

Supplementary material for

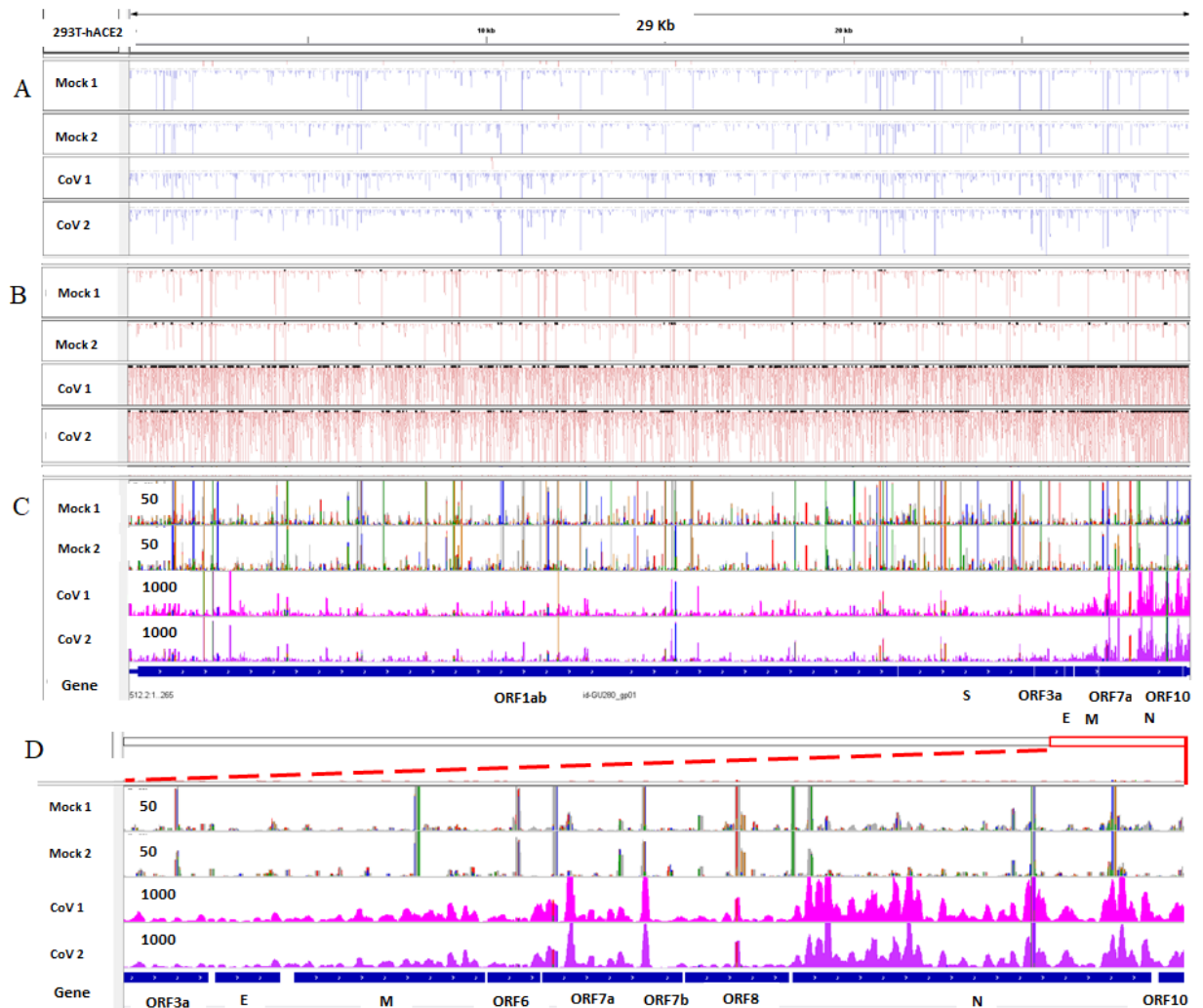
**Viral and host small RNA response to SARS-CoV-2 infection**

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Arumugaswami<sup>3,4</sup>, Yanhong Shi<sup>2,\*</sup>, Arthur D. Riggs<sup>1</sup>

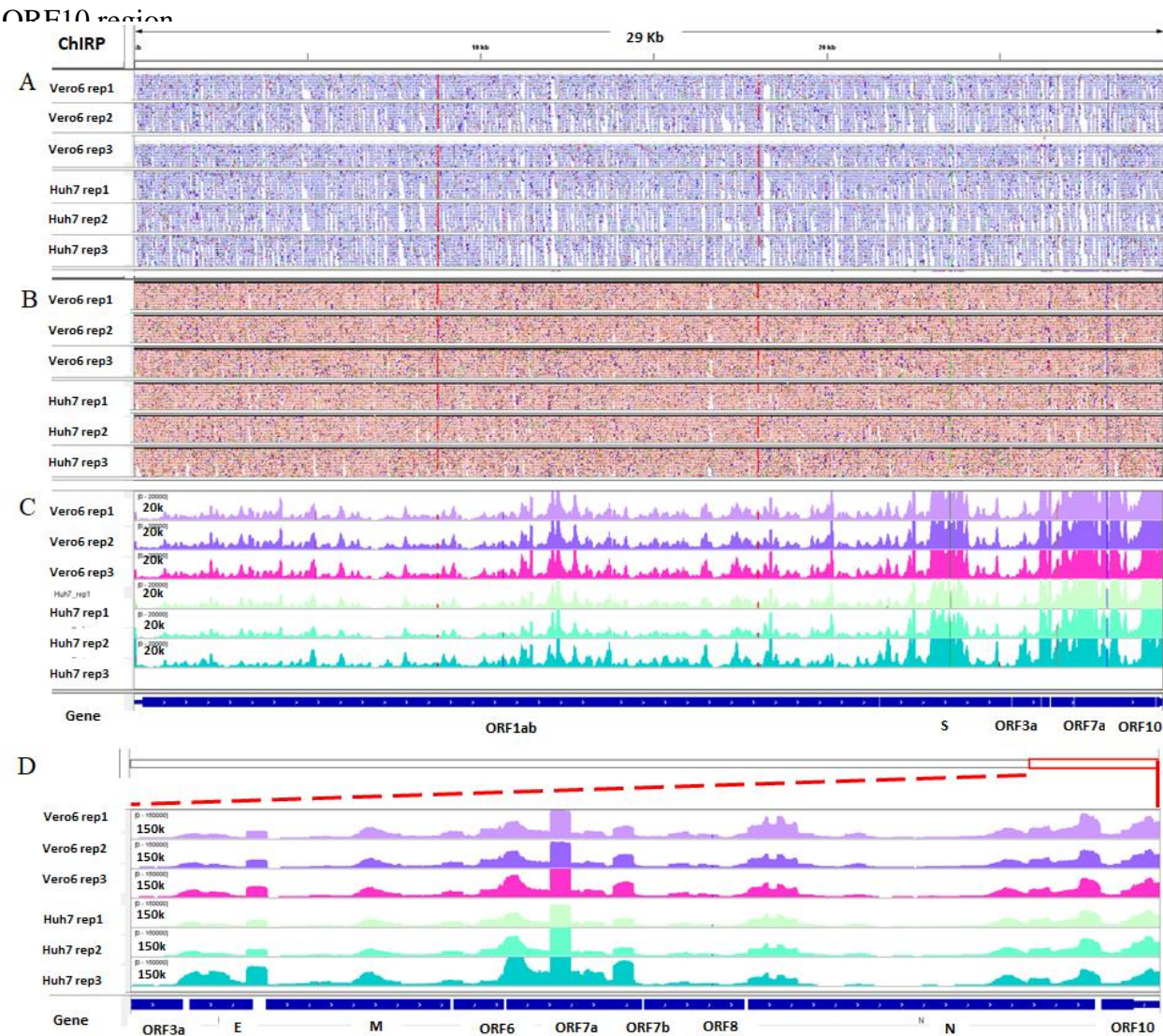
## 1. Supplementary Figures:

**Figure S1. IGV view of small RNAs from SARS-CoV-2 infection of HEK293T-hACE2 cells aligned to the SARS-CoV-2 genome.** (A) Reads mapped to the positive genome of SARS-CoV-2; (B) Reads mapped to the negative genome of SARS-CoV-2; (C) Reads coverage; (D) Reads coverage at the E to ORF10 region. Mock 1& Mock 2: replicates 1 & 2 data for mock infection of HEK293T-hACE2 cells; CoV1 & CoV2: replicates 1 & 2 data for SARS-CoV-2 infection of HEK293T-hACE2 cells.



**Figure S2. IGV view of ChIRP data of SARS-CoV-2-infected Vero E6 and Huh7.5 cells.**

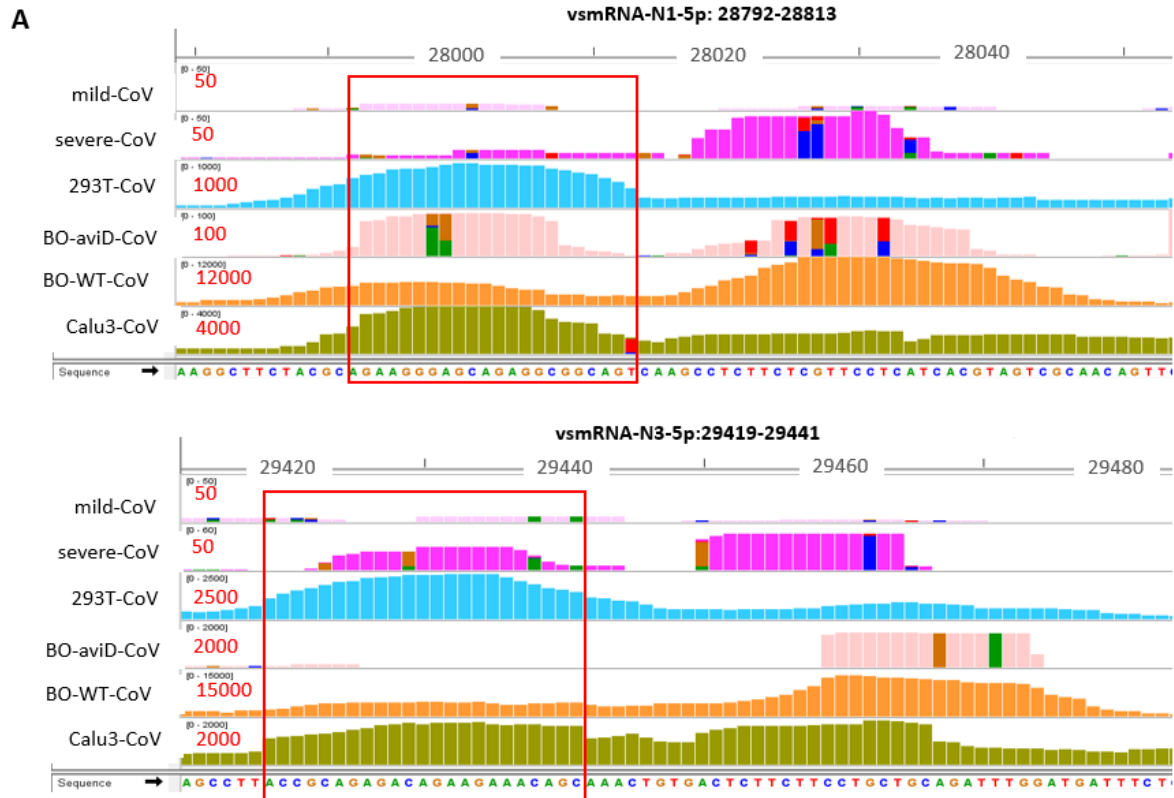
(A) Reads mapped to the negative genome of SARS-CoV-2; (B) Reads mapped to the positive genome of SARS-CoV-2; (C) Reads coverage; (D) Reads coverage at the E to

