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1

2 **SARS-CoV-2 B.1.1.7 sensitivity to mRNA vaccine-elicited, convalescent and monoclonal**
3 **antibodies**

4

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40 **mutation; variant**

41

42 **Abstract**

43 **Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) transmission is**
44 **uncontrolled in many parts of the world, compounded in some areas by higher**
45 **transmission potential of the B.1.1.7 variant now seen in 50 countries. It is unclear**
46 **whether responses to SARS-CoV-2 vaccines based on the prototypic strain will be**
47 **impacted by mutations found in B.1.1.7. Here we assessed immune responses following**
48 **vaccination with mRNA-based vaccine BNT162b2. We measured neutralising antibody**
49 **responses following a single immunization using pseudoviruses expressing the wild-type**
50 **Spike protein or the 8 amino acid mutations found in the B.1.1.7 spike protein. The**
51 **vaccine sera exhibited a broad range of neutralising titres against the wild-type**
52 **pseudoviruses that were modestly reduced against B.1.1.7 variant. This reduction was**
53 **also evident in sera from some convalescent patients. Decreased B.1.1.7 neutralisation**
54 **was also observed with monoclonal antibodies targeting the N-terminal domain (9 out of**
55 **10), the Receptor Binding Motif (RBM) (5 out of 31), but not in neutralising mAbs**
56 **binding outside the RBM. Introduction of the E484K mutation in a B.1.1.7 background**
57 **to reflect newly emerging viruses in the UK led to a more substantial loss of neutralising**
58 **activity by vaccine-elicited antibodies and mAbs (19 out of 31) over that conferred by**
59 **the B.1.1.7 mutations alone. E484K emergence on a B.1.1.7 background represents a**
60 **threat to the vaccine BNT162b.**

61

62 **Introduction**

63 The outbreak of a pneumonia of unknown cause in Wuhan, China in December 2019,
64 culminated in a global pandemic due to a novel viral pathogen, now known to be SARS-CoV-
65 2¹. The unprecedented scientific response to this global challenge has led to the rapid
66 development of vaccines aimed at preventing SARS-COV-2 infection and transmission.
67 Continued viral evolution led to the emergence and selection of SARS-CoV-2 variants with
68 enhanced infectivity/transmissibility^{2,3 4,5} and ability to circumvent drug⁶ and immune
69 control^{7,8}.

70 SARS-CoV-2 vaccines have recently been licensed that target the spike (S) protein,
71 either using mRNA or adenovirus vector technology with protection rates ranging from 62 to
72 95%⁹⁻¹¹. The BNT162b2 vaccine encodes the full-length trimerised S protein of SARS CoV-2
73 and is formulated in lipid nanoparticles for delivery to cells¹². Other vaccines include the
74 Moderna mRNA-1273 vaccine, which is also a lipid nanoparticle formulated S glycoprotein¹³
75 and the Oxford-AstraZeneca ChAdOx1 nCoV-19 vaccine (AZD1222) which is a replication-
76 deficient chimpanzee adenoviral vector ChAdOx1, containing the S glycoprotein¹⁴. The
77 duration of immunity conferred by these vaccines is as yet unknown. These vaccines were
78 designed against the Wuhan-1 isolate discovered in 2019. Concerns have been raised as to
79 whether these vaccines will be effective against newly emergent SARS-CoV-2 variants, such

80 as B.1.1.7 (N501Y.V1), B.1.351 (N501Y.V2) and P1 (N501Y.V3) that originated in the UK,
81 South Africa, and Brazil and are now being detected all over the world¹⁵⁻¹⁷.

82 In clinical studies of the Pfizer-BioNTech BNT162b2 vaccine, high levels of
83 protection against infection and severe disease were observed after the second dose¹⁰.
84 Neutralising geometric mean titre (GMT) was below cut-off in most cases after prime dose,
85 but as anticipated, titres substantially increased after boost immunization¹⁸. In older adults
86 mean GMT was only 12 in a preliminary analysis of 12 participants¹⁹ and increased to 109
87 after the second dose.

88 In this study, we assess antibody responses against the the B.1.1.7 variant after
89 vaccination with the first and second doses of BNT162b2, showing modest reduction in
90 neutralisation against pseudoviruses bearing B.1.1.7 Spike mutations (Δ H69/V70, Δ I144,
91 N501Y, A570D, P681H, T716I, S982A and D1118H). In addition, by using a panel of human
92 neutralising monoclonal antibodies (mAbs) we show that the B.1.1.7 variant can escape
93 neutralisation mediated by most NTD-specific antibodies tested and by a fraction of RBM-
94 specific antibodies. Finally, we show that the recent emergence and transmission of B.1.1.7
95 viruses bearing the Spike E484K mutation results in significant additional loss of
96 neutralisation by BNT162b2 mRNA-elicited antibodies, convalescent sera and mAbs.

97

98 **Results**

99 Thirty seven participants had received the first dose of BNT162b2 mRNA vaccine
100 three weeks prior to blood draw for serum and peripheral blood mononuclear cells (PBMC)
101 collection. Median age was 63.5 years (IQR 47-84) and 33% were female. Serum IgG titres to
102 Nucleocapsid (N) protein, S and the S receptor binding domain (RBD) were assayed by
103 particle based flow cytometry on a Luminex analyser (**Extended Data Fig. 1a**). These data
104 showed S and RBD antibody titres much higher than in healthy controls, but lower than in
105 individuals recovered from COVID-19 and titres observed in therapeutic convalescent
106 plasma. The raised N titres relative to control could be the result of non-specific cross
107 reactivity that is increased following vaccination. However, the antibody response was
108 heterogeneous with almost 100-fold variation in IgG titres to S and RBD across the
109 vaccinated participants.

110 Using lentiviral pseudotyping we studied WT (wild type bearing D614G) and mutant
111 B.1.1.7 S proteins (**Fig. 1a**) on the surface of enveloped virions in order to measure
112 neutralisation activity of vaccine-elicited sera. This system has been shown to give results

113 correlating with replication competent authentic virus^{20,21}. Eight out of 37 participants
114 exhibited no appreciable neutralisation against the WT pseudotyped virus following the first
115 dose of vaccines. The vaccine sera exhibited a range of inhibitory dilutions giving 50%
116 neutralisation (ID50) (**Fig. 1c-d**). The GMT against wild type (WT) following the second
117 dose of vaccine was an order of magnitude higher than after the first dose (318 vs 77) (Fig 1c-
118 f). There was correlation between full length S IgG titres and serum neutralisation titres
119 (**Extended Data Fig. 1b**). A broad range of T cell responses was measured by IFN gamma
120 FluoroSpot against SARS-CoV-2 peptides in vaccinees. These cellular responses did not
121 correlate with IgG S antibody titres (**Extended Data Fig. 1c-d**).

122 We then generated mutated pseudoviruses carrying S protein with mutations N501Y,
123 A570D and the H69/V70 deletion. We observed no reduction in the ability of sera from
124 vaccinees to inhibit either WT or mutant virus (**Extended Data Fig. 2a, b**). A panel of sera
125 from ten recovered individuals also neutralised both wild type and the mutated viruses
126 similarly (**Extended Data Fig. 2c**). We next completed the full set of eight mutations in the S
127 protein present in B.1.1.7 variant (**Fig. 1a**), Δ H69/V70, Δ 144, N501Y and A570D in the S₁
128 subunit and P681H, T716I, S982A and D1118H in the S₂ subunit. All constructs also
129 contained D614G. We found that among 29 individuals with neutralisation activity against the
130 WT three weeks after receiving a single dose of the the BNT162b2 mRNA vaccine, 20
131 showed evidence of reduction in efficacy of antibodies against the B.1.1.7 mutant (**Fig. 1b-c**,
132 **Extended Data Fig. 3**). The mean fold change reduction in sensitivity to first dose vaccine
133 sera of B.1.1.7 compared to WT was approximately 3.2 (SD 5.7). The variation is likely due
134 to the low neutralisation titres following first dose. Following the second dose, GMT was
135 markedly increased compared with first dose titres, and the mean fold change had reduced to
136 1.9 (SD 0.9) (**Fig. 1d-e**). Amongst sera from 27 recovered individuals, the GMT at 50%
137 neutralisation was 1334 for WT, significantly higher than post second dose vaccination (**Fig.**
138 **1f-g**). The fold change in ID50 for neutralisation of B.1.1.7 versus wild type (D614G) was 4.5
139 (**Fig. 1f-g** and **Extended Data Fig. 4**).

140 **B.1.1.7 with spike E484K mutation and neutralization by vaccine and convalescent sera**
141 The E484K substitution (**Fig. 2a**) is antigenically important, and has been reported as an
142 escape mutation for several monoclonal antibodies including C121, C144, REGN10933 and
143 Ly-CoV555²². E484K is also known to be present in the B.1.351 (501Y.V2) and P.1
144 (501Y.V3) lineages in combination with amino acid replacements at N501 and K417. As of

145 10th Feb 2021, twenty three English and two Welsh B.1.1.7 sequences from viral isolates
146 contained the E484K substitution (**Fig. 2b**). The number of B.1.1.7 sequences has been
147 increasing since the start of December 2020 (**Fig. 2c**). Phylogenetic analysis suggests that
148 there have been multiple independent acquisitions, with one lineage appearing to expand over
149 time, indicating active transmission (**Fig. 2b**). This has resulted in Public Health England
150 naming this as a variant of concern (VOC 202102/02)²³, triggering enhanced public health
151 measures. There are as yet no phenotypic data on the sensitivity to neutralisation for this
152 virus or its spike protein.

153 We therefore generated pseudoviruses bearing B.1.1.7 spike mutations with or without
154 additional E484K and tested these against sera obtained after first and second dose mRNA
155 vaccine as well as against convalescent sera. Following second dose, we observed a
156 significant loss of neutralising activity for the pseudovirus with B.1.1.7 spike mutations and
157 E484K (Fig 3d-e). The mean fold change for the E484K B.1.1.7 Spike was 6.7 compared to
158 1.9 for B.1.1.7, relative to WT (**Fig. 3a-c**). Similarly when we tested a panel of convalescent
159 sera with a range of neutralisation titres (Fig. 1f-g), we observed additional loss of activity
160 against the mutant B.1.1.7 spike with E484K, with fold change of 11.4 relative to WT (**Fig.**
161 **3f-g**).

162 **B.1.1.7 variant escape from NTD- and RBM-specific mAb-mediated neutralization.**

163 To investigate the role of the full set of mutations in NTD, RBD and S2 present in the B.1.1.7
164 variant, we tested 60 mAbs isolated from 15 individuals that recovered from SARS-CoV-2
165 infection in early 2020 with an *in-vitro* pseudotyped neutralization assay using VeroE6 target
166 cells expressing Transmembrane protease serine 2 (TMPRSS2, **Extended Data Table 1**). We
167 found that 20 out of 60 (33.3%) mAbs showed a greater than 2-fold loss of neutralising
168 activity of B.1.1.7 variant compared to WT SARS-CoV-2 (**Fig. 4a,b** and **Extended Data Fig.**
169 **5**). Remarkably, the B.1.1.7 mutant virus was found to fully escape neutralization by 8 out of
170 10 NTD-targeting mAbs (80%), and partial escape from an additional mAb (10%) (**Fig. 4c**).
171 We previously showed that the deletion of residue 144 abrogates binding by 4 out of 6 NTD-
172 specific mAbs tested, possibly accounting for viral neutralization escape by most NTD-
173 specific antibodies²⁴. Of the 31 RBM-targeting mAbs, 5 (16.1%) showed more than 100-fold
174 decrease in B.1.1.7 neutralization, and additional 6 mAbs (19.4%) had a partial 2-to-10-fold
175 reduction (**Fig. 4d**). Finally, all RBD-specific non-RBM-targeting mAbs tested fully retained
176 B.1.1.7 neutralising activity (**Fig. 4e**).

177 To address the role of B.1.1.7 N501Y mutation in the neutralization escape from
178 RBM-specific antibodies, we tested the binding of 50 RBD-specific mAbs to WT and N501Y
179 mutant RBD by biolayer interferometry (**Fig. 4f** and **Extended Data Fig. 6**). The 5 RBM-
180 specific mAbs that failed to neutralize B.1.1.7 variant (**Fig. 4d**) showed a complete loss of
181 binding to N501Y RBD mutant (**Fig. 4g-h**), demonstrating a role for this mutation as an
182 escape mechanism for certain RBM-targeting mAbs.

183 The decreased neutralising activity of the immune sera from vaccinees and
184 convalescent patients against B.1.1.7, but not against Δ 69/70-501Y-570D mutant (**Fig. 1** and
185 **Extended Data Fig. 2**), could be the result of a loss of neutralising activity of both RBD- and
186 NTD-targeting antibodies, and suggests that the key mutation is Δ 144. RBD antibodies
187 against N501Y could play a role in decreased neutralisation by sera, with the overall impact
188 possibly modulated by other mutations present in B.1.1.7, as well as the relative dominance
189 of NTD versus RBM antibodies in polyclonal sera.

