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1 **Long-lasting neutralizing antibody responses in SARS-CoV-2 seropositive individuals are**
2 **robustly boosted by immunization with the CoronaVac and BNT162b2 vaccines**

3

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50 **KEYWORDS:** COVID-19; serological response; neutralizing antibody persistence; SARS-CoV-
51 2 vaccines

52

53 **ABSTRACT**

54 The durability of circulating neutralizing antibody (nAb) responses to severe acute respiratory
55 syndrome coronavirus 2 (SARS-CoV-2) infection and their boosting by vaccination remains to be
56 defined. We show that outpatient and hospitalized SARS-CoV-2 seropositive individuals mount a
57 robust neutralizing antibody (nAb) response that peaks at days 23 and 27 post-symptom onset,
58 respectively. Although nAb titers remained higher in hospitalized patients, both study groups
59 showed long-lasting nAb responses that can persist for up to 12 months after natural infection.
60 These nAb responses in previously seropositive individuals can be significantly boosted through
61 immunization with two doses of the CoronaVac (Sinovac) or one dose of the BNT162b2
62 (BioNTech/Pfizer) vaccines, suggesting a substantial induction of B cell memory responses.
63 Noteworthy, three obese previously seropositive individuals failed to mount a booster response
64 upon vaccination, warranting further studies in this population. Immunization of naïve individuals
65 with two doses of the CoronaVac vaccine or one dose of the BNT162b2 vaccine elicited similar
66 levels of nAbs compared to seropositive individuals 4.2 to 13.3 months post-infection with
67 SARS-CoV-2. Thus, this preliminary evidence suggests that both, seropositive and naïve
68 individuals, require two doses of CoronaVac to ensure the induction of robust nAb titers.

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69 **MAIN TEXT**

70 The durability of circulating neutralizing antibody (nAb) responses to severe acute respiratory
71 syndrome coronavirus 2 (SARS-CoV-2) infection or vaccination has become a central question
72 during the current pandemic to determine correlates of protection against disease. Current
73 evidence shows that SARS-CoV-2 spike-specific antibodies decline over time but remain
74 detectable up to 8 months post-symptoms onset[1]. However, additional longitudinal data are
75 needed to characterize the medium- and long-term protective antibody dynamics, starting from
76 the acute phase of disease of patients with mild and moderate/severe outcome, and to determine
77 their nAb memory responses upon immunization with different vaccines currently in use.
78
79 We enrolled 74 individuals (overall mean age 44 years [range 14 to 83, >60 23%]), of whom 37
80 were outpatient (mild disease, mean age 37 years [range 14 to 66]) and 37 were hospitalized
81 (moderate and severe disease, mean age 51 years [range 16 to 83]) with a confirmed SARS-CoV-
82 2 quantitative RT-PCR test (Suppl. Table 1). These individuals were followed longitudinally to
83 determine nAb response for up to one year from the onset of symptoms (demographic and
84 baseline characteristics of the patients are summarized in Suppl. Table 1; samples were collected
85 between 2 to 414 days after the onset of symptoms).
86
87 Regardless of disease severity, infected individuals developed robust nAb responses during the
88 first month. These responses declined over time but were sustained for up to 12 months (Fig. 1A,
89 B), as determined with a microneutralization assay based on a recombinant vesicular stomatitis
90 virus carrying a SARS-CoV-2 spike protein that showed strong correlation (Pearson's r = 0.86,
91 R2 = 0.75, P< 0.001) with authentic SARS-CoV-2 microneutralization (Fig. S1). We performed
92 kinetic analyses with samples from 41 individuals that were sampled weekly during the first

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93 month from symptom onset (Fig. 1A and Fig. S2A-B). In agreement with previous reports,
94 hospitalized individuals had significantly higher neutralization titers as compared to outpatients
95 (Fig. S2C), with peak average nAb responses at day 23 and at day 27 post-symptom onset,
96 respectively (Fig. 1A and Fig. S2A-B). We included longitudinal samples for all participants and
97 performed a nAb titer time decay analysis starting from the respective peak average responses.
98 Fitting our nAb data to a one-phase decay model, the initial decay half time was 42 days (95% CI
99 : 2.21 to 362.4) for outpatients and 84 days (95% CI : 1.7 to indeterminate) for hospitalized
100 individuals, and when we used a continuous decay fit, the half time was 225 (95% CI : 121 to
101 1,648) and 195 (95% CI : 120 to 535) days for these groups, respectively (Fig. 1B). None of the
102 individuals in the study had evidence of re-infections. Hence, albeit the nAb titers remained
103 higher in hospitalized patients than in the outpatients, both study groups showed long-lasting
104 responses of circulating antibodies after natural infection.
105
106 Twenty-seven of the previously seropositive individuals (mean age 45 years [range 17 to 83])
107 included in our longitudinally cohort study were immunized during the study period. Thus, we
108 analyzed the nAb response in these previously infected individuals after immunization with the
109 two main vaccines currently being used in Chile; the CoronaVac (Sinovac) vaccine based on
110 inactivated virus or the BNT162b2 (BioNTech/Pfizer) vaccine based on spike protein-encoding
111 messenger RNA. We compared them to nAbs titers in healthy SARS-CoV-2 naïve (seronegative)
112 individuals immunized with two doses of either vaccine (CoronaVac, 11 participants, mean age
113 34 years [range 21 to 47] or BNT162b2, 10 participants, mean age 37 years [range 23 to 53];
114 Suppl. Table 1). The seropositive individuals were vaccinated between 4.2 to 13.3 months
115 (average 9.9 months) after the onset of symptoms for both, the outpatient (13 participants) and
116 hospitalized groups (14 participants; see arrows in Fig. S3). The average increase in the nAb titers