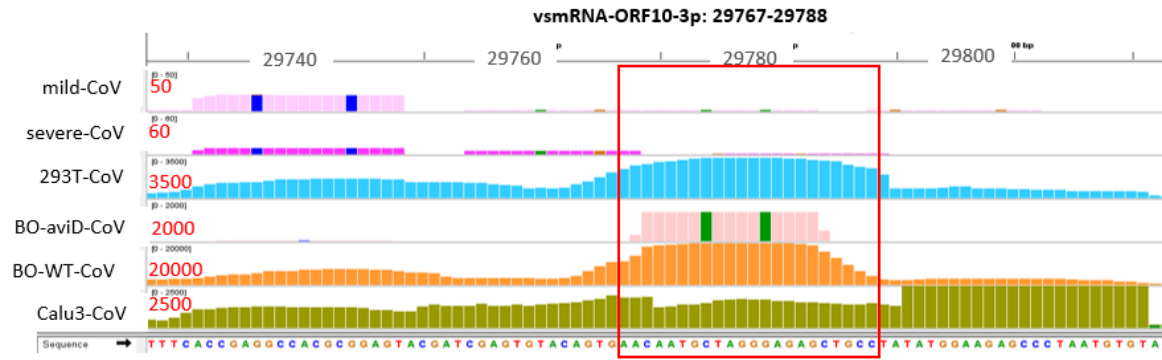


**Figure S3. IGV view, predicted tertiary structure, and sequence isoforms of vsmRNA-N1-5p, -N3-5p, and -ORF10-3p.**

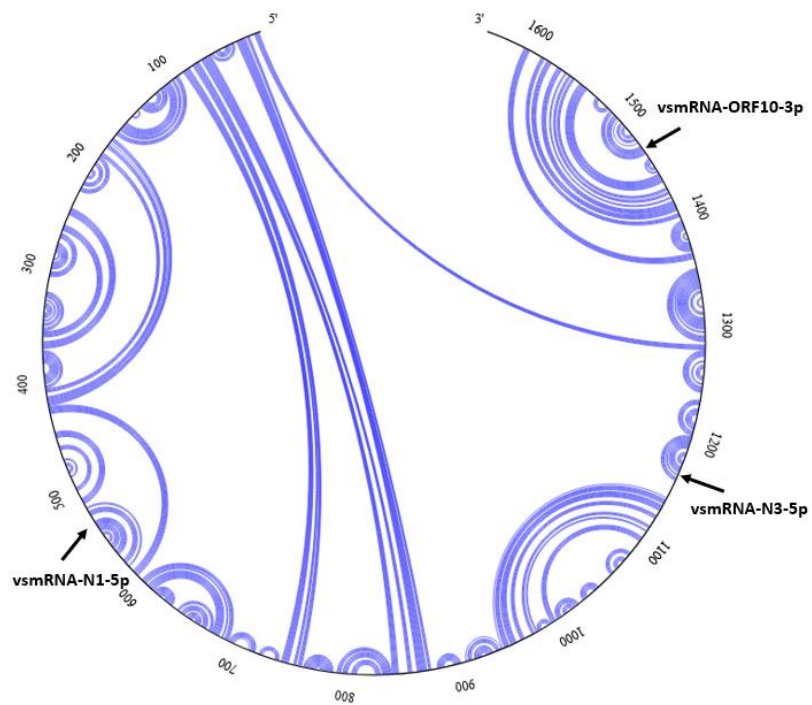
vsmRNAs were choose from datasets of SARS-CoV-2-infected HEK293T-hACE2, BO-WT-CoV, Calu3-EW. vsmRNA-N1-5p (AGA AGG GAG CAG AGG CGG CAG U), located at NC-045512.2:28,792-28,813(+); vsmRNA-N3-5p (ACC GCA GAG ACA GAA GAA ACA GC), located at NC-045512.2:29,419-29441; and vsmRNA-ORF10-3p (AAC AAT GCT AGG GAG AGC TGC C), located at NC-045512.2:29,767-29,788

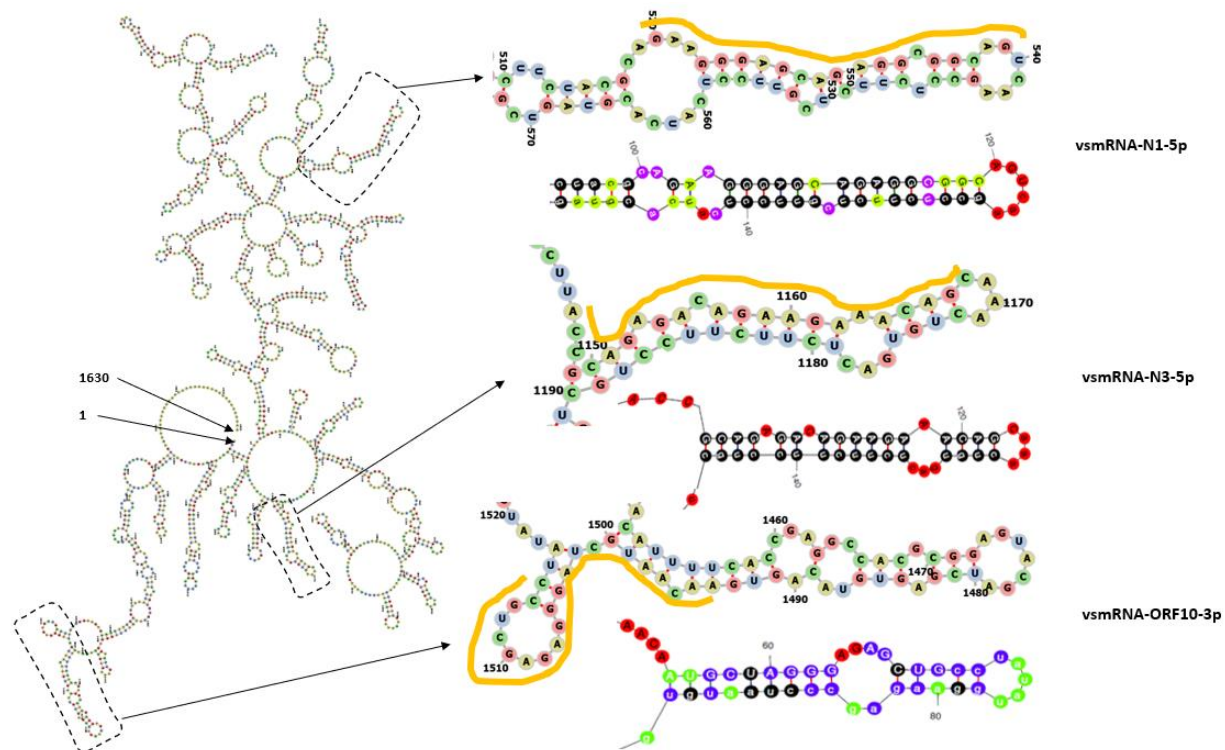
(A) Reads coverage for vsmRNA-N1,-N3, and -ORF10 on the genome of SARS-CoV-2. Mild-CoV: mild cases of COVID-19 patients; Severe-CoV: severe cases of COVID-19 patients; 293T-CoV: SARS-CoV-2 infected HEK293T-hACE2 cells; BO-aviD-CoV: Dicer knock out BO cells overexpressing aviD; BO-WT-CoV: Wild type BO cells overexpressing both endogenous Dicer and aviD; Calu3-CoV: SARS-CoV-2 infection of Calu-3 cells (Calu3-EW).





(B) LinearFold and mFold predicted tertiary structure of N-ORF10-3' UTR, vsmRNA-N1-5p, -N3-5p, and -ORF10-3p.



