

1 **Virological traits of the SARS-CoV-2 BA.2.87.1 lineage**

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22 **Abstract**

23 **The highly mutated SARS-CoV-2 BA.2.87.1 lineage was recently detected in South**
24 **Africa, but its transmissibility is unknown. Here, we report that BA.2.87.1 efficiently**
25 **enters human cells but is more sensitive to antibody-mediated neutralization than the**
26 **currently dominating JN.1 variant. Acquisition of adaptive mutations might thus be**
27 **needed for high transmissibility.**

28

29 **Main text**

30 The emergence and rapid global dominance of the highly mutated Omicron variant in
31 2021 and its sublineage JN.1 (a derivative of BA.2.86) in 2023 reveals that novel, antigenically
32 distinct variants can rapidly reshape the now fading COVID-19 pandemic. At the end of 2023,
33 a novel SARS-CoV-2 lineage, BA.2.87.1, was detected in eight patients in South Africa and
34 one traveler entering the USA. The BA.2.87.1 lineage harbors 65 mutations in the spike (S)
35 protein (relative to the virus that circulated in Wuhan in early 2020 (Figure 1A)), which
36 facilitates viral entry into cells and constitutes the key target for neutralizing antibodies.
37 However, it is unknown whether these mutations are compatible with robust entry into human
38 cells and allow for efficient antibody evasion. We addressed these questions using pseudovirus
39 particles (pp) bearing the SARS-CoV-2 S protein, which adequately model key aspects of
40 SARS-CoV-2 entry into host cells and antibody-mediated neutralization (1). Besides particles
41 bearing BA.2.87.1 S (BA.2.87.1_{pp}), we included particles pseudotyped with the S proteins of
42 the B.1 lineage (B.1_{pp}), which circulated early in the pandemic, the XBB.1.5 lineage
43 (XBB.1.5_{pp}), which served as the target lineage for adaptation of the latest COVID-19 mRNA
44 vaccines (2), and the currently prevailing JN.1 lineage (JN.1_{pp}). All S proteins were efficiently
45 processed and incorporated into particles (Figure 1B).

46

47 **BA.2.87.1 efficiently enters and fuses human cells**

48 All particles efficiently entered a panel of six cell lines (Figure 1C). BA.2.87.1_{pp} entered
49 293T (human, kidney), Huh-7 (human, liver), LoVo (human, colon) and Vero cells (African
50 green monkey, kidney, ± S protein-priming protease TMPRSS2) with similar efficiency as
51 B.1_{pp} and JN.1_{pp}, except for 293T and LoVo cells, which were more susceptible to JN.1_{pp}. For
52 Calu-3 cells (human, lung), entry of B.1_{pp} was highest, followed by JN.1_{pp}, whereas XBB.1.5_{pp}
53 and BA.2.87.1_{pp} entry was less efficient.

54 The ability of the S protein to fuse infected with uninfected cells is believed to contribute
55 to COVID-19 pathogenesis (4-6), which is why we assessed the capacity of BA.2.87.1 S to
56 drive cell-cell fusion using a split beta-galactosidase reporter assay (Figure 1D and
57 Supplementary figure 1C). BA.2.87.1 S displayed significantly higher cell-cell fusion capacity
58 than XBB.1.5 S and JN.1 S, reaching levels observed for B.1 S protein.

59

60 **BA.2.87.1 efficiently utilizes human and animal ACE2 as entry receptors**

61 Next, we analyzed the ability of BA.2.87.1 S to engage the SARS-CoV-2 receptor
62 ACE2 and found that BA.2.87.1 S and XBB.1.5 S bound soluble human ACE2 with comparable
63 efficiency while ACE2 binding of B.1 S and JN.1 S was significantly reduced (Figure 1E).
64 However, antibody-mediated of ACE2 engagement did not reveal major differences in ACE2
65 dependency for cell entry by the different S proteins (Figure 1F). Moreover, all four S proteins
66 could comparably utilize diverse mammalian ACE2 orthologues as entry receptors, with the
67 exception of pangolin ACE2 (highest for B.1_{pp}) and mouse efficiency (lowest for B.1_{pp}) (Figure
68 1G). Thus, the BA.2.87.1 lineage efficiently binds human ACE2 and robustly enters and fuses
69 human cells, although entry into Calu-3 lung cells is reduced compared to JN.1.

