Supplementary Information for

# Detection and characterization of the SARS-CoV-2 lineage B.1.526 in New York

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## Supplementary Methods.

Commands for the program **vdb**, implementing a mutation pattern query language:

Notation

cluster = group of viruses  < > = user input  n = an integer
pattern = group of mutations  [ ] = optional  ( ) = explanation of command
"world" = all viruses in database  -> result

To define a variable for a cluster or pattern: <name> = cluster or pattern
Set operations +, -, and * (intersection) can be applied to clusters or patterns
If no cluster is entered, all viruses will be used ("world")

Filter commands

<cluster> from <country or state>  -> cluster
<cluster> containing [<n>] <pattern>  -> cluster  alias with  (matches for >=n mutations)
<cluster> not containing <pattern>  -> cluster  alias without  (considers whole pattern)
<cluster> before <date>  -> cluster
<cluster> after <date>  -> cluster
<cluster> > or < <n>  -> cluster  (filter by number of mutations)

Commands to find mutation patterns

consensus [for] <cluster or country or state>  -> pattern
patterns [in] [<n>] <cluster>  -> pattern  (lists n patterns)

Listing commands

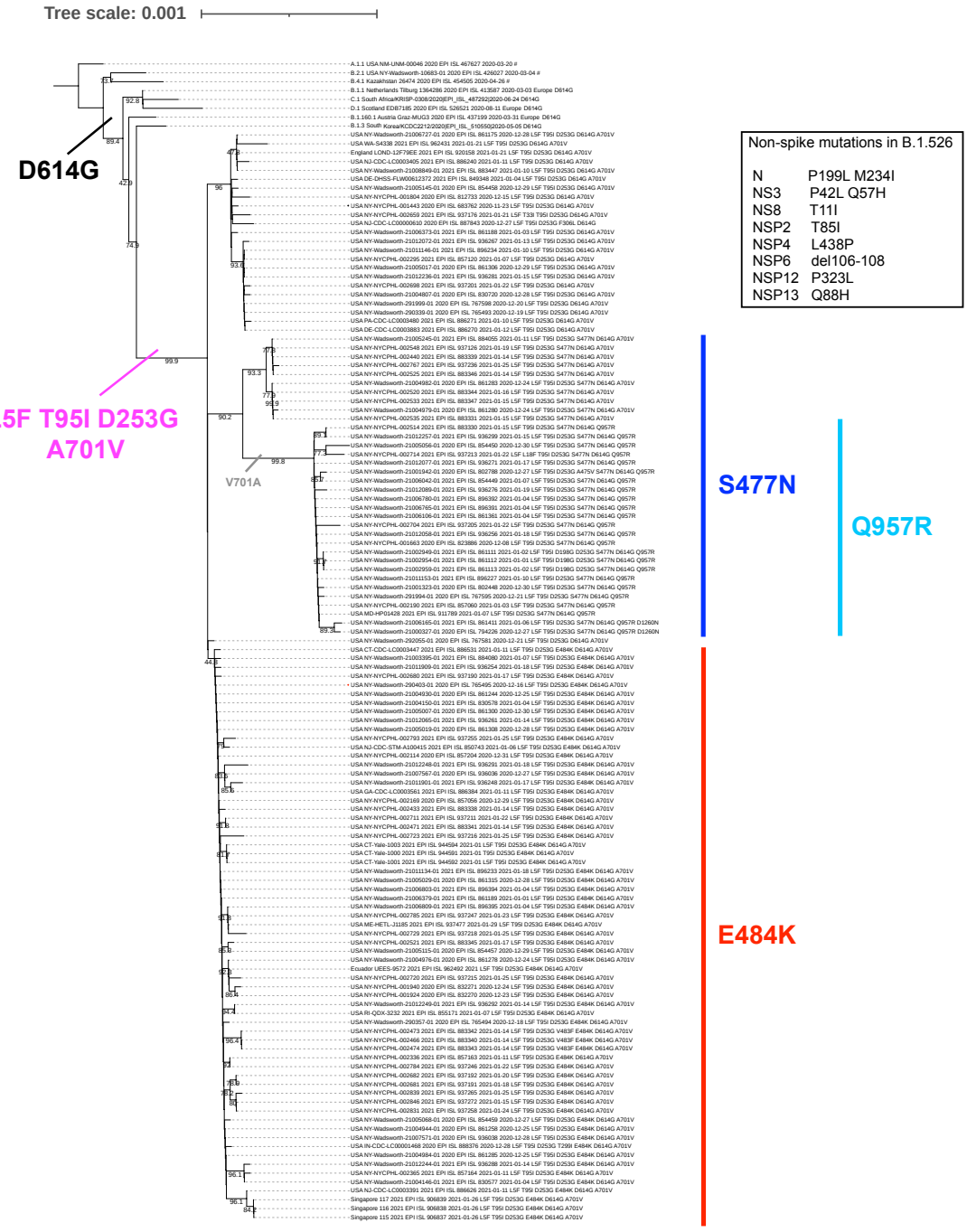
list [<n>] <cluster>
[list] countries [for] <cluster>
[list] states [for] <cluster>
[list] frequencies [for] <cluster>  alias freq  (frequency of individual mutations)
[list] monthly [for] <cluster> [<cluster2>]  (number of viruses per month or week)
[list] weekly [for] <cluster> [<cluster2>]  (as a fraction of number of viruses in cluster2)
[list] patterns  (list built-in and user-defined patterns)
[list] clusters  (list built-in and user-defined clusters)

