Neutralization of N501Y mutant SARS-CoV-2 by BNT162b2 vaccine-elicited sera

Supplementary Material

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Materials and Methods

Construction of isogenic viruses

We prepared an isogenic pair of SARS-CoV-2 containing the N501 or Y501 spike protein (Figure S1). The N501Y mutation was generated by an A-to-T substitution at nucleotide 23,063 of the viral genome using an infectious cDNA clone of clinical strain WA1 (2019-nCoV/USA_WA1/2020).[1] Following a previously reported mutagenesis protocol,[2] we recovered N501 and Y501 viruses with titers of $>10^7$ plaque-forming units (PFU) per ml. The two viruses developed similar plaque morphologies on Vero E6 cells (Fig. S2).

Serum specimens and neutralization assay

The immunization and serum collection regimen is illustrated schematically in Fig. S3. For measuring neutralization titers, each serum was 2-fold serially diluted in culture medium with the first dilution of 1:40 (dilution range of 1:40 to 1:1280). The diluted serum was incubated with 100 PFU of N501 or Y501 virus at 37 °C for 1 h, after which the serum-virus mixtures were inoculated onto Vero E6 cell monolayer in 6-well plates. A conventional (non-fluorescent) plaque reduction neutralization assay was performed to quantify the serum-mediated virus suppression as previously reported.[3] A minimal serum dilution that suppressed >50% of viral plaques is defined as $PRNT_{50}$. A table of the neutralization titers is provided (Table S1). The ratio for each serum of the $PRNT_{50}$ against N501 and Y501 virus is plotted in Fig. S4.

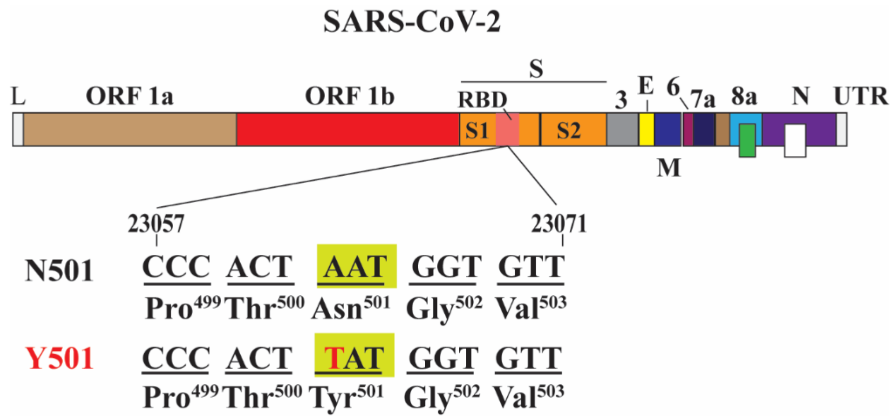

Figure S1. Diagram of the N501Y substitution. L – leader sequence; ORF – open reading frame; RBD – receptor binding domain; S – spike glycoprotein; S1 – N-terminal furin cleavage fragment of S; S2 – C-terminal furin cleavage fragment of S; E – envelope protein; M – membrane protein; N – nucleoprotein; UTR – untranslated region.

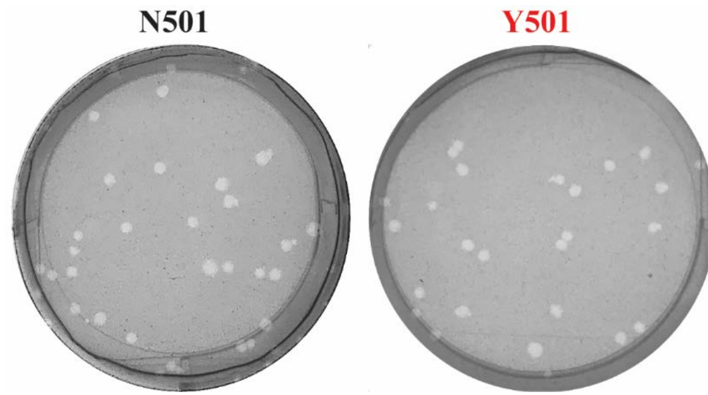

Figure S2. Plaque morphologies of N501 and Y501 SARS-CoV-2 on Vero E6 cells.

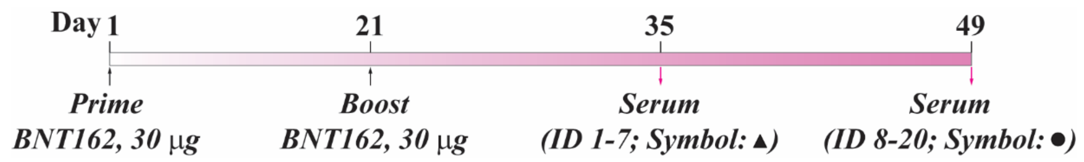

Figure S3. Scheme of the BNT162 vaccination and serum sampling.

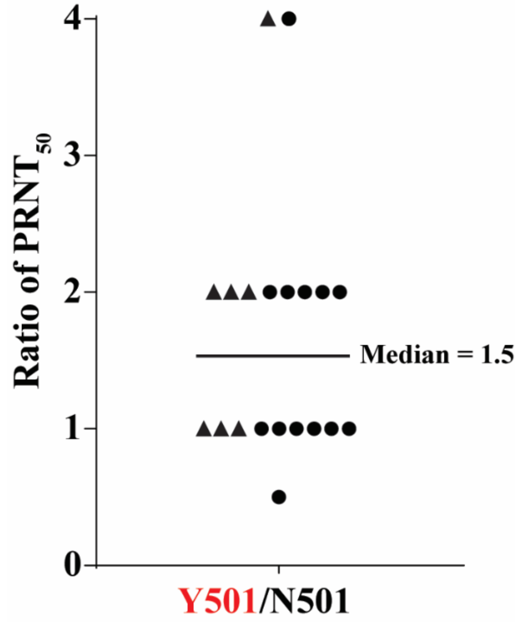

Figure S4. Plot of the ratio of $PRNT_{50}$ between Y501 and N501 viruses. Triangles represent sera drawn two weeks after the second dose; circles represent sera drawn four weeks after the second dose.

Table S1. $PRNT_{50}$ values of 20 BNT162b2 post-immunization sera against N501 and Y501 SARS-CoV-2.

Serum ID	$PRNT_{50}$		$PRNT_{50}$ ratio (Y501/N501)
	N501	Y501	
1	160	640	4
2	160	320	2
3	320	640	2
4	80	160	2
5	160	160	1
6	320	320	1
7	640	640	1
8	160	160	1
9	640	640	1
10	640	1280	2
11	160	640	4
12	320	320	1
13	640	1280	2
14	640	320	0.5
15	320	640	2
16	320	640	2
17	640	640	1
18	640	1280	2
19	640	640	1
20	640	640	1

Supplementary References

1. Xie X, Muruato A, Lokugamage KG, et al. An Infectious cDNA Clone of SARS-CoV-2. Cell Host Microbe 2020;27:841-8 e3.
2. Plante JA, Liu Y, Liu J, et al. Spike mutation D614G alters SARS-CoV-2 fitness. Nature 2020.
3. Muruato AE, Fontes-Garfias CR, Ren P, et al. A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation. Nat Commun 2020;11:4059.