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SARS-CoV-2 mechanistic correlates of protection: insight from modelling response to vaccines

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33

34 **Key words:** SARS-CoV-2, Correlate of protection, Neutralization, Vaccines

35 **One Sentence Summary:** A framework for modelling the immune control of viral dynamics is
36 applied to quantify the effect of several SARS-CoV-2 vaccine platforms and to define
37 mechanistic correlates of protection.

38

39 **Abstract:** The definition of correlates of protection is critical for the development of next
40 generation SARS-CoV-2 vaccine platforms. Here, we propose a new framework for identifying
41 mechanistic correlates of protection based on mathematical modelling of viral dynamics and data
42 mining of immunological markers. The application to three different studies in non-human
43 primates evaluating SARS-CoV-2 vaccines based on CD40-targeting, two-component spike
44 nanoparticle and mRNA 1273 identifies and quantifies two main mechanisms that are a decrease
45 of rate of cell infection and an increase in clearance of infected cells. Inhibition of RBD binding
46 to ACE2 appears to be a robust mechanistic correlate of protection across the three vaccine
47 platforms although not capturing the whole biological vaccine effect. The model shows that
48 RBD/ACE2 binding inhibition represents a strong mechanism of protection which required
49 significant reduction in blocking potency to effectively compromise the control of viral
50 replication.

51 **Main Text:**

52 **INTRODUCTION**

53 There is an unprecedented effort for SARS-CoV-2 vaccine development with 294 candidates
54 currently evaluated (1). However, variants of concern have emerged before the vaccine coverage
55 was large enough to control the pandemics (2). Despite a high rate of vaccine protection, these
56 variants might compromise the efficacy of current vaccines (3–6). Control of the epidemic by
57 mass vaccination may also be compromised by unknown factors such as long-term protection
58 and the need of booster injections in fragile, immuno-compromised, elderly populations, or even
59 for any individual if protective antibody levels wane. Furthermore, the repeated use of some of
60 the currently approved vaccine could be compromised by potential adverse events or by
61 immunity against vaccine viral vectors (7). Finally, the necessity to produce the billions of doses
62 required to vaccinate the world's population also explains the need to develop additional vaccine
63 candidates.

64

65 The identification of correlates of protection (CoP) is essential to accelerate the development of
66 new vaccines and vaccination strategies (8, 9). Binding antibodies to SARS-CoV-2 and *in vitro*
67 neutralization of virus infection are clearly associated with protection (10–13). However, the
68 respective contribution to virus control *in vivo* remains unclear (14), and many other
69 immunological mechanisms may also be involved, including other antibody-mediated functions
70 (antibody-dependent cellular cytotoxicity, antibody-dependent complement deposition, antibody-
71 dependent cellular phagocytosis (11, 15, 16)), as well as T cell immunity (17). Furthermore,
72 correlates of protection may vary between the vaccine platforms (18–21).

73 Non-human primate (NHP) studies offer a unique opportunity to evaluate early markers of
74 protective response (22, 23). Challenge studies in NHP allow the evaluation of vaccine impact on
75 the viral dynamics in different tissue compartments (upper and lower respiratory tract) from for
76 day one to virus exposure (11, 15, 24). Such approaches in animal models may thus help to infer,
77 for example, the relation between early viral events and disease or the capacity to control
78 secondary transmissions.

79 Here, we propose a novel model-based framework to evaluate i) the immune mechanism
80 involved in the vaccine response, and ii) the markers capturing this/these effect(s) leading to
81 identification of mechanisms of protection and definition of mechanistic CoP (25). First, we
82 present a mechanistic approach based on ordinary differential equation (ODE) models reflecting
83 the virus-host interaction (26–29). The proposed model includes several new aspects refining the
84 modeling of viral dynamics *in vivo*, in addition to the integration of vaccine effect. A specific
85 inoculum compartment allows distinguishing the virus coming from the challenge inoculum and
86 the virus produced *de novo*, which is a key point in the context of efficacy provided by antigen
87 specific pre-existing immune effectors induced by the vaccine. Then, an original data mining
88 approach is implemented to identify the immunological biomarkers associated with specific
89 mechanisms of vaccine-induced protection.

90 We apply our approach to a recently published study (30) testing a protein-based vaccine
91 targeting the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein to CD40
92 (α CD40.RBD vaccine). Targeting vaccine antigens to Dendritic Cells via the surface receptor
93 CD40 represents an appealing strategy to improve subunit-vaccine efficacy (31–34) and for
94 boosting natural immunity in SARS-CoV-2 convalescent NHP.

95 We show that immunity induced by natural SARS-CoV-2 infection, as well as vaccine-elicited
96 immune responses contribute to viral load control by i) blocking new infection of target cells and
97 ii) by increasing the loss of infected cells. The modelling showed that antibodies inhibiting
98 binding of RBD domain to ACE2 correlated with blockade of new infections and RBD binding
99 antibodies correlate with the loss of infected cells, reflecting importance of additional antibody
100 functionalities. The role of RBD/ACE2 binding inhibition has been confirmed in two other
101 vaccine platforms.

102

103 **RESULTS**

104 **A new mechanistic model fits the *in vivo* viral load dynamics in nasopharyngeal and** 105 **tracheal compartments**

106 The mechanistic model aims at capturing the viral dynamics following challenge with SARS-
107 CoV-2 virus in NHP. For that purpose, we used data obtained from 18 cynomolgus macaques
108 involved in the vaccine study reported by Marlin et al (30) and exposed to a high dose (1×10^6
109 pfu) of SARS-CoV-2 administered via the combined intra-nasal and intra-tracheal route. The
110 viral dynamics during the primary infections were characterized by a peak of genomic RNA
111 (gRNA) production three days after infection, followed by a decrease toward undetectable levels
112 beyond day 15 (**Figure S1**). At the convalescent phase (median 24 weeks after the primary
113 infection), 12 macaques were challenged with SARS-CoV-2 a second time, four weeks after
114 being randomly selected to receive either a placebo (n=6) or a single injection of the
115 α CD40.RBD vaccine (n=6) (**Figure 1A**). A third group of 6 naïve animals were infected at the
116 same time. Compared to this naïve group, viral dynamics were blunted following the second

117 challenge of convalescent animals with the lowest viral load observed in vaccinated animals
118 **(Figure 1B, S2).**

119 We developed a mathematical model to better characterize the impact of the immune response on
120 the viral gRNA and subgenomic RNA (sgRNA) dynamics, adapted from previously published
121 work (26, 27, 35), which includes uninfected target cells (T) that can be infected (I_1) and produce
122 virus after an eclipse phase (I_2). The virus generated can be infectious (V_i) or non-infectious
123 (V_{ni}). We completed the model by a compartment for the inoculum to distinguish between the
124 injected virus (V_s) and the virus produced *de novo* by the host (V_i and V_{ni}). The viral dynamics in
125 the two compartments, the nasopharynx and the trachea, were jointly considered **(Figure 2A)**.
126 Using the gRNA and sgRNA viral loads, we estimated the viral infectivity (β), the viral
127 production rate (p) and the loss rate of infected cells (δ). We assumed that gRNA and sgRNA
128 were proportional to the free virus and the infected cells, respectively. The duration of the eclipse
129 phase, the clearance of the free virus from the inoculum and produced *de novo* were estimated
130 separately by profile likelihood. The infectivity rate (0.95×10^{-6} (copies/ml) $^{-1}$ day $^{-1}$), the loss rate
131 of infected cells (1.04 day $^{-1}$), the eclipse phase (3 day $^{-1}$) estimations in naïve animals were in the
132 range of previously reported modelling results (26, 27). Here, we distinguished the clearance of
133 the inoculum which was much higher (20 virions day $^{-1}$) as compared to the clearance of the virus
134 produced *de novo* (3 virions day $^{-1}$). Furthermore, the viral production by each infected cells was
135 estimated to be higher in the nasopharyngeal compartment ($12.1 \cdot 10^3$ virions/cell/day) as
136 compared to the tracheal compartment ($0.92 \cdot 10^3$ virions/cell/day). These estimations are in
137 agreement with the observation of the intense production of viral particles by primary human
138 bronchial epithelial cells in culture (36). By allowing parameters to differ between animals
139 (through random effects), the variation of cell infectivity and of the loss rate of infected cells

140 captured the observed variation of the dynamics of viral load. The variation of those parameters
141 could be partly explained by the group to which the animals belong reducing the unexplained
142 variability of the cell infectivity by 66% and of the loss rate of infected cells by 54% (**Table S1**).
143 The model fitted well the observed dynamics of gRNA and sgRNA (**Figure 2B**).