Other commands

sort <cluster>  (by date)
help
history
quit

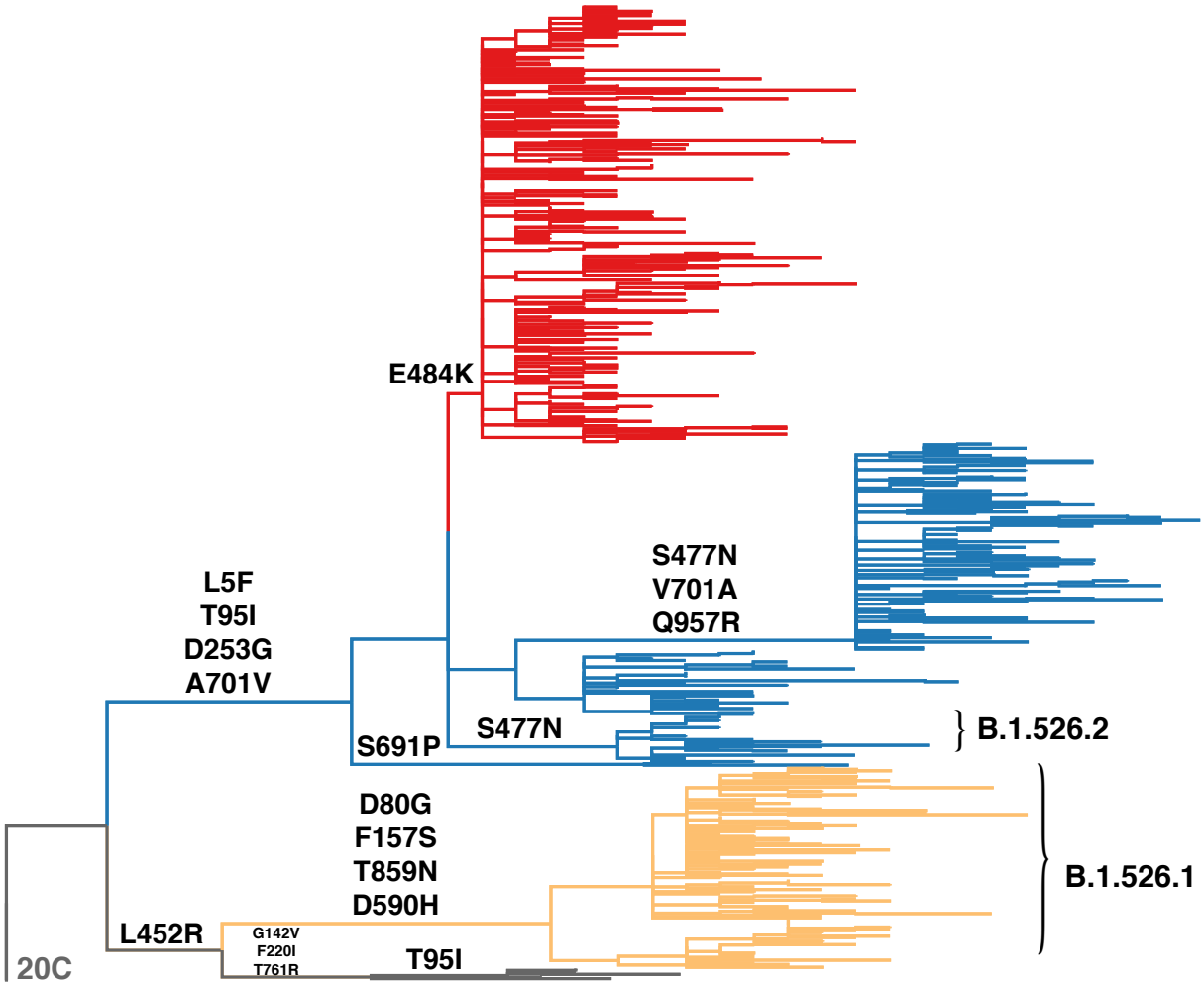
## Supplementary Fig. 1.

