Non-neutralizing antibodies elicited by recombinant Lassa–Rabies vaccine are critical for protection against Lassa fever

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Lassa fever (LF), caused by Lassa virus (LASV), is a viral hemorrhagic fever for which no approved vaccine or potent antiviral treatment is available. LF is a WHO priority disease and, together with rabies, a major health burden in West Africa. Here we present the development and characterization of an inactivated recombinant LASV and rabies vaccine candidate (LASSARAB) that expresses a codon-optimized LASV glycoprotein (coGPC) and is adjuvanted by a TLR-4 agonist (GLA-SE). LASSARAB elicits lasting humoral response against LASV and RABV in both mouse and guinea pig models, and it protects both guinea pigs and mice against LF. We also demonstrate a previously unexplored role for non-neutralizing LASV GPC-specific antibodies as a major mechanism of protection by LASSARAB against LF through antibody-dependent cellular functions. Overall, these findings demonstrate an effective inactivated LF vaccine and elucidate a novel humoral correlate of protection for LF.
Lassa fever (LF) is a viral hemorrhagic fever (VHF) whose etiologic agent is Lassa virus (LASV), a bio-safety level 4 (BSL-4) pathogen. Similar to other VHFs caused by other viruses, such as Ebola virus (EBOV) and Marburg virus (MARV), LF can be highly fatal and no vaccine is currently available. The need to develop vaccines against emerging viral pathogens became starkly apparent during the 2014–2016 West Africa Ebola epidemic1–4. Indeed, reaffirming the urgency and importance of preventive measures, an unprecedented major LF surge, with 25.4% high case fatality rate, is currently unfolding in Nigeria6. Unlike most other BSL-4 agents which cause temporally and geographically confined epidemics, LF is believed to be widespread throughout most of West Africa, with an estimated 100,000–300,000 humans infected annually6,7. As many as 80% of LF exposures are mildly symptomatic and thus go unreported6, however, the case fatality rate of LF has been reported to reach as high as 50%8. Such discrepancy can be dependent on both the contributing strain and the population afflicted (e.g., pregnant women are especially susceptible)9,10. Even among survivors, LF can cause severe neurosensory sequela; it is a leading cause of neurotrophism and improves its safety profile35. A codon-optimized LASV-GPC was cloned into BNSP333 using two unique restriction sites (BsiWI and NheI) that flank a RABV transcription start/stop signal between the RABV N and P genes, and it was designated as LASSARAB (Fig. 1). Utilizing LASRARB, we also constructed LASSARAB-ΔG by deleting the RABV G. For a control vector, we constructed a recombinant RABV-expressing LASV GPC, named LASSARAB, expresses a codon-optimized version of LASV GPC (coGPC) in addition to RABV G. LASSARAB elicits lasting humoral response against LASV and RABV in both mouse and guinea pig models, and it protects both against LF. In developing LASSARAB, we also sought to uncover its mechanism of protection, which our results suggest is dependent on a previously uncharacterized antibody-mediated protection of LASV through effector cell functions of GPC-targeted non-neutralizing antibodies (Non-NAbs).

**Results**

**Generation of rhabdoviral-based vectors expressing LASV GPC.** To generate a recombinant RABV-expressing LASV GPC, we used the previously described vector BNSP33333. BNSP333 is a modified RABV vaccine strain (SAD B19) with an arginine-to-glutamate change at position 333 of RABV G that further reduces neurotropism and improves its safety profile35. A codon-optimized LASV-GPC was cloned into BNSP333 using two unique restriction sites (BsiWI and NheI) that flank a RABV transcription start/stop signal between the RABV N and P genes, and it was designated as LASSARAB (Fig. 1). Utilizing LASSARAB, we also constructed LASSARAB-ΔG by deleting the RABV G. For a control vector, we constructed a recombinant vesicular stomatitis virus (VSV) expressing the same GPC as the RABV vector (rVSV-GPC); similar to LASSARAB-ΔG, it lacks its native glycoprotein (G). In several prior NHP studies, similar rVSV-GPC vectors have been used as live-attenuated (replication-competent) vaccine candidates for LASV with promising results15,16. As an additional control, we used BNSP333-expressing Ebola GP (FILOBR1), a vaccine extensively characterized by our group36–38.

**GPC is transported to the cell surface and incorporated into virions.** Successful utilization of LASSARAB and LASSARAB-ΔG as vaccines depends on LASV GPC expression at the cell surface membrane. VERO cells were infected at a multiplicity of infection (MOI) of 0.1 or 1, and cell surface expression of LASV GPC and RABV G was evaluated by immunofluorescence and flow cytometry at 48 h post-infection (Figs 2a, b). Immunostaining with antibodies directed against either LASV GPC or RABV G detected both LASV GPC and RABV G cells on the cellular surface of VERO cells infected with LASSARAB (Figs 2a, b panel LASSARAB). In cells infected with FILOBR1, only RABV G was detected on the cell surface as expected (Figs 2a, b, panel FILOBR1) whereas for the LASSARAB-ΔG and rVSV-GPC-infected cells, LASV GPC but not RABV G was detected on the cell surface (Fig. 2b panel LASSARAB-ΔG/rVSV-GPC).

Besides direct viral neutralization, antibodies can also lead to effector cell activation and clearance of the viral antigen-expressing cells through antibody-dependent cellular cytotoxicity (ADCC) or phagocytosis (ADCP)29. Through this mechanism, antibodies bound to antigen interact with Fc-y-receptor-bearing immune effector cells, such as macrophages or NK cells, through Fc region cross-linking29 that triggers clearance of the antigen-expressing cell. As such, ADCC/ADCP are among several mechanisms that bridge the adaptive and innate immune responses. ADCC/ADCP has been shown to be highly relevant for protecting against and clearing several different viruses, including HIV, influenza virus, and EBOV30–34. However, the role of ADCC, ADCP, and other antibody-mediated effector functions in LASV infection and disease outcome has not been investigated.

Here we report the use of a rabies virus (RABV)-based vaccine vector as an inactivated dual vaccine for LASV and RABV. This vaccine, named LASSARAB, expresses a codon-optimized version of LASV GPC (coGPC) in addition to RABV G. LASSARAB elicits lasting humoral response against LASV and RABV in both mouse and guinea pig models, and it protects both against LF. In developing LASSARAB, we also sought to uncover its mechanism of protection, which our results suggest is dependent on a previously uncharacterized antibody-mediated protection of LASV through effector cell functions of GPC-targeted non-neutralizing antibodies (Non-NAbs).

