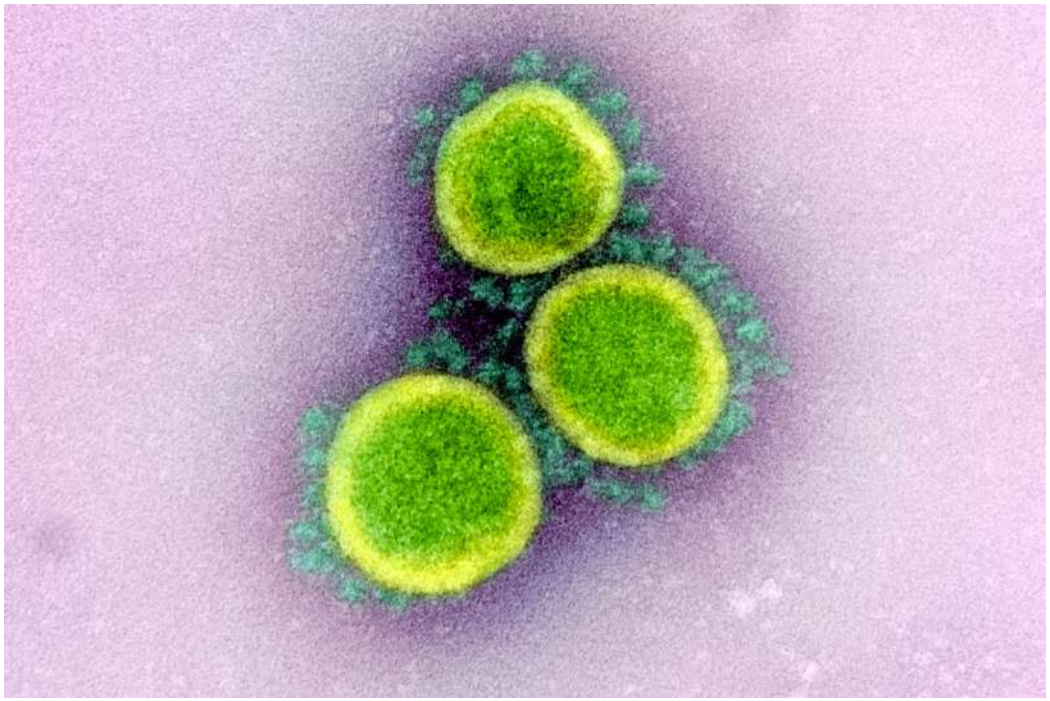
Selection for immune evasion in SARS-CoV-2 revealed by high-resolution epitope mapping combined with viral genome sequence analysis

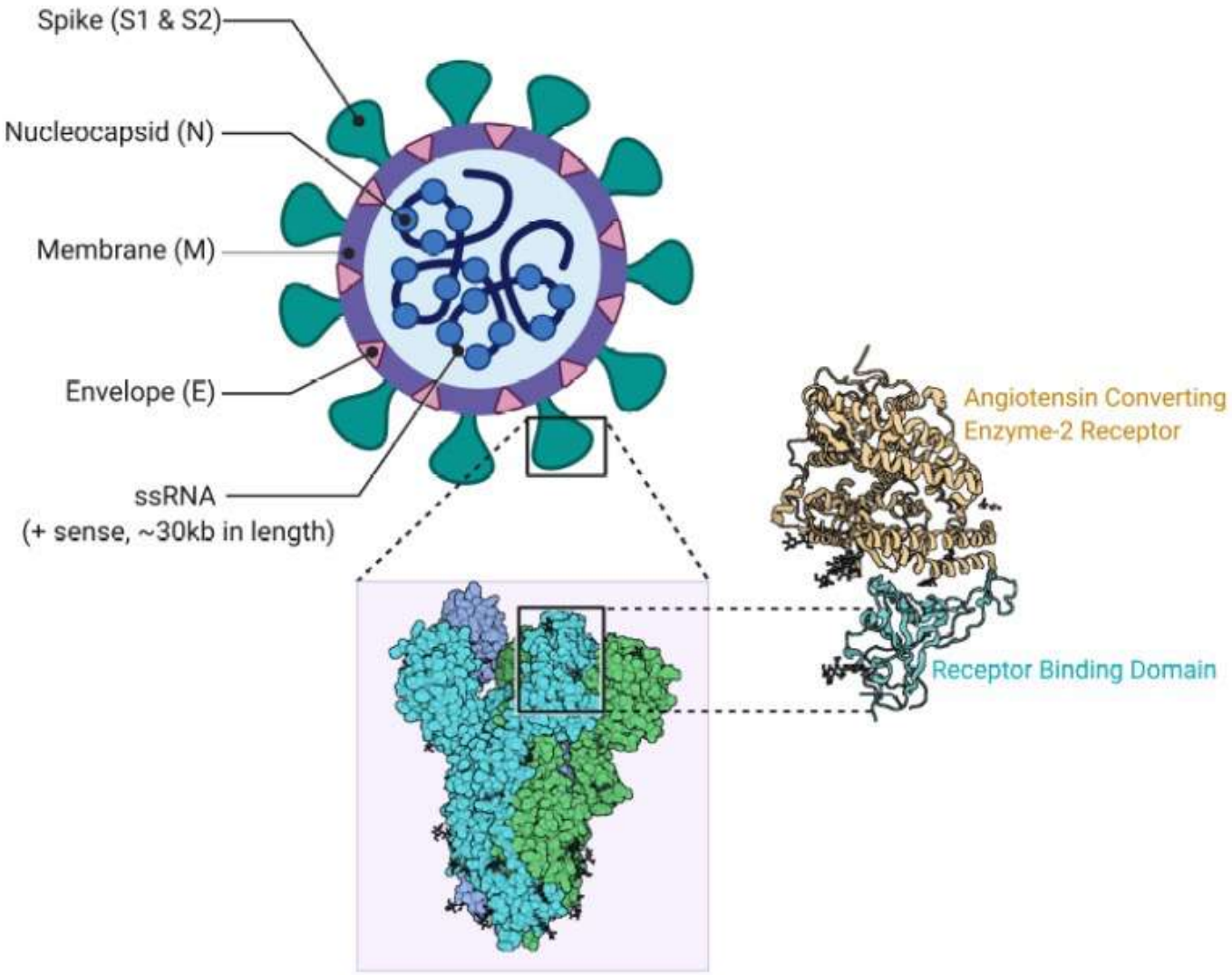


20th April 2022

Jörg H. Fritz & Ciriaco A. Piccirillo

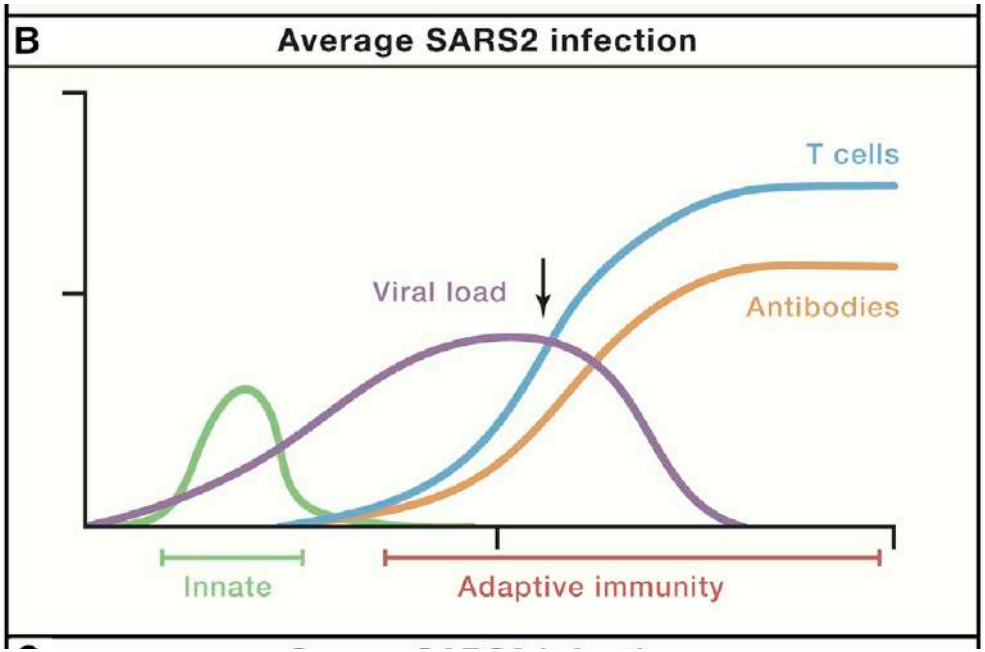
McGill University – Dept. of Microbiology & Immunology

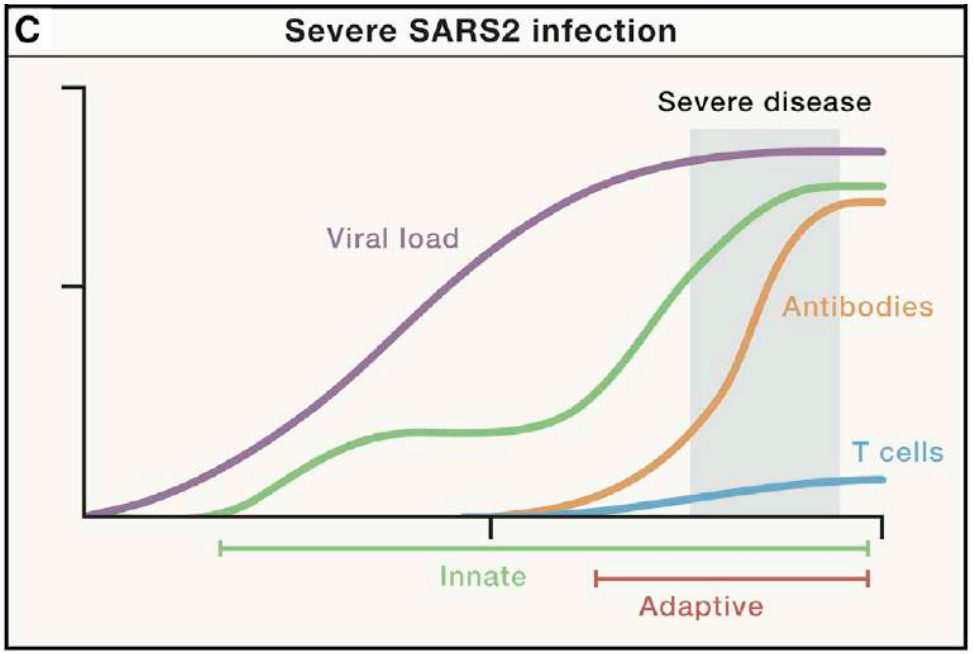
SARS-CoV 2 Structure

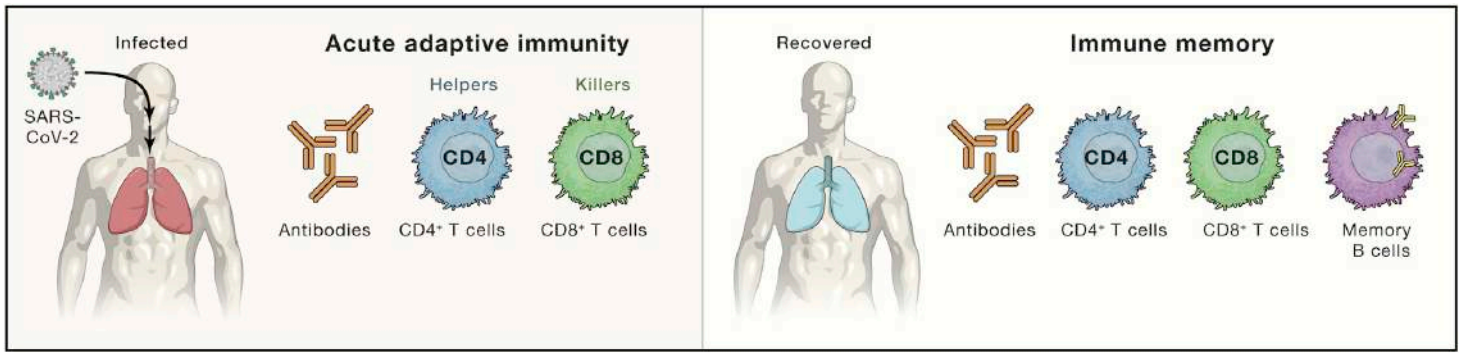


Adaptive Immunity to SARS-CoV-2

(T and B cells)

