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A monoclonal antibody that neutralizes SARS-CoV-2 variants, SARS-CoV, and other sarbecoviruses

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39 **The repeated emergence of highly pathogenic human coronaviruses as well as**
40 **their evolving variants highlight the need to develop potent and broad-spectrum**
41 **antiviral therapeutics and vaccines. By screening monoclonal antibodies (mAbs)**
42 **isolated from COVID-19-convalescent patients, we found one mAb, 2-36, with**
43 **cross-neutralizing activity against SARS-CoV. We solved the cryo-EM structure of**
44 **2-36 in complex with SARS-CoV-2 or SARS-CoV spike, revealing a highly**
45 **conserved epitope in the receptor-binding domain (RBD). Antibody 2-36**
46 **neutralized not only all current circulating SARS-CoV-2 variants and SARS-COV,**
47 **but also a panel of bat and pangolin sarbecoviruses that can use human**
48 **angiotensin-converting enzyme 2 (ACE2) as a receptor. We selected 2-36-escape**
49 **viruses *in vitro* and confirmed that K378T in SARS-CoV-2 RBD led to viral**
50 **resistance. Taken together, 2-36 represents a strategic reserve drug candidate for**
51 **the prevention and treatment of possible diseases caused by pre-emergent**
52 **SARS-related coronaviruses. Its epitope defines a promising target for the**
53 **development of a pan-sarbecovirus vaccine.**

54

55 **Introduction**

56 Coronaviruses are zoonotic pathogens found in avian and mammalian reservoirs, and
57 seven strains have been found to spillover to humans. Among them, four continually
58 circulate in the human population and only cause mild symptoms of the common cold:
59 229E and NL63 belong to the *alpha-coronavirus* genus and OC43 and HKU1 belong to
60 the *beta-coronavirus* genus [1]. The other three human coronaviruses are all highly
61 pathogenic and belong to the *beta-coronavirus* genus: severe acute respiratory

62 syndrome coronavirus 2 (SARS-CoV-2), causing the current COVID-19 pandemic, and
63 SARS-CoV, which caused an outbreak 18 years ago, are members of the subgenus
64 *sarbecovirus*; whereas Middle-East respiratory syndrome coronavirus (MERS-CoV) is a
65 member of the *merbecovirus* subgenus [2].

66

67 Phylogenetic analysis of the entire genomes grouped SARS-CoV-2 and SARS-CoV with
68 some SARS-related coronaviruses found in bats or pangolins, including bat
69 coronaviruses RaTG13, Rs4231, SHC014, and WIV1, as well as pangolin
70 coronaviruses Pangolin Guangdong and Pangolin Guangxi in the *Sarbecovirus*
71 subgenus [2]. Both SARS-CoV-2 and SARS-CoV express a transmembrane
72 glycoprotein termed spike protein, which mediates viral entry into host cells by engaging
73 ACE2 as the receptor [3,4] and is therefore the primary target of virus-neutralizing
74 antibodies. There is also experimental evidence showing that some of these bat or
75 pangolin viruses could enter into human cells expressing ACE2 [5], indicating their
76 pandemic potential.

77

78 SARS-CoV-2 is the causative agent of COVID-19, having infected >238 million people
79 and caused >4.8 million deaths worldwide. Over the past year, several protective
80 vaccines and neutralizing antibody-based therapeutics have become available.
81 However, the emergence of SARS-CoV-2 variants has altered the landscape,
82 threatening the efficacy of these interventions. We and others have shown that some
83 variants such as B.1.351 [6], P.1 [7], B.1.526 [8] and B.1.427/B.1.429 [9] are more
84 resistant to neutralization by some mAbs, as well as by sera from convalescent patients

85 and vaccinees. As an example, a single mutation, E484K, found in several variants
86 could knock out a class of antibodies binding the receptor binding motif (RBM) on the
87 viral spike [6-8]. Therefore, finding a reagent that can target not only the SARS-CoV-2
88 mutant variants but also related sarbecoviruses is of utmost importance.

89

90 Here we describe the isolation of a mAb that cross-reacts and broadly neutralizes
91 SARS-CoV-2 variants, SARS-CoV, and a panel of bat and pangolin sarbecoviruses.
92 Structural analyses and *in vitro* escape mutation selection indicate that this mAb
93 targeting a highly conserved RBD epitope that could be informative for the development
94 of pan-sarbecovirus vaccines and therapeutics.

95

96 **Materials and methods**

97 **Cell lines**

98 HEK293T/17 (cat# CRL-11268) and Vero E6 cells (cat# CRL-1586) were from ATCC,
99 293T-ACE2 cells were kindly provided by J. Sodroski of Harvard Medical School, and
100 they were cultured in 10% fetal bovine serum (FBS, GIBCO cat# 16140071)
101 supplemented Dulbecco's Modified Eagle Medium (DMEM, ATCC cat# 30-2002) at
102 37°C, 5% CO₂. I1 mouse hybridoma cells (ATCC, cat# CRL-2700) were cultured in
103 Eagle's Minimum Essential Medium (EMEM, ATCC cat# 30-2003)) with 20% FBS.

104 **Pseudovirus neutralization assays**

105 Plasmids encoding the single-mutation and the combination of mutations found in
106 SARS-CoV-2 variants were generated by Quikchange II XL site-directed mutagenesis

107 kit (Agilent). Recombinant Indiana vesicular stomatitis virus (VSV) expressing different
108 coronavirus spikes were generated as previously described [10,11]. Briefly, HEK293T
109 cells were grown to 80% confluency before transfection with the spike gene using
110 Lipofectamine 3000 (Invitrogen). Cells were cultured overnight at 37°C with 5% CO₂,
111 and VSV-G pseudo-typed ΔG-luciferase (G*ΔG-luciferase, Kerafast) was used to infect
112 the cells in DMEM at a multiplicity of infection (MOI) of 3 for 2 hrs before washing the
113 cells with 1X DPBS three times. The next day, the transfection supernatant was
114 harvested and clarified by centrifugation at 300 g for 10 min. Each viral stock was then
115 incubated with 20% I1 hybridoma (anti-VSV-G, ATCC: CRL-2700) supernatant for 1 hr
116 at 37°C to neutralize contaminating VSV-G pseudo-typed ΔG-luciferase virus before
117 measuring titers and making aliquots to be stored at -80°C. Neutralization assays were
118 performed by incubating each pseudovirus with serial dilutions of a mAb and scored by
119 the reduction in luciferase gene expression as previously described [10,11]. Briefly,
120 Vero E6 cells (for SARS-CoV-2 and SARS-CoV) or 293T-ACE2 cells (for bat/pangolin
121 coronaviruses) were seeded in 96-well plates (2 ×10⁴ cells per well). Each pseudovirus
122 was incubated with serial dilutions of a mAb in triplicate for 30 min at 37°C. The
123 mixture was added to cultured cells and incubated for an additional 16 hrs.
124 Luminescence was measured using Luciferase Assay System (Promega), and IC₅₀ was
125 defined as the dilution at which the relative light units were reduced by 50% compared
126 with the virus control wells (virus + cells) after subtraction of the background in the
127 control groups with cells only. The IC₅₀ values were calculated using a five-parameter
128 dose-response curve in GraphPad Prism v.8.4.

