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

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Pathogenic New World arenaviruses (NWAs) cause haemorrhagic fevers and can have high mortality rates, as shown in outbreaks in South America. Neutralizing antibodies (Abs) are critical for protection from NWAs. Having shown that the MOPEVAC vaccine, based on a hyperattenuated arenavirus, induces neutralizing Abs 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, prevented the lethality of this virus and induced partially NWA cross-reactive neutralizing Abs. We then developed the pentavalent MOPEVAC$_{NEW}$ vaccine, expressing glycoproteins from all pathogenic South American NWAs. Immunization of cynomolgus monkeys with MOPEVAC$_{NEW}$ induced neutralizing Abs against five NWAs, strong innate followed by adaptive immune responses as detected by transcriptomics and provided sterile protection against Machupo virus and the genetically distant Guanarito virus. MOPEVAC$_{NEW}$ may thus be efficient to protect against existing and potentially emerging NWAs.

New World arenaviruses (NWAs) are causative agents for severe haemorrhagic fevers. Among the four clades of NWAs, clade B contains all the pathogenic strains with the exception of Whitewater Arroyo virus (WWAV), which belongs to clade A/B[1]. These pathogenic strains share the same entry receptor, human transferrin receptor 1 (ref. [2]). Pathogenic NWAs are a public health concern, with Junin virus (JUNV) being 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 US Food and Drug Administration-approved and induces adverse events[5–7]. Machupo virus (MACV) recently re-emerged and

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caused hundreds of cases[8,9]. Consequently, vaccine approaches have been proposed[10,11]. The three other pathogenic NWAs, Sabia virus (SABV), Guanarito virus (GTOV) and Chapare virus (CHAV) have so far emerged only sporadically[12–14]. The evolution of rodent populations, the natural reservoir for NWAs, has been associated with human incidence[15–17]. Depending on the weather, an 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 arenaviruses regularly emerge, such as WWAV in 1999 and 2000 (refs. [18,19]), CHAV[14] in 2003 and 2004 and Lujo virus[20] in 2008.

Treatment of patients infected with JUNV with convalescent plasma is efficient during acute disease[21]. 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 (Abs) are crucial for the control of NWAs[21,22,24]. We previously developed MOPEVAC, a hyperattenuated 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 and a robust Ab response[26]. Thus, we hypothesized that MOPEVAC, which induces neutralizing Abs, could protect against NWAs. We first developed MOPEVAC$_{MAC}$[25], the MOPEVAC platform expressing MACV glycoproteins. Vaccinated animals developed an Ab response with neutralizing Abs 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 Abs 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 pre-emptively protecting against the re-emergence of known NWAs.

MOPEVAC$_{MAC}$ induces MACV-specific Ab responses

Cynomolgus monkeys (CMs) received MOPEVAC$_{MAC}$ vaccine as 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 hyperattenuated MOPV expressing MACV glycoproteins. Activity of the exonuclease virulence factor was abolished by six mutations in the nucleoprotein[25,27–30], ensuring attenuation of MOPV, already known to be non-pathogenic (Fig. 1b). Infection of antigen-presenting cells with MOPEVAC results in immune activation and a lack of viral replication. We previously showed that we can swap the gene encoding glycoproteins (glycoprotein precursor (*GPC*) gene) with any *GPC* gene from arenaviruses[25]. In this study, we demonstrated that equal amounts of glycoproteins were expressed in infected cells by the different MOPEVAC expressing the NWA glycoproteins. The ratio between RNA amounts of GPC at day 3 versus day 0 was similar for all MOPEVAC (Extended Data Fig. 1a). Similar amounts of GP2 protein were contained in all MOPEVAC viruses, except for SABV whose GP2 was not recognized by our Abs (Extended Data Fig. 1b). Replication of MOPEVAC was tested with different NWA GPC. They all replicated equally and were stable over multiple passages (Fig. 1c), as shown previously for some candidates[25]. We sequenced the viruses at passage 2, 5 and 10 to confirm the absence of major changes in the consensus genome sequence after passaging. No mutation was consistently present in all GPC sequences after ten passages, or in the six amino acids mutated to abrogate the exonuclease domain (Supplementary Table 1). Only one or two 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 in particular of the immunogenic antigen and 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 Ab titres rose to 1,000 during the first month (Fig. 1d). The boost resulted in a rapid increase in Ab release, with titres reaching 16,000, the upper limit of our test. By day 14 after the first injection, 6 of 8 animals showed neutralizing Abs, mainly at low titres. By day 30, all animals were positive. The second vaccine injection resulted in neutralizing titres of 100 in all animals. Thus, MOPEVAC$_{MAC}$ promoted a neutralizing Ab response.

MOPEVAC$_{MAC}$ induces sterile protection against MACV

After the immunization period, all animals were challenged with MACV. A clinical score was calculated each day based on the symptoms observed (Supplementary Table 2). This score remained low throughout the experiment for vaccinated animals, with no difference between vaccination regimens. Unvaccinated animals experienced fever from days 4 to 5 (Extended Data Fig. 2); from day 8 they presented with balance disorders, dehydration and reduced interactions. They eventually reached the ethical end point between days 11 and 13 because of the absence of reactivity, intense dehydration and epistaxis. They continuously lost weight from day 4 (Fig. 2a) and experienced profound lymphopenia and thrombocytopenia, with a drop in haemoglobin concentration observed for 2–3 animals (Extended Data Fig. 3a). They also presented increasing levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and plasma urea and a decrease in plasma albumin concentrations (Fig. 2b), suggesting liver and tissue damage and renal injury. No alterations in haematological and biochemical parameters were detected in vaccinated animals.

Control animals presented increasing titres of MACV from day 6 after infection in plasma and oral or nasal swabs (Fig. 2c). At the day of euthanasia, the infection was pantropic and all organs tested were MACV-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, controlled MACV replication. Thus, MOPEVAC$_{MAC}$ induces a sterilizing immunity in CMs.

In vaccinated animals, we did not observe any increase in IgG titres after challenge (Fig. 2d). To ensure that a low increase in Ab levels did not occur, we represented the optical density at a single dilution. A slight decrease occurred for the prime-boost group but did not alter the IgG titre and the values remained stable for the prime-only group. However, neutralizing Ab levels increased after challenge. The titre was finally at 100 for all vaccinated animals with no heterogeneity depending on the vaccination regimen. In the control animals, specific Abs were not 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$_{JUN,GTO,CHA}$, that is, MOPEVAC expressing JUNV, GTOV and CHAV glycoproteins, respectively. Although all plasma samples presented detectable neutralizing Abs 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 neutralizing Abs except for in one animal that did not neutralize GTOV. Although titres were lower than for the homologous virus, MOPEVAC$_{MAC}$ induced cross-neutralizing Abs.

MOPEVAC$_{NEW}$ induces Ab 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; six other animals received the vehicle (Fig. 3a). We did not observe

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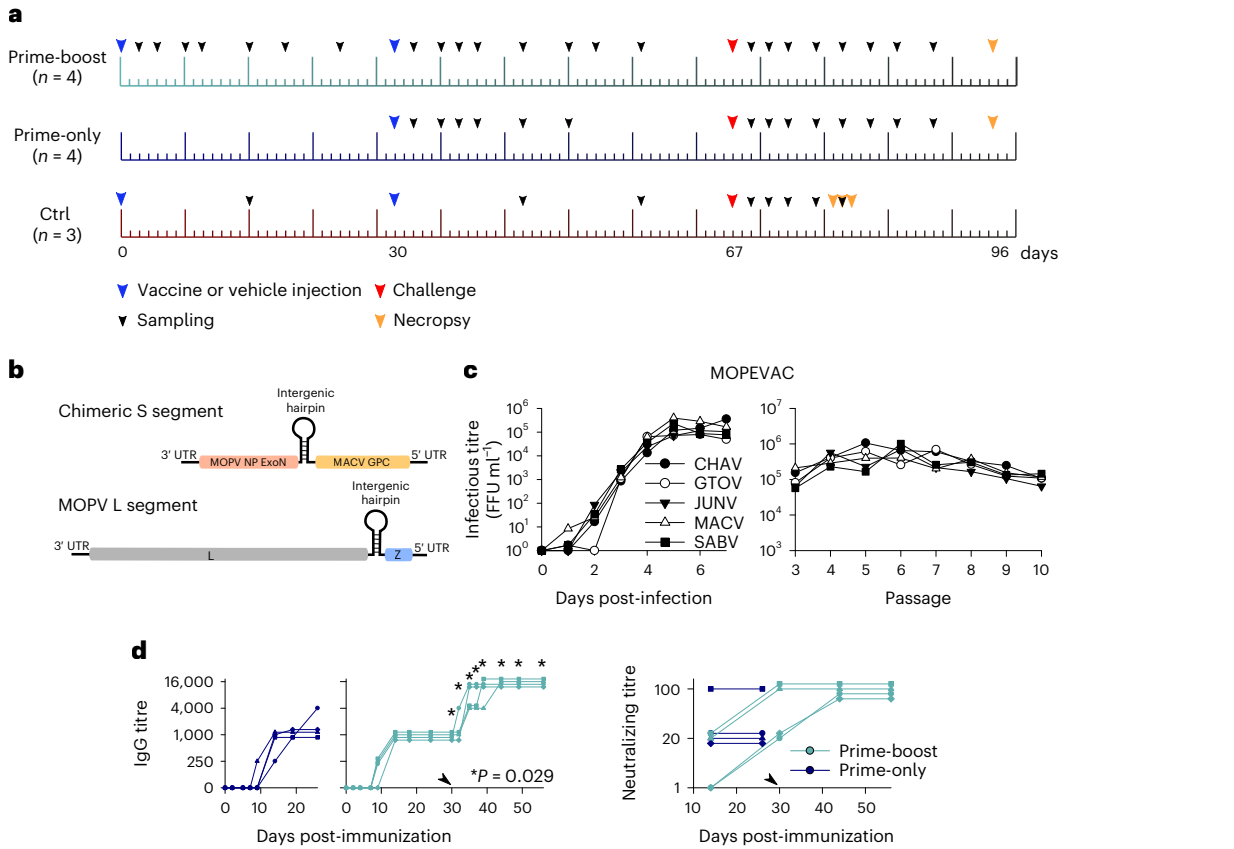

Fig. 1 | CM immunization with MOPEVAC$_{MACV}$ induces Ab responses. **a**, Schematic view of the experiment. The long vertical bars represent each week. The days of vaccination, sampling, challenge and necropsy are indicated with coloured arrows. **b**, Bi-segmented organization of the MOPEVAC$_{MACV}$ genome. The S segment is mutated in the exonuclease domain (ExoN) of the nucleoprotein (NP) and the GPC of MACV replaces the GPC of MOPV. The L segment is that of WT MOPV. L, polymerase; UTR, untranslated region; Z, zinc finger matrix protein. **c**, Vero E6 cells were infected at an MOI = 0.0001 and cell supernatants were collected every day until day 7 and titrated (left). Viral titres of the different MOPEVAC at each passage from passage two to ten (right). The mean of two experiments is represented for both graphs. **d**, Ab response during the immunization period. The IgG titre is the last dilution of the plasma sample that is still positive for MACV-specific IgG. Each dot represents an individual value ($n = 4$). When all samples were negative, dots were not separated. A two-sided Mann–Whitney *U*-test was performed to compare IgG values after boost versus after prime on the same day after injection. *P* values obtained are indicated by a single asterisk (*$P < 0.05$). The neutralization titre corresponds to the last dilution of plasma that still neutralizes more than 50% of WT MACV. Each curve represents an individual value. The arrow indicates the second vaccine injection.

any clinical signs during the immunization period (Extended Data Fig. 4a). We also failed to detect vaccine shedding in the days after the injections (until day 9 after prime and day 5 after boost). The neutralizing titres 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 a higher detection cut-off for this experiment compared to that in the MOPEVAC$_{MAC}$ experiments. Neutralizing Abs against all viruses were finally detectable in all animals after the boost with the strongest response detected against JUNV.

