

1 **Distinct Patterns of SARS-CoV-2 BA.2.87.1 and JN.1 Variants in Immune Evasion,**
2 **Antigenicity and Cell-Cell Fusion**

3
4 Pei Li^{1,2}, Yajie Liu^{1,2}, Julia Faraone^{1,2,3}, Cheng Chih Hsu², Michelle Chamblee², Yi-Min Zheng^{1,2},
5 Claire Carlin⁴, Joseph S. Bednash^{5,6}, Jeffrey C. Horowitz^{5,6}, Rama K. Mallampalli^{5,6},
6 Linda J. Saif^{7,8,9}, Eugene M. Oltz^{10,11}, Daniel Jones¹², Jianrong Li², Richard J. Gumina^{4,12,14},
7 and Shan-Lu Liu^{1,2,9,10*}

8 ¹Center for Retrovirus Research, The Ohio State University, Columbus, OH 43210, USA

9 ²Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210, USA

10 ³Molecular, Cellular, and Developmental Biology Program,
11 The Ohio State University, Columbus, OH 43210, USA

12 ⁴Department of Internal Medicine, Division of Cardiovascular Medicine,
13 The Ohio State University, Columbus, OH 43210, USA

14 ⁵Department of Internal Medicine, Division of Pulmonary, Critical Care, and Sleep Medicine,
15 The Ohio State University, Columbus, OH 43210, USA

16 ⁶Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Wexner
17 Medical Center, Columbus, OH 43210, USA

18 ⁷Center for Food Animal Health, Animal Sciences Department, OARDC,
19 College of Food, Agricultural and Environmental Sciences,
20 The Ohio State University, Wooster, OH 44691, USA

21 ⁸Veterinary Preventive Medicine Department, College of Veterinary Medicine,
22 The Ohio State University, Wooster, OH 44691, USA

23 ⁹Viruses and Emerging Pathogens Program, Infectious Diseases Institute,
24 The Ohio State University, Columbus, OH 43210, USA

25 ¹⁰Department of Microbial Infection and Immunity, The Ohio State University,
26 Columbus, OH 43210, USA

27 ¹¹Pelotonia Institute for Immuno-Oncology, The Ohio State University Comprehensive Cancer
28 Center Arthur G James Cancer Hospital and Richard J Solove Research Institute,
29 Columbus, Ohio, USA.

30 ¹²Department of Pathology, The Ohio State University Wexner Medical Center,
31 Columbus, OH, USA.

32 ¹³Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University
33 Wexner Medical Center, Columbus, OH 43210, USA

34 ¹⁴Department of Physiology and Cell Biology, College of Medicine, The Ohio State University
35 Wexner Medical Center, Columbus, OH
36 43210, USA

37
38
39 *Corresponding author: liu.6244@osu.edu

41 **ABSTRACT**

42 The rapid evolution of SARS-CoV-2 variants presents a constant challenge to the global
43 vaccination effort. In this study, we conducted a comprehensive investigation into two newly
44 emerged variants, BA.2.87.1 and JN.1, focusing on their neutralization resistance, infectivity,
45 antigenicity, cell-cell fusion, and spike processing. Neutralizing antibody (nAb) titers were
46 assessed in diverse cohorts, including individuals who received a bivalent mRNA vaccine booster,
47 patients infected during the BA.2.86/JN.1-wave, and hamsters vaccinated with XBB.1.5-
48 monovalent vaccine. We found that BA.2.87.1 shows much less nAb escape from WT-BA.4/5
49 bivalent mRNA vaccination and JN.1-wave breakthrough infection sera compared to JN.1 and
50 XBB.1.5. Interestingly, BA.2.87.1 is more resistant to neutralization by XBB.15-monovalent-
51 vaccinated hamster sera than BA.2.86/JN.1 and XBB.1.5, but efficiently neutralized by a class III
52 monoclonal antibody S309, which largely fails to neutralize BA.2.86/JN.1. Importantly, BA.2.87.1
53 exhibits higher levels of infectivity, cell-cell fusion activity, and furin cleavage efficiency than
54 BA.2.86/JN.1. Antigenically, we found that BA.2.87.1 is closer to the ancestral BA.2 compared to
55 other recently emerged Omicron subvariants including BA.2.86/JN.1 and XBB.1.5. Altogether,
56 these results highlight immune escape properties as well as biology of new variants and
57 underscore the importance of continuous surveillance and informed decision-making in the
58 development of effective vaccines.

59

60 **KEY WORDS:**

61 SARS-CoV-2, BA.2.87.1, JN.1, Neutralizing antibody, Cell-cell fusion, Furin cleavage, Infectivity

62

63 INTRODUCTION

64 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of
65 the COVID-19 pandemic, continues to evolve despite the global pandemic being declared over.
66 Late 2023 into early 2024 has seen the emergence of highly mutated variants of the virus,
67 heightening new concern over the continued efficacy of current vaccination strategies and other
68 pandemic control measures (1, 2). Among these, the BA.2.86 variant was characterized by around
69 30 mutations and evolved into JN.1 and a series of other subvariants with the spike protein distinct
70 from the previously dominant variant XBB.1.5 (1). While BA.2.86 proved to be a less dominant
71 variant and displayed minimal escape of neutralizing antibodies in mRNA-vaccinated and SARS-
72 CoV-2 infected sera (3, 4), JN.1, which has only an additional L455S mutation in spike compared
73 to BA.2.86, has significantly increased evasion of neutralizing antibodies and become the
74 dominant variant in the United and States and other countries (5, 6).

75 Concern is mounting once more as a new highly mutated variant, BA.2.87.1, has been
76 detected in South Africa (7). This variant contains over 100 mutations relative to XBB.1.5 and
77 JN.1 throughout the genome, with over 30 in spike alone (**Fig. 1a**) (1). Since its initial detection in
78 September of 2023, 9 cases have been recorded in South Africa as of early February 2024 and
79 was recently reported in the wastewater of Southeast Asia. This variant has not yet been detected
80 elsewhere (7). Currently, little is known about this new variant, including critical aspects of virus
81 biology, sensitivity to neutralizing antibodies, and transmissibility. While BA.2.87.1 does not
82 appear to have spread widely now, the fact that the currently dominant JN.1 was derived from a
83 single mutation L455S in the spike in the less-fit BA.2.86 variant raises concerns over similar
84 situations occurring.

85 Here, we investigate the immune escape and biology of the BA.2.87.1 variant in
86 comparison to previously dominant variants JN.1 and XBB.1.5 and ancestral BA.2.86, BA.2 and
87 parental D614G. We characterized the nAb titers in the sera of health care workers (HCWs) that
88 received the wildtype (WT) plus BA.4/5 spike bivalent mRNA vaccine (n=13), sera from hamsters

89 that received the XBB.1.5 monovalent mRNA vaccine (n=15), and sera from patients in the ICU
90 during the BA.2.86/JN.1-wave of infection in Columbus, Ohio, U.S (n=9). We also elucidated the
91 antigenic distance between variants and examined the neutralization of two RBD-targeting
92 monoclonal antibodies S309 and 2B04. Additionally, we studied other aspects of virus biology
93 including viral infectivity in lung airway epithelial cells, spike processing into the S1 and S2
94 subunits, spike surface expression, and cell-cell fusion.

95

96 **RESULTS**

97 ***BA.2.87.1 exhibits comparable infectivity to its ancestral BA.2 in human 293T-ACE2 and***
98 ***lung epithelial CaLu-3 cells.*** We first investigated the infectivity of pseudotyped lentiviral vectors
99 bearing the spike of BA.2.87.1 or others of interest in 293T cells overexpressing human ACE2
100 (293T-ACE2) (**Fig. 1b**) and human lung epithelial cell line CaLu-3 (**Fig. 1c**). In 293T-ACE2 cells,
101 BA.2.87.1 exhibited comparable infectivity to BA.2, but with a 4-fold increase relative to D614G
102 ($p < 0.0001$). In contrast, JN.1 showed an infectivity comparable to D614G but lower than BA.2
103 (3.2-fold, $p < 0.0001$), BA.2.87.1 (3.1-fold, $p < 0.0001$) and XBB.1.5 (2.4-fold, $p < 0.0001$),
104 respectively. The infectivity of JN.1 was even lower than its ancestral BA.2.86, with a 40%
105 decrease ($p < 0.01$), and was among the lowest in all examined Omicron subvariants (**Fig. 1b**).

106 Omicron spikes have been characterized by an overall lower infectivity in CaLu-3 cells,
107 but infectivity increased with some of the recently emerged subvariants (8-12). Here we found
108 that both JN.1 and BA.2.87.1 had titers about 2-fold lower in relative infectivity compared to
109 D614G ($p < 0.0001$), but 1.6-fold ($p < 0.0001$) and 1.7-fold ($p < 0.0001$) higher than JN.1 and
110 XBB.1.5, respectively. Notably BA.2.86 showed an increased infectivity in CaLu-3 cells compared
111 to other Omicron subvariants, similar to previous results (4, 13-15) (**Fig. 1c**).

112

113 ***Bivalent mRNA-vaccinated sera more effectively neutralize BA.2.87.1 than JN.1.*** We next
114 investigated the nAb responses in a series of cohorts (**Fig. 2, Fig. S1**). The first was The Ohio

115 State University (OSU) Wexner Center HCWs that received at least 2 doses of monovalent
116 vaccine (WT) plus a single booster of bivalent vaccine (WT + BA.4/5) (**Table S1**). The samples
117 were collected between December 2022 and January 2023, approximately 23 and 108 days post
118 the bivalent dose administration; the cohort had no breakthrough infection with BA.2.86/JN.1 or
119 BA.2.87.1, but 9 of the 13 were COVID-19 positive with variants prior to the XBB.1.5 wave (see
120 **Table S1**). BA.2.87.1 exhibited an increased sensitivity to neutralization by the bivalent mRNA-
121 vaccine sera, with a titer ~4-fold lower than D614G ($p < 0.05$) as compared to JN.1, which was
122 7.6-fold lower than D614G ($p < 0.001$) (**Fig. 2a, Fig. S1a**). JN.1 exhibited the lowest titers of all
123 variants tested, even relative to its ancestral BA.2.86 and previous XBB.1.5, which were 4.7-fold
124 and 4.8-fold lower than D614G ($p < 0.05$ for both), respectively. However, all variants were
125 effectively neutralized by the bivalent HCW sera, with none falling below the limit of detection for
126 the assay ($NT_{50} = 40$). These results together suggest that bivalent mRNA vaccine could still be
127 effective for BA.2.87.1 but efficiency is reduced for JN.1.

