

1 The BNT162b2 mRNA vaccine against SARS-CoV-2 reprograms both adaptive and 2 innate immune responses

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NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.

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35

36

37 **Summary**

38

39 The mRNA-based BNT162b2 vaccine from Pfizer/BioNTech was the first registered COVID-19
40 vaccine and has been shown to be up to 95% effective in preventing SARS-CoV-2 infections.

41 Little is known about the broad effects of the new class of mRNA vaccines, especially whether
42 they have combined effects on innate and adaptive immune responses. Here we confirmed

43 that BNT162b2 vaccination of healthy individuals induced effective humoral and cellular
44 immunity against several SARS-CoV-2 variants. Interestingly, however, the BNT162b2 vaccine

45 also modulated the production of inflammatory cytokines by innate immune cells upon
46 stimulation with both specific (SARS-CoV-2) and non-specific (viral, fungal and bacterial) stimuli.

47 The response of innate immune cells to TLR4 and TLR7/8 ligands was lower after BNT162b2
48 vaccination, while fungi-induced cytokine responses were stronger. In conclusion, the mRNA

49 BNT162b2 vaccine induces complex functional reprogramming of innate immune responses,
50 which should be considered in the development and use of this new class of vaccines.

51

52

53 **Keywords:** COVID-19, coronaviruses, mRNA vaccines, trained immunity, innate immune

54 tolerance

55

56 **Main text**

57

58 Coronavirus disease 2019 (COVID-19) is a new respiratory tract infection caused by the severe
59 acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which spread worldwide since the end
60 of 2019 causing a global pandemic. The COVID-19 pandemic represents the most important
61 healthcare crisis humanity has encountered since World War II, combined with a devastating
62 societal and economic impact. Confronted with this critical situation, a major effort to develop
63 vaccines against COVID-19 has been initiated in many countries around the world.

64

65 To date, 13 vaccines have been approved for use in humans (“COVID-19 vaccine tracker |
66 RAPS,” n.d.). The scale of the pandemic has led to accelerated development of vaccines based
67 on new technologies, such as mRNA- and viral vector-based vaccines (van Riel & de Wit, 2020).
68 One of the most widely used anti-COVID-19 vaccines in the world was developed by a
69 collaboration between BioNTech and Pfizer (BNT162b2). This vaccine is based on a lipid
70 nanoparticle–formulated, nucleoside-modified mRNA that encodes a prefusion stabilized form
71 of the spike (S)-protein derived from the SARS-CoV-2 strain isolated early on in Wuhan, China
72 (Walsh et al., 2020). Several phase-3 trials have demonstrated that BNT162b2 elicits broad
73 humoral and cellular responses, providing protection against COVID-19 (Sahin et al., 2020;
74 Walsh et al., 2020).

75

76 While global vaccination campaigns against the SARS-CoV-2 infection are rolled out, major
77 challenges remain, especially the spread of novel virus variants (Madhi et al., 2021). One of the
78 most prominent mutations during the pandemic has been the spike D614G substitution to the
79 Wuhan Hu-1 original strain (Korber et al., 2020). With the steadily increasing prevalence of

80 infections, SARS-CoV-2 variants emerged with multiple spike mutations and were first detected
81 in the United Kingdom (B.1.1.7 lineage), South Africa (B.1.351 lineage), and Brazil (P.1 lineage).
82 These variants are of significant concern because of their potential effects on disease severity,
83 viral transmissibility, reinfection rates, and vaccine effectiveness (Abdool Karim & de Oliveira,
84 2021).

85
86 The capacity of BNT162b2 to induce effective humoral and cellular immunity against the new
87 SARS-CoV-2 variants is only now beginning to be understood. Whereas neutralization of B.1.1.7
88 was either similar or just slightly reduced as compared to the standard strain (Muik et al., 2021;
89 Wang, Nair, et al., 2021), neutralizing titers of B.1.351 were markedly diminished (Liu et al.,
90 2021; Planas et al., 2021; Wang, Nair, et al., 2021) after vaccination of healthy volunteers with
91 BNT162b2. In contrast, cellular immunity against the virus variants seems to be less affected
92 (Lilleri et al., 2021; Skelly et al., 2021). In addition, an unexplored area is whether vaccination
93 with BNT162b2 also leads to long-term effects on innate immune responses: this could be very
94 relevant in COVID-19, in which dysregulated inflammation plays an important role in the
95 pathogenesis and severity of the disease (Tahaghoghi-Hajghorbani et al., 2020). The long-term
96 modulation of innate immune responses has been an area of increased interest in the last
97 years: multiple studies have shown that long-term innate immune responses can be either
98 increased (*trained immunity*) or down-regulated (*innate immune tolerance*) after certain
99 vaccines or infections (Netea et al., 2020).