190 To assess the effect of E484K on this panel of mAbs we generated a SARS-CoV-2
191 pseudotype carrying the K417N, E484K and N501Y mutations (TM). The inclusion of the
192 K417N substitution was prompted by the observation that substitutions at this position have
193 been found in 5 sequences from recent viral isolates within the B.1.1.7 lineage (K417 to N, E
194 or R). This is in keeping with convergent evolution of the virus towards an RBD with
195 N501Y, E484K and K417N/T as evidenced by B.1.351 and P.1 lineages (K417N or K417T,
196 respectively) causing great concern globally. It is therefore important to assess this
197 combination going forward.

198 Importantly, mutations at K417 are reported to escape neutralization from mAbs,
199 including the recently approved mAb LY-CoV016^{22,25}. Out of the 60 mAbs tested, 20
200 (33.3%) showed >10 fold loss of neutralising activity of TM mutant compared to WT SARS-
201 CoV-2 (**Fig. 4 a-b** and **Extended Data Fig. 5**), and of these 19 are RBM-specific mAbs. As
202 above, we addressed the role of E484K mutation in escape from RBM-specific antibodies, by
203 testing the binding of 50 RBD-specific mAbs to WT and E484K mutant RBD by biolayer
204 interferometry (**Fig. 4f** and **Extended Data Fig. 7**). Out of the 19 RBM-specific mAbs that
205 showed reduced or loss of neutralization of TM mutant (**Fig. 4d**), 16 showed a complete or
206 partial loss of binding to E484K RBD mutant (**Fig. 4g-h**), consistent with findings that
207 E484K is an important viral escape mutation^{26,39,27}. Three of these 16 mAbs also lost binding
208 to an RBD carrying N501Y, indicating that a fraction of RBM antibodies are sensitive to both
209 N501Y and E484K mutations. Similarly, 3 of the 19 mAbs that lost neutralization of TM

210 mutant (S2D8, S2H7 and S2X128) were previously shown to lose binding and neutralization
211 to the K417V mutant, and here shown to be sensitive to either N501Y or E484K mutations.

212

213 **SARS-CoV-2 B.1.1.7 binds human ACE2 with higher affinity than WT**

214 SARS-CoV-2 and SARS-CoV enter host cells through binding of the S glycoprotein to
215 angiotensin converting enzyme 2 (ACE2)^{1,28}. Previous studies showed that the binding
216 affinity of SARS-CoV for human ACE2 correlated with the rate of viral replication in distinct
217 species, transmissibility and disease severity²⁹⁻³¹. However, the picture is unclear for SARS-
218 CoV-2. To understand the potential contribution of receptor interaction to infectivity, we set
219 out to evaluate the influence of the B.1.1.7 RBD substitution N501Y on receptor engagement.
220 We used biolayer interferometry to study binding kinetics and affinity of the purified human
221 ACE2 ectodomain (residues 1-615) to immobilized biotinylated SARS-CoV-2 B.1.1.7 or WT
222 RBDs. We found that ACE2 bound to the B.1.1.7 RBD with an affinity of 22 nM compared
223 to 133 nM for the WT RBD (**Extended Data Fig. 8**), in agreement with our previous deep-
224 mutational scanning measurements using dimeric ACE2³². Although ACE2 bound with
225 comparable on-rates to both RBDs, the observed dissociation rate constant was slower for
226 B.1.1.7 than for the WT RBD (**Table 1**).

227

228 To understand the impact of TM mutations (K417N, E484K and N501Y), we evaluated
229 binding of ACE2 to the immobilized TM RBD using biolayer interferometry. We determined
230 an ACE2 binding affinity of 64 nM for the TM RBD which is driven by a faster off-rate than
231 observed for the B.1.1.7 RBD but slower than for the WT RBD. Based on our previous deep-
232 mutational scanning measurements using dimeric ACE2, we propose that the K417N
233 mutation is slightly detrimental to ACE2 binding explaining the intermediate affinity
234 determined for the TM RBD compared to the B.1.17 and WT RBDs, likely as a result of
235 disrupting the salt bridge formed with ACE2 residue D30. Enhanced binding of the B.1.1.7
236 RBD to human ACE2 resulting from the N501Y mutation might participate in the efficient
237 ongoing transmission of this newly emergent SARS-CoV-2 lineage, and possibly reduced
238 opportunity for antibody binding. Although the TM RBD mutations found in B.1.351 are
239 known to participate in immune evasion^{33,34}, the possible contribution to transmissibility of
240 enhanced ACE2 binding relative to WT remains to be determined for this lineage.

241 Discussion

242 Serum neutralising activity is a correlate of protection for other respiratory viruses, including
243 influenza³⁵ and respiratory syncytial virus where prophylaxis with monoclonal antibodies has
244 been used in at-risk groups^{36,37}. Neutralising antibody titres appeared to be highly correlated
245 with vaccine protection against SARS-CoV-2 rechallenge in non-human primates, and
246 importantly, there was no correlation between T cell responses (as measured by ELISpot) and
247 protection³⁸. Moreover, passive transfer of purified polyclonal IgGs from convalescent
248 macaques protected naïve macaques against subsequent SARS-CoV-2 challenge³⁹. Coupled
249 with multiple reports of re-infection, there has therefore been significant attention placed on
250 virus neutralisation.

251 This study reports on the neutralisation by sera collected after both the first and second
252 doses of the BNT162b2 vaccine. The participants of this study were older adults, in line with
253 the targeting of this age group in the initial rollout of the vaccination campaign in the UK.
254 Participants showed similar neutralising activity against wild type pseudovirus as in the phase
255 I/II study¹². This is relevant for the UK and other countries planning to extend the gap
256 between doses of mRNA and adenovirus based vaccines from 3 to 12 weeks, despite lack of
257 data for this schedule for mRNA vaccines in particular.

258 The three mutations in S1 (N501Y, A570D, Δ H69/V70) did not appear to impact
259 neutralisation in a pseudovirus assay, consistent with data on N501Y having little effect on
260 neutralisation by convalescent and post vaccination sera⁴⁰. However, we demonstrated that a
261 pseudovirus bearing S protein with the full set of mutations present in the B.1.1.7 variant (i.e.,
262 Δ H69/V70, Δ 144, N501Y, A570D, P681H, T716I, S982A, D1118H) did result in small
263 reduction in neutralisation by sera from vaccinees that was more marked following the first
264 dose than the second dose. This could be related to increased breadth/potency/concentration
265 of antibodies following the boost dose. A reduction in neutralization titres from mRNA-
266 elicited antibodies in volunteers who received two doses (using both mRNA-1273 and
267 BNT162b2 vaccines) was also observed by Wang et al.⁴¹ using pseudoviruses carrying the
268 N501Y mutation. Other studies also reported small reduction of neutralization against the
269 B.1.1.7 variant against sera from individuals vaccinated with two doses of BNT162b2⁴² and
270 mRNA-1273⁴³. Xie et al did not find an effect of N501Y alone in the context of BNT162b2
271 vaccine sera⁴⁴.

272 The reduced neutralising activity observed with polyclonal antibodies elicited by
273 mRNA vaccines observed in this study is further supported by the loss of neutralising activity
274 observed with human mAbs directed to both RBD and, to a major extent, to NTD. In the
275 study by Wang et al., 6 out of 17 RDB-specific mAbs isolated from mRNA-1273 vaccinated
276 individuals showed more than 100-fold neutralisation loss against N501Y mutant, a finding
277 that is consistent with the loss of neutralisation by 5 out of 29 RBM-specific mAbs described in
278 this study. However, the contribution of N501Y to loss of neutralisation activity of polyclonal
279 vaccine and convalescent sera is less clear, and interactions with other mutations likely.

280 Multiple variants, including the 501Y.V2 and B.1.1.7 lineages, harbor multiple
281 mutations as well as deletions in NTD, most of which are located in a site of vulnerability that
282 is targeted by all known NTD-specific neutralising antibodies^{24,45}. The role of NTD-specific
283 neutralising antibodies might be under-estimated, in part by the use of neutralization assays
284 based on target cells over-expressing ACE2 receptor. NTD-specific mAbs were suggested to
285 interfere with viral entry based on other accessory receptors, such as DC-SIGN and L-SIGN⁴⁶,
286 and their neutralization potency was found to be dependent on different in vitro culture
287 conditions²⁴. The observation that 9 out of 10 NTD-specific neutralising antibodies failed to
288 show a complete or near-complete loss of neutralising activity against B.1.1.7 indicates that
289 this new variant may have evolved also to escape from this class of antibodies, that may have
290 a yet unrecognized role in protective immunity. Wibmer et al.³⁴ have also recently reported
291 the loss of neutralization of 501Y.V2 by the NTD-specific mAb 4A8, likely driven by the
292 R246I mutation. This result is in line with the lack of neutralization of B.1.1.7 by the 4A8
293 mAb observed in this study, likely caused by Δ 144 due to loss of binding²⁴. Finally, the role
294 of NTD mutations (in particular, L18F, Δ 242-244 and R246I) was further supported by the
295 marked loss of neutralization observed by Wibmer et al.³⁴ against 501Y.V2 compared to the
296 chimeric pseudotyped viral particle carrying only the RBD mutations K417N, E484K and
297 N501Y. Taken together, the presence of multiple escape mutations in NTD is supportive of
298 the hypothesis that this region of the spike, in addition to RBM, is also under immune
299 pressure.

300 Worryingly, we have shown that there are multiple B.1.1.7 sequences in the UK
301 bearing E484K with early evidence of transmission as well as independent acquisitions. We
302 measured further reduction neutralisation titers by vaccine sera when E484K was present
303 alongside the B.1.1.7 S mutations. Wu and co-authors⁴³ have also shown that variants

304 carrying the E484K mutation resulted in 3-to-6 fold reduction in neutralization by sera from
305 mRNA-1273 vaccinated individuals. Consistently, in this study we found that approximately
306 50% of the RBM mAbs tested lost neutralising activity against SARS-CoV-2 carrying
307 E484K. E484K has been shown to impact neutralisation by monoclonal antibodies or
308 convalescent sera, especially in combination with N501Y and K417N^{16,26,47-49}. Wang et al
309 also showed reduced neutralisation by mRNA vaccine sera against E484K bearing
310 pseudovirus³⁴.

311 Evidence for the importance role of NTD deletions in combination with E484K in immune
312 escape is provided by Andreano *et al.*²⁷ who describe the emergence of Δ 140 in virus co-
313 incubated with potently neutralising convalescent plasma, causing a 4-fold reduction in
314 neutralization titre. This Δ 140 mutant subsequently acquired E484K which resulted in a
315 further 4-fold drop in neutralization titre indicating a two residue change across NTD and
316 RBD represents an effective pathway of escape that can dramatically inhibit the polyclonal
317 response.

318 Our study was limited by modest sample size. Although the spike pseudotyping system has
319 been shown to faithfully represent full length infectious virus, there may be determinants
320 outside the S that influence escape from antibody neutralization either directly or indirectly in
321 a live replication competent system. On the other hand live virus systems allow replication
322 and therefore mutations to occur, and rigorous sequencing at multiple steps is needed.

323 Vaccines are a key part of a long term strategy to bring SARS-CoV-2 transmission under
324 control. Our data suggest that vaccine escape to current Spike directed vaccines designed
325 against the Wuhan strain will be inevitable, particularly given that E484K is emerging
326 independently and recurrently on a B.1.1.7 (501Y.V1) background, and given the rapid global
327 spread of B.1.1.7. Other major variants with E484K such as 501Y.V2 and V3 are also
328 spreading regionally. This should be mitigated by designing next generation vaccines with
329 mutated S sequences and using alternative viral antigens.

330

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350

351 **Author contributions**

352 Conceived study: D.C., RKG, DAC. Designed study and experiments: RKG, DAC, LEM, JB,
353 MW, JT, LCG, GBM, RD, BG, NK, AE, M.P., D.V., L.P., A.D.M, J.B., D.C. Performed
354 experiments: BM, DAC, RD, IATMF, ACW, LCG, GBM. Interpreted data: RKG, DAC, BM,
355 RD, IATMF, ACW, LEM, JB, KGCS, DV. ADM, JB and CSF carried out pseudovirus
356 neutralization assays. DP produced pseudoviruses. MSP, LP, DV and DC designed the
357 experiments. MAT, JB, NS and SJ expressed and purified the proteins. KC, SJ and EC
358 sequenced and expressed antibodies. EC and KC performed mutagenesis for mutant
359 expression plasmids. ACW and S.B. performed binding assays. AR, AFP and CG contributed
360 to donor's recruitment and sample collection related to mAbs isolation. HWV, GS, AL, DV,
361 LP, DV and DC analyzed the data and prepared the manuscript with input from all authors.

362

363 **Competing interests**

364 A.D.M., J.B., D.P., C.S.F., S.B., K.C., N.S., E.C., G.S., S.J., A.L., H.W.V., M.S.P., L.P. and
365 D.C. are employees of Vir Biotechnology and may hold shares in Vir Biotechnology. H.W.V.