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To analyze whether LASV GPC affects RABV growth kinetics, we performed a multi-step growth curve analysis of LASSARAB, LASSARAB-ΔG, and FILORAB1 (Fig. 2c). LASSARAB and FILORAB1 grew similarly and reached titers of 10^8 after 72 h. LASSARAB-ΔG grew to a higher titer than the RABV G-containing construct LASSARAB, indicating that LASV GPC is being functionally expressed. The higher titer achieved by LASSARAB-ΔG might be explained by its shorter genome or its expression of two glycoproteins, or both.

LASSARAB's potential as an inactivated vaccine depends on LASV GPC incorporation in LASSARAB-inactivated virions. As such, sucrose-purified virions from infected VERO cells were analyzed by SDS-PAGE gel, western blotting, and ELISA (Figs 2d, e, and Supplementary Fig. 1). SDS-PAGE protein stain of purified FILORAB1 (control) and LASSARAB virions showed protein migration in the expected size for the RABV G-containing construct LASSARAB, indicating that LASV GPC is being functionally expressed. The higher titer achieved by LASSARAB-ΔG might be explained by its shorter genome or its expression of two glycoproteins, or both.

LASVARAB does not induce LASSV-specific GPC IgGs. We first evaluated immunization with replication competent vaccines. All live-attenuated (replication-competent) RABV-based vaccines will be referred from now on with an rc- suffix (e.g., rc-LASSARAB). rVSV-GPC is always used as replication competent vaccine. C57BL/6 mice were intramuscularly immunized on day 0 with 10^6 pfu rc-LASSARAB, rc-LASSARAB-ΔG, rc-FILORAB1, or 10^6 pfu of rVSV-GPC. Humoral immune responses were analyzed by a newly developed LASV GPC-specific ELISA bi-weekly until day 42 post-immunization (Supplementary Fig. 1 and 2). By day 14, both rc-FILORAB1- and rc-LASSARAB-immunized mice had high titers of RABV-G-specific total IgG, and by day 28, maximum titers were achieved and were maintained until day 42, as seen previously (Supplementary Fig. 2)\(^{36}\), rc-LASSARAB-ΔG and rVSV-GPC immunized mice did not seroconvert to RABV-G. In contrast, LASV GPC-specific titers were detected in pathogenic after IN exposure, was used as a positive control, while FILORAB1 and PBS were used as negative controls because previous studies had demonstrated that they are not virulent\(^{43,44}\). On day 8, RABV-infected animals started to exhibit clinical signs of rabies, particularly weight loss. (Fig. 3a, RABV group). Mice inoculated with LASSARAB or FILORAB1 showed no clinical signs of disease. For the LASSARAB-ΔG IN inoculated group, one mouse died at day 14 without displaying previous clinical signs or weight loss (Fig. 3a, LASSARAB-ΔG group). Two animals inoculated with rVSV-GPC displayed signs of neurological deficits (Fig. 3a, rVSV-GPC group, m2/4/5); two succumbed and one survived, indicating pathogenicity after IN inoculation of this vaccine. None of the animals inoculated through the IP route displayed clinical signs of disease.

We further characterized the safety profile of the infectious LASSARAB vaccine by intracranial inoculation (IC) in both adult BALB/c and adult severe combined immunodeficiency (SCID) mice (3b). Increased pathogenicity was not observed following infections with LASSARAB compared with BNP333 in either Balb/C or SCID mice (Fig. 3b). Finally, to confirm absent or decreased pathogenicity in a more sensitive model\(^{44}\), Swiss Webster suckling mice were IC-exposed with LASSARAB or BNP333 (Fig. 3c). Independent of the virus dose used, LASSARAB or BNP333 suckling mice started to succumb to the infection by day 7.

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**Fig. 2** Evaluation of LASSARAB and LASSARAB-ΔG vectors in cell culture and inactivated virion characterization. 

- **a** LASSARAB, FILORAB1 and uninfected VERO cells were probed for LASV-GPC and RABV-G expression with 37.7H anti-LASV human mAb and 1C5 anti-RABV G mouse mAb and analyzed by flow cytometry 48 h post infection. 
- **b** VERO cells were infected at a MOI of 0.1 with 4 viruses: FILORAB1, LASSARAB, LASSARAB-ΔG, and rVSV-coGPC. 
- **c** LASSARAB infected cells, yellow is observed as the superimposition of LASV GPC surface expression with RABV G. The bar indicates 12 μm. 
- **d** VERO CCL-81 cells were infected with a MOI of 0.01 and media supernatant was collected at 0, 24, 48, 72, and 96 h. Virus titers were measured through foci-forming assay (in Y-axis) and plotted through time (X-axis). 
- **e** LASSARAB and FILORAB1 virions were concentrated through TFF and sucrose purified through ultra-centrifugation. Pellets were resuspended in PBS, BPL inactivated at 1:2000 for 24 h, and 2 μg of each was loaded in a denaturing 10% SDS-PAGE gel. 

The Suppl. Info. includes western blots for LASV GPC incorporation in LASSARAB particles. Uncropped versions are available in supplementary figures.
rc-LASSARAB-ΔG immunized mice only, and only at low titers on days 28 and 42 (Supplementary Fig. 2). rVSV-GPC had a significant LASV GPC-specific immune response (Fig. 4c, purple line).

Inactivated-LASSARAB virions induce humoral response in mice. We also explored the humoral immunogenicity of inactivated LASSARAB virions. Inactivated LASSARAB or FILORAB1 virions will simply be referred as LASSARAB or FILORAB1. We
intramuscularly administered 10 μg of β-propiolactone (BPL)-inactivated LASSARAB or FILORAB1 particles to C57BL/6 mice following the standard three-inoculation RAVB vaccine schedule (Fig. 4a). Both vaccines were further tested in two different formulations: either in PBS only (LASSARAB/FILORAB1 groups), or adjuvanted with TLR4 receptor agonist (Glucopyranosyl Lipid A) in a stable emulsion (LASSARAB+/GLA-SE group)45. GLA-SE is a clinical-trial stage adjuvant that has been shown to enhance the breadth and quality of humoral immune responses for FILORAB1 and influenza virus37,38,46. Blood was collected and the humoral immune response was analyzed periodically until day 42 (Fig. 4 and Supplementary Fig. 2). Analysis of total IgG against LAVS GPC by ELISA indicated seroconversion at day 14 by both LASSARAB and LASSARAB+/GLA-SE groups; by day 28 both achieved statistical significance in comparison to control groups (Fig. 4b). Since endpoint titers of both inactivated LASSARAB and LASSARAB+/GLA-SE had achieved appreciable total IgG responses against LAVS GPC, we examined the quality of this humoral response by IgG2c and IgG1 subtype-specific LAVS GPC ELISA. IgG1/IgG2c ratios lower than 1.0 indicated an increasing Th1-bias response, which is desirable for antiviral responses. LASSARAB+/GLA-SE not only achieved a significantly higher IgG2c response than LASSARAB, but also achieved consistently lower and uniform IgG1/IgG2c ratios (F-test, p < 0.01), thus decreasing the variability of the immune response between mice (Figs 4d, e).

