

1 **Emergence of RBD mutations in circulating SARS-CoV-2 strains enhancing the structural**
2 **stability and human ACE2 receptor affinity of the spike protein**

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19 **Running Head:** RBD mutations enhance human ACE2 receptor affinity

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28 **Abstract**

29 A novel coronavirus SARS-CoV-2 is associated with the current global pandemic of Coronavirus
30 Disease 2019 (COVID-19). Spike protein receptor-binding domain (RBD) of SARS-CoV-2 is the
31 critical determinant of viral tropism and infectivity. To investigate whether the mutations in the RBD
32 have altered the receptor binding affinity and caused these strains more infectious, we performed
33 molecular dynamics simulations of the binding affinity between the mutant SARS-CoV-2 RBDs to
34 date and the human ACE2 receptor. Among 1609 genomes of global SARS-CoV-2 strains, 32
35 non-synonymous RBD mutants were identified and clustered into 9 mutant types under high positive
36 selection pressure. Three mutant types (V367F, W436R, and D364Y) emerging in Wuhan, Shenzhen,
37 Hong Kong, and France, displayed higher human ACE2 affinity, and probably higher infectivity. This
38 is due to the enhanced structural stabilization of the RBD beta-sheet scaffold. High frequencies of
39 RBD mutations were identified: V367F from five France and one Hong Kong mutants, 13 V483A and
40 7 G476S mutants from the U.S.A. This suggested they originated as novel sub-lineages. The
41 enhancement of the binding affinity of the mutant type (V367F) was further validated by the
42 receptor-ligand binding ELISA assay. The molecular dynamics simulations also indicated that it
43 would be difficult for bat SARS-like CoV to infect humans. However, the pangolin CoV is potentially
44 infectious to humans. The analysis of critical RBD mutations provides further insights into the
45 evolutionary history of SARS-CoV-2 under high selection pressure. An enhancement of the
46 SARS-CoV-2 binding affinity to human ACE2 receptor reveals higher infectivity of the mutant
47 strains.

48

49 **Importance**

50 A novel coronavirus SARS-CoV-2 has caused the pandemic of COVID-19. The origin of
51 SARS-CoV-2 was associated with zoonotic infections. The spike protein receptor-binding domain
52 (RBD) is identified as the critical determinant of viral tropism and infectivity. Thus, whether the
53 mutations in the RBD of the circulating SARS-CoV-2 strains have altered the receptor binding
54 affinity and caused these strains more infectious, should be paid more attentions to. Here, 32
55 non-synonymous RBD mutants were identified and clustered into 9 mutant types under high positive

56 selection pressure, suggesting they originated as novel sub-lineages. Three mutant types displayed
57 higher human ACE2 affinity, and probably higher infectivity, one of which (V367F) was validated
58 by wet bench. The RBD mutation analysis provides insights into SARS-CoV-2 evolution. The
59 emergence of RBD mutations with increased human ACE2 affinity reveals higher risk of severe
60 morbidity and mortality during a sustained COVID-19 pandemic, particularly if no effective
61 precautions are implemented.

62

63 **Keywords:** COVID-19; SARS-CoV-2; ACE2; RBD; mutations; affinity; infectivity; spike protein

64

65 **1. Introduction**

66 A novel coronavirus SARS-CoV-2 has caused the outbreaks of Coronavirus Disease 2019
67 (COVID-19) globally since the first report in mid-December 2019 in Wuhan, China (1–4). As of
68 April 14, 2020, SARS-CoV-2 has infected 1,844,863 people world-wide and caused 117,021 deaths
69 (5) with the estimated fatality rate of 6.34%. This on-going pandemic of COVID-19 has become the
70 most serious threat to public health in recent times.

71
72 The origin of SARS-CoV-2 remains elusive. However, the initial cases were largely associated with
73 the seafood market, which indicated this were potential zoonotic infections(2). Although bats and
74 pangolins are most likely the reservoir hosts and the intermediate hosts in the wild, more evidences
75 are in need to support the zoonotic infections and track the origin of this new coronavirus(6–8).

76
77 Angiotensin-converting enzyme 2 (ACE2) is the cellular receptor of SARS-CoV-2 (9), which is the
78 same as for SARS-CoV. The receptor-binding domain (RBD) of the subunit S1 directly interacts
79 with ACE2, which provides for tight binding to the peptidase domain of ACE2. Therefore, RBD is
80 the critical determinant of virus-receptor interaction and reflects viral host range, tropism and
81 infectivity(6, 10–12). Although the RBD sequences of different SARS-CoV-2 strains circulating
82 globally are conserved, mutations have appeared, which might account for differences in viral
83 infectivity and contribute to its spread.

84
85 Meanwhile, S protein participates in antigenic recognition expressed on its protein surface, likely to
86 be immunogenic as for carrying both T-cell and B-cell epitopes. The potential antibody binding
87 sites that have been identified indicates RBD has important B-cell epitopes. The main antibody
88 binding sites substantially overlap with RBD, and the antibody binding to these sites is likely to
89 block viral entry into cells(13, 14).

90
91 To investigate whether these mutations in RBD have altered the receptor binding affinities and
92 whether these strains may have been selected for higher infectivity, the binding dynamics between

93 the SARS-CoV-2 RBDs of the mutant strains to date and human ACE2 receptor were modelled and
94 assessed.

95

96 **2. Results and discussion**

97 **2.1 SARS-CoV-2 RBD mutation mapping**

98 Among the 1609 SARS-CoV-2 strains with whole genome sequences available in the public
99 databases, 32 strains contained non-synonymous amino acid mutations in the RBD (**Supplementary**
100 **Table 1**). These strains were reported from multiple locations, including China, U.K., Finland,
101 France, Belgium, U.S.A., and India (**Fig. 1**). Most mutants deviate from the original reported
102 genome (SARS-CoV-2 Wuhan-Hu-1) by only one amino acid (**Supplementary Figure 1**). These 32
103 mutations parse into 9 mutant types. Mutation V367F was found in six individual strains isolated
104 from four patients: Three in France and one in Hong Kong. Similarly, high frequencies of RBD
105 mutations were also identified the U.S.A: 13 V483A mutants and 7 G476S mutants (**Fig. 1**). This
106 suggested that these mutant strains may have originated as novel sub-lineages.

