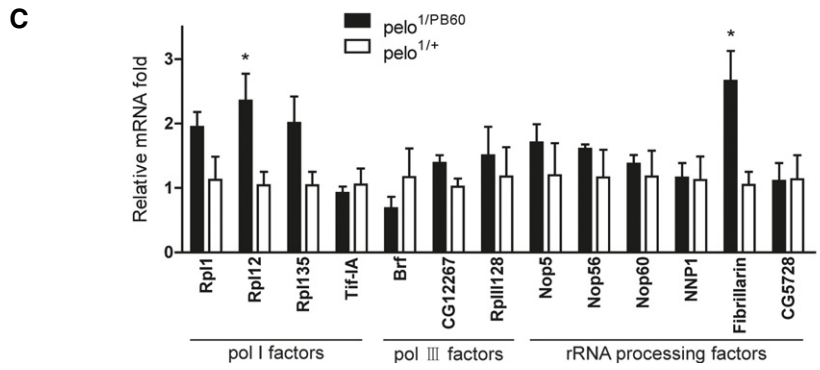


Figure EV3. RNA profiles in wild-type and *pelo* mutant ovaries.

A Reads analysis of total small RNAs from *pelo*$^{1/PB60}$ 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.

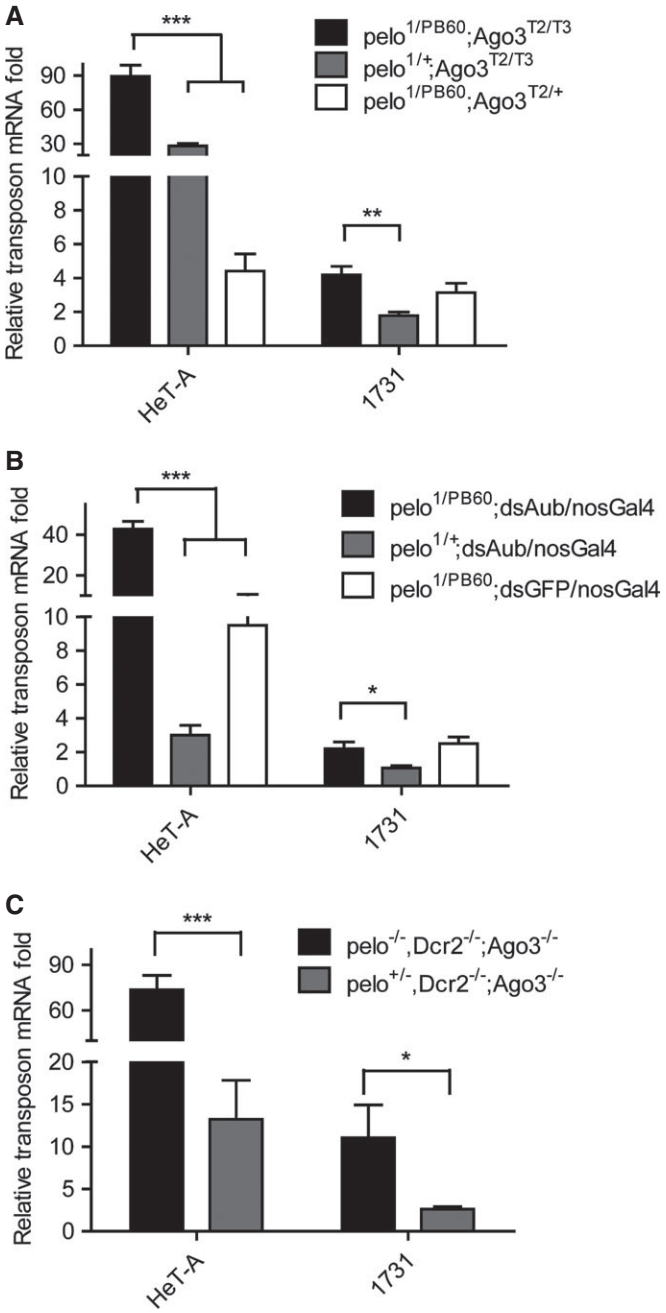


Figure EV4. Pelo-mediated TE silencing in *Ago3* or *Aub* mutant ovaries.

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.