LASSARAB does not induce neutralizing antibodies. The development of NAbS was investigated for LASSARAB using a pseudotyped VSV in vitro assay. This assay utilizes a single round ΔG-rVSV pseudovirus (ppVSV) which expresses both NanoLuc and eGFP as reporter genes38,47. When ppVSV pseudotyped with RABV G was used, the sera of either replication-competent or inactivated LASSARAB achieved high NAbS against RABV G (> 10,000) compared to negative controls (Fig. 5, RABV). Since RABV G NAbS are a correlate of protection against RABV, these results indicated that LASSARAB is a suitable vaccine against RABV. Protection by RABV NAbS was further confirmed by using the WHO standard (Figs 5a, c) in which values > 0.5 IU/ml are considered protective against RABV; every group achieved 1U/ml values much higher than 0.5 IU/ml, indicating that the addition of LAVS GPC in the RABV backbone did not compromise its ability to generate RABV NAbS. Conversely, when ppVSV was pseudotyped with LAVS GPC, we were not able to detect GPC-specific NAbS both in the presence or absence of complement (Supplementary Fig. 3), whereas the control human mAbs (12.1F, 25.10C and 37.7H) exhibited neutralizing activity at similar concentrations as described26, indicating that our assay was functional (Figs. 5a, b).

LASSARAB+/GLA-SE is efficacious in guinea pigs. We evaluated LASSARAB vaccine efficacy using outbred Hartley guinea pigs and the guinea pig-adapted LASV. Six groups of ten Hartley guinea pigs were used (Fig. 6a): three groups were immunized with inactivated LASSARAB/GLA-SE particles once (1), twice (2), or three times (3); two groups were immunized with replication-competent LASSARAB (rc-LASSARAB) or rVSV-GPC, and one group received RabAvert. All groups were challenged 58 days after the primary immunization with 10^5 pfu of the guinea pig-adapted LASV Josiah strain. The animals were monitored for viremia and clinical signs were recorded daily up to day 47 post-challenge (Figs 6b, c). Significant protection was observed for animals immunized three times with LASV GPC ELISA. IgG1/IgG2c ratios lower than 0.18). These data indicated that either NAbS play a minor role in survival or, in the case of the succumbed animals, develop too late in the infection to play a significant role.

LASSARAB induced non-neutralizing antibodies stimulate ADCC. Once we found that a high LAVS GPC-specific IgG titer with low or no NAbS correlated with protection in the LASSARAB+/GLA-SE group, we determined whether non-NAb can mediate protection through cell-mediated mechanisms, such as ADCC or ADCP. For this purpose, we used sera from mice immunized twice (on day 0 and day 28) with LASSARAB+/GLA-SE (LASSARAB sera) or FILORAB1+/GLA-SE (control)
First, we analyzed NK cell-mediated ADCC activity using an in vitro assay modified from a previously described rapid and fluorometric antibody-dependent cellular cytotoxicity (RFADCC) assay. Briefly, we developed a stable 3T3 cell line expressing LASV GPC (3T3-LASV) and used it as target cells, and purified murine C57BL/6 NK cells as effectors, as described in Methods and Supplementary Fig. 3. 3T3-LASV cells were incubated either with LASARAB sera or control, and different ratios of effector cells to target cells (E:T) were used (Fig. 7a and Supplementary Fig. 3). In the presence of LASARAB sera, murine NK cells mediated significantly more killing (p < 0.01) at any E:T compared to controls (Fig. 7a). This effect was reduced to background levels when another 3T3-based cell line expressing an irrelevant viral glycoprotein (3T3-MARV) was used as a target cell (Supplementary Fig. 3).

To determine which antibody isotype is important for ADCC-mediated killing of 3T3-LASV, we isolated IgG from the sera and conducted the assay with 40 µg/ml of either purified IgG or IgG-depleted sera (Fig. 7b and Supplementary Fig. 3). Again, killing of 3T3-LASV was significantly higher in the presence of LASV-specific purified IgG than in the control; in contrast, target cell cytotoxicity was reduced to background levels when IgG-depleted sera were used. Together these findings indicate that ADCC is mediated by the LASV GPC-specific IgG.

Macrophages mediate ADCC after immunization with LASARAB. To examine whether other antibody-dependent cell-mediated mechanisms are involved in the clearance of LASV, we modified our ADCC assay to test if macrophages are involved in ADCC. As seen for the NK cells, peritoneal C57BL/6 macrophages (IC-21) induced 3T3-LASV cell killing compared to control sera when incubated with LASARAB sera (Fig. 7d and Supplementary Fig. 3). Moreover, we observed that peritoneal BALB/c macrophages (J774A.1) internalized 3T3-LASV cells in the presence of LASARAB sera, likely through ADCP (Figs 7c, e). Target cell internalization was confirmed to be dependent upon Fcy-R activation as macrophages incubated with anti-FcyRII mAb (but not anti-FcyRI or anti-Fcy-RIV) abolished 3T3-LASV internalization to background levels (Fig. 7c).
standard starting dilution). IU/ml from test sera is plotted.

Primary immunization, mice were exposed IP with 10^4 pfu of (controls) in a total of four groups (Fig. 8a). On day 42 post-

interferon-α/β were made more susceptible to rVSV-GPC by blocking the was used elsewhere for other VHF viruses51 because it should have a similar tropism to LASV, and such approach has

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murine model utilizing rVSV-GPC (Supplementary Fig. 4), since previous results suggest. To that end, we developed a surrogate LASV murine model utilizing rVSV-GPC (Supplementary Fig. 4), since LASV is a BSL-4 agent with no established LASV murine model. Because rVSV-GPC expresses LASV GPC as its sole glycoprotein, it should have a similar tropism to LASV, and such approach has been a strategy used elsewhere for other VHF viruses51-53. Mice were made more susceptible to rVSV-GPC by blocking the interferon-a/β receptor (IFNAR) with anti-IFNAR mAb followed by an IP exposure of rVSV-GPC 24 h later54.

