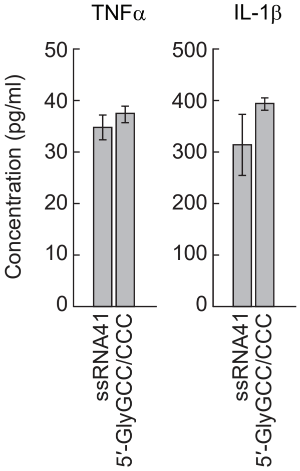

Figure S1. The activity of the 5′-tRNA$^{GlyGCC/CCC}$ half
After RNA transfection into HMDMs using DOTAP, culture medium was subjected to measurement of concentration of the indicated cytokines. Error bars indicate mean ± SD of triplicate measurements.

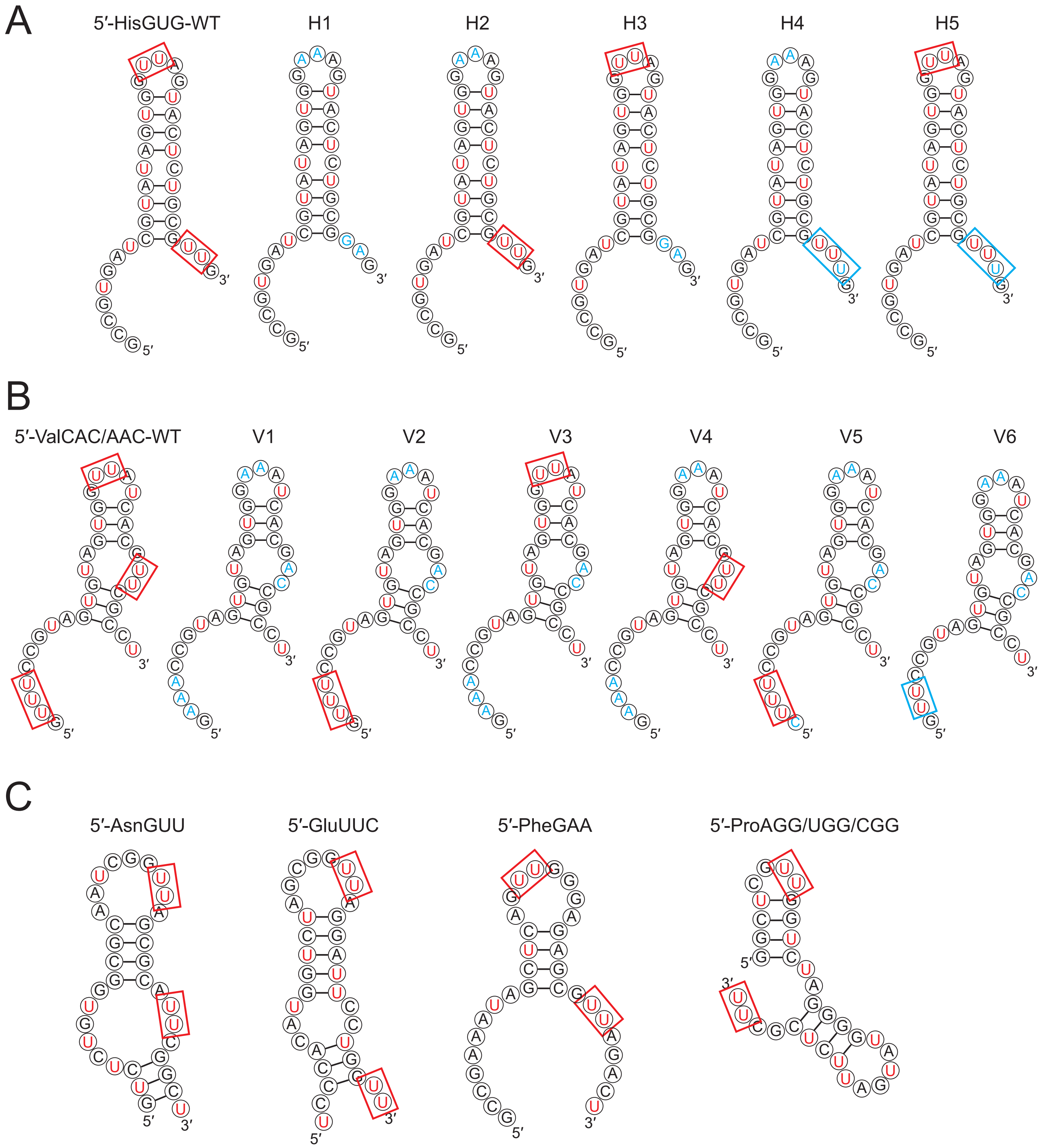

Figure S2. Secondary structure of 5′-tRNA halves and their mutants

(A-C) Secondary structures of the 5′-tRNA$^{\text{HisGUG}}$ half and its mutants (A) or the 5′-tRNA$^{\text{ValCAC/AAC}}$ half and its mutants (B), and other 5′-tRNA halves which possess more than two successive Us in the single-stranded regions (C) are shown. The presence of successive Us are highlighted (Us are shown in red), and the mutated nucleotides are shown in blue.

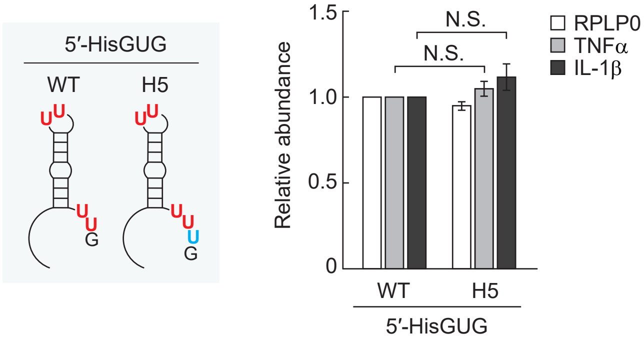

Figure S3. The activity of the mutant 5′-tRNA^{HisGUG} half

The wild-type and H5 mutant of 5′-tRNA^{HisGUG} half were subjected to DOTAP-mediated transfection into HMDMs. Total RNAs from the cells were subjected to RT-qPCR for the indicated mRNAs.

Supplementary Table 1. Abundant 5'-tRNA half species in sequencing data of HMDM-secreted EVs