129 **Authentic SARS-CoV-2 microplate neutralization**

130 The SARS-CoV-2 viruses USA-WA1/2020 (WA1), hCoV-19/USA/CACDC_5574/2020
131 (B.1.1.7), hCoV-19/South Africa/KRISP-K005325/2020 (B.1.351), hCoV-19/Japan/TY7-
132 503/2021 (P.1), and hCoV-19/USA/NY-NP-DOH1/2021 (B.1.526) were obtained from
133 BEI Resources (NIAID, NIH). The viruses were propagated for one passage using Vero
134 E6 cells. Virus infectious titer was determined by an end-point dilution and cytopathic
135 effect (CPE) assay on Vero E6 cells as described previously [10,11]. An end-point-
136 dilution microplate neutralization assay was performed to measure the neutralization
137 activity of purified mAbs. Triplicates of each dilution were incubated with SARS-CoV-2
138 at an MOI of 0.1 in EMEM with 7.5% inactivated FBS for 1 hr at 37°C. Post incubation,
139 the virus-antibody mixture was transferred onto a monolayer of Vero E6 cells grown
140 overnight. The cells were incubated with the mixture for ~70 hrs. CPE was visually
141 scored for each well in a blinded fashion by two independent observers. The results
142 were then converted into percentage neutralization at a given sample dilution or mAb
143 concentration, and the averages \pm SEM were plotted using a five-parameter dose-
144 response curve in GraphPad Prism v.8.4.

145 **SARS-CoV neutralization assay**

146 Antibodies were subjected to successive two-fold dilutions starting from 20 μ g/ml.
147 Quadruplicates of each dilution were incubated with SARS-CoV GZ50 strain (GenBank
148 accession no. AY304495) at MOI of 0.01 in DMEM with 2% inactivated FBS for 1 hr at
149 37°C [12]. After incubation, the virus-antibody mixture was transferred onto a monolayer
150 of Vero E6 cells grown overnight. The cells were incubated with the mixture for 72 hrs.
151 Cytopathogenic effects of viral infection were visually scored for each well in a blinded
152 manner by two independent observers. The results were then converted into the

153 percentage of neutralization at a given monoclonal antibody concentration, and the data
154 were plotted using a five-parameter dose–response curve in GraphPad Prism v.8.4.

155

156 **Protein expression and purification**

157

158 The SARS-CoV-2 and SARS-CoV S2P spike constructs were produced as previously
159 described [3]. The proteins were expressed in HEK293 Freestyle cells (Invitrogen) in
160 suspension culture using serum-free media (Invitrogen) and transfected into HEK293
161 cells using polyethyleneimine (Polysciences). Cell growths were harvested four days
162 after transfection, and the secreted proteins were purified from supernatant by nickel
163 affinity chromatography using Ni-NTA IMAC Sepharose 6 Fast Flow resin (GE
164 Healthcare) followed by size exclusion chromatography on a Superdex 200 column (GE
165 Healthcare) in 10 mM Tris, 150 mM NaCl, pH 7.4. Spike purity was assessed by SDS-
166 PAGE. 2-36 was expressed and purified as previously described [10]. Fab fragments
167 were produced by digestion of IgGs with immobilized papain at 37 °C for 3 hrs in 50 mM
168 phosphate buffer, 120 mM NaCl, 30 mM cysteine, 1 mM EDTA, pH 7. The resulting
169 Fabs were purified by affinity chromatography on protein A, and purity was assessed by
170 SDS-PAGE.

171

172 **ELISA**

173

174 ELISA detection of mAbs binding to SARS-CoV-2 and SARS-CoV spike trimers was
175 performed as previously described [10]. For the competition ELISA, purified mAbs were

176 biotin-labelled using One-Step Antibody Biotinylation Kit (Miltenyi Biotec) following the
177 manufacturer's recommendations and purified using 40K MWCO Desalting Column
178 (ThermoFisher Scientific). Serially diluted competitor antibodies (50 μ l) were added into
179 spike trimer-precoated ELISA plates, followed by 50 μ l of biotinylated antibodies at a
180 concentration that achieves an OD₄₅₀ reading of 1.5 in the absence of competitor
181 antibodies. Plates were incubated at 37°C for 1 hr, and 100 μ l of 500-fold diluted
182 Avidin-HRP (ThermoFisher Scientific) was added into each well and incubated for
183 another 1 hr at 37°C. The plates were washed with PBST between each of the
184 previous steps. The plates were developed afterwards with 3,3',5,5'-
185 tetramethylbenzidine (TMB) and absorbance was read at 450 nm after the reaction was
186 stopped. For the ACE2 competition ELISA, 100 ng of ACE2 protein (Abcam) was
187 immobilized on the plates at 4°C overnight. The unbound ACE2 was washed away by
188 PBST and then the plates were blocked. After washing, 100 ng of S trimer in 50 μ l
189 dilution buffer was added into each well, followed by addition of another 50 μ l of serially
190 diluted competitor antibodies and then incubation at 37°C for 1 hr. The ELISA plates
191 were washed four times with PBST and then 100 μ l of 2,000-fold diluted anti-strep-HRP
192 (Millipore Sigma) was added into each well for another 1 hr at 37°C. The plates were
193 then washed and developed with TMB, and absorbance was read at 450 nm after the
194 reaction was stopped.