Phylogenetic tree of lineage B.1.526 indicating spike mutations. The inset lists non-spike mutations common in this lineage.



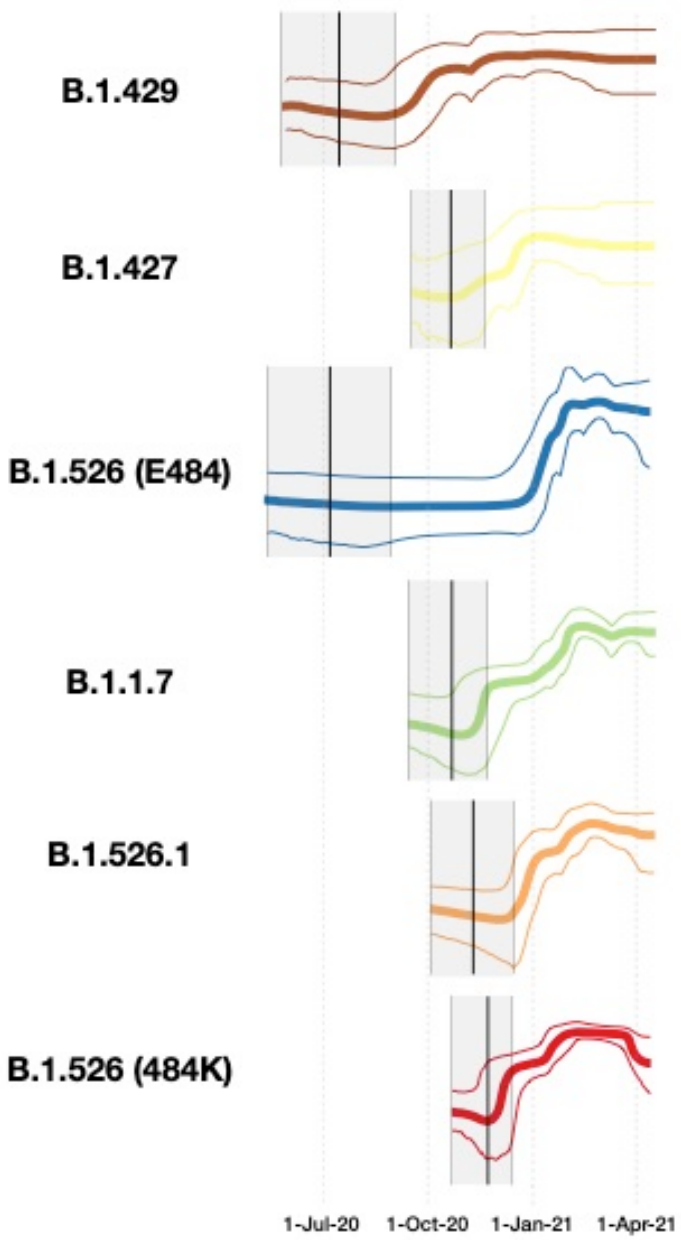
## Supplementary Fig. 2.