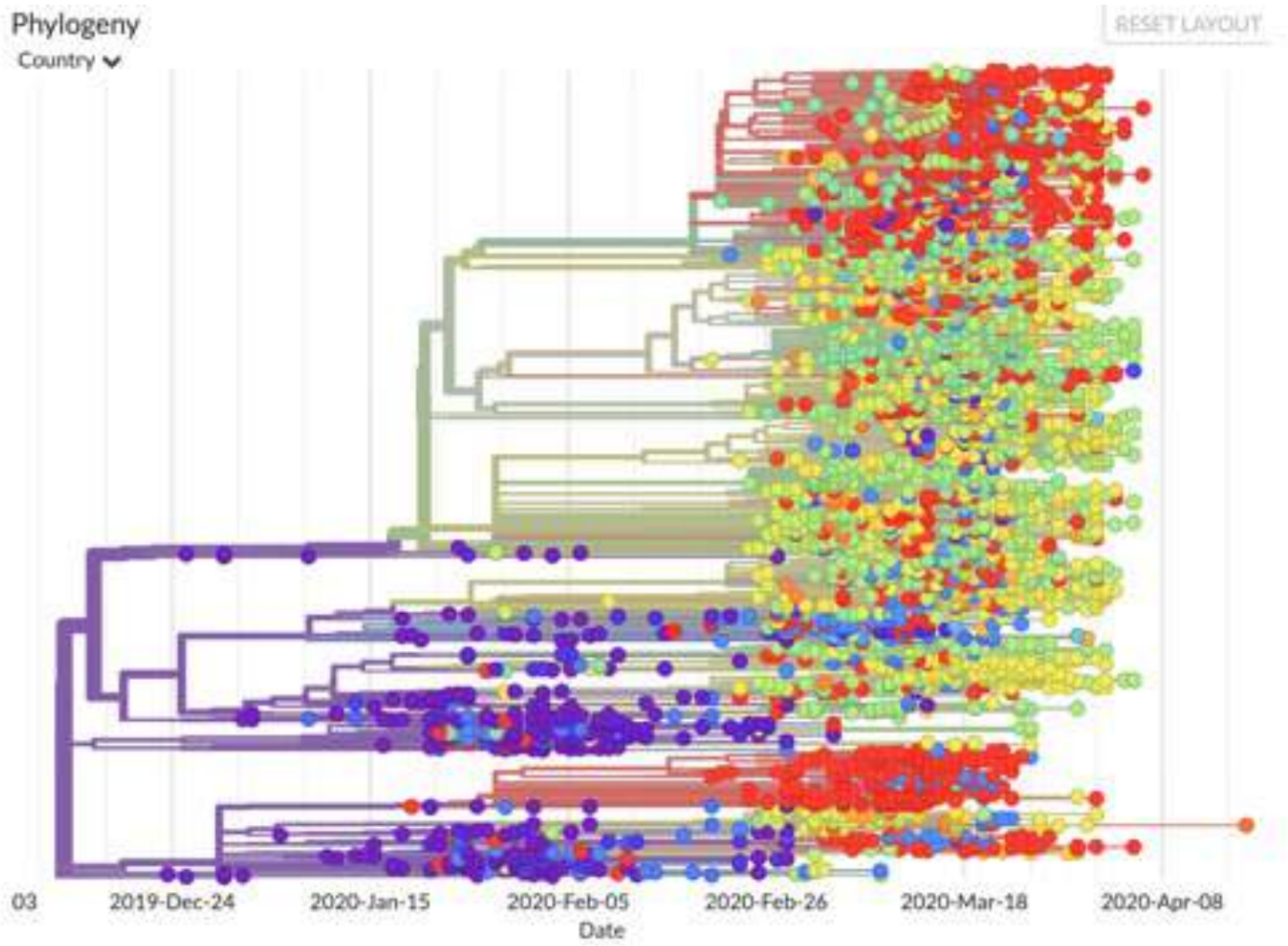




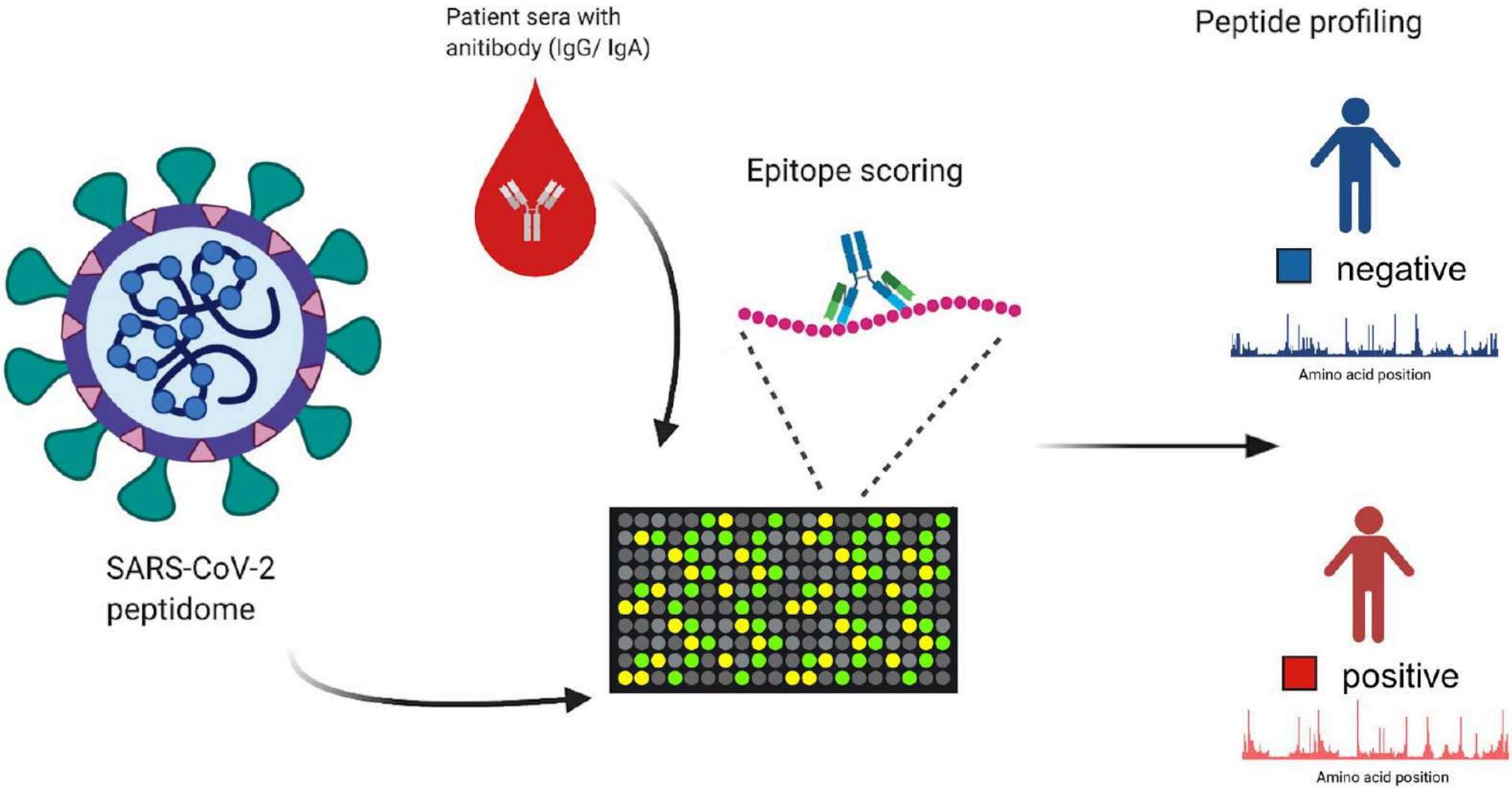


Viral evolution of SARS-CoV-2

(How to generate an assessment tool for public health risk of newly arising SARS-CoV-2 variants)



High density peptide arrays (HDPA) to map epitopes (PEPperPRINT)

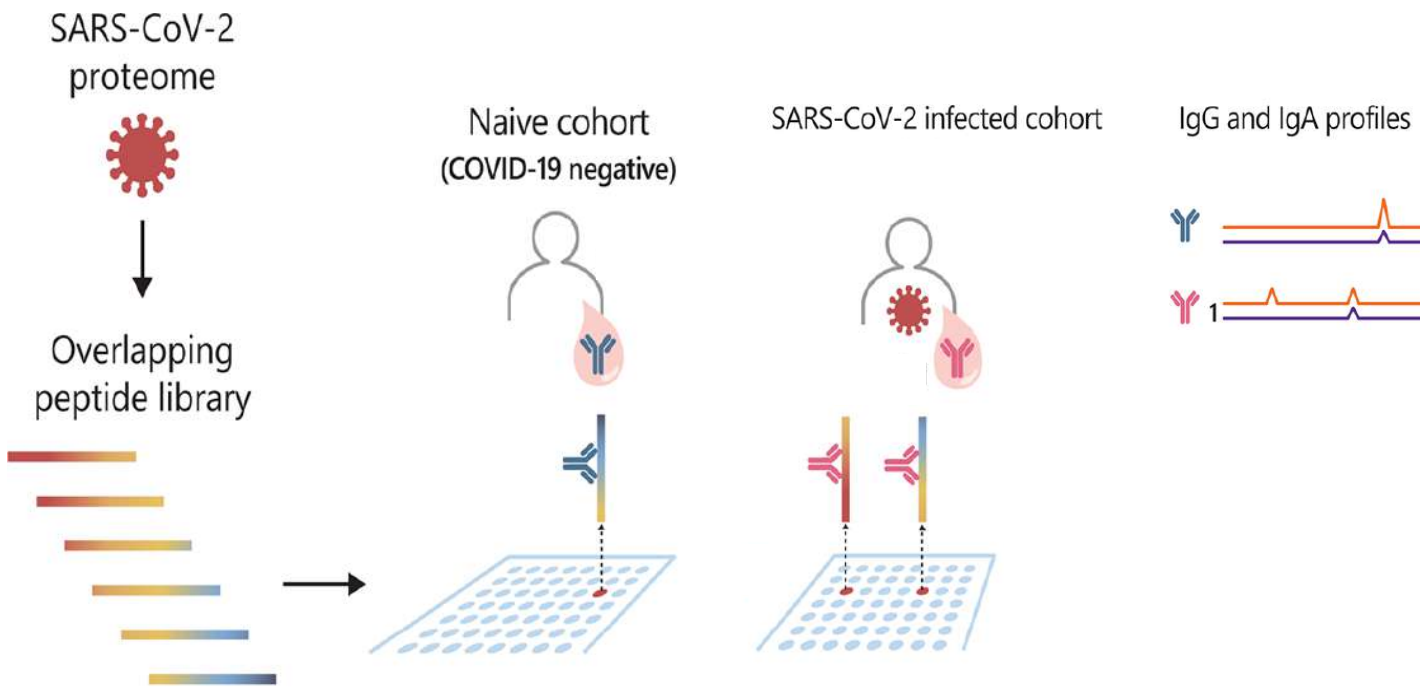


High-Density Peptide Array (HDPA)

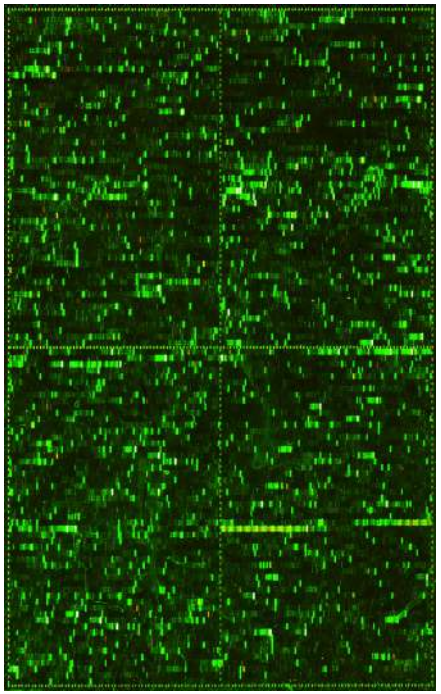
(PEPperPRINT)

