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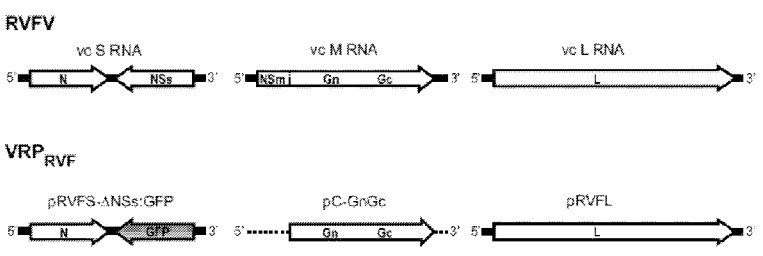
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FIG. 1A



(57) Abstract: Disclosed herein is a robust system for the reverse genetics generation of a Rift Valley fever (RVF) virus replicon particle (VRP_{RVF}) vaccine candidate. VRP_{RVF} can actively synthesize viral RNA and proteins, but lack structural glycoprotein genes, preventing spread within immunized individuals and reducing the risk of vaccine-induced pathogenicity. Is it disclosed herein that VRP_{RVF} immunization is both safe and efficacious, resulting in a robust immune response that is protective against RVF virus challenge within 24 hours of immunization. Provided herein are VRP_{RVF}, methods of producing VRP_{RVF}, and method of using VRP_{RVF} for immunization against RVF virus infection.

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RIFT VALLEY FEVER VIRUS REPLICON PARTICLES AND USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/661,614, filed June 19, 2012, which is herein incorporated by reference in its entirety.

FIELD

This disclosure concerns Rift Valley Fever virus replicon particles (VRPs), methods of making VRPs and their use as vaccines against Rift Valley Fever virus infection.

BACKGROUND

Rift Valley fever virus (RVFV) causes sporadic but devastating outbreaks of severe human disease and widespread morbidity and mortality in livestock. RVFV is a mosquito-borne virus of the *Bunyaviridae* family (genus *Phlebovirus*), and the timing of outbreaks is often closely associated with emergence of floodwater *Aedes* species mosquitoes following periods of extensive heavy rainfall (Schmaljohn and Nichol, Bunyaviridae, p. 1741-1789. *In* D. M. Knipe and P. M. Howley (ed.), Fields Virology, 5 ed. Lippincott Williams and Wilkins, Philadelphia, PA, 2007). Although so far confined to Africa and the Arabian Peninsula, RVFV has the potential to spread to other parts of the world, given the presence and changing distribution of competent vectors throughout Europe and the Americas (Chevalier *et al.*, *Euro. Surveill.* 15:19506, 2010; Elliott, *Clin. Microbiol. Infect.* 15:510-517, 2009; Turell *et al.*, *J. Med. Entomol.* 47:884-889, 2010).

Livestock (*e.g.*, sheep, cattle and goats) are particularly susceptible to RVFV disease; outbreaks are characterized by widespread abortion storms and neonatal mortality approaching 100% (Swanepoel and Coetzer, Rift Valley fever, p. 688-717. *In* J. A. W. Coetzer, G. R. Thomson, and R. C. Tustin (ed.), Infectious Diseases of Livestock with Special Reference to South Africa. Oxford University Press, Cape Town, 1994). Infection in adult animals is associated with lower mortality, but the loss of a large proportion of young animals has serious economic impact. Humans usually become infected after handling aborted materials or other infected animal tissues, or through the bite of an infected mosquito. Although generally self-limiting, human infections can manifest as a serious febrile illness marked by myalgia, arthralgia, photophobia, and severe headache. In a small proportion of individuals, RVFV disease can progress to hepatitis, delayed-onset encephalitis or retinitis, or a hemorrhagic

syndrome. Case fatality in severely afflicted individuals can be as high as 20% (Bird *et al.*, *J. Am. Vet. Med. Assoc.* 234:883-893, 2009). Currently, there are no specific treatments for RVFV infection recommended in humans or other animals.

RVFV has a tripartite negative-sense single-stranded RNA genome. The large (L) segment encodes the viral polymerase. The medium (M) segment encodes the structural glycoproteins, Gn and Gc, as well as non-structural proteins, including a 78 kD protein and NSm, a virulence factor suggested to function by inhibiting apoptosis (Won *et al.*, *J. Virol.* 81:13335-13345, 2007). The ambisense small (S) segment encodes, in the viral sense, the nucleoprotein (NP) that is required for RNA synthesis, and the non-structural NSs protein in the opposite orientation. NSs is the major RVFV virulence factor and functions to inhibit the host immune response (Bouloy *et al.*, *J. Virol.* 75:1371-1377, 2001) by generalized downregulation of host transcription (Billecocq *et al.*, *J. Virol.* 78:9798-9806, 2004; Le May *et al.*, *Cell* 116:541-550, 2004), post-transcriptional degradation of protein kinase R (PKR) (Habjan *et al.*, *J. Virol.* 83:4365-4375, 2009; Ikegami *et al.*, *Ann. N. Y. Acad. Sci.* 1171 Suppl 1:E75-85, 2009), and repression of the interferon-ß (IFN- ß) promoter (Le May *et al.*, *PLoS Pathogens* 4:e13, 2008). Previous work has indicated the importance of both NSm (Bird *et al.*, *Virology* 362:10-15, 2007) and NSs (Barnett *et al.*, *J. Virol.* 84:5975-5985, 2010; Vialat *et al.*, *J. Virol.* 74:1538-1543, 2010) in determining virulence *in vivo*.

The impact of RVFV disease throughout Africa and the Arabian Peninsula, and the potential for viral spread elsewhere, provide strong incentives to develop safe, efficacious, and affordable vaccines. Examples of recently developed candidate vaccines include DNA-vectored (Bhardwaj *et al.*, *PLoS Negl Trop Dis* 4:e725, 2010; Lagerqvist *et al.*, *Virol. J.* 6:6, 2009; Lorenzo *et al.*, *Vaccine* 28:2937-2944, 2010), virus-like particle (VLP) (de Boer *et al.*, *Vaccine* 28:2330-2339, 2010; Mandell *et al.*, *Virology* 397:187-198, 2010; Näslund *et al.*, *Virology* 385:409-415, 2009), replicon particle (Kortekaas *et al.*, *J. Virol.* 85:12622-12630, 2011), and live attenuated vaccines (Bird *et al.*, *J. Virol.* 82:2681-2691, 2008; Dungu *et al.*, *Vaccine* 28:4581-4587, 2010). VLP candidates show promise and remarkable safety, but generally require adjuvant and/or multiple immunizations for complete protection. In comparison, live attenuated vaccines are highly immunogenic, presumably due to viral replication in an immunized host. However, early live attenuated vaccines (Smithburn, MP-12) were associated with teratogenesis and abortion in livestock (Botros *et al.*, *J. Med. Virol.* 78:787-791, 2006; Hunter *et al.*, *Onderstepoort J. Vet. Res.* 69:95-98, 2002). More recently, a naturally occurring RVFV mutant (Dungu *et al.*, *Vaccine* 28:4581-4587, 2010; von Teichman *et al.*, *Vaccine*

29:5771-5777, 2011) and a reverse-genetics derived candidate (Bird *et al.*, *J. Virol.* 85:12901-12909, 2011) have been shown to be both safe and efficacious in livestock.

SUMMARY

Described herein is a replication-competent but non-spreading Rift Valley fever virus, referred to herein as Rift Valley fever virus replicon particles (VRP_{RVF}). VRP_{RVF} are capable of undergoing a single round of infection, but are unable to spread beyond the first infected cell due to the lack of the M genome segment encoding the viral glycoproteins Gn and Gc. It is disclosed herein that immunization with VRP_{RVF} is both safe and efficacious, resulting in a robust immune response that is protective against wild-type RVFV challenge within 24 hours of immunization.

Provided herein are VRP_{RVF} comprising RVF virus Gn and Gc glycoproteins, polymerase protein and nucleoprotein, and RVF virus L and S genome segments, wherein the VRP_{RVF} does not contain an RVF virus M segment or any nucleic acid molecule encoding the Gn and Gc glycoproteins.

Also provided is a method of producing VRP_{RVF} by transfecting a host cell with a plasmid containing an antigenomic copy of an RVF virus L segment; a plasmid containing an antigenomic copy of an RVF virus S segment; and a plasmid encoding RVF virus Gn and Gc glycoproteins; and culturing the cells for a period of time sufficient to produce VRP_{RVF}. VRP_{RVF} produced by such a method are also provided.

Immunogenic compositions comprising the VRP_{RVF} disclosed herein and a pharmaceutically acceptable carrier are also provided by the present disclosure.

Also provided is a method of eliciting an immune response against RVF virus in a subject by administering to the subject an effective amount of a VRP_{RVF} or an immunogenic composition disclosed herein.

Further provided is a method of immunizing a subject against RVF virus infection by administering to the subject an effective amount of a VRP_{RVF} or immunogenic composition disclosed herein.

Methods of using VRP_{RVF} as an adjuvant to enhance immunogenicity of a heterologous vaccine, or as a vaccine vector to elicit an immune response against a heterologous protein, such as a viral antigen or tumor antigen, are further provided by the present disclosure. A method of treating cancer in a subject by administration of the VRP_{RVF} disclosed herein is also provided.

Also provided is a method of differentiating a subject administered VRP_{RVF} from a subject naturally infected with RVF virus by detecting the presence or absence of anti-nucleoprotein antibodies, and either anti-NSs or anti-NSm antibodies.

The foregoing and other objects, features, and advantages of the invention will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1B: Reverse genetics-derived VRP_{RVF} are morphologically indistinguishable from wild-type RVFV. (FIG. 1A) Schematic of Rift Valley fever virus (RVFV) genome (top) and the reverse genetics system as used for virus replicon particle (VRP_{RVF}) production (bottom). (FIG. 1B) Negative stain electron microscopy images demonstrating the morphological similarity of RVFV particles (left) and VRP_{RVF} particles (right).

FIGS. 2A-2B: VRP_{RVF} cannot spread beyond initially infected cells and pass a stringent safety test. (FIG. 2A) VRP_{RVF} (top) and RVFV (bottom) were used to infect VeroE6 monolayers. Over the course of 5 days (left to right), VRP_{RVF} remain confined to the initially infected cells, while RVFV gradually spreads throughout the entire monolayer. Limit of detection: 1 fluorescent focus unit (FFU). (FIG. 2B) Survival curves of newborn suckling mice inoculated intracranially with VRP_{RVF} or RVFV, or mock-inoculated.

FIGS. 3A-3D: Single dose immunization of replicating VRP_{RVF} confers complete protection. (FIG. 3A) Survival curves of C57BL/6 mice in the VRP_{RVF} dose titration experiment. Mice were immunized with VRP_{RVF} and challenged 28 days later with virulent RVFV. (FIG. 3B) Survival curves of mice immunized with VRP_{RVF} or nr-VRP_{RVF} and challenged 28 days later with virulent RVFV. Survival curves are significantly different ($p = 0.005$). (FIG. 3C) Total IgG titers of mice immunized with VRP_{RVF} or nr-VRP_{RVF} 28 days prior to serum collection. Limit of detection 1:100. (FIG. 3D) Virus neutralization titers of mice immunized with VRP_{RVF} or nr-VRP_{RVF} 28 days prior to serum collection. Limit of detection 1:10.

FIGS. 4A-4C: VRP_{RVF} immunization is protective as early as 24 hours post-immunization. Shown are survival curves from early protection studies. C57BL/6 mice were immunized with VRP_{RVF} or nr-VRP_{RVF} and challenged with virulent virus (FIG. 4A) 24 hours,

(FIG. 4B) 48 hours, or (FIG. 4C) 72 hours later. Survival curves were significantly different at 48 hours ($p = 0.008$) and 72 hours ($p = 0.025$).

FIG. 5: VRP_{RVF} stimulate significantly higher antiviral gene expression than non-replicating counterparts. The graphs show fold-change (over mock-immunized) of relevant antiviral cytokine gene expression 12-24 hours post-immunization with VRP_{RVF} (left bar in each pair) or nr-VRP_{RVF} (right bar in each pair). Error bars show standard deviation of fold change. Asterisks indicate significant differences between VRP_{RVF} and nr-VRP_{RVF} (*$p < 0.05$; **$p<0.01$).

FIG. 6: VRP_{RVF} stimulate significantly higher chemokine gene expression than non-replicating counterparts. The graphs show fold-change (over mock-immunized) of relevant chemokine gene expression 12-24 hours post-immunization with VRP_{RVF} (left bar in each pair) or nr-VRP_{RVF} (right bar in each pair). Error bars show standard deviation of fold change. Asterisk indicates significant differences between VRP_{RVF} and nr-VRP_{RVF} (*$p < 0.05$).

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. The Sequence Listing is submitted as an ASCII text file, created on June 11, 2013, 15.2 KB, which is incorporated by reference herein. In the accompanying sequence listing:

SEQ ID NO: 1 is the nucleotide sequence of the antigenomic L segment of the RVFL plasmid.

SEQ ID NO: 2 is the nucleotide sequence of the antigenomic S segment of the RVFS-ΔNSs:GFP plasmid.

SEQ ID NO: 3 is the nucleotide sequence encoding RVFV Gn-Gc from the pC-GnGc expression vector.

DETAILED DESCRIPTION

I. Abbreviations

DIVA	differentiation of naturally infected from vaccinated animals
DMEM	Dulbecco's modified Eagle's medium
dpi	days post-infection
ELISA	enzyme-linked immunosorbent assay
FBS	fetal bovine serum

FFU	fluorescent focus unit
GFP	green fluorescent protein
hpi	hours post-infection
ic	intracranial
IFA	indirect fluorescent-antibody assay
IFN	interferon
L	large genome segment of RVFV
LD50	50% lethal dose
M	medium genome segment of RVFV
NP	nucleoprotein
nr-VRP_{RVF}	non-replicating VRP_{RVF}
NS	non-structural
ORF	open reading frame
PBS	phosphate buffered saline
pfu	plaque forming unit
PKR	protein kinase R
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
S	small genome segment of RVFV
sc	subcutaneous
SM	suckling mice
TCID	50% tissue culture infectious dose
VLP	virus-like particle
VNT_{100}	100% virus neutralization titer
VRP	virus replicon particle
VRP_{RVF}	virus replicon particle of Rift Valley fever

II. Terms and Methods

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

Adjuvant: A substance or vehicle that non-specifically enhances the immune response to an antigen. Adjuvants can include a suspension of minerals (alum, aluminum hydroxide, or phosphate) on which antigen is adsorbed; or water-in-oil emulsion in which antigen solution is emulsified in mineral oil (for example, Freund's incomplete adjuvant), sometimes with the inclusion of killed mycobacteria (Freund's complete adjuvant) to further enhance antigenicity. Immunostimulatory oligonucleotides (such as those including a CpG motif) can also be used as

adjuvants (for example, see U.S. Patent Nos. 6,194,388; 6,207,646; 6,214,806; 6,218,371; 6,239,116; 6,339,068; 6,406,705; and 6,429,199). Adjuvants also include biological molecules, such as costimulatory molecules. Exemplary biological adjuvants include IL-2, RANTES, GM-CSF, TNF-α, IFN-γ, G-CSF, LFA-3, CD72, B7-1, B7-2, OX-40L and 41 BBL. In some embodiments, VRP_{RVF} functions as an adjuvant to enhance the immunogenicity of a heterologous vaccine.

