SUPPLEMENTARY INFORMATION

Supplementary Figure Legends

Figure S1. A schematic representation of specific quantifications of 5′-tRNA half and td-piRNA by TaqMan qRT-PCR

Figure S2. Variant sequences of *Bombyx* cyto tRNA^{AspGUC} (A), tRNA^{HisGUG} (B), tRNA^{GluUUC} (C), and tRNA^{GluCUC} (D).

Indicated variant sequences of *Bombyx* cyto tRNAs were identified using tRNAscan program (50) and aligned using the CLUSTALW algorithm. The sequences were sorted by the number of genome loci and mismatches; pink characters show minor sequences among variants.

Figure S3. BmThg1l amino acid sequences

Sequences of BmThg1l and its homologs were aligned using Clustal X (version 2.1). BmThg1l was aligned with its counterparts from *D. melanogaster* (NP_609737.1), *H. sapiens* (NP_060342.2), *M. musculus* (NP_001074438.1), *X. tropicalis* (XP_002935942.2), and *D. rerio* (NP_001007456.1). The Thg1 and Thg1C domains of BmThg1l, as defined by the Pfam database (http://pfam.sanger.ac.uk/), are indicated.

Figure S4. BmNSun2 amino acid sequences

BmNSun2 was aligned with its counterparts from *D. melanogaster* (NP_652007.1), *H. sapiens* (NP_060225.4), *M. musculus* (NP_663329.3), *X. tropicalis* (NP_001015962.1), and *D. rerio* (NP_956005.1). The Nol1_Nop2_Fmu domain of BmNSun2 as defined by the Pfam database is indicated.

Figure S5. Analyses of td-piR$^{GluUUC/CUC}$

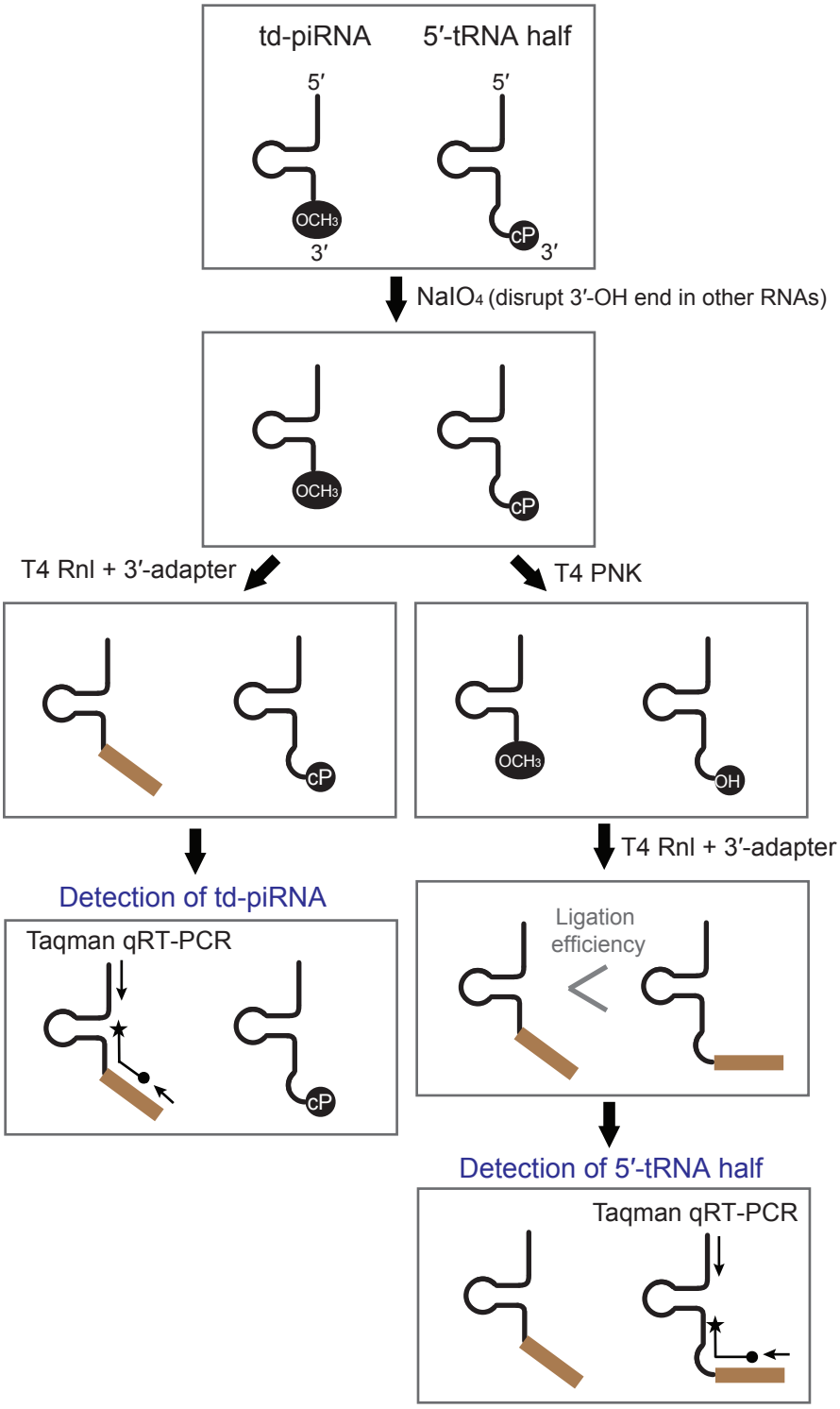
(A) The 5′-terminal position of Siwi-bound td-piR$^{GluUUC/CUC}$ in the mature tRNA.

