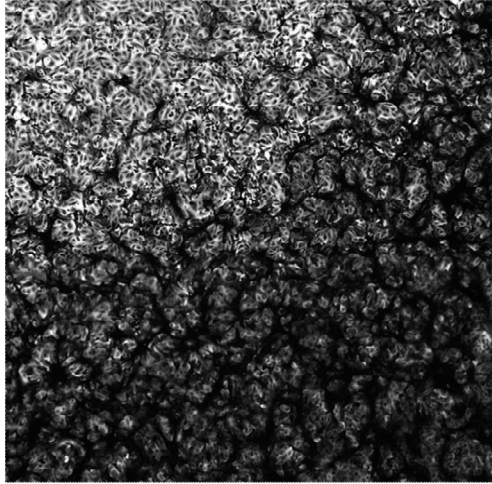


| PHE VEROE6 | | | | | | | | | | | | |
|-----------------|------------------------------|---------|-----|-----|-----|-----|-----|----------|--------|-------|---------------------------|---------|
| POS | REFERENCE | VARIANT | P1 | P2 | P3 | P4A | P4B | CATEGORY | GENE | S/NS? | CONSEQUENCE | PROTEIN |
| 7749 | C | T | 0.1 | 12 | 21 | 16 | 19 | MINOR | ORF1AB | NS | T2495I | NSP3 |
| 8782 | C | T | 97 | 98 | 97 | 97 | 98 | CORE | ORF1AB | S | | |
| 9534 | C | T | 0.1 | 0.1 | 2 | 5 | 5 | MINOR | ORF1AB | NS | T3090I | NSP4 |
| 18488 | T | C | 98 | 97 | 97 | 98 | 96 | CORE | ORF1AB | NS | I6075T | NSP14 |
| 19983 | C | CT | 7 | 6 | 7 | 9 | 8 | MINOR | ORF1AB | NS | FRAMESHIFT. D6576* | NSP15 |
| 21697 | C | T | 13 | 8 | 4 | 24 | 15 | MINOR | ORF1AB | S | | |
| 21761 | GCTATACATGTCTCTGGGACCAATGGTA | G | 0.1 | 0.1 | 2 | 10 | 6 | MINOR | SPIKE | NS | IHVSGTNGT67-76del | SPIKE |
| 21846 | C | T | 17 | 47 | 3 | 0.1 | 0.1 | MINOR | SPIKE | NS | T95I | SPIKE |
| 22100 | G | A | 0.1 | 0.1 | 0.1 | 11 | 15 | MINOR | SPIKE | NS | E180K | SPIKE |
| 22206 | A | G | 0.1 | 0.1 | 0.1 | 8 | 11 | MINOR | SPIKE | NS | D215G | SPIKE |
| 23605 | T | G | 97 | 94 | 0.1 | 0.1 | 0.1 | VARIANT | SPIKE | S | | |
| 23597 | AATTCTCCTCGGGCGGCACGTA | A | 0.6 | 4 | 89 | 94 | 94 | VARIANT | SPIKE | NS | NSPRRARSV679I | SPIKE |
| 25339 | C | T | 0.1 | 7 | 0.1 | 0.1 | 0.1 | MINOR | SPIKE | S | | |
| 26353 | C | T | 0.1 | 0.1 | 7 | 16 | 19 | MINOR | E | NS | L37F | E |
| 26354 | T | G | 0.1 | 0.1 | 0.1 | 5 | 5 | MINOR | E | NS | L37R | E |
| 28144 | T | C | 96 | 98 | 98 | 97 | 97 | CORE | ORF8 | NS | L84S | ORF8 |
| 28833 | C | T | 0.1 | 22 | 0.1 | 0.1 | 0.1 | MINOR | N | NS | S187L | N |
| 29366 | A | T | 7 | 34 | 2 | 0.1 | 0.1 | MINOR | N | NS | P365S | N |
| 29596 | C | G | 98 | 98 | 98 | 98 | 98 | CORE | ORF10 | NS | I13M | ORF10 |
| 29637 | T | C | 0.1 | 0.1 | 0.1 | 6 | 5 | MINOR | ORF10 | NS | I27T | ORF10 |
| 29844 | AT | A | 0.1 | 11 | 0.1 | 0.1 | 0.1 | MINOR | 3' UTR | S | | NC |
| PHE WD-PNECS | | | | | | | | | | | | |
| POS | REFERENCE | VARIANT | P1 | P2 | P3 | P4 | | CATEGORY | GENE | S/NS? | CONSEQUENCE | PROTEIN |
| 241 | C | T | 0.1 | 0.1 | 6 | 0.1 | | MINOR | 5'UTR | S | | |
| 514 | TGTTATG | T | 2 | 7 | 0.1 | 0.1 | | MINOR | ORF1AB | NS | MV85DEL | NSP1 |
| 1593 | C | T | 0.1 | 0.1 | 0.1 | 35 | | MINOR | ORF1AB | NS | S443F | NSP2 |
| 2994 | A | C | 0.1 | 0.1 | 0.1 | 7 | | MINOR | ORF1AB | NS | E910A | NSP3 |
| 4455 | C | T | 0.1 | 15 | 0.1 | 0.1 | | MINOR | ORF1AB | NS | A1397V | NSP3 |
| 4928 | A | T | 0.1 | 0.1 | 0.1 | 46 | | MINOR | ORF1AB | NS | N1555Y | NSP3 |
| 6696 | C | CT | 3 | 8 | 0.1 | 1 | | MINOR | ORF1AB | NS | L2146S* | NSP3 |
| 6750 | A | G | 0.1 | 7 | 0.1 | 0.1 | | MINOR | ORF1AB | NS | N2162S | NSP3 |
