SARS-CoV-2 antibodies cross-react and enhance dengue infection

Kamini Jakhar[†], Sudipta Sonar[†], Gagandeep Singh, Tania Sarkar, Mahima Tiwari, Jaskaran Kaur, Deepak Kumar Rathore, Banwari Lal, Sandeep Kumar, Puneet Srivastav, Satendra Kumar, Vikas Phagna, Lokesh Kumar, Vishal Gupta, Pallavi Kshetrapal, Savita Singh, Nitya Wadhwa, Ramachandran Thiruvengadam, Sreevatsan Raghavan, Mudita Gosain, Tripti Shrivastava, Sankar Bhattacharyya, Jayanta Bhattacharya, Shailendra Asthana and Shailendra Mani[*]

† Contributed equally

*Corresponding author

Corresponding author: Dr Shailendra Mani PhD, Translational Health Science and Technology Institute, Faridabad, NCR Delhi, India +91-129-2876478

Email: shailendramani@thsti.res.in

Author affiliations:

Translational Health Science and Technology Institute, Faridabad, Haryana, India-120001

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Abstract

Dengue disease is highly prevalent in tropical and subtropical regions worldwide. However, its pathogenesis is still incompletely understood, particularly in comparison to other endemic viruses. Antibody-dependent enhancement (ADE) is a well-known phenomenon for dengue viruses. Given the recent surge in dengue cases and potential cross-reactivity with SARS-CoV-2 antibodies, this study explores the impact of anti-SARS-CoV-2 antibodies on DENV-2 infection.

The study assessed the cross-reactivity of SARS-CoV-2 antibodies with the DENV-2 Virus. Human convalescent plasma samples collected during different waves of COVID-19 and monoclonal and polyclonal antibodies raised against SARS-CoV-2 were examined for their potential to cause ADE of DENV-2 infection using cell-based assays. The study found that anti-SARS-CoV-2 antibodies acquired from natural infection in humans or through experimental immunization in animals were cross-reactive with DENV-2 and had the potential to enhance DENV-2 infection in K562 and U937 cells. In-silico and in-vitro studies indicated a strong interaction between SARS-CoV-2 antibodies and DENV-2 E-protein, providing a molecular basis for these findings. This study is the first to demonstrate that anti-SARS-CoV-2 antibodies can cross-react with DENV-2 and can enhance its infection through ADE. These findings have implications for SARS-CoV-2 vaccine development and deployment strategies in regions where dengue is endemic.

Summary:

Antibodies against SARS-CoV-2 (RBD and Spike) showed significant cross reactivity with DENV-2 (E protein). Also, anti-SARS-CoV-2- commercial antibodies, immunised animal sera

and 46 human convalescent plasma samples (from different waves of pandemic) demonstrated antibody-dependent enhancement (ADE) of DENV-2 infection.

Keywords; SARS-CoV-2; Cross-reactivity; Dengue; ADE; Vaccine-development.

Introduction

Dengue virus (DENV) is a positive-sense single-stranded RNA virus with a genome size of ~11kb belonging to the *Flaviviridae* family and causes the most prevalent mosquito-borne disease called dengue. Dengue is endemic to more than 120 countries with Asia contributing 70% of the global burden. The COVID-19 pandemic has had a substantial impact on Dengue cases globally with many countries reporting significant a surge since 2020. In 2021, prevalence of dengue has increased over three times in India (44,585 versus 193,245 cases), 19 times in Bangladesh (1,405 versus 28,429 cases), 2 times in Singapore (5,000 versus 11,000), over 1.8 times in Malaysia (87,000 versus 120,000 cases), and more than seven times (6,016 vs. 52,894 instances) in Pakistan. Taiwan, Sri Lanka, Mexico, and the Philippines have also reported notable increase, while Brazil documented a surge in severe cases and fatalities predominantly impacting younger population under 20 years of age during the period of 2021-2022 [[1], [2], [3], [4]]. DENV circulates as four serotypes (DENV 1-4) each containing multiple distinct genotypes. Infection with any serotype can lead to mild fever or severe forms like Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Primary infection provides lifelong immunity to homotypic secondary infection but gives partial immunity from heterotypic challenge. Such heterotypic secondary infections positively correlate with severe symptoms, as a consequence of higher viremia through Antibody-dependent Enhancement (ADE). ADE is caused by sub-neutralizing antibodies from previous infection binding to DENV virion and promoting FcR-mediated viral entry into the cells bearing Fc-receptor [5]. ADE occurs when the number of bound antibodies support stable attachment to Fc receptors, without reaching the neutralization threshold [6].

Presently there are multiple reports showing modest serological cross-reactivity between SARS-CoV-2 and DENV-2, significantly impacting dengue diagnostics [7] [8] [9]. However, it is yet unclear if this would have any clinical impact on dengue cases [10]. A study reported

79 IgG produced in rabbits upon immunization with purified SARS-CoV-2 S1-RBD, to cross-react
80 with DENV proteins, particularly the Envelope (E), precursor-Membrane (PrM), and Non-
81 structural protein 1 (NS1). However, these antibodies did not enhance DENV infection (ADE)
82 in THP-1 cells which express FcR[11]. Contrarily a study showed *in-vitro* inhibition
83 of dengue infection by sera from convalescent COVID-19 patients sera.
84 In this pretext, our current study aimed to examine if SARS-CoV-2 antibodies have any impact
85 on DENV-2 infection. To the best of our knowledge, this will be the first study to provide a
86 blueprint as to how SARS-CoV-2 antibodies cross-reactive with DENV-2 (particularly E
87 protein) may enhance dengue infection via ADE, using a combination of experimental and
88 computational studies.
89
90 **Methods:**
91 A detailed material and methods section is given as supplementary data. In brief, we selected a
92 group of convalescent plasma samples (n=48) before the Delta wave collected in India that is
93 15th of April 2021 (n=21), post the delta wave (n=17) and during Omicron wave (n=10). To
94 check if the SARS-CoV-2 antibodies enhance dengue virus infection in-vitro, human myeloid
95 cell lines K562 and U937 were used for the study. Along with the patient samples, a panel of
96 commercially available antibodies and animal sera raised against SARS-CoV-2
97 (Supplementary Table-2) were used. In ADE assay, the 4G2 antibody was used as a positive-
98 control while Rabishield®-100 was used as a Negative-control. ADE assay was performed to
99 obtain the percentage of DENV-2 positive cells by flowcytometry. The cross-reactivity of
100 SARS-CoV-2 antibodies with DENV protein E was tested using in-silico approach where
101 fourteen SARS-CoV-2 antibodies mainly targeting SARS-CoV-2 RBD were chosen. They were
102 docked onto the DENV-2 E-protein using multiple docking programs to choose the best pose.
103 The binding free energy of protein and antibody complexes was calculated in Prime MM-GBSA