128

129 ***Sera from JN.1/BA.2.86-wave ICU patients neutralize BA.2.87.1 better compared to JN.1***
130 ***and XBB.1.5.*** The next cohort we investigated were Columbus first-responders and their
131 household contacts ($n=5$, P1 to P5) as well as ICU COVID-19 patients admitted to the OSU
132 Medical Center ($n=4$, P6 to P9) during the BA.2.86/JN.1 wave of infection in Columbus, OH (early
133 2024) (total $n=9$ in this cohort) (**Fig. 2b, Fig. S1b, and Table S1**). Nasal swabs were collected
134 and sequenced, with 1 individual being confirmed to have been infected with BA.2.86, 1 individual
135 confirmed to have been infected with JN.1, and the remaining 7 were assumed to have been
136 infected with JN.1 based on the timing of the cases in Columbus, Ohio after Jan 2024. Of note,
137 all nine patients were vaccinated with different doses of mRNA vaccine, most 357-898 days prior
138 to sample collection, except one (P5), who was vaccinated with XBB.1.5 monovalent vaccine with
139 sample collected 45 days after the vaccination (**Table S1**). Overall, nAb titers varied greatly in this
140 cohort due to its heterogeneity, and were generally lower compared to the bivalent vaccinated

141 cohort, especially against Omicron-lineage variants (**Figs. 2a-b** and **Figs. S1a-b**). Notably,
142 BA.2.87.1 exhibited a modestly increased titer compared to JN.1 (1.3-fold, $p = 0.301$), with only
143 3.3-fold lower than D614G ($p = 0.6778$). Surprisingly, JN.1 showed the lowest neutralization titers,
144 which were similar to the bivalent serum samples (**Fig. 2a**, **Fig. S1a**), with ~ 4.3 -fold lower than
145 D614G ($p = 0.1321$). Notably, despite the limited sample size, 3 of the 4 ICU patients (P6, P8 and
146 P9) exhibited very high neutralization titers compared to the first-responders and household
147 contacts, results of which were in accordance with our previous studies (4, 9, 10, 13). We noticed
148 that one ICU patient (P7, 78-year-old female) and one first-responder and household contact (P1)
149 exhibited extremely low titers, especially against the Omicron variants (**Fig. 2b**, **Fig. S1b**). This
150 was despite that P7 had received 4 doses of monovalent WT mRNA and 2 doses of WT-BA.4/5
151 bivalent vaccine shots prior to the BA.2.86/JN.1-wave in July 2023, without obvious history of
152 immunocompromised conditions.

153

154 ***BA.2.87.1 is less efficiently neutralized by XBB.1.5 monovalent-vaccinated hamster sera***
155 ***compared to JN.1.*** The final cohort we tested was a group of hamsters vaccinated twice with a
156 monovalent XBB.1.5 spike vaccine delivered by recombinant mumps virus ($n=15$). In contrast to
157 the human cohorts that received WT and BA.4/5 bivalent vaccine doses shown above, we found
158 that these hamster serum samples exhibited the highest titers against XBB.1.5 ($NT_{50} = 14,626$),
159 BA.2.86 ($NT_{50} = 10,452$), and JN.1 ($NT_{50} = 9,081$), with D614G showing the lowest titers ($NT_{50} =$
160 916), followed by BA.2.87.1 ($NT_{50} = 1,850$) and BA.2 ($NT_{50} = 3,130$) (**Fig. 2c**, **Fig. S1c**). For this
161 cohort, comparisons were thus made instead to XBB.1.5 rather than D614G, due to the fact that
162 XBB.1.5 is the variant included in the vaccine. Titers against JN.1 were only slightly reduced, with
163 1.6-fold lower than XBB.1.5 ($p = 0.4722$). Titers against BA.2.87.1 were markedly reduced, with
164 7.9-fold lower than XBB.1.5 ($p < 0.0001$). No neutralization escape was evident for this cohort
165 relative to XBB.1.5, though one hamster (XBB.1.5-15) exhibited titers near the limit of detection
166 for both D614G and BA.2.87.1 (**Fig. 2c**, **Fig. S1c**).

167

168 ***Class III monoclonal antibody S309 efficiently neutralizes BA.2.87.1 but not JN.1.*** We next
169 tested the neutralization of BA.2.87.1 and JN.1 by two neutralizing antibodies: the class III
170 monoclonal antibody (mAb) S309 and class I mAb 2B04 (16, 17). S309 targets the epitopes of
171 non-receptor binding motif (RBM) of the spike and has largely maintained efficacy against
172 Omicron variants with the exception of CH.1.1, CA.3.1, BA.2.75.2 and BA.2.86 (9, 18).
173 Interestingly, we found that S309 maintained neutralization against BA.2.87.1, with an IC₅₀ of 0.62
174 µg/mL (**Fig. 2d, Fig. S1d**). However, the neutralizing activity of S309 was lost for JN.1 and greatly
175 reduced for BA.2.86, with an IC₅₀ of 6.22 µg/mL for the latter (**Fig. 2d, Fig. S1d**). Omicron variants
176 have been expected to exhibit a complete escape of mAb 2B04 due to the multitude of mutations
177 contained within the class I RBM epitope (1, 19) (**Fig. 1a**), and JN.1 and BA.2.87.1 were no
178 exception, both having escaped neutralization by this monoclonal antibody (**Figs. S1d-e**).

179

180 ***BA.2.87.1 is antigenically more related to D614G and BA.2 other than recent Omicron***
181 ***subvariants.*** To further analyze our neutralization data, we performed antigenic cartography
182 analysis using a program called Racmacs, which uses principal component analysis to plot the
183 antigenic distance between the variants tested based on the nAb titers. For bivalent-vaccinated
184 human samples, D614G and BA.2 clustered near each other, with an antigenic distance of 0.45,
185 and they were farther away from the cluster of newer variants (**Fig. 2e**). Notably, JN.1 was farthest
186 away from D614G, with antigenic distance of 2.95, which was in accordance with its lowest nAb
187 titers (**Fig. 2a and Fig. S1a**), suggesting that JN.1 is more antigenically distinct from D614G and
188 BA.2 than XBB.1.5, BA.2.86, and BA.2.87.1. Interestingly, BA.2.87.1 clustered closer to D614G
189 and BA.2, with an antigenic distance of 2 and 2.15., respectively, suggesting that despite the 30
190 additional mutations in the spike, it has actually become more antigenically similar to the parental
191 variants (**Fig. 2e**). Because of the heterogeneity as well as the small sample size of JN.1-wave
192 patient samples, we did not perform the antigenic analysis for this cohort.

193 The hamster cohort map was quite distinct from the bivalent mRNA-vaccinated human
194 cohort due to the very different patterns of antigenic exposure. We observed that XBB.1.5,
195 BA.2.86, and JN.1 all clustered together, but with greater antigenic distances of 3.48~4.14 from
196 D614G; whereas BA.2.87.1 was antigenically closer with distances of 1.08 and 2 from D614G
197 and BA.2, respectively (**Fig. 2f**). Overall, these analyses indicate that antigenically, BA.2.87.1 is
198 more closely related to BA.2, the ancestral Omicron variant; however, BA.2.86 and JN.1 are more
199 closely related to XBB.1.5.

200

201 ***BA.2.87.1 spike exhibits increased cell-cell fusion and processing into S1 and S2.*** Given
202 more than 30 amino-acid changes in the spike protein of BA.2.87.1 and JN.1, including some
203 near the furin cleavage site as well as in the S2 subunit (**Fig. 1a**), it is important to examine the
204 furin cleavage efficiency and cell-cell fusion property of these new variants. For cell-cell fusion,
205 we transfected 293T cells with the spikes of interest plus GFP, followed by co-culturing the
206 detached effector 293T cells with target 293T-ACE2 or CaLu-3 cells. In both cell lines, D614G
207 exhibited the highest cell-cell fusion compared to all Omicron variants (**Figs. 3a-d**), as would be
208 expected. Notably, BA.2.87.1 exhibited the highest cell-cell fusion activity of the Omicron variants
209 in both cell lines. While JN.1 exhibited an increased cell-cell fusion relative to BA.2, the level was
210 comparable to its ancestral BA.2.86. XBB.1.5 showed increased fusion activity relative to the
211 ancestral BA.2, which was consistent with our previous results (4, 9), although the level was
212 relatively lower than BA.2.87.1 in both 293T-ACE2 and CaLu3 cells (**Figs. 3a-d**). We validated
213 these results using a syncytia formation assay wherein 293T-ACE2 cells are transfected to
214 produce the spikes of interest and GFP and incubated 24 hours before imaging fusion (**Figs. S2a**
215 **and b**).

216 We next determined the surface expression level of spike proteins in 293T cells used to
217 produce the lentiviral pseudotyped viruses by flow cytometry. We found that XBB.1.5 exhibited
218 the highest expression, followed by D614G and BA.2.86. Interestingly, BA.2, JN.1, an BA.2.87.1

219 all exhibited decreased surface expression relative to D614G, with BA.2.87.1 being the lowest
220 (**Figs. 4a-b**). This pattern is corroborated by western blotting analysis of the lysate of these
221 producer cells which depicts overall less spike expression for all Omicron variants except for
222 XBB.1.5 (upper panel, **Fig. 4c**). The differences in spike protein expression, including on the
223 plasma membrane, were not due to artifacts of transfection efficiency, given the similar levels of
224 HIV-1 lentiviral Gag expression detected by an anti-P24 antibody (middle panel, **Fig. 4c**) and the
225 comparable signals of GAPDH detected by anti-GAPDH (lower panel, **Fig. 4c**). Importantly,
226 despite the relatively low level of expression, BA.2.87.1 and JN.1 both exhibited increased
227 processing of spike into the S1 and S2 subunits as compared to the parental D614G and their
228 ancestral BA.2, as quantified by the S1/S and S2/S ratios (**Fig. 4c**).

