

1 **A prefusion SARS-CoV-2 spike RNA vaccine is highly immunogenic and**
2 **prevents lung infection in non-human primates**

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36 **Abstract**

37 To contain the coronavirus disease 2019 (COVID-19) pandemic, a safe and effective vaccine
38 against the new severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is urgently
39 needed in quantities sufficient to immunise large populations. In this study, we report the
40 design, preclinical development, immunogenicity and anti-viral protective effect in rhesus
41 macaques of the BNT162b2 vaccine candidate. BNT162b2 contains an LNP-formulated
42 nucleoside-modified mRNA that encodes the spike glycoprotein captured in its prefusion
43 conformation. After expression of the BNT162b2 coding sequence in cells, approximately 20%
44 of the spike molecules are in the one-RBD ‘up’, two-RBD ‘down’ state. Immunisation of mice
45 with a single dose of BNT162b2 induced dose level-dependent increases in pseudovirus
46 neutralisation titers. Prime-boost vaccination of rhesus macaques elicited authentic SARS-
47 CoV-2 neutralising geometric mean titers 10.2 to 18.0 times that of a SARS-CoV-2
48 convalescent human serum panel. BNT162b2 generated strong T_H1 type $CD4^+$ and $IFN\gamma^+$ $CD8^+$
49 T-cell responses in mice and rhesus macaques. The BNT162b2 vaccine candidate fully
50 protected the lungs of immunised rhesus macaques from infectious SARS-CoV-2 challenge.
51 BNT162b2 is currently being evaluated in a global, pivotal Phase 2/3 trial (NCT04368728).

52 **Main**

53 **Introduction**

54 Due to the shattering impact of the coronavirus disease 2019 (COVID-19) pandemic on human
55 health and society, multiple collaborative research programs have been launched, leading to
56 new insights and progress towards vaccine development. Soon after it emerged in December
57 2019, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was identified as a β -
58 coronavirus with high sequence similarity to bat-derived SARS-like coronaviruses^{1,2}. The
59 globalised response is mirrored by the upload of over 92,000 viral genome sequences as of
60 August 29, 2020, to GISAID (Global Initiative on Sharing All Influenza Data).

61 The trimeric spike glycoprotein (S) of SARS-CoV-2 binds its cellular receptor, angiotensin
62 converting enzyme 2 (ACE2), through a receptor-binding domain (RBD), which is part of its
63 N-terminal furin cleavage fragment (S1)^{3,4}. S rearranges to translocate the virus into cells by
64 membrane fusion^{5,6}. The C-terminal furin cleavage fragment (S2) contains the fusion
65 machinery⁷. Membrane fusion can be blocked by mutating S residues 986 and 987 to prolines,
66 producing an S antigen stabilised in the prefusion conformation (P2 S)⁸⁻¹⁰. The RBD is a key
67 target for virus neutralising antibodies, with an 'up' conformation, in which more neutralising
68 epitopes are exposed, and a 'down' conformation in which many epitopes are buried^{5,10-12}. In
69 addition, some neutralising antibodies bind S epitopes outside the RBD.

70 During this pandemic, fast vaccine availability is critical. COVID-19 vaccine candidates based
71 on different platforms are already in clinical trials, with the most advanced based on viral vector
72 and nucleic acid technologies¹³⁻¹⁶. We report the preclinical development of BNT162b2, a lipid-
73 nanoparticle (LNP) formulated N¹-methyl-pseudouridine (m¹Ψ) nucleoside-modified mRNA
74 (modRNA) vaccine candidate that encodes P2 S with a native furin cleavage site resulting in
75 the S1 and S2 cleavage fragments (Fig. 1a). The m¹Ψ-modification dampens innate immune
76 sensing, and, together with optimised non-coding sequence elements, increases RNA
77 translation *in vivo*¹⁷⁻¹⁹. ModRNA vaccines have already proven immunogenic for several viral
78 targets^{20,21}. BNT162b2 has been evaluated in phase 1 clinical trials in the US (NCT04368728)
79 and Germany (NCT04380701, EudraCT: 2020-001038-36), and is now being evaluated in a
80 pivotal, global, phase 2/3 safety and efficacy study¹⁵.

81 **Results**

82 BNT162b2 RNA *in vitro* transcribed by T7 polymerase from a plasmid DNA template has a
83 single, sharp-peak microfluidic capillary electrophoresis profile, consistent with its calculated
84 length of 4,283 nucleotides, indicating purity and integrity (Fig. 1b). When HEK293T/17 cells
85 were incubated with BNT162b2 (which is LNP-formulated) or with BNT162b2 RNA mixed
86 with a transfection reagent, robust expression of P2 S was detectable by flow cytometry
87 (Extended Data Fig. 1a).

88 For structural characterisation, P2 S was expressed in Expi293F cells from DNA that encodes
89 the same amino acid sequence as BNT162b2 RNA, with the addition of a C-terminal TwinStrep
90 tag for affinity purification. The trimeric P2 S bound the human ACE2 peptidase domain (PD),
91 and an anti-RBD human neutralising antibody B38 with high affinity (K_D 1 nM, Extended Data
92 Fig. 1b,c)²². Structural analysis by cryo-electron microscopy (cryo-EM) produced a 3.29 Å
93 nominal resolution mass density map, into which a previously published atomic model¹⁰ was
94 fitted and rebuilt (Fig. 1c,d; Extended Data Fig. 2, Extended Data Table 1). The rebuilt model
95 shows good agreement with reported structures of prefusion full-length wild type S and its
96 ectodomain with P2 mutations^{5,10}. Three-dimensional classification of the dataset showed a
97 class of particles that was in the one RBD ‘up’ (accessible for receptor binding), two RBD
98 ‘down’ (closed) conformation and represented 20.4% of the trimeric molecules. The remainder
99 were in the all RBD ‘down’ conformation (Fig. 1e, Extended Data Fig. 2c). The RBD in the
100 ‘up’ conformation was less well resolved than other parts of the structure, suggesting
101 conformational flexibility and a dynamic equilibrium between RBD ‘up’ and RBD ‘down’
102 states as also suggested by others^{5,23}. Nevertheless, the binding and structural analyses indicate
103 that the BNT162b2 RNA sequence encodes a recombinant P2 S that authentically presents the
104 ACE2 binding site and other epitopes targeted by SARS-CoV-2 neutralising antibodies.

105 To characterise BNT162b2-elicited B- and T-cell responses, BALB/c mice were immunized
106 intramuscularly (IM) once with 0.2, 1, or 5 µg BNT162b2 or received a buffer control. S1- and
107 RBD-binding serum IgG developed rapidly at all dose levels in a dose-dependent manner. For
108 S1-binding antibodies, the geometric mean concentration (GMC) in the 5 µg group was 386
109 µg/mL at Day 28 (Fig. 2a, Extended Data Fig. 3a). At Day 28 after immunisation, vaccine-
110 elicited IgG had a strong binding affinity for S1 (geometric mean K_D 12 nM) and the RBD
111 (geometric mean K_D 0.99 nM), with both having a low off-rate (Extended Data Fig. 3b). SARS-
112 CoV-2 neutralising activity in mouse serum was measured by a vesicular stomatitis virus

113 (VSV)-based SARS-CoV-2 pseudovirus neutralisation assay. Fifty percent pseudovirus
114 neutralisation geometric mean titers (pVNT₅₀ GMTs) increased steadily after immunisation to
115 26, 176, and 296 on Day 28 for the 0.2, 1, and 5 µg dose levels, respectively (Fig. 2b, Extended
116 Data Fig. 3c).

117 A high fraction of splenocytes of CD4⁺ and CD8⁺ T-cell phenotype isolated from mice on Days
118 12 and 28 after BNT162b2-immunisation had a strong antigen-specific IFN γ and IL-2 response
119 in ELISpot and intracellular cytokine staining flow cytometry analysis when re-stimulated *ex*
120 *vivo* with a full-length S peptide pool (Fig. 2c-e). Total splenocytes harvested on Day 28 and
121 *ex vivo* re-stimulated with the full-length S peptide pool secreted high levels of the T_H1
122 cytokines IL-2 or IFN γ , but minute amounts of the T_H2 cytokines IL-4, IL-5 and IL-13 as
123 measured in multiplex immunoassays (Fig. 2f).

124 BNT162b2-induced effects on proliferation and dynamics of immune cell populations were
125 assessed in injection site draining lymph nodes (dLNs), which are the principal immune-
126 educated compartments for proficient T- and B-cell priming, and in blood and spleen for
127 evaluation of its systemic effects. Higher numbers of plasma cells, class switched IgG1- and
128 IgG2a-positive B cells, and germinal center B cells were observed in dLNs and spleens of mice
129 12 days after immunisation with 5 µg BNT162b2 than after immunisation with buffer
130 (Extended Data Fig. 4a, b). In Day 7 post-immunisation blood, there were significantly fewer
131 circulating B cells than in blood from buffer-immunised mice (Extended Data Fig. 4c), which
132 may imply that B-cell homing to lymphoid compartments augments B cell counts in dLN and
133 spleen. The dLNs from BNT162b2-immunised mice also have significantly elevated counts of
134 CD8⁺ and CD4⁺ T cells, which was most pronounced for T follicular helper (T_{FH}) cells,
135 including ICOS⁺ subsets essential for germinal center formation (Extended Data Fig. 4a)²⁴.
136 BNT162b2 immunisation increased CD8⁺ T cell counts in the blood and T_{FH} cell counts in the
137 spleen and blood (Extended Data Fig. 4b, c). These data indicate that BNT162b2 concurrently
138 elicits strong SARS-CoV-2 pseudovirus neutralising titers and systemic T_H1-driven CD4⁺ and
139 CD8⁺ T-cell responses.

140 To assess BNT162b2-mediated protection in non-human primates, groups of six male, 2-4 year
141 old rhesus macaques were immunised IM with 30 or 100 µg of BNT162b2 or saline control on
142 Days 0 and 21. S1-binding IgG was readily detectable by Day 21 after Dose 1, and levels
143 increased further after Dose 2 through Day 28 (Fig. 3a). Seven days after Dose 2 (Day 28), the
144 GMCs of S1-binding IgG were 30,339 units (U)/mL (30 µg dose level) and 34,668 U/mL

145 (100 µg dose level). For comparison, the S1-binding IgG GMC of a panel of 38 SARS-CoV-2
146 convalescent human sera was 631 U/mL, substantially lower than the GMCs of the immunised
147 rhesus macaques after one or two doses.

