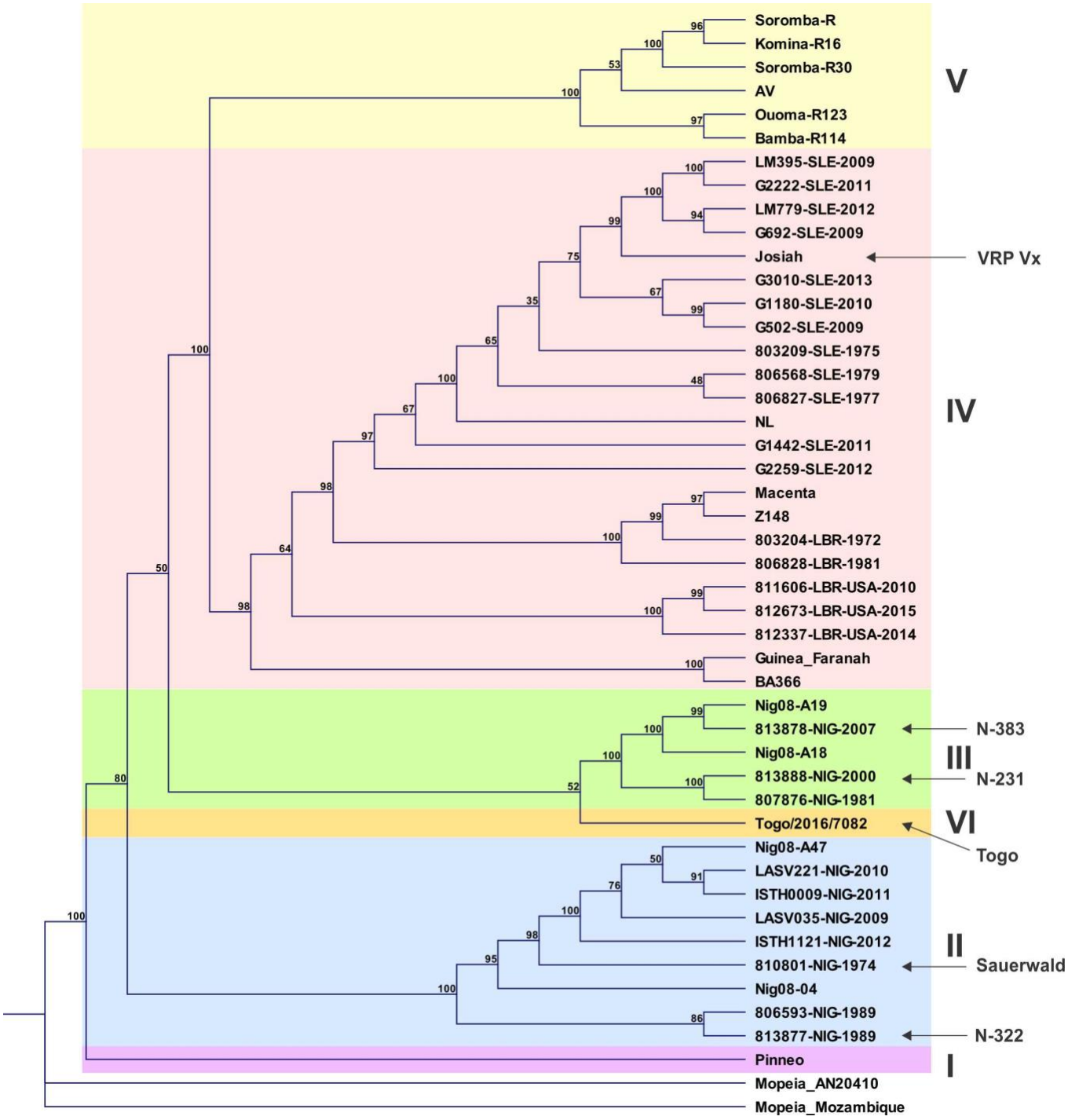

Figure S1. Phylogeny of Lassa virus strains evaluated in Strain 13/N guinea pigs. Phylogenetic analysis of full-length nucleoprotein (NP) nucleotide sequences. Phylogenetic tree was rooted using 2 Mopeia virus (MOPV) isolates, and isolates were grouped by clades. Node labels represent bootstrap values, 1,000 replicates. Indicated are the five LASV strains that were evaluated in strain 13/N guinea pigs (Sauerwald, Nigeria-322, Nigeria-231, Nigeria-383, and Togo) and Josiah (strain that the VRP vaccine (Vx) was based on). LASV clades cluster based on geographical location, and include 6 major lineages. Clades I–III are found in different regions of Nigeria; Clade IV in Sierra Leone, Liberia, and Guinea; Clade V in southern Mali; and Clade VI in Togo.

Lassa virus replicon particle vaccine protects strain 13/N guinea pigs against challenge with geographically and genetically diverse viral strains

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Supplementary tables:

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Table S1. Summary of LASV strains assessed in vitro and in vivo

Strain	Clade	Alternative designation	Source	Origin	Passage history	GenBank/Reference
Sauerwald	II	803213 810801 813884-NIG-1974	Human, fatal	Onitsha, Nigeria, 1974	P3 or P5, 1 pass on Vero, 2 or 4 passes on Vero-E6	MG812680, MG812681 (PMID: 31588039)
Nigeria-322	II	las806322 813877-NIG-1989	Human, outcome unknown	Owerri, Nigeria, 1989	P3 on Vero-E6	AF182226 (NP partial; PMID: 10888638). OM140826, OM140827
Nigeria-231	III	Nigeria 200003231 813888-NIG-2000	Human, fatal	Jos, Nigeria, 2000	P2 on Vero-E6	OM140830, OM140831
Nigeria-383	III	Nigeria 200706383 813878-NIG-2007	Human, fatal	Jos, Nigeria, 2007	P2 on Vero-E6	OM140828, OM140829
Togo	VI	Lassa/H.sapiens-tc/TGO/2016/812939; 201600568 Togo 813889-TGO-2016	Human, survivor	Togo, 2016	P3 on Vero-E6	MF990887 (PMID: 29460758), KU961971