144

145 **Modelling of the dynamics of viral replication argues for the capacity of α CD40.RBD** 146 **vaccine to block virus entry into host cells and to promote the destruction of infected cells**

147 We distinguish the respective contribution of the vaccine effect and post-infection immunity on
148 the reduction of the cell infection rate and the increase of the clearance of infected cells. Because
149 blocking *de novo* infection and promoting the destruction of infected cells would lead to different
150 viral dynamics profile (**Figure S3**), we were able to identify the contribution of each mechanism
151 by estimating the influence of the vaccine compared to placebo or naive animals on each model
152 parameter. The α CD40.RBD vaccine reduced by 99.6% the infection of target cells in the trachea
153 compared to the naïve group. The estimated clearance of infected cells was 1.04 day^{-1} (95% CI
154 $0.75; 1.45$) in naïve macaques. It was increased by 80% ($1.86/\text{day}^{-1}$) in the convalescent
155 macaques vaccinated by α CD40.RBD or not.

156 The mechanistic model allows predicting the dynamics of unobserved compartments. Hence, a
157 very early decrease of the target cells (all cells expressing ACE2) as well as of the viral inoculum
158 which fully disappeared from day 2 onward were predicted (**Figure 2C**). In the three groups, the
159 number of infected cells as well as infectious viral particles increased up to day 2 and then
160 decreased. We show that this viral dynamic was blunted in the vaccinated animals leading to a
161 predicted maximum number of infectious viral particles in the nasopharynx and the trachea
162 below the detection threshold (**Figure 2C**). The number of target cell levels would be decreased

163 by the infection in the naïve and the convalescent groups, whereas it would be preserved in
164 vaccinated animals.

165

166 **The RBD-ACE2 binding inhibition is the main mechanistic CoP explaining the effect of the**
167 **α CD40.RBD vaccine on new cell infection**

168 In our study (30), an extensive evaluation of the immunological response has been performed
169 with quantification of spike binding antibodies, antibodies inhibiting the attachment of RBD to
170 ACE2, antibodies neutralizing infection, SARS-CoV-2-specific CD4⁺ and CD8⁺ T cells
171 producing cytokines and serum cytokine levels (**Figure 3, S4, S5, S6**). Therefore, based on our
172 mechanistic model we investigated if any of these markers could serve as a mechanistic CoP.
173 Such a CoP should be able to capture the effect of the natural immunity following infection,
174 associated or not to the vaccine (group effect) estimated on both the rate of cell infection and the
175 rate of the loss of infected cells. To this aim, we performed a systematic screening by adjusting
176 the model for each marker and we compared these new models to the reference model adjusted
177 for the groups (See supplementary information for a detailed description of the algorithm). We
178 demonstrate that the RBD-ACE2 binding inhibition measure is sufficient to capture most of the
179 effect of the groups on the infection of target cells (**Figure 4A, 4B**). The integration of this
180 marker in the model explains the variability of the cell infection rate with greater certainty than
181 the group of intervention, reducing the unexplained variability by 87% compared to 66% (**Table**
182 **S1**). The marker actually takes into account the variation between animals within the same
183 group. Hence, it suggests that the levels of anti-RBD antibodies induced by the vaccine that
184 block attachment to ACE2 are highly efficient at reflecting the neutralization of new infections *in*
185 *vivo*. Furthermore, when taking into account the information provided by the RBD-ACE2
186 binding inhibition assay, the effect of the group of intervention was no longer significant (**Table**

187 **S1**). Finally, we looked at the estimated infection rate according to the inhibition binding assay
188 in every animal (**Figure 4C**). The values were not overlapping at all, distinguishing clearly the
189 vaccinated and unvaccinated animals.

190

191 In the next step, several markers (IgG binding anti-RBD antibodies, CD8⁺ T cells producing
192 IFN- γ) appeared to be associated to the rate of loss of infected cells (**Figure S7A**). Both specific
193 antibodies and specific CD8⁺ T cells are mechanisms commonly considered important for killing
194 infected cells. We retained the anti-RBD binding IgG Ab that were positively associated to the
195 increase of the loss of infected cells. For unknown reason the IFN- γ response was high in
196 unstimulated conditions in the naïve group. Thus, although this marker was associated with a
197 decrease of the loss rate of infected cells, it appears essentially here as an indicator of the animal
198 group. Further studies would be needed to fully confirm the place of IFN- γ response as a
199 mechanistic marker.

200 A large part of the variation of the infection rate (71%) and loss rate of infected cells (60%) were
201 captured by the two markers of CoP: the RBD-ACE2 binding inhibition and the anti-RBD
202 binding Ab concentration. Using the estimated parameters, the effective reproduction rate could
203 be calculated (R) which is representing the number of cells secondarily infected by virus from
204 one infected cell (**Figure 4D**). When looking at this effective reproduction rate according to the
205 groups, the vaccinated animal presented from the first day of challenge an effective R below 1
206 meaning that no propagation of the infection started within the host. These results were
207 consistent when taking the value of RBD-ACE2 binding inhibition at the time of the challenge
208 without considering the evolution of the inhibition capacity over time (**Figure S7B**). This means
209 that the dynamics of the viral replication is impacted very early during the infection process in

210 immunized animals and that vaccinated animals were protected from the beginning by the
211 humoral response. Then, we looked at the threshold of the markers of interest leading to the
212 control of the within-host infection (as defined by $R < 1$) which was around 30 000 AU for the
213 RBD-ACE2 binding inhibition assay. For the animals in the naive and the convalescent groups,
214 the observed values of binding inhibition measured by ECL RBD (the lower the better) and of
215 IgG anti-RBD binding antibodies (the higher the better) led to $R > 1$, whereas in vaccinated
216 animals, the value of ECL RBD led to $R < 1$. Therefore, our modeling study shows that the
217 inhibition of binding of RBD to ACE2 by antibodies is sufficient to control initial infection of
218 the host (**Figure 4E**). According to the observed value of ECL RBD in vaccinated animals (e.g.,
219 66 AU in **Figure 4E**), a decrease of more than 2 log₁₀ of the inhibition capacity (to reach 81 000
220 AU), due to variant of concern (VoC) or waning of immunity, would have been necessary to
221 impair the control of the within-host infection. Moreover, a decrease of the neutralizing activity
222 (i.e., increased ECL) could be compensated by an increase of cell death as measured by an
223 increase of binding IgG anti-RBD as a surrogate. As an example, increasing IgG anti-RBD from
224 2.5 to 10 in the animal MF7 of the convalescent group would lead to a control of the infection.
225 In conclusion, the α CD40.RBD vaccine-elicited humoral response leads to the blockade of new
226 cell infection that is well captured by measure of the inhibition of attachment of the virus to
227 ACE2 through the RBD domain of the spike protein. Hence, the inhibition of binding of RBD to
228 ACE2 is a promising mechanistic CoP. Indeed, this CoP fulfils the three criteria of leading to the
229 best fit (lower BIC), the best explanation of inter-individual variability, and fully captured the
230 effect of the group of intervention.

231

232 **The model revealed the same CoP related to another protein-based vaccine but not with**
233 **mRNA-1273 vaccine**

234 We took the opportunity of another study testing a two-component spike nanoparticle vaccine
235 performed in the same laboratory and using the same immune and virological assays (37) for
236 applying the proposed model and methodology. In this study, 6 animals were vaccinated and
237 compared to 4 naive animals (**Figure S8A, S8B**). The good fit of the data (**Figure S8C, S8D**)
238 allows for estimating the effect of the vaccine that appeared here also to decrease the
239 transmission rate (by 99%) and increase the clearance of the infected cells by 79%. Looking at
240 the best mechanistic CoP following the previously described strategy, we ended here again with
241 the inhibition of RBD binding to ACE2 as measured by ECL RBD. In fact, this marker measured
242 at baseline before challenge responded to the three criteria: i) it led to the best model in front of a
243 model adjusted for group effect, ii) it rendered the group effect non-significant and iii) it
244 explained around 71% of the transmission rate variability, compared to 65% of variability
245 explained by the groups. Interestingly, here again, the inhibition assay led to a clear separation of
246 the estimated rate of transmission between vaccinees and the placebo group (**Figure S8E**).

