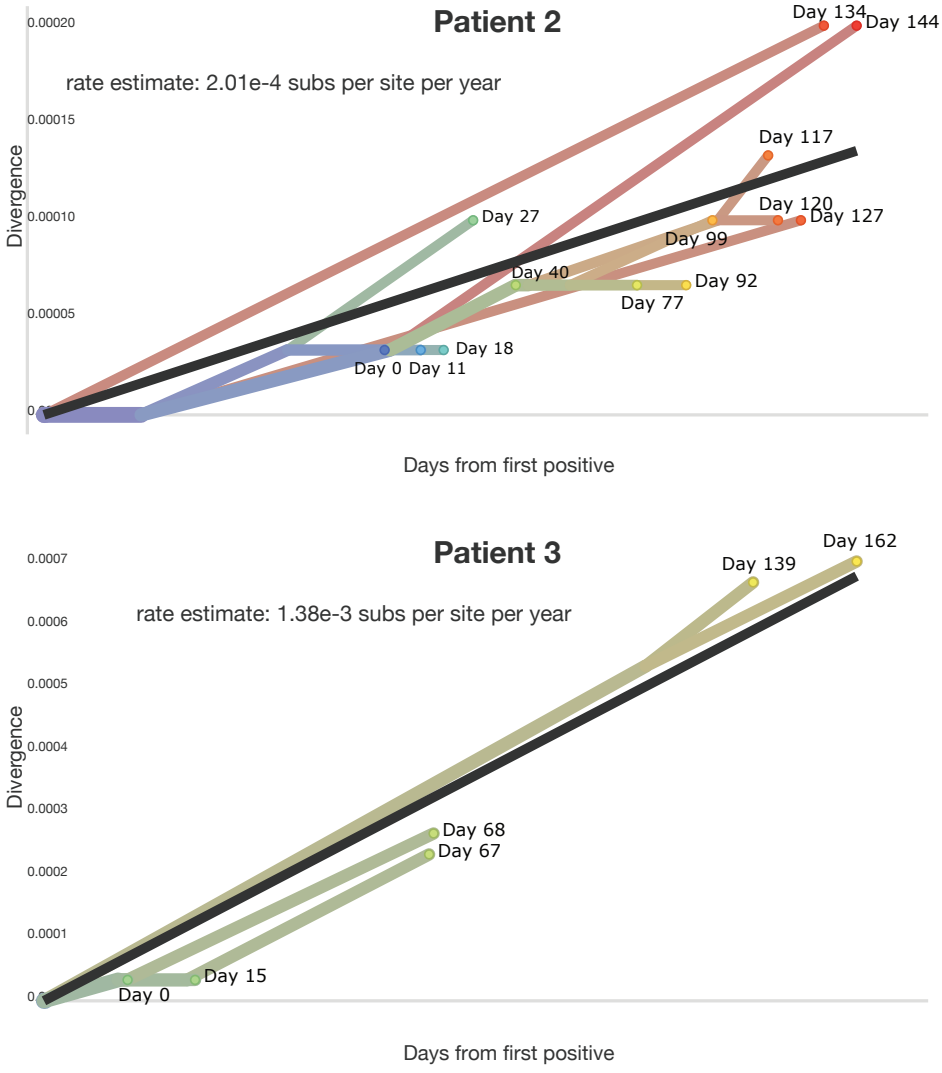
Supplementary figure

Figure S1. A time-resolved evolutionary rate estimation for SARS-CoV-2 in patients with prolonged viral infectivity (patient 2 and patient 3). Collection date for each sample is shown on the x-axis. For patient 2, the estimated evolutionary rate was 2.01 x 10^{-4} substitutions per site. For patient 3, the estimated evolutionary rate was 1.38 X 10^{-3}. The mutation rate is calculated based on differences in major and not minor variants, relative to the consensus difference.



Supplementary tables

Table S1. RT-PCR assay values for detection of SARS-CoV-2 from nasopharyngeal or combined nares/oropharyngeal swabs collected from each patient. Corresponding viral culture assay results are also shown.