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

MOPEVAC$_{NEW}$ induces sterile protection against MACV and GTOV

Three vaccinated and three control animals were each challenged with either MACV or GTOV. MACV and GTOV infection induced illness in control animals (Fig. 4a). The evolution of the disease after MACV infection was highly similar to that of the first experiment. Animals reached the ethical end point between days 12 and 18. One was euthanized despite a clinical score of 13 due to a weight loss of 27%. GTOV infection induced symptoms 2 d later, including reduced activity, gastrointestinal symptoms, weight loss and fever (Extended Data Fig. 4b). One GTOV control animal reached the end point on day 14 (Fig. 4a). The two remaining CMs presented maximum scores of 12 and 13 on day 16. These scores then decreased; by day 29, they were at 5 and 10, respectively. This latter animal presented with a weight loss of 22%, which should have been an end point (Fig. 4a). None of the vaccinated CMs experienced clinical signs; the low score observed was due to diarrhoea, which was also observed for certain animals before the challenge and probably unspecific.

The three MACV-infected controls presented viral replication in fluids and in the organs highly similar to that observed in the first experiment (Fig. 4b and Extended Data Fig. 6a). GTOV-infected controls presented lower infectious titres in the fluids but high viral RNA loads (Fig. 4b). Viral RNA was also measured in the organs of the deceased

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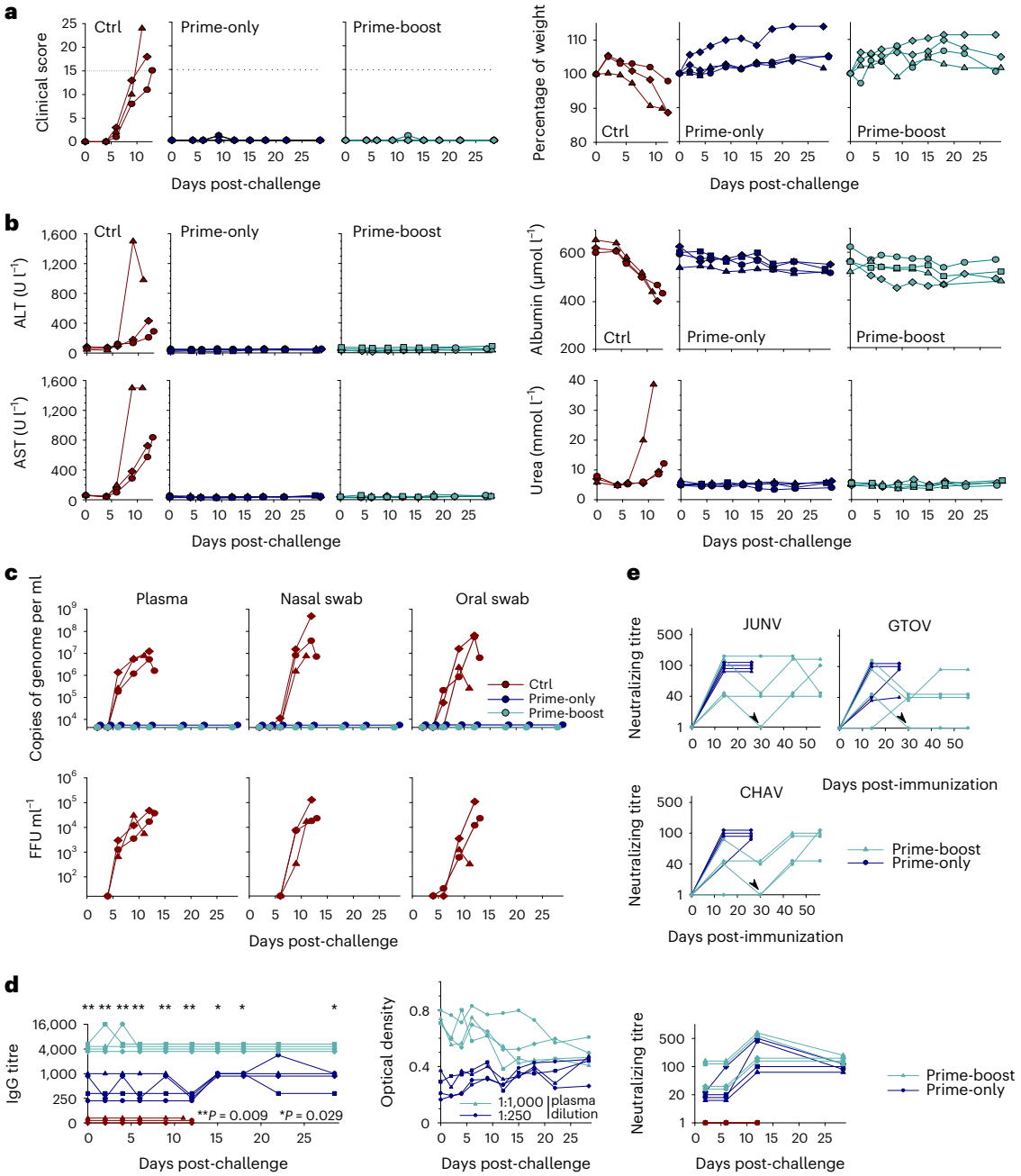

Fig. 2 | Post-challenge monitoring of animals and Ab response. a, Clinical score and percentage of initial body weight at each sampling. **b**, Biochemical parameters measured in plasma samples (ALT/AST). The point represented at day 0 was actually day −9 (receipt of animals). **c**, Viral RNA quantified by RT–qPCR and virus titration performed on positive samples. Negative values are represented at the detection limit. **d**, IgG and neutralization titres are represented as in Fig. 1c. A one-way ANOVA was performed from day 0 to day 12. Significant *P* values were obtained only for the prime-boost group versus controls. From day 15, a two-sided Mann–Whitney *U*-test was performed. $^{*}P < 0.05$, $^{**}P < 0.01$. The optical density obtained by ELISA is represented at dilutions of 1:1,000 and 1:250 for the prime-boost and prime-only groups, respectively. **e**, Cross-neutralization was evaluated on samples after immunization using MOPEVAC viruses. The arrowhead indicates the boost.

GTOV control but at lower titres. Infectious particles were detected mainly in the secondary lymphoid organs, the liver, ovaries and intestine (Extended Data Fig. 6a). At the end of the protocol, the healthiest remaining GTOV control presented the lowest viral titres in the fluids and organs. The other presented viral RNA in many organs but infectious particles were found only in the adrenal gland. We detected viral RNA in all cerebrospinal fluids from control animals at the peak of the disease but only two MACV-infected animals had infectious particles (Extended Data Fig. 6b). This indicates that the virus can cross the blood–brain barrier. The vitreous humour contained viral RNA in one surviving GTOV-infected control. We never detected viral RNA in the organs or fluids of vaccinated animals, showing that the vaccine induced a sterilizing immunity as observed with $MOPEVAC_{MAC}$. Moreover, no significant change was observed in vaccinated animals for haematological and biochemical parameters, unlike control animals (Extended Data Fig. 7a,b).

The IgG titres against GTOV increased after challenge for vaccinated animals and eventually reached the IgG titre measured against

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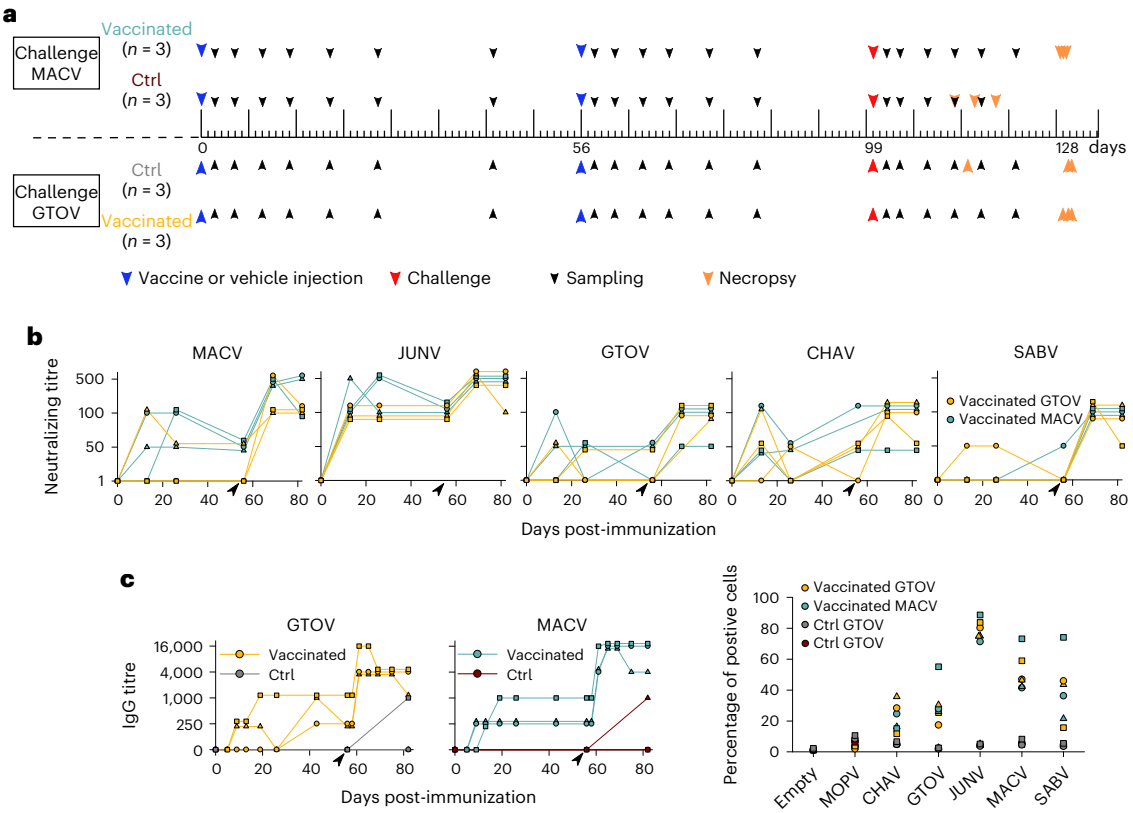

Fig. 3 | Immune responses induced by MOPEVAC$_{NEW}$ in CMs. a, Schematic representation of the protocol. Monkeys were vaccinated or not using a prime-boost strategy ($n = 6$) and then infected with either MACV or GTOV (4 groups, $n = 3$). The representation is as in Fig. 1a. **b**, Neutralizing Abs were measured during the immunization period and quantified for each pathogenic NWA. The representation is as in Fig. 2e. All vaccinated or control animals received the same injections; they are distinguished depending on the future 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 *U*-tests were performed but did not provide significant *P* values ($n = 3$). The arrowhead indicates the second injection of the vaccine. The animals are represented with colours as in **b**. IgG was also tested on 293T cells expressing GPC from NWAs, MOPV and control cells without GPC expression. The percentage of cells that were recognized by IgG from the plasma samples at day 82 post-immunization is represented for each animal.

MACV (Fig. 5a). Two out of the 3 MACV-infected control animals developed specific IgG by their respective end points (day 12 and 18). The GTOV-infected controls that survived showed increasing Ab levels from day 16. The vaccinated CMs boosted the production of neutralizing Abs specific to the virus used for their challenge (Fig. 5b). We did not observe any neutralizing Abs in MACV-infected controls but all GTOV-infected controls showed significant levels of neutralizing Abs at day 12. In one of the animals that survived, the titre rose to 2,000, the highest titre measured. Unexpectedly, one of the MACV controls showed a low neutralizing Ab titre against MOPEVAC$_{CHA}$ and MOVEPAC$_{SAB}$ but not against wild-type (WT) MACV (Fig. 5c). Since we used whole viruses for the experiments, this must have increased the risk of non-specific neutralization.

Immune responses involved in protection

We evaluated the expression profiles of genes related to innate, T cell and B cell responses using a transcriptomic analysis on peripheral blood mononuclear cells (PBMCs) from the MOPEVAC$_{MAC}$ experiment[31]. Control animals were included, with day 30 samples, the day of the second vehicle injection. After immunization, we observed strong and transient activation of the innate immune response in the first 2 d. The boost reactivated the response to a lower extent before significant downregulation from day 5 (Fig. 6a and Supplementary Table 3). The T cell response was also upregulated in a very significant manner from days 4 and 5 after the prime and from day 2 after the boost. Therefore, boost was efficient in reactivating the T cell immune response. At the day 0 time point, expression of B cell response-related genes implicated was heterogeneous, including for controls, but the mean expression in the pathway was comparable. Expression of genes related to the B cell response was quickly and significantly downregulated from day 2 after vaccination until 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 nucleoproteins and glycoproteins. Super-antigen staphylococcal enterotoxin A (SEA) was used as a positive control. This stimulation did not activate the expression of CD154 or CD137, markers of CD4 and CD8 T cell activation respectively, and of interferon-γ (IFN-γ) or granzyme B (indicators of a cytotoxic response), in contrast to the strong activation induced by SEA (Extended Data Figs. 8 and 9a). After challenge, we only observed, after whole-blood stimulation, a slight expression of IFN-γ around day 15 in only 1 out of 8 animals (Extended Data Fig. 9b).