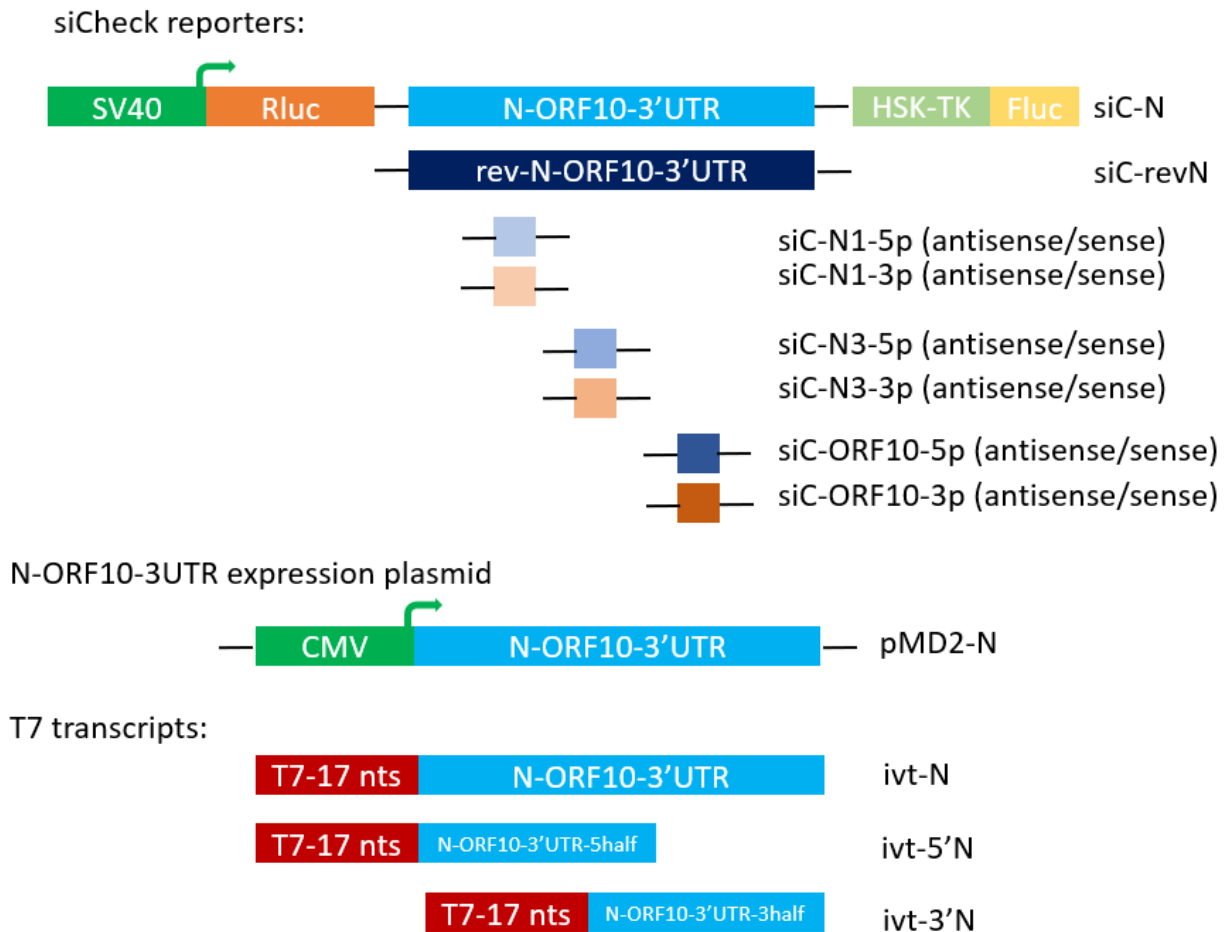


(C) Major sequence isoforms of vsmRNA-N1-5p, -N3-5p, and -ORF10-3p. Total: total reads count of top expressed isoforms. Rest: total reads count of other isoforms excluding the top expressed isoforms.

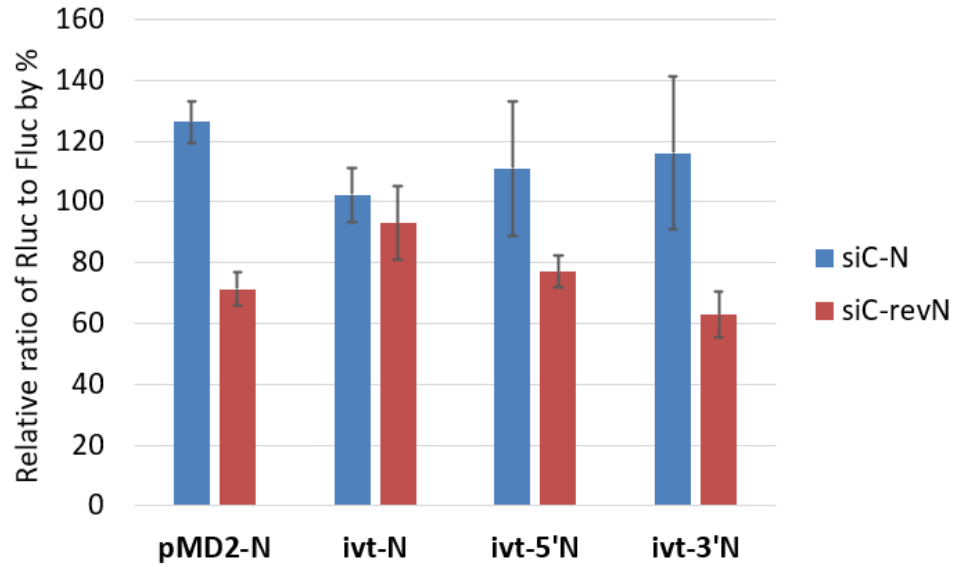
vsmRNA	Sequence	Reads # nts		%
N1	CGCAGAAGGGAGCAGAGGCGGCAGT	45	25	7.61
	AGGGAGCAGAGGCGGCAGT	31	19	5.25
	AAGGGAGCAGAGGCGGCAGT	22	20	3.72
	CTACGCAGAAGGGAGCAGAGGCGGCAGT	18	28	3.05
	GAAGGGAGCAGAGGCGGCAG	16	20	2.71
	AGAAGGGAGCAGAGGCGGCAGT	15	22	2.54
	GAAGGGAGCAGAGGCGGCAG	15	19	2.54
	GAAGGGAGCAGAGGCGGCAGT	15	21	2.54
	ACGCAGAAGGGAGCAGAGGCGGCAG	14	24	2.37
	AGAAGGGAGCAGAGGCGGCAG	14	21	2.37
	Total	205		34.69
	Rest	386		65.31
N3	ACCGCAGAGACAGAAGAAACAGCAAAC	56	28	3.26
	ACCGCAGAGACAGAAGAA	49	18	2.85
	CCGCAGAGACAGAAGAA	48	17	2.79
	ACCGCAGAGACAGAAGAAAC	46	20	2.68
	CCGCAGAGACAGAAGAAAC	40	19	2.33
	CCGCAGAGACAGAAGAAA	34	18	1.98
	ACCGCAGAGACAGAAGAAACAGCAAAC	33	27	1.92
	CCGCAGAGACAGAAGA	32	16	1.86
	AGAGACAGAAGAAACAGC	31	18	1.80
	GCAGAGACAGAAGAAACAGC	31	20	1.80
	Total	400		23.28
	Rest	1318		76.72
ORF10	GAACAATGCTAGGGAGAGCTGCCT	223	24	8.22
	ACAATGCTAGGGAGAGCTGCCT	135	22	4.97
	GTGAACAATGCTAGGGAGAGCTGCCT	116	26	4.27
	TGAACAATGCTAGGGAGAGCTGCCT	116	25	4.27
	AATGCTAGGGAGAGCTGCCT	84	20	3.10
	AGTGAACAATGCTAGGGAGAGCT	84	23	3.10
	ACAGTGAACAATGCTAGGGAGAGCTGCCT	80	29	2.95
	AACAATGCTAGGGAGAGCTGCCT	78	23	2.87
	ACAATGCTAGGGAGAGCTGCC	68	21	2.51
	AGTGAACAATGCTAGGGAGAGCTGCC	67	26	2.47
	Total	1051		38.73
	Rest	1663		61.27