107

108 **2.2 Nucleotide diversity indicates strong positive selective pressure on RBD**

109 Since RBD is the only domain to bind human ACE2 and initiate cell entry, it is believed that the
110 RBD should be highly conserved. However, polymorphism and divergence analysis by DnaSP6
111 (version 6.12.03) (15) showed that the RBD sequences were as diverse as the other regions of the S
112 protein (**Fig. 2**). The peak signals for diversity distribute across the entire S protein, and the multiple
113 peaks in the RBD also reached the P_i value of ~ 0.0008 , similar to P_i values in the other regions.
114 Therefore, we hypothesize that the RBD would be selected to maintain or even improve its binding
115 affinity to human ACE2.

116

117 To test this hypothesis, we investigated the selective pressures of the S gene by calculating
118 nonsynonymous/synonymous substitution rate ratios (dN/dS ratios) for various segments of the S
119 gene in the 1609 SARS-CoV-2 strains. With respect to our hypothesis, the entire S gene exhibited a
120 dN/dS of 4.1197, remarkably greater than 1, showing that the S gene is indeed under positive

121 selective pressure (Table 1). The RBD showed a similar dN/dS (3.3545) as the entire S protein,
122 indicating that high selective pressure was also applied to this essential domain. Therefore, the
123 functional relevance of these RBD mutations may be inferred.

124

125 **2.3 Three mutant types bind human ACE2 receptor with higher affinity**

126 To estimate the functional changes suggested by the RBD mutations, we performed molecular
127 dynamics simulations for the prototype SARS-CoV-2 (Wuhan-Hu-1 strain) and the RBD mutants in
128 order to assess their binding energy to human ACE2, which was performed using GROMACS 2019.
129 The complex structure of the SARS-CoV-2 S-protein RBD domain and human ACE2 was obtained
130 from National Microbiology Data Center (ID: NMDCS0000001) (PDB ID: 6LZG)
131 (<https://www.rcsb.org/structure/6LZG>). Mutant amino acids of the SARS-CoV-2 RBD mutants were
132 directly replaced in the model, and the bat/pangolin CoV RBD domain was modeled using
133 SWISS-MODEL(16). Each simulation was performed at 10ns and each model was simulated in
134 triplicates. All trajectories reached a plateau of RMSD after 2~5ns (Fig. 3A), indicating that their
135 structures reached an equilibrium. All of the subsequent computations on thermodynamics were
136 based on the 5~10ns trajectories. Three RBD mutant types (N354D and D364Y, V367F, W436R)
137 exhibited significantly lowered ΔG , suggesting a significantly increased affinity to human ACE2;
138 the other mutants showed a similar ΔG compared to the prototype (Fig. 3B). The ΔG of these three
139 mutant types were around -58 kJ/mol, approximately 25% lower than the prototype strain (-46.5
140 kJ/mol, calculated from the experimentally measured K_D) (Fig. 3B). Compared to the $K_D = 14.7$ nM
141 of the prototype RBD(17), the equilibrium dissociation constant (K_D) of the three mutants are
142 calculated as 0.12 nM, 0.11 nM, and 0.13 nM, respectively (Fig. 3C), which were two orders of
143 magnitude lower than for the prototype strain, indicating a remarkably increased affinity to the
144 human ACE2 receptor. In the only double amino acid mutant (N354D, D364Y), the N354D
145 substitution decreased the affinity, while the D364Y provided an even higher affinity than the
146 overall double mutations (Fig. 3B). This indicated that the D364Y is the major contributor of the
147 enhanced affinity.

148

149 To validate the change of the binding affinity of the mutant S protein (V367F) experimentally, a
150 receptor-ligand binding ELISA assay of the S proteins and the ACE2 was performed. **Fig. 3D**
151 showed that the V367F mutant significantly lowered the ED50 concentration ($ED_{50} = 0.8 \pm 0.04$
152 $\mu\text{g/ml}$), as compared to the prototype ($ED_{50} = 1.7 \pm 0.14$ $\mu\text{g/ml}$), demonstrating that the V367F
153 mutant has higher affinity than the prototype. This result qualitatively validated our computational
154 simulation.

155

156 In comparison, the bat CoV RBD (strain RaTG13, with the highest genome similarity) showed a
157 much lower binding affinity ($K_D = 1.17 \text{ mM}$; $\Delta G = -17.4 \text{ kJ/mol}$) to human ACE2 than the pangolin
158 CoV ($K_D = 1.89 \mu\text{M}$; $\Delta G = -33.9 \text{ kJ/mol}$). For comparison, the affinity of the pangolin CoV was slightly
159 lower than the SARS-CoV-2 prototype strain ($K_D = 14.7 \text{ nM}$; $\Delta G = -46.5 \text{ kJ/mol}$) (**Fig. 3B, 3C**).

160

161 **2.4 Structural basis for the increased affinity**

162 The 9 mutant types were divided into two groups: the “similar affinity” group (V341I, F342L,
163 R408I, A435S, G476S, V483A), whose affinity is not significantly increased, and the “higher
164 affinity” group (N354D D364Y, V367F, W436R), whose affinity is significantly increased. To
165 explain the structural basis of the increased affinity, we investigated deeper into the dynamics of the
166 residues of these structures. The binding surface of the RBD to ACE2 is largely in random coil
167 conformation, which lacks structural rigidity. Therefore, a firm scaffold should be necessary to
168 maintain this conformation of the interaction surface, and thus may facilitate the binding affinity.
169 The beta-sheet structure scaffold, centered by residues 510-524 (**Fig. 4A**, marked as red), provides
170 this rigidity. “Higher affinity” mutants (N354D D364Y, V367F, and W436R) showed a considerable
171 decrease of the RMSF (Root Mean Square of Fluctuation) at this region, demonstrating a more rigid
172 structure; this was not observed for the “similar affinity” mutants (**Fig. 4B**). Coincidentally, the
173 substitutions that account for the affinity increase (D364Y, V367F, and W436R) are all located near
174 this fragment. Indeed, residues 475-485, which is a random coil near the binding site, showed a
175 remarkably higher RMSF for the “similar affinity” group mutants, in contrast to the “higher
176 affinity” group mutants (**Fig. 4B**). Moreover, the “higher affinity” group exhibited a general

177 decreased ΔG in the binding site region, but not the “similar affinity” group mutants (Fig. 4C). In
178 addition, the D364Y and W436R themselves directly contributed to the ΔG decrease. In contrast,
179 the N354D mutation directly elevated the ΔG , which coincides its consequence (Fig. 4B). The
180 mutation W436R provides a positively charged Arg in the proximity of the complementing highly
181 negative charged ACE2 surface. This potential electrostatic attraction may contribute to the higher
182 affinity (Fig. 4D).