100
101 In this study, we assessed the effect of the BNT162b2 mRNA COVID-19 vaccine on both the
102 innate and adaptive (humoral and cellular) immune responses. We first examined the
103 concentration of RBD- and S-binding antibody isotype concentrations before vaccination

104 (baseline; t1), 3 weeks after the first dose of 30 µg of BNT162b2 (t2), and two weeks after the
105 second dose (t3) (Figure S1A). We calculated fold-changes by comparing concentrations from
106 both t2 and t3 to baseline. BNT162b2 vaccination elicited high anti-S protein and anti-RBD
107 antibody concentrations already after the first vaccination, and even stronger responses after
108 the second dose of the vaccine. As expected, IgG responses were the most pronounced, with
109 RBD-specific median fold changes at t2 and t3 of 56-fold and 1839-fold, and S-specific fold
110 changes of 208-fold and 1100-fold, respectively. The lowest observed fold change increase to
111 pre-vaccination levels of IgG targeting RBD was 14-fold at t2, and 21-fold at t3, respectively. For
112 S-specific IgG, the fold changes were at least 32-fold at t2 and 339-fold at t3. Regarding IgA
113 concentrations, a single dose of the vaccine elicited a 7-fold increase in the RBD-specific
114 concentration and a 35-fold increase in the S-specific concentration. The second dose
115 enhanced the antibody concentrations elicited by the first vaccination by 24-fold and 52-fold,
116 for RBD and S, respectively. Compared to IgG and IgA, the increase in IgM concentrations was
117 considerably lower. RBD-specific concentration only doubled after the first dose, and it did not
118 further increase after the second dose of the vaccine. In contrast, S-specific fold changes were
119 11-fold at t2 and 20-fold at t3 (Figure S1A). These results confirm and extend recent
120 observations reporting strong induction of humoral responses by BNT162b2 vaccination (Sahin
121 et al., 2020).

122

123 To investigate the neutralizing capacity of the serum against SARS-CoV-2 variants, we
124 performed 50% plaque reduction neutralization testing (PRNT50) using sera collected two
125 weeks after the second vaccine administration. All the serum samples neutralized the D614G
126 strain and the B.1.1.7 variant with titers of at least 1:80. However, six subjects (37,5%) had titers
127 lower than 1:80 against the B.1.351 variant. Geometric mean neutralizing titers against the

128 D614G strain, B.1.1.7 and B.1.351 were 381, 397, and 70, respectively (Figure S1B, $p < 0.001$).
129 Similar to our investigation, several studies reported 6 to 14-fold decreased neutralizing activity
130 of post-vaccine sera against the B.1.351 variant, and only slightly reduced activity against
131 B.1.1.7, when compared to the standard strain (Planas et al., 2021; Shen et al., 2021; Wang,
132 Liu, et al., 2021). These data support the evidence that B.1.351, and possibly other variants,
133 may be able to escape vaccine-induced humoral immunity to a certain extent (Kustin et al.,
134 2021). Furthermore, the PRNT titer and the antibody concentrations of IgG after the second
135 dose were strongly correlated (Figure S1C). The correlation was stronger for B.1.1.7 and B.1.351
136 than for the standard strain, both for anti-RBD and anti-S.

137
138 BNT162b2 vaccination has been reported to activate virus-specific CD4+ and CD8+ T cells, and
139 upregulate the production of immune-modulatory cytokines such as IFN- γ (Sahin et al., 2020).
140 Hence, we assessed IFN- γ secretion from peripheral blood mononuclear cells (PBMCs) before
141 and after BNT162b2 vaccination in response to heat-inactivated SARS-CoV-2 strains (Figs 2A-
142 2D). While vaccination with BNT162b2 generally seems to moderately increase specific IFN- γ
143 production after the second dose of the vaccine, this reached statistical significance only upon
144 stimulation with B.1.351 variant (Figure S2A-2C). The same tendency has also been observed
145 by Tarke et al. who used synthetic SARS-CoV-2 variants proteins to induce elevated IFN- γ
146 responses against B.1.351 (Tarke et al., 2021). IFN- γ production was higher by at least 50% in
147 37.5% of the subjects upon stimulation with the standard SARS-CoV-2 strain, in 50% of the
148 subjects upon stimulation with the B.1.1.7 variant and the B.1.351 variant, but only in 18,75%
149 of the subjects upon stimulation with the Bavarian variant (Figure S1D). These findings argue
150 that BNT162b2 vaccination induces better humoral than cellular immune responses. Weak T-
151 cell responses have previously been reported in vaccinees that have received just a single dose

