

Potent neutralizing antibodies in the sera of convalescent COVID-19 patients are directed against conserved linear epitopes on the SARS-CoV-2 spike protein

Chek Meng Poh^{1†}, Guillaume Carissimo^{1†}, Bei Wang^{1†}, Siti Naqiah Amrun¹, Cheryl Yi-Pin Lee¹, Rhonda Sin-Ling Chee¹, Nicholas Kim-Wah Yeo¹, Wen-Hsin Lee¹, Yee-Sin Leo^{2,3,4,5}, Mark I-Cheng Chen^{2,6}, Seow-Yen Tan⁷, Louis Yi Ann Chai^{3,8}, Shirin Kalimuddin^{9,10}, Siew-Yee Thien⁹, Barnaby Edward Young^{2,3,4}, David C. Lye^{2,3,4,5}, Cheng-I Wang¹, Laurent Renia¹, Lisa F.P. Ng^{1,11,12*}

9

- 10 1. Singapore Immunology Network, Agency of Science, Technology and
11 Research, Immunos, Biopolis, 138648, Singapore
- 12 2. National Centre for Infectious Diseases, 16 Jalan Tan Tock Seng, 308442,
13 Singapore
- 14 3. Department of Infectious Diseases, Tan Tock Seng Hospital, 11 Jalan Tan
15 Tock Seng, 308433, Singapore
- 16 4. Lee Kong Chian School of Medicine, Nanyang Technological University, 11
17 Mandalay Road, 308232, Singapore
- 18 5. Yong Loo Lin School of Medicine, National University of Singapore and
19 National University Health System, 10 Medical Drive, 117597, Singapore
- 20 6. Saw Swee Hock School of Public Health, National University of Singapore and
21 National University Health System, 12 Science Drive 2, #10-01, 117549,
22 Singapore
- 23 7. Department of Infectious Diseases, Changi General Hospital, 2 Simei Street 3,
24 529889, Singapore

- 25 8. Department of Medicine, National University Hospital, 5 Lower Kent Ridge
26 Road, 119074, Singapore
- 27 9. Department of Infectious Diseases, Singapore General Hospital, 31 Third
28 Hospital Ave, #03-03 Bowyer Block C, 168753, Singapore
- 29 10. Emerging Infectious Disease Program, Duke-NUS Medical School, 8 College
30 Road, 169857, Singapore
- 31 11. Department of Biochemistry, Yong Loo Lin School of Medicine, National
32 University of Singapore, 8 Medical Drive, 117596, Singapore.
- 33 12. Institute of Infection and Global Health, University of Liverpool, Liverpool, 8
34 West Derby Street, Liverpool L7 3EA, United Kingdom

35 [†] contributed equally to this work

36

37 **Keywords:**

38 SARS-CoV, COVID-19, SARS-CoV-2, convalescent patients, recovered patients,
39 antibodies, IgG, B-cell epitopes, neutralizing capacity

40

41 ***Corresponding author:** Lisa F.P. Ng: Laboratory of Microbial Immunity, Singapore
42 Immunology Network, A*STAR, 8A Biomedical Grove, Immunos #04-06, Singapore
43 138648. Phone: (+65)-64070028. Email: lisa_ng@immunol.a-star.edu.sg

44

45 **Words:** 1148 words

46 **Abstract (69 words)**

47 The ongoing SARS-CoV-2 pandemic demands rapid identification of immunogenic
48 targets for the design of efficient vaccines and serological detection tools. In this
49 report, using pools of overlapping linear peptides and functional assays, we present
50 two immunodominant regions on the spike glycoprotein that were highly recognized
51 by neutralizing antibodies in the sera of COVID-19 convalescent patients. One is
52 highly specific to SARS-CoV-2, and the other is a potential pan-coronavirus target.

53 **Main**

54 In December 2019, a cluster of pneumonia cases of unknown etiology was reported
 55 in the city of Wuhan in the province of Hubei. The previously unidentified pathogen,
 56 which induces symptoms resembling an infection by the Severe Acute Respiratory
 57 Syndrome Coronavirus (SARS-CoV), was later identified as a novel coronavirus,
 58 SARS-CoV-2 [1]. Within a span of four months, there are more than 750,000
 59 laboratory-confirmed cases of human Coronavirus Disease 2019 (COVID-19), with
 60 over 35,000 deaths across 199 countries and territories (For up to date information
 61 consult [https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/)
 62 [reports/](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/)). After being declared a pandemic by World Health Organization (WHO) on
 63 11th March 2020, there is a compelling need to understand and develop effective
 64 therapeutic interventions against SARS-CoV-2.