No.	Sequence (5′–3′)	Length (nt)	Isoacceptor	% to total 5'-tRNA half reads	
				Library 1	Library 2
#1	GUUUCCGUAGUGUAGUGGUUAUCACGUUCGCCU*	33	ValCAC/AAC	17.84	17.43
#2	GUUUCCGUAGUGUAGUGGUUAUCACGUUCGCCUC	34	ValCAC	14.24	13.35
#3	GCCGUGAUCGUAUAGUGGUUAGUACUCUGCGUUG*	34	HisGUG	10.93	14.80
#4	GCAUUGGUGGUUCAGUGGUAGAAUUCUCGCCU*	32	GlyGCC/CCC	9.63	5.08
#5	GUUUCCGUAGUGUAGUGGUUAUCACGUUCGCCUCAC	36	ValCAC	3.56	4.21
#6	GCAUGGGUGGUUCAGUGGUAGAAUUCUCGCCU	32	GlyGCC	2.95	1.31
#7	UCCCUGGUGGUCUAGUGGUUAGGAUUCGGCGCUCUC	36	GluCUC	2.55	2.57
#8	GCAUUGGUGGUUCAGUGGUAGAAUUCUCGCC	31	GlyGCC/CCC	2.54	1.94
#9	UCCCUGGUGGUCUAGUGGUUAGGAUUCGGCGCU	33	GluCUC	2.51	3.06
#10	GCAUUGGUGGUUCAGUGGUAGAAUUCUCGCCUGC	34	GlyGCC	2.48	2.37
#11	GUUUCCGUAGUGUAGUGGUCAUCACGUUCGCCU	33	ValAAC	2.46	3.87
#12	GUUUCCGUAGUGUAGUGGUCAUCACGUUCGCCUAAC	36	ValAAC	2.35	2.62
#13	GUUUCCGUAGUGUAGUGGUUAUCACGUUCGCCUAAC	36	ValAAC	2.28	2.88
#14	UCCCUGGUGGUCUAGUGGUUAGGAUUCGGCGCUCU	35	GluCUC	1.88	1.77
#15	UCCCUGGUGGUCUAGUGGUUAGGAUUCGGCGC	32	GluCUC	1.67	2.49
#16	UCCCUGGUGGUCUAGUGGUUAGGAUUCGGCGCUC	34	GluCUC	1.14	1.04
#17	GCUUCUGUAGUGUAGUGGUUAUCACGUUCGCCU	33	ValCAC	1.02	1.15

