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Supplemental information

Immunostimulatory short non-coding RNAs in the circulation of patients with tuberculosis infection

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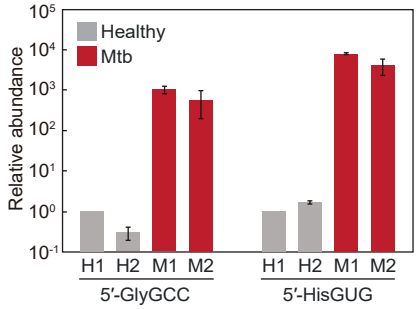

Figure S1. Drastic upregulation of 5′-tRNA halves in plasma samples from Mtb-infected patients
Plasma RNAs were subjected to TaqMan RT-qPCR for specific quantification of 5′-half^{GlyGCC} and 5′-half^{HisGUG}. Spike-in RNA was added during RNA extraction, and its levels were used for normalization. The abundance value of sample H1 was set as 1, and relative abundances for the other samples are shown.

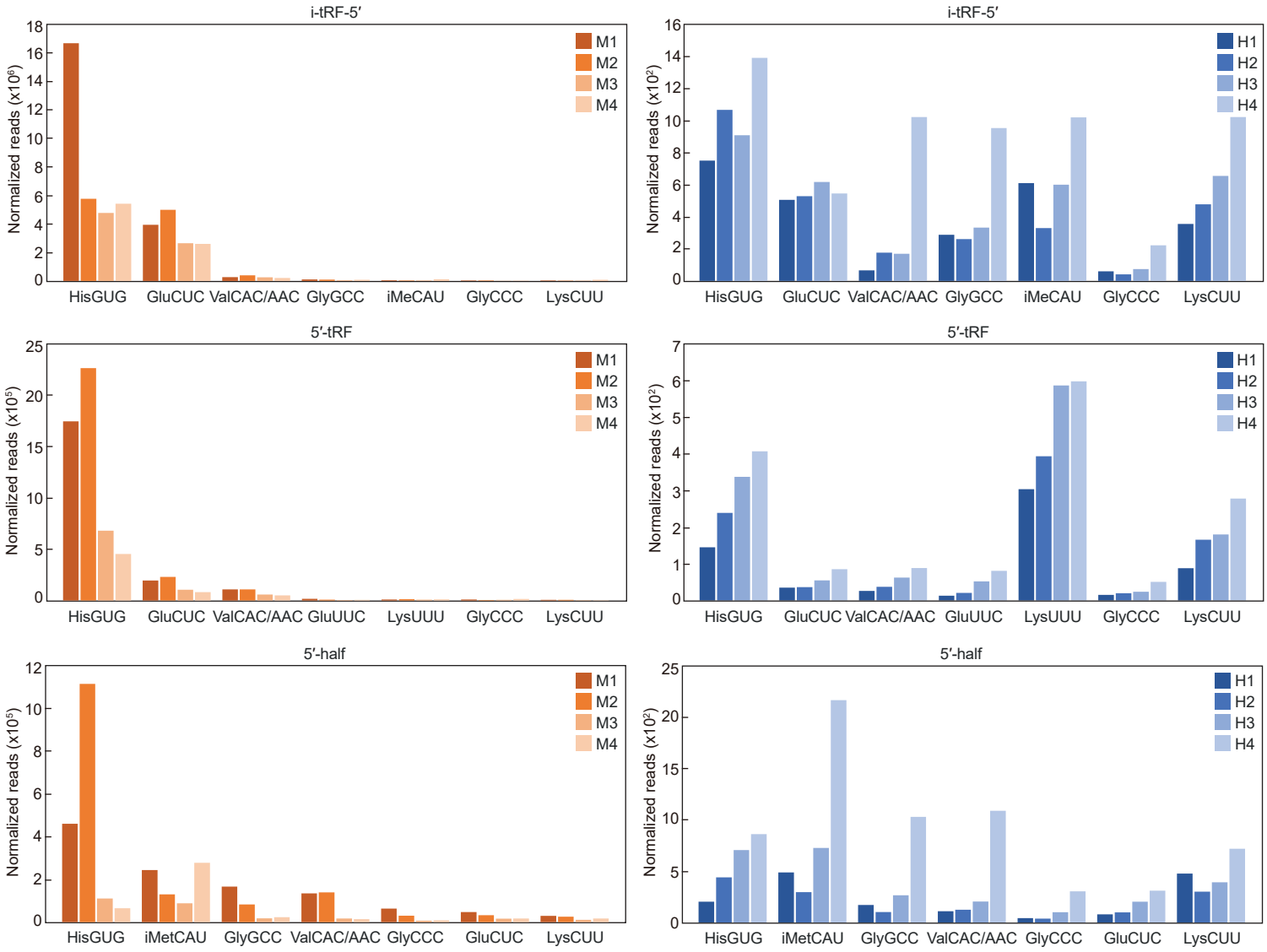

Figure S2. Abundant isoacceptors producing 5′-derivatives of tRNA-derived sncRNAs
Normalized read counts of i-tRFs-5′, 5′-tRFs, and 5′-halves, which originate from the top 6 isoacceptors of each class in Mtb samples and were ordered by abundance in M1.

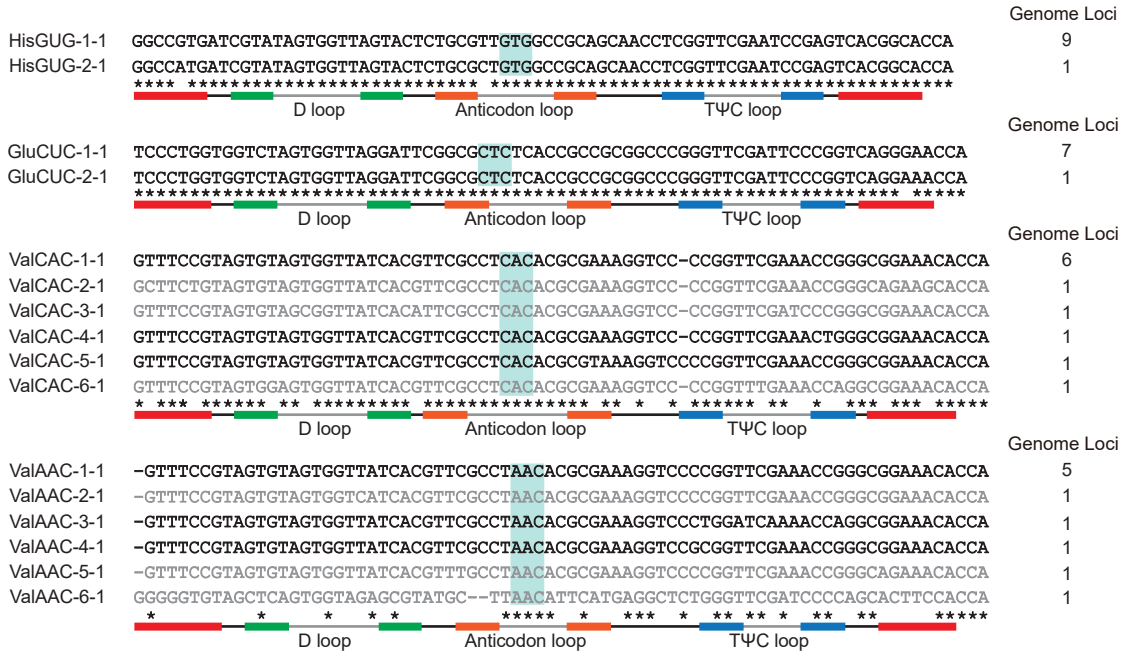

Figure S3. Isodecoder sequences of four cytoplasmic tRNAs

The isodecoders —unique sequences of tRNA distinguished by the gene ID — responsible for generating the abundant sncRNAs shown in Fig. 3A are highlighted in bold black. Sequence alignments were prepared using Clustal Omega. “Genome loci” indicates the number of gene copies on the genome.