(D) siCheck reporters, plasmid and *in vitro* transcription produced transcripts of N-ORF10-3'UTR. siC-N: siCheck reporter carrying the N-ORF10-3'UTR sequence; siC-revN: siCheck reporter carrying the antisense sequence of N-ORF10-3'UTR; siC-N1-5p and 3p: siCheck reporters carrying both antisense or sense sequences of vsmRNA-N1, respectively; siC-N3-5p and 3p: siCheck reporters carrying both antisense or sense sequences of vsmRNA-N3,

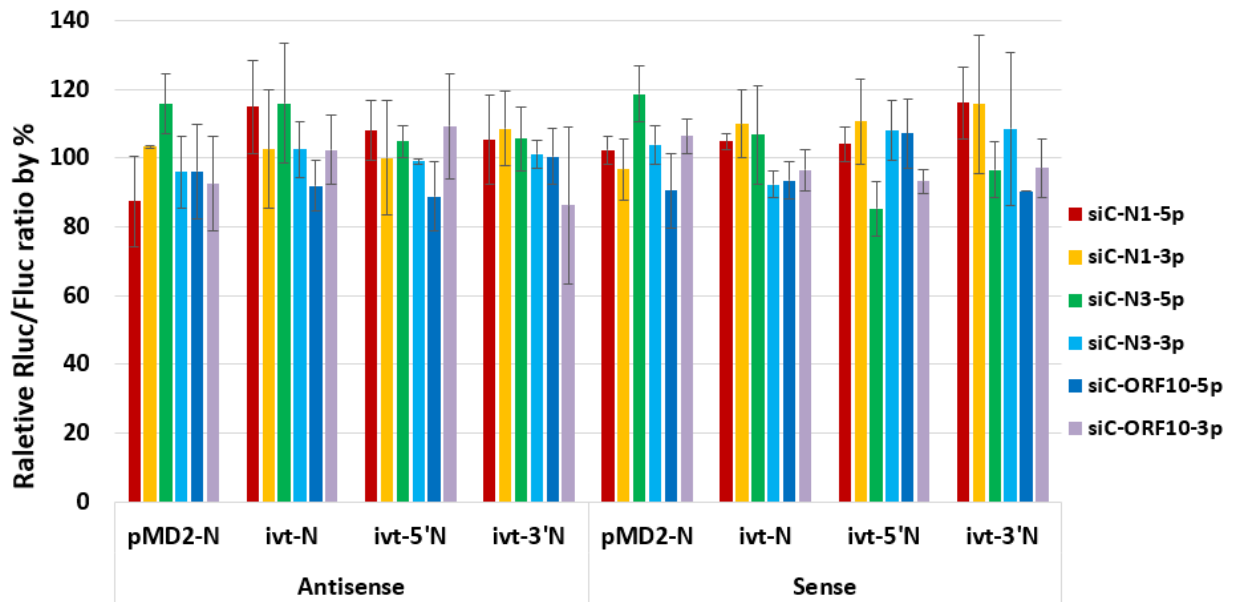
respectively; siC-ORF10-5p and 3p: siCheck reporters carrying both antisense or sense sequences of vsmRNA-ORF10, respectively; pMD2-N: plasmid carrying CMV promoter-driven N-ORF10-3'UTR sequence; ivt-N: T7 *in vitro* transcription produced N-ORF10-3'UTR; ivt-5'N: *in vitro* transcription produced 5' half of N-ORF10-3'UTR; ivt-3'N: *in vitro* transcription produced 3' half of N-ORF10-3'UTR.



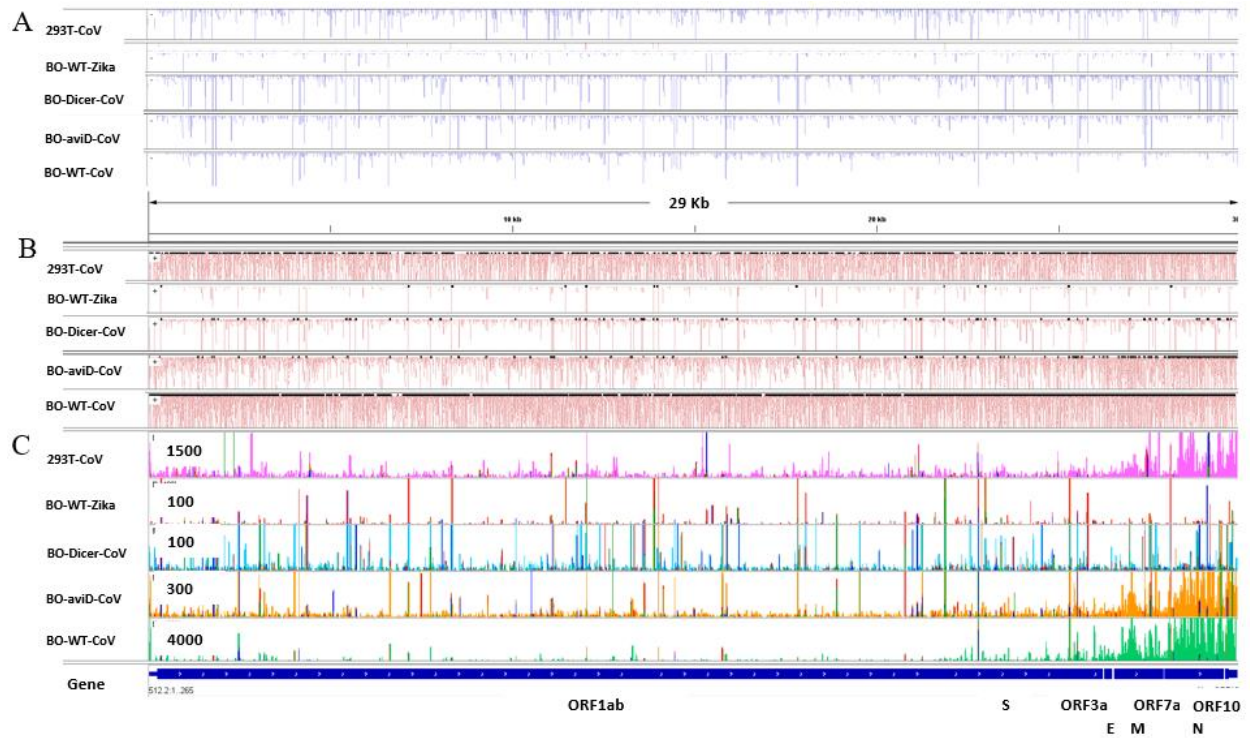
(E) siCheck reporter assay of silencing of siC-N and siC-revN by N-ORF10-3'UTR plasmid or T7 transcripts (ivt).



