Immune escape and replicative capacity of Omicron lineages BA.1, BA.2, BA.5.1, BQ.1, XBB.1.5, EG.5.1 and JN.1.1

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Abstract

In the 5th year after the emergence of SARS-CoV-2, Omicron lineages continue to evolve and cause infections. Here, we used eight authentic SARS-CoV-2 isolates to assess their capacity to escape immunity of different exposure histories and their replicative capacity in polarized human airway epithelial cells (HAE) derived from the nasal and bronchial epithelium.

Using live-virus neutralization assays of 108 human sera or plasma of different immunological backgrounds, progressive immune escape was observed from B.1 (ancestral virus) to EG.5.1, but no significant difference between EG.5.1 and JN.1.1. Vaccinated individuals without natural infection and individuals with a single infection, but no vaccination showed markedly reduced or completely lost neutralization against the latest variants, while in those with hybrid immunity almost all sera showed some neutralization capacity. Furthermore, although absolute titers differed between groups, the pattern of immune escape between the variants remains comparable with strongest loss of neutralization observed for the latest variants.

In vitro studies with HAE at 33°C and 37°C showed some, but minor differences in virus replication and innate immune responses upon infection. Notably, infection with XBB.1.5, EG.5.1 and JN.1.1 showed slightly increased viral growth in nasal HAE at 33°C.

Altogether, these data underscore increasing immune escape across heterogeneous immunological backgrounds with gradually increasing antibody escape of evolving Omicron lineages until variant EG.5.1, but not any further for the latest dominant lineage JN.1.1. They also suggest that viral dynamics within Omicron lineages are driven by a combination of immune evasion and increase in viral replication.

Keywords: SARS-CoV-2, omicron variants, viral replication, immune escape, neutralisation.

Introduction

The emergence of the SARS-CoV-2 in late 2019 has been followed by emergence of numerous virus variants [1]. Two years later, the emergence of the Omicron variant of concern (VOC) marked a significant shift, enhancing immune evasion due to extensive mutations in its Spike region. This has led to widespread infections by overcoming immunity from vaccines and prior natural infection. Omicron's distinct genetic profile, particularly in the Spike region, positions it as a potential new serotype of SARS-CoV-2 [2, 3].

Global observations have documented successive waves of infections since the initial emergence of Omicron, with a huge number of evolving Omicron lineages. Consequently, a complex immunological landscape now exists in the population, where immunity is derived from vaccines, one or more infections due to pre-Omicron or Omicron variants, or a combination therefore, known as hybrid immunity. In most individuals in high income countries, the immunological background of SARS-CoV-2 today consists of multiple vaccinations and multiple exposures to SARS-CoV-2 through natural infection. On the other hand, some individuals have never received a vaccine and were only infected after the circulation of Omicron, in particular younger children, for which there is no vaccine recommendation in many countries. This leads to a heterogeneous immunological background in the population, ranging from both extremes of the exposure spectrum [2-9]. Despite background immunity in the population, SARS-CoV-2 continues to circulate. Multiple new variants have arisen within the Omicron clade, with XBB.1.5, XBB.1.16, EG.5, BA.2.86 and JN.1, being the latest variants of interest (VOIs) designated by WHO. BA.2.86, first identified in August 2023, has a remarkable number of mutations, known to allow antibody evasion, compared to earlier Omicron variants [10]. BA.2.86 did not show strong epidemiological signs of spread, but its decedent did, after BA.2.86 acquired an additional mutation S:L455S in the

83 Spike, which became JN.1. This mutation was shown to be associated with significantly
84 enhanced immune evasion capabilities but also increased transmissibility [5, 11-15]. In early
85 2024, JN.1 and its descendant lineages showed a strong increase globally, outcompeting earlier
86 variants, and remains dominant as of mid 2024.
87 With reduced testing and surveillance and a highly variable immunological background in the
88 population, obtaining data from epidemiology and/or clinical specimen collections has become
89 more challenging. Therefore, an experimental assessment of immune escape and replicative
90 capacity of emerging Omicron lineages *in vitro* is interesting, although it does not fully reflect
91 the *in vivo* situation. Here, we investigated these aspects in SARS-CoV-2 Omicron lineages:
92 BA.1, BA.2, BA.5.1, BQ.1, XBB.1.5, EG.5.1 and JN.1.1 compared to the ancestral SARS-
93 CoV-2 B.1, using live viruses as a widely acknowledged gold standard, first, to compare
94 immune escape capacities using sera from individuals with different immunological
95 backgrounds by neutralization assays and second, to study the infection in relevant primary
96 polarized airway epithelial culture models of the upper and lower respiratory tract.

97 **Methods**

98 **Viruses and cells**

99 Vero-E6 (ATCC CRL-1586) and Vero-E6-TMPRSS (Vero-E6 overexpressing TMPRSS2
100 protease, provided by National Institute for Biological Standards and Controls, NIBSC, Cat.
101 Nr. 100978) cells were cultured as previously described [2, 16, 17]. All SARS-CoV-2 viruses
102 used in this study were isolated from anonymized nasopharyngeal swabs collected at
103 University Hospitals of Geneva (HUG) under an approval that allows the usage of anonymized
104 left-over materials for virus culture. For this study, the following virus isolates were used
105 (according to Pango lineages designation [18]): B.1 (ancestral SARS-CoV-2) and BQ.1,
106 isolated and propagated on Vero-E6; BA.1 and BA.5.1, isolated on Vero-TMPRSS, then

propagated to Vero-E6; BA.2, XBB.1.5, EG.5.1 and JN.1.1, isolated and propagated on Vero-TMPRSS. Both the initial clinical specimen and the obtained virus isolates were fully sequenced (**Table S1**). All virus stocks were titrated on the same cell line on which the virus stock was produced (either Vero-E6 or Vero-TMPRSS).

SARS-CoV-2 infections in HAE

Infections with SARS-CoV-2 Omicron lineages BA.1, BA.2, BA.5.1, BQ.1, XBB.1.5, EG.5.1 and JN.1.1 were performed at 37°C or 33°C at 5% CO_2 at a multiplicity of infection of approximately 0.1 in commercially available polarized tissues "MucilAir™" (Epithelix SARL), *in vitro* reconstituted from human nasal or bronchial (3 donors from each group) epithelial cells of adult healthy donors cultured in an air-liquid interface (ALI) system, as previously described [16, 19, 20]. Viral replication was assessed at 24, 48, 72 and 96 hours post infection (hpi), as previously described [16, 19, 20].

Assessment of host gene response

Induction of interferons IFN-α and IFN-β IFN-λ, ISG15 (Interferon stimulated gene 15), angiotensin-converting enzyme 2 (ACE-2) and Transmembrane Serine Protease 2 (TMPRSS2) was assessed by semi-quantitative real-time PCR for intracellular RNA collected at 96 hpi, as previously described [16, 19, 20].

Human serum and plasma samples

Immunocompetent and healthy individual samples consisted of serum or plasma samples collected after vaccination, infection or a combination of both (hybrid immunity). Plasma or serum samples from vaccinated healthy individuals, vaccinated either with two or three doses (boosted) of BNT162b2 (Pfizer/BioNTech) or mRNA-1273 (Moderna) were available from a prospective observational studies (Ethics approval number: CCER 2021-00430 and CCER

130 2020-02323). Asymptomatic or undetected infections of the vaccinated-only group were
131 excluded in those samples by testing all specimens for antibodies against SARS-CoV-2
132 nucleocapsid (Roche Elecsys anti-SARS-CoV-2 N assay). Specimens of individuals with
133 hybrid immunity were collected from adults vaccinated with BNT162b2 or mRNA-1273
134 vaccines and with one or more documented infections. Breakthrough infection samples were
135 collected from individuals with either 2x or 3x mRNA vaccination, followed by an Omicron
136 BA.1 or BA.2 breakthrough infection, respectively (Ethics approval number: CCER 2020-
137 02323). Serum samples from individuals with XBB breakthrough infection had received either
138 2x (n=2), 3x (n=7) or 4x (n=2) mRNA vaccination, followed by breakthrough infection with
139 one of the XBB sublineages that occurred between March-June 2023 (specimens were left-
140 over samples from a prospective observational study, ethics approval number: CCER 2022-
141 01722). Four of these individuals had a subsequent infection prior to XBB infection. One of
142 the individuals vaccinated with 4x mRNA had received a bivalent vaccine. For individuals with
143 infections from two different VOCs (e.g. Alpha and Omicron or Delta and Omicron) had
144 received either 1x (n=6), 2x (n=10) or 3x (n=2) mRNA vaccine (Ethics approval number:
145 CCER 2020-02323). Convalescent sera from unvaccinated adults and children with confirmed
146 SARS-CoV-2 infection in early 2022 were also available (Ethics approval number: CCER
147 2020-00881). Based on the data from our Swiss national genomic surveillance, the vast
148 majority of the variants circulating at that time was Omicron BA.1 and BA.2, with only few
149 Delta sequences remaining in January 2022 [21]. The infecting variant of each episode was
150 either determined by sequencing of the diagnostic samples or extrapolated by the time of
151 infection according to the information that was self-reported by the participant and/or by their
152 parent (for children), taking the knowledge on variant circulation generated by the Swiss
153 national genomic surveillance program into account [22]. Data on collection time of specimens

154 after vaccination and/or infection are displayed in **Tables 1-5**. Only one serum per individual
155 of a single collection time point was used in this study.

156 A written informed consent was obtained from all adult participants, and from the legally
157 appointed representatives (parents) of all minor participants. All necessary approvals were
158 obtained from the Cantonal Ethical board of the Canton of Geneva, Switzerland (Commission
159 Cantonal d'Ethique de la Recherche, CCER). Since no differences are to be expected in
160 neutralizing capacity between plasma or serum, both sample types were used in parallel.

