# Supplementary information: extended materials and methods

## Generating Vero-LASV-GPCco cell line and cell culture conditions

The stable cell line was generated with the Flp-In kit (Invitrogen). Briefly, TransIT LT1 reagent (Mirus Bio) was used to transfect a subconfluent 12-well of Vero-E6 cells with 1.5 µg of ScaI-linearized plasmid pFRT/lacZeo. Two days later, the cells were split into cell culture dishes and selected with 640 µg/mL Zeocin (Gibco). Clones were picked 11 days later using glass cloning rings and expanded further in the presence of 320 µg/mL Zeocin to obtain Vero-E6 FRT host cell lines. A codon-optimized open reading frame for Lassa Josiah strain GPC was cloned into pcDNA5/FRT of the Flp-In system using In-Fusion HD Cloning Kit (Clontech). This construct (200 ng/well of a 6-well plate) was transfected with the Flp recombinase plasmid pOG44 (1800 ng/well) into Vero-E6 FRT host cells. Vero-LASV-GPCco cell clones were selected with 200 µg/mL hygromycin B (Invitrogen). Vero-E6, A549 (ATCC #CLL-185), and GPC-16 cells (ATCC #CCL-242) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with sodium pyruvate, penicillin/streptomycin (Gibco), and 10% FCS (HyClone).

## Rescue of Lassa VRPs

LASV rescue constructs pLasL and pLasS (described in [11]) were modified to enable rescue of non-spreading VRPs. The GPC ORF in the S segment was replaced with ORF for zsGreen (ZsG) to obtain plasmid pLasSΔGPC+ZsG. For some VRPs, mutations D389A and G392A were made to inactivate NP exonuclease domain. To replace LASV Z ORF in the large segment with that of Mopeia virus Z, plasmid pLasL was first cut with SacI and then partially digested with KflI. A gene synthesis product coding for Mopeia Z (corresponding to NC\_006574, except for a silent mutation to abolish an internal SacI restriction site) in the context of LASV L segment was then excised and ligated into the resulting pLasL fragment. VRPs were rescued by transfecting 6-wells of BSR T7/5 cells with 1 µg each of a pLasL construct, a pLasS construct, and the LASV GPC expression construct pCAGGS-LASV-GPC. Four days later, supernatants were passed onto Vero-LASV-GPCco cells. To grow stocks, Lassa VRPs with no additional mutations (WT-WT VRPs, indicating no mutations in either L or S segment) were passed twice and all other VRPs 3 times on Vero-LASV-GPCco cells. VRP titers were determined on Vero-E6 and Vero-LASV-GPC cells under 1.5% CMC overlay by imaging the monolayers using Cytation3 instrument (BioTek). Virus and VRP stocks were sequenced using MiniSeq instrument with TruSeq chemistry (Illumina). WT-WT VRP stocks displayed the expected sequence, but other VRP stocks typically displayed single mutations in varying positions of the L ORF, most likely due to reduced rescue efficiency and the need for additional passaging before obtaining usable stock titers. No reversions were observed in the exonuclease-inactivating mutations or Mopeia Z sequence. For some experiments, inocula were inactivated using 400 mJ/cm2 UV light (Spectroline Spectrolinker).

## Immunoblotting

Cell monolayers were lysed in a buffer containing 50 mM NaCl, 5 mM EDTA, 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate, and protease and phosphatase inhibitors (Roche). Lysates from infected cells were gamma-irradiated using a high-energy 60Co source. Sample concentrations were normalized using DC Protein Assay (BioRad) and proteins were separated on Novex 4–12% Bis-Tris gels (Invitrogen) with MOPS running buffer. Proteins were blotted onto nitrocellulose membranes (BioRad) and probed with the aforementioned primary antibodies in 1% non-fat dry milk and 0.05% Tween-20 in PBS.

## Immunofluorescence

Formalin-fixed samples were permeabilized with 0.5% Triton X100 in PBS and washed/blocked with 1% BSA/PBS. Antigens were detected with antibodies listed earlier. When using microscope slides, DNA was counterstained by DAPI in ProLong Gold Antifade Mountant (Molecular Probes). Figure 1 images were made on clear bottom/black wall cell culture plates inside the BSL-4 laboratory using Evos FL system (ThermoFisher Scientific). Figure 2A images were made using Zeiss Axio Imager.A1 fluorescence microscope.

