

1 **Title:**

2 **Dichotomy of neutralizing antibody, B cell and T cell**
3 **responses to SARS-CoV-2 vaccination and protection in**
4 **healthy adults**

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31

32 **ABSTRACT**

33 **Heterogeneity in SARS-CoV-2 vaccine responses is not understood. Here, we identify**
34 **four patterns of live-virus neutralizing antibody responses: individuals with hybrid**
35 **immunity (with confirmed prior infection); rare individuals with low responses (paucity**
36 **of S1-binding antibodies); and surprisingly, two further groups with distinct serological**
37 **repertoires. One group – broad responders – neutralize a range of SARS-CoV-2**
38 **variants, whereas the other – narrow responders – neutralize fewer, less divergent**
39 **variants. This heterogeneity does not correlate with Ancestral S1-binding antibody,**
40 **rather the quality of the serological response. Furthermore, IgD^{low}CD27⁺CD137⁺ B cells**
41 **and CCR6⁺ CD4⁺ T cells are enriched in broad responders before dose 3. Notably, broad**
42 **responders have significantly longer infection-free time after their third dose.**
43 **Understanding the control and persistence of these serological profiles could allow**
44 **personalized approaches to enhance serological breadth after vaccination.**

45
46 **Introduction:**

47 Inter-individual heterogeneity in human immune responses is well-described¹⁻⁴. While
48 immunological heterogeneity was previously seen as a “nuisance variable”, over the
49 last 10-20 years, this view has shifted due to new strategies, tools and hypotheses.
50 Today, the study of the variation between individuals provides novel insights in human
51 immunology⁵.

52
53 Alongside the deployment of COVID-19 vaccines as the primary control strategy of
54 the SARS-CoV-2 pandemic, observational studies were established to generate data
55 to inform timing of future doses, and to examine vaccine immunogenicity in vulnerable
56 populations omitted from phase 3 trials. Secondary aims included studying the
57 mechanisms of vaccine responses for mRNA and adenoviral vectored vaccines,
58 vaccine platforms not previously used outside of early phase trials, with profound
59 primary immunological and epidemiological responses⁶⁻⁸. We, with colleagues,
60 established three sentinel UK studies: CAPTURE, studying responses in solid-organ
61 and hematological cancer patients⁹⁻¹¹, NAOMI exploring responses in hemodialysis

62 patients ^{12,13}, and the Legacy study, an observational cohort study of healthy adults
63 undergoing occupational health screening and vaccination for SARS-CoV-2 ^{14–16}.
64 Legacy is a collaboration between the Francis Crick Institute and University College
65 London Hospitals (clinical trials registration NCT04750356). We have previously
66 reported on neutralizing ability of sera after two ^{14,15}, and three doses of vaccine ¹⁶.
67 Here, we explore inter-individual differences in serological and cellular responses
68 before and after a third vaccine dose in 283 Legacy participants (**Fig. 1A**) and show
69 that stratification of individuals based on live-virus neutralization patterns uncovers
70 previously unrecognized immune differences in otherwise healthy individuals.

71

72 **Results:**

73 We hypothesized that inter-individual heterogeneity would exist in the neutralizing
74 antibody responses after SARS-CoV-2 vaccination. We anticipated identifying three
75 groups: firstly, those with strong neutralization capacity due to encounters with Spike
76 during infection episodes, in addition to their vaccinations – so called “hybrid” immunity
77 ¹⁷; secondly, a small number of partial or low responders where their vaccine-induced
78 antibody responses were attenuated; and thirdly, a group of “normal” responders,
79 comprising the bulk of participants. To test this hypothesis, we performed hierarchical
80 clustering of the neutralizing capacity of 282 pre-dose 3 sera against ancestral SARS-
81 CoV-2 and seven variants of concern (VOCs, **Fig. 1B**). The first two doses were either
82 AZD1222 (Oxford/AstraZeneca, n=73) or BNT162b2 (Pfizer-BioNTech, n=209).
83 Surprisingly, unsupervised clustering identified four groups of individuals, which we
84 tentatively assigned: individuals with hybrid responses (n=49, 17%), “low responders”
85 (n=10, 3.5%) and two unexpected further groups, “broad responders” (n=129, 46%)
86 and “narrow responders” (n=94, 33%), mainly separated by their neutralization (or not)
87 of Omicron BA.1 before dose 3. To confirm the biological identities of these clusters,
88 we assessed whether we could identify these four groups using infection history, anti-
89 S1 and anti-N IgG. We proposed that hybrid responses should be readily identifiable
90 with exposure history and widely available binding S antibody and anti-N IgG assays.
91 This strategy confirmed our grouping of individuals with hybrid immunity: 46 individuals
92 (of 49; 94%) had 47 episodes of prior infections confirmed by the presence of
93 symptoms (39 episodes, 87.8%), by a molecular test (31 episodes, 66%), or by the
94 detection of anti-nucleocapsid IgG (44 individuals, 93.6%) (**Fig. 1C**). The ten low
95 responders were separable from the rest of the cohort by low anti-S1 IgG titers (**Fig.**

96 **1D**), and neutralizing activity restricted to ancestral SARS-CoV-2 (**Fig. 1B**). Hybrid and
97 low responders could be identified by binding anti-S1 and anti-N IgG individually or
98 jointly, however these tests distinguished poorly between broad and narrow
99 responders (**Fig. S1**).

100

101 Having confirmed two biologically relevant groups – low and hybrid responders –
102 through exposure history and anti-S1/anti-N titers, we next focused on the broad and
103 narrow responder groups which were not clearly defined by these parameters. We
104 reasoned that these two groups were likely to reflect similarly important, but hitherto
105 unrecognized, biological distinctions and therefore sought to further characterize these
106 groups. From hierarchical clustering with serum drawn just before dose 3, we
107 observed that 119 of 129 [92.3%] broad responders had serum $IC_{50} > 40$ for Omicron
108 BA.1, indicating neutralizing activity against the Omicron BA.1 lineage, whereas only
109 19 of 94 [20.3%] narrow responders had serum $IC_{50} > 40$ for Omicron BA.1 (χ^2 test
110 $P < 2.2 \times 10^{-16}$; **Fig. 1B**). Neutralization titers against Omicron BA.1 before dose 3
111 therefore offer a population-level surrogate. We found a small fraction of the broad
112 group was also anti-N positive (31 of 130, 23.8%; **Fig. 1C**), from prior infection. The
113 vast majority of broad responders was not previously infected by SARS-CoV-2, based
114 on weekly occupational health screening by RT-qPCR for SARS-CoV-2 infection and
115 absence of anti-N IgG (**Fig. S2**). Anti-N IgG positive broad responders were positive
116 from their first serum sample, indicating infection in 2020 (**Fig. S2C**). Focusing on anti-
117 N IgG negative individuals, there were no differences in age and sex between broad
118 and narrow responders (**Fig. 1E-F**). We found that BNT162b2 was more commonly
119 used for doses 1 and 2 than AZD1222 in broad responders, compared to either narrow
120 or low responders (**Fig. 1G**, χ^2 test $P = 0.006$ or 0.002 respectively). We excluded
121 benign explanations for the difference between these two groups: there were no
122 differences in age or gender between broad N seronegative (N-) or seropositive
123 individuals (**Fig. S3A-B**); broad N- individuals were more likely to have been
124 administered BNT162b2 for doses 1 and 2 (**Fig. S3C**); additional spike exposure
125 through infection provided boosting to anti-S1 titers in broad N+ individuals compared
126 to N- individuals (**Fig. S3D**); and there were no differences between any of the four
127 groups in intervals between doses 1 and 2 or between dose 2 and their serum sample
128 (**Fig. S3E-F**).

129

130 Interestingly, we plotted the trajectories of neutralizing titers against 8 different variants
131 between doses 2 and 3, and found, that broad and narrow responder groups followed
132 offset trajectories throughout this period, suggesting that an individual's response is
133 consistently either broad or narrow, across antigen encounters (**Fig. 1H**). Next, we
134 considered whether serological breadth initiated by SARS-CoV-2 would include other
135 coronaviruses. To test this possibility, we performed live-virus microneutralization
136 assays using HCoV-OC43, a seasonal human coronavirus (**Fig. S4**). We found no
137 differences in starting titers between broad or narrow responders, and no boosting
138 effect from SARS-CoV-2 vaccination in either group.

139
140 Given that neutralizing antibody production is a function of the orchestrated response
141 of B and CD4⁺ T cells after vaccination, we anticipated that underlying lymphocyte
142 differentiation might give rise to our observed distinct serological profiles. To
143 determine whether cellular differences contribute to neutralization breadth we
144 performed mass cytometry in individuals with broad or narrow serological profiles
145 (n=11 and 6 respectively), before (median 1d [range 10-0]) and after (median 19d
146 [range 14-21]) their third BNT162b2 dose (**Table S1**). All individuals were anti-N IgG
147 negative at both timepoints, and all preceding samples. Gating, quality control and
148 clustering are described in the Methods (**Fig. S5-7**). First, we assessed whether
149 changes in the B cell compartment were present between individuals with broad and
150 narrow serological profiles (**Fig. 2A and B**). We expected altered utilization of different
151 memory B cell compartments between broad and narrow responders. We therefore
152 compared the pre-dose 3 samples (*i.e.* the long-term memory footprint from dose 2)
153 between broad and narrow responders, and found that IgD^{low}CD27⁻CD137⁺ B cells
154 were more abundant in the broad responders (cluster B3: log₂ fold change 4.3, P_{adj}
155 0.045 and cluster B7: log₂ fold change 4.1, P_{adj} 0.013, **Fig. 2C**). IgD^{low}CD27⁻ B cells
156 are traditionally termed double negative (DN) memory cells, originally described in
157 ageing and chronic infections and now with newer evidence from many groups
158 showing roles in healthy serological responses (reviewed in ¹⁸). Following dose 3,
159 there were no differences in the B cell compartment between broad and narrow
160 responders that reached statistical significance comparing before and after third-dose
161 vaccination (**Fig. 2D**).

162

163 The next cellular comparison was the response to vaccination within each serological
164 profile. Comparing broad responders before and after vaccination, we found a
165 decrease in the abundance of atypical, double-negative memory B cells that express
166 CD137 (IgD^{low}CD27⁻CD137⁺; cluster B3: log₂ fold change -5.1, P_{adj} 0.0008 and cluster
167 B7: log₂ fold change -2.4, P_{adj} 0.02, **Fig. 2E**). In narrow responders, we found no
168 changes in the B cell compartment after vaccination (**Fig. 2F**). For plasmablasts
169 (cluster B2: CD20⁻CD27⁺CD38⁺⁺⁺), we observed an expansion in broad responders,
170 which did not reach our significance threshold (log₂ fold change 1.4, P_{adj} 0.08) and a
171 smaller, non-significant fold-change in narrow responders (log₂ fold change 0.6,
172 $P_{uncorrected}$ 0.42, P_{adj} 0.52). Finally, we tested for differential vaccine responses between
173 each serological profile, and found no significant differences (**Fig. 2G**, cluster B7
174 P_{adj} =0.053).

175
176 Together, these results suggest that broad responders favor a relatively higher
177 proportion of DN-CD137⁺ memory B cells after two doses, which is perturbed by
178 further vaccination. Broad responders also had a tendency towards larger plasmablast
179 responses after dose 3. CD137 expression on human B cells has been shown in
180 several contexts, including CD11c⁺ B cells (a further subgroup of DN memory cells) in
181 healthy donors, lupus, and systemic sclerosis¹⁹; healthy B cells stimulated *in vitro*²⁰;
182 and lymphoma, including on Hodgkin Reed-Sternberg cells^{20,21}. There are reports of
183 rare individuals with de-functioning mutations in *TNFRSF9*, the gene encoding CD137
184 (also called 4-1BB), who display perturbations in B cell biology including a propensity
185 to autoinflammation and lymphomagenesis, vulnerability to respiratory infections, and
186 attenuated responses to vaccination^{22,23}.