Maximum likelihood phylogenetic tree of the B.1.526 lineage (including B.1.526.2) in relation to a sister clade defined by an L452R spike mutation (including B.1.526.1, shown in orange) and the 20C ancestral virus (shown in gray). Tree was rooted using the clade 20C ancestral viruses sampled in NYC. Amino acid substitutions in the spike protein occurring on internal branches are labeled. The B.1.526 lineage is colored blue, except for the clade defined by the E484K mutation, which is highlighted in red.



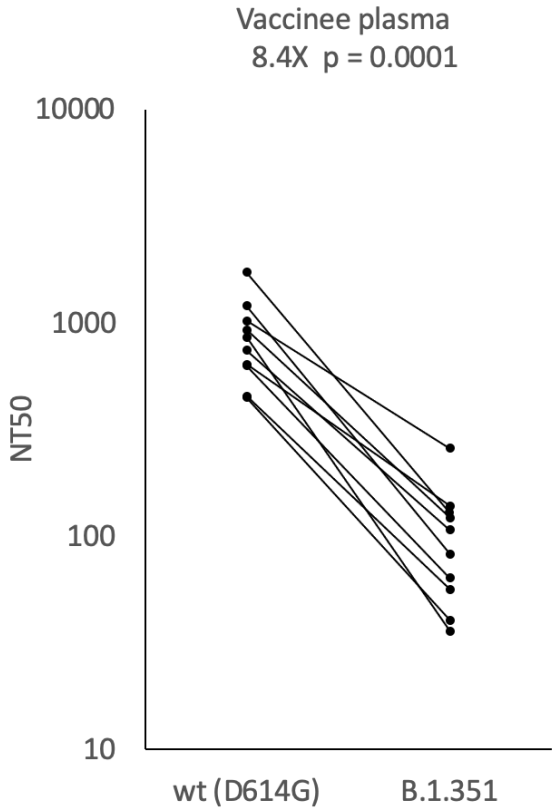
## Supplementary Fig. 3.

Bayesian skyline demographic reconstruction and time of most recent common ancestor (TMRCA) inference for SARS-CoV-2 variants in New York City, sampled December 2020 through April 2021. Thick solid lines represent the median estimate of the effective population size over time for each variant, the thinner solid lines represent the 95% Highest Posterior Density (HPD) intervals. The grey shaded areas represent the 95% HPD for the estimated TMRCAs, the solid grey lines represent the median estimated TMRCA values. B.1.526 (E484) estimate includes B.1.526.2 subclade.



## Supplementary Fig. 4.

Vaccinee plasma neutralizing activity against pseudoviruses with B.1.351 lineage spike mutations or D614G wildtype. SARS-CoV-2 pseudovirus neutralization assays were used to determine neutralization titer (NT50) for COVID-19 vaccinee plasma (n=10). B.1.351 pseudovirus containing spike mutations L18F, D80A, D215G, Δ242-244, R246I, K417N, E484K, N501Y, D614G, and A701V. Statistical significance was determined using paired two-tailed *t*-tests. Fold-difference of means is shown.



## Supplementary Table 1.

List of 124 viral genomes (with their accession number, location, collection date, and spike mutations) in lineage B.1.526. Mutations E484K, S477N, Q957R are highlighted in red, blue, and cyan, respectively.

