Cell Reports Medicine, Volume *1*

Supplemental Information

ReScan, a Multiplex Diagnostic Pipeline, Pans Human Sera for SARS-CoV-2 Antigens

Colin R. Zamecnik, Jayant V. Rajan, Kevin A. Yamauchi, Sabrina A. Mann, Rita P. Loudermilk, Gavin M. Sowa, Kelsey C. Zorn, Bonny D. Alvarenga, Christian Gaebler, Marina Caskey, Mars Stone, Philip J. Norris, Wei Gu, Charles Y. Chiu, Dianna Ng, James R. Byrnes, Xin X. Zhou, James A. Wells, Davide F. Robbiani, Michel C. Nussenzweig, Joseph L. DeRisi, and Michael R. Wilson

Supplementary Material

ReScan, a Multiplex Diagnostic Pipeline, Pans Human Sera for SARS-CoV-2 Antigens

Colin R. Zamecnik[1*], Jayant V. Rajan[2*], Kevin A. Yamauchi[3], Sabrina A. Mann[3,4], Rita P. Loudermilk[1], Gavin M. Sowa[5], Kelsey C. Zorn[4], Bonny D. Alvarenga[1], Christian Gaebler[6], Marina Caskey[6], Mars Stone[7,8], Philip J. Norris[7,8,9], Wei Gu[8], Charles Y. Chiu[8,9], Dianna Ng[9], James R. Byrnes[10], Xin X. Zhou[10], James A. Wells[10,11], Davide F. Robbiani[6,#], Michel C. Nussenzweig[6,12], Joseph L. DeRisi[3,4**], Michael R. Wilson[1**,***]

[*]Authors contributed equally

[**]Authors contributed equally

Affiliations:

[1]Weill Institute for Neurosciences, Department of Neurology, University of California, San Francisco, San Francisco, CA, USA

[2]Division of Experimental Medicine, Department of Medicine, Zuckerberg San Francisco General Hospital, University of California, San Francisco, San Francisco, CA, USA

[3]Chan Zuckerberg Biohub, San Francisco, CA, USA

[4]Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA, USA

[5]University of California, San Francisco School of Medicine, San Francisco, CA, USA

[6]Laboratory of Molecular Immunology, The Rockefeller University, New York, NY 10065, USA

[7]Vitalant Research Institute, San Francisco, CA, USA

[8]Department of Laboratory Medicine, University of California, San Francisco, San Francisco, USA

[9]Department of Medicine, University of California, San Francisco, San Francisco, USA

[10]Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, USA

[11]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, USA

[12]Howard Hughes Medical Institute

[#]present address: Institute for Research in Biomedicine, Università della Svizzera italiana, 6500 Bellinzona, Switzerland

***Corresponding Author Information:

Name: Dr. Michael Wilson

Address: UCSF, Department of Neurology, Division of Neuroimmunology and Glial Biology, 675 Nelson Rising Lane, NS212, Campus Box 3206, San Francisco, CA 94158