(B) The regions from which td-piR$^{GluUUC/CUC}$, starting from np 1, were derived are shown in black in the cloverleaf secondary structure of *Bombyx* cyto tRNA^{GluUUC}-V1 (**Supplementary Fig. S2**). Non-piRNA-derived regions are shown in gray.

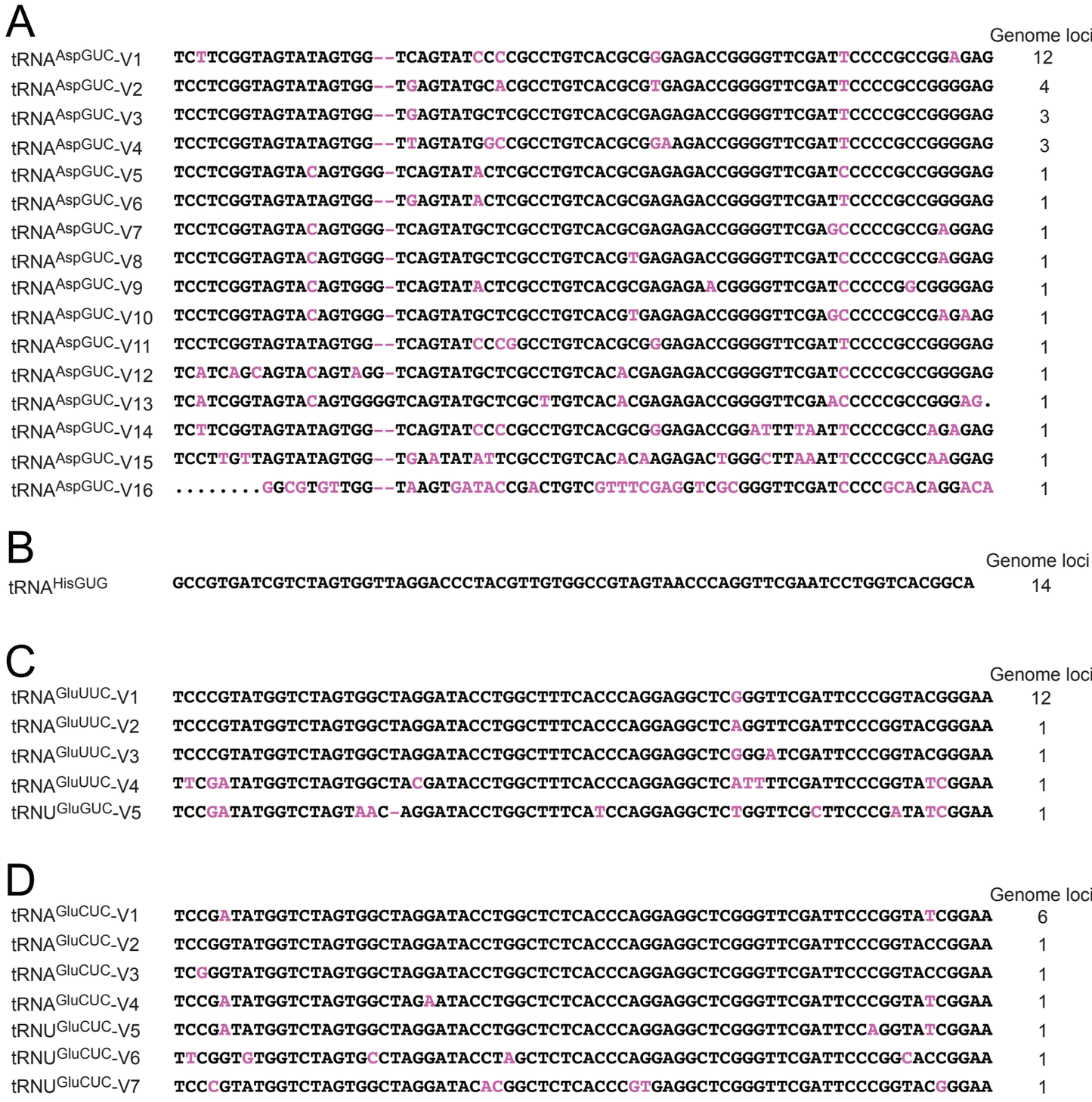
(C) Total RNA from Rluc- or BmNsun2-depleted cells was subjected to Northern blot targeting the 5′-part of mature cyto tRNA^{GluUUC}. 5′-half and td-piRNA, as well as mature tRNA, were all detected. The Northern blot bands were quantified and shown as relative abundance in the right graph. Abundances in Rluc-depleted cells were set as 1, and the averages of three independent experiments with bars showing the SD are shown.

