Mapping the Immunodominance Landscape of SARS-CoV-2 Spike Protein for the Design of Vaccines against COVID-19

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Abstract

The ongoing coronavirus disease 2019 (COVID-19) pandemic is a serious threat to global public health, and imposes severe burdens on the entire human society. The severe acute respiratory syndrome (SARS) coronavirus-2 (SARS-CoV-2) can cause severe respiratory illness and death. Currently, there are no specific antiviral drugs that can treat COVID-19. Several vaccines against SARS-CoV-2 are being actively developed by research groups around the world. The surface S (spike) protein and the highly expressed internal N (nucleocapsid) protein of SARS-CoV-2 are widely considered as promising candidates for vaccines. In order to guide the design of an effective vaccine, we need experimental data on these potential epitope candidates. In this study, we mapped the immunodominant (ID) sites of S protein using sera samples collected from recently discharged COVID-19 patients. The SARS-CoV-2 S protein-specific antibody levels in the sera of recovered COVID-19 patients were strongly correlated with the neutralising antibody titres. We used epitope mapping to determine the landscape of ID sites of S protein, which identified nine linearized B cell ID sites. Four out of the nine ID sites were found in the receptor-binding domain (RBD). Further analysis showed that these ID sites are potential high-affinity SARS-CoV-2 antibody binding sites. Peptides containing two out of the nine sites were tested as vaccine candidates against SARS-CoV-2 in a mouse model. We detected epitope-specific antibodies and SARS-CoV-2-neutralising activity in the immunised mice. This study for the first time provides human serological data for the design of vaccines against COVID-19.

Introduction

The coronavirus disease 2019 (COVID-19), a novel infectious disease caused by severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, first emerged in December 2019 and has since become a worldwide pandemic. COVID-19 infections have so far led to over 2 million cases and more than 0.13 million deaths across more than 200 countries and geographical regions around the world. The World Health Organization has launched a worldwide clinical trial called SOLIDARITY to test antiviral treatment options for the novel coronavirus, but as of April 15, 2020, there are no specific drugs for treating COVID-19[1]. Many research teams worldwide are racing to develop vaccines against SARS-CoV-2 to combat this novel coronavirus pandemic.

Similar to other coronaviruses, SARS-CoV-2 is an enveloped positive-stranded RNA virus with four major structural proteins: S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins[2-4]. The surface S protein is the key that allows SARS-COV-2 to enter into cells[5], as it plays a role in binding to the cellular receptor and membrane fusion[5,6]. The SARS-CoV-2 shares 75.96% homology with the 2003 SARS-CoV[3], and the structure and functional domains of the SARS-CoV-2 S protein have already been identified[7]. The receptor-binding domain (RBD) within the S1 domain binds to angiotensin-converting enzyme 2 (ACE2) to facilitate the entry of SARS-CoV-2 into host cells[6,7].

As of April 8, 2020, there are at least 115 vaccine candidates in active development worldwide[8]. The SARS-CoV-2 S (spike) glycoprotein is the immunogen that is the focus of the majority of the vaccine research. In the absence of *in vivo* and *in vitro* data, researchers have been using *in silico* data based on the IEDB database or other online epitope prediction algorithms[9-14]. However, the *in silico* data is less likely to be useful for vaccine development, because these bioinformatics tools were not optimised for vaccine design. Furthermore, the antigenic properties of S proteins remain elusive, and therefore experimental immunogenic information is urgently needed.

Although a putative epitope that binds to antibodies against SARS-CoV-2 has been reported[15], more epitopes with high affinity need to be identified. Information on these epitopes would be useful, particularly in regard to human immunological bias

and immunodominance (ID), which might restrict the immune response. Immunodominance is the phenomenon of immunogenic variation among distinct immunogens or antigenic sites on the same immunogen, which has been demonstrated in both $CD8^+$ T cells and B cells[16]. This phenomenon has restricted the development of effective vaccines for influenza A and other highly variable viruses[17-19]. All the methods used to overcome or manipulate these viral immunity restrictions rely on the landscape of immunodominance. It is therefore crucial to map the immunogenicity landscape of the potential epitopes of the S protein to accelerate the development of vaccines.

An ideal vaccine against SARS-CoV-2 should induce highly potent neutralising antibodies but should not induce any disease-enhancing antibodies. Previous studies that used the full-length S protein of SARS-CoV to immunise animals resulted in adverse effect after the animals were challenged with SARS-CoV[20]. Jiang et al., identified five linear immunodominant (ID) sites in the S protein that did not induce neutralising antibodies. On the contrary, the RBD was found to contain the major neutralising epitopes in the S protein, but did not have any ID sites[21].

In this study, we aimed to map the landscape of ID sites within the S protein of SARS-CoV-2 and compare these results with those of its counterpart in SARS-CoV. Unlike SARS-CoV, we found the RBD of SARS-CoV-2 had linear ID sites in sera samples collected from recently discharged COVID-19 patients. Subsequent microneutralisation tests demonstrated that mice immunised with peptides containing the ID sites within RBD produced neutralising antibodies. Taken together, the RBD of SARS-CoV-2 has a different ID landscape to its counterpart in SARS-CoV. Furthermore, these data can provide guidance on the design of S protein-based SARS-CoV-2 vaccines that might avoid adverse reactions or disease-enhancing effects.

Materials and Methods

Serum specimens from COVID-19 patients

Serum samples were collected from 39 patients with COVID-19 (22 males and 17 females) between Jan 26, 2020, and March 18, 2020. Twenty-six patients who were discharged from hospital provided written informed consent under UW 13-265. The other 13 hospitalized patients were sampled under UW 13-372, and written informed consent was waived. Average sampling time from the onset date was 28.7 days (range, 14–44 days). The median age of patients was 59.7 years (range, 26-80 years). All patients were diagnosed with COVID-19 by PCR test, and two patients had a severe illness. The diagnostic criteria for SARS-CoV infection followed the clinical description of COVID-19[22]. The initial laboratory confirmation was performed on nasopharyngeal or sputum specimens at the Public Health Laboratory Centre of Hong Kong. The discharge criteria were clinically stable and negative nucleic acid test twice consecutively (sampling interval ≥ 24 hours). Six healthy donors were also sampled under UW 19-470. This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 13-372, UW 13-265, and UW 19-470).