148 Fifty percent virus neutralisation GMTs, measured by an authentic SARS-CoV-2 neutralisation
149 assay²⁵, were detectable in rhesus macaque sera by Day 21 after Dose 1 and peaked at a GMT
150 of 962 (Day 35, 14 days after Dose 2 of 30 µg) or 1,689 (Day 28, 7 days after Dose 2 of 100 µg;
151 Fig. 3b). Robust GMTs of 285 for 30 µg and 310 for 100 µg dose levels persisted to at least
152 Day 56 (most recent time point tested). For comparison, the neutralisation GMT of the human
153 convalescent serum panel was 94.

154 S-specific T-cell responses were analysed in BNT162b2-immunised rhesus macaques and
155 saline-immunised controls by ELISpot and intracellular cytokine staining (ICS). Peripheral
156 blood mononuclear cells (PBMCs) were collected before immunisation and at the times
157 indicated after Doses 1 and 2. Strong IFN γ but minimal IL-4 responses were detected by
158 ELISpot after Dose 2 (Fig. 3c,d, Extended Data Fig. 5). ICS confirmed that BNT162b2 elicited
159 strong S-specific IFN γ producing T-cell responses, including a high frequency of CD4⁺ T cells
160 that produced IFN γ , IL-2, and TNF but a low frequency of CD4⁺ T cells that produced IL-4,
161 indicating a T_{H1}-biased response (Fig. 3e,f). BNT162b2 also elicited S-specific
162 IFN γ ⁺ producing CD8⁺ T cells (Fig. 3g).

163 Six rhesus macaques that had received two immunisations with 100 µg BNT162b2 and three
164 age-matched macaques that had received saline were challenged 55 days after Dose 2 with
165 1.05×10^6 plaque forming units of SARS-CoV-2 (strain USA-WA1/2020), split equally
166 between intranasal and intratracheal routes, as previously described²⁶. Three additional non-
167 immunised, age-matched rhesus macaques (sentinels) were mock-challenged with cell culture
168 medium. Nasal and oropharyngeal (OP) swabs were collected and bronchoalveolar lavage
169 (BAL) was performed at the times indicated, and samples were tested for SARS-CoV-2 RNA
170 (genomic RNA or subgenomic transcripts) by reverse-transcription quantitative polymerase
171 chain reaction (RT-qPCR; Fig. 4). All personnel performing clinical, radiological,
172 histopathological, or RT-qPCR evaluations were blinded to the group assignments of the
173 macaques.

174 Viral RNA was detected in BAL fluid from 2 of the 3 control-immunised macaques on Day 3
175 after challenge and from 1 of 3 on Day 6 (Fig. 4a). At no time point sampled was viral RNA

176 detected in BAL fluid from the BNT162b2-immunised and SARS-CoV-2 challenged macaques
177 (Fig. 4a). The difference in viral RNA detection in BAL fluid between BNT162b2-immunised
178 and control-immunised rhesus macaques after challenge is highly statistically significant (by a
179 nonparametric test, $p=0.0014$).

180 From control-immunised macaques, viral RNA was detected in nasal swabs obtained on Days
181 1, 3, and 6 after SARS-CoV-2 challenge; from BNT162b2-immunised macaques, viral RNA
182 was detected only in nasal swabs obtained on Day 1 after challenge and not in swabs obtained
183 on Day 3 or subsequently (Fig. 4b). The pattern of viral RNA detection from OP swabs was
184 similar to that for nasal swabs (Fig. 4c).

185 In general, virus-challenged animals showed no clinical signs of significant disease. We
186 conclude that the 2-4 year old male rhesus macaque challenge model is primarily a SARS-CoV-
187 2 infection model and not a COVID-19 disease model.

188 **Discussion**

189 We demonstrate that BNT162b2, an LNP-formulated, m1 Ψ nucleoside-modified mRNA
190 encoding SARS-CoV-2 S captured in a prefusion conformation is highly immunogenic in mice
191 and rhesus macaques. Expression from DNA of protein with the BNT162b2-encoded amino
192 acid sequence confirmed the P2 S prefusion conformation by cryo-EM. This analysis confirmed
193 that the antigenically important RBD can assume the ‘up’ conformation, with the receptor
194 binding site, rich in neutralising epitopes, accessible in a proportion of the molecules²⁷. The
195 alternative states observed likely reflect a dynamic equilibrium between RBD ‘up’ and ‘down’
196 positions^{10,23}. Binding of expressed and purified P2 S to ACE2 and a neutralising monoclonal
197 antibody further demonstrates its conformational and antigenic integrity.

198 In mice, a single injection of BNT162b2 elicited high neutralizing titers and strong T_H1 and T_{FH}
199 type CD4⁺ and IFN γ ⁺IL-2⁺ CD8⁺ T-cell responses. Both BNT162b2 induced CD4⁺ T-cell types
200 may support antigen-specific antibody generation and maturation, and potentially protection
201 from infectious challenge. Limitation and clearance of virus infection is promoted by the
202 interplay of neutralising antibodies with CD8⁺ T cells that eliminate intracellular virus
203 reservoirs. CD8⁺ T cells may also reduce the influx of monocytes into infected lung tissue,
204 which can be associated with undesirable IL-6 and TNF production and impaired antigen
205 presentation^{28,29}. The contributions of the immune effector systems to human protection from

206 SARS-CoV-2 is not yet understood. Therefore, it appears prudent to develop COVID-19
207 vaccines that enlist concomitant cognate B cell, CD4⁺ T cell, and CD8⁺ T-cell responses.

208 The immunogenicity of BNT162b2 in rhesus macaques paralleled its immunogenicity in mice.
209 Seven days after Dose 2 of 100 µg, the neutralising GMT reached 18-times that of a human
210 SARS-CoV-2 convalescent serum panel and remained 3.3-times higher than this benchmark
211 five weeks after the last immunisation. The strongly T_H1-biased CD4⁺ T-cell response and
212 IFNγ⁺ CD8⁺ T-cell response to BNT162b2 is a pattern favoured for vaccine safety and efficacy,
213 providing added reassurance for clinical translation³⁰. BNT162b2 protected 2-4 year old rhesus
214 macaques from infectious SARS-CoV-2 challenge, with reduced detection of viral RNA in
215 immunised animals compared to those that received saline and with no evidence of clinical
216 exacerbation. Strong RT-qPCR evidence for lower respiratory tract protection was
217 demonstrated by the absence of detectable SARS-CoV-2 RNA in serial BAL samples obtained
218 starting 3 days after challenge of BNT162b2-immunised rhesus macaques.

219 We recently presented data from immunisation with BNT162b1, a vaccine candidate that has
220 the same LNP-formulated m1Ψ nucleoside-modified RNA platform but expresses a trimerised,
221 secreted RBD (Vogel et al., manuscript in preparation). The pattern, magnitude and durability
222 of humoral and cellular responses to BNT162b1 in mice and macaques were in the range of
223 those elicited by BNT162b2, as was protection of macaques from virus challenge, indicating
224 that these features are largely class-intrinsic for this particular vaccine platform. BNT162b1
225 elicits high SARS-CoV-2 neutralizing titers and strong T_H1-biased CD4⁺ and IFNγ⁺ and IL-2⁺
226 CD8⁺ T cell responses in humans, consistent with the preclinical findings^{15,31,32}.

227 The selection of BNT162b2 over BNT162b1 for further clinical testing was largely driven by
228 greater tolerability of BNT162b2 with comparable immunogenicity in clinical trials¹⁵ and the
229 broader range of T-cell epitopes on the much larger full length spike. A global, pivotal, phase
230 3 safety and efficacy study of immunisation with BNT162b2 (NCT04368728) is now well
231 under way and may answer those open questions that cannot be addressed by preclinical
232 models.

233

234 **Materials and Methods**

235 **Ethics statement.**

236 All mouse studies were performed at BioNTech SE, and protocols were approved by the local
237 authorities (local welfare committee), conducted according to FELASA recommendations and
238 in compliance with the German Animal Welfare Act and Directive 2010/63/EU. Only animals
239 with an unobjectionable health status were selected for testing procedures.

240 Immunisations for the non-human primate (NHP) study were performed at the University of
241 Louisiana at Lafayette-New Iberia Research Center (NIRC), which is accredited by the
242 Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC, Animal
243 Assurance #: 000452). The work was in accordance with USDA Animal Welfare Act and
244 Regulations and the NIH Guidelines for Research Involving Recombinant DNA Molecules, and
245 Biosafety in Microbiological and Biomedical Laboratories. All procedures performed on these
246 animals were in accordance with regulations and established guidelines and were reviewed and
247 approved by an Institutional Animal Care and Use Committee or through an ethical review
248 process. Infectious SARS-CoV-2 challenge for the NHP study was performed at the Southwest
249 National Primate Research Center. Animal husbandry followed standards recommended by
250 AAALAC International and the NIH Guide for the Care and Use of Laboratory Animals. This
251 study was approved by the Texas Biomedical Research Institute Animal Care and Use
252 Committee.

253 **Protein and peptide reagents.**

254 Purified recombinant SARS-CoV-2 S1 subunit including a histidine tag and the RBD tagged
255 with the Fc region of human IgG1 (both Sino Biological) were used in ELISA to detect SARS-
256 CoV-2 S-specific IgG in mice. Purified recombinant SARS-CoV-2 S1 and RBD with a histidine
257 tag (both Sino Biological) were used for surface plasmon resonance (SPR) spectroscopy. An
258 overlapping 15-mer peptide pool of the S protein was used for ELISpot, cytokine profiling and
259 intracellular cytokine staining. An irrelevant peptide control (SPSYVYHQF, derived from gp70
260 AH-1³³) or a CMV peptide pool was used as control for ELISpot assays. All peptides were
261 obtained from JPT Peptide Technologies.

262 **Human convalescent sera.**

263 Human COVID-19 convalescent sera (n=38) were drawn from donors 18-83 years of age at
264 least 14 days after PCR-confirmed diagnosis and at a time when the participants were
265 asymptomatic. Serum donors had symptomatic infections (35/38), or had had been hospitalised
266 (1/38). Sera were obtained from Sanguine Biosciences (Sherman Oaks, CA), the MT group
267 (Van Nuys, CA) and Pfizer Occupational Health and Wellness (Pearl River, NY) and used
268 across different studies as reference benchmark.

269 **Cell culture.**

270 Human embryonic kidney (HEK)293T/17 and Vero 76 cells (both ATCC) were cultured in
271 Dulbecco's modified Eagle's medium (DMEM) with GlutaMAX™ (Gibco) supplemented with
272 10% fetal bovine serum (FBS [Sigma-Aldrich]). Cell lines were tested for mycoplasma
273 contamination after receipt, before expansion and cryopreservation. For studies including NHP
274 samples, Vero 76 and Vero CCL81 (both ATCC) cells were cultured in DMEM (Gibco)
275 containing 2% HyClone fetal bovine and 100 U/mL penicillium/streptomycin (Gibco).
276 Expi293F™ cells were grown in Expi293™ media and transiently transfected using
277 ExpiFectamine™293 (all from Thermo Fisher Scientific).