| 7444 | A | G | 0.1 | 0.1 | 10 | 0.1 | | MINOR | ORF1AB | S | | |
| 7749 | C | T | 0.1 | 7 | 0.1 | 0.1 | | MINOR | ORF1AB | NS | T2495I | NSP3 |
| 7866 | G | T | 0.1 | 0.1 | 6 | 0.1 | | MINOR | ORF1AB | NS | G2534V | NSP3 |
| 8782 | C | T | 97 | 99 | 99 | 99 | | CORE | ORF1AB | S | | |
| 9532 | C | T | 0.1 | 15 | 0.1 | 0.1 | | MINOR | ORF1AB | S | | |
| 11074 | C | CT | 1 | 5 | 0.1 | 0.1 | | MINOR | ORF1AB | NS | L3606F* | NSP6 |
| 12809 | C | T | 0.1 | 0.1 | 0.1 | 5 | | MINOR | ORF1AB | NS | L4182F | NSP9 |
| 12860 | A | G | 0.1 | 0.1 | 10 | 0.1 | | MINOR | ORF1AB | NS | S4199G | NSP9 |
| 13604 | G | A | 0.1 | 0.1 | 7 | 0.1 | | MINOR | ORF1AB | NS | R4447H | NSP12 |
| 16949 | C | T | 0.1 | 0.1 | 13 | 0.1 | | MINOR | ORF1AB | NS | P5562L | NSP13 |
| 17440 | C | T | 0.1 | 35 | 0.1 | 0.1 | | MINOR | ORF1AB | NS | P5726S | NSP13 |
| 18063 | TA | T | 0.1 | 0.1 | 9 | 0.1 | | MINOR | ORF1AB | NS | frameshift | |
| 18488 | T | C | 98 | 97 | 96 | 97 | | CORE | ORF1AB | NS | I6075T | NSP14 |
| 18508 | C | T | 0.1 | 0.1 | 0.1 | 8 | | MINOR | ORF1AB | NS | L6082F | NSP14 |
| 19983 | C | CT | 7 | 7 | 0.1 | 6 | | MINOR | ORF1AB | NS | FRAMESHIFT. D6576* | NSP15 |
| 20178 | C | T | 0.1 | 0.1 | 0.1 | 5 | | MINOR | ORF1AB | S | | |
| 21697 | C | T | 13 | 0.1 | 8 | 0.1 | | MINOR | SPIKE | S | | |
| 21846 | C | T | 17 | 0.1 | 18 | 2 | | MINOR | SPIKE | NS | T95I | Spike |
| 23277 | C | T | 0.1 | 0.1 | 10 | 0.1 | | MINOR | SPIKE | NS | T572I | Spike |
| 23582 | T | C | 0.1 | 0.1 | 9 | 0.1 | | MINOR | SPIKE | NS | T674H | Spike |
| 23597 | AATTCTCCTCGGGCGGCACGTA | A | 6 | 11 | 0 | 2 | | MINOR | SPIKE | NS | NSPRRARSV679I | Spike |
| 23605 | T | G | 97 | 96 | 94 | 95 | | CORE | SPIKE | S | | |
| 26681 | C | T | 0.1 | 0.1 | 7 | 0.1 | | MINOR | M | S | | M |
| 27434 | C | T | 0.1 | 0.1 | 6 | 0.1 | | MINOR | ORF7A | NS | T14I | 7A |
| 27509 | C | T | 0.1 | 0.1 | 6 | 0.1 | | MINOR | ORF7A | NS | T39I | 7A |
| 27814 | TTG | T | 0.1 | 0.1 | 10 | 0.1 | | MINOR | ORF7B | NS | FRAMESHIFT | 7B |
| 28144 | TTG | C | 96 | 97 | 96 | 98 | | CORE | ORF8 | NS | L84S | 8 |
| 28393 | T | C | 0.1 | 11 | 0.1 | 0.1 | | MINOR | ORF8 | S | | |
| 29274 | C | T | 0.1 | 23 | 0.1 | 0.1 | | MINOR | N | NS | T334I | N |
| 29366 | C | T | 7 | 0.1 | 17 | 0.1 | | MINOR | ORF10 | NS | P365S | N |
| 29596 | A | G | 98 | 98 | 98 | 99 | | CORE | 10 | NS | I13M | 10 |
| BT20.1 VERO | | | | | | | | | | | | |
| POS | REFERENCE | VARIANT | P1 | P2 | P3 | P4 | | CATEGORY | GENE | S/NS? | CONSEQUENCE | PROTEIN |
| 241 | C | T | 100 | 100 | 100 | 100 | | CORE | 5' UTR | S | | |
| 635 | C | T | 7 | 0.1 | 0.1 | | | MINOR | ORF1AB | NS | R124C | NSP1 |
| 1420 | C | T | 98 | 99 | 99 | | | CORE | ORF1AB | S | | |
| 1681 | G | A | 99 | 99 | 99 | | | CORE | ORF1AB | S | | |
