Supplemental Information

Loss of detection of sgN precedes viral abridged replication in COVID19-affected patients – a target for SARS-CoV-2 propagation

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Supplementary Figures

Supplementary Figure S1

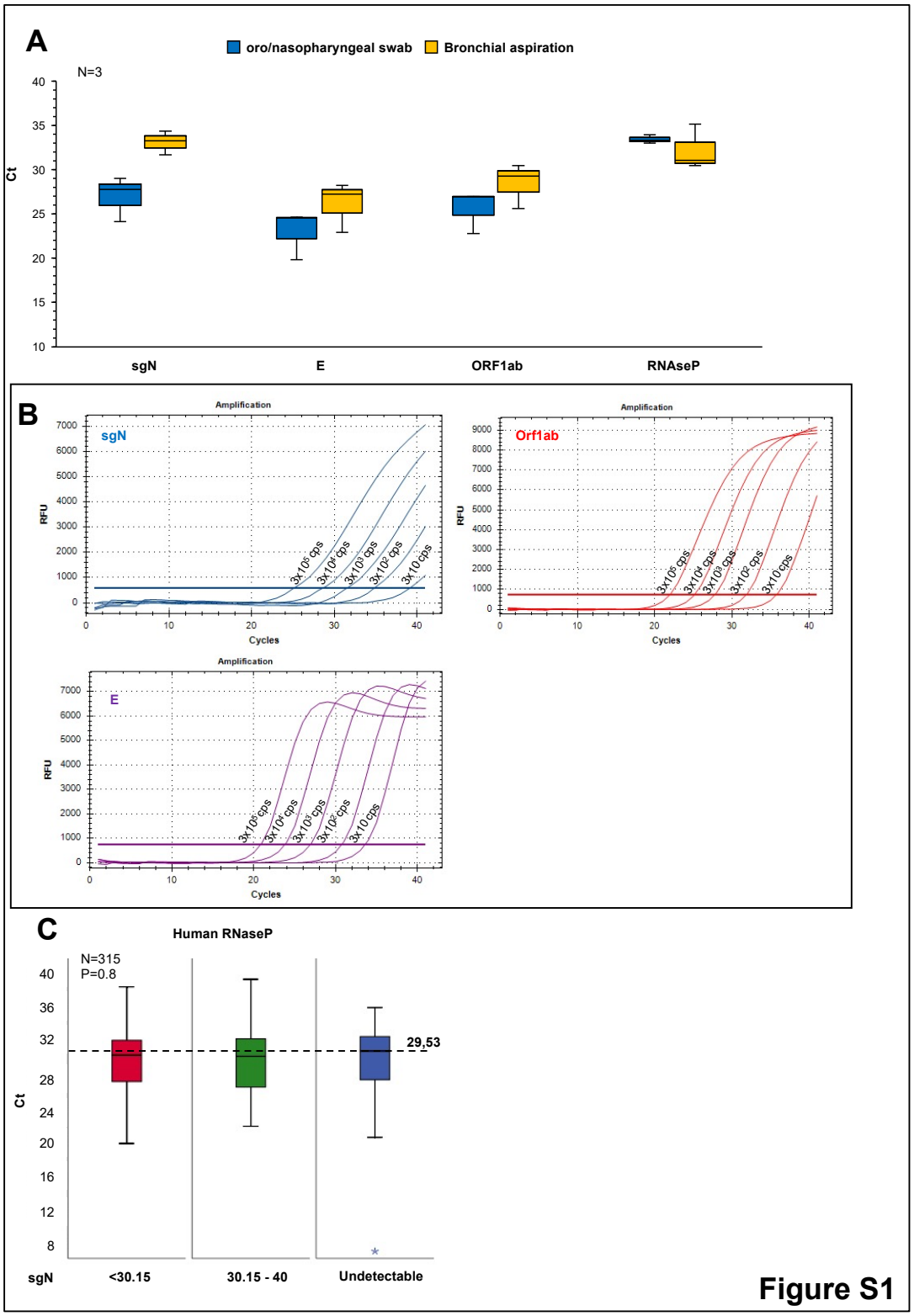

Figure S1

Supplementary Figure S1. SARS-CoV-2 Viral3 kit to detect sgN. Related to Figures 1. (A) Detection of viral sgN, gene E, gene ORF1ab and human RNAse P gene using the SARS-CoV-2 Viral3 kit for oro/nasopharyngeal swabs (orange) and bronchial aspirate samples (orange) in 3 hospitalized COVID19-positive patients. Mean Cq values are shown. **(B)** qPCR assays for the sensitivity of the SARS-CoV-2 Viral3 kit. Human 2019-nCoV strain 2019-nCoV/Italy INMI1 RNA was used as reference to test the sensitivity. Serial dilutions (from 3×10^5 to 3×10) of Human 2019-nCoV strain 2019-nCoV/Italy INMI1 RNA) were used. cps, standard copy particles. **(C)** Samples obtained from oro/nasopharyngeal swabs from COVID19-positive patients (N = 315) were stratified into three groups according to the median Cq values of sgN (sgN Cq median = 33.51). The first group consisted of those samples where Cq values for sgN were below the median value (i.e., Cq <30.51; 99 samples, in red). The second group of samples were characterized by Cq values of sgN ranged from the Cq median value (30.51) to 40 (96 samples, in green). Third group comprised samples in which sgN was not detectable (i.e., Cq >40; 120 samples, in blue). One-way analysis of variance (ANOVA) was used through IBM SPSS Statistics to determine the cut-off for sgN detection. SgN was detected in these samples independent of human RNAseP detection (P <0.8).

Supplementary Figure S2.

