1   **IGHV3-53 antibody abundance drives divergent SARS-CoV-2 immune imprinting**

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Abstract

The mechanisms driving divergent SARS-CoV-2 immune imprinting in populations primed with different COVID-19 vaccines remain unclear. Recipients of inactivated vaccines readily develop Omicron-specific antibodies through repeated breakthrough infections, whereas mRNA-vaccinated individuals exhibit severe ancestral-strain imprinting that suppresses *de novo* Omicron-specific responses. These differences could result in distinct antibody landscapes, leading to regional epidemiological divergence and necessitating region-specific vaccine update strategies. Importantly, conventional wild-type mouse models fail to recapitulate strong human SARS-CoV-2 imprinting, which significantly hinders imprinting-related mechanistic investigation and vaccine update evaluation. Here, we surprisingly found that V(D)J-humanized mice could faithfully recapitulate human severe SARS-CoV-2 immune imprinting phenotypes. Comprehensive antibody repertoire and epitope mapping of 583 monoclonal antibodies from these models revealed that the abundance of pre-existing human IGHV3-53/66-encoded SARS-CoV-2 antibody responses determine imprinting severity following Omicron exposure through antibody-mediated masking of Omicron-specific epitopes. Both passive transfer of IGHV3-53/66 antibodies and knock-in of the human IGHV3-53 gene were sufficient to induce severe SARS-CoV-2 imprinting in wild-type mice. Concordantly, head-to-head comparison also showed that mRNA vaccine recipients retained higher IGHV3-53/66 antibody abundance and thus stronger imprinting than inactivated vaccine recipients. Consequently, compared to NB.1.8.1, XFG exhibits greater immune evasion in mRNA-vaccinated individuals but not in inactivated vaccine recipients. This explains the regional predominance of XFG in mRNA-vaccinated populations, while NB.1.8.1 prevails in inactivated vaccine-dominated countries. Together, these findings demonstrate that the V(D)J germline repertoire—even a single germline-encoded antibody response—can profoundly shape humoral imprinting severity. Accordingly, we constructed a human IGHV3-53 knock-in mouse model that can accurately recapitulate human SARS-CoV-2 antibody landscape, providing a valuable tool for guiding future COVID-19 vaccine updates.

Main

Extensive studies have investigated immune imprinting in SARS-CoV-2 [1], yet the reasons why different vaccine platforms produce markedly different imprinting severity have remained unclear. Recipients of mRNA vaccines exhibit pronounced immune imprinting, persistently recalling ancestral Wuhan-Hu-1 (Wuhan) spike-reactive antibodies even upon repeated Omicron exposures, significantly limiting the development of Omicron-specific neutralizing responses [2–10]. In contrast, individuals only receiving inactivated vaccines, predominantly in China, demonstrate greater adaptability in humoral responses, developing robust Omicron-specific antibodies upon repeated infections or boosters, thus overriding Wuhan immune imprinting [11–14].

This distinct imprinting pattern has become increasingly consequential, as it aligns with the emerging global divergence in SARS-CoV-2 epidemiology: the XFG lineage currently leads in areas with high mRNA vaccine coverage, whereas NB.1.8.1 is dominant in nations that primarily used inactivated vaccines, especially in China (Extended Data Fig. 1a) [15–20].

Understanding the mechanistic basis of this imprinting divergence is critical, not only because it may have directly influenced real-world viral epidemiology—potentially necessitating region-specific vaccine update strategies—but also because these distinct imprinting phenotypes provide a unique opportunity to uncover general principles of humoral immune imprinting applicable to other pathogens, such as influenza.

However, direct head-to-head comparisons of imprinting severity and antibody repertoires between mRNA- and inactivated-vaccine recipients have been limited by challenges in sample collection and international sample shipping. Moreover, conventional wild-type mouse models fail to recapitulate the strong imprinting observed in humans [2,11,13], severely hindering mechanistic studies and preclinical evaluation of SARS-CoV-2 vaccines.

In this paper, we present a head-to-head comparison of antibody repertoires, imprinting severity, and responses to emerging variants in individuals primed with mRNA versus inactivated vaccines. These analyses reveal that differences in immune imprinting are already influencing real-world epidemiology, contributing to the distinct regional dominance of XFG and NB.1.8.1. Using V(D)J-humanized mice, we demonstrate faithful recapitulation of strong human-like imprinting and

75 identify pre-existing human IGHV3-53/66-encoded antibodies as the key driver of imprinting

76 severity via epitope masking. Finally, we establish a human IGHV3-53 knock-in mice model that

77 accurately recapitulate human SARS-CoV-2 antibody responses, offering a practical tool for

78 evaluating future vaccine update strategies.
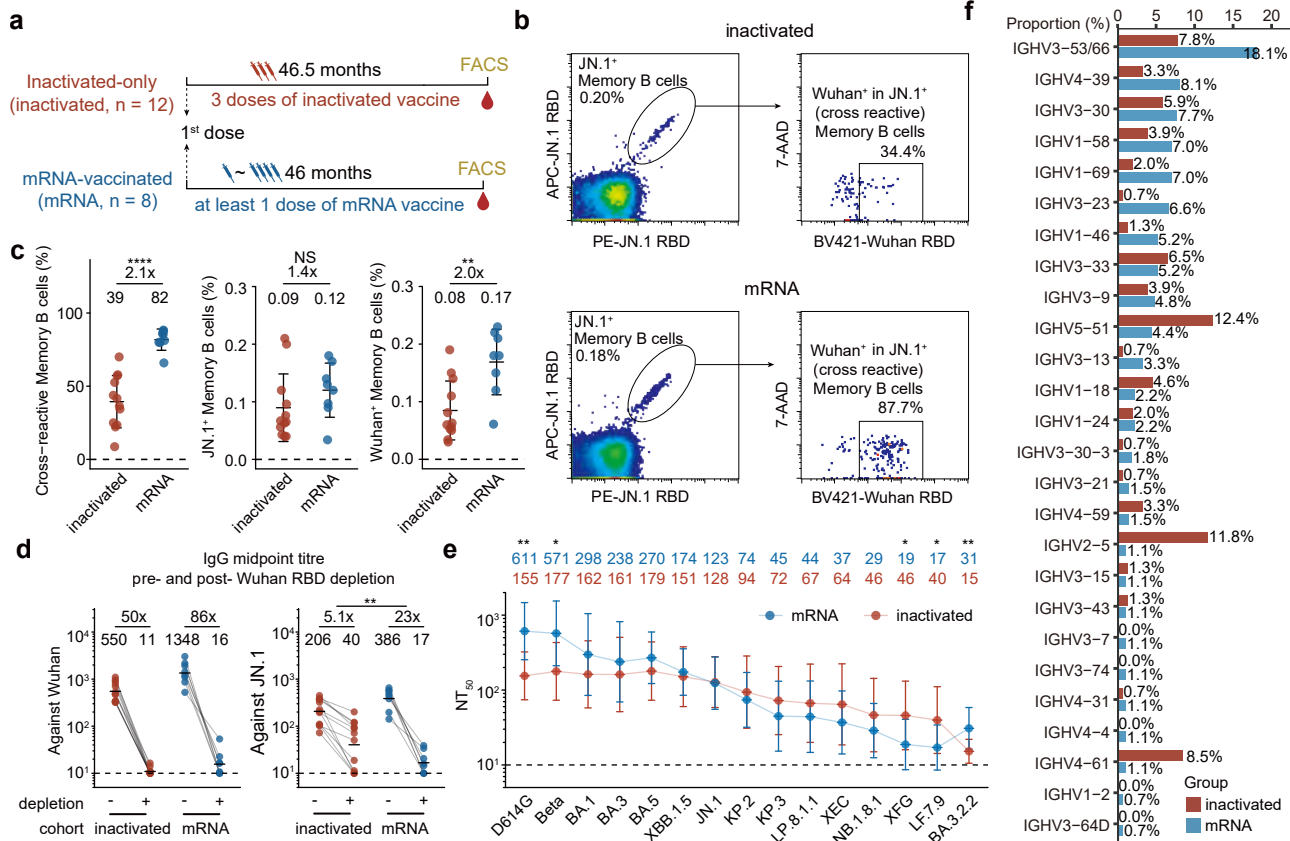
79

80 **Distinct immune imprinting in humans**

81 To directly compare the humoral immunity in individuals primed with mRNA versus inactivated

82 vaccines, we recruited 20 participants stratified into two cohorts based on vaccination history

83 (Supplementary Table 1 and Fig. 1a). Despite the modest sample size, these cohorts present a rare

84 opportunity to directly compare mRNA and inactivated vaccine priming outcomes. All participants

85 had resided in the same region since 2021 and had experienced multiple Omicron infections. The

86 inactivated-only cohort (n = 12) received three doses of CoronaVac, with samples collected 46.5 ±

87 5.4 months after the first vaccine dose. The mRNA-vaccinated cohort (n = 8) received at least one

88 mRNA vaccine dose (BNT162b2 or mRNA-1273), with samples collected 46.0 ± 2.3 months after

89 initial vaccination. Although detailed infection histories were unavailable, widespread Omicron

90 transmission following China's reopening strongly suggests that most participants were exposed to

91 at least BA.5 and JN.1 sublineages.

92 To assess immune imprinting, we first used fluorescence-activated cell sorting (FACS) to quantify

93 cross-reactive memory B cells, defined as the proportion of Wuhan$^+$ cells among JN.1$^+$ memory B

94 cells (Fig. 1b and Supplementary Information Fig. 1a). The mRNA-vaccinated cohort indeed

95 exhibited a significantly higher proportion of cross-reactive memory B cells than the inactivated-

96 only cohort, indicating stronger imprinting (Fig. 1c). Additionally, the mRNA-vaccinated group

97 showed a higher proportion of Wuhan$^+$ memory B cells, reflecting persistent responses to the

98 ancestral strain. In contrast, JN.1$^+$ memory B cell frequencies were comparable between groups,

99 suggesting comparable responses to recent variants despite different priming histories (Fig. 1c).

100 To examine antibody specificity, we depleted Wuhan RBD-binding antibodies from serum and

101 measured the remaining IgG titres against the JN.1 RBD (Fig. 1d). This depletion nearly abolished

102 JN.1-binding activity in the mRNA-vaccinated cohort (23-fold reduction), indicating that the

Figure 1

Figure 1 | mRNA and inactivated vaccine priming induce distinct levels of immune imprinting.

a, Schematic of the SARS-CoV-2-related immune histories and sampling time of the inactivated-only and mRNA-vaccinated cohorts involved in this study. b, Representative flow cytometry dot plots of cross-reactive memory B cells from individuals from the inactivated-only (top) and mRNA-vaccinated (bottom) cohorts. APC, allophycocyanin; PE, phycoerythrin; BV421, Brilliant Violet 421. c, Scatter plots showing the proportion of cross-reactive memory B cells (left), JN.1-reactive memory B cells (middle), and Wuhan-reactive memory B cells (right). d, Serum IgG midpoint titre against Wuhan (left) or JN.1 (right) RBD before and after Wuhan RBD depletion. Statistical significance of the fold-reduction in titres was assessed between two cohorts. Dashed lines indicate the limit of detection (midpoint titre = 10). e, NAb response of the two cohorts against a panel of SARS-CoV-2 variant pseudoviruses. Geometric mean titres (GMTs) are shown on the top. Dashed lines indicate the limit of detection (NT_{50} = 10). f, Frequency of heavy chain V gene usage proportion of the two cohorts. IGHV3-53 and IGHV3-66 were combined for analysis. Two-tailed Wilcoxon rank-sum tests were used in c-e. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; NS, not significant (P > 0.05).

103   response was almost entirely contributed by cross-reactive antibodies. In contrast, the inactivated-

104   only cohort showed a more modest decrease (5.1-fold), consistent with the presence of a subset of

105   JN.1-specific antibodies (Fig. 1d).

106   Neutralization assays against a pseudovirus panel further supported this divergence (Fig. 1e). In the

107   mRNA-vaccinated cohort, 50% neutralization titres (NT_{50}) were highest for D614G and declined

108   rapidly with newer variants [21–25]. In contrast, the inactivated-only cohort maintained relatively stable

109   neutralization, declining only from JN.1 onward [11,13,17,26–29]. Interestingly, the NT_{50} curves

110   intersected at JN.1, beyond which the inactivated-only cohort showed higher neutralization. These

111   data suggest that, owing to stronger immune imprinting, the mRNA-vaccinated cohort's

112   neutralizing responses to emerging variants are strongly confined by the recall and expansion of

113   antibodies originally elicited by ancestral exposure, whereas the more adaptable response in the

114   inactivated-only cohort enables superior neutralization of later-emerging variants.

115   Notably, the mRNA-vaccinated cohort showed a marked reduction in titres against XFG relative to

116   NB.1.8.1, whereas the inactivated-only cohort displayed comparable neutralizing activity against

117   both variants. This disparity provides a serological explanation for the regional predominance of

118   XFG in mRNA-vaccinated populations and NB.1.8.1 in inactivated vaccine-dominated countries [17].

119   Additionally, neutralization titres against BA.3.2.2 were significantly higher in the mRNA-

120   vaccinated cohort (Fig. 1e). This variant carries unique mutations that have been shown to evade

121   Class 1/4 antibodies prevalent in inactivated vaccinees but not the dominant Class 1 antibodies

122   induced by mRNA vaccines [17]. Collectively, these head-to-head comparisons of serological and flow

123   cytometry data confirm robust imprinting in the mRNA-vaccinated cohort and weaker imprinting

124   in the inactivated-only cohort that can be partially overcome. Such imprinting differences have

125   resulted in distinct antibody landscapes, contributing to regional epidemiological divergence and

126   raising the potential necessity for region-specific vaccine update strategies.

127   While the lack of longitudinal samples makes it challenging to trace the evolutionary mechanism of

128   this divergence in humans, distinct signatures within the BCR repertoire may offer critical insights.

129   To explore these correlates, we performed plate-based single B cell V(D)J sequencing to compare

130   heavy-chain V-gene usage of JN.1$^+$ memory B cells between the two cohorts (Fig. 1f) [30]. The

131   mRNA-vaccinated cohort showed enriched usage of IGHV3-53/66—germline genes encoding

132 "public" antibodies targeting the Class 1 epitope, which overlaps with the ACE2-binding site [31–38].

133 These neutralizing antibodies are broadly elicited by Wuhan vaccination or infection and are well

134 known for their potent neutralizing capacity [31,37]. In contrast, the inactivated-only cohort showed

135 greater representation of IGHV5-51 and IGHV2-5, associated with Omicron-specific Class 1/4

136 antibodies [26]. The differences in light chain V-gene usage between the two cohorts were less

137 pronounced, with both groups showing a preference for IGKV1-39, IGKV3-20, and IGKV1-33

138 (Extended Data Fig. 2a).

139 These findings suggest that the IGHV3-53/66–encoded Class 1 antibodies—originally elicited by

140 ancestral Wuhan strain exposure—are preferentially recalled and expanded upon repeated Omicron

141 encounters, and the mRNA-vaccinated cohort exhibits much higher IGHV3-53/66 antibody

142 abundance compared to the inactivated-only cohort, due to its higher priming vaccine

143 immunogenicity. Although our analysis was limited by small cohort sizes and unavoidable

144 demographic heterogeneity (e.g., age, sex, and infection history), the observed immune imprinting

145 patterns align remarkably well with extensive prior studies [2–9,11–14]. Importantly, this parallel

146 analysis enabled us to observe a correlation between IGHV3-53/66-encoded SARS-CoV-2 antibody

147 enrichment and imprinting severity. To move beyond correlation and rigorously establish causality,

148 it is critical to establish a animal model that can control strictly matched immune histories while

149 faithfully recapitulating the severe immune imprinting observed in human mRNA vaccinees.