## qRT-PCR

Nucleic acids were isolated using MagMAX RNA isolation reagents (ThermoFisher Scientific). Genomic DNA was then removed by incubating 30 µL of eluent with 1.5 units of BaseLine-ZERO DNase (Epicentre) in a total volume of 50 µL at 37°C for 2 h. The nuclease was inactivated by 10 min incubation at 65°C in the presence of 1× stop solution from the kit. Quantitative RT-PCR was performed using SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen) and BioRad CFX96 Real Time machinery with 15 min reverse transcription at 50°C, 2 min at 95°C, and 40 cycles of 15 s at 95°C and 30 s at 60°C. To measure LASV and VRP replication, a primer-probe assay was designed for the NP sequence, with primers 5′-GTACTCACATGGGATTGATGTCAC-′, 5′- CTTCCTTGTGATTCAAGGAGTTTC-3′, and 5′-56-FAM/TTCGCTACA/ZEN/CAACCGGGCTTGACC/3IABkFQ/-3′. Guinea pig GAPDH and CCL5/RANTES transcripts were detected using TaqMan Gene Expression Assays with FAM/MGB chemistry (ThermoFisher Scientific Cp03755743\_g1 and Cp03754832\_m1, respectively). To detect guinea pig IFN-β, the following primers and probes were designed by IDT DNA using sequence XM\_003472239 as the template: 5′-GTGTATCCTCCAAATCGCTCTC-3′, 5′-GAATTGCTGCTGCGTTGTT-3′, and 5′-/56-FAM/TGCTGTCCT/ZEN/TCACCACATCTCTTTCC/3IABkFQ/-3′. No-template controls and no-RT controls (template added after reverse transcription) demonstrated that detected signals originated from sample RNA. To determine tissue loads of LASV RNA, a standard curve of in vitro-transcribed RNA was used with tissue-specific correction for input using 18S Ct.

## Animal experiments

All animal procedures were approved by the Centers for Disease Control and Prevention (CDC) Institutional Animal Care and Use Committee (IACUC). The CDC is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Male and female Strain 13/N guinea pigs (aged 2.0–3.5 years), obtained from our breeding, were housed in a BSL-4 laboratory in HEPA-filtered micro-isolator cage systems. Groups of 5 animals, distributed proportionally by age and sex, were vaccinated s.c. with 5 × 105 FFU WT-WT VRPs or WT-Exo(N) VRPs, or mock vaccinated with DMEM. Twenty-eight days post vaccination, blood samples were collected from the cranial vena cava and animals were challenged with a lethal dose (1 × 104 FFU) of LASV Josiah s.c. Animals were monitored for signs of clinical illness, and daily body weight and temperature readings were obtained. Animals were humanely euthanized once clinical illness scores (including, but not limited to, piloerection, ocular discharge, weight loss > 25%, changes in mentation, ataxia, dehydration, dyspnea, or hypothermia) indicated that the animal was in the terminal stages of disease, or at the completion of study (42 d p.i.).

## ELISA

Plasma was separated from EDTA-blood by centrifugation at 6000 rcf and inactivated using 5 million rads of gamma irradiation from a 60Co source. Samples were diluted 1:25 in master plate diluent (5% skim milk powder, 0.5% Tween-20, 0.5% Triton X100, and 0.01 % thimerosal in PBS). For IgG ELISA, samples were pre-absorbed at 4°C overnight using ELISA plates coated with Vero-E6 cell lysate. After pre-absorption, the samples were further diluted in a 4-fold series in serum diluent (5% skim milk powder and 0.1% Tween-20 in PBS) to 1:100, 1:400, 1:1600, and 1:6400. These samples were incubated for 1 h at 37°C on ELISA plates coated with either Vero-E6 lysate or lysate from LASV Josiah-infected Vero-E6 cells. After automatic washing with 0.1% Tween-20 in PBS, the wells were incubated for 1 h at 37°C with HRP-conjugated secondary antibody directed against guinea pig IgG (KPL) in serum diluent. Washed wells were then incubated with ABTS Microwell Peroxidase Substrate (KPL) for 30 min at 37°C, and absorbances read using a plate reader (BioTek). For IgM ELISA, a capture format with a goat anti-guinea pig IgM antibody (ICL Lab #GM-60A) was used. Sample dilutions were incubated on capture antibody-coated plates, followed by cell slurries from mock-infected or LASV Josiah-infected Vero-E6 cells. Bound antigen was detected using an in-house polyclonal antibody against LASV (HMAF 703079) and secondary anti-mouse IgG HRP-conjugate (Pierce 31446). For both IgG and IgM assays, the raw data were first corrected by subtracting the value obtained with unspecific lysate (Vero-E6) from the value obtained using specific lysate (infected cells). Samples were considered positive if: 1) individual well signals were more than 2 (IgG) or 3 standard deviations (IgM) higher than the average of known negative samples (pre-challenge samples from mock-vaccinated animals), and 2) summarized signals from all dilutions for that sample were more than 2-3 standard deviations above the average sum of the known negatives. When 2 repeated measurements gave a positive signal for a sample, the average titer was reported. Otherwise the sample was reported as negative.