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► To cite this version:

Stéphanie Reynard, Xavier Carnec, Caroline Picard, Virginie Borges-Cardoso, Alexandra Journeaux, et al.. A MOPEVAC multivalent vaccine induces sterile protection against New World arenaviruses in non-human primates. *Nature Microbiology*, 2023, 8 (1), pp.64-76. 10.1038/s41564-022-01281-y . hal-03931828

HAL Id: hal-03931828

<https://hal.science/hal-03931828v1>

Submitted on 9 Jan 2023

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8 **A MOPEVAC multivalent vaccine induces sterile protection against New World**

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Arenaviruses in non-human primates

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37 **ABSTRACT**

38 **Pathogenic New World arenaviruses (NWAs) cause hemorrhagic fevers and can have high mortality**
39 **rates, as shown in outbreaks in South America. Neutralizing antibodies (Nabs) are critical for protection**
40 **from NWAs. Having shown that the MOPEVAC vaccine, based on a hyper-attenuated arenavirus, induces**
41 **NAbs against Lassa fever, we hypothesized that expression of NWA glycoproteins in this platform might**
42 **protect against NWAs. Cynomolgus monkeys immunized with MOPEVAC_{MAC}, targeting Machupo virus,**
43 **prevented the lethality of this virus and induced partially NWA-cross-reactive Nabs. We then developed**
44 **the pentavalent MOPEVAC_{NEW} vaccine, expressing glycoproteins from all pathogenic South American**
45 **NWAs. Immunization of cynomolgus monkeys with MOPEVAC_{NEW} induced Nabs against five NWAs,**
46 **strong innate followed by adaptive immune responses as detected by transcriptomics, and provided**
47 **sterile protection against Machupo virus and the genetically distant Guanarito virus. MOPEVAC_{NEW} may**
48 **thus be efficient to protect against existing and, potentially, emerging NWAs.**

49 Introduction

50

51 New World arenaviruses (NWAs) are causative agents for severe hemorrhagic fevers (HFs). Among the
52 four clades of NWAs, clade B contains all the pathogenic strains with the exception of Whitewater Arroyo
53 virus (WWAV), belonging to clade A/B¹. These pathogenic strains share the same entry receptor, human
54 transferrin receptor 1². Pathogenic NWAs are a public health concern, with Junin virus (JUNV) being
55 endemic in Argentina and having caused hundreds of cases each year before the Candid#1 vaccine was
56 introduced^{3,4}. However, this vaccine is not FDA-approved and induces adverse effects⁵⁻⁷. Machupo virus
57 (MACV) recently re-emerged and caused hundreds of cases^{8,9}. Consequently, vaccine approaches have
58 been proposed^{10,11}. The three other pathogenic NWAs, Sabia virus (SABV), Guanarito virus (GTOV), and
59 Chapare virus (CHAV) have so far emerged only sporadically¹²⁻¹⁴. The evolution of rodent populations, the
60 natural reservoir for NWAs, has been associated with human incidence¹⁵⁻¹⁷. Depending on the weather,
61 increase in rodent populations can occur and cause viral emergence. The absence of treatment or
62 prophylaxis for all the NWAs exposes populations to sporadic viral circulation. Moreover, new pathogenic
63 arenaviruses regularly emerge, such as WWAV in 1999/2000^{18,19}, CHAV¹⁴ in 2003/2004, and Lujo virus²⁰ in
64 2008.

65 Treatment of JUNV-infected patients with convalescent plasma is efficient during acute disease²¹.
66 However, late onset encephalitis with high lethality has been reported in animal models^{22,23}, suggesting
67 that this approach can control acute infection but fails to prevent viral persistence at immunologically
68 privileged sites. Nevertheless, neutralizing antibodies (NAbs) are crucial for the control of NWAs^{21,22,24}. We
69 previously developed MOPEVAC, a hyper-attenuated Mopeia virus (MOPV)-based vaccine platform, and
70 showed its efficacy against the Old World arenavirus Lassa virus (LASV) in macaques^{25,26}. MOPEVAC_{LAS}
71 induced a T-cell response, crucial in counteracting LASV infection, as well as a robust antibody response²⁶.

72 We thus hypothesized that MOPEVAC, which induces Nabs, could protect against NWAs. We first
73 developed MOPEVAC_{MAC}²⁵, the MOPEVAC platform expressing MACV glycoproteins. Vaccinated animals
74 developed an antibody response with Nabs and were protected against a lethal challenge with MACV.
75 Then, we developed MOPEVAC_{NEW}, a pentavalent vaccine expressing epitopes from all five pathogenic
76 NWAs known in South America. Immunized animals produced antibodies against the five antigens and
77 they were protected against a challenge with MACV and the phylogenetically distant GTOV. This vaccine
78 could provide a means of preemptively protecting against the re-emergence of known NWAs.

79

80 **Main text**

81

82 **MOPEVAC_{MAC} induces MACV-specific antibody responses**

83 Cynomolgus monkeys (CMs) received MOPEVAC_{MAC} vaccine with a single dose (n=4) or a prime-boost
84 strategy (n=4). Three CMs received the excipient. All were challenged with MACV (Fig. 1a).

85 MOPEVAC_{MAC} comprises a hyper-attenuated MOPV expressing MACV glycoproteins. Activity of the
86 exonuclease virulence factor was abolished by six mutations in the nucleoprotein^{25,27-30}, ensuring
87 attenuation of MOPV, already known to be nonpathogenic (Fig. 1b). Infection of antigen presenting cells
88 with MOPEVAC results in immune activation and in the lack of viral replication. We previously showed that
89 we can swap the gene encoding for glycoproteins (GPC gene) with any GPC gene from arenaviruses²⁵. Here,
90 we demonstrated that equal amounts of glycoproteins were expressed in infected cells by the different
91 MOPEVAC expressing the NWAs glycoproteins. The ratio between RNA amounts of GPC at day 3 *versus*
92 day 0 was similar for all MOPEVAC (Extended data Fig. 1a). Similar amounts of GP2 protein were contained
93 in all MOPEVAC viruses, except for SABV whose GP2 was not recognized by our antibodies (Extended data
94 Fig. 1b). The replication of MOPEVAC was tested with different NWA GPC. They all replicated equally and
95 were stable over multiple passages (fig. 1c), as previously shown for some candidates²⁵. We sequenced
96 the viruses at passage 2, 5 and 10 to confirm the absence of major changes in the consensus genome
97 sequence after passages. No mutation was consistently present in all GPC sequences after ten passages,
98 or in the 6 amino acids mutated to abrogate the exonuclease domain (Supp. Table 1). Only one or two
99 amino acid changes were observed in the genome in vaccine candidates but without consequence for
100 replication. These results confirm the stability of all MOPEVAC_{NEW} components over multiple passages and
101 in particular of the immunogenic antigen and of the attenuation phenotype.

102 MOPEVAC_{MAC} did not induce any appreciable adverse effect *in vivo*. We never detected vaccine shedding:
103 no MOPEVAC RNA was found in plasma, urine, or nasal and oral swabs. We detected MACV-specific IgG
104 from day 9 post-immunization and the antibody titers rose to 1,000 during the first month (fig. 1d). The
105 boost resulted in a rapid increase in antibody release, with titers reaching 16,000, the upper limit of our
106 test. By day 14 after the first injection, 6 of 8 animals showed NABs, mainly at low titers. By day 30, all
107 animals were positive. The second vaccine injection resulted in neutralizing titers of 100 in all animals.
108 Thus, MOPEVAC_{MAC} promotes a NAB response.

109 **MOPEVAC_{MAC} induces sterile protection against MACV**

110 After the immunization period, all animals were challenged with MACV. A clinical score was calculated
111 each day based on the symptoms observed (Supp. Table 2). This score remained low throughout the
112 experiment for vaccinated animals, with no difference between the vaccination regimens. Unvaccinated
113 animals experienced fever from days 4 to 5 (Extended data Fig. 2) and from day 8, they presented with
114 balance disorders, dehydration, and reduced interactions. They eventually reached the ethical endpoint
115 between days 11 and 13 because of the absence of reactivity, intense dehydration, and epistaxis. They
116 continuously lost weight from day 4 (fig. 2a), and experienced profound lymphopenia and
117 thrombocytopenia, with a drop in hemoglobin concentration observed for 2/3 animals (Extended data Fig.
118 3a). They also presented increasing levels of alanine aminotransferase (ALT), aspartate aminotransferase
119 (AST) and plasma urea, and a decrease in plasma albumin concentrations (Fig. 2b), suggesting liver and
120 tissue damage, as well as renal injury. No alterations in hematological and biochemical parameters were
121 detected in the vaccinated animals.

122 Control animals presented increasing titers of MACV from day 6 after infection in plasma and oral/nasal
123 swabs (Fig. 2c). At the day of euthanasia, the infection was pantropic, all the organs tested were MACV-
124 positive (Extended data Fig. 3b). However, we never detected viral RNA in any of the samples of the

125 vaccinated animals, neither in the fluids nor in the organs. Vaccination, even with a single dose, was able
126 to control MACV replication. Thus, MOPEVAC_{MAC} induces a sterilizing immunity in CMs.

127 In the vaccinated animals, we did not observe any increase in IgG titers after challenge (Fig. 2d). To ensure
128 that a low increase in antibody levels did not occur, we represented the optical density at a single dilution.
129 A slight decrease occurred for the prime boost group but did not alter the IgG titer and the values remained
130 stable for prime only group. However, NAb levels increased after challenge. The titer was finally at 100 for
131 all vaccinated animals with no heterogeneity depending on the vaccination regimen. In the control
132 animals, specific antibodies were never detected.

133 We determined whether MOPEVAC_{MAC} could cross-neutralize other NWAs by assaying plasma samples
134 from the immunization period for their ability to neutralize MOPEVAC_{JUN,GTO,CHA}: MOPEVAC expressing
135 respectively JUNV, GTOV and CHAV glycoproteins. Although all plasma samples presented detectable NAb
136 against MACV at the end of the prime period, this was not true with other NWAs (Fig. 2e). The boost
137 injection resulted in an increase in cross-reactive NAb except for in one animal that did not neutralize
138 GTOV. Although titers were lower than for the homologous virus, MOPEVAC_{MAC} induced cross-neutralizing
139 antibodies.

140 **MOPEVAC_{NEW} induces antibody responses against five NWAs**

141 Given the efficacy of MOPEVAC_{MAC}, we considered the generation of a multivalent vaccine protecting
142 against all five pathogenic South American arenaviruses. This pentavalent vaccine, named MOPEVAC_{NEW},
143 includes five MOPEVAC vaccines, each expressing MACV, GTOV, CHAV, SABV, or JUNV GPC.

144 We vaccinated six CMs with MOPEVAC_{NEW} in a prime-boost protocol, and six other animals received the
145 vehicle (Fig. 3a). We did not observe any clinical signs during the immunization period (Extended data Fig.
146 4a). We also failed to detect vaccine shedding in the days following the injections (until day 9 after prime
147 and day 5 after boost). The neutralizing titers of plasma samples were heterogeneous depending on the

148 MOPEVAC viruses targeted (fig. 3b). Some plasma samples did not neutralize all viruses at the end of the
149 prime period. This result could be explained by higher detection cut-off for this experiment compared to
150 that in the MOPEVAC_{MAC} experiments. NAbs against all viruses were finally detectable in all animals after
151 the boost with the strongest response detected against JUNV.

152 The kinetics of these antibody responses were similar to that observed with MOPEVAC_{MAC}. Vaccinated
153 animals produced IgG against MACV and GTOV, with a strong increase observed after the boost. However,
154 one animal from each control group presented low Ab levels at the end of the immunization period (Fig.
155 3c). We did not observe vaccine shedding and vaccinated CMs were not in contact with the controls. This
156 result is therefore probably due to cellular contents present in the mock vaccine and also present in
157 antigen preparations. To check for the presence of antibodies against other NWAs, we incubated plasma
158 samples with 293T cells expressing GPCs. Despite differences in the level of recognition, only plasma from
159 the vaccinated animals recognized NWA GPCs. There was no cross-reactivity with MOPV GPC (Fig 3c,
160 Extended data Fig.5).

161 **MOPEVAC_{NEW} induces sterile protection against MACV and GTOV**

162 Three vaccinated and three control animals were each challenged with either MACV or GTOV. MACV and
163 GTOV infection induced illness in control animals (Fig. 4a). The evolution of the disease after MACV
164 infection was highly similar to that of the first experiment. Animals reached the ethical endpoint between
165 days 12 and 18. One was euthanized despite a clinical score of 13, due to a weight loss of 27%. GTOV
166 infection induced symptoms two days later, including reduced activity, gastrointestinal symptoms, weight
167 loss, and fever (Extended data Fig. 4b). One GTOV-control animal reached the endpoint on day 14 (Fig. 4a).
168 The two remaining CMs presented maximum scores of 12 and 13 at day 16. These scores then decreased
169 and by day 29, were at 10 and 5, respectively. This latter animal presented with a weight loss of 22%, which
170 should have been an endpoint (Fig. 4a). None of the vaccinated CMs experienced clinical signs; the low

171 score observed was due to diarrhea, which was also observed for certain animals before the challenge and
172 probably unspecific.

173 The three MACV infected controls presented viral replication in fluids and in the organs highly similar to
174 that observed in the first experiment (Fig. 4b, Extended data Fig. 6a). GTOV-infected controls presented
175 lower infectious titers in the fluids but high viral RNA loads (fig. 4b). Viral RNA was also measured in the
176 organs of the deceased GTOV control but at lower titers. Infectious particles were detected mainly in the
177 secondary lymphoid organs, the liver, the ovaries and the intestine (Extended data Fig. 6a). At the end of
178 the protocol, the healthiest remaining GTOV-control presented the lowest viral titers in the fluids and
179 organs. The other presented viral RNA in many organs but infectious particles were found only in the
180 adrenal gland. We detected viral RNA in all cerebrospinal fluids (CSF) from control animals at the peak of
181 the disease but only two MACV-infected animals had infectious particles (Extended data Fig. 6b). This
182 indicates that the virus can cross the blood-brain barrier. The vitreous humor contained viral RNA in one
183 surviving GTOV-infected control. We never detected viral RNA in the organs or fluids of vaccinated animals,
184 showing that the vaccine induced a sterilizing immunity as observed with MOPEVAC_{MAC}. Moreover, no
185 significant change was observed in vaccinated animals for hematological and biochemical parameters,
186 unlike control animals (Extended data Fig. 7 a and b, respectively).

187 The IgG titers against GTOV increased after challenge for vaccinated animals and eventually reached the
188 IgG titer measured against MACV (Fig. 5a). Two out of the three MACV-infected control animals had
189 developed specific IgG by their respective endpoints (day 12 and 18). The GTOV-infected controls that
190 survived showed increasing antibody levels from day 16. The vaccinated CMs boosted the production of
191 NAbS specific of the virus used for their challenge (fig. 5b). We did not observe any NAbS in MACV-infected
192 controls but all GTOV-infected controls showed significant levels of Nabs at day 12. In one of the animals
193 that survived, the titer rose to 2,000, the highest titer measured. Unexpectedly, one of the MACV-controls

194 showed a low NAb titer against MOPEVAC_{CHA} and _{SAB}, but not against wild-type MACV (Fig. 5c). As we used
195 whole viruses for the experiments, this must have increased the risk of nonspecific neutralization.

196 **Immune responses involved in protection**

197 We evaluated the expression profiles of genes related to innate, T-cell and B-cell responses using a
198 transcriptomic analysis on PBMCs from the MOPEVAC_{MAC} experiment³¹. Control animals were included,
199 with day 30 samples, the day of the second vehicle injection. After immunization, we observed strong and
200 transient activation of the innate immune response in the first two days. The boost reactivated the
201 response to a lower extent, before significant downregulation from day 5 (Fig. 6a, Supp. Table 3). The T-
202 cell response was also upregulated in a very significant manner from days 4 and 5 after the prime, and
203 from day 2 after the boost. The boost was therefore efficient in reactivating the T-cell immune response.
204 At the day 0 timepoints, expression of B-cell response-related genes implicated was heterogeneous,
205 including for controls, but the mean of expression in the pathway was comparable. Expression of genes
206 related to B-cell response was quickly and significantly downregulated from day 2 after vaccination until
207 day 9 after the prime or day 5 after the boost.

208 We looked for the presence of circulating MACV-specific T cells. After immunization, we stimulated PBMCs
209 with overlapping peptides from NP and GP proteins. Super-antigen staphylococcal enterotoxin A (SEA)
210 was used as a positive control. This stimulation did not activate expression of CD154 or CD137, markers of
211 CD4 and CD8 T cell activation respectively, and of IFN γ , or GrzB (indicators of cytotoxic response), in
212 contrast to the strong activation induced by SEA (Extended data Fig. 8 and 9a). After challenge, we only
213 observed, following whole blood stimulation, a slight expression of IFN γ around day 15 in only one over 8
214 animals (Extended data Fig. 9b).

215 Transcriptomic analysis on PBMCs after challenge showed a strong and early innate immune response in
216 control animals, which lasted until the end of the experiment. The T-cell response was downregulated until

217 day 6, and upregulated from day 9, in the very last days of the illness. The B-cell response was increasingly
218 downregulated during the course of the disease (Fig. 6b, Supp. Table 3).). The day 0 was highly similar to
219 that of the point taken during immunization period. For the vaccinated animals, we observed only a
220 modest regulation of these pathways, more significant in the prime only group.

221 **Discussion**

222

223 Despite currently low incidence of human cases, NWAs are of concern due to the risk of emergence and
224 the severity of the disease³². Immunization with the JUNV Candid#1 vaccine enabled the endemic virus to
225 be efficiently controlled. There are also vaccines for MACV^{10,11,33} at the preclinical stage. Here, we provide
226 a vaccine candidate validated in a non-human primate model able to confer sterile protection against two
227 distant viruses and induce NAbs against all pathogenic South American NWAs. Bivalent vaccines were
228 previously tested but failed to provide sterile protection against different viruses^{10,33}. Moreover, a study
229 of NAbs in the plasma of Candid#1 vaccinated patients showed that there was no cross-neutralization with
230 other NWA³⁴. Yet, both known and unknown arenaviruses present a high risk of emergence or re-
231 emergence. For example, WWAV and CHAV emerged in the last decades, while MACV, first described in
232 the 60's after being responsible for 637 cases³⁵, caused sporadic cases until 2006, and re-emerged to cause
233 more than 200 cases in 2008. CHAV also caused an outbreak in 2019³⁶ and GTOV in 2021³⁷. Despite the
234 quite low incidence of human contaminations, cases of human-to-human transmission were documented,
235 mainly to health care workers and laboratory staff^{35,36,38}. The recent Ebola virus epidemics and SARS-CoV-
236 2 pandemic have shown the importance of preparedness and, concerning vaccines, the necessity of having
237 products fully validated at the preclinical level and ready to go into the clinic. During the 2014-2016 Ebola
238 outbreak, the use of Ervebo[®] vaccine helped to control the outbreak and accelerated licensing of the
239 vaccine³⁹. The low incidence of individual NWAs favors development of an easy to produce, affordable
240 multivalent vaccine. The production of MOPEVAC_{NEW}, composed of five attenuated viruses, represents a
241 challenge, but all viruses replicate similarly and optimization of the production could provide an efficient
242 method suitable for all five viruses. Eventually, this vaccine could protect the entire South American
243 continent against NWAs.

244 We demonstrated that MOPEVAC could protect against NWAs. We were able to fully protect CMs against
245 MACV and GTOV infection, whereas in the LASV experiment, 3/4 CMs experienced fever and low transient
246 replication of the virus after challenge²⁶. NAbS are crucial in protection against NWAs^{21,22,24}, the induction
247 of NAbS following immunization met our expectations. We did not find persistent virus in any of the organs
248 or samples tested, suggesting that the late onset encephalitis should not occur with MOPEVAC. As we
249 detected low neutralizing titers against heterologous viruses after MOPEVAC_{MAC} immunization, we created
250 a multivalent vaccine against all known NWAs from South America. MOPEVAC_{NEW} was efficient against two
251 phylogenetically distant NWAs. Thus, we do not face low specificity and inefficient cross-neutralization as
252 sometimes observed with multivalent vaccines.

253 We observed cross-neutralization between NWAs. Plasma samples from MACV-immunized CMs partially
254 neutralized other NWAs GPCs. Interestingly, such broad NAbS are not always induced after NWA
255 immunization^{34,40}. Studies have demonstrated that NAbS against NWAs did not share the same binding
256 site. JUNV-NAbS mainly used transferrin receptor mimicry while MACV-NAbS did not. However, NAbS able
257 to neutralize both JUNV and MACV were found in plasma from Candid#1 vaccinated donor⁴¹, a conserved
258 domain in the receptor-binding site was identified. Cross-neutralization observed in MACV-vaccinated
259 animals may thus be due to such antibodies. The MOPEVAC_{NEW} vaccine expresses GPs from all NWAs and
260 thus induced many NAbS. We observed an increase in NAb titers specifically against the virus used for the
261 challenge. This suggests that a pool of NAbS is induced and that the infection boosts the synthesis of NAbS
262 that are the most specific for the challenge virus. NAbS therefore seem important for protection although
263 this was not mechanistically shown.

264 The immune responses promoted after immunization and challenge were different from those observed
265 with MOPEVAC_{LAS}²⁶. We did not detect the activation of T cells after peptide stimulation, suggesting that
266 the vaccine did not predominantly induce a Th1 response and/or cytotoxic T cells like MOPEVAC_{LAS}²⁶. This
267 difference is unlikely to be due to a change in primary target cells, since both NWAs and LASV, target

268 antigen-presenting cells. However, the GPC carried by MOPEVAC could affect the response. Indeed,
269 previous studies have linked the presence of N-glycans on GP1 to low neutralization capacity of NAbs⁴².
270 Interestingly, JUNV is the most efficiently neutralized virus and its GP1 is the least glycosylated^{42,43}.

271 Transcriptomic analysis of PBMCs showed that both vaccination and infection of control animals are
272 responsible for a strong IFN response, which is consistent with *in vitro* and *in vivo* studies^{44–46}. Double-
273 stranded RNAs (dsRNA) accumulate in NWA-infected cells and thus activate RIG-I like receptors⁴⁷. In
274 MOPEVAC, the mutations in the exonuclease domain prevent dsRNA degradation. Consistently, both
275 immunization and challenge induce the overexpression of genes implicated in RIG-I like receptor signaling:
276 *IFIH1* (MDA5), *DDX58* (RIG-I), *DHX58* (LGP2) and *DDX60*. *TLR7*, involved in single stranded RNA recognition,
277 was upregulated after challenge. Overall, upregulation of *MX1* and *MX2* genes involved in IFN signaling is
278 observed. After immunization, a significant T-cell response took place from day 4 and was reactivated
279 quickly after the boost injection. Interferon- γ , TNF α , Granzyme B and perforin gene expression did not
280 appear to be significantly regulated, reinforcing the hypothesis of a non-cytotoxic T-cell response. The
281 genes associated with B-cell responses were significantly downregulated in the first days after vaccination.
282 This could be due to the recruitment of B cells to the germinal centers to promote Ag recognition and
283 initiation of the humoral response. The observed T-cell response, associated with B-cell regulation,
284 suggests the induction of a humoral response dependent on T helper cells. This results in the strong
285 induction of antibodies and provides protection with sterilizing immunity. NAbs were previously used as a
286 treatment and succeeded in resolving the acute phase of the disease^{21,22,24}. Here, the presence of NAbs at
287 the time of infection may enable viral elimination before dissemination, particularly into the brain,
288 avoiding the risk of late onset encephalitis.

289 MOPEVAC_{NEW} is an efficacious vaccine against MACV and GTOV, two distinct arenaviruses. We detected
290 cross-reactive NAbs produced in response to MOPEVAC_{MAC} and NAbs against all NWAs tested in response
291 to MOPEVAC_{NEW}. Thus, this vaccine could protect against all NWAs, including some that have still not

292 emerged. We demonstrated that this vaccine is safe. Completion of preclinical and clinical development
293 could provide a ready-to-use solution for a future emergence.

294

295 **Methods**

296

297 **Study design**

298 For the first experiment, 11 male CMs (*Macaca fascicularis*) were injected with vehicle or vaccine. The
299 animals were 2.5 years old and weighed 2.3 to 3.9 kg. There were no significant differences in these
300 parameters between the groups. Immunization was performed in a BSL2 animal facility (SILABE, France).
301 Three animals received the vehicle and four animals received MOPEVAC_{MAC} 67 and 37 days before
302 challenge (controls and Prime/boost group respectively). The four remaining animals received a single
303 dose of MOPEVAC_{MAC} 37 days before challenge (Prime only group). The vaccine consisted of 2 x 10⁶ ffu
304 injected intramuscularly. Blood, urine, and oral and nasal swabs were sampled periodically.

305 Animals were challenged in the BSL4 laboratory (P4 Jean Mérieux-INSERM, Lyon, France) after 10 days of
306 acclimation. They all received 3,000 ffu of MACV (strain Carvallo) subcutaneously. Samples were taken
307 every two days until day 6, every three days from day 6 to day 18, and on day 22. Each sampling was
308 performed under anesthesia (Zoletil 100, 0.1 ml/kg). Each day, the state of the animals was evaluated, and
309 a clinical score was calculated based on behavior, body temperature, dehydration, weight loss, clinical
310 signs, and reactivity. A clinical score ≥ 15 or weight loss $> 20\%$ were defined as an endpoint in the protocol
311 and the animal was euthanized. The end of the protocol was planned for days 28/29. Euthanasia was
312 performed under anesthesia with a 5 ml intracardiac injection of Dolethal or Euthasol and samples then
313 taken as for previous sampling and the organs collected.

314 Animals were implanted with intraperitoneal body temperature recording systems (EMKA technologies)
315 before the beginning of the experimentation. However, most were defective. We implanted new
316 subcutaneous systems before the challenge (Star Oddi) but also experienced a number of issues and could
317 finally obtain the temperature recording after challenge for only seven animals (3 controls, 3 prime only,

318 and 1 prime/boost). Moreover, the new implantation resulted in local inflammation that was still present
319 for some animals at the day of challenge.

320 The second experiment was conducted in the same laboratories using similar protocols and procedures.
321 The body temperature was efficiently recorded throughout the procedure using intraperitoneal loggers
322 (Star Oddi). Twelve female CMs were used. They were almost three years of age and weighed 2.5 to 3.4
323 kg. Six received the vehicle and six were vaccinated with MOPEVAC_{NEW} (2.10^6 ffu i.e 4.10^5 ffu of each
324 valence). The immunization was performed on days 0 and 56. The animals were transferred to the BSL4
325 laboratory on day 89. After a period of acclimation of 10 days, they were challenged with an expected
326 dose of 3,000 ffu. We titered the virus dilution used for challenge and it was actually 4,500 ffu for MACV
327 and 3,000 ffu for GTOV. Six animals were inoculated with each virus: three vaccinated and three
328 unvaccinated. The sampling interval was extended due to the lower weight of the animals.

329 **Ethical statements**

330 The protocol of the first experiment was approved by the “Comité Régional d’Ethique en Matière
331 d’Expérimentation Animale de Strasbourg” for the immunization period and registered with the number
332 APAFIS#18970-2019020616112503 v8 (2019/07/23) and by the ethical committee “CELYNE” (Lyon,
333 France) for the challenge procedure and registered with the number
334 APAFIS#18397_2019011010351235_v4 (2019/03/15).

335 The protocol of the second experiment was approved by the same ethical committees and registered with
336 the numbers: APAFIS#18970-2019020616112503 v8 (2019/07/23) for the immunization protocol and
337 APAFIS#28798_2020122311384240_v2 (2021/02/11) for the challenge procedure.

338 **Cell lines**

339 VeroE6 cells and 293T cells were used in this study, from ATCC (CRL-1586 and CRL-3216 respectively).

340 **Viruses**

341 The MOPEVAC platform consists of a MOPV (strain AN21366; GenBank accession numbers JN561684 and
342 JN561685) that carries the GPC of the virus of interest in place of its own GPC and is mutated in the NP
343 gene to abolish the exonuclease function²⁵. The resulting attenuated virus was produced in VeroE6 cells
344 cultivated in DMEM, 2% FCS. MOPEVAC_{MACV} was then concentrated by centrifugation in filter tubes with a
345 1,000 kDa cutoff. A vehicle solution was prepared with uninfected VeroE6 supernatant under the same
346 conditions and was used in the control animals in place of the vaccine injection.

347 MOPEVAC_{NEW} is a mixture of equivalent quantities of infectious particles of MOPEVAC expressing the GPC
348 of MACV (strain Carvallo; GenBank accession number AY619643), GTOV (strain INH95551; GenBank
349 accession number AY129247), CHAV (strain 810419; GenBank accession number NC_010562), SABV (strain
350 SPH114202; GenBank accession number NC_006317), and JUNV (strain P2045; GenBank accession number
351 DQ854733). It was produced under the same conditions, except for the concentration method. The cell
352 supernatant was precipitated using a PEG solution (Abcam). After overnight incubation at 4°C with gentle
353 agitation, it was centrifuged for 3 h at 4,696 x g and the pellet was resuspended in DMEM, 2% FCS.

354 For stability experiments, Vero E6 cells were used as described above. The stability was tested until
355 passage 10, starting from passage 2. After each passage, viral RNAs harvested in supernatants at day 4
356 were quantified by RT-qPCR. VeroE6 cells were then infected with the supernatant using ten copies of
357 genome per cell. The viruses harvested in supernatants at passages 2, 5, and 10 were sequenced on
358 MiniSeq (Illumina) and analyzed using the public platform Galaxy⁴⁸. Briefly, RNA was extracted from 1ml
359 of supernatant with the QIAamp Viral RNA Mini Kit (Qiagen) according to manufacturer's instructions. The
360 RNAs were rigorously treated with Turbo DNase (Ambion, ThermoFisher) and concentrated by ethanol
361 precipitation. Then, cytoplasmic and mitochondrial ribosomal RNAs were removed using the NEBNext®
362 rRNA depletion kit v2 (human/mouse/rat). The libraries were prepared using the NEBNext® ultra II RNA
363 library prep for illumina® with 6 minutes of RNA fragmentation and 16 cycles of amplification. Finally,

364 quality and the concentration of libraries were determined by using the High Sensitivity D5000 Screentape
365 assay on a TapeStation (Agilent). Sequencing was performed using an illumina Miniseq platform with 150-
366 base paired ends and single indexing for each library. The loading concentration on the flow cell for the
367 sequencing was 1.45pM from a pool of normalized concentration of 18 libraries. For data analysis, reads
368 were trimmed according to the quality score (99%) and length (reads below 80bp were removed) and
369 illumina adapter were deleted using trimmomatic V0.38. Trimmed fastq files were then mapped onto the
370 genome of rescued viruses using bowtie2 V2.4.5 and PCR duplicates were removed using MarkDuplicates.
371 Finally, consensus sequences were called by using ivar consensus and variants were checked on Integrative
372 Genomics Viewer.

373 MACV, strain Caravallo, GTOV, strain INH-95551, and JUNV, strain P2045 were produced in VeroE6 cells in
374 DMEM, 2% FCS. The clarified cell supernatants were diluted in PBS for inoculation of the animals with the
375 virus. The same viruses were used for further experiments on biological samples from the experiments.

376 **GPC expression by MOPEVAC viruses**

377 VeroE6 cells were infected at a moi of 0.001 and cellular RNAs were extracted at day 0 and day 3 post-
378 infection. RNAs coding for GPC were quantified by RT-qPCR using EurobioGreen One Step Lo-Rox kit
379 (Eurobio). The primers were designed to match all NWA (Fwd: GCC TGG WGG TTA TTG TYT and Rev: CTC
380 ARC ATG TCA CAG AAY TC). The GAPDH expression was measured using a RT step with oligo dT primers
381 (Superscript III and Oligo dT from Life Technologies) followed by an amplification step with Taqman Gene
382 Expression Master Mix (Applied Biosystems) and a CM probe / primer mix (Applied Biosystems). The
383 expression of GPC was normalized using GAPDH mRNA expression and the ratio expression at day 3 on
384 expression at day 0 calculated to measure the increase of GPC RNA expression after infection.

385 **Western Blot for GP2 detection**

386 MOPEVAC_{CHA, GTO, JUN, MAC, SAB} were ultra-centrifugated for 1h30 at 450.000 x g. The pellet was lysed in
387 Laemmli buffer. The amount of lysis solution corresponding to 10⁵ ffu was separated on SDS-Page gel 4-
388 12% and transferred on a nitrocellulose membrane. GP2 proteins were detected by staining with the anti-
389 GP2 antibody (KL-AV-2A1⁴⁹) diluted 1/1.000 and anti-mouse HRP diluted 1/20.000 (Jackson
390 ImmunoResearch). Revelation of staining was performed using Super Signal WestDura Extended Duration
391 substrate (ThermoScientific).

392

393 **ELISA**

394 Virus-specific IgG detection was performed on plasma samples. To produce antigens, VeroE6 cells were
395 infected with wild type viruses. The supernatants were collected at day 4, and diluted with 25% of PEG
396 solution (Abcam). After an overnight incubation at 4°C, the media was centrifuged and the pellet
397 resuspended in buffer containing 1% Triton X-100 (Sigma). The solution was sonicated and frozen. Antigen-
398 negative supernatants were made with the same protocol using uninfected cells. These antigens were
399 coated diluted 1/500 or 1/1,000 in PBS on polysorp 96-microwell plates. After an overnight incubation at
400 4°C, the wells were blocked for 1 h with PBS, 2.5% BSA. Plasma samples, diluted from 1/250 to 1/16,000
401 in PBS with 2.5% BSA and 0.5% Tween 20, were added to the wells and the plates incubated for 1 h at 37°C
402 before a final incubation with anti-monkey HRP 1/5,000 (Sigma). Attachment of the conjugated antibody
403 was revealed using TMB and the reaction stopped with orthophosphoric acid. Between each step, the
404 plates were washed three times with PBS, 0.5% Tween 20. The optical density was finally measured and
405 the value obtained from antigen-negative was subtracted from the value of antigen-positive
406 measurements. A sample with a resulting value ≥ 0.1 was defined to be positive. Antibody titers
407 corresponded to the last dilution that was still positive. Statistical differences between conditions were
408 calculated as indicated in figure legends using SigmaPlot 14.5 software (Systat).

409 **IgG detection on 293T cells**

410 293T cells were transfected in 12-well plates with phCMV plasmids coding for GPC gene or the empty
411 vector using Lipofectamine 2000 (Invitrogen). After 2 days of incubation, transfected cells were harvested
412 and divided into 96-well plates. Cells were incubated with Live Dead fixable viability dye (Life Technologies)
413 and plasma samples diluted 1/20 in PBS, 2.5% FCS and 2mM EDTA for 30 min in ice. After two washes in
414 the same buffer, secondary antibody anti-monkey IgG FITC (Southern Biotech) was added to the cells for
415 30 min at +4°C. Two final washes were performed before fixation with paraformaldehyde 2% and analysis
416 by flow cytometry (Fortessa 4L, BD). The percentage of cells that have bound anti-GPC antibodies was
417 determined on live cells (Kaluza software for flow cytometry analysis).

418 **Quantitative RNA analysis**

419 RNA was prepared from liquid samples using the Qlamp viral RNA mini kit (Qiagen), or from cells or tissues
420 using the RNeasy mini kit (Qiagen). Quantitative RT-qPCR was performed using the SensiFAST Probe No-
421 ROX One-Step kit (Bioline) on a LightCycler480 device (Roche). A standard RNA was used for quantification;
422 we were able to detect 4 copies viral RNA/ μ l. We performed a sensitivity test using a different matrix and
423 obtained a limit of quantification of 6 ffu/ml in plasma and oral/nasal swabs for GTOV and 25 ffu/ml in
424 plasma and 625 ffu/ml in swabs for MACV.

425 **Virus titration**

426 Infectious particles were quantified in samples in which viral RNA was detected. For the organs, 10 mg of
427 material was diluted in 100 μ l DMEM, 2% FCS and then dispersed for 10 min at 30 beats per second using
428 metal beads. The solution obtained was centrifuged for 3 min at 500 x g to pellet the debris. The
429 supernatant was used for titration.

430 Samples were serially diluted in DMEM, 2% FCS, added to VeroE6 cells, and the plates incubated for 1 h at
431 37°C. Medium supplemented with carboxymethylcellulose was added and the plates incubated for one

432 week. Cells were then fixed for 20 min with 4% formaldehyde. To determine the number of focus-forming
433 units, plates were permeabilized for 5 min with 0.5 % triton, stained for 1 h with anti-virus antibody, and
434 for an addition hour with HRP-conjugated secondary antibody. The reaction was finally revealed using
435 NBT/BCIP (Thermo Scientific). Focus-forming units were counted. To determine the number of plaque-
436 forming units, cells were colored with crystal violet solution (Sigma-Aldrich) diluted 50% in PBS for 10 min,
437 washed with water, and the plaques counted.

438 MACV and JUNV were revealed using anti-Z MACV and MOPEVAC was stained using anti-Z MOPV. These
439 antibodies were produced in rabbits (Agrobio). For SABV virus, we used an anti-monkey MACV obtained
440 from the USAMRIID. The secondary antibodies were all coupled with HRP (Sigma). We did not obtain
441 reactive antibodies for GTOV but the virus was lytic, and we used crystal violet to reveal plaques. The
442 threshold of detection was 17 ffu/ml for liquid samples and 0.5 ffu/mg for organs.

443 **Seroneutralization**

444 Seroneutralization experiments were conducted using wild type or MOPEVAC viruses, as described in the
445 figure legends. Plasma samples were serially diluted in cell culture medium and a single viral dilution was
446 added to the wells of a 96-well microplate. After a 1 h incubation (37°C/5% CO₂) the plasma/virus mixture
447 was added to cells. The infection was allowed to proceed for 1h and media supplemented with
448 carboxymethylcellulose was added. The cells were incubated for one week before immunostaining of
449 infected cells or crystal violet coloration (cf virus titration). The neutralizing titer was the last dilution that
450 allowed more than a 50% reduction in the number of viral plaques relative to the control condition.

451 **Hematological and biochemical analyses**

452 Hematological parameters were analyzed using a MS9-5s (Melet Schloesing Laboratories) and biochemical
453 analyses were performed on plasma from heparin lithium blood tubes using a Pentra C200 analyzer
454 (Horiba).

455 **Intracellular cytokine staining in PBMCs**

456 Fresh whole blood or PBMCs were stimulated with overlapping peptides of the NP and GPC proteins and
457 stained for flow cytometry analysis, as previously described²⁶. Staphylococcal Enterotoxin A (SEA) was used
458 as a positive control. The gating strategy is presented in Extended data 8.

459 **Transcriptomic analyses**

460 Total RNA from PBMCs was extracted using the RNeasy Mini kit (Qiagen) with an on-column DNase step.
461 RNA samples were then quantified using the QuantifluorRNA system (Promega) and qualified using a
462 Standard Sensitivity kit on an Advanced Analytical (AITI) fragment analyzer. The External RNA Controls
463 Consortium (ERCC) RNA Spike-in Mix 1 (Thermo Fisher Scientific) was added to all samples to limit sample
464 variability in multiple batches and mRNA was poly(A)-captured using NEXTflex Poly(A) beads (Perkin
465 Elmer). The libraries were prepared using the NEXTflex Rapid Directional RNA-seq (RNA sequencing) Kit
466 (Perkin Elmer), were quantified and qualified using a Quantus quantification kit (Promega) and a fragment
467 analyzer. Sequencing was performed on a NextSeq 500 Flow Cell High OutputSR75 instrument (Illumina)
468 with six samples per flow cell.

469 Bioinformatics analysis was performed using the RNA-seq pipeline from Sequana⁵⁰. Reads were cleaned of
470 adapter sequences and low-quality sequences using cutadapt version 1.11⁵¹. Only sequences of at least 25
471 nucleotides in length were considered for further analysis. STAR version 2.5.0a⁵², with default parameters,
472 was used for alignment against the reference genome (Macaca Fascicularis 5 from ENSEMBL version 95).
473 Reads were assigned to genes using featureCounts version 1.4.6-p3⁵³ from the Subreads package
474 (parameters: -t gene -g ID -O -s 2). Data from these transcriptomic analyses are available on Zenodo DOI:
475 [10.5281/zenodo.7229438](https://doi.org/10.5281/zenodo.7229438)⁵⁴.

476

477 Statistical analyses were performed to identify genes for which the expression profiles were significantly
478 different between each pair of biological conditions. Therefore, statistical tests were performed between
479 each time-point compared to their respective baseline (day 0) within each group. For each data set (post-
480 vaccination and post-challenge), genes exhibiting expression lower than one count per million in at least
481 three samples were considered to have a low level of expression and discarded from the analysis.
482 Differential analysis was performed using the DESeq2 R package (v1.24.0)⁵⁵. The model was adjusted for
483 the effect of vaccination status, time-points, and animal identifier. Gene set enrichment analysis was
484 performed for both data sets to identify gene sets and pathways enriched in the various biological
485 conditions using a one-sided Fisher exact test. The overall gene sets over- or under-expression was tested
486 with a 1-way mixed ANOVA on centered and scaled expressions of the gene set, averaged by condition.
487 The fixed part of the model was adjusted on the groups and timepoints, and the random part was adjusted
488 on the gene identifiers. The Tukey's multiple comparison test was used to compare the timepoints to J0
489 within each group.

490

491 **Data availability**

492 The datasets from RNA sequencing generated during the current study are not publicly available due to
493 current analyses still ongoing, but are available from the corresponding author on reasonable request.

494

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497

498 **Acknowledgments**

499 We thank S. Godard, B. Labrosse, C. Leculier, S. Mely, E. Moissonnier, S. Mundweiler, D. Pannetier, A.
500 Pocquet, H. Theoule, and D. Thomas (P4 INSERM–Jean Merieux, US003, INSERM) for BSL4 management
501 during these experiments. We are also grateful to G. Fourcaud, B. Lafoux, and K. Noy for their logistical
502 help. We also thank M. Carroll and R. Hewson (PHE, Porton Down, UK), S. Günther (BNI, Germany) and T.G.
503 Ksiazek (CDC, Atlanta, GA) for providing the Machupo, Guanarito, and Junin viruses and T. G. Ksiazek, P. E.
504 Rollin, and P. Jahrling (Special Pathogens Branch, CDC, Atlanta, GA) for the polyclonal anti-MACV
505 antibodies. Antibody KL-AV-2A1 was a kind gift of F. Krammer (department of Microbiology, Icahn School
506 of Medicine at Mount Sinai, New York). We thank C. Gerke and M-A Dillies for their support in vaccine
507 development and bioinformatics and the “Grand Projet Fédérateur de Vaccinologie” of the Institut Pasteur
508 that funded this project (grant obtained by SB).

509

510 **Data availability**

511 Data are available in public, open access repositories. For transcriptomic analyses, they are available on
512 Zenodo DOI: [10.5281/zenodo.7229438](https://doi.org/10.5281/zenodo.7229438).

513

514 **Author contributions**

515 SR managed and performed the experiments, analyzed the results, and wrote the publication. XC
516 performed the reverse genetics experiments to rescue the vaccine candidates. XC, CP, VBC, AJ, MMA, CG,
517 JH, and SB performed the experiments on samples during the animal experiments. LF and PHM were in
518 charge of the animal experiments in the BSL2. CP, VBC, and LA were responsible for the neutralization
519 assays, AJ performed the ELISA experiments and MMA realized the viral titrations in organs. EP and NP
520 computed all transcriptomic data and performed the related analyses. AV, SBar, AD, OL, OJ, and MMo
521 managed animals in the BSL4 facility. MDi was the referent veterinary of this study. MDa and CLL

522 performed the sequencing for the transcriptomic analyses. HR and CC managed the BSL4 team. SB
523 supervised the entire project.

524 **Competing interests**

525 The authors declare that they have no competing interests. The MOPEVAC vaccine platform described in
526 this study is protected by the U.S. patent 62/245,631, authors listed as co-inventors are SR, SB, XC, MMA,
527 and AJ.

528

529

530

531 **Figure legends**

532

533 **Fig. 1. CM immunization with MOPEVAC_{MACV} induces antibody responses.** **a.** Schematic view of the
534 experiment. The long vertical bars represent each week. The days of vaccination, sampling, challenge, and
535 necropsy are indicated with colored arrows. **b.** Bi-segmented organization of the MOPEVAC_{MACV} genome.
536 The S segment is mutated in the exonuclease domain of the nucleoprotein (NP) and the glycoprotein
537 precursor (GPC) of MACV replaces the GPC of the Mopeia virus (MOPV). The L segment is that of wildtype
538 MOPV (L: polymerase, Z: zinc finger matrix protein, UTR: untranslated regions). **c.** Vero E6 cells were
539 infected at moi = 0.0001 and cell supernatants were collected every day until day 7 and titrated (left
540 graph). Viral titers of the different MOPEVAC at each passage from passage two to ten (right graph). The
541 mean of two experiments is represented for both graphs. **d.** Antibody response during the immunization
542 period. The IgG titer is the last dilution of the plasma sample that is still positive for MACV-specific IgG.
543 Each dot represents an individual value (n = 4). When all samples were negative, dots were not separated.
544 Two-sided Mann-Whitney statistical test was performed to compare IgG values after boost versus after
545 prime at the same day after injection. P values obtained are indicated by an asterisk (*: < 0,05). The
546 neutralization titer corresponds to the last dilution of plasma that still neutralizes more than 50% of wild
547 type MACV. Each curve represents an individual value. The arrow indicates the second vaccine injection.

548

549 **Fig. 2. Post challenge monitoring of animals and antibody response.** **a.** Clinical score and percentage of
550 the initial body weight at each sampling. **b.** Biochemical parameters measured in plasma samples
551 (ALT/AST: alanine/aspartate aminotransferase). The point represented at day 0 was actually day -9
552 (reception of the animals). **c.** Viral RNA quantified by RT-qPCR and virus titration performed on positive
553 samples. Negative values are represented at the detection limit. **d.** IgG and neutralization titers are
554 represented as in fig. 1c. One-way Anova test was performed from day 0 to day 12. Significant p values

555 were obtained only for prime boost group *versus* controls. From day 15, two-sided Mann-Whitney test
556 was performed. Asterisks indicate the p-values (*: <0,05, **<0,01). The optical density obtained by ELISA
557 is represented (at dilution 1/1,000 and 1/250 for prime boost and prime only groups respectively). **e.** Cross-
558 neutralization was evaluated on samples after immunization using MOPEVAC viruses. The arrow indicates
559 the boost.

560
561 **Fig. 3. Immune responses induced by MOPEVAC_{NEW} in CMs.** **a.** Schematic representation of the protocol.
562 Monkeys were vaccinated or not using a prime-boost strategy (n = 6) and then infected with either MACV
563 or GTOV (four groups, n = 3). The representation is as in fig. 1a. **b.** NAbs were measured during the
564 immunization period and quantified for each pathogenic NWA. The representation is as in fig. 2e. All
565 vaccinated or control animals received the same injections; they are distinguished depending on the future
566 challenge they will receive. **c.** IgG against MACV and GTOV were quantified by ELISA in plasma samples
567 throughout the immunization period. Two-sided Mann-Whitney statistical tests were performed but did
568 not provide significant p-values (n=3). The arrow indicates the second injection of the vaccine. The animals
569 are represented with colors like in b. IgG were also tested on 293T cells expressing GPC from NWAs, MOPV
570 and control cells without GPC expression. The percentage of cells that were recognized by IgG from plasma
571 samples at day 82 post-immunization is represented for each animal.

572
573 **Fig. 4. Challenge of vaccinated or unvaccinated CMs with GTOV and MACV.** Unvaccinated CMs were
574 challenged with GTOV (gray, n = 3) or MACV (red, n = 3). Vaccinated CMs received the same challenge with
575 GTOV (orange, n = 3) or MACV (blue, n = 3) **a.** The clinical score and weight loss were evaluated at each
576 sampling as in fig. 2a. **b.** Viral RNA was quantified by RT-qPCR at each sampling. We performed the
577 quantification of infectious viruses on RT-qPCR positive samples. Negative values are represented at the
578 threshold of detection.

579
580 **Fig. 5. Antibody response after challenge.** The colors used are the same as in Fig. 4. **a.** IgG titers were
581 evaluated by ELISA and represent the last dilution of plasma that is still positive. The dots indicate
582 individual values. Two-sided Mann-Whitney statistical tests were performed but did not provide significant
583 p-values. **b.** Neutralization titers were defined against wildtype viruses at days 0, 12, and the date of
584 necropsy. The experiment was performed as for fig. 1c. **c.** MOPEVAC viruses were used to evaluate the
585 neutralization titers. The experiment was performed using the same protocol.

586
587 **Fig. 6. Immune responses after immunization and challenge.** **a.** Transcriptomic analysis of PBMCs after
588 immunization. The heatmaps represent the genes expressions in each pathway. The color of the heatmaps
589 represents the mean of scaled normalized counts for each group and time-point (Vacc: n=4, Ctrl n=3). Box
590 plots show the average of the normalized gene expression of the pathway (central line: median; hinges:
591 first and third quartiles; whiskers: largest or smallest value no further than 1.5 inter-quartile range from
592 the hinge). Outlying points are plotted individually. Significant differences were tested using 1-way mixed
593 ANOVA on centered and scaled expressions of the gene set, averaged by condition. The Tukey's multiple
594 comparison test was used to compare the timepoints to J0 within each group. Asterisks indicate the
595 difference from day 0 (**p < 0.01, *p < 0.05) and exact p values are provided (Supp. Table
596 3). **b.** Transcriptomic analysis of PBMCs after challenge. Data were analyzed and represented as in a (n=3).

597

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601 **References**

602

- 603 1. Charrel, R. N., de Lamballerie, X. & Fulhorst, C. F. The Whitewater Arroyo virus: natural evidence
604 for genetic recombination among Tacaribe serocomplex viruses (family Arenaviridae). *Virology*
605 **283**, 161–166 (2001).
- 606 2. Choe, H., Jemielity, S., Abraham, J., Radoshitzky, S. R. & Farzan, M. Transferrin receptor 1 in the
607 zoonosis and pathogenesis of New World hemorrhagic fever arenaviruses. *Curr. Opin. Microbiol.*
608 **14**, 476–482 (2011).
- 609 3. Ambrosio, A., Saavedra, M., Mariani, M., Gamboa, G. & Maiza, A. Argentine hemorrhagic fever
610 vaccines. *Hum. Vaccin.* **7**, 694–700 (2011).
- 611 4. Enria, D. A., Briggiler, A. M. & Sánchez, Z. Treatment of Argentine hemorrhagic fever. *Antiviral*
612 *Res.* **78**, 132–139 (2008).
- 613 5. Enria, D. A. *et al.* [Candid#1 vaccine against Argentine hemorrhagic fever produced in Argentina.
614 Immunogenicity and safety]. *Medicina (B. Aires).* **70**, 215–22 (2010).
- 615 6. Albariño, C. G. *et al.* The major determinant of attenuation in mice of the Candid1 vaccine for
616 Argentine hemorrhagic fever is located in the G2 glycoprotein transmembrane domain. *J. Virol.*
617 **85**, 10404–8 (2011).
- 618 7. York, J. & Nunberg, J. H. Epistatic Interactions within the Junín Virus Envelope Glycoprotein
619 Complex Provide an Evolutionary Barrier to Reversion in the Live-Attenuated Candid#1 Vaccine. *J.*
620 *Virol.* **92**, (2018).
- 621 8. Aguilar, P. V *et al.* Reemergence of Bolivian hemorrhagic fever, 2007-2008. *Emerg. Infect. Dis.* **15**,

- 622 1526–1528 (2009).
- 623 9. Patterson, M., Grant, A. & Paessler, S. Epidemiology and pathogenesis of Bolivian hemorrhagic
624 fever. *Curr. Opin. Virol.* **5**, 82–90 (2014).
- 625 10. Johnson, D. M. *et al.* Bivalent Junin & Machupo experimental vaccine based on alphavirus RNA
626 replicon vector. *Vaccine* **38**, 2949–2959 (2020).
- 627 11. Koma, T. *et al.* Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is
628 Highly Attenuated and Immunogenic. *J. Virol.* **90**, 1290–1297 (2016).
- 629 12. Coimbra, T. L. . *et al.* New arenavirus isolated in Brazil. *Lancet* **343**, 391–392 (1994).
- 630 13. Tesh, R. B., Jahrling, P. B., Salas, R. & Shope, R. E. Description of Guanarito virus (Arenaviridae:
631 Arenavirus), the etiologic agent of Venezuelan hemorrhagic fever. *Am. J. Trop. Med. Hyg.* **50**,
632 452–459 (1994).
- 633 14. Delgado, S. *et al.* Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic
634 fever case in Bolivia. *PLoS Pathog.* **4**, e1000047 (2008).
- 635 15. Ellis, B. A. *et al.* A Longitudinal Study of Junin Virus Activity in the Rodent Reservoir of Argentine
636 Hemorrhagic Fever. *Am. J. Trop. Med. Hyg.* **47**, 749–763 (1992).
- 637 16. Mills, J. N. *et al.* Junin virus activity in rodents from endemic and nonendemic loci in central
638 Argentina. *Am. J. Trop. Med. Hyg.* **44**, 589–597 (1991).
- 639 17. Mercado, R. Rodent control programmes in areas affected by Bolivian haemorrhagic fever. *Bull.*
640 *World Health Organ.* **52**, 691–6 (1975).
- 641 18. Fulhorst, C. F. *et al.* Isolation and characterization of Whitewater Arroyo virus, a novel North
642 American arenavirus. *Virology* **224**, 114–120 (1996).

- 643 19. Fatal illnesses associated with a new world arenavirus--California, 1999-2000. *MMWR. Morb.*
644 *Mortal. Wkly. Rep.* **49**, 709–711 (2000).
- 645 20. Briese, T. *et al.* Genetic Detection and Characterization of Lujo Virus, a New Hemorrhagic Fever–
646 Associated Arenavirus from Southern Africa. *PLoS Pathog.* **5**, e1000455 (2009).
- 647 21. Enria, D. A., Briggiler, A. M., Fernandez, N. J., Levis, S. C. & Maiztegui, J. I. Importance of dose of
648 neutralising antibodies in treatment of Argentine haemorrhagic fever with immune plasma.
649 *Lancet (London, England)* **2**, 255–256 (1984).
- 650 22. Kenyon, R. H., Green, D. E., Eddy, G. A. & Peters, C. J. Treatment of Junin Virus-Infected Guinea
651 Pigs With Immune Serum: Development of Late Neurological Disease. *J. Med. Virol.* **20**, 207–218
652 (1986).
- 653 23. Avila, M. M., Samoilovich, S. R., Laguens, R. P., Merani, M. S. & Weissenbacher, M. C. Protection
654 of Junín virus-infected marmosets by passive administration of immune serum: association with
655 late neurologic signs. *J. Med. Virol.* **21**, 67–74 (1987).
- 656 24. Maiztegui, J. I., Fernandez, N. J. & de Damilano, A. J. Efficacy of immune plasma in treatment of
657 Argentine haemorrhagic fever and association between treatment and a late neurological
658 syndrome. *Lancet (London, England)* **2**, 1216–1217 (1979).
- 659 25. Carnec, X. *et al.* A Vaccine Platform against Arenaviruses Based on a Recombinant
660 Hyperattenuated Mopeia Virus Expressing Heterologous Glycoproteins. *J. Virol.* **92**, (2018).
- 661 26. Mateo, M. *et al.* Vaccines inducing immunity to Lassa virus glycoprotein and nucleoprotein
662 protect macaques after a single shot. *Sci. Transl. Med.* **11**, (2019).
- 663 27. Luis, M.-S. *et al.* Identification of Amino Acid Residues Critical for the Anti-Interferon Activity of
664 the Nucleoprotein of the Prototypic Arenavirus Lymphocytic Choriomeningitis Virus. *J. Virol.* **83**,

- 665 11330–11340 (2009).
- 666 28. Jiang, X. *et al.* Structures of arenaviral nucleoproteins with triphosphate dsRNA reveal a unique
667 mechanism of immune suppression. *J. Biol. Chem.* **288**, 16949–16959 (2013).
- 668 29. Habjan, M. *et al.* Processing of genome 5' termini as a strategy of negative-strand RNA viruses to
669 avoid RIG-I-dependent interferon induction. *PLoS One* **3**, e2032 (2008).
- 670 30. Hastie, K. M., Kimberlin, C. R., Zandonatti, M. A., MacRae, I. J. & Saphire, E. O. Structure of the
671 Lassa virus nucleoprotein reveals a dsRNA-specific 3' to 5' exonuclease activity essential for
672 immune suppression. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 2396–2401 (2011).
- 673 31. Nakaya, H. I. *et al.* Systems Analysis of Immunity to Influenza Vaccination across Multiple Years
674 and in Diverse Populations Reveals Shared Molecular Signatures. *Immunity* **43**, 1186–1198 (2015).
- 675 32. Frank, M. G. *et al.* South American Hemorrhagic Fevers: A summary for clinicians. *Int. J. Infect. Dis.*
676 **105**, 505–515 (2021).
- 677 33. Golden, J. W. *et al.* An attenuated Machupo virus with a disrupted L-segment intergenic region
678 protects guinea pigs against lethal Guanarito virus infection. *Sci. Rep.* **7**, 4679 (2017).
- 679 34. Leske, A. *et al.* Assessing cross-reactivity of Junín virus-directed neutralizing antibodies. *Antiviral*
680 *Res.* **163**, 106–116 (2019).
- 681 35. Silva-Ramos, C. R., Faccini-Martínez, Á. A., Calixto, O.-J. & Hidalgo, M. Bolivian hemorrhagic fever:
682 A narrative review. *Travel Med. Infect. Dis.* **40**, 102001 (2021).
- 683 36. Escalera-Antezana, J. P. *et al.* Clinical features of fatal cases of Chapare virus hemorrhagic fever
684 originating from rural La Paz, Bolivia, 2019: A cluster analysis. *Travel Med. Infect. Dis.* **36**, 101589
685 (2020).

- 686 37. Rodríguez-Morales, A. J., Bonilla-Aldana, D. K., Risquez, A., Paniz-Mondolfi, A. & Suárez, J. A.
687 Should we be concerned about Venezuelan hemorrhagic fever? - A reflection on its current
688 situation in Venezuela and potential impact in Latin America amid the migration crisis. *New*
689 *microbes new Infect.* **44**, 100945 (2021).
- 690 38. Ellwanger, J. H. & Chies, J. A. B. Keeping track of hidden dangers - The short history of the Sabiá
691 virus. *Rev. Soc. Bras. Med. Trop.* **50**, 3–8 (2017).
- 692 39. Medaglini, D., Harandi, A. M., Ottenhoff, T. H. M., Siegrist, C.-A. & Consortium, V.-E. Ebola vaccine
693 R&D: Filling the knowledge gaps. *Sci. Transl. Med.* **7**, 317ps24-317ps24 (2015).
- 694 40. M., N. W. *et al.* Contrasting Modes of New World Arenavirus Neutralization by Immunization-
695 Elicited Monoclonal Antibodies. *MBio* **0**, e02650-21 (2022).
- 696 41. Clark, L. E. *et al.* Vaccine-elicited receptor-binding site antibodies neutralize two New World
697 hemorrhagic fever arenaviruses. *Nat. Commun.* **9**, 1884 (2018).
- 698 42. Sommerstein, R. *et al.* Arenavirus Glycan Shield Promotes Neutralizing Antibody Evasion and
699 Protracted Infection. *PLOS Pathog.* **11**, e1005276 (2015).
- 700 43. Bonhomme, C. J. *et al.* Glycosylation modulates arenavirus glycoprotein expression and function.
701 *Virology* **409**, 223–233 (2011).
- 702 44. Huang, C. *et al.* Highly Pathogenic New World and Old World Human Arenaviruses Induce Distinct
703 Interferon Responses in Human Cells. *J. Virol.* **89**, 7079–7088 (2015).
- 704 45. Levis, S. C. *et al.* Correlation between endogenous interferon and the clinical evolution of patients
705 with Argentine hemorrhagic fever. *J. Interferon Res.* **5**, 383–389 (1985).
- 706 46. Marta, R. F. *et al.* Proinflammatory cytokines and elastase-alpha-1-antitrypsin in Argentine
707 hemorrhagic fever. *Am. J. Trop. Med. Hyg.* **60**, 85–89 (1999).