70

71 **Lung cell entry of BA.2.87.1 depends on TMPRSS2**

72 Most Omicron sublineages show a reduced capacity to employ TMPRSS2 for cell entry,
73 which has been linked to diminished lung cell entry and reduced virulence (8-10). Therefore,
74 we evaluated the dependency of BA.2.87.1_{pp} on TMPRSS2 for lung cell entry using the
75 cathepsin L inhibitor MDL28170 and the TMPRSS2 inhibitor camostat mesylate (Figure 1H).
76 MDL28170 reduced Vero kidney cell entry of all particles analyzed but had no impact on Calu-
77 3 lung cell entry of B.1_{pp}, JN.1_{pp} and BA.2.87.1_{pp}, while XBB.1.5_{pp} entry into lung Calu-3 cells
78 was diminished. Camostat mesylate inhibited Calu-3 cell entry of all particles, with entry of
79 B.1_{pp}, JN.1_{pp} and BA.2.87.1_{pp} being more affected than entry of XBB.1.5_{pp}. Finally, neither of
80 the inhibitors reduced entry of control particles bearing the vesicular stomatitis virus
81 glycoprotein (VSV-G). Thus, BA.2.78.1 deviates from other Omicron sublineage in its ability
82 to efficiently employ TMPRSS2 for lung cell entry.

83

84 **Few therapeutic monoclonal antibodies neutralize BA.2.87.1**

85 Recombinant monoclonal antibodies (mAb) have been successfully used for COVID-
86 19 therapy but contemporary SARS-CoV-2 lineages developed resistance against most or all of
87 them (11). Using a panel of twelve mAbs that were previously approved for COVID-19 therapy
88 or are currently under development, we found that five of them (Casirivimab, Tixagevimab,
89 Amubarvimab, Regdanvimab and Sotrovimab), displayed neutralizing activity against
90 BA.2.87.1_{pp} and should constitute suitable treatment options (Figure 2A-B and Supplementary
91 figure 2). In comparison, only Sotrovimab was effective against XBB.1.5_{pp} and none of the
92 mAbs neutralized JN.1_{pp}.

93

94 **Less neutralization evasion by BA.2.87.1 compared to JN.1**

95 Finally, we studied the sensitivity of BA.2.87.1 to neutralization by antibodies induced
96 upon vaccination or vaccination plus breakthrough infection. Cohorts 1 and 2 included

97 participants who recently received the XBB.1.5-adapted COVID-19 mRNA vaccine from
98 BioNTech (raxtozinameran). Members of cohort 1 had no history of SARS-CoV-2 infection
99 while members of cohort 2 had documented SARS-CoV-2 infection between 01/2022 and
100 03/2023. Cohorts 3 and 4 included participants without XBB.1.5-booster vaccination, who
101 experienced one (cohort 3) or two (cohort 4) SARS-CoV-2 infections with the most recent
102 infection occurring during the JN.1 wave. Of note, all participants received at least four
103 vaccinations with non-XBB.1.5-adapted COVID-19 vaccines and plasma samples were
104 collected within three months after the last infection or vaccination (Supplementary table 1 and
105 Supplementary figure 3). For all four cohorts, highest neutralizing activity was measured for
106 B.1_{pp} (geometric mean titer = 2797-7289), while neutralization of JN.1_{pp} was lowest (~5-17-
107 fold reduction compared to B.1_{pp}) with the exception of cohort 4 (Figure 2C and Supplementary
108 figure 4). Importantly, although BA.2.87.1_{pp} displayed substantial resistance to antibody-
109 mediated neutralization independent of the cohort analyzed, neutralization evasion was less
110 efficient compared to JN.1_{pp}, with the exception of cohort 4.