The sequences with asterisk (#1, #3, and #4) were selected and analyzed.

Supplementary Table 2. Ratio of the 5'-tRNA half reads derived from respective tRNA isoacceptors in HMDM Evs

No.	Isoacceptor	% to total 5'-tRNA half reads	
		Library 1	Library 2
#1	ValCAC/AAC	47.25	48.92
#2	GlyGCC/CCC	20.07	13.69
#3	HisGUG	13.61	18.29
#4	GluCUC	9.94	11.08
#5	GluUUC	4.62	2.97
#6	LysCUU	2.56	3.30
#7	MetCAU	0.71	0.76
#8	AspGUC	0.58	0.27
#9	ProUGG	0.12	0.06
#10	ProAGG	0.11	0.06
	Others	0.42	0.58
	Total	100.00	100.00

Supplementary Table 3. Sequences of synthetic RNAs used in this study

RNA	Sequence (5′–3′)	Licence plate	tDRname
5′-AsnGUU	GUCUCUGUGGCGCAAUCGGUUAGCGCAUUCGGCU	tRF-34-S998LOW3VHJ9IW	tDR-1:33-Asn-GTT-3
5′-GluUUC	UCCCACAUGGUCUAGCGGUUAGGAUUCCUGGUU	tRF-33-86J8WPMN1E8Y0E	tDR-1:33-Glu-TTC-2
5′-GlyGCC/CCC	GCAUUGGUGGUUCAGUGGUAGAAUUCUCGCCU	tRF-32-PNR8YP9LON4V3	tDR-1:33-Gly-CCC-1-M4
5′-HisGUG	GCCGUGAUCGUAUAGUGGUUAGUACUCUGCGUUG	tRF-34-PW5SVP9N15WV2P	tDR-1:34-His-GTG-1
5′-HisGUG-H1	GCCGUGAUCGUAUAGUGGAAAGUACUCUGCGGAG	nlr-34-PW5SVP9B15WVIS	N/A
5′-HisGUG-H2	GCCGUGAUCGUAUAGUGGAAAGUACUCUGCGUUG	nlr-34-PW5SVP9B15WV2P	N/A
5′-HisGUG-H3	GCCGUGAUCGUAUAGUGGUUAGUACUCUGCGGAG	nlr-34-PW5SVP9N15WVIS	N/A
5′-HisGUG-H4	GCCGUGAUCGUAUAGUGGAAAGUACUCUGCGUUUG	nlr-35-PW5SVP9B15WV7Y	N/A
5′-HisGUG-H5	GCCGUGAUCGUAUAGUGGUUAGUACUCUGCGUUUG	nlr-35-PW5SVP9N15WV7Y	N/A
5′-PheGAA	GCCGAAAUAGCUCAGUUGGGAGAGCGUUAGACU	tRF-33-PUDP4PZKFJ7PW	tDR-1:33-Phe-GAA-1-M2
5′-ProAGG/UGG/CGG	GGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUU	tRF-32-6978WPRLXN4VQ	tDR-1:33-Pro-AGG-1-M5