150

V(D)J-humanized mice mimic strong imprinting

152 Previous studies have shown that wild-type mice do not recapitulate the strong SARS-CoV-2

153 immune imprinting observed in the human mRNA-vaccinated cohort [2]. Given that our human data

154 identified a critical role for the IGHV3-53/66 germline genes in severe SARS-CoV-2 imprinting,

155 we hypothesized that the absence of these specific human V-genes in the murine repertoire explains

156 this discrepancy. Therefore, we employed V(D)J humanized mice for comparative analysis. These

157 BALB/c-derived mice, commonly used in therapeutic antibody discovery, carry human

158 immunoglobulin V(D)J genes for all heavy chains and kappa light chains (Supplementary

159 Information Fig. 4a). Both V(D)J humanized and wild-type mice were primed with three doses of
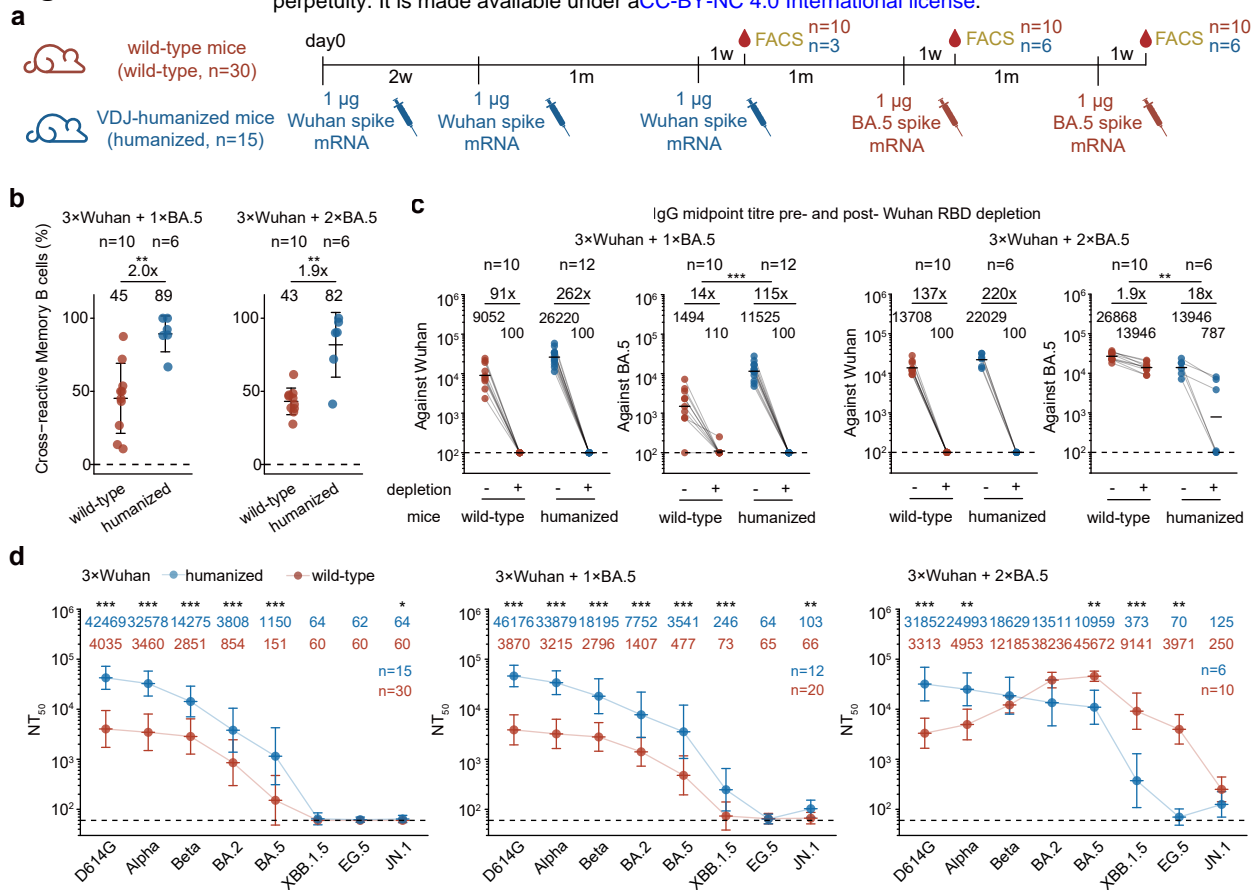
Figure 2

Figure 2 | V(D)J-humanized mice recapitulate severe SARS-CoV-2 imprinting.
a, Schematic of the mouse immunization regimen and sampling timeline for wild-type and V(D)J-humanized mice. The number of mice is indicated at the time point for each endpoint experiment. b, Scatter plots showing the proportion of cross-reactive memory B cells in draining lymph nodes of the two mouse strains after one (left) or two (right) BA.5 boosts. c, Serum IgG midpoint titre of the two mouse strains after one (left) or two (right) BA.5 boosts against Wuhan or BA.5 RBD before and after Wuhan RBD depletion. Statistical significance of the fold-reduction in titres was assessed between humanized and wild-type mice. Dashed lines indicate the limit of detection (midpoint titre = 100). d, Serum neutralization titres (NT_{50}) of the two mouse models after Wuhan priming (left), one BA.5 boost (middle), or two BA.5 boosts (right) against a panel of SARS-CoV-2 variant pseudoviruses. Geometric mean titres (GMTs) are shown on the top. Dashed lines indicate the limit of detection (NT_{50} = 60). Two-tailed Wilcoxon rank-sum tests were used in b-d.

160 ancestral mRNA vaccine (encoding the spike protein of SARS-CoV-2 ancestral strain), followed by

161 two booster doses of BA.5 mRNA vaccine to mimic human exposure history to SARS-CoV-2

162 variants (Fig. 2a). Blood, spleens, and lymph nodes were collected one week after the third ancestral

163 dose and each BA.5 dose for serological and flow cytometry analyses.

164 We first performed FACS analysis on mouse lymph nodes to assess cross-reactivity among memory

165 B cells and germinal-center (GC) B cells (Supplementary Information Fig. 1b). As expected, wild-

166 type mice harbored abundant BA.5-specific memory and GC B cells after each BA.5 booster (Fig.

167 2b and Extended Data Fig. 3a). In striking contrast, the V(D)J-humanized mice exhibited a strong

168 imprinting phenotype, developing a high proportion of cross-reactive memory B cells (89% and 82%

169 after the first and second boosters, respectively) that mirrored the response observed in the human

170 mRNA-vaccinated cohort. Despite similar overall percentages of GC B cells, humanized mice

171 showed a significantly higher frequency of cross-reactive GC B cells after one BA.5 booster,

172 indicating that their GC response was dominated by Wuhan-strain imprinted B cells (Extended Data

173 Fig. 3a and 3b). Additionally, they presented a significantly higher frequency of class-switched

174 memory B cells, which aligns with the preferential recall and expansion of a pre-existing memory

175 population typical of immune imprinting (Extended Data Fig. 3c).

176 The imprinting was also faithfully reflected in the serological response. Wuhan RBD-depletion

177 caused a substantially greater drop in BA.5-binding IgG titres in humanized mice than in wild-type

178 mice (18-fold vs. 1.9-fold), although the magnitude of this effect varied among individual

179 humanized mice (Fig. 2c). Specifically, titres in three humanized mice dropped to undetectable

180 levels, while the remaining three exhibited modest reductions. The post-depletion titre ratio was

181 inversely correlated with the proportion of cross-reactive memory B cells (Supplementary

182 Information Fig. 3a), demonstrating consistency between cellular and serological assays and

183 reflecting individual heterogeneity in imprinting strength.

184 We next compared serum neutralization profiles between the two mouse models (Fig. 2d and

185 Extended Data Fig. 4a). Initially, following Wuhan priming, humanized mice mounted a

186 significantly more potent and broad response than wild-type mice. Two BA.5 boosters successfully

187 shifted the neutralizing antibody (NAb) preferences of wild-type mice toward Omicron variants. In

188 contrast, the neutralization curves of humanized mice closely mirrored those of the mRNA-

189 vaccinated cohort, with titres remaining maximal against D614G despite repeated Omicron

190 exposure, indicating strong immune imprinting that could not be overcome by two doses of BA.5

191 boosting (Extended Data Fig. 4a). Together, these data collectively demonstrate that humanized

192 mice can mirror SARS-CoV-2 imprinting. This divergence between the two mouse models strongly

193 suggests that the presence of human immunoglobulin V(D)J genes is a critical determinant of the

194 imprinting phenotype.

195

196 **Distinct antibody landscapes of mouse models**

197 To dissect the molecular basis of imprinting, we tracked the evolution of antibody repertoire from

198 the two mouse models throughout Wuhan-priming and BA.5-boosting. We sorted RBD-specific

199 memory B cells from spleens of humanized and wild-type mice following Wuhan priming (sorted

200 on Wuhan RBD) and after each BA.5 booster (sorted on BA.5 RBD; Supplementary Information

201 Fig. 1c). Single-cell V(D)J sequencing of paired heavy- and light-chain variable regions generated

202 583 unique monoclonal antibodies (mAbs) from the six mice groups. These mAbs were

203 recombinantly expressed as human IgG1 (Supplementary Table 2), and their half-maximal

204 inhibitory concentration (IC_{50}) measured via pseudovirus neutralization assay (Extended Data Fig.

205 5a). Consistent with the established imprinting signature, enzyme-linked immunosorbent assay

206 (ELISA) revealed that antibodies from humanized mice exhibited a higher proportion of cross-

207 reactivity than those from wild-type mice (Extended Data Fig. 5b).

208 To systematically dissect the divergence in antibody epitope distribution between humanized and

209 wild-type mice, we employed high-throughput yeast-display-based deep mutational scanning (DMS)

210 to map the RBD mutations that could escape the isolated mAbs and define the epitope targeted

211 [13,26,39–41]. We built single-site saturation mutant libraries based on the Wuhan-Hu-1 and BA.5 RBDs

212 and performed DMS for antibodies isolated following Wuhan priming or BA.5 boosting,

213 respectively. The resulting escape profiles of 583 mAbs define the critical residues that mediate

214 immune evasion and facilitating their precise categorization into distinct epitope clusters.

215 IC_{50}-weighted escape profiles show that neutralizing antibodies from wild-type and humanized mice

216 exhibit distinct escape hotspot sites (Fig. 3a-3c and Extended Data Fig. 6a-6c). Following Wuhan

Figure 3

Figure 3 | Distinct antibody landscapes between mRNA vaccinated humanized and wild-type mice.
a–c, Normalized average DMS escape scores for mAbs isolated from humanized (top) and wild-type (bottom) mice following Wuhan priming (a), the first BA.5 booster (b), or the second BA.5 booster (c). Escape scores were aggregated and weighted by the IC_{50} of each individual mAb (To focus on the neutralizing mAbs, lower IC_{50} corresponds to greater weight) against D614G for Wuhan-primed groups or BA.5 for BA.5-boosted groups. Codon constraints were applied (see Methods). In each panel, the ten residues with the highest cumulative escape scores are annotated, with their specific mutational escape profiles visualized as logo plots above the scatter plots. To highlight divergent immune pressure, residues exhibiting pronounced differences between humanized and wild-type strains (as identified in Extended Data Fig. 6) are colored red in both the scatter plots and logo plots.

217 priming, the NAbs elicited in humanized mice substantially focused on Class 1 epitopes, featured

218 by hotspots at residues 417, 456, 460, and 473. Although wild-type mice displayed a comparable

219 landscape after Wuhan priming, they lacked these Class 1 peaks, instead featuring residue 477 as a

220 characteristic hotspot (Fig. 3a).   BA.5 boosting leads to further divergence between the two mouse

221 models. In humanized mice, the prominent Class 1 hotspots (e.g., residue 456) persisted obstinately

222 throughout the boosting regimen. In contrast, the wild-type repertoire diverged significantly upon

223 boosting, characterized by the emergence of distinct Class 1/4 escape peaks, such as residue 504

224 (Fig. 3b-3c).

225 To translate these differences in escape sites into a more intuitive view of epitope-level repertoire

226 differences, we categorized the 583 mAbs into 9 epitope groups (Supplementary Information Fig.

227 2a) [13]. Names of the epitope groups were generally assigned in line with the epitope groups on

228 Wuhan RBD defined previously [42,43]. We then comprehensively analyzed the antibody repertoires

229 elicited in humanized and wild-type mice after each timepoint (Fig. 4a and 4b, Extended Data Fig.

230 7a-7d). Following Wuhan priming, humanized mice uniquely elicited a dominant population of

231 antibodies targeting the A1 epitope (Fig. 4a). Notably, antibodies targeting this epitope are closely

232 related to the IGHV3-53 germline and target key residues such as 456, aligning with the escape

233 hotspots. The foundational divergence between the two mouse models was established immediately

234 after Wuhan priming. After two BA.5 boosting doses, while dynamic shifts occurred—including the

235 abrogation of Group B and the emergence of Group A2—the A1 response remained persistent in

236 humanized mice but was completely absent in wild-type mice (Fig. 4c and Extended Data Fig. 7a

237 and 7c). In contrast, Omicron-specific antibodies in epitope groups B, D, and F3 contribute mostly

238 to the neutralization against BA.5 exclusively in wild-type but not the V(D)J-humanized mice.

239 Overall, DMS analysis demonstrates that Class 1 (A1) antibodies should play a pivotal role in the

240 response to Wuhan priming, serving as a defining characteristic of the V(D)J-humanized mouse

241 model, compared to the wild-type mice. This antibody subset substantially contributed to the

242 polyclonal neutralization landscape and could be robustly recalled upon BA.5 boosting. Notably,

243 these A1 antibodies exhibited remarkable germline convergence, consistently dominated by

244 IGHV3-53 after both Wuhan priming and subsequent BA.5 boosting (Fig. 4a). This mirrors the

245 IGHV3-53/3-66 dominance observed in human mRNA-vaccinated cohort. Collectively, these
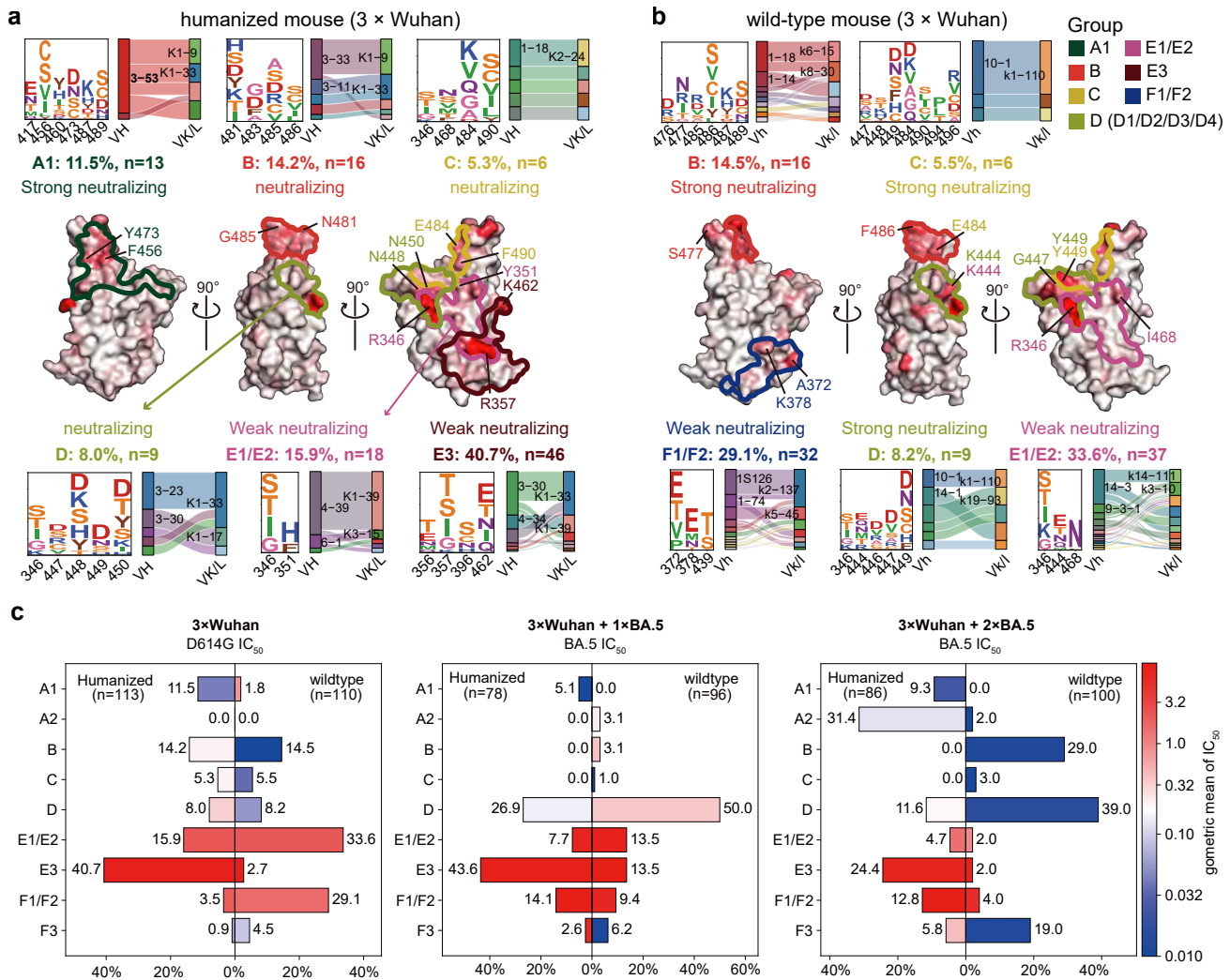
Figure 4

