Repeated Omicron infection alleviates SARS-CoV-2 immune imprinting

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Abstract

The continuous emergence of highly immune evasive SARS-CoV-2 variants, like XBB.1.5[1,2] and XBB.1.16 [3,4], highlights the need to update COVID-19 vaccine compositions. However, immune imprinting induced by wildtype (WT)-based vaccination would compromise the antibody response to Omicron-based boosters[5-8]. Vaccination strategies that can counter immune imprinting are critically needed. In this study, we investigated the degree and dynamics of immune imprinting in mouse models and human cohorts, especially focusing on the role of repeated Omicron stimulation. Our results show that in mice, the efficacy of single Omicron-boosting is heavily limited by immune imprinting, especially when using variants antigenically distinct from WT, like XBB, while the concerning situation could be largely mitigated by a second Omicron booster. Similarly, in humans, we found that repeated Omicron infections could also alleviate WT-vaccination-induced immune imprinting and generate high neutralizing titers against XBB.1.5 and XBB.1.16 in both plasma and nasal mucosa. By isolating 781 RBD-targeting mAbs from repeated Omicron infection cohorts[7], we revealed that double Omicron exposure alleviates immune imprinting by generating a large proportion of highly matured and potent Omicron-specific antibodies. Importantly, epitope characterization using deep mutational scanning (DMS) showed that these Omicron-specific antibodies target distinct RBD epitopes compared to WT-induced antibodies, and the bias towards non-neutralizing epitopes observed in single Omicron exposures due to imprinting was largely restored after repeated Omicron stimulation, together leading to a substantial neutralizing epitope shift. Based on the DMS profiles, we identified evolution hotspots of XBB.1.5 RBD and demonstrated the combinations of these mutations could further boost XBB.1.5's immune-evasion capability while maintaining high ACE2 binding affinity. Our findings suggest the WT component should be abandoned when updating COVID-19 vaccine antigen compositions to XBB lineages, and those who haven't been exposed to Omicron yet should receive two updated vaccine boosters.

Main

SARS-CoV-2 continues to evolve, and new mutants constantly emerge under humoral immune pressure [9,10]. New variants, such as the XBB lineages, are capable of evading antibodies induced by vaccination or infection, resulting in repeated infections among populations [5,7,11,12]. Therefore, it is critical to develop updated vaccines that can elicit strong immune responses against the latest variants.

mRNA vaccine platforms can quickly adapt to new SARS-CoV-2 variants [13-16]. However, since the majority of the population was vaccinated with the ancestral SARS-CoV-2 strain, immune imprinting induced by WT vaccination presents a major challenge to the performance of updated boosters[17,18]. This is because boosting with a variant antigenically distinct from WT would majorly recall memory B cells induced by WT vaccination and masks the *de novo* generation of variant-specific B cells, which would hinder the generation of satisfying humoral immune responses toward new variants [6,7,19-23].

It is crucial to explore vaccination strategies that can counter immune imprinting. In this paper, we investigate the dynamics of immune imprinting in both mouse models and human cohorts, with a particular focus on how repeated exposure to Omicron variants could alleviate immune imprinting.

Alleviation of immune imprinting in mice

First, we investigated the effects of WT-vaccination-induced SARS-CoV-2 immune imprinting in mice. To accomplish this, two doses of 3 μg CoronaVac (an inactivated vaccine derived from the wildtype SARS-CoV-2) were used as primary immunization and variant Spike proteins were used as boosters [24]. All Spike proteins contained six proline substitutions (S6P) and alanine substitutions in the furin cleavage site to stabilize in prefusion conformation [25].

Indeed, mice that received a single booster of 10 μg Spike protein, including BA.1, BA.5, BQ.1.1, XBB, and SARS-CoV-1, had significantly lower serum 50% neutralizing titers (NT50s) (VSV-based pseudovirus) against the boosting variants compared to D614G, revealing substantial immune

78 imprinting at serum level (Fig. 1a and Extended Data Fig.1a). This is consistent with the
79 observations in humans [7,19,20,26], as well as previous findings of immune imprinting in influenza
80 viruses [27,28]. Importantly, as the antigenic distance between the boosting variant and the wildtype
81 increased, the fold-change between the NT50 against the D614G and the boosting variant also
82 increased, and single-dose XBB Spike boosting exhibited relatively low overall neutralizing
83 response (Fig. 1a and Extended Data Fig. 1b). To ensure that the results were not specific to
84 CoronaVac priming, we administered mice with two doses of 10 μg mRNA encoding Spike as
85 primary immunization (Fig. 1b and Extended Data Fig. 2a-b). mRNA vaccines also used S6P and
86 alanine substitutions for prefusion-stablization. Similar results were observed when the mRNA
87 vaccines were used as the priming agent, and there were even larger fold changes between NT50s
88 against D614G and the boosting variants, despite higher absolute titers (Fig. 1b). To investigate
89 whether prolonging the interval between the primary WT immunization and the variant booster
90 could alleviate immune imprinting, we further tested boosting mice 3-month and 6-month after
91 CoronaVac priming (Fig. 1c). It was observed that 3-month and 6-month intervals between WT-
92 priming and variant-boosting slightly increased overall NT50s, but the fold-change between NT50s
93 against D614G and XBB remained high (Fig. 1c). This suggests that longer intervals between the
94 priming and Omicron-boosting, which allows the maturation of WT-induced antibodies, may not be
95 sufficient to alleviate immune imprinting.

96 The efficacy of the first Omicron booster is heavily limited by immune imprinting. It's crucial to
97 examine how a second Omicron booster performs. We started by boosting CoronaVac-primed mice
98 with two doses of the variant Spike protein over a one-month interval (Fig. 1d). Importantly, the
99 second boosters resulted in greatly increased NT50s against the corresponding variants, as well as
100 substantially reduced fold-changes between the D614G and variants, when compared to the first
101 boosters (Fig. 1d). However, NT50s against the variants still remained lower than those against
102 D614G and the neutralizing titers induced by two boosters after two doses of CoronaVac priming
103 were even lower than those induced by two doses of variant priming, clearly indicating the
104 interference caused by immune imprinting (Extended Data Fig. 3a and Fig. 1e). Notably, among the
105 Omicron variants tested, XBB double boosting exhibited the lowest overall titers (Fig. 1d and
106 Extended Data Fig. 3b). Indeed, these variant boosters, whether protein or mRNA, exhibit different

levels of immunogenicity in mice, with XBB demonstrating the lowest (Fig. 1e and Extended Data Fig. 3c).

We also tested whether prolonged intervals between Omicron-boosters would facilitate the maturation of variant-specific B cells and enhance the alleviation of immune imprinting (Data Fig.3d). Indeed, a 3-month interval between Omicron boosters resulted in improvements in NT50s against all the corresponding boosting variants, and the fold-change between the NT50s against D614G and the boosting variants substantially decreased, with the XBB boosting group even exhibiting comparable titers to XBB primary (Fig.1d and Extended Data Fig.3a). Interestingly, we observed that prolonging the interval between the first Omicron-booster and the priming series could also improve the performance of the second Omicron-booster (Extended Data Fig. 4a-e). However, the improvement is not as substantial as the ones observed by increasing the time interval between Omicron-boosters, which highlights that the maturation of B cells induced by Omicron-boosting versus those induced by WT-vaccination are more beneficial for immune imprinting mitigation.

Since mRNA vaccines encoding Spike have proved to be capable of quick adaptation to new variants, it is critical to test how updated mRNA variant boosters perform, especially when the higher immunogenicity of mRNA vaccine might help alleviate immune imprinting when served as Omicron boosters. Therefore, we tested 1 μg or 10 μg mRNA vaccines encoding BA.5, BQ.1.1, and XBB Spike as boosters in replacement of protein boosters (Extended Data Fig. 5a-d). As expected, 1 μg or 10 μg mRNA vaccine demonstrated higher immunogenicity than the protein vaccine (Fig. 1e). Similarly, the performance of one-dose mRNA Omicron-boosters is still heavily interfered by immune imprinting despite higher immunogenicity, while two mRNA Omicron-boosters would significantly increase antibody titers (Fig. 1f and Extended Data Fig. 5a-d). Interestingly, we found that by increasing the dosage of mRNA boosters, repeated mRNA Omicron-boosting can achieve even higher NT50s than 2-dose mRNA priming against the boosting variant, especially demonstrated by XBB mRNA boosting (Extended Data Fig. 5e). This suggests raising the immunogenicity of variant boosters could help to counter immune imprinting brought by WT-vaccination, although this strategy might be practical in the real world due to safety concerns.

Together, our results observed in mice emphasize that the efficacy of the first Omicron boosters is severely limited by immune imprinting, especially for those with long antigenic distance from WT, such as XBB, while a second booster is almost mandatory to alleviate immune imprinting and generate high antibody responses.

Mitigating immune imprinting in humans

To verify whether findings obtained from mice can also be applied to humans, we recruited cohorts with repeated Omicron breakthrough infections (BTIs), including individuals with post-vaccination BA.1 or BA.2 BTI followed by BA.5/BF.7 reinfection (BTI+reinfection) and compared them to previously reported BA.1, BA.2, BA.5, BF.7 one-time BTI cohorts[7,29]. Importantly, we also included individuals who had no history of SARS-CoV-2 vaccination before repeated infection (vaccination-naïve reinfection) as controls. Detailed information about the cohorts can be found in Supplementary Table 1. We first tested neutralizing titers against exposed variants of these cohorts (Fig. 2a). Similar to mice immunization results, plasma neutralizing titers induced by one-time Omicron BTIs against the corresponding variant were significantly lower than those against D614G, consistent with our previous report[7], and the fold changes between the NT50 against D614G and those against corresponding variants also increased as the antigenic distance increases (Fig. 2a). As expected, in the repeated Omicron infection group, with or without SARS-CoV-2 vaccination history, the neutralizing titers against Omicron variants significantly increased compared to one-time BTIs (Fig. 2a-b). More importantly, BA.1 or BA.2 BTI followed by BA.5/ BF.7 reinfections demonstrate comparable NT50 between exposed Omicron variants and D614G, indicating immune imprinting alleviation by the second Omicron exposure (Fig. 2a). However, the NT50s of vaccination-naïve reinfection group against Omicron variants were the highest among these cohorts (Fig. 2a), suggesting that repeated BTIs were still subjected to WT-vaccination induced immune imprinting. Compared to one-time BTIs, repeated Omicron infection also led to an increase in the neutralizing titers against highly immune-evasive CH.1.1, BQ.1.1, XBB, XBB.1.15, XBB.1.16, and XBB.1.5.10 (Fig. 2b and Extended Data Fig. 6a-c), indicating that repeated Omicron infections may broaden the breadth of antibody response. In addition, we found that the nasal swab samples from repeated Omicron infection individuals exhibited exceptionally high neutralizing titers against Omicron variants, suggesting strong nasal mucosal humoral immunity has been established (Fig. 2c

166 and Extended Data Fig. 6d-f).

167 Neutralization data from both mice and human studies underscore the crucial role of secondary
168 Omicron exposure in mitigating immune imprinting and generating potent antibody responses to
169 immune-evasive variants such as XBB and its sublineages. We propose that this is largely
170 attributable to the further expansion of Omicron-specific memory B cells *de novo* generated by the
171 first Omicron exposure. To assess this hypothesis, we first analyzed the Omicron specificity of
172 RBD-specific memory B cells from BTIs, BTIs+reinfection, and vaccine-naive reinfection cohorts
173 through FACS. As we previously reported, in one-time Omicron BTI cohorts, more than 70% of the
174 RBD-binding memory B cells also binded to WT, indicating that post-vaccination Omicron
175 infection mainly recalls cross-reactive memory B cells elicited by WT-based vaccination, but rarely
176 contains BA.1/BA.2-specific B cells (Fig. 3a). Subsequently, following an extended duration of time
177 (8 months) after the first Omicron BTI, the proportion of cross-reactive cells declined while that of
178 Omicron-specific cells increased, suggesting that longer B cell maturation periods elevated the
179 proportion of Omicron-specific memory B cells (Fig. 3b). Nevertheless, at 8 months post-BA.1 BTI,
180 the plasma neutralizing titers were very low due to antibody waning, and thus required a secondary
181 Omicron boosting via either vaccination or infection to increase the antibody levels (Extended Data
182 Fig. 6g). Importantly, for Omicron BTI_reinfection cohorts, the proportion of cross-reactive cells
183 declined further but still remained higher than that observed in the vaccination-naïve reinfection
184 cohort (Fig. 3c-d). These results are highly correlated with the plasma NT50s of the cohorts, which
185 suggests that Omicron-specific antibodies are majorly responsible for the increased antibody
186 breadth and neutralization capability after repeated Omicron infection.

187 To further investigate the potency, breadth, and epitopes of these antibodies, the BA.1 RBD-binding
188 cells and BA.2 RBD-binding cells from above various BA.1/BA.2 infection cohorts were sorted and
189 sequenced by high throughput single-cell V(D)J sequencing. Antibodies were then expressed *in*
190 *vitro* as human IgG1 monoclonal antibodies (mAbs) (Supplementary Table 2). For one-time
191 Omicron BTI cohorts, Enzyme-linked immunosorbent assay (ELISA) confirmed that only
192 approximately 20% of the isolated mAbs specifically bind to the BA.1/BA.2 RBD and were not
193 cross-reactive to the WT RBD, which is consistent with FACS results (Fig. 3e). Furthermore, long-
194 term sampling (8 months) after BA.1 BTI yielded an increased proportion of BA.1 RBD-specific

195 mAbs compared to short-term (2 months) sampling. Moreover, reinfection with BA.5/BF.7 further
196 increased the proportion of BA.1/BA.2 RBD-specific mAbs to around 50%, but this was still lower
197 than those in vaccination-naïve reinfection groups (Fig. 3e). Notably, the somatic hypermutation
198 (SHM) rates of BA.1/BA.2 specific antibodies in BTI+reinfection cohorts were higher than that in
199 one-time BTI cohorts, indicating that BA.1/BA.2 specific antibodies from repeated Omicron
200 infection are more affinity-matured than those without reinfection (Fig. 3f), and the increased
201 affinity-maturation of BA.1/BA.2 specific antibodies in response to repeated Omicron infection may
202 contribute to their increased potency against Omicron variants. Together, these data indicate that
203 long-term maturation after one-time Omicron BTI and repeated Omicron infections could
204 significantly raise the proportion and maturation of Omicron-specific antibodies, greatly
205 contributing to the increased plasma neutralization potency against Omicron variants.

206

207 **Epitope analyses of Omicron-specific mAbs**

208 To further interrogate the composition of antibodies elicited by Omicron BA.5/BF.7 BTI and
209 reinfection, and deciphering the molecular mechanism behind the broadly neutralizing capability of
210 convalescent plasma from reinfection, we determined the binding sites and escaping mutations on
211 RBD of these mAbs using deep mutational scanning (DMS) [30,31]. As the proportion of Omicron-
212 specific antibodies is indispensable in reinfection cohorts, and the last exposure of all cohorts
213 involved in this study is BA.5/BF.7, we built a yeast display mutant library based on BA.5 RBD and
214 performed deep mutational scanning (DMS) for these mAbs in a high-throughput manner, akin to
215 our previously described WT-based methods [31]. To enhance the sampling of Omicron-specific NAbs
216 to facilitate the epitope characterization of these unprecedented antibodies, we specifically isolated
217 an additional panel of RBD-targeting mAbs that do not cross-bind to WT according to the feature
218 barcode counting during the 10x VDJ sequencing and determined their BA.5-based DMS data. We
219 also determined the BA.5-based DMS data for all BA.5-RBD binding mAbs from previous
220 collections isolated from various immune backgrounds (Supplementary Table 2). In total, a
221 comprehensive panel consisting of 1350 mAb BA.5-based DMS is collected.

222

223 By graph-based unsupervised clustering on the determined escape scores over sites on RBD, we
224 identified 12 major epitope groups on BA.5 RBD and embedded the mAbs using UMAP for
225 visualization (Fig. 4a). Names of the epitope groups are generally assigned in line with the epitope
226 groups on WT RBD defined previously [7,26]. Neutralizing activities against SARS-CoV-2 D614G,
227 BA.1, BA.2, BA.5, BA.2.75, BQ.1.1, and XBB.1.5 are determined using VSV-based pseudovirus
228 neutralization assays. In general, neutralization is highly correlated with targeting epitopes of mAbs.
229 Antibodies in epitope groups F3, A1, A2, B, C/D1, D2, D3, D4, and E1/E2.1 target neutralizing
230 epitopes, while antibodies in the other three groups, E2.2, E3, F1, exhibit weak or no neutralization
231 activity (Fig. 4b and Extended Data Fig. 7b). Consistent with the plasma neutralization results, BA.5
232 or BF.7 BTI exhibited substantially imprinted antibody response, leading to over 50% antibodies
233 that target conserved weakly neutralizing epitopes. In contrast, convalescent individuals who
234 experienced BA.5 or BF.7 reinfection after prior BA.1 or BA.2 BTI induce only ~20% antibodies
235 targeting such epitopes, indicating striking alleviation of immune imprinting (Fig. 4c and Extended
236 Data Fig. 7a). Interestingly, prior BA.1 or BA.2 BTI leads to Omicron-specific antibodies targeting
237 distinct epitopes after reinfection. Prior BA.1 BTI induces a higher level of Group D3, while BA.2
238 BTI cohorts consist of more antibodies in Group F3, indicating that the Omicron infection history
239 during repeated Omicron infections would also introduce new Omicron-based immune imprinting.