Transcriptomic analysis of 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 and Supplementary Table 3). Day 0 was highly similar to that of the point taken during the immunization period. For vaccinated animals, we observed only a modest regulation of these pathways, which was more significant in the prime group.

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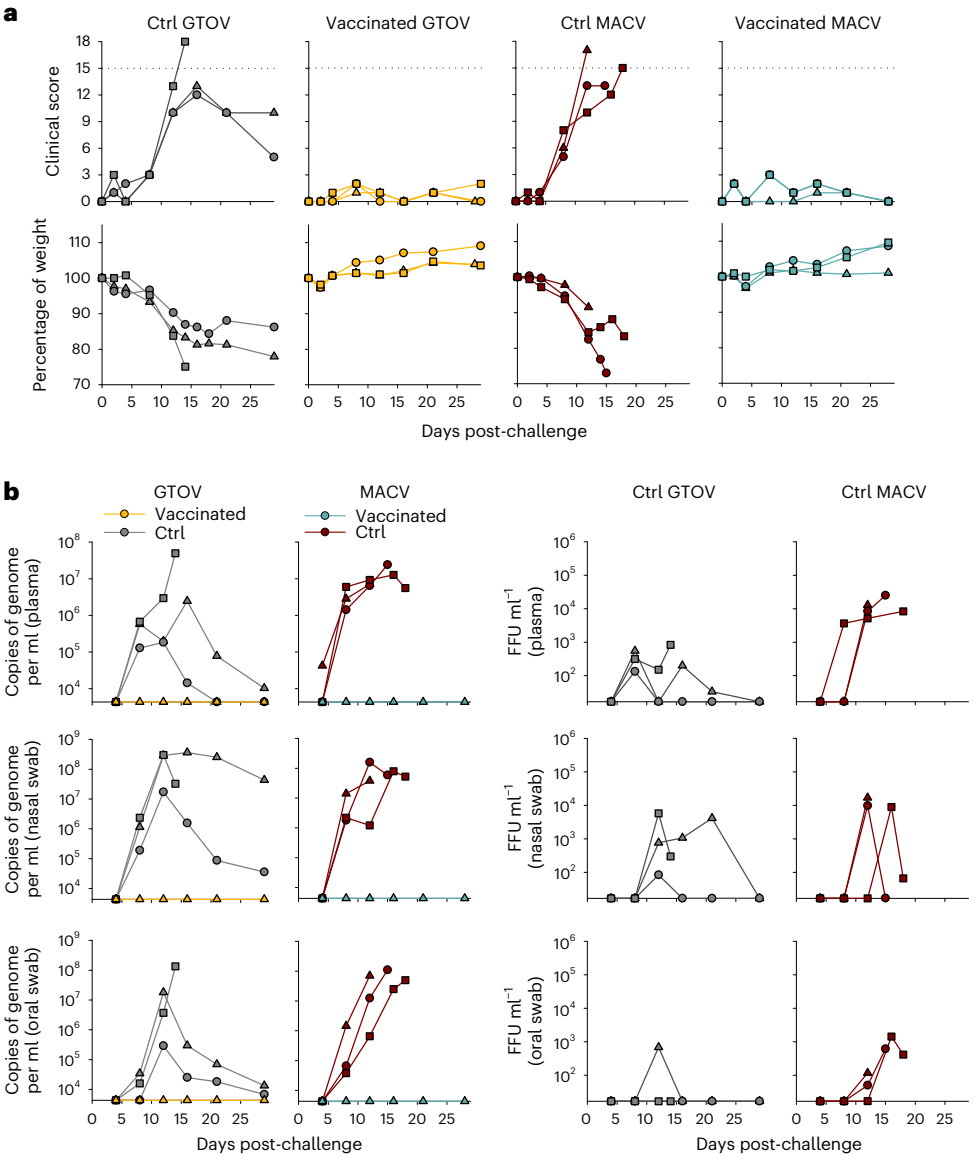

Fig. 4 | Challenge of vaccinated or unvaccinated CMs with GTOV and MACV. **a**, Unvaccinated CMs were challenged with GTOV (grey, $n = 3$) or MACV (red, $n = 3$). Vaccinated CMs received the same challenge with GTOV (orange, $n = 3$) or MACV (turquoise, $n = 3$). The clinical score and weight loss were 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.

Discussion

Despite the currently low incidence of human cases, NWAs are of concern due to the risk of emergence and the severity of the disease[32]. 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. In this study, we provide a vaccine candidate validated in a non-human primate model able to confer sterile protection against two distant viruses and induce neutralizing Abs 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 of neutralizing Abs in the plasma of patients vaccinated with Candid#1 showed that there was no cross-neutralization with other NWAs[34]. Yet, both known and unknown arenaviruses present a high risk of emergence or re-emergence. For example, WWAV and CHAV emerged in the last decades, while MACV, first described in the 1960s after being responsible for 637 cases[35], caused sporadic cases until 2006 and re-emerged to cause more than 200 cases in 2008. CHAV also caused an outbreak in 2019 (ref. [36]) and GTOV in 2021 (ref. [37]). Despite the quite low incidence of human contamination, cases of human-to-human transmission were documented, mainly to healthcare workers and laboratory staff[35,36,38]. The recent Ebola virus epidemics and severe acute respiratory syndrome coronavirus 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 outbreak, the use of the Ervebo vaccine helped to control the outbreak and accelerated licensing of the vaccine[39]. The low incidence of individual NWAs favours development of an easy-to-produce, affordable multivalent vaccine. The production of $MOPEVAC_{NEW}$, composed of five attenuated viruses, represents a challenge, but all viruses replicate similarly and optimization of production could provide an efficient method suitable for all five viruses. Eventually, this vaccine could protect the entire South American continent against NWAs.

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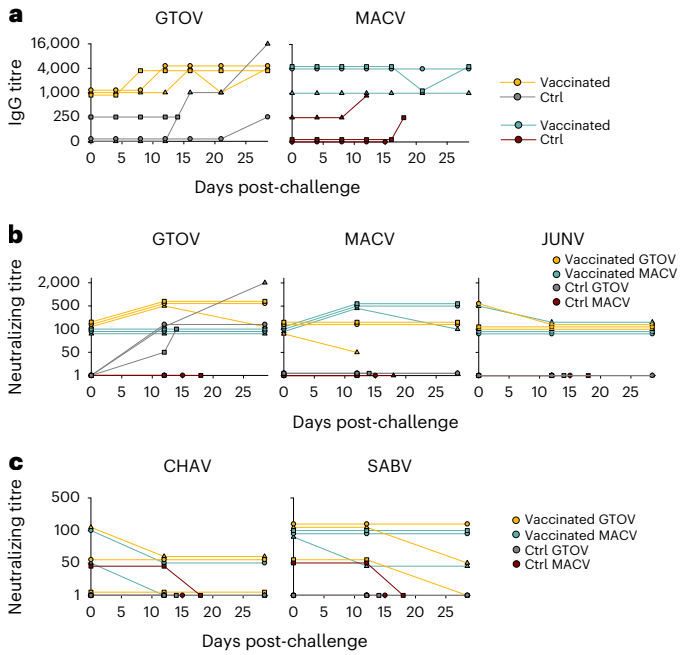

Fig. 5 | Ab response after challenge. The colours used are the same as in Fig. 4. **a**, IgG titres were evaluated by ELISA and represent the last dilution of plasma that was still positive. The dots indicate individual values. Two-sided Mann–Whitney *U*-tests were performed but did not provide significant *P* values. **b**, Neutralization titres were defined against WT viruses on days 0, 12 and the date of necropsy. The experiment was performed as in Fig. 1c. **c**, MOPEVAC viruses were used to evaluate the neutralization titres. The experiment was performed using the same protocol.

We demonstrated that MOPEVAC could protect against NWAs. We were able to fully protect CMs against MACV and GTOV infection, whereas in the LASV experiment, 3 out of 4 CMs experienced fever and low transient replication of the virus after challenge[26]. Neutralizing Abs are crucial in the protection against NWAs[21,22,24], the induction of neutralizing Abs after immunization met our expectations. We did not find persistent virus in any of the organs or samples tested, suggesting that the late-onset encephalitis should not occur with MOPEVAC. Since we detected low neutralizing titres against heterologous viruses after $MOPEVAC_{MAC}$ immunization, we created a multivalent vaccine against all known NWAs from South America. $MOPEVAC_{NEW}$ was efficient against two phylogenetically distant NWAs. Thus, we did not face low specificity and inefficient cross-neutralization as sometimes observed with multivalent vaccines.

We observed cross-neutralization between NWAs. Plasma samples from MACV-immunized CMs partially neutralized other NWAs GPCs. Interestingly, such broad neutralizing Abs are not always induced after NWA immunization[34,40]. Studies have demonstrated that neutralizing Abs against NWAs did not share the same binding site. JUNV neutralizing Abs mainly used transferrin receptor mimicry while MACV neutralizing Abs did not. However, neutralizing Abs able to neutralize both JUNV and MACV were found in plasma from a Candid#1 vaccinated donor[41]; a conserved domain in the receptor binding site was identified. Cross-neutralization observed in MACV-vaccinated animals may thus be due to such Abs. The $MOPEVAC_{NEW}$ vaccine expresses glycoproteins from all NWAs and thus induced many neutralizing Abs. We observed an increase in neutralizing Ab titres specifically against the virus used for the challenge. This suggests that a pool of neutralizing Abs is induced and that the infection boosts the synthesis of neutralizing Abs that are the most specific for the challenge virus. Therefore, neutralizing Abs seem important for protection although 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 T_H1 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 neutralizing Abs[42]. Interestingly, JUNV is the most efficiently neutralized virus and its GP1 is the least glycosylated[42,43].

Transcriptomic analysis of PBMCs showed that both vaccination and infection of control animals are responsible for a strong IFN response, which is consistent with in vitro and in vivo studies[44–46]. Double-stranded RNAs (dsRNAs) accumulate in NWA-infected cells and thus activate RIG-I-like receptors[47]. In MOPEVAC, the mutations in the exonuclease domain prevent dsRNA degradation. Consistently, both immunization and challenge induce the overexpression of genes implicated in RIG-I-like receptor signalling: *IFIH1* (MDA5), *DDX58* (RIG-I), *DHX58* (LGP2) and *DDX60*. *TLR7*, involved in single-stranded RNA recognition, was upregulated after challenge. Overall, upregulation of the *MX1* and *MX2* genes involved in IFN signalling is observed. After immunization, a significant T cell response took place from day 4 and was reactivated quickly after the boost injection. IFN-γ, tumour necrosis factor-α, granzyme B and perforin gene expression did not appear to be significantly regulated, reinforcing the hypothesis of a non-cytotoxic T cell response. The genes associated with B cell responses were significantly downregulated in the first days after vaccination. This could be due to the recruitment of B cells to the germinal centres to promote Ag recognition and initiation of the humoral response. The observed T cell response, associated with B cell regulation, suggests the induction of a humoral response dependent on helper T cells. This results in the strong induction of Abs and provides protection with sterilizing immunity. Neutralizing Abs were previously used as a treatment and succeeded in resolving the acute phase of the disease[21,22,24]. In this study, the presence of neutralizing Abs at the time of infection may enable viral elimination before dissemination, particularly into the brain, avoiding the risk of late-onset encephalitis.

$MOPEVAC_{NEW}$ is an efficacious vaccine against MACV and GTOV, two distinct arenaviruses. We detected cross-reactive neutralizing Abs produced in response to $MOPEVAC_{MAC}$ and neutralizing Abs against all NWAs tested in response 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 could provide a ready-to-use solution for a future emergence.

Methods

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 2.3–3.9 kg. There were no significant differences in these parameters between the groups. Immunization was performed in a Biosafety Level 2 (BSL2) animal facility (SILABE). Three animals received vehicle and four animals received $MOPEVAC_{MAC}$ 67 and 37 d before challenge (controls and prime-boost group, respectively). The four remaining animals received a single dose of $MOPEVAC_{MAC}$ 37 d before challenge (prime-only group). The vaccine consisted of 2×10^6 focus-forming units (FFU) injected intramuscularly. Blood, urine and oral and nasal swabs were sampled periodically.