Patient	Day	Routine RT-PCR		Strand-specific RT-PCR		Subgenomic RT-PCR Ct	Viral culture
		Assay used	Cycle threshold (Ct)[a]	Minus strand Ct	Plus strand Ct		
1	0	Thermo Fisher	15.9	25.2	17.5	22.9	Positive
	13	Thermo Fisher	26.2				Negative
	27	CDC	33.3				
	34	Thermo Fisher	29.0	UND	34.1	UND	
	40	Thermo Fisher	30.7	UND	34.1	UND	
	46	Thermo Fisher	30.7	UND	35.1	UND	Negative
	91	Cepheid	UND				
	116	Thermo Fisher	UND	UND	UND	UND	Negative
	117	Thermo Fisher	37.5	UND	UND	UND	Negative
	145	Thermo Fisher	UND				
2	0	CDC	20.7	31.7	20.2	26.2	Positive
	11	Thermo Fisher	21.4	29.5	22.3	29.6	Positive
	18	Thermo Fisher	16.6	23.9	17.6	23.6	
	27	Thermo Fisher	16.2				Positive
	40	Thermo Fisher	26.1				
	53	Diasorin	23.4 / 23.4				
	65	Diasorin	23.1 / 22.8	35.3	24.6	33.8	
	77	Thermo Fisher	21.8				
	92	Thermo Fisher	17.7	28.5	19.0	25.7	
	99	Thermo Fisher	20.2	29.5	21.8	27.8	Positive
	117	Thermo Fisher	19.3				Positive
	120	Thermo Fisher	18.5	27.5	20.8	25.6	Positive
	127	Thermo Fisher	28.1	UND	27.7	36.8	Positive
	130	Thermo Fisher	23.6				
	134	Thermo Fisher	20.9	28.9	22.5	29.3	Positive
	137	Thermo Fisher	17.2	24.1	19.8	23.4	Positive
	144	Thermo Fisher	21.6	30.7	24.3	31.2	Positive
	156	Thermo Fisher	24.8				
	172	Thermo Fisher	23.1				
3	0	CDC	16.0	23.4	17.5	25.0	Positive
	11	CDC	22.5				
	15	Thermo Fisher	23.5	39.3	25.5	31.9	Negative

	22	Thermo Fisher	21.8	32.1	24.2	32.9	Positive
	36	Diasorin	19.8 / 19.2				
	43	Diasorin	21.2 / 22.3				
	67	Thermo Fisher	19.2	26.7	20.8	27.0	Negative
	68	Thermo Fisher	21.9	30.7	22.8	31.4	
	139	Thermo Fisher	23.0	36.7	33.5	33.0	Positive
	162[b]	Thermo Fisher	20.0	35.8	23.0	27.4	Negative
	196	Thermo Fisher	UND				

UND, undetected

[a] Ct values for the Thermo Fisher Taqpath COVID-19 RT-PCR N target, CDC 2019-Novel Coronavirus Real-Time RT-PCR N1 target, Cepheid Xpert Xpress SARS-CoV-2 E and N2 targets, and Diasorin Simplexa S and ORF1ab targets.

[b] All specimens were collected with a nasopharyngeal swab except for the day 162 specimen from patient 3, which was collected with a combined nares and oropharyngeal swab.

Table S2. Major and minor allele frequency variants in patient 1. Variants with allele frequency below 25% were filtered out. Most samples were processed on an additional platform to verify the variant calls. Variant calls confirmed by another platform are marked in bold. Patient 1, Day 27 sample with a low depth of coverage from Paragon run was replaced with AmpliSeq data. PAF = population allele frequency of variant in over 80,000 complete genome sequences in the Children's Hospital Los Angeles COVID-19 Analysis Research Database (CARD), extracted from and kept updated based on GIDAID, NCBI, and Nextstrain.[1]