Figure 4 | V(D)J germline difference shapes vaccination- induced SARS-CoV-2 antibody epitope distribution.
Epitope distribution of the antibody repertoire generated after Wuhan priming in humanized (a) and wild-type mice (b). The Wuhan RBD structure (PDB: 6m0j) is displayed as a surface map colored by normalized aggregate escape scores, with major epitope groups outlined in distinct colors. Their neutralizing category, antibody count, and percentage are indicated. Neutralizing category is classified based on geometric mean of IC_{50}: Strong neutralizing (<0.1 µg/mL), Neutralizing ($0.1 \leq IC_{50} < 1$ µg/mL), and Weak neutralizing ($1 \leq IC_{50} \leq 10$ µg/mL).Epitope groups accounting for <5% of the total antibodies are not labeled. Key escape residues for each group are displayed as logos, and the sites with the highest escape scores per group are labeled on the structure. Paired heavy and light chain V-gene usage for each epitope is shown in Sankey plots. c, Pyramidal bar charts showing the proportional distribution of epitope groups in antibodies isolated from humanized and wild-type mice after Wuhan priming and after one or two BA.5 boosts. Bars are colored according to the log10 geometric mean IC_{50} of antibodies within each group.

246   findings led us to hypothesize that the dominant, IGHV3-53/66-driven A1 antibody response

247   induced by Wuhan priming is the mechanistic driver of SARS-CoV-2 immune imprinting.

248

249   **Molecular mechanism of imprinting**

250   Given that immune imprinting relies on the recall of pre-existing memory B cells, we then confirmed

251   the cross-reactivity of these IGHV3-53/66-encoded A1 antibodies to determine their potential for

252   reactivation by the Omicron booster (Fig. 5a). 38% of A1 mAbs from Wuhan-primed humanized

253   mice were cross-reactive, constituting a pre-existing memory pool that was efficiently recalled to

254   achieve 100% cross-reactivity upon BA.5 boosting. In contrast, the negligible A1 response in

255   primed wild-type mice was entirely Wuhan-specific and thus completely escaped by BA.5, resulting

256   in the total absence of A1 antibodies following the boosters. Competitive SPR mapping revealed

257   that A1 antibodies compete with ACE2 and the majority of antibodies targeting neutralizing epitopes

258   (A2, B, C, D4, F3). Conversely, they showed minimal to no competition with Group D1 or the

259   weakly or non-neutralizing E and F antibodies (Fig. 5b).

260   These results indicate that A1 antibodies can sterically mask most other major neutralizing sites on

261   Omicron RBD from access by other antibodies and their corresponding B cell receptors. Enabled

262   by the IGHV3-53/66 germline genes, Wuhan priming induces a high-frequency pool of these potent

263   "masking" antibodies, the abundance of which scales directly with the intensity of the priming.

264   Critically, a significant portion of this pool is not escaped by Omicron and remains cross-reactive,

265   making it available for recall upon subsequent Omicron exposure. Therefore, during Omicron

266   vaccination, this pre-existing and cross-reactive memory B cell population is preferentially

267   reactivated and expanded. Such a potent, recalled response could actively outcompete and suppress

268   the *de novo* activation of B cells targeting novel Omicron-specific epitopes by antibody masking,

269   providing a direct mechanistic basis for the strong immune imprinting observed in humanized mice.

270   (Fig. 5c).

271   To test whether these antibodies can indeed drive strong imprinting *in vivo*, we performed passive

272   antibody transfer experiments in wild-type mice (Fig. 5d). Wuhan-primed wild-type mice were

273   infused with BD55-1205 (a representative IGHV3-66-encoded A1 broad neutralizing antibody [44])
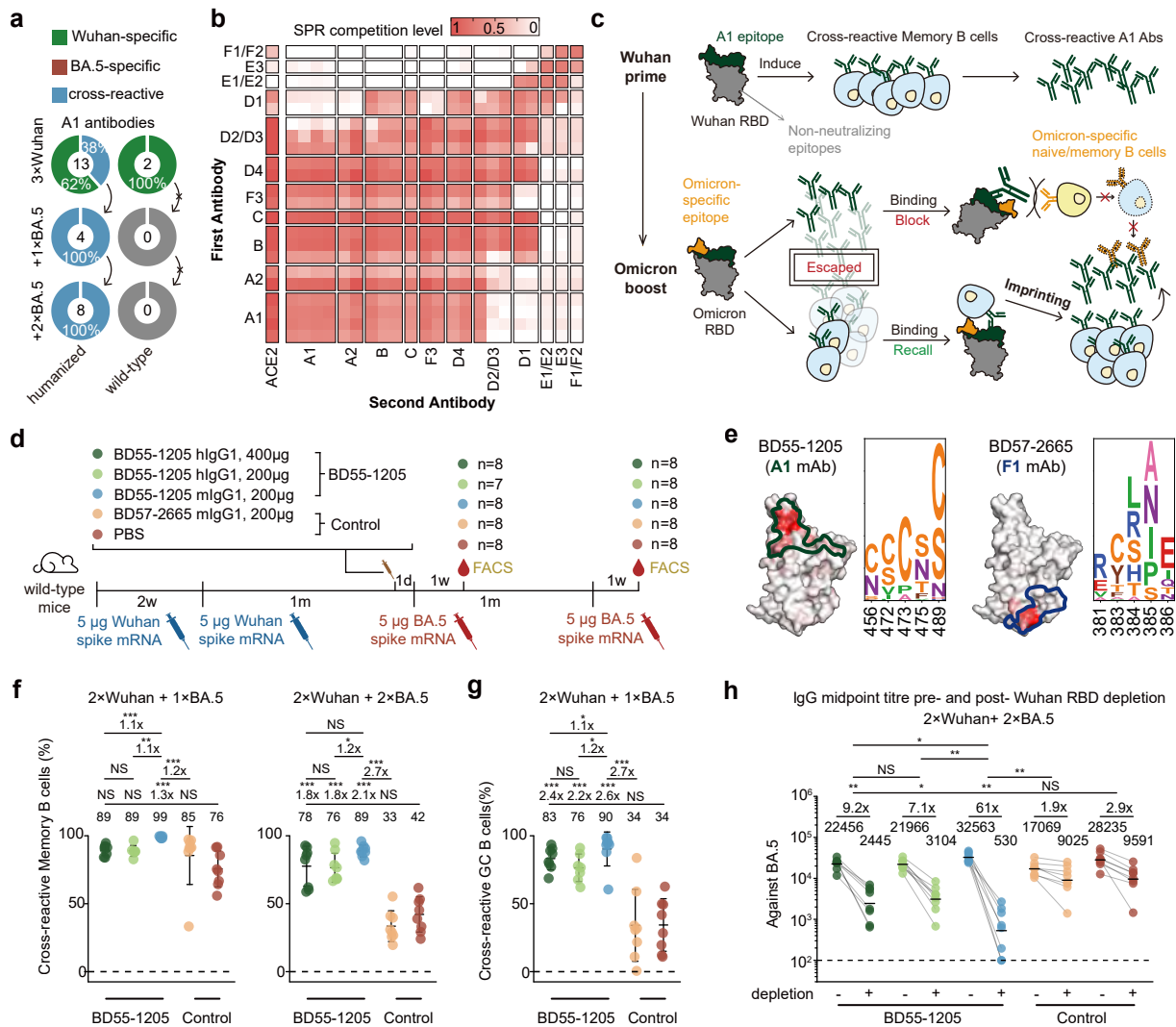
Figure 5

Figure 5 | IGHV3-53/66-encoded Class 1 antibody drives SARS-CoV-2 imprinting through epitope masking.
a, Donut plots showing the cross-reactivity of A1 antibodies from humanized or wild-type mice. The number of antibodies are indicated in the centre of the donut. Antibodies exhibiting ELISA OD450 values > 2 against both WT and BA.5 RBDs (1 µg/mL) were defined as cross-reactive. Those showing an OD450 > 2 for one variant but < 2 for the other were classified as specific. b, Heatmap of competitive SPR for various antibody groups. The definition of the competition score is described in the Methods section. c, Schematic of the molecular mechanism by which pre-existing IGHV3-53/66-encoded A1 antibodies cause strong immune imprinting. d, Schematic of the antibody passive transfer experiment. Timing of mRNA vaccinations, antibody injection, blood collection, and FACS analysis are indicated. Mice were divided into experimental groups (receiving 400 µg BD55-1205 hIgG1, 200 µg BD55-1205 hIgG1, or 200 µg BD55-1205 mIgG1) and control groups (receiving 200 µg BD57-2665 mIgG1 or PBS). The number of mice per group is indicated at the endpoint. e, DMS escape map logoplots for BD55-1205 and BD57-2665 and their projection onto the SARS-CoV-2 Wuhan RBD (PDB: 6m0j). f, Scatter plots showing the proportion of cross-reactive memory B cells in draining lymph nodes after one (left) or two (right) BA.5 boosts. g, Scatter plots showing the proportion of cross-reactive germinal center B cells in draining lymph nodes after one BA.5 boost. h, Serum IgG midpoint titre against BA.5 RBD before and after Wuhan RBD depletion. Statistical significance of the fold-reduction in titres was assessed between groups. Dashed lines indicate the limit of detection (midpoint titre = 100). Two-tailed Wilcoxon rank-sum tests were used in f-h.

274   at various doses and formats (human or mouse IgG1) one day prior to BA.5 boosting (Fig. 5d-5e).

275   Controls received either PBS or a non-neutralizing antibody (BD57-2665, a representative F1

276   antibody targeting the cryptic sites of RBD, Fig. 5e). Flow cytometry analysis of lymph nodes

277   showed that mice receiving 400 μg or 200 μg BD55-1205 hIgG1 and 200 μg BD55-1205 mIgG1

278   developed pronounced imprinting, evidenced by significantly elevated frequencies of cross-reactive

279   memory B cells at both post-boost time points compared with PBS controls (Fig. 5f). After the first

280   BA.5 boost, GC B cells in BD55-1205–treated mice also showed markedly higher cross-reactivity,

281   demonstrating that early maturation and clonal expansion within the GC was dominated by recalled,

282   imprint-driven B cells (Fig. 5g). In contrast, BD57-2665 mIgG1–treated mice showed no significant

283   change in cross-reactive memory B cell or GC B cell frequencies, indicating that non-neutralizing

284   antibody blockade does not induce imprinting, ruling out the possibility that the transferred antibody

285   simply depleted the vaccine antigen and prevented a successful BA.5 immunization (Fig. 5f-5g).

286   Correspondingly, Wuhan-RBD depletion of serum from BD55-1205–treated mice produced a more

287   severe drop in BA.5 IgG titres, whereas BD57-2665 mIgG1 and PBS groups showed minimal titre

288   reduction (Fig. 5h). Notably, the mIgG1 format induced a significantly stronger imprinting

289   phenotype than hIgG1, which is likely attributable to the species-matched Fc region (Fig. 5f-5h).

290   Pharmacokinetic analysis confirmed that the passively transferred antibodies had decayed to near-

291   undetectable levels by the time of analysis, thus eliminating their potential interference with ELISA

292   measurements (Extended Data Fig. 8a-8b). Importantly, we demonstrated that the suppressive effect

293   of BD55-1205 on the BA.5-specific response was dose-dependent, with doses as low as 12.5 μg

294   being sufficient to achieve inhibition (Extended Data Fig. 8c-8d). Together, these results confirm

295   that IGHV3-53/66-encoded A1 neutralizing antibodies cansuppress the emergence of

296   Omicron-specific B cell and antibody responses to induce severe imprinting *in vivo*.

297   This explains why immune imprinting is recapitulated in V(D)J-humanized mice but not in wild-

298   type mice—only the former harbor this human germline gene required to produce such imprinting-

299   prone antibodies. Similarly, consistent with our observation in two human cohorts (Fig. 1f), in

300   mRNA-vaccinated individuals, the potent Wuhan priming response by mRNA vaccination (elicited

301   by monovalent Wuhan vaccines and, in certain populations, bivalent Wuhan/BA.1 or Wuhan/BA.5

302   vaccines) could induce a larger pool of IGHV3-53/66-encoded A1 antibodies, thereby establishing

303   pronounced imprinting. In contrast, recipients of inactivated vaccines typically experience weaker

304   priming responses and, following stringent containment policies during 2021-2022, undergo

305   substantial waning of Wuhan antibodies. This attenuated pre-existing immunity, potentially leading

306   to lower IGHV3-53/66 antibody abundance, fails to fully suppress subsequent Omicron-specific

307   responses, resulting in more flexible and adaptive humoral evolution.

308

309   **IGHV3-53 knock-in mouse model**

310   Since wild-type mice could not recapitulate human SARS-CoV-2 immune imprinting observed in

311   mRNA vaccine recipients, evaluating SARS-CoV-2 vaccine updates and broad-spectrum vaccine

312   design in mice is highly problematic. Although V(D)J-humanized mice could capture human

313   imprinting phenotypes, its significant cost creates a pressing need for a more accessible and cost-

314   effective model for vaccine evaluation. Building on our finding that the human IGHV3-53/66

315   germline is the primary driver of immune imprinting, we engineered a knock-in mouse model by

316   replacing the murine Ighv3-1 gene with human IGHV3-53 (Supplementary Information Fig. 4b).

317   This generated both heterozygous (IGHV3-53$^{+/-}$) and homozygous (IGHV3-53$^{+/+}$) mice. High-

318   throughput Genome-wide Translocation Sequencing (HTGTS) of the naïve B cell repertoire

319   confirmed the successful knock-in, revealing IGHV3-53 usage at 0.5% in heterozygous and 1.5%

320   in homozygous mice, with a corresponding ablation of Ighv3-1 usage (0.1% and 0.0%, respectively,

321   Fig. 6a).

322   To test the model, we immunized wild-type, heterozygous, and homozygous mice using the same

323   regimen of three Wuhan primes followed by two BA.5 boosts (Fig. 6b). Subsequent flow cytometry

324   analysis and depletion assays confirmed that both IGHV3-53$^{+/-}$ and IGHV3-53$^{+/+}$ mice faithfully

325   recapitulated the strong immune imprinting phenotype (Fig. 6c-6d). The serum neutralization

326   profiles of IGHV3-53$^{+/-}$ and IGHV3-53$^{+/+}$ mice also showed a trend remarkably similar to that of

327   the V(D)J-humanized mice (Fig. 6e and Extended Data Fig. 9a). These results demonstrate that both

328   heterozygous and homozygous IGHV3-53 knock-in mice faithfully recapitulate the strong immune

329   imprinting phenotype.