152 of BNT162b2 (Predecki et al., 2021; Stankov, Cossmann, Bonifacius, Dopfer-jablonka, &
153 Morillas, 2021). Intriguingly, the best cellular responses after vaccination were against the
154 B.1.351 variant: the fact that the neutralizing antibody responses against this variant were
155 relatively poor, that may raise the possibility that the protective BNT162b2 vaccine effects
156 against this variant may be mainly reliant on cellular, rather than humoral, responses. No
157 significant differences between the individual variants were observed. The absolute
158 concentrations of the cytokines after stimulations can be found in Supplementary Table 2.

159
160 Interestingly, we observed important heterologous effects of BNT162b2 vaccination on IFN- γ
161 production induced by other stimuli as well (Figures S2E, 2F). BNT162b2 vaccination decreased
162 IFN- γ production upon stimulation with the TLR7/8 agonist R848 (Figure S2F). In contrast, the
163 IFN- γ production induced by inactivated influenza virus tended to be higher two weeks after
164 the second BNT162b2 vaccination, though the differences did not reach statistical significance.
165 We did not find any significant correlation between cellular responses and IgG antibody titers.

166
167 Besides their effects on specific (adaptive) immune memory, certain vaccines such as Bacillus
168 Calmette-Guérin (BCG) and the measles, mumps, and rubella (MMR) vaccine also induce long-
169 term functional reprogramming of cells of the innate immune system. (Netea et al., 2020). This
170 biological process is also termed *trained immunity* when it involves increased responsiveness,
171 or *innate immune tolerance* when it is characterized by decreased cytokine production (Ifrim
172 et al., 2014). Although these effects have been proven mainly for live attenuated vaccines, we
173 sought to investigate whether the BNT162b2 vaccine might also induce effects on innate
174 immune responses against different viral, bacterial and fungal stimuli. One of the trademarks
175 of trained immunity is an elevated production of inflammatory cytokines following a secondary

176 insult (Quintin et al., 2012). Surprisingly, the production of the monocyte-derived cytokines
177 TNF- α , IL-1 β and IL-1Ra tended to be lower after stimulation of PBMCs from vaccinated
178 individuals with either the standard SARS-CoV-2 strain or heterologous Toll-like receptor
179 ligands (Figures 1 and 2). TNF- α production (Figure 1B-1G) following stimulation with the
180 TLR7/8 agonist R848 of peripheral blood mononuclear cells from volunteers was significantly
181 decreased after the second vaccination (Figure 1C). The same trend was observed after
182 stimulation with the TLR3 agonist poly I:C (Figure 1D), although the difference did not reach
183 statistical significance. In contrast, the responses to the fungal pathogen *Candida albicans* were
184 higher after the first dose of the vaccine (Figure 1G). The impact of the vaccination on IL-1 β
185 production was more limited (Figure 2A-2F), though the response to *C. albicans* was
186 significantly increased (Figure 2F). The production of the anti-inflammatory cytokine IL-1Ra
187 (Figure 2G-2L) was reduced in response to bacterial lipopolysaccharide (LPS) and *C. albicans*
188 after the second vaccination (Figure 2K, 2L), which is another argument for a shift towards
189 stronger inflammatory responses to fungal stimuli after vaccination. IL-6 responses were
190 similarly decreased, though less pronounced (data not shown).

191
192 The induction of tolerance towards stimulation with TLR7/8 (R848) or TLR4 (LPS) ligands by
193 BNT162b2 vaccination may indicate a more balanced inflammatory reaction during infection
194 with SARS-CoV-2, and one could speculate whether such effect may be thus useful to regulate
195 the potential over-inflammation in COVID-19, one of the main causes of death (Tang et al.,
196 2020). On the other hand, inhibition of innate immune responses may diminish anti-viral
197 responses. Type I interferons also play a central role in the pathogenesis and response against
198 viral infections, including COVID-19 (Hadjadj et al., 2020). With this in mind, we also assessed
199 the production of IFN- α by immune cells of the volunteers after vaccination. Although the

200 concentrations of IFN- α were below the detection limit of the assay for most of the stimuli, we
201 observed a significant reduction in the production of IFN- α secreted after stimulation with poly
202 I:C and R848 after the administration of the second dose of the vaccine (Figure 1H, 1I). This
203 may hamper the initial innate immune response against the virus, as defects in TLR7 have been
204 shown to result in and increased susceptibility to COVID-19 in young males (Van Der Made et
205 al., 2020). These results collectively demonstrate that the effects of the BNT162b2 vaccine go
206 beyond the adaptive immune system and can also modulate innate immune responses.

