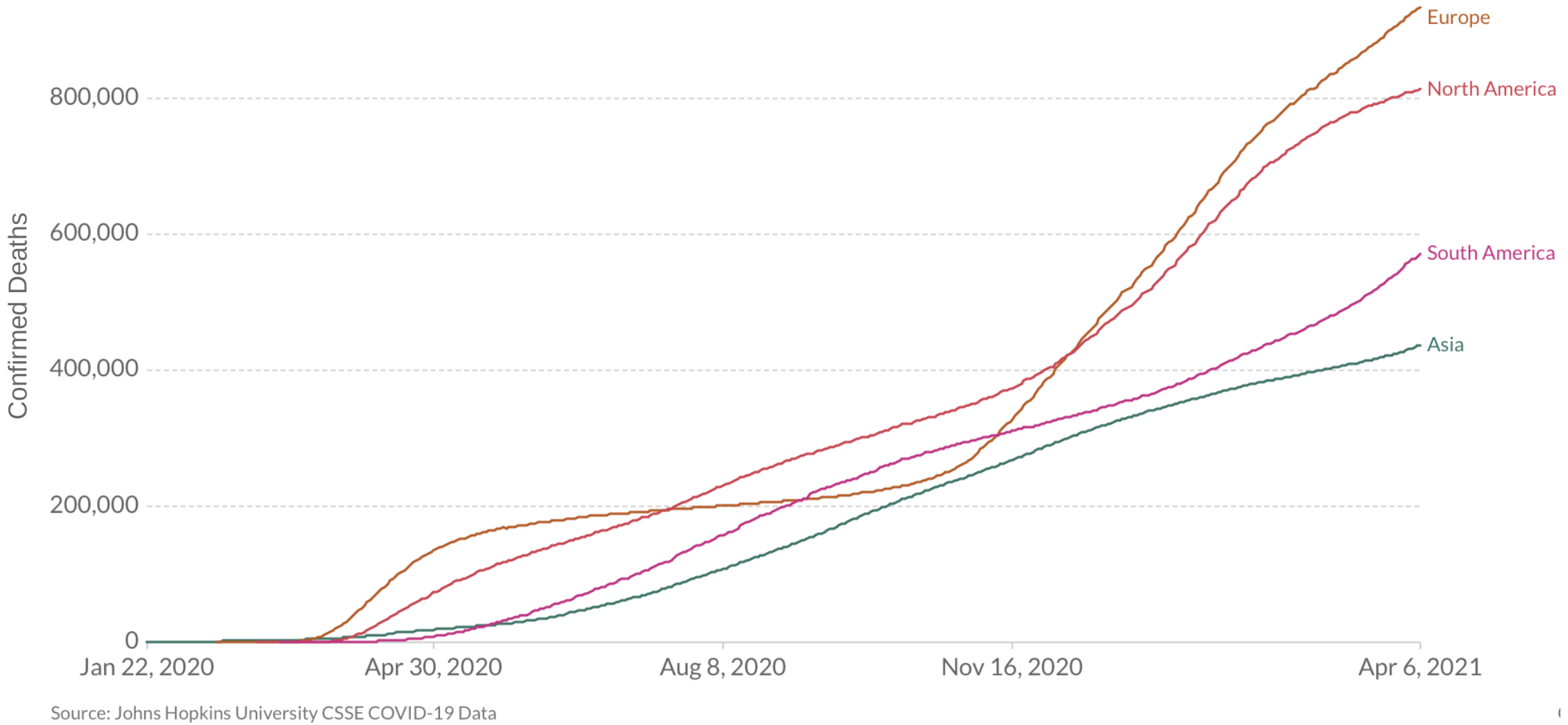
A SITE OF VULNERABILITY IN THE SARS-COV-2 SPIKE N-TERMINAL DOMAIN

MATTHEW MCCALLUM

HIGHER ORDER STRUCTURE (HOS) 2021

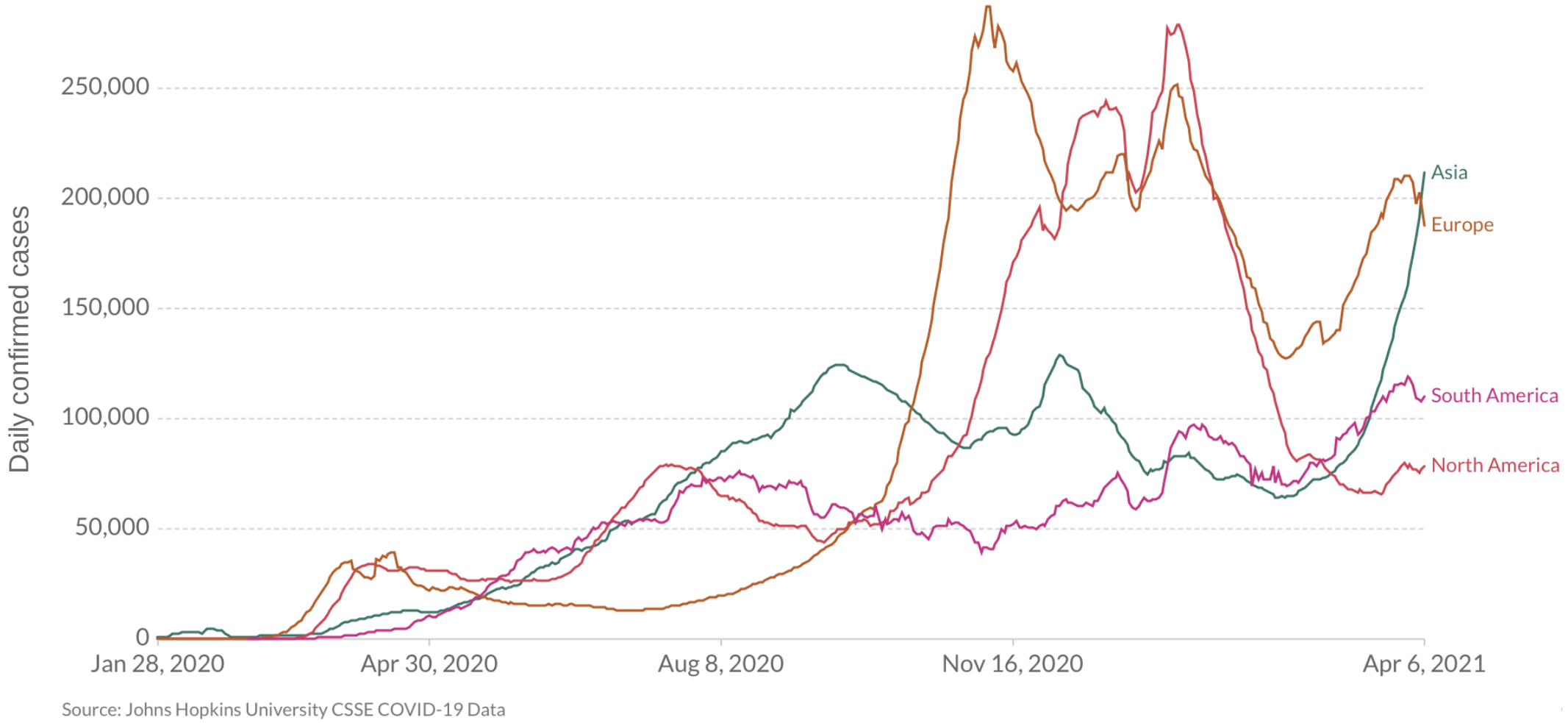
APRIL 15, 2021

THE SARS-CoV-2 PANDEMIC HAS BEEN DEVASTATING



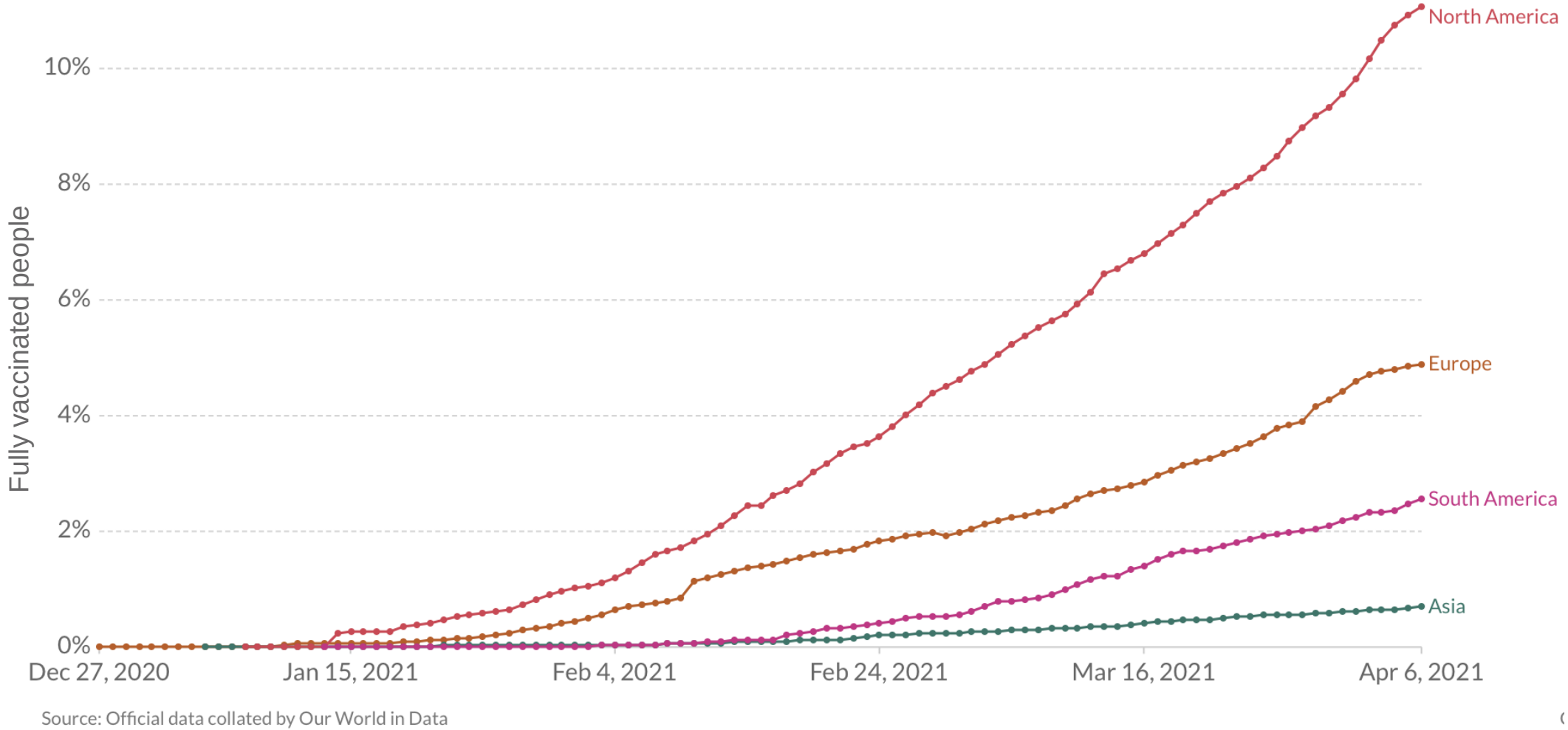
Source: Johns Hopkins University CSSE COVID-19 Data

