



Supplementary information, Figure S5 CAF1 plays a critical role in MIWI/piRNA-mediated target repression.

(A) Co-IP assays of the interaction of Myc-CAF1 and Flag-MIWI in GC-2-spd(ts) cells (lane 1), or in Scr siR- (lane 2) or mouse testicular piRNA-cotransfected GC-2-spd(ts) cells (lane 3).

(B) Co-IP assays of the interaction of Myc-CAF1 and Flag-MILI in GC-2-spd(ts) cells. Top, anti-Myc IP pellets immunoblotted by anti-Flag and anti-Myc antibodies, respectively. Bottom, cell lysates immunoblotted by anti-Flag or anti-Myc antibodies, with β -actin serving as a loading control.

- (C)** Knockdown of *Caf1* 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* (left top, red), piR_027161 on *Cacnalh* (left bottom, blue), piR_013474 on *Zkscan17* (right top, orange), and piR_035327 on *Cnot1* (right bottom, purple) reporter activities. Middle, PAT assays. Right, western blot assays of CAF1 proteins, with β -actin serving as a loading control.
- (D)** Ectopic Myc-CAF1 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 CAF1 proteins, with β -actin serving as a loading control.
- (E)** Knockdown of *Cnot1* (Top) or *Cnot6* (bottom) marginally changed the effect of piR_010111 on repression of *Grk4* reporter in GC-2-spd(ts) cells. Left, dual-luciferase reporter assays. Middle, PAT assays. Right, western blot assays of CNOT1 or CNOT6 proteins, with β -actin serving as a loading control.
- (F)** Western blot assays of the expression of CNOT1, CNOT6 and CAF1 in adult mouse tissues, with GAPDH serving as a loading control.