330   We subsequently utilized this model to simulate the real-world SARS-CoV-2 exposure history
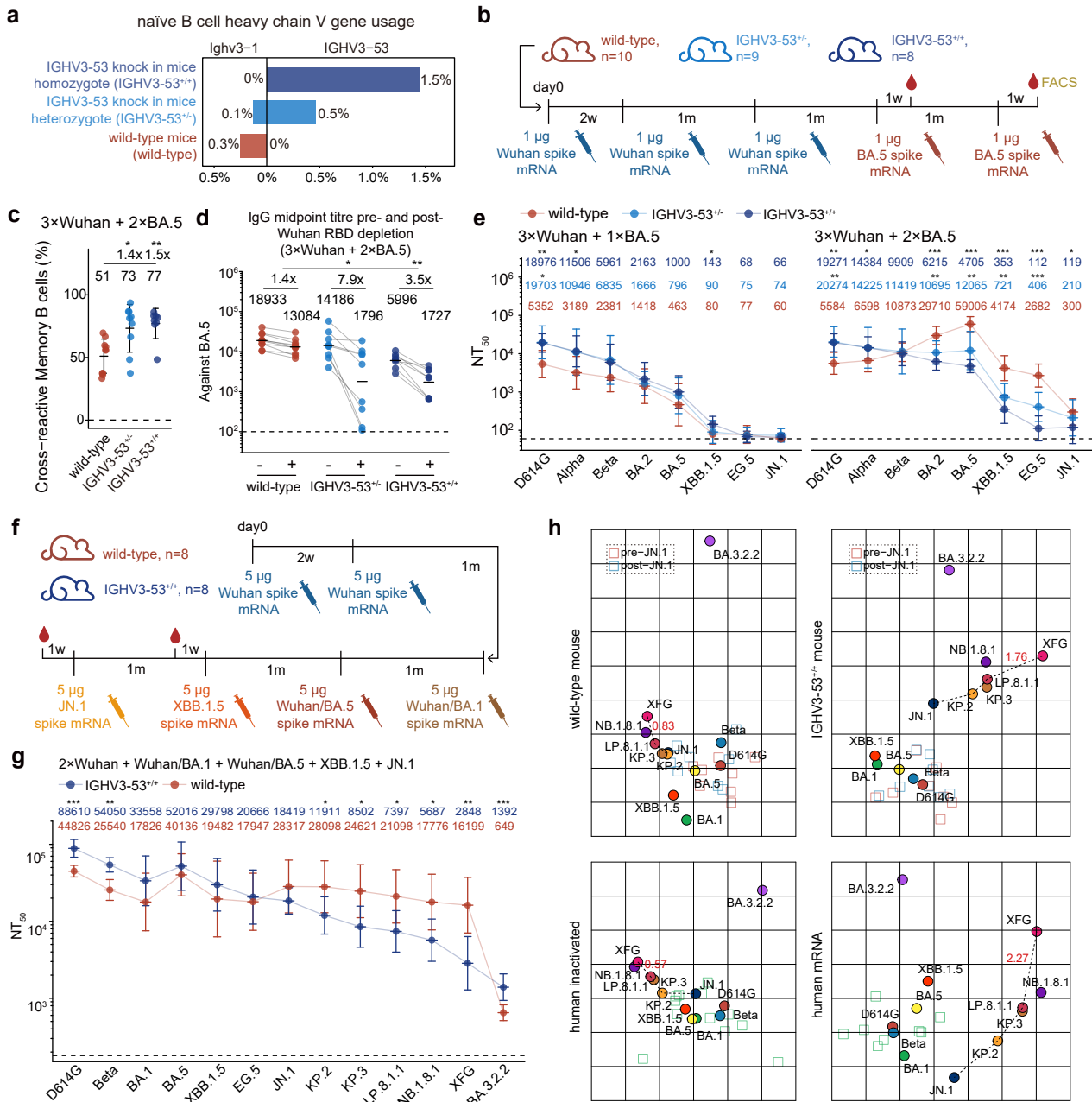
Figure 6

Figure 6 | IGHV3-53 knock-in mice faithfully reflect SARS-CoV-2 antibody map in human.

a, Usage percentage of IGHV3-53 and Ighv3-1 in naïve B cells from HTGTS sequencing of IGHV3-53$^{+/+}$ (balb/c), IGHV3-53$^{+/-}$ (balb/c* C57B6/J), and wild-type (C57B6/J) mice. b, Schematic of the immunization regimen and time points for blood collection and FACS analysis of the three mouse models involved in this study. The number of mice is indicated above the timeline. c, Scatter plots showing the proportion of cross-reactive memory B cells in draining lymph nodes of the three mouse models after two BA.5 boosts. d, Serum IgG midpoint titre of the three mouse strains after two BA.5 boosts against BA.5 RBD before and after Wuhan RBD depletion. Statistical significance of the fold-reduction in titres was assessed between groups. Dashed lines indicate the limit of detection (midpoint titre = 100). e, Serum neutralization titres (NT$_{50}$) of the three mouse strains after one (left) or two (right) BA.5 boosts against a panel of SARS-CoV-2 variant pseudoviruses. Geometric mean titres (GMTs) are shown on the top. Dashed lines indicate the limit of detection (NT50 = 60). f, Schematic of the immunization regimen simulating real-world SARS-CoV-2 exposure history. g, Serum neutralization titres (NT$_{50}$) of IGHV3-53$^{+/+}$ and wild-type mice against a panel of SARS-CoV-2 variant pseudoviruses. Geometric mean titres (GMTs) are shown on the top. Dashed lines indicate the limit of detection (NT$_{50}$ = 180). h, Antigenic cartography was performed using mouse (top) and human (bottom) serum neutralization data. Each square indicates a serum sample, and each circle indicates a SARS-CoV-2 variant. Two-tailed Wilcoxon rank-sum tests were used in c-e and g.

331   characteristic of mRNA-vaccinated populations (Fig. 6f). Specifically, mice received a two-dose

332   Wuhan priming series, mimicking the standard primary course. This was followed by sequential

333   boosters with bivalent Wuhan/BA.1 and Wuhan/BA.5 vaccines, a monovalent XBB.1.5 vaccine,

334   and a monovalent JN.1 vaccine, corresponding to the recommended mRNA booster updates during

335   the Omicron era. These regimens effectively reconstruct the antigenic trajectory of SARS-CoV-2

336   evolution encountered by humans. Compared to wild-type controls, IGHV3-53$^{+/+}$ mice recapitulated

337   a more pronounced immune imprinting phenotype following the six-dose regimen. Specifically,

338   while retaining potent neutralization against D614G and early Omicron variants (antecedent to JN.1,

339   Fig. 6g), IGHV3-53$^{+/+}$ mice exhibited a compromised breadth of neutralization against the newly

340   emerged JN.1 sublineages. Specifically, titres against KP.2, KP.3, LP.8.1.1, NB.1.8.1, and XFG were

341   significantly suppressed compared to those in wild-type mice, indicating an inhibition of *de novo*

342   responses to the newly emerged Omicron-specific epitopes in these variants. (Fig. 6g). Notably,

343   IGHV3-53$^{+/+}$ mice showed higher neutralization titres against BA.3.2.2 than wild-type mice (Fig.

344   6g), mirroring the specific serological feature observed in the human mRNA-vaccinated cohort (Fig.

345   1e). Similar trends were observed after five doses (Extended Data Fig. 10a). Furthermore,

346   comparing neutralization profiles pre- and post-JN.1 boost revealed a strong back-boosting effect

347   in IGHV3-53$^{+/+}$ mice, where JN.1 immunization significantly elevated titres against antecedent

348   strains (pre-JN.1). In contrast, wild-type mice displayed no such effect and even exhibited a

349   significant decline in D614G titres (Extended Data Fig. 10b).

350   Consequently, the two mouse models exhibited distinct susceptibility to viral escape. Following

351   XBB.1.5 immunization, neutralization against EG.5 was significantly reduced compared to

352   XBB.1.5 in IGHV3-53$^{+/+}$ mice, a reduction not seen in wild-type mice (Extended Data Fig. 10c).

353   Similarly, after the JN.1 boost, IGHV3-53$^{+/+}$ mice showed significant susceptibility to escape by

354   KP.2 and KP.3 relative to JN.1, whereas wild-type mice did not. Consistently, the fold-reduction in

355   titres against XFG relative to NB.1.8.1 was greater in IGHV3-53$^{+/+}$ mice than in wild-type mice

356   (Extended Data Fig. 10c). This result faithfully recapitulates the serological divergence observed

357   between the human mRNA-vaccinated and inactivated-only cohorts (Fig. 1e), and matches the

358   variant escaping profiles of V3-53/66 Class 1 NAbs. By leveraging the genetic precision of the

359   IGHV3-53 knock-in model to eliminate confounding immune histories, we definitively confirm that

the imprinted, V3-53/66-dominated response inherently renders mRNA recipients more susceptible to immune evasion by such emerging variants.

To visualize how IGHV3-53^{+/+} mice could help with SARS-CoV-2 vaccine update evaluation, we constructed antigenic cartography using serum neutralization data from both mouse models and human cohorts (Fig. 6h). The resulting maps revealed striking topological differences. Wild-type mice exhibited a 'condensed' antigenic landscape similar to the inactivated-only cohort, characterized by short antigenic distances between all JN.1 sublineages. In contrast, the antigenic map of IGHV3-53^{+/+} mice closely mirrors that of mRNA-vaccinated cohort, with both displaying substantial antigenic distances between JN.1, KP.2, KP.3/LP.8.1.1, and XFG, supporting vaccine antigen updates. Crucially, only the IGHV3-53^{+/+} model faithfully reproduces the substantial antigenic distance between XFG and LP.8.1.1 characteristic of the mRNA-vaccinated cohort. These findings further implicate the IGHV3-53 knock-in mice could faithfully recapitulates human SARS-CoV-2 immune imprinting, and can serve as a superior model for preclinical SARS-CoV-2 vaccine evaluation.

Discussion

Overall, our study establishes that the abundance of pre-existing IGHV3-53/66-encoded Class 1 antibodies is the primary determinant of SARS-CoV-2 immune imprinting severity. Specifically, three converging factors—the inherent potent immunogenicity of mRNA platforms in human [45], the widespread administration of bivalent mRNA boosters containing Wuhan components in mRNA-vaccinated populations, and the substantial antibody waning in inactivated vaccine recipients due to strict containment policies (e.g., zero-COVID in China) [46–48]—sufficiently infer that mRNA vaccinees harbored a significantly higher abundance of IGHV3-53/66-encoded Class 1 antibodies prior to their first Omicron exposure. Consequently, this mRNA-vaccinated population exhibits a more robust humoral imprinting phenotype. As these distinct humoral immune landscapes became established, a parallel divergence in viral evolution emerged, characterized by the regional predominance of XFG and NB.1.8.1. This co-evolution highlights the necessity of incorporating regional immunological differences into vaccine update strategies.

We acknowledge that the sample size of our human cohorts was relatively small, and intrinsic individual heterogeneity could not be fully controlled. However, the findings align well with previous studies and were mechanistically corroborated by our animal models. This consistency confirms the validity of our head-to-head comparisons and the real-world impact of immune imprinting on regional epidemiology. Additionally, we acknowledge the individual heterogeneity within our humanized mouse model, where a part of them exhibited weaker immune imprinting. We hypothesize that the isolated Omicron-specific A2 antibodies likely derived from these mice. Although the pooling of samples during B cell sorting prevents us from retrospectively tracing specific antibodies to individual animals, the existence of these outlier responses does not undermine our core conclusions that humanized mice collectively develop a significantly stronger imprinting phenotype in both serum and memory B cell compartments. These "outlier" mice likely resemble inactivated vaccine recipients, where the abrupt exposure to BA.5 resulted in extensive escape of pre-existing A1 antibodies, leaving insufficient cross-reactive binding to effectively mask Omicron-specific epitopes. In contrast, sequential exposure to BA.1/BA.2 in mRNA vaccinees involved a gradual antigenic shift, allowing A1 antibodies to mature and expand without facing the drastic escape seen with BA.5. This preserved a sufficient cross-reactive pool to effectively mask the *de novo* response upon the subsequent BA.5 exposure.

Importantly, we developed an accessible and robust IGHV3-53 knock-in mouse model that overcomes the limitations of wild-type mice, which fail to recapitulate the SARS-CoV-2 imprinting phenotype. By simulating real-world SARS-CoV-2 exposure histories in this model, we successfully mapped the humoral response landscape of mRNA human vaccinees in mice. Moving forward, this model should be leveraged to conduct parallel evaluations of boosting regimens derived from diverse, newly emerging variants. Such comparative studies will provide the essential data support required to guide the further updating and optimization of SARS-CoV-2 vaccines. Furthermore, as a platform that explicitly exposes the 'imprinted barrier' of mRNA recipients, it would enable the future research of vaccine candidates capable of overcoming such entrenched imprinting.

Data and code availability

416   Information of individuals providing blood samples and isolated mAbs involved in this study have

417   been included in the Supplementary Tables. All data and materials presented in this manuscript are

418   available from the lead contact upon a reasonable request under a completed Material Transfer

419   Agreement. Further information and requests for resources and reagents should be directed to and

420   will be fulfilled by the lead contact, yunlongcao@pku.edu.cn (Y.C.).

425   **Author contributions**

426   Y.C. designed and supervised the study. X.N., F.J. and Y.C. wrote the manuscript with input from

427   all authors. X.N., W.S., R.A. and Y.W. performed B cell sorting, single-cell V(D)J sequencing

428   experiments and data analysis. X.N., Y.L., K.L., S.L., R.K., X.C., R.A., and Y.W. performed FACS

429   analysis. H.S. and F.J. obtained and analyzed the DMS data. J.W. and F.S. performed antibody

430   expression. W.W. constructed mRNA vaccines and conducted mouse immunization. Y.Y.

431   constructed the pseudotyped virus. L.Y. performed the pseudovirus neutralization assays, ELISAs

432   and SPR experiments. Y.H., S.L., X.N. and Y.L. performed the HTGTS-Rep-seq and data analysis.

433   B.Z., T.Z., J.H., and X.T. recruited the patients and collected the blood samples.

434   **Declaration of interests**

435   Provisional patents related to the antibodies mentioned in this paper have been filed. Y.C. is a co-

436   founder of Singlomics Biopharmaceuticals. Other authors declare no competing interests.

437

438   **References**

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557

558   **Methods**

559   **Human serum and PBMC isolation**

560   Blood samples were obtained from convalescent individuals who had received various vaccine

561   platforms (detailed in Supplementary Table 1). The research protocol and the collection of human

562   blood samples were approved by the Institutional Review Broad (IRB) of Shenzhen Institute of

563   Advanced Science, Chinese Academy of Science (Ethics committee archiving no. SIAT-IRB-

564   230715-H0667) and Beijing Ditan Hospital, Capital Medical University (Ethics committee

565   archiving no. DTEC-KY2024-112-01). All participants provided their agreement for the collection,

566   storage and use of their blood samples strictly for research purposes and the subsequent publication

567   of related data.

568   To isolate serum and PBMCs, whole blood was diluted 1:1 with PBS (Invitrogen, C10010500BT)

569   containing 2% (v/v) fetal bovine serum (FBS; Hyclone, SH30406.05) and separated by density

570   gradient centrifugation using Ficoll (Cytiva, 17-1440-03). The upper serum layer was collected,

571   aliquoted, and stored at or below -20°C. Before use in assays, serum samples were heat-inactivated

572   and assessed for neutralizing titres against SARS-CoV-2 variant spike-pseudotyped vesicular

573   stomatitis virus (VSV).