366 is a founder of PierianDx and Casma Therapeutics. Neither company provided funding for
367 this work or is performing related work. D.V. is a consultant for Vir Biotechnology Inc. The
368 Veesler laboratory has received a sponsored research agreement from Vir Biotechnology Inc.
369 The remaining authors declare that the research was conducted in the absence of any
370 commercial or financial relationships that could be construed as a potential conflict of
371 interest. RKG has received consulting fees from UMOVIS Lab, Gilead and ViiV.

372

373 **MATERIALS AND METHODS**

374 *Participant recruitment and ethics*

375 Participants who had received the first dose of vaccine and individuals with COVID-19
376 (Coronavirus Disease-19) were consented into the COVID-19 cohort of the NIHR
377 Bioresource. The study was approved by the East of England – Cambridge Central Research
378 Ethics Committee (17/EE/0025).

379

380 *SARS-CoV-2 serology by multiplex particle-based flow cytometry (Luminex):*

381 Recombinant SARS-CoV-2 N, S and RBD were covalently coupled to distinct carboxylated
382 bead sets (Luminex; Netherlands) to form a 3-plex and analyzed as previously described
383 (Xiong et al. 2020). Specific binding was reported as mean fluorescence intensities (MFI).
384 Linear regression was used to explore the association between antibody response, T cell
385 response and serum neutralisation in Stata 13. The Pearson correlation coefficient was
386 reported.

387

388 *Recombinant expression of SARS-CoV-2-specific mAbs.*

389 Human mAbs were isolated from plasma cells or memory B cells of SARS-CoV-2 immune
390 donors, as previously described⁵⁰⁻⁵². Recombinant antibodies were expressed in ExpiCHO
391 cells at 37°C and 8% CO₂. Cells were transfected using ExpiFectamine. Transfected cells
392 were supplemented 1 day after transfection with ExpiCHO Feed and ExpiFectamine CHO
393 Enhancer. Cell culture supernatant was collected eight days after transfection and filtered
394 through a 0.2 µm filter. Recombinant antibodies were affinity purified on an ÄKTA xpress
395 fast protein liquid chromatography (FPLC) device using 5 mL HiTrap™ MabSelect™
396 PrismA columns followed by buffer exchange to Histidine buffer (20 mM Histidine, 8%
397 sucrose, pH 6) using HiPrep 26/10 desalting columns

398

399 *Generation of S mutants*

400 Amino acid substitutions were introduced into the D614G pCDNA_SARS-CoV-2_S plasmid
401 as previously described⁵³ using the QuikChange Lightning Site-Directed Mutagenesis kit,
402 following the manufacturer's instructions (Agilent Technologies, Inc., Santa Clara, CA).
403 Sequences were checked by Sanger sequencing.
404 Preparation of B.1.1.7 or TM SARS-CoV-2 S glycoprotein-encoding-plasmid used to
405 produce SARS-CoV-2-MLV based on overlap extension PCR. Briefly, a modification of the
406 overlap extension PCR protocol⁵⁴ was used to introduce the nine mutations of the B.1.1.7
407 lineage or the three mutations in TM mutant in the SARS-CoV-2 S gene. In a first step,
408 9 DNA fragments with overlap sequences were amplified by PCR from a plasmid (phCMV1,
409 Genlantis) encoding the full-length SARS-CoV-2 S gene (BetaCoV/Wuhan-Hu-1/2019,
410 accession number mn908947). The mutations (del-69/70, del-144, N501Y, A570D, D614G,
411 P681H, S982A, T716I and D1118H or K417N, E484K and N501Y) were introduced by
412 amplification with primers with similar Tm. Deletion of the C-terminal 21 amino acids was
413 introduced to increase surface expression of the recombinant S⁵⁵. Next, 3 contiguous
414 overlapping fragments were fused by a first overlap PCR (step 2) using the utmost external
415 primers of each set, resulting in 3 larger fragments with overlapping sequences. A final
416 overlap PCR (step 3) was performed on the 3 large fragments using the utmost external
417 primers to amplify the full-length S gene and the flanking sequences including the restriction
418 sites KpnI and NotI. This fragment was digested and cloned into the expression plasmid
419 phCMV1. For all PCR reactions the Q5 Hot Start High fidelity DNA polymerase was used
420 (New England Biolabs Inc.), according to the manufacturer's instructions and adapting the
421 elongation time to the size of the amplicon. After each PCR step the amplified regions were
422 separated on agarose gel and purified using Illustra GFX™ PCR DNA and Gel Band
423 Purification Kit (Merck KGaA).

424

425 *Pseudotype virus preparation*

426 Viral vectors were prepared by transfection of 293T cells by using Fugene HD transfection
427 reagent (Promega). 293T cells were transfected with a mixture of 11ul of Fugene HD, 1µg of
428 pCDNAΔ19spike-HA, 1ug of p8.91 HIV-1 gag-pol expression vector^{56,57}, and 1.5µg of
429 pCSFLW (expressing the firefly luciferase reporter gene with the HIV-1 packaging signal).
430 Viral supernatant was collected at 48 and 72h after transfection, filtered through 0.45um filter

431 and stored at -80°C . The 50% tissue culture infectious dose (TCID₅₀) of SARS-CoV-2
432 pseudovirus was determined using Steady-Glo Luciferase assay system (Promega).

433

434 *Serum/plasma pseudotype neutralization assay*

435 Spike pseudotype assays have been shown to have similar characteristics as neutralisation
436 testing using fully infectious wild type SARS-CoV-2²⁰. Virus neutralisation assays were
437 performed on 293T cell transiently transfected with ACE2 and TMPRSS2 using SARS-CoV-
438 2 spike pseudotyped virus expressing luciferase⁵⁸. Pseudotyped virus was incubated with
439 serial dilution of heat inactivated human serum samples or sera from vaccinees in duplicate
440 for 1h at 37°C . Virus and cell only controls were also included. Then, freshly trypsinized
441 293T ACE2/TMPRSS2 expressing cells were added to each well. Following 48h incubation
442 in a 5% CO₂ environment at 37°C , luminescence was measured using the Steady-Glo or
443 Bright-Glo Luciferase assay system (Promega). Neutralization was calculated relative to virus
444 only controls. Dilution curves were presented as a mean neutralization with standard error of
445 the mean (SEM). ID₅₀ values were calculated in GraphPad Prism. The ID₅₀ within groups
446 were summarised as a geometric mean titre and statistical comparison between groups were
447 made with Wilcoxon ranked sign test. In addition, the impact of the mutations on the
448 neutralising effect of the sera were expressed as fold change (FC) of ID₅₀ of the wild-type
449 compared to mutant pseudotyped virus. Statistical difference in the mean FC between groups
450 was determined using a 2-tailed t-test.

451 *IFN γ FluoroSpot assays*

452 Frozen PBMCs were rapidly thawed, and the freezing medium was diluted into 10ml of
453 TexMACS media (Miltenyi Biotech), centrifuged and resuspended in 10ml of fresh media
454 with 10U/ml DNase (Benzonase, Merck-Millipore via Sigma-Aldrich), PBMCs were
455 incubated at 37°C for 1h, followed by centrifugation and resuspension in fresh media
456 supplemented with 5% Human AB serum (Sigma Aldrich) before being counted. PBMCs
457 were stained with 2ul of each antibody: anti-CD3-fluorescein isothiocyanate (FITC), clone
458 UCHT1; anti-CD4-phycoerythrin (PE), clone RPA-T4; anti-CD8a-peridinin-chlorophyll
459 protein - cyanine 5.5 (PerCP Cy5.5), clone RPA-8a (all BioLegend, London, UK),
460 LIVE/DEAD Fixable Far Red Dead Cell Stain Kit (Thermo Fisher Scientific). PBMC
461 phenotyping was performed on the BD Accuri C6 flow cytometer. Data were analysed with
462 FlowJo v10 (Becton Dickinson, Wokingham, UK). 1.5 to 2.5×10^5 PBMCs were incubated

463 in pre-coated Fluorospot plates (Human IFN γ FLUOROSPOT (Mabtech AB, Nacka Strand,
464 Sweden)) in triplicate with peptide mixes specific for Spike, Nucleocapsid and Membrane
465 proteins of SARS-CoV-2 (final peptide concentration 1 μ g/ml/peptide, Miltenyi Biotech) and
466 an unstimulated and positive control mix (containing anti-CD3 (Mabtech AB),
467 Staphylococcus Enterotoxin B (SEB), Phytohaemagglutinin (PHA) (all Sigma Aldrich)) at
468 37°C in a humidified CO₂ atmosphere for 48 hours. The cells and medium were decanted
469 from the plate and the assay developed following the manufacturer's instructions. Developed
470 plates were read using an AID iSpot reader (Oxford Biosystems, Oxford, UK) and counted
471 using AID EliSpot v7 software (Autoimmun Diagnostika GmbH, Strasberg, Germany). All
472 data were then corrected for background cytokine production and expressed as spot forming
473 units (SFU)/Million PBMC or CD3 T cells. The association between spike Tcell response,
474 spike specific antibody response and serum neutralisation was determined using linear
475 regression and the Pearson correlation coefficient between these variables were determined
476 using Stata 13.

477

478 *Ab discovery and recombinant expression*

479 Human mAbs were isolated from plasma cells or memory B cells of SARS-CoV or SARS-
480 CoV-2 immune donors, as previously described^{48,56-58}. Recombinant antibodies were
481 expressed in ExpiCHO cells at 37°C and 8% CO₂. Cells were transfected using
482 ExpiFectamine. Transfected cells were supplemented 1 day after transfection with ExpiCHO
483 Feed and ExpiFectamine CHO Enhancer. Cell culture supernatant was collected eight days
484 after transfection and filtered through a 0.2 μ m filter. Recombinant antibodies were affinity
485 purified on an ÄKTA xpress FPLC device using 5 mL HiTrap™ MabSelect™ Prisma
486 columns followed by buffer exchange to Histidine buffer (20 mM Histidine, 8% sucrose, pH
487 6) using HiPrep 26/10 desalting columns.

488

489 *MABs pseudovirus neutralization assay*

490 MLV-based SARS-CoV-2 S-glycoprotein-pseudotyped viruses were prepared as previously
491 described (Pinto et al., 2020). HEK293T/17cells were cotransfected with a WT, B.1.1.7 or
492 TM SARS-CoV-2 spike glycoprotein-encoding-plasmid, an MLV Gag-Pol packaging
493 construct and the MLV transfer vector encoding a luciferase reporter using X-tremeGENE
494 HP transfection reagent (Roche) according to the manufacturer's instructions. Cells were
495 cultured for 72 h at 37°C with 5% CO₂ before harvesting the supernatant. VeroE6 stably
496 expressing human TMPRSS2 were cultured in Dulbecco's Modified Eagle's Medium

497 (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin–streptomycin (100 I.U.
498 penicillin/mL, 100 µg/mL), 8 µg/mL puromycin and plated into 96-well plates for 16–24 h.
499 Pseudovirus with serial dilution of mAbs was incubated for 1 h at 37°C and then added to the
500 wells after washing 2 times with DMEM. After 2–3 h DMEM containing 20% FBS and 2%
501 penicillin–streptomycin was added to the cells. Following 48–72 h of infection, Bio-Glo
502 (Promega) was added to the cells and incubated in the dark for 15 min before reading
503 luminescence with Synergy H1 microplate reader (BioTek). Measurements were done in
504 duplicate and relative luciferase units were converted to percent neutralization and plotted
505 with a non-linear regression model to determine IC₅₀ values using GraphPad PRISM
506 software (version 9.0.0).

507

508 *Antibody binding measurements using bio-layer interferometry (BLI)*

509 MAbs were diluted to 3 µg/ml in kinetic buffer (PBS supplemented with 0.01% BSA) and
510 immobilized on Protein A Biosensors (FortéBio). Antibody-coated biosensors were
511 incubated for 3 min with a solution containing 5 µg/ml of WT, N501Y or E484K SARS-
512 CoV-2 RBD in kinetic buffer, followed by a 3-min dissociation step. Change in molecules
513 bound to the biosensors caused a shift in the interference pattern that was recorded in real
514 time using an Octet RED96 system (FortéBio). The binding response over time was used to
515 calculate the area under the curve (AUC) using GraphPad PRISM software (version 9.0.0).

516

517 *Production of SARS-CoV-2 and B.1.1.7 receptor binding domains and human ACE2*

518 The SARS-CoV-2 RBD (BEI NR-52422) construct was synthesized by GenScript into
519 CMVR with an N-terminal mu-phosphatase signal peptide and a C-terminal octa-histidine tag
520 (GHHHHHHHH) and an avi tag. The boundaries of the construct are N₃₂₈RFPN₃₃₁ and
521 ₅₂₈KKST₅₃₁-C⁵⁹. The B.1.1.7 RBD gene was synthesized by GenScript into pCMVR with the
522 same boundaries and construct details with a mutation at N501Y. These plasmids were
523 transiently transfected into Expi293F cells using Expi293F expression medium (Life
524 Technologies) at 37°C 8% CO₂ rotating at 150 rpm. The cultures were transfected using PEI
525 cultivated for 5 days. Supernatants were clarified by centrifugation (10 min at 4000xg) prior
526 to loading onto a nickel-NTA column (GE). Purified protein was biotinylated overnight using
527 BirA (Biotin ligase) prior to size exclusion chromatography (SEC) into phosphate buffered
528 saline (PBS). Human ACE2-Fc (residues 1-615 with a C-terminal thrombin cleavage site and
529 human Fc tag) were synthesized by Twist. Clarified supernatants were affinity purified using
530 a Protein A column (GE LifeSciences) directly neutralized and buffer exchanged. The Fc tag

531 was removed by thrombin cleavage in a reaction mixture containing 3 mg of recombinant
532 ACE2-FC ectodomain and 10 µg of thrombin in 20 mM Tris-HCl pH8.0, 150 mM NaCl and
533 2.5 mM CaCl₂. The reaction mixture was incubated at 25°C overnight and re-loaded on a
534 Protein A column to remove uncleaved protein and the Fc tag. The cleaved protein was
535 further purified by gel filtration using a Superdex 200 column 10/300 GL (GE Life Sciences)
536 equilibrated in PBS.