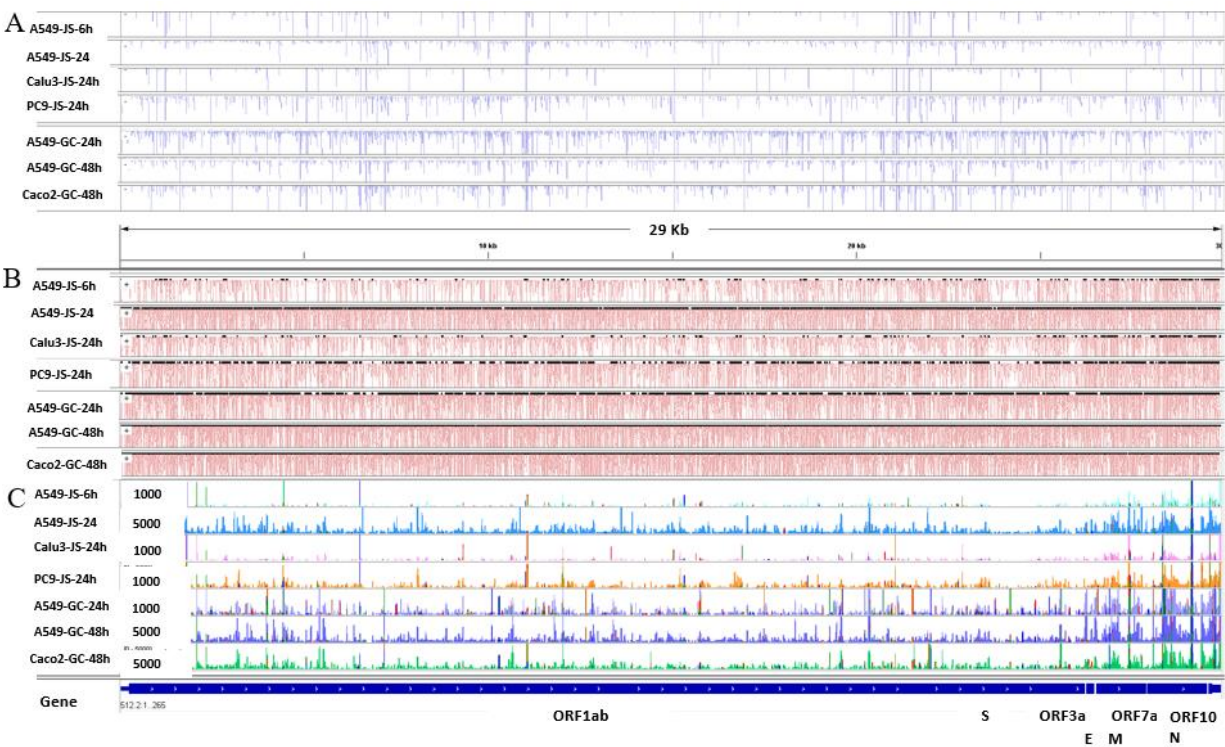
(F) siCheck reporter assay of silencing of 5p and 3p reporters of vsmRNA-N1,-N3, and ORF10 by N-ORF10-3'UTR plasmid or T7 transcripts (ivt).



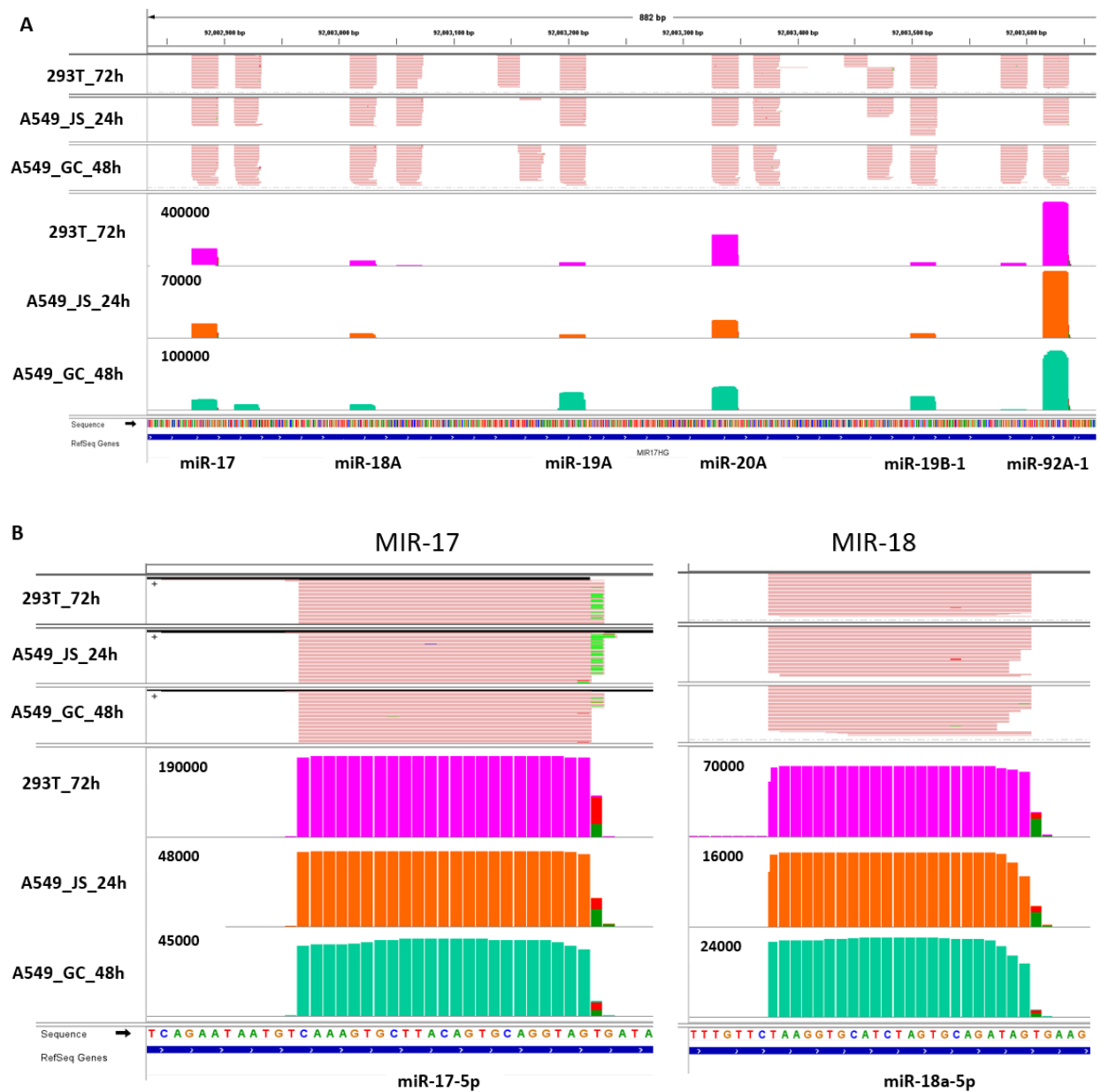
**Figure S4. Comparative IGV view of reads from smRNAseq of HEK293T-hACE2 and mouse brain organoids mapped to the SARS-CoV-2 genome. (A) Reads mapped to the full-length negative genome of SARS-CoV-2; (B) Reads mapped to the full-length positive genome of SARS-CoV-2; (C) Reads coverage on full-length SARS-CoV-2 genome.**