Animals were challenged in the Biosafety Level 4 (BSL4) laboratory (P4 Jean Mérieux-Institut National de la Santé et de la Recherche (INSERM)) after 10 d of acclimation. They all received 3,000 FFU of MACV (Carvallo strain) subcutaneously. Samples were taken every 2 d until day 6, every 3 d from day 6 to day 18, and on day 22. Each sampling

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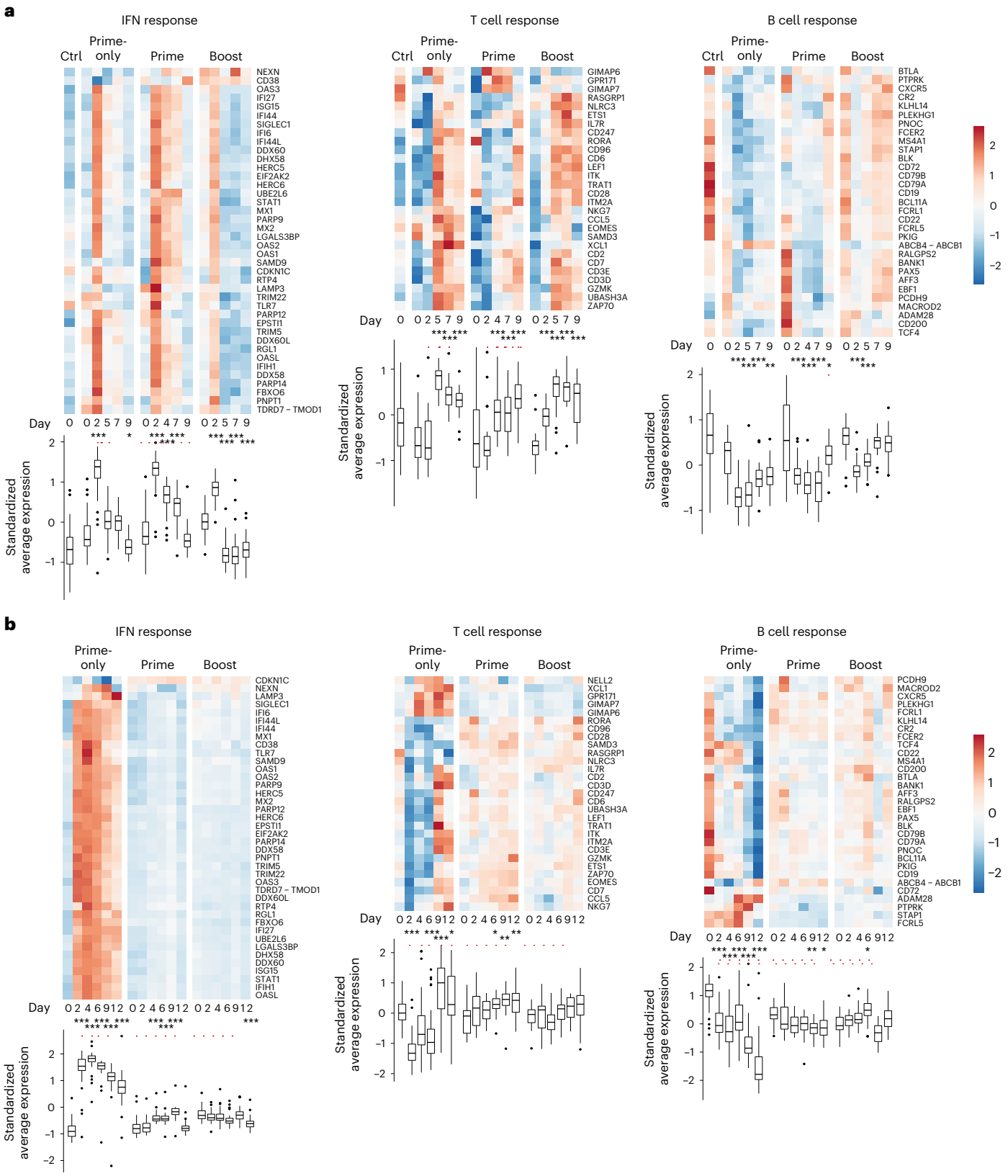

Fig. 6 | Immune responses after immunization and challenge. a, Transcriptomic analysis of PBMCs after immunization. The heatmaps represent gene expression in each pathway. The colour of the heatmaps represents the mean of scaled normalized counts for each group and time point (vaccinated: $n = 4$; controls: $n = 3$). The box plots show the average of the normalized gene expression of the pathway (central line, median; hinges, first and third quartiles; whiskers, largest or smallest value no further than 1.5 times the interquartile range from the hinge). Outliers are plotted individually. Significant differences were tested using a one-way mixed ANOVA on centred and scaled expressions of the gene set, averaged by condition. The Tukey's multiple comparison test was used to compare the time points to J0 in each group. The asterisks indicate the difference from day 0 (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$); exact P values are provided (Supplementary Table 3). **b**, Transcriptomic analysis of PBMCs after challenge. Data were analysed and represented as in **a** ($n = 3$).

was performed under anaesthesia (Zoletil 100, 0.1 ml kg^{-1}). Each day, the state of the animals was evaluated and a clinical score was calculated based on behaviour, body temperature, dehydration, weight loss, clinical signs and reactivity. A clinical score ≥15 or weight loss >20% were defined as an end point in the protocol and the animal was euthanized. The end of the protocol was planned for days 28 and/or 29. Euthanasia was performed under anaesthesia with a 5 ml intracardiac injection of pentobarbital or pentobarbital sodium and phenytoin sodium, samples then taken as for previous sampling and organs collected.

Animals were implanted with intraperitoneal body temperature recording systems (EMKA Technologies) before the beginning of the experiments. 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 (three controls, three prime-only and one prime-boost). Moreover, the new implantation resulted in local inflammation that was still present for some animals at the day of challenge.

The second experiment was conducted in the same laboratories using similar protocols and procedures. The body temperature was efficiently recorded throughout the procedure using intraperitoneal loggers (Star-Oddi). Twelve female CMs were used. They were almost 3 years of age and weighed 2.5–3.4 kg. Six received the vehicle and six were vaccinated with $MOPEVAC_{NEW}$ (2.10^6 FFU, i.e. 4.10^5 FFU of each valence). The immunization was performed on days 0 and 56. The animals were transferred to the BSL4 laboratory on day 89. After a period of acclimation of 10 d, they were challenged with an expected dose of 3,000 FFU. We titrated the virus dilution used for challenge and it was 4,500 FFU for MACV 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.

Ethical statements

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 APAFIS#18970-2019020616112503 v8 (2019/07/23) and by the ethical committee CELYNE for the challenge procedure and registered with the number 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.

Cell lines

Vero E6 cells and 293T cells from ATCC were used in this study (CRL-1586 and CRL-3216, respectively).

Viruses

The MOPEVAC platform consists of a MOPV (AN21366 strain; GenBank accession nos. JN561684 and JN561685) that carries the GPC of the virus of interest in place of its own GPC and is mutated in the nucleoprotein gene to abolish the exonuclease function[25]. The resulting attenuated virus was produced in Vero E6 cells cultivated in DMEM and 2% FCS. $MOPEVAC_{MACV}$ was then concentrated by centrifugation in filter tubes with a 1,000 kDa cut-off. A vehicle solution was prepared with uninfected Vero E6 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 (Carvallo strain; GenBank accession no. AY619643), GTOV (INH95551 strain; GenBank accession no. AY129247), CHAV (810419 strain; GenBank accession no. NC_010562), SABV (SPH114202 strain; GenBank accession no. NC_006317) and JUNV (P2045 strain; GenBank accession no. DQ854733). It was produced under the same conditions, except for the concentration method. The cell supernatant was precipitated using a polyethylene glycol (PEG) solution (Abcam). After overnight incubation at 4 °C with gentle agitation, it was centrifuged for 3 h at 4,696 *g* and the pellet was resuspended in DMEM and 2% FCS.

For the stability experiments, Vero E6 cells were used as described above. The stability was tested until passage 10, starting from passage 2. After each passage, viral RNAs collected in supernatants at day 4 were quantified by quantitative PCR with reverse transcription (RT–qPCR). Vero E6 cells were then infected with the supernatant using ten copies of genome per cell. The viruses collected in supernatants at passages 2, 5 and 10 were sequenced on a MiniSeq platform (Illumina) and analysed using the public platform Galaxy[48]. Briefly, RNA was extracted from 1 ml of supernatant with the QIAamp Viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions. The RNAs were rigorously treated with TURBO DNase (Thermo Fisher Scientific) and concentrated by ethanol precipitation. Then, cytoplasmic and mitochondrial ribosomal RNAs were removed using the NEBNext ribosomal RNA depletion kit v2 (human/mouse/rat). The libraries were prepared using the NEBNext ultra II RNA library prep for Illumina with 6 min of RNA fragmentation and 16 cycles of amplification. Finally, the quality and concentration of libraries were determined by using the High Sensitivity D5000 Screentape assay on a TapeStation (Agilent Technologies). Sequencing was performed using an Illumina MiniSeq platform with 150-base paired ends and single indexing for each library. The loading concentration on the flow cell for the sequencing was 1.45 pM from a pool of normalized concentration of 18 libraries. For data analysis, reads were trimmed according to the quality score (99%) and length (reads below 80 base pairs were removed) and Illumina adaptor were deleted using Trimmomatic v.0.38. Trimmed FASTQ files were then mapped onto the genome of rescued viruses using bowtie2 v.2.4.5 and PCR duplicates were removed using MarkDuplicates v2.18.2.3. Finally, consensus sequences were called by using iVar consensus and variants were checked on the Integrative Genomics Viewer.

MACV, Caravallo strain, GTOV, INH95551 strain, and JUNV, P2045 strain, were produced in Vero E6 cells in DMEM and 2% FCS. The clarified cell supernatants were diluted in PBS for inoculation of the animals with the virus. The same viruses were used for further experiments on biological samples from the experiments.

GPC expression by MOPEVAC viruses

Vero E6 cells were infected at a multiplicity of infection (MOI) of 0.001 and cellular RNAs were extracted at day 0 and day 3 post-infection. RNAs coding for GPC were quantified by RT–qPCR using EurobioGreen One-Step Lo-Rox kit (Eurobio). The primers were designed to match all NWAs (forwards: GCC TGG WGG TTA TTG TYT; reversed: CTC ARC ATG TCA CAG AAY TC). GAPDH expression was measured using a reverse transcription step with oligo dT primers (Superscript III and Oligo dT from Thermo Fisher Scientific) followed by an amplification step with TaqMan Gene Expression Master Mix (Applied Biosystems) and a CM probe/primer mix (Applied Biosystems). The expression of GPC was normalized using GAPDH messenger RNA expression and the ratio expression at day 3 on expression at day 0 calculated to measure the increase of GPC RNA expression after infection.

Western blot for GP2 detection

$MOPEVAC_{CHA,\ GTO,\ JUN,\ MAC,\ SAB}$ were ultracentrifuged for 1 h 30 min at 450,000 *g*. The pellet was lysed in Laemmli buffer. The amount of lysis solution corresponding to 10^5 FFU was separated on SDS-polyacrylamide gel electrophoresis gel 4–12% and transferred on a nitrocellulose membrane. GP2 proteins were detected by staining with the anti-GP2 Ab (KL-AV-2A1 (ref. [49])) diluted 1:1,000 and anti-mouse horseradish peroxidase (HRP) diluted 1:20,000 (Jackson ImmunoResearch). Revelation of staining was performed using Super Signal WestDura Extended Duration substrate (Thermo Fisher Scientific).