537

538 *Protein affinity measurement using bio-layer interferometry*

539 Biotinylated RBD (WT, N501Y, or TM) were immobilized at 5 ng/µL in undiluted 10X
540 Kinetics Buffer (Pall) to SA sensors until a load level of 1.1nm. A dilution series of either
541 monomeric ACE2 or Fab in undiluted kinetics buffer starting at 1000-50nM was used for
542 300-600 seconds to determine protein-protein affinity. The data were baseline subtracted and
543 the plots fitted using the Pall FortéBio/Sartorius analysis software (version 12.0). Data were
544 plotted in Prism.

545

546 Statistical analysis

547 Linear regression was used to explore the association between antibody response, T cell
548 response and serum neutralisation in Stata 13. The Pearson correlation coefficient was
549 reported.

550

551 Neutralisation data analysis

552 Neutralization was calculated relative to virus only controls. Dilution curves were presented
553 as a mean neutralization with standard error of the mean (SEM). IC₅₀ values were calculated
554 in GraphPad Prism. The inhibitory dilution (ID₅₀) within groups were summarised as a
555 geometric mean titre and statistical comparison between groups were made with Wilcoxon
556 ranked sign test. In addition, the impact of the mutations on the neutralising effect of the sera
557 were expressed as fold change of ID₅₀ of the wild-type compared to mutant pseudotyped
558 virus. Statistical difference in the mean FC between groups was determined using a 2-tailed t-
559 test

560

561

562 IFN γ FluoroSpot assay data analysis

563 The association between spike Tcell response, spike specific antibody response and serum
564 neutralisation was determined using linear regression and the Pearson correlation coefficient
565 between these variables were determined using Stata 13.

566

567 *Data availability.*

568 The neutralization and BLI data shown in Fig. 4 and Extended Data Fig. 5-7 can be found in

569 **Source Data Fig. 4.** Other data are available from the corresponding author on request.

570

571

572

573

574 **Table 1. Kinetic analysis of human ACE2 binding to SARS-CoV-2 Wuhan-1, N501Y**
575 **and N501Y/ E484K/ K417N (TM) RBDs by biolayer interferometry.** Values reported
576 represent the global fit to the data shown in Extended Data Fig. 8.

577

		SARS-CoV-2 RBD WT	SARS-CoV-2 RBD N501Y	SARS-CoV-2 RBD TM
K_D (nM)		133	22	64
k_{on} (M⁻¹.s⁻¹)	hACE2	1.3*10 ⁵	1.4*10 ⁵	1.3*10 ⁵
k_{off} (s⁻¹)		1.8*10 ⁻²	3*10 ⁻³	8.5*10 ⁻³

578

579

580

Extended Data Table 1. Neutralization, V gene usage and other properties of tested mAbs.

mAb	Domain (site)	VH usage (% id.)	Source (DSO)	IC50 WT (ng/ml)	IC50 B.1.1.7 (ng/ml)	ACE2 blocking	SARS-CoV	Escape residues	Ref.
4A8	NTD (i)	1-24	N/A	38	-	Neg.	-	S12P; C136Y; Y144del; H146Y; K147T; R246A	⁶⁰
S2L26	NTD (i)	1-24 (97.2)	Hosp. (52)	70	-	Neg.	-	N/A	²⁴
S2L50	NTD (i)	4-59 (95.4)	Hosp. (52)	264	50	Neg.	-	N/A	²⁴
S2M28	NTD (i)	3-33 (97.6)	Hosp. (46)	295	12'207	Neg.	-	P9S/Q; S12P; C15F/R; L18P; Y28C; A123T; C136Y; G142D; Y144del; K147Q/T; R246G; P251L; G252C	²⁴
S2X107	NTD (i)	4-38-2 (97)	Sympt. (75)	388	-	Neg.	-	N/A	²⁴
S2X124	NTD (i)	3-30 (99)	Sympt. (75)	221	-	Neg.	-	N/A	²⁴
S2X158	NTD (i)	1-24 (96.3)	Sympt. (75)	56	-	Neg.	-	N/A	²⁴
S2X28	NTD (i)	3-30 (97.9)	Sympt. (48)	1'399	-	Neg.	-	P9S; S12P; C15W; L18P; C136G/Y; F140S; L141S; G142C/D; Y144C/N; K147T/Q/E; R158G; L244S; R246G	²⁴
S2X303	NTD (i)	2-5 (95.9)	Sympt. (125)	69	-	Neg.	-	N/A	²⁴
S2X333	NTD (i)	3-33 (96.5)	Sympt. (125)	66	-	Neg.	-	P9L; S12P; C15S/Y; L18P; C136G/Y; F140C; G142D; K147T	²⁴
S2D106	RBD (I/RBM)	1-69 (97.2)	Hosp. (98)	27	20	Strong	-	N/A	⁸
S2D19	RBD (I/RBM)	4-31 (99.7)	Hosp. (49)	128	75'200	Moderate	-	N/A	⁸
S2D32	RBD (I/RBM)	3-49 (98.3)	Hosp. (49)	26	11	Strong	-	N/A	⁸
S2D65	RBD (I/RBM)	3-9 (96.9)	Hosp. (49)	24	12	Weak	-	N/A	⁸
S2D8	RBD (I/RBM)	3-23 (96.5)	Hosp. (49)	27	58'644	Strong	-	N/A	⁸
S2D97	RBD (I/RBM)	2-5 (96.9)	Hosp. (98)	20	17	Weak	-	N/A	⁸
S2E11	RBD (I/RBM)	4-61 (98.3)	Hosp. (51)	27	16	Weak	-	N/A	⁸
S2E12	RBD (I/RBM)	1-58 (97.6)	Hosp. (51)	27	31	Strong	-	G476S (3x)	^{8,61}
S2E13	RBD (I/RBM)	1-18 (96.2)	Hosp. (51)	34	77	Strong	-	N/A	⁸
S2E16	RBD (I/RBM)	3-30 (98.3)	Hosp. (51)	36	38	Strong	-	N/A	⁸
S2E23	RBD (I/RBM)	3-64 (96.9)	Hosp. (51)	139	180	Strong	-	N/A	⁸
S2H14	RBD (I/RBM)	3-15 (100)	Sympt. (17)	460	64'463	Weak	-	N/A	^{8,62}
S2H19	RBD (I/RBM)	3-15 (98.6)	Sympt. (45)	239	-	Weak	-	N/A	⁸
S2H58	RBD (I/RBM)	1-2 (97.9)	Sympt. (45)	27	14	Strong	-	N/A	⁸
S2H7	RBD (I/RBM)	3-66 (98.3)	Sympt. (17)	492	573	Weak	-	N/A	⁸
S2H70	RBD (I/RBM)	1-2 (99)	Sympt. (45)	147	65	Weak	-	N/A	⁸
S2H71	RBD (I/RBM)	2-5 (99)	Sympt. (45)	36	9	Moderate	-	N/A	⁸
S2M11	RBD (I/RBM)	1-2 (96.5)	Hosp. (46)	11	4	Weak	-	Y449N; L455F; E484K; E484Q; F490L; F490S; S494P	^{8,61}
S2N12	RBD	4-39 (97.6)	Hosp. (51)	76	40	Strong	-	N/A	⁸

	(I/RBM)								
S2N22	RBD (I/RBM)	3-23 (96.5)	Hosp. (51)	32	21	Strong	-	N/A	⁸
S2N28	RBD (I/RBM)	3-30 (97.2)	Hosp. (51)	72	21	Strong	-	N/A	⁸
S2X128	RBD (I/RBM)	1-69-2 (97.6)	Sympt. (75)	50	112	Strong	-	N/A	⁸
S2X16	RBD (I/RBM)	1-69 (97.6)	Sympt. (48)	45	103	Strong	-	N/A	⁸
S2X192	RBD (I/RBM)	1-69 (96.9)	Sympt. (75)	326	-	Weak	-	N/A	⁸
S2X227	RBD (I/RBM)	1-46 (97.9)	Sympt. (75)	26	14	Strong	-	N/A	
S2X246	RBD (I/RBM)	3-48 (96.2)	Sympt. (75)	35	30	Strong	-	N/A	
S2X30	RBD (I/RBM)	1-69 (97.9)	Sympt. (48)	32	53	Strong	-	N/A	⁸
S2X324	RBD (I/RBM)	2-5 (97.3)	Sympt. (125)	8	23	Strong	-	N/A	
S2X58	RBD (I/RBM)	1-46 (99)	Sympt. (48)	32	47	Strong	-	N/A	⁸
S2H90	RBD (II)	4-61 (96.6)	Sympt. (81)	77	32	Strong	+	N/A	⁸
S2H94	RBD (II)	3-23 (93.4)	Sympt. (81)	123	144	Strong	+	N/A	⁸
S2H97	RBD (V)	5-51 (98.3)	Sympt. (81)	513	248	Weak	+	N/A	
S2K15	RBD (II)	2-26 (99.3)	Sympt. (87)	361	235	0	+	N/A	
S2K21	RBD (II)	3-33 (96.2)	Sympt. (118)	201	189	0	+	N/A	
S2K30	RBD (II)	1-2 (97.2)	Sympt. (87)	185	134	0	+	N/A	
S2K63v2	RBD (II)	3-30-3 (95.6)	Sympt. (118)	144	215	0	+	N/A	
S2L17	RBD (?)	5-10-1 (98.3)	Hosp. (51)	313	127	Moderate	+	N/A	⁸
S2L49	RBD (?)	3-30 (97.9)	Hosp. (51)	24	32	Neg.	+	N/A	⁸
S2X259	RBD (IIa)	1-69 (94.1)	Sympt. (75)	145	91	Moderate	+	N/A	
S2X305	RBD (?)	1-2 (95.1)	Sympt. (125)	34	21	Strong	-	N/A	
S2X35	RBD (IIa)	1-18 (98.6)	Sympt. (48)	140	143	Strong	+	N/A	⁶²
S2X450	RBD (?)	2-26 (96.9)	Sympt. (271)	368	198	Strong	+	N/A	
S2X475	RBD (?)	3-21 (93.8)	Sympt. (271)	1'431	851	Strong	+	N/A	
S2X607	RBD (?)	3-66 (95.4)	Sympt. (271)	41	23	Strong	-	N/A	
S2X608	RBD (?)	1-33 (93.2)	Sympt. (271)	21	35	Strong	-	N/A	
S2X609	RBD (?)	1-69 (93.8)	Sympt. (271)	47	35	Strong	-	N/A	
S2X613	RBD (I)	1-2 (91.7)	Sympt. (271)	28	19	Strong	-	N/A	
S2X615	RBD (I)	3-11 (94.8)	Sympt. (271)	23	17	Strong	-	N/A	
S2X619	RBD (?)	1-69 (92.7)	Sympt. (271)	36	60	Strong	-	N/A	
S2X620	RBD (?)	3-53 (95.1)	Sympt. (271)	34	45	Strong	-	N/A	

id., identity. DSO, days after symptom onset. * as described in Piccoli et al and McCallum et al. N/A, not available; -, not neutralising