65 SARS-CoV-2 uses the spike (S) glycoprotein to bind to the angiotensin-
 66 converting enzyme 2 (ACE2) receptor with a better affinity than SARS-CoV S
 67 glycoprotein for entry [2]. Thus, blocking the binding to ACE2, or blocking host
 68 protease cleavage to release the fusion peptide is an efficient strategy to prevent
 69 coronavirus entry [3-5]. To date, one study has assessed the immunogenicity of
 70 structural domains of recombinant SARS-CoV-2 S protein [6]. At the time of writing,
 71 findings on SARS-CoV-2 linear epitopes remain limited to bioinformatics prediction of
 72 human B and T-cell epitopes using SARS-CoV as a model [7-9]. Five regions on the
 73 S glycoprotein of SARS-CoV (residues 274-306, 510-586, 587-628, 784-803 and
 74 870-893) were predicted to be associated with a robust immune response [7], while
 75 other studies reported candidate epitopes [8, 9] that require validation with human
 76 patient samples.

77 In this brief communication, we report the antibody profiles of COVID-19
78 patients, and the identification of two immunodominant linear B-cell epitopes on the
79 S glycoprotein of SARS-CoV-2 that are crucial in controlling infection. A total of 25
80 convalescence serum samples collected during the current COVID-19 outbreak in
81 Singapore were screened at 1:1000 dilution for neutralizing antibodies against a
82 pseudotyped lentivirus expressing SARS-CoV-2 S glycoprotein tagged with a
83 luciferase reporter (Figure 1a). Of the 25 patients tested, six patients (2, 4, 6, 7, 11,
84 20) with sufficient amount of serum samples that displayed a good neutralizing
85 activity were selected for further functional characterization. Sera from all patients
86 showed similar IC_{50} , ranging from a titre of 694 to 836, except patient 20, who
87 showed the strongest neutralizing activity with an IC_{50} of 1603 (Figure 1b).

88 Next, we assessed the antigenic targets of these sera using a linear B-cell
89 peptide library spanning the entire S protein of either SARS-CoV or SARS-CoV-2
90 with pools of five overlapping peptides (Figure 1c, Supplementary Figure 1b).
91 Interestingly, two distinct peptide pools from SARS-CoV-2 S library, pools S14 and
92 S21, were strongly detected by sera from COVID-19 patients, and not by recovered
93 SARS patients (17 years post disease recovery) (Figure 1c, Supplementary Figure
94 1b). Sera from recalled SARS patients could neutralize SARS-CoV, but not the
95 SARS-CoV-2 pseudotyped lentiviruses (Supplementary Figure 1c). Moreover,
96 COVID-19 patients sera could strongly detect SARS-CoV S library pool S51, which
97 partially overlaps with SARS-CoV-2 pool S21 (Figure 1c, Supplementary Figure 1b).
98 This region encompasses the fusion peptide, which is highly conserved among
99 coronaviruses [10, 11], suggesting a potential pan-coronavirus epitope at this
100 location. Interestingly, targeting this region was also demonstrated to neutralize
101 infection with a pan-coronavirus fusion inhibitor peptide [12]. Surprisingly, no linear

epitope was identified in the receptor binding domain (RBD) which suggest that antibodies targeting that region are mostly conformational epitopes. Further assessment of individual peptides within pools S14 and S21 narrowed down the specific region of interest to peptides S14P5 and S21P2, respectively (Figure 1d). Recognition of these regions was stronger for the peptides of SARS-CoV-2 than SARS-CoV (Figure 1e). Together, these findings suggest that these linear B-cell epitopes are dominant antigenic regions, which corroborated previous bioinformatics predictions [7].

Using a recently published structure of SARS-CoV-2 S protein in prefusion conformation [2], peptide S14P5 is localized in proximity to the RBD (Figure 2a). As such, it is plausible that antibodies binding to this region may sterically hinder binding to ACE2 receptor, thereby abolishing virus infection [13]. Another possibility could be an allosteric effect on ACE2 binding. Similarly, peptide S21P2 contains a part of the fusion peptide sequence, which may potentially affect virus fusion (Figure 2b). In order to assess the importance of these regions in controlling SARS-CoV-2 infection, antibody depletion assays were performed against S14P5 and S21P2 (Figure 2c). Depletion efficiency and specificity was validated by ELISA, showing that only antibodies against the respective peptides were depleted but not other unrelated antibodies (Figure 2d). Interestingly, sera that were depleted for antibodies targeting either peptides S14P5, S21P2, or S14P5+S21P2 led to a significantly reduced ability to neutralize SARS-CoV-2 pseudovirus infection, as compared to the non-depleted sera controls (Figure 2e).