187
188 Since B cell memory development is cued in part by CD4⁺ T_H cells, we next assessed
189 the CD4⁺ T_H cell compartment (**Fig. 3A-B**). Before third doses, we found two clusters
190 were over-represented in narrow responders (cluster H8 CXCR3⁺TCR $\gamma\delta$ ⁺: log₂ fold
191 change -5.6, P_{adj} 3.1 x 10⁻⁶; cluster H11 NCAM⁺CXCR3⁺TCR $\gamma\delta$ ⁺: log₂ fold change -
192 4.3, P_{adj} 5.9x10⁻⁴, **Fig. 3C**), and found a CCR7⁻CD27⁻CD28⁻CD45RA⁺CCR6⁺CD57⁺
193 population (cluster H12) that was “pre-expanded” in broad responders (log₂ fold
194 change 4.5 and P_{adj} 0.01, **Fig. 3C**). For brevity, we refer to these CD27⁻CD28⁻
195 CD45RA⁺CCR6⁺CD57⁺ CD4⁺ T cells as breadth-related T_H cells (brT_H). We found no

196 significant differences comparing CD4⁺ T cells between the two serological profiles
197 after vaccination (**Fig. 3D**). Comparing before and after third doses, we found no
198 significant differences in the abundance of CD4⁺ T_H cell clusters in broad responders
199 (**Fig. 3E**). However, narrow responders showed a significant decrease in two clusters
200 in response to vaccination (cluster H8: log₂ fold change -6.3, P_{adj} 0.0002; cluster H11:
201 log₂ fold change -6.4, P_{adj} 0.0002, **Fig. 3F**). CD4⁺T cells co-expressing TCR $\gamma\delta^+$ and
202 TCR $\alpha\beta^+$ have been recently described²⁴. Clusters H8 and H11 were distinct from *bona*
203 *fide* TCR $\gamma\delta^+$ T cells, which we observed as a distinct population adjacent to CD8 T
204 cells in our PBMC analysis (**Fig. S6**). The only differentially responsive clusters
205 between broad and narrow individuals were clusters 8 and 11 (**Fig. 3G**). brT_H are
206 CCR7-CD45RA⁺ suggesting a terminally differentiated effector memory phenotype
207 (T_{emra})²⁵, and further classifiable within a CD27-CD28- T_{emra} sub-compartment²⁶. A
208 study examining CD4 responses to Dengue virus, reported two subgroups of Dengue-
209 specific CD4⁺ T_{emra}, one of which was CCR6⁺ and lacked the expression of cytotoxic
210 and terminal differentiation markers (perforin and KLRG1) found on CCR6⁻ T_{emra}²⁷.
211 Taken together, these observations suggest that broad responders harbor T_{emra}-like
212 memory populations, which are not terminally differentiated.

213

214 To summarize our multi-dimensional cytometry: examining differences between
215 individuals with broad and narrow serological profiles showed that broad responders
216 are marked by the presence of brT_H cells and DN-CD137⁺ B cells before their third
217 doses. We found a propensity for broad responders to expand their plasmablast
218 population compared to narrow responders. After the immune stimulus of a third
219 mRNA vaccination, there were no cellular differences in either B or CD4⁺T_H cells
220 populations between broad and narrow responders (**Fig. 2D and 3D**). There are
221 several possible explanations for this observation. Firstly, vaccination has been shown
222 to transiently perturb the immune landscape^{1,2}, thus cytometry performed during a
223 time of immune activation may be obfuscated, and overlook intrinsic underlying inter-
224 individual differences. In this situation, a later timepoint, memory analysis of post-
225 vaccination may be more informative, as it lacks the overlaid perturbation from a recent
226 vaccination. Our pre-dose 3 samples provide that retrospective memory assessment
227 of dose 2, once the early cellular changes have resolved, and is it at that timepoint
228 that we observed the most striking cellular differences between broad and narrow

229 responders. Secondly, it is possible that narrow responders represent an accelerated
230 resolution of the cellular changes after doses 1 and 2, with the loss of certain memory
231 lymphocyte sub-populations rapidly (brT_H cells and DN-CD137⁺ B cells), whereas
232 broad responders retain a diversity in their cellular responses until after dose 3. If a
233 third dose were to “reset” the serological profile such that there are no cellular or
234 serological differences between broad or narrow responders (defined before dose 3),
235 then we would anticipate that all individuals would have equal susceptibility to infection
236 after dose 3, as neutralizing antibody is the single best predictor of infection ^{28,29}.

237
238 Thus, we next assessed whether membership of these two serologically-defined
239 groups of broad and narrow responders influenced susceptibility to SARS-CoV-2
240 infection, after their third doses with Omicron BA.2. Because we expected additional
241 antigenic exposures to influence breadth (**Fig. 1B**), we censored individuals with
242 identified BA.1 infection, or who seroconverted to nucleocapsid (at the date of their
243 first positive anti-N IgG result). Individuals from the lowest age quartile (22-33yo) had
244 a tendency towards an increased likelihood of experiencing an infection compared to
245 those from the highest age quartile (53-72yo; **Fig. 4A**). There were no sex-related
246 differences (**Fig. 4B**). We found that our two serological profiles of interest, broad and
247 narrow, were significant predictors of time-to-infection (**Fig. 4C**), with participants older
248 than the median age (>44yo) from the broad group protected from infection relative to
249 their counterparts in the narrow group. The serological effect was attenuated in 22-
250 44yo (**Fig. 4C**). To quantify the effect of breadth across the entire age range, we fitted
251 a Cox proportional hazard model, allowing interactions between breadth and age, and
252 dividing age into two groups: those older (>44yo) or younger (22-44yo) than the
253 median (**Fig. 4D**). This model gave a hazard ratio for broad responders of 0.45 (HR,
254 95% CI 0.22-0.94) in the >44yo age group (the reference age group), implying a ~60%
255 reduction in infection risk during the Omicron BA.2 wave for broad responders
256 compared to narrow responders. The interaction term between age and breadth
257 suggest that the serological effect was attenuated in younger participants
258 approximately two-fold (**Fig. 4D**). A potential limitation of this analysis is that the timing
259 of the BA.2 in the UK was at a time when asymptomatic community and occupational
260 testing was being withdrawn, and when national requirements to isolate after a positive
261 test ceased, so it is possible the exposure risk varied. In summary, we found that
262 serologically defined groups of individuals with altered B and T cell compartments

263 were differentially protected from infection after vaccination, especially among older
264 adults in our cohort.

265

266 **Discussion:**

267 Here, we have used detailed serological profiling to uncover inter-individual
268 heterogeneity in vaccine responses, with corresponding alterations in T and B cell
269 compartments, and investigated the relationship between these differences in
270 immunity and subsequent risk of SARS-CoV-2 infection. Serological profiling with live-
271 virus microneutralization assays identified 4 groups – responders with hybrid
272 immunity, and those with low, narrow, or broad responses. We have focused on the
273 apparent dichotomy between the cohort of broad responders who have serological
274 capacity to neutralize Omicron lineages before their third doses, and the cohort of
275 narrow responders who do not. We have shown that surrogate classification by binding
276 anti-S titers by ELISA is inadequate to define these classes of breadth; a range of
277 neutralization titers against a panel of viruses is required. We have found that broad
278 responders have specific lymphocyte populations in circulation before dose 3,
279 including DN-CD137⁺ B cells and brT_H. Our findings are critical in several contexts.
280 Firstly, to offer personalized risk assessments to current VOCs, or forwards
281 prognostication, anti-S is inadequate. Secondly, the inter-individual heterogeneity
282 appears consistent over ~60-100 days — without additional antigen encounter from
283 infection: evidenced by symptom diaries, PCR screening, anti-N IgG testing (**Fig. S2**).
284 This observation suggests that breadth might be intrinsic to that individual, with
285 implications for other vaccine responses (and design), and perhaps for antibody
286 responses in general including autoimmune contexts. Thirdly, variant-specific booster
287 trials will require careful interpretation: inadvertently unmatched arms between broad
288 and narrow could plausibly reverse or obfuscate a true effect. In conclusion, we show
289 that our serological profiling with high-throughput live-virus microneutralization
290 identifies immunological and epidemiological inter-individual heterogeneity, where
291 breadth of neutralizing response is key to protection. Our data suggest serological
292 breadth of response to vaccination is not a purely stochastic phenomenon in humans,
293 rather it has important underlying cellular correlates with fertile ground for further study
294 to understand both the mechanistic underpinnings and their clinical consequences.

295

296

297 **Materials and Methods**

298

299 **Ethics approvals and study design**

300 The Legacy study (NCT04750356) was established in January 2021 and enrolled two
301 prospective cohorts. The Legacy study was approved by London Camden and Kings
302 Cross Health Research Authority (HRA) Research and Ethics committee (REC) IRAS
303 number 286469 and sponsored by University College London, The study has been
304 described in our prior interim reports^{14–16}. Participants were included if they were an
305 employee of either UCLH or the Francis Crick Institute and had provided at least one
306 swab for qRT-PCR testing via the Crick PCR pipeline. At the commencement of the
307 Legacy study, the Crick PCR pipeline was performing NHS staff and patient testing to
308 support local NHS Trusts and partners. Participants comprised of patient facing
309 healthcare workers at UCLH and Crick staff. Study visits with venipuncture were
310 offered approximately one month after vaccination, and at approximately 3, 6 and 12
311 months. Participants who experienced infection after two (or more) doses of vaccine
312 were invited for a study visit approximately 2 weeks after the start of their infection
313 episode.

314

315 **SARS-CoV-2 RT-qPCR**

316 RNA was extracted from nasopharyngeal swabs taken at time of occupational health
317 screening, as previously described³⁰. Viral RNA was detected by RT-qPCR (TaqPath
318 COVID-19 CE-IVD Kit, ThermoFisher) to confirm SARS-CoV-2 infection. Individuals
319 reporting symptoms, positive lateral flow tests, or positive external PCR testing were
320 invited to perform a study nasopharyngeal swab.

321

322 **Venipuncture and serum processing**

323 Legacy participants were invited for venipuncture before and after (~10-21d)
324 vaccinations, with additional samples planned at approximately 3, 6 and 12 months.
325 After an infection episode, individuals were invited for additional venipuncture after
326 convalescence (~10-21d). Venipuncture was performed into K2-EDTA (for PBMC), or
327 SST (serum) vacutainer tubes (BD). Serum was separated within 24 hours.

328

329 **PBMC isolation**

330 Whole blood was collected in K2-EDTA tubes and samples were processed within 24
331 hours. PBMC and plasma were isolated by density-gradient centrifugation for 30
332 minutes at 1000 x g at RT. Plasma was carefully removed then centrifuged for 10
333 minutes at 4000 x g to remove debris, aliquoted and stored at -80°C. The cell layer
334 was then collected and washed twice in PBS by centrifugation for 10 minutes at 300 x
335 g at RT. PBMC were resuspended in cell freezing medium (Fisher Scientific)
336 containing 10% DMSO, placed overnight in CoolCell freezing containers (Corning) at
337 -80°C and then stored in liquid nitrogen tanks until batched analysis.