183

184

185 **3. Discussion**

186 Due to the lengthening pandemic and evolving nature of the SARS-CoV-2 virus globally, identifying
187 changes in viral infectivity is crucial to containing the COVID-19 spread. Quarantine policies need to
188 be adapted with respect to the changes in virus infectivity. This report provides computational insight
189 into the functional outcome of mutations in RBD: RBD mutants under positive selection pressure,
190 and several mutants acquired increased binding affinity to human ACE2 receptor, implying higher
191 infectivity to humans (noted for one mutant with experimental validation).

192

193 It should be noted that the mutation V367F enhancing the affinity was found in six strains: One in
194 Hong Kong and five in France. As RBD is conserved in SARS-CoV-2, the coincidence of six strains
195 with the same mutation across the geographic distance indicates that this mutant may have evolved
196 to be more robust and that these strains originated as a novel sub-lineage, given the close isolation
197 dates (January 22 and 23, respectively). Combined with the epidemiological data, mutation
198 surveillance is of critical importance as it can reveal more exact transmission routes of the epidemic
199 and provide early warning for additional outbreaks. Emergence of SARS-CoV-2 strains in Hong
200 Kong, France, and other countries with RBD mutations providing higher binding affinity to human
201 ACE2 receptor suggests a higher risk of more severe morbidity and mortality
202 during a sustained pandemic of COVID-19, particularly if no effective precautions
203 are implemented.

204

205 Our analysis of molecular dynamics simulation indicates the remarkable enhancement of the
206 affinity efficiency of mutant S protein. Compared to the prototype strain Wuhan-Hu-1, the ΔG of
207 mutants decreased $\sim 25\%$. Mutants bind ACE2 more stably due to the enhancement of the base
208 rigidity. Potential and recent animal-to-human transmission events of SARS-CoV-2, may explain
209 the strong positive selection and enhancement of the affinity during the pandemic. The viruses have
210 been adapting to transmission and replication in humans; mutation or recombination events in RBD
211 may boost the binding affinity and cause the basic reproduction number (R_0) to climb in theory, i.e.,
212 the human to human transmission more easily.

213

214 The origination of the virus is a constant hot topic since the virus outbreak. Due to the high
215 homology of the bat SARS-like CoV genome and pangolin CoV RBD to the SARS-CoV-2, these
216 wild animals were thought to initiate the infection in human. Our results provided more clues on
217 this postulation. In our study, the binding energy of the bat SARS-like CoV RBD suggests it is too
218 high to bind human ACE2 effectively (K_D in millimolar range). In contrast, the pangolin CoV
219 showed a K_D of binding to human ACE2 at the micromolar range, just $\sim 6x$ higher than that of
220 human SARS virus ($K_D = 0.326\mu M$)(17) (Fig. 3), indicating that the pangolin CoV has the potential
221 to infect human in unprotected close contact. Alignment of the genomic sequences of SARS-CoV-2
222 and pangolin CoV viruses indicated the evidence for recombination events in RBD domain between
223 pangolin and bat viruses. The pangolin CoV has been detected among the smuggled Malayan
224 pangolins in multiple provinces in China(7, 8), suggesting a risk of zoonotic infection from wild
225 animals to human constantly and widely.

226

227 The S protein is also important for antigen recognition. In this survey of 1609 strains, 32 had amino
228 acid mutations in the RBD. High frequencies of RBD mutations were identified: V367F from five
229 France and one Hong Kong mutants, 13 V483A and 7 G476S mutants from the U.S.A. Since the
230 RBD contains important antigenic epitopes, frequent mutations in RBD, especially those which
231 change the amino acid properties, may weaken the binding affinity of the antibody raised against
232 the prototype strain. This may lead to decreased vaccine efficacy and should be further validated.

233

234 In summary, we have identified 32 RBD mutant strains clustering into 9 mutant types under high
235 positive selection pressure. This suggested that they originated as novel sub-lineages. Three mutant
236 types emerging in Asia and Europe displays enhanced structural stability of the spike protein along
237 with higher binding affinities to human ACE2 receptor, which indicates that these mutants may
238 have acquired increased infectivity to humans.

239

240

241 **4. Methods and materials**

242 **4.1 Genome sequence dataset in this study**

243 Full-length protein sequences of S protein RBD were downloaded from the NCBI GenBank
244 Database, China 2019 Novel Coronavirus Resource (<https://bigd.big.ac.cn/ncov>) and GISAID
245 EpiFluTM Database (<http://www.GISAID.org>). 1609 SARS-CoV-2 full-genome sequences were
246 downloaded and the sequences with amino acid mutations in S protein and RBD region were
247 screened. The genome sequences with amino acid mutations in S protein and the RBD were
248 analyzed in this study ([Supplementary Table 1](#)).

249

250 **4.2 Sequences alignment and polymorphism analyses**

251 Alignment of S protein sequences from different sources and comparison of ACE2 proteins among
252 different species were accomplished by MAFFT version 7 online server with default parameter
253 (<https://mafft.cbrc.jp/alignmeloaddnt/server/>) and Bioedit(18, 19). Polymorphism and divergence
254 were analyzed by DnaSP6 (version 6.12.03) (15). Analyses were conducted using the Nei-Gojobori
255 model(20). All positions containing gaps and missing data were eliminated. Evolutionary analyses
256 were conducted in Mega X (version 10.0.2) (21).