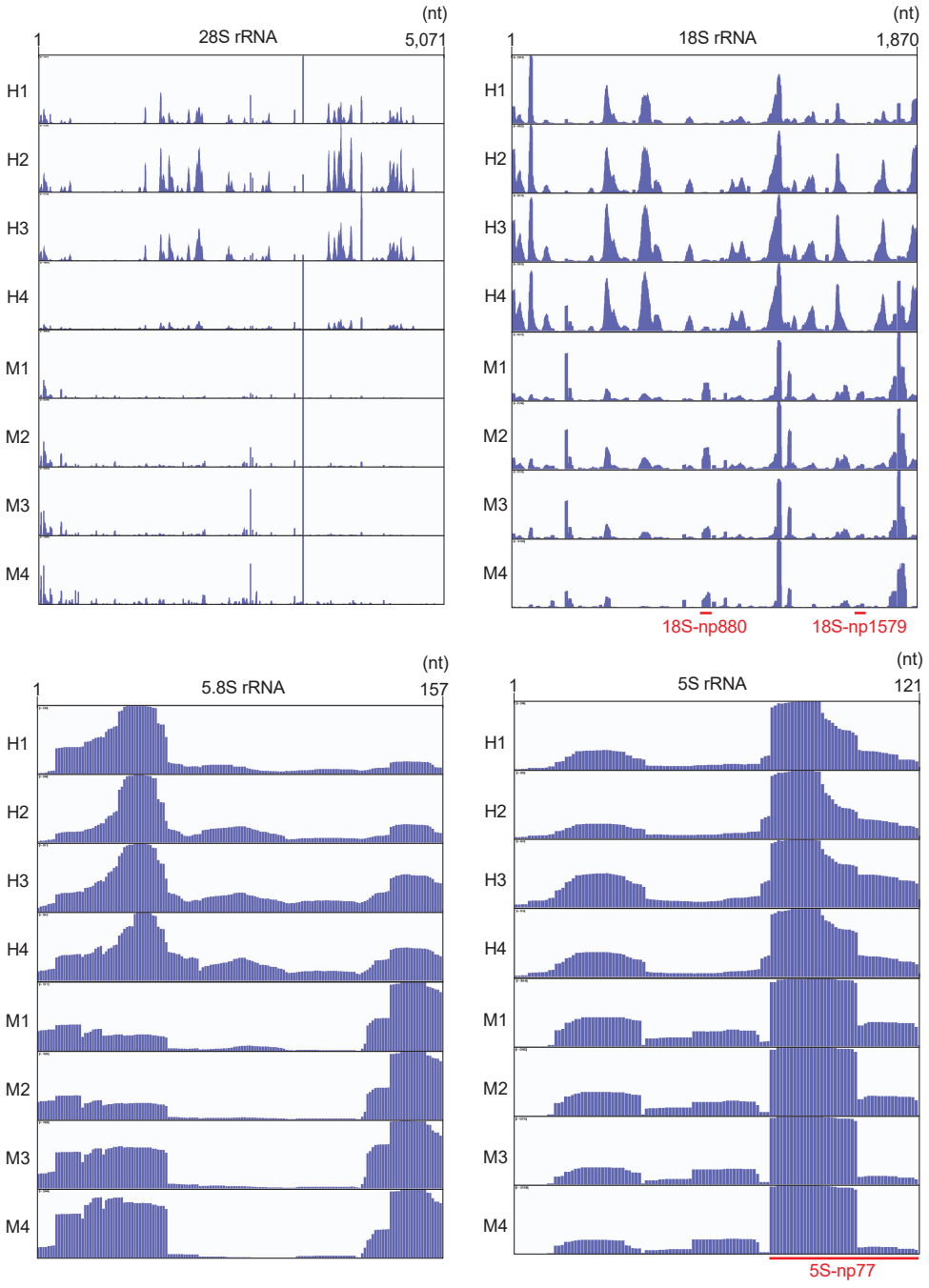

Figure S4. Alignments of rRF reads
The alignments were visualized using the IGV, and the positions of the three rRFs examined in this study are indicated by red lines.