BALB/c (WT) and BALB/c Fcγ−/− mice were immunized twice with either LASSARAB+GLA-SE or FILORAB1+GLA-SE (controls) in a total of four groups (Fig. 8a). On day 42 post-

primary immunization, mice were exposed IP with 10^4 pfu of rVSV-GPC and clinical signs and weight were monitored (Fig. 8b and Supplementary Fig. 4). WT LASSARAB immunized mice mostly resisted infection, with 8/10 mice having only transient weight loss (Fig. 8b and Supplementary Fig. 4, continuous orange line). Meanwhile, all (10/10) of the Fcγ−/− LASSARAB mice quickly lost weight and succumbed to infection by day 5, with some showing signs of hemorrhage (Fig. 8b and Supplementary Fig. 4, dashed orange lines) indicating that Fcγ is essential to control viral infection in LASSARAB immunize mice. In FILORAB1 immunized mice (control), both WT and Fcγ−/− groups had a similar outcome, with 2/5 mice of each group surviving infection until study endpoint (Fig. 8b and Supplementary Fig. 4, gray lines), demonstrating that both WT and Fcγ−/− are equally susceptible to surrogate LASV exposure.

Upon pre-exposure analysis of GPC-specific IgG titers, both WT and Fcγ−/− mice immunized with LASSARAB had significantly higher titers in comparison with FILORAB1 control mice (Fig. 8c and Supplementary Fig. 4c), but no LASV NABs were detected in neutralization assays (Supplementary Fig. 4b). In post-exposure analysis of LASV NABs, surviving LASSARAB immunized mice developed little to no neutralizing antibody (Fig. 8d, orange symbols), while one WT FILORAB1 vaccinated mouse developed modest levels of LASV NABs (Fig. 8d). Overall this data shows that previous LASSARAB immunization is heavily dependent on non-NAB effector function activity in vivo for protection against LASV.

Discussion

The WHO R&D Blueprint for Action to Prevent Epidemics55 defines LF as a priority agent for vaccine development.

Fig. 5 Virus neutralization antibody titers. Day 42 sera from immunized mice was incubated with pseudotyped rVSV-ΔG-NL-GFP. a rVSV-ΔG-NL-GFP was pseudotyped with either RABV-G, LASV-GPC, or EBOV-GP to assay for RABV-G, LASV-GPC, or EBOV-GP NAB titers, respectively. 12.1F, 37.7H, and 25.10c are LASV-GPC neutralizing antibodies used as a positive control for LASV-GPC neutralization26. Y-axis is a represents 50% of inhibitory serum dilution (IC50) titers obtained based on the antibody dilution that has 50% infection percentage of infected cells curves obtained in a. All groups achieved high neutralizing titers against RABV-G except for the groups immunized with virus lacking RABV-G: rc-LASSARAB-RAB-

pseudotyped viruses with 12.1F, 37.7H, and 25.10c had an average IC50 of 1546 ng/ml, 375 ng/ml, and 69 ng/ml, respectively.

Fig. 4 dashed orange lines) indicating that Fcγ is essential to control viral infection in LASSARAB immunize mice. In FILORAB1 immunized mice (control), both WT and Fcγ−/− groups had a similar outcome, with 2/5 mice of each group surviving infection until study endpoint (Fig. 8b and Supplementary Fig. 4, gray lines), demonstrating that both WT and Fcγ−/− are equally susceptible to surrogate LASV exposure.

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Accordingly, preferred vaccine requirements include: (1) a highly favorable risk-benefit profile suitable for all age groups, (2) practicality for non-emergency/preventive scenarios, (3) at least 90% efficacy in preventing disease, (4) high thermostability, and (5) the possibility of co-administration with other vaccines. LASSARAB appears to be the first inactivated LF vaccine to fulfill most of these requirements as demonstrated in our study and based on previous work done with the same platform for other VHF vaccines. Another advantage to LASSARAB, as an inactivated LF vaccine, is that it could potentially be used in pregnant women and immunosuppressed patients, both of which are major risk groups for LF. In addition to LF, LASSARAB also confers...
Fig. 6 LASV challenge of outbred Hartley guinea pigs immunized with several vaccine candidates and control. a Guinea pigs were immunized with either two replication-competent vaccine strains: rSVS-GPC (positive control for survival) and LASSARAB replication-competent at 10^9 f.u. by intraperitoneal injection (IP); or inactivated LASSARAB+GLA-SE with different immunization schedules: day −58 (LASSARAB+GLA-SE (1)), day −58, day −51 (LASSARAB+GLA-SE (2)) and day −58, day −51, and day −30 (LASSARAB+GLA-SE (3)). RabAvert was used as mock immunization (negative control). b Survival curves post IP exposure with 10^4 pfu guinea pig-adapted LASV Josiah strain. Statistical significance is compared against RabAvert group using log-rank (Mantel–Cox) test. c Heat plot representing the clinical score information. X-axis represents days’ post-challenge and Y-axis represents the individual animal number. d Terminal viremia was plotted using LASV RNA copies/ml in Y-axis. Statistical significance was calculated using Kruskal–Wallis one-way ANOVA (not significant). e LASV neutralizing antibody titers are reported as the IC50 (half maximal inhibitory concentration) of serum dilution. The human mAbs 25.10C, 12.1F, and 37.7H25,26 were used as positive LASV neutralization controls. f Pre-challenge titers of LASV GPC specific IgG were performed on sera collected on day −15 prior to challenge by ELISA with LASV GPC antigen and the EC50 (50% effective concentration) of serum dilution was plotted in the Y-axis. Statistical significance (compared to the RabAvert group) was calculated by using one-way ANOVA (post-hoc test Tukey Honest Significant Difference Test). g Post-challenge titers of LASV GPC specific IgG was performed on sera collected on terminal bleeding of both succumbed animals and survivors (day 50 post challenge) and the EC50 of serum dilution is plotted on the Y-axis. Statistical significance reported between survivors and succumbed in e, g was determined by using two-way ANOVA. All error bars represented are the standard error mean (SEM) of 10 animals per group (in triplicates). ****P < 0.0001; ***P < 0.001; **P < 0.01; *P < 0.05)