SARS-CoV-2 proteome-wide IgG and IgA epitope mapping

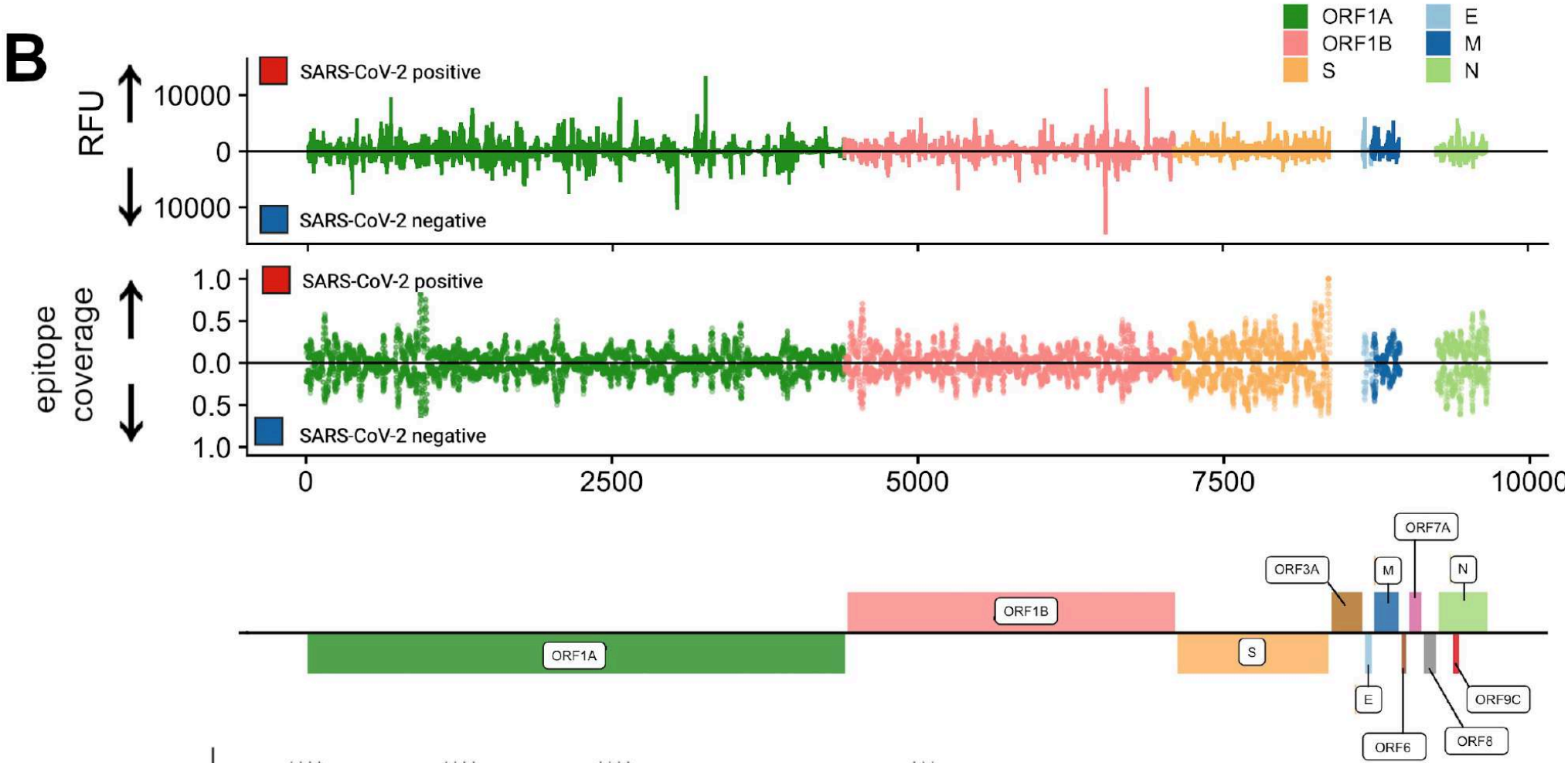
A



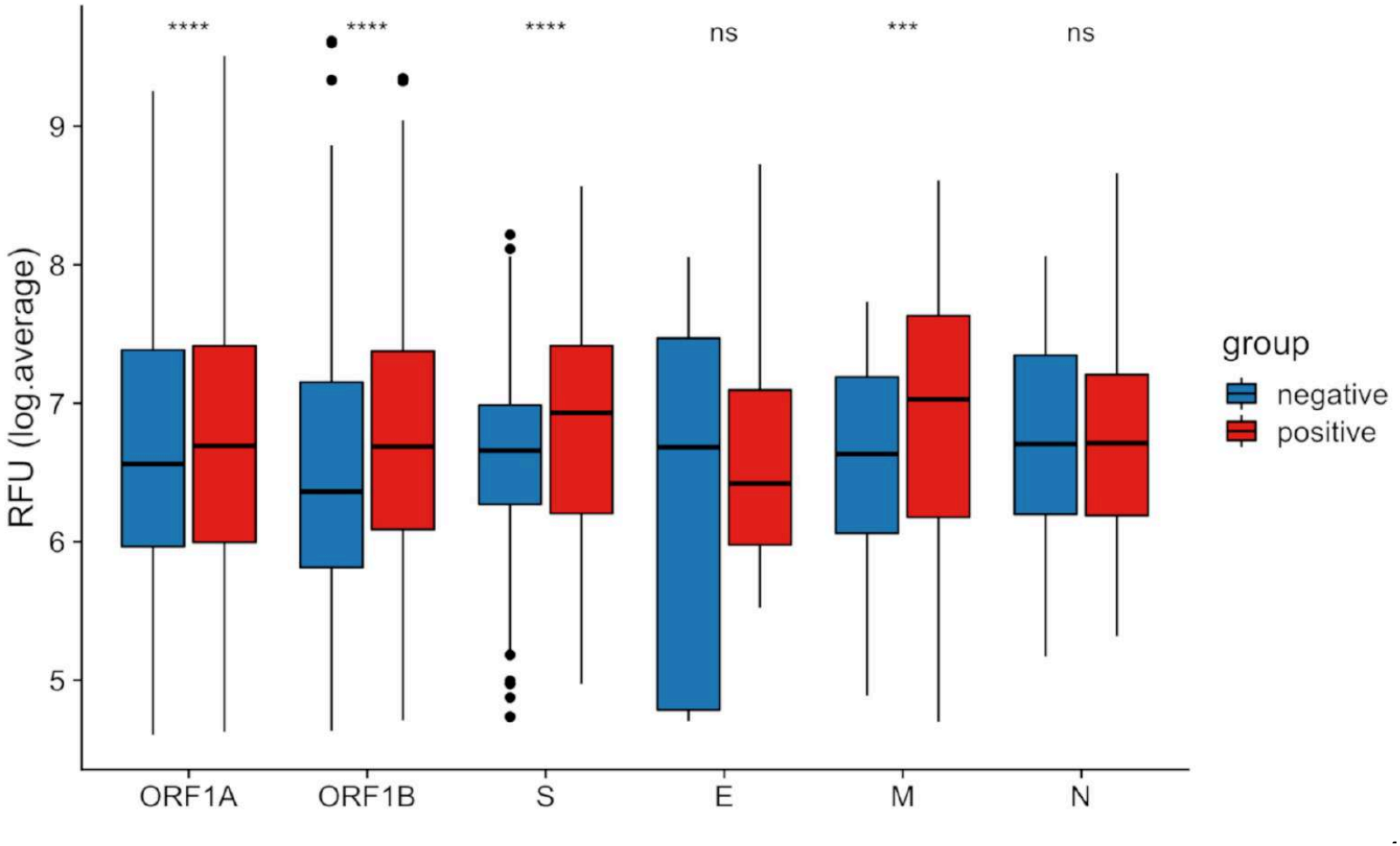
Example of raw data



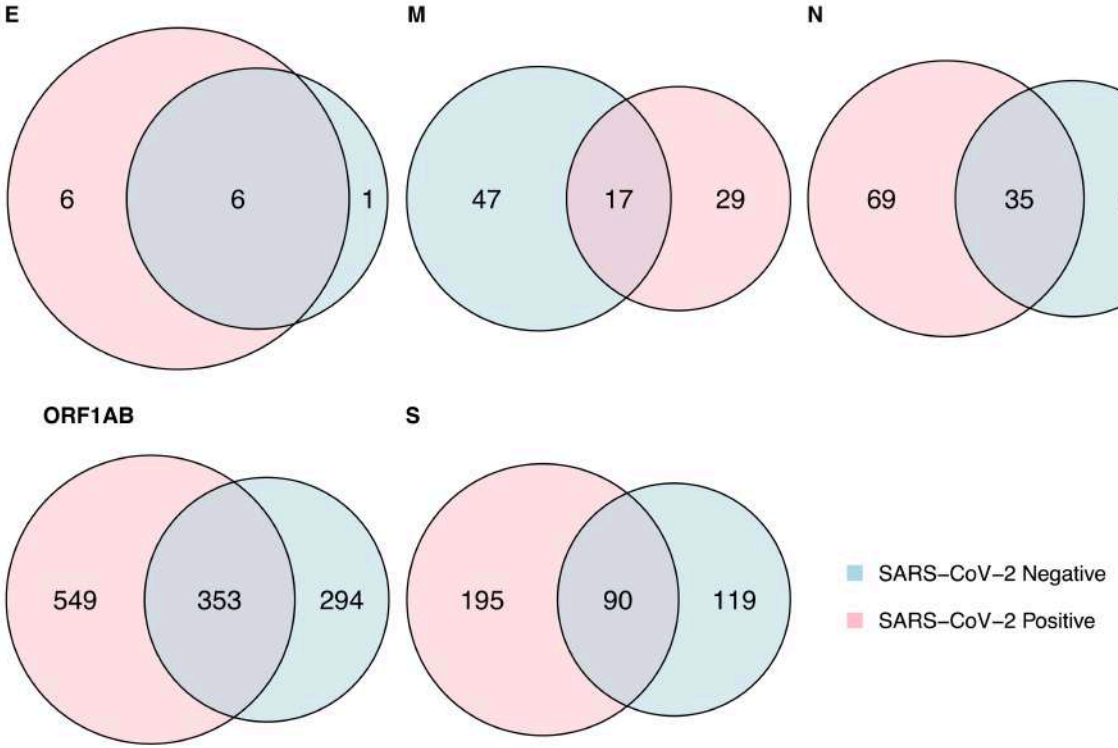
High density peptide arrays (HDPA) to map epitopes (PEPperPRINT)



High density peptide arrays (HDPA) to map epitopes (PEPperPRINT)



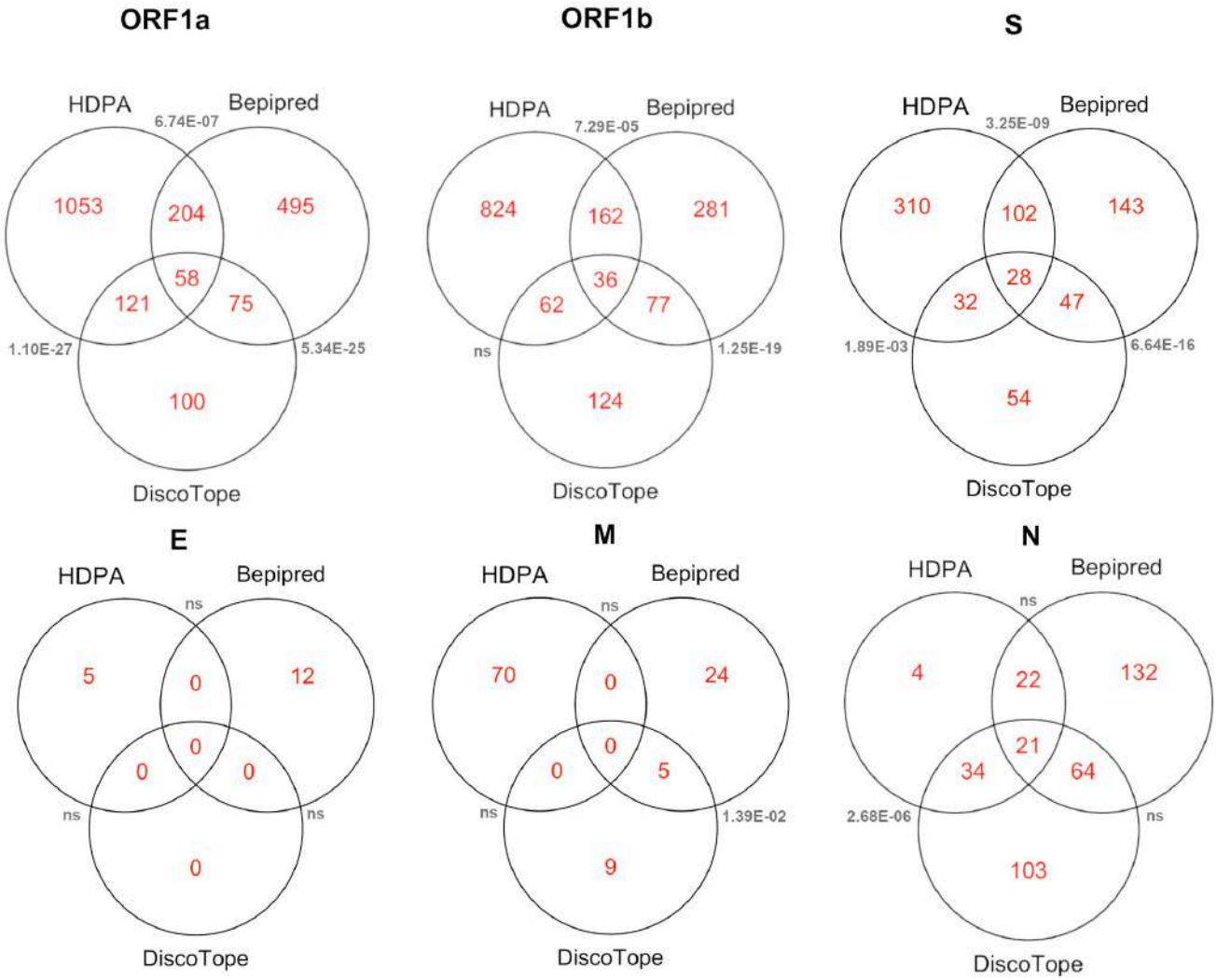
High density peptide arrays (HDPA) to map epitopes (PEPperPRINT)