Variant		Gene	Day			PAF
			0	13	27	
241:C/T	.	5UTR_orf1ab	**1**	**1**	**0.99**	0.001
1059:C/T	p.Thr265Ile	orf1ab	**0.99**	**1**	**0.99**	0.48
3037:C/T	p.Phe924Phe	orf1ab	**1**	**1**	**0.96**	0.916
3042:C/T	p.Pro926Leu	orf1ab	**1**	**0.99**	**1**	<0.001
9967:C/T	p.Leu3234Leu	orf1ab	**1**	**1**	**1**	0.002
11514:C/T	p.Thr3750Ile	orf1ab	**1**	**.**	**.**	0.001
14408:C/T	p.Pro4715Leu	orf1ab	**1**	**1**	**0.99**	0.916
21777:G/A	p.Gly72Glu	S	**1**	**1**	**0.98**	<0.001
22792:C/A	p.Ile410Ile	S	**1**	**1**	**0.49**	<0.001
23403:A/G	p.Asp614Gly	S	**1**	**1**	**1**	0.920
24770:G/T	p.Ala1070Ser	S	**1**	**1**	**0.99**	<0.001
25563:G/T	p.Gln57His	ORF3a	**1**	**0.99**	**0.99**	0.221
27964:C/T	p.Ser24Leu	ORF8	**1**	**1**	**1**	0.042
28824:C/T	p.Ser184Phe	N	**1**	**1**	**0.99**	<0.001

Table S3. Major and minor allele frequency variants in patient 2. Variants with allele frequency below 25% were filtered out. Most samples were processed on an additional platform to verify the variant calls. Variant calls confirmed by another platform are marked in bold. PAF = population allele frequency of variant in over 80,000 complete genome sequences in the Children's Hospital Los Angeles COVID-19 Analysis Research Database (CARD), extracted from and kept updated based on GIDAID, NCBI, and Nextstrain.[1]

Variant		Gene	Day														PAF
			0	11	18	27	40	77	92	99	117	120	127	134	137	144	
241:C/T	.	5UTR_orf1ab	**0.99**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	1	1	0.889
498:C/T	p.Thr78Ile	orf1ab	.	.	.	.	.	.	.	.	.	.	.	.	.	0.47	<0.001
509:G/A	p.Gly82Ser	orf1ab	.	.	.	.	.	.	.	.	**0.74**	**0.39**	.	.	.	.	<0.001
521:G/A	p.Val86Ile	orf1ab	.	.	.	.	.	.	.	**0.47**	.	.	.	.	.	.	<0.001
2939:C/G	p.Pro892Ala	orf1ab	.	.	.	.	.	.	.	.	.	.	.	**0.95**	.	0.48	<0.001
3037:C/T	p.Phe924Phe	orf1ab	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0.99**	**1**	**0.99**	**1**	1	1	0.916
3523:A/G	p.Glu1086Glu	orf1ab	.	.	.	.	.	.	.	.	.	.	**0.43**	.	0.75	.	<0.001
7315:T/C	p.Phe2350Phe	orf1ab	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	1	0.99	<0.001
8290:C/T	p.Leu2675Leu	orf1ab	.	.	.	.	.	.	.	.	.	.	.	.	.	0.57	0.001
8327:C/T	p.Leu2688Phe	orf1ab	.	.	.	.	.	.	.	.	**0.33**	.	.	.	.	.	0.001
9711:C/T	p.Ser3149Phe	orf1ab	.	.	.	.	.	.	.	.	.	.	.	**0.95**	.	0.44	<0.001
10029:C/T	p.Thr3255Ile	orf1ab	.	.	.	.	.	.	.	.	.	.	**0.64**	.	0.99	.	0.001
10319:C/T	p.Leu3352Phe	orf1ab	.	.	.	.	.	.	.	**0.53**	.	.	.	.	.	.	0.024
10834:C/T	p.Ala3523Ala	orf1ab	.	.	.	**0.61**	.	.	.	.	.	.	.	.	.	.	0.001
11230:G/T	p.Met3655Ile	orf1ab	.	.	.	.	**0.99**	**0.78**	**0.99**	**0.96**	**0.93**	**0.99**	**0.36**	.	.	0.58	0.009
12008:C/T	p.Leu3915Phe	orf1ab	.	.	.	.	.	.	**0.40**	**0.28**	**0.85**	**0.94**	0.25	.	.	.	<0.001