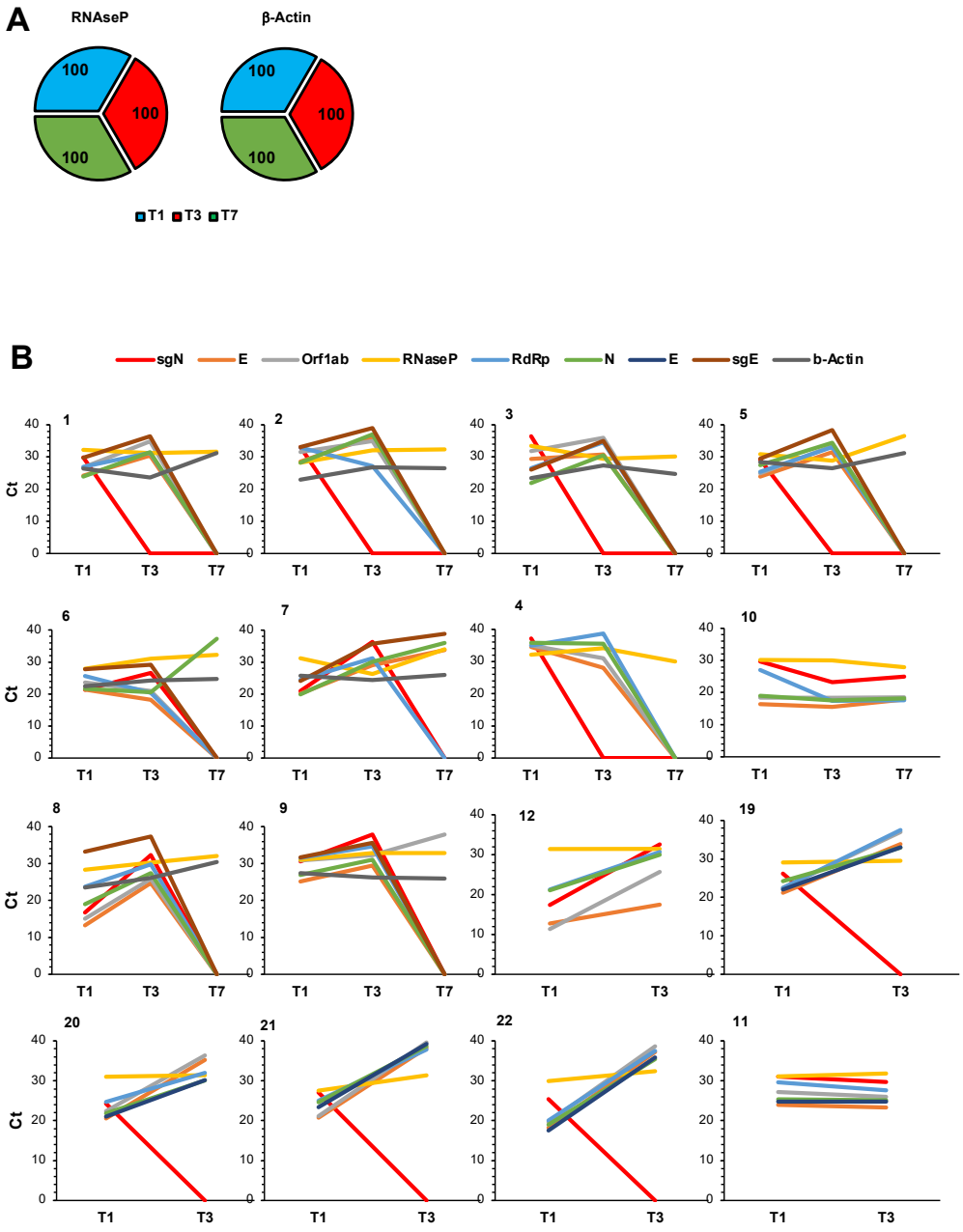

Figure S2

Supplementary Figure S2. Loss of detection of sgN precedes SARS-CoV-2 replication failure in home-isolated COVID19-affected patients. Related to Figures 2A-B. (A) Pie charts showing the proportions (%) of positivity of the oro/nasopharyngeal samples from home-isolated patients to human RNAse P and β-actin genes at the different times (blue, first swab [n = 16]; red, second swab collected after 3 days [n = 16]; green, third swab collected after 7 days [n = 10]). **(B)** Line graphs showing changes over time of the Cq values related to viral sgN (red), sgE (brown), gene N (green), gene E (orange), gene ORF1ab (light gray), RdRp (light blue), and human RNAse P (yellow) and β-actin (dark gray) genes in the oro/nasopharyngeal samples from 16 home-isolated patients.

Supplementary Figure S3.

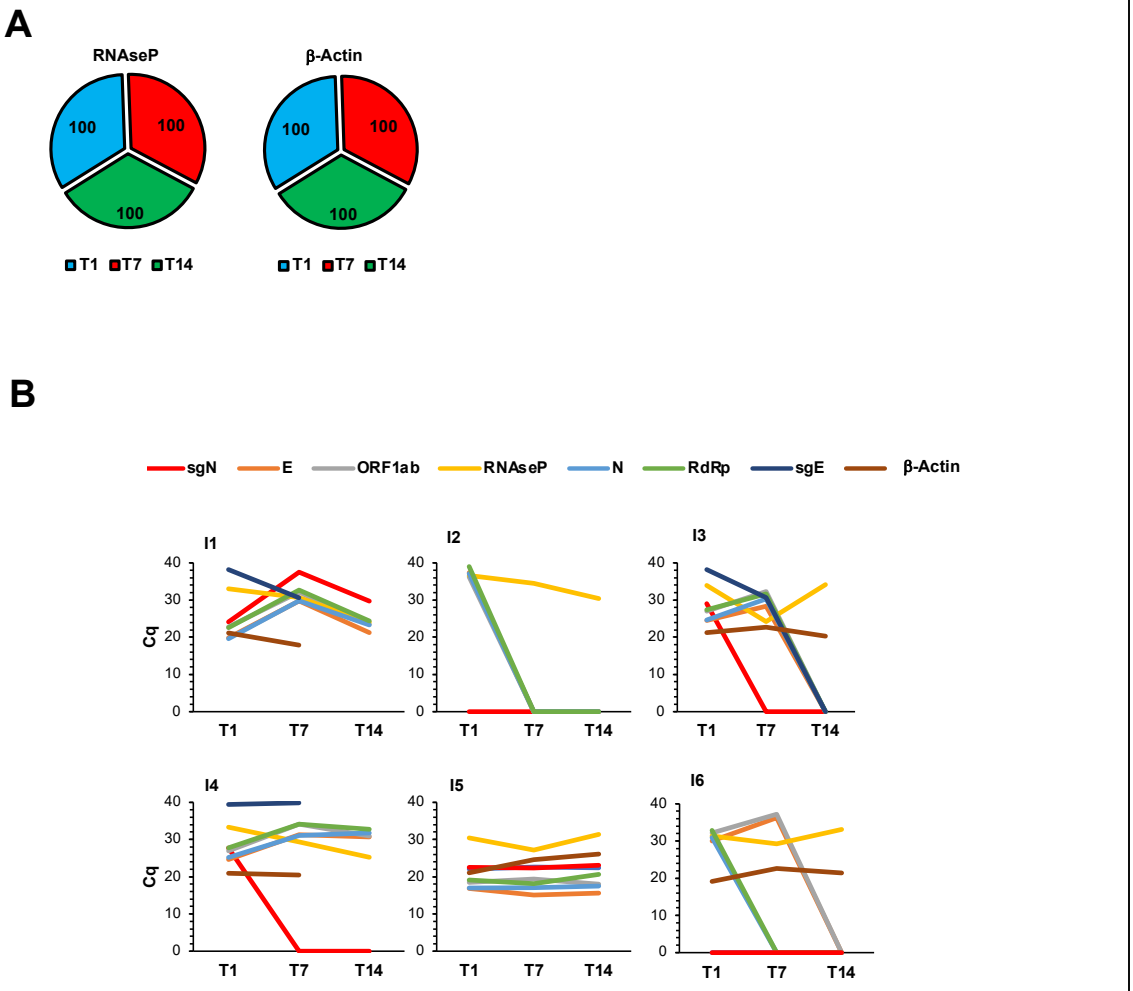

Figure S3

Supplementary Figure S3. Loss of detection of sgN precedes SARS-CoV-2 replication failure in home-isolated COVID19-affected patients. Related to Figures 2C-D. (A) Pie chart showing the proportions (%) of positivity of the oro/nasopharyngeal samples from 6 hospitalized COVID19-positive patients to human RNAse P gene at the different times (blue, first swab; red, second swab collected after 7 days; green, third swab collected after 14 days). **(B)** Line graphs showing the changes over time of the Cq values related to viral sgN (red), gene N (light blue), gene E (orange), gene ORF1ab (light gray), and RdRp (green), human RNAse P gene (yellow), sgE (dark blue and orange) and β-Actin (brown) in the oro/nasopharyngeal samples from 6 hospitalized patients.