Animal experiments

The SPF BALB/c mice were supplied by the Laboratory Animal Unit of the University of Hong Kong. All animal experiments were approved by the Committee on the Use of Live Animals in Teaching & Research, the University of Hong Kong (CULATR 5312-20). The recombinant S protein RBD or peptide was formulated at a ratio of 9:1 with Aluminium hydroxide gel (Alhydrogel, AHG, InvivoGen). These treatments were administered to mice on day 0 and 14. Blood samples were drawn from the tail vein on days 21.

Synthesis of peptides

All peptides were manufactured by GL Biochem (Shanghai) Ltd in the form of a dry powder. The peptides were generated using solid phase synthesis methods and the quality of the products was monitored by mass spectrometry. All peptides were dissolved in pH 7.4 PBS buffer or 8 M pH 7.0 urea Na_2HPO_4/NaH_2PO_4 buffer. Peptides conjugated to keyhole limpet haemocyanin (KLH) carrier proteins were also purchased from GL Biochem (Shanghai) Ltd. These peptide-conjugated proteins were

137 dissolved in a pH 7.4 PBS buffer.

138 **Antibody detection using ELISA**

139 Epitope-specific antibodies were detected by enzyme-linked immunosorbent assay
140 (ELISA). Briefly, all peptides or recombinant proteins at a final concentration of 0.5
141 μg/mL in 50 mM pH 9.6 $Na_2CO_3/NaHCO_3$ buffer were coated on ELISA plates
142 (Nunc, Roskilde, Denmark) and incubated overnight at 4°C. Plates were blocked with
143 TBS-5% (w/v) non-fat milk for 3 h at 37°C and washed four times in 0.05% Tween in
144 TBST. Diluted patient or mice sera were added into the wells and incubated for 1 h at
145 37°C. Plates were washed six times in TBS-0.05% Tween and incubated with HRP-
146 conjugated goat anti-mouse IgG (ThermoFisher, Catalog # 31410), or Goat anti-
147 Human IgM (ThermoFisher, Catalog # A18841), or Goat anti-Human IgA
148 (ThermoFisher, Catalog # A18781) for 1 h at 37°C. The colour was developed using
149 Trimethyl Borane (TMB) solution (Sigma) and absorbance was measured at 450 nm
150 using an ELISA reader. Samples from non-immunised mice or healthy volunteers
151 were used as the controls. The cut-off lines were based on the mean value plus three
152 times the standard deviation.

153 **Identification of T cell epitopes using ELISpot**

154 The T cell responses were detected by enzyme-linked immunosorbent spot (ELISpot)
155 kits (Dakewe Biotech Co., Ltd) following the manufacturer's protocol. Briefly,
156 splenocytes harvested from sacrificed mice were washed and immediately transferred
157 to anti-IFN-γ antibody pre-coated filter plates. For stimulation, splenocytes were co-
158 incubated with distinct epitopes overnight at 37℃. All samples were assayed with
159 positive controls (Phorbol myristate acetate, PMA and Ionomycin) and cells from a
160 reference donor. All images in different wells were captured using a CTL
161 ImmunoSpot ELISpot Analyzer and processed using the ImmunoCapture software
162 (Cellular Technology Ltd., USA).

163 **Microneutralisation tests**

164 Serial two-fold dilutions of heat-inactivated sera (treated at 56ºC for 30 minutes) were
165 prepared from a starting dilution of 1:10. The serum dilutions were mixed with equal
166 volumes of 100 TCID50 of SARS-CoV-2 as indicated. After 1 h of incubation at 37ºC,
167 35 μL of the virus–serum mixture was added to Vero-E6 cell monolayer for SARS-
168 CoV-2 infection in 96-well microtitre plates in quadruplicate. After 1 h of adsorption,

169 an additional 150 μL of culture medium was added to each well and incubated for 3
170 days at 37ºC in 5% CO2 in a humidified incubator. A virus back-titration was
171 performed without immune serum to assess the input virus dose. The CPE was read at
172 3 days post infection. The highest serum dilution that completely protected cells from
173 CPE in half the wells was estimated using the Reed-Muench method and was taken as
174 the neutralising antibody titre. Positive and negative control sera were included to
175 validate the assay.

176 **Statistical analysis**

177 ELISA data were collected by Thermo Scientific VARIOSKAN FLASH 3001
178 (Ref:5250040). ELISpot assay results were captured using a CTL ImmunoSpot
179 ELISpot Analyzer and processed using the ImmunoCapture software (Cellular
180 Technology Ltd., USA). Data were reported as the median (indicating the range from
181 minimum to maximum value occurred) and arithmetic mean ± standard deviation, as
182 specified (R software package, 3.4.1, Microsoft office 365 Excel). All R-squared and
183 t-test were calculated by t-test using Microsoft office 365 Excel, while all results were
184 plotted by Prism 7 (GraphPad Software Inc., CA).

185

186 **Results**

187 **Immune responses and antiviral effects in serum from COVID-19 patients**

188 All serum samples from COVID-19 patients tested positive for SARS-CoV-2 by
189 ELISA assay using plates coated by SARS-CoV-2 lysates (Figure 1A). The profiles of
190 IgG, and IgM, and IgA against S (spike) and N (nucleocapsid) proteins showed that
191 not all patients had elevated antibody titres compared to healthy donors (Figure 1B-G).
192 Generally, high IgG titres against both S and N proteins were present in the sera,
193 samples, and relatively high IgM and IgA responses were also observed in the
194 majority of patients (Figure S1).

195 Our previous work revealed that the correlations between microneutralisation (MN)
196 activity and anti-NP or anti-RBD IgG titres were stronger than the correlation
197 between MN activity and IgM titre[22]. To further investigate the correlations between
198 MN activity and anti-NP or anti-RBD IgG, IgM, and IgA titres, we carefully analysed
199 the sera from recovered patients. We found MN activity in the sera of 26 out of 39
200 COVID-19 patients. The MN titres were adjusted following previous criteria: MN
201 titres less than 10 were re-designated a value of 5 and MN titres greater than 320 were
202 re-designated a value of 640[22]. We identified a very strong correlation between anti-
203 RBD IgG titres and MN activity in recovered patients (R^2 = 0.8009). The correlations
204 between anti-RBD IgM/IgA titres and MN activity were weaker than for IgG (R^2 =
205 0.5130 and 0.5926, respectively) (Figure 2E, G, I). However, we found very poor
206 correlations between anti-NP IgG/IgM/IgA titres and MN activity. These correlations
207 dropped dramatically when MN activity titres were greater than 1:160 (Figure 2D, F,
208 H). These results suggest that anti-RBD antibodies play an important role in the
209 antiviral immune response.