EPI_ISL_683762, USA/NY-NYCPHL-001443/2020-11-23 : L5F T95I D253G D614G A701V
EPI_ISL_823886, USA/NY-NYCPHL-001663/2020-12-08 : L5F T95I D253G S477N D614G Q957R
EPI_ISL_812733, USA/NY-NYCPHL-001804/2020-12-15 : L5F T95I D253G D614G A701V
EPI_ISL_765495, USA/NY-Wadsworth-290403-01/2020-12-16 : L5F T95I D253G E484K D614G A701V
EPI_ISL_765494, USA/NY-Wadsworth-290357-01/2020-12-18 : L5F T95I D253G E484K D614G A701V
EPI_ISL_765493, USA/NY-Wadsworth-290339-01/2020-12-19 : L5F T95I D253G D614G A701V
EPI_ISL_767598, USA/NY-Wadsworth-291999-01/2020-12-20 : L5F T95I D253G D614G A701V
EPI_ISL_767581, USA/NY-Wadsworth-292055-01/2020-12-21 : L5F T95I D253G D614G A701V
EPI_ISL_767595, USA/NY-Wadsworth-291994-01/2020-12-21 : L5F T95I D253G S477N D614G Q957R
EPI_ISL_832270, USA/NY-NYCPHL-001924/2020-12-23 : L5F T95I D253G E484K D614G A701V
EPI_ISL_832271, USA/NY-NYCPHL-001940/2020-12-24 : L5F T95I D253G E484K D614G A701V
EPI_ISL_861278, USA/NY-Wadsworth-21004976-01/2020-12-24 : L5F T95I D253G E484K D614G A701V
EPI_ISL_861280, USA/NY-Wadsworth-21004979-01/2020-12-24 : L5F T95I D253G S477N D614G A701V
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EPI_ISL_861258, USA/NY-Wadsworth-21004944-01/2020-12-25 : L5F T95I D253G E484K D614G A701V
EPI_ISL_861285, USA/NY-Wadsworth-21004984-01/2020-12-25 : L5F T95I D253G E484K D614G A701V
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EPI_ISL_854459, USA/NY-Wadsworth-21005068-01/2020-12-27 : L5F T95I D253G E484K D614G A701V
EPI_ISL_887843, USA/NJ-CDC-LC00000610/2020-12-27 : L5F T95I D253G F306L D614G
EPI_ISL_861315, USA/NY-Wadsworth-21005029-01/2020-12-28 : L5F T95I D253G E484K D614G A701V
EPI_ISL_888376, USA/IN-CDC-LC00001468/2020-12-28 : L5F T95I D253G T299I E484K D614G A701V
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EPI_ISL_944594, USA/CT-Yale-1003/2021-01-01 : L5F T95I D253G E484K D614G A701V

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EPI_ISL_906838, Singapore/116/2021-01-26 : L5F T95I D253G E484K D614G A701V
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EPI_ISL_937477, USA/ME-HETL-J1185/2021-01-29 : L5F T95I D253G E484K D614G A701V

**Supplementary Table 2. Individual vaccinee characteristics and plasma titers**

| | Demographic characteristics | | | | Vaccination details | | | | | Neutralization titer (reciprocal dilution) NT50 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Time between (days) | | | Experiment 1 | | Experiment 2 | | Experiment 3 | |
| Participant ID | Age range (years) | Sex | Race § | Ethnicity | Vaccine platform | # of doses received | 1st & 2nd dose | 1st dose & blood draw | 2nd dose & blood draw | B.1.526 v.1† | D614G | B.1.526 v.2‡ | D614G | B.1.351* | D614G |
| MOD1 | 50-59 | Male | W | Hispanic | Moderna | 2 | 28 | 74 | 46 | 364 | 2176 | 497 | 917 | 64 | 630 |
| MOD2 | 30-39 | Male | W | Non-Hispanic | Moderna | 2 | 34 | 62 | 28 | 265 | 975 | 205 | 573 | 41 | 447 |
| MOD3 | 30-39 | Male | W | Non-Hispanic | Moderna | 2 | 31 | 78 | 47 | 269 | 1412 | 460 | 1762 | 107 | 744 |
| MOD6 | 40-49 | Female | W | Non-Hispanic | Moderna | 2 | 28 | 89 | 61 | 223 | 679 | 201 | 983 | 57 | 454 |
| MOD7 | 60-69 | Male | A | Non-Hispanic | Moderna | 2 | 35 | 84 | 49 | 563 | 2412 | 746 | 2981 | 122 | 927 |
| MOD8 | 60-69 | Female | W | Non-Hispanic | Moderna | 2 | 35 | 96 | 63 | 214 | 1713 | 381 | 1660 | 83 | 1203 |
| PFZ12 | 50-59 | Female | W | Non-Hispanic | Pfizer/BioNTech | 2 | 21 | 83 | 62 | 345 | 1516 | 733 | 1278 | 36 | 857 |
| PFZ16 | 60-69 | Female | W | Non-Hispanic | Pfizer/BioNTech | 2 | 22 | 119 | 97 | 109 | 566 | 188 | 718 | 139 | 639 |
| C001 | 30-39 | Female | W | Non-Hispanic | Pfizer/BioNTech | 2 | 22 | 57 | 35 | 884 | 2652 | 1035 | 1738 | 129 | 1726 |
| C004 | 30-39 | Male | W | Non-Hispanic | Pfizer/BioNTech | 2 | 21 | 91 | 70 | 254 | 1603 | 860 | 1991 | 258 | 1023 |

§ = White (W); Asian (A)

† = B.1.516 v.1 spike mutations L5F, T95I, D253G, E484K, D614G, and A701V

‡ = B.1.526 v.2 spike mutations: L5F, T95I, D253G, S477N, D614G, and Q957R

* = B.1.351 spike mutations: L18F D80A D215G Δ242-244 R246I K417N E484K N501Y D614G A701V

Characteristics from Supplementary Table 1 of Wang, et al., "mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants", Nature (2021)

**Supplementary Table 3. Individual convalescent participant characteristics and plasma titers**

| | | | | | Temporal dynamics (days) | | | | | | | Neutralization titer (reciprocal dilution) NT50 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Timepoint T1 - 1.3 months | | | | Timepoint T2 - 6.2 months | | | |
| | | | | | | | | | | | | Experiment 1 | | Experiment 2 | | Experiment 1 | | Experiment 2 | |
| ID | Age range (years) | Sex | Race | Ethnicity | Sx duration during acute disease | Sx onset to initial visit (T1) | Sx onset to follow-up visit (T2) | Time between visits | # of solicited comorbidities § | Acute disease severity by WHO (0-8) ¶ | Post-acute Sx persistence # | B.1.526 v.1† | D614G | B.1.526 v.2‡ | D614G | B.1.526 v.1† | D614G | B.1.526 v.2‡ | D614G |
| **38** | 50-59 | F | White | Non-Hispanic | 10 | 38 | 211 | 173 | 0 | 2 | N | 27 | 148 | 255 | 385 | 154 | 891 | 775 | 2597 |
| **40** | 40-49 | M | White | Non-Hispanic | 7 | 23 | 195 | 172 | 0 | 2 | N | 13 | 37 | 72 | 90 | N.N. | 10 | N.N. | N.N. |
| **76** | 40-49 | F | White | Non-Hispanic | 28 | 34 | 204 | 170 | 0 | 1 | Y | 33 | 105 | 105 | 184 | 10 | 23 | 13 | 24 |
| **99** | 30-39 | F | White | Non-Hispanic | 13 | 29 | 204 | 175 | 0 | 2 | N | 180 | 973 | 794 | 1055 | 22 | 358 | 176 | 240 |
| **233** | 50-59 | M | White | Non-Hispanic | 20 | 41 | 206 | 165 | 0 | 2 | N | 55 | 194 | 108 | 165 | 19 | 50 | 17 | 46 |
| **328** | 50-59 | F | White | Non-Hispanic | 22 | 62 | 203 | 141 | 0 | 2 | N | 24 | 347 | 50 | 131 | 22 | 135 | 29 | 77 |
| **352** | 40-49 | M | White | Non-Hispanic | 16 | 43 | 197 | 154 | 0 | 2 | N | 129 | 1684 | 587 | 744 | 27 | 148 | 40 | 107 |
| **403*** | 50-59 | M | Asian | Non-Hispanic | 18 | 39 | 174 | 135 | 1 | 4 | Y | 555 | 3223 | 1067 | 2433 | 89 | 463 | 45 | 183 |
| **437** | 40-49 | F | Asian | Non-Hispanic | 14 | 34 | 192 | 158 | 1 | 2 | N | 122 | 525 | 276 | 996 | 56 | 132 | 65 | 270 |
| **537** | 50-59 | M | White | Non-Hispanic | 15 | 45 | 178 | 133 | 2 | 2 | Y | 650 | 3528 | 1610 | 4291 | 240 | 853 | 334 | 957 |

Sx = symptoms, N.N = non-neutralizing

* = hospitalized

§ = Arterial hypertension (HTN), obesity (OB), diabetes mellitus (DM), asthma (A), chronic obstructive pulmonary disease (COPD), coronary artery disease (CAD), cancer (CX)

¶ = WHO Ordinal Scale for Clinical Improvement, COVID-19 Trial Design Synopsis

# = Persistent fatigue, dyspnea, athletic deficit, or ≥ 3 other solicited symptoms beyond 6 weeks from Sx onset

† = B.1.516 v.1 spike mutations L5F, T95I, D253G, E484K, D614G, and A701V

‡ = B.1.526 v.2 spike mutations: L5F, T95I, D253G, S477N, D614G, and Q957R

Characteristics from Supplementary Table 2 of Gaebler, et al., "Evolution of antibody immunity to SARS-CoV-2" Nature 591, 639–644 (2021)

**Supplementary Table 4. Monoclonal antibody neutralizataion of B.1.526**

| | | Neutralization IC50 (μg/mL) | |
|---|---|---|---|
| **Antibody** | **Antibody Class*** | **B.1.526 v.1†** | **D614G** |
| C105[a] | 1 | 0.112 | 0.035 |
| C002[a] | 2 | 53 | 0.006 |
| BG10-19[b] | 3 | 0.008 | 0.004 |
| BG7-15[b] | 2/3 | 0.0002 | 0.002 |

a = Robbiani, D.F. et al. "Convergent antibody responses to SARS-CoV-2 in convalescent individuals" Nature 584, 437–442 (2020)
b = Schied, J.F. et al. "B cell genomics behind cross-neutralization of SARS-CoV-2 variants and SARS-CoV" Cell April 24 (2021)
* = Barnes, C.O. et al. "SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies" Nature 588, 682–687 (2020)
† = B.1.516 v.1 spike mutations L5F, T95I, D253G, E484K, D614G, and A701V

## Supplementary Table 5.

List of primers used to make B.1.526 v.1 and v.2 pseudovirus constructs

| Primer Name | Sequence |
|---|---|
| L5F_1 | GCCACCATGTTCGTGTTCTTCGTACTCCTTCCTTTGGTG |
| L5F_2 | CACCAAAGGAAGGAGTACGAAGAACACGAACATGGTGGC |
| T95I_1 | GGAGTGTACTTCGCGTCCATCGAGAAGAGCAATATCATTC |
| T95I_2 | GAATGATATTGCTCTTCTCGATGGACGCGAAGTACACTCC |
| D253G_1 | CTGACACCGGGCGGCTCAAGTTCCGGA |
| D253G_2 | TCCGGAACTTGAGCCGCCCGGTGTCAG |
| S477N_1 | GGAGATATATCAAGCCGGCAATACACCATGTAATGGTGTG |
| S477N_2 | CACACCATTACATGGTGTATTGCCGGCTTGATATATCTCC |
| E484K_1 | CACCATGTAATGGTGTGAAGGGCTTTAACTGCTAC |
| E484K_2 | GTAGCAGTTAAAGCCCTTCACACCATTACATGGTG |
| A701V_1 | CGATGAGCCTCGGAGTGGAGAACAGCGTCGCG |
| A701V_2 | CGCGACGCTGTTCTCCACTCCGAGGCTCATCG |
| Q957R_1 | CGTGGTGAATCAGAATGCACGGGCACTGAATACCTTGGTTAA |
| Q957R_2 | TTAACCAAGGTATTCAGTGCCCGTGCATTCTGATTCACCACG |