278 ***In vitro* transcription and purification of RNA.**

279 To generate the template for RNA synthesis, a DNA fragment encoding the SARS-CoV-2 P2
280 S protein (based on GenBank: MN908947), including the amino acid exchanges K986P and
281 V987P, was cloned into a starting plasmid vector with backbone sequence elements for
282 improved RNA stability and translational efficiency^{19,34}. Non-coding backbone elements
283 included the regions from the T7 promoter to the 5' and 3' UTR plus a poly(A) tail
284 (100 nucleotides) interrupted by a linker (A30LA70, 10 nucleotides). The DNA was purified,
285 spectrophotometrically quantified, and *in vitro* transcribed by T7 RNA polymerase in the
286 presence of a trinucleotide cap1 analogue ((m₂^{7,3'-O})Gppp(m^{2'-O})ApG; TriLink) and of N¹-
287 methylpseudouridine-5'-triphosphate (m¹ΨTP; Thermo Fisher Scientific) instead of uridine-
288 5'-triphosphate (UTP)³⁵. RNA was purified using magnetic particles³⁶, integrity assessed by
289 microfluidic capillary electrophoresis (Agilent Fragment Analyser), and concentration, pH,
290 osmolality, endotoxin level and bioburden determined.

291 **Lipid-nanoparticle formulation of the RNA.**

292 Purified RNA was formulated into LNPs using an ethanolic lipid mixture of ionisable cationic
293 lipid and transferred into an aqueous buffer system via diafiltration to yield an LNP composition
294 similar to one previously described³⁷. BNT162b2 was stored at -70 °C at a concentration of
295 0.5 mg/mL.

296 **mRNA transfection and P2 S translation.**

297 HEK293T/17 cells were transfected with 1 µg RiboJuice transfection reagent-mixed
298 BNT162b2 RNA or with BNT162b2 (BNT162b2 RNA formulated as LNP) by incubation for
299 18 hours. Non-LNP formulated mRNA was diluted in Opti-MEM medium (Thermo Fisher
300 Scientific) and mixed with the transfection reagents according to the manufacturer's
301 instructions (RiboJuice, Merck Millipore). Transfected HEK293T/17 cells were stained with
302 Fixable Viability Dye (eBioscience). After fixation (Fixation Buffer, BioLegend), cells were
303 permeabilised (Perm Buffer, eBioscience) and stained with a monoclonal SARS-CoV-2 spike
304 S1 antibody (SinoBiological). Cells were acquired on a FACSCanto II flow cytometer (BD
305 Biosciences) using BD FACSDiva software version 8.0.1 and analysed by FlowJo software
306 version 10.6.2 (FlowJo LLC, BD Biosciences).

307 **P2 S expression and purification.**

308 To express P2 S for structural characterisation, a gene encoding the full length of SARS-CoV-
309 2 (GenBank: MN908947) with two prolines substituted at residues 986 and 987 followed with
310 a C-terminal HRV3C protease site and a TwinStrep tag was cloned into a modified
311 pcDNA3.1(+) vector with the CAG promoter. The TwinStrep-tagged P2 S was expressed in
312 Expi293 cells. Purification of the recombinant protein was based on a procedure described
313 previously, with minor modifications⁵. Upon cell lysis, P2 S was solubilized in 1% NP-40
314 detergent. The TwinStrep-tagged protein was then captured with StrepTactin Sepharose HP
315 resin in 0.5% NP-40. P2 S was further purified by size-exclusion chromatography and eluted
316 as three distinct peaks in 0.02 % NP-40 as previously reported⁵. Peak 1, which consists of intact
317 P2 S migrating at around 150 kDa, as well as dissociated S1 and S2 subunits, which co-migrate
318 at just above 75 kDa, was used in the structural characterisation. Spontaneous dissociation of
319 the S1 and S2 subunits mostly occurs throughout the course of the protein purification, starting
320 at the point of detergent-mediated protein extraction.

321 **Biolayer interferometry.**

322 The binding of detergent NP-40 solubilized, purified P2 S to human ACE2 peptidase domain
323 (ACE2 PD) and human neutralising monoclonal antibody B38²² was performed on Octet
324 RED384 (FortéBio) at 25 °C in a running buffer (RB) consisting of 25 mM Tris pH7.5, 150 mM
325 NaCl, 1 mM EDTA and 0.02% NP-40. Avi-tagged ACE2-PD was captured on streptavidin
326 coated sensors and B38 antibody was captured on sensors coated with protein G. After initial
327 baseline equilibration of 120 s, the sensors were dipped in 10 µg/mL solution of Avi-tagged
328 ACE2-PD or B38 mAb for 300 s to achieve capture levels of 1 nM using the threshold function.
329 The sensors were dipped in RB for 120 s for collecting baseline before they were dipped in a
330 concentration series of purified P2 S samples for 300 s (association phase). The sensors were
331 immersed in RB for measuring 600 s (dissociation phase). Data were reference subtracted and
332 fit to a 1:1 binding model with R² value greater than 0.95, to determine kinetics and affinity of
333 binding, using Octet Data Analysis Software v10.0 (FortéBio).

334 **Cryo-electron microscopy sample preparation, data collection and data processing.**

335 For TwinStrep-tagged P2 S, 4 µL purified protein at 0.5 mg/mL were applied to gold Quantifoil
336 R1.2/1.3 300 mesh grids freshly overlaid with graphene oxide. Sample was blotted using a
337 Vitrobot Mark IV for 4 s with a force of -2 before being plunged into liquid ethane cooled by
338 liquid nitrogen. 27,701 micrographs were collected from a two identically prepared grids on a
339 Titan Krios operating at 300 keV equipped with a Gatan K2 Summit direct electron detector in
340 super-resolution mode at a magnification of 165,000x, for a magnified pixel size of 0.435 Å at
341 the specimen level. Data were collected from each grid over a defocus range of -1.2 to -3.4 µm
342 with a total electron dose of 50.32 and 50.12 e⁻/Å², respectively, fractionated into 40 frames
343 over a 6-second exposure for 1.26 and 1.25 e⁻/Å²/frame. On-the-fly motion correction, CTF
344 estimation, and particle picking and extraction with a box size of 450 pixels were performed in
345 Warp³⁸, during which super-resolution data were binned to give a pixel size of 0.87 Å. A total
346 of 1,119,906 particles were extracted. All subsequent processing was performed in RELION
347 3.1-beta³⁹. Particle heterogeneity was filtered out with 2D and 3D classification to filter out bad
348 particles, yielding a set of 73,393 particles, which refined to 3.6 Å with C3 symmetry. 3D
349 classification of this dataset without particle alignment separated out one class with a single
350 RBD up, representing 15,098 particles. The remaining 58,295 particles, in three RBD ‘down’
351 conformation, were refined to give a final model at 3.29 Å. The atomic model from PDB ID
352 6XR8⁵ was rigid-body fitted into the map density, then flexibly fitted to the density using real-
353 space refinement in Phenix⁴⁰ alternating with manual building in Coot⁴¹. The cryo-EM model

354 validation is provided in Extended Data Fig 3b, the full cryo-EM data processing workflow in
355 Extended Data Fig. 3c, and the model refinement statistics in Extended Data Table 1.

356 **Immunisation.**

357 *Mice.* Female BALB/c mice (Janvier; 8-12 weeks) and were randomly allocated to groups.
358 BNT162b2 was diluted in PBS, 300 mM sucrose or saline (0.9% NaCl) and injected IM into
359 the gastrocnemius muscle at a volume of 20 μ L under isoflurane anaesthesia.

360 *Rhesus macaques (Macaca mulatta).* Male rhesus macaques (2–4 years) were randomly
361 assigned to receive either BNT162b2 or saline placebo control in 0.5 mL volume administered
362 by IM injection in the left quadriceps muscle on Days 0 and 21.

363 **Tissue preparation.**

364 *Mice.* Peripheral blood was collected from the retro-orbital venous plexus under isoflurane
365 anaesthesia or *vena facialis* without prior anaesthetisation. Blood was centrifuged for 5 minutes
366 at 16,000 x g, and the serum was immediately used for downstream assays or stored at -20 °C.
367 Spleen single-cell suspensions were prepared in PBS by mashing tissue against the surface of
368 a 70 μ m cell strainer (BD Falcon). Erythrocytes were removed by hypotonic lysis. Popliteal,
369 inguinal and iliac lymph nodes were pooled, cut into pieces, digested with collagenase D
370 (1 mg/mL; Roche) and passed through cell strainers.

371 *Rhesus macaques (Macaca mulatta).* Serum was obtained before immunisation and on Days
372 14, 21, 28, 35, 42, and 56. PBMCs were obtained before immunisation and on Days 7, 28, and
373 42, except that PBMCs were not obtained from the buffer-immunised group on Day 28. Blood
374 for serum and PBMCs was collected in compliance with animal protocol 2017-8725-023
375 approved by the NIRC Institutional Animal Care and Use Committee. Animals were
376 anaesthetised with ketamine HCl (10 mg/kg; IM) during blood collection and immunisation, and
377 monitored for adequate sedation.

378 **S1- and RBD-binding IgG assay.**

379 For mouse sera, MaxiSorp plates (Thermo Fisher Scientific) were coated with recombinant S1
380 or RBD (100 ng/100 μ L) in sodium carbonate buffer, and bound IgG was detected using an
381 HRP-conjugated secondary antibody and TMB substrate (Biotrend). Data collection was
382 performed using a BioTek Epoch reader and Gen5 software version 3.0.9. For concentration
383 analysis, the signal of the specific samples was correlated to a standard curve of an isotype

384 control. For rhesus macaque and human sera, a recombinant SARS-CoV-2 S1 containing a C-
385 terminal Avitag™ (Acro Biosystems) was bound to streptavidin-coated Luminex microspheres.
386 Bound rhesus macaque or human anti-S1 antibodies present in the serum were detected with a
387 fluorescently labelled goat anti-human polyclonal secondary antibody (Jackson
388 ImmunoResearch). Data were captured as median fluorescent intensities (MFIs) using a
389 Bioplex200 system (Bio-Rad) and converted to U/mL antibody concentrations using a reference
390 standard consisting of 5 pooled human COVID-19 convalescent serum samples (obtained >14
391 days PCR diagnosis), diluted in antibody depleted human serum with arbitrary assigned
392 concentrations of 100 U/mL and accounting for the serum dilution factor.