257

258 **4.3 Molecular dynamics (MD) simulation**

259 The complex structure of the SARS-CoV-2 S-protein RBD domain and human ACE2 was obtained
260 from National Microbiology Data Center (ID: NMDCS0000001) (PDB ID: 6LZG). Mutant amino
261 acids of the SARS-CoV-2 RBD mutants were directly replaced in the model, and the bat/pangolin
262 CoV RBD domain was modelled using SWISS-MODEL(16). Molecular dynamics simulation was
263 performed using GROMACS 2019 with the following options and parameters: explicit solvent
264 model, system temperature 37°C, OPLS/AA all-atoms force field, LINCS restraints. With 2fs steps,
265 each simulation was performed at 10ns, and each model was simulated 3 times to generate 3
266 independent trajectory replications. Binding free energy (ΔG) was calculated using MM-PBSA
267 method (software downloaded from GitHub: <https://github.com/Jerkwin/gmxtool>) with the
268 trajectories after structural equilibrium assessed using RMSD (Root Mean Square Deviation)(22).

269 The formula $\Delta G = RT \ln K_D$ was used to calculate between equilibrium dissociation constant (K_D)
270 and ΔG . The estimated ΔG of the RBD mutants were normalized using the ΔG of the prototype
271 strain which was derived from experimental data(23).

272

273 **4.4 Recombinant S protein mutant expression**

274 The SARS-CoV-2 prototype S gene was cloned into pNPM5 vector (Novoprotein, NJ, USA), fused
275 with C-terminal His₆-tag. V367F mutation was introduced using site-directed mutagenesis
276 according to the nucleotide sequence of the actual isolate. These two constructs were transfected
277 into HEK293 cells using polyethyleneimine, respectively. Since the S protein includes the signal
278 peptide in its N-terminal 14 amino acids, the S protein was secreted into the medium. The expressed
279 proteins were purified from filtered cell supernatants by Ni-NTA column. The eluted protein
280 solution was dialyzed in buffer PBS (pH7.4) for downstream assays.

281

282 **4.5 Ligand-receptor binding ELISA assay**

283 The human ACE2 was immobilized in the microtiter plate at 5 $\mu\text{g/ml}$ (100 $\mu\text{l/well}$). The S proteins
284 (prototype and V367F, respectively) was added as ligand at different concentrations, from 0.03
285 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$, and then incubated for 2 hours at 37°C to allow receptor-ligand interaction. The
286 ligand was then washed 3 times. 100 μl of HRP anti-His Tag Antibody (BioLegend, USA) (diluted
287 1:20000) was added to each well for 1 hour. After 3 times washing, the signal was visualized using
288 TMB solution (Sigma-Aldrich, USA). OD450 was recorded using microtiter plate reader.

289

290

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295
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297
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305
306 **Conflict of interest**

307 The authors declare that they have no conflicts of interest.

308
309
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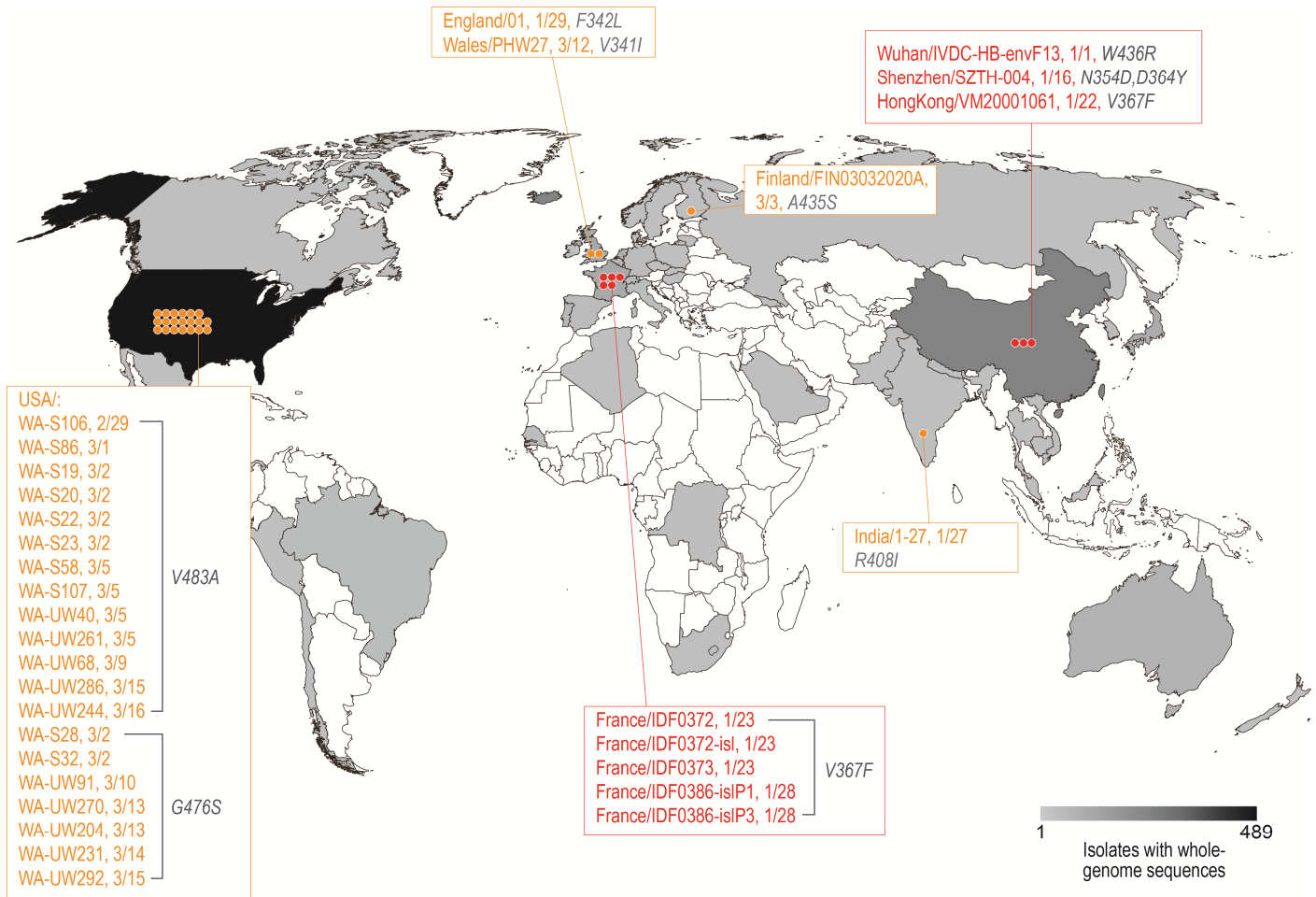
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381 *J Chem Inf Model* 59:4018–4033.