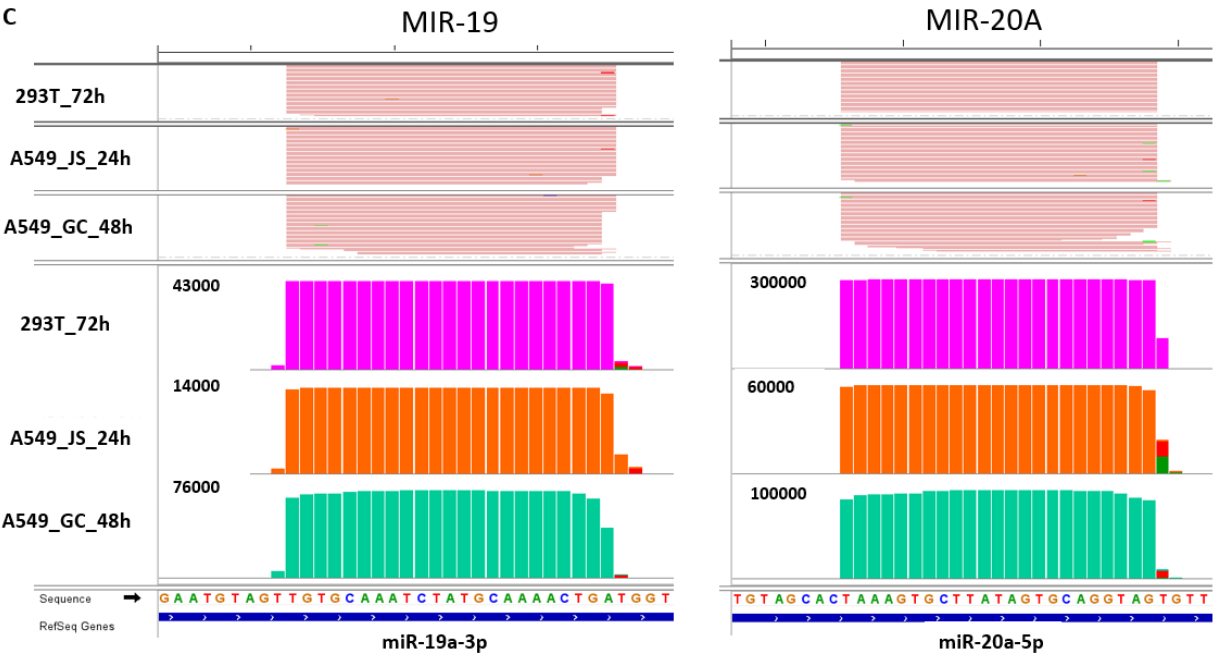
**Figure S5. Comparative IGV view of reads from smRNAseq datasets of GC and JS.** (A) Reads mapped to the full-length negative genome of SARS-CoV-2; (B) Reads mapped to the full-length positive genome of SARS-CoV-2; (C) Reads coverage on full-length SARS-CoV-2 genome.



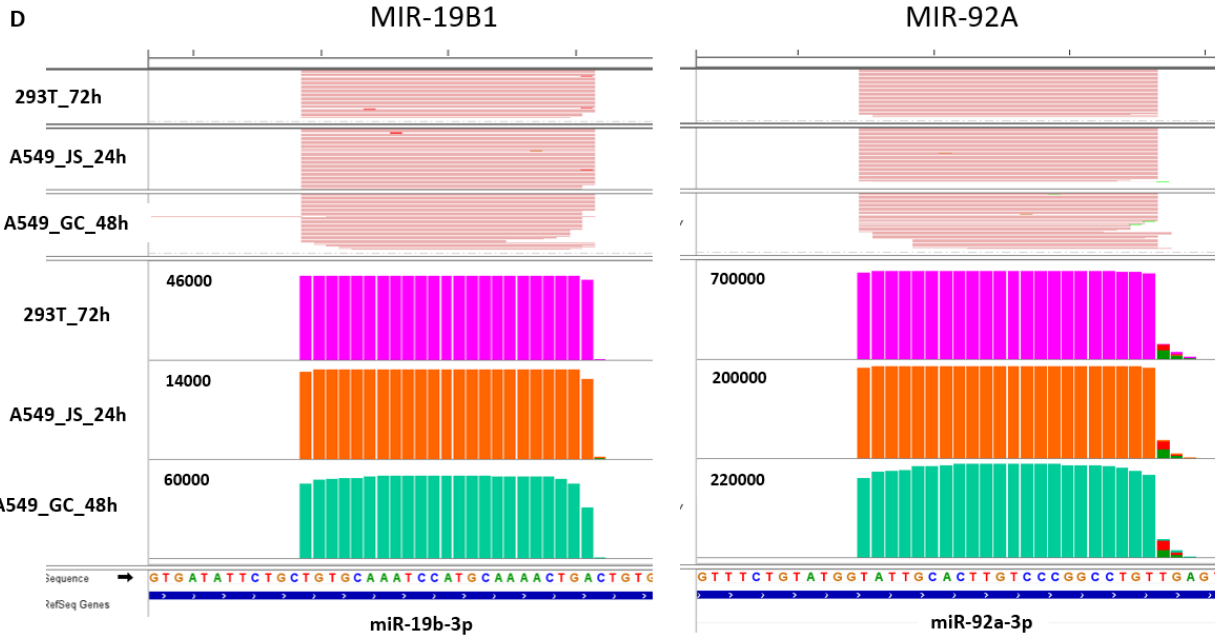
**Figure S6. IGV view of human miRNAs in SARS-CoV-2 infected HEK293T-hACE2 cells and A549-hACE2 cells.** Reads distribution and coverage for human miRNAs in SARS-CoV-2 infected HEK293T-hACE2 at 72hpi (HEK293T\_72h), A549-hACE2 at 24hpi (A549\_JS\_24h), and A549-hACE2 at 48hpi (A549\_GC\_48h). (A) miR-17 cluster: miR-17, miR-18A, miR-19A, miR-20A, miR-19B-1, and miR-92A-1; (B) miR-17-5p and miR-18a-5p; (C) miR-19a-3p and miR-20a-5p; (D) miR-19b-3p and miR-92a-3p; (E) miR-7-5p and miR-29b-3p.