Email: michael.wilson@ucsf.edu

Phone: 415-502-7429

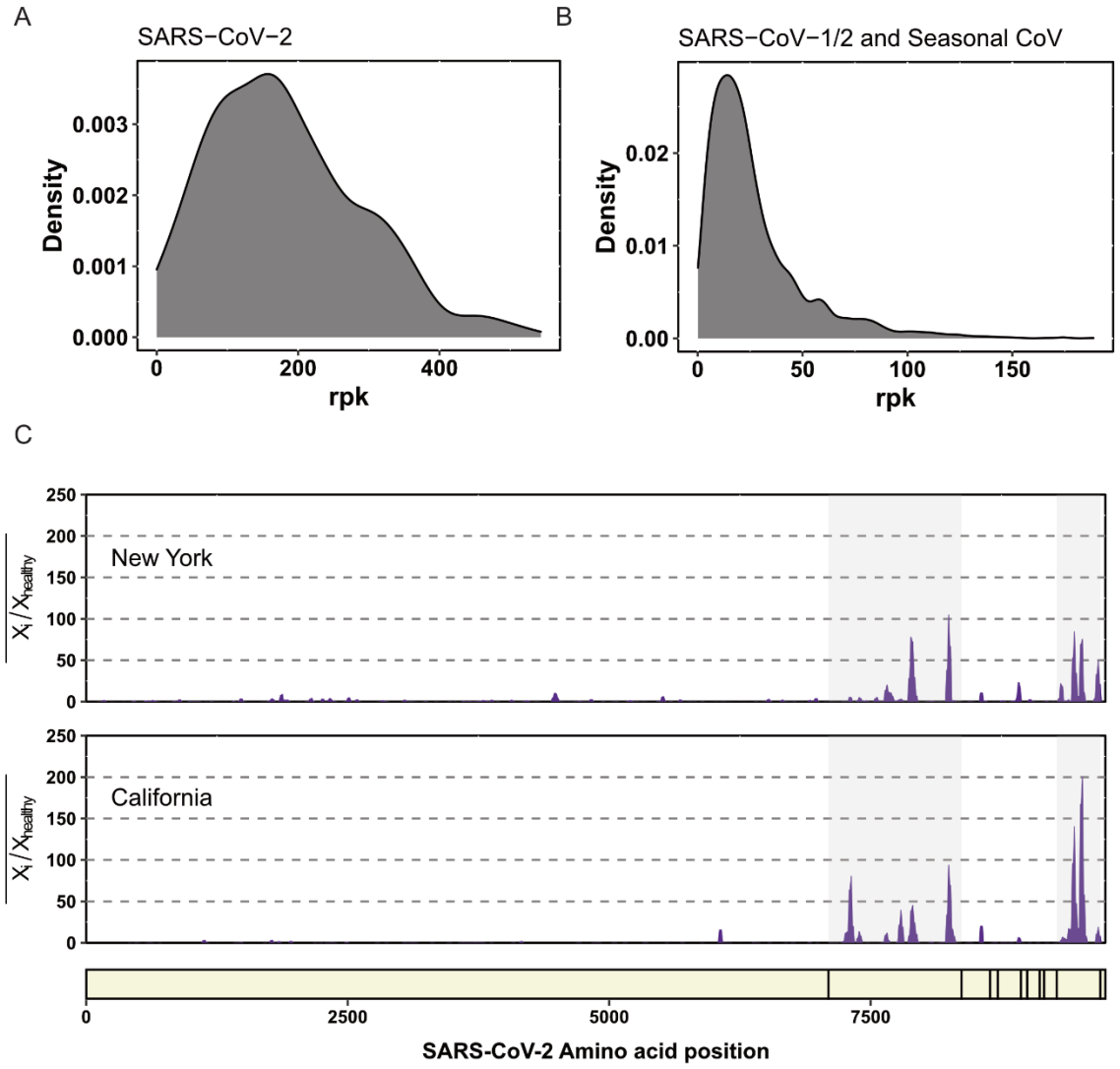
Supplementary Table 1. **SARS-CoV-2 Peptide Sequences in ReScan Microarray – Related to Figure 2.**

Peptide Name (Figure 2)	Peptide Name (Library Design)	Amino acid sequence
SARS2_N_134-171	SARS2__N__YP_009724397.2__frag_8	ATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGF
SARS2_N_153-190	SARS2__N__YP_009724397.2__frag_9	NNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRS
SARS2_N_210-247	SARS2__N__YP_009724397.2__frag_12	MAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVT
SARS2_N_362-399	SARS2__N__YP_009724397.2__frag_20	TFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLPAAD
SARS2_S_552-589	SARS2__S__YP_009724390.1__frag_30	LTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITP
SARS2_S_799-836	SARS2__S__YP_009724390.1__frag_43	GFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQ
SARS2_S_799-836	SARS2__S__YP_009724390.1__frag_44	IEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKF
SARS2_S_819-855	SARS2__S__YP_009724390.1__frag_61	LQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVN
SARS2_ORF3a_172-209	SARS2__ORF3a__YP_009724391.1__frag_10	GDGTTSPISEHDYQIGGYTEKWESGVKDCVVLHSYFTS