THE SARS-CoV-2 PANDEMIC HAS BEEN DEVASTATING



Source: Johns Hopkins University CSSE COVID-19 Data

THE SARS-CoV-2 PANDEMIC HAS BEEN DEVASTATING

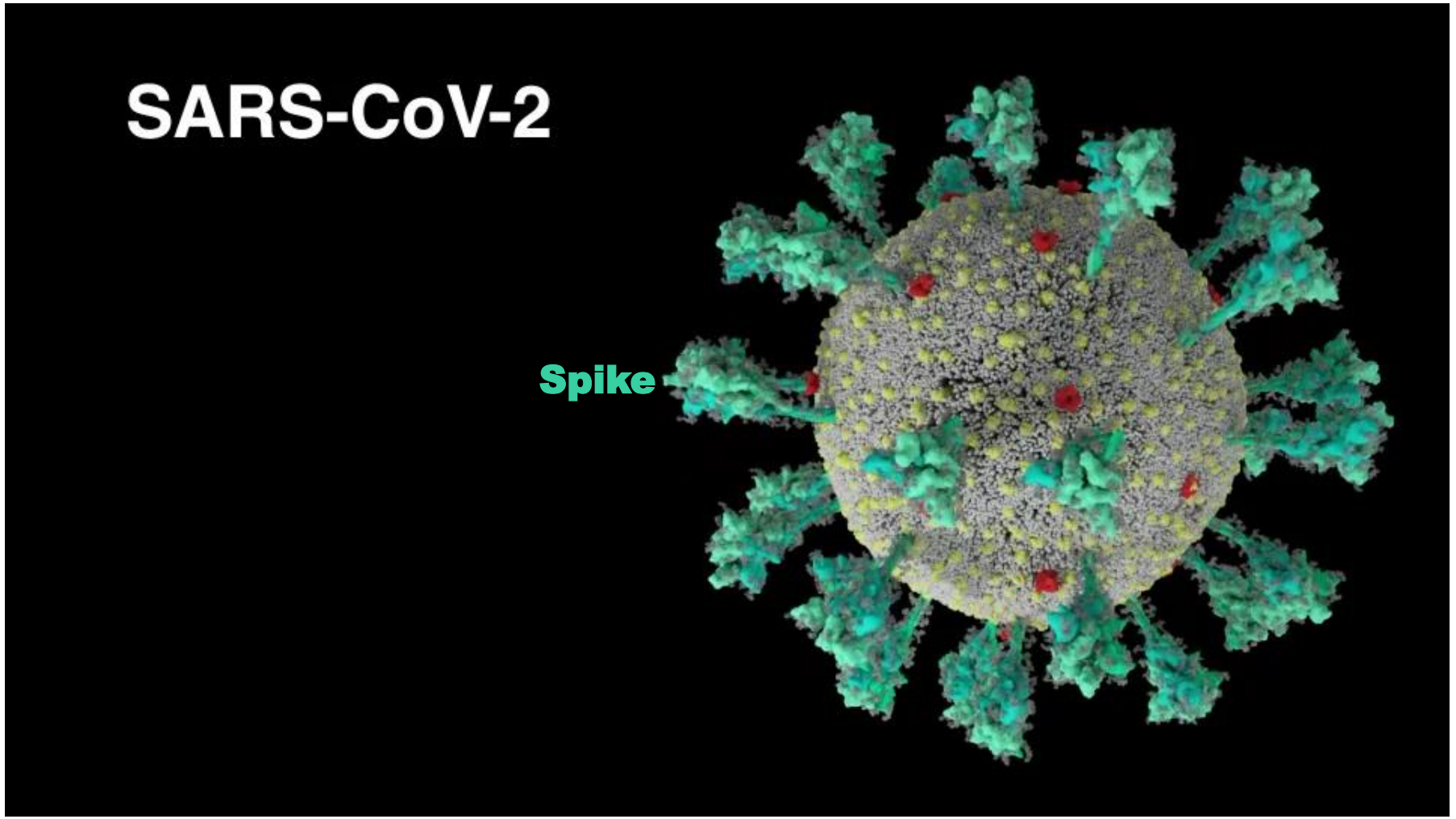


Vaccines work, but roll-out slow

Antibody therapy works, but must used early

Emergence of variants threatens both

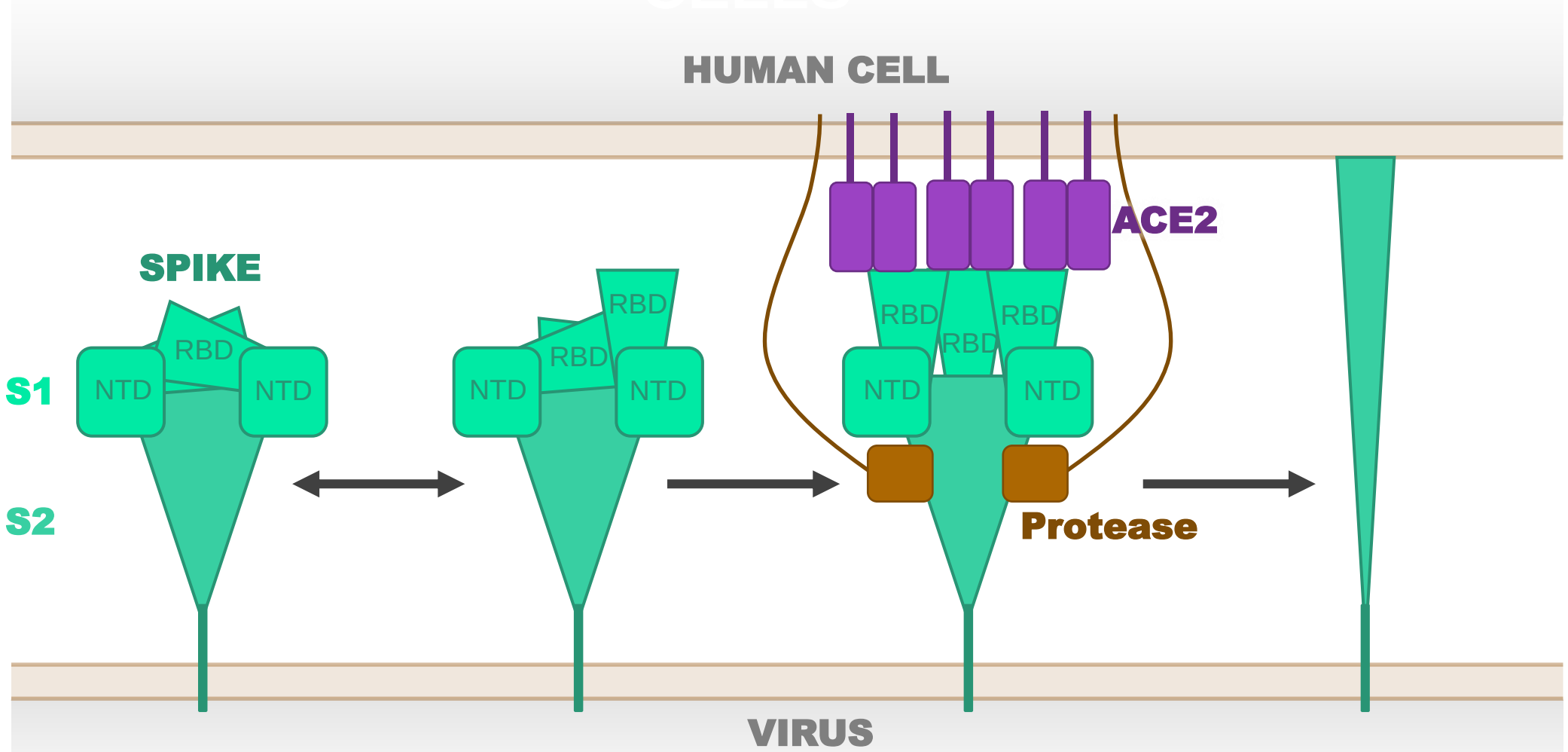
SARS-COV-2 USES A SURFACE SPIKE PROTEIN TO ENTER CELLS



Spike
ACE2
Protease

Source: Janet Iwasa, https://animationlab.utah.edu/cova

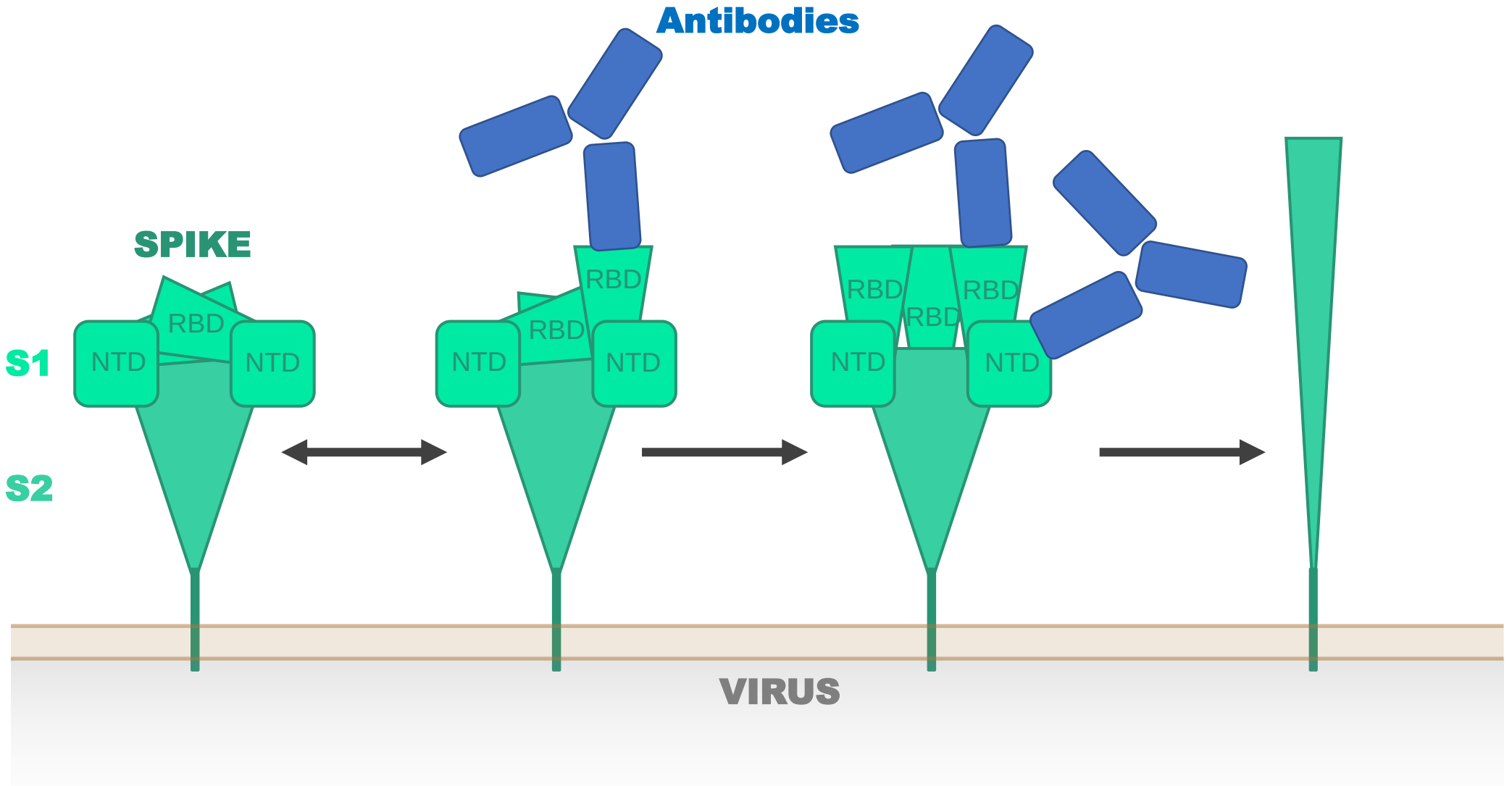
SARS-COV-2 USES A SURFACE SPIKE PROTEIN TO ENTER CELLS



The spike RBD must open to bind ACE2

Opening allows proteases to trigger the fusion process

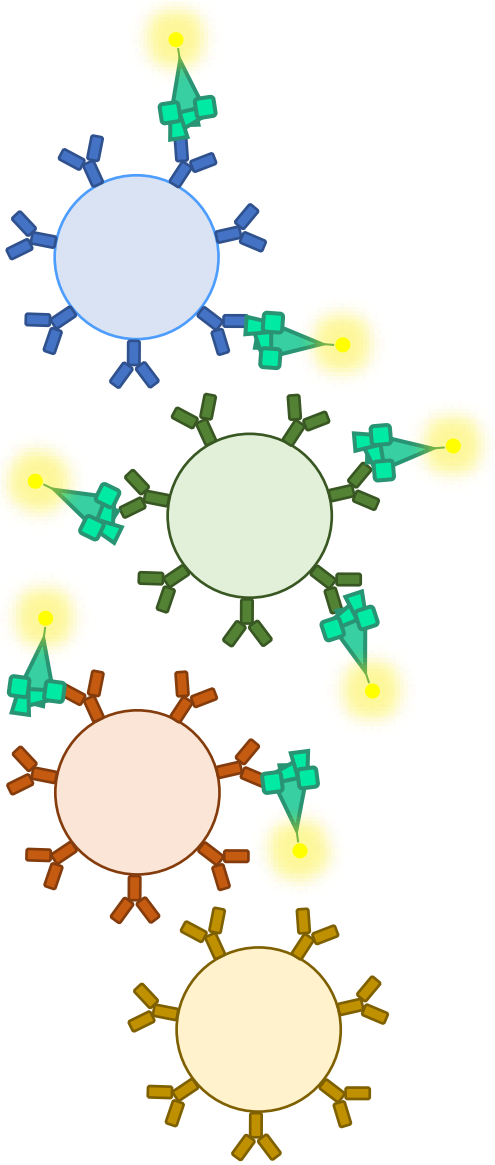
ANTIBODIES CONFER NEUTRALIZING IMMUNITY



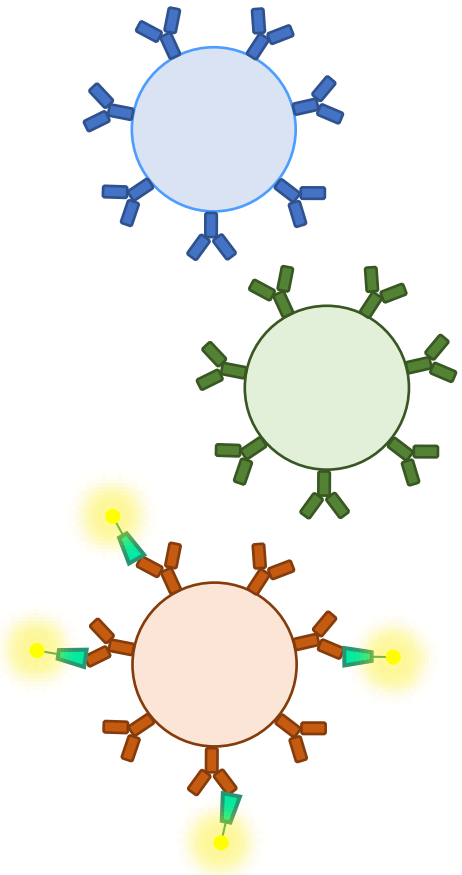
Antibodies that bind to RBD thought to be primarily responsible for immunity

One neutralizing antibody previously shown to bind the NTD – how common is this?