Enzyme-linked immunosorbent assay

Virus-specific IgG detection was performed on plasma samples. To produce antigens, Vero E6 cells were infected with WT viruses. The supernatants were collected at day 4 and diluted with 25% of PEG solution. After an overnight incubation at 4 °C, the media was centrifuged and the pellet resuspended in buffer containing 1% Triton X-100 (Sigma-Aldrich). The solution was sonicated and frozen. Antigen-negative supernatants were made with the same protocol using uninfected cells. These antigens were coated diluted 1:500 or 1:1,000 in PBS on polysorp 96-microwell plates. After an overnight incubation at 4 °C, wells were blocked for 1 h with PBS and 2.5% bovine serum albumin (BSA). Plasma samples, diluted from 1:250 to 1:16,000 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 before a final incubation with anti-monkey HRP 1:5,000 (Sigma-Aldrich). Attachment of the conjugated Ab was revealed using TMB and the reaction stopped with orthophosphoric acid. Between each step, the plates were washed three times with PBS and 0.5% Tween-20. Optical density was finally measured and the value obtained from antigen-negative was subtracted from the value of antigen-positive measurements. A sample with a resulting value ≥0.1 was defined as positive. Ab titres corresponded to the last dilution that was still positive. Statistical differences between conditions were calculated as indicated in the figure legends using the SigmaPlot v.14.5 software (Systat Software).

IgG detection on 293T cells

293T cells were transfected in 12-well plates with phCMV plasmids coding for the *GPC* gene or the empty vector using Lipofectamine 2000 (Invitrogen). After 2 d of incubation, transfected cells were collected and divided into 96-well plates. Cells were incubated with LIVE/DEAD fixable viability dye (Thermo Fisher Scientific) and plasma samples diluted 1:20 in PBS, 2.5% FCS and 2 mM EDTA for 30 min in ice. After two washes in the same buffer, secondary Ab anti-monkey IgG FITC (Southern Biotech) was added to the cells for 30 min at +4 °C. Two final washes were performed before fixation with paraformaldehyde 2% and analysis by flow cytometry (Fortessa 4L; BD). The percentage of cells with bound anti-GPC Abs was determined on live cells (Kaluza software v.2.1 for flow cytometry analysis).

Quantitative RNA analysis

RNA was prepared from liquid samples using the QIamp Viral RNA Mini Kit (QIAGEN) or from cells or tissues using the RNeasy Mini Kit (QIAGEN). 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 detected four copies of viral RNA per microliter. We performed a sensitivity test using a different matrix and obtained a limit of quantification of 6 FFU ml^{-1} in plasma and oral/nasal swabs for GTOV and 25 FFU ml^{-1} in plasma and 625 FFU ml^{-1} in swabs for MACV.

Virus titration

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

Samples were serially diluted in DMEM and 2% FCS, added to Vero E6 cells and the plates incubated for 1 h at 37 °C. Medium supplemented with carboxymethylcellulose was added and the plates were incubated for one week. Cells were then fixed for 20 min with 4% formaldehyde. To determine the number of FFUs, plates were permeabilized for 5 min with 0.5 % Triton X-100, stained for 1 h with anti-virus Ab and for an additional hour with HRP-conjugated secondary Ab. The reaction was finally revealed using NBT/BCIP (Thermo Fisher Scientific). FFUs were counted. To determine the number of plaque-forming units, cells were coloured with crystal violet solution (Sigma-Aldrich) diluted 50% in PBS for 10 min, washed with water and the plaques counted.

MACV and JUNV were revealed using anti-Z MACV and MOPEVAC was stained using anti-Z MOPV. These Abs were produced in rabbits (Agrobio). For the SABV virus, we used an anti-monkey MACV obtained from the United States Army Medical Research Institute of Infectious Diseases. The secondary Abs were all coupled with HRP (Sigma-Aldrich). We did not obtain reactive Abs for GTOV but the virus was lytic; we used crystal violet to reveal plaques. The threshold of detection was 17 FFU ml^{-1} for liquid samples and 0.5 FFU mg^{-1} for organs.

Seroneutralization

Seroneutralization experiments were conducted using WT 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 and 5% CO_2) the plasma and virus mixture was added to cells. The infection was allowed to proceed for 1 h and medium supplemented with carboxymethylcellulose was added. Cells were incubated for one week before immunostaining of infected cells or crystal violet colouration (see Virus titration). The neutralizing titre was the last dilution that allowed more than a 50% reduction in the number of viral plaques relative to the control condition.

Haematological and biochemical analyses

Haematological parameters were analysed 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).

Intracellular cytokine staining in PBMCs

Fresh whole blood or PBMCs were stimulated with overlapping peptides of the nucleoprotein and GPC protein and stained for flow cytometry analysis, as described previously[26]. SEA was used as a positive control. The gating strategy is presented in Extended Data Fig. 8.

Transcriptomic analyses

Total RNA from PBMCs was extracted using the RNeasy Mini Kit with an on-column DNase step. RNA samples were then quantified using the QuantifluorRNA system (Promega Corporation) and qualified using a standard sensitivity kit on an Advanced Analytical Fragment Analyzer. The External RNA Controls Consortium RNA Spike-in Mix 1 (Thermo Fisher Scientific) was added to all samples to limit sample variability in multiple batches and mRNA was poly(A)-captured using NEXTflex poly(A) beads (PerkinElmer). The libraries were prepared using the NEXTflex Rapid Directional RNA-seq Kit (PerkinElmer) and quantified and qualified using a Quantus Quantification Kit (Promega Corporation) and a fragment analyzer. Sequencing was performed on a NextSeq 500 Flow Cell High OutputSR75 instrument (Illumina) with six samples per flow cell.

Bioinformatics analysis was performed using the RNA-seq pipeline from Sequana[50]. Reads were cleaned of adaptor sequences and low-quality sequences using cutadapt v.1.11 (ref. [51]). Only sequences of at least 25 nucleotides in length were considered for further analysis. STAR v.2.5.0a[52], with default parameters, was used for alignment against the reference genome (*M. fascicularis* 5 from ENSEMBL v.95). Reads were assigned to genes using featureCounts v.1.4.6-p3 (ref. [53]) from the Subreads package v.2.0.1 (parameters: -t gene -g ID -O -s 2). Data from these transcriptomic analyses are available on *Zenodo*[54].

Statistical analyses were performed to identify genes for which the expression profiles were significantly different between each pair of biological conditions. Therefore, statistical tests were performed between each time point compared to their respective baseline

(day 0) within each group. For each dataset (post-vaccination and post-challenge), genes exhibiting expression lower than one count per million in at least 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 v.1.24.0 (ref. [55]). The model was adjusted for the effect of vaccination status, time point and animal identifier. Gene set enrichment analysis was performed for both datasets to identify gene sets and pathways enriched in the various biological conditions using a one-sided Fisher exact test. The overall gene set over- or underexpression was tested with a one-way mixed analysis of variance (ANOVA) on centred and scaled expressions of the gene set, averaged by condition. The fixed part of the model was adjusted on the groups and time points and the random part was adjusted on the gene identifiers. The Tukey's multiple comparisons test was used to compare the time points to J0 in each group.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The datasets from the RNA sequencing generated during the current study are not publicly available due to current ongoing analyses but are available from the corresponding author upon reasonable request. S. Baize is the corresponding author for any request or correspondence (sylvain.baize@pasteur.fr). Data are available in public open access repositories. For the transcriptomic analyses, they are available on *Zenodo* (https://zenodo.org/record/7229439).

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Author contributions

S.R. managed and performed the experiments, analysed the results and wrote the publication. X.C. performed the reverse genetics experiments to rescue the vaccine candidates. X.C., C.P., V.B.-C., A.J., M. Mateo, C.G., J.H. and S. Baize performed the experiments on samples during the animal experiments. L.F. and P.-H.M. were in charge of the animal experiments in the BSL2 facility. C.P., V.B.-C. and L.A. were responsible for the neutralization assays. A.J. performed the ELISA experiments and M. Mateo realized the viral titrations in organs. E.P. and N.P. computed all transcriptomic data and performed the related analyses. A.V., S. Barron, A.D., O.L., O.J. and M. Moroso managed the animals in the BSL4 facility. M. Dirheimer was the referent veterinarian of this study. M. Daniau and C.L.-L. performed the sequencing for the transcriptomic analyses. H.R. and C.C. managed the BSL4 team. S. Baize supervised the entire project.

Competing interests

The authors declare no competing interests. The MOPEVAC vaccine platform described in this study is protected by US patent 62/245,631; the authors listed as co-inventors are S.R., S. Baize, X.C., M. Mateo and A.J.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41564-022-01281-y.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41564-022-01281-y.

Correspondence and requests for materials should be addressed to Sylvain Baize.

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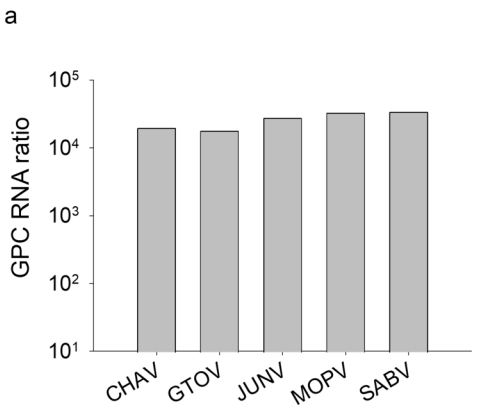

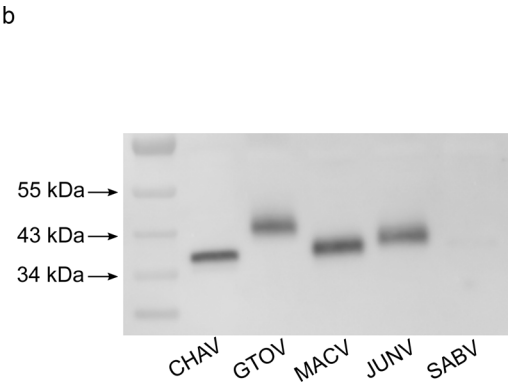

Extended Data Fig. 1 | GPs expression by MOPEVAC viruses. a. VeroE6 cells were infected at a moi of 0.001 and cellular RNAs were extracted at day 0 and day 3 post-infection. The ratio of GPC expression of day 3 relative to day 0 was calculated and represented for each MOPEVAC virus. **b**. Expression 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.

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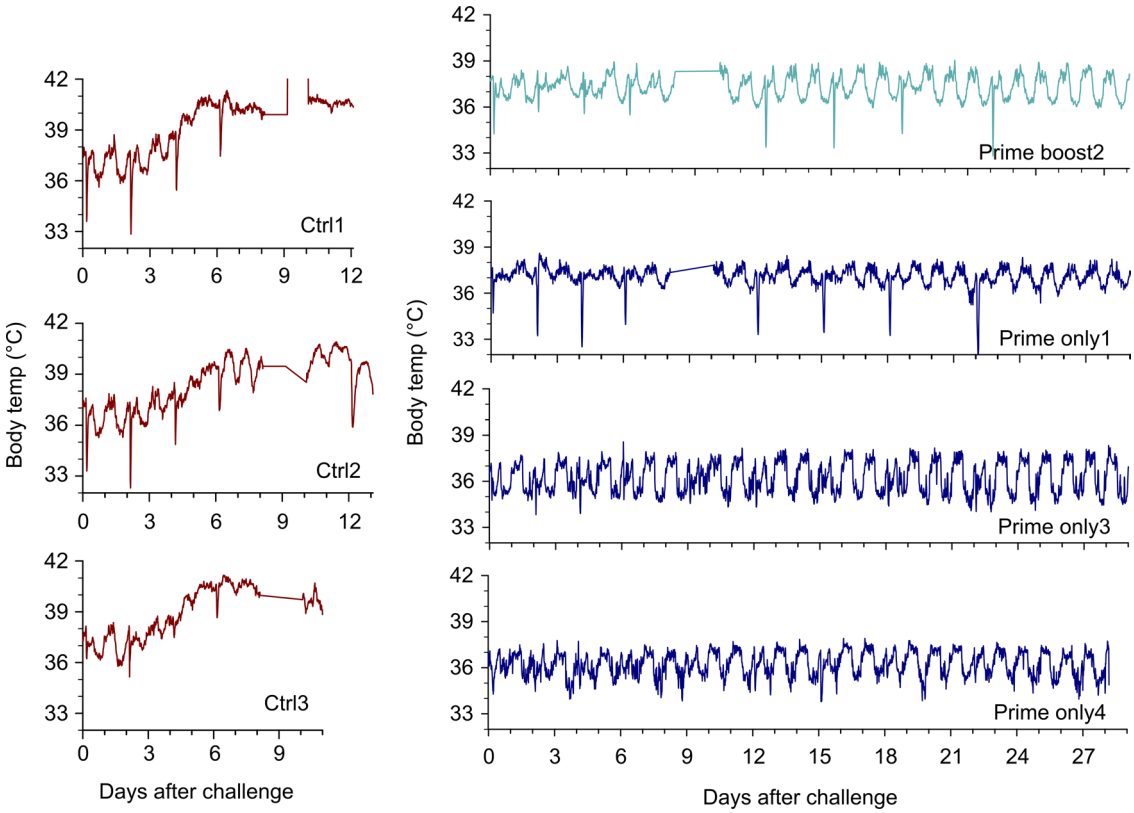

Extended Data Fig. 2 | Real-time recording of body temperature after challenge. Recording systems were implanted in the CMs to evaluate the body temperature throughout the protocol. A number were defective. We thus obtained data for seven CMs: the three controls, three prime only vaccinated animals, and one prime boost. The recording was stopped unintentionally for a small period for five animals, this is clearly visible in the graphs.