161 **Focus reduction neutralization test (FRNT)**

162 FRNT was used to determine the infectious titer after neutralization. Vero-TMPRSS cells were
163 seeded at a density of 4×10^5 cells/mL in 96-well cell culture plates. All sera/plasma and Vero-
164 TMPRSS cells infections were prepared as previously described [2]. After incubation for 16-
165 24 hours at 37 °C, 5% CO_2, the plates were fixed and stained for SARS-CoV-2 nucleocapsid
166 protein as described previously [17, 23]. The 90% reduction endpoint titers ($FRNT_{90}$) were
167 calculated as previously described [2]. For samples that did not reach 90% reduction at a 1:10
168 dilution, we extrapolated the titer until a dilution of 0.5. If the extrapolation reached a titer
169 below 0.5, the sample was given a value of 0.5. All samples with a titer below 1, i.e. undiluted
170 sample are considered negative.

171 Data was recorded in Excel 2019. Geometric means with 95% CI were used for the comparison
172 of $FRNT_{90}$ titers. Statistical analyses for $FRNT_{90}$ were conducted using GraphPad Prism
173 version 9.1.0 software and performed using repeated measures one-way ANOVA with
174 Dunnett's multiple comparisons test with $\log_{10}$ transformed $FRNT_{90}$ titers.

175

Results

1. Virus neutralization to Omicron lineages of sera or plasma after infection, vaccination, and hybrid immunity

A panel of sera/plasma was used from: (i) double or monovalent boosted vaccinated individuals with BNT162b2 or mRNA-1273 without prior or subsequent infection (**Table 1**); (ii) individuals with hybrid immunity after receiving two or three doses of mRNA vaccine followed by a break-through infection with either Omicron BA.1 or BA.2, respectively (**Table 2**); (iii) individuals with hybrid immunity due to XBB-variant breakthrough infection (**Table 3**); (iv) individuals with hybrid immunity receiving between one to three doses of mRNA vaccine and two different documented VOCs infections (either infection with Alpha or Delta, followed by infection with Omicron) (**Table 4**); and (v) a panel of convalescent sera from unvaccinated individuals, adults and children infected between January and March 2022, a period that was characterized by high circulation of Omicron BA.1 and BA.2 (**Table 5**).

2.1. *Neutralizing capacity in vaccinated but never-infected individuals against Omicron lineages*

We investigated a total of 33 individuals' specimens, either double-vaccinated (n=14) or monovalent boosted individuals that had never been infected with SARS-CoV-2 (n=19) for neutralization against ancestral SARS-CoV-2 lineage B.1 as well as SARS-CoV-2 Omicron lineages BA.1, BA.2, BA.5.1, BQ.1, XBB.1.5, EG.5.1 and JN.1.1. For double-vaccinated individuals, the highest neutralizing capacity was observed against the ancestral SARS-CoV-2 virus B.1 with geometric mean $FRNT_{90}$ titers of 251.7 (95%CI: 158.3-400.1) but very reduced titers were observed against all Omicron lineages with $FRNT_{90}$ titers of 5.7 (95%CI: 2.8-11.5) for BA.1, 10.4 (95%CI: 6.6-16.3) for BA.2, 2.9 (95%CI: 1.5-5.5) for BA.5.1, 1.5 (95%CI: 0.8-3.0) for BQ.1, 1.0 (95%CI: 0.6-1.4) for XBB.1.5 and 0.6 (95%CI: 0.5-0.8) for JN.1.1. None of

200 the samples neutralized EG.5.1. Although titers were very reduced compared to B.1, none of
201 the samples failed to neutralize Omicron BA.2. An increasing number of samples with
202 complete failure to neutralize was observed for Omicron BA.1, BA.5.1, BQ.1, XBB.1.5,
203 EG.5.1 and JN.1.1 with 2/14, 4/14, 7/14, 7/14, 14/14 and 12/14, respectively (**Figure 1A**).

204 For boosted individuals, overall geometric mean $FRNT_{90}$ titers were higher against all viruses.
205 Geometric mean $FRNT_{90}$ titers were 357.5 (95%CI: 255.3-500.6), 68.8 (95%CI: 39.7-119.2),
206 27.6 (95%CI: 15.5-49.1), 13.7 (95%CI: 10.7-17.4), 6.2 (95%CI: 3.1-12.3), 2.1 (95%CI: 1.0-
207 4.1), 1.5, 0.8 (95%CI: 0.5-1.3) and 1.0 (95%CI: 0.6-1.6) against B.1, BA.1, BA.2 , BA.5.1,
208 BQ.1, XBB, EG.5.1 and, JN.1.1, respectively. No complete loss of neutralization in this group
209 was observed for variants B.1, BA.1, BA.2, and BA.5.1, while 3/19 samples were not
210 neutralized for BQ.1, 9/19 for XBB.1.5, 16/19 for EG.5.1 and 12/19 for JN.1.1 (**Figure 1B**).

211 *2.2. Neutralizing capacity of vaccinated individuals with breakthrough infection (hybrid*
212 *immunity)*

213 We investigated the impact of BA.1 breakthrough infection in individuals vaccinated with two
214 doses of mRNA vaccine. Geometric mean $FRNT_{90}$ titers in the hybrid immunity group were
215 higher than for vaccinated individuals. They were 865.4 (95%CI: 450.9-1661.0) against B.1,
216 416.5 (95%CI: 175.6-987.8) against BA.1, 56.7 (95%CI: 31.8-100.9) against BA.2, 65.4
217 (95%CI: 30.3-141.4) against BA.5.1, 17.4 (95%CI: 7.6-39.9) against BQ.1, 11.3 (95%CI: 5.1-
218 24.9) against XBB.1.5, 8.4 (95%CI: 3.5-20.3) against EG.5.1 and 7.1 (95%CI: 3.6-14.0)
219 against JN.1.1. Complete loss of neutralization was observed only for 1/11 sample each for
220 XBB.1.5, EG.5.1 and JN.1.1 (**Figure 1C**).

221 For boosted individuals with BA.2 breakthrough infection, geometric mean $FRNT_{90}$ titers were
222 818.7 (95%CI: 541.5-1238.0) for B.1, 266.2 (95%CI: 134.2-528.0) for BA.1; 415.8 (95%CI:
223 250.5-690.1) for BA.2, 85.1 (95%CI: 44.7-161.9) for BA.5.1, 50.2 (95%CI: 24.2-104.0) for

224 BQ.1, 33.6 (95%CI: 16.7-67.4) for XBB.1.5, 7.4 (95%CI: 2.7-20.7) for EG.5.1 and 6.2
225 (95%CI: 2.8-13.5) for JN.1.1. No complete loss of neutralization in this group was observed
226 for variants B.1, BA.1, BA.2, BA.5.1, BQ.1 and XBB.1.5, while 2/11 samples were not
227 neutralized for EG.5.1 and 1/11 for JN.1.1 (**Figure 1D**).

228 Although titers for B.1 were highest in both groups, the second highest neutralization titers
229 were found against the infecting virus, *e.g.*: neutralization of BA.1 was higher in the BA.1-
230 infected group and neutralization of BA.2 was higher in the BA.2.-infected group. No
231 difference was seen between the groups in the neutralization titers for EG.5.1. and JN.1.1 which
232 were both comparably low independent of the infecting virus.

233 We then investigated the impact of breakthrough infections in individuals (n=11) who have
234 been infected with one of the Omicron XBB subvariants between March and June 2023. The
235 geometric mean $FRNT_{90}$ titers were 1984.0 (95%CI: 1109-3547) against B.1, 46.0 (95%CI:
236 18.2-116.4) against XBB.1.5, 31.8 (95%CI: 9.3-108.6) against EG.5.1 and 21.1 (95%CI: 9.1-
237 48.7) against JN.1.1. All sera were able to neutralize in this group (**Figure 2A**).

238 *2.3. Neutralizing capacity in vaccinated individuals with breakthrough infections from two*
239 *antigenically different VOCs (multi-variant hybrid immunity) towards Omicron lineages*

240 We investigated vaccinated individuals (n=18) who subsequently have been exposed to at least
241 two antigenically different variants through two independent infection episodes that included
242 a pre-Omicron VOC (Alpha, n=9 or Delta, n=9) and another infection episode with an Omicron
243 lineage. The geometric mean $FRNT_{90}$ titers were 226.2 (95%CI: 153.8-332.8) against B.1,
244 80.33 (95%CI: 57.3-112.6) against BA.1, 32.3 (95%CI: 20.5-50.7) against Omicron BA.2, 22.4
245 (95%CI: 17.7-28.4) against BA.5.1, 7.6 (95%CI: 3.6-16.1) against BQ.1, 8.1 (95%CI: 4.3-15.3)
246 against XBB.1.5, 2.4 (95%CI: 1.0-5.4) against EG.5.1 and 2.5 (95%CI: 1.2-4.8) against JN.1.1.

247 Loss of neutralization was observed for BQ.1, XBB.1.5, EG.5.1 and JN.1.1 in 2/18, 1/18, 7/18
248 and 6/18 samples, respectively (**Figure 2B**).

249 *2.4. Neutralizing capacity from unvaccinated adults and children infected in early 2022*

250 We also studied neutralization of Omicron variants in 24 sera (adults, n=12; children, n=12) of
251 unvaccinated individuals with a single infection between January and March 2022 (most likely
252 exposed to BA.1 or BA.2). Due to the limited volume of serum available for this group, we
253 only assessed neutralization towards Omicron variants BA.1, BQ.1, XBB.1.5, EG.5.1 and
254 JN.1.1 in this cohort. Geometric mean $FRNT_{90}$ titers in this group for adult individuals were
255 29.1 (95%CI: 13.0-65.1) against BA.1, 1.3 (95%CI: 0.6-2.6) against BQ.1; 0.8 (95%CI: 0.5-
256 1.4) against XBB.1.5 and none of the samples neutralized EG.5.1 and Omicron JN.1.1.
257 Complete loss of neutralization was observed for 7/12 samples for BQ.1, 8/12 samples for
258 XBB.1.5 and all samples for EG.5.1 and JN.1.1 (**Figure 3A**). For children, geometric mean
259 $FRNT_{90}$ titers were 43.3 (95%CI: 18.0-103.7) against BA.1, 2.3 (95%CI: 0.9-5.8) against BQ.1,
260 1.1 (95%CI: 0.5-2.4) against XBB.1.5, 1.2 (95%CI: 0.6-2.5) against EG.5.1 and 0.6 (95%CI:
261 0.4-0.8) against JN.1.1. Of note, complete loss of neutralization was observed for 5/12 samples
262 for BQ.1, 8/12 samples for XBB.1.5, 6/12 samples for EG.5.1 and 11/12 for JN.1.1 (**Figure**
263 **3B**).