240

241 Among the 12 identified epitope groups, A1, D2, E1/E2.1, E2.2, E3, and F1 are similar to their
242 corresponding WT-based groups and mainly consist of WT-reactive antibodies (Fig. 4d-e and
243 Extended Data Fig. 7c-d) [26,32]. As expected, BA.5-based epitope landscape also defines novel groups
244 that mainly comprise Omicron-specific mAbs, including Group A2, D3, D4, and F3. Notably, most
245 antibodies in Group F3 here are not cross-reactive to WT RBD as well, which is different from the
246 rare sarbecovirus-neutralizing broad NAbs in Group F3 from SARS convalescents described
247 previously, such as SA55 and BD55-3372 [33]. Compared to A1, which mainly contains IGHV3-53/3-
248 66 public antibodies (also known as class 1 or site Ia) [34,35], mAbs in Group A2 are susceptible to
249 mutations on 417 and 505, including the reversions. Group D3 and D4 target an epitope near Group
250 D2 (targeted by LY-CoV1404), but exhibited distinct escape profiles or interacting residues [36]. D3
251 is susceptible to N439 and K440 mutations, and thus escaped by WT due to N440, while the

252 footprint of D4 is closer to the receptor-binding motif (RBM), interacting with G447, Y449, and
253 R498 (Fig. 4d-e). Antibodies in WT-based Group B, C, and D1 have been mostly escaped by L452R,
254 E484A, and F486V in BA.5. B and C/D1 here comprise both WT-reactive and Omicron-specific
255 antibodies, where Group B is more focused on N487 and Y489, and C/D1 mainly focus on F490,
256 which is largely escaped by F490S in XBB variants (Fig. 4d-e and Extended Data Fig. 7c-d). Among
257 the 12 groups, A1, A2, B, D3, especially D4 and F3 consist of a substantial proportion of NAbs
258 exhibiting broad neutralization against BQ.1.1 and XBB.1.5 (Fig. 4f). C/D1, D2, and E1/E2.1 also
259 consist of a small proportion of XBB.1.5-neutralizing mAbs (Extended Data Fig. 7f). Considering
260 the recent emergence and prevalence of XBB subvariants harboring F456L (XBB.1.5.10) or K478R
261 (XBB.1.16), which are crucial sites for NAbs in Group A1 and A2, or B and C/D1, respectively, we
262 tested the neutralization of XBB.1.5-neutralizing antibodies from these groups against these two
263 mutants. As expected, F456L escapes or dampens the neutralization of most XBB.1.5-neutralizing
264 antibodies in Group A1 or A2, and XBB.1.16 (E180V+K478R) also escapes a large proportion of
265 NAbs in B and C/D1 (Extended Data Fig. 7e). Overall, these results demonstrate that Omicron
266 repeated infection stimulates a higher level of Omicron-specific neutralizing antibodies targeting
267 neutralizing epitopes compared to one-time Omicron BTI, indicating substantial alleviation of
268 immune imprinted on antibody epitope level. And that these Omicron-specific mAbs have distinct
269 RBD epitopes and escaping mutations compared to WT-induced mAbs would introduce a large
270 neutralizing epitope shift, contributing majorly to the broadly neutralizing capability against
271 XBB.1.5.

272

273 **Evolutionary hotspots on XBB.1.5 RBD**

274 Encouraged by the successful rationalization of the prevalence of F456L and K478R based on DMS,
275 we desire to systematically investigate the evolutionary preference of other RBD mutations. To
276 integratively evaluate the preference of each mutation considering their impacts on neutralizing
277 antibody escape, hACE2 binding, RBD stability, and codon constraints, we previously calculated a
278 weighted preference score for RBD mutations using WT-based DMS profiles and neutralizing
279 activities against BA.5 to predict the convergent evolution of BA.5 RBD [7] (Extended Data Fig. 8).
280 We desire to utilize similar approach with BA.5-based profiles and neutralization against XBB.1.5

281 to identify the evolutionary trends of XBB.1.5 RBD. When considering antibodies from BA.5/BF.7
282 BTI only, the most significant sites include R403S/K, N405K, N417Y, Y453S/C/F, L455W/F/S,
283 F456C/V/L, and Y505H/D, corresponding to escape hotspots of Group A1, A2, and F3 (Fig. 5a).
284 With antibodies from repeated Omicron infection included in the analysis, scores of N439K,
285 K440N/E, K444N/E, and P445S/H/R/L become higher, corresponding to Group D3 and D4, which
286 are consistent with the epitope distributions of mAbs from each cohort (Fig. 5b). Notably, N405D
287 and N417K reversions should hardly appear in the real world due to the potential recovery of
288 previously escaped NAbs in Group F2 and A, respectively. K478 mutations are not identified in the
289 calculation, which is also a limitation of our model due to the low proportion of XBB-neutralizing
290 antibodies in Group B or C/D1 in our cohorts.

291

292 Based on the analysis above, we wonder if the combination of multiple escape mutations against
293 major XBB.1.5-effective epitope groups could essentially evade the broadly neutralizing capability
294 of plasma from repeated Omicron infection while retaining high ACE2 binding affinity. Besides the
295 two emerging mutations K478R and F456L, we selected seven additional substitutions, including
296 H505Y, R403K, K444T, K440N, A484P, Y453F, and N405K, which are sequentially added to
297 XBB.1.5, and constructed seven pseudoviruses named XBB.1.5-S1 to XBB.1.5-S7 (Fig. 6a). The
298 mutations are selected from a larger set of mutation candidates considering their impacts on hACE2-
299 binding affinity as determined by surface plasmon resonance (SPR) and the capability of escaping
300 the neutralization of a panel of 131 potent XBB.1.5-neutralizing antibodies from 8 epitope groups
301 (Fig. 6b-c and Extended Data Fig. 9a). XBB.1.5-S7 successfully escapes the most of NAbs in the
302 panel, except for a small group of broad NAbs from Group F3, A1, and D4, including SA55, a
303 therapeutic antibody under clinical development[33]. Then, we evaluated the neutralization titers of
304 convalescent plasma from individuals who experienced Omicron BTI or repeated Omicron infection
305 against the designed escape mutants. As expected, XBB.1.5-S7 could significantly escape plasma
306 samples from all tested cohorts. Plasma from BA.5 or BF.7 BTI are significantly escaped upon the
307 inclusion of F456L, and nearly negative against XBB.1.5-S7 (Extended Data Fig. 9b). Plasma from
308 repeated Omicron infections is much more resistant to escape mutations. Interestingly, plasma from
309 BA.5/BF.7 reinfection with prior BA.1 BTI or BA.2 BTI exhibited distinct neutralization to different

escape mutants. The former samples are largely evaded by K444T and K440N, but not strongly affected by H505Y, while the latter samples are significantly evaded by H505Y (Fig. 6d-e). This is consistent with the observation that reinfection with prior BA.1 BTI elicits more Group D3 antibodies, while reinfection with prior BA.2 BTI elicits more Group F3 antibodies (Fig. 4c). Unvaccinated reinfection cohorts exhibited higher neutralization against XBB.1.5 compared to vaccinated cohorts, but equivalently escaped by XBB.1.5-S7. The most significant reduction occurs upon the inclusion of H505Y, K440N, and N405K, indicating a high proportion of Omicron-specific antibodies in Group D3 and F3 (Fig. 6f).

In summary, our findings suggest that secondary Omicron exposure is necessary to mitigate the immune imprinting conferred by previous ancestral virus exposure and to elicit higher levels of Omicron-specific antibodies. Accordingly, our recommendation is to administer two booster doses of Omicron-based vaccines to individuals who have not received prior Omicron-based vaccinations or who have not been previously infected with the Omicron variant. Moreover, administering the second booster shot after a prolonged interval can provoke a wider and more efficient immune response, while incorporating the wildtype virus into subsequent vaccine designs may worsen immune imprinting [22]. Furthermore, it is imperative to incorporate the XBB variants into vaccine design to achieve broad-spectrum protection, given its potential to mutate and evade vaccines based on previous Omicron variants.

Recently, several fast-growing XBB lineages, such as the variant of interest (VOI) XBB.1.16 (K478R), XBB.2.3.5 (K478N), and XBB.2.3.4 (K478Q), have acquired RBD mutations on K478. However, the K478 mutation did not emerge in our prediction of evolutionary trends for XBB.1.5 RBD. This contradiction may be attributed to the fact that our mutational prediction model primarily relies on the cohorts we recruited, and we haven't captured the immune background that introduced K478 mutation. One possible background that may give rise to K478 is repeated BA.5/BQ.1.1/XBB exposure, as F486 could mask the immunogenicity of K478. Another potential source of K478 is Delta-imprinted convalescents who experienced BA.5/BQ.1.1/XBB infections, which could result in the generation of abundant K478X-sensitive mAbs, given that Delta carries T478K. This may explain why K478X is mostly observed in India [4,40].

The degree of immune imprinting might be different between mRNA and inactivated vaccination. Recent studies have shown that subsequently exposed to Omicron twice after two doses of WT-based mRNA vaccines still produce significantly low levels of Omicron-specific antibodies, despite the enhanced neutralization breadth against BQ.1.1 and XBB variants [37,38]. Additionally, individuals who have received two doses of mRNA vaccines and experienced two rounds of Omicron infection also have low levels of Omicron-specific antibodies [37]. This indicates that mRNA vaccines may generate a stronger immune imprinting effect compared to inactivated vaccines, potentially due to its stronger primary humoral immune response [8,39]. However, a head-to-head comparison is needed for validation.

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Figure Legends

Fig. 1 | Humoral immune imprinting in mice.

a, Neutralizing antibody response after priming with 2 doses of 3 μg CoronaVac followed by boosting with 10 μg SARS-CoV-1 Spike protein or SARS-CoV-2 variant Spike proteins in mice. **b**, Neutralizing antibody response after priming with 2 doses of 10 μg WT-mRNA followed by

boosting with 10 μg SARS-CoV-2 variant Spike proteins. **c**, Neutralizing antibody response after immunization with 2 doses of 3 μg CoronaVac followed by boosting with 10 μg SARS-CoV-2 variant Spike proteins with 3 months or 6 months time interval in mice. **d**, Neutralizing antibody response after priming with 2 doses of 3 μg CoronaVac followed by boosting twice with 10 μg SARS-CoV-2 variant Spike proteins with different time intervals in mice. Mice were boosted twice with intervals between boosters of either 1 month or 3 months. **e**, Neutralizing antibody response after priming with 2 doses of 10 μg variant Spike proteins or 1 μg or 10 μg variant Spike mRNAs. **f**, Neutralizing antibody response after priming with 2 doses of 3 μg CoronaVac followed by boosting twice with 1 μg or 10 μg of SARS-CoV-2 variant Spike mRNAs. 10 mice were immunized and analyzed in each group (n= 10). All Spike protein and mRNA used S6P prefusion-stabilization. The time interval between the two priming doses is 2 weeks. The interval between priming and boosting is 4 weeks. Sera were collected one week after the last dose. Geometric mean titers (GMT) were labeled. For paired samples in a-d and f, statistical significance were determined using two-tailed Wilcoxon signed-rank tests. For independent samples in e, statistical significance were determined using two-tailed Wilcoxon rank sum tests. *$p < 0.05$, **$p < 0.01$, and not significant (NS) $p > 0.05$. All neutralization assays were conducted in at least two independent experiments.

Fig. 2 | Humoral immune imprinting after repeated Omicron infections in humans.

a, Examination of immune imprinting after Omicron breakthrough infections and repeated Omicron infections. Plasma antibody titers against pseudotyped D614G and variants were measured. Fold changes between titers against variants and D614G were calculated and shown above the line. Statistical significance was determined using the Wilcoxon signed-rank test. BA.1, BA.2, BA.5, BF.7 BTI: post-vaccination Omicron breakthrough infection (BTI). BA.1, BA.2 BTI+ BA.5/BF.7 infection: post-vaccination Omicron breakthrough infection followed by BA.5/BF.7 reinfection. BA.1/BA.2+ BA.5/BF.7 infection: BA.1/BA.2 infection followed by BA.5/BF.7 reinfection with no vaccination history. Blood samples were collected 1-2 months after the last infection. Detailed information about the cohorts is in Supplementary Table 1. **b**, Plasma antibody breadth after repeated Omicron infections. Plasma antibody titers against circulating pseudotyped variants were measured. Fold changes between titers of different cohorts were calculated and shown above the line. Statistical significance was determined using the Wilcoxon rank sum tests. **c**, Nasal swab

492 antibody breath after repeated Omicron infections. Nasal swab antibody titers against pseudotyped
493 variants were measured. Fold changes between titers of different cohorts were calculated and shown
494 above the line. Statistical significance was determined using the Wilcoxon rank sum test. Geometric
495 mean titers (GMT) are labeled in (**a**). Geometric mean ± SD are labeled in (**b, c**). Dashed lines
496 indicate the limit of detection (LOD, NT50 = 20). *$p < 0.05$, **$p < 0.01$, ***$p<0.001$,
497 ****$p<0.0001$, and not significant (NS) $p > 0.05$. All neutralization assays were conducted in at
498 least two independent experiments.
499
500 **Fig. 3 | B cell immune imprinting after repeated Omicron infections.**
501 **a–d**, Flow cytometry analysis of pooled B cells from Omicron infection convalescent individuals.
502 BA.1 (up) and BA.2 (down) RBD double-positive CD20+, IgM-, IgD-, CD27+ B cells were isolated
503 for paired-single-cell V(D)J sequencing. Flow cytometry analyses were performed in cohorts of the
504 following: (**a**) 2 months after BA.1 (up) or BA.2 (down) breakthrough infections, (**b**) 8 months after
505 BA.1 (up) or BA.2 (down) breakthrough infections, (**c**) 1 month after BA.5/BF.7 reinfection after
506 BA.1 (up) and BA.2 (down) breakthrough infections, **(d**) 2-3 months after BA.5/BF.7 reinfection
507 after BA.1 (up) or BA.2 (down) infection without SARS-CoV-2 vaccination history. APC,
508 allophycocyanine; FITC, fluorescein isothiocyanate; PE, phycoerythrin. BV605, Brilliant Violet
509 605. **e**, Proportions of WT-binding and non-WT-binding antibodies from Omicron breakthrough
510 infection and repeated Omicron infection cohorts. Binding specificity was determined by ELISA.
511 The antibodies were expressed in vitro using the sequence of the RBD-binding memory B cells from
512 various cohorts. **f**, The heavy-chain variable domain somatic hypermutation rate of the mAbs from
513 various cohorts. Statistical tests were determined using two-tailed Wilcoxon rank-sum tests. Boxes
514 display the 25th percentile, median and 75th percentile, and whiskers indicate median ± 1.5 times
515 the interquartile range. Violin plots show kernel density estimation curves of the distribution. The
516 numbers and ratios of samples in each group are labeled above the violin plots. *$p < 0.05$, **$p <$
517 0.01, ***$p<0.001$, ****$p<0.0001$, and not significant (NS) $p > 0.05$.
518
519 **Fig. 4 | Epitope distribution and characterization of mAbs elicited by Omicron BTI and**
520 **reinfection**
521 **a**, UMAP embedding of epitope groups of monoclonal antibodies (mAbs) binding BA.5 RBD

isolated from convalescent individuals who experienced BA.5/BF.7 BTI or reinfection (n=1350). **b**, Neutralization activities, denoted as IC50 values, against SARS-CoV-2 D614G (n = 1349), BA.4/5 (n = 1322), and XBB.1.5 (n = 1346) spike-pseudotyped vesicular stomatitis viruses (VSV), as well as ACE2 competition levels determined by ELISA (n = 1344), are projected onto the UMAP embedding space. **c**, Distribution of mAbs across epitope groups is shown for BA.5 Breakthrough Infection (BTI), BF.7 BTI, BA.1 BTI with reinfection, and BA.2 BTI with reinfection. Epitope groups predominantly comprising non-neutralizing or weakly neutralizing mAbs (E2.2, E3, and F1) are highlighted with dashed boxes. The percentage of antibodies in these three groups is labeled on each bar. **d**, Average DMS escape scores of the crucial epitope groups contributing to neutralization against XBB.1.5 are illustrated on the structure model of the SARS-CoV-2 BA.5 RBD (PDB: 7XNS). Key residues with high escape scores for each group are labeled. **e**, The average DMS escape scores for the key epitope groups are represented as sequence logos; residues are depicted using the standard one-letter code and colored based on their chemical properties. The height of each letter corresponds to the escape score of the respective mutation. **f**, Pseudovirus-neutralization activities of mAbs within the six crucial epitope groups (A1 [n = 170], A2 [n = 60], B [n = 33], F3 [n = 129], D3 [n = 155], and D4 [n = 80]) are shown against SARS-CoV-2 D614G, BA.5, BQ.1.1, and XBB.1.5. Geometric mean IC50 values are displayed as bars and labeled above each group of data points.

Fig. 5 | Estimate the evolutionary trends of XBB.1.5 RBD from DMS profiles.