207
208 The effect of the BNT162b2 vaccination on innate immune responses may also indicate a
209 potential to interfere with the responses to other vaccinations, as known for other vaccines to
210 be as ‘vaccine interference’ (Lum et al., 2010; Nolan et al., 2008; Vajo, Tamas, Sinka, &
211 Jankovics, 2010). Future studies are therefore needed to investigate this possibility, especially
212 the potential interaction with the influenza vaccine: in the coming years (including the autumn
213 of 2021) COVID-19 vaccination programs will probably overlap with the seasonal Influenza
214 vaccination, so it is crucial to perform additional studies to elucidate the potential interactions
215 and effects of the COVID-19 vaccines with the current vaccination schedules, especially for
216 immunosuppressed and elderly individuals.

217
218 The generalizability of these results is subject to certain limitations. First, the number of
219 volunteers in this study was relatively small, although in line with earlier immunological studies
220 on the effects of COVID-19 vaccines. Second, our cohort consisted of healthcare workers, who
221 are middle-aged and healthy, and future studies in elderly individuals and people with
222 comorbidities and other underlying risk factors for severe COVID-19 infections need to be
223 performed (Gao et al., 2021). Third, our study is performed only with individuals with a Western

224 European ancestry. Therefore, the conclusions of our study should be tested in populations
225 with different ancestry and alternative lifestyles since the induction of innate and adaptive
226 immune responses is largely dependent on different factors such as genetic background, diet,
227 and exposure to environmental stimuli which largely differ between communities around the
228 globe.

229

230 In conclusion, our data show that the BNT162b2 vaccine induces effects on both the adaptive
231 and the innate branch of immunity and that these effects are different for various SARS-CoV-2
232 strains. Intriguingly, the BNT162b2 vaccine induces reprogramming of innate immune
233 responses as well, and this needs to be taken into account: in combination with strong adaptive
234 immune responses, this could contribute to a more balanced inflammatory reaction during
235 COVID-19 infection, or it may contribute to a diminished innate immune response towards the
236 virus. BNT162b2 vaccine is clearly protective against COVID-19, but the duration of this
237 protection is not yet known, and one could envisage future generations of the vaccine
238 incorporating this knowledge to improve the range and duration of the protection. Our findings
239 need to be confirmed by conducting larger cohort-studies with populations with diverse
240 backgrounds, while further studies should examine the potential interactions between
241 BNT162b2 and other vaccines.

242

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244

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252

253 **Author contributions**

254 Conceptualization: M.G.N, F.K.F, J.t.O, J.H., R.v.C, J.v.d.M., F.v.d.V. and L.A.B.J.; Clinical
255 Investigation: F.K.F.; Experimental work: J.D-A, B.G., G.K., O.B., E.S., B.L.H., G.J.O., C.G.v.K.,
256 H.D., H.L., S.A.S, M.R.; Supervision: R.P.v.R., M.I.d.J., J.D-A. and M.G.N. Writing and correction
257 of the manuscript: all authors.