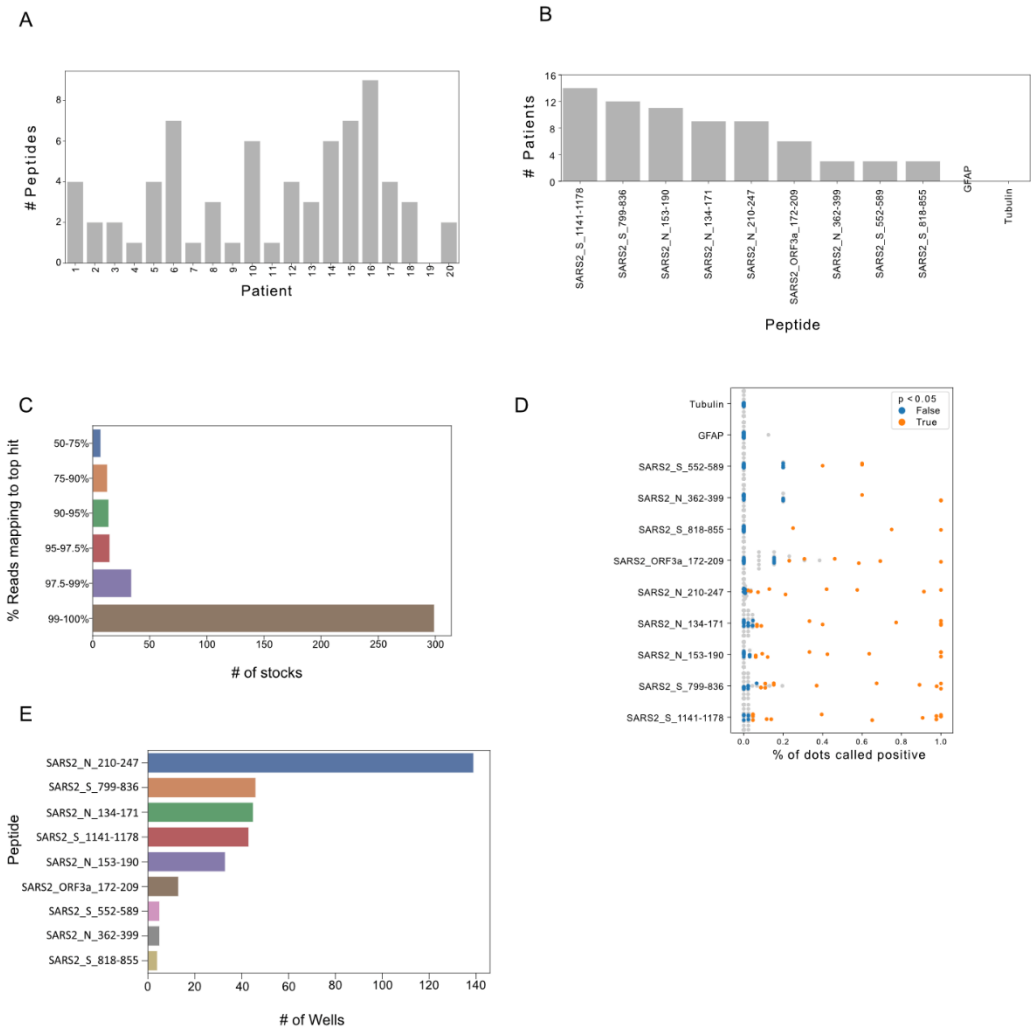
Supplementary Table 2. Test Characteristics of ReScan Peptides and Whole Protein Luminex Assay – Related to Figure 4. Test Performance Characteristics of Luminex Assay (top) using all 9 ReScan peptides and (bottom) 7 peptides excluding ORF3A 179-209 and S protein 552-589.