111

112 **Discussion**

113 Our initial virological assessment of the BA.2.87.1 lineage revealed that it efficiently
114 utilizes human and animal ACE2 orthologues as receptors, and robustly enters human cell lines.
115 However, cell entry of BA.2.87.1_{pp} was found to be reduced compared JN.1_{pp}. Calu-3 lung cell
116 entry of BA.2.87.1_{pp} was highly dependent on the cellular serine protease TMPRSS2, a trait
117 that is shared with lineages dominating the pre-Omicron era and the recently emerged BA.2.86
118 lineage (11). With respect to antibody-mediated neutralization, we found that BA.2.87.1 can be
119 neutralized by Casirivimab, Tixagevimab, Amubarvimab, Regdanvimab and Sotrovimab,
120 which could constitute suitable treatment options in case of BA.2.78.1 spread. In addition,
121 BA.2.87.1_{pp} evaded neutralization by antibodies present in the plasma of individuals with

122 diverse immune backgrounds but antibody evasion was reduced compared to JN.1_{pp}. Based on
123 the data obtained in this study it seems unlikely that BA.2.87.1 will efficiently spread in regions
124 where JN.1 is dominant. However, BA.2.87.1 may still be able to spread in locations where
125 JN.1 prevalence is low and may acquire additional mutations that improve transmissibility
126 and/or immune evasion.

127 Limitations of our study include the lack of data for authentic SARS-CoV-2 lineages
128 and small sample sizes of the cohorts, with the latter precluding an analysis of the impact of
129 biological factors (e.g. age, sex, comorbidities, etc.) on neutralization. Nevertheless, this study
130 provides valuable information on the virological traits of the BA.2.87.1 lineage that support
131 political decision makers and medical personnel to determine whether changes in containment
132 and treatment strategies are required.

133

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160 responsibility to submit for publication.

161

162 **Conflict of interest statement**

163 S.P. and M.H. performed contract research (testing of vaccinee sera for neutralizing activity
164 against SARS-CoV-2) for Valneva unrelated to this work. A.D-J. served as advisor for Pfizer,
165 unrelated to this work. G.M.N.B. served as advisor for Moderna, unrelated to this work. S.P.
166 served as advisor for BioNTech, unrelated to this work. All other authors declare no competing
167 interests.

168

169 **Ethical statement**

170 Plasma samples were collected as part of the COVID-19 Contact (CoCo) Study (German
171 Clinical Trial Registry, DRKS00021152). The CoCo Study and the analysis performed for this

172 study were approved by the Internal Review Board of Hannover Medical School (institutional
173 review board no. 8973_BO-K_2020, last amendment Sep 2023). All study participants
174 provided written informed consent and received no compensation.

175

176 **Data availability**

177 Raw data are available upon request. This study did not generate code. All materials and
178 reagents will be made available upon installment of a material transfer agreement.

179

180 **Authors' contributions**

181 Conceptualization: L.Z and M.H.; Methodology: S.P. and M.H.; Investigation: L.Z., I.N., A.K.,
182 L.G., and M.H.; Formal analysis: L.Z., M.H., and M.V.S.; Resources: A.D.-J., N.C.H., A.C.,
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184 S.P.; Writing – original draft: M.H.; Writing – review & editing: all authors.