195

196 **Surface plasmon resonance (SPR)**

197

198 The antibody binding affinity to SARS-CoV-2 and SARS-CoV spike trimers and RBDs
199 was detected by Biacore T200 SPR system (Cytiva). All experiments were performed at
200 25°C in HBS-EP+ buffer (10 mM HEPES, pH 7.4; 150 mM NaCl; 3.4 mM EDTA; 0.005%
201 (v/v) surfactant P20). The anti-His tag antibodies, diluted at 50 µg/mL in 10 mM sodium
202 acetate, pH 4.5, were immobilized on both the active and reference flow cells surface of
203 the activated CM5 sensor chip using amine coupling method. Approximately 200 RU of
204 His-tagged SARS-CoV-2 and SARS-CoV spike trimers and RBDs were captured onto
205 the chip for the active surface, and anti-His antibody alone served as the reference
206 surface. The antibodies were injected through both flow cells at different concentrations
207 (ranging from 300-1.2 nM in 1:3 successive dilutions) at a flow rate of 30 µL/min for 120
208 s, followed by a 15 s dissociation step. After each assay cycle, the sensor surface was
209 regenerated with a 30 s injection of 10 mM glycine, pH 1.5, at a flow rate of 30 µL/min.
210 Background binding to reference flow cells was subtracted and antibody binding levels
211 were calculated using Biacore T200 evaluation software (GE Healthcare).

212

213 **Cryo-EM grid preparation**

214

215 Samples for cryo-EM grid preparation were produced by mixing purified spike protein to
216 a final trimer concentration of 0.33 mg/mL with 2-36 Fab in a 1:9 molar ratio, followed by
217 incubation on ice for 1 hr. The final buffer for the 2-36 complex was 10 mM sodium
218 acetate, 150 mM NaCl, pH 5.5. n-Dodecyl β-D-maltoside (DDM) at a final concentration
219 of 0.005% (w/v) was added to the mixtures to prevent preferred orientation and
220 aggregation during vitrification. Cryo-EM grids were prepared by applying 3 µL of

221 sample to a freshly glow-discharged carbon-coated copper grid (CF 1.2/1.3 300 mesh);
222 the sample was vitrified in liquid ethane using a Vitrobot Mark IV with a wait time of 30
223 s, a blot time of 3 s, and a blot force of 0.

224

225 **Cryo-EM data collection and analysis**

226

227 Cryo-EM data for single particle analysis was collected on a Titan Krios electron
228 microscope operating at 300 kV, equipped with an energy filter and a Gatan K3-
229 BioQuantum direct detection detector, using the Legion [13] software package.
230 Exposures were taken with a total electron fluence of 41.92 e-/Å² fractionated over 60
231 frames, with a total exposure time of 3 seconds. A defocus range of -0.8 to -2.5 μm was
232 used with a magnification of 81,000x, and a pixel size of 1.07 Å.

233 Data processing was performed using cryoSPARC v2.15 [14]. Raw movies were
234 aligned and dose-weighted using patch motion correction, and the CTF was estimated
235 using patch CTF estimation. Micrographs were picked using blob picker, and a particle
236 set was selected using 2D and 3D classification. The resulting particle set was refined
237 to high resolution using a combination of heterogenous and homogenous refinement,
238 followed by nonuniform refinement. The interface between RBD and 2-36 Fab was
239 locally refined by using a mask that included RBD and the variable domains of the Fab.
240 The final global and local maps were deposited to the EMDB with ID: EMD-24190.

241

242 **Model building and refinement**

243

244 The 2-36-RBD complex model was built starting from template PDB structures 6BE2
245 (Fab) and 7BZ5 (RBD) using Phenix Sculptor. SARS-CoV-2 S2P spike density was
246 modeled starting with PDB entry 6VXX [15]. Automated and manual model building
247 were iteratively performed using real space refinement in Phenix [16] and Coot [17]. 2-
248 36 Fab residues were numbered according to Kabat numbering scheme. Geometry
249 validation and structure quality assessment were performed using Molprobit [18].
250 PDBePISA was used to calculate buried surface area [19]. A summary of the cryo-EM
251 data collection, processing, and model refinement statistics is shown in Table S1. The
252 final model was deposited in the PDB with ID 7N5H.

253

254 **Structure Conservation Analysis**

255

256 The conservation of each RBD residue was calculated using the entropy function of the
257 R package bio3d (H.norm column). The calculation was based on the sequence
258 alignment of SARS-CoV, SARS-CoV-2 and SARS-related bat coronavirus. The
259 visualization of sequence entropy was displayed by PyMol version 2.3.2.

260

261 **In vitro selection for resistant mutations against mAb 2-36**

262

263 SARS-Cov-2 isolate USA-WA1/2020 was mixed with serial five-fold dilutions of 2-36
264 antibody at MOI 0.2 and incubated for 1 hr. Following incubation, the mix was overlaid
265 on 24-well plate to a final volume of 1mL. the plates were incubated at 37°C for 70 hrs
266 till CPE was complete (100%) in virus control wells bearing no antibody. At this time, all

267 wells were scored to determine the 50% inhibition titer (EC50) and supernatant
268 collected from this well was used for subsequent round of selection. Passaging
269 continued till the virus was able to form CPE in the presence of 50 µg/mL of 2-36
270 antibody. At this point, the resulting supernatant was collected, and RNA was extracted
271 using QiaAMP Viral RNA kit (Qiagen). cDNA was obtained using Superscript IV
272 enzyme (Thermo Scientific). Amplification of spike gene from cDNA was performed
273 using nested PCR and sequenced using Sanger sequencing (Genewiz). Multiple
274 clones from limiting dilution nested PCR were sequenced to confirm the dominant
275 mutants in the pool of the resulting progeny viruses and a percentage of their
276 prevalence was calculated from total number sequenced. For passage 4, 9 and 12, a
277 total of 20, 10 and 10 clones were sequenced respectively to confirm the mutations.

278

279 **Data availability**

280

281 The cryo-EM structure of antibody 2-36 in complex with prefusion SARS-CoV-2 spike
282 glycoprotein has been deposited in the PDB ID: 7N5H and EMDB ID: 24190.

283

284 **Results**

285

286 **Identification of a SARS-CoV-2 and SARS-CoV cross-reactive mAb from a COVID- 287 19 patient**

288 By single B-cell sorting and 10X Genomics sequencing, we have previously recovered
289 252 mAb sequences from five COVID-19 patients and isolated 19 potent neutralizing

290 mAbs [10] targeting different epitopes on SARS-CoV-2 spike [10,20-22]. To identify
291 mAbs with broad reactivity against other coronaviruses, we screened these 252 mAb
292 transfection supernatants for neutralization against SARS-CoV pseudovirus in the same
293 way we did for SARS-CoV-2 pseudovirus. Although about one fifth of mAbs showed
294 neutralization activities against SARS-CoV-2 [10], only one, 2-36, had an appreciable
295 neutralization potency against SARS-CoV (Figure 1A).