5′-ValCAC/AAC	GUUUCCGUAGUGUAGUGGUUAUCACGUUCGCCU	tRF-33-79MP9P9NH57SD3	tDR-1:33-Val-AAC-1-M6
5′-ValCAC/AAC modification	GUUUCCGUAGUGΨAGDGGDDAUCACm^{2}GΨUCGCCU	tRF-33-79MP9P9NH57SD3	N/A
5′-ValCAC/AAC-V1	GAAACCGUAGUGUAGUGGAAAUCACGACCGCCU	nlr-33-ODMP9P9BH5OSD3	N/A
5′-ValCAC/AAC-V2	GUUUCCGUAGUGUAGUGGAAAUCACGACCGCCU	nlr-33-79MP9P9BH5OSD3	N/A
5′-ValCAC/AAC-V3	GAAACCGUAGUGUAGUGGUUAUCACGACCGCCU	nlr-33-ODMP9P9NH5OSD3	N/A
5′-ValCAC/AAC-V4	GAAACCGUAGUGUAGUGGAAAUCACGUUCGCCU	nlr-33-ODMP9P9BH57SD3	N/A
5′-ValCAC/AAC-V5	CUUUCCGUAGUGUAGUGGAAAUCACGACCGCCU	nlr-33-N9MP9P9BH5OSD3	N/A
5′-ValCAC/AAC-V6	GUUCCGUAGUGUAGUGGAAAUCACGACCGCCU	nlr-32-7RS3S36B8H0V3	N/A
FL-ValCAC	GUUUCCGUAGUGUAGUGGUUAUCACGUUCGCCUCACACGCGAAAGGUCCCCGGUUCGAAACCGGGCGGAAACACCA	N/A	N/A
miR-122-5p	UGGAGUGUGACAAUGGUGUUUG	iso-22-909U247NP	N/A
miR-548ah-5p	AAAAGUGAUUGCAGUGUUUG	iso-20-B0XNP37Y	N/A
miR-552-5p	GUUUAACCUUUUGCCUGUUGG	iso-21-7X0ZZ19Y0	N/A
Spike-in	GGGAGGCAAGCCCGACGUCGUCCAGAUUGUCCGC	nlr-34-R0P0KUMS8PIW1M	N/A
ssRNA40	GCCCGUCUGUUGUGUGACUC	nlr-20-PSWW9WO9	N/A
ssRNA41	GCCCGACAGAAGAGAGACAC	nlr-20-PS02F2O5	N/A
AS-oligo	mA*mG*mG*mC*mG*mA*mA*mC*mG*mU*G*A*T*A*A*C*C*A*C*T*A*C*A*mC*mU*mA*mC*mG*mG*mA*mA*mA*mC	N/A	N/A
Ctrl-oligo	mC*mA*mA*mA*mA*mG*mU*mC*mU*mC*A*A*A*G*C*A*C*C*C*G*C*A*A*mG*mC*mU*mU*mA*mA*mA*mG*mG*mG	N/A	N/A

Mutated nucleotides are shown in blue.

Supplementary Table 4. The most sbundant seuqneces of EV-5'-tRNA halves derived from the respective isoacceptors and the presence of successive Us