210

211 **The landscape of immunodominant sites on the S protein in COVID-19 patients**

212 To identify the immunodominant (ID) sites on the SARS-CoV-2 S protein, we
213 mapped the epitopes in 42 peptides spanning the entire extra-membrane domain (21-
214 926) of the S protein with three gaps (106-160, 365-374, and 687-741). Each peptide
215 was between 20 and 25 residues in length with a five-residue overlap. We measured
216 ID sites in terms of the positive rate and the percentage of convalescent sera from
217 COVID-19 patients having positive reactions to the epitope. Here, we used the mean
218 response plus three times the standard deviation in healthy donors as the cut-off value
219 to define positive reactions (Figure S2). The epitope mapping showed nine linear ID
220 sites on the S protein located at 21-45(IDa), 221-245(IDb), 261-285(IDc), 330-
221 349(IDd), 375-394(IDe), 450-469(IDf), 480-499(IDg), 522-646(IDh), and 902-
222 926(IDi), respectively (Figure 3), with an average positive rate of ≥50% among all 39
223 patients. We found the SARS-CoV-2 RBD contained four ID sites, IDd, IDe, IDf, and
224 IDg, whereas the SARS-CoV RBD has no ID sites. Considering the SARS-CoV-2 S
225 protein shares 75.96% identity with the SARS-CoV S protein, we found five out of
226 the nine fragments, IDc (79.17%), IDd (90%), IDe (90%), IDh (79.2%), and IDi (96%)
227 were evolutionarily highly conserved in the SARS-CoV S protein. However, only
228 three of the nine ID sites, IDa (63.64%), IDh (79.05%), and IDi (96%) were highly
229 homologous (>50%) to SARS-CoV S protein ID sites. These results suggest that the
230 conserved regions contribute to the immunogenicity of the S protein, whereas the
231 majority of ID sites (6 out of 9) were less likely to be conserved. Interestingly, some
232 epitopes induced personalized immune responses in specific patients. Patient 33 had
233 an extremely strong immune response to epitope S (21-45) with a 40 times increase
234 compared to the cut-off value, whereas the responses of patients 26 to 31 were not
235 significantly different compared to those of healthy donors. A similar response was
236 also observed for S (281-305), which is less likely to be an ID site. (Figure 3A) These
237 results suggest that some unexpected ID sites of the S protein might lead to highly
238 variable immune responses in patients if immunised with certain viral proteins.

239 **Immunodominant sites can generate antiviral protection in a mouse model**

240 To examine if specific ID sites of the S protein can generate immune responses and
241 antiviral effects, we immunised mice with the RBD and two epitopes (S370-395 and
242 S435-479) of the S protein, which were selected based on the results of B cell epitope
243 prediction, toxicity prediction, and allergenicity prediction (Figure 4A, B). The S370-
244 395 and S435-479 epitopes were validated in our epitope mapping assay in patient
245 sera, which showed positive rates of 51.3% and 74.4%, respectively. The ELISA
246 assay showed that mice immunised with the entire RBD, S370-395, and S435-479
247 generated high levels of specific antibodies (Figure 4C, D, E). In the MN test, mice
248 immunised with S370-395, S435-479 or RBD showed viral neutralising titres of
249 1:26.7, 1:16.7, and 1:33.3, respectively (Figure 4F).

250 The landscape of T cell epitopes in the RBD fragment was profiled by ELISpot assay,
251 which revealed a distinct pattern compared to the B cell responses. In the RBD
252 fragment, three of the T cell epitopes, S405-469, S480-499, and S510-521, induced
253 strong adaptive responses after immunisation. Among the epitopes, S370-395, S450-
254 469 and S480-499 were identified as ID sites in human sera (Figure 4G). The S370-
255 395 and S435-479 epitopes are more likely to be both T cell ID sites and B cell ID
256 sites.

257

258 **Immunodominant sites might reveal potent neutralising sites of S protein**

259 To validate the potential functions of ID sites, we highlighted the ID sites in the
260 SARS-CoV-2 S protein structure model containing glycosylation sites[23] (Figure 5A-
261 C). Among ID sites with positive rates ranging from 50% to 60%, five sites were
262 located on the head region of the S protein and two sites were on the stem of the S
263 protein (Figure 5B). Among ID sites with positive rates over 60%, all five epitopes
264 were located on the head of the S protein (Figure 5C). Although the S protein is a
265 glycoprotein, glycosylation modification had limited effects on immunisation. Two
266 glycosylation sites, N331 and N343, were observed in the RBD region, but these sites
267 had no influence on the immune response. In sera from patients, non-glycosylated
268 peptides were able to induce immune responses (Figure 3A, C and Figure 4G, S330-
269 349).

270 The neutralising titre results (Figure 4F) suggest the ID sites have neutralising
271 activities against SARS-CoV-2, although the ID sites in SARS-CoV were unlikely to
272 be neutralising sites[20]. To further validate the ID sites, we compared the binding
273 residues of the S protein to reported monoclonal antibodies with the ID sites of
274 RBD[15,24,25]. Interestingly, some binding residues of SARS-CoV-2-specific
275 neutralising antibodies were highly similar to the ID sites (Figure 5). For CR3022, a
276 monoclonal antibody targeting a highly conserved cryptic epitope[15], 14 out of 28
277 binding residues of SARS-CoV-2 RBD were located in ID sites. For F26G19, a
278 mouse antibody, 11 out of 20 sites were located at ID sites, indicating there was likely
279 a relatively high binding affinity to the RBD fragment[25]. However, for m396, an
280 antibody with relatively low binding affinity to SARS-CoV-2 RBD, only 5 out of 22
281 binding residues were located at ID sites[24]. This was also the case for R80, another
282 low binding affinity antibody, which had no matching binding residues[24]. These
283 results suggest that SARS-CoV-2 ID sites are potent neutralising sites for high-
284 affinity antibodies.