296

297 We then focused on 2-36, by carefully characterizing it on SARS-CoV-2 and SARS-CoV
298 with purified antibody. It neutralized SARS-CoV-2 pseudovirus at $IC_{50} \sim 0.04 \mu\text{g/mL}$ and
299 the authentic virus (WA1 strain) at $IC_{50} \sim 0.1 \mu\text{g/mL}$. Its potency versus SARS-CoV was
300 lower, with $IC_{50} \sim 0.2 \mu\text{g/mL}$ against the pseudovirus and $IC_{50} \sim 7.5 \mu\text{g/mL}$ against the
301 authentic virus (GZ50 strain) (Figure 1B). While 2-36 could bind to both SARS-CoV-2
302 and SARS-CoV spike trimer (Supplementary Figure 1), its binding affinity to SARS-CoV-
303 2 spike measured by SPR was much higher than that to SARS-CoV (Supplementary
304 Figure 2A). As our previous study already showed 2-36 is an RBD-directed mAb [10],
305 we also compared its binding affinity to SARS-CoV-2 and SARS-CoV RBDs, and
306 observed similar trend as seen for the spike proteins (Supplementary Figure 2B), with
307 higher affinity found for SARS-CoV-2 RBD.

308

309 **Cryo-EM structure of 2-36 in complex with SARS-CoV-2 and SARS-CoV spikes**

310 To gain insight into the epitope of 2-36, we first evaluated its competition with other
311 mAbs in binding to SARS-CoV-2 spike trimer by ELISA. Two mAbs isolated from SARS
312 patients and showed cross-reactivity against SARS-CoV-2 by targeting distinct

313 epitopes, CR3022 [23] and S309 [24], together with our SARS-CoV-2 mAb targeting the
314 RBM, 2-4 [10], were used in the competition experiments. 2-36 binding to SARS-CoV-2
315 spike was inhibited by CR3022, but not by S309 or 2-4 (Supplementary Figure 3A),
316 suggesting 2-36 targeted a region similar to the “highly conserved cryptic epitope” of
317 CR3022 [23]. To further investigate the molecular nature of the 2-36 epitope, we
318 determined cryo-EM structures for 2-36 Fab in complex with both SARS-CoV-2 and
319 SARS-CoV spike (S2P-prefusion-stabilized trimers). In the SARS-CoV-2 structure, a
320 single predominant population was observed wherein three 2-36 Fabs were bound per
321 spike in a 3-RBD-up conformation (Figure 2A and Supplementary Figure 4). In the
322 SARS-CoV structure, two 3D classes were observed: one Fab bound per spike in a 1-
323 RBD-up conformation and two Fabs bound per spike in a 2-RBD-up conformation
324 (Figure 2B and Supplementary Figure 5). For the SARS-CoV-2 complex structure, local
325 refinement provided side chain resolution for much of the interface, allowing
326 construction of a molecular model.

327

328 Antibody 2-36 recognizes a region on the ‘inner-side’ of RBD that is buried in the RBD-
329 down conformation of the spike [6]. Thus, 2-36 recognizes RBD only in the up position.
330 This is similar to the antibody epitopes previously defined for antibodies CR3022 [23]
331 and COVA1-16 [25]. The positioning of the 2-36 light chain causes a clash with ACE2 in
332 the spike-ACE2 complex (Figure 2C), which is consistent with competition ELISA data
333 (Supplementary Figure 3B), suggesting that blockage of receptor binding likely accounts
334 for neutralization by 2-36. The 2-36 epitope on RBD is highly conserved among
335 sarbecoviruses that utilize ACE2 for binding, including SARS-CoV-2, SARS-CoV, and a

336 panel of SARS-related coronaviruses (Figure 2D). For example, 24 out of 27 amino
337 acids that contact 2-36 are identical based on the sequence alignment of SARS-CoV-2
338 Wuhan-Hu-1 and SARS-CoV BJ01 (Supplementary Figure 6), consistent with a similar
339 binding mode between SARS-CoV-2 and SARS-CoV (Figure 2A and 2B).

340

341 The interaction of 2-36 with RBD is dominated by CDR H3 (472 Å² buried in the
342 interface), with minor contributions from CDR H1 (176 Å² buried) and CDR L2 (145 Å²
343 buried) (Figure 2E). CDR H3 forms most of the interactions by interacting with a loop on
344 RBD comprising residues 369-385. Residue 99 in the CDR H3 forms backbone
345 hydrogen bonds with RBD residue 379 to extend an RBD β-sheet, much like antibodies
346 C118 and C022 [26] (Figure 2E, bottom right). For 2-36, interactions were primarily
347 hydrophobic, with CDR H3 residues Tyr98, Tyr99, the aliphatic chain of Arg100a, and
348 Tyr100e all making significant contacts with hydrophobic residues on RBD.

349

350 **2-36 broadly neutralizes SARS-CoV-2 variants and SARS-related sarbecoviruses**

351 Given the high conservation of the 2-36 epitope, we went on to test its breadth on the
352 recent emerging SARS-CoV-2 variants first. Our previous studies already showed 2-36
353 retained its activities against SARS-CoV-2 variants B.1.1.7, B.1.351 [6], P.1 [7] and
354 B.1.526 [8], on both authentic viruses and pseudoviruses (replotted in Figure 3A). Here
355 we assessed 2-36 activity on more variants, including pseudoviruses representing the
356 combination of key spike mutations of B.1.427/B.1.429, R.1, B.1.1.1, B.1.525,
357 B.1.617.1, B.1.617.2 and B.1.1.7 with E484K, as well as many pseudoviruses with
358 single spike mutations which are naturally circulating in COVID-19 patients with high

359 frequency and located in the N-terminal domain, RBD, or S2. As shown in Figure 3A, 2-
360 36 could neutralize all the variants tested and maintained its potency against most of
361 the variants with IC₅₀ below 0.1 µg/mL.