104 by Prime module of Schrodinger suite. Biolayer Interferometry (BLI) was performed to study
105 the binding kinetics of two SARS-CoV-2 antibodies with dengue virus envelope and SARS-
106 CoV-2 spike protein. The cross-reactivity was also checked by immunofluorescence assay
107 where C6/36 cells were infected with DENV2 and stained with antibodies and animal sera
108 raised against SARS-CoV-2. Experimental values were represented as mean ± standard
109 deviation (SD). Statistical analysis was done using the ordinary one-way ANOVA followed by
110 Dunnett's multiple comparison test. Differences were considered significant at p value<0·05.
111
112 **Results:**
113 **Antibodies against SARS-CoV-2 cross react with Dengue Virus**
114 Previous studies have suggested considerable serological cross-reactivity between SARS-CoV-
115 2 and DENV. We employed multiple techniques to strengthen such observations. Using
116 confocal microscopy, we observed that the anti-spike polyclonal antibody (P1R) and anti-spike
117 mice serum (MSer) showed a significant cross-reactivity with an average MFI of 12·8 (p-
118 value<0·0001) and 13·4 (p-value<0·0001) (Figure-1A-B) with respect to the Negative-control,
119 Rabishield® whereas, the SARS-CoV-2 monoclonals (1A9 and MM41) also showed cross-
120 reactivity with DENV-2. The positive-control, 4G2 (pan-flavivirus-anti-envelope antibody)
121 showed maximum MFI of 18·28 whereas the Negative-control, anti-Rabies monoclonal
122 (Rabishield®) showed no reactivity with DENV-2. The binding kinetics of 1A9 (SARS-CoV-
123 2 spike antibody) in BLI showed high affinity with a dissociation constant (K_D) of 4·84 nM and
124 22·7 nM with DENV-2 Envelope and SARS-CoV-2 Spike respectively. (Figure-1C ii-iii). C8
125 (DENV-2 Envelope antibody) was used as a positive-control with K_D value of 3·24 nM (Figure-
126 1C (i)).
127
128 **SARS-CoV-2 convalescent plasma enhances Dengue virus infection *in-vitro***

129 Probing the surface expression of FcγR-I, II and III in K562 and U937 cell lines showed both
130 cell lines to predominantly express FcγR-II (Supplementary Figure-1), as reported previously
131 [12]. When samples from convalescent COVID-19 patients (n=48, Supplementary Table-1)
132 were tested for their ability to cause ADE of DENV-infection in K562 cells, a significant
133 augmentation was observed (Figure-2). The percentage of DENV-2 infected K562 cells
134 (Supplementary Figure-2) was increased by 14 out of 21 samples from the pre-delta wave (p-
135 value<0·0001), 15 out 17 samples from the delta wave (p-value<0·0001) and 8 out of 10
136 samples from the Omicron wave (p-value=0·0001), in comparison with untreated virus control
137 (Figure-2A-D). Furthermore, using a subset of the convalescent plasma samples (n=15) similar
138 results were observed in U937 cells, where 9 out 15 serum samples enhanced DENV-2
139 infection. (p-value=0·0001) (Supplementary Figure-3A-B). A quantification of the neutralizing
140 antibody titers against SARS-CoV-2 Wuhan strain in these samples by live-virus neutralization
141 assay (Supplementary Table-2), did not show any association between the neutralizing antibody
142 titer and ADE efficiency (Supplementary Figure-4A-C). Similarly, we did not find any
143 association with severity of the COVID-19 patients (Supplementary Figure-4D).
144 A quantitative-PCR for intracellular DENV genomic RNA normalized to host cell GAPDH
145 transcripts (Supplementary Table-3), showed that 7 out 10 COVID-19 convalescent patient
146 samples caused higher viral replication in K562 cells when compared to control, probably
147 through increasing virus uptake in ADE (p-value<0·0001) (Supplementary Figure-5). This data
148 correlates with the increase in DENV-2 positive cells observed in flowcytometry.
149 Determination of the infectious titer of virus produced from infected cells by focus-forming
150 unit assay also showed a significant increase in the Foci-forming units/ml (FFU/ml) in 2 out of
151 3 COVID-19 convalescent plasma samples as compared to the virus control (p-value<0·0001)
152 (Supplementary figure-6).
153

154 **Monoclonal and polyclonal antibodies raised against SARS-CoV-2 enhance dengue virus**
155 **infection:**
156 Since DENV is endemic to India it is possible that pre-existing anti-DENV antibodies are
157 responsible for the observation of ADE as described above. In order to rule this possibility out,
158 we tested commercially available monoclonal and polyclonal antibodies and sera raised against
159 the SARS-CoV-2 spike and RBD for their potential to enhance the DENV-2 infection in ADE
160 assay using K562 cells (Supplementary Table-4). Interestingly, all of these antibodies enhanced
161 DENV-2 infection (p-value=0·0001) (Figure-3) confirming that antibodies binding to SARS-
162 CoV-2 surface protein cross-react with DENV cause ADE**.** The RBD specific antibody (M5B)
163 showed stronger enhancement compared to antibody against spike (MM41) with dose-
164 dependent increase in DENV-2 infection (Figure-3A-B). Similarly, serum from Hamsters
165 immunized with purified SARS-CoV-2 spike protein also showed ADE of dengue virus
166 infection in K562 cells (Figure-3C).
167 As a control monoclonal antibody against Rabies virus, which do not cross react with DENV,
168 (Negative-control), did not show any ADE of dengue infection in K562 cells suggesting a
169 specific effect of anti-SARS-CoV-2 antibodies (Figure-3C). A similar trend of enhancement
170 in viral infection was observed in U937 cells too using these antibodies (Supplementary figure-
171 7). Three monoclonal antibodies namely 4G2, 1A9 and M4B showed significant ADE, as
172 determined by infectious titer of virus released in the culture supernatant from K562 cells
173 infected in the presence of these antibodies (p-value<0·0001) (Supplementary figure-6).
174 Having established that there is an enhancement of DENV-2 infection due to anti-SARS-CoV-
175 2 antibodies, we next investigated whether it is time-dependent. We assessed the enhancement
176 at 6 h, 18 h and 36 h post infection by virus and virus-antibody complexes, using FACS
177 (Supplementary figure-8). We observed that the mice (Mser) and hamster (Hser) sera, showed
178 a significant enhancement in percentage DENV-2 positive cells with respect to virus control