Normalized average DMS escape scores weighted by IC50 against XBB.1.5 using DMS profiles of mAbs from BA.5/BF.7 BTI (**a**), and mAbs from BA.5/BF.7 BTI and BA.1/BA.2 BTI with BA.5/BF.7 reinfection (**b**). The impacts of each mutation on ACE2 binding and RBD expression, and the codon constraints on each residue, are also considered (see Methods). Residues with high estimated preferences are labeled, and their corresponding mutation scores are shown as logos.

Fig. 6 | Combination of escape mutations evades XBB.1.5-neutralizing antibodies from reinfection.

a, SARS-CoV-2 XBB.1.5-based pseudoviruses harboring combinations of critical mutations identified through analysis of DMS profiles are generated. **b**, hACE2-binding affinity for various

RBD mutants of SARS-CoV-2 is assessed using SPR. Geometric mean dissociation constants (KD) from at least four independent replicates are shown, with statistical significance in comparison to XBB.1.5 RBD's KD labeled above the bars. P-values are determined using a two-tailed t-test on log-transformed KD values. **c**, IC50 values for representative potent XBB.1.5-neutralizing antibodies from different epitope groups against XBB.1.5 variants carrying individual or multiple escape mutations are displayed. Fold changes in IC50 against the mutants relative to XBB.1.5 are presented as a heatmap. **d-f**, Pseudovirus 50% neutralization titers (NT50) for SARS-CoV-2 XBB.1.5-based mutants are shown using plasma from convalescent individuals who experienced BA.5 or BF.7 reinfection: BA.1 BTI prior to BA.5/BF.7 reinfection (n = 26) (**d**); BA.2 BTI prior to BA.5/BF.7 reinfection (n = 19) (**e**); and reinfection with BA.5 or BF.7 after BA.1 or BA.2 infection without vaccination (n = 12) (**f**). Key mutations diminishing neutralization are labeled above their corresponding lines. Dashed lines indicate the limit of detection (LOD, NT50 = 20). Geometric mean titers are labeled above data points. Statistical tests are performed between neighboring mutants. P-values are calculated using two-tailed Wilcoxon signed-rank tests on paired samples. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05.

Methods

Isolation of PBMCs and plasma

Blood samples from vaccinated or unvaccinated individuals who had recovered from Omicron breakthrough infection or reinfection were obtained under study protocols approved by Beijing Ditan Hospital, Capital Medical University (Ethics committee archiving No. LL-2021-024-02) and the Tianjin Municipal Health Commission, and the Ethics Committee of Tianjin First Central Hospital (Ethics committee archiving No. 2022N045KY). All participants have provided written informed consent for the collection of information, storage and use of their clinical samples for research purposes, and publication of data generated from this study.

The whole blood samples were 1:1 diluted with 2% fetal bovine serum (FBS) (Hyclone, SH30406.05) in phosphate buffered saline (PBS) (Invitrogen, C10010500BT) and subjected to Ficoll (Cytiva, 17-1440-03) gradient centrifugation to isolate plasma and PBMCs. Plasma was collected from upper layer after centrifugation. PBMCs were collected at the interface and further prepared through centrifugation, red blood cell lysis (Invitrogen™ eBioscience™ 1X RBC Lysis

Buffer, 00-4333-57) and washing steps. If not used for downstream process immediately, samples were stored in FBS with 10% DMSO (Sigma-Aldrich, D4540) in liquid nitrogen. All PBMC samples were shipped on dry ice and cryopreserved PBMCs were thawed in PBS + 1mM EDTA (Invitrogen, AM9260G) + 2% FBS before use.

mRNA vaccine preparation and mouse immunization

For mRNA vaccine preparation, 5′ untranslated region (UTR), target sequence, and 3'UTR were sequentially inserted after T7 promoter in an empty PSP73 plasmid firstly. The plasmid was then subjected to double digestion to obtain linearized DNA. This DNA served as a template for an in vitro transcription reaction mediated by T7 RNA polymerase to synthesize RNA encoding the SARS-CoV-2 S6P (F817P, A892P, A899P, A942P, K986P, V987P) protein according to the manufacturer's instructions (Vazyme, DD4201). Uridine was fully replaced by N1-methyl-pseudouridine in this process. Transcription products were treated with DNase I to remove DNA templates, and purified using VAHTS RNA Clean Beads (Vazyme, N412-02). Cap 1 structure was added using Vaccinia Capping Enzyme (Vazyme, DD4109) and mRNA Cap 2'-O-Methyltransferase (Vazyme, DD4110), followed by magnetic bead purification. Poly(A) tails were added using E.coli Poly(A) Polymerase (Vazyme, N412-02) and the product was purified again.

The mRNA was encapsulated in a functionalized lipid nanoparticle as described previously [41]. In brief, ionizable lipid, DSPC, cholesterol, and PEG2000-DMG were dissolved in ethanol at the mole ratio of 50:10:38.5:1.5, respectively. mRNA was diluted in RNase free 50 mM citrate buffer (pH 4.0) to obtain a final lipid:mRNA weight ratio of 6:1. The aqueous and ethanol solutions were mixed in a 3:1 volume ratio using a microfluidic apparatus and the obtained LNPs were dialyzed overnight. All of the samples were stored within a week at 2~8 ℃ of use to ensure the chemical stability of the components. The size of LNPs, the particle size distributions, and the encapsulation and concentration of mRNA were determined. The encapsulation in all of the samples was typically 90–99%.

Animal experiments were carried out under study protocols approved by Institute of Biophysics, Chinese Academy of Sciences (SYXK2023300) and HFK Biologics (HFK-AP-20210930). Mice

were immunized according to schemes in figure 1. All inactivated vaccines were administered intraperitoneally at a dose of 3 μg per mouse, while mRNA vaccines were administered intramuscularly at a dose of 10 μg per mouse. Protein subunit vaccines were administered subcutaneously at six sites on the back at a dose of 10 μg per mouse, where complete Freund's adjuvant was used for the prime immunization, and incomplete Freund's adjuvant was used for booster immunizations, with a 1:1 volume ratio of protein subunit and adjuvant. The second immunizations were given 2 weeks after the first dose, with subsequent doses administered at 1-month intervals, unless stated otherwise. Blood samples were collected 1 week after the final immunization.

BCR sequencing, analysis and recombinant antibody expression

$CD19^+$ B cells were enriched from PBMCs using EasySep Human CD19 Positive Selection Kit II (STEMCELL, 17854). Following enrichment, $1x10^6$ B cells in 100 μl buffer were incubated with a panel of antibodies including 3 μl FITC anti-human CD20 antibody (BioLegend, 302304), 3.5 μl Brilliant Violet 421 anti-human CD27 antibody (BioLegend, 302824), 2 μl PE/Cyanine7 anti-human IgD antibody (BioLegend, 348210) and 2 μl PE/Cyanine7 anti-human IgM antibody (BioLegend, 314532). Additionally, fluorophore or oligonucleotide conjugated RBD were added. For FACS, 0.13 μg of biotinylated BA.1 (Sino Biological, 40592-V49H7-B) or BA.2 (customized from Sino Biological) RBD protein conjugated with PE-streptavidin (BioLegend, 405204) and APC-streptavidin (BioLegend, 405207), and 0.13 μg of WT biotinylated RBD protein (Sino Biological, 40592-V27H-B) conjugated with BV605-streptavidin (BioLegend, 405229) were added. For sequencing, BA.1 or BA.2 biotinylated RBD protein conjugated with TotalSeq™-C0971 Streptavidin (BioLegend, 405271) and TotalSeq™-C0972 Streptavidin (BioLegend, 405273), WT biotinylated RBD protein conjugated with TotalSeq™-C0973 Streptavidin (BioLegend, 405275) and TotalSeq™-C0974 Streptavidin (BioLegend, 405277) and biotinylated Ovalbumin (Sino Biological) conjugated with TotalSeq™-C0975 Streptavidin (BioLegend, 405279) were added. After incubation and washing steps, 5 μl of 7-AAD (Invitrogen, 00-6993-50) was included for dead cell exclusion.

Cells negative for 7-AAD, IgM and IgD, but positive for CD20, CD27 and BA.1 or BA.2 were

sorted using a MoFlo Astrios EQ Cell Sorter (Beckman Coulter). FACS data were collected by Summit 6.0 (Beckman Coulter) and analyzed using FlowJo v10.8 (BD Biosciences).

The sorted B cells were processed using the Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1 according to the manufacturer's user guide (10x Genomics, CG000208). Briefly, the cells were resuspended in PBS after centrifugation and then processed to obtain gel beads-in-emulsion (GEMs) using the 10X Chromium controller. The GEMs were subjected to reverse transcription and the products were further purified with a GEM-RT clean up procedure. Preamplification was then performed on the products which were subsequently purified using the SPRIselect Reagent Kit (Beckman Coulter, B23318). The paired V(D)J BCR sequences were enriched with 10X BCR primers, followed by library preparation. Finally, the libraries were sequenced using the Novaseq 6000 platform, running either the Novaseq 6000 S4 Reagent Kit v1.5300 cycles (Illumina, 20028312) or the NovaSeq XP 4-Lane Kit v1.5 (Illumina, 20043131).

10X Genomics V(D)J sequencing data were assembled as BCR contigs and aligned using the Cell Ranger (v6.1.1) pipeline according to the GRCh38 BCR reference. To ensure high quality, only the productive BCR contigs and cells with one heavy chain and one light chain were retained. The IgBlast program (v1.17.1) was utilized to identify and annotate the germline V(D)J genes. The Change-O toolkit (v1.2.0) was employed to detect somatic hypermutation sites in the variable domain of the antibodies.

For expression optimization in human cells, heavy and light chain genes were synthesized by GenScript, inserted separately into plasmids (pCMV3-CH, pCMV3-CL or pCMV3-CK) via infusion (Vazyme, C112), and co-transfected into Expi293F cells (Thermo Fisher, A14527) using polyethylenimine transfection. The cells were cultured at 36.5°C in 5% CO(2) and 175 r.p.m. for 6-10 days. The cell expression fluid was collected and centrifuged. After centrifugation, supernatants containing the monoclonal antibodies were purified using protein A magnetic beads (L00695, Genscript). The purified samples were determined by SDS-PAGE.

Pseudovirus-neutralization assay

Codon-optimized SARS-CoV-2 S gene was inserted into the pcDNA3.1 vector to construct plasmids encoding the spike proteins of SARS-CoV-2 and G*ΔG-VSV was amplified before production of SARS-CoV-2 pseudotyped virus. The 293T cell line (ATCC, CRL-3216) was infected with G*ΔG-VSV virus (VSV G pseudotyped virus, Kerafast) and transfected with the spike protein-expressing plasmids simultaneously. After culturing, the pseudovirus-containing supernatant was collected, filtered, aliquoted, and frozen at −80 °C for future use. Pseudovirus-neutralization assays were conducted on the Huh-7 cell line (Japanese Collection of Research Bioresources (JCRB), 0403).

Monoclonal antibodies or plasma were serially diluted in DMEM (Hyclone, SH30243.01) and incubated with pseudovirus in 96-well plates at 5% CO(2) and 37°C for 1 h. Digested Huh-7 cell (JCRB, 0403) or 293T-hACE2 cells (ATCC, CRL-3216) were seeded and cultured for 24h. Half of the supernatant was then discarded and D-luciferin reagent (PerkinElmer, 6066769) was added to react in the dark. The luminescence value was detected using a microplate spectrophotometer (PerkinElmer, HH3400). IC50 was determined by a four-parameter logistic regression model using PRISM (version 9.0.1).

ELISA

ELISA assays were conducted by pre-coating ELISA plates with RBD (SARS-CoV-2 wild type, SARS-CoV-2 BA.1, SARS-CoV-2 BA.2 RBD, Sino Biological) at concentrations of 0.03 μg ml(−1)and 1 μg ml(−1) in PBS overnight at 4 °C. The plates were then washed and blocked, after which 100 μl of 1 μg ml(−1) antibodies were added to each well and incubated at room temperature for 2 hours. Following incubation, the plates were washed and incubated with 0.25 μg ml(−1) Peroxidase-conjugated AffiniPure goat anti-human IgG (H+L) (JACKSON, 109-035-003) for 1 hour at room temperature. The reaction was developed using tetramethylbenzidine (TMB) (Solarbio, 54827-17-7), and stopped by adding $H_{(2)}SO_{(4)}$. The absorbance was measured at 450 nm using a microplate reader (PerkinElmer, HH3400) and the negative control used was the H7N9 human IgG1 antibody HG1K. (Sino Biological, HG1K).

DMS Library construction

Duplicate single site saturated mutant libraries spanning all 201 amino acids of BA.5 RBD (position N331-T531 by Wuhan-Hu-1 reference numbering) were constructed based on previously reported method[1], in order to ensure the reproducibility and reliability of results. A unique N26 barcode was PCR appended to each RBD variant as an identifier, and the correspondence of variant and N26 barcode was obtained by PacBio sequencing on Sequel ll platform in Peking University throughput sequencing center (HTSC). The BA.5 RBD mutant libraries were assembled into pETcon 2649 vector and amplified in DH10B cells. Above plasmids products were then transformed into Saccharomyces cerevisiae EBY100. Yeasts were screened on SD-CAA plates and further enlarged in SD-CAA liquid media, the resulted libraries were preserved at -80°C after flash frozen in liquid nitrogen.

MACS-based mutation escape profiling

The high-throughput mutation escape profiling for every single antibody was performed as previously described [7,26]. Briefly, unexpressed and non-functional RBD variants were first eliminated from BA.5 mutant libraries by magnetic-activated cell sorting (MACS). The selected yeasts were inoculated into SG-CAA media to induce RBD surface expression by overnight culture. To capture yeast cells that escape specific antibody binding, two rounds of sequential negative selection and one round of positive selection were carried out based on MACS. After overnight amplification, plasmids were extracted from the sorted yeasts using the 96 Well Plate Yeast Plasmid Preps Kit (Coolaber, PE053), then used as template for N26 barcode amplification by PCR. Final PCR products were purified, quantified, and sequenced on Nextseq 550 or MGISEQ-2000 platform.

DMS data analysis and antibody clustering

DMS raw sequencing data were processed as described previously [7,26]. In brief, the detected barcode sequences of both the antibody-screened and reference library were aligned to the barcode-variant dictionary generated using dms_variants (v0.8.9) from PacBio sequencing data of the BA.5 DMS library. Only barcodes that are detected more than 5 times in the reference library are included in the calculation to avoid large sampling error. The escape scores of a variant X that are detected both in the screened and reference library were defined as $F\times(n_{X,ab} / N_{ab}) / (n_{X,ref} / N_{ref})$, where F is a scale

factor to normalize the scores to the 0-1 range, while n and N are the number of detected barcodes for variant X and total barcodes in antibody-screened (ab) or reference (ref) samples, respectively. To assign an escape score to each single substitution on RBD, an epistasis model is fitted using dms_variants (v0.8.9) as described previously [42,43]. For antibodies with multiple replicates of DMS, the final escape score of each mutation is the average over all replicates.

We used graph-based unsupervised clustering and embedding to assign an epitope group for each antibody and visualize them in a two-dimensional space. First, site escape scores (the sum of mutation escape scores on a residue) of each antibody are first normalized to a sum of one and considered as a distribution over RBD residues. The dissimilarity of two antibodies is defined by the Jessen-Shannon divergence of the normalized escape scores. Pair-wise dissimilarities of all antibodies in the dataset are calculated using the SciPy module (scipy.spatial.distance.jensenshannon, v1.7.0). Then, a 12-nearest-neighbor graph is built using python-igraph module (v0.9.6). Leiden clustering is performed to assign a cluster to each antibody [44]. The name of each cluster is annotated manually based on the featured sites on the average escape profiles of a cluster to make it consistent with the definition of our previously published DMS dataset using WT-based library in general[7]. To project the dataset onto a 2D space for visualization, we performed UMAP based on the constructed k-nearest-neighbor graph using umap-learn module (v0.5.2). Figures were generated by R package ggplot2 (v3.3.3).

Estimate the preference of RBD mutations

Similar to the approach in our previous study [7], we incorporated four types of weights in our calculations to account for the impact of each mutation on hACE2-binding affinity, RBD expression, neutralizing activity, and the codon constraints on each residue. The weights for ACE2 binding and RBD expression are determined by $\tanh(S_{\mathrm{bind}}) + 1$ and $\tanh\left(\min\left(0, S_{\mathrm{expr}}\right)\right) + 1$, respectively, where the $\mathrm{S}_{\mathrm{bind}}$ and $\mathrm{S}_{\mathrm{expr}}$ values are from the BA.2-based DMS on ACE2 binding and RBD expression [45]. The function $\tanh(x)$ is employed as a sigmoidal curve to constrain the weights between 0 and 2. For codon constraint weights, mutations that cannot be accessed through single nucleotide mutation are first assigned a weight of zero. To address the intrinsic disparities in the frequency of distinct nucleotide substitutions in SARS-CoV-2, we assign different weights for

759 mutations corresponding to various nucleotide substitutions [46]. Specifically, the weight of the most
760 frequent substitution (C>T) is assigned a value of 0.1, while weights for G>T and G>A are 0.041
761 and 0.035, respectively. To retain the potential of rare mutations, all other substitutions are assigned
762 a weight of 0.03. We use BA.4/5 (EPI_ISL_11207535) and XBB.1.5 (EPI_ISL_17054053) to define
763 weights for codon usage. Regarding the neutralizing activities, the weight is calculated as -
764 $\log_{10}(IC_{50})$. IC_{50} values (μg/mL) less than 0.0005 or greater than 1.0 are considered as 0.0005 or 1.0,
765 respectively. As the dataset specifically enriches for Omicron-specific antibodies, potentially
766 introducing bias when estimating mutation preferences. An additional weighting strategy is applied
767 that assigns higher weights to cross-reactive mAbs, resulting in 89% cross-reactive mAbs for
768 BA.5/BF.7 BTI cohorts and 51% for reinfection cohorts, as determined by unbiased characterization
769 of mAbs using ELISA. The raw escape scores for each antibody are first normalized by the
770 maximum score among all mutants. The weighted score for each antibody and each mutation is
771 obtained by multiplying the normalized scores with the corresponding four weights, and the final
772 mutation-specific weighted score is the sum of scores for all antibodies in the designated set, which
773 is then normalized once more to produce a value between 0 and 1. To visualize the calculated escape
774 maps, sequence logos were created using the Python module logomaker (v0.8).

