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Canadian Intellectual Property Office

CA 2990643 C 2023/10/17

(11)(21) **2 990 643**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) Date de dépôt PCT/PCT Filing Date: 2016/06/23
(87) Date publication PCT/PCT Publication Date: 2016/12/29
(45) Date de délivrance/Issue Date: 2023/10/17
(85) Entrée phase nationale/National Entry: 2017/12/21
(86) N° demande PCT/PCT Application No.: US 2016/038964
(87) N° publication PCT/PCT Publication No.: 2016/210094
(30) Priorité/Priority: 2015/06/23 (US62/183,410)

(51) Cl.Int./Int.Cl. *C07K 14/08* (2006.01), *A61K 39/12* (2006.01), *C12N 15/86* (2006.01)

(72) Inventeurs/Inventors:
MEBATSION, TESHOME, US;
KASSA, AEMRO, US;
KIM, TAEJOONG, US

(73) Propriétaire/Owner:
BOEHRINGER INGELHEIM ANIMAL HEALTH USA INC., US

(74) Agent: SMART & BIGGAR LP

(54) Titre : VECTEURS VIRAUX RECOMBINES CONTENANT UNE PROTEINE MINEURE DE PRRSV ET PROCEDES DE PREPARATION ET D'UTILISATION DE CEUX-CI

(54) Title: PRRSV MINOR PROTEIN-CONTAINING RECOMBINANT VIRAL VECTORS AND METHODS OF MAKING AND USE THEREOF

(57) Abrégé/Abstract:
The present invention encompasses recombinant porcine reproductive and respiratory syndrome virus (PRRSV) vaccines or compositions. In particular, the invention encompasses recombinant adenovirus vectors encoding and expressing PRRSV gp2, gp3, gp4, gp5a, gp5 and/or E antigens, proteins, epitopes or immunogens. Such vaccines or compositions can be used to protect animals from PRRSV.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 December 2016 (29.12.2016)

(10) International Publication Number
WO 2016/210094 A1

(51) International Patent Classification:
C07K 14/08 (2006.01) *A61K 39/12* (2006.01)
C12N 15/86 (2006.01)

(21) International Application Number:
PCT/US2016/038964

(22) International Filing Date:
23 June 2016 (23.06.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/183,410 23 June 2015 (23.06.2015) US

(71) Applicant: **MERIAL, INC.** [US/US]; 3239 Satellite Blvd., Duluth, GA 30096 (US).

(72) Inventors: **MEBATSION, Teshome**; 2670 Bent Tree Lane, Watkinsville, GA 30677 (US). **KASSA, Aemro**; 4390 Meadow Springs Dr., Watkinsville, GA 30677 (US). **KIM, Taejoong**; 1960 Harperfield Way, Bogart, GA 30622-2344 (US).

(74) Agents: **JARECKI-BLACK, Judy** et al.; 3239 Satellite Blvd., Duluth, GA 30096 (US).

(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))*

WO 2016/210094 A1

(54) **Title**: PRRSV MINOR PROTEIN-CONTAINING RECOMBINANT VIRAL VECTORS AND METHODS OF MAKING AND USE THEREOF

(57) **Abstract**: The present invention encompasses recombinant porcine reproductive and respiratory syndrome virus (PRRSV) vaccines or compositions. In particular, the invention encompasses recombinant adenovirus vectors encoding and expressing PRRSV gp2, gp3, gp4, gp5a, gp5 and/or E antigens, proteins, epitopes or immunogens. Such vaccines or compositions can be used to protect animals from PRRSV.

PRRSV MINOR PROTEIN-CONTAINING RECOMBINANT VIRAL VECTORS AND METHODS OF MAKING AND USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to provisional application USSN 62/183,410, filed on 23
5 June 2015.

CITED REFERENCES

[0002] Any foregoing applications and all documents cited therein or during their prosecution ("application cited documents") and all documents cited or referenced in the application cited documents, and all documents cited or referenced herein ("herein cited documents"), and all
10 documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein, may be employed in the practice of the invention. Citation or identification of any such document in this application is not an admission that such document is available as prior art to the present invention and does not reflect any view of the validity, patentability and/or
15 enforceability of such cited patent documents. All sequences referenced herein by GenBank Accession numbers are as set forth in GenBank at as of the filing date of the present application.

[0003]

FIELD OF THE INVENTION

[0004] The present invention encompasses recombinant adenovirus-vectored PRRSV vaccines,
20 compositions and methods of use.

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SUMMARY OF THE INVENTION

[0005] PRRSV is devastating viral infection of pigs with huge economic importance (Derald J. Holtkamp, 2013). There is large variability in the antigenic characteristics of the different isolates and effective measures to prevent infections are limited. There are two major groups of vaccines available for PRRS, which are attenuated modified live virus (MLV) or killed virus vaccine. The MLV vaccines, although effective in a homologues challenge, fail to provide broader protection among the many circulating variants and have the potential to revert to wild-type resulting in fulminant infection. Besides, animals vaccinated with MLV vaccines continue to shed the virus and farms that use this vaccines cannot be PRRSV free. On the other side, the killed virus vaccines are much safer, but less effective than MLV vaccines. Therefore, the current options available to prevent infection are neither safe nor effective (Charerntantanakul, 2012) (Tjeerd G. Kimman, 2009). There has been a concerted effort to develop recombinant vaccines that can address the major drawbacks of current vaccines for much of the last 2 decades (Zhang, 2012). However, despite extensive effort, there is no single recombinant vaccine on the market licensed for prevention of PRRSV infection. Most recombinant vaccines that were evaluated in the past were based on one or combination of viral envelope proteins that are believed to be targets of neutralizing antibody response. However, lack of complete understanding of functional interaction either among the envelope proteins or with receptor on the target cells hampered the rational design of efficacious recombinant vaccines.

[0006] The viral envelope proteins of PRRSV are generally categorized into major and minor proteins based on abundance of proteins in the virion (Dokland, 2010) (Dea S, 2000). The major viral envelope proteins are gp5 (ORF 5) and M (ORF 6) and form a dimer. The minor envelope proteins are gp2 (ORF2), gp3 (ORF3), gp4 (ORF4) and E (ORF2b) and probably a newly identified viral protein gp5a (ORF 5a). The minor envelope proteins are believed to exist as multimers and they are implicated in direct interaction with receptor, CD163, and mediate viral entry (Phani B. Das, 2010).

[0007] Most of the previous attempts to develop recombinant vaccines have focused on major proteins, gp5, M or a combination (Dea, 1998). This is probably due to the fact that antibodies to major proteins are readily detected in PRRSV infected animals and assumed they might present neutralizing targets to the immune system. Besides, there is large degree of sequence variability

in gp5 indicating these proteins are under immune selection pressure. However, depletion of gp5 specific antibodies from neutralizing sera indicated that these antibodies belong largely to a non-neutralizing fraction of the sera (Juan Li, 2012). Therefore, these have indicated to the presence of the primary neutralizing target on viral envelope proteins other than the major proteins and probably on minor proteins. Despite extensive effort to develop the major proteins as antigens in recombinant vaccines, ranging from purified recombinant proteins to vaccines delivered using a variety of vector platforms (Jazmina L.G. Cruza, 2010), none has made it to the market because of failure to afford robust protection.

[0008] Recently, the focus in developing recombinant PRRS vaccine has shifted to the minor proteins (Jing-Qiang Ren, 2014) (Sakthivel Subramaniam, 2014) (Z.S. WANG, 2011). This shift has been primarily driven by three recent findings. First, two of the minor proteins, gp2 and gp4 were shown to bind directly to CD163 receptor. Second, a swap of minor proteins but not major proteins with EAV (Equine arteritis Virus), also an arterivirus, altered the tropism of the virus, indicating the importance of minor proteins in interaction with receptor and directing virus to target cells (Lu Z1, 2012) (Tian D, 2012). Finally, knock-out mutants of CD163, which is the primary receptor for minor proteins, prevented virus infection, whereas similar knock-out for CD169, receptor for major proteins, did not affect viral entry (Randall S. Prather, 2013). Despite the increasing knowledge in the role of minor proteins in virus entry and as relevant target for neutralizing antibody response, none of the recombinant vaccines developed so far based on minor proteins resulted in protection of vaccinated animal from PRRS infection.

[0009] Here, we present that inclusion of another minor protein E to this combination of minor proteins resulted in a dramatically different protective response. Surprisingly, the presence of E protein together with gp2, gp3 and gp4 induced a robust immune response and reduced lung lesion from PRRS challenge. This is the first time that E protein has been shown as a critical component of protein complex that can induce protective immune response. This was achieved not only by identifying E protein as the essential component of the minor protein complex, but also by expressing all four proteins from a single vector platform that promoted formation of protein complex. This new finding will not only serve to further understand the critical interactions among viral proteins and cellular receptor but also paves the way toward achieving a universal recombinant PRRS vaccine that is actually free of live PRRSV.

[0010] In our hands, vaccination of animals with pooled plasmids expressing gp2, gp3 and gp4 failed to generate robust immune response (unpublished observation). The conclusion from this animal trial was that these proteins are presumed to exist as multimers and therefore expression of all the proteins simultaneously within a single cell to promote multimerization is required to form the correct conformation that presents a neutralizing epitope to the immune system. Subsequent biochemical assays also indicated this and all the proteins were placed in single vector to allow simultaneous expression. Surprisingly, in the animal trial reported here, we have found that this is also not sufficient to induce protective immune response. Rather, the critical factor for induction of protective immune response by these antigens was the modification introduced to re-target the proteins from intracellular compartments to the surface of the cells. Such a dramatic difference between the modified and unmodified proteins was entirely unexpected and will open new avenues to address similar challenges with a variety of viral targets. This is also the first time, to our knowledge; the immunogenicity of PRRSV envelope minor proteins was enhanced to a degree it can afford both protection from lung lesion against PRRS challenge as well as reduce level of serum viremia by simultaneously expressing all the minor proteins from a single vector and introducing modifications that enhanced cell surface expression.

[0010a] In an embodiment, there is provided an immunological composition comprising: a. a recombinant viral vector comprising a heterologous nucleotide sequence encoding retargeted porcine reproductive and respiratory syndrome virus (PRRSV) gp2, retargeted PRRSV gp3 and retargeted PRRSV gp4; and b. a pharmaceutically or veterinarily acceptable carrier, wherein the retargeted PRRSV gp2, the retargeted PRRSV gp3, and the retargeted PRRSV gp4 are retargeted by the replacement of their existing cellular localization sequence with a corresponding cell-surface expression determinant sequence from a heterologous gene.

[0010b] In an embodiment, there is provided a recombinant adenovirus 5-porcine reproductive and respiratory syndrome virus (Ad5-PRRSV) vector, wherein the Ad5-PRRSV vector comprises polynucleotides encoding retargeted porcine reproductive and respiratory syndrome virus (PRRSV) gp2, retargeted PRRSV gp3, and retargeted PRRSV gp4, wherein: a. the retargeted PRRSV gp2 has at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 14, the retargeted PRRSV gp3 has at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 16, and the retargeted PRRSV gp4 has at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 18; or b. the retargeted PRRSV gp2 has at least 90% sequence identity to the ectodomain of the sequence as set forth in SEQ ID NO: 14, the retargeted PRRSV gp3 has at least 90% sequence identity to the ectodomain of the sequence as set forth in SEQ ID NO: 16, and

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the retargeted PRRSV gp4 has at least 90% sequence identity to the ectodomain of the sequence as set forth in SEQ ID NO: 18, wherein the retargeted PRRSV gp2, the retargeted PRRSV gp3, and the retargeted PRRSV gp4 are retargeted by the replacement of their existing cellular localization sequence with a corresponding cell-surface expression determinant sequence from a 5 heterologous gene.

[0010c] In an embodiment, there is provided use of the composition as described herein, or a vector as described herein for eliciting an immunological response in a porcine animal in need thereof against PRRSV.

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[0033] The present disclosure provides novel PRRSV vaccine compositions and methods of making and use thereof.

[0034] This disclosure is based, in part, upon the surprising and unexpected finding that inclusion of another PRRSV minor protein (E) to other combinations of minor proteins resulted in a dramatically different protective response. In some embodiments, sufficient portions of the E protein, for example, its transmembrane (TM), amino terminal (NT) or its carboxy terminal (CT) domain, may be used to elicit said protective response.

[0035] Surprisingly, the presence of E protein together with gp2, gp3 and gp4 induced a robust immune response and reduced lung lesion from PRRS challenge. This is the first time that E protein has been shown as a critical component of protein complex that can induce protective immune response.

5 **[0036]** As such, the disclosed vaccines were not merely achieved by identifying E protein as the essential component of the minor protein complex, but also, by expressing all four proteins from a single vector platform that promoted formation of protein complex.

[0037] In another aspect, the disclosure provides recombinant viral vectors expressing chimeric versions of PRRSV minor proteins, which contain different cellular localization determinants, as
10 compared with their corresponding wild-type genes. In particular, a portion of VSV glycoprotein (G) and tissue plasminogen activator protein (tPA) has been added to cause the resulting chimeric gene products to localize to the cell surface. These recombinant vectors elicit safe and effective immune responses in the host animal against PRRSV. As such, modifications introduced to the PRRSV minor proteins to achieve their surface expression produced a similar
15 effect as did co-expressing E protein along with gp2, gp3, and gp4.

[0038] Accordingly, this disclosure thus provides a roadmap for achieving a universal recombinant PRRS vaccine that is 100% free of live PRRSV.

[0039] The present invention more particularly relates to an adenovirus-vectored PRRSV vaccine or composition that comprises one or more engineered, recombinant adenovirus vectors
20 that harbor and express certain PRRSV antigens, and optionally a pharmaceutically or veterinarily acceptable carrier, adjuvant, excipient, or vehicle. The PRRSV may be any strain, as the novel and inventive compositions and methods disclosed herein are universally applicable to all known and yet to be discovered PRRSV strains, for reasons discussed more fully below.

[0040] The PRRSV antigen includes PRRSV minor proteins (e.g. gp2, gp3, gp4, gp5a, gp5 or
25 E), in any combination, and optionally includes additional PRRSV major proteins (e.g. gp5 or M). Similar to the other minor proteins, gp5a is relatively well-conserved, and is envisioned by Applicants to be an effective addition or substitution for the safe and effective recombinant viral vectors of the instant disclosure.

[0041] The PRRSV recombinant vectors may contain and express in an animal host at least the following combinations (in any order, and driven by any promoter element, PE, including the one indicated, and including elements such as IRES and 2A-peptides) of genes or components (*rtg* = re-targeted; CMV = cytomegalovirus promoter; SV40 = simian virus 40 promoter; IRES = internal ribosomal entry site, self-cleaving 2A peptides derived from foot-and-mouth disease (FMD) virus, equine rhinitis A virus, *Thosea asigna* virus or porcine teschovirus-1): 1) (PE)gp2, (PE)gp3, (PE)gp4, (PE)E; 2) (PE)*rtg* gp2, (PE)gp3 and (PE)gp4; 3) (PE)*rtg* gp2, (PE)*rtg* gp3 and (PE)*rtg* gp4; 4) (PE)*rtg* gp2, (PE)*rtg* gp3, (PE)*rtg* gp4 and (PE)E; 5) (PE)*rtg* gp2, (PE)*rtg* gp3, (PE)*rtg* gp4 and (PE)*rtg* E; 6) (PE)*rtg* gp2, (PE)*rtg* gp4 and (PE)*rtg* E; 7) (PE)*rtg* gp2 and (PE)*rtg* gp4, 8) (M-(SV40)-(CMV)-gp5-(IRES)-gp5a; 9) gp2-(SV40)-(CMV)-E; 10) *rtg* gp2-(SV40)-(CMV)-E; 11) *rtg* gp2-(SV40)-(CMV)-*rtg* E; 12) (CMV)-E; 11) E-(p2A)-gp2-(SV40)-(CMV)-gp4; 12) *rtg* E-(p2A)- *rtg* gp2-(SV40)-(CMV)- *rtg* gp4; 13) (PE)gp2-(PE)gp4-(PE)E; 14) (PE)gp2-(PE)E; 15) (PE)gp2; 16) (PE)gp2-(PE)gp3; 16) (PE)gp2-(PE)gp4; 17) (PE)gp2-(PE)gp5a; 18) (PE)E; 19) (PE)E-(PE)gp3; 20) (PE)E-(PE)gp4; 19) (PE)E-(PE)gp5a; 20). In an advantageous embodiment, the vector contains and expresses at minimum (PE)gp2, (PE)gp4 and (PE)E, either wild-type or "rtg" versions thereof. The vector may also advantageously comprise gp2 plus any other gene encoding a PRRSV polypeptide.

[0042] The re-targeting may be accomplished by replacing existing gp2, gp3, gp4, gp5a, gp5 or E proteins transmembrane (TM) and cytoplasmic tail (CT) domains with, respectively, the TM and CT domains of VSV. In an embodiment, the gp5 and M proteins may also be subjected to the re-targeting procedure. The native PRRSV protein sequences may also or alternatively be replaced with the tPA signal sequence and either or both TM and CT of VSV (or those same elements from other suitable surface-expressed polypeptide). Alternatively, the re-targeting may be accomplished by replacing existing gp2, gp3, gp4, gp5a, E, gp5 or M protein CT domains with the CT domains of VSV (i.e. not changing the existing TM domains). Re-targeting of E may also be accomplished by replacing its cellular localization signals with that from a Type II membrane protein, or with VSV-G or combinations thereof, or the TM/CT domains of other surface glycoproteins.

[0043] Applicants further envision many alternative means of presenting the PRRSV antigens to the host animal's immune system. For example, the antigens could be displayed on the surface of virus-like particles (VLPs). In other embodiments, soluble versions of the antigens could be

administered to the host animal, wherein oligomerization (including trimerization) of the proteins with each other, or additionally, with components of VSV-G, or other viral proteins or any oligomerization (including trimerization motifs) (e.g. motifs from bacterial GCN4, and the like). Moreover, the TM/CT domains of Type I viral surface glycoproteins are envisioned to
5 accomplish the same purpose as, and are therefore interchangeable with, the corresponding domains from VSV-G.

[0044] Accordingly, now that the invention has been disclosed, the skilled person will recognize many alternative and functionally equivalent ways to accomplish substantially the same presentation of PRRSV minor proteins, including E, gp2, gp3, gp4, gp5a, major proteins,
10 including gp5 and M, or combinations of minor and/or major proteins, to a host animal's immune system.

[0045] The invention also relates to a method of vaccinating an animal comprising administering to the animal an effective amount of one or more vaccines or compositions which may comprise an effective amount of an adenovirus-vectored PRRSV vaccine and optionally a
15 pharmaceutically or veterinarily acceptable carrier, adjuvant, excipient, or vehicle. The administering may be subcutaneous, intranasal, intramuscular, transdermal, intradermal, mucosal, including oral, or any other administration.

[0046] The invention further relates to administration of the vaccine or composition using prime-boost protocol. The invention further encompasses a kit for performing a method of eliciting or
20 inducing an immune response that may comprise any one of the recombinant Ad5 immunological compositions or vaccines, or inactivated immunological compositions or vaccines, and instructions for performing the method.

[0047] Accordingly, it is an object of the invention to not encompass within the invention any previously known product, process of making the product, or method of using the product such
25 that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that

Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product.

[0048] These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

5 **BRIEF DESCRIPTION OF THE DRAWINGS**

[0049] The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may be best understood in conjunction with the accompanying drawings, in which:

[0050] FIG. 1 presents maps of the inserts used to produce four different recombinant viral 10 vectors expressing porcine reproductive and respiratory syndrome virus (PRRSV) minor viral envelope proteins. vAD3042 expresses codon-optimized, PRRSV gp2, gp3 and gp4 without E (A); vAD3038 expresses codon-optimized, re-targeted ("*rtg*") *rtg*-gp2, *rtg*-gp3 and *rtg*-gp4 without E (B); vAD3041 expresses codon-optimized, gp2, gp3, gp4 with E (C); vAD3067 expresses codon-optimized, *rtg*-gp2, *rtg*-gp3, *rtg*-gp4 with E (D); vAD3046 expresses codon-15 optimized Swine influenza virus hemagglutinin (SIV-HA) (E); vAD3069 expresses codon-optimized Nucleoprotein (Np or N), M, gp5 and gp5a (F); and vAD3064 expresses codon-optimized, rtg-M, rtg-gp5 and rtg-gp5a (G);

[0051] FIG. 2 is a schematic showing the arrangement of PRRSV "major" and "minor" proteins on the surface of a viral membrane;

20 **[0052]** FIG. 3 is a schematic showing the arrangement and interactions of the PRRSV "minor" proteins, as the current and disclosed evidence indicates these proteins are understood to interact with the host cell surface receptors (e.g. CD163);

[0053] FIG. 4 is a gel image showing the PCR amplicon of the region of PRRSV minor protein inserted in vAD3041 passage 3 (A) and vAD3042 passage 3 (B);

25 **[0054]** FIG. 5A presents the scheme used to re-target PRRSV envelope proteins to the cell surface;

[0055] FIGs. 5B-5D present maps of the *rtg*-gp2, *rtg*-gp3 and *rtg*-gp4 proteins, wherein the endogenous TM and CT domains have been replaced with vesicular stomatitis virus-G (VSV-G)

transmembrane (TM) and cytoplasmic tail (CT) domains, the signal sequence has been replaced, epitope tags have been added and linker sequences have been inserted;

[0056] FIG. 6 presents immunofluorescence assay (IFA) images of fixed HEK 293T cells that had been transfected with epitope-tagged *rtg*-gp2, *rtg*-gp3 and *rtg*-gp4 proteins;

5 **[0057]** FIG. 7 shows an anti-VSVG Western Blot (WB) of co-immunoprecipitated (co-IP) lysates from HEK 293T cells transfected with plasmids coding for each of the individual re-targeted envelope proteins;

[0058] FIG. 8 shows several WBs of co-IP lysates from HEK 293T cells transfected with plasmids coding for each of the individual re-targeted envelope proteins or porcine CD16. IP: α-
10 VSV, Wb: α-VSV-HRP (A); IP: α-VSV, Wb: α-CD163 (B); IP: α-CD163, Wb: α-CD163-Biotin (C);

[0059] FIGs. 9A to 9C present dual-immunofluorescence assay (IFA) images of HEK 293 cells infected with vAD3038 (containing codon-optimized *rtg*-gp234); and stained simultaneously with two antibodies specific for indicated proteins and different fluorophore tags. Images were
15 taken from identical optical field using filters specific for each fluorophore. Corresponding images are shown with arrow;

[0060] FIG. 10 is a chart detailing samples collected and time of collection throughout the study;

[0061] FIG. 11 is a graph showing the distribution of lung lesion scores among different groups. vAD3042 (Ad5 expressing codon-optimized, wild-type gp2, wild-type gp3 and wild-type gp4);
20 vAD3041 (Ad5 expressing codon-optimized, wild-type gp2, wild-type gp3, wild-type gp4 and wild-type E); vAD3038 (Ad5 expressing codon-optimized, *rtg*-gp2, *rtg*-gp3 and *rtg*-gp4); and vAD3033 (Ad5 expressing a codon-optimized hemagglutinin (HA) gene of swine influenza virus (SIV), negative control). The median (cross-bar) and mean (+) and boxes represent the range between the 1st and 3rd inter-quartile range. The grey circles indicate the actual lung scores of
25 each individual animal in each group;

[0062] FIG. 12 lists and describes the sequences present in the sequence listing;

[0063] FIG. 13 is a ClustalW alignment of the gp2 polypeptide sequences as set forth in SEQ ID NOs: 34-39;

[0064] FIG. 14 is a ClustalW alignment of the gp3 polypeptide sequences as set forth in SEQ ID NOs: 40-45;

[0065] FIG. 15 is a ClustalW alignment of the gp4 polypeptide sequences as set forth in SEQ ID NOs: 46-51;

5 **[0066]** FIG. 16 is a ClustalW alignment of the E polypeptide sequences as set forth in SEQ ID NOs: 52-58;

[0067] FIG. 17 is a ClustalW alignment of the gp5a polypeptide sequences as set forth in SEQ ID NOs: 62-65;

[0068] FIG. 18 is plot showing lung lesion scores for porcines administered either vAd3038
10 (Gp234-Rtrg + Killed Vaccine) or vAd3046 (SIV-HA);

[0069] FIG. 19 is a plot showing serum viral load for porcines administered either vAd3038 (Gp234-Rtrg + Killed Vaccine) or vAd3046 (SIV-HA);

[0070] FIG. 20 compares the immune responses of Groups 1, 2, 4 and 5, before and after challenge. Western blots were probed with anti-V5 to visualize E protein levels (top left); anti-
15 Flag to detect gp3 (right); and anti-HA to visualize gp4 protein levels (bottom left);

[0071] FIG. 21 is a plot showing lung lesion scores for porcines administered vAD3067 (IM/IM) followed by Killed vaccine, vAD3067 (IN/IM) followed by killed vaccine; vAD3067+vAD3064 (IN/IM) followed by killed vaccine; or vAD3046 followed by placebo. All killed vaccines were given once IM;

20 **[0072]** FIG. 22 is a plot serum viral load for porcines administered vAD3067 (IM/IM) followed by Killed vaccine, vAD3067 (IN/IM) followed by Killed vaccine; vAD3067+vAD3064 (IN/IM) followed by Killed vaccine; or vAD3046 and placebo. All killed vaccines were given once IM;

[0073] FIG. 23 shows the results of the immunoprecipitation study designed to interrogate the possible interaction between E and retargeted gp4 (no interaction observed). In the construct, the
25 Flag tag is attached to gp3; the V5 tag is attached to E; the HA tag is attached to gp4; and, the Myc tag is attached to gp2. WB (Western blot), IP (immunoprecipitation), S (soluble gps) and V (VSV-tagged gps);

[0074] FIG. 24 shows the results of the IP study designed to interrogate the possible interaction between E and retargeted gp3 (no interaction observed).

DETAILED DESCRIPTION

[0075] It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0076] Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a", "an", and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise.

[0077] The term "about," as used herein, means approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10%. In one aspect, the term "about" means plus or minus 20% of the numerical value of the number with which it is being used. Therefore, about 50% means in the range of 45%-55%. Numerical ranges recited herein by endpoints include all numbers and fractions subsumed within that range (e.g. 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.90, 4, and 5). It is also to be understood that all numbers and fractions thereof are presumed to be modified by the term "about."

[0078] In the present invention, adenovirus 5 (Ad5), or another suitable vector, is used to deliver and express *in vivo* in an animal host selected PRRSV envelope proteins, to elicit in the animal a safe and effective immune response against experimental or natural challenge with virulent PRRSV.

