

## 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<sup>AspGUC</sup> (A), tRNA<sup>HisGUG</sup> (B), tRNA<sup>GluUUC</sup> (C), and tRNA<sup>GluCUC</sup> (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. BmThg11 amino acid sequences**

Sequences of BmThg11 and its homologs were aligned using Clustal X (version 2.1). BmThg11 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 BmThg11, 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<sup>GluUUC/CUC</sup>**

(A) The 5'-terminal position of Siwi-bound td-piR<sup>GluUUC/CUC</sup> in the mature tRNA.

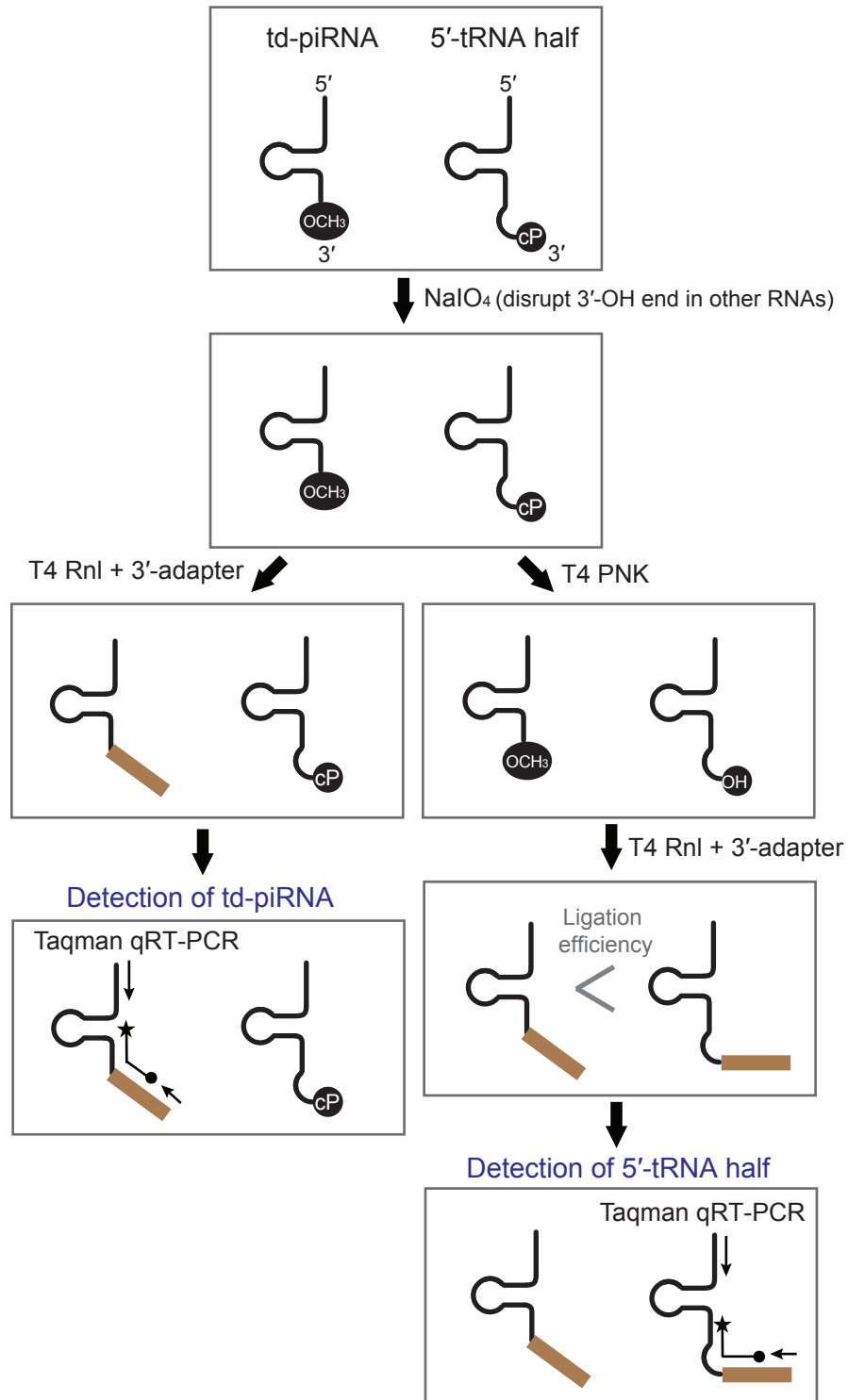
(B) The regions from which td-piR<sup>GluUUC/CUC</sup>, starting from np 1, were derived are shown in black in the cloverleaf secondary structure of *Bombyx cyto* tRNA<sup>GluUUC</sup>-V1 (**Supplementary Fig. S2**). Non-piRNA-derived regions are shown in gray.