185 **References**

- 186 1. Schmidt F, Weisblum Y, Muecksch F, Hoffmann HH, Michailidis E, Lorenzi JCC, et
187 al. Measuring SARS-CoV-2 neutralizing antibody activity using pseudotyped and chimeric
188 viruses. *The Journal of experimental medicine*. 2020 Nov 2;217(11). PubMed PMID:
189 32692348. Pubmed Central PMCID: 7372514.
- 190 2. Gayed J, Diya O, Lowry FS, Xu X, Bangad V, Mensa F, et al. Safety and
191 Immunogenicity of the Monovalent Omicron XBB.1.5-Adapted BNT162b2 COVID-19
192 Vaccine in Individuals ≥ 12 Years Old: A Phase 2/3 Trial. *Vaccines*. 2024 Jan 24;12(2).
193 PubMed PMID: 38400102.
- 194 3. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al.
195 SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically
196 Proven Protease Inhibitor. *Cell*. 2020 Apr 16;181(2):271-80 e8. PubMed PMID: 32142651.
197 Pubmed Central PMCID: 7102627.
- 198 4. Bussani R, Schneider E, Zentilin L, Collesi C, Ali H, Braga L, et al. Persistence of viral
199 RNA, pneumocyte syncytia and thrombosis are hallmarks of advanced COVID-19 pathology.
200 *EBioMedicine*. 2020 Nov;61:103104. PubMed PMID: 33158808. Pubmed Central PMCID:
201 PMC7677597. Epub 20201103.
- 202 5. Braga L, Ali H, Secco I, Chiavacci E, Neves G, Goldhill D, et al. Drugs that inhibit
203 TMEM16 proteins block SARS-CoV-2 spike-induced syncytia. *Nature*. 2021
204 Jun;594(7861):88-93. PubMed PMID: 33827113. Pubmed Central PMCID: 7611055.
- 205 6. Rajah MM, Bernier A, Buchrieser J, Schwartz O. The Mechanism and Consequences
206 of SARS-CoV-2 Spike-Mediated Fusion and Syncytia Formation. *J Mol Biol*. 2022 Mar
207 30;434(6):167280. PubMed PMID: 34606831. Pubmed Central PMCID: PMC8485708. Epub
208 20211001.
- 209 7. Qu P, Faraone JN, Evans JP, Zheng YM, Carlin C, Anghelina M, et al. Enhanced
210 evasion of neutralizing antibody response by Omicron XBB.1.5, CH.1.1, and CA.3.1 variants.
211 *Cell reports*. 2023 May 30;42(5):112443. PubMed PMID: 37104089. Pubmed Central PMCID:
212 10279473.
- 213 8. Hui KPY, Ho JCW, Cheung MC, Ng KC, Ching RHH, Lai KL, et al. SARS-CoV-2
214 Omicron variant replication in human bronchus and lung ex vivo. *Nature*. 2022
215 Mar;603(7902):715-20. PubMed PMID: 35104836. Epub 20220201.
- 216 9. Shuai H, Chan JF, Hu B, Chai Y, Yuen TT, Yin F, et al. Attenuated replication and
217 pathogenicity of SARS-CoV-2 B.1.1.529 Omicron. *Nature*. 2022 Mar;603(7902):693-9.
218 PubMed PMID: 35062016. Epub 20220121.
- 219 10. Meng B, Abdullahi A, Ferreira I, Goonawardane N, Saito A, Kimura I, et al. Altered
220 TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. *Nature*. 2022
221 Mar;603(7902):706-14. PubMed PMID: 35104837. Pubmed Central PMCID: 8942856.
- 222 11. Zhang L, Kempf A, Nehlmeier I, Cossmann A, Richter A, Bdeir N, et al. SARS-CoV-
223 2 BA.2.86 enters lung cells and evades neutralizing antibodies with high efficiency. *Cell*. 2024
224 Feb 1;187(3):596-608 e17. PubMed PMID: 38194966.

225 **Figure legends**

226 **Figure 1: Host cell entry properties of the SARS-CoV-2 BA.2.87.1 lineage.**

227 **(A)** S protein mutations of B.1, XBB.1.5, BA.2, JN.1 and BA.2.87.1 compared to the Wuhan-
228 Hu-01 isolate. Abbreviations: NTD, N-terminal domain; RBD, receptor-binding domain, pre-
229 S1/S2, region between RBD and S1/S2 cleavage site. **(B)** Processing and particle incorporation
230 of the BA.2.87.1 S protein. Presented are representative data from a single biological replicate
231 and results were confirmed in five additional biological replicates. **(C)** Entry efficiency of the
232 BA.2.87.1 lineage. Pseudotype particles harboring the indicated S proteins were inoculated onto
233 the indicated cell lines and entry was analyzed. Presented are mean data from six biological
234 replicates, conducted with four technical replicates, with cell entry normalized against particles
235 harboring the B.1 S protein (set as 1). Error bars represent the standard error of the mean (SEM).
236 **(D)** Cell-cell fusion capacity of the BA.2.87.1 lineage. Presented are the mean data from four
237 biological replicates, conducted with three technical replicates. Fusion driven by the B.1 S
238 protein was set as 1. Error bars indicate the SEM. **(E)** Soluble human ACE2 binding by the
239 BA.2.87.1 S protein. Presented are mean ACE2 binding data from six biological replicates,
240 conducted with a single technical replicate, and ACE2 binding was corrected for S protein cell
241 surface expression and normalized using the B.1 S protein as reference (= 1). Error bars indicate
242 the SEM. **(F)** Impact of ACE2 blockade on cell entry of the BA.2.87.1 lineage. Pseudotype
243 particles harboring the indicated S proteins were inoculated onto Vero cells that were
244 preincubated with different concentration of an ACE2-blocking antibody and entry was
245 analyzed. Presented are mean data from three biological replicates, conducted with four
246 technical replicates, with cell entry in the absence of antibody used as reference (set as 100%).
247 Error bars represent the SEM. **(G)** Utilization of mammalian ACE2 orthologues by the
248 BA.2.87.1 lineage. Particles bearing the indicated S proteins were inoculated onto BHK-21
249 cells expressing the indicated ACE2 orthologues following transfection and entry efficiency