	Peptide				**Protein**			
	FNR (FN/TP)	**% (95% CI)**	**FPR (FP/TN)**	**% (95% CI)**	**FNR (FN/TP)**	**% (95% CI)**	**FPR (FP/TN)**	**% (95% CI)**
Test	11/98	11.2 (5.1-18.4)	2/20	10.0 (0.0-25.0)	3/98	3.1 (0.0-7.1)	6/20	30.0 (10.0-50.0)
Validation	3/34	8.8 (0.0-20.6)	3/45	6.7 (0.0-15.6)	2/34	5.9 (0.0-14.7)	5/45	11.1 (2.2-20.0)

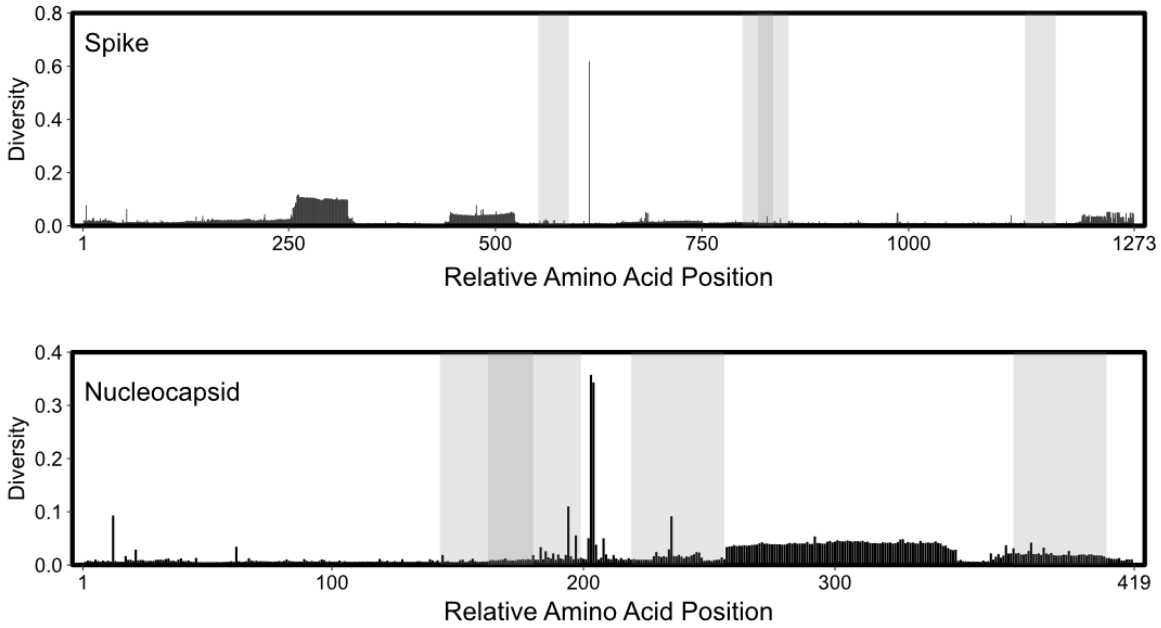
	Peptide				**Protein**			
	FNR (FN/TP)	**% (95% CI)**	**FPR (FP/TN)**	**% (95% CI)**	**FNR (FN/TP)**	**% (95% CI)**	**FPR (FP/TN)**	**% (95% CI)**
Test	11/98	11.2 (5.1-18.4)	2/20	10.0 (0.0-25.0)	3/98	3.1 (0.0-7.1)	6/20	30.0 (10.0-50.0)
Validation	3/34	8.8 (0.0-20.6)	1/45	2.2 (0-6.7)	2/34	5.9 (0.0-14.7)	5/45	11.1 (2.2-20.0)