581

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583

584 References

585

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Figure 1

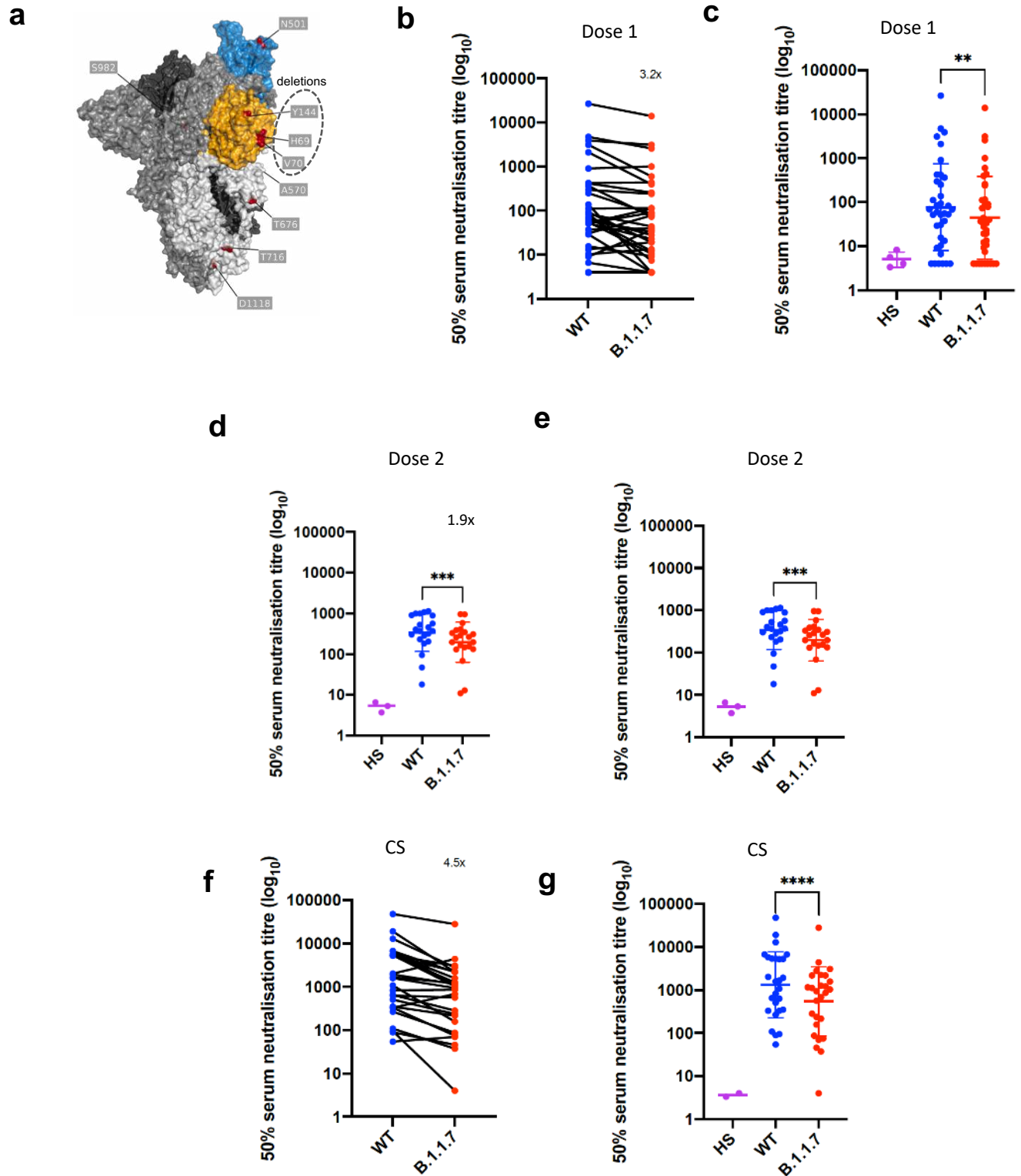


Figure 1. Neutralization by first and second dose mRNA vaccine sera against wild type and B.1.1.7 Spike mutant SARS-CoV-2 pseudotyped viruses. **a**, Spike in open conformation with a single erect RBD (PDB: 6ZGG) in trimer axis vertical view with the locations of mutated residues highlighted in red spheres and labelled on the monomer with erect RBD. Vaccine first dose (**b-c**, $n=37$), second dose (**d-e**, $n=21$) and convalescent sera, Conv. (**f-g**, $n=27$) against WT and B.1.1.7 Spike mutant with N501Y, A570D, Δ H69/V70, Δ 144/145, P681H, T716I, S982A and D1118H. GMT with s.d presented of two independent experiments each with two technical repeats. Wilcoxon matched-pairs signed rank test p-values * <0.05 , ** <0.01 , *** <0.001 , **** <0.0001 , ns not significant HS – human AB serum control. Limit of detection for 50% neutralization set at 10.

Figure 2

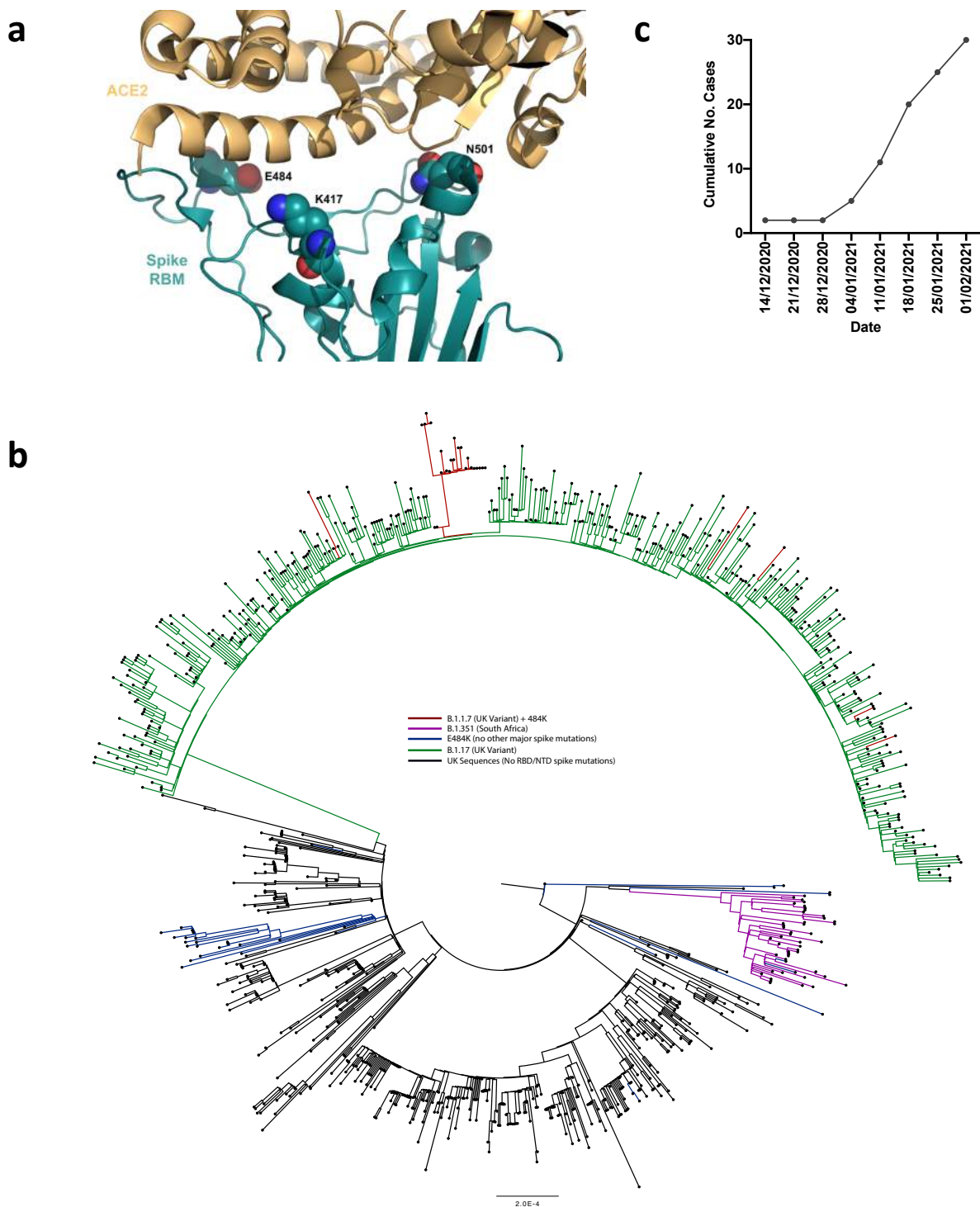


Figure 2. E484K appearing in background of B.1.1.7 with evidence of transmission a. Representation of Spike RBM:ACE2 interface (PDB: 6M0J) with residues E484, N501 and K417 highlighted as spheres coloured by element **b**. Maximum likelihood phylogeny of a subset of sequences from the United Kingdom bearing the E484K mutation (green) and lineage B.1.1.7 (blue), with background sequences without RBD mutations in black. As of 11th Feb 2021, 30 sequences from the B.1.1.7 lineage (one cluster of 25 at top of phylogenetic tree) have acquired the E484K mutation (red). **c.** Sequence accumulation over time in GISAID for UK sequences with B.1.1.7 and E484K. RBD – receptor binding domain; NTD – N terminal domain.

Figure 3

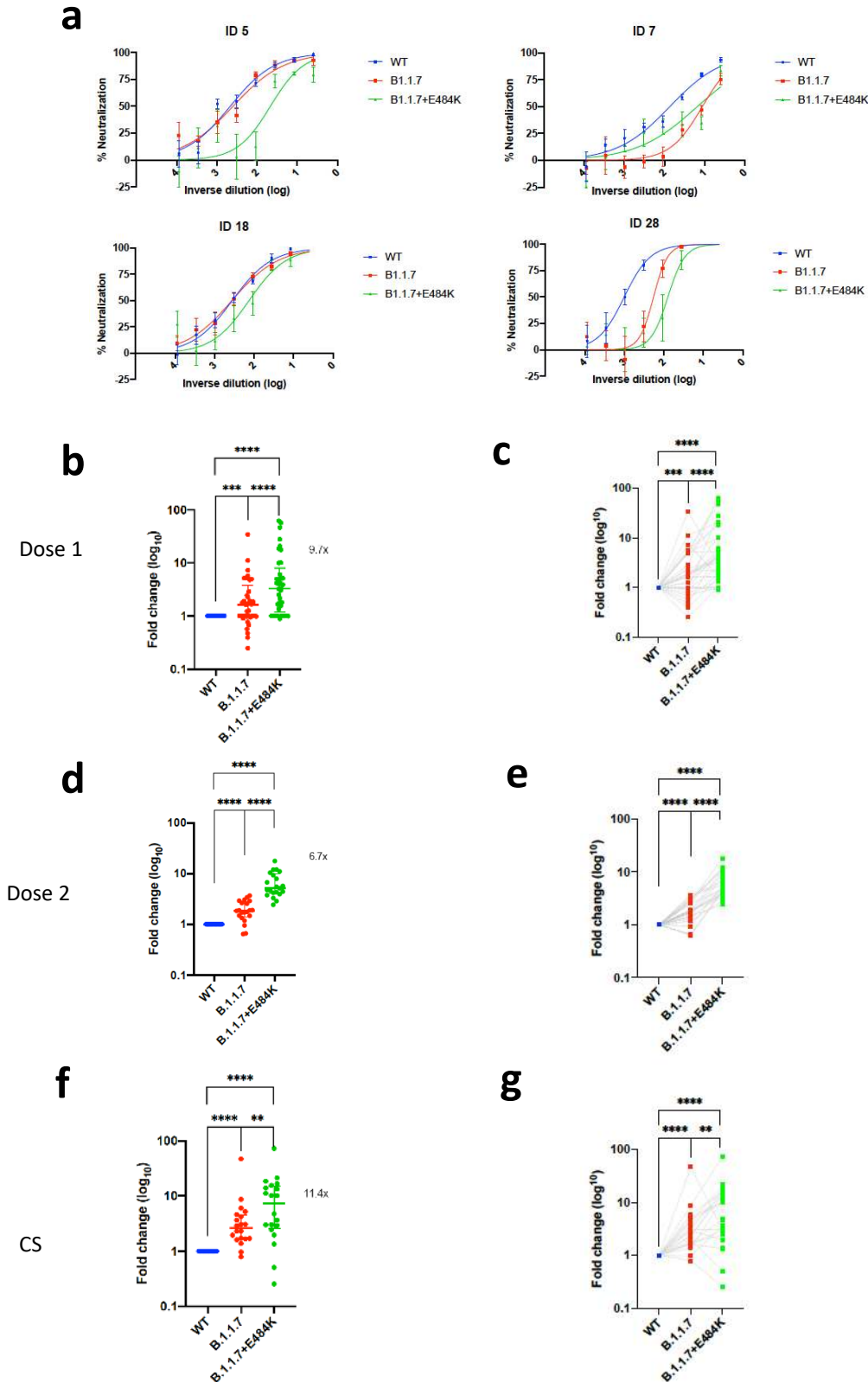


Figure 3. Neutralization potency of mRNA vaccine sera and convalescent sera (pre SARS-CoV-2 B.1.1.7) against pseudotyped virus bearing Spike mutations in the B.1.1.7 lineage with and without E484K in the receptor binding domain (all In Spike D614G background). a, Example neutralization curves for vaccinated individuals. Data points represent mean of technical replicates with standard error and are representative of two independent experiments (**b-g**). 50% neutralisation titre for each virus against sera derived (**b,c**, n=37) following first vaccination (**d,e**, n=21) following second vaccination and (**f,g**, n=20) convalescent sera (CS) expressed as fold change relative to WT. Data points are mean fold change of technical replicates and are representative of two independent experiments. Central bar represents mean with outer bars representing s.d. Wilcoxon matched-pairs signed rank test p-values * <0.05 , ** <0.01 , *** <0.001 , **** <0.0001 ; ns not significant. Limit of detection for 50% neutralization set at 10.