247 Finally, we applied our approach to a published NHP study performed to evaluate several doses
248 of mRNA-1273 vaccine (24). Using available data, we compared the viral dynamics in the 100
249 μg , 10 μg and placebo group. We started from the same model as defined previously. We
250 estimated a reduction of transmission rate by 97% but we did not find any additional effect.
251 Looking at potential mechanistic CoP, we retained neutralization as measured on live cells with
252 Luciferase marker. Although this marker led to the best fit and replaced the group effect (which
253 was non-significant after adjustment for the marker), it explained only 15% of the variability of
254 estimated transmission rate, while 19% were explained by the groups.

255 In conclusion, we demonstrated, based upon challenge studies in NHP vaccinated with two
256 different protein-based vaccine platforms that both vaccines lead to the blockade of new cell
257 infection. Neutralizing antibodies likely represent a consistent mechanistic correlate of
258 protection. This could change across vaccine platforms especially because mechanisms of action
259 are different.

260

261 **DISCUSSION**

262 We propose a novel framework to explore the mechanistic effects of vaccines and to assess the
263 quality of markers as mechanistic CoP (mCoP) that we applied to SARS-CoV-2 vaccines. This
264 model showed that neutralizing and binding antibodies, elicited by a non-adjuvanted protein-
265 based vaccine targeting the RBD of spike to the CD40 receptor of antigen presenting cells are
266 reliable mCoP. Interestingly, we found the simpler and easier to standardize and realize binding
267 inhibition assay may be more relevant to use as a correlate of protection than cell-culture
268 neutralization assays. This result has been replicated in another study testing a nanoparticle spike
269 vaccine. The model was able to capture the effect of the vaccines on the reduction of the rate of
270 infection of target cells and identified additional effects of vaccines beyond neutralizing
271 antibodies. This latter consisted of increasing the loss rate of infected cells which was better
272 reflected by the IgG binding antibodies and CD8⁺ T cell responses in the case of the CD40-
273 targeting vaccine. One limitation of our study is that the prediction potential of our model relies
274 on the range of the immune markers measured. However, our approach would allow a full
275 exploitation of the data generated as in systems serology where non-neutralizing Ab functions,
276 such as Ab-dependent cellular cytotoxicity (ADCC), Ab-dependent cellular phagocytosis
277 (ADCP), Ab-dependent complement deposition (ADCD), and Ab-dependent respiratory burst

278 (ADRB) are explored (38). The role of ADCC in natural infection has been previously shown
279 (39), ADCD in DNA vaccine recipients (11) and with Ad26 vaccine (40). Here, we extended
280 significantly these data by modelling the viral dynamic, showing that two other protein-based
281 vaccines exert an additional effect on infected cell death which relied on the level of IgG anti-
282 RBD binding antibodies especially for the CD40.RBD targeting vaccine. Measurements of other
283 non-neutralizing Ab functions would probably also capture this additional effect.

284
285 The next question after determining which marker is a valid mCoP is to define the concentration
286 that leads to protection, looking for a threshold effect that will help to define an objective (10,
287 41). In the context of SARS-CoV-2 virus, several emerged variants are leading to a significant
288 reduction of viral neutralization as measured by various approaches. However, a 20-fold
289 reduction of viral neutralization might not translate in 20-fold reduction of vaccine efficacy (42).
290 First, there are many steps between viral neutralization and the reduction of transmission or the
291 improvement of clinical symptoms. Second, the consequences of a reduction of viral
292 neutralization could be alleviated by other immunological mechanisms not compromised by the
293 variant. In the context of natural immunity, when the level of neutralizing antibodies was below
294 a protective threshold, the cellular immune response appeared to be critical (17, 43). We showed
295 with our model that an improvement of infected cell destruction could help to control the within-
296 host infection and is quantitatively feasible.

297
298 The control of viral replication is the key for reducing transmission (44, 45) as well as disease
299 severity (46–48). According to our non-linear model linking the neutralization to the viral
300 replication, a decrease of 4 to 20 fold in neutralization as described for the variants of concern (4,

301 6) is not enough, especially in the context of the response to CD40.RBD targeting vaccine, to
302 compromise the control of viral replication. This potential limited impact of variants on the host
303 viral dynamics should be associated to a reduced transmission of escape variants in vaccinated
304 population as compared to wild type virus in the unvaccinated population (49). The results
305 showing a conserved effectiveness of mRNA vaccines in humans infected by the alpha or beta
306 variants (50), although a decrease of neutralization has been reported (4), are consistent with this
307 hypothesis. However, this is highly dependent upon the mode of action of currently used
308 vaccines and if there is no new VoC compromising the neutralization in a much higher scale than
309 what has been described to date (51, 52). This may globally have an impact on the global burden
310 of the pandemic, since the occurrence of variants within host is probably a rare event (53) more
311 likely occurring in specific conditions (54) and therefore the strongest selection for vaccine-
312 escape mutants occurs by transmission (49). In the case of delta variants, a marked decrease of
313 neutralization has been described (55) but the impact on vaccine effectiveness is less clear (56).

314

315 The analysis performed extended significantly the observation of associations between markers
316 as previously reported for SARS-CoV-2 vaccine (11) and other vaccines (57) because it allows a
317 more causal interpretation of the effect of immune markers. However, our modelling approach
318 requires the *in vivo* identification of the biological parameters under specific experimentations.
319 On the other hand, the estimation of parameters included in our model also provided information
320 on some aspect of the virus pathophysiology. Notably, we found an increased capacity of virion
321 production in nasopharynx compared to the trachea which could be explained by the difference
322 in target cells according to the compartment (58).

323

324 In conclusion, the framework presented here based on a mathematical model of viral dynamics
325 should help in better evaluating the effect of vaccines and defining mechanistic CoP. The
326 application to two new promising SARS-CoV-2 vaccines revealed a combination of effects with
327 a blockade of new cell infections and the destruction of infected cells. For these two vaccines,
328 the antibody inhibiting the attachment of RBD to ACE2, appeared to be a very good surrogate of
329 the vaccine effect on the rate of infection of new cells and therefore could be used as a
330 mechanistic CoP. This modelling framework participates to the improvement of the
331 understanding of the immunological concepts by adding a quantitative evaluation of the
332 contributions of different mechanisms of control of viral infection. In terms of acceleration of
333 vaccine development, our results may help to develop vaccines for “hard-to-target pathogens”, or
334 to predict their efficacy in aging and particular populations (59). It should also help in choosing
335 vaccine dose, for instance at early development (60) as well as deciding if and when boosting
336 vaccination is needed in the face of waning protective antibody levels (61, 62).

337

338 **MATERIALS AND METHODS**

339 **Experimental model and subjects details**

340 *Cynomolgus* macaques (*Macaca fascicularis*), aged 37-66 months (18 females and 13 males) and
341 originating from Mauritian AAALAC certified breeding centers were used in this study. All
342 animals were housed in IDMIT facilities (CEA, Fontenay-aux-roses), under BSL2 and BSL-3
343 containment when necessary (Animal facility authorization #D92-032-02, Préfecture des Hauts
344 de Seine, France) and in compliance with European Directive 2010/63/EU, the French
345 regulations and the Standards for Human Care and Use of Laboratory Animals, of the Office for
346 Laboratory Animal Welfare (OLAW, assurance number #A5826-01, US). The protocols were

347 approved by the institutional ethical committee “Comité d’Ethique en Expérimentation Animale
348 du Commissariat à l’Energie Atomique et aux Energies Alternatives” (CEtEA #44) under
349 statement number A20-011. The study was authorized by the “Research, Innovation and
350 Education Ministry” under registration number APAFIS#24434-2020030216532863v1.

351

352 **Evaluation of anti-Spike, anti-RBD and neutralizing IgG antibodies**

353 *Anti-Spike IgG were titrated by multiplex bead assay.* Briefly, Luminex beads were coupled to
354 the Spike protein as previously described (63) and added to a Bio-Plex plate (BioRad). Beads
355 were washed with PBS 0.05% tween using a magnetic plate washer (MAG2x program) and
356 incubated for 1h with serial diluted individual serum. Beads were then washed and anti-NHP
357 IgG-PE secondary antibody (Southern Biotech, clone SB108a) was added at a 1:500 dilution for
358 45 min at room temperature. After washing, beads were resuspended in a reading buffer 5 min
359 under agitation (800 rpm) on the plate shaker then read directly on a Luminex Bioplex 200 plate
360 reader (Biorad). Average MFI from the baseline samples were used as reference value for the
361 negative control. Amount of anti-Spike IgG was reported as the MFI signal divided by the mean
362 signal for the negative controls.

363

364 *Anti-RBD and anti-Nucleocapside (N) IgG* were titrated using a commercially available
365 multiplexed immunoassay developed by Mesoscale Discovery (MSD, Rockville, MD) as
366 previously described (64). Briefly, antigens were spotted at 200–400 µg/mL in a proprietary
367 buffer, washed, dried and packaged for further use (MSD® Coronavirus Plate 2). Then, plates
368 were blocked with MSD Blocker A following which reference standard, controls and samples
369 diluted 1:500 and 1:5000 in diluent buffer were added. After incubation, detection antibody was

370 added (MSD SULFO-TAG™ Anti-Human IgG Antibody) and then MSD GOLD™ Read
371 Buffer B was added and plates read using a MESO QuickPlex SQ 120MM Reader. Results were
372 expressed as arbitrary unit (AU)/mL.

373

374 *Anti-RBD and anti-N IgG* were titrated by ELISA. The Nucleocapsid and the Spike RBD domain
375 (Genbank # NC_045512.2) were cloned and produced in *E. Coli* and CHO cells, respectively, as
376 previously described (31). Antigens were purified on C-tag column (Thermo Fisher) and quality-
377 controlled by SDS-PAGE and for their level of endotoxin. Antigens were coated in a 96 wells
378 plates Nunc-immuno Maxisorp (Thermo Fisher) at 1 µg/mL in carbonate buffer at 4°C overnight.
379 Plates were washed in TBS tween 0.05% (Thermo Fisher) and blocked with PBS 3% BSA for 2
380 hours at room temperature. Samples were then added, in duplicate, in serial dilution for 1 hour at
381 RT. Non-infected NHP sera were used as negative controls. After washing, anti-NHP IgG
382 coupled with HRP (Thermo Fisher) was added at 1:20,000 for 45 min at RT. After washing,
383 TMB substrate (Thermo Fisher) was added for 15 min at RT and the reaction was stopped with
384 1M sulfuric acid. Absorbance of each well was measured at 450 nm (reference 570 nm) using a
385 Tristar2 reader (Berthold Technologies). The EC₅₀ value of each sample was determined using
386 GraphPad Prism 8 and antibody titer was calculated as log (1/EC₅₀).

387

388 *The MSD pseudo-neutralization assay* was used to measure antibodies neutralizing the binding
389 of the spike protein to the ACE2 receptor. Plates were blocked and washed as above, assay
390 calibrator (COVID- 19 neutralizing antibody; monoclonal antibody against S protein; 200
391 µg/mL), control sera and test sera samples diluted 1:10 and 1:100 in assay diluent were added to
392 the plates. Following incubation of the plates, an 0.25 µg/mL solution of MSD SULFO-TAG™

393 conjugated ACE-2 was added after which plates were read as above. Electro-
394 chemoluminescence (ECL) signal was recorded.

395

396 **Viral dynamics modelling**

397 The mechanistic approach we developed to characterize the impact of the immune response on
398 the viral gRNA and sgRNA dynamics relies on a mechanistic model divided in three layers:
399 firstly, we used a mathematical model based on ordinary differential equations to describe the
400 dynamics in the two compartments, the nasopharynx and the trachea. Then we used a statistical
401 model to take into account both the inter-individual variability and the effects of covariates on
402 parameters. Finally, we considered an observation model to describe the observed \log_{10} viral
403 loads in the two compartments.

404 For the mathematical model, we started from previously published models (26, 27, 35) where
405 nasopharynx and trachea were described by target cell limited models and we completed the
406 model by adding a compartment for the inoculum to be able to distinguish between the injected
407 virus (V_s) and the virus produced *de novo* (V_i and V_{ni}). Consequently, for each of the two
408 compartments, the model included uninfected target cells (T) that can be infected (I_1) either by
409 infectious viruses (V_i) or inoculum (V_s) at an infectivity rate β . After an eclipse phase, infected
410 cells become productively infected cells (I_2) that can produce virions at rate P and be lost at a per
411 capita rate δ . The virions generated can be infectious (V_i) with proportion μ while the $(1-\mu)$
412 remaining virions are non-infectious (V_{ni}). Finally, free *de novo* produced virions and free
413 virions from inoculum are respectively cleared at a rate c and c_i . The model can be written as the
414 following set of differential equations, where the superscript X denotes the compartment of
415 interest (N, nasopharynx or T, trachea):

$$\left(\begin{array}{l}
\frac{dT^X}{dt} = -\beta^X V_i^X T^X - \mu \beta^X V_s^X T^X \\
\frac{dI_1^X}{dt} = \beta^X V_i^X T^X + \mu \beta^X V_s^X T^X - k I_1^X \\
\frac{dI_2^X}{dt} = k I_1^X - \delta^X I_2^X \\
\frac{dV_i^X}{dt} = \mu P^X I_2^X - c V_i^X - \beta^X V_i^X T^X \\
\frac{dV_{ni}^X}{dt} = (1 - \mu) P^X I_2^X - c V_{ni}^X \\
\frac{dV_s^X}{dt} = -c_i V_s^X - \mu \beta^X V_s^X T^X \\
T^X(t=0) = T_0^X ; I_1^X(t=0) = 0 ; I_2^X(t=0) = 0 \\
V_i^X(t=0) = 0 ; V_{ni}^X(t=0) = 0 ; V_s^X(t=0) = V_{S,0}^X
\end{array} \right. \quad \# \quad (1)$$

416 where $T^X(t=0)$, $I_1^X(t=0)$, $I_2^X(t=0)$, $V_i^X(t=0)$, $V_{ni}^X(t=0)$ and $V_s^X(t=0)$ are the initial
417 conditions at the time of exposure. The initial concentration of target cells, that are the epithelial
418 cells expressing the ACE2 receptor, is expressed as $T_0^X = \frac{T_0^{X,nbc}}{W^X}$ where $T_0^{X,nbc}$ is the initial
419 number of cells and W^X is the volume of distribution of the compartment of interest (see
420 “Consideration of the volume of distribution”). Each animal was exposed to 1×10^6 pfu of SARS-
421 CoV-2 representing 2.19×10^{10} virions. Over the total inoculum injected (5 mL), 10% (0.5 mL)
422 and 90% (4.5 mL) of virions were respectively injected by the intra-nasal route and the intra-
423 tracheal route leading to the following initial concentrations of the inoculum within each
424 compartment : $V_{S,0}^N = \frac{0.10 \times \text{Inoc}_0}{W^N}$ and $V_{S,0}^T = \frac{0.90 \times \text{Inoc}_0}{W^T}$, with Inoc_0 the number virions injected via
425 the inoculum.

426 Using the gRNA and sgRNA viral loads, we estimated the viral infectivity, the viral production
427 rate and the loss rate of infected cells (**Table S2**). To account for inter-individual variability and
428 covariates, each of those three parameters was described by a mixed-effect model and jointly
429 estimated between the two compartments as follows:

$$\left\{ \begin{array}{l} \log_{10}(\beta_i^N) = \beta_0 + \phi_{conv}^\beta \times \mathbb{I}_{group=conv} + \phi_{CD40}^\beta \times \mathbb{I}_{group=CD40} + u_i^\beta \\ \beta_i^T = \beta_i^N \times \exp(f_\beta^T) \\ \log(\delta_i^N) = \log(\delta_0) + \phi_{conv}^\delta \times \mathbb{I}_{group=conv} + \phi_{CD40}^\delta \times \mathbb{I}_{group=CD40} + u_i^\delta \\ \delta_i^T = \delta_i^N \times \exp(f_\delta^T) \\ \log(P_i^N) = \log(P_0) + \phi_{conv}^P \times \mathbb{I}_{group=conv} + \phi_{CD40}^P \times \mathbb{I}_{group=CD40} + u_i^P \\ P_i^T = P_i^N \times \exp(f_P^T) \end{array} \right. \quad (2)$$

430 with $u_i^\beta \sim \mathcal{N}(0, \omega_\beta^2)$, $u_i^\delta \sim \mathcal{N}(0, \omega_\delta^2)$ and $u_i^P \sim \mathcal{N}(0, \omega_P^2)$, where $\beta_0, \log(\delta_0)$ and $\log(P_0)$ are the
 431 fixed effects, $\{\phi_{conv}^\theta | \theta \in \{\beta, \delta, P\}\}$ and $\{\phi_{CD40}^\theta | \theta \in \{\beta, \delta, P\}\}$ are respectively the regression
 432 coefficients related to the effects of the group of convalescent and α CD40.RBD vaccinated
 433 animals for the parameters β, δ and P , and u_i^θ is the individual random effect for the parameter θ ,
 434 which supposedly normally distributed with variance ω_θ^2 .

435 In practice, after selection (see ‘‘Parameter estimation’’), only random effects and group effects
 436 on the parameters β and δ were kept, fixing $\omega_P = 0, \phi_{conv}^P = 0$ and $\phi_{CD40}^P = 0$. In addition, the
 437 estimation of several models identified the viral production rate P as the single parameter taken
 438 different values in nasopharynx and trachea ($f_\beta^T = f_\delta^T = 0$). For the observation model, the
 439 \log_{10} -transformed genomic and subgenomic viral loads of the i th animal at the j th time point in
 440 the compartment X (nasopharynx or trachea), labelled $gRNA_{ij}^X$ and $sgRNA_{ij}^X$ respectively, were
 441 described by the following equations:

$$\begin{cases} gRNA_{ij}^X = \log_{10}[V_i^X + V_{ni}^X + V_s^X](\Theta_i^X, t_{ij}) + \varepsilon_{ij,g}^X & \varepsilon_{ij,g}^X \sim \mathcal{N}(0, \sigma_{gX}^2) \\ sgRNA_{ij}^X = \alpha_{sgRNA} \times \log_{10}[I_1^X + I_2^X](\Theta_i^X, t_{ij}) + \varepsilon_{ij,sg}^X & \varepsilon_{ij,sg}^X \sim \mathcal{N}(0, \sigma_{sgX}^2) \end{cases} \quad (3)$$

442 where Θ_i^X is the set of parameters of the subject i for the compartment X and ε are the additive
 443 normally distributed measurement errors.

444

445

446 **Consideration of the volume of distribution**

447 To define the concentration of inoculum within each compartment after injection,
448 nasopharyngeal and tracheal volumes of distribution, labelled W^N and W^T respectively, were
449 requested. Given the estimated volumes of the trachea and the nasal cavities in four monkeys
450 similar to our 18 macaques (**Figure S9A-C**) and the well documented relationship between the
451 volume of respiratory tract and animal weights (65), the volume of distribution of each
452 compartment was defined as a step function of NHP weights:

$$\begin{aligned} W_i^N &= \begin{cases} 4 & \text{if Weight}_i \leq 4.5 \\ 5.5 & \text{otherwise} \end{cases} \\ W_i^T &= \begin{cases} 2 & \text{if Weight}_i \leq 4.5 \\ 3 & \text{otherwise} \end{cases} \end{aligned} \quad (4)$$

453 Where Weight_i is the weight of the monkey i in kgs. Using equation (4) and weights of our 18
454 NHPs (mean= 4.08 ; [Q1 ; Q3] = [3.26 ; 4.77]), we estimated $W^T = 2$ and $W^N = 4\text{mL}$ for a third
455 of them (n=12) (**Figure S9D**), leading to the initial concentration of target cells T_0^X (see “Viral
456 dynamics modeling” for equation) fixed at $3.13 \times 10^4 \text{ cells.mL}^{-1}$ and $1.13 \times 10^4 \text{ cells.mL}^{-1}$ in
457 nasopharynx and trachea respectively. Similarly, their initial concentrations of challenge
458 inoculum $V_{S,0}^X$ were fixed at $5.48 \times 10^8 \text{ copies.mL}^{-1}$ and $9.86 \times 10^9 \text{ copies.mL}^{-1}$ in nasopharynx and
459 trachea resp. For the last third of NHPs (n=6), $W^T = 3$ and $W^N = 5.5 \text{ mL}$ leading to T_0^X fixed at
460 $2.27 \times 10^4 \text{ cells.mL}^{-1}$ in nasopharynx and $7.50 \times 10^3 \text{ cells.mL}^{-1}$ in trachea while $V_{S,0}^X$ was fixed at
461 $3.98 \times 10^8 \text{ copies.mL}^{-1}$ in nasopharynx and $6.57 \times 10^9 \text{ copies.mL}^{-1}$ in trachea. Through this
462 modeling, we assumed a homogenous distribution of injected virions and target cells within
463 nasopharyngeal and tracheal compartments. In addition, the natural downward flow of inoculum
464 towards lungs, at the moment of injection, was indirectly taken into account by the parameter of
465 inoculum clearance, c_i .

466

467

468 **Parameter estimation**

469 Among all parameters involved in the three layers of the mechanistic model, some of them have
470 been fixed based on experimental settings and/or literature. That is the case of the proportion of
471 infectious virus (μ) that has been fixed at 1/1000 according to previous work (28) and additional
472 work (results not shown) evaluating the stability of the model estimation according to the value
473 of this parameter. The initial number of target cells, that are the epithelial cells expressing the
474 ACE2 receptor, $T_0^{X,nbc}$ was fixed at 1.25×10^5 cells in the nasopharynx and 2.25×10^4 cells in
475 trachea (28) (**Table S2**). The duration of the eclipse phase ($1/k$), the clearance of the inoculum
476 (c_i) and the clearance of the virus produced *de novo* (c) were estimated by profile likelihood.
477 Although available data did not allow the direct estimation of these three parameters, the use
478 profile likelihood enabled the exploration of various potential values for k , c and c_i . In a first
479 step, we explored the 18 models resulting from the combination of 3 values of $k \in \{1,3,6\} \text{ day}^{-1}$
480 and 6 values for $c \in \{1,5,10,15,20,30\} \text{ day}^{-1}$, assuming that the two parameters of virus
481 clearance were equal, as first approximation. As shown in **Table S3**, an eclipse phase of 8 hours
482 ($k=3$) and virus clearance higher than 15 virions per day led to lowest values of -2log-likelihood
483 (-2LL, the lower the better). In a second step, we fixed the parameter k at 3 day^{-1} and estimated
484 the 70 models resulting from the combination of 10 values for $c \in \{1,2,3,4,5,10,15,20,25,30\}$
485 day^{-1} and 7 values for $c_i \in \{1,5,10,15,20,25,30\} \text{ day}^{-1}$ (**Table S4**). The distinction of the two
486 parameters of free virus clearance enabled to find much lower half-life of inoculum (~50
487 minutes) than half-life of virus produced *de novo* (~5.55 hours), with $c=3 \text{ day}^{-1}$ compared to
488 $c_i=20 \text{ day}^{-1}$.

489

490 Once all these parameters have been fixed, the estimation problem was restricted to the
491 determination of the viral infectivity β , the viral production rate P , the loss rate of infected cells δ
492 for each compartment, the parameter α_{vlsq} in the observation model, regression coefficients for
493 groups of intervention (ϕ_{conv}, ϕ_{DC40}) and standard deviations for both random effects (ω) and
494 error model (σ). The estimation was performed by Maximum likelihood estimation using a
495 stochastic approximation EM algorithm implemented in the software Monolix
496 (<http://www.lixoft.com>). Selection of the compartment effect on parameters (β, δ, P) as well as
497 random effects and covariates on the statistical model (2) was performed by the estimation of
498 several models that were successively compared according to the corrected Bayesian information
499 criterion (BICc) (to be minimized). After the removal of random effect on the viral production
500 ($\omega_P = 0$) allowing the reduction of the variance on the two other random effects, all
501 combinations of compartment effects were evaluated, leading to the final selection of a single
502 effect on P ($f_\beta^T = f_\delta^T = 0$). Then, the effect of group intervention was independently added on
503 model parameters among β, δ, P and c . Once the group effect on the viral infectivity identified as
504 the best one, the addition of a second effect on the remaining parameters was tested, resulting in
505 the selection of the loss rate of infected cells. Finally, the irrelevance of the addition of a third
506 effect was verified.

507

508 **Exchange of viruses between nasopharynx and trachea compartments**

509 The possibility for viruses to migrate from nasopharyngeal to tracheal compartment and vice
510 versa was tested. To this end, equations of infectious (V_i) and non-infectious (V_{ni}) viruses in
511 equation (1) between the two compartments were linked as follows:

$$\begin{aligned}
\frac{dV_i^T}{dt} &\mapsto \frac{dV_i^T}{dt} - g_{TN}V_i^T + g_{NT}V_i^N & \frac{dV_{ni}^T}{dt} &\mapsto \frac{dV_{ni}^T}{dt} - g_{TN}V_{ni}^T + g_{NT}V_{ni}^N \\
\frac{dV_i^N}{dt} &\mapsto \frac{dV_i^N}{dt} + g_{TN}V_i^T - g_{NT}V_i^N & \frac{dV_{ni}^N}{dt} &\mapsto \frac{dV_{ni}^N}{dt} + g_{TN}V_{ni}^T - g_{NT}V_{ni}^N
\end{aligned} \tag{5}$$

512 with the arrow symbolizing the modification of the equations defined in (1) and where g_{TN} and
513 g_{NT} are the positive constant rates of exchange from trachea to nasopharynx and vice versa,
514 respectively. Data described in the main article were too much sparse to estimate either
515 bidirectional or at least one of the two unidirectional transfers defined by g_{TN} and g_{NT} , additional
516 data were used. Two naive macaques were exposed to the same dose (1×10^6 pfu) of SARS-CoV-
517 2 than our 18 monkeys but were inoculated via intra-gastric route (4.5mL) instead of intra-
518 tracheal route. Similarly to our study, the viral gRNA dynamics in both tracheal and
519 nasopharyngeal compartments were repeatedly measured during the 20 days following the
520 challenge (**Figure S9E**). The model resulting from equation (5) was used to fit these data,
521 considering all parameters as fixed (see **Table S2**), except for g_{TN} and g_{NT} . The estimation of
522 multiple models on those 2 animals tended to conclude that only an unidirectional transfer of
523 viruses from the nasopharyngeal to the tracheal compartment should be explored, with an
524 estimation of g_{TN} ranging from 0.9 to 2.5 day^{-1} . However, the use of those fixed values in the
525 estimation of the model on our 18 animals led irremediably to the degradation of the model with
526 an increase of more than 2 points of BICc. An estimation of this parameter by profile likelihood
527 (results not shown), resulting in a strictly decreasing profile of the likelihood (the higher the
528 better), was not more conclusive. Consequently, we fixed the values of g_{TN} and g_{NT} at 0 day^{-1} .

529

530 **Algorithm for automatic selection of biomarkers as CoP**

531 After identifying the effect of the group of intervention on both the viral infectivity (β) and the
532 loss rate of infected cells (δ), we aimed at determining whether some immunological markers

533 quantified in the study could capture this effect. To this end, we developed a classic stepwise
534 data-driven automatic covariate modelling method (**Figure S10**). The specificity of this method
535 is the possibility to add either time-dependent or constant covariates in the model.

536 At the initialization step ($k=0$) (see **Figure S10**), the algorithm requests 3 inputs: (1) a set of
537 potential M covariates, labelled Marker m for $m \in \{1, \dots, M\}$ (e.g., immunological markers) ; (2)
538 a set of P parameters on which covariates could be added, labelled θ_p for $p \in \{1, \dots, P\}$ (e.g. β
539 and δ) ; and (3) an initial model (e.g., the model without covariates), labelled M^0 , with θ_p^0 the
540 definition of the parameter θ_p . At each step $k>0$, we note M^{k-1} the current model resulting in the
541 model built in the step $k-1$. Then each combination of markers and parameters that have not
542 already been added in M^{k-1} , labelled r ($r \in \{\text{Marker } m \otimes \theta_p \notin M^{k-1} \mid m \in \{1, \dots, M\}, p \in$
543 $\{1, \dots, P\}\}$), are considered and tested in an univariate manner (each relation r is independently
544 added in M^{k-1} and ran). To this end, the parameter θ_p involved in this relationship r is modified
545 as $\theta_p^k(t) = \theta_p^{k-1}(t) \times \exp(\phi_m^p \times \text{Marker}_m(t))$, where ϕ_m^p is the regression coefficient related
546 the marker, while other parameters remain unchanged ($\forall \theta_q \notin r, \theta_q^k(t) = \theta_q^{k-1}(t)$). Once all
547 these models evaluated, the one with the optimal value of a given criteria defining the quality of
548 the fits (e.g., the lowest BICc value) is selected and compared to the model M^{k-1} . If its criteria
549 value is better than the one found for M^{k-1} , then this model is defined as the new current model,
550 M^k , and the algorithm moves to the step $k+1$. Otherwise, the algorithm stops. The algorithm can
551 also be stopped at the end of a fixed number of step K .

552 The objective of this algorithm being to identify mechanistic correlates of protection, at each
553 step, the selected model should respect, in addition to the best fits criteria, the 2 other criteria
554 defining mCoP meaning the ability to capture the effect of the group of intervention and the

555 ability to better explain the variability on individual parameters than the model adjusted on the
556 group effect. To this end, we verify that in the selected model additionally adjusted on the group
557 of intervention, the group effect appears as non-significantly different from 0 using a Wald-test.
558 Then, we check that the variances of random effects in the selected model are well lower or
559 equal to the ones obtained in the model adjusted only on the group effect.

560

561 **Quantification and statistical analysis**

562 Statistical significance of the effect of groups in model estimation is indicated in the tables by
563 stars: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ and were estimated by Wald test (Monolix®
564 software version 2019R1). In addition, statistical significance between viral loads in the two
565 published studies (Brouwer et al, Cell 2021 ; Marlin et al., Nat Com 2021) in the control group
566 were estimated by Welch two-sample t-test (R version 3.6.1) and are indicated in the
567 supplementary file by p value. Model parameters were estimated with the SAEM algorithm
568 (Monolix® software version 2019R1).

569 Graphs were generated using R version 3.6.1 and Excel 2016 and details on the statistical
570 analysis for the experiments can be found in the accompanying figure legends. Horizontal red
571 dashed lines on graphs indicate assay limit of detection.

572 **Supplementary Materials**

573 Fig. S1. Viral dynamics after the first exposure to SARS-CoV-2 and biomarker measurements
574 from the first to the second exposure to SARS-CoV-2.

575 Fig. S2. Subgenomic viral dynamics after the second exposure to SARS-CoV-2.

576 Fig. S3. Modelling of the viral dynamics using mechanistic model.

577 Fig. S4. Antibody measurements after the second exposure to SARS-CoV-2.

578 Fig. S5. Antigen-specific T-cell responses in NHPs after the second exposure to SARS-CoV-2.

579 Fig. S6. Cytokines and chemokines in the plasma in NHPs after the second exposure to SARS-
580 CoV-2.

581 Fig. S7. Immune markers selection and Basic reproduction number.

582 Fig. S8. The second study testing two-component spike nanoparticle vaccine.

583 Fig. S9. Modelling of the dynamics of viral replication.

584 Fig. S10. Flow chart of the algorithm for automatic selection of covariate.

585 Table S1. Criteria defining RBD-ACE2 binding inhibition or neutralization measured on live
586 cells with luciferase marker as mechanistic correlate of protection of the effect of the vaccine on
587 new cell infection.

588 Table S2. Model parameters for viral dynamics in both the nasopharynx and the trachea
589 estimated by the model adjusted for groups of intervention.

590 Table S3. Values of -2LL estimated on models with viral clearance ($c=c_i$) and eclipse phase rate
591 k fixed at different values.

592 Table S4. Values of -2LL estimated on models with inoculum clearance c_i and clearance of virus
593 de novo produced c fixed at different values.

594

595

596 **References and Notes**

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963 **Author contributions:**

964 Conceptualization: MA, RT, RLG, YL, RM

965 Methodology: MA, MP, RT

966 Software: MA, MP

967 Validation: MA, RT, MP

968 Investigation: RM, SC, NK, SC, TN, BD, MS, NDB, MC, PM, CL, AW

969 Resources: RM, SC, NK, SC, TN, BD, MS, NDB, MC, PM, CL, AW, OS, RWS, RLG, YL, MA

970 Writing – Original draft: RT, MA, YL, RLG, RM, MP

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977 **Competing interests:** Authors declare that they have no competing interests

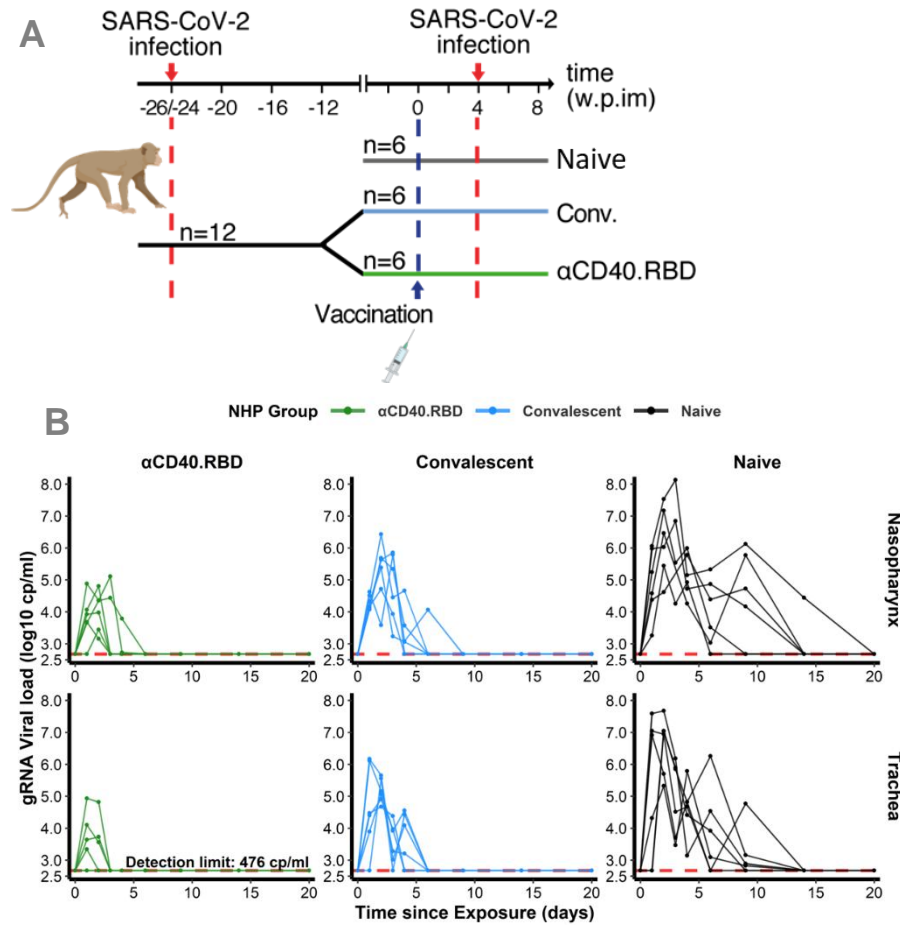
978 **Data and materials availability:** No unique reagents were generated for this study.

979 Data from the studies ³⁰ and ³⁷ are available upon request. Data from the study ²⁴ are available as

980 supplementary material online.

981 The original Monolix code is available and free-of-cost on github (Inria SISTM Team) at the

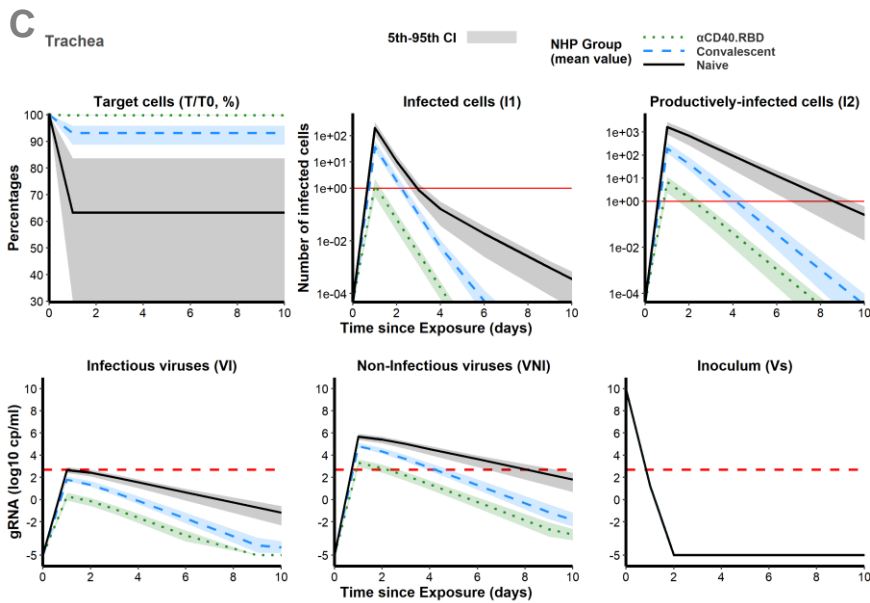
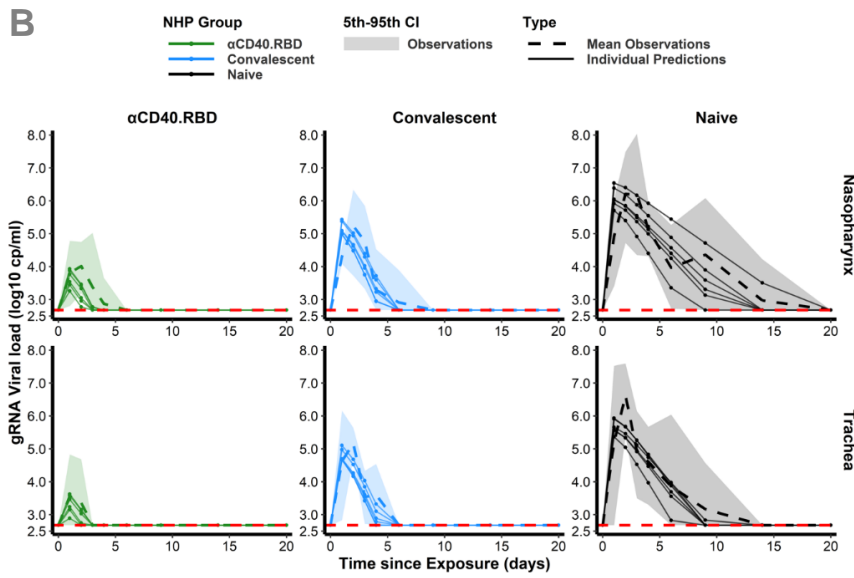
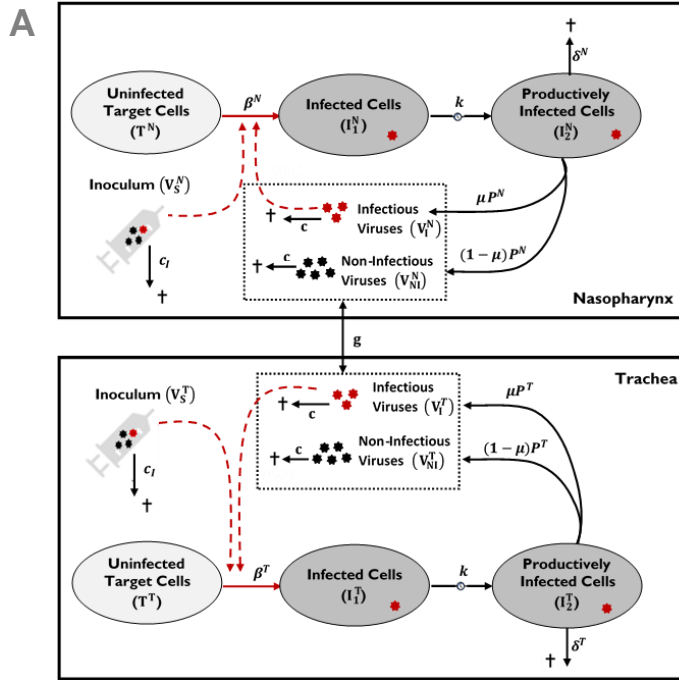
982 following link: <https://github.com/sistm/SARSCoV2modelingNHP>.