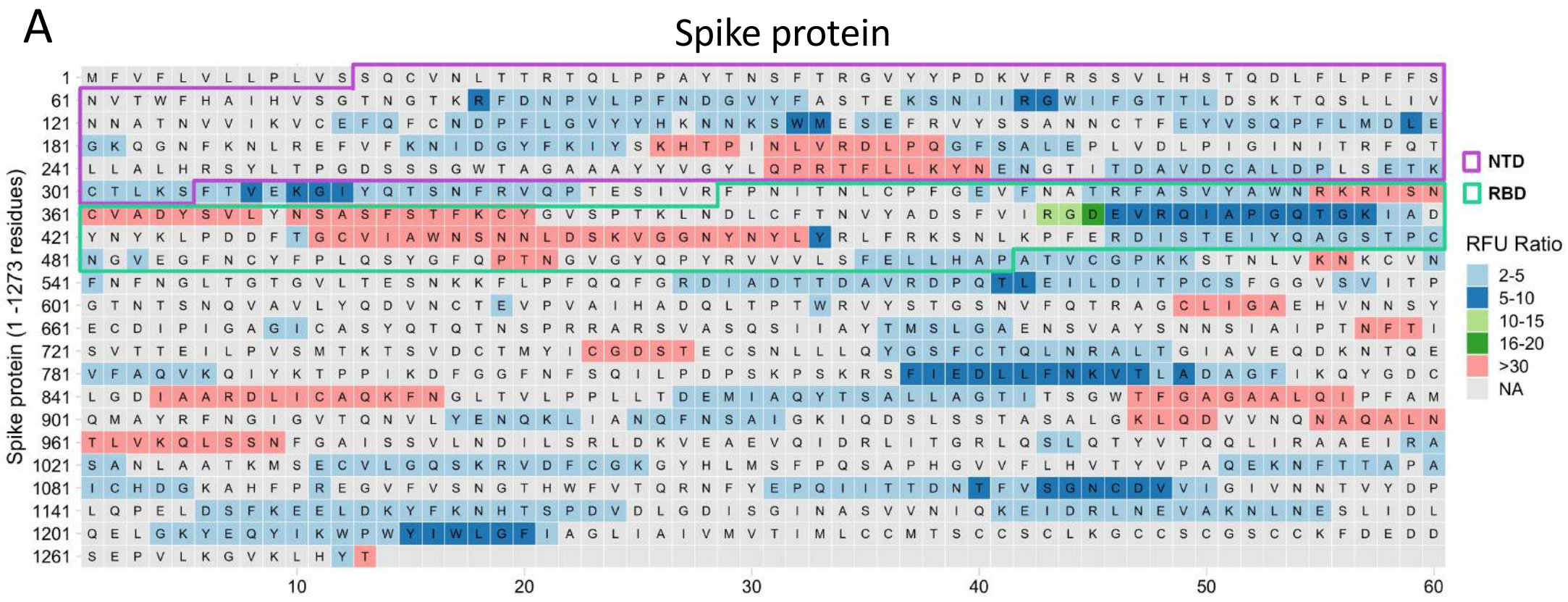
SARS-CoV2-specific Peptides

	Spike	Nucleocapsid	Envelope	Membrane	ORF1ab	TOTAL
SARS-CoV-2 negative	119	41	1	47	294	502
SARS-CoV-2 positive	195	69	6	29	549	848
Overlap	90	35	6	17	353	501
Total	404	145	13	93	1196	1851

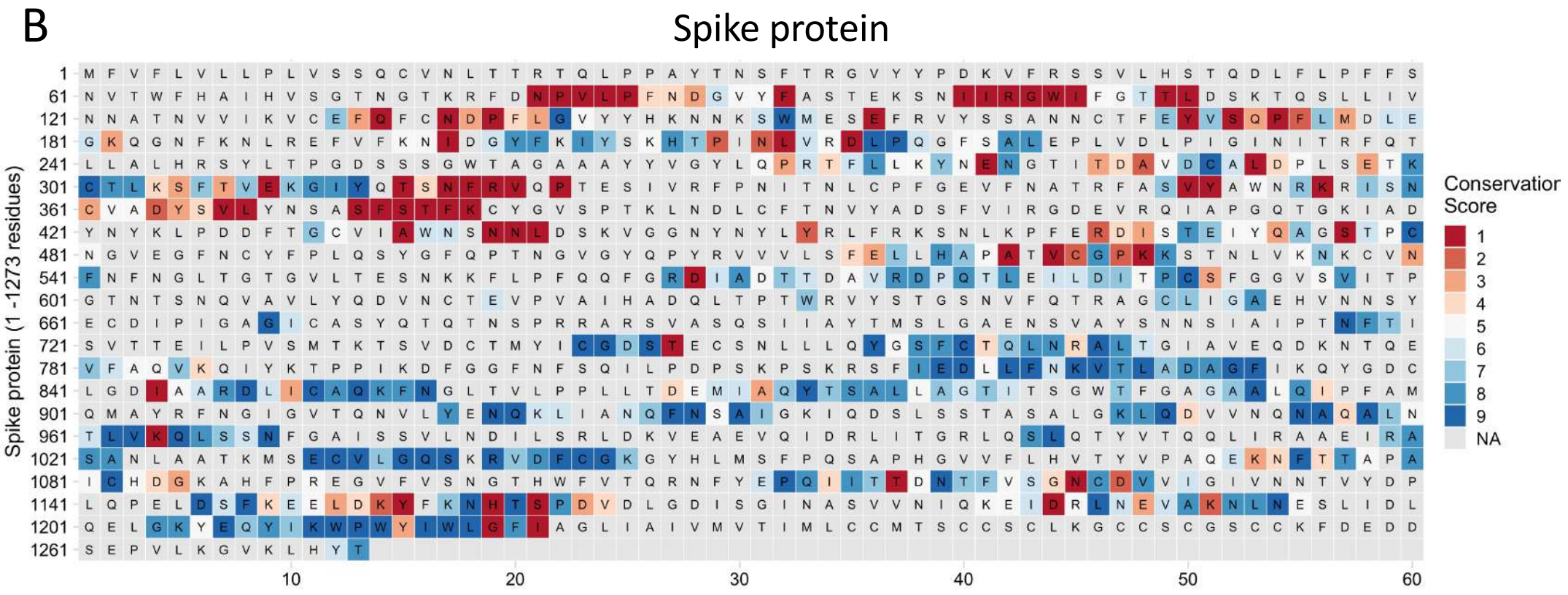
Comparison HDPA (PEPperPRINT) with prediction tools (Bepipred, DiscoTope)



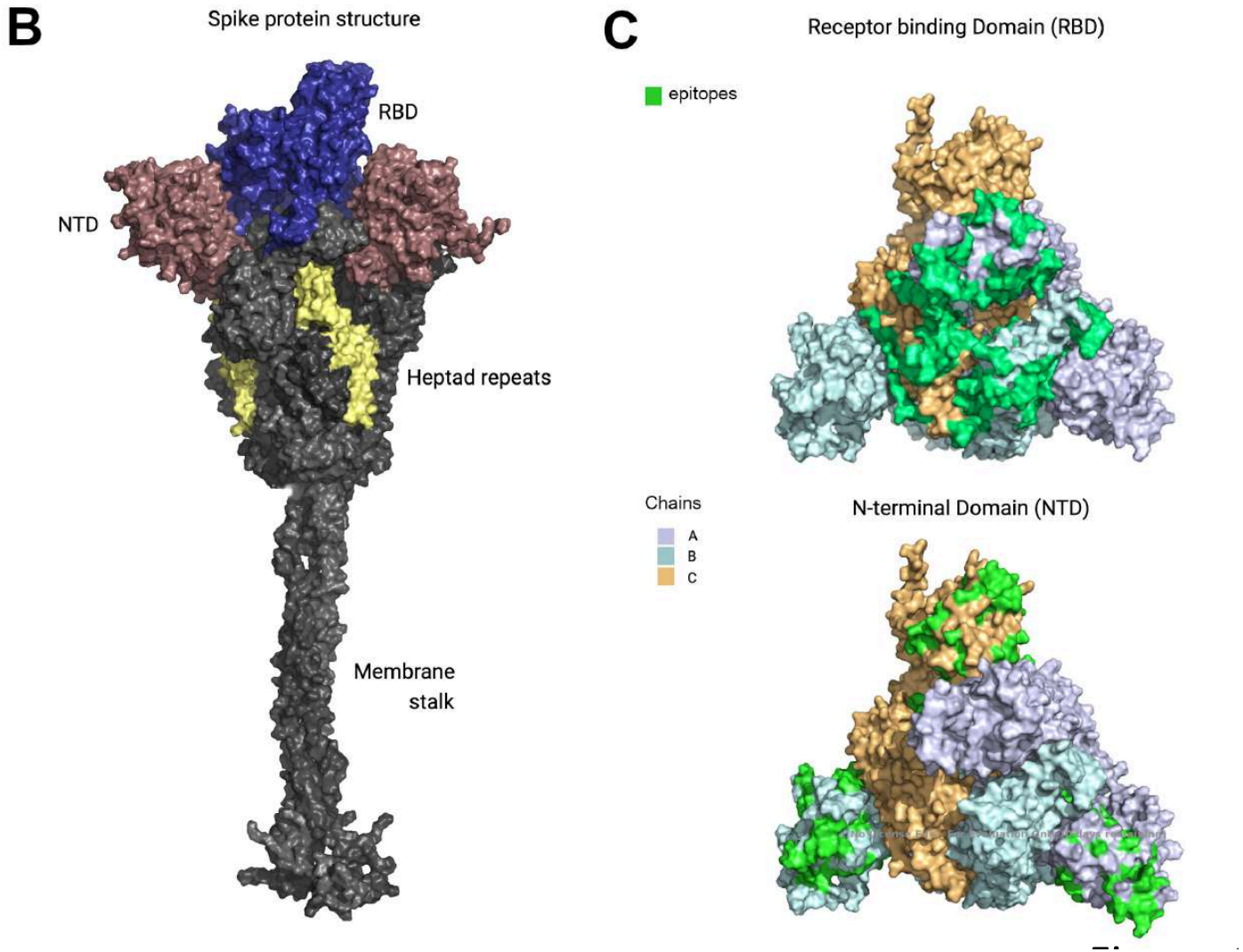
High density peptide arrays (HDPA) to map epitopes (PEPperPRINT)



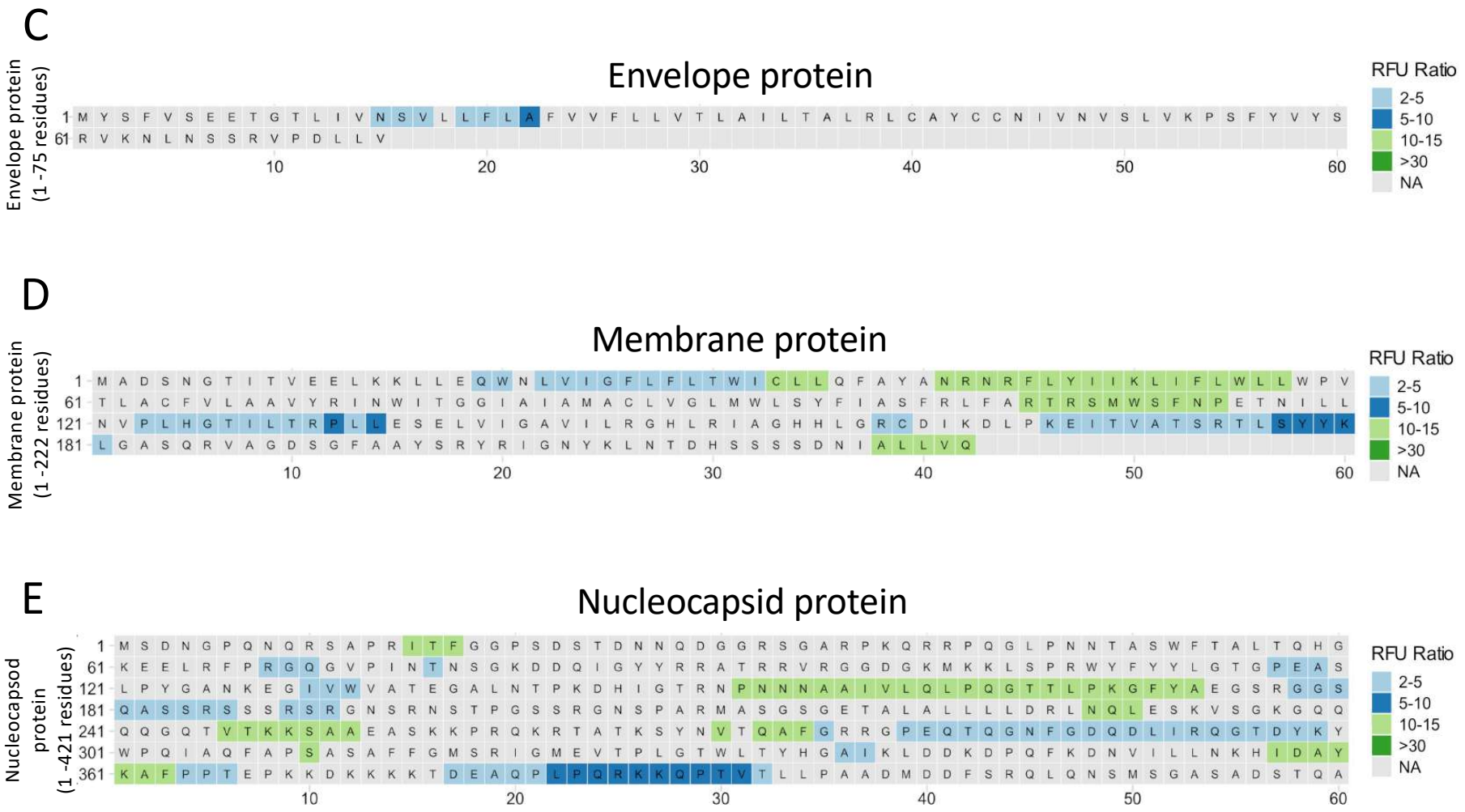
High density peptide arrays (HDPA) to map epitopes (PEPperPRINT)