179 (adjusted p-values Mser=0·0071 and Hser=0·0001), even at 6 hpi, Although, we didn't observe
180 a significant enhancement due to 4G2 and anti-Spike monoclonals M3S and M2G at 6 hpi. At
181 18 hpi, there was a significant increase in the DENV-2 positive cells with respect to virus
182 control in 4G2 (Adjusted p-value=0·0024) as well as M2G (Adjusted p-value=0·0101), which
183 further enhanced significantly at 36 hpi (Adjusted p-values 4G2=0·0001 and M2G=0·0035).
184 The Hser showed highest increase in DENV-2 positive cells at 6 h which remained almost
185 constant till 36 h. (Adjusted p-value<0·0001).
186
187 **Cross recognition of DENV-E protein by anti-Spike and anti-RBD SARS-CoV-2**
188 **antibodies *in-silico***
189 In parallel, in order to assess the cross-reactivity of SARS-CoV-2 antibodies with DENV-2 E-
190 protein dimer (Figure-4A) and to underpin the residues critically involved, we considered an
191 *in-silico* approach (Figure-4B). Since the structures for the antibodies used for *in-vitro* assays
192 weren't available, we selected a panel of reported SARS-CoV-2 antibodies, based on their
193 structural (extent of their characterization, availability of the structure information of their co-
194 crystals with low resolution, etc.) and biological (neutralization potential against SARS-CoV-
195 2, target epitopes, etc.) relevance (Supplementary Table-5).
196 For the identification of epitopes of DENV-2 E-protein, based on interaction fingerprinting, an
197 antibody against E-protein dimer i.e. C8 (Fab region, PDB: 4UTA) was used to characterize the
198 interaction site using its co-crystal. For the selected SARS-CoV-2 antibodies, in order to rule
199 out any biases, the protein-protein (PP) blind docking was carried out with the whole DENV-2
200 E dimer, for identification of the cross-reactive antibodies forming most promising complexes
201 with the E-protein. The consensus docked pose between Piper and HDOCK was used for post-
202 docking qualitative and quantitative analysis such as pose selection and pose-filtering criteria.
203 The top three SARS-CoV-2 antibodies which formed the best complexes with DENV-2

evaluated in terms of docking energy, interaction fingerprinting with co-crystal and MM-GBSA scores are CR3022 (Fab region, PDB:6W7Y), S2E12 ab (Fab region, PDB:7K45) and DMAb 2196 (Fab region, PDB:8D8R) (Figure-4C). These complexes were picked for further analysis. For the C8 antibody (4UTA) binding with DENV-2 E-protein, the residues in the interacting epitopes were, T68, T70, E84, R99, G102, G104, K247, Q248, S274, K310 and E311 (formed hydrogen bonds and/or salt-bridges); E71, S72, R73, C74, N103 and T155 (formed hydrophobic contacts and Vander Waal interactions etc.), and were majorly localized in the Domain-II (Figure-4D). The binding sites of CR3022, S2E12 ab and DMAb 2196 antibodies were found to overlap nicely with the C8 binding sites, as it revealed well by the binding site analysis (Figure-4D).

Furthermore, to quantitate their binding association at the residue level, the major contributing amino acids were underscored in the form of residue-wise decomposition analysis using the cut-off value of -1·5 kcal/mol. The contributing residues for CR3022 were E71, S72, R73, Q77, S81, Q86 and N153 (Majorly in Domain-II); for S2E12ab, were S72, R73, Q77, S81, N83, R99, N103, G152, D154 and T155 (Majorly in Domain-II including Fusion loop); and for DMAb 2196 the residues S72, R73, C74, Q77, E79, N83, E84, R99, and E311 (Majorly in Domain-II including Fusion loop) were involved (Figure-4E, F). From residue-wise mapping it was interesting to note that there were few overlapping residues in the interaction of E-protein with C8 and the top three SARS-CoV-2 interacting antibodies [13]. The residue R73 appeared to be the most common residue among all antibodies. Further in the pair-wise interaction of SARS-CoV-2 and DENV-2 antibodies, the common residues between C8 and CR3022 were T70, E71, S72, G102 and N103; between C8 and DMAb2196, were T70, S72, C74 and R99; and between C8 and S2E12 ab were E71, R99, N103, T155 and K247 (Figure-4E and 4F). Furthermore, we also performed the mining of paratope residues of SARS-CoV-2 antibodies which have shown common interaction with DENV-2 E with equal and/or increased interaction energy. The

229 paratope residues involved in the interaction of DENV-2-E residues as well with CR3022 are
230 Y27(H), Q65(H), S100(H), G101(H) and E111 (L); with DMAb2196 residues are Y32(L),
231 V52(H), S54(H), Y91(L), S93(L), I100(H) and C102(H); and with S2E12, the residues are
232 V2(H), V3(L), L4 (H), L48(L), S53(L), I59(L), G65(L), V93(L), Q102(L) and C106(H)
233 (Supplementary Figure-9).
234 Overall, the interaction maps and per-residue energetic decompositions, underpinned that there
235 were several overlapping residues involved in the interaction of DENV-2-E with C8 and
236 CR3022, S2E12 ab and DMAb 2196 (Figure-4G and 4H). The epitope and paratope analysis
237 followed by thermodynamic energy (ΔG) calculations clearly indicated a high chance that
238 SARS-CoV-2 antibodies possibly interact with DENV-2 E-protein (Figure-4G and 4H). The
239 most cross-reactive residues involved in the interacting epitopes of C8, CR3022, S2E12 ab and
240 DMAb 2196 were also elucidated (Supplementary Table-6)
241
242 **Cross reactivity and enhancement of DENV-2 infection due to CR3022 antibody**
243 Based on the computational outcomes, one of the SARS-CoV-2 antibodies, CR3022 was further
244 investigated *in-vitro* for its cross-reactivity and ability to cause ADE. Confocal microscopy
245 images of CR3022 showed a statistically significant cross-reactivity with an average MFI of
246 7·68 (p-value<0·0001) w.r.t. the Negative-control, Rabishield® (Figure-5A). In BLI, it showed
247 high affinity with a dissociation constant (K_D) of <1·0pM with Spike protein (Figure-5B (i)),
248 and 2·83 nM with DENV-2 E-protein (Figure-5B (ii)). We observed a significant augmentation
249 in the percentage of infected cells at 2 µg and 4 µg concentrations of antibody (p-value=0·01)
250 in comparison with untreated virus control (Figure-5C).
251
252 **Discussion:**

253 In this study, we demonstrated that SARS-CoV-2 antibodies and plasma cross-react with
254 DENV-2. Also, SARS-CoV-2 spike, and RBD-directed antibodies have a very strong binding
255 affinity with stabilized DENV-2 E-protein dimer *in-vitro* with the dissociation constant in nM
256 range) as well as *in-silico*. We used the DENV-2 E-protein for BLI and computational studies
257 since it has been reported to have maximum cross-reactivity with SARS-CoV-2 S1 specific
258 antibodies [7]. Additionally, it is critical for viral entry into cells and is the major target not
259 only for neutralizing antibodies but also for infection-enhancing antibodies [14]. We did the
260 docking studies using dimeric DENV-2 E-protein since we intended to study the interaction
261 of DENV-2 with SARS-CoV-2 antibodies, which would happen before the viral entry,
262 extracellularly and it's known that the E-protein exists as a head-to-tail oriented homodimer
263 on the surface of mature-virions [15].
264 We further assessed whether the SARS-CoV-2 antibodies could enhance DENV-2 infection *in-*
265 *vitro.* We found that convalescent plasma samples of qRT-PCR confirmed SARS-CoV-2
266 infected individuals, from the pre-delta, delta and post-delta waves, significantly enhanced the
267 DENV-2 infection in cultured Fc-receptor positive K562 and U937 cells. Also, the
268 commercially available anti-Spike and anti-RBD SARS-CoV-2 antibodies and Spike-
269 immunized mice and hamster sera showed an increased DENV-2 infection in both the model
270 systems.
271 Our docking results indicated that there is a significant interaction of SARS-CoV-2 antibodies
272 with DENV-2 dimeric E-protein with the binding free energy of three of them, CR3022
273 (6W7Y), S2E12 ab (7K45) and DMAb2196 (8D8R), being comparable to the DENV-2 dimer
274 specific antibody, C8 (4UTA). These top three binders shared their interacting interface with
275 C8 antibody. The residues involved in the interaction of C8 antibody with DENV-2 E that were
276 also involved in the interaction of at-least one of the top three binders with E-protein were: R73,
277 E84, R99, G102 and E311 (Localized in Domain-II and Fusion-loop). The contributing residues

278 which were found to be majorly stabilizing the interaction of these top three SARS-CoV-2
279 antibodies with E-protein were S72, R73, Q77, S81, N83 and R99 (involved in interacting
280 epitopes of at least two out of the three). All the residues involved in the interaction of these
281 three SARS-CoV-2 antibodies with E-protein when mapped, were majorly found to lie in the
282 Domain-II and fusion-loop of E-protein. It is well known that the antibodies against two
283 epitopes of DENV are strong mediators of ADE: prM epitopes[16] and fusion loop epitopes
284 [17]**.** The fusion loop epitopes could be the ones, potentially involved in ADE. Multiple residues
285 have been identified from *in-silico* interaction mining, among them few residues such as R73
286 (common among all), T70, S72 (Domain-II), R99 and N103 (Fusion loop) and E311 (Domain-
287 III) seems critical as they are contributing with DENV-2 (C8:4UTA) and at least two antibodies
288 of SARS-CoV-2 CR3022:6W7Y; S2E12ab:7K45; and DMAb 2196:8D8R; as well. These
289 residues put forth the possible avenue to explore further for epitope-based therapeutic
290 interventions.
291 There are several limitations to our study. First, our study doesn't provide the mechanistic
292 insights into the pathway involved in ADE of DENV-2 infection due to SARS-CoV-2
293 antibodies. The extrinsic pathway, involves an enhanced FcR-mediated entry of the virus into
294 the host cells leading to an increase in the number of infected cells, whereas the intrinsic
295 pathway, involves a suppression of the host antiviral immune response causing increased viral
296 replication and would boost virus production per infected cell (burst size) [18] [19]. Although,
297 here we briefly did a time-course based kinetic study of DENV-2 infection upon treatment of
298 virus with SARS-CoV-2 antibodies and convalescent plasma. We observed an enhancement in
299 the percentage of DENV-2 infected cells, even at 6 h, indicating enhanced virus internalization,
300 since this is before E-protein from replicated new virions is expected [14] [15] which may
301 possibly indicate the presence of at least extrinsic pathway for ADE. However, it needs a lot of
302 further exploration for confirmation.

303 Secondly, the DENV-2 used for the assay was prepared in C6/36 cells and wasn't characterized
304 for the proportions of immature and mature-virions. It is well known that the virus prepared *in-*
305 *vitro* in mosquito cells has considerably a greater amount of immature virions as compared to
306 mammalian cells, probably due to differential furin-expression [20] [21] . Also, it is recognized
307 that laboratory adapted DENV strains are more dynamic than clinical strains and expose cryptic
308 epitopes more easily [22]. Thus our virus preparation might have major proportion of immature
309 virions but this probably won't change the fact that the ADE of DENV-infection would happen
310 since the viral heterogeneity in response to the natural DENV-infections is also well known
311 The antibody repertoire in the natural infections is known to have an abundance of poorly
312 neutralizing antibodies which are majorly generated in response to the immature conformations
313 [23], which is a result of incomplete furin processing, resulting in egress of immature-forms
314 along with and mature-forms of virions post-assembly [24] [25]. Also, the fully mature-virions
315 have been reported to be less susceptible to neutralization by cross-reactive and heterotypic
316 immune sera than partially mature variants [23]**.** Thus, the heterogeneity in the DENV-2
317 preparation used for the *in-vitro* assays can be probably closely related to the natural infection
318 scenario. Further, our results from the BLI and docking studies are based on the dimeric form
319 of E-protein (indicating mature-form). Thus, one may then ask that the cross-reactive epitopes
320 defined here, might not be all the interacting epitopes that would possibly be involved and
321 interactions with other conformations must also be considered. Although it can be challenging
322 to map the exact cross-reactive epitopes since DENV is known to have multiple transient states,
323 involving different arrangements and oligomeric states of E-proteins that which governs the
324 antigenic landscape or accessibility of DENV [26]. Still, a docking study with a heterotrimer of
325 E-protein (consisting of un-cleaved prM), being a crystallized transient state [27] may give a
326 better indication of more of the cross-reactive epitopes involved.