[0079] While Ad5 was used to deliver the PRRSV proteins in the instant disclosure, any other suitable vector could be used. For example, baculovirus, poxvirus, including fowl poxvirus and canarypox virus may be used to deliver the novel and inventive combinations of genes disclosed herein. In another embodiment, porcine cytomegalovirus (PCMV), which is a herpesvirus found
5 in the tissues throughout the body including the nose of newborn piglets where it causes inflammation (rhinitis), may be used as the vector.

[0080] The present invention thus relates to a vaccine or immunological composition that may comprise an effective amount of one or more engineered Ad5 vectors, or other suitable vectors, and optionally, a pharmaceutically or veterinarily acceptable carrier, adjuvant, excipient, or
10 vehicle.

[0081] Accordingly, the present invention encompasses an engineered Ad5 vector, or other suitable vector, expressing PRRSV envelope protein(s), polypeptide(s), antigen(s), epitope(s) or immunogen(s), which elicit an immunogenic response in an animal. The PRRSV protein, polypeptide, antigen, epitope or immunogen includes at least one PRRSV minor protein,
15 polypeptide, antigen, epitope or immunogen, selected from PRRSV gp2, gp3, gp4, gp5a and E.

[0082] As used herein, the term "PRRSV minor polypeptide, antigen, epitope or immunogen" refers to any minor polypeptide, antigen, epitope or immunogen of a porcine reproductive and respiratory syndrome virus. Currently, the minor polypeptides or components thereof include gp2, gp3, gp4, gp5a and E proteins, but there may be other proteins associated with the currently
20 known minor proteins that could also be used effectively in the practice of the disclosed invention. In general, and as used herein, the term "ectodomain" refers to the domain or domains of a membrane protein that extend into the extracellular space. As such, any reference to percent identity to the ectodomain of a given protein is not intended to include a comparison to non-ectodomains, including transmembrane domains (TMDs) and cytoplasmic domains (CTDs), of
25 said protein.

[0083] By "animal" is intended mammals, human, birds, and the like. The animal may be selected from the group consisting of equine (e.g., horse), canine (e.g., dogs, wolves, foxes, coyotes, jackals), feline (e.g., lions, tigers, domestic cats, wild cats, other big cats, and other feline including cheetahs and lynx), ovine (e.g., sheep), bovine (e.g., cattle, cow, buffalo), swine
30 (pig), avian (e.g., chicken, duck, goose, turkey, quail, pheasant, parrot, finches, hawk, crow,

ostrich, emu and cassowary), primate (e.g., prosimian, tarsier, monkey, gibbon, ape), and fish. The term "animal" also includes an individual animal in all stages of development, including embryonic and fetal stages.

[0084] In the current invention, immunological protection of porcine animals against porcine
5 reproductive and respiratory syndrome virus is of primary importance. However, the concepts disclosed herein will apply equally well to other viruses where, as here, the relatively low or limited expression of key "cell-entry-mediating" surface proteins renders vaccine development especially challenging. Accordingly, as disclosed herein, the re-targeting and/or chaperoning of such "minor envelope proteins" to a cell's surface has broad-reaching applications to all
10 enveloped viruses.

[0085] In one embodiment, the Ad5 immunological composition or vaccine comprises one or more engineered Ad5 vectors, and optionally a pharmaceutical or veterinary acceptable excipient, adjuvant, carrier or vehicle. The engineered Ad5 vector may comprise a polynucleotide encoding a PRRSV minor protein, polypeptide, antigen, epitope or immunogen.
15 The PRRSV protein, polypeptide, antigen, epitope or immunogen may be a gp2, gp3, gp4, gp5a, E, or any fragment thereof.

[0086] As used herein, the term "antigen" or "immunogen" means a substance that induces a specific immune response in a host animal. The antigen may comprise a whole organism, killed, attenuated or live; a subunit or portion of an organism; a recombinant vector containing an insert
20 expressing an epitope, polypeptide, peptide, protein, or fragment thereof with immunogenic properties; a piece or fragment of nucleic acid capable of inducing an immune response upon presentation to a host animal; a protein, a polypeptide, a peptide, an epitope, a hapten, or any combination thereof. Alternately, the immunogen or antigen may comprise a toxin or antitoxin.

[0087] The term "immunogenic protein or peptide" as used herein also includes peptides and
25 polypeptides that are immunologically active in the sense that once administered to the host, it is able to evoke an immune response of the humoral and/or cellular type directed against the protein. Preferably the protein fragment is such that it has substantially the same immunological activity as the complete, intact native protein. Thus, a protein fragment according to the invention comprises or consists essentially of or consists of at least one epitope or antigenic
30 determinant. The term epitope, also known as antigenic determinant, is the part of a

macromolecule recognized by the immune system and able to induce an immune reaction of the humoral type (B cells) and/or cellular type (T cells).

[0088] The term "immunogenic protein or peptide" further contemplates deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein. In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic--aspartate and glutamate; (2) basic--lysine, arginine, histidine; (3) non-polar--alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar--glycine, asparagine, glutamine, cysteine, serine threonine and tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. It is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, or vice versa; an aspartate with a glutamate or vice versa; a threonine with a serine or vice versa; or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the reference molecule but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of the protein are, therefore, within the definition of the reference polypeptide.

[0089] The term "epitope" refers to the part of a macromolecule recognized by the immune system and able to induce an immune reaction of the humoral type (B cells) and/or cellular type (T cells). The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site." Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen.

[0090] An "immunological response" to a composition or vaccine is the development in the host of a cellular and/or antibody-mediated immune response to a composition or vaccine of interest. More often than not, an "immunological response" includes, but is not limited to, one or more of the following effects: the production of antibodies, B cells, helper T cells, and/or cytotoxic T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or protective immunological

response such that resistance to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host, a quicker recovery time and/or a lowered viral titer in the infected host.

5 **[0091]** The term "immunogenic" protein or polypeptide as used herein also refers to an amino acid sequence which elicits an immunological response as described above. An "immunogenic" protein or polypeptide, as used herein, includes the full-length sequence of the protein, analogs thereof, or immunogenic fragments thereof. By "immunogenic fragment" is meant a fragment of a protein which includes one or more epitopes and thus elicits the immunological response
10 described above. Such fragments can be identified using any number of epitope mapping techniques, well known in the art. See, e.g., Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66 (Glenn E. Morris, Ed., 1996).

[0092] For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein
15 molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Such techniques are known in the art and described in, e.g., U.S. Pat. No. 4,708,871; Geysen et al., 1984; Geysen et al., 1986. Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols, *supra*.

20 **[0093]** Synthetic antigens are also included within the definition, for example, polyepitopes, flanking epitopes, and other recombinant or synthetically derived antigens. Immunogenic fragments, for purposes of the present invention, will usually include at least about 3 amino acids, about 5 amino acids, about 10-15 amino acids, about 15-25 amino acids or more amino acids, of the molecule. There is no critical upper limit to the length of the fragment, which could
25 comprise nearly the full-length of the protein sequence, or even a fusion protein comprising at least one epitope of the protein.

[0094] Accordingly, a minimum structure of a polynucleotide expressing an epitope is that it comprises or consists essentially of or consists of nucleotides to encode an epitope or antigenic determinant of PRRSV protein or polypeptide. A polynucleotide encoding a fragment of the total
30 protein or polypeptide comprises or consists essentially of or consists of a minimum of 15

nucleotides, advantageously about 30-45 nucleotides, and preferably about 45-75, at least 57, 87 or 150 consecutive or contiguous nucleotides of the sequence encoding the total protein or polypeptide. Epitope determination procedures, such as, generating overlapping peptide libraries (Hemmer et al., 1998), Pepscan (Geysen et al., 1984; Geysen et al., 1985; Van der Zee R. et al., 5 1989; Geysen, 1990; Multipin.RTM. Peptide Synthesis Kits de Chiron) and algorithms (De Groot et al., 1999), can be used in the practice of the invention, without undue experimentation.

[0095] A "polynucleotide" is a polymeric form of nucleotides of any length that contains deoxyribonucleotides, ribonucleotides, and analogs in any combination. Polynucleotides may have three-dimensional structure, and may perform any function, known or unknown. The term 10 "polynucleotide" includes double-, single-, and triple-stranded helical molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide encompasses both the double stranded form and each of two complementary forms known or predicted to make up the double stranded form of either the DNA, RNA or hybrid molecule.

15 **[0096]** The term "codon optimization" refers to the process of optimally configuring the nucleic acid sequence encoding a protein, polypeptide, antigen, epitope, domain or fragment for expression/translation in a selected host. In general, gene expression levels depend on many factors, such as promoter sequences and regulatory elements. One of the most important factors is the adaptation of the codon usage of the transcript gene to the typical codon usage of the host 20 (Lithwich, G. and Margalit, H., Genome Res. 13, 2665-2673, 2003). Therefore, highly expressed genes in prokaryotic genomes under translational selection have a pronounced codon usage bias. This is because they use a small subset of codons that are recognized by the most abundant tRNA species (Ikemura, T., J. Mol. Biol. 151, 389-409, 1981). The force that modulates this codon adaptation is called translational selection and its strength is important in fast-growing 25 bacteria (Rocha, E.P., Genome Res. 14, 2279-2286, 2004; Sharp, P.M. et al., Nucleic Acids Res. 33, 1141-1153). If a gene contains codons that are rarely used by the host, its expression level will not be maximal. This may be one of the limitations of heterologous protein expression (Gustafsson, C. et al., Trends Biotechnol. 22, 346-353, 2004) and the development of DNA vaccines (Ivory, C. and Chadee, K., Genet. Vaccines Ther. 2, 17, 2004). A high number of 30 synthetic genes have been re-designed to increase their expression level. The Synthetic Gene Database (SGDB) (Wu, G. et al., Nucleic Acids Res. 35, D76-D79, 2007) contains information

from more than 200 published experiments on synthetic genes. In the design process of a nucleic acid sequence that will be inserted into a new host to express a certain protein in optimal amounts, codon usage optimization is usually one of the first steps (Gustafsson, C., Trends Biotechnol. 22, 346-353, 2004). Codon usage optimization basically involves altering the rare codons in the target gene so that they more closely reflect the codon usage of the host without modifying the amino acid sequence of the encoded protein (Gustafsson, C., Trends Biotechnol. 22, 346-353, 2004). The information usually used for the optimization process is therefore the DNA or protein sequence to be optimized and a codon usage table (reference set) of the host.

[0097] There are several public web servers and stand-alone applications that allow some kind of codon optimization by anyone skilled in the art. '*GeneDesign*' (Richardson, S.M. et al., Genome Res. 16, 550-556, 2006), '*Synthetic Gene Designer*' (Wu, G. et al., Protein Expr. Purif. 47, 441-445, 2006) and '*Gene Designer*' (Villalobos, A. et al., BMC Bioinformatics 7, 285, 2006) are packages that provide a platform for synthetic gene design, including a codon optimization step. With regard to the methods for codon usage optimization available in each server or program, the first programs developed used only the 'one amino acid–one codon' approach. More recent programs and servers now include further methods to create some codon usage variability. This variability reflects the codon usage variability of natural highly expressed genes and enables additional criteria to be introduced (such as the avoidance of restriction sites) in the optimization process. Most applications and web servers described herein provide three methods of codon optimization: a complete optimization of all codons, an optimization based on the relative codon usage frequencies of the reference set that uses a Monte Carlo approach and a novel approaches designed to maximize the optimization with the minimum changes between the query and optimized sequences.

[0098] In one embodiment, the nucleic acid sequence encoding the recombinant PRRSV minor protein, antigen, peptide, polypeptide, fragment, domain, or epitope is codon optimized for expression in animal. In another embodiment, the codon optimized sequences encode porcine PRRSV minor envelope proteins, antigens, peptides, polypeptides, fragments, domains, or epitopes for animal expression. In yet another embodiment, the codon optimized sequences encode PRRSV gp2, gp3, gp4, gp5a, gp5 or E proteins, antigens, peptides, polypeptides, fragments, domains, or epitopes for animal expression.

[0099] The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, siRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide may comprise modified
5 nucleotides, such as methylated nucleotides and nucleotide analogs, uracil, other sugars and linking groups such as fluororibose and thiolate, and nucleotide branches. The sequence of nucleotides may be further modified after polymerization, such as by conjugation, with a labeling component. Other types of modifications included in this definition are caps, substitution of one or more of the naturally occurring nucleotides with an analog, and
10 introduction of means for attaching the polynucleotide to proteins, metal ions, labeling components, other polynucleotides or solid support. The polynucleotides can be obtained by chemical synthesis or derived from a microorganism.

[0100] The term "gene" is used broadly to refer to any segment of polynucleotide associated with a biological function. Thus, genes include introns and exons as in genomic sequence, or just
15 the coding sequences as in cDNAs and/or the regulatory sequences required for their expression. For example, gene also refers to a nucleic acid fragment that expresses mRNA or functional RNA, or encodes a specific protein, and which includes regulatory sequences.

[0101] The invention further comprises a complementary strand to a polynucleotide encoding a PRRSV minor envelope protein, antigen, epitope or immunogen. The complementary strand can
20 be polymeric and of any length, and can contain deoxyribonucleotides, ribonucleotides, and analogs in any combination thereof.

[0102] The terms "protein", "peptide", "polypeptide" and "polypeptide fragment" are used interchangeably herein to refer to polymers of amino acid residues of any length. The polymer can be linear or branched, it may comprise modified amino acids or amino acid analogs, and it
25 may be interrupted by chemical moieties other than amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling or bioactive component.

[0103] An "isolated" polynucleotide or polypeptide is one that is "substantially free" of the
30 materials with which it is associated in its native environment. By "substantially free," it is

meant that the polynucleotide or polypeptide is at least 50%, at least 70%, at least 80%, at least 90%, or at least 95% free of these materials. If the "isolated" polynucleotide or polypeptide is designated as being "nearly entirely free of contaminants," it is meant that the isolated polynucleotide or polypeptide is at least 98% free of these materials.

5 **[0104]** The invention further encompasses polynucleotides encoding functionally equivalent variants and derivatives of the PRRSV polypeptides and functionally equivalent fragments thereof that may enhance, decrease or not significantly affect inherent properties of the polypeptides encoded thereby. These functionally equivalent variants, derivatives, and fragments display the ability to retain the activity. For instance, changes in a DNA sequence that do not 10 change the encoded amino acid sequence, as well as those that result in conservative substitutions of amino acid residues, one or a few amino acid deletions or additions, and substitution of amino acid residues by amino acid analogs are those which will not significantly affect properties of the encoded polypeptide. In one embodiment, the variants have at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at 15 least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% homology or identity to the PRRSV polynucleotide or polypeptide of interest.

[0105] In one aspect, the present invention provides PRRSV polypeptides, particularly PRRSV minor envelope polypeptides. In another aspect, the present invention provides a polypeptide 20 having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139, or variants or fragments thereof.

[0106] In another aspect, the present invention provides a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to PRRSV gp2, gp3, gp4, gp5a, gp5 or E polypeptide of the invention, 25 particularly to the polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139.

[0107] In yet another aspect, the present invention provides fragments and variants of the PRRSV gp2, gp3, gp4, gp5a, gp5 or E polypeptides identified above (SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139) which 30 may readily be prepared by one of skill in the art using well-known molecular biology

techniques. Variants are homologous polypeptides having an amino acid sequence at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the antigenic polypeptides of the invention, particularly to the amino acid sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139.

5 **[0108]** An immunogenic fragment of a PRRSV gp2, gp3, gp4, gp5a, gp5 or E polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids, at least 21 amino acids, at least 23 amino acids, at least 25 amino acids, or at least 30 amino acids of the PRRSV gp2, gp3, gp4, gp5a, gp5 or E polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139, or variants
10 thereof. In another embodiment, a fragment of the PRRSV gp2, gp3, gp4, gp5a, gp5 or E polypeptide includes a specific antigenic epitope found on a full-length PRRSV gp2, gp3, gp4, gp5a, gp5 or E polypeptide.

[0109] In another aspect, the present invention provides a polynucleotide encoding a PRRSV gp2, gp3, gp4, gp5a, gp5 or E polypeptide, such as a polynucleotide encoding a polypeptide
15 having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139. In yet another aspect, the present invention provides a polynucleotide encoding a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39,
20 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. The polynucleotide encoding the PRRSV gp2, gp3, gp4, gp5a, gp5 or E polypeptide may be codon-optimized for expression in a specific animal species.

25 **[0110]** In another aspect, the present invention provides a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 9, 10, 11, 12, 13, 15, 17, 19, 21-24, 30, 67, 69, 70, 72, 74, 76, or 78, or a variant thereof. In yet another aspect, the present invention provides a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polynucleotide having a

sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 9, 10, 11, 12, 13, 15, 17, 19, 21-24, 30, 67, 69, 70, 72, 74, 76, or 78, or a variant thereof.

[0111] In one aspect, the present invention provides PRRSV polypeptides, particularly PRRSV E polypeptide. In another aspect, the present invention provides a polypeptide having a sequence as set forth in SEQ ID NO: 7, 20, 52-58, or 130-139, and variant or fragment thereof.

[0112] In another aspect, the present invention provides a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a PRRSV E polypeptide of the invention, particularly to the polypeptides having a sequence as set forth in SEQ ID NO: 7, 20, 52-58, or 130-139.

[0113] In yet another aspect, the present invention provides fragments and variants of the PRRSV E polypeptides identified above (SEQ ID NO: 7, 20, 52-58, or 130-139) which may readily be prepared by one of skill in the art using well-known molecular biology techniques.

[0114] Variants are homologous polypeptides having an amino acid sequence at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the antigenic polypeptides of the invention, particularly to the amino acid sequence as set forth in SEQ ID NO: 7, 20, 52-58, or 130-139.

[0115] An immunogenic fragment of a PRRSV E polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids, at least 21 amino acids, at least 23 amino acids, at least 25 amino acids, or at least 30 amino acids of the PRRSV E polypeptide having a sequence as set forth in SEQ ID NO: 7, 20, 52-58, or 130-139, or variants thereof. In another embodiment, a fragment of a PRRSV E polypeptide includes a specific antigenic epitope found on a full-length PRRSV E polypeptide.

[0116] In another aspect, the present invention provides a polynucleotide encoding a PRRSV E polypeptide, such as a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 7, 20, 52-58, or 130-139. In yet another aspect, the present invention provides a polynucleotide encoding a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 7, 20, 52-58, or 130-139, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at

least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. The polynucleotide encoding the PRRSV E polypeptide may be codon-optimized for expression in a specific animal species.

[0117] In another aspect, the present invention provides PRRSV polypeptides, particularly
5 PRRSV gp2 polypeptide. In another aspect, the present invention provides a polypeptide having a sequence as set forth in SEQ ID NO: 1, 14, 34-39, or 80-89, and variant or fragment thereof.

[0118] In another aspect, the present invention provides a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a PRRSV gp2 polypeptide of the invention, particularly to the polypeptides
10 having a sequence as set forth in SEQ ID NO: 1, 14, 34-39, or 80-89.

[0119] In yet another aspect, the present invention provides fragments and variants of the PRRSV gp2 polypeptides identified above (SEQ ID NO: 1, 14, 34-39, or 80-89) which may readily be prepared by one of skill in the art using well-known molecular biology techniques.

[0120] Variants are homologous polypeptides having an amino acid sequence at least about
15 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the antigenic polypeptides of the invention, particularly to the amino acid sequence as set forth in SEQ ID NO: 1, 14, 34-39, or 80-89.

[0121] An immunogenic fragment of a PRRSV gp2 polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids, at least 21 amino acids, at least 23 amino acids, at least 25 amino acids,
20 or at least 30 amino acids of the PRRSV gp2 polypeptide having a sequence as set forth in SEQ ID NO: 1, 14, 34-39, or 80-89, or variants thereof. In another embodiment, a fragment of a PRRSV gp2 polypeptide includes a specific antigenic epitope found on a full-length PRRSV gp2 polypeptide.

[0122] In another aspect, the present invention provides a polynucleotide encoding a PRRSV
25 gp2 polypeptide, such as a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 1, 14, 34-39, or 80-89. In yet another aspect, the present invention provides a polynucleotide encoding a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 1, 14, 34-39, or 80-89, or a conservative variant,

an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. The polynucleotide encoding the PRRSV gp2 polypeptide may be codon-optimized for expression in a specific animal species.

5 **[0123]** In another aspect, the present invention provides PRRSV polypeptides, particularly PRRSV gp3 polypeptide. In another aspect, the present invention provides a polypeptide having a sequence as set forth in SEQ ID NO: 3, 16, or 40-45, and variant or fragment thereof.

[0124] In another aspect, the present invention provides a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99%
10 sequence identity to a PRRSV gp3 polypeptide of the invention, particularly to the polypeptides having a sequence as set forth in SEQ ID NO: 3, 16, or 40-45.

[0125] In yet another aspect, the present invention provides fragments and variants of the PRRSV gp3 polypeptides identified above (SEQ ID NO: 3, 16, or 40-45) which may readily be prepared by one of skill in the art using well-known molecular biology techniques.

15 **[0126]** Variants are homologous polypeptides having an amino acid sequence at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the antigenic polypeptides of the invention, particularly to the amino acid sequence as set forth in SEQ ID NO: 3, 16, or 40-45.

[0127] An immunogenic fragment of a PRRSV gp3 polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids, at least 21 amino acids, at least 23 amino acids, at least 25 amino acids,
20 or at least 30 amino acids of the PRRSV gp3 polypeptide having a sequence as set forth in SEQ ID NO: 3, 16, or 40-45, or variants thereof. In another embodiment, a fragment of a PRRSV gp3 polypeptide includes a specific antigenic epitope found on a full-length PRRSV gp3 polypeptide.

[0128] In another aspect, the present invention provides a polynucleotide encoding a PRRSV gp3 polypeptide, such as a polynucleotide encoding a polypeptide having a sequence as set forth
25 in SEQ ID NO: 3, 16, or 40-45. In yet another aspect, the present invention provides a polynucleotide encoding a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 3, 16, or 40-45, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at least ten

consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. The polynucleotide encoding the PRRSV gp3 polypeptide may be codon-optimized for expression in a specific animal species.

[0129] In another aspect, the present invention provides PRRSV polypeptides, particularly PRRSV gp4 polypeptide. In another aspect, the present invention provides a polypeptide having a sequence as set forth in SEQ ID NO: 5, 18, or 46-51, and variant or fragment thereof.

[0130] In another aspect, the present invention provides a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a PRRSV gp4 polypeptide of the invention, particularly to the polypeptides having a sequence as set forth in SEQ ID NO: 5, 18, or 46-51.

[0131] In yet another aspect, the present invention provides fragments and variants of the PRRSV gp4 polypeptides identified above (SEQ ID NO: 5, 18, or 46-51) which may readily be prepared by one of skill in the art using well-known molecular biology techniques.

[0132] Variants are homologous polypeptides having an amino acid sequence at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the antigenic polypeptides of the invention, particularly to the amino acid sequence as set forth in SEQ ID NO: 5, 18, or 46-51.

[0133] An immunogenic fragment of a PRRSV gp4 polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids, at least 21 amino acids, at least 23 amino acids, at least 25 amino acids, or at least 30 amino acids of the PRRSV gp4 polypeptide having a sequence as set forth in SEQ ID NO: 5, 18, or 46-51, or variants thereof. In another embodiment, a fragment of a PRRSV gp4 polypeptide includes a specific antigenic epitope found on a full-length PRRSV gp4 polypeptide.

[0134] In another aspect, the present invention provides a polynucleotide encoding a PRRSV gp4 polypeptide, such as a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 5, 18, or 46-51. In yet another aspect, the present invention provides a polynucleotide encoding a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 5, 18, or 46-51, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides.

The polynucleotide encoding the PRRSV gp4 polypeptide may be codon-optimized for expression in a specific animal species.

[0135] In another aspect, the present invention provides PRRSV polypeptides, particularly PRRSV gp5a polypeptide. In another aspect, the present invention provides a polypeptide having
5 a sequence as set forth in SEQ ID NO:31 or 62-65, and variant or fragment thereof.

[0136] In another aspect, the present invention provides a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a PRRSV gp5a polypeptide of the invention, particularly to the polypeptides having a sequence as set forth in SEQ ID NO:31 or 62-65.

10 **[0137]** In yet another aspect, the present invention provides fragments and variants of the PRRSV gp5a polypeptides identified above (SEQ ID NO:31 or 62-65) which may readily be prepared by one of skill in the art using well-known molecular biology techniques.

[0138] Variants are homologous polypeptides having an amino acid sequence at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the antigenic polypeptides of
15 the invention, particularly to the amino acid sequence as set forth in SEQ ID NO:31 or 62-65.

[0139] An immunogenic fragment of a PRRSV gp5a polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids, at least 21 amino acids, at least 23 amino acids, at least 25 amino acids, or at least 30 amino acids of the PRRSV gp5a polypeptide having a sequence as set forth in SEQ ID NO: 31 or 62-65, or variants thereof. In another embodiment, a fragment of a PRRSV
20 gp5a polypeptide includes a specific antigenic epitope found on a full-length PRRSV gp5a polypeptide.

[0140] In another aspect, the present invention provides a polynucleotide encoding a PRRSV gp5a polypeptide, such as a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 31 or 62-65. In yet another aspect, the present invention provides a
25 polynucleotide encoding a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 31 or 62-65, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides.

The polynucleotide encoding the PRRSV gp5a polypeptide may be codon-optimized for expression in a specific animal species.

[0141] In another aspect, the present invention provides a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 9, 10, 11, 12, 13, 15, 17, 19, 21-24, 30, 67, 69, 70, 72, 74, 76, or 78, or a variant thereof. In yet another aspect, the present invention provides a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 95%, 96%, 97%, 98% or 99% sequence identity to one of a polynucleotide having a sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 9, 10, 11, 12, 13, 15, 17, 19, 21-24, 30, 67, 69, 70, 72, 74, 76, or 78, or a variant thereof.

[0142] In some embodiments, the invention provides a safe and effective immunological or vaccine composition comprising: one or more recombinant viral vectors, comprising one or more heterologous polynucleotides, encoding one or more porcine reproductive and respiratory syndrome virus (PRRSV) gp2, gp3, gp4, gp5a, gp5 or E antigen, polypeptide, ectodomain, or variant thereof; and a pharmaceutically or veterinarily acceptable carrier. "Variant thereof" is intended to encompass immunologically equivalent versions of the antigens, polypeptides and ectodomains, including, for example, retargeted variants of the proteins as disclosed herein. "Immunologically equivalent" means the "variant thereof" is capable of eliciting a substantially similar immune response—as compared with the original comparator antigen, polypeptide or ectodomain—including a protective immune response.