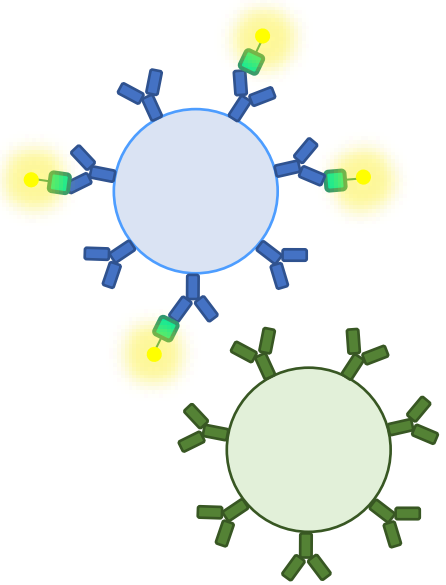
SORTING B-CELLS BY SPIKE-BINDING



Does not bind Spike



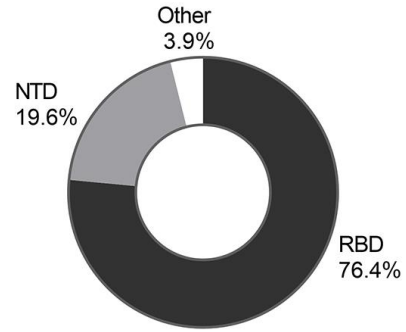
Binds RBD



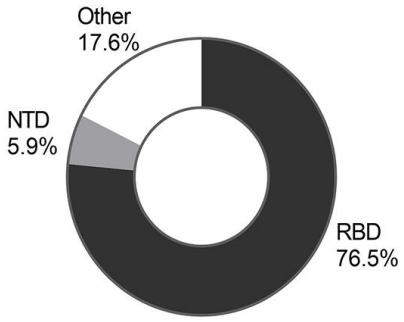
Binds NTD

Binds Spike but not RBD or NTD

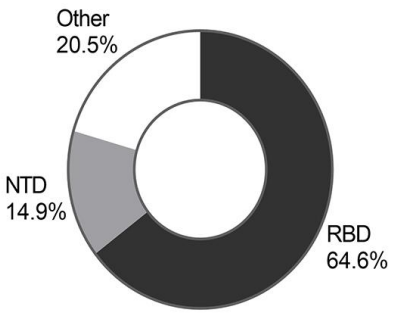
IDENTIFYING NTD-BINDING ANTIBODIES FROM THREE DONORS



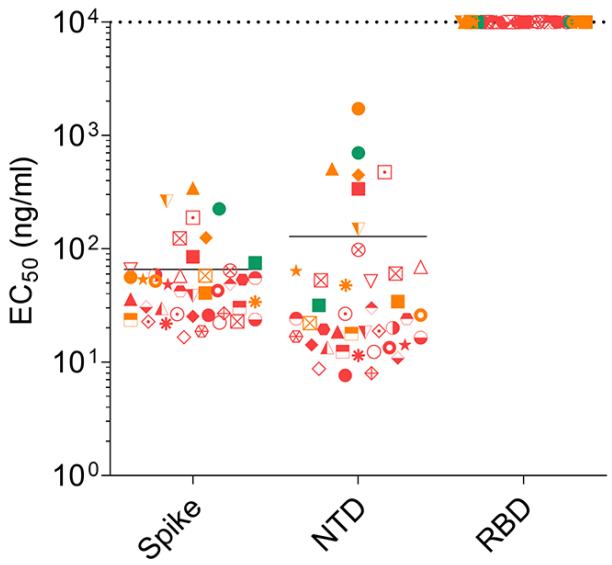
Donor L
Total=51

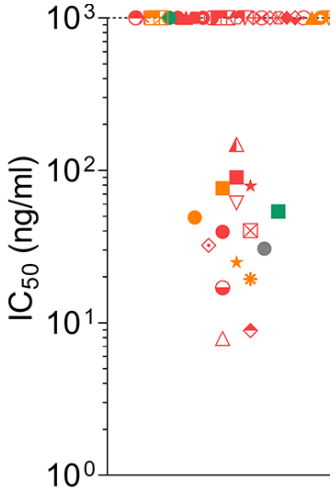


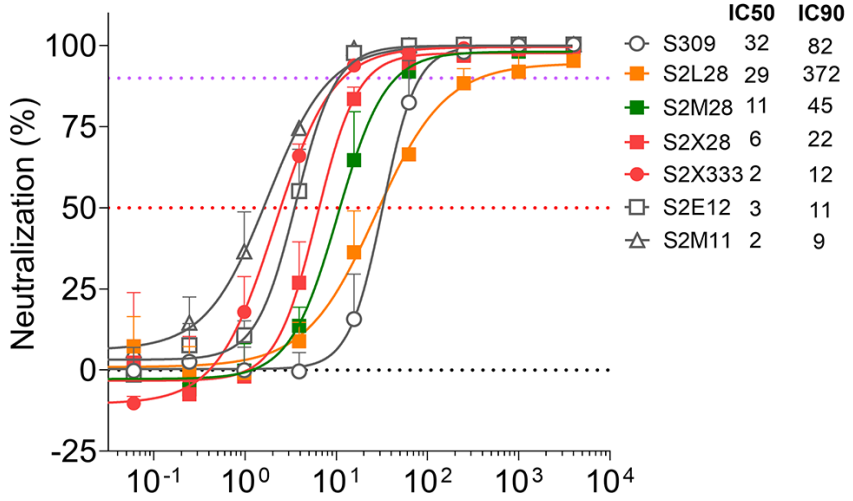
Donor M
Total=34



Donor X
Total=193



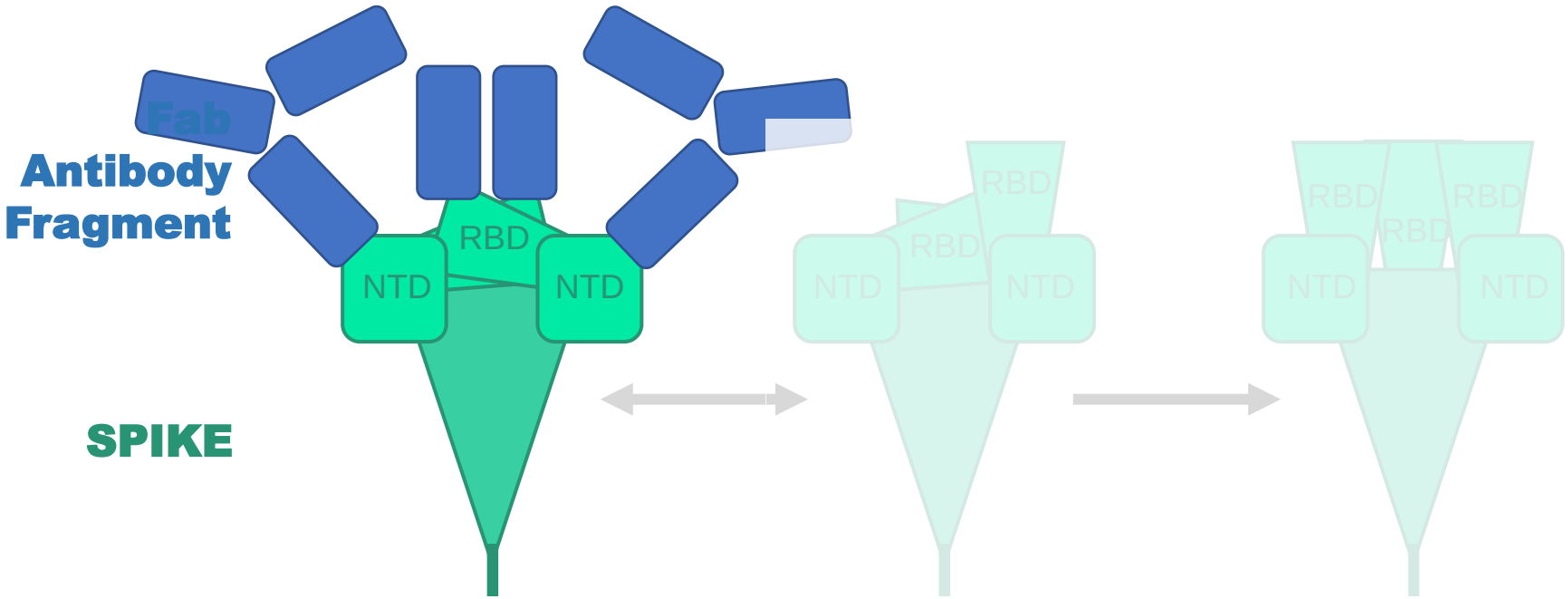




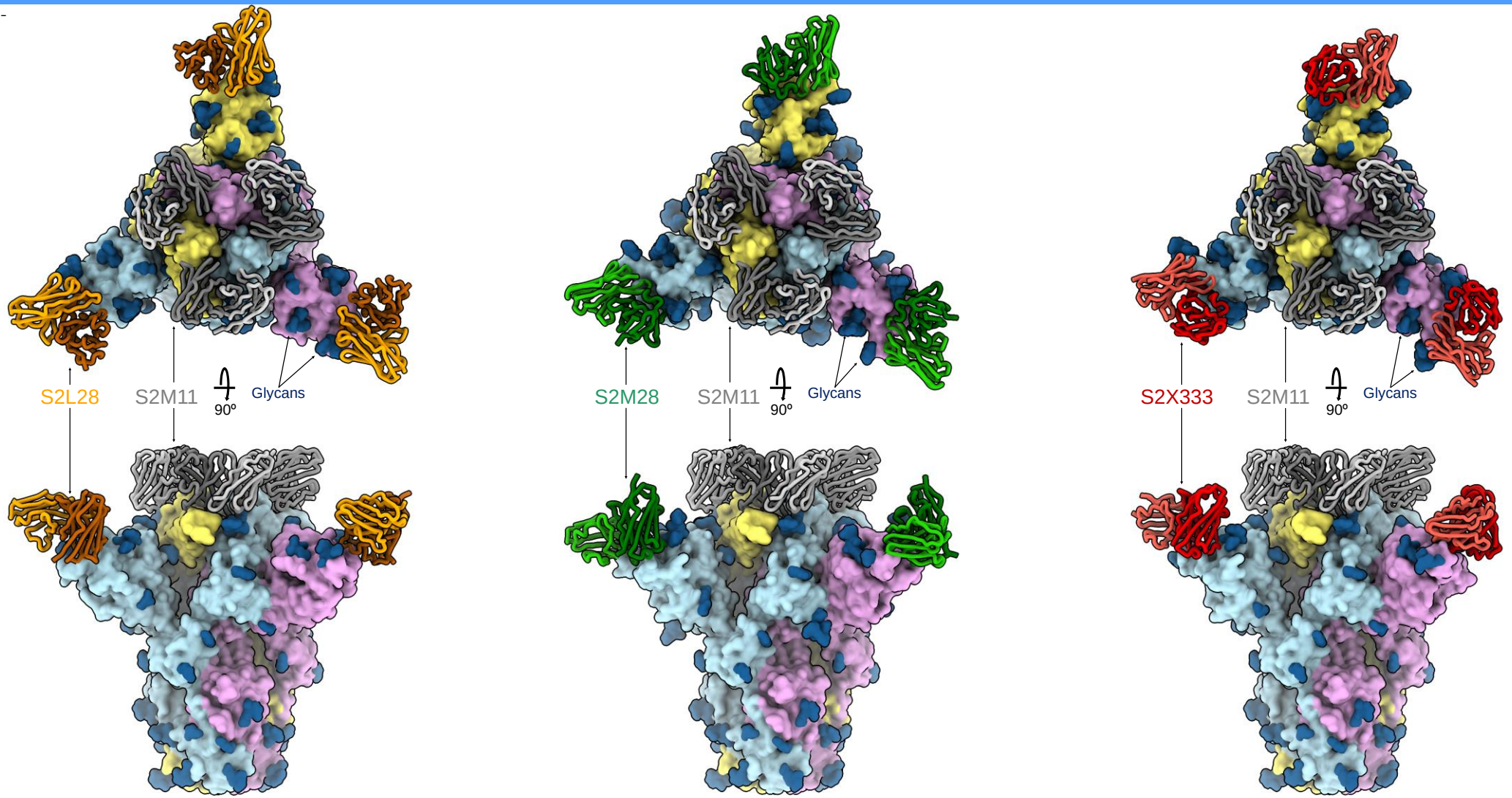
Each patient harbored potent neutralizing NTD antibodies

Anna De Marco (McCallum & De Marco *et al.* 2021. Cell *In press*)

SAMPLE PREPARATION FOR ELECTRON CRYOMICROSCOPY



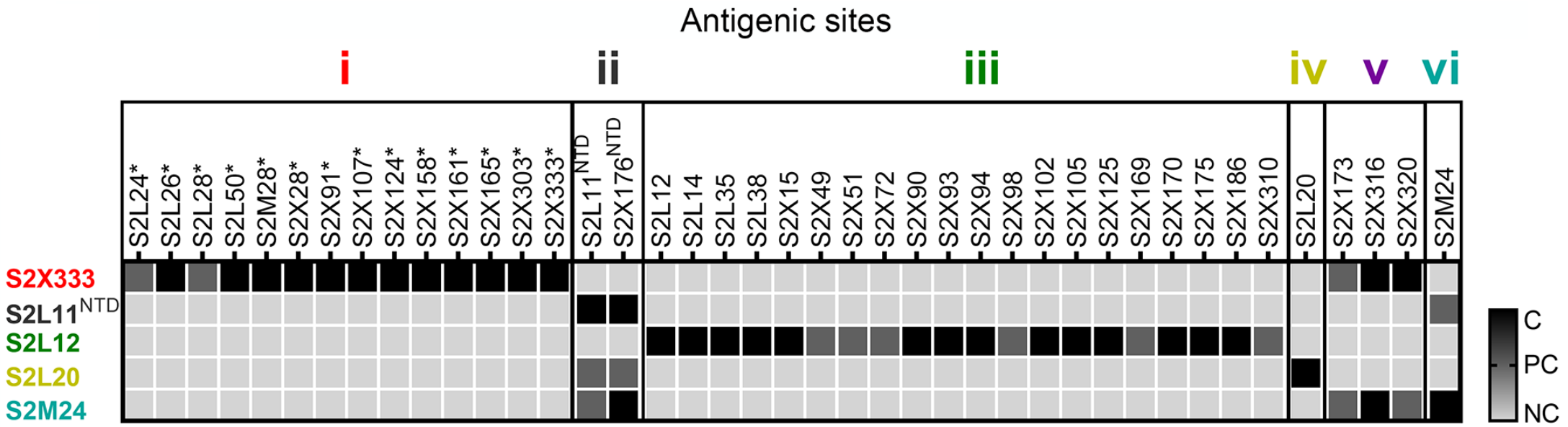
STRUCTURE OF NEUTRALIZING FAB FROM EACH DONOR



S2L28, S2M28, and S2X333 bind the same site or "supersite"

McCallum & De Marco *et al.* 2021. Cell *In press*

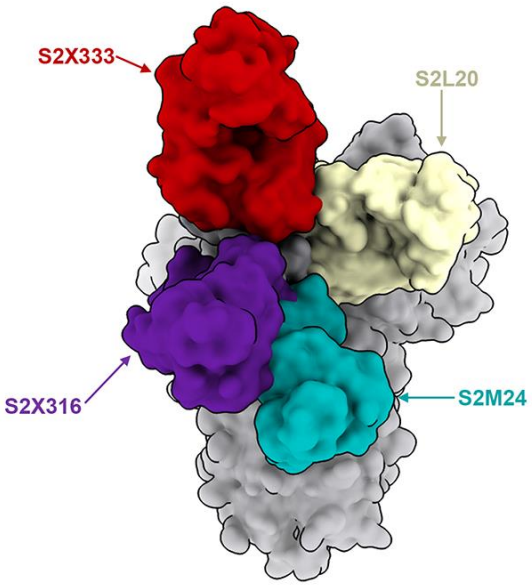
MAPPING NTD ANTIGENIC SITES BY BINDING COMPETITION

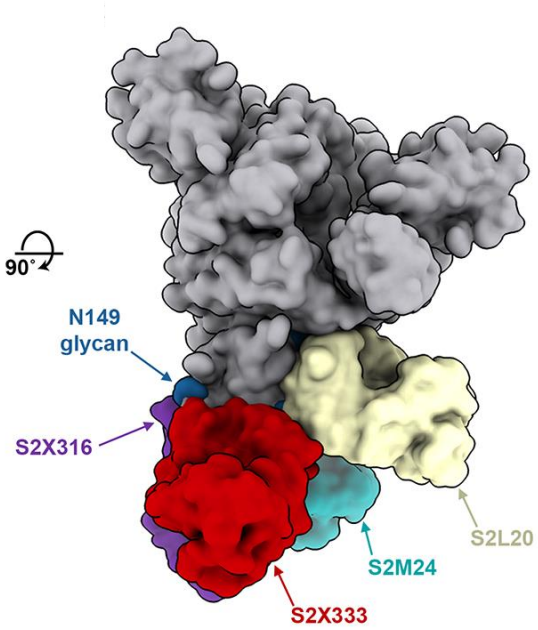


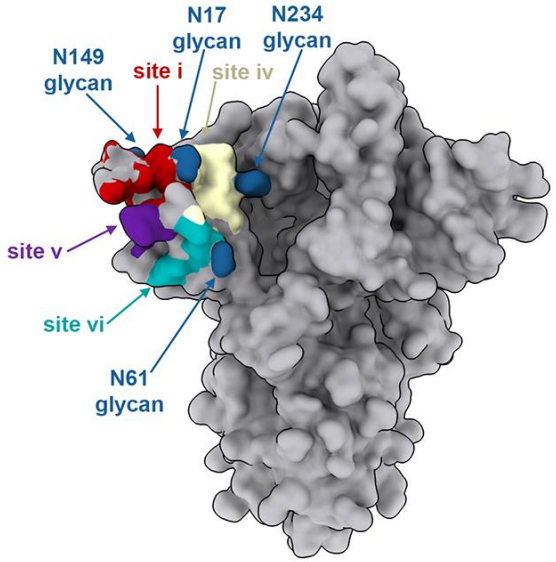
All neutralizing antibodies bind the supersite,

