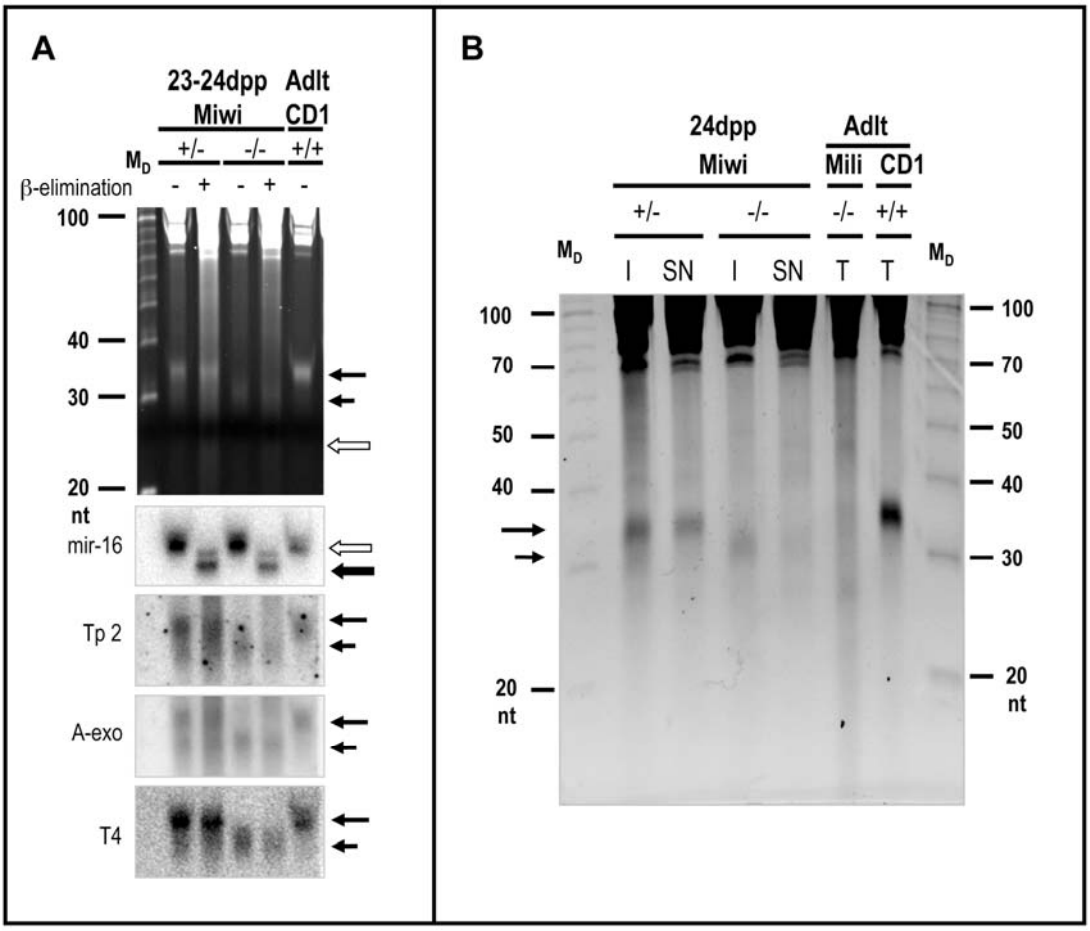

Supplementary information, Figure S2 piRNAs observed in the Miwi-/- testis are intact MILI-associated piRNAs.

A, β-elimination reaction shows that terminal nucleotides of MILI-associated piRNAs retain their 2`-O-methyl group in the absence of MIWI. 40μg total RNA samples from 23-24dpp Miwi+/- and Miwi-/- testes were either subjected to β-elimination reaction or not, and resolved with 20% Urea-PAGE. miRNA mir-16 was used as an internal loading control and positive control for the β-elimination reaction. Ethidium bromide staining and northern blotting were used to assess the piRNAs at the global and individual levels respectively. Tp2: Transposonic 2, A-exo: Anti-sense exonic, T4: piRNA-T4, mir-16: miRNA-16. The annotations indicate the genomic regions from which piRNAs are derived (e.g; "anti-sense exonic" is a piRNA that corresponds to the antisense strand of an exon). Long and short arrows denote piRNAs of MIWI and MILI respectively. Blank arrow indicates the location of mir-16. β-elimination caused mir-16 to shorten by one nucleotide, with β-eliminated mir-16 indicated by a thick black arrow. In contrast, no β-eliminatted form of Tp2, A-exo, and T4 piRNA was detected, indicating that the 3' end of these piRNAs was modified. B, The small RNAs observed in Miwi-/- testes were depleted upon immunoprecipitation of MILI, which confirms that the observed small RNAs are MILI-associated piRNAs. Shown is the negative image of the ethidium bromide stained samples on a 15% Urea-PAG. I: Input, SN: Supernatant, T: 70μg Total testicular RNA, Adlt: Adult. The immunoprecipitates (IPs) were analyzed in Figure 3C. Adult Mili-/- testis was used as the negative control for MIWI-associated piRNAs as well as MILI-associated piRNAs since the spermatogenic arrest occurs before MIWI expression starts.