338

339 **Virus variants and culture**

340 The Alpha, Delta and Omicron BA.1 isolates used were the same as previously, and
341 our viral culture technique is unchanged^{14–16}. The SARS-CoV-2 B.1.1.7 isolate
342 (“Alpha”) was hCoV-19/England/204690005/2020, which carries the D614G, Δ69-70,
343 Δ144, N501Y, A570D, P681H, T716I, S982A and D1118H mutations in Spike³¹, and
344 was obtained from Public Health England (PHE), UK, through Prof. Wendy Barclay,
345 Imperial College London, London, UK via the Genotype-to-Phenotype National
346 Virology Consortium (G2P-UK). The B.1.617.2 (“Delta”) isolate was MS066352H
347 (GISAID accession number EPI_ISL_1731019), which carries the T19R, K77R,
348 G142D, Δ156-157/R158G, A222V, L452R, T478K, D614G, P681R, D950N mutations
349 in Spike, and was kindly provided by Prof. Wendy Barclay, Imperial College London,
350 London, UK via the Genotype-to-Phenotype National Virology Consortium (G2P-UK).
351 The BA.1 (“Omicron”) isolate was M21021166, which carries the A67V, Δ69-70, T95I,
352 Δ142-144, Y145D, Δ211, L212I, G339D, S371L, S373P, S375F, K417N, N440K,
353 G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K,
354 D614G, H655Y, N679K, P681H, A701V, N764K, D796Y, N856K, Q954H, N969K, and
355 L981F mutations in Spike, and was kindly provided by Prof. Gavin Screaton, University
356 of Oxford, Oxford, UK via G2P-UK. The Omicron BA.2 isolate carries the T19I, Δ24-
357 26, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S,
358 K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G,
359 H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K mutations in Spike and
360 was obtained from a Legacy study participant. The Omicron BA.2.12.1 isolate carries
361 the L452Q and S704L mutations in Spike, in addition to the BA.2 mutations listed
362 previously, and was kindly provided by Prof. Gavin Screaton, University of Oxford,
363 Oxford, UK. The Omicron BA.5 isolate carries the T19I, Δ24-26, A27S, Δ69-70,

364 G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N,
365 N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G,
366 H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K mutations in Spike was
367 obtained from the laboratory of Alex Sigal, Africa Health Research Institute, Durban,
368 South Africa.

369
370 All viral isolates were propagated in Vero V1 cells (a gift from Stephen Goodbourn).
371 Briefly, 50% confluent monolayers of Vero V1 cells were infected with the given SARS
372 CoV-2 strains at an MOI of approx. 0.001. Cells were washed once with DMEM
373 (Sigma; D6429), then 5 ml virus inoculum made up in DMEM was added to each T175
374 flask and incubated at room temperature for 30 minutes. DMEM + 1% FCS (Biosera;
375 FB-1001/500) was added to each flask. Cells were incubated at 37° C, 5% CO₂ for 4
376 days until extensive cytopathogenic effect was observed. Supernatant was harvested
377 and clarified by centrifugation at 2000 rpm for 10 minutes in a benchtop centrifuge.
378 Supernatant was aliquoted and frozen at -80°C.

379

380 **High-throughput live virus microneutralization assay**

381 High-throughput live virus microneutralisation assays were performed as previously
382 described ¹⁴. In brief, Vero E6 cells (Institut Pasteur) at 90-100% confluency were
383 infected with given SARS-CoV-2 variants in 384-well format, in the presence of serial
384 dilutions of patient serum samples. After infection, cells were fixed with 4% final
385 Formaldehyde, permeabilized with 0.2% TritonX-100, 3% BSA in PBS (v/v), and
386 stained for SARS-CoV-2 N protein using Biotin-labelled-CR3009 antibody produced
387 in-house together with a Streptavidin-Alexa488 (S32354, Invitrogen) and cellular DNA
388 using DAPI (10236276001, Merck). Whole-well imaging at 5x was carried out using
389 an Opera Phenix (Perkin Elmer) and fluorescent areas and intensity calculated using
390 the Phenix-associated software Harmony (Perkin Elmer). Inhibition was estimated
391 from the measured area of infected cells/total area occupied by all cells and expressed
392 as percentage of maximal (virus only wells). The inhibitory profile of each serum
393 sample was estimated by fitting a 4-parameter dose response curve executed in
394 SciPy. Neutralizing antibody titers are reported as the fold-dilution of serum required
395 to inhibit 50% of viral replication (IC₅₀), and are further annotated if they lie above the
396 quantitative (complete inhibition) range, below the quantitative range but still within the
397 qualitative range (i.e. partial inhibition is observed but a dose-response curve cannot

398 be fit because it does not sufficiently span the IC50), or if they show no inhibition at
399 all. Human coronavirus OC43 (HCoV-OC43) neutralization was performed as above,
400 except Vero E6 cells were substituted for Mv1Lu cells.

401

402 **ELISA and other serological testing**

403 Anti-S1 was performed as described previously³². To minimize variation across ELISA
404 plates, we re-scaled serum OD405 measurements by (i.) subtracting the *plate-wide*
405 average negative control, (ii.) dividing by the *plate-wide* average positive control and
406 then (iii.) multiplying by the *study-wide* median of the plate-averaged positive controls.

407

408 **Anti-nucleocapsid IgG detection**

409 Anti-nucleocapsid IgG was measured using the Elecsys Anti-SARS-COV-2 assay
410 (Roche; 09203095190) run on a Cobas e411 analyser (Roche) in accordance with the
411 manufacturer's instructions. Serum was used for this immunoassay and results
412 reported as reactive (positive) or non-reactive (negative), with a semi-quantitative titer.
413 To separate participants into anti-N positive and negative groups, we used their most
414 recent anti-N result.

415

416 **Mass cytometry sample processing**

417 Peripheral blood mononuclear cells were thawed in the presence of benzonase (Merck
418 70746-3, used at 1µl/ml), cells were counted and up to 3 x 10⁶ cells were processed
419 for mass cytometry. Mass cytometry staining was performed using the MaxPar Direct
420 Immune Profiling Assay (Fluidigm, now Standard Biotools), in line with the
421 manufacturer's instructions, with T cell expansion panel 3. Once stained and fixed,
422 cells were stored at -80C and processed in batches. After thawing, cells were stained
423 with Iridium as per the manufacturer's instructions (Standard Biotools). Events were
424 collected using a CyTOF XT (Standard Biotools), and were bead-normalized using the
425 in-built algorithm.

426

Target antigen	Clone	Isotope
CD45	HI30	89Y
CCR6 (CD196)	G034E3	141Pr
CD123	6H6	143Nd

CD19	HIB19	144Nd
CD4	RPA-T4	145Nd
CD8a	RPA-T8	146Nd
CD11c	Bu15	147Sm
CD16	3G8	148Nd
CD45RO	UCHL1	149Sm
CD45RA	HI100	150Nd
CD161	HP-3G10	151Eu
CCR4 (CD194)	L291H4	152Sm
CD25	BC96	153Eu
CD27	O323	154Sm
CD57	HNK-1	155Gd
CXCR3 (CD183)	G025H7	156Gd
CXCR5 (CD185)	J252D4	158Gd
CD28	CD28.2	160Gd
CD38	HB-7	161Dy
NCAM (CD56)	NCAM16.2	163Dy
TCRgd	B1	164Dy
CD294	BM16	166Er
CCR7 (CD197)	G043H7	167Er
CD14	63D3	168Er
CD3	UCHT1	170Er
CD20	2H7	171Yb
CD66b	G10F5	172Yb
HLA-DR	LN3	173Yb
IgD	IA6-2	174Yb
CD127	A019D5	176Yb
Live/dead intercalator		103Rh
DNA1		191Ir
DNA2		193Ir
CD134/OX40	ACT35	142Nd
TIGIT	MBSA43	159Tb

CD69	FN50	162Dy
CD279/PD-1	EH12.2H7	165Ho
CD366/Tim-3	F38-2E2	169Tm
CD278/ICOS	C398.4A	175Lu
CD137/4-1BB	4B4-1	209Bi

427

428 **Mass cytometry gating strategy**

429 Events from bead-normalized FCS files were gated as shown in Figs. S5 & S6. This
430 was performed using R v4.0.0, the following packages: flowCore v2.2.0 ³³,
431 flowWorkspace v4.2.0, openCyto v2.2.0 ³⁴ and ggcyto v1.18.0 ³⁵.

432

433 Gated samples were re-saved as FCS files (this allowed the parallel processing of
434 samples). Gated FCS files were analyzed using CATALYST ³⁶. As a quality control
435 step to further filter out dead cells, or debris, we performed clustering within
436 CATALYST (which itself uses rounds of flowSOM ³⁷ aggregated using
437 ConsensusClusteringPlus ³⁸), using the live/dead, DNA1 and DNA2 channels only
438 (Fig. S7). This returned 3 meta-clusters, one of which was DNA1- and DNA2- negative,
439 one was dead+ and the largest cluster contained DNA1+, DNA2+,live events. The
440 DNA1+, DNA2+, live cluster was selected and used for all downstream analyses.
441 PBMC were then re-clustered using CATALYST and the following markers (selected
442 to define “types” of cells in PBMC): CD3, CD20, CD19, CD14, CD16, CD161, CD56,
443 CD45RA, CD45RO, CD4, CD8a, CD11c, live/dead and DNA1 and DNA2. The PBMC
444 dataset was sub-sampled to 500 cells/sample for visualization with UMAP-embedding
445 and summary heatmap of marker expression. For differential abundance analyses,
446 edgeR ³⁹ was used via diffcyt ⁴⁰. For differential state analyses, we used limma ⁴¹ via
447 diffcyt. For each population of PBMCs, events were filtered and then re-clustered using
448 CATALYST and differential expression analyses as before. The optimum number of
449 meta-clusters was determined by visual inspection of UMAP projections and
450 heatmaps for each cell type.

451

452 **Time to event analysis**

453 Time to event analysis was performed in R using the survival package. Individuals
454 were left censored on the day they received their third dose. Individuals were right

455 censored on the date of an infection with a variant that was Omicron BA.2, at the last
456 visit date (participants are asked if they have experienced COVID-19 symptoms in the
457 interim, and an anti-N IgG level is tested), or at the date of dose 4. Omicron BA.2
458 infection was confirmed by viral sequencing, by S gene target presence, or if no
459 nucleic acid testing was available, based upon calendar date. Infection >14d after
460 dose 3 was considered the event of interest and included infections were the
461 BA.1/BA.2 BA.2-BA.4/5 assignment was date-based. Days of entry, exit and event
462 were calculated with respect to the earliest date for dose 3 in the study. Data are
463 presented as cumulative incidence plots, with at numbers at risk shown. Cox
464 proportional hazard models were used as described in the text.

465

466 **Data analysis**

467 Study data were collected and managed using REDCap electronic data capture tools
468 hosted at University College London ^{42,43}. Data were exported from REDCap into R
469 for visualization and analysis, similar to previously ¹⁶. Neutralizing antibody titers are
470 reported as IC₅₀ values. As described above, for each serum sample, four dilutions
471 (1:40, 1:160, 1:640, 1:2560) are assayed in duplicate. All 8 points are used to fit a 4
472 parameter curve, and the IC₅₀ (the fold-dilution corresponding to 50% viral inhibition),
473 is reported. IC₅₀ values below 40 and above 2560 are reported as 'weak' or 'complete'
474 inhibition. For plotting and analysis, winsorizing was used: IC₅₀ values above the
475 quantitative limit of detection of the assay (>2560) were recoded as 5120; IC₅₀ values
476 below the quantitative limit of the assay (<40) but within the qualitative range were
477 recoded as 10; data below the qualitative range (i.e. no response observed) were
478 recoded as 5.

479 All data analysis was performed in R. The statistical tests used are described in the
480 relevant section of the methods, figure legends or text.

481

482 **Online supplemental material**

483 Fig S1 shows receiver operating characteristics for anti-S1 and anti-N IgG for the
484 prediction of serological profile. Fig S2 displays longitudinal PCRs, symptom diaries
485 and anti-N IgG to confirm seronaive individuals. Fig S3 shows the demographics,
486 vaccine usage and dosing intervals for broad anti-N IgG seropositive or seronegative
487 individuals. Fig S4 shows the neutralization of HCoV-OC43 is not augmented by
488 SARS-CoV-2 Spike exposure (infections or vaccinations). Figure S5-S7 show the

489 mass cytometry gating strategy, illustrative gating and quality control. Figure S8 shows
490 PBMC-level analysis of mass cytometry.

491

492 **Data availability**

493 Requests for de-anonymized data will be considered by the Legacy Governance
494 Board, via covid-19-legacy-study@crick.ac.uk, to ensure the request is from a genuine
495 researcher and that legal and ethical obligations are maintained.