| 3037 | C | T | 98 | 99 | 99 | | | CORE | ORF1AB | S | | |
| 6255 | C | T | 96 | 99 | 96 | | | CORE | ORF1AB | NS | A1997V | NSP3 |
| 10870 | G | T | 0.1 | 90 | 96 | | | VARIANT | ORF1AB | S | | |
| 14318 | C | T | 0.1 | 85 | 96 | | | VARIANT | ORF1AB | NS | T4685I | NSP12 |
| 14408 | C | T | 98 | 97 | 98 | | | CORE | ORF1AB | NS | P4715L | NSP12 |
| 16293 | CATACGT | C | 0.1 | 0.1 | 7 | | | MINOR | ORF1AB | NS | CHANGE | |
| 19983 | C | CT | 8 | 6 | 7 | | | MINOR | ORF1AB | NS | FRAMESHIFT. D6576* | NSP15 |
| 22311 | C | T | 6 | 0.1 | 0.1 | | | MINOR | SPIKE | NS | T205I | SPIKE |
| 23403 | A | G | 97 | 98 | 99 | | | CORE | SPIKE | NS | D614G | SPIKE |
| 23997 | C | G | 0.1 | 84 | 96 | | | VARIANT | SPIKE | NS | P812R | SPIKE |
| 25314 | G | T | 3 | 5 | 0.1 | | | MINOR | SPIKE | NS | G1251V | SPIKE |
| 25317 | C | G | 3 | 5 | 7 | | | MINOR | SPIKE | NS | S1252C | SPIKE |
| 25521 | C | T | 18 | 0.1 | 7 | | | MINOR | ORF3A | S | | |
| 27208 | C | T | 8 | 0.1 | 0.1 | | | MINOR | ORF6 | NS | H3Y | ORF6 |
| 27213 | C | T | 9 | 2 | 0.1 | | | MINOR | ORF6 | S | | |
| 27671 | TTCAAG | T | 100 | 96 | 96 | | | CORE | ORF7A | NS | FRAMESHIFT and truncation | ORF7A |
| 28253 | C | T | 19 | 93 | 99 | | | VARIANT | ORF8 | S | | |
| 28881 | G | A | 97 | 98 | 99 | | | CORE | N | NS | R203K | N |
| 28882 | G | A | 96 | 99 | 99 | | | CORE | N | S | | |
| 28883 | G | C | 99 | 99 | 100 | | | CORE | N | NS | G204R | N |
| BT20.1 WD-PNECS | | | | | | | | | | | | |
| POS | REFERENCE | VARIANT | P1 | P2 | P3 | P4 | | CATEGORY | GENE | S/NS? | CONSEQUENCE | PROTEIN |
| 241 | C | T | 99 | 100 | 99 | | | CORE | 5' UTR | S | | |
| 1420 | C | T | 99 | 100 | 97 | | | CORE | ORF1AB | S | | |
| 1681 | G | A | 97 | 98 | 99 | | | CORE | ORF1AB | S | | |
| 3037 | C | T | 98 | 99 | 99 | | | CORE | ORF1AB | S | | |
| 6255 | C | T | 91 | 89 | 93 | | | CORE | ORF1AB | NS | A1997V | NSP3 |
| 6683 | AATT | A | 46 | 45 | 52 | | | VARIANT | ORF1AB | NS | | |
| 14408 | C | T | 99 | 98 | 98 | | | CORE | ORF1AB | NS | P4715L | NSP12 |
| 19983 | C | CT | 7 | 6 | 8 | | | MINOR | ORF1AB | NS | FRAMESHIFT. D6576 | NSP15 |
| 21101 | G | GT | 1 | 5 | 0.1 | | | MINOR | ORF1AB | NS | FRAMESHIFT. F6948 | NSP16 |
| 23403 | A | G | 97 | 99 | 98 | | | CORE | SPIKE | NS | D614G | SPIKE |
| 25314 | G | T | 0.1 | 6 | 1 | | | MINOR | SPIKE | NS | G1251V | SPIKE |
| 25317 | C | G | 0.1 | 6 | 1 | | | MINOR | SPIKE | NS | S1252C | SPIKE |
| 27671 | TTCAAG | T | 100 | 98 | 100 | | | CORE | ORF7A | NS | | |
| 28881 | G | A | 99 | 97 | 99 | | | CORE | N | NS | R203K | N |
| 28882 | G | A | 96 | 97 | 99 | | | CORE | N | S | | |
| 28883 | G | C | 99 | 99 | 99 | | | CORE | N | NS | G204R | N |
| 29418 | T | A | 0.1 | 5 | 0.1 | | | MINOR | N | NS | L382* | N |