229

230 **DISCUSSION**

231 The continued tracking and characterization of emerging variants of SARS-CoV-2 has
232 proven critical to maintaining pandemic control strategies including vaccination. In accordance
233 with the variants' swift rise to dominance, in this work we showed that JN.1 exhibits the lowest
234 nAb titers for both bivalent-vaccinated individuals and first-responders/ICU-admitted COVID-19
235 patients. The decrease in neutralization titers against JN.1 relative to BA.2.86 is consistent with
236 data published by others, and also explains, at least in part, why JN.1 has become a globally
237 dominant variant compared to its ancestral BA.2.86 (6, 14, 20). Interestingly, we discovered that
238 the newly emerged BA.2.87.1 variant possesses an increased sensitivity to neutralization by
239 these sera compared to JN.1, implying that this variant may not be able to outcompete the current
240 JN.1 and become predominant. However, given that a single L455S mutation in the spike of JN.1
241 can dramatically increase the nAb evasion of BA.2.86 (3, 14, 21), there is a possibility that
242 additional mutations in BA.2.87.1 could similarly result in new variants that dramatically enhance
243 the nAb escape.

244 It is currently unclear what amino acid changes in the BA.2.87.1 spike are responsible for
245 the enhanced neutralization by nAb generated by the bivalent mRNA vaccine and JN.1-wave
246 infection. However, given the differences in spike between BA.2.87.1 and others including BA.2
247 and JN.1 shown in **Fig. 1a**, we speculate that two N-terminal deletions, specifically 15-25del and
248 136-146del, might have contributed to the comparatively higher nAb titers against BA.2.87.1
249 compared to BA.2.86/JN.1 and XBB.1.5 — both lacking these deletions. Moreover, BA.2, which
250 serves as the precursor to recent Omicron subvariants and is devoid of these two deletions,
251 demonstrates approximately a 3.2-fold increased titer against BA.2.87.1 (**Fig. 2a**). These findings
252 collectively support for a potential role of these deletions in nAb evasion, which was confirmed by
253 a recent preprint (32). Beyond the N-terminal deletions, the presence of eight amino acid changes
254 in the Receptor Binding Domain (RBD), along with seven amino acid modifications in the furin
255 cleavage site and S2 of the spike (**Fig. 1a**), could alter the ACE2 binding and/or viral membrane
256 fusion capabilities of BA.2.87.1, thus contributing to the varied entry efficiency of BA.2.87.1 (**Fig.**
257 **1b-c**). These amino acid changes could also explain the loss of sensitivity of BA.2.87.1 to mAb
258 2B04 yet re-gain of its neutralizing by S309 (**Fig. 2d** and **Fig. S1e**). Nevertheless, it's crucial to
259 acknowledge that the replication of BA.2.87.1 diverges from entry mechanisms, and mutations in
260 non-spike regions of the genome could also hold significant roles. Therefore, a comprehensive
261 analysis of the replication of authentic BA.2.87.1 will provide insights into the impact of spike
262 mutations on immune evasion and replication.

263 In this work, we found that antibodies elicited by BA.2.86/JN.1-wave infection did not
264 effectively neutralize BA.2.86/JN.1 compared to D614G, potentially due to immune imprinting,
265 which has been observed for BA.4/5 and XBB.1.5 variants by ours and other groups (22-24).
266 Immune imprinting arises through two general mechanisms, one is that the immune system
267 prioritizes a recalled response over a new one ("antigenic seniority"), and the other is that new
268 response is actively suppressed ("primary addiction") (25, 26). Importantly, SARS-CoV-2 infection

269 and vaccination can both cause immune imprinting, resulting in decreased vaccine efficacy (22).
270 For example, vaccinated individuals who had breakthrough infection with different variants mount
271 nAb response primarily towards the wildtype spike protein (9, 10, 14, 18, 21, 26, 27). In this study,
272 all JN.1 patients in the infected cohort had received some doses of vaccine containing the WT
273 spike (**Table S1**). We suspect that this could explain the relatively low titers of these patient sera
274 against JN.1 as compared to D614G (**Fig. 2b** and **Fig. S1b**) (25, 26, 28). A single antigenic
275 exposure to an Omicron subvariant such as JN.1 may not be sufficient to overcome immune
276 imprinting driven by the monovalent WT vaccines (18, 28-32).

277 The neutralization pattern of XBB.1.5-monovalent-vaccinated hamster sera against
278 BA.2.87.1 is somewhat surprising. These samples exhibited robust titers against XBB.1.5,
279 BA.2.86 and JN.1 yet showed low titers against D614G, which emphasize the need to move away
280 from WT spike-containing vaccines. Interestingly, the titers against BA.2.87.1 were notably lower
281 than those of other Omicron variants in this cohort, raising the possibility that XBB.1.5 monovalent
282 vaccine may not be able to effectively protect against infection by this new variant in SARS-CoV-
283 2 naïve individuals. However, this concern might be diminished, given that a majority of the world
284 population has been vaccinated and/or infected by SARS-CoV-2, unlike the naïve hamsters in
285 this cohort; this hybrid immunity could offer potential broader protection against emerging
286 variants, including JN.1 and BA.2.87.1 (30-32). Indeed, despite JN.1 exhibiting an enhanced
287 ability to evade the COVID-19 vaccine compared to BA.2.86, recent studies (29, 33, 34) have
288 shown that the monovalent XBB.1.5 vaccine can generate effective nAbs against JN.1,
289 contributing to the control of the rapid JN.1 transmission. Unfortunately, we were unable to confirm
290 the result of hamster serum samples in XBB.1.5 monovalent-vaccinated human population with
291 no prior history of exposure to COVID-19 vaccination or SARS-CoV-2 infection, because XBB.1.5
292 monovalent vaccination is only allowed as booster to those who had been previously vaccinated.
293 In addition, our finding that BA.2.87.1 does not cluster with the other more recent Omicron
294 variants, but instead resembles D614G and BA.2, further highlights the distinctive antigenic nature

295 of BA.2.87.1, underscoring the need to continue monitoring the SARS-CoV-2 variants and
296 updating the COVID-19 vaccines.

297 In addition to its distinct antigenic phenotype, BA.2.87.1 spike also displayed changes in
298 spike protein biology. Most noticeably, we found that the BA.2.87.1 spike has increased cell-cell
299 fusion and processing as compared to the other Omicron variants including JN.1. While both
300 phenotypes still fall below the levels of D614G, we cannot rule out the possibility that the
301 pathogenicity and/or tissue tropism of this variant may be altered. Experiments using infectious
302 virus to investigate these biological properties will be necessary. Although viral replication fitness
303 is not a focus of this work, it is important to emphasize that differences exist between immunized
304 and immunologically naïve individuals, which can shape the emergence of new SARS-CoV-2
305 variants and disease pathogenesis. In immunized individuals, viral replication may be controlled
306 more efficiently in immunized subjects due to the quicker and targeted immune response, leading
307 to faster viral clearance and reduced severity of the infection. However, the immune system's
308 selective pressure in immunized individuals may also drive the evolution of the virus towards
309 variants that can escape immune recognition, although the replication fitness of these escape
310 variants may vary, and they may not always outcompete the original strains in terms of
311 transmissibility or virulence.

312

313 **ACKNOWLEDGEMENTS**

314 We thank the members of Liu lab, especially Panke Qu, for helpful discussion. We also
315 thank the Clinical Research Center/Center for Clinical Research Management of The Ohio State
316 University Wexner Medical Center and The Ohio State University College of Medicine in
317 Columbus, Ohio, specifically J. Brandon Massengill, Francesca Madiari, Dina McGowan, Breona
318 Edwards, Evan Long, and Trina Wemlinger, for collection and processing of samples. We thank
319 Tongqing Zhou at NIH for providing the S309 monoclonal antibody. In addition, we thank Sarah

320 Karow, Madison So, Preston So, Daniela Farkas, and Finny Johns in the clinical trials team of
321 The Ohio State University for sample collection and other supports. We thank Moemen Eltobgy
322 for assistance in sample processing. We specially thank Ashish R. Panchal, Soledad Fernandez,
323 Mirela Anghelina, and Patrick Stevens for their assistance in providing the sample information of
324 the first responders and their household contacts. We thank Peng Ru and Lauren Masters for
325 sequencing and Xiaokang Pan for bioinformatic analysis. S.-L.L., D. J., R.J.G., L.J.S. and E.M.O.
326 were supported by the National Cancer Institute of the NIH under award no. U54CA260582. The
327 content is solely the responsibility of the authors and does not necessarily represent the official
328 views of the National Institutes of Health. This work was also supported by a fund provided by an
329 anonymous private donor to OSU. NIH R01AI090060. M.C. was supported by an NIH T32 training
330 grant (T32AI165391). J. L. was supported by NIH R01AI090060. M.C. was supported by an NIH
331 T32 training grant (T32AI165391). J.S.B. was supported by award number grants UL1TR002733
332 and KL2TR002734 from the National Center for Advancing Translational Sciences. R.J.G. was
333 additionally supported by the Robert J. Anthony Fund for Cardiovascular Research and the JB
334 Cardiovascular Research Fund, and L.J.S. was partially supported by NIH R01 HD095881.