Supplementary Figure S4.

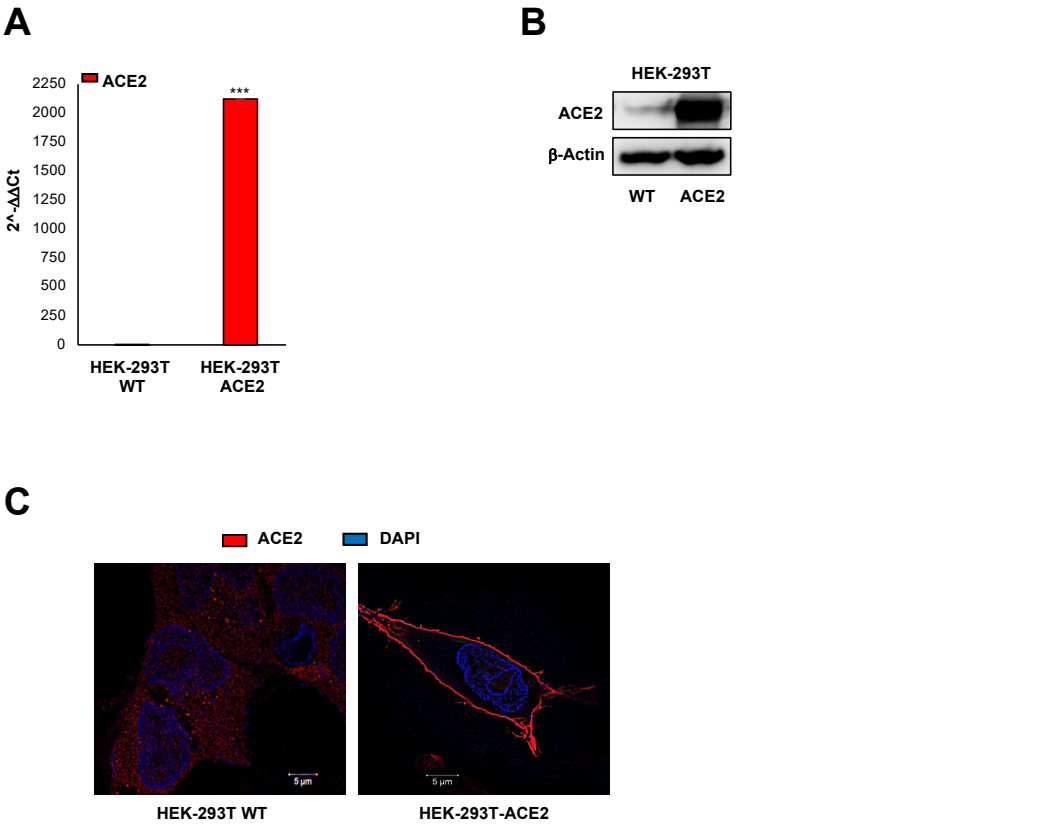

Figure S4

Supplementary Figure S4. HEK-293T overexpressing ACE2 cellular model. Related to Figures 3C-E, 4A-B. (A) Quantification of mRNA abundance relative to that of HEK-293T cells ($2^{-\Delta\Delta Ct}$) for the ACE2 gene. RT-PCR analysis with SYBR Green of RNA extracted from HEK-293T wild-type (WT) and HEK-293T–overexpressing ACE2 cells. Data are means ±SD. ***P <0.001 (unpaired two-tailed Student's t test; n = 3 independent experiments per group). **(B)** Representative immunoblotting (using antibodies against the indicated proteins) of human HEK-293T cells and HEK-293T stable clones overexpressing ACE2. B-Actin was used as the loading control. (**C)** Immunofluorescence staining with an antibody against the human ACE2 (red) protein in human HEK-293T cells and HEK-293T stable clones overexpressing ACE2. DAPI was used for nuclei. The SIM image was acquired with Elyra 7 and processed with the Zeiss ZEN software (blue edition). Magnification, ×63. Scale bars: 5 μm.

Supplementary Figure S5.