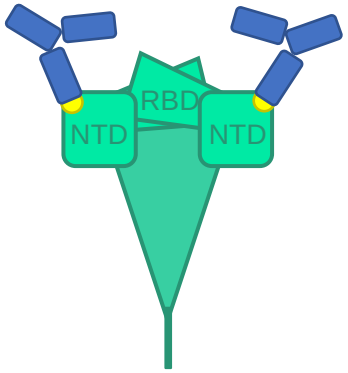
while all non-neutralizing antibodies bind other sites

MAPPING NTD ANTIGENIC SITES BY CRYOEM







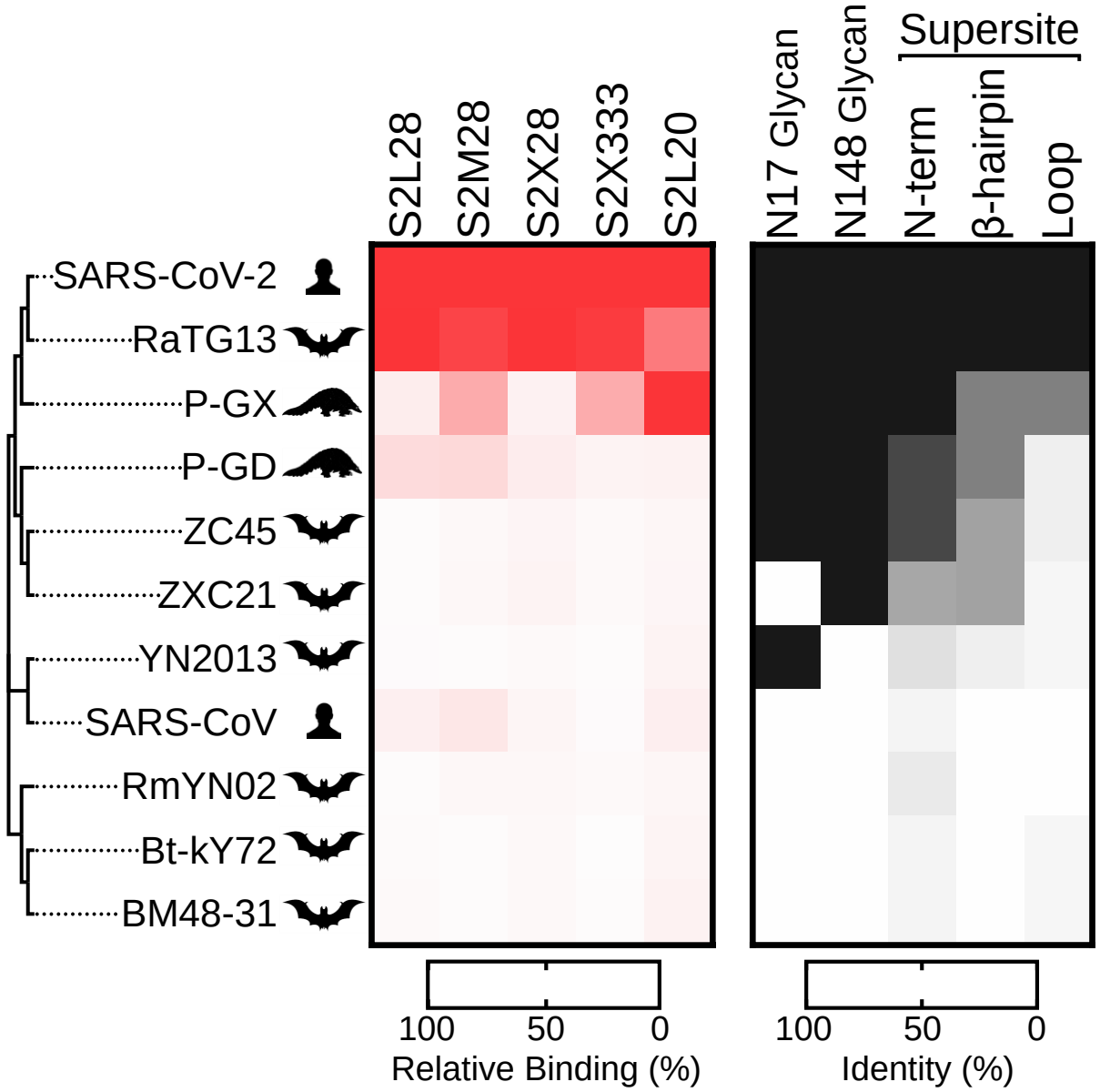


All neutralizing antibodies bind the supersite,

while all non-neutralizing antibodies bind other sites

McCallum & De Marco *et al.* 2021. Cell *In press*

BINDING (FLOW-CYTOMETRY) OF OTHER CORONAVIRUSES

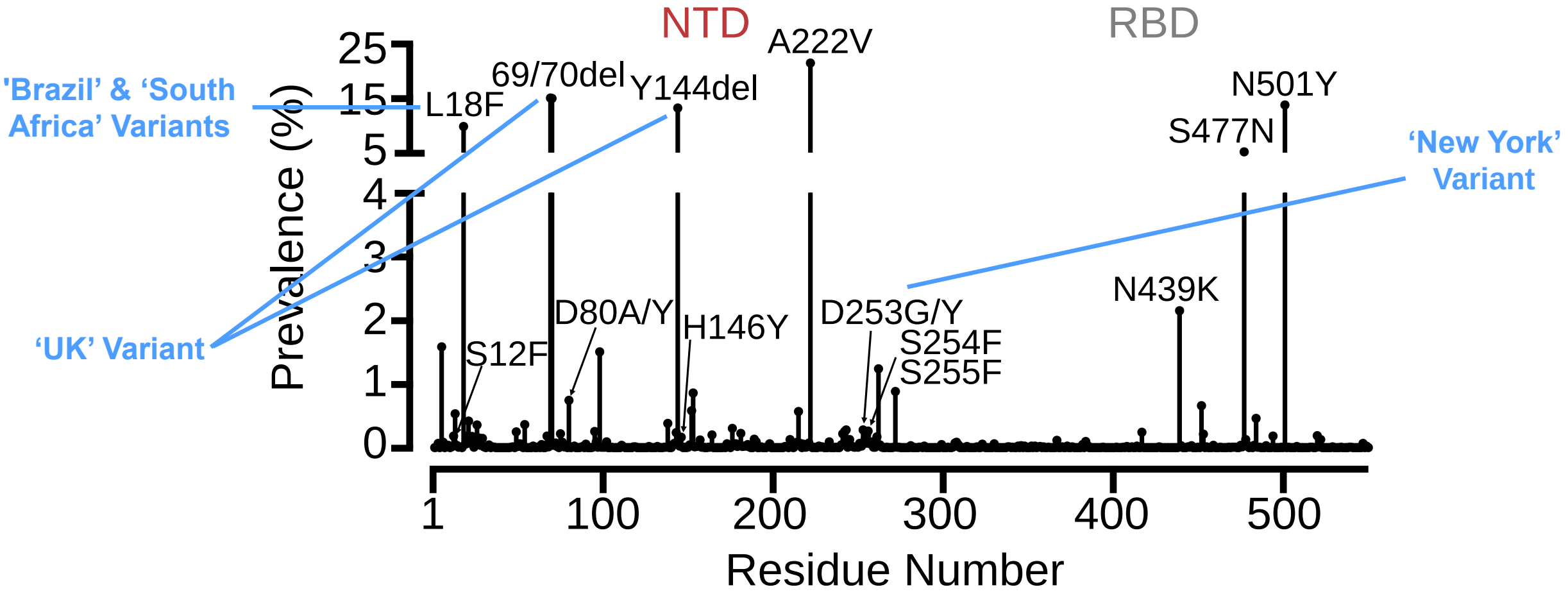


The supersite is not conserved

so binding is low outside of close relatives

Anna De Marco (McCallum & De Marco *et al.* 2021. Cell *In press*)

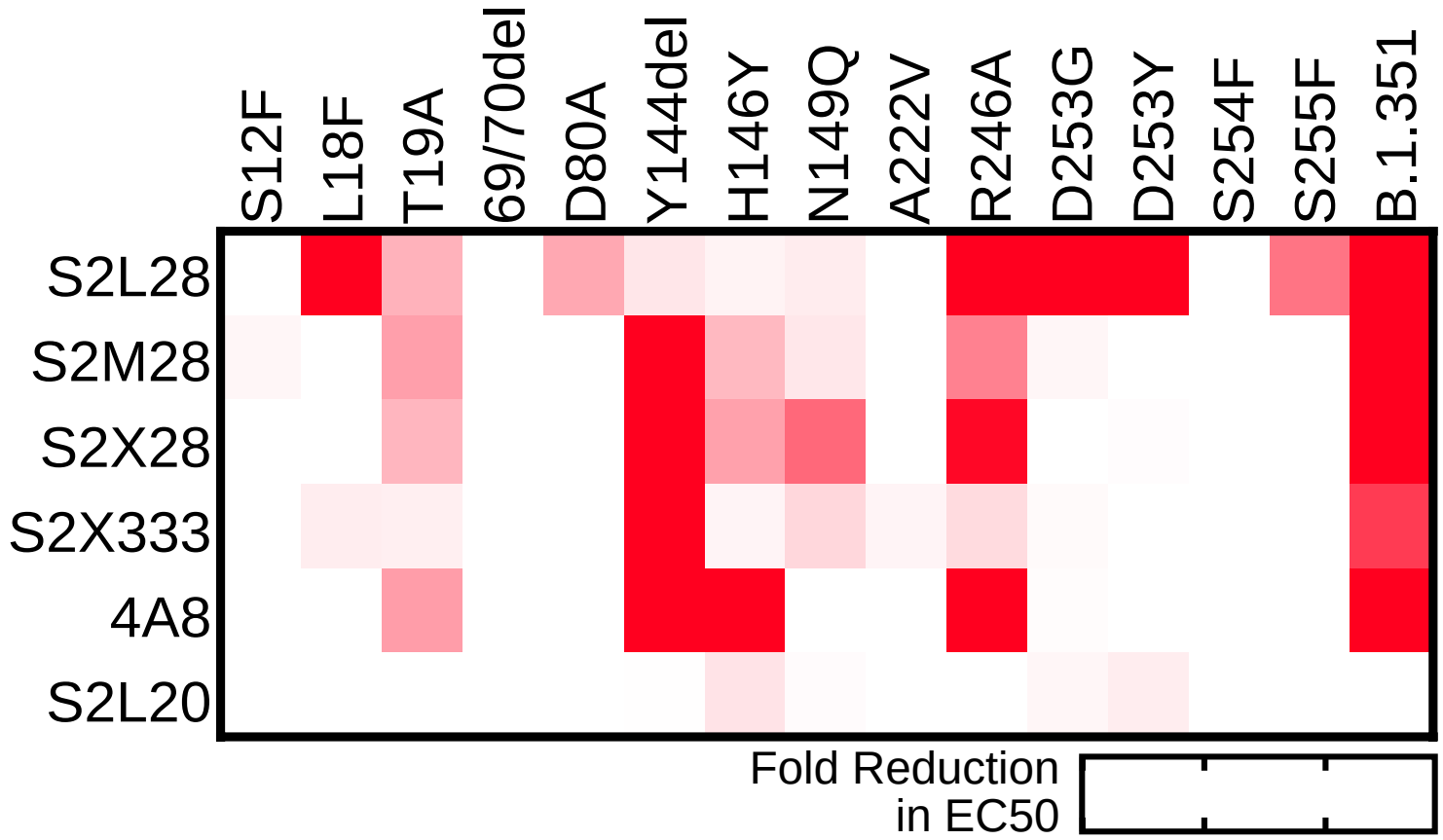
IDENTIFICATION OF POLYMORPHISMS IN THE SPIKE



The NTD is the most variable segment of the spike
The supersite is especially variable:
L18F, Y144del, H146Y, D253G/Y, S254F, and S255F have > 0.1 % prevalence