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117 was four times for outpatients (pre-vaccine mean titer = 188.9 [range 98.7 to 273.6], first dose
118 mean titer = 666.3 [range 80.9 to 1,143]) and three times for hospitalized individuals (pre-vaccine
119 mean titer = 8,836 [range 1.0 to 29,389], first dose mean titer = 27,562 [range = 124.7 to 91,571])
120 after one dose (Fig. 1C). After the second dose, the average increase was 13 times (pre-vaccine
121 mean titer = 528.7 [range 10.9 to 1,791], mean titer = 6,597 [range 261.6 to 38,912) and 179
122 times (pre-vaccine mean titer = 649.1 [range 78.0 to 1,360], mean titer = 116,012 [range 31.0 to
123 1,000,000]), respectively (Fig. 1C). Except for four cases, all participants showed an increase in
124 the nAb titer after receiving one or two doses of the vaccines, suggesting a significant induction
125 of B cell memory response at 4.2 to 13.3 months after onset of symptoms. One corresponded to
126 an outpatient (Fig. S3C, dark yellow patient) who had been vaccinated two days earlier and may
127 therefore not have had sufficient time to mount a booster response. The other three participants
128 (age range 29 to 63 years) were obese outpatient (Fig. S3C, light green patient) or hospitalized
129 (Fig. S3D, grey and cyan patients) individuals for whom we only had a previous sample 5.6 to
130 10.7 months prior to vaccination (Fig. S3C, D), and hence no clear conclusions can be drawn
131 about the trajectory of their nAb titers. Noteworthy, one of these participants had a marked
132 decreased nAb titer after two doses of the vaccine (IC50 563.9 to 31.0; Fig. 1C). Due to the high
133 prevalence of obesity in severe coronavirus disease 2019 (COVID-19)[2], further studies to monitor
134 the induction of nAbs after vaccination in this population are required.
135
136 The induction of nAbs in seropositive individuals vaccinated with one dose as compared to the
137 naïve individuals vaccinated with BNT162b2 was on average 18 times higher (previously
138 infected first dose mean titer = 14,099 [range 80.91 to 91,571], naïve first dose mean titer = 791.0
139 [range 137.0 to 2,663]). When comparing them with naïve individuals vaccinated with one dose
140 of CoronaVac, their nAb titers were on average 492 times higher (naïve first dose mean titer =

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141 28.64 [range 1.0 to 71.6]). Importantly, we did not detect a statistical difference between nAb
142 titers of seropositive individuals 0.9 to 10.1 months post-infection (mean titer = 1,860 [range 1.0
143 to 29,389]) with those of naïve individuals vaccinated with two doses of CoronaVac (mean titer =
144 1,595 [range 56.9 to 6,262]), and higher titers were observed in those individuals with two doses
145 of BNT162b2 (mean titer = 16,648 [range 3,500 to 44,719]), suggesting the induction of robust
146 nAbs responses in naïve individuals with both vaccines (Fig. 1D). Immunization of previously
147 infected individuals with CoronaVac (24 participants), showed no significant differences in nAb
148 titers after one dose or when comparing the first and second dose. Significantly increased nAb
149 titers were only observed after both doses ($P<0.01$) of this vaccine (Fig. 1D), differing from
150 vaccination of seropositive or naïve individuals with mRNA and adenovirus-based vaccines that
151 induce high nAb titers after the first dose[3,4].
152
153 We found long lasting nAb titers that persist for over 12 months after the onset of symptoms in
154 both, outpatient and hospitalized individuals. Neutralizing activity in seropositive individuals was
155 boosted significantly after two doses of the CoronaVac or BNT162b2 vaccines, regardless of the
156 time interval since the onset of COVID-19 symptoms, suggesting that infection induces a robust
157 B-cell memory response. The correlates of protection against SARS-CoV-2 are currently
158 unknown. However, current evidence of re-infections remains limited, and they appear to be
159 infrequent, suggesting that natural infection provides significant protection against COVID-19 in
160 most individuals[5]. While this is a small cohort study, our results indicate that natural infection
161 induces long-lasting nAb responses that can be significantly boosted through vaccination, and that
162 immunization of naïve individuals with two doses of the CoronaVac vaccine or one dose of the
163 BNT162b2 vaccine elicit similar levels of nAbs compared to seropositive individuals 4.2 to 13.3
164 months post-infection with SARS-CoV-2. Our preliminary evidence suggests that both,

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165 seropositive and naïve individuals, require two doses of CoronaVac to generate a robust induction
166 of nAb titers. Further studies to determine the long-term duration of vaccine-induced nAbs
167 against SARS-CoV-2 are warranted.

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168 **DISCLOSURE:** The authors reported no potential conflict of interest. The Icahn School of
169 Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays
170 and NDV-based SARS-CoV-2 vaccines which list Florian Krammer as co-inventor. Mount Sinai
171 has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. Florian Krammer
172 has consulted for Merck and Pfizer (before 2020), and is currently consulting for Pfizer, Seqirus
173 and Avimex. The Krammer laboratory is also collaborating with Pfizer on animal models for
174 SARS-CoV-2. Kartik Chandran is a member of the scientific advisory boards of Integrum
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176

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191 **Author Contribution:**

192 NAM and TGS collected and analyzed data, made figures and tables, interpreted data, and wrote
193 the paper. CPR, EFS, JL, MJA, LIA, EP, and SS processed samples, performed experiments,
194 analyzed data, and revised the paper. GV, ES, CG, AR, recruited patients, collected clinical
195 metadata, and revised the paper. RJ, KC, DH, MED, generated rVSV viral stocks, analyzed data
196 and revised the paper. FK analyzed serological data, advised on data interpretation, and provided
197 funding for the study and revised the paper. NT designed the study, collected, analyzed, and
198 interpreted data, provided funding for the study, and wrote the paper. RAM conceived the
199 longitudinal cohort design, recruited patients; collected, analyzed, and interpreted data, provided
200 funding for the study, and wrote the paper.