(C) Total RNA from Rluc- or BmNsun2-depleted cells was subjected to Northern blot targeting the 5'-part of mature cyto tRNA<sup>GluUUC</sup>. 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<sup>Lys<sup>CUU</sup></sup> are indicated by a black line.



*Honda et al. Figure S1*

# A

		Genome loci
tRNA <sup>Asp</sup> GUC_V1	TC <b>TTCGGTAGTATAGTGG</b> --TCAGTAT <b>CCCGCCTGTCACGCGGAGACCGGGGTTTCGAT</b> TCCCCGCCGG <b>AGAG</b>	12
tRNA <sup>Asp</sup> GUC_V2	TCCTCGGTAGTATAGTGG-- <b>TGAGTATGCACGCCTGTCACGCGTGAGACCGGGGTTTCGAT</b> TCCCCGCCGGGGAG	4
tRNA <sup>Asp</sup> GUC_V3	TCCTCGGTAGTATAGTGG-- <b>TGAGTATGCTCGCCTGTCACGCGAGAGACCGGGGTTTCGAT</b> TCCCCGCCGGGGAG	3
tRNA <sup>Asp</sup> GUC_V4	TCCTCGGTAGTATAGTGG-- <b>TTAGTATGGCCGCCTGTCACGCGAAGACCGGGGTTTCGAT</b> TCCCCGCCGGGGAG	3
tRNA <sup>Asp</sup> GUC_V5	TCCTCGGTAGTACAGTGGG-TCAGTAT <b>ACTCGCCTGTCACGCGAGAGACCGGGGTTTCGAT</b> CCCCGCCGGGGAG	1
tRNA <sup>Asp</sup> GUC_V6	TCCTCGGTAGTATAGTGG-- <b>TGAGTATACTCGCCTGTCACGCGAGAGACCGGGGTTTCGAT</b> TCCCCGCCGGGGAG	1
tRNA <sup>Asp</sup> GUC_V7	TCCTCGGTAGTACAGTGGG-TCAGTATGCTCGCCTGTCACGCGAGAGACCGGGGTT <b>CGAGC</b> CCCCGCC <b>AGGAG</b>	1
tRNA <sup>Asp</sup> GUC_V8	TCCTCGGTAGTACAGTGGG-TCAGTATGCTCGCCTGTCACG <b>TGAGAGACCGGGGTTTCGAT</b> CCCCGCC <b>AGGAG</b>	1
tRNA <sup>Asp</sup> GUC_V9	TCCTCGGTAGTACAGTGGG-TCAGTAT <b>ACTCGCCTGTCACGCGAGAGAACGGGGTTCGAT</b> CCCCG <b>CGGGGAG</b>	1
tRNA <sup>Asp</sup> GUC_V10	TCCTCGGTAGTACAGTGGG-TCAGTATGCTCGCCTGTCACG <b>TGAGAGACCGGGGTTTCGAGC</b> CCCCGCC <b>AGAAG</b>	1
tRNA <sup>Asp</sup> GUC_V11	TCCTCGGTAGTATAGTGG--TCAGTAT <b>CCCGCCTGTCACGCGGAGACCGGGGTTTCGAT</b> TCCCCGCCGGGGAG	1
tRNA <sup>Asp</sup> GUC_V12	<b>TCATCAGCAGTACAGTAGG</b> -TCAGTATGCTCGCCTGTCAC <b>ACGAGAGACCGGGGTTTCGAT</b> CCCCGCCGGGGAG	1
tRNA <sup>Asp</sup> GUC_V13	<b>TCATCGGTAGTACAGTGGGGT</b> CAGTATGCTCGC <b>TTGTACACGAGAGACCGGGGTTTCGAA</b> CCCCGCCGGGGAG.	1
tRNA <sup>Asp</sup> GUC_V14	TC <b>TTCGGTAGTATAGTGG</b> --TCAGTAT <b>CCCGCCTGTCACGCGGAGACCGGATTTTAAT</b> TCCCCGCC <b>AGAGAG</b>	1
tRNA <sup>Asp</sup> GUC_V15	TCCT <b>TGT</b> TTAGTATAGTGG-- <b>TGAATATATT</b> CGCCTGTCAC <b>CAAGAGACTGGGC</b> TT <b>AAAT</b> TCCCCGCC <b>AAGGAG</b>	1
tRNA <sup>Asp</sup> GUC_V16	..... <b>GGCGTGTGG</b> -- <b>TAAGTGATACCGACTGTCGTTTCGAGGTCGCGGGTTCGAT</b> CCCC <b>GCACAGGACA</b>	1