Table S1. Frequency of variants in reference to Wuhan-Hu-1 identified in this study. Variants only shown where there was at least one instance of frequency >5%. Where undetectable an arbitrary value of 0.1 was assigned. Frequency data is highlighted by colour (green for higher, yellow for lower). Mutations have been assigned status of core, variant or mi-

nor. “Variant” mutations, which are shown in the main figures, have been highlighted in bold text. Additionally, for each variant, data for nucleotide location, reference and variant nucleotides, gene & protein location, and consequence (e.g., synonymous [S] or non-synonymous [NS]) are shown.

a.



b.

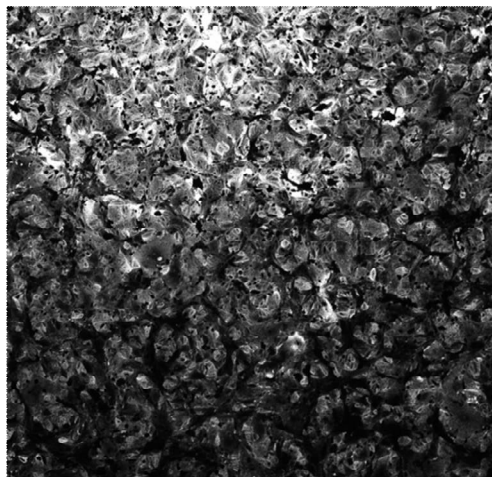


Figure S1. Fusogenicity of PHE and BT20.1 P4 on Vero cells. Higher magnification images of plaque visualisation of PHE (a) and BT20.1 (b) P4 on Vero cells from the same images shown in Figure 1.

a.