201

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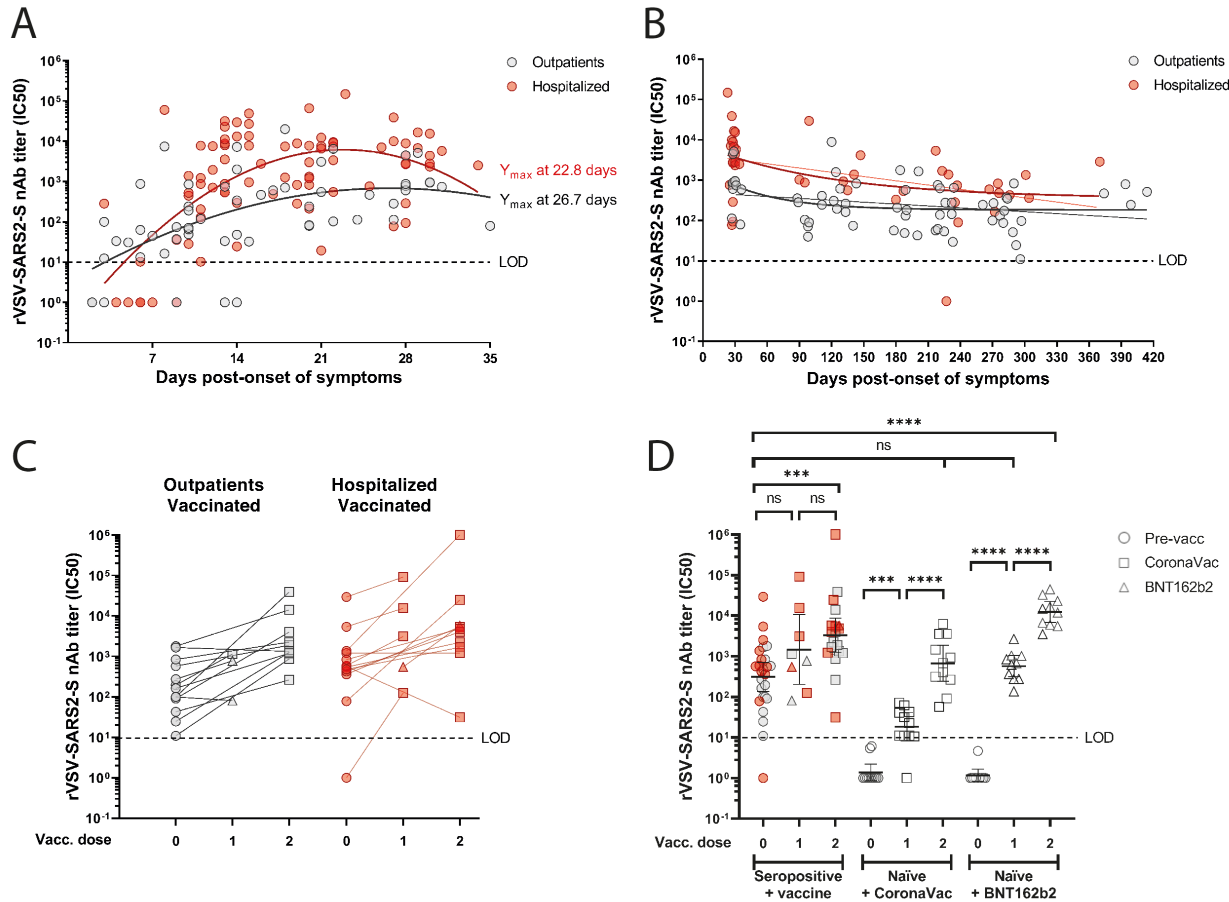
213 **FIGURE**



214

215 **Figure 1. Neutralizing antibody responses to SARS-CoV-2 in seropositive and naïve**
216 **individuals before and after CoronaVac or BNT162b2 vaccination.** (Panels A to D) The half-
217 maximum inhibitory concentration (IC50) of sera was determined by microneutralization assay of
218 recombinant vesicular stomatitis virus carrying SARS-CoV-2 spike protein (rVSV-SARS2-S).
219 (Panel A) Neutralizing antibody (nAb) titers (IC50) from 15 outpatients (57 samples; grey circles)
220 and 26 hospitalized (84 samples; red circles) at 2 to 36 days post-symptom onset. Second order
221 polynomial (quadratic) curve fitting was used to establish the days at which peak titers occurred
222 (Ymax). (Panel B) Longitudinal nAb titers from 36 outpatients (66 samples) and 31 hospitalized
223 (44 samples) taken from day 27 (outpatients) or day 23 (hospitalized) until day 414 post-symptom
224 onset. One-phase decay fit is indicated as a bold line, while continuous decay fit is shown with
225 the thinner line in red and gray for the corresponding patient group. (Panel C) nAb titers from 13
226 outpatient (26 samples) or 14 hospitalized (28 samples) individuals immunized with one or two
227 doses of CoronaVac (24 participants) or one or two doses of BNT162b2 (3 participants) vaccines.
228 (Panel D) nAb titers from naïve individuals after the first and second dose of CoronaVac (11
229 participants) or BNT162b2 (10 participants) vaccines, compared to seropositive individuals who
230 were not vaccinated (26 participants) or received one dose (8 samples) or two doses (20 samples)
231 of the indicated vaccines. Geometric means with 95% confidence intervals are shown. Circles,
232 non-vaccinated; squares, vaccinated with CoronaVac; triangles, vaccinated with BNT162b2.
233 Dashed line indicates the limit of detection (LOD) of the microneutralization assay. Statistics

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234 were performed using unpaired two-tailed Mann-Whitney test. ***$P<0.001$; ****$P<0.0001$; ns,
235 non-significant.

