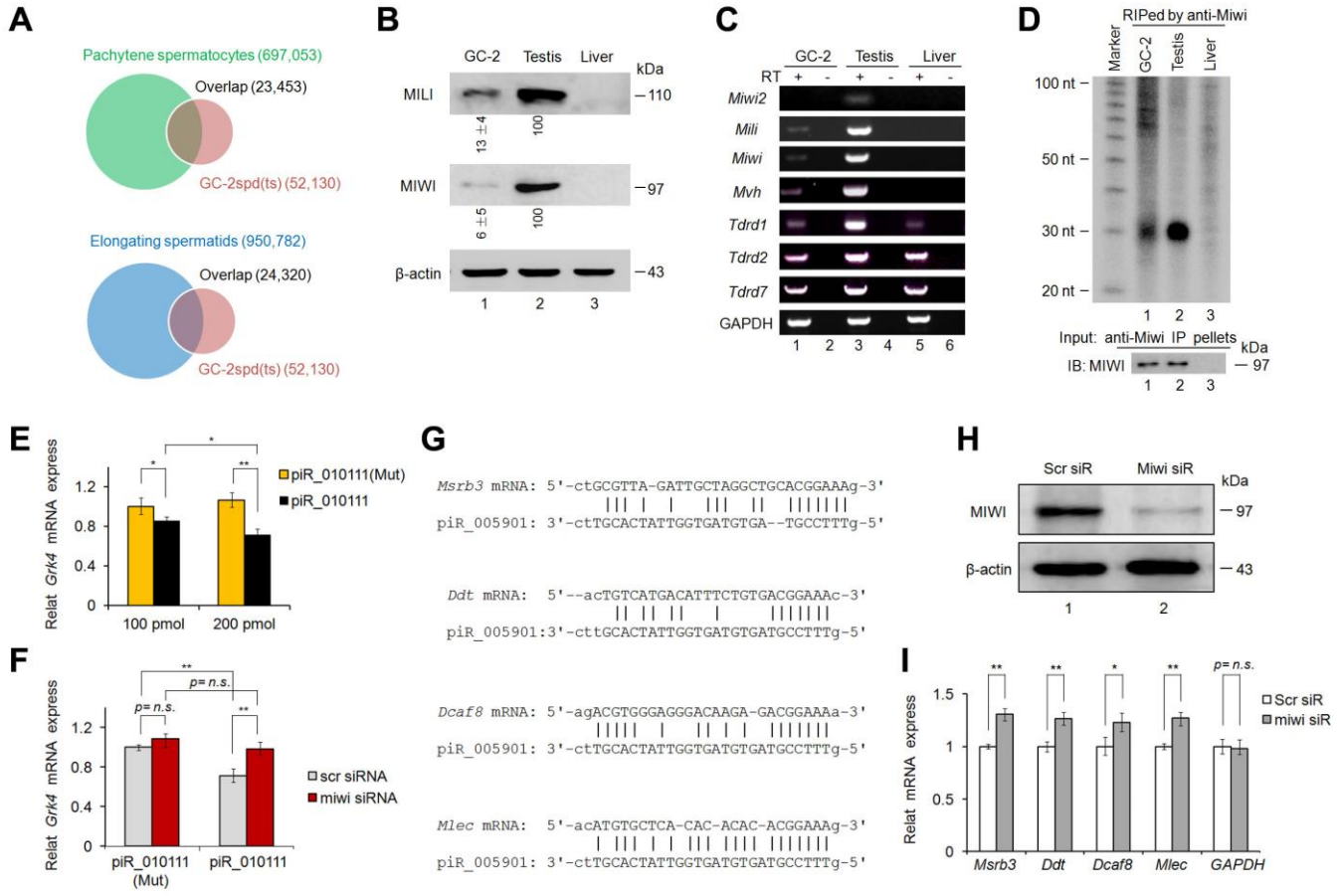


**Supplementary information, Figure S2** Characterzation of piRNA pathway components of GC-2-spd(ts) cells.

**(A)** Venn diagram showing the cross of piRNA reads commonly detected in GC-2spd(ts) cells and pachytene spermatocytes (top) and piRNAs commonly in GC-2spd(ts) and elongating spermatids (bottom). Our deep sequencing on small RNAs identified a total of 52,130 piRNA reads in GC-2spd(ts) cells, of which ~45% (23,453 piRNA reads) and ~46% (24,320 piRNA reads) are respectively detected in mouse pachytene spermatocytes (compared to the piRNA profile in pachytene spermatocytes reported by Gan et al., *RNA*, 2011) and elongating spermatids (compared to our piRNA profile in elongating spermatids, see Table S5 blow), indicating that some pachytene piRNAs are expressed in GC-2spd(ts) cells. Nevertheless, the piRNAs that we previously selected for reporter assays (such as piR_010111, piR_013474, piR_027161, piR_035327, and etc), appeared to be absent in GC-2spd(ts) cells.

**(B)** Western blot assays of MIWI and MILI proteins in GC-2spd(ts) cells (lane 1), adult mouse testes (lane 2) and livers (lane 3), with β-actin served as a loading control. The relative expression of MIWI and MILI proteins in GC-2spd(ts) cells in comparison with those in testes is indicated in parentheses (the one in mouse testes is set as 100%). Error bar is given as the SD of three independent experiments.

**(C)** Semi-quantitative RT-PCR assays of the transcripts of genes that are involved in piRNA pathways,

including *Miwi2*, *Mili*, *Miwi*, *Mvh*, *Tdrd1*, *Tdrd2*, and *Tdrd7* in GC-2spd(ts) cells, with *GAPDH* served as an internal control.

**(D)** RNA co-IP assays of MIWI associated-piRNAs (top) in GC-2spd(ts) cells (lane 1), adult mouse testes (lane 2) and livers (lane 3), with anti-MIWI IB as loading references(bottom).

**(E)** qRT-PCR assays of the effect of piR_010111 on *Grk4* mRNA level in GC-2spd(ts) cells. Total RNAs for qRT-PCR were extracted from GC-2spd(ts) cells transfected with 100 pmol or 200 pmol of piR_010111. piR_010111(Mut) transfection served as a negative control.

**(F)** qRT-PCR assays of the effect of *Miwi* knockdown on *Grk4* mRNA level in piR_010111 or piR_010111(Mut)-transfected GC-2spd(ts) cells, with the non-target GAPDH transcript serving as an internal control. **P*<0.05, ***P*<0.01. Results shown are representative of three independent experiments.

**(G)** Predicted piR_005901 regulatory elements at 3′UTRs of *Msrb3*, *Ddt*, *Dcaf8* and *Mlec*.

**(H)** Western bolt assays of MIWI protein in GC-2spd(ts) cells transfected with Scrambled siRNA (lane 1) or *Miwi* siRNA (lane 2).

**(I)** qRT-PCR assays of the effect of *Miwi* knockdown on the expression of 4 predicted target genes, with the non-target *GAPDH* transcript serving as an internal control. **P*<0.05, ***P*<0.01. Results shown are representative of three independent experiments.