362

363 We further explored 2-36's potential as a broadly neutralizing antibody against bat or
364 pangolin coronaviruses (Figure 3B) in the SARS-CoV-2-related lineage (bat coronavirus
365 RaTG13, pangolin coronavirus Guangdong and pangolin coronavirus Guangxi) and
366 SARS-CoV-related lineage (bat WIV1, SHC014, LYRa11, Rs7327, Rs4231 and
367 Rs4084), each of which can use human ACE2 as receptor [2,5]. For comparison, we
368 also tested COVA1-16, CR3022 and S309 in parallel with 2-36. As indicated by the
369 heatmap in Figure 3C and neutralization curves in Supplementary Figure 7, 2-36 could
370 neutralize all these sarbecoviruses. COVA1-16, with a similar epitope as 2-36 (Figure
371 2D), neutralized most of the viruses but with lower potency. S309 and CR3022 could
372 only neutralize part of this panel of viruses. None of the four antibodies could neutralize
373 MERS (*merbecovirus*) or 229E (*alpha-coronavirus*), reflecting their longer genetic
374 distance from SARS-CoV-2 and SARS-CoV (Figure 3B and C).

375

376 ***In vitro* selection of 2-36 escape virus**

377 To test whether SARS-CoV-2 can escape from 2-36 neutralization, we co-incubated the
378 authentic SARS-CoV-2 (WA1 strain) with serially diluted 2-36, and repeatedly passaged
379 the virus from wells showing 50% cytopathic effect (CPE) again in serial dilutions of the
380 antibody. 2-36 retained its neutralization activities on the serially passaged viruses until
381 passage 10 (Supplementary Figure 8), and then at passage 12, the virus became

382 resistant to the antibody (Figure 4A). Sequence analyses of the passage 12 virus
383 revealed four single point spike mutations (T284I, K378T, H655Y, V1128A), all of which
384 were found at 100% frequency. And when we went back to sequence the viruses from
385 earlier passages, only H655Y, which has also been found in other circulating variants
386 such as P.1 [27,28], and V1128A were found. The T284I and K378T mutations
387 appeared only in the 2-36-resistant virus (Figure 4B). We localized the selected
388 mutations in the model of 2-36 in complex with SARS-CoV-2 S trimer (Figure 4C), only
389 K378 resided in RBD and showed a strong van der Waals contact with Y98 of the 2-36
390 heavy chain, while all the other three residues were distal from the antibody interface.
391 Indeed, when these mutations were introduced into pseudoviruses and tested for their
392 sensitivity to 2-36, only K378T alone or in combination with the other mutations was
393 found to be resistant to 2-36, whereas viruses with T284I, H655Y, or V1128A alone
394 remained sensitive (Figure 4D). Interestingly, although the K378 position in SARS-CoV-
395 2 spike can be mutated to other residues at very low frequency, we could not find any
396 K378T mutation circulating in patient viruses to date (Figure 4E), further demonstrating
397 the conserved nature of the region recognized by 2-36.

398

399 **Discussion**

400 We have seen the repeated emergence of novel highly pathogenic human
401 coronaviruses that seriously threaten public health and the global economy in the last
402 two decades. While we are still in the midst of the COVID-19 pandemic, other animal
403 coronaviruses with the potential to cross the species-barrier to infect humans need to be
404 considered as potential threats in the future. Therefore, the development of potent and

405 broad-spectrum antiviral interventions against current and emerging coronaviruses is
406 critical. Here, in this study, we described a mAb, 2-36, that exhibited broadly
407 neutralizing activity against not only SARS-CoV-2 variants and SARS-CoV but also a
408 panel of related sarbecoviruses.

409
410 Several other studies have also reported the discovery of broadly neutralizing mAbs
411 against sarbecoviruses in addition to the controls used in this study, COVA1-16 and
412 S309. For example, H014 [29] and Ey6a [30] were reported to neutralize SARS-CoV
413 and SARS-CoV-2. Wec et al isolated several antibodies from a SARS survivor that
414 neutralized SARS-CoV, SARS-CoV-2, and the bat SARS-CoV-like virus WIV1 with
415 modest potency [31]. In addition, antibodies C118 and C022 were also shown to
416 neutralize another bat SARS-CoV-like virus SHC014 [26]. Compared to these studies,
417 we have included a much larger panel of viruses for testing the neutralization breadth of
418 2-36, with 4 viruses in the SARS-CoV-2-related lineage and 7 viruses in the SARS-CoV-
419 related lineage (Figure 3C). Very recently, additional antibodies with broad activities,
420 such as S2X259 [32] and DH1047 [33], targeting a similar surface on the inner side of
421 RBD as 2-36 (Supplementary figure 9), have been reported, again highlighting that the
422 conserved region of the inner face of RBD could represent a target for a pan-
423 sarbecovirus vaccine.

424
425 Apart from these RBD-directed mAbs, antibodies targeting the conserved S2 stem-helix
426 region of the coronavirus spike fusion machinery have been shown to possess even
427 broader reactivity against more beta-coronaviruses including MERS [34,35]. However,

428 their relatively low neutralization potency may limit their clinical utility. Using an in vitro
429 affinity maturation strategy, CR3022 has been re-engineered to neutralize SARS-CoV-2
430 more potently [36]. Similarly, Rappazzo et al engineered a SARS-CoV-2 and SARS-
431 CoV mAb into a better version, ADG-2, with enhanced neutralization breadth and
432 potency [37]. Perhaps 2-36 and S2-specific mAbs could be improved in the same way
433 without sacrificing neutralization breadth.

434

435 The cryo-EM structures of 2-36 in complex with both SARS-CoV-2 and SARS-CoV
436 spike trimers revealed a highly conserved epitope. This region on the inner side of RBD
437 is also targeted by several other mAbs [25,26,30]. The structural information from these
438 studies of broadly neutralizing antibodies isolated from natural infection could be
439 valuable in guiding immunogen design for the development of pan-sarbecovirus
440 vaccines. Such vaccines and 2-36-like broadly neutralizing antibodies could be
441 developed and stockpiled to prevent or mitigate future outbreaks of sarbecoviruses.

442

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452

453

454 **Author contributions:** The study was conceptualized by D.D.H. The biological
455 experiments and analyses were carried out by P.W., M.S.N., J.Y., M.W., J.F.-W.C., S.I.,
456 L.L., Z.C., K-Y.Y., and Y.H. The structural experiment and analysis were carried out by
457 R.G.C., G.C., Y.G., Z.S., P.D.K., and L.S. The manuscript was written by P.W., R.G.C.,
458 L.S., and D.D.H. and reviewed, commented, and approved by all the authors.

459

460 **Competing interests:** P.W., J.Y., M.N., Y.H., L.L., and D.D.H. are inventors on a
461 provisional patent application on mAbs to SARS-CoV-2.

462

463 **Figure legends**

464 **Figure 1. 2-36 neutralizes both SARS-CoV-2 and SARS-CoV.**

465 **(A)** Screening of mAb transfection supernatant for neutralizing activity against SARS-
466 CoV-2 and SARS-CoV pseudoviruses.