574   The PBMC layer was harvested from the interface and processed further. Following red blood cell

575   lysis (Invitrogen eBioscience 1X RBC Lysis Buffer, 00-4333-57) and washing, PBMCs were either

576   used immediately or cryopreserved in FBS with 10% (v/v) DMSO (Solarbio, D8371) for storage in

577   liquid nitrogen. All PBMC samples were transported on dry ice. For B cell isolation, cryopreserved

578   PBMCs were thawed in PBS supplemented with 5% (v/v) FBS. B cells were subsequently enriched

579   by immunomagnetic positive selection using the EasySep™ Human CD19 Positive Selection Kit II

580   (STEMCELL, 17854) following the manufacturer's protocol. The resulting purified B cells were

581   resuspended in PBS with 2% (v/v) FBS, and cell counts and viability were determined using 0.4%

582   (w/v) trypan blue staining (Invitrogen, T10282) on a Countess Automated Cell Counter (Invitrogen).

583   **Mice**

584   All animal experiments were conducted under protocols approved by the Animal Welfare Ethics

585   Committee of HFK Biologics (Approval No. HFK-AP-20250313). The humanized mouse model

586   (NeoMab-IgG), provided by NeoMab Biotechnology Co., Ltd., was created by in situ replacing the

587   mouse heavy chain variable region genes and kappa light chain variable region genes with human

588   genes in BALB/c mouse background. The mouse constant region genes were retained, making sure

589   the Fc of the immunoglobulins can interact with the Fc receptors expressed on other immune cells

590   normally, supporting standard immune system development and response. The IGHV3-53$^{+/+}$ or

591   IGHV3-53$^{+/-}$ mice were generated by Cyagen Biosciences (Suzhou, China). To generate these mice,

592   the targeting construct replaced the sequence from ATG start codon to exon 3 of mouse Ighv3-1 by

593   the sequence from ATG start codon to exon 2 of the human IGHV3-53, including the introns. The

594   Cas9 protein, construct and guide RNAs (gRNA-A1: 5'-TGAGAGTGCTGATTCTTTTGTGG-3',

595   gRNA-A2:   5'-TGCAGTGCTGCTCTGCAAGGAGG-3'   and   gRNA-B1:   5'-

596   TGGCTGTTCACAGCCTTTCCTGG-3', gRNA-B2: 5'-TGGTGCAGTGCTGCTCTGCAAGG-3')

597   were microinjected into zygotes from BALB/cAnCya wild-type mice. The embryos were

598 transferred to recipient female mice to obtain F0 mice. The genotype of IGHV3-53$^{+/+}$ or IGHV3-

599 53$^{+/-}$ mice was confirmed by PCR using two pairs of primers (F4: 5'-

600 CACCATCTCCAGAGACAATTCCAAG-3', R3: 5'-GTTCTGGATACCAATGTGCCTTCAG-3'

601 and F3: 5'-TGCAGTCAGTAGCCACCTCGCCAAT-3', R4: 5'-

602 CATCAGACAGGATACCTTCAGAGAAG-3') and sequencing. Mice of all strains (6–8 weeks old)

603 were housed under specific-pathogen-free (SPF) conditions, maintained at 22 ± 2°C with 50–60%

604 humidity on a 12-hour light/dark cycle. All animals had *ad libitum* access to standard chow and

605 sterile water. Due to the limited availability of the IGHV3-53 knock-in mice, the sample size and

606 sex distribution varied slightly across the experimental groups shown in Figure 6. Female mice were

607 used for the VDJ-humanized and wild-type BALB/c models, while both sexes were used for the

608 IGHV3-53 knock-in models. Sex distribution was 4 males and 4 females for IGHV3-53$^{+/+}$ mice, 3

609 males and 6 females for IGHV3-53$^{+/-}$ mice in figure 6b, and 4 males and 4 females for IGHV3-53$^{+/+}$

610 mice in figure 6f.

611 **mRNA-LNP Synthesis and Formulation**

612 A PSP73 plasmid bearing the antigen insert followed by a 120-nt poly(T) tract was linearized with

613 the appropriate restriction enzyme. This DNA served as a template for *in vitro* transcription process

614 to generate RNA that encoded the SARS-CoV-2 Wuhan, BA.1, BA.5, XBB.1.5 and JN.1 S6P (F817P,

615 A892P, A899P, A942P, K986P, V987P, R683A and R685A) protein. Linearized DNA template (1

616 µg) was transcribed for 2 hours at 37°C using the EasyCap T7 Co-transcription Kit with CAG

617 Trimer (Vazyme, DD4203). Following transcription, the DNA template was digested by incubation

618 with 1 U of RNase-free DNase I for 15 minutes at 37°C. The resulting mRNA was purified with

619 VAHTS RNA Clean Beads (Vazyme, N412-03). The concentration and purity of the purified mRNA

620 were determined by UV spectrophotometry (absorbance at 260 nm and A260/A280), and its

621 integrity was verified by agarose gel electrophoresis.

622 The mRNA was encapsulated in a functionalized lipid nanoparticle (LNP) as described previously

623 [49]. The lipid mixture (oil phase) was prepared by dissolving SM-102, DSPC, cholesterol, and DMG-

624 PEG2000 in 100 % ethanol at a molar ratio of 50:10:38.5:1.5 to a total concentration of 9.04 mg/mL.

625 The filter-sterilized (0.22 µm) aqueous phase, consisting of 50 mM sodium citrate buffer (pH 4.0),

626 and the purified mRNA was diluted within it to a concentration of 133 µg/mL. Oil and aqueous

627 phases were then rapidly mixed at a 3:1 volume ratio using a staggered herringbone micromixer,

628 operating at a total flow rate of 12 mL/min to induce LNP self-assembly. The crude LNP suspension

629 underwent buffer exchange and purification via overnight dialysis at 4°C against a solution of 10

630 mM Tris-HCl (pH 7.4) with 8-10% (w/v) sucrose, using 10 kDa MWCO dialysis cassettes. The

631 dialyzed LNPs were subsequently concentrated by centrifugation at 1,500-2,500 × g for 10-15

632 minutes at 4°C using 10 kDa MWCO ultrafiltration units (ULRC0100150P). The final formulation

633 was adjusted to a sucrose concentration of 8.7% (w/v), aliquoted, flash-frozen in liquid nitrogen,

634 and stored at -80°C.

635 The final product was subjected to rigorous quality control. Key attributes including particle size

636 (by dynamic light scattering), RNA encapsulation efficiency (by RiboGreen assay), mRNA integrity

637 (by capillary electrophoresis), osmolality, and endotoxin levels (by LAL assay) were assessed. All

638 manufactured batches were required to meet the following release criteria: a particle diameter of 80-

639 100 nm, ≥90% RNA encapsulation, ≥80% mRNA integrity, and an endotoxin level below 1 EU/mL.

640 **Mouse immunization and passive antibody transfer**

641 For immunization, mice were administered mRNA-LNP vaccines. The specific immunization

642 regimens, including vaccine type, dosage, and timelines, are detailed in the schematics of Figure 2a,

643 5d, 6b, 6f and Extended Data figure 11a and 11c. On the day of administration, LNP-mRNA vials

644 were thawed on ice and diluted in sterile 1× PBS to the appropriate concentration. Each mouse was

645 injected intramuscularly (i.m.) into the quadriceps muscle with 100 µL of the vaccine solution using

646 a 29 G insulin syringe. To prevent leakage of the inoculum, the needle was held in place for 3–5

647 seconds post-injection.

648 For passive antibody transfer, purified mAbs (BD55-1205-mIgG1, BD55-1205-hIgG1, or BD57-

649 2665-mIgG1) with low endotoxin levels (<0.02 EU/mg) were used. The antibodies were diluted in

650 sterile PBS to the desired concentration for injection. Female BALB/c mice (6–8 weeks old)

651 received a 200 µL dose via intraperitoneal (i.p.) injection in the left lower quadrant using a 26 G

652 needle. Mice were monitored for at least 5 minutes following the injection, and any subsequent

653 doses were administered according to the specified experimental schedules.

654 **Pseudovirus preparation and neutralization assay**

655   We generated SARS-CoV-2 variant spike protein pseudovirus as described previously [13,17,26–28,50].

656   Plasmids encoding a codon-optimized SARS-CoV-2 Spike (S) protein were constructed by inserting

657   the S gene into the pcDNA3.1 vector. To produce pseudovirus, 293T cells (ATCC, CRL-3216) were

658   transfected with the S protein expressing plasmids with Lipofectamine 3000 (Invitrogen, L3000015)

659   and subsequently infected with G*ΔG-VSV (Kerafast, EH1020-PM). After 24 hours, the

660   supernatant containing the pseudovirus was harvested, filtered through a 0.45 μm filter (Millipore),

661   aliquoted, and stored at -80°C.

662   Neutralization assays were performed using the Huh-7 cell line (JCRB, 0403). Monoclonal

663   antibodies or serum samples were serially diluted in DMEM (Hyclone, SH30243.01) and incubated

664   with the pseudovirus in 96-well plates for 1 hour at 37°C with 5% CO_2. Following incubation, Huh-

665   7 cells were seeded into the wells (2×10^4 cells per well) and cultured for an additional 24 hours at

666   37°C with 5% CO_2. To assess infection levels, the culture supernatant was removed and left 100 μ

667   l in each well. The Bright-Lite Luciferase Assay Substrate was reconstituted with its corresponding

668   Assay Buffer (Vazyme, DD1209-03-AB), and this mixture was added to the wells. After incubating

669   in the dark for 2 minutes, luminescence was measured using a microplate spectrophotometer

670   (PerkinElmer, HH3400). The NT_{50} or IC_{50} values were determined using a three-parameter logistic

671   regression model.

672   **Mouse tissue processing and B cell extraction**

673   Following euthanasia, the spleen, inguinal lymph nodes, and popliteal lymph nodes were harvested

674   and placed in RPMI 1640 culture medium (Invitrogen, C11875500CP) containing 5% (v/v) FBS.

675   Single-cell suspensions were prepared by mechanical disruption using the plunger of a syringe. The

676   popliteal and inguinal lymph nodes from each mouse were pooled, ground, and filtered through a

677   40 μm cell strainer. For the spleen, tissue was processed by the same grinding method and passed

678   through a 70 μm cell strainer, followed by centrifugation and lysis of red blood cells using $1\times$ RBC

679   Lysis Buffer (Invitrogen eBioscience, 00-4333-57). After washing steps and centrifugation, the

680   resulting cell pellets were resuspended in PBS containing 2% (v/v) FBS.

681   Splenic B cells were enriched from the splenic single-cell suspensions via immunomagnetic

682   negative selection with the EasySep™ Mouse Pan-B Cell Isolation Kit (STEMCELL, 19844).

683   Following the manufacturer's protocol, the untouched, purified B cells were collected and washed

684   in PBS with 2% (v/v) FBS. The cell numbers of total lymph node cells and purified splenic B cells

685   were determined using 0.4% (w/v) trypan blue stain (Invitrogen, T10282) and a Countess

686   Automated Cell Counter (Invitrogen).

687   **Flow cytometry analysis and antigen-specific B cell sorting**

688   For the isolation of antigen-specific human B cells, enriched B cell populations from PBMCs were

689   prepared for fluorescence-activated cell sorting (FACS). The staining panel included FITC anti-

690   human CD20 (BioLegend, 302304), Brilliant Violet 605™ anti-human CD27 (BioLegend, 302824),

691   PE/Cyanine7 anti-human IgM (BioLegend, 314532), and PE/Cyanine7 anti-human IgD (BioLegend,

692   348210). Antigen-specific cells were detected using biotinylated JN.1 RBD (Sino Biological,

693   40592-V49H16-B) conjugated with PE-streptavidin (BioLegend, 405204) and APC-streptavidin

694   (BioLegend, 405207), and Wuhan RBD (Sino Biological, 40592-V27H-B) conjugated with BV421-

695   streptavidin (BioLegend, 405225). The viability dye 7-AAD (Invitrogen, 00-6993-50) was used to

696   exclude dead cells. A gating strategy was applied to sort single, viable (7-AAD$^-$), class-switched

697   (IgM$^-$ and IgD$^-$), CD20$^+$CD27$^+$ memory B cells that were positive for JN.1 RBD.

698   For the characterization of B cell responses in immunized mice, single-cell suspensions from the

699   inguinal and popliteal lymph nodes were stained for flow cytometry analysis. The cells were stained

700   with a panel including PE/Cyanine7 anti-mouse CD38 (BioLegend, 102718), Brilliant Violet 605™

701   anti-mouse/human B220 (BioLegend, 103244), APC/Cyanine7 anti-mouse IgD (BioLegend,

702   405716), Brilliant Violet 711™ anti-mouse IgM (BioLegend, 406539), and FITC anti-mouse/human

703   GL7 (BioLegend, 144603). The antigen probe cocktail consisted of biotinylated BA.5 RBD (Sino

704   Biological, 40592-V49H9-B) conjugated with PE- and APC-streptavidin, and Wuhan RBD

705   conjugated with BV421-streptavidin. Data for lymph node analysis were acquired on a Symphony

706   A5SE cytometer (BD Biosciences).

707   For the isolation of antigen-specific mouse splenic B cells, purified splenic B cells were stained

708   using the identical panel of antibodies and RBD probes as described for the lymph node analysis.

709   MoFlo Astrios EQ Cell Sorter (Beckman Coulter) was used for all sorting experiments, targeting

710   live (7-AAD$^-$), B220$^+$, CD38$^+$, class-switched (IgM$^-$ and IgD$^-$), non-germinal center (GL7$^-$) B cells

711   that bound to the BA.5 RBD.

712   For all procedures, data were collected via Summit 6.0 software (Beckman Coulter). Data from all

713   experiments were uniformly analyzed using FlowJo v10.8 (BD Biosciences).

714   **Single-cell V(D)J sequencing**

715   For the 10X Genomics workflow, sorted antigen-specific B cells, suspended in PBS with 10% (v/v)

716   FBS, were processed with the Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1 (10X

717   Genomics, CG000208). The cell suspension was loaded onto a 10X Chromium Controller to

718   generate Gel Beads-in-Emulsion (GEMs), which facilitate the barcoding of mRNA and subsequent

719   reverse transcription within individual droplets. Following cDNA synthesis, the product was

720   purified using a SPRIselect Reagent Kit (Beckman Coulter, B23318) and pre-amplified. Targeted

721   enrichment of paired V(D)J sequences was then achieved using 10X-specific BCR primers, and the

722   resulting products were used for sequencing library construction. These final libraries were

723   sequenced on an Illumina NovaSeq 6000 platform with a NovaSeq 6000 S4 Reagent Kit v1.5 (300

724   cycles; Illumina, 20028312). The 10X Genomics V(D)J Illumina sequencing data were assembled

725   as B cell receptor contigs and aligned to the B cell V(D)J reference using Cell Ranger (v.6.1.1)

726   pipeline. For human-source IGH and IGK contigs, we use GRCh38 as reference. For mouse-source

727   IGL contigs, we use GRCm38 as reference. Only the productive contigs and B cells with one heavy

728   chain and one light chain were kept to remove doublets. The germline V(D)J genes were identified

729   and annotated using IgBlast (v1.17.1) [51]. SHM nucleotides and residues in the antibody variable

730   domain were detected using Change-O toolkit (v.1.2.0) [52].

