Supplementary Information

Supplementary Figure 1. Gel source images.

Supplementary Table 1. Euchromatic *P*-element insertions in *Harwich* strain, as determined by DNA-seq analysis.

Supplementary Table 2. Description of *Harwich*-derived stocks.

Supplementary Table 3. List of crosses.

Supplementary Table 4. RNA FISH probes.

Supplementary Table 5. List of primers.

Supplementary Note 1

In contrast to Gypsy, for which splicing changes are accompanied with mRNA accumulation changes in piRNA mutants (Figure 4, Extended data Figure 7), *P*-element mRNA steady-state levels under dysgenic conditions or in piRNA pathway mutants in the Harwich background are not up-regulated despite loss of K9me3 marks (Figure 2-3; Extended data Figure 2,4, and 6).We interpret this result as consistent with the lack of sufficient promoter and enhancer activity of the endogenous *P*-elements and with the fact that the expression of cellular genes containing *P*-element insertions is invariable regardless to the methylation status of the resident element (Figure 3e-f, Extended data Figure 4e-g and 6a). Overall, the IVS3-*lacZ-neo* reporter, which is under the control of the HSP83 promoter, behaves similarly to the endogenous *P*-element insertions (i.e. changes are restricted to splicing regulation). Total transcript steady-state levels show a slight change in dysgenic crosses, as well as *aub* and *vas* mutants, with large changes being restricted to IVS3 splicing (Extended data Figure 3). In relation to the Panx/Silencio

mutant analyses, we observed that the effect on RNA levels and splicing was dis-proportionally affected, with RNA levels changing ~10-fold and splicing level changes of ~100-fold (Extended data Figure 3b). These data strongly suggest that the piRNA pathway mediates chromatin-dependent regulation of alternative splicing and that is largely independent of its previously described effects on the regulation of RNA transcription and RNA stability.