264 *2.5. Heatmap of neutralization data across different immunological backgrounds*

265 To summarize the findings across the cohorts, we have displayed the fold change of geometric
266 mean $FRNT_{90}$ titers in comparison to the ancestral virus B.1 for all cohorts (**Figure 3**). Across
267 cohorts, a consecutive loss in neutralization was observed from B.1 to BA.1/BA.2. to BA.5.1
268 to BQ.1 to XBB.1.5. and to EG.5.1/JN.1. The effect was the strongest for sera from individuals
269 that were only vaccinated but never infected, and the least pronounced for individuals exposed
270 to more than one natural infection with different variants. The differences between EG.5.1 and

271 JN.1. were only subtle across groups, and JN.1.1 did not show an enhanced immune escape
272 compared to EG.5.1. In vaccinated but never infected individuals, there was even a tendency
273 for better neutralization of JN.1.1 compared to EG.5.1.

274 **2. Replicative capacity and innate immune responses of Omicron sublineages in nasal and**
275 **bronchial HAE**

276 To understand if Omicron lineages differ in their ability to replicate, we infected polarized
277 HAE of nasal (3 donors) and bronchial (3 donors) origin at different temperatures (**Figure 1**)
278 with different Omicron lineages. Comparison across lineages overall revealed similar kinetics
279 and replication range, with a rapid increase in viral RNA, reaching peak viral loads at 24/48hpi
280 followed by a slight decline at 72 and 96hpi. An increase in viral RNA was observed at 24h for
281 almost all lineages under all conditions. In nasal HAE, virus replication was slightly lower in
282 the physiological conditions (33°C) than at 37°C, where the peak of replication, reached at 48h,
283 varied from 9.6 log10 RNAc/mL (for BA.2), to 10.9 log10RNA c/mL (for XBB). The most
284 recent subvariants XBB.1.5, EG.5.1 and JN.1.1 showed better replication efficacy (higher than
285 10.5 log10 RNAc/mL). At 37°C, the peak was reached at 1dpi, except for BA.2, BA.5.1 and
286 BQ.1. Similar patterns were found in bronchial HAE with comparable levels of replication at
287 both temperatures. BA.2 again showed the lowest level of replication (10.1 log10 RNAc/mL
288 at 33°C and 10.0 log10 RNAc/mL at 37°C). The three most recent subvariants all showed
289 earlier replication peaks with a stronger increase of viral RNA at 37°C.

290 To compare innate immune responses after infection between Omicron lineages, we studied
291 IFN-α and -β and IFN-λ interferon responses and the induction of downstream interferon-
292 stimulated genes 15 (ISG15) at the end of the infection experiments (96hpi) in the infected and
293 non-infected HAE cultures (**Figure 6**). IFN-α by all variant's induction was barely observed
294 (less than 1log increase versus non-infected cells) at 33°C in infected nasal and bronchial HAE

295 and at 37°C in only in bronchial tissues. For all IFNs and ISG15, the lowest and highest
296 inductions were found in infected nasal HAE at 33°C and bronchial HAE at 37°C, respectively.
297 Weak induction (less than 0.5 log log10FC) of IFN-α and -β and ISG15 was observed in
298 bronchial HAE at 37°C, except for the IFN-β induction by the recent subvariants XBB.1.5,
299 EG.5.1 and JN.1.1 (0.65, 0.48 and 0.84 log10 FC, respectively).

300 IFN-λ showed the highest level of induction with all variants (except BA.2 in nasal HAE at
301 33°C) and the most pronounced variability between subvariants. While low induction was
302 observed in BA.1-, BA.2- and BQ.1- infected nasal tissues at 33°C, XBB.1.5, EG.5.1 and
303 JN.1.1 induced higher induction levels (2.17, 1.57- and 1.92-log10 FC, respectively). Inversely,
304 the latter showed lower induction levels in bronchial HAE at 33°C (from 0.58 to 0.81 log10
305 FC versus from 1.19 to 2.34 log10 FC for BA.1, BA.2, BA.5.1 and BQ.1) and at 37°C from
306 0.76 to 0.95 $\log_{10}$ FC versus from 1.63 to 1.84 $\log_{10}$ FC for BA.1, BA.2, BA.5.1 and BQ.1).
307 Induction of the main entry host factors, ACE-2 and TMPRSS2, was not enhanced in all
308 conditions of Omicron lineages' infections (**Figure S2**).

309 Altogether, our data showed, regardless of inter-donor variability, comparable replicative
310 capacity in both tissue origins under both temperature conditions, although lower for BA.2 and
311 slightly better for the most recent subvariants in nasal HAE.

312 Omicron lineages showed low induction of host responses, with an overall only slight
313 difference between variants.

314 **Discussion**

315 With the emergence of Omicron in late 2021, the COVID-19 pandemic has entered a new
316 phase. It has previously been shown that Omicron, compared to earlier VOCs, could overcome
317 immunity from various exposures, including prior infections and/or vaccinations, and showed

318 a distinct phenotype in *ex-vivo* infections compared to previous variants [2]. In the continuous
319 emergence of new Omicron lineages, intrinsic transmissibility and immune pressure are
320 considered as the main drivers of viral evolution [24]. A range of studies have shown increasing
321 escape from prior immunity for Omicron lineages, particularly those that are currently
322 designated as VOIs such as XBB.1.5 like and BA.2.86.

323 Omicron BA.2.86, with its highly mutated spike carrying over 30 mutations compared to other
324 Omicron lineages and a genetic distance that is comparable to that of the first Omicron lineages
325 BA.1 to that of Delta, was initially suspected to have potentially enhanced immune escape
326 properties [25]. Multiple neutralization studies showed similar or slightly diminished immunity
327 evasion compared to other Omicron variants [10, 14, 26-33]. Despite its first detection in mid-
328 2023, the prevalence of BA.2.86 remained low, possibly due to presumed lower viral fitness
329 observed in cell culture studies and reduced pathogenicity in animal models compared to other
330 Omicron lineages [14, 27, 34].

331 In line with clinical observations, previous studies including ours recently confirmed the faster
332 but shorter replication of Omicron BA.1 compared to previous SARS-CoV-2 variants in nasal
333 HAE [16]. We here confirmed this typical replication for BA.1 and extended this observation
334 to more recent subvariants relative to Omicron, despite modest differences (especially with
335 BA.2) in nasal and bronchial HAE models. A sustained phenotype of Omicron lineages has
336 also been shown when looking at their intrinsic host response mainly involving IFN-λ. Even
337 with an overall equal replicative capacity at upper and lower respiratory tract temperatures, as
338 previously shown for Omicron but not the previous ancestral (B.1) and Alpha variants [35],
339 little differences in IFN induction were observed at 37°C compared to 33°C. Higher replication
340 efficiency in *in vitro* (Calu3) and *in vivo* (Balb/c mice) of BA.5, compared to BA.1, in lung
341 tissues/cells have been reported [36].

342 More *ex-vivo* (explant) and *in vivo* (mice) studies suggested an association between the milder
343 severity of Omicron lineages and their enhanced replication efficacy in the upper, compared to
344 the lower, respiratory tract, in contrast to Delta variant [37-39]. One study found increased
345 replicative capacity and infectivity of BA.5 in comparison to BA.1 and the ancestral virus in
346 human nasal and airway organoids at 37°C [40]. In HAE, Zaderer *et al.* showed that, compared
347 to the Deta variant, there was a decreased replication efficiency with Omicron subvariants
348 BA.1, BA.2, BA.5 and BQ.1.1, and a superficial localization into the pseudostratified tissue
349 with less pronounced anti-viral response [41]. The more recently emerging lineages, like the
350 XBB, EG.5 and JN.1 benefit from an additional fitness advantage, as experimental data
351 obtained with live virus in cell culture/primary cell cultures infection studies. We showed a
352 small replication advantage and slightly higher IFN-λ response [19, 20] in nasal epithelia with
353 these variants, in line with Planas *et al* [14].

354 In comparison with the parent lineage BA.2.86, JN.1 has an additional L455S substitution in
355 the spike protein that was described to be associated with increased escape from humoral
356 immunity as well as transmissibility [5, 11-15]. However, in contrast to its parental lineage
357 BA.2.86, JN.1 rapidly outcompeted earlier variants in late 2023 and became domiant,
358 associated with a wave of infections worldwide [11, 15]. Limited data on the immune escape
359 assessment of JN.1 are available, but it demonstrated lower geometric mean neutralization
360 titers and lower fold change values compared to earlier Omicron variants [15, 42].