285 **Discussion**

286 In this study, we profiled IgG/IgM/IgA levels against the S protein and N protein in
287 the sera of COVID-19 patients (Figure 1). All convalescent sera from the COVID-19
288 patients contained specific antibodies against recombinant SARS-CoV-2 N protein,
289 but not all sera had specific antibodies for the RBD fragment of the S protein. The
290 relatively high immunogenicity of SARS-CoV-2 N protein during infection showed it
291 has potential as an antigen for developing COVID-19 diagnostics (Figure 1). However,
292 amounts of the different antibodies varied across patients. We found that IgM
293 contributed 5%-34% of N protein-specific antibodies, whereas anti-RBD IgM
294 contributed 10%-49% of RBD-specific antibodies. Patients with acute SARS-CoV-2
295 infection displayed highly diverse immune responses, and this diversity remained
296 until convalescence. These diverse immune responses highlight safety concerns such
297 as secondary infections that need to be considered.

298 We also analysed the correlation between S or N protein-specific antibody levels and
299 SARS-CoV-2 neutralising titres. The Spike RBD-specific antibody level displayed a
300 strong linear correlation with MN titres, but not with the N-specific antibody level
301 (Figure 2). This observation suggests that RBD-specific antibodies in the sera of
302 recovered patients might provide antiviral protection mainly through neutralising
303 activity rather than non-neutralising antibodies against the N protein. This suggests
304 that manipulating the RBD-induced immune responses might be more effective for
305 developing COVID-19 vaccines.

306 This is the first reported mapping of the landscape of the ID sites in the S protein of
307 SARS-CoV-2 using sera from COVID-19 patients (Figure 3). The personalized
308 immune response pattern needs to be further investigated as unexpected and highly
309 variable immune responses in some individuals might lead to adverse events or
310 disease-enhancing responses to certain viral proteins used as vaccines. We further
311 tested if the ID sites in the RBD fragment could be used as potential vaccines in mice.
312 We found that epitopes/protein-specific antibody titres exceeded the
313 microneutralisation titres by several orders of magnitude. Considering the relatively
314 low neutralising antibody levels in the recovered patients in this study and in a
315 previous report[26], it is reasonable to expect significant differences between specific
316 antibodies and neutralising antibodies. We found there was equivalent antiviral
317 activity from immunisation with epitopes compared to immunisation with the entire

RBD fragment (Figure 4). This provides evidence that epitope-based vaccines could offer comparable protection compared to subunit vaccines, but with the potentially better safety.

We also compared binding residues of previously identified SARS-CoV-2 neutralising antibodies with ID epitopes. The ID sites in the RBD were found to be potent neutralising sites for SARS-CoV-2 (Figure 5). Previous research suggests that it might be better to overcome the immunodominance of non-neutralising antigenic epitopes for SARS-CoV[20,27], influenza virus A[16], dengue fever virus[28], and human immunodeficiency virus[19], which can potentially enhance the disease. Indeed a prior study on SARS-CoV implicated ID sites in antibody-dependent enhancement (ADE)[27,29]. Recent research showed that passive immunisation with early convalescent COVID-19 serum in hamsters resulted in significantly lower viral loads in the respiratory tract without apparent differences in the clinical signs and histopathological changes[30]. Even so, these results provide limited information on ID. We still do not know how to effectively target immunodominance, and we still need to understand the mechanism underlying this phenomenon.

Several candidate vaccines against SARS-CoV-2 including mRNA, inactivated virus, and recombinant adenovirus vaccines have started phase I clinical trials in the US and China[8]. However, there are no reports on the targets of these candidate vaccines, and they might have disease-enhancing effects. Our findings provide evidence for using specific linear antigenic epitopes of S protein instead of the entire S protein for vaccines against SARS-CoV-2 with better safety.

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Author Contributions

BZ Zhang, YF Hu, LL Chen, YG Tong, TCC Yau, KKW To, KY Yuen, and JD Huang designed the study. IFN Hung recruited all the patients. BZ Zhang, YF Hu, KH Chan, and LL Chen performed the experiments. JC Hu, JP Cai, Y Dou, J Deng, HR Gong, C Kuwentrai, WJ Li, XL Wang, H Chu, and CH Su participated in the study. BZ Zhang, YF Hu, LL Chen, YG Tong, TCC Yau, KKW To, KY Yuen, and JD Huang analysed the data. BZ Zhang, YF Hu, and JD Huang wrote the manuscript.

Competing Interests

The authors declare that they have no competing interests.

Data and Materials Availability

All data used to draw the conclusions in the paper are presented in the paper and/or the supplementary materials.

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440 **Figure Legends and Table**

441 **Figure 1. Detection of antibodies specific for SARS-CoV-2 proteins in early**
442 **convalescent sera from COVID-19 patients by ELISA.** (A) Total proteins from
443 SARS-CoV-2 lysates were used as the coated antigen. (B-D) The recombinant N
444 protein was used as the coated antigen. (E-G) The recombinant SP_RBD protein was
445 used as the coated antigen. (H-I) Antibody isotyping of N and SP_RBD binding
446 antibodies in early convalescent sera from COVID-19 patients. Sera from 39 COVID-
447 19 patients, and 6 healthy blood donors were tested at a dilution of 1:100. The dashed
448 lines represent cut-off values (the mean absorbance at 450 nm of sera from healthy
449 blood donors plus three times the standard deviation).

450

451 **Figure 2. Correlations between S or N protein-specific antibody titres and**
452 **microneutralisation antibody titres.** (A-C) N protein or RBD fragment of S protein-
453 specific IgG levels and microneutralisation (MN) assay results of recovered patients'
454 antibody titres. (D-E) Correlation between N protein or RBD fragment of S protein-
455 specific IgG levels and microneutralisation antibody titres. (F-G) Correlation between
456 N protein or RBD fragment of S protein-specific IgM levels and microneutralisation
457 antibody titres. (H-I) Correlation between N protein or RBD fragment of S protein-
458 specific IgA levels and microneutralisation antibody titres.

459

460 **Figure 3. Epitope landscape of SARS-CoV-2 S proteins in convalescent sera from**
461 **COVID-19 patients by ELISA.** (A) The landscape of adjusted epitope-specific
462 antibody levels in each patient. The ELISA results of absorbance at 450 nm were
463 normalized to the aforementioned cut-off values. (B) Schematic representation of
464 SARS-CoV-2 S protein and identified immunodominant sites. Here, only epitopes
465 with positive rates greater than 50% are immunodominant. (C) Positive rates of
466 distinct epitopes of SARS-CoV-2 S protein.