382

383 **Figure**

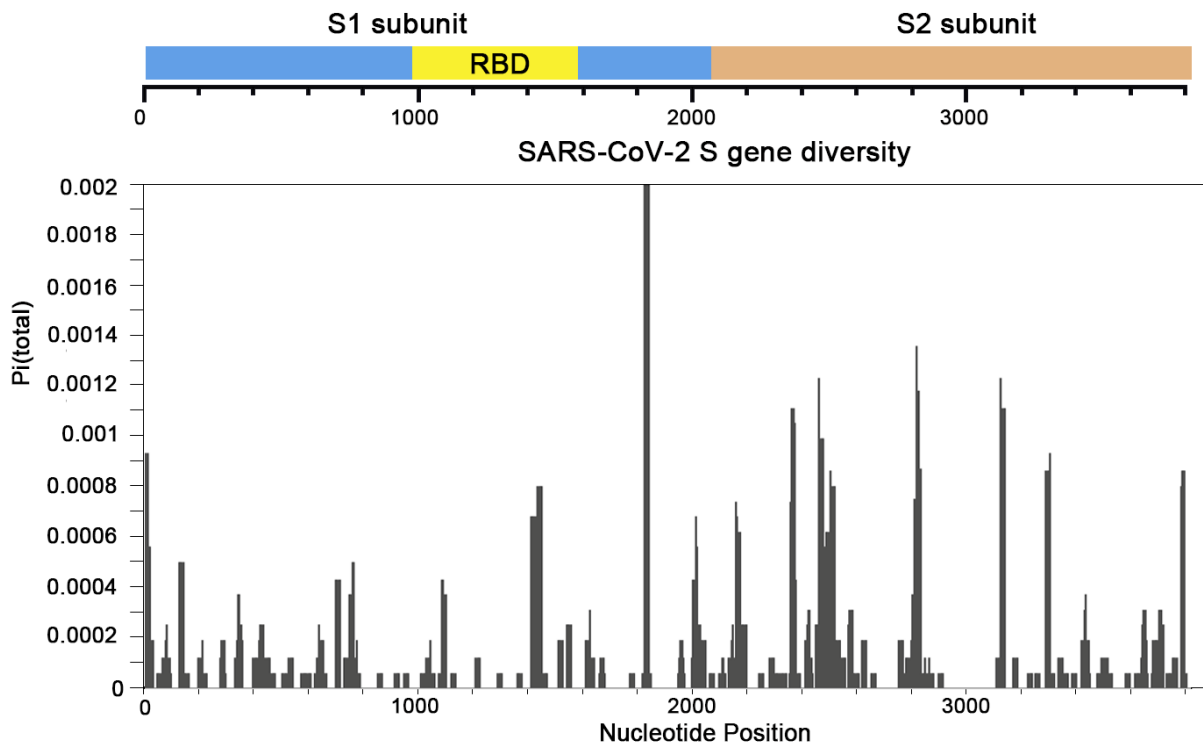
384 **Fig. 1: Distribution of the SARS-CoV-2 strains mutant in the RBD of the S protein.** The
385 geographic distribution of the 32 RBD mutant strains clustering into 9 mutant types is displayed.
386 The strains with names highlighted in red are mutants with the enhanced binding affinity. The
387 strains with names noted in yellow are mutants with similar binding affinities.