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236 **Long-lasting neutralizing antibody responses in SARS-CoV-2 seropositive individuals are**
237 **robustly boosted by immunization with the CoronaVac and BNT162b2 vaccines**

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283 **SUPPLEMENTARY APPENDIX**

284 **Table of content**

285 Supplemental Material and Methods

286 Supplemental References to Methods

287 **Table 1**. Demographic and baseline characteristics of COVID-19 patients and vaccinated controls

288 **Figure S1**. Correlation of the neutralizing activity of convalescent sera in neutralization assays
289 with rVSV-SARS2-S and to the authentic SARS-CoV-2 virus.

290 **Figure S2**. Longitudinal dynamics of humoral responses to the spike protein of SARS-CoV-2 of
291 mild and hospitalized seropositive individuals.

292 **Figure S3**. Longitudinal responses of convalescent individuals with or without CoronaVac and
293 BNT162b2 vaccination.

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294 **Supplemental Material and Methods**

295

296 **Study population and clinical metadata**

297 74 individuals with a confirmed diagnosis for SARS-CoV-2 infection were recruited between
298 March 5 and October 22, 2020. Patient clinical and epidemiological data, along with their clinical
299 specimens were collected after informed written consent was obtained under protocols 16-066
300 and 200829003 which were reviewed and approved by the Scientific Ethics Committee at
301 Pontificia Universidad Católica de Chile (PUC). The analysis were performed considering two
302 major groups of individuals, hospitalized and outpatients: **Hospitalized** individuals were either
303 severe patients**,** defined as those who developed pneumonia with one of the following three
304 conditions: (1) acute respiratory failure that required invasive mechanical ventilation or a high-
305 flow nasal cannula (HFNC) with prone position, (2) septic shock or (3) multiple organ
306 dysfunction; moderate cases consisted of inpatients with pneumonia without these conditions.
307 **Outpatients** were individuals that had mild symptoms of COVID-19 but did not meet the above-
308 mentioned criteria. Peripheral blood samples, nasopharyngeal swabs and sputum samples were
309 collected between 2 and 414 days after the onset of symptoms. Healthy individuals were recruited
310 as controls and received the two doses of the CoronaVac (Sinovac Life Sciences Co., LTD,
311 Beijing, China) or the BNT162b2 (Pfizer Manufacturing Belgium NV, Puurs, Belgium) vaccines.
312 Samples were collected between 20-30 days after first dose but prior to the second dose and 13-
313 19 days after second dose. Demographic data for all patients and controls, obtained by a clinical
314 questionnaire, are shown in Supplementary Table 1.

315

316 **Plasma and serum collection**

317 Peripheral blood was collected in both plasma separating (EDTA/purple top) and serum
318 separating (red top) tubes and was processed by centrifugation at 3000 rpm for 5 min. Plasma and
319 serum samples were aliquoted and stored at −80°C. Serum samples were heated at 56°C for 1h
320 before use to eliminate the risk of any potential residual virus.

321

322 **SARS-CoV-2 spike ELISA**

323 Overnight, 96-well plates (Immulon 4 HBX; Thermo Fisher Scientific) were coated at 4°C with
324 50 μL per well of a 2 mg/mL solution recombinant SARS-CoV-2 spike protein suspended in
325 phosphate-buffered saline (PBS, Gibco), as previously described[1,2]. The next morning, the coating

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326 solution was removed and 100 µL per well of 3% non-fat milk prepared in PBS with 0.1% Tween
327 20 (PBST) was added to the plates as a blocking solution and incubated at room temperature for
328 1h. Serial dilutions of serum and antibody samples were prepared in 1% non-fat milk prepared in
329 PBST. The blocking solution was removed and 100 µL of each serial dilution was added to the
330 plates for 2 h at room temperature. Plates were washed three times with 250 µL per well of 0.1%
331 PBST. Next, a 1:3,000 dilution of goat anti-human IgG–horseradish peroxidase (HRP) conjugated
332 secondary antibody (Thermo Fisher Scientific) was prepared in 0.1% PBST and 100 µL of this
333 secondary antibody was added to each well for 1 h. Plates were again washed three times with
334 0.1% PBST. Once completely dry, 100 µL SIGMAFAST OPD (o-phenylenediamine
335 dihydrochloride; Sigma–Aldrich) solution was added to each well. This substrate was left on the
336 plates for 10 min and then the reaction was stopped by the addition of 50 µL per well of 3 M
337 hydrochloric acid. The optical density at 490 nm (OD490) was measured using a Synergy 4
338 (BioTek) plate reader. The background value was set at an OD490 of 0.11 and the area under the
339 curve (AUC) was calculated. AUC values below 1 were assigned a value of 0.5 for plotting and
340 calculation purposes. In some cases, end-point titers were calculated, with the end-point titer
341 being the last dilution before reactivity dropped below an OD490 of <0.11. CR3022, a human
342 monoclonal antibody reactive to the RBD of both SARS-CoV-1 and SARS-CoV-2[3,4], was used as
343 control.
344

