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Supplemental information

SARS-CoV-2 spike L452R variant evades cellular immunity and increases infectivity

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Supplementary Information

An emerging SARS-CoV-2 mutant evading cellular immunity and increasing viral infectivity

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Supplementary Figures S1-S2
Supplementary Tables S2-S6

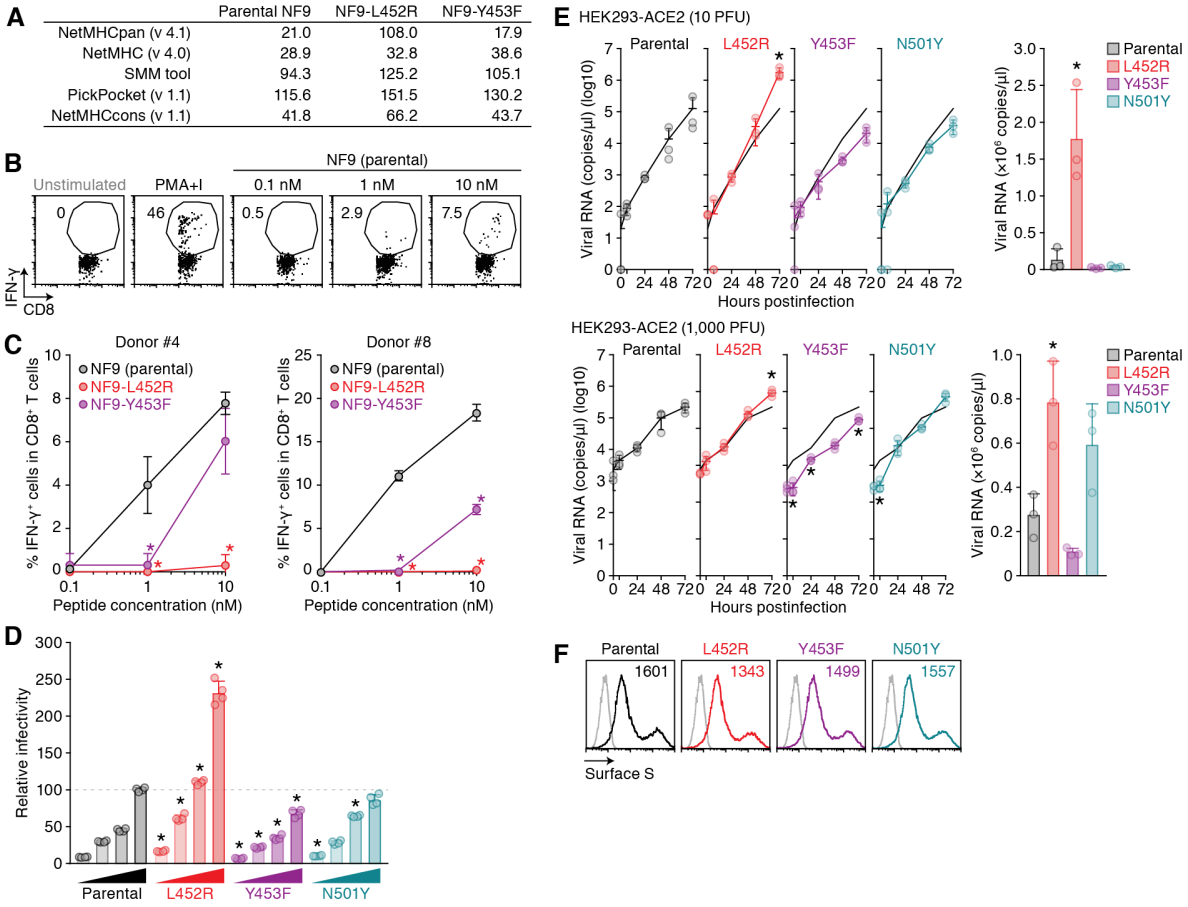

Figure S1. Immunological and virological phenotypes of the L452R and Y453 mutants (Related to Figures 1 and 2)

(**A**) Predicted binding affinity of the peptides and HLA-A24. Each value indicates the IC_{50} (nM) estimated.

(**B and C**) IFN-γ production of HLA-A24-restricted NF9-specific $CD8^+$ T cells by parental NF9 but not by its derivatives. HLA-A24-restricted NF9-specific $CD8^+$ T cells were stimulated with the C1R-A2402 cells pulsed with 0.1, 1 and 10 nM of NF9 peptide or PMA (10 ng/ml) and Ionomycin (1 μg/ml) (PMA+I). (**A**) Representative plots of donor #4. The numbers in the plots indicate the percentage of IFN-$γ^+$ cells in $CD8^+$ T cells. (**B**) The sensitivity of HLA-A24-restricted NF9-specific $CD8^+$ T cells towards parental NF9 peptide and its derivatives (NF9-L452R and NF9-Y453F). The assay was performed in triplicate, and the means with SDs are shown. Statistically significant differences (*, $P < 0.05$) versus parental NF9 at the same dose are determined by Student's *t* test.

(**D**) Pseudovirus assay. The HIV-1-based reporter virus pseudotyped with the parental SARS-CoV-2 S or its derivatives (L452R, Y453F and N501Y) was inoculated into HEK293 cells transiently expressing mink ACE2 and human TMPRSS2 at 4 different doses (1, 3, 5 and 10 ng p24 antigens). Percentages of

infectivity compared to the virus pseudotyped with parental S (10 ng p24 antigen) are shown. Statistically significant differences (*, $P < 0.05$) versus parental S at the same dose are determined by ANOVA, with multiple comparisons by Bonferroni correction.