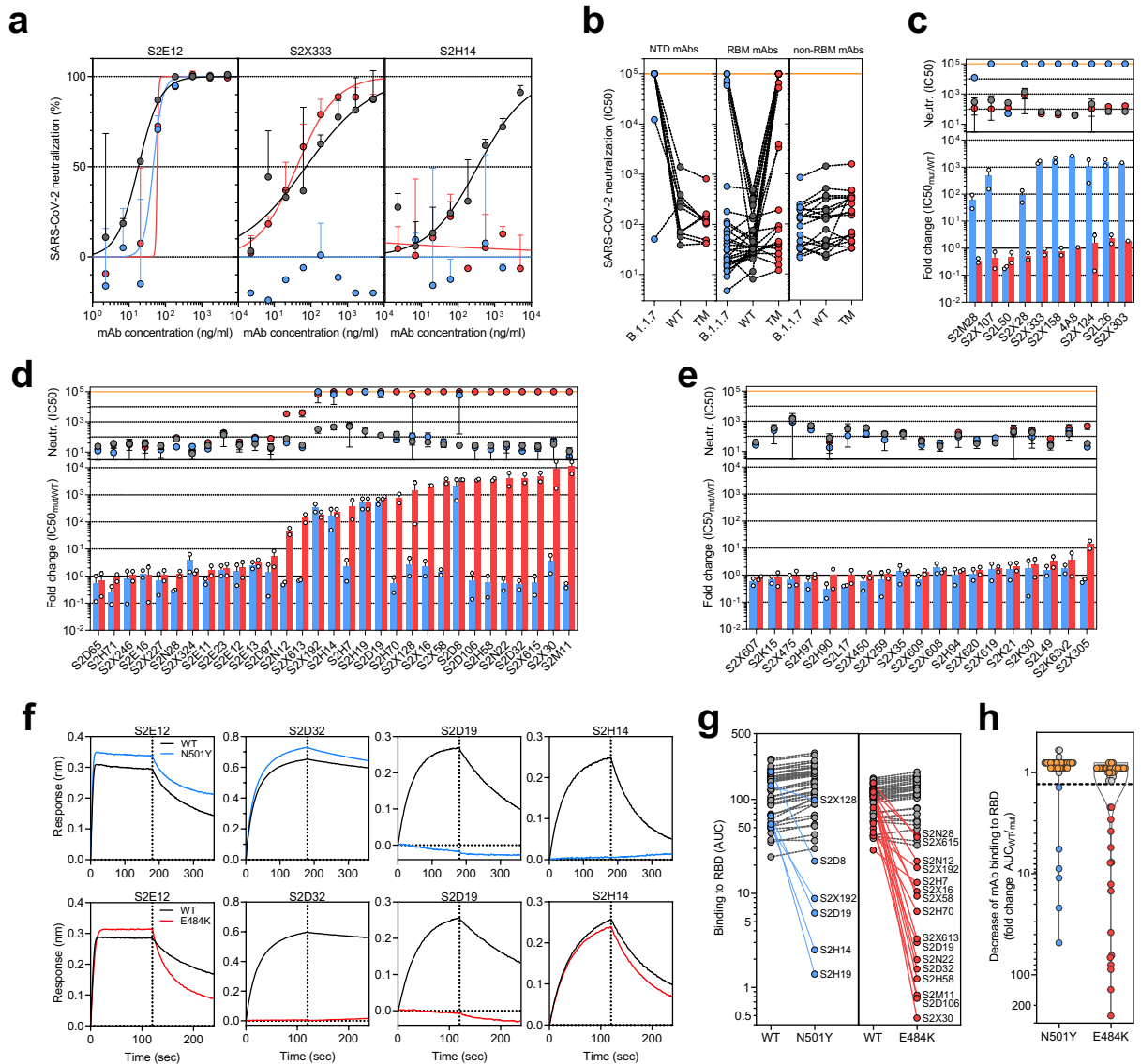
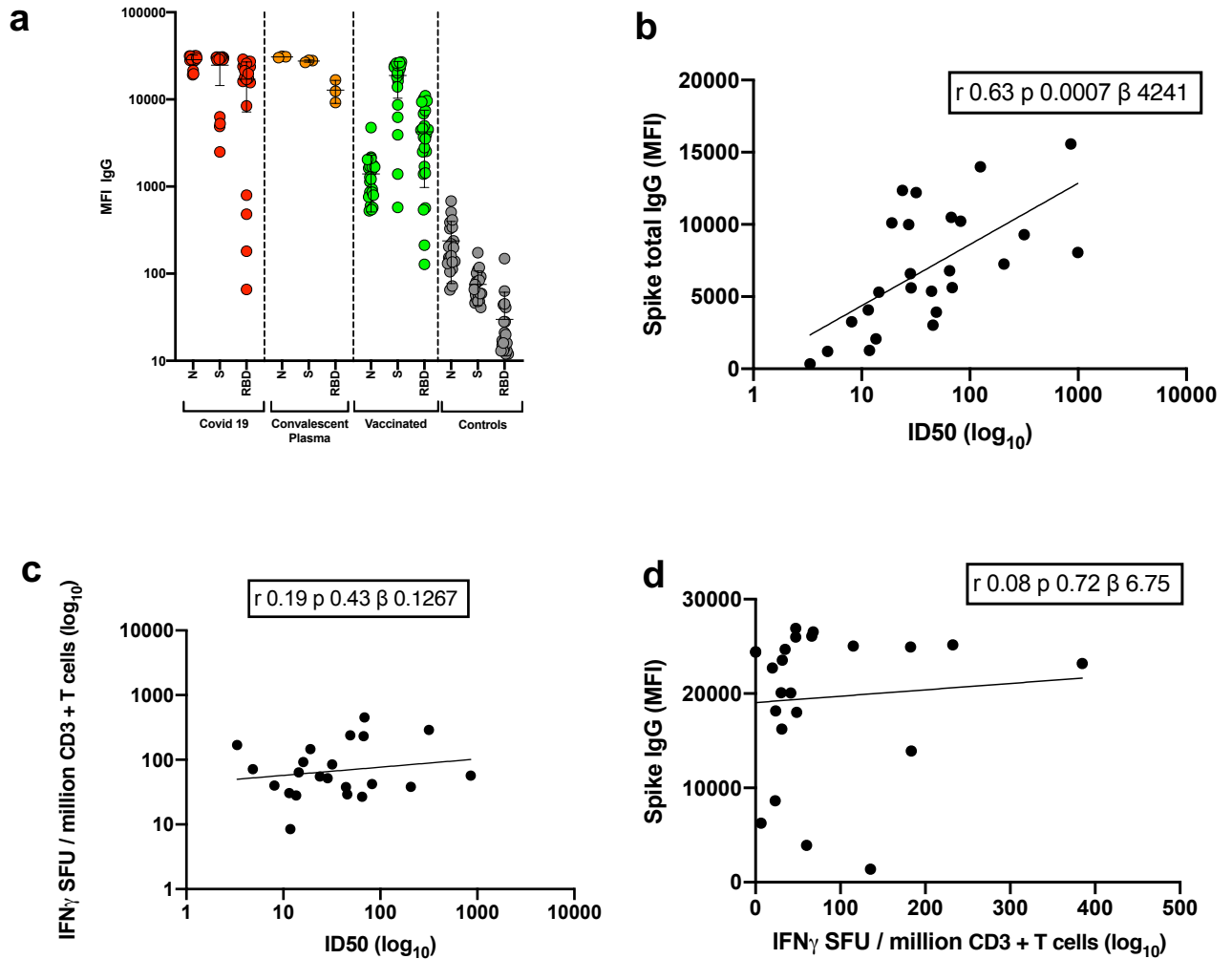
Figure 4

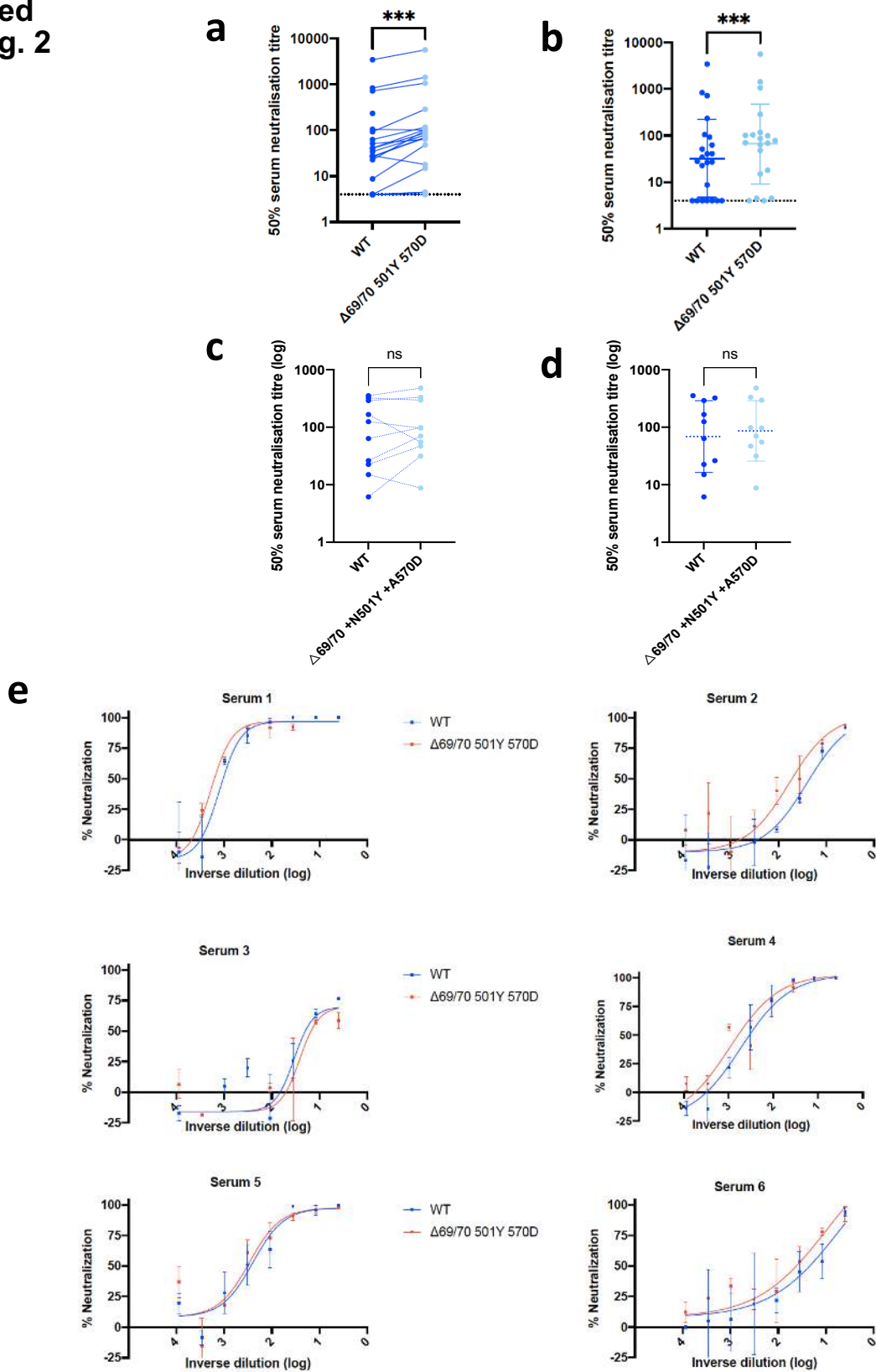
Figure 4. Neutralization and binding by a panel of NTD- and RBD-specific mAbs against WT, B.1.1.7 and RBD mutant SARS-CoV-2 viruses. **a**, Neutralization of WT D614G (black), B.1.1.7 (blue) and a triple mutant (TM, carrying RBD mutations K417N/E484K/N501Y) (red) pseudotyped SARS-CoV-2-MLVs by 3 selected mAbs (S2E12, S2X333 and S2H14) from one representative experiment. Shown is the mean \pm s.d. of 2 technical replicates. **b**, Neutralization of WT (D614G), B.1.1.7 and TM SARS-CoV-2-MLVs by 60 mAbs targeting NTD (n=10), RBM (n=31) and non-RBM sites in the RBD (n=19). Shown are the mean IC₅₀ values (ng/ml) of n=2 independent experiments. **c-e**, Neutralization shown as mean IC₅₀ values (upper panel) and mean fold change of B.1.1.7 (blue) or TM (red) relative to WT (lower panel) of NTD (c), RBM (d) and non-RBM (e) mAbs. Lower panel shows IC₅₀ values from 2 independent experiments. **f-h**, Kinetics of binding of mAbs to WT (black), N501Y (blue) and E484K (red) RBD as measured by bio-layer interferometry (BLI). Shown in (f) are the 4 RBM-targeting mAbs with no reduced binding to N501Y or E484K RBD. Area under the curve (AUC) (g) and AUC fold change (h) of 50 mAbs tested against WT, N501Y and E484K RBD. mAbs with a >1.3 AUC fold change shown in blue and red. mAbs: monoclonal antibodies. NTD: N- terminal domain