258

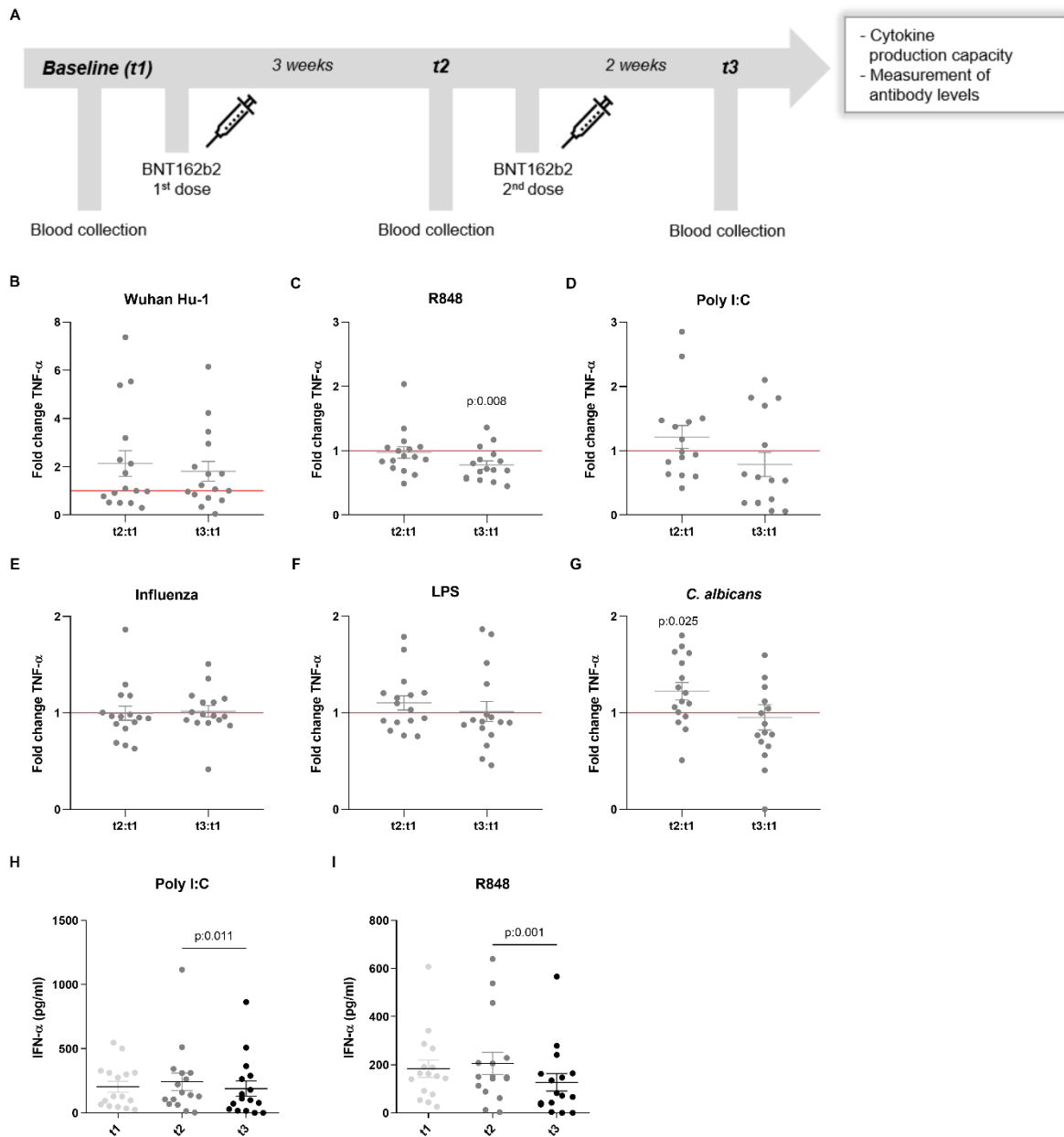
259 **Declaration of Interests**

260 M.G.N and L.A.B.J are scientific founders of Trained Therapeutix and Discovery.

261

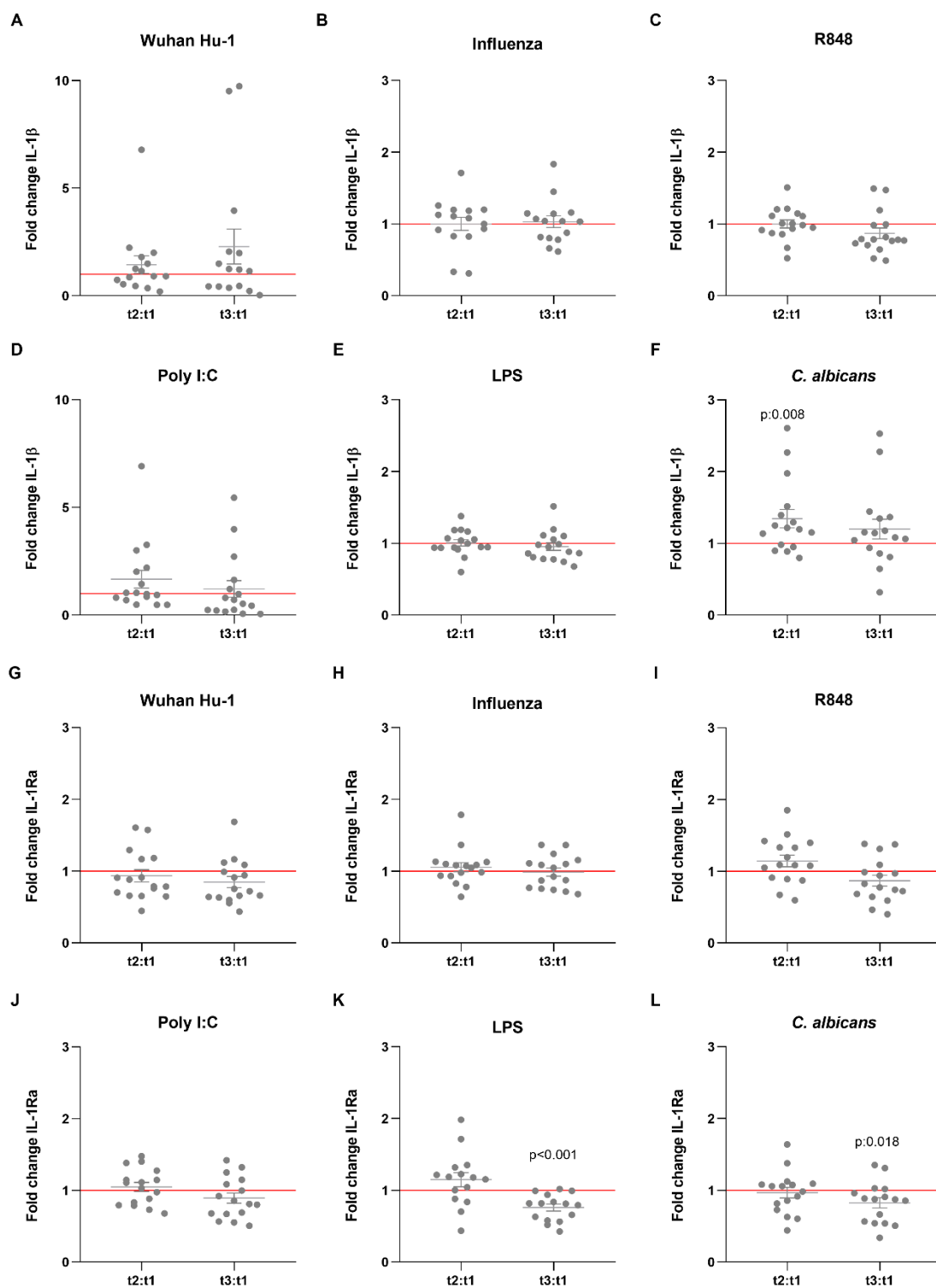
262

263 Main figures



264

265 **Figure 1. TNF- α and IFN- α production in response to heterologous stimuli in PBMCs isolated**
266 **from vaccinated subjects. (A) Description of the study: vaccination and blood collection days.**
267 (B-G) Fold change values of TNF- α production are calculated individually for each subject by
268 division of t2:t1 and t3:t1. Data are presented as fold changes \pm SEM (n=15-16) and analysed
269 by Wilcoxon's matched-pairs signed-rank test comparing each ratio to t1=1 (red line). (H-I)
270 IFN- α production (pg/ml) at t1, t2 and t3. Data are presented as cytokine concentration \pm SEM
271 (n=15-16) and analysed by Wilcoxon's matched-pairs signed-rank test.



272

273 **Figure 2. IL-1 β and IL-1Ra production in response to heterologous stimuli in PBMCs isolated**
274 **from vaccinated subjects. IL-1 β (A-F) and IL-1Ra (G-L) Fold change values are calculated**
275 **individually for each subject by division of t2:t1 and t3:t1. Data are presented as fold changes**
276 **\pm SEM (n=15-16) and analysed by Wilcoxon's matched-pairs signed-rank test comparing each**
277 **ratio to t1=1 (red line).**

278 **Methods**

279

280 **Resource availability**

281 **Lead contact**

282 Further information and requests for resources and reagents should be directed to and will be