345 **SARS-CoV-2 microneutralization assay**

346 Vero E6 cells were seeded at a density of 20,000 cells per well in a 96-well cell culture plate in
347 complete Dulbecco's Modified Eagle Medium (cDMEM). The following day, heat-inactivated
348 serum samples (dilution of 1:10) were serially diluted threefold in 1× MEM (10% 10× minimal
349 essential medium (Gibco), 2 mM L-glutamine, 0.1% sodium bicarbonate (wt/vol; Gibco), 10 mM
350 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; Gibco), 100 U/mL penicillin, 100
351 mg/mL streptomycin (Gibco) and 0.2% bovine serum albumin (MP Biomedicals). As previously
352 described[5], the authentic SARS-CoV-2 (USA-WA1/2020; GenBank: MT020880) was diluted to a
353 concentration of 100 TCID50 (50% tissue culture infectious dose) in 1× MEM. Then, 80 µL of
354 each serum dilution and 80 µL of the virus dilution were added to a 96-well cell culture plate and
355 allowed to incubate for 1h at room temperature. cDMEM was removed from Vero E6 cells and
356 120 µL of the virus–serum mixture was added to the cells. Then, the cells were incubated at 37°C
357 for 1h. After the 1 h incubation, the virus–serum mixture was removed from the cells and 100 µL

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358 of each corresponding serum dilution and 100 µL of 1× MEM containing 1% fetal bovine serum
359 (FBS, Corning) was added to the cells. The cells were incubated for 48 h at 37°C and then fixed
360 with 10% paraformaldehyde (Polysciences) for 24 h at 4°C. Following fixation, the
361 paraformaldehyde was removed, and the cells were washed with 200 µL PBS. The cells were then
362 permeabilized by the addition of 150 µL PBS containing 0.1% Triton X-100 for 15 min at room
363 temperature. The plates were then washed three times with PBS containing PBST and blocked in
364 [3% milk (American Bio) in PBST] solution for 1 h at room temperature. After blocking, 100 µL
365 of mAb 1C7 (anti-SARS nucleoprotein antibody generated in-house) at a dilution of 1:1,000 was
366 added to all wells and the plates were allowed to incubate for 1h at room temperature. The plates
367 were then washed three times with PBST before the addition of goat anti-mouse IgG–HRP
368 (Rockland Immunochemicals), diluted 1:3,000 in blocking solution for 1 h at room temperature.
369 Plates were then washed three times with PBST, and SIGMAFAST OPD (Sigma–Aldrich) was
370 added. After a 10 min incubation at room temperature, the reaction was stopped by adding 50 µL
371 3 M hydrochloric acid to the mixture. The OD490 was measured on a Synergy 4 plate reader
372 (BioTek). A cut-off value of the average of the optical density values of blank wells plus three
373 standard deviations was established for each plate and used to calculate the microneutralization
374 titer. Microneutralization assays were performed in a facility with a biosafety level of 3.
375
376 **rVSV SARS-CoV-2 spike protein (rVSV-SARS2-S) microneutralization assay**
377 To determine the nAb titers of patient sera, the replication-competent recombinant vesicular
378 stomatitis virus previously described by Dieterle et al. (2020) was used, in whose genome the
379 native glycoprotein gene has been replaced by that encoding the spike glycoprotein of the SARS-
380 CoV-2 and whose genome further encodes an enhanced green fluorescent protein (eGFP)[6]. This
381 system has the advantage of generating high viral titers (~10^7), an easy score of cell infection by
382 GFP fluorescence, correlates of convalescent serum-mediated neutralization with that of authentic
383 SARS-CoV-2, enters cells through pathways of SARS-CoV-2, and does not require high
384 biosafety containment infrastructure for manipulation[5]. Briefly, Vero E6 cells (ATCC) grown in
385 1X MEM supplemented with 10% FBS (Thermo Scientific) were transfected with plasmid
386 pCEP4-myc-ACE2 (Addgene catalog # 141185) and stable clones selected in presence of
387 hygromycin (400 µg/mL) based on the presence of high levels of hACE2 at the plasma
388 membrane. To assay nAb titers, serial dilutions of serum samples were mixed with rVSV-
389 SARS2-S and incubated for 1 h at 37°C. The serum-virus was subsequently added to Vero E6

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390 hACE2 cells seeded the day before in optical bottom 96-well plates (Thermo Scientific) at 80%
391 confluence and adsorbed for 2 h at 37°C. Next, the mixture was replaced by culture media and
392 infection allowed to proceed for 20 h at 37ºC, 5% CO2 and 80% humidity. Next, the cells were
393 fixed with 4% formaldehyde (Thermo Scientific), washed with PBS, stained in with 4′,6-
394 diamidino-2-phenylindole (DAPI) 300 nM (Thermo Scientific) and stored in PBS at 4ºC until
395 acquiring fluorescence data. Viral infectivity was either quantified by automated enumeration of
396 GFP-positive cells (normalizing against cells stained with DAPI) using a Cytation5 automated
397 fluorescence microscope (BioTek) and segmentation algorithms applied from the ImageJ
398 program. Alternatively, total GFP fluorescence per well was acquired using the Cytation5
399 fluorescence lector (wavelength for DAPI 360 nm for absorption, 460 nm for emission and for
400 GFP, 485 nm for absorption, 526 nm for emission) and normalized against DAPI fluorescence.
401 The half-maximum inhibitory concentration (IC50) of the sera, calculated using non-linear
402 regression analysis and curve fitting using second-order polynomial (quadratic), one phase decay
403 and linear regression models (using log10 IC50 transformed data) were done with GraphPad
404 Prism 5 software.
405
406 **Computational and statistical analysis**
407 Categorical variables were expressed as numbers or percentages. Association between categorical
408 variables was examined with Chi-squared or Fisher's exact test. Continuous variables were
409 expressed in mean, geometric mean and range and compared with unpaired two-tailed Mann-
410 Whitney test. Correlation was evaluated calculating the Pearson correlation coefficient. GraphPad
411 Prism 8 was used for statistical analysis. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$.