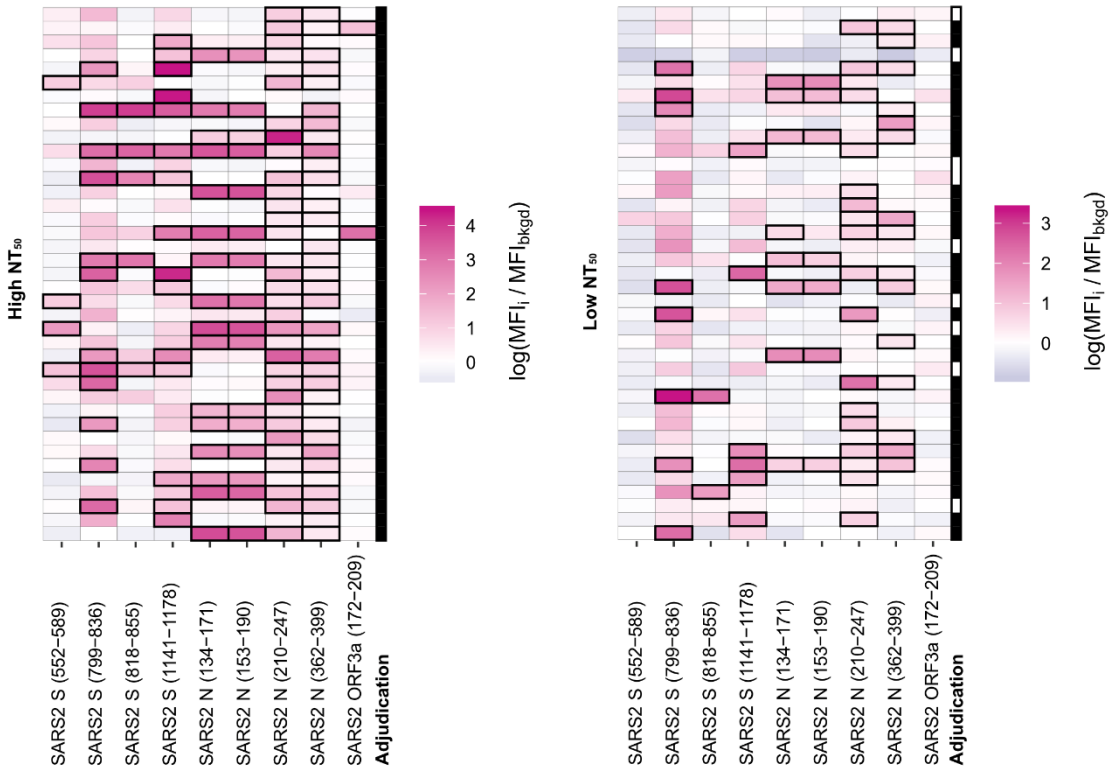
Supplementary Data Figure 1. T7 Phage Display Library Benchmarking and Geographic Differences in Peptide Enrichments (Related to Figure 1). (A) Distribution of peptides by reads per 100,000 reads (rpk) in the SARS-CoV-2 library used to identify antigens for ReScan. (B) Distribution of peptide rpk in the combined SARS-CoV-2, SARS-CoV-1, and Seasonal CoV library used for VirScan. (C) Mean enrichments of COVID-19 patient sera separated by sample geographic origin, from New York area (n=78) and SF Bay Area (n=20).



Supplementary Data Figure 2. ReScan Benchmarking and Quality Control (Related to Figure 2). (A) Number of peptide hits for each patient on ReScan and (B) number of times each peptide was called as significant across the COVID-19 cohort (n=20, p<0.05, Fisher's exact test comparing positive signal to healthy distribution). (C) Monoclonality of all wells on ReScan array. (D) Fraction of dots that are positive for each peptide on all strips from COVID-19 positive sera. Strips that are significantly enriched for positive dots over healthy control strips ($p < 0.05$ by Fisher's exact test) are colored orange and strips that were not significantly enriched are colored blue. All healthy control strips are plotted in gray. (E) Number of wells passing quality filters that were mapped to each peptide identity.



Supplementary Data Figure 3. ReScan Peptides Located in Areas of Low Mutagenicity (Related to Figure 2). Top: Multiple sequence alignments of all available S and N protein sequences (9,950 spike protein sequences and 9,866 nucleoprotein sequences) to the reference Wuhan genome used in the generation of the library, with overlaid ReScan peptides in grey (overlapping sequences in dark grey). Diversity represented as Shannon entropies, where gaps were not included as an additional residue.



Supplementary Data Figure 4. ReScan Assay Performance by NT50 (Related to Figure 4). ReScan peptide assay performance broken down by high (518.9-10433.3, left) and low (5.0-494.9, right) NT50 values from respective sera.