Our results demonstrate that the two B-cell linear epitopes identified in this study are immunodominant. Depletion assays functionally validated that antibodies targeting S14P5 and S21P2 peptides possess significant neutralizing roles against

127 SARS-CoV-2 pseudotyped lentiviruses. Importantly, we also assessed the potential
128 presence of mutations in the peptide regions and found a low rate of mutations for
129 S14P5 and S21P2 with low to moderate impact on the viral sequence (2 and 24
130 mutations out of 2596 viral genome sequences respectively, supplementary table 3)
131 [14]. These results are essential to guide the design and evaluation of efficient and
132 specific serological assays, as well as help prioritize vaccine target designs during
133 this unprecedented crisis.

Acknowledgements

Authors would like to thank Professor Yee-Joo Tan (Department of Microbiology, NUS) who kindly provided CHO-ACE2 cells and pXJ3'-S plasmid, and Assoc. Prof. Brendon Hanson (Defence Science Organization National Laboratories) who kindly provided pTT5LnX-CoV-SP plasmid. We would also like to thank the study participants who donated their blood samples to this project, and the healthcare workers who are caring for patients with COVID-19.

Author contributions

CMP, GC, SNA, CYPL conceptualized, designed, acquired, analyzed, interpreted the data and wrote the manuscript. BW acquired, analyzed, interpreted the data and wrote the manuscript. RSLC, WHL and NKWY acquired and analyzed the data. YSL, MICC, SYT, LC, SK, SYT, BEY, and DCL designed and supervised sample collection. CIW, LR, LFPN conceptualized, designed, analyzed and wrote the manuscript. All authors revised and approved the final version of the manuscript.

Competing interests

The authors declare no conflict of interest.

Funding

This work was supported by core research grants provided to Singapore Immunology Network by the Biomedical Research Council (BMRC), and by the A*ccelerate GAP-funded project (ACCL/19-GAP064-R20H-H). Subject recruitment and sample collection was funded by the National Medical Research Council (NMRC) COVID-19 Research fund (COVID19RF-001).

159 **References**

- 160 1. Cohen J, Normile D. New SARS-like virus in China triggers alarm. *Science*.
161 2020;367(6475):234-5. doi: 10.1126/science.367.6475.234. PubMed PMID: 31949058.
- 162 2. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM
163 structure of the 2019-nCoV spike in the prefusion conformation. *Science*.
164 2020;367(6483):1260-3. doi: 10.1126/science.abb2507. PubMed PMID: 32075877.
- 165 3. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veasler D. Structure,
166 Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*. 2020. doi:
167 10.1016/j.cell.2020.02.058. PubMed PMID: 32155444.
- 168 4. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage
169 for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol*. 2020. doi:
170 10.1038/s41564-020-0688-y. PubMed PMID: 32094589.
- 171 5. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al.
172 SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically
173 Proven Protease Inhibitor. *Cell*. 2020. doi: 10.1016/j.cell.2020.02.052. PubMed PMID:
174 32142651.
- 175 6. Lv H, Wu NC, Tsang OT-Y, Yuan M, Perera RAPM, Leung WS, et al. Cross-reactive
176 antibody response between SARS-CoV-2 and SARS-CoV infections. *bioRxiv*.
177 2020:2020.03.15.993097. doi: 10.1101/2020.03.15.993097.
- 178 7. Grifoni A, Sidney J, Zhang Y, Scheuermann RH, Peters B, Sette A. A Sequence
179 Homology and Bioinformatic Approach Can Predict Candidate Targets for Immune
180 Responses to SARS-CoV-2. *Cell Host Microbe*. 2020. doi: 10.1016/j.chom.2020.03.002.
181 PubMed PMID: 32183941.
- 182 8. Zheng M, Song L. Novel antibody epitopes dominate the antigenicity of spike
183 glycoprotein in SARS-CoV-2 compared to SARS-CoV. *Cell Mol Immunol*. 2020. doi:
184 10.1038/s41423-020-0385-z. PubMed PMID: 32132669.
- 185 9. Ahmed SF, Quadeer AA, McKay MR. Preliminary Identification of Potential Vaccine
186 Targets for the COVID-19 Coronavirus (SARS-CoV-2) Based on SARS-CoV Immunological
187 Studies. *Viruses*. 2020;12(3). doi: 10.3390/v12030254. PubMed PMID: 32106567.
- 188 10. Madu IG, Roth SL, Belouzard S, Whittaker GR. Characterization of a highly
189 conserved domain within the severe acute respiratory syndrome coronavirus spike protein S2
190 domain with characteristics of a viral fusion peptide. *J Virol*. 2009;83(15):7411-21. doi:
191 10.1128/JVI.00079-09. PubMed PMID: 19439480; PubMed Central PMCID:
192 PMCPMC2708636.
- 193 11. Alsaadi EAJ, Neuman BW, Jones IM. A Fusion Peptide in the Spike Protein of
194 MERS Coronavirus. *Viruses*. 2019;11(9). doi: 10.3390/v11090825. PubMed PMID:
195 31491938; PubMed Central PMCID: PMCPMC6784214.
- 196 12. Xia S, Yan L, Xu W, Agrawal AS, Algaissi A, Tseng CK, et al. A pan-coronavirus
197 fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Sci Adv*.
198 2019;5(4):eaav4580. doi: 10.1126/sciadv.aav4580. PubMed PMID: 30989115; PubMed
199 Central PMCID: PMCPMC6457931.
- 200 13. Tian X, Li C, Huang A, Xia S, Lu S, Shi Z, et al. Potent binding of 2019 novel
201 coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody.
202 *Emerg Microbes Infect*. 2020;9(1):382-5. doi: 10.1080/22221751.2020.1729069. PubMed
203 PMID: 32065055; PubMed Central PMCID: PMCPMC7048180.
- 204 14. Zhao WM, Song SH, Chen ML, Zou D, Ma LN, Ma YK, et al. The 2019 novel
205 coronavirus resource. *Yi Chuan*. 2020;42(2):212-21. doi: 10.16288/j.yczz.20-030. PubMed
206 PMID: 32102777.