361 The present study aimed at the assessment of emerging SARS-CoV-2 variants in an authentic
362 virus neutralization assay against a panel of human sera and plasma. To add to the complexity
363 of the underlying immunity, we used cohorts with immunity at both ends of the exposure
364 spectrum, which reflects the complex situation in the population in the fifth year of the
365 pandemic. Here we show that recently emerged SARS-CoV-2 Omicron variants, namely BQ.1,
366 XBB.1.5, EG.5.1 and JN.1.1, display pronounced immune evasion to earlier Omicron variants,

367 but that JN.1.1, does not show additional immune escape compared to EG.5.1. We also showed
368 that earlier findings on hybrid immunity remain valid; specifically, hybrid immunity continues
369 to offer higher neutralization titers compared to monovalent vaccination or natural infection.
370 The use of authentic live virus isolates and $FRNT_{90}$ to assess the neutralization titers of a large
371 number of serum/plasma samples with a heterogeneous immunological background of the
372 population adds strength to our findings. Indeed, according to the reported data, results may be
373 different using a pseudovirus instead of a live virus, underscoring the importance of having
374 data with authentic live viruses. It should be noted that our study has some limitations. First,
375 we had a low number of sera that were available for the individual groups, especially for those
376 that were exposed to more than one variant. It is important to note that vaccine sera from
377 bivalent vaccines or updated vaccine formulations were not included in the analysis, which
378 could impact the comprehensiveness of the findings regarding vaccine efficacy against
379 emerging variants.

380 In summary, our data show that continuous assessment of newly evolving SARS-CoV-2, taking
381 the different groups within the population into account, remains crucial to understand viral
382 strategies to overcome existing immunity. In the case of JN.1, that showed a rapid global
383 increase but no enhanced immune escape, other factors than immune escape seem to be the
384 driving force behind this variant success. Collectively, this comparative study of the fitness and
385 the immune escape capacity of the most relevant SARS-CoV-2 Omicron lineages, using
386 pertinent cell models, authentic viruses and human specimens from immunized individuals,
387 highlights the role of both virus fitness and adaptive immune response pressure on the evolution
388 of Omicron lineages. It hence contributes to the better understanding of SARS-CoV-2
389 dynamics including its main driving forces as well as its phenotypical impact on viral
390 properties.

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Authorship contributions

M.Bek., and M.E.L performed the experiments and conducted the analysis; K.A., C.A., P.S.R, D.D. and M.Bel helped to carry out some experiments; M.Bek, K.H.F, C.S.E., M.E.Z, S.S., I.G., O.P., M.Bel., and L.K. conducted the clinical studies and/or helped with clinical sample collection; I.E., M.Bek designed and supervised the study; M.Bek., M.E.L and I.E wrote the main manuscript. All authors have contributed to the final version of the manuscript.

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548

549 **Table 1.** Characteristic of vaccinated individuals' samples.

Vaccine	Number of individuals	Sample type	Gender *(M/F)*	Age *mean*	WPLV *Mean weeks (range)*	Date of last vaccination
2x mRNA vaccine	14	Plasma	2/12	51 (36-62)	4 (4-5)	March-May 2021
3x mRNA vaccine	19	Serum	9/10	43 (26-63)	13 (3-22)	November 2021-January 2022

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551

552 **Table 2.** Characteristic of hybrid immunity (breakthrough with BA.1 or BA.2) individuals' samples.

Vaccination/infection status	Number of individuals	Sample type	Gender *(M/F)*	Age *mean*	WPLV *Mean weeks (range)*	WPLP *Mean weeks (range)*	Interval vaccination – infection Mean weeks (range)	Variant Identification[1]
2x mRNA vaccine & BA.1 breakthrough	11	Serum	5/6	39 (25-55)	34 (12-52)	15 (2-15)	27 (8-41)	Sequencing (n=11)
3x mRNA vaccine & BA.2 breakthrough	11	Serum	5/6	39 (25-62)	22 (15-35)	7 (3-10)	15 (5-32)	Sequencing (n=11)

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556 **Table 3.** Characteristic of hybrid immunity (breakthrough with XBB) individuals' samples.

Vaccination[2]/infection status[3]	Number of individuals	Sample type	Gender (M/F)	Age mean	Number of vaccinations	WPLV Mean weeks (range)	WPLP Mean weeks (range)	Variant[1] identification
Vaccination & XBB breakthrough infection	11	Serum	1/10	44 (24-61)	2, 3 or 4	73 (36-93)	10 (2-19)	Sequencing (n=11)

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560 **Table 4.** Characteristic of multi-VOC infected individuals' samples.

Vaccination[4]/infection status	Number of individuals	Sample type	Gender *(M/F)*	Age *mean*	WPLV *Mean weeks (range)*	WPLD *Mean weeks (range)*	Interval vaccination – last infection Mean weeks (range)	Variant identification[1] Alpha or Delta	Omicron
Vaccination + Alpha & Omicron breakthrough	9	Serum	5/4	41 (25-50)	42 (30-64)	17 (10-29)	25 (12-44)	Sequencing (n=3)	Sequencing (n=5)
Vaccination + Delta & Omicron breakthrough	9	Serum	3/6	40 (29-51)	53 (27-87)	21 (4-41)	31 (18-55)	Sequencing (n=6)	Sequencing (n=1)

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565 **Table 5.** Characteristic of convalescent children and adults' individuals' samples.

Infecting virus[5]	Number of individuals	Sample type	Gender *(M/F)*	Age *mean*	WPLD Mean days (range)	Infection period	Variant Identification[1]
Omicron in adults	12	Serum	2/10	40 (28-46)	15 (9-21)	January-March 2022	Extrapolated (n=12)
Omicron in children	12	Serum	6/6	5 (2-7)	17 (12-21)	January-March 2022	Extrapolated (n=12)

566

567 *WPLV* weeks post last dose of vaccine; *WPLP* weeks post last positive RT-PCR; *WPLD* weeks post last positive diagnosis;

568 [1]The infecting variant of each episode was either determined by sequencing of the diagnostic samples or extrapolated according to the dominance of the variant
569 at that time based on the data from our Swiss national genomic surveillance (**Figure S1**).

570 [2]Vaccination consisted of either 2 (n=2), 3 (n=7) or 4 (n=2) doses of BNT162b2 or mRNA-1273 vaccine. One of the individuals vaccinated with 4 doses of
571 mRNA had received a bivalent vaccine.

572 [3]Infection consisted of either 1 (n=7) or 2 (n=4) infection including one of the Omicron XBB variants infection.

573 [4]Vaccination consisted of either 1 (n=6), 2 (n=10) or 3 (n=2) doses of BNT162b2 or mRNA-1273 vaccine.

574 [5]Individuals were infected between January and March 2022 when the predominant circulation of Omicron BA.1 and BA.2 were taking place (<90%).

575 **Figures**

576 **Figure 1.** Neutralization in vaccine and hybrid immunity-derived blood specimens against
577 eight authentic isolates of SARS-CoV-2 variants (B.1 and Omicron lineages including BA.1,
578 BA.2, BA.5.1, BQ.1, XBB.1.5, EG.5.1 and JN.1.1). Bars represent geometric mean titers
579 (GMT) of 90% reduction endpoint titers ($FRNT_{90}$) with 95% confidence interval. **A**–**D** Cohorts
580 of individuals with **A)** double-dose mRNA vaccination (n=14), **B)** boosted mRNA vaccination
581 (n=19), **C)** BA.1 breakthrough infection following double mRNA vaccination (n=11) and **D)**
582 BA.2 breakthrough infection following 3 mRNA vaccination (n=11). Coloured numbers below
583 each bar represent the number of specimens with complete loss of neutralization ($FRNT_{90}$
584 titer < 1). Repeated measures one-way ANOVA with Dunnett's multiple comparisons test
585 using log_{10} transformed $FRNT_{90}$ titers was performed to analyze the statistical significance.

586 **Figure 2. A)** Neutralization in XBB exposure-derived blood specimens against four authentic
587 isolates of SARS-CoV-2 variants (B.1 and Omicron lineages including XBB.1.5, EG.5.1 and
588 JN.1.1). Cohort of specimens after XBB-derived infections with one of Omicron XBB variant.
589 Bars represent geometric mean titers (GMT) of 90% reduction endpoint titers ($FRNT_{90}$) with
590 95% confidence interval. Coloured numbers below each bar represent the number of specimens
591 with complete loss of neutralization ($FRNT_{90}$ titer < 1). Repeated measures one-way ANOVA
592 with Dunnett's multiple comparisons test using log_{10} transformed $FRNT_{90}$ titers was performed
593 to analyze the statistical significance. **B)** Neutralization in hybrid immunity-derived blood
594 specimens against eight authentic isolates of SARS-CoV-2 variants (B.1 and Omicron lineages
595 including BA.1, BA.2, BA.5.1, BQ.1, XBB.1.5, EG.5.1 and JN.1.1). Bars represent geometric
596 mean titers (GMT) of 90% reduction endpoint titers ($FRNT_{90}$) with 95% confidence interval.
597 Cohort of vaccinated individuals with dual SARS-CoV-2 infections (e.g. Alpha and Omicron
598 (n=9) or Delta and Omicron (n=9)). Coloured numbers below each bar represent the number
599 of specimens with complete loss of neutralization ($FRNT_{90}$ titer < 1). Repeated measures one-
600 way ANOVA with Dunnett's multiple comparisons test using log_{10} transformed $FRNT_{90}$ titers
601 was performed to analyze the statistical significance.

602 **Figure 3.** Neutralization in infection-derived blood specimens against five authentic isolates
603 of SARS-CoV-2 variants (omicron lineages including BA.1, BQ.1, XBB.1.5, EG.5.1 and
604 JN.1.1). Bars represent geometric mean titers (GMT) of 90% reduction endpoint titers
605 ($FRNT_{90}$) with 95% confidence interval. Cohorts of convalescent specimens that are derived
606 from **A)** unvaccinated adult individuals (n=12) and **B)** unvaccinated children individuals

607 (n=12) with confirmed SARS-CoV-2 infection in early 2022 (thus, probably Omicron BA.1 or
608 BA.2). Coloured numbers below each bar represent the number of specimens with complete
609 loss of neutralization ($FRNT_{90}$ titer < 1). Repeated measures one-way ANOVA with Dunnett's
610 multiple comparisons test using log_{10} transformed $FRNT_{90}$ titers was performed to analyze the
611 statistical significance.