Administer: As used herein, administering a composition to a subject means to give, apply or bring the composition into contact with the subject. Administration can be accomplished by any of a number of routes, such as, for example, topical, oral, subcutaneous, intramuscular, intraperitoneal, intravenous, intrathecal and intramuscular.

Ambisense: Refers to a genome or genomic segments having both positive sense and negative sense portions. For example, the S segment of a *Phlebovirus*, such as Rift Valley fever virus, is ambisense, encoding nucleoprotein in the negative sense and the non-structural protein NSs in the positive sense.

Animal: Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and bird. Mammals include, but are not limited to, humans, non-human primates, dogs, cats, horses, sheep, goats and cows. The term mammal includes both human and non-human mammals.

Antibody: An immunoglobulin molecule produced by B lymphoid cells with a specific amino acid sequence. Antibodies are evoked in humans or other animals by a specific antigen (immunogen). Antibodies are characterized by reacting specifically with the antigen in some demonstrable way, antibody and antigen each being defined in terms of the other. "Eliciting an antibody response" refers to the ability of an antigen or other molecule to induce the production of antibodies.

Antigen: A compound, composition, or substance that can stimulate the production of antibodies or a T-cell response in an animal, including compositions that are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous immunogens. In one embodiment, an antigen is a RVF virus antigen.

Antigenomic: As used herein, "antigenomic" refers to a genomic segment of a *Phlebovirus* (such as RVF virus) in the orientation opposite to the viral genome. For example, *Phleboviruses* are negative-sense RNA viruses. Thus, "antigenomic" refers to the positive-sense

orientation (or virus complementary sense), while "genomic" refers to the negative-sense orientation of a gene segment.

Attenuated: In the context of a live virus, the virus is attenuated if its ability to infect a cell or subject and/or its ability to produce disease is reduced (for example, eliminated) compared to a wild-type virus. Typically, an attenuated virus retains at least some capacity to elicit an immune response following administration to an immunocompetent subject. In some cases, an attenuated virus is capable of eliciting a protective immune response without causing any signs or symptoms of infection.

Biological sample: A sample obtained from a subject (such as a human or veterinary subject). Biological samples include, for example, fluid, cell and/or tissue samples. In some embodiments herein, the biological sample is a fluid sample. Fluid sample include, but are not limited to, serum, blood, plasma, urine, feces, saliva, cerebral spinal fluid (CSF) or other bodily fluid. Biological samples can also refer to cells or tissue samples, such as biopsy samples or tissue sections.

Cancer, tumor, neoplasia and malignancy: A neoplasm is an abnormal growth of tissue or cells that results from excessive cell division. Neoplastic growth can produce a tumor. The amount of a tumor in an individual is the "tumor burden" which can be measured as the number, volume, or weight of the tumor. A tumor that does not metastasize is referred to as "benign." A tumor that invades the surrounding tissue and/or can metastasize is referred to as "malignant." Malignant tumors are also referred to as "cancer."

Hematologic cancers are cancers of the blood or bone marrow. Examples of hematological (or hematogenous) cancers include leukemias, including acute leukemias (such as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma (indolent and high grade forms), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia. In some cases, lymphomas are considered solid tumors.

Solid tumors are abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumors can be benign or malignant. Different types of solid tumors are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). Examples of solid tumors, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma,

liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, seminoma, bladder carcinoma, melanoma, and CNS tumors (such as a glioma (such as brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme) astrocytoma, CNS lymphoma, germinoma, medulloblastoma, Schwannoma craniopharyogioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, neuroblastoma, retinoblastoma and brain metastasis).

Contacting: Placement in direct physical association; includes both in solid and liquid form. "Contacting" is often used interchangeably with "exposed." In some cases, "contacting" includes transfecting, such as transfecting a nucleic acid molecule into a cell. In other examples, "contacting" refers to incubating a molecule (such as an antibody) with a biological sample.

Hepatitis delta virus ribozyme: A non-coding, catalytic RNA from the hepatitis delta virus. Ribozymes catalyze the hydrolysis of their own phosphodiester bonds or those of other RNA molecules.

Heterologous: As used herein a "heterologous protein" or "heterologous virus" is a protein or virus derived from a source *other than* Rift Valley fever virus.

Host cell: In the context of the present disclosure, a "host cell" is a cell of use with the RVF virus replicon system described herein. A suitable host cell is one that is capable of transfection with and expression of the plasmids of the RVF virus replicon system. In one embodiment, the host cell is a cell expressing the T7 polymerase, such as, but not limited to BSR-T7/5 cells (Buchholz *et al.*, *J. Virol.* 73(1):251-259, 1999).

Immune response: A response of a cell of the immune system, such as a B-cell, T-cell, macrophage or polymorphonucleocyte, to a stimulus such as an antigen. An immune response can include any cell of the body involved in a host defense response, including for example, an epithelial cell that secretes an interferon or a cytokine. An immune response includes, but is not limited to, an innate immune response or inflammation. As used herein, a protective immune response refers to an immune response that protects a subject from infection (prevents infection

or prevents the development of disease associated with infection). **Immunize:** To render a subject protected from an infectious disease, such as by vaccination.

Immunogen: A compound, composition, or substance which is capable, under appropriate conditions, of stimulating an immune response, such as the production of antibodies or a T-cell response in an animal, including compositions that are injected or absorbed into an animal. Accordingly, an "immunogenic protein" is a protein capable of stimulating an immune response in a subject, such as a human or animal subject. As used herein, an "immunogenic composition" is a composition comprising an immunogen.

Immunogenic composition: A composition useful for stimulating or eliciting a specific immune response (or immunogenic response) in a vertebrate. In some embodiments, the immunogenic composition includes RVF_{VRP}. In some embodiments, the immunogenic response is protective or provides protective immunity, in that it enables the subject to better resist infection with or disease progression from the pathogen against which the immunogenic composition is directed (*e.g.*, Rift Valley fever virus). One specific example of a type of immunogenic composition is a vaccine.

Immunomodulatory protein: Any protein capable of modifying or regulating one or more functions of the immune system. Cytokines and chemokines are two exemplary types of immunomodulatory proteins. Specific immunomodulatory proteins include, but are not limited to, IFN-α, IFN-β, IFN-γ and TNF-α.

Isolated: An "isolated" biological component (such as a nucleic acid, protein, virus or virus particle) has been substantially separated or purified away from other biological components (such as cell debris, or other proteins or nucleic acids). Biological components that have been "isolated" include those components purified by standard purification methods. The term also embraces recombinant nucleic acids, proteins, viruses or virus particles, as well as chemically synthesized nucleic acids or peptides.

Livestock: Domesticated animals reared in an agricultural setting as a source of food or to provide labor. The term "livestock" includes, but is not limited to, cattle, deer, donkeys, goats, horses, mules, rabbits and sheep.

ORF (open reading frame): A series of nucleotide triplets (codons) coding for amino acids without any termination codons. These sequences are usually translatable into a peptide.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the

promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

Pharmaceutically acceptable carrier: The pharmaceutically acceptable carriers (vehicles) useful in this disclosure are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of one or more therapeutic compounds or molecules, such as one or more RVF virus replicon particles, and additional pharmaceutical agents.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Plasmid: A circular nucleic acid molecule capable of autonomous replication in a host cell.

Polypeptide: A polymer in which the monomers are amino acid residues which are joined together through amide bonds. When the amino acids are alpha-amino acids, either the L-optical isomer or the D-optical isomer can be used. The terms "polypeptide" or "protein" as used herein are intended to encompass any amino acid sequence and include modified sequences such as glycoproteins. The term "polypeptide" is specifically intended to cover naturally occurring proteins, as well as those which are recombinantly or synthetically produced. The term "residue" or "amino acid residue" includes reference to an amino acid that is incorporated into a protein, polypeptide, or peptide.

Conservative amino acid substitutions are those substitutions that, when made, least interfere with the properties of the original protein, that is, the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. Examples of conservative substitutions are shown below.

Original Residue	Conservative Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

The substitutions which in general are expected to produce the greatest changes in protein properties will be non-conservative, for instance changes in which (a) a hydrophilic residue, for example, seryl or threonyl, is substituted for (or by) a hydrophobic residue, for example, leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, for example, lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, for example, glutamyl or aspartyl; or (d) a residue having a bulky side chain, for example, phenylalanine, is substituted for (or by) one not having a side chain, for example, glycine.

Preventing, treating or ameliorating a disease: "Preventing" a disease refers to inhibiting the full development of a disease. "Treating" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. "Ameliorating" refers to the reduction in the number or severity of signs or symptoms of a disease.

Promoter: A promoter is an array of nucleic acid control sequences which direct transcription of a nucleic acid. A promoter includes necessary nucleic acid sequences near the start site of transcription. A promoter also optionally includes distal enhancer or repressor

elements. A "constitutive promoter" is a promoter that is continuously active and is not subject to regulation by external signals or molecules. In contrast, the activity of an "inducible promoter" is regulated by an external signal or molecule (for example, a transcription factor). In some embodiments herein, the promoter is a T7 promoter (from bacteriophage T7).

Purified: The term "purified" does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified peptide, protein, virus, virus particle or other active compound is one that is isolated in whole or in part from naturally associated proteins and other contaminants. In certain embodiments, the term "substantially purified" refers to a peptide, protein, virus, virus particle or other active compound that has been isolated from a cell, cell culture medium, or other crude preparation and subjected to fractionation to remove various components of the initial preparation, such as proteins, cellular debris, and other components.

Recombinant: A recombinant nucleic acid, protein, virus or virus particle is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques. In some embodiments, a recombinant RVF virus particle is generated using the VRP system described herein.

Reporter gene: A reporter gene is a gene operably linked to another gene or nucleic acid sequence of interest (such as a promoter sequence). Reporter genes are used to determine whether the gene or nucleic acid of interest is expressed in a cell or has been activated in a cell. Reporter genes typically have easily identifiable characteristics, such as fluorescence, or easily assayed products, such as an enzyme. Reporter genes can also confer antibiotic resistance to a host cell or tissue. Reporter genes include, for example, GFP (or eGFP) or other fluorescence genes, luciferase, β-galactosidase and alkaline phosphatase.

Reverse genetics: Refers to the process of introducing mutations (such as deletions, insertions or point mutations) into the genome of an organism or virus, such as to determine the phenotypic effect of the mutation or to engineer a specific mutation or alteration.

Rift Valley fever (RVF) virus (RVFV): A virus belonging to the family *Bunyaviridae* and genus *Phlebovirus*. RVF virus has a single-stranded, negative-sense genome composed of three genome segments, S, M and L. The S segment is an ambisense genome segment, meaning it encodes proteins in both the positive-sense and negative-sense orientations. The RVF virus genome encodes both structural and non-structural proteins. A "structural" protein is a protein found in the virus particle, whereas a "non-structural" protein is only expressed in a virus-infected cell. RVF virus

structural proteins include nucleoprotein (NP or N, used interchangeably), two glycoproteins (Gn and Gc) and the viral RNA-dependent RNA polymerase (L protein). Non-structural RVF virus proteins include NSs, NSm and the NSm+Gn fusion protein. As used herein, a "full-length" RVF virus genome segment is one containing no deletions. Full-length genome segments can contain mutations or substitutions, but retain the same length as the wild-type virus. A "complete deletion" of an ORF of a RVF virus genome segment means either every nucleotide encoding the ORF is deleted from genome segment, or nearly every nucleotide encoding the ORF is deleted such that no proteins are translated from the ORF. Thus, a "complete deletion" includes genome segments retaining up to ten nucleotides encoding the ORF, such as one, two, three, four, five, six, seven, eight, nine or ten nucleotides. A number of RVF virus strains have been identified. In one embodiment described herein, the RVF virus strain is ZH501.

Sequence identity: The similarity between amino acid or nucleic acid sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are. Homologs or variants of a given gene or protein will possess a relatively high degree of sequence identity when aligned using standard methods.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988; Higgins and Sharp, *Gene* 73:237-244, 1988; Higgins and Sharp, *CABIOS* 5:151-153, 1989; Corpet *et al.*, *Nucleic Acids Research* 16:10881-10890, 1988; Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988; and Altschul *et al.*, *Nature Genet.* 6:119-129, 1994.

The NCBI Basic Local Alignment Search Tool (BLAST™) (Altschul *et al.*, *J. Mol. Biol.* 215:403-410, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx.

Subject: Living multi-cellular vertebrate organisms, a category that includes both human and non-human mammals. Subjects include, but are not limited to veterinary subjects, including livestock such as cows, goats, sheep, and non-human primates, and human subjects.

Therapeutically effective amount or effective amount: A quantity of a specified agent sufficient to achieve a desired effect in a subject being treated with that agent. For example, this may be the amount of VRP_{RVF} useful for eliciting an immune response in a subject and/or for

preventing or inhibiting infection by RVF virus. Ideally, in the context of the present disclosure, a therapeutically effective amount of a VRP_{RVF} (or an immunogenic composition thereof) is an amount sufficient to increase resistance to, prevent, ameliorate, and/or treat infection caused by RVF virus in a subject without causing a substantial cytotoxic effect in the subject. The effective amount of VRP_{RVF} useful for increasing resistance to, preventing, ameliorating, and/or treating infection in a subject will be dependent on, for example, the subject being treated, the manner of administration of the therapeutic composition and other factors.

Transformed: A transformed cell is a cell into which has been introduced a nucleic acid molecule by molecular biology techniques. As used herein, the term transformation encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

Tumor antigen: An antigen that can stimulate tumor-specific T-cell immune responses. Exemplary tumor antigens include, but are not limited to, RAGE-1, tyrosinase, MAGE-1, MAGE-2, NY-ESO-1, Melan-A/MART-1, glycoprotein (gp) 75, gp100, beta-catenin, PRAME, MUM-1, WT-1, CEA, and PR-1. Additional tumor antigens are known in the art (for example see Novellino *et al.*, *Cancer Immunol Immunother* 54(3):187-207, 2005).

Vaccine: A preparation of immunogenic material capable of stimulating an immune response, administered for the prevention, amelioration, or treatment of infectious or other types of disease. The immunogenic material may include attenuated or killed microorganisms (such as attenuated viruses), or antigenic proteins, peptides or DNA derived from them. Vaccines may elicit both prophylactic (preventative) and therapeutic responses. Methods of administration vary according to the vaccine, but may include inoculation, ingestion, inhalation or other forms of administration. Inoculations can be delivered by any of a number of routes, including parenteral, such as intravenous, subcutaneous or intramuscular. Vaccines may be administered with an adjuvant to boost the immune response.