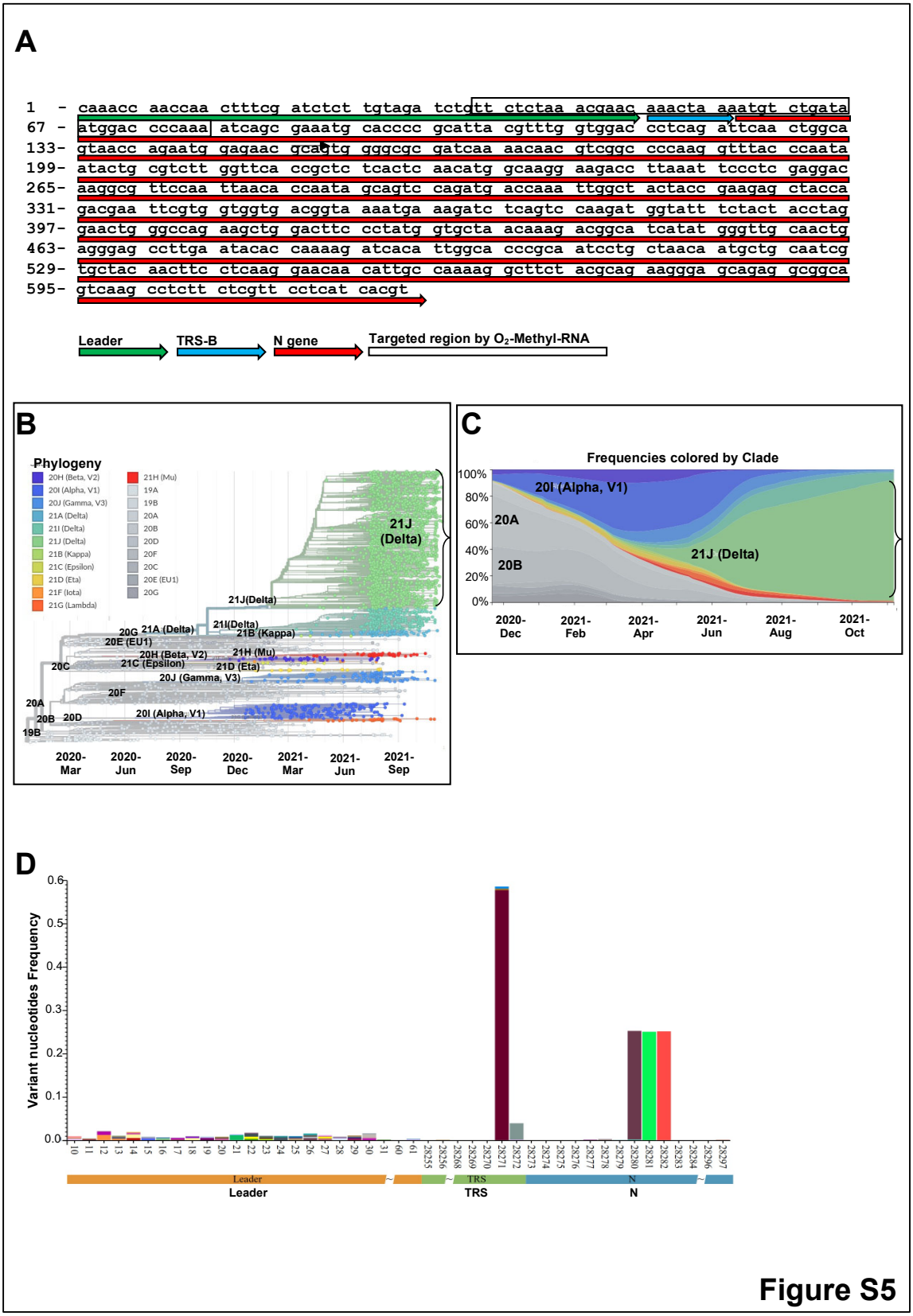

Figure S5

Supplementary Figure S5. 2'-*O*-methyl RNA against sgN sequence. Related to Figure 3A-B. (A) Sanger sequencing of the sgN region of SARS-CoV-2. Green arrow indicates the leader sequence, blue arrow indicates the TRS upstream gene N, and red arrow indicates gene N. The region recognized by 2'-*O*-methyl antisense RNA is highlighted in yellow. **(B, C)** Clade 21J is now the predominant form of Delta, with an estimated ~90% global frequency. **(D)** Frequencies of variant nucleotides calculated on a collection of about 350,000 sequences. Dataset downloaded from Global Initiative on Sharing Avian Influenza Data (GISAID) on November 15, 2021.

Supplementary Tables

Supplementary Table S1: Related to Figure 1. Cq values obtained for 50 oro/nasopharyngeal swabs from COVID19-positive patients according to the SARS-CoV-2 Viral3 kit and the Allplex 2019-nCoV assay.

Code	Cq value						
	SARS-CoV-2 Viral3 kit				Allplex 2019-nCoV assay		
	E gene	sgN transcript	ORF1ab gene	RNAse P gene	E gene	N gene	RdRP/S gene
S8	16.36	20.37	15.83	33.28	13.84	15.58	14.16
S48	17.78	25.60	16.79	30.22	15.51	16.66	15.62
S4	15.15	18.56	14.54	30.56	12.08	13.38	15.65
S82	20.66	28.96	19.76	36.21	17.06	18.20	16.05
S37	19.38	24.17	18.49	30.75	13.30	15.67	16.64
S94	19.04	30.32	19.50	30.84	13.50	14.83	16.69
S16	16.31	22.26	16.20	31.43	13.79	15.73	16.74
S162	19.17	35.75	19.19	33.42	17.55	18.53	17.07
S33	18.88	23.82	17.82	30.80	17.34	18.56	17.18
S36	16.28	24.12	15.33	35.34	13.69	16.79	17.29
S35	17.26	24.02	16.62	31.80	16.60	16.48	17.33
S46	17.84	25.59	17.80	34.92	16.84	18.22	17.42
S43	19.37	24.63	18.52	31.58	16.88	18.78	17.46
S188	20.87	38.78	24.66	29.88	15.99	15.95	17.63
S6	16.54	19.28	16.00	31.48	16.58	17.68	17.67
S5	18.67	18.82	17.66	32.43	17.03	18.00	17.97
S11	15.80	20.82	16.31	31.15	15.78	15.92	18.34
S32	20.59	23.60	20.54	30.31	18.59	19.18	18.43
S23	18.47	22.73	17.53	32.01	16.74	16.91	18.53
S10	18.45	20.66	19.65	24.36	17.95	18.14	18.57
S27	20.58	23.31	19.50	31.98	19.04	19.77	18.75
S3	16.73	17.83	16.03	31.71	17.20	17.73	18.82
S25	18.71	23.09	19.06	34.34	18.68	19.87	18.88
S7	18.05	19.90	17.58	34.08	17.03	17.27	18.94
S62	21.45	26.65	23.51	38.09	19.23	20.55	19.09
S113	22.67	31.48	22.24	33.66	19.30	20.53	19.16
S59	22.20	26.50	21.13	34.97	19.56	21.16	19.28
S28	18.31	23.41	17.27	29.76	17.58	18.50	19.30
S29	18.75	23.56	19.09	34.11	17.90	17.60	19.68
S69	18.38	27.49	18.18	29.52	17.82	18.52	19.71
S42	19.15	24.57	19.19	32.09	19.07	20.46	20.07
S34	19.01	23.86	19.17	32.48	18.68	18.33	20.19
S100	22.07	30.67	21.77	33.91	20.54	21.73	20.37
S64	20.18	26.96	19.73	34.76	19.08	19.38	20.67
S9	18.78	20.63	18.21	31.34	18.63	18.67	20.70
S60	21.18	26.51	19.89	35.12	20.82	19.64	20.71
S70	21.18	27.65	21.16	38.10	18.84	19.21	20.94
S12	19.46	20.96	19.64	31.86	18.83	19.03	21.21

S61	19.59	26.54	19.72	33.18	19.43	19.54	21.25
S67	21.28	27.30	21.02	34.16	19.69	19.86	21.39
S73	26.40	27.75	26.50	35.30	24.31	24.95	23.14
S84	24.14	29.04	24.19	32.04	23.95	24.12	25.81
S120	28.05	31.87	28.04	36.70	28.22	28.68	26.32
S106	25.72	31.05	25.38	32.90	25.08	26.34	26.45
S163	30.14	35.75	29.64	36.23	28.30	27.76	26.56
S121	31.98	31.90	30.87	38.98	28.59	27.68	26.91
S156	29.34	35.06	29.15	35.59	28.79	28.89	27.15
S119	27.28	31.84	27.44	34.63	25.27	25.79	27.16
S96	28.64	30.49	28.91	27.29	26.11	25.69	28.21
S161	30.29	35.39	30.30	33.30	27.03	27.30	28.22

Supplementary Table S2. Related to Figure 1. Cq values obtained for 12 oro/nasopharyngeal swabs from negative COVID19-tested people according to the SARS-CoV-2 Viral3 kit and the Allplex 2019-nCoV assay.

Code	Cq values						
	SARS-CoV-2 Viral3 kit				Allplex 2019-nCoV assay		
	E gene	sgN transcript	ORF1ab gene	RNAse P gene	E gene	N gene	RdRP/S gene
N1	N.A.	N.A.	N.A.	38.26	N.A.	N.A.	N.A.
N2	N.A.	N.A.	N.A.	34.59	N.A.	N.A.	N.A.
N3	N.A.	N.A.	N.A.	34.82	N.A.	N.A.	N.A.
N4	N.A.	N.A.	N.A.	31.12	N.A.	N.A.	N.A.
N6	N.A.	N.A.	N.A.	37.75	N.A.	N.A.	N.A.
N7	N.A.	N.A.	N.A.	25.58	N.A.	N.A.	N.A.
N8	N.A.	N.A.	N.A.	28.54	N.A.	N.A.	N.A.
N9	N.A.	N.A.	N.A.	23.17	N.A.	N.A.	N.A.
N10	N.A.	N.A.	N.A.	26.42	N.A.	N.A.	N.A.
N11	N.A.	N.A.	N.A.	30.46	N.A.	N.A.	N.A.
N12	N.A.	N.A.	N.A.	26.49	N.A.	N.A.	N.A.