Table S2. LASV and Mopeia virus sequences used for phylogenetic analysis

Clade	Country	Isolated	Strain Name	Genbank# S	Genbank# L
1	Nigeria	1969	Pinneo	KM822128.1	KM822127.1
2	Nigeria	1974	810801-NIG-1974	MG812681	MG812680
2	Nigeria	1989	806593-NIG-1989	KU978811.1	KU978812.1
2	Nigeria	1989	813877-NIG-1989	OM140827	OM140826
2	Nigeria	2008	Nig08-04	GU481068.1	GU481069.1
2	Nigeria	2008	Nig08-A47	GU481078.1	GU481079.1
2	Nigeria	2009	LASV035-NIG-2009	KM822004	KM822003
2	Nigeria	2010	LASV221-NIG-2010	KM822022	KM822021
2	Nigeria	2011	ISTH0009-NIG-2011	KM821912.1	KM821911.1
2	Nigeria	2012	ISTH1121-NIG-2012	KM821945.1	KM821944.1
3	Nigeria	1981	807876-NIG-1981	MG812635	MG812634
3	Nigeria	2000	813888-NIG-2000	OM140831	OM140830
3	Nigeria	2007	813878-NIG-2007	OM140829	OM140828
3	Nigeria	2008	Nig08-A18	GU481070.1	GU481071.1
3	Nigeria	2008	Nig08-A19	GU481072.1	GU481073.1
4	Liberia	1972	803204-LBR-1972	MG812648	MG812649
4	Sierra Leone	1975	803209-SLE-1975	MG812647	MG812646
4	Sierra Leone	1976	Josiah	AY628203.1	AY628202.1
4	Sierra Leone	1977	806827-SLE-1977	MG812639	MG812638
4	Sierra Leone	1979	806568-SLE-1979	MG812643	MG812642
4	Liberia	1980	Z148	AY628205.1	AY628204.1
4	Liberia	1981	806829-LBR-1981	MG812636	MG812637
4	Guinea	1981	Macenta	AY628201.1	AY628200.1
4	Guinea	1996	Guinea Faranah	KU978807.1	KU978808.1
4	Sierra Leone	2000	NL	AY179173.1	AY179172.1
4	Sierra Leone	2009	G502-SLE-2009	KM821773.1	KM821772.1
4	Sierra Leone	2009	G692-SLE-2009	KM821783.1	KM821782.1
4	Sierra Leone	2009	L395-SLE-2009	KM822115	KM822114
4	Liberia	2010	811606-LBR-USA-2010	MG812679	MG812678
4	Sierra Leone	2010	G1180-SLE-2010	KM821794.1	KM821793.1
4	Sierra Leone	2011	G1442-SLE-2011	KM821800.1	KM821799.1
4	Sierra Leone	2011	G2222-SLE-2011	KM821832.1	KM821831.1
4	Sierra Leone	2012	G2259-SLE-2012	KM821835.1	KM821834.1
4	Sierra Leone	2012	LM779-SLE-2012	KM822126	KM822125
4	Sierra Leone	2013	G3010-SLE-2013	KM821882.1	KM821881.1
4	Liberia	2014	812337-LBR-USA-2014	MG812658	MG812659
4	Liberia	2015	812673-LBR-USA-2015	MG812650	MG812651
5	Ghana, CI, BF	2000	AV	AF246121.2	AY179171.1
5	Mali	2012	Bamba-R114	KF478766.1	KF478761.1
5	Mali	2012	Komina-R16	KF478767.1	KF478760.1
5	Mali	2012	Ouoma-R123	KF478768.1	KF478764.1
5	Mali	2012	Soromba-R	KF478765.1	KF478762.1
5	Mali	2012	Soromba-R30	KF478769.1	KF478763.1
6	Togo	2016	Togo/2016/7082	KU961971.1	KU961972.2
NA	Mozambique	1972	Mopeia AN20410	AY772170.1	AY772169.1
NA	Mozambique	UNK	Mopeia Mozambique	DQ328874.1	DQ328875.1
NA	NA	NA	rJosiah	HQ688673.1	HQ688675.1

Sequences used for phylogenetic analyses depicted in Figure S1. CI, Côte d'Ivoire; BF, Burkina Faso; UNK, unknown; NA, not applicable.

Table S3. Clinical scoring and euthanasia criteria for LASV-infected strain 13/N guinea pigs

Score	Clinical sign
0	BAR QAR
1	QDR +/- interest in enrichment Hunched back/ruffled coat vs. normal Huddling/burrowing
2	Mild/moderate weakness Dehydration (eye recession) Ataxia, circling, tremors, and/or head tilt Weight loss >15%
3	Severe weakness Abnormal breathing Anemia
12	Paralysis Frank hemorrhage/Bleeding Moribund Weight loss > 25%

Clinical signs were scored based on 14 parameters. Weight change % calculated from baseline at -1 dpi. Each row within the score category is additive, i.e., if an animal exhibited mild weakness and had weight loss >15%, the total score would equal 4. End-point criteria reached at score of ≥ 12. BAR, bright, alert, and responsive; QAR, quiet, alert, and responsive; QDR, quiet, dull, but responsive.

Table S4. Summary of clinical parameters, viral RNA detection, and serology in LASV-infected strain 13/N guinea pigs