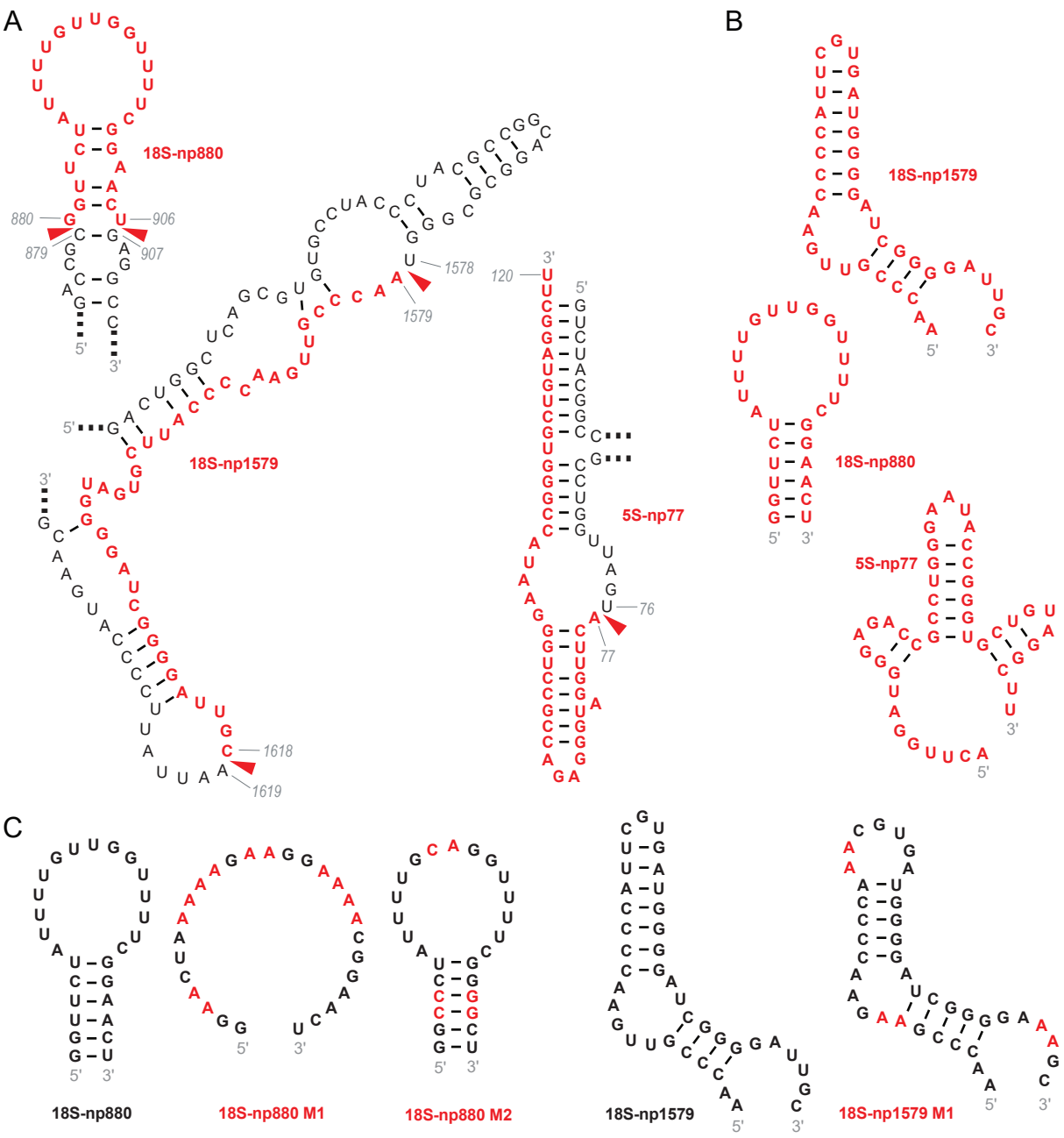

Figure S5. Secondary structures of the characterized rRFs

(A) Secondary structures of rRFs within their parent rRNAs before cleavage, with the rRF sequences highlighted in red. (B) Secondary structures of the rRFs, predicted using RNAfold. (C) Secondary structures of both the wild type and mutant forms of 18S-np880 and 18S-np1579.

Table S1. Fold change and P-value of the sncRNAs highlighted in this study, obtained by DESeq2