731   For the plate-based method, single antigen-specific B cells were sorted directly into the individual

732   wells of 96-well plates, each containing 4 µL of lysis buffer (0.095% Triton X-100, 2.5 µM oligo-

733   dT   primer: 5'-

734   AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3',

735   2.5 mM dNTPs, 1 U/µL RNase Inhibitor). The plates were immediately processed by vortexing,

736   centrifugation, and incubation at 72°C for 3 min to denature RNA secondary structures, followed

737   by rapid chilling on ice. Reverse transcription was performed in each well by adding 5.7 µL of an

738   RT master mix (final concentrations: 1× HiScript III first-strand buffer, 10 U/µL HiScript III

739 reverse transcriptase, 2 U/μL RNase inhibitor, 5 mM DTT, 1 M betaine, 6 mM MgCl$_2$, 1 μM TSO

740 primer: 5'-AAGCAGTGGTATCAACGCAGAGTACATrGrG+G-3'). The RT reaction was

741 incubated at 37°C for 60 minutes, followed by enzyme inactivation at 85°C for 5 seconds. The

742 resulting cDNA served as a template for amplifying full-length immunoglobulin heavy and light

743 chain V(D)J sequences via a two-round nested PCR strategy using Phanta Max Master Mix (Vazyme,

744 P515). The first PCR round employed a multiplex primer pool targeting all VH, Vκ, and Vλ gene

745 families, along with constant region-specific reverse primers. A second, nested PCR round was then

746 performed with internal primers to enhance specificity and yield. Final amplicons were purified and

747 subsequently analyzed by Sanger sequencing. The germline V(D)J genes were identified and

748 annotated using IgBlast (v1.22.0) [51].

749 **HTGTS-Rep-seq**

750 0.5-4 μg of genomic DNA from purified splenic B cells was used for generating HTGTS-Rep-seq

751 libraries as previously described [53]. Four bait primers that target mouse JH1 (5'-

752 TGACATGGGGAGATCTGAGA-3'), JH2 (5'-CCCCAACAAATGCAGTAAAATCT-3'), JH3 (5'-

753 GAGAATCTTGGTCCTGAAGGC-3'), and JH4 (5'-CTGCAATGCTCAGAAAACTCC-3') were

754 mixed to capture all heavy chain (HC) repertoire in one library. All primers carried a 5′ biotin

755 modification (5BiosG). These HTGTS-Rep-seq libraries were sequenced on DNBSEQ-T7. Data

756 were analyzed with the HTGTS-Rep-seq pipeline [53].

757 **Monoclonal antibody expression and purification**

758 The sequences for the antibody heavy and light chains were initially codon-optimized for expression

759 in human cells (GenScript). The variable regions (VH and VL) were then separately inserted into

760 corresponding expression vectors (pCMV3-CH, pCMV3-CL or pCMV3-CK). Plasmids of the

761 heavy and light chain constructs were transformed into *Escherichia coli* DH5α competent cells

762 (Tsingke, TSC-C01-96). After overnight incubation at 37°C, single colonies were picked for colony

763 PCR identification. Plasmid DNA of expanded cultures was extracted (CWBIO,CW2105) after

764 verified by Sanger sequencing.

765 For protein production, heavy and light chain plasmids were co-transfected into Expi293F cells

766 (Thermo Fisher, A14527) using polyethylenimine (PEI; Yeasen, 40816ES03). The plasmid-PEI

767 complexes were prepared in sterile 0.9% NaCl solution before being added to the cell culture. The

768 transfected cells were cultured at 36.5°C with 5% CO_2 and 175 rpm shaking for 6–10 days. A

769 nutrient supplement (OPM Biosciences, F081918-001) was added to each culture at 24 hours post-

770 transfection and every 48 hours thereafter.

771 To purify the antibodies, the culture supernatant was first clarified by centrifugation (3,000 × g, 10

772 minutes). The supernatant was then incubated with Protein A magnetic beads (GenScript, L00695)

773 for 2 hours to allow antibody binding. The beads were subsequently washed, and the bound

774 antibodies were eluted using a KingFisher automated purification system (Thermo Fisher). The

775 concentration of the purified antibody was determined using a NanoDrop spectrophotometer

776 (Thermo Fisher, 840-317400), and its purity was assessed by SDS-PAGE (LabLead, P42015).

777 **RBD depletion of serum**

778 To deplete RBD-specific antibodies from serum, 50 µL of Dynabeads™ MyOne™ Streptavidin T1

779 (Invitrogen, 65601) were washed once with PBS. 10 µg of biotinylated SARS-CoV-2 Wuhan RBD

780 was incubated with the washed beads for 1 hours with gentle rotation to allow binding via

781 streptavidin-biotin interaction. The beads were then collected using a magnetic rack for 2-3 min, the

782 supernatant was discarded, and the beads were washed three times with PBS to remove unbound

783 proteins. Subsequently, 200-400 µL of serum was incubated with the RBD-conjugated beads for

784 1 hours with gentle rotation to allow specific antibody binding. Finally, the tubes were placed on the

785 magnetic rack, and the supernatant representing the RBD-depleted serum was carefully collected

786 for downstream analyses.

787 **Enzyme-linked immunosorbent assays**

788 High-binding 96-well plates (NEST, 504201) were coated overnight at 4°C with SARS-CoV-2

789 Wuhan (Sino Biological, 40592-V27H-B) or BA.5 RBD proteins (Sino Biological, 40592-V49H9-

790 B). The following day, plates were washed three times with 1×PBST (Solarbio, P1033) and blocked

791 with 250 µL 3–5% bovine serum albumin (BSA; Solarbio, A8020) in 1×PBST for 2 hours at 37°C

792 to prevent non-specific binding. After three additional washes, 100 µL of serially diluted antibodies

793 or serum samples were added to the wells and incubated for 30 minutes at room temperature.

794 Unbound antibodies were removed by five washes with 1×PBST. Subsequently, 100 µL of HRP-

795   conjugated Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (Invitrogen, G21040)

796   or Peroxidase AffiniPure Goat Anti-Human IgG (H+L) (Jackson Immunoresearch, 109-035-003)

797   was added and incubated for 30 minutes at room temperature. Following a final five washes, the

798   signal was developed by adding 100 μL of TMB substrate (Solarbio, PR1200) to each well and

799   incubating for 8 minutes in the dark. The reaction was terminated by adding 50 μL of stop solution

800   (Solarbio, C1058). The optical density (OD) was measured at 450 nm with a reference wavelength

801   of 630 nm using a Multiskan FC microplate reader (Thermo Scientific). Final absorbance values

802   were obtained by subtracting the OD630 reading from the OD450 reading for each well.

803   **Surface plasmon resonance**

804   Surface plasmon resonance (SPR) experiments were conducted using a Biacore 8K+ system (Cytiva)

805   at room temperature. For competitive binding assays, His-tagged SARS-CoV-2 BA.5 RBD protein

806   (5 μg/mL) was immobilized onto an anti-His-tagged CM5 sensor chip (Cytiva) for 1 minute.

807   Subsequently, a saturating concentration of the first antibody (Ab1, 20 μg/mL) was injected for 2

808   minutes, immediately followed by the injection of the second antibody (Ab2, 20 μg/mL) for another

809   2 minutes. The sensor surface was regenerated between cycles using a glycine solution (pH 1.5).

810   All binding data were recorded and processed using Biacore 8K Evaluation Software (v4.0.8.20368).

811   The degree of epitope competition was calculated using the following formula:

812
$$score_{Ab2-Ab1} = 1 - \frac{response_{Ab2\ after\ Ab1}}{\overline{response_{Ab2\ alone}}}$$

813   Where $response_{Ab2\ after\ Ab1}$ represents the response units when Ab2 serves as the second

814   antibody and Ab1 as the first antibody, whereas $\overline{response_{Ab2\ alone}}$ denotes the mean response

815   units when Ab2 acts as the first antibody.

816   **DMS library construction**

817   Replicate deep mutational scanning (DMS) libraries of the SARS-CoV-2 BA.5 RBD (residues

818   N331–T531; Wuhan-Hu-1 numbering) were generated based on established protocols [13,39] with a

819   modification. Rather than using a pooled primer mix, we performed 201 individual PCR reactions,

820   each using a specific NNS primer pair to introduce all possible amino acid substitutions at a single

821   target residue. The resulting products from each single-site mutagenesis reaction were then

822　combined to form the final comprehensive library. Each unique RBD variant was subsequently

823　tagged with a distinct 26-nucleotide (N26) barcode via PCR. The mutagenized and barcoded RBD

824　sequences were then cloned into pETcon 2649 vector and the resulted plasmid libraries were

825　amplified in electrocompetent *E. coli* DH10B cells. The association between every RBD variant and

826　its corresponding N26 barcode was established by preparing PacBio sequencing libraries and

827　performing long-read sequencing on the Sequel II platform. Amplified DMS plasmid libraries were

828　transformed into *Saccharomyces cerevisiae* strain EBY100. Transformed yeast cells were initially

829　selected on SD-CAA agar plates. Positive clones were then expanded by culturing in SD-CAA liquid

830　media. The resulting comprehensive DMS yeast libraries were preserved by flash-freezing in liquid

831　nitrogen and stored at -80 °C.

832　Magnetic beads-based antibody mutation escape profiling

833　High-throughput mutation escape profiling for mAbs was performed using magnetic beads based

834　on previously established protocols [13,39]. DMS yeast libraries (Wuhan and BA.5) first underwent

835　functional pre-screening. Non-functional or misfolded RBD variants were removed using ACE2-

836　biotin conjugate (Sino Biological, 10108-H08H-B) bound to streptavidin magnetic beads (Thermo

837　Fisher, 11533D). ACE2-bound yeast cells were washed with PBS containing 0.1% (v/v) BSA,

838　released, expanded in SD-CAA liquid medium, and cryopreserved at -80°C as functional libraries.

839　For Antibody Escape Selection, thawed functional libraries were cultured overnight in SD-CAA

840　with shaking, then back-diluted into SG-CAA medium to induce RBD surface expression. Escape

841　variants of each mAb were isolated using a sequential selection strategy, with 2 rounds of negative

842　selection to deplete antibody-binding variants and 1 round of positive selection to capture the

843　antibody-escaping population using anti-c-Myc magnetic beads (Thermo Fisher, 88843). The final

844　sorted yeasts were washed, regrown overnight and subjected to plasmid extraction using a 96-well

845　kit (Coolaber, PE053). The unique N26 barcodes appended to each RBD variant were amplified by

846　PCR using extracted plasmid as template. PCR products were purified with Ampure XP beads

847　(Beckman Coulter, A63882) and subjected to high-throughput single-end sequencing (NextSeq

848　500/550 platforms or MGI200 platforms).

849　Antibody DMS data analysis

850 The raw sequencing data from the DMS were processed as previously described [13]. Specifically, the

851 barcode sequences detected from both the antibody-screened and reference libraries were aligned

852 with a barcode-variant dictionary derived from PacBio sequencing data of the Wuhan and BA.5

853 DMS libraries using the alignparse (v.0.6.2) and dms_variants (v.1.4.3) tools. Ambiguous barcodes

854 were excluded during the merging of yeast libraries. Only barcodes detected more than five times

855 in the reference library were considered for further analysis. The escape score for a variant X,

856 present in both the screened and reference libraries, was calculated as $F \times (n_{X,ab}/N_{ab})/(n_{X,ref}/N_{ref})$,

857 where F is a scaling factor to normalize the scores to a 0–1 range, and n and N represent the numbers

858 of detected barcodes for variant X and the total barcodes in the antibody-screened (ab) or reference

859 (ref) samples, respectively. For antibodies subjected to DMS with multiple replicates using different

860 mutant libraries, the final escape score for each mutation was averaged for subsequent analyses.

861 We used graph-based unsupervised clustering and embedding to assign an epitope group to each

862 antibody and visualize them in a two-dimensional space. Initially, site escape scores (sum of

863 mutation escape scores per residue) for each antibody were normalized to a sum of one, representing

864 a distribution over RBD residues. The dissimilarity between two antibodies is defined based on the

865 Pearson's correlation coefficient of their escape score vectors using numpy (v1.25.2). A k-nearest-

866 neighbour graph was constructed using the python-igraph module (v.0.9.6), and Leiden clustering

867 was applied to assign a cluster to each antibody [54]. Cluster names were manually annotated on the

868 basis of the characteristic sites in the average escape profiles of each cluster, using the same

869 nomenclature as our previously published DMS dataset [13]. To visualize the dataset in two

870 dimensions, uniform manifold approximation and projection was performed based on the k-nearest-

871 neighbour graph using umap-learn module (v.0.5.2), and figures were generated using R package

872 ggplot2 (v.3.3.3).

873 To compute the average immune pressure or identify escape hotspots using a collection of mAb

874 DMS profiles, we incorporating two types of weight to account for the impact of each mutation on

875 neutralizing activity and codon constraints at each residue. For codon usage constraints, mutations

876 inaccessible through single nucleotide changes were assigned a weight of zero, whereas others

877 received a weight of 1.0. We used Wuhan/D614G (Wuhan-Hu-1 reference genome) and BA.4/5

878 (EPI_ISL_11207535) to define one-nucleotide-accessible amino acid mutations. Neutralizing

879   activity weights were calculated as $-\log10$ (IC_{50}), with IC_{50} values below 0.0005 or above 1.0

880   adjusted to 0.0005 or 1.0, respectively. Raw escape scores for each antibody were normalized by

881   the maximum score across all mutants. The weighted score for each antibody and mutation was

882   obtained by multiplying the normalized scores by the corresponding two weights, and the final

883   mutation-specific weighted score was the sum of scores for all antibodies in the designated set,

884   subsequently normalized to a 0–1 range. To visualize the calculated escape maps, sequence logos

885   were generated using the Python module logomaker (v.0.8).

886

887   **Figure legends**

888   **Figure 1 | mRNA and inactivated vaccine priming induce distinct levels of immune imprinting.**

889   **a,** Schematic of the SARS-CoV-2-related immune histories and sampling time of the inactivated-

890   only and mRNA-vaccinated cohorts involved in this study. **b,** Representative flow cytometry dot

891   plots of cross-reactive memory B cells from the inactivated-only (top) and mRNA-vaccinated

892   (bottom) cohorts. APC, allophycocyanin; PE, phycoerythrin; BV421, Brilliant Violet 421. **c,** Scatter

893   plots showing the proportion of cross-reactive memory B cells (left), JN.1-reactive memory B cells

894   (middle), and Wuhan-reactive memory B cells (right). **d,** Serum IgG midpoint titre against Wuhan

895   (left) or JN.1 (right) RBD before and after Wuhan RBD depletion. Statistical significance of the

896   fold-reduction in titres was assessed between two cohorts. Dashed lines indicate the limit of

897   detection (midpoint titre = 10). **e,** NAb response of the two cohorts against a panel of SARS-CoV-2

898   variant pseudoviruses. Geometric mean titres (GMTs) are shown on the top. Dashed lines indicate

899   the limit of detection ($NT_{50} = 10$). **f,** Frequency of heavy chain V gene usage of the two cohorts.

900   IGHV3-53 and IGHV3-66 were combined for analysis. Two-tailed Wilcoxon rank-sum tests were

901   used in **c-e**. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$; NS, not significant ($P > 0.05$).

902   **Figure 2 | V(D)J-humanized mice recapitulate severe SARS-CoV-2 imprinting.**

903   **a,** Schematic of the immunization regimen and sampling timeline for wild-type and V(D)J-

904   humanized mice. The number of mice is indicated at the time point for each endpoint experiment.

905   **b,** Scatter plots showing the proportion of cross-reactive memory B cells in draining lymph nodes

906   of the two mouse strains after one (left) or two (right) BA.5 boosts. **c,** Serum IgG midpoint titre of

907   the two mouse strains after one (left) or two (right) BA.5 boosts against Wuhan or BA.5 RBD before

908   and after Wuhan RBD depletion. Statistical significance of the fold-reduction in titres was assessed

909   between humanized and wild-type mice. Dashed lines indicate the limit of detection (midpoint

910   titre = 100). **d,** Serum neutralization titres (NT_{50}) of the two mouse models after Wuhan priming

911   (left), one BA.5 boost (middle), or two BA.5 boosts (right) against a panel of SARS-CoV-2 variant

912   pseudoviruses. Geometric mean titres (GMTs) are shown on the top. Dashed lines indicate the limit

913   of detection ($NT_{50} = 60$). Two-tailed Wilcoxon rank-sum tests were used in **b-d**.