# B

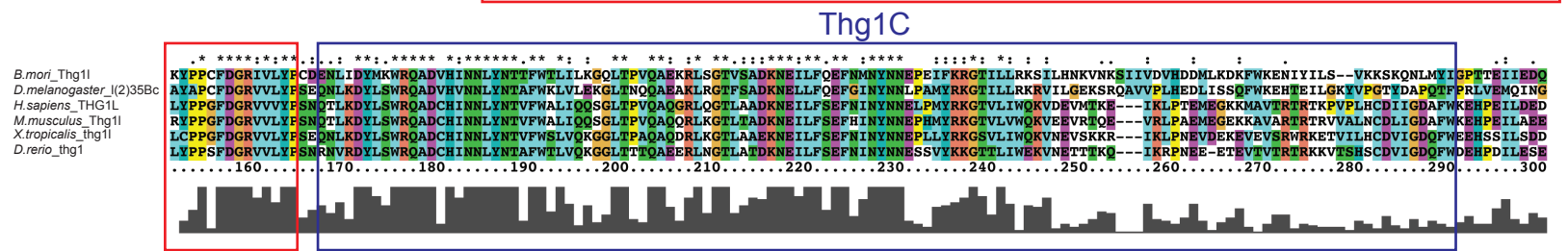
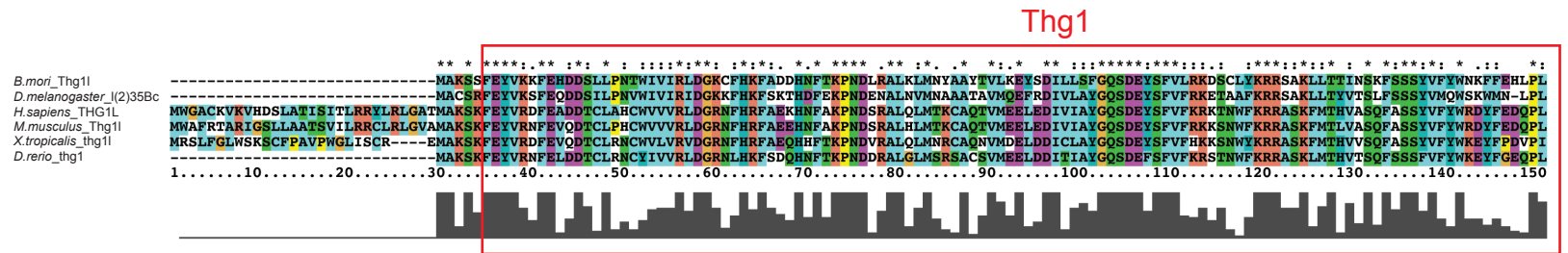
		Genome loci
tRNA <sup>His</sup> GUG	<b>GCCGTGATCGTCTAGTGGTTAGGACCCTACGTTGTGGCCGTAGTA</b> AACCAGGTT <b>CGAATCCTGGTCACGGCA</b>	14