Type	Isoacceptor/Gene	Class/Name	Sequence	Fold change	Adjusted P-value
tRNA	HisGUG	i-tRF-5'	CGUAUAGUGGUUAGUACUCUGCGU	6836	3.09E-92
tRNA	HisGUG	i-tRF-5'	CGUAUAGUGGUUAGUACUCUGCGC	5582	5.78E-65
tRNA	HisGUG	i-tRF-5'	CGUGAUCGUAUAGUGGUUAGU	6330	2.47E-105
tRNA	HisGUG	5'-tRF	GCCGUGAUCGUAUAGUGGUU	4030	6.70E-113
tRNA	HisGUG	5'-tRF	GCCGUGAUCGUAUAGUGGU	5906	2.47E-105
tRNA	HisGUG	5'-tRF	GCCGUGAUCGUAUAGUGGUUAGU	1106	3.90E-39
tRNA	HisGUG	5'-tRF	GCCGUGAUCGUAUAGUGGUUAGUACU	1842	9.60E-58
tRNA	HisGUG	5'-half	GCCGUGAUCGUAUAGUGGUUAGUACUCUGCGUUG	403	2.78E-21
tRNA	HisGUG	5'-half	GCCGUGAUCGUAUAGUGGUUAGUACUCUGCGUU	772	2.33E-33
tRNA	HisGUG	5'-half	GCCGUGAUCGUAUAGUGGUUAGUACUCUGCGU	1335	1.14E-49
tRNA	GluCUC	i-tRF-5'	AGGAUUCGGCGCUCU	5832	7.22E-102
tRNA	GluCUC	i-tRF-5'	AGUGGUUAGGAUUCGGC	6329	1.88E-127
tRNA	GluCUC	i-tRF-5'	AGGAUUCGGCGCUCUC	1177	4.03E-80
tRNA	GluCUC	i-tRF-5'	GGUCUAGUGGUUAGGAU	4021	5.16E-72
tRNA	GluCUC	i-tRF-5'	GGUGGUCUAGUGGUUAGGAU	6293	1.73E-108
tRNA	GluCUC	5'-tRF	UCCCUGGUGGUCUAGUGGUUAGGAU	4664	2.44E-77
tRNA	GluCUC	5'-tRF	UCCCUGGUGGUCUAGUGGUUAGGAUUCGGC	3969	2.09E-49
tRNA	GluCUC	5'-half	UCCCUGGUGGUCUAGUGGUUAGGAUUCGGCGC	121	3.21E-15
tRNA	GluCUC	5'-half	UCCCUGGUGGUCUAGUGGUUAGGAUUCGGCGCU	161	4.40E-31
tRNA	ValCAC/AAC	5'-tRF	GUUUCCGUAGUGUAGUGGUU	4863	3.24E-38
tRNA	ValCAC/AAC	5'-tRF	GUUUCCGUAGUGUAGUGGU	3446	4.22E-24
tRNA	ValCAC/AAC	5'-half	GUUUCCGUAGUGUAGUGGUUAUCACGUUCGCCU	318	1.67E-16
tRNA	ValCAC/AAC	5'-half	GUUUCCGUAGUGUAGUGGUUAUCACGUUCGCC	73	9.17E-08
rRNA	18S	18S-np880-906	GCUUAAUACGACUCACUAUAGGUUCUA	41	7.89E-06
rRNA	18S	18S-np1579-1618	CCUGCAGUAAUACGACUCACUAUAGGGAGAUCAACGGGUUCUGAUGAGUCCGUGAGGAC	492	7.64E-38
rRNA	5S	5S-np77-120	CCUGCAGUAAUACGACUCACUAUAGGGAGACCAUCCAAGUCUGAUGAGUCCGUGAGGAC	16	2.24E-12

Table S2. Human plasma samples

Name	ID	Age	Sex
H1	BRH1552217	33	Male
H2	BRH1552218	30	Male
H3	BRH1552219	32	Male
H4	BRH1552220	34	Male
M1	BRH1576558	30	Male
M2	BRH1576559	33	Male
M3	BRH1576560	35	Male
M4	BRH1576561	33	Male

Table S3. Sequences of spike-in RNAs

Name	Sequence (5′-to-3′)
1.P	UGCCUCGAAUUGAGCAUGACGCGCGGUUCUUUC/3Phos/
2.P	GCUGGGAAUGGUUUUGAUCGUUCCUUGCUC/3Phos/