N.A., not amplified (i.e., Cq value >40).

Supplementary Table S3. Related to Figure 1A-B. Cq values obtained from the 315 oro/nasopharyngeal swabs from COVID19-positive patients with the SARS-CoV-2 Viral3 kit.

Code	Cq value				Code	Cq value			
	E	sgN	ORF1ab	RNAse P		E	sgN	ORF1ab	RNAse P
S1	16.18	15.12	16.41	28.45	**S159**	26.61	35.31	26.44	27.75
S2	20.43	17.02	21.45	29.23	**S160**	26.61	35.31	26.44	27.75
S3	16.73	17.83	16.03	31.71	**S161**	30.29	35.39	30.30	33.30
S4	15.15	18.56	14.54	30.56	**S162**	19.17	35.75	19.19	33.42
S5	18.67	18.82	17.66	32.43	**S163**	30.14	35.75	29.64	36.23
S6	16.54	19.28	16.00	31.48	**S164**	28.31	36.57	29.10	26.02
S7	18.05	19.90	17.58	34.08	**S165**	28.31	36.57	29.10	26.02
S8	16.36	20.37	15.83	33.28	**S166**	29.16	36.59	28.94	28.64
S9	18.78	20.63	18.21	31.34	**S167**	30.27	36.62	30.73	29.67
S10	18.45	20.66	19.65	24.36	**S168**	14.74	36.77	14.09	22.18
S11	15.80	20.82	16.31	31.15	**S169**	14.74	36.77	14.09	22.18
S12	19.46	20.96	19.64	31.86	**S170**	25.85	36.94	25.22	28.00
S13	15.12	21.79	17.46	26.59	**S171**	29.05	36.98	29.18	26.40
S14	15.12	21.79	17.46	26.59	**S172**	26.98	37.00	26.67	32.00
S15	15.16	22.05	14.65	27.00	**S173**	26.66	37.19	25.42	23.01
S16	16.31	22.26	16.20	31.43	**S174**	26.66	37.19	25.42	23.01
S17	17.70	22.34	18.73	26.82	**S175**	29.90	37.24	30.46	25.64
S18	17.70	22.34	18.73	26.82	**S176**	29.90	37.24	30.46	25.64
S19	17.35	22.41	17.52	24.75	**S177**	27.62	37.42	27.43	32.00
S20	17.35	22.41	17.52	24.75	**S178**	30.77	37.56	31.18	31.61
S21	20.04	22.61	20.32	26.64	**S179**	30.77	37.56	31.18	31.61
S22	20.04	22.61	20.32	26.64	**S180**	27.13	37.60	27.27	32.08
S23	18.47	22.73	17.53	32.01	**S181**	33.59	37.72	34.24	30.88
S24	23.13	23.07	23.73	28.47	**S182**	30.40	37.80	31.35	31.67
S25	18.71	23.09	19.06	34.34	**S183**	25.01	37.89	25.46	29.65
S26	17.70	21.43	17.94	29.25	**S184**	25.01	37.89	25.46	29.65
S27	20.58	23.31	19.50	31.98	**S185**	28.16	37.98	28.84	31.13
S28	18.31	23.41	17.27	29.76	**S186**	28.16	37.98	28.84	31.13
S29	18.75	23.56	19.09	34.11	**S187**	22.38	38.25	23.04	26.27
S30	18.87	23.59	19.13	26.55	**S188**	20.87	38.78	24.66	29.88
S31	18.87	23.59	19.13	26.55	**S189**	28.56	38.85	28.01	28.93
S32	20.59	23.60	20.54	30.31	**S190**	26.06	38.88	25.22	30.53
S33	18.88	23.82	17.82	30.80	**S191**	30.17	38.90	29.02	32.24
S34	19.01	23.86	19.17	32.48	**S192**	32.64	38.99	32.48	34.09
S35	17.26	24.02	16.62	31.80	**S193**	30.14	39.02	30.21	33.30
S36	16.28	24.12	15.33	35.34	**S194**	30.34	39.12	30.15	32.11
S37	19.38	24.17	18.49	30.75	**S195**	27.54	39.19	26.83	33.10
S38	20.05	24.22	20.97	30.60	**S196**	24.32	N.A.	23.17	25.21
S39	20.05	24.22	20.97	30.60	**S197**	26.50	N.A.	25.73	31.21
S40	21.42	24.40	22.02	26.14	**S198**	26.52	N.A.	26.52	26.43
S41	21.42	24.40	22.02	26.14	**S199**	26.63	N.A.	25.84	28.65
S42	19.15	24.57	19.19	32.09	**S200**	26.88	N.A.	27.61	33.86
S43	19.37	24.63	18.52	31.58	**S201**	26.94	N.A.	26.39	30.14
S44	20.51	25.03	20.63	28.18	**S202**	27.02	N.A.	25.57	25.00