283 fulfilled by the Lead Contact Mihai G. Netea (mihai.netea@radboudumc.nl).

284

285 **Materials availability**

286 This study did not generate new unique reagents.

287

288 **Data and code availability**

289 Data from this study are available upon request.

290

291 **Experimental model and subject details**

292 **Human subject collection**

293 The study was conducted in compliance with ethical principles of the Declaration of Helsinki,
294 approved by the Arnhem-Nijmegen Institutional Review Board (protocol NL76421.091.21) and
295 registered in the European Clinical Trials Database (2021-000182-33). Health care workers from
296 the Radboudumc Nijmegen were enrolled who received the BNT162b2 mRNA Covid-19 vaccine
297 as per national vaccination campaign and provided informed consent. Subjects (n = 16) were
298 26-59 years of age, both male and female, and healthy (demographic data presented in
299 Supplementary Table 1). Key exclusion criteria included a medical history of COVID-19. Sera and
300 blood samples were collected before the first administration of BNT162b2, three weeks after
301 the first dose (right before the second dose), and two weeks after the second dose. A high

302 percentage (56.3%) of individuals had been vaccinated with BCG in the past 12 months due to
303 the fact that many participants participated in parallel in a BCG-trial. One individual was
304 removed from the dataset after detecting high concentrations of antibodies against SARS-CoV-
305 2 N-antigen at baseline.