protection to rabies (Fig. 5b), which is a major health burden in Africa.56

Most LASV vaccine studies have characterized the role of humoral response against LASV as either a secondary mechanism of protection or even detrimental to survival.11 Such correlations were drawn based on results measuring antibody responses against LASV nucleoprotein (NP) or nonspecific LASV antigens.11,16,57 Although NP is highly immunogenic, it is neither expressed on the surface of cells nor virions. As such, antibodies directed against LASV NP should only have diagnostic value. Meanwhile, GPC has been shown to be the most effective LASV immunogen but, to our knowledge, no attempts were made to correlate GPC-specific humoral response with LASV protection.16,58–60 Thus, as part of LASSARAB characterization, we were compelled to develop a GPC-specific antigen that is expressed in its native conformation (Supplementary Fig. 1). Throughout the development of LASSARAB, we observed that replication-competent LASSARAB and replication-competent LASSARABAG were poor inducers of GPC-specific antibodies, despite being able to induce RABV protective response (Figs. 4c, 5, and Supplementary Fig. 2). In contrast, when inactivated LASSARAB immunizations were combined with a late boost (day 28 post-prime), high levels of LASV GPC-specific antibodies were induced at later time points, especially when administered with a TLR-4 agonist (GLA-SE). This contrast might be attributed to the fact that LASV GPC is a poor immunogen25,61 and, as such, induction of antibodies against GPC might be dependent on replication-competent vectors that achieve high or persistent viral loads post immunization. Given that inactivated LASSARAB incorporates LASV GPC, it can safely be administered in higher dosages in a prime/boost regimen and, as such, more antigen might be available to prime follicular B helper T cells and B cell response. The high effectiveness of a TLR-4 agonist in inducing higher levels of anti-LASV GPC antibodies with higher quality (IgG2c bias) further corroborates recent findings by Galan-Navarro et al.64 indicating that inactivated LASV vaccines might benefit of TLR-4 agonists. Nonetheless, no NAb against LASV pseudotypes were detected in either replication competent or inactivated approaches (Figs 4, 5 and Supplementary Fig. 3 and 4).

Because it has been the case with vaccines for some other viruses,44,62,63 it might be expected that an effective LF vaccine protects through NAb. Sommerstein et al. have elegantly demonstrated that LASV exposure or immunization in mice does not induce LASV NAb due to the LASV GPC’s glycan shield.25,65 Additionally, as recently shown by the important works of Robinson JE et al. and Hastie et al., most potent LASV NAb (such as 37.7H) require very specific quaternary epitopes bridging LASV GP1 and GP2, making it challenging to elicit through immunization. Interestingly, these NAb, instead of blocking GPC receptor binding, achieve neutralization by stabilizing LASV GPC in its pre-fusion conformation.25,26,42 The lack of NAb induced with the several vaccine candidates, either replication competent or inactivated, in our study (Fig. 5a) and in previous published vaccine candidates, further corroborates this expectation.11,23 Even after LASV exposure, only a small fraction of human and animal survivors produce NAb, findings that our study further confirmed (Figs 6, 8)11,26,28. Additionally, we showed that guinea pigs that succumbed to disease also had NAb, suggesting either that NAb by themselves play a minor role in protection or that they develop too late during infection to impact outcome. Studies by Mire et al. have recently shown that some LASV NAb can mediate protection in NHPs and guinea pigs when administrated prophylactically25,26. Although providing evidence that GPC specific mAbs can mediate protection against Lassa Fever, the role of antibody-dependent effector cellular functions was not evaluated and GPC-specific non-NAb were not used. Furthermore, LASV neutralizing potency in vitro did not necessarily correlate with protection.27 Together with the findings in our study (Fig. 6), this raises the question whether GPC-specific non-NAb play a role in protection through other mechanisms, such as ADCC, since guinea pig survival post-LASV exposure was correlated with high levels of GPC-specific non-NAb independent of NAb titer.

In several other viruses (e.g., Influenza, LCMV), antibody Fc-FcyR interactions leading to ADCC and ADCP are important for protection, playing a critical role both in viral clearance and in preventing chronic infection regardless of neutralizing ability.29,30,32,34,64 Through our in vitro studies, we showed that sera from LASSARAB-immunized mice with high GPC-specific antibodies (Supplementary Fig. 4) did not neutralize LASV but elicited significant ADCC and ADCP of 3T3 cells expressing LASV GPC (Figs 5, 7). Interestingly, the Fcy-RIV blockade did not reduce ADCP activity by macrophages (Fig. 7e), despite having a high affinity for IgG2 subclass-dependent ADCP. This suggests that GPC-specific IgG1 might be mediating ADCP,65 nevertheless, in contrast with IgG2 subclass, GPC-specific IgG1 titers were almost non-existent in the purified IgG used (S3c).

To corroborate the relevance of Fcy-R effector functions in LASSARAB-induced protection in vivo, we used an Fcy-KO mouse model challenged with surrogate LASV exposure (Fig. 8)50. This approach permitted us to dissect the role that LASSARAB induced non-NAb play in protection against surrogate LASV exposure in the context of a similar immunogenic response. Despite similar levels of antibody titers and isotype to both RABV G and LASV GPC as detected by ELISA (Fig. 8c and...
Supplementary Fig. 5a and b), LASSARAB immunized Fcγ-KO mice quickly succumbed to surrogate LASV exposure, in contrast to the WT mice. However, some differences exist between human and mouse Fcγ-Rs, and future studies using humanized knock-in models would be of interest66. Curiously, besides the critical role that Fcγ-R effector functions played in protection against LASV, our results from Fig. 8 indicated (but not significantly) that Fcγ−/− mice immunized with LASSARAB seemed more susceptible to surrogate LASV infection than control mice (Fig. 8 and Supplementary Fig. 5). Although based on a contrived model, this makes us question whether, beyond viral clearance, pre-existing GPC-directed non-NAbs might also work as immune regulators in LASV infection.

By the end of our guinea pig exposure study (Fig. 6d), we observed that ~20% to ~40% of survivors had low (below the LOQ) but detectable levels of LASV RNA in the blood 50 days'
post-exposure in all groups, except LASSARAB and RabAvert. This has been reported in the literature for LASV in NHPs. This result suggests a chronic asymptomatic infection that, after reactivation, may explain some of the late deaths and clinical signs observed in both the LASSARAB+GLA-SE and rVSV-GPC groups. As such, future studies should consider possible LASV reactivation, as well as the importance and crucial task for LF vaccine development. Ideally, a LF vaccine should be protective, safe, and confer a long-lasting humoral immunity that can be easily measured and identified as a correlate of protection. As our results demonstrate, LASSARAB induces high LASV GPC-specific IgG titers that correlate with protection prior to LASV exposure, in the absence of LASV NAb. This could potentially become a LF correlate of protection that would provide easy screening for vaccine efficacy post immunization. Additionally, the finding that GPC-specific non-NABs play a crucial role in protecting mice against a LASV infection.

