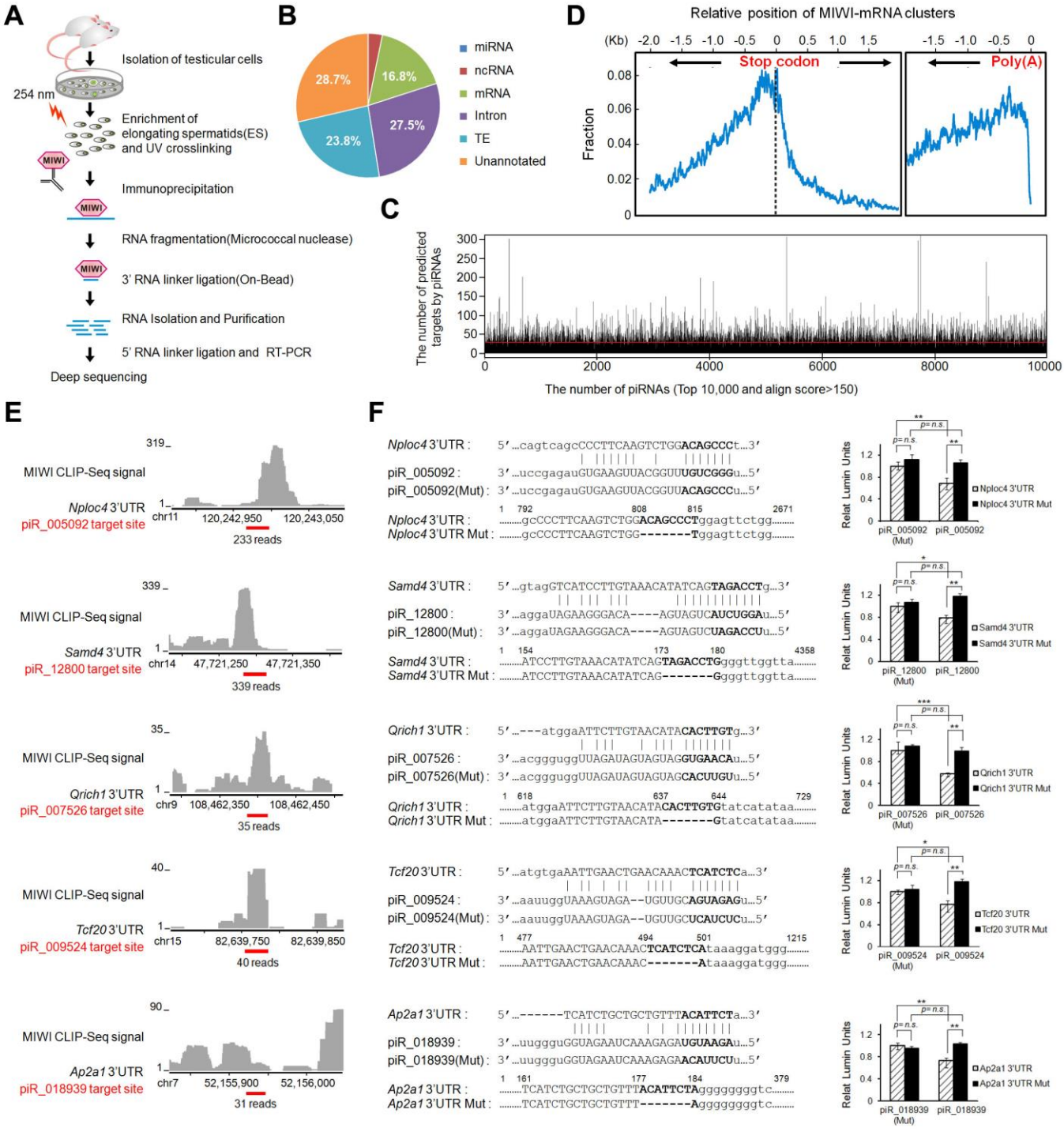

Supplementary information, Figure S3 Verification of piRNA targets by MIWI CLIP-seq.

(A) Strategy of MIWI CLIP-seq in elongating spermatids.

(B) Genome annotation for MIWI CLIPed RNAs >33 nt.

(C) The number of predicted targets for each top 10,000 MIWI-associated piRNAs. X axis shows individual MIWI-CLIPed 9,975 piRNAs containing in predicted mRNA:piRNA interactions. Y axis represents the predicted target numbers for each MIWI-CLIPed piRNA, with miRanda alignscore >150. This resulted in a predicted set of 287,355 piRNA:mRNA interactions that involve 9,975 piRNAs identified in the MIWI complex, with each piRNA having the potential to target ~28.8 mRNAs on average.

(D) The distribution of MIWI-binding tags on target mRNAs.

(E) The clusters formed by MIWI-mRNA CLIP tags in each of the 5 genes (i.e. *Nploc4*, *Samd4*, *Qrich1*, *Tcf20*, and *Ap2a1*) non-responsive to the selected piRNAs in previous reporter assays. The red thick lines (bottom) indicated the predicted piRNA target sites. The scales in genes were numbered following the dimension of their respective chromosomes. The indicated numbers at top left or under red thick lines respectively represent the highest reads of CLIPed mRNA tags for cognate genes or the reads of MIWI-mRNA CLIP tags that perfectly matched to predicted target sites.

(F) Validation of predicted piRNA:mRNA interaction by reporter assays in GC-2spd(ts) cells. Left, predicted piRNA regulatory elements at 3′UTRs of *Nploc4*, *Samd4*, *Qrich1*, *Tcf20*, and *Ap2a1*, and the sequences of synthetic piRNAs and their mutated forms (piR_xx (Mut)), wild-type (3′UTR) and mutated 3′UTR-*Renilla* luciferase reporters (3′UTR Mut). Right, dual-luciferase reporter assays of the effect of piRNAs on reporter activities in GC-2spd(ts) cells, with cognate piRNA(Mut) serving as negative controls.