McCallum & De Marco *et al.* 2021. Cell *In press*

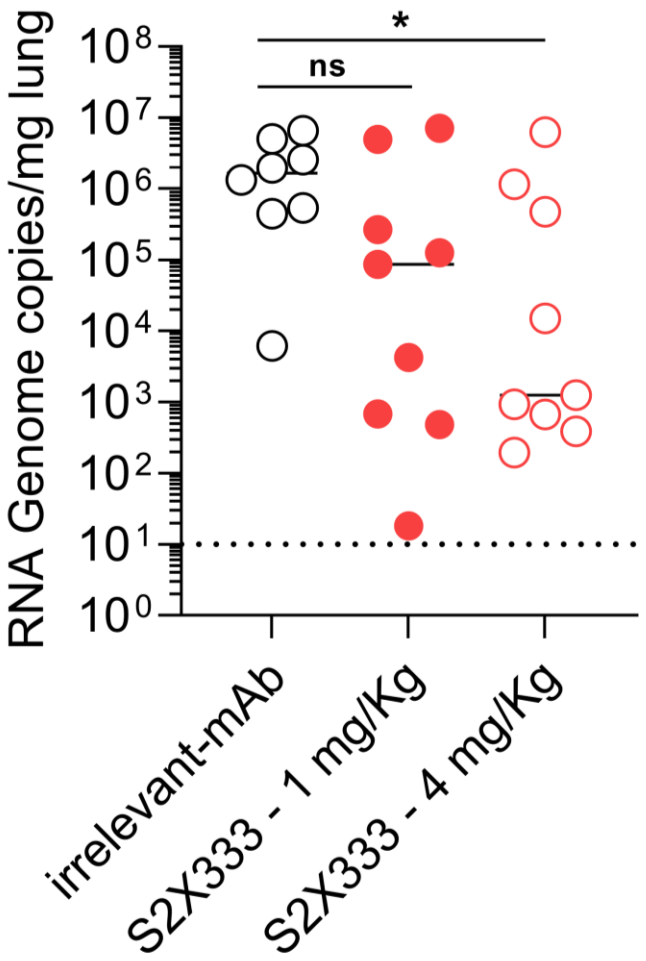
BINDING (ELISA) OF POLYMORPHISMS IN CIRCULATING VARIANTS

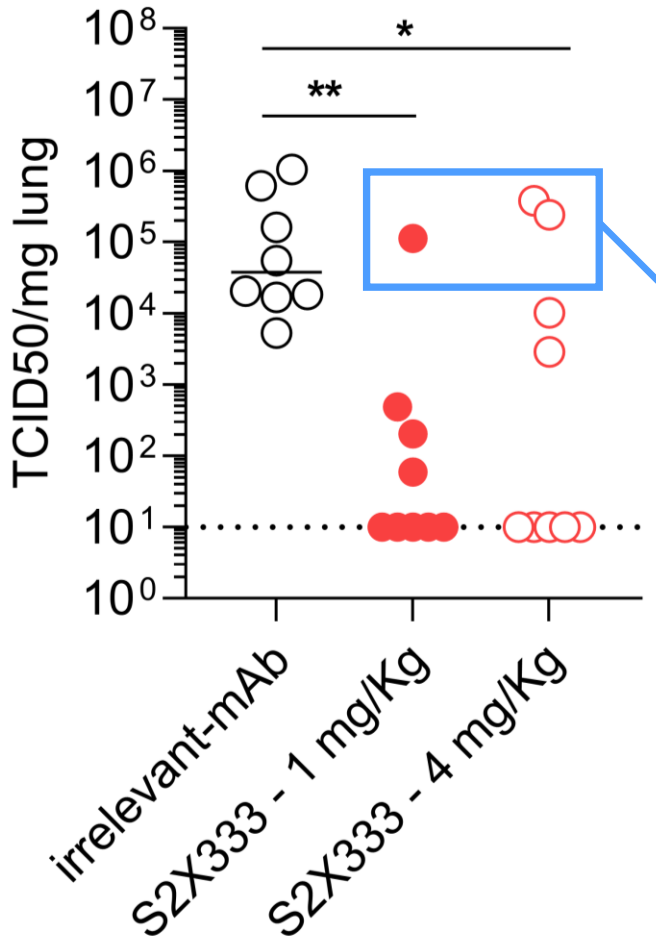


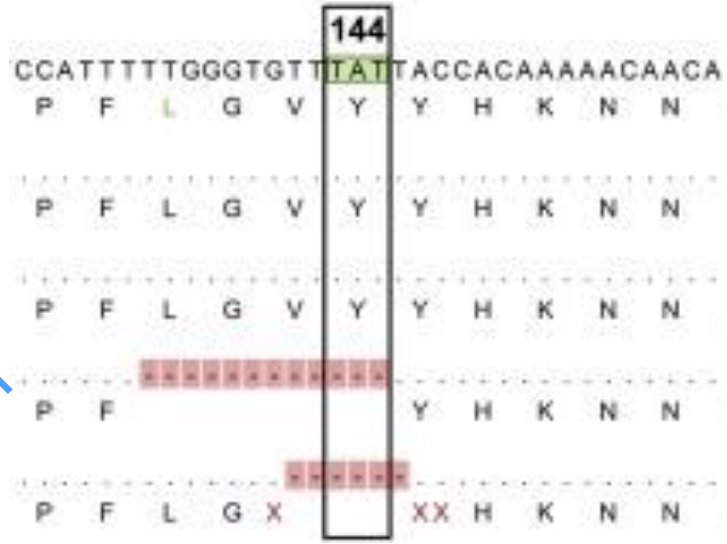
Escape mutations are evidence that the virus is under pressure from NTD antibodies, which supports that the NTD antibodies are a key component of immunity

McCallum & De Marco *et al.* 2021. Cell *In press*

CHALLENGING HAMSTERS WITH SARS-CoV-2 INFECTION







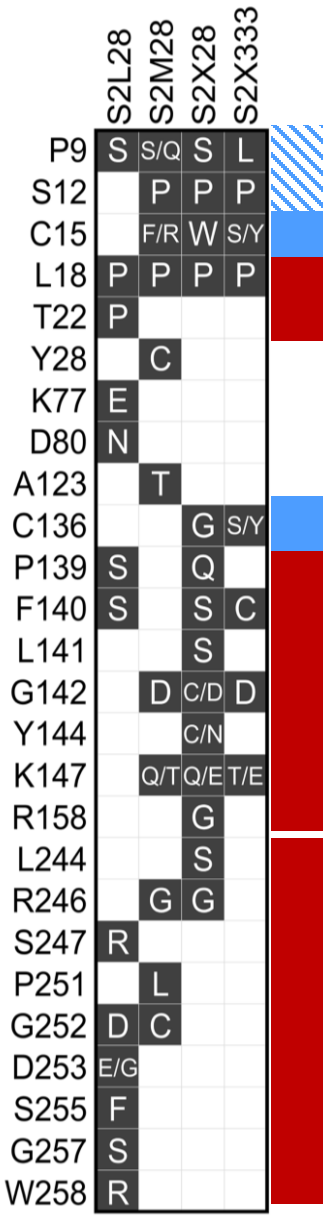
Y144del is in 'UK' Variant

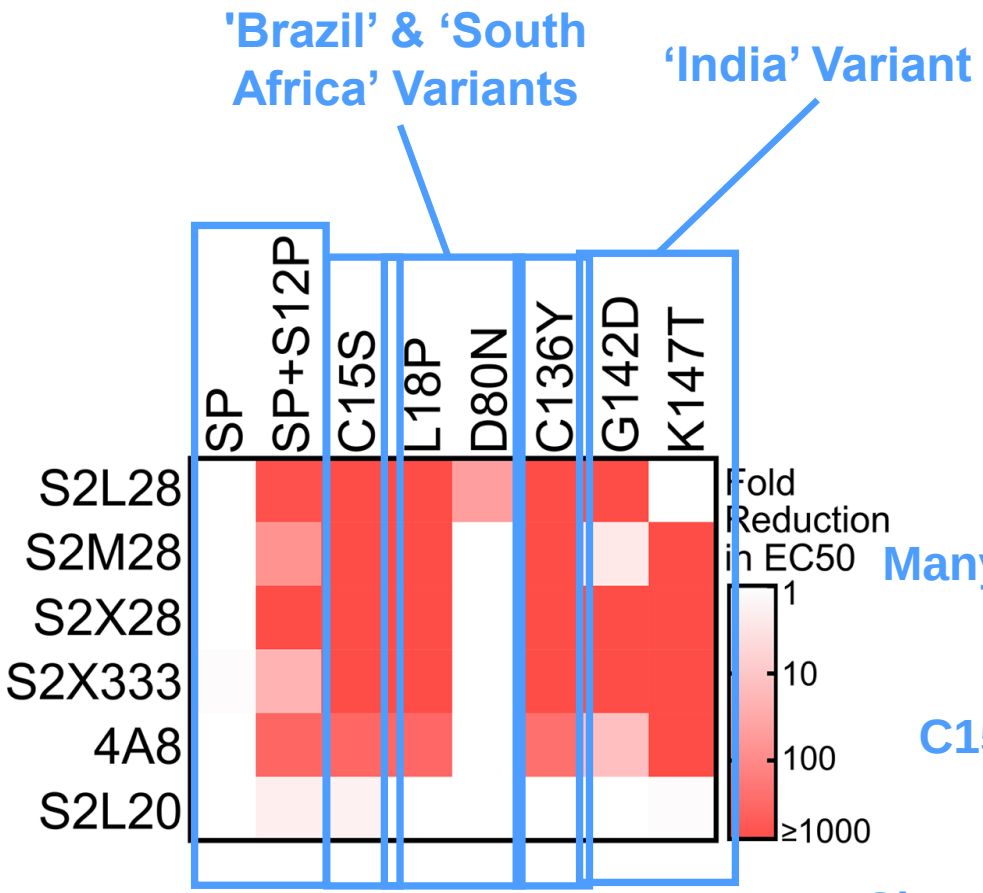
S2X333 anti-NTD antibody protects hamsters from SARS-CoV-2 challenge

Deletions including Y144 were selected for; these hamsters were not protected

Davide Corti (McCallum & De Marco *et al.* 2021. Cell *In press*)

VSV-PSEUDOTYPE SELECTION EXPERIMENTS





Mutations mostly stick to the supersite

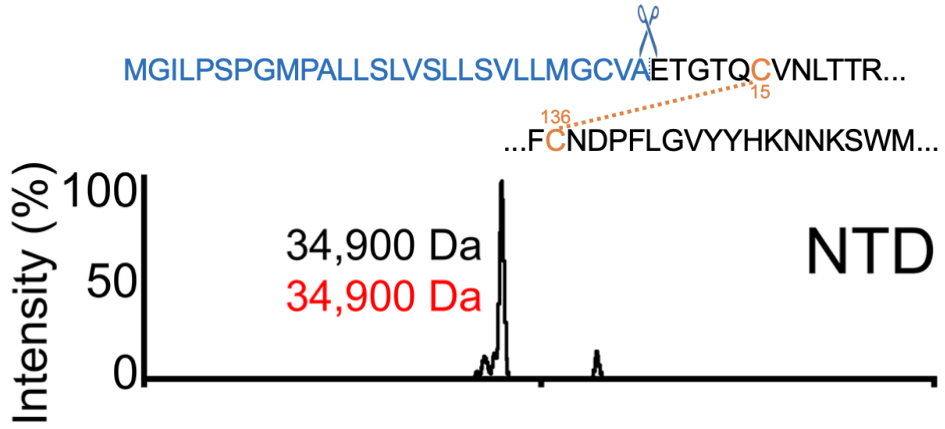
Many mutations in circulating virus and variants

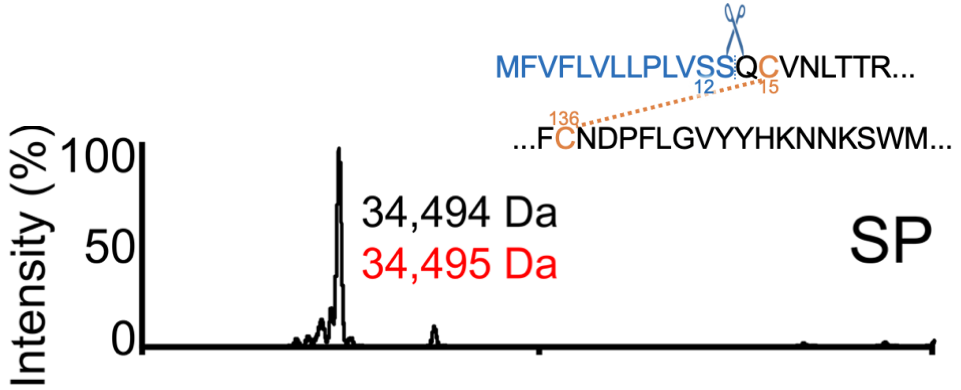
C15-C136 mutants escape all NTD antibodies

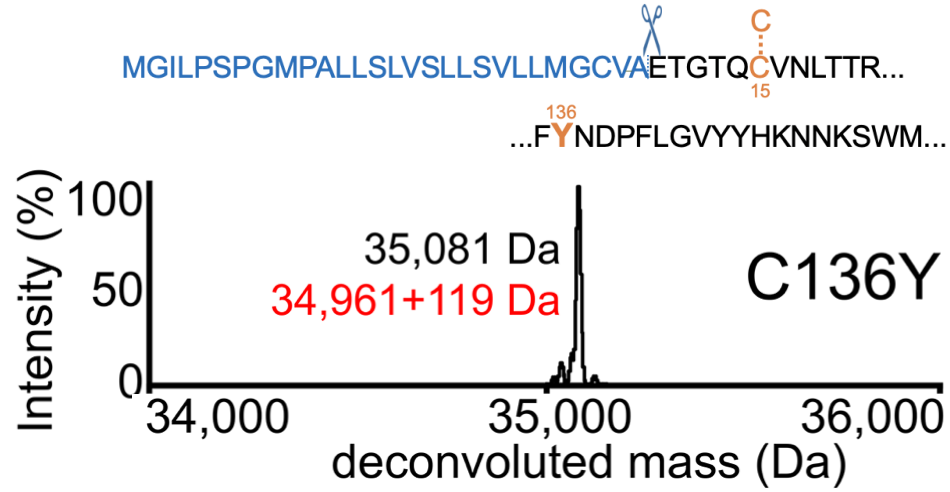
Signal peptide mutant escapes all NTD antibodies

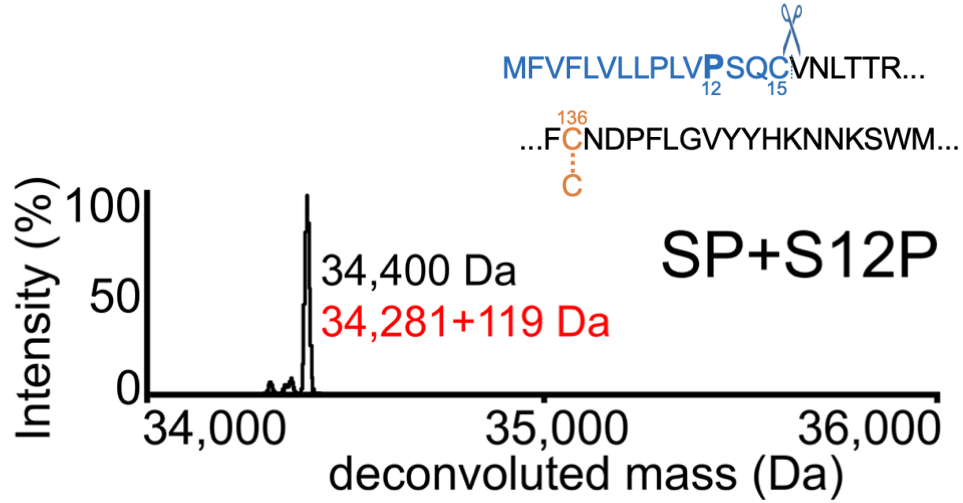
McCallum & De Marco *et al.* 2021. Cell *In press*