393 **Binding kinetics of antigen-specific IgGs using surface plasmon resonance spectroscopy**

394 Binding kinetics of murine S1- and RBD-specific serum IgGs was determined using a Biacore
395 T200 device (Cytiva) with HBS-EP running buffer (BR100669, Cytiva) at 25 °C. Carboxyl
396 groups on the CM5 sensor chip matrix were activated with a mixture of 1-ethyl-3-(3-
397 dimethylaminopropyl) carbodiimidehydrochloride (EDC) and N-hydroxysuccinimide (NHS) to
398 form active esters for the reaction with amine groups. Anti-mouse-Fc-antibody (Jackson
399 ImmunoResearch) was diluted in 10 mM sodium acetate buffer pH 5 (30 µg/mL) for covalent
400 coupling to immobilisation level of ~10,000 response units (RU). Free N-hydroxysuccinimide
401 esters on the sensor surface were deactivated with ethanolamine.

402 Mouse serum was diluted 1:50 in HBS-EP buffer and applied at 10 µL/min for 30 seconds to
403 the active flow cell for capture by immobilised antibody, while the reference flow cell was
404 treated with buffer. Binding analysis of captured murine IgG antibodies to S1-His or RBD-His
405 (Sino Biological) was performed using a multi-cycle kinetic method with concentrations
406 ranging from 25 to 400 nM or 1.5625 to 50 nM, respectively. An association period of 180
407 seconds was followed by a dissociation period of 600 seconds with a constant flow rate of 40
408 µL/min and a final regeneration step. Binding kinetics were calculated using a global kinetic fit
409 model (1:1 Langmuir, Biacore T200 Evaluation Software Version 3.1, Cytiva).

410 **VSV-SARS-CoV-2 spike variant pseudovirus neutralisation.**

411 A recombinant replication-deficient vesicular stomatitis virus (VSV) vector that encodes GFP
412 instead of VSV-G (VSVΔG-GFP) was pseudotyped with SARS-CoV-2 S protein according to
413 published pseudotyping protocols^{42,43}. In brief, HEK293T/17 monolayers transfected to express
414 SARS-CoV-2 S truncated of the C-terminal cytoplasmic 19 amino acids (SARS-CoV-2-S-

415 CA19) were inoculated with VSV Δ G-GFP vector. After incubation for 1 hour at 37 °C, the
416 inoculum was removed and cells were washed with PBS before medium supplemented with
417 anti-VSV-G antibody (clone 8G5F11, Kerafast Inc.) was added to neutralise residual input
418 virus. VSV/SARS-CoV-2 pseudovirus-containing medium was harvested 20 hours after
419 inoculation, 0.2 μ m filtered and stored at -80 °C.

420 Serial dilutions of mouse serum samples were prepared and pre-incubated for 10 minutes at
421 room temperature with VSV/SARS-CoV-2 pseudovirus suspension (4.8×10^3 infectious units
422 [IU]/mL) before transferring the mix to Vero 76 cells. Inoculated Vero-76 cells were incubated
423 for 20 hours at 37 °C. Plates were placed in an IncuCyte Live Cell Analysis system (Sartorius)
424 and incubated for 30 minutes prior to the analysis (IncuCyte 2019B Rev2 software). Whole
425 well scanning for brightfield and GFP fluorescence was performed using a 4 \times objective. The
426 50% pseudovirus neutralisation titre (pVNT₅₀) was reported as the reciprocal of the first serum
427 dilution yielding a 50% reduction in GFP-positive infected cell number per well compared to
428 the mean of the no serum pseudovirus positive control. Each serum sample dilution was tested
429 in duplicates.

430 **IFN γ and IL-4 ELISpot.**

431 Murine ELISpot assays were performed with mouse IFN γ ELISpot^{PLUS} kits according to the
432 manufacturer's instructions (Mabtech). A total of 5×10^5 splenocytes was *ex vivo* restimulated
433 with the full-length S peptide mix (0.1 μ g/mL final concentration per peptide) or controls
434 (gp70-AH1 [SPSYVYHQF]³³, 4 μ g/mL; Concanavalin A [ConA], 2 μ g/mL [Sigma]).
435 Streptavidin-alkaline phosphatase (ALP) and BCIP/NBT-plus substrate were added, and spots
436 counted using an ELISpot plate reader (ImmunoSpot® S6 Core Analyzer [CTL]). Spot numbers
437 were evaluated using ImmunoCapture Image Acquisition Software V7.0 and ImmunoSpot
438 7.0.17.0 Professional. Spot counts denoted too numerous to count by the software were set to
439 1,500. For T-cell subtyping, CD8⁺ T cells and CD4⁺ T cells were isolated from splenocyte
440 suspensions using MACS MicroBeads (CD8a [Ly-2] and CD4 [L3T4] [Miltenyi Biotec])
441 according to the manufacturer's instructions. 1×10^5 CD8⁺ or CD4⁺ T cells were subsequently
442 restimulated with 5×10^4 syngeneic bone marrow-derived dendritic cells loaded with full-
443 length S peptide mix (0.1 μ g/mL final concentration per peptide) or cell culture medium as
444 control. Purity of isolated T-cell subsets was determined by flow cytometry.

445 Rhesus macaque PBMCs were tested with commercially available NHP IFN γ and IL-4 ELISpot
446 assay kits (Mabtech, Sweden). Cryopreserved rhesus macaque PBMCs were thawed in pre-

447 warmed AIM-V media (Thermo Fisher Scientific, US) with Benzonase (EMD Millipore, US).
448 For IFN γ ELISpot, 1.0×10^5 PBMCs and 2.5×10^5 PBMCs for IL-4 ELISpot were stimulated
449 *ex vivo* with 1 μ g/mL of the full-length S overlapping peptide mix. Tests were performed in
450 triplicate wells and media-DMSO, a CMV peptide pool and PHA (Sigma) were included as
451 controls. After 24 hours for IFN γ and 48 hours for IL-4, Streptavidin-HRP and AEC substrate
452 (BD Bioscience) were added, and spots counted using a CTL ImmunoSpot S6 Universal
453 Analyzer (CTL, US). Results shown are background (Media-DMSO) subtracted and
454 normalized to SFC/ 10^6 PBMCs.

455 **Flow cytometry for analysis of cell mediated immunity.**

456 For mouse T-cell analysis in peripheral blood, erythrocytes from 50 μ L freshly drawn blood
457 were lysed (ACK lysing buffer [Gibco]), and cells were stained with Fixable Viability Dye
458 (eBioscience) and primary antibodies in the presence of Fc block in flow buffer (DPBS [Gibco]
459 supplemented with 2% FCS, 2 mM EDTA [both Sigma] and 0.01% sodium azide [Morphisto]).
460 After staining with secondary biotin-coupled antibodies in flow buffer, cells were stained
461 extracellularly against surface markers with directly labelled antibodies and streptavidin in
462 Brilliant Stain Buffer Plus (BD Bioscience) diluted in flow buffer. Cells were washed with 2%
463 RotiHistofix (Carl Roth), fixed (Fix/Perm Buffer, FoxP3/Transcription Factor Staining Buffer
464 Set [eBioscience]) and permeabilised (Perm Buffer, FoxP3/Transcription Factor Staining
465 Buffer Set [eBioscience]) overnight. Permeabilised cells were intracellularly treated with Fc
466 block and stained with antibodies against transcription factors in Perm Buffer.

467 For mouse T-cell analysis in lymphoid tissues, 1.5×10^6 lymph node and 4×10^6 spleen cells
468 were stained for viability and extracellular antigens with directly labelled antibodies. Fixation,
469 permeabilisation and intracellular staining was performed as described for blood T-cell staining.

470 For mouse B-cell subtyping in lymphoid tissues, 2.5×10^5 lymph node and 1×10^6 spleen cells
471 were treated with Fc block, stained for viability and extracellular antigens as described for blood
472 T-cell staining and fixed with 2% RotiHistofix overnight.

473 For mouse intracellular cytokine staining in T cells, 4×10^6 spleen cells were *ex vivo*
474 restimulated with 0.5 μ g/mL final concentration per peptide of full-length S peptide mix or cell
475 culture medium (no peptide) as control in the presence of GolgiStop and GolgiPlug (both BD
476 Bioscience) for 5 hours. Cells were stained for viability and extracellular antigens as described

477 for lymphoid T-cell staining. Cells were fixed with 2% RotiHistofix and permeabilised
478 overnight. Intracellular staining was performed as described for blood T-cell staining.

479 Mouse cells were acquired on a BD Symphony A3 or BD Celesta (B-cell subtyping) flow
480 cytometer (BD Bioscience) using BD FACSDiva software version 9.1 or 8.0.1.1, respectively,
481 and analysed with FlowJo 10.6 (FlowJo LLC, BD Biosciences).

482 For rhesus macaques intracellular cytokine staining in T cells, 1.5×10^6 PBMCs were stimulated
483 with the full-length S peptide mix at 1 $\mu\text{g}/\text{mL}$, Staphylococcus enterotoxin B (SEB; 2 $\mu\text{g}/\text{mL}$)
484 as positive control, or 0.2% DMSO as negative control. GolgiStop and GolgiPlug (both BD
485 Bioscience) were added. Following 37 °C incubation for 12 to 16 h, cells were stained for
486 viability and extracellular antigens after blocking Fc binding sites with directly labelled
487 antibodies. Cells were then fixed and permeabilized with BDCytoFix/CytoPerm solution (BD
488 Bioscience), intracellular staining was performed in perm buffer for 30 min at RT. Cells were
489 washed, resuspended in 2% FBS/PBS buffer and acquired on a LSR Fortessa. Data analyzed
490 by FlowJo (10.4.1). Results shown are background (Media-DMSO) subtracted.

491 **Cytokine profiling.**

492 Mouse splenocytes were re-stimulated for 48 hours with full-length S peptide mix (0.1 $\mu\text{g}/\text{mL}$
493 final concentration per peptide) or cell culture medium (no peptide) as control. Concentrations
494 of IFN γ , IL-2, IL-4, IL-5 and IL-13 in supernatants were determined using a bead-based, 11-
495 plex T_{H1}/T_{H2} mouse ProcartaPlex multiplex immunoassay (Thermo Fisher Scientific)
496 according to the manufacturer's instructions. Fluorescence was measured with a Bioplex200
497 system (Bio-Rad) and analysed with ProcartaPlex Analyst 1.0 software (Thermo Fisher
498 Scientific). Values below the lower limit of quantification (LLOQ) were set to zero.