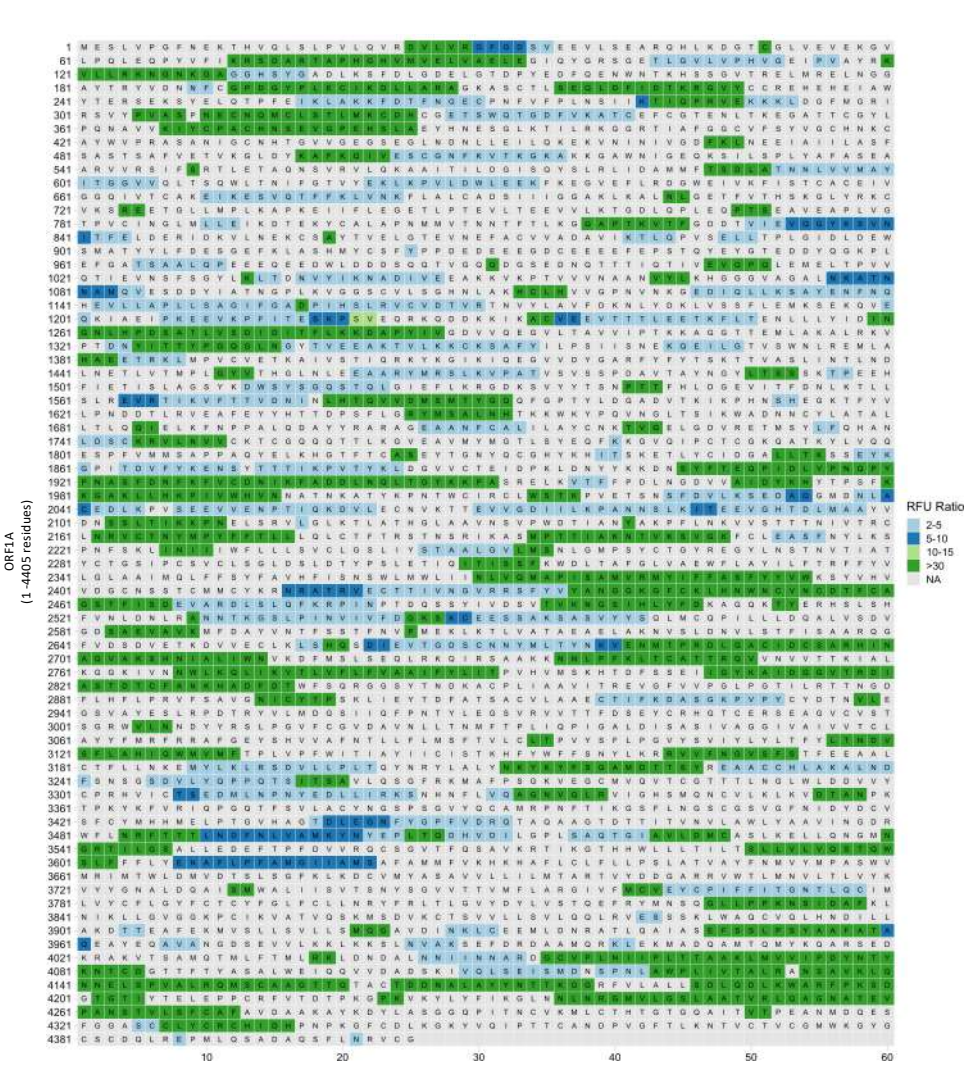
High density peptide arrays (HDPA) to map epitopes (PEPperPRINT)



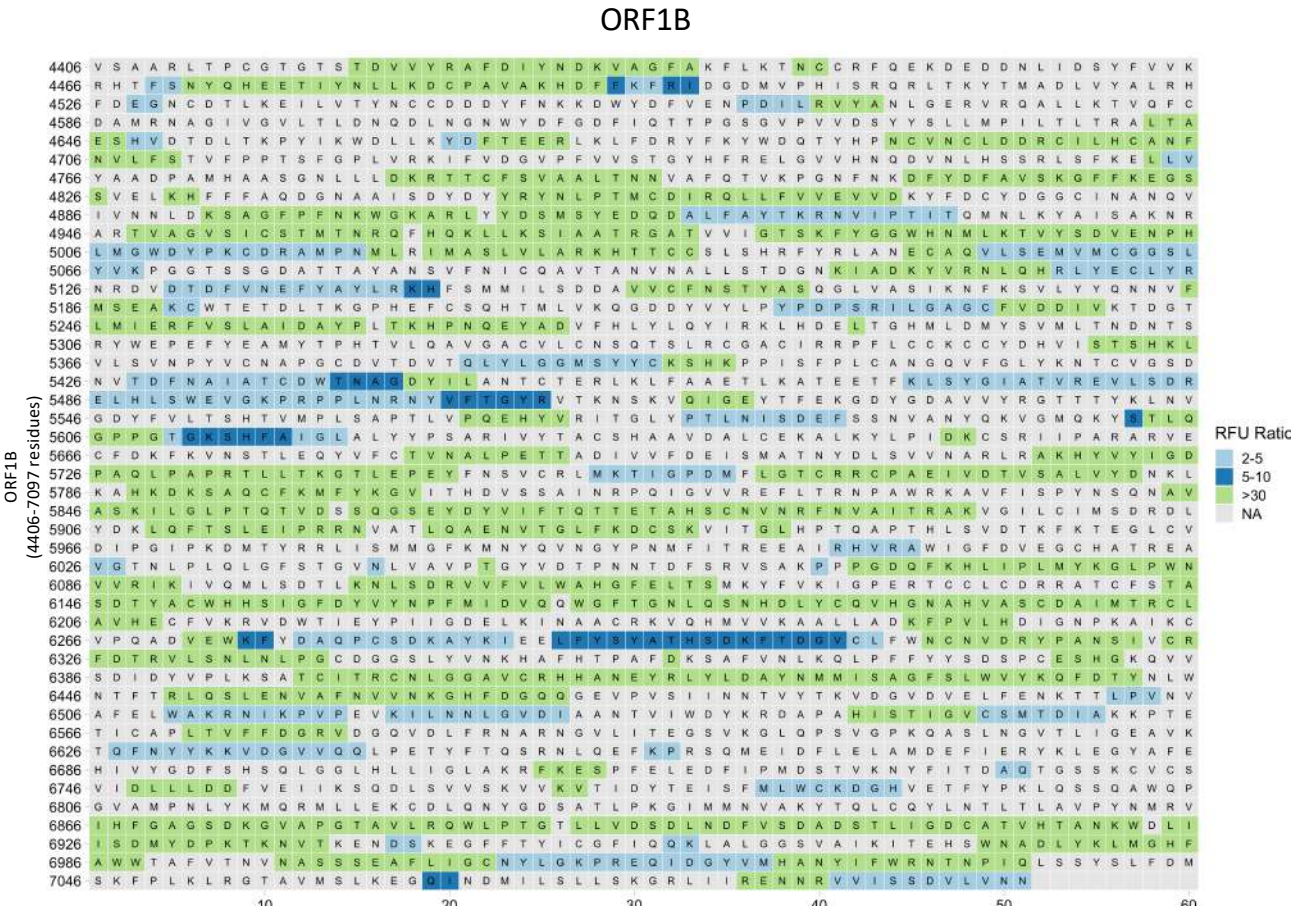
High density peptide arrays (HDPA) to map epitopes (PepperPrint)



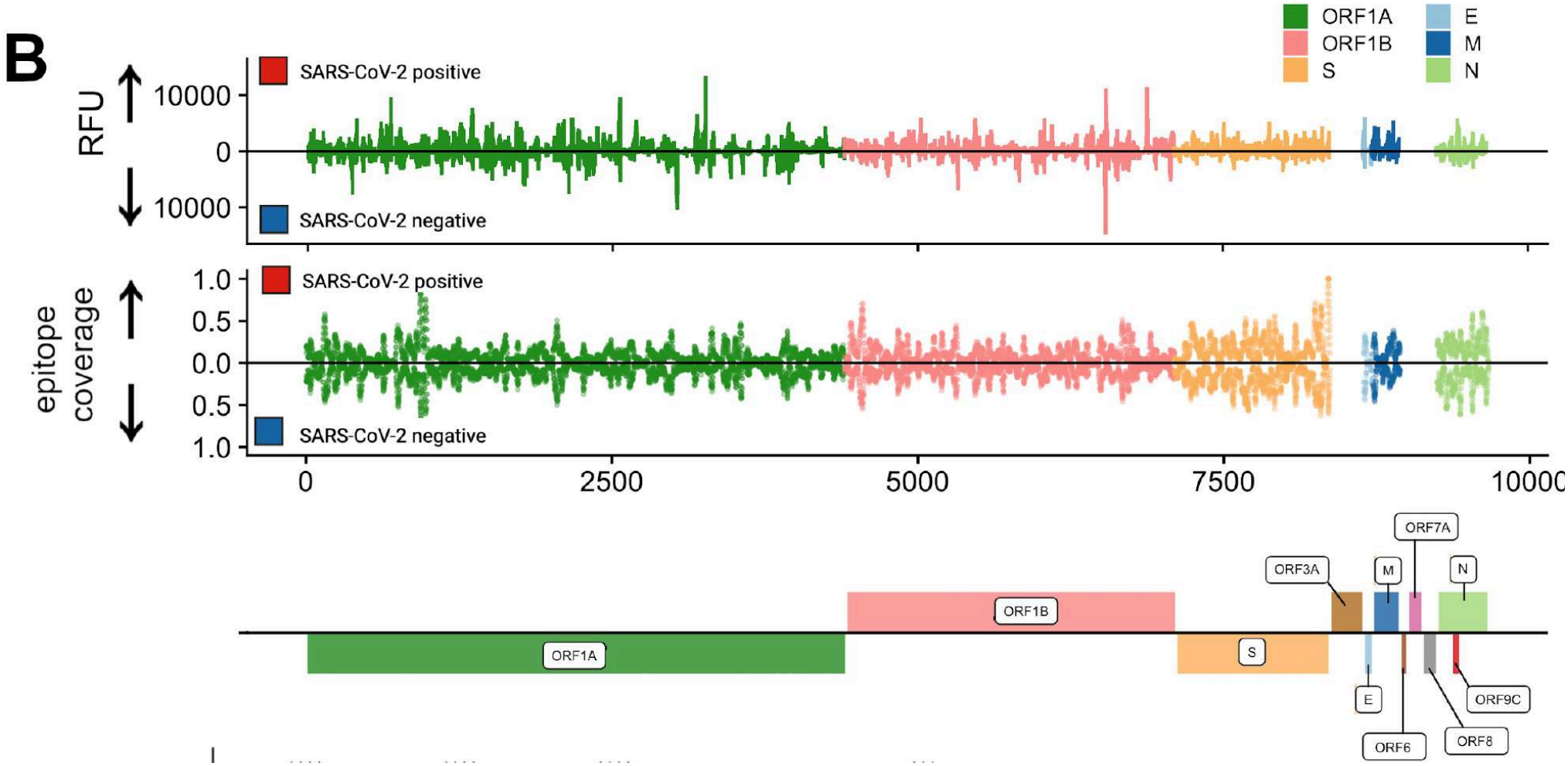
High density peptide arrays (HDPA) to map epitopes (PepperPrint)



High density peptide arrays (HDPA) to map epitopes (PepperPrint)



High density peptide arrays (HDPA) to map epitopes (PepperPrint)



The Case for Pre-existing Immunity

(asymptomatic infections)

Cite as: R. Li *et al*., *Science*
10.1126/science.abb3221 (2020).

Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV2)

Ruiyun Li[1]*, Sen Pei[2]*†, Bin Chen[3]*, Yimeng Song[4], Tao Zhang[5], Wan Yang[6], Jeffrey Shaman[2]†

[1]MRC Centre for Global Infectious Disease Analysis, Department of Infectious Disease Epidemiology, School of Public Health, Faculty of Medicine, Imperial College London, London W2 1PG, UK. [2]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY 10032, USA. [3]Department of Land, Air and Water Resources, University of California, Davis, Davis, CA 95616, USA. [4]Department of Urban Planning and Design, The University of Hong Kong, Hong Kong. [5]Ministry of Education Key Laboratory for Earth System Modeling, Department of Earth System Science, Tsinghua University, Beijing 10084, P. R. China. [6]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032, USA.

***These authors contributed equally to this work.**

†Corresponding author. Email: sp3449@cumc.columbia.edu (S.P.); jls106@cumc.columbia.edu (J.S.)

Estimation of the prevalence and contagiousness of undocumented novel coronavirus (SARS-CoV2) infections is critical for understanding the overall prevalence and pandemic potential of this disease. Here we use observations of reported infection within China, in conjunction with mobility data, a networked dynamic metapopulation model and Bayesian inference, to infer critical epidemiological characteristics associated with SARS-CoV2, including the fraction of undocumented infections and their contagiousness. We estimate 86% of all infections were undocumented (95% CI: [82%–90%]) prior to 23 January 2020 travel restrictions. Per person, the transmission rate of undocumented infections was 55% of documented infections ([46%–62%]), yet, due to their greater numbers, undocumented infections were the infection source for 79% of documented cases. These findings explain the rapid geographic spread of SARS-CoV2 and indicate containment of this virus will be particularly challenging.