14313:T/C	p.Asp4683Asp	orf1ab	.	.	.	.	.	.	.	.	.	.	.	**0.72**	.	0.43	0.001
14408:C/T	p.Pro4715Leu	orf1ab	**0.99**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	1	0.99	0.916
14809:C/T	p.Arg4849Cys	orf1ab	.	.	.	.	**0.98**	**0.74**	**0.98**	**0.96**	**0.92**	**0.99**	**0.36**	.	.	.	<0.001
15828:A/C	p.Glu5188Asp	orf1ab	.	.	.	.	.	.	.	.	**0.37**	**0.28**	.	.	.	.	<0.001
16877:C/T	p.Thr5538Ile	orf1ab	.	.	.	.	.	.	.	.	.	.	.	.	.	0.59	<0.001
20384:C/T	p.Ala6707Val	orf1ab	.	.	.	**0.46**	.	.	.	.	.	.	**0.68**	.	0.98	.	<0.001
21627:C/T	p.Thr22Ile	S	.	.	.	.	.	.	.	.	.	.	.	.	.	0.60	0.001
21846:C/T	p.Thr95Ile	S	.	.	.	.	.	.	.	.	.	.	**0.51**	.	0.76	.	0.002
21981:TTTTGGGTGTTTA/T[a]	p.Leu141_Tyr144del	S	.	.	.	.	.	.	.	.	.	.	.	.	0.78	.	<0.001
21990:TTTA/T[a]	p.Tyr145del	S	.	.	.	.	.	.	.	.	.	**0.43**	**0.33**	**0.97**	.	0.44	<0.001
22193:AATT/A	p.Asn211_Leu212delinsIle	S	.	.	.	.	.	.	.	.	.	.	**0.57**	.	0.95	.	<0.001
22264:C/T	p.Asn234Asn	S	.	.	.	.	.	.	.	.	.	.	.	.	.	0.58	0.001
23403:A/G	p.Asp614Gly	S	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	1	1	0.920
24138:C/A	p.Thr859Asn	S	.	.	.	.	.	.	.	.	**0.34**	**0.29**	.	.	.	.	0.001
25904:C/T	p.Ser171Leu	ORF3a	.	.	.	.	.	.	.	.	.	.	.	**0.76**	.	0.36	0.004
26511:T/C		E-M	.	.	.	.	.	.	.	.	.	.	**0.49**	.	0.98	.	<0.001
27112:G/C	p.Ser197Thr	M	.	.	.	.	.	.	.	.	.	.	.	**0.53**	.	0.29	<0.001
27707:C/T	p.Ala105Val	ORF7a	.	.	.	.	**0.94**	**0.99**	**1**	**1**	**1**	**1**	**0.99**	**0.98**	1	0.98	0.001
28321:G/A	p.Thr16Thr	N	.	.	.	.	.	**0.36**	.	.	.	.	.	**0.77**	.	0.96	0.001
29679:C/T	.	ORF10_3UTR	.	.	.	.	.	.	.	.	**0.29**	.	.	.	.	.	<0.001
29700:A/T	.	ORF10_3UTR	.	.	.	.	.	.	.	.	.	.	.	.	0.85	.	<0.001

29742:G/T	.	ORF10_3UTR	.	.	.	**0.53**	.	.	.	.	.	.	.	.	.	.	0.003

[a] Leu141_Tyr144del and Tyr145del are in a region where similar mutations have been found in other immunocompromised patients.[2,3]

Table S4. Major and minor allele frequency variants in patient 3. Variants with allele frequency below 25% were filtered out. Most samples were processed on an additional platform to verify the variant calls. Variant calls confirmed by another platform are marked in bold. PAF = population allele frequency of variant in over 80,000 complete genome sequences in the Children's Hospital Los Angeles COVID-19 Analysis Research Database (CARD), extracted from and kept updated based on GIDAID, NCBI, and Nextstrain.[1]