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication (DNA sequences that participate in initiating DNA synthesis). A vector may also include one or more selectable marker genes and other genetic elements known in the art.

Virus replicon particle (VRP): In the context of the present disclosure, a RVF virus replicon particle (VRP_{RVF}) is a viral particle that contains the RVF virus Gn, Gc, polymerase

and nucleoproteins, and packaged within the particle are the RVF virus S and L genome segments. VRP_{RVF} do not contain the RVF virus M segment or any nucleic acid molecule encoding the Gn and Gc glycoproteins. Thus, VRP_{RVF} are capable of a single round of infection, including viral RNA expression and *de novo* viral protein synthesis, but cannot form new particles (due to the lack of Gn and Gc) and therefore cannot spread to neighboring cells.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Hence "comprising A or B" means including A, or B, or A and B. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

III. Introduction

Rift Valley fever virus (RVFV) causes outbreaks of severe disease in people and livestock throughout Africa and the Arabian Peninsula. The potential for RVFV introduction outside this endemic area highlights the need for fast-acting, safe, and efficacious vaccines. Disclosed herein is a robust system for the reverse genetics generation of a RVF virus replicon particle (VRP_{RVF}) vaccine candidate. Using a mouse model, it is shown that VRP_{RVF} immunization provides the optimal balance of safety and single dose robust efficacy. VRP_{RVF} can actively synthesize viral RNA and proteins, but lack structural glycoprotein genes, preventing spread within immunized individuals and reducing the risk of vaccine-induced pathogenicity.

VRP_{RVF} were completely safe following intracranial inoculation of suckling mice, a stringent test of vaccine safety. Although highly attenuated, single dose VRP_{RVF} subcutaneous immunization completely protected mice against a virulent RVFV challenge 100,000-fold

greater than the LD_{50}. Robust protection from lethal challenge was observed by 24 hours post-vaccination, with 100% protection induced in as little as 96 hours. A single subcutaneous VRP_{RVF} immunization initiated a systemic antiviral state followed by an enhanced adaptive response. The data provided in the present disclosure contrast sharply with the much reduced survivability and immune responses observed among animals immunized with non-replicating viral particles, indicating that replication, even confined to the initially infected cells, contributes substantially to protective efficacy at early and late time points post-immunization.

IV. Overview of Several Embodiments

Disclosed herein is the development and characterization of RVF virus replicon particles (VRP_{RVF}). VRP_{RVF} are replication-competent but non-spreading virus particles. It is disclosed herein that VRP_{RVF} are a safe and rapidly efficacious immunogenic composition (such as a vaccine) for protection against RVFV infection. In particular embodiments, VRP_{RVF} contain full-gene deletions of the critical virulence factors NSs and NSm. As an additional safety measure, VRP_{RVF} do not carry the genes for structural glycoproteins, and are therefore unable to produce new particles from infected cells, preventing spread within the immunized host and eliminating the risk of vaccine-induced pathogenicity.

The data disclosed herein demonstrate that VRP_{RVF} immunization is both safe and efficacious against virulent RVFV challenge in a relevant animal model as early as 1 day after vaccination. Immunization with non-replicating VRP_{RVF} (nr-VRP_{RVF}) resulted in significantly lower survival following RVFV challenge at both early and late time points. Relative to mock and nr-VRP_{RVF}-immunized animals, VRP_{RVF} animals developed a stronger systemic antiviral and subsequent adaptive response following immunization, indicating that VRP_{RVF} RNA and protein synthesis, even when confined to the initially infected cells, are critical for stimulating robust immunity and subsequent protection.

The RVF virus replicon particles (VRP_{RVF}) disclosed herein include a virus particle that contains a RVF virus L genome segment and a RVF virus S genome segment, but does not contain a RVF virus M genome segment (or any nucleic acid that encodes the RVF virus Gn and Gc proteins). The protein components of the VRP_{RVF} include RVF virus Gn and Gc glycoproteins, polymerase protein and nucleoprotein.

Provided herein is a VRP_{RVF}, comprising (i) RVF virus Gn and Gc glycoproteins; (ii) RVF virus polymerase protein; (iii) RVF virus nucleoprotein; (iv) an RVF virus L genome

segment; and (v) an RVF virus S genome segment, wherein the VRP_{RVF} does not contain an RVF virus M segment or any nucleic acid molecule encoding the Gn and Gc glycoproteins.

In some embodiments, the S genome segment of the VRP_{RVF} comprises a deletion, such as a complete deletion, of the NSs open reading frame (ORF). In particular examples, the NSs ORF is replaced by an ORF encoding a heterologous protein, such as a reporter gene ORF. Any gene that produces a protein with a functional readout can be used as the reporter gene. Reporter genes include, but are not limited to genes encoding fluorescent proteins, antibiotic resistance or enzymes (*e.g.*, β-galactosidase or alkaline phosphatase). In some examples, the reporter gene is a GFP, such as enhanced GFP.

The VRP_{RVF} disclosed herein can also be used as vaccine vectors to elicit an immune response against a heterologous protein. Thus, in some examples in which the NSs ORF is replaced by an ORF encoding a heterologous protein, the heterologous protein comprises an immunogenic protein from a heterologous virus (any virus other than Rift Valley fever virus), or from any other infectious organism against which an immune response is desired. In some cases, the heterologous protein is a vaccine or a component of a vaccine for an infectious organism, such as a bacterial or viral organism. In yet other examples, the heterologous protein is a tumor antigen.

In some embodiments, the VRP_{RVF} does not contain an RVF virus NSm protein.

The RVF virus from which the RVF virus genome segments and proteins are derived can be any strain of RVF virus. Exemplary strains of RVF virus include, for example, ZH501, ZH548, MP-12, 35/74, SA75 and SPB 9800523. In specific examples, the RVF virus is RVF virus strain ZH501. In some examples, the RVF virus is not RVF virus strain 35/74.

The sequences of the S and L genome segments of the ZH501 strain are deposited under GenBank Accession No. DQ380149 and DQ375406, respectively. The nucleotide sequences of the S and L segments need not be 100% identical to a known RVF virus (such as ZH501) sequence. In some examples, the S and L segments are at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to a known or disclosed RVF virus S or L segment, such as the S and L segments of ZH501. In some embodiments, the RVF virus S and L segments are at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to an S or L segment disclosed in PCT Publication No. WO 2009/082647 or U.S. Patent Application Publication No. 2011-0123567, which are herein incorporated by reference in their entirety.

Further provided herein is a method of producing VRP_{RVF}, comprising (i) transfecting a host cell with a plasmid containing an antigenomic copy of an RVF virus L segment; a plasmid containing an antigenomic copy of an RVF virus S segment; and a plasmid encoding RVF virus Gn and Gc glycoproteins; and (ii) culturing the cells for a period of time sufficient to produce VRP_{RVF}. In some embodiments, the method further includes collecting the VRP_{RVF} from the cell culture supernatant. In some examples, the method further includes isolating or purifying the VRP_{RVF} from the cell culture supernatant.

In some embodiments of the method, the S genome segment comprises a deletion, such as a complete deletion, of the NSs ORF. In some examples, the NSs ORF is replaced by a reporter gene ORF. In particular examples, the reporter gene is a GFP, such as enhanced GFP. However, the reporter gene can be any gene that produces a protein with a functional readout, including genes that encode a fluorescent protein, antibiotic resistance or an enzyme.

In some embodiments, none of the plasmids used for producing the VRP_{RVF} encode an RVF virus NSm protein.

In some embodiments, the plasmid containing an antigenomic copy of the S segment and the plasmid containing an antigenomic copy of the L segment further comprise a T7 promoter and a hepatitis delta virus ribozyme.

In some embodiments, the host cells express T7 polymerase. In particular examples, the host cells are BSR-T7/5 cells (Buchholz *et al.*, *J. Virol.* 73(1):251-259, 1999).

The RVF virus genome segments and proteins used with the disclosed methods can be derived from any strain of RVF virus, including, but not limited to ZH501, ZH548, MP-12, 35/74, SA75 and SPB 9800523. In specific examples, the RVF virus is RVF virus strain ZH501. In some examples, the RVF virus is not RVF virus strain 35/74.

In specific examples, the plasmid containing an antigenomic copy of the L segment comprises a nucleotide sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 1. In one non-limiting embodiment, the nucleotide sequence of the plasmid containing an antigenomic copy of the L segment comprises SEQ ID NO: 1.

In other specific examples, the plasmid containing an antigenomic copy of the S segment comprises a nucleotide sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 2. In one non-limiting example, the nucleotide sequence of the plasmid containing an antigenomic copy of the S segment comprises SEQ ID NO: 2.

In other specific examples, the plasmid encoding RVF virus Gn and Gc glycoproteins comprises a nucleotide sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 3. In one non-limiting example, the nucleotide sequence of the plasmid encoding RVF virus Gn and Gc glycoproteins comprises SEQ ID NO: 3.

Also provided are VRP_{RVF} produced according to a method disclosed herein.

Further provided are immunogenic compositions comprising the VRP_{RVF} disclosed herein and a pharmaceutically acceptable carrier. Suitable pharmaceutical carriers are described herein and are well known in the art. The pharmaceutical carrier used depends on a variety of factors, including the route of administration. In one embodiment, the immunogenic composition further comprises an adjuvant. The adjuvant can be any substance that improves the immune response to the VRP_{RVF}.

Also provided is the use of VRP_{RVF} as an adjuvant to enhance the immunogenicity of a heterologous protein, such as a heterologous vaccine. Thus, in some embodiments, the immunogenic compositions provided herein further include an immunogenic protein from a heterologous virus (any virus other than Rift Valley fever virus), or any other infectious microorganism.

Also provided is a method of eliciting an immune response against RVF virus in a subject. In some embodiments, the method includes administering to the subject an effective amount of VRP_{RVF} or an immunogenic composition disclosed herein. Further provided is a method of immunizing a subject against RVF virus infection by administering to the subject an effective amount of VRP_{RVF} or an immunogenic composition disclosed herein. In some embodiments, the subject is a human. In other embodiments, the subject is livestock, such as, for example, sheep or cattle.

In some embodiments of the methods, the VRP_{RVF} or immunogenic composition is administered in a single dose. In another embodiment, the VRP_{RVF} or immunogenic composition is administered in multiple doses, such as two, three or four doses. When administered in multiple doses, the time period between doses can vary. In some cases, the time period is days, weeks or months. The VRP_{RVF} or immunogenic composition can be administered using any suitable route of administration. In some embodiments, the VRP_{RVF} or immunogenic composition is administered intravenously, intramuscularly or subcutaneously.

A suitable does of VRP_{RVF} (or immunogenic composition comprising the VRP_{RVF}) can be selected by a skilled practitioner. In some embodiments, the VRP_{RVF} is administered at a

dose of about 10^2 to about 10^6 $TCID_{50}$. In some examples, the VRP_{RVF} is administered at a dose of about 10^4 to about 10^5 $TCID_{50}$. In particular examples, the VRP_{RVF} is administered at a dose of about 10^2, about 10^3, about 10^4, about 10^5 or about 10^6 $TCID_{50}$.

Further provided is a method of enhancing an immune response against an immunogenic protein, such as an immunogenic viral protein, in a subject by administering the immunogenic protein to the subject and further administering VRP_{RVF}.

Also provided is a method of eliciting an immune response against a heterologous protein in a subject, by administering to the subject VRP_{RVF} in which the NSs ORF of the S genome segment is replaced by an ORF encoding the heterologous protein. In some embodiments, the heterologous protein is a protein from an infectious organism, such as a heterologous virus (any virus other than Rift Valley fever virus). In other embodiments, the heterologous protein is a tumor antigen.

Further provided is a method of treating cancer in a subject by selecting a subject with cancer and administering to the subject a therapeutically effective amount of the VRP_{RVF} disclosed herein. For example, the VRP_{RVF} can be administered systemically, such as intravenously, or can be administered directly into a tumor to promote destruction of tumor cells. In some embodiments, the NSs ORF of the S genome segment of the VRP_{RVF} is replaced by an ORF encoding a tumor antigen, or other biologically active immunomodulatory protein, such as IFN-α, IFN-β, IFN-γ, TNF-α or the like. In some embodiments, the subject is further administered an anti-cancer agent and/or other therapeutic treatment for cancer, such as surgical removal of the tumor of a portion thereof. Exemplary anti-cancer agents include, but are not limited to, chemotherapeutic agents, such as, for example, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, anti-survival agents, biological response modifiers, anti-hormones (*e.g.* anti-androgens) and anti-angiogenesis agents. Other anti-cancer treatments include radiation therapy and antibodies that specifically target cancer cells.

Also provided is a method of differentiating a subject administered the VRP_{RVF} disclosed herein from a subject naturally infected with RVF virus. In some embodiments, the method includes obtaining a biological sample from the subject; and detecting the presence or absence of anti-nucleoprotein antibodies and either anti-NSm or anti-NSs antibodies in the sample. The presence of anti-nucleoprotein and anti-NSm and/or anti-NSs antibodies in the sample indicates that the subject was naturally infected with RVF virus. The presence of anti-nucleoprotein

antibodies and the absence of anti-NSm and/or anti-NSs antibodies in the sample indicates that the subject was administered the VRP_{RVF}.

In some embodiments, the biological sample is a bodily fluid sample. In particular examples, the bodily fluid sample is a blood or serum sample.

In some embodiments of the differential diagnosis method, the subject is human. In other embodiments, the subject is livestock.

V. Administration of Rift Valley Fever Virus Replicon Particles (VRP_{RFV})

VRP_{RFV}, or immunogenic compositions thereof, can be administered to a subject by any of the routes normally used for introducing virus or virus particles into a subject. Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, parenteral, intravenous, subcutaneous, vaginal, rectal, intranasal, inhalation or oral. Parenteral administration, such as subcutaneous, intravenous or intramuscular administration, is generally achieved by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Administration can be systemic or local.

Immunogenic compositions are administered in any suitable manner, such as with pharmaceutically acceptable carriers. Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions of the present disclosure.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base-addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

Administration can be accomplished by single or multiple doses. The dose administered to a subject in the context of the present disclosure should be sufficient to induce a beneficial therapeutic response in a subject over time, or to inhibit or prevent RVF virus infection. The dose required will vary from subject to subject depending on the species, age, weight and general condition of the subject, the severity of the infection being treated, the particular immunogenic composition being used and its mode of administration. An appropriate dose can be determined by one of ordinary skill in the art using only routine experimentation.