# C

		Genome loci
tRNA <sup>Glu</sup> UUC_V1	<b>TCCCGTATGGTCTAGTGGCTAGGATACCTGGCTTTCACCCAGGAGGCTC</b> GGGTT <b>CGATTCCC</b> GGTACGGG <b>AA</b>	12
tRNA <sup>Glu</sup> UUC_V2	<b>TCCCGTATGGTCTAGTGGCTAGGATACCTGGCTTTCACCCAGGAGGCTC</b> AGGTT <b>CGATTCCC</b> GGTACGGG <b>AA</b>	1
tRNA <sup>Glu</sup> UUC_V3	<b>TCCCGTATGGTCTAGTGGCTAGGATACCTGGCTTTCACCCAGGAGGCTC</b> GGG <b>ATCGATTCCC</b> GGTACGGG <b>AA</b>	1
tRNA <sup>Glu</sup> UUC_V4	<b>TT<b>CGAT</b>ATGGTCTAGTGGCTAC</b> GATACCTGGCTTTCACCCAGGAGGCT <b>CATTTTCGATTCCC</b> GGT <b>TCGGAA</b>	1
tRNA <sup>Glu</sup> GUC_V5	<b>TCC<b>GAT</b>ATGGTCTAGT<b>AA</b>C</b> -AGGATACCTGGCTTTC <b>ATCCAGGAGGCTC</b> TGGTT <b>CGCTTCCC</b> G <b>ATTCGGAA</b>	1

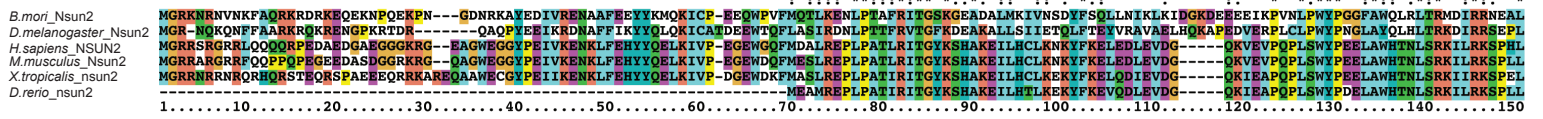
# D

		Genome loci
tRNA <sup>Glu</sup> CUC_V1	<b>TCCG<b>A</b>TATGGTCTAGTGGCTAGGATACCTGGCTCTC</b> ACCCAGGAGGCTCGGGTT <b>CGATTCCC</b> GGT <b>ATTCGGAA</b>	6
tRNA <sup>Glu</sup> CUC_V2	<b>TCCGGTATGGTCTAGTGGCTAGGATACCTGGCTCTC</b> ACCCAGGAGGCTCGGGTT <b>CGATTCCC</b> GGT <b>ACCGGAA</b>	1
tRNA <sup>Glu</sup> CUC_V3	<b>TC<b>GGG</b>TATGGTCTAGTGGCTAGGATACCTGGCTCTC</b> ACCCAGGAGGCTCGGGTT <b>CGATTCCC</b> GGT <b>ACCGGAA</b>	1
tRNA <sup>Glu</sup> CUC_V4	<b>TCCG<b>A</b>TATGGTCTAGTGGCTAG<b>A</b>ATACCTGGCTCTC</b> ACCCAGGAGGCTCGGGTT <b>CGATTCCC</b> GGT <b>ATTCGGAA</b>	1
tRNA <sup>Glu</sup> CUC_V5	<b>TCCG<b>A</b>TATGGTCTAGTGGCTAGGATACCTGGCTCTC</b> ACCCAGGAGGCTCGGGTT <b>CGATTCC<b>AGG</b>TA</b> TCGG <b>AA</b>	1
tRNA <sup>Glu</sup> CUC_V6	<b>TT<b>CGGT</b><b>G</b>TGGTCTAGT<b>G</b>C</b> TAGGATACCT <b>AGCTCTC</b> ACCCAGGAGGCTCGGGTT <b>CGATTCCC</b> GG <b>C</b> ACCG <b>GAA</b>	1
tRNA <sup>Glu</sup> CUC_V7	<b>TCC<b>C</b>GATATGGTCTAGTGGCTAGGATAC<b>AC</b>GGCTCTC</b> ACCC <b>G</b> TGAGGCTCGGGTT <b>CGATTCCC</b> GGT <b>ACGGGAA</b>	1