Science. 2020 May 1;368(6490):489-493

The Case for Pre-existing Immunity

(asymptomatic infections)

21.7 – 85%

Table 2. Antibody Testing

Study or Report	Tested, *n**	Random Sampling*	SARS-CoV-2–Positive, *n* (%)	Asymptomatic, *n* (%)
England residents (55)	**365 104**	**Yes**	17 576 (4.8)	5694 (32.4)
Spain residents (56)	**61 075**	**Yes**	3053 (5.0)	1008 (33.0)
Detroit, Michigan, hospital staff (57)	**20 614**	No	1818 (8.8)	798 (43.9)
Wuhan, China, hospital staff (58)	8553	No	424 (5.0)	148 (34.9)
Bavaria, Germany, children aged 1–18 y (59)	4859	**Yes**	47 (1.0)	22 (46.8)
Louisiana residents (60)	4778	**Yes**	311 (6.5)	147 (47.3)
Munich, Germany, hospital staff (61)	4554	No	108 (2.4)	28 (25.9)
Cairo, Egypt, hospital staff (62)	4040	No	170 (4.2)	116 (68.2)
Health care personnel at 13 U.S. medical centers (63)	3248	No	194 (6.0)	56 (28.9)
Maranhão, Brazil, residents (64)	3156	**Yes**	1167 (37.0)	320 (27.4)
Ischgl, Austria, residents (65)	1473	No	622 (42.2)	529 (85.0)
Wuhan dialysis patients (66)	1027	No	99 (9.6)	50 (50.5)
Buenos Aires, Argentina, residents (67)	873	No	466 (53.4)	396 (85.0)
Connecticut residents (68)	567	**Yes**	23 (4.1)	5 (21.7)
Sweden nursing home staff (69)	459	No	86 (18.7)	40 (46.5)
London, England, dialysis patients (70)	356	No	129 (36.2)	52 (40.3)
Nashville, Tennessee, hospital staff (71)	249	No	19 (7.6)	8 (42.1)
London maternity unit staff (72)	200	No	29 (14.5)	10 (34.5)

SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.
* Boldface indicates details that increase the likelihood of higher-quality evidence.

Ann Intern Med. 2020 Sep 1;173(5):362-367

The Case for Pre-existing Immunity

(seasonal human Coronaviruses)

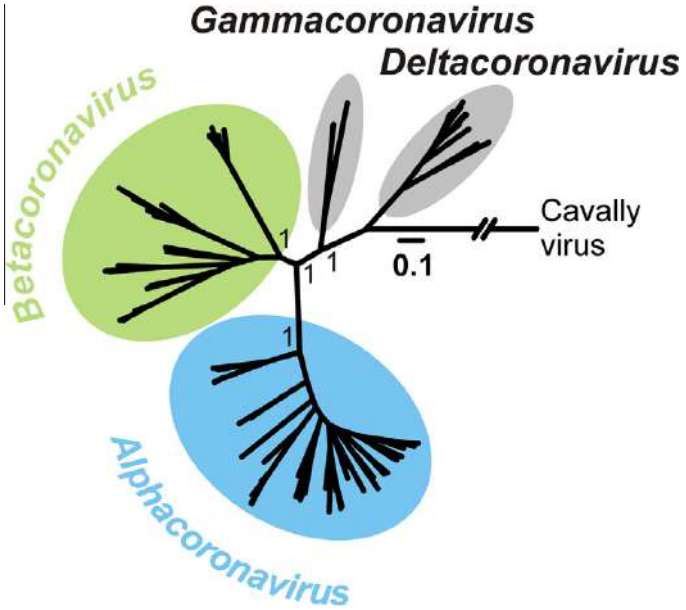


Fig. 2. Phylogenetic relationships in the subfamily *Coronavirinae*. Bayesian phylogeny of an 816-nucleotide *RNA-dependent RNA polymerase* fragment, as described previously (Drexler et al., 2010) of the subfamily *Coronavirinae* using MrBayes V3.1 (Ronquist and Huelsenbeck, 2003) under assumption of a GTR + G + I substitution model, using 2,000,000 trees sampled every 100 steps, annotated with a burn-in of 25% using TreeAnnotator V1.7.4 and visualized using FigTree V1.4 from the BEAST package (Drummond et al., 2012). Cavally virus (Zirkel et al., 2011) was used as an outgroup. Values at deep nodes indicate statistical support from Bayesian posterior probabilities, scale bar genetic distance.

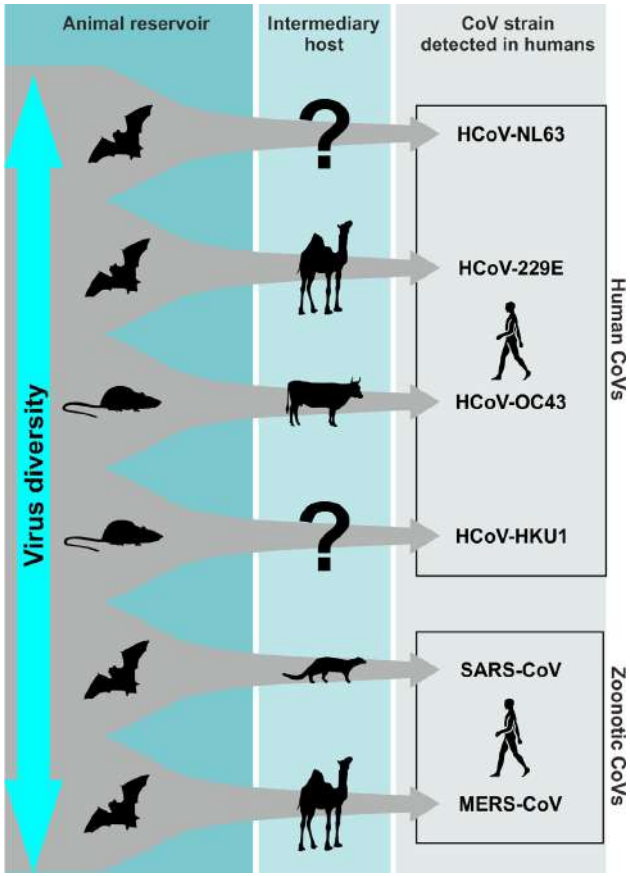
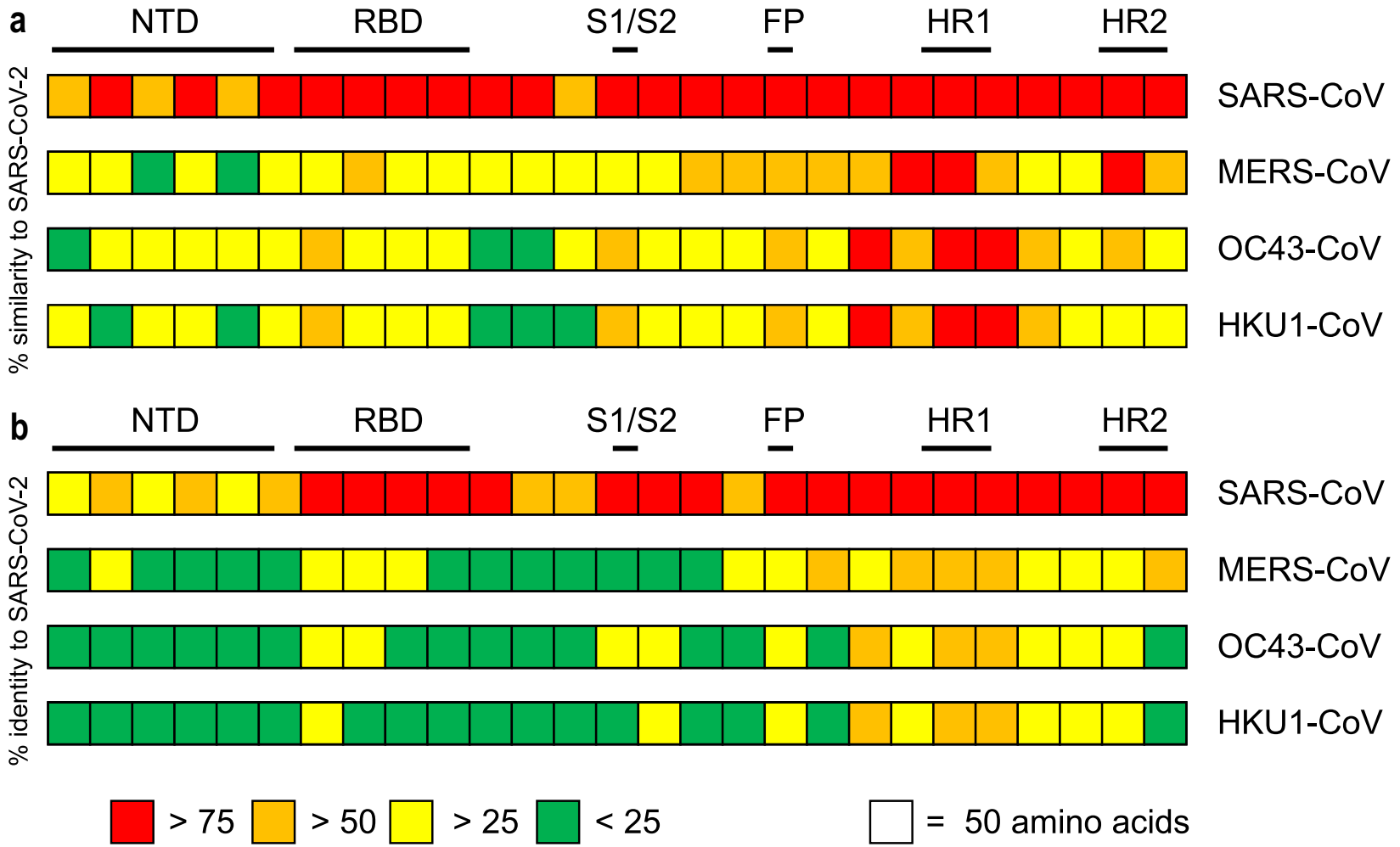


Fig. 1 Summary diagram of the animal groups representing natural hosts and the putative intermediate hosts for the six CoVs found in humans.

Adv Virus Res. 2018;100:163-188

The Case for Pre-existing Immunity

(seasonal human Coronaviruses)



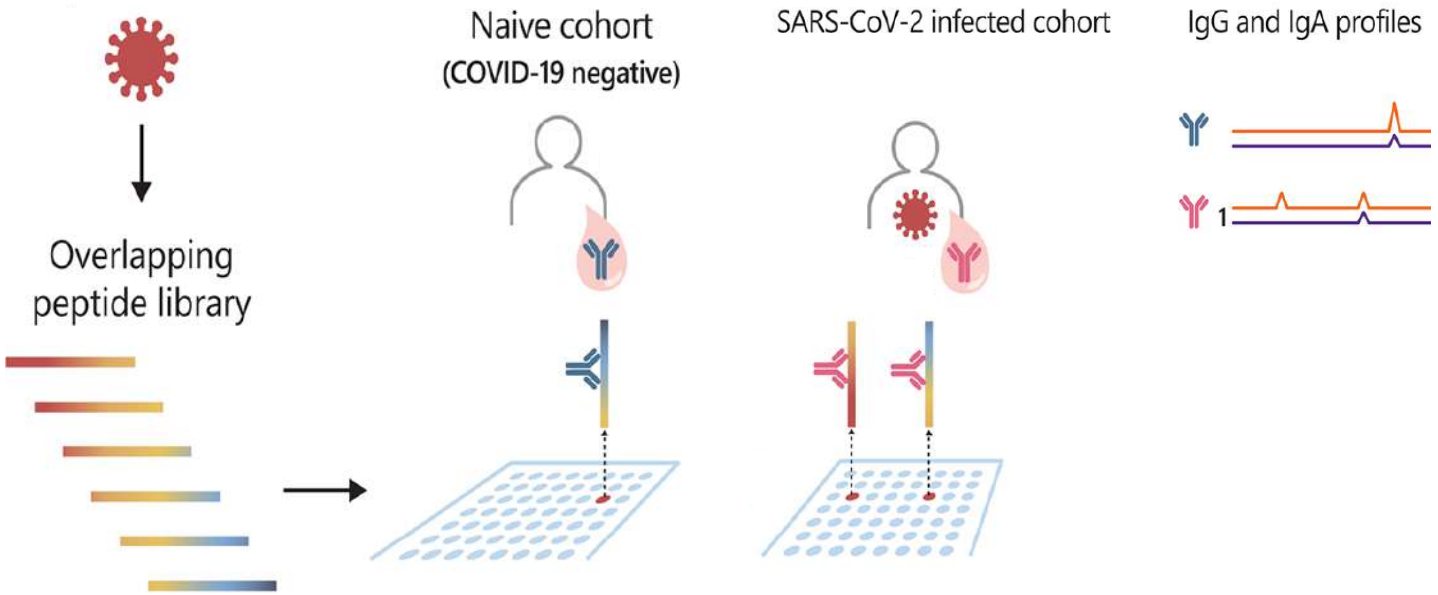
High-Density Peptide Array (HDPA)

(PEPperPRINT)

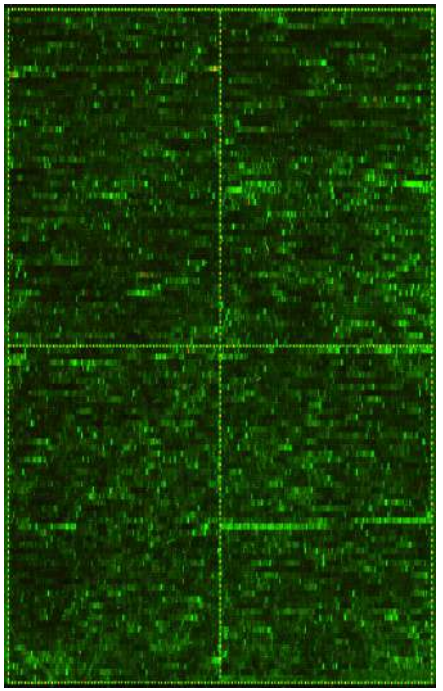
Seasonal coronavirus (hCoV) proteome-wide IgG and IgA epitope mapping (OC43, NL63, 229E, HKU1)

B

hCoV
(OC43, NL63, HKU1, 229E)
proteome



Example of raw data



How conserved are identified epitopes?