Variant		Gene	Day						PAF
			0	15	67	68	139	162	
241:C/T	.	5UTR_orf1ab	**1**	**1**	**1**	**1**	**1**	**1**	0.889
379:C/A	p.Val38Val	orf1ab	**1**	**0.99**	**1**	**0.99**	**1**	**1**	0.003
635:C/T	p.Arg124Cys	orf1ab	.	.	.	.	.	**0.44**	<0.001
1059:C/T	p.Thr265Ile	orf1ab	**1**	**0.99**	**0.99**	**0.99**	**1**	**1**	0.148
1753:T/C	p.Tyr496Tyr	orf1ab	.	.	.	.	.	**0.47**	<0.001
2424:G/T	p.Cys720Phe	orf1ab	.	.	.	.	.	**0.51**	<0.001
2655:A/C	p.Glu797Ala	orf1ab	.	.	.	.	**1**	**1**	<0.001
3037:C/T	p.Phe924Phe	orf1ab	**1**	**1**	**1**	**1**	**1**	**1**	0.916
3421:A/G	p.Val1052Val	orf1ab	.	.	.	.	**1**	**1**	<0.001
4763:C/T	p.His1500Tyr	orf1ab	.	.	.	.	**1**	**1**	<0.001
4891:C/T	p.Thr1542Thr	orf1ab	.	.	.	**0.62**	.	.	<0.001
9165:C/T	p.Thr2967Ile	orf1ab	.	.	.	.	**1**	**1**	0.001
10276:A/G	p.Gln3337Gln	orf1ab	.	.	.	.	.	**0.49**	<0.001
11082:TG/T	p.Leu3606fs	orf1ab	.	.	.	.	.	0.27	<0.001
11083:G/T	p.Leu3606Phe	orf1ab	.	.	.	.	**0.47**	**0.73**	0.065
11270:A/G	p.Met3669Val	orf1ab	.	.	.	.	**1**	**1**	<0.001
12025:C/T	p.Ser3920Ser	orf1ab	**1**	**1**	**1**	**1**	**1**	**1**	0.002

13860:C/T	p.Asp4532Asp	orf1ab	.	.	.	**0.62**	.	.	0.001
14408:C/T	p.Pro4715Leu	orf1ab	**0.99**	**1**	**1**	**1**	**1**	**1**	0.916
15714:C/T	p.Leu5150Leu	orf1ab	.	.	.	.	.	**0.51**	<0.001
16014:G/T	p.Arg5250Arg	orf1ab	.	.	.	**0.59**	.	.	<0.001
16374:T/C	p.Asn5370Asn	orf1ab	.	.	.	.	**0.99**	**0.51**	<0.001
16421:A/G	p.Gln5386Arg	orf1ab	.	.	.	**0.41**	.	.	<0.001
16466:C/T	p.Pro5401Leu	orf1ab	.	.	.	**0.44**	.	.	<0.001
16949:C/T	p.Pro5562Leu	orf1ab	.	.	.	**0.60**	.	.	<0.001
17278:G/T	p.Val5672Leu	orf1ab	.	.	.	**0.40**	.	.	0.002
17895:T/C	p.Ala5877Ala	orf1ab	.	.	.	.	**1**	**0.56**	<0.001
18318:G/T	p.Gly6018Gly	orf1ab	.	.	.	.	.	**0.37**	<0.001
18870:T/A	p.Thr6202Thr	orf1ab	.	.	.	.	**1**	**1**	<0.001
19398:G/T	p.Glu6378Asp	orf1ab	.	.	**0.99**	.	.	.	<0.001
21600:G/T	p.Ser13Ile	S	.	.	.	.	**1**	**1**	0.001
21658:C/T	p.Phe32Phe	S	.	.	.	**0.52**	.	.	0.001
21770:G/T[a]	p.Val70Phe	S	.	.	.	.	.	**0.42**	<0.001
21852:A/T	p.Lys97Met	S	.	.	.	.	**1**	**0.51**	<0.001
21980:TTTTTGGGTG/T[c]	p.Leu141_Val143del	S	.	.	.	.	**0.99**	**0.99**	<0.001
22131:G/A	p.Arg190Lys	S	.	.	.	.	**1**	**1**	<0.001
22152:T/C	p.Ile197Thr	S	.	.	.	**0.58**	.	.	<0.001
22195:T/G	p.Asn211Lys	S	.	.	.	.	.	**0.56**	<0.001
22843:T/C	p.Asp427Asp	S	.	.	.	**0.52**	.	.	<0.001
22882:T/A[b]	p.Asn440Lys	S	.	.	.	.	.	**0.51**	<0.001
23010:T/C	p.Val483Ala	S	.	.	.	.	**1**	**1**	<0.001
23012:G/C	p.Glu484Gln	S	.	.	.	.	**1**	**0.45**	<0.001
23403:A/G	p.Asp614Gly	S	**1**	**1**	**1**	**1**	**1**	**1**	0.920