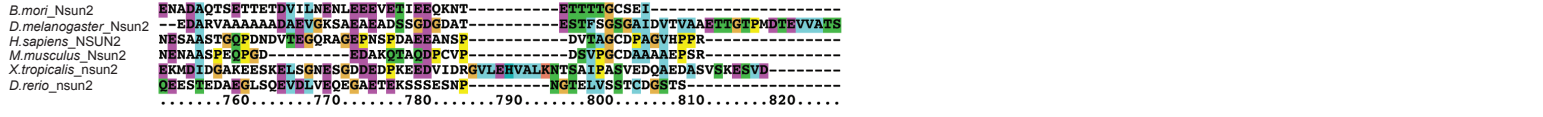
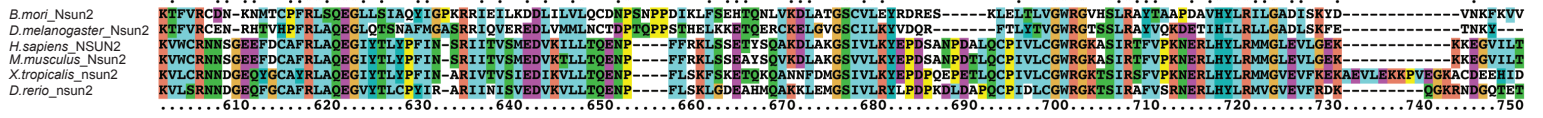
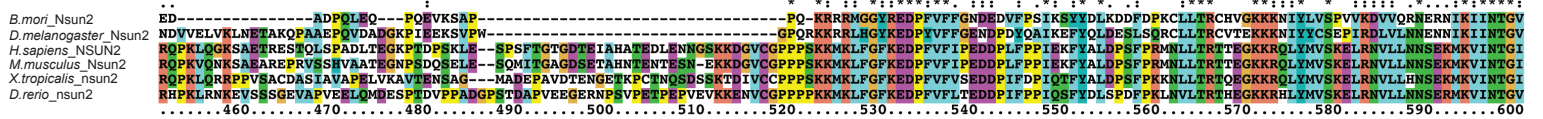


<i>B.mori_Thg1</i>	LSKY <b>ES</b> -----HLEA--	316
<i>D.melanogaster_(2)35Bc</i>	KDD <b>NEA</b> EE <b>P</b> ONLAG <b>TS</b>	316
<i>H.sapiens_Thg1L</i>	S-----	316
<i>M.musculus_Thg1</i>	N-----	316
<i>X.tropicalis_thg1</i>	S-----	316
<i>D.reio_thg1</i>	QC-----	316
	.....310.....	