Sequence (5′–3′)	Length (nt)	% to total 5'-half reads	Isoacceptor	UU(U) in single-stranded regions	
				Loop	Other
GGGGAAUUAGCUCAAAUGGUAGAGCGCUCGCUU	33	0.0007	AlaAGC	1	1
GGGGAUGUAGCUCAGUGGUAGAGCGCGCGC	30	0.0025	AlaCGC	0	0
GGGGAUGUAGCUCAGUGGUAGAGCGCAUGCUUUGCAC	37	0.0001	AlaUGC	0	1
GGGCCAGUGGCGCAAUGGAUAACGCGUCUGACU	33	0.0055	ArgACG	0	0
GACCCAGUGGCCUAAUGGAUAAGGCAUCAGCCU	33	0.0116	ArgCCG	0	0
GCCCCAGUGGCCUAAUGGAUAAGGCACUGGCCUCCU	36	0.0003	ArgCCU	0	0
GACCGCGUGGCCUAAUGGAUAAGGCGUCUGACU	33	0.0047	ArgUCG	0	0
GGCUCCGUGGCGCAAUGGAUAGCGCAUUGGACU	33	0.0297	ArgUCU	0	1
GUCUCUGUGGCGCAAUCGGUUAGCGCAUUCGGCU*	34	0.0021	AsnGUU	2	0
UCCUCGUUAGUAUAGUGGUGAGUAUCCCCGCCUGUC	36	0.4176	AspGUC	0	1
GGGGGUAUAGCUCAGUGGGUAGAGCAUUUGACU	33	0.0003	CysGCA	0	1
GGUUCCAUGGUGUAAUGGUUAGCACUCUGGACUCU	35	0.0190	GlnCUG	1	1
GGCCCCAUGGUGUAAUGGUUAGCACUCUGGACU	33	0.0006	GlnUUG	1	0
UCCCUGGUGGUCUAGUGGUUAGGAUUCGGCGCU	33	3.0580	GluCUC	0	2
UCCCACAUGGUCUAGCGGUUAGGAUUCCUGGUU*	33	1.0244	GluUUC	1	2
GCAUUGGUGGUUCAGUGGUAGAAUUCUCGCCU*	33	9.6276	GlyGCC/CCC	0	3
GCGUUGGUGGUAUAGUGGUGAGCAUAGCUGCCUUCC	36	0.0008	GlyUCC	0	2
GCCGUGAUCGUAUAGUGGUUAGUACUCUGCGUUG*	34	14.7964	HisGUG	1	1
GGCCGGUUAGCUCAGUCGGCUAGAGCGUGGUGCUA	35	0.0002	IleAAU	0	1
GGUAGCGUGGCCGAGCGGUCUAAGGCGCUGGAUU	34	0.0025	LeuAAG	0	1
GUCAGGAUGGCCGAGUGGUCUAAGGCGCCAGAC	33	0.0013	LeuCAA	0	0
GUCAGGAUGGCCGAGCGGUCUAAGGCGCUGCGUUC	35	0.1067	LeuCAG	0	1
GUUAAGAUGGCAGAGCCUGGUAAUUGCAUAAAACUUA	37	0.0235	LeuUAA	0	3
GGUAGUGUGGCCGAGCGGUCUAAGGCGCUGGAUU	34	0.0004	LeuUAG	0	1
GCCCGGCUAGCUCAGUCGGUAGAGCAUGAGACU	33	0.8703	LysCUU	0	0
GCCCGGAUAGCUCAGUCGGUAGAGCAUCAGACU	33	0.0441	LysUUU	0	0
AGCAGAGUGGCGCAGCGGAAGCGUGCUGGGCCC	33	0.4089	MetCAU	0	0
GCCGAAAUAGCUCAGUUGGGAGAGCGUUAGACU*	33	0.0003	PheGAA	1	1
GGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUU*	32	0.2057	ProAGG/UGG/CGG	1	2
GCCCGGAUGAUCCUCAGUGGUCUGGGGUGCAGGCUUC	37	0.0300	SeCUCA	0	1
GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGAC	32	0.0001	SerAGA	1	0
GCUGUGAUGGCCGAGUGGUUAAGGUGUUGGACU	33	0.0000	SerCGA	0	2
GACGAGGUGGCCGAGUGGUUAAGGCGAUGGAC	32	0.0004	SerGCU	0	1
GCAGCGAUGGCCGAGUGGUUAAGGCGUUGGACUUGA	36	0.0000	SerUGA	0	3
GGCAGAGUGGUGCAGCGGAAGCGUGCUGGGC	31	0.0001	ThrCGU	0	0
GGCUCCAUAGCUCAGGGGUUAGAGCACUGGUCU	33	0.0021	ThrUGU	1	0
GACCUCGUGGCGCAACGGUAGCGCGUCUGACUCC	34	0.0012	TrpCCA	0	0
CCUUCGAUAGCUCAGCUGGUAGAGCGGAGGACUGUAG	37	0.0000	TyrGUA	0	1
GUUUCCGUAGUGUAGUGGUUAUCACGUUCGCCU*	33	16.6442	ValCAC/AAC	2	1
GGUUCCAUAGUGUAGUGGUUAUCACGUCUGCUUU	34	0.0568	ValUAC	1	2

The sequences with asterisk were selected and analyzed.