207

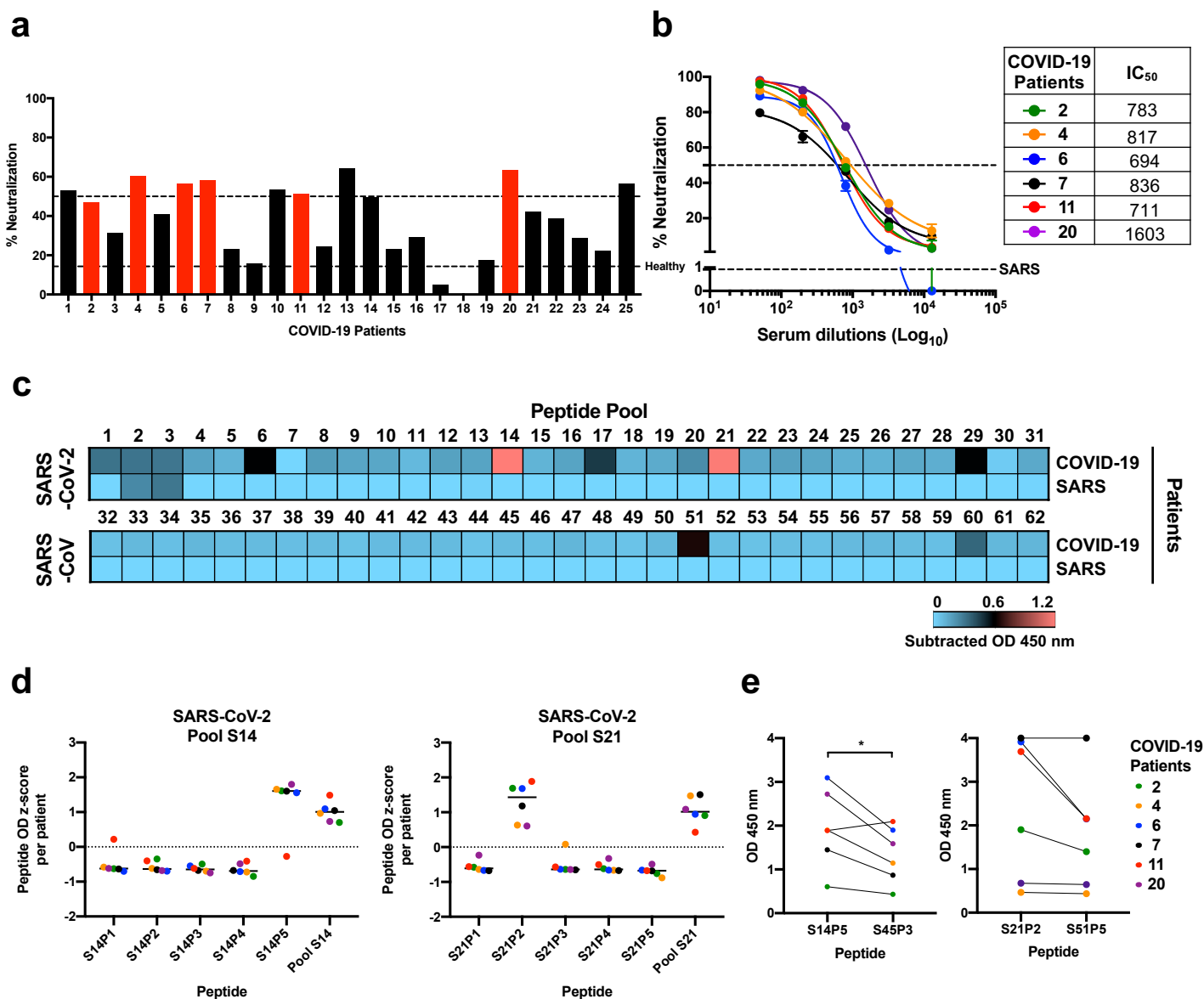


Figure 1. COVID-19 patients elicit neutralizing and specific antibody response against SARS-CoV-2. (a-b) Sera of COVID-19 patients (n=25) were mixed with pseudotyped lentiviruses expressing SARS-CoV-2 spike (S) glycoprotein protein, prior to incubation with CHO-ACE2 cells for 48 h. Infection levels were determined by luciferase assay, and percentage of infected cells were analyzed. Healthy control and recovered SARS patients (n=5) were also conducted in parallel. **(a)** Percentage of virus neutralization at 1:1000 sera dilution. IC₅₀ titer is the reciprocal of sera dilution at which half-maximal neutralization was observed (dotted lines). **(b)** Dose-response neutralization kinetics from sera (1:50 to 1:12800 dilutions) of selected COVID-19 patients (n=6). Dotted lines indicate 50% neutralization or percentage neutralization of SARS patients (n=5) at 1:100 sera dilution. **(c)** Preliminary mapping of linear B cell epitopes within SARS-CoV-2 and SARS-CoV S protein. Sera of COVID-19 (n=6) and SARS patients (n=5) at 1:1000 dilution were subjected to peptide-based ELISA for IgG using peptide pools of the S protein of SARS-CoV-2 (pools S1-31) and SARS-CoV (pools S32-62) in duplicates. Sera of pooled healthy donors (n=13) were carried out in parallel. Data were normalized against healthy controls and the subtracted OD values are presented in a heat-map. Blue and pink indicate low and high OD values, respectively. **(d-e)** Determination of SARS-CoV-2 specific and pan-CoV linear B cell epitopes on S protein. **(d)** Sera of COVID-19 patients (n=6) were subjected to peptide-based ELISA for IgG detection using individual peptides of SARS-CoV-2 S peptide pools 14 and 21. The z-score values of patient were calculated using the formula $[\text{value} - \text{average (OD value of patient)}] / \text{standard deviation (OD value of patient)}$. Data shown are from two independent experiments and presented as mean. **(e)** Peptide binding response of COVID-19 patients on SARS-CoV-2 peptides S14P5 and S21P2, and the corresponding regions on SARS-CoV peptides S45P3 and S51P5, respectively. Statistical analysis was carried out with paired parametric two-tailed t-test (* $p < 0.05$).