Provided herein are pharmaceutical compositions (also referred to as immunogenic compositions) which include a therapeutically effective amount of the VRP_{RFV} alone or in combination with a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition can be sterile, and the formulation suits the mode of administration. The composition can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, and magnesium carbonate. Any of the common pharmaceutical carriers, such as sterile saline solution or sesame oil, can be used. The medium can also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. Other media that can be used with the compositions and methods provided herein are normal saline and sesame oil.

The VRP_{RFV} described herein can be administered alone or in combination with other therapeutic agents to enhance antigenicity. For example, the VRP_{RFV} can be administered with an adjuvant, such as Freund incomplete adjuvant or Freund's complete adjuvant.

Optionally, one or more cytokines, such as IL-2, IL-6, IL-12, RANTES, GM-CSF, TNF-α, or IFN-γ, one or more growth factors, such as GM-CSF or G-CSF; one or more molecules such as OX-40L or 41 BBL, or combinations of these molecules, can be used as biological adjuvants (see, for example, Salgaller *et al.*, 1998, *J. Surg. Oncol.* 68(2):122-38; Lotze *et al.*, 2000, *Cancer J. Sci. Am.* 6(Suppl 1):S61-6; Cao *et al.,* 1998, *Stem Cells* 16(Suppl 1):251-60; Kuiper *et al.,* 2000, *Adv. Exp. Med. Biol.* 465:381-90). These molecules can be administered systemically (or locally) to the host.

VI. Use of VRP_{RVF} and Immunogenic Compositions Thereof

The VRP_{RVF} and immunogenic compositions comprising VRP_{RVF} disclosed herein can be used, for example, to elicit an immune response in a subject or to immunize a subject against RVF virus. In some embodiments, the immunogenic compositions disclosed herein are used as vaccines to prevent or inhibit infection by RVF virus. The disclosed VRP_{RVF} and immunogenic compositions can be used for example, to prevent or inhibit RVF infection in humans or in livestock, such as cattle, sheep or goats.

The VRP_{RVF} and immunogenic compositions disclosed herein can be targeted towards veterinary medical use, and thus indirectly prevent human RVF disease; however, the candidate vaccines can also provide effective prophylactic protection for humans, such as those in high risk occupational settings, or in recognized risk groups following natural or intentional introduction of RVF virus into previously unaffected areas.

Efforts to prevent RVF virus infection via vaccination began shortly after the first isolation of the virus in 1931 (Findlay and Daubney, *Lancet* ii:1350-1351, 1931). These earliest vaccines (MacKenzie, *J. Pathol. Bacterol.* 40:65-73, 1935) and several that followed, including the currently available TSI-GSD-200 preparation, relied on formalin inactivation of live wild-type virus (Pittman *et al.*, *Vaccine* 18:181-189, 1999; Randall *et al.*, *J. Immunol.* 89:660-671, 1962). While capable of eliciting protective immune responses among livestock and humans, these inactivated vaccines typically require a series of 2 or 3 initial inoculations, followed by regular booster vaccinations to achieve and maintain protection (Pittman *et al.*, *Vaccine* 18:181-189, 1999; Swanepoel *et al.*, "Rift Valley fever" *in* Infectious Diseases of livestock with special reference to South Africa, pages 688-717, Oxford university Press, Cape Town). However,

multiple dosing and annual vaccination regimens are logistically difficult to implement and expensive to maintain, and thus are of limited practical value in resource-poor settings, especially in regard to control of RVF virus infection in livestock in enzootic settings. In addition, there have been problems in the past with quality control and "inactivated" vaccines causing disease.

In an effort to eliminate the necessity of booster inoculations, several live-attenuated vaccine candidates were developed for RVF virus with some, such as the Smithburn neurotropic strain, being employed in Africa. These vaccine candidates have relied upon the random introduction of attenuating mutations via serial passage in suckling mouse brain or tissue culture, *in vitro* passage in the presence of chemical mutagens, such as 5-flurouracil, or as naturally occurring virus isolates (such as the Smithburn neurotropic strain, the Kenyan-IB8 strains, MP-12, or the Clone 13 isolate) (Caplen *et al.*, *J. Gen. Virol.* 66:2271-2277, 1985; Coackley, *J. Pathol. Bacteriol.* 89:123-131, 1965; Moussa *et al.*, *Am. J. Trop. Med. Hyg.* 35:660-662, 1986; Muller *et al.*, *Am J. Trop. Med. Hyg.* 53:405-411, 1995; Rossi and Turell, *J. Gen. Virol.* 69:817-823, 1988; Smithburn, *Br. J. Exp. Pathol.* 30:1-16, 1949).

Due to the technical limitations of these procedures, and the lack of complete genome sequence for many of the historically derived RVF virus vaccines, the exact underlying molecular mechanisms of attenuation for many of these live-attenuated RVF virus vaccines is either unknown (Smithburn neurotropic strain or Kenyan-IB8) or reliant on the combinatorial effects of multiple nucleotide or amino acid substitutions (MP-12) (Saluzzo and Smith, *Vaccine* 8:369-375, 1990; Takehara *et al.*, *Virology* 169:452-457, 1989). Experimental and field experience with existing live-attenuated RVF virus vaccines demonstrated that in certain instances these vaccines retain the ability to cause teratogenic effects, abortion, and neural pathology in livestock or animal models. Thus, widespread use of these live-attenuated vaccines is problematic, especially in non-endemic areas, or during inter-epizootic/epidemic periods (Hunter *et al.*, *Onderstepoort J. Vet. Res.* 69:95-98, 2002; Morrill *et al.*, *Am. J. Vet. Res.* 58:1104-1109, 1997; Morrill *et al.*, *Am. J. Vet. Res.* 58:1110-1114, 1997; Morrill and Peters, *Vaccine* 21:2994-3002, 2003).

Several distinct disadvantages exist among live attenuated RNA virus vaccines prepared by the traditional techniques discussed above. Live-attenuated vaccines reliant on single or multiple nucleotide substitutions are at increased risk for reversion to virulent phenotypes due to the inherently high rate of viral RNA polymerase errors. The loss of attenuation via this mechanism among livestock and human live vaccines has been documented (Berkhout *et al., J.*

Virol. 73:1138-1145, 1999; Catelli *et al.*, Vaccine 24:6476-6482, 2006; Halstead *et al.*, Am. *J. Trop. Med. Hyg.* 33:672-678, 1984; Hopkins and Yoder, *Avian Dis.* 30:221-223, 1986; Rahimi *et al.*, *J. Clin. Virol.* 39:304-307, 2007).

The potential for a similar reversion event among live RVF virus vaccines dependent on attenuating nucleotide mutation was illustrated by a genomic analyses of RVF virus that revealed an overall molecular evolution rate (~$2.5x10^{-4}$ nucleotide substitutions/site/year) similar to other single-stranded RNA viruses (Bird *et al.*, *J. Virol.* 81:2805-2816, 2007). Due to error-prone polymerases, live-attenuated RNA virus vaccines prepared by multiple serial passage techniques involved in virus attenuation inherently consist of a complex mixture of genomic micro-variants. In contrast, the origin of reverse genetics derived virus vaccine candidates (including the VRP_{RVF} disclosed herein) is advantageous in that vaccine stocks can be generated directly from precisely defined DNA plasmids. This approach allows for the simple production of virus vaccines following good manufacturing processes (GMP) with higher levels of genetic homogeneity.

Another significant drawback of all previously generated live-attenuated RVF virus vaccines is that they do not allow for differentiation of naturally infected from vaccinated animals (DIVA). This ability is important to augment efforts to contain an accidental or intentional release of wild-type RVF virus in previously unaffected areas (Henderson, *Biologicals* 33:203-209, 2005). As a high consequence pathogen, RVF virus has been classified as a category A Select Agent as defined by the United States Department of Health and Human Services and the United States Department of Agriculture (USDA), and is listed as a high consequence agent with potential for international spread (List A) by the Office International des Epizooties (OIE) (Le May *et al.*, *Cell* 116:541-550, 2004) of the World Organization for Animal Health (WOAH), thus greatly increasing the consequences for international livestock trade following the introduction of RVF virus into previously unaffected countries or epizootics in enzootic areas (USDA, 7 CFR Part 331 and 9 CFR Part 121, Federal Register RIN 0579-AB47:13241-13292, 2005). Currently, OIE regulations require surveillance and absence of RVF virus activity for 2 years following an outbreak before resumption of disease free status and the subsequent easing of import/export trade restrictions (International Office of Epizootics, Terrestrial Animal Health Code, XI:2.2.14.1, 2007). The use of any current commercially available livestock vaccines does not permit the differentiation of vaccinated from naturally infected livestock, thus contraindicating the use of prophylactic vaccination in countries wishing to retain disease free status, or in those with ongoing/endemic RVF virus activity.

The VRP_{RVF} disclosed herein do contain or encode the NSs or NSm proteins, which allows for the serologic differentiation of naturally infected and vaccinated animals by presence/absence of anti-RVF NP/anti-RVF NSs antibodies.

Thus, the VRP_{RVF} disclosed herein have several distinct advantages over prior RVF virus vaccines – they exhibit rapid and robust efficacy, enhanced safety, are incapable of reverting to a virulent virus, and infection by VRP_{RVF} can be distinguished from natural RVF virus infection (DIVA).

The VRP_{RVF} disclosed herein can also be used as adjuvants to enhance the immunogenicity of another vaccine. As described herein, VRP_{RVF} elicit rapid and robust immunogenicity, indicating these particles are capable of functioning as a strong adjuvant.

VRP_{RVF} can further be used as vaccine vectors to deliver a heterologous protein as a means of eliciting an immune response against the heterologous protein. The data disclosed herein demonstrate that replacement of the NSs ORF with GFP resulted in robust GFP expression within VRP-infected cells. It is further disclosed herein that VRP_{RVF} infection *in vivo* resulted in a strong innate immune response.

Thus, provided herein is a method of eliciting an immune response against a heterologous protein in a subject by administering to the subject VRP_{RVF} in which the NSs ORF of the S genome segment is replaced by an ORF encoding the heterologous protein. In some embodiments, the heterologous protein is a protein from an infectious organism, such as a heterologous virus (any virus other than Rift Valley fever virus). In other embodiments, the heterologous protein is a tumor antigen.

In particular embodiments, provided herein is a method of eliciting an immune response against a heterologous viral protein in a subject by administering to the subject VRP_{RVF} in which the NSs ORF of the S genome segment is replaced by an ORF encoding a heterologous viral protein. In other particular embodiments, provided herein is a method of eliciting an immune response against a tumor antigen by administering to the subject VRP_{RVF} in which the NSs ORF of the S genome segment is replaced by an ORF encoding a tumor antigen.

The VRPRVF disclosed herein can also be used as anti-cancer agents to promote destruction of tumor cells. Thus, in some embodiments, provided is a method of treating cancer in a subject by selecting a subject with cancer and administering to the subject a therapeutically effective amount of the VRP_{RVF} disclosed herein. In some examples, the VRP_{RVF} are administered systemically, such as intravenously, for example by infusion. Systemic administration is particularly useful for metastatic cancer. In other examples, the VRP_{RVF} are

administered directly into a tumor. In some embodiments, the NSs ORF of the S genome segment of the VRP_{RVF} is replaced by an ORF encoding a tumor antigen.

In some embodiments, the subject is further administered a second anti-cancer agent and/or other therapeutic treatment for cancer, such as surgical removal of the tumor of a portion thereof. Exemplary anti-cancer agents include, but are not limited to, chemotherapeutic agents, such as, for example, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, anti-survival agents, biological response modifiers, anti-hormones (*e.g.* anti-androgens) and anti-angiogenesis agents. Other anti-cancer treatments include radiation therapy and antibodies that specifically target cancer cells.

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the disclosure to the particular features or embodiments described.

EXAMPLES

Example 1: Materials and Methods

This example describes the experimental procedures for the studies described in Example 2.

Cell culture and biosafety

BSR-T7/5 and VeroE6 cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and penicillin-streptomycin (Invitrogen). BSR-T7/5 cells were maintained under selection with G418 every other passage (1 mg/mL; Geneticin; Invitrogen). All animals were housed within BSL-4 or BSL-3+ laboratories in microisolator pans in HEPA filtration racks, following standard barrier techniques.

Animal immunization and infection

For the suckling mouse safety test, 2-day-old CD-1 (ICR) mice (Charles River Laboratories) were inoculated intracranially (ic) with 1.0×10^4 50% tissue culture infective dose ($TCID_{50}$) VRP_{RVF}, or with 1.0×10^4 plaque forming units (pfu) of recombinant RVFV ZH501 (RVFV) in a total volume of 10 µL DMEM. Mock-immunized mice were inoculated with 10 µL

DMEM ic. Mice were evaluated daily for 28 days post-immunization (dpi), and animals were euthanized if found in distress or moribund.

Four experiments used 10-12-week-old female C57BL/6 mice (Jackson Laboratory): (i) VRP_{RVF} dose titration for minimum effective immunization dose; (ii) vaccine efficacy and immunogenicity of VRP_{RVF} and nr-VRP_{RVF} at 28 dpi; (iii) VRP_{RVF} and nr-VRP_{RVF} efficacy at 1-4 dpi; and (iv) systemic immune responses of VRP_{RVF} and nr-VRP_{RVF} immunized mice during the first 24 hours post-immunization (hpi). In these experiments, mice were immunized subcutaneously (sc) with VRP_{RVF} or nr-VRP_{RVF} in doses ranging from 10 to 1.0×10^5 $TCID_{50}$ prepared in a total volume of 100 µL DMEM. Mock immunized controls were inoculated with 100 µL DMEM. Mice were challenged sc with 1.0×10^5 pfu RVFV in 100 µL DMEM. Animals were evaluated at least once daily for 28 dpi. All animals were euthanized according to a pre-determined clinical illness scoring algorithm or if found in acute distress or moribund.

Plasmid construction

Construction of the full length RVFL, RVFM, and RVFS plasmids, and the RVFM-ΔNSm and RVFS-ΔNSs:GFP plasmids has been described previously (Bird *et al.*, *Virology* 362:10-15, 2007). The plasmids contain the viral antigenome flanked by the T7 promoter (5′ terminus), and the hepatitis Δ virus ribozyme and T7 polymerase terminator motifs (3′ terminus). For this study, the open reading frame (ORF) encoding the RVFV glycoproteins, Gn and Gc, was amplified by PCR (nt 408-3614, as in GenBank Accession No. DQ380200) and cloned into the pCAGGS expression plasmid (Niwa *et al.*, *Gene* 108:193-199, 1991) using standard cloning techniques (pC-GnGc). Two silent mutations were introduced into the Gn/Gc ORF used for VRP_{RVF} generation to differentiate this ORF from that of wild-type virus.