467

468 **Figure 4. B Cell and T Cell responses to distinct epitopes in SARS-CoV-2**
469 **immunised mice.** (A-B) Schematic representation of the mouse immunisation
470 schedule. Balb/C mice (n = 5 per group) were immunised subcutaneously (s.c) with
471 25 μg of rRBD and KLH-conjugated peptides S370-395 and S435-479 mixed with

472 aluminium hydroxide gel (AHG). (C-E) rRBD-specific antibody responses in
473 immunised mice. rRBD-specific IgG antibody responses in mouse sera collected at 7
474 days after the second vaccination. (F) Virus microneutralisation antibody titres were
475 measured against SARS-CoV-2 in classical BSL3. (G) Number of IFN-γ-secreting
476 splenocytes in response to stimulation with the 12 RBD peptide pools of 20-mer
477 peptides. SFU: spot-forming units. Student's t-test was used for the statistical analysis.
478 Data was expressed as mean ± SD.

479

480 **Figure 5. Structural representation of immunodominant and potential**
481 **neutralising sites in SARS-CoV-2 RBD.** (A) Top view and side view of the S (spike)
482 protein in trimer form with labelled RBD region and glycosylation sites. (B) Top view
483 and side view of the S (spike) protein with immunodominant sites with positive rates
484 ranging from 50% to 60%. (C) Top view and side view of the S (spike) protein with
485 immunodominant sites with positive rates greater than 60%. (D) Amino acid sequence
486 alignment of SARS-CoV-2 and SARS-CoV RBD sequences. Related neutralising
487 antibody binding sites, ACE2 binding sites, prominent alterations, and
488 immunodominant sites of SARS-CoV-2 and SARS-CoV are shown.

489

Figure 1

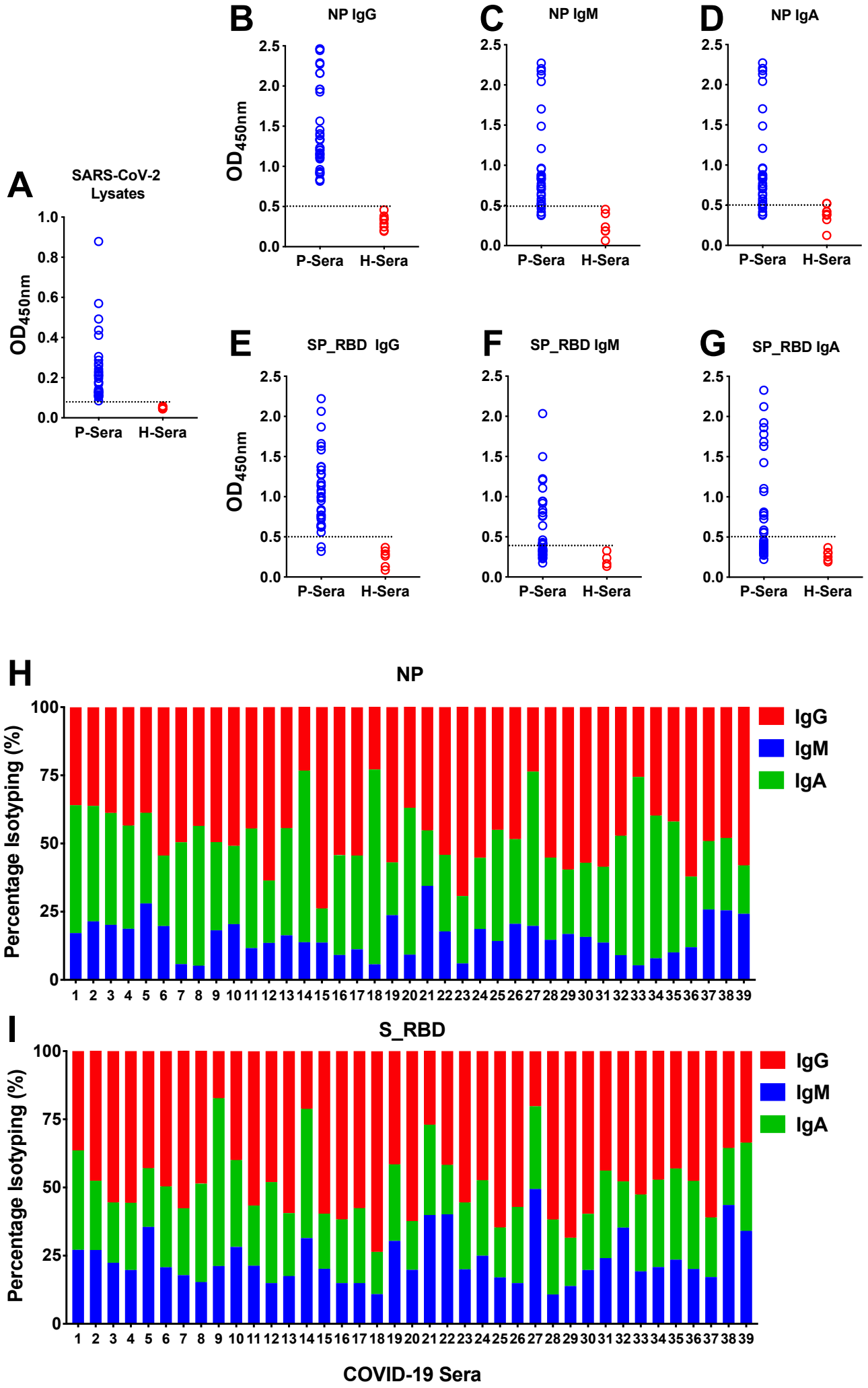

A
SARS-CoV-2 Lysates
OD450nm
P-Sera
H-Sera
B
NP IgG
C
NP IgM
D
NP IgA
E
SP_RBD IgG
F
SP_RBD IgM
G
SP_RBD IgA
H
NP
I
S_RBD
Percentage Isotyping (%)
IgG
IgM
IgA
COVID-19 Sera