[0143] In some embodiments of the composition the one or more vectors comprise a recombinant adenovirus 5 PRRSV (Ad5-PRRSV) vector, a recombinant baculovirus PRRSV vector, a recombinant porcine cytomegalovirus PRRSV vector or a recombinant poxvirus PRRSV vector.

[0144] In some embodiments, the one or more vectors comprise either: a nucleotide sequence encoding a PRRSV E antigen, polypeptide, ectodomain or variant thereof; or, a nucleotide sequence encoding a modified PRRSV gp2, gp3, gp4, gp5a, gp5 or M antigen, polypeptide, ectodomain, or variant thereof, wherein an existing cellular localization sequence of gp2, gp3, gp4, gp5a, gp5 or M has been replaced with a cell-surface expression determinant sequence from an heterologous gene. In some embodiments, the one or more vectors comprise a mixture of two

vectors, a first vector expressing retargeted PRRSV minor proteins, and a second vector expressing re-targeted PRRSV major proteins.

[0145] In some embodiments, the recombinant vector(s) comprise a polynucleotide encoding an antigen, polypeptide or ectodomain having: at least 90% sequence identity to any one or more of SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139; or, at least 90% sequence identity to an ectodomain sequence as set forth in a subsequence of SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139.

[0146] In some embodiments, the recombinant Ad5-PRRSV vector comprises a polynucleotide having: at least 90% sequence identity to SEQ ID NO: 2, 4, 6, 8, 9, 10, 11, 12, 13, 15, 17, 19, 21-24, 30, 67, 69, 70, 72, 74, 76, or 78; or, at least 90% sequence identity to an ectodomain sequence encoded by a subsequence of SEQ ID NO: 2, 4, 6, 8, 9, 10, 11, 12, 13, 15, 17, 19, 21-24, 30, 67, 69, 70, 72, 74, 76, or 78.

[0147] In some embodiments, the composition or vaccine comprises one or two Ad5-PRRSV vectors. In some embodiments, the Ad5-PRRSV may expresses gp2 and E; gp2, gp4 and E; gp2, gp3, gp4 and E; rtg-gp2, rtg-gp3 and rtg-gp4; rtg-gp2 and E; rtg-gp2, rtg-gp4 and E; rtg-gp3 and E; rtg-gp4 and E; E alone; rtg-E alone; rtg-gp5, rtg-M.

[0148] In some embodiments, the Ad5-PRRSV recombinant vector comprises a polynucleotide encoding an antigen, polypeptide or ectodomain having at least 90% sequence identity to SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139; or, comprises a polynucleotide encoding an ectodomain having at least 90% sequence identity to an ectodomain as set forth in a subsequence of SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139.

[0149] In some embodiments, the recombinant Ad5-PRRSV vector comprises a polynucleotide having at least 90% sequence identity to SEQ ID NO: 2, 4, 6, 8, 9, 10, 11, 12, 13, 15, 17, 19, 21-24, 30, 67, 69, 70, 72, 74, 76, or 78; or, comprises a polynucleotide having at least 90% identity to an ectodomain sequence encoded by a subsequence of SEQ ID NO: 2, 4, 6, 8, 9, 10, 11, 12, 13, 15, 17, 19, 21-24, 30, 67, 69, 70, 72, 74, 76, or 78.

[0150] In some embodiments, the recombinant Ad5-PRRSV vector comprises one or more polynucleotides encoding one or more PRRSV gp2, gp3, gp4, gp5a, gp5 or E antigen, polypeptide, ectodomain, or variants thereof, or combinations thereof.

[0151] In some embodiments, the recombinant Ad5-PRRSV vector comprises one or more polynucleotides encoding one or more antigen, polypeptide or ectodomain having: (a) at least 90% sequence identity to a sequence set forth in SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139; or, (b) at least 90% sequence identity to the ectodomain(s) encompassed by a sequence set forth in SEQ ID NO: 1, 3, 5, 7, 14, 16, 18, 20, 31, 34-39, 40-45, 46-51, 52-58, 59-61, 62-66, 68, 71, 73, 75, 77, or 79-139. By "ectodomain(s) encompassed by," it is intended that only the extracellular portion (i.e. not the transmembrane or cytoplasmic portion) of a given SEQ ID NO is to be subjected to the percent sequence identity limitation. For example, if a polypeptide consisting of 200 amino acids has an ectodomain spanning amino acids # 20 to 100, a comparator polypeptide need only be 90% identical (i.e. in the case of 90% sequence identity language) across amino acids # 20 to 100. Now that the invention has been disclosed, Applicants envision that the skilled person may routinely select from a wide variety of TMDs and CTDs to combine with the ectodomains of the disclosed individual and combinations of protective PRRSV polypeptides.

[0152] In some embodiments, the one or more polynucleotides have at least 90% sequence identity to a sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 9, 10, 11, 12, 13, 15, 17, 19, 21-24, 30, 67, 69, 70, 72, 74, 76, or 78; or, the polynucleotides have at least 90% sequence identity across the length of an ectodomain encoded by a sequence as set forth in a subsequence of SEQ ID NO: 2, 4, 6, 8, 9, 10, 11, 12, 13, 15, 17, 19, 21-24, 30, 67, 69, 70, 72, 74, 76, or 78. The skilled person using routine techniques can comprehend or ascertain which polynucleotide sequences encode ectodomains.

[0153] In some embodiments, the Ad5-PRRSV vector comprises a polynucleotide encoding a PRRSV gp2 polypeptide having: (a) at least 90% sequence identity to a sequence as set forth in SEQ ID NO: 1, 14, 34-39, or 80-89 (gp2 protein); or (b) at least 90% sequence identity to an ectodomain sequence as set forth in a subsequence of SEQ ID NO: 1, 14, 34-39, or 80-89.

[0154] In some embodiments, the Ad5-PRRSV vector comprises a polynucleotide encoding a PRRSV E polypeptide having: (a) at least 90% sequence identity to a sequence as set forth in

SEQ ID NO: 7, 20, 52-58, or 130-139 (E protein); or (b) at least 90% sequence identity to an ectodomain sequence as set forth in a subsequence of SEQ ID NO: 7, 20, 52-58, or 130-139.

[0155] In some embodiments, the Ad5-PRRSV vector comprises a polynucleotide encoding a PRRSV gp3 polypeptide having: (a) at least 90% sequence identity to a sequence as set forth in
5 SEQ ID NO: 5, 18, 40-45, or 90-99 (gp3 protein); or (b) at least 90% sequence identity to an ectodomain sequence as set forth in a subsequence of SEQ ID NO: 5, 18, 40-45, or 90-99.

[0156] In some embodiments, the Ad5-PRRSV vector comprises two polynucleotides encoding PRRSV gp2 and E polypeptides having: (a) at least 90% sequence identity to one of the sequences as set forth in SEQ ID NO: 1, 14, 34-39, or 80-89 (gp2 protein) and one of the
10 sequences as set forth in SEQ ID NO: 7, 20, 52-58, or 130-139 (E protein); or (b) at least 90% sequence identity to an ectodomain sequence as set forth in a subsequence of SEQ ID NO: 1, 14, 34-39, or 80-89 (gp2 protein) and an ectodomain sequence as set forth in a subsequence of SEQ ID NO: 7, 20, 52-58, or 130-139 (E protein).

[0157] In some embodiments, the Ad5-PRRSV vector comprises polynucleotides encoding
15 PRRSV gp2, E and gp4 polypeptides having: (a) at least 90% sequence identity to one of the sequences as set forth in SEQ ID NO: 1, 14, 34-39, or 80-89 (gp2 protein), one of the sequences as set forth in SEQ ID NO: 7, 20, 52-58, or 130-139 (E protein) and one of the sequences as set forth in SEQ ID NO: 5, 18, 40-45, 90-99 (gp3 protein); or (b) at least 90% sequence identity to an ectodomain encompassed by one of the sequences as set forth in SEQ ID NO: 1, 14, 34-39, or
20 80-89 (gp2 protein), an ectodomain encompassed by one of the sequences as set forth in SEQ ID NO: 7, 20, 52-58, or 130-139 (E protein) and an ectodomain encompassed by one of the sequences as set forth in SEQ ID NO: 5, 18, 40-45, 90-99 (gp3 protein).

[0158] In another aspect, the disclosure provides a method of eliciting a protective immune response in an animal in need thereof against PRRSV comprising administering to the animal a
25 recombinant Ad5-PRRSV vector expressing at least one gp2, gp3, gp4, gp5a, gp5 or E PRRSV antigen, and, a pharmaceutically or veterinarily acceptable carrier, adjuvant, excipient or vehicle.

[0159] In some embodiments of the method, the Ad5-PRRSV vector comprises one or more polynucleotides encoding one or more polypeptides having: (a) at least 90% sequence identity to one of the sequences as set forth in SEQ ID NO: 1, 14, 34-39, or 80-89 (gp2 protein) and SEQ

ID NO: 7, 20, 52-58, or 130-139 (E protein); or (b) at least 90% sequence identity to the gp2 protein or E protein ectodomain(s) encompassed by the corresponding foregoing SEQ ID NOs.

[0160] The method of claim 24, wherein the Ad5-PRRSV vector comprises one or more polynucleotides encoding one or more polypeptides having at least 90% sequence identity to one of the sequences as set forth in SEQ ID NO: 1, 14, 34-39, or 80-89 (gp2 protein), one of the sequences as set forth in SEQ ID NO: 7, 20, 52-58, or 130-139 (E protein) and one of the sequences as set forth in SEQ ID NO: 5, 18, 40-45, 90-99 (gp3 protein); or (b) at least 90% sequence identity to gp2, E and gp3 ectodomains encompassed by the corresponding foregoing SEQ ID NOs.

[0161] In some embodiments, the administration is by oro-nasal, spray, drinking water, intramuscular, or subcutaneous administration, intradermal, transdermal. In some embodiments, the administration is a prime-boost. In some embodiments, the first vaccination is a mixture of two Ad5 vectors, the first expressing re-targeted PRRSV minor proteins and the second expressing PRRSV major proteins; and the boost comprises or consists essentially of either both vectors of the first vaccination, or either vector alone. In some embodiments, the animal in need of protection is a porcine animal.

[0162] In general, comparison of amino acid sequences is accomplished by aligning an amino acid sequence of a polypeptide of a known structure with the amino acid sequence of a polypeptide of unknown structure. Amino acids in the sequences are then compared and groups of amino acids that are homologous are grouped together. This method detects conserved regions of the polypeptides and accounts for amino acid insertions and deletions. Homology between amino acid sequences can be determined by using commercially available algorithms (see also the description of homology above). In addition to those otherwise mentioned herein, mention is made of the programs BLAST, gapped BLAST, BLASTN, BLASTP, and PSI-BLAST, provided by the National Center for Biotechnology Information. These programs are widely used in the art for this purpose and can align homologous regions of two amino acid sequences.

[0163] Alternatively or additionally, the term "homology" or "identity", for instance, with respect to a nucleotide or amino acid sequence, can indicate a quantitative measure of homology between two sequences. The percent sequence identity can be calculated as $(N_{ref} - N_{dif})*100/N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and

wherein N_{ref} is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC (N_{ref} = 8; N_{dif}=2).

[0164] Alternatively or additionally, "homology" or "identity" with respect to sequences can refer to the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur et al., 1983), for instance, using a window size of 20 nucleotides, a word length of 4 nucleotides, and a gap penalty of 4, and computer-assisted analysis and interpretation of the sequence data including alignment can be conveniently performed using commercially available programs (e.g., Vector NTI Software ™, Invitrogen Inc. CA, USA). When RNA sequences are said to be similar, or have a degree of sequence identity or homology with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence. Thus, RNA sequences are within the scope of the invention and can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences. And, without undue experimentation, the skilled artisan can consult with many other programs or references for determining percent homology.

[0165] The invention further encompasses the PRRSV polynucleotides contained in a vector molecule or an expression vector and operably linked to a promoter element and optionally to an enhancer.

[0166] A "vector" refers to a recombinant DNA or RNA plasmid, bacteriophage, or virus that comprises a heterologous polynucleotide to be delivered to a target cell, either *in vitro* or *in vivo*. The heterologous polynucleotide may comprise a sequence of interest for purposes of prevention or therapy, and may optionally be in the form of an expression cassette. As used herein, a vector needs not be capable of replication in the ultimate target cell or subject. The term "vector" includes vectors for cloning as well as viral vectors.

[0167] The term "engineered" or "recombinant" means a polynucleotide of semi-synthetic, or synthetic origin that either does not occur in nature or is linked to another polynucleotide in an arrangement not found in nature.

[0168] "Heterologous" means derived from a genetically distinct entity from the rest of the entity to which it is being compared. For example, a polynucleotide may be incorporated by genetic engineering techniques into a plasmid or vector derived from a different source, and is thus a heterologous polynucleotide. A promoter removed from its native coding sequence and 5 operatively linked to a coding sequence other than the native sequence is a heterologous promoter.

[0169] The polynucleotides of the invention may comprise additional sequences, such as additional encoding sequences within the same transcription unit, controlling elements such as promoters, ribosome binding sites, 5'UTR, 3'UTR, transcription terminators, polyadenylation 10 sites, additional transcription units under control of the same or a different promoter, sequences that permit cloning, expression, homologous recombination, and transformation of a host cell, and any such construct as may be desirable to provide embodiments of this invention.

[0170] Elements for the expression of a PRRSV polypeptide, antigen, epitope or immunogen are advantageously present in an inventive vector. In minimum manner, this comprises, consists 15 essentially of, or consists of an initiation codon (ATG), a stop codon and a promoter, and optionally also a polyadenylation sequence for certain vectors such as plasmid and certain viral vectors. When the polynucleotide encodes a polypeptide fragment, e.g. a PRRSV peptide, advantageously, in the vector, an ATG is placed at 5' of the reading frame and a stop codon is placed at 3'. Other elements for controlling expression may be present, such as enhancer 20 sequences, stabilizing sequences, such as intron and or untranslated 5' or 3' sequences and signal sequences permitting the secretion of the protein.

[0171] Methods for making and/or administering a vector or recombinants or plasmid for expression of gene products of the invention either *in vivo* or *in vitro* can be any desired method, e.g., a method which is by or analogous to the methods disclosed in documents cited in: U.S. 25 Patent Nos. 4,603,112; 4,769,330; 4,394,448; 4,722,848; 4,745,051; 4,769,331; 4,945,050; 5,494,807; 5,514,375; 5,744,140; 5,744,141; 5,756,103; 5,762,938; 5,766,599; 5,990,091; 5,174,993; 5,505,941; 5,338,683; 5,494,807; 5,591,639; 5,589,466; 5,677,178; 5,591,439; 5,552,143; 5,580,859; 6,130,066; 6,004,777; 6,130,066; 6,497,883; 6,464,984; 6,451,770; 6,391,314; 6,387,376; 6,376,473; 6,368,603; 6,348,196; 6,306,400; 6,228,846; 6,221,362; 30 6,217,883; 6,207,166; 6,207,165; 6,159,477; 6,153,199; 6,090,393; 6,074,649; 6,045,803;

6,033,670; 6,485,729; 6,103,526; 6,224,882; 6,312,682; 6,348,450; 6,312,683, and 6,596,279; U.S. patent application Serial No.12/753,597; WO 90/01543; W091/11525; WO 94/16716; WO 96/39491; WO 98/33510; EP 265785; EP 0 370 573.

[0172] The present invention also relates to a composition or vaccine comprising vectors, such as expression vectors. The composition or vaccine can comprise, consist essentially of, or consist of one or more vectors, e.g., expression vectors, such as *in vivo* expression vectors, comprising, consisting essentially or consisting of (or expressing) one or more of PRRSV polypeptides, antigens, epitopes or immunogens. The vector contains and expresses a polynucleotide that comprises, consists essentially of, or consists of a polynucleotide coding for (or expressing) a PRRSV antigen, epitope or immunogen, in a pharmaceutically or veterinarily acceptable carrier, adjuvant, excipient or vehicle.

[0173] According to another embodiment, the vector or vectors in the composition or vaccine comprise, or consist essentially of, or consist of polynucleotide(s) encoding one or more proteins or fragment(s) thereof a PRRSV polypeptide, antigen, epitope or immunogen. The inventive composition or vaccine comprises, consists essentially of, or consists of, one or more vectors comprising, consisting essentially of, or consisting of, and advantageously also expressing, *in vivo* under appropriate conditions or suitable conditions or in a suitable host cell, polynucleotides from different PRRSV isolates encoding the same proteins and/or for different proteins.

[0174] The term plasmid covers any DNA transcription unit comprising a polynucleotide according to the invention and the elements necessary for its *in vivo* expression in a cell or cells of the desired host or target; and, in this regard, it is noted that a supercoiled plasmid and all of its topoisomers, open-circular plasmid, as well as linear forms of the plasmid, are intended to be within the scope of the invention.

[0175] Each plasmid comprises or contains or consists essentially of, in addition to the heterologous polynucleotide encoding a recombinant protein, antigen, epitope or immunogen, optionally fused with a polynucleotide encoding a heterologous peptide sequence, variant, analog or fragment, operably linked to a promoter or under the control of a promoter or dependent upon a promoter. In general, it is advantageous to employ a strong promoter that is functional in eukaryotic cells. The preferred strong promoter is the immediate early cytomegalovirus promoter (CMV-IE) of human or murine origin, or optionally having another origin such as the rat or

guinea pig. The CMV-IE promoter can comprise the actual promoter segment, which may or may not be associated with the enhancer segment. Reference can be made to EP-A-260 148, EP-A-323 597, U.S. Patents Nos. 5,168,062, 5,385,839, and 4,968,615, as well as to PCT Application No WO87/03905. The CMV-IE promoter is advantageously a human CMV-IE (Boshart et al., 1985) or murine CMV-IE.

[0176] In more general terms, the promoter is either of a viral or a cellular origin. A strong viral promoter other than CMV-IE that may be usefully employed in the practice of the invention is the early/late promoter of the SV40 virus or the LTR promoter of the Rous sarcoma virus. A strong cellular promoter that may be usefully employed in the practice of the invention is the promoter of a gene of the cytoskeleton, such as e.g. the desmin promoter (Kwissa et al., 2000), or the actin promoter (Miyazaki et al., 1989).

[0177] Functional sub-fragments of these promoters, i.e., portions of these promoters that maintain an adequate promoting activity, are included within the present invention, e.g. truncated CMV-IE promoters according to PCT Application No. WO98/00166 or U.S. Patent No. 6,156,567. A promoter in the practice of the invention consequently includes derivatives and sub fragments of a full-length promoter that maintain an adequate promoting activity and hence function as a promoter, preferably promoting activity substantially similar to that of the actual or full-length promoter from which the derivative or sub fragment is derived, e.g., akin to the activity of the truncated CMV-IE promoters of U.S. Patent No. 6,156,567 to the activity of full-length CMV-IE promoters. Thus, a CMV-IE promoter in the practice of the invention can comprise or consist essentially of or consist of the promoter portion of the full-length promoter and/or the enhancer portion of the full-length promoter, as well as derivatives and sub-fragments.

[0178] Preferably, the plasmids comprise or consist essentially of other expression control elements. It is particularly advantageous to incorporate stabilizing sequence(s), e.g., intron sequence(s), preferably the first intron of the hCMV-IE (PCT Application No. WO89/01036), the intron II of the rabbit β-globin gene (van Ooyen et al., 1979).

[0179] As to the polyadenylation signal (polyA) for the plasmids and viral vectors other than poxviruses, use can more be made of the poly(A) signal of the bovine growth hormone (bGH) gene (see U.S. Patent No. 5,122,458), or the poly(A) signal of the rabbit β-globin gene or the poly(A) signal of the SV40 virus.

[0180] According to another embodiment of the invention, the expression vectors are expression vectors used for the *in vitro* expression of proteins in an appropriate cell system. The expressed proteins can be harvested in or from the culture supernatant after, or not after secretion (if there is no secretion a cell lysis typically occurs or is performed), optionally concentrated by
5 concentration methods such as ultrafiltration and/or purified by purification means, such as affinity, ion exchange or gel filtration-type chromatography methods.

[0181] A "host cell" denotes a prokaryotic or eukaryotic cell that has been genetically altered, or is capable of being genetically altered by administration of an exogenous polynucleotide, such as a recombinant plasmid or vector. When referring to genetically altered cells, the term refers both
10 to the originally altered cell and to the progeny thereof. Host cells include, but are not limited to, baby hamster kidney (BHK) cells, colon carcinoma (Caco-2) cells, COS7 cells, HEK 293 cells, MCF-7 cells, MCF-10A cells, Madin-Darby canine kidney (MDCK) lines, mink lung (Mv1Lu) cells, MRC-5 cells, U937 cells, Chinese hamster ovary (CHO) cells, monkey Vero cells (cell line with the origin of the kidney of an African green monkey), quail (Quail muscle cell line QM7),
15 chicken cell line DF1, and VERO cells. Polynucleotides comprising a desired sequence can be inserted into a suitable cloning or expression vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification. Polynucleotides can be introduced into host cells by any means known in the art. The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including direct
20 uptake, endocytosis, transfection, f-mating, electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is infectious, for instance, a retroviral vector). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

25 **[0182]** In one embodiment of the present invention, the vector is an Ad5 vector as described in US 2010/0255029.

[0183] Advantages of PRRSV vaccines based on the Ad5 vector include, but are not limited to, (1) induce a broad immunity, including humoral, cellular and mucosal responses (2) do not express all PRRSV proteins and therefore is compatible with the DIVA (differentiate infected

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from vaccinated animals) strategy, (3) induce rapid onset of immunity, and (4) production poses less risk for the environment than inactivated vaccines in case of accidental release.

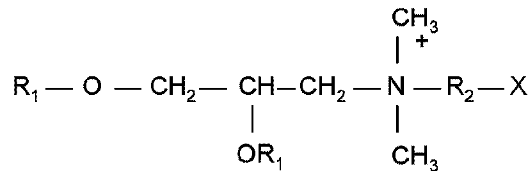
[0184] One aspect of the invention relates to engineered or recombinant Ad5 vectors expressing PRRSV antigens. The antigen may be PRRSV minor envelope proteins, such as gp2, gp3, gp4, 5 gp5a, or E protein, aforementioned. The engineered Ad5 vector may comprise one or more polynucleotides encoding one or more PRRSV antigens. In another aspect, the engineered Ad5 vector comprises one or more polynucleotides encoding a PRRSV gp2 antigen or variant thereof, a PRRSV E antigen or variant thereof, a PRRSV gp3 antigen or variant thereof, a PRRSV antigen or variant thereof, gp4 antigen or variant thereof, or a combination thereof.

10 **[0185]** In one embodiment, the invention provides for the administration of a therapeutically effective amount of a formulation for the delivery and expression of a protein, antigen, epitope or immunogen in a target cell. Determination of the prophylactically or therapeutically effective amount is routine experimentation for one of ordinary skill in the art. In another embodiment, the formulation comprises an expression vector comprising a polynucleotide that expresses a 15 PRRSV minor envelope antigen, epitope or immunogen and a pharmaceutically or veterinarily acceptable carrier, vehicle, adjuvant or excipient. In another embodiment, the pharmaceutically or veterinarily acceptable carrier, vehicle, adjuvant or excipient facilitates transfection and/or improves preservation of the vector or protein.

[0186] The pharmaceutically or veterinarily acceptable carriers or vehicles or adjuvant or 20 excipients are well known to the one skilled in the art. For example, a pharmaceutically or veterinarily acceptable carrier or vehicle or adjuvant or excipient can be sterile water, a 0.9% NaCl (e.g., saline) solution or a phosphate buffer. Other pharmaceutically or veterinarily acceptable carrier or vehicle or adjuvant or excipients that can be used for methods of this invention include, but are not limited to, poly-(L-glutamate) or polyvinylpyrrolidone. The 25 pharmaceutically or veterinarily acceptable carrier or vehicle or adjuvant or excipients may be any compound or combination of compounds facilitating the administration of the vector (or protein expressed from an inventive vector *in vitro*); advantageously, the carrier, vehicle or adjuvant or excipient may facilitate transfection and/or improve preservation of the vector (or protein). Doses and dose volumes are herein discussed in the general description and can also be

determined by the skilled artisan from this disclosure read in conjunction with the knowledge in the art, without any undue experimentation.

[0187] The cationic lipids containing a quaternary ammonium salt which are but not exclusively suitable for plasmids, are those having the following formula:



5 in which R1 is a saturated or unsaturated straight-chain aliphatic radical having 12 to 18 carbon atoms, R2 is another aliphatic radical containing 2 or 3 carbon atoms and X is an amine or hydroxyl group, e.g. the DMRIE. In another embodiment the cationic lipid can be associated with a neutral lipid, e.g. the DOPE.

[0188] Among these cationic lipids, preference is given to DMRIE (N-(2-hydroxyethyl)-N,N-
10 dimethyl-2,3-bis(tetradecyloxy)-1-propane ammonium; WO96/34109), advantageously associated with a neutral lipid, advantageously DOPE (dioleoyl-phosphatidyl-ethanol amine; Behr, 1994), to form DMRIE-DOPE.

[0189] The plasmid mixture with the adjuvant is formed extemporaneously and/or contemporaneously with administration of the preparation or shortly before administration of the
15 preparation; for instance, shortly before or prior to administration, the plasmid-adjuvant mixture is formed, advantageously so as to give enough time prior to administration for the mixture to form a complex, e.g. between about 10 and about 60 minutes prior to administration, such as approximately 30 minutes prior to administration.

[0190] When DOPE is present, the DMRIE:DOPE molar ratio may be about 95:about 5 to about
20 5:about 95, or about 1:about 1, e.g., 1:1. The DMRIE or DMRIE-DOPE adjuvant: plasmid weight ratio can be between about 50:about 1 and about 1:about 10, such as about 10:about 1 and about 1:about 5, and advantageously about 1:about 1 and about 1:about 2, e.g., 1:1 and 1:2.