985 **Fig. 1. Design of the study 1 and viral dynamics.**

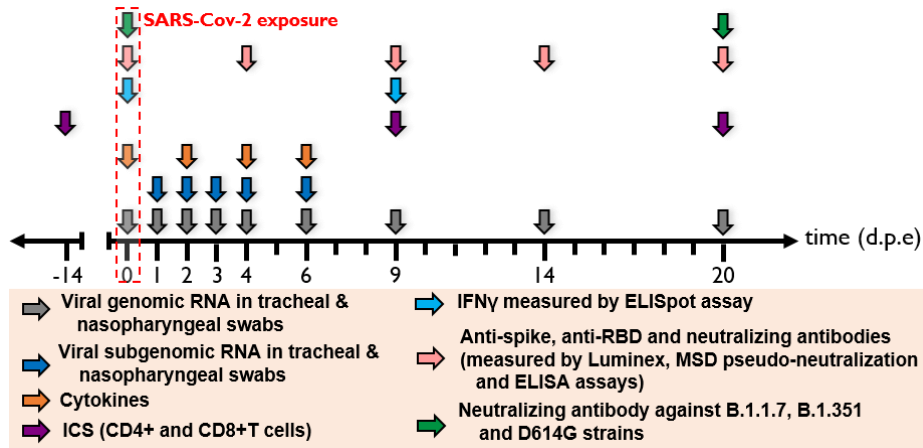
986 **(A)** *Study design.* Cynomolgus macaques (*Macaca fascicularis*), aged 37-58 months (8 females
 987 and 13 males). 24-26 weeks post infection with SARS-CoV-2, twelve of these animals were
 988 randomly assigned in two experimental groups. The convalescent vaccinated group (n=6)
 989 received 200 μg of αCD40.RBD vaccine. The other six convalescent animals were used as
 990 controls. Additional six age matched (43.7 months +/-6.76) cynomolgus macaques from same
 991 origin were included in the study as controls naive from any exposure to SARS-CoV-2. Four
 992 weeks after immunization, all animals were exposed to a total dose of 10⁶ pfu of SARS-CoV-2

993 virus via the combination of intra-nasal and intra-tracheal routes. **(B)** Individual log₁₀
994 transformed gRNA viral load dynamics in nasopharyngeal swabs (top) and tracheal swabs
995 (bottom) after the initial exposure to SARS-CoV-2 in naive macaques (black, right) and after the
996 second exposure in convalescent (blue, middle) and α CD40.RBD-vaccinated convalescent
997 (green, left) groups. Horizontal red dashed lines indicate the limit of quantification.



999 **Fig. 2. Mechanistic modelling.**

1000 **(A)** Description of the model in the two compartments: the nasopharynx and the trachea. **(B)**
1001 Model fit to the log₁₀ transformed observed gRNA viral loads in nasopharyngeal (top) and
1002 tracheal (bottom) compartments after the initial exposure to SARS-CoV-2 in naive macaques
1003 (black, right) and after the second exposure in convalescent (blue, middle) and vaccinated (green,
1004 left) animals. Solid thin lines indicate individual dynamics predicted by the model adjusted on
1005 the effect of group. Thick dashed lines indicate mean viral loads over time. Shaded areas indicate
1006 the 95% confidence interval. Horizontal red dashed lines indicate the limit of quantification. **(C)**
1007 Model predictions of unobserved quantities in the tracheal compartment for naive (black, solid
1008 lines), convalescent (blue, dashed lines) and vaccinated (green, dotted lines) animals: target cells
1009 as percentage of the value at the challenge (top, left), infected cells (top, middle), productively
1010 infected cells (top, right), inoculum (bottom, right), infectious (bottom, left) and non-infectious
1011 virus (bottom, middle). Thick lines indicate mean values over time within each group. Shaded
1012 areas indicate the 95% confidence interval. Horizontal dashed red lines indicate the limit of
1013 quantification and horizontal solid red lines highlight the threshold of one infected cell.



1014

1015 **Fig. 3. Harvest times and measurements.**

1016 Nasopharyngeal and tracheal fluids, were collected at 0, 1, 2, 3, 4, 6, 9, 14 and 20 days post
 1017 exposure (d.p.e) while blood was taken at 0, 2, 4, 6, 9, 14 and 20 d.p.e. Genomic and subgenomic
 1018 viral loads were measured by RT-qPCR. Anti-Spike IgG sera were titrated by multiplex bead
 1019 assay, Anti-RBD and anti-Nucleocapside (N) IgG were titrated using a commercially available
 1020 multiplexed immunoassay developed by Mesoscale Discovery (MSD, Rockville, MD). The
 1021 MSD pseudo-neutralization assay was used to measure antibodies neutralizing the binding of the
 1022 spike protein and RBD to the ACE2 receptor. Neutralizing antibodies against B.1.1.7, B.1.351
 1023 and D614G strains were measured by S-Fuse neutralization assay and expressed as ED50
 1024 (Effective dose 50%). T-cell responses were characterized as the frequency of PBMC expressing
 1025 cytokines (IL-2, IL-17 a, IFN- γ , TNF-a, IL-13, CD137 and CD154) after stimulation with S or N
 1026 sequence overlapping peptide pools. IFN- γ ELISpot assay of PBMCs were performed on PBMC
 1027 stimulated with RBD or N sequence overlapping peptide pools and expressed as spot forming
 1028 cell (SFC) per 1.0×10^6 PBMC.

Fig. 4. Immune markers.

(A) Dynamics of biomarker selected as mCoP. Quantification of antibodies inhibiting RBD-ACE2 binding, measured by the MSD pseudo-neutralization assay (ECL, in AU) (top) and anti-RBD IgG titrated by ELISA assay (in IgG titer) (bottom). Thin lines represent individual values. Thick lines indicate medians within naïve (black, solid line), convalescent (blue, dashed line) and α CD40.RBD-vaccinated convalescent (green, dotted line) animals. Shaded areas indicate 5th-95th confidence intervals. **(B) Systematic screening of effect of the markers.** For every single marker, a model has been fitted to explore whether it explains the variation of the parameter of interest better or as well than the group indicator. Parameters of interest were β , the infection rate of ACE2+ target cells, and δ , the loss rate of infected cells. Models were compared according to the Bayesian Information Criterion (BIC), the lower being the better. The green line represents the reference model that includes the group effect (naïve/convalescent/vaccinated) without any adjustment for immunological marker (see **Figure 3** for more details about measurement of immunological markers). **(C) Thresholds of inhibition of RBD-ACE2 binding.** Estimated infection rate (in $(\text{copies/mL})^{-1} \text{ day}^{-1}$) of target cells according to the quantification of antibodies inhibiting RBD-ACE2 (in ECL) at exposure. Thin dotted lines and circles represent individual values of infection rates (right axis) and neutralizing antibodies (left axis). Shaded areas delimit the pseudo-neutralization / viral infectivity relationships within each group. **(D) Reproduction rate over time.** Model predictions of the reproduction rate over time in the trachea (right) and nasopharynx (left). The reproduction rate is representing the number of infected cells from one infected cell if target cells are unlimited. Below one, the effective reproduction rate indicates that the infection is going to be cured. Horizontal solid red lines highlight the threshold of one. Same legend than A). **(E) Conditions for controlling the infection.** Basic reproduction rate at the time of

the challenge according to the levels of antibodies inhibiting RBD-ACE2 binding (the lower the better) and of anti-RBD IgG binding antibodies (the higher the better) assuming they are mechanistic correlates of blocking new cell infection and promoting infected cell death, respectively. The red area with $R > 1$ describes a situation where the infection is spreading. The green area with $R < 1$ describes a situation where the infection is controlled. The dotted red line delimitates the two areas. Black long dashed lines represent the values of neutralizing and binding antibodies measured at exposure. Observed values for three different animals belonging to the naive (bottom, right), convalescent (bottom, left) and vaccinated (top, left) groups are represented.