No	Virus strain	Clade	Sex	Age at d0 (d)	End (dpi)	Max weight loss (%)	Peak temp (dpi)	Max clin score	Anti-NP reactivity*	Liver	Spleen	Repro	Kidney	Heart	Lung	Eye	Brain	Blood
1	Togo	VI	F	1093	42	-9.4	12	1	++	−	1.0E+05	−	−	−	−	−	4.3E+01	5.4E+02
2	Togo	VI	M	926	24†	-25.7	12	12	+	3.3E+05	6.8E+07	−	6.2E+04	6.9E+07	1.2E+05	1.4E+07	6.6E+04	1.1E+04
3	Togo	VI	M	966	42	-2.8	12	1	++	−	6.4E+03	−	5.5E+01	−	−	−	−	−
4	Togo	VI	M	667	42	-1.6	11	1	++	−	2.6E+03	−	−	−	−	−	−	−
5	Togo	VI	F	431	42	2.0	11	1	+++	−	4.8E+03	−	−	−	−	−	−	−
6	Sauerwald	II	F	1093	42	-10.6	11	4	++	1.7E+03	3.6E+04	−	−	4.6E+02	−	4.6E+02	−	1.2E+03
7	Sauerwald	II	M	234	42	-8.8	12	2	+++	2.8E+02	8.1E+04	−	4.2E+01	−	−	9.5E+02	−	5.4E+02
8	Sauerwald	II	M	939	42	4.3	10	1	+++	−	2.3E+04	−	−	2.3E+01	−	−	−	−
9	Sauerwald	II	M	666	42	0.9	11	2	+++	−	6.0E+04	−	−	−	−	3.9E+02	−	−
10	Sauerwald	II	F	431	42	-0.5	10	1	+++	−	1.5E+05	−	−	−	−	−	−	4.1E+02
11	Nigeria 322	II	F	971	42	-12.3	NA	0	++	−	−	−	−	−	−	−	−	−
12	Nigeria 322	II	M	903	42	-6.3	NA	1	+++	−	−	−	−	−	−	−	−	−
13	Nigeria 322	II	M	757	42	-20.6	NA	5	+++	−	−	−	−	−	−	−	−	−
14	Nigeria 322	II	M	631	42	6.1	NA	2	+++	−	−	−	−	−	−	−	−	−
15	Nigeria 322	II	F	434	42	-0.7	NA	0	+++	−	−	−	−	−	−	−	−	−
16	Nigeria 231	III	F	971	42	-11.1	14	2	++	−	6.4E+04	−	−	−	−	1.0E+03	−	3.1E+02
17	Nigeria 231	III	M	903	21†	-20.6	12	12	−	6.8E+07	1.1E+08	2.3E+06	8.6E+04	3.8E+04	4.8E+07	7.8E+07	1.4E+05	8.8E+05
18	Nigeria 231	III	M	669	42	-8.8	15	2	++	4.3E+02	1.5E+05	−	−	−	1.7E+02	5.0E+03	4.3E+03	−
19	Nigeria 231	III	M	631	42	-4.9	12	1	++	−	3.8E+04	−	−	−	−	1.5E+03	−	−
20	Nigeria 231	III	F	424	42	-11.6	12	5	++	−	1.6E+05	−	−	−	−	5.4E+04	−	5.6E+02
21	Nigeria 383	III	F	939	42	-17.7	13	3	+	5.7E+03	1.5E+05	7.4E+03	−	−	2.2E+03	2.2E+05	−	5.4E+03
22	Nigeria 383	III	M	755	42	-20.9	12–13	4	+	6.0E+03	1.4E+05	2.8E+02	3.8E+03	−	−	1.4E+05	−	1.3E+03
23	Nigeria 383	III	M	757	42	-4.8	12	2	+++	−	4.1E+04	−	−	NA	−	−	−	−
24	Nigeria 383	III	M	667	42	-2.7	12–14	1	+++	−	2.6E+04	−	−	−	−	2.3E+03	−	2.4E+03
25	Nigeria 383	III	F	1093	42	-4.9	12, 14	3	++	1.8E+03	1.0E+05	−	−	−	−	1.8E+05	1.8E+04	1.3E+03

Groups of 5 animals were inoculated SC with a target dose of 1×10^4 FFU with 1 of 5 LASV strains. All blood and tissue samples were collected at study endpoint. Viral RNA load reported in copy number per µL of RNA. †, euthanasia due to severe disease; −, below the limit of detection; *, level of IgG reactivity in plasma to Josiah NP by immunofluorescent assay (-, not detected; +, low; ++, moderate; +++, high). NA, not applicable.