S45	20.52	25.43	21.19	30.08	**S203**	27.06	N.A.	26.25	31.53
S46	17.84	25.59	17.80	34.92	**S204**	27.10	N.A.	26.11	27.56
S47	19.26	23.94	19.15	30.60	**S205**	27.15	N.A.	26.35	30.88
S48	17.78	25.60	16.79	30.22	**S206**	28.01	N.A.	28.08	21.13
S49	20.33	25.71	21.10	29.35	**S207**	28.12	N.A.	26.85	22.56
S50	19.37	25.78	19.73	25.70	**S208**	28.15	N.A.	29.20	31.10
S51	17.01	25.88	17.19	22.79	**S209**	28.54	N.A.	27.03	23.46
S52	17.01	25.88	17.19	22.79	**S210**	29.44	N.A.	28.43	21.68
S53	25.46	25.98	25.57	30.39	**S211**	29.47	N.A.	29.67	32.39
S54	19.61	26.00	20.50	30.43	**S212**	29.48	N.A.	29.80	32.75
S55	20.33	26.11	21.56	28.38	**S213**	29.66	N.A.	29.36	31.44
S56	20.15	26.15	20.81	28.35	**S214**	30.18	N.A.	30.60	28.63
S57	17.47	26.39	17.99	27.70	**S215**	30.35	N.A.	31.17	34.03
S58	17.47	26.39	17.99	27.70	**S216**	30.73	N.A.	29.51	25.68
S59	22.20	26.50	21.13	34.97	**S217**	30.77	N.A.	31.03	32.09
S60	21.18	26.51	19.89	35.12	**S218**	30.86	N.A.	29.52	31.64
S61	19.59	26.54	19.72	33.18	**S219**	31.10	N.A.	32.20	29.02
S62	21.45	26.65	23.51	38.09	**S220**	31.22	N.A.	31.75	32.61
S63	20.04	26.70	20.05	31.00	**S221**	31.29	N.A.	31.42	31.98
S64	20.18	26.96	19.73	34.76	**S222**	31.39	N.A.	32.22	24.57
S65	21.38	27.24	22.25	26.96	**S223**	31.39	N.A.	31.47	32.37
S66	21.38	27.24	22.25	26.96	**S224**	31.45	N.A.	31.36	32.47
S67	21.28	27.30	21.02	34.16	**S225**	31.47	N.A.	31.92	31.60
S68	22.07	27.40	21.94	31.11	**S226**	31.51	N.A.	32.14	33.78
S69	18.38	27.49	18.18	29.52	**S227**	31.63	N.A.	31.82	34.04
S70	21.18	27.65	21.16	38.10	**S228**	31.69	N.A.	31.58	32.29
S71	21.60	27.70	21.03	30.09	**S229**	31.71	N.A.	31.85	30.55
S72	18.59	27.74	25.70	27.47	**S230**	31.95	N.A.	30.63	23.50
S73	26.40	27.75	26.50	35.30	**S231**	32.00	N.A.	32.02	26.08
S74	20.46	27.88	20.10	31.23	**S232**	32.15	N.A.	31.31	23.07
S75	20.67	28.11	22.61	29.13	**S233**	32.16	N.A.	33.77	26.18
S76	20.15	28.13	20.94	28.82	**S234**	32.20	N.A.	32.54	35.03
S77	20.15	28.13	20.94	28.82	**S235**	32.22	N.A.	32.35	30.50
S78	19.28	28.20	19.32	27.46	**S236**	32.40	N.A.	33.78	34.06
S79	24.13	28.64	24.52	29.42	**S237**	32.40	N.A.	33.23	33.86
S80	18.59	28.75	17.64	25.18	**S238**	32.53	N.A.	32.19	27.44
S81	18.59	28.75	17.64	25.18	**S239**	32.69	N.A.	32.55	23.08
S82	20.66	28.96	19.76	36.21	**S240**	32.75	N.A.	32.02	31.48
S83	16.36	29.02	16.64	19.82	**S241**	32.75	N.A.	32.76	33.57
S84	24.14	29.04	24.19	32.04	**S242**	32.89	N.A.	33.02	27.95
S85	24.92	29.23	24.69	31.00	**S243**	32.98	N.A.	33.18	31.03
S86	18.45	29.29	26.88	28.45	**S244**	33.05	N.A.	31.81	27.02
S87	18.75	29.52	19.69	20.76	**S245**	33.10	N.A.	32.11	31.61
S88	24.25	29.53	24.07	31.00	**S246**	33.12	N.A.	33.02	28.33
S89	23.79	29.66	23.34	31.90	**S247**	33.13	N.A.	33.20	31.20
S90	24.32	29.82	24.83	27.84	**S248**	33.15	N.A.	33.43	30.83
S91	18.52	30.18	17.72	25.35	**S249**	33.15	N.A.	32.97	32.37
S92	18.52	30.18	17.72	25.35	**S250**	33.23	N.A.	32.75	32.23
S93	22.44	30.24	22.75	30.25	**S251**	33.25	N.A.	32.22	26.29
S94	19.04	30.32	19.50	30.84	**S252**	33.30	N.A.	33.04	33.50

S95	16.89	30.42	18.91	19.71	**S253**	33.34	N.A.	34.05	29.96
S96	28.64	30.49	28.91	27.29	**S254**	33.38	N.A.	33.56	30.59
S97	24.37	30.49	24.43	32.00	**S255**	33.44	N.A.	33.68	25.23
S98	23.24	30.49	22.81	32.08	**S256**	33.49	N.A.	33.07	27.39
S99	22.06	30.51	30.21	29.12	**S257**	33.67	N.A.	33.03	34.98
S100	22.07	30.67	21.77	33.91	**S258**	33.70	N.A.	32.72	21.89
S101	25.61	30.69	25.58	33.29	**S259**	33.74	N.A.	33.96	31.10
S102	21.08	30.76	20.53	29.81	**S260**	33.84	N.A.	34.09	26.58
S103	23.30	30.76	23.85	30.39	**S261**	33.85	N.A.	32.48	29.74
S104	21.36	30.84	22.62	22.84	**S262**	33.95	N.A.	33.95	26.49
S105	22.56	31.05	23.08	31.70	**S263**	34.09	N.A.	33.69	34.35
S106	25.72	31.05	25.38	32.90	**S264**	34.10	N.A.	33.74	28.07
S107	19.69	31.06	29.08	28.42	**S265**	34.10	N.A.	33.35	30.66
S108	28.16	31.07	28.53	32.00	**S266**	34.14	N.A.	35.26	34.66
S109	22.14	31.11	27.65	29.21	**S267**	34.19	N.A.	35.11	28.51
S110	23.80	31.27	23.59	30.10	**S268**	34.23	N.A.	34.87	30.28
S111	18.95	31.29	19.45	23.45	**S269**	34.25	N.A.	34.61	22.51
S112	26.14	31.42	25.98	28.36	**S270**	34.25	N.A.	32.92	31.49
S113	22.67	31.48	22.24	33.66	**S271**	34.25	N.A.	35.98	28.15
S114	21.48	31.58	22.66	25.70	**S272**	34.26	N.A.	35.01	30.14
S115	22.10	31.65	21.05	21.73	**S273**	34.33	N.A.	33.62	26.48
S116	22.10	31.65	21.05	21.73	**S274**	34.36	N.A.	35.03	32.93
S117	24.28	31.68	25.58	31.35	**S275**	34.37	N.A.	34.51	20.43
S118	25.25	31.70	25.24	32.01	**S276**	34.48	N.A.	35.23	32.24
S119	27.28	31.84	27.44	34.63	**S277**	34.50	N.A.	34.41	31.16
S120	28.05	31.87	28.04	36.70	**S278**	34.50	N.A.	34.60	31.16
S121	31.98	31.90	30.87	38.98	**S279**	34.59	N.A.	34.40	33.33
S122	25.74	31.98	26.20	30.02	**S280**	34.68	N.A.	33.89	30.21
S123	24.21	32.09	23.83	30.06	**S281**	34.73	N.A.	34.49	34.39
S124	23.43	32.25	23.94	28.28	**S282**	34.73	N.A.	35.13	33.30
S125	26.33	32.25	26.27	31.44	**S283**	34.90	N.A.	35.58	31.01
S126	20.19	32.36	20.10	27.42	**S284**	35.06	N.A.	34.38	27.45
S127	25.41	32.40	24.54	29.38	**S285**	35.12	N.A.	33.81	21.71
S128	21.69	32.46	24.69	23.94	**S286**	35.12	N.A.	37.19	30.61
S129	26.89	32.53	26.45	29.14	**S287**	35.16	N.A.	34.67	7.06
S130	20.78	32.58	19.59	25.15	**S288**	35.18	N.A.	35.74	25.19
S131	20.78	32.58	19.59	25.15	**S289**	35.20	N.A.	36.04	30.15
S132	24.49	32.68	26.02	28.34	**S290**	35.34	N.A.	36.49	31.28
S133	26.83	32.83	31.92	33.50	**S291**	35.40	N.A.	35.57	26.87
S134	18.68	32.85	18.97	26.55	**S292**	35.47	N.A.	35.91	35.66
S135	29.20	32.97	29.47	38.03	**S293**	35.76	N.A.	36.34	32.81
S136	27.45	33.04	29.30	29.65	**S294**	35.83	N.A.	37.33	28.48
S137	27.57	33.06	27.97	30.07	**S295**	35.94	N.A.	38.10	30.48
S138	25.67	33.32	26.17	31.17	**S296**	36.00	N.A.	36.58	32.74
S139	25.67	33.32	26.17	31.17	**S297**	36.08	N.A.	34.14	32.90
S140	23.63	33.35	23.65	23.13	**S298**	36.13	N.A.	37.50	29.16
S141	23.63	33.35	23.65	23.13	**S299**	36.16	N.A.	36.35	31.19
S142	24.67	33.44	31.25	33.90	**S300**	36.18	N.A.	36.31	30.95
S143	24.86	33.45	23.99	30.00	**S301**	36.21	N.A.	36.70	31.10
S144	27.30	33.53	27.19	28.84	**S302**	36.28	N.A.	35.74	34.68