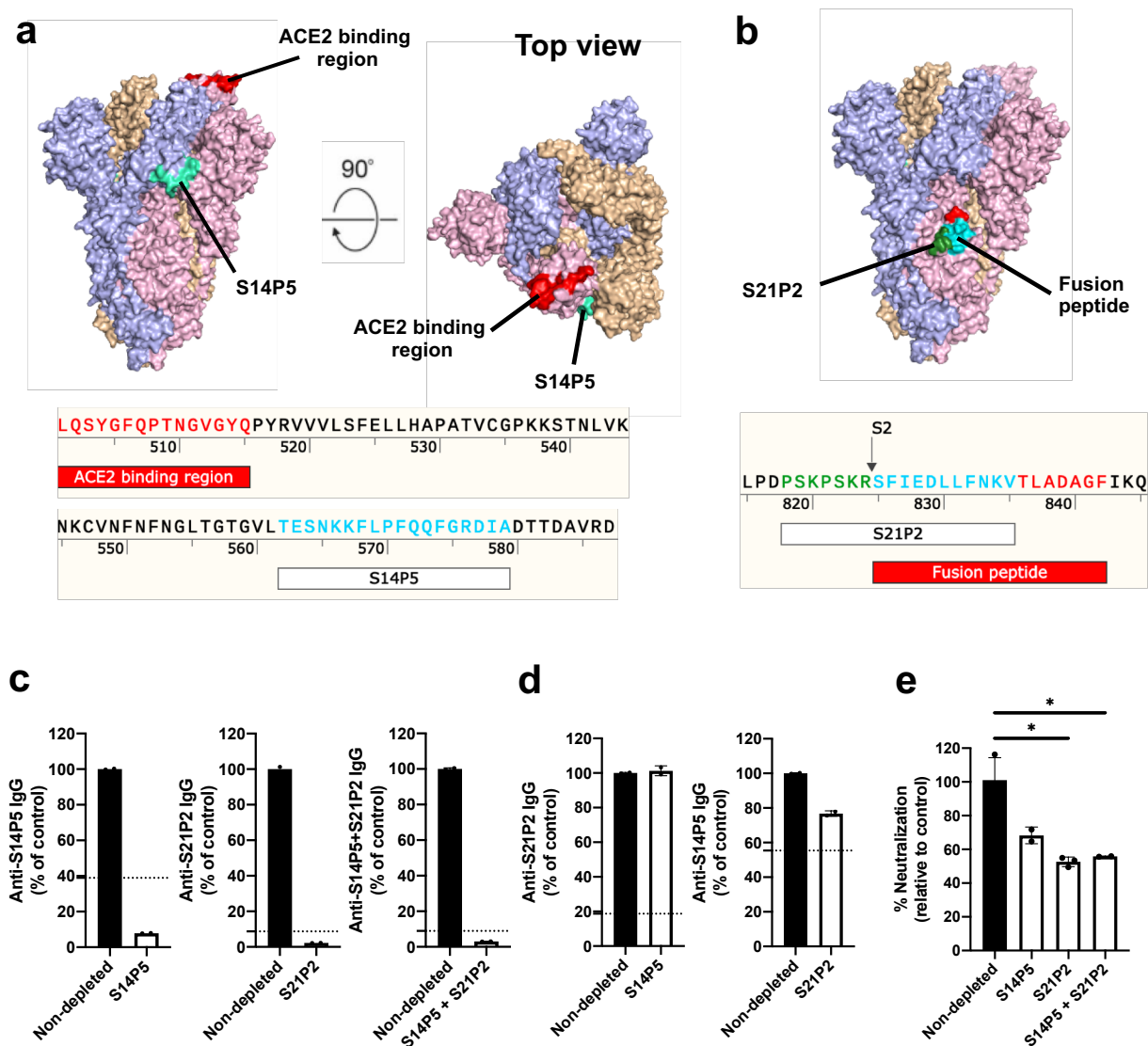


Figure 2. Antibodies against S14P5 and S21P2 linear B-cell epitopes neutralize SARS-CoV-2. (a-b) Localization and sequences of (a) SARS-CoV-2 specific S14P5 and (b) pan-CoV S21P2 epitopes on spike (S) protein (PDB: 6VSB) are shown. Each S monomer is denoted as either pink, blue or orange. (c-e) Pooled sera of COVID-19 patients (n=6) were added to plates coated with the corresponding peptides to deplete specific antibodies. (c) Non-depleted and peptide-specific antibody-depleted sera were then subjected to peptide-based ELISA of the peptide (d) or were cross-assayed. Data of depleted sera (white) were normalized to percentages of the non-depleted sera (black). Signal from pooled healthy donor sera were displayed as black dotted lines and data are shown as mean $\pm$ SD. (e) Non-depleted and peptide-specific antibody-depleted pooled sera were then mixed with SARS-CoV-2 pseudovirus before incubating with CHO-ACE2 cells for 48 h. The percentage of neutralization against pseudovirus infection, relative to the non-depleted sera, are shown. Data are presented as mean $\pm$ SD, and signal from pooled healthy donor sera were displayed as black dotted lines. Statistical analysis was carried out with one-sample t test (* p <0.05, ** p <0.01).