914   **Figure 3 | Distinct antibody landscapes between mRNA vaccinated humanized and wild-type**

915   **mice.**

916   **a–c**, Normalized average DMS escape scores for mAbs isolated from humanized (top) and wild-

917   type (bottom) mice following Wuhan priming (**a**), the first BA.5 booster (**b**), or the second BA.5

918   booster (**c**). Escape scores were aggregated and weighted by the IC_{50} of each individual mAb (To

919   focus on the neutralizing mAbs, lower IC_{50} corresponds to greater weight) against D614G for

920   Wuhan-primed groups or BA.5 for BA.5-boosted groups. Codon constraints were applied (see

921   Methods). In each panel, the ten residues with the highest cumulative escape scores are annotated,

922   with their specific mutational escape profiles visualized as logo plots above the scatter plots. To

923   highlight divergent immune pressure, residues exhibiting pronounced differences between

924   humanized and wild-type strains (as identified in Extended Data Fig. 6) are colored red in both the

925   scatter plots and logo plots.

926

927   **Figure 4 | V(D)J germline difference shapes vaccination- induced SARS-CoV-2 antibody**

928   **epitope distribution.**

929   Epitope distribution of the antibody repertoire generated after Wuhan priming in humanized (**a**) and

930   wild-type mice (**b**). The Wuhan RBD structure (PDB: 6m0j) is displayed as a surface map colored

931   by normalized aggregate escape scores, with major epitope groups outlined in distinct colors. Their

932   neutralizing category, antibody count, and percentage are indicated. Neutralizing category is

933   classified based on geometric mean of IC_{50}: Strong neutralizing (<0.1 µg/mL), Neutralizing (0.1 ≤

934   IC_{50} < 1 µg/mL), and Weak neutralizing (1 ≤ IC_{50} ≤ 10 µg/mL). Epitope groups accounting for <5%

935   of the total antibodies are not labeled. Key escape residues for each group are displayed as logos,

936   and the sites with the highest escape scores per group are labeled on the structure. Paired heavy and

937   light chain V-gene usage for each epitope is shown in Sankey plots. **c,** Pyramidal bar charts showing

938   the proportional distribution of epitope groups in antibodies isolated from humanized and wild-type

939   mice after Wuhan priming and after one or two BA.5 boosts. Bars are colored according to the log10

940   geometric mean IC_{50} of antibodies within each group.

941   **Figure 5 | IGHV3-53/66-encoded Class 1 antibody drives SARS-CoV-2 imprinting through**

942   **epitope masking.**

a, Donut plots showing the cross-reactivity of A1 antibodies from humanized or wild-type mice. The number of antibodies are indicated in the centre of the donut. Antibodies exhibiting ELISA OD450 values > 2 against both WT and BA.5 RBDs (1 μg/mL) were defined as cross-reactive. Those showing an OD450 > 2 for one variant but < 2 for the other were classified as specific. **b,** Heatmap of competitive SPR for various antibody groups. The definition of the competition score is described in the Methods section. **c,** Schematic of the molecular mechanism by which pre-existing IGHV3-53/66-encoded A1 antibodies cause strong immune imprinting. **d,** Schematic of the antibody passive transfer experiment. Timing of mRNA vaccinations, antibody injection, blood collection, and FACS analysis are indicated. Mice were divided into experimental groups (receiving 400 μg BD55-1205-hIgG1, 200 μg BD55-1205-hIgG1, or 200 μg BD55-1205-mIgG1) and control groups (receiving 200 μg BD57-2665-mIgG1 or PBS). The number of mice per group is indicated at the endpoint. **e,** DMS escape map logoplots for BD55-1205 and BD57-2665 and their projection onto the SARS-CoV-2 Wuhan RBD (PDB: 6m0j). **f,** Scatter plots showing the proportion of cross-reactive memory B cells in draining lymph nodes after one (left) or two (right) BA.5 boosts. **g,** Scatter plots showing the proportion of cross-reactive germinal center B cells in draining lymph nodes after one BA.5 boost. **h,** Serum IgG midpoint titre against BA.5 RBD before and after Wuhan RBD depletion. Statistical significance of the fold-reduction in titres was assessed between groups. Dashed lines indicate the limit of detection (midpoint titre = 100). Two-tailed Wilcoxon rank-sum tests were used in **f-h.**

Figure 6 | IGHV3-53 knock-in mice faithfully reflect SARS-CoV-2 antibody map in human.

a, Usage percentage of IGHV3-53 and Ighv3-1 in naïve B cells from HTGTS sequencing of IGHV3-53$^{+/+}$ (balb/c), IGHV3-53$^{+/-}$ (balb/c* C57B6/J), and wild-type (C57B6/J) mice. **b,** Schematic of the immunization regimen and time points for blood collection and FACS analysis of the three mouse models involved in this study. The number of mice is indicated above the timeline. **c,** Scatter plots showing the proportion of cross-reactive memory B cells in draining lymph nodes of the three mouse models after two BA.5 boosts. **d,** Serum IgG midpoint titre of the three mouse strains after two BA.5 boosts against BA.5 RBD before and after Wuhan RBD depletion. Statistical significance of the fold-reduction in titres was assessed between groups. Dashed lines indicate the limit of detection (midpoint titre = 100). **e,** Serum neutralization titres (NT$_{50}$) of the three mouse strains after one (left)

972   or two (right) BA.5 boosts against a panel of SARS-CoV-2 variant pseudoviruses. Geometric mean

973   titres (GMTs) are shown on the top. Dashed lines indicate the limit of detection ($NT_{50} = 60$). **f,**

974   Schematic of the immunization regimen simulating real-world SARS-CoV-2 exposure history. **g,**

975   Serum neutralization titres (NT_{50}) of IGHV3-53$^{+/+}$ and wild-type mice against a panel of SARS-

976   CoV-2 variant pseudoviruses. Geometric mean titres (GMTs) are shown on the top. Dashed lines

977   indicate the limit of detection ($NT_{50} = 180$). **h,** Antigenic cartography was performed using mouse

978   (top) and human (bottom) serum neutralization data. Each square indicates a serum sample, and

979   each circle indicates a SARS-CoV-2 variant. Two-tailed Wilcoxon rank-sum tests were used in **c-e**

980   and **g**.

981   **Extended Data Figure 1 | Different epidemiological landscape of XFG and NB.1.8.1 in 2025.**

982   **a,** Prevalence of XFG and NB.1.8.1 in China and the rest of the world as of October 5, 2025. The

983   asterisk (*) denotes the specific lineage and its sublineages. Data were retrieved from CovSpectrum

984   (https://cov-spectrum.org/).

985   **Extended Data Figure 2 | Enrichment of IGHV3-53/66 and high SHM rate characterize the**

986   **mRNA-imprinted antibody repertoire.**

987   **a,** Clonal analysis of memory B cells from individuals in the mRNA-vaccinated cohort. Single

988   clones are colored white. The numbers of B cells analyzed are labelled in the centre of the donut

989   plots. **b,** Paired heavy and light chain V-gene usage for memory B cells from the mRNA-vaccinated

990   and inactivated-only cohorts. **c, d,** Violin plots comparing somatic hypermutation (SHM) rates

991   between the two cohorts, shown for heavy and light chains (**c**) and representative heavy chain V-

992   genes (**d**). The number of sequences (n) is indicated above each plot. Two-tailed Wilcoxon rank-

993   sum tests were used in **d**.

994   **Extended Data Figure 3 | V(D)J-humanized mice exhibit a cellular imprinting phenotype**

995   **dominated by cross-reactive GC and memory B cells.**

996   **a-c,** Scatter plots showing the proportion of cross-reactive GC B cells (**a**), total GC B cells (**b**),

997   and class-switched memory B cells (**c**). The indicated percentages reflect the frequency relative to

998   their respective parental gates defined in Supplementary Information Fig. 1b.

999 **Extended Data Figure 4 | Repeated Omicron boosters fail to elicit robust Omicron-specific**

1000 **neutralization in imprinted humanized mice.**

1001 **a,** Line plots showing longitudinal pseudovirus neutralization titres (NT_{50}) against a panel of

1002 SARS-CoV-2 variants in paired humanized and wild-type mice across Wuhan priming and BA.5

1003 booster timepoints; lines connect data from the same mouse. Dashed lines indicate the limit of

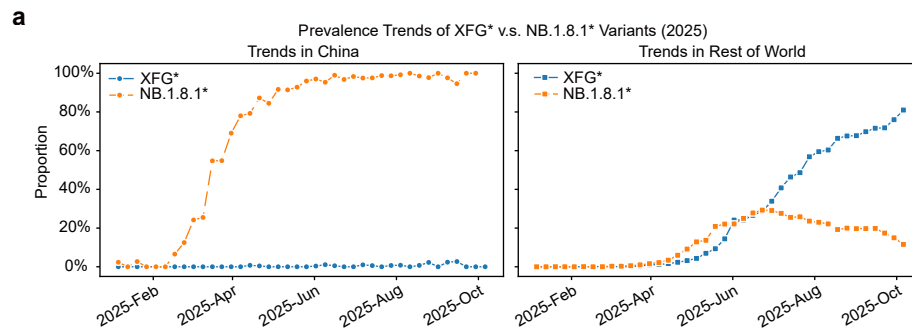1004 detection ($NT_{50} = 60$). Two-tailed Wilcoxon rank-sum tests were used.

1005 **Extended Data Figure 5 | Neutralization potency and cross-reactivity of mAbs from**

1006 **humanized and wild-type mice.**

1007 **a,b,** Comparison of antibody IC_{50} values against D614G and BA.5(**a**) and cross-reactivity

1008 proportion (**b**) from humanized and wild-type mice. Two-tailed Wilcoxon rank-sum tests were

1009 used in **a**. Antibodies exhibiting ELISA OD450 values > 2 against both WT and BA.5 RBDs (1

1010 μg/mL) were defined as cross-reactive. Those showing an OD450 > 2 for one variant but < 2 for

1011 the other were classified as specific.

1012 **Extended Data Figure 6 | Differential escape hotspots in humanized versus wild-type mice.**

1013 **a–c,** Scatter plots comparing the normalized average DMS escape scores of neutralizing

1014 antibodies isolated from humanized (x-axis) versus wild-type (y-axis) mice following Wuhan

1015 priming (**a**), the first BA.5 booster (**b**), and the second BA.5 booster (**c**). Residues falling along the

1016 diagonal (y=x) indicate shared immune pressure between the two models. To identify divergent

1017 hotspots—including those with low-to-moderate scores in one strain that are absent in the other—

1018 a shaded tolerance region was defined by the boundaries y = 1.4x + 0.3 and y = 0.6x - 0.18. Points

1019 falling outside this shaded region represent distinct escape hotspots. Among these outliers, the five

1020 residues with the highest escape scores in each group are highlighted in red. Related to Fig. 3.

1021 **Extended Data Figure 7 | Distinct SARS-CoV-2 antibody epitope distribution after**

1022 **sequential BA.5 boosts.**

1023 **a-d,** Epitope distribution of the antibody repertoire generated after one BA.5 boost (**a, b**) or two

BA.5 boosts (**c, d**) in humanized mice (**a, c**) and wild-type mice (**b, d**). Related to Figure 4. **e,**
Pyramidal bar charts showing the proportional distribution of epitope groups in antibodies isolated
from humanized and wild-type mice after one or two BA.5 boosts. Bars are colored according to
the log10 geometric mean IC_{50} of antibodies within each group.

**Extended Data Figure 8 | Pharmacokinetics and dose-dependent suppressive effects of
antibodies used in passive transfer.**

a, Schematic of the *in vivo* monoclonal antibody pharmacokinetic study. **b,** Serum IgG titres over
time following antibody injection (mIgG1 forms were detected using an anti-mouse Fc secondary
antibody, and hIgG1 forms using an anti-human Fc secondary antibody). Dashed lines indicate the
limit of detection (midpoint titre = 100). **c,** Schematic of the BD55-1205 dose-ranging experiment.
d, Corresponding serum IgG titres before and after Wuhan RBD depletion. Statistical significance
of the fold-reduction in titres was assessed between groups. Dashed lines indicate the limit of
detection (midpoint titre = 100). Two-tailed Wilcoxon rank-sum tests were used in **d**.

**Extended Data Figure 9 | IGHV3-53 knock-in mice exhibit diminished Omicron
neutralization boosting compared to wild-type mice.**

a, Line plots showing longitudinal pseudovirus neutralization titres (NT_{50}) against a panel of
SARS-CoV-2 variants in paired IGHV3-53$^{+/+}$ (top), IGHV3-53$^{+/-}$ (middle), and wild-type (bottom)
mice across BA.5 booster timepoints; lines connect data from the same mouse. Dashed lines
indicate the limit of detection (NT_{50} = 60). Two-tailed Wilcoxon rank-sum tests were used.

**Extended Data Figure 10 | Strong back-boosting of Wuhan immunity in IGHV3-53 KI mice
restricts the breadth of neutralization against emerging variants.**

a, Serum neutralization titres (NT_{50}) of IGHV3-53$^{+/+}$ and wild-type mice against a panel of SARS-
CoV-2 variant pseudoviruses following the fifth dose (XBB.1.5). Geometric mean titres (GMTs) are
shown on the top. Dashed lines indicate the limit of detection (NT_{50} = 180). **b,** Radar plot illustrating
the back-boosting effect of the JN.1 booster on neutralization titres against pre-JN.1 variants. **c,**
Neutralization profiles of IGHV3-53$^{+/+}$ and wild-type mice following XBB.1.5 and JN.1 boosters.

1050   Fold changes and statistical significance between highlighted variants are indicated. Lines connect

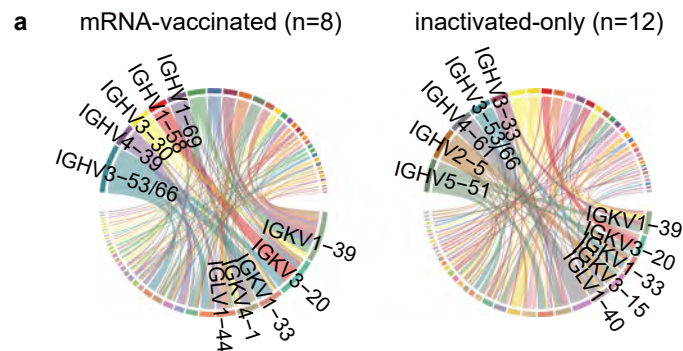1051   data from the same mouse. Two-tailed Wilcoxon rank-sum tests were used in **a-c**.

Extended Data figure 1

a

Extended Data Figure 1 | Different epidemiological landscape of XFG and NB.1.8.1 in 2025.
a, Prevalence of XFG and NB.1.8.1 in China and the rest of the world as of October 5, 2025. The asterisk (*) denotes the specific lineage and its sublineages. Data were retrieved from CovSpectrum (https://cov-spectrum.org/).

Extended Data figure 2

a

Extended Data Figure 2 | Paired heavy and light chain V-gene usage for human memory B cells
a, Paired heavy and light chain V-gene usage for memory B cells from the mRNA-vaccinated and inactivated-only cohorts.

Extended Data figure 3

Extended Data Figure 3 | V(D)J-humanized mice exhibit a cellular imprinting phenotype dominated by cross-reactive GC and memory B cells.

a-c, Scatter plots showing the proportion of cross-reactive GC B cells (a), total GC B cells (b), and class-switched memory B cells (c). The indicated percentages reflect the frequency relative to their respective parental gates defined in Supplementary Information Fig. 1b.

Extended Data figure 4

Extended Data Figure 4 | Repeated Omicron boosters fail to elicit robust Omicron-specific neutralization in imprinted humanized mice.