388

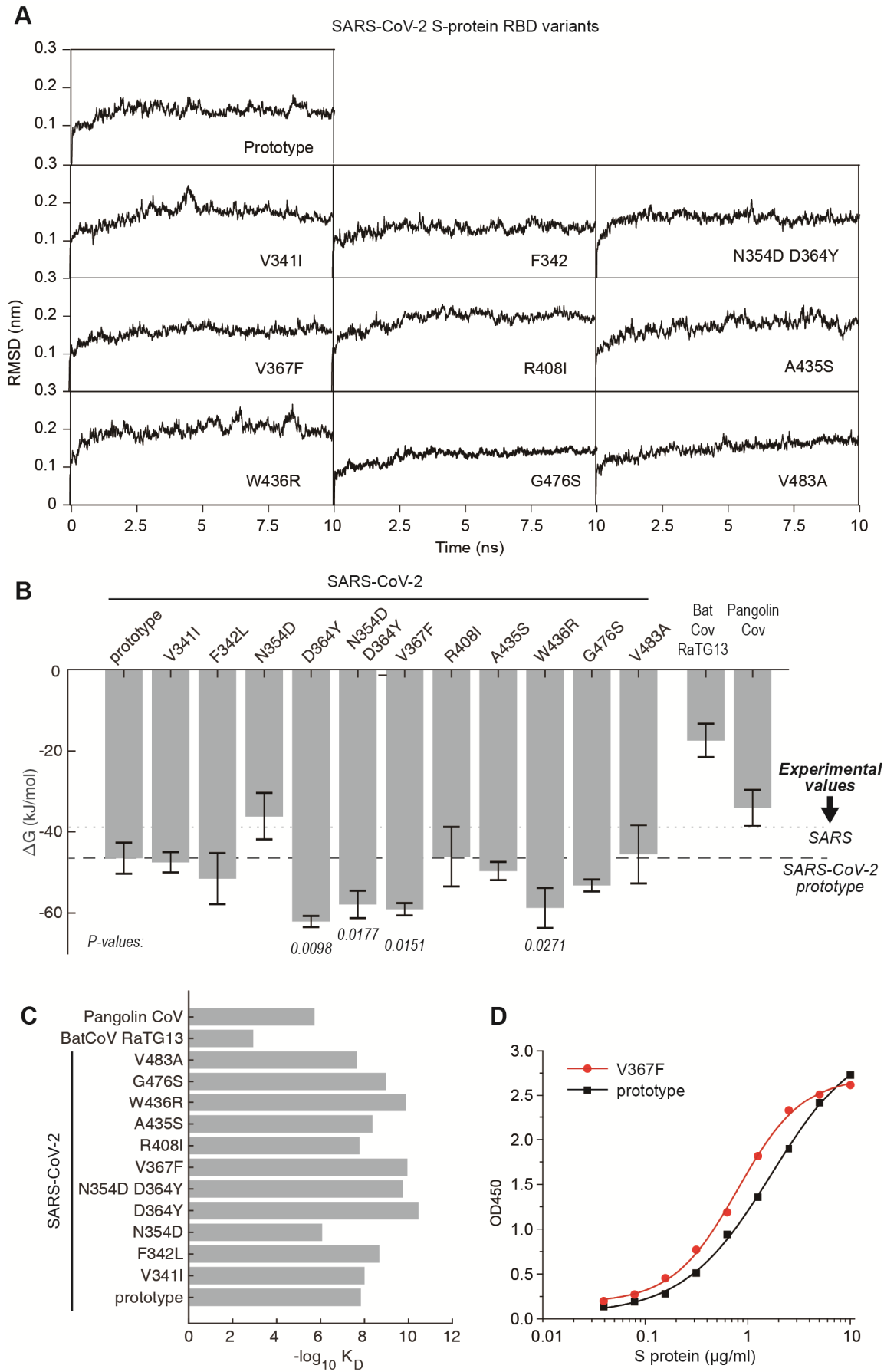
389

390 **Fig. 2: Polymorphism and divergence graph of SARS-CoV-2 S gene.** Polymorphism and
391 divergence were analyzed by DnaSP6 (version 6.12.03). Analyses were conducted using the
392 Nei-Gojobori model. All positions containing gaps and missing data were eliminated. Structural
393 domains are annotated. The Pi values are calculated with window size: 20 nt, step size: 5.
394