327 In summary, our findings demonstrated that the SARS-CoV-2 antibodies can enhance the
328 DENV-2 infection. A recent report by Nath *et. al.* demonstrated serological cross-reactivity of
329 SARS-CoV-2 positive serum samples with DENV1 and that the sera neutralized DENV1 [28]
330 but contrastingly, we didn't observe neutralization of DENV-2 at any antibody concentration
331 used. Also Panting *et. al.*, in a recent retrospective matched cohort study of pediatric patients,
332 diagnosed with SARS-CoV-2 and dengue coinfection, in Philippines, reported that the co-
333 infection predominantly presented with typical dengue symptoms more case fatality rate in
334 coinfection than mono-infection (6·7% versus 5%) [29], which could possibly be due to an
335 ADE of DENV-infection by SARS-CoV-2 antibodies. Further, it would be interesting to
336 investigate if the antibodies induced in response to various SARS-CoV-2 vaccines also possess
337 the potential to enhance DENV-infection. This finding can have significant implications
338 keeping in view the co-circulation of both DENV-2 and SARS-CoV-2 in several countries and
339 thus the vaccine development strategies against both the agents might need an inevitable
340 change. The findings suggest the need to consider DENV seasonality when deploying SARS-
341 CoV-2 vaccines to prevent severe infections, particularly in countries where DENV is endemic.
342 Finally, the study also identifies the cross-reactive epitopes and paratopes and lists some critical
343 residues involved in the interaction of SARS-CoV-2 antibodies with DENV-2 E-protein, which
344 can have prophylactic and therapeutic implications.
345

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348 Coronavirus 2, Isolate USA-WA1/2020, NR-52281, Monoclonal Anti-SARS-Related
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350 Anti-SARS-Related Coronavirus 2 Spike Glycoprotein Receptor Binding Domain-(RBD),
351 Clone 2TP1C3 (produced *in-vitro*), NR-55296, Polyclonal Anti-SARS-Related Coronavirus 2

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Data availability:

All the data are presented in this manuscript. The virus strains used in this study have the following accession numbers: original Wuhan (USA-WA1/2020, Genbank: MN985325), B·1·617·2 (δ-variant) known as THSTI_287 (GenBank: MZ356566·1), and Omicron BA·1 (GISAID accession no.: EPI_ISL_8764350) Source data are provided with this paper.

Author Contributions:

SM conceptualized the idea. SM, SB, SA and TrS coordinated the research activity. KJ and SS contributed to designing and execution of viral assays. KJ, SS, GS, TS, MT, JK, DKR, BL, SK, PS, SK, VP, LK and VG contributed to data curation, investigation and methodology. KJ, SS,

GS, SB, SA, TrS and SM contributed to formal analysis. SS, SR, MG, PK, NW, RT, SB, JB, TrS, SA and SM provided resources. KJ, SS, TrS, PK, NW, RT, SB, JB, SA and SM contributed to writing, review & editing the draft. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Ethics statement

We obtained ethical approval from the Institutional Bio-Safety Committee of THSTI prior to conducting all studies: (IBSC#495/2023) and RCGM (No.-BT/IBKP/137/2020).

Disclosure statement

The Author declares no conflict of interest.

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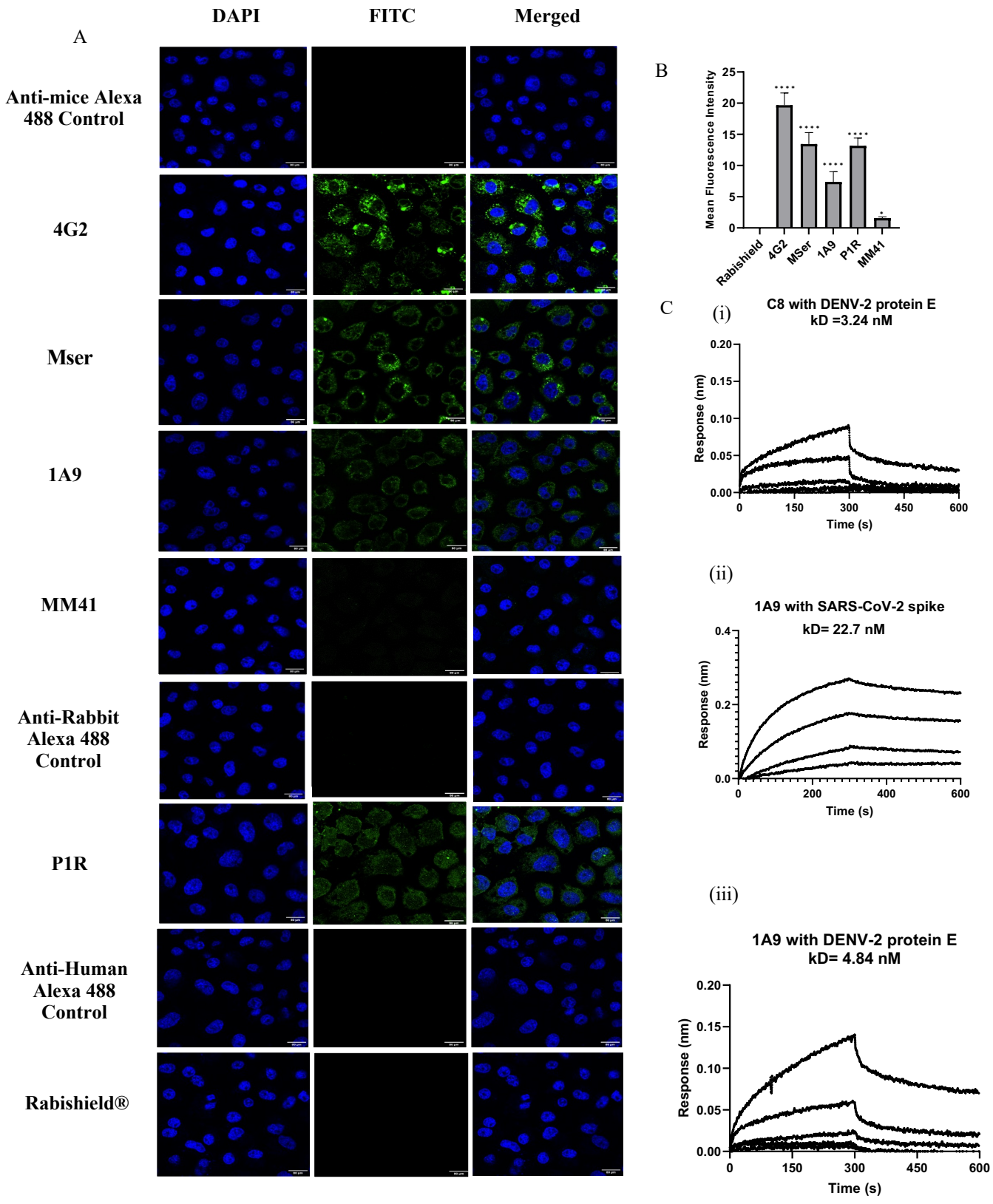

Figure 1: Cross-reactivity of antibodies and sera raised against SARS-CoV-2 Spike with DENV-2 (NGC) proteins.

(A) Confocal, digital images of DENV-2 (NGC) infected C6/36 cells reacted with 4G2, antibodies and sera raised against SARS-CoV-2 after 48 hpi. Cell nuclei are stained with DAPI while the other antibodies are detected using FITC-labeled respective secondary antibodies.