Figure S6. Northern blot detection of mature tRNAs and 5′-tRNA halves

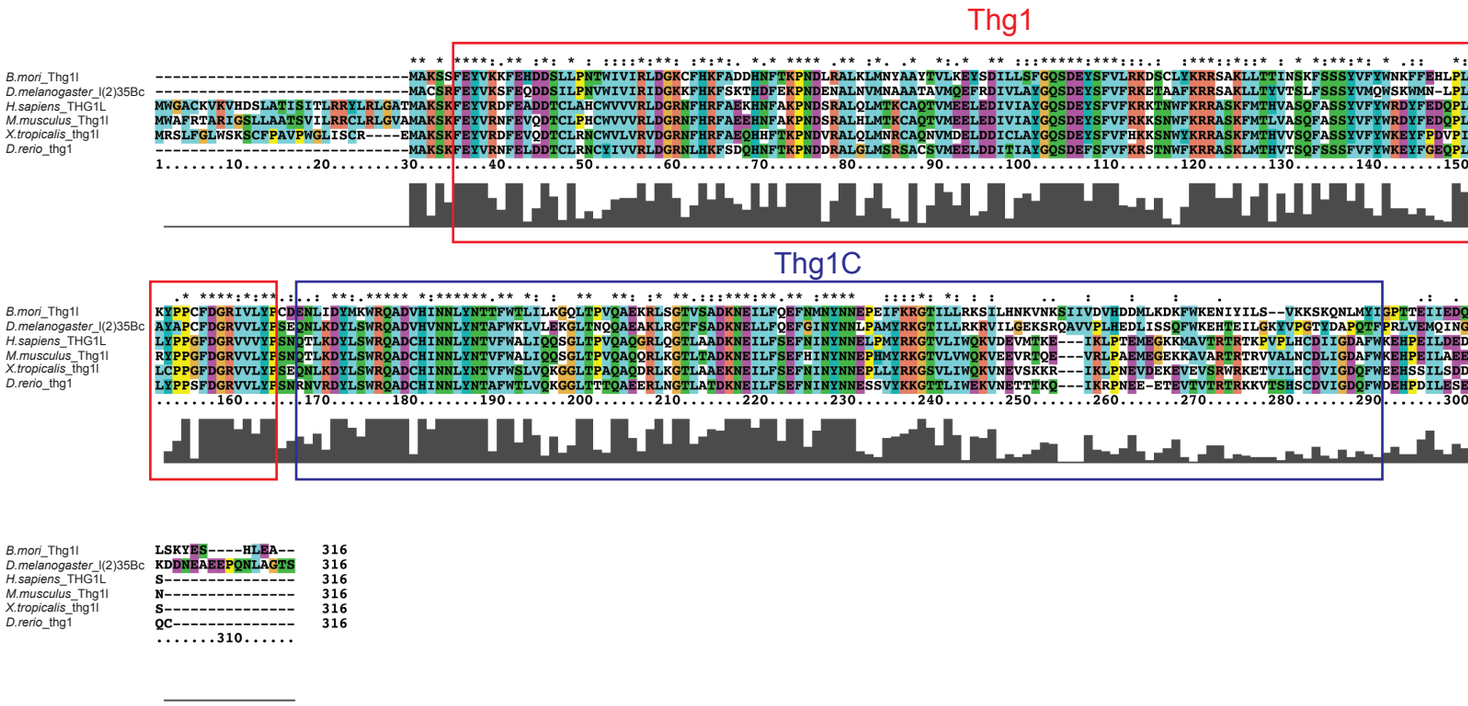
BmN4 total RNA was subjected to Northern blots targeting 5′-part of the indicated cyto tRNAs. Detected 5′-halves^{LysCUU} are indicated by a black line.