335 The authors have no competing interests to disclose.

336 S.-L.L. conceived and directed the project. R.J.G. led the clinical study and experimental
337 design and implementation. P.L. performed all experiments with assistance from Y.L. P.L.
338 performed data processing and analyses with help from J.N.F and Y.-M.Z. C.C.H and J.L.
339 provided the hamster serum samples. D.J. led SARS-CoV-2 variant genotyping and DNA
340 sequencing analyses. C.C., J.S.B, J.C.H., R.M., and R.J.G provided clinical samples and
341 associated information. P.L., J.N.F, and S.-L.L. wrote the paper. J.L., L.J.S., and E.M.O. provided
342 insightful discussion and revision of the manuscript.

343

344 **FIGURE LEGENDS**

345

346 **FIG 1 Infectivity of BA.2.87.1 and JN.1 in 293T-ACE2 and CaLu-3 cells.** (a) A schematic
347 depiction comparing spike mutations in the studied variants including BA.2.87.1 and JN.1 by
348 amino acid numbers. NTD = N-terminal domain, RBD = receptor binding domain; S2: the S2
349 subunit region. (b-c) Relative infectivity of lentiviral pseudotypes bearing each of the listed spikes
350 in (b) 293T cells expressing human ACE2 (293T-ACE2) and (c) human lung cell line CaLu-3.
351 Relative luminescence readouts were normalized to D614G (D614G = 1.0). Bars in (b-c)
352 represent means \pm standard error from triplicates of transfection. Significance relative to D614G
353 was analyzed by a one-way repeated measures ANOVA with Bonferroni's multiple testing
354 correction (n = 6). p values are displayed as ns p > 0.05, ***p < 0.001, and ****p < 0.0001.

355

356 **FIG 2 Neutralization of BA.2.87.1 and JN.1 by bivalent-vaccinated human sera, JN.1-wave**
357 **human sera, XBB.1.5-vaccinated hamster sera, and monoclonal antibody S309.** (a-c) NAb
358 titers were determined using lentiviruses bearing the indicated spike proteins, with the titer of
359 D614G as a control. All were compared against D614G or XBB.1.5 unless otherwise specified.
360 The three cohorts included sera from 13 HCWs who had at least 2 monovalent doses of mRNA
361 vaccine and 1 dose of bivalent mRNA vaccine (n = 13) (a), sera from Columbus first-
362 responder/household contact cohort (P1 to P5) and ICU patients admitted to OSU Wexner
363 Medical Center (P6 to P9) during when the BA.2.86/JN.1 variants were predominantly circulating
364 in Columbus, Ohio (b) (n=9 total), and sera from Golden Syrian hamsters inoculated with two
365 doses of XBB.1.5 monovalent vaccine (recombinant mumps virus expressing the spike of
366 XBB.1.5, 1.5×10^5 PFU per hamster, three weeks apart) (n=15), with blood being collected 5
367 weeks after inoculation (c). Geometric mean NT₅₀ values for each variant are shown on the top.
368 Bars represent geometric means with 95% confidence intervals. Statistical significance was
369 analyzed with log₁₀ transformed NT₅₀ values. Comparisons between multiple groups were
370 performed using a one-way ANOVA with Bonferroni post-test. Dashed lines represent the
371 threshold of detection, i.e., NT₅₀ = 40. p values are shown as ns p > 0.05, *p < 0.05, **p < 0.01,

372 ****p < 0.0001. (d) Neutralization by mAb S309 was assessed, with representative plot curves
373 displayed. Bars represent means \pm standard deviation. (e–f) Antigenic maps for neutralization
374 titers from Fig. 2a (bivalent-vaccinated human sera) and Fig. 2c (XBB.1.5-monovalent-vaccinated
375 hamster sera) were made using the Racmacs program (1.1.35) (see Methods). Squares represent
376 the individual sera sample and circles represent variants. One square on the grid represents one
377 antigenic unit squared.

378

379 **FIG 3 Cell-Cell fusion of BA.2.87.1 and JN.1 spikes alongside other SARS-CoV-2 variants**
380 **in 293T-ACE2 and CaLu-3 cells.** HEK293T cells were co-transfected with plasmids of indicated
381 spikes together with GFP and were cocultured with 293T-ACE2 (a-b) or human lung epithelial
382 CaLu-3 cells (c-d) for 6.5 h (HEK293-ACE2) or 4h (CaLu-3). Cell-cell fusion was imaged and GFP
383 areas of fused cells were quantified (see Methods). D614G and no spike served as positive and
384 negative control, respectively. Comparisons of the extent of cell-cell fusion were made for each
385 Omicron subvariant against D614G. Scale bars represent 150 μ M. Bars in (b and d) represent
386 means \pm standard error. Dots represent three images from two biological replicates. Statistical
387 significance relative to D614G was determined using a one-way repeated measures ANOVA with
388 Bonferroni's multiple testing correction (n = 3). p values are displayed as ns p > 0.05, *p < 0.05,
389 and ****p < 0.0001.

390

391 **FIG 4 Surface expression and processing of BA.2.87.1, JN.1, and other spike proteins.** (a-
392 b) Cell surface expression of spike proteins. HEK293T cells used for production of pseudotyped
393 lentiviral vectors bearing indicated spikes of interest were fixed and stained for spike with an anti-
394 S1 specific antibody T62 followed by flow cytometric analyses. (a) Histogram plots of anti-S1
395 signals in transfected cells. (b) Mean fluorescence intensities of individual subvariants from (a).
396 (c) Spike expression and processing. HEK293T cells used to produce pseudotyped vectors were
397 lysed and probed with anti-S1, anti-S2, anti-GAPDH, or anti-p24 antibodies; spike processing was

398 quantified using NIH ImageJ to determine the S1/S or S2/S ratio and normalized to D614G
399 (D614G = 1.0). Bars in (b) represent means \pm standard error. Dots represent three biological
400 replicates from one typical experiment. Significance relative to D614G was determined using a
401 one-way repeated measures ANOVA with Bonferroni's multiple testing correction ($n = 3$). p values
402 are displayed as ns $p > 0.05$, ** $p < 0.01$, and **** $p < 0.0001$.
403

404

Table S1. Bivalent-vaccinated HCW and BA.2.86/JN.1-wave first responder cohorts

	Bivalent HCWs (n=13)	BA.2.86-JN.1 Wave Patients (n=9)
Age in Years at Sample Collection [Median (Range)]	37 (25-48)	53(35-78)
Gender [n (% of Total)]		
Male	8 (61.5%)	4 (44.4%)
Female	5 (38.5%)	5 (55.6%)
Sample Collection Window	Dec. 2022- Jan.2023	Nov. 2023-Feb.2024
Vaccine status [n (% of Total)]		
2-dose Moderna	NA	3 (11.1%)
1-dose Moderna+1-dose Pfizer bivalent	NA	1 (11.1%)
3-dose Pfizer +1-dose Moderna bivalent	1 (7.7%)	0
2-dose Pfizer+1-dose Pfizer bivalent	1 (7.7%)	0
4-dose Pfizer+1-dose Pfizer bivalent	1 (7.7%)	0
3-dose Pfizer+1-dose Pfizer bivalent	3 (23.1%)	0
3-dose Moderna+1-dose Moderna bivalent	6 (46.2%)	1 (11.1%)
2-dose Moderna+1 Pfizer+1-dose Pfizer bivalent	1 (7.7%)	0
3-dose Moderna+1-dose Pfizer bivalent+1-dose Moderna bivalent	0	1 (11.1%)
2-dose Pfizer +1-dose Moderna	0	1 (11.1%)
1-dose Pfizer	0	1 (11.1%)
3-dose Moderana+1-dose XBB.1.5 Moderna monovalent	0	1 (11.1%)
Sample Collection Timing [Median (Range)]		
Days from last vaccination	NA	656 (45-898)
Days post the bivalent dose for recipients	66 (23-108)	NA
COVID-19 positive [n (% of Total)]	9 (69.2%)	9 (100%)
Days before sample collection [Median (Range)]	324 (182-994)	7 (1-10)
Infected Variants		
JN.1/BA.2.86	0	2 (22.2%)
Undetermined	NA	7 (77.8%)

405

406 Summary of the demographic information for two cohorts used for neutralization experiments
407 depicted in Figure 2. “NA” means the category is not applicable to the cohort.

408 MATERIALS AND METHODS

409 *Study cohorts*

410 Bivalent Vaccinated HCWs (n=13): These sera were collected from HCWs at the Ohio State
411 Wexner Medical Center that received at least 2 doses of monovalent vaccine (WT) and 1 dose of
412 bivalent vaccine (WT+BA.4/5) under the approved IRB protocols 2020H0228, 2020H0527, and
413 2017H0292. 11 individuals received 3 doses of monovalent vaccine (Pfizer or Moderna
414 formulations) and 1 bivalent booster dose (Pfizer). 1 person received 4 doses of monovalent
415 vaccine (Pfizer) and 1 bivalent booster dose (Pfizer). 1 person received 2 doses of Pfizer
416 monovalent vaccine and 1 bivalent booster dose (Pfizer). This cohort ranged from 25-48 years of
417 age and included 8 males and 5 females. Blood was collected between 23-108 days post-bivalent
418 booster dose (see details in **Table S1**).

419

420 ICU patients infected in BA.2.86/JN.1 wave (n=9): These sera were collected from ICU patients
421 in the OSU Wexner Medical Center or symptomatic participants in the first responder/household
422 contact STOP-COVID cohort who had reverse transcription PCR positivity for SARS-CoV-2
423 between the dates of 11/23/2024-2/16/2024 during which the BA.2.86/JN.1 variants were
424 predominantly circulating in Columbus, Ohio, U.S (**Table S1**). Samples were collected under the
425 approved IRBs protocols 2020H0527, 2020H0531, 2020H0240, and 2020H0175. Variant type
426 was confirmed in a subset of samples with available nasopharyngeal swabs by SARS-CoV-2
427 complete genome next-generation sequencing using Artic v5.3.2 (IDT, Coralville, IA) and Artic
428 v4.1 primer sets (Illumina, San Diego, CA).