499 **SARS-CoV-2 neutralisation.**

500 The SARS-CoV-2 neutralisation assay used a previously described strain of SARS-CoV-2
501 (USA_WA1/2020) that had been rescued by reverse genetics and engineered by the insertion
502 of an mNeonGreen (mNG) gene into open reading frame 7 of the viral genome²⁵. This reporter
503 virus generates similar plaque morphologies and indistinguishable growth curves from wild-
504 type virus. Viral master stocks were grown in Vero 76 cells as previously described⁴⁴. When
505 testing human convalescent serum specimens, the fluorescent neutralisation assay produced
506 comparable results as the conventional plaque reduction neutralisation assay. Serial dilutions

507 of heat-inactivated sera were incubated with the reporter virus (2×10^4 PFU per well) to yield
508 approximately a 10-30% infection rate of the Vero CCL81 monolayer for 1 hour at 37 °C before
509 inoculating Vero CCL81 cell monolayers (targeted to have 8,000 to 15,000 cells in the central
510 field of each well at the time of seeding, one day before infection) in 96-well plates to allow
511 accurate quantification of infected cells. Cell counts were enumerated by nuclear stain (Hoechst
512 33342) and fluorescent virally infected foci were detected 16-24 hours after inoculation with a
513 Cytation 7 Cell Imaging Multi-Mode Reader (Biotek) with Gen5 Image Prime version 3.09.
514 Titers were calculated in GraphPad Prism version 8.4.2 by generating a 4- parameter (4PL)
515 logistical fit of the percent neutralisation at each serial serum dilution. The 50% neutralisation
516 titre (VNT₅₀) was reported as the interpolated reciprocal of the dilution yielding a 50%
517 reduction in fluorescent viral foci.

518 **SARS-CoV-2 challenge of rhesus macaques (*Macaca mulatta*).**

519 The SARS-CoV-2 inoculum was obtained from a stock of 2.1×10^6 PFU/mL previously
520 prepared at Texas Biomedical Research Institute (San Antonio, TX), aliquoted into single use
521 vials, and stored at -70 °C. The working virus stock was generated from two passages of the
522 SARS-CoV-2 USA-WA1/2020 isolate (a 4th passage seed stock purchased from BEI Resources;
523 NR-52281) in Vero 76 cells. The virus was confirmed to be SARS-CoV-2 by deep sequencing
524 and identical to the published sequence (GenBank accession number MN985325.1).
525 BNT162b2-immunised (n=6) and age-matched saline control-immunised (n=3) male rhesus
526 macaques (control) were challenged with 1.05×10^6 plaque forming units of SARS-CoV-2
527 USA-WA1/2020 isolate, split equally between the intranasal (IN; 0.25 mL) and intratracheal
528 (IT; 0.25 mL) routes as previously described²⁶. The challenge was performed 55 days after the
529 second immunisation. A separate sentinel group of non-immunised age- and sex-matched
530 animals (n=3) was mock challenged with DMEM supplemented with 10% FCS IN (0.25 mL)
531 and IT (0.25 mL). Approximately two weeks prior to challenge, animals were moved to the
532 ABSL-3 facility at Southwest National Primate Research Center (SNPRC; San Antonio, TX).
533 Animals were monitored regularly by a board-certified veterinary clinician for rectal body
534 temperature, weight and physical examination. Specimen collection was performed under
535 tiletamine zolazepam (Telazol) anaesthesia as described²⁶. Nasal and oropharyngeal swabs
536 were collected from all macaques at Day 0, 1, 3, and 6 (relative to the day of challenge), from
537 BNT162b2-immunised macaques on Day 7 or 8, and from control and sentinel macaques on

538 Day 10. Bronchoalveolar lavage (BAL) was performed on all macaques the week before
539 challenge and on Days 3 and 6 post-challenge and on BNT162b2-immunised macaques on Day
540 7 or 8. BAL was performed by instilling four times 20 mL of saline. These washings were
541 pooled, aliquoted and stored frozen at -70 °C. Necropsy was performed on BNT162b2-
542 immunised animals on Day 7 or 8. Control and sentinel animals were not necropsied to allow
543 re-challenge (control) or challenge (sentinel) on a subsequent day.

544 **Reverse-transcription quantitative polymerase chain reaction.**

545 To detect and quantify SARS-CoV-2 in NHP, viral RNA was extracted from nasal swabs, OP
546 swabs, and BAL specimens as previously described⁴⁵⁻⁴⁷ and tested by RT-qPCR as previously
547 described²⁶. Briefly, 10 µg yeast tRNA and 1×10^3 PFU of MS2 phage (*Escherichia coli*
548 bacteriophage MS2, ATCC) were added to each thawed sample, and RNA extraction performed
549 using the NucleoMag Pathogen kit (Macherey-Nagel). The SARS-CoV-2 RT-qPCR was
550 performed on extracted RNA using a CDC-developed 2019-nCoV_N1 assay on a QuantStudio
551 3 instrument (Applied Biosystems). The cut-off for positivity (limit of detection, LOD) was
552 established at 10 gene equivalents (GE) per reaction (800 GE/mL). Samples were tested in
553 duplicate. On day 6, one BAL specimen from the control group and one day 1 nasal swab from
554 the BNT162b1-immunised group had, on repeated measurements, viral RNA levels on either
555 side of the LLOD. These specimens were categorised as indeterminate and excluded from the
556 graphs and the analysis.

557 **Statistics and reproducibility.**

558 No statistical methods were used to predetermine group and samples sizes (n). All experiments
559 were performed once. P-values reported for RT-qPCR analysis were determined by
560 nonparametric analysis (Friedman's test) based on the ranking of viral RNA shedding data
561 within each day. PROC RANK and PROC GLM from SAS® 9.4 were used to calculate the p-
562 values. Samples from post challenge days (Days 3, 6 and end of protocol [EOP] for BAL; Days
563 1, 3, 6 and 10 [control and sentinel] or EOP [BNT162b2-immunised] for nasal and
564 oropharyngeal swabs) were included in the analysis. Indeterminate results were excluded from
565 this analysis. All remaining analyses were carried out using GraphPad Prism 8.4.

566 **Data availability.**

567 The data that support the findings of this study are available from the corresponding author
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584 **Author Contributions**

585 U.S. conceived and conceptualised the work and strategy. S.He., S.C.D., C.K. and M.C.G.
586 designed primers and cloned all constructs. T.Z., S.F., J.S. and A.N.K. developed, planned,
587 performed and supervised RNA synthesis and analysis. E.H.M. purified P2 S. P.V.S. developed
588 and performed biolayer interferometry. J.A.L. and S.H. performed electron microscopy and
589 solved the structure of the complex. Y.C. supervised the structural and biophysical
590 characterisation and analysed the structures. A.M. and B.G.L. performed surface plasmon
591 resonance spectroscopy. A.G. and S.A.K. planned, performed and analysed *in vitro* studies.
592 F.B., T.K., C.R. managed formulation strategy. A.B.V., M.V., L.M.K. designed mouse studies
593 and analysed and interpreted data. A.P., S.E., D.P. and G.S. performed and analysed the S1-
594 binding IgG assays. R.C., Jr. and K.J.A. performed and analysed viral RT-qPCR data. A.M.,

595 B.S., A.W., C.F.-G. and P.-Y.S. performed and analysed pVNT and VNT assays. D.E., D.S.,
596 B.J., Y.F., H.J. performed *in vivo* studies and ELISpot assays. A.B.V., K.C.W., J.L., M.S.M. and
597 M.V. planned, analysed and interpreted ELISpot assays. L.M.K., J.L., D.E., Y.F., H.J., A.P.H.
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601 I.L.S. and W.K. oversaw NHP immunogenicity and serology testing. S.H.-U. and K.B. provided
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605 **Competing interests**

606 The authors declare: U.S. and Ö.T. are management board members and employees at
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628 **Additional Information**

629 Supplementary Information is available for this study.

630 Correspondence and requests for materials should be addressed to Ugur Sahin.

631

632

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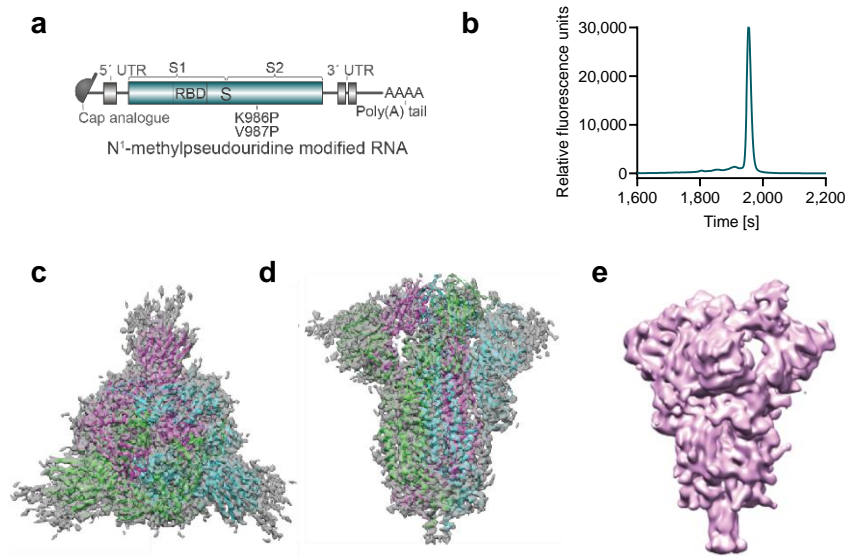


Figure 1. Vaccine design and characterisation of the expressed antigen.

a, BNT162b2 RNA structure. UTR, untranslated region; S, SARS-CoV-2 S glycoprotein; S1, N-terminal furin cleavage fragment; S2, C-terminal furin cleavage fragment; RBD, receptor-binding domain. Positions of the P2 mutation (K986P and V897P) are indicated. **b**, Liquid capillary electropherogram of *in vitro* transcribed BNT162b2 RNA. **c**, A 3.29 Å cryoEM map of P2 S, with fitted and refined atomic model, viewed down the three-fold axis toward the membrane. **d**, Cryo-EM map and model of (d) viewed perpendicular to the three-fold axis. **e**, Mass density map of TwinStrep-tagged P2 S produced by 3D classification of images extracted from cryo-EM micrographs with no symmetry averaging. This class, in the one-RBD ‘up’, two RBD ‘down’ positioning, represents 20.4% of the population.