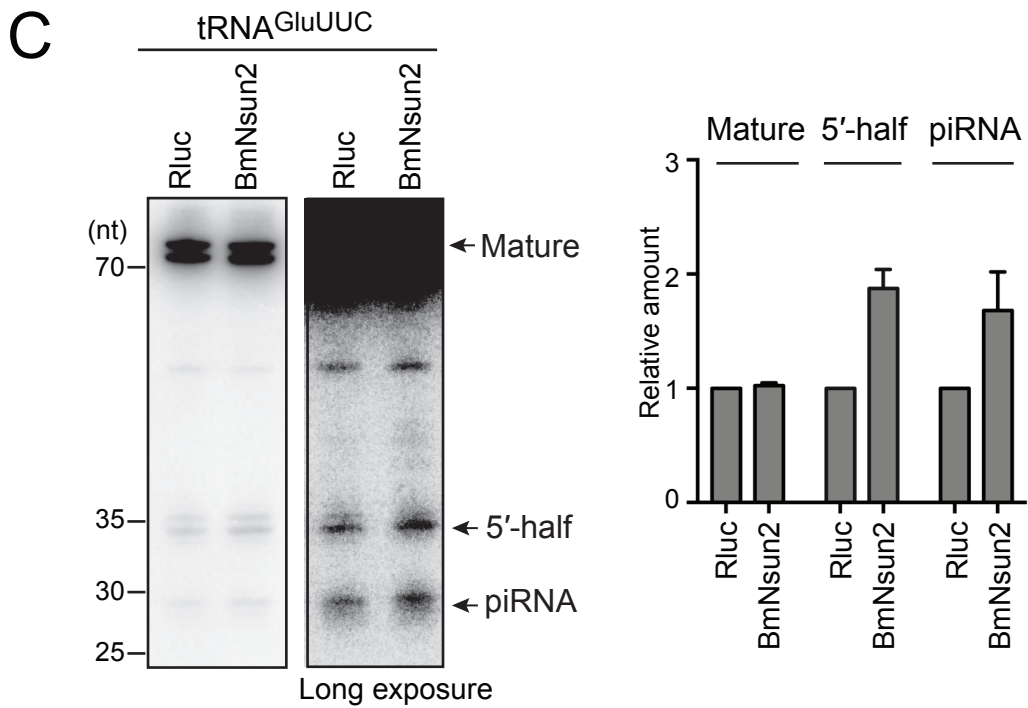
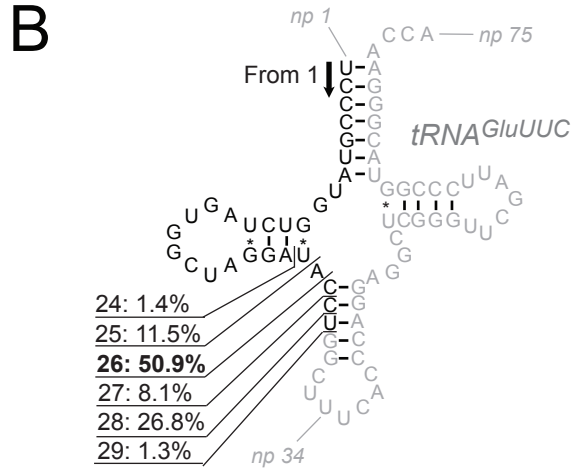
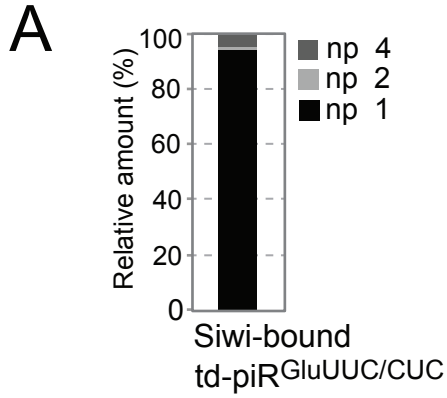
Honda et al. Figure S3