429

430 Hamster cohorts vaccinated with monovalent XBB.1.5 vaccine (n=15)

431 Fifteen 4-week-old golden Syrian hamsters (Envigo, Indianapolis, IN) were immunized
432 intranasally with 1.5×10^5 PFU per animal of XBB.1.5 spike-based monovalent vaccine

433 (recombinant mumps virus expressing spike of XBB.1.5). Three weeks later, hamsters were
434 boosted with the same vaccine at the same dose. Blood was collected at week 5 after initial
435 immunization (week 2 after booster immunization).

436

437 *Cell lines*

438 The cell lines utilized in this investigation comprised human epithelial kidney cells (HEK293T,
439 ATCC CRL-11268, RRID: CVCL_1926) and HEK293T cells overexpressing human ACE2 (BEI:
440 NR-52511, RRID: CVCL_A7UK). Additionally, we employed the human epithelial lung carcinoma
441 cell line CaLu-3. HEK293T cell lines were cultured in DMEM Gibco (11965-092) supplemented
442 with 10% fetal bovine serum (Sigma, F1051) and 0.5% penicillin/streptomycin (HyClone,
443 SV30010). CaLu-3 cells (RRID: CVCL_0609) were cultured in EMEM (ATCC, 30-2003) under the
444 same conditions. Cell cultures were maintained at 37°C with 5.0% CO₂ and sub-cultured by
445 washing with PBS (Sigma, D5652-10X1L) followed by detachment using 0.05% trypsin + 0.53
446 mM EDTA (Corning, 25-052-CI).

447

448 *Plasmids*

449 All spike constructs are encoded within the pcDNA3.1 backbone and flanked by C-terminal FLAG
450 tags. They were cloned using KpnI and EcoRI restriction sites. D614G, BA.2, BA.2.86, and
451 BA.2.87.1 plasmids were all synthesized by GenScript Biotech (Piscataway, NJ). The BA.2.87.1
452 spike sequence was generated based on the consensus of the first few reported

453 Isolates: hCoV-19/SouthAfrica/NICD-R13200/2023 EPI_ISL_18849984; hCoV
454 19/SouthAfrica/NICD-N56614/2023 EPI_ISL_18849985; hCoV-19/SouthAfrica/NICD-
455 N56836/2023 EPI_ISL_18849986; hCoV-19/SouthAfrica/NICD-N57176/2023
456 EPI_ISL_18849987; hCoV-19/SouthAfrica/NICD-N57208/2023 EPI_ISL_18849988; hCoV-
457 19/SouthAfrica/NICD-N57216/2023 EPI_ISL_18849989; hCoV-19/SouthAfrica/NICD-
458 N57440/2023 EPI_ISL_18849990; hCoV-19/SouthAfrica/NICD-N57469/2023

459 EPI_ISL_18849991;hCoV-19/South Africa/NICD-R13515/2023 EPI_ISL_18845398; while
460 XBB.1.5 and JN.1 were generated through site-directed mutagenesis of XBB and BA.2.86,
461 respectively. The lentiviral vector used was an HIV-1 based vector called Pnl4-3 with an Env
462 deletion that encodes a *Gaussia luciferase* reporter gene.

463

464 *Pseudotyped lentiviral production and infectivity*

465 Pseudotyped lentiviral vectors were generated following established protocols. Briefly, 293T cells
466 were co-transfected using PEI (Transporter 5 Transfection Reagent, Polysciences) at a 2:1 ratio
467 with the Pnl4-3_inGluc vector and the spike plasmid under investigation. Pseudovirus was
468 harvested by collecting media from the cells at 48 and 72 hours post-transfection. The media was
469 then clarified by centrifugation, and equal volumes were utilized to infect the target cells.
470 Luciferase activity was measured by combining 20UI of infected cell culture media with 20UI of
471 *Gaussia luciferase* substrate (0.1 M Tris Ph 7.4, 0.3 M sodium ascorbate, 10 μ M coelenterazine)
472 and immediately quantifying luminescence using a BioTek Cytation plate reader. These values
473 were normalized relative to D614G, with D614G set as 1.

474

475 *Virus neutralization assay*

476 The pseudotyped lentiviral vector neutralization assay was performed as described previously
477 (10). Briefly, sera samples are serially diluted 4-fold at a starting dilution of 1:40 for 5 total dilutions
478 (1:40, 1:160, 1:640, 1:2560, 1:10240), with one well left without sera. Pseudotyped viruses are
479 diluted based on infectivity readouts in order to normalize them then placed in equal volumes on
480 the diluted sera and incubated 1 hour at 37°C. The sera/virus mixture is then used to infect 293T-
481 ACE2 cells. As described for infectivity, luminescence readouts are collected at 48 and 72 hours
482 and used to determine a neutralization titer at 50% (NT₅₀) using least squares fit non-linear
483 regression normalized to the no serum value using GraphPad Prism 9 (San Diego, CA).

484

485 *Cell-cell fusion*

486 Direct spike-mediated cell to cell fusion assays were performed by first co-transfecting 293T cells
487 with spike and GFP. 293T cells were incubated 24 hours then detached and reseeded in a plate
488 containing one of two detached target cells; 293T-ACE2 or CaLu-3. 293T-ACE2 cells were
489 incubated for 6.5 hours and CaLu-3 cells 4 hours then fusion was imaged using a Leica Dmi8
490 microscope. Areas of fusion were quantified using the Leica X Applications Suite software to
491 outline the edges of fields of GFP and quantify then areas. Three images from duplicate wells
492 were randomly taken. Scale bars represent 150 μ M and one representative image was selected
493 for presentation.

494

495 *Syncytia formation assay*

496 To validate the cell-cell fusion results, a syncytia formation assay was also performed. 293T-
497 ACE2 cells were co-transfected with the spike of interest and GFP and incubated 24 hours before
498 imaging syncytia using a Leica Dmi8 microscope. The images were processed and displayed the
499 same way as the cell-cell fusion results.

500

501 *Spike protein surface expression detected by flow cytometry*

502 A portion of 293T cells used to produce the lentiviral vectors were collected by detaching with
503 PBS + 5Mm EDTA and fixed in 3.7% formaldehyde for 10 minutes and room temperature. Cells
504 were then stained with polyclonal anti-SARS-CoV-2 S1 antibody (Sino Biological, 40591-T62;
505 RRID: AB_2893171) followed by anti-Rabbit-IgG-FITC (Sigma, F9887, RRID: AB_259816)
506 secondary to visualize on a Life Technologies Attune NxT flow cytometer. FlowJo v10 (Ashland,
507 OR) is used to analyze data.

508

509 *Spike protein processing*

510 The remaining 293T cells used to produce lentiviral vectors are lysed in RIPA buffer (Sigma-
511 Aldrich, R0278) supplemented with protease inhibitor (Sigma, P8340) for 40 minutes on ice.
512 Lysate is collected and a portion is used for SDS-PAGE on a 10% poly-acrylamide gel and
513 transferred to a PVDF membrane for western blotting. Blots were probed with polyclonal anti-
514 SARS-CoV-2 S1 (Sino Biological, 40591-T62; RRID:AB_2893171), anti-S2 (Sino Biological,
515 40590; RRID:AB_2857932), anti-p24 (NIH HIV Reagent Program, ARP-1513), and anti-GAPDH
516 (Santa Cruz, Cat# sc-47724, RRID: AB_627678). Secondary antibodies used were Anti-Rabbit-
517 IgG-HRP (Sigma, A9169; RRID:AB_258434) and Anti-Mouse (Sigma, Cat# A5278, RRID:
518 AB_258232). Blots were visualized via Immobilon Crescendo Western HRP substrate (Millipore,
519 WBLUR0500) and exposed on a GE Amersham Imager 600. Band intensities were quantified
520 using NIH Image J analysis software (Bethesda, MD).

521

522 *Antigenic mapping*

523 Antigenic cartography was performed using the Racmacs program (v1.1.35) by following
524 instructions provided on their GitHub (<https://github.com/acorg/Racmacs/tree/master>). Briefly, the
525 program is run in R (Vienna, Austria) and works by taking raw neutralization titers and log2
526 transforming them to create a distance table for the individual antigens (spike protein) and sera
527 samples. The program then uses this table to perform multidimensional scaling to plot the
528 individual antigen and sera samples as single points where distance between the points directly
529 correlates to antigenic differences. 1 antigenic distance unit (AU), represented by one side of a
530 square in the plots, is equivalent to a 2-fold change in neutralization titers. Optimization settings
531 were kept on default (2 dimensions, 500 optimizations, minimum column basis “none”). Maps
532 were saved as images via the “view(map)” function and labeled using Microsoft Office
533 PowerPoint.

534

535 *Statistical analysis*

536 Statistical analyses were conducted using GraphPad Prism 9. Error bars in the figures represent
537 means with standard error. In Figs 1b and 1c, Figs. 3b and 3d, Fig. 4b, and Fig. S2b, comparisons
538 between viruses were made using a one-way ANOVA with Bonferroni post-test. Neutralization
539 titers were determined using least-squares non-linear regression. In Figs 2a-c, error bars
540 represent geometric means with 95% confidence intervals. Comparisons between viruses in
541 these figures were made using a repeated measures one-way ANOVA with Bonferroni post-test.
542 To better approximate normality, comparisons were conducted using log₁₀ transformed NT₅₀
543 values. Error bars in Fig. 2d represent means ± standard deviation. Cell-cell fusion and syncytia
544 formation shown in Figs. 3a, 3c, and Fig. S2a was quantified using the Leica X Applications Suite
545 software. Spike processing shown in Fig. 4c was quantified by NIH ImageJ; the values are then
546 set relative to D614G, with D614G = 1.0.

547

548 **RESOURCE AVAILABILITY**

549 Data reported in this paper will be shared by the lead contact upon request, Dr. Shan-Lu Liu
550 (liu.6244@osu.edu). Any additional information required to reanalyze the data reported in this
551 paper is available from the lead contact upon request.