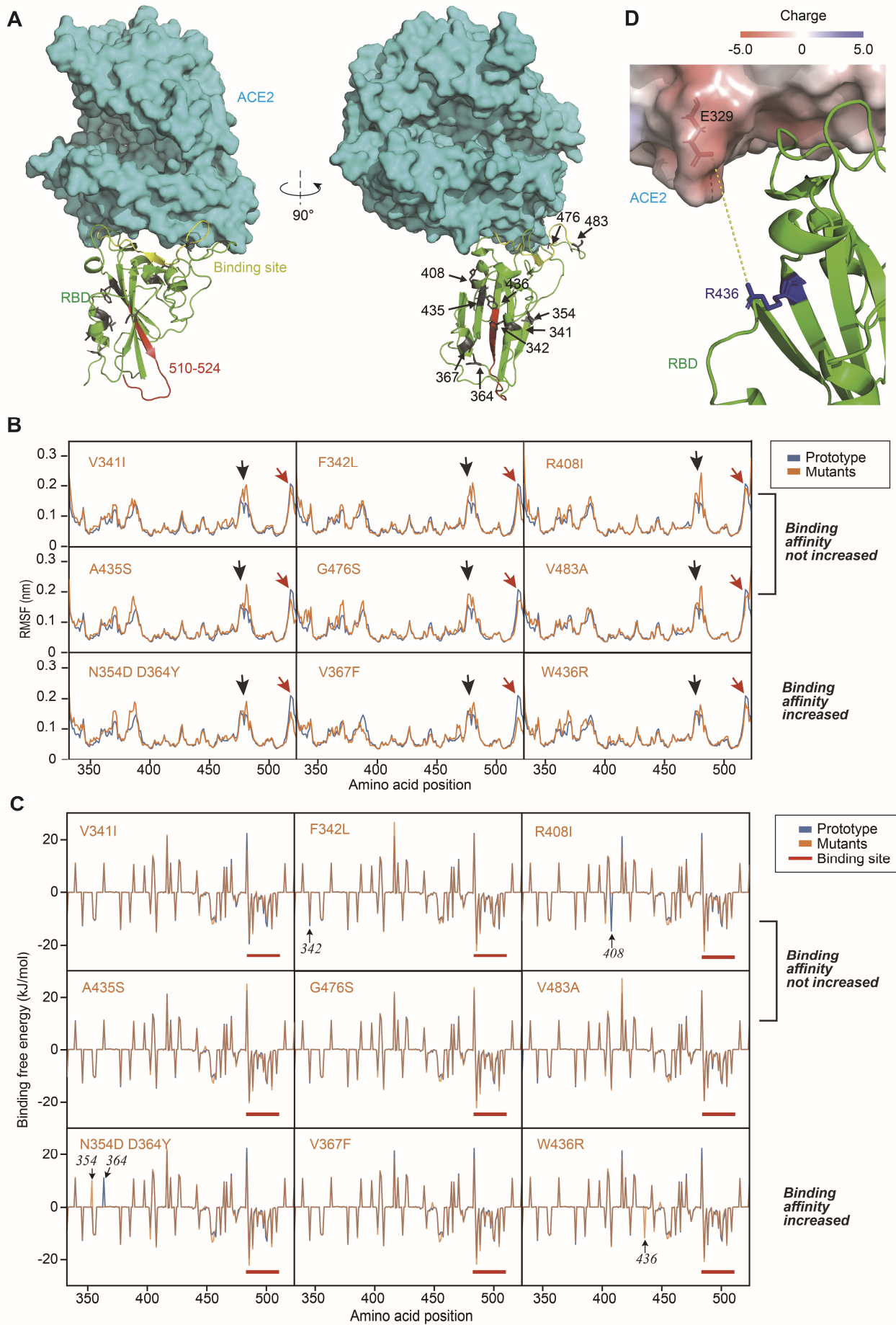


395
396
397

398 **Fig. 3: Binding free energy calculated for the SARS-CoV-2 S-RBD to human ACE2. (A)** RMSD
399 of typical MD trajectories of the SARS-CoV-2 prototype and the mutant strains. **(B)** Comparison of
400 the binding free energy (ΔG) of the RBDs and the human ACE2. Note, the ΔG is inversely
401 proportional to the binding affinity. Data are presented as mean \pm SD. *P*-values were calculated using
402 single-tailed student t-test. The *P*-values are shown for those with $P < 0.05$. The ΔG calculated from
403 experimental K_D values of SARS and SARS-CoV-2 prototype are marked in dotted and dashed
404 lines, respectively. **(C)** Comparison of the equilibrium dissociation constants (K_D) as calculated
405 with the ΔG . **(D)** Comparison of the binding affinity of prototype S protein and V367F mutant to
406 human ACE2 by ligand-receptor binding ELISA assay.



408 **Fig. 4: Structural analysis of RBD mutants and the effects on their binding affinity. (A)** Spatial
409 location of the mutant amino acids and the fragment 510-524. **(B)** RMSF of the nine mutants were
410 compared to that of the prototype. Red arrows denote the fragment of residues 510-524. Black
411 arrows denote the fragment of residues 475-485. **(C)** Contribution of each amino acid to the binding
412 free energy. Red bars denote the binding site. **(D)** View of the interaction surface of ACE2, with
413 charge density noted. The arginine of the W436R mutant is in the proximity of the negatively
414 charged amino acids. The electrostatic surface charges of the ACE2 are calculated using Pymol,
415 with the charge unit K_bT/e_c , as noted in the Pymol manual.



417 **Table 1: Nucleotide substitution rates and selection pressures for S gene.**

418 The numbers of nonsynonymous and synonymous differences per sequence from averaging over all
419 sequence pairs are shown. Analyses were conducted using the Nei-Gojobori model. The analysis
420 involved 1609 SARS-CoV-2 S gene sequences. All positions containing gaps and missing data were
421 discarded.

422

Gene	Length(bp)	Mean Non-synonymous Substitutions/site	Mean Synonymous Substitutions/site	dN/dS
S	3822	0.7726	0.1875	4.1197
S1	2043	0.6207	0.0571	10.8636
S1-RBD	585	0.0458	0.0137	3.3545
S2	1779	0.1519	0.1304	1.1646

423

424 **Supplementary data**

425 **Supplementary Table 1: Meta data of the strains with non-synonymous mutations in the RBD of spike glycoprotein.**

GISAID Virus name	RBD mutation	Collection date	Location	Gender	Age	Specimen source	Additional information	Accession ID
hCoV-19/Wuhan/IVDC-HB-envF 13/2020	W436R	2020/1/1	Asia/China/Hubei/Wuhan	Unknown	Unknown	Environment	HuananSeafoodMarket	EPI_ISL_408511
hCoV-19/Shenzhen/SZTH-004/20 20	N354D, D364Y	2020/1/16	Asia/China/Guandong/Shenzhen	Male	63	Alveolarlavage fluid		EPI_ISL_406595
hCoV-19/HongKong/VM2000106 1/2020	V367F	2020/1/22	Asia/HongKong	Male	39	Nasopharyngealaspirate&Throatswab		EPI_ISL_412028
hCoV-19/France/IDF0372/2020	V367F	2020/1/23	Europe/France/Ile-de-France/Paris	Female	31	Oro-Pharyngealswab		EPI_ISL_406596
hCoV-19/France/IDF0372-is1/2020	V367F	2020/1/23	Europe/France/Ile-de-France/Paris	Female	31	Oro-Pharyngealswab		EPI_ISL_410720
hCoV-19/France/IDF0373/2020	V367F	2020/1/23	Europe/France/Ile-de-France/Paris	Male	32	Orao-pharungealswab		EPI_ISL_406597
hCoV-19/India/1-27/2020	R408I	2020/1/27	Asia/India/Kerala	Female	20	Throatswab	TravelhistorytoChina	EPI_ISL_413522
hCoV-19/France/IDF0386-is1P1/2020	V367F	2020/1/28	Europe/France/Ile-de-France/Paris	Female	30	Naso-pharyngealrelatedtoEPI_ISL_406596 alswab		EPI_ISL_411219
hCoV-19/France/IDF0386-is1P3/2020	V367F	2020/1/28	Europe/France/Ile-de-France/Paris	Female	30	Naso-pharyngealrelatedtoEPI_ISL_406596 alswab		EPI_ISL_411220
hCoV-19/England/01/2020	F342L	2020/1/29	Europe/England	Female	50	swab	Englandclusterpatient1	EPI_ISL_407071
hCoV-19/USA/WA-S106/2020	V483A	2020/2/29	NorthAmerica/USA/Washington	Unknown	Unknown			EPI_ISL_417159
hCoV-19/USA/WA-S86/2020	V483A	2020/3/1	NorthAmerica/USA/Washington	Unknown	Unknown			EPI_ISL_417139
hCoV-19/USA/WA-S19/2020	V483A	2020/3/2	NorthAmerica/USA/Washington	Unknown	Unknown			EPI_ISL_417072