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**Fig. 8** Evaluation of in vivo relevance of non-NABs LASV GPC specific antibodies induced by LASSARAB + GLA-SE vaccination. **a** 8- to 10-week-old Balb/c (WT) or Balb/c with Fcy chain KO (Fcy−/−) female mice were immunized with 10 µg of inactivated particles of either LASSARAB or FILORAB1 (mock control) on day 0 and boosted on day 28. All four groups in total were adjuvanted with 5 µg of GLA in a 2% SE with each vaccination. One day before exposure (day 41) animals were injected with 1.25 mg of anti-Ifnar mAb (MAR1-5A3, Leinco technologies) through intra-peritoneal injection (IP). On day 42, mice were exposed to 10^4 rVSV-GPC virus IP and general health (weights and clinical observation) was recorded until endpoint criteria were reached or end of study (supplemental). **b** Survival curves post-exposure of rVSV-GPC. Significance is compared between the WT LASSARAB vaccinated and the Fcy−/− vaccinated using the log-rank (Mantel-Cox) test. **c** Pre-exposure total IgG titers anti LASV GPC were measured by ELISA on day 35 post-prime and ELISA curves were plotted according to OD490 reading value (Y-axis) and serum dilution (X-axis). On the right, EC50 (half maximal effective concentration) of serum dilution of both LASSARAB groups (WT and Fcγ−/−) is plotted on Y-axis on a log scale; statistical significance was calculated using one-way ANOVA. **d** Virus neutralization assay using pseudotyped VSV-GFP-NanoLuc with LASV GPC. On the right, percentage of cells infected is plotted against the serum dilution (survivors on day 14 post-exposure) of each respective group. On the right, the IC50 (half maximal inhibitory concentration) of serum dilution is plotted individually and significance was calculated using one-way ANOVA. Error bars represent Standard Error Mean (SEM) and include all mice (n = 10 per group [WT and KO] in LASSARAB and n = 5 per group [WT and KO] in FILORAB1 control) in pre-challenge and survivor mice in post-challenge. (****P < 0.0001; ***P < 0.001; **P < 0.01; *P < 0.05)
surrogate exposure suggests that non-NAb cellular effector functions should be further investigated as a correlate of protection in both LF vaccine development and mAb antibody therapy.

**Methods**

**Generation and recovery of Rhabdovirus vaccine vectors.** To generate the vaccine vectors, LASSARABAG and rSVS-GPC, the ORF of LASSAR GPC Josiah strain was codon-optimized for mammalian codon usage and synthesized by GenScript (Genbank, Accession Number MH778559). LASSV GPC was cloned between BsiWI and Nhel restriction sites between BSNPS33, generating LASSARAB. LASSARABAG was generated by removing the RABV glycoprotein (G) from the LASSARAB DNA using the Pael and Smal restriction sites and subsequent re-ligation after treatment with Klenow Fragment (Promega).

rSVS-GPC was generated by replacing the native VSV G, through MluI and Nhel restriction sites and cloned in VSV-XN vector.22. The correct sequence of the three plasmids was confirmed by sequencing using RP95, RP952, VPS, and VP6 primers.

Recombinant RABV and VSV were recovered as described previously.25,26, Briefly, X-tremeGENE 9 (Roche) was used to transfect the respective full-length viral cDNA clones along with the plasmids encoding RABV N, P, G, or L and VSV N, P, L, proteins, and pCAGGS plasmids expressing T7 RNA polymerase in Vero cells in 6-well plates (RABV), or 293T cells in T25 flasks (VSV). The supernatants of RABV transfected cells were harvested after 7 days and after 3 days for VSV. Presence of infectious virus was detected by immunostaining for RABV N with 1:200 dilution of FITC anti-rabies globulin (Fujirebio, product # 800-092) or for virus-induced cytopathic effect (CPE) in the case of VSV.

**Request for material.** Upon reasonable request all utilized antibodies, plasmids, and viruses are available from the authors pending on an executed MTA as well as biosafety approval of the requesting institution(s).

**Cell culture.** Vero (ATCC® CCL81™), 293T (ATCC® CRL-3216™), and BSR (available from our laboratory) cells were cultured using DMEM (Corning®) and 5% FBS (Atlanta-Biologicals®). The cells were seeded at a density of 3 × 10^5 cells/cm^2, and cultured in 5% CO_2 at 37°C.

**Viruses and ELISA antigen characterization.** Viruses and purified LASSV GPC were denatured with Urea Sample Buffer (125 mM Tris-HCl [pH 6.8], 8 M urea, 4% sodium dodecyl sulfate, 50 mM dithiothreitol, 0.02% bromophenol blue) at 95°C for 5 min. 2 μg of protein were run on 10% SDS-polyacrylamide gel and stained O/N with SYPRO Red (Thermofisher) for total protein analysis. For western blot analysis SDS-PAGE gel was transferred into a nitrocellulose membrane in Towbin buffer (192 mM glycine, 25 mM Tris, 2% methanol) then blocked in 5% milk dissolved in PBS-T (0.05% Tween 20) at room temperature for 1 h. Next, the membrane was incubated O/N with either rabbit pAb anti-LASSV GPC or 9E9 mAb anti-LASSV GPC at a dilution of 1:1000 in 5% bovine serum albumin (BSA). Rabbit G and P proteins were confirmed with a rabbit anti-G and P polyclonal antibody used at 1:1000.27 After washing, the blot was incubated for 1 h with 1:1000 diluted anti-rabbit secondary antibodies conjugated to horseradish peroxidase (Jackson ImmunoResearch® catalogue numbers: 115-035-146; 111-035-144) at 1:50,000 in 1% milk PBS-T. Proteins were detected with SuperSignal West Dura Chemiluminescent substrate ( Pierce®).

**Animals ethics statement.** Mice and guinea pigs used in this study were handled in adherence to both the recommendations described in the Guide for the Care and Use of Laboratory Animals, and the guidelines of the National Institutes of Health and the Office of Animal Welfare. Animal care was approved by the Institutional Animal Care and Use Committee (IACUC) of Thomas Jefferson University (TJU) or the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of Clinical Research Animal Care and Use Committee for experiments performed at each respective facility. Animal procedures at TJU were conducted under 3% isoflurane/O_2 gas anesthesia. Mice were housed with five individuals per cage, under controlled conditions of humidity, temperature, and light (12 h light/12 h dark cycles). Food and water were available ad libitum.