Extended Data Figure 1



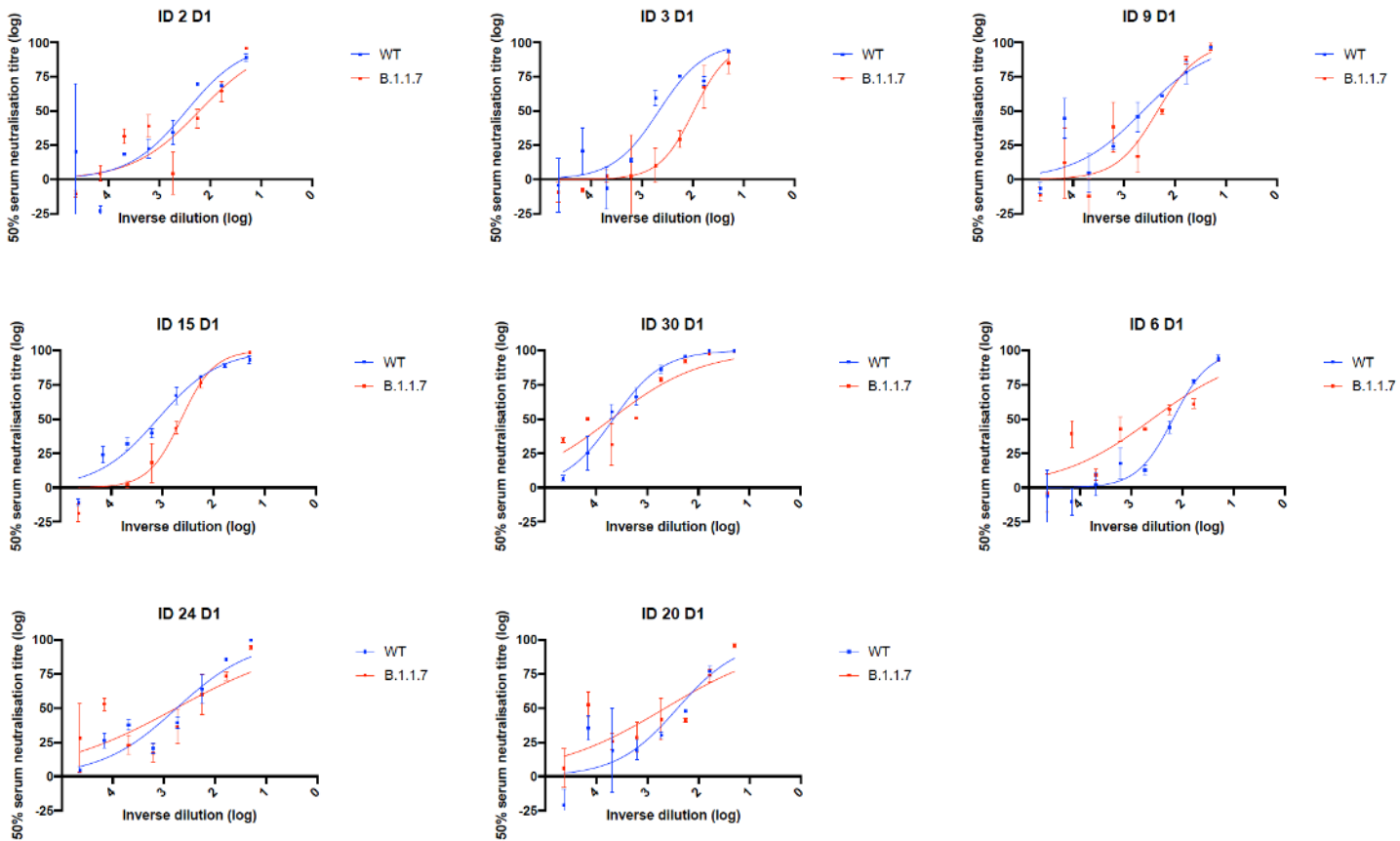
Extended Data Figure 1: Immune responses three weeks after first dose of Pfizer SARS-CoV-2 vaccine BNT162b2 **a**, Serum IgG responses against N protein, Spike and the Spike Receptor Binding Domain (RBD) from first vaccine participants (green), recovered COVID-19 cases (red), 3 convalescent plasma units and healthy controls (grey) as measured by a flow cytometry based Luminex assay. MFI, mean fluorescence intensity. Geometric mean titre (GMT with standard deviation (s.d) of two technical repeats presented. **b**, Relationship between serum IgG responses as measured by flow cytometry and serum neutralisation ID50. **c**, Relationship between serum neutralisation ID50 and T cell responses against SARS-CoV-2 by IFN gamma ELISpot. SFU: spot forming units. **d**, Relationship between serum IgG responses and T cell responses. Simple linear regression is presented with Pearson correlation (r), P-value (p) and regression coefficient/slope (β).

Extended Data Fig. 2



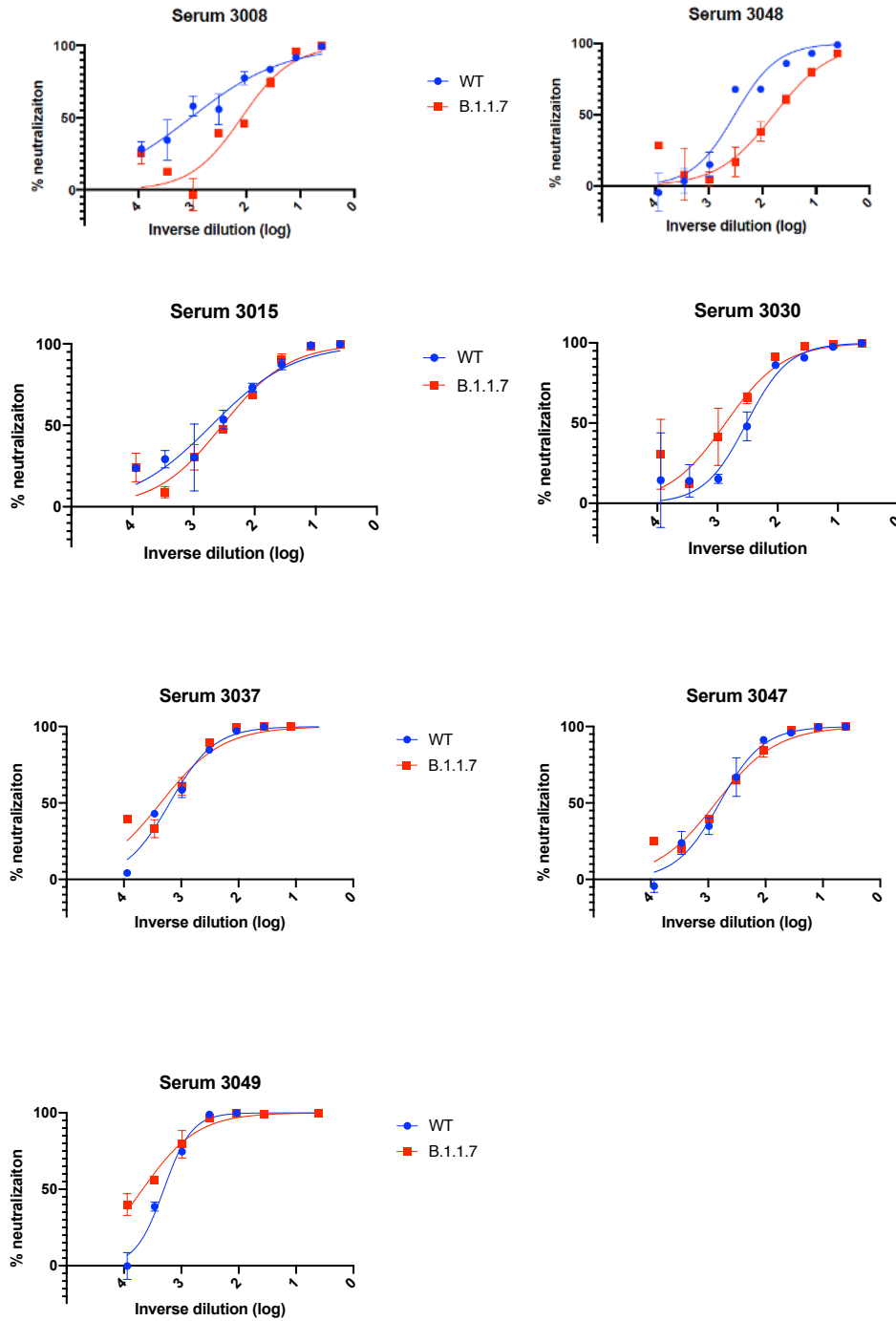
Extended data Fig 2. Neutralization by first dose BNT162b2 vaccine and convalescent sera against wild type and mutant (N501Y, A570D, Δ H69/V70) SARS-CoV-2 pseudotyped viruses: (a-b) Vaccine sera dilution for 50% neutralization against WT and Spike mutant with N501Y, A570D, Δ H69/V70. Geometric mean titre (GMT) + s.d. of two independent experiments with two technical repeats presented. (c-d) Convalescent sera dilution for 50% neutralization against WT and Spike mutant with N501Y, A570D, Δ H69/V70. GMT + s.d. of representative experiment with two technical repeats presented. e, Representative curves of convalescent serum log₁₀ inverse dilution against % neutralization for WT v N501Y, A570D, Δ H69/V70. Where a curve is shifted to the right this indicates the virus is less sensitive to the neutralizing antibodies in the serum. Data are means of technical replicates and error bars represent standard error of the mean. Data are representative of 2 independent experiments. Limit of detection for 50% neutralization set at 10.

Extended Data Fig. 3



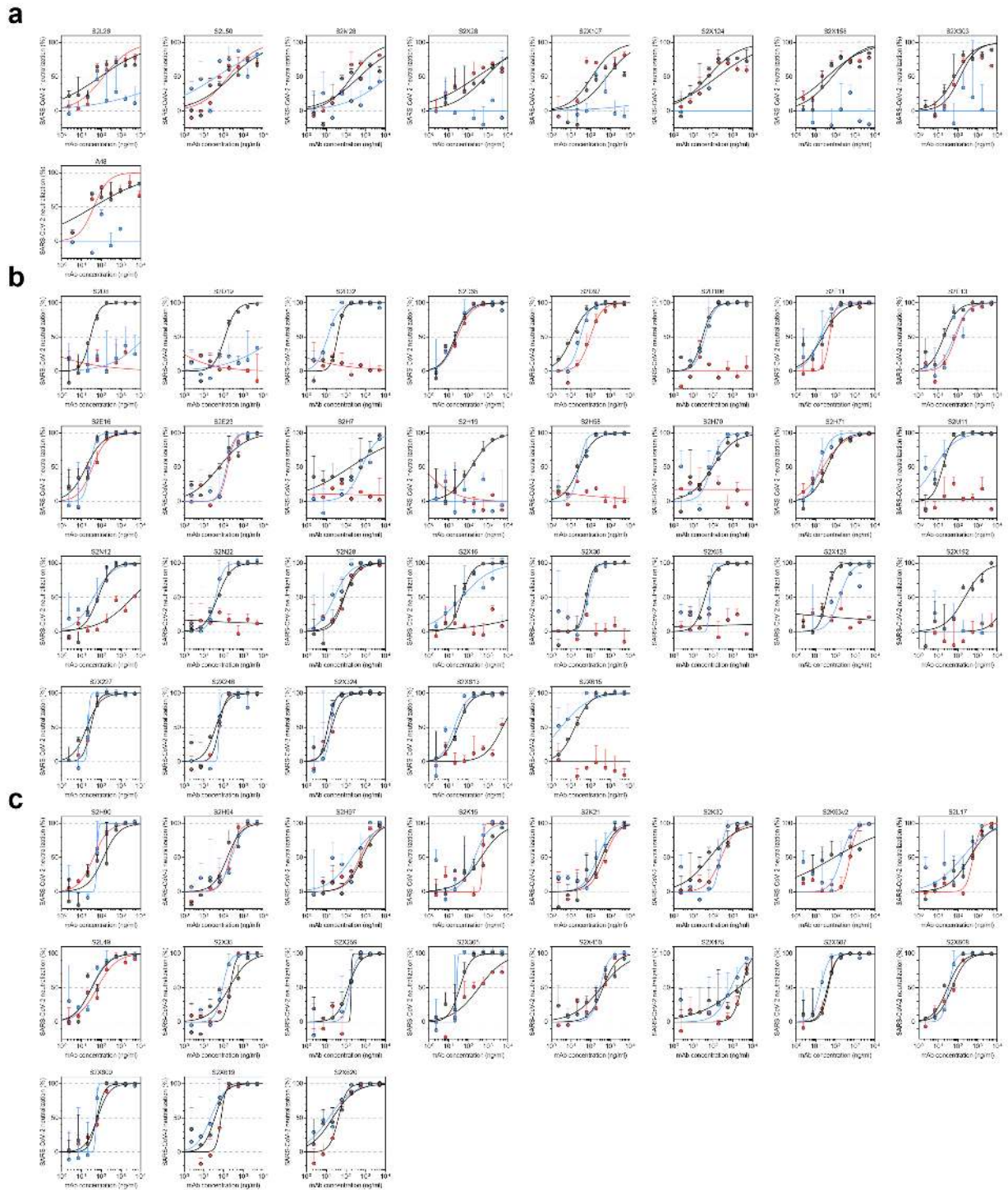
Extended Data Fig. 3. Representative neutralization curves of BNT162b2 vaccine sera against pseudovirus bearing eight Spike mutations present in B.1.1.7 versus wild type (all In Spike D614G background). Indicated is serum \log_{10} inverse dilution against % neutralization. Where a curve is shifted to the right this indicates the virus is less sensitive to the neutralizing antibodies in the serum. Data are for first dose of vaccine (D1). Data points represent means of technical replicates and error bars represent standard error of the mean. Limit of detection for 50% neutralization set at 10.