MASS SPECTROMETRY ANALYSIS OF S12P MUTANT







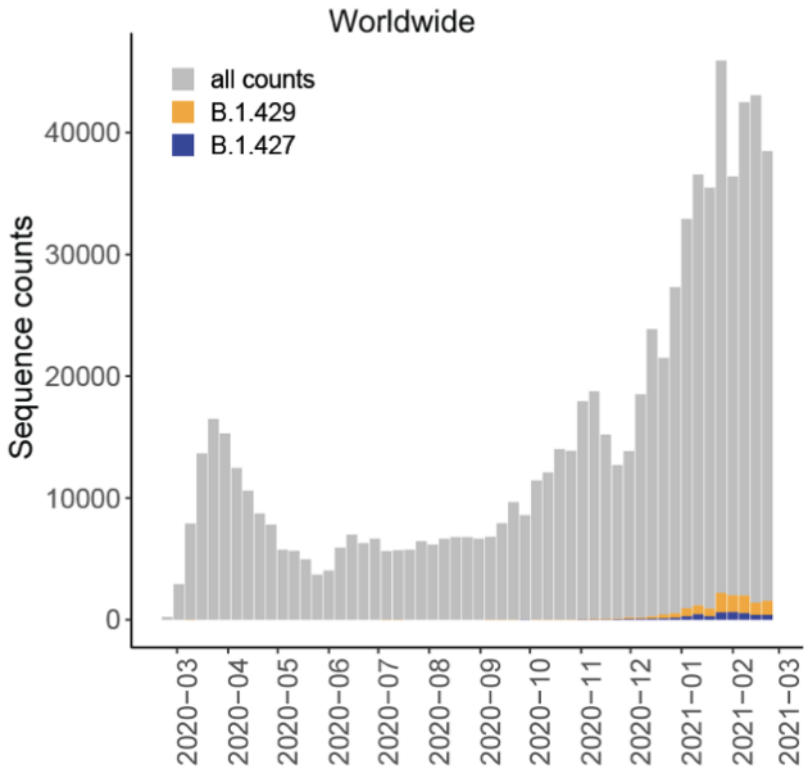


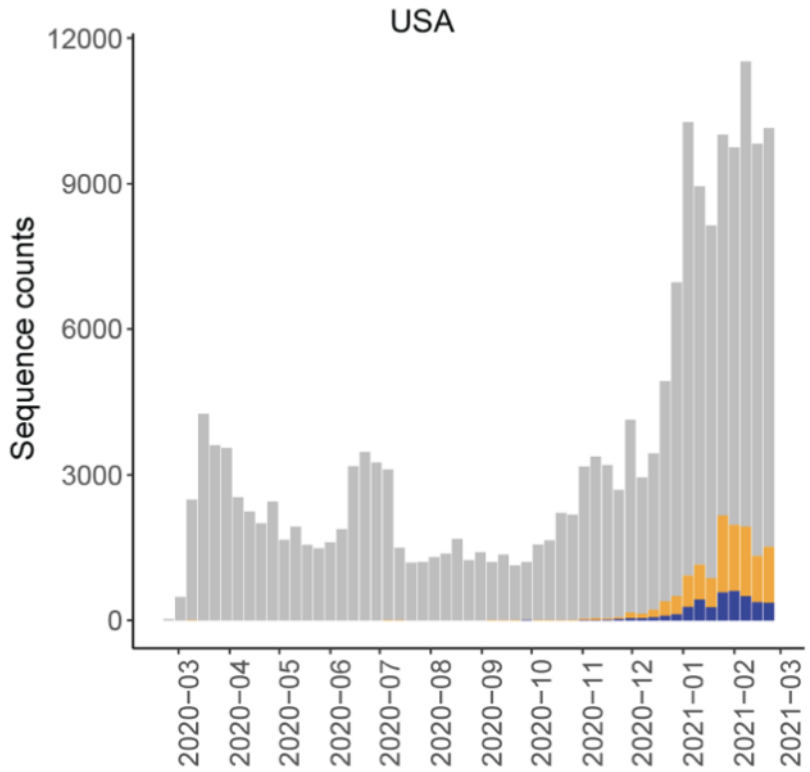
S12P delays signal peptide cleavage, causing a *de facto* C15del mutation

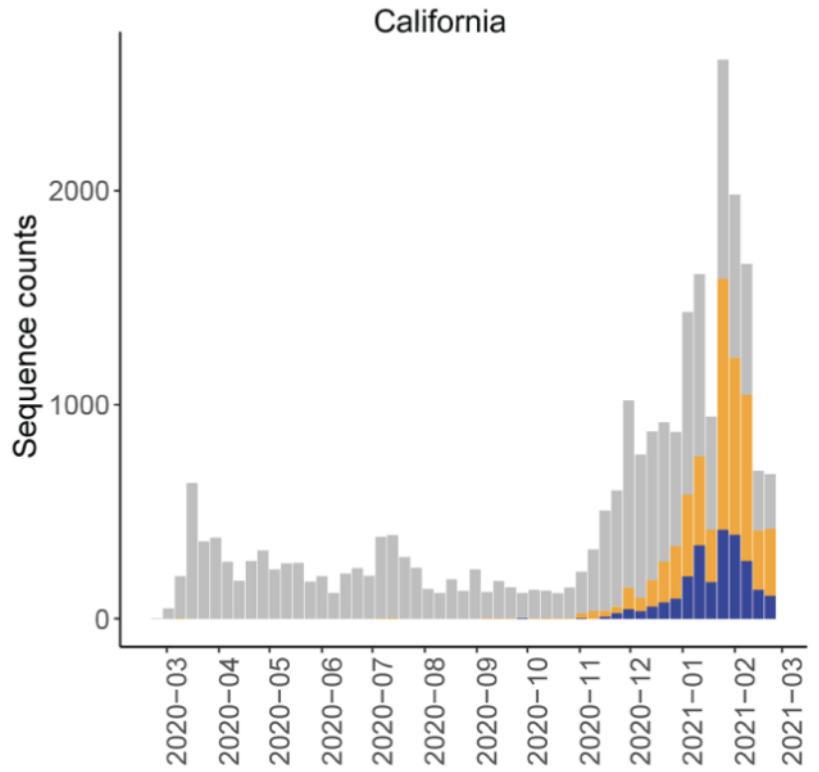
Should we expect signal peptide mutations in circulation?

Alex Chen (McCallum & De Marco *et al.* 2021. Cell *In press*)

THE CALIFORNIA (B.1.429/B.1.427) VARIANT PREVALENCE



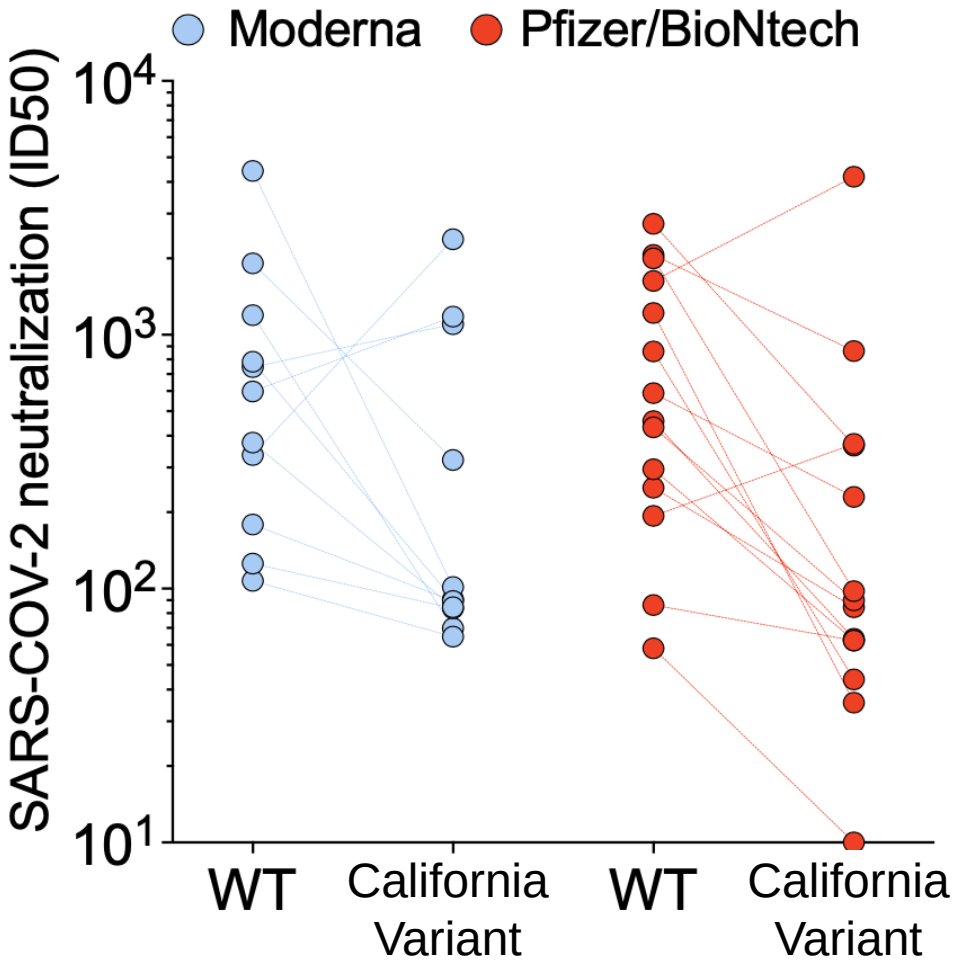




The B.1.429/B.1.427 variant is taking over in California

Julia Di Iulio (McCallum, Bassi, De Marco, Chen, Walls, *et al.* 2021. BioRxiv)

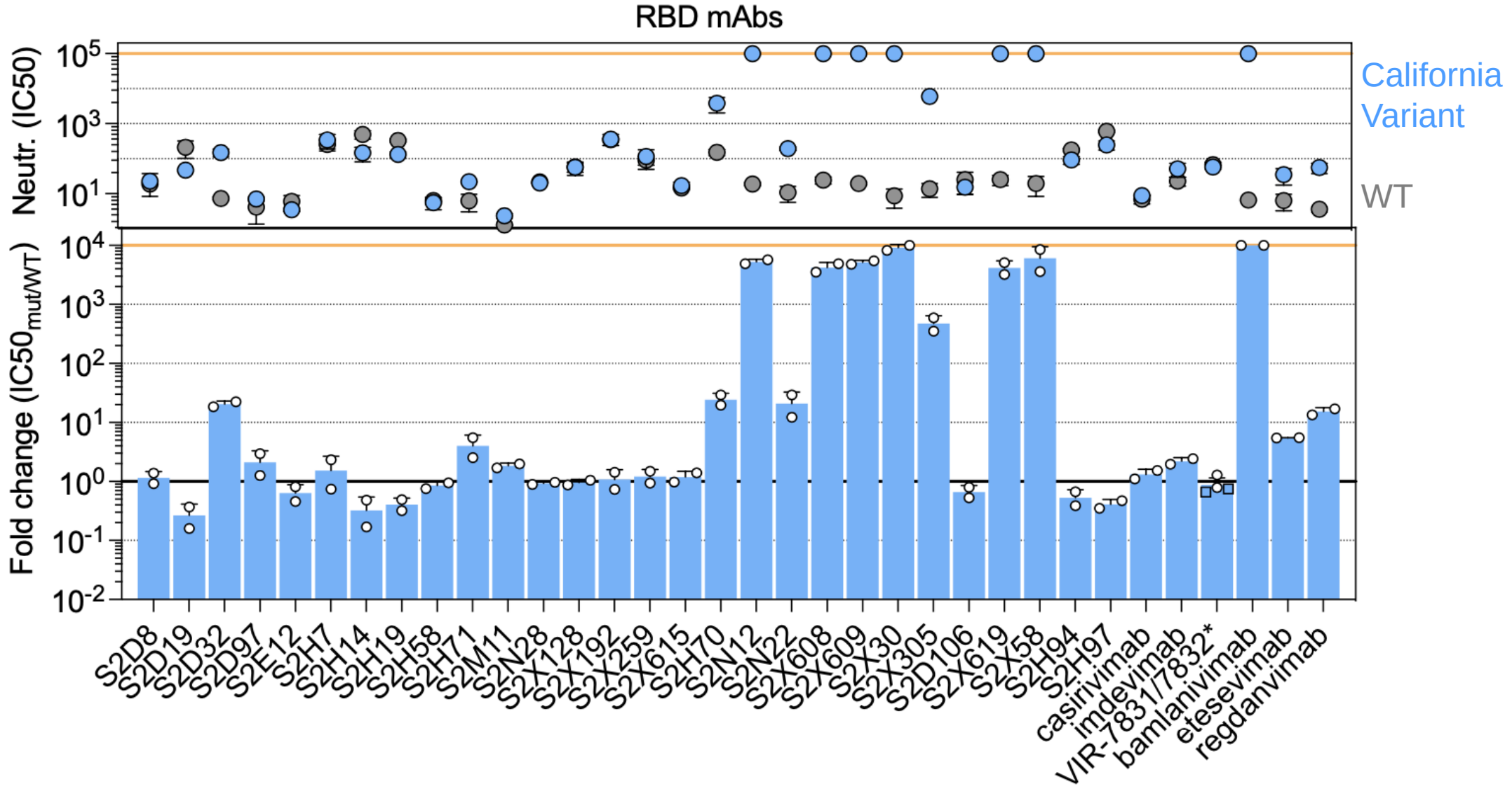
CALIFORNIA VARIANT NEUTRALIZATION



The California variant is comparable to the South Africa in terms of neutralization evasion

Mary-Jane Navarro (McCallum, Bassi, De Marco, Chen, Walls, *et al.* 2021. BioRxiv)