VRP_{RVF} and RVFV production

VRP_{RVF}, wild-type RVFV, and RVFV-ΔNSm/ΔNSs:GFP viruses were produced using an established 3-plasmid RVFV reverse genetics system. As described previously, rescue of recombinant wild-type RVFV ZH501 (RVFV) was accomplished using RVFL, RVFM, and RVFS plasmids (Bird *et al.*, *Virology* 362:10-15, 2007), and RVFV-ΔNSm/ΔNSs:GFP was rescued using RVFL, RVFM-ΔNSm, and RVFS-ΔNSs:GFP plasmids (Bird *et al.*, *J. Virol.* 82:2681-2691, 2008; U.S. Patent Application Publication No. 2011-0123567). VRP_{RVF} were rescued similarly using RVFL, RVFS-ΔNSs:GFP, and pC-GnGc plasmids. Briefly, BSR-T7/5 cells were seeded in a 6-well plate format. Cells were transfected at approximately 75%

confluency with 1 μg of each plasmid and LT1 transfection reagent (Mirus) in a ratio of 1 μg:4 μl LT1. Rescue of VRP_{RVF} resulted only from cells transfected with all 3 plasmids. Supernatants were harvested at 4 days post-transfection, subjected to low-speed centrifugation to clear cellular debris, and stored at -80°C. Non-replicating VRP_{RVF} (nr-VRP_{RVF}) were generated by exposing VRP_{RVF} to γ-irradiation (5×10^6 rads), following a standard CDC protocol for removing antigen preparations from BSL-4 containment for diagnostic testing in a BSL-2 laboratory. This protocol completely abolishes viral (or VRP_{RVF}) replication while preserving the antigenicity of the sample.

VRP_{RVF} titration and one-step growth curve

To determine VRP_{RVF} titer and production kinetics, BSR-T7/5 transfection supernatants were harvested from individual wells of a 6-well plate 1, 2, 3, 4, or 5 days post-transfection, clarified by low-speed centrifugation and frozen at -80 °C. VRP_{RVF} titers were determined as $TCID_{50}$ on VeroE6 cells. Initial titration of VRP_{RVF}-infected cells was based on eGFP fluorescence and confirmed by indirect fluorescent-antibody assay (IFA) using an anti-RVF primary antibody.

Total anti-RVFV IgG enzyme-linked immunosorbent assay (ELISA)

Total anti-RVFV IgG ELISA testing was completed using whole cell lysates from RVFV infected Vero E6 cells or uninfected Vero E6 cells at 1:2000 following standard CDC Viral Special Pathogens Branch diagnostic protocols as described previously (Bird *et al.*, *J. Virol.* 85:12901-12909, 2011).

Virus neutralization titers (VNT_{100})

RVFV stock was diluted to 3000 $TCID_{50}$ in 50 μL DMEM without FBS. Sera from VRP_{RVF}, nr-VRP_{RVF}, and mock-immunized mice were heat-inactivated at 56°C for 30 minutes. In a 96-well plate, 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, and 1:640 serum dilutions were made in 50 μL DMEM. An equal volume of diluted RVFV was added to diluted sera and incubated for 1 hour at 37°C. A suspension of approximately 3×10^4 VeroE6 cells was added to each well, and the plates were incubated for 36 hours before formalin fixation. Cells were visualized with an IFA using an anti-RVFV primary antibody. VNT_{100} were defined as the highest dilution that permitted complete (100%) neutralization of virus input.

Electron microscopy

VRP_{RVF} and RVFV samples from the supernatants of transfected BSRT7/5 cells were taken 3 days post-transfection, fixed with 4% paraformaldehyde, and inactivated by γ-irradiation for removal from containment laboratory. Each sample was incubated overnight on 400 mesh nickel grids at 4°C. Grids were rinsed once and stained with 5% ammonium molybdate and 0.1% trehalose. Specimens were viewed at 120 kV on a Tecnai FEI electron microscope (FEI).

In vivo safety assessment

A total of 30 2-day-old suckling mice (SM) were inoculated with 1.0×10^4 $TCID_{50}$ VRP_{RVF}. 10 SM were inoculated with 1.0×10^4 pfu RVFV (positive control), and 20 SM were inoculated with 10 μL DMEM (negative control).

VRP_{RVF} dose titration

A total of 25 mice were immunized sc in groups of 5 with 1.0×10^5, 1.0×10^4, 1.0×10^3, 1.0×10^2, or 10 $TCID_{50}$ VRP_{RVF}, and 5 mice were mock immunized with DMEM. Mice were evaluated once daily for clinical signs. At 28 dpi, all mice were challenged with a lethal dose of 1.0×10^5 pfu RVFV sc.

Efficacy and immunogenicity of VRP_{RVF} and nr-VRP_{RVF} at 28 dpi

Mice were immunized sc in 5 groups: (i) 1.0×10^5 $TCID_{50}$ VRP_{RVF} (n = 10); (ii) 1.0×10^4 $TCID_{50}$ VRP_{RVF} (n = 13); (iii) 1.0×10^5 $TCID_{50}$ nr-VRP_{RVF} (n = 10); (iv) 1.0×10^4 $TCID_{50}$ nr-VRP_{RVF} (n = 13); and (v) sham-immunized with DMEM. At 28 dpi, 5 mice from each group were challenged with a lethal dose of 1.0×10^5 pfu RVFV sc and evaluated daily for 28 days. The remaining mice in each group were anesthetized with isoflurane for serum collection for determination of total anti-RVFV IgG and neutralizing antibody titers.

VRP_{RVF} and nr-VRP_{RVF} efficacy at early time points

A total of 100 mice were immunized sc in 5 groups of 20: (i) 1.0×10^5 $TCID_{50}$ VRP_{RVF}; (ii) 1.0×10^4 $TCID_{50}$ VRP_{RVF}; (iii) 1.0×10^5 $TCID_{50}$ nr-VRP_{RVF}; (iv) 1.0×10^4 $TCID_{50}$ nr-VRP_{RVF}; and (v) mock-immunized with DMEM. On each of days 1, 2, 3, and 4, a subset of 5 mice from each group (25 total) were challenged sc with 1.0×10^5 pfu virulent RVFV and evaluated twice daily for clinical signs of disease.

Comparison of early immune response of VRP_{RVF} and nr-VRP_{RVF} immunized mice

A total of 24 mice were immunized sc in 3 groups of 8: (i) 1.0×10^4 $TCID_{50}$ VRP_{RVF}; (ii) 1.0×10^4 $TCID_{50}$ nr-VRP_{RVF}; or (iii) mock-immunized with 100 µL DMEM. At 12 and 24 hpi, 4 mice from each group were anesthetized with isoflurane and perfused with PBS. Approximately 100 µg samples from the perfused liver and brain were placed in RNA*later*™ (Ambion Inc.) and frozen at -80 °C until used for RNA extraction.

RNA extraction

To extract mRNA from tissues, tissue samples were removed from RNA*later*™, and placed in 1 mL of Tripure™ isolation reagent (Roche Applied Science). RNA was extracted using the Qiagen RNeasy™ mini kit per manufacturer's instructions, including the RNase-free DNase step (Qiagen).

Antiviral assays

Antiviral Response quantitative PCR arrays (SABiosciences) were used to determine up- or down-regulation of a select panel of 84 antiviral genes in mice immunized with VRP_{RVF} or nr-VRP_{RVF}, relative to mock immunized mice. Assays were run on liver and brain samples from 3 VRP_{RVF}-immunized mice, 3 nr-VRP_{RVF}-immunized mice, and 3 mock-immunized mice at each time point. For each sample, cDNA was synthesized from 0.8-1.0 µg of RNA using the RT^2 first strand kit (SABioscience). Arrays were run on an ABI 7500 using RT^2 SYBR™ Green/ROX PCR master mix according to the manufacturer's instructions (SABioscience).

Statistical analyses

For efficacy experiments, the Gehan-Breslow-Wilcoxon test was used to determine whether survival curves were significantly different (GraphPad Prism, GraphPad Software Inc.). A student's t-test was used to determine statistical significance of VNT_{100} and IgG titers.

In the antiviral array analysis, the mean value of each gene was calculated for each set of replicate tissue samples using the $\Delta\Delta C_t$ method, and normalized to the average of 5 housekeeping genes (Gus-ß, Hprt, HSP-90AB1, GAPDH, and ß-actin). The p values were calculated based on a student's t-test of the replicate $2^{-\Delta Ct}$ values (SABioscience) for each gene in the VRP_{RVF} and nr-VRP_{RVF} groups.

Example 2: Single dose immunization with virus replicon particles confers rapid robust protection against Rift Valley fever virus challenge

This example describes the finding that a single dose of VRP_{RVF} provides complete protection against RVFV challenge, and that complete protection is conferred within 96 hours of vaccination.

VRP_{RVF} high yield production and growth kinetics

Efficient VRP_{RVF} production was accomplished with an established RVFV reverse genetics system and simultaneous transfection of 3 plasmids: RVFL (wild-type L segment; SEQ ID NO: 1), RVFS-ΔNSs:GFP (S segment with GFP replacing NSs; SEQ ID NO: 2), and pC-GnGc (expression vector carrying glycoprotein genes; SEQ ID NO: 3) (FIG. 1A). VRP_{RVF} were produced from cells transfected with all 3 plasmids and rescued directly from BSR-T7/5 transfection media. Total VRP_{RVF} production increased from 1 day through 4 days after transfection, and declined at 5 days. In several independent experimental replicates, VRP_{RVF} were rescued with 100% efficiency with titers ranging from 1.0×10^6 to 5.0×10^7 $TCID_{50}$/mL (average titer = 1.2×10^7 $TCID_{50}$/mL). Recombinant wild-type RVFV ZH501 and RVFV-ΔNSm/ΔNSs:GFP, a GFP-expressing virus with full-gene deletions of NSs and NSm, were also successfully rescued as described previously (Bird *et al.*, *Virology* 362:10-15, 2007; U.S. Patent Application Publication No. 2011-0123567).

VRP_{RVF} are morphologically indistinguishable from RVFV

To compare the morphology of replicon particles to virus particles, VRP_{RVF} and RVFV particles were harvested from BSRT7/5 cells at 3 days after transfection for electron microscopy analysis. VRP_{RVF} and classic RVFV particles ranged in size between 80-100 nm, were round to slightly pleiomorphic, and indistinguishable from one another (FIG. 1B).

VRP_{RVF} do not spread beyond initially infected cells

To illustrate that VRP_{RVF} only undergo one round of infection, VeroE6 cell monolayers were infected with VRP_{RVF} and RVFV-ΔNSm/ΔNSs:GFP, and monitored daily for 5 days. While RVFV spreads rapidly throughout the cell monolayer, the VRP_{RVF} do not spread beyond the initially infected cells (FIG. 2A).

VRP_{RVF} is completely safe in suckling mouse infections

In a stringent vaccine safety test, newborn (2-day-old) suckling mice were inoculated ic with 1.0×10^4 $TCID_{50}$ VRP_{RVF}, 1.0×10^4 pfu RVFV, or 10 μL DMEM. All RVFV-inoculated mice died 2 days after infection. All mice inoculated with VRP_{RVF} or DMEM survived with no indication of clinical signs (FIG. 2B).

Single dose immunization with VRP_{RVF} is completely protective from virulent virus challenge

A dose titration study was conducted to determine the minimum VRP_{RVF} immunization that confers protection from virulent virus challenge. Mice were immunized sc in groups of 5 with 10, 1.0×10^2, 1.0×10^3, 1.0×10^4, or 1.0×10^5 $TCID_{50}$ VRP_{RVF}, or mock-immunized with DMEM, and challenged sc with 1.0×10^5 pfu RVFV. Previous experiments indicated the 50% lethal dose (LD_{50}) of RVFV ZH501 in adult C57BL/6 mice is less than 1 pfu; therefore the virus challenge for all experiments in this study was 100,000-fold higher than the LD_{50}. Results showed a dose-dependent effect on survival. A single dose immunization with 1.0×10^5 or 1.0×10^4 $TCID_{50}$ VRP_{RVF} conferred 100% protection against virus challenge, whereas 1.0×10^3 $TCID_{50}$ VRP_{RVF} protected 60% of mice. Although there were no survivors in groups given lower VRP_{RVF} doses, mortality was delayed in mice receiving 1.0×10^2 $TCID_{50}$ (FIG. 3A).

Active replication of VRP_{RVF} is important for complete protection

To evaluate the relative importance of VRP_{RVF} replication for vaccine efficacy, mice were immunized with replicating VRP_{RVF} or non-replicating (nr)VRP_{RVF} at doses of 1.0×10^5 or 1.0×10^4 $TCID_{50}$, or mock-immunized with DMEM, and were challenged with 1.0×10^5 pfu virulent RVFV. As seen in the earlier experiment, all mice immunized with a single dose of 1.0×10^5 or 1.0×10^4 $TCID_{50}$ VRP_{RVF} survived lethal virus challenge with no clinical signs. In contrast, only 60% and 20% of the mice immunized with 1.0×10^5 or 1.0×10^4 $TCID_{50}$ nr-VRP_{RVF}, respectively, survived challenge (FIG. 3B).

Replicating VRP_{RVF} induce IgG response by 28 dpi

The IgG antibody responses of mice immunized with 1.0×10^5 $TCID_{50}$ VRP_{RVF}, 1.0×10^5 $TCID_{50}$ nr-VRP_{RVF}, or DMEM were evaluated 28 dpi. Mice immunized with VRP_{RVF} had total anti-RVF IgG titers ranging from 1600 (1/5) to 6400 (4/5). None of the mice immunized with nr-VRP_{RVF} had detectable IgG response (limit of detection 1:100; FIG. 3C).

VRP_{RVF} induce a significantly stronger neutralizing antibody response than nr-VRP_{RVF}

Also at 28 dpi, the neutralizing antibody response of mice immunized with 1.0×10^4 $TCID_{50}$ VRP_{RVF} or nr-VRP_{RVF} was assessed. Seven of the 8 VRP_{RVF}-immunized mice had detectable levels of neutralizing antibodies, as compared with 2 of the 8 nr-VRP_{RVF}-immunized mice. VNT_{100} were significantly higher in VRP_{RVF}-immunized mice than nr-VRP_{RVF}–immunized mice ($p < 0.05$; FIG. 3D).

Single dose VRP_{RVF} immunization provides protection from virulent virus challenge by 24 hpi

To determine vaccine efficacy at early times post-immunization, mice were immunized with a single dose of 1.0×10^5 or 1.0×10^4 $TCID_{50}$ VRP_{RVF}, or 1.0×10^5 or 1.0×10^4 $TCID_{50}$ nr-VRP_{RVF} and challenged with virulent RVFV 1, 2, 3 or 4 dpi. When challenged 24 hours after immunization, 60% of the higher dose VRP_{RVF}-immunized mice survived, and 80% survived challenge administered on days 2 and 3, irrespective of dose (FIGS. 4A-4C). All mice immunized with VRP_{RVF} survived challenge given 4 dpi. Mice immunized with nr-VRP_{RVF} displayed lower levels of protection; regardless of immunization dose, 20% of mice challenged 1 or 2 dpi survived. The highest survivorship of nr-VRP-$_{RVF}$-immunized mice observed was 40% on 3 dpi (FIGS. 4A-4C).