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Author contributions

Y.C. designed and supervised the study. A.Y., F.J., W.S., Q.G., X.S.X, and Y.C. wrote the manuscript with inputs from all authors. A.Y., W.S., S.Y., R.A., Yao W., and X.N. performed B-cell sorting, single-cell VDJ sequencing, and antibody sequence analyses. J.W. (BIOPIC), F.J., H.S. and L.Z. performed and analyzed the DMS data. Y.Y. and Youchun W. constructed the pseudotyped virus. N.Z., P.W., L.Y., T.X. and F.S. performed the pseudotyped virus neutralization assays. W.S. and A.Y. analyzed the neutralization data. Y.X., X.C., Z.S. and R.J. recruited the SARS-CoV-2 vaccinees and convalescents. J.W. (Changping Laboratory), L.Y. and F.S. performed the antibody expression.

Conflicts of interest

X.S.X. and Y.C. are inventors on the provisional patent applications of BD series antibodies, which include BD55-5514 (SA55) and mAbs from Omicron infection convalescents. X.S.X. and Y.C. are founders of Singlomics Biopharmaceuticals. Other authors declare no competing interests.

Data and code availability

Information of SARS-CoV-2 RBD-targeting mAbs is included in Supplementary Table 2. Processed mutation escape scores, and custom scripts for processing and analyzing DMS data can be downloaded at https://github.com/jianfcpku/SARS-CoV-2-reinfection-DMS. Raw sequencing data of DMS are available on China National GeneBank with Project accession CNP0004294. PDB 7XNS is used for the structural model of SARS-CoV-2 RBD.

Extended Data Figure Legends

Extended Data Fig. 1 | Neutralizing antibody response after CoronaVac primary and spike protein booster

a, Comparison of neutralizing antibody responses among mouse immunization groups against pseudotyped D614G, SARS, and additional SARS-CoV-2 variants. Mice sera antibody titers after priming with two doses of 3 μg CoronaVac followed by boosting with 10 μg SARS-CoV-1 Spike protein or SARS-CoV-2 variant Spike proteins in mice, or no boosting were measured. Fold changes between groups receiving different boosters and the primary-only group were calculated and shown above the line. Statistical significance was determined using the Wilcoxon rank sum test. **b**, Antibody breadth after priming with CoronaVac followed by boosting with different Spike proteins or no boosting. The neutralizing titer data used is the same as in a. Fold changes between titers against SARS or SARS-CoV-2 variants, and D614G were calculated and shown above the line. Statistical significance was determined using the Wilcoxon signed-rank test. *$p < 0.05$, **$p < 0.01$, ***$p<0.001$, ****$p<0.0001$, and not significant (NS) $p > 0.05$.

Extended Data Fig. 2 | Neutralizing antibody response with mRNA vaccine as primary or booster

a, Comparison of neutralizing antibody responses among mouse immunization groups against pseudotyped D614G, SARS, and additional SARS-CoV-2 variants. Mice sera antibody titers after priming with 2 doses of 10 μg WT-mRNA followed by boosting with 10 μg SARS-CoV-2 variant Spike proteins in mice, or no boosting were measured. Fold changes between groups receiving different boosters and the primary-only group were calculated and shown above the line. Statistical significance was determined using the Wilcoxon rank sum test. **b**, Antibody breadth after priming with WT-mRNA followed by boosting with SARS-CoV-2 variant Spike proteins in mice, or no boosting. The neutralizing titer data used is the same as in a. Fold changes between titers against SARS or SARS-CoV-2 variants, and D614G were calculated and shown above the line. Statistical significance was determined using the Wilcoxon signed-rank test. Fold changes between titers against SARS or SARS-CoV-2 variants and D614G were calculated and shown above the line.

Statistical significance was determined using the Wilcoxon signed-rank test. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05.

Extended Data Fig. 3 | Comparison of neutralizing antibody responses with different intervals between the first and second Spike protein boosters.

a, Comparison of neutralizing antibody responses among different groups of mice immunized with 2 doses of CoronaVac, followed by two BA.5/BQ.1.1/XBB Spike protein boosters administered with one-month or three-month intervals between the first and second boosters, and compared to the primary immunization. Statistical significance was determined using the Wilcoxon rank sum test. **b,** Antibody breadth after priming with CoronaVac followed by boosting with 2 doses of BA.5/BQ.1.1/XBB Spike proteins with a one-month interval between the first and second boosters. Statistical significance was determined using the Wilcoxon signed-rank test. **c,** Antibody breadth after priming with 2 doses of BA.5/BQ.1.1/XBB Spike proteins. Statistical significance was determined using the Wilcoxon signed-rank test. **d,** Antibody breadth after priming with CoronaVac followed by boosting with 2 doses of BA.5/BQ.1.1/XBB Spike proteins with a three-month interval between the first and second boosters. Statistical significance was determined using the Wilcoxon signed-rank test. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05.

Extended Data Fig. 4 | Comparison of neutralizing antibody responses with different intervals between primary vaccination and the first booster.

a, Comparison of neutralizing antibody responses among different groups of mice immunized with 2 doses of CoronaVac, followed by two additional doses of SARS-CoV-2 Spike protein vaccine at different intervals. Statistical significance was determined using the Wilcoxon signed-rank test. **b-c,** Comparison of neutralizing antibody responses among different groups of mice immunized with 2 doses of CoronaVac, followed by one dose of BQ.1.1(b) or XBB(c) Spike protein vaccine at different intervals. Statistical significance was determined using the Wilcoxon rank sum test. **d-e,** Comparison of neutralizing antibody responses among different groups of mice immunized with 2 doses of CoronaVac, followed by two additional doses of BQ.1.1(d) or XBB(e) Spike protein vaccine at different intervals. Statistical significance was determined using the Wilcoxon rank sum

882 test. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05.

883

884 **Extended Data Fig. 5 | Comparison of neutralizing antibody responses with different mRNA**
885 **vaccine booster dosages.**

886 **a-b,** Antibody breadth after priming with 2 doses of 3 μg CoronaVac followed by boosting with 1
887 dose of 1μg (a) or 10μg (b) BA.5/BQ.1.1/XBB Spike proteins in mice. Statistical significance was
888 determined using the Wilcoxon signed-rank test. **c-d,** Antibody breadth after priming with 2 doses
889 of 3 μg CoronaVac followed by boosting with 2 doses of 1μg (c) or 10μg (d) BA.5/BQ.1.1/XBB
890 Spike proteins in mice. Statistical significance was determined using the Wilcoxon signed-rank test.
891 **e,** Comparison of neutralizing antibody responses among different groups of mice immunized with
892 2 doses of CoronaVac, followed by 2 doses of BA.5/BQ.1.1/XBB mRNA boosters at different doses
893 and compared to the primary immunization. Statistical significance was determined using the
894 Wilcoxon rank sum test. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS)
895 p > 0.05.

896

897 **Extended Data Fig. 6 | Antibody breadth of plasma and nasal swab after repeated Omicron**
898 **infections.**

899 **a-c**, Plasma antibody titers against pseudotyped D614G and variants after (a) BA.1 BTI + BA.5/BF.7
900 infection (n = 26), (b) BA.2 BTI + BA.5/BF.7 infection (n = 19), (c) BA.1/BA.2 + BA.5 infection
901 (n = 12). Fold changes between titers against variants and D614G were calculated and shown above
902 the line. Statistical significance was determined using the Wilcoxon signed-rank test.

903 **d-f**, Nasal swab antibody titers against pseudotyped D614G and variants after (d) BA.1 BTI +
904 BA.5/BF.7 infection (n = 26), (e) BA.2 BTI + BA.5/BF.7 infection (n = 19), (f) BA.1/BA.2 + BA.5
905 infection (n = 12). Fold changes between titers against variants and D614G were calculated and
906 shown above the line. Statistical significance was determined using the Wilcoxon signed-rank test.
907 *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05.

908

909 **Extended Data Fig. 7 | Characteristics of BA.5-reactive mAbs elicited by BA.5/BF.7 BTI or**
910 **reinfection.**

911 **a,** Source of the antibodies are projected onto the UMAP embedding space. Antibodies from BA.5

BTI (n=445), BF.7 BTI (n=243), BA.1 BTI with reinfection (n=284), and BA.2 BTI with reinfection (n=232) are colored blue in the corresponding panel, and other antibodies are gray. **b**, Neutralization activities, denoted as IC50 values, against SARS-CoV-2 BA.1 (n = 1260), BA.2 (n = 1238), BA.2.75 (n=1238), BQ.1.1 (n = 1335) and XBB (n = 1341) spike-pseudotyped VSV are projected onto the UMAP embedding space. **c**, Average escape scores of epitope groups that are not shown in Fig. 4d (C/D1, D2, E1/E2.1, E2.2, E3, and F1) are illustrated on the structure model of the SARS-CoV-2 BA.5 RBD (PDB: 7XNS). Key residues with high escape scores for each group are labeled. **d**, Average DMS escape scores for these epitope groups are represented as sequence logos; residues are depicted using the standard one-letter code and colored based on their chemical properties. The height of each letter corresponds to the escape score of the respective mutation. **e**, Pseudovirus-neutralization activities of XBB.1.5-neutralizing mAbs in groups A1 (n=70) and A2 (n=23) against XBB.1.5 and XBB.1.5.10; and mAbs in groups B (n=15) and C/D1 (n=13) against XBB.1.5 and XBB.1.16. Fold changes in IC50 are labeled. P-values are calculated using two-tailed Wilcoxon signed-rank test of paired samples. **f,** Pseudovirus-neutralization activities of mAbs within the six crucial epitope groups (C/D1 [n = 76], D2 [n = 86], E1/E2.1 [n = 100], E2.2 [n = 124], E3 [n = 101], and F1 [n = 236]) are shown against SARS-CoV-2 D614G, BA.5, BQ.1.1, and XBB.1.5. Geometric mean IC50 values are displayed as bars and labeled above each group of data points.

Extended Data Fig. 8 | Workflow of calculating weighted escape scores of each mutation on RBD.

Weights for ACE2 binding and RBD expression, neutralization activity, and codon usage are sequentially applied on the calculation to achieve informative results. Mutation preferences of BA.5 RBD under the pressure of NAbs from BA.5 or BF.7 BTI are shown.

Extended Data Fig. 9 | SPR sensorgrams for affinity of hACE2 and SARS-CoV-2 mutants RBD

Representative sensorgram of at least four replicates is shown for each RBD. Geometric mean kinetic constants k_a, k_d, and dissociation equilibrium constant K_D are labeled in each panel.

Extended Data Fig. 10 | NAbs from BTI and reinfection are escaped by constructed mutants

a, IC50 values for representative potent XBB.1.5-neutralizing antibodies from different epitope groups against XBB.1.5 variants carrying individual or multiple escape mutations are shown. The order of antibodies is the same as that in Fig. 6c. **b**, Pseudovirus NT50 for SARS-CoV-2 XBB.1.5-based mutants are shown using plasma from convalescent individuals who experienced BA.5 (n=36) or BF.7 BTI (n = 30). Statistical tests are performed between neighboring mutants. P-values are calculated using two-tailed Wilcoxon signed-rank tests on paired samples. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, and $p > 0.05$ (NS).

Figure 1

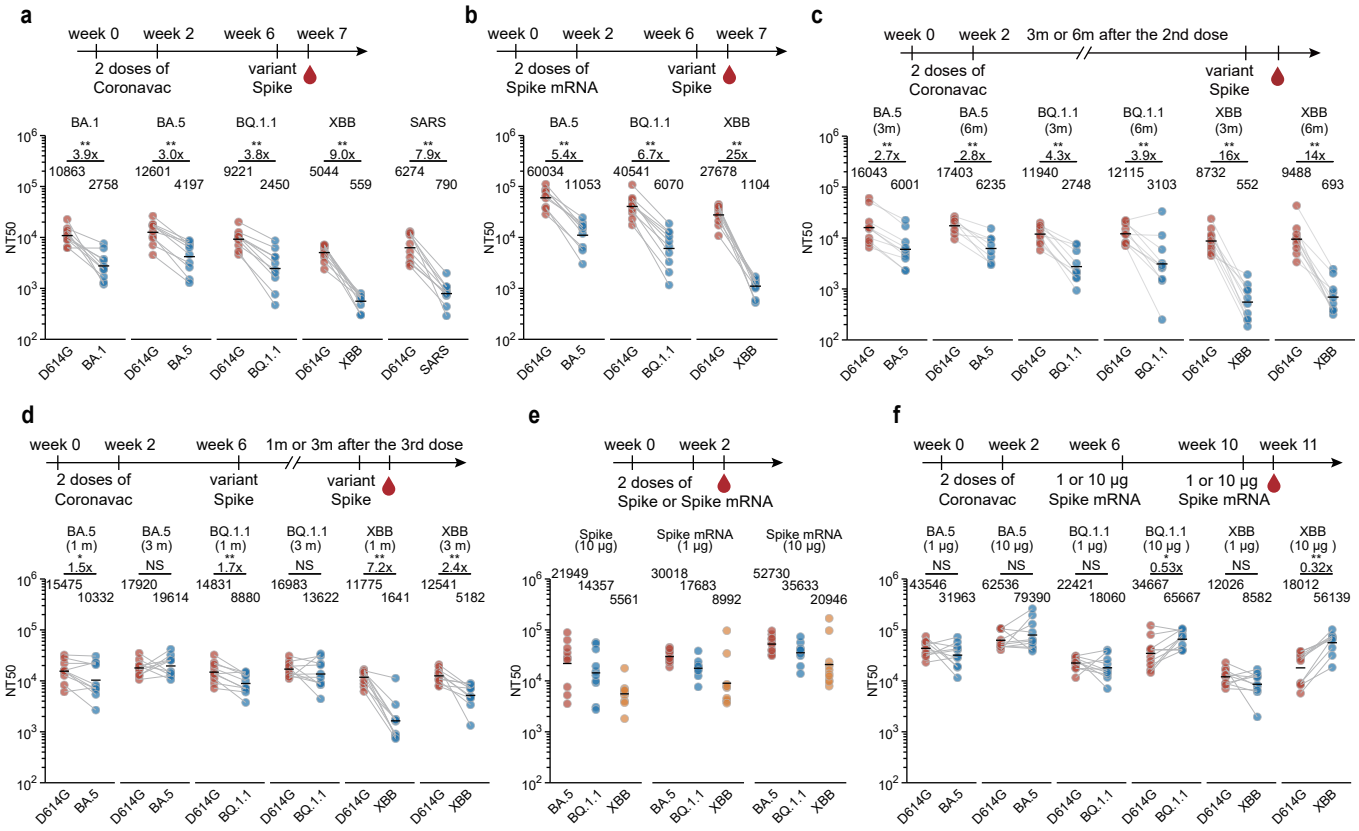


Fig.1| Humoral immune imprinting in mice.

a, Neutralizing antibody response after priming with 2 doses of 3 μg CoronaVac followed by boosting with 10 μg SARS-CoV-1 Spike protein or SARS-CoV-2 variant Spike proteins in mice. b, Neutralizing antibody response after priming with 2 doses of 10 μg WT-mRNA followed by boosting with 10 μg SARS-CoV-2 variant Spike proteins. c, Neutralizing antibody response after immunization with 2 doses of 3 μg CoronaVac followed by boosting with 10 μg SARS-CoV-2 variant Spike proteins with 3 months or 6 months time interval in mice. d, Neutralizing antibody response after priming with 2 doses of 3 μg CoronaVac followed by boosting twice with 10 μg SARS-CoV-2 variant Spike proteins with different time intervals in mice. Mice were boosted twice with intervals between boosters of either 1 month or 3 months. e, Neutralizing antibody response after priming with 2 doses of 10 μg variant Spike proteins or 1 μg or 10 μg variant Spike mRNAs. f, Neutralizing antibody response after priming with 2 doses of 3 μg CoronaVac followed by boosting twice with 1 μg or 10 μg of SARS-CoV-2 variant Spike mRNAs. 10 mice were immunized and analyzed in each group (n= 10). All Spike protein and mRNA used S6P prefusion-stabilization. The time interval between the two priming doses is 2 weeks. The interval between priming and boosting is 4 weeks. Sera were collected one week after the last dose. Geometric mean titers (GMT) were labeled. For paired samples in a-d and f, statistical significance were determined using two-tailed Wilcoxon signed-rank tests. For independent samples in e, statistical significance were determined using two-tailed Wilcoxon rank sum tests. *$p < 0.05$, **$p < 0.01$, and not significant (NS) $p > 0.05$. All neutralization assays were conducted in at least two independent experiments.