496

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529
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553

554

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- 662

663 **Supplemental Material**

664

665 Table S1

666 Figs. S1-8 [*at end of PDF – Figs S1-8 after Figs 1-4*]

667

668

669 **Table S1 – Demographics and vaccine characteristics of the mass cytometry**
 670 **cohort**

671 The primary doses, third doses, age, sex and interval between dose 2 and 3, and
 672 cumulative anti-N IgG status are summarized for individuals in the mass cytometry
 673 dataset

Characteristic	broad, N = 11¹	narrow, N = 6¹
doses 1 and 2		
AZD1222	0 (0%)	0 (0%)
BNT162b2	11 (100%)	6 (100%)
mRNA1272	0 (0%)	0 (0%)
others	0 (0%)	0 (0%)
dose 3		
AZD1222	0 (0%)	0 (0%)
BNT162b2	11 (100%)	6 (100%)
mRNA1272	0 (0%)	0 (0%)
others	0 (0%)	0 (0%)
Sex		
Female	9 (82%)	3 (50%)
Male	2 (18%)	3 (50%)
χ^2 test $P=0.4$		
Age		
	56 [54-61]	62 [48-64]
2 tailed Mann Whitney $P=0.6$		
Interval between dose 3 and 2 [days]		
	193.0 [188.5-195.0]	192.5 [189.0-198.2]
2 tailed Mann Whitney $P=0.6$		
anti-N IgG results up-to 28d after dose 3		
all negative	11 (100%)	6 (100%)

674 ¹n (%); Median [25%-75%]

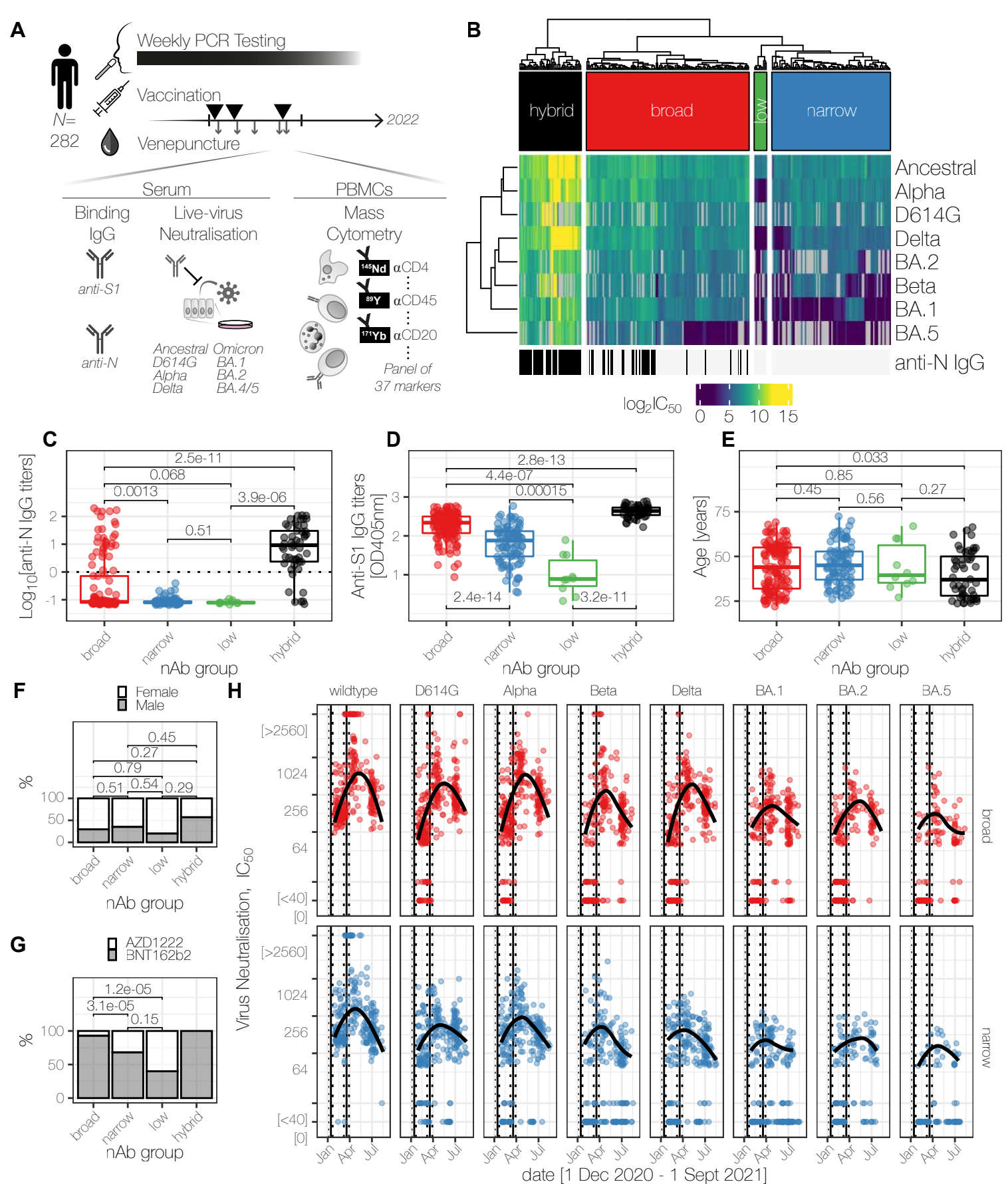


Figure 1. Serological profiling of Legacy participants identifies heterogeneity in patterns of SARS-CoV-2 neutralization
(A) Study design, longitudinal sampling and assays performed for 282 healthcare or laboratory workers. **(B)** Hierarchical clustering of live-virus neutralizing antibody titers before dose 3. Each individual is represented by a column and each SARS-CoV-2 variant by a row. The $\log_2 IC_{50}$ is shown by the color bar, and missing data are in grey. Both rows and columns are clustered using Euclidean distances and anti-N IgG status is indicated. In the color bar above the heatmap, the label of each groups is shown: hybrid, broad, low, and narrow responders in black, red, green and blue respectively. **(C)** anti-N IgG titers. **(D)** anti-S1 IgG titers. **(E)** participant age at enrollment. **(F)** Participant sex, and **(G)** vaccines used for doses 1 and 2, for anti-N negative individuals. **(H)** Trajectory neutralizing antibody titers between doses 1 and 3 of anti-N negative individuals from broad and narrow clusters. In (C)-(E) and (F)-(G) *P* values are from two-tailed unpaired Mann-Whitney tests or χ^2 tests respectively. In (H), smoothed splines were restricted to data within the quantitative range of the assay, and vertical solid and dashed lines represent the median and inter-quartile ranges of the dates of doses 1 and 2.

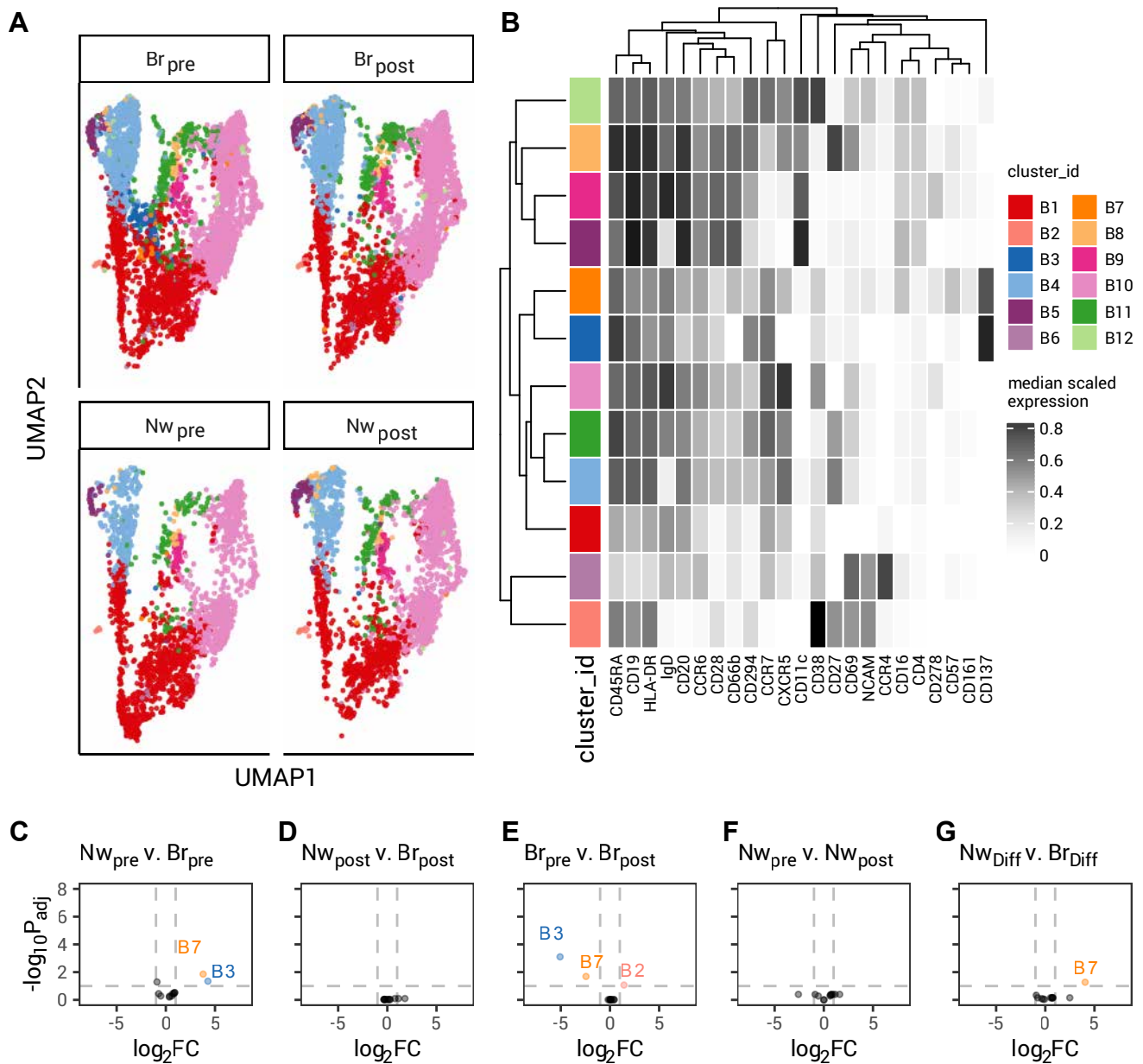


Figure 2. Mass cytometry demonstrates altered B cell sub-populations between broad and narrow responders before and after third doses

(A) UMAP embedding of B cells separated by breadth (Nw narrow, n=6; Br broad, n=11) and before (pre) and after (post) vaccination. 12 clusters identified by FlowSOM and ConsensusClusteringPlus are shaded. **(B)** Heatmap of surface expression of selected markers for the clusters shown in (A), and their color key is shared. Columns reflect the labelled cell surface marker. Scaled expression is shown from white (low/no expression) to black (high expression). **(C)-(G)** Differential abundance analysis for the 12 B cell clusters shown in (A) and (B), for the comparisons indicated: narrow pre vs broad pre in (C); narrow post vs broad post in (D); broad pre vs. broad post in (E); narrow pre vs. narrow post in (F); and the difference between (narrow pre vs. narrow post) and (broad pre vs. broad post) in (G). For (C-G), log₂ fold change ± 1 and $P_{adj}=0.01$ are shown by dashed lines. Color keys are shared (A-G).