VRP_{RVF}-immunized mice show significant upregulation in antiviral gene expression

Antiviral gene expression, including IFN-ß, was quantified in VRP_{RVF}- and nr-VRP_{RVF}-immunized mice relative to mock-immunized mice 12 and 24 hpi. Genes significantly upregulated are shown in Table 1 (*$p < 0.05$; **$p < 0.001$). The only gene significantly downregulated relative to mock-immunized mice was cFOS, in the livers of VRP_{RVF}-immunized mice 12 hpi, and in the brains of both VRP_{RVF}- and nr-VRP_{RVF}-immunized mice 24 hpi. Several genes were significantly upregulated in only VRP_{RVF}-immunized mice, including CD40, CCL3, CCL5, CXCL9, CXCL10, RIG-I, IRF3, JUN, MX1, STAT1, TLR9, TNF, and TRADD. Both VRP_{RVF}- and nr-VRP_{RVF}-immunized mice had significant upregulation of IRF7, ISG15, and LGP2 genes relative to mock-immunized mice. Significant increases of Nlrp3 and CARD9 were apparent in VRP_{RVF}-immunized mice 12 hours earlier than in nr-VRP_{RVF}-immunized mice. All genes upregulated in nr-VRP_{RVF}- relative to mock-immunized mice were also upregulated in VRP_{RVF}-immunized mice.

Table 1

Fold-change in expression of selected antiviral genes in VRP_{RVF}-immunized or nr-VRP_{RVF}-immunized relative to mock-immunized controls

	VRP_{RVF}	nr-VRP_{RVF}	VRP_{RVF}	nr-VRP_{RVF}	VRP_{RVF}	nr-VRP_{RVF}	VRP_{RVF}	nr-VRP_{RVF}
GENE	12 h Liver		24 h Liver		12 h Brain		24 h Brain	
CARD9	0.8	0.7	1.2	1.8 *	2.2 **	1.5	1.0	0.8
CD40	2.2*	1.6	1.5	1.0	1.2	0.9	1.0	1.1
CCL3	0.9	1.0	3.0 *	1.0	1.2	1.0	1.9	0.5
CCL5	1.2	1.4	2.0 **	1.6	1.0	0.3	0.7	1.0
CXCL9	1.0	0.8	4.5 *	0.8	2.6 *	3.2	2.4	0.3
CXCL10	3.7 *	1.8	3.7 *	1.9	1.8	1.7	3.5	1.0
cFOS	0.09 *	0.5	0.4	0.6	1.5 *	1.3	0.4 *	0.3 *
IRF3	0.9	0.9	0.9	0.6	2.6 **	1.6	0.4	0.7
IRF7	5.0 **	2.1 **	11.2	3.6 **	3.2	2.2	6.0	1.3
ISG15	16.1 *	3.3 *	14.1 *	2.9 *	1.2	0.9	6.3	0.6
JUN	0.4	0.7	0.4	1.0	2.6 *	1.5	0.6	0.4
LPG2	5.1 **	2.0 **	4.4 **	1.9 *	2.7	2.3	2.3	1.0
MX1	1.7	0.4	7.7 **	2.1	0.6	0.6	1.9 *	0.8
NLRP3	0.8	0.8	1.6	2.0	2.2 *	1.2	0.4	0.7
RIG-I	2.0 **	1.0	1.7	0.8	0.7	0.9	2.4	1.0
STAT1	2.7 **	1.3	2.6 *	1.2	0.7	0.8	4.0	0.8
TLR9	3.1	1.4	1.5	0.8	2.9 *	1.3	0.7	0.8
TNF	2.7 *	1.2	4.0	1.8	N/A	N/A	2.0	0.9
TRADD	1.1	1.0	1.0	1.3	1.6 **	1.2	0.8	0.9

Significant fold change: *$p < 0.05$; **$p<0.01$

Several genes were expressed at significantly higher levels in VRP_{RVF}-immunized mice relative to nr-VRP_{RVF}-immunized mice. In the liver, RIG-I, LGP2, IRF7, and ISG15 expression was elevated in VRP_{RVF}-immunized mice by 12 hpi, and STAT1 and MX1 expression was significantly higher by 24 hpi (FIG. 5). Chemokines CCL3, CCL4, and CXCL9 were significantly elevated in the livers of VRP_{RVF}-immunized mice by 24 hpi (FIG. 5). Also by 24 hpi, MDA5 and CXCL10 were upregulated in the brains of VRP_{RVF}- relative to nr-VRP_{RVF}-immunized mice (FIG. 5 and FIG. 6).

Discussion

Large outbreaks of RVFV can have a devastating impact on human and animal health; however, there are currently no approved vaccines for use outside of the endemic areas within

Africa. In these areas, widespread use of available livestock vaccines has been limited due to safety concerns or poor immunogenicity. Early live attenuated constructs were associated with abortion or teratogenesis in pregnant animals (*e.g.*, Smithburn and MP12) (Botros *et al.*, *J. Med. Virol.* 78:787-791, 2006; Hunter *et al.*, *Onderstepoort J. Vet. Res.* 69:95-98, 2002). Inactivated VLP-like vaccines are much safer, but require the use of adjuvant or multiple boosters for complete protection. Recently, a rationally designed, reverse genetics-derived vaccine candidate that is safe and efficacious in livestock was developed (Bird *et al.*, *J. Virol.* 85:12901-12909, 2011). As an additional countermeasure against RVFV, the robust efficacy of this vaccine was paired with the enhanced safety inherent in non-replicating constructs. The resulting VRP_{RVF} undergo only one round of infection and are biologically confined to the initially infected cells, but can actively synthesize viral RNA and express viral nucleoprotein and polymerase.

VRP_{RVF} particles are morphologically indistinguishable from wild-type virus, but lack 4 genes, those encoding virulence factors NSs and NSm and structural proteins Gn and Gc. Deletions of NSm (Bird *et al.*, *Virology* 362:10-15, 2007) and NSs (Vialat *et al.*, *J. Virol.* 74:1538-1543, 2010) have been shown to reduce virulence of RVFV in adult rodents. The NSs protein inhibits the host immune response to RVFV infection through multiple mechanisms (Billecocq *et al.*, *J. Virol.* 78:9798-9806, 2004; Habjan *et al.*, *J. Virol.* 83:4365-4375, 2009; Ikegami *et al.*, *Ann. N. Y. Acad. Sci.* 1171 Suppl 1:E75-85, 2009; Le May *et al.*, *Cell* 116:541-550, 2004; Le May *et al.*, *PLoS Pathogens* 4:e13, 2008), and therefore its absence or mutation is a common feature of many RVF vaccine candidates (Bird *et al.*, *J. Virol.* 82:2681-2691, 2008; Dungu *et al.*, *Vaccine* 28:4581-4587, 2010). Additional full-length deletions of genes encoding the structural proteins Gn and Gc confine VRP_{RVF} replication to the initially infected cells. The resulting inability to spread within the host dramatically reduces the chance of vaccine-induced pathogenicity and likely explains the safety of VRP_{RVF} infections in suckling mice, particularly given the rapid and uniform mortality seen with intracranial inoculation of suckling mice with RVF viruses.

Although extremely attenuated, VRP_{RVF}, like RVFV, contain the polymerase and nucleoprotein, the two factors required for viral replication, allowing for viral RNA expression and *de novo* viral protein synthesis in the target cells. Intracellular replication of single-stranded RNA viruses (including members of the *Bunyaviridae*) initiates a strong innate immune response via Toll-like receptors and/or the cytoplasmic RIG-I-like helicases, culminating in the production of important antiviral proteins, including interferon (IFN) (Elliott and Weber, *Viruses* 1:1003-1021, 2009; Kawai and Akira, *Ann. N. Y. Acad. Sci.* 1143:1-20, 2008). In wild-type

RVFV infection, the NSs protein abolishes these host responses. However, immunization with replicating VRP_{RVF} lacking the NSs would allow for unobstructed production of IFN and IFN-stimulated genes (ISGs), thus preserving critical aspects of the antiviral response. Indeed, in multiple experiments, a significantly stronger immune response and associated protective efficacy was observed in VRP_{RVF}-immunized mice relative to nr-VRP_{RVF}- and mock-immunized mice.

Mice immunized with VRP_{RVF} produced significantly higher levels of total IgG and neutralizing antibodies than nr-VRP_{RVF}-immunized mice, and were completely protected from virulent virus challenge at 28 dpi, suggesting that replication is critical for robust immunity and subsequent protection. As early as 12 hpi, clear differences in host response were already apparent between mice immunized with VRP_{RVF} and nr-VRP_{RVF}. Relative to both nr-VRP_{RVF}- and mock-immunized mice, VRP_{RVF} immunization resulted in significant systemic induction of IFN-inducible genes, including STAT1, IRF7, ISG15, RIG-I, LPG2, and MDA5. These genes stimulate the expression of important players in the cellular defense against viruses, including PKR, OAS, IRFs, MX1, and MHC classes I and II (Katze *et al.*, *Nat. Rev. Immunol.* 2:675-687, 2002). Activation of ISGs, particularly MHC, provides a mechanism for the improved antibody response and protection seen after immunization with replicating VRP_{RVF}. Additionally, induction of very early cell-mediated and subsequent adaptive immune responses in VRP_{RVF} immunized mice was evident from the significant upregulation of CCL4 (MIP-1β) and CXCL9 (MIG) expression in the liver, and CCL3 (MIP-1α) and CXCL10 (IP-10) expression in the liver and brain. These chemokines play important roles in attracting various immune cells, including monocytes/macrophages, NK cells and T cells, and in mediating T cell activation, aiding in initiation of cell-mediated and humoral adaptive immunity.

Rapid onset of a systemic antiviral response suggested that VRP_{RVF} immunization could confer early protection. VRP_{RVF} were found to be highly efficacious against virulent virus challenge within days of immunization; a single dose of VRP_{RVF} provided 60% protection by just 1 dpi and complete protection by 4 dpi. This early efficacy indicates that VRP_{RVF} will be a valuable control measure in the field. If RVFV was introduced into an area with large naïve populations, immunization with VRP_{RVF} early in the outbreak could prevent rapid viral spread throughout and beyond the affected region. Furthermore, the low genetic diversity and single serotype of the virus suggests that a VRP_{RVF} vaccine would be broadly protective against all known strains of RVFV.

The efficacy of VRP_{RVF} immunization against a virulent virus challenge 100,000-fold higher than the LD_{50} at early and late time points was remarkable. This protection likely hinges on the ability of the VRP_{RVF}, administered subcutaneously and in a single dose, to elicit a robust immune response in distant tissues within hours of immunization. This systemic response to VRP_{RVF} inoculation is clearly illustrated by the upregulation of antiviral genes in the liver and brain after vaccination. To explain the host-wide effect of localized VRP_{RVF} immunization, it was hypothesized that immunization results in VRP_{RVF} infection of resident macrophages or dendritic cells in the skin. Recent work has demonstrated that macrophages and dendritic cells are permissive to replication and are important targets of RVFV infection (Lozach *et al.*, *Cell Host Microbe* 10:75-88, 2011; McElroy and Nichol, *Virology* 422(1):6-12, 2011; Smith *et al.*, *Virology* 407:256-267, 2010). Given the absence of the NSs protein in the VRP_{RVF} construct, active replication within these cell types should stimulate a strong IFN response, as shown *in vitro* (McElroy and Nichol, *Virology* 422(1):6-12, 2011), leading to a systemic antiviral response. At the time points tested in these experiments, upregulation of the tested IFN subtypes was not detected. However, IFN must clearly have been produced within the host to induce the downstream expression of ISGs that were detected in the liver and brain. The bulk of IFN synthesis may occur at the site of immunization in locally infected macrophages or dendritic cells and then be dispersed systemically. Alternatively, IFN induction might be detectable in the liver and brain only at earlier time points, or as subtypes not evaluated here. RVFV is highly sensitive to IFN, and the rapid onset of a strong IFN response associated with VRP_{RVF} immunization provides a plausible explanation for early protection against challenge.

Replication-competent particles are a safe vaccine approach, much like inactivated or VLP-like constructs, yet stimulate a stronger immune response and, therefore, provide higher levels of protection from virulent challenge with just a single immunization. The data described herein show that VRP_{RVF} immunization rapidly and systemically initiates a strong cytokine and chemokine response with resulting protection seen as early as 24 hours post immunization. Further, it is demonstrates that the active replication that defines VRP_{RVF}, and distinguishes these particles from classical VLP, is critical for strong innate and adaptive immune responses and subsequently, complete protection from challenge.

In view of the many possible embodiments to which the principles of the disclosed invention may be applied, it should be recognized that the illustrated embodiments are only preferred examples of the invention and should not be taken as limiting the scope of the

invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

CLAIMS

1. A Rift Valley fever (RVF) virus replicon particle (VRP_{RVF}), comprising:

(i) RVF virus Gn and Gc glycoproteins;

(ii) RVF virus polymerase protein;

(iii) RVF virus nucleoprotein;

(iv) an RVF virus L genome segment; and

(v) an RVF virus S genome segment,

wherein the VRP_{RVF} does not contain an RVF virus M segment or any nucleic acid molecule encoding the Gn and Gc glycoproteins.

2. The VRP_{RVF} of claim 1, wherein the S genome segment comprises a deletion of the NSs open reading frame (ORF).

3. The VRP_{RVF} of claim 2, wherein the NSs ORF is replaced by an ORF encoding a heterologous protein.

4. The VRP_{RVF} of claim 3, wherein the ORF encoding a heterologous protein comprises a reporter gene ORF.

5. The VRP_{RVF} of claim 3, wherein the heterologous protein comprises an immunogenic protein from a heterologous virus, a tumor antigen or an immunomodulatory protein.

6. The VRP_{RVF} of any one of claims 1-5, wherein the VRP_{RVF} does not contain an RVF virus NSm protein.

7. The VRP_{RVF} of any one of claims 1-6, wherein the RVF virus is RVF virus strain ZH501.

8. A method of producing VRP_{RVF}, comprising:

transfecting a host cell with a plasmid comprising:

an antigenomic copy of an RVF virus L segment;

a plasmid comprising an antigenomic copy of an RVF virus S segment; and

a plasmid encoding RVF virus Gn and Gc glycoproteins; and

culturing the cells for a period of time sufficient to produce VRP_{RVF}.

9. The method of claim 8, further comprising collecting the VRP_{RVF} from the cell culture supernatant.

10. The method of claim 8 or claim 9, wherein the S genome segment comprises a deletion of the NSs ORF.

11. The method of claim 10, wherein the NSs ORF is replaced by an ORF encoding a heterologous protein.

12. The method of any one of claims 8-11, wherein none of the plasmids encode an RVF virus NSm protein.

13. The method of any one of claims 8-12, wherein the plasmid comprising an antigenomic copy of the S segment and the plasmid comprising an antigenomic copy of the L segment further comprise a T7 promoter and a hepatitis delta virus ribozyme.