Figure 2

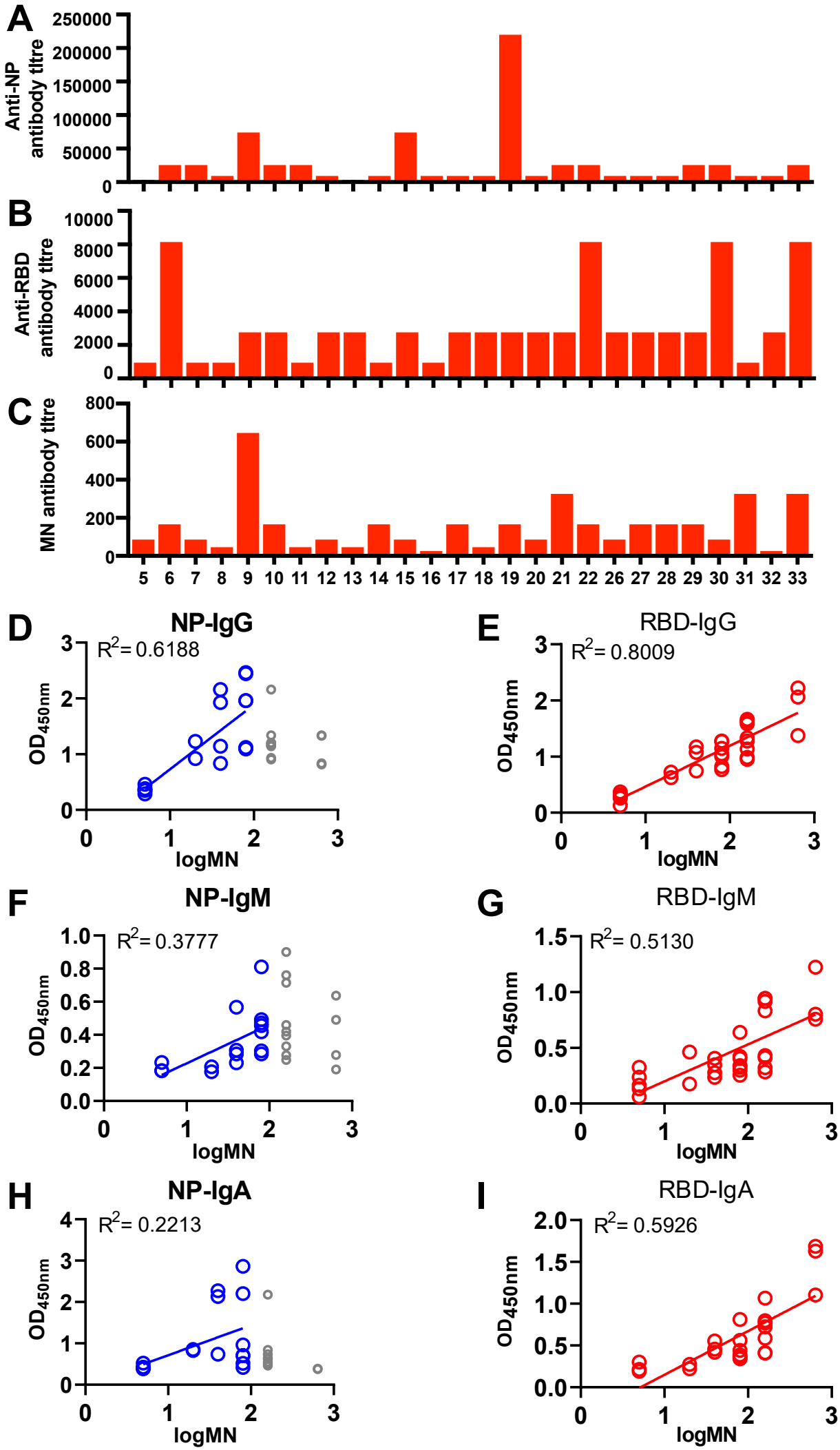

A
Anti-NP antibody titre
250000
200000
150000
100000
50000
0
B
Anti-RBD antibody titre
10000
8000
6000
4000
2000
0
C
MN antibody titre
800
600
400
200
0
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 26 27 28 29 30 31 32 33
D
NP-IgG
R2= 0.6188
OD450nm
logMN
E
RBD-IgG
R2= 0.8009
OD450nm
logMN
F
NP-IgM
R2= 0.3777
OD450nm
logMN
G
RBD-IgM
R2= 0.5130
OD450nm
logMN
H
NP-IgA
R2= 0.2213
OD450nm
logMN
I
RBD-IgA
R2= 0.5926
OD450nm
logMN

Figure 3

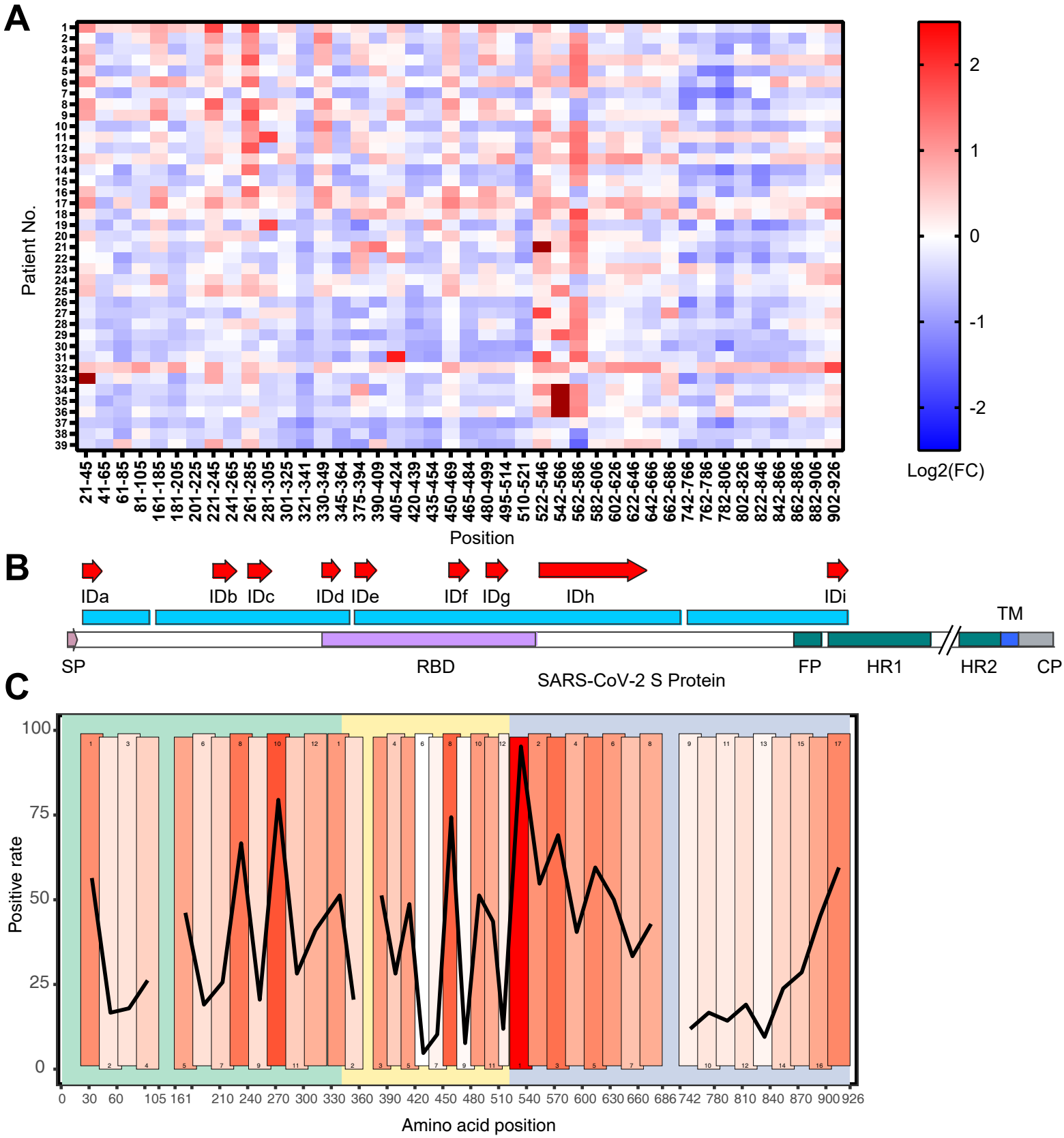
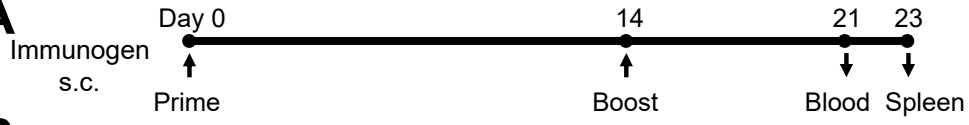