(**E**) Growth kinetics of parental SARS-CoV-2 and SARS-CoV-2 mutants. Parental SARS-CoV-2 and the L452R, Y453F and N501Y mutants (10 or 1,000 PFU) were inoculated into HEK293-ACE2 cells and the copy number of viral RNA in the culture supernatant was quantified by real-time RT-PCR. (Left) The growth curve of the viruses inoculated. The result for the parental virus is shown in all panels as a black line. (Right) The amount of viral RNA in the culture supernatant at 72 h postinfection. Assays were performed in triplicate, and statistically significant differences (*, $P < 0.05$) versus parental virus are determined by Student's *t* test.

(**F**) Representative FACS plots showing surface expression of parental S and its derivatives (L452R, Y453F and N501Y). The number in the plot represents the mean fluorescence intensity of surface S, and gray lines represent the histograms of isotype control.

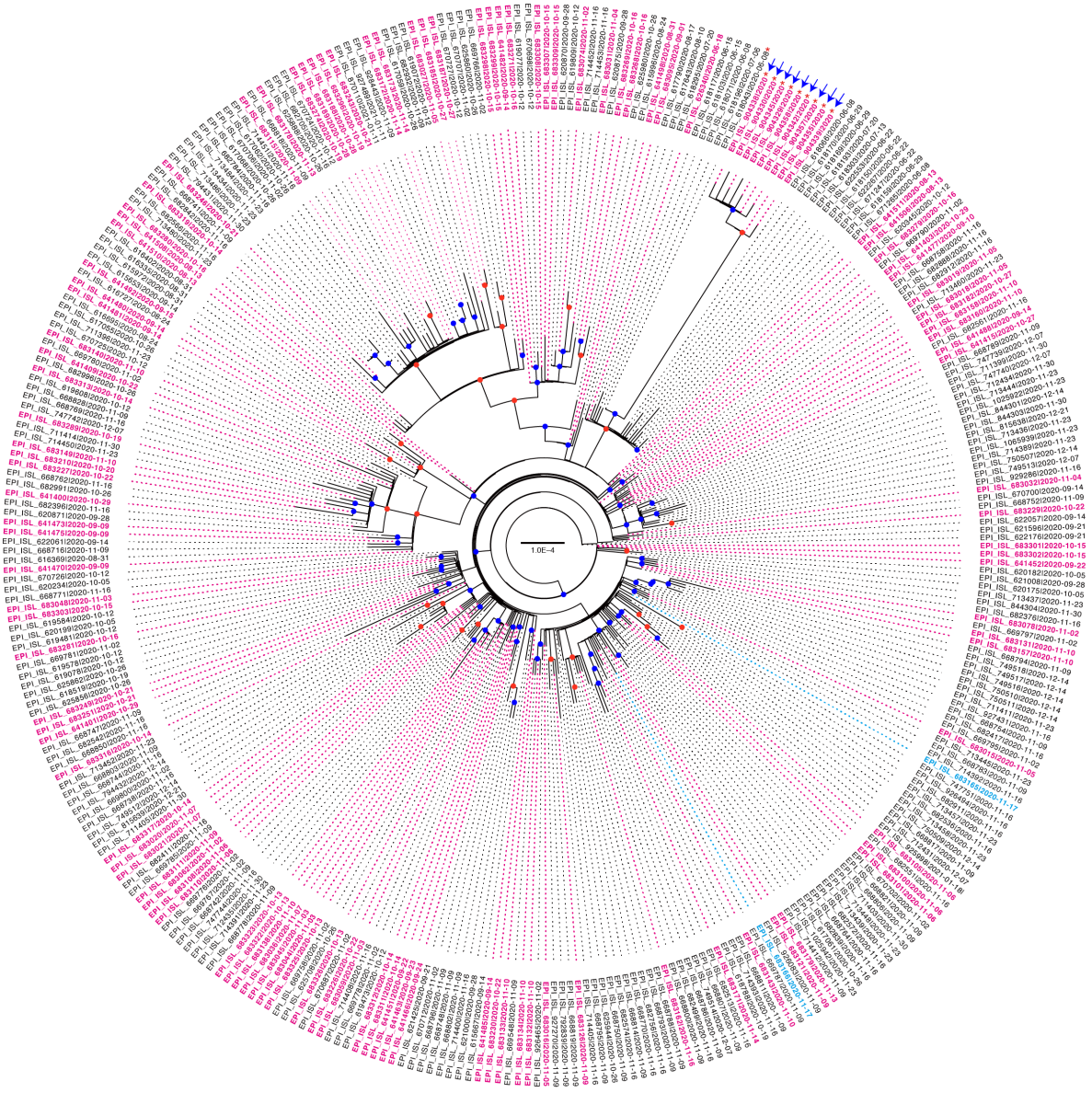

Figure S2. A maximum-likelihood based phylogenetic tree of the representative 345 SARS-CoV-2 sequences that belongs to the B.1.1.298 lineage (Related to Figure 3)

GISAID ID and sampling date of the representative 345 SARS-CoV-2 sequences belonging to the B.1.1.298 lineage were noted in each terminal node. The colors (black, magenta or cyan) indicates their hosts (humans, minks or cats). Red asterisks indicate the sequences that encode Y453 in SARS-CoV-2 S [i.e., no nonsynonymous (Y453F) mutation]. Blue arrows indicate the sequences that were isolated in Netherlands (i.e., the others were isolated in Denmark). Red or blue circles denote the bootstrap value were ≥80 or ≥50 (n=100).