14. The method of any one of claims 8-13, wherein the RVF virus is RVF virus strain ZH501.

15. The method of any one of claims 8-14, wherein:

(i) the plasmid comprising an antigenomic copy of the L segment comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 1;

(ii) the plasmid comprising an antigenomic copy of the S segment comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 2; and/or

(iii) the plasmid encoding RVF virus Gn and Gc glycoproteins comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 3.

16. The method of claim 15, wherein:

(i) the nucleotide sequence of the plasmid comprising an antigenomic copy of the L segment comprises SEQ ID NO: 1;

(ii) the nucleotide sequence of the plasmid comprising an antigenomic copy of the S segment comprises SEQ ID NO: 2; and/or

(iii) the nucleotide sequence of the plasmid encoding RVF virus Gn and Gc glycoproteins comprises SEQ ID NO: 3.

17. An immunogenic composition comprising the VRP_{RVF} of any one of claims 1-7, and a pharmaceutically acceptable carrier.

18. The immunogenic composition of claim 17, further comprising an immunogenic protein from a heterologous virus.

19. A method of eliciting an immune response against RVF virus in a subject, comprising administering to the subject an effective amount of the VRP_{RVF} of any one of claims 1-7, or the immunogenic composition of claim 17.

20. A method of immunizing a subject against RVF virus infection, comprising administering to the subject an effective amount of the VRP_{RVF} of any one of claims 1-7, or the immunogenic composition of claim 17.

21. The method of claim 19 or claim 20, wherein the subject is human or livestock.

22. The method of any one of claims 19-21, wherein the VRP_{RVF} or immunogenic composition is administered in a single dose.

23. The method of any one of claims 19-22, wherein the VRP_{RVF} or immunogenic composition is administered intravenously, intramuscularly or subcutaneously.

24. A method of enhancing an immune response against an immunogenic viral protein in a subject, comprising administering the immunogenic viral protein to the subject and further administering the VRP_{RVF} of any one of claims 1-3 and 5-7.

25. A method of eliciting an immune response against a viral protein in a subject, comprising administering to the subject the VRP_{RVF} of claim 5 or the immunogenic composition of claim 18.

26. A method of eliciting an immune response against a tumor antigen in a subject, comprising administering to the subject the VRP_{RVF} of claim 5.

27. A method of treating cancer in a subject, comprising selecting a subject with cancer and administering to the subject a therapeutically effective amount of the VRP_{RVF} of any one of claims 1-3 and 5-7.

28. The method of claim 27, wherein the VRP_{RVF} are administered systemically.

29. The method of claim 27, wherein the VRP_{RVF} are administered by direction injection into a tumor.

30. A method of differentiating a subject administered the VRP_{RVF} of any one of claims 1-7 from a subject naturally infected with RVF virus, comprising:

obtaining a biological sample from the subject; and

detecting the presence or absence of anti-NSm and anti-nucleoprotein antibodies in the sample, wherein the presence of both anti-nucleoprotein and anti-NSm antibodies in the sample indicates that the subject was naturally infected with RVF virus, and wherein the presence of anti-nucleoprotein antibodies and absence of anti-NSm antibodies in the sample indicates that the subject was administered the VRP_{RVF}.

31. A method of differentiating a subject administered the VRP_{RVF} of any one of claims 2-7 from a subject naturally infected with RVF virus, comprising:

obtaining a biological sample from the subject; and

detecting the presence or absence of anti-NSs and anti-nucleoprotein antibodies in the sample, wherein the presence of both anti-nucleoprotein and anti-NSs antibodies in the sample indicates that the subject was naturally infected with RVF virus, and wherein the presence of anti-nucleoprotein antibodies and absence of anti-NSs antibodies in the sample indicates that the subject was administered the VRP_{RVF}.

32. The method of claim 30 or claim 31, wherein the subject is human or livestock.

FIG. 1A

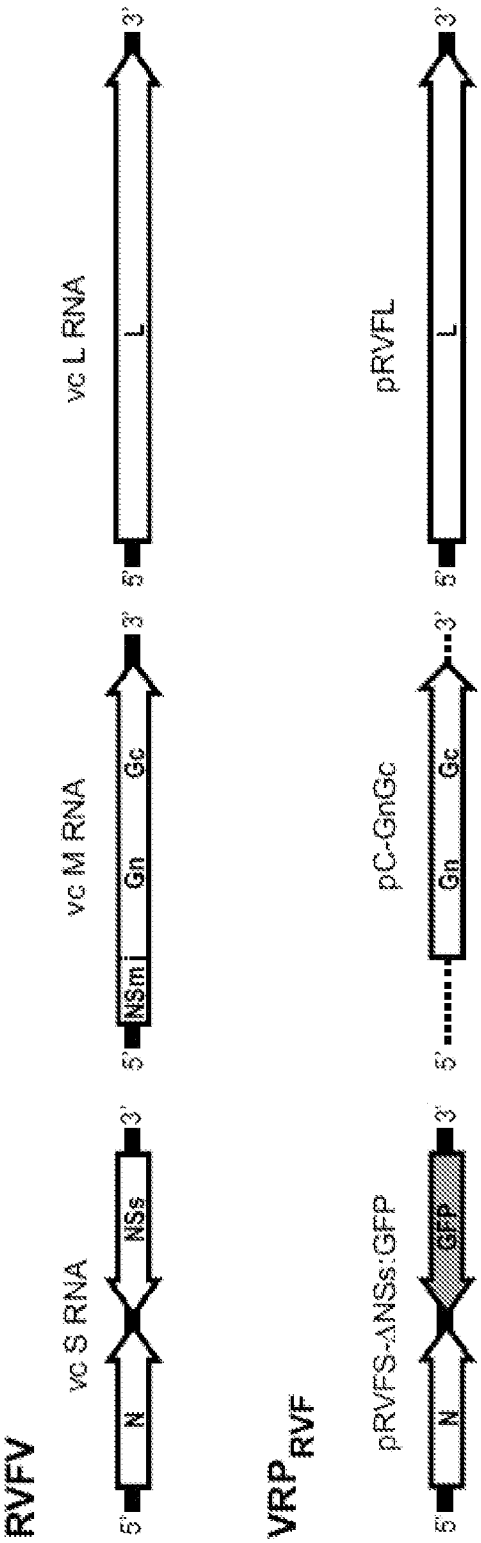

RVFV
vc S RNA
N
NSs
5'
3'
vc M RNA
NSmi
Gn
Gc
vc L RNA
L
VRP RVF
pRVFS-ΔNSs:GFP
GFP
pC-GnGc
pRVFL