Figure 2

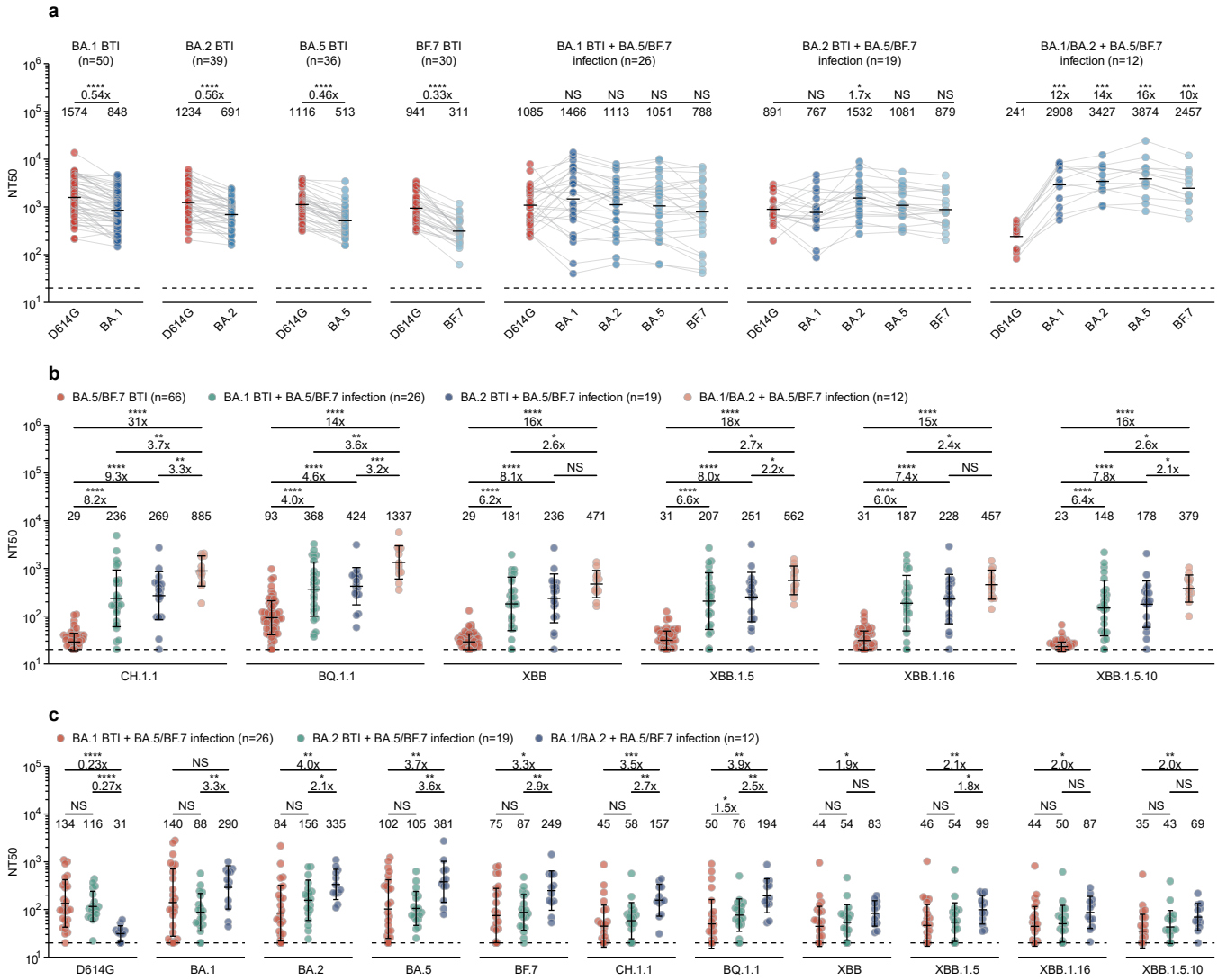


Fig.2| Humoral immune imprinting after repeated Omicron infections in human.
a, Examination of immune imprinting after Omicron breakthrough infections and repeated Omicron infections. Plasma antibody titers against pseudotyped D614G and variants were measured. Fold changes between titers against variants and D614G were calculated and shown above the line. Statistical significance was determined using the Wilcoxon signed-rank test. BA.1, BA.2, BA.5, BF.7 BTI: post-vaccination Omicron breakthrough infection (BTI). BA.1, BA.2 BTI+ BA.5/BF.7 infection: post-vaccination Omicron breakthrough infection followed by BA.5/BF.7 reinfection. BA.1/BA.2+ BA.5/BF.7 infection: BA.1/BA.2 infection followed by BA.5/BF.7 reinfection with no vaccination history. Blood samples were collected 1-2 months after the last infection. Detailed information about the cohorts is in Supplementary Table 1. b, Plasma antibody breadth after repeated Omicron infections. Plasma antibody titers against circulating pseudotyped variants were measured. Fold changes between titers of different cohorts were calculated and shown above the line. Statistical significance was determined using the Wilcoxon rank sum tests. c, Nasal swab antibody breath after repeated Omicron infections. Nasal swab antibody titers against pseudotyped variants were measured. Fold changes between titers of different cohorts were calculated and shown above the line. Statistical significance was determined using the Wilcoxon rank sum test. Geometric mean titers (GMT) are labeled in (a). Geometric mean ± SD are labeled in (b, c). Dashed lines indicate the limit of detection (LOD, NT50 = 20). *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05. All neutralization assays were conducted in at least two independent experiments.

Figure 3

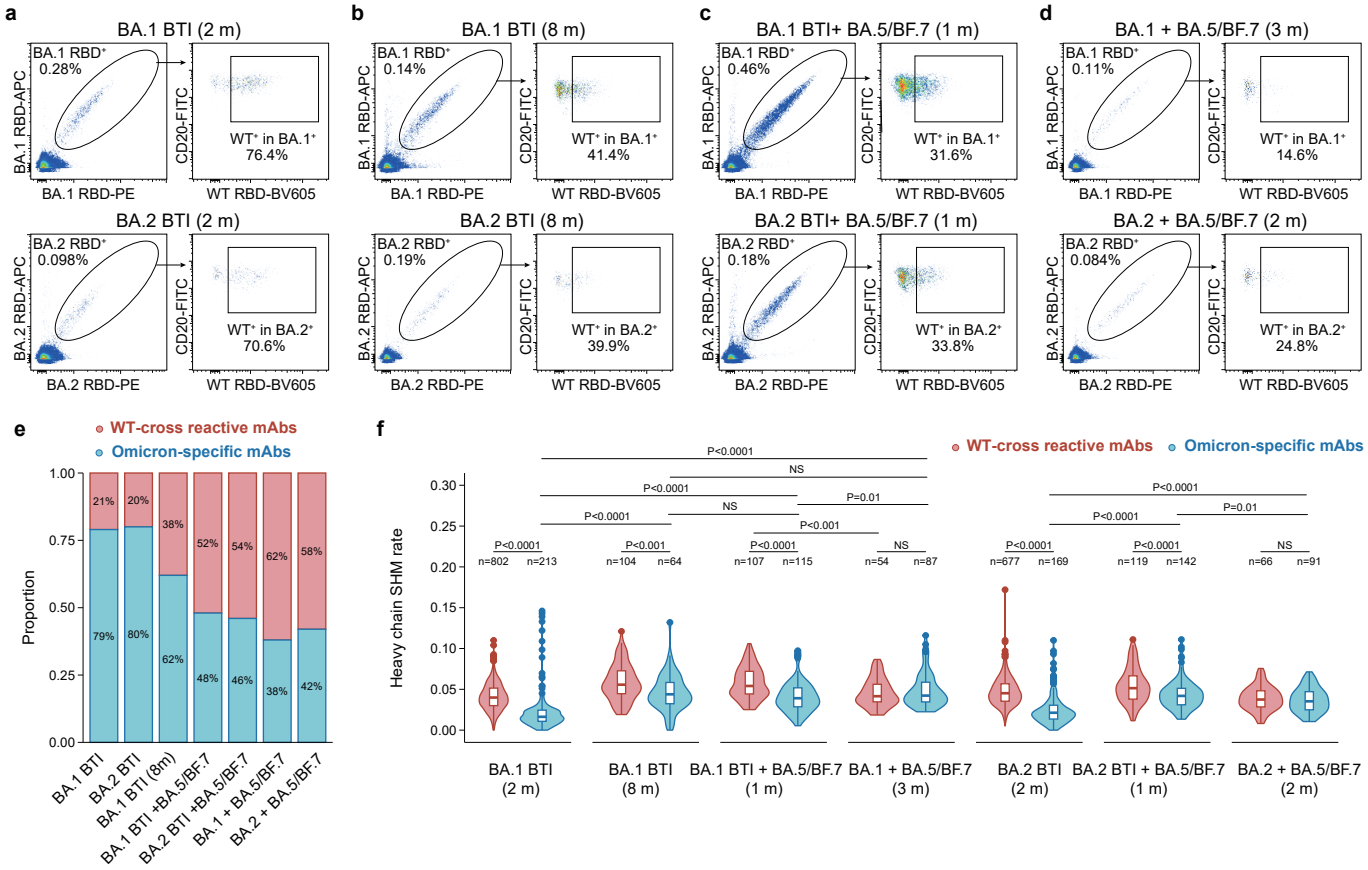


Fig.3 | B cell immune imprinting after repeated Omicron infections.
a–d, Flow cytometry analysis of pooled B cells from Omicron infection convalescent individuals. BA.1 (up) and BA.2 (down) RBD double-positive CD20+, IgM-, IgD-, CD27+ B cells were isolated for paired-single-cell V(D)J sequencing. Flow cytometry analyses were performed in cohorts of the following: (a) 2 months after BA.1 (up) or BA.2 (down) breakthrough infections, (b) 8 months after BA.1 (up) or BA.2 (down) breakthrough infections, (c) 1 month after BA.5/BF.7 reinfection after BA.1 (up) and BA.2 (down) breakthrough infections, (d) 2-3 months after BA.5/BF.7 reinfection after BA.1 (up) or BA.2 (down) infection without SARS-CoV-2 vaccination history. APC, allophycocyanine; FITC, fluorescein isothiocyanate; PE, phycoerythrin. BV605, Brilliant Violet 605. e, Proportions of WT-binding and non-WT-binding antibodies from Omicron breakthrough infection and repeated Omicron infection cohorts. Binding specificity was determined by ELISA. The antibodies were expressed in vitro using the sequence of the RBD-binding memory B cells from various cohorts. f, The heavy-chain variable domain somatic hypermutation (SHM) rate of the mAbs from various cohorts. Statistical tests were determined using two-tailed Wilcoxon rank-sum tests. Boxes display the 25th percentile, median and 75th percentile, and whiskers indicate median ± 1.5 times the interquartile range. Violin plots show kernel density estimation curves of the distribution. The numbers and ratios of samples in each group are labeled above the violin plots.

Figure 4

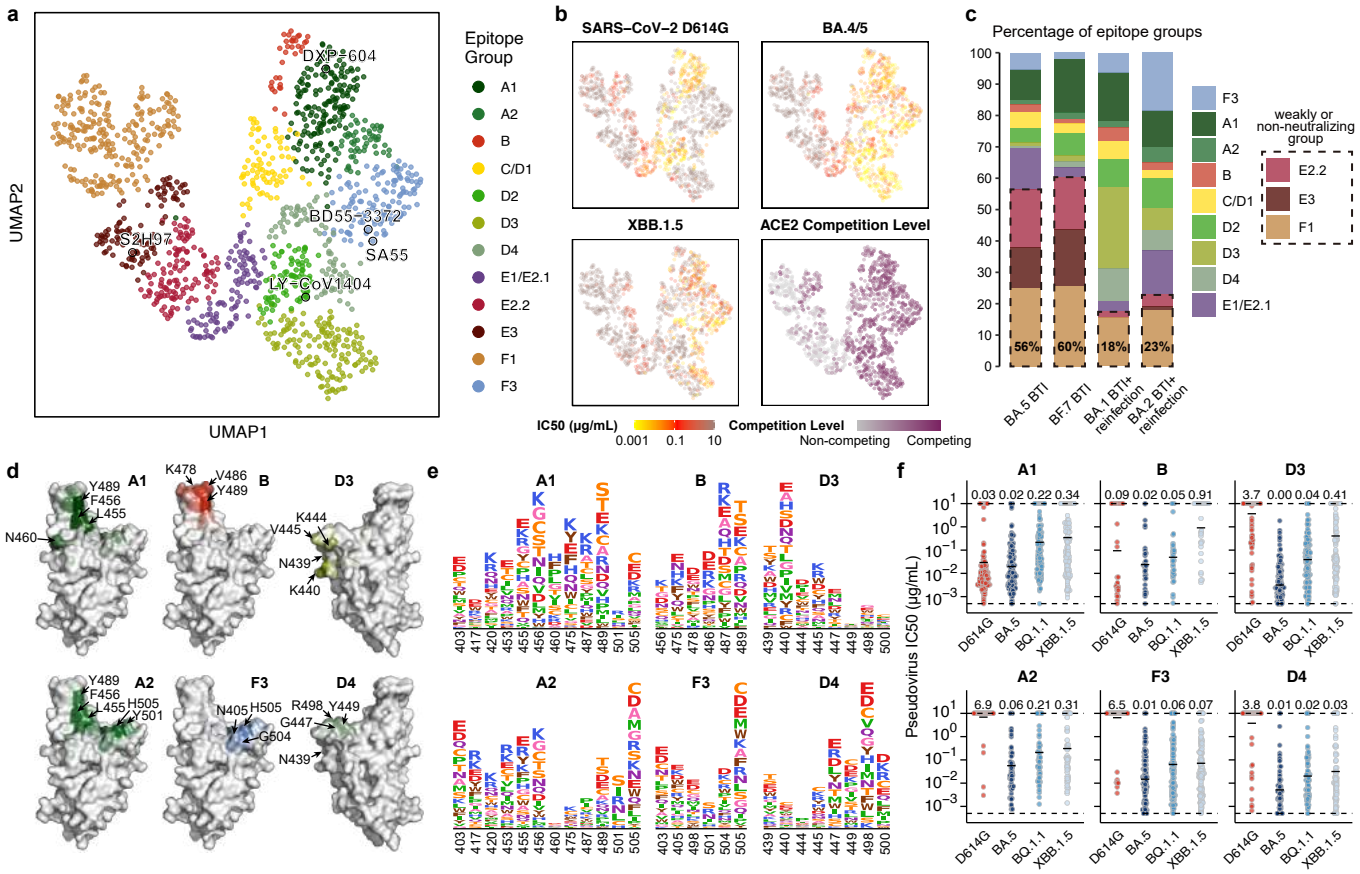


Fig. 4 | Epitope distribution and characterization of mAbs elicited by Omicron BTI and reinfection.
a, UMAP embedding of epitope groups of monoclonal antibodies (mAbs) binding BA.5 RBD isolated from convalescent individuals who experienced BA.5/BF.7 BTI or reinfection (n=1350). b, Neutralization activities, denoted as IC50 values, against SARS-CoV-2 D614G (n = 1349), BA.4/5 (n = 1322), and XBB.1.5 (n = 1346) spike-pseudotyped vesicular stomatitis viruses (VSV), as well as ACE2 competition levels determined by ELISA (n = 1344), are projected onto the UMAP embedding space. c, Distribution of mAbs across epitope groups is shown for BA.5 Breakthrough Infection (BTI), BF.7 BTI, BA.1 BTI with reinfection, and BA.2 BTI with reinfection. Epitope groups predominantly comprising non-neutralizing or weakly neutralizing mAbs (E2.2, E3, and F1) are highlighted with dashed boxes. The percentage of antibodies in these three groups is labeled on each bar. d, Average DMS escape scores of the crucial epitope groups contributing to neutralization against XBB.1.5 are illustrated on the structure model of the SARS-CoV-2 BA.5 RBD (PDB: 7XNS). Key residues with high escape scores for each group are labeled. e, The average DMS escape scores for the key epitope groups are represented as sequence logos; residues are depicted using the standard one-letter code and colored based on their chemical properties. The height of each letter corresponds to the escape score of the respective mutation. f, Pseudovirus-neutralization activities of mAbs within the six crucial epitope groups (A1 [n = 170], A2 [n = 60], B [n = 33], F3 [n = 129], D3 [n = 155], and D4 [n = 80]) are shown against SARS-CoV-2 D614G, BA.5, BQ.1.1, and XBB.1.5. Geometric mean IC50 values are displayed as bars and labeled above each group of data points.