467 **(B)** 2-36 neutralization IC_{50} ($\mu\text{g/mL}$) against SARS-CoV-2 and SARS-CoV
468 pseudoviruses (PV) and live viruses (LV).

469

470 **Figure 2. Cryo-EM structure of 2-36 in complex with SARS-CoV-2 and SARS-CoV**
471 **Spike.**

472 **(A)** Cryo-EM reconstruction of 2-36 Fab in complex with the SARS-CoV-2 S trimer at
473 3.4 Å. RBD is colored in green, the 2-36 Fab heavy chain in darker blue, the light chain
474 in lighter blue, and the rest of the spike is colored in light purple with glycans in darker
475 purple.

476 **(B)** Cryo-EM reconstructions of 2-36 Fab in complex with the SARS-CoV S trimer reveal
477 two major classes: one 2-36 Fab bound to spike with 1-RBD up, and two 2-36 Fabs
478 bound to spike with 2-RBD up. Reconstructions are shown in two different orientations.

479 **(C)** The 2-36 interface model superposed onto an ACE2-RBD complex model (pdb:
480 6M0J) shows an ACE2 clash with the light chain.

481 **(D)** Conservation analysis on the RBD among SARS-CoV-2, SARS-CoV-1, and bat and
482 pangolin sarbecoviruses show 2-36 binding site is highly conserved, with regions of high
483 conservation in grey and low conservation in red. The 2-36 epitope is outlined in dark
484 blue, and the epitope for COVA1-16 is outlined in cyan.

485 **(E)** The interface model depicted in ribbon representation, with the CDR1 loops in gold,
486 CDR2 loops in orange, and CDR3 loops in red. The interface residue contacts are show
487 for CDR H1, H3, and L2 complemented with the electron density map for a 4.1 Å local
488 reconstruction.

489

490 **Figure 3. 2-36 neutralizes SARS-CoV-2 variants and SARS-CoV-like**
491 **coronaviruses.**

492 **(A)** 2-36 neutralization IC₅₀ (µg/mL) against SARS-CoV-2 variants.

493 **(B)** Phylogenetic tree of SARS-CoV-2- and SARS-CoV-related lineages and other
494 coronaviruses constructed via MEGA7 and maximum likelihood analysis of spike amino
495 acid sequences extracted from the NCBI and GISAID database. Representative viruses
496 selected for further testing are denoted in color same as in **(C)**.

497 **(C)** Heatmap showing the neutralization IC₅₀ values of the indicated antibodies against
498 SARS-CoV-2, SARS-CoV and their related sarbecoviruses.

499

500 **Figure 4. In vitro selection of 2-36 escape viruses.**

501 **(A)** Neutralizing activity of 2-36 against viruses at different passages.

502 **(B)** Spike mutations found in viruses at different passages

503 **(C)** Model of 2-36 in complex with the SARS-CoV-2 S trimer highlighting mutations in
504 red. CDR H3 Tyrosine 98 makes van der Waals contacts with RBD residue 378 as
505 shown as electron density map mesh in the subpanel.

506 **(D)** The selected mutations were introduced into pseudoviruses and then tested for
507 neutralization sensitivity to 2-36.

508 **(E)** The frequency of the selected mutations in circulating in infected patients (data
509 updated to Oct 13th, 2021).

510

511

512 **Supplementary Figure 1.** Binding of 2-36 to SARS-CoV-2 and SARS-CoV spike as
513 determined by ELISA.

514 **Supplementary Figure 2.** 2-36 binding affinity to SARS-CoV-2 and SARS-CoV (A)
515 spike or (B) RBD as measured by SPR.

516 **Supplementary Figure 3.** 2-36 binding to SARS-CoV-2 spike is inhibited by CR3022;
517 2-36 inhibits hACE2 binding to SARS-CoV-2 spike.

518 **Supplementary Figure 4. Cryo-EM data processing for antibody 2-36 in complex**
519 **with the SARS-CoV-2 S trimer.**

520 **(A)** Representative micrograph, power spectrum, and contrast transfer function (CTF)
521 fit.

522 **(B)** Representative 2D class averages showing spike particles.

523 **(C)** Global consensus refinement Fourier Shell Correlation (FSC) curve and particle
524 projection viewing angle distribution.

525 **(D)** Local focused refinement FSC curve and viewing direction distribution.

526 **(E)** Local resolution estimation mapped on surface density for global refinement.

527 **(F)** Local resolution estimation mapped on surface density for local refinement.

528 **Supplementary Figure 5. Cryo-EM data processing for antibody 2-36 in complex**
529 **with the SARS-CoV S trimer.**

530 **(A)** Representative micrograph, power spectrum, and contrast transfer function (CTF)
531 fit.

532 **(B)** Representative 2D class averages showing spike particles.

533 **(C)** Global consensus refinement Fourier Shell Correlation (FSC) curve and particle
534 projection viewing angle distribution.

535 **(D)** Local resolution estimation mapped on surface density for global consensus
536 refinement.

537 **Supplementary Figure 6. Sequence alignment for SARS-CoV-2 and SARS-CoV**
538 **RBD binding interface of 2-36.** The dots represent the conserved residues in SARS-
539 CoV-1 compared to SARS-CoV-2. The interface residues are colored in red, residues
540 form hydrogen bond with 2-36 are labeled by underline.

541 **Supplementary Figure 7.** 2-36 Neutralizes SARS-like coronaviruses using hACE2.

542 **Supplementary Figure 8.** 2-36 neutralization IC₅₀ (µg/mL) on the serially passaged
543 virus.

544 **Supplementary Figure 9.** Structural comparison between antibody 2-36 in complex
545 with SARS CoV-2 RBD and other published antibody structures.

546 (A) Molecular models for COVA1-16 (dark blue), 2-36 (teal), and S2X259 (orange),
547 aligned based on RBD, all bind to a similar face on the inner part of RBD.

548 (B) Close up of the aforementioned antibody CDRH3 loops all target the same beta-
549 strand on the surface of the inner face of RBD.

550 (C) Comparison of binding footprints for published broadly neutralizing antibodies that
551 bind to the same inner face of RBD.

552 **Supplementary Table 1.** Cryo-EM data collection, processing, and model refinement
553 and validation statistics. Related to Figures 2 and 4.