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429

430 **Supplementary Table 1. Demographic and baseline characteristics of COVID-19 patients and vaccinated controls**

	Outpatients (37 participants)	Hospitalized (37 participants)	P value (Outpatients vs. Hospitalized)	CoronaVac (11 participants)	BNT162b2 (10 participants)	P value (CoronaVac vs. BNT162b2)
Characteristics						
Male, n (%)	17 (45.9)	25 (67.6)	0.0998	4 (36.4)	2 (20)	0.6351
Age, mean (range)	37 (14-66)	51 (16-83)	**0.0004**	34 (21-47)	37 (23-53)	0.6981
>60 years, n (%)	5 (13.5)	12 (32.4)	0.0956	0	0	-
Symptoms						
Respiratory						
Cough, n (%)	27 (73)	31 (83.8)	0.3975			
Dyspnea, n (%)	6 (16.2)	19 (51.4)	**0.0028**			
Odynophagia, n (%)	21 (56.8)	6 (16.2)	**0.0006**			
Chest discomfort, n (%)	3 (8.1)	5 (13.5)	0.7106			
Constitutional						
Fever, n (%)	22 (59.5)	31 (83.8)	**0.0377**			
Headache, n (%)	32 (86.5)	14 (37.8)	**< 0.0001**			
Myalgia, n (%)	25 (67.6)	18 (48.6)	0.157			
Severe fatigue, n (%)	0	20 (54.1)	**< 0.0001**			
Altered mental status, n (%)	0	3 (8.1)	0.2397			
Gastrointestinal						
Diarrhea, n (%)	12 (32.4)	10 (27)	0.7997			
Nausea/Vomiting, n (%)	6 (16.2)	9 (24.3)	0.5642			
Sensorial						
Ageusia, n (%)	18 (48.6)	5 (13.5)	**0.0022**			
Anosmia, n (%)	24 (64.9)	8 (21.6)	**0.0004**			
Comorbidities or conditions						
Obesity (BMI ≥ 30), n (%)	5 (13.5)	14 (37.8)	**0.0317**	2 (18.2)	4 (40)	0.3615
Hypertension, n (%)	3 (8.1)	13 (35.1)	**0.0095**	0	2 (20)	0.2143
Metabolic conditions*, n (%)	4 (10.8)	12 (32.4)	**0.0459**	1 (9.1)	1 (10)	1
Hyperlipidemia, n (%)	4 (10.8)	7 (18.9)	0.5151	0	2 (20)	0.2143
Cardiovascular disease, n (%)	0	3 (8.1)	0.2397	0	0	-

Chronic pulmonary disease, n (%)	4 (10.8)	3 (8.1)	1	0	0	-
Asthma, n (%)	6 (16.2)	2 (5.4)	0.2611	1 (9.1)	1 (10)	1
Rheumatologic disease, n (%)	0	3 (8.1)	0.2397	0	1 (10)	0.4762
Immunocompromised, n (%)	0	5 (13.5)	0.0541	0	1 (10)	0.4762
Allergy**, n (%)	16 (43.2)	6 (16.2)	**0.0209**	7 (63.6)	2 (20)	0.0805
Neurologic disease, n (%)	0	4 (10.8)	0.1148	0	0	-
Smoker, n (%)	8 (21.6)	9 (24.3)	1	1 (9.1)	3 (30)	0.3108

Abbreviation: BMI, Body mass index. *Metabolic conditions include insulin resistance, prediabetes, type 1/2 diabetes, non-alcoholic steatohepatitis and obstructive sleep apnea; **Allergy considered self-reported allergic rhinitis (by seasonal, perennial/year-round, or episodic allergens) and food allergy.

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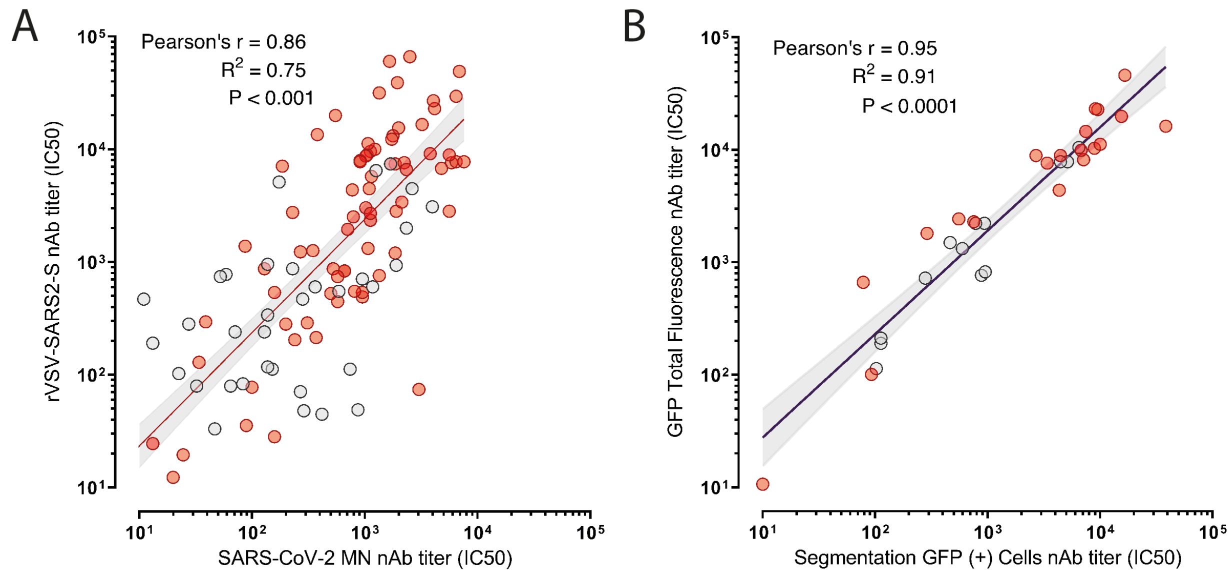