Table S4. Unique ID for the sncRNAs investigated in this study

Name	Sequence (5′-to-3′)	License plate	tDRname
GlyGCC 5'-half	GCAUUGGUGGUUCAGUGGUAGAAUUCUCGCCUGC	tRF-34-PNR8YP9LON4VHM	tDR-1:35-Gly-GCC-2-M3
HisGUG 5'-half	GCCGUGAUCGUAUAGUGGUUAGUACUCUGCGUUG	tRF-34-PW5SVP9N15WV2P	tDR-1:34-His-GTG-1
ValCAC/AAC 5'-half	GUUUCCGUAGUGUAGUGGUUAUCACGUUCGCCU	tRF-33-79MP9P9NH57SD3	tDR-1:33-Val-AAC-1-M6
ValCAC/AAC 5'-tRF	GUUUCCGUAGUGUAGUGGUU	tRF-20-79MP9P9N	tDR-1:20a-Val-AAC-1-M9
18S-np880-906	GGUUCUAUUUUGUUGGUUUUCGGAACU	rRF-27-R9VZ9Y7ZMB3	(N/A)
18S-np1579-1618	AACCCGUUGAACCCCAUUCGUGAUGGGGAUCGGGGAUUGC	rRF-40-BR7U0RISX4REMKIV	(N/A)
5S-np77-120	ACUUGGAUGGGAGACCGCCUGGGAAUACCGGGUGCUGUAGGCUU	rRF-44-EY585DL7RBUSRV9PIQ	(N/A)
18S-np880-906 M1	GGAACUAAAAAGAAGGAAAACGGAACU	nlr-27-6DUBF0OBMB3	(N/A)
18S-np880-906 M2	GGCCCUAUUUUGCAGGUUUUCGGGGCU	nlr-27-6RVZXP7ZMK3	(N/A)
18S-np1579-1618 M1	AACCCGAAGAACCCCAAACGUGAUGGGGAUCGGGGAAAGC	nlr-40-BRO20RBHX4REMKBJ	(N/A)

Table S5. Sequences of the oligos used in this study

Experiment	Target	Type	Sequence (5′-to-3′)
TaqMan qPCR	5′-HisGUG	Forward primer	GCTCGCCGTGATCGTATAGT
		TaqMan probe	/5HEX/TAGTACTCT/ZEN/GCGTTGGAACACTGCGTTTGC/3IABkFQ/
	5′-GlyGCC	Forward primer	GCATTGGTGGTTCAGTGGT
		TaqMan probe	/56-TAMN/ATTCTCGCCTGCGAACACTGCG/3IAbRQSp/
	5'-ValCAC/AAC	Forward primer	GTTTCCGTAGTGTAGTGGT
		TaqMan probe	/56-FAM/ACGTTCGCC/ZEN/TGAACACTGCGTT/3IABkFQ/
	R-Luc	Forward primer	GAGGCAAGCCCGACGT
		TaqMan probe	/56-FAM/GATTGTCCG/ZEN/CGAACACTGCGT/3IABkFQ/
In vitro transcription	ValCAC/AAC 5'-half	Forward primer	CCTGCAGTAATACGACTCACTATAGGGAGACTACGGAAACCTGATGAGTCCGTGAGGAC
		Reverse primer	mAmGGCGAACGTGATAACCACTACACTACGGAAACGACGGTACCGGGTACCGTTTCGTCCTCACGGACT
	ValCAC/AAC 5'-tRF	Forward primer	CCTGCAGTAATACGACTCACTATAGGGAGACTACGGAAACCTGATGAGTCCGTGAGGAC
		Reverse primer	mAmACCACTACACTACGGAAACGACGGTACCGGGTACCGTTTCGTCCTCACGGACT
	18S-np880-906	Forward primer	GCTTAATACGACTCACTATAGGTTCTA
		Reverse primer	mAmGTTCCGAAAACCAACAAAATAGAACC
	18S-np1579-1618	Forward primer	CCTGCAGTAATACGACTCACTATAGGGAGATCAACGGGTTCTGATGAGTCCGTGAGGAC
		Reverse primer	mGmCAATCCCCGATCCCCATCACGAATGGGGTTCAACGGGTTGACGGTACCGGGTACCGTTTCGTCCTCACGGACT
	5S-np77-120	Forward primer	CCTGCAGTAATACGACTCACTATAGGGAGACCATCCAAGTCTGATGAGTCCGTGAGGAC
		Reverse primer	mAmAGCCTACAGCACCCGGTATTCCCAGGCGGTCTCCCATCCAAGTGACGGTACCGGGTACCGTTTCGTCCTCACGGACT

A universal reverse primer (5'-GATCGTCGGACTGTAGAACTC-3') was used for all TaqMan RT-qPCR.