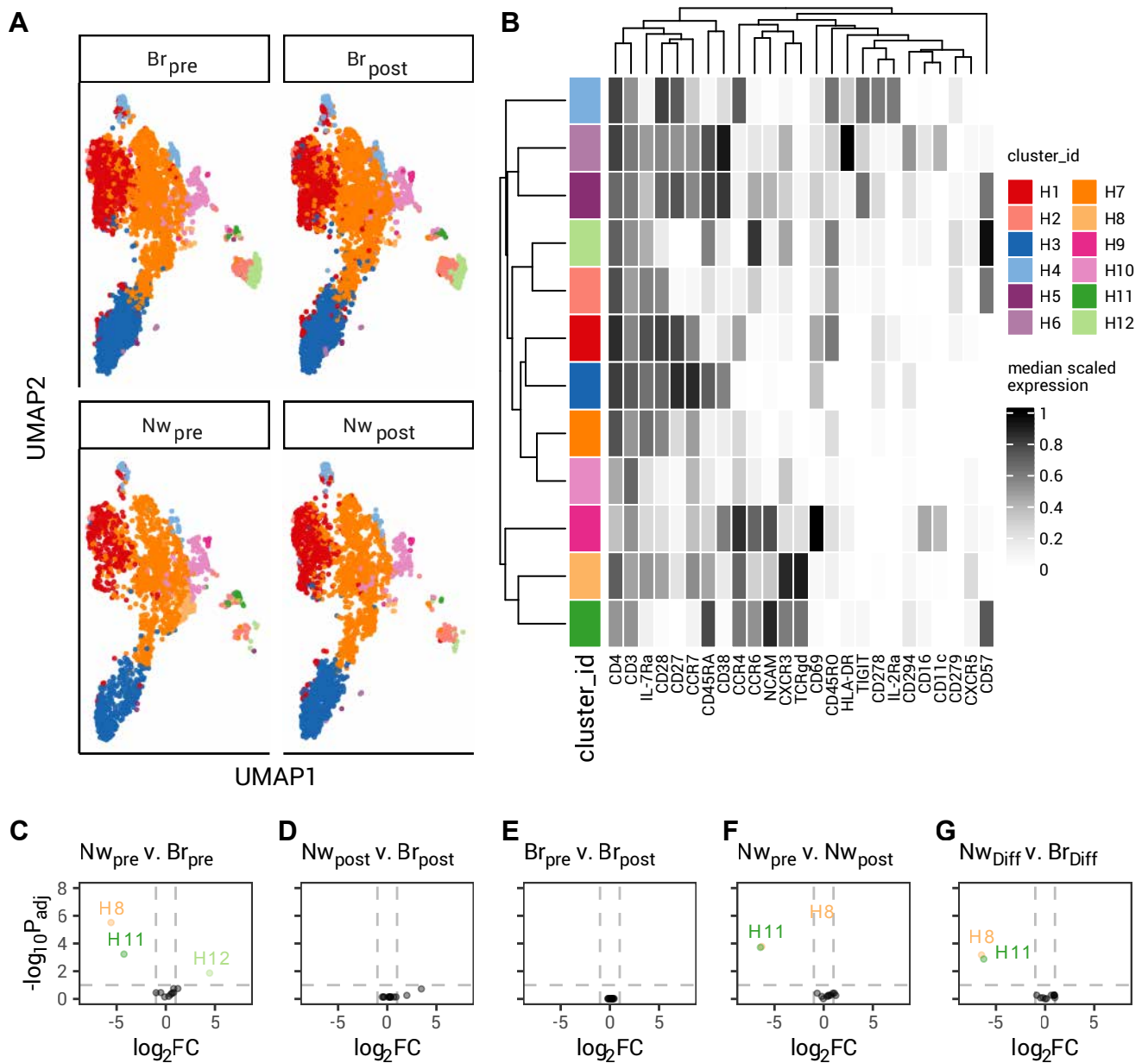


Figure 3. Perturbations in the CD4⁺ T cell compartment between broad and narrow responders before and after third doses

(A) UMAP embedding of CD4⁺ T cells separated by breadth (Nw narrow, n=6; Br broad, n=11) and before (pre) and after (post) vaccination. 12 clusters identified by FlowSOM and ConsensusClusteringPlus are shaded. **(B)** Heatmap of surface expression of selected markers for the clusters shown in (A). Rows represent the clusters shown in (A), and their color key is shared. Columns reflect the labelled cell surface marker. Scaled expression is shown from white (low/no expression) to black (high expression). **(C)-(G)** Differential abundance analysis for the 12 CD4⁺ T cell clusters shown in (A) and (B), for the comparisons indicated: narrow pre vs broad pre in (C); narrow post vs broad post in (D); broad pre vs. broad post in (E); narrow pre vs. narrow post in (E); and the difference between (narrow pre vs. narrow post) and (broad pre vs. broad post) in (G). For (C-G), \log_2 fold change ± 1 and $P_{adj}=0.01$ are shown by dashed lines. Color keys are shared (A-G).

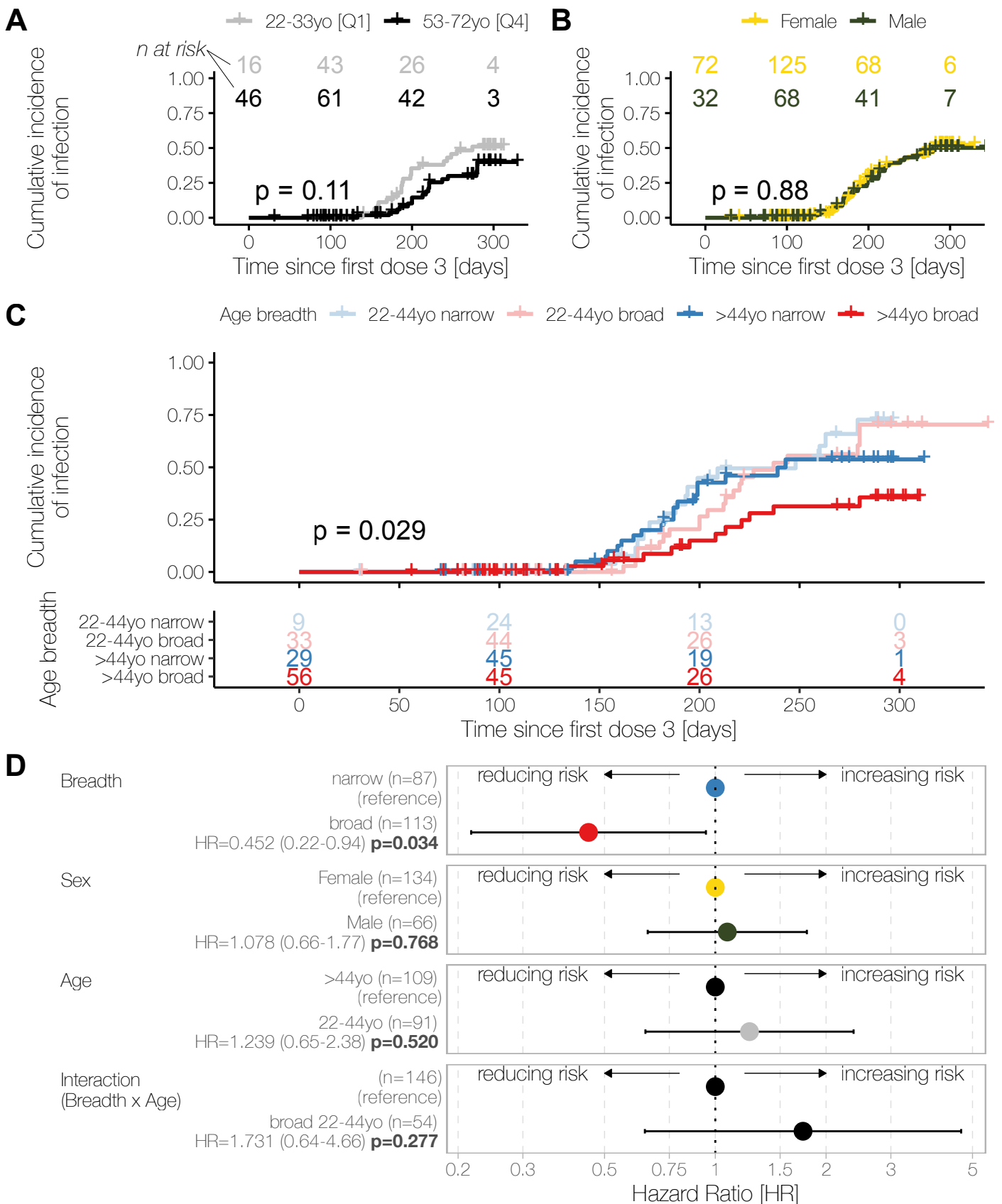


Figure 4. Individuals with broad neutralizing responses are relatively protected from Omicron BA.2 infection
(A) and **(B)** Time-to-event analysis for the acquisition of an Omicron BA.2 infection >14d after dose 3 in individuals in the first and last age quartiles: 22-33 years old (yo; Q1, grey) or 53-72 yo (Q4, black) (A); or in female (yellow) or male (green) participants (B). **(C)** Time-to-event analysis for the acquisition of an Omicron BA.2 infection >14d after dose 3 with two age groups (22-44yo, Q1-Q2; 44-72yo, >44yo, Q3-Q4), and by breadth of neutralization responses before dose 3. **(D)** Forest plot of proportional hazard ratios from a Cox proportional hazard model, with breadth, sex, age groups and the interaction term between breadth and age as predictors. For age, >44yo is used as the reference group (hazard ratio, HR=1); for breadth and sex, narrow and female are used as the respective reference group (HR=1). In (A)-(D), left-censoring occurs on the day of that individual's third dose and right censored with a non-BA.2 infection, or their last study visit before their fourth dose. In (A)-(C), the x-axis is the time in days since the earliest third dose, and P values are the log likelihood ratio test from a Cox model. The numbers at risk are shown for each group within the graph (A) and (B), or tabulated below (C).

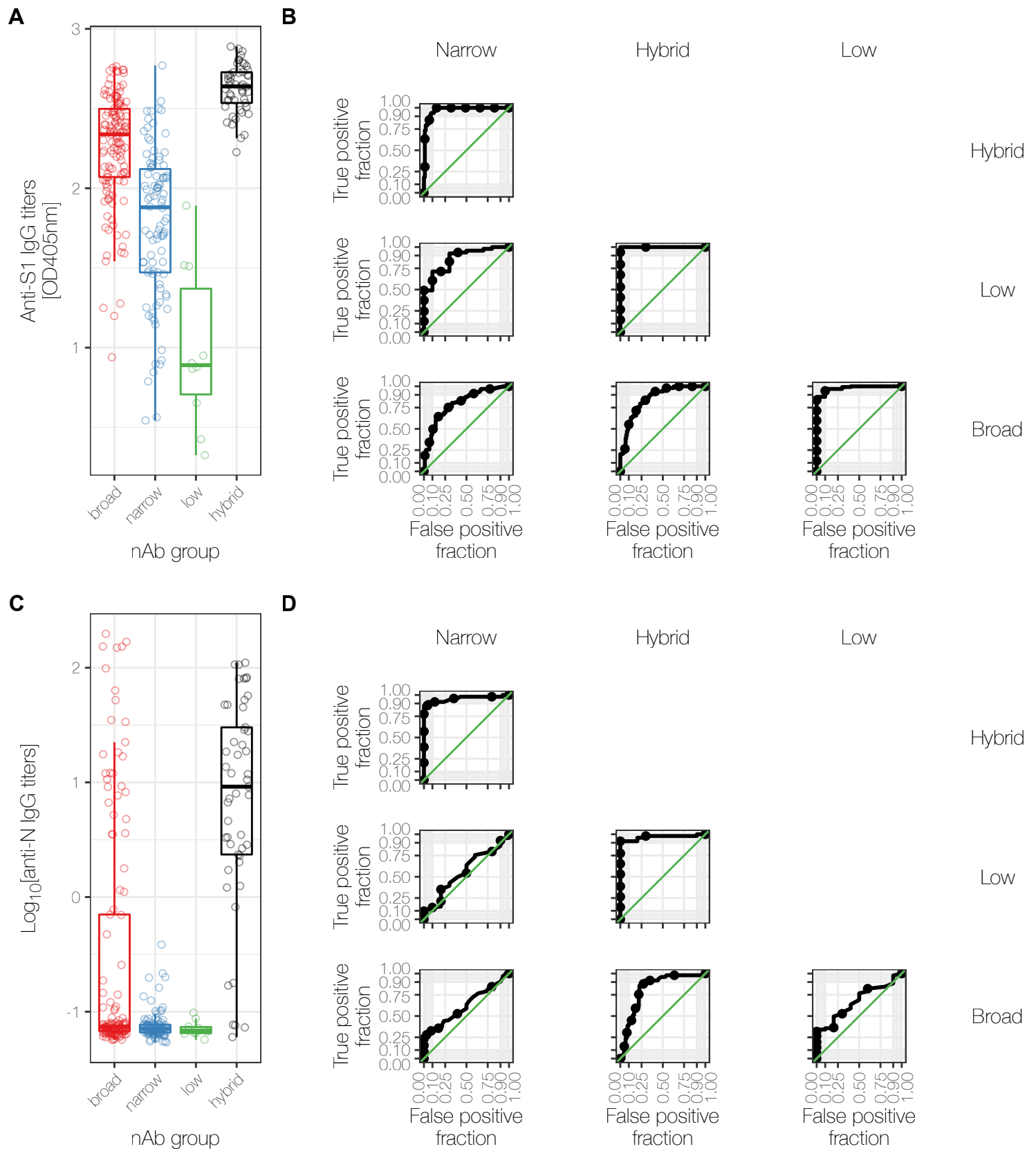


Figure S1. **Receiver operating characteristic for anti-S1 and anti-N IgG predicting serological profile**
(A) Titres of anti-S1 IgG, reported as scaled absorbance at OD405nm, for all 4 serological profile groups. **(B)** Receiver operating characteristic (ROC) curves for anti-S1 IgG titres in (A), between the indicated serological profile groups. **(C)** Decimal logarithm of anti-N IgG titres for all 4 serological groups. **(D)** As in (B), using anti-N IgG titres from (C).