(seasonal human Coronaviruses)

OC43-specific Peptides

	Spike	Nucleocapsid	Envelope	Membrane	ORF1ab	TOTAL
SARS-CoV-2 negative	126	37	6	4	293	466
SARS-CoV-2 positive	209	70	10	27	508	824
Overlap	104	35	2	8	280	429
Total	439	142	18	39	1081	1719

HKU1-specific Peptides

	Spike	Nucleocapsid	Envelope	Membrane	ORF1ab	TOTAL
SARS-CoV-2 negative	104	43	7	17	293	464
SARS-CoV-2 positive	220	105	7	23	503	858
Overlap	90	35	2	10	254	391
Total	414	183	16	50	1050	1713

NL63-specific Peptides

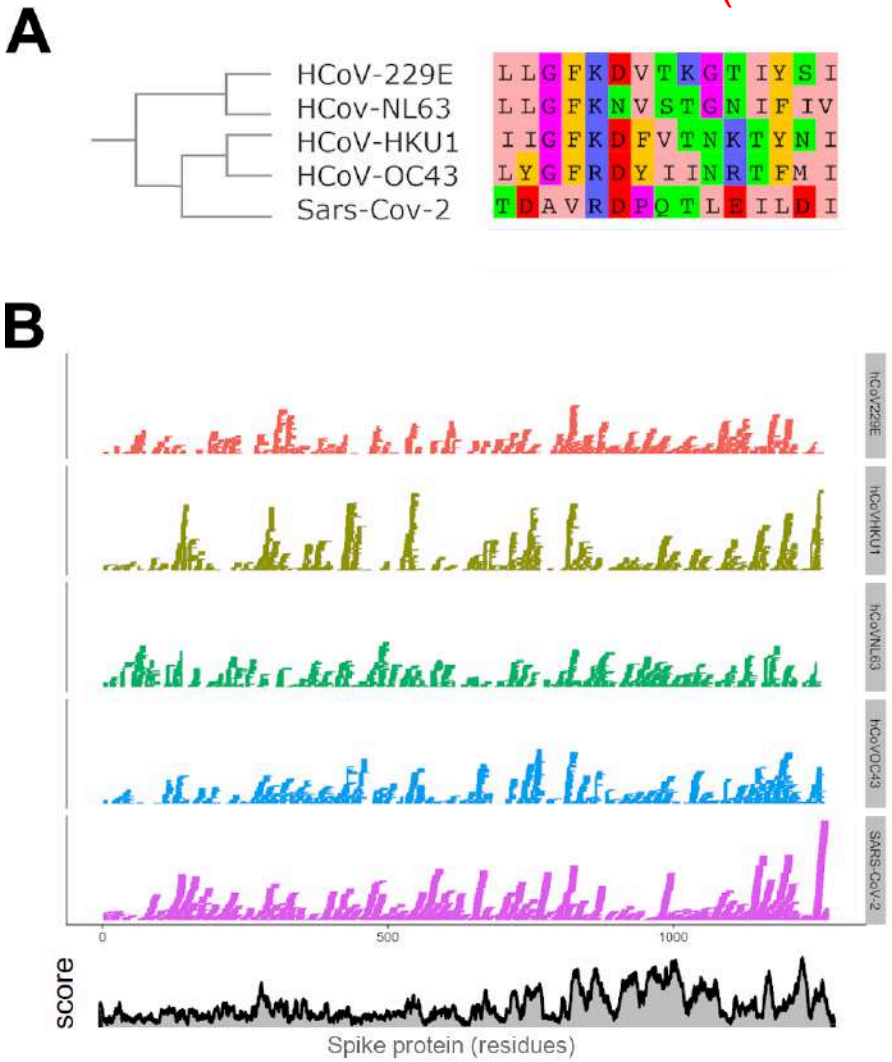
	Spike	Nucleocapsid	Envelope	Membrane	ORF1ab	TOTAL
SARS-CoV-2 negative	139	54	12	17	296	518
SARS-CoV-2 positive	183	77	8	24	571	863
Overlap	70	56	3	14	269	412
Total	392	187	23	55	1136	1793

229E-specific Peptides

	Spike	Nucleocapsid	Envelope	Membrane	ORF1ab	TOTAL
SARS-CoV-2 negative	116	43	3	15	306	483
SARS-CoV-2 positive	158	99	7	38	592	894
Overlap	72	46	2	12	325	457
Total	346	188	12	65	1223	1834

How conserved are identified epitopes?

(seasonal human Coronaviruses)



To evaluate conservation of epitopes:

- Aligned protein sequences of viral strains and calculated conservation score based on physico-chemical properties

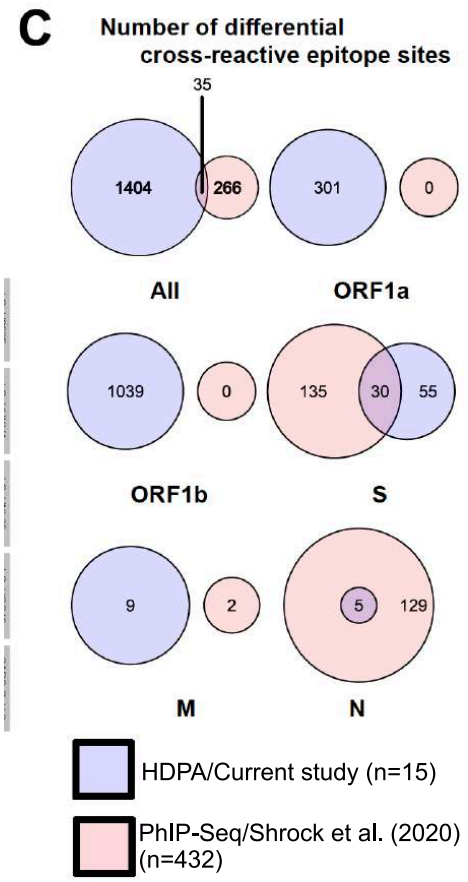
- Defined cross-reactivity per amino acid sites within 15-mer peptides

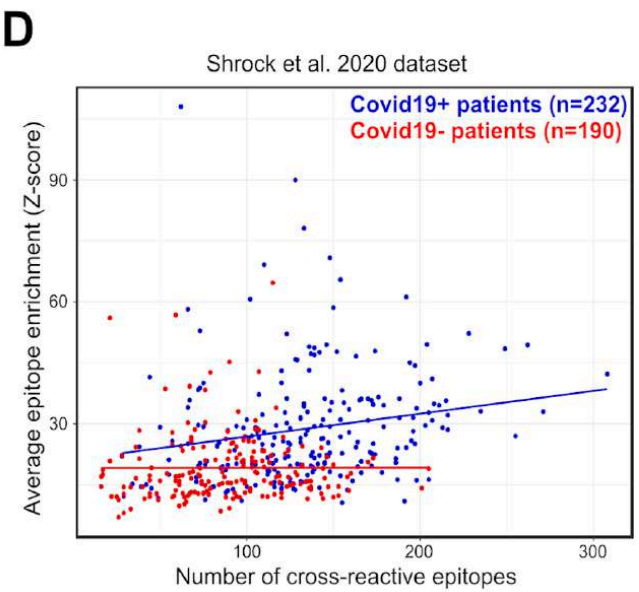
- Sites with conservation score > 6 for which Ab response to SARS-CoV-2 and at least one shCoV were considered cross-reactive

- ~ 27% of the pool of detected epitope sites are cross-reactive

- Local alignment of HDPA response for S protein of all 5 viruses shown on the left

Are cross-reactive epitope sites that particularly important for the humoral immune response after exposure to SARS-CoV-2?





CORONAVIRUS

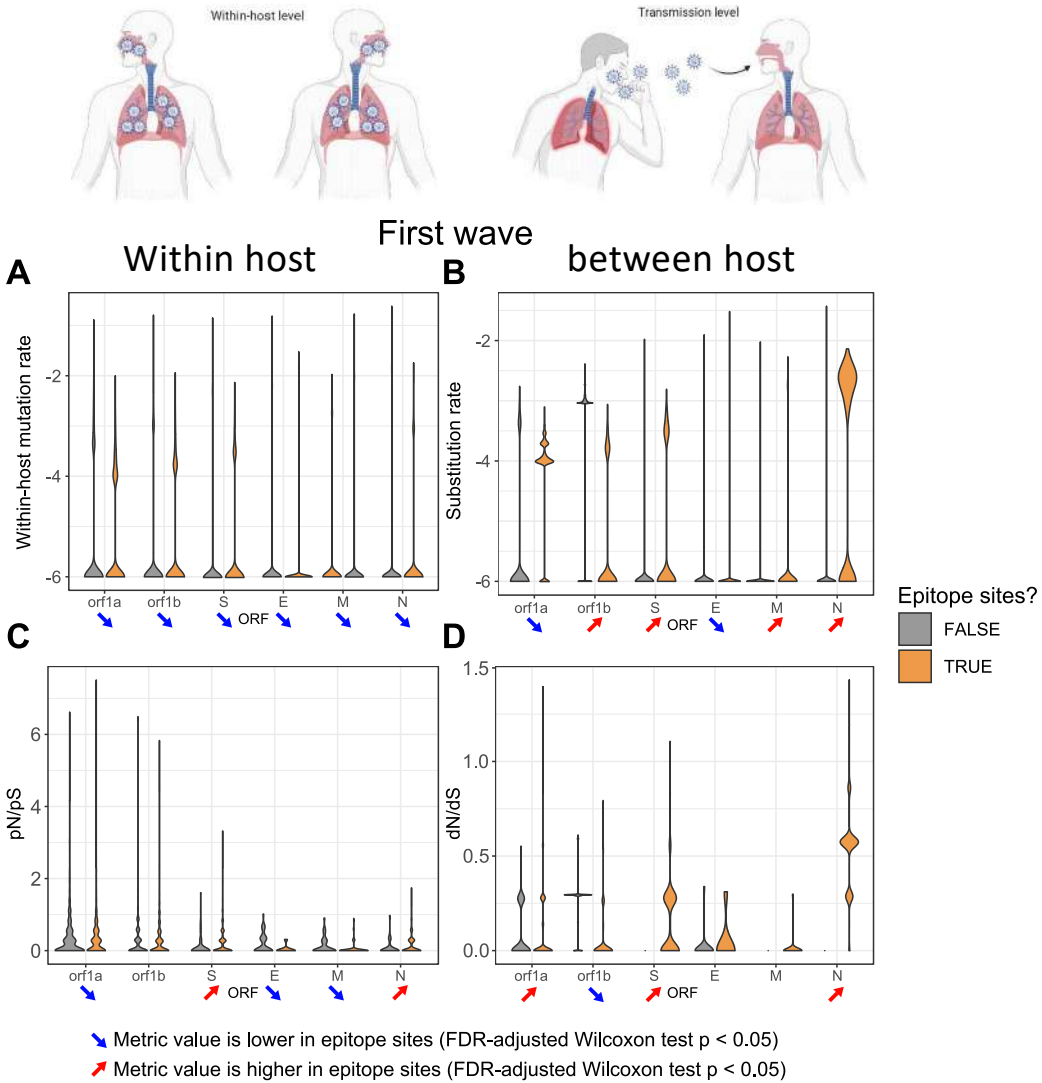
Viral epitope profiling of COVID-19 patients reveals cross-reactivity and correlates of severity

Ellen Shrock*, Eric Fujimura*, Tomasz Kula†, Richard T. Timms†, I-Hsiu Lee, Yumei Leng, Matthew L. Robinson, Brandon M. Sie, Mamie Z. Li, Yuezhou Chen, Jennifer Logue, Adam Zuiani, Denise McCulloch, Felipe J. N. Lelis, Stephanie Henson, Daniel R. Monaco, Meghan Travers, Shaghayegh Habibi, William A. Clarke, Patrizio Caturegli, Oliver Laeyendecker, Alicja Piechocka-Trocha, Jonathan Z. Li, Ashok Khatri, Helen Y. Chu, MGH COVID-19 Collection & Processing Team, Alexandra-Chloé Villani, Kyle Kays, Marcia B. Goldberg, Nir Hacohen, Michael R. Filbin, Xu G. Yu, Bruce D. Walker, Duane R. Wesemann, H. Benjamin Larman, James A. Lederer, Stephen J. Elledge‡