Extended Data Fig. 4



Extended Data Fig. 4. Representative neutralization curves of convalescent sera against wild type and B.1.1.7 Spike mutant SARS-CoV-2 pseudoviruses. Indicated is serum \log_{10} inverse dilution against % neutralization. Where a curve is shifted to the right this indicates the virus is less sensitive to the neutralizing antibodies in the serum. Data points represent means of technical replicates and error bars represent standard error of the mean. Limit of detection for 50% neutralization set at 10.

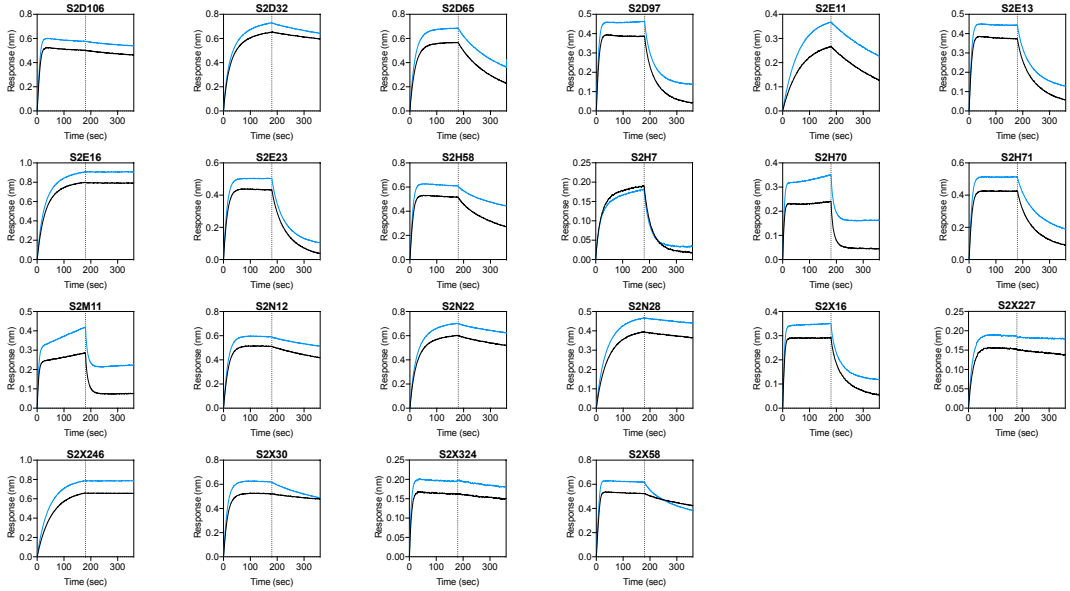
Extended Data Fig. 5



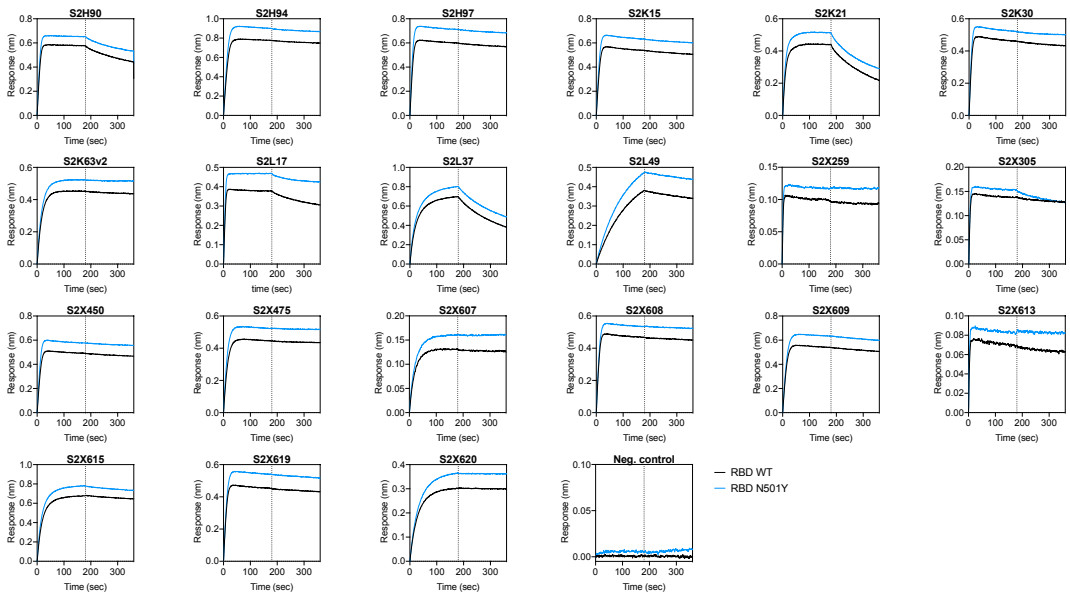
Extended Data Fig. 5. Neutralisation of WT (D614G), B.1.1.7 and TM (N501Y, E484K, K417N) SARS-CoV-2 Spike pseudotyped virus by a panel of 57 monoclonal antibodies (mAbs). **a-c**, Neutralisation of WT (black), B.1.1.7 (blue) and TM (red) SARS-CoV-2-MLV by 9 NTD-targeting (a), 29 RBM-targeting (b) and 19 non-RBM-targeting (c) mAbs.

Extended Data Fig. 6

a



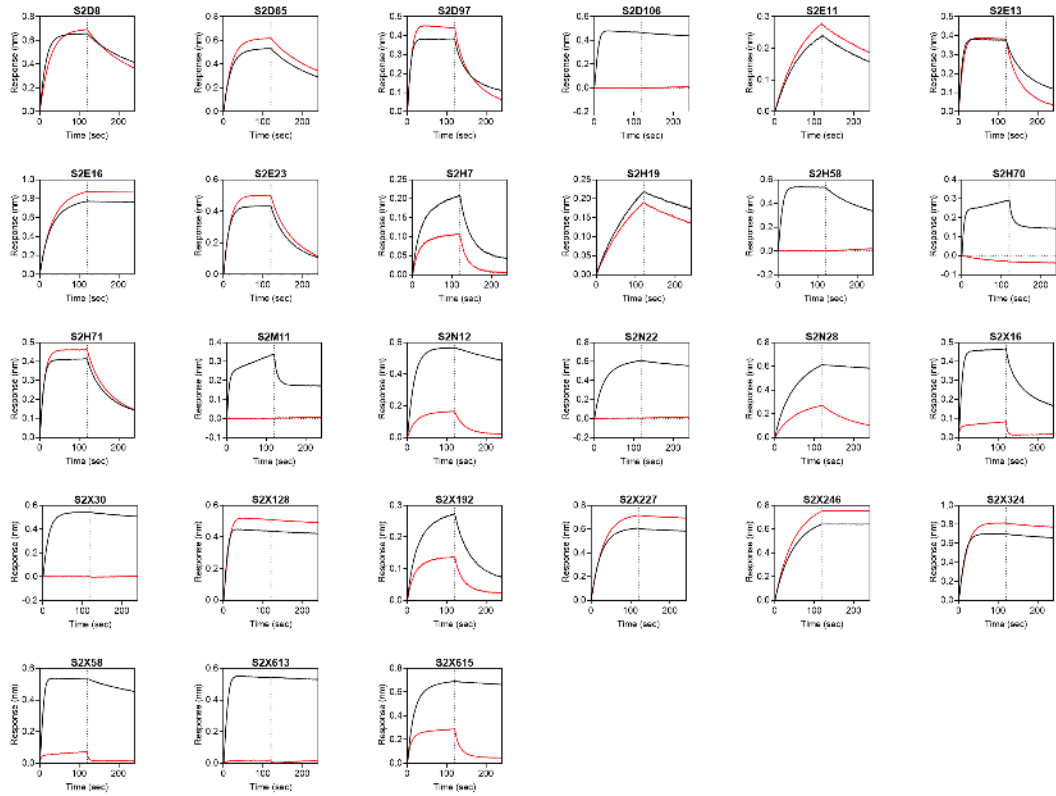
b



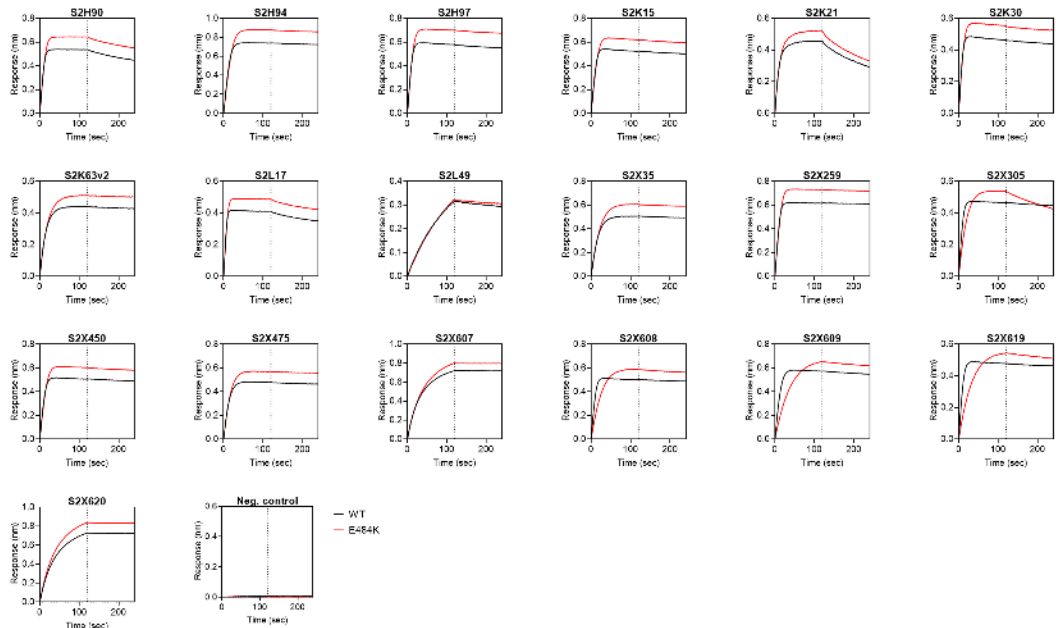
Extended Data Fig. 6. Kinetics of binding to WT and N501Y SARS-CoV-2 RBD of 43 RBD-specific mAbs. a-b, Binding to WT (black) and N501Y (blue) RBD by 22 RBM-targeting (a) and 21 non-RBM-targeting (b) mAbs. An antibody of irrelevant specificity was included as negative control. mAbs: monoclonal antibodies

Extended Data Fig. 7

a

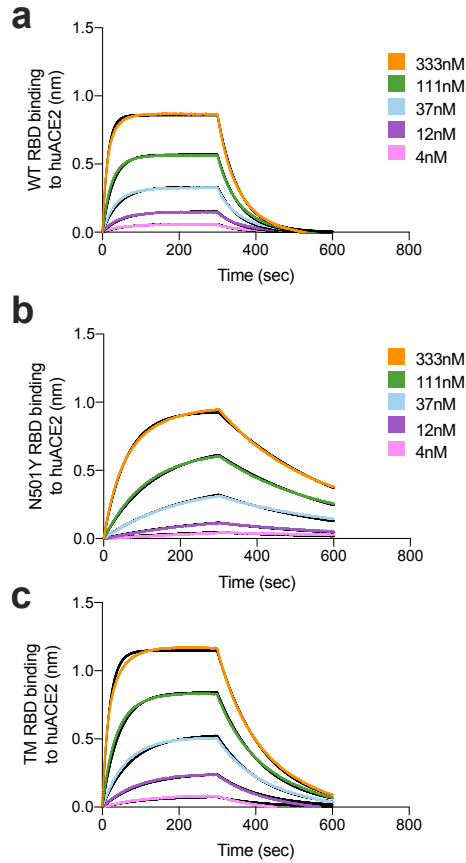


b



Extended Data Fig. 7. Kinetics of binding to WT and E484K SARS-CoV-2 RBD of 46 RBD-specific mAbs. a-b, Binding to WT (black) and E484K (red) RBD by 27 RBM-targeting (a) and 19 non-RBM-targeting (b) mAbs. An antibody of irrelevant specificity was included as negative control. mAbs: monoclonal antibodies

Extended Data Fig. 8



Extended Data Fig. 8. Binding of human ACE2 to SARS-CoV-2 WT, N501Y, TM (N501Y, E484K, K417N) RBDs. a-b. BLI binding analysis of the human ACE2 ectodomain (residues 1-615) to immobilized SARS-CoV-2 WT RBD (a) and B.1.1.7 RBD (c). Black lines correspond to a global fit of the data using a 1:1 binding model. RBD: receptor binding domain.