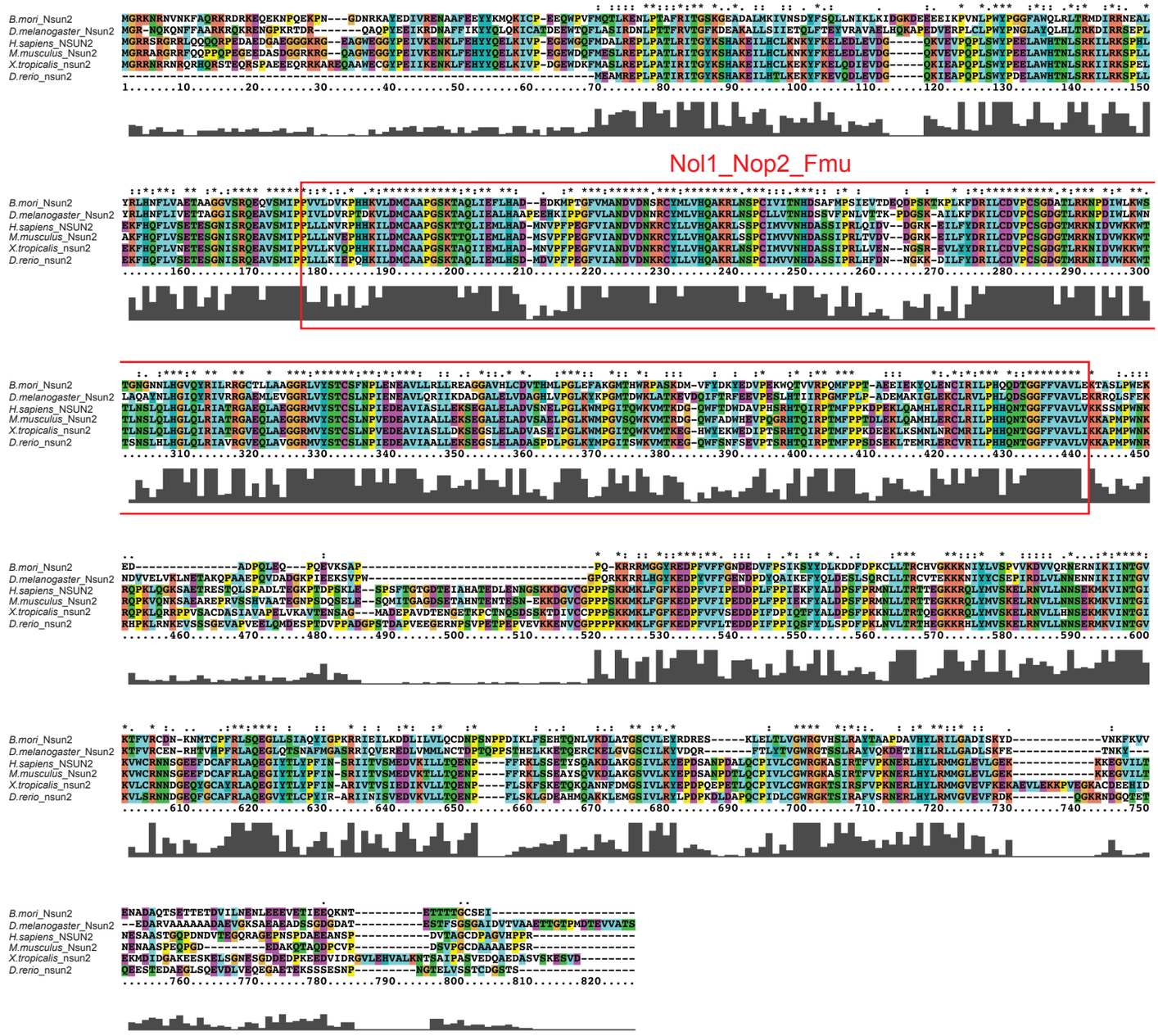
Honda et al. Figure S1



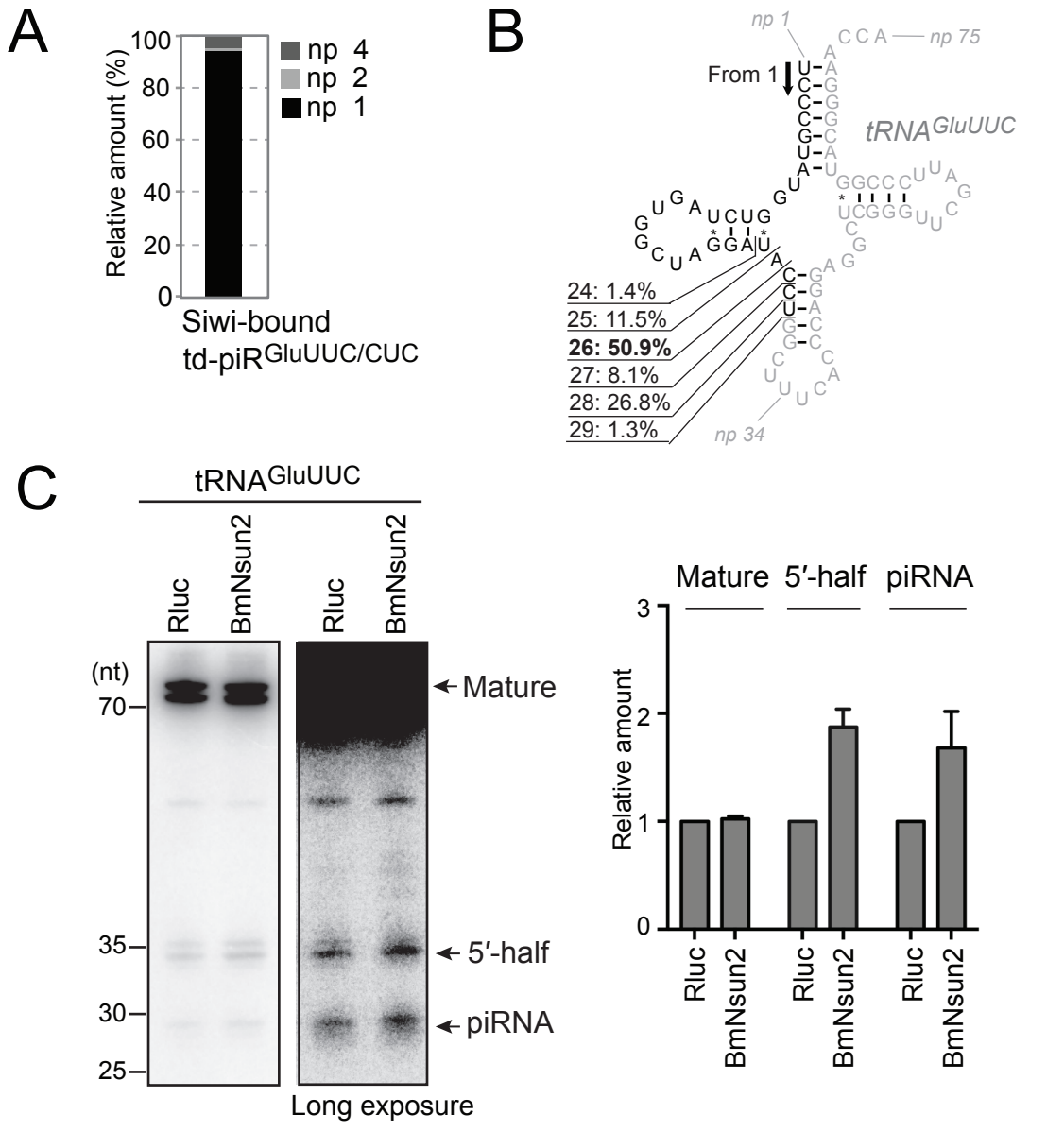
Honda et al. Figure S2



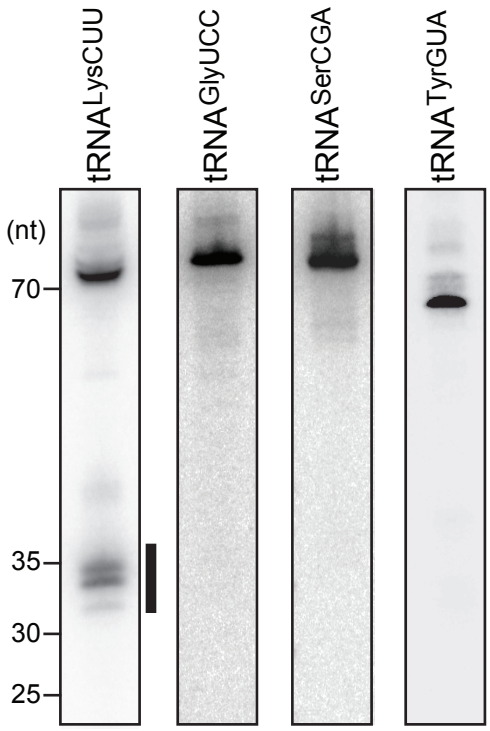
Honda et al. Figure S3



Honda et al. Figure S4



Honda et al. Figure S5



Honda et al. Figure S6

Table S1. Sequences of primers and TaqMan probes for quantification of 5′-tRNA halves and td-piRNAs by TaqMan qRT-PCR