554

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- 649

Figure 1

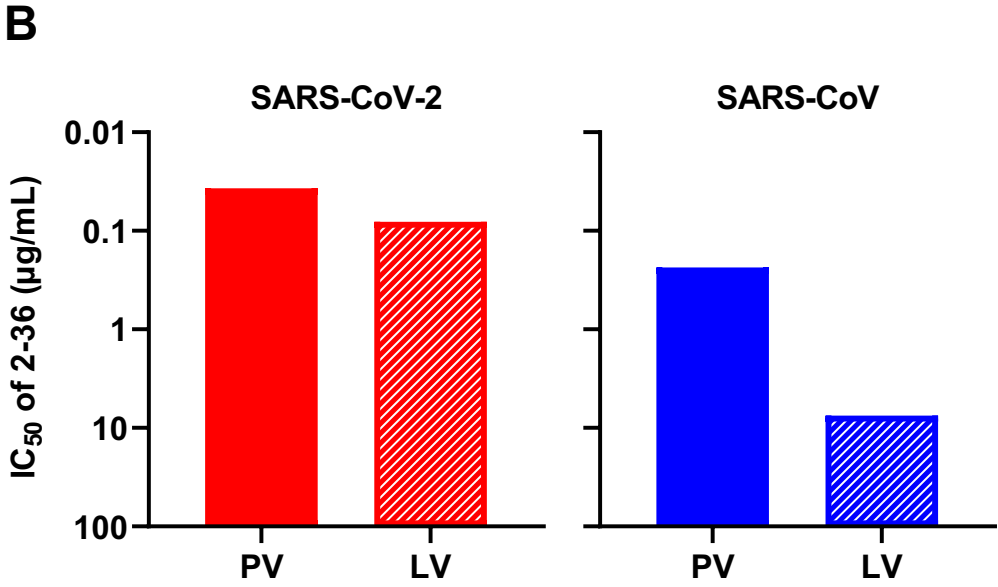
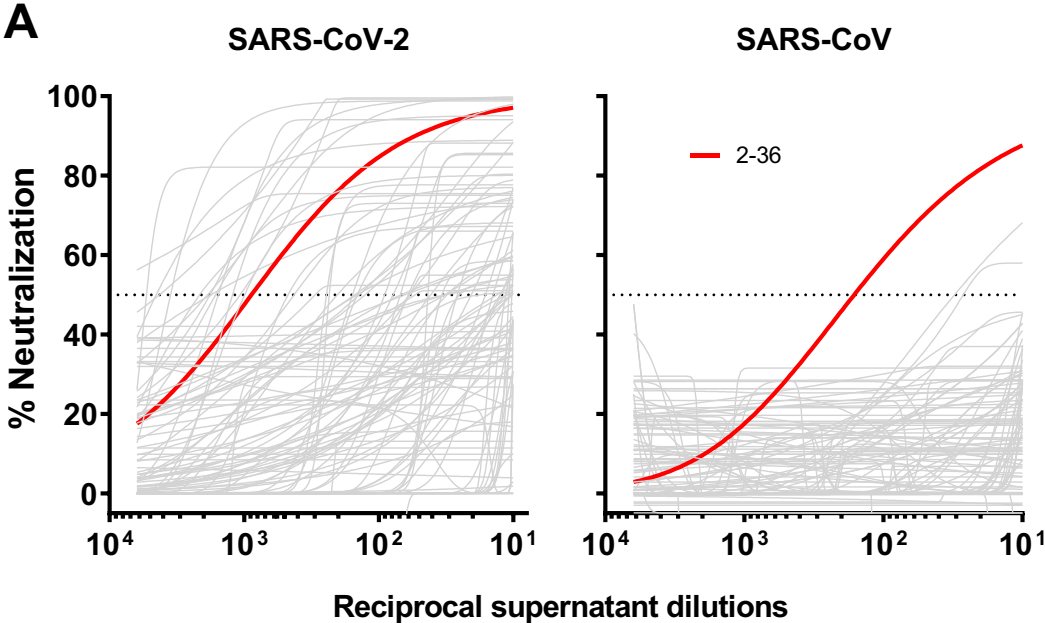