[0191] In another embodiment, pharmaceutically or veterinarily acceptable carrier, adjuvant, excipient, or vehicle may be a water-in-oil emulsion. Examples of suitable water-in-oil
25 emulsions include oil-based water-in-oil vaccinal emulsions which are stable and fluid at 4°C containing: from 6 to 50 v/v % of an antigen-containing aqueous phase, preferably from 12 to 25 v/v %, from 50 to 94 v/v % of an oil phase containing in total or in part a non-metabolizable oil

(e.g., mineral oil such as paraffin oil) and/or metabolizable oil (e.g., vegetable oil, or fatty acid, polyol or alcohol esters), from 0.2 to 20 p/v % of surfactants, preferably from 3 to 8 p/v %, the latter being in total or in part, or in a mixture either polyglycerol esters, said polyglycerol esters being preferably polyglycerol (poly)ricinoleates, or polyoxyethylene ricin oils or else
5 hydrogenated polyoxyethylene ricin oils. Examples of surfactants that may be used in a water-in-oil emulsion include ethoxylated sorbitan esters (e.g., polyoxyethylene (20) sorbitan monooleate (TWEEN 80®), available from AppliChem, Inc., Cheshire, CT) and sorbitan esters (e.g., sorbitan monooleate (SPAN 80®), available from Sigma Aldrich, St. Louis, MO). In addition, with respect to a water-in-oil emulsion, see also US Patent No. 6,919,084. In some embodiments,
10 the antigen-containing aqueous phase comprises a saline solution comprising one or more buffering agents. An example of a suitable buffering solution is phosphate buffered saline. In one embodiment, the water-in-oil emulsion may be a water/oil/water (W/O/W) triple emulsion (see, e.g., U.S. Patent No. 6,358,500). Examples of other suitable emulsions are described in U.S. Patent No. 7,371,395.

15 **[0192]** The immunological compositions and vaccines according to the invention may comprise or consist essentially of one or more adjuvants. Suitable adjuvants for use in the practice of the present invention are (1) polymers of acrylic or methacrylic acid, maleic anhydride and alkenyl derivative polymers, (2) immunostimulating sequences (ISS), such as oligodeoxyribonucleotide sequences having one or more non-methylated CpG units (Klinman et al., 1996; WO98/16247),
20 (3) an oil in water emulsion, such as the SPT emulsion described on p 147 of "Vaccine Design, The Subunit and Adjuvant Approach" published by M. Powell, M. Newman, Plenum Press 1995, and the emulsion MF59 described on p183 of the same work, (4) cation lipids containing a quaternary ammonium salt, e.g., DDA (5) cytokines, (6) aluminum hydroxide or aluminum phosphate, (7) saponin or (8) other adjuvants discussed in any document cited, or (9) any
25 combinations or mixtures thereof.

[0193] The oil in water emulsion (3), which is especially appropriate for viral vectors, can be based on: light liquid paraffin oil (European pharmacopoeia type), isoprenoid oil such as squalane, squalene, oil resulting from the oligomerization of alkenes, e.g. isobutene or decene, esters of acids or alcohols having a straight-chain alkyl group, such as vegetable oils, ethyl
30 oleate, propylene glycol, di(caprylate/caprate), glycerol tri(caprylate/caprate) and propylene glycol dioleate, or esters of branched, fatty alcohols or acids, especially isostearic acid esters.

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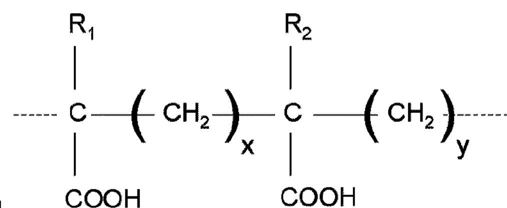
[0194] The oil is used in combination with emulsifiers to form an emulsion. The emulsifiers may be nonionic surfactants, such as: esters of on the one hand sorbitan, mannide (e.g. anhydromannitol oleate), glycerol, polyglycerol or propylene glycol and on the other hand oleic, isostearic, ricinoleic or hydroxystearic acids, said esters being optionally ethoxylated, or
5 polyoxypropylene-polyoxyethylene copolymer blocks, such as Pluronic, e.g., L121.

[0195] Among the type (1) adjuvant polymers, preference is given to polymers of cross linked acrylic or methacrylic acid, especially cross linked by polyalkenyl ethers of sugars or polyalcohols. These compounds are known under the name carbomer (Pharmeuropa, vol. 8, no. 2, June 1996). One skilled in the art can also refer to U.S. Patent No. 2,909,462, which provides
10 such acrylic polymers cross linked by a polyhydroxyl compound having at least three hydroxyl groups, preferably no more than eight such groups, the hydrogen atoms of at least three hydroxyl groups being replaced by unsaturated, aliphatic radicals having at least two carbon atoms. The preferred radicals are those containing 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals can also contain other substituents,
15 such as methyl. Products sold under the name Carbopol (BF Goodrich, Ohio, USA) are especially suitable. They are cross linked by allyl saccharose or by allyl pentaerythritol. Among them, reference is made to Carbopol 974P, 934P and 971P.

[0196] As to the maleic anhydride-alkenyl derivative copolymers, preference is given to EMA (Monsanto), which are straight-chain or cross linked ethylene-maleic anhydride copolymers and
20 they are, for example, cross linked by divinyl ether. Reference is also made to J. Fields et al., 1960.

[0197] With regard to structure, the acrylic or methacrylic acid polymers and EMA are preferably formed by basic units having the following formula:

[0198] 

25 [0199] in which:

[0200] R1 and R2, which can be the same or different, represent H or CH3

[0201] x = 0 or 1, preferably x = 1

[0202] y = 1 or 2, with x + y = 2.

[0203] For EMA, x = 0 and y = 2 and for carbomers x = y = 1.

[0204] These polymers are soluble in water or physiological salt solution (20 g/l NaCl) and the pH can be adjusted to 7.3 to 7.4, e.g., by soda (NaOH), to provide the adjuvant solution in which the expression vector(s) can be incorporated. The polymer concentration in the final immunological or vaccine composition can range between 0.01 and 1.5% w/v, 0.05 to 1% w/v or 0.1 to 0.4% w/v.

[0205] The cytokine or cytokines (5) can be in protein form in the immunological or vaccine composition, or can be co-expressed in the host with the immunogen or immunogens or epitope(s) thereof. Preference is given to the co-expression of the cytokine or cytokines, either by the same vector as that expressing the immunogen or immunogens or epitope(s) thereof, or by a separate vector thereof.

[0206] The invention comprehends preparing such combination compositions; for instance by admixing the active components, advantageously together and with an adjuvant, carrier, cytokine, and/or diluent.

[0207] Cytokines that may be used in the present invention include, but are not limited to, granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage colony stimulating factor (GM-CSF), interferon α (IFNα), interferon β (IFNβ), interferon γ, (IFNγ), interleukin-1α(IL-1α), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11), interleukin-12 (IL-12), tumor necrosis factor α (TNFα), tumor necrosis factor β (TNF□β), and transforming growth factor β (TGF□β). It is understood that cytokines can be co-administered and/or sequentially administered with the immunological or vaccine composition of the present invention. Thus, for instance, the vaccine of the instant invention can also contain an exogenous nucleic acid molecule that expresses *in vivo* a suitable cytokine, e.g., a cytokine matched to this host to be vaccinated or in which an immunological response is to be elicited (for instance, a feline cytokine for preparations to be administered to a feline).

[0208] In another embodiment, the composition of the present invention may be prepared using the chemical or physical procedure as described by Stauffer *et al.* (Recent Patents on Anti-Infective Drug Discovery, 1, 291-296, 2006). Some of the inactivation techniques are summarized in the table below.

5 Table 1. Inactivation techniques

Chemical	Physical	Combined
Ascorbic Acid		Ascorbic Acid + UV
b-Propiolactone	Heat	Beta Propiolactone + UV
b-aminophenylketone	Pressure	Formalin + Heat
Diethylpyrocarbonate	UV	Formalin + UV
Ethylenimine	Non Ionic Detergents	Heat + Low Pressure
Formalin/Formaldehyde		Pressure + Heat or Cold
Phenol		Psoralen + UV

[0209] The immunological composition and/or vaccine according to the invention comprise or consist essentially of or consist of an effective quantity to elicit a protective or therapeutic response of one or more expression vectors and/or polypeptides as discussed herein; and, an
10 effective quantity can be determined from this disclosure, and the knowledge in the art, without undue experimentation.

[0210] The compositions or vaccines of the present invention may be administered to an animal via drinking water, oro-nasal, sprays, aerosols, intranasal instillation, transdermal, subcutaneous, or intramuscular injection. Advantageously, the vaccines are administered by transdermal, oro-
15 nasal, subcutaneous, intramuscular, spray or drinking water.

[0211] The present invention contemplates at least one administration to an animal of an efficient amount of the therapeutic composition made according to the invention. The therapeutic composition according to the invention can be administered by a needleless apparatus (as, for example with a Pigjet, Dermojet, Biojector, Vetjet or Vitajet apparatus (Bioject, Oregon, USA)).

20 [0212] In one embodiment of the invention, a prime-boost regimen can be employed, which is comprised of at least one primary administration and at least one booster administration using at least one common protein, polypeptide, antigen, epitope or immunogen. The immunological composition or vaccine used in primary administration is different in nature from those used as a booster. However, it is noted that the same composition can be used as the primary

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administration and the boost administration. This administration protocol is called “prime-boost”.

[0213] In another aspect of the prime-boost protocol of the invention, a composition comprising the engineered Ad5 PRRSV vaccine or composition is administered followed by the
5 administration of vaccine or composition comprising a recombinant viral vector that contains and expresses a PRRSV antigen *in vivo*, or an inactivated viral vaccine or composition comprising the PRRSV antigen, or a vaccine or composition comprising a PRRSV subunit (protein), or a DNA plasmid vaccine or composition that contains or expresses a PRRSV antigen. Likewise, a prime-boost protocol may comprise the administration of vaccine or composition comprising a
10 recombinant viral vector that contains and expresses a PRRSV antigen *in vivo*, or an inactivated viral vaccine or composition comprising the PRRSV antigen, or a vaccine or composition comprising a PRRSV subunit (protein), or a DNA plasmid vaccine or composition that contains or expresses a PRRSV antigen, followed by the administration of a composition comprising the engineered Ad5 PRRSV vaccine or composition. It is noted that both the primary and the
15 secondary administrations may comprise the composition comprising the engineered Ad5 PRRSV vaccine or composition. It is further noted that both the primary and the secondary administrations may comprise one or more compositions comprising the engineered vectors of the present invention.

[0214] A prime-boost protocol comprises at least one prime-administration and at least one boost
20 administration using at least one common antigen. The vaccine or composition used in prime-administration may be different in nature from those used as a later booster vaccine or composition. The prime-administration may comprise one or more administrations. Similarly, the boost administration may comprise one or more administrations.

[0215] The various administrations are preferably carried out about 1 to about 6 weeks apart, or
25 about 2 to about 4 weeks apart. Repeated booster every 2 to 6 weeks or an annual booster is also contemplated. The animals are preferably at least one day old at the time of the first administration.

[0216] The immunological composition and/or vaccine contains per dose from about 10^4 to about 10^{11}, advantageously from about 10^5 to about 10^{10} and more advantageously from about
30 10^6 to about 10^9 viral particles of recombinant adenovirus expressing a PRRSV antigen, epitope

or immunogen. In the case of immunological composition and/or vaccine based on a poxvirus, a dose can be between about 10^2 pfu and about 10^9 pfu. The immunological composition and/or vaccine contains per dose from about 10^2 to about 10^7, advantageously from about 10^3 to about 10^5 pfu of poxvirus or herpesvirus recombinant expressing the PRRSV antigen, epitope or immunogen.

[0217] The viral vector may be an attenuated avipox expression vector. In one embodiment, the avipox expression vector may be a fowlpox vector, for example, TROVAC®. In another embodiment, the avipox expression vector may be a canarypox vector, for example, ALVAC®. In still another embodiment, a baculovirus expression platform may be used. For example, the antigens may be produced in a baculovirus expression system using insect cell cultures as host, and the resulting recombinant polypeptides may be administered to the animals. Alternatively, the entire recombinant baculovirus may be administered as a vaccine. In general, the PRRSV antigen, epitope or immunogen may be a PRRSV minor envelope protein, such as gp2, gp3, gp4, gp5a, gp5 or E. Other viruses that may be used in methods of the invention include, but are not limited to, vaccinia viruses, such as an attenuated vaccinia virus, for instance NYVAC, adenoviruses and herpesviruses, including porcine CMV.

[0218] The efficacy of the vaccines may be tested about 2 to 4 weeks after the last immunization by challenging animals with a virulent strain of PRRSV. Both homologous and heterologous strains may be used for challenge to test the efficacy of the vaccine. The animal may be challenged by spray, intra-nasal, IM, intra-tracheal, and/or oral. The challenge viral challenge may be about 10^3 to about 10^9 virions or infectious units per dose, in a volume depending upon the route of administration. For example, if the administration is by spray, a virus suspension is aerosolized to generate about 1 to 200 μm droplets, if the administration is intra-nasal, intra-tracheal or oral, the volume of the challenge virus is about 0.05 to about 5 ml. Animals may be observed daily for 14 days following challenge for clinical signs and mortality. In addition, the groups of animals may be euthanized and evaluated for pathological findings. Oropharyngeal, tracheal or cloacal swabs may be collected from all animals post challenge for virus detection. The presence or absence of viral antigens in tissues may be evaluated by immunohistochemistry, viral isolation or titration, or nucleic acid detection such as reverse-transcriptase polymerase chain reaction (RT-PCR). Blood samples may be collected post-challenge and may be analyzed for the presence of anti-PRRSV gp2, gp3, gp4, gp5a, E virus-specific antibody. Alternatively,

when the engineered vectors contain epitope tags, tag-specific antibodies may be used to detect the presence and location of recombinant vaccine polypeptides.

[0219] It should be understood by one of skill in the art that the disclosure herein is provided by way of example and the present invention is not limited thereto. From the disclosure herein and
5 the knowledge in the art, the skilled artisan can determine the number of administrations, the administration route, and the doses to be used for each immunization protocol, without any undue experimentation.

[0220] Another embodiment of the invention is a kit for performing a method of inducing an immunological or protective response against PRRSV in an animal comprising a recombinant
10 Ad5 immunological composition or vaccine or an inactivated PRRSV immunological composition or vaccine and instructions for performing the method of delivery in an effective amount for eliciting an immune response in the animal.

[0221] Unless otherwise specifically recited, construction of nucleic acid inserts, plasmids and recombinant viral vectors was carried out using the standard molecular biology techniques
15 known in the art, for example, described by J. Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989).

[0222] Particularly as to subject matter eligibility, the vectors disclosed herein do not result in the expression in the vaccinated animal of naturally-occurring levels of PRRSV proteins. Each
20 gene's expression is driven by non-native heterologous promoter elements, and so, the ultimate amount of each cognate protein expressed will not be equivalent to that produced during natural PRRSV infection. Moreover, one important purpose of the disclosed expression system is to produce relatively high levels of PRRSV minor envelope proteins (native, modified or engineered), and to properly present the minor proteins to the host animal's immune system, to
25 elicit in the animals a safe and protective immune response. The levels and presentation of the PRRSV minor envelope proteins typical of natural PRRSV infection fail to elicit a safe and effective immune response against the PRRSV minor proteins. Accordingly, both the disclosed vaccine compositions, and their ultimate disposition within the vaccinated animal, differ significantly in *structure* and *function* when compared to their closest naturally-occurring
30 counterparts.

[0223] The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1 - Construction and testing of plasmids expressing PRRSV genes

[0224] In order to increase visibility to the immune system, the PRRSV envelope proteins were re-targeted to the cell surface from intracellular compartments by introducing multiple changes while maintaining the extracellular domain (putative antibody binding site). The re-targeting of the envelope genes was initially attempted by removing the cytoplasmic and transmembrane domains of the native protein, which is probable site for the retention signal, and replacing them with similar domains from vesicular Stomatitis Virus glycoprotein (VSV-G), another viral protein known for cell surface expression. The signal sequence of the native envelope genes was also replaced with the signal sequence from tissue plasminogen activator (tPA), a well-characterized secretory protein, to promote entry of the modified proteins to the secretory pathway and eventual expression on the cell surface. Specific epitope tags were also inserted into each of the re-targeted proteins to track the expression and translocation of the proteins within the cell. The epitope tags Myc, Flag and HA flanked with linker sequences were inserted into gp2, gp3 and gp4, respectively (FIGs. 5A-5D).

[0225] Surface expression of re-targeted proteins. Each of the re-targeted genes was synthesized in its entirety and cloned into the expression plasmid with CMV promoter. The plasmids were transfected into HEK 293T cells and expression was detected in fixed cells by immunofluorescence assay (IFA) (FIG. 6). Cell surface and total protein expression was readily detected in cells transfected with both gp3-Flag-VSV and gp4-HA-VSV. However, expression in gp2-Myc-VSV-transfected cells was detected only after permeabilization of the cells, indicating the modifications introduced in gp2 were not sufficient to re-target the protein to the cells surface. Moreover, upon permeabilization, the staining for gp2-Myc-VSV was distinctly different from that of gp3 or gp4 modified (re-targeted) proteins. In the case of gp2-myc-VSV, the staining was more focal and intense, while in the gp3-Flag-VSV and gp4-HA-VSV it was diffuse throughout the cell. This indicated that the gp2-VSV-Myc protein was expressed, but might have folded improperly, becoming trapped in some sub-cellular compartment. There can be several reasons for inability of the modified gp2 to fold properly. First, these can be the requirement of other parts of the protein for proper folding, such as signal sequence, trans-

membrane or cytoplasmic tail that were removed in the process of modifying for surface expression. Second, it can also be due to incomplete removal of domains of gp2 that has still contained retention signal. Third, the misfolding might have been induced due to the presence of myc tag, which is not present in either modified gp3 or gp4. Fourth, it has been shown that the lack of expression of one of the minor proteins abrogates incorporation of all of the minor proteins into the virion; therefore, gp2 may require the presence of gp3 and gp4 to achieve proper folding.

[0226] Re-targeted proteins interact to form oligomers. Interaction among minor proteins has been implicated by a functional assay and directly demonstrated by a biochemical assay. Plasmids coding for each of the re-targeted proteins were co-transfected to HEK-293T cells and interaction among the minor proteins was tested by co-immunoprecipitation (Co-IP) assay. As shown in FIG. 7, the anti-HA antibody pulls down specifically gp4-HA-VSV (lane 3) but not gp3-flag-VSV (lane 2) or gp2-myc-VSV (lane 1). However, when all the modified proteins were co-transfected, the same anti-HA antibody pulled down additional protein band other than gp4-HA-VSV (lane 4, red dot), indicating that the additional protein has direct interaction with gp4-HA-VSV but not the anti-HA antibody. The size of this band is similar to the gp2-Myc-VSV (lane 6) or gp3-Flag-VSV (lane 7), indicating that this protein interacting with gp4 can be gp2, gp3 or both. A subsequent probe of the additional band in the co-IP (lane 4) with anti-Flag or anti-Myc antibody turned out to be positive for both (not shown), indicating that this band contains both gp2 and gp3 proteins. Therefore, the conclusion from this and additional experiments is that the modifications introduced for surface expression of the gps did not alter their quaternary structure.

[0227] Re-targeted proteins maintain interaction with CD163 receptor after modification. The next step in ensuring the proper folding of the re-targeted protein was to show that they still maintain their capacity to interact with the receptor, porcine CD163. Each of the plasmids expressing the re-targeted proteins were co-transfected with plasmid expressing CD163 (domains 4-9), previously shown to be sufficient to mediate entry of virus into target cells. One portion of the cell lysate was immunoprecipitated with anti-VSV antibody (specific for the envelope proteins) and the other portion was immunoprecipitated with anti-CD163 antibody. The lysate precipitated with anti-CD163 antibody was probed with anti-CD163 antibody conjugated with Biotin to control for the input CD163 into each co-IP reaction (FIG. 8C). The lysate

immunoprecipitated with anti-VSV was run in duplicates and one membrane was probed with anti-VSV-HRP (FIG. 8A), to measure the amount of modified gp, and the other membrane was probed with anti-CD163 (FIG. 8B) to measure the amount of CD163 co-immunoprecipitated with the modified envelope glycoproteins.

[0228] All the modified minor envelope glycoproteins do interact with CD163, whereas the modified gp5, a major glycoprotein used as negative control, had a much weaker or undetectable interaction with CD163.

Example 2 - Animal vaccination with pooled PRRSV envelope gene-expressing plasmids

[0229] Thirty-two, 3 weeks pigs were divided into 4 groups, of 8 animals each (Table 2).

Table 2. Study details.

Group	No. Animals	Group	Immunization (Days)					Killed/DNA	Challenge
				0	14	28	42	63	84
1	8	Wild-type PRRSV Gps	1A	X	X	X	X	DNA (3)	X
			1B	X	X	X	X	Killed (5)	X
2	8	Recombinant PRRSV Gps	2A	X	X	X	X	DNA (3)	X
			2B	X	X	X	X	Killed (5)	X
3	8	Mock DNA Imm. (Rabies G)	3A	X	X	X	X	DNA (3)	X
			3B	X	X	X	X	Killed (3)	X
4	8	Un-vaccinated							X

[0230] The wild-type group received pool of 3 plasmids expressing the non-targeted gps, the recombinant group received pool of three plasmids expressing the re-targeted gps (i.e. FIGs. 5B to 5D), the Mock group received plasmid coding for the Rabies glycoprotein, while the unvaccinated group received only Tris-EDTA buffer. Each plasmid was at a concentration of about 1 µg/µL, and about 400 µg of each plasmid was administered at 200 µl per each ear lobe. After 4 immunizations, each group was further divided and boosted with either Killed vaccine, in TS6 adjuvant (US 7,371,395 B2, to Merial), or received a 5th round of DNA immunization.

[0231] While there appeared to be a *trend* toward increased protection against lung lesions in animals vaccinated with either of the pooled plasmids, when compared to the rabies-G or unvaccinated groups, the mean among all groups was not statistically different. There was also no significant difference between groups receiving targeted vs. re-targeted plasmids.

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[0232] Therefore, Applicants next set out to put all the genes within a single vector, to enable simultaneous expression within a single cell, to facilitate interaction/oligomerization of the PRRSV envelope proteins.

Example 3 - Construction and testing of viral vectors expressing PRRSV genes

5 **[0233]** Cells and Media. HEK 293 cells (ATCC) were maintained in MEM (Gibco #11095) with 10% Fetal Bovine serum (Moregate Batch #81827101) at 37 °C in 5% CO2. These cells were used to rescue the recombinant adenovirus (vAD3041, vAD3042, vAD3038, vAD3033, and vAD3067) and make virus stocks.

[0234] Construction of Viral vectors and Immunogens. The minor envelope proteins of 10 PRRSV include gp2 (ORF2), gp3 (ORF3), gp4 (ORF4) and E (ORF2b). The DNA sequence of each of these proteins was obtained from GenBank Accession # U87392 (VR2332, PRRSV Type II). VR2332 (North American strain) represents one of two known major serotypes of PRRSV (Done et al., 1996). The other, prototype Lelystad, is representative of at least most strains that have been isolated in Western Europe. The codon-optimized sequences of each protein when 15 constructed with appropriate promoter to express all proteins from single viral vector (FIG. 1). In each case, SV40 (Simian virus 40) and CMV (Cytomegalovirus) promoters drive expression of gp2 and gp4, respectively, in opposite directions, as indicated by arrows. It is envisioned that these promoters could be exchanged, such that SV40 could drive expression of gp4 and CMV could drive expression of gp2. Such variations will be obvious to the skilled person. Importantly, 20 because of the disclosed critical role played by the PRRSV minor proteins in eliciting a safe and protective immune response, Applicants fully expect the following approaches to apply equally well to <u>all</u> PRRSV strains. Accordingly, codon-optimized versions of the Lelystad minor proteins may be prepared by routine methods, and the resulting sequences cloned into the recombinant vectors of the instant disclosure.

25 **[0235]** In all Ad5 PRRSV constructs, the expression of minor envelope glycoprotein gp3 is promoted by an <u>I</u>nternal <u>R</u>ibosome <u>E</u>ntry <u>S</u>ite (IRES). Expression of minor envelope glycoprotein E in vAD3041 and vAD3067 (FIGs. 1C & 1D) is enabled by the presence of self-cleavage peptide (p2A), situated in the Ad5 constructs immediately following the gp2 coding region.

[0236] Further, the half-life of transcripts from SV40 and CMV promoters is enhanced by addition of poly A tails (pA) from SV40 or thymidine kinase (TK). The attL1 and attL2 sites (far left and right of each insert shown in FIG. 1) were used to insert the entire synthetic fragments into the adenoviral genome by LR recombination, Gateway Technology (Invitrogen) (thereby
5 creating vAD3042, vAD3038, vAD3041 and vAD3067. The inserts of FIG. 1 were chemically synthesized (Genscript) to contain the appropriate restriction sites for cloning into the expression clone to generate recombinant Ad5 (Gateway Technology, Invitrogen). Once more, variations as to which element promotes expression of which particular PRRSV gene are contemplated, and are well within the reach of the skilled artisan reading this disclosure.

10 [0237] Accordingly, multiple combinations of minor proteins were assembled for recombination into the Ad5 vector: one containing only three of the minor proteins without E (vAD3042) (FIG. 1A; SEQ ID NO: 2); one containing *rtg*-gp2, *rtg*-gp3, *rtg*-gp4 proteins without E (vAD3038) (FIG. 1B; SEQ ID NO: 3); one containing all four codon-optimized minor proteins gp2, gp3, gp4 and E (vAD3041) (FIG. 1C; SEQ ID NO: 3); and one containing all four codon-optimized minor
15 proteins *rtg*-gp2, *rtg*-gp3, *rtg*-gp4 and E (vAD3067) (FIG. 1D; SEQ ID NO: 4).

Table 3. Locations of features within the constructs

Construct	Feature	Location
vAD3041 insert (4662 bp)	attL1	1-96
	SV40 poly A	97-314 (complementary)
	E ORF	341-562 (complementary)
	P2A	568-633 (complementary)
	gp2 ORF	642-1412 (complementary)
	SV40 promoter	1418-1785(complementary)
	CMV promoter	1806-2393
	gp4 ORF	2406-2942
	IRES	2949-3511
	gp3 ORF	3518-4282
	TK poly A	4295-4566
	attL2	4567-4662
vAD3042 insert (4662 bp)	attL1	1-96
	SV40 poly A	97-314 (complementary)
	gp2 ORF	341-1111 (complementary)
	SV40 promoter	1117-1484(complementary)
	CMV promoter	1505-2092
	gp4 ORF	2105-2641
	IRES	2648-3210

	gp3 ORF	3217-3981
	TK poly A	3994-4265
	attL2	4266-4361
vAD3038 insert (re-targeted vector)	attL1	1-96
	SV40 poly A	97-314 (complementary)
	gp2-Myc-VSV ORF	333-1151 (complementary)
	SV40 promoter	1163-1530(complementary)
	CMV promoter	1551-2138
	gp4-HA-VSV ORF	2148-2864
	IRES	2865-3427
	gp3-Flag-VSV ORF	3431-4192
	TK poly A	4199-4470
	attL2	4471-4566
vAD3067 insert (FIG. 1D)	attL1	1-96
	SV40 poly A	97-314 (complementary)
	E ORF	341-562 (complementary)
	P2A	568-633 (complementary)
	gp2-Myc-VSV ORF	642-1460 (complementary)
	SV40 promoter	1472-1839 (complementary)
	CMV promoter	1860-2447
	gp4-HA-VSV ORF	2457-3173
	IRES	3174-3736
	gp3-Flag-VSV ORF	3740-4501
	TK poly A	4508-4779
	attL2	4480-4575
pAd/PL-DEST (Above transgene cassette inserts were placed between the attR1 and attR2 sites of pAD/PL-DEST)	Human Adenovirus 5 sequences	(wild type 1-458; includes 5'L-ITR and packaging signal): 1-458
	attR1 site	512-636
	attR2 site	2092-2216
	Human Adenovirus 5 sequences	(wild type 3513-35935; E3 region deleted, includes 3'R-ITR): 2234-32782
	PacI restriction site	32788 and 34862
	Plasmid backbone region	32959-34705 including pUC origin, Ampicillin resistance gene

[0238] Production of virus. The expression clones were generated by LR recombination of entry vector with destination vector using Gateway technology (Invitrogen). Recombinant adenovirus vAD3041, vAD3042 and vAD3038 were generated by transfection of linearized expression clones in HEK 293 cells with transfection reagent. After rescue of, each virus was

harvested by freeze-thaw cycle and clarification the cell debris by centrifugation. For passage, each virus was inoculated into monolayer of HEK 293 cells and approximately 3-4 days post infection, virus was harvested by freeze-thaw cycle and clarification by centrifugation. Three passages were conducted to make virus stock, which was stored at -80 °C. As a negative control, codon-optimized hemagglutinin (HA) gene of Swine Influenza Virus (SIV) was assembled similarly in Ad5 viral vectors (vAD3033).

[0239] Viral Titer. HEK 293 cells were plated at a density of 7 x 10^5 cells per plate in three 96 well plates with MEM (Gibco #11095) media containing 2% FBS (Moregate Batch #81827101), non-essential amino acid (Gibco #11140), antibiotics-antimycotics (Gibco #15240). On the day of infection, each plate was infected with 100 µl per well of diluted virus from 10^{-3} to 10^{-10}. Virus titers were read on day 10 post infection and the average of three plates was used to calculate the titer. The Passage 3 stock titer of vAD3041 P.3 was $10^{9.03}$ $TCID_{50}$ per ml, and that of vAD3042 P.3 was $10^{8.90}$ $TCID_{50}$ per ml. The Passage 3 stock titer of vAD3038 P.3 was $10^{9.93}$ $TCID_{50}$ per ml, and that of another batch of vAD3042 P.3 was $10^{9.97}$ $TCID_{50}$ per ml.

[0240] Viral DNA was extracted from each virus stock and amplified with primers pAd Forward (5'-GAC TTT GAC CGT TTA CGT GGA GAC-3') (SEQ ID NO: 26) and pAd Reverse (5'-CCT TAA GCC ACG CCC ACA CAT TTC-3') (SEQ ID NO: 27) using platinum PCR supermix High Fidelity (Invitrogen #12532) as directed. The PCR amplicons were the same size as expected: e.g. 4709 bp for vAD3041; 4408 bp for vAD3042 (FIG. 4). The nucleotide sequences of PCR amplicons from each recombinant adenovirus were identical as constructed in the entry vectors (described in FIG. 1), and there was no change in nucleotide sequence of transgene cassettes (PRRSV genes and promoter and poly A tails).

[0241] Expression of re-targeted minor envelope proteins from recombinant adenovirus. The simultaneous expression of each of the modified envelope proteins from the recombinant adenovirus within a single cell was confirmed by using dual-Immunofluorescence assay. The recombinant vAD3038 was used to infect confluent HEK293 monolayer at high MOI and cells were fixed after 48 hours and visualized by IFA for expression of the recombinant antigens. All the proteins were shown to express well including on the cell surface (FIGs. 9A & 9B).

[0242] Importantly, the expression of gp2, which was defective when expressed alone, shown earlier as intense focal intracellular expression with no detectable surface expression, has

improved with diffuse intracellular expression and distinct cell surface expression (FIG. 9C). This indicated that the proper folding and transport of modified gp2 might depend upon the co-expression of gp3 and/or gp4. This result suggests formation of the neutralizing epitope requires formation of higher order structure by interaction among the minor proteins.

5 **Example 4 – Clinical Trial Testing Safety and Efficacy of the Ad5 PRRSV Vaccines**

[0243] Sixty (60) pigs were randomly divided into 4 groups, each containing 15 animals (Table 3). Group 3 received vAD3042, which expresses only gp2, gp3 and gp4, whereas Group 2 received vAD3041, which further expresses E. Group 1 received vAD3038, which expresses re-targeted gp2, gp3 and gp4, and Group 4 received vAD3033 that expresses SIV HA (negative
10 control). Groups that received the adenoviral vaccines were primed by administering 1 ml of the preparation in each nostril, total 2 mL, approximately at a concentration of 10^{8-9} $TCID_{50}$/mL. These groups were boosted after 21 days by the same preparation administered intramuscularly. After 42 days of initiation of the experiment, all animals were challenged with PRRSV NADC20 strain intranasally. All animals were sacrificed after 2 weeks of challenge and examined for
15 lesions in the lung and samples were collected for analysis of virus titer in tissues and sera, as indicated in FIG. 9.

Table 3. Vaccination trial scheme

Group #	# /group	Prime	Boost	Challenge
		Day 0	Day 21	Day 42
1	15	vAD3038	vAD3038	NADC20
2	15	vAD3041	vAD3041	NADC20
3	15	vAD3042	vAD3042	NADC20
4	15	vAD3033	vAD3033	NADC20

[0244] In general, the data demonstrate that while vaccination with a single vector encoding the
20 minor envelope proteins gp2, gp3 and gp4 (vAD3042) does not confer any significant advantage compared to the negative control, addition of E minor protein (vAD3041) makes a significant difference in protection against lung lesion from a PRRSV challenge. Moreover, *re-targeting* of the minor proteins (vAD3038) also makes a significant difference (Fig. 11).

[0245] Accordingly, the data and results disclosed herein support a generally-applicable model,
25 wherein protection against PRRSV challenge is provided by antibodies directed against either one of the surface proteins (e.g. gp2), or the oligomeric structure of the surface formed and

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presented by the ternary/quaternary structure/arrangement of proteins. As such, these protective antibodies function, at least in part, by blocking the PRRSV infection by interfering with binding of the viral proteins to the cellular receptor(s).

[0246] Prior to this disclosure, the interaction of E protein with the rest of the minor proteins or other proteins in the virion was not known to be a prerequisite for elicitation of protective immunity. The instant vaccination trial has thus revealed a surprising and unexpected role for minor protein E, either alone or in combination with one or more of gp2, gp3 and gp4, in eliciting from porcine animals significantly higher protection against virulent PRRSV challenge.

[0247] It is envisioned by the Applicants, for example, that a neutralizing epitope may be, for example, located directly on the E protein, or it may induced by any one or combination of minor proteins in the presence of E protein. In view of the prior art references, this finding is entirely unexpected and surprising. Accordingly, this serendipitous discovery has not only identified a PRRSV-protective antigen composition, which serves as a basis to develop live-PRRSV-free vaccine, but it also opens up new areas of PRRSV research to elucidate protein-protein / virus-cell receptor interactions.

[0248] In view of the data and results, Applicants envision that other combinations of E + minor protein (e.g. E + gp2; E + gp2 + gp3; E + gp2 + gp4; and the like) will similarly overcome the problem of presenting a "neutralizing epitope" (defined herein as an epitope that is capable of eliciting in an animal a protective immune response, including the production of virus-neutralizing antibodies) to an animal's immune system. Moreover, the results indicate that *re-targeting* of the PRRSV minor proteins elicits a similarly surprising safe and protective immunity.

[0249] Applicants have thus revealed two major, yet related, approaches for overcoming the inability of separately-expressed gp2, gp3, and gp4 to present a virus-neutralizing epitope to a host animal's immune system, and elicit a protective immune response against virulent PRRSV challenge.

[0250] Moreover, this application discloses, for the first time, that the immunogenicity of PRRSV envelope minor proteins may be enhanced sufficiently to elicit protective immune responses. These inventive approaches are envisioned to have broad applicability to other

viruses, particularly where cell localization plays a role in preventing virus neutralizing epitopes from being presented to the host's immune system.

Example 4 – Clinical Trial Testing Safety and Efficacy of the Ad5 PRRSV Vaccines

[0251] Another study was conducted using the methods disclosed in Example 3, and Table 4
5 provides an overview. The adenoviral vectors had inserts according to the following: **vAD3038** (Gp234-Rtrg); **vAD3067** (Gp234-Rtrg+E-opt); **vAD3064** (M-gp5-gp5a-Rtrg); **vAD3041** (Gp234E); **vAD3069** (Np-M-gp5-gp5a); **vAD3046** (SIV-HA).

Table 4. Vaccination trial scheme (IM=intramuscular; IN=intranasal)

Group #	**# per group**	**Prime**	**Boost**	**Killed Vaccine**
		Day 0	Day 14	Day 28
1	12	vAD3038 (IN)	vAD3038 (IM)	Yes
2	8	vAD3067 (IM)	vAD3067 (IM)	Yes
3	12	vAD3067 (IN)	vAD3067 (IM)	Yes
4	12	(vAD3067+vAD3064) (IN)	(vAD3067+vAD3064) (IM)	Yes
5	12	(vAD3041+vAD3069) (IN)	(vAD3041+vAD3069) (IM)	Yes
6	12	vAD3038 (IN)	vAD3038 (IM)	No
7	12	vAD3046 (IN)	vAD3046 (IM)	No

10 **[0252]** *Summary*. The data demonstrated that vector-expressed, retargeted PRRSV minor envelope proteins boosted with killed vaccine lowered serum virus load in porcines and elicited in significant protection from lung lesion (FIGs. 18 & 19). These data could not have been predicted in advance of this study, even in view of the data presented in Example 3. Now that this study has been conducted, Applicants envision that the surprising protection from lung
15 lesion and reduction in serum viral load may be attributable to a strong priming effect of the retargeted minor envelope proteins (FIG. 20). Also unpredictable was the finding that addition of E to *retargeted* minor envelope proteins showed no significant protection from lung lesion (FIGs. 21 & 22), in contrast to the opposite result disclosed in Example 3 (i.e. administration of the adeno construct containing E+Wt minor envelope proteins significantly reduced lung lesion).
20 In view of the interaction data depicted in FIGs. 23 & 24, Applicants envision that this loss of protection from lung lesion could be caused by wild-type E negatively interacting with the retargeted minor envelope proteins (i.e. owing to the altered TM & CT domains, present in the retargeted proteins).

* * *

[0253] Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without 5 departing from the spirit or scope of the present invention.

[0254] All documents cited or referenced in the application cited documents, and all documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein, may be employed in 10 the practice of the invention.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 84131674 Seq 01-MAR-18 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

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CLAIMS:

1. An immunological composition comprising:

    a. a recombinant viral vector comprising a heterologous nucleotide sequence encoding retargeted porcine reproductive and respiratory syndrome virus (PRRSV) gp2, retargeted PRRSV gp3 and retargeted PRRSV gp4; and

    b. a pharmaceutically or veterinarily acceptable carrier,

    wherein the retargeted PRRSV gp2, the retargeted PRRSV gp3, and the retargeted PRRSV gp4 are retargeted by the replacement of their existing cellular localization sequence with a corresponding cell-surface expression determinant sequence from a heterologous gene.

2. The composition of claim 1, wherein the recombinant viral vector comprises a recombinant adenovirus PRRSV vector.

3. The composition of claim 1, wherein the recombinant viral vector comprises a recombinant adenovirus 5-porcine reproductive and respiratory syndrome virus (Ad5-PRRSV) vector.

4. The composition of claim 3, wherein the composition comprises a further Ad5-PRRSV vector comprising a heterologous nucleotide sequence encoding retargeted PRRSV M, retargeted PRRSV gp5, and retargeted PRRSV gp5a.

5. A recombinant adenovirus 5-porcine reproductive and respiratory syndrome virus (Ad5-PRRSV) vector, wherein the Ad5-PRRSV vector comprises polynucleotides encoding retargeted porcine reproductive and respiratory syndrome virus (PRRSV) gp2, retargeted PRRSV gp3, and retargeted PRRSV gp4, wherein:

    a. the retargeted PRRSV gp2 has at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 14, the retargeted PRRSV gp3 has at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 16, and the retargeted PRRSV gp4 has at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 18; or

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b. the retargeted PRRSV gp2 has at least 90% sequence identity to the ectodomain of the sequence as set forth in SEQ ID NO: 14, the retargeted PRRSV gp3 has at least 90% sequence identity to the ectodomain of the sequence as set forth in SEQ ID NO: 16, and the retargeted PRRSV gp4 has at least 90% sequence identity to the ectodomain of the sequence as set forth in SEQ ID NO: 18,

wherein the retargeted PRRSV gp2, the retargeted PRRSV gp3, and the retargeted PRRSV gp4 are retargeted by the replacement of their existing cellular localization sequence with a corresponding cell-surface expression determinant sequence from a heterologous gene.

6. The immunological composition of claim 1, further comprising a recombinant viral vector comprising a heterologous nucleotide sequence encoding retargeted PRRSV M, retargeted PRRSV gp5, and retargeted PRRSV gp5a, wherein the retargeted PRRSV M, the retargeted PRRSV gp5, and the retargeted PRRSV gp5a are retargeted by the replacement of their existing cellular localization sequence with a corresponding cell-surface expression determinant sequence from a heterologous gene.

7. Use of the composition of any one of claims 1 to 4 and 6, or the vector of claim 5 for eliciting an immunological response in a porcine animal in need thereof against PRRSV.

8. The use of claim 7, wherein the composition is for administration by oro-nasal, spray, drinking water, intramuscular, subcutaneous, intradermal, or transdermal administration.

9. The use of claim 7 or 8 wherein the use is a prime-boost.

10. The use of claim 9, wherein the prime is a mixture of two adenovirus 5-porcine reproductive and respiratory syndrome virus (Ad5-PRRSV) vectors, the first Ad5-PRRSV vector expressing retargeted PRRSV gp2, retargeted PRRSV gp3, and retargeted PRRSV gp4 and the second Ad5-PRRSV vector expressing retargeted PRRSV M, retargeted PRRSV gp5, and retargeted PRRSV gp5a; wherein the boost comprises either both vectors of the prime, or either vector alone; and wherein the retargeted PRRSV M, the retargeted PRRSV gp5, and the retargeted PRRSV gp5a are retargeted by the replacement of their existing cellular localization sequence with a corresponding cell-surface expression determinant sequence from a heterologous gene.

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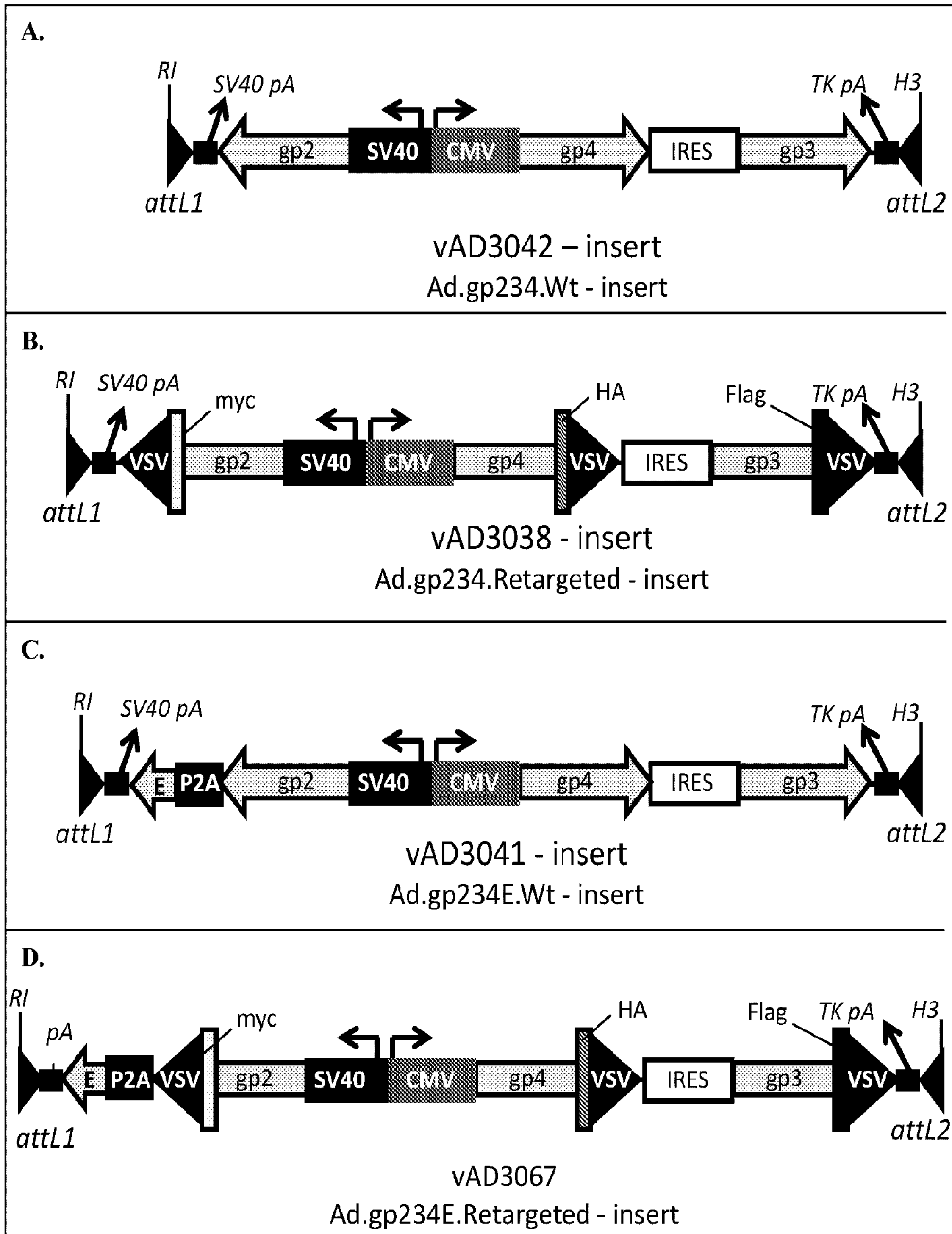

FIG. 1

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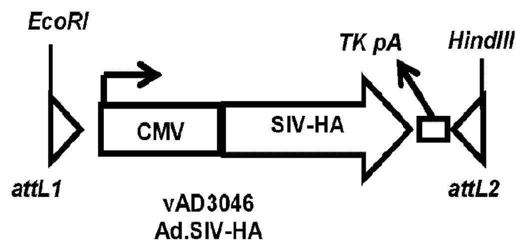
E.



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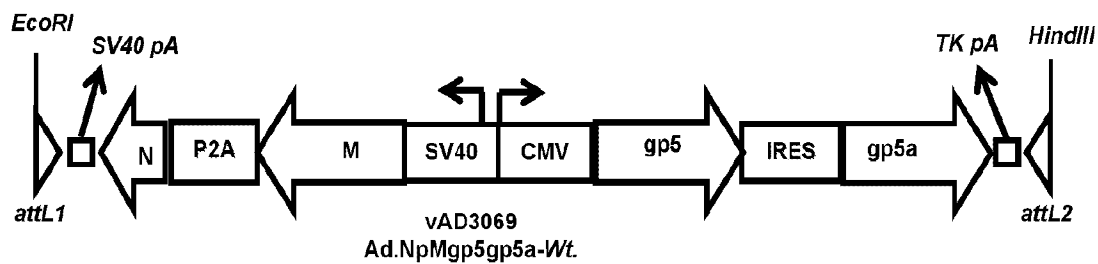

G.

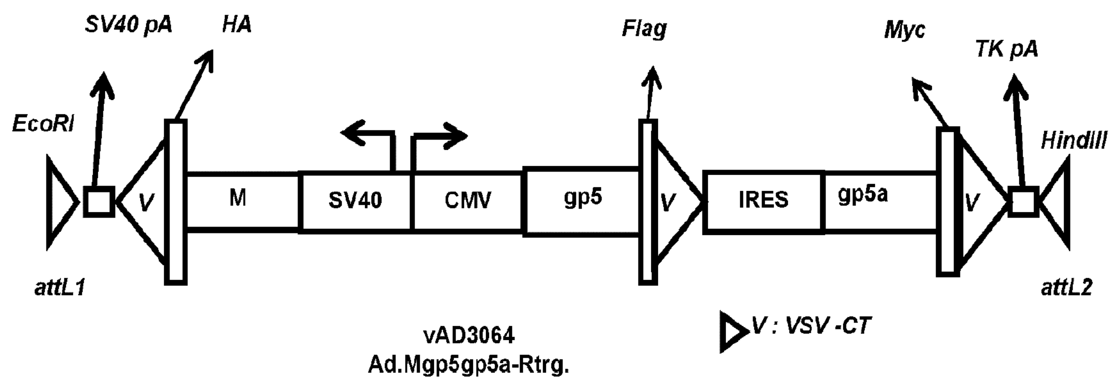

FIG. 1 (Continued)

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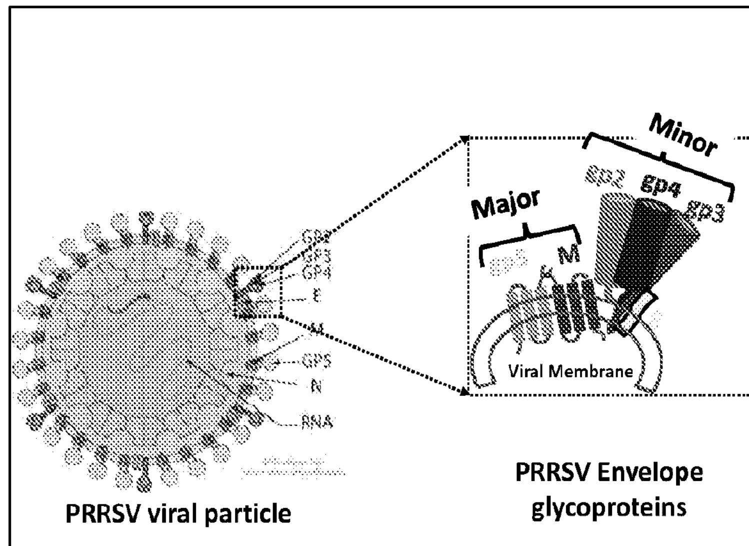

FIG. 2

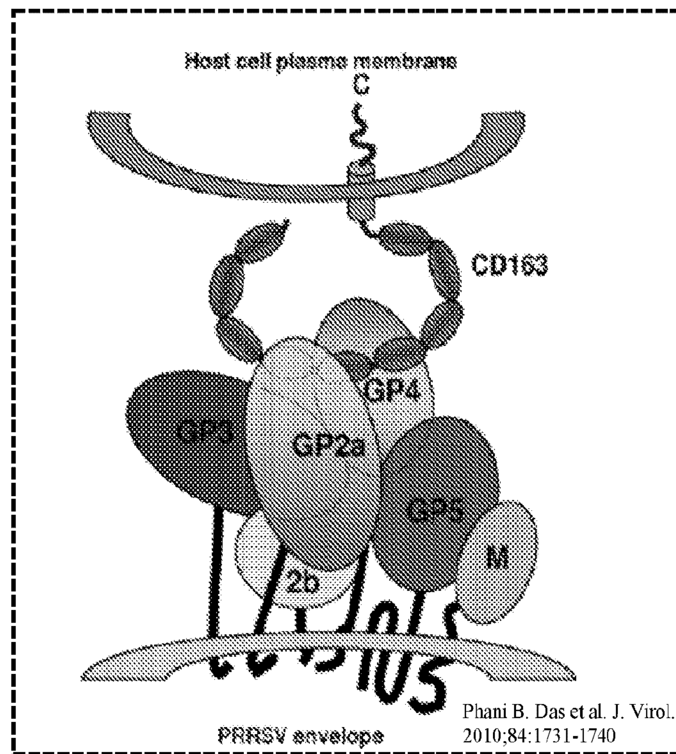

FIG. 3

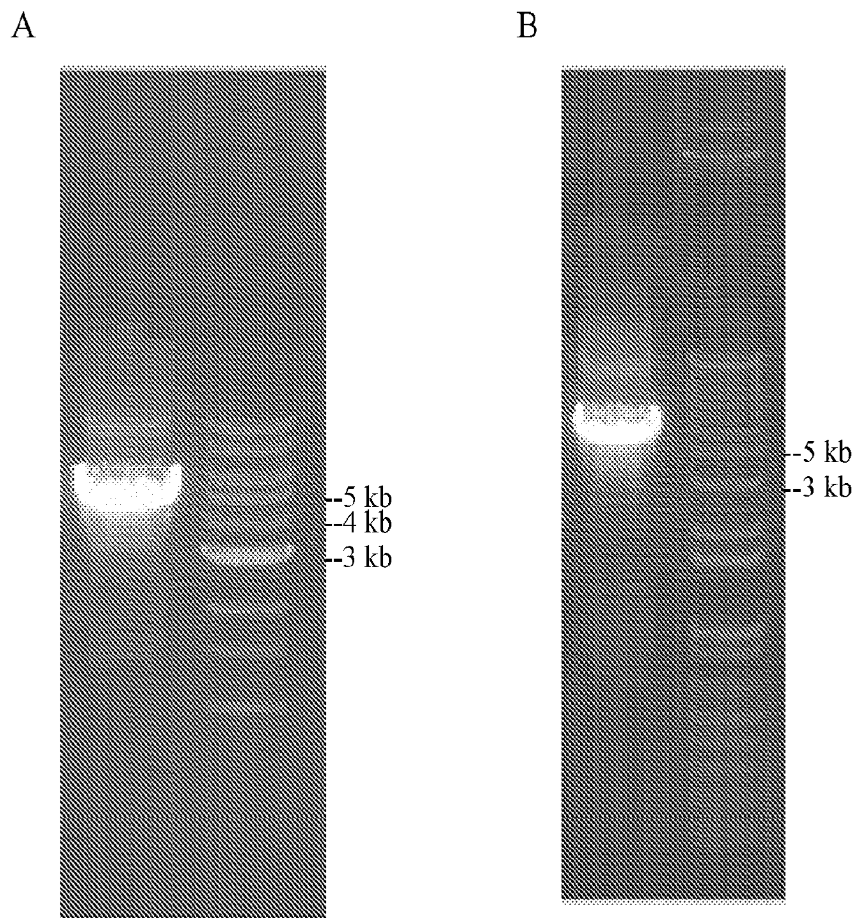

FIG. 4

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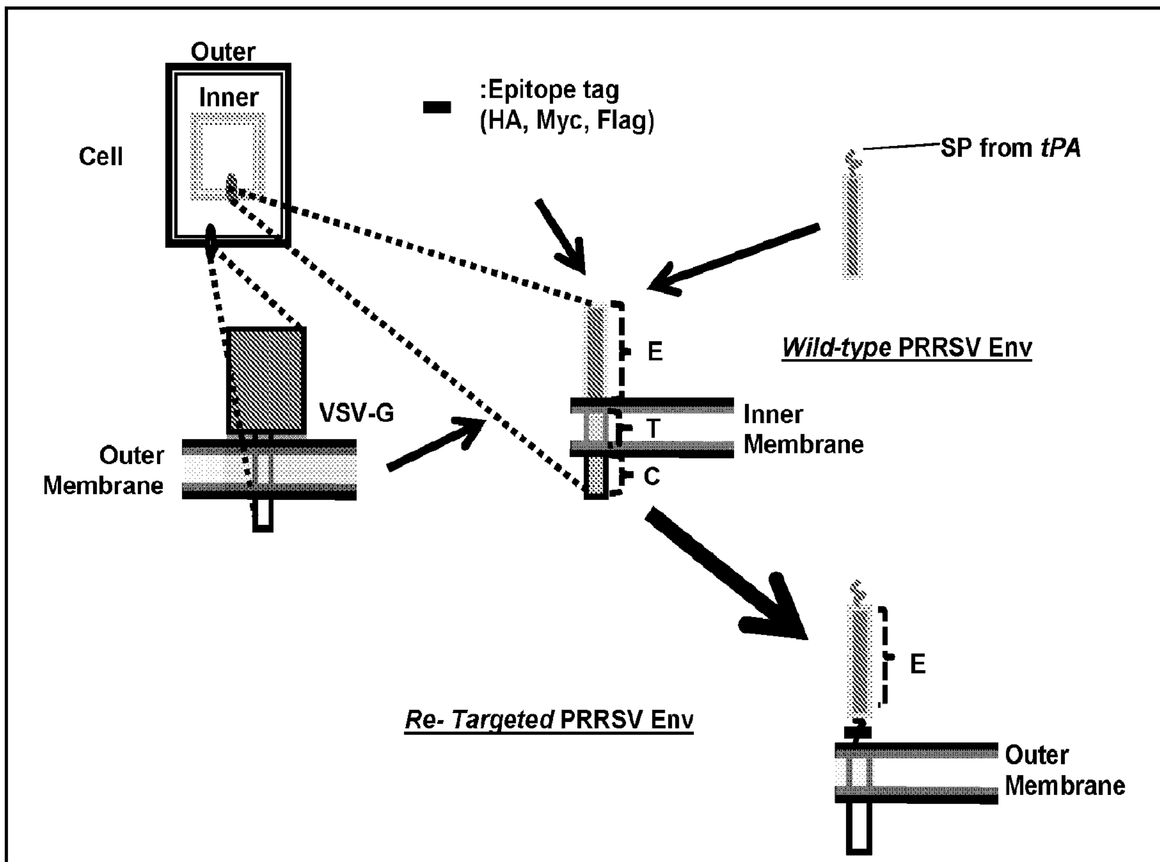

FIG. 5A

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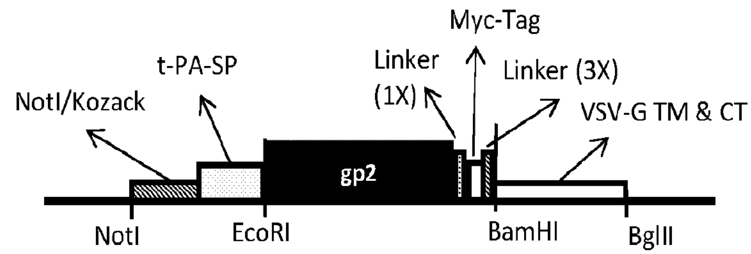

FIG. 5B

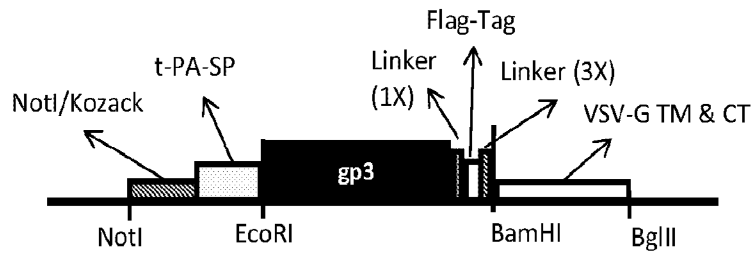

FIG. 5C

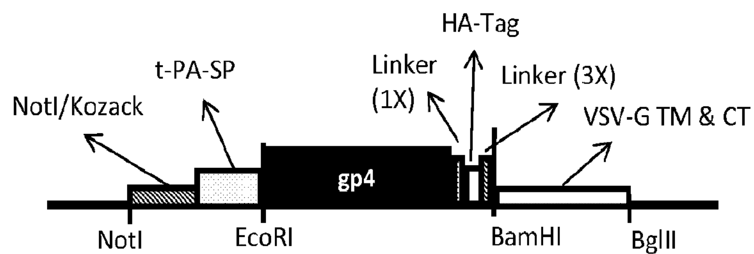

FIG. 5D

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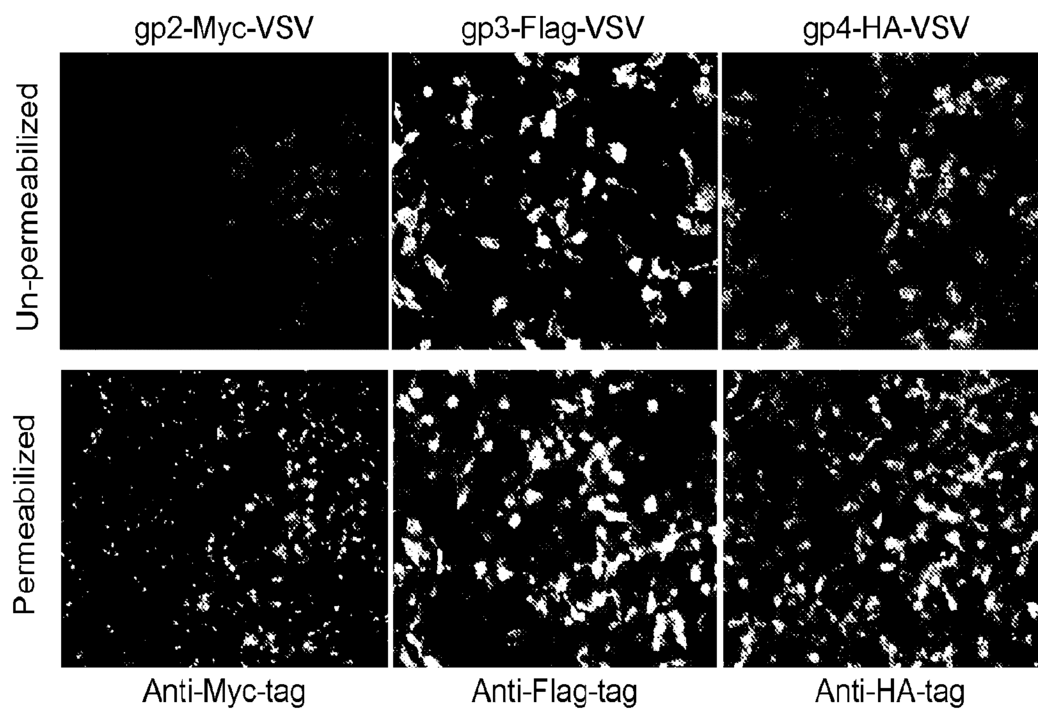

FIG. 6

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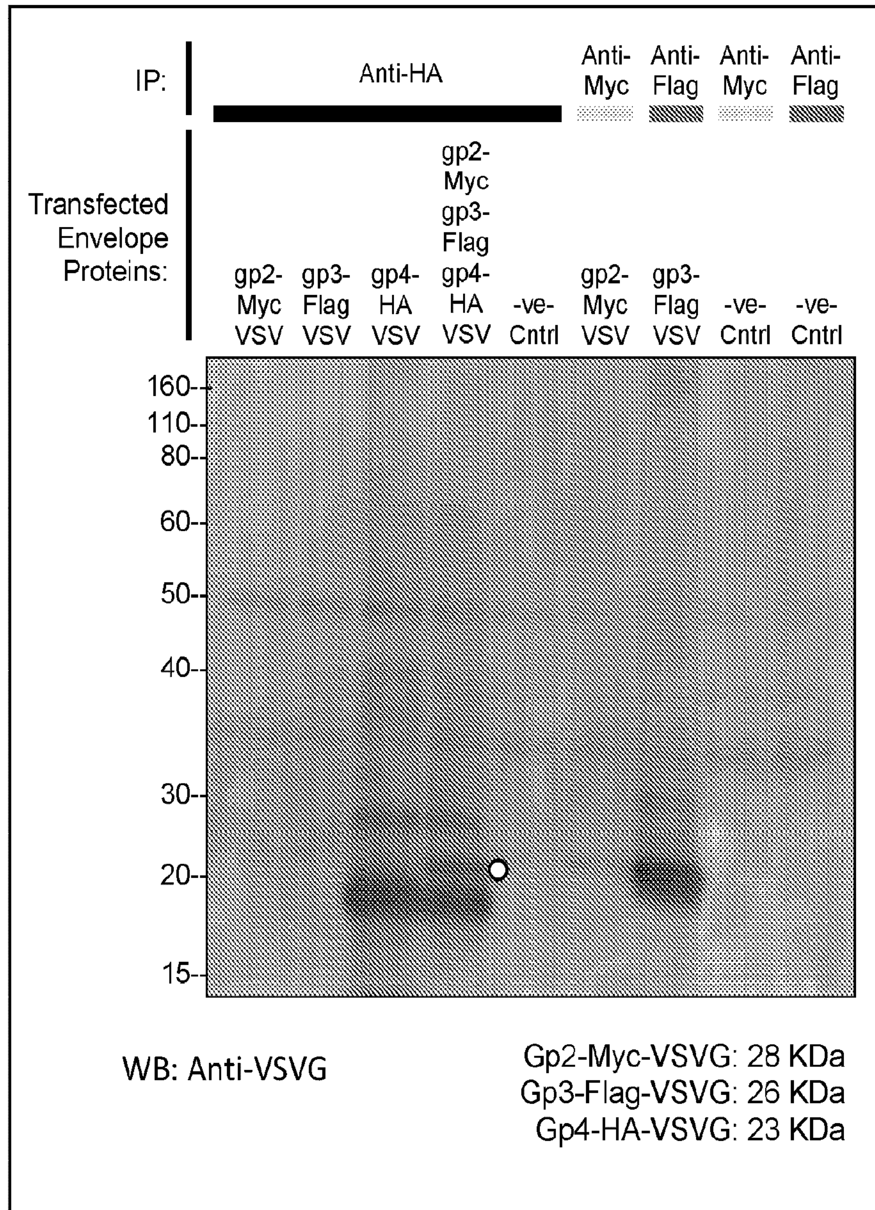

FIG. 7

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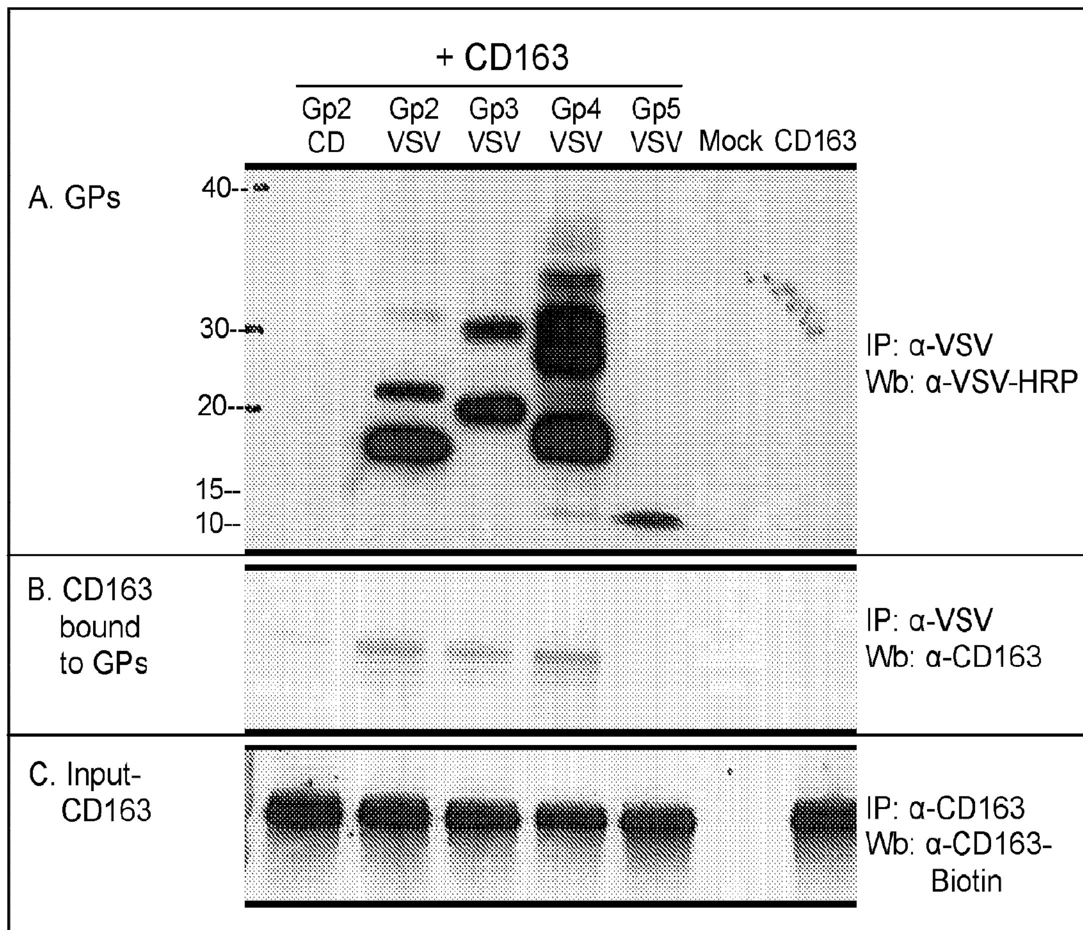

FIG. 8

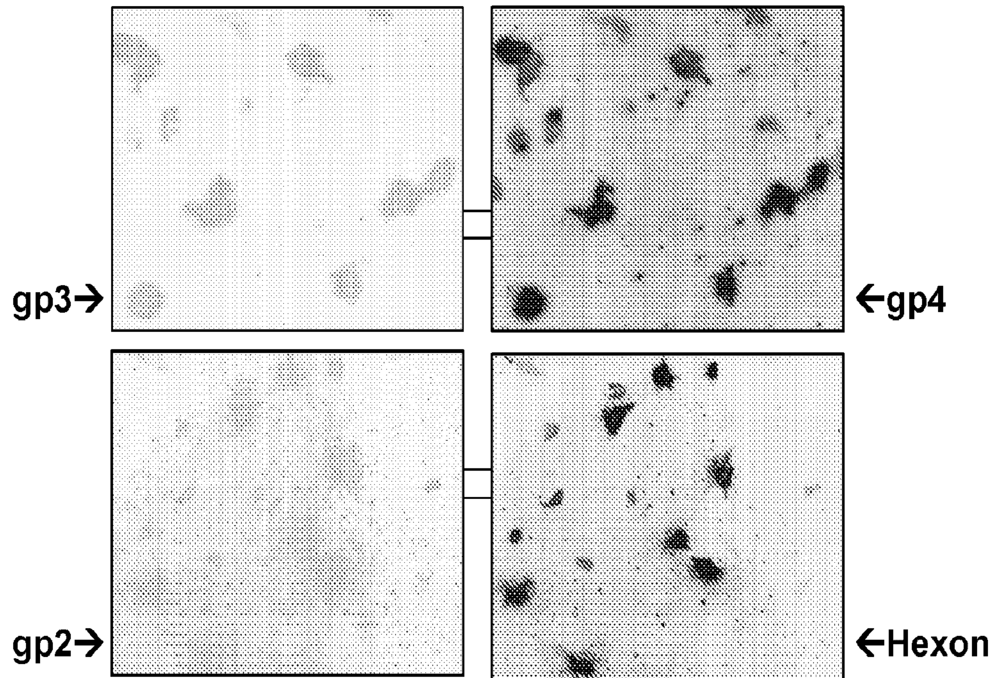

FIG. 9A

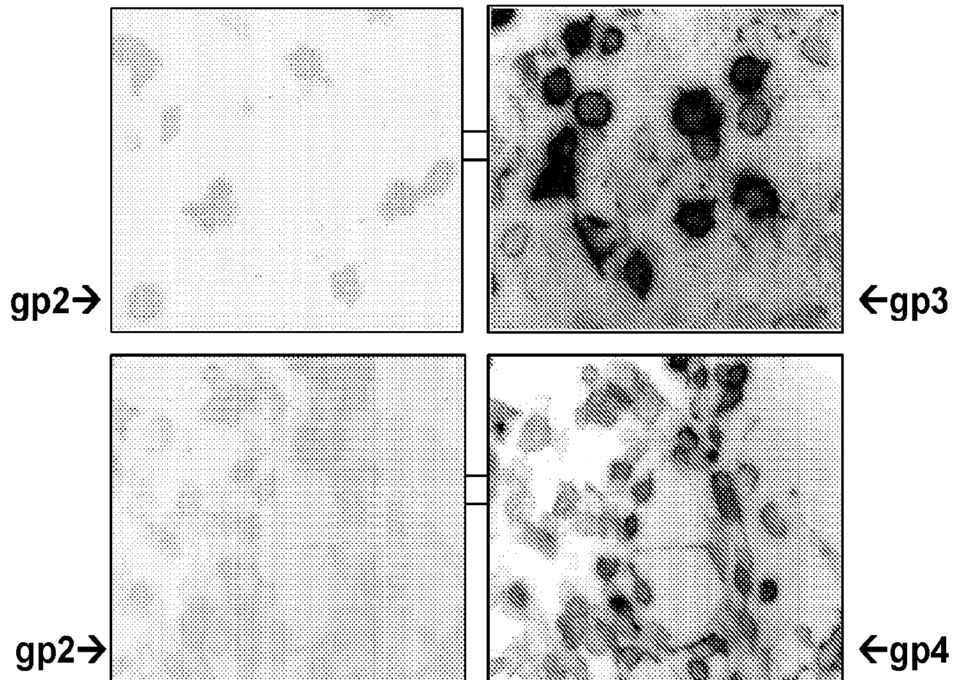

FIG. 9B

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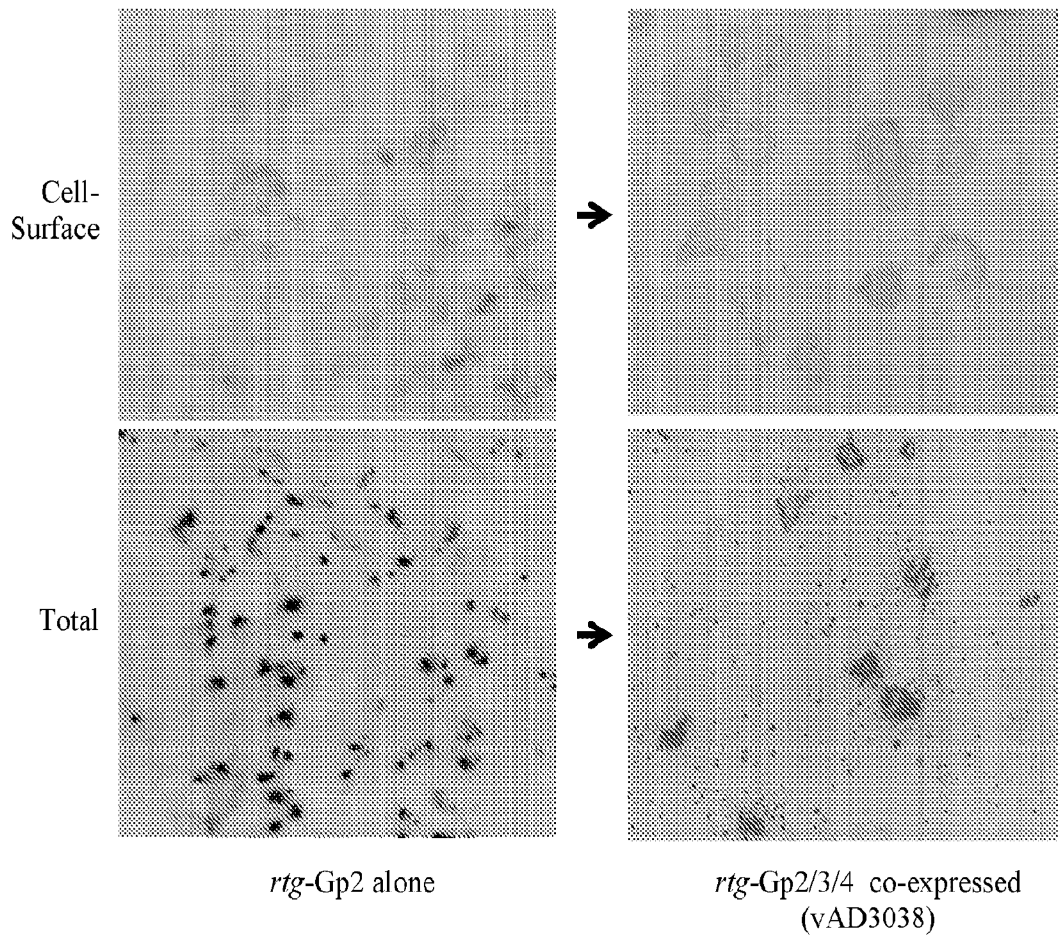

FIG. 9C

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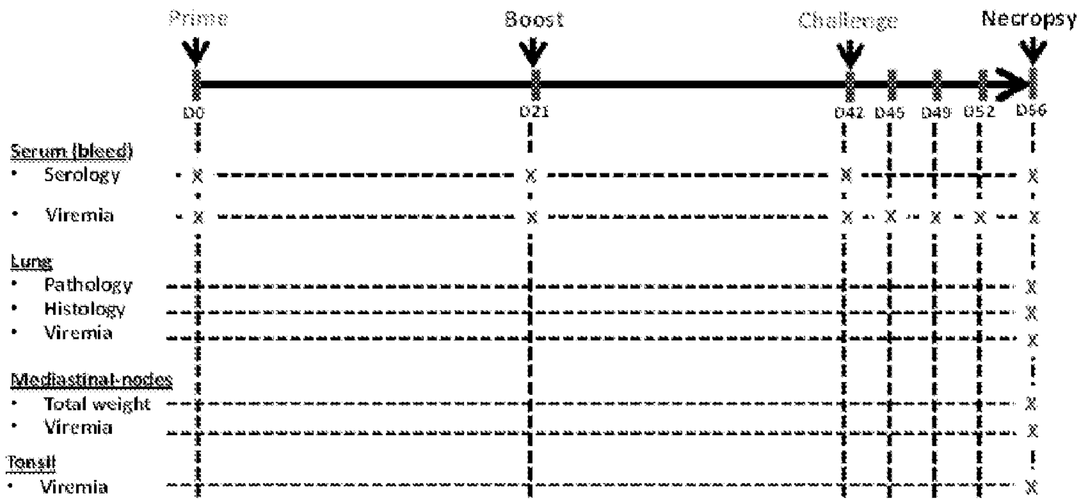

FIG. 10

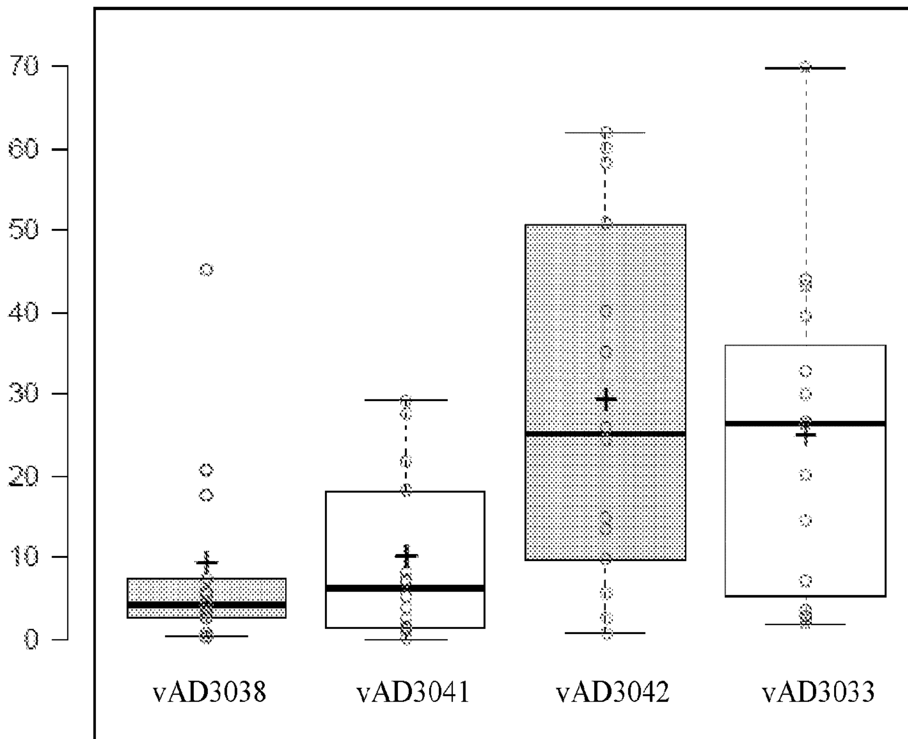

FIG. 11

SEQ ID #	Type	Description
1	Polypeptide	PRRSV gp2 polypeptide, from VR2332, PRRSV Type II (entire viral sequence provided by Accession #:U87392.3)
2	DNA/RNA	VR2332 PRRSV gp2 (12073..12843 of VR2332)
3	Polypeptide	VR2332 PRRSV gp3 polypeptide
4	DNA/RNA	VR2332 PRRSV gp3 (12696..13460 of VR2332)
5	Polypeptide	VR2332 PRRSV gp4 polypeptide
6	DNA/RNA	VR2332 PRRSV gp4 (13241..13777 of VR2332)
7	Polypeptide	VR2332 PRRSV E polypeptide
8	DNA/RNA	VR2332 PRRSV E (12078..12299 of VR2332)
9	DNA/RNA	VR2332 PRRSV gp2 (codon-optimized)
10	DNA/RNA	VR2332 PRRSV gp3 (codon-optimized)
11	DNA/RNA	VR2332 PRRSV gp4 (codon-optimized)
12	DNA/RNA	VR2332 PRRSV E (codon-optimized)
13	DNA/RNA	VR2332 PRRSV *rtg*-gp2 DNA (codon-optimized, re-targeted)
14	Polypeptide	VR2332 PRRSV *rtg*-gp2 polypeptide (gp2-myc-VSV)
15	DNA/RNA	VR2332 PRRSV *rtg*-gp3 DNA (codon-optimized, re-targeted)
16	Polypeptide	VR2332 PRRSV *rtg*-gp3 polypeptide (gp3-Flag-VSV)
17	DNA/RNA	VR2332 PRRSV *rtg*-gp4 DNA (codon-optimized, re-targeted)
18	Polypeptide	VR2332 PRRSV *rtg*-gp4 polypeptide (gp4-HA-VSV)
19	DNA/RNA	VR2332 PRRSV *rtg*-E (codon-optimized, re-targeted)
20	Polypeptide	VR2332 PRRSV *rtg*-E polypeptide
21	DNA/RNA	vAD3038 pre-recombination insert
22	DNA/RNA	vAD3041 pre-recombination insert
23	DNA/RNA	vAD3042 pre-recombination insert
24	DNA/RNA	vAD-*rtg*-gp234-E pre-recombination insert
25	DNA/RNA	vAD3033 pre-recombination insert
26	DNA/RNA	pAd5 Forward primer
27	DNA/RNA	pAd5 Reverse primer
28	DNA/RNA	Entire VR2332, PRRSV Type II sequence
29	DNA/RNA	Entire Lelystad PRRSV sequence (GenBank: A26843.1)
30	DNA/RNA	pAd/PL-DEST vector; attR1 site: 512-636; attR2 site: 2092-2216
31	Polypeptide	PRRSV gp5a
32	Polypeptide	VR2332 PRRSV M (matrix protein)
33	Polypeptide	VR2332 PRRSV N (nucleocapsid protein)

FIG. 12

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SEQ ID #	Type	Description
34	Polypeptide	ABO40192.1 PRRSV gp2
35	Polypeptide	ACF93748.1 PRRSV gp2
36	Polypeptide	AHA83141.1 PRRSV gp2
37	Polypeptide	CAA01838.1 PRRSV gp2
38	Polypeptide	AAE74522.1 PRRSV gp2
39	Polypeptide	AAB54503.1 PRRSV gp2
40	Polypeptide	AAE68461.1 PRRSV gp3
41	Polypeptide	AAQ51784.1 PRRSV gp3
42	Polypeptide	AAE74530.1 PRRSV gp3
43	Polypeptide	CAA01839.1 PRRSV gp3
44	Polypeptide	ABH73414.1 PRRSV gp3
45	Polypeptide	AAE74526.1 PRRSV gp3
46	Polypeptide	AAE74537.1 PRRSV gp4
47	Polypeptide	AAE74538.1 PRRSV gp4
48	Polypeptide	AAE74533.1 PRRSV gp4
49	Polypeptide	CAA01840.1 PRRSV gp4
50	Polypeptide	ABH73415.1 PRRSV gp4
51	Polypeptide	AAE68462.1 PRRSV gp4
52	Polypeptide	AGX46781.1 PRRSV E
53	Polypeptide	AED17147.1 PRRSV E
54	Polypeptide	AED17148.1 PRRSV E
55	Polypeptide	AGX46783.1 PRRSV E
56	Polypeptide	AED17156.1 PRRSV E
57	Polypeptide	AIS76359.1 PRRSV E
58	Polypeptide	ABU49670.1 PRRSV E
59	Polypeptide	VR2332 PRRSV gp5
60	Polypeptide	CAA01841.1 PRRSV gp5
61	Polypeptide	ADA15222.1 PRRSV gp5
62	Polypeptide	AFS30909.1 PRRSV gp5a
63	Polypeptide	AGK45334.1 PRRSV gp5a
64	Polypeptide	AFU75332.1 PRRSV gp5a
65	Polypeptide	AGW23843.1 PRRSV gp5a
66	Polypeptide	*rtg*-gp5 of VR2332 PRRSV
67	DNA/RNA	*rtg*-gp5 of VR2332 PRRSV
68	Polypeptide	*rtg*-M of VR2332 PRRSV
69	DNA/RNA	*rtg*-M of VR2332 PRRSV

FIG. 12 (Continued)

Date Reçue/Date Received 2022-03-29

SEQ ID #	Type	Description
70	DNA/RNA	Gp2 of PRRSV; Lelystad strain (portion of GenBank M96262.2)
71	Polypeptide	Gp2 of PRRSV; Lelystad strain
72	DNA/RNA	Gp3 of PRRSV; Lelystad strain (portion of GenBank M96262.2)
73	Polypeptide	Gp3 of PRRSV; Lelystad strain
74	DNA/RNA	Gp4 of PRRSV; Lelystad strain (portion of GenBank M96262.2)
75	Polypeptide	Gp4 of PRRSV; Lelystad strain
76	DNA/RNA	Gp4 of PRRSV; Lelystad strain (portion of GenBank M96262.2)
77	Polypeptide	Gp4 of PRRSV; Lelystad strain
78	DNA/RNA	M of PRRSV; Lelystad strain (portion of GenBank M96262.2)
79	Polypeptide	M of PRRSV; Lelystad strain
80	Polypeptide	Gp2 of PRRSV related to Lelystad strain
81	Polypeptide	Gp2 of PRRSV related to Lelystad strain
82	Polypeptide	Gp2 of PRRSV related to Lelystad strain
83	Polypeptide	Gp2 of PRRSV related to Lelystad strain
84	Polypeptide	Gp2 of PRRSV related to Lelystad strain
85	Polypeptide	Gp2 of PRRSV related to Lelystad strain
86	Polypeptide	Gp2 of PRRSV related to Lelystad strain
87	Polypeptide	Gp2 of PRRSV related to Lelystad strain
88	Polypeptide	Gp2 of PRRSV related to Lelystad strain
89	Polypeptide	Gp2 of PRRSV related to Lelystad strain
90	Polypeptide	Gp3 of PRRSV related to Lelystad strain
91	Polypeptide	Gp3 of PRRSV related to Lelystad strain
92	Polypeptide	Gp3 of PRRSV related to Lelystad strain
93	Polypeptide	Gp3 of PRRSV related to Lelystad strain
94	Polypeptide	Gp3 of PRRSV related to Lelystad strain
95	Polypeptide	Gp3 of PRRSV related to Lelystad strain
96	Polypeptide	Gp3 of PRRSV related to Lelystad strain
97	Polypeptide	Gp3 of PRRSV related to Lelystad strain
98	Polypeptide	Gp3 of PRRSV related to Lelystad strain
99	Polypeptide	Gp3 of PRRSV related to Lelystad strain
100	Polypeptide	Gp4 of PRRSV related to Lelystad strain
101	Polypeptide	Gp4 of PRRSV related to Lelystad strain
102	Polypeptide	Gp4 of PRRSV related to Lelystad strain
103	Polypeptide	Gp4 of PRRSV related to Lelystad strain

FIG. 12 (Continued)

Date Reçue/Date Received 2022-03-29

SEQ ID #	Type	Description
104	Polypeptide	Gp4 of PRRSV related to Lelystad strain
105	Polypeptide	Gp4 of PRRSV related to Lelystad strain
106	Polypeptide	Gp4 of PRRSV related to Lelystad strain
107	Polypeptide	Gp4 of PRRSV related to Lelystad strain
108	Polypeptide	Gp4 of PRRSV related to Lelystad strain
109	Polypeptide	Gp4 of PRRSV related to Lelystad strain
110	Polypeptide	Gp5 of PRRSV related to Lelystad strain
111	Polypeptide	Gp5 of PRRSV related to Lelystad strain
112	Polypeptide	Gp5 of PRRSV related to Lelystad strain
113	Polypeptide	Gp5 of PRRSV related to Lelystad strain
114	Polypeptide	Gp5 of PRRSV related to Lelystad strain
115	Polypeptide	Gp5 of PRRSV related to Lelystad strain
116	Polypeptide	Gp5 of PRRSV related to Lelystad strain
117	Polypeptide	Gp5 of PRRSV related to Lelystad strain
118	Polypeptide	Gp5 of PRRSV related to Lelystad strain
119	Polypeptide	Gp5 of PRRSV related to Lelystad strain
120	Polypeptide	M of PRRSV related to Lelystad strain
121	Polypeptide	M of PRRSV related to Lelystad strain
122	Polypeptide	M of PRRSV related to Lelystad strain
123	Polypeptide	M of PRRSV related to Lelystad strain
124	Polypeptide	M of PRRSV related to Lelystad strain
125	Polypeptide	M of PRRSV related to Lelystad strain
126	Polypeptide	M of PRRSV related to Lelystad strain
127	Polypeptide	M of PRRSV related to Lelystad strain
128	Polypeptide	M of PRRSV related to Lelystad strain
129	Polypeptide	M of PRRSV related to Lelystad strain
130	Polypeptide	E of PRRSV related to Lelystad strain
131	Polypeptide	E of PRRSV related to Lelystad strain
132	Polypeptide	E of PRRSV related to Lelystad strain
133	Polypeptide	E of PRRSV related to Lelystad strain
134	Polypeptide	E of PRRSV related to Lelystad strain
135	Polypeptide	E of PRRSV related to Lelystad strain
136	Polypeptide	E of PRRSV related to Lelystad strain
137	Polypeptide	E of PRRSV related to Lelystad strain
138	Polypeptide	E of PRRSV related to Lelystad strain
139	Polypeptide	E of PRRSV related to Lelystad strain

FIG. 12 (Continued)

Date Reçue/Date Received 2022-03-29

ClustalW alignment of PRRSV gp2 polypeptide sequences

```
34 MKWGLCKAFSTKLANFLWMLSRNFWCPLLISSYFWPFCLASQSQVGWWSSVSDWFAPRYS 60
36 MKWGPYKAFLTKLANFLWMLSRSSWCPLLISLYFWPFCLASPSPVGWWSFASDWFAPRYS 60
35 MKWGLCKASLTKLANFLWMLSRNFWCPLLISSYFWPFCLASPSPVGWWSFASDWFAPRYS 60
38 MQWGPCKAFLTRSVNFLWMLSRSSWCPLLISSYFWPFCLASPLPAGWWSFASDWFAPRYS 60
37 MQWGHCG---VKSASCSWTPSLSSLLVWLILPFSLPYCLGSPSQDGYWSFFSEWFAPRFS 57
39 MQWGHCG---VKSASCSWTPSLSSLLVWLILXFSLPYCLGSPSQDGYWSFFSEWFAPRFS 57
   *:**      .: ..  *  * .     **  :  *:**.*    *:**  *:*****:*
34 VRALPFTLSNYRRSYEAFLSQCQVDIPTWGTKHPLGMFWHHKVSTLIDEMVSRRMYRIME 120
36 VRALPFTLSNYRRSYEAFLSQCQVDIPTWGTKHPLGMFWHHKVSTLIDEMVSRRMYRIME 120
35 VRALPFTLSNYRRSYEAFLSQCQVDIPTWGFKHPLGMLWHHKVSTLIDEMVSRRMYRTME 120
38 VRALPFTLSNYRRSYEAFLSQCQVDIPAWGTRHPLGMLWHHKVSTLIDEMVSRRMYRIME 120
37 VRALPFTLPNYRRSYEGLLPNCRPDVPQFAVKHPLGMFWHMRVSHLIDEMVSRRIYQTME 117
39 VRALPFTLPNYRRSYEGLLPNCRPDVPQFAVKHPLXMFWHMRVSHLIDEXVSRRIYQTME 117
   ********.*******.:*.:*: *:* :. :*** *:** :** **** ****:*: **
34 KAGQAAWKQVVSEATLSRISSLDVVAHFQHLAAIEAETCKYLASRLPMLHNLRMTGSNVT 180
36 KAGQAAWKQVVSEATLSRISSLDVVAHFQHLAAIEAETCKYLASRLPMLHNLRMTGSNVT 180
35 KAGQAAWKQVVSEATLSRISGLDVVAHFQHLAAIEAETCKYLASRLPMLHNLRMTGSNVT 180
38 KAGQAAWKQVVSEATLSRISGLDVVAHFQHLAAIEAETCKYLASRLPMLHNLRITGSNVT 180
37 HSGQAAWKQVVGEATLTKLSGLDIVTHFQHLAAVEADSCRFLSSRLVMLKNLAVG--NVS 175
39 HSGQAAWKQVVGEATLTKLSGLDIVTHFQHLAAVEADSCRFLSSRLVMLKNLAVG--NVS 175
   ::*********.****:::*.**:*:*******:**::*::*:*** **:** :   **:
34 IVYNSTLEQVVAIFPTPGSRPKLHDFQQWLIAVHSSIFSSVAASCTLFVVLWLRIPMLRT 240
36 IVYNSTLSQVFAIFPTPGSRPKLHDFQQWLIAVHSSIFSSVAASCTLFVVLWLRVPILHT 240
35 IVYNSTSNQVFAIFPTPGSRPKRHDFQQWLIAVHSSIFSSVAASCTLFVVLWLRIPMLRS 240
38 IVHNSTLNQVFAIFPTPGSRPKLHDFQQWLIAVHSSISSSVAASCTLFVVLWLRMPMLRS 240
37 LQYNTTLDRVELIFPTPGTRPKLTDFRQWLISVHASIFSSVASSVTLFIVLWLRIPALRY 235
39 LQYNTTLDRVELIFPTPGTRPKLTDFRQWLISVHASIFSSVASSVTLFIVLWLRIPALRY 235
   : :*:* .:*  ******:***  **:****:**:** ****:* ***:*****:* *:
34 VFGFHWLGAIFLSNSQ 256
36 VFGFRWLGAIFLSNSQ 256
35 VFGFRWLGAIFLLNSR 256
38 VFGFRWLGAIFPSSSW 256
37 VFGFHWPTATHHSS-- 249
39 VFGFHWPTATHHSS-- 249
   ****:*  * .  .
(34:36) Aligned. Score: 92.97
(34:35) Aligned. Score: 93.75
(34:38) Aligned. Score: 61.85
(34:37) Aligned. Score: 88.67
(34:39) Aligned. Score: 61.04
(36:35) Aligned. Score: 92.58
(36:38) Aligned. Score: 61.45
(36:37) Aligned. Score: 90.23
(36:39) Aligned. Score: 60.64
(35:38) Aligned. Score: 59.84
(35:37) Aligned. Score: 91.02
(35:39) Aligned. Score: 59.44
(38:37) Aligned. Score: 61.85
(38:39) Aligned. Score: 98.80
(37:39) Aligned. Score: 61.04
```

FIG. 13

ClustalW alignment of PRRSV gp3 polypeptide sequences

```
40 MVNSCTFLHIFLCCSFLYSLCCAVVAGSNTTYCFWFPLVRGNFSFELTVNYTVCPPCLTR 60
45 MANSCTFLYIFLCCSFLYSFCCAVVAGSNATYCFWFPLVRGNFSFELTVNYTVCPPCLTR 60
41 MANSCTFLYIFLCCSFLYSFCCAVVAGSNATYCFWFPLVRGNFSFELTVNYTVCPPCLTR 60
42 MANSCTFLHILLCCSFLYSFCCVVVTDANATFCFWFPLVRGNFSFELMVNYTVCPPCLTR 60
43 MAHQCARFHFFLCGFICYLVHSALASNSSSTLCFWFPLAHGNTSFELTINYTICMPCSTS 60
44 MAHQCARFHFFLCGFICYFVHSALASNSSSTLCFWFPLAHGNTSFELTINYTVCMPCPTS 60
   *.:.*: ::::**   : *  .  ..:.:.:.:* ******.:** **** :***:* ** *
40 QAAAEAYEPGRSLWCRIGYDRCGEDDHDELGFMVPSGLSSEGHLTSVYAWLAFLSFSYTA 120
45 QAATEAYEPGRSLWCRIGYDRCGEDDHDELGFVVPSGLSSEGHLTSVYAWLAFLSFSYTA 120
41 QAAAEAYEPGRSLWCRIGHDRCGEDDHDELGFVVPSGLSSEGHLTSAYAWLASLSFSYTA 120
42 QAAAQIYEPNRSLWCRIGNDRCGEDDHDELGFTVPPGLSKEVHLTSVYAWLAFLSFSYTA 120
43 QAARQRLEPGRNMWCKIGHDRCEERDHDELLMSIPSGYG-QLKLEGYYAWLAFLSFSYAA 119
44 QAALQRLEPGRNMWCKIGHDRCEERDQDELLMSIPSGYD-NLKLEGYYAWLAFLSFSYAA 119
   *** :   **.*.:**:** *** * *:*** : :*.* . : :*  . ***** *****:*
40 QFHPEIFGIGNVSRVYVDIEHQLICAEHDGQNTTLPRHDNISAVFQTYYQHQVDGGNWFH 180
45 QFHPEIFGIGNVSQVYVDIRHQFICAVHDGQNATLPRHDNISAVFQTYYQHQVDGGNWFH 180
41 QFHPEIFGIGNVSRVYVDIKHQFICAVHDGQNTTLPHHDNISAVLQTYYQHQVDGGNWFH 180
42 QFHPEIFGIGNVSKVYVDINHQLICAVHDGQNTTLPRHDNISAVFQTYYQHQVDGGNWFH 180
43 QFHPELFGIGNVSRVFVDKRHQFICAEHDGHNSTVSTGHNISALYAAYYHHQIDGGNWFH 179
44 QFHPELFGIGNVSRVFVDKWHQFICAEHDGSNSTVSTGHNISALYAAYYHHQIDGGNWFH 179
   *****:*******:*:**  **:*** *** *:*:.  .****:  :**:**:*******
40 LEWLRPFFSSWLVLNVSWFLRRSPANHVSVRVLQTLRPTPPQRQALLSSKTSVALGIATR 240
45 LEWLRPFFSSWLVLNVSWFLRRSPASHVSVRVLQTLRPTPPQRQALLSSKTSVALGIATR 240
41 LEWVRPFFSSWLVLNVSWFLRRSPASHVSVRVFQTSRPTPPQRQALLSSKTSVALGIATR 240
42 LEWLRPFFSSWLVLNVSWFLRRSPASHVSVRVFQTSRPTPPRQQISLSSRTSAALGMATR 240
43 LEWLRPLFSSWLVLNISWFLRRSPVSPVSRRIYQILRPTRPRLPVSWSFRTSIVSDLTGS 239
44 LEWLRPFFSSWLVLNISWFLRRSPVSPVSRRIYQILRPTRPQLPVSWSFRTSIVSDLMRS 239
   ***:**:********:********.. ** *: *  *** *:      * :** . .:
40 PLRR--FAKS-----------LSAVRR 254
45 PLRR--FAKS-----------LSVVRR 254
41 PLRR--FAKS-----------LSAARR 254
42 PLRR--FAKS-----------LSAARR 254
43 QQRKRKFPSESRPNVVKPSVLPSTSR 265
44 QQRKGKFPSGSRPNAVKPSALPNISR 265
     *:  *..           *.   *
```

```
Sequences (1:2) Aligned. Score: 92.91
Sequences (1:3) Aligned. Score: 87.40
Sequences (1:4) Aligned. Score: 56.30
Sequences (1:5) Aligned. Score: 57.09
Sequences (1:6) Aligned. Score: 94.88
Sequences (2:3) Aligned. Score: 87.80
Sequences (2:4) Aligned. Score: 55.91
Sequences (2:5) Aligned. Score: 56.69
Sequences (2:6) Aligned. Score: 94.49
Sequences (3:4) Aligned. Score: 55.12
Sequences (3:5) Aligned. Score: 55.12
Sequences (3:6) Aligned. Score: 87.40
Sequences (4:5) Aligned. Score: 92.83
Sequences (4:6) Aligned. Score: 56.69
```

FIG. 14

Sequences (5:6) Aligned. Score: 57.09

ClustalW alignment of PRRSV gp4 polypeptide sequences

```
47 MAASLLFLMVGFKCLLVSQAFACKPCFSSSLADIKTNTTAAASFAVLQDISCLR-HRNSA 59
51 MAASLLFLMVGFKCLLVSQAFACKPCFSSSLADIKTNTTAAASFAVLQDISCLR-HRNSA 59
46 MASSLLFLMVGFKCLLVSQAFACKPCFSSSLADIKTNTTAAASFAVLQDIGCLR-HRDSA 59
48 MGASLLFLLVGFKCLLVSQAFACKPCFSSSLSDIKTNTTAAAGFAVLQDISCLR-HRNSA 59
49 MAAATLFFLAGAQHIMVSEAFACKPCFSTHLSDIETNTTAAAGFMVLQDINCFRPHGVSA 60
50 MAAAILFLLAGAQHIMVSEAFACKPCFSTHLSDIKTNTTAAAGFMVLQDINCFRPHEVSA 60
   *.:: **::.* : ::**:*********: *:**:*******.* *****.*:* *  **

47 SE---AIRKIPQCRTAIGTPMYITITANVTDENYLHSSDLLMLSSCLFYASEMSEKGFEV 116
51 SE---AIRKIPQCRTAIGTPVYITTTANVTDENYLHSSDLLMLSSCLFYASEMSEKGFKV 116
46 SE---AIRKIPQCRTAIGTPVYITITANVTDENYLHSSDLLMLSSCLFYASEMSEKGFKV 116
48 SE---AIRKVPQCRTAIGTPVYITVTANVTDENYLHSSDLLMLSSCLFYASEMSEKGFKV 116
49 AQEKISFGKSSQCREAVGTPQYITITANVTDESYLYNADLLMLSACLFYASEMSEKGFKV 120
50 TQREIPFRKSSQCREAVGTPQYITITANVTDESYLYNADLLMLSACLFYASEMSEKGFKV 120
   ::   .: * .*** *:*** *** *******.**:.:******:*************:*

47 VFGNVSGIVAVCVNFTSYVQHVREFTQR-SLMVDHVRLLHFMTPETMRWATVLACLFAIL 175
51 VFGNVSGIVAVCVNFTSYVQHVREFTQR-SLMVDHVRLLHFMTPETMRWATVLACLFAIL 175
46 VFGNVSGIVAVCVNFTSYVQHVREFTQR-SLVVDHVRLLHFMTPETMRWATVLACLFAIL 175
48 VFGNVSGIVAVCVNFTSYVQHVKEFTQR-SLVVDHVRLLHFMTPETMRWATVLACLFTIL 175
49 IFGNVSGVVSACVNFTDYVAHVTQHTQQHHLVIDHIRLLHFLTPSAMRWATTIACLFAIL 180
50 IFGNVSGVVSACVNFTDYVAHVTQHTQQHHLVIDHIRLLHFLTPSTMRWATTIACLFAIL 180
   :******:*:.*****.** ** :.**:  *::**:*****:**.:*****.:****:**

47 LAI 178
51 LAI 178
46 LAI 178
48 LAI 178
49 LAI 183
50 LAI 183
   ***
```

Sequences (1:2) Aligned. Score: 96.63
Sequences (1:3) Aligned. Score: 93.82
Sequences (1:4) Aligned. Score: 67.42
Sequences (1:5) Aligned. Score: 69.66
Sequences (1:6) Aligned. Score: 97.19
Sequences (2:3) Aligned. Score: 93.82
Sequences (2:4) Aligned. Score: 66.85
Sequences (2:5) Aligned. Score: 69.10
Sequences (2:6) Aligned. Score: 98.31
Sequences (3:4) Aligned. Score: 67.98
Sequences (3:5) Aligned. Score: 70.22
Sequences (3:6) Aligned. Score: 94.94
Sequences (4:5) Aligned. Score: 94.54
Sequences (4:6) Aligned. Score: 66.85
Sequences (5:6) Aligned. Score: 69.10

FIG. 15

Date Reçue/Date Received 2022-03-29

ClustalW alignment of PRRSV E polypeptide sequences

```
 53 MGSIQSLFDKIGQLFVDAFTEFLVSIVDIIIFLAILFGFTIAGWLVVFCIRLVCSAVFRA 60
 54 MGSIQSLFDKIGQLFVDAFTEFLVSIVDIIIFLAILFGFTIAGWLVVFCIRLVSSAVFRA 60
 52 MGSMQSLFDKIGQLFVDAFTEFLVSIVDIIIFLAILFGFTIAGWLVVFCIRLVCSAILRT 60
 55 MGSMQSLFDKIGQLFVDAFTEFLVSIVDIIIFLAILFGFTIAGWLVVFCIRLVCSALRRP 60
 57 MGSMQSLFDKIGQLFVDAFTEFLVSIVDIIIFLAILFGFTVAGWLVVFCIRLVFSAVLRA 60
 56 MG---SLWSKISQLFVDAFTEFLVSVVDIAIFLAILFGFTVAGWLLVFLLRVVCSALLRS 57
 58 MG---SLWSKISQLFVDAFTEFLVSVVDIVIFLAILFGFTVAGGLLVFFLRVVCSAILRS 57
    **   **:.**.************:***  *********:** *:** :*:* **: *.

 53 RPAIHPEQLQKIL 73
 54 RPAIHPEQLQKIL 73
 52 RPAIHPEQLQKIL 73
 55 ----AH-EQLQKIL 69
 57 RSTVHPEQLQKIL 73
 56 RSAIHSPELSKVL 70
 58 RSAIHSPELSKIL 70
       *  :*.*:*
```

```
Sequences (1:2) Aligned. Score: 94.52
Sequences (1:3) Aligned. Score: 93.15
Sequences (1:4) Aligned. Score: 82.61
Sequences (1:5) Aligned. Score: 72.86
Sequences (1:6) Aligned. Score: 90.41
Sequences (1:7) Aligned. Score: 74.29
Sequences (2:3) Aligned. Score: 98.63
Sequences (2:4) Aligned. Score: 81.16
Sequences (2:5) Aligned. Score: 70.00
Sequences (2:6) Aligned. Score: 90.41
Sequences (2:7) Aligned. Score: 70.00
Sequences (3:4) Aligned. Score: 79.71
Sequences (3:5) Aligned. Score: 68.57
Sequences (3:6) Aligned. Score: 90.41
Sequences (3:7) Aligned. Score: 68.57
Sequences (4:5) Aligned. Score: 63.77
Sequences (4:6) Aligned. Score: 89.86
Sequences (4:7) Aligned. Score: 60.87
Sequences (5:6) Aligned. Score: 71.43
Sequences (5:7) Aligned. Score: 92.86
Sequences (6:7) Aligned. Score: 71.43
```

FIG. 16

Date Reçue/Date Received 2022-03-29

ClustalW alignment of PRRSV gp5a polypeptide sequences

```
63 MFKYVGELLDRGLLLAIAFFVVYRAVLFYCARQRQRKQQLLLPVDLQLDAM 51
64 MFKYVGEMLDRGLLLAIAFFVVYRAVLFHCARRRQRQQQLSSAIDLQLDAM 51
62 MFKYVGEVLDRVLLLAIAFFVVYRAVLSCCARQRQQQQQLSYSVDL----- 46
65 MFKYVGEMLDRGLLLTIAFFVVYRAVLVCCARQSRKRQQLPLTVDI----- 46
   *******:*** ***:***********  ***: :::***  .:*:
```

```
Sequences (1:2) Aligned. Score: 80.43
Sequences (1:3) Aligned. Score: 80.43
Sequences (1:4) Aligned. Score: 73.91
Sequences (2:3) Aligned. Score: 84.31
Sequences (2:4) Aligned. Score: 76.09
Sequences (3:4) Aligned. Score: 71.74
```

FIG. 17

Date Reçue/Date Received 2022-03-29

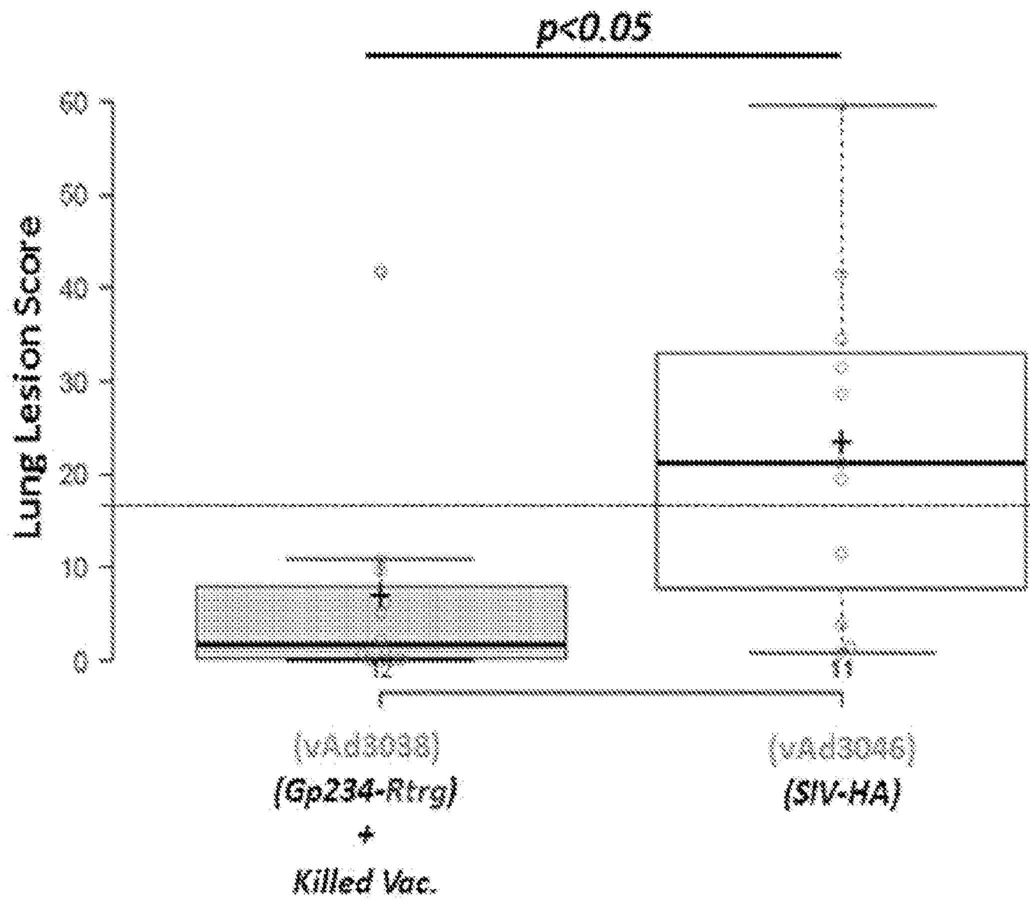


FIG. 18

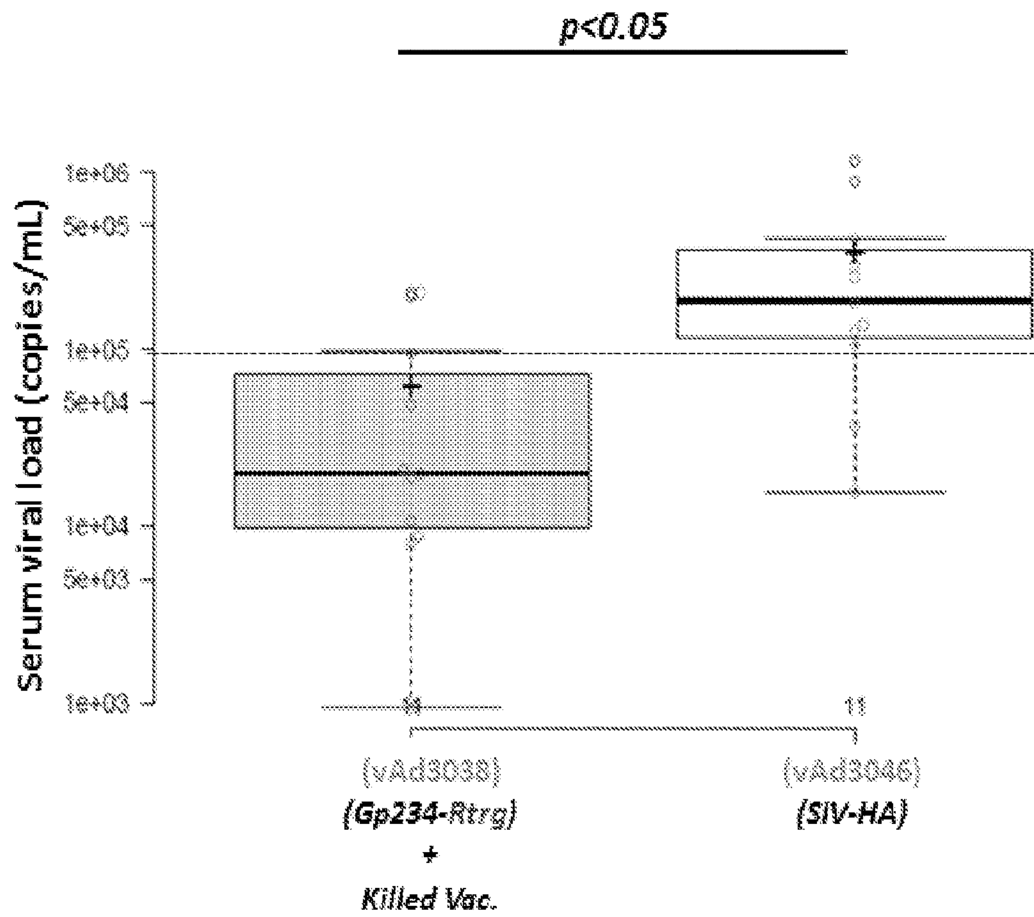


FIG. 19

Date Reçue/Date Received 2022-03-29

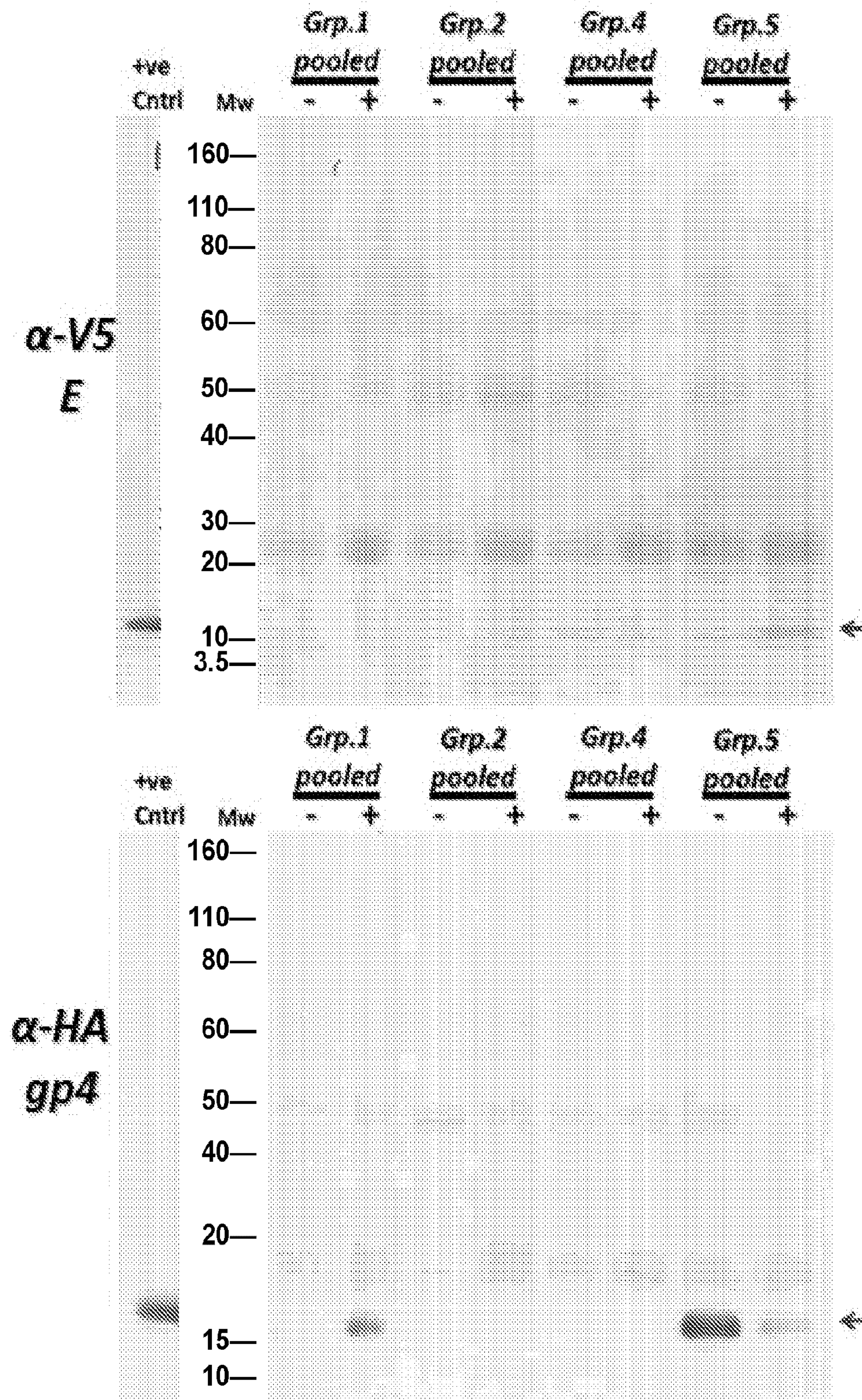
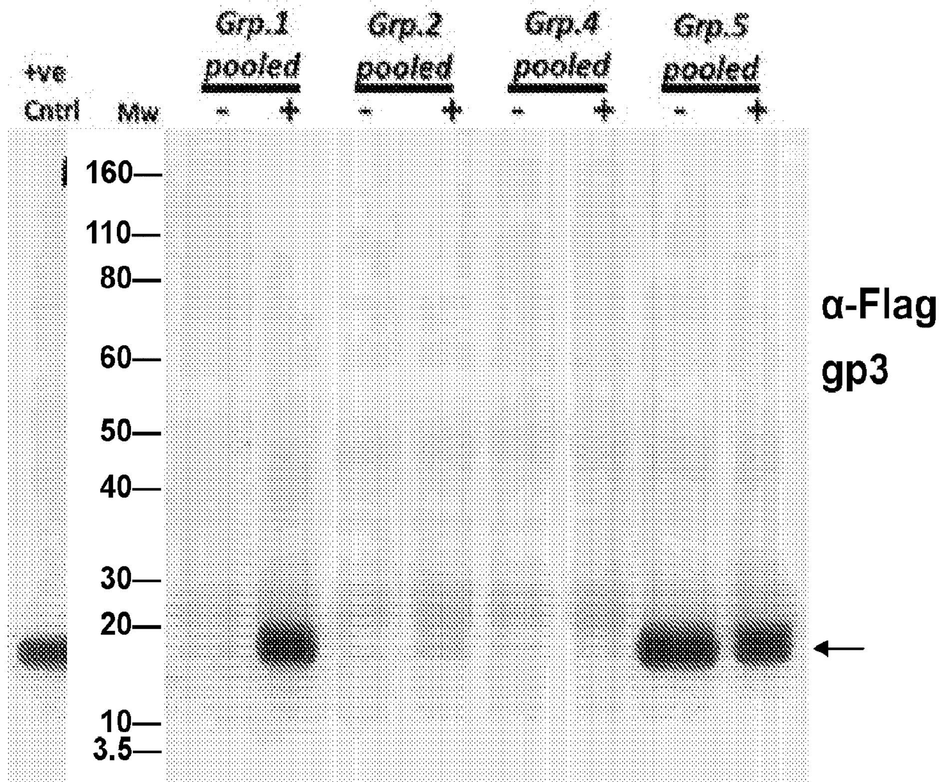


FIG. 20

Date Reçue/Date Received 2022-03-29



Grp.1 → Gp234-Rtrg.

Grp.1 → Gp234-Rtrg.

Grp.1 → Gp234-Rtrg.

Grp.1 → Gp234-Rtrg.

- : before challenge

+ : after challenge

Cell lysate (HEK-293T) co-transfected with sgp2, sgp3, sgp4 and E-v5 were IPed with pooled sera (50 µL/mL of lysate) and probed with anti-tag antibodies

FIG. 20 (Continued)

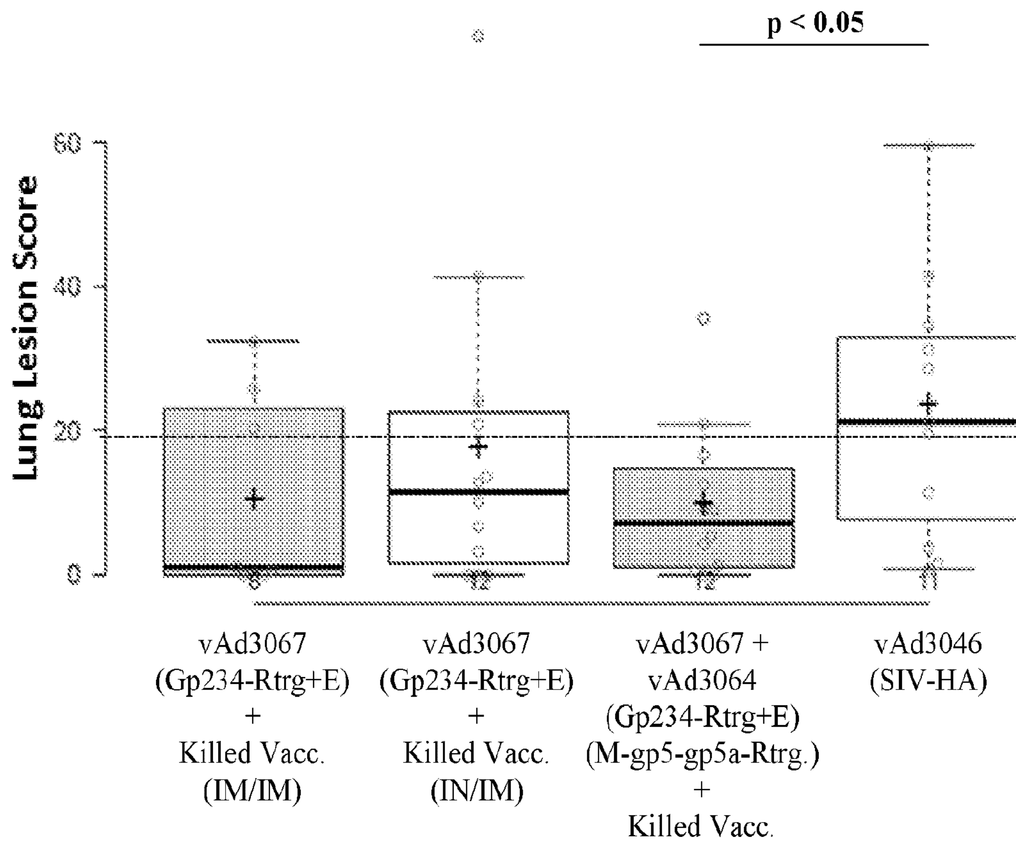


FIG. 21

Date Reçue/Date Received 2022-03-29

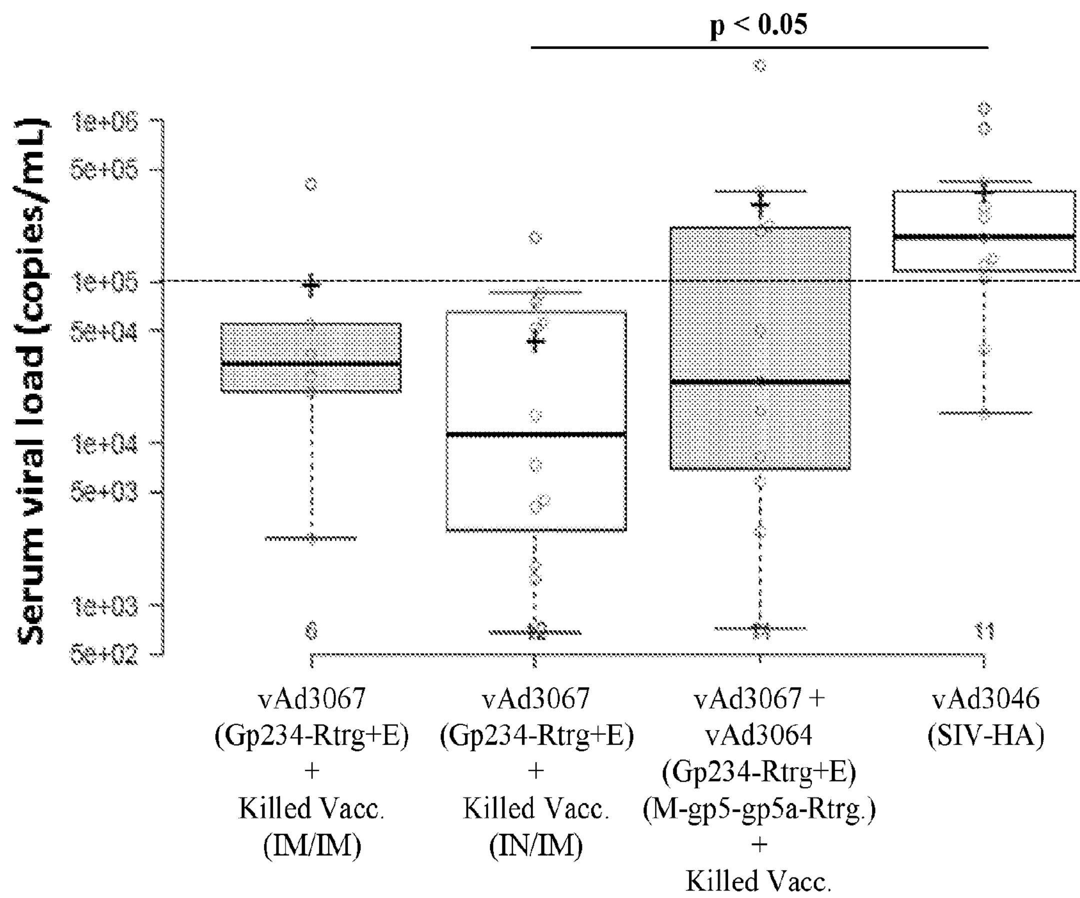
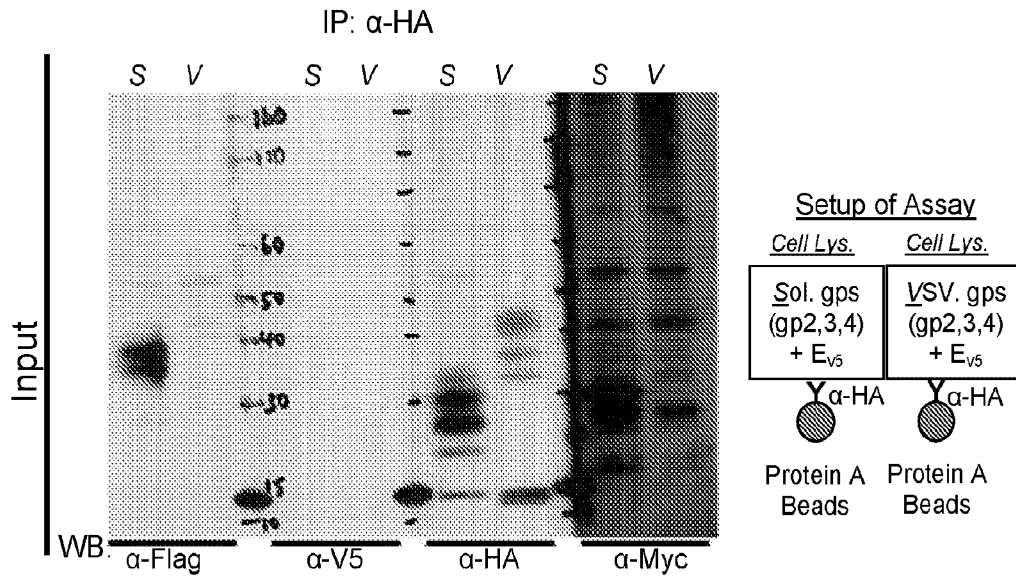


FIG. 22

Date Reçue/Date Received 2022-03-29



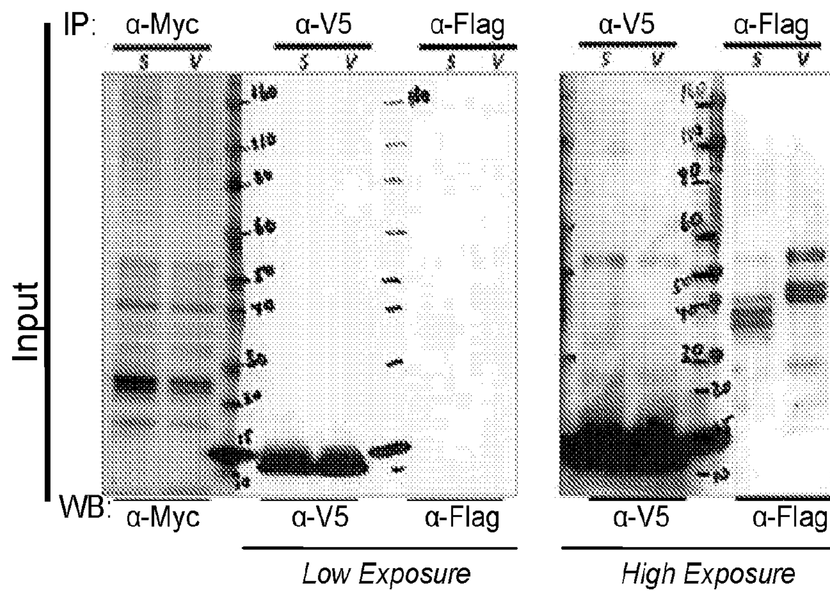


FIG. 23

Date Reçue/Date Received 2022-03-29

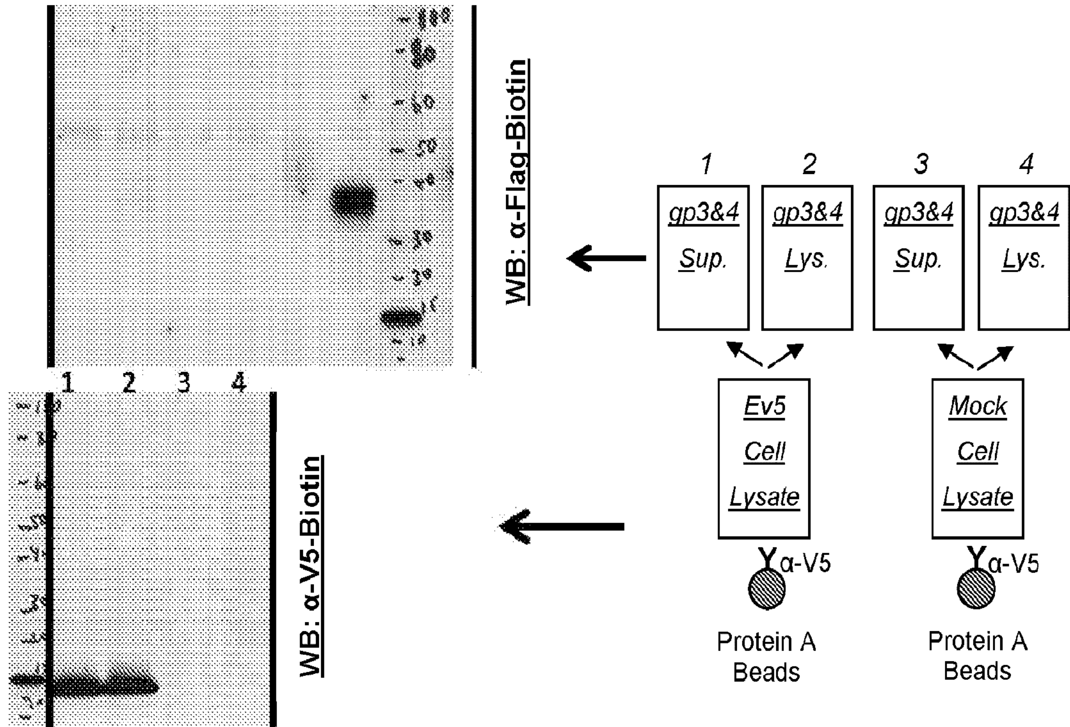


FIG. 24

Date Reçue/Date Received 2022-03-29