23638:C/T	p.Ile692Ile	S	.	.	**0.97**	.	.	.	0.001
24885:A/C	p.Asn1108Thr	S	.	.	.	.	.	**0.39**	<0.001
25563:G/T	p.Gln57His	ORF3a	**1**	**0.99**	**0.99**	**1**	**1**	**1**	0.221
26228:C/T	.	ORF3a-E	.	.	.	**0.43**	.	.	0.001
26333:C/T	p.Thr30Ile	E	.	.	.	.	**1**	**0.59**	<0.001
26386:A/G	p.Asn48Asp	E	.	.	.	.	**1**	.	<0.001
26393:G/T	p.Ser50Ile	E	.	.	.	.	**0.96**	.	<0.001
26895:C/T	p.His125Tyr	M	.	.	.	.	**1**	**1**	0.002
27393:C/T	.	ORF6-ORF7a	.	.	.	.	**1**	**1**	<0.001
27925:C/T	p.Thr11Ile	ORF8	.	.	.	.	**1**	**0.89**	<0.001
28232:C/T	p.Asp113Asp	ORF8	.	.	.	**0.32**	.	.	<0.001
28253:C/T	p.Phe120Phe	ORF8	.	.	**0.99**	.	.	.	0.005
28255:T/C	p.Ile121Thr	ORF8	**0.83**	**0.84**	.	**0.77**	**0.86**	**0.79**	<0.001
28300:G/T	p.Gln9His	N	.	.	**0.99**	.	.	.	0.003
28715:A/G	p.Thr148Ala	N	.	.	.	.	.	**0.59**	<0.001
28887:C/T	p.Thr205Ile	N	**0.96**	**0.74**	**1**	**0.61**	**1**	**1**	0.008

[a] Val70Phe overlaps the Δ69-70 deletion which has been reported in the B.1.1.7 variant and has been associated with other receptor binding domain changes.[4]

[b] Asn440Lys was also reported by Weisblum et al. and Liu et al. by passaging SARS-CoV-2 in the presence of a monoclonal antibody. This mutation also emerged in an adult immunocompromised patient reported in Choi et al.[2,5,6]

[c] Leu141_Val143del is in a region where similar mutations have been found in other immunocompromised patients.[2,7]

Table S5. Confirmation of variant calls by alternate whole genome sequencing methods

Primary Paragon protocol was confirmed by either Ampliseq or Twist protocols.

Sample	Platform
Patient 1, Day 0	Twist
Patient 1, Day 13	AmpliSeq
Patient 1, Day 27	n/a
Patient 2, Day 0	Twist
Patient 2, Day 11	Twist
Patient 2, Day 18	Twist
Patient 2, Day 27	Twist
Patient 2, Day 40	AmpliSeq
Patient 2, Day 77	Twist
Patient 2, Day 92	Twist

Patient 2, Day 99	Twist
Patient 2, Day 117	Twist
Patient 2, Day 120	Twist
Patient 2, Day 127	AmpliSeq
Patient 2, Day 134	Twist
Patient 2, Day 137	n/a
Patient 2, Day 144	n/a
Patient 3, Day 0	Twist
Patient 3, Day 15	AmpliSeq
Patient 3, Day 67	Twist
Patient 3, Day 68	Twist
Patient 3, Day 139	AmpliSeq
Patient 3, Day 162	Twist