**Virulopathogenicity analysis.** Five groups of five to eight-week-old female Swiss Webster mice were either intranasally (IN) or intraperitoneally (IP) infected with 10^6 PFU/FFU of each of the respective viruses diluted in 20 μl phosphate-buffered saline (PBS). Mice were weighed daily and monitored for signs of disease until day 28 post-infection. Mice that lost more than 20% weight or showed severe neurological symptoms were humanely euthanized. Intracranial challenge (IC) was performed in 48, 6- to 8-week-old Balb/c mice were anesthetized using isoflurane to secure the animal followed by IC injection of 10^6 FFU/ml of live or 51 of virus exposure, Group 5 RABV-LASV-GPC (30 μg BPL-inactivated virus (3 doses at 0, 7, and 28 days) formulated in either PBS or GLA-SE adjuvant (see Fig. 4 and adjuvant formulation below). All IM immunizations were performed by administering 50 μl of live or BPL-inactivated virus into each hind leg muscle. For serum collection, retro-orbital bleeds were performed under isoflurane anesthesia on days 0, 7, 14, 21, 28, and 35, with the final bleed on day 42 or 63.

**LASSV challenge on outbred Hartley guinea pigs.** Six groups of ten Hartley guinea pigs with PinPorts for blood withdrawal (Charles River Laboratory) were vaccinated as follows: Group 1: Mock (PBS), Group 2 rSVS-GPC 10^7 FFU, Group 3 RABV-LASSV-GPC 10^7 FFU, Group 4 RABV-LASSV-GPC (30 μg) + GLA-SE (7.5 μg) on day −58 of virus exposure, Group 5 RABV-LASSV-GPC (30 μg) + GLA-SE (7.5 μg) on days −58 and −51 of virus exposure, Group 6 RABV-LASSV-GPC (30 μg) + GLA-SE (7.5 μg) on days −58, −51, and −30 of virus exposure. All subjects were challenged with 10,000 PFU of guinea pig-adapted LASSV (GPa-LASSV (IRF0205)); L segment GenBank KY425651.1; S segment GenBank KY425650.1) by IP route.28 Subjects were monitored to at least once daily throughout the experiment and at least twice daily following virus exposure until clinical signs of disease abated. Blood withdrawals were performed at days −65, −58, −51, −30, 0, 16 and study end day at 42 post-exposure. All LASSV experiments were performed in a biosafety level 4 environment and subjects were anesthetized using isoflurane/O_2 gas.
Enzyme-linked immunosorbent assay (ELISA) was used to analyze sera collected on day 42 from two groups of five mice each immunized with 10 µg BLP-inactivated LASV and 10 mg of Nara
cel (Na2CO3 [pH 9.6]) at a concentration of 500 ng/ml and then plated in 96-well 
plates with RABV G, and EBOV GP. Antigens were resuspended in coating buffer (50 mM 
Tris, 10 mM EDTA pH7.6) containing 2% OGP (Octyl 
trifuged at 3000 g, and the supernatant was collected and further centrifuged at 
4°C for 30 min at 56 °C. For IgG puri
cation of LASV GPC glycoprotein and insert the NanoLuc ORF 
through mechanical methods and strained through a 35 µm mesh. Then, the mouse 
NK cell isolation protocol (4) was used to isolate murine NK cells were collected in RPMI (10% FBS, 
Murine NK cell (effector cells) isolation and purification. Murine splenocytes obtained from naïve C57BL/6 mouse spleens were made in a single cell suspension through mechanical methods and strained through a 35 µm mesh. Then, the mouse 
NK Cell Isolation Kit II (Macs-Milenyi Biotech) was used following the manu-
faturer’s protocol. Puri
ced murine NK cells were collected in RPMI (10% FBS, 
0.5 µM (H1–L), Fc specific (heavy chain), IgG2c, 
IgG2a, and IgG1; or goat anti-guinea pig Fc-specific (heavy chain) secondary 
antibody (Jackson ImmunoResearch® cat
loupe: 100221; 108709; 137611; 101323), and by 1:1000 dilution of Zombie 
Dead® (BioLegend) and characterized by 
flow cytometry (BD LSFRFortessa) to confirm NK cell purity and Fcy-Receptor III expression 7.

Macrophage effector cells. IC-21 or J774A.1 macrophages were cultured as per 
above. At 24 h before an ADCP or ADCC assay, macrophages were scraped in a single cell suspension, centrifuged at 200 g and resuspended in sterile cell culture 
PBS. For ADCP assays the internal cellular dye CellTrace 
Far Red (Invitrogen®) was added following the manufacturer’s recommendations. Macrophages were resuspended in serum-free cell culture media containing 5 ng/ml of mGM-CSF 
(cell signaling technology) and used in the following day for ADCP/ADCC assays 
and phenotypic analysis. To confirm macrophage phenotype and expression of all Fc-receptors, 
macrophages were stained with 1:200 dilution of F4/80, CD64, 

RT-PCR analysis for LASV viral loads. See also ref.29, 200 µl of whole blood was 
lysed for RNA extraction using Trizol LS at a 3:1 vol/vol ratio. RNA samples were 
then extracted using the QIAamp Viral RNA Mini Kit (QIAGEN) and eluted in 
50 µl Buffer AVE (QIAGEN). 5 µl of extracted RNA per reaction was added to 2X 
Maxima H Minus First Strand cDNA Synthesis Kit (ThermoFisher 
Scientific) in which the LASV-GPC ORF was amplified by MP3 and MP4 primers (Supplemen
tary Table 1) and added between the Ecorl and Xhol restriction digest sites thus gener-
ating MSCV-GPC-IREs-GFP. Briefly, MSCV-GPC-IREs-GFP was co-transfected 
with a pcAGGS-WSV G with Xyreme-Gene 9 in a Gryphon packaging cell line 
(Albany Molecular Research) and infective retroviral viruses were harvested 48 h post 
transfection. Next, low passage 3T3 murine cell line was transduced with viral 
supernatant and 8 µg/ml of polybrene and centrifuged at 800g for 30 min. After 
72 h, 3T3 cells were enriched by GFP expression through BD FACSaria II. 
Confirmation of LASV GPC expression was done by immunofluorescence by using 50 µg of total cell lysate with anti-EGFP antibody. The target 
cell line (3T3-MARV) was generated through similar methods but with a Marburg 
virus GP (Angola strain) expressing MSCV (kind gift from Rohan Keshwara, 
Thomas Jefferson University).