S145	20.64	33.71	21.34	24.45	**S303**	36.39	N.A.	37.25	26.97
S146	23.56	33.72	23.53	30.08	**S304**	36.71	N.A.	38.21	26.99
S147	20.34	33.77	19.20	23.64	**S305**	36.74	N.A.	38.25	35.29
S148	20.34	33.77	19.20	23.64	**S306**	36.98	N.A.	36.69	28.51
S149	24.95	33.92	25.60	33.28	**S307**	37.13	N.A.	36.05	31.11
S150	27.78	33.96	28.00	31.88	**S308**	37.19	N.A.	38.09	31.35
S151	27.72	34.07	27.81	30.14	**S309**	37.61	N.A.	36.69	28.06
S152	28.87	34.18	29.79	33.89	**S310**	38.07	N.A.	36.32	30.28
S153	22.87	34.24	21.34	21.81	**S311**	38.30	N.A.	40.20	25.99
S154	22.87	34.24	21.34	21.81	**S312**	39.79	N.A.	38.81	35.17
S155	22.07	34.58	21.88	31.90	**S313**	39.79	N.A.	37.27	29.62
S156	29.34	35.06	29.15	35.59	**S314**	40.23	N.A.	40.40	30.22
S157	19.16	35.14	19.35	26.84	**S315**	40.26	N.A.	40.08	30.52
S158	19.16	35.14	19.35	26.84					

N.A., not amplified (i.e., Cq >40)

Supplementary Table S4. Related to Figure 1D,F,H. Cq values for sgE and β-actin obtained from the single cohort of 122 oro/nasopharyngeal swabs from COVID19-positive patients analyzed using Taqman qPCR.

Code	Cq value		Code	Cq value		Code	Cq value		Code	Cq value	
	sgE	β-actin		sgE	β-actin		sgE	β-actin		sgE	β-actin
S44	27.37	26.50	**S312**	39.40	26.95	**S261**	N.A.	24.48	**S274**	N.A.	25.44
S45	27.50	27.75	**S191**	39.49	26.34	**S239**	N.A.	26.2	**S267**	N.A.	27.54
S49	26.92	25.39	**S200**	39.65	28.35	**S250**	N.A.	30.57	**S276**	N.A.	28.45
S54	26.19	27.27	**S199**	39.74	29.76	**S241**	N.A.	30.78	**S266**	N.A.	33.50
S55	27.36	25.92	**S219**	39.88	28.03	**S270**	N.A.	28.09	**S291**	N.A.	23.98
S56	26.80	25.39	**S243**	38.04	27.35	**S249**	N.A.	29.90	**S283**	N.A.	28.03
S71	30.75	28.07	**S256**	38.25	32.94	**S242**	N.A.	29.51	**S302**	N.A.	27.18
S74	40.4	30.28	**S212**	38.57	29.13	**S246**	N.A.	27.11	**S288**	N.A.	25.24
S98	31.27	28.53	**S215**	38.81	29.84	**S257**	N.A.	24.47	**S292**	N.A.	33.38
S105	37.98	28.06	**S213**	39.17	29.19	**S252**	N.A.	26.87	**S271**	N.A.	25.56
S112	35.15	27.05	**S280**	39.45	27.76	**S247**	N.A.	29.69	**S289**	N.A.	26.91
S127	36.95	30.51	**S227**	38.73	30.58	**S237**	N.A.	30.55	**S307**	N.A.	28.49
S137	37.45	27.42	**S234**	38.34	31.15	**S265**	N.A.	29.04	**S300**	N.A.	27.74
S144	40.10	28.67	**S203**	N.A.	28.14	**S248**	N.A.	30.38	**S310**	N.A.	24.03
S149	33.92	27.80	**S201**	N.A.	29.33	**S254**	N.A.	27.94	**S293**	N.A.	30.41
S150	37.49	28.96	**S198**	N.A.	27.84	**S273**	N.A.	25.37	**S299**	N.A.	27.48
S151	34.30	26.84	**S218**	N.A.	27.33	**S263**	N.A.	31.51	**S290**	N.A.	22.25
S166	38.85	26.63	**S217**	N.A.	30.21	**S264**	N.A.	27.97	**S306**	N.A.	30.13
S167	40.10	26.15	**S224**	N.A.	30.52	**S262**	N.A.	24.45	**S301**	N.A.	28.23
S180	41.10	30.08	**S221**	N.A.	27.62	**S259**	N.A.	25.81	**S286**	N.A.	28.38
S190	38.03	26.79	**S223**	N.A.	29.47	**S253**	N.A.	26.29	**S303**	N.A.	27.48
S238	28.94	25.97	**S228**	N.A.	29.21	**S260**	N.A.	25.81	**S294**	N.A.	22.79
S206	31.03	28.27	**S220**	N.A.	27.22	**S297**	N.A.	30.51	**S298**	N.A.	27.30
S275	34.37	26.10	**S244**	N.A.	29.69	**S284**	N.A.	29.72	**S308**	N.A.	28.20
S282	36.72	28.70	**S229**	N.A.	25.35	**S279**	N.A.	27.47	**S295**	N.A.	27.27
S208	37.82	27.04	**S225**	N.A.	30.02	**S277**	N.A.	30.19	**S304**	N.A.	25.69
S205	38.18	28.11	**S231**	N.A.	30.60	**S281**	N.A.	33.87	**S305**	N.A.	30.99
S211	38.47	27.09	**S240**	N.A.	29.44	**S278**	N.A.	27.52	**S315**	N.A.	29.07
S197	38.87	28.86	**S245**	N.A.	28.46	**S287**	N.A.	25.94	**S311**	N.A.	31.67
S236	39.40	30.65	**S226**	N.A.	30.72	**S268**	N.A.	26.54			
S235	N.A.	29.60	**S272**	N.A.	29.32	**S314**	N.A.	25.23			

N.A., not amplified (i.e., Cq >40)

Supplementary Table S5. Related to Figure 2A-B. Cq values obtained from a cohort of 16 oro/nasopharyngeal swabs from home-isolated COVID19-infected patients analyzed according to scheduled times (i.e., 3-day intervals from the first swab) with the SARS-CoV-2 Viral3 kits (sgN, E gene, ORF1ab gene) and the Allplex 2019-nCoV assay (E, N, RdRp/S genes) and by Taqman qPCR (sgE, β-actin genes).