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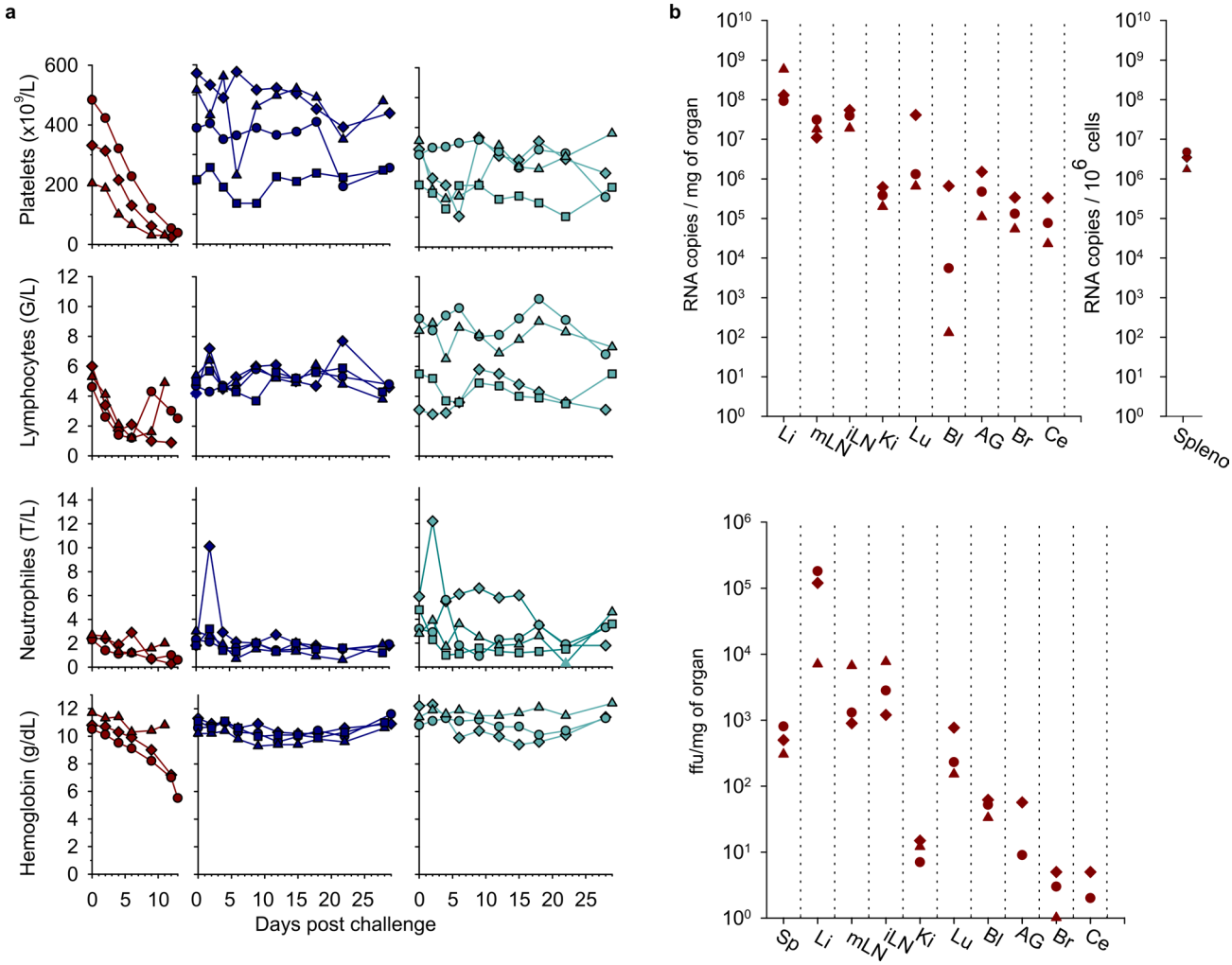

Extended Data Fig. 3 | Hematological parameters and viral loads in the organs at the day of necropsy. a. Cell counts and hemoglobin concentrations in whole blood were measured at each sampling. **b.** Viral RNA was quantified by RT-qPCR from crushed organs or cells. RT-qPCR-positive samples were evaluated 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.

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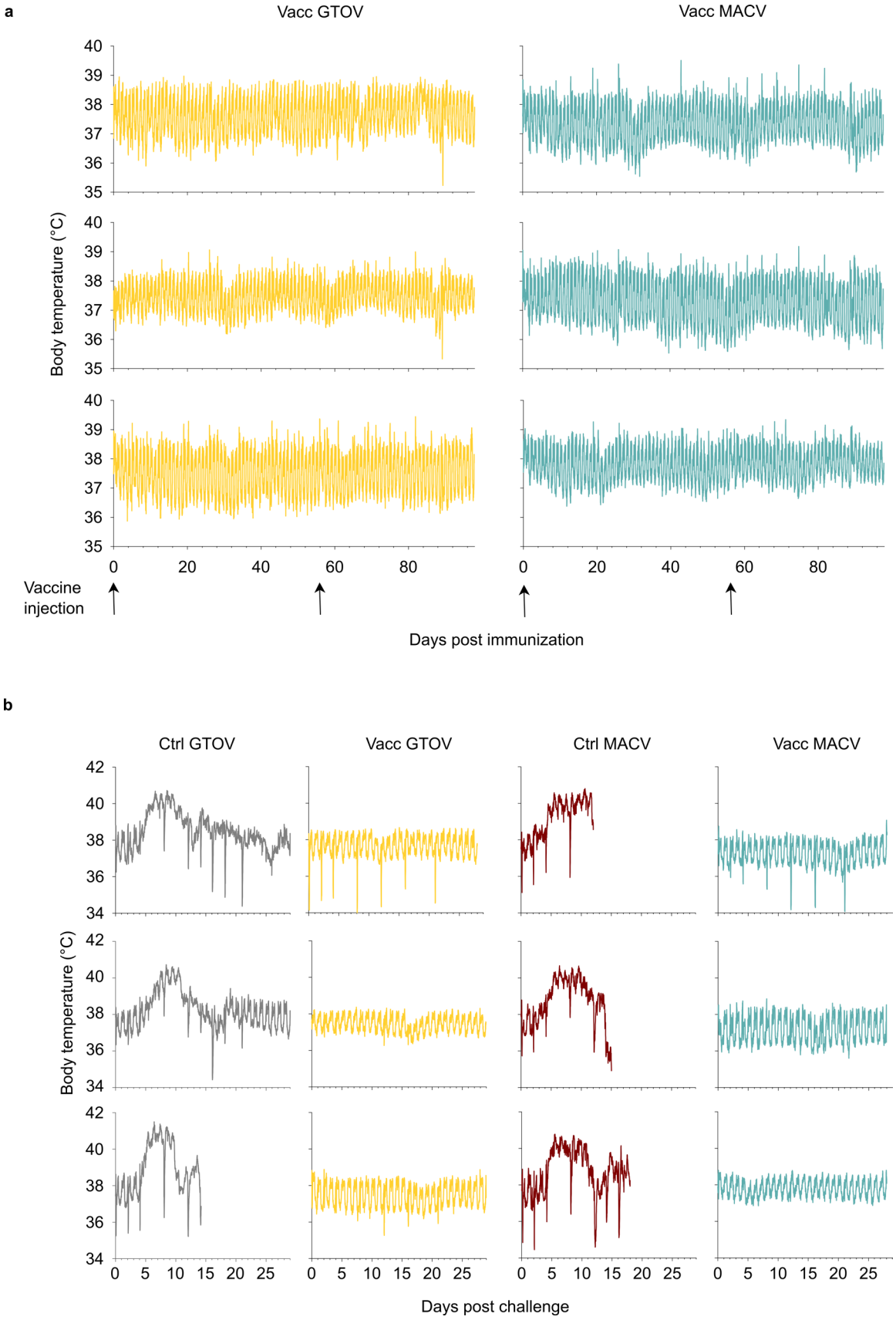

Extended Data Fig. 4 | Body temperature before and after challenge in the MOPEVAC$_{NEW}$ experiment. Intraperitoneal implants recorded the body temperature throughout the experiment at 15-min intervals. **a**. Post immunization period in vaccinated CMs. All received the same vaccine, but the color indicates the virus used for the challenge. **b**. Body temperature of vaccinated and control animals after challenge.

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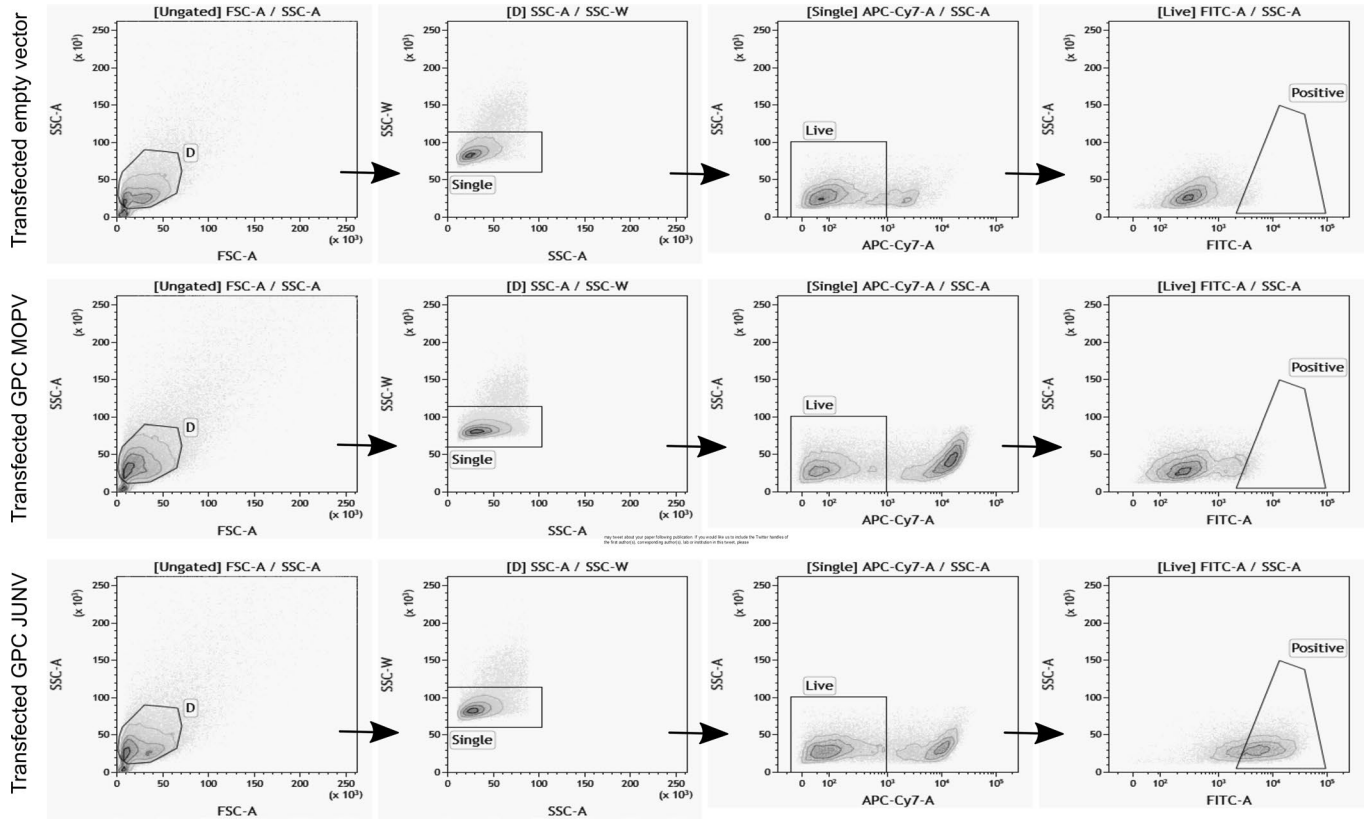

Extended Data Fig. 5 | Gating strategy for determination of IgG fixation on GPs. The gates used to quantify the cells expressing or not GPs that fixed IgG from plasma are presented. FSC / SSC was used to gate cells, then singlets were determined using SSC / SSC-W and live cells were gated: Live Dead 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.