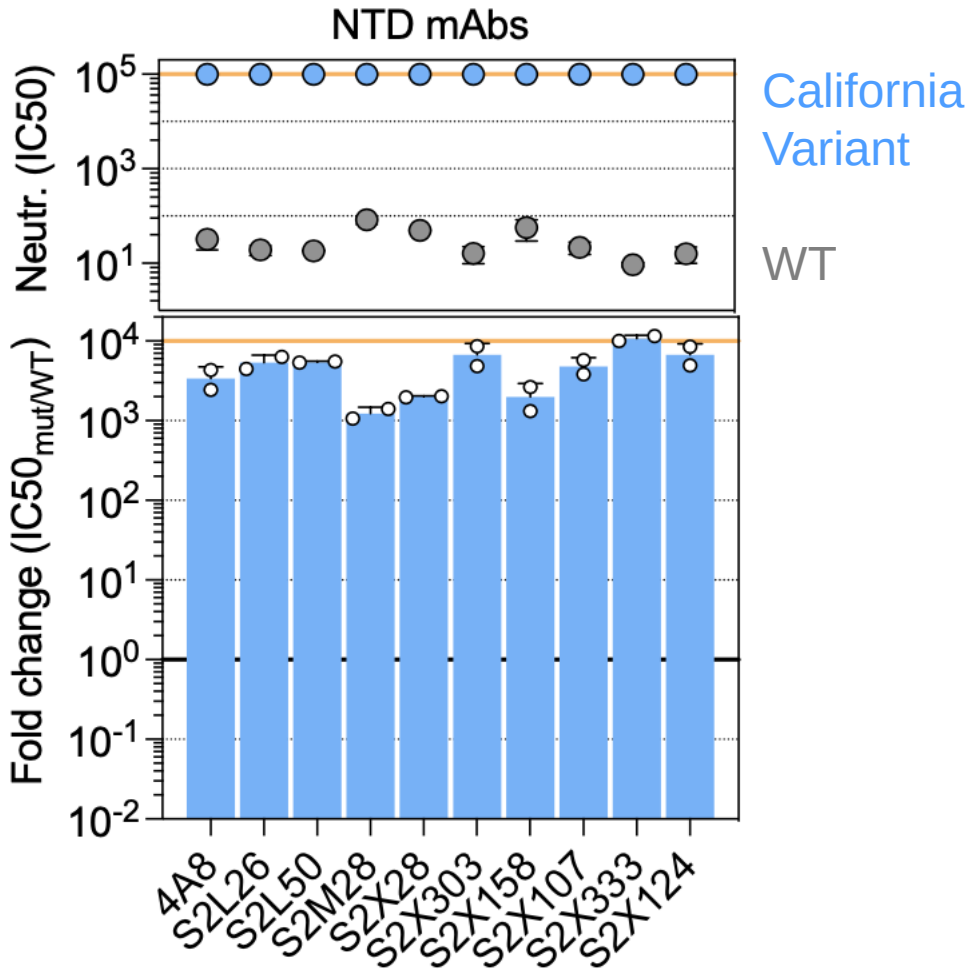
CALIFORNIA VARIANT ANTI-RBD NEUTRALIZATION EVASION

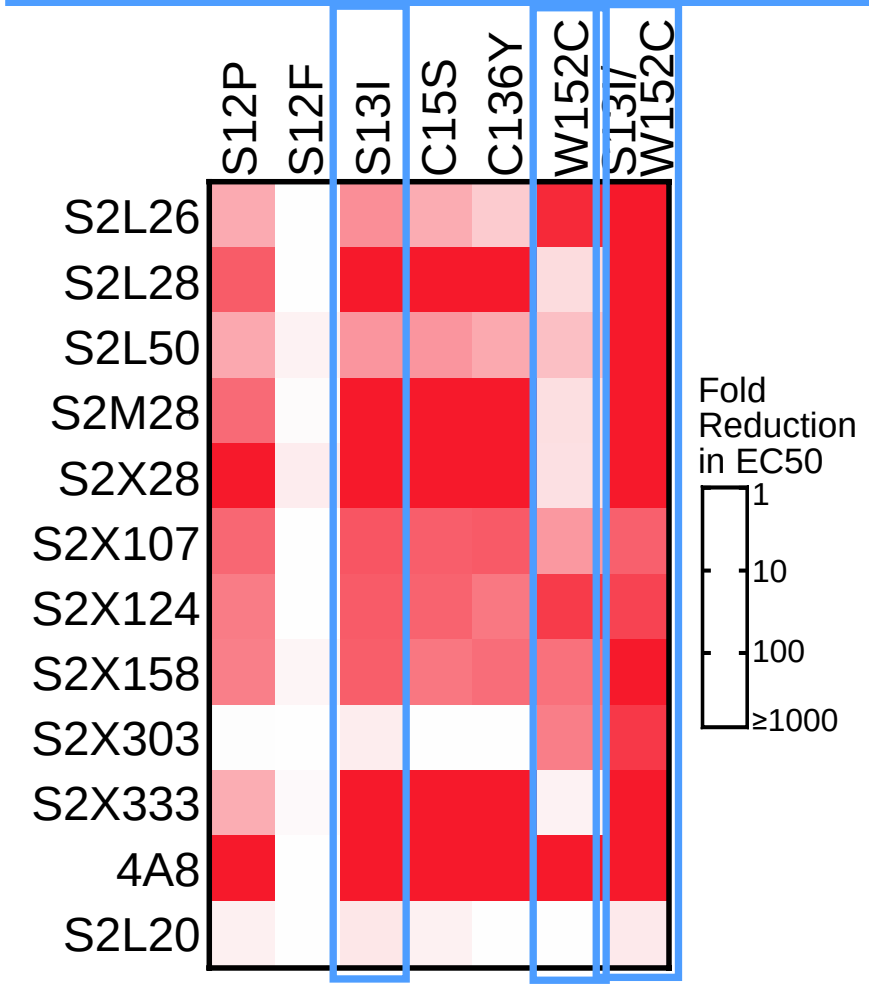


Variant evades some but not most anti-RBD neutralizing antibodies (owing to L452R mutation in RBD)

McCallum, Bassi, De Marco, Chen, Walls, *et al.* 2021. BioRxiv

CALIFORNIA VARIANT ANTI-NTD NEUTRALIZATION EVASION

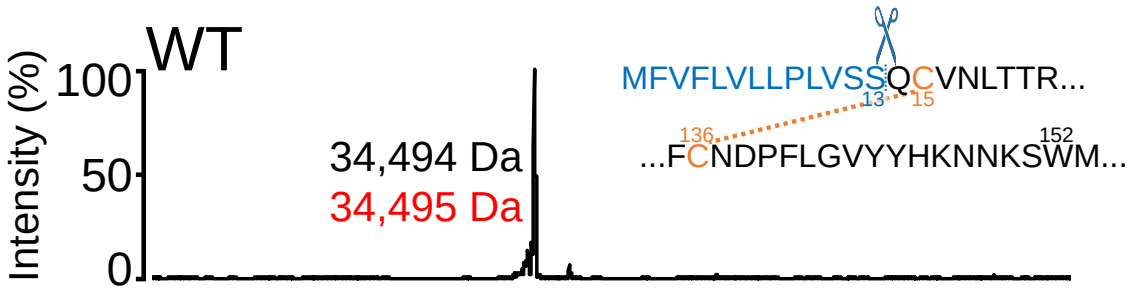


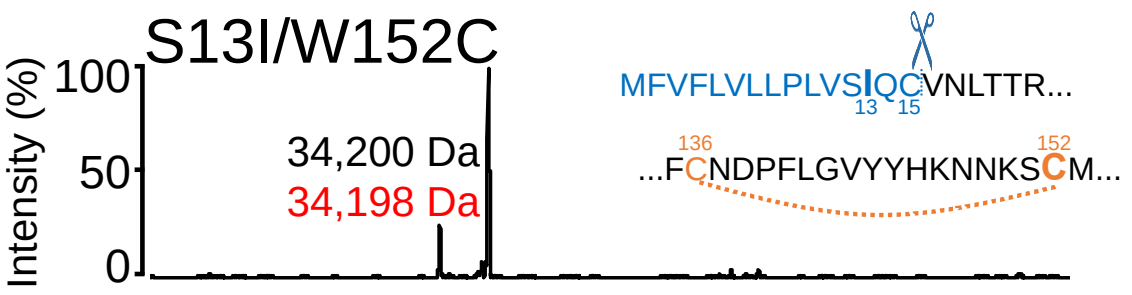


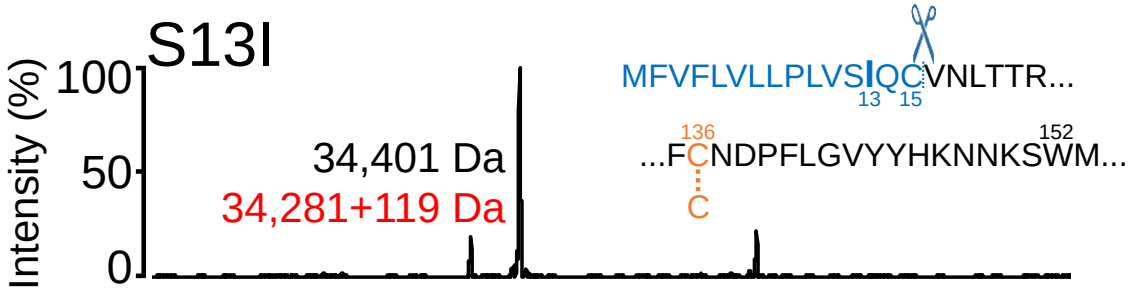
Variant evades ALL anti-NTD neutralizing antibodies (owing to S13I and W152C mutations in NTD)

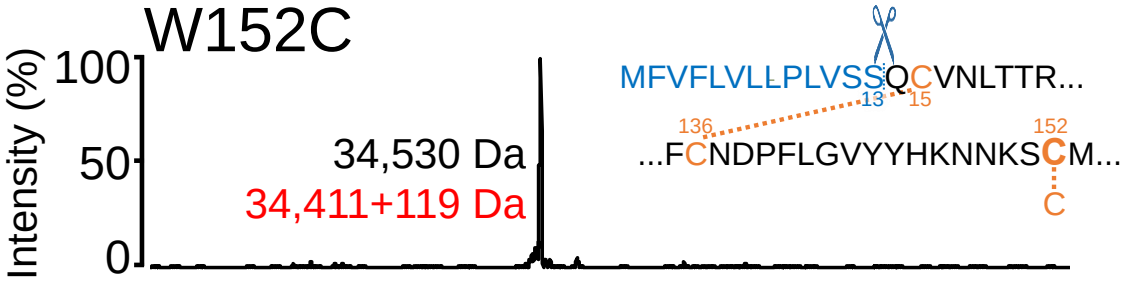
McCallum, Bassi, De Marco, Chen, Walls, *et al.* 2021. BioRxiv

MASS SPECTROMETRY ANALYSIS OF S13I/W152C MUTANT





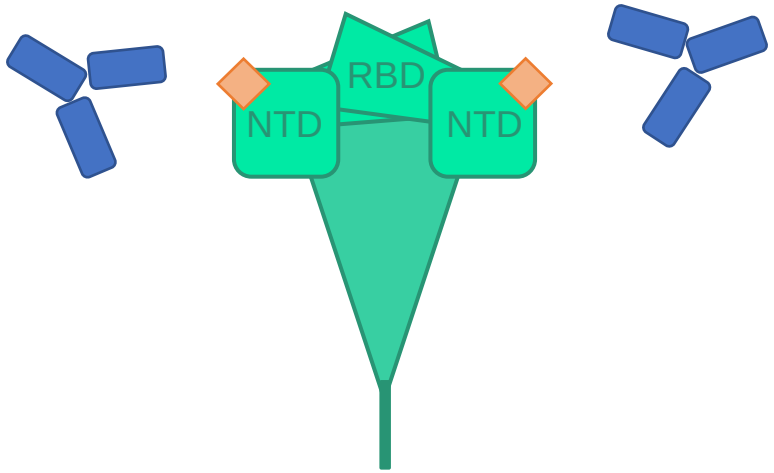




S13I delays the signal peptide cleavage

This breaks C15-C136 disulfide bond

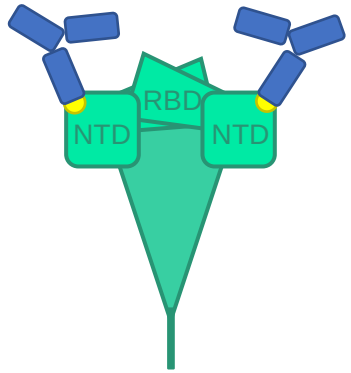
The W152C mutation forms a new disulfide bond with C136



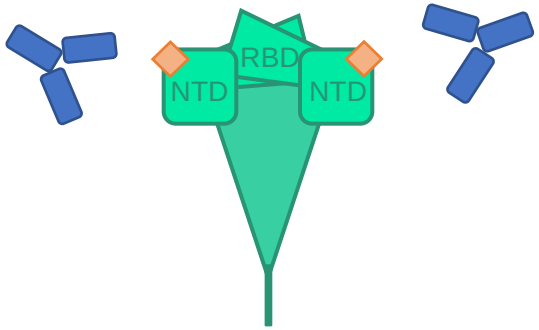
Alex Chen (McCallum, Bassi, De Marco, Chen, Walls, *et al.* 2021. BioRxiv)

CONCLUSIONS

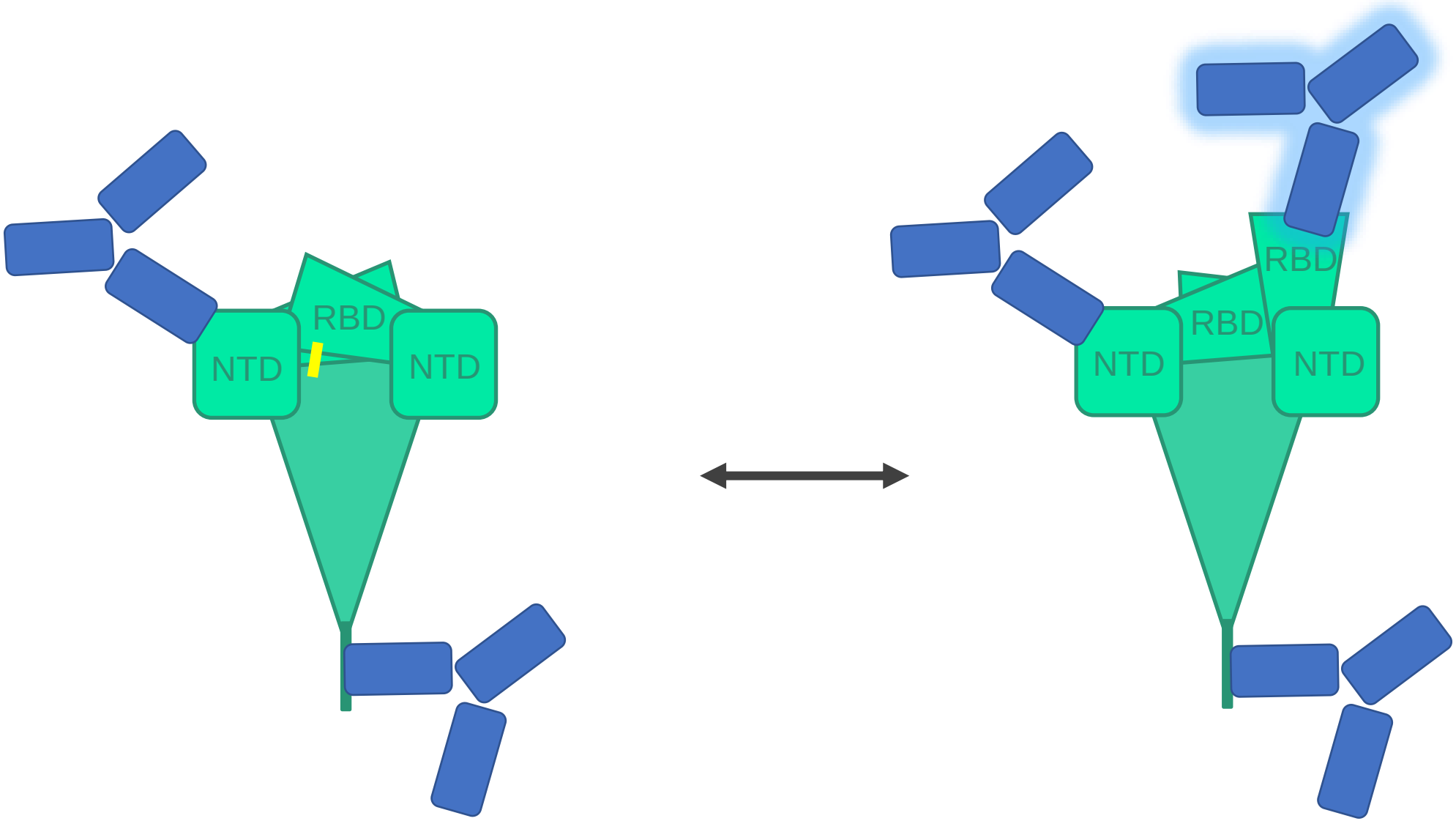
The NTD supersite is a key component of immunity