Figure 2

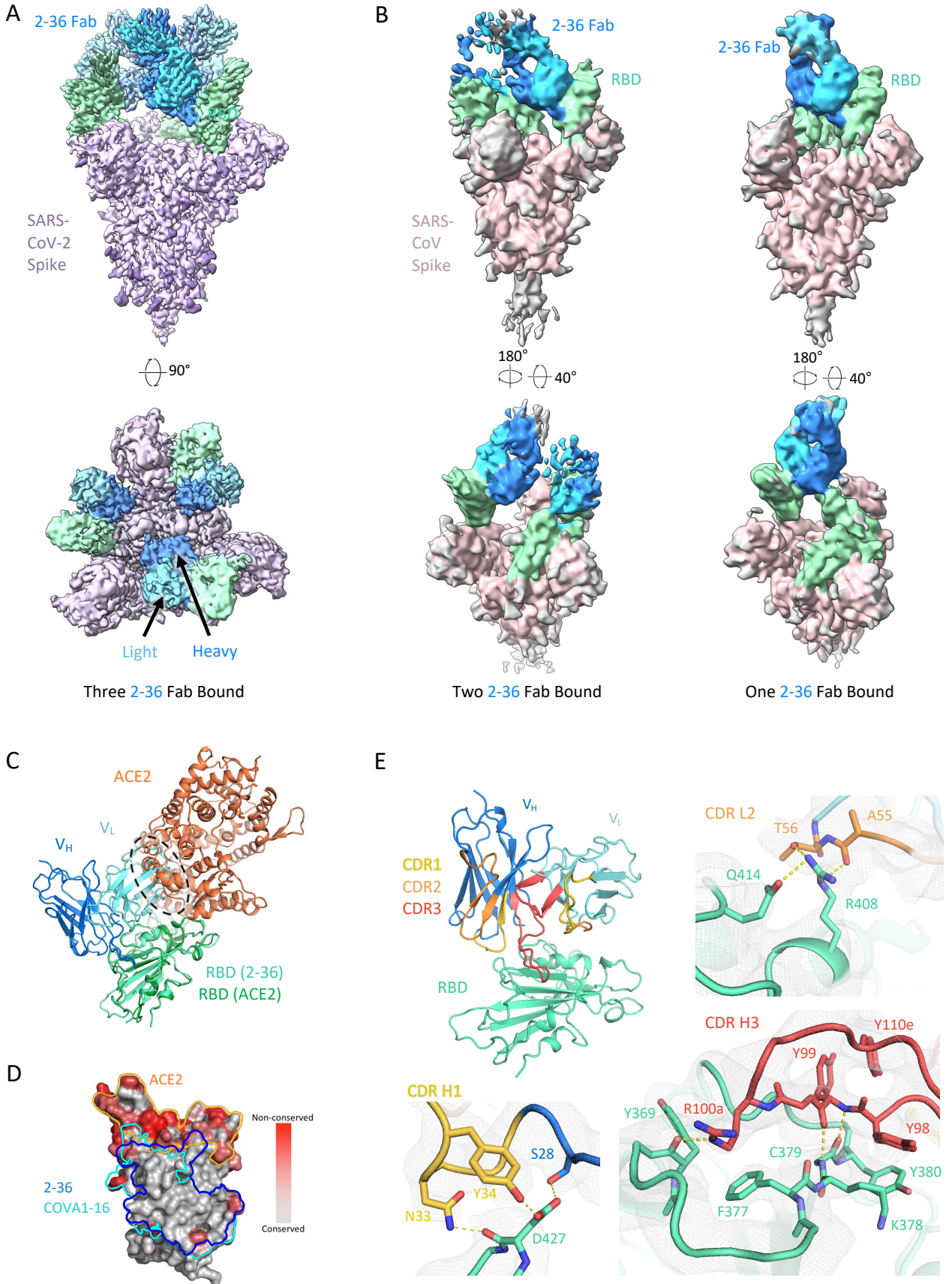
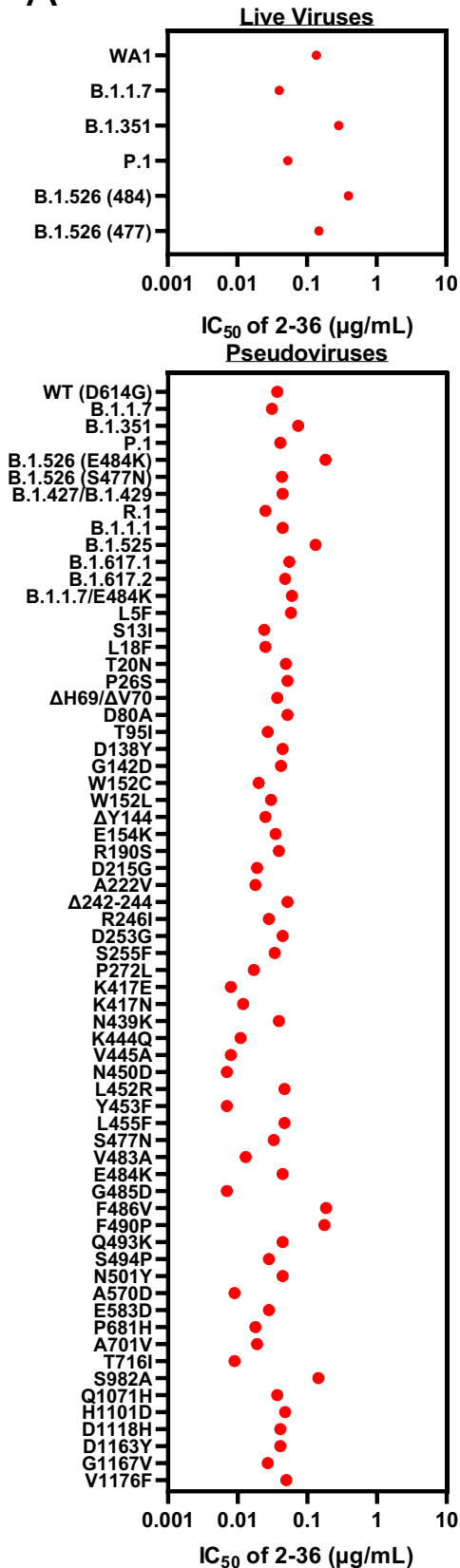
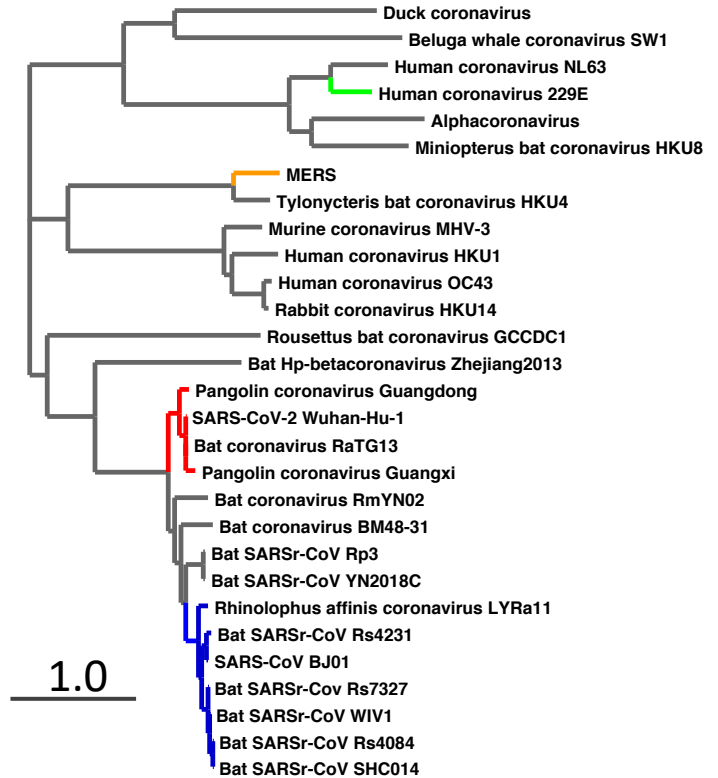


Figure 3

A



B



C

	2-36	COVA1-16	S309	CR3022	
229E	>20	>20	>20	>20	IC₅₀ (µg/mL) <0.01 0.01-0.1 0.1-1 1-20 >20
MERS-CoV	>20	>20	>20	>20	
SARS-CoV-2	0.029	0.178	0.007	>20	
GDPangolin	0.025	0.040	0.024	>20	
GXPangolin	0.073	0.053	>20	>20	
RaTG13	0.003	0.007	>20	3.553	
SARS-CoV	0.224	15.116	0.020	1.494	
WIV1	0.020	1.888	0.047	0.668	
SHC014	0.025	1.062	>20	>20	
LYRa11	0.016	1.275	0.011	>20	
Rs7327	0.658	>20	>20	>20	
Rs4231	0.002	1.179	0.141	>20	
Rs4084	1.041	1.183	>20	>20	

Figure 4