612 **Figure 4.** Heatmap of fold-reduction in neutralization based on $FRNT_{90}$ data. Values of fold-
613 reduction in neutralization ($FRNT_{90}$) of B.1 and Omicron sublineages including BA.1, BA.2,
614 BA.5.1, BQ.1, XBB.1.5, EG.5.1 and JN.1.1 are presented as heat maps with lighter colors
615 implying greater changes. The immune sera/plasma were organized into cohorts of individuals
616 with double-dose mRNA vaccination (n=14), boosted individuals with three doses of mRNA
617 vaccine (n=19), BA.1 breakthrough infection of double-vaccinated individuals (n=11), BA.2
618 breakthrough infection individuals following 3 doses of mRNA vaccine (n=11), vaccinated
619 individual with XBB breakthrough infection (n=11) and vaccinated Individuals with dual
620 SARS-CoV-2 Infections (n=18).

621 **Figure 5.** Replication of SARS-CoV-2 Omicron lineages in HAE**.** Nasal (**A** and **B)** and
622 Bronchial (**C** and **D**) HAE were infected with SARS-CoV-2 Omicron lineages BA.1, BA.2,
623 BA.5.1, BQ.1, XBB.1.5, EG.5.1 and JN.1.1 at 33°C (**A** and **C**) and 37°C (**B** and **D**). Viral
624 replication was assessed by the quantification of viral RNA from apical washes collected at
625 3hpi (baseline), 24hpi, 48hpi, 72hpi and 96hpi. For each cell origin, HAE from 3 donors have
626 been tested. Data are expressed as mean of the log of viral RNA copies/mL (log_{10} RNA/mL)
627 and SEM.

628 **Figure 6. Induction of HAE intrinsic host response during by SARS-CoV-2 Omicron**
629 **lineages**
630 Nasal and Bronchial HAE infected with SARS-CoV-2 Omicron lineages BA.1, BA.2, BA.5.1,
631 BQ.1, XBB.1.5, EG.5.1 and JN.1.1 at 33°C (the same shown **Figure 1**) were lysed at 96hpi.
632 Induction of IFN-α (A) IFN-β (B), IFN-λ (C) and ISG15 (D) was assessed by semi-quantitative
633 real time RT-PCR using intracellular RNA and expressed in fold change relative to non-
634 infected and normalized to RNAseP. Data are represented as mean and SEM (n=3 donors), as
635 for each cell origin.

636 **Figure S1. Trend of SARS-CoV-2 variants in Switzerland over time.** From December 2020
637 until September 2023: from left to right, Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta
638 (B.1.617), BA.1, BA.2, BA.5, BQ.1, XBB.1.5, EG.5.1 and JN.1.1 SARS-CoV-2 variants
639 (https://cov-spectrum.org/explore/Switzerland/AllSamples/Past6M).

640 **Figure S2. Induction entry host factor in HAE during infection by SARS-CoV-2 Omicron**
641 **lineages**
642 Nasal and Bronchial HAE infected with SARS-CoV-2 Omicron lineages BA.1, BA.2, BA.5.1,
643 BQ.1, XBB.1.5, EG.5.1 and JN.1.1. at 33°C (as in **Figures 5** and **6**) were lysed at 96hpi.
644 Induction of host ACE-2 receptor (A) and TMPRSS2 protease (B), involved in SARS-CoV-2
645 entry during infection, was assessed by semi quantitative real time PCR using total RNA from
646 the cell and expressed in fold change relative to non-infected and normalized to RNAseP. Data
647 are represented as mean and SEM (n=3 epithelia from 3 different donors tested for each cell
648 origin, nasal/bronchial).

Figure 1

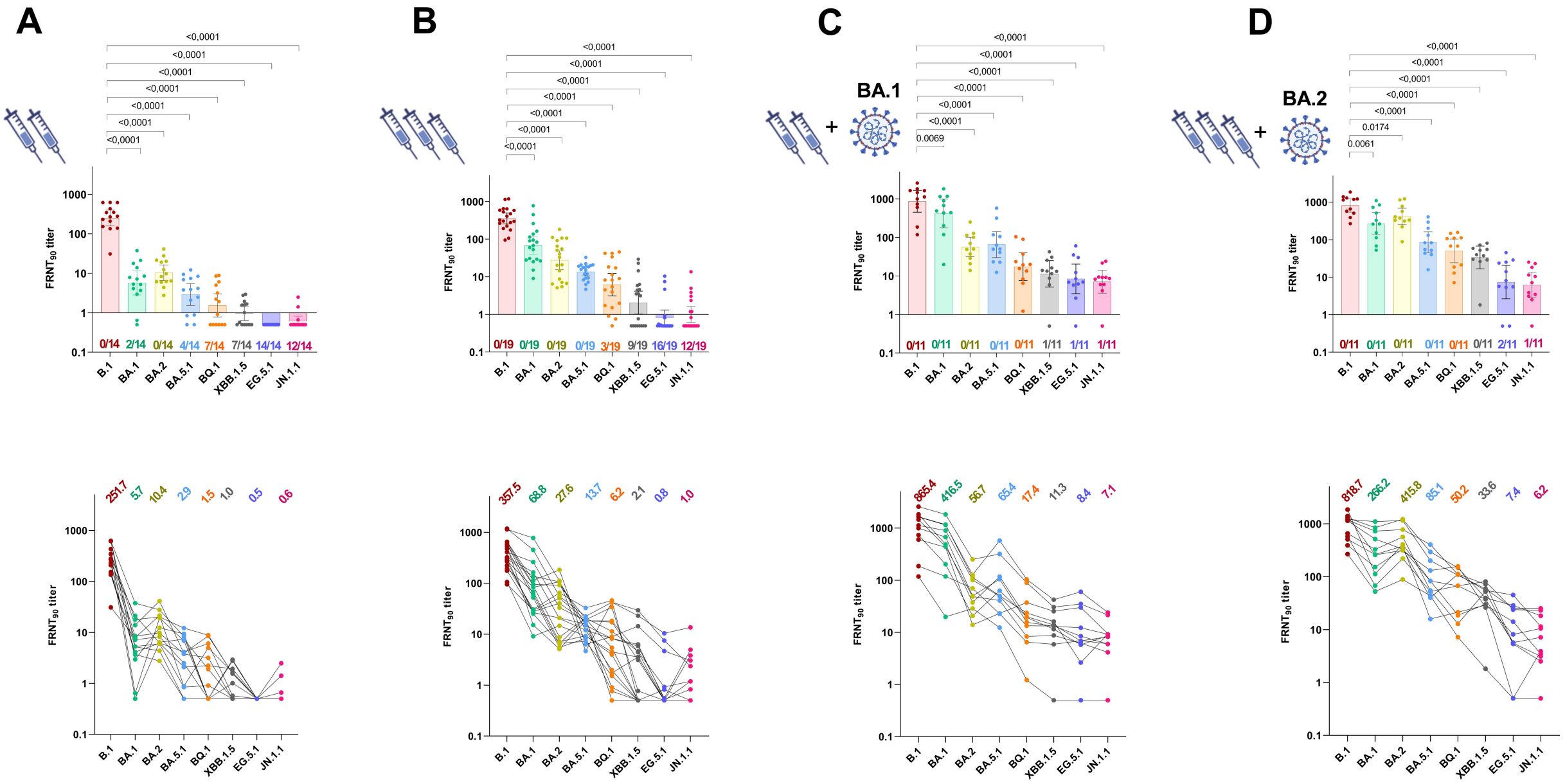

A
B
C
D
BA.1
BA.2
<0,0001
0.0069
0.0174
0.0061
FRNT90 titer
0/14 2/14 0/14 4/14 7/14 7/14 14/14 12/14
0/19 0/19 0/19 0/19 3/19 9/19 16/19 12/19
0/11 0/11 0/11 0/11 0/11 1/11 1/11 1/11
0/11 0/11 0/11 0/11 0/11 0/11 2/11 1/11
251.7 5.7 10.4 2.9 1.5 1.0 0.5 0.6
357.5 68.8 27.6 13.7 6.2 2.1 0.8 1.0
865.4 416.5 56.7 65.4 17.4 11.3 8.4 7.1
818.7 266.2 415.8 85.1 50.2 33.6 7.4 6.2
B.1 BA.1 BA.2 BA.5.1 BQ.1 XBB.1.5 EG.5.1 JN.1.1