C

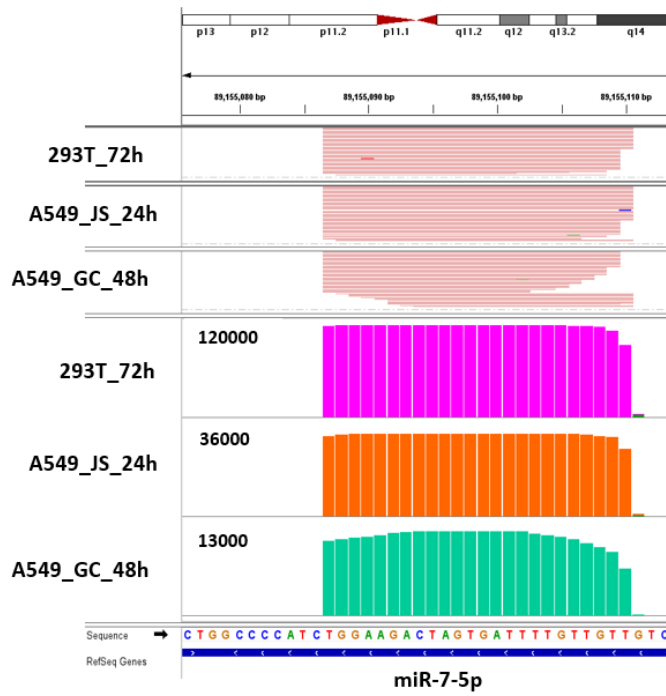


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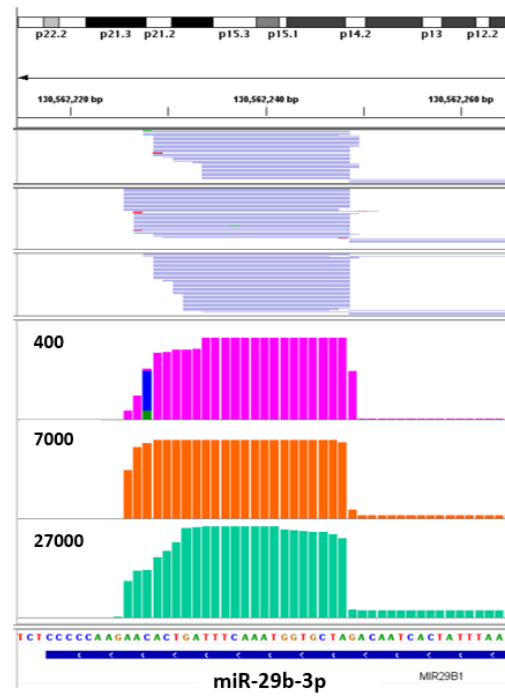


E

## MIR-7-2



## MIR-29B-1





## 2. Supplementary tables

**Table S1.** Oligos used in the study.

Name	Sequence (5' to 3')	Note
Rev-polyT	CAGTGCAGGGTCCGAGGT	smRNA qRT-PCR
f-210-3p	CTGTGCGTGTGACAGCG	
rt-210-3p	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTTTCAGCC	
f-222-3p	AGCTACATCTGGCTACTGGG	
rt-222-3p1	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTTAGACCC	
rt-222-3p2	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTGAGACC	
rt-222-3p3	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTAGAGAC	
f-30d-5p	TGTAAACATCCCGACTGGAAG	
rt-30d-5p1	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTGCTTCC	
rt-30d-5p2	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTAGCTTC	
f-874-3p	CTGCCCTGGCCCGAGGGA	
rt-874-3p1	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTGTCCGT	
rt-874-3p2	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTAGTCGG	
f-Asp-GTC-2-8	CGCGGGAGACCGGGGTTTC	
rt-Asp-GTC-2-8	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTTGGCTC	
f-Glu-CTC-1-6	agTGGTTAGGATTCGGCGC	
rt-Glu-CTC-1-6	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTGCGCGC	
f-Glu-CTC-2-1	TCCCTGGTGGTCTAGTGGT	
rt-Glu-CTC-2-1-p1	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTATCCTA	
rt-Glu-CTC-2-1-p2	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTGAGAGC	
f-hY4	GGCTGGTCCGATGGTAGT	
rt-hY4-p1	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTGTCTG	
rt-hY4-p2	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTAGTTCT	
rt-hY4-p3	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTAAGTTC	
f-10a-5p	gatccTACCCTGTAGATCCGAATT	
rt-10a-5p	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTAATTCG	
f-484	TCAGGCTCAGTCCCCTCC	
rt-484-p1	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTGGGAGG	
rt-484-p2	CAGTGCAGGGTCCGAGGTGACAGCCACCTGGGCAATTTTTTTTTTTATCGGG	
5N28791-5p	tcgaGAACGAGAAGAGGCTTGACTGCCGCCTCTGCTCCCTTCTGCGTAGAAGCCTTTTGG	siC-N1-5p
3N28791-5p	ctagccaaaagccttctacgcAGAAGGAGCAGAGGCGGCAGTcaagcctctctcgttc	siC-N1-3p
5N28791-3p	tcgaGGAGTTGAATTTCTTGAAGTGTGCGACTACGTGATGAGGAACGAGAAGAGGCTTG	
3N28791-3p	ctagcaagcctcttctcgttctctcatcacgtagtcgcaacagttcaagaaattcaactcc	siC-N3-5p
5N29419-5p	tcgaAGAAGAGTCACAGTTTGCTGTTTCTTCTGTCTCTGCGGTAAGGCTTGAGTTTCATC	
3N29419-5p	ctaggatgaaaactcaagccttACCAGCAGAGACAGAAGAAACAGCaaactgtgactcttct	siC-N3-3p
5N29419-3p	tcgaTTGCAATTGTTTGGAGAAATCATCCAAATCTGCAGCAGGAAGAAGAGTCACAGTTT	
3N29419-3p	ctagaaaactgtgactcttcttctcgtgctgcagatttgatgatttctccaaacaattgcaa	siC-ORF10-5p
5ORF10-29767-5p	tcgaTAGGGCTCTTCCATATAGGCAGCTCTCCCTAGCATTGTTCACTGTACACTCGATCG	
3ORF10-29767-5p	ctagcgatcgagtgtacagtgAACAAATGCTAGGGAGAGCTGCCtatatggaagagcccta	siC-ORF10-3p
5ORF10-29767-3p	tcgaTCACATGGGGATAGCACTACTAAATTAATTTTACACATTAGGGCTCTTCCATATA	
3ORF10-29767-3p	ctagtatatggaagagccctaagtgtgtaaaattaatttttagtagtgctatccccatgtga	

### 3. Supplementary data files

File\_S1A\_ HEK293T-hACE2-smRNAseq\_miRNA and tRFs read count and DESeq2 results infected vs uninfected\_miRge3.xlsx

File\_S1B\_ HEK293T-hACE2-smRNAseq\_miRNA read count and DESeq2 results infected vs uninfected\_miRge3.xlsx

File\_S1C\_ HEK293T-hACE2-smRNAseq\_miRNA read count and DESeq2 results infected vs uninfected\_miRge2.xlsx

File\_S2A\_ raw miRNA read count\_Calu3\_EW-Plasma-II of h-m-s-COVID-19.xlsx

File\_S2B\_ HEK293T vs GC JS datasets-DESeq2 results of log2FC-p-adj-baseMean.xlsx

File\_S3A\_miRDB predicted miRNAs targeting the SARS-CoV-2 -- list and rank.docx

File\_S3B\_miRDB predicted host miRNA seed on SARS-CoV-2 genome.docx

File\_S4\_normalized read count of human miRNAs from the second plasma dataset of COVID-19 patients.xlsx

File\_S5\_Filtered HEK293T miRNA count.xlsx contains miRge analyzed original raw miRNA counts in HEK293T-hACE2 cells.xlsx

File\_S6\_GC and JS datasets- DESeq2 results infected vs uninfected.xlsx