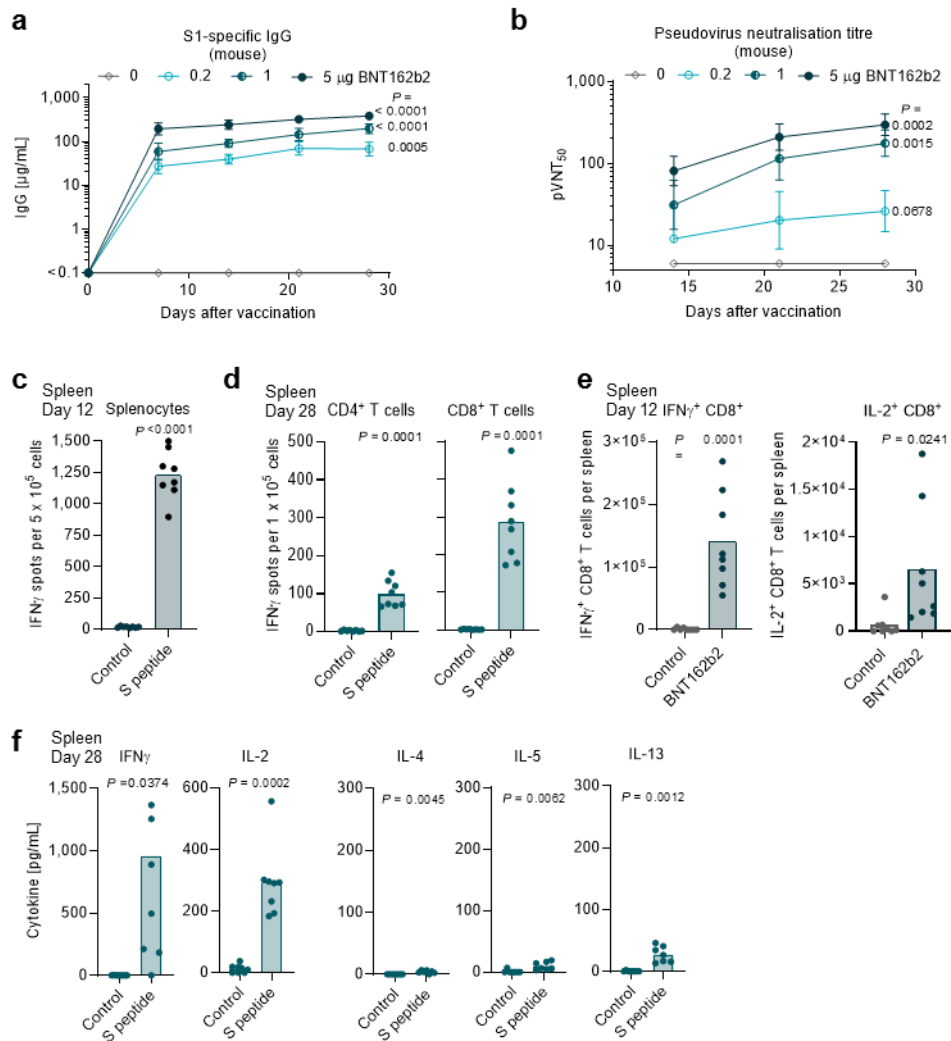


Figure 2. Mouse immunogenicity.

BALB/c mice (n=8 per group unless otherwise specified) were immunised intramuscularly (IM) with a single dose of with 0.2, 1 or 5 µg BNT162b2 or buffer. Geometric mean of each group \pm 95% CI, P-values compare Day 28 to non-immunised (0 µg; n=8) baseline sera (multiple comparison of mixed-effect analysis using Dunnett's multiple comparisons test) (a, b). **a**, S1-binding IgG responses in sera obtained 7, 14, 21 and 28 days after immunisation with 0, 0.2, 1, or 5 µg BNT162b2, determined by ELISA. For day 0 values, a pre-screening of randomly selected animals was performed (n=4). **b**, VSV-SARS-CoV-2 pseudovirus 50% serum neutralising titers (pVNT₅₀) of sera from (a). **c-f**, Splenocytes of BALB/c mice immunised IM with BNT162b2 or buffer (control) were *ex vivo* re-stimulated with full-length S peptide mix or negative controls ([c], [e], [f]: no peptide; [d]: irrelevant peptide). Individual values and mean of each group, P-values were determined by a two-tailed paired t-test. **c**, IFN γ ELISpot of splenocytes collected 12 days after immunisation with 5 µg BNT162b2. **d**, IFN γ ELISpot of isolated splenic CD4⁺ or CD8⁺ T cells collected

28 days after immunisation with 1 μg BNT162b2. **e**, CD8⁺ T-cell specific cytokine release by splenocytes collected 12 days after immunisation with 5 μg BNT162b2 or buffer (Control), determined by flow cytometry. S-peptide specific responses are corrected for background (no peptide). **f**, Cytokine production by splenocytes collected 28 days after immunisation with 1 μg BNT162b2, determined by bead-based multiplex analysis (n=7 for IL-4, IL-5 and IL-13, one outlier removed via routs test [Q=1%] for the S peptide stimulated samples).

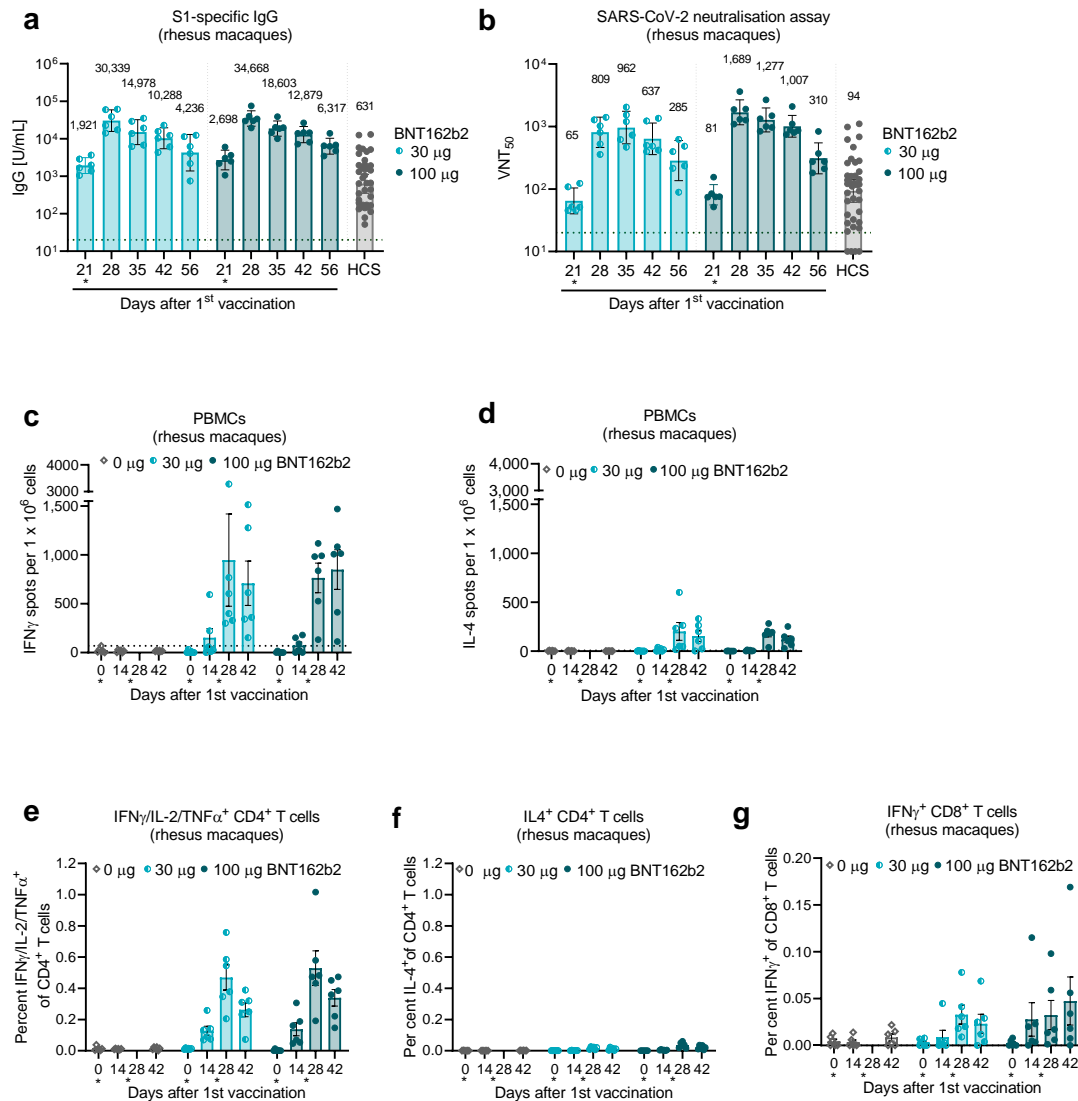


Figure 3. Rhesus macaque immunogenicity.

Rhesus macaques (n=6 per group) were immunised on Days 0 and 21 with 30 μg or 100 μg BNT162b2 or buffer. Sera and PBMCs were collected at the times indicated. Human convalescent sera (HCS) were obtained from SARS-CoV-2-infected patients at least 14 days after PCR-confirmed diagnosis and at a time when acute COVID-19 symptoms had resolved (n=38). **a**, Concentration, in arbitrary units, of IgG binding recombinant SARS-CoV-2 S1. **b**, SARS-CoV-2 50% virus neutralisation titers (VNT₅₀). **c-g**, PBMCs collected on Day 42 were *ex vivo* re-stimulated with full-length S peptide mix. **c**, IFN_γ, and **d**, IL-4 ELISpot. **e**, **f**, CD4⁺ T-cell specific, and **g**, CD8⁺ T-cell specific cytokine release, determined by flow cytometry. Heights of bars indicate the geometric (a-b) or arithmetic (c-g) means for each group. Whiskers indicate 95% confidence intervals (CI's; a-b) or standard errors of means (SEMs; c-g). Every symbol represents one animal. Horizontal dotted lines mark the LLODs. Values below the LLOD set to ½ the LLOD. Asterisks below the x-axis indicate the day of Dose 2.