552

553 **REFERENCES**

- 554 1. Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C, du Plessis L, Pybus OG.
555 2020. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic
556 epidemiology. *Nature Microbiology* 5:1403-1407.
- 557 2. CDC. 2022. COVID Data Tracker, Variant Proportions.
- 558 3. Wang Q, Guo Y, Liu L, Schwanz LT, Li Z, Nair MS, Ho J, Zhang RM, Iketani S, Yu J, Huang
559 Y, Qu Y, Valdez R, Lauring AS, Huang Y, Gordon A, Wang HH, Liu L, Ho DD. 2023.
560 Antigenicity and receptor affinity of SARS-CoV-2 BA.2.86 spike. *Nature* 624:639-644.
- 561 4. Qu P, Xu K, Faraone JN, Goodarzi N, Zheng Y-M, Carlin C, Bednash JS, Horowitz JC,
562 Mallampalli RK, Saif LJ, Oltz EM, Jones D, Gumina RJ, Liu S-L. 2024. Immune evasion,
563 infectivity, and fusogenicity of SARS-CoV-2 BA.2.86 and FLip variants. *Cell* 187:585-
564 595.e6.
- 565 5. Liu Z, Zhou J, Wang W, Zhang G, Xing L, Zhang K, Wang Y, Xu W, Wang Q, Man Q, Wang
566 Q, Ying T, Zhu Y, Jiang S, Lu L. 2024. Neutralization of SARS-CoV-2 BA.2.86 and JN.1 by
567 CF501 adjuvant-enhanced immune responses targeting the conserved epitopes in
568 ancestral RBD. *Cell Rep Med* doi:10.1016/j.xcrm.2024.101445:101445.
- 569 6. Yang S, Yu Y, Xu Y, Jian F, Song W, Yisimayi A, Wang P, Wang J, Liu J, Yu L, Niu X, Wang
570 J, Wang Y, Shao F, Jin R, Wang Y, Cao Y. 2024. Fast evolution of SARS-CoV-2 BA.2.86
571 to JN.1 under heavy immune pressure. *Lancet Infect Dis* 24:e70-e72.
- 572 7. CDC. 2/9/2024 2024. CDC Tracks New SARS-CoV-2 Variant, BA.2.87.1.
- 573 8. Zeng C, Evans JP, Qu P, Faraone J, Zheng YM, Carlin C, Bednash JS, Zhou T, Lozanski
574 G, Mallampalli R, Saif LJ, Oltz EM, Mohler P, Xu K, Gumina RJ, Liu SL. 2021.
575 Neutralization and Stability of SARS-CoV-2 Omicron Variant. *bioRxiv*
576 doi:10.1101/2021.12.16.472934.
- 577 9. Qu P, Faraone JN, Evans JP, Zheng Y-M, Carlin C, Anghelina M, Stevens P, Fernandez
578 S, Jones D, Panchal AR, Saif LJ, Oltz EM, Zhang B, Zhou T, Xu K, Gumina RJ, Liu S-L.
579 2023. Enhanced evasion of neutralizing antibody response by Omicron XBB.1.5, CH.1.1,
580 and CA.3.1 variants. *Cell Reports* 42:112443.
- 581 10. Faraone JN, Qu P, Goodarzi N, Zheng Y-M, Carlin C, Saif LJ, Oltz EM, Xu K, Jones D,
582 Gumina RJ, Liu S-L. 2023. Immune Evasion and Membrane Fusion of SARS-CoV-2 XBB
583 Subvariants EG.5.1 and XBB.2.3. *Emerging Microbes and Infections* 12(2):2270069.
- 584 11. Evans JP, Zeng C, Qu P, Faraone J, Zheng YM, Carlin C, Bednash JS, Zhou T, Lozanski
585 G, Mallampalli R, Saif LJ, Oltz EM, Mohler PJ, Xu K, Gumina RJ, Liu SL. 2022.
586 Neutralization of SARS-CoV-2 Omicron sub-lineages BA.1, BA.1.1, and BA.2. *Cell Host*
587 *Microbe* 30:1093-1102.e3.
- 588 12. Qu P, Evans JP, Faraone JN, Zheng YM, Carlin C, Anghelina M, Stevens P, Fernandez S,
589 Jones D, Lozanski G, Panchal A, Saif LJ, Oltz EM, Xu K, Gumina RJ, Liu SL. 2023.
590 Enhanced neutralization resistance of SARS-CoV-2 Omicron subvariants BQ.1, BQ.1.1,
591 BA.4.6, BF.7, and BA.2.75.2. *Cell Host Microbe* 31:9-17.e3.
- 592 13. Zeng C, Evans JP, Pearson R, Qu P, Zheng YM, Robinson RT, Hall-Stoodley L, Yount J,
593 Pannu S, Mallampalli RK, Saif L, Oltz E, Lozanski G, Liu SL. 2020. Neutralizing antibody
594 against SARS-CoV-2 spike in COVID-19 patients, health care workers, and convalescent
595 plasma donors. *JCI Insight* 5.
- 596 14. Zhang L, Kempf A, Nehlmeier I, Cossmann A, Richter A, Bdeir N, Graichen L,
597 Moldenhauer AS, Dopfer-Jablonka A, Stankov MV, Simon-Loriere E, Schulz SR, Jäck HM,
598 Čičin-Šain L, Behrens GMN, Drosten C, Hoffmann M, Pöhlmann S. 2024. SARS-CoV-2
599 BA.2.86 enters lung cells and evades neutralizing antibodies with high efficiency. *Cell*
600 187:596-608.e17.
- 601 15. Wang X, Lu L, Jiang S. 2024. SARS-CoV-2 evolution from the BA.2.86 to JN.1 variants:
602 unexpected consequences. *Trends Immunol* 45:81-84.

- 603 16. Chen RE, Winkler ES, Case JB, Aziati ID, Bricker TL, Joshi A, Darling TL, Ying B, Errico
604 JM, Shrihari S, VanBlargan LA, Xie X, Gilchuk P, Zost SJ, Droit L, Liu Z, Stumpf S, Wang
605 D, Handley SA, Stine WB, Jr., Shi PY, Davis-Gardner ME, Suthar MS, Knight MG, Andino
606 R, Chiu CY, Ellebedy AH, Fremont DH, Whelan SPJ, Crowe JE, Jr., Purcell L, Corti D,
607 Boon ACM, Diamond MS. 2021. In vivo monoclonal antibody efficacy against SARS-CoV-
608 2 variant strains. *Nature* 596:103-108.
- 609 17. Errico JM, Zhao H, Chen RE, Liu Z, Case JB, Ma M, Schmitz AJ, Rau MJ, Fitzpatrick JAJ,
610 Shi PY, Diamond MS, Whelan SPJ, Ellebedy AH, Fremont DH. 2021. Structural
611 mechanism of SARS-CoV-2 neutralization by two murine antibodies targeting the RBD.
612 *Cell Reps* 37:109881.
- 613 18. Yang S, Yu Y, Jian F, Song W, Yisimayi A, Chen X, Xu Y, Wang P, Wang J, Yu L, Niu X,
614 Wang J, Xiao T, An R, Wang Y, Gu Q, Shao F, Jin R, Shen Z, Wang Y, Cao Y. 2023.
615 Antigenicity and infectivity characterisation of SARS-CoV-2 BA.2.86. *Lancet Infect Dis*
616 23:e457-e459.
- 617 19. Alsoussi WB, Turner JS, Case JB, Zhao H, Schmitz AJ, Zhou JQ, Chen RE, Lei T, Rizk
618 AA, McIntire KM, Winkler ES, Fox JM, Kafai NM, Thackray LB, Hassan AO, Amanat F,
619 Krammer F, Watson CT, Kleinstein SH, Fremont DH, Diamond MS, Ellebedy AH. 2020. A
620 Potently Neutralizing Antibody Protects Mice against SARS-CoV-2 Infection. *J Immunol*
621 205:915-922.
- 622 20. Uriu K, Ito J, Kosugi Y, Tanaka YL, Mugita Y, Guo Z, Hinay AA, Jr., Putri O, Kim Y, Shimizu
623 R, Begum MM, Jonathan M, Saito A, Ikeda T, Sato K. 2023. Transmissibility, infectivity,
624 and immune evasion of the SARS-CoV-2 BA.2.86 variant. *Lancet Infect Dis* 23:e460-e461.
- 625 21. Tamura T, Mizuma K, Nasser H, Deguchi S, Padilla-Blanco M, Oda Y, Uriu K, Tolentino
626 JEM, Tsujino S, Suzuki R, Kojima I, Nao N, Shimizu R, Wang L, Tsuda M, Jonathan M,
627 Kosugi Y, Guo Z, Hinay AA, Jr., Putri O, Kim Y, Tanaka YL, Asakura H, Nagashima M,
628 Sadamasu K, Yoshimura K, Saito A, Ito J, Irie T, Tanaka S, Zahradnik J, Ikeda T, Takayama
629 K, Matsuno K, Fukuhara T, Sato K. 2024. Virological characteristics of the SARS-CoV-2
630 BA.2.86 variant. *Cell Host Microbe* 32:170-180.e12.
- 631 22. Addetia A, Piccoli L, Case JB, Park YJ, Beltramello M, Guarino B, Dang H, de Melo GD,
632 Pinto D, Sprouse K, Scheaffer SM, Bassi J, Silacci-Fregni C, Muoio F, Dini M, Vincenzetti
633 L, Acosta R, Johnson D, Subramanian S, Saliba C, Giurdanella M, Lombardo G, Leoni G,
634 Culap K, McAlister C, Rajesh A, Dellota E, Jr., Zhou J, Farhat N, Bohan D, Noack J, Chen
635 A, Lempp FA, Quispe J, Kergoat L, Larrous F, Cameroni E, Whitener B, Giannini O, Cippà
636 P, Ceschi A, Ferrari P, Franzetti-Pellanda A, Biggiogero M, Garzoni C, Zappi S, Bernasconi
637 L, Kim MJ, Rosen LE, Schnell G, et al. 2023. Neutralization, effector function and immune
638 imprinting of Omicron variants. *Nature* 621:592-601.
- 639 23. Reynolds CJ, Pade C, Gibbons JM, Otter AD, Lin KM, Muñoz Sandoval D, Pieper FP,
640 Butler DK, Liu S, Joy G, Forooghi N, Treibel TA, Manisty C, Moon JC, Semper A, Brooks
641 T, McKnight Á, Altmann DM, Boyton RJ, Abbass H, Abiodun A, Alfarih M, Alldis Z, Altmann
642 DM, Amin OE, Andiapen M, Artico J, Augusto JB, Baca GL, Bailey SNL, Bhuva AN, Boulter
643 A, Bowles R, Boyton RJ, Bracken OV, O'Brien B, Brooks T, Bullock N, Butler DK, Captur
644 G, Carr O, Champion N, Chan C, Chandran A, Coleman T, Couto de Sousa J, Couto-
645 Parada X, Cross E, Cutino-Moguel T, D'Arcangelo S, et al. 2022. Immune boosting by
646 B.1.1.529 (Omicron) depends on previous SARS-CoV-2 exposure. *Science*
647 377:eabq1841.
- 648 24. Yisimayi A, Song W, Wang J, Jian F, Yu Y, Chen X, Xu Y, Yang S, Niu X, Xiao T, Wang J,
649 Zhao L, Sun H, An R, Zhang N, Wang Y, Wang P, Yu L, Lv Z, Gu Q, Shao F, Jin R, Shen
650 Z, Xie XS, Wang Y, Cao Y. 2024. Repeated Omicron exposures override ancestral SARS-
651 CoV-2 immune imprinting. *Nature* 625:148-156.
- 652 25. Faraone JN, Liu SL. 2023. Immune imprinting as a barrier to effective COVID-19 vaccines.
653 *Cell Rep Med* 4:101291.

- 654 26. Wang Q, Guo Y, Tam AR, Valdez R, Gordon A, Liu L, Ho DD. 2023. Deep immunological
655 imprinting due to the ancestral spike in the current bivalent COVID-19 vaccine. *Cell*
656 *Reports Medicine*.
- 657 27. Qu P, Faraone J, Evans JP, Zou X, Zheng YM, Carlin C, Bednash JS, Lozanski G,
658 Mallampalli RK, Saif LJ, Oltz EM, Mohler PJ, Gumina RJ, Liu SL. 2022. Neutralization of
659 the SARS-CoV-2 Omicron BA.4/5 and BA.2.12.1 Subvariants. *N Engl J Med* 386:2526-
660 2528.
- 661 28. Yisimayi A, Song W, Wang J, Jian F, Yu Y, Chen X, Xu Y, Yang S, Niu X, Xiao T, Wang J,
662 Zhao L, Sun H, An R, Zhang N, Wang Y, Wang P, Yu L, Gu Q, Shao F, Jin R, Shen Z, Xie
663 XS, Wang Y, Cao Y. 2023. Repeated Omicron infection alleviates SARS-CoV-2 immune
664 imprinting. *bioRxiv* doi:10.1101/2023.05.01.538516:2023.05.01.538516.
- 665 29. Wang Q, Guo Y, Bowen A, Mellis IA, Valdez R, Gherasim C, Gordon A, Liu L, Ho DD.
666 2024. XBB.1.5 monovalent mRNA vaccine booster elicits robust neutralizing antibodies
667 against XBB subvariants and JN.1. *Cell Host Microbe* doi:10.1016/j.chom.2024.01.014.
- 668 30. Wang X, Jiang S, Ma W, Li C, Liu C, Xie F, Zhu J, Zhan Y, Jiang S, Li M, Zhang Y, Wang
669 P. 2024. Robust Neutralization of SARS-CoV-2 Variants Including JN.1 and BA.2.87.1 by
670 Trivalent XBB Vaccine-Induced Antibodies. *bioRxiv*
671 doi:10.1101/2024.02.16.580615:2024.02.16.580615.
- 672 31. Lasrado N, Rössler A, Rowe M, Collier A-rY, Barouch DH. 2024. Neutralization of SARS-
673 CoV-2 Omicron subvariant BA.2.87.1. Vaccine
674 doi:<https://doi.org/10.1016/j.vaccine.2024.03.007>.
- 675 32. Yang S, Yu Y, Jian F, Yisimayi A, Song W, Liu J, Wang P, Xu Y, Wang J, Niu X, Yu L, Wang
676 Y, Shao F, Jin R, Wang Y, Cao Y. 2024. Antigenicity assessment of SARS-CoV-2 saltation
677 variant BA.2.87.1. *bioRxiv* doi:10.1101/2024.03.07.583823 %J
678 *bioRxiv:2024.03.07.583823*.
- 679 33. Chalkias S, McGhee N, Whatley JL, Essink B, Brosz A, Tomassini JE, Girard B, Edwards
680 DK, Wu K, Nasir A, Lee D, Avena LE, Feng J, Deng W, Montefiori DC, Baden LR, Miller
681 JM, Das R. 2024. Interim report of the reactogenicity and immunogenicity of SARS-CoV-
682 2 XBB-containing vaccines. *J Infect Dis* doi:10.1093/infdis/jiae067.
- 683 34. Jain S, Kumar S, Lai L, Linderman S, Malik AA, Ellis ML, Godbole S, Solis D, Sahoo MK,
684 Bechnak K, Paredes I, Tanios R, Kazzi B, Dib SM, Litvack MB, Wimalasena ST, Ciric C,
685 Rostad C, West R, Teng IT, Wang D, Edupuganti S, Kwong PD, Roupheal N, Pinsky BA,
686 Douek DC, Wrammert J, Moreno A, Suthar MS. 2024. XBB.1.5 monovalent booster
687 improves antibody binding and neutralization against emerging SARS-CoV-2 Omicron
688 variants. *bioRxiv* doi:10.1101/2024.02.03.578771.

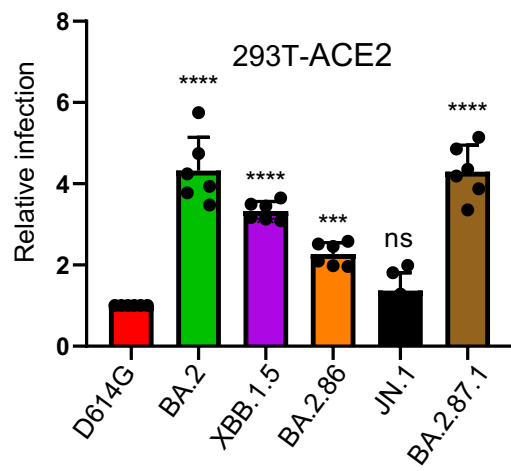
689

a

	NTD																																							
	15	16	17	18	19	20	21	50	69	70	75	83	98	126	127	136	137	138	139	140	141	142	143	144	145	146	152	157	168	183	190	211	212	213	215	216	245	252	264	
BA.2	C	V	N	L	I	T	R	S	H	V	G	V	S	V	V	C	N	D	P	F	L	D	V	Y	Y	H	W	F	R	Q	R	N	L	G	D	L	H	G	A	
BA.2.87.1	D	.	F	A	L	.	.	S	.	.	.	G
XBB.1.5	A	Q	.	.	E	.	.	E	.	.	.	V	.	.
BA.2.86	T	L	F	S	G	.	.	I	.	.	F	N	.	D	.	.
JN.1	T	L	F	S	G	.	.	I	.	.	F	N	.	D	.	.

	RBD																	S2																			
	332	339	346	356	368	403	417	444	445	446	450	452	455	460	478	481	483	484	486	490	493	554	570	621	642	670	679	681	691	791	796	936	939	1143			
BA.2	I	D	R	K	L	R	N	K	V	G	N	L	L	N	K	N	V	A	F	F	R	E	A	P	V	I	K	H	S	T	Y	D	S	P			
BA.2.87.1	T	N	G	.	.	M	.	K	T	K	Q	.	.	S	G	.	R	.	P	I	H	G	
XBB.1.5	.	H	T	.	I	.	.	.	P	S	.	.	.	K	P	S	Q
BA.2.86	V	H	.	T	.	K	.	.	H	S	D	W	.	K	.	K	-	K	P	.	Q	K	V	S	.	V	.	R	F	L	.	.	
JN.1	V	H	.	T	.	K	.	.	H	S	D	W	S	K	.	K	-	K	P	.	Q	K	V	S	.	V	.	R	F	L	.	.	