250 was analyzed. Net plots present the mean data from three biological replicates, conducted with
251 four technical replicates, and data were normalized to human ACE2 (set as 1). **(H)** Dependency
252 of BA.2.87.1 lung cell entry on TMPRSS2. Pseudotype particles harboring the indicated S
253 proteins were inoculated onto Vero and Calu-3 cells that were preincubated with MDL28170
254 or camostat mesylate and entry was analyzed. Presented are mean data from three biological
255 replicates, conducted with four technical replicates, with cell entry in the absence of inhibitor
256 used as reference (set as 100%). Error bars represent the SEM. For panels C, D and E statistical
257 significance was analyzed by two-tailed students' t-test with Welch correction, while for panels
258 F and H, statistical significance was analyzed by two-way ANOVA with Dunnett's posttest (p
259 > 0.05 , not significant [ns]; $p \leq 0.05$, *; $p \leq 0.01$, **; $p \leq 0.001$, ***)

260

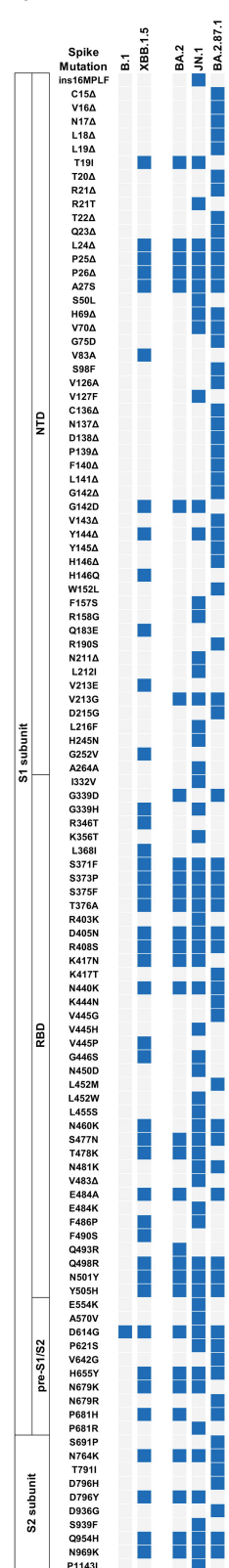
261 **Figure 2: Neutralization sensitivity of the SARS-CoV-2 BA.2.87.1 lineage.**

262 **(A)** Sensitivity of the BA.2.87.1 lineage to neutralization by monoclonal antibodies (mAb).
263 Pseudotype particles harboring the indicated S proteins were incubated with different
264 concentrations of the indicated mAbs, before being inoculated onto Vero cells and cell entry
265 was analyzed at 16–18 h post inoculation by measuring firefly luciferase activity in cell lysates.
266 Presented are the mean data from three biological replicates, conducted with four technical
267 replicates, and cell entry was normalized against entry in the absence of mAb (set as 0%
268 inhibition). **(B)** Net plots indicate the effective dose 50 (EC50) values calculated from the data
269 presented in panel A. **(C)** Sensitivity of the JN.1 lineage to neutralization by antibodies in the
270 blood plasma of individuals with different immunization background. Pseudotype particles
271 harboring the indicated S proteins were incubated with different dilutions of plasma, before
272 being inoculated onto Vero cells and cell entry was analyzed at 16–18 h post inoculation by
273 measuring firefly luciferase activity in cell lysates. Cell entry was further normalized against
274 entry in the absence of plasma (set as 0% inhibition) and the neutralizing titer 50 values were