A
Patient No.
Position
Log2(FC)
2
1
0
-1
-2
B
IDa
IDb
IDc
IDd
IDe
IDf
IDg
IDh
IDi
TM
SP
RBD
FP
HR1
HR2
CP
SARS-CoV-2 S Protein
C
Positive rate
Amino acid position

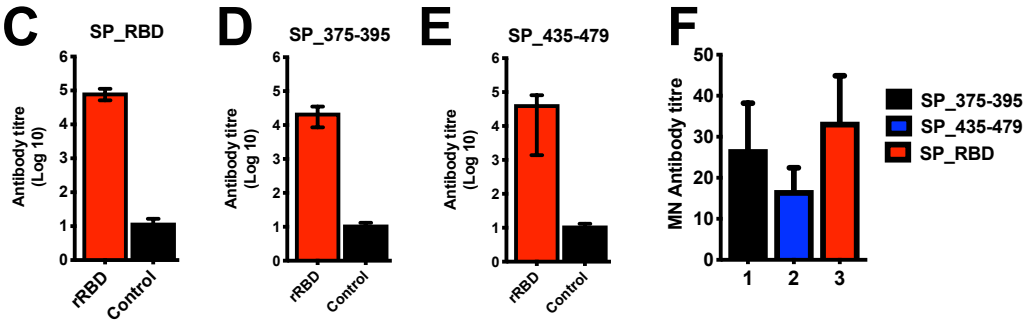
Figure 4

A



B

Group	Dose	Adjuvant
rRBD	25 µg	AHG
S370-395 - KLH	25 µg	AHG
S435-479 - KLH	25 µg	AHG
PBS buffer	-	AHG