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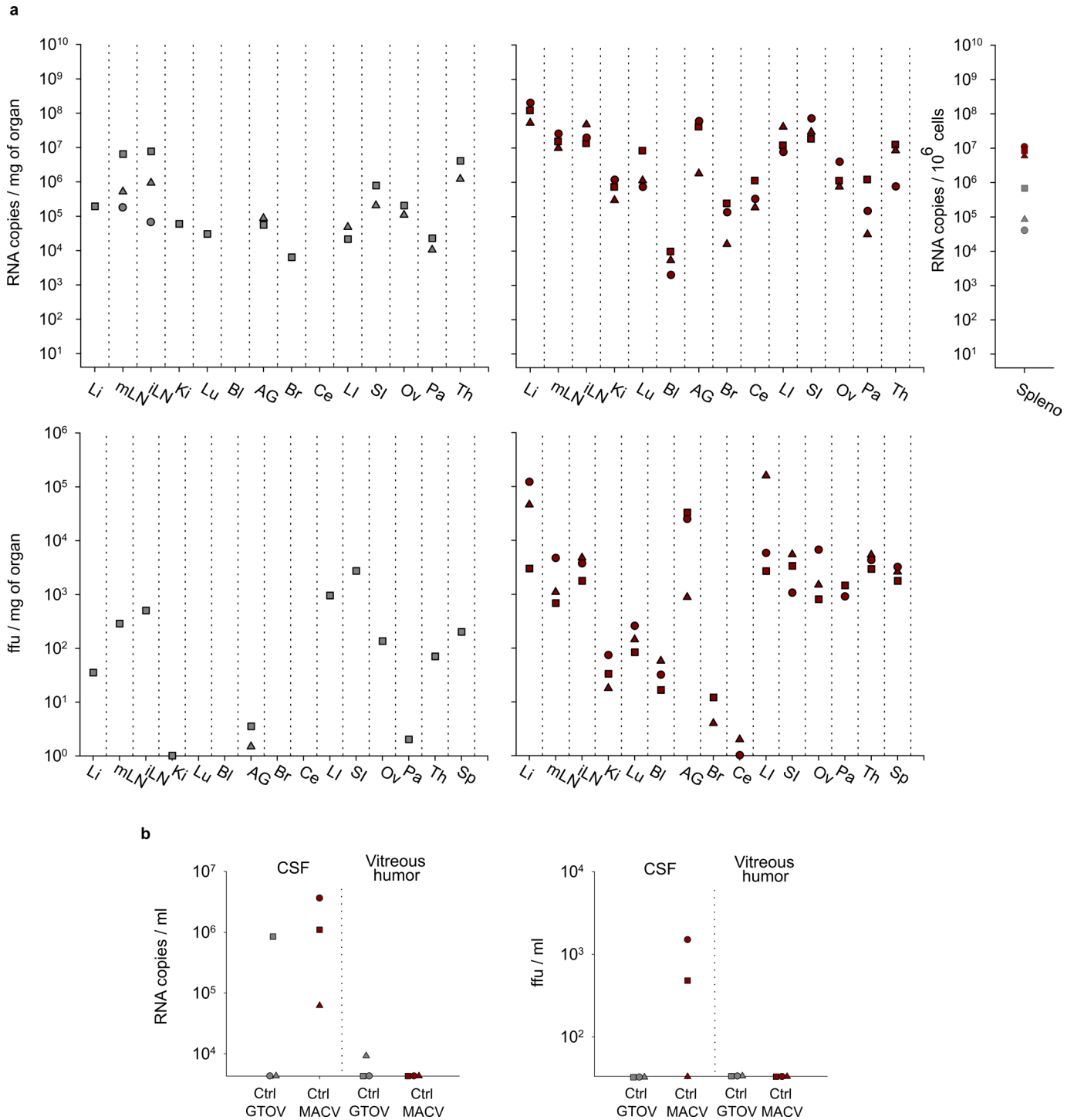

Extended Data Fig. 6 | Viral loads in organs and immune-preserved compartments. a. Viral RNA was quantified by RT-qPCR from crushed organs or cells. RT-qPCR-positive samples were evaluated for infectious virus titers. Grey: GTOV-infected controls. Red: MACV-infected controls. **b**. Viral RNA was quantified 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.

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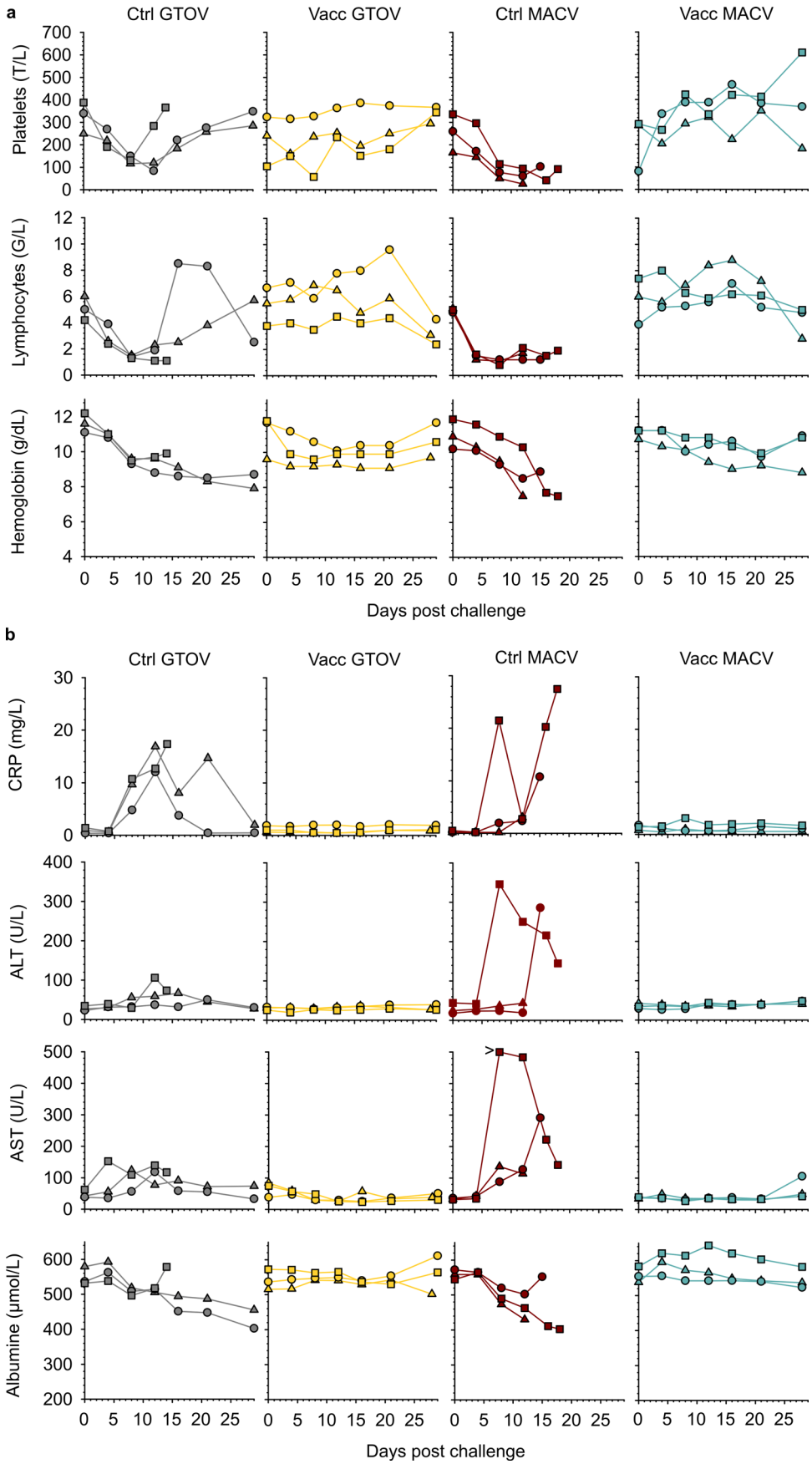

Extended Data Fig. 7 | Hematological and biochemical parameters after challenge in the MOPEVAC$_{NEW}$ experiment. a. Cell counts and hemoglobin concentrations were measured at each sampling after challenge. **b.** Biochemical parameters were assayed in plasma at each sampling. C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and plasmatic albumin levels are presented.

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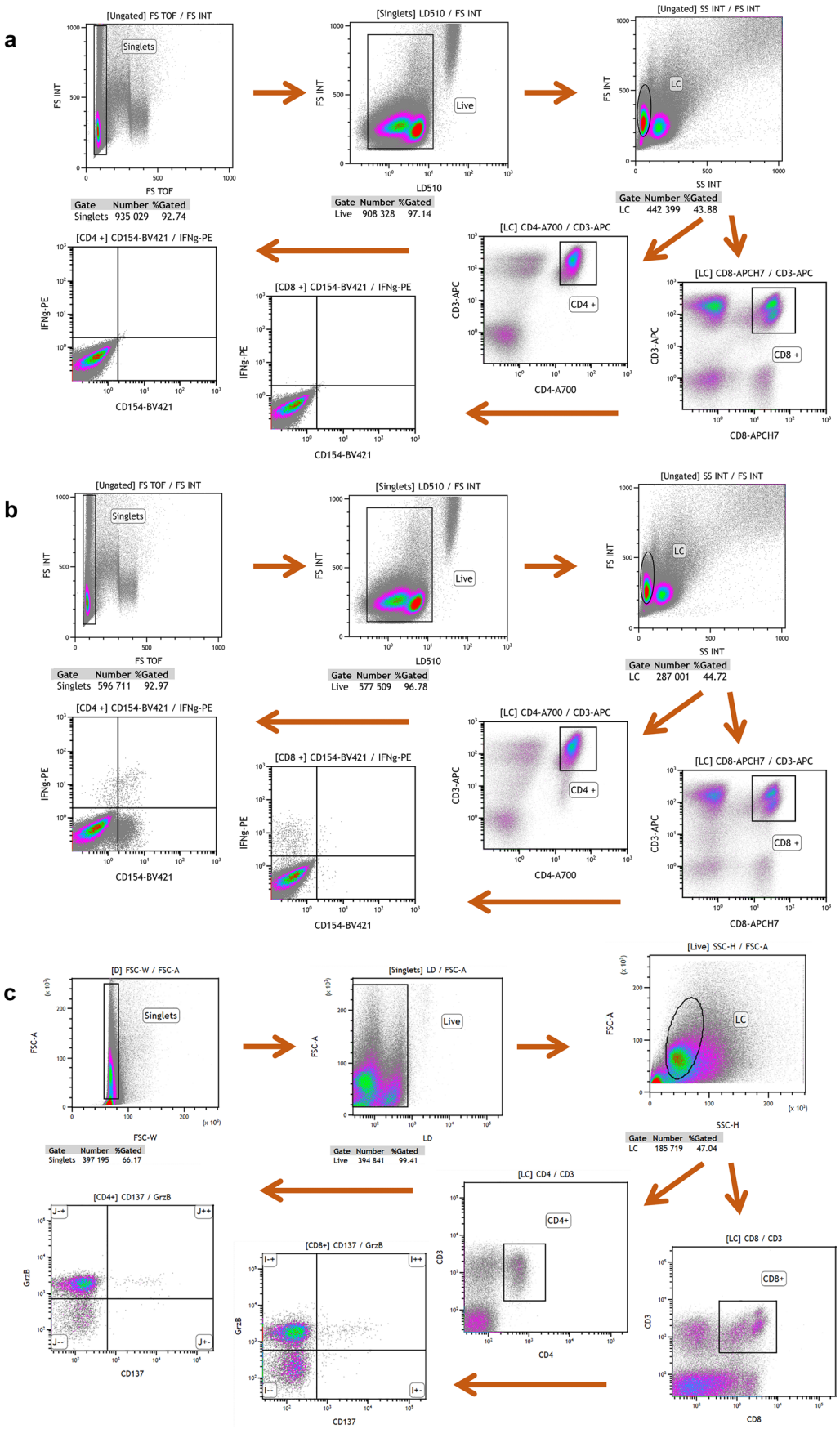

Extended Data Fig. 8 | See next page for caption.

https://doi.org/10.1038/s41564-022-01281-y

Extended Data Fig. 8 | Gating strategy for flow cytometry analysis. The gates used to quantify IFNγ-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 IFNγ-producing and CD154-expressing CD4+ and CD8+ T cells is determined using a quadrant in the IFNγ/CD154 dotplot. **c**. A similar strategy was applied for CD137 and GrzB detection.

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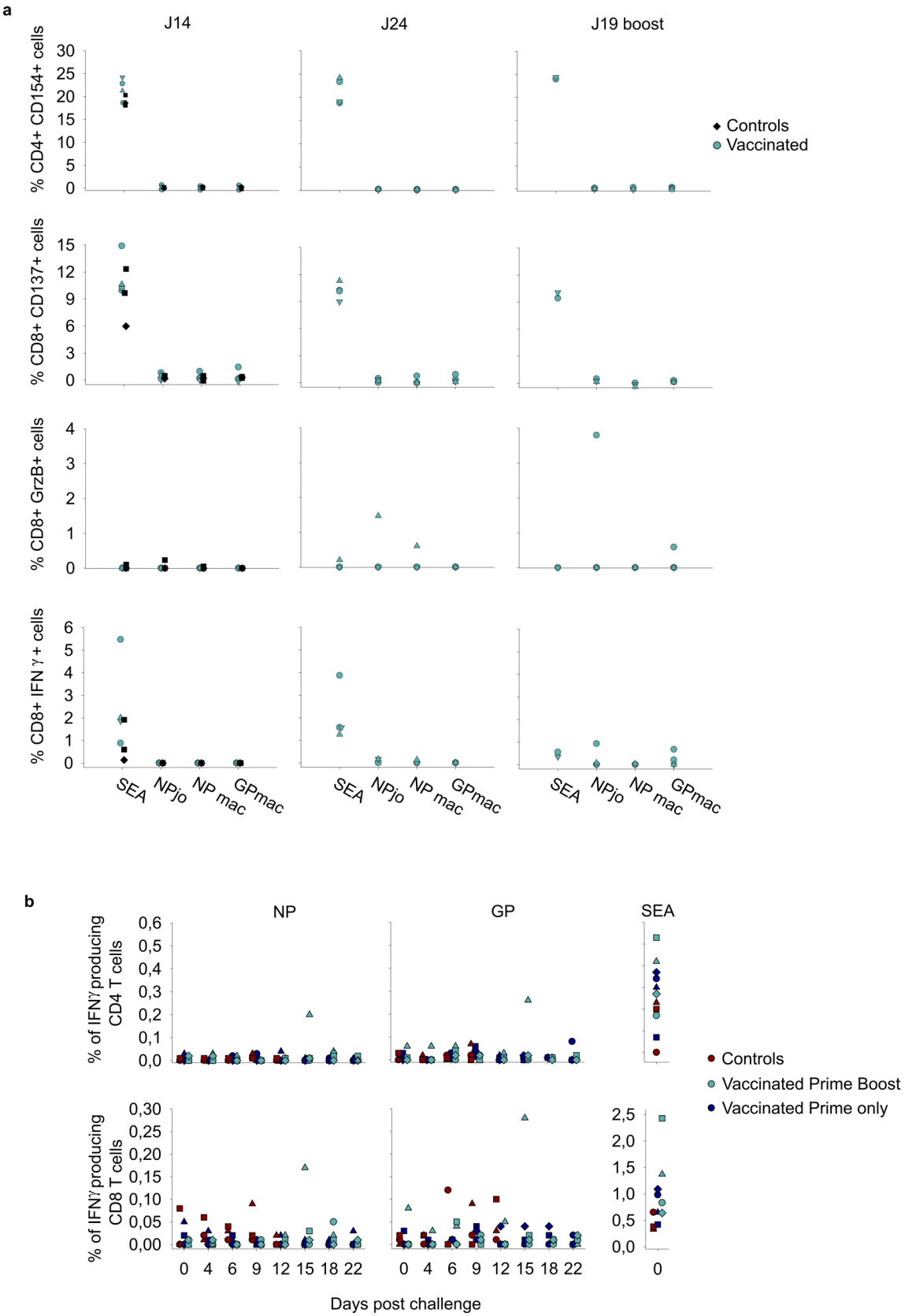

Extended Data Fig. 9 | Activation of T cells in response to peptide stimulation. **a**. PBMCs sampled at days 14 and 24 post-prime and day 19 post-boost were stimulated with overlapping peptides covering MACV NP and GP and LASV strain Josiah NP. SEA was used as a positive control. After an overnight incubation, the cells were stained with conjugated antibodies and analyzed by flow cytometry for the expression of CD154, CD137, GrzB and IFNγ. 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.

nature portfolio

Corresponding author(s): Dr. Sylvain BAIZE

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Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	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). RT-qPCR were were performed with a LightCycler480 (Roche). Sequencing of RNA from PBMCs was performed on a NextSeq 500 Flow Cell High OutputSR75 instrument (Illumina). Sequencing of RNA for virus stability experiments was performed on a MiniSeq (Illumina). Flow cytometry data were collected using a LSR 4L (BD Biosciences) with Diva software V9.0.1 after immunization and a Gallios cyometer (Beckman Coulter) after challenge.
Data analysis	Graphs and statistical analyses were performed using SigmaPlot 14.5 software (Systat) except for transcriptomic data that were analyzed and represented using R software, version 4.0.4. RT-qPCR were analyzed with the LC480 software, 1.5.0 SP4 version (Roche). Flow cytometry analyzes were performed using Kaluza Analysis 2.1 software (Beckman Coulter). Sequences for virus stability were analyzed using the Galaxy platform (https://usegalaxy.org), tool Trimmomatic V 0.38.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Sequences of viruses used in this study are disponible in Genebank: MOPV JN561684 and JN561685. For GPC sequences, the Genebank accession numbers are MACV AY619643, GTOV AY129247, CHAV NC_010562, SABV NC_006317 and JUNV DQ854733. Data from RNA seq and used for transcriptomic analyses are disponible on Zenodo DOI: 10.5281/zenodo.7229438 .

Field-specific reporting

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☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was adapted to be at least n=3 and to respect the maximum of twelve animals for an experiment in the BSL4 facility. With the aim to respect the 3R rule in animal studies (Reduce, Refine, Replace) we stated that a minimum of n=3 should be sufficient to provide confident results on vaccine efficacy in comparison with control animals non-vaccinated and infected with a virus that induce accute haemorragic fever.
Data exclusions	One animal from each group of immunized-NHP have been removed from transcriptomic analyzes after challenge because of local inflammation at the site of implantation of loggers recording body temperature (see methods). Nevertheless, the comparisons were performed with n=3 for each group, allowing statistically relevant analyses.
Replication	All attempts of replication of the data are presented in the study and we never obtained results that would not fit the results presented here.
Randomization	Animals were allocated to their groups because of their social interactions with the others and there was no significant differences in their age or weight.
Blinding	Blinding was not possible in these experiments because of biosafety constraints in the BSL4 animal facilities. Indeed, as the animals were accomodated by 3 in the same cage, it was not possible to mix animals receiving different treatments to avoid cross-contamination.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Anti-monkey-HRP antibody ref SAB3700766 (Sigma-Aldrich) was used in ELISA assays, diluted 1/5,000. Anti-rabbit and anti-monkey alkaline phosphatase, ref A3687 and A1929 respectively (Sigma-Aldrich) were used as secondary antibodies for titration of viruses, diluted 1/2.000. Polyclonal anti-Z MACV and anti-Z MOPEVAC were made to order (Agrobio) and used 1/1,000, anti-MACV from monkey was obtained from the USAMRIID and used 1/50. Cytometry analyzes were performed using anti-CD3 clone SP34-2 ref 557597, anti-CD4 clone L200 ref 560836, anti-CD8 clone SK1 ref 560179, anti-IFNg clone B27 ref 559327, anti-GrzB PE clone GB11

ref 561142 (BD Biosciences); and anti-CD154 clone 5C8 ref 130-113-609 and anti-CD137 clone 4B4-1 ref 130-119-886 (Miltenyi Biotec). Anti-monkey IgG ref 4700-02 (Southern biotech) was used 1/20 to detect the fixation of IgG from the plasma samples by flow cytometry. Detection of GP2 proteins by western blot was performed using KL454 AV-2A1 antibody diluted 1/500 (a kind gift of F. Krammer, department of Microbiology, Icahn School of Medicine at Mount Sinai, New York) and anti-mouse HRP diluted 1/20,000 (Jackson Immunoresearch).

Validation

Primary antibodies to MACV Z protein and anti-MACV from the USAMRIID were previously tested for their ability to detect the viruses of interest and in a titration experiment of known virus stock to determine the optimal dilution for detection. Anti-Z for MOPEVAC was previously described in Carnec et al, 2018. Flow cytometry antibodies were described in Mateo et al 2019 and Mateo et al 2021. KL454 AV-2A1 was described in Amanat et al 2018. Anti-monkey IgG HRP was tested in house to verify the reactivity with cynomolgus antibodies and evaluate the best dilution. Anti-rabbit and anti-monkey alkaline phosphatase antibodies are in house validated for titration experiments. Anti-mouse HRP is provided by the manufacturer for western blot applications and validated in house to provide efficient signal. Anti-monkey IgG was provided as suitable for flow cytometry by the manufacturer and all controls in the experiment confirmed this.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	VeroE6 cells ref CRL-1586 and 293T ref CRL-3216 were obtained from ATCC.
Authentication	As provided by ATCC, no further authentification was performed
Mycoplasma contamination	VeroE6 cells were used to produce viral stocks that were tested negative for mycoplasma (Mycoalert, Lonza).
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	In the first experiment, 11 male cynomolgus monkeys (Macaca fascicularis) were included. They were 2.5 years old and weighed 2.3 to 3.9 kg. In the second experiment, twelve female cynomolgus monkeys were used. They were almost three years of age and weighed 2.5 to 3.4 kg. For both experiments, there was no significant difference in age and weight parameters between groups.
Wild animals	The study does not involve wild animals.
Field-collected samples	The study does not involve samples collected from the field.
Ethics oversight	The protocols for both experiments were approved by the “Comité Régional d'Ethique en Matière d'Expérimentation Animale de Strasbourg” for the immunization period and by the ethical committee “CELYNE” (Lyon, France) for the challenge procedure. Registration numbers : APAFIS#18970-2019020616112503 v8, APAFIS#18397_2019011010351235_v4, APAFIS#28798_2020122311384240_v2

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

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Methodology

Sample preparation	MACV-specific T cells were analyzed from 200 ul of fresh whole blood or from isolated PBMC. PBS-EDTA (2 mM final concentration) supplemented with Live-Dead reagent (Molecular Probes) was added to samples before staining for CD3, CD4, and CD8 (BD Biosciences). Red blood cells were then lysed using PharmLyse (BD Biosciences). Cells were fixed and permeabilized using the FoxP3 staining buffer set (Miltenyi) before intracellular staining with antibody to IFNg, CD154, CD137 and/or GrzB (BD Biosciences or Miltenyi).
Instrument	LSR-4L (BD Biosciences) and Gallios cyometer (Beckman Coulter)
Software	Diva was used to collect data from LSR4 instrument and Kaluza for Gallios instrument.
Cell population abundance	For Fig3 cell cytometry experiment, 293T were transfected to induce GPC expression. This step increases the mortality rate,

Cell population abundance	so Live Dead staining was used to analyze only live cells (~50%). For extended data fig 9, when whole blood was used we considered that all cells were alive and when frozen PBMCs were used with used LIve Dead to analyze only live cells (between 75-100% viability depending on samples).
Gating strategy	Singlets were gate using FSC int/FSC tof, then dead cells were excluded with Live/Dead staining. Lymphocytes were gated using FSC/SSC parameters, CD3+ cells were gated to identify CD4+ and CD8+ T cells. These populations were then analyzed for IFNg, CD154, CD137 and GrzB staining.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.