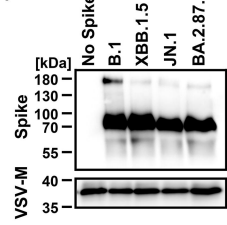
275 calculated based on a nonlinear regression model. Presented are the geometric mean titers
276 (GMT) from a single biological replicate, conducted with four technical replicates. Information
277 above the graphs include response rates (proportion of plasma samples with neutralizing
278 activity), GMT values, and median fold GMT changes compared to particles bearing the B.1 S
279 protein. Please also see Supplementary table 1 and supplementary figures 3 and 4 for additional
280 information. Statistical significance was assessed by Wilcoxon matched-pairs signed rank test
281 ($p > 0.05$, ns; $p \leq 0.05$, *; $p \leq 0.01$, **; $p \leq 0.001$, ***).

Figure 1

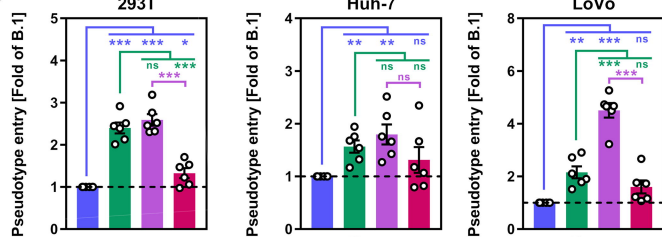
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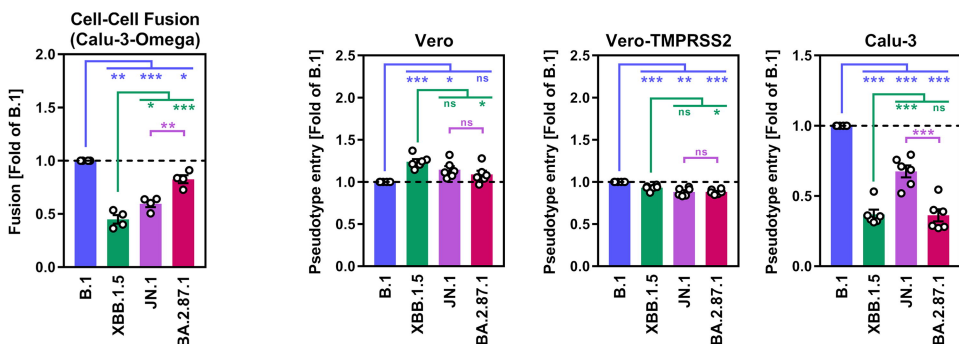
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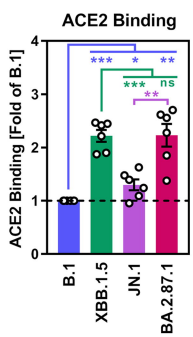
C)



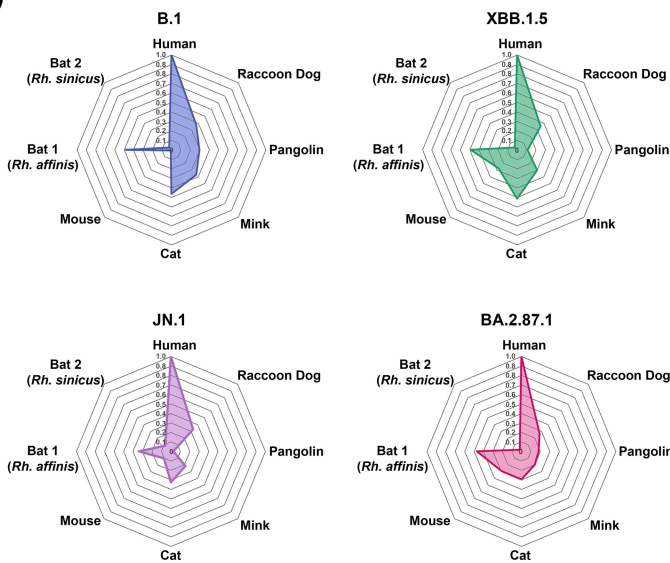
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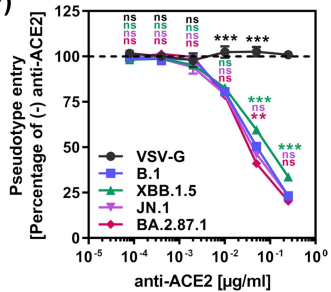
E)