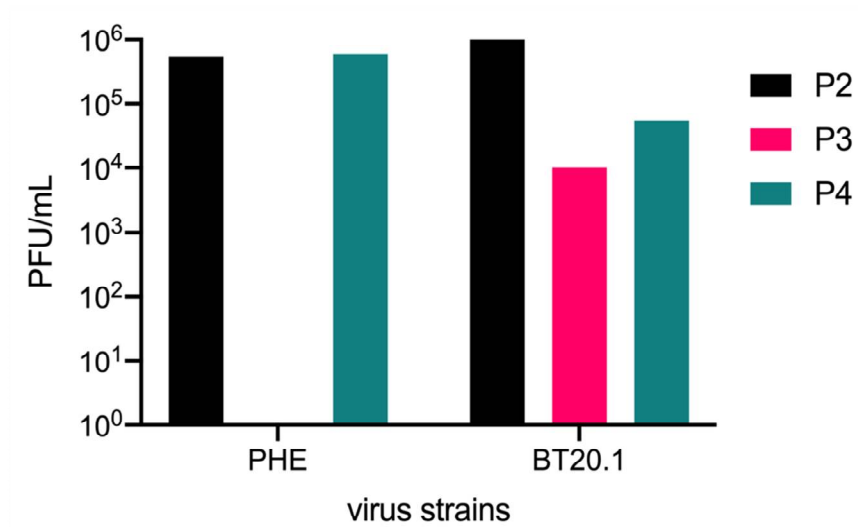
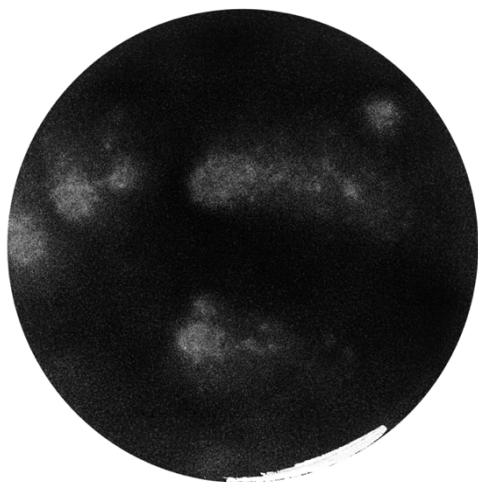
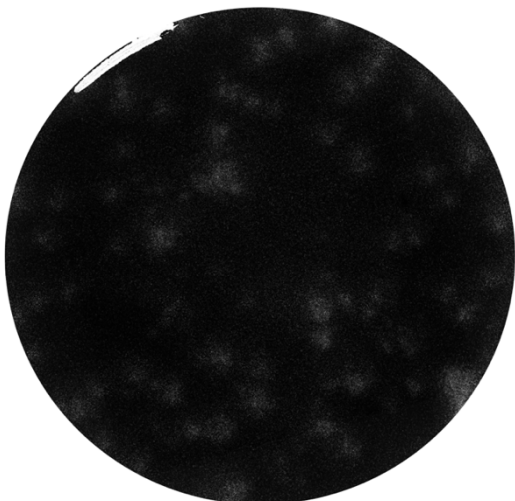


Figure S2. Growth kinetics of PHE and BT20.1 during passage in WD-PNECs. Infectivity titres for material generated from isolation/passage of PHE and BT20.1 on WD-PNECs.

a.



b.



c.



Figure S3. Plaque morphology of SARS-CoV-2 grown in WD-PNECs. Plaque visualisation of PHE (a) and BT20.1 (b) P4 on Vero cells. Higher magnification images of plaque visualisation of BT20.1 (c) P4 on Vero cells from the same images shown (b).

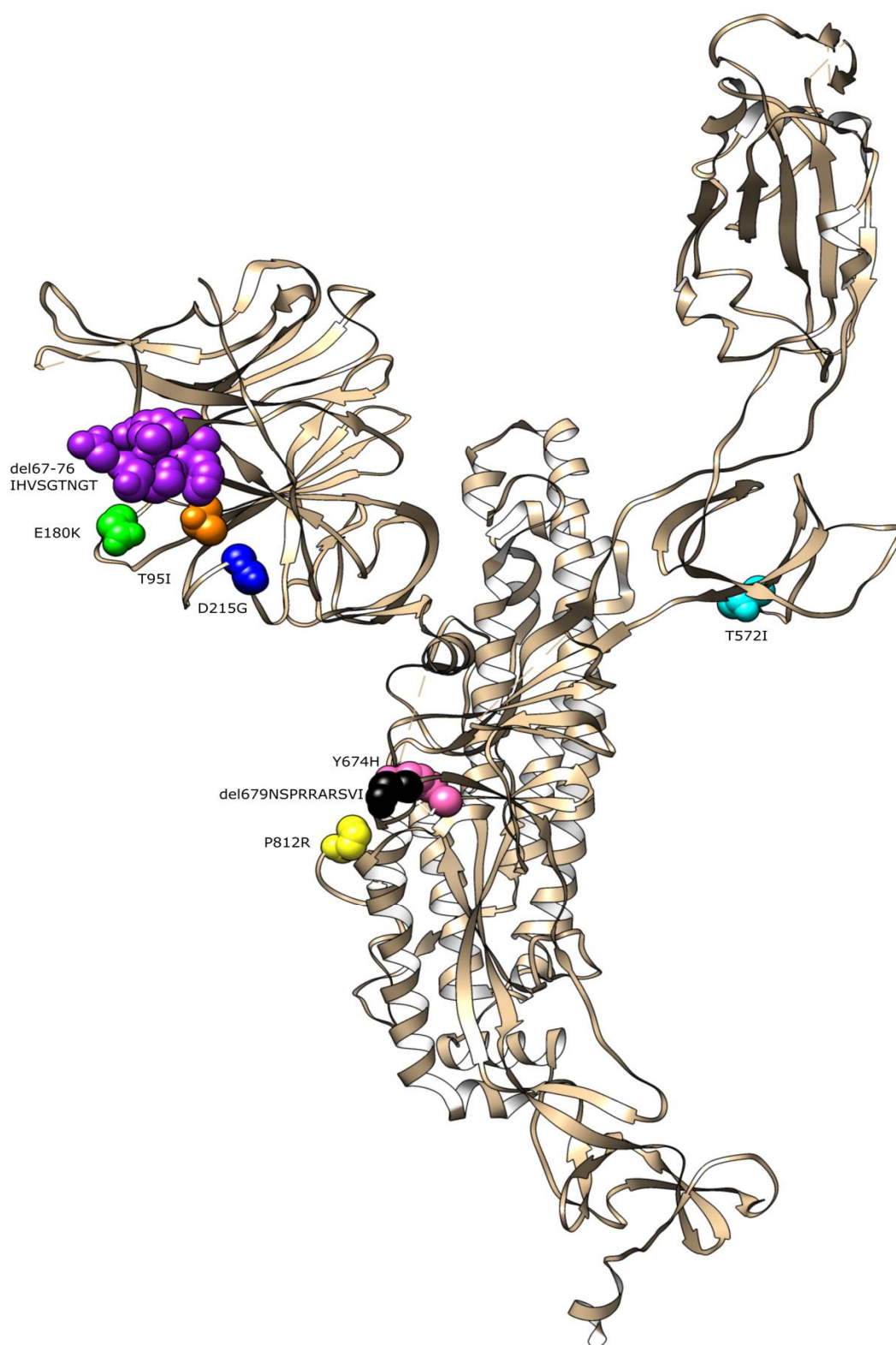


Figure S4. Location of Spike mutant variants observed in this study on model structure of a single Spike monomer in the pre-fusion state (PDB 7C2L from [40]). Variants identified in the Spike cytoplasmic tail (G1251V and S1252C) are not shown.