Murine NK cell (effector cells) isolation and purification. Mouse splenocytes
were stained with 1:200 dilution of F4/80, CD64. Then, the mouse 

Generation of VSV pseudovirions (ppVSV). To generate ppVSV, the cDNA 
plasmid backbone of rVSV-GPC was digested with MluI and Nhel restriction 
enzymes to remove the LASV GPC glycoprotein and insert the NanoLuc ORF 
(Promega). To enable GPC expression, the EGFPR ORF plus a VSV start stop 
signal was inserted in XhoI and NheI cloning sites. Viruses were recovered as described 
above and purified on sucrose gradients, centrifuged at 200 g and resuspended in sterile cell culture 
PBS. For ADCP assays the internal cellular dye CellTrace 
Far Red (Invitrogen®) was added following the manufacturer’s recommendations. Macrophages were resuspended in serum-free cell culture media containing 5 ng/ml of mGM-CSF 
(cell signaling technology) and used in the following day for ADCP/ADCC assays 
and phenotypic analysis. To confirm macrophage phenotype and expression of all Fc-receptors, 
macrophages were stained with 1:200 dilution of F4/80, CD64, 

pSVS-121-GFP was added to the cells at an MOI of 1 and viral supernatant was 
collected 24 and 48 h later.

Virus neutralization assay (VNA). For VNA using animal sera (mouse or guinea 
pig), the serum was heat inactivated at 56 °C for 30 min to ensure complement 
deactivation. Next, heat-inactivated serum was diluted two fold starting at 1:10 
dilution (1:100 in RABV G pseudotyped assays) in Opti-MEM (Gibco), and 104 
ppVSV-121-GFP particles were added to each dilution series. Control mAbs (12.1F, 
25.1E, 1A5, and 9E9, a panel of antibodies selected based on neutralizing activity) were added starting at 30 µg/ml and 2 U/ml, respectively. The sera/ 
antibody+ virus mixture was incubated for 2 h at 34 °C with 5% CO2 and transferred to 
a previously seeded monolayer of Vero cells in a 96 well plate and further incubated for 2 h at 34 °C with 5% CO2. Next, the virus/serum mixture was replaced by complete 
DMEM media. At 18–22 h later, cells were lysed with passive lysis buffer (Promega) 
and added to a 1:1 mixture of luciferase substrate (Promega) and Substrate Plus (Sub-
strate Mega) added following the manufacturer’s recommendations. Optical lumines-
cence units were normalized to 100% infectivity signal as measured by no sera 
control (maximum signal). Half maximal inhibition (IC50) values were calculated by 
GraphPad® Prism 7 using a sigmoidal nonlinear fit model (4PL regression curve). Values that were above 100% infectivity were converted to 100%.
CD32/16, and CD16.2 fluorescence-conjugated antibodies (BioLegend, catalogue numbers: 403233, 139303, 123115, 149513) and characterized by flow cytometry, (BD LSRRFortessa).

ADCC/ADCP assays. Either 1:100 of heat-inactivated sera from immunized mice (see immunizations section), 40 μg/ml of purified IgG from the sera (see immunizations section) or 40 μg/ml of control mAbs (4C8, 9E9 and 5A3) were added to previously seeded 2 × 10^5 3T3-LASV GPC target cells or control target cells and incubated for 30 min at 37°C in 5% CO2. For Fcy receptor blockade 100 μg/ml of either anti-CD64, CD32/16, or CD16.2 (BioLegend) was added to effector cells (see above) for 30 min. Next, effector cells were added to target cells at different effector to target cell ratios and incubated for 4 h. Target cells were then dissociated from the plate with Cellstripper® solution (Corning), washed, and resuspended in 200 μl of IFAC-SIF (5% PBS) with 30 μg/ml of propidium iodine (PI) viability dye. Cells were then immediately analysed by flow cytometry (BD LSRRFortessa).

ADCP confocal microscopy analysis. For confocal analysis ADCC assay was conducted in the same conditions as described above but adapted for later microscopy analysis. Briefly, 3T3-LASV GPC target cells were seeded in glass cover-slips and incubated with the respective sera conditions, and then J774A.1 macrophages previously stained with 1:1000 CellTrace® Far Red (see above) were added at a 1:1 Target to effector cell ratio to allow easy visualization. After 4 h, coverslips were washed and mounted in slides with DAPI containing mounting media (VECTASHIELD) and allowed to solidify O/N. Next, day samples were analyzed in a Nikon confocal microscope and further compiled through ImageJ software.

Gating strategy and ADCC and ADCP analysis. All flow cytometry data were collected using the FACSDiva (BD) software. Laser voltage settings were adjusted for each analysis by running single color controls. For ADCC analysis, cells were first gated for size using the side scatter (SS) and forward scatter (FS) and selecting the 3T3 population (Supplementary Fig. 3). Next, using the histogram function GFP+ cells were gated and based on this gate a total of 5000 GFP+ events were captured. Due to size variability, ADCC analysis was performed by excluding PI+ events and collecting a total of 10,000 APC+ events (macrophages). For data analysis FlowJo 10 (BD) software was used. The percentage of cytotoxicity (ADCC) was measured by the percentage of PI- cells of the total GFP+ population after size gating. Since PI is a continuous dye in apoptotic cells [9], PI+ histogram gating was based by defining a 10% PI+ population gate on the control 3T3-LASV GPC cells (no effector cells and sera) as the background. ADCP percentage was measured by measuring the percentage of GFP+APC+ of the total APC+ population. After defining gating strategy on control cells all gating was applied uniformly to all samples.

Statistical analysis. All statistical analysis was performed by using the Graphpad 7 (Prism). To determine the statistical test to be used the population was first analysed to check whether it followed a normal distribution (Gaussian curve) by applying a D’Agostino-Pearson omnibus normality test. If so a parametric two-tailed T-test was used for comparison within two groups. For grouped analysis, a one-way ANOVA or two-way ANOVA test was used and a post-Hoc analysis using either Sidak or Tukey honest significant difference Test with a 95% confidence interval to test significance within groups. Non-parametric tests were used if the population did not follow a normal distribution (indicated in the figure legends).

Data availability
All relevant data are available from the corresponding author upon request. Sequences of LASV GPC are available at Genbank under accession number MF778559.

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