(B) Bar graph showing MFI of 4G2, MSer, 1A9, P1R (normalized w.r.t. the respective secondary antibody controls) with standard deviation. Analysis was performed using ImageJ and statistical significance was carried out using One-way ANOVA followed by Dunnett's test in GraphPad Prism 8·4·2. Asterisk (*) indicates statistically significant difference in MFI w.r.t. Rabishield® (Negative control).P value = 0·1234 (ns), 0·0332 (*), 0·0021 (**), 0·0002 (***), <0·0001 (****)

(C) Binding kinetics of C8 (DENV-E protein specific) and 1A9 (SARS-CoV-2 spike specific) antibodies with DENV-2 protein E and SARS-CoV-2 spike protein. Antibodies were loaded onto the sensors and serial dilutions of the proteins were used to study binding kinetics. The binding responses were calculated by subtracting data from reference and fitting globally with a 1:1 binding model using ForteBio's Data Analysis software 10·1. The data was considered validated if $\chi 2$ <0·5.

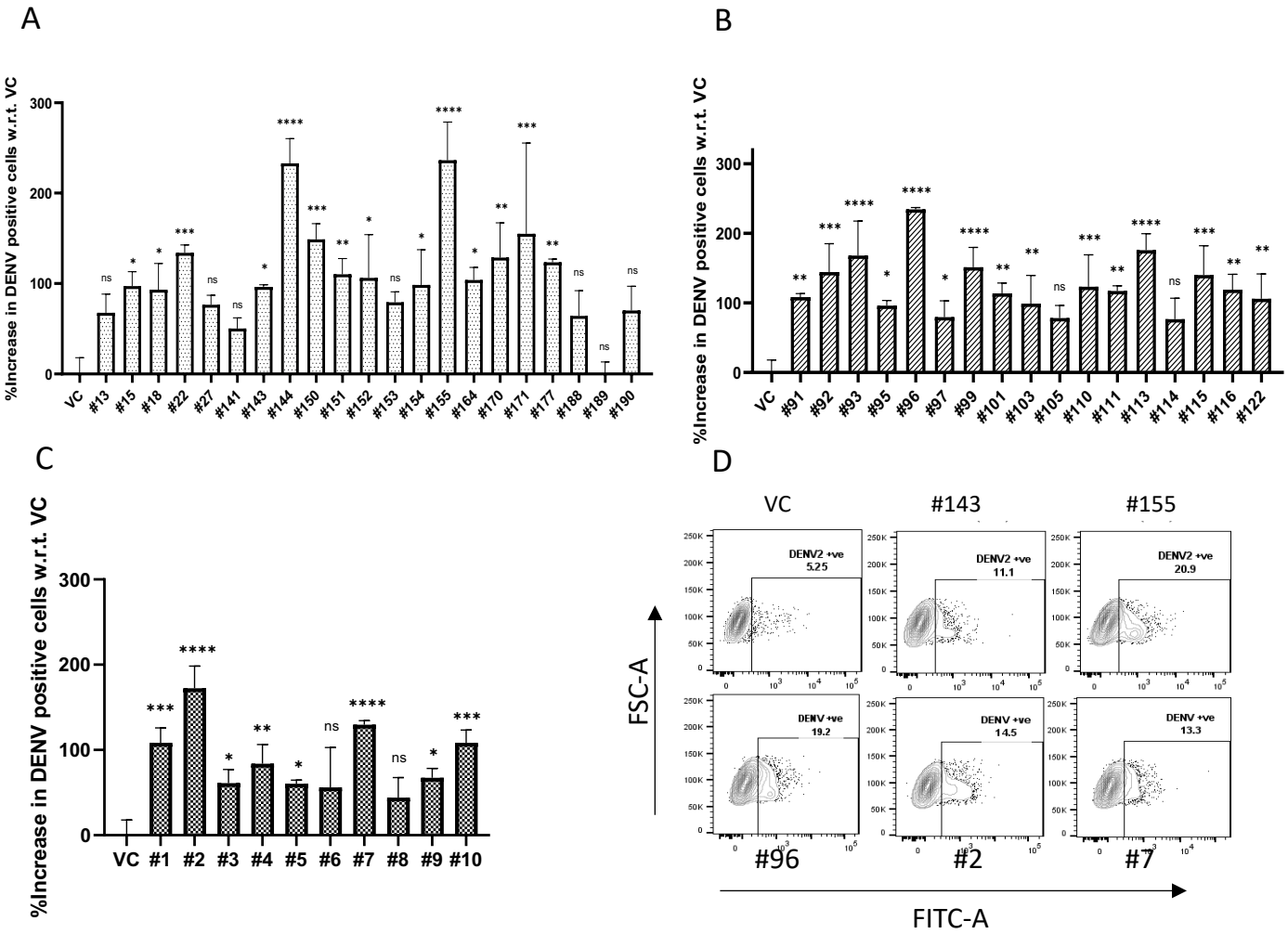

Figure 2: ADE of dengue virus infection by the SARS-CoV-2 positive patients' sera in K562 cells.
ADE due to convalescent plasma samples collected in the (A) pre-delta wave, n=21, (B) delta wave, n=17, and (C) Omicron wave, n=10. The bar graph represents an average percentage increase in DENV positive cells w.r.t. VC for each sample with standard deviation for duplicates. Statistical significance was carried out using One-way ANOVA followed by Dunnett's test in GraphPad Prism 8·4·2. Asterisk (*) indicates that the difference between the Virus control and the serum samples is statistically significant. P value = 0·1234 (ns), 0·0332 (*), 0·0021 (**), 0·0002 (***), <0·0001 (****)
(D) Representative dot plots of patient samples from Pre –delta wave (#143, #155), Delta wave (#96), and Omicron wave (#2, #7). Data was analyzed on FlowJo version 10·8·1.

