



Supplementary information, Figure S4 MIWI, but not MILI or Ago2, is required for piRNA-mediated target repression.

(A) Knockdown of *Miwi* attenuated the effect of piRNAs on repression of their target reporters in GC-2-spd(ts) cells. Left, dual-luciferase reporter assays of the effect of piR_013474 on *Sox6* (1st panel, red), piR_027161 on *Cacna1h* (2nd panel, blue), piR_013474 on *Zkscan17* (3rd panel, orange), and piR_035327 on *Cnot1* (4th panel, purple) reporter activities. Middle, PAT assays. Right, western blot assays of MIWI proteins, with β -actin serving as a loading control.

(B) Neither knockdown of *Mili* or *Ago2* altered the effect of piRNAs on repression of their target reporters in GC-2-spd(ts) cells. Left, dual-luciferase reporter assays. The respective treatments were indicated in parentheses. Right, western blot assays of MILI and AGO2 proteins, with β -actin serving as a loading control.

(C) Northern blotting assays of anti-Flag co-IPed RNAs for piR_010111 (lanes 2-5), piR_010111(Mut) (lanes 7-10), or Scr siR (lanes 12-15). GC-2spd(ts) cells were co-transfected with indicated Flag-tagged proteins and piR_010111 (left), or piR_010111(Mut) (middle), or Scr siR (right), respectively. Total RNAs extracted from the respective small RNA-transfected cells used as positive controls (lanes 1, 6, and 11). The results showed that both chemically synthesized 30-nt piR_010111 and piR_010111(Mut) were significantly loaded onto co-transfected Flag-HIWI and Flag-MIWI (lanes 2, 3, 7 and 8) but little onto Flag-MILI (lanes 4 and 9) and Flag-Ago2 (lanes 5 and 10). In sharp contrast, the 19-nt scrambled siRNA (Scr siR) was specifically associated with Flag-Ago2 (lane 15) but not Flag-Piwi proteins (lanes 12-14).

(D) Ectopic Flag-MIWI promoted the effect of piRNAs on repression of their target reporters in GC-2-spd(ts) cells. Left, dual-luciferase reporter assays. Middle, PAT assays. Right, western blot assays of MIWI proteins, with β -actin serving as a loading control.

(E) Northern blotting assays of anti-Flag co-IPed RNAs for piR_010111. GC-2spd(ts) cells were co-transfected with piR_010111 and indicated Flag-tagged Piwi proteins, respectively. Total RNAs extracted from piR_010111-transfected cells used as positive controls (lane 1). The results showed that piR_010111 was significantly loaded onto co-transfected Flag-tagged MIWI (lane 2), HIWI (lane 3) and HIWI ADH mutant (lane 4), but not HIWI Y345/346A mutant (lane 5).