Table S5. Summary of clinical parameters, viral RNA detection, and serology in VRP-vaccinated and mock-vaccinated LASV-infected strain 13/N guinea pigs

No	Vx	Virus strain	Cd	Sx	Age at d0 (d)	End (dpi)	Max weight loss (%)	Peak temp (dpi)	Max clin. score	Pre Cx Anti-NP react.*	Post Cx Anti-NP react.*	Liver	Spleen	Repro	Kidney	Heart	Lung	Eye	Brain	Blood
1	+	Sauerwald	II	F	843	42	2.1	26	0	−	+++	-	-	-	-	-	-	-	-	-
2	+	Sauerwald	II	F	717	42	-4.5	34	0	−	+	-	-	-	-	-	-	-	-	-
3	+	Sauerwald	II	M	520	42	-0.4	28-29	0	+	+++	-	-	-	-	-	-	-	-	-
4	+	Sauerwald	II	M	273	42	4.9	29	0	+	+++	-	-	-	-	-	-	-	-	-
5	+	Sauerwald	II	F	280	42	0.0	9, 35	0	−	+++	-	-	-	-	-	-	-	-	-
6	+	Nigeria-231	III	F	841	42	-1.3	10, 34	0	−	+++	-	-	-	-	-	-	-	-	-
7	+	Nigeria-231	III	M	398	42	3.5	34	0	+	+++	-	-	-	-	-	-	-	-	-
8	+	Nigeria-231	III	M	516	42	4.9	32	0	+	+++	-	-	-	-	-	-	-	-	-
9	+	Nigeria-231	III	M	278	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	+	Nigeria-231	III	F	280	42	1.7	23	0	++	+++	-	-	-	-	-	-	-	-	-
11	-	Sauerwald	II	F	752	42	-5.5	11-12	0	−	++	-	3.3E+04	-	-	9.8E+02	-	-	-	-
12	-	Sauerwald	II	M	510	42	-2.7	11	2	−	+++	-	6.3E+04	-	-	-	-	-	-	-
13	-	Sauerwald	II	F	520	42	0.2	11	1	−	++	-	5.3E+04	NA	-	-	-	-	-	-
14	-	Sauerwald	II	M	278	42	-0.8	12	2	−	+++	-	1.7E+04	-	-	-	-	3.3E+02	-	-
15	-	Sauerwald	II	F	273	42	-0.9	11	2	−	++	-	1.0E+04	-	-	-	-	-	-	-
16	-	Nigeria-231	III	F	749	27†	-18.6	12-13	12	−	NA	7.2E+06	1.9E+06	4.7E+05	-	1.6E+04	3.2E+05	1.7E+07	3.0E+04	NA
17	-	Nigeria-231	III	M	398	42	-11.9	12-13	2	−	++	-	2.9E+03	-	-	-	-	2.0E+04	-	-
18	-	Nigeria-231	III	M	398	42	-2.0	11	1	−	+++	-	2.8E+04	-	-	-	-	-	-	-
19	-	Nigeria-231	III	M	273	42	-8.2	12-12	2	−	++	-	4.1E+04	-	-	-	-	9.9E+01	-	-
20	-	Nigeria-231	III	F	267	42	-9.4	11	1	−	++	3.9E+02	4.5E+04	-	-	-	-	7.7E+03	-	-

Animal no. 9 was removed from study prior to challenge (at 4 days post vaccination) due to study independent health reasons (urinary obstruction). Pre-challenge (Pre Cx) blood samples were collected from the cranial vena cava at 28 days post vaccination, just prior to infection (Day 0); all post challenge (Post Cx) blood (intra-cardiac) and tissue samples were collected at study endpoint. Viral RNA load reported in copy number per µL of RNA. †, succumbed to disease, no terminal blood sample was obtained. *, level of IgG reactivity in plasma to Josiah NP by immunofluorescent assay (-, not detected; +, low; ++, moderate; +++, high). Cx, challenge; NA, not applicable or sample not available; VRP, LASV replicon particle; Vx, indicates VRP vaccination (+, present; -, absent).