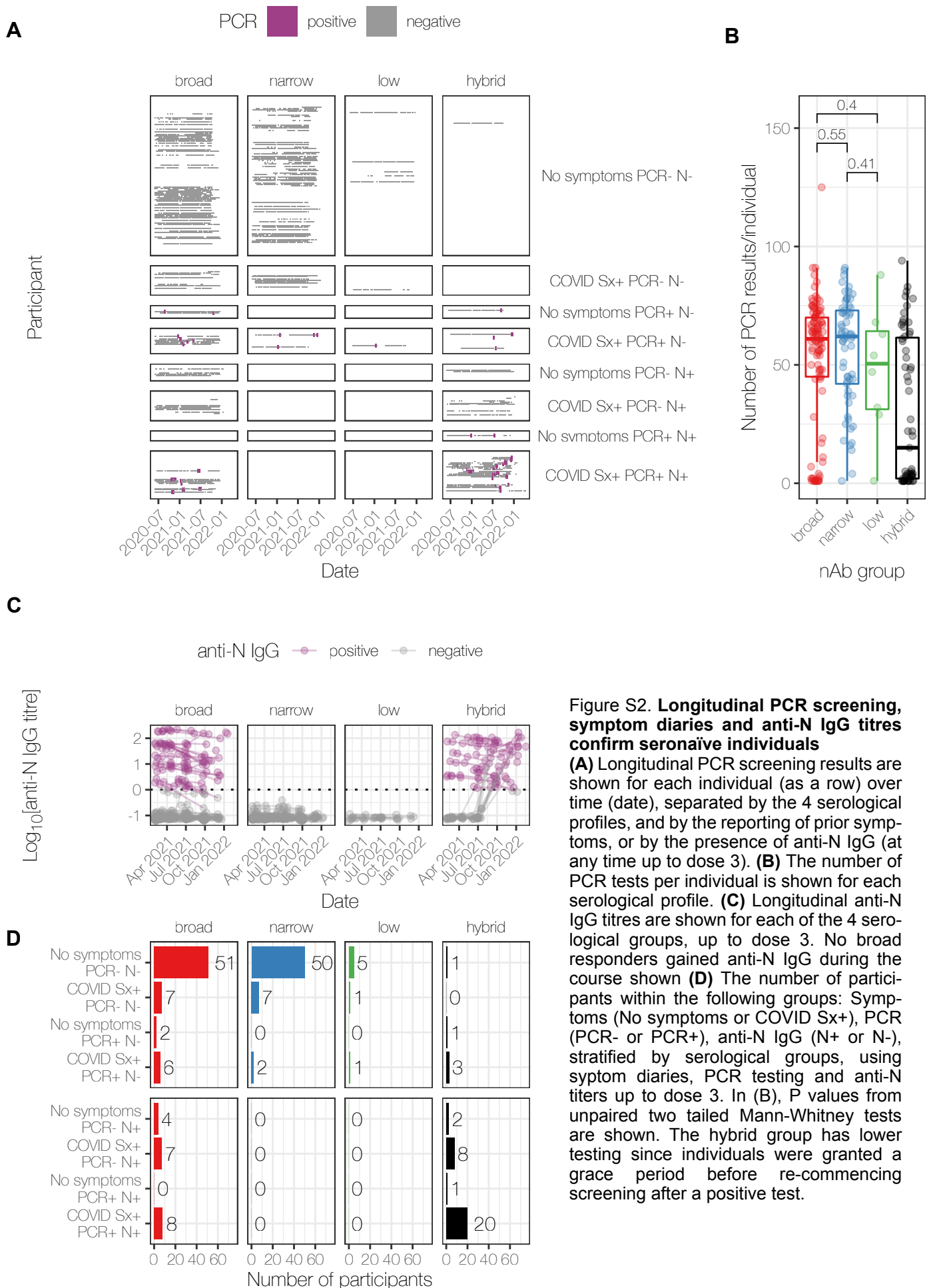


Figure S2. Longitudinal PCR screening, symptom diaries and anti-N IgG titres confirm seronegative individuals

(A) Longitudinal PCR screening results are shown for each individual (as a row) over time (date), separated by the 4 serological profiles, and by the reporting of prior symptoms, or by the presence of anti-N IgG (at any time up to dose 3). **(B)** The number of PCR tests per individual is shown for each serological profile. **(C)** Longitudinal anti-N IgG titres are shown for each of the 4 serological groups, up to dose 3. No broad responders gained anti-N IgG during the course shown **(D)** The number of participants within the following groups: Symptoms (No symptoms or COVID Sx+), PCR (PCR- or PCR+), anti-N IgG (N+ or N-), stratified by serological groups, using symptom diaries, PCR testing and anti-N titers up to dose 3. In (B), P values from unpaired two tailed Mann-Whitney tests are shown. The hybrid group has lower testing since individuals were granted a grace period before re-commencing screening after a positive test.

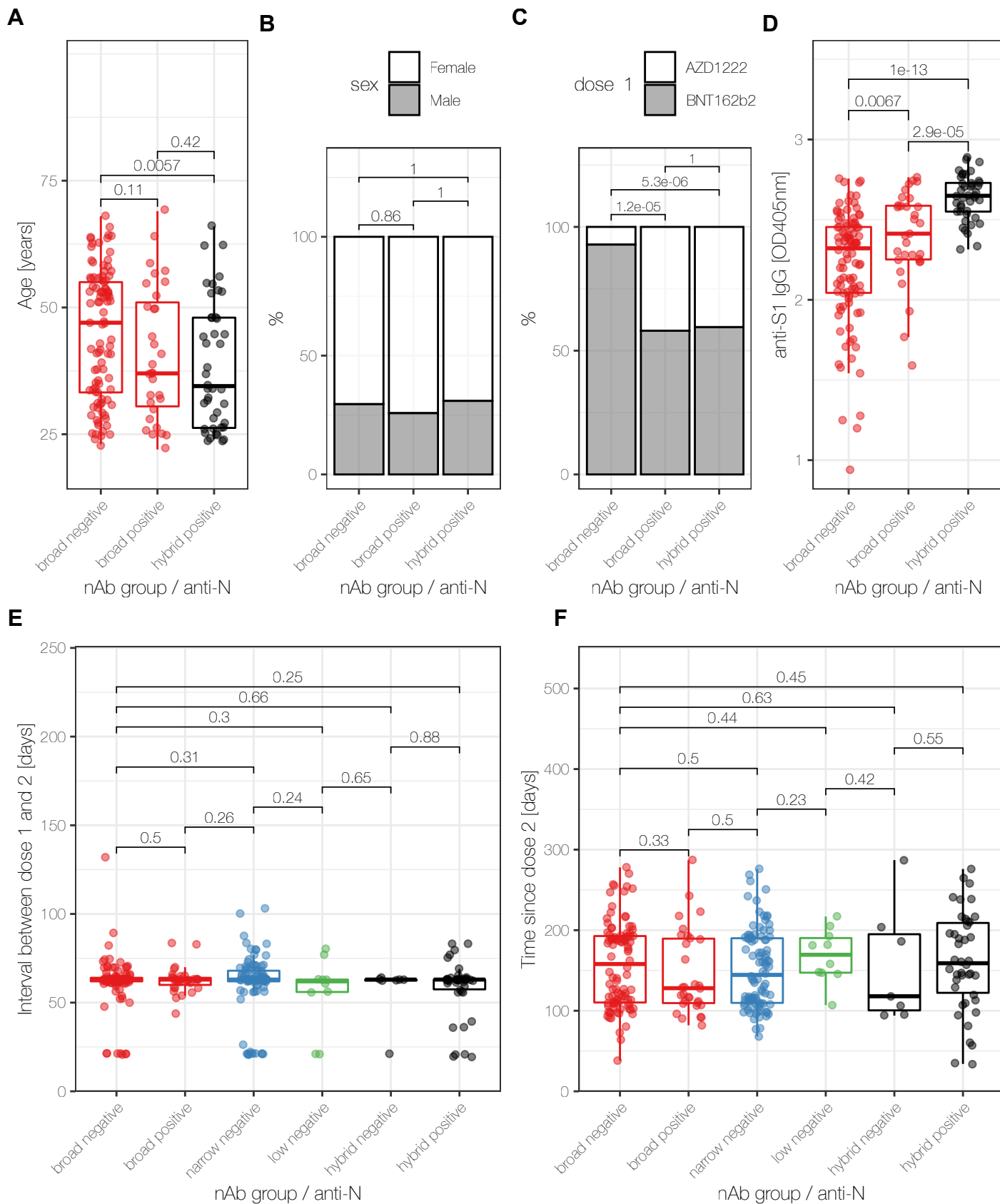


Figure S3. Demographics, vaccine usage and dose intervals for broad anti-N IgG seropositive or seronegative individuals

(A)-(D) Age in years (A) or sex (B) or vaccine used for doses 1 and 2 (C) or anti-S1 binding titres (D) for broad anti-N seropositive and seronegative individuals, compared to hybrid responders.

(E)-(F) Time interval in days between doses 1 and 2 (E) or serum sampling time from dose 2 (F) for all serological groups, stratified by anti-N result.

In (A, D-F), P values shown are from two tailed unpaired Mann-Whitney tests, without multiple correction testing. In (B-C) P values are from χ^2 tests.