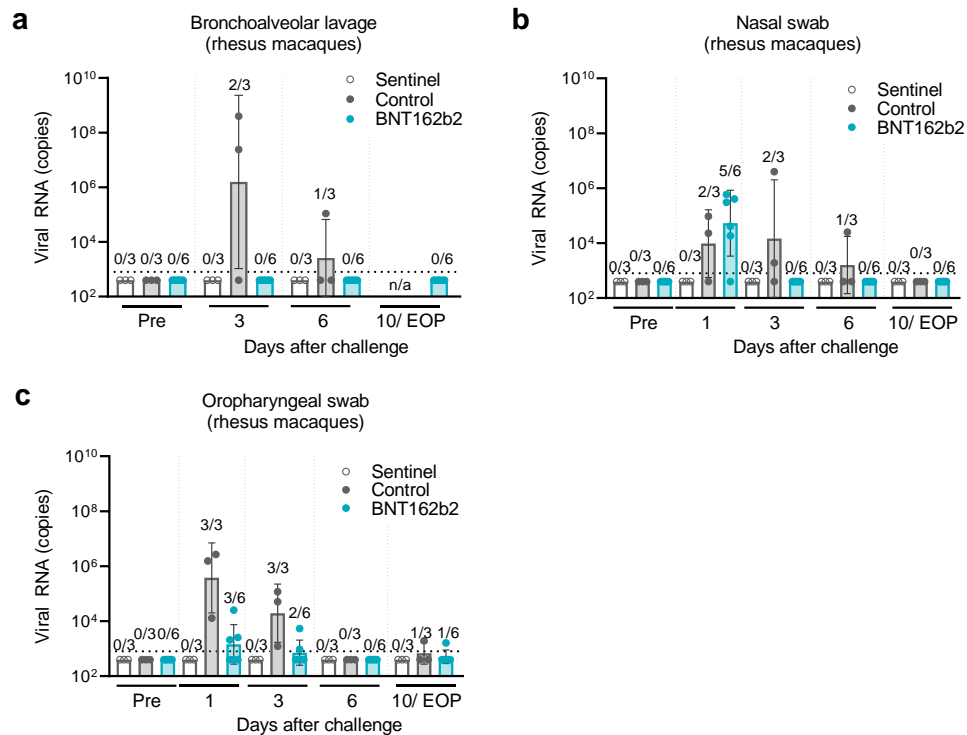
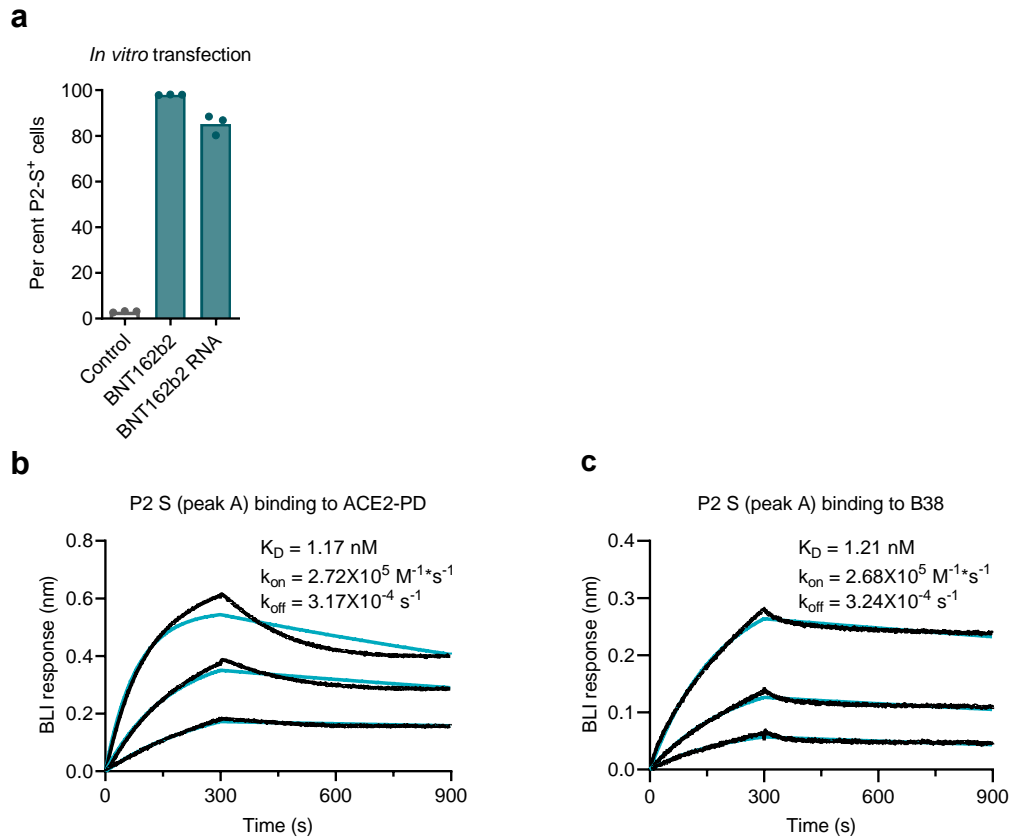


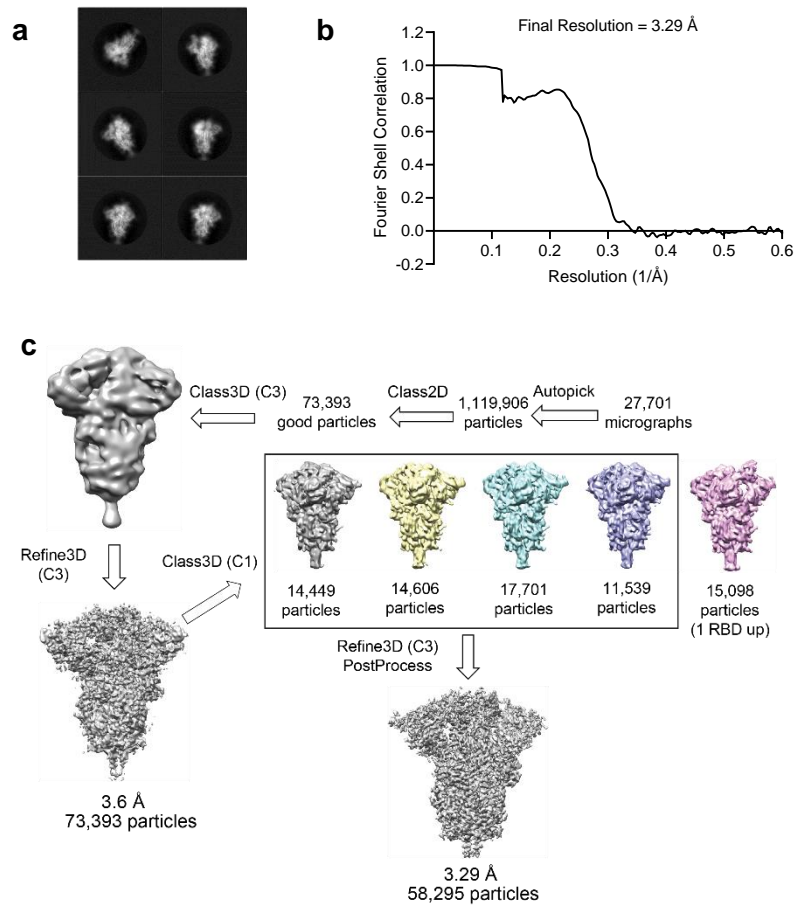
Figure 4. Protection of rhesus macaques from challenge with infectious SARS-CoV-2.

Fifty-five days after the Dose 2 of 100 μ g BNT162b2 (n=6) or saline control (n=3), rhesus macaques were challenged with 1.05×10^6 total pfu of SARS-CoV-2 split equally between the IN and IT routes. Non-immunised rhesus macaques (n=3) were mock-challenged with cell culture medium (sentinel). Viral RNA levels were detected by RT-qPCR. BAL was performed and nasal and oropharyngeal (OP) swabs obtained at the indicated time points. Final collection of samples was on Day 10 relative to challenge for the sentinel and control groups and at the end of protocol (EOP) on Day 7 or 8 for the BNT162b2-immunised group. Ratios above data points indicate the number of viral RNA positive animals among all animals per group. Heights of bars indicate geometric means. Whiskers indicate geometric standard deviations. Every symbol represents one animal. Dotted lines indicate the lower limits of detection (LLOD). Values below the LLOD were set to $\frac{1}{2}$ the LLOD. **a**, Viral RNA in bronchoalveolar lavage (BAL) fluid. **b**, Viral RNA in nasal swabs. **c**, Viral RNA in OP swabs. The statistical significance by a non-parametric test (Friedman's test) of differences in viral RNA detection between control-immunised and BNT162b2-immunised animals after challenge was $p=0.0014$ for BAL fluid, $p=0.2622$ for nasal swabs, and $p=0.0007$ for OP swabs. n/a – not available.

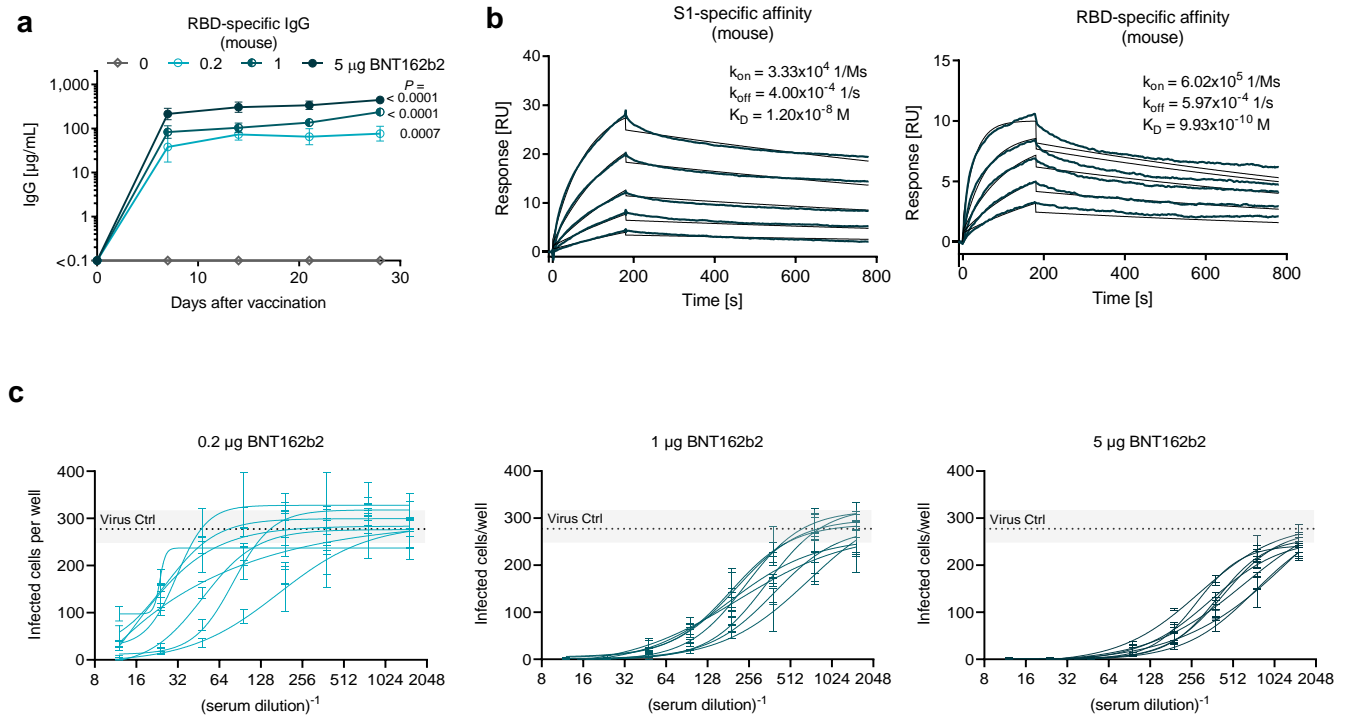


Extended Data Figure 1. P2 S expression and receptor affinity.