Figure 2

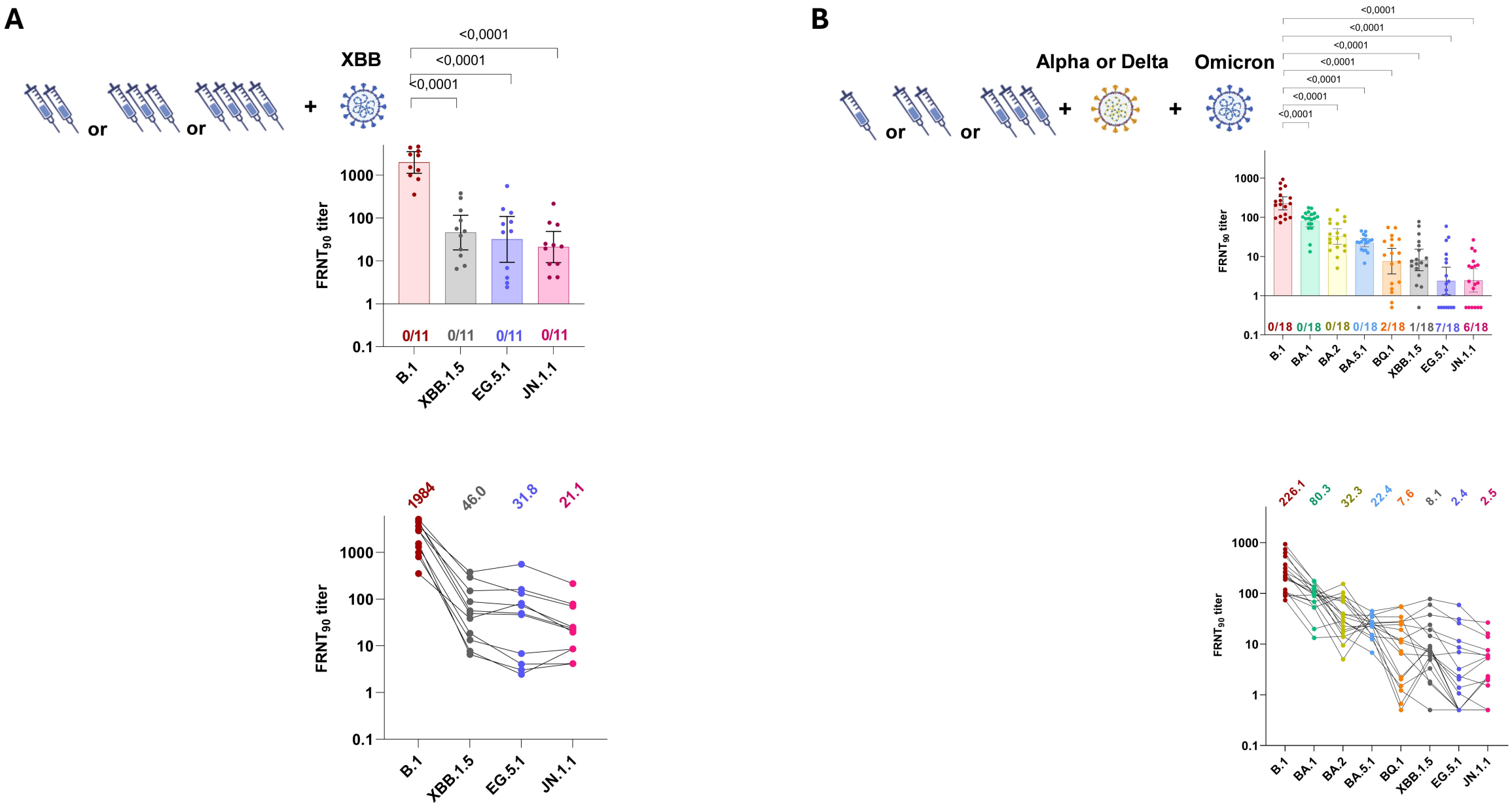

A
or
or
+
XBB
<0,0001
<0,0001
<0,0001
FRNT90 titer
1000
100
10
1
0.1
0/11
0/11
0/11
0/11
B.1
XBB.1.5
EG.5.1
JN.1.1
1984
46.0
31.8
21.1
B
or
or
+
+
Alpha or Delta
Omicron
<0,0001
<0,0001
<0,0001
<0,0001
<0,0001
<0,0001
<0,0001
0/18
0/18
0/18
0/18
2/18
1/18
7/18
6/18
B.1
BA.1
BA.2
BA.5.1
BQ.1
XBB.1.5
EG.5.1
JN.1.1
226.1
80.3
32.3
22.4
7.6
8.1
2.4
2.5

Figure 3

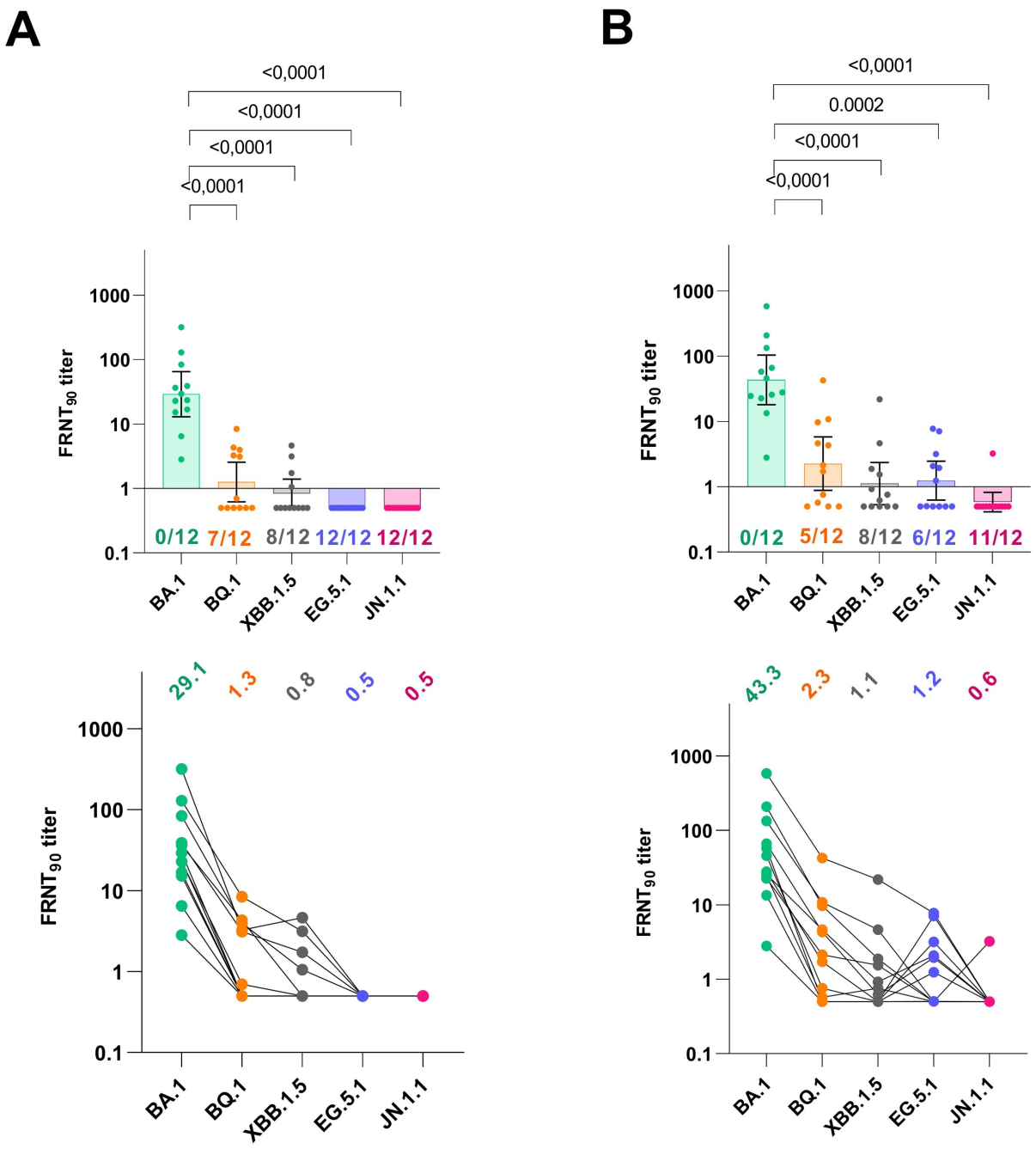

A
B
<0,0001
<0,0001
<0,0001
<0,0001
0.0002
FRNT90 titer
1000
100
10
1
0.1
0/12
7/12
8/12
12/12
12/12
5/12
6/12
11/12
BA.1
BQ.1
XBB.1.5
EG.5.1
JN.1.1
29.1
1.3
0.8
0.5
0.5
43.3
2.3
1.1
1.2
0.6