Supplementary Table 5. Primer sequences for the synthesis of dsDNA templates that were used for *in vitro* RNA synthesis

RNA	Primer	Sequence (5′–3′)
5′-AsnGUU	Forward	GCTTAATACGACTCACTATAGTCTCTGTGGCGCAA
	Reverse	mAmGCCGAATGCGCTAACCGATTGCGCCACAGAGAC
5′-GluUUC	Forward	CCTGCAGTAATACGACTCACTATAGGGAGACCATGTGGGACTGATGAGTCCGTGAGGACGAAACG
	Reverse	mAmACCAGGAATCCTAACCGCTAGACCATGTGGGAGACGGGTACCGGGTACCGTTTCGTCC
5′-GlyGCC/CCC	Forward	GCTTAATACGACTCACTATAGCATTGGTGGTTCAG
	Reverse	mAmGGCGAGAATTCTACCACTGAACCACCAATGC
5′-HisGUG	Forward	GCTTAATACGACTCACTATAGCCGTGATCGTATAG
	Reverse	mCmAACGCAGAGTACTAACCACTATACGATCACGGC
5′-HisGUG-H1	Forward	GCTTAATACGACTCACTATAGCCGTGATCGTATAG
	Reverse	mCmUCCGCAGAGTACTTTCCACTATACGATCACGGC
5′-HisGUG-H2	Forward	GCTTAATACGACTCACTATAGCCGTGATCGTATAG
	Reverse	mCmAACGCAGAGTACTTTCCACTATACGATCACGGC
5′-HisGUG-H3	Forward	GCTTAATACGACTCACTATAGCCGTGATCGTATAG
	Reverse	mCmUCCGCAGAGTACTAACCACTATACGATCACGGC
5′-HisGUG-H4	Forward	GCTTAATACGACTCACTATAGCCGTGATCGTATAG
	Reverse	mCmAAACGCAGAGTACTTTCCACTATACGATCACGGC
5′-HisGUG-H5	Forward	GCTTAATACGACTCACTATAGCCGTGATCGTATAG
	Reverse	mCmAAACGCAGAGTACTAACCACTATACGATCACGGC
5′-PheGAA	Forward	GCTTAATACGACTCACTATAGCCGAAATAGCTCAG
	Reverse	mAmGTCTAACGCTCTCCCAACTGAGCTATTTCGGC
5′-ProAGG/UGG/CGG	Forward	GCTTAATACGACTCACTATAGGCTCGTTGGTCTAG
	Reverse	mAmAGCGAGAATCATACCCCTAGACCAACGAGCC
5′-ValCAC/AAC	Forward	GCTTAATACGACTCACTATAGTTTCCGTAGTGTAG
	Reverse	mAmGGCGAACGTGATAACCACTACACTACGGAAAC
5′-ValCAC/AAC-V1	Forward	GCTTAATACGACTCACTATAGAAACCGTAGTGTAG
	Reverse	mAmGGCGGTCGTGATTTCCACTACACTACGGTTTC
5′-ValCAC/AAC-V2	Forward	GCTTAATACGACTCACTATAGTTTCCGTAGTGTAG
	Reverse	mAmGGCGGTCGTGATTTCCACTACACTACGGAAAC
5′-ValCAC/AAC-V3	Forward	GCTTAATACGACTCACTATAGAAACCGTAGTGTAG
	Reverse	mAmGGCGGTCGTGATAACCACTACACTACGGTTTC
5′-ValCAC/AAC-V4	Forward	GCTTAATACGACTCACTATAGAAACCGTAGTGTAG
	Reverse	mAmGGCGAACGTGATTTCCACTACACTACGGTTTC
5′-ValCAC/AAC-V5	Forward	CCTGCAGTAATACGACTCACTATAGGGAGACTACGGAAAGCTGATGAGTCCGTGAGGAC
	Reverse	mAmGGCGGTCGTGATTTCCACTACACTACGGAAAGGACGGGTACCGGGTACCGTTTCGTCC
5′-ValCAC/AAC-V6	Forward	GCTTAATACGACTCACTATAGTTCCGTAGTGTAGT
	Reverse	mAmGGCGGTCGTGATTTCCACTACACTACGGAAC
FL-ValCAC	Forward	GCTTAATACGACTCACTATAGTTTCCGTAGTGTAGTGGTTATCACGTTCGCCTCACAC
	Reverse	mUmGGTGTTTCCGCCCGGTTTCGAACCGGGGACCTTTCGCGTGTGAGGCG
miR-122-5p	Forward	CCTGCAGTAATACGACTCACTATAGGGAGATCACACTCCACTGATGAGTCCGTGAGGACGAAA
	Reverse	mCmAAACACCATTGTCACACTCCAGACGGGTACCGGGTACCGTTTCGTCCTC
miR-548ah-5p	Forward	CCTGCAGTAATACGACTCACTATAGGGAGAAATCACTTTTCTGATGAGTCCGTGAGGACGAAA
	Reverse	mCmAAACACTGCAATCACTTTTGACGGGTACCGGGTACCGTTTCGTCCTC
miR-552-5p	Forward	GCTTAATACGACTCACTATAGTTTAACCTT
	Reverse	mCmCAACAGGCAAAAGGTTAAAC
ssRNA41	Forward	GCTTAATACGACTCACTATAGCCCGA
	Reverse	mGmUGTCTCTCTTCTGTCGGGCTATAGT