a, Fraction of HEK293T/17 cells expressing P2 S determined by flow cytometry after incubation with BNT162b2 RNA formulated as LNP (BNT162b2), BNT162b2 RNA formulated with a transfection reagent (BNT162b2 RNA), or no RNA (Control), determined by flow cytometry. **b**, **c**, P2 S with a C-terminal TwinStrep tag, expressed in Expi293F cells, was detergent solubilized and purified by affinity and size exclusion chromatography. Protein from the first peak of a size exclusion column, containing intact P2 S and dissociated S1 and S2 fragments, was assayed by biolayer interferometry. Sensorgram of the binding kinetics of TwinStrep-tagged P2 S to immobilised **b**, human ACE2-PD and **c**, B38 monoclonal antibody.

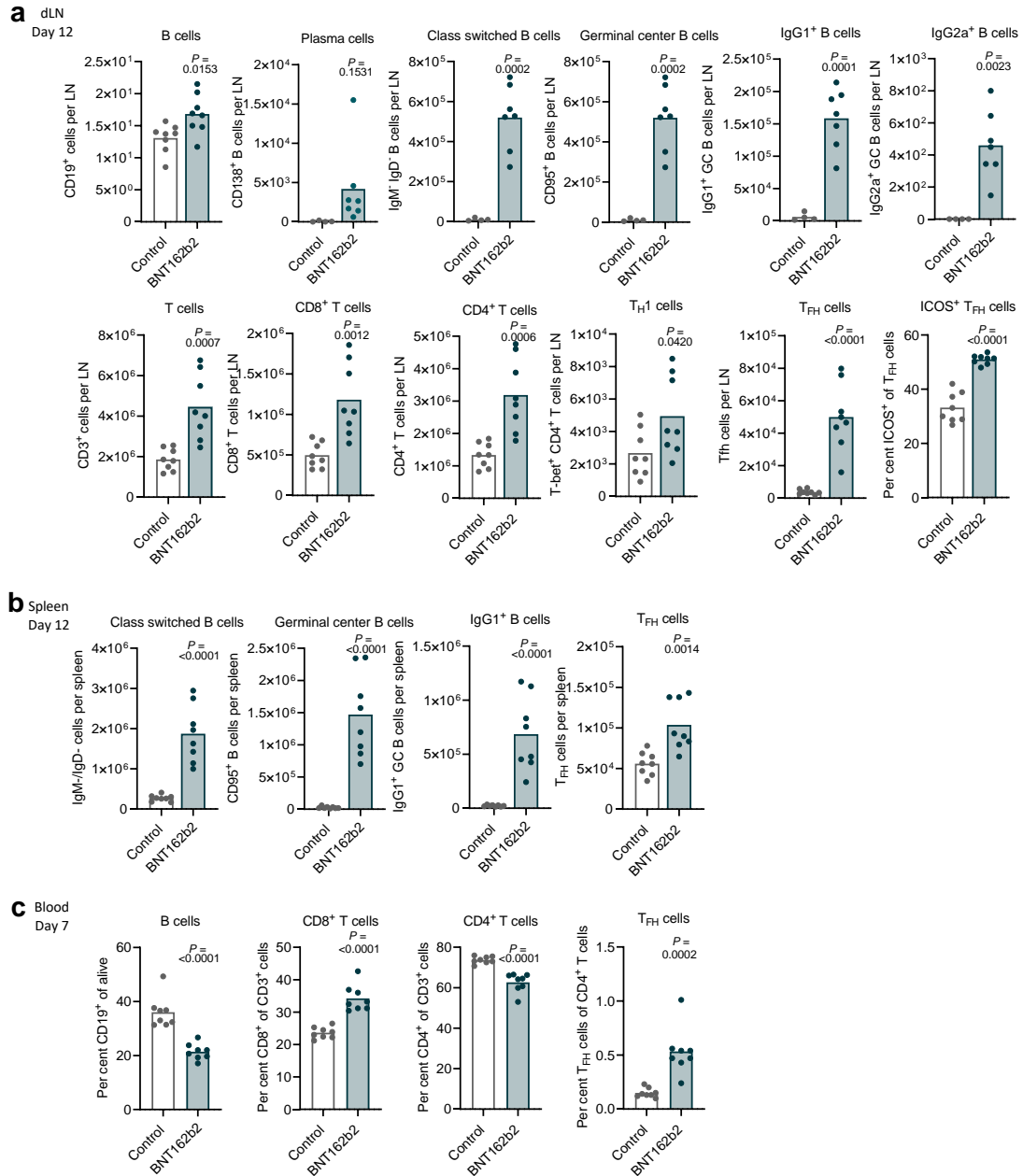


Extended Data Figure 2. Structure analysis of BNT162b2-encoded P2 S by cryo-electron microscopy
a, 2D class averages of TwinStrep-tagged P2 S particles extracted from cryo-EM micrographs.. Box edge: 39.2 nm in each dimension. **b**, Fourier shell correlation curve from RELION gold-standard refinement of the P2 S trimer. **c**, Flowchart for cryo-EM data processing of the complex.



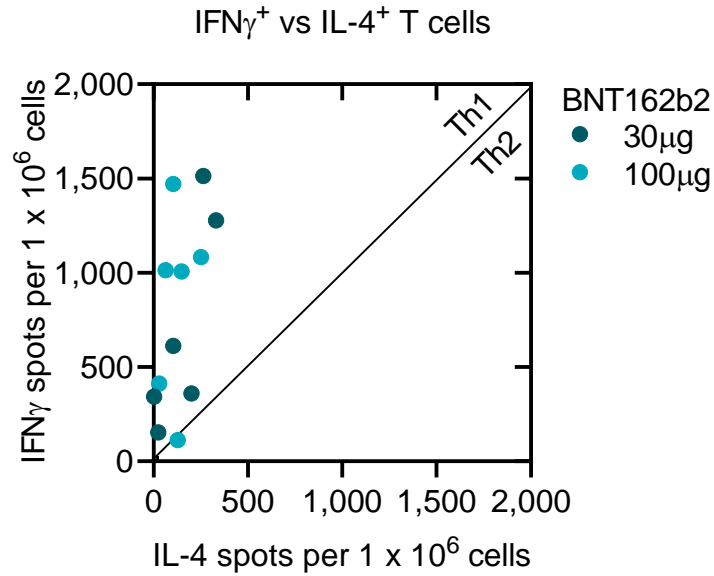
Extended Data Figure 3. BNT162b2-elicited antibody responses in mice.

BALB/c mice (n=8 per group) were immunised intramuscularly (IM) with 0.2, 1 or 5 µg of BNT162b2 or buffer. **a**, RBD-binding IgG responses in sera obtained 7, 14, 21 and 28 days after immunisation, determined by ELISA. For day 0, a pre-screening of randomised animals was performed (n=4). Geometric mean of each group is shown. **b**, Representative surface plasmon resonance sensorgram of the binding kinetics of recombinant S1 (*left*) and RBD (*right*) to immobilised mouse IgG from serum 28 days after immunisation with 5 µg BNT162b2 (n=8). Actual binding (dark blue) and the best fit of the data to a 1:1 binding model (thin line in black). **c**, Number of infected cells per well in pseudovirus neutralisation assays conducted with serially diluted mouse serum samples obtained 28 days after immunisation with BNT162b2 are shown (n=8 per group, see also Figure 2b).



Extended Data Figure 4. B-cell and T-cell phenotyping in lymph nodes, spleen and blood of BNT162b2 immunised mice.

Mice (n=8 per group) were immunised with 5 µg BNT162b2 or buffer (Control). P-values were determined by an unpaired two-tailed t-test. **a+b**, B-cell and T-cell numbers 12 days after immunisation in (a) draining lymph nodes (dLN; for B-cell subtyping only: n=4 for control, n=7 for BNT162b2 immunised group) or (b) the spleen, determined by flow cytometry. The percentage of ICOS⁺ cells among T follicular helper cells (T_{FH}) in dLNs is depicted on the lower right in (a). **c**, B- and T-cell fractions in the blood seven days after immunisation.



Extended Data Figure 5. Scatterplot of IL-4 vs. IFN γ ELISpot of S peptide stimulated PBMCs collected on Day 42.

Rhesus macaques (n=6 per group) were immunised on Days 0 and 21 with 30 μ g or 100 μ g BNT162b2 (see Fig. 3c,d) and individual animal values are shown.

Extended Data Table 1. Electron cryomicroscopy data collection, 3D reconstruction and refinement statistics.

Data collection		
EM equipment	Titan Krios (Thermo Fisher Scientific)	
Voltage (keV)	300	
Detector	K2 Summit	
Energy filter	Gatan GIF, 20 eV slit	
Nominal Magnification	165,000 x	
Pixel size (Å)	0.435 (super-resolution)	
	Grid 1	Grid 2
Electron dose ($e^-/\text{Å}^2$)	50.32	50.12
Dose rate ($e^-/\text{Å}^2/\text{sec}$)	8.4	8.33
Defocus range (μm)	-1.2 to -3.4	-1.2 to -3.4
Number of collected micrographs	10422	17279
3D Reconstruction		
Software	Warp, Relion	
Number of used particles		
Symmetry imposed	C3	
Global Resolution (Å)		
FSC 0.143	3.29	
Applied B factor (Å^2)	-50	
Refinement		
Software	Phenix, Coot	
Protein residues	22,051	
Map Correlation Coefficient	0.82	
RMSD		
Bond length (Å)	0.011	
Bond angles (°)	0.962	
Ramachandran plot statistics (%):		
Preferred	90.4	
Allowed	9.59	
Outlier	0	
Poor Rotamers (%)	11.06	
MolProbity Score	2.96	
Clashscore (all atoms)	13.23	