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Extended Data figure 5

Extended Data Figure 5 | Neutralization potency and cross-reactivity of mAbs from humanized and wild-type mice.
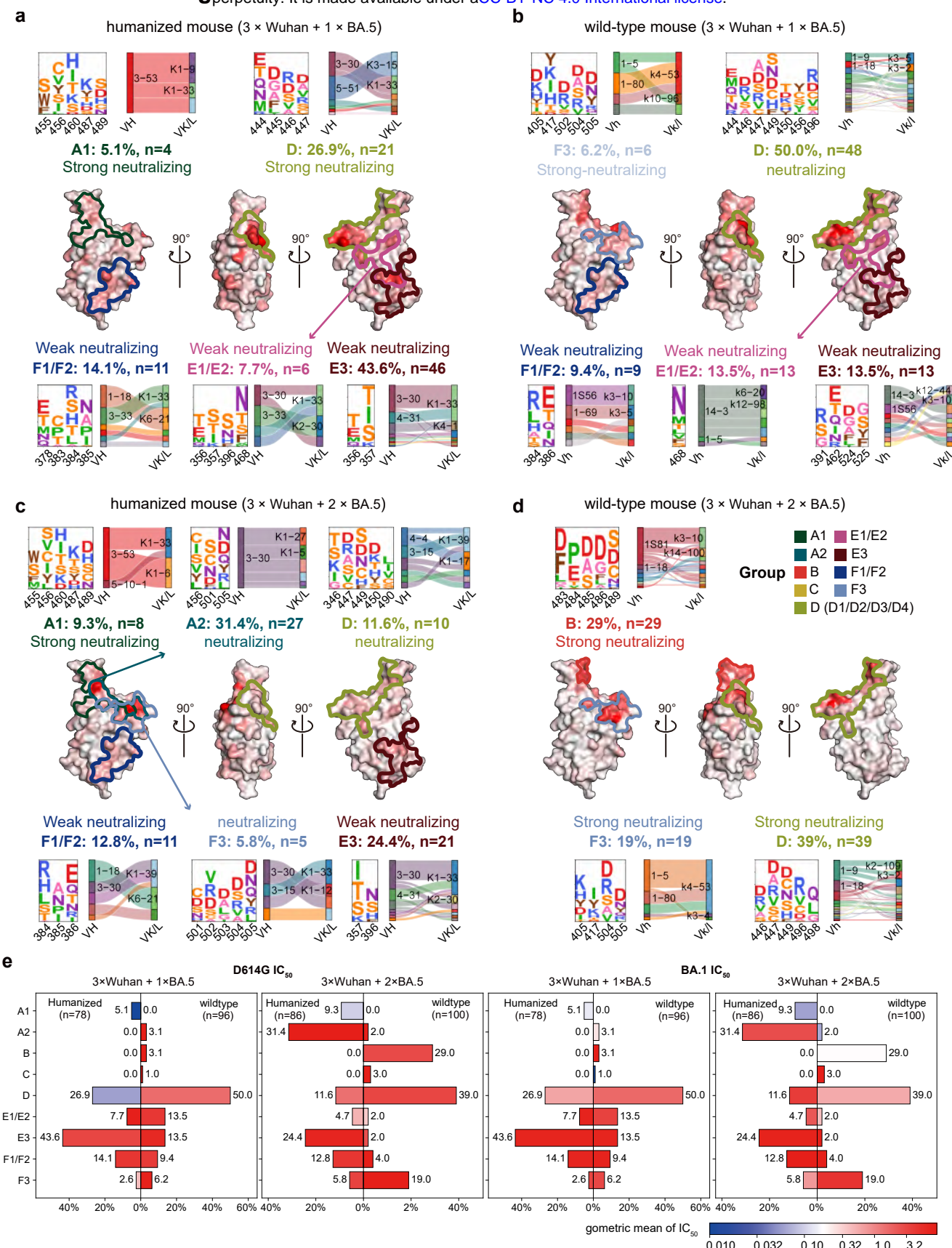
a,b, Comparison of antibody IC$_{50}$ values against D614G and BA.5(a) and cross-reactivity proportion (b) from humanized and wild-type mice. Two-tailed Wilcoxon rank-sum tests were used in a. Antibodies exhibiting ELISA OD450 values > 2 against both WT and BA.5 RBDs (1 μg/mL) were defined as cross-reactive. Those showing an OD450 > 2 for one variant but < 2 for the other were classified as specific.

Extended Data figure 6

Extended Data Figure 6 | Differential escape hotspots in humanized versus wild-type mice.
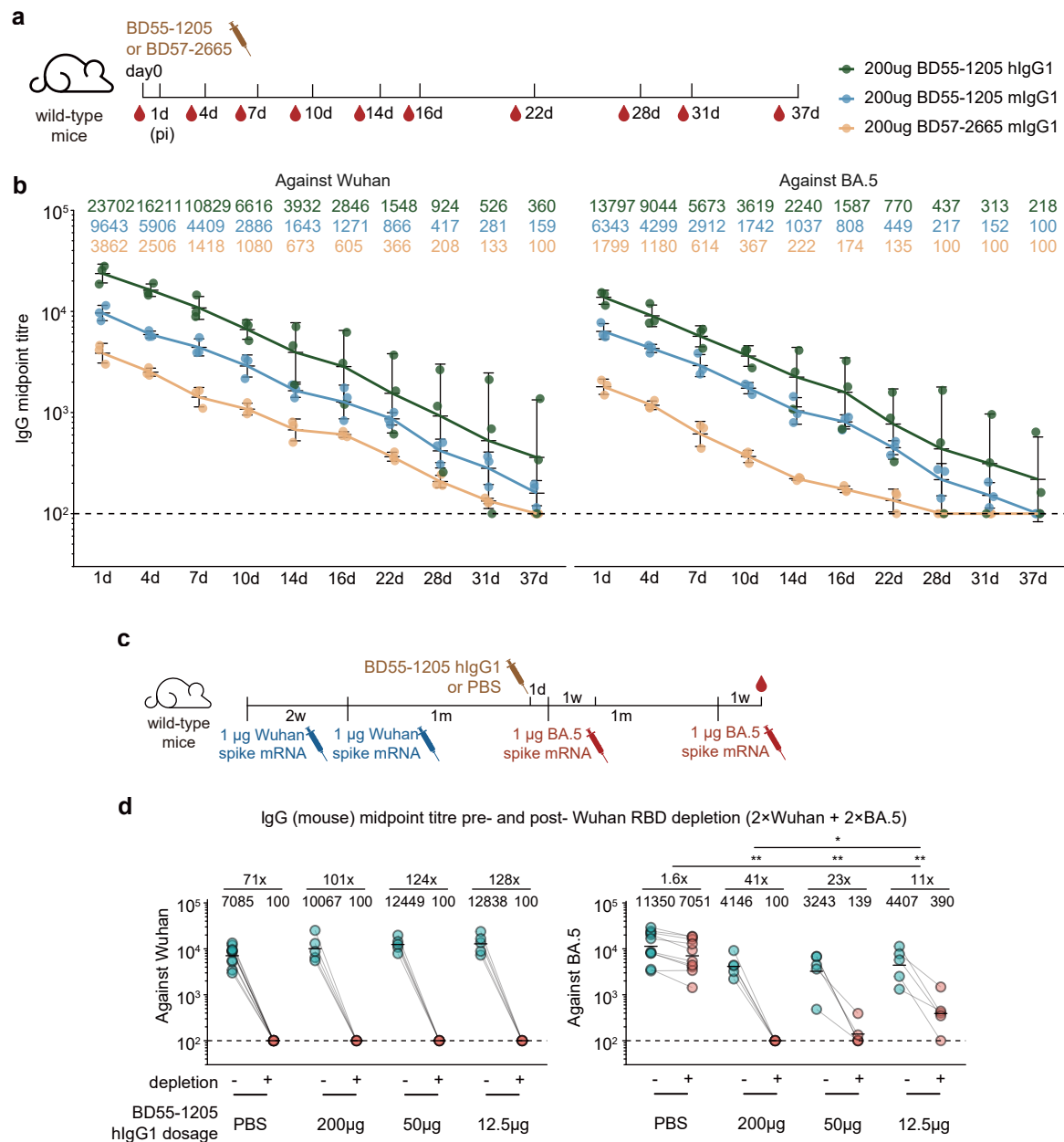
a–c, Scatter plots comparing the normalized average DMS escape scores of neutralizing antibodies isolated from humanized (x-axis) versus wild-type (y-axis) mice following Wuhan priming (a), the first BA.5 booster (b), and the second BA.5 booster (c). Residues falling along the diagonal (y=x) indicate shared immune pressure between the two models. To identify divergent hotspots—including those with low-to-moderate scores in one strain that are absent in the other—a shaded tolerance region was defined by the boundaries y = 1.4x + 0.3 and y = 0.6x - 0.18. Points falling outside this shaded region represent distinct escape hotspots. Among these outliers, the five residues with the highest escape scores in each group are highlighted in red. Related to Fig. 3.

Extended Data figure 7

Extended Data Figure 7 | Distinct SARS-CoV-2 antibody epitope distribution after sequential BA.5 boosts.
a-d, Epitope distribution of the antibody repertoire generated after one BA.5 boost (a, b) or two BA.5 boosts (c, d) in humanized mice (a, c) and wild-type mice (b, d). Related to Figure 4. e, Pyramidal bar charts showing the proportional distribution of epitope groups in antibodies isolated from humanized and wild-type mice after one or two BA.5 boosts. Bars are colored according to the log10 geometric mean IC$_{50}$ of antibodies within each group.

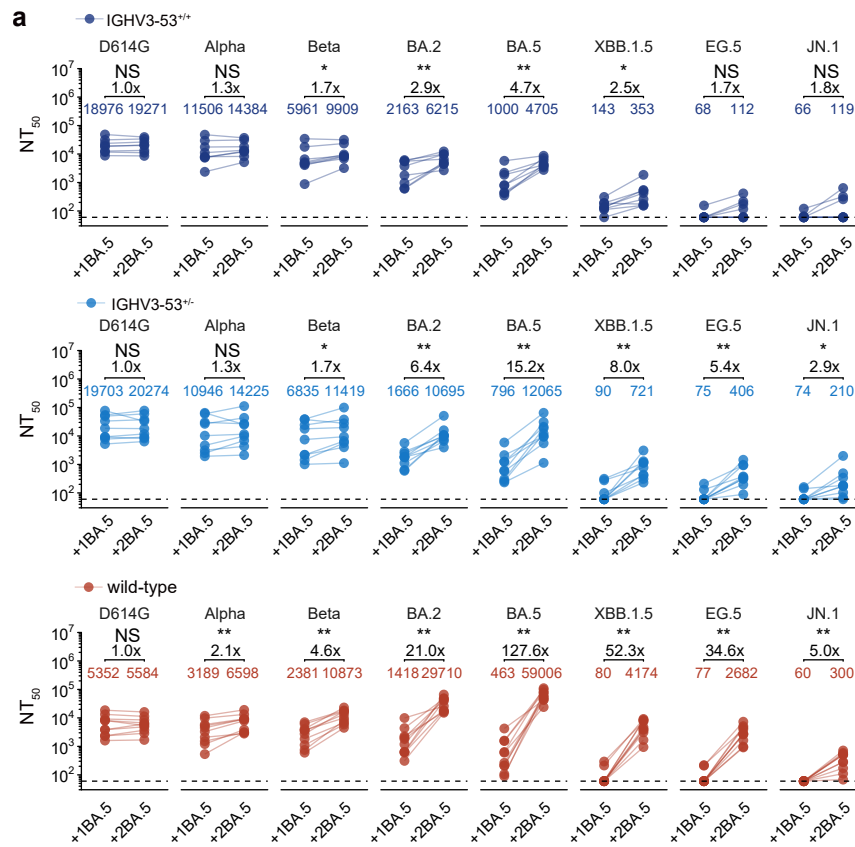# Extended Data figure 8

Extended Data Figure 8 | Pharmacokinetics and dose-dependent suppressive effects of antibodies used in passive transfer.
a, Schematic of the in vivo monoclonal antibody pharmacokinetic study. b, Serum IgG titres over time following antibody injection (mIgG1 forms were detected using an anti-mouse Fc secondary antibody, and hIgG1 forms using an anti-human Fc secondary antibody). Dashed lines indicate the limit of detection (midpoint titre = 100). c, Schematic of the BD55-1205 dose-ranging experiment. d, Corresponding serum IgG titres before and after Wuhan RBD depletion. Statistical significance of the fold-reduction in titres was assessed between groups. Dashed lines indicate the limit of detection (midpoint titre = 100). Two-tailed Wilcoxon rank-sum tests were used in d.
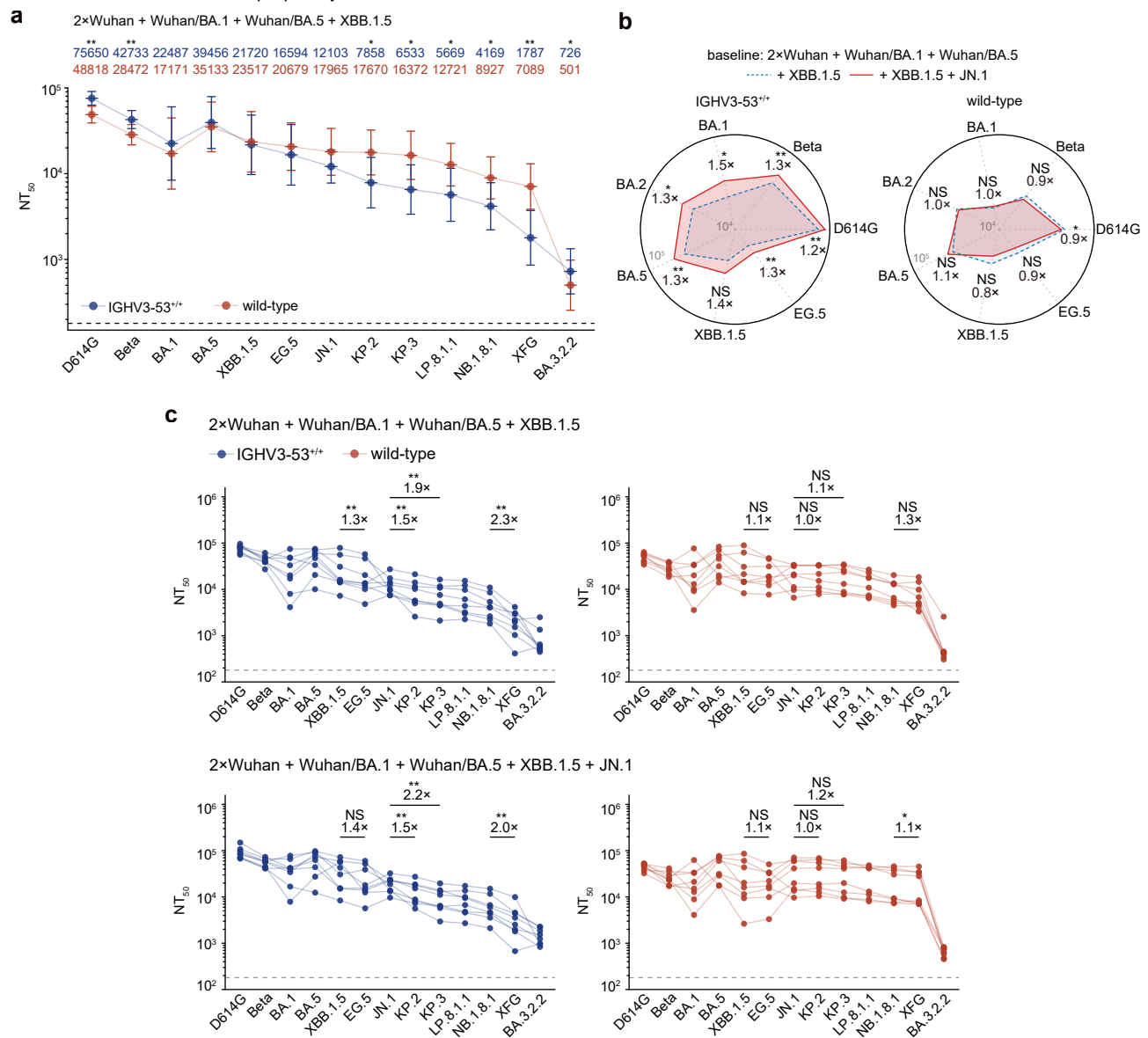
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