G)



F)



H)

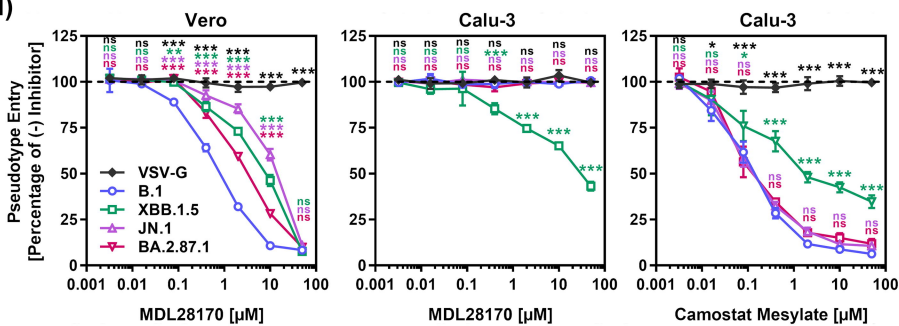
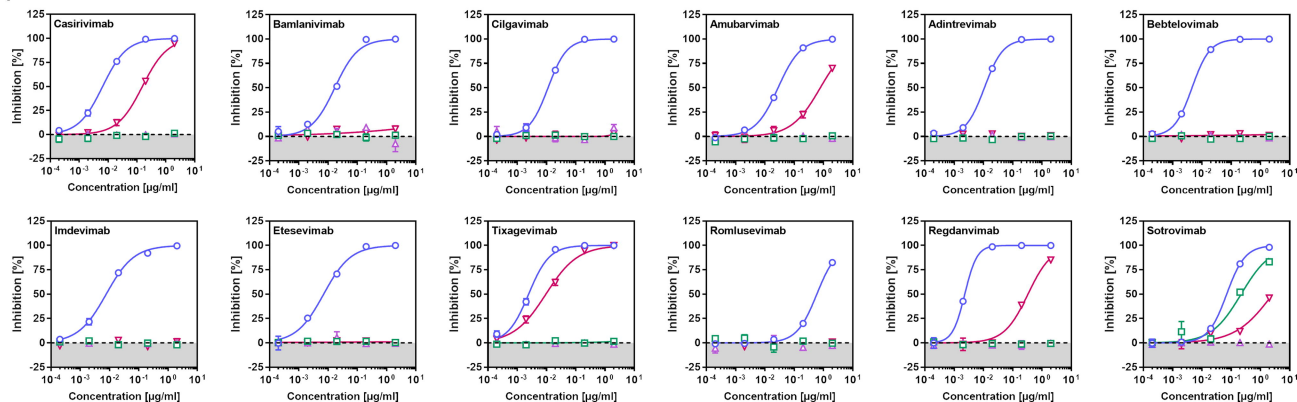
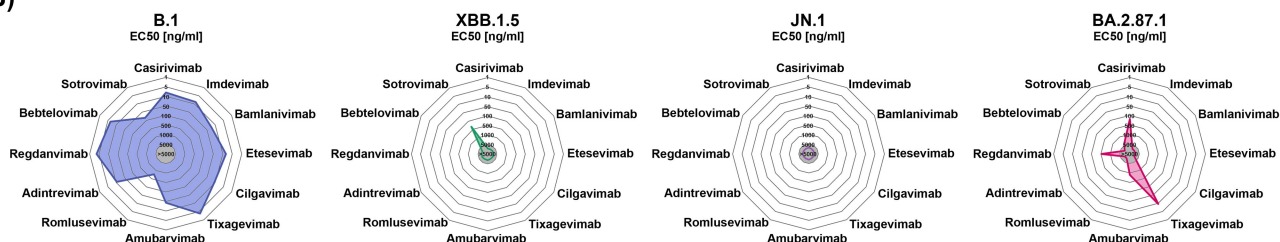


Figure 2

A)



B)



C)