b



c

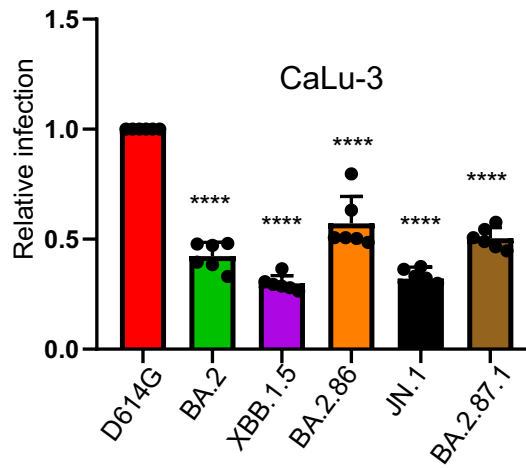


FIG 1

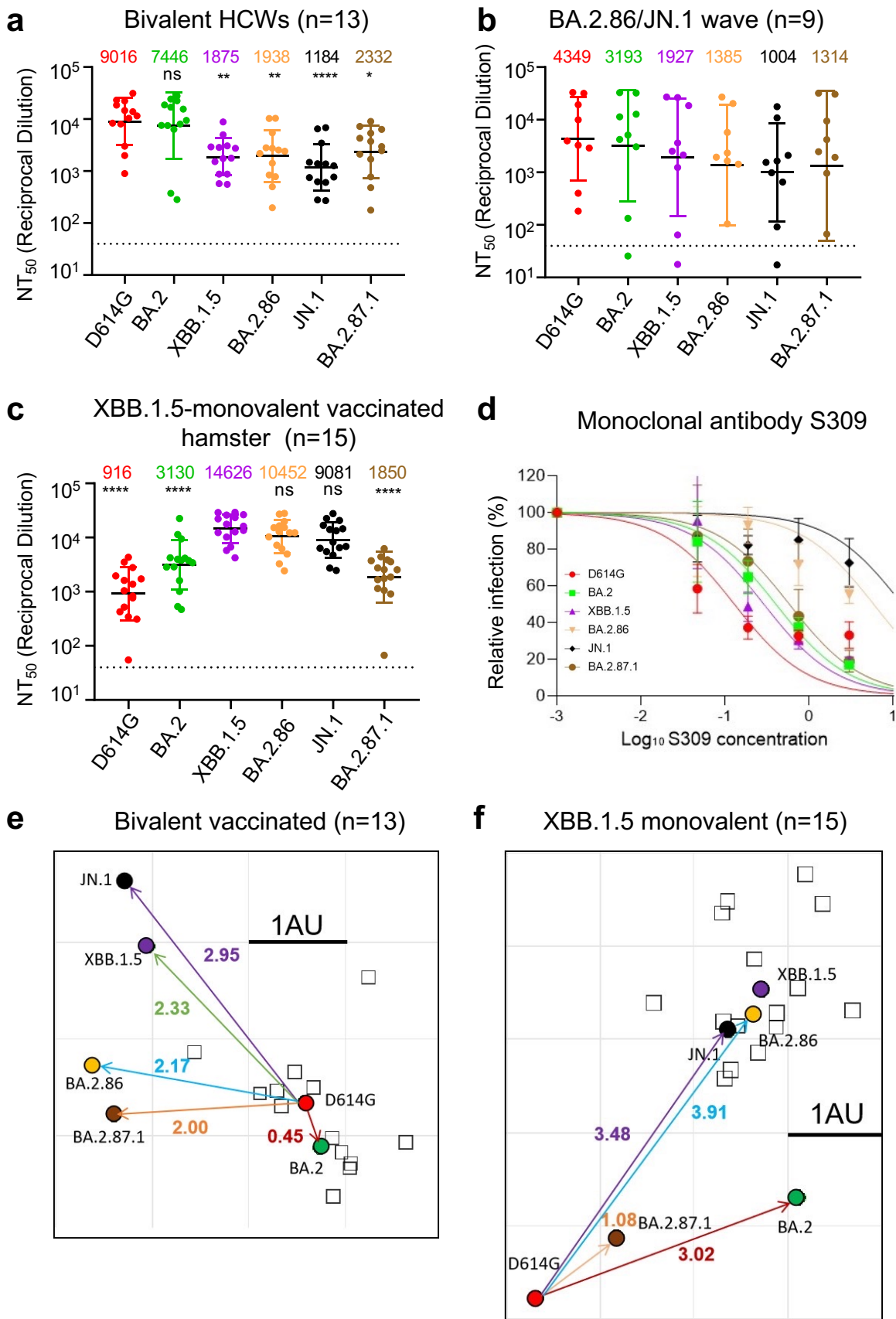


FIG 2

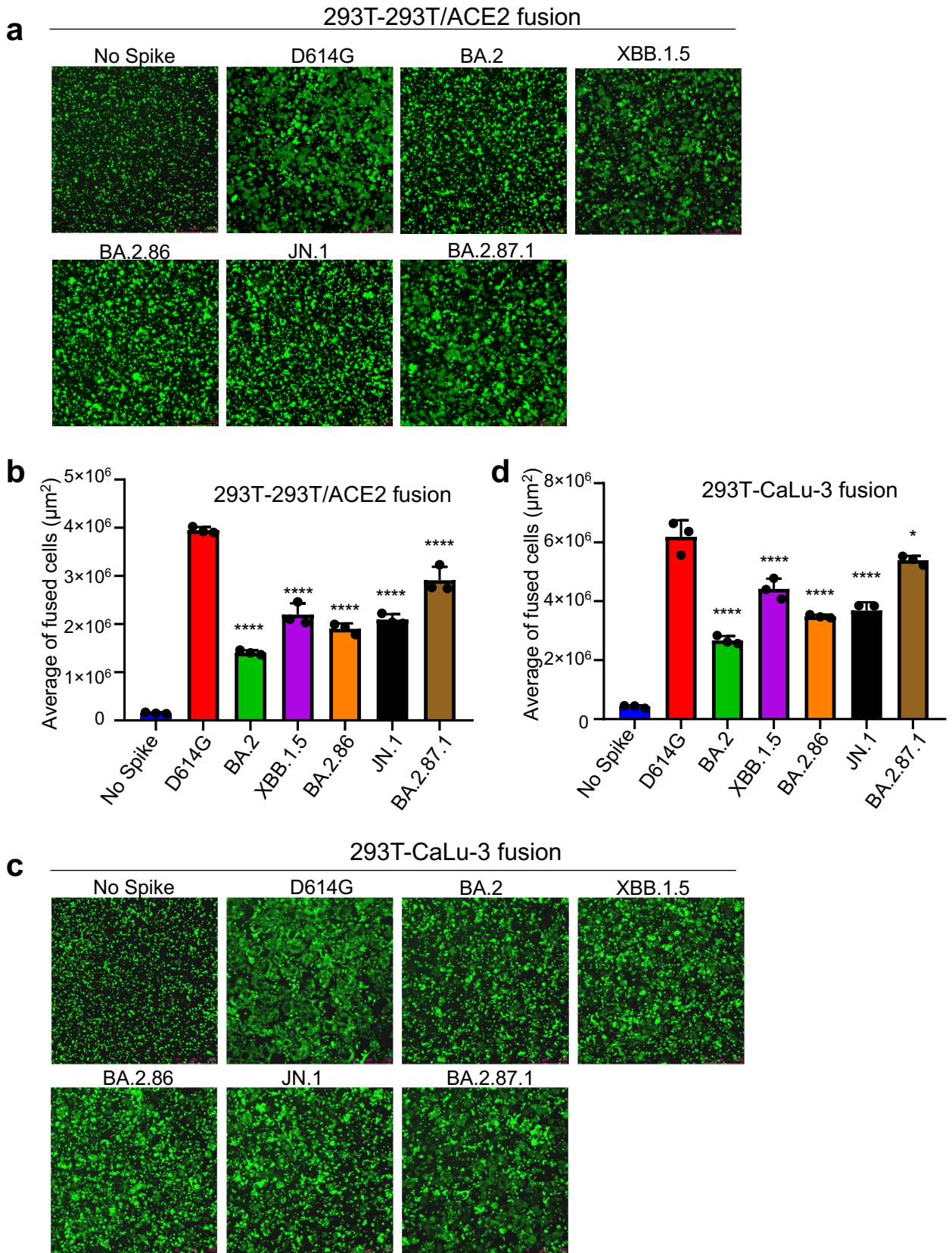


FIG 3

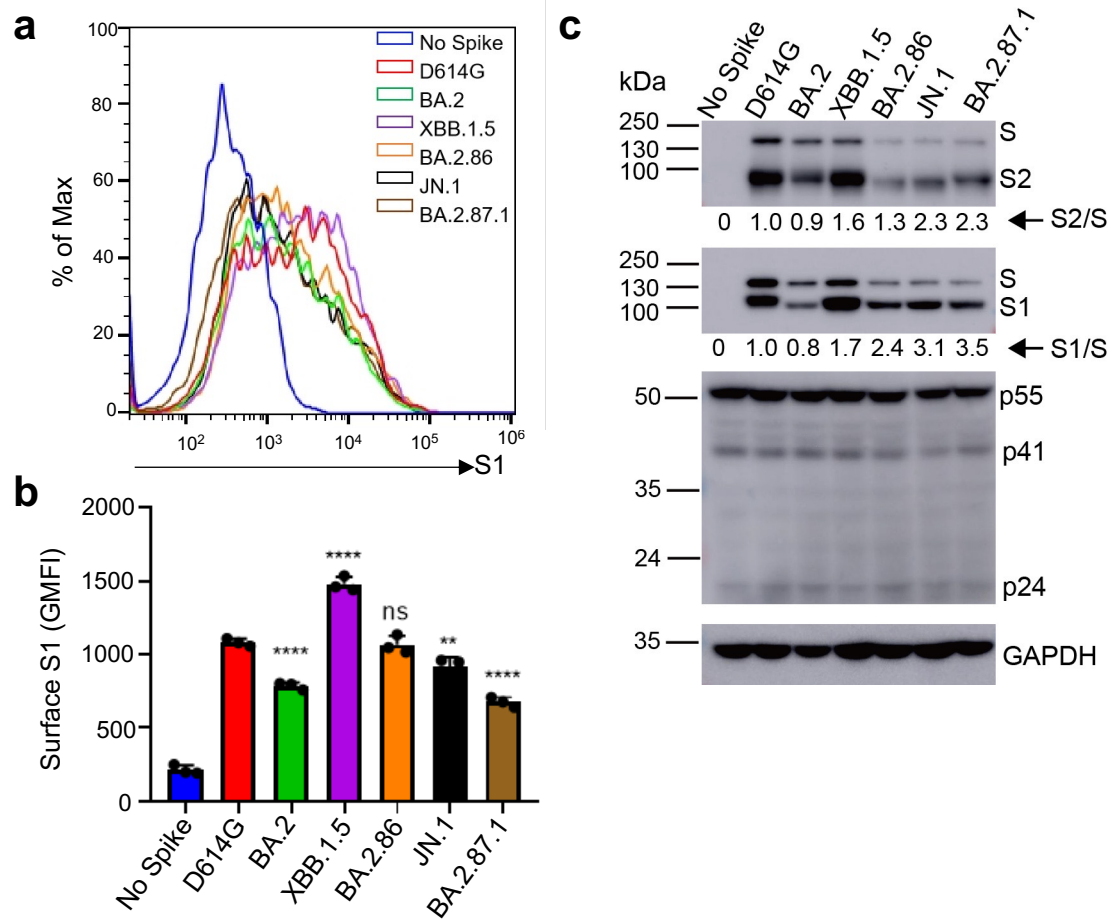


FIG 4