306

307 **Virus isolation and sequencing**

308 Viruses were isolated from diagnostic specimen at the department of Viroscience, Erasmus MC,
309 and subsequently sequenced to rule out additional mutations in the S protein: D614G
310 (BetaCoV/Munich/BavPat1/2020, European Virus Archive 026V-03883), B.1.1.7 (GISAID: hCov-
311 465 19/Netherlands/ZH-EMC-1148) and B.1.351 (GISAID: hCov-19/Netherlands/ZH-EMC-
312 1461). SARS-CoV-2 isolate BetaCoV/Munich/BavPat1/2020 (European Virus Archive 026V-
313 03883), was kindly provided by Prof. C. Drosten. SARS-CoV-2 Wuhan Hu-1 strain was kindly
314 provided by Prof. Heiner Schaal (Dusseldorf University, Germany). The B.1.1.7 and B.1.351
315 isolates were isolated from diagnostic specimens on Calu-3 lung adenocarcinoma cells for three
316 passages. Passage 3 BavPat1, B.1.1.7 and B.1.351 variants were used to infect Vero E6 cells at
317 an MOI of 0.01 in DMEM, high glucose (Thermo Fisher Scientific, USA, cat #11965092)
318 supplemented with 2% fetal bovine serum (Sigma-Aldrich, Germany, cat #F7524), 20 mM
319 HEPES (Thermo Fisher Scientific, USA, cat #15630056) and 50 U/mL penicillin-50 µg/mL
320 streptomycin (Thermo Fisher Scientific, USA, cat #15070063) at 37 °C in a humidified 5% CO₂
321 incubator. At 72 h post infection, the culture supernatant was centrifuged for 5 min at 1500 x
322 g and filtered through an 0.45 µm low protein binding filter (Sigma-Aldrich, Germany, cat
323 #SLHPR33RS). To further purify the viral stocks, the medium was transferred over an Amicon
324 Ultra-15 column with 100 kDa cutoff (Sigma-Aldrich, Germany, cat #UFC910008), which was
325 washed 3 times using Opti-MEM supplemented with GlutaMAX (Thermo Fisher Scientific, USA,

326 cat #51985034). Afterwards the concentrated virus on the filter was diluted back to the original
327 volume using Opti-MEM and the purified viral aliquots were stored at -80 °C. The infectious
328 viral titers were measured using plaque assays as described (Varghese et al., 2021) and stocks
329 were heat inactivated for 60 min at 56 °C for use in stimulation experiments.

330

331 **Measurement of antibody levels against RBD and Spike protein**

332 To measure the levels of antibodies against RBD and Spike protein, a fluorescent-bead-based
333 multiplex immunoassay (MIA) was developed as previously described by (Fröberg et al., 2021).
334 The stabilized pre-fusion conformation of the ectodomain of the Spike protein (amino acids 1
335 – 1,213) fused with the trimerization motif GCN4 (S-protein) and the receptor binding domain
336 of the S-protein (RBD) were each coupled to beads or microspheres with distinct fluorescence
337 excitation and emission spectra. Serum samples were diluted and incubated with the antigen-
338 coupled microspheres. Following incubation, the microspheres were washed and incubated
339 with phycoerythrin-conjugated goat anti-human, IgG, IgA and IgM. The data were acquired on
340 the Luminex FlexMap3D System. Mean Fluorescent Intensities (MFI) were converted to
341 arbitrary units (AU/ml) by interpolation from a log-5PL-parameter logistic standard curve and
342 log-log axis transformation, using Bioplex Manager 6.2 (Bio-Rad Laboratories) software.

343

344 **Plaque reduction neutralization assay**

345 A plaque reduction neutralization test (PRNT) was performed. Viruses used in the assay were
346 isolated from diagnostic specimen at the department of Viroscience, Erasmus MC, cultured
347 and subsequently sequenced to rule out additional mutations in the S protein: D614G
348 (GISAID: hCov-19/Netherlands/ZH-EMC-2498), B.1.1.7 (GISAID: hCov-19/Netherlands/ZH-
349 EMC-1148) and B.1.351 (GISAID: hCov-19/Netherlands/ZH-EMC-1461). Heat-inactivated sera

350 were 2-fold diluted in Dulbecco modified Eagle medium supplemented with NaHCO₃, HEPES
351 buffer, penicillin, streptomycin, and 1% fetal bovine serum, starting at a dilution of 1:10 in 60
352 µL. We then added 60 µL of virus suspension (400 plaque-forming units) to each well and
353 incubated at 37°C for 1h. After 1 hour incubation, we transferred the mixtures on to Vero-E6
354 cells and incubated for 8 hours. After incubation, we fixed the cells with 10% formaldehyde
355 and stained the cells with polyclonal rabbit anti-SARS-CoV antibody (Sino Biological) and a
356 secondary peroxidase-labeled goat anti-rabbit IgG (Dako). We developed signal by using a
357 precipitate forming 3,3',5,5'-tetramethylbenzidine substrate (True Blue; Kirkegaard and Perry
358 Laboratories) and counted the number of infected cells per well by using an ImmunoSpot
359 Image Analyzer (CTL Europe GmbH). The serum neutralization titer is the reciprocal of the
360 highest dilution resulting in an infection reduction of >50% (PRNT₅₀). We considered a titer
361 >20 to be positive based on assay validation

362

363 **Isolation of peripheral blood mononuclear cells**

364 Blood samples from subjects were collected into EDTA-coated tubes (BD Bioscience, USA) and
365 used as the source of peripheral blood mononuclear cells (PBMCs) after sampling sera from
366 each individual. Blood is diluted 1:1 with PBS (1X) without Ca⁺⁺, Mg⁺⁺ (Westburg, The
367 Netherlands, cat #LO BE17-516F) and PBMCs were isolated via density gradient centrifuge
368 using Ficoll-PaqueTM-plus (VWR, The Netherlands, cat #17-1440-03P). The tubes used for the
369 isolation was specialized SepMate-50 tubes (Stem Cell Technologies, cat #85450) to ensure
370 better separation. Cells counts were determined via Sysmex XN-450 hematology analyzer.
371 Afterwards, PBMCs were frozen using Recovery Cell Culture Freezing (Thermo Fisher
372 Scientific, USA, cat #12648010) in the concentration of 15x10⁶/mL.

373

374 **Simulation experiments**

375 The PBMCs were thawed and washed with 10mL Dutch modified RPMI 1640 medium (Roswell
376 Park Memorial Institute; Invitrogen, USA, cat # 22409031) containing 50 µg/mL Gentamicine
377 (Centrafarm, The Netherlands), 1 mM Sodium-Pyruvate (Thermo Fisher Scientific, USA, cat
378 #11360088), 2 mM Glutamax (Thermo Fisher Scientific, USA, cat #35050087) supplemented
379 with 10% Bovine Calf Serum (Fisher Scientific, USA, cat #11551831) twice, and afterwards the
380 cells were counted via Sysmex XN-450. PBMCs (4×10^5 cells/well) stimulated in sterile round
381 bottom 96-well tissue culture treated plates (VWR, The Netherlands, cat #734-2184) in Dutch
382 modified RPMI 1640 medium containing 50 µg/mL Gentamicine, 1 mM Sodium-Pyruvate, 2
383 mM Glutamax supplemented with 10% human pooled serum. Stimulations were done with
384 heat-inactivated SARS-CoV-2 Wuhan Hu-1 strain (3.3×10^3 TCID₅₀/mL), SARS-CoV-2 B.1.1.7
385 (3.3×10^3 TCID₅₀/mL), SARS-CoV-2 B.1.351 (3.3×10^3 TCID₅₀/mL), and SARS-CoV-2 Bavarian
386 (3.3×10^3 TCID₅₀/mL) variants, Influenza (3.3×10^5 TCID₅₀/mL), 10 µg/mL Poly I:C (Invivogen,
387 USA, cat #tlrl-pic), 3 µg/mL R848 (Invivogen, USA, cat #tlrl-r848), 10 ng/mL *E. coli* LPS, and $1 \times$
388 10^6 /mL *C. albicans*. The PBMCs were incubated with the stimulants for 24 hours to detect IL-
389 1β, TNF-α, IL-6, IL-1Ra and 7 days to detect IFN-γ. Supernatants were collected and stored in -
390 20°C. Secreted cytokine levels from supernatants were quantified by ELISA (IL-1β cat # DLB50,
391 TNF-α cat # STA00D, IL-6 cat # D6050, IL-1Ra cat # DRA00B, IFN-γ cat #DY285B, R&D Systems,
392 USA).

393

394 **Statistical analysis**

395 Graphpad Prism 8 was used for all statistical analyses. Outcomes between paired groups were
396 analyzed by Wilcoxon's matched-pairs signed-rank test. Three or more groups were
397 compared using Kruskal-Wallis Test - Dunnet's multiple comparison. P-value of less than 0.05

398 was considered statistically significant. Spearman correlation was used to determine
399 correlation between groups.

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