Target	Primer/ probe	Sequence (5′–3′)
5′-half^{AspGUC}	Forward primer	CGGGTCTTCGGTAGTATAGT
	Reverse primer	GATCGTCGGACTGTAGAACTC
	TaqMan probe	/56FAM/TATCCCCGC/ZEN/CTGGAACACTGCGTTT/3IABkFQ/
5′-half^{HisGUG}	Forward primer	GCTCGCCGTGATCGTCTAGT
	Reverse primer	GATCGTCGGACTGTAGAACTC
	TaqMan probe	/56-FAM/TAGGACCCT/ZEN/ACGTTGGAACACTGCGTTTGC/3IABkFQ/
td-piR^{AspGUC}	Forward primer	CGGGTCTTCGGTAGTATA
	Reverse primer	GATCGTCGGACTGTAGAACTC
	TaqMan probe	/56-FAM/CAGTATCCC/ZEN/GAACACTGCGTTTGC/3IABkFQ/
td-piR^{HisGUG}	Forward primer	ACATGCCGTGATCGTC
	Reverse primer	GATCGTCGGACTGTAGAACTC
	TaqMan probe	/56-FAM/TTAGGACCG/ZEN/AACACTGCGTTTGC/3IABkFQ/

Table S2. Sequences of probes for Northern blot

Target	Sequence (5′–3′)
5′-half^{AspGUC}	GGGATACTGACCACTATACTACCGAAGA
3′-half^{AspGUG}	GGCGGGGAATCGAACCCCGGTCTCC
5′-half^{HisGUG}	GGGTCCTAACCACTAGACGATCACGGC
3′-half^{HisGUG}	GGATTCGAACCTGGGTTACT
5′-half^{GluUUC}	GGTATCCTAGCCACTAGACCATACG
5′-half^{LysCUU}	GTCATGCTCTACCGACTGAGCTAG
tRNA^{GlyUCC}	GTATGCTGACCATTACACCACCAACGC
tRNA^{SerCGA}	GACGCCTTAACCACTCGGCCACGACTG
tRNA^{TyrGUA}	GCTCTACCAACTGAGCT
piR-1	GTTCGAAACCAATCCGTTAGTTTTTGA
piR-2	GCCGCAGACAGCAAATTCTCATGCTTTT
let-7	GTACTATACAACCTACTACCTCA
5S rRNA	GCTTGACTTCGGTGATCGGACGAGAAC

Locked Nucleic Acid (LNA)-modified probes were used for the detection of 3′-half^{HisGUG} (underlined letters designate LNA).

Table S3. Sequences of primers for the production of DNA templates for *in vitro* dsRNA synthesis

Target	Primer	Sequence (5′–3′)
Rluc (control)	Forward	TAATACGACTCACTATAGGGGTCGGCCATGATTGGGGTGCTTGTTTG
	Reverse	TAATACGACTCACTATAGGGCCCATTTCATCAGGTGCATCTTCTTGC
BmThg1l	Forward	TAATACGACTCACTATAGGGTCGTCATCGTATGTTTTCTACTGG
	Reverse	TAATACGACTCACTATAGGGTTCCCCTCTTGAATATTTCTGG
BmNSun2	Forward	TAATACGACTCACTATAGGGAGGCATTGTATCGGCTTCATAA
	Reverse	TAATACGACTCACTATAGGGACGGTACGTCGCACAATATCC

Table S4. Sequences of primers for mRNA quantification by qRT-PCR

Target	Primer	Sequence (5′–3′)
BmRp49	Forward	GGATCGCTATGACAAACTTAAGAGG
	Reverse	TATGACGGGTCTTCTTGTTGGA
BmThg1l	Forward	AAATGGCAAAGAGTTCCTTCG
	Reverse	TCGTTAGGCTTCGTAAAGTTGTG
BmNsun2	Forward	CTGACGCTCTAATGAAGATTGTGAA
	Reverse	CTCCAGGGTACCAAGGCAAA

Table S5. Sequences of primers for the qRT-PCR using a stem-loop primer

Target	Primer	Sequence (5′–3′)
piR-1	Forward	CCGCTCAAAAACTAACGGATTG
	SL-Reverse	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTTCGA
piR-2	Forward	CGCCAAAAGCATGAGAATTTGC
	SL-Reverse	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCGCAG
let-7	Forward	CGGGTGAGGTAGTAGGTTG
	SL-Reverse	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTACTAT
5S rRNA	Forward	TAATGGTGACCGCCTGGGAACACC
	SL-Reverse	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAGCCA