

A MOPEVAC multivalent vaccine induces sterile protection against New World arenaviruses in non-human primates

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9	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 protect against NWAs. Cynomolgus monkeys immunized with MOPEVAC_{MAC}, targeting Machupo virus, 42 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

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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 transferrin receptor 1². Pathogenic NWAs are a public health concern, with Junin virus (JUNV) being 54 55 endemic in Argentina and having caused hundreds of cases each year before the Candid#1 vaccine was introduced^{3,4}. However, this vaccine is not FDA-approved and induces adverse effects^{5–7}. Machupo virus 56 (MACV) recently re-emerged and caused hundreds of cases^{8,9}. Consequently, vaccine approaches have 57 been proposed^{10,11}. The three other pathogenic NWAs, Sabia virus (SABV), Guanarito virus (GTOV), and 58 Chapare virus (CHAV) have so far emerged only sporadically ^{12–14}. The evolution of rodent populations, the 59 natural reservoir for NWAs, has been associated with human incidence^{15–17}. Depending on the weather, 60 61 increase in rodent populations can occur and cause viral emergence. The absence of treatment or prophylaxis for all the NWAs exposes populations to sporadic viral circulation. Moreover, new pathogenic 62 arenaviruses regularly emerge, such as WWAV in 1999/2000^{18,19}, CHAV¹⁴ in 2003/2004, and Lujo virus²⁰ in 63 2008. 64

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

We thus hypothesized that MOPEVAC, which induces Nabs, could protect against NWAs. We first developed MOPEVAC_{MAC²⁵}, the MOPEVAC platform expressing MACV glycoproteins. Vaccinated animals developed an antibody response with Nabs and were protected against a lethal challenge with MACV. Then, we developed MOPEVAC_{NEW}, a pentavalent vaccine expressing epitopes from all five pathogenic NWAs known in South America. Immunized animals produced antibodies against the five antigens and they were protected against a challenge with MACV and the phylogenetically distant GTOV. This vaccine could provide a means of preemptively protecting against the re-emergence of known NWAs.

80 Main text

81

82 MOPEVAC_{MAC} induces MACV-specific antibody responses

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

MOPEVAC_{MAC} comprises a hyper-attenuated MOPV expressing MACV glycoproteins. Activity of the 85 exonuclease virulence factor was abolished by six mutations in the nucleoprotein^{25,27–30}, ensuring 86 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 we can swap the gene encoding for glycoproteins (GPC gene) with any GPC gene from arenaviruses²⁵. Here, 89 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 were stable over multiple passages (fig. 1c), as previously shown for some candidates²⁵. We sequenced 95 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 replication. These results confirm the stability of all MOPEVAC_{NEW} components over multiple passages and 100 101 in particular of the immunogenic antigen and of the attenuation phenotype.

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

vaccinated animals, neither in the fluids nor in the organs. Vaccination, even with a single dose, was able
 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.

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

140 MOPEVAC_{NEW} induces antibody responses against five NWAs

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

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

MOPEVAC viruses targeted (fig. 3b). Some plasma samples did not neutralize all viruses at the end of the prime period. This result could be explained by higher detection cut-off for this experiment compared to that in the MOPEVAC_{MAC} experiments. NAbs against all viruses were finally detectable in all animals after 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 samples with 293T cells expressing GPCs. Despite differences in the level of recognition, only plasma from 158 159 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 score observed was due to diarrhea, which was also observed for certain animals before the challenge andprobably 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).

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

- showed a low NAb titer against MOPEVAC_{CHA} and _{SAB}, but not against wild-type MACV (Fig. 5c). As we used
 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 transcriptomic analysis on PBMCs from the MOPEVAC_{MAC} experiment³¹. Control animals were included, 198 199 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.

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

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

day 6, and upregulated from day 9, in the very last days of the illness. The B-cell response was increasingly
downregulated during the course of the disease (Fig. 6b, Supp. Table 3).). The day 0 was highly similar to
that of the point taken during immunization period. For the vaccinated animals, we observed only a
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 be efficiently controlled. There are also vaccines for MACV^{10,11,33} at the preclinical stage. Here, we provide 225 a vaccine candidate validated in a non-human primate model able to confer sterile protection against two 226 227 distant viruses and induce NAbs against all pathogenic South American NWAs. Bivalent vaccines were previously tested but failed to provide sterile protection against different viruses^{10,33}. Moreover, a study 228 229 of NAbs in the plasma of Candid#1 vaccinated patients showed that there was no cross-neutralization with other NWA³⁴. Yet, both known and unknown arenaviruses present a high risk of emergence or re-230 231 emergence. For example, WWAV and CHAV emerged in the last decades, while MACV, first described in the 60's after being responsible for 637 cases³⁵, caused sporadic cases until 2006, and re-emerged to cause 232 more than 200 cases in 2008. CHAV also caused an outbreak in 2019³⁶ and GTOV in 2021³⁷. Despite the 233 234 quite low incidence of human contaminations, cases of human-to-human transmission were documented, mainly to health care workers and laboratory staff^{35,36,38}. The recent Ebola virus epidemics and SARS-CoV-235 236 2 pandemic have shown the importance of preparedness and, concerning vaccines, the necessity of having products fully validated at the preclinical level and ready to go into the clinic. During the 2014-2016 Ebola 237 outbreak, the use of Ervebo® vaccine helped to control the outbreak and accelerated licensing of the 238 vaccine³⁹. The low incidence of individual NWAs favors development of an easy to produce, affordable 239 240 multivalent vaccine. The production of MOPEVAC_{NEW}, composed of five attenuated viruses, represents a challenge, but all viruses replicate similarly and optimization of the production could provide an efficient 241 242 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 immunization^{34,40}. Studies have demonstrated that NAbs against NWAs did not share the same binding 255 256 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.

The immune responses promoted after immunization and challenge were different from those observed with MOPEVAC_{LAS}²⁶. We did not detect the activation of T cells after peptide stimulation, suggesting that the vaccine did not predominantly induce a Th1 response and/or cytotoxic T cells like MOPEVAC_{LAS}²⁶. This difference is unlikely to be due to a change in primary target cells, since both NWAs and LASV, target antigen-presenting cells. However, the GPC carried by MOPEVAC could affect the response. Indeed,
 previous studies have linked the presence of N-glycans on GP1 to low neutralization capacity of NAbs⁴².
 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}. Doublestranded RNAs (dsRNA) accumulate in NWA-infected cells and thus activate RIG-I like receptors⁴⁷. In 273 274 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 treatment and succeeded in resolving the acute phase of the disease^{21,22,24}. Here, the presence of NAbs at 286 287 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

- 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.

295 Methods

296

297 Study design

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

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

and 1 prime/boost). Moreover, the new implantation resulted in local inflammation that was still presentfor 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 kg. Six received the vehicle and six were vaccinated with MOPEVAC_{NEW} (2.10⁶ ffu i.e 4.10⁵ ffu of each 323 valence). The immunization was performed on days 0 and 56. The animals were transferred to the BSL4 324 325 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 unvaccinated. The sampling interval was extended due to the lower weight of the animals. 328

329 Ethical statements

330 The protocol of the first experiment was approved by the "Comité Régional d'Ethique en Matière d'Expérimentation Animale de Strasbourg" for the immunization period and registered with the number 331 332 APAFIS#18970-2019020616112503 v8 (2019/07/23) and by the ethical committee "CELYNE" (Lyon, 333 France) for the challenge registered procedure and with the number 334 APAFIS#18397 2019011010351235 v4 (2019/03/15).

The protocol of the second experiment was approved by the same ethical committees and registered with the numbers: APAFIS#18970-2019020616112503 v8 (2019/07/23) for the immunization protocol and 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

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

MOPEVAC_{NEW} is a mixture of equivalent quantities of infectious particles of MOPEVAC expressing the GPC of MACV (strain Carvallo; GenBank accession number AY619643), GTOV (strain INH95551; GenBank accession number AY129247), CHAV (strain 810419; GenBank accession number NC_010562), SABV (strain SPH114202; GenBank accession number NC_006317), and JUNV (strain P2045; GenBank accession number DQ854733). It was produced under the same conditions, except for the concentration method. The cell supernatant was precipitated using a PEG solution (Abcam). After overnight incubation at 4°C with gentle 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 MiniSeq (Illumina) and analyzed using the public platform Galaxy⁴⁸. Briefly, RNA was extracted from 1ml 358 359 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 library prep for illumina[®] with 6 minutes of RNA fragmentation and 16 cycles of amplification. Finally, 363

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 NWAs (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

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

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

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

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

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

451 Hematological and biochemical analyses

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

455 Intracellular cytokine staining in PBMCs

Fresh whole blood or PBMCs were stimulated with overlapping peptides of the NP and GPC proteins and
stained for flow cytometry analysis, as previously described²⁶. Staphylococcal Enterotoxin A (SEA) was used
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. RNA samples were then quantified using the QuantifluorRNA system (Promega) and qualified using a 461 462 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 analyzer. Sequencing was performed on a NextSeq 500 Flow Cell High OutputSR75 instrument (Illumina) 467 468 with six samples per flow cell.

Bioinformatics analysis was performed using the RNA-seq pipeline from Sequana⁵⁰. Reads were cleaned of
adapter sequences and low-quality sequences using cutadapt version 1.11⁵¹. Only sequences of at least 25
nucleotides in length were considered for further analysis. STAR version 2.5.0a⁵², with default parameters,
was used for alignment against the reference genome (Macaca Fascicularis 5 from ENSEMBL version 95).
Reads were assigned to genes using featureCounts version 1.4.6-p3⁵³ from the Subreads package
(parameters: -t gene -g ID -O -s 2). Data from these transcriptomic analyses are available on Zenodo DOI:
10.5281/zenodo.7229438⁵⁴.

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. Differential analysis was performed using the DESeq2 R package (v1.24.0)⁵⁵. The model was adjusted for 482 483 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

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

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509

510 Data availability

511 Data are available in public, open access repositories. For transcriptomic analyses, they are available on
512 Zenodo DOI: <u>10.5281/zenodo.7229438</u>.

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 MOPEVACMACV 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 type MACV. Each curve represents an individual value. The arrow indicates the second vaccine injection. 547

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

were obtained only for prime boost group *versus* controls. From day 15, two-sided Mann-Whitney test was performed. Asterisks indicate the p-values (*: <0,05, **<0,01). The optical density obtained by ELISA is represented (at dilution 1/1,000 and 1/250 for prime boost and prime only groups respectively). **e**. Crossneutralization was evaluated on samples after immunization using MOPEVAC viruses. The arrow indicates 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 throughout the immunization period. Two-sided Mann-Whitney statistical tests were performed but did 567 568 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.

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

Fig. 5. Antibody response after challenge. The colors used are the same as in Fig. 4. **a**. IgG titers were evaluated by ELISA and represent the last dilution of plasma that is still positive. The dots indicate individual values. Two-sided Mann-Whitney statistical tests were performed but did not provide significant p-values. **b**. Neutralization titers were defined against wildtype viruses at days 0, 12, and the date of necropsy. The experiment was performed as for fig. 1c. **c**. MOPEVAC viruses were used to evaluate the 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 JO within each group. Asterisks indicate the 595 difference from day 0 (***p < 0.001, **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).

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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. <i>Hum. Vaccin.</i> 7 , 694–700 (2011).
611	4.	Enria, D. A., Briggiler, A. M. & Sánchez, Z. Treatment of Argentine hemorrhagic fever. Antiviral
612		<i>Res.</i> 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. Epistastic Interactions within the Junín Virus Envelope Glycoprotein
619		Complex Provide an Evolutionary Barrier to Reversion in the Live-Attenuated Candid#1 Vaccine. J.
620		<i>Virol.</i> 92 , (2018).
621	8.	Aguilar, P. V et al. Reemergence of Bolivian hemorrhagic fever, 2007-2008. Emerg. Infect. Dis. 15,

- 1526-1528 (2009).
- 623 9. Patterson, M., Grant, A. & Paessler, S. Epidemiology and pathogenesis of Bolivian hemorrhagic fever. Curr. Opin. Virol. 5, 82-90 (2014). 624
- 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 Tesh, R. B., Jahrling, P. B., Salas, R. & Shope, R. E. Description of Guanarito virus (Arenaviridae: 13.
- 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 fever case in Bolivia. PLoS Pathog. 4, e1000047 (2008). 634
- Ellis, B. A. et al. A Longitudinal Study of Junin Virus Activity in the Rodent Reservoir of Agrentine 635 15. 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).

- Fatal illnesses associated with a new world arenavirus--California, 1999-2000. *MMWR. Morb. Mortal. Wkly. Rep.* 49, 709–711 (2000).
- Briese, T. *et al.* Genetic Detection and Characterization of Lujo Virus, a New Hemorrhagic Fever–
 Associated Arenavirus from Southern Africa. *PLoS Pathog.* 5, e1000455 (2009).
- Enria, D. A., Briggiler, A. M., Fernandez, N. J., Levis, S. C. & Maiztegui, J. I. Importance of dose of
 neutralising antibodies in treatment of Argentine haemorrhagic fever with immune plasma. *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
- Pigs With Immune Serum: Development of Late Neurological Disease. J. Med. Virol. 20, 207–218
 (1986).
- Avila, M. M., Samoilovich, S. R., Laguens, R. P., Merani, M. S. & Weissenbacher, M. C. Protection
 of Junín virus-infected marmosets by passive administration of immune serum: association with
 late neurologic signs. *J. Med. Virol.* 21, 67–74 (1987).
- Maiztegui, J. I., Fernandez, N. J. & de Damilano, A. J. Efficacy of immune plasma in treatment of
 Argentine haemorrhagic fever and association between treatment and a late neurological
 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
- the Nucleoprotein of the Prototypic Arenavirus Lymphocytic Choriomeningitis Virus. J. Virol. 83,

- 11330–11340 (2009).
- Jiang, X. *et al.* Structures of arenaviral nucleoproteins with triphosphate dsRNA reveal a unique
 mechanism of immune suppression. *J. Biol. Chem.* 288, 16949–16959 (2013).
- Habjan, M. *et al.* Processing of genome 5' termini as a strategy of negative-strand RNA viruses to
 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
- 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).
- Silva-Ramos, C. R., Faccini-Martínez, Á. A., Calixto, O.-J. & Hidalgo, M. Bolivian hemorrhagic fever:
 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).
- Medaglini, D., Harandi, A. M., Ottenhoff, T. H. M., Siegrist, C.-A. & Consortium, V.-E. Ebola vaccine
 R&D: Filling the knowledge gaps. *Sci. Transl. Med.* 7, 317ps24-317ps24 (2015).
- 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).
- 43. Bonhomme, C. J. *et al.* Glycosylation modulates arenavirus glycoprotein expression and function.
 701 *Virology* **409**, 223–233 (2011).
- 44. Huang, C. *et al.* Highly Pathogenic New World and Old World Human Arenaviruses Induce Distinct
- 703Interferon Responses in Human Cells. J. Virol. 89, 7079–7088 (2015).
- 45. Levis, S. C. *et al.* Correlation between endogenous interferon and the clinical evolution of patients
 with Argentine hemorrhagic fever. *J. Interferon Res.* 5, 383–389 (1985).
- 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. <i>mSphere</i> 3 , e00189-18 (2018).
715	50.	Cokelaer, T., Desvillechabrol, D., Legendre, R. & Cardon, M. 'Sequana': a Set of Snakemake NGS
716		pipelines. <i>J. Open Source Softw.</i> 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. <i>et al.</i> STAR: ultrafast universal RNA-seq aligner. <i>Bioinformatics</i> 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. <i>Bioinformatics</i> 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. <i>Genome Biol.</i> 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	Filename	Figure Legend
	One sentence only	This should be the name the file is saved as when it is uploaded to our system. Please include the file extension. i.e.: Smith_ED_Fig1.jpg	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	Ext_data_Fig1.pdf	a. VeroE6 cells were infected at a moi of 0.001 and cellular
	MOPEVAC viruses		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	ategy for tion of IgGExt_data_Fig5.pdfThe gates used to quantify the cells expressing fixed IgG from plasma are presented. FSC / SSC gate cells, then singlets were determined using and live cells were gated: Live Dead negative c that fixed IgG and the secondary anti-IgG FITC with the gate "Positive". Three conditions of th sample are presented for comparison: empty v expressing GPs of MOPV and cells expressing G	
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, BI: 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 IFNy-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 IFNy-producing and CD154-expressing CD4+ and CD8+ T cells is determined using a quadrant in the IFNy/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 IFNy. 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		

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.: Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.
Supplementary Information	Choose an item.		
Reporting Summary	Choose an item.		
Peer Review Information	Choose an item.	OFFICE USE ONLY	

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Туре	Number 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_</i> <i>Supplementary_Video_1.mov</i>	Legend or Descriptive Caption Describe the contents of the file
			Evolution of vaccine candidate
			genomes during serial passages
			in VeroE6 cells. The consensus
			genome sequences of the five
Supplementary Table		Supp Tables	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.
			Parameters used to establish the
			clinical score after challenge and
Supplementary Table	2	Supp_Tables	their respective values
			Exact p values corresponding to
			figure 6. For each condition the
			comparison was made with the
Supplementary Table	3	Supp_Tables	day 0 timepoint.
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	Unmodified_Gels_Fig1.pdf	
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Extended Data Fig. 1