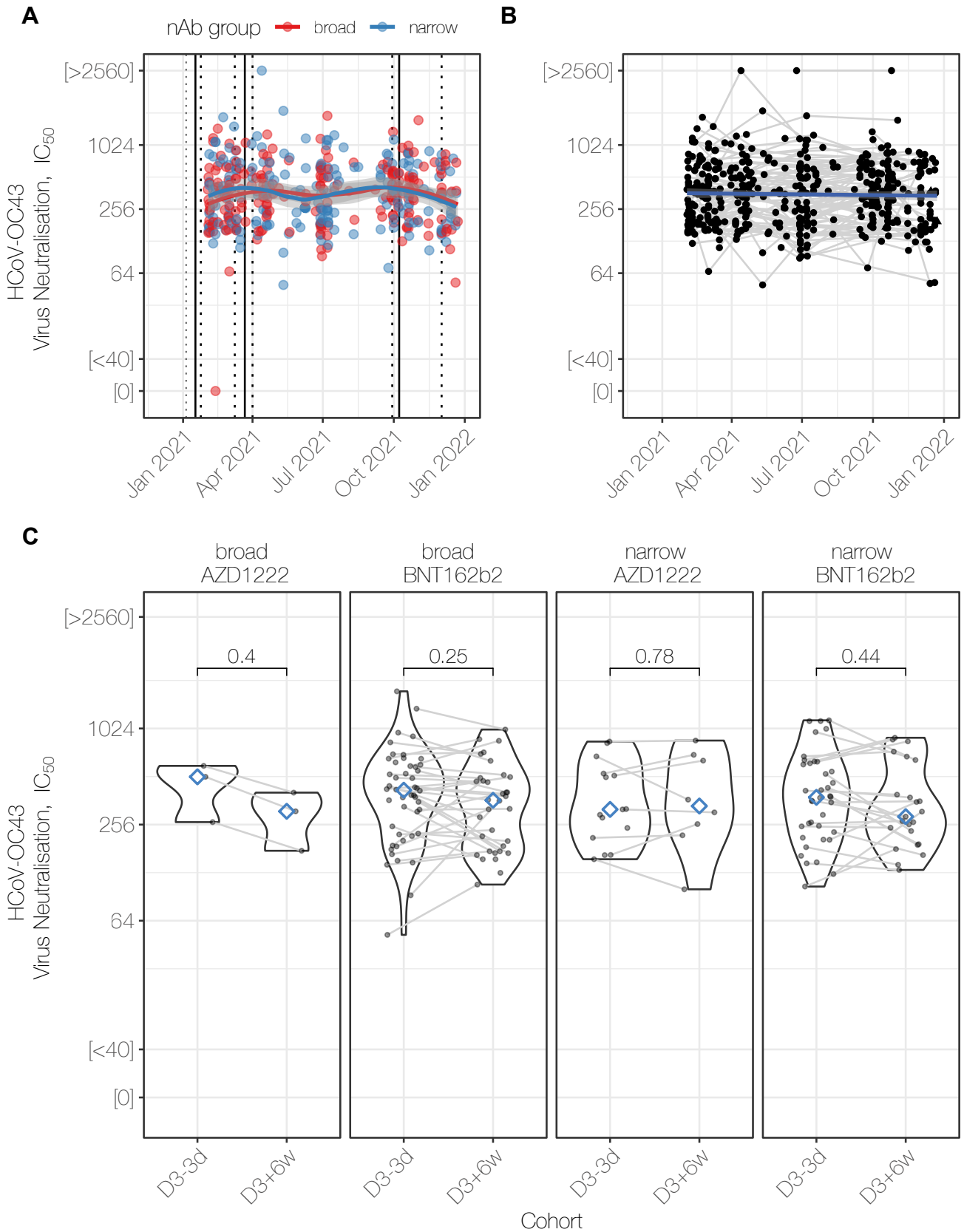


Figure S4. Neutralization of the seasonal human coronavirus HCoV-OC43 is not augmented by SARS-CoV-2 vaccination or infection.

(A) HCoV-OC43 live-virus microneutralization titer trajectories for broad and narrow responders. The median dates of vaccine doses and their interquartile ranges are shown by the vertical solid and dashed lines respectively. Neutralization titers are expressed as reciprocal of dilution with 50% inhibition of viral infection (IC_{50}). **(B)** As in (A), with a linear regression fit to demonstrate rate of waning **(C)** HCoV-OC43 neutralization before (median -91d; IQR 9-77d) and up to 6 weeks after (median 23d, IQR 18-31d) dose 3 in broad and narrow responders, stratified by their primary vaccination course.

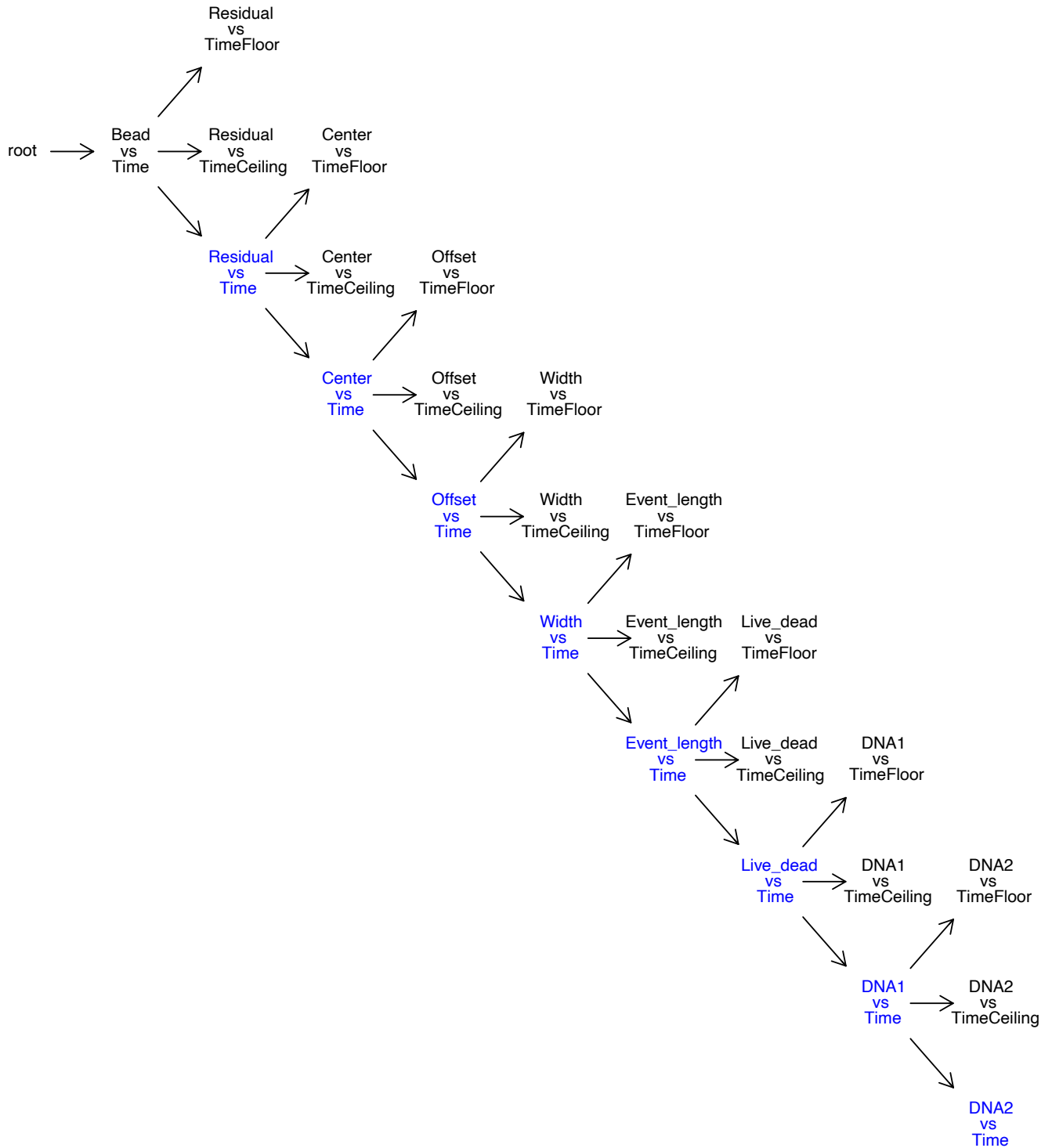


Figure S5. **Mass Cytometry gating tree**

Gating hierarchy progressing from raw events to processed single cells for downstream analysis. See Figure S6 for illustrative gates, and Figure S7 for flowSOM based quality control before biological clustering.

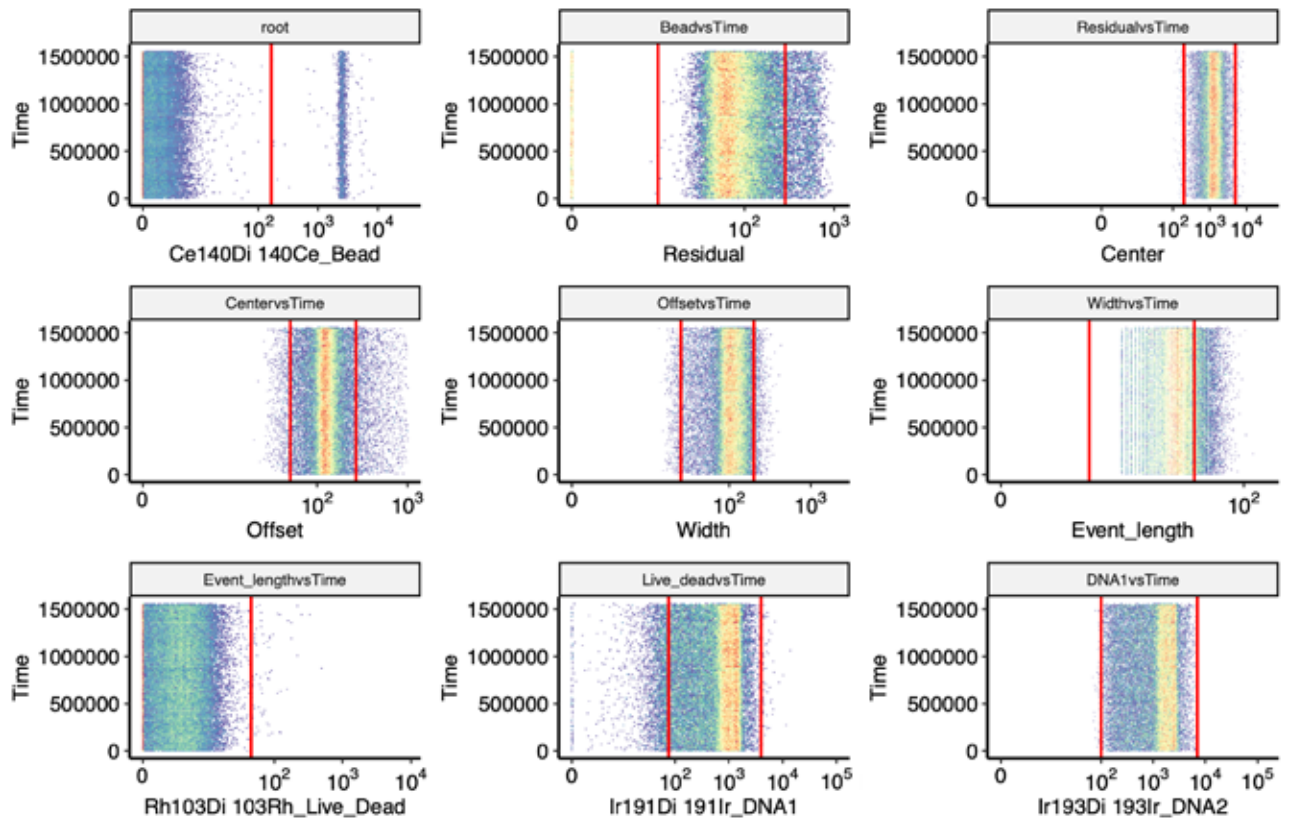
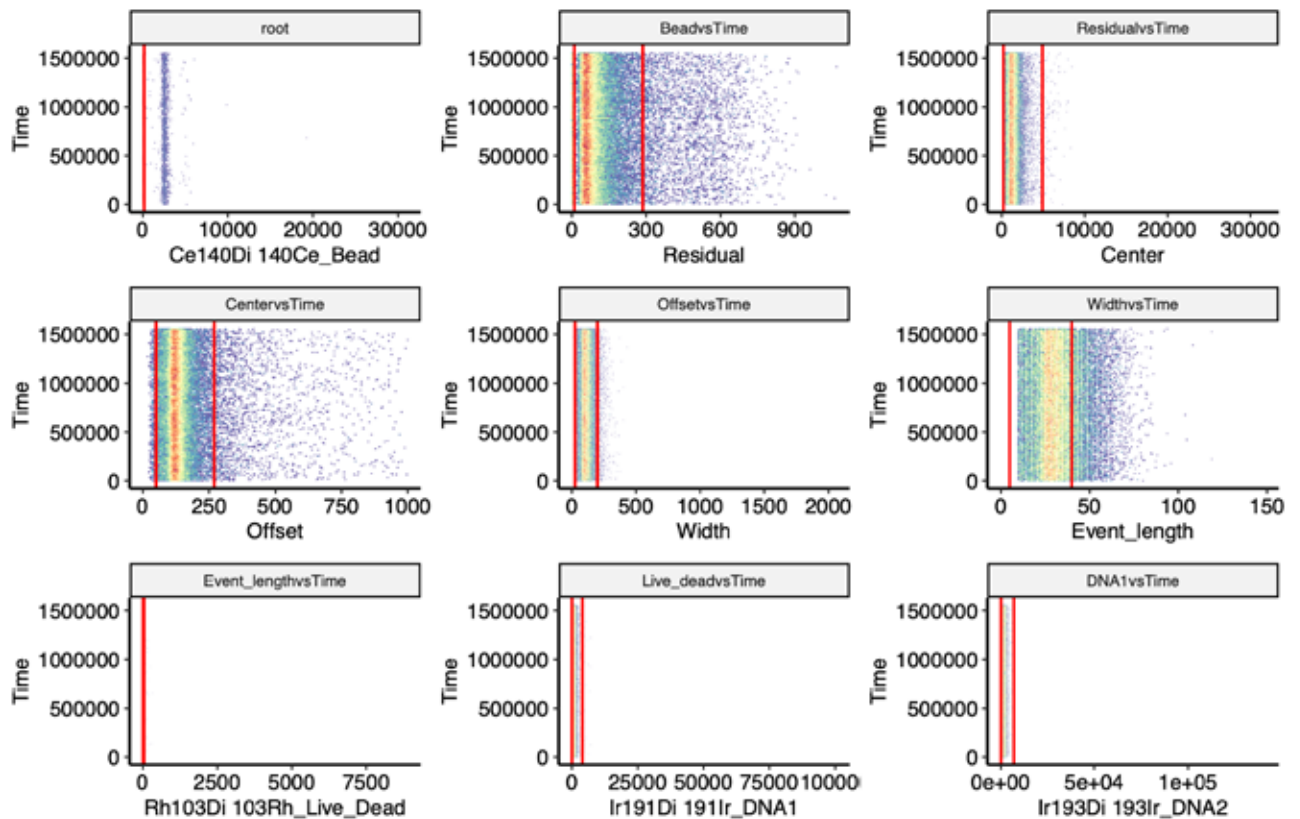
A**B**

Figure S6. **Mass Cytometry gating strategy**

(A) and **(B)** Sequential gating of events. First QC-beads are gated out (top left) and samples progress through the gating hierarchy (Figure S5), by row left-right to processed single cells.

In **(A)** parameters are plotted after inverse hyperbolic sine transformation (fasinH) and in **(B)** the same parameters are plotted as a linear transform. Details of the gating algorithm are described in the Methods.

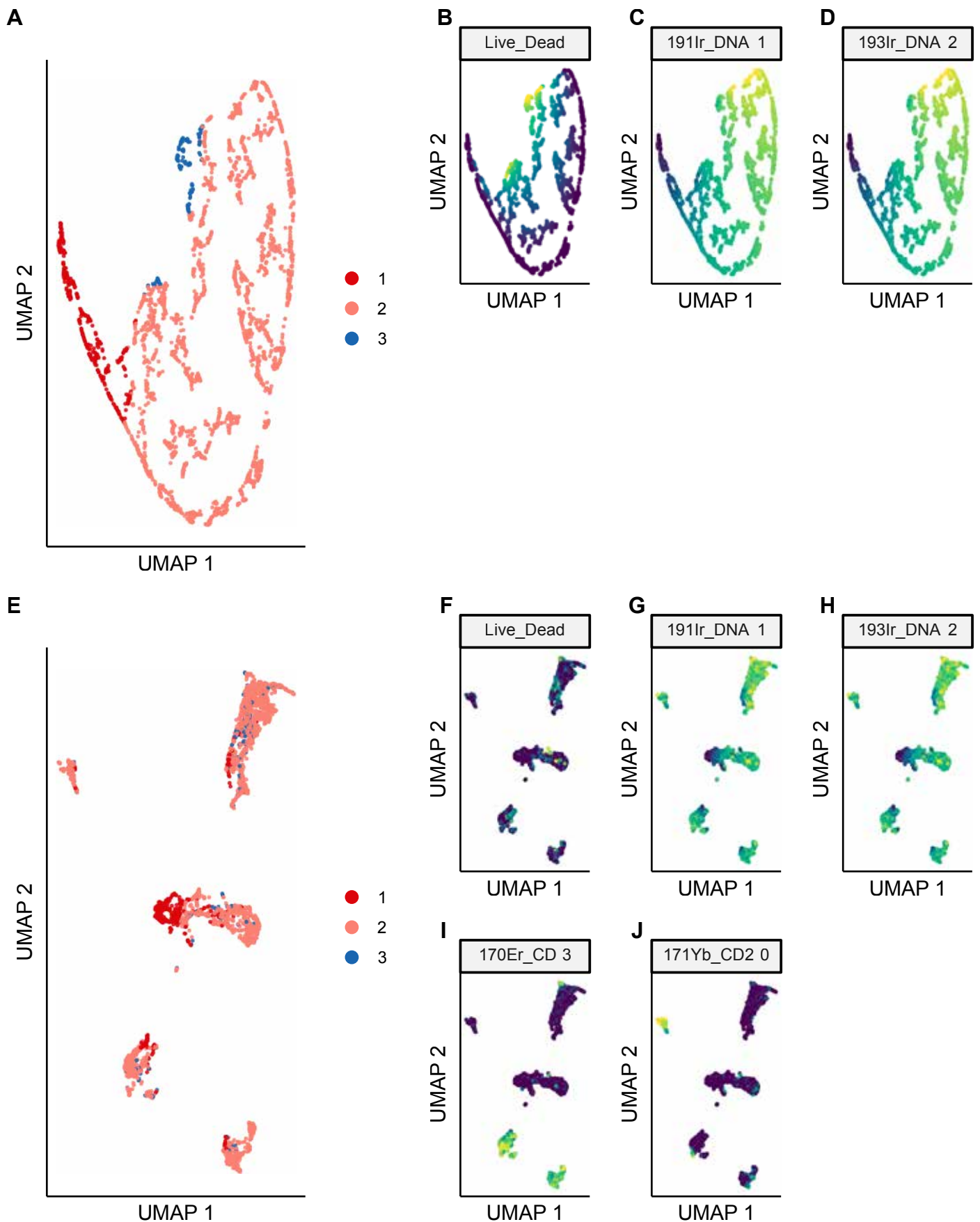


Figure S7. **Mass cytometry quality control using live/dead and DNA1 + DNA2**

(A) UMAP embedding of gated events, after flowSOM and ConsensusClusterPlus performed with the following channels: live/dead exclusion, DNA1 and DNA2.

(B)-(D) UMAP embedding from (A) shaded by the intensity of signal from each channel: live/dead exclusion (B), DNA1 (C) and DNA2 (D). **(E)** UMAP embedding of gated events, after flowSOM and ConsensusClusterPlus performed with the following channels: CD3, CD20, CD19, CD14, CD16, CD161, CD56, CD45RA, CD45RO, CD4, CD8a, CD11c, live/dead and DNA1 and DNA2. The shading reflects the clustering in (A).

(F)-(J) UMAP embedding from (E) shaded by the indicated marker.

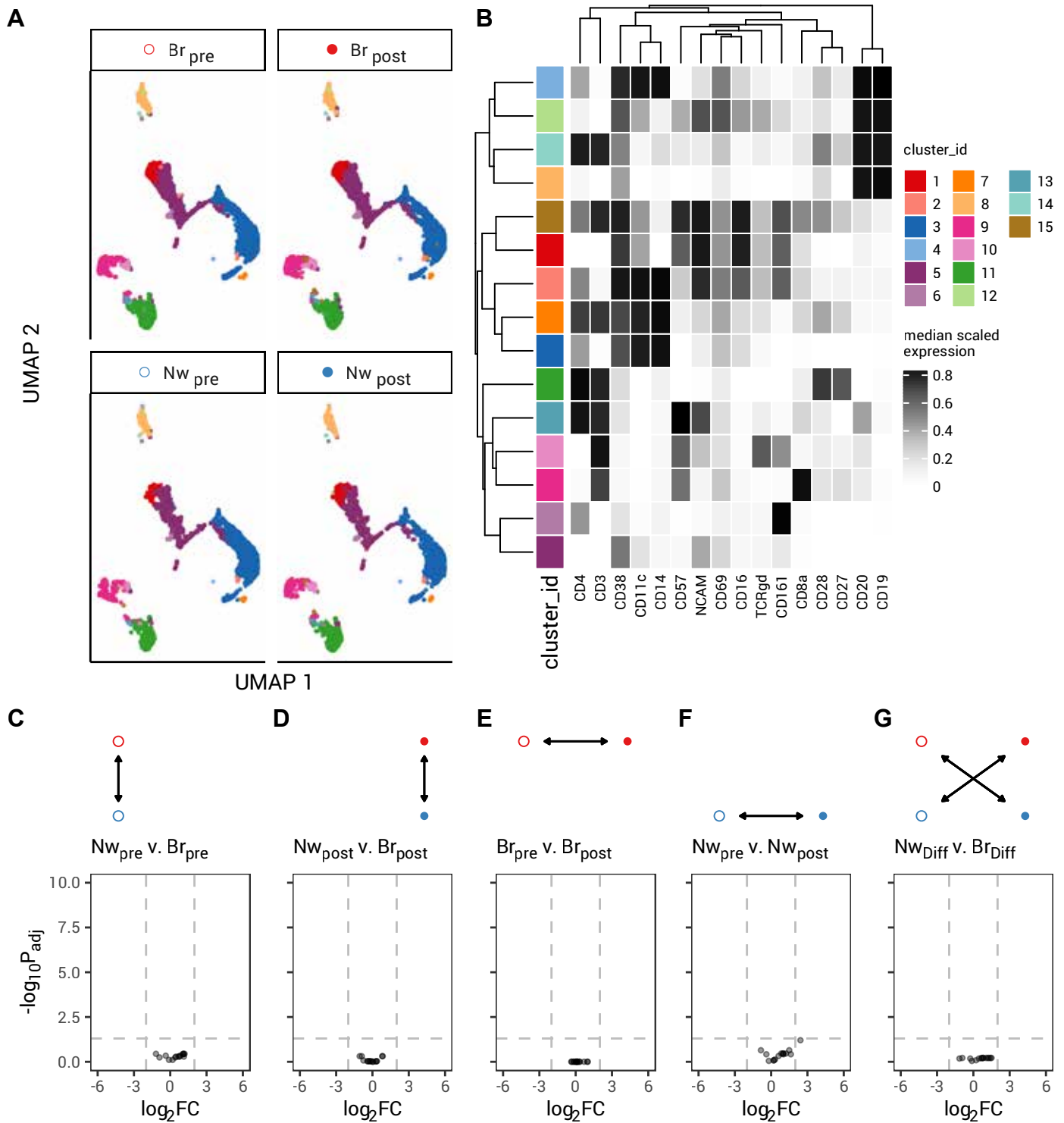


Figure S8. **PBMC-level mass cytometry analysis**

(A) UMAP embedding of peripheral blood mononuclear cells (PBMC) separated by breadth (Nw narrow, n=6; Br broad, n=11) and before (pre) and after (post) vaccination. 15 clusters identified by FlowSOM and ConsensusClusteringPlus are shaded.

(B) Heatmap of surface expression of selected markers for the clusters shown in (A). Rows represent the clusters shown in (A), and their color key is shared. Columns reflect the labelled cell surface marker. Scaled expression is shown from blue (low/no expression) to yellow (high expression). Cluster 9 are CD8⁺ T cells, with the adjacent cluster 10 being TCR $\gamma\delta^+$ T cells.

(C)-(G) Differential abundance analysis for the 15 PBMC clusters shown in (A) and (B), for the comparisons indicated: narrow pre vs broad pre in (C); narrow post vs broad post in (D); broad pre vs. broad post in (E); narrow pre vs. narrow post in (F); and the difference between (narrow pre vs. narrow post) and (broad pre vs. broad post) in (G). \log_2 fold change ± 1 and $P_{adj}=0.05$ are shown by dashed lines.