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434
435 **Supplementary Figure 1. Correlation of the neutralizing activity of convalescent sera in**
436 **neutralization assays with rVSV-SARS2-S and to the authentic SARS-CoV-2 virus.** (Panel
437 A) Linear regression and Pearson correlation analysis comparing neutralizing activities of
438 seropositive sera with authentic SARS-CoV-2 and rVSV-SARS2-S (122 samples; outpatients,
439 grey circles; hospitalized, red circles). (Panel B) Linear regression and Pearson correlation
440 analysis from two different quantification methods to measure neutralizing activity against rVSV-
441 SARS2-S; GFP (+) cell counting by using ImageJ segmentation analysis and total GFP
442 measurement normalized to DAPI counterstaining (33 samples).

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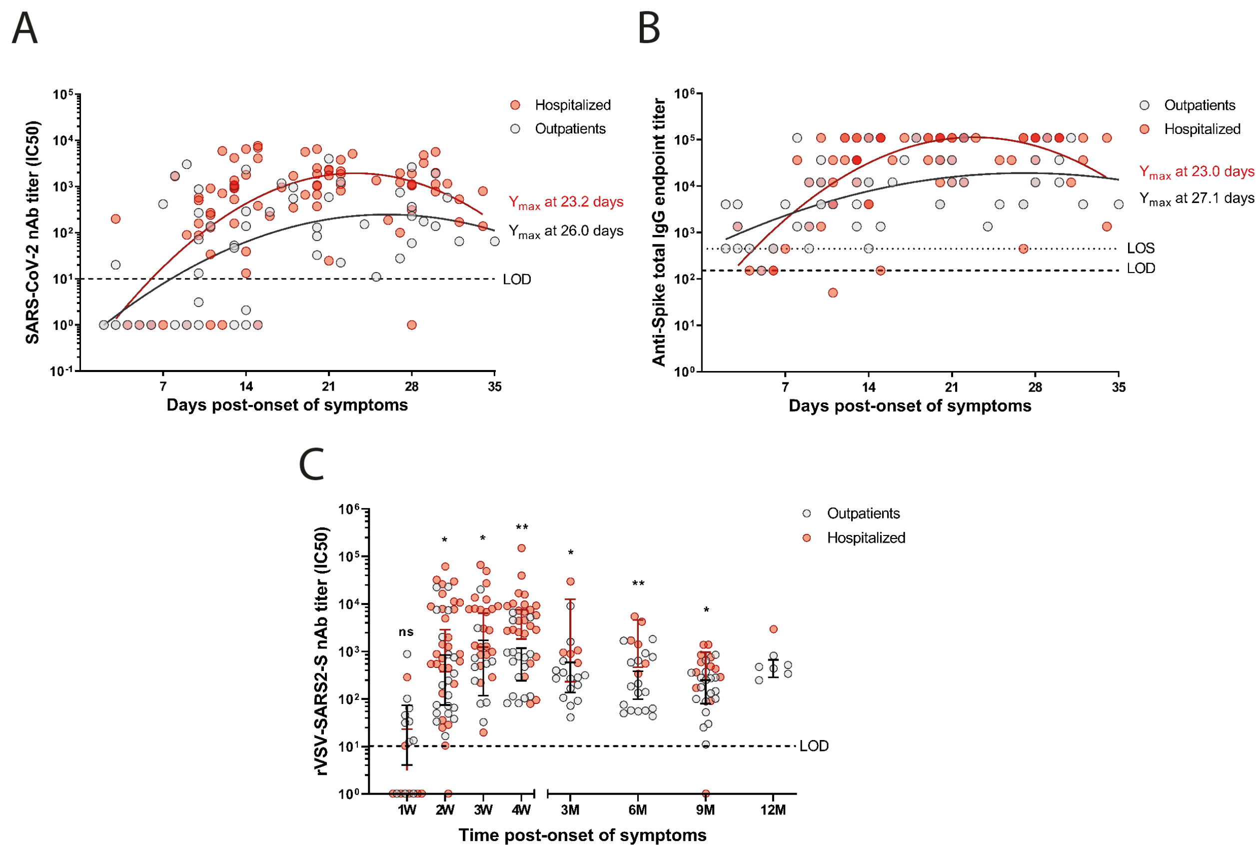