Marker	Day	Cq value															
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#19	#20	#21	#22
SARS-CoV-2 Viral3 kit																	
sgN transcript	1	29.87	33.08	36.46	37.16	29.21	21.38	20.92	16.7	30.58	29.67	31.07	17.38	26.22	24.26	27.07	25.41
	3	N.A.	N.A.	N.A.	N.A.	N.A.	26.59	36.35	32.29	37.87	23.25	29.71	32.58	N.A.	N.A.	N.A.	N.A.
	7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	24.95	-	-	-	-	-	-
E gene	1	24.22	28.52	29.34	34.5	23.89	21.38	20.15	13.3	25.13	16.44	23.96	12.75	21.23	20.57	20.77	18.25
	3	30.43	36.28	30.76	28.04	31.49	18.16	29.03	24.76	29.5	15.54	23.32	17.48	33.87	35.23	38.28	37.23
	7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	33.78	N.A.	N.A.	17.83	-	-	-	-	-	-
Orf1ab gene	1	26.77	31.55	31.88	35.04	24.84	23.62	23.18	15.05	30.85	18.47	27.18	11.34	22.51	22.39	21.15	19.68
	3	34.84	34.94	35.95	30.98	33.37	20.98	35.83	25.87	32.32	18.37	25.99	25.67	37.03	36.38	39.65	38.64
	7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	37.91	18.52	-	-	-	-	-	-
RNAse P gene	1	32.23	28.27	33.49	32.14	30.91	27.89	31.21	28.34	31	30.13	31.11	31.42	29.11	31.04	27.53	29.93
	3	31.2	32.07	29.46	34.11	28.87	31.07	26.31	30.24	32.87	30.02	31.86	31.49	29.56	31.39	31.37	32.42
	7	31.7	32.4	30.23	30.01	36.56	32.25	34	32.1	32.87	27.87	-	-	-	-	-	-
Allplex 2019-nCoV assay																	
RdRp/S gene	1	26.93	32.94	26.55	34.94	25.45	25.64	24.46	23.78	31.55	27.03	29.61	21.26	22.47	24.71	25.03	20.05
	3	31.34	27.15	34.6	38.74	33.12	20.38	31.23	29.84	34.64	17.41	27.64	30.67	37.51	31.98	37.86	37.49
	7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	17.66	-	-	-	-	-	-
N gene	1	23.96	28.35	21.91	35.81	27.44	21.60	19.90	18.94	26.92	19.03	25.38	21.09	24.25	21.85	24.6	19.02
	3	31.5	37.01	30.55	35.56	34.42	20.59	30.17	27.42	31.05	17.6	25.14	29.98	32.97	30.09	38.71	35.45
	7	N.A.	N.A.	N.A.	N.A.	N.A.	37.23	36	N.A.	N.A.	18.21	-	-	-	-	-	-
E gene	1	24.55	29.38	21.74	34.37	24.98	22.30	21.19	19.04	26.62	20.71	24.79	20.14	22.06	21.01	23.37	17.54
	3	32.29	38.74	31.03	35.52	31.26	20.54	31.23	25.6	31.39	17.3	24.78	32.35	33.06	30.18	39.22	35.86
	7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	36.05	N.A.	N.A.	17.94	-	-	-	-	-	-
Taqman qPCR																	
sgE transcript	1	29.84	33.08	26.11	-	29.55	27.78	24.11	33.17	31.63	-	-	-	-	-	-	-
	3	36.42	38.97	35.01	-	38.4	29.18	35.68	37.35	35.64	-	-	-	-	-	-	-
	7	N.A.	N.A.	N.A.	-	N.A.	N.A.	38.81	N.A.	N.A.	-	-	-	-	-	-	-
β-actin gene	1	26.42	22.96	23.42	-	28.45	22.42	25.68	23.52	27.42	-	-	-	-	-	-	-
	3	23.63	26.83	27.39	-	26.53	24.21	24.39	26.11	26.18	-	-	-	-	-	-	-
	7	31.2	26.58	24.72	-	31.23	24.73	25.99	30.44	25.97	-	-	-	-	-	-	-

N.A., not amplified (i.e., Cq >40)

Supplementary Table S6. Related to Figure 2C-D. Cq values obtained from a cohort of 6 oro/nasopharyngeal swabs from hospitalized COVID19-positive patients analyzed according to scheduled times (i.e., 7-day intervals from the first swab) with the SARS-CoV-2 Viral3 kits (sgN, E gene, ORF1ab gene) and the Allplex 2019-nCoV assay (E, N, RdRp/S genes) and by Taqman qPCR (sgE, β-actin genes).

Marker	Days	Cq value					
		I1	I2	I3	I4	I5	I6
SARS-CoV-2 Viral3 kits							
sgN transcript	1	24.13	N.A.	29.01	27.76	22.06	N.A.
	7	37.47	N.A.	N.A.	N.A.	22.62	N.A.
	14	29.63	N.A.	N.A.	N.A.	22.44	N.A.
E gene	1	19.85	37.36	24.56	24.66	16.86	30.04
	7	29.76	N.A.	28.32	31.32	15.09	36.27
	14	21.24	N.A.	N.A.	30.67	15.64	N.A.
ORF1ab gene	1	22.79	36.2	26.95	26.98	18.47	32.16
	7	32.11	N.A.	32.31	34.18	19.37	37.2
	14	23.49	N.A.	N.A.	31.03	18.01	N.A.
RNAseP	1	33.01	36.6	33.93	33.31	30.44	31.34
	7	30.63	34.5	24.21	29.39	27.2	29.24
	14	24.05	30.37	34.12	25.22	31.42	33.14
Allplex 2019-nCoV assay							
E gene	1	20.55	38.46	25.44	25.85	17.26	30.44
	7	30.27	N.A.	29.34	32.1	16.26	37.76
	14	21.96	N.A.	N.A.	30.79	18	N.A.
N gene	1	19.57	37.1	24.61	25.21	17.01	30.92
	7	29.8	N.A.	30.31	31.17	17.11	N.A.
	14	23.25	N.A.	N.A.	31.77	17.52	N.A.
RdRp/S gene	1	22.54	39	27.43	27.8	19.12	32.88
	7	32.69	N.A.	31.64	34.16	18.13	N.A.
	14	24.32	N.A.	N.A.	32.71	20.67	N.A.
Taqman qPCR							
sgE transcript	1	38.15	-	39.13	39.45	22.58	39.55
	7	30.59	-	39.88	39.86	22.34	N.A.
	14	-	-	N.A.	-	23.09	N.A.
β-actin gene	1	21.15	-	21.22	20.93	21.07	19.16
	7	17.87	-	22.68	20.49	24.61	22.64
	14	-	-	20.28	-	26.08	21.41

N.A., not amplified (i.e., Cq values >40)