- 708 47. Mateer, E. J., Maruyama, J., Card, G. E., Paessler, S. & Huang, C. Lassa Virus, but Not Highly
709 Pathogenic New World Arenaviruses, Restricts Immunostimulatory Double-Stranded RNA
710 Accumulation during Infection. *J. Virol.* **94**, e02006-19 (2020).
- 711 48. Afgan, E. *et al.* The Galaxy platform for accessible, reproducible and collaborative biomedical
712 analyses: 2018 update. *Nucleic Acids Res.* **46**, W537–W544 (2018).
- 713 49. Amanat, F. *et al.* Antibodies to the Glycoprotein GP2 Subunit Cross-React between Old and New
714 World Arenaviruses. *mSphere* **3**, e00189-18 (2018).
- 715 50. Cokelaer, T., Desvillechabrol, D., Legendre, R. & Cardon, M. ‘Sequana’: a Set of Snakemake NGS
716 pipelines. *J. Open Source Softw.* **2**, 352 (2017).
- 717 51. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads.
718 *EMBnet.journal; Vol 17, No 1 Next Gener. Seq. Data Anal.* - 10.14806/ej.17.1.200 (2011).
- 719 52. Dobin, A. *et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 (2013).
- 720 53. Liao, Y., Smyth, G. K. & Shi, W. featureCounts: an efficient general purpose program for assigning
721 sequence reads to genomic features. *Bioinformatics* **30**, 923–930 (2014).
- 722 54. Baize, S. The MOPEVAC multivalent vaccine induces sterile protection against New World
723 Arenaviruses in non-human primates. (2022) doi:10.5281/ZENODO.7229439.
- 724 55. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-
725 seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).

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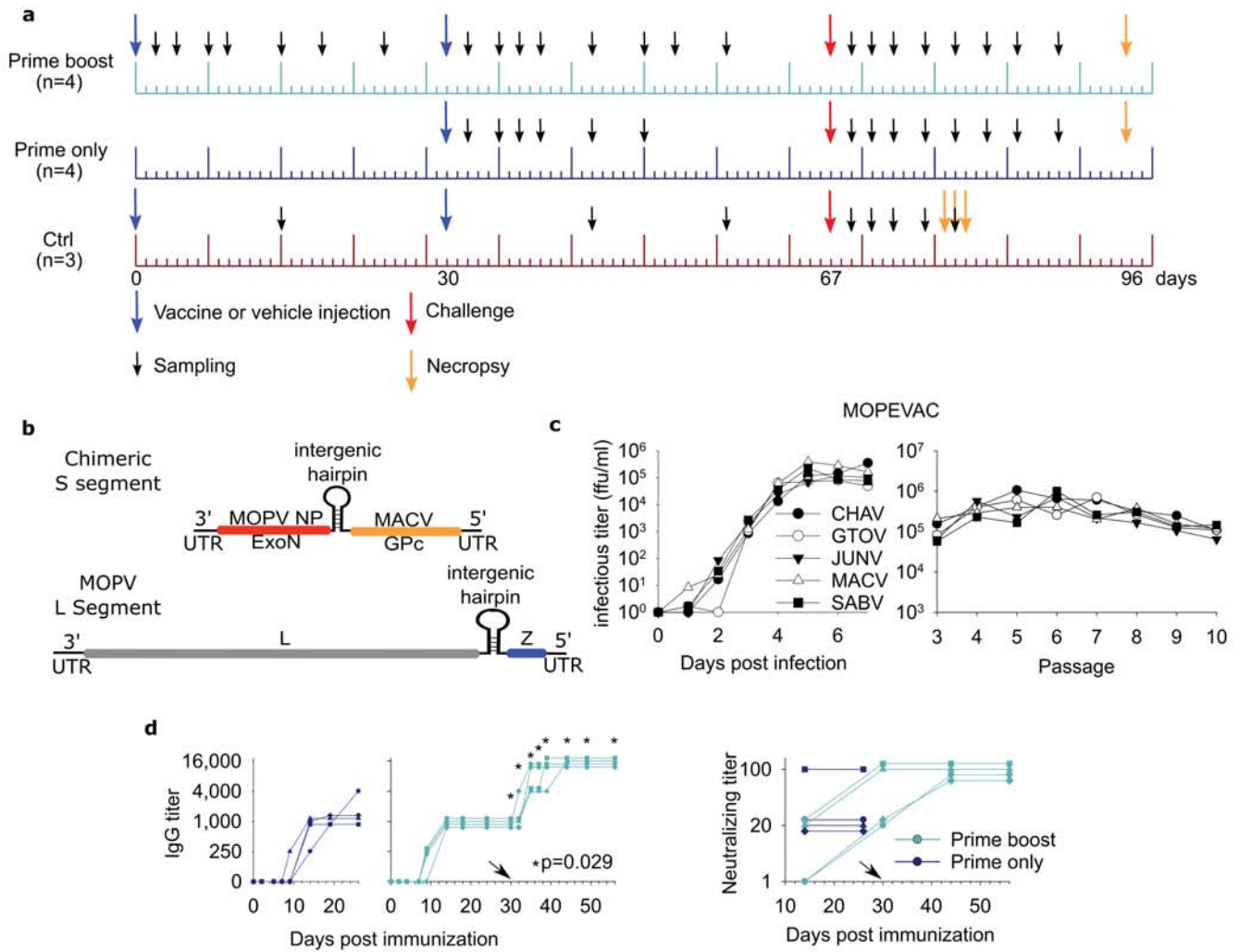


Fig. 1: CM immunization with MOPEVACMACV induces Ab responses.

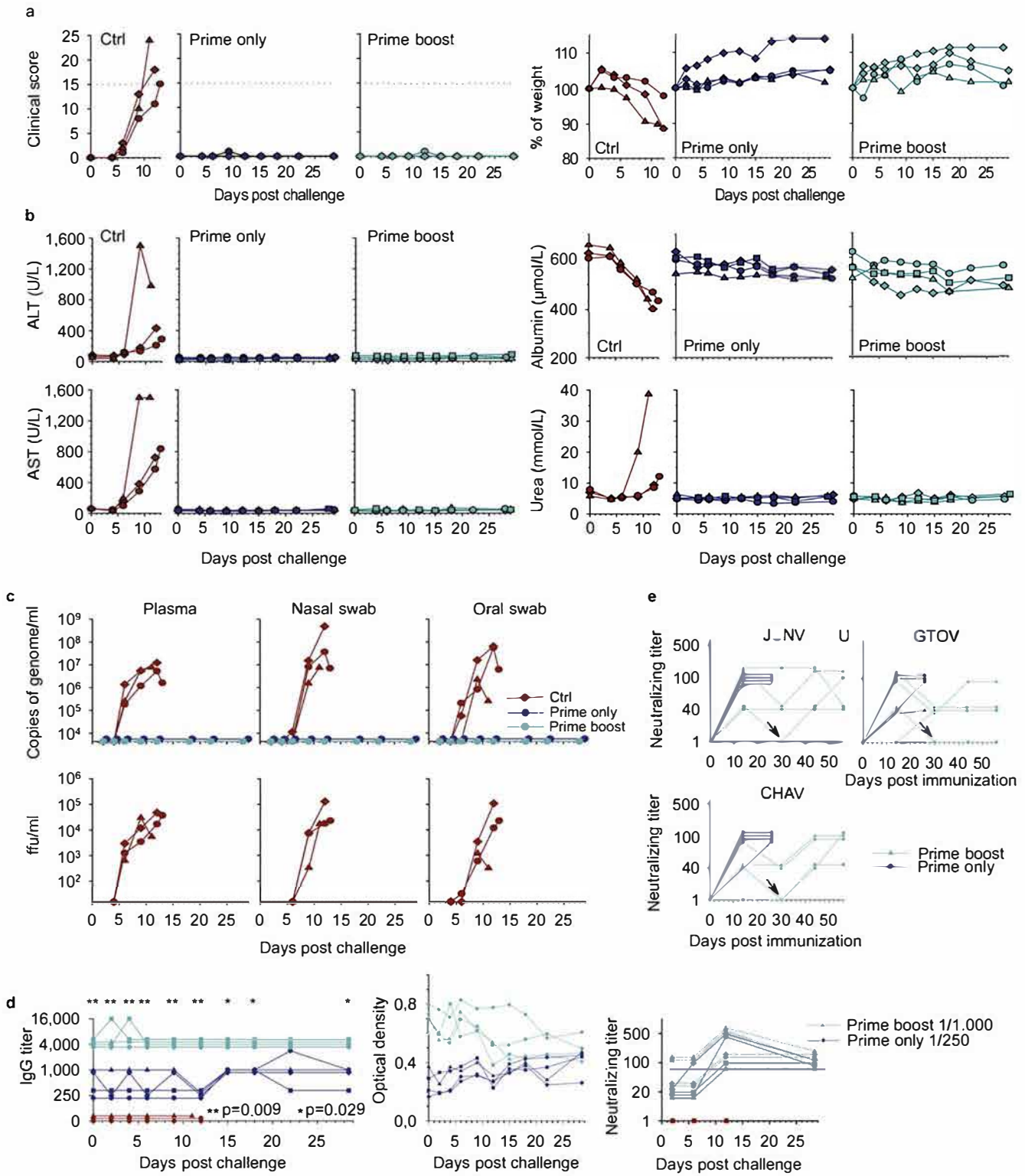


Fig. 2. Post challenge monitoring of animals and antibody response

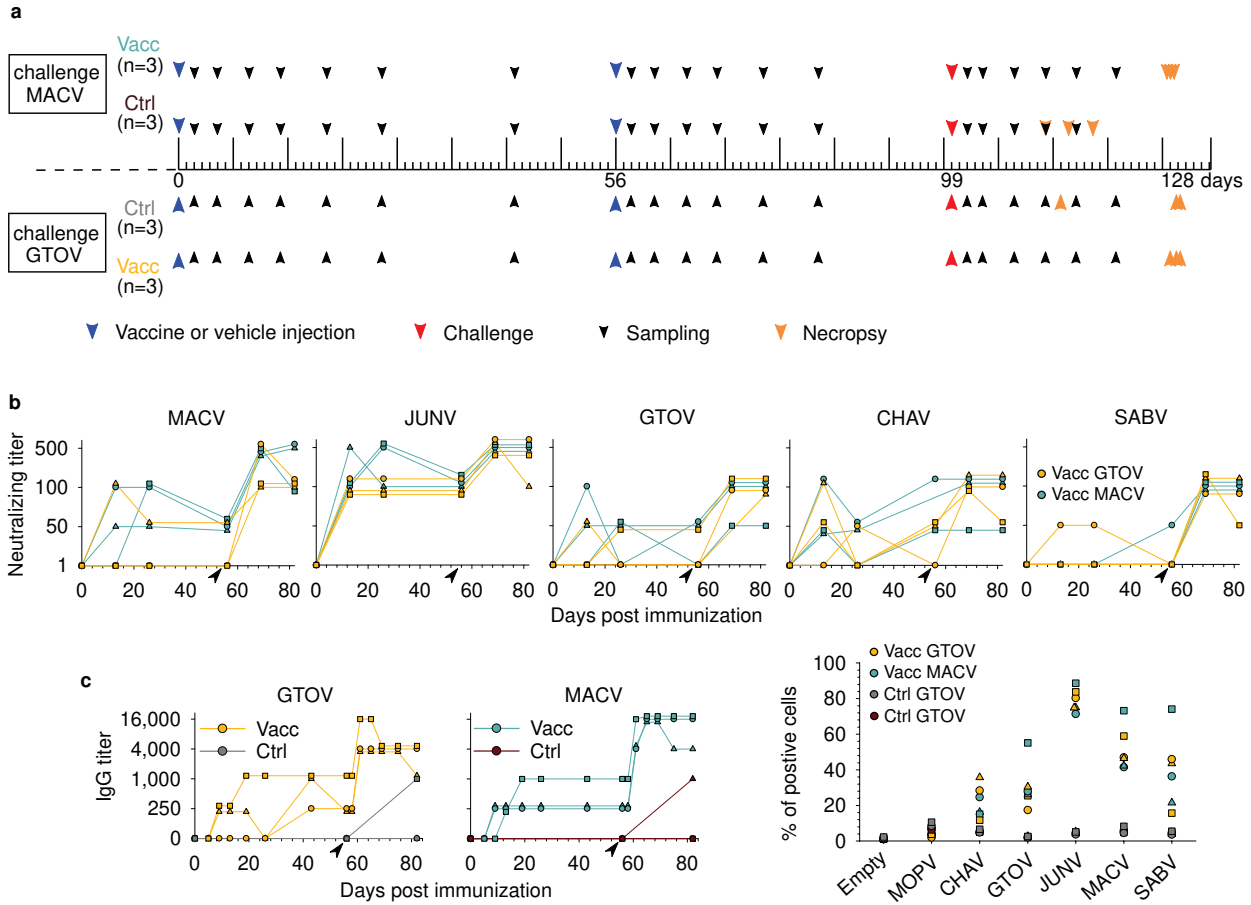


Fig. 3: Immune responses induced by MOPEVACNEW in CMs

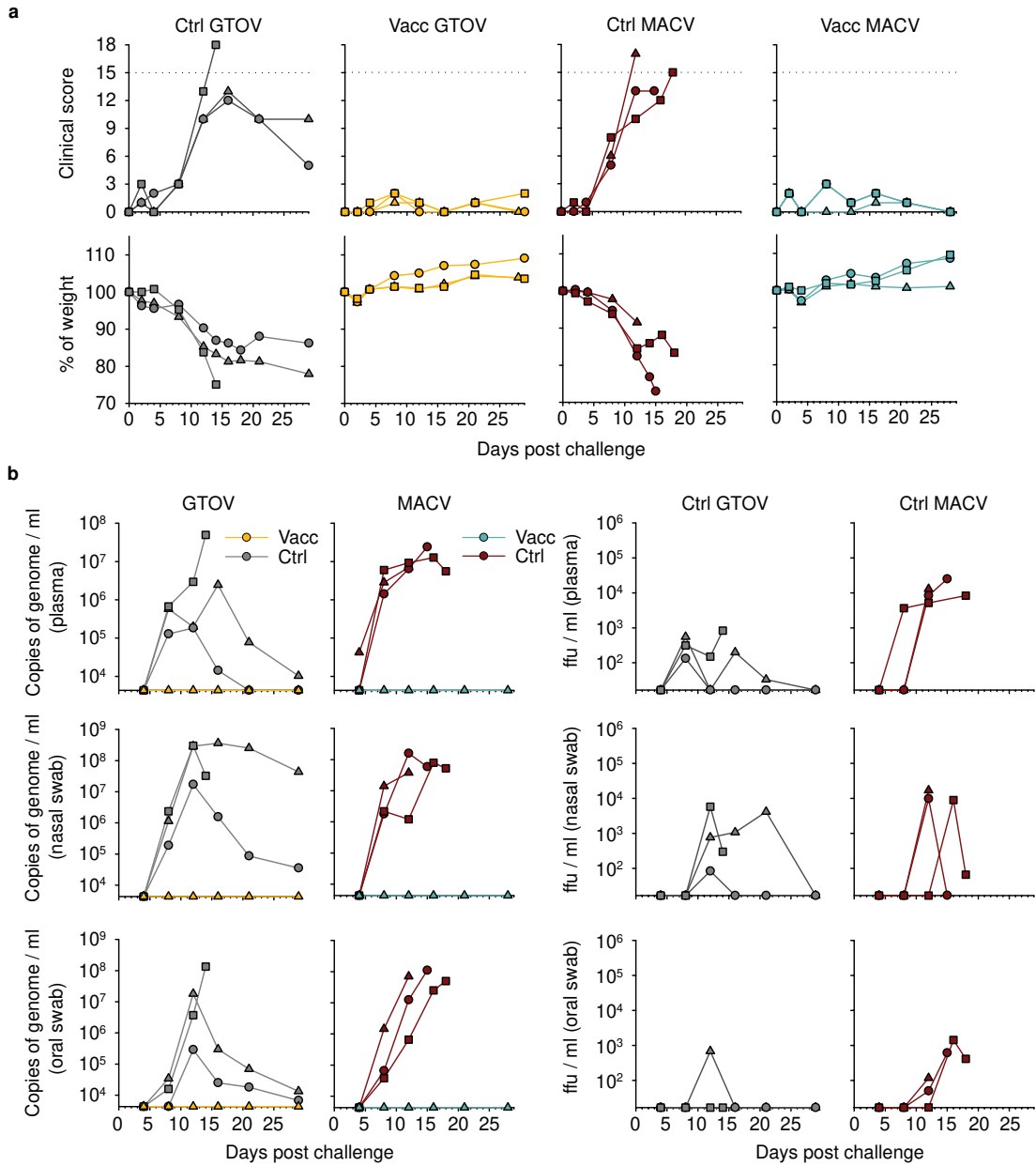


Fig. 4. Challenge of vaccinated or unvaccinated CMs with GTOV and MACV

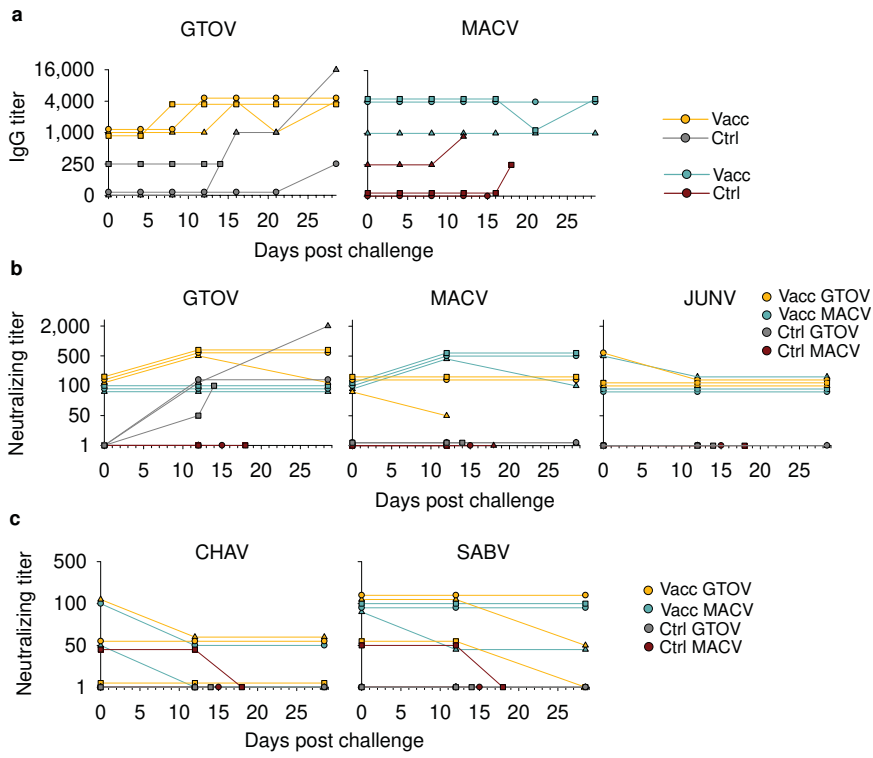


Fig. 5. Antibody response after challenge

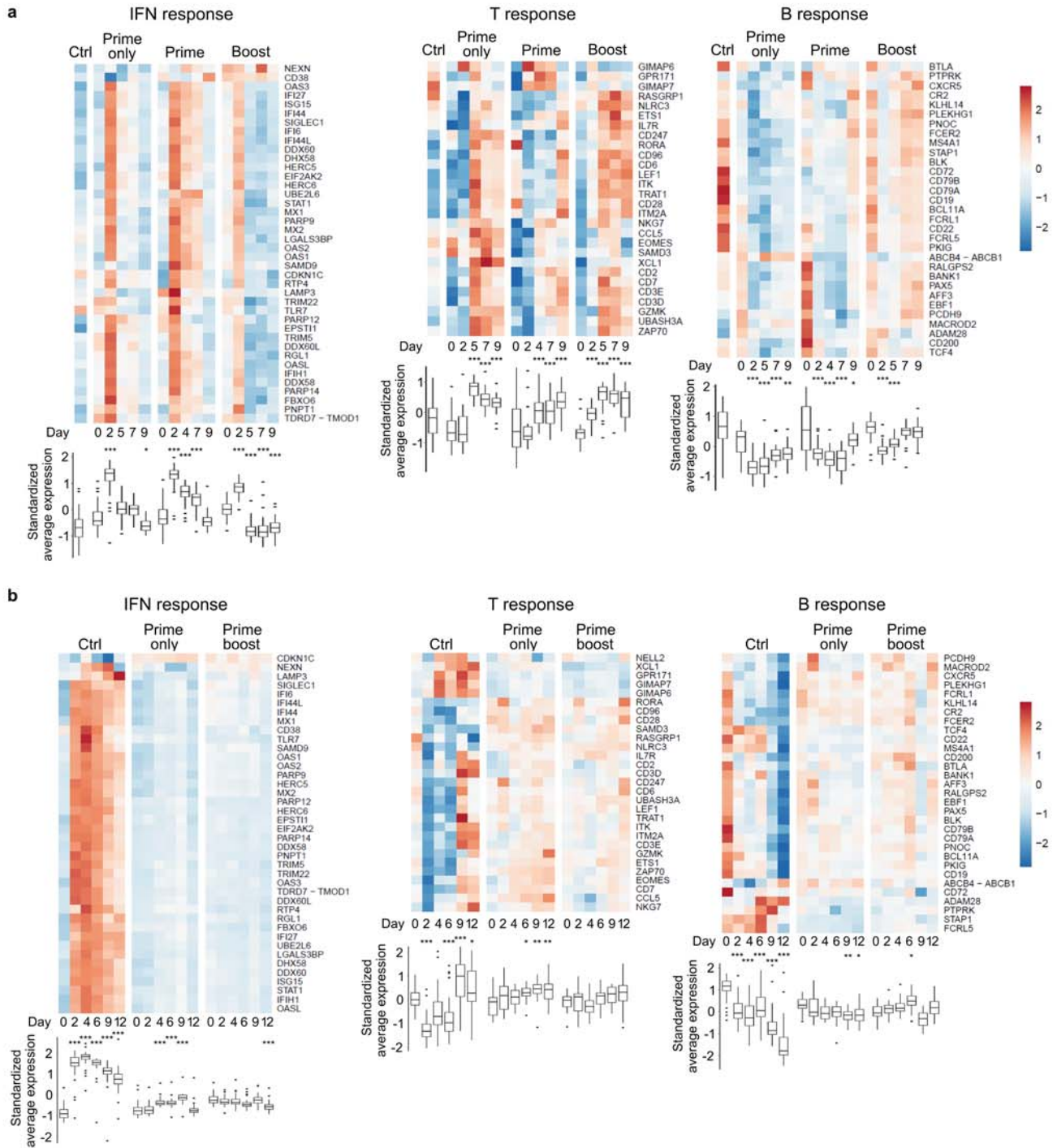


Fig. 6. Immune responses after immunization and challenge

Figure #	Figure title One sentence only	Filename This should be the name the file is saved as when it is uploaded to our system. Please include the file extension. i.e.: <i>Smith_ED_Fig1.jpg</i>	Figure Legend If you are citing a reference for the first time in these legends, please include all new references in the main text Methods References section, and carry on the numbering from the main References section of the paper. If your paper does not have a Methods section, include all new references at the end of the main Reference list.
Extended Data Fig. 1	GPs expression by MOPEVAC viruses	Ext_data_Fig1.pdf	a. VeroE6 cells were infected at a moi of 0.001 and cellular RNAs were extracted at day 0 and day 3 post-infection. The ratio of GPC expression of day 3 relative to day 0 was calculated and represented for each MOPEVAC virus. b. Expression of GP2 detected by KL-AV-2A1 ⁴⁹ antibody. GP2 protein expression was detected by western blot from 10 ⁵ ffu of MOPEVAC viruses, in a single experiment. The antibody has been described to detect JUNV, GTOV and MACV but its binding on CHAV and SABV was not known. These results show the expression by the different MOPEVAC viruses of the GP2 proteins of all NWA except the one of SABV, probably because of a lack of cross-reactivity of the antibody.
Extended Data Fig. 2	Real-time recording of body temperature after challenge	Ext_data_Fig2.pdf	Recording systems were implanted in the CMs to evaluate the body temperature throughout the protocol. A number were defective. We thus obtained data for seven CMs: the three controls, three prime only vaccinated animals, and one prime boost. The recording was stopped unintentionally for a small period for five animals, this is clearly visible in the graphs.
Extended Data Fig. 3	Hematological parameters and viral loads in the organs at the day of necropsy	Ext_data_Fig3.pdf	a. Cell counts and hemoglobin concentrations in whole blood were measured at each sampling. b. Viral RNA was quantified by RT-qPCR from crushed organs or cells. RT-qPCR-positive samples were evaluated for infectious virus titers. Li: liver, mLN: mesenteric lymph node, iLN: inguinal lymph node, Ki:

			kidney, Lu: lung, Bl: bladder, AG: adrenal gland, Br: brain, Ce: cerebellum, Sp: spleen, Spleno: splenocytes.
Extended Data Fig. 4	Body temperature before and after challenge in the MOPEVAC_{NEW} experiment	Ext_data_Fig4.pdf	Intraperitoneal implants recorded the body temperature throughout the experiment at 15-min intervals. a. Post immunization period in vaccinated CMs. All received the same vaccine, but the color indicates the virus used for the challenge. b. Body temperature of vaccinated and control animals after challenge.
Extended Data Fig. 5	Gating strategy for determination of IgG fixation on GPs	Ext_data_Fig5.pdf	The gates used to quantify the cells expressing or not GPs that fixed IgG from plasma are presented. FSC / SSC was used to gate cells, then singlets were determined using SSC / SSC-W and live cells were gated: Live Dead negative cells. The cells that fixed IgG and the secondary anti-IgG FITC were defined with the gate "Positive". Three conditions of the same plasma sample are presented for comparison: empty vector, cells expressing GPs of MOPV and cells expressing GPs of JUNV.
Extended Data Fig. 6	Viral loads in organs and immune-preserved compartments	Ext_data_Fig6.pdf	a. Viral RNA was quantified by RT-qPCR from crushed organs or cells. RT-qPCR-positive samples were evaluated for infectious virus titers. b. Viral RNA was quantified from cerebrospinal fluid (CSF) and eye vitreous humor and infectious virus titration was also performed. Li: liver, mLN: mesenteric lymph node, iLN: inguinal lymph node, Ki: kidney, Lu: lung, Bl: bladder, AG: adrenal gland, Br: brain, Ce: cerebellum, LI: large intestine, SI: small intestine, Ov: ovary, Pa: pancreas, Th: thymus, Sp: spleen, Spleno: splenocytes.
Extended Data Fig. 7	Hematological and biochemical parameters after challenge in the	Ext_data_Fig7.pdf	a. Cell counts and hemoglobin concentrations were measured at each sampling after challenge. b. Biochemical parameters were assayed in plasma at each sampling. C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate

	MOPEVAC_{NEW} experiment		aminotransferase (AST), and plasmatic albumin levels are presented.
Extended Data Fig. 8	Gating strategy for flow cytometry analysis	Ext_data_Fig8.pdf	The gates used to quantify IFN γ -producing and CD154-expressing T cells are presented for an unstimulated sample (a) and for the same sample stimulated with staphylococcus-enterotoxin A (SEA), as a positive control (b). FSCint/FSCtof was used to select singlets (singlets gate). Then, dead cells are excluded using live-dead staining (live gate). Lymphocytes were selected using FSCint/SSCint parameters (LC gate). Then, CD4+ and CD8+ T cells were selected using CD3/CD4 and CD3/CD8 staining (CD4+ and CD8+ gates). Finally, the percentage of IFN γ -producing and CD154-expressing CD4+ and CD8+ T cells is determined using a quadrant in the IFN γ /CD154 dotplot. c. A similar strategy was applied for CD137 and GrzB detection.
Extended Data Fig. 9	Activation of T cells in response to peptide stimulation	Ext_data_Fig9.pdf	a. PBMCs sampled at days 14 and 24 post-prime and day 19 post-boost were stimulated with overlapping peptides covering MACV NP and GP and LASV NP. SEA was used as a positive control. After an overnight incubation, the cells were stained with conjugated antibodies and analyzed by flow cytometry for the expression of CD154, CD137, GrzB and IFN γ . Expression values represent the difference between stimulated and non-stimulated cells. Light blue dots represent animals vaccinated with a prime-boost strategy (n=4, except for J19 boost where SEA n=2, NP LASV n=3) and black dots the control animals (n=3). The dots were not separated when the expression values were close to 0. b. After challenge, peptide stimulation was performed on whole blood. GPC and NP specific T cell responses were evaluated. The difference from

			the non-stimulated condition is represented (Ctrl: n=3, Vacc: n=4). The SEA control at day 0 is presented for comparison.
Extended Data Fig. 10			

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Item	Present?	Filename	A brief, numerical description of file contents.
		This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. The extension must be .pdf	i.e.: <i>Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.</i>
Supplementary Information	Choose an item.		
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Type	Number	Filename	Legend or Descriptive Caption
	If there are multiple files of the same type this should be the numerical indicator. i.e. "1" for Video 1, "2" for Video 2, etc.	This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. i.e.: <i>Smith_Supplementary_Video_1.mov</i>	Describe the contents of the file
Supplementary Table	1	Supp_Tables	Evolution of vaccine candidate genomes during serial passages in VeroE6 cells. The consensus genome sequences of the five vaccine candidates were determined at passages 2, 5, and

			10 and compared to the initial sequences (P2). The changes in codon sequences were indicated as well as the position of the mutation in the genome. The amino acid changes are indicated for non synonymous mutations whereas synonymous mutations are colored in green. The passage after which the mutation has been detected is indicated by the presence of a colored box.
Supplementary Table	2	Supp_Tables	Parameters used to establish the clinical score after challenge and their respective values
Supplementary Table	3	Supp_Tables	Exact p values corresponding to figure 6. For each condition the comparison was made with the day 0 timepoint.
Choose an item.			
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Parent Figure or Table	Filename	Data description
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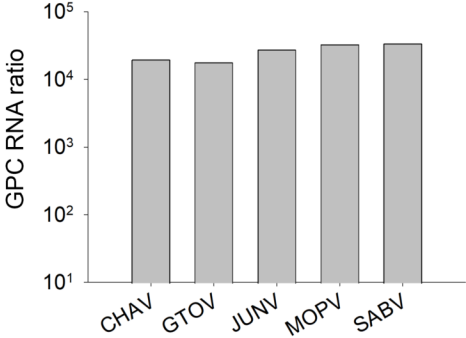
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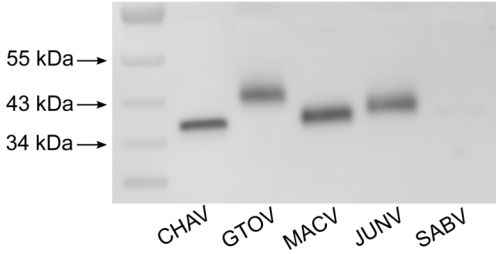
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Extended Data Fig. 1

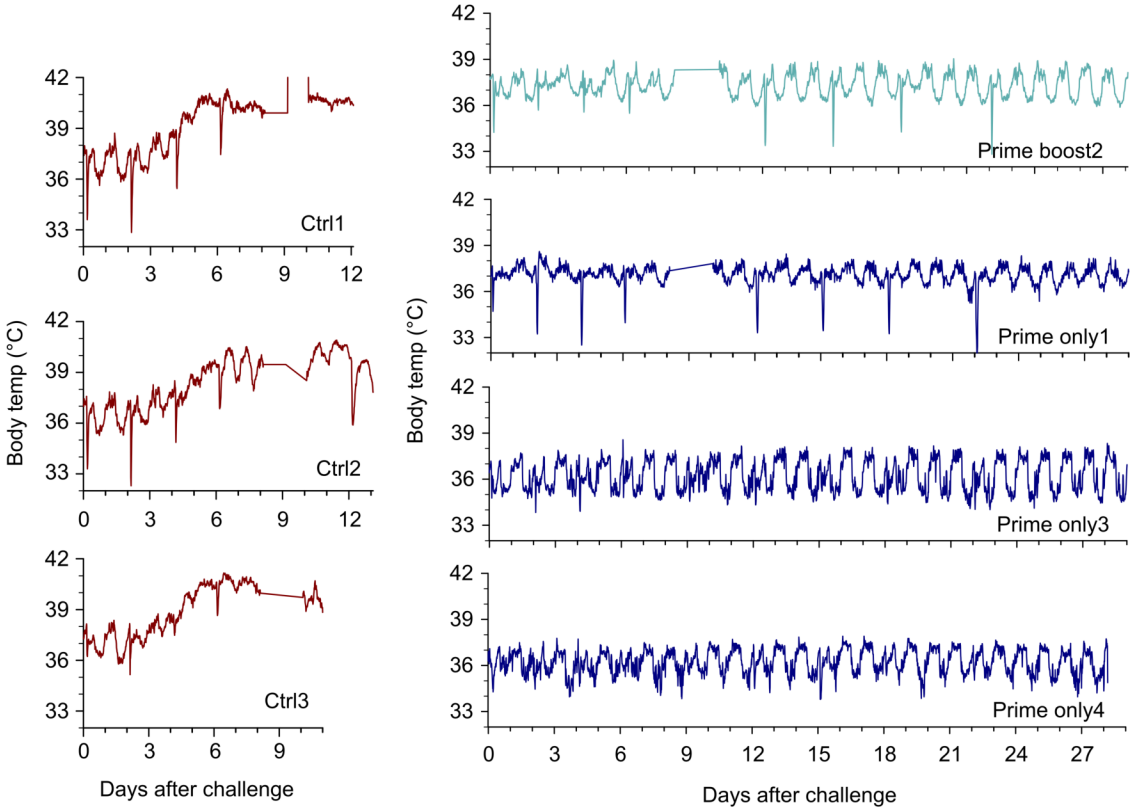
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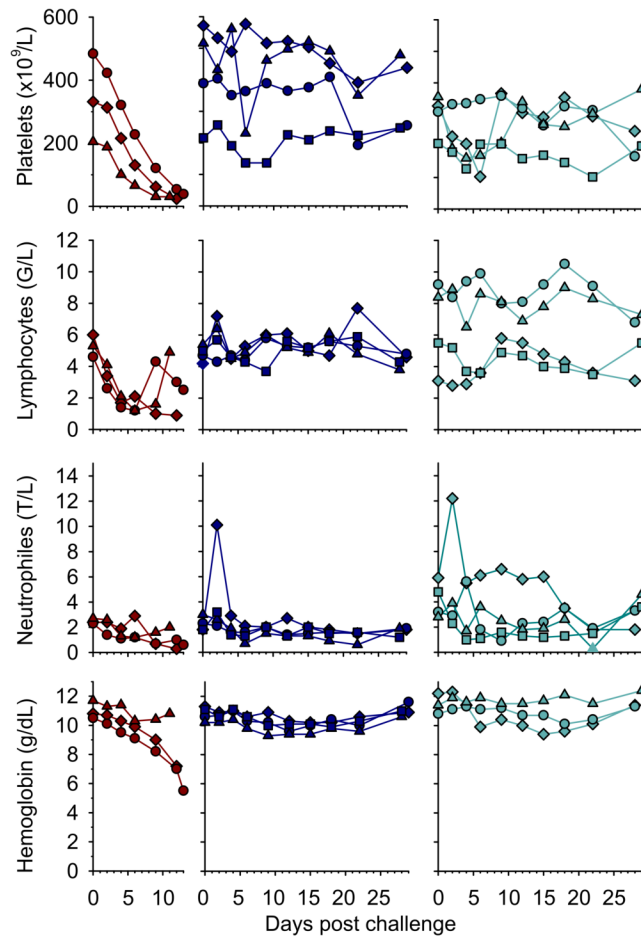


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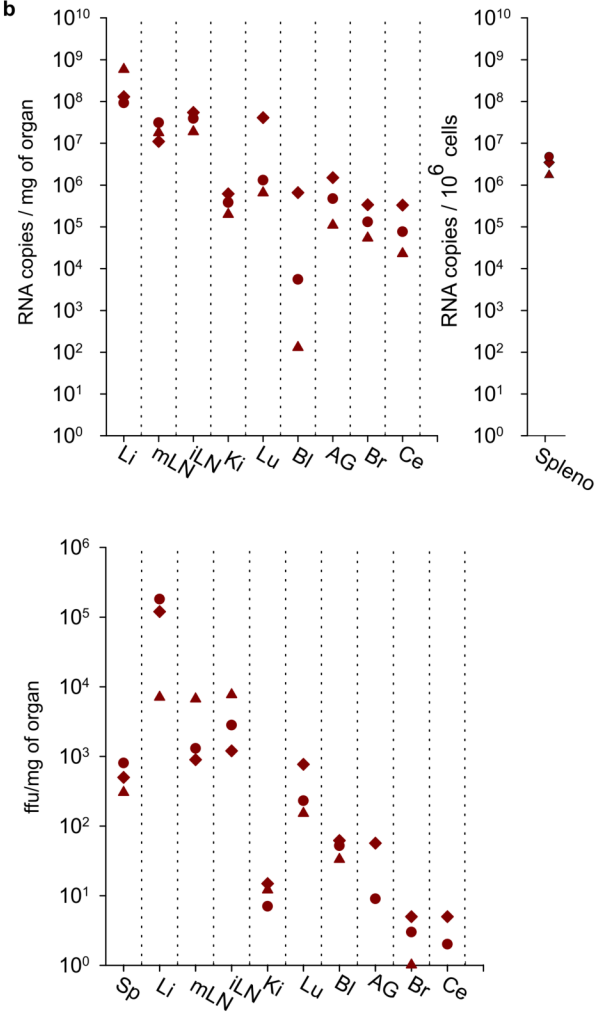


Extended Data Fig. 3

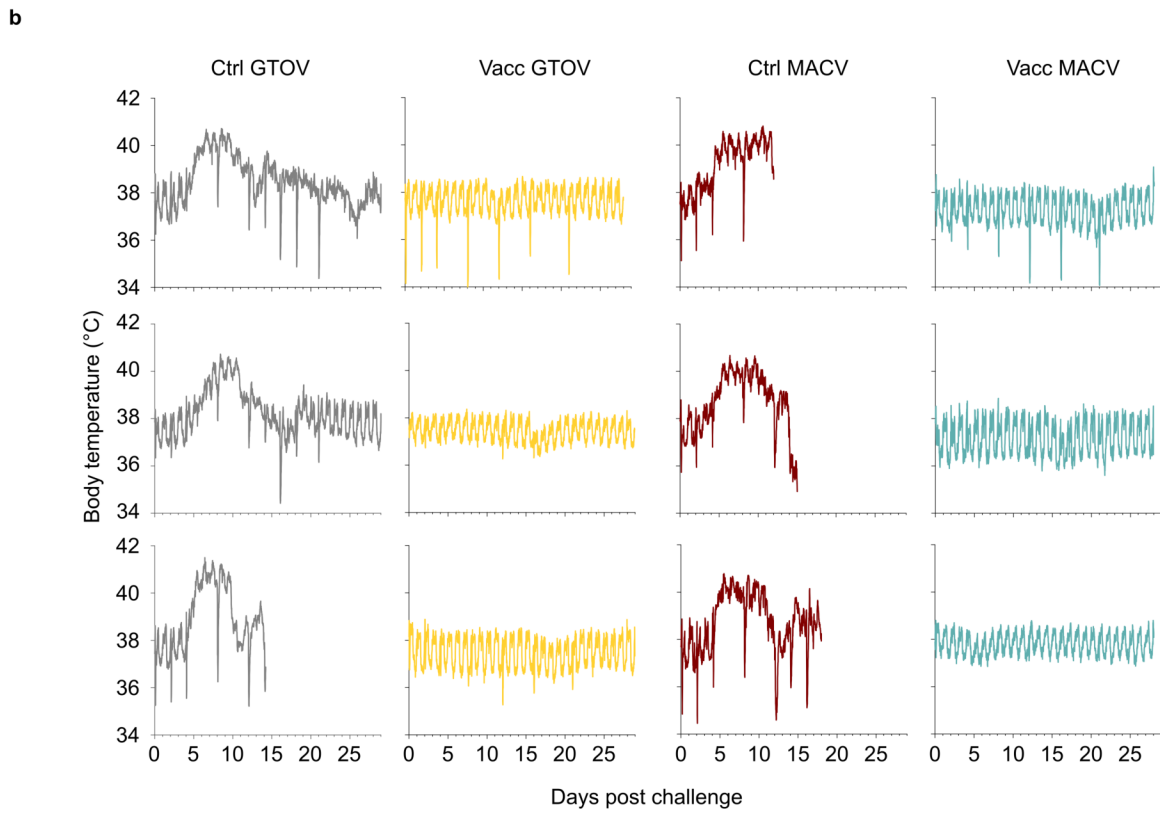
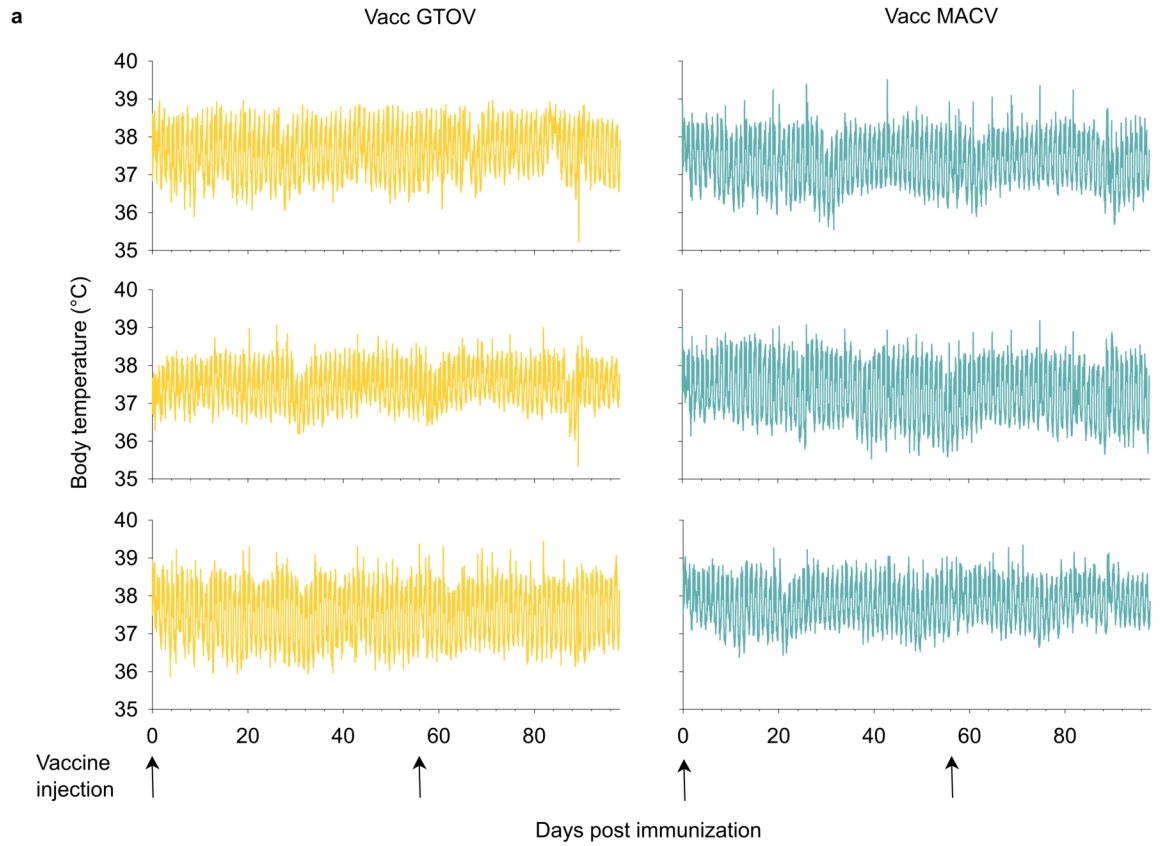
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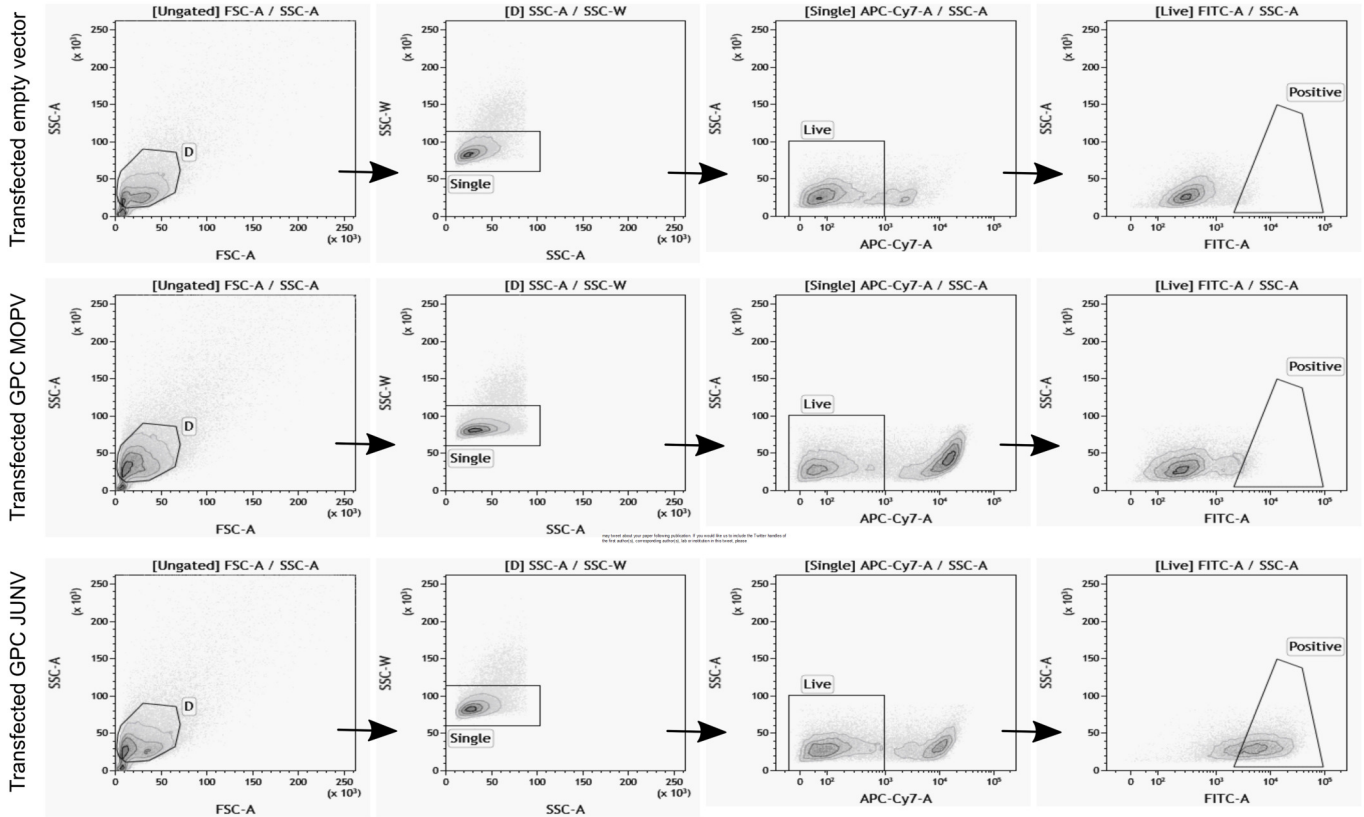
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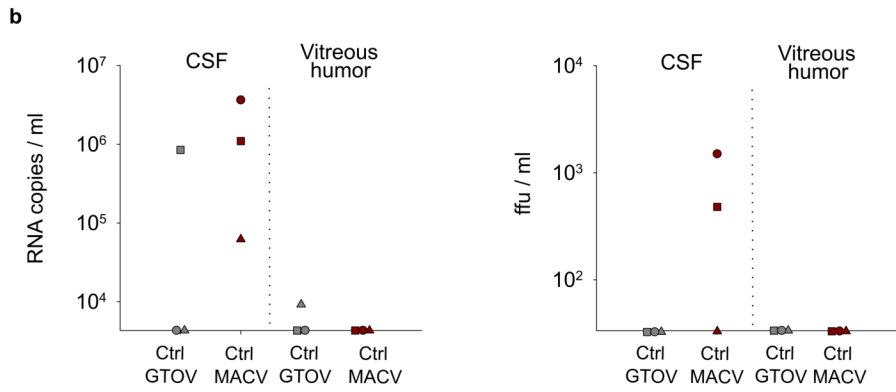
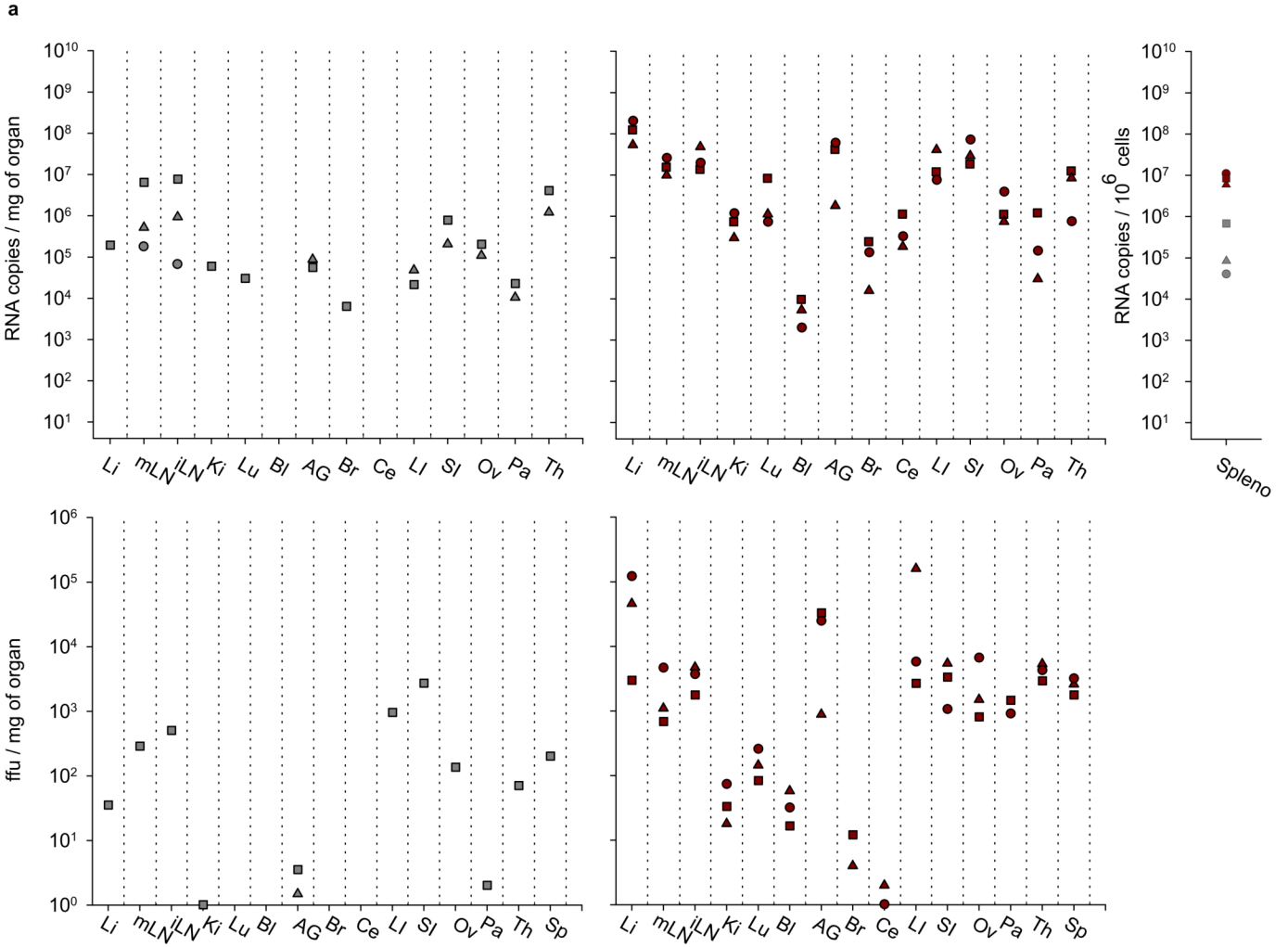
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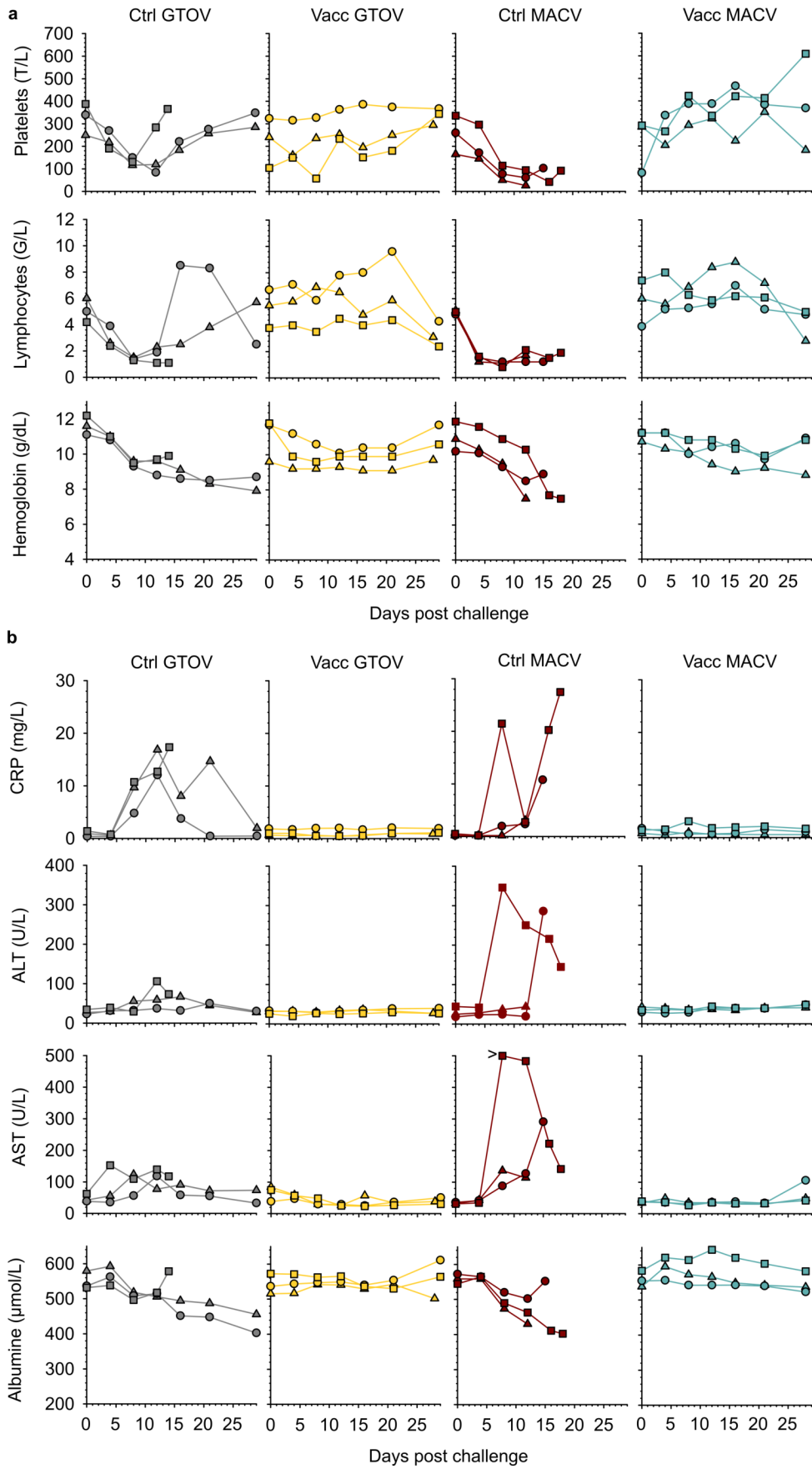
Extended Data Fig. 5



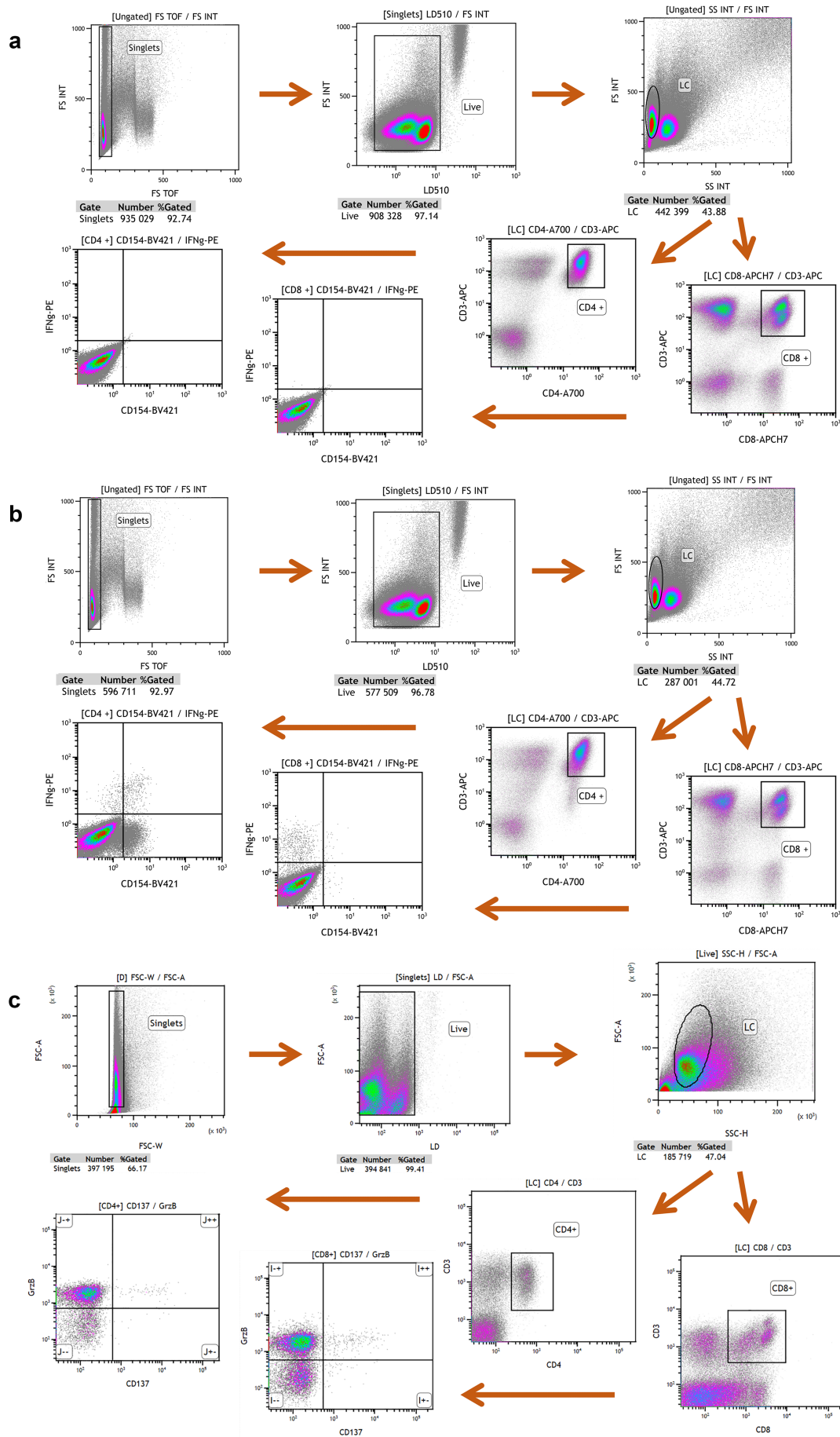
Extended Data Fig. 6



Extended Data Fig. 7



Extended Data Fig. 8



Extended Data Fig. 9