G

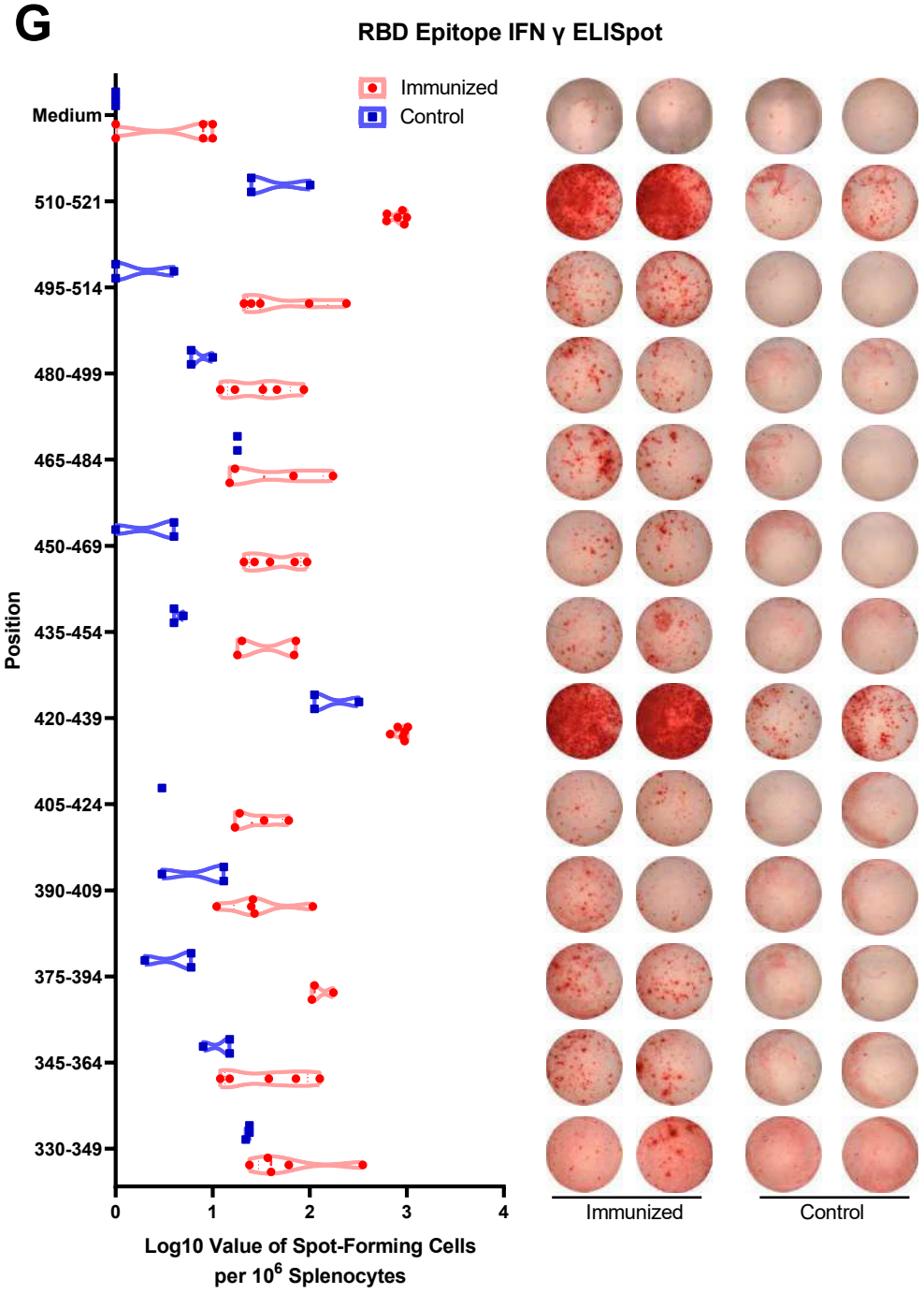
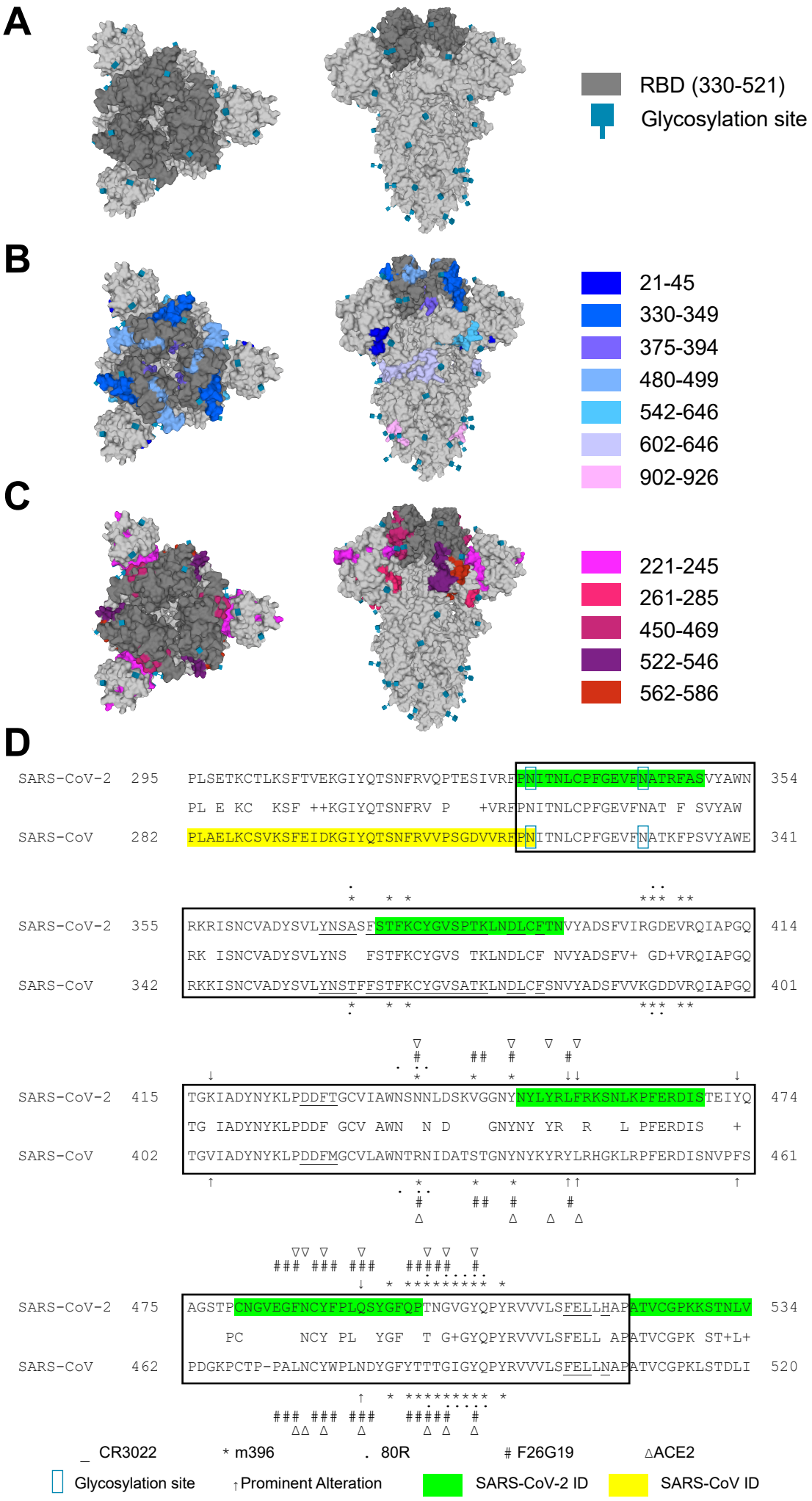


Figure 5


A
RBD (330-521)
Glycosylation site
B
21-45
330-349
375-394
480-499
542-646
602-646
902-926
C
221-245
261-285
450-469
522-546
562-586
D
SARS-CoV-2 295 PLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWN 354
PL E KC KSF ++KGIYQTSNFRV P +VRFPNITNLCPFGEVFNAT F SVYAW
SARS-CoV 282 PLAELKCSVKSFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWE 341
SARS-CoV-2 355 RKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQ 414
RK ISNCVADYSVLYNS FSTFKCYGVS TKLNDLCF NVYADSFV+ GD+VRQIAPGQ
SARS-CoV 342 RKKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQ 401
SARS-CoV-2 415 TGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQ 474
TG IADYNYKLPDDF GCV AWN N D GNYNY YR R L PFERDIS +
SARS-CoV 402 TGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFS 461
SARS-CoV-2 475 AGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLV 534
PC NCY PL YGF T G+GYQPYRVVVLSFELL APATVCGPK ST+L+
SARS-CoV 462 PDGKPCTP-PALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLI 520
_ CR3022
* m396
. 80R
F26G19
ΔACE2
Glycosylation site
Prominent Alteration
SARS-CoV-2 ID
SARS-CoV ID