sample	Sequencer	Total useful reads*	rRNAs sense	rRNAs anti- sense	tRNAs sense	tRNAs anti- sense	snRNAs sense	snRNAs anti- sense	Mitochond rial genome	Used for analysis	Data used for Fig.
Twilp-IP	GAII	34959335	1133041	5622	771751	1473	13178	159	69643	32964468	1
TWI1_KO_3h	GAII	24967491	915849	1947	749782	836	4306	145	151125	23143501	2,3
HA-Twilp IP	HiSeq2000	41322677	32721	2400	27154	1324	475	198	1359	41260968	2F
HA-Twilp- hAGO2Lmut IP	HiSeq2000	39352231	215056	2819	27318	1083	483	163	2022	39107352	2F

\*longer than 18 nt, did not match to the mixed phiX174 phage DNA, and no non-ACGT bases in the first 25 nt

## Table S1. Basic analysis of sequence reads from small RNA cDNA libraries



Supplementary Figure S1. Treatment of total RNA with the terminator 5'-monophosphate specific exonuclease.

Total RNA from the wild-type cells at 4 h or 8 h post-mixing was combined with a 21-nt oligoribonucleotide possessing a 5'-hydroxyl and was incubated with (+) or without (-) Terminator Exonuclease. Positions of the scnRNA, the 5S rRNA (possessing a 5'-triphosphate), and the 21-nt oligoribonucleotide are marked with an arrow, a single arrowhead, and double arrowheads, respectively.



## Supplementary Figure S2. Comparison of HA-Twi1p-associated and HA-Twi1p-hAGO2Lmut-associated small RNAs

(A, B) Size distribution of the HA-Twi1p-associated (A) and Twi1p-hAGO2Lmut-associated (B) small RNAs. The number of sequenced small RNAs (reads per million sequences (RPM)) of each size is shown.
(C, D) Base composition of the HA-Twi1p-associated (C) and Twi1p-hAGO2Lmut-associated (C) 29-nt RNAs.