hCoV-19/USA/WA-S20/2020	V483A	2020/3/2	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_417073
hCoV-19/USA/WA-S22/2020	V483A	2020/3/2	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_417075
hCoV-19/USA/WA-S23/2020	V483A	2020/3/2	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_417076
hCoV-19/USA/WA-S28/2020	G476S	2020/3/2	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_417081
hCoV-19/USA/WA-S32/2020	G476S	2020/3/2	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_417085
hCoV-19/Finland/FIN03032020A/ 2020	A435S	2020/3/3	Europe/Finland/Helsinki	Male	40	EPI_ISL_413602
hCoV-19/USA/WA-S58/2020	V483A	2020/3/5	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_417111
hCoV-19/USA/WA-S107/2020	V483A	2020/3/5	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_417160
hCoV-19/USA/WA-UW40/2020	V483A	2020/3/5	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_415605
hCoV-19/USA/WA-UW261/2020	V483A	2020/3/5	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_418046
hCoV-19/USA/WA-UW68/2020	V483A	2020/3/9	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_415596
hCoV-19/USA/WA-UW91/2020	G476S	2020/3/10	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_416447
hCoV-19/Wales/PHW27/2020	V341I	2020/3/12	Europe/UnitedKingdom/Wales	Male	49	EPI_ISL_415655
hCoV-19/USA/WA-UW270/2020	G476S	2020/3/13	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_418055
hCoV-19/USA/WA-UW204/2020	G476S	2020/3/13	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_417353
hCoV-19/USA/WA-UW231/2020	G476S	2020/3/14	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_417380
hCoV-19/USA/WA-UW286/2020	V483A	2020/3/15	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_418071
hCoV-19/USA/WA-UW292/2020	G476S	2020/3/15	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_418077
hCoV-19/USA/WA-UW244/2020	V483A	2020/3/16	NorthAmerica/USA/Washington	Unknown	Unknown	EPI_ISL_418029

427 **Supplementary Figure 1: Multiple alignments of the RBD amino acid sequences. SARS-CoV-2**
428 **Wuhan-Hu-1, the first reported genome, is used as reference. A bat and a pangolin SARS-like**
429 **coronavirus are also included. Amino acid substitutions are marked. Dots indicate identical**
430 **amino acids.**
431

432	330	340	350	360	370	
433					
434	PNITNLC	PFGEVFNATR	FASVYAWN	RKRISNCVADY	SVLYNSASFSTFKC	SARS-CoV-2 Wuhan-Hu-1
435	F.....	SARS-CoV-2 France/IDF0372
436	F.....	SARS-CoV-2 France/IDF0373
437	F.....	SARS-CoV-2 France/IDF0372-isl
438	F.....	SARS-CoV-2 France/IDF0386-islP1
439	F.....	SARS-CoV-2 France/IDF0386-islP3
440	F.....	SARS-CoV-2 Hong Kong/VM20001061
441	SARS-CoV-2 Wuhan/IVDC-HB-envF13
442	D.....	Y.....	SARS-CoV-2 Shenzhen/SZTH-004
443	SARS-CoV-2 Finland/FIN03032020A
444	I.....	SARS-CoV-2 Wales/PHW27
445	L.....	SARS-CoV-2 England/01
446	SARS-CoV-2 India/1-27
447	SARS-CoV-2 USA/WA-UW31
448	SARS-CoV-2 USA/WA-S19
449	SARS-CoV-2 USA/WA-S20
450	SARS-CoV-2 USA/WA-S22
451	SARS-CoV-2 USA/WA-S23
452	SARS-CoV-2 USA/WA-S58
453	SARS-CoV-2 USA/WA-S86
454	SARS-CoV-2 USA/WA-S106
455	SARS-CoV-2 USA/WA-S107
456	SARS-CoV-2 USA/WA-UW68
457	SARS-CoV-2 USA/WA-UW244
458	SARS-CoV-2 USA/WA-UW286
459	SARS-CoV-2 USA/WA-UW40
460	SARS-CoV-2 USA/WA-UW292
461	SARS-CoV-2 USA/WA-S28
462	SARS-CoV-2 USA/WA-S32
463	SARS-CoV-2 USA/WA-UW91
464	SARS-CoV-2 USA/WA-UW270
465	SARS-CoV-2 USA/WA-UW204
466	SARS-CoV-2 USA/WA-UW231
467	T.....	T.....	Bat SARS-like Yunnan/RaTG13
468	T.....	T.....	Pangolin SARS-like Guandong/1
469						
470						

510	430	440	450	460	470					
511									
512	TGC	VI	AWNSNNL	DSK	VGG	NYNLYRLFRKSNL	KPFERDISTE	IYQAGSTP	SARS-CoV-2	Wuhan-Hu-1
513	SARS-CoV-2	France/IDF0372
514	SARS-CoV-2	France/IDF0373
515	SARS-CoV-2	France/IDF0372-isl
516	SARS-CoV-2	France/IDF0386-islP1
517	SARS-CoV-2	France/IDF0386-islP3
518	SARS-CoV-2	Hong Kong/VM20001061
519	R.	SARS-CoV-2	Wuhan/IVDC-HB-envF13
520	SARS-CoV-2	Shenzhen/SZTH-004
521	S.	SARS-CoV-2	Finland/FIN03032020A
522	SARS-CoV-2	Wales/PHW27
523	SARS-CoV-2	England/01
524	SARS-CoV-2	India/1-27
525	SARS-CoV-2	USA/WA-UW31
526	SARS-CoV-2	USA/WA-S19
527	SARS-CoV-2	USA/WA-S20
528	SARS-CoV-2	USA/WA-S22
529	SARS-CoV-2	USA/WA-S23
530	SARS-CoV-2	USA/WA-S58
531	SARS-CoV-2	USA/WA-S86
532	SARS-CoV-2	USA/WA-S106
533	SARS-CoV-2	USA/WA-S107
534	SARS-CoV-2	USA/WA-UW68
535	SARS-CoV-2	USA/WA-UW244
536	SARS-CoV-2	USA/WA-UW286
537	SARS-CoV-2	USA/WA-UW40
538	S.	SARS-CoV-2	USA/WA-UW292
539	S.	SARS-CoV-2	USA/WA-S28
540	S.	SARS-CoV-2	USA/WA-S32
541	S.	SARS-CoV-2	USA/WA-UW91
542	S.	SARS-CoV-2	USA/WA-UW270
543	S.	SARS-CoV-2	USA/WA-UW204
544	S.	SARS-CoV-2	USA/WA-UW231
545	KHI.A.E.F.	Bat SARS-like	Yunnan/RaTG13
546	Pangolin SARS-like	Guandong/1
547										
548										