444
445 **Supplementary Figure 2. Longitudinal dynamics of humoral responses to the spike protein**
446 **of SARS-CoV-2 from outpatient and hospitalized seropositive individuals.** (Panel A) nAbs
447 titers against authentic SARS-CoV-2 of sera obtained from 41 seropositive individuals (142
448 samples) during 35 days since symptom onset. (Panel B) Anti-Spike total IgG endpoint titer of
449 serially diluted sera from 41 seropositive individuals (145 samples) during 36 days since
450 symptom onset determined by an enzyme-linked immunosorbent assay (ELISA). Second order
451 polynomial (quadratic) curve fitting was used to establish the days at which peak titers occurred
452 (Ymax). (Panel C) Neutralizing antibody (nAb) titers (IC50) obtained using rVSV-SARS2-S
453 microneutralization assay for 37 outpatients (111 samples; grey circles) and 37 hospitalized (108
454 samples; red circles) grouped by weeks (W) or months (M) post-symptom onset. The bars
455 indicate geometric mean titers with 95% confidence intervals. Dotted line represents the limit of
456 sensitivity (LOS) of ELISA. Dashed line represents the limit of detection (LOD) of each assay.
457 Statistics were calculated at the indicated time points between nAb titers of outpatient and
458 hospitalized using the unpaired two-tailed Mann-Whitney test (*P<0.05; **P<0.01).

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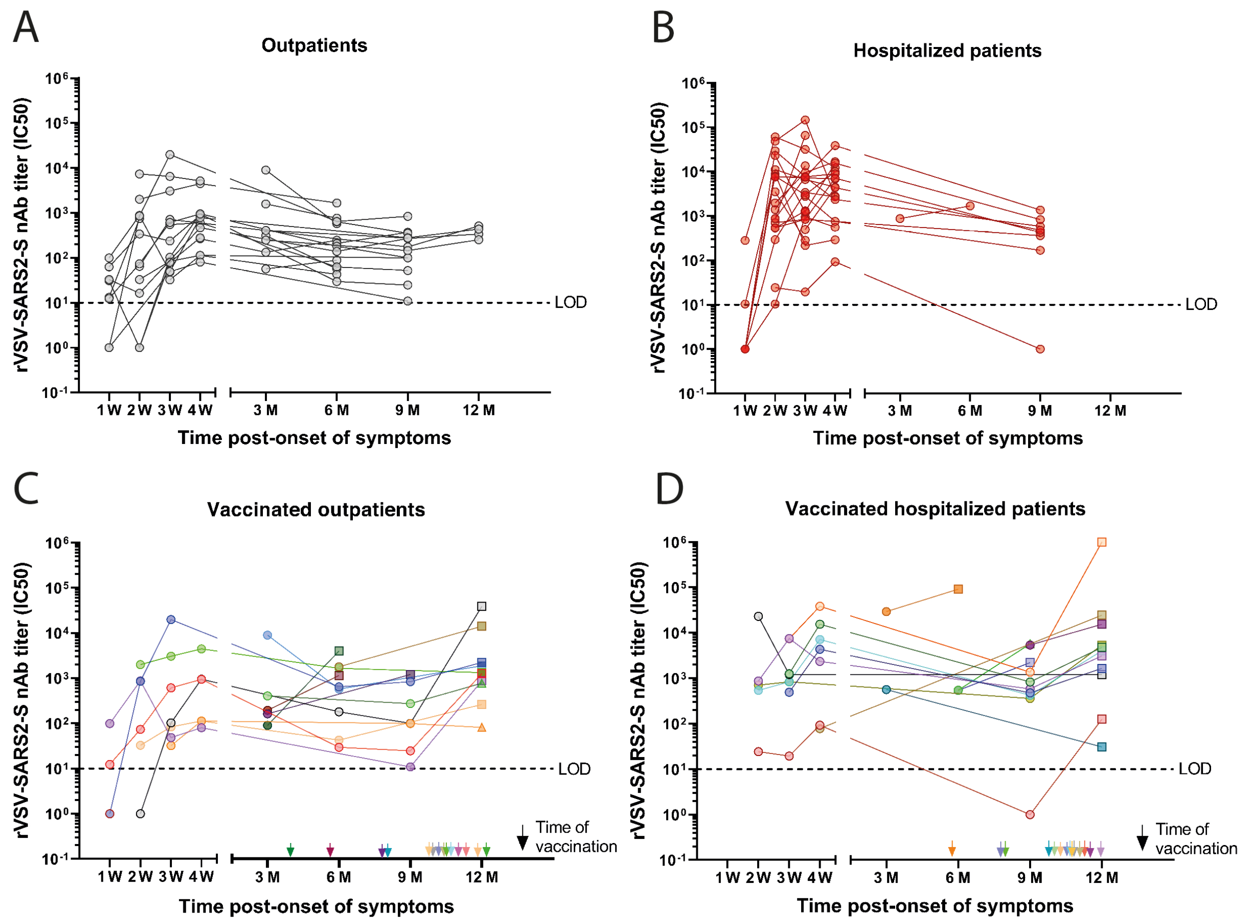

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462 **Supplementary Figure 3. Longitudinal responses of seropositive individuals with or without**
463 **CoronaVac and BNT162b2 vaccination.** Longitudinal neutralizing antibody (nAb) titers (IC50)
464 obtained using an rVSV-SARS2-S microneutralization assay for seropositive outpatients (Panel
465 A; 24 participants), hospitalized patients (Panel B; 25 participants), vaccinated seropositive
466 outpatients (Panel C; 13 participants) or vaccinated hospitalized patients (Panel D; 14
467 participants) at different time points grouped by weeks (W) or months (M) post-symptom onset
468 (serum samples from: 3M = 46-135 days; 6M = 136-225 days; 9M = 226-315 days and 12M =
469 316-414 days). The arrows indicate time of vaccination post-onset of symptoms. Circles, non-
470 vaccinated; squares, vaccinated with CoronaVac; triangles, vaccinated with BNT162b2. Dashed
471 line indicates the limit of detection (LOD) of the microneutralization assay.