All synthetic probes and primes used in this study were synthesized by Integrated DNA Technologies. “mN” designates 2′-*O*-methylated nucleotide.

Supplementary Table 6. Sequences of TaqMan probes and primers for TaqMan RT-qPCR

Target	Probe/primer	Sequence (5′–3′)
5′-HisGUG	Forward	GCTCGCCGTGATCGTATAGT
	Reverse	GATCGTCGGACTGTAGAACTC
	TaqMan	/5HEX/TAGTACTCT/ZEN/GCGTTGGAACACTGCGTTTGC/3IABkFQ/
5′-ValCAC/AAC	Forward	GTTTCCGTAGTGTAGTGGT
	Reverse	GATCGTCGGACTGTAGAACTC
	TaqMan	/56-FAM/ACGTTCGCC/ZEN/TGAACACTGCGTT/3IABkFQ/
5S rRNA	Forward	TACGGCCATACCACCCTGAAC
	Reverse	GATCGTCGGACTGTAGAACTC
	TaqMan	/56-FAM/CGGGTGCTG/ZEN/TAGGCTTTGAACACTGCGTT/3IABkFQ/
Spike-in	Forward	GAGGCAAGCCCGACGT
	Reverse	GATCGTCGGACTGTAGAACTC
	TaqMan	/56-FAM/GATTGTCCG/ZEN/CGAACACTGCGT/3IABkFQ/

TaqMan probes contain Hexachlorofluorescein (HEX), 6-carboxyfluorescein (FAM), ZEN, and Iowa Black (IABkFQ) as the fluorophore or quencher.

Supplementary Table 7. Sequences of primers for standard RT-qPCR

Target	Primer	Sequence (5′–3′)
TNFα	Forward	GAGCACTGAAAGCATGATCC
	Reverse	CGAGAAGATGATCTGACTGCC
IL-1β	Forward	CAGGCTGCTCTGGGATTCTC
	Reverse	CCTGGAAGGAGCACTTCATCT
RPLP0	Forward	CTATCATCAACGGGTACAAACGAG
	Reverse	CAGATGGATCAGCCAAGAAGG
GAPDH	Forward	GTCTTCACCACCATGGAGAAGG
	Reverse	ATGATCTTGAGGCTGTTGTCAT