Figure 5

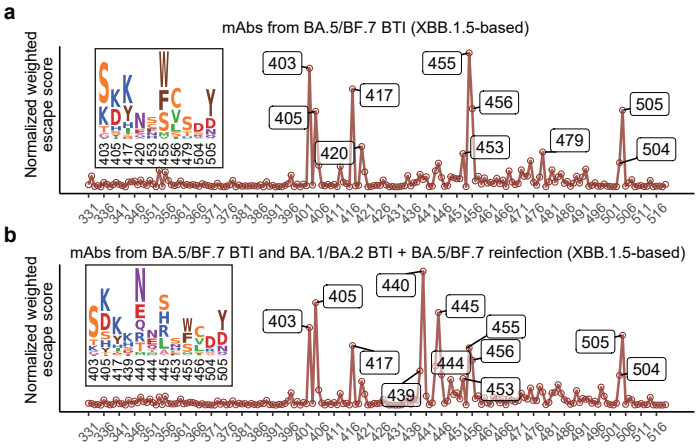


Fig. 5 | Estimate the evolutionary trends of XBB.1.5 RBD from DMS profiles.
Normalized average DMS escape scores weighted by IC50 against XBB.1.5 using DMS profiles of mAbs from BA.5/BF.7 BTI (a), and mAbs from BA.5/BF.7 BTI and BA.1/BA.2 BTI with BA.5/BF.7 reinfection (b). The impacts of each mutation on ACE2 binding and RBD expression, and the codon constraints on each residue, are also considered (see Methods). Residues with high estimated preferences are labeled, and their corresponding mutation scores are shown as logos.

Figure 6

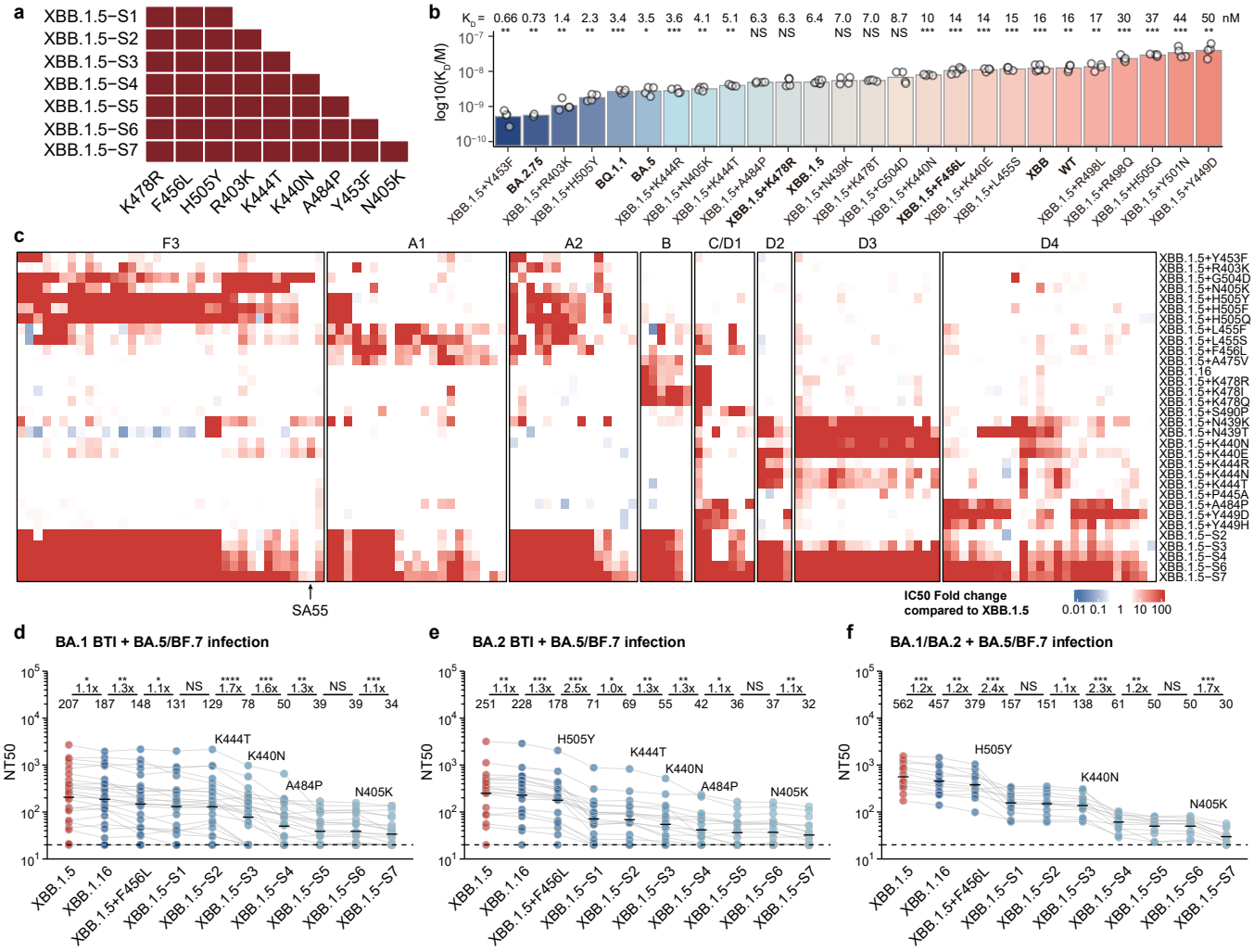
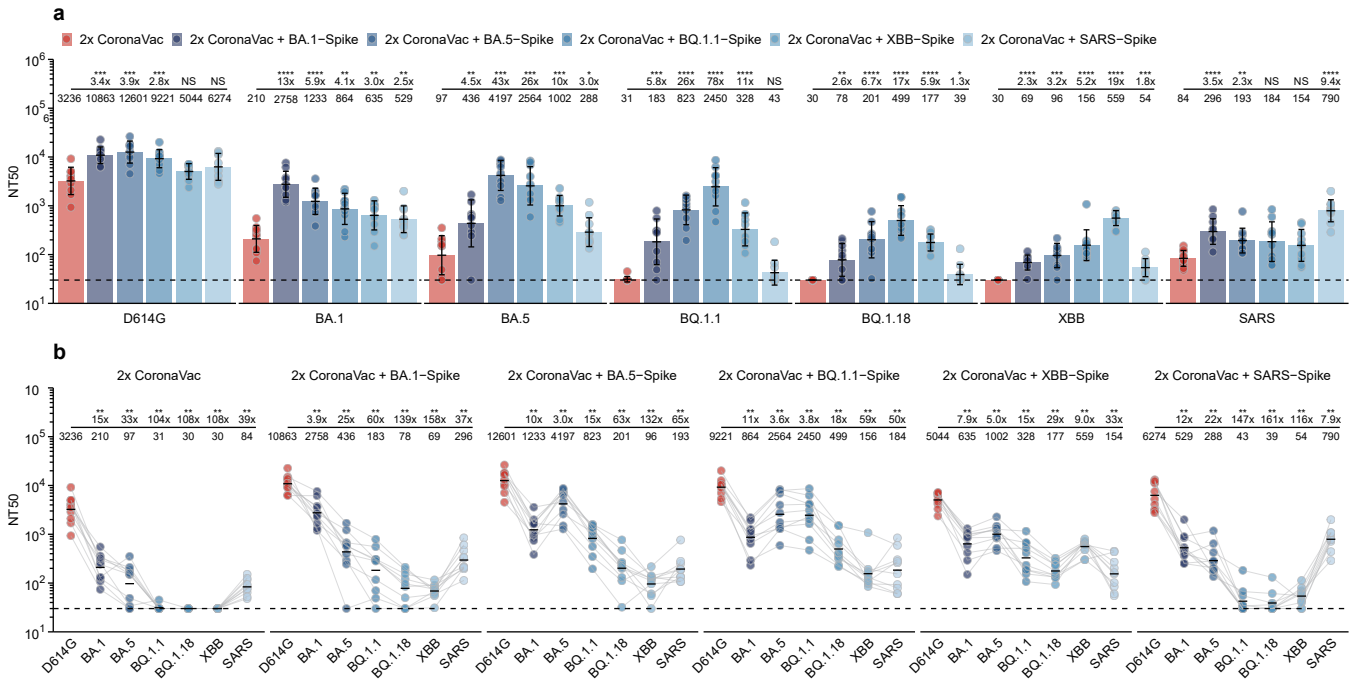


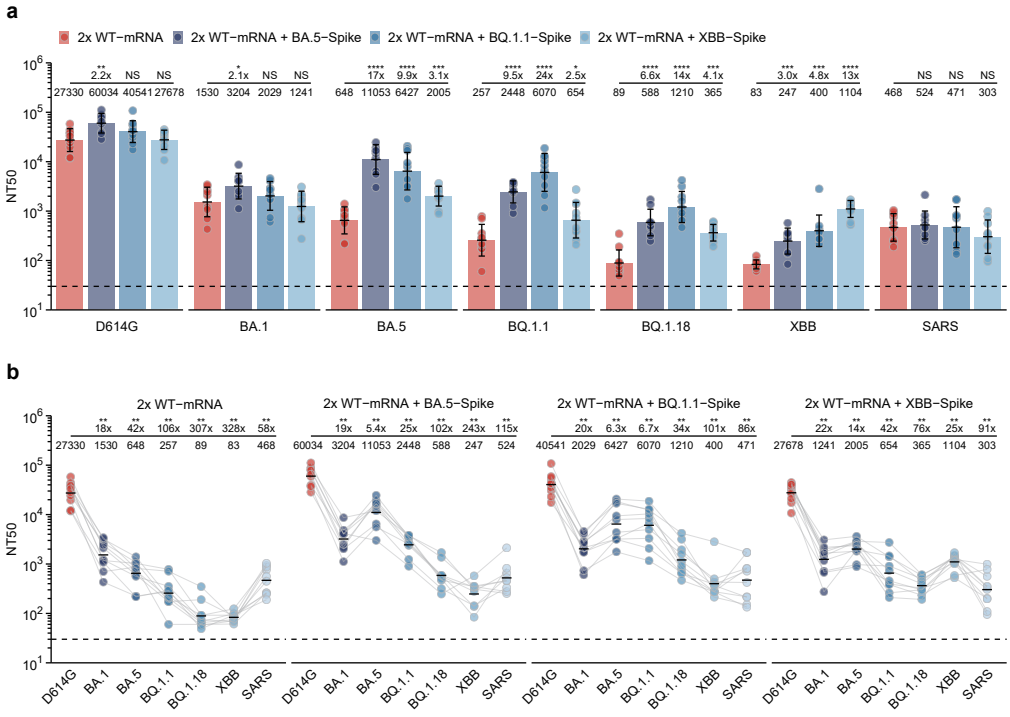
Fig. 6 | Combination of escape mutations evades XBB.1.5-neutralizing antibodies.
a, SARS-CoV-2 XBB.1.5-based pseudoviruses harboring combinations of critical mutations identified through analysis of DMS profiles are generated. b, hACE2-binding affinity for various RBD mutants of SARS-CoV-2 is assessed using SPR. Geometric mean dissociation constants (KD) from at least four independent replicates are shown, with statistical significance in comparison to XBB.1.5 RBD's KD labeled above the bars. P-values are determined using a two-tailed t-test on log-transformed KD values. c, IC50 values for representative potent XBB.1.5-neutralizing antibodies from different epitope groups against XBB.1.5 variants carrying individual or multiple escape mutations are displayed. Fold changes in IC50 against the mutants relative to XBB.1.5 are presented as a heatmap. d-f, Pseudovirus 50% neutralization titers (NT50) for SARS-CoV-2 XBB.1.5-based mutants are shown using plasma from convalescent individuals who experienced BA.5 or BF.7 reinfection: BA.1 BTI prior to BA.5/BF.7 reinfection (n = 26) (d); BA.2 BTI prior to BA.5/BF.7 reinfection (n = 19) (e); and reinfection with BA.5 or BF.7 after BA.1 or BA.2 infection without vaccination (n = 12) (f). Key mutations diminishing neutralization are labeled above their corresponding lines. Dashed lines indicate the limit of detection (LOD, NT50 = 20). Geometric mean titers are labeled above data points. Statistical tests are performed between neighboring mutants. P-values are calculated using two-tailed Wilcoxon signed-rank tests on paired samples. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05.

Extended Data Figure 1



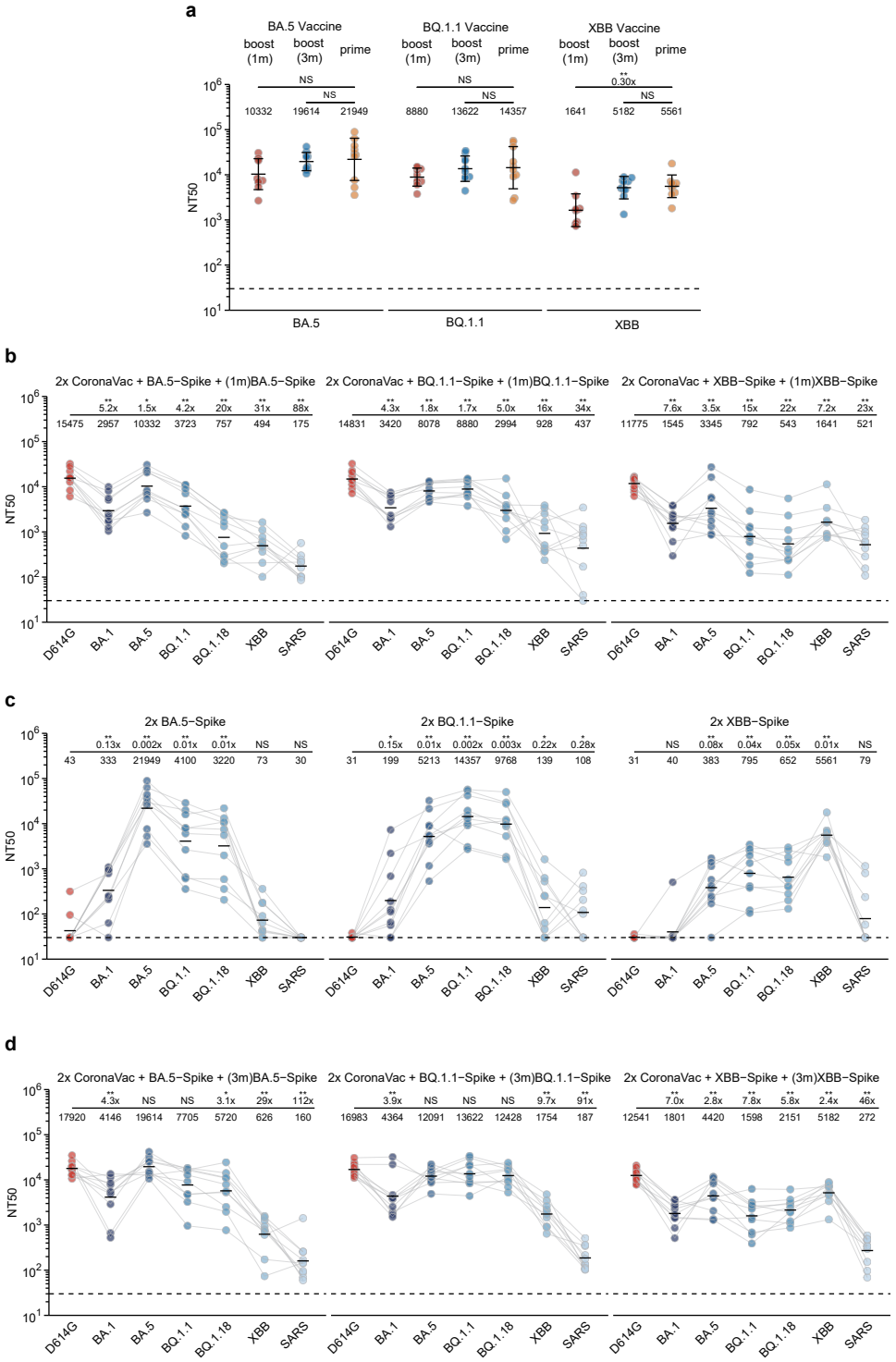
Extended Data Fig.1 | Neutralizing antibody response after CoronaVac primary and spike protein booster.
a, Comparison of neutralizing antibody responses among mouse immunization groups against pseudotyped D614G, SARS, and additional SARS-CoV-2 variants.. Mice sera antibody titers after priming with two doses of 3 μg CoronaVac followed by boosting with 10 μg SARS-CoV-1 Spike protein or SARS-CoV-2 variant Spike proteins in mice, or no boosting were measured. Fold changes between groups receiving different boosters and the primary-only group were calculated and shown above the line. Statistical significance was determined using the Wilcoxon rank sum test. b, Antibody breadth after priming with CoronaVac followed by boosting with different Spike proteins or no boosting. The neutralizing titer data used is the same as in a. Fold changes between titers against SARS or SARS-CoV-2 variants, and D614G were calculated and shown above the line. Statistical significance was determined using the Wilcoxon signed-rank test. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05.