FIG. 1B

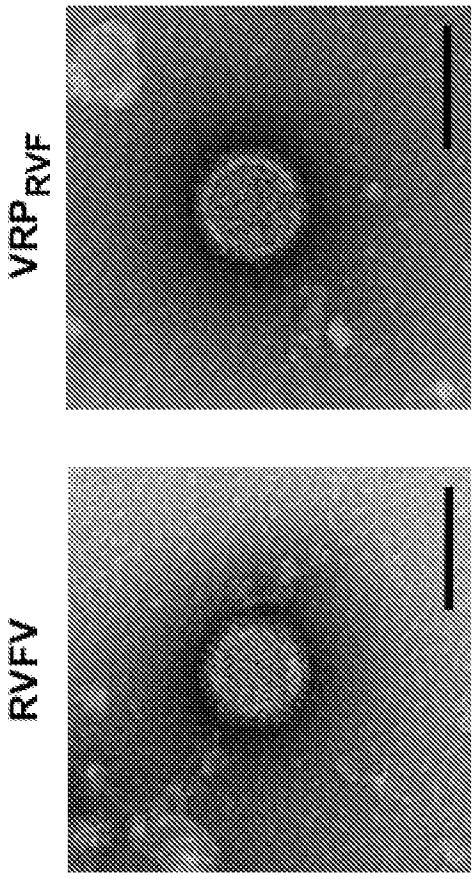

RVFV
VRP RVF

FIG. 2A

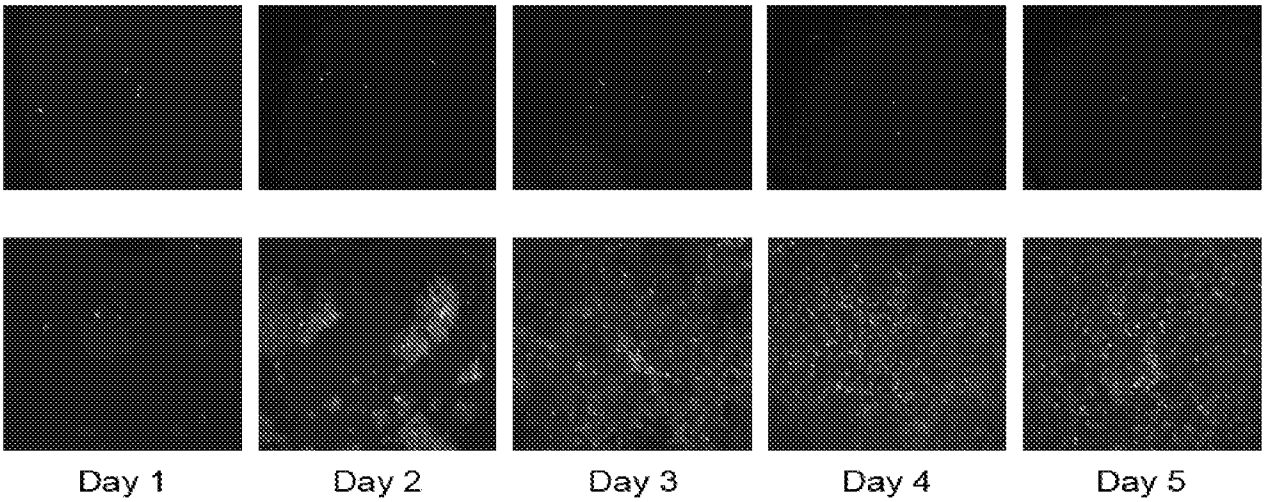

Day 1
Day 2
Day 3
Day 4
Day 5

FIG. 2B

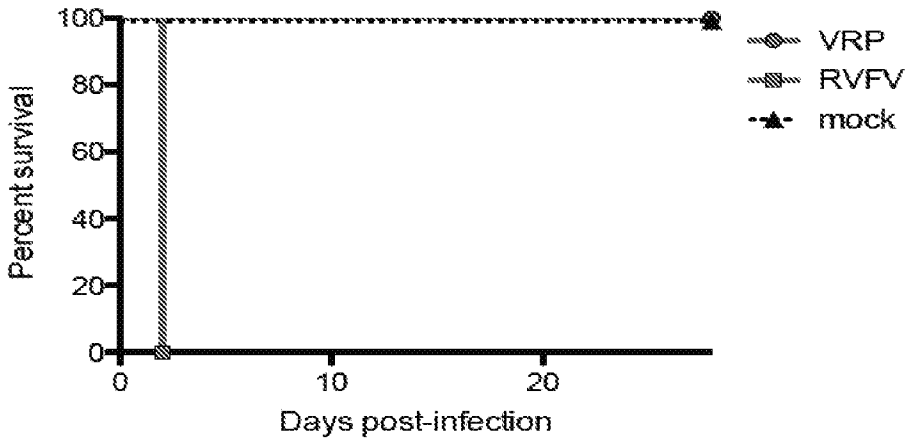

100
80
60
40
20
0
Percent survival
0
10
20
Days post-infection
VRP
RVFV
mock

FIG. 3A

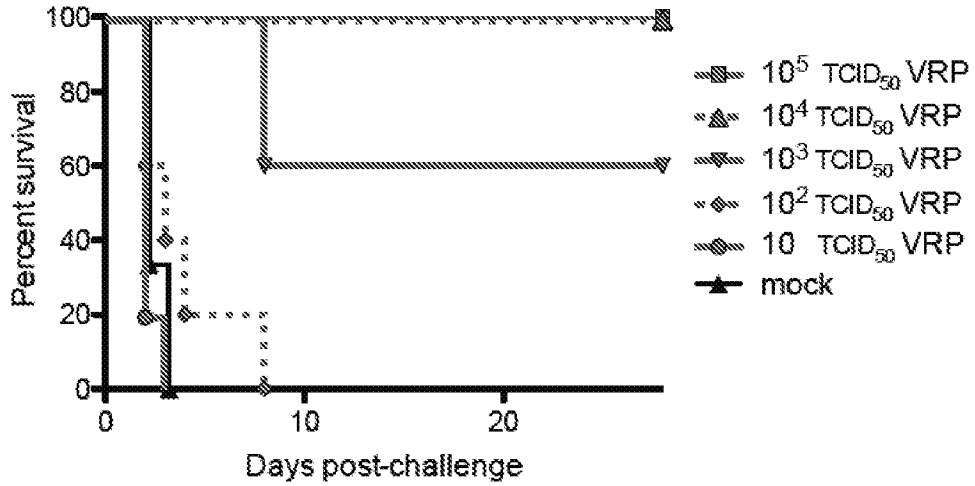


FIG. 3B

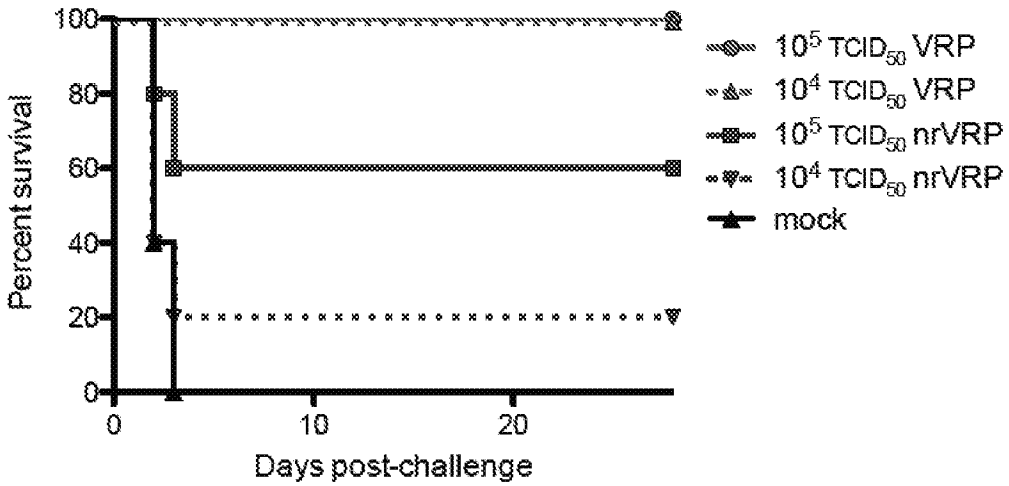


FIG. 3C

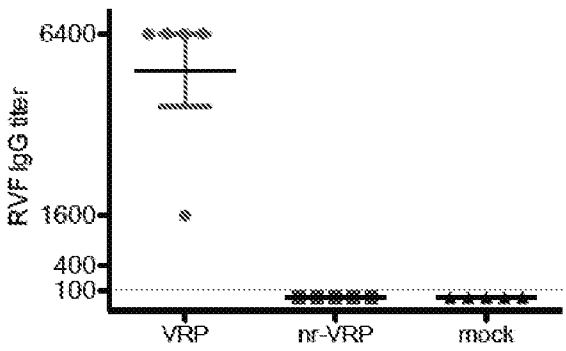


FIG. 3D

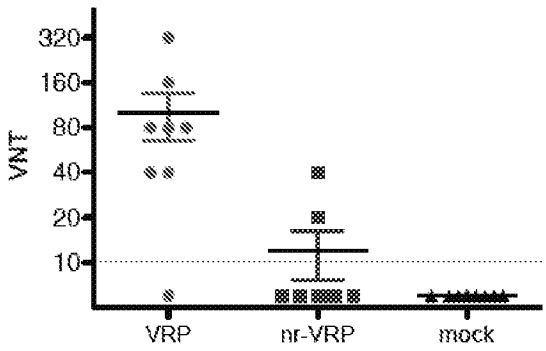


FIG. 4A

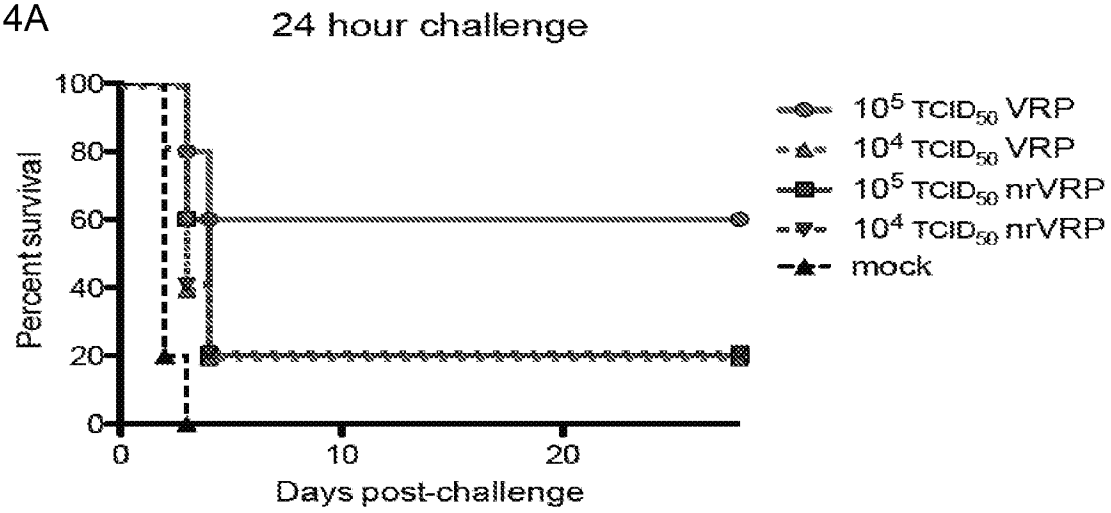


FIG. 4B

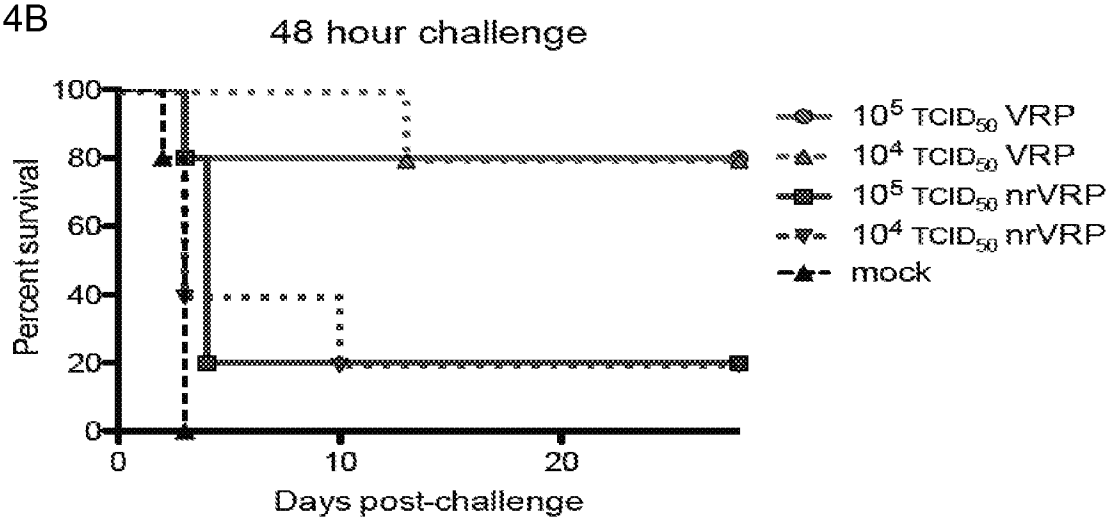


FIG. 4C

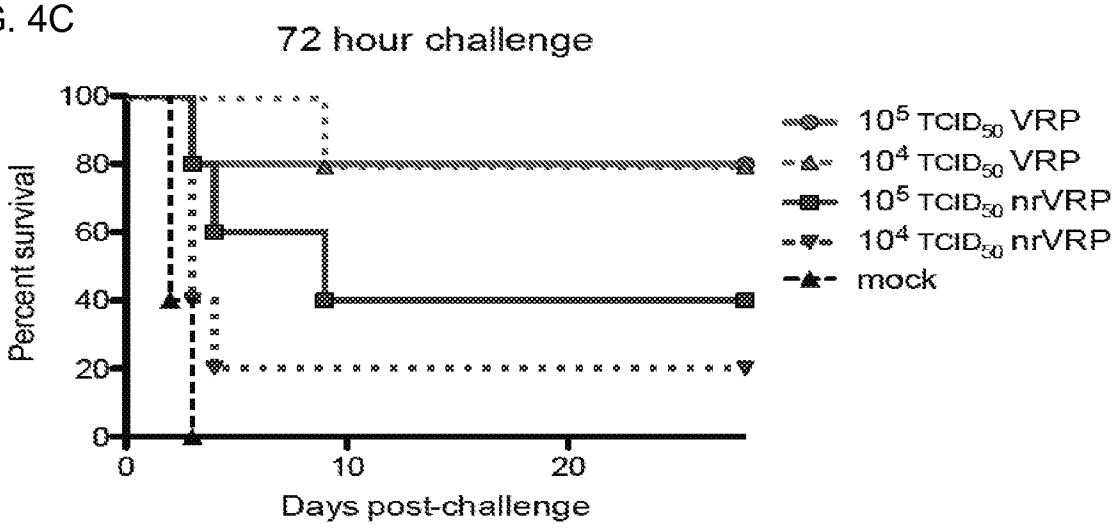
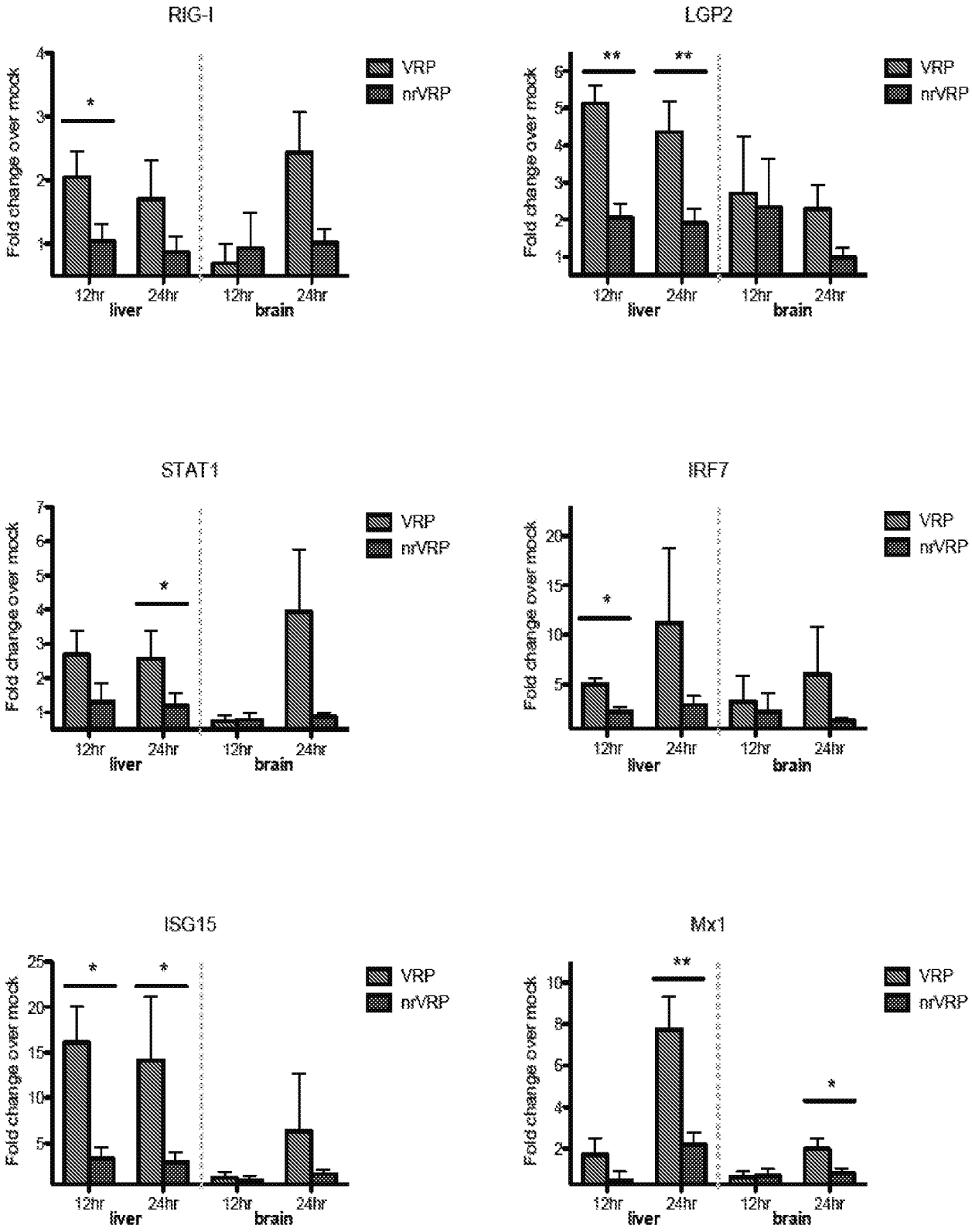


FIG. 5


RIG-I
LGP2
STAT1
IRF7
ISG15
Mx1
Fold change over mock
VRP
nrVRP
12hr
24hr
liver
brain
*
**

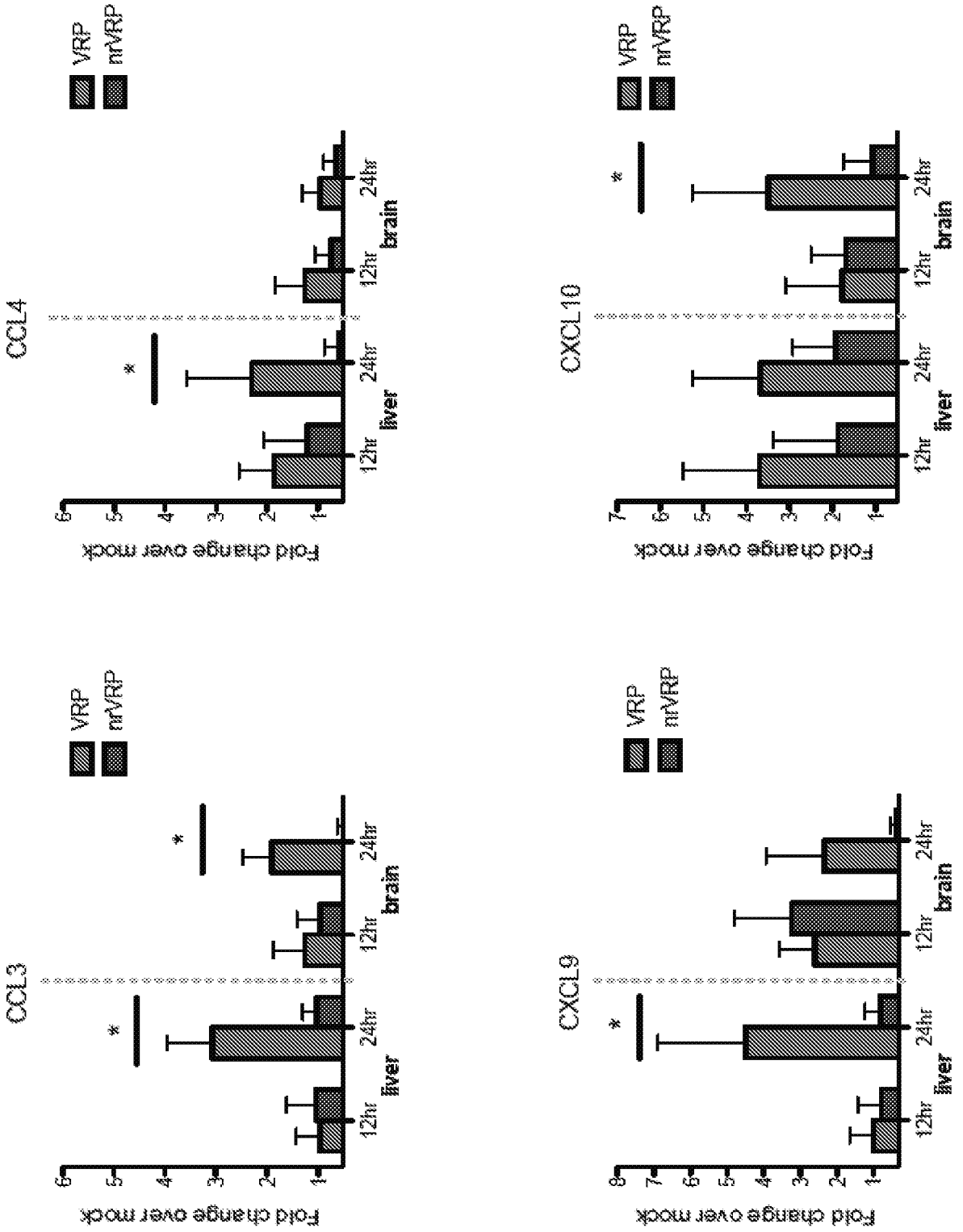

CCL4
CXCL10
CCL3
CXCL9
VRP
mVRP
Fold change over mock
12hr
24hr
liver
brain
*

FIG. 6