Figure 4

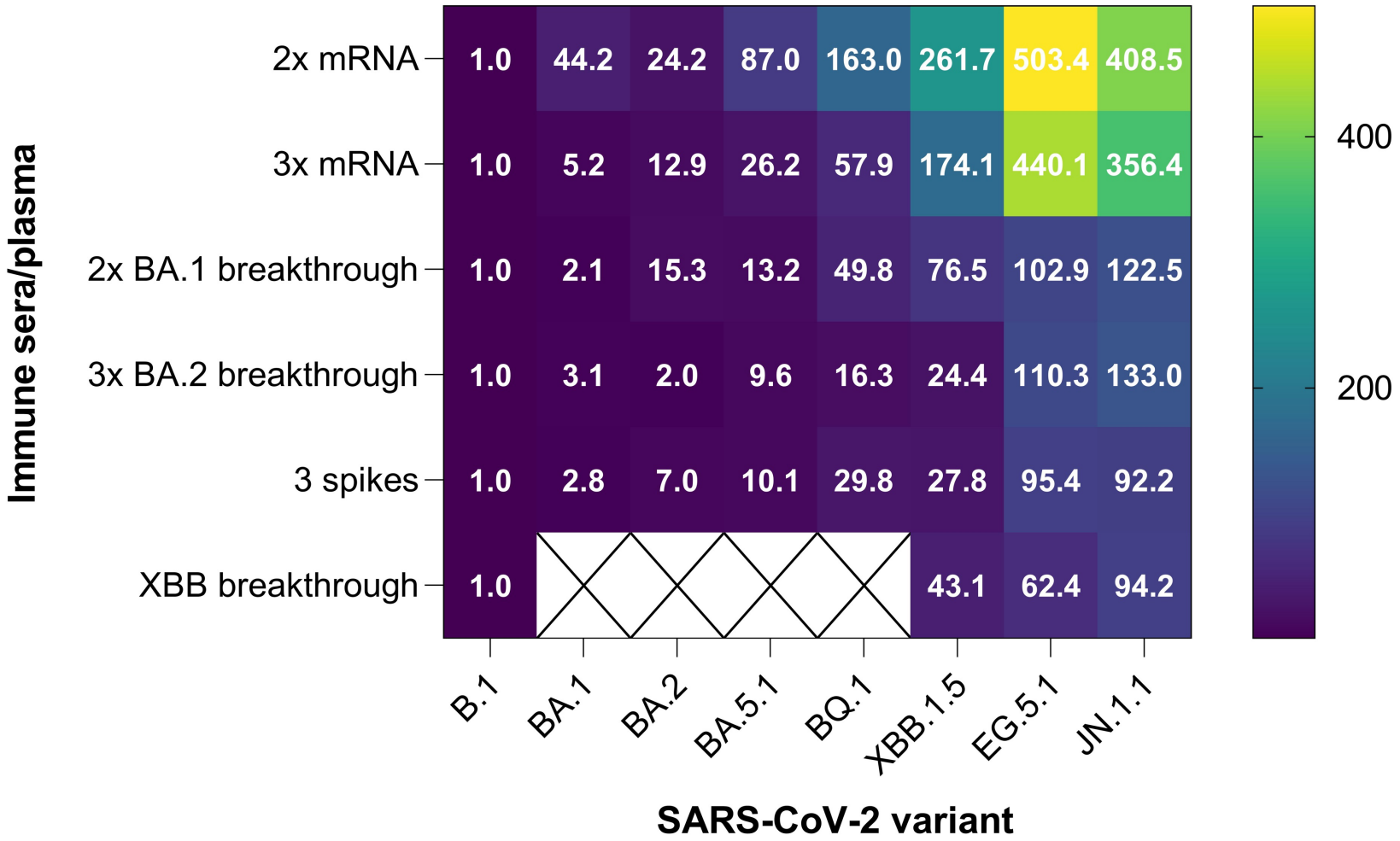

Immune sera/plasma
2x mRNA
3x mRNA
2x BA.1 breakthrough
3x BA.2 breakthrough
3 spikes
XBB breakthrough
1.0 44.2 24.2 87.0 163.0 261.7 503.4 408.5
1.0 5.2 12.9 26.2 57.9 174.1 440.1 356.4
1.0 2.1 15.3 13.2 49.8 76.5 102.9 122.5
1.0 3.1 2.0 9.6 16.3 24.4 110.3 133.0
1.0 2.8 7.0 10.1 29.8 27.8 95.4 92.2
1.0 43.1 62.4 94.2
B.1
BA.1
BA.2
BA.5.1
BQ.1
XBB.1.5
EG.5.1
JN.1.1
SARS-CoV-2 variant
400
200

Figure 5

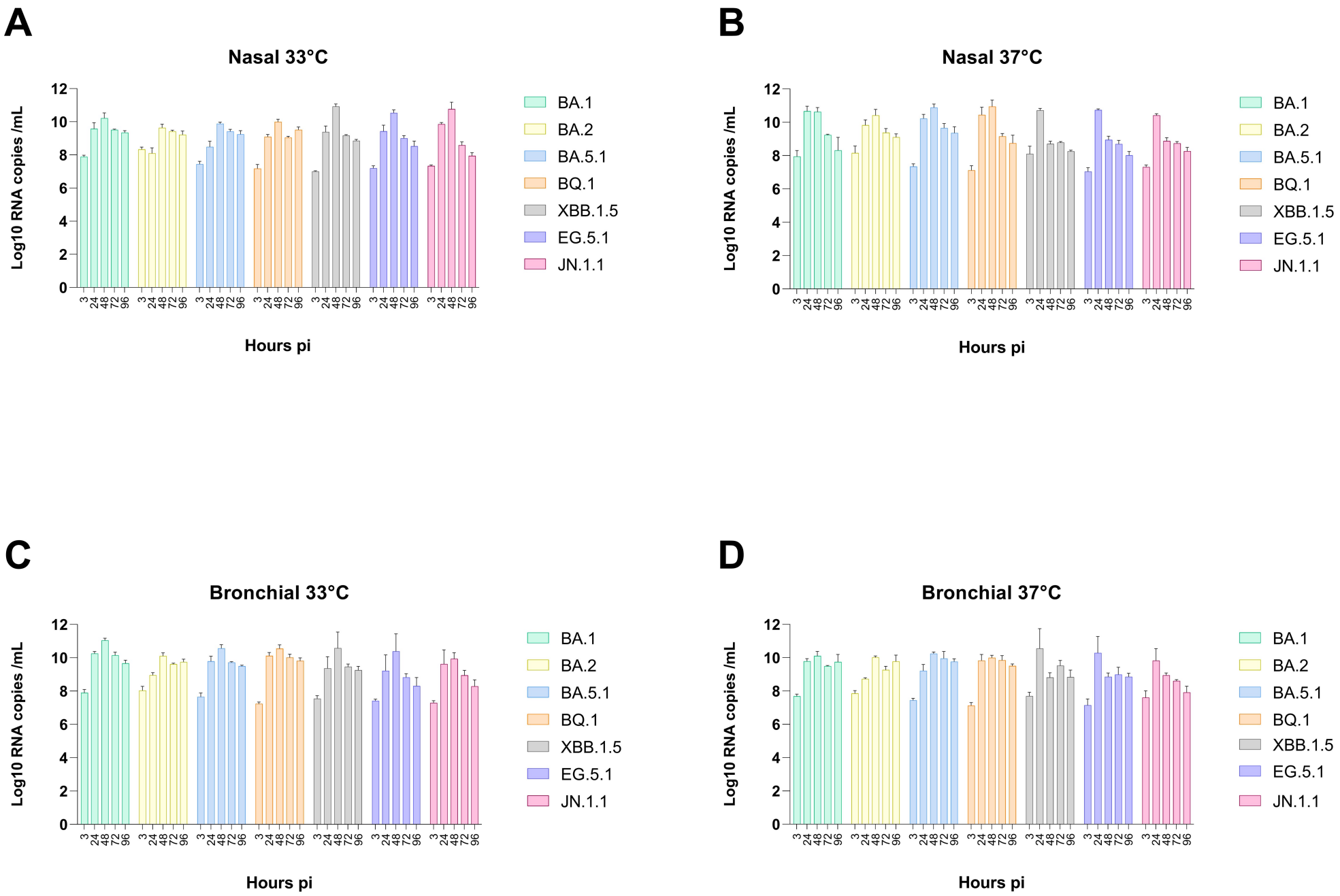

A
Nasal 33°C
B
Nasal 37°C
C
Bronchial 33°C
D
Bronchial 37°C
Log10 RNA copies /mL
Hours pi
BA.1
BA.2
BA.5.1
BQ.1
XBB.1.5
EG.5.1
JN.1.1

Figure 6

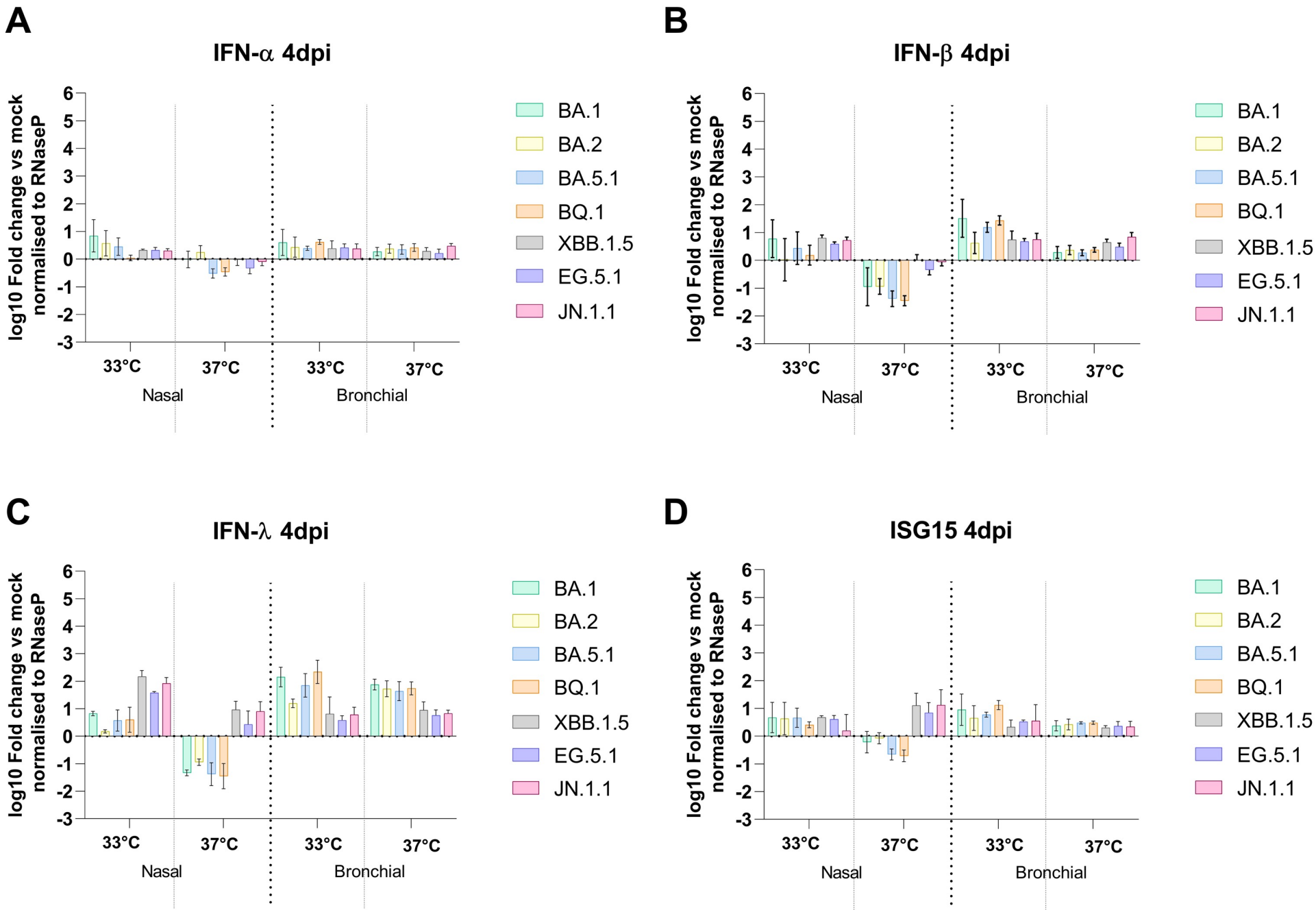

A
IFN-α 4dpi
log10 Fold change vs mock normalised to RNaseP
6
5
4
3
2
1
0
-1
-2
-3
33°C
37°C
33°C
37°C
Nasal
Bronchial
BA.1
BA.2
BA.5.1
BQ.1
XBB.1.5
EG.5.1
JN.1.1
B
IFN-β 4dpi
log10 Fold change vs mock normalised to RNaseP
33°C
37°C
33°C
37°C
Nasal
Bronchial
BA.1
BA.2
BA.5.1
BQ.1
XBB.1.5
EG.5.1
JN.1.1
C
IFN-λ 4dpi
log10 Fold change vs mock normalised to RNaseP
33°C
37°C
33°C
37°C
Nasal
Bronchial
BA.1
BA.2
BA.5.1
BQ.1
XBB.1.5
EG.5.1
JN.1.1
D
ISG15 4dpi
log10 Fold change vs mock normalised to RNaseP
33°C
37°C
33°C
37°C
Nasal
Bronchial
BA.1
BA.2
BA.5.1
BQ.1
XBB.1.5
EG.5.1
JN.1.1