Extended Data Figure 2



Extended Data Fig.2 | Neutralizing antibody response with mRNA vaccine as primary or booster.
a, Comparison of neutralizing antibody responses among mouse immunization groups against pseudotyped D614G, SARS, and additional SARS-CoV-2 variants. Mice sera antibody titers after priming with 2 doses of 10 μg WT-mRNA followed by boosting with 10 μg SARS-CoV-2 variant Spike proteins in mice, or no boosting were measured. Fold changes between groups receiving different boosters and the primary-only group were calculated and shown above the line. Statistical significance was determined using the Wilcoxon rank sum test. b, Antibody breadth after priming with WT-mRNA followed by boosting with SARS-CoV-2 variant Spike proteins in mice, or no boosting. The neutralizing titer data used is the same as in a. Fold changes between titers against SARS or SARS-CoV-2 variants, and D614G were calculated and shown above the line. Statistical significance was determined using the Wilcoxon signed-rank test. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05.

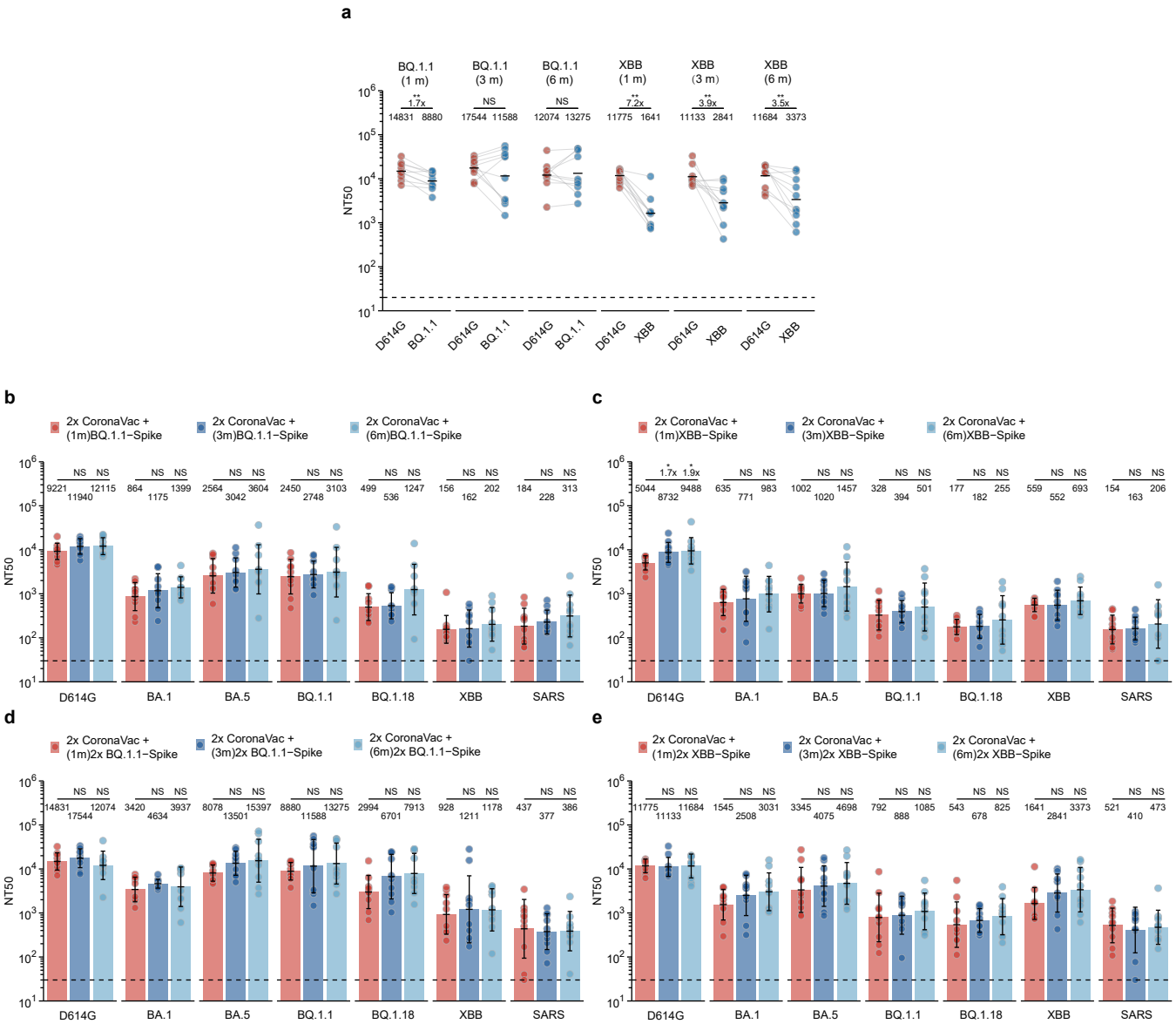
Extended Data Figure 3



Extended Data Fig.3 | Comparison of neutralizing antibody responses with different intervals between the first and second Spike protein boosters.

a, Comparison of neutralizing antibody responses among different groups of mice immunized with 2 doses of CoronaVac, followed by two BA.5/BQ.1.1/XBB Spike protein boosters administered with one-month or three-month intervals between the first and second boosters, and compared to the primary immunization. Statistical significance was determined using the Wilcoxon rank sum test. b, Antibody breadth after priming with CoronaVac followed by boosting with 2 doses of BA.5/BQ.1.1/XBB Spike proteins with a one-month interval between the first and second boosters. Statistical significance was determined using the Wilcoxon signed-rank test. c, Antibody breadth after priming with 2 doses of BA.5/BQ.1.1/XBB Spike proteins. Statistical significance was determined using the Wilcoxon signed-rank test. d, Antibody breadth after priming with CoronaVac followed by boosting with 2 doses of BA.5/BQ.1.1/XBB Spike proteins with a three-month interval between the first and second boosters. Statistical significance was determined using the Wilcoxon signed-rank test. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05.

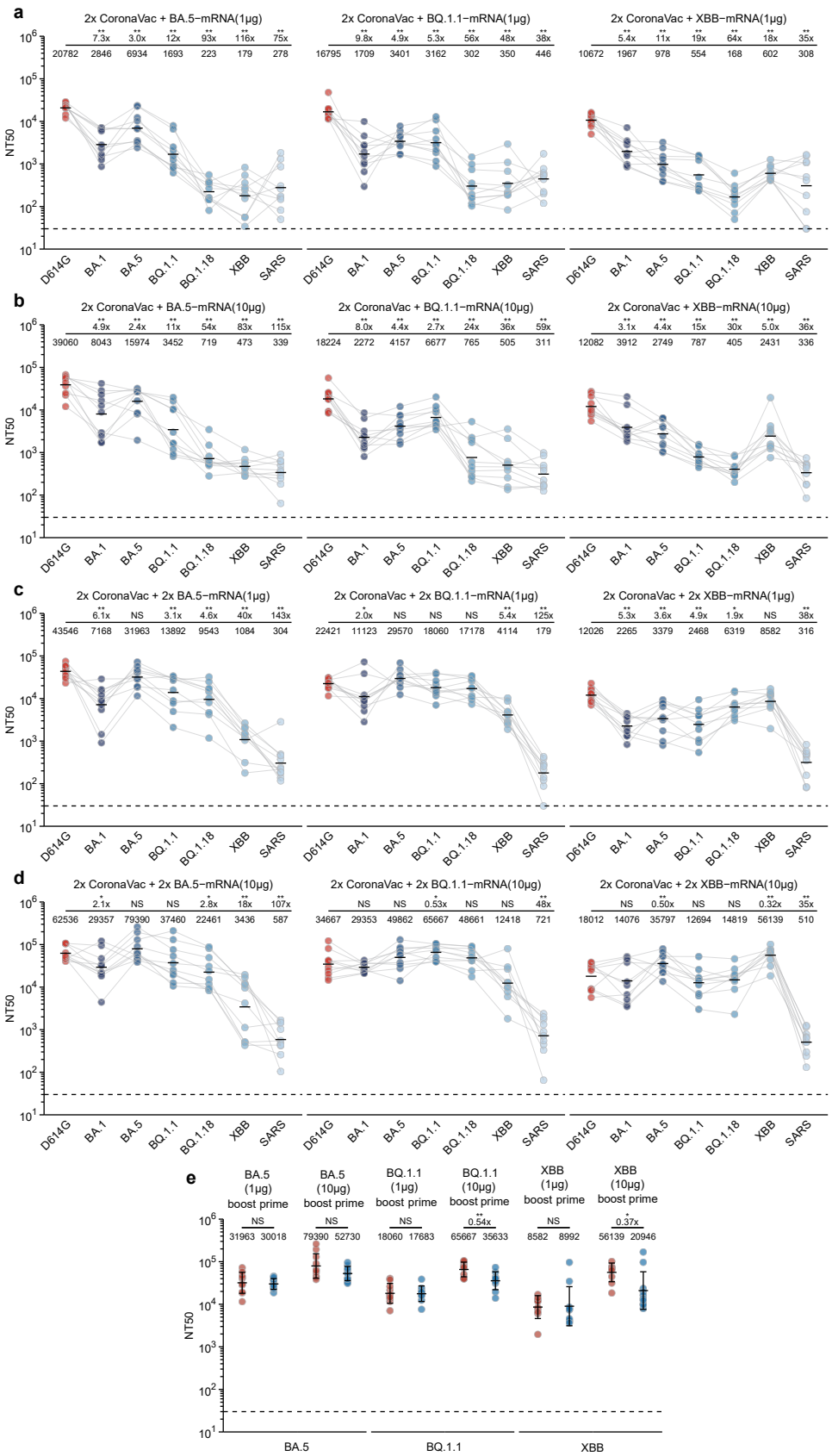
Extended Data Figure 4



Extended Data Fig.4 | Comparison of neutralizing antibody responses with different intervals between primary vaccination and the first booster.

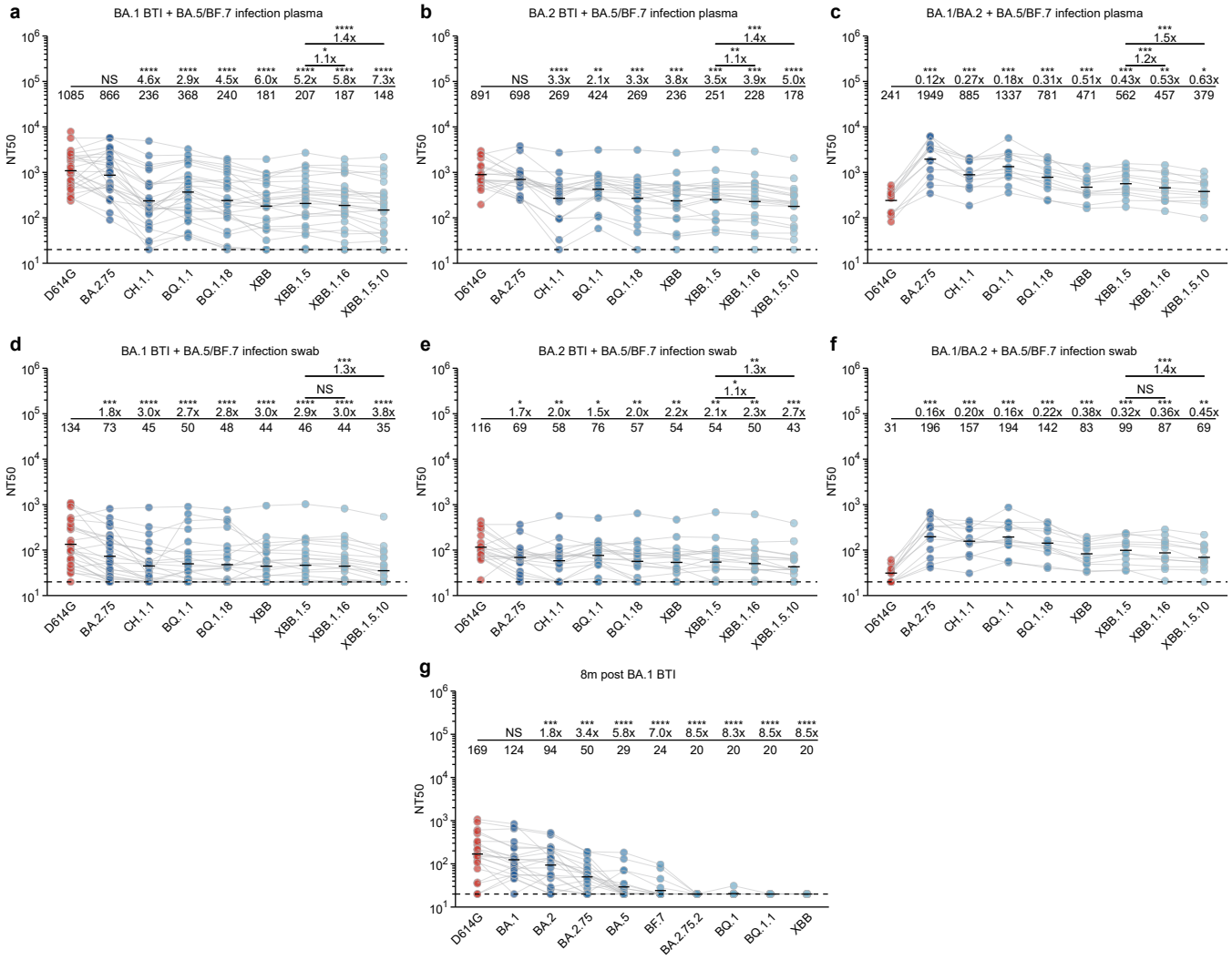
a, Comparison of neutralizing antibody responses among different groups of mice immunized with 2 doses of CoronaVac, followed by two additional doses of SARS-CoV-2 Spike protein vaccine at different intervals. Statistical significance was determined using the Wilcoxon signed-rank test. b-c, Comparison of neutralizing antibody responses among different groups of mice immunized with 2 doses of CoronaVac, followed by one dose of BQ.1.1(b) or XBB(c) Spike protein vaccine at different intervals. Statistical significance was determined using the Wilcoxon rank sum test. d-e, Comparison of neutralizing antibody responses among different groups of mice immunized with 2 doses of CoronaVac, followed by two additional doses of BQ.1.1(d) or XBB(e) Spike protein vaccine at different intervals. Statistical significance was determined using the Wilcoxon rank sum test. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05.

Extended Data Figure 5



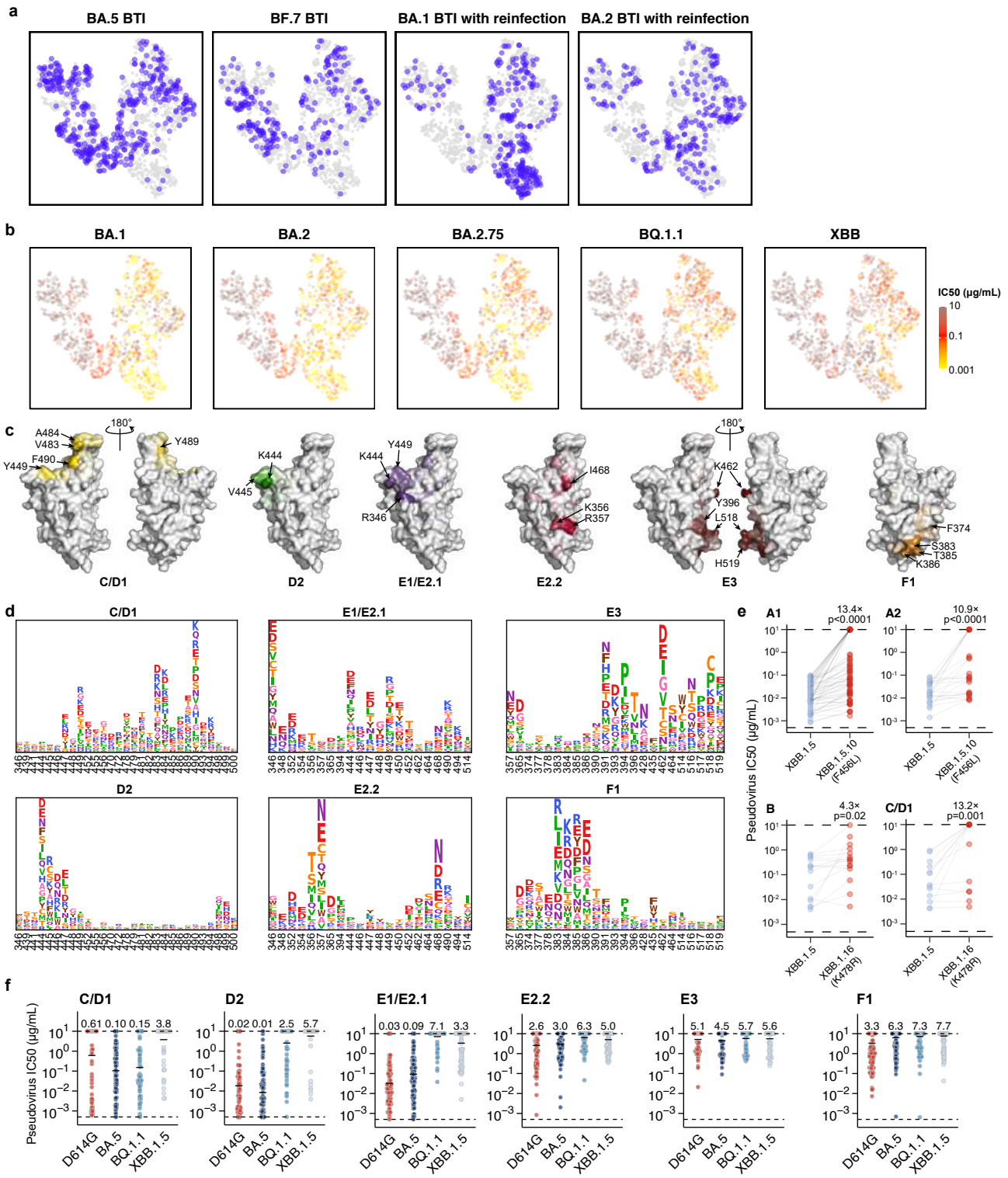
Extended Data Fig.5 | Comparison of neutralizing antibody responses with different mRNA vaccine booster dosages. a-b, Antibody breadth after priming with 2 doses of 3 μg CoronaVac followed by boosting with 1 dose of 1μg (a) or 10μg (b) BA.5/BQ.1.1/XBB Spike proteins in mice. Statistical significance was determined using the Wilcoxon signed-rank test. c-d, Antibody breadth after priming with 2 doses of 3 μg CoronaVac followed by boosting with 2 doses of 1μg (c) or 10μg (d) BA.5/BQ.1.1/XBB Spike proteins in mice. Statistical significance was determined using the Wilcoxon signed-rank test. e, Comparison of neutralizing antibody responses among different groups of mice immunized with 2 doses of CoronaVac, followed by 2 doses of BA.5/BQ.1.1/XBB mRNA boosters at different doses and compared to the primary immunization. Statistical significance was determined using the Wilcoxon rank sum test. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05.