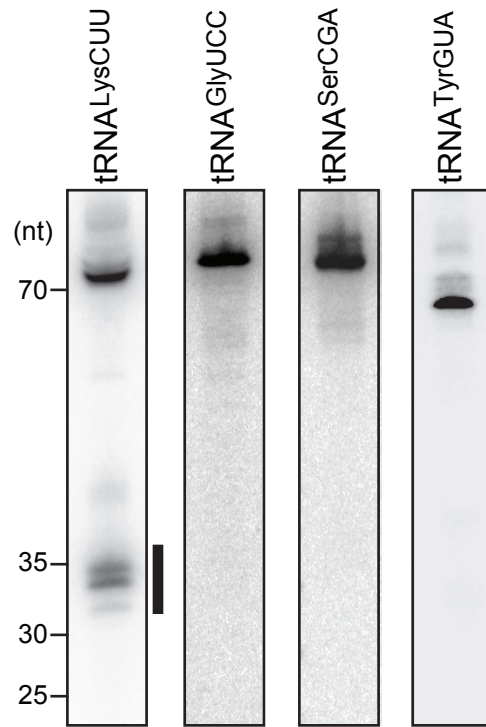
Nol1\_Nop2\_Fmu



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 <sup>AspGUC</sup>	Forward primer	CGGGTCTTCGGTAGTATAGT
	Reverse primer	GATCGTCGGACTGTAGAACTC
	TaqMan probe	/56FAM/TATCCCCGC/ZEN/CTGGAACACTGCGTTT/3IABkFQ/
5'-half <sup>HisGUG</sup>	Forward primer	GCTCGCCGTGATCGTCTAGT
	Reverse primer	GATCGTCGGACTGTAGAACTC
	TaqMan probe	/56-FAM/TAGGACCCT/ZEN/ACGTTGGAACACTGCGTTTGC/3IABkFQ/
td-piR <sup>AspGUC</sup>	Forward primer	CGGGTCTTCGGTAGTATA
	Reverse primer	GATCGTCGGACTGTAGAACTC
	TaqMan probe	/56-FAM/CAGTATCCC/ZEN/GAACACTGCGTTTGC/3IABkFQ/
td-piR <sup>HisGUG</sup>	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 <sup>Asp</sup> GUC	GGGATACTGACCACTATACTACCGAAGA
3'-half <sup>Asp</sup> GUG	GGCGGGGAATCGAACCCCGGTCTCC
5'-half <sup>His</sup> GUG	GGGTCCTAACCACTAGACGATCACGGC
3'-half <sup>His</sup> GUG	GG <u>ATT</u> CGA <u>ACCT</u> GGGTT <u>ACT</u>
5'-half <sup>Glu</sup> UUC	GGTATCCTAGCCACTAGACCATAACG
5'-half <sup>Lys</sup> CUU	GTCATGCTCTACCGACTGAGCTAG
tRNA <sup>Gly</sup> UCC	GTATGCTGACCATTACACCACCAACGC
tRNA <sup>Ser</sup> CGA	GACGCCTTAACCACTCGGCCACGACTG
tRNA <sup>Tyr</sup> GUA	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<sup>His</sup>GUG (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	TAATACGACTCACTATAGGGGTCGGCCATGATTGGGGTGCTTGTTG
	Reverse	TAATACGACTCACTATAGGGCCCATTTTCATCAGGTGCATCTTCTTGC
BmThg11	Forward	TAATACGACTCACTATAGGGTCGTCATCGTATGTTTTCTACTGG
	Reverse	TAATACGACTCACTATAGGGTTCCTCCTTGAATATTTCTGG
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
BmThg11	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	CCGCTCAAAAATAACGGATTG
	SL-Reverse	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTTCTGA
piR-2	Forward	CGCCAAAAGCATGAGAATTTGC
	SL-Reverse	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCGCAG
let-7	Forward	CGGGTGAGGTAGTAGGTTG
	SL-Reverse	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTACTAT
5S rRNA	Forward	TAATGGTGACCGCCTGGGAACACC
	SL-Reverse	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAGCCA