Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2967720 C 2024/01/02

(11)(21) **2 967 720**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) **Date de dépôt PCT/PCT Filing Date:** 2015/11/12
(87) **Date publication PCT/PCT Publication Date:** 2016/05/19
(45) **Date de délivrance/Issue Date:** 2024/01/02
(85) **Entrée phase nationale/National Entry:** 2017/05/12
(86) **N° demande PCT/PCT Application No.:** EP 2015/076458
(87) **N° publication PCT/PCT Publication No.:** 2016/075250
(30) **Priorité/Priority:** 2014/11/13 (US62/079,493)

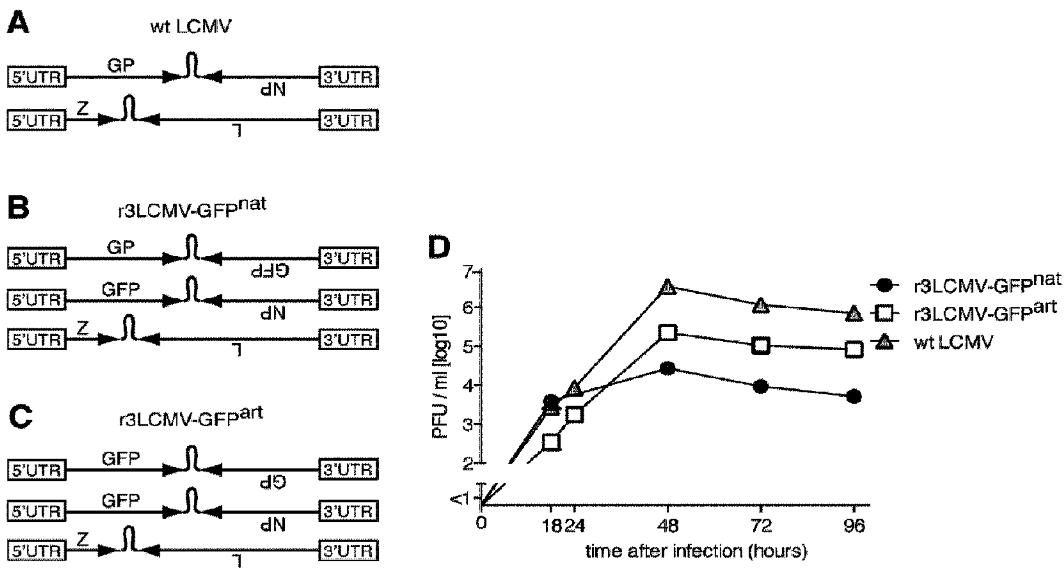
(51) **Cl.Int./Int.Cl.** ***C12N 15/86*** (2006.01), ***A61K 35/76*** (2015.01), ***A61K 39/00*** (2006.01)

(72) **Inventeurs/Inventors:**
PINSCHEWER, DANIEL DAVID, CH;
MERKLER, DORON, CH;
KALLERT, SANDRA MARGARETE, CH;
KREUTZFELDT, MARIO, CH;
DARBRE ABDELRAHMAN, STEPHANIE GABRIELLE, CH;
PAGE, NICOLAS JEAN, FR

(73) **Propriétaire/Owner:**
UNIVERSITE DE GENEVE, CH

(74) **Agent:** OSLER, HOSKIN & HARCOURT LLP

(54) **Titre : ARENAVIRUS TRI-SEGMENTES EN TANT QUE VECTEURS DE VACCINS**
(54) **Title: TRI-SEGMENTED ARENAVIRUSES AS VACCINE VECTORS**



(57) **Abrégé/Abstract:**
The present application relates to arenaviruses with rearrangements of their open reading frames ("ORF") in their genomes. In particular, described herein is a modified arenavirus genomic segment, wherein the arenavirus genomic segment is engineered to carry a viral ORF in a position other than the wild-type position of the ORF. Also described herein are trisegmented arenavirus particles comprising one L segment and two S segments or two L segments and one S segment. The arenavirus, described herein may be suitable for vaccines and/or treatment of diseases and/or for the use in immunotherapies.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 May 2016 (19.05.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/075250 A1

(51) International Patent Classification:
C12N 15/86 (2006.01) *A61K 39/00* (2006.01)
A61K 35/76 (2015.01)

(21) International Application Number:
PCT/EP2015/076458

(22) International Filing Date:
12 November 2015 (12.11.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/079,493 13 November 2014 (13.11.2014) US

(71) Applicant: UNIVERSITÉ DE GENÈVE [CH/CH]; Rue du Général Dufour 24, CH-1211 Genève 4 (CH).

(72) Inventors: PINSCHEWER, Daniel David; Im Zehntenfrei 21a, CH-4102 Binningen (CH). MERKLER, Doron; Rue Monnier 1, CH-1206 Geneva (CH). KALLERT, Sandra Margarete; Delsbergerallee 50, CH-4053 Basel (CH). KREUTZFELDT, Mario; Avenue Industrielle 9, CH-1227 Carouge (CH). DARBRE ABDELRAHMAN, Stephanie Gabrielle; Chemin de la Chaumière 1, CH-1010 Lausanne (CH). PAGE, Nicolas Jean; allée du Clos Charlemagne 89, F-74130 Bonneville (FR).

(74) Agent: JONES DAY; Prinzregentenstraße 11, 80538 München (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title**: TRI-SEGMENTED ARENAVIRUSES AS VACCINE VECTORS

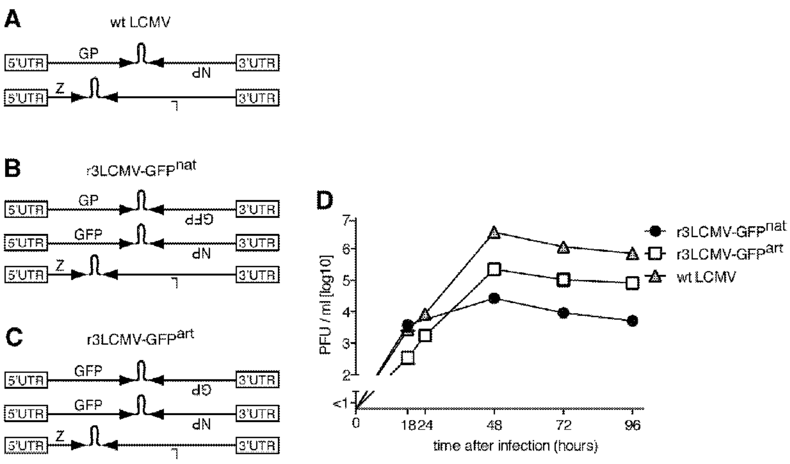


Figure 1

(57) **Abstract**: The present application relates to arenaviruses with rearrangements of their open reading frames ("ORF") in their genomes. In particular, described herein is a modified arenavirus genomic segment, wherein the arenavirus genomic segment is engineered to carry a viral ORF in a position other than the wild-type position of the ORF. Also described herein are trisegmented arenavirus particles comprising one L segment and two S segments or two L segments and one S segment. The arenavirus, described herein may be suitable for vaccines and/or treatment of diseases and/or for the use in immunotherapies.

TRI-SEGMENTED ARENAVIRUSES AS VACCINE VECTORS

1. INTRODUCTION

[0001] The present application relates to arenaviruses with rearrangements of their open reading frames ("ORF") in their genomes. In particular, described herein is a modified arenavirus genomic segment, wherein the arenavirus genomic segment is engineered to carry a viral ORF in a position other than the wild-type position of the ORF.

[0002] Also described herein are tri-segmented arenavirus particles comprising one L segment and two S segments or two L segments and one S segment. The arenavirus, described herein may be suitable for vaccines and/or treatment of diseases and/or for the use in immunotherapies.

2. BACKGROUND

2.1 Lymphocytic Choriomeningitis Virus Research and Human Disease

[0003] Lymphocytic choriomeningitis virus (LCMV), a member of the family arenaviridae, is a prototypic mouse model virus in research on viral infections. Since its isolation in the 1930s (Rivers and McNair Scott, 1935, Science, 81(2105): 439-440) studies using this virus have uncovered many key concepts in viral immunology and pathogenesis (summarized in Zinkernagel, 2002, Curr Top Microbiol Immunol, 263:1-5; Oldstone, 2002, Curr Top Microbiol Immunol, 263:83-117). LCMV has been extensively used to investigate viral molecular biology and immune responses particularly in the context of persistent infection. The natural host of LCMV are mice, however, several reports revealed that LCMV might also be a neglected human pathogen (Barton, 1996, Clin. Infect. Dis, 22(1):197; Wright *et al.,* 1997, Pediatrics 100(1): E9). Moreover, numerous other members of the arenavirus family have been found in rodent populations around the world. In addition to the Old World arenavirus Lassa virus (LASV), which can be found in Africa, several New World arenaviruses like Junin (JUNV), Guanarito or Machupo are prevalent in diverse rodent populations of South America (Johnson *et al.,* 1966, Am J Trop Med Hyg, 15(1): 103-106; Tesh *et al.,* 1993, Am J Trop Med Hyg 49(2):227-235; Mills *et al.,* 1994, Trop Med Hyg 51(5): 554-562). Upon transmission to humans, many of those viruses can cause viral hemorrhagic fever associated with high mortality (Geisbert and Jahrling, 2004, Nat Med 10(12 Suppl): S110-121).

Date Reçue/Date Received 2022-02-07

2.2 Genomic Organization of Lymphocytic Choriomeningitis Virus

[0004] Arenaviruses are enveloped viruses. Their genome consists of two segments of single-stranded RNA of negative sense (L: 7.2 kb, S: 3.4 kb). Each segment encodes for two viral genes in opposite orientations. The short segment (S segment) encodes the viral glycoprotein (GP) precursor (GP-C; 75 kDa) and the nucleoprotein (NP; 63 kDa) (Salvato *et al.,* 1988, Virology 164(2): 517-522). The long segment (L segment) expresses the RNA-dependent RNA polymerase (RdRp; L protein; approximately 200 kDa) and the matrix protein Z (protein Z), a RING finger protein (11 kDa) (Fig. 1A) (Salvato *et al.,* 1988, Virology 164(2): 517-522). The GP precursor GP-C is post-translationally cleaved into GP-1 and GP-2, which remain non-covalently associated (Buchmeier and Oldstone 1979, Virology 99(1): 111-120). Trimers of GP-1 and GP-2 are assembled as spikes on the surface of virions and are essential for mediating entry into the host cells by interaction with the cellular surface receptors. Binding and entry of the virus into host cells was long claimed to be mediated by interaction of the LCMV GP with the cellular receptor α-Dystroglycan as the only cellular receptor for LCMV (Cao *et al.,* 1998, Science, 282(5396):2079-2081). Only very recently three additional human molecules (Axl and Tyro3 from the TAM family and dendritic cell-specific intracellular adhesion molecule 3-grabbing nonintegrin) were postulated as additional receptors for LCMV and LASV, a close relative of LCMV, which enable entry of LCMV into cells independently of α-Dystroglycan (Shimojima and Kawaoka 2012, J Vet Med, 74(10):1363-1366; Shimojima *et al.,* 2012, J Virol 86(4):2067-2078). NP binds to the viral RNA, forming the nucleocapsid, which serves as a template for the viral L protein. The nucleocapsid associated with the viral L protein forms the so-called ribonucleoprotein complex, which is active both in replication and transcription and represents the minimum unit of viral infectivity. It has been shown, that NP and the L protein are the minimal trans-acting factors necessary for viral RNA transcription and replication (Lee *et al.,* 2000, J Virol 74(8): 3470-3477). The two genes on each segment are separated by a non-coding intergenic region (IGR) and flanked by 5' and 3' untranslated regions (UTR). The IGR forms a stable hairpin structure and has been shown to be involved in structure-dependent termination of viral mRNA transcription (Pinschewer *et al.,* 2005, J Virol 79(7): 4519-4526). The terminal nucleotides of the UTR show a high degree of complementarity, resulting in the formation of secondary structures. These panhandle structures are known to serve as the viral promoter for transcription and replication, and their analysis by site-directed mutagenesis has revealed sequence- and structure-dependence, tolerating not even minor sequence changes (Perez and de la Torre, 2003, Virol 77(2): 1184-1194).

2.3 Reverse Genetic System

[0005] Isolated and purified RNAs of negative-strand viruses like LCMV cannot directly serve as mRNA *i.e.,* cannot be translated when introduced into cells. Consequently transfection of cells with viral RNA does not lead to production of infectious viral particles. In order to generate infectious viral particles of negative-stranded RNA viruses from cDNA in cultured permissive cells, the viral RNA segment(s) must be trans-complemented with the minimal factors required for transcription and replication. With the help of a minigenome system which has been published several years ago, viral cis-acting elements and transacting factors involved in transcription, replication and formation of viral particles could finally be analyzed (Lee *et al.,* 2000, J Virol 74(8): 3470-3477; Lee *et al.,* 2002, J Virol 76(12): 6393-6397; Perez and de la Torre 2003, J Virol 77(2): 1184-1194; Pinschewer *et al.,* 2003, J Virol 77(6): 3882-3887; Pinschewer *et al.,* 2005, J Virol 79(7): 4519-4526.). Also for other arenaviruses like LASV and Tacaribe virus reverse genetic systems have been established (Lopez *et al.,* 2001, J Virol 75(24): 12241-12251; Hass *et al.,* 2004, J Virol 78(24): 13793-13803). Two publications showed the recovery of infectious LCMV entirely from cDNA using pol-I/-II or T7/pol-II-driven plasmids, respectively (referred to as "viral rescue") (Flatz *et al.,* 2006, Proc Natl Acad Sci U S A 103(12): 4663-4668; Sanchez and de la Torre, 2006, Virology 350(2): 370-380).

2.4 Recombinant LCMV Expressing Genes of Interest

[0006] The generation of recombinant negative-stranded RNA viruses expressing foreign genes of interest has been pursued for a long time. Different strategies have been published for other viruses (Garcia-Sastre *et al.,* 1994, J Virol 68(10): 6254-6261; Percy *et al.,* 1994, J Virol 68(7): 4486-4492; Flick and Hobom, 1999, Virology 262(1): 93-103; Machado *et al.,* 2003, Virology 313(1): 235-249). In the past it has been shown that it is possible to introduce additional foreign genes into the genome of bi-segmented LCMV particles (Emonet *et al.,* 2009, PNAS, 106(9):3473-3478). Two foreign genes of interest were inserted into the bi-segmented genome of LCMV, resulting in tri-segmented LCMV particles (r3LCMV) with two S segments and one L segment. In the tri-segmented virus, published by Emonet *et al.,* (2009), both NP and GP were kept in their respective natural position in the S segment and thus were expressed under their natural promoters in the flanking UTR (Fig. 1B). However, the present application reveals that the tri-segmented LCMV particle disclosed by Emonet *et al.,* assembles predominately bi-segmented particles (*i.e.,* the arenavirus only packages one instead of two S segments), resulting in attenuated growth and strong selection pressure to recombine the two S segments. As further shown in the present application, such recombination is reproducibly found and results in phenotypic reversion to wild-type virus and transgene loss.

2.5 Replication-defective Arenavirus

[0007] Recently, it has been shown that an infectious arenavirus particle can be engineered to contain a genome with the ability to amplify and express its genetic material in infected cells but unable to produce further progeny in normal, not genetically engineered cells (*i.e.,* an infectious, replication-deficient arenavirus particle) (International Publication No.: WO 2009/083210 A1 and International Publication No.: WO 2014/140301 A1).

3. SUMMARY OF THE INVENTION

[0008] The present application, relates to arenaviruses with rearrangements of their ORFs in their genomes. In particular, the present application relates to an arenavirus genomic segment that has been engineered to carry an arenavirus ORF in a position other than the wild-type position. The present application also provides a tri-segmented arenavirus particle comprising one L segment and two S segments or two L segments and one S segment that do not recombine into a replication-competent bi-segmented arenavirus particle. The present application demonstrates that the tri-segmented arenavirus particle can be engineered to improve genetic stability and ensure lasting transgene expression.

[0009] In certain embodiments, a viral vector as provided herein is infectious, *i.e.*, is capable of entering into or injecting its genetic material into a host cell. In certain more specific embodiments, a viral vector as provided herein is infectious, *i.e.*, is capable of entering into or injecting its genetic material into a host cell followed by amplification and expression of its genetic information inside the host cell. In certain embodiments, the viral vector is an infectious, replication-deficient arenavirus viral vector engineered to contain a genome with the ability to amplify and express its genetic information in infected cells but unable to produce further infectious progeny particles in normal, not genetically engineered cells. In certain embodiments, the infectious arenavirus viral vector is replication-competent and able to produce further infectious progeny particles in normal, not genetically engineered cells. In certain more specific embodiments, such a replication-competent viral vector is attenuated relative to the wild type virus from which the replication-competent viral vector is derived.

3.1 Non-natural Open Reading Frame

Accordingly, in one aspect, provided herein is an arenavirus genomic segment. In certain embodiments, the genomic segment is engineered to carry a viral ORF in a position other than the wild-type position of the ORF. In some embodiments, the arenavirus genomic segment is selected from the group consisting of:

(i) an S segment, wherein the ORF encoding the NP is under control of an arenavirus 5' UTR;

(ii) an S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 5' UTR;

(iii) an S segment, wherein the ORF encoding the L protein is under control of an arenavirus 5' UTR;

(iv) an S segment, wherein the ORF encoding the GP is under control of an arenavirus 3' UTR;

(v) an S segment, wherein the ORF encoding the L protein is under control of an arenavirus 3' UTR;

(vi) an S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR;

(vii) an L segment, wherein the ORF encoding the GP is under control of an arenavirus 5' UTR;

(viii) an L segment, wherein the ORF encoding the NP is under control of an arenavirus 5' UTR;

(ix) an L segment, wherein the ORF encoding the L protein is under control of an arenavirus 5' UTR;

(x) an L segment, wherein the ORF encoding the GP is under control of an arenavirus 3' UTR;

(xi) an L segment, wherein the ORF encoding the NP is under control of an arenavirus 3' UTR; and

(xii) an L segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR.

[0010] In some embodiments, the arenavirus 3' UTR is the 3' UTR of the arenavirus S segment or the arenavirus L segment. In certain embodiments, the arenavirus 5' UTR is the 5' UTR of the arenavirus S segment or the arenavirus L segment.

[0011] Also provided herein is an isolated cDNA of an arenavirus genomic segment provided herein. Also provided herein, is a DNA expression vector comprising a cDNA of the arenavirus genomic segment.

[0012] Also provided herein, is a host cell comprising the arenavirus genomic segment, a cDNA of the arenavirus genomic segment, or the vector comprising a cDNA of the arenavirus genomic segment.

[0013] Also provided herein, is an arenavirus particle comprising the arenavirus genomic segment and a second arenavirus genomic segment so that the arenavirus particle comprises an S segment and an L segment.

[0014] In certain embodiments, the arenavirus particle is infectious and replication competent. In some embodiments, the arenavirus particle is attenuated. In other embodiments, the arenavirus particle is infectious but unable to produce further infectious progeny in non-complementing cells.

[0015] In certain embodiments, at least one of the four ORFs encoding GP, NP, Z protein, and L protein is removed or functionally inactivated.

[0016] In certain embodiments, at least one of the four ORFs encoding GP, NP, Z protein and L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In other embodiments, only one of the four ORFs encoding GP, NP, Z protein and L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In a more specific embodiment, the ORF encoding GP is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In other embodiments, the ORF encoding NP is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In some embodiments, the ORF encoding the Z protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In other embodiments, the ORF encoding the L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus.

[0017] In certain embodiments, the heterologous ORF encodes a reporter protein. In some embodiments, the heterologous ORF encodes an antigen derived from an infectious organism, tumor, or allergen. In other embodiments, the heterologous ORF encoding an antigen is selected from human immunodeficiency virus antigens, hepatitis C virus antigens, hepatitis B surface antigen, varizella zoster virus antigens, cytomegalovirus antigens, mycobacterium tuberculosis antigens, and tumor associated antigens.

[0018] In certain embodiments, the growth or infectivity of the arenavirus particle is not affected by the heterologous ORF from an organism other than an arenavirus.

[0019] Also provided herein is a method of producing the arenavirus genomic segment. In certain embodiments, the method comprises transcribing the cDNA of the arenavirus genomic segment.

[0020] Also provided herein is a method of generating the arenavirus particle. In certain embodiments the method of generating the arenavirus particle comprises:

(i) transfecting into a host cell the cDNA of the arenavirus genomic segment;

(ii) transfecting into the host cell a plasmid comprising the cDNA of the second arenavirus genomic segment;

(iii) maintaining the host cell under conditions suitable for virus formation; and

(iv) harvesting the arenavirus particle.

[0021] In certain embodiments, the transcription of the L segment and the S segment is performed using a bidirectional promoter.

[0022] In certain embodiments, the method further comprises transfecting into a host cell one or more nucleic acids encoding an arenavirus polymerase. In yet more specific embodiments, the polymerase is the L protein. In other embodiments, the method further comprises transfecting into the host cell one or more nucleic acids encoding the NP.

[0023] In certain embodiments, transcription of the L segment, and the S segment are each under the control of a promoter selected from the group consisting of:

(i) a RNA polymerase I promoter;

(ii) a RNA polymerase II promoter; and

(iii) a T7 promoter.

[0024] In another embodiment, provided herein is a vaccine comprising an arenavirus particle, wherein at least one of the four ORFs encoding GP, NP, Z protein, and L protein is removed or functionally inactivated; or wherein at least one ORF encoding GP, NP, Z protein, and L protein is removed and replaced with a heterologous ORF from another organism other than an arenavirus; or wherein only one of the four ORFs encoding GP, NP, Z protein, and L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In more specific embodiments, the vaccine further comprises a pharmaceutically acceptable carrier.

[0025] In another embodiment, provided herein is a pharmaceutical composition comprising an arenavirus particle, wherein at least one of the four ORFs encoding GP, NP, Z protein, and L protein is removed or functionally inactivated; or wherein at least one ORF encoding GP, NP, Z protein, and L protein is removed and replaced with a heterologous ORF from another organism other than an arenavirus; or wherein only one of the four ORFs encoding GP, NP, Z protein, and L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In more specific embodiments, the pharmaceutically acceptable carrier further comprises a pharmaceutically acceptable carrier.

[0026] In certain embodiments, the arenavirus genomic segment or the arenavirus particle is derived from LCMV. In some embodiments, the arenavirus genomic segment or arenavirus particle is derived from the LCMV MP strain, Armstrong strain, or Armstrong Clone 13 strain. In other embodiments, the arenavirus genomic segment or the arenavirus particle is derived from Junin virus vaccine Candid #1, or Junin virus vaccine XJ Clone 3 strain.

3.2 Tri-segmented arenavirus

[0027] In one aspect, provided herein is a tri-segmented arenavirus particle comprising one L segment and two S segments. In some embodiments, propagation of the tri-segmented arenavirus particle does not result in a replication-competent bi-segmented viral particle after 70 days of persistent infection in mice lacking type I interferon receptor, type II interferon receptor and recombination activating gene 1 (RAG1), and having been infected with 10^4 PFU of the tri-segmented arenavirus particle. In certain embodiments, inter-segmental recombination of the two S segments, uniting two arenavirus ORFs on only one instead of two separate segments, abrogates viral promoter activity.

[0028] In another aspect, provided herein is a tri-segmented arenavirus particle comprising two L segments and one S segment. In certain embodiments, propagation of the tri-segmented arenavirus particle does not result in a replication-competent bi-segmented viral particle after 70 days of persistent infection in mice lacking type I interferon receptor, type II interferon receptor and recombination activating gene 1 (RAG1), and having been infected with 10^4 PFU of the tri-segmented arenavirus particle. In certain embodiments, inter-segmental recombination of the two L segments, uniting two arenavirus ORFs on only one instead of two separate segments, abrogates viral promoter activity.

[0029] In certain embodiments, one of the two S segments is selected from the group consisting of:

(i) an S segment, wherein the ORF encoding the NP is under control of an arenavirus 5' UTR

(ii) an S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 5' UTR;

(iii) an S segment, wherein the ORF encoding the L protein is under control of an arenavirus 5' UTR;

(iv) an S segment, wherein the ORF encoding the GP is under control of an arenavirus 3' UTR;

(v) an S segment, wherein the ORF encoding the L protein is under control of an arenavirus 3' UTR; and

(vi) an S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR.

[0030] In certain embodiments, one of the two L segments is selected from the group consisting of:

(i) an L segment, wherein the ORF encoding the GP is under control of an arenavirus 5' UTR;

(ii) an L segment, wherein the ORF encoding the NP is under control of an arenavirus 5' UTR;

(iii) an L segment, wherein the ORF encoding the L protein is under control of an arenavirus 5' UTR;

(iv) an L segment, wherein the ORF encoding the GP is under control of an arenavirus 3' UTR;

(v) an L segment, wherein the ORF encoding the NP is under control of an arenavirus 3' UTR; and

(vi) an L segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR.

[0031] In certain embodiments, the tri-segmented arenavirus particle 3' UTR is the 3' UTR of the arenavirus S segment or the arenavirus L segment. In other embodiments, the tri-segmented arenavirus particle 5' UTR is the 5' UTR of the arenavirus S segment or the arenavirus L segment.

[0032] In certain embodiments, the two S segments comprise (i) one or two heterologous ORFs from an organism other than an arenavirus; or (ii) one or two duplicated arenavirus ORFs; or (iii) one heterologous ORF from an organism other than an arenavirus and one duplicated arenavirus ORF.

[0033] In certain embodiments, the two L segments comprise (i) one or two heterologous ORFs from an organism other than an arenavirus; or (ii) one or two duplicated arenavirus ORFs; or (iii) one heterologous ORF from an organism other than an arenavirus and one duplicated arenavirus ORF.

[0034] In certain embodiments, the heterologous ORF encodes an antigen derived from an infectious organism, tumor, or allergen. In other embodiments, the heterologous ORF encoding an antigen is selected from human immunodeficiency virus antigens, hepatitis C virus antigens, hepatitis B surface antigen, varizella zoster virus antigens, cytomegalovirus antigens, mycobacterium tuberculosis antigens, and tumor associated antigens.

[0035] In certain embodiments, at least one heterologous ORF encodes a fluorescent protein. In other embodiments the fluorescent protein is a green fluorescent protein (GFP) or red fluorescent protein (RFP).

[0036] In certain embodiments, the tri-segmented arenavirus particle comprises all four arenavirus ORFs. In some embodiments the tri-segmented arenavirus particle is infectious and replication competent.

[0037] In certain embodiments, the tri-segmented arenavirus particle lacks one or more of the four arenavirus ORFs. In other embodiments, the tri-segmented arenavirus particle is infectious but unable to produce further infectious progeny in non-complementing cells.

[0038] In certain embodiments, the tri-segmented arenavirus particle lacks one of the four arenavirus ORFs, wherein the tri-segmented arenavirus particle is infectious but unable to produce further infectious progeny in non-complementing cells.

[0039] In some embodiments, the tri-segmented arenavirus particle lacks the GP ORF.

[0040] In a further aspect, provided herein is a tri-segmented arenavirus particle comprising one L segment and two S segments. In certain embodiments, a first S segment is engineered to carry an ORF encoding GP in a position under control of an arenavirus 3' UTR and an ORF encoding a first gene of interest in a position under control of an arenavirus 5' UTR. In some embodiments, a second S segment is engineered to carry an ORF encoding the NP in a position under control of an arenavirus 3' UTR and an ORF encoding a second gene of interest in a position under control of an arenavirus 5' UTR.

[0041] In yet another aspect, provided herein, is a tri-segmented arenavirus particle comprising one L segment and two S segments. In certain embodiments, a first S segment is engineered to carry an ORF encoding GP in a position under control of an arenavirus 5' UTR and an ORF encoding a first gene of interest in a position under control of an arenavirus 3' UTR. In some embodiments, a second S segment is engineered to carry an ORF encoding NP in a position under control of an arenavirus 5' UTR and an ORF encoding a second gene of interest in a position under control of an arenavirus 3' UTR.

[0042] In certain embodiments, the gene of interest encodes an antigen derived from an infectious organism, tumor, or allergen. In other embodiments, the gene of interest encodes an antigen selected from human immunodeficiency virus antigens, hepatitis C virus antigens, hepatitis B surface antigen, varizella zoster virus antigens, cytomegalovirus antigens, mycobacterium tuberculosis antigens, and tumor associated antigens. In yet another embodiment, at least one gene of interest encodes a fluorescent protein. In a specific embodiment, the fluorescent protein is GFP or RFP.

[0043] Also provided herein is an isolated cDNA of the genome of the tri-segmented arenavirus particle. Also provided herein, is a DNA expression vector comprising a cDNA of the genome of the tri-segmented arenavirus particle. Also provided herein is one or more

DNA expression vectors comprising either individually or in their totality the cDNA of the tri-segmented arenavirus.

[0044] Also provided herein, is a host cell comprising the tri-segmented arenavirus particle, the cDNA of the genome of the tri-segmented arenavirus particle, or the vector comprising the cDNA of the genome of the tri-segmented arenavirus particle.

[0045] In certain embodiments, the tri-segmented arenavirus particle is attenuated

[0046] Also provided herein is a method of generating the tri-segmented arenavirus particle. In certain embodiments the method of generating the arenavirus particle comprises:

(i) transfecting into a host cell one or more cDNAs of one L segment and two S segments;

(ii) maintaining the host cell under conditions suitable for virus formation; and

(iii) harvesting the arenavirus particle.

[0047] Also provided herein is a method of generating the tri-segmented arenavirus particle. In certain embodiments the method of generating the tri-segmented arenavirus particle comprises:

(i) transfecting into a host cell one or more cDNAs of two L segments and one S segment;

(ii) maintaining the host cell under conditions suitable for virus formation; and

(iii) harvesting the arenavirus particle.

[0048] In certain embodiments, the transcription of the one L segment and two S segment is performed using a bidirectional promoter. In some embodiments, the transcription of the two L segments and one S segment is performed using a bidirectional promoter.

[0049] In certain embodiments, the method further comprises transfecting into a host cell one or more nucleic acids encoding an arenavirus polymerase. In yet more specific embodiments, the polymerase is the L protein. In other embodiments, the method further comprises transfecting into the host cell one or more nucleic acids encoding the NP protein.

[0050] In certain embodiments, transcription of the one L segment, and two S segments are each under the control of a promoter selected from the group consisting of:

(i) a RNA polymerase I promoter;

(ii) a RNA polymerase II promoter; and

(iii) a T7 promoter.

[0051] In certain embodiments, transcription of the two L segments, and one S segment are each under the control of a promoter selected from the group consisting of:

(i) a RNA polymerase I promoter;

(ii) a RNA polymerase II promoter; and

(iii) a T7 promoter.

[0052] In certain embodiments, the tri-segmented arenavirus particle has the same tropism as the bi-segmented arenavirus particle. In other embodiments, the tri-segmented arenavirus particle is replication deficient.

[0053] In another embodiment, provided herein is a vaccine comprising a tri-segmented arenavirus particle and a pharmaceutically acceptable carrier.

[0054] In another embodiment, provided herein is a pharmaceutical composition comprising a tri-segmented arenavirus particle and a pharmaceutically acceptable carrier.

[0055] In certain embodiments, the tri-segmented arenavirus particle is derived from LCMV. In some embodiments, the tri-segmented arenavirus particle is derived from the LCMV MP strain, Armstrong strain, or Armstrong Clone 13 strain. In other embodiments, the tri-segmented arenavirus particle is derived from Junin virus vaccine Candid #1, or Junin virus vaccine XJ Clone 3 strain.

3.3 Conventions and Abbreviations

Abbreviation	Convention
APC	Antigen presenting cell
art	Artificial
CAT	Chloramphenicol acetyltransferase
CMI	cell-mediated immunity
CD8	Cluster of differentiation 8
CD4	Cluster of differentiation 4
GFP	Green fluorescent protein
GP	Glycoprotein
IGR	Intergenic region
JUNV	Junin virus
LCMV	Lymphocytic choriomeningitis virus

Abbreviation	Convention
L protein	RNA-dependent RNA polymerase
L segment	Long segment
MHC	Major Histocompatibility Complex
Z protein	Matrix protein Z
nat	Natural
NP	Nucleoprotein
ORF	Open reading frame
RFP	Red fluorescent protein
r2JUNV	Recombinant bi-segmented JUNV
r3JUNV	Recombinant tri-segmented JUNV
r2LCMV	Recombinant bi-segmented LCMV
r3LCMV	Recombinant tri-segmented LCMV
S segment	Short segment
UTR	Untranslated region
VSV	Vesicular Stomatitis Virus

4. BRIEF DESCRIPTION OF THE FIGURES

[0056] Figure 1: Recombinant tri-segmented viruses show impaired growth compared to wild-type LCMV independently of the position of the GP ORF in the genome. (A-C) Schematic representation of the genomic organization of bi- and tri-segmented LCMV. The bi-segmented genome of wild-type LCMV consists of one S segment encoding the GP and NP and one L segment encoding the Z protein and the L protein (A). Both segments are flanked by the respective 5' and 3' UTRs. The genome of recombinant tri-segmented LCMVs (r3LCMV) consists of one L and two S segments with one position where to insert a gene of interest (here GFP) into each one of the S segments. (B) r3LCMV-GFP^{natural} (nat) has all viral genes in their natural position whereas the GP ORF in r3LCMV-GFP^{artificial} (art) is artificially juxtaposed to and expressed under control of the 3' UTR (C). (D) Growth kinetics of the indicated viruses in BHK-21 cells, infected at a multiplicity of infection (moi) of 0.01 (wild-type LCMV: grey triangles; r3LCMV-GFP^{nat}: black circles; r3LCMV-GFP^{art}: white squares). Supernatant was taken at the indicated time points after infection and viral titers were determined by focus forming assay. Symbols and bars represent the mean±SEM of three replicates per group. Error bars are hidden in the symbol size.

[0057] Figure 2: Tri-segmented virus preparations contain a majority of bi-segmented replication-deficient particles (r2LCMV). (A) r2LCMV (white bars), r3LCMV-GFP/RFP^{art} (black bars, GFP-GP, RFP-NP) and r3LCMV-GFP/RFP^{nat} (grey bars, GP-GFP, RFP-NP) were grown on wild-type BHK-21 cells and the infectivity of supernatant was determined on wild-type non-complementing BHK-21 cells (BHK21), GP-expressing (BHK-GP) or NP-expressing (BHK-NP) BHK-21 cells. Titers on BHK-21 and BHK-GP cells were determined by staining NP-positive viral foci. Titers on NP-complementing BHK-21 cells were determined by counting GP-positive foci. Titers were normalized to the average titer obtained when assessed on BHK-21 cells, and thus are expressed as a multiple thereof. Bars represent the mean±SEM of six replicates per group. ns.: not statistically significant ($p \geq 0.05$); **: $p < 0.01$ by 1-way ANOVA followed by Dunnett's post-test using r2LCMV as a reference. (B) r2LCMV (left plot) or r3LCMV-GFP/RFP^{art} (middle and right plot) were grown on wild-type BHK-21 cells (BHK21; left and middle plot) or NP-expressing BHK-21 cells (BHK-NP, right plot) and fluorescence was assessed 12 hours after infection by flow cytometry. r2LCMV infected cells were used as gating control. One representative plot per condition is shown. (C) Quantification of GFP+, RFP+ or GFP+RFP+ double positive cells 12 hours after infection with r3LCMV-GFP/RFP^{art} on BHK-21 or BHK-NP cells. Bars represent the mean±SEM of three replicates per group. ns.: not statistically significant ($p \geq 0.05$); ***: $p < 0.001$ by unpaired two-tailed student's *t* test.

[0058] Figure 3: Design and growth kinetics of recombinant tri-segmented viruses carrying a partially codon-optimized GP ORF or a genetic tag in the IGR of the S segment. (A) Schematic of genetically engineered S segment wherein the 255 C-terminal base pairs of GP are codon-optimized and NP is replaced for GFP (GP ORF referred to as "WE/WET"). Growth kinetics of the tri-segmented r3LCMV-WEWET/GFP^{nat} consisting of two S and one L segment as detailed in Fig. 1B, with modification of the GP-containing S segment as shown in (A) were performed on BHK-21 cells. Supernatant was taken at the indicated time points after infection at moi = 0.01 and viral titers were determined by focus forming assay (B). Symbols and bars represent the mean±SEM of three replicates per group. Error bars are hidden in the symbol size. (C) Schematic of the NP-encoding S segment wherein one base pair of the IGR has been deleted in order to genetically "tag" this non-coding RNA element. The deleted G residue (indicated by an arrow) lies outside the critical stem-loop structure of the IGR. (D) Comparative growth kinetics of tri-segmented viruses with or without genetic tag in the IGR of the NP-encoding S segment (r3LCMV-GFP^{nat}: black circles; r3LCMV-GFP^{nat} IGR*: white circles) were performed on BHK-21 cells at a moi of 0.01. Supernatant was collected at the indicated time points after infection and viral titers were determined by focus forming assay. Symbols and bars represent the mean±SEM of three replicates per group. Representative data from one of two independent experiments are shown.

[0059] Figure 4: r3LCMV-GFP^{nat} but not r3LCMV-GFP^{art} persistent infection in immunodeficient mice reaches viremia levels equivalent to bi-segmented wild-type virus and

Date Reçue/Date Received 2022-02-07

results in loss of GFP expression. (A) AGRAG mice were infected intravenously with 1x10^4 PFU of r3LCMV-GFP^{nat} (black circles), r3LCMV-GFP^{art} (white squares) or control bi-segmented r2LCMV (grey triangles) and viremia was monitored over time. Symbols represent the mean±SEM of 3-7 mice per group. (B) LCMV viremia on day 127 after intravenous infection of AGRAG mice with 1x10^4 PFU of r3LCMV-GFP^{nat} or r3LCMV-GFP^{art} is shown. Immunofocus assays were performed to detect either nucleoprotein NP (grey circles) or GFP (white circles). Symbols represent individual mice. ns.: not statistically significant ($p \geq 0.05$); ***: $p < 0.001$ (unpaired two-tailed student's t test). (C-E) Blood from AGRAG mice infected with r3LCMV-GFP^{nat}, r3LCMV-GFP^{art} or r2LCMV was analyzed on day 120 after infection by flow cytometry for the presence of GFP+ cells. Monocytes and Macrophages were identified using the gating strategy outlined in (C). One representative FACS plot for each group and one representative histogram overlay of the GFP expression is shown in (D). (E) Quantification of the GFP+ population within the CD11b+ GR1- monocytes/macrophage population. Symbols represent individual mice.

[0060] Figure 5: r3LCMV-GFP^{nat} persistent infection of mice results in S-segment recombination and loss of functional full-length transgenes. Viral RNA was isolated from the serum of AGRAG mice on day 127 after intravenous infection with 1x10^4 PFU r3LCMV-GFP^{nat} or r3LCMV-GFP^{art}. Viral RNA was reverse transcribed and cDNA carrying both NP as well as GP sequences was PCR-amplified with appropriate gene-specific primers. (A) DNA electrophoresis of PCR products obtained subsequent to (+RT, lanes 1-8) or without prior reverse transcription of RNA template (-RT, negative control, lanes 9-12). Serum of a naive animal was used as a separate negative control (n, lane 8) and a plasmid DNA encoding a wild-type LCMV S segment as positive control (p, lane 17). Amplicons of lanes 1-3 were subject to Sanger sequencing. (B) Representative cDNA sequence obtained from animal #3 (r3LCMV-GFP^{nat} #3) revealing a recombined S segment combining NP and GP sequences, two IGRs (bold) and a C-terminal GFP portion (grey highlight) (SEQ ID NO: 17). (C) Schematic of three recombined viral S segment sequences isolated on day 127 after infection, each of them dominating the viral population in one representative AGRAG mouse. The tagged IGR originating from the NP-carrying S segment is marked with a star (*). The stretch that has been sequenced is indicated by a double-arrow (<-->). Base pair (bp) length indications describe the above GFP remnant and truncated (shortened) IGR elements.

[0061] Figure 6: Growth kinetics of recombined virus with two IGRs on the S segment are similar to bi-segmented virus. BHK-21 cells were infected at moi of 0.01 with either bi-segmented LCMV (grey triangles) carrying a wild type S segment, with tri-segmented r3LCMV-GFP^{nat} (black circles) or with r2LCMV_2IGRs (white diamonds) carrying one S segment corresponding to the recombination product recovered from an infected AGRAG mouse (compare Fig. 5). Supernatant was taken at the indicated time points and viral titers were determined by focus forming assay. Symbols and bars represent the mean±SEM of three replicates per group. Error bars and are hidden in the symbol size. ns.: not statistically significant ($p \geq 0.05$); ***: $p < 0.001$ (1-way ANOVA followed by Bonferroni's post-test for multiple comparisons).

[0062] Figure 7: Model for the recombination events accountable for r3LCMV-GP^{nat} transgene loss and postulated mechanism of r3LCMV-GP^{art} genetic stability. This model bases itself upon sequence data of LCMV transcription termination (Meyer and Southern, 1993, J Virol, 67(5):2621-2627) combined with reverse genetic evidence for the IGR as transcription termination signal (Pinschewer *et al.,* 2005, J Virol, 79(7):4519-4526). Together, these findings suggested structure-dependent polymerase pausing when completing the hairpin structure of the IGR. The GFP remnant between the two IGRs in recombined S segments was found to originate from either one or both S segments, fostering the model that polymerase template switch (also referred to as copy-choice) occurred when the polymerase paused, either during genome or antigenome synthesis (below scenarios A and B, respectively). (A) During antigenome synthesis the RNA dependent RNA polymerase (RdRp) initiates at the 3'UTR of a genomic S segment template and then reads through the NP ORF and IGR. At the end of the IGR the polymerase pauses due to the secondary structure ("structure-dependent polymerase pausing"). Stalling of the polymerase facilitates copy choice and continuation of RNA replication on an alternative template (here: GP-encoding S segment genome). Template switch must occur upstream of the GP stop codon, and apparently is most likely to target sequences close to or at the base of the IGR hairpin. Continuing its read through the C-terminus of the second template's GFP, the polymerase then synthesizes a second IGR, the GP ORF and the 5'UTR. (B) During genome synthesis the RdRp initiates RNA synthesis at the 3' end of an antigenomic S segment template containing GP, synthesizes the 5'UTR, GP and most or all of the IGR, followed by structure-dependent polymerase pausing. Copy choice occurs, switching into the C-terminal portion of the GFP ORF near the IGR of an NP-containing S segment. Replication thus adds a fragment of GFP, followed by an IGR in full length, the NP and 3'UTR. (C – D) Template switch analogously to scenarios (A) and (B) can also occur during genome or antigenome synthesis of r3LCMV-GFP^{art}. This process also can combine NP and GP ORFs onto one RNA segment. The latter is, however, made up of two 3' UTRs instead of a 3'UTR and a 5'UTR, which only together form a functional viral promoter. Such molecules can therefore not be amplified by the RdRp and thus do not form recombined replication-competent virus.

[0063] Figure 8: An r3LCMV-OVA^{art} vaccine vector with a genome organization analogous to r3LCMV-GFP^{art} was generated (see Fig. 1C) but with two ovalbumin (OVA) genes instead of the respective GFP genes in the latter virus. C57BL/6 mice were immunized intramuscularly (i.m.) with either 10^4 PFU of r3LCMV-OVA^{art} or with 108 particles of a replication-deficient E1-deleted adenovirus 5-based vector expressing OVA. 8 days later the animals were euthanized and the T cell response elicited in response to the vaccination was analyzed. A: The frequency of OVA-specific CD8+ T cells in spleen was determined using SIINFEKL peptide-loaded MHC class I tetramers. Epitope-specific cell frequencies were determined amongst B220-negative CD8+ lymphocytes. B: The functionality of OVA-specific CD8+ T cells was analyzed by intracellular cytokine assays using SIINFEKL peptide for restimulation. Bars represent the mean+/-SEM of five mice per group. *: $p<0.05$; **: $p>0.01$ by unpaired two-tailed student's t test.

[0064] Figure 9: Trisegmented LCMV induces polyfunctional memory CD8+ T cells. C57BL/6 mice were infected i.v. with $1x10^5$ PFU r3LCMV-OVA or $1x10^8$ PFU rAd-OVA. Spleens were taken 25 days after infection and the functionality of OVA-specific CD8+ T cells was analyzed by intracellular cytokine staining. The cytokine profile (IFN-γ, TNF-α and IL-2) of OVA-specific T cells induced by r3LCMV-OVA (black bars) or rAd-OVA (white bars) is shown as percent of CD8+ T cells (A) or as absolute numbers per spleen (B). Symbols and bars represent the mean±SEM of five mice per group. Unpaired two-tailed student's *t* test was used for statistical analysis, resulting p values were corrected for multiple comparisons by multiplication with the number of comparisons (n=7). One representative of two similar experiments is shown.

[0065] Figure 10: Antigen-encoding LCMV induces specific T cell responses to foreign and autoantigens. C57BL/6 mice were infected i.v. with $1x10^5$ PFU r3LCMV encoding for rat, human or mouse Her2 peptide (A, B and C, respectively). Spleens were taken nine days after infection and the induction of functional antigen-specific CD8+ T cells was analyzed by intracellular cytokine staining and flow cytometry. The cytokine profile (IFN-γ, TNF-α and IL-2) of Her2-specific CD8+ T cells induced by r3LCMV is shown in % of CD8+ T cells. Symbols and bars represent the mean±SEM of three mice per group.

[0066] Figure 11: Interferon-α is induced upon r3LCMV infection but not upon infection with recombinant Adeno- or Vacciniavirus. C57BL/6 mice were infected i.v. with $1x10^5$ PFU r3LCMV-OVA, $1x10^8$ PFU rAd-OVA or $1x10^6$ PFU rVacc-OVA. Blood was collected on the indicated time points after infection and levels of Interferon-α in the serum were determined by ELISA. Symbols and bars represent the mean±SEM of four mice per group. ***: $p < 0.001$ (2-way ANOVA followed by Bonferroni's post-test for multiple comparisons). Representative data from one out of two independent experiments are shown.

[0067] Figure 12: Cell culture growth of r3JUNV-GFP^{art} in comparison to r3JUNV-GFP^{nat} and r2JUNV-wt. r3JUNV-GFP^{art} and r3JUNV-GFP^{nat} were constructed analogously to the respective r3LCMV vectors schematically outlined in Figure 1. To compare their cell culture growth properties 293T cells were infected at multiplicity of infection (MOI) of 0.01 with r2LCMV-wt, r3JUNV-GFP^{art} , and r3JUNV-GFP^{nat} , and supernatant was harvested at the indicated time points. Infectious units (FFU) in supernatant were determined by immunofocus assay. Symbols and bars represent the mean±SEM of three replicates per group and are hidden in the symbol size.

[0068] Figure 13: Trisegmented JUNV are dramatically attenuated in vivo and only lead to detectable viremia upon loss of GFP. (A) AGRAG mice were infected i.v. with 7×10^4 PFU of r3JUNV-GFP^{nat} (grey squares), r3JUNV-GFP^{art} (white triangles) or control bi-segmented r2JUNV strain Candid#1 (black circles), and viremia was monitored over time. Symbols represent individual mice (n=3-7 per group). (B) JUNV viremia was determined on day 120 after intravenous infection of AGRAG mice with 7x104 PFU of r3JUNV-GFP^{nat} or r3JUNV-GFP^{art}. Immunofocus assays were performed to detect either nucleoprotein NP (grey circles) or GFP (white circles). Viral stocks used to inoculate mice were used as a staining control in the assay. Symbols represent individual mice and inocula, respectively.

[0069] Figure 14: Homologous and heterologous prime-boost combinations of trisegmented LCMV- and JUNV-based vaccine vectors induce strong P1A autoantigen-specific CD8+ T cells responses. (A) On day 0 and 35 of the experiment BALB/c mice were immunized with 8.5×10^4 PFU of r3JUNV-P1A^{art} (r3JUNV-P1A) and r3LCMV-P1A^{art} (r3LCMV-P1A) intravenously in the homologous or heterologous combinations indicated in the chart. Epitope-specific CD8+ T cells were stained using P1A epitope-loaded MHC class I tetramers in combination with anti-CD8a antibody. The frequency of P1A-tetramer-binding cells within the CD8+ T cell compartment in peripheral blood (A) and the absolute number of P1A tetramer-binding CD8+ T cells per microliter of peripheral blood (B) was calculated. Symbols represent the mean+/-SEM of 3-5 mice per group and time point.

DETAILED DESCRIPTION OF THE INVENTION

4.1 Arenaviruses with an Open Reading Frame in a Non-natural Position

[0070] Provided herein are arenaviruses with rearrangements of their ORFs. In certain embodiments, such arenaviruses are replication competent and infectious. Genomic sequences of such arenaviruses are provided herein. In one aspect, provided herein is an arenavirus genomic segment, wherein the arenavirus genomic segment is engineered to carry an arenavirus ORF in a position other than the position in which the respective gene is found in viruses isolated from the wild, such as LCMV-MP (see SEQ ID NOs: 4 and 5) (referred to herein as “wild-type position”) of the ORF (*i.e.,* a non-natural position). In one embodiment, the arenavirus particle is an LCMV.

[0071] The wild-type arenavirus genomic segments and ORFs are known in the art. In particular, the arenavirus genome consists of an S segment and an L segment. The S segment carries the ORFs encoding the GP and the NP. The L segment encodes the L protein and the Z protein. Both segments are flanked by the respective 5’ and 3’ UTRs (see Figure 1A). Illustrative wild-type arenavirus genomic segments are provided in SEQ ID NOs: 1-10.

[0072] In certain embodiments, an arenavirus genomic segment can be engineered to carry two or more arenavirus ORFs in a position other than the wild-type position. In other embodiments, the arenavirus genomic segment can be engineered to carry two arenavirus ORFs, or three arenavirus ORFs, or four arenavirus ORFs in a position other than the wild-type position.

[0073] In certain embodiments, an arenavirus genomic segment provided herein can be:

(i) an arenavirus S segment, wherein the ORF encoding the NP is under control of an arenavirus 5’ UTR;

(ii) an arenavirus S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 5’ UTR;

(iii) an arenavirus S segment, wherein the ORF encoding the L protein is under control of an arenavirus 5’ UTR;

(iv) an arenavirus S segment, wherein the ORF encoding the GP is under control of an arenavirus 3’ UTR;

(v) an arenavirus S segment, wherein the ORF encoding the L protein is under control of an arenavirus 3' UTR;

(vi) an arenavirus S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR;

(vii) an arenavirus L segment, wherein the ORF encoding the GP is under control of an arenavirus 5' UTR;

(viii) an arenavirus L segment, wherein the ORF encoding the NP is under control of an arenavirus 5' UTR;

(ix) an arenavirus L segment, wherein the ORF encoding the L protein is under control of an arenavirus 5' UTR;

(x) an arenavirus L segment, wherein the ORF encoding the GP is under control of an arenavirus 3' UTR;

(xi) an arenavirus L segment, wherein the ORF encoding the NP is under control of an arenavirus 3' UTR; and

(xii) an arenavirus L segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR.

[0074] In certain embodiments, the ORF that is in the non-natural position of the arenavirus genomic segment described herein can be under the control of an arenavirus 3' UTR or an arenavirus 5' UTR. In more specific embodiments, the arenavirus 3' UTR is the 3' UTR of the arenavirus S segment. In another specific embodiment, the arenavirus 3' UTR is the 3'UTR of the arenavirus L segment. In more specific embodiments, the arenavirus 5' UTR is the 5' UTR of the arenavirus S segment. In other specific embodiments, the 5' UTR is the 5' UTR of the L segment.

[0075] In other embodiments, the ORF that is in the non-natural position of the arenavirus genomic segment described herein can be under the control of the arenavirus conserved terminal sequence element (the 5'- and 3'-terminal 19-20-nt regions) (see *e.g.*, Perez & de la Torre, 2003, J Virol. 77(2): 1184–1194).

[0076] In certain embodiments, the ORF that is in the non-natural position of the arenavirus genomic segment can be under the control of the promoter element of the 5' UTR (see *e.g.,* Albarino *et al.,* 2011, J Virol., 85(8):4020-4). In another embodiment, the ORF that is in the non-natural position of the arenavirus genomic segment can be under the control of the promoter element of the 3' UTR (see *e.g.,* Albarino *et al.,* 2011, J Virol., 85(8):4020-4). In more specific embodiments, the promoter element of the 5' UTR is the 5' UTR promoter element of the S segment or the L segment. In another specific embodiment, the promoter element of the 3' UTR is the 3' UTR the promoter element of the S segment or the L segment.

[0077] In certain embodiments, the ORF that is in the non-natural position of the arenavirus genomic segment can be under the control of a truncated arenavirus 3' UTR or a truncated arenavirus 5' UTR (see *e.g.,* Perez & de la Torre, 2003, J Virol. 77(2): 1184–1194; Albarino *et al.,* 2011, J Virol., 85(8):4020-4). In more specific embodiments, the truncated 3' UTR is the 3' UTR of the arenavirus S segment or L segment. In more specific embodiments, the truncated 5' UTR is the 5' UTR of the arenavirus S segment or L segment.

[0078] Also provided herein, is an arenavirus particle comprising a first genomic segment that has been engineered to carry an ORF in a position other than the wild-type position of the ORF and a second arenavirus genomic segment so that the arenavirus particle comprises an S segment and an L segment. In specific embodiments, the ORF in a position other than the wild-type position of the ORF is one of the arenavirus ORFs.

[0079] In certain specific embodiments, the arenavirus particle can comprise a full complement of all four arenavirus ORFs. In specific embodiments, the second arenavirus genomic segment has been engineered to carry an ORF in a position other than the wild-type position of the ORF. In another specific embodiment, the second arenavirus genomic segment can be the wild-type genomic segment (*i.e.,* comprises the ORFs on the segment in the wild-type position).

[0080] In certain embodiments, the first arenavirus genomic segment is an L segment and the second arenavirus genomic segment is an S segment. In other embodiments, the first arenavirus genomic segment is an S segment and the second arenavirus genomic segment is an L segment.

[0081] Non-limiting examples of the arenavirus particle comprising a genomic segment with an ORF in a position other than the wild-type position of the ORF and a second genomic segment are illustrated in Table 1.

Table 1

Arenavirus particle

*Position 1 is under the control of an arenavirus S segment 5' UTR; Position 2 is under the control of an arenavirus S segment 3' UTR; Position 3 is under the control of an arenavirus L segment 5' UTR; Position 4 is under the control of an arenavirus L segment 3' UTR.

Position 1	Position 2	Position 3	Position 4
GP	NP	L	Z
GP	Z	L	NP
GP	Z	NP	L
GP	L	NP	Z
GP	L	Z	NP
NP	GP	L	Z
NP	GP	Z	L
NP	L	GP	Z
NP	L	Z	GP
NP	Z	GP	L
NP	Z	L	GP
Z	GP	L	NP
Z	GP	NP	L
Z	NP	GP	L
Z	NP	L	GP
Z	L	NP	GP
Z	L	GP	NP
L	NP	GP	Z
L	NP	Z	GP
L	GP	Z	NP
L	GP	NP	Z
L	Z	NP	GP
L	Z	GP	NP

[0082] Also provided herein, is a cDNA of the arenavirus genomic segment engineered to carry an ORF in a position other than the wild-type position of the ORF. In more specific embodiments, provided herein is a cDNA or a set of cDNAs of an arenavirus genome as set forth in Table 1.

[0083] In certain embodiments, a cDNA of the arenavirus genomic segment that is engineered to carry an ORF in a position other than the wild-type position of the ORF is part of or incorporated into a DNA expression vector. In a specific embodiment, a cDNA of the arenavirus genomic segment that is engineered to carry an ORF in a position other than the wild-type position of the ORF is part of or incorporated into a DNA expression vector that facilitates production of an arenavirus genomic segment as described herein. In another embodiment, a cDNA described herein can be incorporated into a plasmid. More detailed description of the cDNAs or nucleic acids and expression systems are provided is Section

4.5.1. Techniques for the production of a cDNA are routine and conventional techniques of molecular biology and DNA manipulation and production. Any cloning technique known to the skilled artesian can be used. Such as techniques are well known and are available to the skilled artesian in laboratory manuals such as, Sambrook and Russell, Molecular Cloning: A laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory N.Y. (2001).

[0084] In certain embodiments, the cDNA of the arenavirus genomic segment that is engineered to carry an ORF in a position other than the wild-type position of the ORF is introduced (*e.g.,* transfected) into a host cell. Thus, in some embodiments provided herein, is a host cell comprising a cDNA of the arenavirus genomic segment that is engineered to carry an ORF in a position other than the wild-type position of the ORF (*i.e.,* a cDNA of the genomic segment). In other embodiments, the cDNA described herein is part of or can be incorporated into a DNA expression vector and introduced into a host cell. Thus, in some embodiments provided herein is a host cell comprising a cDNA described herein that is incorporated into a vector. In other embodiments, the arenavirus genomic segment described herein is introduced into a host cell.

[0085] In certain embodiments, described herein is a method of producing the arenavirus genomic segment, wherein the method comprises transcribing the cDNA of the arenavirus genomic segment. In certain embodiments, a viral polymerase protein can be present during transcription of the arenavirus genomic segment *in vitro* or *in vivo*.

[0086] In certain embodiments transcription of the arenavirus genomic segment is performed using a bi-directional promoter. In other embodiments, transcription of the arenavirus genomic segment is performed using a bi-directional expression cassette (see *e.g.,* Ortiz-Riaño *et al.,* 2013, J Gen Virol., 94(Pt 6): 1175–1188). In more specific embodiments the bi-directional expression cassette comprises both a polymerase I and a polymerase II promoter reading from opposite sides into the two termini of the inserted arenavirus genomic segment, respectively. In yet more specific embodiments the bi-directional expression cassette with pol-I and pol-II promoters read from opposite sides into the L segment and S segment

[0087] In other embodiments, transcription of the cDNA of the arenavirus genomic segment described herein comprises a promoter. Specific examples of promoters include an RNA polymerase I promoter, an RNA polymerase II promoter, an RNA polymerase III promoter, a T7 promoter, an SP6 promoter or a T3 promoter.

[0088] In certain embodiments, the method of producing the arenavirus genomic segment can further comprise introducing into a host cell the cDNA of the arenavirus genomic segment. In certain embodiments, the method of producing the arenavirus genomic segment can further comprise introducing into a host cell the cDNA of the arenavirus genomic segment, wherein the host cell expresses all other components for production of the arenavirus genomic segment; and purifying the arenavirus genomic segment from the supernatant of the host cell. Such methods are well-known to those skilled in the art.

[0089] Provided herein are cell lines, cultures and methods of culturing cells infected with nucleic acids, vectors, and compositions provided herein. More detailed description of nucleic acids, vector systems and cell lines described herein is provided in Section 4.5.

[0090] In certain embodiments, the arenavirus particle as described herein results in an infectious and replication competent arenavirus particle. In specific embodiments, the arenavirus particle described herein is attenuated. In a particular embodiment, the arenavirus particle is attenuated such that the virus remains, at least partially, able to spread and can replicate *in vivo*, but can only generate low viral loads resulting in subclinical levels of infection that are non-pathogenic. Such attenuated viruses can be used as an immunogenic composition. Provided herein, are immunogenic compositions that comprise an arenavirus with an ORF in a non-natural position as described in Section 4.7.

4.1.1 Replication-Defective Arenavirus Particle with an Open Reading Frame in a Non-natural Position

[0091] In certain embodiments, provided herein is an arenavirus particle in which (i) an ORF is in a position other than the wild-type position of the ORF; and (ii) an ORF encoding GP, NP, Z protein, and L protein has been removed or functionally inactivated such that the resulting virus cannot produce further infectious progeny virus particles. An arenavirus particle comprising a genetically modified genome in which one or more ORFs has been deleted or functionally inactivated can be produced in complementing cells (*i.e.,* cells that express the arenavirus ORF that has been deleted or functionally inactivated). The genetic material of the resulting arenavirus particle can be transferred upon infection of a host cell into the host cell, wherein the genetic material can be expressed and amplified. In addition, the genome of the genetically modified arenavirus particle described herein can encode a heterologous ORF from an organism other than an arenavirus particle.

[0092] In certain embodiments, at least one of the four ORFs encoding GP, NP, Z protein, and L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In another embodiment, at least one ORF, at least two ORFs, at least three ORFs, or at least four ORFs encoding GP, NP, Z protein and L protein can be removed and replaced with a heterologous ORF from an organism other than an arenavirus. In specific embodiments, only one of the four ORFs encoding GP, NP, Z protein, and L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus particle. In more specific embodiments, the ORF that encodes GP of the arenavirus genomic segment is removed. In another specific embodiment, the ORF that encodes the NP of the arenavirus genomic segment is removed. In more specific embodiments, the ORF that encodes the Z protein of the arenavirus genomic segment is removed. In yet another specific embodiment, the ORF encoding the L protein is removed.

[0093] Thus, in certain embodiments, the arenavirus particle provided herein comprises a genomic segment that (i) is engineered to carry an ORF in a non-natural position; (ii) an ORF encoding GP, NP, Z protein, or L protein is removed; (iii) the ORF that is removed is replaced with a heterologous ORF from an organism other than an arenavirus.

[0094] In certain embodiments, the heterologous ORF is 8 to 100 nucleotides in length, 15 to 100 nucleotides in length, 25 to 100 nucleotides in length, 50 to 200 nucleotide in length, 50 to 400 nucleotide in length, 200 to 500 nucleotide in length, or 400 to 600 nucleotides in length, 500 to 800 nucleotide in length. In other embodiments, the heterologous ORF is 750 to 900 nucleotides in length, 800 to 100 nucleotides in length, 850 to 1000 nucleotides in length, 900 to 1200 nucleotides in length, 1000 to 1200 nucleotides in length, 1000 to 1500 nucleotides or 10 to 1500 nucleotides in length, 1500 to 2000 nucleotides in length, 1700 to 2000 nucleotides in length, 2000 to 2300 nucleotides in length, 2200 to 2500 nucleotides in length, 2500 to 3000 nucleotides in length, 3000 to 3200 nucleotides in length, 3000 to 3500 nucleotides in length, 3200 to 3600 nucleotides in length, 3300 to 3800 nucleotides in length, 4000 nucleotides to 4400 nucleotides in length, 4200 to 4700 nucleotides in length, 4800 to 5000 nucleotides in length, 5000 to 5200 nucleotides in length, 5200 to 5500 nucleotides in length, 5500 to 5800 nucleotides in length, 5800 to 6000 nucleotides in length, 6000 to 6400 nucleotides in length, 6200 to 6800 nucleotides in length, 6600 to 7000 nucleotides in length, 7000 to 7200 nucleotides in lengths, 7200 to 7500 nucleotides in length, or 7500 nucleotides in length. In some embodiments, the heterologous ORF encodes a peptide or polypeptide that is 5 to 10 amino acids in length, 10 to 25 amino acids in length, 25 to 50 amino acids in length, 50 to 100 amino acids in length, 100 to 150 amino acids in length, 150 to 200 amino acids in length, 200 to 250 amino acids in length, 250 to 300 amino acids in length, 300 to 400 amino acids in length, 400 to 500 amino acids in length, 500 to 750 amino acids in length, 750 to 1000 amino acids in length, 1000 to 1250 amino acids in length, 1250 to 1500 amino acids in length, 1500 to 1750 amino acids in length, 1750 to 2000 amino acids in length, 2000 to 2500 amino acids in length, or more than 2500 or more amino acids in length. In some embodiments, the heterologous ORF encodes a polypeptide that does not exceed 2500 amino acids in length. In specific embodiments the heterologous ORF does not contain a stop codon. In certain embodiments, the heterologous ORF is codon-optimized. In certain embodiments the nucleotide composition, nucleotide pair composition or both can be optimized. Techniques for such optimizations are known in the art and can be applied to optimize a heterologous ORF.

[0095] Any heterologous ORF from an organism other than an arenavirus may be included in an arenavirus genomic segment. In one embodiment, the heterologous ORF encodes a reporter protein. More detailed description of reporter proteins are described in Section 4.3. In another embodiment, the heterologous ORF encodes an antigen for an infectious pathogen or an antigen associated with any disease that is capable of eliciting an immune response. In specific embodiments the antigen is derived from an infectious organism, a tumor (*i.e.,* cancer), or an allergen. More detailed description on heterologous ORFs is described in Section 4.3.

[0096] In certain embodiments, the growth and infectivity of the arenavirus particle is not affected by the heterologous ORF from an organism other than an arenavirus.

[0097] Techniques known to one skilled in the art may be used to produce an arenavirus particle comprising an arenavirus genomic segment engineered to carry an arenavirus ORF in a position other than the wild-type position. For example, reverse genetics techniques may be used to generate such arenavirus particle. In other embodiments, the replication-defective arenavirus particle (*i.e.,* the arenavirus genomic segment engineered to carry an arenavirus ORF in a position other than the wild-type position, wherein an ORF encoding GP, NP, Z protein, L protein, has been deleted) can be produced in a complementing cell.

[0098] In certain embodiments, the arenavirus genomic segment or the arenavirus particle using according to the present application can be Old World Viruses, for example, LCMV.

[0099] In certain embodiments, the present application relates to the arenavirus particle as described herein suitable for use as a vaccine and methods of using such arenavirus particle in a vaccination and treatment or prevention of, for example, infections or cancers. More detailed description of the methods of using the arenavirus particle described herein is provided in Section 4.6

[00100] In certain embodiments, provided herein is a kit comprising, in one or more containers, one or more cDNAs described herein. In a specific embodiment, a kit comprises, in one or two or more containers an arenavirus genomic segment or an arenavirus particle as described herein. The kit may further comprise one or more of the following: a host cell suitable for rescue of the arenavirus genomic segment or the arenavirus particle, reagents suitable for transfecting plasmid cDNA into a host cell, a helper virus, plasmids encoding viral proteins and/or one or more primers specific for an modified arenavirus genomic segment or arenavirus particle or cDNAs of the same.

[00101] In certain embodiments, the present application relates to the arenavirus particle as described herein suitable for use as a pharmaceutical composition and methods of using such arenavirus particle in a vaccination and treatment or prevention of, for example, infections and cancers. More detailed description of the methods of using the arenavirus particle described herein is provided in Section 4.7.

4.2 Tri-segmented Arenavirus Particle

[00102] Provided herein are tri-segmented arenavirus particles with rearrangements of their ORFs. In one aspect, provided herein is a tri-segmented arenavirus particle comprising one L segment and two S segments or two L segments and one S segment. In certain embodiments, the tri-segmented arenavirus particle does not recombine into a replication competent bi-segmented arenavirus particle. More specifically, in certain embodiments, two of the genomic segments (*e.g.*, the two S segments or the two L segments, respectively) cannot recombine in a way to yield a single viral segment that could replace the two parent segments. In specific embodiments, the tri-segmented arenavirus particle comprises an ORF in a position other than the wild-type position of the ORF. In yet another specific embodiment, the tri-segmented arenavirus particle comprises all four arenavirus ORFs. Thus, in certain embodiments, the tri-segmented arenavirus particle is replication competent and infectious. In other embodiments, the tri-segmented arenavirus particle lacks one of the four arenavirus ORFs. Thus, in certain embodiments, the tri-segmented arenavirus particle is infectious but unable to produce further infectious progeny in non-complementing cells.

[00103] In certain embodiments, the ORF encoding GP, NP, Z protein, or the L protein of the tri-segmented arenavirus particle described herein can be under the control of an arenavirus 3' UTR or an arenavirus 5' UTR. In more specific embodiments, the tri-segmented arenavirus 3' UTR is the 3' UTR of an arenavirus S segment(s). In another specific embodiment, the tri-segmented arenavirus 3' UTR is the 3' UTR of a tri-segmented arenavirus L segment(s). In more specific embodiments, the tri-segmented arenavirus 5' UTR is the 5' UTR of an arenavirus S segment(s). In other specific embodiments, the 5' UTR is the 5' UTR of the L segment(s).

[00104] In other embodiments, the ORF encoding GP, NP, Z protein, or the L protein of tri-segmented arenavirus particle described herein can be under the control of the arenavirus conserved terminal sequence element (the 5'- and 3'-terminal 19-20-nt regions) (see *e.g.,* Perez & de la Torre, 2003, J Virol. 77(2): 1184–1194).

[00105] In certain embodiments, the ORF encoding GP, NP, Z protein or the L protein of the tri-segmented arenavirus particle can be under the control of the promoter element of the 5' UTR (see *e.g.,* Albarino *et al.,* 2011, J Virol., 85(8):4020-4). In another embodiment, the ORF encoding GP, NP Z protein, L protein of the tri-segmented arenavirus particle can be under the control of the promoter element of the 3' UTR (see *e.g.,* Albarino *et al.,* 2011, J Virol., 85(8):4020-4). In more specific embodiments, the promoter element of the 5' UTR is the 5' UTR promoter element of the S segment(s) or the L segment(s). In another specific embodiment, the promoter element of the 3' UTR is the 3' UTR the promoter element of the S segment(s) or the L segment(s).

[00106] In certain embodiments, the ORF that encoding GP, NP, Z protein or the L protein of the tri-segmented arenavirus particle can be under the control of a truncated arenavirus 3' UTR or a truncated arenavirus 5' UTR (see *e.g.,* Perez & de la Torre, 2003, J Virol. 77(2): 1184–1194; Albarino *et al.,* 2011, J Virol., 85(8):4020-4). In more specific embodiments, the truncated 3' UTR is the 3' UTR of the arenavirus S segment or L segment. In more specific embodiments, the truncated 5' UTR is the 5' UTR of the arenavirus S segment(s) or L segment(s).

[00107] Also provided herein, is a cDNA of the tri-segmented arenavirus particle. In more specific embodiments, provided herein is a DNA nucleotide sequence or a set of DNA nucleotide sequences encoding a tri-segmented arenavirus particle as set forth in Table 2 or Table 3.

[00108] In certain embodiments, the nucleic acids encoding the tri-segmented arenavirus genome are part of or incorporated into one or more DNA expression vectors. In a specific embodiment, nucleic acids encoding the genome of the tri-segmented arenavirus particle is part of or incorporated into one or more DNA expression vectors that facilitate production of a tri-segmented arenavirus particle as described herein. In another embodiment, a cDNA described herein can be incorporated into a plasmid. More detailed description of the cDNAs and expression systems are provided is Section 4.5.1. Techniques for the production of a cDNA routine and conventional techniques of molecular biology and DNA manipulation and production. Any cloning technique known to the skilled artesian can be used. Such techniques are well known and are available to the skilled artesian in laboratory manuals such as, Sambrook and Russell, Molecular Cloning: A laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory N.Y. (2001).

[00109] In certain embodiments, the cDNA of the tri-segmented arenavirus is introduced (*e.g.,* transfected) into a host cell. Thus, in some embodiments provided herein, is a host cell comprising a cDNA of the tri-segmented arenavirus particle (*i.e.,* a cDNA of the genomic segments of the tri-segmented arenavirus particle). In other embodiments, the cDNA described herein that is part of or can be incorporated into a DNA expression vector and introduced into a host cell. Thus, in some embodiments provided herein is a host cell comprising a cDNA described herein that is incorporated into a vector. In other embodiments, the tri-segmented arenavirus genomic segments (*i.e.,* the L segment and/or S segment or segments) described herein is introduced into a host cell.

[00110] In certain embodiments, described herein is a method of producing the tri-segmented arenavirus particle, wherein the method comprises transcribing the cDNA of the tri-segmented arenavirus particle. In certain embodiments, a viral polymerase protein can be present during transcription of the tri-segmented arenavirus particle *in vitro* or *in vivo*. In certain embodiments, transcription of the arenavirus genomic segment is performed using a bi-directional promoter.

[00111] In other embodiments, transcription of the arenavirus genomic segment is performed using a bi-directional expression cassette (see *e.g.,* Ortiz-Riaño *et al.,* 2013, J Gen Virol., 94(Pt 6): 1175–1188). In more specific embodiments the bi-directional expression cassette comprises both a polymerase I and a polymerase II promoter reading from opposite sides into the two termini of the inserted arenavirus genomic segment, respectively.

[00112] In other embodiments, transcription of the cDNA of the arenavirus genomic segment described herein comprises a promoter. Specific examples of promoters include an RNA polymerase I promoter, an RNA polymerase II promoter, an RNA polymerase III promoter, a T7 promoter, an SP6 promoter or a T3 promoter.

[00113] In certain embodiments, the method of producing the tri-segmented arenavirus particle can further comprise introducing into a host cell the cDNA of the tri-segmented arenavirus particle. In certain embodiments, the method of producing the tri-segmented arenavirus particle can further comprise introducing into a host cell the cDNA of the tri-segmented arenavirus particle, wherein the host cell expresses all other components for production of the tri-segmented arenavirus particle; and purifying the tri-segmented arenavirus particle from the supernatant of the host cell. Such methods are well-known to those skilled in the art.

[00114] Provided herein are cell lines, cultures and methods of culturing cells infected with nucleic acids, vectors, and compositions provided herein. More detailed description of nucleic acids, vector systems and cell lines described herein is provided in Section 4.5.

[00115] In certain embodiments, the tri-segmented arenavirus particle as described herein results in an infectious and replication competent arenavirus particle. In specific embodiments, the arenavirus particle described herein is attenuated. In a particular embodiment, the tri-segmented arenavirus particle is attenuated such that the virus remains, at least partially, replication-competent and can replicate *in vivo*, but can only generate low viral loads resulting in subclinical levels of infection that are non-pathogenic. Such attenuated viruses can be used as an immunogenic composition.

[00116] In certain embodiments, the tri-segmented arenavirus particle has the same tropism as the bi-segmented arenavirus particle.

[00117] Also provided herein is a kit comprising, in one or more containers, one or more cDNAs described herein. In a specific embodiment, a kit comprises, in one or two or more containers a tri-segmented arenavirus particle as described herein. The kit may further comprise one or more of the following: a host cell suitable for rescue of the tri-segmented arenavirus particle, reagents suitable for transfecting plasmid cDNA into a host cell, a helper virus, plasmids encoding viral proteins and/or one or more oligonucleotide primers specific for a modified arenavirus genomic segment or arenavirus particle or nucleic acids encoding the same.

[00118] Also provided herein are immunogenic compositions that comprise the tri-segmented arenavirus particle as described in Section 4.6 and 4.7.

4.2.1 Tri-segmented Arenavirus Particle comprising one L segment and two S segments

[00119] In one aspect, provided herein is a tri-segmented arenavirus particle comprising one L segment and two S segments. In certain embodiments, propagation of the tri-segmented arenavirus particle comprising one L segment and two S segments does not result in a replication-competent bi-segmented viral particle. In specific embodiments, propagation of the tri-segmented arenavirus particle comprising one L segment and two S segments does not result in a replication-competent bi-segmented viral particle after at least 10 days, at least 20 days, at least 30 days, at least 40 days, at least 50 days, at least 60 days, at least 70 days, at least 80 days, at least 90 days, or at least 100 days of persistent infection in mice lacking type I interferon receptor, type II interferon receptor and recombination activating gene (RAG1), and having been infected with 10^4 PFU of the tri-segmented arenavirus particle (see Section

4.8.13). In other embodiments, propagation of the tri-segmented arenavirus particle comprising one L segment and two S segments does not result in a replication-competent bi-segmented viral particle after at least 10 passages, at least 20 passages, at least 30 passages, at least 40 passages, or at least 50 passages.

[00120] The tri-segmented arenavirus particle with all viral genes in their respective wild-type position is known in the art (*e.g.,* Emonet *et al.,* 2011 J. Virol., 85(4):1473; Popkin *et al.,* 2011, J. Virol, 85(15):7928). In particular, the tri-segmented arenavirus genome consists of one L segment and two S segments, in which a heterologous ORF (*e.g.,* a GFP) is inserted into one position on each S segment. More specifically, one S segment encodes GP and GFP, respectively. The other S segment encodes GFP and NP, respectively. The L segment encodes the L protein and Z protein. All segments are flanked by the respective 5' and 3' UTRs.

[00121] In certain embodiments, inter-segmental recombination of the two S segments of the tri-segmented arenavirus particle, provided herein, that unities the two arenaviral ORFs on one instead of two separate segments results in a non functional promoter (*i.e.,* a genomic segment of the structure: 5' UTR-----------5' UTR or a 3' UTR------------3' UTR), wherein each UTR forming one end of the genome is an inverted repeat sequence of the other end of the same genome.

[00122] In certain embodiments, the tri-segmented arenavirus particle comprising one L segment and two S segments has been engineered to carry an arenavirus ORF in a position other than the wild-type position of the ORF. In other embodiments, the tri-segmented arenavirus particle comprising one L segment and two S segments has been engineered to carry two arenavirus ORFs, or three arenavirus ORFs, or four arenavirus ORFs, or five arenavirus ORFs, or six arenavirus ORFs in a position other than the wild-type position. In specific embodiments, the tri-segmented arenavirus particle comprising one L segment and two S segments comprises a full complement of all four arenavirus ORFs. Thus, in some embodiments, the tri-segmented arenavirus particle is an infectious and replication competent tri-segmented arenavirus particle. In specific embodiments, the two S segments of the tri-segmented arenavirus particle have been engineered to carry one of their ORFs in a position other than the wild-type position. In more specific embodiments, the two S segments comprise a full complement of the S segment ORF's. In certain specific embodiments, the L segment has been engineered to carry an ORF in a position other than the wild-type position or the L segment can be the wild-type genomic segment.

[00123] In certain embodiments, one of the two S segments can be:

(i) an arenavirus S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 5' UTR;

(ii) an arenavirus S segment, wherein the ORF encoding the L protein is under control of an arenavirus 5' UTR;

(iii) an arenavirus S segment, wherein the ORF encoding the NP is under control of an arenavirus 5' UTR;

(iv) an arenavirus S segment, wherein the ORF encoding the GP is under control of an arenavirus 3' UTR;

(v) an arenavirus S segment, wherein the ORF encoding the L is under control of an arenavirus 3' UTR; and

(vi) an arenavirus S segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR.

[00124] In certain embodiments, the tri-segmented arenavirus particle comprising one L segment and two S segments can comprise a duplicate ORF (*i.e.,* two wild-type S segment ORFs *e.g.,* GP or NP). In specific embodiments, the tri-segmented arenavirus particle comprising one L segment and two S segments can comprise one duplicate ORF (*e.g.,* (GP, GP)) or two duplicate ORFs (*e.g.,* (GP, GP) and (NP, NP)).

[00125] Table 2A, below, is an illustration of the genome organization of a tri-segmented arenavirus particle comprising one L segment and two S segments, wherein intersegmental recombination of the two S segments in the tri-segmented arenavirus genome does not result in a replication-competent bi-segmented viral particle and abrogates arenaviral promoter activity (*i.e.,* the resulting recombined S segment is made up of two 3'UTRs instead of a 3' UTR and a 5' UTR).

Table 2A

Tri-segmented arenavirus particle comprising one L segment and two S segments

Position 1 is under the control of an arenavirus S segment 5' UTR; Position 2 is under the control of an arenavirus S segment 3' UTR; Position 3 is under the control of an arenavirus S segment 5' UTR; Position 4 under the control of an arenavirus S segment 3' UTR; Position 5 is under the control of an arenavirus L segment 5' UTR; Position 6 is under the control of an arenavirus L segment 3' UTR.

*ORF indicates that a heterologous ORF has been inserted.

Position 1	Position 2	Position 3	Position 4	Position 5	Position 6
*ORF	GP	*ORF	NP	Z	L
*ORF	NP	*ORF	GP	Z	L
*ORF	NP	*ORF	GP	L	Z
*ORF	NP	*ORF	Z	L	GP
*ORF	NP	Z	GP	*ORF	Z
*ORF	NP	Z	GP	Z	*ORF
*ORF	NP	*ORF	L	Z	GP
*ORF	L	*ORF	NP	Z	GP
*ORF	L	Z	NP	*ORF	GP
*ORF	L	*ORF	GP	Z	NP
*ORF	L	Z	GP	*ORF	NP
*ORF	Z	L	NP	*ORF	GP
*ORF	Z	*ORF	GP	L	NP
*ORF	Z	L	GP	*ORF	NP
L	GP	*ORF	NP	*ORF	Z
L	GP	*ORF	*ORF	Z	NP
L	GP	*ORF	Z	*ORF	NP
L	*ORF	Z	GP	*ORF	NP
L	GP	*ORF	NP	*ORF	Z
L	GP	*ORF	Z	*ORF	NP
L	GP	Z	NP	*ORF	*ORF
L	GP	Z	NP	*ORF	*ORF
L	*ORF	Z	NP	*ORF	GP
L	NP	*ORF	Z	*ORF	GP
L	NP	Z	*ORF	GP	*ORF
L	*ORF	Z	*ORF	GP	NP
L	NP	Z	GP	*ORF	*ORF
L	NP	*ORF	Z	*ORF	GP
L	*ORF	Z	NP	*ORF	GP
L	Z	*ORF	GP	*ORF	NP
L	Z	*ORF	NP	*ORF	GP
Z	GP	*ORF	NP	*ORF	L
Z	GP	*ORF	*ORF	L	NP
Z	GP	*ORF	L	*ORF	NP
Z	*ORF	L	GP	*ORF	NP
Z	GP	*ORF	NP	*ORF	L
Z	GP	*ORF	L	*ORF	NP
Z	GP	L	NP	*ORF	*ORF
Z	GP	L	NP	*ORF	*ORF
Z	*ORF	L	NP	*ORF	GP
Z	NP	*ORF	*ORF	L	GP
Z	NP	*ORF	GP	*ORF	L
Z	NP	*ORF	*ORF	L	GP
Z	NP	*ORF	L	*ORF	GP
Z	NP	L	GP	*ORF	*ORF
Z	*ORF	L	GP	*ORF	NP
Z	NP	*ORF	GP	*ORF	L
Z	NP	*ORF	L	*ORF	GP
Z	*ORF	L	NP	*ORF	GP
Z	L	*ORF	GP	*ORF	NP

[00126] In certain embodiments, the IGR between position one and position two can be an arenavirus S segment or L segment IGR; the IGR between position two and three can be an arenavirus S segment or L segment IGR; and the IGR between the position five and six can be an arenavirus L segment IGR. In a specific embodiment, the IGR between position one and position two can be an arenavirus S segment IGR; the IGR between position two and three can be an arenavirus S segment IGR; and the IGR between the position five and six can be an arenavirus L segment IGR. In certain embodiments, other combinations are also possible. For example, a tri-segmented arenavirus particle comprising one L segment and two S segments, wherein intersegmental recombination of the two S segments in the tri-segmented arenavirus genome does not result in a replication-competent bi-segmented viral particle and abrogates arenaviral promoter activity (*i.e.,* the resulting recombined S segment is made up of two 5'UTRs instead of a 3' UTR and a 5' UTR).

[00127] In certain embodiments, intersegmental recombination of an S segment and an L segment in the tri-segmented arenavirus particle comprising one L segment and two S segments, restores a functional segment with two viral genes on only one segment instead of two separate segments. In other embodiments, intersegmental recombination of an S segment and an L segment in the tri-segmented arenavirus particle comprising one L segment and two S segments does not result in a replication-competent bi-segmented viral particle.

[00128] Table 2B, below, is an illustration of the genome organization of a tri-segmented arenavirus particle comprising one L segment and two S segments, wherein intersegmental recombination of an S segment and an L segment in the tri-segmented arenavirus genome does not result in a replication-competent bi-segmented viral particle and abrogates arenaviral promoter activity (*i.e.,* the resulting recombined S segment is made up of two 3'UTRs instead of a 3' UTR and a 5' UTR).

Table 2B

Tri-segmented arenavirus particle comprising one L segment and two S segments

Position 1 is under the control of an arenavirus S segment 5' UTR; Position 2 is under the control of an arenavirus S segment 3' UTR; Position 3 is under the control of an arenavirus S segment 5' UTR; Position 4 under the control of an arenavirus S segment 3' UTR; Position 5 is under the control of an arenavirus L segment 5' UTR; Position 6 is under the control of an arenavirus L segment 3' UTR.

*ORF indicates that a heterologous ORF has been inserted.

Position 1	Position 2	Position 3	Position 4	Position 5	Position 6
L	GP	*ORF	NP	Z	*ORF

Position 1	Position 2	Position 3	Position 4	Position 5	Position 6
L	GP	Z	*ORF	*ORF	NP
L	GP	*ORF	NP	Z	*ORF
L	GP	Z	*ORF	*ORF	NP
L	NP	*ORF	GP	Z	*ORF
L	NP	Z	*ORF	*ORF	GP
L	NP	*ORF	GP	Z	*ORF
L	NP	Z	*ORF	*ORF	GP
Z	GP	*ORF	NP	L	*ORF
Z	GP	L	*ORF	*ORF	NP
Z	GP	*ORF	NP	L	*ORF
Z	NP	L	*ORF	*ORF	GP
Z	NP	*ORF	GP	L	*ORF
Z	NP	L	*ORF	*ORF	GP

[00129] In certain embodiments, the IGR between position one and position two can be an arenavirus S segment or L segment IGR; the IGR between position two and three can be an arenavirus S segment or L segment IGR; and the IGR between the position five and six can be an arenavirus L segment IGR. In a specific embodiment, the IGR between position one and position two can be an arenavirus S segment IGR; the IGR between position two and three can be an arenavirus S segment IGR; and the IGR between the position five and six can be an arenavirus L segment IGR. In certain embodiments, other combinations are also possible. For example, a tri-segmented arenavirus particle comprising one L segment and two S segments, wherein intersegmental recombination of the two S segments in the tri-segmented arenavirus genome does not result in a replication-competent bi-segmented viral particle and abrogates arenaviral promoter activity (*i.e.,* the resulting recombined S segment is made up of two 5'UTRs instead of a 3' UTR and a 5' UTR).

[00130] In certain embodiments, one of skill in the art could construct an arenavirus genome with an organization as illustrated in Table 2A or 2B and as described herein, and then use an assay as described in Section 4.8 to determine whether the tri-segmented arenavirus particle is genetically stable, *i.e.*, does not result in a replication-competent bi-segmented viral particle as discussed herein.

4.2.2 Tri-segmented Arenavirus Particle comprising two L segments and one S segment

[00131] In one aspect, provided herein is a tri-segmented arenavirus particle comprising two L segments and one S segment. In certain embodiments, propagation of the tri-segmented arenavirus particle comprising two L segments and one S segment does not result in a replication-competent bi-segmented viral particle. In specific embodiments, propagation of the tri-segmented arenavirus particle comprising two L segments and one S segment does not result in a replication-competent bi-segmented viral particle after at least 10 days, at least 20 days, at least 30 days, at least 40 days, or at least 50 days, at least 60 days, at least 70 days, at least 80 days, at least 90 days, at least 100 days of persistent in mice lacking type I interferon receptor, type II interferon receptor and recombination activating gene (RAG1), and having been infected with 10^4 PFU of the tri-segmented arenavirus particle (see Section 4.8.13). In other embodiments, propagation of the tri-segmented arenavirus particle comprising two L segments and one S segment does not result in a replication-competent bi-segmented viral particle after at least 10 passages, 20 passages, 30 passages, 40 passages, or 50 passages.

[00132] In certain embodiments, inter-segmental recombination of the two L segments of the tri-segmented arenavirus particle, provided herein, that unities the two arenaviral ORFs on one instead of two separate segments results in a non functional promoter (*i.e.*, a genomic segment of the structure: 5' UTR-----------5' UTR or a 3' UTR------------3' UTR), wherein each UTR forming one end of the genome is an inverted repeat sequence of the other end of the same genome.

[00133] In certain embodiments, the tri-segmented arenavirus particle comprising two L segments and one S segment has been engineered to carry an arenavirus ORF in a position other than the wild-type position of the ORF. In other embodiments, the tri-segmented arenavirus particle comprising two L segments and one S segment has been engineered to carry two arenavirus ORFs, or three arenavirus ORFs, or four arenavirus ORFs, or five arenavirus ORFs, or six arenavirus ORFs in a position other than the wild-type position. In specific embodiments, the tri-segmented arenavirus particle comprising two L segments and one S segment comprises a full complement of all four arenavirus ORFs. Thus, in some embodiments, the tri-segmented arenavirus particle is an infectious and replication competent tri-segmented arenavirus particle. In specific embodiments, the two L segments of the tri-segmented arenavirus particle have been engineered to carry one of their ORFs in a position other than the wild-type position. In more specific embodiments, the two L segments comprise a full complement of the L segment ORF's. In certain specific embodiments, the S segment has been engineered to carry one of their ORFs in a position other than the wild-type position or the S segment can be the wild-type genomic segment.

[00134] In certain embodiments, one of the two L segments can be:

(i) an L segment, wherein the ORF encoding the GP is under control of an arenavirus 5' UTR;

(ii) an L segment, wherein the ORF encoding NP is under control of an arenavirus 5' UTR;

(iii) an L segment, wherein the ORF encoding the L protein is under control of an arenavirus 5' UTR;

(iv) an L segment, wherein the ORF encoding the GP is under control of an arenavirus 3' UTR;

(v) an L segment, wherein the ORF encoding the NP is under control of an arenavirus 3' UTR; and

(vi) an L segment, wherein the ORF encoding the Z protein is under control of an arenavirus 3' UTR.

[00135] In certain embodiments, the tri-segmented arenavirus particle comprising one L segment and two S segments can comprise a duplicate ORF (*i.e.,* two wild-type L segment ORFs *e.g.,* Z protein or L protein). In specific embodiments, the tri-segmented arenavirus particle comprising two L segments and one S segment can comprise one duplicate ORF (*e.g.,* (Z protein, Z protein)) or two duplicate ORFs (*e.g.,* (Z protein, Z protein) and (L protein, L protein)).

[00136] Table 3, below, is an illustration of the genome organization of a tri-segmented arenavirus particle comprising two L segments and one S segment, wherein intersegmental recombination of the two L segments in the tri-segmented arenavirus genome does not result in a replication-competent bi-segmented viral particle and abrogates arenaviral promoter activity (*i.e.,* the putatively resulting recombinant L segment would be made up of two 3'UTRs or two 5' UTRs instead of a 3' UTR and a 5' UTR). Based on Table 3 similar combinations could be predicted for generating an arenavirus particle made up of two 5' UTRs instead of a 3' UTR and a 5' UTR.

Table 3

Tri-segmented arenavirus particle comprising two L segments and one S segment

*Position 1 is under the control of an arenavirus L segment 5' UTR; position 2 is under the control of an arenavirus L segment 3' UTR; position 3 is under the control of an arenavirus L segment 5' UTR; position 4 is under the control of an arenavirus L segment 3' UTR; position 5 is under the control of an arenavirus S segment 5' UTR; position 6 is under the control of an arenavirus S segment 3' UTR.

* ORF indicates that a heterologous ORF has been inserted.

Position 1	Position 2	Position 3	Position 4	Position 5	Position 6
ORF*	Z	ORF*	L	NP	GP
ORF*	Z	ORF*	L	GP	NP
ORF*	Z	GP	L	ORF*	NP
ORF*	Z	ORF*	GP	NP	L
ORF*	Z	GP	ORF*	NP	L
ORF*	Z	NP	ORF*	GP	L
ORF*	ORF*	NP	Z	GP	L
ORF*	Z	GP	NP	ORF*	L
ORF*	Z	NP	GP	ORF*	L
ORF*	L	ORF*	Z	NP	GP
ORF*	L	ORF*	Z	GP	NP
ORF*	L	ORF*	GP	NP	Z
ORF*	L	GP	Z	ORF*	NP
ORF*	L	ORF*	GP	NP	Z
ORF*	L	NP	Z	ORF*	GP
ORF*	L	GP	NP	ORF*	Z
ORF*	L	NP	GP	ORF*	Z
ORF*	GP	ORF*	L	NP	Z
ORF*	GP	NP	L	ORF*	Z
ORF*	GP	ORF*	Z	NP	L
ORF*	GP	NP	Z	ORF*	L
ORF*	NP	ORF*	L	GP	Z
ORF*	NP	GP	L	ORF*	Z
ORF*	NP	GP	Z	ORF*	L
ORF*	NP	ORF*	Z	GP	L
ORF*	L	ORF*	Z	NP	GP
ORF*	L	ORF*	Z	GP	NP
ORF*	L	ORF*	NP	GP	Z
ORF*	L	ORF*	GP	NP	Z
ORF*	L	NP	Z	ORF*	GP
ORF*	Z	ORF*	GP	NP	L
ORF*	Z	GP	L	ORF*	NP
ORF*	Z	NP	GP	ORF*	L
ORF*	Z	GP	NP	ORF*	L
ORF*	GP	ORF*	L	NP	Z
ORF*	GP	ORF*	L	Z	NP
ORF*	GP	ORF*	Z	GP	L
ORF*	GP	NP	L	ORF*	Z
GP	L	ORF*	Z	ORF*	NP
GP	L	ORF*	NP	ORF*	Z
GP	Z	ORF*	L	ORF*	NP
GP	Z	ORF*	L	ORF*	NP
GP	Z	ORF*	NP	ORF*	L
GP	NP	ORF*	Z	ORF*	L
NP	L	ORF*	Z	ORF*	GP
NP	L	ORF*	GP	ORF*	Z
NP	L	ORF*	Z	ORF*	GP

[00137] In certain embodiments, the IGR between position one and position two can be an arenavirus S segment or L segment IGR; the IGR between position two and three can be an arenavirus S segment or L segment IGR; and the IGR between the position five and six can be an arenavirus S segment or L segment IGR. In a specific embodiment, the IGR between position one and position two can be an arenavirus L segment IGR; the IGR between position two and three can be an arenavirus L segment IGR; and the IGR between the position five and six can be an arenavirus S segment IGR. In certain embodiments, other combinations are also possible.

[00138] In certain embodiments intersegmental recombination of an L segment and an S segment from the tri-segmented arenavirus particle comprising two L segments and one S segment restores a functional segment with two viral genes on only one segment instead of two separate segments. In other embodiments, intersegmental recombination of an L segment and an S segment in the tri-segmented arenavirus particle comprising two L segments and one S segment does not result in a replication-competent bi-segmented viral particle..

[00139] Table 3B, below, is an illustration of the genome organization of a tri-segmented arenavirus particle comprising two L segments and one S segment, wherein intersegmental recombination of an L segment and an S segment in the tri-segmented arenavirus genome does not result in a replication-competent bi-segmented viral particle and abrogates arenaviral promoter activity (*i.e.,* the resulting recombined S segment is made up of two 3'UTRs instead of a 3' UTR and a 5' UTR).

Table 3B

Tri-segmented arenavirus particle comprising two L segments and one S segment

*Position 1 is under the control of an arenavirus L segment 5' UTR; position 2 is under the control of an arenavirus L segment 3' UTR; position 3 is under the control of an arenavirus L segment 5' UTR; position 4 is under the control of an arenavirus L segment 3' UTR; position 5 is under the control of an arenavirus S segment 5' UTR; position 6 is under the control of an arenavirus S segment 3' UTR.

* ORF indicates that a heterologous ORF has been inserted.

Position 1	Position 2	Position 3	Position 4	Position 5	Position 6
NP	Z	*ORF	GP	L	*ORF
NP	Z	GP	*ORF	*ORF	L
NP	Z	*ORF	GP	L	*ORF
NP	Z	GP	*ORF	*ORF	L
NP	L	*ORF	GP	Z	*ORF
NP	L	GP	*ORF	*ORF	Z

Position 1	Position 2	Position 3	Position 4	Position 5	Position 6
NP	L	*ORF	GP	Z	*ORF
NP	L	GP	*ORF	*ORF	Z
GP	Z	*ORF	NP	L	*ORF
GP	Z	NP	*ORF	*ORF	L
GP	Z	*ORF	NP	L	*ORF
GP	L	NP	*ORF	*ORF	Z
GP	L	*ORF	NP	Z	*ORF
GP	L	NP	*ORF	*ORF	Z

[00140] In certain embodiments, the IGR between position one and position two can be an arenavirus S segment or L segment IGR; the IGR between position two and three can be an arenavirus S segment or L segment IGR; and the IGR between the position five and six can be an arenavirus S segment or L segment IGR. In a specific embodiment, the IGR between position one and position two can be an arenavirus L segment IGR; the IGR between position two and three can be an arenavirus L segment IGR; and the IGR between the position five and six can be an arenavirus S segment IGR. In certain embodiments, other combinations are also possible.

[00141] In certain embodiments, one of skill in the art could construct an arenavirus genome with an organization as illustrated in Table 3A or 3B and as described herein, and then use an assay as described in Section 4.8 to determine whether the tri-segmented arenavirus particle is genetically stable, *i.e.*, does not result in a replication-competent bi-segmented viral particle as discussed herein.

4.2.3 Replication-Defective Tri-segmented Arenavirus Particle

[00142] In certain embodiments, provided herein is a tri-segmented arenavirus particle in which (i) an ORF is in a position other than the wild-type position of the ORF; and (ii) an ORF encoding GP, NP, Z protein, or L protein has been removed or functionally inactivated such that the resulting virus cannot produce further infectious progeny virus particles (*i.e.,* is replication defective). In certain embodiments, the third arenavirus segment can be an S segment. In other embodiments, the third arenavirus segment can be an L segment. In more specific embodiments, the third arenavirus segment can be engineered to carry an ORF in a position other than the wild-type position of the ORF or the third arenavirus segment can be the wild-type arenavirus genomic segment. In yet more specific embodiments, the third arenavirus segment lacks an arenavirus ORF encoding GP, NP, Z protein, or the L protein.

[00143] In certain embodiments, a tri-segmented genomic segment could be a S or a L segment hybrid (*i.e.,* a genomic segment that can be a combination of the S segment and the L segment). In other embodiments, the hybrid segment is an S segment comprising an L segment IGR. In another embodiment, the hybrid segment is an L segment comprising an S segment IGR. In other embodiments, the hybrid segment is an S segment UTR with and L segment IGR. In another embodiment, the hybrid segment is an L segment UTR with an S segment IGR. In specific embodiments, the hybrid segment is an S segment 5' UTR with an L segment IGR or an S segment 3' UTR with an L segment IGR. In other specific embodiments, the hybrid segment is an L segment 5' UTR with an S segment IGR or an L segment 3' UTR with an S segment IGR.

[00144] A tri-segmented arenavirus particle comprising a genetically modified genome in which one or more ORFs has been deleted or functionally inactivated can be produced in complementing cells (*i.e.,* cells that express the arenavirus ORF that has been deleted or functionally inactivated). The genetic material of the resulting arenavirus particle can be transferred upon infection of a host cell into the host cell, wherein the genetic material can be expressed and amplified. In addition, the genome of the genetically modified arenavirus particle described herein can encode a heterologous ORF from an organism other than an arenavirus particle.

[00145] In certain embodiments, at least one of the four ORFs encoding GP, NP, Z protein, and L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In another embodiment, at least one ORF, at least two ORFs, at least three ORFs, or at least four ORFs encoding GP, NP, Z protein and L protein can be removed and replaced with a heterologous ORF from an organism other than an arenavirus. In specific embodiments, only one of the four ORFs encoding GP, NP, Z protein, and L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus particle. In more specific embodiments, the ORF that encodes GP of the arenavirus genomic segment is removed. In another specific embodiment, the ORF that encodes the NP of the arenavirus genomic segment is removed. In more specific embodiments, the ORF that encodes the Z protein of the arenavirus genomic segment is removed. In yet another specific embodiment, the ORF encoding the L protein is removed.

[00146] In certain embodiments, provided herein is a tri-segmented arenavirus particle comprising one L segment and two S segments in which (i) an ORF is in a position other than the wild-type position of the ORF; and (ii) an ORF encoding GP or NP has been removed or functionally inactivated, such that the resulting virus is replication-defective and not infectious. In a specific embodiment, one ORF is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In another specific embodiment, two ORFs are removed and replaced with a heterologous ORF from an organism other than an arenavirus. In other specific embodiments, three ORFs are removed and replaced with a heterologous ORF from an organism other than an arenavirus. In specific embodiments, the ORF encoding GP is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In other specific embodiments, the ORF encoding NP is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In yet more specific embodiments, the ORF encoding NP and the ORF encoding GP are removed and replaced with one or two heterologous ORFs from an organism other than an arenavirus particle. Thus, in certain embodiments the tri-segmented arenavirus particle comprises (i) one L segment and two S segments; (ii) an ORF in a position other than the wild-type position of the ORF; (iii) one or more heterologous ORFs from an organism other than an arenavirus.

[00147] In certain embodiments, provided herein is a tri-segmented arenavirus particle comprising two L segments and one S segment in which (i) an ORF is in a position other than the wild-type position of the ORF; and (ii) an ORF encoding the Z protein, and/or the L protein has been removed or functionally inactivated, such that the resulting virus replication-defective and not infectious. In a specific embodiment, one ORF is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In another specific embodiment, two ORFs are removed and replaced with a heterologous ORF from an organism other than an arenavirus. In specific embodiments, the ORF encoding the Z protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In other specific embodiments, the ORF encoding the L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus. In yet more specific embodiments, the ORF encoding the Z protein and the ORF encoding the L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus particle. Thus, in certain embodiments the tri-segmented arenavirus particle comprises (i) two L segments and one S segment; (ii) an ORF in a position other than the wild-type position of the ORF; (iii) a heterologous ORF from an organism other than an arenavirus.

[00148] Thus, in certain embodiments, the tri-segmented arenavirus particle provided herein comprises a tri-segmented arenavirus particle (*i.e.,* one L segment and two S segments or two L segments and one S segment) that i) is engineered to carry an ORF in a non-natural position; ii) an ORF encoding GP, NP, Z protein, or L protein is removed); iii) the ORF that is removed is replaced with one or more heterologous ORFs from an organism other than an arenavirus.

[00149] In certain embodiments, the heterologous ORF is 8 to 100 nucleotides in length, 15 to 100 nucleotides in length, 25 to 100 nucleotides in length, 50 to 200 nucleotide in length, 50 to 400 nucleotide in length, 200 to 500 nucleotide in length, or 400 to 600 nucleotides in length, 500 to 800 nucleotide in length. In other embodiments, the heterologous ORF is 750 to 900 nucleotides in length, 800 to 100 nucleotides in length, 850 to 1000 nucleotides in length, 900 to 1200 nucleotides in length, 1000 to 1200 nucleotides in length, 1000 to 1500 nucleotides or 10 to 1500 nucleotides in length, 1500 to 2000 nucleotides in length, 1700 to 2000 nucleotides in length, 2000 to 2300 nucleotides in length, 2200 to 2500 nucleotides in length, 2500 to 3000 nucleotides in length, 3000 to 3200 nucleotides in length, 3000 to 3500 nucleotides in length, 3200 to 3600 nucleotides in length, 3300 to 3800 nucleotides in length, 4000 nucleotides to 4400 nucleotides in length, 4200 to 4700 nucleotides in length, 4800 to 5000 nucleotides in length, 5000 to 5200 nucleotides in length, 5200 to 5500 nucleotides in length, 5500 to 5800 nucleotides in length, 5800 to 6000 nucleotides in length, 6000 to 6400 nucleotides in length, 6200 to 6800 nucleotides in length, 6600 to 7000 nucleotides in length, 7000 to 7200 nucleotides in lengths, 7200 to 7500 nucleotides in length, or 7500 nucleotides in length. In some embodiments, the heterologous ORF encodes a peptide or polypeptide that is 5 to 10 amino acids in length, 10 to 25 amino acids in length, 25 to 50 amino acids in length, 50 to 100 amino acids in length, 100 to 150 amino acids in length, 150 to 200 amino acids in length, 200 to 250 amino acids in length, 250 to 300 amino acids in length, 300 to 400 amino acids in length, 400 to 500 amino acids in length, 500 to 750 amino acids in length, 750 to 1000 amino acids in length, 1000 to 1250 amino acids in length, 1250 to 1500 amino acids in length, 1500 to 1750 amino acids in length, 1750 to 2000 amino acids in length, 2000 to 2500 amino acids in length, or more than 2500 or more amino acids in length. In some embodiments, the heterologous ORF encodes a polypeptide that does not exceed 2500 amino acids in length. In specific embodiments the heterologous ORF does not contain a stop codon. In certain embodiments, the heterologous ORF is codon-optimized. In certain embodiments the nucleotide composition, nucleotide pair composition or both can be optimized. Techniques for such optimizations are known in the art and can be applied to optimize a heterologous ORF.

[00150] Any heterologous ORF from an organism other than an arenavirus may be included in the tri-segmented arenavirus particle. In one embodiment, the heterologous ORF encodes a reporter protein. More detailed description of reporter proteins are described in Section 4.3. In another embodiment, the heterologous ORF encodes an antigen for an infectious pathogen or an antigen associated with any disease and where the antigen is capable of eliciting an immune response. In specific embodiments the antigen is derived from an infectious organism, a tumor (*i.e.,* cancer), or an allergen. More detailed description on heterologous ORFs is described in Section 4.3

[00151] In certain embodiments, the growth and infectivity of the arenavirus particle is not affected by the heterologous ORF from an organism other than an arenavirus.

[00152] Techniques known to one skilled in the art may be used to produce an arenavirus particle comprising an arenavirus genomic segment engineered to carry an arenavirus ORF in a position other than the wild-type position. For example, reverse genetics techniques may be used to generate such arenavirus particle. In other embodiments, the replication-defective arenavirus particle (*i.e.,* the arenavirus genomic segment engineered to carry an arenavirus ORF in a position other than the wild-type position, wherein an ORF encoding GP, NP, Z protein, L protein, has been deleted) can be produced in a complementing cell.

[00153] In certain embodiments, the tri-segmented arenavirus particle using according to the present application can be Old World viruses, for example, LCMV.

[00154] In certain embodiments, the present application relates to the arenavirus particle as described herein suitable for use as a vaccine and methods of using such arenavirus particle in a vaccination and treatment or prevention of, for example, infections and cancers. More detailed description of the methods of using the arenavirus particle described herein is provided in Section 4.6.

[00155] In certain embodiments, the present application relates to the arenavirus particle as described herein suitable for use as a pharmaceutical composition and methods of using such arenavirus particle in a vaccination and treatment or prevention of, for example, infections or cancers. More detailed description of the methods of using the arenavirus particle described herein is provided in Section 4.6.

4.3 Arenavirus Particle or Tri-segmented Arenavirus Particle Expressing a Heterologous ORF

[00156] In certain embodiments, the arenavirus genomic segment, and the respective arenavirus particle or tri-segmented arenavirus particle can comprise a heterologous ORF. In other embodiments, the arenavirus genomic segment and the respective arenavirus particle or tri-segmented arenavirus particle can comprise a gene of interest. In more specific embodiments, the heterologous ORF or the gene of interest encodes an antigen. In more specific embodiments, the heterologous ORF or the gene or interest encodes a reporter protein or a fluorescent protein.

[00157] In certain embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle can comprise one or more heterologous ORFs or one or more genes of interest. In other embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle can comprise at least one heterologous ORF, at least two heterologous ORFs, at least three heterologous ORFs, or more heterologous ORFs. In other embodiments, the arenavirus particle or the tri-segmented arenavirus particle comprises at least one gene of interest, at least two genes of interest, at least three genes of interest, or more genes of interest.

[00158] A wide variety of antigens may be expressed by the arenavirus genomic segment, arenavirus particle or the tri-segmented arenavirus particle of the present application. In one embodiment, the heterologous ORF encodes an antigen of an infectious pathogen or an antigen associated with any disease that is capable of eliciting an immune response. In certain embodiments, the heterologous ORF can encode an antigen derived from a virus, a bacterium, a fungus, a parasite, or can be expressed in a tumor or tumor associated disease (*i.e.,* cancer), an autoimmune disease, a degenerative disease, an inherited disease, substance dependency, obesity, or an allergic disease.

[00159] In some embodiments, the heterologous ORF encodes a viral antigen. Non-limiting examples of viral antigens include antigens from adenoviridae (*e.g.,* mastadenovirus and aviadenovirus), herpesviridae (*e.g.,* herpes simplex virus 1, herpes simplex virus 2, herpes simplex virus 5, herpes simplex virus 6, Epstein-Barr virus, HHV6-HHV8 and cytomegalovirus), leviviridae (*e.g.,* levivirus, enterobacteria phase MS2, allolevirus), poxyiridae (*e.g.,* chordopoxyirinae, parapoxvirus, avipoxvirus, capripoxvirus, leporiipoxvirus, suipoxvirus, molluscipoxvirus, and entomopoxyirinae), papovaviridae (*e.g.,* polyomavirus and papillomavirus), paramyxoviridae (*e.g.,* paramyxovirus, parainfluenza virus 1, mobillivirus (*e.g.,* measles virus), rubulavirus (*e.g.,* mumps virus), pneumonovirinae (*e.g.,* pneumovirus, human respiratory syncytial virus), human respiratory syncytial virus and metapneumovirus (*e.g.,* avian pneumovirus and human metapneumovirus), picornaviridae (*e.g.,* enterovirus, rhinovirus, hepatovirus (*e.g.,* human hepatitis A virus), cardiovirus, and apthovirus), reoviridae (*e.g.,* orthoreovirus, orbivirus, rotavirus, cypovirus, fijivirus, phytoreovirus, and oryzavirus), retroviridae (*e.g.,* mammalian type B retroviruses, mammalian type C retroviruses, avian type C retroviruses, type D retrovirus group, BLV-HTLV retroviruses, lentivirus (e.g. human immunodeficiency virus (HIV) 1 and HIV-2 (*e.g.,* HIV gp160), spumavirus), flaviviridae (*e.g.,* hepatitis C virus, dengue virus, West Nile virus), hepadnaviridae (*e.g.,* hepatitis B virus), togaviridae (*e.g.,* alphavirus (*e.g.,* sindbis virus) and rubivirus (*e.g.,* rubella virus)), rhabdoviridae (*e.g.,* vesiculovirus, lyssavirus, ephemerovirus, cytorhabdovirus, and necleorhabdovirus), arenaviridae (*e.g.,* arenavirus, lymphocytic choriomeningitis virus, Ippy virus, and lassa virus), and coronaviridae (*e.g.,* coronavirus and torovirus). In a specific embodiment the viral antigen, is HIV gp120, gp41, HIV Nef, RSV F glycoprotein, RSV G glycoprotein, HTLV tax, herpes simplex virus glycoprotein (*e.g.,* gB, gC, gD, and gE) or hepatitis B surface antigen, hepatitis C virus E protein or coronavirus spike protein. In one embodiment, the viral antigen is not an HIV antigen.

[00160] In other embodiments, the heterologous ORF encodes a bacterial antigen (*e.g.,* bacterial coat protein). In other embodiments, the heterologous ORF encodes parasitic antigen (*e.g.,* a protozoan antigen). In yet other embodiments, a heterologous nucleotide sequence encodes a fungal antigen.

[00161] Non-limiting examples of bacterial antigens include antigens from bacteria of the Aquaspirillum family, Azospirillum family, Azotobacteraceae family, Bacteroidaceae family, Bartonella species, Bdellovibrio family, *Campylobacter* species, *Chlamydia* species (*e.g., Chlamydia pneumoniae*), *clostridium*, Enterobacteriaceae family (*e.g., Citrobacter* species, *Edwardsiella, Enterobacter aerogenes, Envinia* species, *Escherichia coli, Hafnia* species, *Klebsiella* species, *Morganella* species, *Proteus vulgaris, Providencia, Salmonella* species, *Serratia marcescens*, and *Shigella flexneri*), Gardinella family, *Haemophilus influenzae*, Halobacteriaceae family, Helicobacter family, Legionallaceae family, *Listeria* species, Methylococcaceae family, mycobacteria (*e.g., Mycobacterium tuberculosis*), Neisseriaceae family, Oceanospirillum family, Pasteurellaceae family, Pneumococcus species, *Pseudomonas* species, Rhizobiaceae family, Spirillum family, Spirosomaceae family, *Staphylococcus* (*e.g.,* methicillin resistant *Staphylococcus aureus* and *Staphylococcus* pyrogenes), *Streptococcus* (*e.g., Streptococcus enteritidis*, *Streptococcus* fasciae, and *Streptococcus pneumoniae*), *Vampirovibr Helicobacter* family, *Yersinia* family, *Bacillus antracis* and *Vampirovibrio* family.

[00162] Non-limiting examples of parasite antigens include antigens from a parasite such as an amoeba, a malarial parasite, *Plasmodium, Trypanosoma cruzi*. Non-limiting examples of fungal antigens include antigens from fungus of *Absidia* species (*e.g., Absidia corymbifera and Absidia ramosa*), *Aspergillus* species, (*e.g., Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger*, and *Aspergillus terreus), Basidiobolus ranarum, Blastomyces dermatitidis, Candida* species (*e.g., Candida albicans, Candida glabrata, Candida kern, Candida krusei, Candida parapsilosis, Candida pseudotropicalis, Candida quillermondii, Candida rugosa, Candida stellatoidea,* and *Candida tropicalis*), *Coccidioides* *immitis, Conidiobolus species, Cryptococcus neoforms, Cunninghamella* species, *dermatophytes, Histoplasma capsulatum, Microsporum gypseum, Mucor pusillus, Paracoccidioides brasiliensis, Pseudallescheria boydii, Rhinosporidium seeberi, Pneumocystis carinii, Rhizopus* species (*e.g., Rhizopus arrhizus, Rhizopus oryzae, and Rhizopus microsporus*), *Saccharomyces species, Sporothrix schenckii, zygomycetes*, and classes such as Zygomycetes, Ascomycetes, the Basidiomycetes, Deuteromycetes, and Oomycetes.

[00163] In some embodiments, a heterologous ORF encodes a tumor antigen or tumor associated antigen. In some embodiments, the tumor antigen or tumor associated antigen includes antigens from tumor associated diseases including acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, childhood adrenocortical carcinoma, AIDS-Related Cancers, Kaposi Sarcoma, anal cancer, appendix cancer, astrocytomas, atypical teratoid/rhabdoid tumor, basal-cell carcinoma, bile duct cancer, extrahepatic (see cholangiocarcinoma), bladder cancer, bone osteosarcoma/malignant fibrous histiocytoma, brainstem glioma, brain cancer, brain tumor, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma brain tumor, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, breast cancer, bronchial adenomas/carcinoids, burkitt's lymphoma, carcinoid tumor, carcinoid gastrointestinal tumor, carcinoma of unknown primary, central nervous system lymphoma, primary, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, cervical cancer, childhood cancers, chronic bronchitis, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, emphysema, endometrial cancer, ependymoma, esophageal cancer, ewing's sarcoma in the Ewing family of tumors, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, germ cell tumor: extracranial, extragonadal, or ovarian gestational trophoblastic tumor, glioma of the brain stem, glioma, childhood cerebral astrocytoma, childhood visual pathway and hypothalamic, gastric carcinoid, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, intraocular melanoma, islet cell carcinoma (endocrine pancreas), kaposi sarcoma, kidney cancer (renal cell cancer), laryngeal cancer, acute lymphoblastic lymphoma, acute lymphocytic leukemia, acute myelogenous leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, lip and oral cavity cancer, liposarcoma, liver cancer (primary), lung cancer, non-small cell, small cell, AIDS-related lymphoma, Burkitt lymphoma, cutaneous T-cell lymphoma, hodgkin lymphoma, non-hodgkin lymphoma, lymphoma, primary central nervous system, macroglobulinemia, Waldenström, male breast cancer, malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma, melanoma, intraocular (eye), merkel cell cancer, mesothelioma, adult malignant, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, myelogenous leukemia, chronic, myeloid leukemia, adult acute, myeloid leukemia, childhood acute, myeloma, multiple (cancer of the bone-marrow), myeloproliferative disorders, chronic, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-small cell lung cancer, oligodendroglioma, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer (surface epithelial-stromal tumor), ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, islet cell, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary adenoma, plasma cell neoplasia/multiple myeloma, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma (kidney cancer), renal pelvis and ureter, transitional cell cancer, retinoblastoma, rhabdomyosarcoma, childhood, salivary gland cancer, sarcoma, Ewing family of tumors, Kaposi sarcoma, soft tissue sarcoma, uterine sarcoma, sézary syndrome, skin cancer (non-melanoma), skin cancer (melanoma), merkel cell skin carcinoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma – see skin cancer (non-melanoma), squamous neck cancer with occult primary, metastatic, stomach cancer, supratentorial primitive neuroectodermal tumor, T-Cell lymphoma, cutaneous – see Mycosis Fungoides and Sézary syndrome, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, childhood transitional cell cancer of the renal pelvis and ureter, gestational trophoblastic tumor, unknown primary site, carcinoma of, adult unknown primary site, cancer of childhood, ureter and renal pelvis, transitional cell cancer, rethral cancer, uterine cancer, endometrial uterine sarcoma, bronchial tumor, central nervous system embryonal tumor; childhood chordoma, colorectal cancer, craniopharyngioma, ependymoblastoma, langerhans cell histiocytosis, acute lymphoblastic leukemia, acute myeloid leukemia (adult / childhood), small cell lung cancer, medulloepithelioma, oral cavity cancer, papillomatosis, pineal parenchymal tumors of intermediate differentiation, pituary tumor, respiratory tract carcinoma involving the NUT gene on chromosome 15, spinal cord tumor, thymoma, thyroid cancer, vaginal Cancer; vulvar Cancer, and Wilms Tumor.

[00164] Non-limiting examples of tumor or tumor associated antigens include Adipophilin, AIM-2, ALDH1A1, BCLX (L), BING-4, CALCA, CD45, CPSF, cyclin D1, DKK1, ENAH (hMena), EpCAM, EphA3, EZH2, FGF5, glypican-3, G250 /MN/CAIX, HER-2/neu, IDO1, IGF2B3, IL13Ralpha2, Intestinal carboxyl esterase, alpha-fetoprotein, Kallikrein 4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RU2AS, secernin 1, SOX10, STEAP1, survivinn, Telomerase, VEGF, or WT1, EGF-R, CEA, CD52, gp 100 protein, MELANA/MART1, NY-ESO-1, p53 MAGE1, MAGE3 and CDK4, alpha-actinin-4, ARTC1, BCR-ABL fusion protein (b3a2), B-RAF, CASP-5, CASP-8, beta-catenin, Cdc27, CDK4, CDKN2A, CLPP, COA-1, dek-can fusion protein, EFTUD2, Elongation factor 2, ETV6-AML1 fusion protein, FLT3-ITD, FN1, GPNMB, LDLR-fucosyltransferaseAS fusion protein, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras, N-ras, RBAF600, SIRT2, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, TGF-betaRII, Triosephosphate isomerase, Lengsin, M-CSF, MCSP, or mdm-2.

[00165] In some embodiments, the heterologous ORF encodes a respiratory pathogen antigen. In a specific embodiment, the respiratory pathogen is a virus such as RSV, coronavirus, human metapneumovirus, parainfluenza virus, hendra virus, nipah virus, adenovirus, rhinovirus, or PRRSV. Non-limiting examples of respiratory viral antigens include Respiratory Syncytial virus F, G and M2 proteins, Coronavirus (SARS, HuCoV) spike proteins (S), human metapneumovirus fusion proteins, Parainfluenza virus fusion and hemagglutinin proteins (F, HN), Hendra virus (HeV) and Nipah virus (NiV) attachment glycoproteins (G and F), Adenovirus capsid proteins, Rhinovirus proteins, and PRRSV wild type or modified GP5 and M proteins.

[00166] In a specific embodiment, the respiratory pathogen is a bacteria such as *Bacillus anthracis, mycobacterium tuberculosis, Bordetella pertussis, streptococcus pneumoniae, yersinia pestis, staphylococcus aureus, Francisella tularensis, legionella pneumophila, chlamydia pneumoniae, pseudomonas aeruginosa, neisseria meningitides, and haemophilus influenzae*. Non-limiting examples of respiratory bacterial antigens include Bacillus anthracis Protective antigen PA, Mycobacterium tuberculosis mycobacterial antigen 85A and heat shock protein (Hsp65), Bordetella pertussis pertussis toxoid (PT) and filamentous hemagglutinin (FHA), Streptococcus pneumoniae sortase A and surface adhesin A (PsaA), Yersinia pestis F1 and V subunits, and proteins from Staphylococcus aureus, Francisella tularensis, Legionella pneumophila, Chlamydia pneumoniae, Pseudomonas aeruginosa, Neisseria meningitides, and Haemophilus influenzae.

[00167] In some embodiments, the heterologous ORF encodes a T-cell epitope. In other embodiments, the heterologous ORF encodes a cytokine or growth factor.

[00168] In other embodiments, the heterologous ORF encodes an antigen expressed in an autoimmune disease. In more specific embodiments, the autoimmune disease can be type I diabetes, multiple sclerosis, rheumatoid arthritis, lupus erythmatosus, and psoriasis. Non-limiting examples of autoimmune disease antigens include Ro60, dsDNA, or RNP.

[00169] In other embodiments, ORF encodes an antigen expressed in an allergic disease. In more specific embodiments, the allergic disease can include but is not limited to seasonal and perennial rhinoconjunctivitis, asthma, and eczema. Non-limiting examples of allergy antigens include Bet v 1 and Fel d 1.

[00170] In other embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle further comprises a reporter protein. The reporter protein is capable of expression at the same time as the antigen described herein. Ideally, expression is visible in normal light or other wavelengths of light. In certain embodiments, the intensity of the effect created by the reporter protein can be used to directly measure and monitor the arenavirus particle or tri-segmented arenavirus particle.

[00171] Reporter genes would be readily recognized by one of skill in the art. In certain embodiments, the arenavirus particle is a fluorescent protein. In other embodiments, the reporter gene is GFP. GFP emits bright green light when exposed to UV or blue like.

[00172] Non-limiting examples of reporter proteins include various enzymes, such as, but not to β-galactosidase, chloramphenicol acetyltransferase, neomycin phosphotransferase, luciferase or RFP.

[00173] In certain embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle expressing a heterologous ORF has desirable properties for use as a vector for vaccination (see *e.g.,* Section 4.6) . In another embodiment, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle expressing a heterologous ORF is capable of inducing an immune response in a host (*e.g.,* mouse rabbit, goat, donkey, human). In other embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle expressing a heterologous ORF described herein induces an innate immune response. In other embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle expressing a heterologous ORF induces an adaptive immune response. In more specific embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle expressing a heterologous ORF both an innate and adaptive immune response.

[00174] In another embodiment, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle expressing a heterologous ORF induces a T cell response. In yet more specific embodiments, the arenavirus genomic segment, the arenavirus particle or tri-segmented arenavirus particle expressing a heterologous ORF induces a CD8+T cell response. In other embodiments, the arenavirus particle carrying a foreign gene of interest induces a potent CD8+ T cell response of high frequency and functionality. In other embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen induces CD8+ T cells specific to one or multiple epitopes of the corresponding foreign gene of interest.

[00175] In certain embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle expressing a heterologous ORF can induce T helper 1 differentiation, memory formation of CD4+ T cells and/or elicit durable antibody responses. These antibodies can be neutralizing, opsonizing, toxic to tumor cells or have other favorable biological features. In other embodiments, the arenavirus genomic segment, the arenavirus particle or tri-segmented arenavirus particle expressing a heterologous ORF has a strong tropism for dendritic cells and activates them upon infection. This potentiates presentation of the antigen by antigen presenting cells.

[00176] In certain embodiments, the arenavirus genomic segment, the arenavirus particle or the tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen induces low or undetectable neutralizing antibody titers against LCMV and high protective neutralizing antibody responses to the respective foreign transgene. In some embodiments, the arenavirus backbone forming the particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen has low capacity for inducing immunity to the arenaviral backbone components.

4.4 Generation of an arenavirus particle and a tri-segmented arenavirus particle

[00177] Generally, arenavirus particles can be recombinantly produced by standard reverse genetic techniques as described for LCMV (see Flatz *et al.,* 2006, Proc Natl Acad Sci USA 103:4663-4668; Sanchez *et al.,* 2006, Virology 350:370; Ortiz-Riano *et al.,* 2013, J Gen Virol. 94:1175-88). To generate the arenavirus particles provided herein, these techniques can be applied as described below. The genome of the viruses can be modified as described in Section 4.1 and Section 4.2, respectively.

4.4.1 Non-natural Position Open Reading Frame

[00178] The generation of an arenavirus particle comprising a genomic segment that has been engineered to carry a viral ORF in a position other than the wild-type position of the ORF can be recombinantly produced by any reverse genetic techniques known to one skilled in the art.

(i) *Infectious and Replication Competent Arenavirus Particle*

[00179] In certain embodiments, the method of generating the arenavirus particle comprises (i) transfecting into a host cell the cDNA of the first arenavirus genomic segment; (ii) transfecting into a host cell the cDNA of the second arenavirus genomic segment; (iii) transfecting into a host cell plasmids expressing the arenavirus' minimal trans-acting factors NP and L; (iv) maintaining the host cell under conditions suitable for virus formation; and (v) harvesting the arenavirus particle. In certain more specific embodiments, the cDNA is comprised in a plasmid.

[00180] Once generated from cDNA, arenavirus particles (*i.e.,* infectious and replication competent) can be propagated. In certain embodiments, the arenavirus particle can be propagated in any host cell that allows the virus to grow to titers that permit the uses of the virus as described herein. In one embodiment, the host cell allows the arenavirus particle to grow to titers comparable to those determined for the corresponding wild-type.

[00181] In certain embodiments, the arenavirus particle may be propagated in host cells. Specific examples of host cells that can be used include BHK-21, HEK 293, VERO or other. In a specific embodiment, the arenavirus particle may be propagated in a cell line.

[00182] In certain embodiments, the host cells are kept in culture and are transfected with one or more plasmid(s). The plasmid(s) express the arenavirus genomic segment(s) to be generated under control of one or more expression cassettes suitable for expression in mammalian cells, *e.g.,* consisting of a polymerase I promoter and terminator.

[00183] Plasmids that can be used for the generation of the arenavirus particle can include: i) a plasmid encoding the S genomic segment *e.g.,* pol-I S, ii) a plasmid encoding the

Date Reçue/Date Received 2022-02-07

L genomic segment *e.g.,* pol-I L. In certain embodiments, the plasmid encoding an arenavirus polymerase that direct intracellular synthesis of the viral L and S segments can be incorporated into the transfection mixture. For example, a plasmid encoding the L protein and/or a plasmid encoding NP (pC-L and pC-NP, respectively) can be present. The L protein and NP are the minimal trans-acting factors necessary for viral RNA transcription and replication. Alternatively, intracellular synthesis of viral L and S segments, together with NP and L protein can be performed using an expression cassette with pol-I and pol-II promoters reading from opposite sides into the L and S segment cDNAs of two separate plasmids, respectively.

[00184] In certain embodiments, the arenavirus genomic segments are under the control of a promoter. Typically, RNA polymerase I-driven expression cassettes, RNA polymerase II-driven cassettes or T7 bacteriophage RNA polymerase driven cassettes can be used. In certain embodiments, the plasmid(s) encoding the arenavirus genomic segments can be the same, *i.e.,* the genome sequence and transacting factors can be transcribed by a promoter from one plasmid. Specific examples of promoters include an RNA polymerase I promoter, an RNA polymerase II promoter, an RNA polymerase III promoter, a T7 promoter, an SP6 promoter or a T3 promoter.

[00185] In addition, the plasmid(s) can feature a mammalian selection marker, *e.g.,* puromycin resistance, under control of an expression cassette suitable for gene expression in mammalian cells, *e.g.,* polymerase II expression cassette as above, or the viral gene transcript(s) are followed by an internal ribosome entry site, such as the one of encephalomyocarditis virus, followed by the mammalian resistance marker. For production in E.coli, the plasmid additionally features a bacterial selection marker, such as an ampicillin resistance cassette.

[00186] Transfection of a host cell with a plasmid(s) can be performed using any of the commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation. A few days later the suitable selection agent, *e.g.,* puromycin, is added in titrated concentrations. Surviving clones are isolated and subcloned following standard procedures, and high-expressing clones are identified using Western blot or flow cytometry procedures with antibodies directed against the viral protein(s) of interest.

[00187] For recovering the arenavirus particle described herein, the following procedures are envisaged. First day: cells, typically 80% confluent in M6-well plates, are transfected with a mixture of the plasmids, as described above. For this one can exploit any commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation.

[00188] 3-5 days later: The cultured supernatant (arenavirus vector preparation) is harvested, aliquoted and stored at 4 °C, -20 °C, or -80 °C, depending on how long the arenavirus vector should be stored prior use. The arenavirus vector preparation's infectious titer is assessed by an immunofocus assay. Alternatively, the transfected cells and supernatant may be passaged to a larger vessel (*e.g.,* a T75 tissue culture flask) on day 3-5 after transfection, and culture supernatant is harvested up to five days after passage.

[00189] The present application furthermore relates to expression of a heterologous ORF, wherein a plasmid encoding the genomic segment is modified to incorporated a heterologous ORF. The heterologous ORF can be incorporated into the plasmid using restriction enzymes.

(ii) *Infectious, Replication-Defective Arenavirus Particle*

[00190] Infectious, replication-defective arenavirus particles can be rescued as described above. However, once generated from cDNA, the infectious, replication-deficient arenaviruses provided herein can be propagated in complementing cells. Complementing cells are cells that provide the functionality that has been eliminated from the replication-deficient arenavirus by modification of its genome (*e.g.,* if the ORF encoding the GP protein is deleted or functionally inactivated, a complementing cell does provide the GP protein).

[00191] Owing to the removal or functional inactivation of one or more of the ORFs in arenavirus vectors (here deletion of the glycoprotein, GP, will be taken as an example), arenavirus vectors can be generated and expanded in cells providing *in trans* the deleted viral gene(s), *e.g.,* the GP in the present example. Such a complementing cell line, henceforth referred to as C-cells, is generated by transfecting a cell line such as BHK-21, HEK 293, VERO or other with one or more plasmid(s) for expression of the viral gene(s) of interest (complementation plasmid, referred to as C-plasmid). The C-plasmid(s) express the viral gene(s) deleted in the arenavirus vector to be generated under control of one or more expression cassettes suitable for expression in mammalian cells, *e.g.,* a mammalian polymerase II promoter such as the EF1alpha promoter with a polyadenylation signal. In addition, the complementation plasmid features a mammalian selection marker, *e.g.,* puromycin resistance, under control of an expression cassette suitable for gene expression in mammalian cells, *e.g.,* polymerase II expression cassette as above, or the viral gene transcript(s) are followed by an internal ribosome entry site, such as the one of encephalomyocarditis virus, followed by the mammalian resistance marker. For production in E. coli, the plasmid additionally features a bacterial selection marker, such as an ampicillin resistance cassette.

[00192] Cells that can be used, *e.g.,* BHK-21, HEK 293, MC57G or other, are kept in culture and are transfected with the complementation plasmid(s) using any of the commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation. A few days later the suitable selection agent, *e.g.,* puromycin, is added in titrated concentrations. Surviving clones are isolated and subcloned following standard procedures, and high-expressing C-cell clones are identified using Western blot or flow cytometry procedures with antibodies directed against the viral protein(s) of interest. As an alternative to the use of stably transfected C-cells transient transfection of normal cells can complement the missing viral gene(s) in each of the steps where C-cells will be used below. In addition, a helper virus can be used to provide the missing functionality *in trans*.

[00193] Plasmids can be of two types: i) two plasmids, referred to as TF-plasmids for expressing intracellularly in C-cells the minimal transacting factors of the arenavirus, is derived from *e.g.,* NP and L proteins of LCMV in the present example; and ii) plasmids, referred to as GS-plasmids, for expressing intracellularly in C-cells the arenavirus vector genome segments, *e.g.,* the segments with designed modifications. TF-plasmids express the NP and L proteins of the respective arenavirus vector under control of an expression cassette suitable for protein expression in mammalian cells, typically *e.g.,* a mammalian polymerase II promoter such as the CMV or EF1alpha promoter, either one of them preferentially in combination with a polyadenylation signal. GS-plasmids express the small (S) and the large (L) genome segments of the vector. Typically, polymerase I-driven expression cassettes or T7 bacteriophage RNA polymerase (T7-) driven expression cassettes can be used, the latter preferentially with a 3'-terminal ribozyme for processing of the primary transcript to yield the correct end. In the case of using a T7-based system, expression of T7 in C-cells must be provided by either including in the recovery process an additional expression plasmid, constructed analogously to TF-plasmids, providing T7, or C-cells are constructed to additionally express T7 in a stable manner. In certain embodiments, TF and GS plasmids can be the same, *i.e.,* the genome sequence and transacting factors can be transcribed by T7, polI and polII promoters from one plasmid.

[00194] For recovering of the arenavirus vector, the following procedures can be used. First day: C-cells, typically 80% confluent in M6-well plates, are transfected with a mixture of the two TF-plasmids plus the two GS-plasmids. In certain embodiments, the TF and GS plasmids can be the same, *i.e.,* the genome sequence and transacting factors can be transcribed by T7, polI and polII promoters from one plasmid. For this one can exploit any of the commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation.

[00195] 3-5 days later: The culture supernatant (arenavirus vector preparation) is harvested, aliquoted and stored at 4 °C, -20 °C or -80 °C depending on how long the arenavirus vector should be stored prior to use. Then the arenavirus vector preparation's infectious titer is assessed by an immunofocus assay on C-cells. Alternatively, the transfected cells and supernatant may be passaged to a larger vessel (*e.g.*, a T75 tissue culture flask) on day 3-5 after transfection, and culture supernatant is harvested up to five days after passage.

[00196] The invention furthermore relates to expression of a antigen in a cell culture wherein the cell culture is infected with an infectious, replication-deficient arenavirus expressing a antigen. When used for expression of a antigen in cultured cells, the following two procedures can be used:

[00197] i) The cell type of interest is infected with the arenavirus vector preparation described herein at a multiplicity of infection (MOI) of one or more, *e.g.,* two, three or four, resulting in production of the antigen in all cells already shortly after infection.

[00198] ii) Alternatively, a lower MOI can be used and individual cell clones can be selected for their level of virally driven antigen expression. Subsequently individual clones can be expanded infinitely owing to the non-cytolytic nature of arenavirus vectors. Irrespective of the approach, the antigen can subsequently be collected (and purified) either from the culture supernatant or from the cells themselves, depending on the properties of the antigen produced. However, the invention is not limited to these two strategies, and other ways of driving expression of antigen using infectious, replication-deficient arenaviruses as vectors may be considered.

4.4.2 Generation of a Tri-segmented Arenavirus Particle

[00199] A tri-segmented arenavirus particle can be recombinantly produced by reverse genetic techniques known in the art, for example as described by Emonet *et al.,* 2008, PNAS, 106(9):3473-3478; Popkin *et al.,* 2011, J. Virol., 85 (15):7928–7932. The generation of the tri-segmented arenavirus particle provided herein can be modified as described in Section 4.2.

Date Reçue/Date Received 2022-02-07

(i) *Infectious and Replication Competent Tri-segmented arenavirus Particle*

[00200] In certain embodiments, the method of generating the tri-segmented arenavirus particle comprises (i) transfecting into a host cell the cDNAs of the one L segment and two S segments or two L segments and one S segment; (ii) transfecting into a host cell plasmids expressing the arenavirus' minimal trans-acting factors NP and L; (iii) maintaining the host cell under conditions suitable for virus formation; and (iv) harvesting the arenavirus particle.

[00201] Once generated from cDNA, the tri-segmented arenavirus particle (*i.e.,* infectious and replication competent) can be propagated. In certain embodiments tri-segmented arenavirus particle can be propagated in any host cell that allows the virus to grow to titers that permit the uses of the virus as described herein. In one embodiment, the host cell allows the tri-segmented arenavirus particle to grow to titers comparable to those determined for the corresponding wild-type.

[00202] In certain embodiments, the tri-segmented arenavirus particle may be propagated in host cells. Specific examples of host cells that can be used include BHK-21, HEK 293, VERO or other. In a specific embodiment, the tri-segmented arenavirus particle may be propagated in a cell line.

[00203] In certain embodiments, the host cells are kept in culture and are transfected with one or more plasmid(s). The plasmid(s) express the arenavirus genomic segment(s) to be generated under control of one or more expression cassettes suitable for expression in mammalian cells, *e.g.,* consisting of a polymerase I promoter and terminator.

[00204] In specific embodiments, the host cells are kept in culture and are transfected with one or more plasmid(s). The plasmid(s) express the viral gene(s) to be generated under control of one or more expression cassettes suitable for expression in mammalian cells, *e.g.,* consisting of a polymerase I promoter and terminator.

[00205] Plasmids that can be used for generating the tri-segmented arenavirus comprising one L segment and two S segments can include: i) two plasmids each encoding the S genome segment *e.g.,* pol-I S, ii) a plasmid encoding the L genome segment *e.g.,* pol-I L. Plasmids needed for the tri-segmented arenavirus comprising two L segments and one S segments are: i) two plasmids each encoding the L genome segment *e.g.,* pol-L, ii) a plasmid encoding the S genome segment *e.g.,* pol-I S.

[00206] In certain embodiments, plasmids encoding an arenavirus polymerase that direct intracellular synthesis of the viral L and S segments can be incorporated into the transfection mixture. For example, a plasmid encoding the L protein and a plasmid encoding NP (pC-L and pC-NP, respectively). The L protein and NP are the minimal trans-acting factors necessary for viral RNA transcription and replication. Alternatively, intracellular synthesis of viral L and S segments, together with NP and L protein can be performed using an expression cassette with pol-I and pol-II promoters reading from opposite sides into the L and S segment cDNAs of two separate plasmids, respectively.

[00207] In addition, the plasmid(s) features a mammalian selection marker, *e.g.,* puromycin resistance, under control of an expression cassette suitable for gene expression in mammalian cells, *e.g.,* polymerase II expression cassette as above, or the viral gene transcript(s) are followed by an internal ribosome entry site, such as the one of encephalomyocarditis virus, followed by the mammalian resistance marker. For production in E.coli, the plasmid additionally features a bacterial selection marker, such as an ampicillin resistance cassette.

[00208] Transfection of BHK-21 cells with a plasmid(s) can be performed using any of the commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation. A few days later the suitable selection agent, *e.g.,* puromycin, is added in titrated concentrations. Surviving clones are isolated and subcloned following standard procedures, and high-expressing clones are identified using Western blot or flow cytometry procedures with antibodies directed against the viral protein(s) of interest.

[00209] Typically, RNA polymerase I-driven expression cassettes, RNA polymerase II-driven cassettes or T7 bacteriophage RNA polymerase driven cassettes can be used, , the latter preferentially with a 3'-terminal ribozyme for processing of the primary transcript to yield the correct end. In certain embodiments, the plasmids encoding the arenavirus genomic segments can be the same, *i.e.,* the genome sequence and transacting factors can be transcribed by T7, polI and polII promoters from one plasmid.

[00210] For recovering the arenavirus the tri-segmented arenavirus vector, the following procedures are envisaged. First day: cells, typically 80% confluent in M6-well plates, are transfected with a mixture of the plasmids, as described above. For this one can exploit any commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation.

[00211] 3-5 days later: The cultured supernatant (arenavirus vector preparation) is harvested, aliquoted and stored at 4 °C, -20 °C, or -80 °C, depending on how long the arenavirus vector should be stored prior use. The arenavirus vector preparation's infectious titer is assessed by an immunofocus assay. Alternatively, the transfected cells and supernatant may be passaged to a larger vessel (*e.g.*, a T75 tissue culture flask) on day 3-5 after transfection, and culture supernatant is harvested up to five days after passage.

[00212] The present application furthermore relates to expression of a heterologous ORF and/or a gene of interest, wherein a plasmid encoding the genomic segment is modified to incorporated a heterologous ORF and/or a gene of interest. The heterologous ORF and/or gene of interest can be incorporated into the plasmid using restriction enzymes.

(ii) *Infectious, Replication-Defective Tri-segmented Arenavirus Particle*

[00213] Infectious, replication-defective tri-segmented arenavirus particles can be rescued as described above. However, once generated from cDNA, the infectious, replication-deficient arenaviruses provided herein can be propagated in complementing cells. Complementing cells are cells that provide the functionality that has been eliminated from the replication-deficient arenavirus by modification of its genome (*e.g.*, if the ORF encoding the GP protein is deleted or functionally inactivated, a complementing cell does provide the GP protein).

[00214] Owing to the removal or functional inactivation of one or more of the ORFs in arenavirus vectors (here deletion of the glycoprotein, GP, will be taken as an example), arenavirus vectors can be generated and expanded in cells providing *in trans* the deleted viral gene(s), *e.g.*, the GP in the present example. Such a complementing cell line, henceforth referred to as C-cells, is generated by transfecting a mammalian cell line such as BHK-21, HEK 293, VERO or other (here BHK-21 will be taken as an example) with one or more plasmid(s) for expression of the viral gene(s) of interest (complementation plasmid, referred to as C-plasmid). The C-plasmid(s) express the viral gene(s) deleted in the arenavirus vector to be generated under control of one or more expression cassettes suitable for expression in mammalian cells, *e.g.*, a mammalian polymerase II promoter such as the CMV or EF1alpha promoter with a polyadenylation signal. In addition, the complementation plasmid features a mammalian selection marker, *e.g.*, puromycin resistance, under control of an expression cassette suitable for gene expression in mammalian cells, *e.g.*, polymerase II expression cassette as above, or the viral gene transcript(s) are followed by an internal ribosome entry site, such as the one of encephalomyocarditis virus, followed by the mammalian resistance marker. For production in E. coli, the plasmid additionally features a bacterial selection marker, such as an ampicillin resistance cassette.

[00215] Cells that can be used, *e.g.*, BHK-21, HEK 293, MC57G or other, are kept in culture and are transfected with the complementation plasmid(s) using any of the commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation. A few days later the suitable selection agent, *e.g.*, puromycin, is added in titrated concentrations. Surviving clones are isolated and subcloned following standard procedures, and high-expressing C-cell clones are identified using Western blot or flow cytometry procedures with antibodies directed against the viral protein(s) of interest. As an alternative to the use of stably transfected C-cells transient transfection of normal cells can complement the missing viral gene(s) in each of the steps where C-cells will be used below. In addition, a helper virus can be used to provide the missing functionality *in trans*.

[00216] Plasmids of two types can be used: i) two plasmids, referred to as TF-plasmids for expressing intracellularly in C-cells the minimal transacting factors of the arenavirus, is derived from *e.g.,* NP and L proteins of LCMV in the present example; and ii) plasmids, referred to as GS-plasmids, for expressing intracellularly in C-cells the arenavirus vector genome segments, *e.g.,* the segments with designed modifications. TF-plasmids express the NP and L proteins of the respective arenavirus vector under control of an expression cassette suitable for protein expression in mammalian cells, typically *e.g.,* a mammalian polymerase II promoter such as the CMV or EF1alpha promoter, either one of them preferentially in combination with a polyadenylation signal. GS-plasmids express the small (S) and the large (L) genome segments of the vector. Typically, polymerase I-driven expression cassettes or T7 bacteriophage RNA polymerase (T7-) driven expression cassettes can be used, the latter preferentially with a 3'-terminal ribozyme for processing of the primary transcript to yield the correct end. In the case of using a T7-based system, expression of T7 in C-cells must be provided by either including in the recovery process an additional expression plasmid, constructed analogously to TF-plasmids, providing T7, or C-cells are constructed to additionally express T7 in a stable manner. In certain embodiments, TF and GS plasmids can be the same, *i.e.,* the genome sequence and transacting factors can be transcribed by T7, polI and polII promoters from one plasmid.

[00217] For recovering of the arenavirus vector, the following procedures can be used. First day: C-cells, typically 80% confluent in M6-well plates, are transfected with a mixture of the two TF-plasmids plus the two GS-plasmids. In certain embodiments, the TF and GS plasmids can be the same, *i.e.,* the genome sequence and transacting factors can be transcribed by T7, polI and polII promoters from one plasmid. For this one can exploit any of the commonly used strategies such as calcium-phosphate, liposome-based protocols or electroporation.

[00218] 3-5 days later: The culture supernatant (arenavirus vector preparation) is harvested, aliquoted and stored at 4 °C, -20 °C or -80 °C depending on how long the arenavirus vector should be stored prior to use. Then the arenavirus vector preparation's infectious titer is assessed by an immunofocus assay on C-cells. Alternatively, the transfected cells and supernatant may be passaged to a larger vessel (*e.g.*, a T75 tissue culture flask) on day 3-5 after transfection, and culture supernatant is harvested up to five days after passage.

[00219] The invention furthermore relates to expression of an antigen in a cell culture wherein the cell culture is infected with an infectious, replication-deficient tri-segmented arenavirus expressing a antigen. When used for expression of a CMV antigen in cultured cells, the following two procedures can be used:

[00220] i) The cell type of interest is infected with the arenavirus vector preparation described herein at a multiplicity of infection (MOI) of one or more, *e.g.,* two, three or four, resulting in production of the antigen in all cells already shortly after infection.

[00221] ii) Alternatively, a lower MOI can be used and individual cell clones can be selected for their level of virally driven antigen expression. Subsequently individual clones can be expanded infinitely owing to the non-cytolytic nature of arenavirus vectors. Irrespective of the approach, the antigen can subsequently be collected (and purified) either from the culture supernatant or from the cells themselves, depending on the properties of the antigen produced. However, the invention is not limited to these two strategies, and other ways of driving expression of CMV antigen using infectious, replication-deficient arenaviruses as vectors may be considered.

4.5 Nucleic Acids, Vector Systems and Cell Lines

[00222] In certain embodiments, provided herein are cDNAs comprising or consisting of the arenavirus genomic segment or the tri-segmented arenavirus particle as described in Section 4.1 and Section 4.2, respectively.

4.5.1 Non-natural Position Open Reading Frame

[00223] In one embodiment, provided herein are nucleic acids that encode an arenavirus genomic segment as described in Section 4.1. In more specific embodiments, provided herein is a DNA nucleotide sequence or a set of DNA nucleotide sequences as set forth in Table 1. Host cells that comprise such nucleic acids are also provided Section 4.1.

[00224] In specific embodiments, provided herein is a cDNA of the arenavirus genomic segment engineered to carry an ORF in a position other than the wild-type position of the

ORF, wherein the arenavirus genomic segment encodes a heterologous ORF as described in Section 4.1.

[00225] In one embodiment, provided herein is a DNA expression vector system that encodes the arenavirus genomic segment engineered to carry an ORF in a position other than the wild-type position of the ORF. Specifically, provided herein is a DNA expression vector system wherein one or more vectors encodes two arenavirus genomic segments, namely, an L segment and an S segment, of an arenavirus particle described herein. Such a vector system can encode (one or more separate DNA molecules).

[00226] In another embodiment, provided herein is a cDNA of the arenavirus S segment that has been engineered to carry an ORF in a position other than the wild-type position is part of or incorporated into a DNA expression system. In other embodiments, a cDNA of the arenavirus L segment that has been engineered to carry an ORF in a position other than the wild-type position is part of or incorporated into a DNA expression system. In certain embodiments, is a cDNA of the arenavirus genomic segment that has been engineered to carry (i) an ORF in a position other than the wild-type position of the ORF; and (ii) and ORF encoding GP, NP, Z protein, or L protein has been removed and replaced with a heterologous ORF from an organism other than an arenavirus.

[00227] In certain embodiments, the cDNA provided herein can be derived from a particular strain of LCMV. Strains of LCMV include Clone 13, MP strain, Arm CA 1371, Arm E-250, WE, UBC, Traub, Pasteur, 810885, CH-5692, Marseille #12, HP65-2009, 200501927, 810362, 811316, 810316, 810366, 20112714, Douglas, GR01, SN05, CABN and their derivatives. In specific embodiments, the cDNA is derived from LCMV Clone 13. In other specific embodiments, the cDNA is derived from LCMV MP strain.

[00228] In certain embodiments, the vector generated to encode an arenavirus particle or a tri-segmented arenavirus particle as described herein may be based on a specific strain of LCMV. Strains of LCMV include Clone 13, MP strain, Arm CA 1371, Arm E-250, WE, UBC, Traub, Pasteur, 810885, CH-5692, Marseille #12, HP65-2009, 200501927, 810362, 811316, 810316, 810366, 20112714, Douglas, GR01, SN05, CABN and their derivatives. In certain embodiments, an arenavirus particle or a tri-segmented arenavirus particle as described herein may be based on LCMV Clone 13. In other embodiments, the vector generated to encode an arenavirus particle or a tri-segmented arenavirus particle as described herein LCMV MP strain. The sequence of the S segment of LCMV Clone 13 is listed as SEQ ID NO: 2. In certain embodiments, the sequence of the S segment of LCMV Clone 13 is the sequence set forth in SEQ ID NO: 1. The sequence of the L segment of LCMV Clone

13 is listed as SEQ ID NO: 5. The sequence of the S segment of LCMV strain MP is listed as SEQ ID NO: 53. The sequence of the L segment of LCMV strain MP is listed as SEQ ID NO: 4.

[00229] In another embodiment, provided herein is a cell, wherein the cell comprises a cDNA or a vector system described above in this section. Cell lines derived from such cells, cultures comprising such cells, methods of culturing such cells infected are also provided herein. In certain embodiments, provided herein is a cell, wherein the cell comprises a cDNA of the arenavirus genomic segment that has been engineered to carry an ORF in a position other than the wild-type position of the ORF. In some embodiments, the cell comprises the S segment and/or the L segment.

4.5.2 Tri-segmented Arenavirus Particle

[00230] In one embodiment, provided herein are nucleic acids that encode a tri-segmented arenavirus particle as described in Section 4.2. In more specific embodiments, provided herein is a DNA nucleotide sequence or a set of DNA nucleotide sequences, for example, as set forth in Table 2 or Table 3. Host cells that comprise such nucleic acids are also provided Section 4.2.

[00231] In specific embodiments, provided herein is a cDNA consisting of a cDNA of the tri-segmented arenavirus particle that has been engineered to carry an ORF in a position other than the wild-type position of the ORF. In other embodiments, is a cDNA of the tri-segmented arenavirus particle that has been engineered to (i) carry an arenavirus ORF in a position other than the wild-type position of the ORF; and (ii) wherein the tri-segmented arenavirus particle encodes a heterologous ORF as described in Section 4.2.

[00232] In one embodiment, provided herein is a DNA expression vector system that together encode the tri-segmented arenavirus particle as described herein. Specifically, provided herein is a DNA expression vector system wherein one or more vectors encode three arenavirus genomic segments, namely, one L segment and two S segments or two L segments and one S segment of a tri-segmented arenavirus particle described herein. Such a vector system can encode (one or more separate DNA molecules).

[00233] In another embodiment, provided herein is a cDNA of the arenavirus S segment(s) that has been engineered to carry an ORF in a position other than the wild-type position, and is part of or incorporated into a DNA expression system. In other embodiments, a cDNA of the arenavirus L segment(s) that has been engineered to carry an ORF in a position other than the wild-type position is part of or incorporated into a DNA expression system. In certain embodiments, is a cDNA of the tri-segmented arenavirus particle that has been engineered to carry (i) an ORF in a position other than the wild-type position of the ORF; and (ii) an ORF encoding GP, NP, Z protein, or L protein has been removed and replaced with a heterologous ORF from an organism other than an arenavirus.

[00234] In certain embodiments, the cDNA provided herein can be derived from a particular strain of LCMV. Strains of LCMV include Clone 13, MP strain, Arm CA 1371, Arm E-250, WE, UBC, Traub, Pasteur, 810885, CH-5692, Marseille #12, HP65-2009, 200501927, 810362, 811316, 810316, 810366, 20112714, Douglas, GR01, SN05, CABN and their derivatives. In specific embodiments, the cDNA is derived from LCMV Clone 13. In other specific embodiments, the cDNA is derived from LCMV MP strain.

[00235] In certain embodiments, the vector generated to encode an arenavirus particle or a tri-segmented arenavirus particle as described herein may be based on a specific strain of LCMV. Strains of LCMV include Clone 13, MP strain, Arm CA 1371, Arm E-250, WE, UBC, Traub, Pasteur, 810885, CH-5692, Marseille #12, HP65-2009, 200501927, 810362, 811316, 810316, 810366, 20112714, Douglas, GR01, SN05, CABN and their derivatives. In certain embodiments, an arenavirus particle or a tri-segmented arenavirus particle as described herein may be based on LCMV Clone 13. In other embodiments, the vector generated to encode an arenavirus particle or a tri-segmented arenavirus particle as described herein LCMV MP strain. The sequence of the S segment of LCMV Clone 13 is listed as SEQ ID NO: 2. In certain embodiments, the sequence of the S segment of LCMV Clone 13 is the sequence set forth in SEQ ID NO: 1. The sequence of the L segment of LCMV Clone 13 is listed as SEQ ID NO: 5. The sequence of the S segment of LCMV strain MP is listed as SEQ ID NO: 53. The sequence of the L segment of LCMV strain MP is listed as SEQ ID NO: 4.

[00236] In another embodiment, provided herein is a cell, wherein the cell comprises a cDNA or a vector system described above in this section. Cell lines derived from such cells, cultures comprising such cells, methods of culturing such cells infected are also provided herein. In certain embodiments, provided herein is a cell, wherein the cell comprises a cDNA of the tri-segmented arenavirus particle. In some embodiments, the cell comprises the S segment and/or the L segment.

4.6 Methods of Use

[00237] Vaccines have been successful for preventing and/or treating infectious diseases, such as those for polio virus and measles. However, therapeutic immunization in the setting of established, chronic disease, including both chronic infections and cancer has been less successful. The ability to generate an arenavirus particle and/or a tri-segmented arenavirus particle represents a new novel vaccine strategy.

[00238] In one embodiment, provided herein are methods of treating an infection and/or cancer in a subject comprising administering to the subject one or more types of arenavirus particles or tri-segmented arenavirus particles, as described herein or a composition thereof. In a specific embodiment, a method for treating an infection and/or cancer described herein comprises administering to a subject in need thereof an effective amount of one or more arenavirus particles or tri-segmented arenavirus particles, described herein or a composition thereof. The subject can be a mammal, such as but not limited to a human being, a mouse, a rat, a guinea pig, a domesticated animal, such as, but not limited to, a cow, a horse, a sheep, a pig, a goat, a cat, a dog, a hamster, a donkey. In a specific embodiment, the subject is a human. The human subject might be male, female, adults, children, seniors (65 and older), and those with multiple diseases (*i.e.,* a polymorbid subject). In certain embodiments, subjects are those whose disease has progressed after treatment with chemotherapy, radiotherapy, surgery, and/or biologic agents.

[00239] In another embodiment, provided herein are methods for inducing an immune response against an antigen derived from an infectious organism, tumor, or allergen in a subject comprising administering to the subject an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, tumor, or allergen or a composition thereof.

[00240] In another embodiment, the subjects to whom an arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, tumor, or allergen described herein or a composition thereof is administered have, are susceptible to, or are at risk for a infection, development of cancer or a allergy, or exhibit a pre-cancerous tissue lesion. In another specific embodiment, the subjects to whom a arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, tumor, or allergen described herein or a composition thereof is administered are infected with, are susceptible to, are at risk for, or diagnosed with an infection, cancer, pre-cancerous tissue lesion, or allergy.

[00241] In another embodiment, the subjects to whom an arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, tumor, or allergen described herein or a composition thereof is administered are suffering from, are susceptible to, or are at risk for, an infection, a cancer, a pre-cancerous lesion, or an allergy in the pulmonary system, central nervous system, lymphatic system, gastrointestinal system, or circulatory system among others. In a specific embodiment, the subjects to whom an arenavirus particle or tri-segmented arenavirus particle expressing an antigen derive from an infectious organism, tumor, or allergen described herein or a composition thereof is administered are suffering from, are susceptible to, or are at risk for, an infection, a cancer, or an allergy in one or more organs of the body, including but not limited to the brain, liver, lungs, eyes, ears, intestines, esophagus, uterus, nasopharynx or salivary glands.

[00242] In another embodiment, the subjects to whom an arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen described herein or a composition thereof is administered to a subject suffering from symptoms including but not limited to fever, night sweats, tiredness, malaise, uneasiness, sore throat, swollen glands, joint pain, muscle pain, loss of appetite, weight loss, diarrhea, gastrointestinal ulcerations, gastrointestinal bleeding, shortness of breath, pneumonia, mouth ulcers, vision problems, hepatitis, jaundice, encephalitis, seizures, coma, pruritis, erythema, hyperpigmentation, changes in lymph node, or hearing loss.

[00243] In another embodiment, an arenavirus or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen as described herein or a composition thereof is administered to a subject of any age group suffering from, are susceptible to, or are at risk for, an infection, a cancer, or an allergy. In a specific embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen as described herein or a composition thereof is administered to a subject with a compromised immune system, a pregnant subject, a subject undergoing an organ or bone marrow transplant, a subject taking immunosuppressive drugs, a subject undergoing hemodialysis, a subject who has cancer, or a subject who is suffering from, are susceptible to, or are at risk for, an infection, a cancer, or an allergy. In a more specific embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen as described herein or a composition thereof is administered to a subject who is a child of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 , 13, 14, 15, 16, or 17 years of age suffering from, are susceptible to, or are at risk for, an infection, a cancer, or an allergy. In yet another specific embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen described herein or a composition thereof is administered to a subject who is an infant suffering from, is susceptible to, or is at risk for, an infection, cancer or an allergy. In yet another specific embodiment, an arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen described herein or a composition thereof is administered to a subject who is an infant of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months of age suffering from, is susceptible to, or is at risk for, an infection, cancer, or an allergy. In yet another specific embodiment, an arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen described herein or a composition thereof is administered to an elderly subject who is suffering from, is susceptible to, or is at risk for, an infection, cancer, or an allergy. In a more specific embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen described herein or a composition thereof is administered to a subject who is a senior subject of 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90 years of age.

[00244] In another embodiment, an arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen described herein or a composition thereof is administered to subjects with a heightened risk of disseminated infection, a cancer, or an allergy. In a specific embodiment, arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen described herein or a composition thereof is administered to subjects in the neonatal period with a neonatal and therefore immature immune system.

[00245] In another embodiment, an arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen as described herein or a composition thereof is administered to a subject having a dormant infection, cancer, or allergy. In a specific embodiment, an arenavirus particle or a tri-segmented arenavirus expressing an antigen derived from an infectious organism, a cancer, or an allergen described herein or a composition thereof is administered to a subject having a dormant infection, a dormant cancer, or a dormant allergy which can reactivate upon immune system compromise. Thus, provided herein is a method for preventing reactivation of an infection, a cancer, or an allergy.

[00246] In another embodiment, an arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen as described herein or a composition thereof is administered to a subject having a recurrent infection, a cancer, or an allergy.

[00247] In another embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen as described herein or a composition thereof is administered to a subject with a genetic predisposition for an infection, a cancer, or an allergy. In another embodiment, an arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen as described herein or a composition thereof is administered to a subject. In another embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen is administered to a subject with risk factors. Exemplary risk factors include, aging, tobacco, sun exposure, radiation exposure, chemical exposure, family history, alcohol, poor diet, lack of physical activity, or being overweight.

[00248] In another embodiment, administering an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen reduces a symptomatic infection, cancer, or allergy. In another embodiment, administering an arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen reduces an asymptomatic infection, cancer, or allergy.

[00249] In another embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism described herein or a composition thereof is administered to subjects or animals infected with one or more strains of influenza virus, infectious bursal disease virus, rotavirus, infectious bronchitis virus, infectious laryngotracheitis virus, chicken anemia virus, Marek's disease virus, avian leukosis virus, avian adenovirus, or avian pneumovirus, SARS-causing virus, human respiratory syncytial virus, human immunodeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, poliovirus, rabies virus, Hendra virus, Nipah virus, human parainfluenza 3 virus, measles virus, mumps virus, Ebola virus, Marburg virus, West Nile disease virus, Japanese encephalitis virus, Dengue virus, Hantavirus, Rift Valley fever virus, Lassa fever virus, herpes simplex virus and yellow fever virus.

[00250] In another embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from a cancer described herein or a composition thereof is administered to subjects who suffer from one or more types of cancers. In other embodiments, any type of a cancer susceptible to treatment with the vaccines described herein might be targeted. In a more specific embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from a cancer described herein or a composition thereof is administered to subjects suffering from, for example, melanoma, prostate carcinoma, breast carcinoma, lung carcinoma, neuroblastoma, hepatocellular carcinoma, cervical carcinoma, and stomach carcinoma, burkitt lymphoma; non-Hodgkin lymphoma; Hodgkin lymphoma; nasopharyngeal carcinoma (cancer of the upper part of the throat behind the nose), leukemia, mucosa-associated lymphoid tissue lymphoma.

[00251] In another embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an allergen described herein or a composition thereof is administered to subjects who suffer from one or more allergies. In a more specific embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an allergen described herein or a composition thereof is administered to subjects suffering from, for example, a seasonal allergy, a perennial allergy, rhinoconjunctivitis, asthma, eczema, a food allergy.

[00252] In another embodiment, administering an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen as described herein or a composition thereof to subjects confer cell-mediated immunity (CMI) against an infection, a cancer, or an allergen. Without being bound by theory, in another embodiment, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, an allergen as described herein or a composition thereof infects and expresses antigens of interest in antigen presenting cells (APC) of the host (*e.g.,* macrophages, dendritic cells, or B cells) for direct presentation of antigens on Major Histocompatibility Complex (MHC) class I and II. In another embodiment, administering an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, an allergen as described herein or a composition thereof to subjects induces plurifunctional cytolytic as well as IFN-γ and TNF-α co-producing CMV-specific CD4+ and CD8+ T cell responses of high magnitude to treat or prevent an infection, a cancer, or an allergy.

[00253] In another embodiment, administering an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen or a composition thereof reduces the risk that an individual will develop an infection, a cancer, an allergy by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the risk of developing an infection, a cancer, or an allergy in the absence of such treatment.

[00254] In another embodiment, administering an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen or a composition thereof reduces the symptoms of an infection, a cancer, or an allergy by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the manifestation of the symptoms of an infection, a cancer, an allergy in the absence of such treatment.

[00255] In certain embodiments, the arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen is preferably administered in multiple injections (*e.g.,* at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30, 40, 45, or 50 injections) or by continuous infusion (*e.g.,* using a pump) at multiple sites (*e.g.,* at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, or 14 sites). In certain embodiments, the arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen is administered in two or more separate injections over a 6-month period, a 12-month period, a 24-month period, or a 48-month period. In certain embodiments, the arenavirus particle or tri-segmented arenavirus particle expressing an antigen derived from a infectious organism, a cancer, or an allergen is administered with a first dose at an elected date, a second dose at least 2 months after the first dose, and a third does 6 months after the first dose.

[00256] In one example, cutaneous injections are performed at multiple body sites to reduce extent of local skin reactions. On a given vaccination day, the patient receives the assigned total dose of cells administered from one syringe in 3 to 5 separate intradermal injections of the dose (*e.g.,* at least 0.4 ml, 0.2 ml, or 0.1 ml) each in an extremity spaced at least about 5 cm (*e.g.,* at least 4.5, 5, 6, 7, 8, 9, or cm) at needle entry from the nearest neighboring injection. On subsequent vaccination days, the injection sites are rotated to different limbs in a clockwise or counter-clockwise manner.

[00257] In another embodiment, administering an infectious, replication-deficient arenavirus expressing a CMV antigen or a composition thereof in subjects with a neonatal and therefore immune system induces a cell-mediated immune (CMI) response against an infection, a cancer, or an allergy, exceeding by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, the CMI response against an infection, a cancer, or a allergy in the absence of such a treatment.

[00258] In certain embodiments, administrating to a subject an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen, as described herein induces a detectable antibody titer for a minimum of at least four weeks. In another embodiment, administering to a subject an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen, as describe herein increases the antibody titer by at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000%.

[00259] In certain embodiments, primary antigen exposure elicits a functional, (neutralizing) and minimum antibody titer of at least 50%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000% of mean control sera from infection-immune human subjects. In more specific embodiments, the primary neutralizing geometric mean antibody titer increases up to a peak value of at least 1:50, at least 1:100, at least 1:200, or at least 1:1000 within at least 4 weeks post-immunization. In another embodiment, immunization with an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergy, as described herein produces high titers of antibodies that last for at least 4 weeks, at least 8 weeks, at least 12 weeks, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, or at least 5 years post-immunization following a single administration of the vaccine, or following two or more sequential immunizations.

[00260] In yet another embodiment, secondary antigen exposure increases the antibody titer by at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000%. In another embodiment, secondary antigen exposure elicits a functional, (neutralizing) and minimum antibody titer of at least 50%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000% of mean control sera from infection-immune human subjects. In more specific embodiments, the secondary neutralizing geometric mean antibody titer increases up to a peak value of at least 1:50, at least 1:100, at least 1:200, or at least 1:1000 within at least 4 weeks post-immunization. In another embodiment, a second immunization with an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergy, as described herein produces high titers of antibodies that last for at least 4 weeks, at least 8 weeks, at least 12 weeks, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, or at least 5 years post-immunization.

[00261] In yet another embodiment, a third boosting immunization increases the antibody titer by at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000%. In another embodiment, the boosting immunization elicits a functional, (neutralizing) and minimum antibody titer of at least 50 %, at least 100 %, at least 200 %, at least 300%, at least 400%, at least 500%, or at least 1000% of mean control sera from infection-immune human subjects. In more specific embodiments, the third boosting immunization elicits a functional, (neutralizing), and minimum antibody titer of at least 50%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000% of mean control sera from infection-immune human subjects. In another embodiment, a third boosting immunization prolongs the antibody titer by at least 4 weeks, at least 8 weeks, at least 12 weeks, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, or at least 5 years post-immunization

[00262] In certain embodiments, the arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergy, elicits a T cell independent or T cell dependent response. In other embodiments, arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergy, elicits a T cell response. In other embodiments, an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergy, as described herein elicits a T helper response. In another embodiment, arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergy, as described herein elicits a Th1-orientated response or a Th2-orientated response.

[00263] In more specific embodiments, the Th1-orientated response is indicated by a predominance of IgG1 antibodies versus IgG2. In other embodiments the ratio of IgG1:IgG2 is greater than 1:1, greater than 2:1, greater than 3:1, or greater than 4:1. In another embodiment the infectious, arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergy, as described herein is indicated by a predominance of IgG1, IgG2, IgG3, IgG4, IgM, IgA, IgD or IgE antibodies.

[00264] In some embodiments, the infectious, replication-deficient arenavirus expressing a CMV antigen or a fragment thereof elicits a CD8+ T cell response. In another embodiment, the arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergy elicits both CD4+ and CD8+ T cell responses, in combination with antibodies or not.

[00265] In certain embodiments, the arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergy, as described herein elicits high titers of neutralizing antibodies. In another embodiment, the arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergy, as described herein elicits higher titers of neutralizing antibodies than expression of the protein complex components individually.

[00266] In another embodiment, the arenavirus particle or a tri-segmented arenavirus particle expressing one, two, three, four, five, or more antigen derived from an infectious organism, a cancer, or an allergy elicits higher titers of neutralizing antibodies than an arenavirus particle or a tri-segmented arenavirus particle expressing one expressing one antigen derived from an infectious organism, a cancer, or an allergen.

[00267] In certain embodiments, the methods further comprise co-administration of the arenavirus particle or tri-segmented arenavirus particle and at least one additional therapy. In certain embodiments, the co-administration is simultaneous. In another embodiment, the arenavirus particle or tri-segmented arenavirus particle is administered prior to administration of the additional therapy. In other embodiments, the arenavirus particle or tri-segmented arenavirus particle is administered after administration of the additional therapy. In certain embodiments, the administration of the arenavirus particle or tri-segmented arenavirus particle and the additional therapy is about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, or about 12 hours. In certain embodiments, the interval between administration of the arenavirus particle or tri-segmented arenavirus particle and said additional therapy is about 1 day, 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks. In certain embodiments, the interval between administration of the arenavirus particle or tri-segmented arenavirus particle and the additional therapy is about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months.

[00268] In certain embodiments, administering an arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen or a composition thereof reduces the number of antibodies detected in a patient blood sample, or serum sample. In certain embodiments, administering an arenavirus particle expressing an antigen derived from an infectious organism, a cancer, or an allergen composition thereof reduces the amount of the infectious organism, cancer, or allergy detected in urine, saliva, blood, tears, semen, exfoliated cell sample, or breast milk.

[00269] In another embodiment, the arenavirus particle or the tri-segmented arenavirus particle expressing an antigen derived from an infection organism, a cancer, or an allergen as described herein or a composition may further comprise a reporter protein. In a more specific embodiment, the , the arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infection organism, a cancer, or an allergen and reporter protein as described herein or a composition is administered to subjects for treating and/or preventing an infection, a cancer, or an allergy. In yet another specific embodiment, the reporter protein can be used for monitoring gene expression, protein localization, and vaccine delivery, *in vivo*, *in situ* and in real time.

[00270] In another embodiment, the arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infection organism, a cancer, or an allergen as described herein or a composition may further comprise a fluorescent protein. In a more specific embodiment, the arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infection organism, a cancer, or an allergen and reporter protein as described herein or a composition is administered to subjects for treating and/or preventing an infection, a cancer, or an allergy. In yet another specific embodiment, the fluorescent protein can be the reporter protein can be used for monitoring gene expression, protein localization, and vaccine delivery, *in vivo*, *in situ* and in real time.

[00271] Changes in the CMI response function against an infection, a cancer, or an allergy induced by administering an arenavirus particle or a tri-segmented arenavirus particle expressing an antigen derived from an infectious organism, a cancer, an allergen or a composition thereof in subjects can be measured by any assay known to the skilled artisan including, but not limited to flow cytometry (see, *e.g.,* Perfetto S.P. *et al.,* 2004, Nat Rev Immun., 4(8):648-55), lymphocyte proliferation assays (see, *e.g.,* Bonilla F.A. *et al.,* 2008, Ann Allergy Asthma Immunol, 101:101-4; and Hicks M.J. *et al.,* 1983, Am J Clin Pathol., 80:159-63), assays to measure lymphocyte activation including determining changes in surface marker expression following activation of measurement of cytokines of T lymphocytes (see, *e.g.,* Caruso A. *et al.,* Cytometry. 1997;27:71-6), ELISPOT assays (see, *e.g.,* Czerkinsky C.C. *et al.,* 1983, J Immunol Methods, 65:109-121; and Hutchings P.R. *et al.,* 1989, J Immunol Methods, 120:1-8), or Natural killer cell cytotoxicity assays (see, *e.g.,* Bonilla F.A. *et al.,* 2006, Ann Allergy Asthma Immunol., 94(5 Suppl 1):S1-63).

[00272] Successful treatment of a cancer patient can be assessed as prolongation of expected survival, induction of an anti-tumor immune response, or improvement of a particular characteristic of a cancer. Examples of characteristics of a cancer that might be improved include tumor size (*e.g.,* T0, T is, or T1-4), state of metastasis (*e.g.,* M0, M1), number of observable tumors, node involvement (*e.g.,* N0, N1-4, Nx), grade (*i.e.,* grades 1, 2, 3, or 4), stage (*e.g.,* 0, I, II, III, or IV), presence or concentration of certain markers on the cells or in bodily fluids (*e.g.,* AFP, B2M, beta-HCG, BTA, CA 15-3, CA 27.29, CA 125, CA 72.4, CA 19-9, calcitonin, CEA, chromgrainin A, EGFR, hormone receptors, HER2, HCG, immunoglobulins, NSE, NMP22, PSA, PAP, PSMA, S-100, TA-90, and thyroglobulin), and/or associated pathologies (*e.g.,* ascites or edema) or symptoms (*e.g.,* cachexia, fever, anorexia, or pain). The improvement, if measureable by percent, can be at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, or 90% (*e.g.,* survival, or volume or linear dimensions of a tumor).

[00273] In another embodiment, described herein, is a method of use with an arenavirus particle (*e.g.,* LCMV) expressing an antigen derived from an infectious organism, a cancer, or an allergen as described herein in which the at least one of the ORF encoding the GP, NP, Z protein, and L protein is substituted with a nucleotide sequence encoding an infectious a nucleotide sequence encoding an antigen derived from an infectious organism, a cancer, an allergen, or an antigenic fragment thereof.

4.7 Compositions, Administration, and Dosage

[00274] The present application furthermore relates to vaccines, immunogenic compositions (*e.g.,* vaccine formulations), and pharmaceutical compositions comprising an arenavirus particle or a tri-segmented arenavirus particle as described herein. Such vaccines, immunogenic compositions and pharmaceutical compositions can be formulated according to standard procedures in the art.

[00275] It will be readily apparent to one of ordinary skill in the relevant arts that suitable modifications and adaptations to the methods and applications described herein can be obvious and can be made without departing from the scope of the scope or any embodiment thereof.

[00276] In another embodiment, provided herein are compositions comprising an arenavirus particle or a tri-segmented arenavirus particle described herein. Such compositions can be used in methods of treatment and prevention of disease. In a specific embodiment, the compositions described herein are used in the treatment of subjects infected with, or susceptible to, an infection. In other embodiments, the compositions described herein are used in the treatment of subjects susceptible to or exhibiting symptoms characteristic of cancer or tumorigenesis or are diagnosed with cancer. In another specific embodiment, the immunogenic compositions provided herein can be used to induce an immune response in a host to whom the composition is administered. The immunogenic compositions described herein can be used as vaccines and can accordingly be formulated as pharmaceutical compositions. In a specific embodiment, the immunogenic compositions described herein are used in the prevention of infection or cancer of subjects (*e.g.,* human subjects). In other embodiments, the vaccine, immunogenic composition or pharmaceutical composition are suitable for veterinary and/or human administration.

[00277] In certain embodiments, provided herein are immunogenic compositions comprising an arenavirus vector as described herein. In certain embodiments, such an immunogenic composition further comprises a pharmaceutically acceptable excipient. In certain embodiments, such an immunogenic composition further comprises an adjuvant. The adjuvant for administration in combination with a composition described herein may be administered before, concomitantly with, or after administration of said composition. In some embodiments, the term "adjuvant" refers to a compound that when administered in conjunction with or as part of a composition described herein augments, enhances and/or boosts the immune response to a arenavirus particle or tri-segmented arenavirus particle and, most importantly, the gene products it vectorises, but when the compound is administered alone does not generate an immune response to the arenavirus particle or tri-segmented arenavirus particle and the gene products vectorised by the latter. In some embodiments, the adjuvant generates an immune response to the arenavirus particle or tri-segmented arenavirus particle and the gene products vectorised by the latter and does not produce an allergy or other adverse reaction. Adjuvants can enhance an immune response by several mechanisms including, *e.g.,* lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages or dendritic cells. When a vaccine or immunogenic composition of the invention comprises adjuvants or is administered together with one or more adjuvants, the adjuvants that can be used include, but are not limited to, mineral salt adjuvants or mineral salt gel adjuvants, particulate adjuvants, microparticulate adjuvants, mucosal adjuvants, and immunostimulatory adjuvants. Examples of adjuvants include, but are not limited to, aluminum salts (alum) (such as aluminum hydroxide, aluminum phosphate, and aluminum sulfate), 3 De-O-acylated monophosphoryl lipid A (MPL) (see GB 2220211), MF59 (Novartis), AS03 (GlaxoSmithKline), AS04 (GlaxoSmithKline), polysorbate 80 (Tween® 80; ICL Americas, Inc.), imidazopyridine compounds (see International Application No.

Date Reçue/Date Received 2022-02-07

PCT/US2007/064857, published as International Publication No. WO2007/109812), imidazoquinoxaline compounds (see International Application No. PCT/US2007/064858, published as International Publication No. WO2007/109813) and saponins, such as QS21 (see Kensil *et al.,* 1995, in Vaccine Design: The Subunit and Adjuvant Approach (eds. Powell & Newman, Plenum Press, NY); U.S. Pat. No. 5,057,540). In some embodiments, the adjuvant is Freund's adjuvant (complete or incomplete). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune stimulants, such as monophosphoryl lipid A (see Stoute *et al.,* 1997, N. Engl. J. Med. 336, 86-91).

[00278] The compositions comprise the arenaviruses particle or tri-segmented arenavirus particle described herein alone or together with a pharmaceutically acceptable carrier. Suspensions or dispersions of the arenavirus particle or tri-segmented arenavirus particle , especially isotonic aqueous suspensions or dispersions, can be used. The pharmaceutical compositions may be sterilized and/or may comprise excipients, *e.g.,* preservatives, stabilizers, wetting agents and/or emulsifiers, solubilizers, salts for regulating osmotic pressure and/or buffers and are prepared in a manner known per se, for example by means of conventional dispersing and suspending processes. In certain embodiments, such dispersions or suspensions may comprise viscosity-regulating agents. The suspensions or dispersions are kept at temperatures around 2 °C to 8 °C, or preferentially for longer storage may be frozen and then thawed shortly before use, or alternatively may be lyophilized for storage. For injection, the vaccine or immunogenic preparations may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[00279] In certain embodiments, the compositions described herein additionally comprise a preservative, *e.g.,* the mercury derivative thimerosal. In a specific embodiment, the pharmaceutical compositions described herein comprise 0.001% to 0.01% thimerosal. In other embodiments, the pharmaceutical compositions described herein do not comprise a preservative.

[00280] The pharmaceutical compositions comprise from about 10^3 to about 10^{11} focus forming units of the arenavirus particle or tri-segmented arenavirus particle.

[00281] In one embodiment, administration of the pharmaceutical composition is parenteral administration. Parenteral administration can be intravenous or subcutaneous administration. Accordingly, unit dose forms for parenteral administration are, for example, ampoules or vials, *e.g.,* vials containing from about 10^3 to 10^{10} focus forming units or 10^5 to 10^{15} physical particles of the arenavirus particle or tri-segmented arenavirus particle.

[00282] In another embodiment, a vaccine or immunogenic composition provided herein is administered to a subject by, including but not limited to, oral, intradermal, intramuscular, intraperitoneal, intravenous, topical, subcutaneous, percutaneous, intranasal and inhalation routes, and via scarification (scratching through the top layers of skin, *e.g.,* using a bifurcated needle). Specifically, subcutaneous or intravenous routes can be used.

[00283] For administration intranasally or by inhalation, the preparation for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.,* dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.,* gelatin for use in an inhaler or insufflators may be formulated containing a powder mix of the compound and as suitable powder base such as lactose or starch.

[00284] The dosage of the active ingredient depends upon the type of vaccination and upon the subject, and their age, weight, individual condition, the individual pharmacokinetic data, and the mode of administration. In certain embodiments, an *in vitro* assay is employed to help identify optimal dosage ranges. Effective doses may be extrapolated from dose response curves derived from *in vitro* or animal model test systems.

[00285] In certain embodiments, the vaccine, immunogenic composition, or pharmaceutical composition comprising an arenavirus particle or the tri-segmented arenavirus particle can be used as a live vaccination. Exemplary doses for a live arenavirus particle may vary from 10-100, or more, PFU of live virus per dose. In some embodiments, suitable dosages of an arenavirus particle or the tri-segmented arenavirus particle are 10^2, 5×10^2, 10^3, 5×10^3, 10^4, 5×10^4, 10^5, 5×10^5, 10^6, 5×10^6, 10^7, 5×10^7, 10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 5×10^{10}, 1×10^{11}, 5×10^{11} or 10^{12} pfu, and can be administered to a subject once, twice, three or more times with intervals as often as needed. In another embodiment, a live arenavirus is formulated such that a 0.2-mL dose contains $10^{6.5}$-$10^{7.5}$ fluorescent focal units of live arenavirus particle. In another embodiment, an inactivated vaccine is formulated such that it contains about 15 μg to about 100 μg, about 15 μg to about 75 μg, about 15 μg to about 50 μg, or about 15 μg to about 30 μg of an arenavirus

[00286] In certain embodiments, for administration to children, two doses of an arenavirus particle or a tri-segmented arenavirus particle described herein or a composition thereof, given at least one month apart, are administered to a child. In specific embodiments for administration to adults, a single dose of the arenavirus particle or tri-segmented arenavirus particle described herein or a composition thereof is given. In another embodiment, two doses of an arenavirus particle or a tri-segmented arenavirus particle described herein or a composition thereof, given at least one month apart, are administered to an adult. In another embodiment, a young child (six months to nine years old) may be administered an arenavirus particle or a tri-segmented arenavirus particle described herein or a composition thereof for the first time in two doses given one month apart. In a particular embodiment, a child who received only one dose in their first year of vaccination should receive two doses in the following year. In some embodiments, two doses administered 4 weeks apart are preferred for children 2-8 years of age who are administered an immunogenic composition described herein, for the first time. In certain embodiments, for children 6-35 months of age, a half dose (0.25 ml) may be preferred, in contrast to 0.5 ml which may be preferred for subjects over three years of age..

[00287] In certain embodiments, the compositions can be administered to the patient in a single dosage comprising a therapeutically effective amount of the arenavirus particle or the tri-segmented arenavirus particle. In some embodiments, the arenavirus particle or tri-segmented arenavirus particle can be administered to the patient in a single dose comprising a therapeutically effective amount of an arenavirus particle or tri-segmented arenavirus particle and, one or more pharmaceutical compositions, each in a therapeutically effective amount.

[00288] In certain embodiments, the composition is administered to the patient as a single dose followed by a second dose three to six weeks later. In accordance with these embodiments, the booster inoculations may be administered to the subjects at six to twelve month intervals following the second inoculation. In certain embodiments, the booster inoculations may utilize a different arenavirus or composition thereof. In some embodiments, the administration of the same composition as described herein may be repeated and separated by at least 1 day, 2 days, 3 days, 4 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or at least 6 months.

[00289] Also provided herein, are processes and to the use the arenavirus particle or the tri-segmented arenavirus particle for the manufacture of vaccines in the form of pharmaceutical preparations, which comprise the arenavirus particle or tri-segmented arenavirus particle as an active ingredient. The pharmaceutical compositions of the present application are prepared in a manner known per se, for example by means of conventional mixing and/or dispersing processes.

4.8 Assays

4.8.1 Arenavirus Detection Assays

[00290] The skilled artesian could detect an arenavirus genomic segment or tri-segmented arenavirus particle, as described herein using techniques known in the art. For example, RT-PCR can be used with primers that are specific to an arenavirus to detect and quantify an arenavirus genomic segment that has been engineered to carry an ORF in a position other than the wild-type position of the ORF or a tri-segmented arenavirus particle. Western blot, ELISA, radioimmunoassay, immuneprecipitation, immunecytochemistry, or immunocytochemistry in conjunction with FACS can be used to quantify the gene products of the arenavirus genomic segment or tri-segmented arenavirus particle.

4.8.2 Assay to Measure Infectivity

[00291] Any assay known to the skilled artisan can be used for measuring the infectivity of an arenavirus vector preparation. For example, determination of the virus/vector titer can be done by a "focus forming unit assay" (FFU assay). In brief, complementing cells, *e.g.,* MC57 cells are plated and inoculated with different dilutions of a virus/vector sample. After an incubation period, to allow cells to form a monolayer and virus to attach to cells, the monolayer is covered with Methylcellulose. When the plates are further incubated, the original infected cells release viral progeny. Due to the Methylcellulose overlay the spread of the new viruses is restricted to neighboring cells. Consequently, each infectious particle produces a circular zone of infected cells called a Focus. Such Foci can be made visible and by that countable using antibodies against LCMV- NP or another protein expressed by the arenavirus particle or the tri-segmented arenavirus particle and a HRP-based color reaction. The titer of a virus / vector can be calculated in focus-forming units per milliliter (FFU/mL).

4.8.3 Growth of an Arenavirus Particle

[00292] Growth of an arenavirus particle described herein can be assessed by any method known in the art or described herein (*e.g.,* cell culture). Viral growth may be determined by inoculating serial dilutions of an arenavirus particle described herein into cell cultures (*e.g.,* Vero cells or BHK-21 cells). After incubation of the virus for a specified time, the virus is isolated using standard methods.

4.8.4 Serum ELISA

[00293] Determination of the humoral immune response upon vaccination of animals (*e.g.,* mice, guinea pigs) can be done by antigen-specific serum ELISA's (enzyme-linked immunosorbent assays). In brief, plates are coated with antigen (*e.g.,* recombinant protein), blocked to avoid unspecific binding of antibodies and incubated with serial dilutions of sera. After incubation, bound serum-antibodies can be detected, *e.g.,* using an enzyme-coupled anti-species (*e.g.,* mouse, guinea pig)-specific antibody (detecting total IgG or IgG subclasses) and subsequent color reaction. Antibody titers can be determined as, *e.g.,* endpoint geometric mean titer.

4.8.5 Assay to Measure the Neutralizing Activity of Induced Antibodies

[00294] Determination of the neutralizing antibodies in sera is performed with the following cell assay using ARPE-19 cells from ATCC and a GFP-tagged virus. In addition supplemental guinea pig serum as a source of exogenous complement is used. The assay is started with seeding of $6.5x10^3$ cells/well (50µl/well) in a 384 well plate one or two days before using for neutralization. The neutralization is done in 96-well sterile tissue culture plates without cells for 1 h at 37 °C. After the neutralization incubation step the mixture is added to the cells and incubated for additional 4 days for GFP-detection with a plate reader. A positive neutralizing human sera is used as assay positive control on each plate to check the reliability of all results. Titers (EC50) are determined using a 4 parameter logistic curve fitting. As additional testing the wells are checked with a fluorescence microscope.

4.8.6 Plaque Reduction Assay

[00295] In brief, plaque reduction (neutralization) assays for LCMV can be performed by use of a replication-competent or –deficient LCMV that is tagged with green fluorescent protein, 5% rabbit serum may be used as a source of exogenous complement, and plaques can be enumerated by fluorescence microscopy. Neutralization titers may be defined as the highest dilution of serum that results in a 50%, 75%, 90% or 95% reduction in plaques, compared with that in control (pre-immune) serum samples.

[00296] qPCR LCMV RNA genomes are isolated using QIAamp Viral RNA mini Kit (QIAGEN), according to the protocol provided by the manufacturer. LCMV RNA genome equivalents are detected by quantitative PCR carried out on an StepOnePlus Real Time PCR System (Applied Biosystems) with SuperScript® III Platinum® One-Step qRT-PCR Kit (Invitrogen) and primers and probes (FAM reporter and NFQ-MGB Quencher) specific for part of the LCMV NP coding region or another genomic stretch of the arenavirus particle or the tri-segmented arenavirus particle. The temperature profile of the reaction may be : 30 min at 60 °C, 2 min at 95 °C, followed by 45 cycles of 15 s at 95 °C, 30 s at 56 °C. RNA can be quantified by comparison of the sample results to a standard curve prepared from a log10 dilution series of a spectrophotometrically quantified, in vitro-transcribed RNA fragment, corresponding to a fragment of the LCMV NP coding sequence or another genomic stretch of the arenavirus particle or the tri-segmented arenavirus particle containing the primer and probe binding sites.

4.8.7 Western Blotting

[00297] Infected cells grown in tissue culture flasks or in suspension are lysed at indicated timepoints post infection using RIPA buffer (Thermo Scientific) or used directly without cell-lysis. Samples are heated to 99 °C for 10 minutes with reducing agent and NuPage® LDS Sample buffer (NOVEX) and chilled to room temperature before loading on 4-12% SDS-gels for electrophoresis. Proteins are blotted onto membranes using Invitrogens iBlot® Gel transfer Device and visualized by Ponceau staining. Finally, the preparations are probed with a primary antibodies directed against proteins of interest and alkaline phosphatase conjugated secondary antibodies followed by staining with 1-Step NBT/BCIP solution (INVITROGEN).

4.8.8 MHC-Peptide Multimer Staining Assay for Detection of Antigen-Specific CD8+ T-cell proliferation

[00298] Any assay known to the skilled artisan can be used to test antigen-specific CD8+ T-cell responses. For example, the MHC-peptide tetramer staining assay can be used (see, *e.g.,* Altman J.D. *et al.,* Science. 1996; 274:94-96; and Murali-Krishna K. *et al.,* Immunity. 1998; 8:177-187). Briefly, the assay comprises the following steps, a tetramer assay is used to detect the presence of antigen specific T-cells. In order for a T-cell to detect the peptide to which it is specific, it must both recognize the peptide and the tetramer of MHC molecules custom made for a defined antigen specificity and MHC haplotype of T-cells (typically fluorescently labeled). The tetramer is then detected by flow cytometry via the fluorescent label.

4.8.9 ELISPOT Assay for Detection of Antigen-Specific CD4+ T-cell Proliferation.

[00299] Any assay known to the skilled artisan can be used to test antigen-specific CD4+ T-cell responses. For example, the ELISPOT assay can be used (see, *e.g.,* Czerkinsky C.C. *et al.,* J Immunol Methods. 1983; 65:109-121; and Hutchings P.R. *et al.,* J Immunol Methods. 1989; 120:1-8). Briefly, the assay comprises the following steps: An immunospot plate is coated with an anti-cytokine antibody. Cells are incubated in the immunospot plate. Cells secrete cytokines and are then washed off. Plates are then coated with a second biotyinlated-anticytokine antibody and visualized with an avidin-HRP system.

Date Reçue/Date Received 2022-02-07

4.8.10 Intracellular Cytokine Assay for Detection of Functionality of CD8+ and CD4+ T-cell Responses.

[00300] Any assay known to the skilled artisan can be used to test the functionality of CD8+ and CD4+ T cell responses. For example, the intracellular cytokine assay combined with flow cytometry can be used (see, *e.g.,* Suni M.A. *et al.,* J Immunol Methods. 1998; 212:89-98; Nomura L.E. *et al.,* Cytometry. 2000; 40:60-68; and Ghanekar S.A. *et al.,* Clinical and Diagnostic Laboratory Immunology. 2001; 8:628-63). Briefly, the assay comprises the following steps: activation of cells via specific peptides or protein, an inhibition of protein transport (*e.g.,* brefeldin A) is added to retain the cytokines within the cell. After a defined period of incubation, typically 5 hours, a washing steps follows, and antibodies to other cellular markers can be added to the cells. Cells are then fixed and permeabilized. The flurochrome-conjugated anti-cytokine antibodies are added and the cells can be analyzed by flow cytometry.

4.8.11 Assay for Confirming Replication-Deficiency of Viral Vectors

[00301] Any assay known to the skilled artisan that determines concentration of infectious and replication-competent virus particles can also be used as a to measure replication-deficient viral particles in a sample. For example, FFU assays with non-complementing cells can be used for this purpose.

[00302] Furthermore, plaque-based assays are the standard method used to determine virus concentration in terms of plaque forming units (PFU) in a virus sample. Specifically, a confluent monolayer of non-complementing host cells is infected with the virus at varying dilutions and covered with a semi-solid medium, such as agar to prevent the virus infection from spreading indiscriminately. A viral plaque is formed when a virus successfully infects and replicates itself in a cell within the fixed cell monolayer, and spreads to surrounding cells (see, *e.g.,* Kaufmann, S.H.; Kabelitz, D. (2002). Methods in Microbiology Vol.32:Immunology of Infection. Academic Press. ISBN 0-12-521532-0). Plaque formation can take 2 – 14 days, depending on the virus being analyzed. Plaques are generally counted manually and the results, in combination with the dilution factor used to prepare the plate, are used to calculate the number of plaque forming units per sample unit volume (PFU/mL). The PFU/mL result represents the number of infective replication-competent particles within the sample. When C-cells are used, the same assay can be used to titrate replication-deficient arenavirus particles or tri-segmented arenavirus particles.

4.8.12 Assay for Expression of Viral Antigen

[00303] Any assay known to the skilled artisan can be used for measuring expression of viral antigens. For example, FFU assays can be performed. For detection, mono- or polyclonal antibody preparation(s) against the respective viral antigens are used (transgene-specific FFU).

4.8.13 Animal Models

[00304] To investigate recombination and infectivity of an arenavirus particle described herein *in vivo* animal models can be used. In certain embodiments, the animal models that can be used to investigate recombination and infectivity of a tri-segmented arenavirus particle include mouse, guinea pig, rabbit, and monkeys. In a preferred embodiment, the animal models that can be used to investigate recombination and infectivity of an arenavirus include mouse. In a more specific embodiment, the mice can be used to investigate recombination and infectivity of an arenavirus particle are triple-deficient for type I interferon receptor, type II interferon receptor and recombination activating gene 1 (RAG1).

[00305] In certain embodiments, the animal models can be used to determine arenavirus infectivity and transgene stability. In some embodiments, viral RNA can be isolated from the serum of the animal model. Techniques are readily known by those skilled in the art. The viral RNA can be reverse transcribed and the cDNA carrying the arenavirus ORFs can be PCR-amplified with gene-specific primers. Flow cytometry can also be used to investigate arenavirus infectivity and transgene stability.

5. EXAMPLES

[00306] These examples demonstrate that LCMV virus-based vector technology can be used to successfully develop (1) an arenavirus genomic segment with a viral ORF in a position other than the wild-type position of the ORF, and (2) a tri-segmented arenavirus particle that does not result in a replication competent bi-segmented viral particle.

5.1 Materials and Methods

5.1.1 Cells

[00307] BHK-21 cells were cultured in high-glucose Dulbecco's Eagle medium (DMEM; Sigma) supplemented with 10 % heat-inactivated fetal calf serum (FCS; Biochrom), 10 mM HEPES (Gibco), 1 mM sodium pyruvate (Gibco) and 1x tryptose phosphate broth. MC57 cells were maintained in Minimum Essential Medium (MEM; Sigma) complemented with 5 % heat-inactivated FCS, 2 mM L-glutamine (Gibco) and penicillin-streptomycin (100'000

U/ml penicillin and 50 mg/l streptomycin; Gibco). Both cell lines were cultured at 37 °C in a humidified 5 % CO2 incubator.

[00308] NP-expressing BHK-21 cells were generated by transfecting BHK-21 cells with a plasmid expressing NP under the control of the eukaryotic EF1-alpha promoter and encoding the puromycin resistance gene according to the manufacturer's protocol. 48 hours after transfection, 4 μg/ml puromycin was added to the medium. Another 48 hours later, cells were passaged into T150 flasks. Once separate clones became visible, cells were harvested and serially diluted into a 96-well plate to obtain single clones. Wells were checked optically for the growth of cell populations from single clones and respective cells were passaged into 6-well plates once they formed a confluent monolayer. NP-expressing BHK-21 cells were cultured in BHK-21 medium in the presence of 4 μg/ml puromycin.

[00309] GP-expressing BHK-21 cells have previously been described. Briefly, BHK-21 cells were stably transfected with a plasmid that expresses a codon-optimized LCMV-GP cDNA and the puromycin resistance cassette. GP-expressing clones were selected by the addition of 4 μg/ml puromycin to the medium and single clones were obtained by serial dilutions as described for the NP-expressing BHK-21 cells.

5.1.2 Plasmids

[00310] The pol-I L, pC-NP and pC-L plasmids have previously been described. For the generation of pol-I S plasmids encoding for GFP or RFP as reporter genes and either NP or GP, we used a pol-I Bbs/Bsm cloning plasmid as a basis (pol-I 5'-BsmBI_IGR_BbsI_3'). This plasmid encodes for the 5' untranslated region (5' UTR) of the viral S segment followed by two BsmBI restriction sites, the intergenic region (IGR), an NP rest and CAT open reading frame (ORF) flanked by BbsI restriction sites and the 3' UTR of the S segment. The pol-I S plasmids encoding for GP in its natural 5' and GFP in antisense orientation at the 3' position (pol-I 5'-GP_IGR_GfP-3') were cloned by inserting GP by BsmBI site-specific restriction and ligation into the pol-I Bbs/Bsm plasmid. In a second step GFP was inserted by BbsI digestion and ligation. In order to obtain pol-I S plasmids encoding for GP in the artificial 3' orientation (pol-I 5'-GFP_IGR_GP-3'), GP was inserted by BbsI digest at the 3' position into the pol-I Bbs/Bsm plasmid and GFP with BsmBI restriction/ligation at the 5' position. pol-I S encoding for GFP or RFP and NP (pol-I 5'-GFP_IGR_NP-3' or pol-I 5'RFP_IGR_NP-3') were cloned by inserting NP by BbsI digestion and ligation into the pol-I Bbs/Bsm cloning plasmid and GFP or RFP by BsmBI cloning. The pol-I plasmid with GP of LCMV strain WE and NP of LCMV strain Clone 13 (Cl13) were cloned by inserting the respective genes by Bbs and Bsm site-specific restriction/ligation at the respective sites in the pol-I Bbs/Bsm cloning plasmid.

[00311] The S segment encoding for the WE/WET fusion GP was obtained by replacing the last 255 base pairs of the WE ORF with a codon-optimized sequence named "WET". This was achieved by PCR amplifying in a first step a fragment of WE GP with one WE specific primer (SEQ ID NO: 11) and a WE specific fusion-primer carrying an overhang complementary to the WET sequence (SEQ ID NO: 12). In parallel the WET sequence was amplified by PCR using a WET-specific primer (SEQ ID NO: 13) and a WET-specific fusion-primer complementary to the WE sequence (SEQ ID NO: 14). In a third PCR reaction the two PCR products were fused by PCR fusion using the two mentioned fusion-primers. The resulting WE/WET fusion fragment was digested with BsmBI and ligated into a pol-I BsmBI_IGR_GFP-3' plasmid that had been digested with the same restriction enzyme.

[00312] The pol-I plasmid encoding for the recombined S segment of the in vivo recombined virus r3LCMV-GFP^{nat} #3 was cloned by inserting the synthesized DNA fragment (gene synthesis by GenScript) by site-specific restriction/ligation with SacI and XmaI into a plasmid encoding a wild-type S-segment under the control of a pol-I promoter (pol-I GP_IGR_NP) resulting in pol-I GP_IGR_GFPrest_IGR_NP.

5.1.3 DNA transfection of cells and rescue of recombinant viruses

[00313] BHK-21 cells were seeded into 6-well plates at a density of 4x105 cells/well and transfected 24 hours later with different amounts of DNA using either lipofectamine (3 µl/µg DNA; Invitrogen) or jetPRIME (2 µl/µg DNA; Polyplus) according to the manufacturer's instructions. For rescue of recombinant bi-segmented viruses entirely from plasmid DNA, the two minimal viral trans-acting factors NP and L were delivered from pol-II driven plasmids (0.8 µg pC-NP, 1 µg pC-L) and were co-transfected with 1.4 µg of pol-I L and 0.8 µg of pol-I S. In case of rescue of tri-segmented r3LCMV consisting of one L and two S segments, 0.8 µg of both pol-I driven S segments were included in the transfection mix. 72 hours after transfection the supernatant was harvested and passaged on BHK-21 cells for further amplification of the virus. Viral titers in the supernatant were determined by focus forming assay.

5.1.4 Viruses and growth kinetics of viruses

[00314] Wild-type Cl13 LCMV, originally derived from wild-type LCMV Armstrong, has previously been described. Stocks of wild-type and recombinant viruses were produced by infecting BHK-21 cells at a multiplicity of infection (moi) of 0.01 and supernatant was harvested 48 hours after infection. Growth curves of viruses were done in vitro in a 6-well format. BHK-21 cells were seeded at a density of 6×105 cells/well and infected 24 hours later by incubating the cells together with 500 µl of the virus inoculum at a moi of 0.01 for 90 minutes on a rocker plate at 37°C and 5% CO2. Fresh medium was added and cells incubated at 37°C / 5% CO2 for 72 to 96 hours. Supernatant was taken at given time points (normally 18, 24, 48, 72 hours) and viral titers analyzed by focus forming assay.

5.1.5 Focus forming assay

[00315] Next, titers of LCMV are determined by focus forming assay. LCMV is a non-cytolytic virus that does not lyse its host cells and as such does not create plaques. Nevertheless, units in this work will be expressed in the more commonly used term plaque forming units (PFU) instead of the correct term focus forming units (FFU). MC57 cells were used for focus forming assay if not stated otherwise. Cells were seeded at a density of 1.6x105 cells per well in a 24-well plate and mixed with 200 µl of 10-fold serial dilutions of virus prepared in MEM/ 2 % FCS. After 2-4 hours of incubation at 37 °C, 200 µl of a viscous medium (2 % Methylcellulose in 2x supplemented DMEM) were added per well to ensure spreading of viral particles only to neighboring cells. After 48 hours at 37 °C the supernatant was flicked off and cells were fixed by adding 200 µl of 4 % paraformaldehyde (PFA) in PBS for 30 minutes at room temperature (all following steps are performed at room temperature). Cells were permeabilised with 200 µl per well of BSS/ 1 % Triton® X-100 (Merck Millipore) for 20 minutes and subsequently blocked for 60 minutes with PBS/ 5 % FCS. For anti-NP staining a rat anti-LCMV-NP monoclonal antibody was used as a primary staining antibody at a dilution of 1:30 in PBS/ 2.5 % FCS for 60 minutes. For anti-GFP staining purified rat-anti-GFP antibody (Biolegend 338002) was used at a dilution of 1:2000 in PBS/ 2.5 % FCS. Plates were washed three times with tap water and the secondary HRP-goat-anti-rat-IgG was added at a dilution of 1:100 in PBS/ 2.5 % FCS and incubated for 1 hour. The plate was again washed three times with tap water. The color reaction (0.5 g/l DAB (Sigma D-5637), 0.5 g/l Ammonium Nickel sulfate in PBS/ 0.015 % H2O2) was added and the reaction was stopped after 10 minutes with tap water. Stained foci were counted manually and the final titer calculated according to the dilution.

[00316] For anti-GP staining of cells, plates were fixed with 50 % MeOH/ 50 % Acetone for 5 minutes and washed with PBS. Blocking was done as described. As primary antibody anti-GP GP83.4 (produced from hybridomas) was diluted 1:10 in PBS/ 2.5 % FCS and incubated for 60 minutes. After three washes with tap water, the secondary HRP-rabbit-anti-mouse IgG antibody was added at a dilution of 1:50 in PBS/ 2.5 % FCS and incubated for 60 minutes. After another three washes with tap water the color reaction was added as described above.

[00317] In order to determine the viremia of mice in blood, one drop of blood (corresponding to 50 µl volume) was collected in 950 µl of BSS-heparin (Na-heparin, Braun, 1 IE/ml final), mixed by inverting and stored at -80 °C until further use.

5.1.6 Mice

[00318] AGRAG mice (IFNα/βR-/-, IFNγR-/-, RAG-/-) have previously been described and were bred and housed under specific pathogen-free (SPF) conditions. They were bred at the Institut für Labortierkunde of the University of Zurich, Switzerland. All animal experiments were performed at the Universities of Geneva and Basel in accordance with the Swiss law for animal protection and the permission of the respective responsible cantonal authorities of Geneva and Basel. Infection of the mice was done intravenously at a dose of 1×104 PFU per mouse.

Date Reçue/Date Received 2022-02-07

5.1.7 Preparation of viral RNA and Sequencing

[00319] Viral RNA was extracted from cell culture supernatant or from the serum of infected mice using the QIAamp Viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions. The reverse-transcription reaction was done with ThermoScript RT-PCR System (Invitrogen) and a primer specific for LCMV NP (SEQ ID NO: 15) following the manufacturer's protocol. Amplification by PCR was done by using 2 µl of the cDNA from the RT step and NP- and GP-specific primers (SEQ ID NO: 16). The PCR reaction was done using Phusion High-Fidelity DNA Polymerase (NEB). Amplified products were analyzed on and excised from a 2 % agarose gel, purified using QIAquick Gel Extraction Kit (QIAGEN) and sent for DNA Sanger Sequencing (Microsynth) using the NP- and GP-specific primers.

5.1.8 Flow Cytometry

[00320] Blood was stained with antibodies against CD11c (N418), CD11b (M1/70), CD19 (6D5), NK1.1 (PK136), CD90.2 (30-H12) and GR-1 (RB6-8C5). The expression of surface molecules stained with specific antibodies as well as GFP and RFP expression was analyzed on a BD LSR Fortessa flow cytometer using FlowJo software (Tree Star, Ashland, OR).

5.1.9 Statistical Analysis

[00321] Statistical significance was determined by two-tailed unpaired t test or 1-way ANOVA followed by Dunnett's or Bonferroni's post-test for multiple comparisons using Graphpad Prism software (version 6.0d). p values of $p > 0.5$ were considered not significant (ns), whereas p values of $p < 0.5$ were considered significant (*) with gradations of $p < 0.01$ (**) and $p < 0.001$ (***) being highly significant.

5.2 Results

5.2.1 Recombinant tri-segmented viruses grow to lower titers than wild-type LCMV

[00322] The genome of wild-type LCMV consists of two single-stranded RNA segments of negative polarity (one L, one S segment) (Fig 1A). In recent years it has been shown that it is possible to introduce additional foreign genes into the normally bi-segmented genome found in LCMV particles. The NP and GP genes are segregated onto two S segment analogues, and genes of interest are inserted into each resulting S segment of LCMV resulting in replication-competent viral particles with three RNA segments (two S + one L). The only currently published strategy keeps both NP and GP in their natural position in the S segment, thus placing GFP or other transgenes in the respective free sites (r3LCMV-GFP^{nat})

(Fig 1B). This was the intuitive approach aimed at minimizing the likely risk that genetic reshuffling of the S segment abrogates the resulting genome's viability. However, this study hypothesized that it should also be possible to juxtapose the GP to the 3'UTR, expressing it from the promoter element that normally drives the NP (r3LCMV-GFP^{art}; Fig. 1C). Respective expression plasmids were generated by recombinant cDNA cloning and all three viral constructs were rescued entirely from plasmid DNA. Comparative growth curves were performed with the three viruses (Fig. 1D). All three viruses showed highest titers 48 hours after infection, with peak titers of tri-segmented viruses 10-100 fold lower than wild-type virus. Wild-type LCMV reached 3.4×10^6 PFU/ml, r3LCMV-GFP^{nat} peaked at 2.7×10^4 PFU/ml and r3LCMV-GFP^{art} at 2.2×10^5 PFU/ml. Irrespective of its similarly reduced peak titers, r3LCMV-GFP^{nat} exhibited somewhat higher cell-free infectivity during early time points than r3LCMV-GFP^{art}.

5.2.2 Packaging of tri-segmented viral particles is less efficient than of bi-segmented virus

[00323] These observations suggested that the addition of a second S segment impaired and delayed viral growth. It was hypothesized that this reduction in viral fitness might be due to inefficient packaging of all three RNA segments into viral particles, and that an excess of bi-segmented particles were formed, which failed to productively replicate when infecting fresh cells. For these experiments r3LCMVs with two different reporter genes *i.e.,* GFP together with GP on one S segment, and NP next to RFP on the second S segment were used. This resulted in two viruses named r3LCMV-GFP/RFP^{nat} and r3LCMV-GFP/RFP^{art}, which differed only in the arrangement of GFP and GP on the respective S segment. BHK-21 cells were infected with r3LCMV-GFP-RFP^{nat} or bi-segmented r2LCMV and focus forming assays were performed on normal BHK-21 cells or, in parallel, with stably transfected BHK-21 cells expressing either GP (BHK-GP) or NP (BHK-NP) as cell substrate to trans-complement viral genomes lacking the respective genes. Wild-type and GP-complementing cells were stained for nucleoprotein-expressing viral foci, whereas NP-complementing cells were stained for GP-positive foci. Thereby, immunofocus formation on wild-type BHK-21 cells detected only tri-segmented virions. Without being limited by theory, BHK-GP cells should replicate tri-segmented virions as well as bi-segmented ones containing the L segment in combination with the NP-expressing S segment (but devoid of the GP-expressing S). Conversely, BHK-NP cells should replicate tri-segmented LCMV and additionally NP-deficient virions consisting of the L and the GP-expressing S segment (but devoid of the NP-expressing S segment). Infectious titers of both r3LCMV-GFP/RFP^{nat} and r3LCMV-GFP/RFP^{art}, were consistently higher when assessed on BHK-GP or BHK-NP cells than when infectivity was tested on wt BHK-21 cells. Conversely, titers of r2LCMV were similar, irrespective of the cell substrate used to assess its infectivity. In order to correct for potential intrinsic differences in permissiveness of each cell line to LCMV, each virus' titer on BHK-21 cells was normalized to one, for display and BHK-GP as well as BHK-NP titers were expressed as a multiple thereof. Thus reflecting cell clone-related titer differences relating to potential clone-intrinsic differences in viral permissiveness (Fig. 2A). On either one of the complementing cells, an approximately five to ten-fold titer difference was observed for r3LCMV-GFP/RFP^{nat} and r3LCMV-GFP/RFP^{art}, which was significantly higher than for r2LCMV. This suggested that a majority of the viral particles, which were formed by the two tri-segmented viruses, contained only one of the two S-segments, encoding either only the NP-(NP-only particles) or the GP-expressing S segment (GP-only particles), respectively . The 5-fold or greater difference in titer suggested that both, NP-only and also GP-only particles outnumbered tri-segmented particles approximately five-fold each, and that tri-segmented particles made up for less than 10 percent of virions only, which was compatible with delayed growth and a reduction in viral peak titers when grown on non-complementing cells (Fig. 1D). These findings were further validated by flow cytometry. Non-complementing BHK-21 cells or BHK-NP cells were infected with r3LCMV-GFP/RFP^{art} or r2LCMV as gating control and fluorescence intensities of GFP and RFP were assessed with a flow cytometer (Fig. 2B). Since the minimal transacting factors are not provided by wild-type BHK-21 cells, only virions containing at least an L segment together with the NP-expressing S segment can initiate an infectious cycle after cell entry, resulting in fluorescence signal (RFP). Accordingly, a population of RFP+GFP- cells was observed upon infection of BHK-21 cells, reflecting NP-only particles. RFP+GFP+ double-positive cells were evidence of bona fide tri-segmented particles. According to the gating RFP-GFP+ cells were also observed, yet had a higher RFP MFI than RFP-GFP- cells, suggesting that they represented early stages of infection by trisegmented particles, an interpretation that is also supported by the continuity of this population and the RFP+GFP+ double positive one. However, when growing tri-segmented r3LCMV-GFP/RFP^{art} on BHK-NP cells, thus substituting for this minimal transacting factor, we observed a more than 10-fold higher number of RFP-GFP+ cells as compared to infection of non-complementing BHK-21 cells. Conversely, RFP+GFP- (evidence of NP-only particles) and GFP+RFP+ double-positive cells (tri-segmented particles) were detected in comparable abundance (Fig. 2C). These results confirmed at the single-cell level the findings obtained by focus forming assay, thus corroborating that tri-segmented virus preparations contain a majority of bi-segmented replication-deficient particles. These findings offered a likely explanation for attenuated growth of r3LCMV-GFP/RFP^{nat} and r3LCMV-GFP/RFP^{art} providing insight into an apparently quite inefficient random packaging of tri-segmented viruses.

5.2.3 Cloning and rescue of recombinant viruses to track recombination *in vivo*

[00324] Since tri-segmented viruses show impaired growth kinetics as seen in Fig 1, it was hypothesized that there should be high selection pressure on the viruses to recombine their genetic information for NP and GP on only one S segment. Inter-segmental recombination of arenaviruses is postulated to have led to the phylogenetic evolution of the North American clade, and thus seemed a potential mechanism whereby tri-segmented viruses could re-establish a functional bi-segmented genome. Without being limited by theory, looking at the genomic organization of the two tri-segmented viruses it was postulated that the selection pressure on r3LCMV-GFP^{nat} might favor recombination events in the area of the IGR, to bring GP and NP together on the same segment, while getting rid of GFP. In the population of r3LCMV-GFP^{art} selection pressure should be equally high, however, the reshuffling of GP and its positioning next to the 3'UTR should render it very difficult if not impossible for this virus to combine its two S segments into one functional segment (see Fig. 7 below). In account of the caveats for the identification of RNA recombination and to firmly discriminate it from potential cDNA contamination, we cloned an S segment carrying GFP together with a recombinant GP ORF in which the terminal 255 nucleotides were codon-optimized. The resulting GP had a different nucleotide sequence but identical translation product as the wild-type WE strain GP (WE/WET-GP, Fig. 3A). This recombinant WE/WET GP ORF did not, however, exist as an infectious bi-segmented virus nor did the laboratory possess a cDNA construct where it was associated with NP. Any potential bi-segmented virus containing WE/WET on the same segment as NP was therefore deemed clear evidence of intersegmental recombination, differentiating such viruses from potentially contaminating cDNA or RNA in the respective assays. To test whether the chimeric GP had an effect on viral fitness, cell culture growth curves of the recombinant tri-segmented virus carrying the WE/WET fusion GP (r3LCMV-WEWET/GFP^{nat}) were performed in comparison with a tri-segmented virus carrying the wild-type WE GP (r3LCMV-WE/GFP^{nat}) (Fig. 3B). Growth kinetics and peak titers of the two viruses were comparable (r3LCMV-WE/GFP^{nat}: 1.7×10^6 PFU/ml, r3LCMV-WEWET/GFP^{nat}: 2.3×10^6 PFU/ml). Thus the chimeric WEWET glycoprotein did not detectably impact viral growth.

[00325] To test whether potential recombination events could happen between the NP and GP genes of the S segment that would involve the IGR. Hence a single nucleotide deletion was introduced in the intergenic region of the NP-encoding S segment, to serve as a genetic tag. The choice of this nucleotide deletion was made because it is situated in a stretch that unlike most of the S segment IGR is not conserved between strains, neither in sequence nor in length. In case of a recombination event this "tagged" (marked as * throughout, both in figures and text) intergenic region should allow the identification of the genetic origin of the S segment IGR sequences. The position of the deleted cytosine (marked with an arrow) and a schematic of the resulting NP carrying S segment is depicted in Figure 3C. In order to test whether the introduced deletion in the IGR had an impact on viral growth, recombinant r3LCMV-GFP^{nat} with or without the single nucleotide deletion was rescued. Growth curve experiments were performed on BHK-21 cells (moi= 0.01). A tri-segmented virus with a wild-type IGR (r3LCMV-GFP^{nat}) and its comparator with the mutated IGR (r3LCMV-GFP^{nat} IGR*) grew at a similar rate and reached indistinguishable peak titers (Figure 3D). Consequently the tag of the IGR on the NP-carrying S segment did not have a detectable impact on viral fitness, thus validating its use for subsequent experimentation *in vivo*.

5.2.4 r3LCMV-GFP^{nat} but not r3LCMV-GFP^{art} persistent infection in mice reaches viremia levels equivalent to bi-segmented wt virus and results in loss of GFP expression

[00326] Upon rescue of the recombinant r3LCMV-GFP^{nat} an aim was to investigate whether tri-segmented viruses recombined *in vivo*. For this purpose AGRAG mice were infected with r3LCMV-GFP^{nat}, r3LCMV-GFP^{art} or a bi-segmented r2LCMV as control. AGRAG mice carry targeted deletions in the genes encoding for the Interferon-α/β receptor, the Interferon-γ receptor and RAG1, leading to an immuno-deficient phenotype and establishment of chronic viremia after infection with tri-segmented LCMV. Blood samples were taken over time and viral titers were assessed by focus forming assay (Fig. 4A). Carriers of bi-segmented LCMV showed high titer viremia in the range of 5×10^5 PFU/ml blood within 5 days after infection, with subsequently stable viremia in the 10^4 - 10^5 PFU/ml range until at least day 50 post infection. Mice infected with tri-segmented LCMV showed viral loads of about 5×10^3 PFU/ml blood until day 20, in line with attenuated growth in cell culture (compared to Fig. 1D). From day 30 onwards, carriers of r3LCMV-GFP^{nat} displayed a rise in viral loads, which was not observed in animals infected with r3LCMV-GFP^{art}, resulting in more than a 10-fold difference in viremia on day 50. To determine whether the dominating virus population still carried the GFP reporter gene, thus resulting in GFP expression in infected cells, viral focus formation assays with blood samples of r3LCMV-GFP^{nat} and r3LCMV-GFP^{art} carriers taken on day 127 after infection were performed and stained for the nucleoprotein or the reporter gene GFP (Fig. 4B). Whereas staining of blood isolated from r3LCMV-GFP^{art} carriers resulted in equal amounts of foci with anti-NP and anti-GFP antibody detection (both assessments independently indicating viral titers in the 10^3 PFU/ml range) at least 100-fold higher numbers of total (NP+) r3LCMV-GFP^{nat} foci were evident than foci expressing GFP. Viral titers of at least 10^4 PFU/ml were measured based on anti-NP detection, whereas two out of three mice failed to show any detectable GFP-positive infectivity and one mouse had a residual fraction of GFP-positive foci in the 100 PFU/ml range, corresponding to the lower limit of detection of our assays. GFP expression of infected cells was also assessed by fluorescence microscopy (data not shown). GFP-fluorescent foci were virtually undetectable when assaying blood from r3LCMV-GFP^{nat} carriers whereas manual counts of GFP-positive foci from r3LCMV-GFP^{art} carrier blood matched the titer results obtained with anti-NP focus forming assay. Reporter gene expression was further verified by flow cytometric analysis of PBMCs of infected mice on day 120 after infection. We found that more than 10 % of CD11b+GR1- monocytes/macrophages were positive for GFP in r3LCMV-GFP^{art} infected animals whereas blood from r3LCMV-GFP^{nat} evidenced only background levels of GFP, which was comparable to animals infected with non-fluorescent r2LCMV (Fig. 4C-E). This finding further supported the hypothesis that tri-segmented viruses with GP in their natural position lose reporter gene expression over time whereas transposition of the GP in the artificial 3'UTR juxtaposition prevented transgene loss.

5.2.5 Tri-segmented viruses with GP in the natural position can recombine their two S segments resulting in a single S segment with partial or complete IGR duplications flanking a transgene sequence rudiment

[00327] Figure 4 showed elevated viremia and loss of reporter gene expression in mice infected with r3LCMV-GFP^{nat}. Therefore, it was hypothesized that a recombination event could account for this experimental outcome. Intersegmental recombination should combine GP and NP on the same S segment, obviating the need for a second S segment in the viral replication cycle. Such an event could then have explained viremia at the level of wild-type virus, in combination with loss of reporter gene expression. To test this possibility viral RNA from the serum of infected mice was isolated and a pair of primers binding to NP and GP sequences, respectively, were used to selectively amplify by RT-PCR only the putatively recombined RNA molecules, carrying both NP and GP ORFs in ambisense orientation on one RNA segment. The resulting PCR fragments were analyzed by gel electrophoresis (Fig. 5A). The sera of all r3LCMV-GFP^{nat} carriers gave rise to RT-dependent PCR products, whereas r3LCMV-GFP^{art} carriers and naïve controls did not show specific bands. Control PCR reactions were performed on mock-RT-treated RNA samples to rule out cDNA contaminations as a source of PCR product. Sequencing results of three individual r3LCMV-GFP^{nat} carriers are schematically represented in Fig. 5C. The three mice contained viral RNA segments of distinct sequences yet with a similar pattern: C-terminal portions of GP and NP were found in ambisense orientation on one RNA segment. Between them, both intergenic regions, *i.e.,* the one of the NP-expressing and the one of the original GP-expressing segment were at least partially retained, separated by a fragment from either one or both GFP reporter genes in the parental S segments of the trisegmented virus. The direction and length of the GFP fragment varied between the three RNA species recovered from individual mice, which was indicative of independent recombination events. In further support of this notion, the exact same recombined RNA sequence was recovered from two consecutive samples taken from the same mouse with more than three weeks interval between sampling. Based on the recombined S segment sequences obtained, we proposed a molecular mechanism, as schematically outlined in Fig. 7 and described in the figure's legend, whereby r3LCMV-GFP^{nat} recombines its two S segments, resulting in transgene loss and phenotypic reversion to wild-type virus. The schematics in Fig. 7 also explain why, according to the proposed mechanism of S segment recombination, r3LCMV-GFP^{art} cannot recombine and bring together its NP and GP ORFs on one functional S segment.

5.2.6 Recombinant r2LCMV with two IGRs on the S segment is viable and grows to similar titers as bi-segmented LCMV with only one IGR in the S segment.

[00328] The above sequencing data revealed a consistent pattern of viral genetic elements in recombined S segments amongst which the (at least partial) duplication of the IGR was particularly noteworthy and characteristic. However, arenaviruses with repeats of intergenic regions on one S segment were not known. A dual stem loop is, however, naturally found in the Old World arenavirus Mopeia. Hence, we cloned the rearranged S segment of r3LCMV-GFP^{nat} carrier #3 with the two IGRs and the remnant of GFP into a pol-I driven S segment expression plasmid and rescued the respective virus. Growth kinetics of this virus (r2LCMV_2IGRs) on BHK-21 cells were compared to tri-segmented r3LCMV-GFP^{nat} and bi-segmented r2LCMV (Fig. 6). Infectious cell-free titers of r2LCMV_2IGRs exceeded those of r3LCMV-GFP^{nat} already at early time points and reached identical peak titers as r2LCMV (1.7×10^7 PFU/ml vs. 1.6×10^7 PFU/ml, respectively). Importantly, r2LCMV_2IGRs grew to considerably higher peak titers than its parental tri-segmented r3LCMV-GFP^{nat} attesting to the selective advantage of intersegmental recombination despite duplication of the IGR during this process.

5.2.7 Recombinant r3LCMV expressing ovalbumin (OVA) induces a rapid, strong and polyfunctional OVA-specific CD8+ T cell response.

[00329] To test the utility of the r3LCMV^{art} vector delivery technology for vaccination purposes we generated the r3LCMV-OVA^{art} vaccine vector with a genome organization analogous to r3LCMV-GFP^{art} (Fig. 1C) but with two ovalbumin (OVA) genes instead of the respective GFP genes in the latter virus. We immunized C57BL/6 mice intramuscularly (i.m.) with 10^4 PFU of r3LCMV-OVA^{art} and eight days later we analyzed the T cell response in spleen. For comparison to a widely used vector platform we immunized a second group of C57BL/6 mice with 10^8 particles of a replication-deficient E1-deleted adenovirus 5-based vector also expressing OVA (rAd5-OVA). The frequency of OVA-specific CD8+ T cells recognizing the immunodominant OVA-derived SIINFEKL epitope was in the 10% range of CD8+ T cells in the r3LCMV-OVA^{art} vaccine group, which was significantly higher than in the rAd5-OVA group (Fig. 8A). r3LCMV-OVA^{art} induced CD8+ T cell responses were not only of high magnitude but also highly functional as determined by intracellular cytokine assays, revealing that most SIINFEKL-reactive r3LCMV-OVA^{art} induced CD8+ T cells produced IFN-γ in response to peptide stimulation, and that a fair proportion co-produced TNF-α and/or IL-2. This demonstrated the utility of the r3LCMV-OVA^{art} vector technology for vaccine delivery.

5.2.8 Trisegmented LCMV induces polyfunctional memory CD8+ T cells.

[00330] To address the question whether r3LCMV vectors induce functional CD8+ T cell memory we immunized C57BL/6 mice with 10e5 PFU of r3LCMV-OVA^{art} i.v. and analyzed OVA-specific (SIINFEKL-specific) CD8+ T cell responses in spleen on day 25. A reference control group of mice was vaccinated with 10e8 viral particles (vp) of recombinant E1-deleted adenoviral vector (rAd) expressing OVA by the same route. OVA-specific CD8+ T cells producing IFN-γ, TNF-α and/or IL-2 upon peptide stimulation were assessed in standard intracellular cytokine assays upon SIINFEKL peptide stimulation. The frequency (Figure 9A) and absolute number (Figure 9B) of cytokine producing cells as indicated in the chart was determined. r3LCMV-OVA^{art}-immune mice exhibited significantly higher frequencies and numbers of polyfunctional IFN-γ / TNF-α and IFN-γ / TNF-α / IL-2 co-producing OVA-specific CD8+ T cells than rAd-OVA-immune mice.

5.2.9 Antigen-encoding LCMV induces specific T cell responses to foreign and self antigens.

[00331] To investigate whether r3LCMV^{art} vectors can be exploited to induce CD8+ T cell responses against tumor-expressed self antigens, we immunized BALB/c mice with r3LCMV^{art} vectors expressing either rat (TYVPANASL), human (TYLPTNASL) or mouse (TYLPANASL) Her2-derived CD8+ T cell epitopes (Figure 10). Nine days later we measured specific CD8+ T cells producing IFN-γ, TNF-α and/or IL-2 upon stimulation with the respective peptides in intracellular cytokine assays. Figure 10 displays the frequencies of epitope-specific CD8+ T cells as the percentage of CD8+ T cells producing the indicated cytokine combination upon stimulation with the cognate peptide. Frequencies of cytokine-producing CD8+ T cells upon restimulation with medium only were insignificant. The results document that r3LCMV^{art} vectors have the capacity to induce substantial frequencies of tumor self-antigen-reactive CD8+ T cell responses.

5.2.10 Interferon-α is induced upon r3LCMV^{art} infection but not upon infection with recombinant Adeno- or Vaccinia virus vectors.

[00332] Type I interferons can have multiple immunostimulatory and anti-tumoral effects. Hence, type I interferon induction can represent a favorable feature of a virally vectored vaccine. We performed ELISA measurements to determine interferon-alpha concentrations in the serum of mice immunized with r3LCMV-OVA^{art}, rAd-OVA or recombinant vaccinia virus expressing OVA (rVacc) 24, 48 or 72 hours previously (Figure 11). r3LCMV^{art} but neither rAd nor rVacc induced a detectable and sustained (at least 48 hours) systemic interferon-alpha response. This attested to the capacity of r3LCMV^{art} vectors to induce strong innate immune responses.

5.2.11 Cell culture growth of r3JUNV-GFP^{art} in comparison to r3JUNV-GFP^{nat} and parental Junin strain Candid#1.

[00333] By analogy to the r3LCMV-GFP^{nat} and r3LCMV-GFP^{art} vectors, carrying a genome as outlined in Fig 1B we engineered r3JUNV-GFP^{nat} and r3JUNV-GFP^{art}, consisting of trisegmented Junin vaccine strain Candid#1-based vectors carrying GFP genes in each one of their respective two S segments (r3JUNV-GFP^{nat} and r3JUNV-GFP^{art}). We tested their growth properties in 293T cells, which we infected at multiplicity of infection of 0.01 and collected supernatant over time (Fig. 12). We found that r3JUNV-GFP^{art} grew more slowly than its parental bisegmented Junin vaccine strain Candid#1 (Fig. 12). However, it grew more quickly than r3JUNV-GFP^{nat},(Fig. 12). This differential growth behavior of trisegmented Junin virus-based vectors paralleled the growth rates of r3LCMV-GFP^{nat} and r3LCMV-GFP^{art} vectors (Fig. 1D).

5.2.12 Trisegmented JUNV are dramatically attenuated *in vivo,* and r3JUNV-GFP^{nat} but not r3JUNV-GFP^{art} loses GFP expression upon prolonged *in vivo* replication.

[00334] To investigate the genetic stability of r3JUNV-GFP^{nat} and r3JUNV-GFP^{art} we infected AGRAG mice (IFNα/βR-/-, IFNγR-/-, RAG-/-) with 7x10e4 PFU of either of these GFP-expressing vectors. For the purpose of comparison, a third group was infected with the wild type bisegmented Candid#1 virus. The latter virus was readily detected in the blood of all infected mice by day 20 after infection (Figure 13A), whereas the trisegmented viruses remained undetectable for at least 40 days. This finding documented attenuated *in vivo* growth as a result of genome reorganization, extending our findings with r3LCMV-GFP vectors in Fig. 4A to Junin-based vectors. After day 40, also r3JUNV-GFP^{nat} and r3JUNV-GFP^{art} became detectable in several animals in each group (Figure 13A). Importantly, however, some of the r3JUNV-GFP^{nat}-infected mice reached viral loads in the range of wild type Candid#1-infected mice whereas viremic r3JUNV-GFP^{art}-infected mice retained lower viral load than Candid#1-infected controls.

[00335] To determine whether the dominating virus population in these viremic animals still carried the GFP reporter gene, thus resulting in GFP expression in infected cells, we performed viral focus formation assays with blood samples of r3JUNV-GFP^{nat} and r3JUNV-GFP^{art} carriers taken on day 120 after infection. We compared infectious titers of viruses retaining GFP expression (anti-GFP, Fig. 13B) and total Junin virus infectivity (anti-NP, Fig. 13B). r3JUNV-GFP^{art} titers were in similar ranges when determined by either anti-GFP or anti-NP Immunofocus assay documenting that the majority of the virus population retained GFP expression. Conversely, in the blood of the four r3JUNV-GFP^{nat} infected animals with highest viremia (comparable to wildtype Candid#1) the anti-GFP infectious titer was at least 10 fold lower than the total infectious titer as determined by NP staining. This documented that r3JUNV-GFP^{art} but not r3JUNV-GFP^{nat} stably retained the GFP transgene *in vivo.*

5.2.13 Homologous and heterologous prime-boost combinations of trisegmented LCMV- and JUNV-based vaccine vectors induce strong P1A autoantigen-specific CD8+ T cells responses.

[00336] Next we investigated whether r3LCMV^{art}- and r3JUNV^{art}-based vectors can be used in homologous and heterologous prime-boost combinations for inducing tumor autoantigen-specific CD8+ T cell responses. We constructed r3LCMV^{art} and r3JUNV^{art}-based vectors expressing the P815 mouse mastocytoma-derived self antigen P1A (SEQ ID NO: 24) (r3LCMV-P1A^{art} (SEQ ID NOs: 18, 19, 20) and r3JUNV-P1A^{art} (SEQ ID NOs: 21, 22, 23)). These vaccine constructs were used to immunize BALB/c mice i.v. in homologous and heterologous prime-boost combinations as outlined in Figure 14. Both, r3LCMV-P1A^{art} and r3JUNV-P1A^{art} induced P1A epitope-specific CD8+ T cells when administered in homologous prime-boost vaccination, as determined from blood using H-2L^{d}-tetramers loaded with the LPYLGWLVF peptide (P1A epitope 35-43). Mean frequencies of epitope-specific CD8+ T cells on day 63 of the experiment were 1.2% (r3JUNV-P1A^{art}) and 3.9% (r3LCMV-P1A^{art}), respectively. Additionally, animals primed with r3JUNV-P1A^{art} and boosted with r3LCMV-P1A^{art} in a heterologous fashion mounted even higher responses with average epitope-specific CD8+ T cell frequencies of 19.5% on day 63. Frequencies of r3LCMV-P1A^{art}-primed and r3JUNV-P1A^{art}-boosted animals (3.1%) were comparable to those undergoing r3LCMV-P1A^{art} homologous prime-boost vaccination.

6. EQUIVALENTS

[00337] The viruses, nucleic acids, methods, host cells, and compositions disclosed herein are not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the viruses, nucleic acids, methods, host cells, and compositions in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

7. SEQUENCE LISTING

SEQ ID NO.	Description	Sequence
1	LCMV segment S, complete sequence.	cgcaccgggg atcctaggct ttttggattg cgctttcctc tagatcaact gggtgtcagg 60

Date Reçue/Date Received 2022-02-07

SEQ ID NO.	Description	Sequence
	The genomic segment is RNA, the sequence in SEQ ID NO: 1 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:1 for uridines ("U") provides the RNA sequence.	ccctatccta cagaaggatg ggtcagattg tgacaatgtt tgaggctctg cctcacatca 120 tcgatgaggt gatcaacatt gtcattattg tgcttatcgt gatcacgggt atcaaggctg 180 tctacaattt tgccacctgt gggatattcg cattgatcag tttcctactt ctggctggca 240 ggtcctgtgg catgtacggt cttaagggac ccgacattta caaaggagtt taccaattta 300 agtcagtgga gtttgatatg tcacatctga acctgaccat gcccaacgca tgttcagcca 360 acaactccca ccattacatc agtatgggga cttctggact agaattgacc ttcaccaatg 420 attccatcat cagtcacaac ttttgcaatc tgacctctgc cttcaacaaa aagacctttg 480 accacacact catgagtata gtttcgagcc tacacctcag tatcagaggg aactccaact 540 ataaggcagt atcctgcgac ttcaacaatg gcataaccat ccaatacaac ttgacattct 600 cagatcgaca aagtgctcag agccagtgta gaacccttcag aggtagagtc ctagatatgt 660 ttagaactgc cttcgggggg aaatacatga ggagtggctg ggctggaca ggctcagatg 720 gcaagaccac ctggtgtagc cagacgagtt accaatacct gattatacaa aatagaacct 780 gggaaaacca ctgcacatat gcaggtcctt ttgggatgtc caggattctc ctttcccaag 840 agaagactaa gttcttcact aggagactag cgggcacatt cacctggact ttgtcagact 900 cttcaggggt ggagaatcca ggtggttatt gcctgaccaa atggatgatt cttgctgcag 960 agcttaagtg tttcgggaac acagcagttg cgaaatgcaa tgtaaatcat gatgccgaat 1020 tctgtgacat gctgcgacta attgactaca acaaggctgc tttgagtaag ttcaaagagg 1080 acgtagaatc tgccttgcac ttattcaaaa caacagtgaa ttctttgatt tcagatcaac 1140 tactgatgag gaaccacttg agagatctga tgggggtgcc atattgcaat tactcaaagt 1200 tttggtacct agaacatgca aagaccggcg aaactagtgt ccccaagtgc tggcttgtca 1260 ccaatggttc ttacttaaat gagacccact tcagtgatca aatcgaacag gaagccgata 1320 acatgattac agagatgttg aggaaggatt acataaagag gcaggggagt acccccctag 1380 cattgatgga ccttctgatg ttttccacat ctgcatatct agtcagcatc ttcctgcacc 1440 ttgtcaaaat accaacacac aggcacataa aaggtggctc atgtccaaag ccacaccgat 1500 taaccaacaa aggaatttgt agttgtggtg catttaaggt gcctggtgta aaaaccgtct 1560 ggaaaagacg ctgaagaaca gcgcctccct gactctccac ctcgaaagag gtggagagtc 1620 agggaggccc agagggtctt agagtgtcac aacatttggg cctctaaaaa ttaggtcatg 1680 tggcagaatg ttgtgaacag ttttcagatc tgggagcctt gctttggagg cgctttcaaa 1740 aatgatgcag tccatgagtg cacagtgcgg ggtgatctct ttcttctttt tgtcccttac 1800 tattccagta tgcatcttac acaaccagcc atatttgtcc cacactttgt cttcatactc 1860 cctcgaagct tccctggtca tttcaacatc

SEQ ID NO.	Description	Sequence
		gataagctta atgtccttcc tattctgtga 1920 gtccagaagc tttctgatgt catcggagcc ttgacagctt agaaccatcc cctgcggaag 1980 agcacctata actgacgagg tcaacccggg ttgcgcattg aagaggtcgg caagatccat 2040 gccgtgtgag tacttggaat cttgcttgaa ttgtttttga tcaacgggtt ccctgtaaaa 2100 gtgtatgaac tgcccgttct gtggttggaa aattgctatt tccactggat cattaaatct 2160 accctcaatg tcaatccatg taggagcgtt ggggtcaatt cctcccatga ggtcttttaa 2220 aagcattgtc tggctgtagc ttaagcccac ctgaggtgga cctgctgctc caggcgctgg 2280 cctgggtgaa ttgactgcag gtttctcgct tgtgagatca attgttgtgt tttcccatgc 2340 tctccccaca atcgatgttc tacaagctat gtatggccat ccttcacctg aaaggcaaac 2400 tttatagagg atgttttcat aagggttcct gtccccaact tggtctgaaa caaacatgtt 2460 gagttttctc ttggccccga gaactgcctt caagaggtcc tcgctgttgc ttggcttgat 2520 caaaattgac tctaacatgt tacccccatc caacagggct gcccctgcct tcacggcagc 2580 accaagacta aagttatagc cagaaatgtt gatgctggac tgctgttcag tgatgacccc 2640 cagaactggg tgcttgtctt tcagcctttc aagatcatta agatttggat acttgactgt 2700 gtaaagcaag ccaaggtctg tgagcgcttg tacaacgtca ttgagcggag tctgtgactg 2760 tttggccata caagccatag ttagacttgg cattgtgcca aattgattgt tcaaaagtga 2820 tgagtctttc acatcccaaa ctcttaccac accacttgca ccctgctgag gctttctcat 2880 cccaactatc tgtaggatct gagatctttg gtctagttgc tgtgttgtta agttccccat 2940 atataccccct gaagcctggg gcctttcaga cctcatgatc ttggccttca gcttctcaag 3000 gtcagccgca agagacatca gttcttctgc actgagcctc cccactttca aaacattctt 3060 ctttgatgtt gactttaaat ccacaagaga atgtacagtc tggttgagac ttctgagtct 3120 ctgtaggtct ttgtcatctc tcttttcctt cctcatgatc ctctgaacat tgctgacctc 3180 agagaagtcc aacccattca gaaggttggt tgcatcctta atgacagcag ccttcacatc 3240 tgatgtgaag ctctgcaatt ctcttctcaa tgcttgcgtc cattggaagc tcttaacttc 3300 cttagacaag gacatcttgt tgctcaatgg tttctcaaga caaatgcgca atcaaatgcc 3360 taggatccac tgtgcg
2	LCMV clone 13 segment S, complete sequence (GenBank: DQ361065.2). The genomic segment is RNA, the sequence in SEQ ID NO: 2 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO: 2 for	gcgcaccggg gatcctaggc tttttggatt gcgctttcct ctagatcaac tgggtgtcag 60 gccctatcct acagaaggat gggtcagatt gtgacaatgt ttgaggctct gcctcacatc 120 atcgatgagg tgatcaacat tgtcattatt gtgcttatcg tgatcacggg tatcaaggct 180 gtctacaatt ttgccacctg tgggatattc gcattgatca gtttcctact tctggctggc 240 aggtcctgtg gcatgtacgg tcttaaggga cccgacattt acaaaggagt ttaccaattt 300 aagtcagtgg agtttgatat gtcacatctg

SEQ ID NO.	Description	Sequence
	uridines ("U") provides the RNA sequence.	aacctgacca tgcccaacgc atgttcagcc 360 aacaactccc accattacat cagtatgggg acttctggac tagaattgac cttcaccaat 420 gattccatca tcagtcacaa cttttgcaat ctgacctctg ccttcaacaa aaagaccttt 480 gaccacacac tcatgagtat agtttcgagc ctacacctca gtatcagagg gaactccaac 540 tataaggcag tatcctgcga cttcaacaat ggcataacca tccaatacaa cttgacattc 600 tcagatgcac aaagtgctca gagccagtgt agaaccttca gaggtagagt cctagatatg 660 tttagaactg ccttcggggg gaaatacatg aggagtggct ggggctggac aggctcagat 720 ggcaagacca cctggtgtag ccagacgagt taccaatacc tgattataca aaatagaacc 780 tgggaaaacc actgcacata tgcaggtcct tttgggatgt ccaggattct cctttcccaa 840 gagaagacta agttcctcac taggagacta gcgggcacat tcacctggac tttgtcagac 900 tcttcagggg tggagaatcc aggtggttat tgcctgacca aatggatgat tcttgctgca 960 gagcttaagt gtttcgggaa cacagcagtt gcgaaatgca atgtaaatca tgatgaagaa 1020 ttctgtgaca tgctgcgact aattgactac aacaaggctg ctttgagtaa gttcaaagag 1080 gacgtagaat ctgccttgca cttattcaaa acaacagtga attctttgat ttcagatcaa 1140 ctactgatga ggaaccactt gagagatctg atgggggtgc catattgcaa ttactcaaag 1200 ttttggtacc tagaacatgc aaagaccggc gaaactagtg tccccaagtg ctggcttgtc 1260 accaatggtt cttacttaaa tgagacccac ttcagtgacc aaatcgaaca ggaagccgat 1320 aacatgatta cagagatgtt gaggaaggat tacataaaga ggcaggggag taccccccta 1380 gcattgatgg accttctgat gttttccaca tctgcatatc tagtcagcat cttcctgcac 1440 cttgtcaaaa taccaacaca caggcacata aaaggtggct catgtccaaa gccacaccga 1500 ttaaccaaca aaggaatttg tagttgtggt gcatttaagg tgcctggtgt aaaaaccgtc 1560 tggaaaagac gctgaagaac agcgcctccc tgactctcca cctcgaaaga ggtggagagt 1620 cagggaggcc cagagggtct tagagtgtca caacatttgg gcctctaaaa attaggtcat 1680 gtggcagaat gttgtgaaca gttttcagat ctgggagcct tgctttggag gcgctttcaa 1740 aaatgatgca gtccatgagt gcacagtgcg gggtgatctc tttcttcttt ttgtccctta 1800 ctattccagt atgcatctta cacaaccagc catatttgtc ccacactttg tcttcatact 1860 ccctcgaagc ttccctggtc atttcaacat cgataagctt aatgtccttc ctattctgtg 1920 agtccagaag ctttctgatg tcatcggagc cttgacagct tagaaccatc ccctgcggaa 1980 gagcacctat aactgacgag gtcaacccgg gttgcgcatt gaagaggtcg gcaagatcca 2040 tgccgtgtga gtacttggaa tcttgcttga attgtttttg atcaacgggt tccctgtaaa 2100 agtgtatgaa ctgcccgttc tgtggttgga aaattgctat ttccactgga tcattaaatc 2160

SEQ ID NO.	Description	Sequence
		taccctcaat gtcaatccat gtaggagcgt tggggtcaat tcctcccatg aggtctttta 2220 aaagcattgt ctggctgtag cttaagccca cctgaggtgg acctgctgct ccaggcgctg 2280 gcctgggtga attgactgca ggtttctcgc ttgtgagatc aattgttgtg ttttcccatg 2340 ctctccccac aatcgatgtt ctacaagcta tgtatggcca tccttcacct gaaaggcaaa 2400 ctttatagag gatgttttca taagggttcc tgtccccaac ttggtctgaa acaaacatgt 2460 tgagttttct cttggccccg agaactgcct tcaagaggtc ctcgctgttg cttggcttga 2520 tcaaaattga ctctaacatg ttaccccccat ccaacagggc tgcccctgcc ttcacggcag 2580 caccaagact aaagttatag ccagaaatgt tgatgctgga ctgctgttca gtgatgaccc 2640 ccagaactgg gtgcttgtct ttcagccttt caagatcatt aagatttgga tacttgactg 2700 tgtaaagcaa gccaaggtct gtgagcgctt gtacaacgtc attgagcgga gtctgtgact 2760 gtttggccat acaagccata gttagacttg gcattgtgcc aaattgattg ttcaaaagtg 2820 atgagtcttt cacatcccaa actcttacca caccacttgc accctgctga ggctttctca 2880 tcccaactat ctgtaggatc tgagatcttt ggtctagttg ctgtgttgtt aagttcccca 2940 tatatacccc tgaagcctgg ggcctttcag acctcatgat cttggccttc agcttctcaa 3000 ggtcagccgc aagagacatc agttcttctg cactgagcct ccccactttc aaaacattct 3060 tctttgatgt tgactttaaa tccacaagag aatgtacagt ctggttgaga cttctgagtc 3120 tctgtaggtc tttgtcatct ctcttttcct tcctcatgat cctctgaaca ttgctgacct 3180 cagagaagtc caacccattc agaaggttgg ttgcatcctt aatgacagca gccttcacat 3240 ctgatgtgaa gctctgcaat tctcttctca atgcttgcgt ccattggaag ctcttaactt 3300 ccttagacaa ggacatcttg ttgctcaatg gtttctcaag acaaatgcgc aatcaaatgc 3360 ctaggatcca ctgtgcg
3	LCMV clone 13 segment L, complete sequence (GenBank: DQ361066.1). The genomic segment is RNA, the sequence in SEQ ID NO: 3 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO: 3 for uridines ("U") provides the RNA sequence.	gcgcaccggg gatcctaggc gtttagttgc gctgtttggt tgcacaactt tcttcgtgag 60 gctgtcagaa gtggacctgg ctgatagcga tgggtcaagg caagtccaga gaggagaaag 120 gcaccaatag tacaaacagg gccgaaatcc taccagatac cacctatctt ggccctttaa 180 gctgcaaatc ttgctggcag aaatttgaca gcttggtaag atgccatgac cactaccttt 240 gcaggcactg tttaaacctt ctgctgtcag tatccgacag gtgtcctctt tgtaaatatc 300 cattaccaac cagattgaag atatcaacag ccccaagctc tccacctccc tacgaagagt 360 aacaccgtcc ggccccggcc ccgacaaaca gcccagcaca agggaaccgc acgtcaccca 420 acgcacacag acacagcacc caacacagaa cacgcacaca cacacacaca cacacccaca 480 cgcacgcgcc cccaccaccg gggggcgccc ccccccgggg ggcggccccc cgggagcccg 540 ggcggagccc cacggagatg cccatcagtc gatgtcctcg gccaccgacc cgcccagcca 600

SEQ ID NO.	Description	Sequence
		atcgtcgcag gacctcccct tgagtctaaa cctgcccccc actgtttcat acatcaaagt 660 gctcctagat ttgctaaaac aaagtctgca atccttaaag gcgaaccagt ctggcaaaag 720 cgacagtgga atcagcagaa tagatctgtc tatacatagt tcctggagga ttacacttat 780 ctctgaaccc aacaaatgtt caccagttct gaatcgatgc aggaagaggt tcccaaggac 840 atcactaatc ttttcatagc cctcaagtcc tgctagaaag actttcatgt ccttggtctc 900 cagcttcaca atgatatttt ggacaaggtt tcttccttca aaaagggcac ccatctttac 960 agtcagtggc acaggctccc actcaggtcc aactctctca aagtcaatag atctaatccc 1020 atccagtatt cttttggagc ccaacaactc aagctcaaga gaatcaccaa gtatcaaggg 1080 atcttccatg taatcctcaa actcttcaga tctgatatca aagacaccat cgttcacctt 1140 gaagacagag tctgtcctca gtaagtggag gcattcatcc aacattcttc tatctatctc 1200 acccttaaag aggtgagagc atgataaaag ttcagccaca cctggattct gtaattggca 1260 cctaaccaag aatatcaatg aaaatttcct taaacagtca gtattattct gattgtgcgt 1320 aaagtccact gaaattgaaa actccaatac cccttttgtg tagttgagca tgtagtccca 1380 cagatccttt aaggatttaa atgcctttgg gtttgtcagg ccctgcctaa tcaacatggc 1440 agcattacac acaacatctc ccattcggta agagaaccac ccaaaaccaa actgcaaatc 1500 attcctaaac ataggcctct ccacattttt gttcaccacc tttgagacaa atgattgaaa 1560 ggggcccagt gcctcagcac catcttcaga tggcatcatt tctttatgag ggaaccatga 1620 aaaattgcct aatgtcctgg ttgttgcaac aaattctcga acaaatgatt caaaatacac 1680 ctgttttaag aagttcttgc agacatccct cgtgctaaca acaaattcat caaccagact 1740 ggagtcagat cgctgatgag aattggcaag gtcagaaaac agaacagtgt aatgttcatc 1800 ccttttccac ttaacaacat gagaaatgag tgacaaggat tctgagttaa tatcaattaa 1860 aacacagagg tcaaggaatt taattctggg actccacctc atgtttttg agctcatgtc 1920 agacataaat ggaagaagct gatcctcaaa gatcttggga tatagccgcc tcacagattg 1980 aatcacttgg ttcaaattca ctttgtcctc cagtagcctt gagctctcag gctttcttgc 2040 tacataatca catgggttta agtgcttaag agttaggttc tcactgttat tcttcccttt 2100 ggtcggttct gctaggaccc aaacacccaa ctcaaaagag ttgctcaatg aaatacaaat 2160 gtagtcccaa agaagaggcc ttaaaggca tatatgatca cggtgggctt ctggatgaga 2220 ctgtttgtca caaatgtaca gcgttatacc atcccgattg caaactcttg tcacatgatc 2280 atctgtggtt agatcctcaa gcagcttttt gatatacaga ttttccctat ttttgtttct 2340 cacacacctg cttcctagag ttttgcaaag gcctataaag ccagatgaga tacaactctg 2400 gaaagctgac ttgttgattg cttctgacag

SEQ ID NO.	Description	Sequence
		cagcttctgt gcaccccttg tgaatttact 2460 acaaagtttg ttctggagtg tcttgatcaa tgatgggatt ctttcctctt ggaaagtcat 2520 cactgatgga taaaccacct tttgtcttaa aaccatcctt aatgggaaca tttcattcaa 2580 attcaaccag ttaacatctg ctaactgatt cagatcttct tcaagaccga ggaggtctcc 2640 caattgaaga atggcctcct ttttatctct gttaaatagg tctaagaaaa attcttcatt 2700 aaattcacca tttttgagct tatgatgcag tttccttaca agctttctta caacctttgt 2760 ttcattagga cacagttcct caatgagtct ttgtattctg taacctctag aaccatccag 2820 ccaatctttc acatcagtgt tggtattcag tagaaatgga tccaaaggga aattggcata 2880 ctttaggagg tccagtgttc tcctttggat actattaact agggagactg ggacgccatt 2940 tgcgatggct tgatctgcaa ttgtatctat tgtttcacaa agttgatgtg gctctttaca 3000 cttgacattg tgtagcgctg cagatacaaa ctttgtgaga agagggactt cctcccccca 3060 tacatagaat ctagatttaa attctgcagc gaacctccca gccacacttt ttgggctgat 3120 aaatttgttt aacaagccgc tcagatgaga ttggaattcc aacaggacaa ggacttcctc 3180 cggatcactt acaaccaggt cactcagcct cctatcaaat aaagtgatct gatcatcact 3240 tgatgtgtaa gcctctggtc tttcgccaaa gataacacca atgcagtagt tgatgaacct 3300 ctcgctaagc aaaccataga agtcagaagc attatgcaag attccctgcc ccatatcaat 3360 aaggctggat atatgggatg gcactatccc catttcaaaa tattgtctga aaattctctc 3420 agtaacagtt gtttctgaac ccctgagaag ttttagcttc gacttgacat atgatttcat 3480 cattgcattc acaacaggaa aggggacctc gacaagctta tgcatgtgcc aagttaacaa 3540 agtgctaaca tgatctttcc cggaacgcac atactggtca tcacctagtt tgagattttg 3600 tagaaacatt aagaacaaaa atgggcacat cattggtccc catttgctgt gatccatact 3660 atagtttaag aacccttccc gcacattgat agtcattgac aagattgcat tttcaaattc 3720 cttatcattg tttaaacagg agcctgaaaa gaaacttgaa aaagactcaa aataatcttc 3780 tattaacctt gtgaacattt ttgtcctcaa atctccaata tagagttctc tatttccccc 3840 aacctgctct ttataagata gtgcaaattt cagccttcca gagtcaggac ctactgaggt 3900 gtatgatgtt ggtgattctt ctgagtagaa gcacagattt ttcaaagcag cactcataca 3960 ttgtgtcaac gacagagctt tactaaggga ctcagaatta ctttccctct cactgattct 4020 cacgtcttct tccagtttgt cccagtcaaa tttgaaattc aagccttgcc tttgcatatg 4080 cctgtatttc cctgagtacg catttgcatt catttgcaac agaatcatct tcatgcaaga 4140 aaaccaatca ttctcagaaa agaactttct acaaaggttt tttgccatct catcgaggcc 4200 acactgatct ttaatgactg aggtgaaata caaaggtgac agctctgtgg aaccctcaac 4260

SEQ ID NO.	Description	Sequence
		agcctcacag ataaatttca tgtcatcatt ggttagacat gatgggtcaa agtcttctac 4320 taaatggaaa gatatttctg acaagataac ttttcttaag tgagccatct tccctgttag 4380 aataagctgt aaatgatgta gtccttttgt atttgtaagt ttttctccat ctccctttgtc 4440 attggccctc ctacctcttc tgtaccgtgc tattgtggtg ttgacctttt cttcgagact 4500 tttgaagaag cttgtctctt cttctccatc aaaacatatt tctgccaggt tgtcttccga 4560 tctccctgtc tcttctccct tggaaccgat gaccaatcta gagactaact tggaaacttt 4620 atattcatag tctgagtggc tcaacttata cttttgtttt cttacgaaac tctccgtaat 4680 ttgactcaca gcactaacaa gcaatttgtt aaagtcatat tccagaagtc gttctccatt 4740 tagatgctta ttaaccacca cacttttgtt actagcaaga tctaatgctg tcgcacatcc 4800 agagttagtc atgggatcta ggctgtttag cttcttctct cctttgaaaa ttaaagtgcc 4860 gttgttaaat gaagacacca ttaggctaaa ggcttccaga ttaacacctg gagttgtatg 4920 ctgacagtca atttctttac tagtgaatct cttcatttgc tcatagaaca cacattcttc 4980 ctcaggagtg attgcttcct tggggttgac aaaaaaacca aattgacttt tgggctcaaa 5040 gaacttttca aaacatttta tctgatctgt tagcctgtca ggggtctcct ttgtgatcaa 5100 atgacacagg tatgacacat tcaacataaa tttaaatttt gcactcaaca acaccttctc 5160 accagtacca aaaatagttt ttattaggaa tctaagcagc ttatacacca ccttctcagc 5220 aggtgtgatc agatcctccc tcaacttatc cattaatgat gtagatgaaa aatctgacac 5280 tattgccatc accaaatatc tgacactctg tacctgcttt tgatttctct ttgttgggtt 5340 ggtgagcatt agcaacaata gggtcctcag tgcaacctca atgtcggtga gacagtcttt 5400 caaatcagga catgatctaa tccatgaaat catgatgtct atcatattgt ataagacctc 5460 atctgaaaaa attggtaaaa agaacctttt aggatctgca tagaaggaaa ttaaatgacc 5520 atccgggcct tgtatggagt agcaccttga agattctcca gtcttctggt ataataggtg 5580 gtattcttca gagtccagtt ttattacttg gcaaaacact tctttgcatt ctaccacttg 5640 atatctcaca gaccctattt gattttgcct tagtctagca actgagctag ttttcatact 5700 gtttgttaag gccagacaaa cagatgataa tcttctcagg ctctgtatgt tcttcagctg 5760 ctctgtgctg ggttggaaat tgtaatcttc aaacttcgta taatacatta tcgggtgagc 5820 tccaattttc ataaagttct caaattcagt gaatggtatg tggcattctt gctcaaggtg 5880 ttcagacagt ccgtaatgct cgaaactcag tcccaccact aacaggcatt tttgaatttt 5940 tgcaatgaac tcactaatag atgccctaaa caattcctca aaagacacct ttctaaacac 6000 ctttgacttt tttctattcc tcaaaagtct aatgaactcc tctttagtgc tgtgaaagct 6060 taccagccta tcattcacac tactatagca

SEQ ID NO.	Description	Sequence
		acaacccacc cagtgtttat catttttttaa 6120 ccctttgaat ttcgactgtt ttatcaatga ggaaagacac aaaacatcca gatttaacaa 6180 ctgtctcctt ctagtattca acagtttcaa actcttgact ttgtttaaca tagagaggag 6240 cctctcatat tcagtgctag tctcacttcc cctttcgtgc ccatgggtct ctgcagttat 6300 gaatctcatc aaaggacagg attcgactgc ctccctgctt aatgttaaga tatcatcact 6360 atcagcaagg ttttcataga gctcagagaa ttccttgatc aagccttcag ggtttacttt 6420 ctgaaagttt ctctttaatt tcccactttc taaatctctt ctaaacctgc tgaaaagaga 6480 gtttattcca aaaaccacat catcacagct catgttgggg ttgatgcctt cgtggcacat 6540 cctcataatt tcatcattgt gagttgacct cgcatctttc agaattttca tagagtccat 6600 accggagcgc ttgtcgatag tagtcttcag ggactcacag agtctaaaat attcagactc 6660 ttcaaagact ttctcatttt ggttagaata ctccaaaagt ttgaataaaa ggtctctaaa 6720 tttgaagttt gcccactctg gcataaaact attatcataa tcacaacgac catctactat 6780 tggaactaat gtgacacccg caacagcaag gtcttccctg atgcatgcca atttgttagt 6840 gtcctctata aatttcttct caaaactggc tggagtgctc ctaacaaaac actcaagaag 6900 aatgagagaa ttgtctatca gcttgtaacc atcaggaatg ataagtggta gtcctgggca 6960 tacaattcca gactccacca aaattgtttc cacagactta tcgtcgtggt tgtgtgtgca 7020 gccactcttg tctgcactgt ctatttcaat gcagcgtgac agcaacttga gtccctcaat 7080 cagaaccatt ctgggttccc tttgtcccag aaagttgagt ttctgccttg acaacctctc 7140 atcctgttct atatagttta aacataactc tctcaattct gagatgattt catccattgc 7200 gcatcaaaaa gcctaggatc ctcggtgcg 7229
4	LCMV strain MP segment L, complete sequence. The genomic segment is RNA, the sequence in SEQ ID NO:4 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:4 for uridines ("U") provides the RNA sequence.	gcgcaccggg gatcctaggc atttttgttg cgcattttgt tgtgttattt gttgcacagc 60 ccttcatcgt gggaccttca caaacaaacc aaaccaccag ccatgggcca aggcaagtcc 120 aaagagggaa gggatgccag caatacgagc agagctgaaa ttctgccaga caccacctat 180 ctcggacctc tgaactgcaa gtcatgctgg cagagatttg acagtttagt cagatgccat 240 gaccactatc tctgcagaca ctgcctgaac ctcctgctgt cagtctccga caggtgccct 300 ctctgcaaac atccattgcc aaccaaactg aaaatatcca cggccccaag ctctccaccc 360 ccttacgagg agtgacgccc cgagccccaa caccgacaca aggaggccac caacacaacg 420 cccaacacgg aacacacaca cacacaccca cacacacatc cacacacacg cgcccccaca 480 acgggggcgc ccccccgggg gtggcccccc gggtgctcgg gcggagcccc acggagaggc 540 caattagtcg atctcctcga ccaccgactt ggtcagccag tcatcacagg acttgccctt 600 aagtctgtac ttgcccacaa ctgtttcata catcaccgtg ttctttgact tactgaaaca 660

SEQ ID NO.	Description	Sequence
		tagcctacag tctttgaaag tgaaccagtc aggcacaagt gacagcggta ccagtagaat 720 ggatctatct atacacaact cttggagaat tgtgctaatt tccgacccct gtagatgctc 780 accagttctg aatcgatgta gaagaaggct cccaaggacg tcatcaaaat ttccataacc 840 ctcgagctct gccaagaaaa ctctcatatc cttggtctcc agtttcacaa cgatgttctg 900 aacaaggctt cttccctcaa aaagagcacc cattctcaca gtcaagggca caggctccca 960 ttcaggccca atcctctcaa aatcaaggga tctgatcccg tccagtattt tccttgagcc 1020 tatcagctca agctcaagag agtcaccgag tatcaggggg tcctccatat agtcctcaaa 1080 ctcttcagac ctaatgtcaa aaacaccatc gttcaccttg aagatagagt ctgatctcaa 1140 caggtggagg cattcgtcca agaaccttct gtccacctca cctttaaaga ggtgagagca 1200 tgataggaac tcagctacac ctggaccttg taactggcac ttcactaaaa agatcaatga 1260 aaacttcctc aaacaatcag tgttattctg gttgtgagtg aaatctactg taattgagaa 1320 ctctagcact ccctctgtat tatttatcat gtaatcccac aagtttctca aagacttgaa 1380 tgcctttgga tttgtcaagc cttgtttgat tagcatggca gcattgcaca caatatctcc 1440 caatcggtaa gagaaccatc caaatccaaa ttgcaagtca ttcctaaaca tgggcctctc 1500 catatttttg ttcactactt ttaagatgaa tgattggaaa ggccccaatg cttcagcgcc 1560 atcttcagat ggcatcatgt ctttatgagg gaaccatgaa aaacttccta gagttctgct 1620 tgttgctaca aattctcgta caaatgactc aaaatacact tgttttaaaa agtttttgca 1680 gacatccctt gtactaacga caaattcatc aacaaggctt gagtcagagc gctgatggga 1740 atttacaaga tcagaaaata gaacagtgta gtgttcgtcc ctcttccact taactacatg 1800 agaaatgagc gataaagatt ctgaattgat atcgatcaat acgcaaaggt caaggaattt 1860 gattctggga ctccatctca tgttttttga gctcatatca gacatgaagg gaagcagctg 1920 atcttcatag attttagggt acaatcgcct cacagattgg attacatggt ttaaacttat 1980 cttgtcctcc agtagccttg aactctcagg cttccttgct acataatcac atgggttcaa 2040 gtgcttgagg cttgagcttc cctcattctt ccctttcaca ggttcagcta agacccaaac 2100 acccaactca aaggaattac tcagtgagat gcaaatatag tcccaaagga ggggcctcaa 2160 gagactgatg tggtcgcagt gagcttctgg atgactttgc ctgtcacaaa tgtacaacat 2220 tatgccatca tgtctgtgga ttgctgtcac atgcgcatcc atagctagat cctcaagcac 2280 ttttctaatg tatagattgt ccctattttt atttctcaca catctacttc ccaaagtttt 2340 gcaaagacct ataaagcctg atgagatgca actttgaaag gctgacttat tgattgcttc 2400 tgacagcaac ttctgtgcac ctcttgtgaa cttactgcag agcttgttct ggagtgtctt 2460 gattaatgat gggattcttt cctcttggaa

SEQ ID NO.	Description	Sequence
		agtcattact gatggataaa ccactttctg 2520 cctcaagacc attcttaatg ggaacaactc attcaaattc agccaattta tgtttgccaa 2580 ttgacttaga tcctcttcga ggccaaggat gtttcccaac tgaagaatgg cttccttttt 2640 atccctattg aagaggtcta agaagaattc ttcattgaac tcaccattct tgagcttatg 2700 atgtagtctc cttacaagcc ttctcatgac cttcgtttca ctaggacaca attcttcaat 2760 aagcctttgg attctgtaac ctctagagcc atccaaccaa tccttgacat cagtattagt 2820 gttaagcaaa aatgggtcca agggaaagtt ggcatatttt aagaggtcta atgttctctt 2880 ctggatgcag tttaccaatg aaactggaac accatttgca acagcttgat cggcaattgt 2940 atctattgtt tcacagagtt ggtgtggctc tttacactta acgttgtgta atgctgctga 3000 cacaaatttt gttaaaagtg ggacctcttc cccccacaca taaaatctgg atttaaattc 3060 tgcagcaaat cgccccacca cacttttcgg actgatgaac ttgttaagca agccactcaa 3120 atgagaatga aattccagca atacaaggac ttcctcaggg tcactatcaa ccagttcact 3180 caatctccta tcaaataagg tgatctgatc atcacttgat gtgtaagatt ctggtctctc 3240 accaaaaatg acaccgatac aataattaat gaatctctca ctgattaagc cgtaaaagtc 3300 agaggcatta tgtaagattc cctgtcccat gtcaatgaga ctgcttatat gggaaggcac 3360 tattcctaat tcaaaatatt ctcgaaagat tctttcagtc acagttgtct ctgaacccct 3420 aagaagtttc agctttgatt tgatatatga tttcatcatt gcattcacaa caggaaaagg 3480 gacctcaaca agtttgtgca tgtgccaagt taataaggtg ctgatatgat cctttccgga 3540 acgcacatac tggtcatcac ccagtttgag attttgaagg agcattaaaa acaaaaatgg 3600 gcacatcatt ggcccccatt tgctatgatc catactgtag ttcaacaacc cctctcgcac 3660 attgatggtc attgatagaa ttgcattttc aaattctttg tcattgttta agcatgaacc 3720 tgagaagaag ctagaaaaag actcaaaata atcctctatc aatcttgtaa acatttttgt 3780 tctcaaatcc ccaatataaa gttctctgtt tcctccaacc tgctctttgt atgataacgc 3840 aaacttcaac cttccggaat caggaccaac tgaagtgtat gacgttggtg actcctctga 3900 gtaaaaacat aaattcttta aagcagcact catgcatttt gtcaatgata gagccttact 3960 tagagactca gaattacttt ccctttcact aattctaaca tcttcttcta gtttgtccca 4020 gtcaaacttg aaattcagac cttgtctttg catgtgcctg tatttccctg agtatgcatt 4080 tgcattcatt tgcagtagaa tcattttcat acacgaaaac caatcaccct ctgaaaaaaa 4140 cttcctgcag aggtttttttg ccatttcatc cagaccacat tgttctttga cagctgaagt 4200 gaaatacaat ggtgacagtt ctgtagaagt ttcaatagcc tcacagataa atttcatgtc 4260 atcattggtg agacaagatg ggtcaaaatc ttccacaaga tgaaaagaaa tttctgataa 4320

SEQ ID NO.	Description	Sequence
		gatgaccttc cttaaatatg ccattttacc tgacaatata gtctgaaggt gatgcaatcc 4380 ttttgtattt tcaaacccca cctcattttc cccttcattg gtcttcttgc ttctttcata 4440 ccgctttatt gtggagttga ccttatcttc taaattcttg aagaaacttg tctcttcttc 4500 cccatcaaag catatgtctg ctgagtcacc ttctagtttc ccagcttctg tttctttaga 4560 gccgataacc aatctagaga ccaactttga aaccttgtac tcgtaatctg agtggttcaa 4620 tttgtacttc tgctttctca tgaagctctc tgtgatctga ctcacagcac taacaagcaa 4680 tttgttaaaa tcatactcta ggagccgttc cccatttaaa tgtttgttaa caaccacact 4740 tttgttgctg gcaaggtcta atgctgttgc acacccagag ttagtcatgg gatccaagct 4800 attgagcctc ttctcccctt tgaaaatcaa agtgccattg ttgaatgagg acaccatcat 4860 gctaaaggcc tccagattga cacctggggt tgtgcgctga cagtcaactt ctttcccagt 4920 gaacttcttc atttggtcat aaaaaacaca ctcttcctca ggggtgattg actctttagg 4980 gttaacaaag aagccaaact cacttttagg ctcaaagaat ttctcaaagc atttaatttg 5040 atctgtcagc ctatcagggg tttcctttgt gattaaatga cacaggtatg acacattcaa 5100 catgaacttg aactttgcgc tcaacagtac cttttcacca gtcccaaaaa cagttttgat 5160 caaaaatctg agcaatttgt acactacttt ctcagcaggt gtgatcaaat cctccttcaa 5220 cttgtccatc aatgatgtgg atgagaagtc tgagacaatg gccatcacta aatacctaat 5280 gttttgaacc tgtttttgat tcctctttgt tgggttggtg agcatgagta ataatagggt 5340 tctcaatgca atctcaacat catcaatgct gtccttcaag tcaggacatg atctgatcca 5400 tgagatcatg gtgtcaatca tgttgtgcaa cacttcatct gagaagattg gtaaaaagaa 5460 cctttttggg tctgcataaa aagagattag atggccattg ggaccttgta tagaataaca 5520 ccttgaggat tctccagtct tttgatacag caggtgatat tcctcagagt ccaattttat 5580 cacttggcaa aatacctctt tacattccac cacttgatac cttacagagc ccaattggtt 5640 ttgtcttaat ctagcaactg aacttgtttt catactgttt gtcaaagcta gacagacaga 5700 tgacaatctt ttcaaactat gcatgttcct taattgttcc gtattaggct ggaaatcata 5760 atcttcaaac tttgtataat acattatagg atgagttccg gacctcatga aattctcaaa 5820 ctcaataaat ggtatgtggc actcatgctc aagatgttca gacagaccat agtgcccaaa 5880 actaagtccc accactgaca agcacctttg aacttttaaa atgaactcat ttatggatgt 5940 tctaaacaaa tcctcaagag atacctttct atacgccttt gactttctcc tgttccttag 6000 aagtctgatg aactcttcct tggtgctatg aaagctcacc aacctatcat tcacactccc 6060 atagcaacaa ccaacccagt gcttatcatt ttttgaccct ttgagtttag actgtttgat 6120 caacgaagag agacacaaga catccaaatt

SEQ ID NO.	Description	Sequence
		cagtaactgt ctccttctgg tgttcaataa 6180 ttttaaactt ttaactttgt tcaacataga gaggagcctc tcatactcag tgctagtctc 6240 acttcctctc tcataaccat gggtatctgc tgtgataaat ctcatcaaag gacaggattc 6300 aactgcctcc ttgcttagtg ctgaaatgtc atcactgtca gcaagagtct cataaagctc 6360 agagaattcc ttaattaaat ttccggggtt gattttctga aaactcctct tgagcttccc 6420 agtttccaag tctcttctaa acctgctgta aagggagttt atgccaagaa ccacatcatc 6480 gcagttcatg tttgggttga caccatcatg gcacattttc ataatttcat cattgtgaaa 6540 tgatcttgca tctttcaaga ttttcataga gtctataccg gaacgcttat caacagtggt 6600 cttgagagat tcgcaaagtc tgaagtactc agattcctca aagactttct catcttggct 6660 agaatactct aaaagtttaa acagaaggtc tctgaacttg aaattcaccc actctggcat 6720 aaagctgtta tcataatcac accgaccatc cactattggg accaatgtga tacccgcaat 6780 ggcaaggtct tctttgatac aggctagttt attggtgtcc tctataaatt tcttctcaaa 6840 actagctggt gtgcttctaa cgaagcactc aagaagaatg agggaattgt caatcagttt 6900 ataaccatca ggaatgatca aaggcagtcc cgggcacaca atcccagact ctattagaat 6960 tgcctcaaca gatttatcat catggttgtg tatgcagccg ctcttgtcag cactgtctat 7020 ctctatacaa cgcgacaaaa gtttgagtcc ctctatcaat accattctgg gttctctttg 7080 ccctaaaaag ttgagcttct gccttgacaa cctctcatct tgttctatgt ggtttaagca 7140 caactctctc aactccgaaa tagcctcatc cattgcgcat caaaaagcct aggatcctcg 7200 gtgcg 7205
5	LCMV strain MP segment S, complete sequence. The genomic segment is RNA, the sequence in SEQ ID NO:5 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO:5 for uridines ("U") provides the RNA sequence.	cgcaccgggg atcctaggct ttttggattg cgctttcctc agctccgtct tgtgggagaa 60 tgggtcaaat tgtgacgatg tttgaggctc tgcctcacat cattgatgag gtcattaaca 120 ttgtcattat cgtgcttatt atcatcacga gcatcaaagc tgtgtacaat ttcgccacct 180 gcgggatact tgcattgatc agctttcttt ttctggctgg caggtcctgt ggaatgtatg 240 gtcttgatgg gcctgacatt tacaaagggg tttaccgatt caagtcagtg gagtttgaca 300 tgtcttacct taacctgacg atgcccaatg catgttcggc aaacaactcc catcattata 360 taagtatggg gacttctgga ttggagttaa ccttcacaaa tgactccatc atcacccaca 420 acttttgtaa tctgacttcc gccctcaaca agaggacttt tgaccacaca cttatgagta 480 tagtctcaag tctgcacctc agcattagag gggtccccag ctacaaagca gtgtcctgtg 540 attttaacaa tggcatcact attcaataca acctgtcatt ttctaatgca cagagcgctc 600 tgagtcaatg taagaccttc agggggagag tcctggatat gttcagaact gcttttggag 660 gaaagtacat gaggagtggc tggggctgga caggttcaga tggcaagact acttggtgca 720 gccagacaaa ctaccaatat ctgattatac

SEQ ID NO.	Description	Sequence
		aaaacaggac ttgggaaaac cactgcaggt 780 acgcaggccc tttcggaatg tctagaattc tcttcgctca agaaaagaca aggtttctaa 840 ctagaaggct tgcaggcaca ttcacttgga ctttatcaga ctcatcagga gtggagaatc 900 caggtggtta ctgcttgacc aagtggatga tcctcgctgc agagctcaag tgttttggga 960 acacagctgt tgcaaagtgc aatgtaaatc atgatgaaga gttctgtgat atgctacgac 1020 tgattgatta caacaaggct gctttgagta aattcaaaga agatgtagaa tccgctctac 1080 atctgttcaa gacaacagtg aattctttga tttctgatca gcttttgatg agaaatcacc 1140 taagagactt gatgggagtg ccatactgca attactcgaa attctggtat ctagagcatg 1200 caaagactgg tgagactagt gtccccaagt gctggcttgt cagcaatggt tcttatttga 1260 atgaaaccca tttcagcgac caaattgagc aggaagcaga taatatgatc acagaaatgc 1320 tgagaaagga ctacataaaa aggcaaggga gtacccctct agccttgatg gatctattga 1380 tgttttctac atcagcatat ttgatcagca tctttctgca tcttgtgagg ataccaacac 1440 acagacacat aaagggcggc tcatgcccaa aaccacatcg gttaaccagc aagggaatct 1500 gtagttgtgg tgcatttaaa gtaccaggtg tggaaaccac ctggaaaaga cgctgaacag 1560 cagcgcctcc ctgactcacc acctcgaaag aggtggtgag tcagggaggc ccagagggtc 1620 ttagagtgtt acgacatttg gacctctgaa gattaggtca tgtggtagga tattgtggac 1680 agttttcagg tcggggagcc ttgccttgga ggcgctttca aagatgatac agtccatgag 1740 tgcacagtgt ggggtgacct ctttcttttt cttgtccctc actattccag tgtgcatctt 1800 gcatagccag ccatatttgt cccagacttt gtcctcatat tctcttgaag cttctttagt 1860 catctcaaca tcgatgagct taatgtctct tctgttttgt gaatctagga gtttcctgat 1920 gtcatcagat ccctgacaac ttaggaccat tccctgtgga agagcaccta ttactgaaga 1980 tgtcagccca ggttgtgcat tgaagaggtc agcaaggtcc atgccatgtg agtatttgga 2040 gtcctgcttg aattgttttt gatcagtggg ttctctatag aaatgtatgt actgcccatt 2100 ctgtggctga aatattgcta tttctaccgg gtcattaaat ctgccctcaa tgtcaatcca 2160 tgtaggagcg ttagggtcaa tacctcccat gaggtccttc agcaacattg tttggctgta 2220 gcttaagccc acctgaggtg ggcccgctgc cccaggcgct ggtttgggtg agttggccat 2280 aggcctctca tttgtcagat caattgttgt gttctcccat gctctcccta caactgatgt 2340 tctacaagct atgtatggcc acccctcccc tgaaagacag actttgtaga ggatgttctc 2400 gtaaggattc ctgtctccaa cctgatcaga aacaaacatg ttgagtttct tcttggcccc 2460 aagaactgct ttcaggagat cctcactgtt gcttggctta attaagatgg attccaacat 2520 gttacccccca tctaacaagg ctgcccctgc tttcacagca gcaccgagac tgaaattgta 2580

SEQ ID NO.	Description	Sequence
		gccagatatg ttgatgctag actgctgctc agtgatgact cccaagactg ggtgcttgtc 2640 tttcagcctt tcaaggtcac ttaggttcgg gtacttgact gtgtaaagca gcccaaggtc 2700 tgtgagtgct tgcacaacgt cattgagtga ggtttgtgat tgtttggcca tacaagccat 2760 tgttaagctt ggcattgtgc cgaattgatt gttcagaagt gatgagtcct tcacatccca 2820 gaccctcacc acaccatttg cactctgctg aggtctcctc attccaacca tttgcagaat 2880 ctgagatctt tggtcaagct gttgtgctgt taagttcccc atgtagactc cagaagttag 2940 aggcctttca gacctcatga ttttagcctt cagtttttca aggtcagctg caagggacat 3000 cagttcttct gcactaagcc tccctacttt tagaacattc ttttttgatg ttgactttag 3060 gtccacaagg gaatacacag tttggttgag gcttctgagt ctctgtaaat ctttgtcatc 3120 cctcttctct ttcctcatga tcctctgaac attgctcacc tcagagaagt ctaatccatt 3180 cagaaggctg gtggcatcct tgatcacagc agctttcaca tctgatgtga agccttgaag 3240 ctctctcctc aatgcctggg tccattgaaa gcttttaact tctttggaca gagacatttt 3300 gtcactcagt ggatttccaa gtcaaatgcg caatcaaaat gcctaggatc cactgtgcg 3359
6	Amino acid sequence of the NP protein of the MP strain of LCMV.	Met Ser Leu Ser Lys Glu Val Lys Ser Phe Gln Trp Thr Gln Ala Leu Arg Arg Glu Leu Gln Gly Phe Thr Ser Asp Val Lys Ala Ala Val Ile Lys Asp Ala Thr Ser Leu Leu Asn Gly Leu Asp Phe Ser Glu Val Ser Asn Val Gln Arg Ile Met Arg Lys Glu Lys Arg Asp Asp Lys Asp Leu Gln Arg Leu Arg Ser Leu Asn Gln Thr Val Tyr Ser Leu Val Asp Leu Lys Ser Thr Ser Lys Lys Asn Val Leu Lys Val Gly Arg Leu Ser Ala Glu Glu Leu Met Ser Leu Ala Ala Asp Leu Glu Lys Leu Lys Ala Lys Ile Met Arg Ser Glu Arg Pro Leu Thr Ser Gly Val Tyr Met Gly Asn Leu Thr Ala Gln Gln Leu Asp Gln Arg Ser Gln Ile Leu Gln Met Val Gly Met Arg Arg Pro Gln Gln Ser Ala Asn Gly Val Val Arg Val Trp Asp Val Lys Asp Ser Ser Leu Leu Asn Asn Gln Phe Gly Thr Met Pro Ser Leu Thr Met Ala Cys Met Ala Lys Gln Ser Gln Thr Ser Leu Asn Asp Val Val Gln Ala Leu Thr Asp Leu Gly Leu Leu Tyr Thr Val Lys Tyr Pro Asn Leu Ser Asp Leu Glu Arg Leu Lys Asp Lys His Pro Val Leu Gly Val Ile Thr Glu Gln Gln Ser Ser Ile Asn Ile Ser Gly Tyr Asn Phe Ser Leu Gly Ala Ala Val Lys Ala Gly Ala Ala Leu Leu Asp Gly Gly Asn Met Leu Glu Ser Ile Leu Ile Lys Pro Ser Asn Ser Glu Asp Leu Leu Lys Ala Val Leu Gly Ala Lys Lys Lys Leu Asn Met Phe Asp Arg Asn Pro Tyr Glu Asn Ile Leu Tyr Lys Val Cys Leu Ser Gly Glu Gly Trp Pro Tyr Ile Ala Cys Arg Thr Ser Val Val Gly Arg Ala Trp Glu Asn Thr Thr Ile Asp Leu Thr Asn Glu Arg Pro Met Ala Asn Ser Pro Lys Pro Ala Pro Gly Ala Ala Gly Pro Pro Gln Val

SEQ ID NO.	Description	Sequence
		Gly Leu Ser Tyr Ser Gln Thr Met Leu Leu Lys Asp Leu Met Gly Gly Ile Asp Pro Asn Ala Pro Thr Trp Ile Asp Ile Glu Gly Arg Phe Asn Asp Pro Val Glu Ile Ala Ile Phe Gln Pro Gln Asn Gly Gln Tyr Ile His Phe Tyr Arg Glu Pro Thr Asp Gln Lys Gln Phe Lys Gln Asp Ser Lys Tyr Ser His Gly Met Asp Leu Ala Asp Leu Phe Asn Ala Gln Pro Gly Leu Thr Ser Ser Val Ile Gly Ala Leu Pro Gln Gly Met Val Leu Ser Cys Gln Gly Ser Asp Asp Ile Arg Lys Leu Leu Asp Ser Gln Asn Arg Arg Asp Ile Lys Leu Ile Asp Val Glu Met Thr Lys Glu Ala Ser Arg Glu Tyr Glu Asp Lys Val Trp Asp Lys Tyr Gly Trp Leu Cys Lys Met His Thr Gly Ile Val Arg Asp Lys Lys Lys Lys Glu Val Thr Pro His Cys Ala Leu Met Asp Cys Ile Ile Phe Glu Ser Ala Ser Lys Ala Arg Leu Pro Asp Leu Lys Thr Val His Asn Ile Leu Pro His Asp Leu Ile Phe Arg Gly Pro Asn Val Val Thr Leu
7	Amino acid sequence of the GP protein of the MP strain of LCMV.	Met Gly Gln Ile Val Thr Met Phe Glu Ala Leu Pro His Ile Ile Asp Glu Val Ile Asn Ile Val Ile Ile Val Leu Ile Ile Ile Thr Ser Ile Lys Ala Val Tyr Asn Phe Ala Thr Cys Gly Ile Leu Ala Leu Ile Ser Phe Leu Phe Leu Ala Gly Arg Ser Cys Gly Met Tyr Gly Leu Asp Gly Pro Asp Ile Tyr Lys Gly Val Tyr Arg Phe Lys Ser Val Glu Phe Asp Met Ser Tyr Leu Asn Leu Thr Met Pro Asn Ala Cys Ser Ala Asn Asn Ser His His Tyr Ile Ser Met Gly Thr Ser Gly Leu Glu Leu Thr Phe Thr Asn Asp Ser Ile Ile Thr His Asn Phe Cys Asn Leu Thr Ser Ala Leu Asn Lys Arg Thr Phe Asp His Thr Leu Met Ser Ile Val Ser Ser Leu His Leu Ser Ile Arg Gly Val Pro Ser Tyr Lys Ala Val Ser Cys Asp Phe Asn Asn Gly Ile Thr Ile Gln Tyr Asn Leu Ser Phe Ser Asn Ala Gln Ser Ala Leu Ser Gln Cys Lys Thr Phe Arg Gly Arg Val Leu Asp Met Phe Arg Thr Ala Phe Gly Gly Lys Tyr Met Arg Ser Gly Trp Gly Trp Thr Gly Ser Asp Gly Lys Thr Thr Trp Cys Ser Gln Thr Asn Tyr Gln Tyr Leu Ile Ile Gln Asn Arg Thr Trp Glu Asn His Cys Arg Tyr Ala Gly Pro Phe Gly Met Ser Arg Ile Leu Phe Ala Gln Glu Lys Thr Arg Phe Leu Thr Arg Arg Leu Ala Gly Thr Phe Thr Trp Thr Leu Ser Asp Ser Ser Gly Val Glu Asn Pro Gly Gly Tyr Cys Leu Thr Lys Trp Met Ile Leu Ala Ala Glu Leu Lys Cys Phe Gly Asn Thr Ala Val Ala Lys Cys Asn Val Asn His Asp Glu Glu Phe Cys Asp Met Leu Arg Leu Ile Asp Tyr Asn Lys Ala Ala Leu Ser Lys Phe Lys Glu Asp Val Glu Ser Ala Leu His Leu Phe Lys Thr Thr Val Asn Ser Leu Ile Ser Asp Gln Leu Leu Met Arg Asn His Leu Arg Asp Leu Met Gly Val Pro Tyr Cys Asn Tyr Ser Lys Phe Trp Tyr Leu Glu His Ala Lys Thr Gly Glu Thr Ser Val Pro Lys

SEQ ID NO.	Description	Sequence
		Cys Trp Leu Val Ser Asn Gly Ser Tyr Leu Asn Glu Thr His Phe Ser Asp Gln Ile Glu Gln Glu Ala Asp Asn Met Ile Thr Glu Met Leu Arg Lys Asp Tyr Ile Lys Arg Gln Gly Ser Thr Pro Leu Ala Leu Met Asp Leu Leu Met Phe Ser Thr Ser Ala Tyr Leu Ile Ser Ile Phe Leu His Leu Val Arg Ile Pro Thr His Arg His Ile Lys Gly Gly Ser Cys Pro Lys Pro His Arg Leu Thr Ser Lys Gly Ile Cys Ser Cys Gly Ala Phe Lys Val Pro Gly Val Glu Thr Thr Trp Lys Arg Arg
8	Amino acid sequence of the L protein of the MP strain of LCMV.	Met Asp Glu Ala Ile Ser Glu Leu Arg Glu Leu Cys Leu Asn His Ile Glu Gln Asp Glu Arg Leu Ser Arg Gln Lys Leu Asn Phe Leu Gly Gln Arg Glu Pro Arg Met Val Leu Ile Glu Gly Leu Lys Leu Leu Ser Arg Cys Ile Glu Ile Asp Ser Ala Asp Lys Ser Gly Cys Ile His Asn His Asp Asp Lys Ser Val Glu Ala Ile Leu Ile Glu Ser Gly Ile Val Cys Pro Gly Leu Pro Leu Ile Ile Pro Asp Gly Tyr Lys Leu Ile Asp Asn Ser Leu Ile Leu Leu Glu Cys Phe Val Arg Ser Thr Pro Ala Ser Phe Glu Lys Lys Phe Ile Glu Asp Thr Asn Lys Leu Ala Cys Ile Lys Glu Asp Leu Ala Ile Ala Gly Ile Thr Leu Val Pro Ile Val Asp Gly Arg Cys Asp Tyr Asp Asn Ser Phe Met Pro Glu Trp Val Asn Phe Lys Phe Arg Asp Leu Leu Phe Lys Leu Leu Glu Tyr Ser Ser Gln Asp Glu Lys Val Phe Glu Glu Ser Glu Tyr Phe Arg Leu Cys Glu Ser Leu Lys Thr Thr Val Asp Lys Arg Ser Gly Ile Asp Ser Met Lys Ile Leu Lys Asp Ala Arg Ser Phe His Asn Asp Glu Ile Met Lys Met Cys His Asp Gly Val Asn Pro Asn Met Asn Cys Asp Asp Val Val Leu Gly Ile Asn Ser Leu Tyr Ser Arg Phe Arg Arg Asp Leu Glu Thr Gly Lys Leu Lys Arg Ser Phe Gln Lys Ile Asn Pro Gly Asn Leu Ile Lys Glu Phe Ser Glu Leu Tyr Glu Thr Leu Ala Asp Ser Asp Asp Ile Ser Ala Leu Ser Lys Glu Ala Val Glu Ser Cys Pro Leu Met Arg Phe Ile Thr Ala Asp Thr His Gly Tyr Glu Arg Gly Ser Glu Thr Ser Thr Glu Tyr Glu Arg Leu Leu Ser Met Leu Asn Lys Val Lys Ser Leu Lys Leu Leu Asn Thr Arg Arg Arg Gln Leu Leu Asn Leu Asp Val Leu Cys Leu Ser Ser Leu Ile Lys Gln Ser Lys Leu Lys Gly Ser Lys Asn Asp Lys His Trp Val Gly Cys Cys Tyr Gly Ser Val Asn Asp Arg Leu Val Ser Phe His Ser Thr Lys Glu Glu Phe Ile Arg Leu Leu Arg Asn Arg Arg Lys Ser Lys Ala Tyr Arg Lys Val Ser Leu Glu Asp Leu Phe Arg Thr Ser Ile Asn Glu Phe Ile Leu Lys Val Gln Arg Cys Leu Ser Val Val Gly Leu Ser Phe Gly His Tyr Gly Leu Ser Glu His Leu Glu His Glu Cys His Ile Pro Phe Ile Glu Phe Glu Asn Phe Met Arg Ser Gly Thr His Pro Ile Met Tyr Tyr Thr Lys Phe Glu Asp Tyr Asp Phe Gln Pro Asn Thr Glu Gln Leu Arg Asn Met His Ser Leu Lys Arg Leu

SEQ ID NO.	Description	Sequence
		Ser Ser Val Cys Leu Ala Leu Thr Asn Ser Met Lys Thr Ser Ser Val Ala Arg Leu Arg Gln Asn Gln Leu Gly Ser Val Arg Tyr Gln Val Val Glu Cys Lys Glu Val Phe Cys Gln Val Ile Lys Leu Asp Ser Glu Glu Tyr His Leu Leu Tyr Gln Lys Thr Gly Glu Ser Ser Arg Cys Tyr Ser Ile Gln Gly Pro Asn Gly His Leu Ile Ser Phe Tyr Ala Asp Pro Lys Arg Phe Phe Leu Pro Ile Phe Ser Asp Glu Val Leu His Asn Met Ile Asp Thr Met Ile Ser Trp Ile Arg Ser Cys Pro Asp Leu Lys Asp Ser Ile Asp Asp Val Glu Ile Ala Leu Arg Thr Leu Leu Leu Leu Met Leu Thr Asn Pro Thr Lys Arg Asn Gln Lys Gln Val Gln Asn Ile Arg Tyr Leu Val Met Ala Ile Val Ser Asp Phe Ser Ser Thr Ser Leu Met Asp Lys Leu Lys Glu Asp Leu Ile Thr Pro Ala Glu Lys Val Val Tyr Lys Leu Leu Arg Phe Leu Ile Lys Thr Val Phe Gly Thr Gly Glu Lys Val Leu Leu Ser Ala Lys Phe Lys Phe Met Leu Asn Val Ser Tyr Leu Cys His Leu Ile Thr Lys Glu Thr Pro Asp Arg Leu Thr Asp Gln Ile Lys Cys Phe Glu Lys Phe Phe Glu Pro Lys Ser Glu Phe Gly Phe Phe Val Asn Pro Lys Glu Ser Ile Thr Pro Glu Glu Glu Cys Val Phe Tyr Asp Gln Met Lys Lys Phe Thr Gly Lys Glu Val Asp Cys Gln Arg Thr Thr Pro Gly Val Asn Leu Glu Met Met Val Ser Ser Phe Asn Asn Gly Thr Leu Ile Phe Lys Arg Leu Asn Ser Leu Asp Pro Met Thr Asn Ser Gly Cys Ala Thr Ala Leu Asp Leu Ala Ser Asn Lys Ser Val Val Val Asn Lys His Leu Asn Gly Glu Arg Leu Leu Glu Tyr Asp Phe Asn Lys Leu Leu Val Ser Ala Val Ser Gln Ile Thr Glu Ser Phe Met Arg Lys Gln Lys Tyr Lys Leu Asn His Ser Asp Tyr Glu Tyr Lys Val Ser Lys Leu Val Ser Arg Leu Val Ile Gly Ser Lys Glu Thr Glu Ala Gly Lys Leu Glu Gly Asp Ser Ala Asp Ile Cys Phe Asp Gly Glu Glu Glu Thr Ser Phe Phe Lys Asn Leu Glu Asp Lys Val Asn Ser Thr Ile Lys Arg Tyr Glu Arg Ser Lys Lys Thr Asn Glu Gly Glu Asn Glu Val Gly Phe Glu Asn Thr Lys Gly Leu His His Leu Gln Thr Ile Leu Ser Gly Lys Met Ala Tyr Leu Arg Lys Val Ile Leu Ser Glu Ile Ser Phe His Leu Val Glu Asp Phe Asp Pro Ser Cys Leu Thr Asn Asp Asp Met Lys Phe Ile Cys Glu Ala Ile Glu Thr Ser Thr Glu Leu Ser Pro Leu Tyr Phe Thr Ser Ala Val Lys Glu Gln Cys Gly Leu Asp Glu Met Ala Lys Asn Leu Cys Arg Lys Phe Phe Ser Glu Gly Asp Trp Phe Ser Cys Met Lys Met Ile Leu Leu Gln Met Asn Ala Asn Ala Tyr Ser Gly Lys Tyr Arg His Met Gln Arg Gln Gly Leu Asn Phe Lys Phe Asp Trp Asp Lys Leu Glu Glu Asp Val Arg Ile Ser Glu Arg Glu Ser Asn Ser Glu Ser Leu Ser Lys Ala Leu Ser Leu Thr Lys Cys Met Ser Ala Ala Leu Lys Asn Leu Cys Phe Tyr Ser Glu Glu Ser Pro Thr Ser Tyr Thr Ser Val Gly Pro Asp Ser

SEQ ID NO.	Description	Sequence
		Gly Arg Leu Lys Phe Ala Leu Ser Tyr Lys Glu Gln Val Gly Gly Asn Arg Glu Leu Tyr Ile Gly Asp Leu Arg Thr Lys Met Phe Thr Arg Leu Ile Glu Asp Tyr Phe Glu Ser Phe Ser Ser Phe Phe Ser Gly Ser Cys Leu Asn Asn Asp Lys Glu Phe Glu Asn Ala Ile Leu Ser Met Thr Ile Asn Val Arg Glu Gly Leu Leu Asn Tyr Ser Met Asp His Ser Lys Trp Gly Pro Met Met Cys Pro Phe Leu Phe Leu Met Leu Leu Gln Asn Leu Lys Asp Asp Gln Tyr Val Arg Ser Gly Lys Asp His Ile Ser Thr Leu Leu Thr Trp His Met His Lys Leu Val Glu Val Pro Phe Pro Val Val Asn Ala Met Met Lys Ser Tyr Ile Lys Ser Lys Leu Lys Leu Leu Arg Gly Ser Glu Thr Thr Val Thr Glu Arg Ile Phe Arg Glu Tyr Phe Glu Leu Gly Ile Val Pro Ser His Ile Ser Ser Leu Ile Asp Met Gly Gln Gly Ile Leu His Asn Ala Ser Asp Phe Tyr Gly Leu Ile Ser Glu Arg Phe Ile Asn Tyr Cys Ile Gly Val Ile Phe Gly Glu Arg Pro Glu Ser Tyr Thr Ser Ser Asp Asp Gln Ile Thr Leu Phe Asp Arg Arg Leu Ser Glu Leu Val Asp Ser Asp Pro Glu Glu Val Leu Val Leu Leu Glu Phe His Ser His Leu Ser Gly Leu Leu Asn Lys Phe Ile Ser Pro Lys Ser Val Val Gly Arg Phe Ala Ala Glu Phe Lys Ser Arg Phe Tyr Val Trp Gly Glu Glu Val Pro Leu Leu Thr Lys Phe Val Ser Ala Ala Leu His Asn Val Lys Cys Lys Glu Pro His Gln Leu Cys Glu Thr Ile Asp Thr Ile Ala Asp Gln Ala Val Ala Asn Gly Val Pro Val Ser Leu Val Asn Cys Ile Gln Lys Arg Thr Leu Asp Leu Leu Lys Tyr Ala Asn Phe Pro Leu Asp Pro Phe Leu Leu Asn Thr Asn Thr Asp Val Lys Asp Trp Leu Asp Gly Ser Arg Gly Tyr Arg Ile Gln Arg Leu Ile Glu Glu Leu Cys Pro Ser Glu Thr Lys Val Met Arg Arg Leu Val Arg Arg Leu His His Lys Leu Lys Asn Gly Glu Phe Asn Glu Glu Phe Phe Leu Asp Leu Phe Asn Arg Asp Lys Lys Glu Ala Ile Leu Gln Leu Gly Asn Ile Leu Gly Leu Glu Glu Asp Leu Ser Gln Leu Ala Asn Ile Asn Trp Leu Asn Leu Asn Glu Leu Phe Pro Leu Arg Met Val Leu Arg Gln Lys Val Val Tyr Pro Ser Val Met Thr Phe Gln Glu Glu Arg Ile Pro Ser Leu Ile Lys Thr Leu Gln Asn Lys Leu Cys Ser Lys Phe Thr Arg Gly Ala Gln Lys Leu Leu Ser Glu Ala Ile Asn Lys Ser Ala Phe Gln Ser Cys Ile Ser Ser Gly Phe Ile Gly Leu Cys Lys Thr Leu Gly Ser Arg Cys Val Arg Asn Lys Asn Arg Asp Asn Leu Tyr Ile Arg Lys Val Leu Glu Asp Leu Ala Met Asp Ala His Val Thr Ala Ile His Arg His Asp Gly Ile Met Leu Tyr Ile Cys Asp Arg Gln Ser His Pro Glu Ala His Cys Asp His Ile Ser Leu Leu Arg Pro Leu Leu Trp Asp Tyr Ile Cys Ile Ser Leu Ser Asn Ser Phe Glu Leu Gly Val Trp Val Leu Ala Glu Pro Val Lys Gly Lys Asn Glu Gly Ser Ser Ser Leu Lys His Leu Asn Pro Cys Asp Tyr Val

SEQ ID NO.	Description	Sequence
		Ala Arg Lys Pro Glu Ser Ser Arg Leu Leu Glu Asp Lys Ile Ser Leu Asn His Val Ile Gln Ser Val Arg Arg Leu Tyr Pro Lys Ile Tyr Glu Asp Gln Leu Leu Pro Phe Met Ser Asp Met Ser Ser Lys Asn Met Arg Trp Ser Pro Arg Ile Lys Phe Leu Asp Leu Cys Val Leu Ile Asp Ile Asn Ser Glu Ser Leu Ser Leu Ile Ser His Val Val Lys Trp Lys Arg Asp Glu His Tyr Thr Val Leu Phe Ser Asp Leu Val Asn Ser His Gln Arg Ser Asp Ser Ser Leu Val Asp Glu Phe Val Val Ser Thr Arg Asp Val Cys Lys Asn Phe Leu Lys Gln Val Tyr Phe Glu Ser Phe Val Arg Glu Phe Val Ala Thr Ser Arg Thr Leu Gly Ser Phe Ser Trp Phe Pro His Lys Asp Met Met Pro Ser Glu Asp Gly Ala Glu Ala Leu Gly Pro Phe Gln Ser Phe Ile Leu Lys Val Val Asn Lys Asn Met Glu Arg Pro Met Phe Arg Asn Asp Leu Gln Phe Gly Phe Gly Trp Phe Ser Tyr Arg Leu Gly Asp Ile Val Cys Asn Ala Ala Met Leu Ile Lys Gln Gly Leu Thr Asn Pro Lys Ala Phe Lys Ser Leu Arg Asn Leu Trp Asp Tyr Met Ile Asn Asn Thr Glu Gly Val Leu Glu Phe Ser Ile Thr Val Asp Phe Thr His Asn Gln Asn Asn Thr Asp Cys Leu Arg Lys Phe Ser Leu Ile Phe Leu Val Lys Cys Gln Leu Gln Gly Pro Gly Val Ala Glu Phe Leu Ser Cys Ser His Leu Phe Lys Gly Glu Val Asp Arg Arg Phe Leu Asp Glu Cys Leu His Leu Leu Arg Ser Asp Ser Ile Phe Lys Val Asn Asp Gly Val Phe Asp Ile Arg Ser Glu Glu Phe Glu Asp Tyr Met Glu Asp Pro Leu Ile Leu Gly Asp Ser Leu Glu Leu Glu Leu Ile Gly Ser Arg Lys Ile Leu Asp Gly Ile Arg Ser Leu Asp Phe Glu Arg Ile Gly Pro Glu Trp Glu Pro Val Pro Leu Thr Val Arg Met Gly Ala Leu Phe Glu Gly Arg Ser Leu Val Gln Asn Ile Val Val Lys Leu Glu Thr Lys Asp Met Arg Val Phe Leu Ala Glu Leu Glu Gly Tyr Gly Asn Phe Asp Asp Val Leu Gly Ser Leu Leu Leu His Arg Phe Arg Thr Gly Glu His Leu Gln Gly Ser Glu Ile Ser Thr Ile Leu Gln Glu Leu Cys Ile Asp Arg Ser Ile Leu Leu Val Pro Leu Ser Leu Val Pro Asp Trp Phe Thr Phe Lys Asp Cys Arg Leu Cys Phe Ser Lys Ser Lys Asn Thr Val Met Tyr Glu Thr Val Val Gly Lys Tyr Arg Leu Lys Gly Lys Ser Cys Asp Asp Trp Leu Thr Lys Ser Val Val Glu Glu Ile Asp
9	amino acid sequence of the Z protein of the MP strain of LCMV.	Met Gly Gln Gly Lys Ser Lys Glu Gly Arg Asp Ala Ser Asn Thr Ser Arg Ala Glu Ile Leu Pro Asp Thr Thr Tyr Leu Gly Pro Leu Asn Cys Lys Ser Cys Trp Gln Arg Phe Asp Ser Leu Val Arg Cys His Asp His Tyr Leu Cys Arg His Cys Leu Asn Leu Leu Leu Ser Val Ser Asp Arg Cys Pro Leu Cys Lys His Pro Leu Pro Thr Lys Leu Lys Ile Ser Thr Ala Pro Ser Ser Pro Pro Pro Tyr Glu Glu

SEQ ID NO.	Description	Sequence
10	LCMV clone 13 S-Segment encoding HCMV strain Merlin gB; full-length wildtype. The genomic segment is RNA, the sequence in SEQ ID No. 10 is shown for DNA; however, exchanging all thymidines ("T") in SEQ ID NO: 10 for uridines ("U") provides the RNA sequence.	gcgcaccggg gatcctaggc tttttggatt gcgctttcct ctagatcaac tgggtgtcag 60 gccctatcct acagaaggat ggaatccagg atctggtgcc tggtagtctg cgttaacttg 120 tgtatcgtct gtctgggtgc tgcggtttcc tcatcttcta ctcgtggaac ttctgctact 180 cacagtcacc attcctctca tacgacgtct gctgctcact ctcgatccgg ttcagtctct 240 caacgcgtaa cttcttccca aacggtcagc catggtgtta acgagaccat ctacaacact 300 accctcaagt acggagatgt ggtgggggtc aataccacca agtaccccta tcgcgtgtgt 360 tctatggccc agggtacgga tcttattcgc tttgaacgta atatcgtctg cacctcgatg 420 aagcccatca atgaagacct ggacgagggc atcatggtgg tctacaaacg caacatcgtc 480 gcgcacacct ttaaggtacg agtctaccag aaggttttga cgtttcgtcg tagctacgct 540 tacatccaca ccacttatct gctgggcagc aacacggaat acgtggcgcc tcctatgtgg 600 gagattcatc atatcaacag ccacagtcag tgctacagtt cctacagccg cgttatagca 660 ggcacggttt tcgtggctta tcatagggac agctatgaaa acaaaaccat gcaattaatg 720 cccgacgatt attccaacac ccacagtacc cgttacgtga cggtcaagga tcaatggcac 780 agccgcggca gcacctggct ctatcgtgag acctgtaatc tgaattgtat ggtgaccatc 840 actactgcgc gctccaaata tccttatcat tttttcgcca cttccacggg tgacgtggtt 900 gacatttctc ctttctacaa cggaaccaat cgcaatgcca gctactttgg agaaaacgcc 960 gacaagtttt tcatttttcc gaactacact attgtctccg actttggaag accgaattct 1020 gcgttagaga cccacaggtt ggtggctttt cttgaacgtg cggactcggt gatctcctgg 1080 gatatacagg acgaaaagaa tgtcacttgt caactcactt tctgggaagc ctcggaacgc 1140 accattcgtt ccgaagccga ggactcgtat cacttttctt ctgccaaaat gaccgccact 1200 ttcttatcta agaagcaaga ggtgaacatg tccgactctg cgctggactg cgtacgtgat 1260 gaggctataa ataagttaca gcagattttc aatacttcat acaatcaaac atatgaaaaa 1320 tatggaaacg tgtccgtctt tgaaaccact ggtggtttgg tagtgttctg gcaaggtatc 1380 aagcaaaaat ctctggtgga actcgaacgt ttggccaacc gctccagtct gaatcttact 1440 cataatagaa ccaaaagaag tacagatggc aacaatgcaa ctcatttatc caacatggaa 1500 tcggtgcaca atctggtcta cgcccagctg cagttcacct atgacacgtt gcgcggttac 1560 atcaaccggg cgctggcgca aatcgcagaa gcctggtgtg tggatcaacg gcgcacccta 1620 gaggtcttca aggaactcag caagatcaac ccgtcagcca ttctctcggc catttacaac 1680 aaaccgattg ccgcgcgttt catgggtgat gtcttgggcc tggccagctg cgtgaccatc 1740 aaccaaacca gcgtcaaggt gctgcgtgat atgaacgtga aggagtcgcc aggacgctgc 1800 tactcacgac ccgtggtcat ctttaatttc

SEQ ID NO.	Description	Sequence
		gccaacagct cgtacgtgca gtacggtcaa 1860 ctgggcgagg acaacgaaat cctgttgggc aaccaccgca ctgaggaatg tcagcttccc 1920 agcctcaaga tcttcatcgc cgggaactcg gcctacgagt acgtggacta cctcttcaaa 1980 cgcatgattg acctcagcag tatctccacc gtcgacagca tgatcgccct ggatatcgac 2040 ccgctggaaa ataccgactt cagggtactg gaactttact cgcagaaaga gctgcgttcc 2100 agcaacgttt ttgacctcga agagatcatg cgcgaattca actcgtacaa gcagcgggta 2160 aagtacgtgg aggacaaggt agtcgacccg ctaccgccct acctcaaggg tctggacgac 2220 ctcatgagcg gcctgggcgc cgcgggaaag gccgttggcg tagccattgg ggccgtgggt 2280 ggcgcggtgg cctccgtggt cgaaggcgtt gccaccttcc tcaaaaaccc cttcggagcg 2340 ttcaccatca tcctcgtggc catagctgta gtcattatca cttatttgat ctatactcga 2400 cagcggcgtt tgtgcacgca gccgctgcag aacctctttc cctatctggt gtccgccgac 2460 gggaccaccg tgacgtcggg cagcaccaaa gacacgtcgt tacaggctcc gccttcctac 2520 gaggaaagtg tttataattc tggtcgcaaa ggaccgggac caccgtcgtc tgatgcatcc 2580 acggcggctc cgccttacac caacgagcag gcttaccaga tgcttctggc cctggcccgt 2640 ctggacgcag agcagcgagc gcagcagaac ggtacagatt ctttggacgg acggactggc 2700 acgcaggaca agggacagaa gcccaaccta ctagaccgac tgcgacatcg caaaaacggc 2760 taccgacact tgaaagactc tgacgaagaa gagaacgtct gaagaacagc gcctccctga 2820 ctctccacct cgaaagaggt ggagagtcag ggaggcccag agggtcttag agtgtcacaa 2880 catttgggcc tctaaaaatt aggtcatgtg gcagaatgtt gtgaacagtt ttcagatctg 2940 ggagccttgc tttggaggcg ctttcaaaaa tgatgcagtc catgagtgca cagtgcgggg 3000 tgatctcttt cttctttttg tcccttacta ttccagtatg catcttacac aaccagccat 3060 atttgtccca cactttatct tcatactccc tcgaagcttc cctggtcatt tcaacatcga 3120 taagcttaat gtccttccta ttttgtgagt ccagaagctt tctgatgtca tcggagcctt 3180 gacagcttag aaccatcccc tgcggaagag cacctataac tgacgaggtc aacccgggtt 3240 gcgcattgaa gaggtcggca agatccatgc cgtgtgagta cttggaatct tgcttgaatt 3300 gtttttgatc aacgggttcc ctgtaaaagt gtatgaactg cccgttctgt ggttggaaaa 3360 ttgctatttc cactggatca ttaaatctac cctcaatgtc aatccatgta ggagcgttgg 3420 ggtcaattcc tcccatgagg tcttttaaaa gcattgtctg gctgtagctt aagcccacct 3480 gaggtggacc tgctgctcca ggcgctggcc tgggtgagtt gactgcaggt ttctcgcttg 3540 tgagatcaat tgttgtgttt tcccatgctc tccccacaat cgatgttcta caagctatgt 3600 atggccatcc ttcacctgaa aggcaaactt tatagaggat gttttcataa gggttcctgt 3660

SEQ ID NO.	Description	Sequence
		ccccaacttg gtctgaaaca aacatgttga gttttctctt ggccccgaga actgccttca 3720 agagatcctc gctgttgctt ggcttgatca aaattgactc taacatgtta cccccatcca 3780 acagggctgc ccctgccttc acggcagcac caagactaaa gttatagcca gaaatgttga 3840 tgctggactg ctgttcagtg atgaccccca gaactgggtg cttgtctttc agcctttcaa 3900 gatcattaag atttggatac ttgactgtgt aaagcaagcc aaggtctgtg agcgcttgta 3960 caacgtcatt gagcggagtc tgtgactgtt tggccataca agccatagtt agacttggca 4020 ttgtgccaaa ttgattgttc aaaagtgatg agtctttcac atcccaaact cttaccacac 4080 cacttgcacc ctgctgaggc tttctcatcc caactatctg taggatctga gatctttggt 4140 ctagttgctg tgttgttaag ttccccatat atacccctga agcctggggc ctttcagacc 4200 tcatgatctt ggccttcagc ttctcaaggt cagccgcaag agacatcagt tcttctgcac 4260 tgagcctccc cactttcaaa acattcttct ttgatgttga ctttaaatcc acaagagaat 4320 gtacagtctg gttgagactt ctgagtctct gtaggtcttt gtcatctctc ttttccttcc 4380 tcatgatcct ctgaacattg ctgacctcag agaagtccaa cccattcaga aggttggttg 4440 catccttaat gacagcagcc ttcacatctg atgtgaagct ctgcaattct cttctcaatg 4500 cttgcgtcca ttggaagctc ttaacttcct tagacaagga catcttgttg ctcaatggtt 4560 tctcaagaca aatgcgcaat caaatgccta ggatccactg tgcg 4604
11	WE-specific primer	5’AATCGTCTCTAAGGATGGGTCAGATTGTGACAATG-3’
12	WE specific fusion-primer carrying an overhang complementary to the WET-specific primer	5’AATCGTCTCTAAGGATGGGTCAGATTGTGACAATG-3’
13	WET-specific primer	5’CTCGGTGATCATGTTATCTGCTTCTTGTTCGATTTGA-3’
14	WET-specific fusion-primer complementary to the WE-sequence	5’AATCGTCTCTTTCTTTATCTCCTCTTCCAGATGG-3’
15	Primer specific for LCMV NP	5’-GGCTCCCAGATCTGAAAACTGTT-3’
16	NP- and GP-specific primers; NP-specific: same as in RT reaction, GP-specific: 5’	5’-GCTGGCTTGTCACTAATGGCTC-3’
17	Representative cDNA sequence obtained from animal #3 (r3LCMV-GFPnat #3) revealing a recombined S segment combining NP and GP sequences	aagaagcaga taacatgatc accgagatgc tgaggaagga ctacatcaag agacagggca 60 gcacccccct ggccctcatg gatctgctca tgttcagcac cagcgcctac ctcatcagca 120 tcttcctgca cctggtgaag atccccaccc acagacacat caagggcggc agctgcccca 180 agccccacag actcaccaac aagggcatct gcagctgcgg cgccttcaag gtgcccggcg 240 taaaaaccat ctggaagagg agataaagaa cagcgcctcc ctgactctcc acctcgaaag 300

SEQ ID NO.	Description	Sequence
		aggtggagag tcagggaggc ccagagggtc ttacttgtac agctcgtcca tgccgagagt 360 gatcccggcg gcggtcacga actccagcag gaagaacagc gcctccctga ctctccacct 420 cgaaagaggt ggagagtcag ggaggcccag aggtcttaga gtgtcacaac atttgggcct 480 ctaaaaatta ggtcatgtgg cagaatgttg tgaacagttt tcagatctgg gagcc 535
18	S segment 1 of r3LCMV-P1A (containing NP)	gcgcaccggg gatcctaggc tttttggatt gcgctttcct ctagatcaac tgggtgtcag 60 gccctatcct acagaaggat gagcgacaac aagaagcccg acaaggccca ctctggcagc 120 ggcggagatg gcgacggcaa cagatgtaac ctgctgcaca gatacagcct ggaagagatc 180 ctgccctacc tgggctggct ggtgttcgcc gtcgtgacaa caagcttcct ggccctgcag 240 atgttcatcg acgccctgta cgaggaacag tacgagaggg acgtggcctg gatcgccaga 300 cagagcaaga gaatgagcag cgtggacgag gacgaggatg atgaggacga cgaagatgac 360 tactacgacg atgaggatga cgacgacgac gccttctacg atgacgagga cgatgaagag 420 gaagaactgg aaaacctgat ggacgacgag tccgaggatg aggccgagga agagatgagc 480 gtggaaatgg gcgctggcgc cgaagagatg ggagccggcg ctaactgtgc ttgcgtgcca 540 ggacaccacc tgagaaagaa cgaagtgaag tgccggatga tctacttctt ccacgacccc 600 aactttctgg tgtccatccc cgtgaacccc aaagaacaga tggaatgcag atgcgagaac 660 gccgacgaag aggtggccat ggaagaagaa gaggaagagg aagaagaaga agaagaggaa 720 gaaatgggca accccgacgg cttcagcccc tgaagaacag cgcctccctg actctccacc 780 tcgaaagagg tggagagtca gggaggccca gagggtctta gagtgtcaca acatttgggc 840 ctctaaaaat taggtcatgt ggcagaatgt tgtgaacagt tttcagatct gggagccttg 900 ctttggaggc gctttcaaaa atgatgcagt ccatgagtgc acagtgcggg gtgatctctt 960 tcttcttttt gtcccttact attccagtat gcatcttaca caaccagcca tatttgtccc 1020 acactttatc ttcatactcc ctcgaagctt ccctggtcat ttcaacatcg ataagcttaa 1080 tgtccttcct attttgtgag tccagaagct ttctgatgtc atcggagcct tgacagctta 1140 gaaccatccc ctgcggaaga gcacctataa ctgacgaggt caacccgggt tgcgcattga 1200 agaggtcggc aagatccatg ccgtgtgagt acttggaatc ttgcttgaat tgtttttgat 1260 caacgggttc cctgtaaaag tgtatgaact gcccgttctg tggttggaaa attgctattt 1320 ccactggatc attaaatcta ccctcaatgt caatccatgt aggagcgttg gggtcaattc 1380 ctcccatgag gtcttttaaa agcattgtct ggctgtagct taagcccacc tgaggtggac 1440 ctgctgctcc aggcgctggc ctgggtgagt tgactgcagg tttctcgctt gtgagatcaa 1500 ttgttgtgtt ttcccatgct ctccccacaa tcgatgttct acaagctatg tatggccatc 1560 cttcacctga aaggcaaact ttatagagga

SEQ ID NO.	Description	Sequence
		tgttttcata agggttcctg tccccaactt 1620 ggtctgaaac aaacatgttg agttttctct tggccccgag aactgccttc aagagatcct 1680 cgctgttgct tggcttgatc aaaattgact ctaacatgtt acccccatcc aacagggctg 1740 cccctgcctt cacggcagca ccaagactaa agttatagcc agaaatgttg atgctggact 1800 gctgttcagt gatgaccccc agaactgggt gcttgtcttt cagcctttca agatcattaa 1860 gatttggata cttgactgtg taaagcaagc caaggtctgt gagcgcttgt acaacgtcat 1920 tgagcggagt ctgtgactgt ttggccatac aagccatagt tagacttggc attgtgccaa 1980 attgattgtt caaaagtgat gagtctttca catcccaaac tcttaccaca ccacttgcac 2040 cctgctgagg ctttctcatc ccaactatct gtaggatctg agatctttgg tctagttgct 2100 gtgttgttaa gttccccata tatacccctg aagcctgggg cctttcagac ctcatgatct 2160 tggccttcag cttctcaagg tcagccgcaa gagacatcag ttcttctgca ctgagcctcc 2220 ccactttcaa aacattcttc tttgatgttg actttaaatc cacaagagaa tgtacagtct 2280 ggttgagact tctgagtctc tgtaggtctt tgtcatctct cttttccttc ctcatgatcc 2340 tctgaacatt gctgacctca gagaagtcca acccattcag aaggttggtt gcatccttaa 2400 tgacagcagc cttcacatct gatgtgaagc tctgcaattc tcttctcaat gcttgcgtcc 2460 attggaagct cttaacttcc ttagacaagg acatcttgtt gctcaatggt ttctcaagac 2520 aaatgcgcaa tcaaatgcct aggatccact gtgcg 2555
19	S segment 2 of r3LCMV-P1A (containing GP)	gcgcaccggg gatcctaggc tttttggatt gcgctttcct ctagatcaac tgggtgtcag 60 gccctatcct acagaaggat gagcgacaac aagaagcccg acaaggccca ctctggcagc 120 ggcggagatg gcgacggcaa cagatgtaac ctgctgcaca gatacagcct ggaagagatc 180 ctgccctacc tgggctggct ggtgttcgcc gtcgtgacaa caagcttcct ggccctgcag 240 atgttcatcg acgccctgta cgaggaacag tacgagaggg acgtggcctg gatcgccaga 300 cagagcaaga gaatgagcag cgtggacgag gacgaggatg atgaggacga cgaagatgac 360 tactacgacg atgaggatga cgacgacgac gccttctacg atgacgagga cgatgaagag 420 gaagaactgg aaaacctgat ggacgacgag tccgaggatg aggccgagga agagatgagc 480 gtggaaatgg gcgctggcgc cgaagagatg ggagccggcg ctaactgtgc ttgcgtgcca 540 ggacaccacc tgagaaagaa cgaagtgaag tgccggatga tctacttctt ccacgacccc 600 aactttctgg tgtccatccc cgtgaacccc aaagaacaga tggaatgcag atgcgagaac 660 gccgacgaag aggtggccat ggaagaagaa gaggaagagg aagaagaaga agaagaggaa 720 gaaatgggca accccgacgg cttcagcccc tgaagaacag cgcctccctg actctccacc 780 tcgaaagagg tggagagtca gggaggccca

SEQ ID NO.	Description	Sequence
		gagggtctca gcgtcttttc cagacggttt 840 ttacaccagg caccttaaat gcaccacaac tacaaattcc tttgttggtt aatcggtgtg 900 gctttggaca tgagccacct tttatgtgcc tgtgtgttgg tattttgaca aggtgcagga 960 agatgctgac tagatatgca gatgtggaaa acatcagaag gtccatcaat gctagggggg 1020 tactcccctg cctctttatg taatccttcc tcaacatctc tgtaatcatg ttatcggctt 1080 cctgttcgat ttggtcactg aagtgggtct catttaagta agaaccattg gtgacaagcc 1140 agcacttggg gacactagtt tcgccggtct ttgcatgttc taggtaccaa aactttgagt 1200 aattgcaata tggcaccccc atcagatctc tcaagtggtt cctcatcagt agttgatctg 1260 aaatcaaaga attcactgtt gttttgaata agtgcaaggc agattctacg tcctctttga 1320 acttactcaa agcagccttg ttgtagtcaa ttagtcgcag catgtcacag aattcttcat 1380 catgatttac attgcatttc gcaactgctg tgttcccgaa acacttaagc tctgcagcaa 1440 gaatcatcca tttggtcagg caataaccac ctggattctc cacccctgaa gagtctgaca 1500 aagtccaggt gaatgtgccc gctagtctcc tagtggagaa cttagttttc tcttgggaaa 1560 ggagaatcct ggacatccca aaaggacctg catatgtgca gtggttttcc caggttctat 1620 tttgtataat caggtattgg taactcgtct ggctacacca ggtggtcttg ccatctgagc 1680 ctgtccagcc ccagccactc ctcatgtatt tccccccgaa ggcagttcta aacatatcta 1740 ggactctacc tctgaaggtt ctacactggc tctgagcact ttgtgcatct gagaatgtca 1800 agttgtattg gatggttatg ccattgttga agtcgcagga tactgcctta tagttggagt 1860 tccctctgat actgaggtgt aggctcgaaa ctatactcat gagtgtgtgg tcaaaggtct 1920 ttttgttgaa ggcagaggtc agattgcaaa agttgtgact gatgatggaa tcattggtga 1980 aggtcaattc tagtccagaa gtccccatac tgatgtaatg gtgggagttg ttggctgaac 2040 atgcgttggg catggtcagg ttcagatgtg acatatcaaa ctccactgac ttaaattggt 2100 aaactccttt gtaaatgtcg ggtcccttaa gaccgtacat gccacaggac ctgccagcca 2160 gaagtaggaa actgatcaat gcgaatatcc cacaggtggc aaaattgtag acagccttga 2220 tacccgtgat cacgataagc acaataatga caatgttgat cacctcatcg atgatgtgag 2280 gcagagcctc aaacattgtc acaatctgac ccatcttgtt gctcaatggt ttctcaagac 2340 aaatgcgcaa tcaaatgcct aggatccact gtgcg 2375
20	L segment of r3LCMV-P1A	gcgcaccggg gatcctaggc gtttagttgc gctgtttggt tgcacaactt tcttcgtgag 60 gctgtcagaa gtggacctgg ctgatagcga tgggtcaagg caagtccaga gaggagaaag 120 gcaccaatag tacaaacagg gccgaaatcc taccagatac cacctatctt ggccctttaa 180 gctgcaaatc ttgctggcag aaatttgaca

SEQ ID NO.	Description	Sequence
		gcttggtaag atgccatgac cactaccttt 240 gcaggcactg tttaaacctt ctgctgtcag tatccgacag gtgtcctctt tgtaaatatc 300 cattaccaac cagattgaag atatcaacag ccccaagctc tccacctccc tacgaagagt 360 aacaccgtcc ggccccggcc ccgacaaaca gcccagcaca agggaaccgc acgtcaccca 420 acgcacacag acacagcacc caacacagaa cacgcacaca cacacacaca cacacccaca 480 cgcacgcgcc cccaccaccg gggggcgccc ccccccgggg ggcggccccc cgggagcccg 540 ggcggagccc cacggagatg cccatcagtc gatgtcctcg gccaccgacc cgcccagcca 600 atcgtcgcag gacctcccct tgagtctaaa cctgcccccc actgtttcat acatcaaagt 660 gctcctagat ttgctaaaac aaagtctgca atccttaaag gcgaaccagt ctggcaaaag 720 cgacagtgga atcagcagaa tagatctgtc tatacatagt tcctggagga ttacacttat 780 ctctgaaccc aacaaatgtt caccagttct gaatcgatgc aggaagaggt tcccaaggac 840 atcactaatc ttttcatagc cctcaagtcc tgctagaaag actttcatgt ccttggtctc 900 cagcttcaca atgatatttt ggacaaggtt tcttccttca aaaagggcac ccatctttac 960 agtcagtggc acaggctccc actcaggtcc aactctctca aagtcaatag atctaatccc 1020 atccagtatt cttttggagc ccaacaactc aagctcaaga gaatcaccaa gtatcaaggg 1080 atcttccatg taatcctcaa actcttcaga tctgatatca aagacaccat cgttcacctt 1140 gaagacagag tctgtcctca gtaagtggag gcattcatcc aacattcttc tatctatctc 1200 acccttaaag aggtgagagc atgataaaag ttcagccaca cctggattct gtaattggca 1260 cctaaccaag aatatcaatg aaaatttcct taaacagtca gtattattct gattgtgcgt 1320 aaagtccact gaaattgaaa actccaatac cccttttgtg tagttgagca tgtagtccca 1380 cagatccttt aaggatttaa atgcctttgg gtttgtcagg ccctgcctaa tcaacatggc 1440 agcattacac acaacatctc ccattcggta agagaaccac ccaaaaccaa actgcaaatc 1500 attcctaaac ataggcctct ccacattttt gttcaccacc tttgagacaa atgattgaaa 1560 ggggcccagt gcctcagcac catcttcaga tggcatcatt tctttatgag ggaaccatga 1620 aaaattgcct aatgtcctgg ttgttgcaac aaattctcga acaaatgatt caaaatacac 1680 ctgttttaag aagttcttgc agacatccct cgtgctaaca acaaattcat caaccagact 1740 ggagtcagat cgctgatgag aattggcaag gtcagaaaac agaacagtgt aatgttcatc 1800 ccttttccac ttaacaacat gagaaatgag tgacaaggat tctgagttaa tatcaattaa 1860 aacacagagg tcaaggaatt taattctggg actccacctc atgttttttg agctcatgtc 1920 agacataaat ggaagaagct gatcctcaaa gatcttggga tatagccgcc tcacagattg 1980 aatcacttgg ttcaaattca ctttgtcctc cagtagcctt gagctctcag gctttcttgc 2040

SEQ ID NO.	Description	Sequence
		tacataatca catgggttta agtgcttaag agttaggttc tcactgttat tcttcccttt 2100 ggtcggttct gctaggaccc aaacacccaa ctcaaaagag ttgctcaatg aaatacaaat 2160 gtagtcccaa agaagaggcc ttaaaaggca tatatgatca cggtgggctt ctggatgaga 2220 ctgtttgtca caaatgtaca gcgttatacc atcccgattg caaactcttg tcacatgatc 2280 atctgtggtt agatcctcaa gcagcttttt gatatacaga ttttccctat ttttgtttct 2340 cacacacctg cttcctagag ttttgcaaag gcctataaag ccagatgaga tacaactctg 2400 gaaagctgac ttgttgattg cttctgacag cagcttctgt gcaccccttg tgaatttact 2460 acaaagtttg ttctggagtg tcttgatcaa tgatgggatt ctttcctctt ggaaagtcat 2520 cactgatgga taaaccacct tttgtcttaa aaccatcctt aatgggaaca tttcattcaa 2580 attcaaccag ttaacatctg ctaactgatt cagatcttct tcaagaccga ggaggtctcc 2640 caattgaaga atggcctcct ttttatctct gttaaatagg tctaagaaaa attcttcatt 2700 aaattcacca tttttgagct tatgatgcag tttccttaca agctttctta caacctttgt 2760 ttcattagga cacagttcct caatgagtct ttgtattctg taacctctag aaccatccag 2820 ccaatctttc acatcagtgt tggtattcag tagaaatgga tccaaaggga aattggcata 2880 ctttaggagg tccagtgttc tcctttggat actattaact agggagactg ggacgccatt 2940 tgcgatggct tgatctgcaa ttgtatctat tgtttcacaa agttgatgtg gctctttaca 3000 cttgacattg tgtagcgctg cagatacaaa ctttgtgaga agagggactt cctcccccca 3060 tacatagaat ctagatttaa attctgcagc gaacctccca gccacacttt ttgggctgat 3120 aaatttgttt aacaagccgc tcagatgaga ttggaattcc aacaggacaa ggacttcctc 3180 cggatcactt acaaccaggt cactcagcct cctatcaaat aaagtgatct gatcatcact 3240 tgatgtgtaa gcctctggtc tttcgccaaa gataacacca atgcagtagt tgatgaacct 3300 ctcgctaagc aaaccataga agtcagaagc attatgcaag attccctgcc ccatatcaat 3360 aaggctggat atatgggatg gcactatccc catttcaaaa tattgtctga aaattctctc 3420 agtaacagtt gtttctgaac ccctgagaag ttttagcttc gacttgacat atgatttcat 3480 cattgcattc acaacaggaa aggggacctc gacaagctta tgcatgtgcc aagttaacaa 3540 agtgctaaca tgatctttcc cggaacgcac atactggtca tcacctagtt tgagattttg 3600 tagaaacatt aagaacaaaa atgggcacat cattggtccc catttgctgt gatccatact 3660 atagtttaag aacccttccc gcacattgat agtcattgac aagattgcat tttcaaattc 3720 cttatcattg tttaaacagg agcctgaaaa gaaacttgaa aaagactcaa aataatcttc 3780 tattaacctt gtgaacattt ttgtcctcaa atctccaata tagagttctc tatttccccc 3840 aacctgctct ttataagata gtgcaaattt

SEQ ID NO.	Description	Sequence
		cagccttcca gagtcaggac ctactgaggt 3900 gtatgatgtt ggtgattctt ctgagtagaa gcacagattt ttcaaagcag cactcataca 3960 ttgtgtcaac gacagagctt tactaaggga ctcagaatta ctttccctct cactgattct 4020 cacgtcttct tccagtttgt cccagtcaaa tttgaaattc aagccttgcc tttgcatatg 4080 cctgtatttc cctgagtacg catttgcatt catttgcaac agaatcatct tcatgcaaga 4140 aaaccaatca ttctcagaaa agaactttct acaaaggttt tttgccatct catcgaggcc 4200 acactgatct ttaatgactg aggtgaaata caaaggtgac agctctgtgg aaccctcaac 4260 agcctcacag ataaatttca tgtcatcatt ggttagacat gatgggtcaa agtcttctac 4320 taaatggaaa gatatttctg acaagataac ttttcttaag tgagccatct tccctgttag 4380 aataagctgt aaatgatgta gtccttttgt atttgtaagt ttttctccat ctcctttgtc 4440 attggccctc ctacctcttc tgtaccgtgc tattgtggtg ttgacctttt cttcgagact 4500 tttgaagaag cttgtctctt cttctccatc aaaacatatt tctgccaggt tgtcttccga 4560 tctccctgtc tcttctccct tggaaccgat gaccaatcta gagactaact tggaaacttt 4620 atattcatag tctgagtggc tcaacttata cttttgtttt cttacgaaac tctccgtaat 4680 ttgactcaca gcactaacaa gcaatttgtt aaagtcatat tccagaagtc gttctccatt 4740 tagatgctta ttaaccacca cacttttgtt actagcaaga tctaatgctg tcgcacatcc 4800 agagttagtc atgggatcta ggctgtttag cttcttctct cctttgaaaa ttaaagtgcc 4860 gttgttaaat gaagacacca ttaggctaaa ggcttccaga ttaacacctg gagttgtatg 4920 ctgacagtca atttctttac tagtgaatct cttcatttgc tcatagaaca cacattcttc 4980 ctcaggagtg attgcttcct tggggttgac aaaaaaacca aattgacttt tgggctcaaa 5040 gaacttttca aaacatttta tctgatctgt tagcctgtca ggggtctcct ttgtgatcaa 5100 atgacacagg tatgacacat tcaacataaa tttaaatttt gcactcaaca acaccttctc 5160 accagtacca aaaatagttt ttattaggaa tctaagcagc ttatacacca ccttctcagc 5220 aggtgtgatc agatcctccc tcaacttatc cattaatgat gtagatgaaa aatctgacac 5280 tattgccatc accaaatatc tgacactctg tacctgcttt tgatttctct ttgttgggtt 5340 ggtgagcatt agcaacaata gggtcctcag tgcaacctca atgtcggtga gacagtcttt 5400 caaatcagga catgatctaa tccatgaaat catgatgtct atcatattgt ataagacctc 5460 atctgaaaaa attggtaaaa agaacctttt aggatctgca tagaaggaaa ttaaatgacc 5520 atccgggcct tgtatggagt agcaccttga agattctcca gtcttctggt ataataggtg 5580 gtattcttca gagtccagtt ttattacttg gcaaaacact tctttgcatt ctaccacttg 5640 atatctcaca gaccctattt gattttgcct tagtctagca actgagctag ttttcatact 5700

SEQ ID NO.	Description	Sequence
		gtttgttaag gccagacaaa cagatgataa tcttctcagg ctctgtatgt tcttcagctg 5760 ctctgtgctg ggttggaaat tgtaatcttc aaacttcgta taatacatta tcgggtgagc 5820 tccaattttc ataaagttct caaattcagt gaatggtatg tggcattctt gctcaaggtg 5880 ttcagacagt ccgtaatgct cgaaactcag tcccaccact aacaggcatt tttgaatttt 5940 tgcaatgaac tcactaatag atgccctaaa caattcctca aaagacacct ttctaaacac 6000 ctttgacttt tttctattcc tcaaaagtct aatgaactcc tctttagtgc tgtgaaagct 6060 taccagccta tcattcacac tactatagca acaacccacc cagtgtttat catttttttaa 6120 ccctttgaat ttcgactgtt ttatcaatga ggaaagacac aaaacatcca gatttaacaa 6180 ctgtctcctt ctagtattca acagtttcaa actcttgact ttgtttaaca tagagaggag 6240 cctctcatat tcagtgctag tctcacttcc cctttcgtgc ccatgggtct ctgcagttat 6300 gaatctcatc aaaggacagg attcgactgc ctccctgctt aatgttaaga tatcatcact 6360 atcagcaagg ttttcataga gctcagagaa ttccttgatc aagccttcag ggtttacttt 6420 ctgaaagttt ctctttaatt tcccactttc taaatctctt ctaaacctgc tgaaaagaga 6480 gtttattcca aaaaccacat catcacagct catgttgggg ttgatgcctt cgtggcacat 6540 cctcataatt tcatcattgt gagttgacct cgcatctttc agaattttca tagagtccat 6600 accggagcgc ttgtcgatag tagtcttcag ggactcacag agtctaaaat attcagactc 6660 ttcaaagact ttctcatttt ggttagaata ctccaaaagt ttgaataaaa ggtctctaaa 6720 tttgaagttt gcccactctg gcataaaact attatcataa tcacaacgac catctactat 6780 tggaactaat gtgacacccg caacagcaag gtcttccctg atgcatgcca atttgttagt 6840 gtcctctata aatttcttct caaaactggc tggagtgctc ctaacaaaac actcaagaag 6900 aatgagagaa ttgtctatca gcttgtaacc atcaggaatg ataagtggta gtcctgggca 6960 tacaattcca gactccacca aaattgtttc cacagactta tcgtcgtggt tgtgtgtgca 7020 gccactcttg tctgcactgt ctatttcaat gcagcgtgac agcaacttga gtccctcaat 7080 cagaaccatt ctgggttccc tttgtcccag aaagttgagt ttctgccttg acaacctctc 7140 atcctgttct atatagttta aacataactc tctcaattct gagatgattt catccattgc 7200 gcatcaaaaa gcctaggatc ctcggtgcg 7229
21	S segment 1 of r3JUNV-P1A (containing NP)	gcgcaccggg gatcctaggc gattttggtt acgctataat tgtaactgtt ttctgtttgg 60 acaacatcaa aaacatccat tgcacaatga gcgacaacaa gaagcccgac aaggcccact 120 ctggcagcgg cggagatggc gacggcaaca gatgtaacct gctgcacaga tacagcctgg 180 aagagatcct gccctacctg ggctggctgg tgttcgccgt cgtgacaaca agcttcctgg 240

SEQ ID NO.	Description	Sequence
		ccctgcagat gttcatcgac gccctgtacg aggaacagta cgagagggac gtggcctgga 300 tcgccagaca gagcaagaga atgagcagcg tggacgagga cgaggatgat gaggacgacg 360 aagatgacta ctacgacgat gaggatgacg acgacgacgc cttctacgat gacgaggacg 420 atgaagagga agaactggaa aacctgatgg acgacgagtc cgaggatgag gccgaggaag 480 agatgagcgt ggaaatgggc gctggcgccg aagagatggg agccggcgct aactgtgctt 540 gcgtgccagg acaccacctg agaaagaacg aagtgaagtg ccggatgatc tacttcttcc 600 acgaccccaa ctttctggtg tccatccccg tgaaccccaa agaacagatg gaatgcagat 660 gcgagaacgc cgacgaagag gtggccatgg aagaagaaga ggaagaggaa gaagaagaag 720 aagaggaaga aatgggcaac cccgacggct tcagcccctg agacctcctg agggtcccca 780 ccagcccggg cactgcccgg gctggtgtgg ccccccagtc cgcggcctgg ccgcggactg 840 gggaggcact gcttacagtg cataggctgc cttcgggagg aacagcaagc tcggtggtaa 900 tagaggtgta ggttcctcct catagagctt cccatctagc actgactgaa acattatgca 960 gtctagcaga gcacagtgtg gttcactgga ggccaacttg aagggagtat ccttttccct 1020 ctttttctta ttgacaacca ctccattgtg atatttgcat aagtgaccat atttctccca 1080 gacctgttga tcaaactgcc tggcttgttc agatgtgagc ttaacatcaa ccagtttaag 1140 atctcttctt ccatggaggt caaacaactt cctgatgtca tcggatcctt gagtagtcac 1200 aaccatgtct ggaggcagca agccgatcac gtaactaaga actcctggca ttgcatcttc 1260 tatgtccttc attaagatgc cgtgagagtg tctgctacca tttttaaacc ctttctcatc 1320 atgtggtttt ctgaagcagt gaatgtactg cttacctgca ggttggaata atgccatctc 1380 aacagggtca gtggctggtc cttcaatgtc gagccaaagg gtgttggtgg ggtcgagttt 1440 ccccactgcc tctctgatga cagcttcttg tatctctgtc aagttagcca atctcaaatt 1500 ctgaccgttt ttttccggct gtctaggacc agcaactggt ttccttgtca gatcaatact 1560 tgtgttgtcc catgacctgc ctgtgatttg tgatctagaa ccaatataag gccaaccatc 1620 gccagaaaga caaagtttgt acaaaaggtt ttcataagga tttctattgc ctggtttctc 1680 atcaataaac atgccttctc ttcgtttaac ctgaatggtt gattttatga gggaagagaa 1740 gttttctggg gtgactctga ttgtttccaa catgtttcca ccatcaagaa tagatgctcc 1800 agcctttact gcagctgaaa gactgaagtt gtaaccagaa atattgatgg agctttcatc 1860 tttagtcaca atctgaaggc agtcatgttc ctgagtcagt ctgtcaaggt cacttaagtt 1920 tggatacttc acagtgtata gaagcccaag tgaggttaaa gcttgtatga cactgttcat 1980 tgtctcacct ccttgaacag tcatgcatgc aattgtcaat gcaggaacag agccaaactg 2040 attgtttagc tttgaagggt ctttaacatc

SEQ ID NO.	Description	Sequence
		ccatatcctc accacaccat ttcccccagt 2100 cccttgctgt tgaaatccca gtgttctcaa tatctctgat cttttagcaa gttgtgactg 2160 ggacaagtta cccatgtaaa cccccctgaga gcctgtctct gctcttctta tcttgttttt 2220 taatttctca aggtcagacg ccaactccat cagttcatcc ctccccagat ctcccacctt 2280 gaaaactgtg tttcgttgaa cactcctcat ggacatgagt ctgtcaacct ctttattcag 2340 gtccctcaac ttgttgagat cttcttcccc ctttttagtc tttctgagtg cccgctgcac 2400 ctgtgccact tggttgaagt cgatgctgtc agcaattagc ttggcgtcct tcaaaacatc 2460 tgacttgaca gtctgagtga attggctcaa acctctcctt aaggactgag tccatctaaa 2520 gcttggaacc tccttggagt gtgccatgcc agaagttctg gtgatttga tctagaatag 2580 agttgctcag tgaaagtgtt agacactatg cctaggatcc actgtgcg 2628
22	S segment 2 of r3JUNV-P1A (containing GP)	gcgcaccggg gatcctaggc gattttggtt acgctataat tgtaactgtt ttctgtttgg 60 acaacatcaa aaacatccat tgcacaatga gcgacaacaa gaagcccgac aaggcccact 120 ctggcagcgg cggagatggc gacggcaaca gatgtaacct gctgcacaga tacagcctgg 180 aagagatcct gccctacctg ggctggctgg tgttcgccgt cgtgacaaca agcttcctgg 240 ccctgcagat gttcatcgac gccctgtacg aggaacagta cgagagggac gtggcctgga 300 tcgccagaca gagcaagaga atgagcagcg tggacgagga cgaggatgat gaggacgacg 360 aagatgacta ctacgacgat gaggatgacg acgacgacgc cttctacgat gacgaggacg 420 atgaagagga agaactggaa aacctgatgg acgacgagtc cgaggatgag gccgaggaag 480 agatgagcgt ggaaatgggc gctggcgccg aagagatggg agccggcgct aactgtgctt 540 gcgtgccagg acaccacctg agaaagaacg aagtgaagtg ccggatgatc tacttcttcc 600 acgaccccaa ctttctggtg tccatccccg tgaaccccaa agaacagatg gaatgcagat 660 gcgagaacgc cgacgaagag gtggccatgg aagaagaaga ggaagaggaa gaagaagaag 720 aagaggaaga aatgggcaac cccgacggct tcagcccctg agacctcctg agggtcccca 780 ccagcccggg cactgcccgg gctggtgtgg ccccccagtc cgcggcctgg ccgcggactg 840 gggaggcact gcatggggca gttcattagc ttcatgcaag aaataccaac ctttttgcag 900 gaggctctga acattgctct tgttgcagtc agtctcattg ccatcattaa gggtatagtg 960 aacttgtaca aaagtggttt attccaattc tttgtattcc tagcgcttgc aggaagatcc 1020 tgcacagaag aagctttcaa aatcggactg cacactgagt tccagactgt gtccttctca 1080 atggtgggtc tcttttccaa caatccacat gacctacctt tgttgtgtac cttaaacaag 1140 agccatcttt acattaaggg gggcaatgct tcatttcaga tcagctttga tgatattgca 1200 gtattgttgc cacagtatga tgttataata

SEQ ID NO.	Description	Sequence
		caacatccag cagatatgag ctggtgttcc 1260 aaaagtgatg atcaaatttg gttgtctcag tggttcatga atgctgtggg acatgattgg 1320 catctagacc caccatttct gtgtaggaac cgtgcaaaga cagaaggctt catctttcaa 1380 gtcaacacct ccaagactgg tgtcaatgga aattatgcta agaagtttaa gactggcatg 1440 catcatttat atagagaata tcctgaccct tgcttgaatg gcaaactgtg cttaatgaag 1500 gcacaaccta ccagttggcc tctccaatgt ccactcgacc acgttaacac attacacttc 1560 cttacaagag gtaaaaacat tcaacttcca aggaggtcct tgaaagcatt cttctcctgg 1620 tctttgacag actcatccgg caaggatacc cctggaggct attgtctaga agagtggatg 1680 ctcgtagcag ccaaaatgaa gtgttttggc aatactgctg tagcaaaatg caatttgaat 1740 catgactctg aattctgtga catgttgagg ctctttgatt acaacaaaaa tgctatcaaa 1800 accctaaatg atgaaactaa gaaacaagta aatctgatgg ggcagacaat caatgccctg 1860 atatctgaca atttattgat gaaaaacaaa attagggaac tgatgagtgt cccttactgc 1920 aattacacaa aattttggta tgtcaaccac acactttcag gacaacactc attaccaagg 1980 tgctggttaa taaaaaacaa cagctatttg aacatctctg acttccgtaa tgactggata 2040 ttagaaagtg acttcttaat ttctgaaatg ctaagcaaag agtattcgga caggcagggt 2100 aaaactcctt tgactttagt tgacatctgt atttggagca cagtattctt cacagcgtca 2160 ctcttccttc acttggtggg tataccctcc cacagacaca tcaggggcga agcatgccct 2220 ttgccacaca ggttgaacag cttgggtggt tgcagatgtg gtaagtaccc caatctaaag 2280 aaaccaacag tttggcgtag aggacactaa gccagaagtt ctggtgattt tgatctagaa 2340 tagagttgct cagtgaaagt gttagacact atgcctagga tccactgtgc g 2391
23	L segment of r3JUNV-P1A	gcgcaccggg gatcctaggc gtaacttcat cattaaaatc tcagattctg ctctgagtgt 60 gacttactgc gaagaggcag acaaatgggc aactgcaacg gggcatccaa gtctaaccag 120 ccagactcct caagagccac acagccagcc gcagaattta ggagggtagc tcacagcagt 180 ctatatggta gatataactg taagtgctgc tggtttgctg ataccaattt gataacctgt 240 aatgatcact acctttgttt aaggtgccat cagggtatgt taaggaattc agatctctgc 300 aatatctgct ggaagcccct gcccaccaca atcacagtac cggtggagcc aacagcacca 360 ccaccatagg cagactgcac agggtcagac ccgacccccc gggggcccc catggggacc 420 ccccgtgggg gaaccccggg ggtgatgcgc cattagtcaa tgtctttgat ctcgactttg 480 tgcttcagtg gcctgcatgt caccccttttc aatctgaact gcccttgggg atctgatatc 540 agcaggtcat ttaaagatct gctgaatgcc accttgaaat ttgagaattc caaccagtca 600 ccaaatttat caagtgaacg gatcaactgc

SEQ ID NO.	Description	Sequence
		tctttgtgta gatcataaac gaggacaaag 660 tcctcttgct gaaataatat tgtttgtgat gttgttttta gataaggcca tagttggctt 720 aataaggttt ccacactatc aatgtcctct agtgctccaa ttgccttgac tatgacatcc 780 ccagacaact caactctata tgttgacaac ctttcattac ctctgtaaaa gataccctct 840 ttcaagacaa gaggttctcc tgggttatct ggcccaatga ggtcatatgc atacttgtta 900 cttagttcag aataaaagtc accaaagttg aacttaacat ggctcagaat attgtcatca 960 tttgtcgcag cgtagcctgc atcaataaac aagccagcta ggtcaaagct ctcatggcct 1020 gtgaacaatg gtaggctagc gataaccagt gcaccatcca acaatgagtg gcttccctca 1080 gacccagaaa cacattgact cattgcatcc acattcagct ctaattcagg ggtaccgaca 1140 tcatccactc ctagtgaact gacaatggtg taactgtaca ccatctttct tctaagttta 1200 aattttgtcg aaactcgtgt gtgttctact tgaatgatca attttagttt cacagcttct 1260 tggcaagcaa cattgcgcaa cacagtgtgc aggtccatca tgtcttcctg aggcaacaag 1320 gagatgttgt caacagagac accctcaagg aaaaccttga tattatcaaa gctagaaact 1380 acataaccca ttgcaatgtc ttcaacaaac attgctcttg atactttatt attcctaact 1440 gacaaggtaa aatctgtgag ttcagctaga tctacttgac tgtcatcttc tagatctaga 1500 acttcattga accaaaagaa ggatttgaga cacgatgttg acatgactag tgggtttatc 1560 atcgaagata agacaacttg caccatgaag ttcctgcaaa cttgctgtgg gctgatgcca 1620 acttcccaat ttgtatactc tgactgtcta acatgggctg aagcgcaatc actctgtttc 1680 acaatataaa cattattatc tcttactttc aataagtgac ttataatccc taagttttca 1740 ttcatcatgt ctagagccac acagacatct agaaacttga gtcttccact atccaaagat 1800 ctgttcactt gaagatcatt cataaagggt gccaaatgtt cttcaaatag tttggggtaa 1860 tttcttcgta tagaatgcaa tacatggttc atgcctaatt ggtcttctat ctgtcgtact 1920 gctttgggtt taacagccca gaagaaattc ttattacata agaccagagg ggcctgtgga 1980 ctcttaatag cagaaaacac ccactcccct aactcacagg catttgtcag caccaaagag 2040 aagtaatccc acaaaattgg tttagaaaat tggttaactt ctttaagtga tttttgacag 2100 taaataactt taggctttct ctcacaaatt ccacaaagac atggcattat tcgagtaaat 2160 atgtccttta tatacagaaa tccgccttta ccatccctaa cacacttact ccccatactc 2220 ttacaaaacc caatgaagcc tgaggcaaca gaagactgaa atgcagattt gttgattgac 2280 tctgccaaga tcttcttcac gcctttgtg aaatttcttg acagcctgga ctgtattgtc 2340 cttatcaatg ttggcatctc ttctttctct aacactcttc gacttgtcat gagtttggtc 2400 ctcaagacca acctcaagtc cccaaagctc gctaaattga cccatctgta gtctagagtt 2460

SEQ ID NO.	Description	Sequence
		tgtctgattt catcttcact acacccggca tattgcagga atccggataa agcctcatcc 2520 cctcccctgc ttatcaagtt gataaggttt tcctcaaaga ttttgcctct cttaatgtca 2580 ttgaacactt tcctcgcgca gttccttata aacattgtct ccttatcatc agaaaaaata 2640 gcttcaattt tcctctgtag acggtaccct ctagacccat caacccagtc tttgacatct 2700 tgttcttcaa tagctccaaa cggagtctct ctgtatccag agtatctaat caattggttg 2760 actctaatgg aaatctttga cactatatga gtgctaaccc cattagcaat acattgatca 2820 caaattgtgt ctatggtctc tgacagttgt gttggagttt tacacttaac gttgtgtaga 2880 gcagcagaca caaacttggt gagtaaagga gtctcttcac ccatgacaaa aatcttgac 2940 ttaaactcag caacaaaagt tcctatcaca ctctttgggc tgataaactt gtttaattta 3000 gaagataaga attcatggaa gcacaccatt tccagcagtt ctgtcctgtc ttgaaacttt 3060 tcatcactaa ggcaaggaat ttttataagg ctaacctggt catcgctgga ggtataagtg 3120 acaggtatca catcatacaa taagtcaagt gcataacaca gaaattgttc agtaattagc 3180 ccatataaat ctgatgtgtt gtgcaagatt ccctggccca tgtccaagac agacattata 3240 tggctgggga cctggtccct tgactgcaga tactggtgaa aaaactcttc accaacacta 3300 gtacagtcac aacccattaa acctaaagat ctcttcaatt tccctacaca gtaggcttct 3360 gcaacattaa ttggaacttc aacgacctta tgaagatgcc atttgagaat gttcattact 3420 ggttcaagat tcacctttgt tctatctctg ggattcttca attctaatgt gtacaaaaaa 3480 gaaaggaaaa gtgctgggct catagttggt ccccatttgg agtggtcata tgaacaggac 3540 aagtcaccat tgttaacagc cattttcata tcacagattg cacgttcgaa ttccttttct 3600 gaattcaagc atgtgtattt cattgaacta cccacagctt ctgagaagtc ttcaactaac 3660 ctggtcatca gcttagtgtt gaggtctccc acatacagtt ctctatttga gccaacctgc 3720 tccttataac ttagtccaaa tttcaagttc cctgtatttg agctgatgct tgtgaactct 3780 gtaggagagt cgtctgaata gaaacataaa ttccgtaggg ctgcatttgt aaaataactt 3840 ttgtctagct tatcagcaat ggcttcagaa ttgctttccc tggtactaag ccgaacctca 3900 tcctttagtc tcagaacttc actggaaaag cccaatctag atctacttct atgctcataa 3960 ctacccaatt tctgatcata atgtccttga attaaaagat acttgaagca ttcaaagaat 4020 tcatcttctt ggtaggctat tgttgtcaaa tttttaata acaaacccaa agggcagatg 4080 tcctgcggtg cttcaagaaa ataagtcaat ttaaatggag atagataaac agcatcacat 4140 aactctttat acacatcaga cctgagcaca tctggatcaa aatccttcac ctcatgcatt 4200 gacacctctg ctttaatctc tctcaacact ccaaaagggg cccacaatga ctcaagagac 4260 tctcgctcat caacagatgg attttttgat

SEQ ID NO.	Description	Sequence
		ttcaacttgg tgatctcaac ttttgtcccc 4320 tcactattag ccatcttggc tagtgtcatt tgtacgtcat ttctaatacc ctcaaaggcc 4380 cttacttgat cctctgttaa actctcatac atcactgata attcttcttg attggttctg 4440 gttcttgaac cggtgctcac aagacctgtt agatttttta atattaagta gtccatggaa 4500 tcaggatcaa gattatacct gccttttgtt ttaaacctct cagccatagt agaaacgcat 4560 gttgaaacaa gtttctcctt atcataaaca gaaagaatat ttccaagttc gtcgagcttg 4620 gggattacca cacttttatt gcttgacaga tccagagctg tgctagtgat gttaggcctg 4680 tagggattgc ttttcagttc acctgtaact ttaagtcttc ctctattgaa gagagaaatg 4740 cagaaggaca aaatctcttt acacactcct ggaatttgag tatctgagga agtcttagcc 4800 tctttggaaa agaatctgtc caatcctctt atcatggtgt cctcttgttc cagtgttaga 4860 ctcccactta gagggggtt tacaacaaca caatcaaact tgactttggg ctcaataaac 4920 ttctcaaaac actttatttg atctgtcagg cgatcaggtg tctctttggt taccaagtga 4980 cacagataac taacatttaa tagatattta aaccttcttg caaagtaaag atctgcatct 5040 tccccttcac ccaaaattgt ctggaaaagt tccacagcca tcctctgaat cagcacctct 5100 gatccagaca tgcagtcgac ccttaacttt gacatcaaat ccacatgatg gatttgattt 5160 gcatatgcca tcaagaaata tcttagacct tgtaaaaatg tctggttcct tttggaaggg 5220 gaacagagta cagctaacac taacaatctt aatattggcc ttgtcattgt catgagttcg 5280 tggctaaaat ccaaccagct ggtcatttcc tcacacattt caattaacac atcctccgaa 5340 aatataggca ggaaaaatct ctttggatca cagtaaaaag agccttgttc ttccaatacc 5400 ccattgatgg atagatagat agaatagcac cttgacttct cacctgtttt ttggtaaaac 5460 aagagaccaa atgtattctt tgtcagatga aatctttgta cataacactc tcttagtcta 5520 acattcccaa aatatctaga atactctctt tcattgatta acaatcggga ggaaaatgat 5580 gtcttcatcg agttgaccaa tgcaagggaa atggaggaca aaatcctaaa taatttcttc 5640 tgctcacctt ccactaagct gctgaatggc tgatgtctac agattttctc aaattccttg 5700 ttaatagtat atctcatcac tggtctgtca gaaacaagtg cctgagctaa aatcatcaag 5760 ctatccatat cagggtgttt tattagtttt tccagctgtg accagagatc ttgatgagag 5820 ttcttcaatg ttctggaaca cgcttgaacc cacttggggc tggtcatcaa tttcttcctt 5880 attagtttaa tcgcctccag aatatctaga agtctgtcat tgactaacat taacatttgt 5940 ccaacaacta ttcccgcatt tcttaacctt acaattgcat catcatgcgt tttgaaaaga 6000 tcacaaagta aattgagtaa aactaagtcc agaaacagta aagtgtttct cctggtgttg 6060 aaaactttta gacctttcac tttgttacac acggaaaggg cttgaagata acacctctct 6120

SEQ ID NO.	Description	Sequence
		acagcatcaa tagatataga attctcatct gactggcttt ccatgttgac ttcatctatt 6180 ggatgcaatg cgatagagta gactacatcc atcaacttgt ttgcacaaaa agggcagctg 6240 ggcacatcac tgtctttgtg gcttcctaat aagatcaagt catttataag cttagacttt 6300 tgtgaaaatt tgaatttccc caactgcttg tcaaaaatct ccttcttaaa ccaaaacctt 6360 aactttatga gttcttctct tatgacagat tctctaatgt ctcctctaac cccaacaaag 6420 agggattcat ttaacctctc atcataaccc aaagaattct ttttcaagca ttcgatgttt 6480 tctaatccca agctctggtt ttttgtgttg gacaaactat ggatcaatcg ctggtattct 6540 tgttcttcaa tattaatctc ttgcataaat tttgatttct ttaggatgtc gatcagcaac 6600 caccgaactc tttcaacaac ccaatcagca aggaatctat tgctgtagct agatctgcca 6660 tcaaccacag gaaccaacgt aatccctgcc cttagtaggt cggactttag gtttaagagc 6720 tttgacatgt cactcttcca ttttctctca aactcatcag gattgaccct aacaaaggtt 6780 tccaatagga tgagtgtttt ccctgtgagt ttgaagccat ccggaatgac ttttggaagg 6840 gtgggacata gtatgccata gtcagacagg atcacatcaa caaacttctg atctgaattg 6900 atctgacagg cgtgtgcctc acaggactca agctctacta aacttgacag aagtttgaac 6960 ccttccaaca acagagagct ggggtgatgt tgagataaaa agatgtccct ttggtatgct 7020 agctcctgtc tttctggaaa atgctttcta ataaggcttt ttatttcatt tactgattcc 7080 tccatgctca agtgccgcct aggatcctcg gtgcg 7115
24	Amino acid sequence of a P815 mouse mastocytoma-derived self antigen P1A	Met Ser Asp Asn Lys Lys Pro Asp Lys Ala His Ser Gly Ser Gly Gly Asp Gly Asp Gly Asn Arg Cys Asn Leu Leu His Arg Tyr Ser Leu Glu Glu Ile Leu Pro Tyr Leu Gly Trp Leu Val Phe Ala Val Val Thr Thr Ser Phe Leu Ala Leu Gln Met Phe Ile Asp Ala Leu Tyr Glu Glu Gln Tyr Glu Arg Asp Val Ala Trp Ile Ala Arg Gln Ser Lys Arg Met Ser Ser Val Asp Glu Asp Glu Asp Asp Glu Asp Asp Glu Asp Asp Tyr Tyr Asp Asp Glu Asp Asp Asp Asp Asp Ala Phe Tyr Asp Asp Glu Asp Asp Glu Glu Glu Glu Leu Glu Asn Leu Met Asp Asp Glu Ser Glu Asp Glu Ala Glu Glu Glu Met Ser Val Glu Met Gly Ala Gly Ala Glu Glu Met Gly Ala Gly Ala Asn Cys Ala Cys Val Pro Gly His His Leu Arg Lys Asn Glu Val Lys Cys Arg Met Ile Tyr Phe Phe His Asp Pro Asn Phe Leu Val Ser Ile Pro Val Asn Pro Lys Glu Gln Met Glu Cys Arg Cys Glu Asn Ala Asp Glu Glu Val Ala Met Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Met Gly Asn Pro Asp Gly Phe Ser Pro

The embodiments of the present invention for which an exclusive property or privilege is claimed are defined as follows:

1. A tri-segmented arenavirus particle comprising one L segment and two S segments, wherein one of the two S segments is selected from the group consisting of:

(i) an S segment, wherein the open reading frame ("ORF") encoding the nucleoprotein ("NP") is under control of an arenavirus genomic 5' untranslated region ("UTR");

(ii) an S segment, wherein the ORF encoding the matrix protein Z ("Z protein") is under control of an arenavirus genomic 5' UTR;

(iii) an S segment, wherein the ORF encoding the RNA dependent RNA polymerase L ("L protein") is under control of an arenavirus genomic 5' UTR;

(iv) an S segment, wherein the ORF encoding the glycoprotein ("GP") is under control of an arenavirus genomic 3' UTR;

(v) an S segment, wherein the ORF encoding the L protein is under control of an arenavirus genomic 3' UTR; and

(vi) an S segment, wherein the ORF encoding the Z protein is under control of an arenavirus genomic 3' UTR.

2. A tri-segmented arenavirus particle comprising two L segments and one S segment, wherein one of the two L segments is selected from the group consisting of:

(i) an L segment, wherein the open reading frame ("ORF") encoding the glycoprotein ("GP") is under control of an arenavirus genomic 5' untranslated region ("UTR");

(ii) an L segment, wherein the ORF encoding the nucleoprotein ("NP") is under control of an arenavirus genomic 5' UTR;

Date Reçue/Date Received 2022-12-15

(iii) an L segment, wherein the ORF encoding the RNA dependent RNA polymerase L ("L protein") is under control of an arenavirus genomic 5' UTR;

(iv) an L segment, wherein the ORF encoding the GP is under control of an arenavirus genomic 3' UTR;

(v) an L segment, wherein the ORF encoding the NP is under control of an arenavirus genomic 3' UTR; and

(vi) an L segment, wherein the ORF encoding the matrix protein Z ("Z protein") is under control of an arenavirus genomic 3' UTR.

3. The tri-segmented arenavirus particle of claim 1, wherein inter-segmental recombination of the two S segments, uniting two arenavirus ORFs on only one instead of two separate segments, abrogates viral promoter activity.

4. The tri-segmented arenavirus particle of claim 2, wherein inter-segmental recombination of the two L segments, uniting two arenavirus ORFs on only one instead of two separate segments, abrogates viral promoter activity.

5. The tri-segmented arenavirus particle of any one of claims 1 to 4, wherein propagation of the tri-segmented arenavirus particle does not result in a replication-competent bi-segmented viral particle after 70 days of persistent infection in mice lacking type I interferon receptor, type II interferon receptor and recombination activating gene 1 (RAG1) and having been infected with 10^4 PFU of the tri-segmented arenavirus particle.

6. The tri-segmented arenavirus particle of any one of claims 1 to 5, wherein the arenavirus genomic 3' UTR is the 3' UTR of the arenavirus S segment or the arenavirus L segment, and wherein the arenavirus genomic 5' UTR is the 5' UTR of the arenavirus S segment or the arenavirus L segment.

7. The tri-segmented arenavirus particle of claim 1 or 3, wherein the two S segments comprise (i) one or two heterologous ORFs from an organism other than an arenavirus; or (ii)

Date Reçue/Date Received 2022-12-15

one or two duplicated arenavirus ORFs; or (iii) one heterologous ORF from an organism other than an arenavirus and one duplicated arenavirus ORF.

8. The tri-segmented arenavirus particle of claim 2 or 4, wherein the two L segments comprise (i) one or two heterologous ORFs from an organism other than an arenavirus; or (ii) one or two duplicated arenavirus ORFs; or (iii) one heterologous ORF from an organism other than an arenavirus and one duplicated arenavirus ORF.

9. The tri-segmented arenavirus particle of claim 7 or 8, wherein at least one heterologous ORF encodes an antigen derived from an infectious organism, tumor, or allergen.

10. The tri-segmented arenavirus particle of claim 9, wherein the at least one heterologous ORF encodes an antigen selected from the group consisting of: human immunodeficiency virus antigens, hepatitis C virus antigens, hepatitis B virus antigens, papillomavirus antigens, varizella zoster virus antigens, cytomegalovirus antigens, mycobacterium tuberculosis antigens, and tumor associated antigens.

11. The tri-segmented arenavirus particle of claim 7 or 8, wherein at least one heterologous ORF encodes a fluorescent protein.

12. The tri-segmented arenavirus particle of claim 11, wherein the fluorescent protein is green fluorescent protein or red fluorescent protein.

13. The tri-segmented arenavirus particle of any one of claims 1 to 12, wherein the tri-segmented arenavirus particle comprises all four arenavirus ORFs, and wherein the tri-segmented arenavirus particle is infectious and replication competent.

14. The tri-segmented arenavirus particle of any one of claims 1 to 12, wherein the tri-segmented arenavirus particle lacks one or more of the four arenavirus ORFs, wherein the tri-segmented arenavirus particle is infectious but unable to produce further infectious progeny in non-complementing cells.

15. The tri-segmented arenavirus particle of any one of claims 1 to 12, wherein the tri-segmented arenavirus particle lacks one of the four arenavirus ORFs, wherein the tri-segmented

Date Reçue/Date Received 2022-12-15

arenavirus particle is infectious but unable to produce further infectious progeny in non-complementing cells.

16. The tri-segmented arenavirus particle of claim 14 or 15, wherein the arenavirus particle lacks the GP ORF.

17. A tri-segmented arenavirus particle comprising one L segment and two S segments, wherein a first S segment is engineered to carry an open reading frame ("ORF") encoding the glycoprotein ("GP") in a position under control of an arenavirus genomic 3' untranslated region ("UTR") and an ORF encoding a first gene of interest in a position under control of an arenavirus genomic 5' UTR and a second S segment is engineered to carry an ORF encoding the nucleoprotein ("NP") in a position under control of an arenavirus genomic 3' UTR and an ORF encoding a second gene of interest in a position under control of an arenavirus genomic 5' UTR.

18. A tri-segmented arenavirus particle comprising one L segment and two S segments, wherein a first S segment is engineered to carry an open reading frame ("ORF") encoding the glycoprotein ("GP") in a position under control of an arenavirus genomic 5' untranslated region ("UTR") and an ORF encoding a first gene of interest in a position under control of an arenavirus genomic 3' UTR and a second S segment is engineered to carry an ORF encoding the nucleoprotein ("NP") in a position under control of an arenavirus genomic 5' UTR and an ORF encoding a second gene of interest in a position under control of an arenavirus genomic 3' UTR.

19. The tri-segmented arenavirus particle of claim 17 or 18, wherein the first gene of interest encodes an antigen derived from an infectious organism, tumor, or allergen, and wherein the second gene of interest encodes an antigen derived from an infectious organism, tumor, or allergen.

20. The tri-segmented arenavirus particle of claim 19, wherein the first or second gene of interest encodes an antigen selected from the group consisting of: human immunodeficiency virus antigens, hepatitis C virus antigens, hepatitis B virus antigens, papillomavirus antigens, varizella zoster virus antigens, cytomegalovirus antigens, mycobacterium tuberculosis antigens, and tumor associated antigens.

Date Reçue/Date Received 2022-12-15

21. The tri-segmented arenavirus particle of claim 17 or 18, wherein at least one of the two genes of interest encodes a fluorescent protein.

22. The tri-segmented arenavirus particle of claim 21, wherein the fluorescent protein is green fluorescent protein or red fluorescent protein.

23. A cDNA encoding the tri-segmented arenavirus particle genome of any one of claims 1 to 22.

24. A DNA expression vector comprising the cDNA of claim 23.

25. A host cell comprising the tri-segmented arenavirus particle of any one of claims 1 to 22, the cDNA of claim 23, or the DNA expression vector of claim 24.

26. The tri-segmented arenavirus particle of any one of claims 1 to 13 or 17 to 22, wherein the tri-segmented arenavirus particle is attenuated.

27. A method of generating the tri-segmented arenavirus particle of any one of claims 1, 3, 17 and 18, wherein the method comprises:

(i) transfecting into a host cell one or more cDNAs of the one L segment and two S segments;

(ii) maintaining the host cell under conditions suitable for virus formation; and

(iii) harvesting the arenavirus particle.

28. A method of generating the tri-segmented arenavirus particle of claim 2 or 4, wherein the method comprises:

(i) transfecting into a host cell one or more cDNAs of the two L segments and one S segment;

(ii) maintaining the host cell under conditions suitable for virus formation; and

(iii) harvesting the arenavirus particle.

Date Reçue/Date Received 2022-12-15

29. The method of claim 27, wherein transcription of the one L segment and two S segments is performed using a bidirectional promoter.

30. The method of claim 28, wherein transcription of the two L segments and one S segment is performed using a bidirectional promoter.

31. The method of claim 27 or 28, wherein the method further comprises transfecting into the host cell one or more nucleic acids encoding an arenavirus polymerase.

32. The method of claim 31, wherein the arenavirus polymerase is the L protein.

33. The method of any one of claims 27 to 32, wherein the method further comprises transfecting into the host cell one or more nucleic acids encoding the NP.

34. The method of claim 27, wherein transcription of the L segment, and the two S segments are each under the control of a promoter selected from the group consisting of:

(i) a RNA polymerase I promoter;

(ii) a RNA polymerase II promoter; and

(iii) a T7 promoter.

35. The method of claim 28, wherein transcription of the two L segments, and the S segment are each under the control of a promoter selected from the group consisting of:

(i) a RNA polymerase I promoter;

(ii) a RNA polymerase II promoter; and

(iii) a T7 promoter.

36. The tri-segmented arenavirus particle of claim 5, wherein the tri-segmented arenavirus particle has the same tropism as the bi-segmented arenavirus particle.

37. The tri-segmented arenavirus particle of any one of claims 1 to 12 or 14 to 22, wherein the tri-segmented arenavirus particle is replication deficient.

Date Reçue/Date Received 2022-12-15

38. A vaccine comprising the tri-segmented arenavirus particle of any one of claims 1 to 22, 26, 36, or 37 and a pharmaceutically acceptable carrier.

39. A pharmaceutical composition comprising the tri-segmented arenavirus particle of any one of the claims 1 to 22, 26, 36, or 37 and a pharmaceutically acceptable carrier.

40. The tri-segmented arenavirus particle of any one of claims 1 to 22, 26, 36, or 37, wherein the tri-segmented arenavirus particle is derived from lymphocytic choriomeningitis virus ("LCMV").

41. The tri-segmented arenavirus particle of claim 40, wherein the LCMV is Molomut-Padnos strain, Armstrong strain, or Armstrong Clone 13 strain.

42. The tri-segmented arenavirus particle of any one of claims 1 to 22, 26, 36, or 37, wherein the tri-segmented arenavirus particle is derived from Junin virus vaccine Candid #1, or Junin virus vaccine XJ Clone 3 strain.

43. An arenavirus genomic segment, wherein the genomic segment is engineered to carry a viral open reading frame ("ORF") in a position other than the wild-type position of the ORF, wherein the arenavirus genomic segment is selected from the group consisting of:

(i) an S segment, wherein the ORF encoding the nucleoprotein ("NP") is under control of an arenavirus genomic 5' untranslated region ("UTR");

(ii) an S segment, wherein the ORF encoding the matrix protein Z ("Z protein") is under control of an arenavirus genomic 5' UTR;

(iii) an S segment, wherein the ORF encoding the RNA dependent RNA polymerase L ("L protein") is under control of an arenavirus genomic 5' UTR;

(iv) an S segment, wherein the ORF encoding the glycoprotein ("GP") is under control of an arenavirus genomic 3' UTR;

(v) an S segment, wherein the ORF encoding the L protein is under control of an arenavirus genomic 3' UTR;

Date Reçue/Date Received 2022-12-15

(vi) an S segment, wherein the ORF encoding the Z protein is under control of an arenavirus genomic 3’ UTR;

(vii) an L segment, wherein the ORF encoding the GP is under control of an arenavirus genomic 5’ UTR;

(viii) an L segment, wherein the ORF encoding the NP is under control of an arenavirus genomic 5’ UTR;

(ix) an L segment, wherein the ORF encoding the L protein is under control of an arenavirus genomic 5’ UTR;

(x) an L segment, wherein the ORF encoding the GP is under control of an arenavirus genomic 3’ UTR;

(xi) an L segment, wherein the ORF encoding the NP is under control of an arenavirus genomic 3’ UTR; and

(xii) an L segment, wherein the ORF encoding the Z protein is under control of an arenavirus genomic 3’ UTR.

44. The arenavirus genomic segment of claim 43, wherein the arenavirus genomic 3’ UTR is the 3’ UTR of the arenavirus S segment or the arenavirus L segment, and wherein the arenavirus genomic 5’ UTR is the 5’ UTR of the arenavirus S segment or the arenavirus L segment.

45. A cDNA encoding the arenavirus genomic segment of claim 43.

46. A DNA expression vector comprising the cDNA of claim 45.

47. A host cell comprising the arenavirus genomic segment of claim 43, the cDNA of claim 45, or the DNA expression vector of claim 46.

48. An arenavirus particle comprising the arenavirus genomic segment of claim 43 or 44 and a second arenavirus genomic segment so that the arenavirus particle comprises an S segment and an L segment.

Date Reçue/Date Received 2022-12-15

49. The arenavirus particle of claim 48, wherein the arenavirus particle is infectious and replication competent.

50. The arenavirus particle of claim 48, wherein the arenavirus particle is attenuated.

51. The arenavirus particle of claim 48, wherein the arenavirus particle is infectious but unable to produce further infectious progeny in non-complementing cells.

52. The arenavirus particle of claim 51, wherein at least one of the four ORFs encoding GP, NP, Z protein, and L protein is removed or functionally inactivated.

53. The arenavirus particle of claim 51, wherein at least one of the four ORFs encoding GP, NP, Z protein, and L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus.

54. The arenavirus particle of claim 51, wherein only one of the four ORFs encoding GP, NP, Z protein and L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus.

55. The arenavirus particle of claim 53 or 54, wherein the ORF encoding GP is removed and replaced with a heterologous ORF from an organism other than an arenavirus.

56. The arenavirus particle of claim 53 or 54, wherein the ORF encoding NP is removed and replaced with a heterologous ORF from an organism other than an arenavirus.

57. The arenavirus particle of claim 53 or 54, wherein the ORF encoding the Z protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus.

58. The arenavirus particle of claim 53 or 54, wherein the ORF encoding the L protein is removed and replaced with a heterologous ORF from an organism other than an arenavirus.

59. The arenavirus particle of any one of claims 53 to 58, wherein the heterologous ORF encodes a reporter protein.

60. The arenavirus particle of any one of claims 53 to 58, wherein the heterologous ORF encodes an antigen derived from an infectious organism, tumor, or allergen.

Date Reçue/Date Received 2022-12-15

61. The arenavirus particle of claim 60, wherein the heterologous ORF encodes an antigen selected from the group consisting of: human immunodeficiency virus antigens, hepatitis C virus antigens, hepatitis B virus antigens, papillomavirus antigens, varizella zoster virus antigens, cytomegalovirus antigens, mycobacterium tuberculosis antigens, and tumor associated antigens.

62. The arenavirus particle of anyone of claims 53 to 61, wherein the growth or infectivity of the arenavirus particle is not affected by the heterologous ORF from an organism other than an arenavirus.

63. A method of producing the arenavirus genomic segment of claim 43, wherein said method comprises transcribing the cDNA of claim 45.

64. A method of generating the arenavirus particle of claim 48, wherein the method comprises:

(i) transfecting into a host cell the cDNA of claim 45;

(ii) transfecting into the host cell a plasmid comprising the cDNA of the second arenavirus genomic segment;

(iii) maintaining the host cell under conditions suitable for virus formation; and

(iv) harvesting the arenavirus particle.

65. The method of claim 64, wherein transcription of the L segment and the S segment is performed using a bidirectional promoter.

66. The method of claim 64, wherein the method further comprises transfecting into the host cell one or more nucleic acids encoding an arenavirus polymerase.

67. The method of claim 66, wherein the arenavirus polymerase is the L protein.

68. The method of claim 64 or 66, wherein the method further comprises transfecting into the host cell one or more nucleic acids encoding the NP.

69. The method of claim 64, wherein transcription of the L segment, and the S segment are each under the control of a promoter selected from the group consisting of:

Date Reçue/Date Received 2022-12-15

(i) a RNA polymerase I promoter;

(ii) a RNA polymerase II promoter; and

(iii) a T7 promoter.

70. A vaccine comprising the arenavirus particle of any one of claims 48 to 62 and a pharmaceutically acceptable carrier.

71. A pharmaceutical composition comprising the arenavirus particle of any one of claims 48 to 62 and a pharmaceutically acceptable carrier.

72. The arenavirus genomic segment of claim 43 or the arenavirus particle of claim 48, wherein the arenavirus genomic segment or arenavirus particle is derived from lymphocytic choriomeningitis virus ("LCMV").

73. The arenavirus genomic segment or arenavirus particle of claim 72, wherein the LCMV is Molomut-Padnos strain, Armstrong strain, or Armstrong Clone 13 strain.

74. The arenavirus genomic segment of claim 43 or the arenavirus particle of claim 48, wherein the arenavirus genomic segment or arenavirus particle is derived from Junin virus vaccine Candid #1, or Junin virus vaccine XJ Clone 3 strain.

Date Reçue/Date Received 2022-12-15

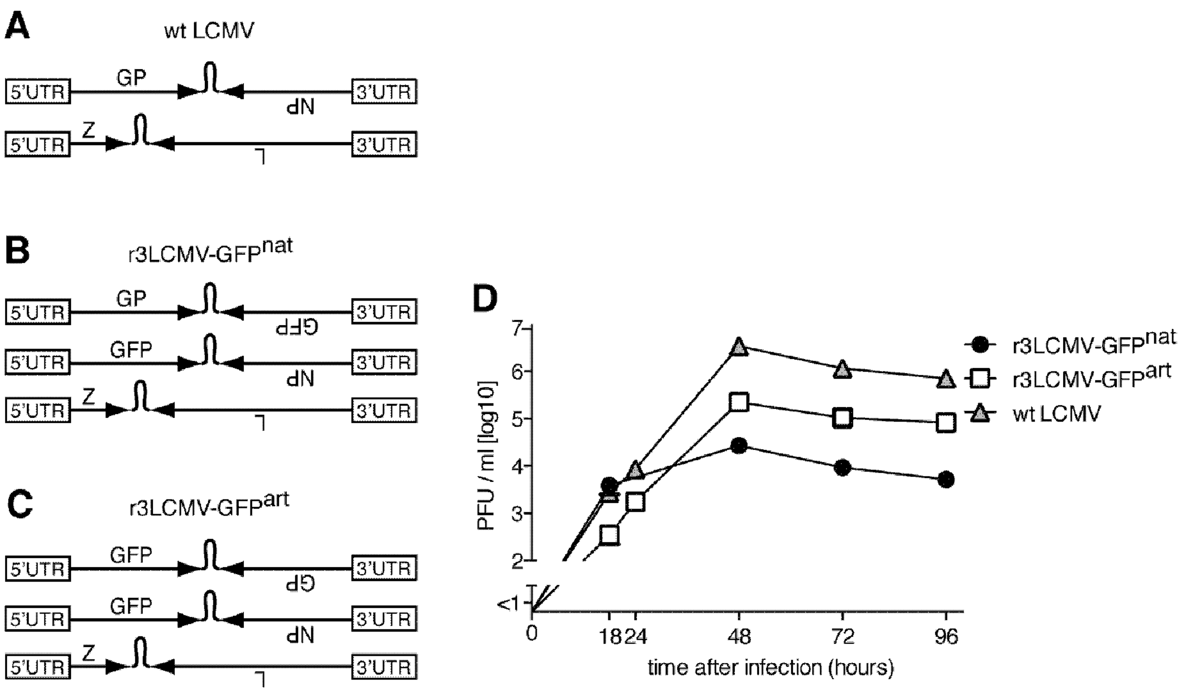

A
wt LCMV
5'UTR
GP
NP
3'UTR
5'UTR
Z
L
3'UTR
B
r3LCMV-GFPnat
5'UTR
GP
GFP
3'UTR
5'UTR
GFP
NP
3'UTR
5'UTR
Z
L
3'UTR
C
r3LCMV-GFPart
5'UTR
GFP
GP
3'UTR
5'UTR
GFP
NP
3'UTR
5'UTR
Z
L
3'UTR
D
PFU / ml [log10]
7
6
5
4
3
2
<1
0
18
24
48
72
96
time after infection (hours)
r3LCMV-GFPnat
r3LCMV-GFPart
wt LCMV

Figure 1

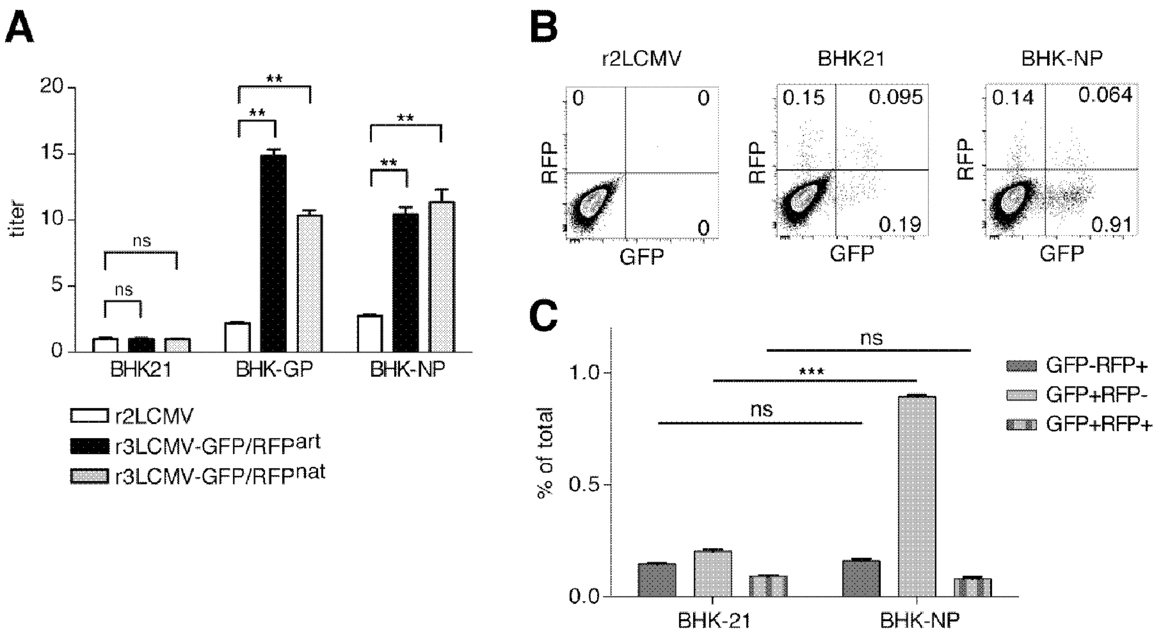

A
titer
20
15
10
5
0
ns
ns
**
**
**
**
BHK21
BHK-GP
BHK-NP
r2LCMV
r3LCMV-GFP/RFPart
r3LCMV-GFP/RFPnat
B
r2LCMV
BHK21
BHK-NP
RFP
GFP
0
0
0
0.15
0.095
0.19
0.14
0.064
0.91
C
% of total
1.0
0.5
0.0
ns
***
ns
GFP-RFP+
GFP+RFP-
GFP+RFP+
BHK-21
BHK-NP

Figure 2

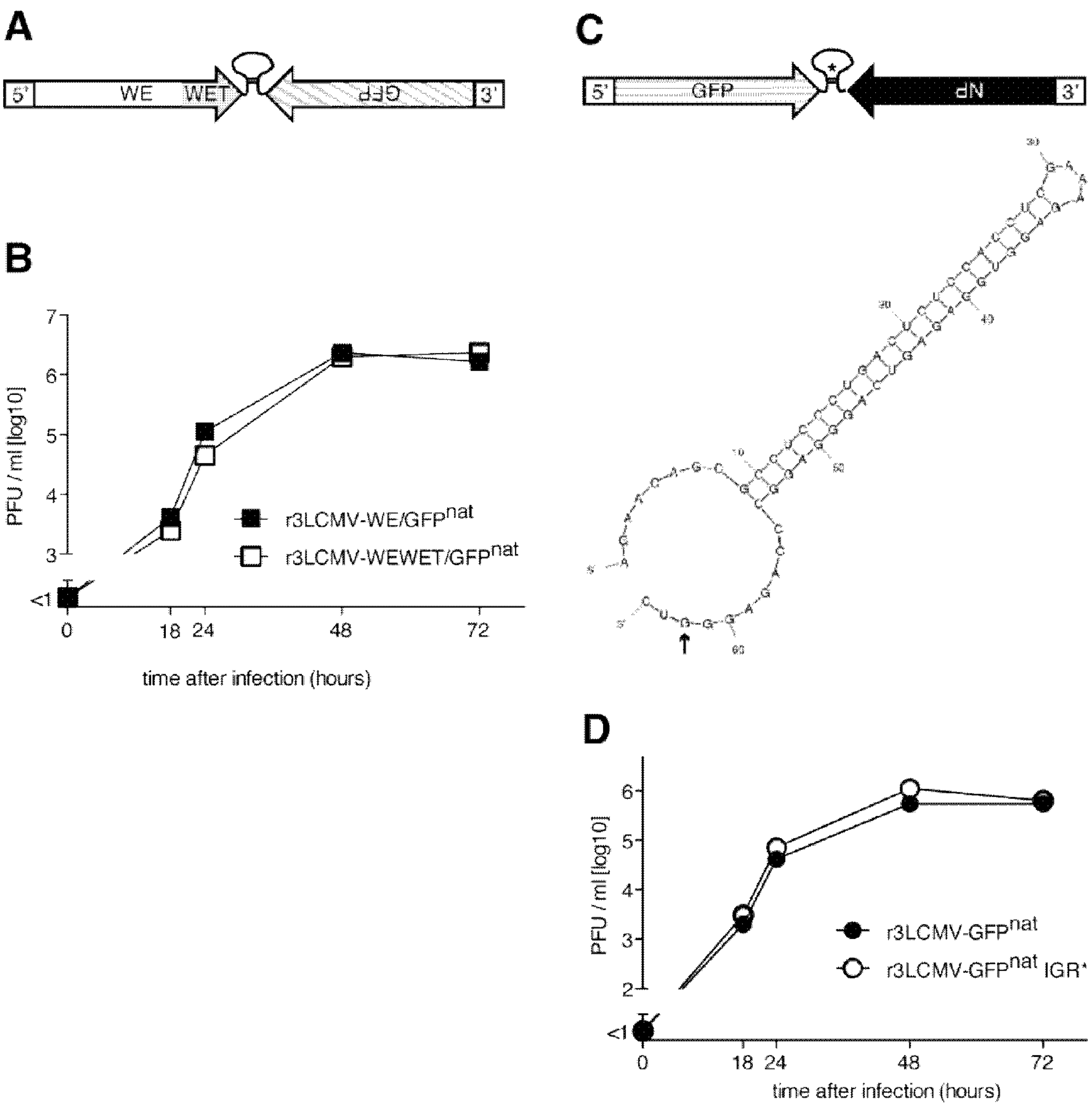

A
5'
WE
WET
GFP
3'
B
PFU / ml [log10]
r3LCMV-WE/GFP^nat
r3LCMV-WEWET/GFP^nat
time after infection (hours)
C
5'
GFP
NP
3'
D
PFU / ml [log10]
r3LCMV-GFP^nat
r3LCMV-GFP^nat IGR*
time after infection (hours)

Figure 3

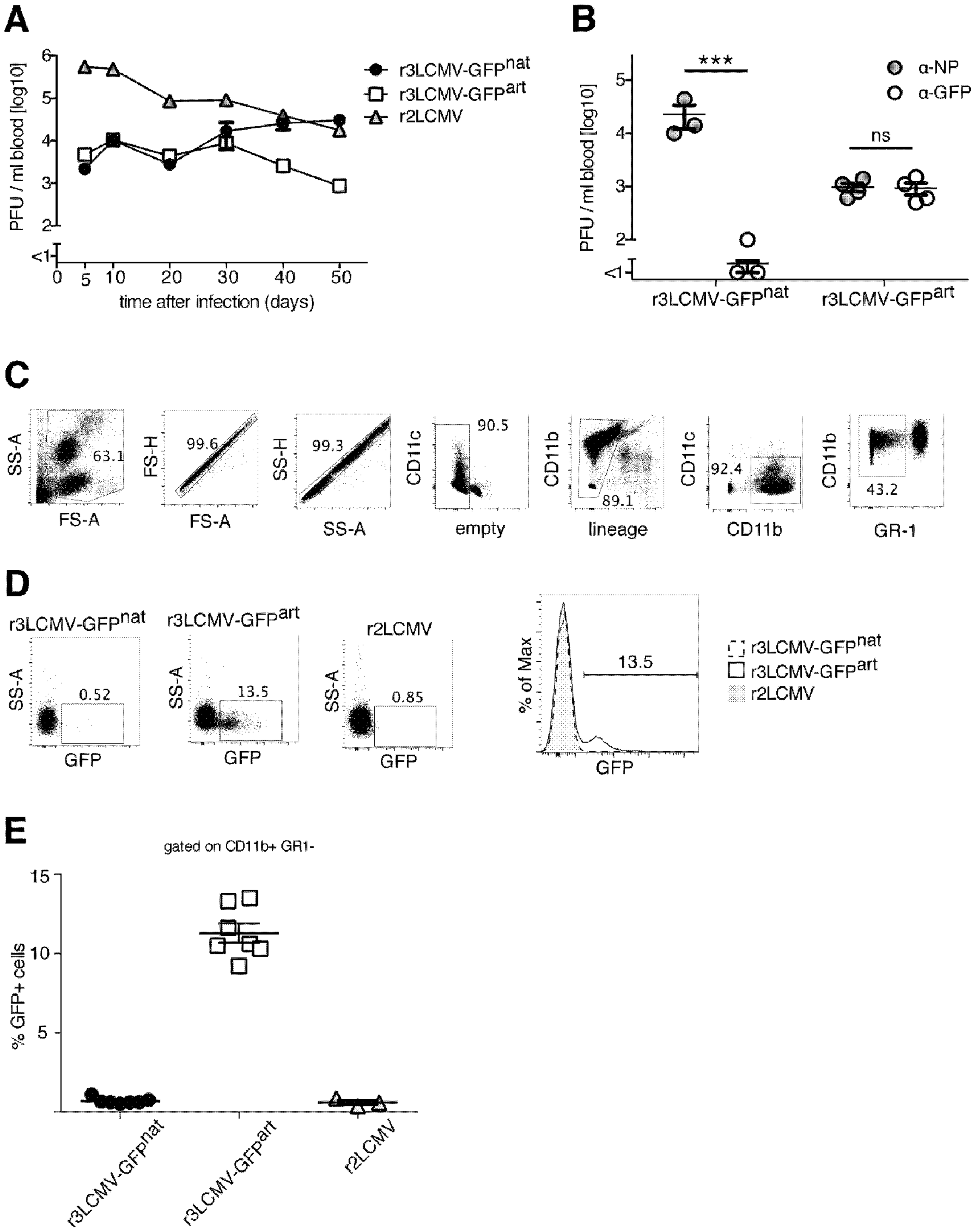
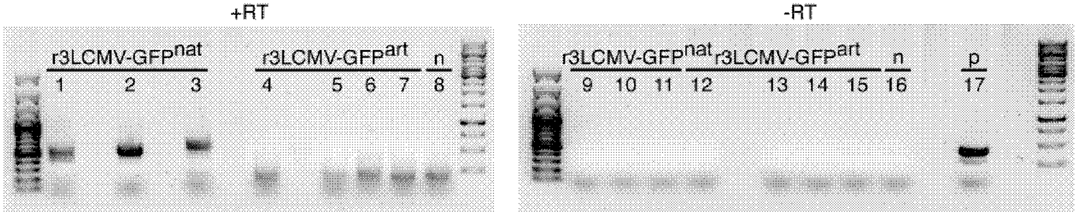

A
PFU / ml blood [log10]
time after infection (days)
r3LCMV-GFPnat
r3LCMV-GFPart
r2LCMV
B
***
ns
α-NP
α-GFP
PFU / ml blood [log10]
r3LCMV-GFPnat
r3LCMV-GFPart
C
SS-A
FS-A
63.1
FS-H
99.6
SS-H
99.3
CD11c
empty
90.5
CD11b
lineage
89.1
CD11c
CD11b
92.4
CD11b
GR-1
43.2
D
r3LCMV-GFPnat
r3LCMV-GFPart
r2LCMV
SS-A
GFP
0.52
13.5
0.85
% of Max
GFP
13.5
r3LCMV-GFPnat
r3LCMV-GFPart
r2LCMV
E
gated on CD11b+ GR1-
% GFP+ cells
r3LCMV-GFPnat
r3LCMV-GFPart
r2LCMV

Figure 4

A



B

r3LCMV-GFPnat #3

```
  1 AAGAAGCAGA TAACATGATC ACCGAGATGC TGAGGAAGGA CTACATCAAG AGACAGGGCA
 61 GCACCCCCCT GGCCCTCATG GATCTGCTCA TGTTCAGCAC CAGCGCCTAC CTCATCAGCA
121 TCTTCCTGCA CCTGGTGAAG ATCCCCACCC ACAGACACAT CAAGGGCGGC AGCTGCCCCA
181 AGCCCCACAG ACTCACCAAC AAGGGCATCT GCAGCTGCGG CGCCTTCAAG GTGCCCGGCG
241 TGAAAACCAT CTGGAAGAGG AGATAAAGAA CAGCGCCTCC CTGACTCTCC ACCTCGAAAG
301 AGGTGGAGAG TCAGGGAGGC CCAGAGGGTC TTACTTGTAC AGCTCGTCCA TGCCGAGAGT
361 GATCCCGGCG GCGGTCACGA ACTCCAGCAG GAAGAACAGC GCCTCCCTGA CTCTCCACCT
421 CGAAAGAGGT GGAGAGTCAG GGAGGCCCAG AGGTCTTAGA GTGTCACAAC ATTTGGGCCT
481 CTAAAAATTA GGTCATGTGG CAGAATGTTG TGAACAGTTT TCAGATCTGG GAGCC
```

C

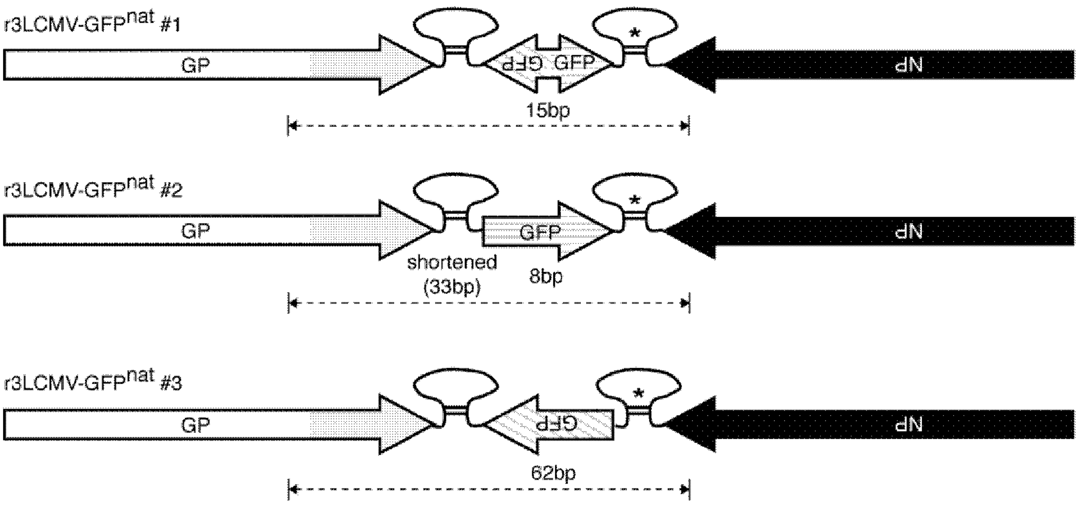


Figure 5

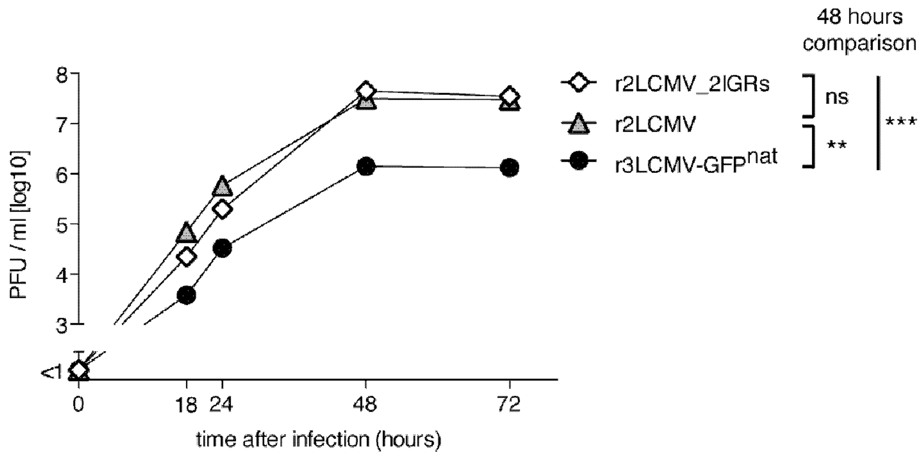

48 hours comparison
r2LCMV_2IGRs
r2LCMV
r3LCMV-GFPnat
ns
**
***
PFU / ml [log10]
8
7
6
5
4
3
<1
0
18
24
48
72
time after infection (hours)

Figure 6

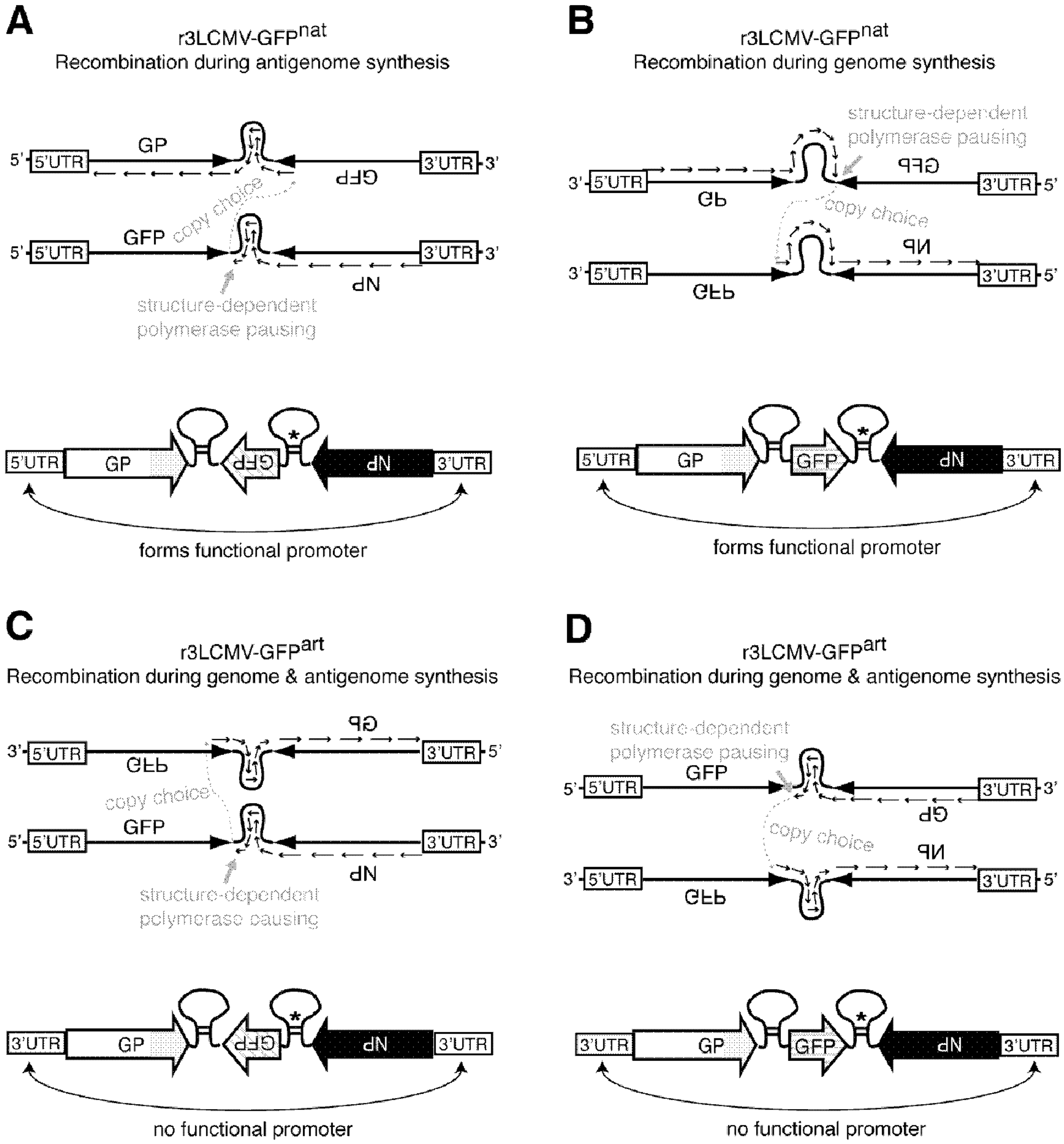

A
r3LCMV-GFPnat
Recombination during antigenome synthesis
5' 5'UTR GP GFP 3'UTR 3'
copy choice
5' 5'UTR GFP NP 3'UTR 3'
structure-dependent polymerase pausing
5'UTR GP GFP NP 3'UTR
forms functional promoter
B
r3LCMV-GFPnat
Recombination during genome synthesis
structure-dependent polymerase pausing
3' 5'UTR GP GFP 3'UTR 5'
copy choice
3' 5'UTR GFP NP 3'UTR 5'
5'UTR GP GFP NP 3'UTR
forms functional promoter
C
r3LCMV-GFPart
Recombination during genome & antigenome synthesis
3' 5'UTR GFP GP 3'UTR 5'
copy choice
5' 5'UTR GFP NP 3'UTR 3'
structure-dependent polymerase pausing
3'UTR GP GFP NP 3'UTR
no functional promoter
D
r3LCMV-GFPart
Recombination during genome & antigenome synthesis
structure-dependent polymerase pausing
5' 5'UTR GFP GP 3'UTR 3'
copy choice
3' 5'UTR GFP NP 3'UTR 5'
3'UTR GP GFP NP 3'UTR
no functional promoter

Figure 7

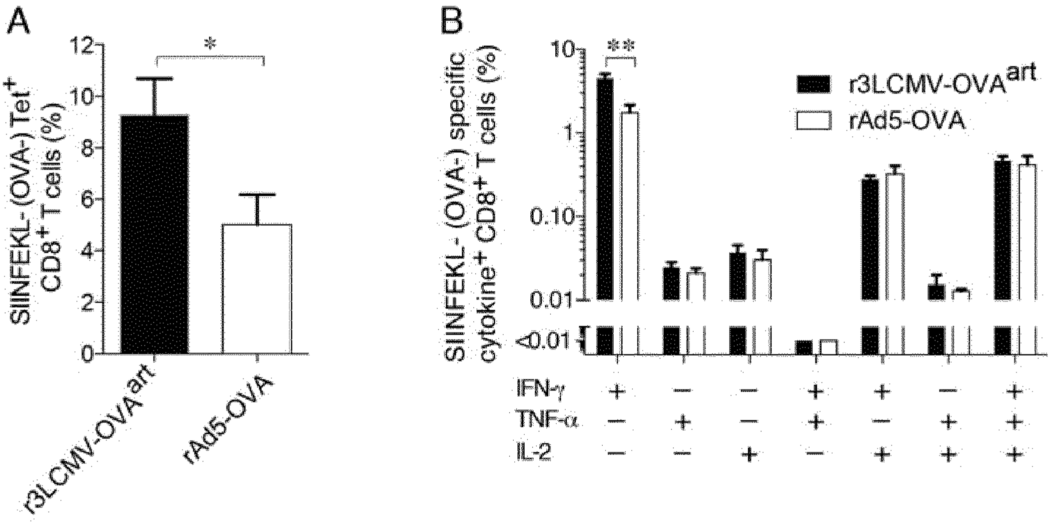

A
SIINFEKL- (OVA-) Tet+ CD8+ T cells (%)
12
10
8
6
4
2
0
*
r3LCMV-OVAart
rAd5-OVA
B
SIINFEKL- (OVA-) specific cytokine+ CD8+ T cells (%)
10
1
0.10
0.01
<0.01
**
r3LCMV-OVAart
rAd5-OVA
IFN-γ + − − + + − +
TNF-α − + − + − + +
IL-2 − − + − + + +

Figure 8

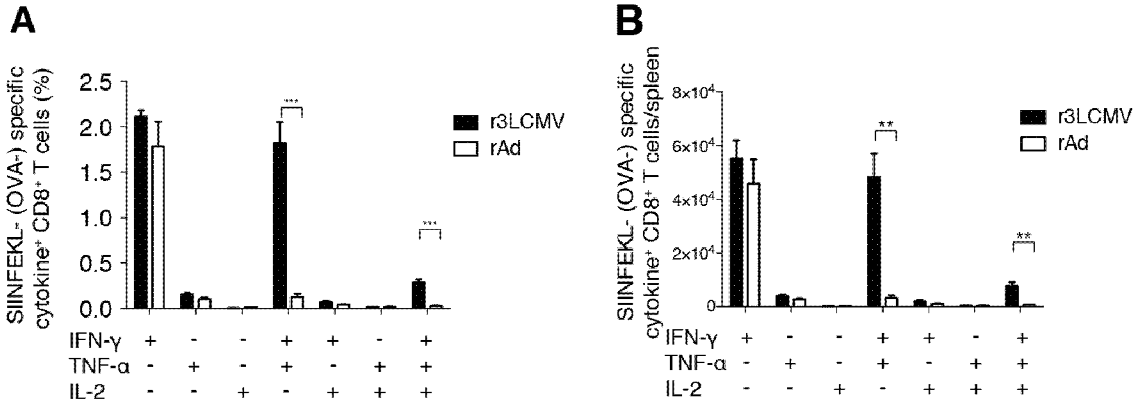

A
SIINFEKL- (OVA-) specific cytokine+ CD8+ T cells (%)
2.5
2.0
1.5
1.0
0.5
0.0
r3LCMV
rAd
IFN-γ
TNF-α
IL-2
B
SIINFEKL- (OVA-) specific cytokine+ CD8+ T cells/spleen
8x10^4
6x10^4
4x10^4
2x10^4
0
**
r3LCMV
rAd
IFN-γ
TNF-α
IL-2

Figure 9

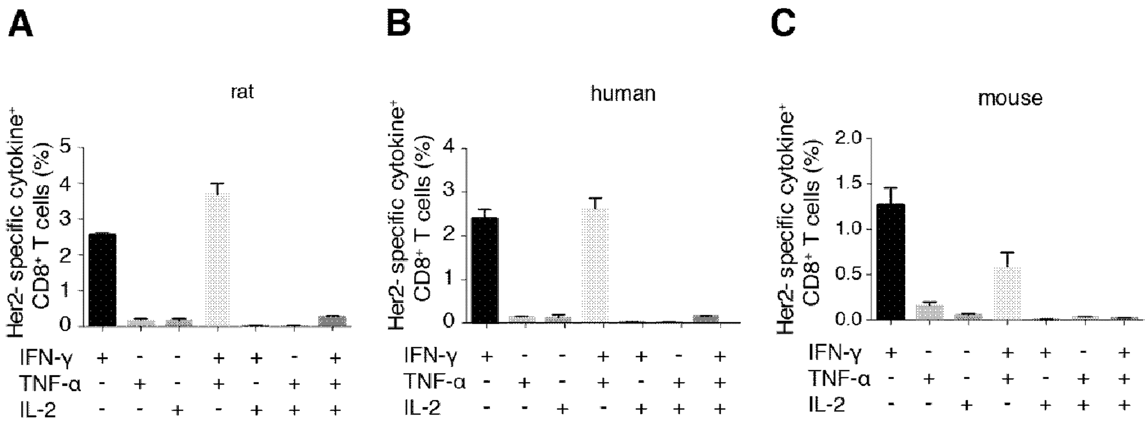
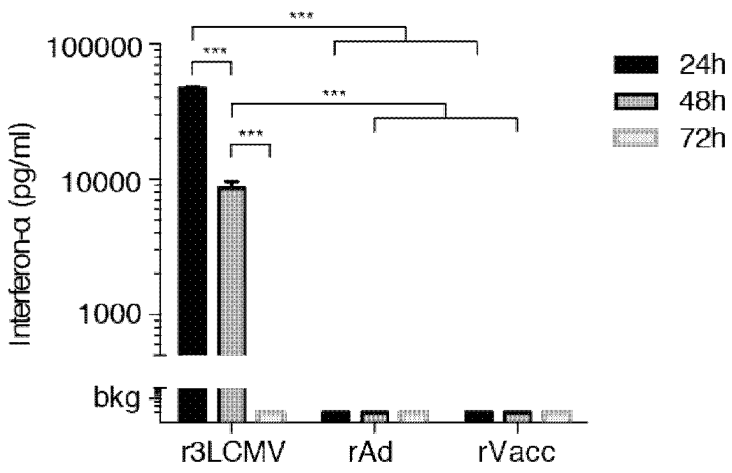

A
B
C
rat
human
mouse
Her2- specific cytokine+ CD8+ T cells (%)
IFN-γ
TNF-α
IL-2

Figure 10



100000
10000
1000
bkg
Interferon-α (pg/ml)
***
***
***
***
24h
48h
72h
r3LCMV
rAd
rVacc

Figure 11

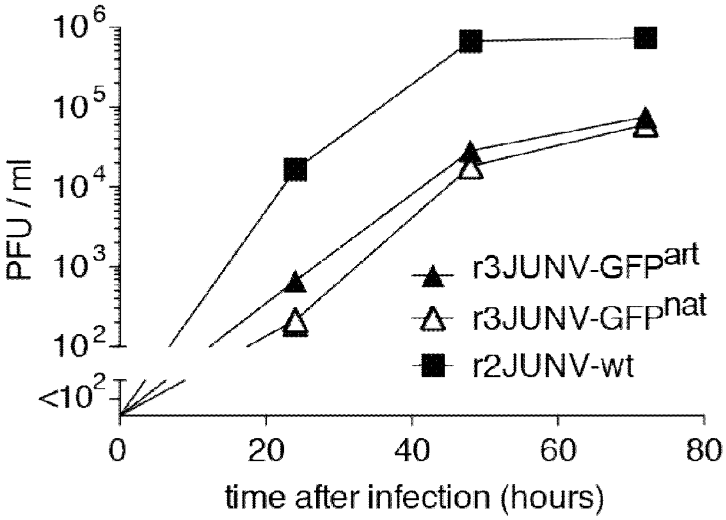

PFU / ml
10^6
10^5
10^4
10^3
10^2
<10^2
0
20
40
60
80
time after infection (hours)
r3JUNV-GFP^art
r3JUNV-GFP^nat
r2JUNV-wt

Figure 12

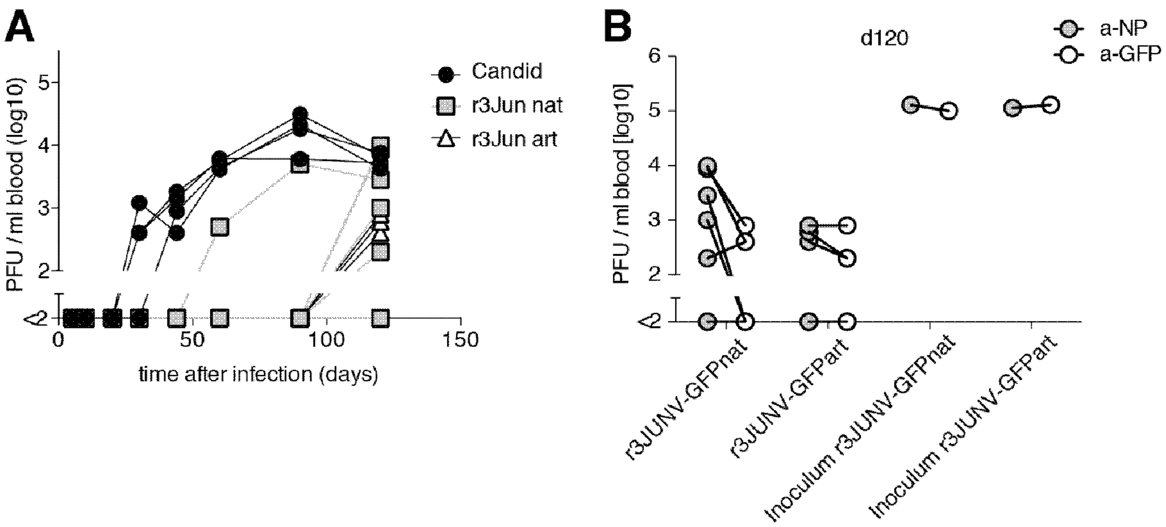
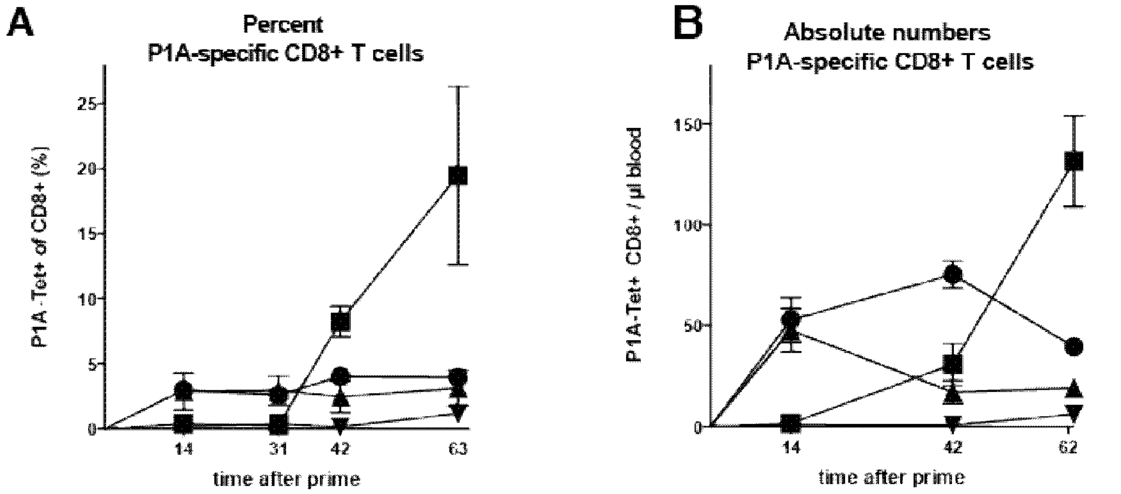
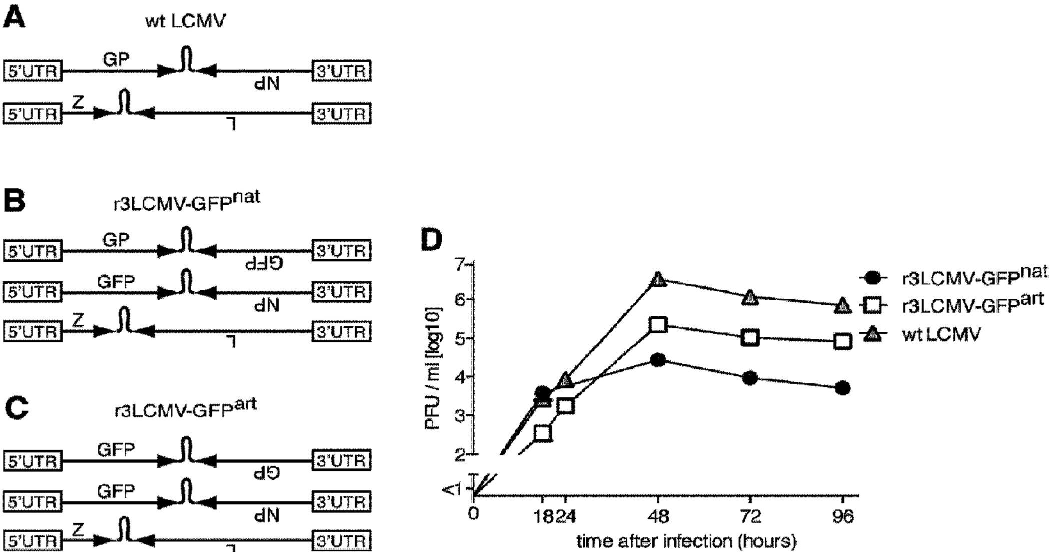

A
PFU / ml blood (log10)
time after infection (days)
Candid
r3Jun nat
r3Jun art
B
d120
a-NP
a-GFP
PFU / ml blood [log10]
r3JUNV-GFPnat
r3JUNV-GFPart
Inoculum r3JUNV-GFPnat
Inoculum r3JUNV-GFPart

Figure 13



	Prime day 0	Boost day 35
■	r3JUNV P1A	r3LCMV P1A
●	r3LCMV-P1A	r3LCMV-P1A
▲	r3LCMV-P1A	r3JUNV- P1A
▼	r3JUNV- P1A	r3JUNV- P1A

Figure 14


A
wt LCMV
5'UTR
GP
3'UTR
NP
Z
L
B
r3LCMV-GFPnat
GFP
C
r3LCMV-GFPart
D
PFU / ml [log10]
7
6
5
4
3
2
<1
0
18
24
48
72
96
time after infection (hours)
r3LCMV-GFPnat
r3LCMV-GFPart
wt LCMV