- analyzed if the humoral immune response to SARS-CoV-2 epitopes correlated with the number of cross-reactive epitopes identified.
- to what extent is the response to SARS-CoV-2 predictable based on cross-reactivity to other endemic hCoVs?
- We defined cross-reactive epitopes as peptide sequences with at least five cross-reactive epitope sites
- 16 epitopes being cross-reactive

Shrock *et al.*, *Science* **370**, 1058 (2020) 27 November 2020

Viral Genome Sequencing

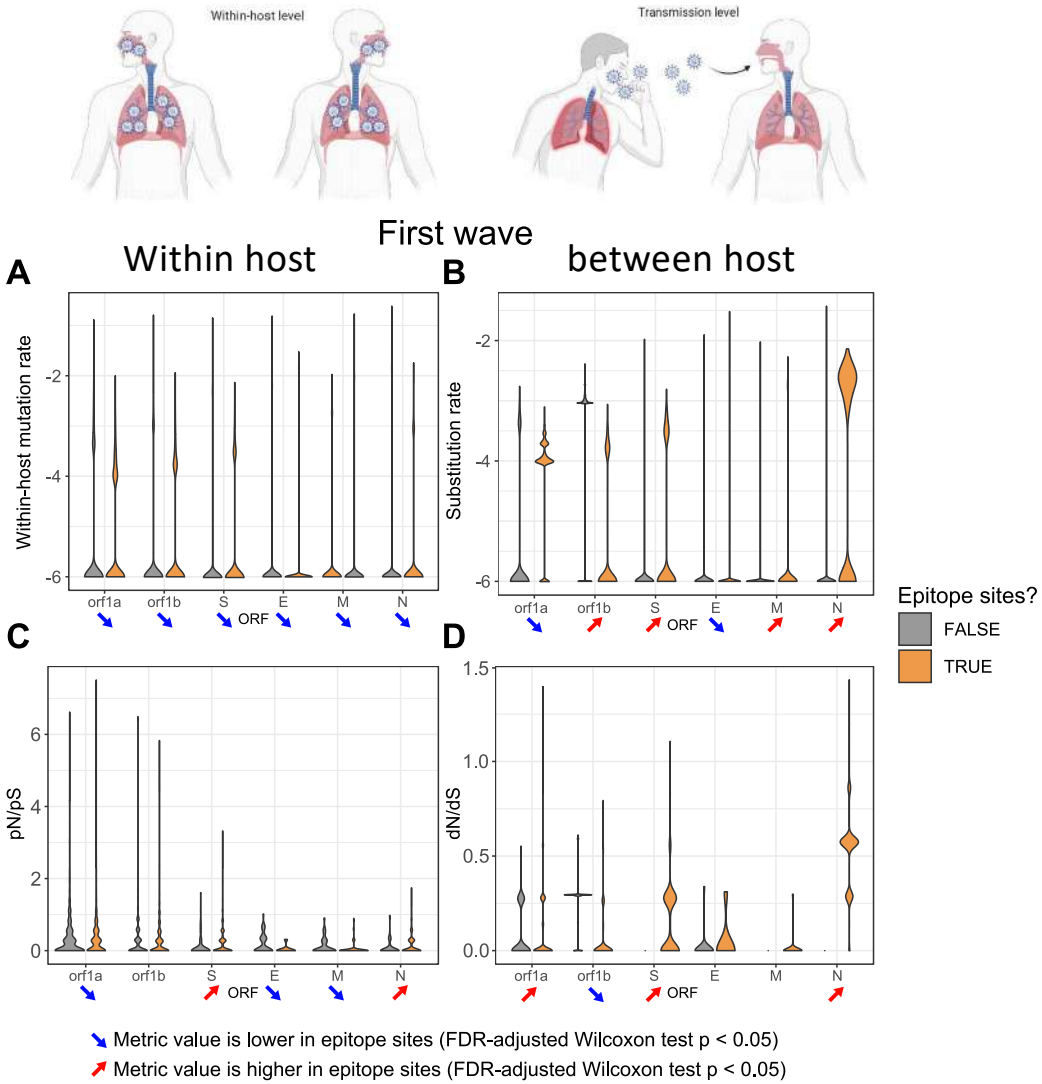


- We tracked the evolution of identified SARS-CoV-2 B cell epitopes using single nucleotide variants (SNVs) identified in 38,685 SARS-CoV-2 genome sequences from the NCBI sequence read archive (Wave 1: 01-07/2020; Wave 2: 08-12/2020) sequenced using Illumina paired-end amplicons with a minimum average depth of coverage of 200x and fewer than 10,000 sites with a depth of coverage lower than 100x. Combined with additional filters to remove sequencing errors

- Such deep coverage allowed us to identify SNVs that are polymorphic within patients, reflecting within-patient evolution, as well as those that are shared between the consensus sequences of different patients.

- Mutations in epitope sites or non-epitope sites for within host and between host genomic viral sequences

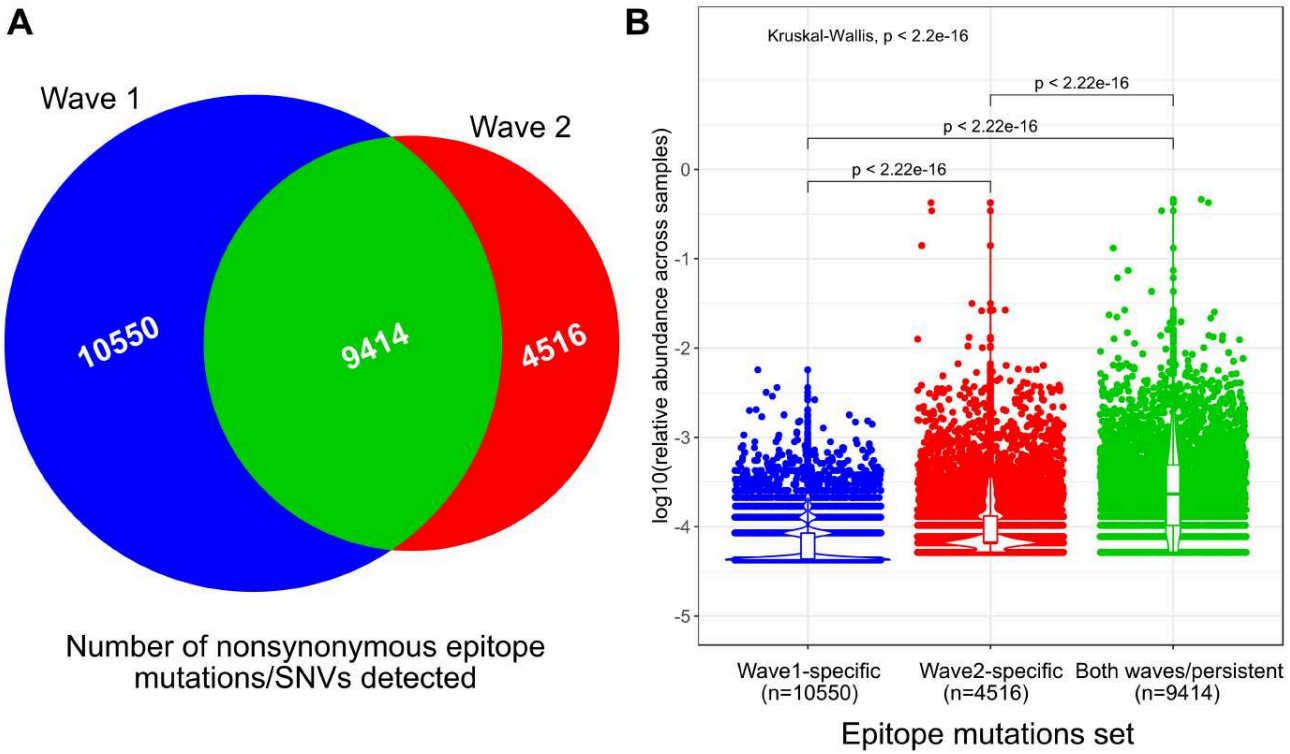
Viral Genome Sequencing



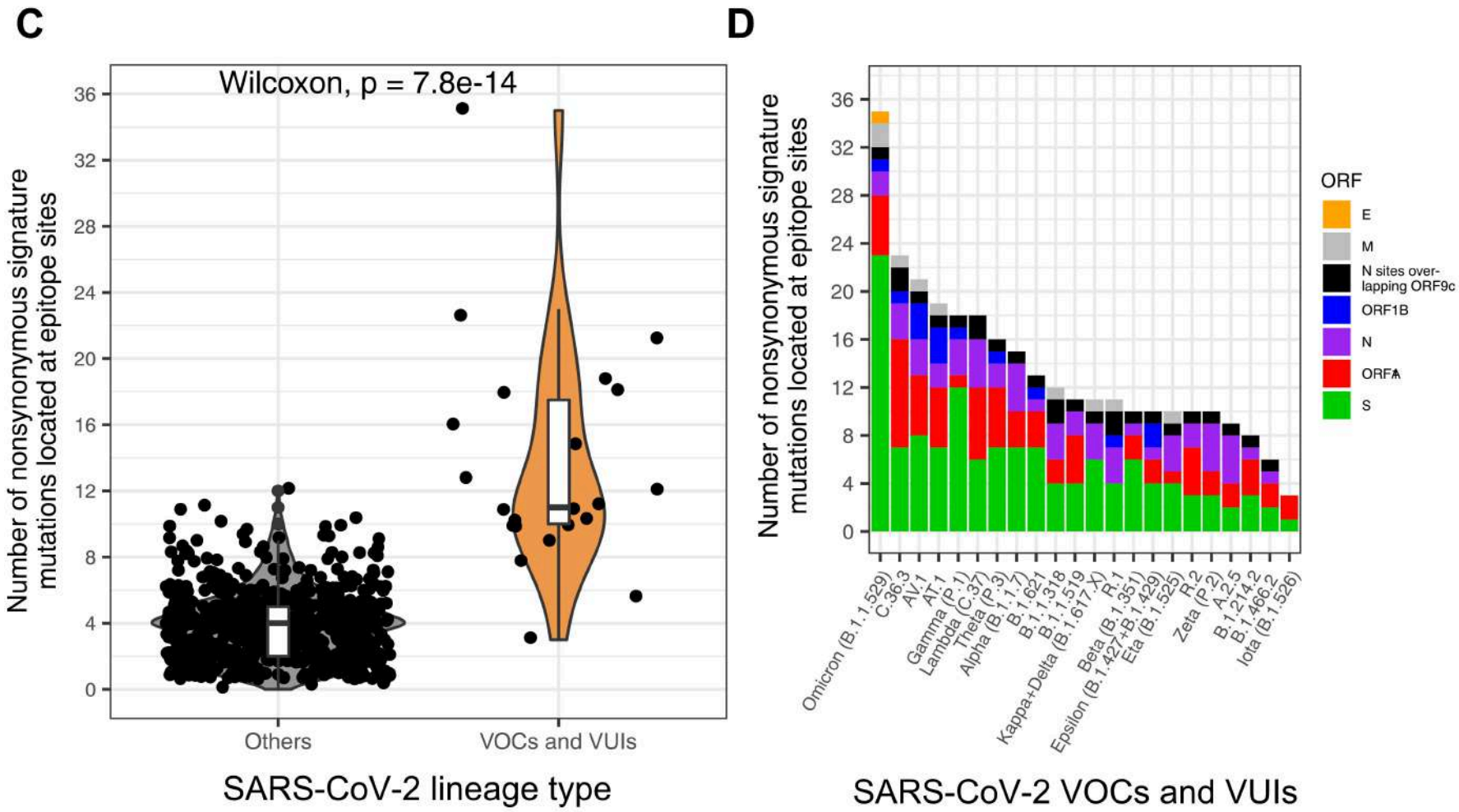
These observations indicate that nonsynonymous substitutions in S and N epitope sites accumulate most rapidly upon transmission, rather than within patients.

Taken together these results support the notion that most of the selective pressure for immune evasion of SARS-CoV-2 occurs upon transmission between hosts, consistent with the asynchrony model

Assessing Immune Evasion Potential of SARS-CoV-2 Variants



Assessing Immune Evasion Potential of SARS-CoV-2 Variants



Acknowledgments

Carsten Haber and Volker Stadler, PEPperPRINT GmbH, Heidelberg, Germany

Arnaud N'Guessan and Jesse Shapiro, Department of Microbiology and Immunology, McGill University and McGill Genome Centre

Senthil Kumar Duraikannu Kailasam and Guillaume Bourque, Canadian Center for Computational Genomics, and Department of Human Genetics, McGill University

Raphael Poujol, Jean-Christophe Grenier, Fatima Mostefai, Julie Hussin, Research Centre, Montreal Heart Institute, and Département de Médecine, Université de Montréal

Paola Contini and Raffaele De Palma, Department of Internal Medicine, University of Genoa and IRCCS IST-Ospedale San Martino, Genoa, Italy

Jörg H. Fritz and Ciriaco A. Piccirillo, Department of Microbiology and Immunology, McGill University and McGill University Research Center on Complex Traits (MRCCT), McGill University

McGill Interdisciplinary Initiative in Infection and Immunity (MI4) for financial support for our study