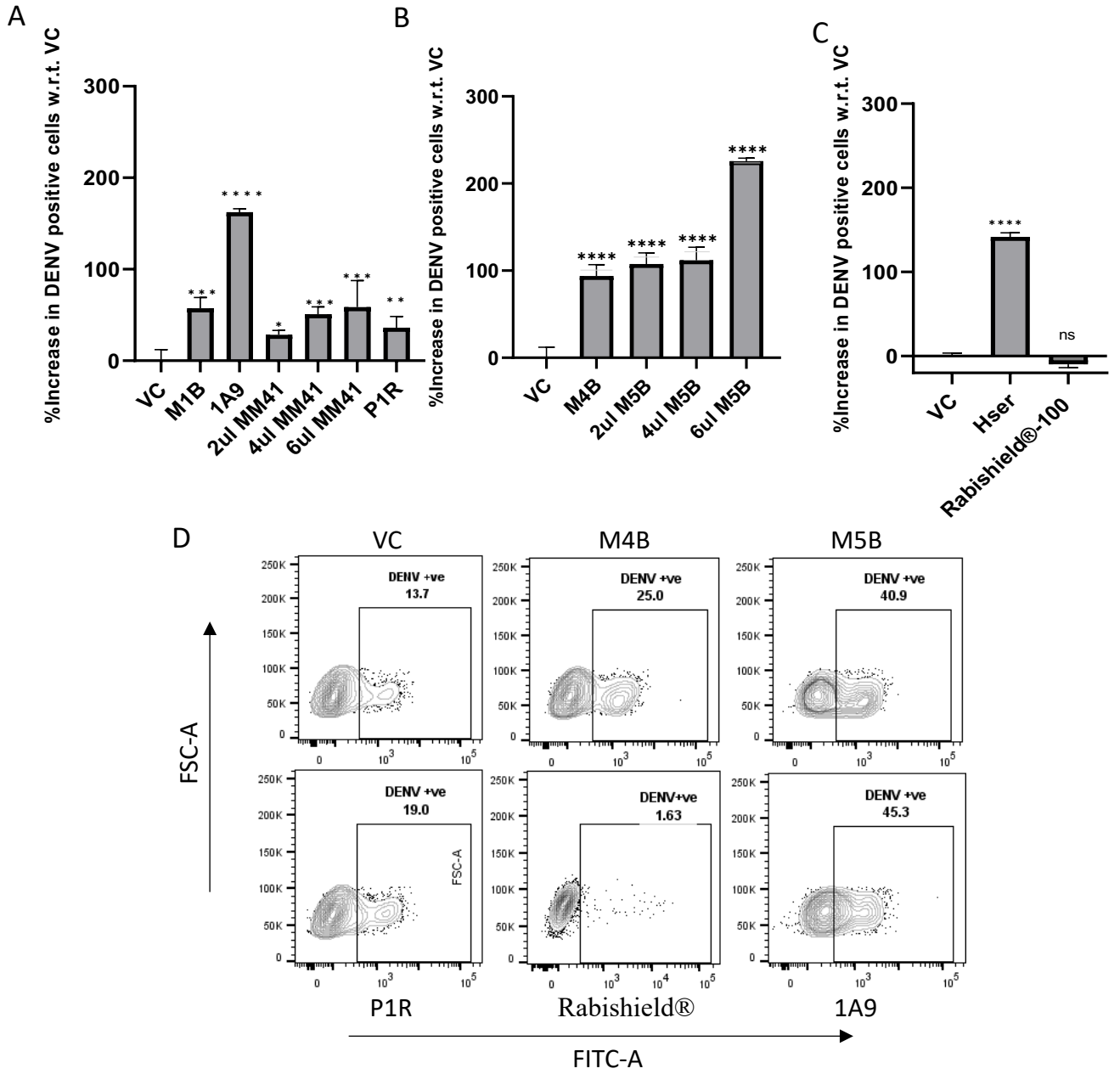

Figure 3: ADE of dengue virus infection by the monoclonal and polyclonal antibodies and serum against SARS-CoV-2.

ADE due to (A) anti-spike monoclonal antibodies, M1B, 1A9, and different volumes of MM41 (from 2 µl to 6 µl) and anti-spike polyclonal antibody raised in rabbit, P1R, (B) anti-RBD monoclonal antibodies, M4B, and different volumes of M5B (from 2 µl to 6 µl), (C) hamster serum immunized with SARS-CoV-2 spike and negative control (Rabishield®). Statistical significance was calculated using One-way ANOVA followed by Dunnett's test in GraphPad Prism 8·4·2. Asterisk (*) indicates that the difference between the Virus control and the serum samples is statistically significant. P value = 0·1234 (ns), 0·0332 (*), 0·0021 (**), 0·0002 (***), <0·0001 (****).

(D) Representative dot plots. Data was analyzed using FlowJo version 10·8·1.