Figure S1

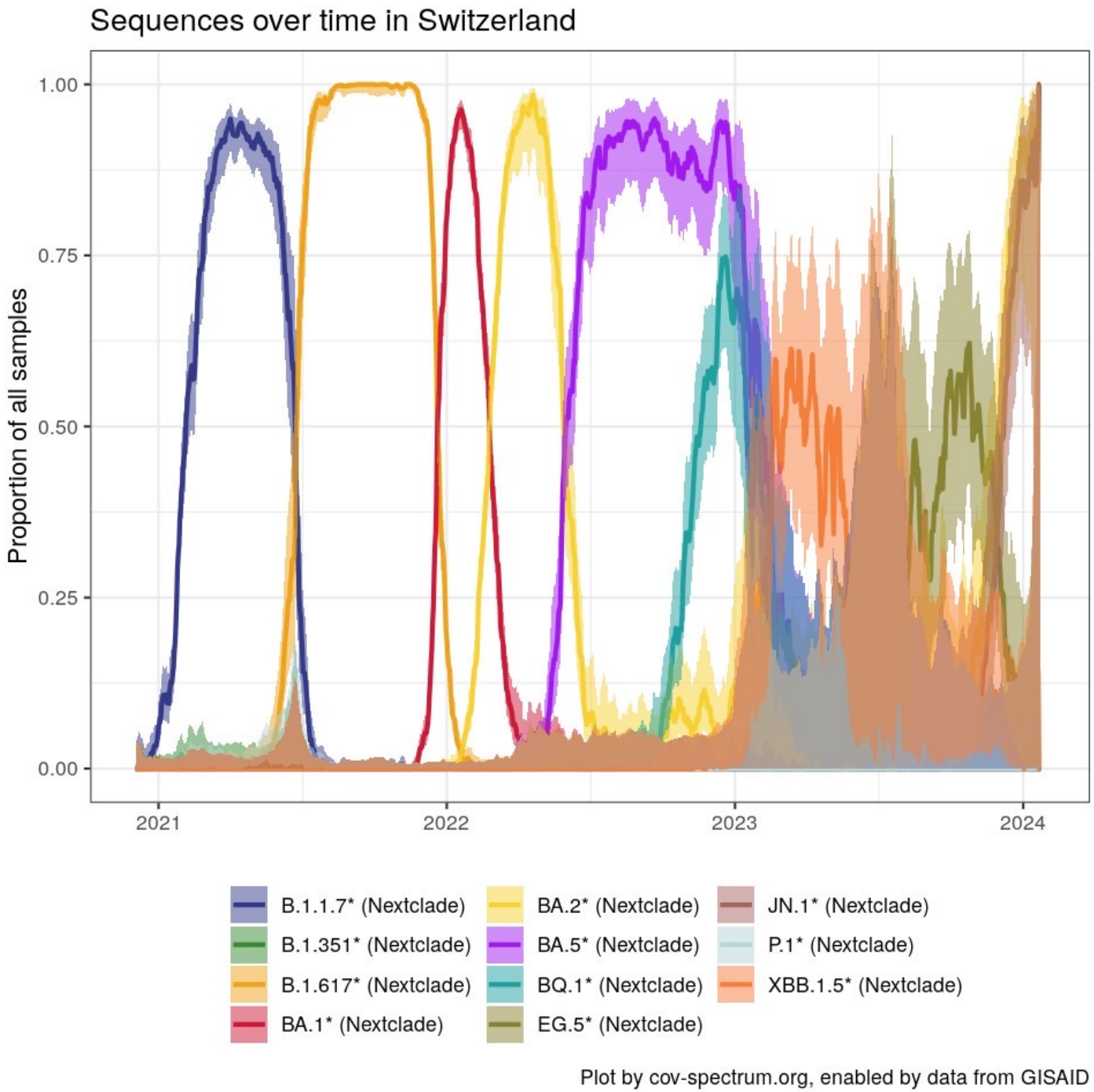
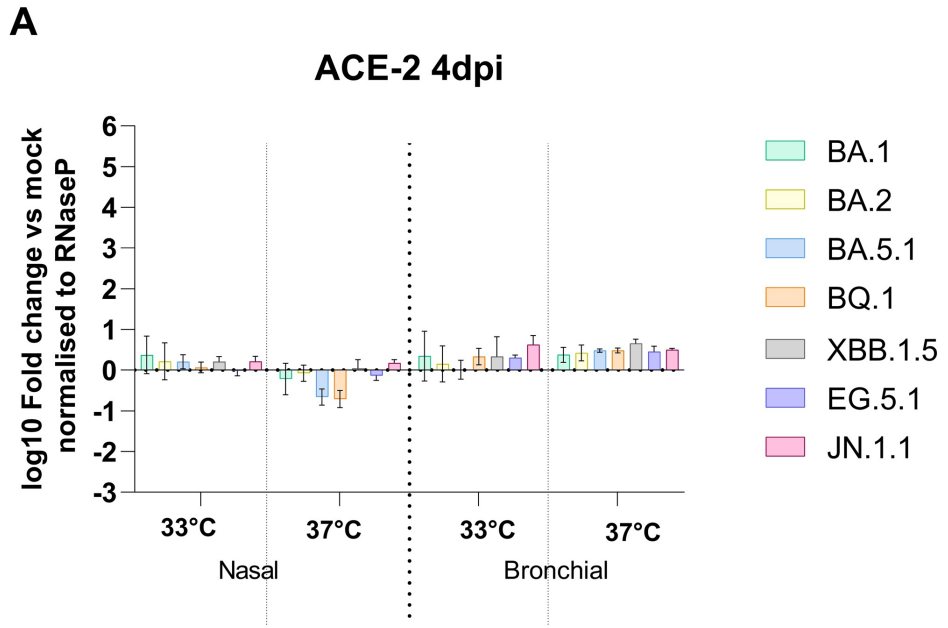
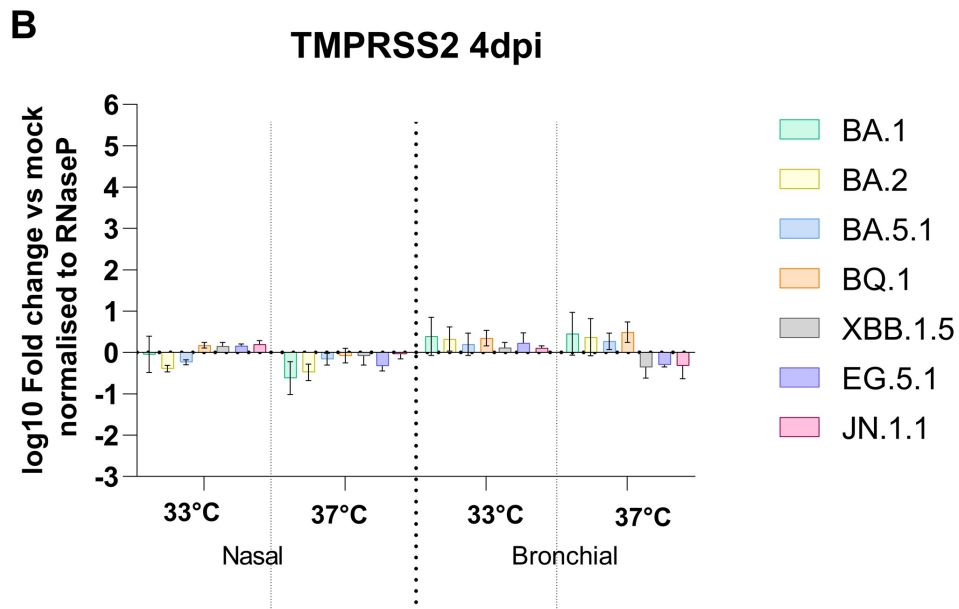

Sequences over time in Switzerland
Proportion of all samples
1.00
0.75
0.50
0.25
0.00
2021
2022
2023
2024
B.1.1.7* (Nextclade)
B.1.351* (Nextclade)
B.1.617* (Nextclade)
BA.1* (Nextclade)
BA.2* (Nextclade)
BA.5* (Nextclade)
BQ.1* (Nextclade)
EG.5* (Nextclade)
JN.1* (Nextclade)
P.1* (Nextclade)
XBB.1.5* (Nextclade)
Plot by cov-spectrum.org, enabled by data from GISAID

Figure S2


A
ACE-2 4dpi
log10 Fold change vs mock normalised to RNaseP
6
5
4
3
2
1
0
-1
-2
-3
33°C
37°C
33°C
37°C
Nasal
Bronchial
BA.1
BA.2
BA.5.1
BQ.1
XBB.1.5
EG.5.1
JN.1.1


B
TMPRSS2 4dpi
log10 Fold change vs mock normalised to RNaseP
6
5
4
3
2
1
0
-1
-2
-3
33°C
37°C
33°C
37°C
Nasal
Bronchial
BA.1
BA.2
BA.5.1
BQ.1
XBB.1.5
EG.5.1
JN.1.1