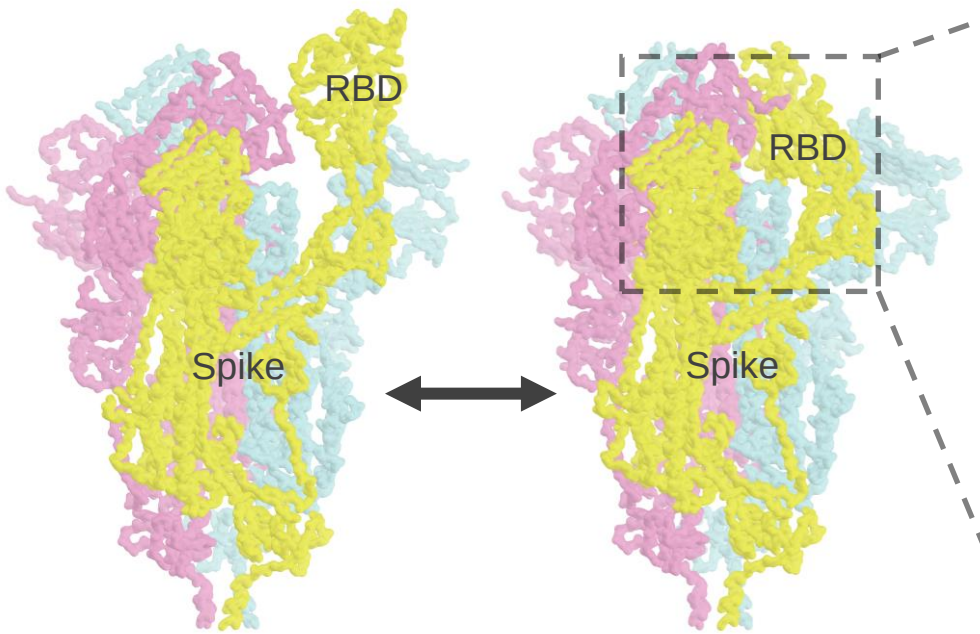
Concerning antibody escape mutations have emerged altering the supersite

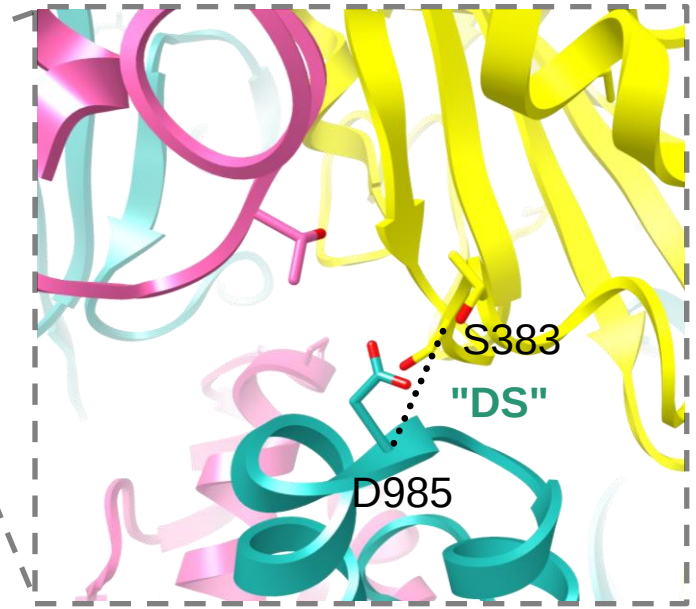


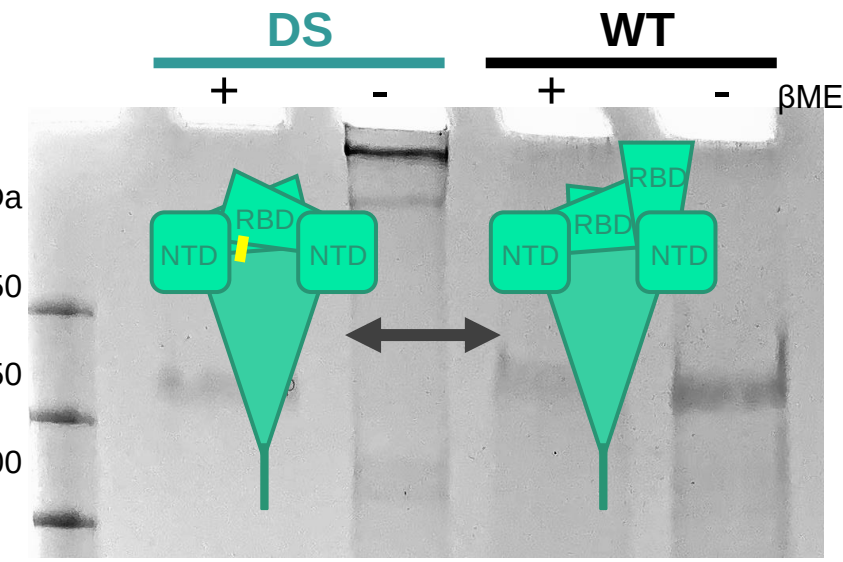
FUTURE DIRECTIONS: TARGETING NON-RBD SITES WITH VACCINATION



INTRODUCING A DISULFIDE BOND TO CLOSE THE SPIKE



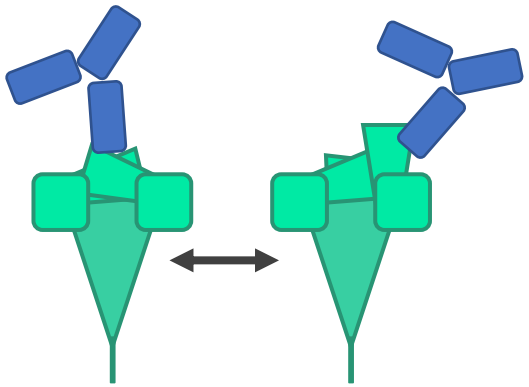


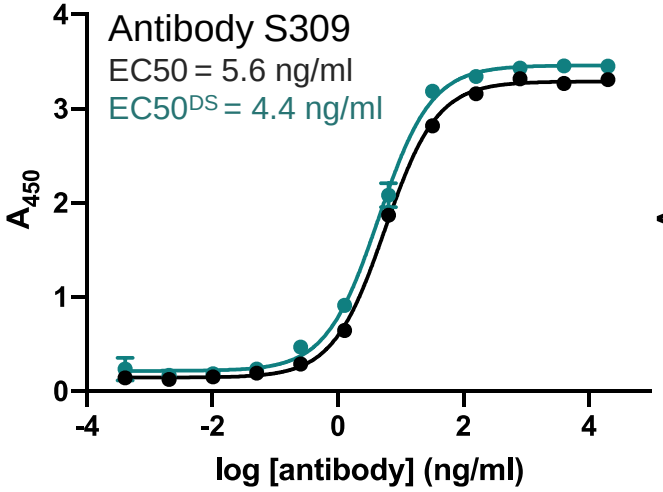


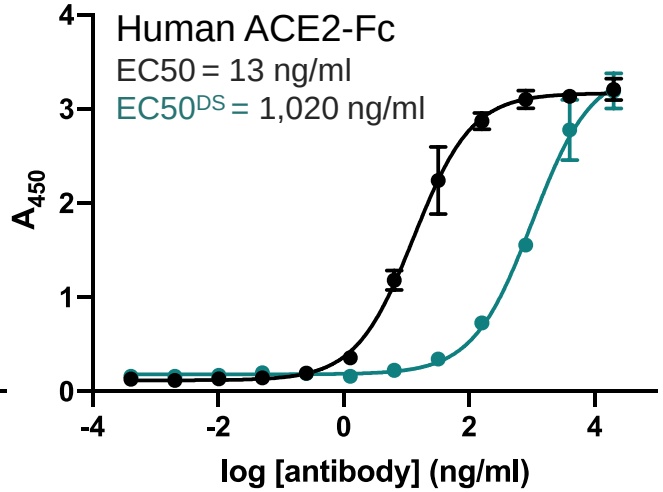
Disulfide bond could be introduced stapling the RBDs closed

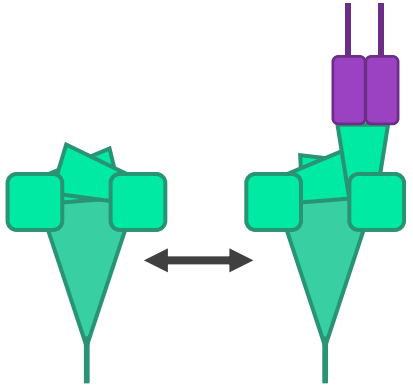
McCallum, et al. 2020. Nat Struct Mol Biol 27, 942–949.

BINDING ASSAYS (ELISAS) WITH DISULFIDE STAPLED SPIKE









Stapled closed spikes have reduced binding to some RBD binding antibodies

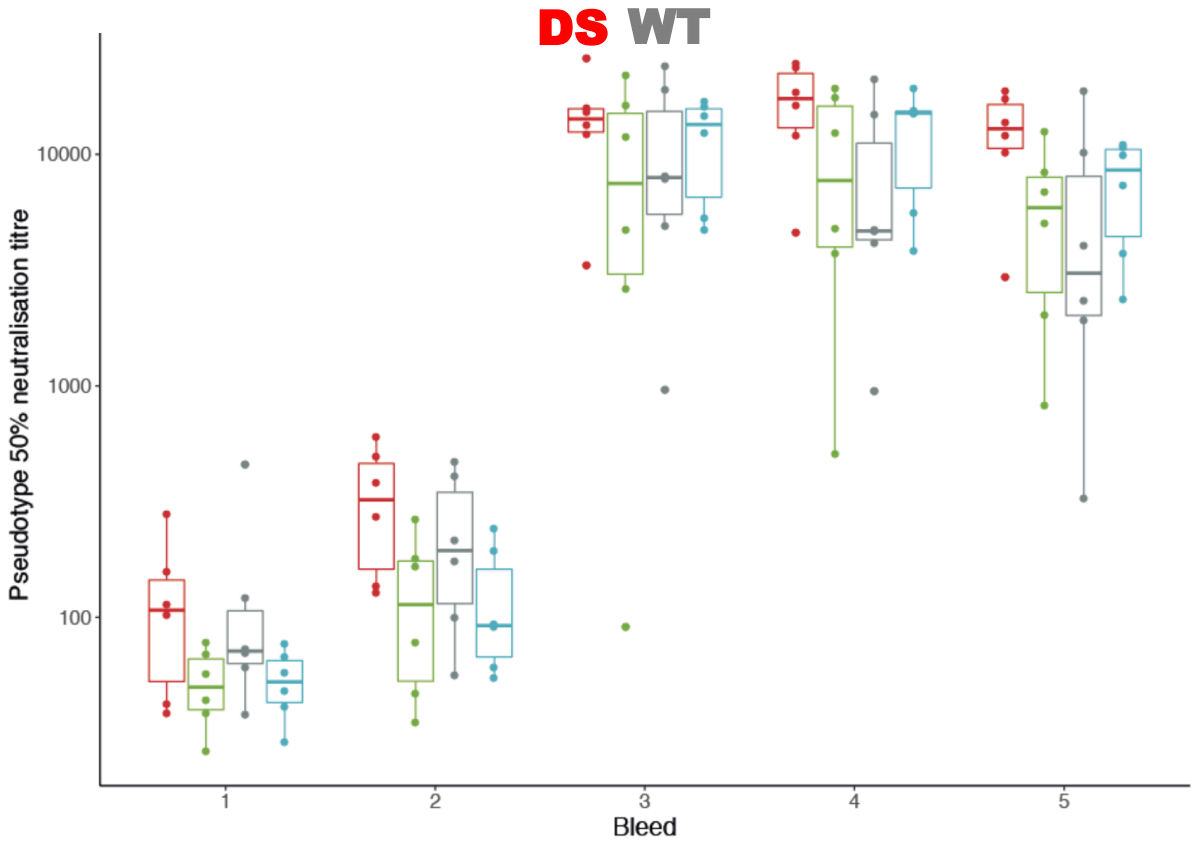
McCallum, et al. 2020. Nat Struct Mol Biol 27, 942–949.

VACCINATION OF MICE WITH A DISULFIDE STAPLED CLOSED SPIKE

SARS-CoV-2 spike protein arrested in the closed state induces potent neutralizing responses

George W. Carnell, Katarzyna A. Ciazynska, David A. Wells, Xiaoli Xiong, Ernest T. Aguinam, Stephen H. McLaughlin, Donna Mallery, Soraya Ebrahimi, Lourdes Ceron-Gutierrez, Leo C. James, Rainer Doffinger, Jonathan L. Heeney, John A. G. Briggs

doi: https://doi.org/10.1101/2021.01.14.426695



Excellent neutralization elicited by stapled closed spike (DS)

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