**Supplementary Figure Legends**

**Figure S1. Trans-silencing of lacZ by P{lArB} derived apiRNAs.**  (A) Ovaries of strain P1152, which carries the P{lArB} insertion in TAS. (B) Ovaries of strain BC69 show lacZ expression from a euchromatic transgene. (C) Trans-silencing of lacZ expression in F1 ovaries of a cross between P1152 females and BC69 males. Note the slightly different levels of repression within different cells of the same ovary.

**Figure S2. RT-PCR analysis of *rosy* transcripts.** Reverse transcription with primer specific to sense strand of *rosy* transcripts was performed on total RNA from ovaries of strain P-1152 (TAS-inserted transgene), BC69 (same transgene inserted into euchromatin) or Oregon-R (wild type). (A) Position of PCR primers flanking 3rd and 2nd introns of *rosy* transcript. (B) Presence of longer PCR product indicates accumulation of non-spliced *rosy* transcripts in ovaries of P-1152, but not BC69 and Oregon flies.

**Figure S3. Features of apiRNAs in mouse.** (A) The 1U bias of apiRNAs mapping to the insertion cassette (cass) is compared to native piRNAs from another cluster on chr9. Sequences are derived from total RNA, MILI and MIWI immunoprecipitations (indicated). (B) Size distributions of native piRNAs mapping to a cluster on chr9 (upper panel) compared to apiRNAs mapping to the insertion cassette (lower panel). Sequences are derived from total RNA, MILI and MIWI immunoprecipitations (indicated).

**Figure S4. piRNA profiles over wild-type and transgenic piRNA clusters in flies and mice** (A) Read densities of piRNAs bound to MIWI are plotted along the cluster on chr 17 on the plus and minus strand (indicated). The site of the GFP cassette insertion is indicated with an asterix. (B) Read densities of piRNAs bound to MILI are plotted along the cluster on chr 17 on the plus and minus strand (indicated). (C) Read densities of piRNAs from total RNA are plotted along *flamenco* on the plus strand. The portion of the cluster contained in the BAC is indicated as ‘transgenic’.

**Figure S5. apiRNAs in mouse are preferentially bound by MILI.** (A) Read counts of apiRNAs bound to MILI are plotted along the inserted GFP sequence on the plus and minus strand (indicated). (B) Read counts of apiRNAs bound to MIWI are plotted along the inserted GFP sequence on the plus and minus strand (indicated). (C) Read counts of apiRNAs from total RNA are plotted along the inserted GFP sequence on the plus and minus strand (indicated).