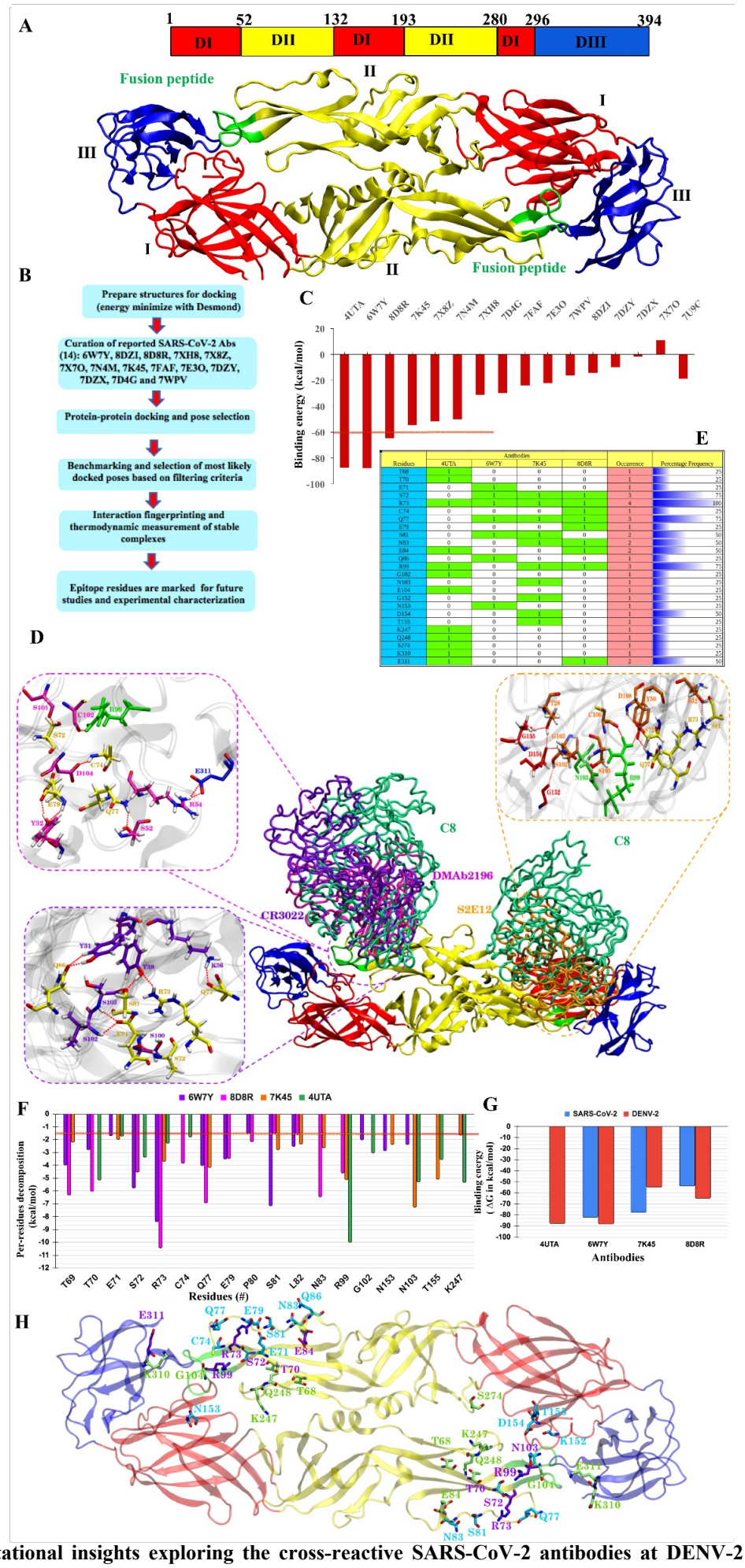

Figure 4. Computational insights exploring the cross-reactive SARS-CoV-2 antibodies at DENV-2 E protein: (A) Sequence and structural organization of the DENV-2 E dimer (PDB 1OAN). (B) Overview of the computational pipeline. (C) The binding free energy in kcal/mol of selected SARS-CoV-2 antibodies. The most likely complexes were generated using Protein-protein dockings. (D) The interaction map of top three antibodies (CR3022: 6W7Y: violet, S2E12 neutralizing antibody Fab fragment: 7K45: orange and DMAb 2196: 8D8R: magenta) along with the native antibody of DENV-2 E as control (C8:4UTA: green). The residue-wise zoom-out view is shown to highlight the critical residues mainly forming the interactions such as Hbonds and SBs. The DENV-2 antibody interactions are shown in green-colored residues and SARS-CoV-2 antibody interactions are highlighted in the pink-colored residues. (E) The matrix of critical residues involved in either establishing either H-bonds or in SBs along with their occurrence. (F) The per-residue decomposition of top three antibodies indicating the thermodynamic contributions of epitope residues. (G) The net binding energy between identified antibodies and DENV-2 E which claims as potential in both cases. (H) The 3D positioning of identified epitope residues at dimer of DENV. The residues which only interact with SARS-CoV-2 antibodies are shown in cyan, the residues interacting with only DENV-2 antibody are shown in green color. The residues which are interacting with both DENV-2 E as well as with SARS-CoV-2 RBD are shown in violet color. All the residues are rendered in licorice and atom-wise C:cyan/green/violet, N:blue, O:red and S:yellow.

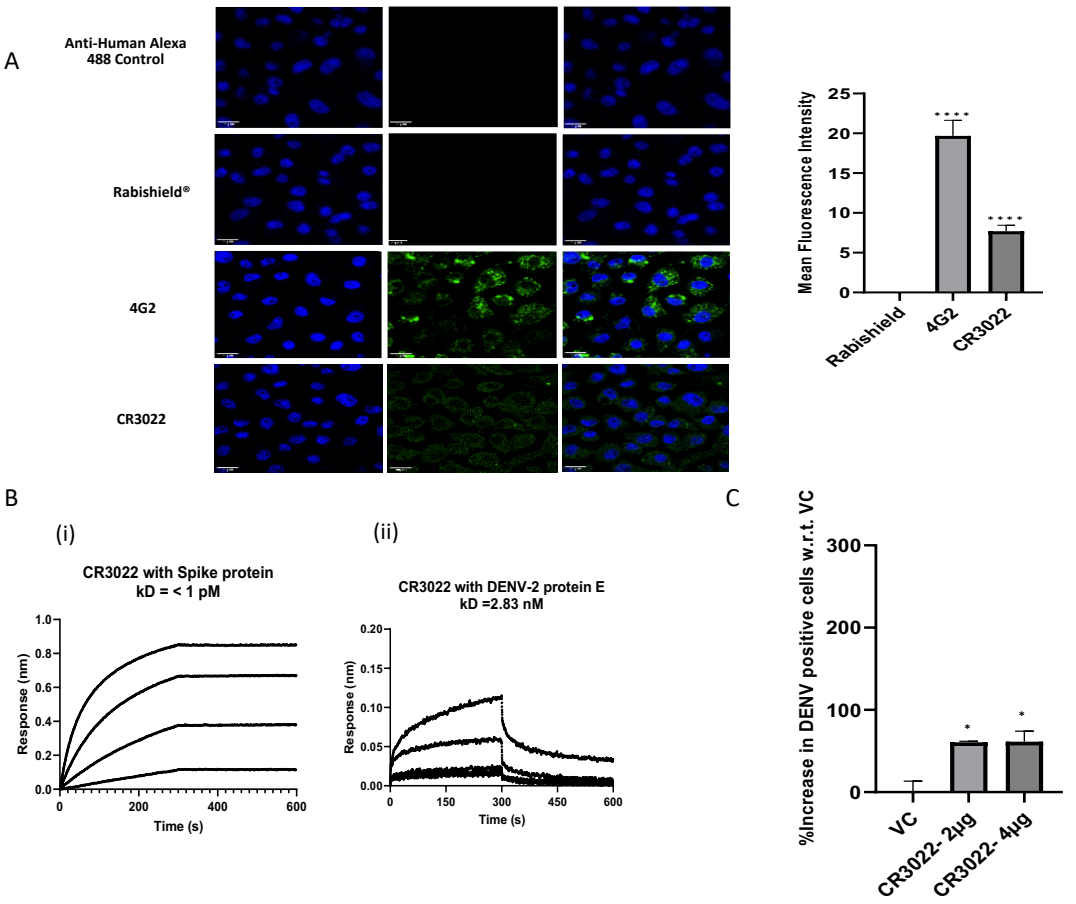

Figure 5: Cross-reactivity and ADE of Dengue virus infection by the SARS-CoV-2 anti-RBD antibody CR3022.
(A) Confocal, digital images of DENV-2 (NGC) infected C6/36 cells stained with CR3022 along with the controls, 4G2 and Rabishield® (taken from Figure 1 to show the comparison) and the Mean Fluorescence Intensity (MFI).
(B) Binding kinetics of CR3022 with SARS-CoV-2 spike protein (i) and DENV-2 protein E (ii).
(C) ADE of DENV-2 infection by different concentrations of CR3022 in K562 cells by flowcytometry. Statistical significance was checked using One-way ANOVA followed by Dunnett's test. Asterisk (*) indicates a statistically significant difference between the control and treatment. P value = 0·1234 (ns), 0·0332 (*), 0·0021 (**), 0·0002 (***), <0·0001 (****).