Extended Data Figure 6



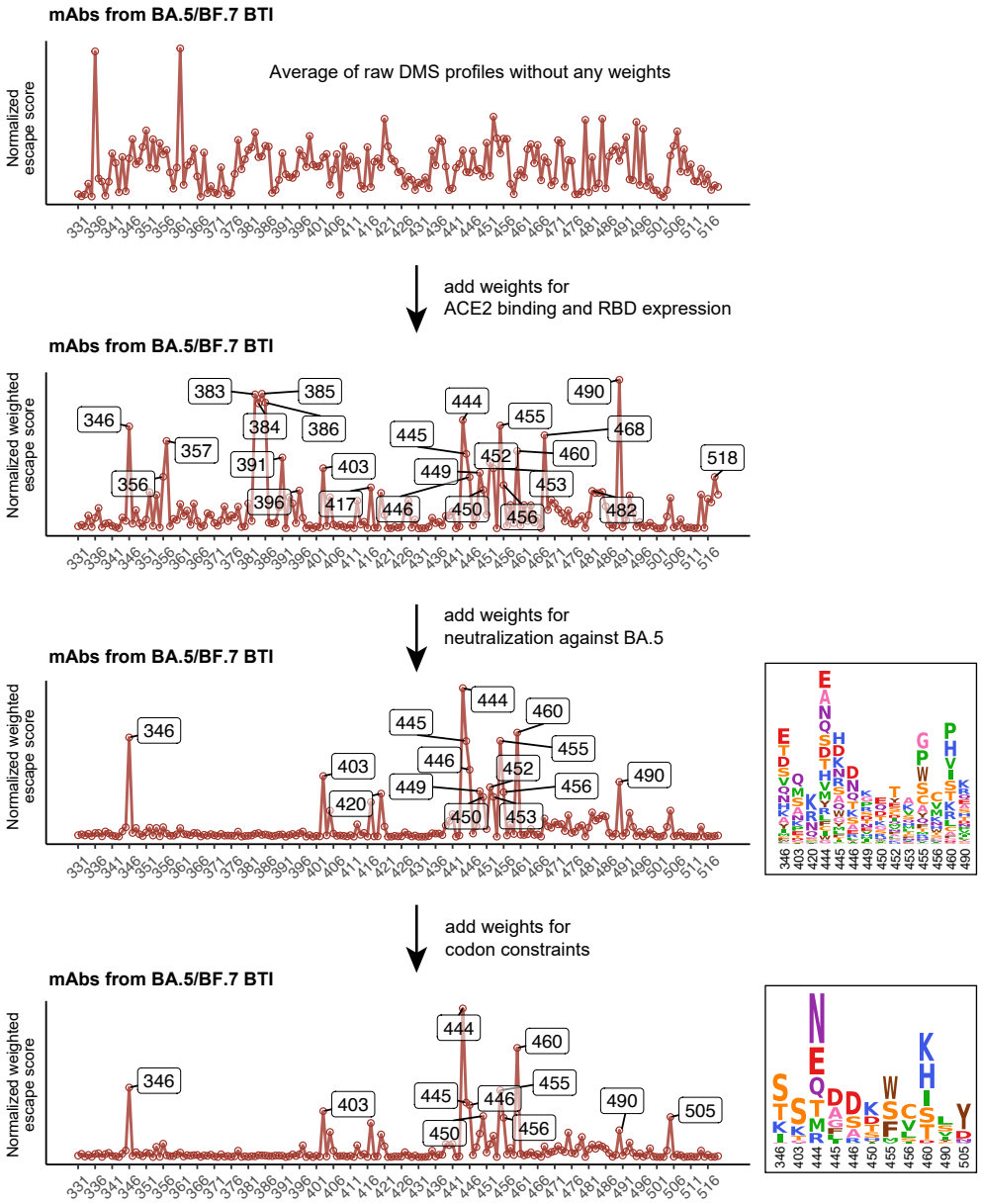
Extended Data Fig.6 | Antibody breadth of plasma and nasal swab after repeated Omicron infections.
a-c, Plasma antibody titers against pseudotyped D614G and variants after (a) BA.1 BTI + BA.5/BF.7 infection (n = 26), (b) BA.2 BTI + BA.5/BF.7 infection (n = 19), (c) BA.1/BA.2 + BA.5 infection (n = 12). d-f, Nasal swab antibody titers against pseudotyped D614G and variants after (d) BA.1 BTI + BA.5/BF.7 infection (n = 26), (e) BA.2 BTI + BA.5/BF.7 infection (n = 19), (f) BA.1/BA.2 + BA.5 infection (n = 12). g, Plasma antibody titers against pseudotyped D614G and variants after BA.1 BTI (n = 22). Fold changes between titers against variants and D614G were calculated and shown above the line in a-g. All statistical significance was determined using the Wilcoxon signed-rank test. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, and not significant (NS) p > 0.05..

Extended Data Figure 7



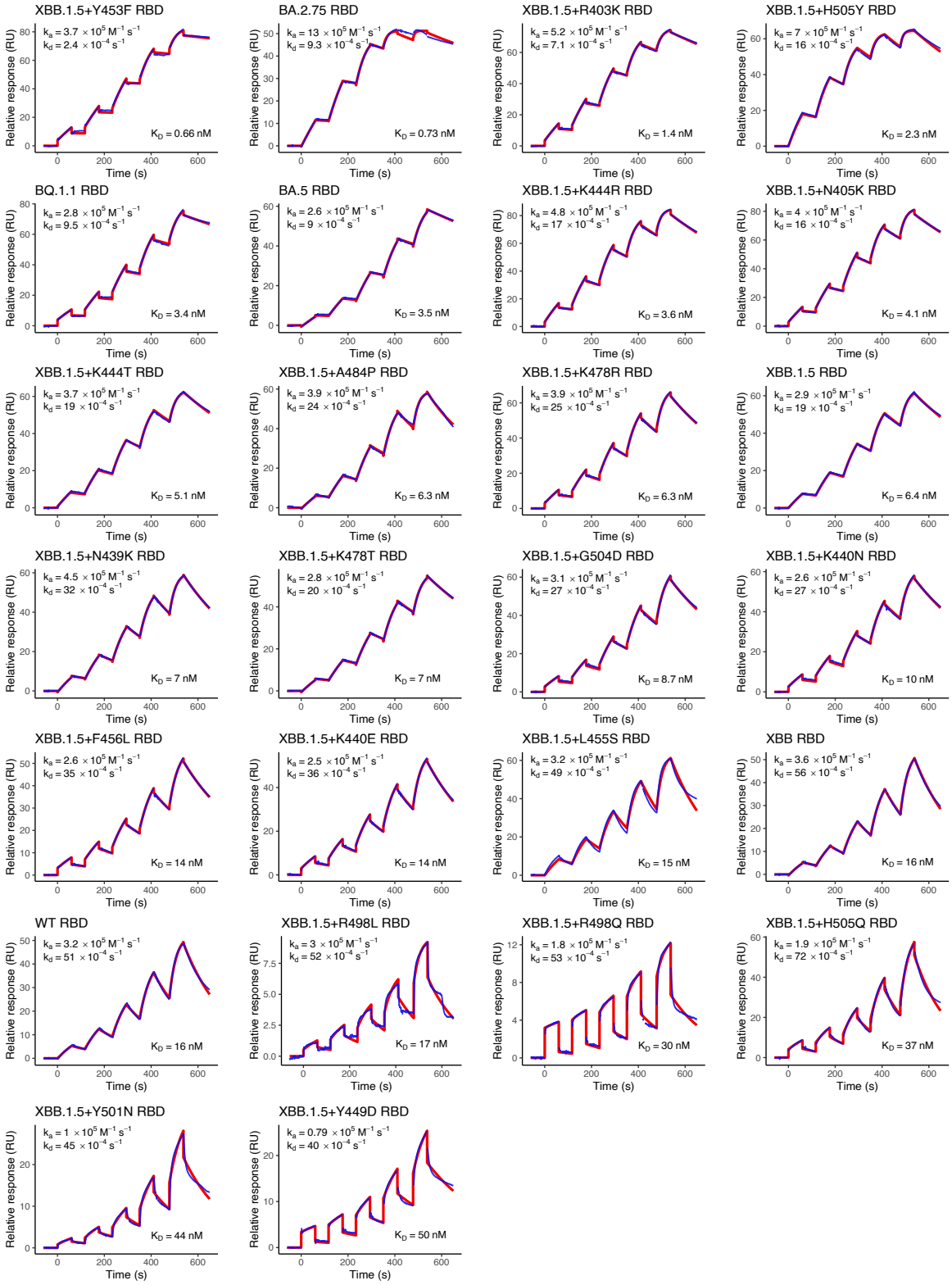
Extended Data Fig. 7 | Characteristics of BA.5-reactive mAbs elicited by BA.5/BF.7 BTI or reinfection.
a, Source of the antibodies are projected onto the UMAP embedding space. Antibodies from BA.5 BTI (n=445), BF.7 BTI (n=243), BA.1 BTI with reinfection (n=284), and BA.2 BTI with reinfection (n=232) are colored blue in the corresponding panel, and other antibodies are gray. b, Neutralization activities, denoted as IC50 values, against SARS-CoV-2 BA.1 (n = 1260), BA.2 (n = 1238), BA.2.75 (n=1238), BQ.1.1 (n = 1335) and XBB (n = 1341) spike-pseudotyped VSV are projected onto the UMAP embedding space. c, Average escape scores of epitope groups that are not shown in Fig. 4d (C/D1, D2, E1/E2.1, E2.2, E3, and F1) are illustrated on the structure model of the SARS-CoV-2 BA.5 RBD (PDB: 7XNS). Key residues with high escape scores for each group are labeled. d, Average DMS escape scores for these epitope groups are represented as sequence logos; residues are depicted using the standard one-letter code and colored based on their chemical properties. The height of each letter corresponds to the escape score of the respective mutation. e, Pseudovirus-neutralization activities of XBB.1.5-neutralizing mAbs in groups A1 (n=70) and A2 (n=23) against XBB.1.5 and XBB.1.5.10; and mAbs in groups B (n=15) and C/D1 (n=13) against XBB.1.5 and XBB.1.16. Fold changes in IC50 are labeled. P-values are calculated using two-tailed Wilcoxon signed-rank test of paired samples. f, Pseudovirus-neutralization activities of mAbs within the six crucial epitope groups (C/D1 [n = 76], D2 [n = 86], E1/E2.1 [n = 100], E2.2 [n = 124], E3 [n = 101], and F1 [n = 236]) are shown against SARS-CoV-2 D614G, BA.5, BQ.1.1, and XBB.1.5. Geometric mean IC50 values are displayed as bars and labeled above each group of data points.

Extended Data Figure 8



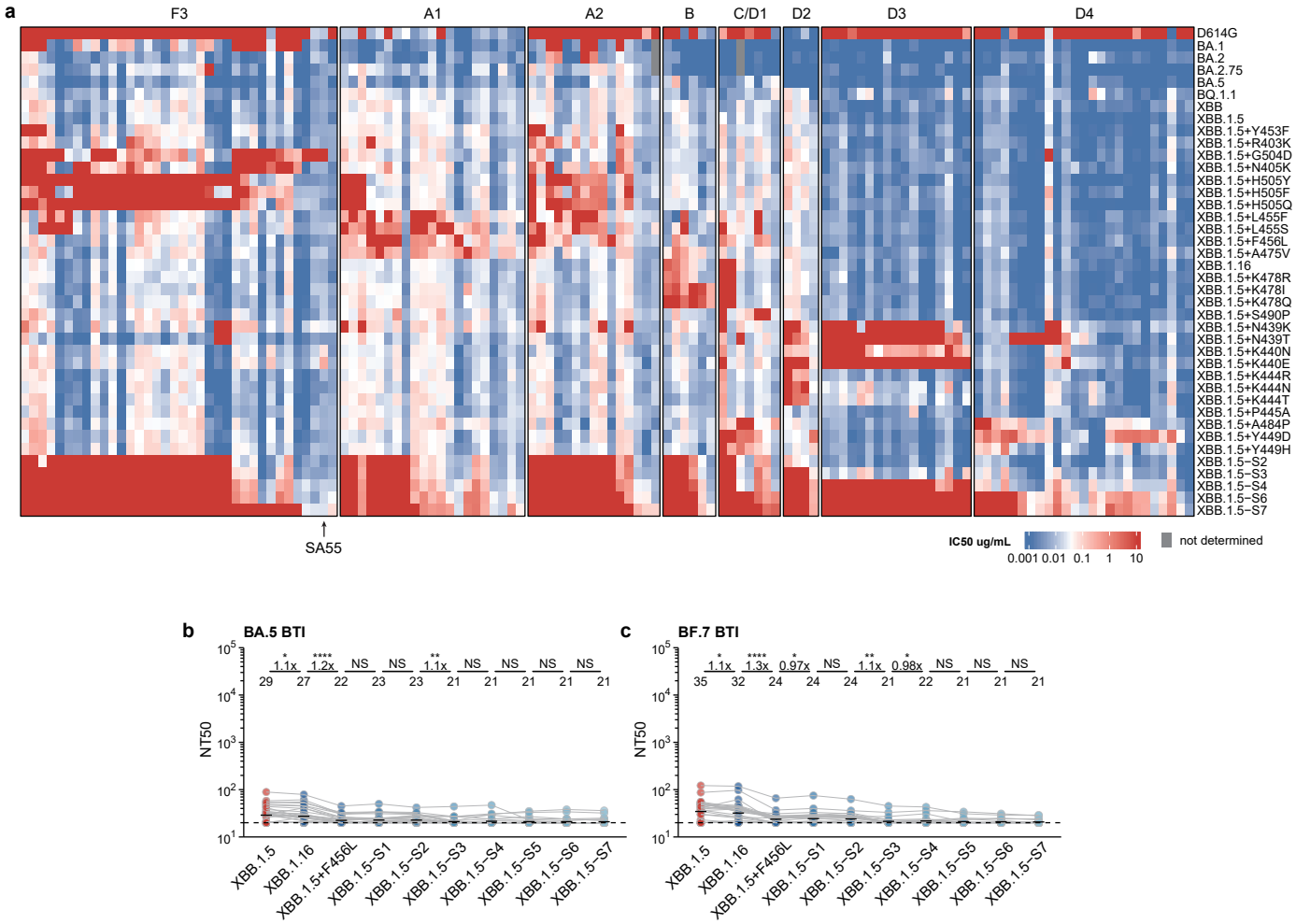
Extended Data Fig. 8 | Workflow of calculating weighted escape scores of each mutation on RBD. Weights for ACE2 binding and RBD expression, neutralization activity, and codon usage are sequentially applied on the calculation to achieve informative results. Mutation preferences of BA.5 RBD under the pressure of NAbs from BA.5 or BF.7 BTI are shown.

Extended Data Figure 9



Extended Data Fig. 9 | SPR sensorgrams for affinity of hACE2 and SARS-CoV-2 mutants RBD
Representative sensorgram of at least four replicates is shown for each RBD. Geometric mean kinetic constants k_a, k_d, and dissociation equilibrium constant (K_D) are labeled in each panel.

Extended Data Figure 10



Extended Data Fig. 10 | NAbs from BTI and reinfection are escaped by constructed mutants.
a, IC50 values for representative potent XBB.1.5-neutralizing antibodies from different epitope groups against XBB.1.5 variants carrying individual or multiple escape mutations are shown. The order of antibodies is the same as that in Fig. 6c. b, Pseudovirus NT50 for SARS-CoV-2 XBB.1.5-based mutants are shown using plasma from convalescent individuals who experienced BA.5 (n=36) or BF.7 BTI (n = 30). Statistical tests are performed between neighboring mutants. P-values are calculated using two-tailed Wilcoxon signed-rank tests on paired samples. *p < 0.05, **p < 0.01, ****p < 0.0001, and p > 0.05 (NS).