Lassa virus replicon particle vaccine protects strain 13/N guinea pigs against challenge with geographically and genetically diverse viral strains

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Supplementary materials and methods

Biosafety

All work with infectious virus or infected animals was conducted in a biosafety level 4 (BSL-4) laboratory at the Centers for Disease Control and Prevention (CDC) following established BSL-4 standard operating procedures approved by the Institutional Biosafety Committee. All animal procedures were approved by the CDC Institutional Animal Care and Use Committee. CDC is fully accredited by AAALAC-International. All recombinant virus work was approved by the Centers for Disease Control and Prevention Institutional Biosafety Committee.

Guinea pigs

Healthy strain 13/N guinea pigs were selected from the CDC breeding colony and distributed proportionally by sex and age into experimental groups. Guinea pigs were housed individually on a bedding mixture of pelleted paperchip (Lab Supply, Fort Worth, TX), crinkle paper (Crink-l'Nest, The Andersons Lab Bedding, Maumee, OH), and natural soft cellulose bedding

(BioFresh Comfort bedding, Patterson, NY) in HEPA filtered IVC caging (Maxi-Miser® PIV System, Thoren Caging Systems), and provided unrestricted water and pelleted diet (LabDiet 5025, Land O'Lakes, St. Louis, MO) with daily timothy hay and supplemental vitamin C-rich fresh vegetables. Environmental parameters were maintained within a temperature range of 68–79°F and 30–70% relative humidity on a 12:12 hour light:dark cycle. Annual health monitoring of the colony includes serology (Guinea Pig Basic Opti-Spot, IDEXX BioResearch, Columbia, MO) and baseline complete blood counts and clinical chemistry analyses on a subset of animals. All evaluated animals were free of *Clostridium piliforme*, guinea pig parainfluenza virus 3, lymphocytic choriomeningitis virus, murine pneumonia virus, Sendai virus, and *Encephalitozoon cuniculi*, as determined using serological screening tests.

Clinical monitoring of guinea pigs

Animals' body temperatures were monitored using implanted microchip transponders (BMDS IPTT-300). Water consumption was measured using graduated bottles and calculated as percentage of water consumed overnight. Clinical signs were scored based on 14 parameters: 1 point each for quiet, dull, responsive (QDR) disposition, hunched back or ruffled coat, huddling or burrowing; 2 points each for mild or moderate weakness, dehydration (eye recession), ataxia, circling, tremors, head tilt, weight loss of >15%; 3 points each for severe weakness, abnormal breathing, or anemia; 12 points each for paralysis, frank hemorrhage or bleeding, moribund state, or weight loss of >25% (Table S3). Animals were humanely euthanized when end-point criteria were reached (clinical score ≥ 12), or at study completion (42 dpi).

Serology

Antibodies against LASV NP in guinea pig plasma were detected as previously described [4][5]. Briefly, GPC-16 cells were transfected with a plasmid constitutively expressing LASV NP (strain Josiah), or a non-expressing control plasmid. Two days later, the cells were fixed and gamma-

irradiated EDTA plasma samples were used as primary antibodies at dilution 1:200, followed by detection with an Alexa488-conjugated anti-guinea pig secondary antibody. The resulting staining was scored as negative or as positive with a three-step scale.

Next-generation sequencing

RNA from cell culture supernatants was clarified by low-speed centrifugation and inactivated with TRIPURE (Roche) reagent; total RNA from was extracted using the Direct-zol RNA purification kit (Zymo Research) according to manufacturer's instructions. Ribosomal RNA was removed, and cDNA libraries were prepared using the KAPA RNA HyperPrep Kit with RiboErase (HMR) (Roche). NGS was performed using paired-end 2 × 150bp chemistry on an Illumina MiniSeq instrument. NGS data, including read mapping, contig assembly, and sequence alignments was done using CLC Genomics Workbench 21. Phylogenetic trees were constructed (neighbor-joining method, Jukes-Cantor nucleotide distance measure; bootstrap analysis based on 1,000 replicates) in CLC Genomics Workbench 21.